Home Office

Fingerprint Source Book v2.0 (second edition)

CAST Publication 081/17

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November 2017

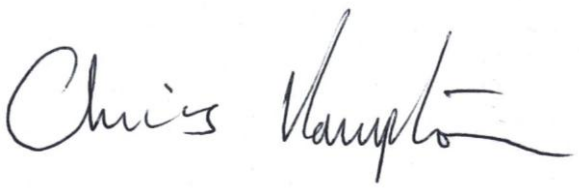
Authorisation

This Fingerprint Source Book has been signed off to my satisfaction and is authorised for circulation, as appropriate.

CAST recognises that the information contained within it, while believed to be correct at the time of writing, may be subject to change as more information becomes available. We would welcome feedback from those using the book, which will be subject to regular review to incorporate appropriate changes.

Chris Rampton, Chief Technical Officer (Home Office CAST)

Signature



Date 24th October 2017

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Preface to the Source Book v2.0 (January 2017)

Since the publication of the *Fingerprint Source Book (v1.0)* in October 2011, the forensic landscape has changed. The Forensic Science Service, at that point the largest UK forensic service provider, was closed in 2012. None of the remaining forensic service providers in the UK market offer a service in fingermark visualisation, nor do they conduct research and development in this area. As a consequence, operational fingermark visualisation work is now conducted almost exclusively by police laboratories.

Police laboratories themselves have undergone a period of change. Several forces have responded to reducing budgets by collaborating and amalgamating laboratory facilities, resulting in a reduction in the number of operational facilities. Reducing budgets has also resulted in police laboratories conducting a wider range of other activities (such as DNA swabbing) in house, increasing the emphasis on developing integrated strategies for forensic recovery. The laboratories that remain have now all achieved ISO 17025 accreditation for fingermark enhancement, and CAST itself achieved this accreditation in 2012, demonstrating that it was possible to validate and receive accreditation for all of the principal processes within the *Fingermark Visualisation Manual*. CAST retired from this accreditation in December 2016 following discussions with UKAS and the Forensic Science Regulator, because accreditation is not required for research and development activities and CAST do not conduct sufficient operational work to meet the requirements of the standard.

CAST itself will be undergoing organisational change with the announcement in January 2017 that CAST will merge with the Defence Science and Technology Laboratory (Dstl) of the Ministry of Defence (MOD). This merger will take place over the next couple of years and offers synergies between CAST's current research activities and operational forensic work that Dstl conducts for MOD.

In January 2014, CAST published the *Fingermark Visualisation Manual*. This was initially to have been the *Manual of Fingerprint Development Techniques (3rd edition),* but it was decided that the significant change in content and approach taken merited a re-launch under a new name. The *Fingermark Visualisation Manual has* now been rolled out to and implemented by all UK police forces. This revision to the *Fingerprint Source Book (v2.0)* has been conducted with the intention of providing consistency between the two publications, for example all visualisation processes appearing in the *Fingermark Visualisation Manual* are covered in the *Fingerprint Source Book v2.0*, and the chapters in the *Fingerprint Source Book v2.0* have been reordered to make them consistent with where they appear in the *Fingermark Visualisation Manual.*

In common with the comments made in the Preface of v1.0 of the *Fingerprint Source Book*, research continues to be conducted worldwide and it cannot be guaranteed that the information contained in this document is entirely up to date. Due to the extensive nature of

the external review required for the *Fingerprint Source Book*, further reviews to this document will be by exception rather than part of a regular review process.

Finally, the authors would like to express their gratitude to all of their colleagues in the fingerprint community that have assisted with the peer reviews of versions 1.0 and 2.0 of the *Fingerprint Source Book*. Without their time, patience and helpful comments it would not have been possible to produce this document. Particular thanks are due to:

v1.0
Dr Karen Stow (then Scientific Support Manager, Derbyshire Constabulary, currently at Forensic Focus, and President of the Fingerprint Society)
Terry Kent (former Sector Manager, Crime Investigation, Police Scientific Development Branch and Editor of the *Manual of Fingerprint Development Techniques*, 1st and 2nd editions)
Dr Della Wilkinson, Forensic Science & Identification Services
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v2.0
Terry Kent (former Sector Manager, Crime Investigation, Police Scientific Development Branch and Editor of the *Manual of Fingerprint Development Techniques*, 1st and 2nd editions)
Nick Marsh (Metropolitan Police Service)
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Dr Ben Jones (Abertay University)
Dr Melanie Bailey (University of Surrey)
Paul Deacon (Forensic Practitioner – Dundee)

1. Introduction

The Fingerprint Source Book is primarily intended to provide the background and validation for the techniques currently (up to 2016) recommended by the Home Office Centre for Applied Science and Technology (CAST), and to publish, in some cases for the first time, data collected over 45 years of research. It will therefore often present information in an 'CAST-centric' way, emphasising research that was carried out at Sandridge or Horseferry House, possibly sometimes at the expense of research carried out elsewhere. It is not the intention of the authors to ignore the significant contributions made by other research groups and apologies are made in advance if this sometimes appears to be the case. The document is also aimed at providing the UK Forensic Science Regulator and the United Kingdom Accreditation Service (UKAS), which has carried out ISO 17025 accreditation in the UK, with the background evidence behind the advice given in the Fingermark Visualisation Manual .

The priorities of CAST in issuing and supporting the Fingermark Visualisation Manual are to provide processes that are highly effective, safe to use, and can be applied by staff who are not necessarily highly-qualified scientists. When developing formulations the approach is to maintain the effectiveness of the process while minimising or eliminating any components that have health and safety issues associated with them. In some cases, more effective formulations or processes may be available, but if they are not felt to be safe to use they will not be recommended.

It should be emphasised that all testing and optimisation of processes by CAST has been carried out under UK climatic conditions. It is recognised that in many parts of the world the conditions of temperature and humidity will differ significantly from those in the UK and in some cases this may affect performance. It is likely that optimised formulations in different countries may differ for this very reason.

Throughout the report, references are made to the two main fingerprint research groups in the UK, CAST and the Forensic Science Service (FSS), now closed. These organisations changed names several times in the 45 years covered, and in the text reference is usually made to the name of the group at that time. However, inconsistencies may arise. In summary, previous names for each organisation have been:

CAST:

Police Research and Development Branch (PRDB) 1969 -1971

Police Scientific Development Branch (PSDB) 1971 - 1981

Home Office Scientific Research and Development Branch (HO SRDB) 1981 -1991

Police Scientific Development Branch (PSDB) 1991 - 2004

Home Office Scientific Development Branch (HOSDB) 2004 - 2011

FSS:

Home Office Central Research Establishment (HO CRE) 1967 – 1988

Home Office Forensic Science Service (FSS), Central Research and Support Establishment (CRSE) 1988 - 1992

Aldermaston Laboratory closed (1992), Metropolitan Police Forensic Support Laboratory (MPFSL) absorbed by the FSS (1996). Fingerprint research subsequently split between FSS Lambeth and FSS Trident House, Birmingham and ceased in 2012 on closure of the Forensic Science Service.

A significant amount of early research was also conducted by the Atomic Weapons Research Establishment (AWRE) under contract to CAST.

Colour separation processes (multispectral imaging, monochromatic illumination and colour filtration)

1. History

1.1 Multispectral imaging is a technology originally developed for aerial photography, and describes a system capable of simultaneously capturing spectral as well as spatial information. The spectral information can be used to distinguish between areas of nominally similar appearance, e.g. identifying different types of crops or vegetation by the differences between their reflected light spectra. More recently, the technique has been applied to other scientific disciplines, in particular medical imaging. In this application, the spectral information captured by multispectral imaging has been used to differentiate between different types of cell/tissue stained with coloured or fluorescent markers. Multiple stains can be used on a single sample and multispectral imaging used to identify the distribution of each in turn.

1.2 The potential of the technique for forensic applications became recognised in the late 1990s. Exline *et al.* [1] demonstrated that:

- chemically treated fingermarks could be imaged in both absorption and fluorescence modes using multispectral imaging systems; and
- the improved spectral resolution obtained revealed more ridge detail than conventional imaging routes.

In some cases, latent untreated fingermarks could be detected on coloured paper by multispectral imaging alone. Later studies by the same group demonstrated that the technique could be applied to a wide range of treated fingermarks, and faint ninhydrin marks in particular could be significantly enhanced by this method [2].

1.3 Initial forensic studies utilised multispectral imaging systems operating in the visible/near infrared (IR) regions of the spectrum. Later studies [3,4,5] demonstrated that multispectral imaging systems operating further into the IR region could also be used to resolve fingermarks, in this case using specific chemical vibrations from species present in fingermark ridges to resolve the print against a patterned background.

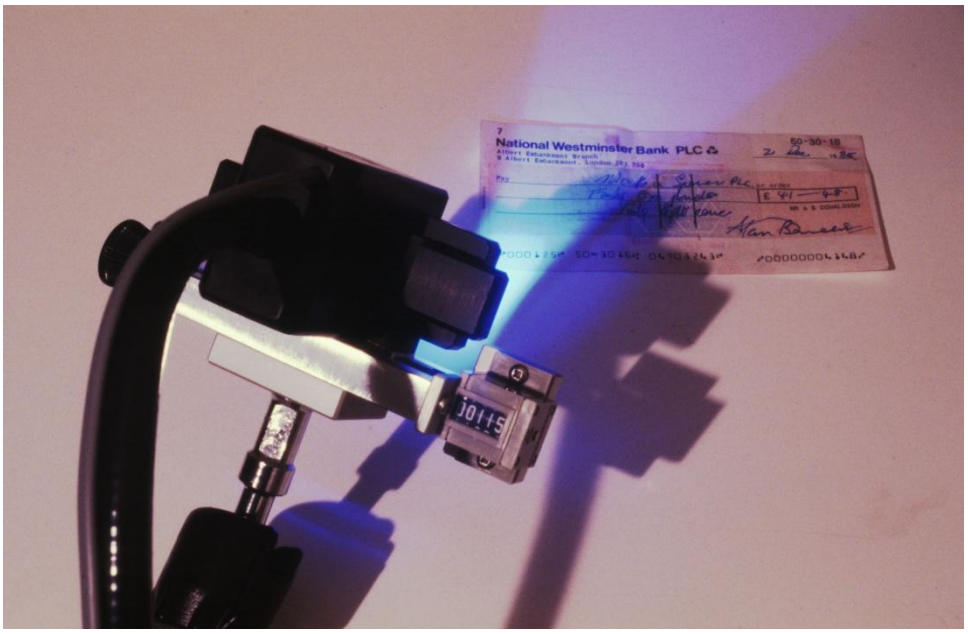
1.4 Subsequent studies using both visible and IR multispectral imaging demonstrated that the technique could also be applied to document examination, including:

- ink discrimination;
- paint analysis;
- detection of gunshot residue and fibre analysis; and
- recently the detection, identification and age estimation of blood stains [6,7,8,9,10,11].

1.5 The Home Office Centre for Applied Science and Technology (CAST) purchased a multispectral imaging system in 2006 and initially confirmed the results of the Australian researchers for a range of different

fingermark development techniques [12]. The technique has since been applied to some operational cases, demonstrating that fingermarks can be successfully resolved against coloured/patterned backgrounds by means of their characteristic spectral response.

1.6 Monochromatic illumination is a refinement of simple colour filtration. It is closely related to multispectral imaging in terms of using spectral differences to differentiate between chemically treated fingermarks and similarly coloured backgrounds. Monochromators function by splitting white light into narrow spectral bands, and may utilise prisms, variable diffraction gratings or variable interference filters to achieve this. In the fingermark imaging application, a monochromator is used in conjunction with a white light source to illuminate an exhibit with a narrow portion of the visible spectrum. By choosing a region of the spectrum that matches and suppresses reflected light from the background, fingermarks may be resolved. CAST first investigated this approach for the imaging of fingermarks developed using ninhydrin against the patterned backgrounds of cheques in the late 1980s [13] and developed the Quaserchrome monochromator accessory for use with the Quaser 100 and Quaser 40 at about the same time. More recently, monochromators have been provided as an integral part of the Integrated Rapid Imaging System (IRIS) workstation [14] developed and manufactured by CAST.



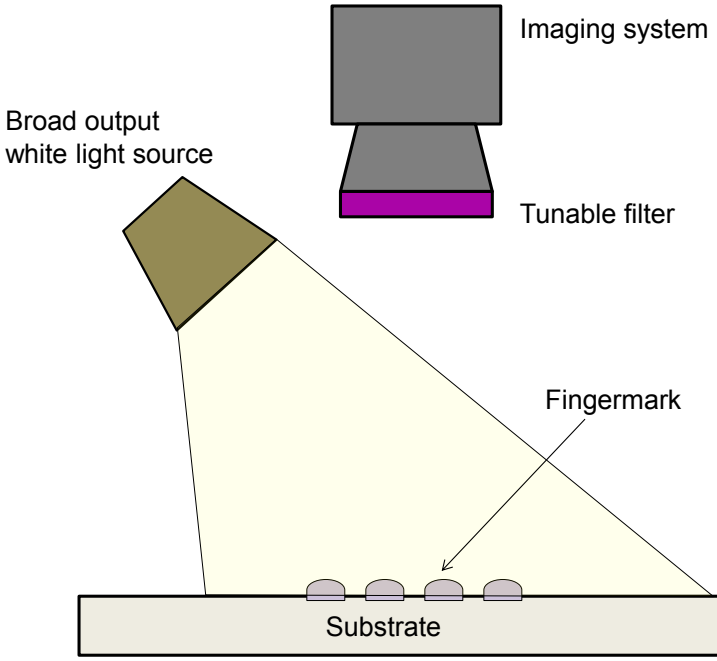
Prototype monochromator under test.

2. Theory

2.1 Multispectral imaging describes a range of techniques that all ultimately result in the capture of a digital image with spectral information associated with each pixel of that image. Such information may be obtained using either a single sensor capturing spectral information,

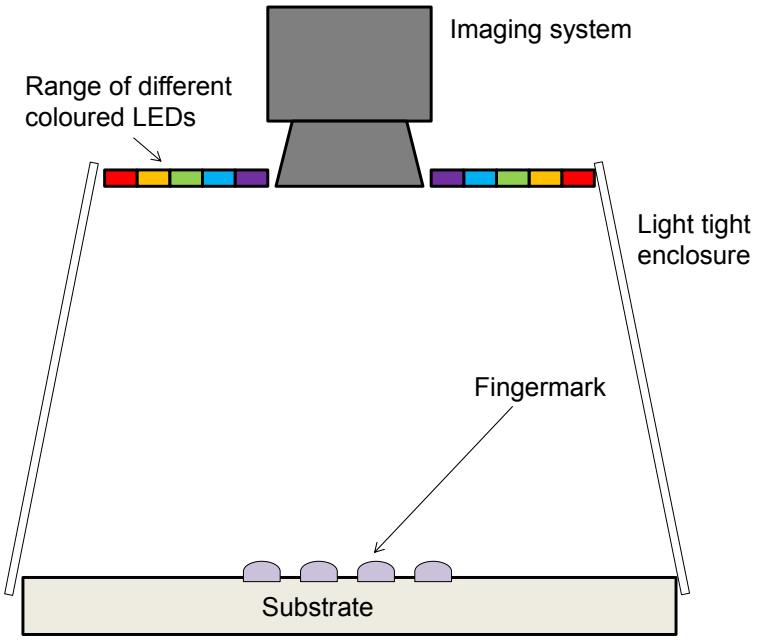
which is then scanned across the area of interest [3], or an array of such sensors capable of capturing spatial and spectral information simultaneously [15,16,17,18]. These approaches are most often used for multispectral imaging in the IR region of the spectrum.

2.2 An alternative approach, more commonly used for multispectral imaging in the visible – near-IR – region, is to use a monochrome sensor array in combination with a tunable filter. Different tunable filter technologies are available, including liquid crystal and acousto-optical, but both types operate in essentially the same way. The tunable filter is a narrow bandwidth bandpass filter (typically with bandwidth in the range 2 to 20 nm) for which the centre point of the bandpass can be controlled within the selected wavelength range. When carrying out multispectral imaging, the exhibit is illuminated with an appropriate light source and the tunable filter programmed to capture monochrome images at set wavelength intervals over the selected wavelength range.



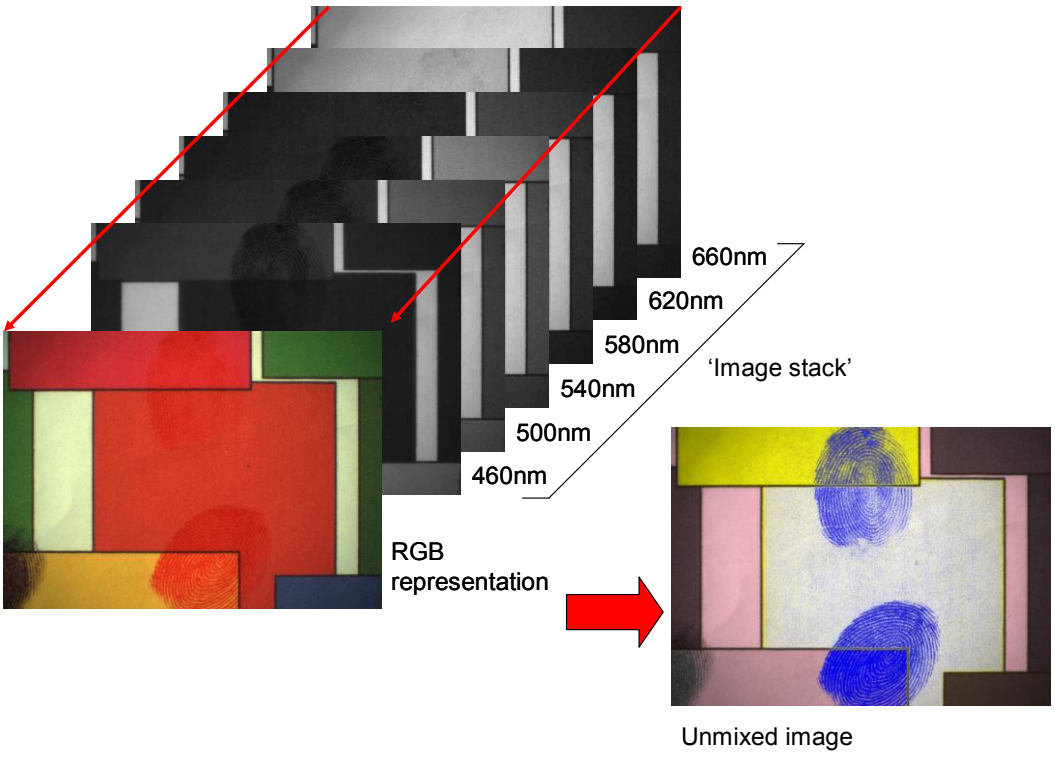
Schematic diagram of the arrangement typically used for multispectral imaging using a system incorporating a tunable filter.

2.3 Alternatively, a similar set of images can be produced by illuminating the sample with a series of LEDs outputting at different wavelengths, controlling the light so that only the wavelengths from the LEDs falls onto the item. This may not be able to obtain the fine discrimination between wavelengths obtained using tunable filters, but can cover larger wavelength ranges with less restriction on the area that can be imaged.



Schematic diagram of the arrangement typically used for multispectral imaging using a system incorporating a range of different wavelength LEDs.

2.4 The series of monochrome images thus collected are known as an 'image cube' (or 'image stack') and can be interpreted by software to give a red-green-blue (RGB), i.e. a colour representation of the exhibit. An example is illustrated below.



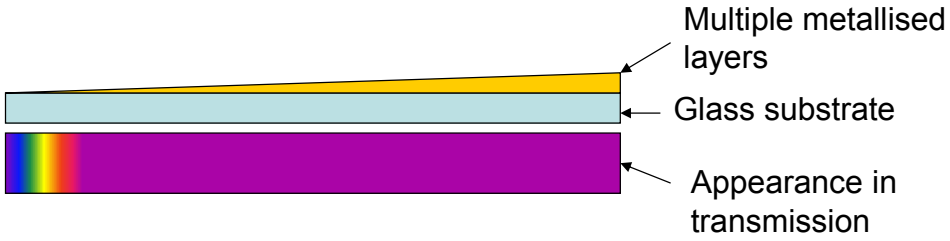
An example of an 'image cube' collected by a multispectral imager, the corresponding red-green-blue representation and unmixed image.

2.5 Once the image cube has been obtained, a range of processing techniques can be applied to the spatial and spectral information to extract the desired information. In the simplest form of analysis, regions with the desired spectrum (e.g. ninhydrin) can be identified, as can regions of unwanted background colour/pattern. These can be assigned false colours and the image unmixed to show the fingermark in greater contrast. Alternatively, the regions corresponding to each colour channel can be viewed individually to see if any of these show the fingermark more clearly than the unmixed image.

2.6 For images with multiple colours in the background and the fingermark crossing several colour boundaries, other approaches can be adopted, such as:

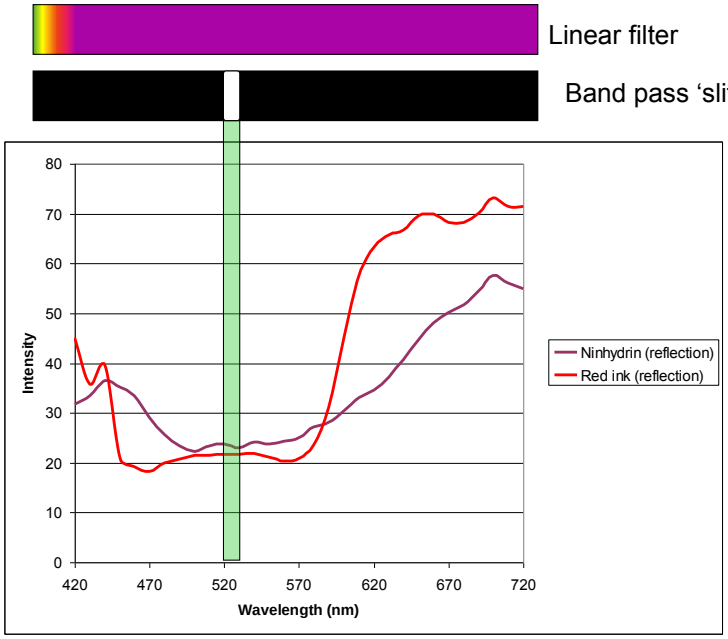
- principal component analysis (PCA), to separate out the major spectral responses from the image; or
- a pure spectrum, calculated from a subtraction from regions of background and mixed background/fingermark spectrum.

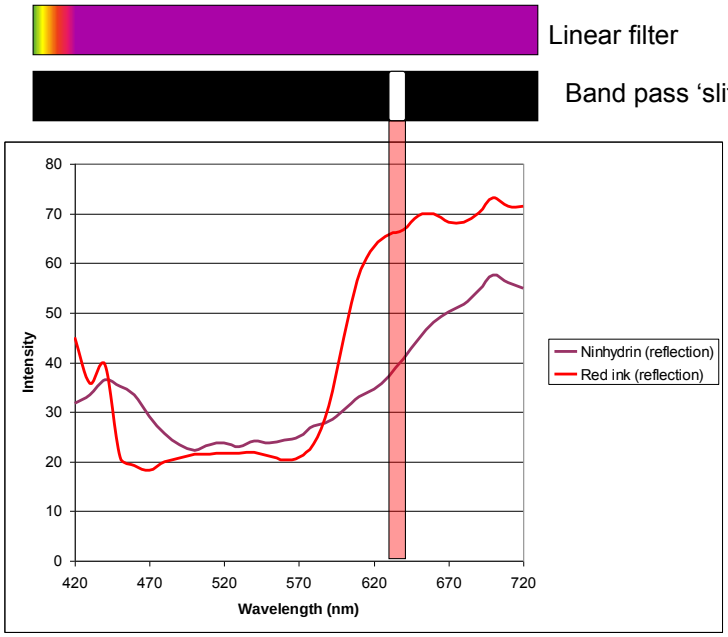
2.7 Monochromatic illumination is an older and less versatile technique for revealing fingermarks on coloured backgrounds. In this technique a high intensity white light source is used in combination with a narrow bandwidth (~25 nm) variable interference filter (typically a glass filter coated with a progressively thinner metal layer, giving a different transmitted colour along its length, e.g. the Schott glass Veril filter).



Schematic diagram showing the typical construction of a linear filter.

2.8 The colour of the incident light can be controlled by moving the filter in front of a slit in the path of a light guide leading from the white light source. The objective is to match the incident colour with that of the printed background, thus suppressing it. If there is sufficient colour difference between the fingermark and the background, this technique can be particularly effective.





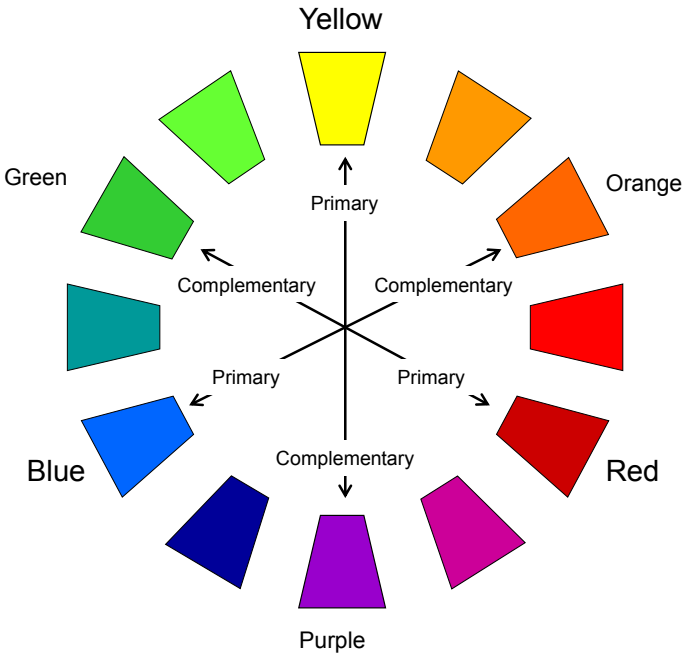
Schematic diagrams showing how monochromatic illumination in the (top) *green region of the spectrum gives little discrimination between a purple ninhydrin mark and a red background, and* (bottom) *red illumination gives better contrast between the reflectivity of the two colours present.*

a) b) c) d)

Mark in ninhydrin developed on patterned background viewed a) *with red monochromatic illumination* b) *with orange monochromatic illumination* c) *with green monochromatic illumination and* d) *white light illumination.*

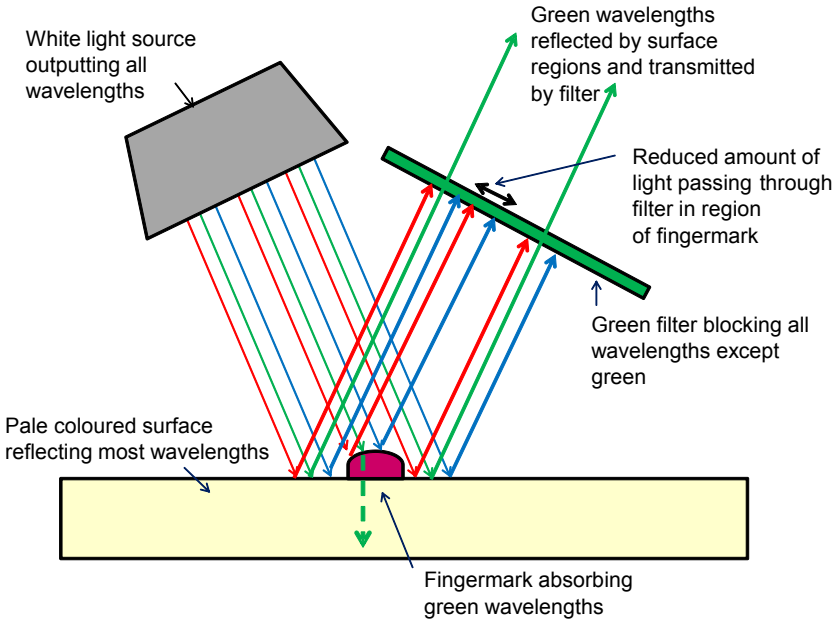
2.9 Disadvantages of monochromatic illumination compared with multispectral imaging are that the monochromator can only suppress one colour at a time and on multicoloured surfaces not all of the background will be removed. In addition, the bandwidth of the monochromator is generally broader than that achievable with tunable filters, and spectral differentiation will be correspondingly less.

2.10 The oldest and simplest of the processes that utilise colour to increase contrast between fingermarks and the background is colour filtration. Colour filtration uses the concept of a colour wheel based on three primary colours (red, blue, yellow) and their complementary colours (green, orange and purple, formed by combining the other two primary colours).



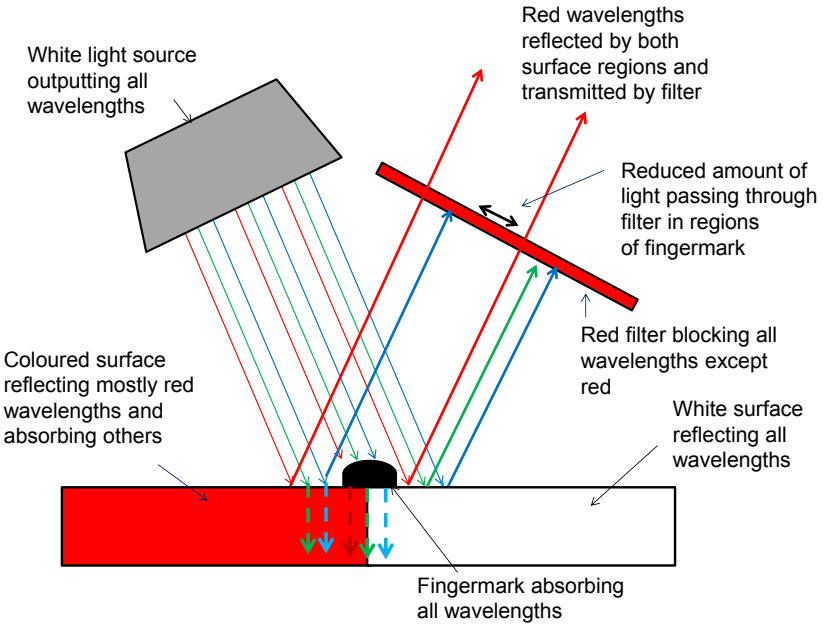
Schematic diagram of a colour wheel.

2.11 The colour wheel can be used to identify coloured filters that complement or contrast with the colours present in either the fingermark or the background, making the mark easier to discriminate. For coloured marks on a light background, filters that contrast with the colour of the mark can be used to make it darker in relation to the background.



Schematic diagram showing the darkening of a coloured mark on a light surface using a filter of contrasting colour.

2.12 Similar contrast enhancement can be produced where light coloured marks are present on a coloured background. A filter of contrasting colour to the background can be used to darken it in relation to the light coloured mark, making it more visible. Filters can also be of benefit where dark coloured marks are present on multicoloured backgrounds. By using a filter that matches one of the background colours, the continuity of the fingermark ridges across colour boundaries can be better discriminated.



Schematic diagram showing the discrimination of a dark coloured mark on a multicoloured background using a filter of complementary colour to the background.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014). The purpose of this manual is to report the history, theory and validation work associated with the process. Multispectral imaging, monochromatic illumination and colour filtration are all included in the manual, primarily as methods for increasing the amount of visible ridge detail in developed marks. The use of these processes (and other optical methods) is actively encouraged after each chemical or physical visualisation process in order to enhance the visibility of any marks.

3.2 CAST is in the process of conducting a comparison of multispectral imaging processes for the capture of developed fingermarks. In the studies to date (2016), the Cambridge Research and Instrumentation (CRi) 'Nuance' system has been utilised. This consists of a 1 megapixel digital camera integrated with a CRi 'Varispec' liquid crystal tunable filter with 7 nm bandwidth and wavelength range 420 to 720 nm. The 'Nuance' system has been fitted with a C-F mount adaptor and Nikon 105 mm macro lens, bringing the camera sufficiently close to the exhibit such that the capture resolution is in excess of 500 ppi. The exhibit is illuminated with a tungsten light source, and brought into sharp focus using the lens, viewing the image of the article on screen in the 'live' mode. The wavelength range of interest is selected, and the tunable filter set to collect a spectrum at 10 nm intervals throughout the selected range. The filter is then set to calculate automatically the optimum exposure for each wavelength, and finally set to acquire the image cube. The operator can then carry out a variety of analysis tasks on the data contained in the

image cube, such as PCA or calculation of pure spectra in order to separate the fingermark from the background. For evidential purposes, it is recommended that the RGB representation of the exhibit, the unmixed image, the image associated with each spectral channel and the spectral information are retained. The wavelength range and step interval between spectra can be refined to the particular region of interest once the initial image cube has been obtained.

3.3 Comparative studies are currently being conducted with the Videometer VideometerLab3, which has a larger (>4 megapixel) sensor allowing capture of larger areas at high resolution. This utilises a series of LEDs operating at 20 different wavelengths to supply controlled lighting conditions to the item. Although this results in an image cube containing fewer individual images, the wavelength range covered by the LEDs extends from the long wave ultraviolet to the near IR, allowing the collection of data over a wider wavelength range. Like the Nuance, the Videometer requires the use of software functions to produce the best discrimination between the fingermark and background. The process instruction in the *Fingermark Visualisation Manual* is written in a generic way that covers the use of either piece of equipment, and the user will need to develop an understanding of which functions give the best results on the particular instrument that they are using.

3.4 For monochromatic illumination using the Quaserchrome, the accessory should be fitted to the end of the light guide running from the Quaser light source. The light source should be set to the white light illumination mode, using the appropriate key to override the interlock. The dial on the Quaserchrome can then be rotated to move the filter in front of the slit in the path of the light guide, and the change in colour output can be observed by eye. Because the output is only a small region of the visible spectrum, the power is much reduced from normal Quaser excitation filters and consequently it is not necessary to wear viewing goggles. The mark to be imaged is viewed by eye as the illumination colour is varied, and the colour giving optimum contrast between the fingermark and the background is selected for any subsequent photography.

3.5 The procedure using the integrated monochromator on IRIS is very similar. The light source selector switches should be set to the Quaser 2000, which should have ‘white light’ selected as the excitation band. The monochromator dial should then be turned until the orange light on the front of the Quaser 2000 is illuminated. Pressing the orange light illuminates the Quaser in the violet region of the spectrum; the illumination colour can be varied by continuing to turn the monochromator dial. The optimum illumination colour is either determined by eye or by viewing the semi-live image on the computer monitor.

3.6 Colour filtration is straightforward and is generally most appropriate for use on digital single lens reflex (SLR) cameras where the filter can be directly attached to the lens. Selection of the filter is carried out by direct

observation of the surface and the fingermark, selecting a filter of contrasting or complementary colour depending on the effect being aimed for. Yellow, orange and red filters are widely available in the form of the long pass filters used in fluorescence examination, other coloured filters giving contrast with different portions of the visible spectrum can be obtained in colours including green, blue and magenta.

4. Critical issues

4.1 For multispectral imaging, it is essential to ensure that the sample is as evenly lit as possible and that the light source used to illuminate it has a continuous output across the spectral range of interest. Best results for spectral discrimination are obtained from systems with narrow bandwidth filters and by collecting images at many closely spaced wavelengths.

5. Application

5.1 Suitable surfaces: The principal application for multispectral imaging and monochromatic illumination is in revealing developed fingermarks on articles with patterned and/or multicoloured backgrounds, as may be encountered on exhibits such as banknotes. The best results are obtained when the development process itself produces marks of a characteristic colour.

Mark developed using ninhydrin on the patterned, multicoloured background of a £20 note.

5.2 Both techniques can be used to exploit differences between the colour spectrum of the developed fingermark and the background printing. Monochromatic illumination is effective if only one background colour is prevalent, whereas multispectral imaging can be applied where many colours are present.

5.3 Both techniques are applied after chemical treatment to produce a coloured or fluorescent product. They are non-destructive, only involving illumination of the exhibit with a relatively low power light source.

6. Alternative formulations and processes

6.1 It is also possible to remove regular patterned backgrounds by means of fast Fourier transforms, a digital filtering technique. A Fourier transform is taken of the digital image and the features associated with the patterned background are identified. These are then masked and the inverse transform is taken, which suppresses the background pattern in the image and may reveal additional detail in the more irregular fingermark [19,20]. However, fast Fourier transforms are most effective where the pattern is regular, and this is often not the case for many regions of banknotes. In these situations multispectral imaging or monochromatic illumination may yield better results.

6.2 The fact that modern digital SLR cameras capture images in three colour channels (red, blue and green) also presents the possibility of applying a form of colour filtration to the image post-capture by the use of packages such as Photoshop. This can negate the need to use a physical filter in front of the camera. A skilled practitioner is required to get the optimum results from this type of image processing, and it will be essential to keep an audit trail of all of the processing steps applied to the original image.

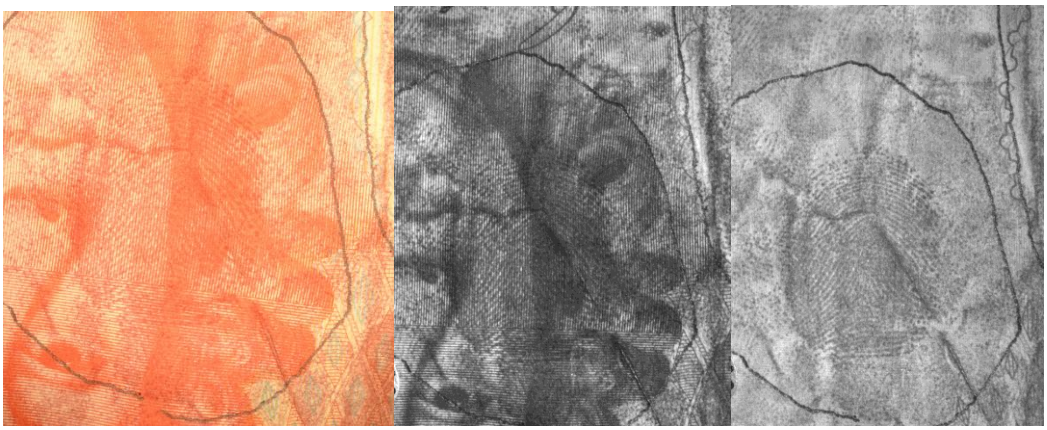
7. Post-treatments

7.1 There are no post-treatments used with monochromatic illumination or colour filtration. The principal post-treatment used with multispectral imaging is the analysis software used to extract the spectrum of the fingermark reagent from those of the background.

8. Validation and operational experience

8.1 Multispectral imaging, monochromatic illumination and colour filtration are non-destructive optical techniques and can be used during a sequential process without detriment to subsequent treatments. As a consequence there is less of a requirement to conduct comparative laboratory experiments with other techniques or operational trials before implementing them on operational work.

8.2 Monochromatic illumination has been in use for 20 years on operational work. It is known to work well with ninhydrin marks on the Bank of England £50 note, the former ‘Edward Elgar’ £20 note and on some regions of the £10 note.



a) b) c)

Fingermark developed using ninhydrin on a £50 note a) *colour image illuminated with white light* b) *monochrome image illuminated with green monochromatic light and* c) *monochrome image illuminated with red monochromatic light.*

8.3 Since 2007 multispectral imaging has been used on selected operational cases where marks have been developed but cannot be resolved by monochromatic illumination, IR imaging or digital filtering techniques such as fast Fourier transforms. In several of these cases entire marks or additional ridge detail have been revealed, leading to successful identifications and convictions.

a) b)

Fingermark in coloured contaminant found on a £10 note a) *red-green-blue image and* b) *unmixed image showing fingermark extracted from the background by spectral characteristics* (reproduced courtesy of Nick Marsh, Metropolitan Police Service)*.*

9. References

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Fluorescence examination

1. History

1.1 The use of fluorescence examination for the enhancement of developed fingermarks was being considered as early as the 1930s, with materials such as anthracene and zinc sulphide being proposed for use as fingermark development powders. Both of these powders photoluminesce when illuminated with ultraviolet (UV) radiation and this effect could be used to enhance the contrast of a mark against the background. In the case of zinc sulphide (in practice impure zinc sulphide containing a dopant), the longer term phosphorescence of the material could also be utilised by imaging the mark once the light source had been removed. In 1954, Cherrill [1] described the use of barrier filters in combination with fluorescent powders to reduce the interfering effect of reflected light (and possible autofluorescence) from the background.

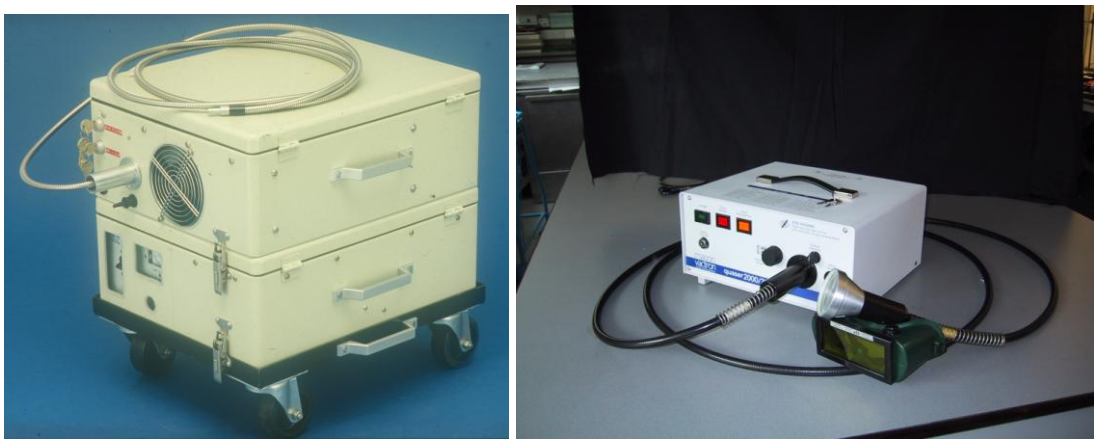
1.2 Most early investigators used UV radiation to produce fluorescence in fingermarks. In 1970 Ohki [2] carried out an investigation into the constituents of fingermarks and found several components that had inherent fluorescence when illuminated with UV radiation. Developments in chemical reagents for fingermark development in the mid-1970s identified several systems that produced reaction products with fluorescence in the visible region when illuminated in the UV, including fluorescamine and o-phthalaldehyde. However, these reagents did not provide any advantages in performance over ninhydrin and were not widely adopted.

1.3 The most significant development in fingermark detection by fluorescence examination was the observation by Dalrymple *et al.* [3] that latent, apparently natural untreated fingermarks could be detected on a range of substrates using a 1.5 W argon ion laser line at 514.5 nm with an appropriate barrier filter. Duff and Menzel were working in the Xerox Mississauga research laboratory and were also involved in using dye lasers in the same laboratory. It is now thought that the high success rates initially observed in these studies may have been attributable to some latent fingermarks being contaminated with laser dyes, however the initial observation was still valid. Fluorescence was also observed by the same authors using a filtered xenon arc lamp, but this light source was of lower power (filtered output measured at 0.5 W) and the fluorescence was correspondingly weaker. The authors recognised the future potential of fluorescent stains and powders, and attempted to enhance marks by powdering with the fluorescent Coumarin 6. Initially this did not reveal any ridge detail, but subsequent spraying with methanol and laser examination showed some powder preferentially adhering to the ridges. Thornton [4] was more successful, dissolving Coumarin 6 in ethanol, mixing with a black powder and then evaporating the ethanol to produce a fluorescent tagged dusting powder.

1.4 Prompted by the promising results of the early work the Ontario Police and the FBI bought large frame argon ion lasers and early results were disappointing; this prompted a search for ways of tagging fingerprints with fluorescent powders or dyes compatible with laser examination to make better use of these expensive laser systems. Menzel and Duff investigated a range of fluorescent powders and chemical reagents [5] and the use of phosphorescent powders in combination with a light chopper to reduce the interfering effect of background fluorescence [6]. This 'time resolved imaging' approach was later explored using a range of substances with long fluorescence decay times.

1.5 Laser examination was also becoming used on operational work and in 1979 Dalrymple [7] was able to record casework successes where laser examination had revealed marks by fluorescence that were not subsequently developed by chemical treatment. Dalrymple also identified the potential of fluorescing the background to enhance the contrast with a non-fluorescent fingermark.

1.6 The published papers attracted worldwide interest in the technique and in 1979 a Police Scientific Development Branch (PSDB) delegation visited Duff and Menzel at the Xerox laboratories and the FBI [8] to assess the technique on a range of test substrates and to discuss its operational applications. Fluorescent marks were detected on low density polyethylene and matt aluminium, and some fainter marks seen on paper and adhesive tape. Overall the process performed poorly in comparison with traditional methods on 19 surfaces and 300 fingermarks. The PSDB group concluded that the coherent output of the laser was not essential to promote fluorescence, just the output power and wavelength specificity, and this initiated the programme of work into filtered light sources that ultimately led to the production of the Quaser 30 in the early 1980s, followed in turn by the Quaser 80, 100, 40 and finally the Quaser 2000.



a) b)

Different generations of Quaser, a) *Quaser 80 and* b) *Quaser 2000.*

1.7 PSDB demonstrated a prototype lamp system to Professor Warrener from Australia and John Watkin from Canada in 1980/81. These other groups also recognised the potential and the significantly lower cost of filtered light sources and research programmes to develop these systems were initiated in both Canada [9] and Australia [10,11]. Comparisons carried out between a filtered light source and a laser [11] indicated little difference in performance between the systems available at that time.

1.8 The PSDB research programme investigated the constituents of fingermarks, in particular those fluorescing under UV and green light [12,13]. Several constituents that fluoresced under laser illumination were detected although it was not possible to conclusively identify all of them. PSDB (by now part of the Home Office Scientific Research and Development Branch) also began a comprehensive programme to assess the optimum excitation and viewing filter combinations for the Quaser filtered light sources, and to devise health and safety guidelines for use with all types of light sources that could be used for fluorescence examination. This included work in close consultation with leading ophthalmologists to ensure that the safety guidance given was directly relevant to the light sources used and to the end application. The culmination of this work was *Fingerprint Detection by Fluorescence Examination – A Guide to Operational Implementation* [14].

1.9 Another advance in fluorescence examination was made by Herod and Menzel [15] during studies into techniques for enhancing fingermarks developed using ninhydrin. It was already known that complexes formed between some metal ions and ninhydrin could result in colour changes to the mark (see Chapter 3, Chemical and Physical Processes, Ninhydrin, reference 13), but not that some of these products were fluorescent. Herod and Menzel found that spraying the ninhydrin marks with zinc chloride resulted in a colour change in the fingermark from purple to orange, and the formation of a fluorescent reaction product excited by the 488 nm line of the argon laser. Subsequent studies showed that zinc toning used after ninhydrin detected significant numbers of additional marks and this became an important sequential processing technique until somewhat superseded by the development of reagents such as 1,8-diazafluoren-9-one (DFO) and 1,2 indandione, which yielded inherently fluorescent reaction products.

1.10 Other refinements to the fluorescence examination process considered were the use of a scanning laser spot to build up a fluorescence intensity map of a surface by Herod and Menzel [16] and the use of narrow bandpass filters in combination with a long-pass barrier filter to improve the contrast between the fluorescing mark and the background by Dalrymple [17].

1.11 Other types of laser were also considered for fluorescence examination and reviews of the options available were made by Menzel [18a,19]. By the early 1980s copper vapour and neodymium:yttrium aluminium garnet

(Nd:YAG) lasers had become available; a number of police forces in the US installed high power copper vapour lasers [18b] and although the copper vapour laser had similar attributes to the argon laser and had the advantage of an additional examination wavelength in the yellow region of the spectrum, the Nd:YAG laser was more portable (albeit with much lower output power at that time) and could be taken to crime scenes.

1.12 Research was also conducted into the use of time-resolved fluorescence imaging, combined with the assessment of reagents capable of developing marks with a longer fluorescence decay time compatible with this technology (PSDB carried out a brief study with the Royal Institution to establish the decay times for natural latent fingerprint fluorescence which proved very short, making time resolved enhancement difficult or impossible for this type of mark). Europium compounds [20] and other lanthanides [21] were proposed by Menzel for producing marks with longer decay times, the potential advantage of the technique being that background fluorescence can be separated from the fluorescence of the fingermark by the difference in decay time.

1.13 Since the early 1980s, advances in fingermark development techniques have meant that fluorescence examination has become an integral process in sequential treatment regimes, being used to detect latent fingermarks and to enhance developed fingermarks. Fluorescent dyes have been developed for use with superglue, DFO is available as a technique for developing fluorescent marks on porous surfaces, acid yellow 7 can be used as a fluorescent blood dye on dark surfaces and marks developed using basic violet 3 may also be enhanced by fluorescence. All of these processes are described in greater detail in Chapter 3, Chemical and Physical Processes.

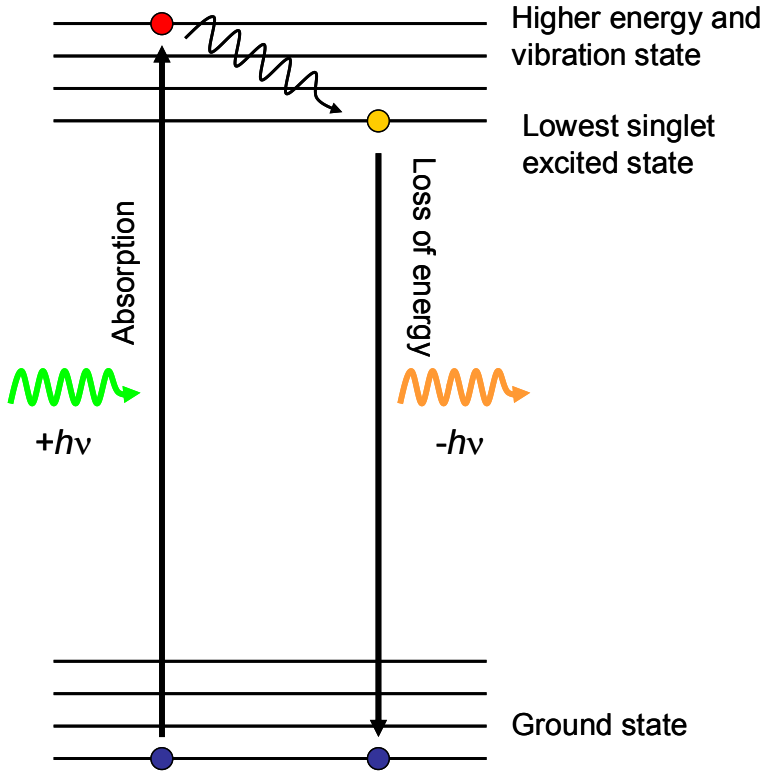
1.14 The advances made in light emitting diode (LED) technology have now resulted in hand-held ‘torches’ with equivalent output power equivalent to or even exceeding some filtered arc lamp systems. However, the output spectra of LEDs (which may have long tails around the peak value) may require additional filtering to avoid reflected light from the light source passing through the viewing filter, which is both a health and safety concern and will result in loss of contrast, possibly leading to marks being missed. Further increases in LED power are anticipated and such systems have become useful tools in fluorescence examination.

1.15 Lasers have also become more lightweight and portable, and scene portable, suitcase-sized Nd:YAG (and semiconductor diode) green lasers with output powers up to 8 W are available. Semiconductor lasers with outputs of 2 W in the blue and 5 W in the yellow regions of the spectrum have also been developed; both of these have been demonstrated to detect marks (and other types of forensic evidence) not found by the green laser. The use of as many different light sources as possible is therefore recommended to maximise evidence recovery.

2. Theory

2.1 Fluorescence is one of a number of processes that are broadly described by the term ‘luminescence’, which means that a substance emits light in response to an external stimulus. Other examples include chemiluminescence, where light is emitted as a result of a chemical reaction (as in the blood detection technique luminol) and triboluminescence, where light is emitted as a result of a material being rubbed, broken or abraded.

2.2 With fluorescent chemicals, light of a very specific colour (wavelength) is absorbed and some of this absorbed energy is subsequently emitted as light of a different colour and longer wavelength. This can occur when a molecule has potential electronic energy excited states with levels compatible with the absorption and emission of visible/UV or IR light. The mechanism of fluorescence is shown schematically below.

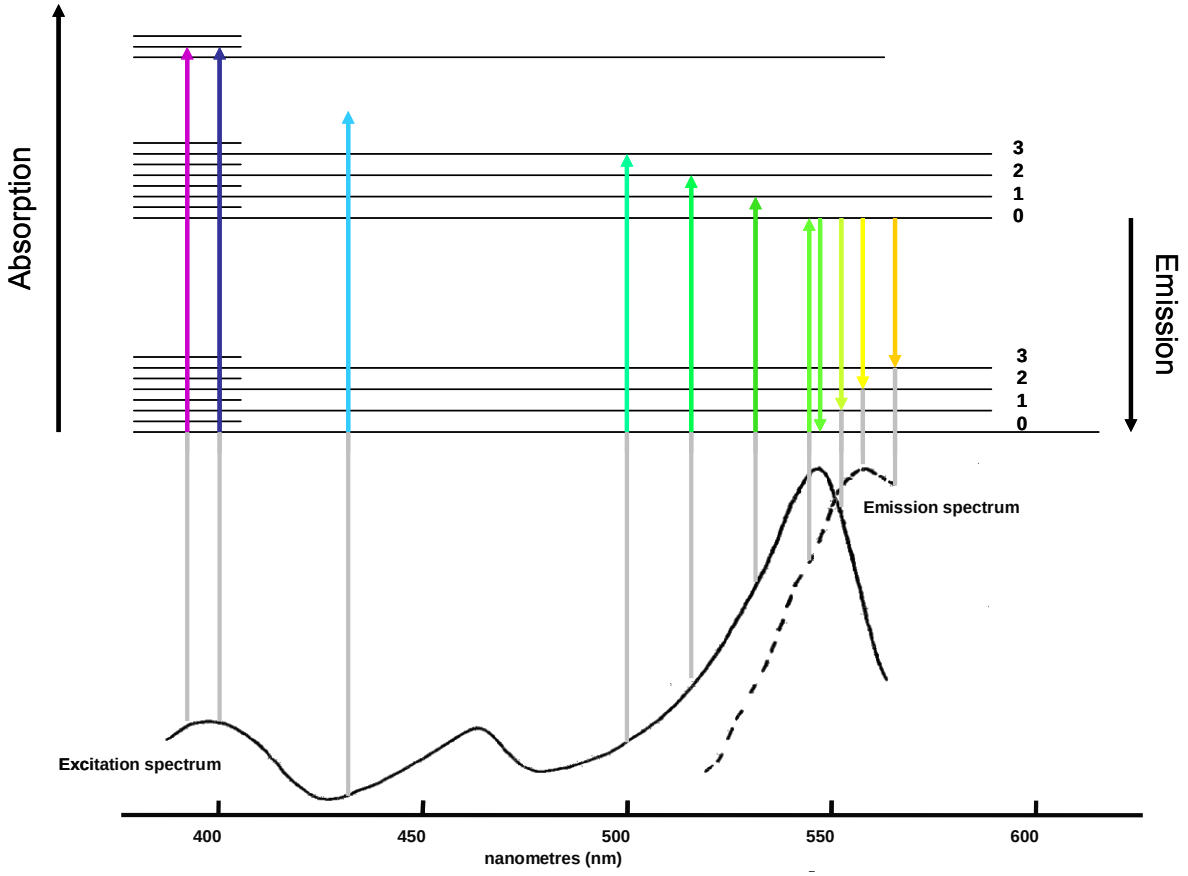


Schematic diagram showing the mechanism by which fluorescence occurs.

2.3 When a fluorescent molecule is excited with light of an appropriate wavelength, the electrons absorb energy from the light and are promoted from the ground state to a higher energy electronic state. Radiationless energy loss transitions result in the vibrational energy level of the excited electron dropping. The electron then drops to a lower electronic level with the emission of a photon of a lower energy and longer wavelength than the original absorbed photon. If the transition between excitation

and emission takes place in less than 10^{-8} seconds it is generally regarded as fluorescence. There are other 'delayed fluorescence' and phosphorescence mechanisms that can occur in some molecules, in addition to the phenomenon of 'Anti-Stokes' fluorescence, where the molecule absorbs energy from two photons of low energy (longer wavelength) and on decay emits a single higher energy (shorter wavelength) photon.

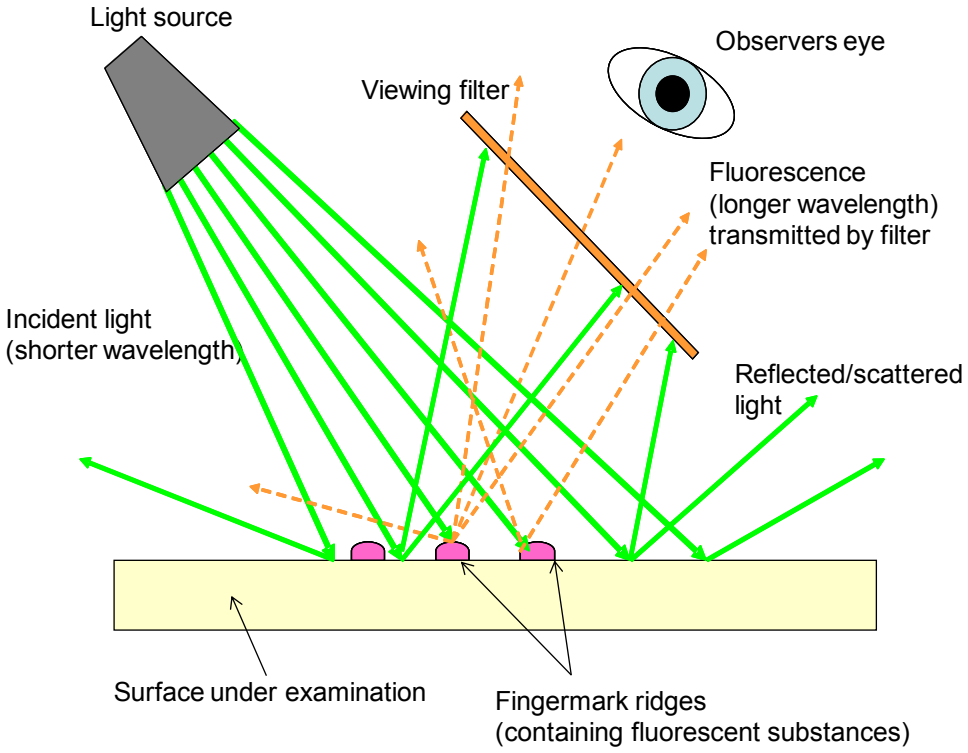
2.4 In some molecules there may be several excited states to which electrons can be promoted and several ground states to which they can return, so that absorption and emission may actually occur over a range of the electromagnetic spectrum. A schematic illustration of a representative emission and absorption spectrum and the corresponding excited states is shown below.



Representation of excitation/emission spectra of a chemical with corresponding transitions between excited and ground states.

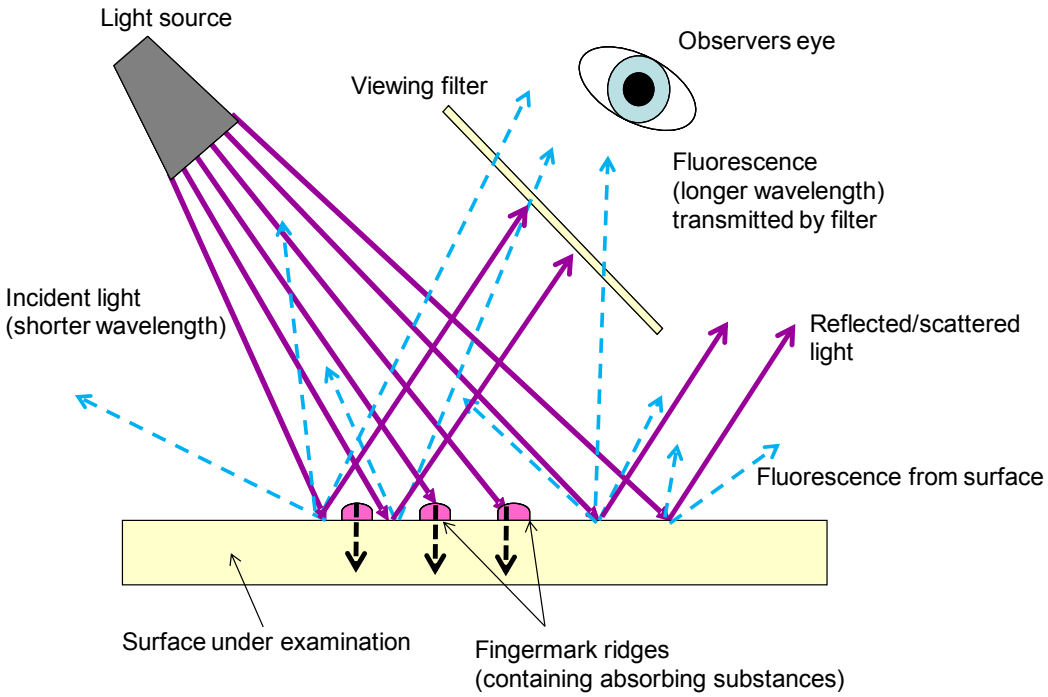
2.5 Typically, light in the UV, blue, green or yellow regions of the spectrum is used to excite fluorescence, which may result in the emission of light in the yellow, orange, red or infra-red (IR) regions. In Anti-Stokes fluorescence, infrared radiation is used to promote fluorescence in the visible region of the spectrum, generally in the green region. Most of the illuminating light is not absorbed but scattered or reflected from the

surface being examined. Filters that transmit the fluorescence but not the illuminating light are therefore placed in front of the eye and/or image capture device to enable the fluorescence to be seen and recorded. This is shown schematically below and selection and implementation of these filters forms a crucial part of a fluorescence detection system.



Schematic diagram illustrating the viewing of fluorescence from fingermark ridges containing a fluorescent species.

2.6 An alternative application is when the fingermark itself may not be fluorescent, but the substrate it has been deposited onto may be. In such situations with appropriate illumination the background fluorescence of the substrate may provide sufficient contrast with the absorbing fingermark for it to be detected. This scenario is shown schematically below.

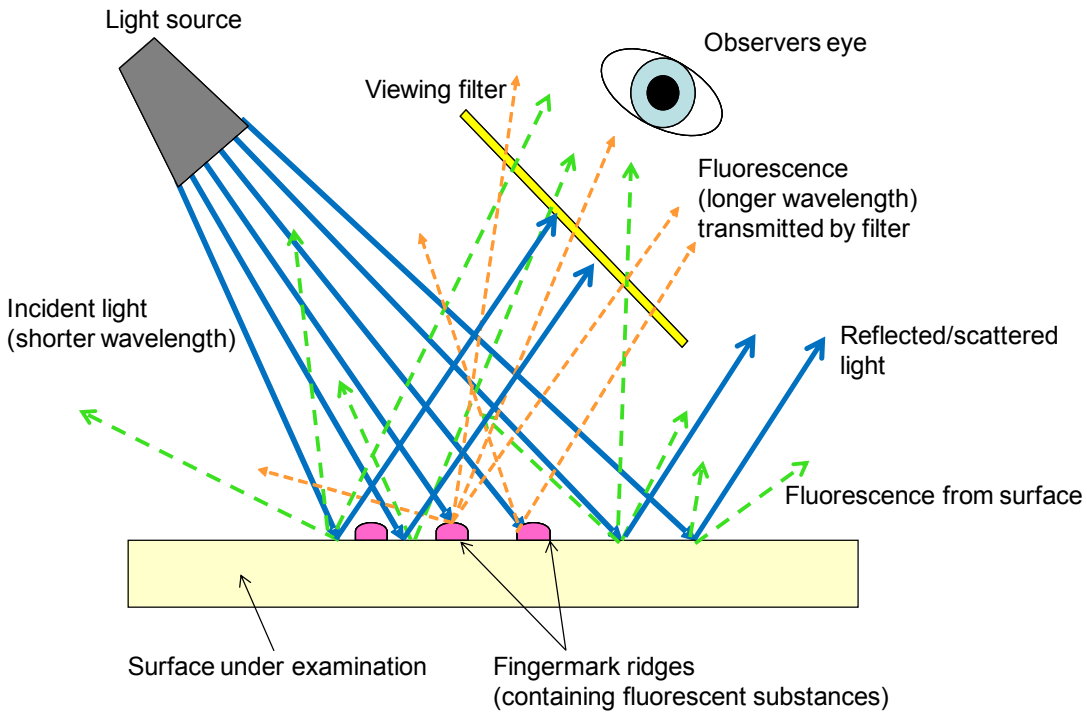


Schematic diagram illustrating the viewing of absorbing fingermark ridges on a substrate containing a fluorescent species.

2.7 In optimising fluorescence examination it is essential to ensure that the illuminating wavelengths of light correspond closely to the excitation of the fluorescent species present (if known), and that the viewing filter used blocks illuminating wavelengths and transmits the maximum emission of the fluorescent species. In many circumstances these wavelengths will not be known so must be established pragmatically.

2.8 Known fluorescence spectra and pragmatic observation have been used to determine the excitation wavelengths and corresponding viewing filters recommended in the 'CAST processes' section below.

2.9 In many scenarios, it is possible that both the fingermark and the substrate may fluoresce to some extent and that it may be difficult to discriminate the fluorescence of the mark from that of the background. This situation is illustrated schematically below, and possible means of overcoming such issues are discussed in the 'CAST processes' section.



Schematic diagram illustrating the viewing of fluorescent fingermark ridges on a substrate also containing a fluorescent species, resulting in difficulties in discriminating the mark.

3. Centre for Applied Science and Technology processes

3.1 The most recent version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. Comprehensive descriptions of the processes used for fluorescence examination are given in the CAST publication *Fingerprint Detection by Fluorescence Examination – A Guide to Operational Implementation* [14] and the *Fingermark Visualisation Manual* [22]. A shorter summary is given here. This section does not cover the use of UV radiation for the detection of fingermarks, which is dealt with in greater detail in Chapter 2, Optical Processes, Ultraviolet imaging.

3.2 In common with visual examination, there are many ways in which fluorescence examination can used to detect fingermarks. There are two principal forms of examination, the initial examination for untreated fingermarks and the examination of developed marks with known fluorescent properties, both of which are described below.

3.3 <u>Initial examination of untreated fingermarks</u>

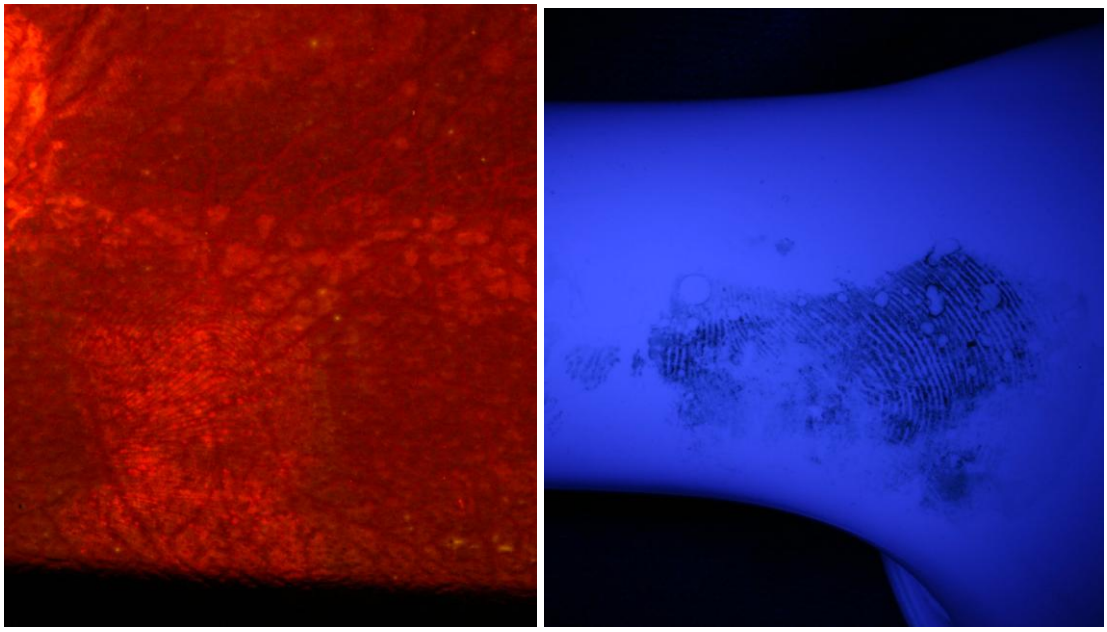
3.3.1 Fluorescence examination is an essentially non-destructive process and may be used as the initial stage in a sequential processing regime. There

are several types of untreated fingermark which may be revealed during initial examination [22].

- Fingermarks may be detected due to the inherent fluorescence of constituents that may be present in sweat. In practice only a small proportion of the population naturally secrete sufficient fluorescent constituents for such marks to be detected.

- Contaminants present in the fingermark, such as ink, drugs or grease, may exhibit enhanced fluorescence over those consisting of sweat alone.

- Some surfaces, such as paper or cardboard, may exhibit background fluorescence, which can improve the contrast of fingermarks contaminated with substances such as blood or dirt that absorb light and appear dark against the light fluorescing background.



a) b)

Marks detected during initial examination a) *mark detected by inherent fluorescence of fingermark and* b) *mark in blood revealed against fluorescent background.*

3.4 Enhancement of developed fingermarks

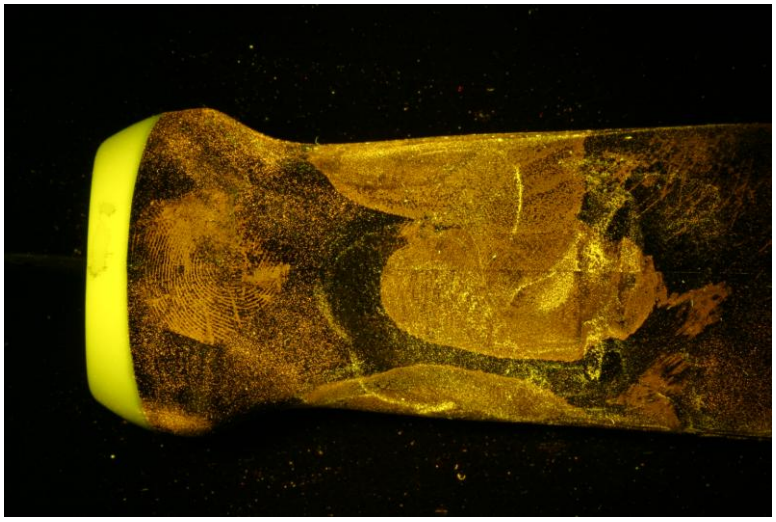
3.4.1 Fluorescence examination may also be used as a method of improving the contrast of fingermarks developed with other processes. There are four principal types of fingermark to which fluorescence examination can be applied.

- The treated fingermark may itself fluoresce because the chemical used to stain it is fluorescent, examples being basic violet 3 and acid yellow 7. In this way, fingermarks that are faint or invisible under normal light may be revealed by fluorescence. However, heavy blood deposits or heavily stained fingermarks may quench fluorescence.

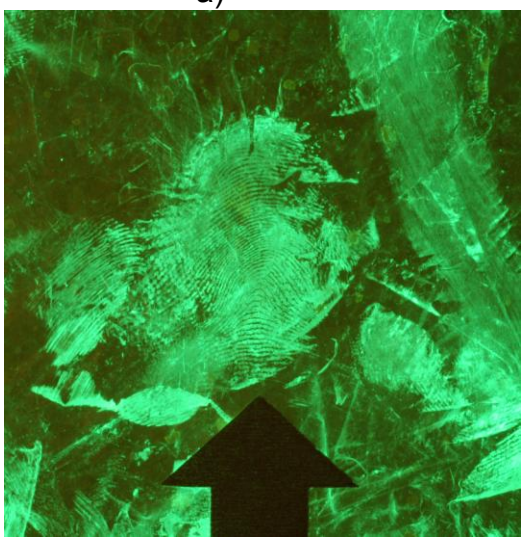
- The substrate may fluoresce and the fingermark may absorb or scatter the incident light and appear black. Examples of this may be seen for ninhydrin, physical developer and the acid dyes, acid black 1 and acid violet 17.

- The treated fingermark may be treated with a secondary reagent or stain prior to fluorescence examination. This converts it from a non-fluorescent to a fluorescent mark, thus improving the detail that can be imaged. Examples of this include the zinc salt toning of ninhydrin marks and the staining of superglue marks with fluorescent dyes.

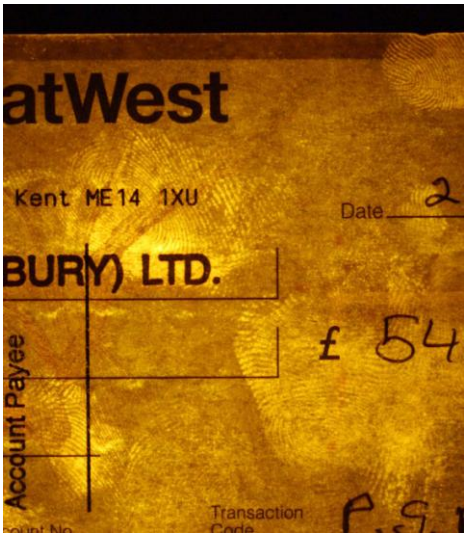
- The reagent used may react directly with fingermark constituents to form a fluorescent product, e.g. DFO and 1,2 indandione. Fluorescent fingerprint powders also fall into this category.



a)



b)

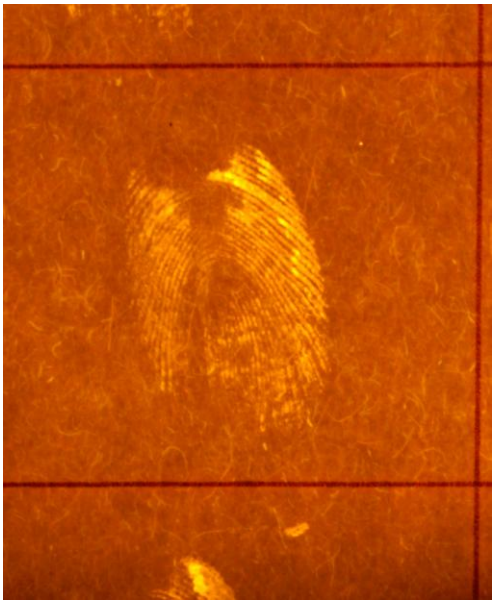


c)

Fingermarks enhanced using fluorescence examination a) *mark in blood enhanced using acid yellow 7* b) *superglue stained with basic yellow 40 and* c) *fingermarks developed using 1,8-diazafluoren-9-one.*

3.4.2 More recently, work on the recovery of fingermarks from arson scenes has revealed a further category of mark that can be revealed or enhanced by fluorescence examination.

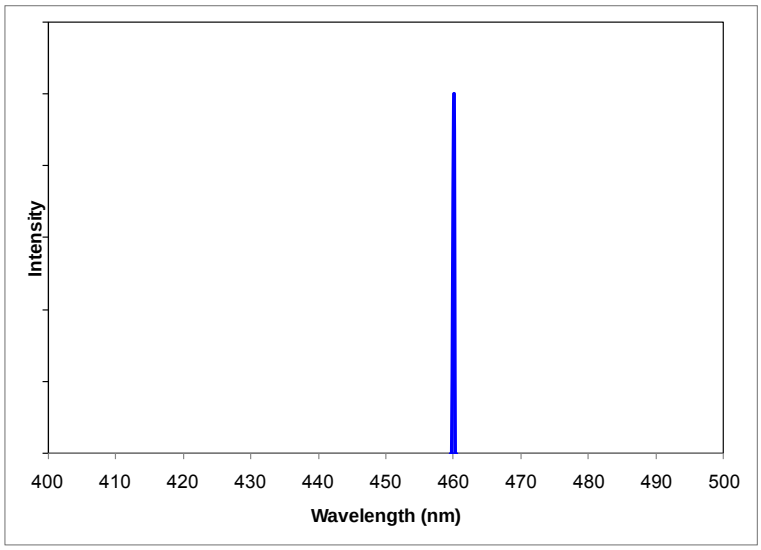
- Thermal effects on a latent fingermark may result in the formation of fluorescent products. This has since been exploited in a commercial product for enhancement of fingermarks (see Chapter 3, Chemical and Physical Processes, Thermal development)



Eccrine mark on paper becoming fluorescent after exposed to 150ºC.

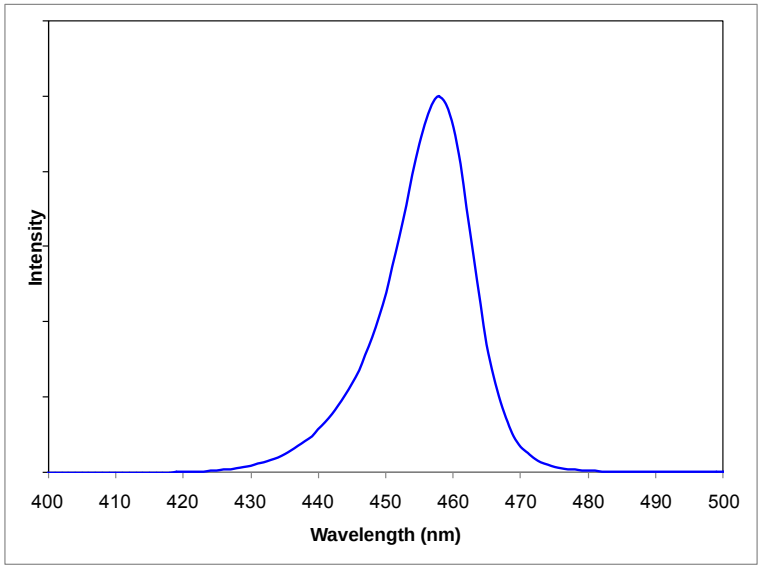
3.4.3 Many different types of light source are available for fluorescence examination, the principal types being lasers, filtered arc lamps and LEDs [22]. These light sources have different output characteristics, and a single light source is highly unlikely to be suitable for the detection of all marks that are present therefore optimum results are obtained by the use of multiple light sources with complimentary properties. A brief summary is given below.

3.4.4 Lasers: Single wavelength output, high output power but generally highest cost and potentially heavier than other light sources (although this may change with ongoing product development).



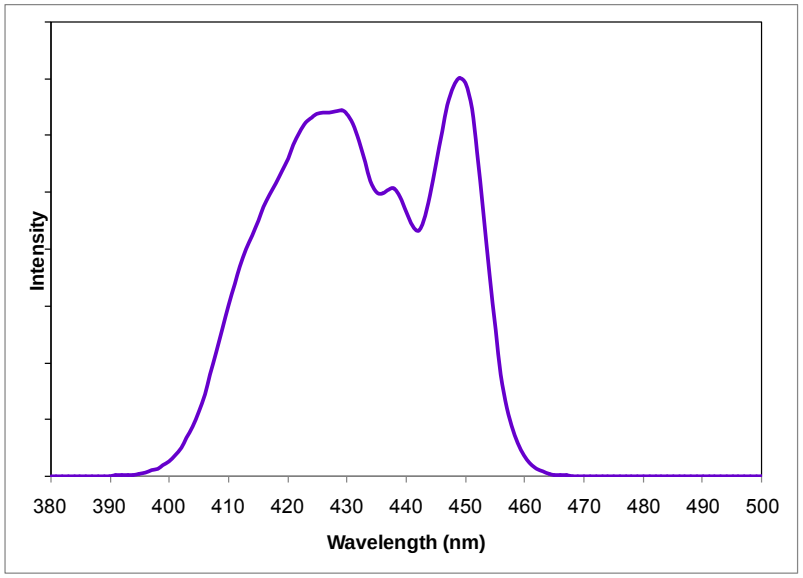
The output spectrum of a blue laser.

3.4.5 LEDs: Output over a range around a peak wavelength, medium output power, lower cost and highly portable.



The output spectrum of a blue LED light source.

3.4.5 Filtered arc lamps: Output over several, tightly defined wavelength ranges, medium output power, medium cost and reasonably portable.



The output spectrum of a violet/blue filter set for a filtered arc lamp light source.

3.4.6 The excitation and viewing conditions recommended for detection of untreated marks and the enhancement of developed marks by CAST for a variety of light sources are summarised below.

3.5 <u>Nd:YAG laser (single wavelength)</u>

3.5.1 Initial examination and enhancement of developed fingermarks

Application	**Excitation wavelength (nm)**	**Schott viewing filter**
Examination of all surfaces	532	OG570
DFO	532	OG570
Superglue dyed with basic red 14	532	OG570

Data given are for the Coherent 'Tracer' green laser, and the Laser Innovations 'Revelation' green laser.

3.6 Yellow semiconductor laser (single wavelength)

3.6.1 Initial examination and enhancement of developed fingermarks.

Application	**Excitation wavelength (nm)**	**Schott viewing filter***
Examination of all surfaces	577	RG610
Basic violet 3	577	RG610
DFO (on backgrounds highly fluorescing under green illumination)	577	RG610

* Pink/purple band blocking filters specifically designed to block 575-579nm are also available and give good results with this laser

Data given are for the Coherent 'Tracer' yellow laser.

3.7 Blue semiconductor laser (single wavelength)

3.7.1 Initial examination and enhancement of developed fingermarks.

Application	**Excitation wavelength (nm)**	**Schott viewing filter**
Examination of all surfaces	460	GG495
Marks contaminated with body fluids (e.g. fluorescence of semen, strong absorption by blood)	460	GG495
Superglue dyed with basic yellow 40	460	GG495

Data given are for the Coherent 'Tracer' blue laser.

3.8 Argon Ion Laser (multiple, selectable wavelengths)

3.8.1 Initial examination.

Application	**Excitation wavelength (nm)**	**Schott viewing filter**
Examination of all surfaces	514.5	OG550
Surfaces with low background fluorescence	488.0	OG530
Fingermarks in dark materials, e.g.	488.0	OG530

blood, where fluorescence of background may improve contrast		

3.8.2Enhancing developed fingermarks.

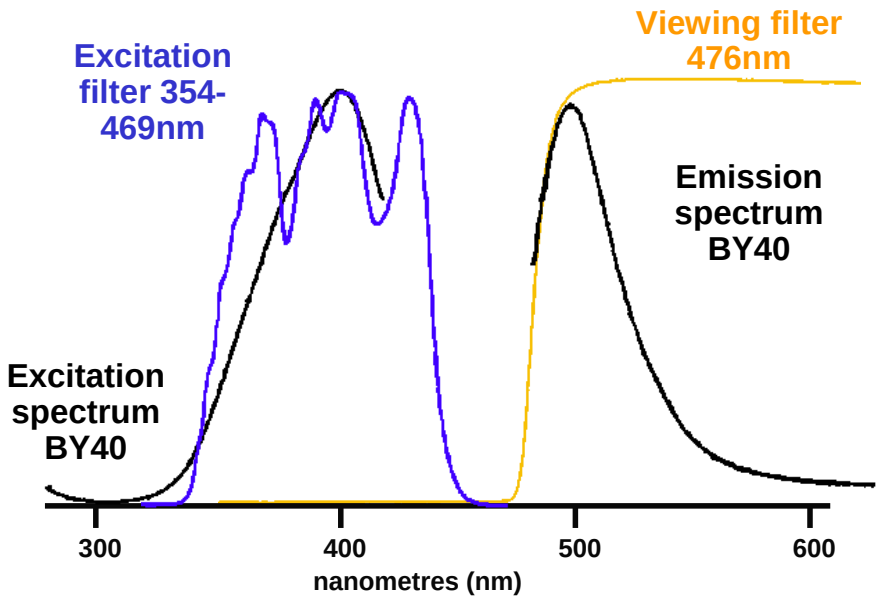
Application	**Excitation wavelength (nm)**	**Schott viewing filter**
Absorbing treatments, e.g. ninhydrin, acid black 1, acid violet 17, Powders (background fluorescence)	457.9 or 476.5 or 488.0	GG495 OG515 OG530
Acid yellow 7	514.5	OG550
DFO	514.5	OG550
Basic violet 3	528.7 or 514.5	RG610 OG550
Ninhydrin toned with zinc salts	488.0	OG530
Superglue dyed with basic yellow 40	457.9 or 476.5 or 488.0	GG495 OG515 OG530
Superglue dyed with basic red 2	514.5	OG550

Where alternative wavelengths are given, users should investigate which is the best combination for their particular laser. The wavelengths of the argon ion laser are not necessarily the optimum for any of the applications listed above.

Data obtained from Hardwick *et al.* [14].

3.9 Quaser (multiple, selectable excitation bands)

3.9.1An example of excitation and viewing filter selection for different applications is illustrated below.



Selection of appropriate Quaser filters to fit with excitation/emission of basic yellow 40 dye.

3.9.2 Initial examination.

Application	**Excitation filter (nm)**		**Viewing filter (1% transmission point)**	
Examination of all surfaces. Background fluorescence may obscure some fingermarks	Blue	352–509 385–509 354–519 385–519 400–519	Yellow/ Orange Orange	510 or 515 529
Reduces background fluorescence	Blue/ Green	468–526	Orange	529
Reduces background fluorescence further	Green	473–548	Orange	549
Detects some fingermarks on polythene packaging and possibly other surfaces	Green	491–548	Orange	549
Fingermarks in dark materials, e.g. blood, where background fluorescence may improve contrast	Violet/ Blue	350–469 385–469 400–469	Yellow	476
Fingermarks in dark materials, e.g. blood, where background fluorescence may improve contrast. Some fingermarks in oils and greases and some absorbing fingermarks on glossy papers	Ultra-violet	280–413 340–413	Yellow	415

Where multiple excitation filters are listed, the set supplied will depend on the Quaser system used.

3.9.3Enhancing developed fingermarks.

Application	**Excitation filter (nm)**		**Viewing filter (1% transmission point)**	
Absorbing treatments, e.g. ninhydrin, acid black 1, acid violet 17, powders (background fluorescence)	Violet/ Blue	350–469 385–469 400–469	Yellow	476
Acid yellow 7	Blue	352–509 385–509 354–519 385–519 400–519	Yellow/ Orange Orange	510 or 515 529
DFO for maximum contrast on most types of paper	Green	473–548	Orange	549
DFO to reduce background fluorescence	Green	491–548	Orange	549
DFO to reduce background fluorescence further	Green/ Yellow	503–591	Red	591
Basic violet 3	Green/	503–591	Red	591

	Yellow			
Ninhydrin toned with zinc salts	Blue/ Green	468–526	Orange	529
Superglue dyed with basic yellow 40	Violet/ Blue	350–469 385–469 400–469	Yellow	476
Superglue dyed with basic red 14	Green	473–548	Orange	549

Where multiple excitation filters are listed, the set supplied will depend on the Quaser system used.

Data obtained from Hardwick *et al.* [14] and from the CAST *Fingermark Visualisation Manual* [22].

3.10 LED light sources

3.10.1 Data given are for Foster and Freeman 'Crime-lite 80S' range, correct as of 28/01/2010. These have since been superseded by the 82S series, which have essentially the same range of output colours but with slightly different wavelength bands.

3.10.2 Initial examination.

Application	**Excitation filter (nm)**		**Viewing filter (1% transmission point)**	
Examination of all surfaces. Background fluorescence may obscure some fingermarks	Blue	430–470	Yellow	476
Reduces background fluorescence	Blue/ Green	460–510	Orange	529
Reduces background fluorescence further	Green	500–550	Orange	549
Detects some fingermarks on polythene packaging and possibly other surfaces	Green	500–550	Orange	549
Fingermarks in dark materials, e.g. blood, where background fluorescence may improve contrast	Violet/ Blue	395–425 430–470	Yellow	476

3.10.3 Enhancing developed fingermarks.

Application	**LED colour and excitation (nm)**		**Viewing filter (1% transmission point)**	
Absorbing treatments, e.g. ninhydrin, acid black 1, acid violet 17, powders (background fluorescence)	Violet	395–425	Yellow	476
Acid yellow 7	Blue	430–470	Yellow	476

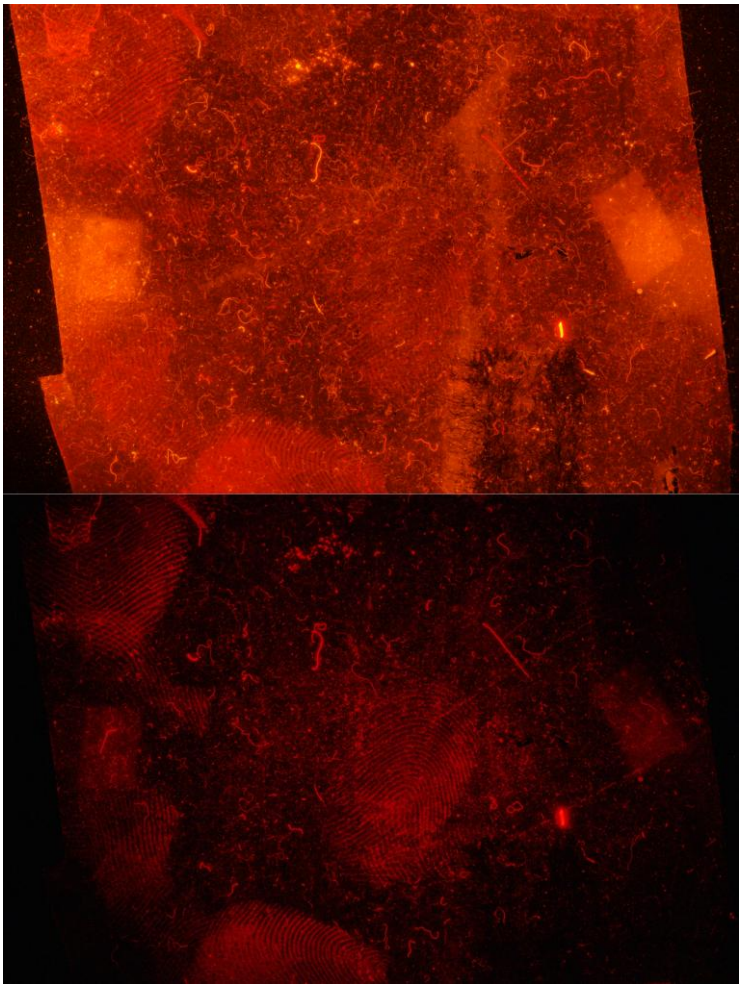
DFO for maximum contrast on most types of paper	Green	500–550	Orange	549
Ninhydrin toned with zinc salts	Blue/ Green	460–510	Orange	529
Superglue dyed with basic yellow 40	Blue	430–470	Yellow	476
Superglue dyed with basic red 14	Green	500–550	Orange	549

3.11 Reducing background fluorescence

3.11.1 There are many situations where both the fingermarks and the background fluoresce under the illumination wavelengths used, with the consequence that it is difficult to discriminate the fingermark from the background. If this occurs, there are several methods that are recommended to try to overcome this:

- Keeping the excitation wavelengths of the light source the same and increasing the cut-on transmission value of the viewing filter (e.g. using an OG590 Schott glass filter in place of an OG570 filter)
- Narrowing the excitation wavelengths used and maintaining the cut-on transmission value of the viewing filter (e.g. using a laser in place of an LED or filtered white light source)
- Increasing the excitation wavelength used and correspondingly increasing the cut-on transmission value of the viewing filter (e.g. using a yellow laser with an RG610 filter in place of a green laser with an OG570 filter).
- Using a bandpass filter in combination with a long pass viewing filter

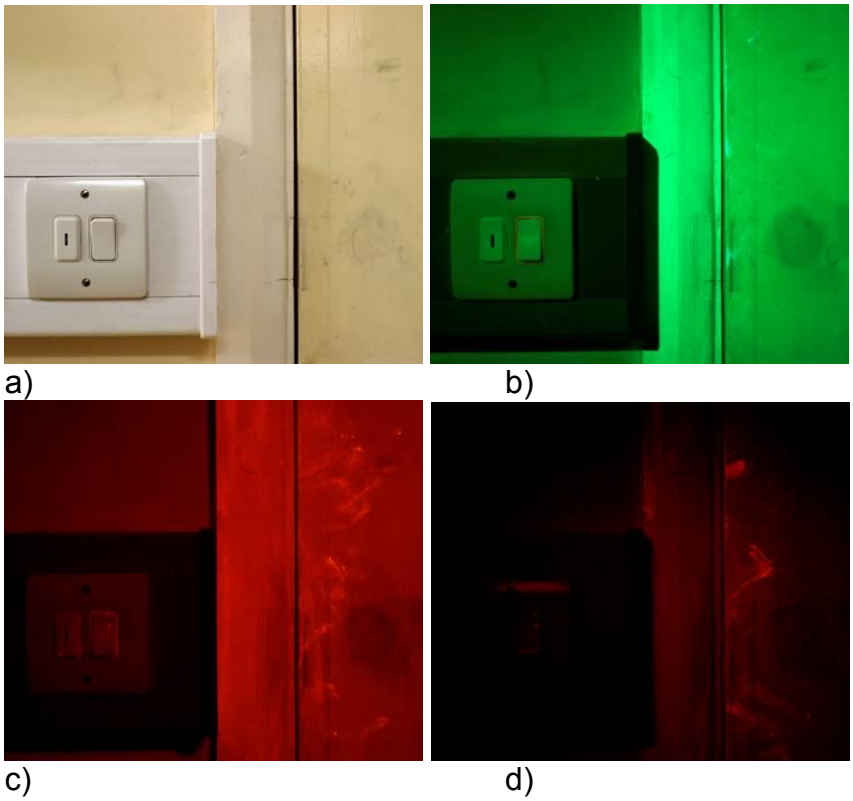
3.11.2 An example of the benefits of this approach can be seen in the images below.



Fingermarks developed using basic violet 3 on an adhesive tape, (top) *fluorescence examination conducted using a green 532 nm laser* (bottom) *fluorescence examination conducted using a yellow 577 nm laser, showing much reduced background fluorescence and better discrimination of marks.*

3.12 Use of multiple light sources

3.12.1 It is suggested that fluorescence examination is conducted using a series of light sources with different output wavelengths, particularly when searching for untreated marks. This is because different substrates and contaminants fluoresce across different wavelength ranges and in addition to reducing background fluorescence changing the illumination wavelength can reveal previously unseen marks. An example of the effect that changing illumination wavelength can have on the appearance of a scene is shown in the series of images below.



An interior scene showing a range of substrates and contaminants with different fluorescent properties viewed under a) *white light,* b) *fluorescence examination using blue light,* c) *fluorescence examination using green light,* d) *fluorescence examination using yellow light*

4. Critical issues

4.1 There are several issues that need to be considered and addressed when performing fluorescence examination, some concerned with technique effectiveness and other relating to health and safety.

4.2 The light source used must output at a wavelength or spectral bandwidth that overlaps with the absorption bands of the dyes/contaminants in the fingermark and thus excite the fingermark into a state where it can fluoresce.

4.3 The light source used must have an effective radiated power that produces a sufficient intensity of fluorescence in the mark for it to be detected and captured.

4.4 The filters used for both viewing and capture of fluorescent marks must be correctly designed and selected so that they block all of the wavelengths output by the illumination source, and transmit as much of the fluorescence output from the mark as possible.

4.5 The potential background fluorescence of the surface should be considered. It may be necessary to move to other wavelengths or wavebands to reduce the impact of background fluorescence, even

though these may not be optimum for exciting the fluorescent constituents in the mark (see above). Many common background materials such as wood, paper, cardboard and some paints show significant mid-range visible fluorescence when illuminated in the UV/violet/purple/blue (350 to 470 nm) part of the spectrum which can mask fingerprint fluorescence. For this reason excitation around 500 nm or higher may be more effective.

4.6 It is essential to carry out a full safety assessment prior to carrying out fluorescence examination to ensure that all operators are wearing appropriate protective eyewear and that unprotected personnel cannot be accidentally exposed to harmful levels of stray light. Safety procedures should be put in place to enforce this.

4.7 The light levels needed to detect weakly fluorescent materials present serious hazards to the eyes and in some cases the skin. It is possible to cause retinal burns in less than the eye blink response time of ¼ second. Some of the light source and filter systems marketed are potentially hazardous and may have inadequate or incorrect safety advice. Those providing safety features to avoid accidental exposure to harmful levels of light are preferable.

4.8 Changes to the wavelength, power, barrier filter or light delivery optical system may dramatically affect the risks to human eyes. The only effective way to carry out a safety assessment of a system is by calculation, and/or by measurement of the radiance levels for visible light systems and irradiance for UV or IR systems.

4.9 The safety of light sources is critically dependent not only on emitted wavelength and power but the radiance of the source. This is controlled by the size and angular distribution of the light guide or lens system and can only be measured with specialist knowledge and equipment.

5. Application

5.1 Suitable surfaces: Fluorescence examination can be used for detection of latent fingermarks on all types of untreated surface, but success rates are higher on non-porous articles. Fluorescence examination is also useful on all types of surface after chemical treatment provided that an appropriate fluorescent chemical has been used to develop the fingermark, or the surface has appreciable background fluorescence while the chemically treated print absorbs.

5.2 Fluorescence examination has two principal applications in fingermark detection, firstly in the detection of latent fingermarks prior to commencing a sequence of chemical treatments and secondly in the enhancement of marks that have either been treated to produce fluorescent products or are absorbing on a fluorescent background.

5.3 In the first role, fluorescence can be an invaluable tool because it may detect marks that contain small quantities of fluorescent contaminants. Because fingermark development processes primarily target natural secretions, many of these marks will never be found during subsequent chemical treatment.

5.4 Fluorescence examination may also detect marks present in contaminants, such as blood. Many surfaces fluoresce when excited by high-intensity light in the UV and violet regions of the spectrum. This is coincidently where the haem group in blood is most absorbent, with a peak around 421 nm (known as the Soret Band) [23] and why blood-contaminated fingermarks will appear dark against a light background. Fluorescence examination may be used before any other fingermark enhancement techniques as it is non-destructive, and if long-wave UV or violet light (350 to 450nm) is used then DNA typing is also unaffected.

5.5 In the enhancement of chemically treated fluorescent marks, fluorescence examination is used to reveal marks that may not be visible to the eye and to enhance the contrast between ridges and background. More powerful light sources (of the correct excitation wavelength) will cause even very weakly developed marks to fluoresce sufficiently for imaging.

5.6 Marks in blood may also be detected by fluorescence, even though the original chemical treatment is not intended to produce fluorescence. If haem-specific enhancement processes are used, the use of a strong organic acid in conjunction with hydrogen peroxide breaks up the haem group so that it is no longer as effective at absorbing light. When subsequently excited by green (500 to 550 nm) light it will fluoresce orange. This effect has also been noted as blood ages.

5.7 Wherever possible, fluorescence examination should be carried out in a darkened room free of highly fluorescent articles and surfaces, and users should allow themselves to become suitably dark adapted before commencing examination. All safety precautions appropriate to the light source being used should be taken [14] to ensure the safety of both the operator and others in the vicinity. The light source should be passed slowly over the article to be examined, taking care to minimise exposure time on articles that may be damaged by the heat associated with some high-power light sources. Handling of the article during examination should be minimised to avoid damage to any marks present on the surface. Any marks detected should be photographed using an imaging system fitted with an appropriate barrier filter. Ideally, users should have exceptionally good close range vision to enable them to distinguish ridge detail and if appropriate wear glasses under the protective goggles.

5.8 Fluorescence examination can be carried out both in a laboratory and at a crime scene, provided that appropriate health and safety precautions are taken. For optimum results, it is essential that the area to be examined is blacked out and the operator takes time to become fully

dark adapted before commencing examination. Further detail on dark adaptation and when it may be required is provided in section 8.4.

Examination of a crime scene using a portable laser.

6. Alternative formulations and processes

6.1 There are many suppliers of light sources, covering the range of lasers, filtered arc lamps and LEDs, but regardless of which is selected the essential examination process is the same. The light source should be selected to provide maximum illumination in the excitation region of the fluorescent chemical (if known), and the viewing filter selected to block the illumination wavelengths and transmit the excitation wavelengths of the chemical. Provided that this approach is adopted, many different combinations of fluorescent dye, illumination light source and viewing filters can be successfully employed in fluorescence examination.

7. Post-treatments

7.1 There are no post-treatments used in fluorescence examination.

8. Validation and operational experience

8.1 Because fluorescence examination is essentially a non-destructive examination technique and is recommended for use as the second stage in a sequential treatment (after visual examination), its operational implementation for this purpose should not require extensive validation.

8.2 Laboratory trials

8.2.1 Few laboratory trials have been conducted using deliberately deposited fingermarks. This is because it is known that the proportion of marks that will be detected in this way is low, but this is accepted for operational use because fluorescence examination is non-destructive and will find marks in contaminants not developed by chemical reagents.

8.2.2 A limited study has been carried out by CAST to compare the effectiveness of fluorescence examination with other non-destructive examination techniques, including short-wave UV imaging on porous surfaces. These results are reported in Chapter 2, Optical Processes, Ultraviolet imaging, and illustrate that both green and yellow lasers will detect marks not found by any other light source, although they are not particularly effective on most porous surfaces.

8.3 Pseudo-operational trials and operational experience

8.3.1 Data are available that demonstrate the benefit of fluorescence examination in sequential treatments. As early as 1979, the FBI reported that from 1,500 articles examined using an argon ion laser, 76 fingermarks were found that were not subsequently developed by any other process [8].

8.3.2 Creer reported early results from the use of an argon ion laser at the Serious Crime Unit of the Metropolitan Police in 1983 [24], stating that from 396 exhibits examined, 121 identifiable fingermarks had been found. Many of these exhibits had been considered unsuitable for other treatments due to surface scratches or patterned backgrounds, which would make conventional photography difficult. Creer also noted that in some cases on plastic bags, the laser detected marks that were totally different to those subsequently developed by vacuum metal deposition. The broader forensic applications of the laser were also presented.

8.3.3 CAST purchased a 5 W, 532 nm green laser in the late 2000s and have loaned it to police forces for trials on operational work. The laser has been compared with the Quaser 100 operating in the green excitation band for both initial fluorescence examination and for examination of marks developed using DFO [25]. The results of this trial, conducted on articles from over 70 cases, are summarised below.

Application	**Total number of marks found**	
	Quaser 100	**Nd:YAG laser**
Initial examination	10	52
DFO enhancement	70	77

Summary of results obtained using a green (532 nm) laser compared with a Quaser 100.

8.3.4 It can be seen that the higher power and higher wavelength specificity of the laser compared with the Quaser 100 provide benefits in the number of marks detected using fluorescence examination. Similar successes have been reported from the use of the laser at crime scenes.

8.3.5 On a limited number of operational cases processed by CAST [26], similar observations to those of Creer [24] were made in that marks were detected on plastic bags using fluorescence examination that differed totally from those developed by subsequent vacuum metal deposition. One set were identified to a householder, the other to a suspect. This type of result demonstrates that fluorescence examination is a complementary tool to chemical treatments, and fully justifies its position within a sequential treatment process.

8.3.6 As prototypes of lasers operating at different wavelengths became available, CAST carried out a small-scale pseudo-operational trial examining items recovered from waste bins and in and around the laboratory. These items were examined using four different light sources and the number of fingermarks recorded. The results of this study are recorded below.

	Blue laser (460 nm)	**Green laser (532 nm)**	**Yellow laser (577 nm)**	**Quaser 101 (503–587 nm)**
Items	56	56	56	56
Total fingermarks	2	15	20	16
Common fingermarks	1	13	12	16
Missed fingermarks	0	0	4	0
Unique fingermarks	1	3	8	0

Summary of results obtained from different light sources in a laboratory trial.

8.3.7 These results suggested that the green and yellow lasers were effective in detecting fingermarks, with the yellow laser finding more fingermarks, and more unique fingermarks overall. Even though the blue laser found very few fingermarks, it was still capable of finding marks not detected by other light sources.

8.3.8 More recently, studies have been carried out by Hampshire Constabulary and the Metropolitan Police [27], using a wide range of light sources to examine exhibits prior to chemical treatments. Both of these studies incorporated fluorescence examination at different wavelengths using a range of light sources. Results confirmed that fluorescence examination will detect marks that are not developed by subsequent chemical treatment. In the Hampshire study, reported in Chapter 2, Optical

Processes, Visual examination, fluorescence examination was the sole means of detection for approximately 8% of marks recovered from 361 exhibits over a period of 6 months. In contrast to the earlier CAST study, the green laser was found to be most effective in detecting marks on operational exhibits, although both green and yellow lasers found marks not detected by other techniques.

8.3.9 CAST also included fluorescence examination as the initial stage in a pseudo-operational trial to establish the optimum processing sequence for plastic bags. In this study, 100 plastic bags of varying types (e.g. supermarket bags, black bin bags, clear magazine wrappings) were divided into quarters and each quarter was assessed using a different fluorescence examination regime followed by a different chemical treatment sequence. The total number of fingermarks and the number of fingermarks unique to each process were recorded. The fluorescence examination regimes used were: exclusively laser examination, using green, yellow (and blue when available) lasers; exclusively Quaser examination, using each waveband of a Quaser 101; solely LED examination, using a green Crime-lite 80S; and finally a full examination using all of the light sources available. The results of this exercise are summarised below.

Light source(s)	**Total fingermarks found with light source**	**Total developed chemically**	**Unique fingermarks to light source**
Laser sequence (460, 532, 577 nm)	46	379	24
Quaser 101	34	335	10
Green Crime-lite	19	392	5
Full (Quaser, Crime-lite, lasers)	65	380	21

Summary of results obtained using fluorescence examination during a pseudo-operational trial on 100 plastic bags.

8.3.10 It can be seen that on plastic bags, fluorescence examination sequences utilising lasers typically recover about 10 to 15% of the marks found overall, with up to 50% of the marks found by fluorescence examination not being subsequently developed by any other process. The lower power Quaser and Crime-lite sources are less effective, but still find several unique marks.

8.3.11 Lasers of different wavelength continue to be developed and tested for forensic applications. Lasers are available that operate at 520 nm and 445 nm, and a prototype orange (594 nm) laser has been produced. All of these lasers offer the potential for finding different marks, although indications from preliminary testing are that the 520 nm laser is less

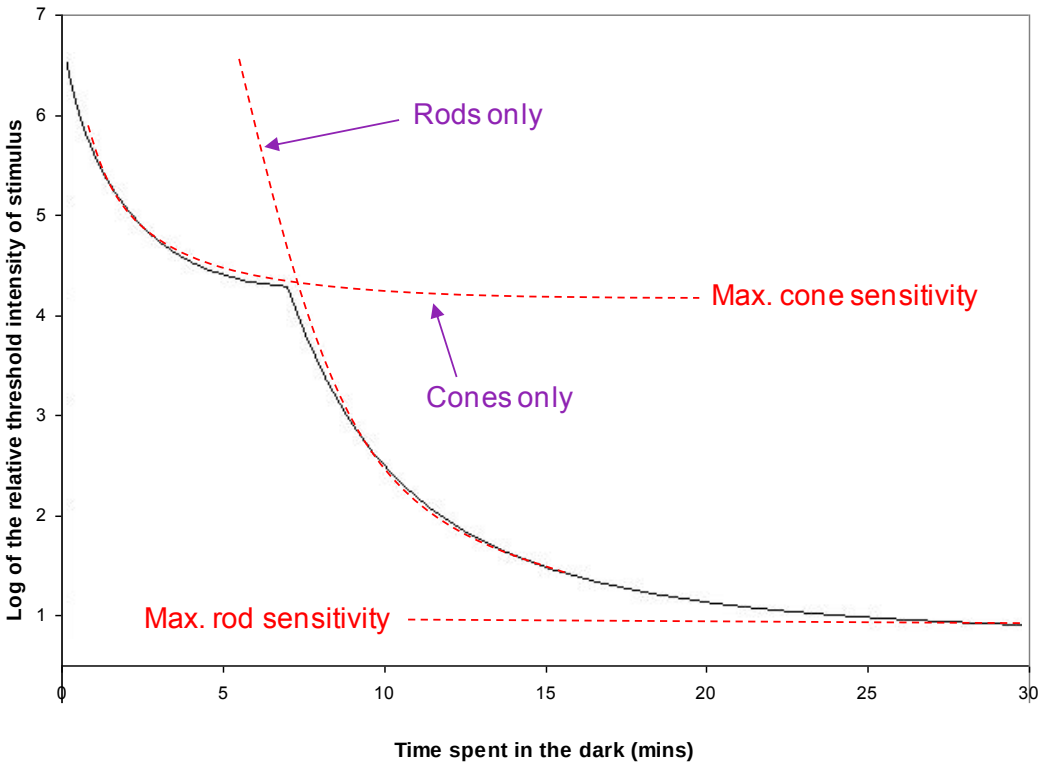
effective than 532 nm, and the 445 nm laser more effective than 460 nm, although more documented evaluations are required.

8.4 Dark adaptation

8.4.1 The numbers of fingermarks recorded during searches with lasers loaned by CAST to UK police forces indicated that there were significant differences between success rates from different forces, suggesting that human factors are a major part in the success of the fluorescence examination process. The trials reported above indicate that around 50% of the fingerprints visualized by light sources were not subsequently developed by chemical techniques, so it is important to optimise the process to locate as many marks as possible. A contributing factor to process optimisation is the need for the operator to properly dark adapt when circumstances require it. The extent of the contribution is dependent upon both the brightness and wavelength of the emission. For example, fluorescence emission from dyes such as basic yellow 40 is very bright and minimal, if any dark adaptation is necessary, but fluorescence from latent marks can be very dim and dark adaptation (together with properly blacking out the examination area) may be essential to obtain the best results. Detection of shorter wavelength emissions (such as that from luminol and some latent marks) may benefit more from dark adaptation than longer wavelength emissions (such as basic violet 3) although it may still be beneficial to some extent for all weakly fluorescing marks.

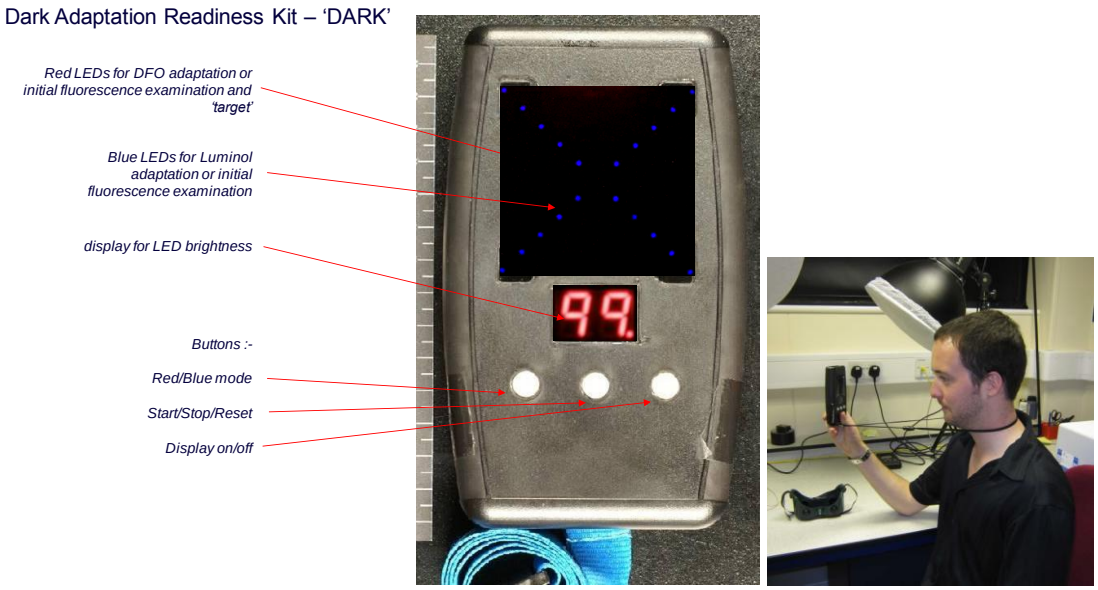
8.4.2 The term ‘dark adaptation’ describes the adjustments (both chemical and physical) that occur in the eye when going from a well lit environment to one of much lower light levels. Therefore by allowing the eye to progressively dark adapt the observer should be more sensitive to low light levels and should be able to see more fingermarks during fluorescence examination.

8.4.3 The sensors in the eye consist of both rods and cones. These sensors are not uniformly distributed, with cones being concentrated around the macula (the central area of the retina), and rods being more evenly distributed across the rest of the retina. At high light intensities both rods and cones function, however the rods are bleached out and so vision is predominantly due to the response from the cones. At low light levels only the rods function. Only the cones provide colour information and visual acuity, so at low light levels colour vision is not possible and peripheral vision (provided by the rods) is better (although ability to see fine detail is lost). As time spent in a darkened environment increases, the eye progressively adapts so that the responses from the rods begins to dominate that from the cones so that the eye can begin to see detail at low light levels.



A 'dark adaptation' curve showing how the response from the rods progressively replaces that from the cones as time in the dark increases, and ability to see in a darkened environment increases over time.

8.4.4 CAST conducted a series of experiments to determine how different conditions affected the level of dark adaptation, with the objective of producing guidelines for practitioners on how long dark adaptation should take place for, and what conditions of 'darkness' were required. An essential starting point for this work was to develop equipment that could give repeatable, empirical measurements of dark adaptation that could be used to identify trends and monitor the impact of different conditioning factors. This equipment was based around a series of LEDs that was observed by the operator in a fully darkened room and became progressively brighter over a short period of time. The operator stopped the test at the 'brightness' level where the LED emission was just perceptible. The equipment is capable of testing dark adaptation in the blue and red part of the spectrum. All of the following data is collected using the blue LED setting as this closely replicated the brightness and emission wavelengths of luminol – a forensic search tool used for locating blood and the focus of CAST studies at that time.



The 'DARK' system and the experimental set up for the tests (which took place with the lights out). Note that the lanyard allows the equipment to be held at a consistent distance from the eyes.

8.4.5 The test methodology used to measure a relative dark adaptation for each experiment was:

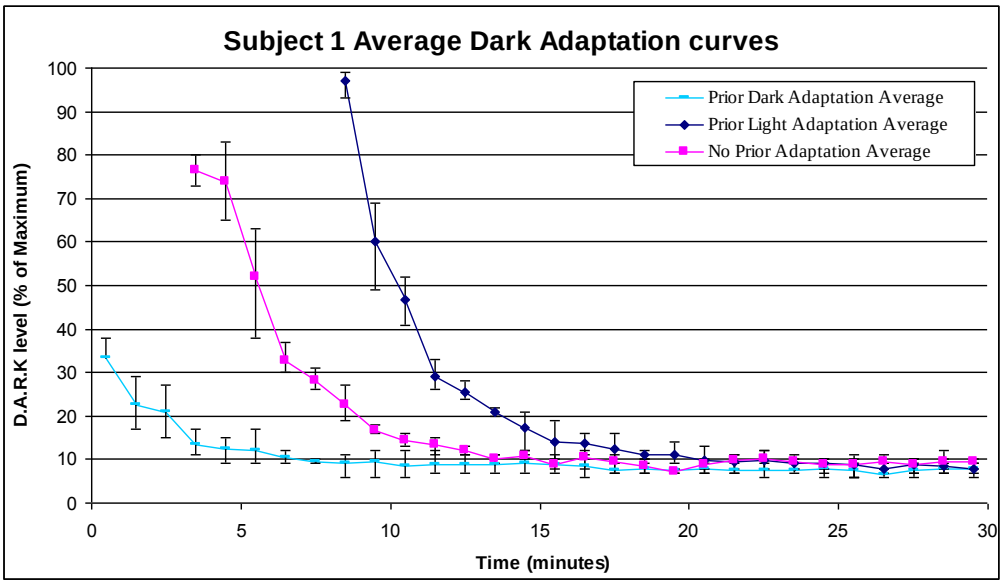
- Turn off lights and sit in the dark
- Every minute press 'start' on the device
- Blue LEDs will activate, gradually getting brighter (1% to 99% intensity) over 30 seconds
- Press stop and record the intensity % at which the blue LEDs become perceptible
- Repeat

8.4.6 Initial experiments used three conditions of prior adaptation:

- No adaptation: Entering test room from the office environment (measured light level approximately 500 lux)
- Prior light adaptation: Reading under diffuse white lights (measured light level approximately 8000 lux) for 10 minutes
- Prior dark adaptation: Wearing 593/630 nm long-pass filters for 20 minutes

For reference, the light level on an overcast day is approximately 1000 lux, and full daylight approximately 10,000 lux.

8.4.7 An example of the typical results from a single subject is given below, showing that the time taken to reach the optimum level of dark adaptation is significantly affected by the conditions the subject has been exposed to immediately before commencing the test.

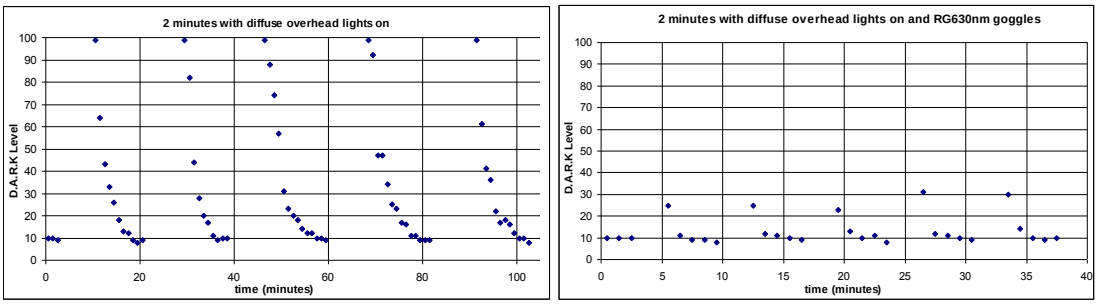


Dark adaptation curve for a single subject, showing differences in time required to become dark adapted according to prior conditioning.

8.4.8 Initial tests were also conducted to assess the impact of interruptions (such as turning on room lights) on dark adaptation and how quickly adaptation could be recovered after such an event. This experiment simulated the common scenario where a mark may be found during fluorescence examination but lights are then turned on so that it can be marked up and recorded. The experiments considered the following variables:

- Type of light being turned on (500 lux room lights, or 8000 lux diffuse lights)
- Length of time of exposure to light (1 minute and 2 minutes)
- Eye protection (no goggles, goggles with long pass red filters (593 nm or 630 nm))

Results indicated that the time required to recover a reasonable level of dark adaptation was significantly reduced when red goggles were worn during periods of exposure to light environments.



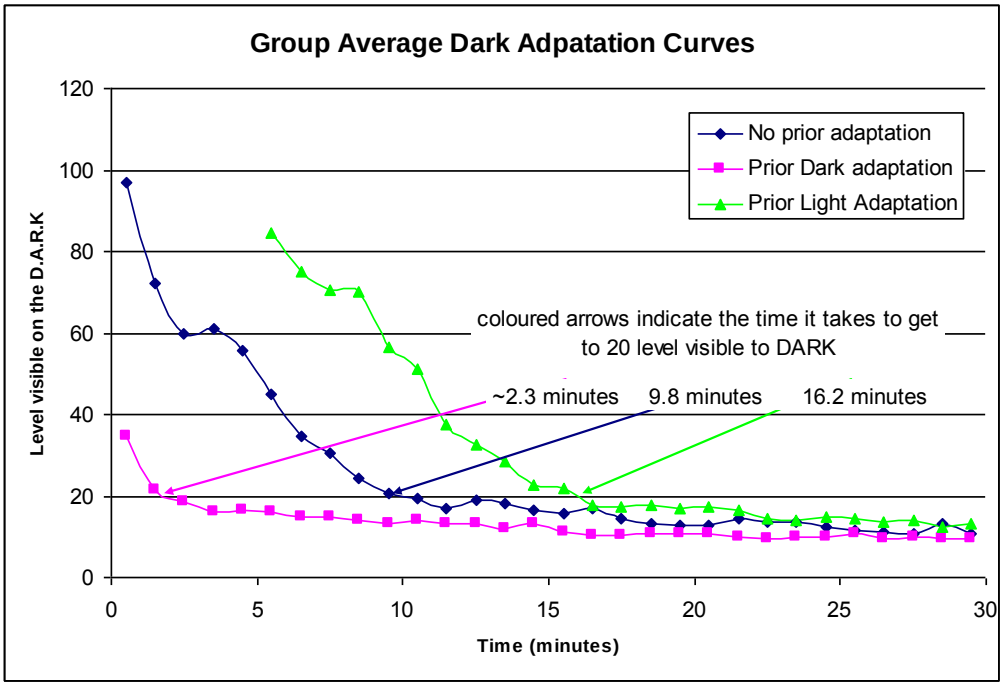
Results from initial studies into the effect of light interruptions on dark adaptation (left – exposure to room lights for 2 minutes without goggles, right – exposure to the same conditions but wearing red 630 nm long pass goggles)

8.4.9 The initial studies into the effect of the pre-examination environment and interruptions on dark adaptation were then repeated using a wider pool of 14 subjects. Of these 14 subjects, around 50% claimed to have good eyesight and the remainder stated they were either long or short sighted. All short or long sighted subjects wore their vision correction (glasses or contact lenses) during the test. The first 4 volunteers repeated each experiment 3 times. The age range of the subjects is summarised in the table below.

Age group	**No. of donors**
20-30	6
30-40	5
40-50	2
50-60	1

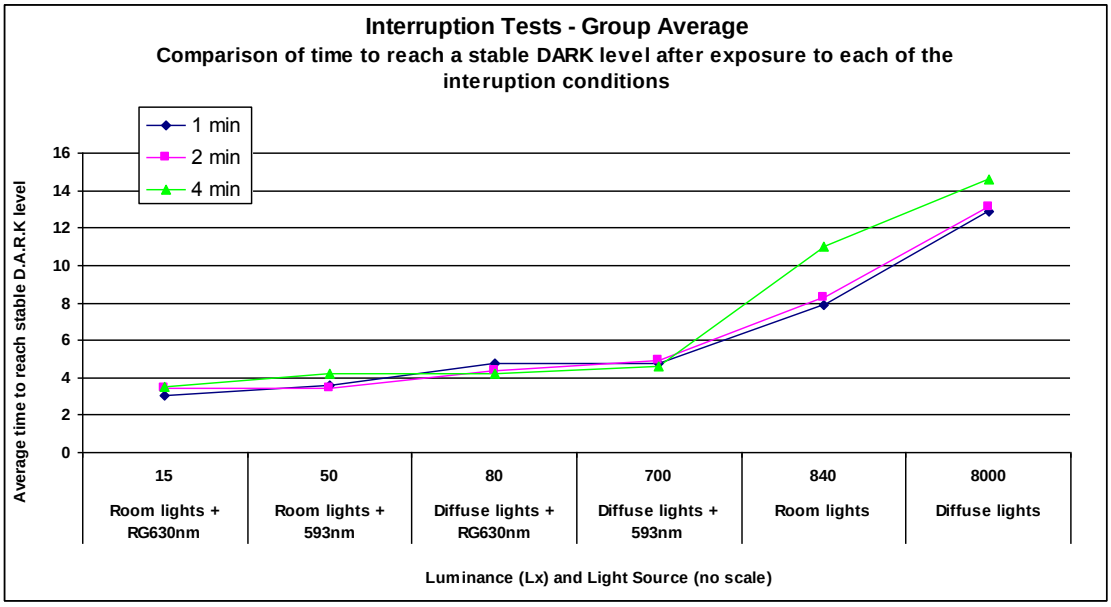
Ages of the subjects involved in the dark adaptation studies.

8.4.10 The averaged curves obtained for all donors across all repeat experiments reiterate that the conditions the subject is exposed to prior to entering a dark environment impact significantly on the time taken to dark adapt. The times taken to reach a reasonable level of dark adaptation are illustrated below, it can be seen that the time taken to reach the optimum level of sensitivity is longer still.



Averaged dark adaptation data for 14 subjects exposed to different pre-conditioning environments.

8.4.11 The interruptions study was extended to investigate interruption times of 1, 2 and 4 minutes, and using both 593 nm and 630 nm long pass goggles under both room lights and diffuse white lights, giving a total of 6 different lighting environments with different levels of lux. The results clearly show that simply turning room lights on and off is bad for dark adaptation, even if the exposure is for short time periods. The impact can be minimised by reducing the intensity of the light the eye is exposed to, and by restricting exposure to wavelengths at the red end of the spectrum.



Time taken to recover a reasonable level of dark adaptation (as measured by DARK equipment) as a function of the type of interruption, its duration and any protective measures used.

8.4.12 It was concluded that the brighter the pre-examination environment, the longer it takes to dark adapt. Wearing red goggles before entering the dark room speeds up the adaptation process, and wearing red goggles when lights are turned on helps to retain dark adaptation through interruptions. A similar effect would be achieved by leaving room lights turned off, but using a red torch or safe light for recording notes and marking up. Little difference was observed between interruptions of 1, 2 or 4 minutes with regards to how long it takes to re-establish maximum adaptation. This suggests the damage from light interruption is done quickly (less than 1 minute).

8.4.13 It is worth considering that these studies were conducted using blue LEDs only. The data clearly shows the impact on the dark adaptation process for realistic scenarios encountered in the forensic environment, and is focused towards providing guidance for luminol examinations. It does not provide definitive guidance on the impact of dark adaptation for fingermark visualisation, where the emission intensity and wavelength can vary, although it does provide a useful insight into the important factors that can impact on dark adaptation times. The advice

given for dark adaptation in the Fingermark Visualisation Manual is based on these observations, suggesting dark adaptation times of 15 to 30 minutes for situations where faint fluorescent marks may be present. This could be significantly reduced (or omitted altogether) where brightly fluorescent fingermarks are being examined.

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Infra-red imaging

1. History

1.1 The existence of infra-red (IR) radiation was discovered in 1800 by William Herschel, building on Newton's observation that sunlight could be separated into different colours by refraction through a glass prism. Herschel was investigating the theory that different colours could contain different levels of heat, and confirmed this by placing thermometers in different colour ranges of the spectrum. Herschel observed that the measured temperature increased from the violet to the red end of the spectrum, but made the additional observation that the measured temperature continued to increase beyond the red portion of the spectrum indicating that non-visible radiation was present. This radiation was termed 'calorific rays' by Herschel, with the term 'infra-red' being adopted in the late 19th century.

1.2 The practical applications of IR radiation were limited until the development of the first detector materials towards the end of World War I. Military imaging applications and astronomy continued to be the main driver for the development of IR detectors and imaging systems for several decades, with the first IR imaging devices developed in the 1940s.

1.3 Although forensic applications of IR imaging such as footwear imaging and visualisation of blood stains were explored as early as the 1930s [1,2], such photography required use of specialised IR sensitive film [3] and was often speculative, carried out under the assumption that a feature of interest would be present. The process did not begin to be more widely explored until the advent of detectors linked to video displays, allowing live imaging in the IR region of the spectrum. With live imaging capability, investigators could see whether there were any IR reflection or fluorescence effects occurring and subsequent photography could be targeted appropriately. Some early experiments were carried out in the Metropolitan Police Forensic Science laboratory and elsewhere using first generation military image converter tubes utilising S1 photocathodes and subsequently S20 photocathodes.

1.4 The principal application of IR imaging in forensic science was in document examination and from the 1950s onwards various researchers [4,5,6,7] used IR sensitive vidicons to investigate the potential of reflected IR and IR fluorescence for applications such as detection of forgeries, revelation of erased writing and ink comparison. As the cost of image converters and IR detectors reduced, bench-top document examination equipment became available in the late 1970s/early 1980s, enabling routine examination of documents in forensic laboratories. IR imaging systems utilising vidicons based on those developed in the Birmingham Forensic Science laboratory [6] were commercialised by Foster and Freeman and marketed as the Video Spectral Comparator (VSC).

1.5 In addition to document examination, IR photography was also found to be a valuable tool for the imaging of blood spatter patterns on surfaces appearing dark under visible light [1,8]. Similarly, IR photography has also been used in the imaging of injuries (such as bruising) on skin [9].

1.6 The potential use of IR imaging for visualisation of fingermarks was being considered in the 1940s [10] although does not appear to have entered routine use until much later. Wilkinson [11] makes a reference in the late 1970s to the use of IR microscopy to enhance a powdered mark (IR opaque) on a dark green bottle (IR translucent) and a US Patent issued around the same time [12] makes specific reference to the IR responsive nature of a fingermark powder. This does not appear to have resulted in a significant increase in the use of IR imaging for fingermark applications. However, the Metropolitan Police was using reflected IR for photography of marks revealed using physical developer in the mid-1980s [13]. The same group subsequently reported the use of IR long-pass filters to detect IR fluorescence from latent fingermarks illuminated with an argon ion laser at 514.5 nm [14]. Use of longer IR wavelengths was also considered for fingermark enhancement. In the early 1980s CAST conducted trials at the laboratories of STC Harlow on the use of a scanning thermal imaging microscope for the detection of finger marks on difficult surfaces such as adhesive tape and some metals, hoping to exploit differences in emissivity. This was not pursued at the time due to the limited resolution of the systems available and low contrast observed.

1.7 Later research using a live capture digital imaging system demonstrated that in addition to IR fluorescence of latent prints, existing fingermark reagents such as basic violet 3 (Gentian Violet) exhibited some fluorescence in the IR region of the spectrum. This fluorescence could be used to aid visualisation of the developed mark [15].

1.8 Subsequent work by the Home Office Centre for Applied Science and Technology (CAST) [16] demonstrated that IR reflection was an effective technique in suppressing background patterns when metallic or inorganic development reagents (e.g. vacuum metal deposition, powders, powder suspensions and physical developer) were used, although marks developed using organic reagents such as ninhydrin became transparent and could not be seen in the IR. CAST also demonstrated that IR fluorescence can be observed for the protein dyes acid black 1 and acid violet 17 [17]. Although IR fluorescence is observed for the pure acid violet 17 dye, much stronger fluorescence is seen for batches of acid violet 17 mixed with dextrin and this may offer a route for producing IR fluorescent reagents in the future.

1.9 All of the above forensic applications (with the exception of work with a scanning thermal imaging microscope) utilise the near IR region of the spectrum where the interactions of the incident radiation with the fingermark residue and the substrate are very similar to those occurring in the visible region. Further into the IR region the incident radiation can

promote molecular vibrations, such as bond stretching and rotation, and characteristic absorption peaks associated with these motions can be used to characterise chemical species present in the fingermark. This approach was applied in a study to compare fingermark residues of males, females and children, investigating compositional differences between these groups and between eccrine and sebaceous deposits [18]. This study used spectromicroscopy and focused on a small portion of an individual ridge. A later study looked at the same technique to detect and identify particles trapped in the fingermark ridges, such as illicit drugs [19].

1.10 More recently the use of the longer wavelength, short- and mid-wave IR regions of the spectrum has been considered for the imaging of fingermarks, using wavelengths where absorption mechanisms characteristic of chemical species in the fingermark ridges occur. The systems used to image fingermarks in this way are currently (in 2016) highly specialised pieces of equipment more suited to research than operational work, mostly using Fourier Transform IR (FTIR) spectroscopy techniques. A FTIR focal plane array detector has been used to scan a fingermark on an Australian banknote in a series of lines, stitching these together to form the final image [20]. Other researchers have used FTIR imaging to detect both latent and treated fingermarks on a range of porous and non-porous surfaces [21]. In an alternative approach researchers have used arrays of FTIR sensors in the attenuated total reflection (ATR) mode [22], which significantly reduced the time taken to produce the image, but there is still the potential to increase the size of the array and reduce the imaging time for the whole area of the fingermark. The limitation of this approach has been that the sample bearing the fingermark needs to be flat and in intimate contact with the detector. This limits the type of exhibit that can be examined using ATR-FTIR equipment, but to overcome this limitation the lifting of marks and other forensic evidence from exhibits using gel lifters and tape has been investigated [23,24]. This technique offers the potential to bring marks back from a crime scene for subsequent laboratory analysis.

1.11 Other regions of the IR spectrum may also offer potential for fingermark detection, and one approach that has been proposed is to pass humidified air over latent fingermarks and utilise an IR thermography camera to detect temperature differences between the fingermark ridges and the substrate [25]. This does not appear to have been progressed further in recent years.

2. Theory

2.1 IR imaging is a broad subject area and there are many processes by which contrast may be obtained between the fingermark ridges and the background. In the near IR regions these include fluorescence, absorption and reflection. At longer IR wavelengths there are other mechanisms that can be used to distinguish fingermarks, including IR absorption characteristics associated with chemical species present in

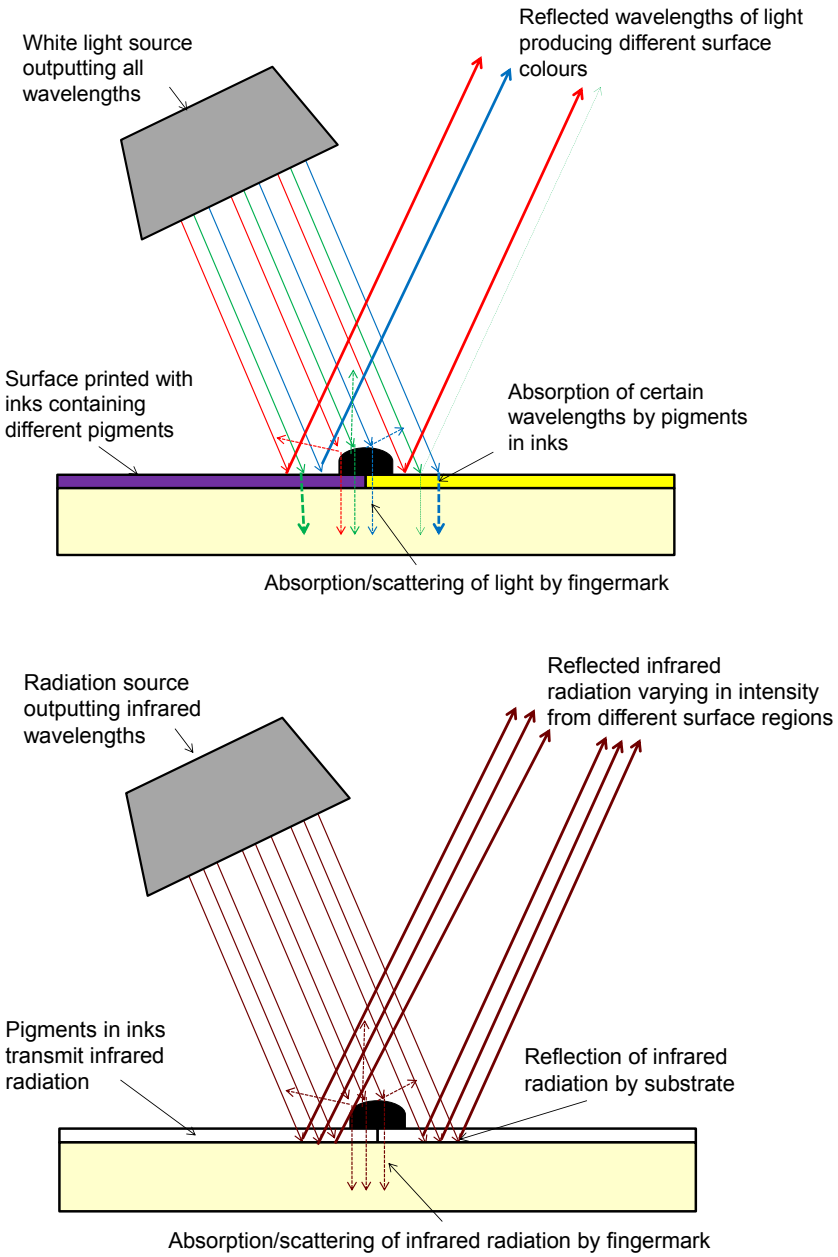
the fingermark but not in the substrate, and thermography using differences in emissivity between the fingermark and the substrate. Each of these processes are described in greater detail below.

2.2 Near IR (700–1,400 nm) (as defined by the International Commission on Illumination (CIE))

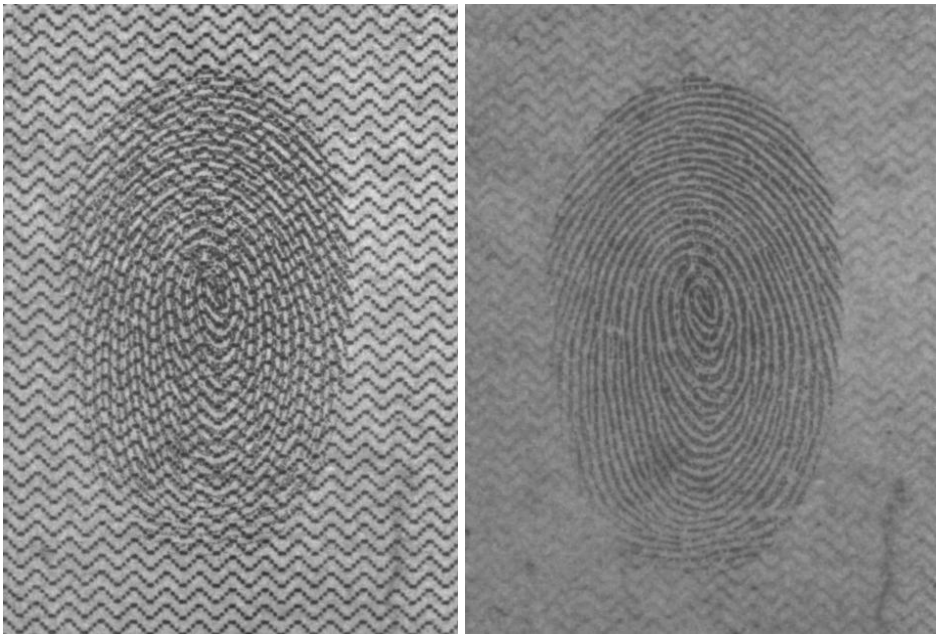
2.2.1 In this region of the IR spectrum, the mechanisms used for fingermark visualisation are essentially the same as those used in the visible and ultraviolet (UV) regions, namely fluorescence and absorption/reflection. The principal difference from imaging in the visible region is that many of the organic pigments used in printing inks are IR transparent, and surfaces that appear highly patterned and/or coloured under 'daylight' conditions in the visible spectrum may appear devoid of printing when viewed in the near IR. This can be a significant advantage when trying to resolve minutiae in fingermarks developed on articles like banknotes.

2.2.2 It has already been established that some fingermark reagents do have some fluorescence in the IR when illuminated with green/yellow and yellow light, most notably basic violet 3 and acid violet 17 (although much of the emission of basic violet 3 is actually in the red spectral region). However, the use of longer wavelength illumination such as orange, red and IR, and the resultant fluorescence from existing reagents has not yet been extensively explored. The potential to develop IR fluorescent dyes and reagents for fingermark detection clearly exists and could be exploited in future. Fingerprint powders with infrared fluorescent properties have recently begun to become available (Foster & Freeman's Natural 1, Natural 2 and the pigment Egyptian Blue). Preliminary investigations have indicated that there may be some constituents of latent fingermarks that have fluorescence in the near IR, but optimum illumination conditions have not yet been identified.

2.2.3 For fingermark imaging using IR reflectivity, a radiation source emitting in the near IR is required. As stated above, many inks used for printing are IR transparent and highly patterned surfaces may be suppressed. To date, it does not seem that latent fingermarks can be detected in this way. It has not yet been established whether there are any fingermark constituents that have characteristic absorption mechanisms in this region, but the background fluorescence of most surfaces is low and the contrast between the substrate and ridges is insufficient for fingermark visualisation. However, it is possible to image some developed fingermarks in IR reflection mode. Many organic reagents and dyes, including ninhydrin, solvent black 3 and superglue, are transparent in this region of the IR spectrum and developed marks are not visible. Those developed using inorganic or metallic processes, including vacuum metal deposition, powders and powder suspensions, either absorb or scatter IR more than the background and developed marks remain visible when imaged in the IR.



Schematic diagram showing the way in which the difference between the reflectivity of pigments in the (top) *visible and* (bottom) *near infrared regions of the spectrum can be used to increase the contrast between the fingermark and the background.*



a) b)

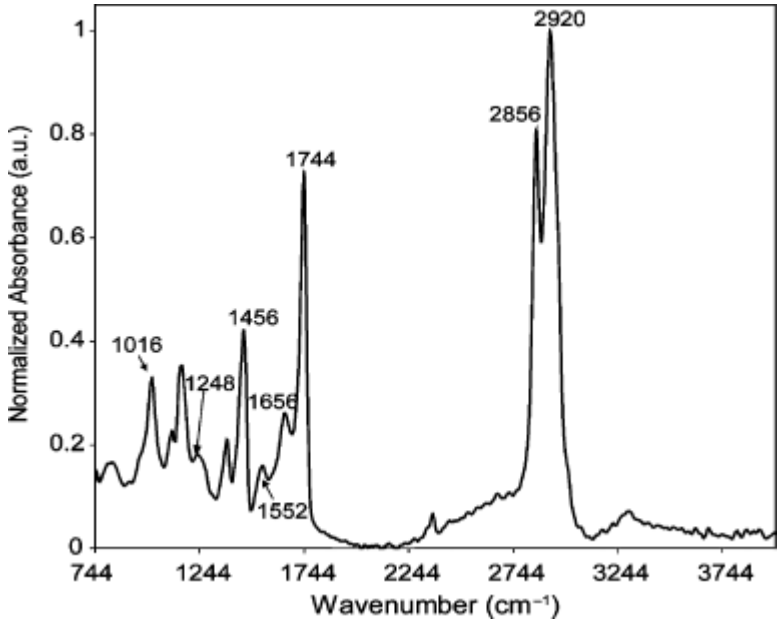
Images of a fingermark developed using physical developer on patterned background a) *imaged in the visible region under tungsten illumination and* b) *imaged under tungsten illumination using an infra-red long-pass filter (Schott glass RG780).*

2.3 Short-wave IR (1,400–3,000 nm)

2.3.1 This region of the IR spectrum has not yet been extensively studied for the detection of fingermarks and there is no established process for fingermark detection. However, there are some known processes, such as water absorption bands at around 1,500 nm and 1,900 nm that could be exploited in future.

2.4 Medium-wave–long-wave IR (3,000 nm–1000 μm)

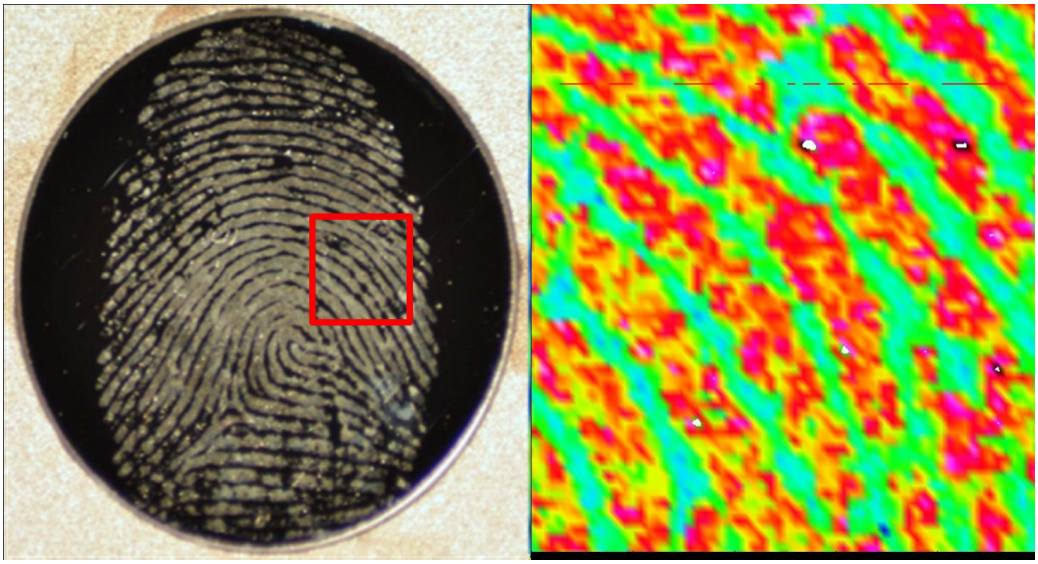
2.4.1 Further into the IR spectrum, many organic compounds have characteristic absorption peaks associated with vibration of organic sidegroups or stretching of chemical bonds. This chemical specificity can be utilised to discriminate different compounds using techniques such as FTIR spectroscopic imaging.



Wavenumber (cm^{-1})	Assignment
1,016	Asymmetric O–C–C stretch, ester
1,248	Asymmetric C–C–O stretch, ester (C bonded to the O included in the carbonyl)
1,456	CH_2 scissors
1,552	N–H bend combined with C–N stretch, protein amide II feature
1,656	C=O stretch, protein amide I feature
1,744	C=O stretch, saturated ester
2,856	Methylene C–H stretch
2,920	Methyl C–H stretch

Fourier Transform infra-red spectrum from fingermark residue and molecular motions associated with peaks [21].

2.4.2 Researchers have begun to investigate the potential of this for fingermark detection. It is possible to use a peak wavelength characteristic of a particular constituent of the fingermark residue or fingermark development reagent (but not present in the substrate) to obtain an image giving enhanced contrast between the fingermark and the background.



Attenuated total reflection-Fourier transform infrared image of a fingermark, showing distribution of lipid components.

2.4.3 This has been successfully demonstrated for fingermarks developed using cyanoacrylate fuming on the highly patterned background of an Australian banknote. The technique may also be able to detect the presence of other characteristic compounds (e.g. drugs, explosives) in fingermark residues, giving investigators additional information about the person depositing the fingermark.

2.4.4 At longer wavelengths, other mechanisms can be utilised to image fingermarks. IR imaging systems in this region can be used in non-destructive evaluation applications, such as thermography. There is the possibility of obtaining a contrast between fingermark ridges and the substrate by exploiting differences in emissivity or differences in thermal conductivity between the two materials. By applying a pulse of heat or humidified air to the region of interest and observing the response of the surface using a thermal camera, it may be possible to resolve fingermark ridges. This approach is routinely used for defect detection in aerospace materials and has been considered as a fingermark detection technique, although a practical system has not yet been produced.

3. Centre for Applied Science and Technology processes

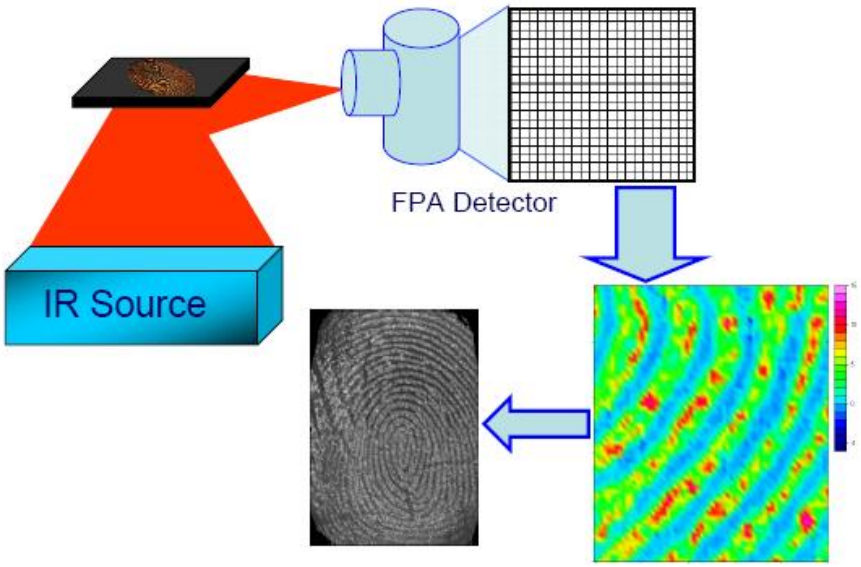
3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. IR reflection is included in the *Fingermark Visualisation Manual* as a process to aid the visualisation of fingermarks developed using other processes, in particular physical developer.

3.2 IR imaging is recommended in the CAST newsletter on arson [26], where it has been demonstrated that IR imaging can reveal fingermarks treated with physical developer on charred paper exhibits. Because IR imaging is an essentially non-destructive technique it can be inserted at appropriate stages of any sequential treatment without detriment to subsequent treatments.

3.3 The procedure currently (2016) recommended is to utilise a digital imaging system without an IR blocking filter bonded to the chip. Previously this option was mostly confined to scientific grade, machine vision cameras, although some digital single lens reflex (SLR) cameras with IR sensitivity were available as early as 1992. SLR cameras built without UV/IR blocking filters over the sensor are now becoming much more widely available for forensic imaging applications. For IR reflection imaging, a radiation source emitting in the near IR is required. Standard tungsten bulbs are appropriate for this purpose, although the range of LEDs with IR outputs is increasing. To view the reflected IR radiation and block the visible region of the spectrum, IR cut-on filters are used in front of the camera. A range of Schott glass filters are available giving cut-on wavelengths between 645 and 1000 nm. Although the lower wavelength filters do have some use in document examination, those of most use in suppression of patterned/coloured backgrounds and charred substrates in fingermark imaging are RG715, RG780, RG850 and RG1000.

3.4 The same range of camera filters can be used when imaging in IR fluorescence. However, the light sources and fingermark development reagents for this application have not yet been optimised.

3.5 ATR-FTIR is also included in the *Fingermark Visualisation Manual* as an emerging process with the potential to add contextual information to marks recovered by other means, such as gelatin lifting. Detailed process instructions are not included because these will vary according to the type of instrument being used and the purposes of the analysis.



Schematic diagram showing the use of ATR-FTIR using a sensor array to produce a composition map of an area of a fingermark.

4. Critical issues

4.1 For near IR reflection imaging to be effective, imaging devices that are sensitive in the IR region of the spectrum must be used. Conventional charge coupled devices (CCD) and complemetary metal oxide semiconductor (CMOS) sensors used in digital cameras are sensitive in this region but usually have an UV/IR blocking filter bonded to them to confine response to the visible region of the spectrum. Specialist models of digital camera are becoming more widely available for IR imaging with this blocking filter either removed altogether, or that have IR blocking filters that can be readily removed when IR imaging is required.

4.2 An appropriate light source must also be used. For IR reflection imaging the light source must output in the near-IR region. Tungsten lamps are suitable for this purpose, fluorescent tubes and light emitting diodes (LEDs), unless specifically produced for IR emission, are not. The most appropriate wavelengths for exciting IR fluorescence are not yet known.

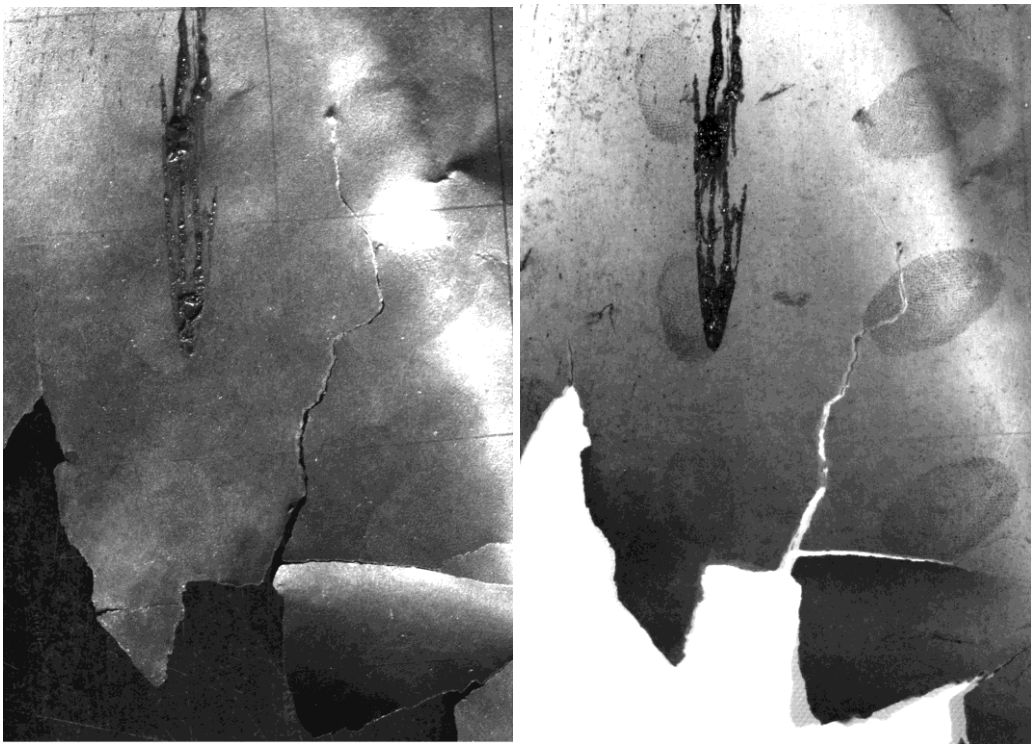
5. Application

5.1 Suitable surfaces: Reflected IR imaging is suitable for use on any coloured, patterned surface provided that the fingermarks present have been developed using a process that leaves metallic or inorganic material on the fingermark ridges (e.g. powders, vacuum metal

deposition, powder suspensions). The fingermarks then remain visible in the IR region of the spectrum and organic dyes and inks are typically transparent. This has been found to be effective on printed paper, fabrics and printed plastic bags.

5.2 The principal application for IR imaging has been in the suppression of patterned backgrounds on porous substrates where marks have been developed using physical developer. This is of particular benefit on articles such as banknotes, where the printing is multicoloured and patterned. Although regular background patterns can be removed using digital techniques such as fast Fourier transforms, in many regions of banknotes the pattern is not regular and this approach cannot be used. The most commonly used technique for porous surfaces, ninhydrin, produces marks of a similar colour to the £20 note and makes imaging of features difficult. In these cases, using physical developer followed by IR imaging can produce fingermarks that cannot be visualised by other techniques.

5.3 The other application where IR imaging has been proven to be of benefit is on charred articles, again where physical developer has first been used to develop any marks present [26].



a) b)

Photograph of fingermarks developed using physical developer on charred paper a) *imaged in the visible region under tungsten illumination and* b) *imaged under tungsten illumination using infra-red long-pass filter (Schott glass RG780).*

6. Alternative formulations and processes

6.1 There are many regions of the IR spectrum that can be utilised for IR imaging of fingermarks, and the different techniques for imaging fingermarks have been described in the preceding sections.

7. Post-treatments

7.1 There are no post-treatments used with IR imaging.

8. Validation and operational experience

8.1 IR imaging is used as a non-destructive post-treatment to aid in the visualisation of marks developed using other processes and therefore an extensive validation study has not been conducted. Studies have been carried out on a range of UK and European banknotes (one of the principal surfaces where IR imaging could give benefits) to demonstrate which features in the printed areas may be suppressed under IR imaging conditions. A further small-scale study has been carried out by CAST to demonstrate which fingermark development processes give marks that are still visible when imaged in the near IR [16].

8.2 There are known operational cases where police forces have utilised IR imaging to suppress backgrounds on exhibits where marks have been developed using physical developer. One police force treated a batch of £20 notes with ninhydrin and although several marks were developed these were in patterned regions where it was not possible to resolve minutiae. CAST recommended re-treating the exhibits with physical developer followed by IR imaging and several identifiable marks were produced.

8.3 Another force carrying out a cold case review treated a 25-year-old postal order that had been wetted with physical developer. A mark was developed but some minutiae were obscured by printing. IR imaging successfully suppressed the printing and revealed some additional minutiae. However, there was still insufficient detail for an identification.

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Scanning electron microscopy

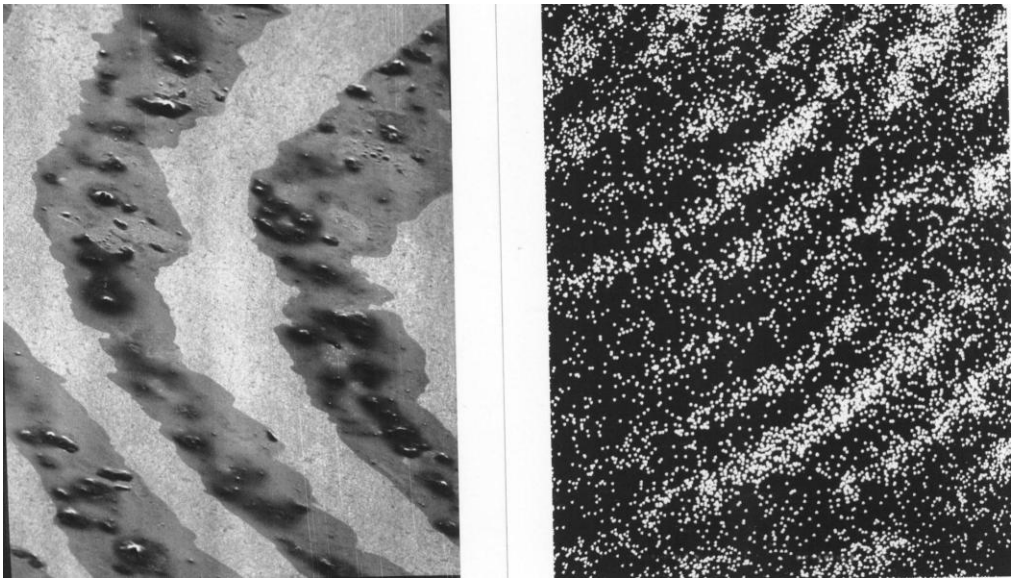
1. History

1.1 As early as the 1920s it was recognised that beams of electrons could be focused by means of electrostatic or magnetic fields and that the short wavelength of electron beams offered significant improvements in both resolution and depth of field compared with light microscopy [1].

1.2 It was not until the 1950s and 1960s that practical electron microscopes began to emerge, utilising both transmission and scanning modes to provide images of materials. The potential applications of electron microscopy (in particular the scanning electron microscope) in forensic science were first explored in the late 1960s. Van Essen [2] reported the use of scanning electron microscopy in combination with energy dispersive x-ray spectroscopy for the analysis of paint and metal fragments, ink composition and for studying hair and fibres.

1.3 It was also recognised that scanning electron microscopy could be used for imaging of fingermarks, and Garner *et al.* [3] demonstrated that latent fingermarks could be detected on both glass and metal substrates. On the non-conductive, glass surface a gold coating was required to prevent charging and it was also observed that older marks were more difficult to image than freshly deposited marks.

1.4 The Police Scientific Development Branch (PSDB) began studies into the use of electron microscopy in the late 1970s [4,5], and installed a JEOL 35C scanning electron microscope with an energy dispersive x-ray spectrometer specifically to explore imaging of fingermarks. Various imaging modes were investigated [4] including specimen current imaging of latent marks and mapping of silver distribution in marks developed using physical developer. The microscope was also used as a research tool to study the secondary electron escape depth from fingermarks [5]. Both latent and treated fingermarks were used in these studies, the differences in elemental deposition occurring using vacuum metal deposition being utilised to reveal fingermark ridges crossing boundaries between printed and unprinted regions of the substrate.



a) b)

Early scanning electron microscopy images of fingermarks a) *latent fingermark in secondary electron imaging mode and* b) *mark developed using vacuum metal deposition in elemental mapping mode*

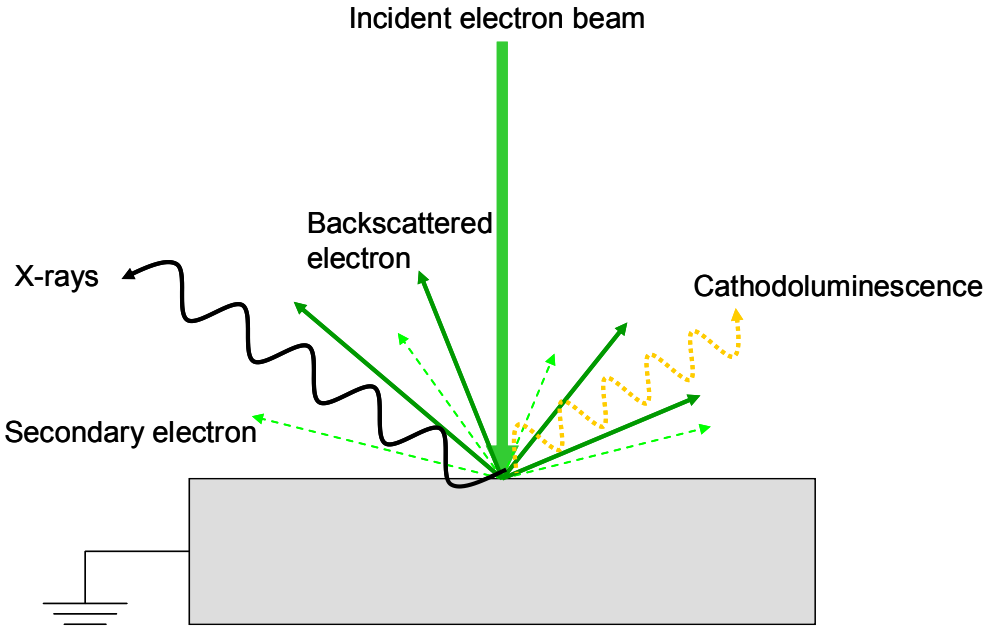
1.5 Although potentially effective for distinguishing fingermark ridges against obscuring backgrounds, scanning electron microscopy and its associated analytical modes have been more useful in providing information about the mechanisms of other development processes and the composition of powders and reaction products. Scanning electron microscopy has been extensively used by PSDB in the characterisation of fingermark powders, brushes and powder suspensions [6,7,8,9,10,11] and has also provided useful images of the reaction products from superglue, vacuum metal deposition, and physical developer processes, as well as acid dye developed marks in blood and interactions of successive development processes [12,13].

1.6 In practical terms, scanning electron microscopy is little used for casework because its application often requires a small area to be cut from the exhibit and coated with a conductive material to prevent the sample charging. However, there are situations where it may provide additional information and it remains an invaluable tool for understanding the interactions between fingermarks and the surfaces they are deposited onto [14,15].

1.7 In its standard form scanning electron microscopy is a high vacuum technique. This may have some detrimental effect on the fingermark and its subsequent development [16]. However, some modern variants such as variable pressure (VP) and environmental SEM both reduce the level of vacuum required and remove the need to apply a thin metal layer as a conductive material [12].

2. Theory

2.1 When an energetic beam of electrons is focused onto a surface there are a number of interactions that can occur [1]. These include transmission and diffraction, which are of most interest for transmission electron microscopy and are therefore not discussed further here. The interactions of principal interest for scanning electron microscopy are illustrated schematically below.



Principal interactions between electron beam and sample in scanning electron microscopy.

2.2 Discussing each mechanism in turn, backscattered electrons occur where the incident electron undergoes a series of inelastic collisions with atoms in the sample and are scattered backwards out of the surface and towards the detector. These electrons are relatively high energy and the number of them occurring will be related to the atomic density of the surface being examined.

2.3 Secondary electrons occur during the inelastic collisions between the primary electrons and the atoms and some have sufficient energy to escape the surface towards the detector. They are of lower energy than backscattered electrons.

2.4 X-rays are also emitted, in a process analogous to fluorescence in the visible region of the spectrum. Electrons promoted into excited states by the interaction with the electron beam decay into their ground states, with the emission of an x-ray of energy/wavelength characteristic of the element present.

2.5 For certain materials, the emission of energy as the electrons decay back into their ground state occurs at an energy/wavelength

corresponding to the visible region of the spectrum. This is known as cathodoluminescence.

2.6 Of all these mechanisms, secondary electron imaging is most useful for examining the morphology of powders, brushes and reaction products. In secondary imaging, a positive charge is applied to the detector, which attracts most of the negatively charged electrons emitted from the surface. As a result, the signal received at the detector is relatively high and the image is not 'noisy'. The electron beam is scanned across the surface in a series of lines known as a raster, and the signal level recorded at each pixel represented on a screen.

2.7 Backscattered electron imaging is most useful where the elemental composition of the fingermark ridge and the background differs, especially if one contains an element of a significantly higher atomic number. Because the number of backscattered electrons is a function of atomic density, areas of high atomic density will produce more backscattered electrons and appear brighter. Backscattered electron imaging can be carried out by biasing the detector with a slight positive charge, thus repelling the low energy secondary electrons and only allowing the higher energy backscattered electrons to reach the detector. Because fewer electrons reach the detector, backscattered images may be more noisy, but may be capable of resolving fingermarks developed using techniques such as vacuum metal deposition and iodine [12].

2.8 X-ray spectroscopy can be carried out in a static mode, to determine the elemental composition of a particular location on the sample. X-rays can be separated and analysed according to their characteristic wavelength or energy. In practice the energy dispersive detectors are more compact (although not as suitable for quantitative analysis) and are more commonly fitted to electron microscopes. Energy dispersive x-ray spectroscopy can also be used in mapping mode, scanning the beam across the surface and recording the types of x-rays emitted at each point. If a characteristic element is present in the fingermark ridges, it is possible to resolve the ridges from the background in this way.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not recommend the process for routine operational work because it will normally be destructive to the exhibit, involving cutting an area small enough to fit inside the chamber of a scanning electron microscope and coating with a conductive element to prevent charging. However, it is recognised that scanning electron microscopy has the potential to image the continuity of ridge detail (particularly of developed marks) in situations where other processes are ineffective, and therefore in high profile cases there may be benefit in its use. It is therefore included as a Category B process in the *Fingermark Visualisation Manual*. These processes may be detrimental to subsequent analysis for

other types of forensic evidence. However, recent advances in electron microscope design may mean that larger samples can be examined and a conductive coating may not always be required. In some circumstances scanning electron microscopy and associated analytical techniques may be capable of providing additional information about a fingermark and its use should not be discounted. Suitable microscopes can be found in most universities.

3.2 Scanning electron microscopy is a useful research tool for investigating fingermark development techniques and has primarily been used for this purpose in recent years, in some cases augmented by transmission electron microscopy [11] and atomic force microscopy [15] for cases where very high magnifications are required.

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Specialist imaging techniques (Secondary ion mass spectrometry; scanning Kelvin probe; matrix assisted laser desorption/ionisation and surface assisted laser desorption/ionisation)

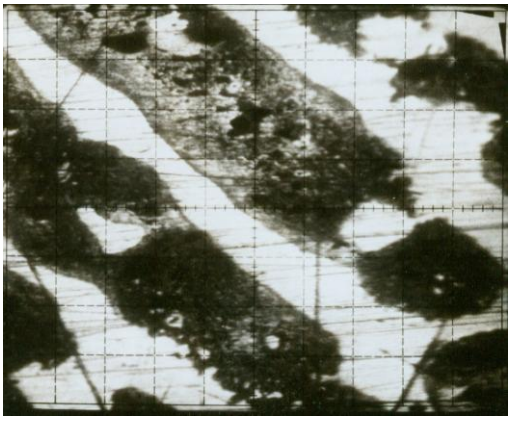
Secondary ion mass spectrometry

1. History

1.1 Secondary ion mass spectrometry (SIMS) has been used for many years as a technique for performing high sensitivity elemental analysis. It operates by bombarding a surface with a high energy beam of particles and analysing the mass of the secondary ions emitted. The process was originally not suitable for surface analysis because the high energy beam progressively removed layers of material. However, by the 1980s higher sensitivity detection systems were available that allowed the use of lower energy primary beam currents, and hence caused considerably less damage to the surface. Researchers began to explore the applications of SIMS for surface analysis, utilising the technique to identify the composition of surface coatings and small surface features [1]. SIMS was also used in an imaging mode, scanning the surface and detecting locations that specific molecular fragments were emitted from.

1.2 Bentz [2] applied SIMS to the analysis of fingermark residues, in particular to the detection of traces of contaminants in the fingermark. Many 'natural' fingermarks contained traces of silicones, and it was also demonstrated that small traces (nanograms) of illicit substances could theoretically be detected by the technique.

1.3 The Home Office Scientific Research and Development Branch (SRDB) funded an investigation of the use of the SIMS technique for both analysing fingermark residues and mapping their distribution using the scanning mode [3]. These studies used fingermarks from six different donors, and confirmed the presence of sodium (Na^+) and chloride (Cl^-) ions in varying quantities. Spectra from all donors contained peaks indicative of the presence of both long- and short-chain aliphatic materials, and also peaks characteristic of silicones. Fragments representative of alkoxy and phenoxy groups were detected and, more specifically, spectra from all donors contained the main negative ion from myristic, palmitic and oleic acids. One donor also gave the stearate ion. The imaging mode was also successful in distinguishing between the composition of fingermark ridges and that of the background.



a)

b)

c)

Images obtained using scanning secondary ion mass spectrometry for surface analysis a) *ion induced secondary electron image,* b) Na^+ *secondary ion image and* c) $C_3H_5^+$ *secondary ion image.*

1.4 As the instrumentation available for SIMS has advanced and desktop SIMS systems have been produced making SIMS more accessible, other researchers have reported the use of the technique for fingermark imaging and determining the distribution of principal constituents [4,5,6]. Follow on studies by the same research groups [7,8] have demonstrated that SIMS can also be used to detect exogenous substances of interest in fingermark residues, including drugs and gunshot residues.

1.5 More recently, SIMS has been explored as a method of determining the order of deposition for fingermarks and printing inks [9,10], a potentially important piece of contextual information that can be used to support an account of events. SIMS has also been found to be a potentially valuable method of revealing surviving ridge detail where other processes fail, particularly on samples exposed to extreme environments such as water immersion and burial [11]. It has been compared with a range of other analytical techniques for exploring fingermark composition, and found to offer several attractive features for this purpose in terms of:

- the chemical species detected;
- the sensitivity to them; and
- the ability to resolve fine detail [12].

However, it is recognised that the vacuum required to conduct a conventional SIMS analysis can modify the fingermark composition, accelerating the loss of certain constituents [13].

1.6 In contrast MeV-SIMS is a variant of SIMS, which employs a mega electron Volt (MeV) ion beam and allows analysis of exhibits in ambient pressure rather than in a vacuum. The technique is under development by a number of laboratories worldwide and is supported by the International Atomic Energy Agency. MeV-SIMS has been used to depth profile the penetration of fingermarks into porous surfaces to determine the deposition order of fingermarks and inks [14].

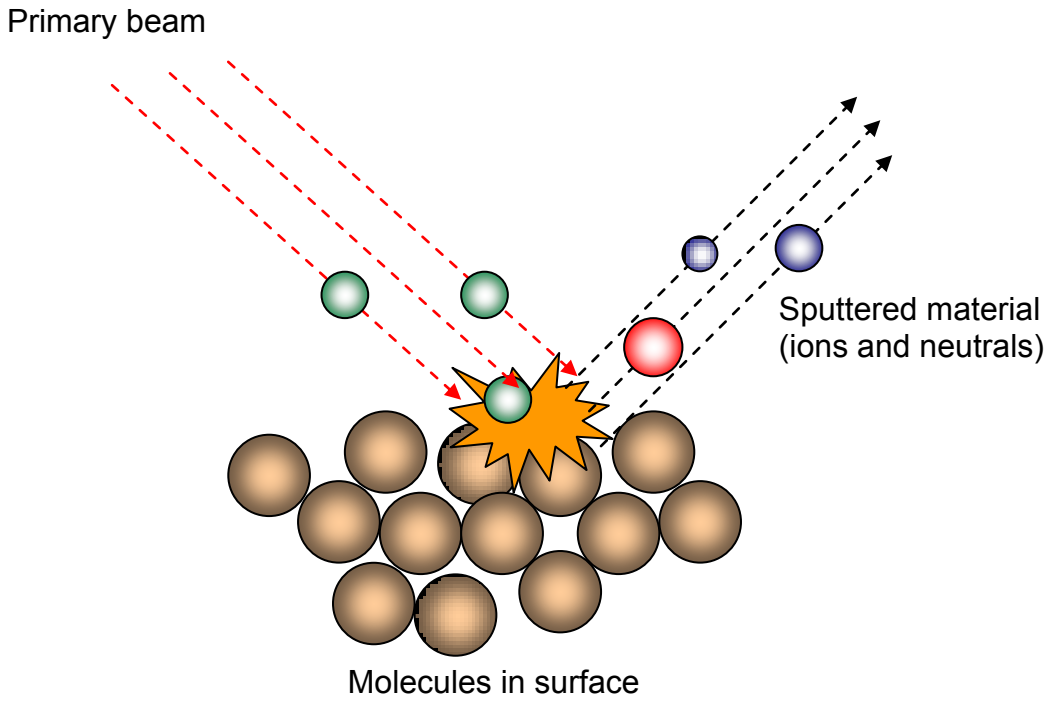
1.7 It is unlikely that SIMS will become a primary fingermark detection and/or imaging technique, but it can provide valuable information about:

- fingermark composition;
- contamination present in the fingermark; and
- contextual data.

It may therefore be appropriate to use the technique in special cases.

2. Theory

2.1 The theory of SIMS is that an energetic beam of particles is used to bombard a surface in a vacuum. The collisions between the incident particles and the molecules in the surface layer produce a number of charged atoms, molecules and molecular fragments, which are ejected from the surface. This process is known as sputtering, and the ejected species are known as secondary ions. The secondary ions may be positively or negatively charged.



Schematic diagram showing secondary ions ejected from surface by the action of the primary beam.

2.2 The secondary ions ejected from the surface can be focused into a mass spectrometer where they are separated and identified according to their mass to charge ratio. Under appropriate conditions, minimal fragmentation of the surface molecules occurs and the molecular ions present can be more readily identified.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Centre for Applied Science and Technology (CAST) does not recommend the process for routine operational work because it will normally be destructive to the exhibit; it involves cutting an area small enough to fit inside the chamber of a SIMS instrument. However, in some circumstances SIMS may be capable of providing additional information about a fingermark and/or previously undetected ridge detail and its use should not be discounted. As a consequence, the SIMS process is included as a Category C process in the *Fingermark Visualisation Manual.* Suitable instruments for the SIMS technique can be found in some universities.

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Scanning Kelvin probe

1. History

1.1 The scanning Kelvin probe technique was developed for the detection of corrosion occurring on metal surfaces. However, it was noted by Williams *et al*. [1] that electrochemical interactions may also occur between fingermark deposits and metal surfaces and they subsequently investigated the application of the scanning Kelvin probe technique to fingermark detection. Initial results were promising, with fingermarks being imaged on metal surfaces heated to 600ºC and beneath layers of insulating films.

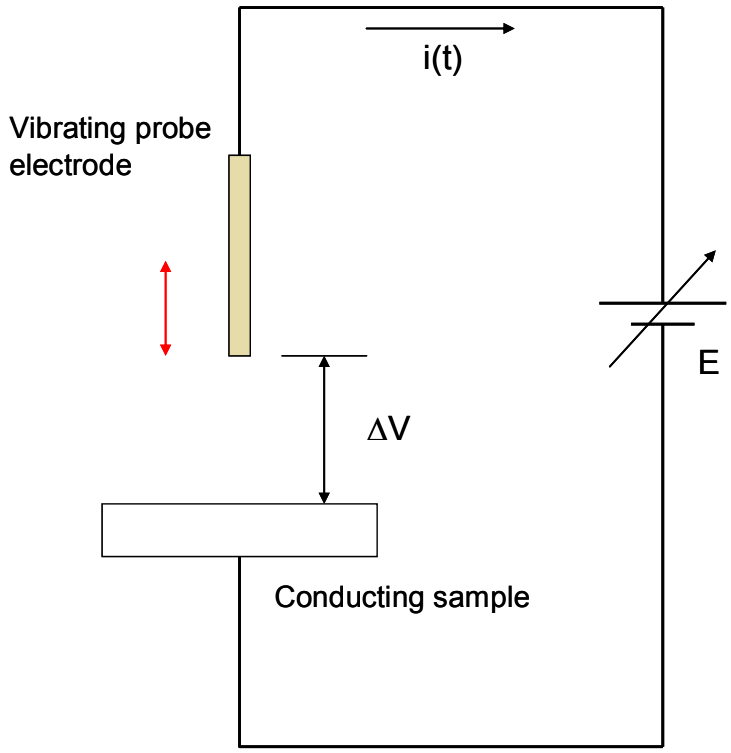
1.2 Subsequent research by the same authors showed that the process was applicable to a range of metal surfaces and could still detect traces of fingermarks on surfaces where the residue had been rubbed away with a tissue. The technique was also applied to practical situations and an apparatus was constructed for scanning cylindrical items such as cartridge casings [2].

1.3 The technique has the advantage that it is non-contact and non-destructive. It could, in theory, be used as the initial stage in a sequential treatment process. The effect of environmental conditions and storage time on the effectiveness of scanning Kelvin probe was investigated in PhD studies part-funded by CAST at Swansea University [3]. The process was also compared with routinely used processes such as superglue fuming and processes specifically intended for use on metal surfaces, such as palladium deposition. These PhD studies also

considered the sequential use of scanning Kelvin probe with other processes, with the prior application of vacuum metal deposition being beneficial in enhancing the contrast of the mark in some circumstances [4].

2. Theory

2.1 The scanning Kelvin probe consists of a fine, vibrating gold electrode brought into close proximity to the surface being examined. The vibrating probe tip and conducting sample surface form the two plates of a parallel plate capacitor, with the space between them (predominantly air but possibly including any non-conducting layers on the surface) forming the dielectric. If there is a Volta potential difference (ΔV) between the probe and sample surface, the periodic capacitance change caused by the vibrating probe generates an alternating current, i(t), in the external circuit. The Kelvin probe measurement is made by applying a direct current bias voltage E until the value of ΔV, and hence i(t) is zero. The circuit is illustrated below.



Schematic diagram showing principle of operation of the scanning Kelvin probe.

2.2 It can therefore be seen that any slight changes to the conducting sample or the dielectric between the probe tip and sample surface will result in changes to ΔV and therefore the resultant Kelvin probe measurement. Both eccrine and sebaceous fingermarks can change the

surface and dielectric sufficiently for changes in ΔV to be detected, giving contrast between areas of ridge and background when the probe is scanned across the surface. In the case of eccrine marks there may be an electrochemical reaction between the mark residue and the metal that changes surface potential, whereas in the case of sebaceous marks an additional layer of dielectric material is deposited on the surface.

3. Reasons technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Centre for Applied Science and Technology (CAST) does not currently (2016) recommend the scanning Kelvin probe process for fingermark detection because as a single process it is less effective than some of the other options available for treating metal surfaces. However, the process is non-destructive, both for subsequent fingermark development techniques, DNA recovery and the examination of firing and rifling marks. Therefore, there is no reason why it should not be utilised if the situation warrants it. The process is relatively slow, taking several hours to scan a single cartridge casing at high resolution, but for serious cases may provide valuable information. As a consequence, the scanning Kelvin probe process is included as a Category B process in the *Fingermark Visualisation Manual.*

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Matrix assisted laser desorption/ionisation and surface assisted laser desorption/ionisation

1. History

1.1 The concept of matrix assisted laser desorption/ionisation (MALDI) as a method for the chemical analysis of surfaces was first reported in the 1980s [1,2], with a matrix material being applied to the surface to

enhance the effect of the laser in initiating the desorption/ionisation process. It was later observed that the surface structure resulting from the application of micro-sized inorganic powders could also aid the subsequent laser analysis, and this alternative method was termed surface assisted laser desorption/ionisation (SALDI).

1.2 The first reported application of these related analytical techniques to fingermark enhancement was the development and patenting of functionalised nanoparticles by Prof. Fred Rowell in 2007. These were proposed for use as a dusting powder for fingermark development, with the chemistry and structure of the powders enabling subsequent chemical analysis of the developed mark. These powders were shown to be capable of detecting both drugs of abuse and their metabolites in developed marks in studies by Rowell *et al.* [3].

1.3 The MALDI process was found to be suited to the analysis of untreated fingermarks [4], although for this application it would be necessary to know where the mark was located. The same research group also identified that a ground powder of the MALDI matrix material could be used both to develop fingermarks and to assist in their subsequent chemical analysis [5]. The naturally occurring substance curcumin was later identified to be similarly effective in the dual role of fingermark development powder and matrix enhancer [6]. In all these applications, the ability of the MALDI process to be operated in an imaging mode enabled the fingermark ridge detail to be captured in addition to the spatial distribution of the chemical species present.

1.4 In recent years there has been much interest in exploring the potential of both MALDI and SALDI to obtain additional contextual information from fingermarks. The processes have been successfully demonstrated to be capable of separating overlapping fingermarks that differ in chemical composition [7]. They have been used to detect and reveal the distribution of a range of contaminants within fingermarks including condom lubricants [8], drugs [9], drug metabolites [10] and explosives [11]. Researchers have looked at the feasibility of recovering contextual information about naturally occurring substances and contaminants of interest from marks previously treated with other chemical and physical processes [12,13]. MALDI has also been proposed as a direct method for confirming the presence of human blood in fingermarks and other crime scene stains [14].

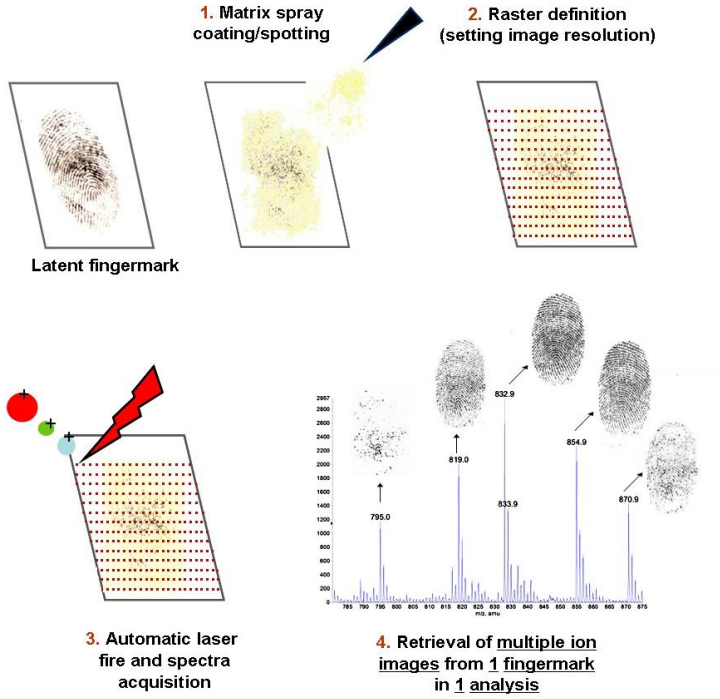
2. Theory

2.1 Both MALDI and SALDI involve the use of a laser to eject characteristic molecular species from the surface being analysed. These species are subsequently collected and analysed using mass spectrometry based methods. MALDI requires a matrix material to be first sprayed over the surface. Matrix materials are selected so that they can first dissolve and then co-crystallise with the molecules of interest on the surface, and also

be capable of strongly absorbing the laser energy to cause desorption and ionisation. The materials used in SALDI are generally deposited onto the surface as a dry powder or a liquid powder suspension, resulting in a layer of these fine powders being deposited across the surface. There is no requirement for the powders to dissolve or interact with the molecules in the surface, but they do need to be able to absorb and enhance the effect of the incident laser.

2.2 Once the matrix or surface enhancer has been applied to the surface, it is irradiated with a laser operating in the ultraviolet region of the spectrum. The effect of the laser on the surface is magnified by the enhancing material, which absorbs the energy of the laser and directs it into the surface where ablation of the surface layers occurs. The ablation process produces a heated plume of matrix molecules and related species close to the surface. This heated plume then contributes to the ionisation of the molecules from the surface.

2.3 By scanning the laser beam across the surface in a raster, mass spectra can be obtained for each point on the surface being analysed. These can be used to produce distribution maps for each of the constituents present.



Schematic diagram showing the different stages in the process of creating distribution maps for different constituents of a fingermark using matrix assisted laser desorption/ionisation.

3. Reasons why technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Centre for Applied Science and Technology (CAST) does not recommend the process for routine operational work because of:

- the additional time taken to perform the analysis; and
- the number of scenarios where additional contextual information is necessary may be limited.

However, in some circumstances MALDI may be capable of providing additional information about a fingermark and/or previously undetected ridge detail and its use should not be discounted. As a consequence, the MALDI process is included as a Category C process in the *Fingermark Visualisation Manual.* Suitable instruments for the MALDI process can be found in some universities.

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Ultraviolet imaging

1. History

1.1 The existence of ultraviolet (UV) radiation was discovered by Johann Ritter in 1801. He found that emissions beyond the violet region of the electromagnetic spectrum were capable of darkening silver chloride in the same way that visible light at the blue end of the spectrum could. Ritter originally called these rays 'de-oxidising rays' although the term 'chemical rays' was adopted soon after and was in use throughout most of the 1800s. 'Chemical rays' was eventually dropped in favour of the current term 'ultraviolet radiation'.

1.2 By 1931 the forensic applications of UV radiation were already being explored, with UV fluorescence being widely used for document examination and glass identification [1,2]. The results of investigations into the fluorescence of body fluids and drugs under UV illumination were also reported [2]. About the same time, fingerprint powders that produced fluorescence or phosphorescence under (long wave) UV radiation were identified (see Chapter 3, Chemical and Physical Processes, Powders) and by the 1970s these were widely available for treating and photographing marks on multicoloured surfaces.

1.3 In 1970, Ohki carried out an investigation into the potential of UV examination for the detection of latent fingermarks without the need for chemical development [3]. These experiments involved collecting secretions from the human skin by means of gauze wrapped around the hands and feet of several subjects, followed by analysis of these secretions to see if any characteristic UV absorption or fluorescent properties were observed. In these experiments, absorption was observed at 277 nm and fluorescence between 300 and 400 nm, depending on the solvent used to take the extract. Ohki was able to utilise the UV absorption characteristics of latent fingermarks to capture pictures of untreated latent fingermarks on paper and PVC, those on paper only being visible using a 253 nm interference filter but both being visible using a 365 nm filter.

1.4 Although the technique was not widely adopted, research continued worldwide to establish the range of surfaces that latent fingermarks could be detected on [4], and to investigate the use of UV image-intensifier viewers for real time observation of latent prints [5,6]. PSDB had demonstrations of some of these early viewing systems. UV-sensitive charge-coupled device (CCD) cameras were also being used for the direct imaging of latent prints by the mid-1990s [7].

1.5 By the 1990s, both long-wave (365 nm) and short-wave UV (254 nm) imaging techniques were in operational use by the Metropolitan Police [7]. Long-wave UV was found to be useful on glossy magazines, where the fingermark ridges absorbed and the background fluoresced, and also on stipple surface photographs, where the photographic emulsion

absorbed and the fingermark ridges reflected. Short-wave UV found application in enhancing marks on patterned backgrounds, where printing absorbed the radiation and the ridges reflected it. Experimental work by the Metropolitan Police group established that the intensity of natural fluorescence in fingermarks was superior in the UV region to that obtained in the visible region [8], with most fluorescence being observed in sebaceous prints. A 266 nm neodymium:yttrium aluminium garnet (Nd:YAG) laser was used in these studies, and the level of fluorescence in the fingermark was observed to decrease with increased exposure time.

1.6 Around the same time, the Rofin company, in conjunction with Israeli researchers, were also developing a short-wave UV-imaging system based on a specially modified, Peltier cooled CCD camera and used this system to image fingermarks in both fluorescence and absorption modes. They also carried out experiments to establish the sensitivity of the system to fluorescence from tyrosine and tryptophan, the amino acids believed to be primarily responsible for the natural UV fluorescence from latent prints [9]. The same group also considered the use of a lower cost imaging system for long-wave UV imaging alone, with the principal applications being the detection of latent fingermarks on smooth surfaces, such as mirrors, and the enhancement of marks developed using superglue without application of fluorescent dyes [10]. The same group also carried out further studies into UV fluorescence [11], showing that for practical casework there were far fewer fluorescent prints present than suggested in the Metropolitan Police study [8], possibly because most prints on paper exhibits are primarily eccrine in character. However, fluorescence was observed in older prints than was suggested in the earlier study. The use of UV imaging for detection of other body fluids was suggested.

1.7 The majority of the imaging systems developed by the Metropolitan Police and the Israel National Police were laboratory-based and not capable of being transported to crime scenes. The US Army Crime Laboratory carried out further experiments with UV image-intensifier systems, which resulted in the commercial production of a scene-portable Reflected Ultraviolet Imaging System (RUVIS) [12]. Several scene-portable RUVIS systems are now available through different manufacturers and reports have been published regarding their practical application to casework [13,14].

1.8 With regard to short-wave UV fluorescence imaging of fingermarks, work has continued in Japan using a tunable laser as the irradiation source and time-resolved imaging to improve fingermark definition [15]. The equipment used was a laboratory-based imaging system and not suited for use at scenes. The results of the study essentially confirmed the observations of previous researchers regarding optimum excitation wavelengths and the types of fingermarks detected.

1.8 The Home Office Centre for Applied Science and Technology (CAST) has carried out intermittent research into UV imaging. In the mid-1990s a prototype RUVIS system was developed, based on a DEP-Photonis intensifier tube linked to a Nikon 105 mm UV lens and a rotatable filter wheel containing a range of different UV filters. This was not pursued any further as a commercial product. A collaborative study was also conducted with the Israeli research group in the late 1990s although this did not result in operational implementation of the process in the UK. Work has also been carried out by CAST to develop safety and best practice guidelines for long-wave UV photography [16], with the focus being on the capture of injury marks and 'smart water' dyed suspects and articles. More recently work has resumed to design and manufacture a laboratory-based UV imaging system based on a UV-sensitive CCD camera (the Alta Apogee U-47 UV) capable of imaging at wavelengths from the near infrared down to 240 nm and operated using the software developed for the Integrated Rapid Imaging System (IRIS) digital imaging workstation [17-19].

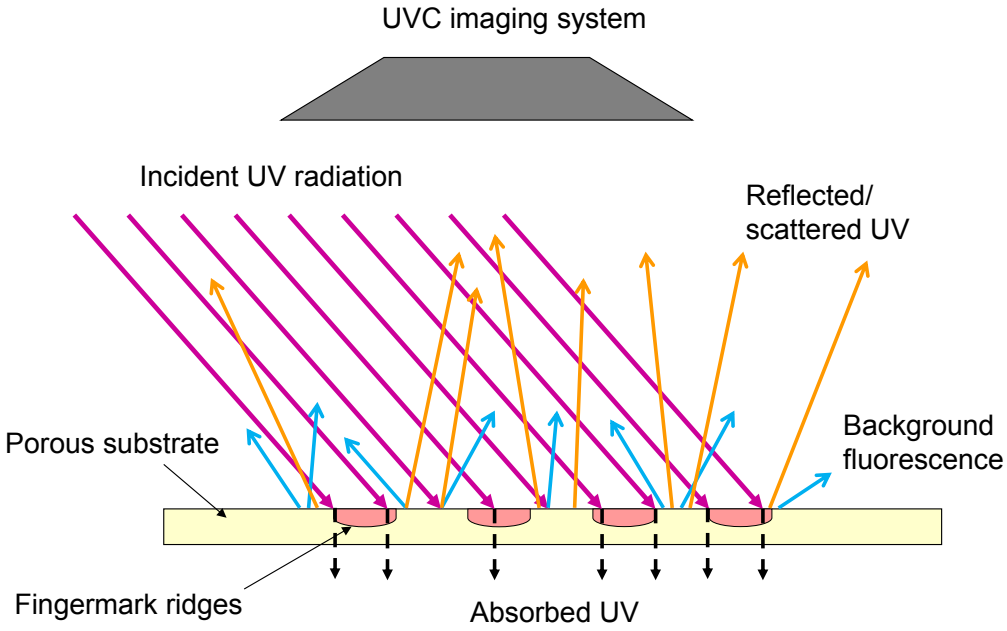
2. Theory

2.1 UV imaging is a broad subject area and there are many processes by which contrast may be obtained between the fingermark ridges and the background. These include fluorescence, absorption and reflection. Each of these processes is described in greater detail below.

2.2 UV fluorescence. The theory associated with UV fluorescence is identical to that for fluorescence in the visible region of the spectrum. The fingermark residue is irradiated with short wavelength UV radiation, which promotes electrons within the molecules of certain fingermark constituents into excited states. These electrons cannot remain in this excited state and drop back to their original electron shell, losing the excess energy by emitting radiation at a longer wavelength (in this case as longer wave UV or into the visible region) than the original excitation. In the case of latent fingermarks, the amino acids tyrosine and tryptophan are believed to contribute most to this fluorescence. UV fluorescence is more applicable to the detection and imaging of latent fingermarks on porous surfaces than to detection of latent fingermarks on non-porous surfaces. However, UV fluorescence is more often used for the enhancement of marks developed using superglue and a range of UV fluorescent dyes are commercially available that can be utilised for this purpose. UV fluorescent powders can also be used for superglue enhancement where use of liquid dyes is not practical.

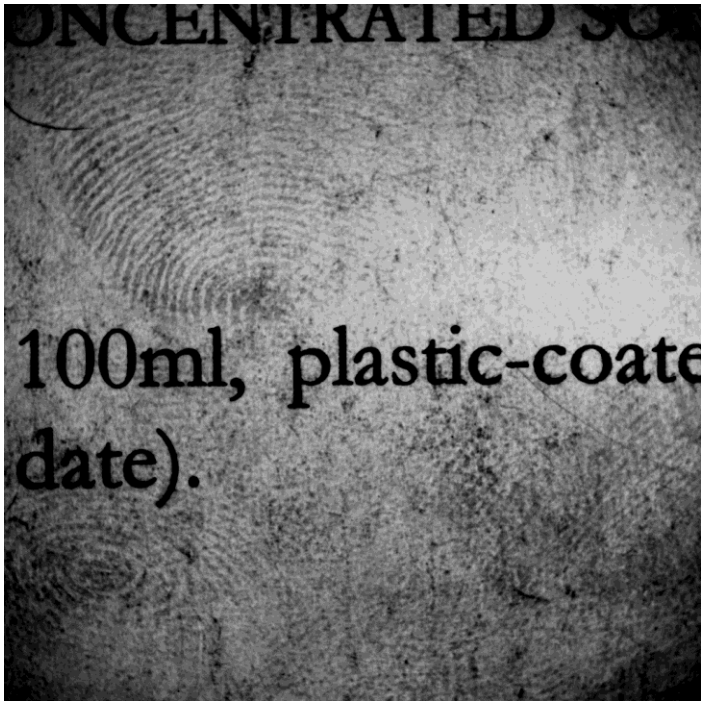
2.3 UV absorption. It is known that fingermark residues absorb strongly at 277 nm [3] , primarily due to absorption by fatty acids, and in cases where fingermarks are deposited on surfaces that either fluoresce under or reflect UV radiation this phenomenon may be sufficient to provide

contrast between fingermark ridges and the background. This is shown schematically in the figure below.



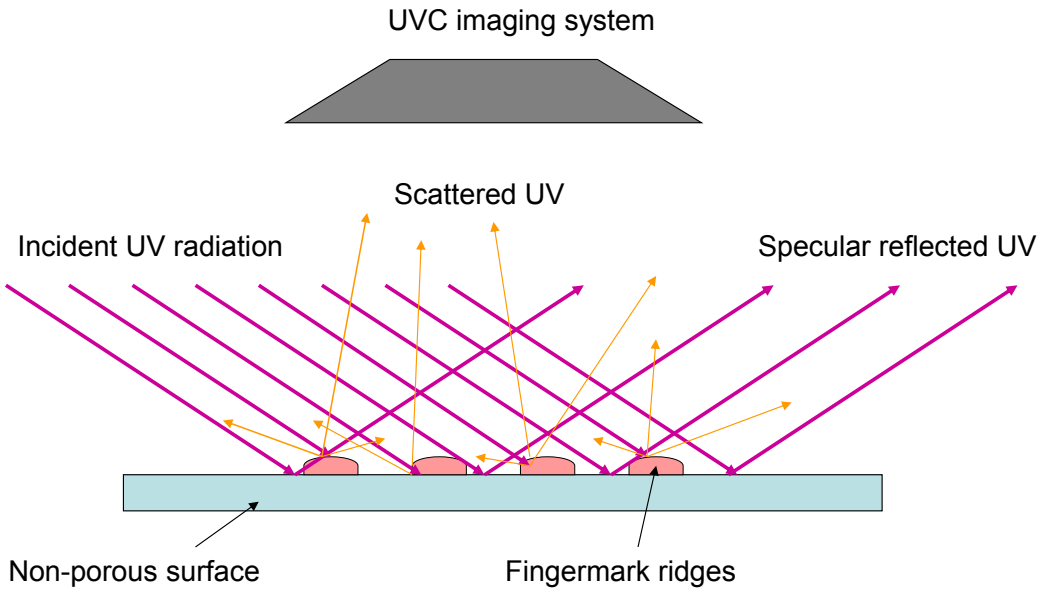
Schematic diagram showing how ridge contrast of latent marks can be obtained by ultraviolet reflection/absorption.

2.4 UV absorption is most applicable to the detection of fingermarks on porous surfaces, in particular on white paper, where optical brighteners may fluoresce under UV radiation and provide a stronger contrast with the absorbing fingermark ridges.



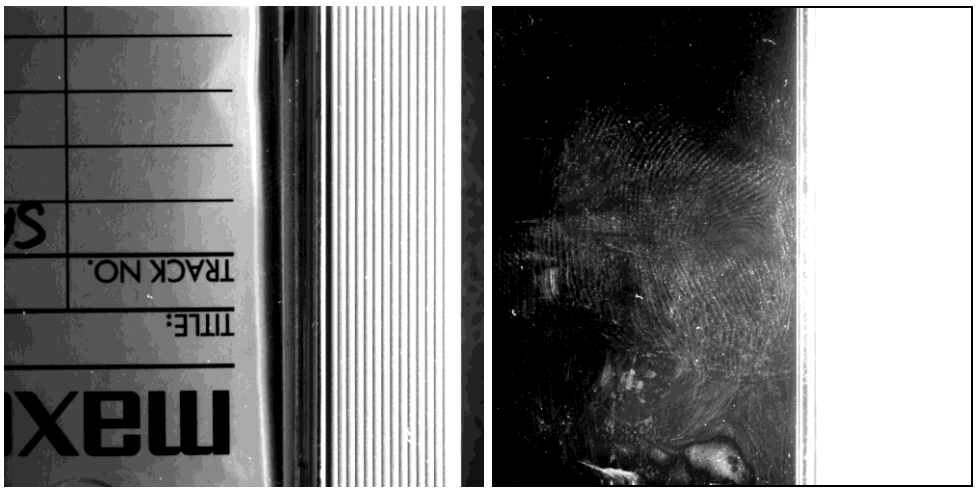
Latent fingermarks detected on glossy paper by reflected short-wave ultraviolet imaging.

2.5 UV reflection. Reflected UV provides contrast between the fingermark ridges and the background by means of a greater reflection or scatter from the fingermark ridges than from the background. This may be due to the fact the background absorbs UV more strongly than the fingermark deposits, or by the fingermark residues being rougher in texture than the background and scattering more UV radiation towards the detection system. This effect is more pronounced in the UV region of the spectrum because the wavelength of the radiation is of a similar scale to the height of the fingermark ridges, and hence is scattered more strongly than light in the visible region. This is shown schematically below. A short-wave UV band-pass filter may be used in front of the camera to block fluorescence and any reflected visible light emitted by the light source.



Schematic diagram showing how ridge contrast of latent and superglue treated marks can be obtained by ultraviolet reflection/scattering.

2.6 UV reflection is most useful for the detection of latent fingermarks on smooth surfaces (especially those that are opaque to short wave UV radiation), and for the enhancement of marks developed using superglue, where the noodle-like structure of the developed mark scatters strongly.



a) b)

Latent fingermark on CD case imaged using reflected short-wave ultraviolet a) *no filter and* b) *short-wave ultraviolet band-pass filter.*

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and

validation work associated with the process. CAST has now included the UVC reflection process (utilising the absorption and reflection mechanisms outlined above in conjunction with short wave UV radiation) in the recently published *Fingermark Visualisation Manual* [20] but recommends caution in its use. This is because of the health and safety issues associated with short-wave (in this case UVC) radiation. However, provided that personnel are suitably trained in both UV safety and fluorescence examination, and appropriate precautions are taken in terms of eye and skin protection, there is no reason why UV imaging should not be carried out in a laboratory or at a crime scene.

3.2 Long-wave UV imaging is provided as an option on the IRIS workstation, using either a 365 nm low pressure mercury vapour lamp/tube or the 340 to 413 nm excitation band of the Quaser 2000. IRIS is fitted with a 415 nm long-pass (Schott GG435) viewing and camera filter, and will reveal fingermarks fluorescing under these irradiation conditions. Skin protection (e.g. a pair of latex gloves) is recommended when using the long-wave UV imaging function.

4. Critical issues

4.1 All wavelengths of UV radiation are capable of causing damage to skin and eyes, and personnel using the process should ensure that they are fully trained and aware of the health and safety issues associated with it. Appropriate protective clothing must be worn.

4.2 Exposure to UV radiation (particularly UVC) will cause progressive damage to DNA and this must be taken into account if it is intended to recover DNA subsequently from the exhibit.

4.3 Exposure to UV, especially for prolonged periods of time, may also result in photochemical decomposition of the mark and/or substrate. The possibility of this should be recognised and watched for during examination.

4.4 As for fluorescence examination, selection of appropriate combinations of irradiation source and viewing filter are essential. The outputs of different sources vary significantly and the band pass characteristics of the filters used need to be selected to be compatible. Some additional information is given in the *Fingermark Visualisation Manual* [20] but this is by no means exhaustive.

4.5 A specially modified camera is required to capture images in the UV region of the spectrum, or an image intensifier is required to convert UV to a visible image that can be captured.

5. Application

5.1 Suitable surfaces: In reflection mode, UV imaging is most appropriate for use on smooth, non-porous surfaces, where the scattering from the ridges is greater than the scattering from the background texture. It is particularly effective on glass where the glass strongly absorbs UV, giving greater contrast between scattering from the ridges and the background. It is also effective on glossy paper surfaces, where fingermark deposits absorb and the paper surface reflects. In fluorescence mode, UV imaging is capable of detecting fingermarks on all types of surface where UV-fluorescent contaminants are present.

5.2 The main applications of short-wave UV imaging are in the detection and capture of latent fingermarks prior to the application of any chemical treatment and imaging of marks developed using superglue fuming on visually opaque non-porous surfaces such as glass. Fingermarks can be detected on both porous and non-porous surfaces by the range of processes outlined above, typically using equipment such as RUVIS for a speculative search of a scene or article and then using specialist equipment to capture marks at the high resolution required. The advantages of using this technique prior to chemical treatment are that it is non-contact and therefore non-destructive to fingermarks (although if exposure is more than a few minutes it is detrimental to DNA[21,22,23]) and that some of the marks revealed will be in the contaminant, and will never be developed by any chemical process. As mentioned above, short-wave UV is destructive to DNA and the process should not be used if DNA recovery is being considered.

5.3 Long-wave UV imaging is more suited to searching for traces of body fluids and other contaminants such as oils, fats or petrol, but may be capable of revealing marks in these types of contaminant. Latent marks may be revealed by their fluorescence on thermal receipts when illuminated with long-wave UV. Long wave UV is also a useful wavelength range for promoting background fluorescence, because of the use of optical brighteners in substrates such as paper and fabric. Marks in absorbing contaminants such as blood or dirt may be enhanced in this way.

6. Alternative formulations and processes

6.1 There are no alternative processes used for UV imaging in addition to those outlined in the sections above.

7. Post-treatments

7.1 There are no post-treatments used with UV imaging.

8. Validation and operational experience

8.1 CAST has not conducted an extensive study on the effectiveness of UV imaging in operational work. However, the Metropolitan Police has been using UV examination and imaging on operational work for over 20 years. It has been demonstrated that in several cases UV imaging can reveal marks that are not subsequently developed by chemical treatment. It is believed that many of these marks are in contaminants that will not be targeted by chemical or physical development techniques, and hence UV imaging is a valuable tool for operational work. Studies that have been conducted under the control of CAST are outlined below.

8.2 Laboratory validation

8.2.1 CAST has conducted some studies of the relative effectiveness of UV imaging in comparison with other development techniques, including a limited investigation to compare UVC reflection with fluorescence examination on porous surfaces [17]. This study looked at single fingermarks deposited by 36 different donors on 5 different paper types, with the fingermarks aged for 1 day and 1 week. The results are summarised below.

Paper type	Light source	Number of fingermarks detected	Number of identifiable marks	Number of unique marks
Pukka Pad lined paper	Laser (532 nm)	3	1	2
	Laser (577 nm)	0	0	0
	UV (254 nm)	9	3	8
Niceday A4 printer paper	Laser (532 nm)	0	0	0
	Laser (577 nm)	0	0	0
	UV (254 nm)	3	1	3
Hello Silk semi-glossy paper	Laser (532 nm)	19	9	1
	Laser (577 nm)	14	7	1
	UV (254 nm)	28	18	10
Brown envelope	Laser (532 nm)	5	2	1
	Laser (577 nm)	6	0	2
	UV (254 nm)	3	0	0
White envelope	Laser (532 nm)	1	0	1
	Laser (577 nm)	0	0	0
	UV (254 nm)	4	1	4

Results for one-day-old marks using different light sources.

Paper type	Light source	Number of fingermarks detected	Number of identifiable marks	Number of unique marks
Pukka Pad lined paper	Laser (532 nm)	4	2	1
	Laser (577 nm)	3	0	0
	UV (254 nm)	10	3	7

Niceday A4 printer paper	Laser (532 nm)	1	0	1
	Laser (577 nm)	0	0	0
	UV (254 nm)	3	0	3
Hello Silk semi-glossy paper	Laser (532 nm)	24	12	1
	Laser (577 nm)	18	10	0
	UV (254 nm)	29	15	5
Brown envelope	Laser (532 nm)	7	0	1
	Laser (577 nm)	10	0	4
	UV (254 nm)	0	0	0
White envelope	Laser (532 nm)	0	0	0
	Laser (577 nm)	1	0	0
	UV (254 nm)	3	1	2

Results for one-week-old marks using different light sources.

8.2.2 The results demonstrate that short-wave UV reflection is a highly effective process for detection of untreated fingermarks on glossy papers, but less so on rougher paper types. It can also be seen that short-wave UV reflection does detect marks that are not found by fluorescence examination and is a complementary technique for non-contact examination of porous exhibits in cases where chemical treatment is not possible.

8.2.3 A further study looked at the effectiveness of short-wave UV reflection in detection of latent fingermarks on a wider range of surfaces [18,19]. In this study a depletion series of ten fingermarks were laid by ten different donors, and the marks graded. Marks were examined in a Digital Enclosed Ultraviolet System (DEUS), custom built by the CAST workshops. The radiation sources used were two 8 W 254 nm mercury vapour lamps, and the imaging system was an Alta Apogee U47-UV camera with a Resolve Optics 60 mm forensic lens. For the glass substrate the experiment was repeated three times to give a total of 300 graded marks. The results of this study are summarised below.

Grade	**Substrate (number of marks assessed), percentage at each grade**				
	Glass (300)	Red painted metal (100)	White ceramic tile (100)	Brown parcel tape (100)	Glossy paper (100)
4	56	6	12	0	7
3	20	24	30	13	7
2	9	10	4	14	7
1	13	18	8	22	21
0	2	42	46	51	58

Results of marks found using ultraviolet imaging on a range of substrates.

8.2.4 It can be seen that the proportion of identifiable marks that are detected by the technique ranges from 13 to 76% according to the substrate, demonstrating that the process is relatively effective for a non-contact technique.

8.2.5 A study was also conducted on the effect of changing the illumination wavelength. A series of samples were illuminated with long-, mid- and short-wave UV radiation in the DEUS imaging chamber [18]. Two 8 W tubes were used for each wavelength, the tubes being incorporated into the same mountings for each wavelength. These results of examining depletion series of 10 marks from 10 different donors (i.e. a total of 100 marks) are summarised below.

Wavelength	**Number of marks detected using optimum filter**	
	Paper	**Glass**
365nm (UVA)	1	45
302nm (UVB)	6	83
254nm (UVC)	10	98

Results of marks found using ultraviolet imaging at different wavelengths.

8.2.6 The results show the increased effectiveness in fingermark detection as the wavelength of illumination decreases, and demonstrates why UVC is preferred if fingermark detection is the priority.

8.3 Pseudo-operational trials and operational experience

8.3.1 CAST has not conducted any pseudo-operational trials using short- or long-wave UV imaging. The Metropolitan Police routinely uses long-wave UV to search crime scenes, and has recently conducted an analysis of the number of unique marks detected by light source examination, including long-wave UV, white light and laser examination [24]. This demonstrated that light source examination accounted for approximately 8% of unique marks detected, although the proportion of those that were uniquely identified using long-wave UV was not identified.

8.3.2 The Metropolitan Police also uses short-wave UV reflection under controlled conditions in a laboratory, and there are several documented examples of where it has detected marks not subsequently developed by chemical techniques.

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Visual examination

1. History

1.1. Visual examination was the first technique proposed for the detection of fingermarks, with Henry Faulds suggesting the use of finger marks in blood, impressions in clay or marks left on glass for identification of criminals in his letter to the journal *Nature* in 1880. Many of the early landmark cases in fingerprint identification involved marks detected visually [1]; in Argentina in 1892 Vucetich used a mark deposited in blood on a door frame to disprove an account of a murder; in 1897 fingermarks in blood on a book cover were used to identify a murderer in India; and in 1902 impressions of fingermarks in paint were used to identify a burglar in the first trial using fingerprint evidence in the UK.

1.2 Detection of a mark by visual examination did not necessarily mean that it could be easily captured. In many cases the lighting conditions required to detect the mark were difficult to recreate and maintain for photography, but as the use of fingerprint evidence increased a range of techniques were developed or adapted for the photography of both developed and latent marks. Those described for operational use in 1954 [2] included transmitted light, vertical/specular illumination, dark ground illumination, oblique illumination, oblique top illumination and duo filtering.

1.3 Practical examples of the use of backlighting, vertical/specular illumination and oblique illumination were presented in subsequent publications [3,4]. The detection of fingermarks in both grease and dust was demonstrated using the range of lighting techniques above. Olsen [3] also recommended visual examination of metals and firearm articles for latent marks that may not be developed by powdering, with marks occasionally being etched into metal by the fingermark constituents or ridge impressions left in the oil coatings often found on firearms. Pfister subsequently reported the application of specular lighting techniques using a semi-silvered mirror for the capture of latent fingermarks on glossy surfaces [5].

1.4 Other photographic techniques such the use of polarising filters [6] began to be employed in the imaging of latent fingermarks, improving the contrast between the fingermark ridges and the background by suppressing the reflections from the background regions. A combination of polarisation and specular reflection techniques has recently been suggested for the detection of latent fingermarks [7]. The use of specialist tilt/shift lenses has also been demonstrated for the capture of marks on mirrors, where the image of the mark may otherwise be obscured by background reflections [8].

1.5 It has also been proposed that marks detected by visual examination need not always be photographed in situ; if it is considered that powdering or chemical development would be of no benefit and direct

photography is difficult (for example if the mark is in an inaccessible position and/or in a contaminant not targeted by chemical techniques), lifting of the mark may be carried out using either transparent lifting tape or gelatine lifters (black, white or transparent) [9]. Lifting of latent marks, either after visual examination or as a speculative technique, should not be carried out as an alternative to treatments such as powdering if the application of a development technique is feasible. The Home Office Centre for Applied Science and Technology (CAST) has recently carried out a comparison of the effectiveness of gel lifting and powdering for development/capture of latent marks [10] and has demonstrated that powdering is the more effective process. The individual circumstances of the mark (including location, type of surface, medium mark is in) should be considered before deciding whether direct photography, lifting, powdering or chemical treatment is most appropriate.

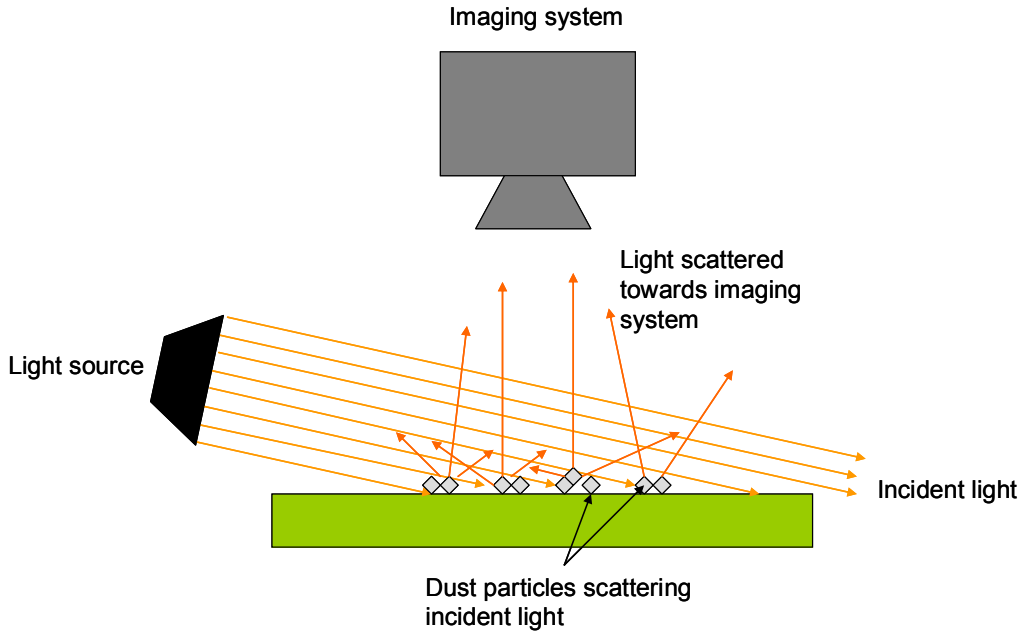
1.6 It has long been recognised that in some circumstances latent fingermarks may be developed by the environment they have been exposed to and fingermarks developed by heat have been found on paper articles at arson scenes [3]. Recent studies by CAST and others have found that there are a wide range of mechanisms by which fingermarks can be developed by the soot and heat at arson scenes [11,12,13,14], and visual examination of articles recovered from such scenes is essential.

2. Theory

2.1 The principle of visual examination is to utilise lighting in such a way as to provide as much contrast as possible between fingermark ridges and the background, if possible suppressing any patterned backgrounds. For the initial detection of marks this is done by trying different lighting angles, but once a mark has been located there are several techniques that can be used to capture images of it in the optimum way. Some of these are described below, together with the situations that they are most appropriate for. It should be noted that there is no firm naming convention for each of these techniques and the descriptions given here may not be consistent with other publications. Reference to the lighting diagrams is important to establish what conditions are being used.

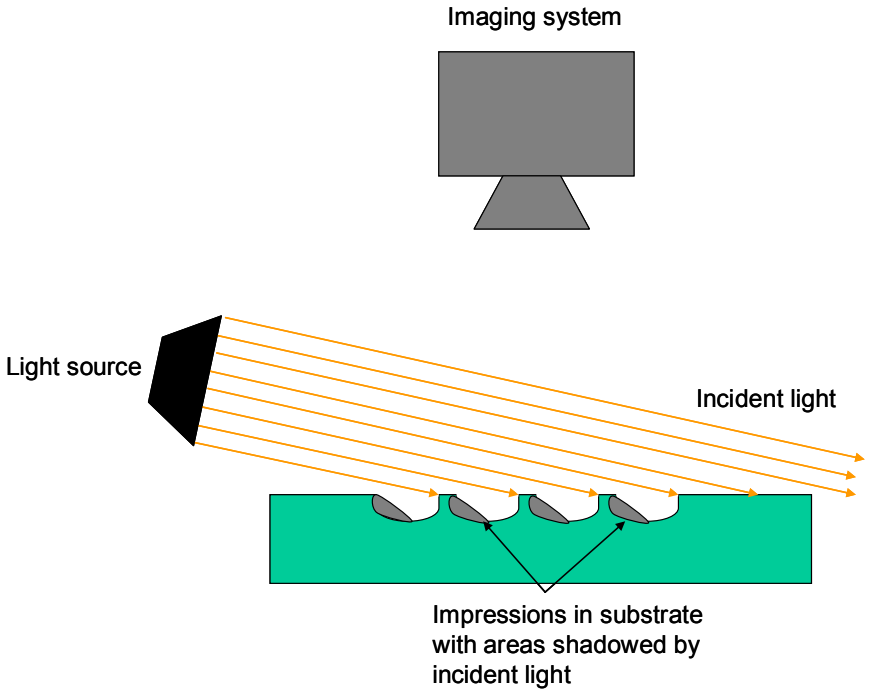
2.2 Oblique illumination

Oblique illumination may be used to capture fingermarks deposited in dust. The low angle illumination is scattered by particles of dust on the surface being examined, resulting in more light reaching the imaging system from these regions than in areas where no dust is present.



Schematic diagram illustrating the use of oblique lighting to detect marks deposited in dust.

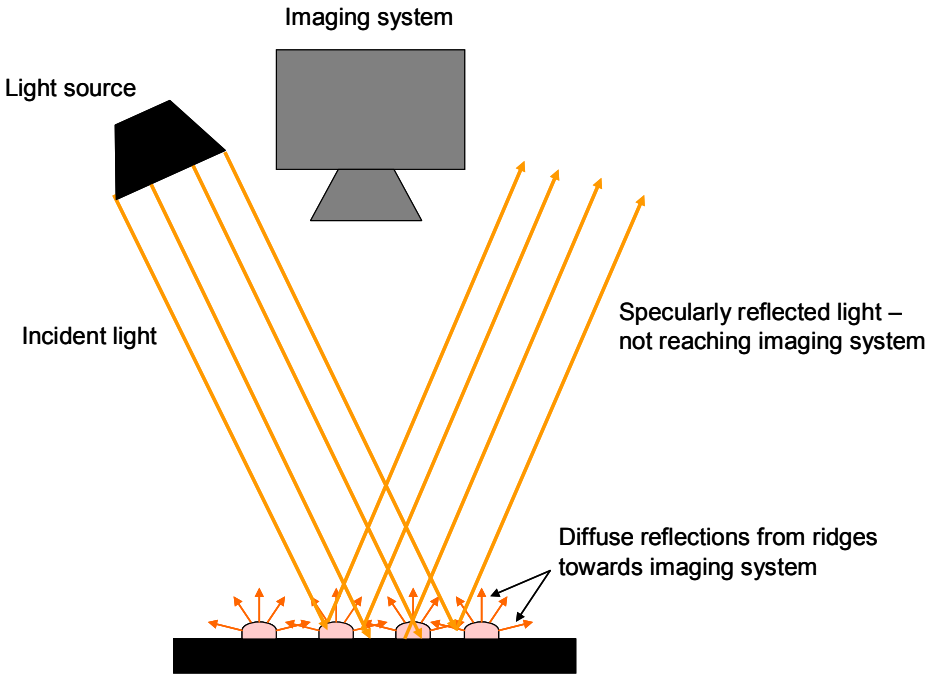
2.3 Oblique illumination can also be used in the capture of fingermark impressions in wax or putty. In this case the low angle illumination casts shadows in the depressions left by the fingerprint ridges, thus aiding in their visualisation. The actual angle of illumination remains critical in obtaining optimum results and varying light source position is important.



Schematic diagram illustrating the use of oblique lighting to detect marks left as impressions in a soft surface.

2.4 Hard specular (oblique top) illumination

Hard specular illumination can be used for latent marks or marks in contaminant on reflective surfaces. It is essentially the opposite of oblique illumination, with the light source being placed at a high illumination angle in close proximity to the imaging system. Where light falls upon a reflective region of the background, it is specularly reflected at an angle where the reflected light does not reach the imaging system. Where light falls upon fingermark ridges, it is either scattered or diffusely reflected, resulting in some light being reflected to the imaging system. The ridges will therefore appear lighter than the background in the image.

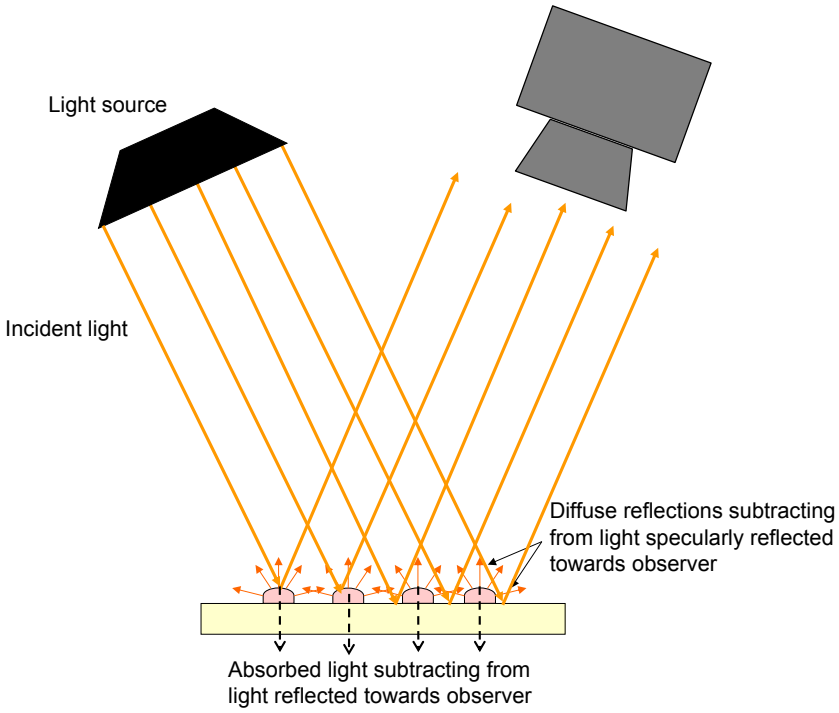


Schematic diagram illustrating the use of hard specular illumination to detect marks on smooth, reflective surfaces.

2.5 The principle of specular reflection is utilised in the BVDA GLScan system, developed for the imaging of trace evidence lifted on black gelatin lifters [15], in this case with two directly opposed light sources providing full specular lighting.

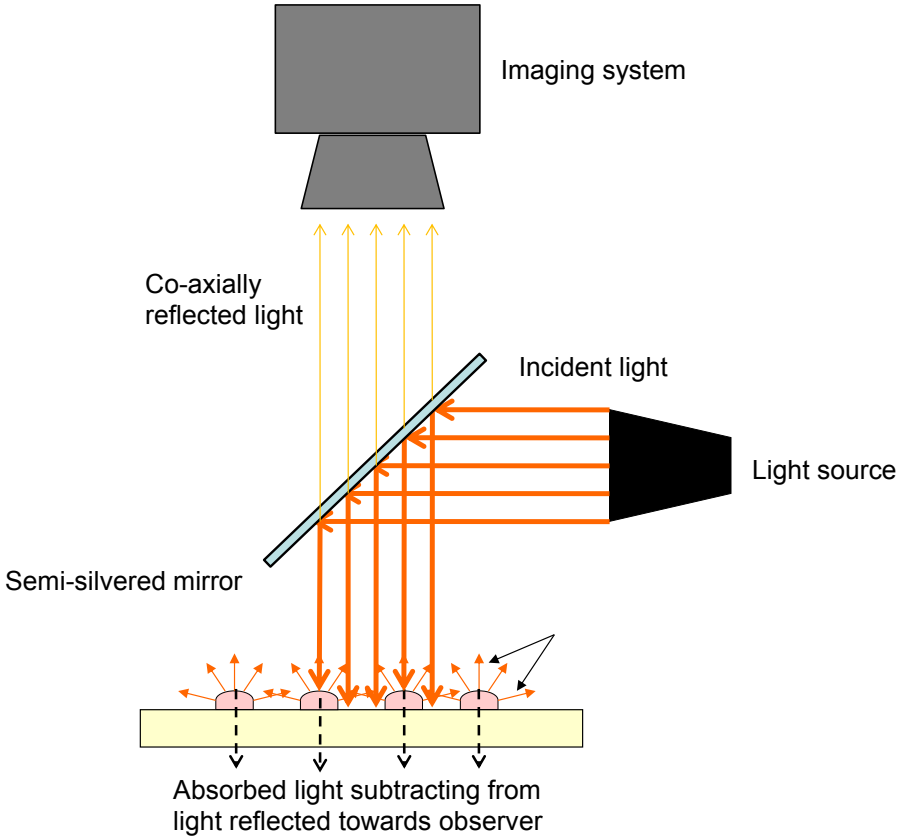
2.6 Soft specular illumination

Soft specular illumination can be used where fingermarks are present on very smooth surfaces, especially where contaminants that absorb or scatter light are present in the mark. The technique requires a light source producing even, diffuse output that is angled so that the reflection of the diffuse light is directed towards the imaging system. Where no surface contaminants are present, light is specularly reflected, whereas the ridges absorb or scatter the light. As a consequence, the ridges appear dark against the light background of the reflection of the light source. The technique can be very effective in suppressing the distracting effects of patterned backgrounds.



Schematic diagram illustrating the use of soft specular illumination to detect marks on smooth, reflective surfaces.

2.7 A variation on soft specular lighting is co-axial, also known as epitaxial or episcopic illumination. Co-axial illumination can be used where a latent mark or a mark in contaminant is present on a patterned, reflective background. A semi-silvered mirror at 45º to the axis of the imaging system is used essentially to provide co-axial illumination. The incident light is reflected downwards onto the sample. Where it meets the reflective surface it is strongly reflected and some passes through the semi-silvered mirror to reach the imaging system. Where the light hits ridges, it is scattered or a diffuse reflection occurs. The amount of light reflected back towards the imaging system from these regions is correspondingly less, and the fingermark will appear as dark ridges against a light background.

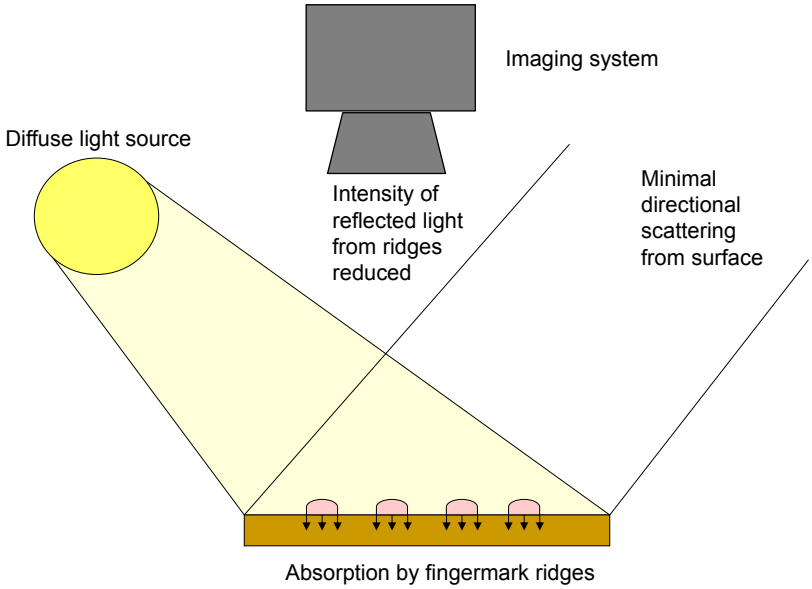


Schematic diagram illustrating the use of co-axial illumination to detect marks on smooth, reflective surfaces.

2.8 Several commercial systems have been developed incorporating co-axial or epitaxial illumination although these are mostly marketed for machine vision applications where imaging conditions are fixed and none has been widely adopted for fingermark detection and imaging where it may be necessary to vary the distance between target and light source.

2.9 <u>Normal diffuse lighting</u>

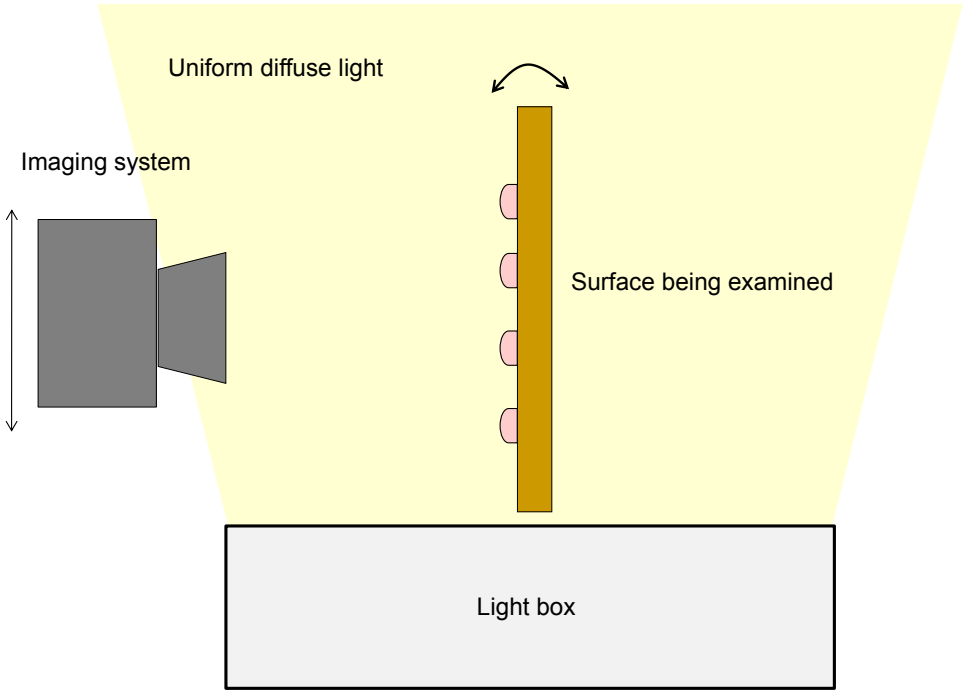
Normal diffuse lighting is suited to situations where marks with some colour contrast with the background, particularly where the surface texture makes the mark difficult to see. The objective of the technique is to use a soft, diffuse light source to give soft, even illumination of the surface with minimal specular reflections, thus making surface texture less apparent and the mark more readily visible. Although good results can be produced with a single light source, the effect is improved with two light sources lighting the surface from each side, and better still using a ring light which gives the most even illumination. The diagram below shows the set up for a single light source for ease of illustration, although in practice the closer the light source is to being perpendicular to the surface the light source is, the better the result. Again, ring lights that fit around the camera lens are useful in providing close to perpendicular lighting in addition to the even lighting conditions discussed above.



Schematic diagram illustrating the use of normal diffuse illumination to enhance coloured marks on light coloured, textured surfaces.

2.10 Uniform diffuse illumination

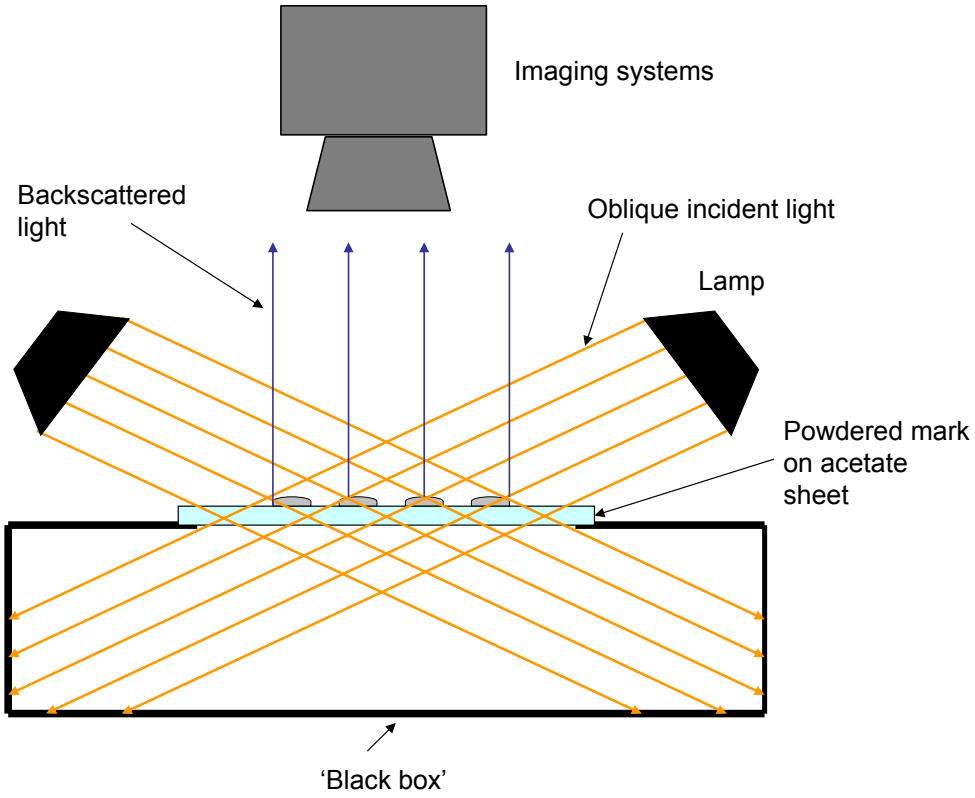
Uniform diffuse illumination is a technique that can be used to reduce problematic speckles and specular reflections when imaging marks on curved, reflective surfaces. The technique uses a large, diffuse light source such as a light box placed underneath the sample so that a flat, even reflection of the light source is cast across the surface facing the imaging system, making the discontinuities of the fingermark ridges easier to see.



Schematic diagram illustrating the use of uniform diffuse illumination to enhance marks on curved, reflective surfaces.

2.11 Dark field reflected illumination

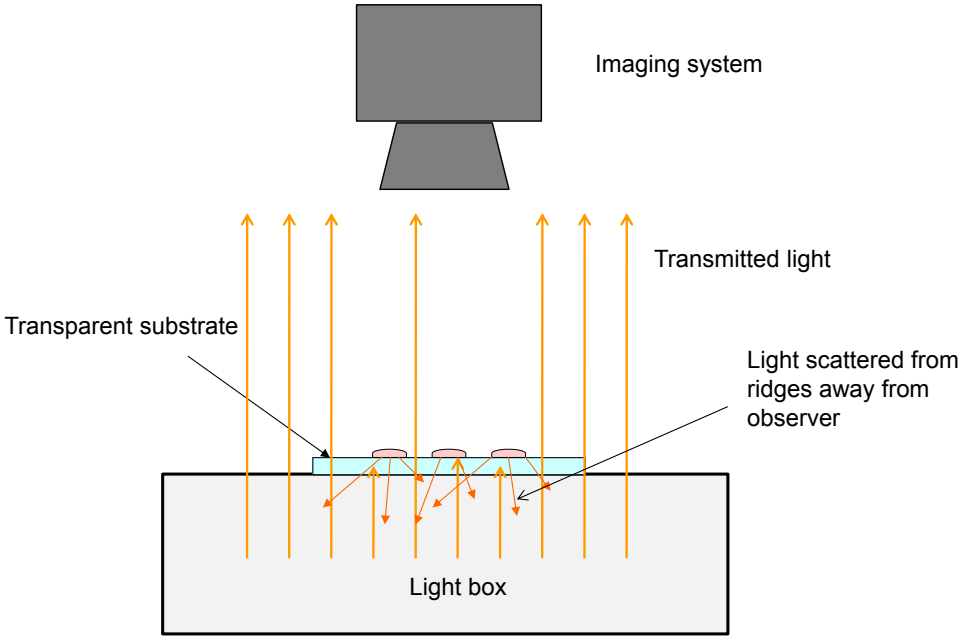
Dark field reflected illumination is a technique used to enhance marks on transparent substrates, particularly where those marks have previously been developed using a reflective material such as a metal powder. The transparent surface is placed over a relatively deep, light absorbing cavity (sometimes referred to as a 'black box') and illuminated from oblique angles above the surface. In regions where no marks are present, light passes through the substrate and is absorbed whereas the marks reflect or scatter the light towards the imaging system. Fingermarks therefore appear lighter than the transparent substrate.



Schematic diagram illustrating the use of dark field reflected illumination to enhance marks on transparent substrates.

2.12 Bright field transmitted illumination

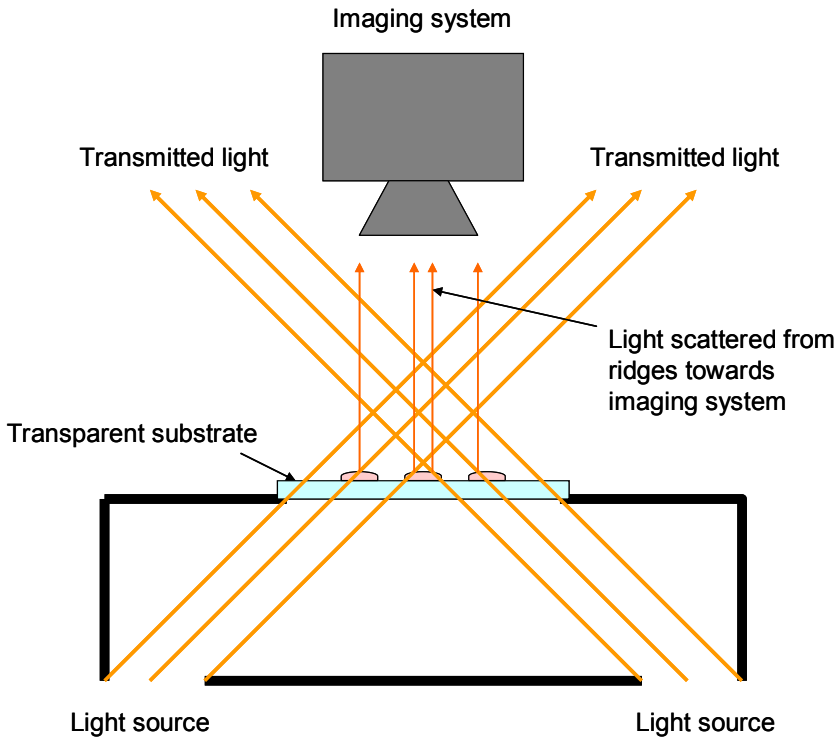
Bright field transmitted illumination is a simple technique suited to enhancing reflective or absorbing marks on transparent substrates. The substrate is illuminated from underneath with an even, diffuse light source such as a light box. Where no marks are present, light passes through to the imaging system. Where marks are present, the contaminants reflect or absorb the light, meaning that the marks appear dark against a light background.



Schematic diagram illustrating the use of bright field transmitted illumination to enhance marks on transparent substrates.

2.13 Dark field transmitted illumination

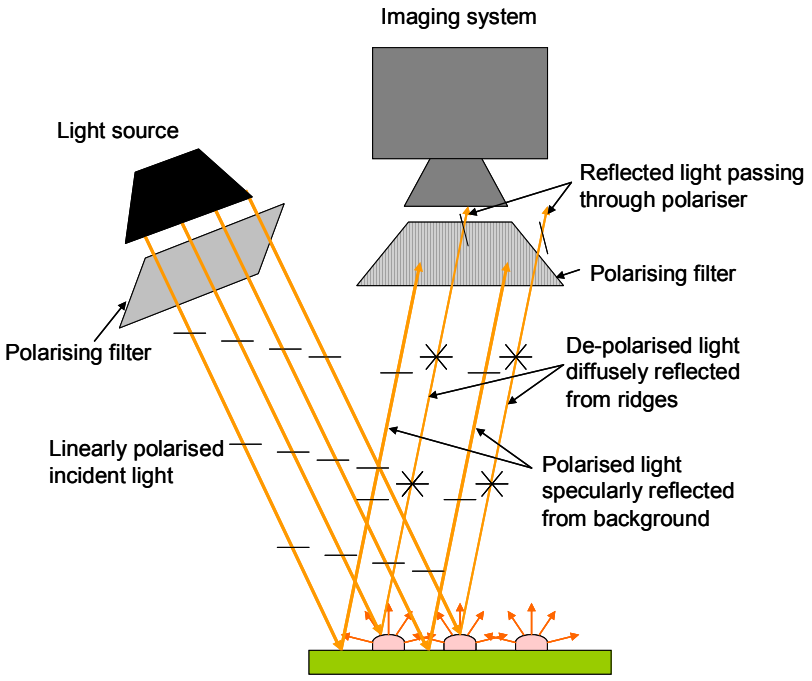
Dark field transmitted illumination is suited to cases where fingermarks in sweat, oil or grease are present on transparent substrates, such as glass or plastic packaging. The sample is illuminated from underneath at oblique angles. In regions with no fingermark deposit, light is transmitted and does not reach the imaging system Where there is a fingermark deposit present the light is scattered, some of it reaching the imaging system. The resultant image shows light fingermark ridges against a dark background.



Schematic diagram illustrating the use of dark field transmitted illumination to detect marks on transparent substrates.

2.14 Polarised light

Polarised light can also be used to detect a latent mark or a mark in contaminant on a reflective background. A linear polarising filter is used in front of the light source to produce linearly polarised light. When this reaches the reflective surface it is reflected and retains its polarisation. Where it hits the fingermark ridges it may be scattered or diffusely reflected, resulting in a depolarised component of light being reflected from the surface. A cross-polarised filter is placed in front of the imaging system, which blocks the specularly reflected light and allows a component of the de-polarised light through, resulting in an image with light ridges against a dark background.



Schematic diagram illustrating use of cross-polarised light to detect marks on reflective backgrounds.

3. Centre for Applied Science and Technology processes

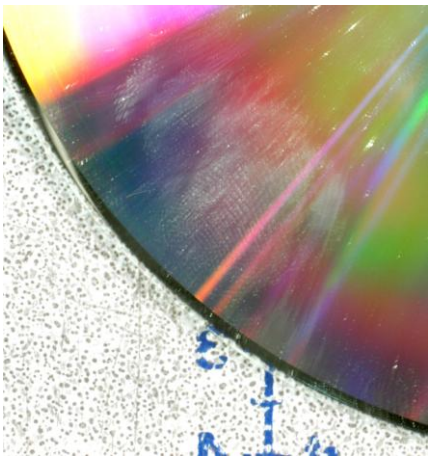
3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The CAST *Fingermark Visualisation Manual* [9] identifies several generic types of untreated fingermark that may be visible.

- where the fingermark is present in a semi-transparent material, such as sweat, oil or grease.
- where the fingermark is deposited in a coloured material, such as blood, ink or paint.
- where the fingermark is in dust.
- where the fingermark is present as a result of a reaction between a fingermark and the surface, e.g. fingermarks visible on ferrous, silver and copper articles as a result of surface corrosion or tarnishing.
- where there are fingermark impressions in wax or putty.

Subsequent to the work carried out on articles recovered from an arson scene [11,12,13,14], a further type was identified .

- where fingermarks have been developed by the effects of an environment the article has been exposed to, e.g. fingermarks developed on paper by the action of heat.

Examples of all these types of mark are illustrated below.

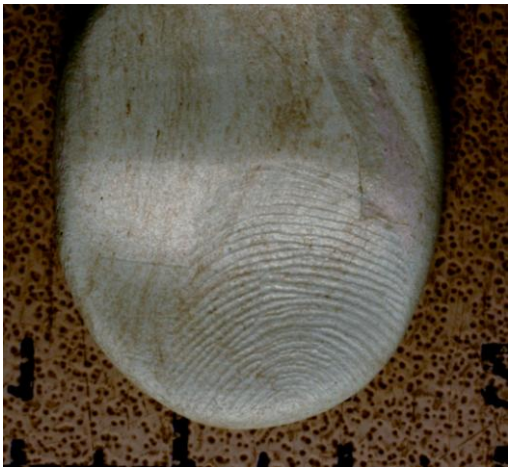


a)

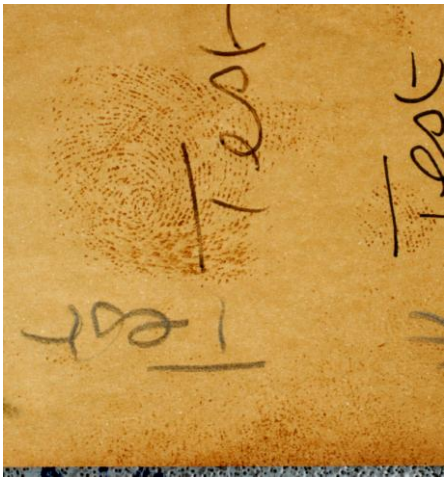
b)

c)

d)



e)



f)

Different types of marks that may be detected by visual examination a) *Type 1 mark in grease on CD* b) *Type 2 mark in soot on mug* c) *Type 3*

mark in dust d) *Type 4 mark on metal sheet* e) *Type 5 mark in plasticine* f) *Type 6 mark developed by heat on paper.*

3.2 The process recommended by CAST for all of these types of marks consists of examination under natural light, turning the article so that illumination falls on it from different angles. This should be followed by an examination using at least one type of even, white light source (e.g. a ball light or a diffuse torch), again altering the angle of illumination from perpendicular to the exhibit to oblique.

3.3 Any fingermarks detected using this examination process should be imaged using the most appropriate technique outlined in the 'Theory' section above.

4. Critical issues

4.1 Visual examination must be performed before commencing any other form of examination or chemical treatment because potentially useful marks may otherwise be missed.

5. Application

5.1 Suitable surfaces: Visual examination is applicable to all types of surface, but will yield most marks on non-porous surfaces.

5.2 Visual examination can be applied to all types of articles, including examination of surfaces at crime scenes. Because it is a non-destructive technique and marks detected in this way may not be subsequently developed by any chemical/physical process, it should be the first stage in any sequential treatment process and any marks found should be imaged before proceeding.

5.3 Because there are a wide range of mechanisms by which latent marks or impressions may occur on articles, a thorough examination using different lighting conditions should be carried out, using both natural light and an even illumination from a white light held at different angles.

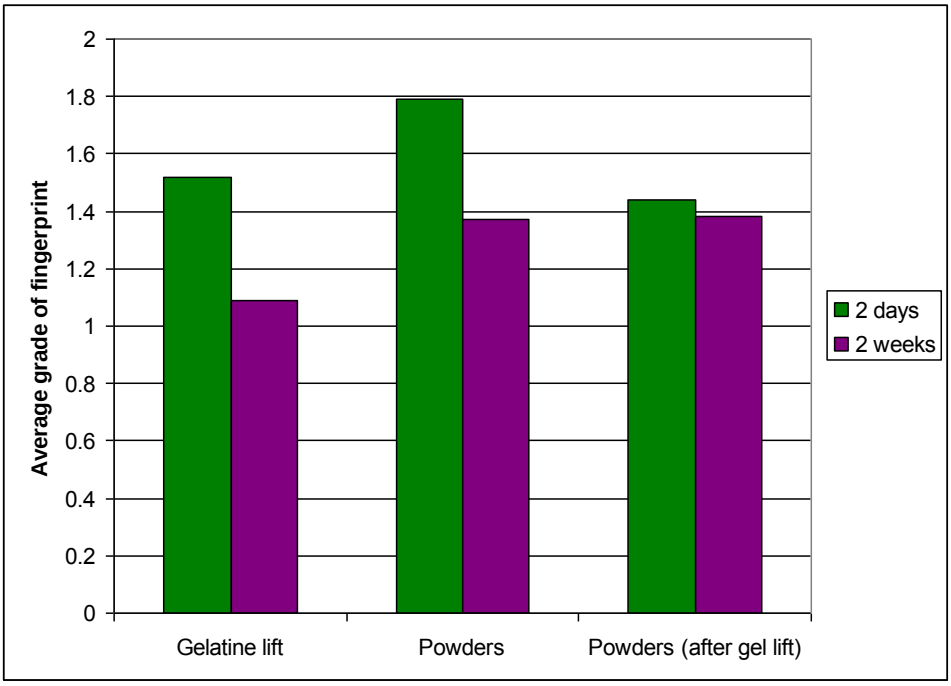
6. Alternative formulations and processes

6.1 There are no alternative treatments or processes to those described in this section.

7. Post-treatments

7.1 If the latent mark detected is thought to be eccrine or sebaceous in nature, appropriate chemical/physical development techniques should be selected from the *Fingermark Visualisation Manual* [9] taking into account the surface it has been deposited on. Similarly, if the mark is thought to be in blood or another contaminant that could be developed by techniques in the manual, an appropriate sequential treatment regime should be selected.

7.2 For other types of contaminant/particulate, marks found by visual examination may be lifted using adhesive tape or gelatine lifts. However, this should only be carried out if the type of mark or surface precludes the use of subsequent development techniques, and/or the mark has already been captured, or cannot be captured in situ. The results of a comparative study between gel-lifting of latent marks and powdering are illustrated below, based on 1,260 graded marks.



Relative effectiveness of powders and gelatin lifts for fingermark recovery from a range of surfaces [10].

7.3 It can be seen that gel-lifting latent marks is less effective than powdering and can be detrimental to subsequent powder application, especially on fresher marks where the deposits are more easily lifted by the gel. On older marks where the deposits are more robust, gel-lifting appears to be less detrimental to subsequent powdering but in general gel-lifting should only be carried out in exceptional circumstances.

7.4 Impressed marks can also be cast and lifted using silicone rubber casting compounds, and the ridges of such casts enhanced by the application of black ink.

7.5 For marks on paper that have been developed by heat, subsequent fluorescence examination using the Quaser 473 to 548 nm excitation band and 549 nm viewing/camera filters may reveal additional detail [12,13,14].

8. Validation and operational experience

8.1 Because visual examination is a non-destructive process and should be used as the first stage in any sequential treatment regime, few documented operational trials have been carried out. There are many reported examples of where visual examination has revealed fingermarks at crime scenes and on articles in laboratories, and it is not considered necessary to extensively validate what should be an intuitive process.

8.2 Recently, studies have been carried out by Hampshire Constabulary and the Metropolitan Police [16], using a wide range of light sources to examine exhibits prior to chemical treatments. Both of these studies incorporated white light sources and visual examination. Results indicate that visual examination will detect marks that are not found by any other light source or developed by subsequent chemical treatment. In the Hampshire study 11% of marks were only detected by a combination of visual and fluorescence examination, and of this visual examination using white light was the sole means of detection for 3% of marks.

8.3 A summary of the results obtained from the study on operational work at Hampshire Constabulary is given in the tables below.

Surface type	Articles	White light	Quaser 2500	Laser (532 nm)	Laser (577 nm)	Chemical treatment
Porous	169	3	10	42	15	240
Non-porous	192	43	36	34	54	277
All	361	46	46	76	69	517

Summary of the performance of different light sources on porous and non-porous surfaces.

8.4 The types of article that marks were detected on using visual examination included cowlings and knife blades for non-porous items, and marks in dirt on paper for porous items. The results indicate that, as expected, subsequent chemical treatment develops appreciably more marks. However, it is also of interest to consider the number of unique

fingermarks attributable to each process. In this analysis, the following information is obtained.

Light source	Total fingermarks	Not developed chemically	Unique fingermarks to process
White light	46	18	18
Quaser 2500	46	27	8
Laser (532 nm)	76	46	36
Laser (577 nm)	69	39	24

Detailed analysis of fingermarks detected by different light sources.

8.5 As stated above, it is evident that although visual examination detects comparatively few fingermarks (less than 10% of all marks detected), 40% of the marks that are detected by visual examination are unique to that process and it is therefore an essential element in a sequential treatment regime.

8.6 The Metropolitan Police study indicated that use of light sources accounted for around 8% of all marks detected on over 1,000 exhibits, although this included white light, long-wave ultraviolet and laser examination. On some non-porous surfaces (e.g. vehicle bodywork), the number of unique marks found by visual examination with a white light source was much higher than the average value above and reinforces the recommendation that visual examination should ideally be carried out before commencing any chemical treatment sequence.

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X-ray imaging

1. History

1.1 The properties of the x-ray were first observed by Wilhelm Roentgen in 1895, during experiments into the effect of passing electricity through bottles containing gas. Roentgen observed that rays emitted from the bottles had the ability to take pictures of objects hidden under or within other objects, and took a picture of his wife's hand that revealed her bones and her wedding ring.

1.2 Although rapidly adopted for medical applications such as the imaging of the interior bones, x-rays were not seriously considered for forensic imaging until the mid-1960s, when Graham and Gray at the Victoria Infirmary, Glasgow, began experimenting with the technique of electronography [1,2], initially with the intention of revealing the watermark of stamps attached to documents. In the electronography technique a metal irradiated with a high energy, monochromatic x-ray beam emits its own characteristic x-rays, which cause a photographic film in intimate contact with the sample to darken. This is outlined in more detail in the 'Theory' section below.

1.3 Graham [2,3] next considered using powdered lead to reveal indented writing, carrying out electronography to enhance the indentations the lead had preferentially settled into. Graham and Gray considered that fingermarks could be developed in a similar way [4], powders already being extensively used for fingermark development. Magnetic powders with the Magna-brush were considered, but the emission from iron was not found as effective as that from lead and subsequent studies utilised lead powdering in combination with electronography. The first application proposed for electronography was the revelation of fingermarks deposited on patterned backgrounds. Once the fingermark had been developed using the lead powder, only the developed areas emitted during subsequent electronography and the resultant fingermark image was free of background. Test fingermarks were resolved on magazine covers and postage stamps.

1.4 Electronography was also proposed to image fingermarks on dead human skin, again using lead powdering to develop the mark and electronography to enhance the image and remove the background of skin texture, hairs, etc. [5]. There was reasonable interest in the technique for this purpose, with no satisfactory development technique being available at that time. The Police Scientific Development Branch (PSDB) placed a contract with Graham in the early 1970s to investigate the development of fingermarks on limbs using lead powder and electronography. The technique was adopted in some laboratories in the USA [6,7], and refinements were proposed to make the technique easier to apply both in the laboratory and in the field [6]. Later adaptations were proposed within the UK [8], and the use of lead powder with electronography was proposed as an alternative to vacuum metal

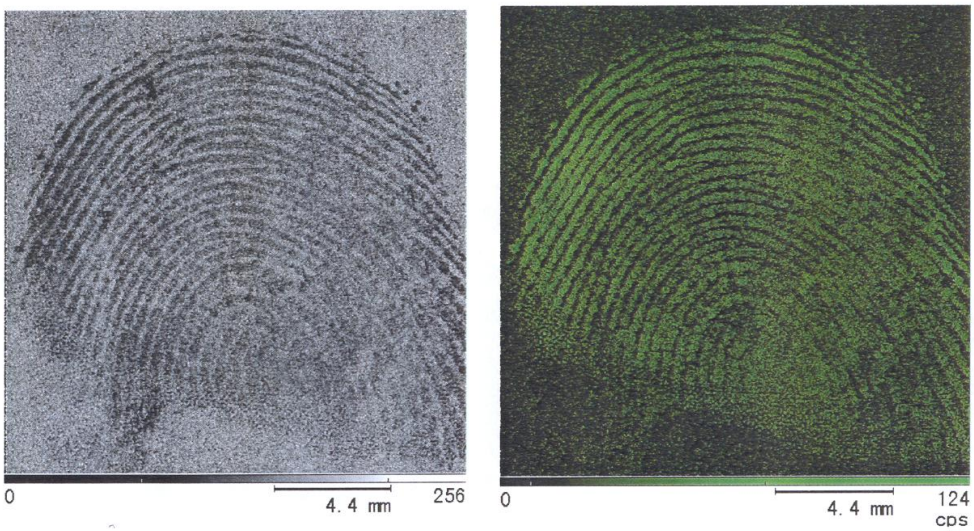
deposition (VMD) for developing marks on polythene [9]. VMD was found to be far more effective than lead powdering for this purpose, and after the late 1970s the technique seems to have gradually faded from use.

1.5 X-rays can also be used to image fingermarks in other ways. X-rays are also emitted from samples bombarded by electron beams in electron microscopes, and the characteristic x-rays thus emitted can be used to build elemental maps of a surface. This is described in greater detail in Chapter 2, Optical Processes, Scanning electron microscopy.

1.6 Another way in which x-rays can be emitted is by x-ray fluorescence, irradiating a sample with monochromatic x-rays and causing characteristic x-rays to be emitted in a process directly analogous to fluorescence in the visible region of the spectrum. More recently, researchers have used an x-ray fluorescence instrument to scan surfaces and detect fingermarks by mapping characteristic elements within latent fingermarks and within contaminants that may be present on fingers such as sun cream [10]. Potential advantages of x-ray fluorescence over x-ray mapping within a scanning electron microscope are that larger areas can be examined, the sample does not have to be under a vacuum and the sample does not have to be coated with a conductive coating to prevent charging.

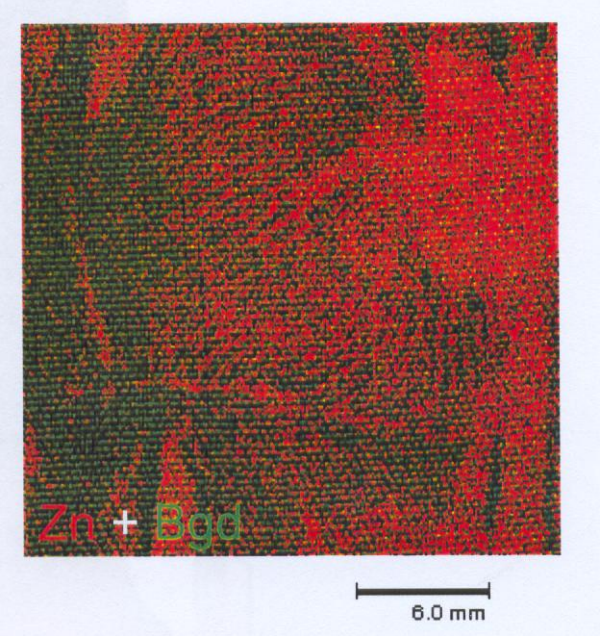
1.7 The Home Office Centre for Applied Science and Technology (CAST) has also carried out some initial studies into the x-ray fluorescence technique, in this case looking at fingermarks developed using techniques that result in characteristic elements being present in fingermark ridges, such as physical developer, vacuum metal deposition and metal toning of ninhydrin [11]. It was shown that the technique had potential for revealing fingermarks on patterned backgrounds, such as magazines, and also on fabrics. The instrument used in these studies also had a transmitted x-ray mode and for fingermarks containing heavy elements, such as iodine, this was also found to be effective for distinguishing ridges from the background.

Overview of fingermark treated with physical developer on magazine page, subsequently toned with potassium iodide.



a) b)

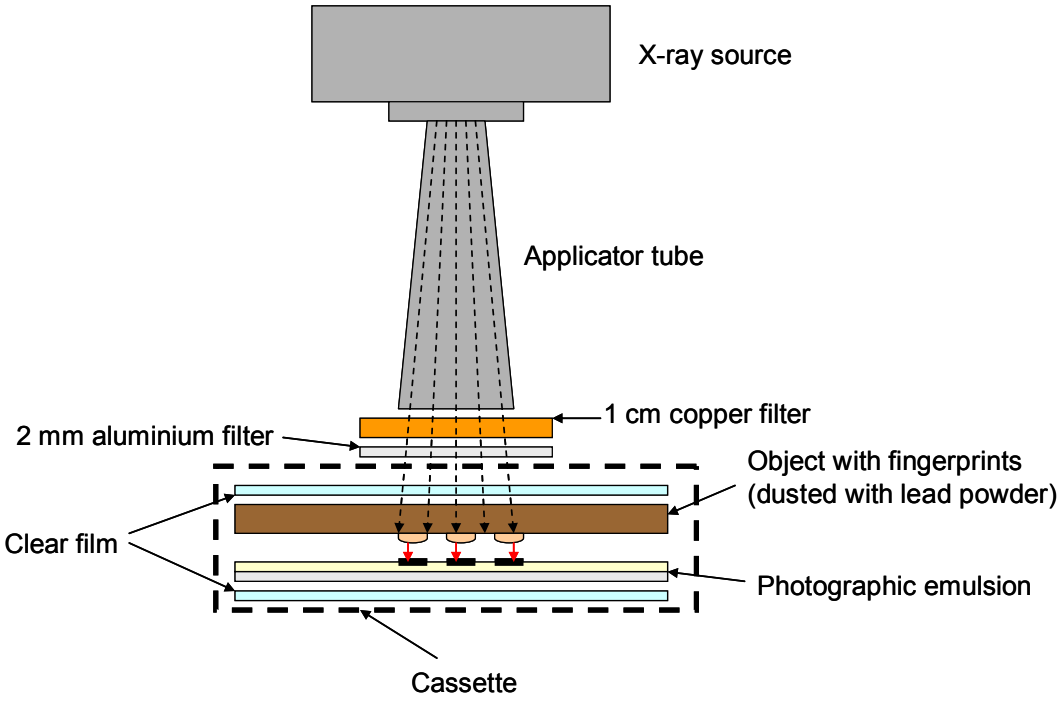
Closer view of x-ray images a) *image of mark in x-ray transmission mode and* b) *image formed from characteristic x-rays from iodine.*



X-ray image from mark developed on fabric using vacuum metal deposition, red signal = zinc from metal deposition, green signal = fabric background.

2. Theory

2.1 The practical apparatus used by Graham and Gray is illustrated schematically below, and the theory of electronography outlined subsequently.

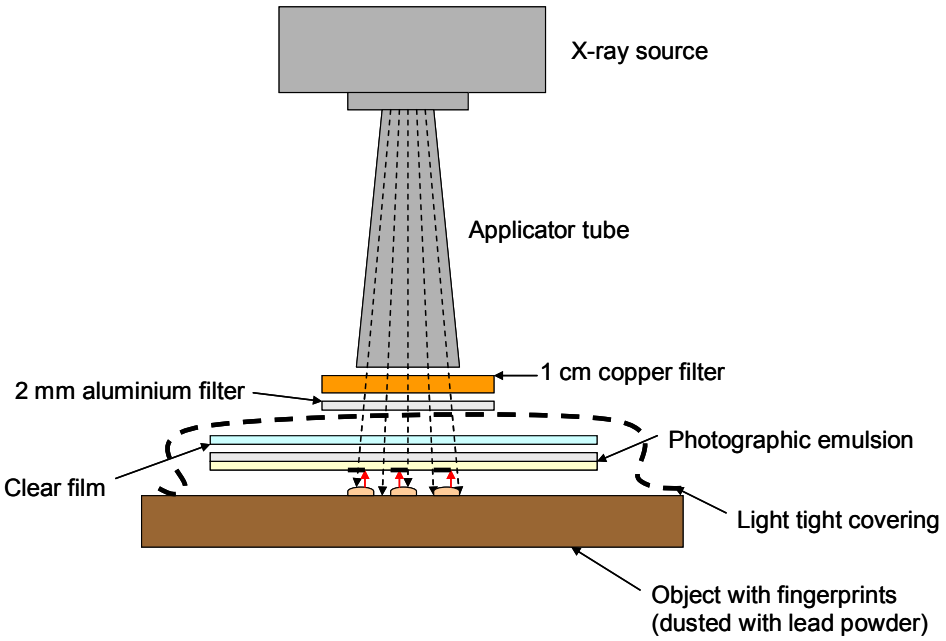


Electronography apparatus proposed for thin exhibits such as documents.

2.2 All metallic elements, when irradiated by a high kilovoltage beam, emit both electrons and x-rays characteristic to that element. These characteristic x-rays and electrons cause the silver halides of a photographic film emulsion to convert to silver, leaving a black image of the areas containing the characteristic metal element.

2.3 For this to be effective, the original, incident x-rays must have a negligible effect on the photographic emulsion and it is therefore necessary to filter the original broad spectrum of wavelengths emitted by the x-ray source. The longer wavelength x-rays that cause film fogging are filtered out by passing the beam through a 1 cm block of copper. The characteristic copper x-rays emitted as the primary beam passes through the copper filter are in turn removed by a further 2 mm aluminium filter, and the x-rays emitted from the aluminium filtered out by a clear plastic film. The short-wave x-rays pass through the object under examination and hit the lead particles adhering to the fingermark ridges, promoting emission of x-rays and electrons that develop an image of the fingermark on the photographic film in intimate contact with the surface. A further clear film is used below the photographic film to absorb scatter and emission from other areas within the cassette.

2.4 For articles that were not flat or could not be fitted inside a cassette, an adaptation of the method was proposed.



Electronography apparatus proposed for solid exhibits such as bodies.

2.5 In this adaptation, x-rays are allowed to pass through the photographic film and fall upon the surface being examined. The x-rays from the surface are emitted backwards onto the film and the clear film, film and surface are enclosed within a light-tight covering.

2.6 The theory of x-ray fluorescence is exactly analogous to fluorescence in the visible region of the spectrum. A short wavelength beam of x-rays is used to irradiate a surface, promoting electrons into excited states. As these electrons decay back to ground states, they emit x-rays at longer wavelengths with an energy characteristic to the particular elements present in the surface. By scanning the x-ray probe across the surface, a map can be produced of all locations where a particular characteristic element is present. If such an element is known to be specific to the ridges of the fingermark, x-ray fluorescence can be used to reveal fingermark detail.

2.7 X-ray imaging can also be carried out in transmission mode. In this mode it is the atomic density of an area that determines the intensity of x-rays transmitted through a sample. If a high atomic number element is present, fewer x-rays are transmitted and the area appears dark in the developed/collected image. If fingermark ridges (or the background) can be preferentially doped with a high atomic number element, it may be possible to obtain contrast between the fingermark and its background. This has been demonstrated using potassium iodide toning of a mark treated with physical developer and to a lesser extent with a mark powdered with bismuth salts.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not recommend electronography for operational use in police force fingermark laboratories because of the hazards associated with the use of x-rays and the harmful nature of lead powder. In addition, no comparative studies have been carried out to demonstrate that electronography is more effective than other techniques for any of the applications for which it has been proposed.

3.2 X-ray fluorescence and x-ray transmission may be useful for practical application and the development of fingermark reagents designed for x-ray functionality is feasible. They may be able to resolve marks developed using heavy elements such as iodine in circumstances where other more conventional imaging techniques are ineffective. As a consequence, the XRF process is included as a Category C process in the *Fingermark Visualisation Manual.* However, the cost of analytical equipment is high and beyond the reach of most police forces. If the technique is to be used operationally it is likely that it will be confined to special cases, utilising equipment at establishments such as universities.

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Alternative blood enhancement techniques

1. History

1.1 The history of the development of blood dyes is outlined in Chapter 3, Chemical and Physical Processes, Acid dyes, of this *Fingerprint Source Book*.

2. Theory

2.1 General theory

2.1.1 The theory associated with the action of protein stains (in particular the acid dyes), in enhancing traces of blood is described in Chapter 3, Chemical and Physical Processes, Acid dyes (which describes acid black 1, acid violet 17, acid yellow 7).

2.1.2 There are other reagents that react with the amines present in blood to give coloured or fluorescent products, the most well known of these being ninhydrin and 1,8-diazafluoren-9-one (DFO). They both react similarly with amino acids to form products that contain two deoxygenated molecules of the starting product bridged by a nitrogen atom, which is donated from the amine [1,2]. These processes are described in greater detail in this *Fingerprint Source Book* (see Chapter 3, Chemical and Physical Processes, Ninhydrin and DFO respectively).

2.1.3 While the reaction mechanisms and products have similarities, the method of their visualisation is entirely different. Ninhydrin, under the right conditions, produces an intensely coloured product called 'Ruhemann's purple' after the discoverer and DFO a pale pink, extremely fluorescent product. Ruhemann's purple can be made to fluoresce by complexing it with metal salts but this additional process is still not as sensitive as DFO [3]. DFO requires heat for the reaction to proceed [4] while ninhydrin will react at room temperature provided moisture is available, although the process proceeds much faster at elevated temperatures and humidities. These techniques are not specific to blood and will detect other amine-containing substances (such as urine), including latent fingermark deposits.

2.1.4 There are several ways of positively identifying blood using spectroscopic methods [5,6] but they are all carried out ex situ, so are of no use in the enhancement of blood-contaminated fingermarks.

2.1.5 Haemoglobin strongly absorbs light throughout the ultraviolet, visible and near infra-red parts of spectrum and this property can be utilised to detect and enhance blood (see Chapter 2, Optical Processes, Other Optical Processes for the use of multispectral imaging in this application), although once again this cannot be regarded as a way of confirming that it is blood that is present. Where deposits of blood are heavy or are present on light coloured surfaces a good white light may

suffice to enable enough detail to be observed. However for pale or insubstantial deposits it may be necessary to use high-intensity light sources to enhance the contrast between the blood and the surface.

2.1.6 The use of fluorescence to enhance fingermarks in blood can be extremely effective in these circumstances. There are two ways this may be achieved:

- by exciting fluorescence of the background surface on which the blood is deposited;
- by treatment with a process that either breaks the haem group or turns the blood into a fluorescent species, or does both of these.

2.1.7 Many materials fluoresce when excited by high-intensity light in the ultraviolet and violet regions of the spectrum. This is coincidently where the haem group is most absorbent, with a peak around 421 nm (known as the Soret Band) [5,7,8] and why blood-contaminated fingermarks will appear dark against a light background. Fluorescence examination may be used before any other fingermark enhancement techniques as it is non-destructive and if long-wave ultraviolet or violet/blue light (350 to 450 nm) [9] is used then DNA typing is also unaffected [10]. The use of ninhydrin, acid black 1 or acid violet 17 can further intensify the contrast between the fingermark and the background by increasing the light absorption properties of the blood.

2.1.8 The use of a strong organic acid in conjunction with hydrogen peroxide [11,12] breaks up the haem group so that it is no longer effective at absorbing light. After such treatment, blood will fluoresce orange when excited by green light (500 to 550 nm). This effect has also been noted as blood ages.

2.1.9 DFO produces fluorescent species with blood, which can be excited by green (510 to 570 nm) light. This can be less effective on heavy deposits of blood as the haem group retains its ability to absorb both the excitation wavelengths of light and that emitted as fluorescence.

2.1.10 There are three kinds of tests for blood detection that use the haem group in haemoglobin: crystal tests; catalytic tests; and antibody tests. The sensitivity of these techniques is limited by their effectiveness to lyse blood cells, so releasing the haem-containing proteins that are only present within the red blood cells.

2.1.11 The Teichmann test [13] results in the formation of brown rhombohedral crystals of haematin and the Takayama test [14] in red-pink crystals of pyridine haemochromogen. Both of these tests have to be carried out ex situ so are of no use for fingermark enhancement as the ridge detail is inevitably destroyed as the blood is removed, unless an area containing no ridge detail, such as a smear, alongside the fingermark is used.

2.1.12 There are a number of advantages to the Takayama test, as compared with the Teichmann test. Heating is not required to obtain results within a reasonable amount of time in the Takayama test; and even if heat is applied, the test is not subject to being ruined by over-heating. The test

also yields positive results under some of the circumstances where the Teichmann test fails.

2.1.13 The catalytic tests are only presumptive or infer the presence of haem, as they only use the haem to facilitate another reaction and are subject to both false positive and false negative reactions caused by a variety of non-blood substances. Consequently individual results require careful interpretation by experts.

2.1.14 These tests all rely on the 'peroxidase activity' of the haem group. Enzymes that catalyse the peroxide-mediated oxidation of organic compounds in vivo are called peroxidases; haemoglobin and the other compounds that show this catalytic property are thus said to have 'peroxidase activity'. This peroxidase activity may be utilised to cause the oxidation of colourless reduced dyes, such as phenolphthalein, leucocrystal violet, tetramethyl-benzidine and fluorescein, which when oxidised form their coloured, or in the case of the latter, fluorescent, counterparts. In general, the reaction below applies:

$$H_2O_2 + \text{colourless reduced dye} \rightarrow H_2O + \text{coloured oxidised dye} \quad [15]$$

2.1.15 The luminol test also relies on the peroxidase activity of the haem group, but can be used with either hydrogen peroxide [16] or sodium perborate [17]. When in the presence of blood a product which chemiluminesces is produced. The bluish-white chemiluminescence is faint and must be viewed in the dark by an operator who is fully dark-adapted to gain the best evidence from this test. However, even with careful application of luminol using equipment such as fine mist sprayers it is extremely easy to damage the fine detail of the blood-contaminated fingermark ridges on both porous and non-porous surfaces, especially when repeat applications may be require to first locate and then image the evidence. Therefore this technique is not recommended for fingermarks and should only be used when fine detail is not required and when other techniques might be compromised by surface type or impracticality, such as dark or patterned carpets [11].

2.1.16 The major concern with the catalytic tests for blood is that they can produce false-positive results in the presence of chemical oxidants and catalysts, salts of heavy metals such as copper, nickel and iron, and plant peroxidases such as those found in horseradish, citrus fruits, and numerous root vegetables [18]. A two-stage test can help to stop false positives from true peroxidases. The reduced colourless dye is applied initially and if no colour change is observed then the hydrogen peroxide added. A colour change at this point is more likely to indicate the presence of blood rather than a peroxidase, although contamination by metal salts is not distinguished.

2.1.17 It is generally accepted that a negative result with a catalytic test proves the absence of blood, however strong reducing agents such as

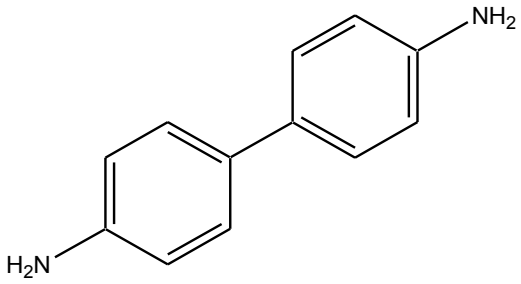
ascorbic acid [19] and active oxygen cleaning products [20] may inhibit such tests.

2.1.18 The antibody tests [21, 22] like the crystal tests are confirmatory for blood, but as they use anti-human Hb antibodies they are also specific for human blood. Currently (2016), they have to be used ex situ so are of no use for fingermark enhancement, and it remains to be seen whether these tests can be used after the more effective enhancement techniques [22] to prove that what is being enhanced is human blood.

2.2 Specific reagents

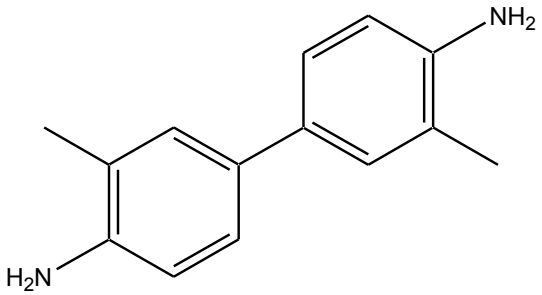
2.2.1 A review of blood enhancement agents has recently been conducted by Powell [23,24] and the relevant information below is extracted from these documents. Although the purpose of the review was for footwear enhancement, there is direct read-across to fingermarks because the contaminant being targeted is the same.

2.2.2 Benzidine: Benzidine was first used in 1904 and was the first reagent that utilised the peroxidase activity of haem. Benzidine is colourless in its reduced form and will turn dark blue when oxidised in the presence of haem or haem derivatives. It caused the entire surface being treated to be stained a light brown colour but was used on a variety of porous and non-porous surfaces. Due to its high sensitivity and dramatic colour change benzidine found widespread operational applications until health and safety concerns curtailed its use.



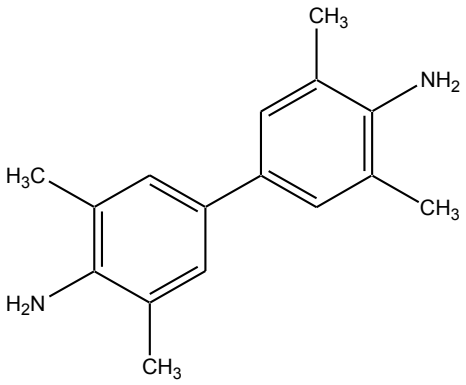
Structure of benzidine.

2.2.3 Ortho-tolidine: Ortho-tolidine is structurally related to benzidine, and is also colourless in its reduced form and dark blue when oxidised. It was first used in 1912 and again was widely employed due to its sensitivity and pronounced colour change. It was initially suggested as a possible alternative to benzidine. A sensitivity comparison of blood enhancement techniques rated ortho-tolidine second only to benzidine and suggested that it could be used providing that all health and safety precautions are taken.



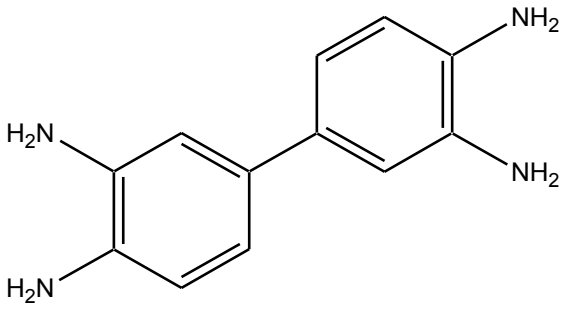
Structure of ortho-tolidine.

2.2.4 Tetramethyl-benzidine: As the commonly used reagents such as benzidine and ortho-tolidine were found to be carcinogenic thoughts were turned to find a new reagent of equal specificity but without the associated health and safety problems. There was some evidence that the issue was the participation of ortho-hydroxy derivatives of aromatic amines in the carcinogenic action, therefore the use of 3,5,3',5-tetramethylbenzidine (TMB) was suggested where ortho-hydroxylation is impossible. A mark developed by TMB would turn green/blue.



Structure of 3,5,3',5- tetramethyl-benzidine.

2.2.5 Diaminobenzidine: Diaminobenzidine (DAB) undergoes a chemical polymerase reaction converting blood marks to an insoluble brown product. Its alternative name is tetraamino-biphenyl (TAB).



Structure of diaminobenzidine.

2.2.6 DAB is a derivative of benzidine and was thought to be a suitable substitute reagent for the enhancement of blood marks, as it is used as an aqueous solution and does not employ any organic solvents. The working solution is mixed just prior to use and involves the addition of a phosphate buffer solution to an aqueous solution of DAB. The reaction is initiated by hydrogen peroxide.

2.2.7 A widely used formulation is given below and involves the addition of a phosphate buffer working solution to the aqueous solution of DAB.

Solution A – fixing solution: Dissolve 20 g 5-sulphosalicylic acid in 1 L of distilled water.

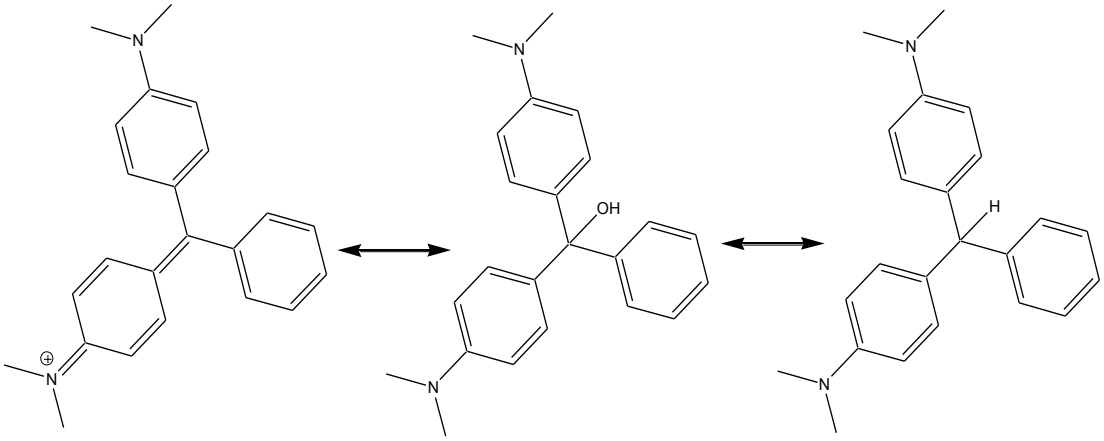
Solution B – buffer solution: Mix 100 mL of 1M phosphate buffer (pH 7.4) with 100 mLof distilled water.

Solution C – DAB: Dissolve 1 g of 3,3'-diaminobenzidine tetrahydrochloride in 100 mL of distilled water.

Working solution: Mix 180 mL of solution B with 20 mL of solution C and add 1 mL of 30% hydrogen peroxide. The fixing solution is applied prior to the working solution.

2.2.8 Leuco-dyes: These are catalytic tests for blood and will bind with the proteins found in blood limiting the leaching and running of the developed impression. The hydrogen peroxide solutions will catalyse oxidation of the haemoglobin and its derivatives, producing a blue/green colour for leucomalachite green (LMG) and violet for leucocrystal violet (LCV).

2.2.9 Leucomalachite green: LMG is oxidised to form its coloured product when in contact with the haem group in blood.



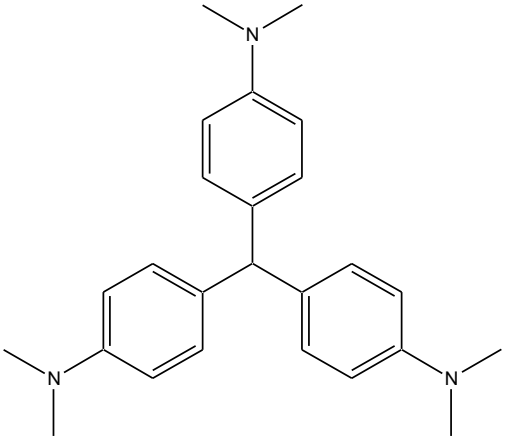
Changes occurring between the leuco- and coloured forms of malachite green (left – coloured form, centre – carbinol base, right – colourless leuco form).

2.2.10 There are several formulations of LMG in the literature; they all contain LMG, diethyl ether, glacial acetic acid and hydrogen peroxide, the only difference being the quantity of each reagent. For optimum results the reagent must be prepared immediately prior to use. A green colour indicates that blood is present. The formulation given below is one used by the Royal Canadian Mounted Police.

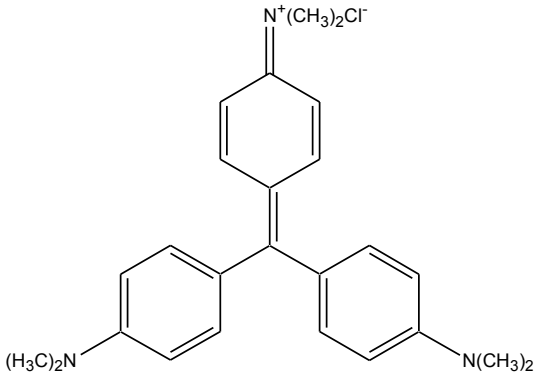
> Place 0.2 g of LMG in a clean glass beaker, and add 67 mL of methanol. Once the LMG is dissolved add 33 mL of glacial acetic and 0.67 g of sodium perborate and stir well until dissolved. Pour into a 1 L beaker and add 300 mL of 1-methoxynonafluorobutane (HFE 7100). Store in a dark glass bottle until required. The marks can be fixed by submersion in ethanol.

Although stated above that marks can be fixed, other researchers [25] have found that fixing marks in blood prior to application of LMG resulted in weaker development.

2.2.11 Leucocrystal violet: LCV is the completely reduced form of crystal violet and is colourless. The reaction is initiated by hydrogen peroxide and when LCV comes into contact with the haem in blood the reaction is catalysed and the clear solution is converted to a purple/violet colour.



Crystal violet

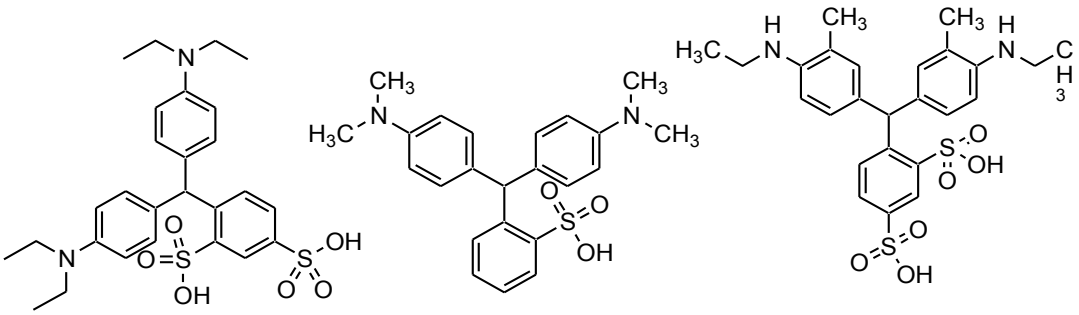


Leucocrystal violet

2.2.12 LCV is applied to the enhancement area via a spray method. The most common formulation is given below.

Dissolve 10 g of 5-sulphosalicylic acid in 500 mL of 3% hydrogen peroxide. Add and dissolve 3.7 g sodium acetate. Add and dissolve 1g of leucocrystal violet with a magnetic stirrer. Store in dark-coloured glassware and refrigerate.

2.2.13 Alternative leuco dyes: Powell [24] studied a range of alternative leuco dyes to investigate whether issues with sensitivity and carcinogenicity of the existing leuco dyes could be overcome. The first alternative dye investigated was leuco patent blue (LPB). LPB is an acidic peroxidase dye compared with LCV, which is basic. As the fixing agent precipitates basic proteins, the acidic peroxidase reagent would then dye the basic proteins in a manner analogous to the protein stains. Two other similar systems, leuco berbelin blue (LBB) and leuco xylene cyanole (LXC) were also evaluated.



Structures of leuco patent blue, leuco berbelin blue , and leuco xylene cyanole.

2.2.14 Formulations for these reagents are given below.

0.1042 g of leuco patent blue is dissolved in 10 mL of water; 4 mL of acetic acid and 1 mL of 3% hydrogen peroxide are then added.

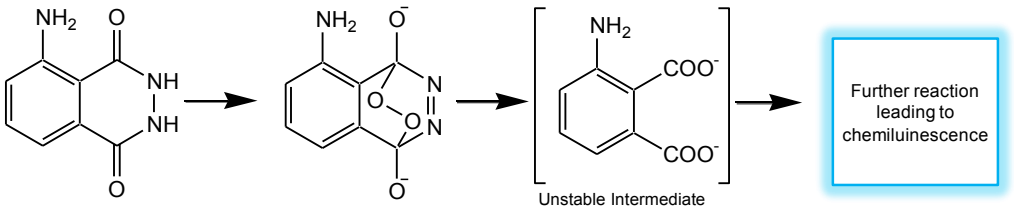
0.072 g of leuco berbelin blue is dissolved in 10 mL of water; 4 mL of acetic acid and 1 mL of 3% hydrogen peroxide are then added.

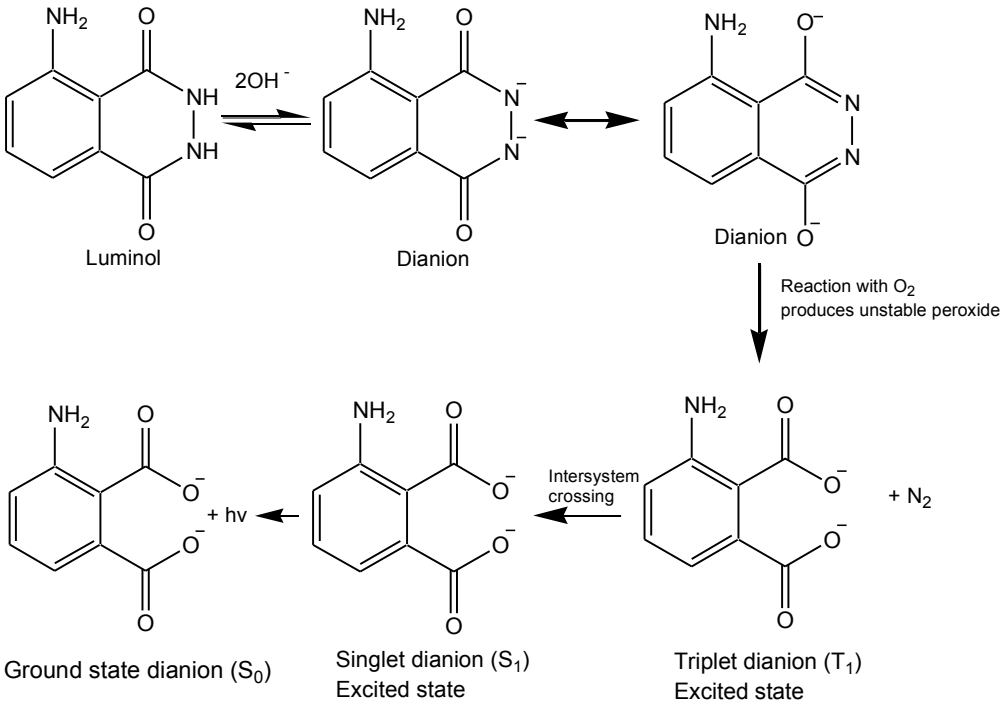
0.091g of leuco xylene cyanole is dissolved in 10 mL of water; 10 mL of acetic acid with 2 mL of hydrogen peroxide are then being added.

The use of the leuco form of the dye rhodamine 6G has also been proposed. This can be produced from rhodamine 6G by first reducing it to the leuco form over zinc and then using it as for other peroxidase reagents [26].

2.2.15 Luminol: The active chemicals in this generic class of blood detection reagents are luminol ($C_8H_7O_3N_3$) and hydrogen peroxide (H_2O_2). The hydrogen peroxide and the luminol react in alkaline conditions to produce chemiluminescence (in this case a blue/white glow), with the reaction being catalysed by the iron present in haemoglobin.

2.2.16 In the resultant oxidation reaction, the luminol molecule loses nitrogen and hydrogen atoms and gains oxygen atoms, resulting in a compound called 3-aminophthalate. The reaction leaves the 3-aminophthalate in an excited state with the electrons in the oxygen atoms being promoted to higher energy levels. The electrons quickly fall back to a lower energy level, emitting the extra energy as a light photon (observed as a blue chemiluminescence).





Schematic diagrams showing the mechanisms associated with the chemiluminescent reaction between luminol and blood.

2.2.17 Two published formulations for luminol are given below, and proprietary pre-prepared products (e.g. Bluestar) are also available.

Grodsky:

3.5 g sodium perborate is dissolved in 500 mL distilled water, 0.5 g luminol and 25 g sodium carbonate are added and dissolved. Solution is left to stand for five minutes before being used immediately.

Weber:

Stock solution A: 8 g sodium hydroxide dissolved in 500 mL distilled water.

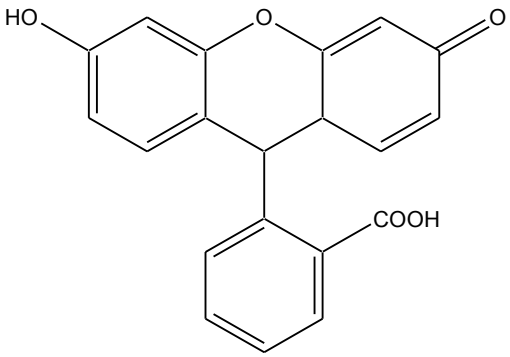
Stock solution B: 10 mL 30% hydrogen peroxide in 490 mL distilled water.

Stock solution C: 0.354 g luminol dissolved in 62.5 mL of solution A and made up to final volume of 500 mL with water.

Working solution: 10 mL solution A + 10 mL of solution B + 10 mL of solution C + 70 mL distilled water.

2.2.18 Fluorescein: Fluorescein is a presumptive test for blood that utilises the peroxidase activity of the haem group. The reduced form of the chemical, fluorescin, is colourless and when sprayed onto the target area it is oxidised to fluorescein, a coloured/fluorescent product, by the presence of blood associated proteins and iron ions found in the haemoglobin

molecule. Even minute traces will fluoresce when excited with a light source between 425 to 485 nm and viewed through a yellow to orange barrier filter.



Structure of fluorescein.

2.2.19 Fluorescein is usually applied in a two-step process – the application of fluorescein alone will develop the yellow coloration, however an overspray of hydrogen peroxide is also used to reduce background fluorescence and false-positive reactions.

2.2.20 The preparation of fluorescein is quite a lengthy process and the reduced fluorescin has a very short shelf life – the recommended usage is within 24 hours. The original formulation is as follows.

A 10% sodium hydroxide (NaOH) stock solution is prepared by dissolving 10 g NaOH in 100 mL deionised water.

1.0 g fluorescein is dissolved in 100 mL of the 10% NaOH stock solution and placed on a hot plate and heated gently.

10.0 g zinc powder is then added and heated to a gentle boil.

The solution is allowed to cool and the un-dissolved zinc to settle.

The cooled solution is then decanted to remove any un-dissolved zinc, which is then neutralised before disposal using hydrochloric acid followed by sodium carbonate.

The fluorescein reagent solution is then made by mixing 50 mL of the decanted solution with 950 mL of deionised water. This reagent must then be kept in dark glassware.

The hydrogen peroxide overspray is made by mixing 100 mL of 30% hydrogen peroxide with 200 mL deionised water (i.e. a 10% solution of hydrogen peroxide).

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Home Office Centre for Applied Science and Technology (CAST) does not recommend the use of haem-specific, reactive blood dyes for general use because they are not as sensitive as the protein stains recommended as Category A processes in the *Fingermark Visualisation Manual*[10]. This is intuitive – there is a far greater quantity of proteinaceous material present to interact with the dye than there is haem and therefore the protein stains will remain effective on far smaller quantities of blood residue than reactive dyes. This is supported by the sensitivity testing conducted by Sears *et al.* [11] when developing the formulations for acid black 1, acid yellow 7 and acid violet 17.

3.2 It is recognised that there will be circumstances where the use of haem-specific dyes will be preferable, e.g. where there is other proteinaceous contamination present and a more specific dye will more clearly identify the blood, and for this reason Leuco crystal violet is included as an option in the Category B processes in the *Fingermark Visualisation Manual*. Reactive dyes are also more suited to speculative searching of scenes, and can be more easily spray applied. However, this approach is more suited to development of footwear marks, especially on carpets, than to fingermark visualisation. A range of the alternative blood enhancement agents (protein stains and reactive dyes) is outlined below, with some comments on those most commonly proposed for operational use.

3.3 Benzidine: Benzidine was found to be a highly effective blood-enhancing reagent but was later recognised as a known carcinogen and there are reports in the literature stating forensic analysts developed bladder cancer due to the use of this reagent. It is now known to be extremely hazardous and breathing its vapours or touching the chemical or its salts could cause cancer to develop. It is not recommended for use by CAST and is included in this review for historical purposes only. It is listed among the Category F processes (not recommended for health and safety reasons) in the *Fingermark Visualisation Manual*.

3.4 Ortho-tolidine: Although ortho-tolidine was originally proposed as a safer alternative to benzidine, there are several reports in literature stating that workers suffered from prolonged headaches and skin burns after using ortho-tolidine when safety precautions were not taken. Ortho-tolidine is now also a known carcinogen and its use is therefore not recommended by CAST. Although not specifically listed in the *Fingermark Visualisation Manual,* it would be regarded as a Category F process.

3.5 3,5,3',5 Tetramethylbenzidine (TMB): Sensitivity studies carried out in comparison with acid black 1 show TMB to be significantly less sensitive. It therefore appears in Category E (no known operational benefits) in the *Fingermark Visualisation Manual*. There are also concerns about TMB

being a possible carcinogen and mutagen and its use is therefore not recommended by CAST.

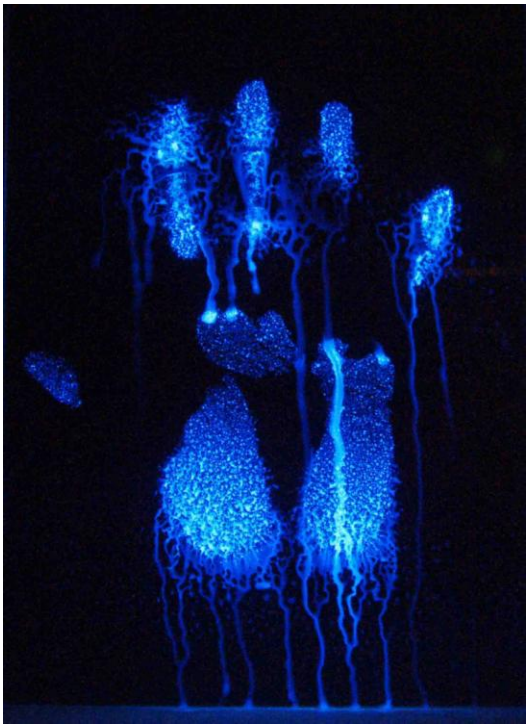
3.6 3,3' Diaminobenzidine tetra hydrochloride dehydrate (DAB): Sensitivity studies carried out in comparison with acid black 1 show DAB to be significantly less sensitive. It therefore appears in Category E (no known operational benefits) in the *Fingermark Visualisation Manual*. The colour formed during the reaction is light brown, which is similar to dried blood and not ideal for enhancement of bloody fingermarks, whereas a protein dye such as acid black 1 will stain the mark a dark colour, which will aid with contrast against the background. There are also reports on the suspected carcinogenic activity of DAB, and therefore it is not recommended for use by CAST.

3.7 Leucomalachite green: LMG has been found to be less sensitive than acid black 1 and does not produce as vivid a colour change as some other reagents studied. It was also found to be less consistent in performance than LCV. It therefore appears in Category E (no known operational benefits) in the *Fingermark Visualisation Manual*.

3.8 Leucocrystal violet: LCV has been shown to be an effective treatment for marks in blood, albeit less sensitive than protein stains. If a haem-specific reagent were to be recommended by CAST, LCV would be the preferred option but only under controlled conditions in a laboratory. The purple coloured form crystal violet is now classified a known carcinogen which makes large scale spraying at scenes undesirable. However, as mentioned above the potential for use in niche applications is recognised and it appears as a Category B process in the *Fingermark Visualisation Manual*.

3.9 Alternative leuco dyes: Of the alternative leuco dyes evaluated, LBB gave high background staining and although LPB and LXC were effective in preliminary studies, the cost of the dyes is prohibitive for operational use. They have only been investigated experimentally and therefore are not listed in the *Fingermark Visualisation Manual.*

3.10 Luminol: Luminol and related compounds are not recommended for fingermark detection because they are spray applied and could cause diffusion of marks. It therefore appears in Category E (no known operational benefits) in the *Fingermark Visualisation Manual*. Because luminol relies on a chemiluminescent reaction to produce blue fluorescence that fades with time, multiple applications may be required to first locate and then photograph any fingermarks. However, it has been demonstrated that repeat applications will ultimately cause diffusion of ridge detail, especially on non-porous surfaces, and therefore the use of a reagent giving a coloured or conventionally fluorescent mark is preferred.



Palm print in blood on glass, with ridge detail diffused by excessive spraying.

3.11 Fluorescein: Fluorescein has been found to be lower in sensitivity to most of the other dyes outlined here and the acid dyes recommended in the *Fingermark Visualisation Manual* [10]. It therefore appears in Category E (no known operational benefits).

3.12 Alternative protein stains: In addition to reactive dyes, CAST has considered a wide range of alternative protein stains that were evaluated in comparative studies with acid black 1, acid yellow 7 and acid violet 17 [27,28]. These dyes were rejected on the basis of lack of sensitivity, lack of availability or poor visibility of the developed mark. A summary of those systems evaluated is given in the table below. Some of these dyes (especially those that have been used operationally) are listed in Category E (no known operational benefits) of the *Fingermark Visualisation Manual*.

Colour Index name	**Colour Index number**	**Comments**
Acid blue 92	13390	Plasma stain
Acid red 88	15620	Plasma stain
Acid red 29	16570	
Acid red 1	18050	Plasma stain
Acid yellow 23	19140	Collagen stain (protein)
Direct yellow 12	24895	Plasma stain (in pathology)
Acid red 71	27165	Cytoplasmic stain
Acid red 112	27195	Stain basic tissue

		elements
Acid blue 1/acid blue 3	42045/42051	
Basic violet 4	42600	
Acid blue 90	42655	Protein stain
Acid blue 83	42660	Protein stain
Acid violet 19	42685	Plasma stain
Acid dye	43535	
Basic blue 11	44040	
Acid red 87	45380	Plasma stain
Basic dye	51140, 51145	
Direct red 148	52005	
Acid blue 74	73015	
Quinacrine	-	
Lucifer Yellow (CH & VS)	-	
Rivanol	-	

Alternative protein stains evaluated by CAST but not recommended for operational use.

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Acid dyes (acid black 1, acid violet 17, acid yellow 7)

1. History

1.1 Fingermarks may be deposited in a number of contaminants at crime scenes, and of all these blood is the most commonly observed. This is possibly because, when present even in small quantities, it is easily seen as it strongly absorbs light throughout the visible spectrum. However, when present in minute amounts, or on dark, patterned or multicoloured confusing backgrounds, the blood may require enhancement to make it more useful for evidential purposes. Additionally, proof that a stain is actually blood rather than an innocuous substance may be important in assessing guilt or innocence, and may even be a matter of life or death in some cases.

1.2 The history of proving the presence of blood evidence in forensic investigation dates back over 150 years using chemical means, and further still when microscopical methods are considered. Anton van Leeuwenhoek was said to be the first person to describe and illustrate blood cells in the latter part of the 17th century, although this is disputed.

1.3 The earliest tests for blood were of two types, both relying on the presence of the haem group present in the red blood cells. The early tests included those that reacted with haem to produce crystals and those that relied on its catalytic nature. More recently (1999) a third test relying on antibodies has been introduced.

1.4 The crystal or confirmatory tests were formulated by Teichmann in 1853 [1], producing crystals of haematin, and by Takayama in 1912 [2], producing crystals of haemochromogen. However, these tests require that the blood be scraped from the surface, and therefore they can only be used where blood is easily observed, and cannot be used speculatively. Having to scrape blood also gives no regard to other forms of physical evidence that may be present, such as fingermarks, footwear impressions or splash patterns.

1.5 Catalytic or presumptive tests that attempted to keep much of the physical evidence intact were produced by Van Deen in 1862 based on guaiacum [3], Schönbein in 1863 using hydrogen peroxide [4] and by Alder and Alder in 1904 using benzidine [5]. They also pioneered the use of leucomalachite green in 1904 [5]; their method being later modified by Medinger in 1933 [6] to make it more sensitive.

1.6 In 1901 Kastle and Shedd [7] developed another catalytic test using phenolphthalein, which Meyer in 1903 [8] modified to detect blood. Further investigation by Kastle and Amos in 1906 [9] proved the phenolphthalein to be reacting with haemoglobin present in blood. This test is known as the Kastle-Meyer Test.

1.7 Other presumptive tests for blood were developed for forensic use by Ruttan and Hardisty in 1912 using o-tolidine [10]; by Specht in 1937 using luminol (3-amino-phthalhydrazide); [11] and by Gershenfeld in 1939 using o-toluidine [12].

1.8 In 1911 Abderhalden and Schmidt [13] reported the development of fingermarks on the bottle label of triketohydrindene hydrate (ninhydrin). This discovery was not exploited for the detection of fingermarks or blood until 1954 when Oden

[14] produced his ninhydrin formulation based on acetone. The use of this method for the enhancement of fingermarks in blood revolutionised thinking in this area of forensic investigation. The emphasis was shifted away from presumptive tests for haem, which generally require expert opinion to interpret the test results correctly, to easier to use reagents, which produce intensely coloured products with other components of blood, usually protein or its breakdown products.

1.9 Use of the protein dye amido black (acid black 1) quickly became popular with forensic investigators. Its use by the Metropolitan Police Laboratory, in a solvent base of methanol and acetic acid, was discussed at a forensic science symposium in 1961 by Godsell [15]. This formulation, with a change away from the fixing of the mark by the use of heat to immersion in methanol in 1981 [16], along with a water-based formulation of the same dye [17] continued to be recommended for the enhancement of fingermarks in blood by the UK Home Office until 2004 [18] when a new formulation by Sears and Prizeman [19] was adopted.

1.10 Many other protein stains for the enhancement of both fingermarks and footwear impressions in blood have also been proposed; coomassie blue (acid blue 83) and Crowle's double stain (acid blue 83 and acid red 71) by Norkus and Noppinger in1986 [20], fuchsin acid (acid violet 19, Hungarian Red), patent blue V (acid blue 1) and tartrazine (acid yellow 23) by Barnett *et al.* in 1988 [21], benzoxanthene yellow and acid violet 17 by Sears *et al.* in 2001 [22] and acid yellow 7 by Sears *et al.* in 2005 [23].

1.11 Although the use of protein dyes became most popular for enhancing fingermarks in blood, research on presumptive enhancement methods continued and in 1976 Garner *et al.* [24] proposed the use of tetramethyl-benzidene (TMB) as a safer and more effective technique than benzidine. Suggestions for other presumptive tests continued; tetraamino-biphenyl (TAB, also known as diaminobenzidine, DAB) in 1989 by Hussain and Pounds [25], fluorescein in 1995 by Cheeseman and DiMeo [26] and leucocrystal violet (LCV) in 1996 by Bodziak [27].

1.12 In addition there have been many modifications made to ninhydrin formulations to increase its effectiveness and safety by Crown in 1969 [28] and Morris and Goode in 1974 [29]. Further changes were forced on the fingerprint community because of The Montreal Protocol on Substances That Deplete the Ozone Layer in 1987 and new formulations were proposed by Watling and Smith in 1993 [30] and Hewlett *et al*. in 1997 [31]. The use of transition metal toners to change the colour or make the reaction product between amines and ninhydrin fluoresce have also been proposed by Morris in 1978 [32], Everse and Menzel 1986 [33] and Stoilovic *et al.* in 1986 [34].

1.13 It was also suggested that the use of one of several ninhydrin analogues would improve sensitivity and many have been proposed; benzo[f]ninhydrin in 1982 by Almog *et al.* [35], 5-methoxyninhydrin by Almog and Hirshfield in 1988 [36], 1,8-diazafluoren-9-one (DFO) in 1990 by Grigg *et al.* [37] and 1,2 indandione by Ramotowski *et al.* in 1997 [38]. All of these techniques, although primarily intended to target with amino acids in latent fingermarks on porous surfaces, will react strongly with the proteins present in blood to form coloured and/or fluorescent products.

1.14 More recently in 1999 Hochmeister *et al.* [39] validated a one-step immunochromatographic test for using anti-human Hb antibodies to prove the presence of human blood. However, this method requires the removal of blood from the surface so it cannot be used to enhance the physical evidence in situ, although if this test could be carried out after the application of the more sensitive protein dyes this would then cover all issues. In 2008 Johnston *et al.* [40] compared several of these tests with luminol and concluded the latter was more sensitive.

1.15 It was observed from the earliest times that blood strongly absorbed light and a number of researchers in the mid- to late-19th century tried to use this as a way to identify that a stain was blood. Among them were Hoppe in 1862 [41], who investigated the spectral properties of the colouring matter in blood; Stokes in 1864 [42], who was able to recognise the difference between haemoglobin and oxy-haemoglobin; and Soret in 1883 [43], who characterised the absorption bands of haemoglobin in the violet and ultraviolet (UV) regions of the spectrum. In 1865 Sorby [44] studied the spectra of various haemoglobin derivatives and proposed these as a means of identification for blood stains.

1.16 In the late 1970s and early 1980s it was observed by those developing high-intensity light sources that one of their most useful properties was that shorter wavelengths of light in the UV and violet make surfaces fluoresce strongly and this can give extra detail if a fingermark is in a strongly light-absorbing material [45]. This is an especially valuable method for the enhancement of fingermarks in blood as the haem group absorbs light throughout much of the visible part of the spectrum [46,47].

1.17 All these developments meant that by the late 1990s there were so many reagents and formulations existing for the enhancement of blood-contaminated fingermarks and footwear impressions with little or no comparative data that they were causing immense confusion among practitioners. Also the emergence of DNA analysis heaped even more uncertainty over which techniques could or should be used for the enhancement of blood. Vital evidence was likely to be lost by the wrong choices. Therefore the UK Home Office set out to clarify the situation and began a programme of work to review and compare the most commonly used of these techniques [19, 22, 23]. Resulting from this colossal task there were a number of key findings that were incorporated in a comprehensive update to *The Manual of Fingerprint Development Techniques* in 2004 [18], which included the current formulations for acid black 1, acid yellow 7 and acid violet 17.

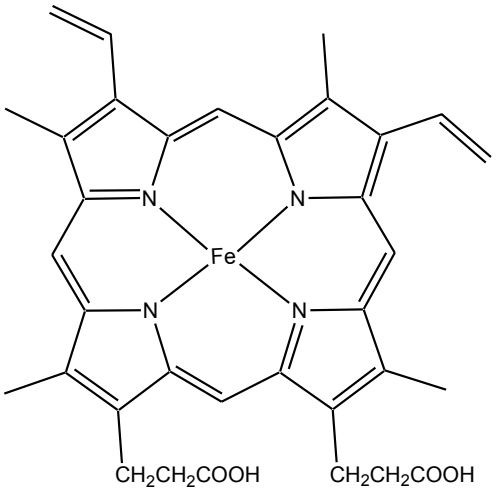
1.18 In addition to the protein stains and haem reagents now most commonly used for blood enhancement, a range of other techniques have been recently reported that may be of assistance in enhancement and interpretation of marks deposited in blood. Au *et al.* [48] report the use of a white powder suspension based on titanium dioxide to enhance marks, with the process revealing additional ridge detail when used sequentially after acid yellow 7. It was recognised that this process was not specific to blood but could enhance traces of it, and was therefore complimentary to the existing protein stains. Both Edelman *et al.* [49] and Li *et al.* [50] have revisited the optical reflectivity of blood, proposing hyperspectral imaging to detect and discriminate blood from coloured backgrounds by means of its characteristic reflectivity spectrum and

also to age it. Frascione *et al.* [51] proposed a more specific means of enhancing blood traces, utilising tagged nanoparticles as a powder to give highly specific binding to blood. Bradshaw *et al.* [52], have more recently shown the potential of mass spectrometry-based techniques to confirm the presence of human blood in fingermarks, even after use of other blood enhancement methods such as acid dyes.

2. Theory

2.1 Blood consists of red cells (erythrocytes), white cells (leukocytes) and platelets (thrombocytes) in a proteinaceous fluid called plasma, which makes up roughly 55% of the whole blood volume. The red cells principally contain the haemoglobin protein, but also have specific surface proteins (agglutinogens) that determine blood group. The white cells, which form part of the immune system, have a nucleus that contains DNA.

2.2 Haemoglobin makes up roughly 95% of red cells' protein content and is made of four protein sub-units each containing a haem group. The haem group is made of a flat porphyrin ring and a conjugated ferrous ion.



Chemical structure of haem.

2.3 As mentioned above, chemical blood enhancement methods fall broadly into two types; those that react with the haem grouping and those that interact with proteins or their breakdown products. The last type are not at all specific for blood; however, because of the high proportion of protein and its products present in blood, and the fact that they do not rely on the effectiveness of cell lysation (as do the haem-specific type) the techniques that interact with proteinous material are the most sensitive available to the forensic investigator [23].

2.4 Many researchers measure the sensitivity of their techniques by diluting blood with water [23,26,53,54,55]. This method favours techniques that utilise the haem as all the red cells would be lysed because of osmotic pressure during dilution, something that will not happen when these techniques are used operationally. Dilution with a buffer at the same osmotic pressure as blood serum would give a clearer indication of ultimate technique sensitivity.

2.5 There is also one other major advantage of the protein staining techniques, in that they generally incorporate a stage that either denatures or fixes proteins to the surface; as most proteins, including haemoglobin, are water soluble, the blood-contaminated fingermark is not then diffused during treatment.

2.6 There are two types of techniques that can be used to target proteins in blood; those that react with amines (e.g. ninhydrin), and those that stain proteinaceous material (e.g. acid dyes). It is this class of protein dyes that constitute the processes recommended by the Centre for Applied Science and Technology (CAST), i.e. acid black 1, acid violet 17 and acid yellow 7.

2.7 As stated above, the protein dyes used by CAST for the enhancement of fingermarks in blood are a group known as acid dyes. They are often characterised by the presence of one or more sulphonate ($-SO_3$) groups, usually the sodium (Na^+) salt. These groups function in two ways; firstly to provide solubility in water or alcohol, the favoured major solvents from which to apply these dyes, and secondly by virtue of their negative charge (anionic). If acidic conditions are used (acetic acid being the favoured option), the blood protein molecules acquire a positive charge (cationic) and this attracts the acid dye anions. Hydrogen bonding and other physical forces such as Van der Waals bonds may also play a part in the affinity of acid dyes to protein molecules [56].

2.8 Protein stains are applied via a three-stage process.

- Firstly the marks are fixed using a solution of 5-sulphosalicylic acid in water; this precipitates the basic proteins and thus prevents diffusion of the marks and any associated loss of detail. This fixing stage gives the protein dyes another advantage over the presumptive tests for fingermark development because as well as being more sensitive, it is often found that the fingermark ridges are more sharply defined and the detail is clearer.

- The marks are then treated with an acidic protein stain that dyes the precipitated basic proteins in the manner described above to give a coloured product.

- A washing stage is required post-staining. On non-porous surfaces this just removes excess dye, however on porous surfaces this also acts as a de-stainer, removing dye that has been absorbed by the background surface. The wash solution has to be carefully constructed so that it dissolves the dye, does not either diffuse or wash away the dyed fingermark and retains the intensity of colour of the dye in the fingermark. For this reason the same solvent mix as that used for the dyeing process, or some small variation of it, is generally most effective in this application [11].

2.9 Fluorescence examination can also assist in the subsequent visualisation of marks developed using the acid dyes. The use of acid black 1 or acid violet 17 can further intensify the contrast between the fingermark and the background by increasing the light absorption properties of the blood, and this may aid visualisation of developed marks during fluorescence examination.

2.10 Acid yellow 7 stains blood with a fluorescent species that can be excited by blue (420 to 485 nm) light. The resultant fluorescence from the stained mark can be less pronounced on heavy deposits of blood as the haem group retains its ability to absorb both the wavelengths of the excitation light and those that are emitted as fluorescence.

2.11 It has also been observed that acid violet 17 has weak fluorescence in the deep red and near infra-red (IR) regions of the spectrum when excited with green/yellow and yellow wavelengths, and this fluorescence could also be utilised to view developed marks.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. CAST recommends the use of a number of fingermark development and blood enhancement processes for use on fingermarks in blood, the ultimate process selection being dependent on the characteristics of the surface the blood is present on [18]. Three acid dyes (acid black 1 [naphthlene black, naphthol blue black, CI 20470], acid violet 17 [Coomassie brilliant violet R150, CI 42650] and acid yellow 7 [brilliant sulphoflavine, CI 56205]) are recommended only for use on blood. DFO and ninhydrin will also develop marks in blood, but are also the most sensitive techniques for the development of latent fingermarks on porous surfaces [19,22,23].

3.2 A holistic approach has been adopted for the acid dyes: the formulations for fixing, staining and de-staining have been very carefully constructed so that the blood is fixed effectively, then it is kept from diffusing during the staining and de-staining stages, and finally the strong coloration from the dye is retained during de-staining [19].

3.3 The most effective formulation for the three recommended acid dyes is as follows [23]:

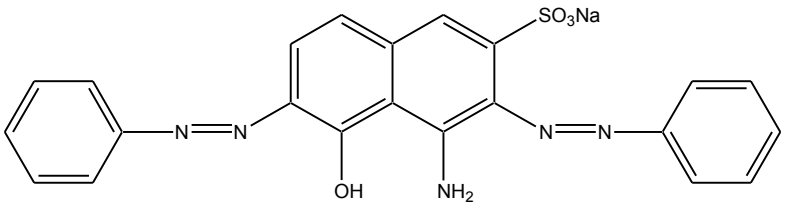
fixing solution – 23 g 5-sulphosalicylic acid dihydrate dissolved in 1 L water;

staining solution – 1 g acid dye (acid black 1, acid violet 17 or acid yellow 7) dissolved in 700 mL distilled water, 250 mL ethanol and 50 mL acetic acid;

washing solution – 700 mL water, 250 mL ethanol and 50 mL acetic acid.

3.4 If acid dye formulations are applied directly to fingermarks in blood without a fixing stage, the blood will dissolve and the ridges will either diffuse or be completely washed away. A number of different fixing agents have been investigated, but the most effective are 5-sulphosalicylic acid and methanol. Which fixing agent is used will depend upon the major solvent used in the dyeing process; in the current (post-2004) formulations where water is the main solvent, a solution of 5-sulphosalicylic acid is most effective. However, in the previously recommended formulations where the main dyeing solvent was methanol, methanol was found to be the best fixing agent [19]. These fixing agents act in different ways; 5-sulphosalicylic acid precipitates basic proteins and methanol dehydrates the blood. All-in-one formulations that stain and fix are generally not stable for more than a day or two and are not as effective as a two-stage process, both in fixing and dyeing.

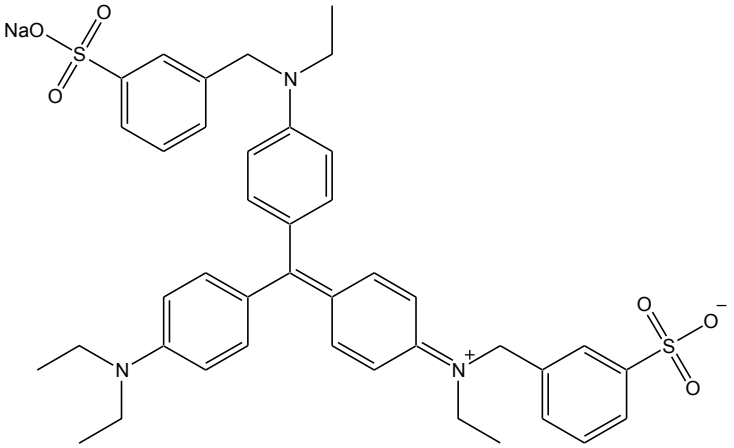
3.5 Acid black 1 (also commonly known as amido black) is a protein stain that dyes the proteins present in blood to give a blue/black colour. It can be absorbed by some porous surfaces so an area away from the mark to be enhanced needs to be tested first to ensure that there is no background staining.



Structure of acid black 1.

Fingermarks in blood on paper enhanced using acid black 1.

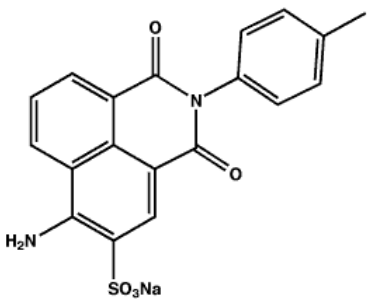
3.6 Acid violet 17 is a protein dye that stains the proteins present in blood to give a bright violet product. It can also be absorbed by some porous surfaces, therefore an area of the substrate away from the target enhancement area should be tested to assess background staining.



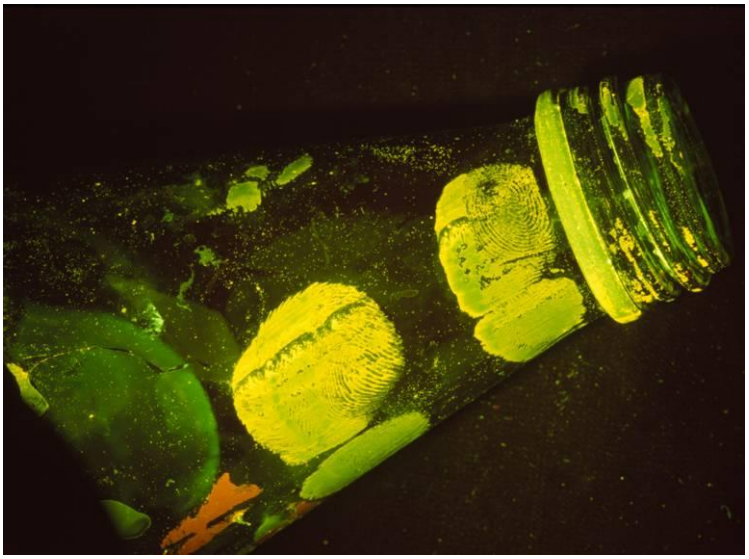
Structure of acid violet 17.

Fingermarks in blood on a wooden handle enhanced using acid violet 17.

3.7 Acid yellow 7 stains the proteins present in blood to give a pale yellow product that fluoresces bright yellow when viewed under broadband blue 385 to 509 nm illumination. The haem group acts as an energy sink that improves the enhancement of lighter marks. Acid yellow 7 is recommended in the *Fingermark Visualisation Manual* [57] for use on dark non-porous surfaces only because in general it cannot easily be removed from the background of porous surfaces, although reasonable results have been obtained for footwear marks on cotton fabrics [58].



Structure of acid yellow 7.



Fingermarks in blood on a dark glass bottle enhanced using acid yellow 7.

3.8 It was found that concentrations of these dyes of less than 0.1w/v resulted in less effective staining [19] and therefore the dye concentration used in the formulation above is selected to minimise dye content yet retain staining effectiveness.

3.9 The presence of a short chain alcohol in the dyeing solution helps to prevent the blood from diffusing during the dyeing stage [19]. Ethanol is preferred as this offers lower toxicity and flammability than methanol. Acetic acid provides the acidic conditions required for the blood protein molecules to acquire a positive charge, which consequently attracts the acid dye anions. The use of water as the major solvent gives the solution a flash point of around 30°C enabling this formulation, containing water, ethanol and acetic acid, to be used at scenes of crime with a few simple precautions [18].

4. Critical issues

4.1 The entire scope of blood evidence (blood pattern analysis, footwear enhancement, DNA recovery) should be taken into account before deciding on a treatment for fingermark evidence alone. In some cases the correct sequence of application will be essential in order to maximise evidential opportunities and the use of protein stains may affect other forms of evidence.

4.2 The protein stains should not be used as the sole means of determining whether a mark is in blood, because they give positive reactions with a number of other protein-containing substances (e.g. egg white). Other presumptive tests should be used to confirm the presence of blood (preferably using an area that does not contain ridge detail) before proceeding to enhancement with protein stains.

4.3 The fixing stage is essential for the process to be effective. If a fixative is not used, the blood marks will diffuse as the dye solution is applied to them, possibly destroying the ridge detail.

4.4 The current (post-2004) solutions are flammable, with a flash point of 30°C. The solutions should not be used in situations where the flash point is likely to be exceeded or where sources of ignition are present.

5. Application

5.1 Suitable surfaces: The three protein dyes recommended are suitable for use on all non-porous surfaces where blood contamination is suspected to be present. Acid black 1 and acid violet 17 are also suitable for use on porous surfaces contaminated with blood, whereas acid yellow 7 is not recommended for porous surfaces because it is more difficult to wash the dye out of the background, making fingermarks more difficult to see.

5.2 Currently (2016) it is considered that combinations of fluorescence examination, two amino acid reagents and three acid dyes are the most effective means of enhancing fingermarks in blood [23]. The most appropriate and effective techniques to use, either individually or in a sequence, depend on the porosity of the surface to be treated. This applies to both latent fingermark development and enhancement of blood-contaminated fingermarks.

5.3 Fluorescence examination of the surface should always be carried out before any other technique to see if any marks are revealed as dark absorbing ridges against a fluorescing background. High-intensity light sources with outputs between 350 and 450 nm are most effective.

5.4 When the blood-contaminated or latent fingermarks are on porous surfaces the most effective sequence of techniques is DFO, ninhydrin, either acid black 1 or acid violet 17, after carrying out a spot test to see which is most suitable, and then finally physical developer [23].

5.5 When the blood-contaminated or latent fingermarks are on non-porous surfaces the most effective sequence of techniques is vacuum metal deposition (VMD), powders, acid yellow 7, acid violet 17 then finally either powder suspensions or solvent black 3 (Sudan Black). Superglue may be used instead of VMD or powders but this will inhibit the dyeing process for blood by sealing the surface and preventing the dye reaching the blood [23].

5.6 The three recommended acid dyes, acid black 1, acid violet 17 and acid yellow 7, should all be applied to blood that has been fixed for at least five minutes with a solution of 5-sulphosalicylic acid. Dyeing of fixed blood is most effective if immersed in the dyeing solution for at least three minutes for acid black 1 and acid violet 17 whereas acid yellow 7 requires at least 5 minutes. Areas heavily contaminated with blood need longer dyeing times. If it is not possible to immerse the bloodied fingermarks then the dyeing solution should be applied above the area of interest and allowed to flow down over it, keeping the area damp for the specified time. A well may be constructed around the area of

interest on horizontal surfaces, which may be flooded and drained as appropriate, or tissues soaked in dye may be applied to the surface [59]. Ethanol-containing staining or de-staining solutions should never be sprayed because this lowers the flash point by at least 100°C making it impossible to work without creating a flammable atmosphere.

5.7 Areas of interest will then need to be washed or de-stained to remove excess dye. The most effective solution for doing this is the same solvent composition as the dye solution, washing as required to remove dye or de-stain the background.

5.8 High-intensity light sources capable of delivering output wavelengths between 420 and 485 nm must be used to excite fluorescence from blood dyed with acid yellow 7. The fluorescence emitted is between 480 and 550 nm. The use of shorter wavelengths between 350 and 450 nm to excite background fluorescence after acid black 1 or acid violet 17 treatment may be beneficial.

5.9 Work carried out by CAST has demonstrated that positive DNA identification may be made after fluorescence examination and any single chemical treatment provided simple guidelines are followed. If more than one fingermark development technique is used in sequence then the chances of successfully carrying out DNA identification are much reduced [18].

6. Alternative formulations and processes

6.1 There are a great number of blood reagents, only some of which have been mentioned above, and there can be many different formulations for each of those reagents to consider. Some of these will be described in more detail in Chapter 3, Chemical and Physical Processes, Alternative Blood Reagents. The water-based formulation of the acid dyes are probably the most practical alternative formulations because they can be used at all times, although methanol-based solutions might prove beneficial under some specialised circumstances.

	Water-based method	**Methanol-based method**
Fixing solution	20 g 5-sulphosalicylic acid 1 L distilled water	Methanol (99%+)
Staining solution	2 g acid dye 20 g citric acid or 5% v/v acetic acid 1 L distilled water	2 g acid dye 900 mL methanol 100 mL acetic acid
De-staining solution 1	Distilled water (5% v/v acetic acid helps to retain	900 mL methanol

	coloration)	100 mL acetic acid
De-staining solution 2	Distilled water (5% v/v acetic acid helps to retain coloration)	950 mL distilled water 50 mL acetic acid

Methanol-based and water-based acid dye and de-staining formulations.

6.2 Originally these formulations were developed for use with acid black 1, but both can be used equally well with acid violet 17 and acid yellow 7.

6.3 Advantages of methanol-based and water-based acid dye formulations

6.3.1 The water-based formula does not use flammable or toxic solvents and can therefore be used safely regardless of the temperature at the scene of a crime. It can also be used in a laboratory if extraction is not available. It is an easy process to use and cheap to carry out.

6.3.2 The methanol-based formula is very effective, cheap and an easy method to use for enhancing fingermarks in blood. It gives good ridge definition, little background staining and produces highly stained fingermarks. It is also the best formulation to use as a corrective action if it becomes necessary to re-process an item already treated with superglue fuming for marks in blood, the methanol providing better penetration of the polycyanoacrylate deposits.

6.4 Disadvantages of methanol-based and water-based acid dye formulations

6.4.1 The water-based formula does not always produce optimum results as it may give diffuse fingermark ridges and weaker coloration with less contrast, especially on porous surfaces. More coloration may be retained by the inclusion of 5% v/v acetic acid in the de-staining solutions. Also on porous surfaces, the contrast between the fingermark and the background can sometimes be poorer than that achieved when using the methanol-based formulation because of relatively high background staining and the lower colour intensity of the developed ridges.

6.4.2 The methanol-based solutions are toxic by ingestion and skin absorption. Methanol is also a highly flammable solvent. Although this formulation can be used safely in a laboratory, its use at scenes of crime is not recommended due to potential ignition or the possibility of absorption of methanol through the skin. Leaching of blood from heavy deposits also occurs with this formulation unless long fixing times (> 10 minutes) are used. The methanol-based formulation may also soften or destroy some surfaces including paints, varnishes and some plastics, damaging or obliterating ridge detail.

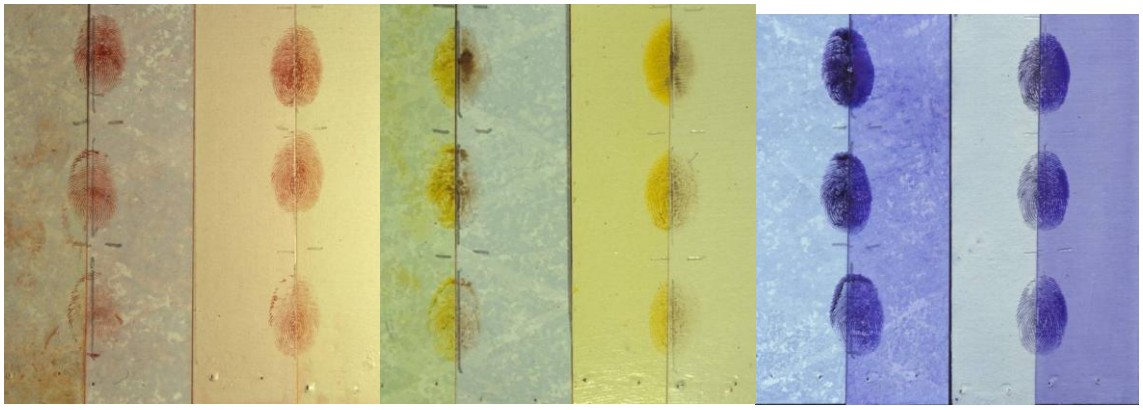
6.5 Rejected dyes and techniques

6.5.1 The CAST blood enhancement project investigated many dyes and reactive techniques that proved less effective, and considered many others that were not ultimately studied because of health and safety concerns. The dyes and techniques that were investigated in practical experiments are listed below in categories.

6.5.2 Protein dyes [22] : Acid blue 74 (indigo carmine), acid blue 83 (Coomassie brilliant blue R250), acid blue 90 (Coomassie brilliant blue G250), acid blue 92 (Coomassie blue R), acid blue 147 (xylene cyanol FF), cid red 1 (amido naphthol red G), acid red 71 (Crocein scarlet 7B), acid red 87 (eosin y), acid red 88 (roccellin), acid red 112 (Ponceau S), acid violet 19 (fuchsin acid, Hungarian Red), acid yellow 23 (tartrazine), benzoxanthene yellow (Hœchst 2495), brilliant sulphaflavine, Crowles double-stain *(acid blue 83 and acid red 71)*, direct yellow 12 (chrysophenine), MBD (7-[p-methoxybenylamino] -4-nitro-2,1,3-benzoxadiazole).

6.5.3 Haem-specific reactive techniques [23]: Azino-di-benzthiazoline sulphonic acid (ABTS); diaminobenzidine (DAB) or tetraamino-biphenyl (TAB); guaiacol; leucocrystal violet (LCV); leucomalachite green (LMG); luminol; organic acid (formic or acetic) and hydrogen peroxide (hæmatoporphyrin); fluorescein.

6.5.4 Amine and protein reactive techniques [23]: ATTO-TAG™ CBQCA; ATTO-TAG™ FQ; fluorescamine; Lucifer Yellow vinyl sulphone (VS) ; SYPRO® Ruby Protein Blot Stain.



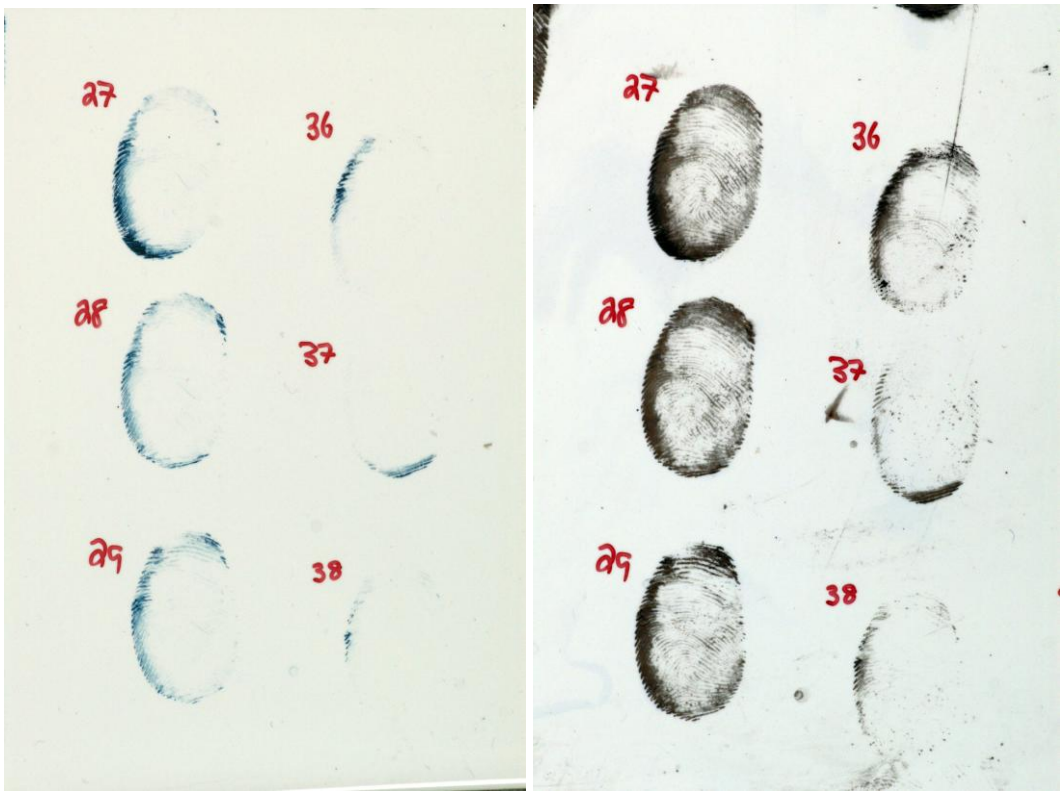
Examples of split depletion experiments carried out on wallpaper and painted wall surfaces using a range of alternative protein stains.

7. Post-treatments

7.1 Fluorescence examination is the most notable post-treatment process and this has been discussed fully above in sections 2.9 and 2.10.

7.2 However, it appears from more recent studies on footwear marks that powder suspensions may have an affinity for blood and can be used as an enhancement technique after the protein dyes [59,60]. It should be noted that

the current (post-2009) application methods will cause potentially disastrous over-development on heavy blood deposits, but on faint fingermarks on non-porous surfaces there may be significant enhancement. Powder suspensions are not specific for blood and cannot be used to determine that any additional ridge detail is in blood.



Finger marks in blood in a depletion series on a ceramic tile showing deposited marks numbers 27,28,29,36,37,38,39, a) *enhanced using acid black 1 and* b) *subsequently treated using iron oxide-based powder suspension.*

8. Validation and operational experience

8.1 The validation of blood dyes is carried out both in terms of the number of graded marks, and also in terms of sensitivity to diluted blood. The first test will give an indication of how far down a depletion series the blood reagent will work (i.e. how many multiple contacts from a single finger contaminated with blood at normal concentration can be detected) and the second will indicate how sensitive the technique is to dilute traces of blood (as may be experienced where efforts have been made to clean a crime scene). Because blood is being targeted as a contaminant, the results obtained for fingermarks will be applicable to development of other types of blood evidence, such as footwear marks. There will be some exceptions to this, e.g. luminol is recommended as a footwear development process for carpets, a surface for which there is no recommended fingermark development process, but would not be recommended as a primary technique for development of fingermarks because the requirement for spray application without fixing may diffuse fingermark ridges and destroy evidence.

8.2 Laboratory trials

8.2.1 During the late 1990s and early 2000s, CAST conducted a series of experiments to optimise the acid black 1 formulation and to identify alternative blood enhancement agents with potentially improved performance [19,22,23]. Experiments to assess the effectiveness of protein dyes were carried out by using series of 6 split depleted blood-contaminated fingermarks on 9 or 15 surface types, depending on whether or not the technique was appropriate for both porous and non-porous surfaces. However, it became obvious that this experiment was not sufficient to resolve the differences in sensitivity of some fluorescent dyes on non-porous surfaces, so the number of depletions was increased to 18.

8.2.2 Additionally, in the literature it is common to compare the sensitivity of blood enhancement techniques by diluting blood with distilled water. Accordingly it was decided to assess techniques in this manner so a series of 12 dilutions from 1/100 to 1/100,000 were used along with a distilled water control. These tests were carried out on photocopy paper and glass using 5 μL of solution for each spot.

8.2.3 Of the 17 protein stains investigated, 2 absorbing (acid violet 17 and acid violet 19) and 2 fluorescent (brilliant sulphaflavine and benzoxanthene yellow) dyes were identified for further study. Ultimately the original fluorescent dyes became unavailable and acid yellow 7 (brilliant sulphoflavine) was identified as a suitable substitute. Further comparisons showed that acid violet 19 was less effective than both acid black 1 and acid violet 17. The lighter coloration of marks stained with acid violet 19 produced ridge detail with less contrast with the background than the other two dyes.

8.2.4 On porous surfaces acid violet 17 proved to be more effective than both the water- and methanol-based formulations of acid black 1, and was very similar in performance to the newly developed water/ethanol/acetic acid (WEAA) formulation of acid black 1.

8.2.5 Experiments on a further 24 porous surfaces failed to show conclusively whether one of these dyes was more effective than the other. However, there were some surfaces where one dye performed better than the other. It proved impossible to define before treatment whether the acid violet 17 or the acid black 1 would give greatest contrast.

8.2.6 Some of the results of comparing and grading fingermarks developed using acid black 1, acid violet 17 and acid yellow 7 across eight different non-porous surfaces are shown below.

Grade	24 Hours after deposition		2 Weeks after deposition	
	Acid black 1	Acid violet 17	Acid black 1	Acid violet 17
0	0	0	0	0
1	0	0	0	0
2	42	46	46	49
3	35	26	31	20
4	167	160	166	174

Grade	24 Hours after deposition		2 Weeks after deposition	
	Acid black 1	**Acid yellow 7**	**Acid black 1**	**Acid yellow 7**
0	0	0	0	0
1	0	0	0	0
2	50	46	51	70
3	39	40	33	38
4	185	188	191	167

Examples of comparative grading exercises carried out between acid black 1, acid violet 17 and acid yellow 7 on non-porous surfaces including glass, ceramic tile, polymers and metals.

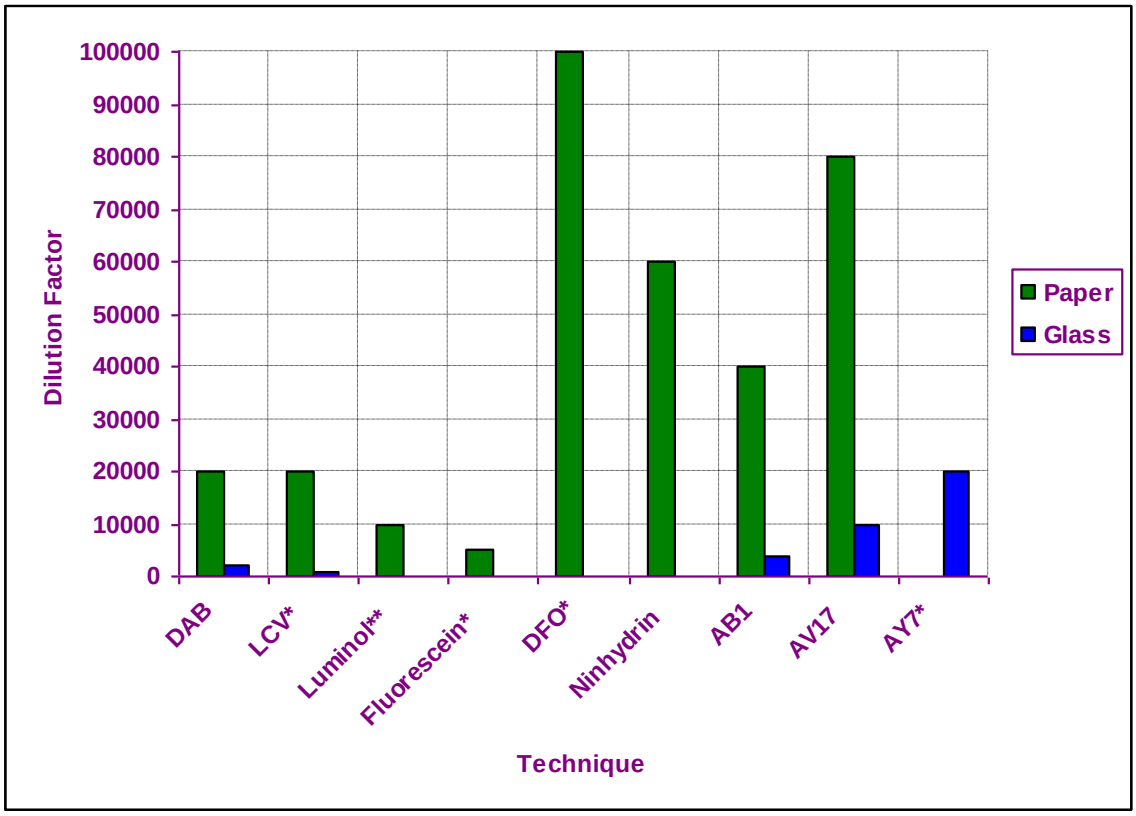
8.2.7 It can be seen that the performance of each of the recommended acid dyes is closely equivalent and the three dyes can be used interchangeably according to which dye will give the best contrast on the particular surface.

8.2.8 The comparative performance of the recommended protein dyes with other types of blood enhancement techniques and with alternative formulations of the same dyes on split depleted fingermarks are shown in the table below.

Technique	**Type (H = haem, A = amine, P = protein)**	**Subjective performance assessment ***** = excellent, * = poor**		
		Porous	**Semi-porous**	**Non-porous**
DAB	H	**	**	*
LCV	H	**	**	-
Acid Violet 19 + organic acid/peroxide	H	**	**	*
Fluorescein	H	*	*	-
DFO	A	*****	**	-
Ninhydrin	A	****	**	-
SYPRO ruby protein blot stain	A	***	**	**
Acid Black 1 (methanol)	P	***	***	****
Acid Black 1 (water)	P	***	***	**
Acid Black 1 (water/ethanol/acetic acid)	P	****	***	***
Acid Violet 17 (water/ethanol/acetic acid)	P	****	***	***
Acid Yellow 7 (water/ethanol/acetic acid)	P	-	-	*****

Summary table showing subjective overview of the comparative effectiveness of several regularly used blood enhancement agents.

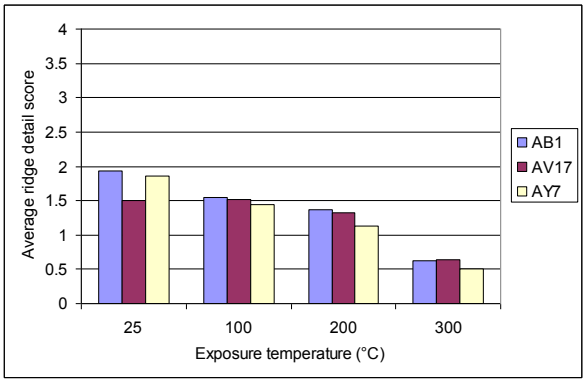
8.2.9 The graph below illustrates the sensitivity of each dye at developing the diluted blood spots on photocopy paper and glass. The sensitivity achieved with diluted blood is not always consistent with the results of experiments with depleted fingermarks, so it is believed that comparative dye performance cannot be measured using dilution series alone. The results below do not take into account the contrast between the stained spots and the background. If spots could be seen then they were counted, even if the contrast between them and the background was very poor.

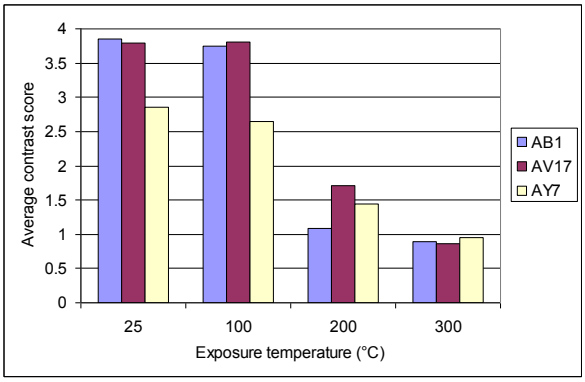


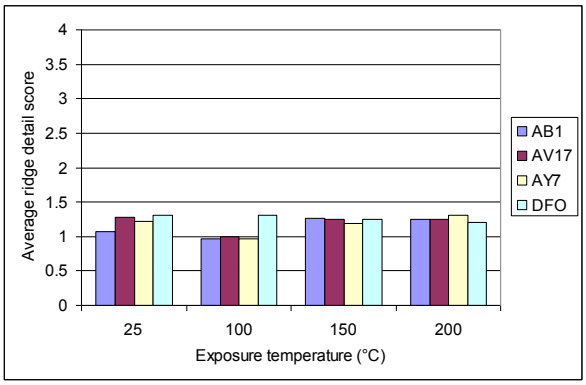
Graph to show the relative performance of various blood enhancement agents in the spot dilution sensitivity test. = visualised by fluorescence, ** = visualised by chemiluminescence.*

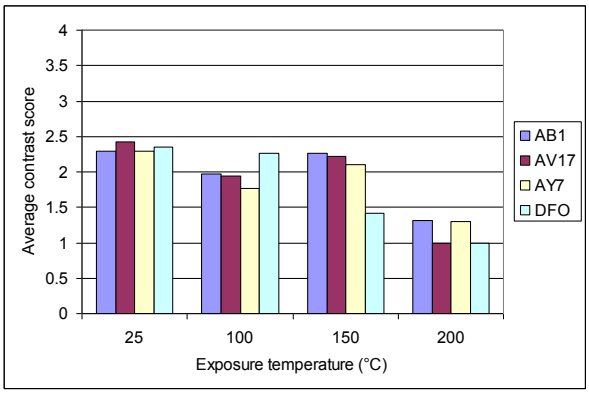
8.2.10 It should be noted that the graph above shows the sensitivity of luminol to be relatively poor. This may be because the viewing conditions used were not optimised. Subsequent research to investigate enhancement of footwear marks in blood has shown that dark adaption and optimised viewing conditions are essential and that the sensitivity of luminol may be far greater than is represented here, for example good results for fluorescein and luminol were observed for footwear marks in blood on dark fabrics [61].

8.2.11 Other laboratory trials that have been carried out using the acid dyes include an assessment of the technique's effectiveness on marks in blood that had been exposed to elevated temperatures [62]. In these studies marks were deposited on a range of surfaces and exposed to temperatures in the range 100 to 300°C for periods between one and eight hours. Marks were graded in terms of both quality and contrast, because it was observed that the contrast of the developed mark decreased as exposure time and temperature decreased.

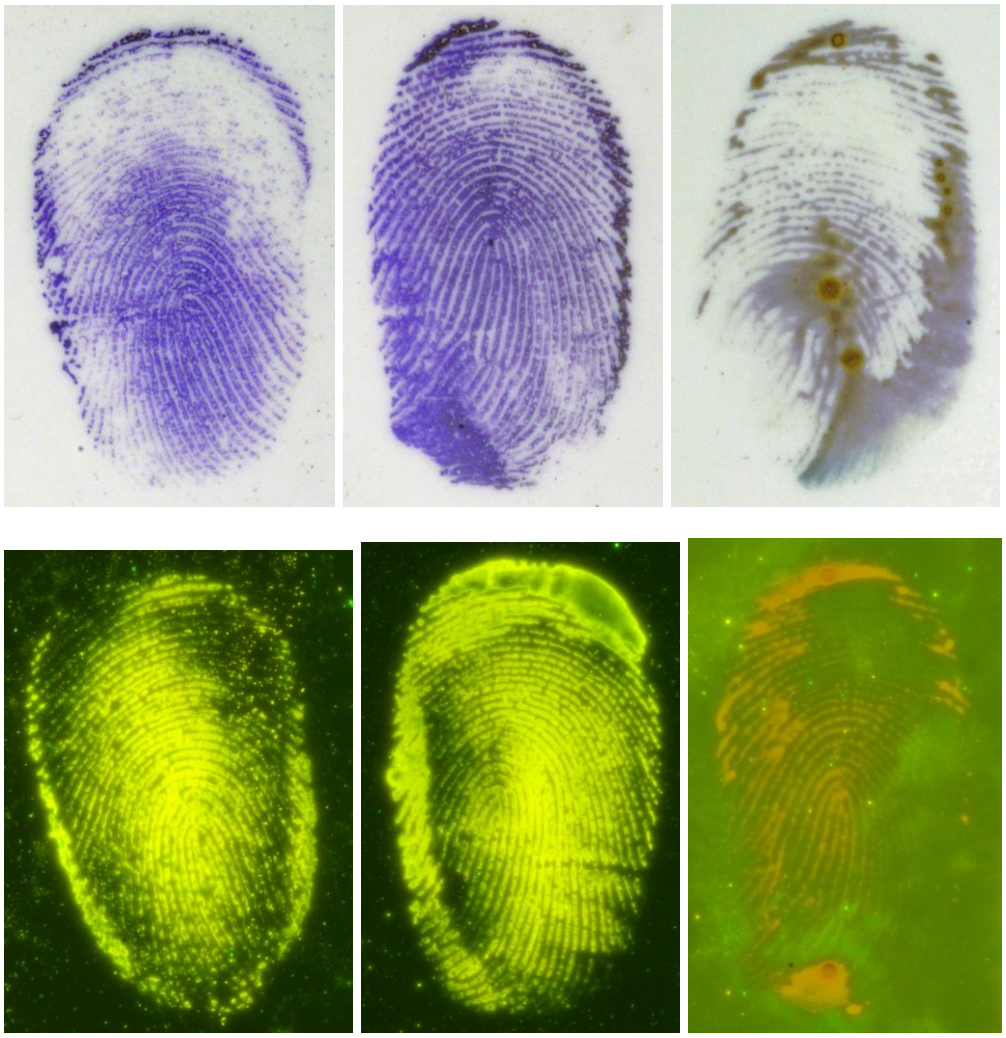








Recorded results of fingermark quality and contrast for marks enhanced using blood dyes on a ceramic tile (top row) *and white card* (bottom row)*, after exposure to different temperatures.*



a) b) c)

Series of images for acid violet 17 (top row) *and acid yellow 7* (bottom row) *showing how quality of developed mark and contrast degrade with increased exposure temperature and exposure time,* a) *control,* b) *8 hours at 100°C and* c) *8 hours at 200°C.*

8.2.12 The results of this study demonstrated that the acid dyes were capable of developing marks exposed to 200°C for eight hours, albeit with reduced effectiveness. Once again, there was little significant difference between the performance of the three recommended dyes. A further important observation from the study was that the haem specific reagent leucocrystal violet had stopped enhancing marks after exposure to temperatures of 150°C, further supporting the recommendation of the acid dyes for operational use in scenarios such as arson scenes.

8.3 Pseudo-operational trials and operational experience

8.3.1 Pseudo-operational trials have not been conducted on the acid dye formulations because this is not practical with articles contaminated with blood. Because the contaminant is known, unlike 'real' fingermarks that are variable in composition, the performance in operational use will be the same as that in laboratory tests. Since the introduction of the new formulation of acid black 1 and the new dyes acid violet 17 and acid yellow 7 in 2005, feedback from operational work has been favourable. Feedback has been especially good for acid yellow 7, which has resulted in, for the first time, the capability of enhancing blood-contaminated fingermarks on dark non-fluorescing surfaces. The new dye has been successfully used to develop marks on exhibits including a black Maglite torch and a dark wood banister, surfaces for which no previous treatment would have been effective. The dyes have also been used for the successful enhancement of footwear marks on large areas of non-porous flooring.

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Basic violet 3 (Gentian Violet)

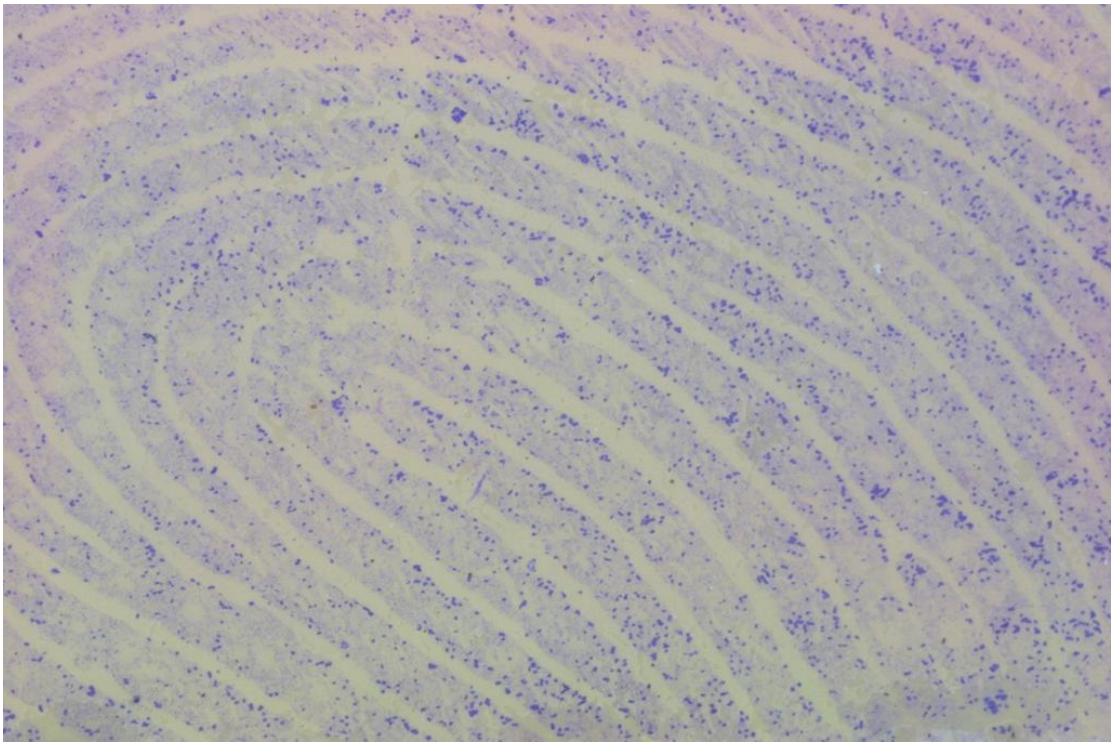
1. History

1.1 The history of basic violet 3 begins with the discovery of the first synthetic dye, 'Mauve', by W. H. Perkin in 1856. In the following years a series of aniline dyes were synthesised for the dyeing of textiles, including methyl violet (basic violet 1) by Lauth in 1861 [1]. A range of closely related compounds were subsequently synthesised including basic violet 3 (also known by several alternative names including gentian violet and crystal violet).

1.2 The applications of these dyes were not confined to the textile industry and microbiologists began to explore the potential of synthetic dyes for the staining of biological sections. The German biologist Paul Ehrlich used aniline water and gentian violet to stain bacteria cells, the gentian violet targeting the lipids in the cell walls to give a purple stain. In 1884 the Danish physician Hans Christian Joachim Gram further developed this staining process for selectively staining bacteria and providing information about the structure of the cell walls. The test is still known as Gram staining to this date. Basic violet 3 has since been used for a variety of medical applications, including treatments for ringworm and scabies, where the ability of the dye to inhibit bacterial action is beneficial.

1.3 Aniline dyes (of which basic violet 3 is one) have been proposed as fingermark reagents since the early part of the 20th century. In 1917 Bock [2] patented a process for recording latent fingermarks by brushing the fingermark with a powder of aniline dye and then fixing the mark by heating. In 1920 Mitchell was reporting the use of aniline dyestuffs in powder form as a means of detecting fingermarks [3], with the observation that basic dyestuffs were preferable.

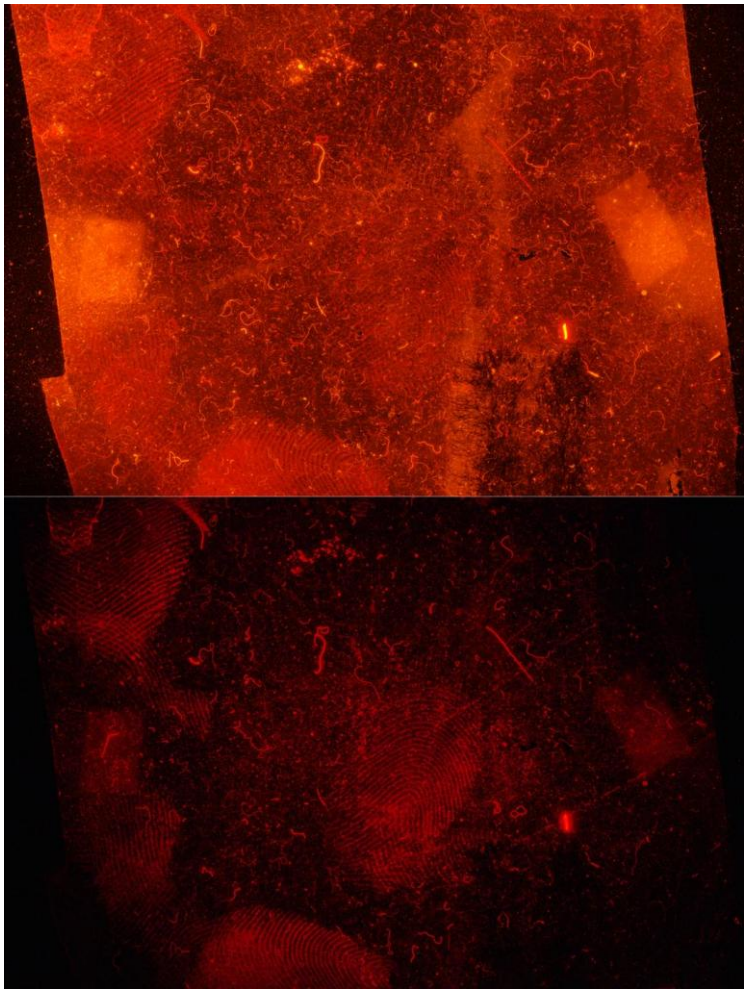
1.4 As research work into the constituents of fingermarks progressed in the 1960s, reagents were proposed that targeted particular components of fingermark residues. Basic violet 3 was proposed as a technique for the selective staining of epithelial cells and fatty components of fingermark residues. Epithelial cells are most likely to be present on the adhesive side of tapes, where a layer of dead cells may be pulled off the fingermark ridges when the tape is touched. The use of basic violet 3 in this application was reported by the Italian Police in the late 1960s and it their recommended phenolic formulation was adopted by PSDB and some forces in the UK during the late 1970s [4,5].



Photograph of adhesive side of tape sample treated with basic violet 3, showing violet staining of lipids in ridges and of epithelial cells in particular.

1.5 Basic violet 3 continued to be used worldwide for development of latent fingermarks on adhesive surfaces [6] but although good results were obtained for a wide range of tapes the detection of marks on black tapes remained problematic, the only technique available for visualisation being photography under oblique lighting. This was overcome by the development of the transfer process by the Police Scientific Development Branch (PSDB) [7,8] and others [9]. This process involved the sandwiching of the tape between sheets of photographic paper, resulting in the transfer of the purple stain from the developed fingermark to the surface of the white paper.

1.6 It has been found that marks developed using basic violet 3 on adhesive tapes are also fluorescent, and can be visualised using green/yellow light to excite the fluorescence and a deep red viewing filter [10]. It was found that the fluorescence had a peak at 720 nm in the deep red region of the spectrum and extended to a small degree into the near infra-red region [11]. More recently, yellow (577 nm) lasers have become commercially available and studies have shown that this gives excellent results when used to image fluorescent marks developed using basic violet 3 [12].

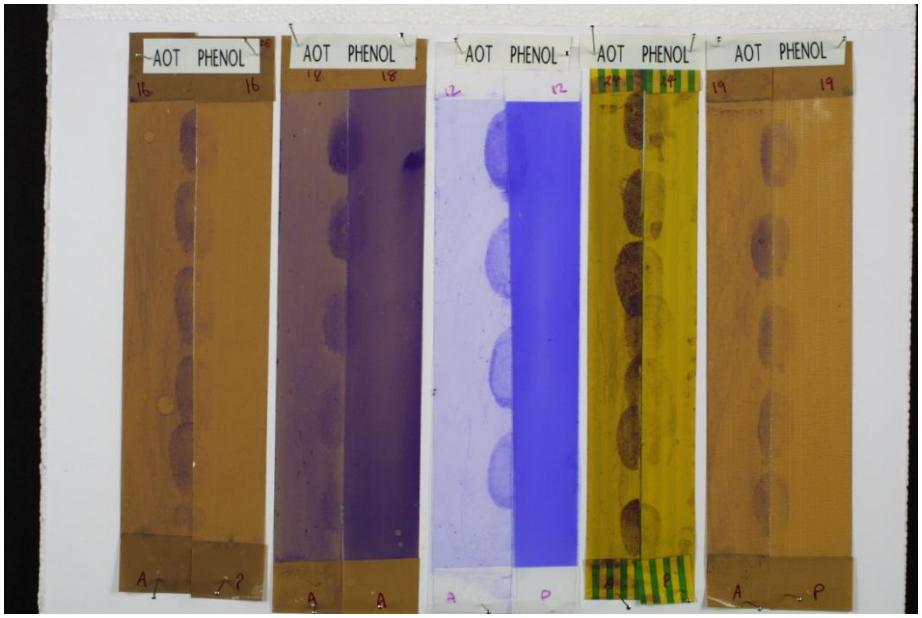


Photograph of basic violet 3 fluorescence in fingermarks developed on adhesive tape, imaged using a 5W 532nm green laser (top) *and a 5W 577nm yellow laser* (bottom).

1.7 Basic violet 3 can also be used for detection of fingermarks on a wide range of non-porous surfaces and can be especially useful where contamination may be present on the surface.

1.8 The work carried out by CAST on basic violet 3 includes the development of the transfer process for black tapes in the late 1970s. More recently, concerns about the toxicity of phenol have prompted in-depth studies into the development of an effective phenol-free formulation for basic violet 3 [13] and a comparative study between basic violet 3 and a possible alternative dye, basic violet 2 [14]. These studies have culminated in the recent issue of a revised formulation of basic violet 3 based on Aerosol OT™, also known by its chemical name of dioctyl-sulfosuccinate, sodium salt (DOSS)[15], which in laboratory trials has consistently out-performed the phenol formulation in terms of number, quality and contrast of marks developed, and has exhibited a reduced amount of background staining. However, recent reclassification of chemicals has resulted in basic violet 3 itself being classed as a

suspect carcinogen and both phenol and DOSS-based formulations must be used under controlled conditions.

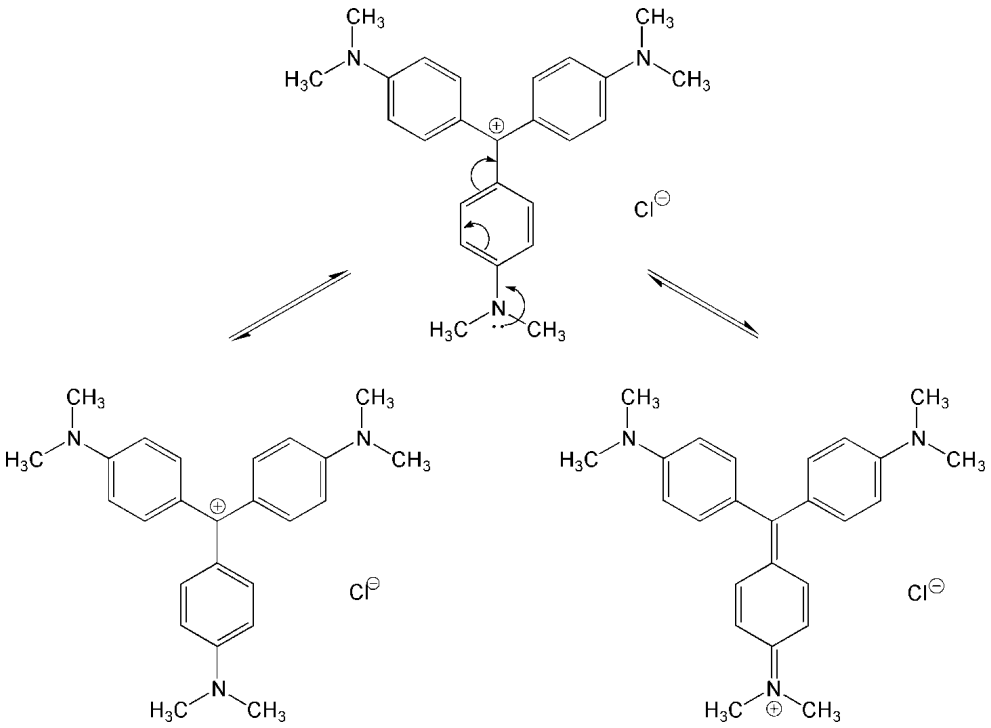


Photograph of different adhesive tapes, showing difference in fingermark development between phenol and DOSS-based basic violet 3 formulations.

2. Theory

2.1 The exact mechanism by which basic violet 3 selectively dyes fingermark deposits is not known, nor has it been determined which individual fingermark constituents are targeted by the dye. However, it is thought that the principal interactions are with water insoluble lipids.

2.2 Resonance structures for the basic violet 3 molecule are shown below:



Resonance structures for basic violet 3.

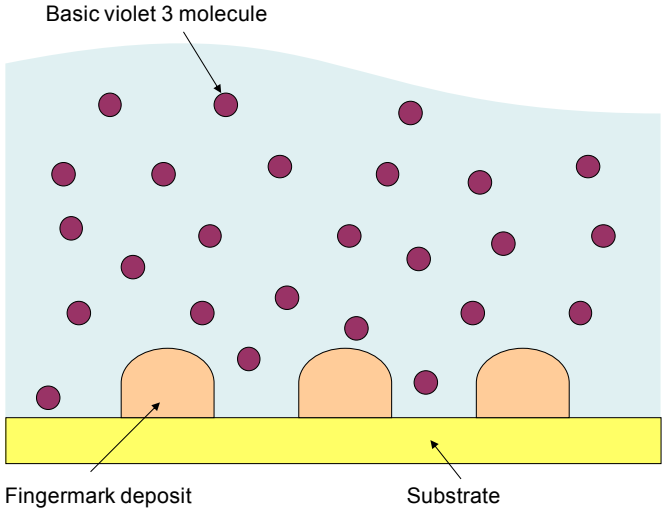
2.3 Gurr [16] proposed that the basic groups such as amines of neutral dyes could form a chemical union with the acidic group of the lipids being stained, but whether this is true for basic 3 has not been established. Phenol is also thought to play an important role in the staining of the lipid constituents, which makes the actual staining mechanism complex and difficult to determine.

2.4 Basic violet 3 does not only stain the lipids present in fingermarks; it is also very effective in staining any epithelial cells that may present, especially those pulled from the fingertip through contact with adhesive surfaces. In this case the staining mechanism is well established, the positively charged basic violet 3 molecule is attracted by ionic forces to the negatively charged surface of the skin cells and covalent bonds may be formed between the dye molecule and certain proteins present.

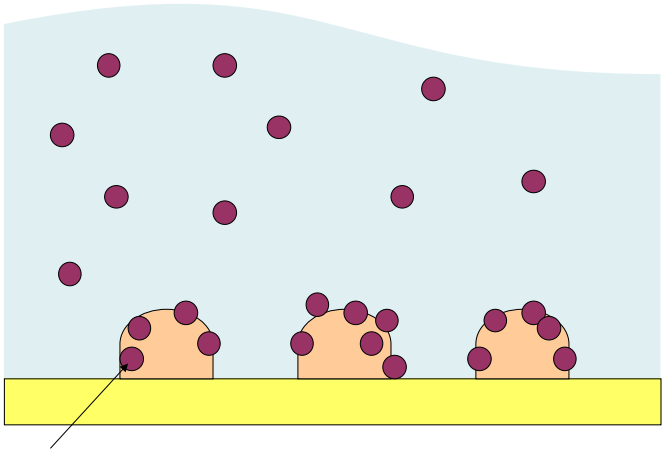
2.5 It is also known that the basic violet 3 molecule is fluorescent, but when basic violet 3 is used as a development reagent on non-porous surfaces fluorescence is not observed in most cases. However, on adhesive tapes fluorescence is observed, and weak marks that are not visible under conventional lighting may be revealed by these means. The fluorescence observed on adhesive surfaces is attributed to the fact that for fluorescence to occur the structure of the compound must be rigid [18]. It is thought that the adhesive promotes fluorescence by binding with the dye molecule and making it more rigid. This theory has been investigated by spraying non-fluorescent marks developed using basic violet 3 with spray adhesive. In these studies a significant increase in fluorescence

was observed [13]. It is thought that additional marks are revealed by fluorescence examination because the more strongly developed, visible marks 'self quench', i.e. the dye absorbs the fluorescence from the fingermark, whereas for the weakly coloured marks that are not visible by eye the fluorescence is not re-absorbed and the marks are detected.

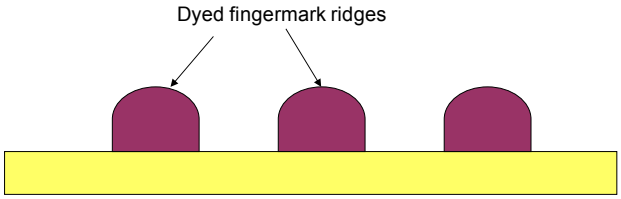
2.6 The fingermark development process using basic violet 3 is shown schematically in the series of figures below.



a)



b)



c)

Schematic illustration of the basic violet 3 development process a) *basic violet 3 molecules in solution,* b) *basic violet 3 molecules preferentially binding to skin cells and lipids in fingermark ridges, and* c) *dried mark leaving dyed fingermark ridges.*

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. There are two formulations recommended for use by CAST, one based on phenol and the other based on DOSS.

3.2 The phenol formulation is produced by first mixing a stock solution comprising 5 g basic violet 3 and 10 g of phenol dissolved into 50 mL of 96% ethanol.

3.3 A working solution is produced by measuring 1 mL of stock solution and progressively adding distilled water until the gold film formed on the surface of the solution disappears.

3.4 The role of basic violet 3 in the formulation is to selectively stain the fingermark deposits. The quantity used is sufficient to produce a supersaturated solution of basic violet 3, thus promoting the transfer of the dye into the lipids in the fingermark.

3.5 The role of phenol in the formulation is not fully understood. The presence of phenol has been found to promote the staining ability of basic violet 3 and appears to make it more specific to fingermark constituents. Several theories have been proposed [13], including:

- the pH change due to the addition of the mildly acidic phenol aids staining;
- phenol aids the wetting of the lipids;
- phenol increases the solubility of the dye, forming a supersaturated solution;
- phenol replaces the dye anion forming a phenolate, which acts as a dye carrier and aids penetration of the fats;
- phenol disaggregates dye molecules, increasing their diffusion rates.

3.6 Experiments have been carried out to investigate some of these theories and while these did not provide conclusive evidence it is thought more likely that phenol acts by affecting the solution properties, either making it supersaturated or by changing its surface tension and increasing staining.

3.7 The ethanol component of the formulation provides a common solvent for both phenol and basic violet 3.

3.8 The DOSS formulation of basic violet 3 is produced by first producing a stock solution by dissolving 5 g of basic violet 3 in 50 mL of absolute ethanol. A separate 1% w/v DOSS solution is then produced by dissolving DOSS in distilled water, stirring for at least 12 hours to allow the DOSS to dissolve. The working solution is produced by placing 1 mL of concentrated stock solution into a clean, dry beaker, then adding 25 mL of DOSS solution.

3.9 Similarly to phenol, the role of DOSS in the formulation is not fully understood. DOSS is an unusual detergent, being preferentially soluble in non-polar solvents and forming reverse micelles. One theory is that basic violet 3 molecules could become contained within the reverse micelles, which are in turn preferentially soluble in the fingermark lipids compared with the polar water/ethanol solution [13].

4. Critical issues

4.1 Basic violet 3 is classified as being carcinogenic and phenol (a major constituent in one of the formulations) is mutagenic. Although the solution can be used safely in a laboratory environment if the procedures outlined in the *Fingermark Visualisation Manual* [17] are followed, it should not be used in the uncontrolled environment of a crime scene.

4.2 If a gold-coloured film forms on the surface of the basic violet 3 working solution it should be discarded because this may give a high background staining on the surface being treated.

4.3 In general strongly stained fingermarks either do not fluoresce or fluoresce only weakly, this is believed to be due to quenching effects. Fluorescence is therefore most valuable for detection of weakly stained fingermarks. However, this means that on dark tapes strongly dyed fingermarks may be missed unless a transfer technique is used.

5. Application

5.1 Suitable surfaces: Basic violet 3 is suitable for use on the adhesive side of adhesive tapes and on surfaces contaminated with fats. It is also suitable for use on all non-porous surfaces as the final process in a

sequential treatment. Its use should be restricted to small articles because of issues with carcinogenicity of the solution.

5.2 The principal application for basic violet 3 is in the development of fingermarks on the adhesive side of adhesive tapes, where it can be used as a single treatment or in sequence to develop additional marks after powder suspensions or superglue [19].

5.3 The DOSS-based basic violet 3 formulation can be used on tapes of any colour and also on tapes with both acrylic and rubber-based adhesive. However, if used as a single treatment, laboratory trials indicate that it is less effective than powder suspensions and superglue and it is more appropriate for use as part of a sequential processing regime.

5.4 Basic violet 3 is also recommended as a treatment for contaminated surfaces, where its specificity as a lipid dye may be capable of selectively dyeing the fingermark ridges without background staining of the contaminant. This is only recommended for small articles because of the toxicity issues associated with the phenol-based formulation. Solvent black 3 can be considered as an alternative treatment for contaminated surfaces and although laboratory trials indicate that solvent black 3 may be more effective than basic violet 3 on latent prints, the most effective treatment on contaminated surfaces has not been conclusively identified.

5.5 Basic violet 3, used in the form of the former Forensic Science Service (FSS) crystal violet formulation, see below, has also been proposed as a treatment for soot-covered articles retrieved from arson scenes, where the phenol in the formulation was believed to assist in lifting surface soot and developing the fingermark. More recent experiments indicate that other chemical treatments and soot removal techniques may be more effective in this application [20].

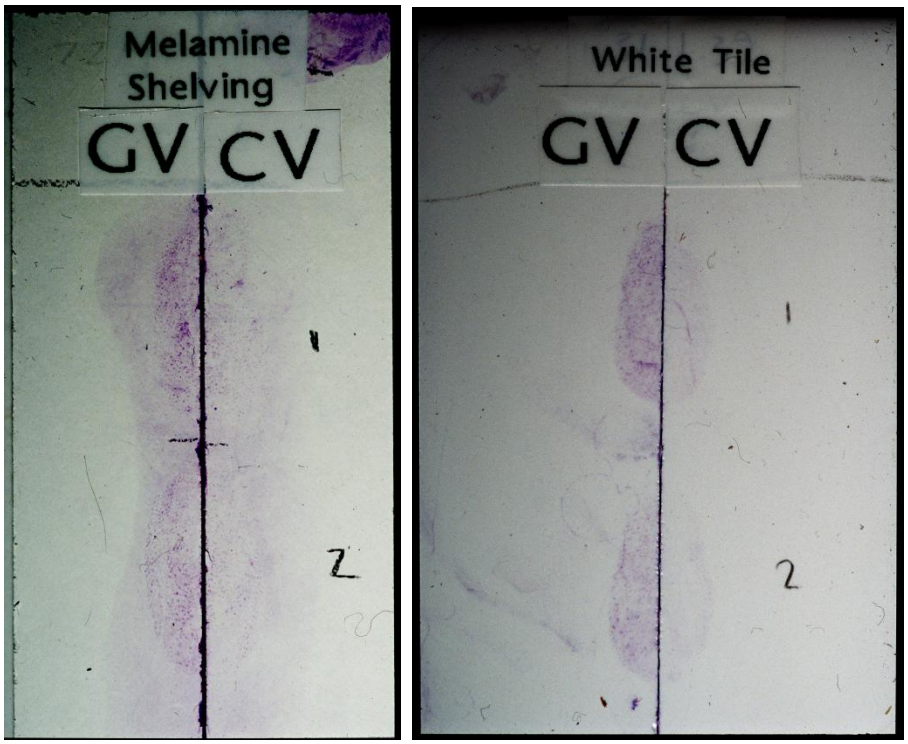
5.6 Most recently, studies on plastic packaging materials show that basic violet 3 will develop additional marks if used as the final stage in a sequential treatment regime, and it is now recommended for these purposes on plastic packaging and non-porous surfaces.

6. Alternative formulations and processes

6.1 An alternative composition based on basic violet 3 was used in the UK by the FSS [21]. This formulation (known as the FSS crystal violet formulation) consists of the following:

- 50 g of basic violet 3 dissolved into 2.5 L of ethanol (min. 95% assay) to form a stock solution;
- 200 mL of stock solution added to 4.8 L of water to form a working solution.

6.2 This formulation was tested against the phenolic formulation in the *Fingermark Visualisation Manual* [17] on a range of substrates, including clear, black and white polythene sheet, laminate, ceramic tiles, melamine and white hardboard using split depletion series. In this comparison [22] it was found that the formulation in the manual produced stronger staining and more ridge detail than the FSS crystal violet formulation.



Images showing relative effectiveness of Home Office Centre for Applied Science and Technology basic violet 3 formulation (GV) and Forensic Science Service crystal violet formulation (CV) on non-porous surfaces.

6.3 CAST has also conducted an extensive evaluation of alternatives to phenol in the formulation, including disinfectants and antibacterial agents, substances with similar chemical structures, properties or functional groups, detergents and surfactants, and substances used as phenol replacements in other formulations [13]. These are summarised in the table below.

Disinfectants / antibacterials	**Similar structure, properties, functional groups**	**Detergents / surfactants**	**Other phenol replacements**
Hexachlorophene	Cyclohexanol	Aerosol OT (DOSS)	Ammonium hydroxide
Benzalkonium Chloride	Phenylalanine	Aerosol 22	Sodium chloride
Cetrimide	Aspargine	1-pentane sulfonic acid	Ammonium oxalate

Chlorhexidine	Arginine	1-hexane sulfonic acid	Pyridoxamine.2HCl
Chlorhexidine diacetate monohydrate	L-Ascorbic acid	1-heptane sulfonic acid	Pyridoxine.HCl
Chlorhexidine digluconate	Salicylic acid	1-octane sulfonic acid	Pyridoxal.HCl
2,4,6-Trichlorophenol	Sulfosalicylic acid	1-decane sulfonic acid	
	Oxalic acid	Cholic acid	
		Deoxycholic acid	
		Aurocholic acid	
		Dehydrocholic acid	
		Alginic acid	
		Caprylic acid	
		N-Lauryl Sarcosine	
		LOC High Suds	
		Arylan PWS	

Alternatives to phenol investigated for basic violet 3 formulations.

6.4 Of these, only DOSS gave performance equivalent to or better than the phenol-based formulation and hence was the only compound considered in further, more focused studies. These subsequent studies [14,23] confirmed the observation that the DOSS formulation consistently out-performed the phenol formulation in laboratory trials and this formulation was ultimately recommended for operational use on adhesive tapes after a brief operational trial.

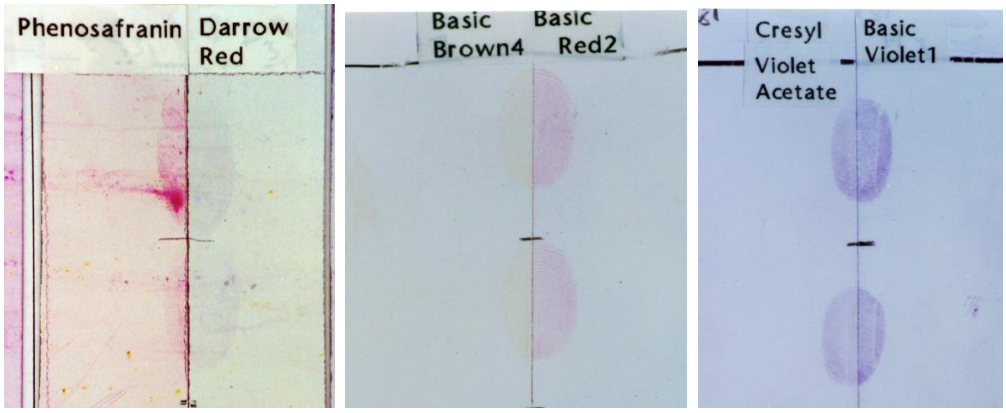
6.5 CAST has also assessed a wide range of alternative lipid dyes, some water soluble, some ethanol soluble and some soluble in both solvents. In addition, some other dyes containing NH_2 groups were also evaluated because this characteristic appeared to be important in the staining of fingermarks. The full list of dyes evaluated is given in the table below.

Common name	**Colour Index name**	**Colour Index number**
2,7-Dichlorofluorescein	–	–
Basic fuschin	Basic red 9	42500
Bismark brown R	Basic brown 4	21010
Cresyl violet acetate	–	–
Darrow red	–	–
Indophenol blue	–	49700
Lucifer yellow CH	–	–
Methyl violet	Basic violet 1	42535
Methylene blue	Basic blue 9	52015
Neutral red	Basic red 5	50040
New fuschin	Basic violet 2	42520

Nigrosin	Solvent black 5	50415
Nile blue Chloride	Basic blue 12	51180
Nile red	–	–
Oil blue N	Solvent blue 14	61555
Oil red O	Solvent red 27	26125
Phenosafranine	–	50200
Primulin	Direct yellow 59	49000
Pyronine B	–	45010
Rose Bengal	Acid red 94	45440
Safranine O	Basic red 2	50240
Sudan green 4B	Solvent green 3	61565
Sudan III	Solvent red 23	26100
Sudan orange G	Solvent orange 1	11920
Solvent violet R	Disperse violet 1/solvent violet 11	61100
Thiazol yellow G	Direct yellow 9	19540
Thionin	–	52000
–	Acid black 48	65005

Dyes investigated as possible alternatives to basic violet 3.

6.6 Of this selection of dyes, basic red 5, direct yellow 59, phenosafranine, basic red 2, cresyl violet acetate, basic violet 2 and basic violet 1 were considered worthy of further investigation. Optimised formulations based on basic violet 2 were ultimately developed, but in comparative trials with an experimental formulation of basic violet 3, the basic violet 2 formulation was found to be less effective [14].



Examples of some of the split depletion experiments conducted using alternative lipid dyes.

7. Post-treatments

7.1 The principal post-treatment used for fingermarks treated with basic violet 3 is the transfer process [7], used for fingermarks on the adhesive side of dark tapes where the violet colour of the dye cannot be seen. In

this process the tape is placed in contact with the glossy surface of photographic paper and pressed. Dye is transferred to the surface of the white paper and the violet dye can be easily visualised. Another advantage of this process is that the fluorescence of the marks is generally increased because the concentration of the dye transferred is less than that present in the original developed fingermark, reducing the self-quenching effect of the dye.

8. Validation and operational experience

8.1 Fundamental studies

8.1.1 The natural constituents and greasy contaminants targeted by basic violet 3 and the way that it stains fingermarks were investigated in studies carried out in 2011.

8.1.2 Garrett et al [23] carried out spot tests using substances typical of those found in the sebaceous component of fingermark residue deposited on a white ceramic tile and then enhanced using the phenol-based formulation of basic violet 3. The substances used included 10 fatty acids ranging from carbon chain lengths C_6 to C_{24} (hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, octadecanoic acid, eicosanoic acid, docosanoic acid, and tetracosanoic acid); cholesterol; two cholesterol esters (cholesteryl acetate and cholesteryl benzoate); squalene; and two triglycerides (glyceryl tripalmitate and glyceryl tristearate). Spots of contaminants such as handcream, butter, vegetable spread and olive oil were also investigated.

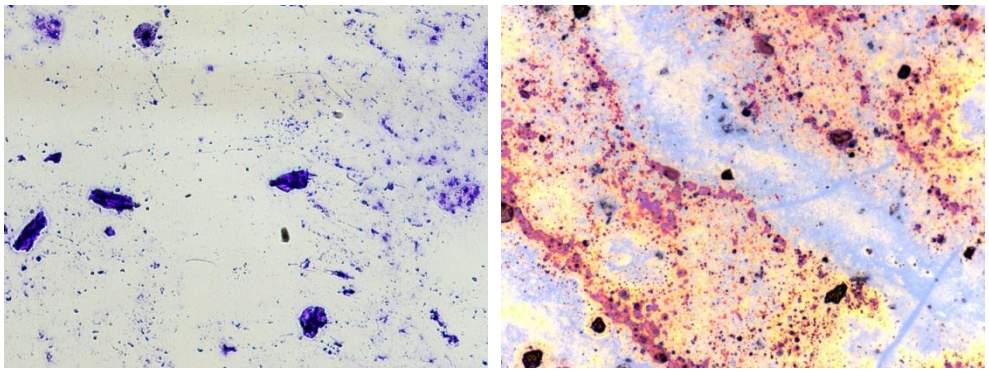
Component	Basic violet 3 (phenol-based) (White light)	Basic violet 3 (phenol-based) (Fluorescence examination)
Hexanoic acid	*	
Octanoic acid	*	
Decanoic acid	*	
Dodecanoic acid	**	
Myristic acid	*	
Palmitic acid		
Octadecanoic acid		
Eicosanoic acid	*	*
Docosanoic acid	***	
Tetracosanoic acid		
Cholesterol	**	***
Cholesteryl acetate	*	**
Cholesteryl benzoate	*	**
Squalene	*	***
Glyceryl tripalmitate		***
Glyceryl tristearate	*	**
Hand cream	***	*
Butter	**	***
Vegetable spread		**
Olive oil	*	*

-	= no evidence of staining/fluorescence
*	= partial or weaker staining/fluorescence seen
**	= strong staining/fluorescence seen
***	= very intense staining/fluorescence seen

Summary of data obtained from spot tests with the phenol-based basic violet 3 formulation [23].

8.1.3 The sebaceous fingermark constituents stained by basic violet 3 differ from those targeted by solvent black 3, indicating the potential to use the two dyes in sequence with each other. It can also be seen that some constituents that are only very faintly visibly stained with basic violet 3 are actually more readily visible in fluorescence mode, indicating the importance of using fluorescence examination in addition to visual examination when examining surfaces treated with basic violet 3. Basic violet 3 was observed to stain all fatty contaminants used in this study, although vegetable spread was only detected using fluorescence examination.

8.1.4 The way in which fingermarks are stained by basic violet 3 was also investigated in the same study. Fingermarks were deposited on clean microscope slides, allowed to age for a selected period of time, and then stained using the phenol-based basic violet 3 formulation. The stained fingermarks were then examined using high magnification optical microscopy.

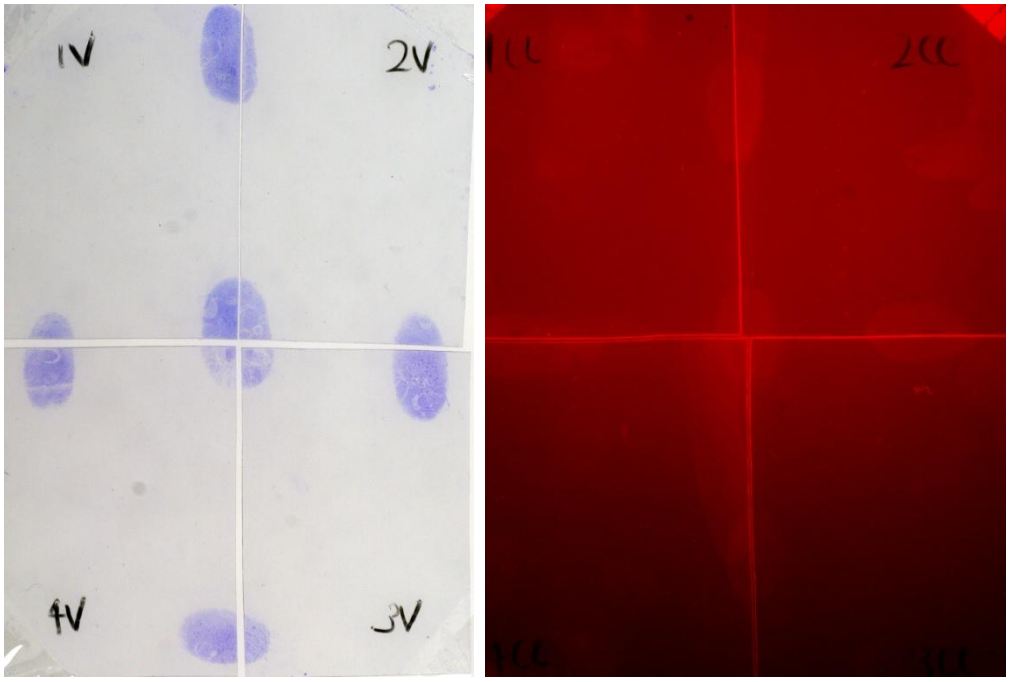


High magnification optical micrographs of two different marks stained with basic violet 3, (left) *transmitted light* (right) *reflected light.*

8.1.5 Microscopy again showed that the staining characteristics of basic violet 3 differ from those of solvent black 3. In many cases the staining of the ridges is reasonably uniform and of low intensity, with the most intensely stained features being shed skin cells within the mark. In other marks there was more heterogeneous staining of different regions, but not to the extent observed for solvent black 3.

8.1.6 Tests were also conducted to evaluate the sensitivity of the phenol-based basic violet 3 formulation to gross changes in the dye concentration. These tests used a quartered fingermark technique,

dividing a series of deposited fingermarks into four and processing each quarter with a different concentration of dye in the formulation. The four dye concentrations used were 1x, 0.75x, 0.5x, and 0.25x the quantity of dye in the current phenol-based basic violet 3 formulation. Deliberately sebaceous fingermarks, produced by wiping the finger against the side of the nose and the forehead, were used in this experiment so that there would be sufficient material in the mark for the dyes to interact with. Typical results are illustrated in the images below.



Quartered fingermark experiments using different concentrations of basic violet 3 on marks deposited by different donors showing (left) *appearance under white light and* (right) *appearance under fluorescence examination.*

8.1.7 It was shown that the formulation is reasonably robust to variations in dye concentration, and although reducing the dye content by 25% was observed to decrease the intensity of the visible purple staining, there were suggestions that marks developed using this formulation were marginally more fluorescent. This is possible due to the fact that intensely stained marks may re-absorb some of the fluorescence.

8.1.8 A broader investigation of the types of contaminant that can be detected with basic violet 3 was conducted by Gaskell et al [24]. A total of 35 different contaminants were used, selected as typical of substances that may be encountered in ‘kitchen’, ‘bathroom’ and ‘garage’ environments, and are listed in the table below.

Environment		
'Kitchen'	**'Bathroom'**	**'Garage'**
Sunflower oil	Garnier hand cream	WD40
Olive oil	Oil of Olay face cream	'3 in 1' drip oil
'Flora light' spread	Nivea sun cream	Hydraulic fluid
Butter	Boots No. 17 tinted moisturiser	Silicone grease
Lard	Ulta foundation	RS anti-seize compound
Coca Cola	Ulta eyeshadow	Shell motor oil
Stella Artois lager	Ulta lipstick	Swarfega
Gaymers cider	Johnsons baby oil	Unleaded petrol
Red wine	Vaseline	Brake pad residue
Orange juice	Buttercup cough syrup	Castrol grease
Tomato ketchup		Used engine oil
Mayonnaise		
Used cooking oil		

Table summarising the initial range of contaminants assessed in terms of their interactions with fingermark enhancement processes [24].

8.1.9 Marks were deposited on a clean ceramic tile by dipping the finger into the contaminant, dabbing it free of any excess, then leaving it for 1 day or 1 week prior to enhancement. These tests utilised the DOSS-based basic violet 3 formulation and are therefore not directly comparable to the spot tests reported above that utilised the phenol-based formulation. Results from the tests on contaminants are shown in the table below.

Contaminant	**BV3**	
	1 day	**1 week**
Natural print	0	0
Deliberately sebaceous	1	2
Sunflower oil	2	3
Olive oil	1	3
Flora light	4	4
Butter	1	0
Lard	2	1
Coca cola	0	0
Stella Artois	0	0
Gaymers cider	0	0

Red Wine	0	0
Orange juice	1	2
Ketchup	0	0
Mayonnaise	2	4
Used cooking oil	4	4
Hair wax - Brylcream	1	1
Garnier hand cream	0	0
Face cream - oil of olay	0	0
Nivea spf 15 suncream	3	2
Tinted moisturiser	0	1
Powder foundation	0	0
Eyeshadow	0	0
Lipstick	2	2
Baby oil	1	0
Vaseline	0	0
Buttercup cough syrup	0	0
WD40	3	1
3 in 1 multipurpose oil	0	0
Hydraulic fluid	0	0
Silicon grease	0	0
RS anti seize compounds	3	3
Shell motor oil	0	0
Swarfega	3	3
Petrol	0	0
Brake pad residue	0	0
Castrol grease	0	0
Dipstick residue	3	3

Table summarising the effectiveness of basic violet 3 on a range of contaminants, on marks aged for 1 day and 1 week [24].

8.1.10 Basic violet 3 was found to be less effective than solvent black 3 in staining broad classes of greasy contaminant, with the most intense staining observed for mayonnaise, used cooking oil and vegetable spread. The differences in performance observed on certain types of contaminant suggest that there may be merit in using the two stains in

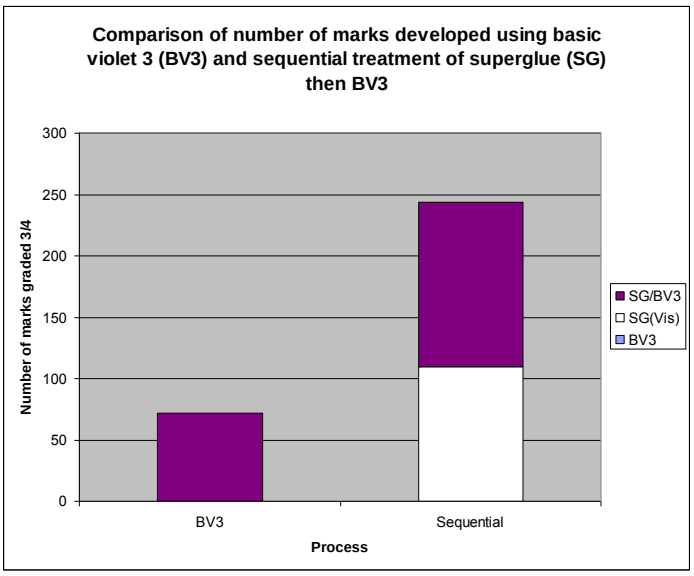
sequence if greasy contaminants are suspected to be present to maximise the number of marks recovered.

8.2 Laboratory trials

8.2.1 Extensive laboratory trials have indicated the following.

- Basic violet 3 alone is not the most effective treatment for any type of adhesive tape.
- Basic violet 3 can be effectively used as a final sequential treatment for adhesive tapes after either superglue or powder suspensions.
- The DOSS-based basic violet 3 formulation appears to develop better quality fingermarks with better contrast, causes less background staining and has fewer health and safety issues associated with it than the phenol-based formulation.
- Basic violet 3 can also be used on contaminated, non-porous surfaces but it has not been conclusively shown whether basic violet 3 or solvent black 3 (or indeed iodine or powder suspensions) are the optimum treatment in these circumstances. Basic violet 3, solvent black 3 and iodine all stain fats, whereas powder suspensions will not stain fats but will develop latent fingermarks laid on contaminated surfaces.

8.2.2 The experiments conducted to support the statements above are summarised below. During studies on optimum treatments for adhesive tapes, basic violet 3 was compared with superglue as a single treatment, and as a secondary treatment after superglue [19]. It was also investigated as a secondary treatment after carbon-based powder suspensions. In total, over 1,000 marks were graded during this study. The results are shown graphically below.



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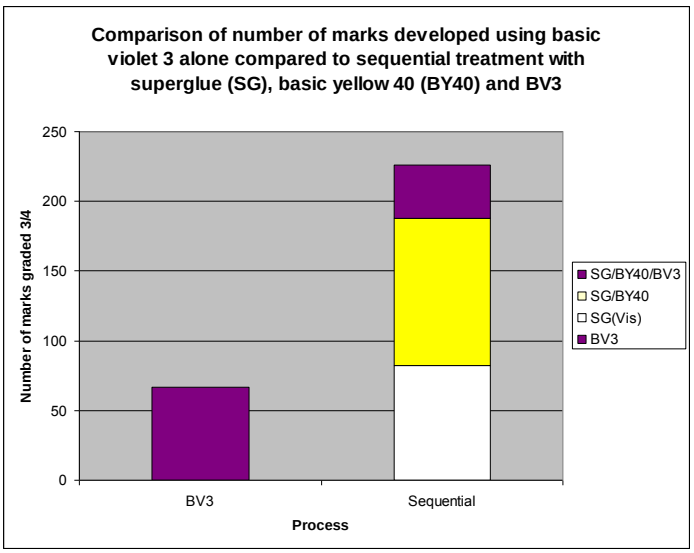

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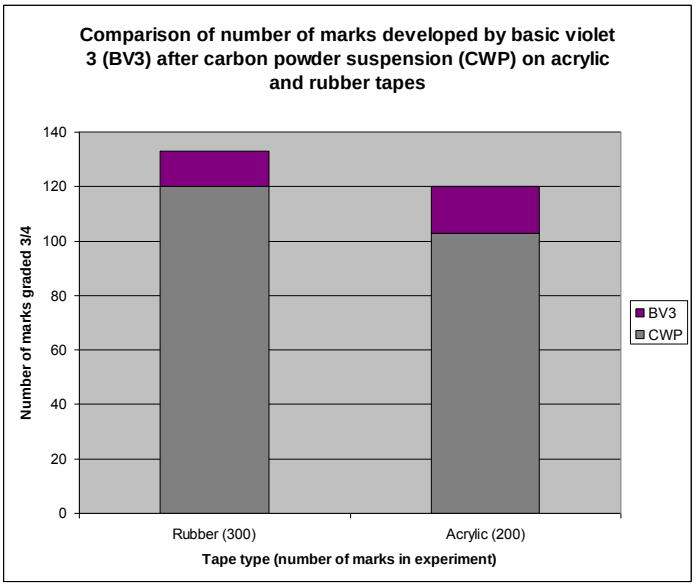
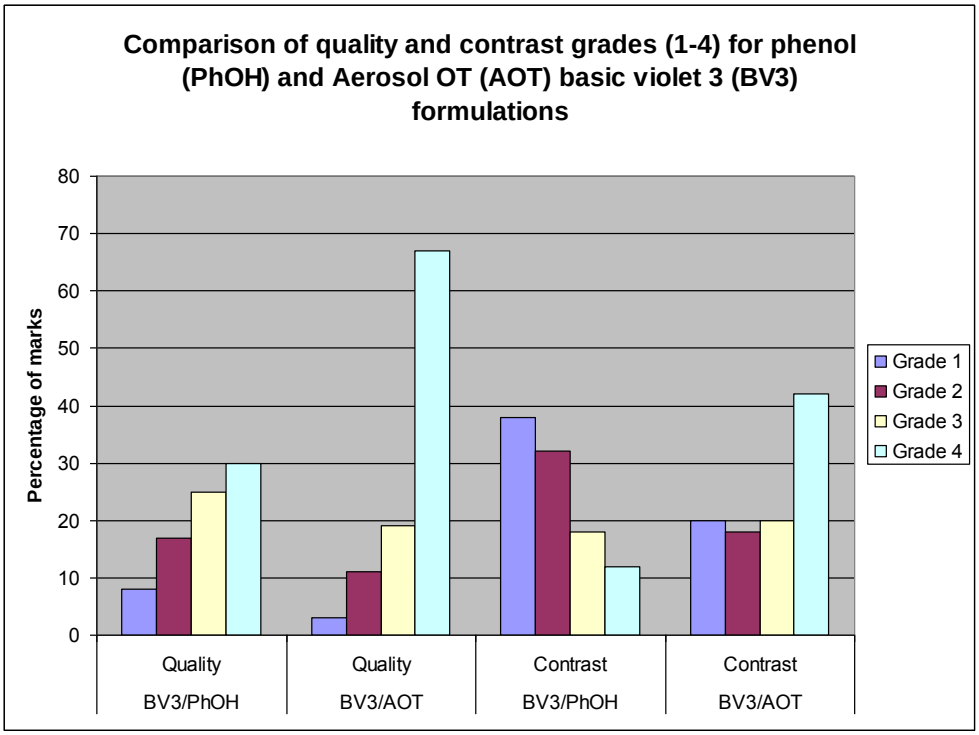


c)

Graphs showing additional marks developed using basic violet 3 (BV3) in sequence on adhesive tapes, a) *comparison of BV3 alone with superglue (SG) followed by BV3,* b) *comparison of BV3 alone with superglue followed by basic yellow 40 (BY40), followed by BV3, and* c) *BV3 after carbon powder suspension (CWP) on both acrylic and rubber-based adhesives [19].*

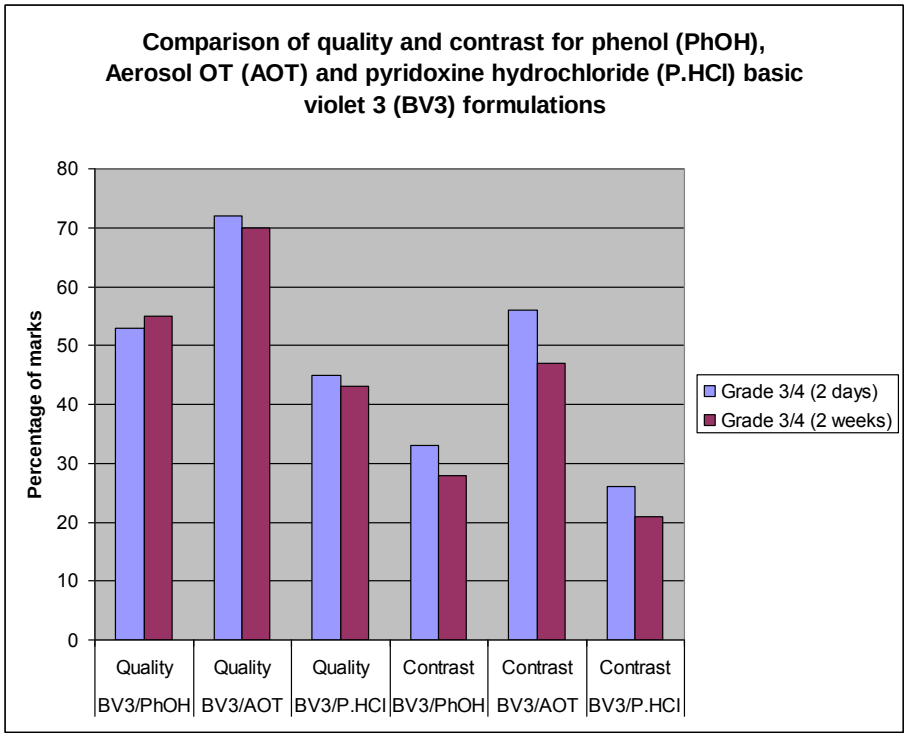
8.2.3 It can be seen that basic violet 3 is not as effective as superglue, but does develop additional marks after both superglue and powder suspensions on adhesive tapes.

8.2.4 Research into phenol replacements evaluated a range of surfactants, of which DOSS gave performance equivalent to, or better than, the phenol-based formulation and hence was the only compound considered in further, more focused studies on adhesive tapes.

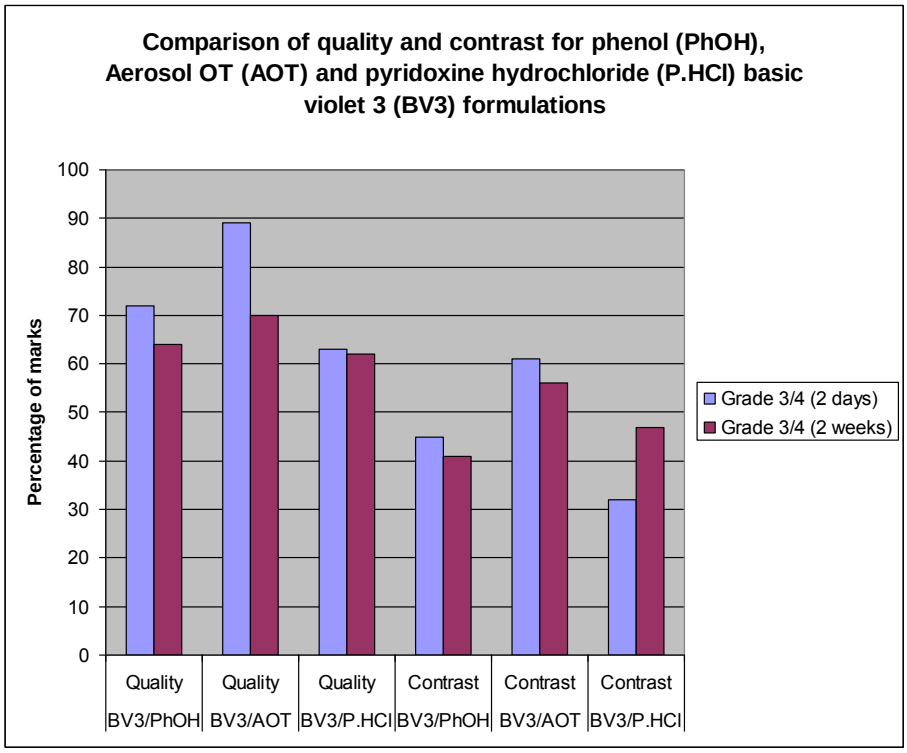


Graph comparing effectiveness of phenol (PhOH) and Aerosol OT (DOSS)-based basic violet 3 (BV3) formulations, results based on grading of 1,920 half marks.

8.2.5 These subsequent studies [14,25] incorporated further phenol alternatives and confirmed the observation that the DOSS formulation consistently out-performed the phenol and pyridoxine hydrochloride formulations in laboratory trials and this formulation was ultimately recommended for operational use on adhesive tapes after a brief operational trial.

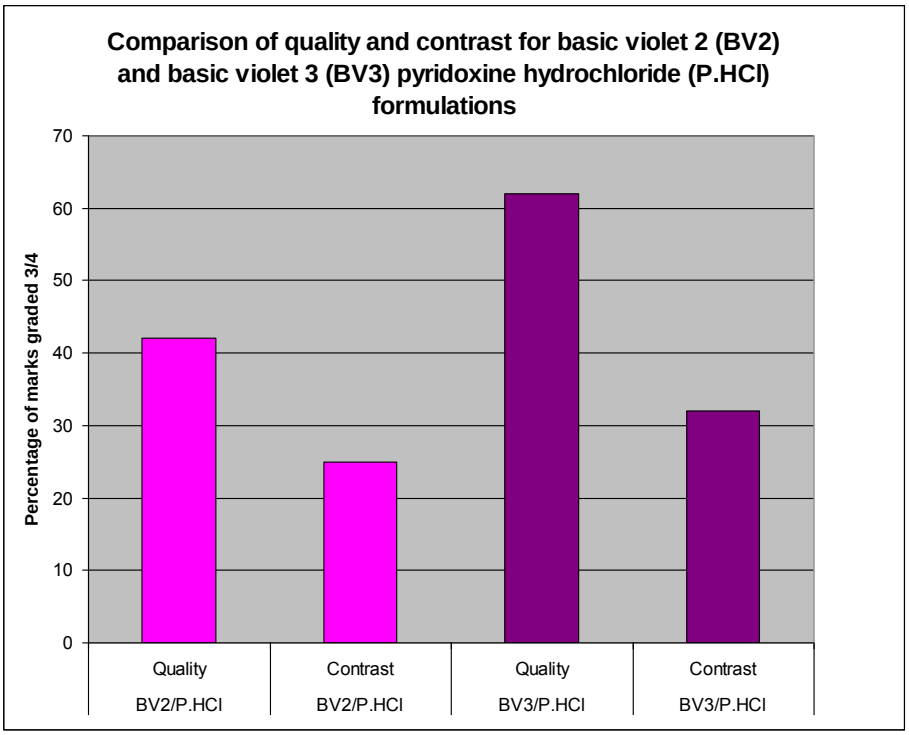


Three-way comparison of phenol, DOSS (Aerosol OT) and pyridoxine hydrochloride basic violet 3 formulations on tapes peeled off plastic bags, based on 4,494 half marks [14].



Three-way comparison of phenol, DOSS (AOT) and pyridoxine hydrochloride basic violet 3 formulations on tapes removed from plastic bags using freezer spray, based on 1,798 half marks [14].

8.2.6 Of all of the alternative lipid dyes evaluated, the most promising was basic violet 2. Comparative studies were conducted between basic violet 2 and basic violet 3, the results indicating that basic violet 2 was inferior in performance based on the colour of marks developed and hence no further research was carried out on this dye.

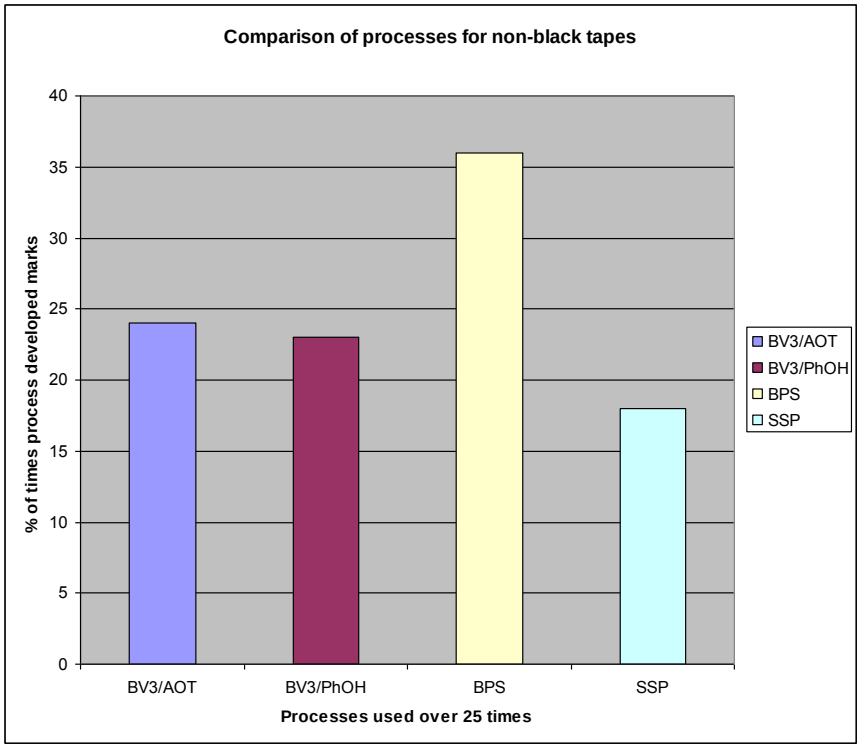


Comparison of pyridoxine hydrochloride-based basic violet 2 and basic violet 3 formulations (pyridoxine hydrochloride-based basic violet 3 subsequently found to be less effective than phenol and DOSS-based basic violet 3). Results obtained from grading 300 half fingermarks [14].

8.2.7 Comparative studies were also carried out between basic violet 3 and solvent black 3 on a range of non-porous surfaces. These are reported in Chapter 3, Chemical and Physical Processes, Solvent Black 3 and show that there is no clear difference between the two processes in this application.

8.3 Pseudo-operational trials and operational experience

8.3.1 An operational trial was conducted, comparing the effectiveness of both phenol- and DOSS-based basic violet 3 formulations with CAST-formulated powder suspensions and a commercial powder suspension. Results were obtained on both black tapes (where a white powder suspension was used) and light tapes (where black powder suspensions were used). These were conducted with police forces traditionally receiving large numbers of tape exhibits over a period of 18 months. However, tapes are not common exhibits and it took a considerable time to generate sufficient data for a reasonable comparison to be made.



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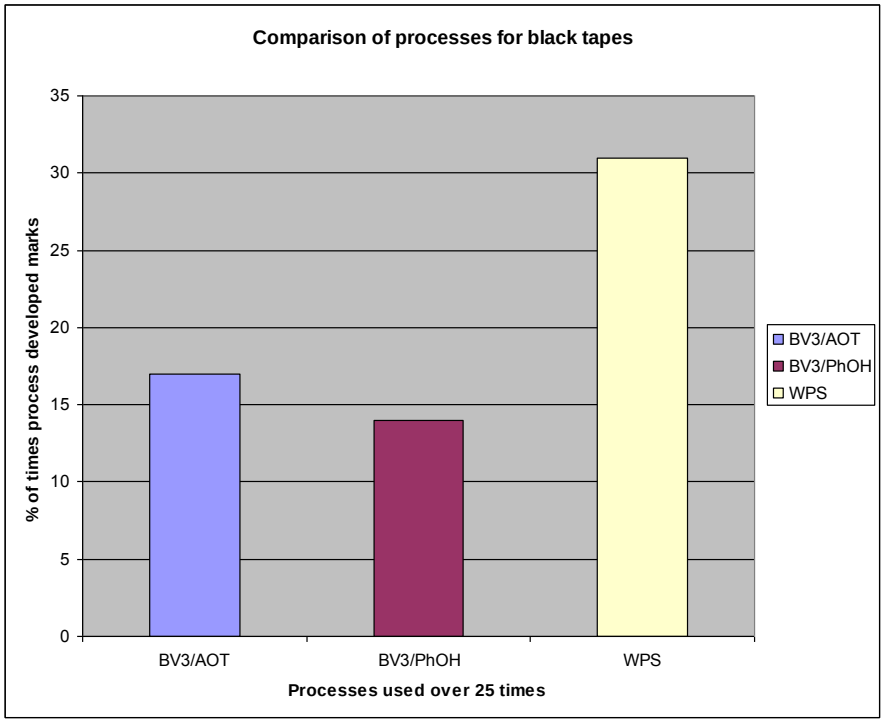

b)

Results of operational trial on adhesive tapes, comparing DOSS (AOT) and phenol-based Basic Violet 3 formulations with a) *Home Office Centre for Applied Science and Technology black powder suspension and Sticky-Side Powder on non-black tapes, and* b) *Home Office Centre for Applied Science and Technology white powder suspension on black tapes.*

8.3.2 The trial effectively confirmed the results of the laboratory trials in that the DOSS-based formulation was more effective than the phenol-based formulation on adhesive tapes and that powder suspensions were more effective than basic violet 3 as a single treatment. As a consequence, formulations for DOSS-basic violet 3 and black and white powder suspensions were issued by CAST in 2006 [15].

8.3.3 A pseudo-operational trial was recently conducted on plastic wrapping materials, which incorporated basic violet 3 as the final process in a sequential treatment scheme [26]. The results of this trial are more fully reported in Chapter 3, Chemical and Physical Processes, Powder suspensions, and demonstrate that basic violet 3 develops up to 10% additional marks in both visual and fluorescence modes after sequences involving other treatments. As a consequence, basic violet 3 has been incorporated as the final stage in the processing treatments for non-porous surfaces and plastic packaging materials.

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1,8-Diazafluoren-9-one (DFO)

1. History

1.1 1,8-Diazafluoren-9-one (DFO) was first synthesised by Druey and Schmidt in the CIBA laboratories in Switzerland in 1950 [1]. The potential of the chemical for the labelling of amino acids and detection of fingermarks was not recognised until the late 1980s, when the Central Research Establishment (CRE) of the then Home Office Forensic Science Service (FSS) placed a contract with Queens University, Belfast to investigate ninhydrin analogues. During the course of this research DFO was identified as a highly promising alternative to ninhydrin, producing marks of a reddish colour when viewed under normal light. The most significant feature of the product formed by the reaction between the DFO reagent and fingermark residues was that it was inherently fluorescent and eliminated the need for toning with metal salts, an additional process required to make ninhydrin marks fluorescent.

1.2 Before open publication of information on the effectiveness of DFO, quantities were sent to selected fingerprint research laboratories worldwide for evaluation. Operational trials were also conducted at two UK police forces, Surrey [2] and the Metropolitan Police [3]. A comparative assessment of DFO with ninhydrin and 5-methoxyninhydrin was carried out in both Israel [4] and New Zealand [5]. The Israeli study looked at results obtained on a series of paper samples and banknotes and found DFO to out-perform both forms of ninhydrin. The New Zealand researchers investigated sequential treatment and found that DFO could be used before ninhydrin, and did not affect the subsequent use of physical developer. However, ninhydrin used after DFO was far less effective and did not produce any additional marks. The fluorescence of DFO was also superior to that of both ninhydrin forms after toning with zinc chloride. Another observation made by the New Zealand group was that DFO also enhanced blood, and could be used in sequential treatments before amido black (acid black 1).

1.3 With all researchers reporting significant improvements in the number of marks developed using DFO over the numbers found with ninhydrin, the first information on the new reagent was published in open literature in 1990 [6,7,8]. The initial formulation issued was based on the chlorofluorocarbon (CFC) 1,1,2-trifluorotrichloroethane (CFC113) solvent, with small quantities of methanol and acetic acid, and required the exhibits to be dipped twice in the solution, allowing them to dry each time before finally heating in a dry oven at 100ºC for 10 minutes to develop the marks. Excitation and emission spectra for DFO were also presented, with the UK laboratories initially using an argon ion laser to promote fluorescence. However, it was also found that a high intensity light source (i.e. the high intensity filtered light sources then becoming available) could also be used to produce fluorescence [9,10].

1.4 Fundamental research into DFO continued, with studies carried out into the reaction products formed between DFO and amino acids [11]. Assessments of the relative sensitivity of DFO and different ninhydrin analogues were also carried out [12], looking at their relative detection limits for serine. This study indicated that DFO was similarly sensitive to ninhydrin in colorimetric mode and as sensitive as 5-methoxyninhydrin toned with zinc chloride (the best of the ninhydrin analogues) in fluorescence mode.

1.5 The issue of the first DFO formulation and the subsequent commercial availability of the reagent prompted further investigations worldwide, with assessments being carried out of alternative solvents to CFC113 including petroleum ether [13] and a petroleum ether/xylene mixture [14]. Sequential treatments were also reassessed, with Masters *et al.* [14] studying a range of different paper types and finding that the DFO-ninhydrin sequence was far superior to ninhydrin-DFO. Corson [13] also investigated sequential treatment and indicated that occasionally DFO could develop additional marks after ninhydrin, but did not state which sequence was best. Masters *et al.*[14] also studied a range of different light sources and filter combinations for excitation and viewing of the fluorescent fingermarks. A red camera/viewing filter was recommended to reduce the background fluorescence that was sometimes observed on coloured papers and from some writing inks.

1.6 The Home Office Scientific Research and Development Branch (HO SRDB) studies into DFO also began in 1990, initially looking at the components of the formulation and the dipping and heating stages. In a split depletion comparison carried out over seven different paper types using five donors, it was found that there was no benefit in dipping the article twice. The purpose of double-dipping was stated to be to increase the uptake of DFO by the fingermark, but the HO SRDB study showed no difference in either the visible appearance or the intensity of fluorescence between single-dipped and double-dipped articles. Single-dipped articles, in particular cheques, showed less evidence of background staining and therefore single dipping was recommended. Heating experiments were also conducted, monitoring the change in fluorescence with increasing exposure time in an oven using a luminance meter. At 100ºC, optimum fluorescence was reached after 20 to 30 minutes, whereas at 50ºC development took several hours [15]. Temperatures in excess of 100ºC were not considered because of potential charring to the paper, although development rates were increased; Australian researchers suggested that development occurred in approximately 20 to 30 seconds at 160˚C [16]. A dry oven was found to be more effective than a heat gun in delivering the optimum heating conditions. It was considered important that the oven used in processing had a laminar air flow across each shelf as opposed to being a convection oven, because paper articles were loaded on cardboard in the same way as used for the processing of ninhydrin treated articles. Further studies also investigated alternative solvent systems and reductions in the amount of DFO in the formulation. It was found that the

quantity of DFO could be reduced from 0.5 g to 0.25 g without any detriment to the intensity of fluorescence produced. This also overcame issues with instability of the working solution, where DFO precipitated rapidly, sometimes before processing had commenced. Operational trials were conducted between the original and revised formulation and processing conditions, with the revised formulation giving marginally better performance. A summary of these studies was published in 1993 [15].

1.7 Other extensive studies of DFO, its reactions and optimum viewing conditions were conducted by Cantu *et al.* [17] and Stoilovic [16]. Cantu *et al.* compared the effectiveness of DFO with a range of ninhydrin analogues on the amino acid glycine and concluded that DFO was the only compound acting as a fluorescer without secondary treatment, with the intensity of fluorescence exceeding that of any of the zinc or cadmium complexes formed with ninhydrin. Cantu *et al.* also demonstrated that the presence of acetic acid in the formulation was essential for fluorescence to occur. Formic acid will also produce a good reaction, but when used in combination with methanol the two constituents react rapidly to produce the unwanted methyl formate. Stoilovic also investigated changes to the formulation, adding chloroform and reducing the methanol and acetic acid components in order to reduce the detrimental effect of inks running when treating documents. He also conducted a sensitivity study and concluded that DFO was equivalent in sensitivity to ninhydrin toned with zinc chloride. Samples were treated by heating with an ironing press at 160ºC, which was thought to give superior results to oven heating (although the oven used in this case was convection, rather than a laminar flow oven). The optimum excitation and viewing conditions were also investigated using a filtered high intensity light source (Polilight).

1.8 The introduction of the Montreal Protocol on Substances that Deplete the Ozone Layer in 1987 and the subsequent prohibition on the use of ozone-depleting solvents, including CFC113, meant that from the mid-1990s efforts were directed towards an ‘ozone-friendly’ DFO formulation. In 1995 Lennard [18] proposed petroleum ether, which was in wide scale use in the US as a solvent for ninhydrin, as a replacement for CFC113, but it was also desirable to identify a solvent replacement without the associated issues of high flammability. During the period 1994 to 1997, PSDB evaluated a range of candidate replacement solvents including hydrochlorofluorocarbons (HCFCs), hydrofluorocarbons (HFCs) and hydrofluoroethers (HFEs). Of these HFC4310 and 1-methoxynonafluorobutane (HFE7100) [19] both showed promise, but required other additives to produce the same level of reaction as CFC113. HFE7100 had also been supplied to French researchers for evaluation, and they too developed a DFO formulation based on this solvent [20]. CAST carried out an operational trial of the most promising new DFO formulations, comparing them with the existing CFC113 formulation and an optimised 1,2 indandione formulation based on HFE7100. In this trial, conducted on 650 articles in an operational police

laboratory, the HFE7100-based DFO formulation gave the best results [21] and was therefore recommended for operational use in the UK. A fuller description of the alternative formulations investigated by PSDB was later published by the researchers [22].

1.9 Further fundamental research was carried out on the DFO system. Wilkinson studied the reaction mechanism between DFO and amino acids [23] and the synthesis of DFO analogues [24]. Conn *et al.* [25] investigated whether metal salt treatment of DFO would give any further benefits in fluorescence but concluded that, in contrast to ninhydrin, there was little effect on the fluorescent product.

1.10 The impact of DFO on other types of forensic evidence was also studied. The emergence of DNA and its importance as an identification tool prompted studies into the effect of DFO treatment of blood on the subsequent recovery of DNA profiles [26]. The authors concluded that DFO had no detrimental effect on DNA. PSDB and the FSS also showed that DFO treatment had little impact on the recovery of DNA from latent fingermark residues [27]. Strzelczyk [28] considered the effects of DFO treatment on subsequent document examination, comparing the PSDB HFE7100 formulation with the CFC113 formulation. The HFE7100 formulation was found to be less detrimental to handwriting evidence.

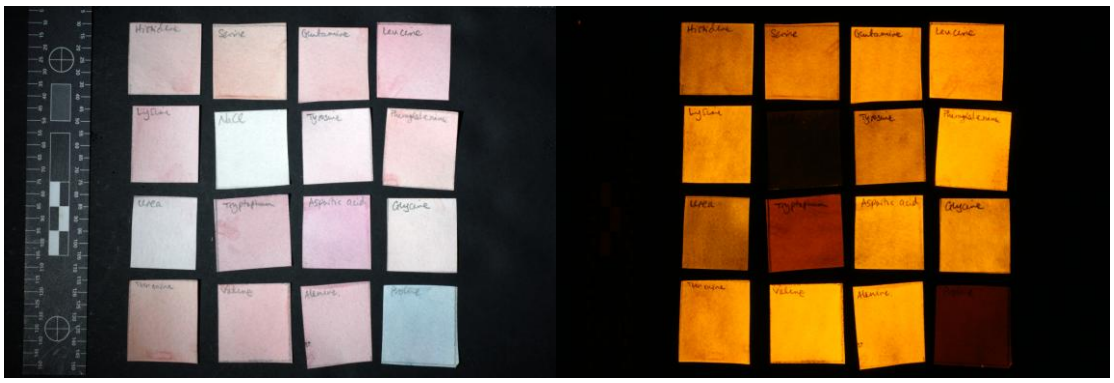
1.11 A survey of fingermark development processes for porous surfaces conducted in 2004 [29] showed that DFO had become the second most widely used reagent for this surface worldwide, with 86% of those responding to the survey saying that they used it in their laboratory.

1.12 More recently, the development of formulations of 1,2 indandione incorporating zinc salts have resulted in claims that 1,2 indandione-zinc is actually more effective than DFO. As a consequence several groups of researchers have carried out further comparative work [30-32]. To date the results of these have given conflicting results with most favouring 1,2 indandione-zinc but some favouring DFO. The most recent studies by CAST [33] have shown clear benefits in the use of 1,2 indandione-zinc over DFO on cardboard and brown paper surfaces and it is likely that DFO will be supplanted for this application. Before DFO can be fully replaced on all porous surfaces work is required to assess the overall effectiveness of sequential treatment routines incorporating DFO and 1,2 indandione-zinc. Some studies into sequential processing have already been reported [34], showing little significant differences between sequences including either reagent, although with 1,2 indandione-zinc being the single most effective process.

1.13 CAST are continuing studies aimed towards wider operational implementation of 1,2 indandione-zinc in place of DFO, including optimisation of formulations and processing parameters for both reagents. It is anticipated that these will be completed in 2017.

2. Theory

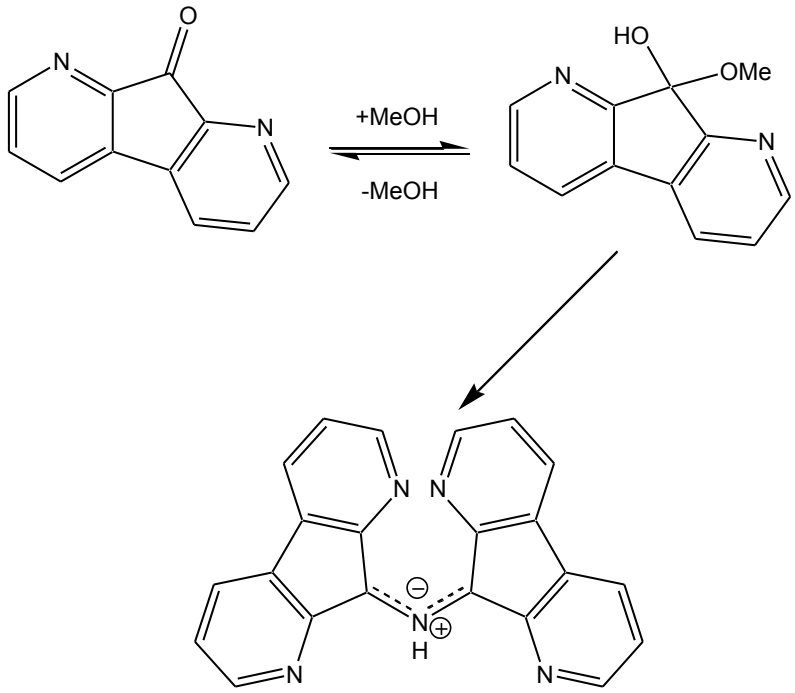
2.1 The reaction mechanism for DFO has been studied by both Grigg *et al.* [11] and Wilkinson [23, 24]. Grigg *et al.* isolated the red reaction product between DFO and various α-amino acids and found it to be closely related to the protonated Ruhemann's purple structure developed with ninhydrin.



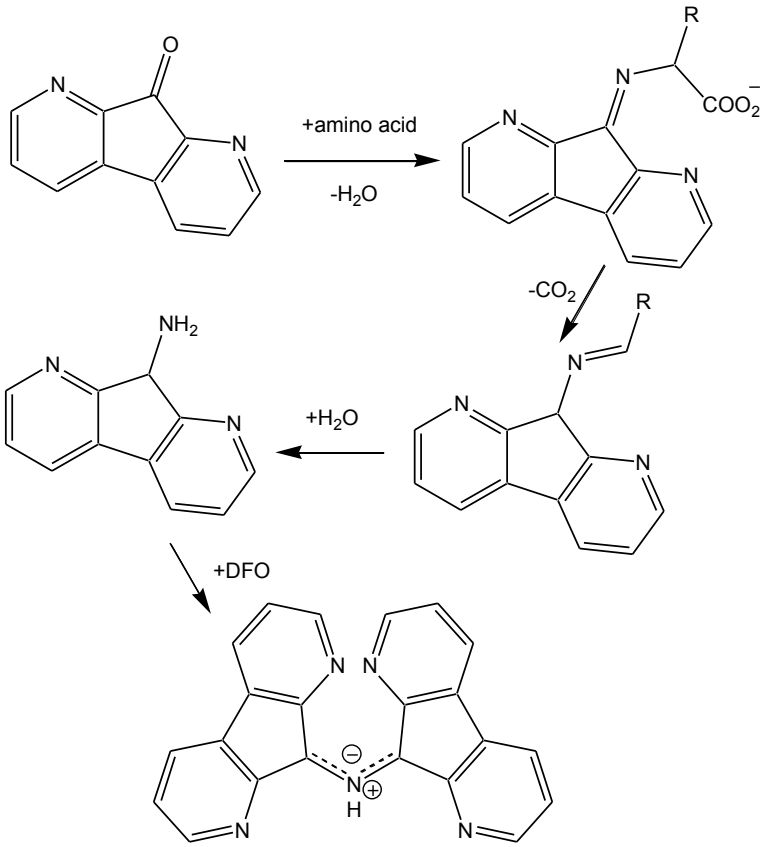
a) b)

Reaction products formed between 1,8-diazafluoren-9-one (DFO) and 0.1M solutions of amino acids and other fingermark constituents a) *visible, and* b) *fluorescence.*

2.2 The analytical studies carried out by Wilkinson used a range of techniques including nuclear magnetic resonance spectroscopy (NMR) and gas chromatography – mass spectrometry (GC-MS) to isolate and identify reaction products. A reaction mechanism was proposed, which is illustrated below.



Proposed mechanism for formation of hemiketal.



Proposed reaction path of 1,8-diazafluoren-9-one (DFO) with amino acids [24].

2.3 Wilkinson [24] proposed that the DFO reaction follows a very similar path to that of ninhydrin with amino acids. DFO reacts with the methanol in the solvent mixture to form a hemiketal, which has a higher reactivity with amino acids than the DFO molecule. The nitrogen atom of the amino acid is able to attack the hemiketal at the electron deficient carbon in the polarised carbonyl, with the loss of water. This forms an aromatic imine, which retains the alkyl fragment of the amino acid and undergoes decarboxylation to form a further intermediate product. Hydrolysis then occurs at the nitrogen-carbon double bond, which forms an aromatic amine and acetaldehyde. The aromatic amine finally reacts with another DFO molecule to form the red, fluorescent reaction product identified in this and previous studies [11, 24]. X-ray crystallography carried out on the reaction product between DFO and L-alanine [23] indicated that the structure of the reaction product consisted of two DFO molecules linked by a bridging nitrogen atom, and was therefore in close agreement with Grigg *et al.*'s original predictions [11]. In the crystalline product analysed, molecules of the reaction product were shown to be linked by hydrogen-bonded bridges with water molecules.

2.4 The reaction between DFO and amino acids is not thought to proceed to completion, which accounts for the observation that ninhydrin will develop additional marks when used after DFO. Alternatively (or additionally), there may not be sufficient DFO to completely react in a 2:1 ratio with all amino acids present.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The process currently (as of 2016) recommended by CAST is to add 30 mL of methanol and 20 mL of acetic acid to 0.25 g of DFO, stirring to produce a yellow solution. To this is then added 275 mL of HFE71DE followed by 725 mL of HFE7100, stirring together to produce a working solution.

3.2 Working solution is poured into a shallow tray, and articles to be treated drawn slowly through the solution with forceps, then removed and allowed to dry on a sheet of tissue. Alternatively, DFO solution may be applied with a soft brush.

3.3 Once dry, articles are heated in a non-humidified oven at 100ºC for 20 minutes, followed by examination in white light (where developed marks may be detected due to their pale pink colour) and subsequent fluorescence examination.

3.4 The role of DFO in the formulation is to react with amino acids present in fingermark residues to give a fluorescent reaction product. The CAST

formulation makes the assertion that the primary purpose of DFO is to produce a fluorescent product, and therefore the presence of any coloured reaction product is of secondary importance. The formulation uses 0.25 g of DFO per litre, found to give the maximum intensity of fluorescence. Any increase in DFO content will make the coloured product more intense (although still far less visible than the purple of ninhydrin) but does not enhance fluorescence. Quantities of > 0.2 g DFO are essential for the reaction to occur, and quantities of > 0.75 g cannot be dissolved.

3.5 Methanol is an essential component of the DFO formulation, its presence allowing DFO to form hemiketals, which in turn have greater reactivity with amino acids. Longer chain alcohols are not as effective, using ethanol, propan-1-ol or propan-2-ol reduces the yield and fluorescence of developed fingermarks and *t*-butanol inhibits the reaction completely. Studies have shown that 30% of DFO reacts with methanol, whereas only 10% reacts with ethanol. The formulation uses the minimum amount of methanol possible due to its toxic nature.

3.6 Acetic acid is added to acidify the solution. If acidification is not carried out, virtually no fingermarks are developed. Propanoic acid can be used in place of acetic acid but has no benefit, whereas formic acid rapidly esterifies with the methanol component of the formulation, producing water as an unwanted by-product. The presence of water causes phase separation of the solution, reducing the amount of DFO in the non-polar phase available for fingermark development, although a small amount of water is essential for the reaction to take place. Dried solutions are brown in colour and do not produce fluorescent marks if used to treat fingermarks.

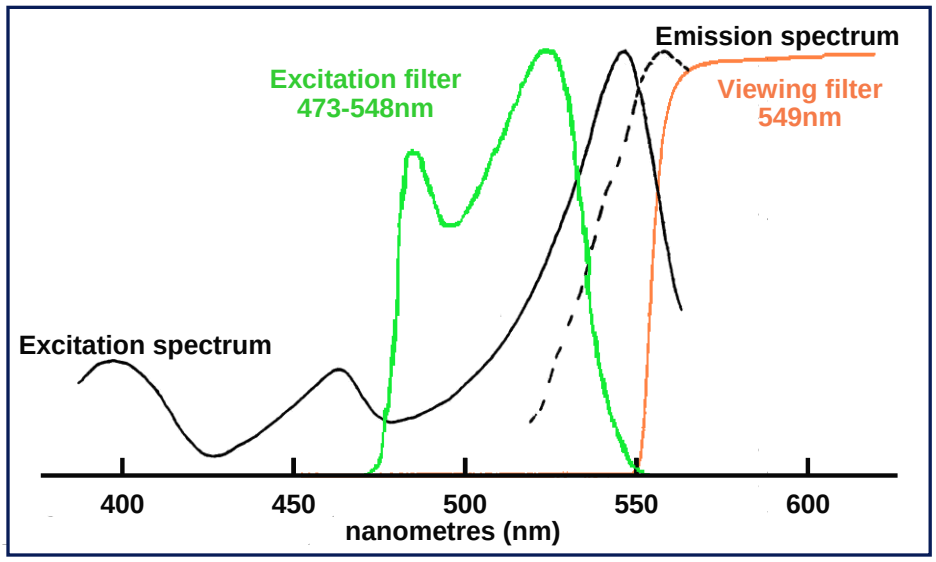
3.7 HFE7100 is used as the principal carrier solvent for DFO. However, during reformulation work it was found that it could not be used as a straight replacement for CFC113 because CFC113 appeared to catalyse the reaction between DFO and amino acids in some way, whereas HFE7100 did not. If HFE7100 was used on its own, the developed fingermarks appeared noticeably less fluorescent and fewer in number. The addition of *trans*-1,2-dichloroethylene as a co-solvent (i.e. in the HFE71DE component of the formulation) is essential for the development of greater quantities of brighter fluorescent fingermarks.

3.8 CAST recommends only a single dip in the DFO working solution. Early studies indicated that double dipping had no benefit in terms of number or intensity of marks developed, and may lead to increased background staining.

3.9 The heating temperature of 100ºC is selected to give a combination of a reasonably short development time combined with a low risk of damage to exhibits, such as paper charring and melting of plastic windows in envelopes. It is also compatible with the upper temperature limit of the ninhydrin oven, enabling a single piece of equipment to be used for both

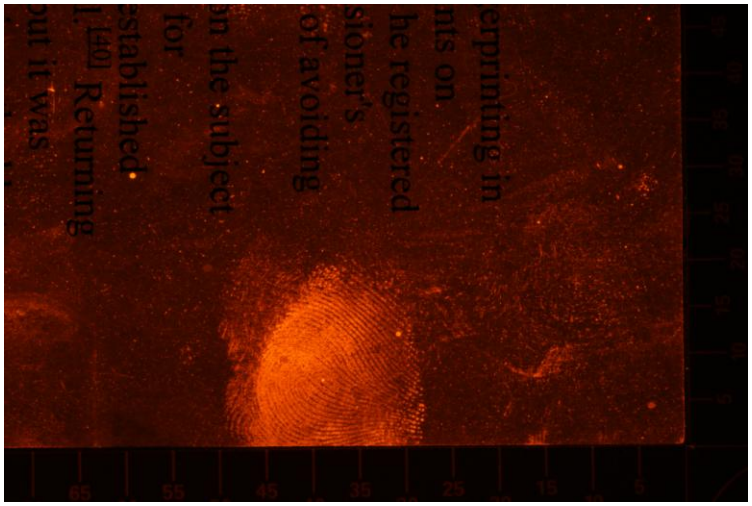
processes. Early studies using a luminance meter showed that optimum fluorescence was obtained after 20 minutes for a significant majority of exhibits and this was therefore recommended in place of the original 10-minute period.

3.10 CAST recommends the viewing of marks developed with DFO using excitation in the green region of the spectrum (the 473 to 548 nm excitation band of the Quaser series of light sources) and viewing fluorescence through an orange, Schott glass OG570 (549 nm long-pass) filter. This gives the optimum match with the excitation and emission spectra for DFO, with the illumination waveband overlapping the DFO excitation and the viewing filter transmitting close to the optimum emission wavelength.



Emission and excitation for 1,8-diazafluoren-9-one, overlaid with the Quaser excitation waveband used and the corresponding transmission of the viewing/camera filters recommended.

3.11 In some circumstances, such as coloured papers, background fluorescence from the paper or ink may make the developed marks more difficult to visualise and in these situations the narrower green excitation waveband of the Quaser (491 to 548nm) should be used instead, in combination with a 593 nm (Schott RG610) filter to cut background fluorescence. More recently, green neodymium:yttrium aluminium garnet (Nd:YAG) lasers with output at 532 nm have become more widely available. This output is further towards the optimum excitation wavelengths for DFO, and being single wavelength will cause far less background fluorescence. Therefore, 532 nm lasers in combination with 549 nm (Schott OG570) long-pass filters are recommended for optimum viewing of fluorescent marks developed using DFO.



Fingermarks developed using 1,8-diazafluoren-9-one, illuminated with green (532 nm) light and viewed using a 549nm long-pass (Schott glass OG570) filter.

3.12 The broad excitation and emission spectra of DFO means that for surfaces where background fluorescence is appreciable when illuminated with light in the green region of the spectrum, better results may be obtained using yellow illumination sources (such as the new 577 nm laser) in conjunction with 593 nm long-pass filters. DFO will still fluoresce under these conditions whereas the background fluorescence may be considerably reduced. This is particularly relevant for many types of brown and coloured paper.

4. Critical issues

4.1 The presence of methanol and *trans*-1,2-dichloroethylene in the formulation is essential for the optimum operational effectiveness. Formulations that substitute or omit these constituents will develop less highly fluorescent marks and fewer marks overall.

4.2 Heating of DFO-treated exhibits should be carried out in a dry oven with even heating via laminar airflow across each shelf; high levels of humidity equivalent to those used for ninhydrin are not beneficial for the reaction. Water is required for the reaction to occur, but a sufficient quantity is generated from the initial DFO reaction with amino acids and excess water is undesirable.

4.3 Appropriate excitation wavelengths and viewing filters must be selected when visualising developed marks. These are detailed in paragraphs 3.10 to 3.12 above. Light sources with higher output powers (e.g. lasers) will detect more marks.

4.4 If any separation of the working solution into oily droplets is observed, the solution should be discarded and not used for processing.

5. Application

5.1 Suitable surfaces: DFO is suitable for use on all porous surfaces, including paper, cardboard, raw wood and matt painted walls.

5.2 The principal application of DFO is in the development of fingermarks on porous items, in particular paper. It has been found to be the single most effective treatment for this surface and can be used as the first process in a sequential processing routine consisting of DFO – ninhydrin – physical developer. The use of DFO does not destroy marks that could have been developed by ninhydrin or physical developer and both processes can reveal further marks that have not been developed by DFO.

5.3 DFO is not as widely used as ninhydrin because it requires access to a forensic light source and appropriate viewing filters to see many of the marks developed. Consequently, ninhydrin is the method of choice for many laboratories processing volume crime exhibits because the marks are visible under normal lighting conditions and can be easily captured. However, ninhydrin is a less effective process (DFO typically develops 1.6 times more marks) and potential marks will be missed if it is used as a sole treatment.

5.4 DFO is also an effective blood dye, reacting strongly with the protein constituents in the blood to produce highly fluorescent marks. Heavy deposits of blood will reabsorb the fluorescence making this process less effective in these areas. It can therefore be used to enhance marks in blood on porous surfaces, but is not specific to the 'haem' component of blood and cannot be used to determine whether a mark is blood or not. The application of DFO has been shown not to affect subsequent recovery of DNA from marks deposited in blood [26].

5.5 DFO is applied in the laboratory by solution dipping, passing the exhibit through a shallow tray containing the DFO working solution, allowing it to dry then heating it in an oven at 100ºC for 20 minutes. Neither the exhibit nor the oven are humidified in any way. For larger items, such as boxes, DFO can be applied as a solution using a soft brush, again allowing the exhibit to dry before placing it in an oven.

5.6 DFO cannot be effectively used at scenes of crime. Although the solution can be applied using a brush, the conditions of temperature required to develop fingermarks in a reasonable time are not compatible with working at scenes. It is possible to apply heat locally using equipment such as a heat gun, but this is less effective than oven treatment and will still require long periods of heat application to develop marks, depending on the particular system used. Some heat guns are capable of heating to several hundred degrees centigrade and must therefore be used with caution.

6. Alternative formulations and processes

6.1 Since 1990 and the introduction of DFO, several different formulations have been investigated. Many of these were prompted by the search for alternative solvents after the banning of CFCs. A summary of some significant alternative formulations proposed is given below.

6.2 Bratton and Juhala [35] proposed a variation of the DFO formulation and process called 'DFO-Dry', which involved impregnating sheets of filter paper with a solution of DFO, allowing them to dry, then sandwiching paper exhibits between the impregnated sheets and applying heat from a steam iron filled with 5% acetic acid solution. Samples were then placed in a dry press at 110ºC for 10 minutes to complete development. The formulation used to impregnate the filter papers sheets was:

200 mL methanol, 200 mL ethyl acetate, 40 mL acetic acid, 1 g DFO.

6.3 Marks developed in this way were equal in intensity to those developed using a solution dipping process using the same formulation diluted with petroleum ether. The principal advantages of the dry process were that there was no ink run, no background staining and no background fluorescence.

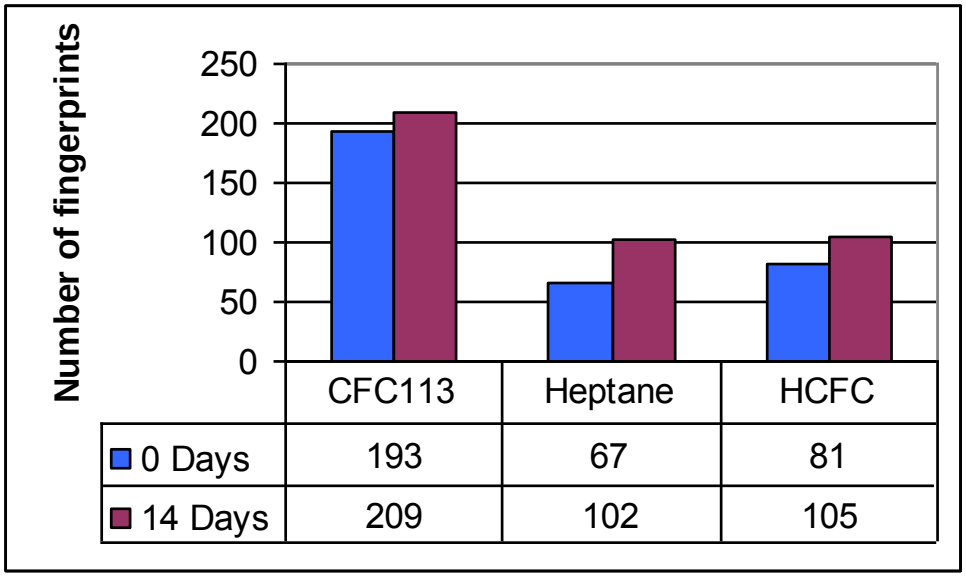
6.4 Petroleum ether was also proposed as a replacement solvent for CFC113 [14,18] but CAST would not recommend the use of this, or any other, highly flammable solvent in a laboratory because of the fire and explosion risks. It was found during testing by CAST that the formulation proposed by Masters *et al.*[14], containing propan-2-ol, xylene and acetone in addition to petroleum ether, developed brightly fluorescent fingermarks but caused significant damage to writing inks and was unstable when stored.

6.5 CAST carried out extensive studies into the identification of replacement solvents for CFC113, using a range of different solvent types including hydrocarbons, HCFCs and HFCs [22]. During these studies, initial evaluations were carried out using split depletions. Any formulations showing promise were taken forward to more detailed trials involving the treatment of a batch of 75 fraudulently passed cheques, using each formulation and counting all developed fingermarks with more than eight minutiae visible.

6.6 The best performing hydrocarbon and HCFC formulations are given below, together with their performance relative to the CFC113 formulation of batches of 75 cheques.

	CFC113	**Hydrocarbon**	**HCFC**
DFO	0.25 g	0.25 g	0.25 g
Methanol	30 mL		25 mL
Acetic acid	20 mL	20 mL	20 mL

Ethanol		100 mL	
Ethyl acetate		50 mL	
Methyl acetate			5 mL
Heptane		850 mL	
CFC113	1 L		
HCFC141b			1 L



Formulations based on hydrocarbons (heptane) and hydrochlorofluorocarbons (HCFCs) and their performance relative to the 1,1,2-trifluorotrichloroethane (CFC113) formulation.

6.7 Despite promising results from laboratory split depletion tests, neither of these formulations performed well when compared with the CFC113 formulation in a realistic trial.

6.8 Another non-CFC formulation evaluated was provided by the Bundeskriminalamt (BKA), Weisbaden, Germany, and consisted of:

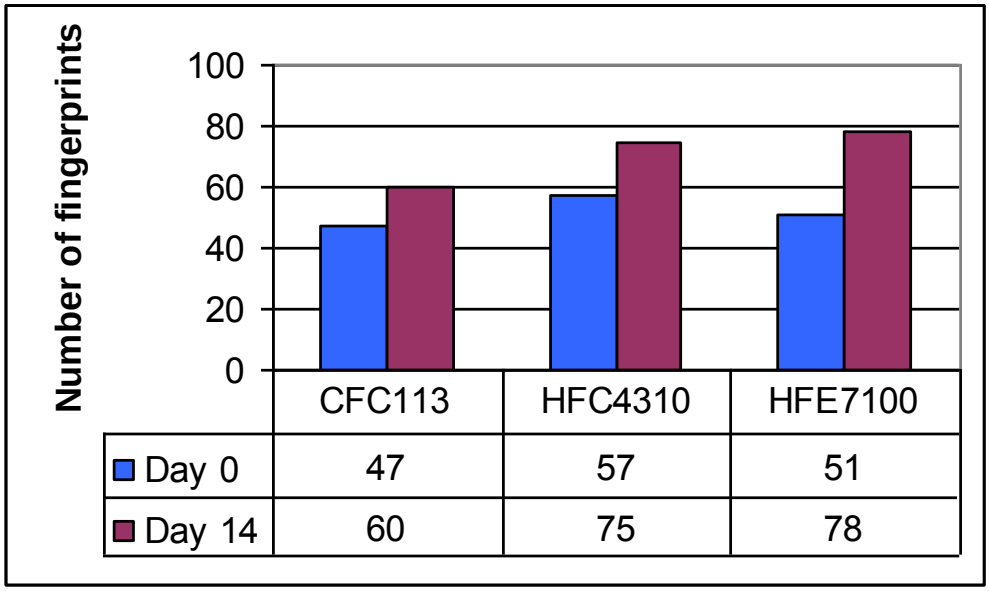
0.5 g DFO, 40 mL methanol, 20 mL acetic acid, 1 L *t*-butyl methyl ether.

6.9 This gave more fluorescent prints than the heptane formulation, but caused significant ink running. The solvent posed an explosion risk, and did not perform as well as the CFC formulation in comparative trials on batches of cheques.

6.10 The final class of solvents evaluated were HFCs, the most suitable of those evaluated being HFE7100 and HFC4310mee. The formulations were trialled against CFC113 and the results are shown below.

	CFC113	**HFC4310mee**	**HFE7100**
DFO	0.25 g	0.25 g	0.25 g
Methanol	30 mL	30 mL	30 mL

Acetic acid	20 mL	20 mL	20 mL
trans–1,2 dichloro-ethylene		100 mL	150 mL
HFC4310mee		1 L	
HFE7100			850 mL
CFC113	1 L		



Formulations based on hydrofluorocarbons and hydrofluoroethers and their performance relative to the CFC113 formulation.

6.11 Both formulations appeared to give superior performance to the CFC113 system and were taken to a full operational trial alongside it [21]. From this trial, the HFE7100-based formulation (with minor modifications) was ultimately recommended for operational use and is described in more detail in the CAST processes section above.

6.12 There are several DFO formulations in operational use worldwide. A survey of these has recently been conducted by Wallace-Kunkel *et al.* [29], the most commonly used being summarised in the table below.

% usage	DFO (g)	Methanol (mL)	Ethyl acetate (mL)	Acetic acid (mL)	Dichloromethane (mL)	Petroleum ether (mL)	HFE7100 (mL)	HFE71DE (mL)
18	0.25	40		20			940	
14	0.5	40		20			940	
11	0.5	100	100	20		780		
7*	0.25	30		20			725	275
4	0.2	50		20	50	880		

* currently (2016) recommended CAST formulation.

Compositions representative of 1,8-diazafluoren-9-one formulations used worldwide.

6.13 The two most commonly used formulations use HFE7100, but do not incorporate *trans*–1,2 dichloroethylene. CAST has found that formulations without this component are less effective and would therefore recommend its inclusion.

6.14 Formulations based on petroleum ether are not recommended by CAST because of the fire and explosion hazards associated with the solvent, and CAST would seek to minimise use of dichloromethane where possible due to health and safety concerns.

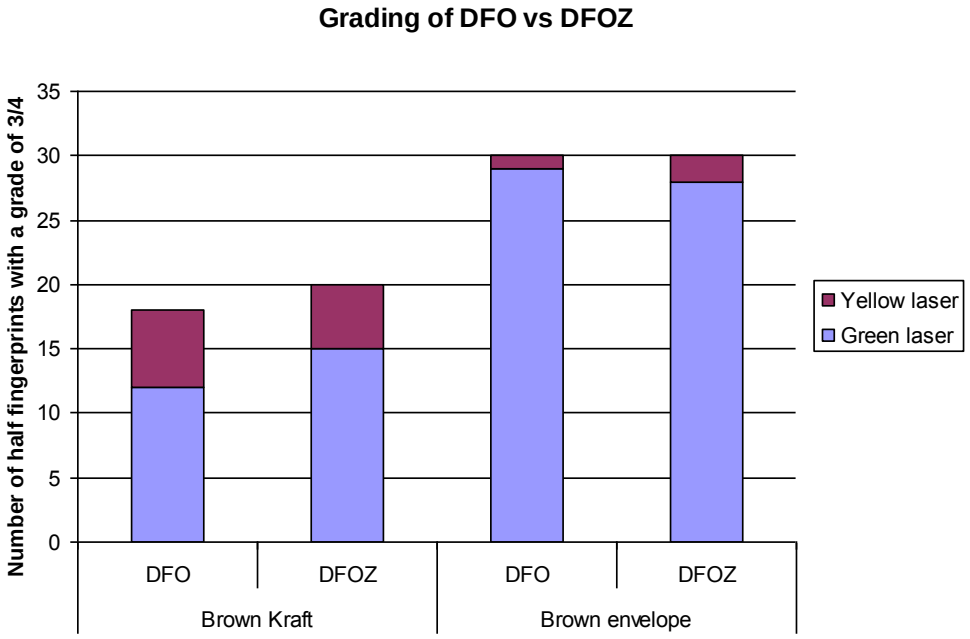
6.15 A modified formulation has been proposed by CAST for the treatment of thermal receipts [27]. When thermal receipts are treated with DFO they blacken due to reaction between acetic acid and the thermal ink layer, blackening also occurring due to the heat in the oven used to develop marks. To counteract this, CAST carried out trials and devised a formulation with the amount of methanol increased to 60 mL. This dissolves away the thermal ink layer and significantly reduces subsequent blackening. The thermal paper is retained in the dip bath until all black deposit is removed from the surface of the paper, then placed into the oven. In practice, this did reduce the problems associated with blackening of thermal receipts but as ink compositions changed it did not prove possible to easily remove all of the ink layer in this way. Pre-dipping the receipt in ethanol until all text disappears and then allowing it to dry prior to dipping in a solution of the standard formulation has proved more effective [36]. The CAST *Fingermark Visualisation Manual* recommends the use of acetone as a pre-dipping solution.

6.16 Although initial work by Conn et al [25] indicated that metal salt post treatments had little influence on the effectiveness of DFO, the success of adding zinc chloride directly into 1,2 indandione formulations prompted work by CAST to explore whether a similar approach could be used for DFO. The resultant DFO-zinc chloride (DFOZ) formulation devised by CAST [33] is as follows:

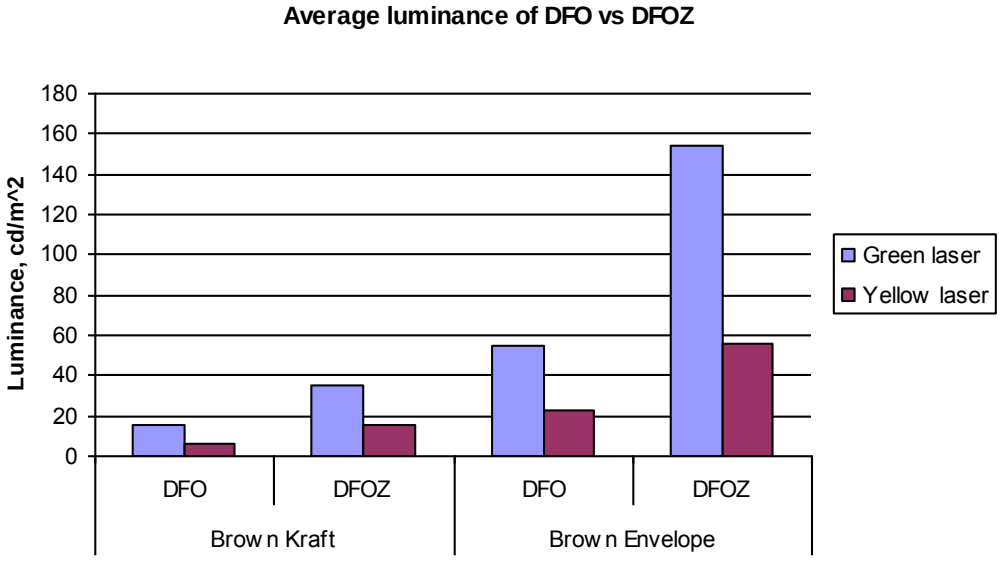
0.125 g DFO
15 mL Methanol
10 mL Acetic acid
137.5 mL HFE 71DE
362.5 mL HFE 7100
0.25 mL zinc chloride solution (0.2 g zinc chloride in 5 mL methanol)

6.17 Comparisons were conducted between this formulation and the standard DFO formulation using single sheets of brown kraft paper and brown kraft envelope [33]. 30 different donors were asked to provide two single fingermarks on each substrate. The fingermarks were aged for 4 days then cut vertically through into strips each containing 10 half fingermarks. One half of the strip was processed with the original DFO formulation and the other half processed with the novel DFOZ formulation, then examined and graded under illumination from both a green (532 nm) and yellow (577 nm) laser. In addition to grading the quality of the mark,

measurements of the intensity of the resultant fluorescence were also made, the results being illustrated below.



Results obtained from grading of fingermarks on brown kraft papers treated with DFO and DFOZ formulations [33].



Results obtained from measuring luminance of fingermarks on brown kraft papers treated with DFO and DFOZ formulations [33].

6.18 The results indicate that DFOZ has the potential to develop a greater number of high quality marks with more intense fluorescence than the conventional DFO formulation. This does make the formulation of interest for further research, but some subsequent studies indicate that the formulation is sensitive to fluctuations in laboratory temperature and

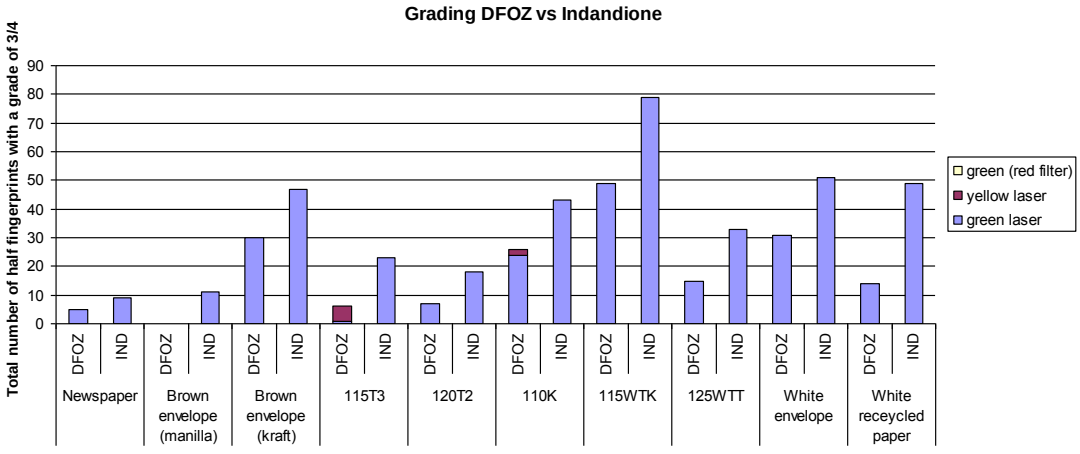
can precipitate out rapidly when the temperature is too low. For this reason, most comparative studies have focused on 1,2 indandione rather than refinements to DFO. This decision has been reinforced by comparative tests conducted between DFOZ and 1,2 indandione-zinc [33], as summarised below.

6.19 In the comparison between DFOZ and 1,2 indandione-zinc a wide range of surfaces was used, these being:

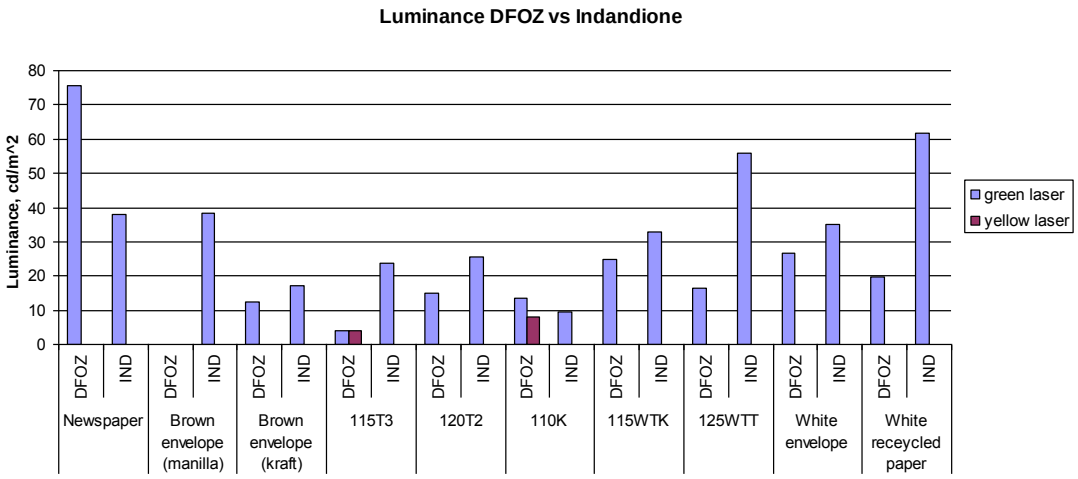
110 g/m^2 Kraft (brown, mostly virgin cellulose fibres)
120 g/m^2 Test 2 (high content of recycled fibres)
115 g/m^2 Test 3 (enitrely recycled fibres, recycled multiple times)
115 g/m^2 White Top Kraft (WTK)
125 g/m^2 White Top Test (WTT).
Brown envelope (kraft)
Brown envelope (manilla).
White envelope
Newspaper
White recycled paper.

The white envelope, newspaper and white recycled paper were also included in this experiment to determine whether either of the two reagents would provide an improvement in fluorescence on a wider variety of substrates.

6.20 20 donors deposited split depletions on each of the 10 surfaces, giving 280 half fingermarks on each substrate in total. After splitting, one half of the substrate was processed using DFOZ and the other with 1,2 indandione-zinc. Developed marks were assessed in terms of both quality and intensity of fluorescence.



Results obtained from grading of fingermarks on various types of paper treated with DFOZ and 1,2 indandione formulations [33].



Results obtained from measuring fluorescence intensity of fingermarks on various types of paper treated with DFOZ and 1,2 indandione formulations [33].

6.21 It was observed that 1,2 indandione-zinc outperformed DFOZ on the majority of surfaces in terms of both the mark quality and the luminance.

7. Post-treatments

7.1 There are no post-treatments used with DFO other than the examination of the developed mark using fluorescence, which is described above. Toning using metal salts is ineffective and does not increase the fluorescence of the mark.

8. Validation and operational experience

8.1 Laboratory trials

8.1.1 Although laboratory trials were conducted during the initial development of DFO formulations in the early 1990s, most of these results no longer survive. It has been found from experience that planted prints rarely give operationally representative results in such trials, typically performing worse than seen on casework. This is possibly because perpetrators of crimes may be under increased stress and sweat more, giving more eccrine prints than seen in the laboratory. As a consequence, development of revised formulations at CAST is usually carried out using small-scale comparative tests until best performing formulations are identified, after which testing proceeds to pseudo-operational trials using realistic items such as bundles of cheques.

8.1.2 One exception to this is the recent comparison between DFO and 1,2 indandione/zinc, carried out using split depletions on a range of different substrate types. This study showed closely equivalent performance between DFO and the 1,2 indandione/zinc formulation studied, and is

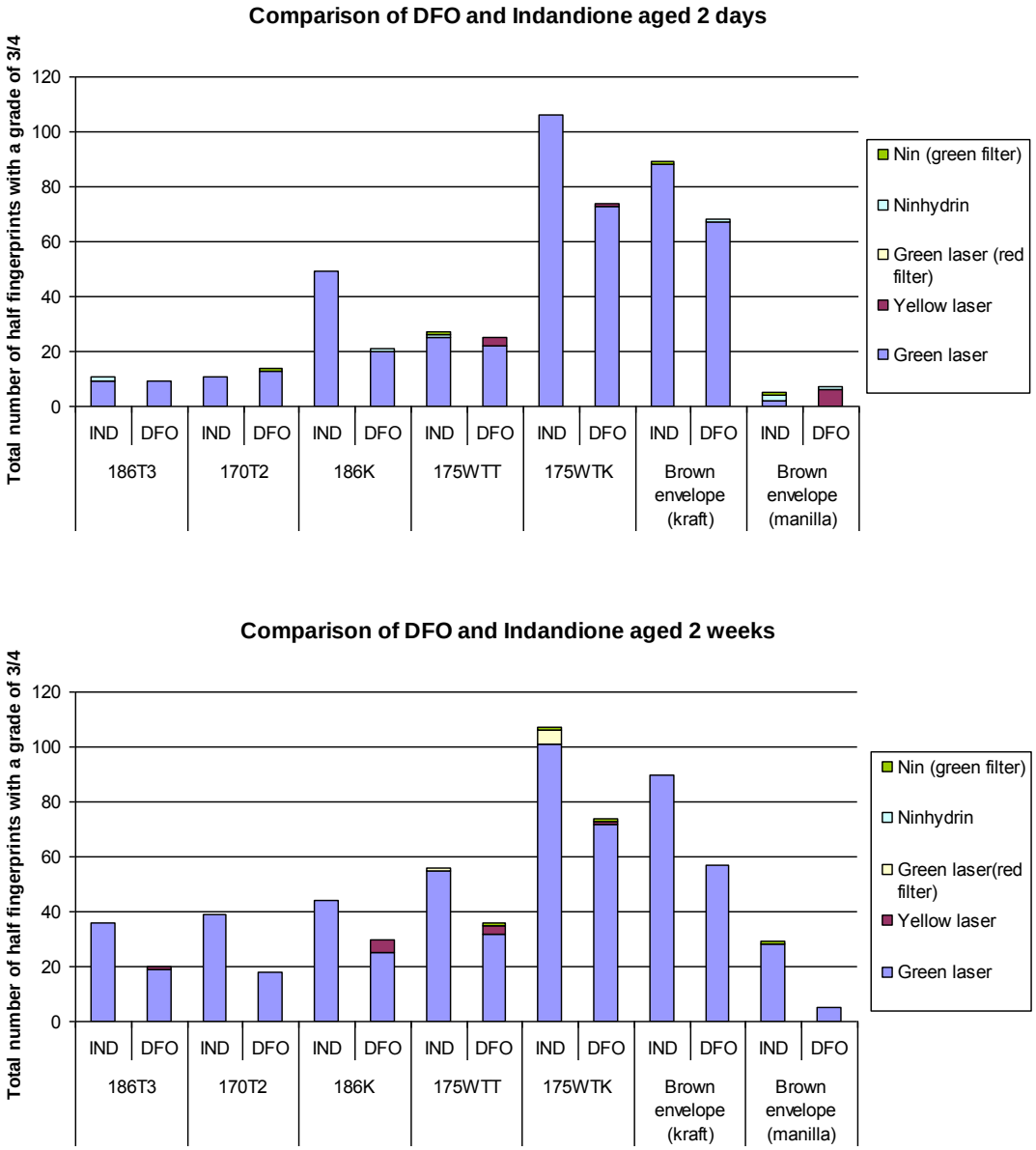
more fully reported in Chapter 3, Chemical and Physical Processes, 1,2 Indandione.

8.1.3 The trial referred to above did make the observation that one type of porous surface that 1,2 indandione consistently outperformed DFO on was brown paper. As a consequence, a focused study was conducted to compare the effectiveness of DFO and 1,2 indandione on brown paper and cardboard [33].

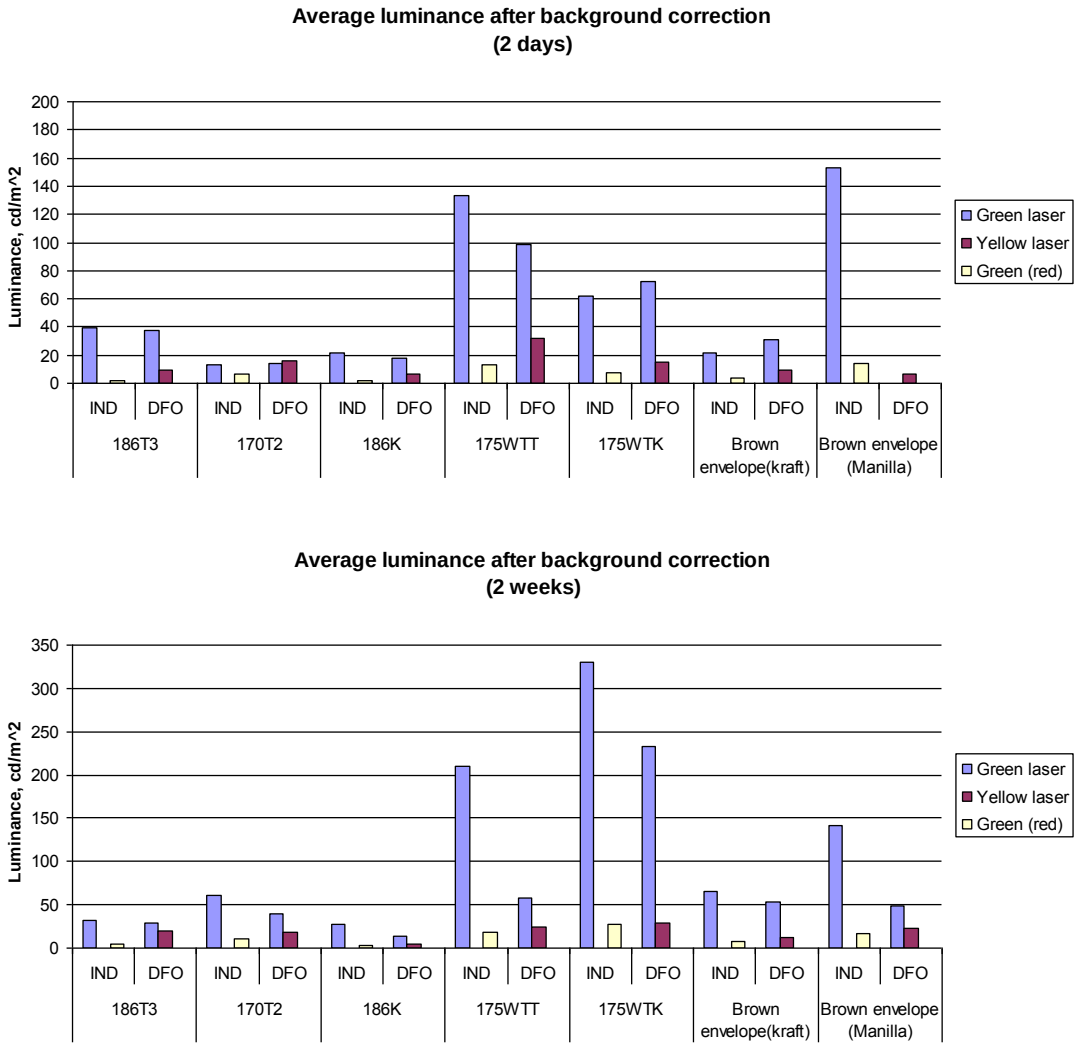
8.1.4 In this study, split depletion series of marks were deposited by 20 people consisting of a mixture of good, medium and poor donors on the following types of cardboard/kraft paper surfaces:
110 g/m^2 Kraft (brown, mostly virgin cellulose fibres)
120 g/m^2 Test 2 (high content of recycled fibres)
115 g/m^2 Test 3 (enitrely recycled fibres, recycled multiple times)
115 g/m^2 White Top Kraft (WTK)
125 g/m^2 White Top Test (WTT).
Brown envelope (kraft)
Brown envelope (manilla).

8.1.5 The surfaces included white top papers for cardboard boxes to obtain information about the effect of the colour of the substrates (the fundamental composition being similar to the brown papers). Fingermarks were aged for 2 days and 2 weeks before being split, one half being treated with the Home Office 1,2 indandione-zinc formulation (as described in the 1,2 Indandione chapter, 5.7) and the other with the standard DFO formulation. Marks were graded using illumination from both a green (532 nm) and yellow (577 nm) laser with appropriate viewing filters.

8.1.6 Both the strips initially processed with DFO and 1,2-indandione were subsequently processed with ninhydrin. The fingermarks were re-graded and any additional grade 3/4 fingermarks were recorded, results being shown below in terms of both quality of developed marks and their intensity of fluorescence.



Results obtained from grading of fingermarks on various kraft papers treated with DFO and 1,2 indandione formulations, (top) *for 2 day old marks, and* (bottom) *for 2 week old marks [33].*



Results obtained from measuring fluorescence intensity of fingermarks on various kraft papers treated with DFO and 1,2 indandione formulations, (top) *for 2 day old marks, and* (bottom) *for 2 week old marks [33].*

8.1.7 Although the results from 2 day old fingermarks were less conclusive, it is apparent that on this type of substrate 1,2-indandione-zinc consistently outperforms DFO in terms of both quality and fluorescence of the marks developed.

8.2 Pseudo-operational trials and operational experience

8.2.1 Several pseudo-operational trials were conducted on alternative DFO formulations during research into a replacement solvent for CFC113. The results of these have been summarised in the section on 'Alternative formulations' above. The outcome of these studies was that the formulation based on HFE7100 solvent was selected for comparative trials with the CFC113-based DFO formulation.

8.2.2 There have also been several pseudo-operational and operational trials conducted to establish the relative effectiveness of the DFO and ninhydrin techniques and also to establish the best sequence of treatment. Before publication of the initial reports on DFO, operational trials were conducted at Surrey Police and the Metropolitan Police Serious Crimes Unit.

8.2.3 The trial at Surrey [2] involved treatment of the exhibits using DFO followed by laser examination, then ninhydrin treatment. An assessment was made of the number and quality of the marks developed using each process. The results of this trial were:

DFO > Ninhydrin 139 articles (69.8%);
Ninhydrin > DFO 13 articles (6.5%);
DFO = Ninhydrin 47 articles (23.6%).

8.2.4 The Metropolitan Police trial [3] involved a direct comparison of the effectiveness of DFO and ninhydrin when used as a single process on casework, and also looked at the impact of zinc chloride treatment on marks developed using ninhydrin. The results are summarised below:

DFO – 510 marks from 168 articles;
Ninhydrin – 1,135 marks from 1,356 articles;
Ninhydrin + zinc chloride – 1,249 marks from 1,356 articles.

8.2.5 Both these trials indicated significant benefits in the use of DFO, with more marks being developed than found using ninhydrin. DFO was found superior to ninhydrin even after zinc chloride toning had been used to make marks fluorescent.

8.2.6 HO SRDB also conducted pseudo-operational trials in 1990 [37], looking at the numbers of marks developed on batches of brown and white envelopes using DFO, ninhydrin and the DFO-ninhydrin sequence. Articles were examined visually and then using fluorescence examination to enhance the DFO marks. The results of this exercise are tabulated below.

Visible examination				
	Ninhydrin		**DFO**	
	Brown	White	Brown	White
Articles	93	93	93	93
Fingermarks	18	24	6	16
Articles with fingermarks	11	14	6	11
% Articles with fingermarks	12	15	6	12

Fluorescence				
	Ninhydrin		DFO	
	Brown	White	Brown	White
Articles	93	93	93	93
Fingermarks	19	24	60	91
Articles with fingermarks	12	14	33	50
% Articles with fingermarks	13	15	35	54

Ninhydrin after DFO				
	New fingermarks		Overall	
	Brown	White	Brown	White
Articles	93	93	93	93
Fingermarks	9	10	15	26
Articles with fingermarks	7	8	12	16
% Articles with fingermarks	8	9	13	17

Results obtained from pseudo-operational trial on batches of envelopes.

8.2.7 Hardwick *et al.* [15] also carried out trials at PSDB in the early 1990s, comparing the original formulation issued by Pounds *et. al*. [7] with revisions to the process suggested by PSDB, including reductions in the amount of DFO, single dipping and increasing the heat treatment time to 20 minutes. The study looked at 200 cheques, 100 from each of two banks, divided into two sets with 50 cheques from each bank. In this trial, both formulations developed just over 200 marks with >8 points ridge detail and so the reduction in DFO (and therefore in the cost of the formulation) was not felt to be detrimental to performance and was recommended operationally. Subsequent treatment of these exhibits with ninhydrin developed an additional 10% of marks.

8.2.8 A direct comparison of the effectiveness of ninhydrin and the revised DFO formulation was also carried out. This study looked at 300 cheques, 100 from each of three banks, divided into batches containing 50 cheques from each bank. In this study DFO gave 60% more fingermarks than ninhydrin, in accordance with all previous studies.

8.2.9 All the studies above utilised DFO and ninhydrin formulations based on CFC113. As this solvent was being withdrawn from operational use, operational trials were conducted to compare the effectiveness of the replacement solvent formulations with CFC113, also to compare the effectiveness of DFO with 1,2 indandione, a new reagent being proposed as an alternative one-step fluorescent treatment for porous surfaces (see

Chapter 3, Chemical and Physical Processes, 1,2 Indandione for further details).

8.2.10 Merrick *et al.* [21] carried out an operational trial at West Midlands Police in conjunction with PSDB. This was carried out over 7 weeks, examining over 650 articles at an average of 2.26 articles per case and counting fingermarks containing >8 points. The trial compared the CFC113 DFO formulation, the DFO formulations based on HFC4310mee and HFE7100 described in the section above, and a 1,2 indandione formulation based on HFE7100. The results are summarised in the tables below.

Formulation		**Week**						
		1	**2**	**3**	**4**	**5**	**6**	**7**
DFO (CFC)	Marks	86	91	109	132	156	201	214
	Cases	67						
DFO (HFC)	Marks	46	59	76	99	104	158	171
	Cases	66						
DFO (HFE)	Marks	93	97	130	144	174	213	218
	Cases	70						
IND (HFE)	Marks	70	89	92	105	116	149	164
	Cases	68						

Cumulative number of identifiable fingermarks developed with 1,8-diazafluoren-9-one and 1,2 indandione formulations, and total number of cases processed.

Formulation	**Week**						
	1	**2**	**3**	**4**	**5**	**6**	**7**
DFO (CFC)	69.6	61.3	61.5	59.2	57.9	61.5	59.7
DFO (HFC)	56.0	60.6	61.0	58.8	55.2	59.1	59.7
DFO (HFE)	78.3	71.0	69.2	65.3	63.2	66.2	62.5
IND (HFE)	52.4	51.6	46.5	49.0	49.1	55.4	54.2

Cumulative proportion of cases producing identifiable fingermarks.

8.2.11 The results showed that the HFE7100-based formulation gave equivalent, if not better, performance to the CFC113 formulation and this was therefore recommended for operational use by PSDB.

8.2.12 A similar trial was carried out by the Royal Canadian Mounted Police (RCMP) [38], assessing the CAST DFO formulation based on HFE7100, an alternative DFO formulation based on HFE7100 but without *trans*-1,2-dichloroethylene, and a 1,2 indandione formulation based on HFE7100.

8.2.13 Preliminary trials were conducted on 80 cheques, which indicated that the CAST formulation gave the best results. The study then proceeded to

an operational field trial, the interim results of which are summarised below:

DFO (alternative HFE7100 formulation): 303 exhibits, 66 identifiable marks;
DFO (CAST HFE7100 formulation): 440 exhibits, 126 identifiable marks;
1,2 indandione (HFE7100-based):165 exhibits, 7 identifiable marks.

8.2.14 The PSDB DFO formulation was therefore adopted by RCMP for operational work. However, pseudo-operational trials between DFO and 1,2 indandione conducted on batches of 1000 cheques by Israeli researchers around the same time [39] indicated that 1,2 indandione gave better performance and the reagent was increasingly adopted in favour of DFO in Israel.

8.2.15 More recently there have been several papers reporting reformulations of 1,2 indandione to incorporate zinc salts as an integral constituent of the dip solution rather than as a post-treatment. Research has been conducted to compare the effectiveness of these revised formulations with DFO [30,31,32]. To some extent the results of these have been conflicting, with some researchers [30, 32] finding 1,2 indandione performing better, and others [31] finding DFO to give marginally better performance. Further refinements have since been made to the 1,2 indandione-zinc formulations and it is becoming evident that this reagent may now give improved performance over DFO under UK conditions. Further validation work was required to demonstrate this, and the overall impact of replacing DFO with 1,2 indandione on the total number of marks recovered during sequential processing has been assessed. This study is more fully reported in Chapter 3, Chemical and Physical Processes, 1,2 Indandione.

8.2.16 Another pseudo-operational trial that was conducted by CAST has been the comparison of DFO, ninhydrin and 4-dimethylaminocinnamaldehyde (DMAC) for the development of marks on thermal receipts [40]. In this study DFO was found to significantly outperform the other two processes, yielding almost twice the number of marks. This study is more fully reported in Chapter 3, Chemical and Physical Processes, 4-Dimethylaminocinnamaldehyde (DMAC).

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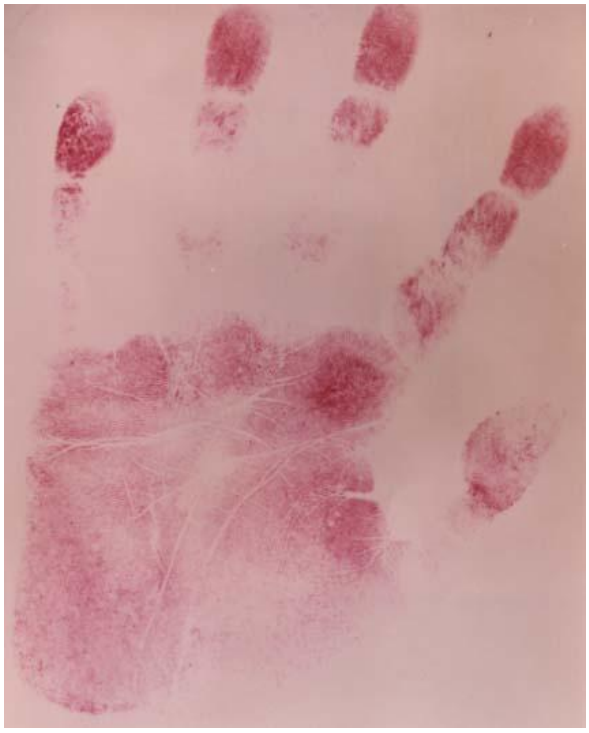
4-Dimethylaminocinnamaldehyde (DMAC)

1. History

1.1 4-Dimethylaminocinnamaldehyde (DMAC) was first proposed as a fingermark development reagent in the UK by Morris *et al.* in 1973 [1] and was believed to react with the urea present in eccrine fingermark secretions. In the initial work conducted at AWRE, DMAC appeared to be more sensitive than the ninhydrin formulations and processing conditions then in use, and it was decided to proceed to operational implementation in 1973. For operational use DMAC was dissolved in a mixed ethanol/ chlorofluorocarbon (CFC) solvent and the articles to be treated immersed in the solution until visible marks developed. When DMAC reacts with urea under acidic conditions it gives a magenta coloured product within two minutes, the developed mark providing good contrast with the background.



Palm mark developed using 4-dimethylaminocinnamaldehyde solution.

1.2 The operational trials in the UK were conducted in a limited number of police forces and abandoned after only a few months as the performance of DMAC was found to be poor in terms of fingermark yield compared to ninhydrin. Many of the marks that were developed were also diffuse and lacking in ridge detail. As a consequence the use of DMAC as a solution dipping process was discontinued in the UK by the mid-1970s.

1.3 Soon afterwards, Sasson and Almog [2] conducted comparative tests between DMAC and ninhydrin and concluded that although ninhydrin was a more general and versatile reagent, DMAC was preferable to

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ninhydrin on fresh marks (up to 72 hours old) in situations where the application of heat is not possible but detrimental diffusion was observed for marks older than this. Van Enckevort [3] found DMAC, when sprayed or dipped, to be useful on a wide range of substrates in laboratory trials, particularly those that showed a high background development with ninhydrin. However, he too found the reagent to be less successful in operational trials with the marks visualised showing blurred ridge detail, which was attributed to the diffusion of urea. He observed that useful marks were only obtained up to three to ten days after deposition and consequently found little use for the reagent when used as a solution-based reagent developing visible marks. It should be noted that none of these researchers studied developed marks using fluorescence examination.

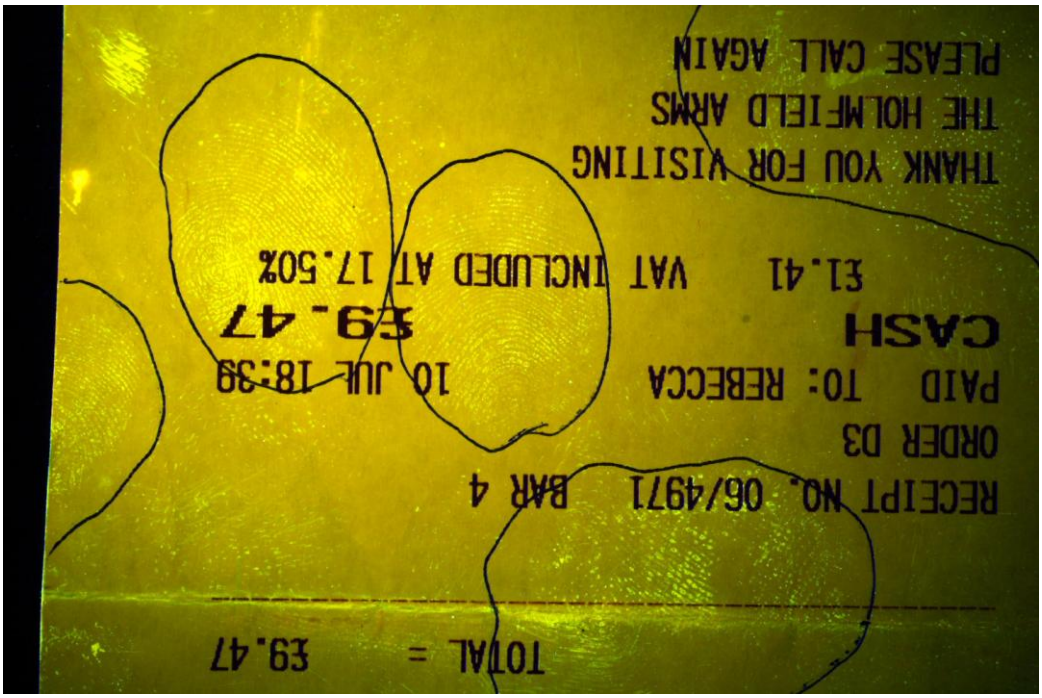
1.4 DMAC was later investigated as a fuming agent and was found by Brennan *et al.* [4] to give good ridge detail visualisation on a wide selection of substrates, with potential to be included in routine sequential examination procedures. Katzung [5] reported that marks developed using DMAC fuming showed yellow fluorescence under excitation using 360nm light sources and that he had managed to detect four-week-old marks using this method.

1.5 Although vapour phase fuming can offer an answer to problems associated with solvent based fingermark techniques, some researchers have described the limitations and scope of the reagent's ability to produce visible marks. Brennan [6] reported that for cases involving porous items other than thermal papers, all the marks developed by DMAC were subsequently developed by 1,8-diazafluoren-9-one (DFO), ninhydrin or physical developer and concluded that DMAC fuming was less effective than existing processes on such articles. On thermal papers, however, marks were developed on the thermal surface that would otherwise have been lost using other methods. This study was further reported by the Metropolitan Police Serious Crimes Unit [7] which emphasised the potential of vapour phase fuming with DMAC and subsequent visualisation of the fluorescence using a laser as a powerful non-destructive technique that does not interfere with following sequential treatments. It was regarded as having particular potential for detecting marks on thermal papers.

1.6 In the mid-1990s, the use of DMAC as a 'contact transfer' development process was proposed by Ramotowski [8] for development of fingermarks on paper. This approach involves pressing an exhibit between two sheets of paper that have been soaked with DMAC solution and subsequently dried, resulting in a pale yellow colouration to the paper and barely visible marks that give yellow fluorescence when illuminated with green light.

1.7 Experiments have also been carried out to investigate the use of the contact transfer process on the polymer banknotes used in Australia, looking at different temperatures and exposure times [9]. Results

indicated that contact transfer at room temperature was not particularly successful, with results demonstrating poor contrast between the notes and marks treated up to four hours. They also found that heat contact transfer at various temperatures using an ironing press for 20 seconds developed high background luminescence and the contrast between the developed fingermark and background was very low. The contact transfer technique has since been proposed for development of fingermarks on thermal papers with the stated advantages that it leaves the printed text intact and does not cause the thermal receipt to blacken during processing.

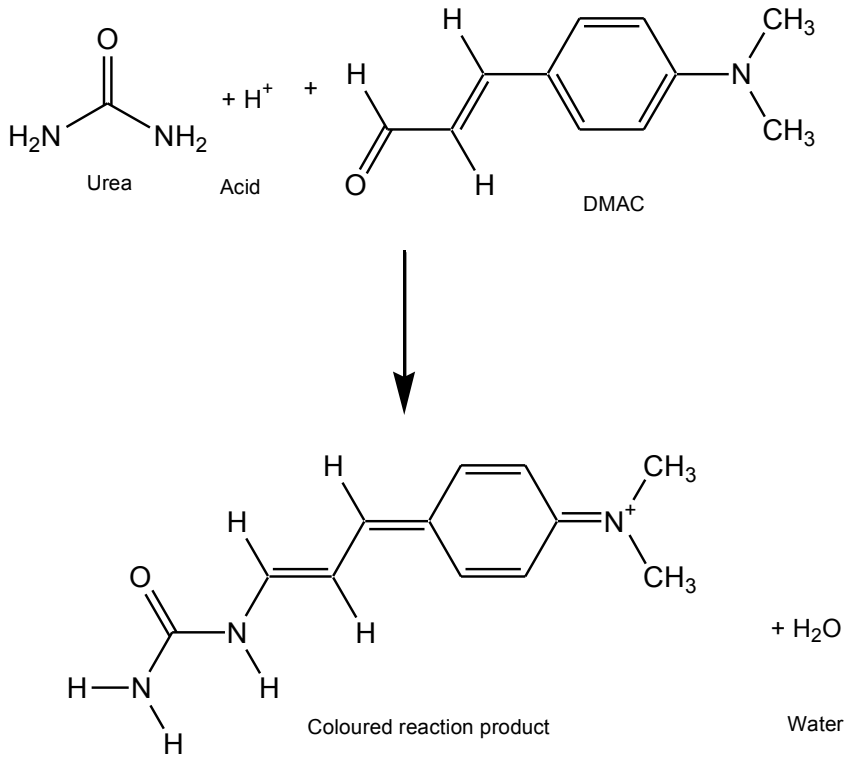


Fingermarks developed using contact transfer 4-dimethylaminocinnamaldehyde process.

1.8 DMAC and the closely related compound p-dimethylaminobenzaldehyde (DMAB) have been more recently reassessed by other workers [10,11,12]. Although DMAB was found to be less effective than DMAC and therefore not considered further for operational use, a revised formulation of DMAC was produced. This was found to reliably develop latent fingermarks on both standard paper and thermal paper surfaces, and to develop fingermarks up to 6 months after deposition. In comparative trials with a 1,2 indandione/zinc formulation, DMAC was marginally less sensitive overall.

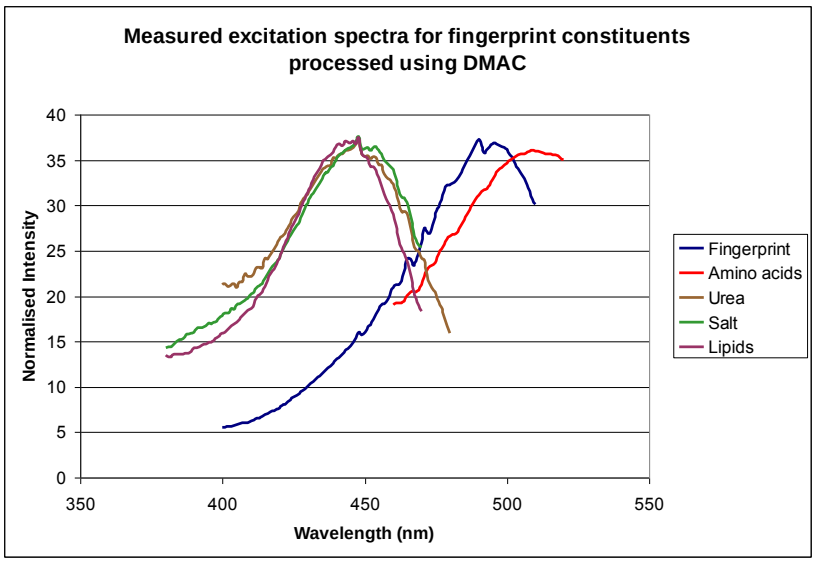
2. Theory

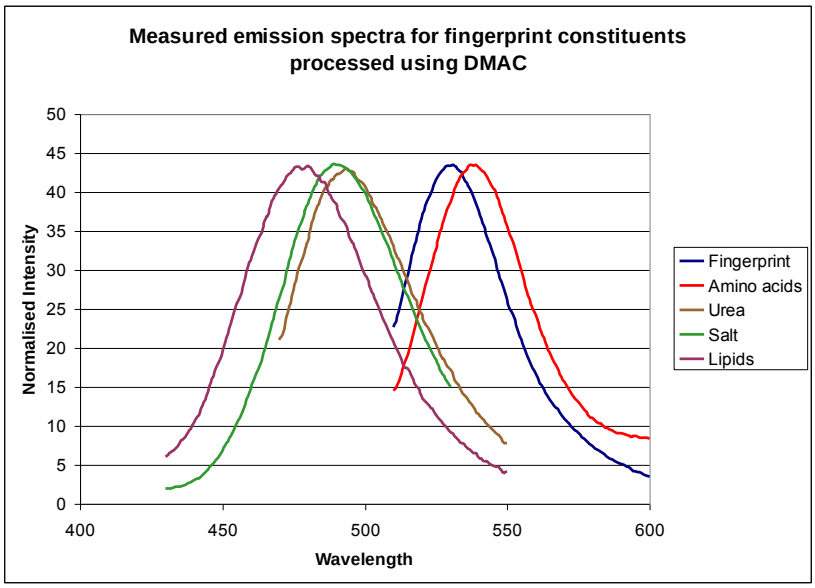
2.1 The reaction mechanism for the original solution treatment form of DMAC was the formation of a coloured Schiff base by the reaction between DMAC and urea under acidic conditions.



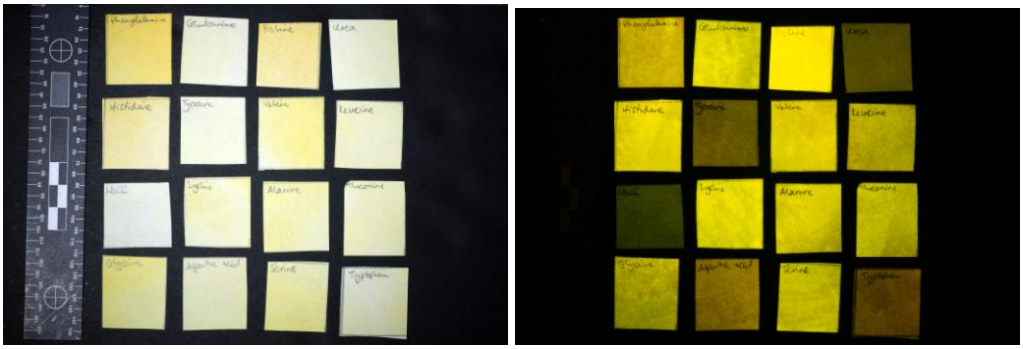
Proposed mechanism for formation of coloured product from reaction between 4-dimethylaminocinnamaldehyde and urea under acid conditions.

2.2 The precise mechanism by which fluorescence occurs in the contact transfer process is not known, but spectroscopy has been carried out by the Home Office Centre for Applied Science and Technology (CAST), which indicates that when used as a contact transfer process DMAC interacts with amino acid constituents (and potentially other sources of primary amines) in the fingermark rather than urea. Similar results were obtained by other researchers [12]. A more detailed analysis by Fritz et al [12] indicated small shifts in the maximum emission for the reaction product formed with different amino acids, and it is possible that a range of reaction products are in fact formed.





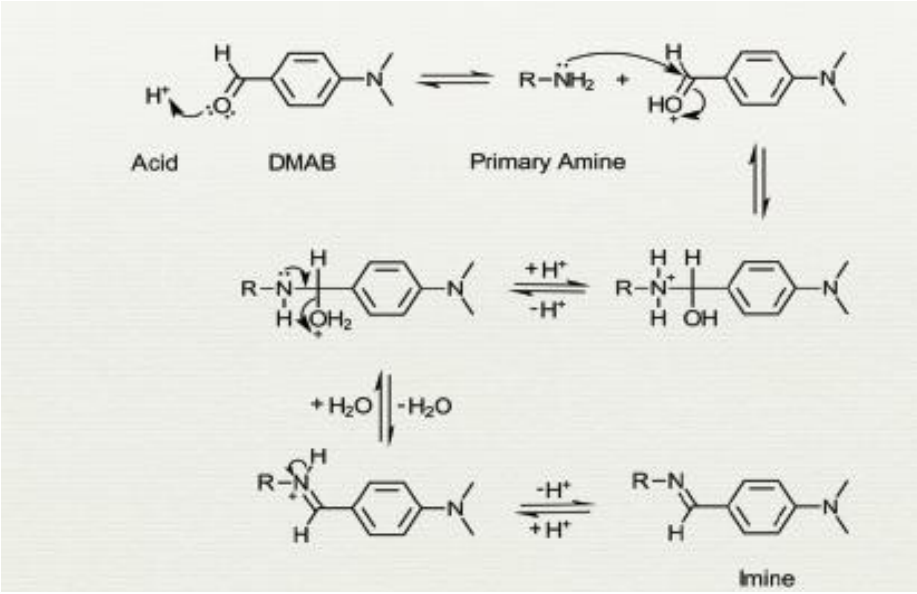
Excitation and emission spectra obtained for filter paper pad impregnated with fingermark deposits and model fingermark constituents, then treated with the 4-dimethylaminocinnamaldehyde contact transfer process.



a) b)

Reaction products formed between 4- dimethylaminocinnamaldehyde and 0.1M solutions of amino acids and other fingermark constituents a) *visible, and* b) *fluorescence.*

2.3 The nature of the fluorescent reaction products formed has not been conclusively determined, but Fritz et al [12] propose a generalised reaction scheme for DMAC and DMAB with primary amines to form imines, and provide a suggestion as to where the colour (and luminescence arises) from on reaction of these compounds with primary amines under the appropriate conditions.



Proposed reaction scheme for DMAB (and DMAC) with primary amines, resulting in the formation of imines [12].

2.3 The formulation originally used for solution dipping was a two-part system made up as follows.

Solution A: mix 650 mL of 1,1,2-trifluorotrichloroethane (CFC113) with 350 mL of absolute ethanol. Take 750 mL of the mixed solvent, add 5 g

of DMAC and stir until dissolved, then make up to 1 L with remainder of solvent, filter and store in a brown bottle.

Solution B: mix 650 mL of CFC113 with 350 mL of absolute ethanol. Add 20 g of 5-sulphosalicylic acid and stir until dissolved.

2.4 A working solution is made by mixing together equal proportions of solutions A and B, and articles are then dipped. Spray application is possible, but in this case the surface to be treated is first sprayed with solution A, followed by a second spray of solution B. More recent formulations since the ban on the use of CFCs utilise ethanol as the single solvent for both DMAC and 5-sulphosalicylic acid.

2.5 The contact transfer process utilises sheets of paper immersed in a solution of 0.25 g of DMAC dissolved in 100 mL of ethanol. Initially 1 g of 5-sulphosalicylic acid was also added to this solution, but it is not critical to the process and often now omitted. The sheets are then allowed to dry. The article to be treated is sandwiched between two sheets of impregnated paper, placed in a press and left overnight.

2.6 The revised wet contact process proposed by Fritz et al [12] contains 0.028 g of DMAC in 0.84 mL of glacial acetic acid, 6.2 mL of ethyl acetate and 993 mL of petroleum ether. This is stated to develop luminescent ridge detail within 3 hours of treatment, without the application of heat.

2.7 The DMAB formulation used by Frick et al [10] consists of:

Concentrated solution:	
DMAB	1 g
Ethyl acetate	22 mL
Acetic acid	3 mL

Working solution:	
Concentrated solution	10 mL
HFE7100	90 mL

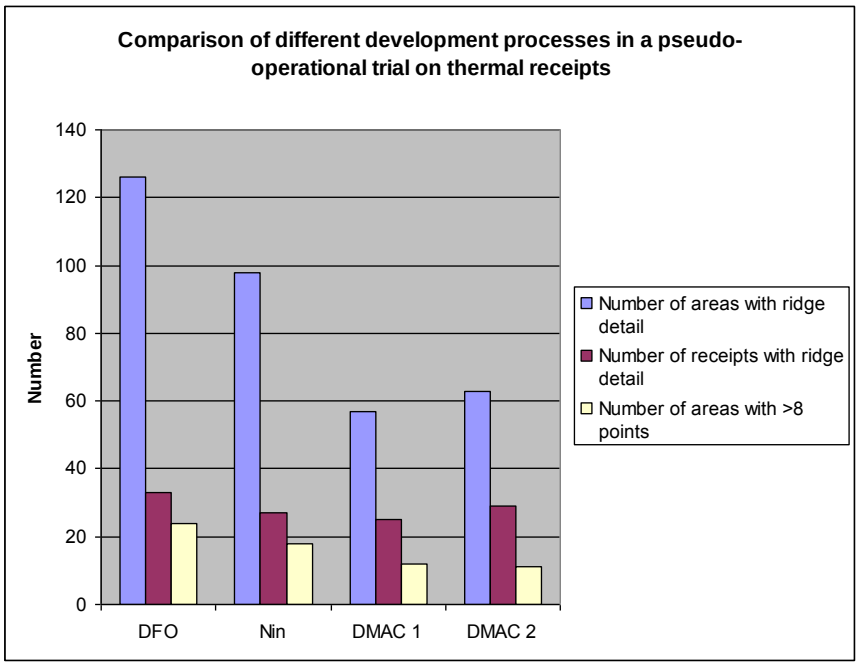
Articles are dipped in the working solution, allowed to dry the heated in a dry oven at 150°C for 20 minutes or in a hot press at ~160°C for 20 seconds.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

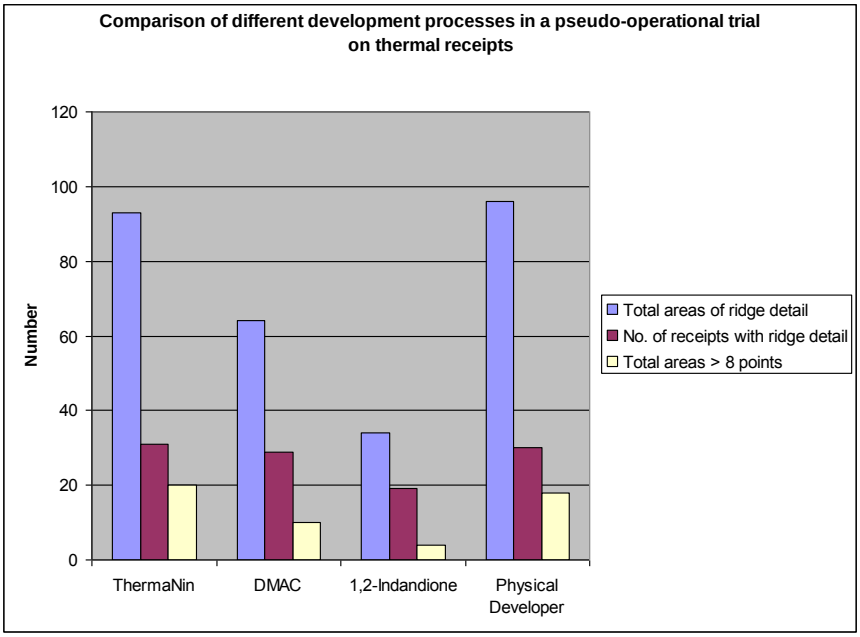
3.1 DMAC is not recommended as a Category A process by CAST in either solution dipping or contact transfer form. Operational experience in the 1970s demonstrated that the solution dipping process was not suitable for marks more than a few days old because of the rapid diffusion of the urea constituent. The original solution dipping formulation is based on CFCs and would not be acceptable for use without reformulation to a

less ozone-depleting solvent, and formulations that just omit CFC and use ethanol as solvent are highly flammable.

3.2 More recently, CAST conducted experiments to compare the effectiveness of DMAC against DFO and ninhydrin for cases where it is not necessary to retain printed text on thermal receipts. A further comparison was conducted with ThermaNin, 1,2-indandione and physical developer for cases where it is necessary to retain printed text on thermal receipts. In both these cases, pseudo-operational trials confirmed laboratory experiments, and in neither case was DMAC found to be as effective as processes currently recommended by CAST [13,14,15].



a)



b)

Results of pseudo-operational trials conducted on batches of thermal receipts comparing the effectiveness of the contact transfer 4-dimethylaminocinnamaldehyde process with a) *techniques removing printed text, and* b) *techniques leaving printed text visible.*

3.3 The contact transfer DMAC process is included in the *Fingermark Visualisation Manual* [16] as a Category B process. It is recognised that the ability of the process to retain printed text on the receipt whilst developing marks may be of benefit in some applications and therefore outline processing instructions are provided.

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Electrochemical techniques

Etching and electrodeposition

1. History

1.1 Untreated metal surfaces present an unusual problem for fingermark development. While the majority of non-porous surfaces received in laboratories are effectively inert, in the case of metals there is the potential for chemical reactions to occur between constituents of the fingermark (e.g. salts) and the metal surface. In extreme circumstances this can result in a permanent record of the fingermark being etched into the metal surface. However, the interactions that occur are very dependent on the metals present, their previous environmental exposure (which will influence formation of surface oxide films) and the particular constituents in the fingermark. Reactions will only occur if conditions are favourable. In many cases the metal will be alloyed with other elements, e.g. chromium in 'stainless steel', or coated with other elements, e.g. galvanizing with zinc, for the purposes of surface protection against corrosion. In both instances, the composition of the surface (upon which the fingermark is deposited) may be quite different from the composition of the bulk.

1.2 It is possible to utilise the chemical reactions that can occur between a metal, the fingermark constituents and a chemical solution to visualise fingermarks on this type of surface. Essentially, there are two generic types of technique that can be applied, etching and electrodeposition. The common feature is that the fingermark deposit provides spatial selectivity of reaction. In etching techniques, material is selectively dissolved from the surface and into a solution. If the fingermark constituents either enhance or inhibit the rate of etching at the fingermark ridge relative to that of the background, there may be sufficient contrast produced to enable the fingermark to be visualised. In electrodeposition the reverse is true. Material is deposited from the solution onto the surface; if the presence of the fingermark constituents inhibits or accelerates growth of the deposit on the ridges relative to the rate of growth on the background, contrast will again be produced.

1.3 The primary sources of untreated metal surfaces are cartridge cases, which have always presented a problem for fingermark development because of the conditions they are exposed to. High temperatures, abrasion and deposition of propellant residue all reduce the chances of recovering fingermarks and a variety of techniques have been considered. Given [1] investigated powdering techniques on brass and nickel-plated cartridges, but also included nitric acid fuming as a technique for selectively etching the metal. It was considered that sebaceous marks would protect the metal surface from corrosion, thus producing contrast.

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1.4 Around the same time, Belcher was experimenting with techniques for developing fingermarks of different metals after heating [2,3]. He proposed dipping copper into solutions of brown photographic toner, and steel samples into liquid gun-blueing solution [2], later recommending potassium permanganate solution for cartridge casings with thin copper coatings [3]. In 1977 Belcher wrote to New Scotland Yard to propose the operational use of these techniques on articles recovered from terrorist incidents and this prompted an investigation by the Police Scientific Development Branch (PSDB) into related methods [4]. Among the chemicals investigated were:

- nitric acid, which showed some preferential etching of nickel-based cases;
- 5% selenic acid, which gave the 'gun blueing' effect on brass with some results on steel and nickel;
- copper sulphate, which etched nickel;
- sodium sulphide, which gave reasonable results on brass;
- a solution of antimony in hydrochloric acid, which plated antimony onto the metal surfaces;
- hydrochloric, sulphuric and hydroiodic acids gave no useful results.

Vacuum metal deposition was noted to give reasonable results on most metal surfaces.

1.5 Interest in techniques for the development of fingermarks on cartridges revived in the mid-1990s, with several papers on the subject being presented at the International Symposium on Fingerprint Detection and Identification in Israel in 1995. Saunders and Cantu [5] investigated the use of a modified physical developer, acidified silver nitrate and gun blueing for unfired cartridge casings. They also compared superglue and gun blueing on a range of fired cases. They found that the most effective combination was superglue, followed by gun blueing, although the success rates on operational work were not as good as those observed experimentally.

1.6 Wiesner *et al.* [6] considered the effects of firing conditions on fingermark development and compared gun-blueing, silver nitrate and superglue. The effects of gunpowder residue, friction and heating to high temperatures were studied. Of the techniques investigated gun blueing again exhibited most promise.

1.7 Migron *et al*. [7,8] considered the electrodeposition of palladium for the development of latent fingermarks and assessed a range of palladium compounds for this purpose. Good results were obtained for fingermarks on unfired cartridges and in some cases a preliminary etch of the surface using iodine also produced good images of the fingermark. However, it proved difficult to develop marks on fired cartridges using this technique.

1.8 Bentsen *et al.* [9] tested a variety of electrodeposition techniques on fired cartridge cases using solutions of copper, nickel, chromium and tin sulphate at different concentrations. They compared the results with

those obtained by other techniques, including 4% selenious acid (the principal constituent of gun blueing solutions). Selenious acid had a higher sensitivity than the other electrodeposition techniques and therefore these other techniques were not studied further.

1.9 One issue sometimes experienced with the use of gun blue solutions was the overdevelopment of the blue surface coating formed. Cantu *et al.* [10] demonstrated that acidified hydrogen peroxide could be used to prevent overdevelopment and that the same solution could also be used to visualise sebaceous marks on metal surfaces by selectively etching the background.

1.10 There was a general consensus among researchers that gun blueing, either used singly or in combination with other processes such as superglue, was one of the most effective processes in revealing marks on brass surfaces. For other types of metal such as aluminium, where a thin, tenacious and unreactive layer of aluminium oxide is present on the surface, alternative formulations such as aluminium black were investigated [11,12]. These still contain selenious acid as the principal active constituent, but with a range of other chemicals making them more suited for use on aluminium (e.g. alkalis to dissolve the oxide film).

1.11 More recent studies involving electrochemical techniques include an extensive comparative investigation conducted by the Bundeskriminalamt (BKA), Germany [13] and an investigation conducted in the laboratories of Strathclyde Police [14,15]. The conclusions from both these studies indicate that optimum treatments may vary from metal to metal and that there may be some merit in combining techniques such as superglue and palladium deposition.

1.12 Electroless deposition of silver onto copper and copper-based alloys has also been considered as a simple, rapid process for the development of fingermarks. This is actively being researched at Leicester University. The formulations being investigated are based on those previously reported for silver film deposition for other applications [16,17].

1.13 Metal deposition is not the only electrochemical method that has been considered for fingermark enhancement. It is also possible to deposit conducting polymers selectively from solution, and this method of fingermark visualisation was first proposed by Bersellini *et al*. in 2001 [18]. In these studies a solution containing pyrrole monomer was used to deposit a polypyrrole film onto metallic surfaces. More recently, researchers at Leicester University have demonstrated that a wider range of conducting polymers can be deposited to delineate fingermarks on metal surfaces, including polyaniline and poly(3,4-ethylenedioxythiophene) (PEDOT) [19,20,21]. It has also been demonstrated that the electrochromic nature of these polymers can be exploited to change the contrast between the fingermark and the substrate, and that the polymer film colour can be varied by using

copolymers [22]. They found that variable composition enables the film colour to be tailored for optimum visual contrast with the substrate.

2. Theory

2.1 The chemical reactions associated with the principal electrochemical techniques are outlined below. The electrochemical treatments are based on *redox* reactions, in which one chemical species is *red*uced and another is *ox*idised. Oxidation is the removal of electrons from a substance, to create a more positively charged entity. Reduction is the addition of electrons to a substance, to create a more negatively (or less positively) charged entity. Simple arguments about conservation of charge mean that the supply and demand of electrons must be balanced – the notion of a *redox* reaction that couples a *red*uction and an *ox*idation process. In the examples that follow, a reactive metal surface may be oxidized by a suitable chemical reagent in solution (which accepts the electrons and is thereby reduced) or the electrons may be conducted away from the surface by the application of a voltage. Silver nitrate treatment is an example of the former. Polymer deposition exploits the latter.

2.2 Silver nitrate: For silver nitrate on brass or copper surfaces, a redox reaction occurs between the silver ions (the oxidizing agent) in solution and the copper metal in exposed regions of the surface. The copper reduces the silver ions, resulting in deposition of silver metal (as a grey deposit) on the surface.

$$2Ag^{+} + Cu \leftrightarrow 2Ag + Cu^{2+}$$

2.3 Gun blueing: The principal reaction occurring with gun blueing is associated with the reaction of selenious acid with metals. The selenium in selenious acid is in a more oxidized state and is reduced by acceptance of the electrons, as explicitly shown below.

$$H_2SeO_3 + 4H^{+} + 4e^{-} \leftrightarrow Se + 3H_2O \text{ (general reaction)}$$

This example includes a common feature of processes involving oxygen in protic media (a solvent that has a hydrogen bound to an oxygen or nitrogen atom, most obviously water, H_2O), namely the involvement of protons as a means of restoring charge balance. In such instances, solution pH may play a significant role in determining the products or the rate of the process.

2.4 Although selenious acid will work on a range of different metals, it is most suited to brass where parallel reactions occur between copper and zinc, and between selenious acid and zinc, resulting in the formation of the black CuSe product on the surface.

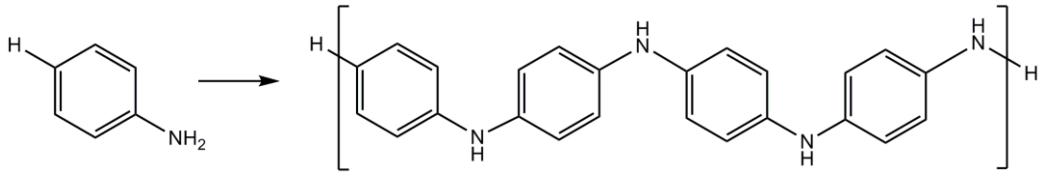
$Cu^{2+} + Zn \rightarrow Cu + Zn^{2+}$
$H_2SeO_3 + 4H^+ + 2Zn \rightarrow Se + 3H_2O + 2Zn^{2+}$
$Se + Cu \rightarrow CuSe_{(black)}$

In this instance, comparison with the previous reaction shows that metallic zinc is the source of the electrons.

2.5 Palladium deposition: Several different palladium (Pd) compounds were investigated for palladium deposition and the reactions of those found most suited for this purpose with brass are shown below. The reagents are Na_2PdCl_4 and K_2PdCl_6, but in solution they dissociate to generate active species containing palladium in a high oxidation state, $PdCl_4^{2-}$ or $PdCl_6^{2-}$. These are able to oxidize the copper and zinc components of the brass, and themselves become reduced. The outcome is a coating of grey palladium metal on the surface; the copper and zinc ions and the liberated chloride ligands are released into the solution.

$Zn/Cu + 2PdCl_4^{2-} \rightarrow Pd + Zn^{2+} + Cu^{2+} + 8Cl^-$
$2Zn/Cu + 2PdCl_6^{4-} \rightarrow Pd + 2Zn^{2+} + 2Cu^{2+} + 8Cl^-$

2.6 Polymer deposition: Several different conducting polymers (and combinations of them) have been proposed for the electrochemical deposition of polymers onto metal surfaces. In general, all processes involve setting up an electrochemical cell with a solution of monomer and setting the conditions so that the selective deposition and polymerisation of the monomer on the exposed regions of the metal surface is favoured. The deposition of the polymer involves an irreversible oxidation of the monomer, shown below; polymer deposition cannot readily be reversed. Subsequent variation of the applied voltage (at a lower level) results in reversible oxidation and reduction of the polymer, with consequent variations in colour (*electrochromism*).



The polymerisation reaction of polyaniline.

3. Reasons technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Centre for Applied Science and Technology (CAST) does not currently (2016) recommend any electrochemical processes as Category A processes for fingermark detection in the *Fingermark Visualisation Manual* because their relative effectiveness has not been fully

established. In addition, some of the chemicals used in the processes are highly corrosive and there are health and safety issues associated with their use. However, such processes may prove to be more effective than the techniques currently recommended and as a consequence several of these processes are included as optional, additional processes for the treatment of metals in the *Fingermark Visualisation Manual*. These include gun blueing and palladium deposition, which are listed as Category B processes, and electrochromic development and electroless silver deposition, which are included as Category C processes.

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Heating and electrostatic powdering

1. History

1.1 A recent addition to the range of techniques that can be utilised for the visualisation of fingermarks on cartridge casing (and other metals) is the heating and electrostatic powdering method developed by Dr John Bond when at Northamptonshire Police [1,2,3,4]. This visualisation technique utilises the fact that salts and other components of fingermark residue are capable of causing metals and their alloys to corrode. In the technique the metal surface is heated to promote further corrosion and oxidation of the surface, the combination of which may produce sufficient distinction in colour between the fingermark ridges and the uncorroded metal for the mark to be seen without any further treatment. Further enhancement of the mark can be obtained by applying an electrostatic charge of 2.5 kV to the surface, then applying carbon-coated spherical beads, as used in the electrostatic detection apparatus (ESDA) process (see Chapter 3, Chemical and Physical Processes, ESDA), to the surface.

1.2 The technique was shown to work for a range of different metals and alloys [1,2] and to continue to develop marks after surfaces had been cleaned with water and acetone. The technique has attracted much interest worldwide and has been used on operational casework dating back several years [3]. Research is ongoing to determine the corrosion mechanisms that operate in producing the fingermark images [2,4,5], to look at the physical and chemical changes occurring at the surface, and also to measure anion and cation concentrations in eccrine sweat.

1.3 Further research has also been conducted into the use of heating alone to visualise fingermarks on metals [6]. Heating was successfully applied to several different types of metal; in some cases the oxide films that formed on the metal surface during heating gave good contrast with the regions where the fingermark was present.

2. Theory

2.1 The theory associated with the process is that corrosion is locally initiated on the metal surface by the action of chloride ions in the fingermark residues. In general, the process operating is:

$$M^{Z+} + ZCl^{-} + ZH_2O \rightarrow M(OH)_Z + Z(H^{+} + Cl^{-})$$

2.2 This process results in pitting corrosion penetrating into the metal surface. This localised pitting corrosion is then enhanced by the subsequent exposure to heat, where the colour change of the metal surface caused by oxidation may also aid visualisation of the fingermark.

2.3 The corroded areas of the metal surface involve oxide films whose electronic properties (generally insulating or semiconducting) result in a surface potential to the uncorroded metal (which is conductive). It is these differences that are exploited by electrostatic charging and subsequent powdering.

3. Reasons why technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Home Office Centre for Applied Science and Technology (CAST) does not currently (2016) designate the process as a Category A process in the *Fingermark Visualisation Manual* because its relative effectiveness has not been compared with both currently recommended processes and processes that are still under development. Commercial equipment (called CERA) was produced to conduct the electrostatic powdering process and as a consequence CERA was included as an optional, additional Category C process for the treatment of metals in the *Fingermark Visualisation Manual*.

3.2 When the process was used on operational casework in the UK, it was observed to cause detrimental effects to the striations in the surface that are used for ballistic analysis. This resulted in the use of the technique being suspended. These detrimental effects were attributed to the high temperature used for ‘developing’ the corrosion in the surface, and this temperature was subsequently reduced to overcome this issue. In practice, although commercial equipment was produced for electrostatic powdering it did not prove possible to reproduce fully the results obtained in the laboratory and the original electrostatic powdering CERA equipment is no longer available.

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Electrostatic detection apparatus (ESDA)

1. History

1.1 The Police Scientific Development Branch (PSDB) set up a general investigative contract with the London College of Printing in the early 1970s, with the purpose of exploring novel fingermark detection methods and also methods for taking the fingerprints of prisoners [1]. During this contract the electrostatic detection apparatus (ESDA) was proposed, originally for the detection of fingermarks on fabrics [2,3]. In-house work at PSDB had indicated that the decay time for charged fingermarks on most materials was very short and that this precluded the use of direct charging and toning as an effective detection technique. The researchers at the London College of Printing overcame this by covering the surface being examined with a thin layer of Mylar® (a polyester) and producing the charge image on this thin polymer film. The thin film was exposed to a corona charging device and then treated with an electrostatic image developer, in this case carrier beads mixed with a cascade toner.

1.2 At around the same time, Japanese researchers also demonstrated that electrostatic images of fingermarks could be transferred to thin polymer films from paper exhibits by sandwiching the paper between the polymer films and holding them in a steel press [4]. Upon separation, the electrostatic image on the polymer sheet was developed by scattering dielectric powders of sulphur, lead oxide and toner over the surface. However, this approach does not appear to have been progressed further and no practical apparatus appeared from this research.

1.3 Further PSDB-sponsored research demonstrated that the process was capable of developing fingermarks on surfaces, including papers and fabrics. Tests were conducted on a range of fabric types, including both natural (e.g. cotton, silk) and synthetic (e.g. nylon, rayon, terylene) fibres and different weave types. The primary factor affecting the quality of the developed image was found to be the density of weave and diameter of the thread, with tightly woven fabrics with thread diameters less than 40 μm giving the best results. It was also found in these tests that decreasing the thickness of the Mylar® film used to overlay the surface from 12 μm to 3.5 μm improved the quality of the electrostatic charge patterns produced. However, it was also noted that good development of fingermarks on fabrics was confined to fresh marks and those over 24 hours old did not generally produce acceptable images (although better results could be obtained on paper). Attempts to improve sensitivity were unsuccessful and therefore the work on fingermarks was terminated. However, during the course of these studies it had been observed that the technique was capable of revealing indented writing on paper and could give results superior to other techniques then available, such as oblique lighting [5,6]. A further contract was placed by PSDB to develop apparatus specifically for enhancement of indenting writing and this was subsequently developed and manufactured as a commercial system by

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Foster and Freeman in the UK, with other manufacturers taking up the concept worldwide.

1.4 HO SRDB did revisit the electrostatic detection apparatus (ESDA) in the early 1980s to establish whether it was possible to explain some of the phenomena associated with the process and also to see if any advances in technology could be used to improve the speed or sensitivity of the process [7]. An experimental system utilising a scanning probe was developed during the course of these studies but was not progressed further. A large format ESDA system was also built with the intention of investigating the technique to screen large areas of fabric for contact areas that could then be targeted using other, more sensitive techniques such as radioactive sulphur dioxide. This had limited success and was not taken forwards to production.

1.5 Although the ESDA system was primarily adopted for document analysis, research was carried out to establish an integrated forensic approach for document examination by examining whether treatment with ESDA could be detrimental to subsequent development of fingermarks. Initial results by Heath in 1983 [8] appeared to indicate that ESDA in general was detrimental to subsequent treatment with ninhydrin and that pre-humidification for five minutes prior to ESDA and ninhydrin treatment actually improved the quality of the fingermarks. This was contradicted in later studies by Moore [9] who found that pre-humidification of documents was detrimental to the development of fingermarks with ninhydrin, and that exposures for longer than 5–15 minutes were to be avoided. The pre-humidification effect was thought to be cumulative and repeat exposures of documents to pre-humidification and ESDA were to be avoided where possible. When it became known that pre-humidification enhanced the performance of ESDA for indented writing, HO SRDB almost immediately issued warnings that this could be detrimental to the detection of amino acids in fingermarks, particularly on some types of paper. A later study by Azoury *et al.* [10] looked at the effects of pre-humidification on fingermark development by other amino acid reagents, including 1,8-diazafluoren-9-one (DFO) and 1,2 indandione. The results of Moore were confirmed and it was also shown that pre-humidification was detrimental to subsequent treatment with 1,2 indandione and less so to DFO, although exposures of over 60 minutes also began to degrade DFO development.

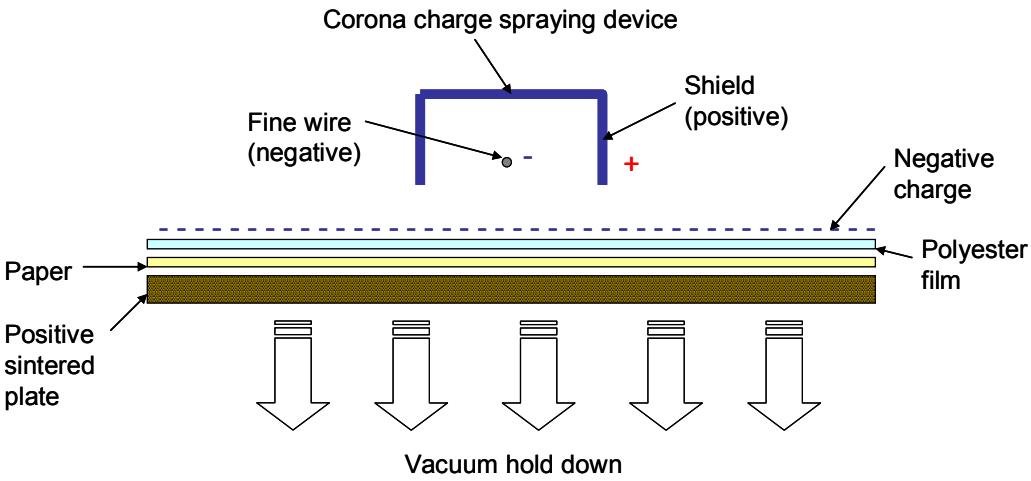
1.6 Although ESDA is found today in most UK police fingermark enhancement laboratories, it is primarily used as a document analysis technique and if fingermarks are detected by the technique during document processing this is regarded as a bonus rather than an expected outcome.

1.7 Watson *et al.* [11] have more recently demonstrated the use of an electrical potential sensor to scan an insulating surface and image the fingermark by means of its residual electrical charge. This has been shown to have the potential to give an indication of the timeline of

fingermark deposition, but also to be capable of imaging fingermarks at a resolution suitable for identification. The sensor technology reported may also have the potential to produce images of the charge maps produced by ESDA, although this has not been investigated.

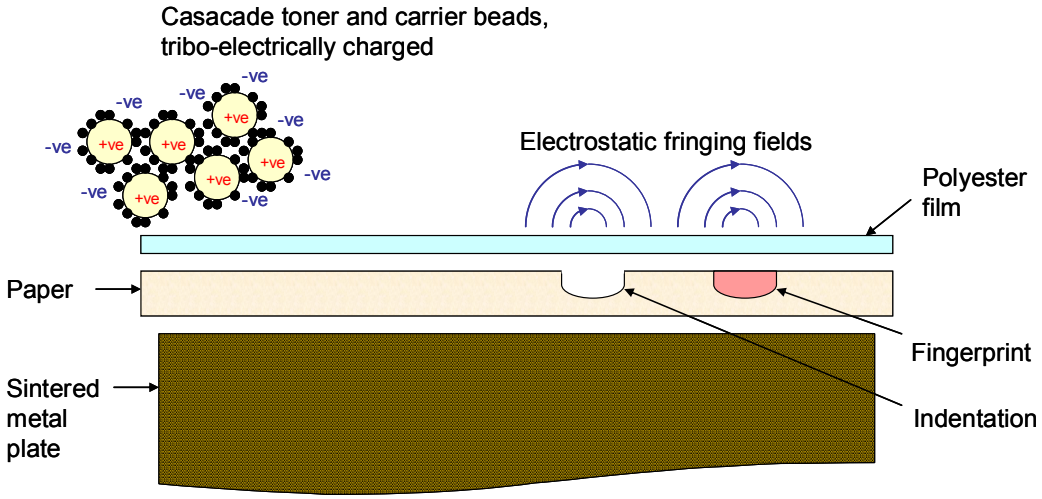
2. Theory

2.1 The mechanism of ESDA has not been conclusively established, but it is possible to describe the stages in the process. The porous exhibit to be treated is first held down on a sintered plate using a vacuum, and a thin (~3.5 μm) film of Mylar® laid over the top of it. This film is then negatively charged by passing a charge spraying device known as a corotron above the surface.

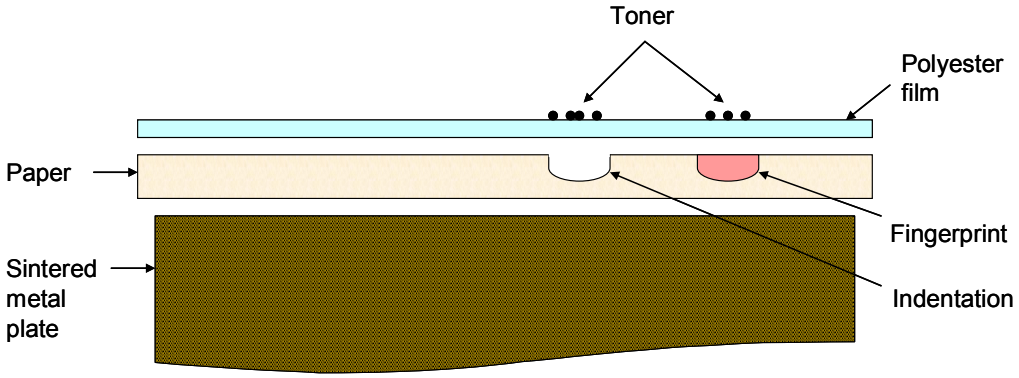


Schematic diagram of the general charging procedure for electrostatic detection apparatus.

2.2 The charging process sets up electrostatic fringing fields around features in the exhibit (the exact mechanisms of which are not precisely known). A mixture of carrier beads (fine glass spheres) and toner particles (carbon black) are cascaded across the surface, and the toner selectively adheres to areas where the fringing fields are present. This is illustrated schematically below.



a)



b)

Schematic diagrams showing toner development of electrostatic images a) *development of electrostatic fringing fields on the polymer film and* b) *selective adherence of toner particles to regions where fields are present.*

2.3 It was originally proposed that the fringing fields could be explained by a simple capacitance theory [6]. The indentations cause a local increase in capacitance due to a reduction in the distance between the charged surfaces and fingermarks, causing a local increase in capacitance because of the water in the fingermark increasing the local dielectric constant. However, capacitance variations cannot be the only mechanism because it is noted that very deep impressions sometimes do not develop with ESDA.

2.4 It was later proposed that the indented writing effect could be explained by damage and abrasion of surface fibres caused by lateral movement between sheets of paper during the writing process [7]. The poor

performance often observed with glossy papers in the ESDA process may be explained by the fact that such papers are sized, calendared or highly loaded with inorganic filler.

2.5 Another theory proposed to explain the improved performance of ESDA often observed after pre-humidification of the article was termed 'surface variation theory' [12], which considered that after humidification the paper no longer behaved as a dielectric but as a conductor. In this theory the variation of electrostatic potential on the polymer film is a function of the degree of close contact between the polymer film and the paper, and also variation in surface features of the paper, such as glossiness and smoothness (which may also be modified by the presence of fingermark residue). This could explain why deep indentations, where the film does not contact the paper, do not produce results using ESDA. As fingermark residues are absorbed into the porous surface, their effect on the surface will reduce, which may explain the poor development observed on marks over 24 hours old. However, none of these mechanisms has been conclusively proven.

Fingermarks developed by electrostatic detection apparatus while processing a document.

3. Centre for Applied Science and Technology processes

3.1 ESDA is included as a Category A process in the *Fingermark Visualisation Manual* [13]. The process instruction directs the user to

follow the manufacturer's instructions for the particular make of commercial equipment available. ESDA does not explicitly appear in any of the processing charts for porous surfaces because it is less sensitive than other techniques for developing fingermarks, and is ineffective on marks more than 24 hours old. However, it may reveal fingermarks when used as part of an integrated strategy for retrieval of forensic evidence, ESDA being mostly non-destructive to fingermark evidence unless pre-humidification is used (note that this may be required to obtain optimum results for indented writing, so some prioritisation of evidential recovery may be required). It is also capable of developing marks not found by chemical or optical methods, and its non-contact, non-destructive nature means that it could be included in sequences before chemical processes such as DFO and ninhydrin. ESDA is therefore proposed as an optional, additional process for small, flat porous surfaces.

4. Critical issues

4.1 If ESDA is being considered as part of a fingermark enhancement sequence, the pre-humidification stage used in its application for document examination should be omitted because this has been shown to be detrimental to subsequent processing using techniques such as DFO and ninhydrin.

4.2 The effectiveness of the ESDA process is known to decrease rapidly as the age of the mark increases. It is also less effective on thick, glossy paper types than on thinner papers with a slightly rougher texture such as printer paper.

5. Application

5.1 ESDA can be used as an additional process on thin, flat, porous surfaces such as letters and envelopes. It can be used in sequence after the optical processes visual and fluorescence examination, and before chemical techniques such as DFO and ninhydrin, where it may detect additional, unique marks. It can also be considered for use in situations where it is important not to cause damage to the document being processed, for example where the document is valuable.

5.2 The process is conducted by drawing the item flat on a vacuum stage, covering it with a thin polyester (Mylar®) film which is also drawn down into intimate contact with the item. An electrostatic charge is then generated on the fingermark by passing a charge spraying device called a corotron over the surface of the polyester film. This charge pattern is then visualised by cascading a toner powder across the surface, the particles preferentially adhering to areas of high charge.

6. Alternative formulations and processes

6.1 There are no alternative formulations and processes

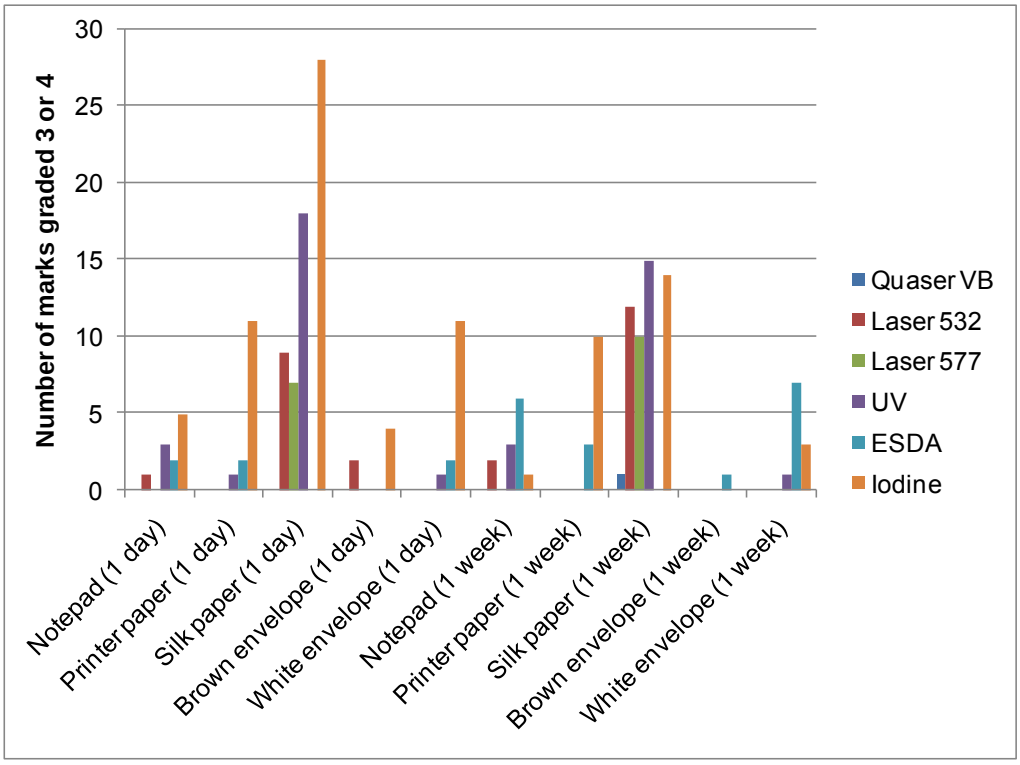
7. Post-treatments

7.1 There are no post-treatments to enhance developed marks. The marks may be visualised by directly imaging them with the document *in situ*, by capturing the charge image using clear adhesive sheet pressed into contact with the polyester film, or by taking a white gelatin lift.

8. Validation and operational experience

8.1 Few comparative studies have been conducted between ESDA and other fingermark enhancement processes because since the late 1970s the primary application of ESDA has been in document examination. It is known that ESDA is less effective than chemical development methods, but is capable of visualising additional marks if used sequentially before them. The non-destructive nature of the process means that it has no impact on subsequent fingermark processes (unless pre-humidification is used) and therefore the level of validation required should be lower.

8.2 A limited study was conducted by CAST to evaluate the effectiveness of ESDA against other non-destructive and low impact processes on a range of different paper types. In this study, 36 different donors placed a single fingermark on five different paper types, which were aged for 1 day and one week prior to examination using light sources (violet-blue Quaser, green and yellow lasers), short wave ultraviolet reflection, ESDA and iodine fuming.



Number of marks (out of 36) detected by various non-destructive/low impact visualisation processes on different paper types.

8.3 It can be seen that on all paper types except the smooth 'Silk' paper, ESDA detects more high quality marks than all of the fluorescence examination processes investigated and UVC reflection. ESDA continues to develop marks 1 week old, and on some types of surface gives better results than iodine fuming for marks of this age. The results show that ESDA is capable of detecting marks not found by other methods and is therefore potentially useful as an additional process in sequential routines. It is for this reason that it has been included as a Category A process in the *Fingermark Visualisation Manual*.

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Fuming techniques

1. History

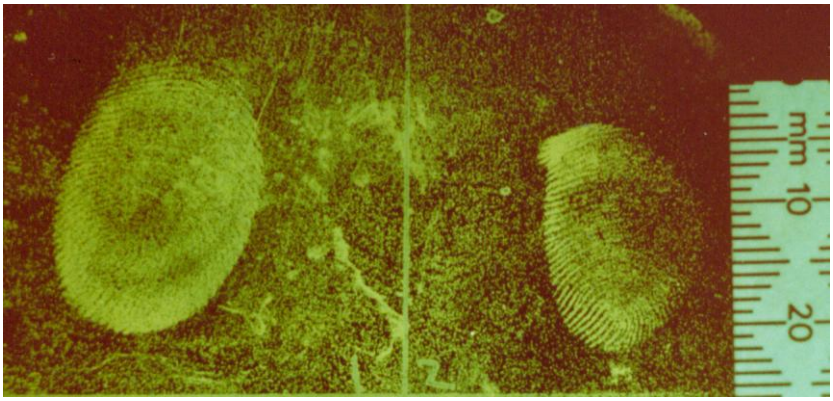
1.1 The development of fingermarks using fuming processes has been utilised since the early days of fingerprint identification. Iodine and osmium tetroxide were already known to develop fingermarks on porous surfaces by the 1920s and the fuming of a range of other substances has been investigated since then.

1.2 Several of the processes described in other chapters either involve fuming, e.g. superglue, or have been investigated as fuming techniques, e.g. 4-dimethylaminocinnamaldehyde (DMAC). Fuming has the potential advantages that it does not wet the article, which may be a benefit if subsequent document analysis is required, will permeate porous exhibits, and impinge upon all available surfaces for non-porous exhibits.

1.3 Fuming can be used to develop fingermarks in several ways, described in more detail in the section below. In a review of techniques for development of latent prints issued in 1974 [1], Micik lists three fuming techniques: iodine; hydrogen fluoride (for etching fingermarks on glass); and the burning of substances, including camphor and magnesium, to produce fumes that selectively deposited particles on fingermark ridges.

1.4 Almog and Gabay [2] carried out an investigation into the development of fingermarks on paper by fuming several fluorescent chemicals. Good results were reported for anthranilic acid (for fresh marks), anthracene (for older marks) and antimony trichloride. In some cases the fluorescent chemical was selectively deposited on the ridges, in other cases deposition occurred on the background only.

1.5 The Home Office Scientific Research and Development Branch (HO SRDB) conducted a subsequent study into the anthracene fuming process [3], first investigating the optimisation of fuming conditions using fingermarks deposited on glass slides and then applying the optimised process to fingermarks on different types of plastic and metal surfaces. The potential benefits of vacuum deposition of anthracene were also explored. It was found that sublimation in air gave better results than vacuum deposition and although the process did develop fingermarks on plastics, it was not as effective as other processes already available. Results on metals were more promising and anthracene fuming was found to be more effective than iodine over a range of different metal surfaces.



Photograph of fingermarks on metal developed by anthracene fuming.

1.6 Haque [4] considered the fuming of naphthalene and camphor, followed by iodine fuming and dusting with magnetic powder. This multi-step process appeared to give excellent sensitivity on plastic bag substrates. The selective attack of polymer surfaces using the fumes of halogenated hydrocarbons such as dichloromethane and chloroform was also studied. The technique worked well on polystyrene, but was ineffective on vinyl and thermoset plastics, and did not work at all on polyethylene.

1.7 Fuming has also been reported in combination with other processes for the revelation of fingermarks. Meylan *et al.* described the fuming of ammonium hydrogen carbonate after exposure of a paper exhibit to a corona discharge [5]. This combined treatment produced fluorescent fingermarks that could be excited by ultraviolet light. This technique was further investigated by Davies *et al.* [6]; they carried out an analysis of the fluorescent products and suggested that lipid derivatives were responsible for the fluorescence observed.

1.8 In addition to the hydrofluoric acid fuming process mentioned by Micik for developing fingermarks on glass, other acid fuming techniques have been considered. Bentsen *et al*. [7] trialled nitric acid fuming for development of fingermarks on brass cartridge cases and Broniek and Knaap [8] proposed hydrochloric acid fuming as a technique for revealing fingermarks on thermal receipts. The highly corrosive nature of these substances meant that such techniques were not widely adopted for operational use because of the precautions required for their use.

Fingermarks developed on a thermal receipt by hydrochloric acid fuming.

1.9 A novel process that has been recently reported by Kelly *et. al*. is the use of disulphur dinitride, allowed to sublime under a static vacuum [9]. This has been shown to be capable of developing fingermarks on a wide range of surfaces, including paper, fabric, clingfilm and metals, possibly by formation of the blue-black sulphur-nitrogen backbone (SN_x) polymer.

2. Theory

2.1 Because many different types of substance have been used as fuming techniques for the development of fingermarks, there is no single mechanism that applies to all chemicals. A range of mechanisms may operate and some of these are outlined below.

2.2 Absorption of coloured vapours into fingermark residues – this is the mechanism that occurs for iodine (and other halogens, such as bromine).

2.3 Chemical reaction between fumes and fingermark residues to form a coloured or fluorescent reaction product, e.g. the black product formed by osmium tetroxide fumes.

2.4 Catalysis of a polymerisation reaction by fingermark residues, promoting growth of a solid phase from gaseous fumes – this is the case for the superglue process and also possibly the recently reported disulphur dinitride process [9].

2.5 Selective deposition of particulates on fingermark ridges (or background) – this is observed for fuming of anthracene, camphor and naphthalene.

2.6 Selective etching/attack of ridges (or background) by fumes of acid or other substance – this can be seen for hydrogen fluoride on glass, nitric acid on brass, and chloroform on polystyrene.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST did recommend two fuming processes, iodine and superglue (Chapter 3, Chemical and Physical Processes, Iodine and Superglue respectively in this book), in the *Manual of Fingerprint Development Techniques* [10]. Other fuming techniques (e.g. anthracene) were not recommended because they are either less effective than other techniques or there are health and safety issues associated with their use. In particular, there are concerns about the fuming of concentrated acids because they are highly corrosive. In general, all fuming processes need to be well-contained and carried out in areas with good ventilation. The updated *Fingermark Visualisation Manual* [11] includes several fuming processes in Categories B-F, and there is the possibility that some of these may increase in maturity to the extent that they appear in Category A in future.

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Gelatin lifting

1. History

1.1 Gelatin lifting has been used for the recovery of fingermarks from the early 20th century. The concept was first proposed in 1913 for lifting of marks powdered with lead acetate and subsequently treated with hydrogen sulphide. The lifting medium used in this case was a paper coated with a gelatin/glycerol mix [1]. Gelatin lifting was not widely adopted for fingermark lifting at that time, but the lifting concept was further investigated for the recovery of footwear marks [2]. By the late 1970s several rubber- and gelatin-based lifters were commercially available for the lifting of footwear marks, including latent marks in dust and dried contaminant, and marks developed by powdering. Experiments conducted by the Police Scientific Development Branch (PSDB) in the early 1970s utilised gelatin films to lift marks developed using vacuum metal deposition from patterned surfaces [3]. Physical developer was then used to intensify the images of the lifted marks. This was reasonably successful, but a high contrast mark developed using vacuum metal deposition was required as a starting point.

1.2 There has been subsequent research into the broader forensic applications of gelatin lifts. The mildly adhesive nature of the gelatin lift combined with a degree of flexibility and compressibility makes them well suited for the lifting of trace evidence from a range of surfaces, without causing significant damage to the surface itself.

1.3 As a consequence of these studies, gelatin lifts are now marketed for the lifting of footwear marks [4,5,6], the lifting of paint and other micro traces [4], recording patterns around bullet holes [4] and the lifting of blood traces from surfaces [4]. They have also been shown to be effective in detecting indented writing, and in comparisons with the electrostatic detection apparatus (ESDA) technique (see Chapter 3, Chemical and Physical Processes, ESDA) have shown better performance than ESDA on thick, glossy paper types, and to be capable of being used sequentially after ESDA on documents [7].

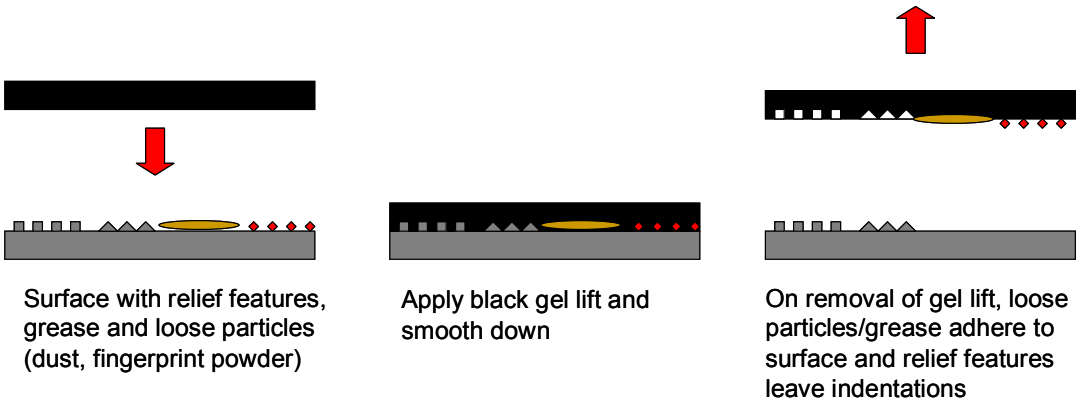
1.4 The principal application of gelatin lifts has remained the lifting of fingerprint and footwear evidence, both latent marks and marks developed using processes such as powders and superglue. Gelatin lifts are currently (2016) available in black, white and clear forms, and because they are flexible and can be compressed against a surface on application, they are better suited to lifting of powdered marks from textured surfaces than some types of tape. The colour of the lift can be selected to give optimum contrast with the powder used, and the lifts are most suited for lifting and subsequent imaging of marks powdered with granular and magnetic powders [4].

1.5 The gelatin lifting process has also been shown to be a potentially useful technique in recovering marks for subsequent chemical analysis. The gelatin lift acts as a transfer medium for marks lifted from a scene to be transported back to a laboratory for subsequent compositional analysis [8].

1.6 The development of specialist imaging equipment for the enhancement of marks lifted on black gelatin lifts circa 2005 (GLScan, produced by BVDA, Haarlem, Netherlands) has increased interest in the use of gelatin lifts for the recovery of latent fingermarks prior to chemical development. Several police forces in the UK have proposed the use of the technique as an alternative to powdering. This chapter deals with the application of gelatin lifting as the sole recovery process for latent fingermarks, as opposed to a lifting process for marks developed using other processes, such as powdering or superglue.

2. Theory

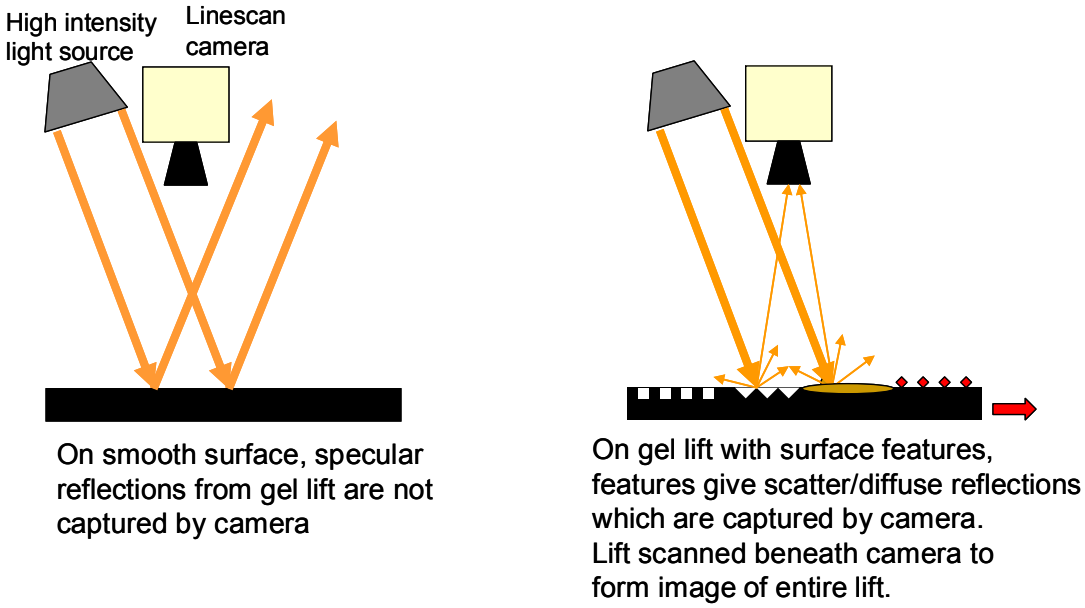
2.1 The theory behind the gelatin-lifting technique is that the gelatin is able to deform to the surface contours during application and smoothing in place. The slight adhesive nature of the surface also means that on removal of the gelatin lift, some of the loose particulate matter and any grease on the surface will be transferred to the surface of the gel. The gel may also retain some impression of the contours of the surface it has been applied to.



Schematic diagram showing how gelatin lifts can lift and reproduce surface features.

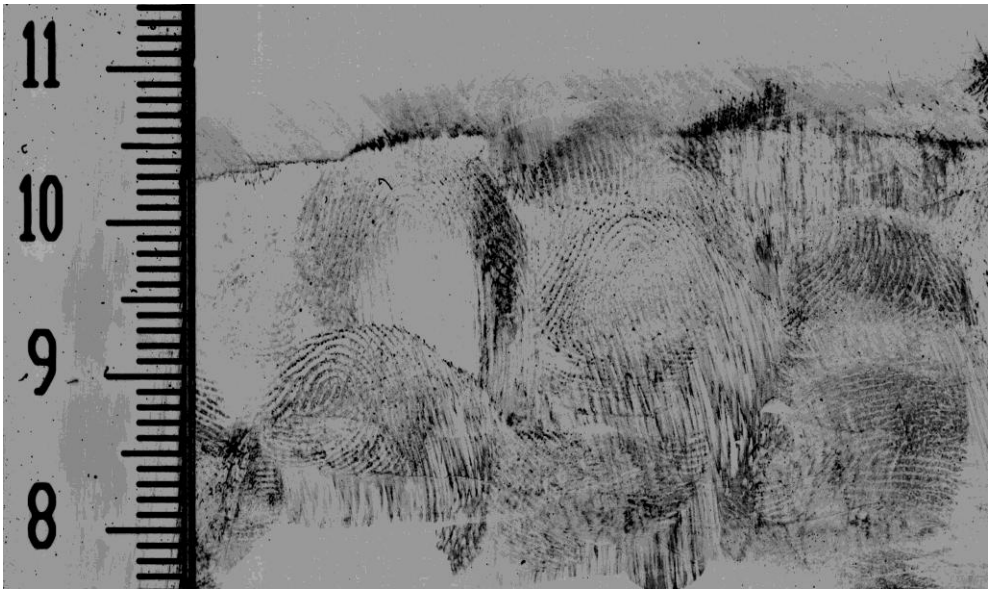
2.2 The surface features retained on the lift are then imaged in a way that maximises the contrast between the surface feature and the black background of the lift. This can be carried out using photography in a dark room with the light source perpendicular to the surface and close to the imaging system (see specular imaging in Chapter 2, Optical Processes, Visual Examination). Alternatively a specialist imaging system such as the BVDA GLScan may be used. The GLScan system consists of a line scan camera combined with high intensity white light

illuminating the gel at an angle close to perpendicular to the surface. The gel itself is mounted on a vacuum stage drawing it flat, and then scanned slowly by moving the vacuum stage underneath the fixed focus position of the line scan camera. The principle common to both imaging methods is that with nothing on the gel, the specular reflection from the surface means that no light is reflected into the camera and the background appears black. The particulates and grease on the surface scatter light and produce diffuse reflections, meaning that some light reaches the camera and those regions appear lighter.



Schematic diagram showing the way in which images are produced in the GLScan system.

2.3 An example of a section of a gelatin lift taken from a door handle and scanned on GLScan equipment is illustrated below.



Example of a series of latent marks lifted from a door handle using a gelatin lift and imaged on a GLScan system (greyscale inverted).

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The Home Office Centre for Applied Science and Technology (CAST) recommends using the process in accord with the gel manufacturer's instructions, peeling the acetate from the gelatin lift and applying it to the surface being treated. The gel is then smoothed in place to remove air bubbles. It may be beneficial to leave the gel in place for several minutes or to warm it slightly, but CAST has no data to conclusively demonstrate the benefit of either of these approaches.

3.2 'Gelatin' lifts can be obtained from more than one manufacturer, the principal supplier being BVDA (Haarlem, Netherlands). A rubber-based lifter was available from Dycem (Bristol, UK) for the same applications and there are other producers of similar products worldwide. It is not possible to recommend a single type of lifter for all applications. In general the BVDA lifts have been found to have higher tack and be more effective than the Dycem lifts, but in some cases the higher tack of the BVDA lift may cause damage to the surface. The ultimate selection of lifter by the user must take these factors into account.

3.3 Once lifted, the gelatin lift should be stored without a cover material, if at all possible, and imaged as soon as it is retrieved to the laboratory. This is because any lifted latent marks will progressively degrade and the reapplication of a cover material exacerbates this.

3.4 Imaging of the lift should be carried out under the conditions outlined in the 'Theory' section above. However, they should also be examined under oblique lighting. This is because any lifted marks in dust are best visualised under oblique light but may not be so prominent under the specular lighting conditions used to capture greasy deposits.

4. Critical issues

4.1 The temperature of the surface to be lifted must be below 40°C otherwise the gelatin lift may melt on the surface. This applies for all true gelatin lifts where gelatin is the principal constituent, but may not be so important for lifters based on other materials such as silicone rubber.

4.2 The gelatin lift must be smoothed in place to eliminate air bubbles, enabling all parts of the surface to come into contact with the lifting material.

4.3 The lift should ideally be stored without a cover and imaged as soon as possible after lifting, to reduce degradation in the quality of the lifted marks.

5. Application

5.1 Suitable surfaces: Gelatin lifts are suitable for use on all smooth non-porous surfaces where they can be readily formed to the shape of the surface. They can also be used on surfaces where a layer of contaminant is present. It is possible to use gelatin lifts on textured, semi-porous and porous surfaces, but their effectiveness is considerably reduced.

5.2 There are no special application methods for the gelatin lifts other than those recommended by the manufacturer [4]. The lifts may be cut to shape to suit the article or surface they are being applied to.

5.3 Gelatin lifting is recommended for situations where the primary processes in the *Fingermark Visualisation Manual* [9] may not be applicable, primarily as an alternative to powdering. Such circumstances may include the following.

- Heavily contaminated surfaces where marks are visible in the contaminant, but cannot be imaged in situ and chemical development is not feasible.
- Articles that cannot be chemically treated and/or the application of powders may leave permanent traces or have a risk of damage (e.g. electrical equipment such as laptops, valuable antiques, etc.).

- Areas that are not easy to reach using powdering and where any developed marks would be difficult to see (e.g. on the inside of door handles).

6. Alternative formulations and processes

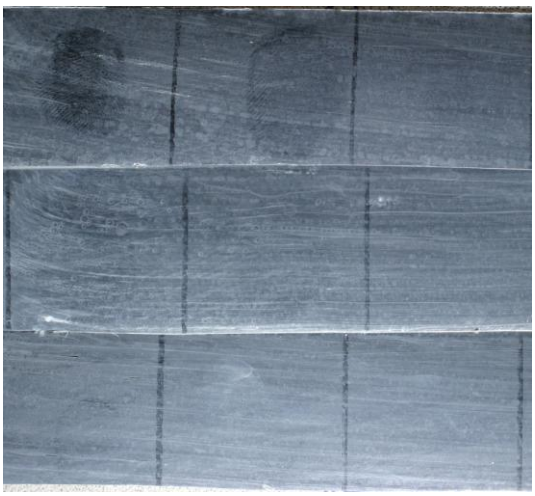
6.1 As alluded to above, there are several different types of gelatin lifter on the market. The only ones evaluated by CAST are the BVDA Black Gelatin Lift and the Dycem High Performance Evidence Lifter. Both of these have advantages and disadvantages and the user is encouraged to make a judgement on which lift to use according to the individual circumstances of the scene.

6.2 Silicone casting compounds have also been used to lift latent marks from surfaces, but in this case the lifted marks are not imaged directly on the surface, but are first developed using another enhancement process such as superglue [10].

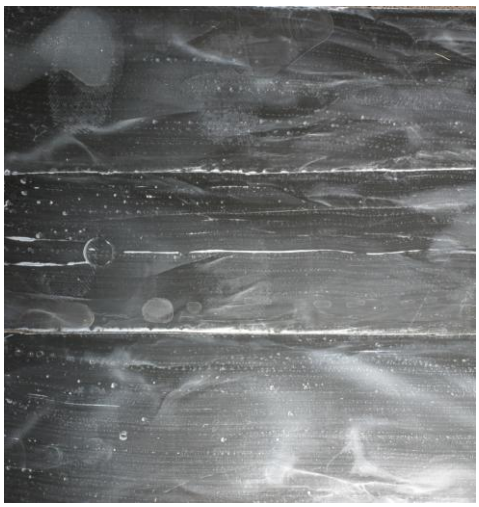
7. Post-treatments

7.1 In some circumstances it may be able to enhance the latent marks lifted by a secondary chemical process. Attempts have been made to enhance marks lifted on both Dycem and BVDA lifters using white powder suspensions and superglue, which were selected to give maximum contrast with the black lift.

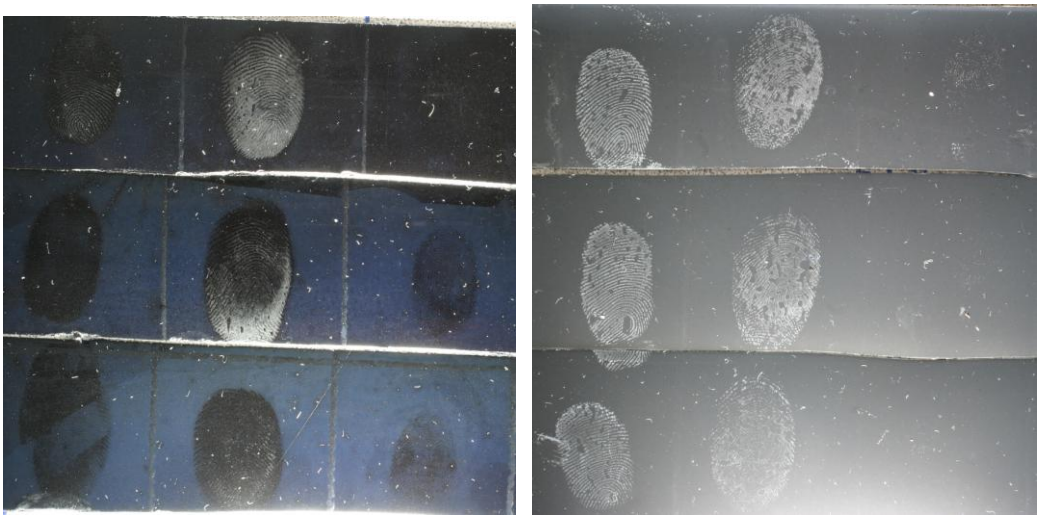
7.2 The results obtained for some donors on post-treated lifts are shown below.



a)



b)



c) d)

Post-recovery enhancement of marks on gelatin lifts a) *white powder suspension on BVDA lift* b) *white powder suspension on Dycem lift* c) *superglue on BVDA lift and* d) *superglue on Dycem lift.*

7.3 The results suggest that although there is little benefit in applying subsequent chemical treatments to BVDA gels, chemical treatment (superglue in particular) of Dycem gel lifts may improve marks in some cases or even develop additional marks, in particular for superglue treatment. This is in accordance with observations made by other researchers using silicone rubber-based casting compounds [10].

8. Validation and operational experience

8.1 Laboratory trials

8.1.1 CAST has carried out a direct comparison of the effectiveness of gelatin lifting with powdering [11]. This study compared gelatin lifting using black gelatin lifts produced by BVDA with the powdering process found to be most appropriate to the particular surface type being studied, according to guidelines published by CAST [12]. In this study six surfaces, representative of those found at crime scenes, were used:

- glass;
- u-PVC;
- painted metal;
- laminate (fake textured granite);
- laminate (fake ash);
- silk painted plasterboard.

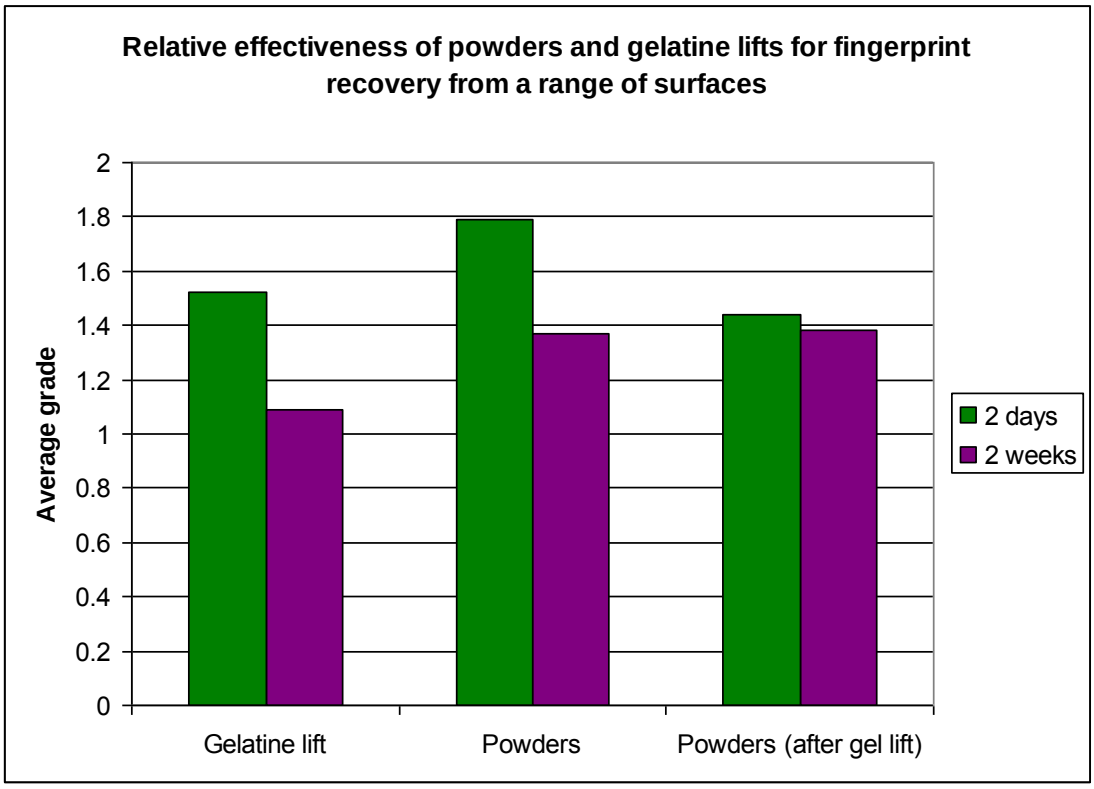
8.1.2 In this trial 70 separate donors were used, each depositing three fingermarks on each of the six surfaces. Donors were asked to wait at least 30 minutes after washing their hands before deposition of the marks, rubbing their hands together before deposition to evenly distribute

the sweat over the entire surface. No 'grooming' of marks (i.e. rubbing fingers on nose or forehead) was permitted.

8.1.3 The surfaces were then aged for periods of two days and two weeks. The marks were processed by three different routes:

- gelatin lift of the latent mark and subsequent imaging on the GLScan;
- application of fingerprint powder according to recommendations of the CAST *Fingerprint Powders Guidelines* [12];
- application of fingerprint powder as above, but on the same surface previously treated with the gelatin lift.

8.1.4 The results of the experiment to compare relative process effectiveness are shown below, with the average grade of developed mark across all 70 donors being compared for powdering and gel lifting.



Results of the experiment to compare the effectiveness of gelatin lifting and powdering for fingermark recovery [11].

8.1.5 The information depicted in the graph shows that for both powdering and gelatin lifting used as a single process there is a drop in the average grade of marks developed as the age of the mark increases from two days to two weeks. This is consistent with trends seen in previous studies of the powdering process.

8.1.6 It can also be seen that for both ages of fingermark, powdering gives superior results to gelatin lifting.

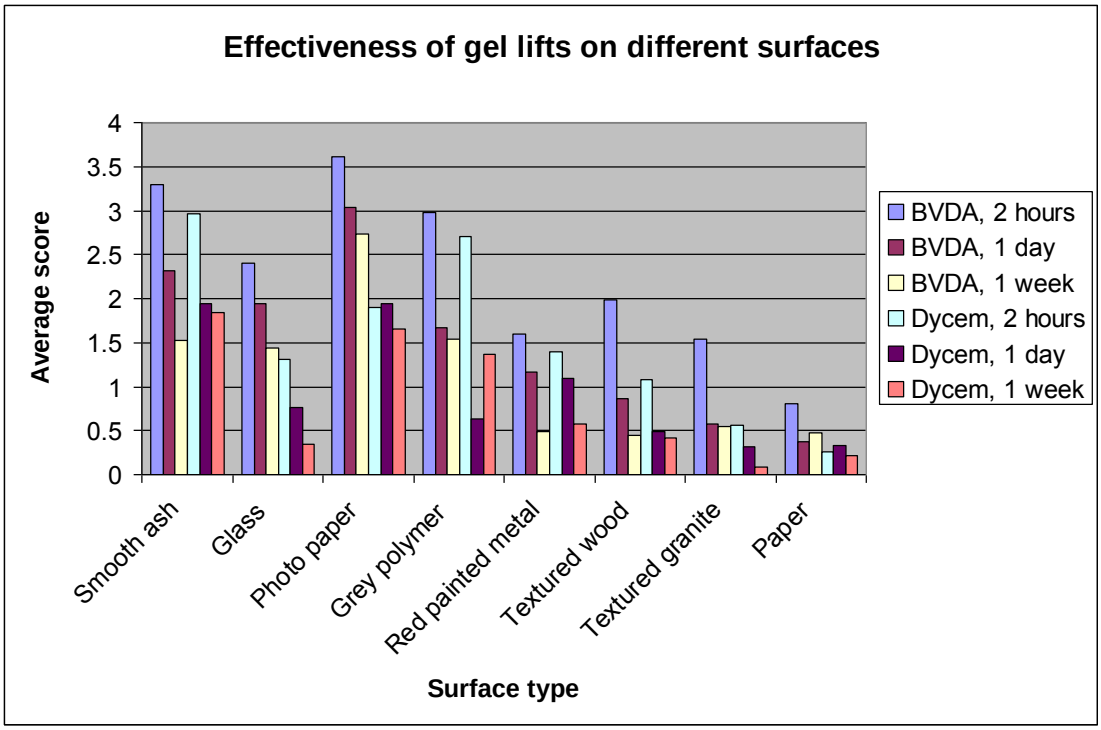
8.1.7 For two-day-old marks, the average grade of powdered marks is reduced by 20% if the gelatin lift is applied prior to powdering. Gelatin lifting transfers some of the residue to the lift, hence reducing the amount of material left on the surface for powders to adhere to. For two-week-old marks, application of the gelatin lift prior to powdering is far less detrimental because the mark has hardened and a smaller quantity of residue is transferred.

8.1.8 A second study [13] was carried out to establish the relative effectiveness of two types of lifter (BVDA and Dycem) across surfaces ranging from smooth non-porous through rough non-porous to porous surfaces. The following surfaces were used in the study:

- glass;
- glossy photographic paper;
- laminate (fake smooth ash);
- grey polypropylene polymer;
- red painted metal (car paint scheme);
- laminate (fake textured oak);
- laminate (fake textured granite);
- printer paper.

8.1.9 Depletion series of nine marks were deposited by a range of six to seven donors (depending on the size of the surface used for deposition) using the same process described above. Marks were aged for two hours, one day and one week prior to gel lifting.

8.1.10 The results of this study are summarised in the graph below, which shows the average score across all marks deposited.



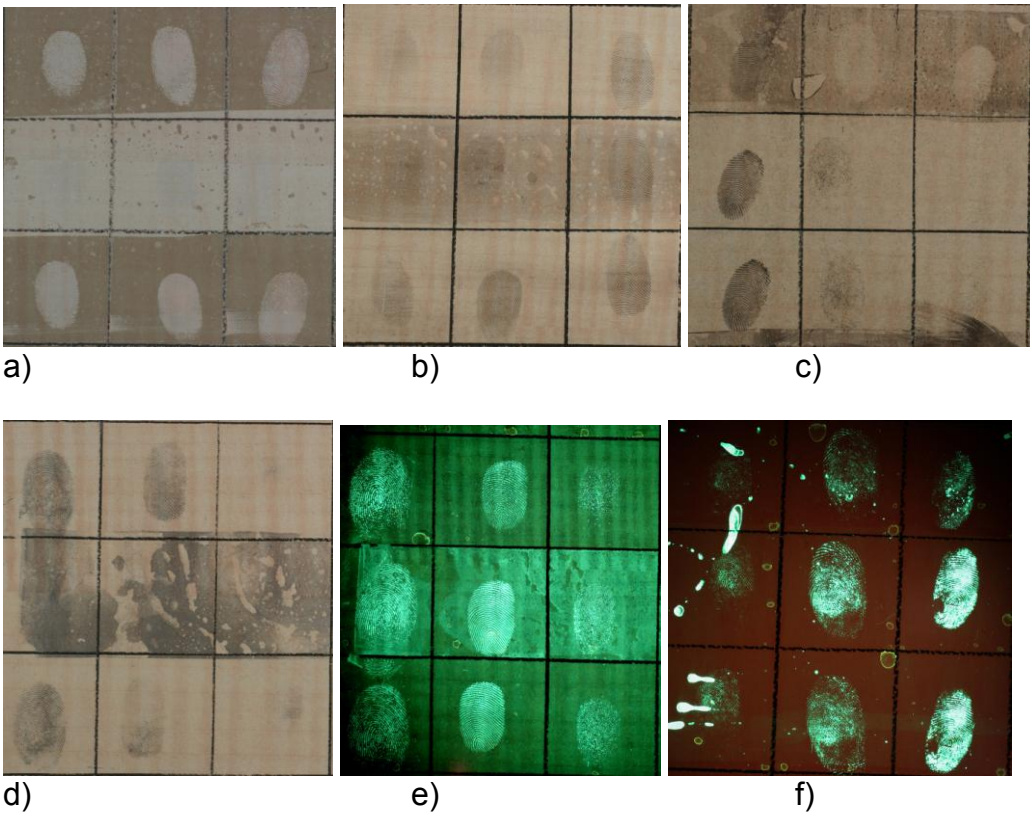
Graph showing the effectiveness of gelatin lifting on different surfaces and on marks of different age [13].

8.1.11 The BVDA gel lifts were found to be more effective than Dycem lifters on all the surfaces studied at that time (2008), and this is consistent with the greater surface tack of the BVDA gel when the protective acetate sheet is removed. However, the potential of the higher tack BVDA lifts to cause surface damage should be recognised.

8.1.12 The effectiveness of gel lifts was seen to decrease as surface roughness and porosity increases.

8.1.13 In accordance with the initial study above, it can be seen that the effectiveness of gelatin lifting decreases as the age of the mark increases, and that significant degradation in the average score of lifted marks actually occurs in the period between two hours and one day after deposition.

8.1.14 Further experiments were carried out to establish if gelatin lifting could be used in sequence with other processes. It was shown that both types of lifter could be detrimental to subsequent treatment, but that it was not always possible to tell which combination of lifting material, surface and subsequent development technique would cause problems. The use of gelatin lifting as a development process is therefore not recommended if further treatments are likely to be carried out to the surface.



a) b) c)

d) e) f)

The effects of gelatin lifting on subsequent fingermark development processes a) *vacuum metal deposition on fake ash laminate* b) *black magnetic powder on fake ash laminate* c) *black powder suspensions on fake ash laminate* d) *Magneta flake powder on fake ash laminate* e) *superglue/basic yellow 40 on fake ash laminate and* f) *superglue/basic yellow 40 on painted metal. Top row = lifted with BVDA lift, middle row = lifted with Dycem lift and bottom row = control (no lifter applied).*

8.2 Pseudo-operational trials and operational experience

8.2.1 No fully recorded pseudo-operational trials have been conducted on gelatin lifting, although small-scale exercises have been conducted on 'real' surfaces by CAST to see what types of item the technique can recover marks from. These were articles and surfaces tested during a tour around the laboratories and common areas at CAST, without any pre-planting of marks. Surfaces that marks were successfully lifted from included: soft drinks cans, coffee mugs, door handles and push plates, glass windows, wooden pool cue handle, bench top, credit card, pens, guns and glossy magazine covers.

8.2.2 Operationally there are few situations where the process should be used in preference to powdering using the optimum brush/powder combination, but there are some police forces using the technique routinely for lifting of latent marks. One widely publicised success was obtained from gelatin lifting a mark in grease from the ceiling of an abattoir. This mark could not be powdered because of contamination,

could not be chemically treated because of the difficulties in washing chemicals over the ceiling, and was difficult to photograph in situ because of it being on a white background.

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1,2 Indandione

1. History

1.1 1,2 Indandione (also referred to as 1,2-indanedione) was first proposed as a fingermark development reagent in 1997 [1,2], following observations by researchers at the University of Pennsylvania that it reacted with amino acids to give products that were both coloured (pink) and fluorescent. A range of analogues were also developed in this study, but only 1,2 indandione has been extensively researched since.

1.2 1,2 Indandione is applied in a very similar way to 1,8-diazafluoren-9-one (DFO) and ninhydrin by drawing the exhibit through a bath of solution, allowing it to dry and then placing it in an oven to develop the marks. The initial observations of both coloured and fluorescent reaction products prompted more detailed investigations of the reagent in comparison to the ninhydrin and DFO formulations then in common use [3,4]. Both of these studies indicated that 1,2 indandione merited further study, with results equivalent to DFO being obtained in laboratory tests. However, it was also observed that sequential treatments using combinations of ninhydrin and 1,2 indandione were not particularly effective [3].

1.3 Further studies were carried out in both Israel [5] and by the Police Scientific Development Branch (PSDB) in the UK [6] to establish the optimum processing conditions for 1,2 indandione, although the two groups arrived at different conclusions. The Israeli researchers found that a formulation free of acetic acid gave the best results, and suggested processing conditions of 20 minutes at 100ºC and 60% relative humidity. However, the UK research identified an optimal level of acetic acid to promote fluorescence and suggested processing for 10 minutes at 100ºC and 0% relative humidity. Variable results have since been obtained from 1,2 indandione at different laboratories worldwide. It has been concluded that humidity is very important in the development process; variations in local humidity conditions affect the results obtained unless additions are made to the formulation.

1.4 However, both the Israeli and UK research provided further evidence that 1,2 indandione justified operational trials:

- PSDB (*ibid.*)[6] finding it gave equivalent results to DFO on batches of 75 cheques and a range of representative porous items; and
- the Israelis [5] reporting an improved performance over DFO in a pseudo-operational trial conducted over batches of 500 cheques per process.

Once again, it was found that using ninhydrin in sequence after 1,2 indandione developed few, if any, additional marks.

1.5 Based on these results, the process was adopted for operational use in Israel and taken forward into a full operational trial in the UK [7]. In the UK operational trials, the performance of 1,2 indandione was the least effective of the formulations under test and was consequently not

recommended for operational use. A similar operational trial in Canada [8] arrived at similar conclusions and in both countries DFO remained the technique of choice.

1.6 1,2 Indandione has become more widely used in Australia and Israel and to some extent in the USA, and further research into the reagent has been conducted in all three countries. Stimac [9] proposed a formulation of 1,2 indandione for use with thermal papers and Azoury *et al.* [10] reported that the treatment of exhibits with 1,2 indandione is not detrimental to subsequent DNA profiling. However, a survey conducted into the usage of chemical treatments worldwide demonstrated that at that time (2004) 1,2 indandione was still not in widespread use in many countries and in some cases the respondents were not aware of it at all [11].

1.7 The lack of widespread use may partly be attributed to the variable results that were initially obtained worldwide. In some cases different cities in the same country obtained very different results according to local weather conditions. As a consequence, optimised formulations differed according to local humidity and environment (and possibly, also, local substrates). More recently, a solution to this problem has been identified. In early assessments of 1,2 indandione it was noted that the fluorescence may be enhanced by toning with metal salts in a similar manner to ninhydrin [3]. It has been shown that by adding zinc salts to the treatment solution the fluorescence of the mark can be enhanced without the need for a post-treatment and the resultant formulation is considerably more resilient to local fluctuations in humidity and environment [12,13]. Optimised formulations were developed for use under Australian conditions, with the best results claimed after hot-pressing at 165ºC for 10 seconds. The development of this formulation prompted further studies by the Home Office Centre for Applied Science and Technology (CAST) to see whether this offered a credible alternative to or replacement for DFO in the UK [14,15]. Comparative studies were carried out on the formulations recommended by Australian and US researchers in 2007 alongside the PSDB formula used in the late 1999 comparison with DFO, with zinc salts added into the formula. In this study the modified CAST formulation was found to be most effective and was compared with DFO in a further experiment. The results of this experiment are reported below, and showed the performance of 1,2 indandione-zinc to be closely equivalent to DFO.

1.8 Further work has since been carried out by the Australian and US research teams in further optimising formulations. Bicknell and Ramotowski in the USA further refined the reagent formulation and found it to outperform DFO [16]. They also observed that, although the stability of the 1,2 indandione-zinc system to humidity fluctuations was much improved, the humidity level in the paper after dipping did have an influence on the subsequent development route. For papers below a critical humidity level (approximately 70%), treatment in a humidity oven using ninhydrin processing conditions was recommended, whereas for

papers with humidity content above this level a dry oven and DFO processing conditions gave best results.

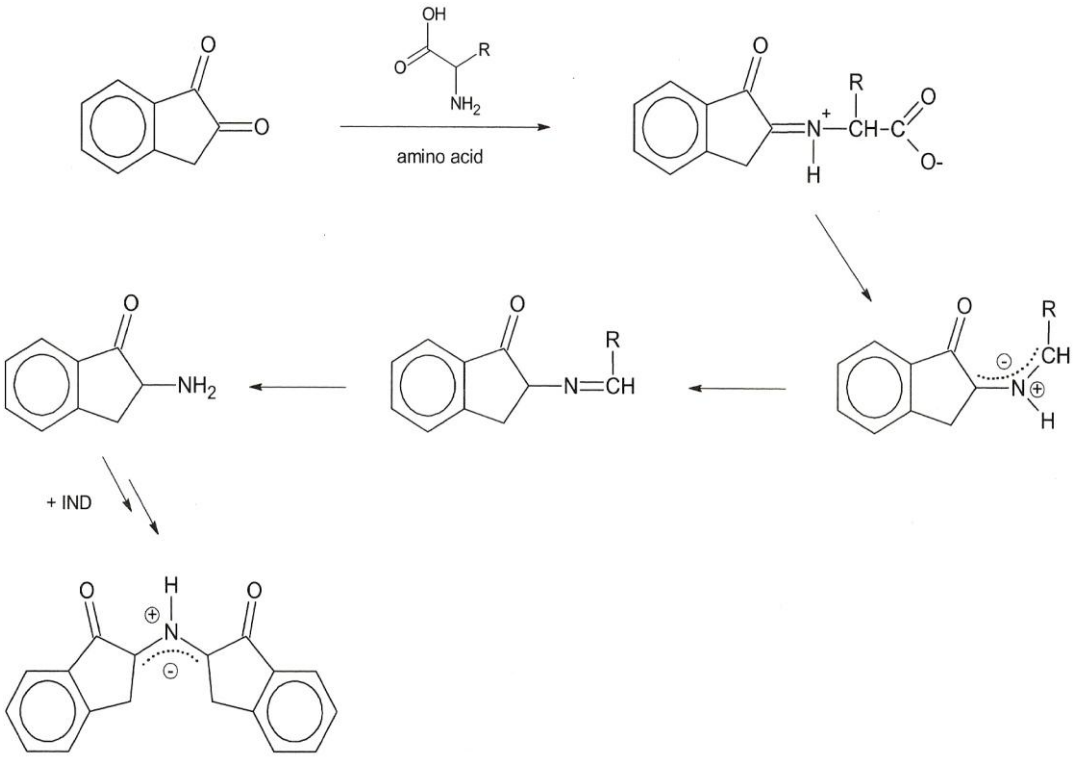
1.9 CAST is aware of several unpublished comparative studies between 1,2 indandione and DFO that have been conducted in different countries around the world since 2009. These have consistently indicated that 1,2 indandione gives better quality, more fluorescent marks on all paper types, with several alternative formulations for 1,2 indandione being proposed in addition to those reported in the papers above. The observations made by CAST during the 2007 comparison [14,15] were that 1,2 indandione was noticeably better than DFO on brown papers. This was investigated in detail in a focused study on different types of brown paper and card [17]. The results of this study (summarised in Chapter 3, Chemical and Physical Processes, DFO) confirmed the earlier observations, with 1,2 indandione consistently giving better results than DFO on this type of substrate.

1.10 Recent results have been consistent in showing the latest 1,2 indandione formulations to outperform DFO as a single process on most types of paper studied. However, the issue of whether processing sequences incorporating 1,2 indandione outperform those incorporating DFO still needs to be addressed before DFO can be fully phased from use. In one small-scale study conducted using single prints from a range of donors, the sequence DFO-ninhydrin was found to develop more marks overall than the sequence 1,2 indandione-ninhydrin [18]. However, a more comprehensive study using pseudo-operational material and more extensive processing sequence (i.e. including physical developer followed by Nile Red) found little difference between the two reagents, with the sequence incorporating 1,2 indandione recovering marginally more marks overall [19]. Nile Red did not develop any additional fingermarks at the end of the sequence, although it was suspected that the cumulative effect of the solvents used in DFO, 1,2 indandione and ninhydrin is detrimental to the constituents targeted by this reagent. Further work is still required in the area of optimised sequential processing.

1.11 CAST has recognised that 1,2 indandione will ultimately replace DFO, initially on brown paper and card and then on other types of paper. With this in mind, work is ongoing to ensure that an optimised formulation is introduced into operational use, which includes observation of the development of fluorescence under different processing conditions [20]. The results of this were used to inform an optimised formulation and processing conditions that have been used in recent comparative trials.

2. Theory

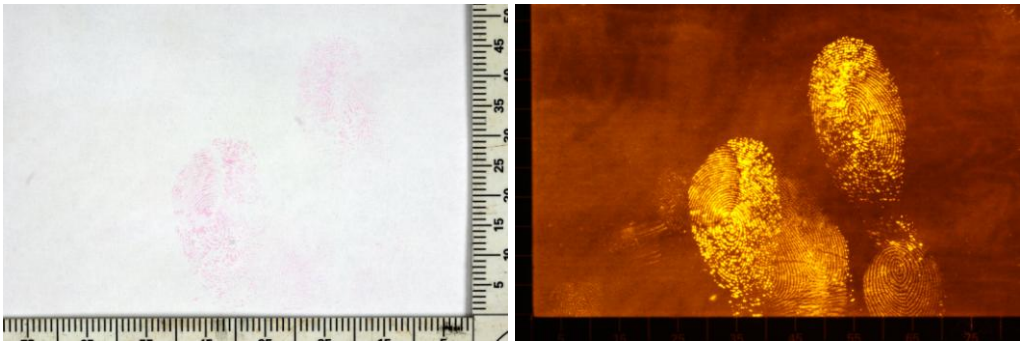
2.1 1,2 Indandione is closely related to ninhydrin and it has been proposed that its reaction with amino acids follows a very similar pathway, one suggested reaction path being illustrated below.



Proposed reaction pathway for 1,2 indandione with amino acids (Petrovskaia et al., *2001)[21].*

2.2 The proposed formation of a Ruhemann's purple analogue as shown above accounts for the pink coloration (now widely referred to as Joullie's Pink) seen for prints developed from formulations using high 1,2 indandione concentrations. All evidence to date indicates that the same product is responsible for the fluorescence observed.

2.3 Unlike DFO, methanol is not necessary for the reaction to proceed and was thought to inhibit it. This is because 1,2 indandione forms a stable hemiketal with methanol and this prevents the reaction with amino acids taking place. However, ongoing CAST research now indicates that methanol may actually be beneficial in promoting the formation of marks with optimum fluorescence [20] and a closer study of its role in formulations is underway.



a) *b)*

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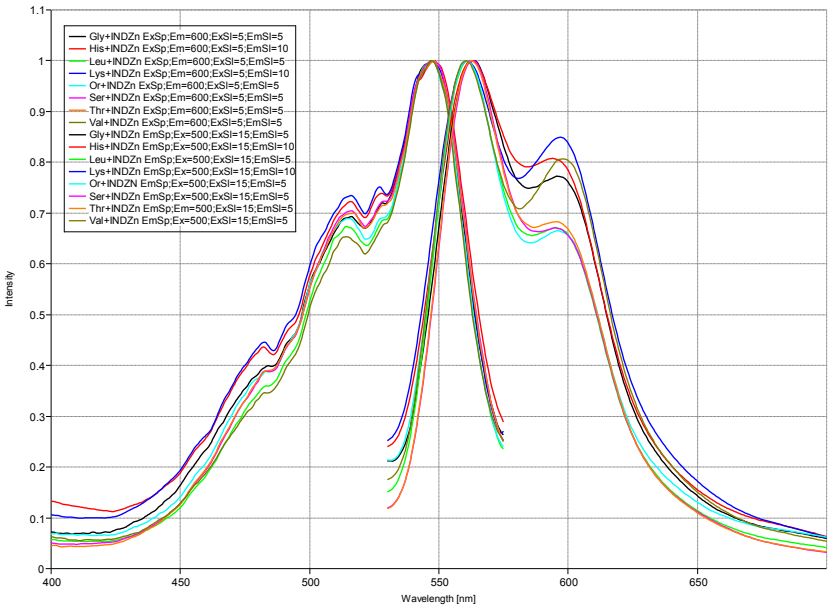
Fingermarks on paper developed using 1,2 indandione and imaged in a) *reflected light and* b) *fluorescence mode.*



Absorption and emission spectra measured for 1,2 indandione-zinc reacted with a range of amino acids (Spindler et al.*, 2009)[22].*

2.4 The addition of zinc to the formulation has been shown to give reaction products that are consistent in their excitation and emission spectra across a wide range of amino acids. This was not true of 1,2 indandione formulations without zinc salt additions [22] and it was proposed that the zinc ions (Zn^{2+}) present in the solution has a catalytic effect in driving the formation of the fluorescent reaction product. Subsequent research indicated that the Zn^{2+} was stabilising a critical reaction intermediate compound, driving the reaction towards the desired product, Jouillié's Pink [23].

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not currently (2016) recommend the use of 1,2 indandione as a Category A process because although work to produce an optimised UK formulation for comparative trials has been completed, it has not yet been published in open literature. It is recognised that there are already circumstances where the use of 1,2 indandione could give operational improvements over DFO, and therefore 1,2 indandione is currently listed as a Category B process in the *Fingermark Visualisation Manual.* It is also recognised that the formulation given in the manual will be superseded by the time the process is upgraded into Category A in the next update. There have also been suggestions in previous published work that 1,2 indandione may not be as effective as DFO

when used as part of sequential treatments [3,5,18] although other studies indicated that 1,2 indandione may develop more fingermarks than the DFO-ninhydrin sequence [12,19]. The most recent testing conducted by CAST indicates that sequences using the 1,2 indandione formulation proposed for operational implementation in the UK followed by ninhydrin do outperform the DFO-ninhydrin sequence currently recommended[24].

3.2 A summary of the experiments performed and the results on which these conclusions are based is given below.

3.3 In the late 1990s/early 2000s, PSDB began a programme of work to optimise the 1,2 indandione formulation for use in UK conditions [6]. Observations from this work included the following.

- The optimum fluorescence level in the developed mark is given by 0.25g 1,2 indandione per litre of solution. Higher concentrations can give a more intense pink colour, but in common with the DFO formulation, CAST regards fluorescence as the most important characteristic.
- The optimum fluorescence of fingermarks without increasing undesirable background fluorescence to a level where it begins to obscure marks is given by 10 mL of acetic acid per litre of solution.
- The 90 mL of ethyl acetate per litre of solution is present to act as a co-solvent.
- The solution is made up to 1 litre with 1-methoxynonafluorobutane (HFE7100), selected as a proven non-flammable, non-toxic solvent for fingermark formulations.

3.4 It was also determined that the optimum processing conditions for maximum fluorescence were:

- heating for 10 minutes at 100ºC without humidity; and
- storing processed exhibits in the dark to maximise the subsequent development of marks and to retain fluorescence.

More recently, other researchers have suggested that equivalent (if not better) performance can be obtained by heating at higher temperatures (~160°C) for shorter times [12,13]. This has recently been investigated by CAST, with results indicating that the rate of heat transfer as opposed to temperature alone may be the most important factor in obtaining optimum fluorescence [20].

3.5 Pseudo-operational trials were then conducted on batches of 75 cheques, comparing the optimised 1,2 indandione formulation with DFO. The results are shown in the graphs below.

Chart 1

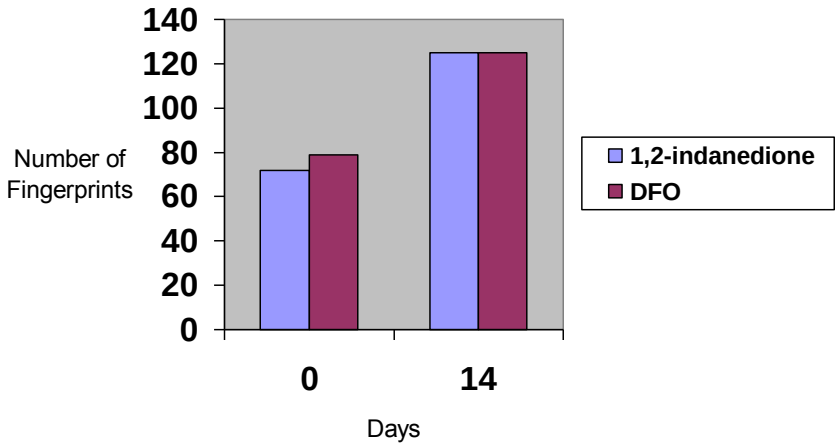
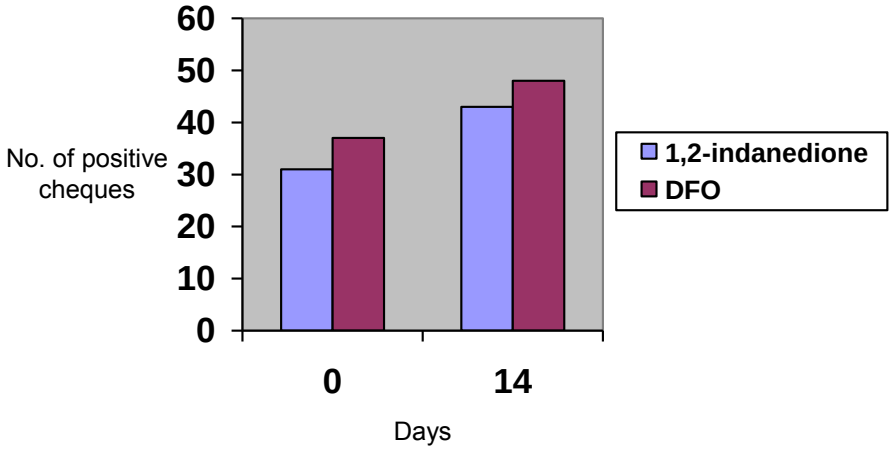


Chart 2



Number of fingermarks detected on cheques developed using 1,2 indandione and 1,8-diazafluoren-9-one processes and number of cheques yielding positive results [6].

3.6 These results indicated that the two systems were closely equivalent in performance, with DFO developing slightly more marks. A further pseudo-operational trial was conducted on a range of different porous exhibits, the results being tabulated below.

Article	Number of articles	Number of developed fingermarks	
		DFO	1,2 Indandione
White envelopes	20	41	44
Brown envelopes	15	17	33
Photocopy paper	20	92	82
Newspaper	20	2	2
Receipts	20	5	7
Train tickets	19	10	6
Total	**114**	**167**	**174**

Results of pseudo-operational trial on samples typical of porous exhibits encountered in casework [6].

3.7 These results were sufficiently encouraging to justify the inclusion of 1,2 indandione in an operational trial of two ozone-friendly DFO formulations against the chlorofluorocarbon (CFC) 1,1,2-trifluorotrichloroethane (CFC113)-based DFO formulation [7]. In these trials, the 1,2 indandione formulation proved least effective on operational work and was not pursued further. Similar results were obtained from an operational trial in Canada [8].

3.8 However, more recent publications from Australia [12,13] suggest stabilisation of the 1,2 indandione system to humidity by the addition of zinc salts (zinc chloride, $ZnCl_2$) in the solution. This necessitated a re-evaluation of the process and CAST carried out a further work programme with the objectives of identifying the optimum formulation with zinc salt additions and carrying out a comparative trial with DFO. In this trial, 18 different porous substrates were used, covering a range of different paper types.

Substrate	Brand	Size	Weight	Description
1	Tesco value	A4	75gsm	White copier paper
2	Woolworths	A4	80gsm	Multipurpose paper
3	WH Smith	A4	100gsm	Premium inkjet paper
4	XEROX	A4	80gsm	Laser copier paper
5	HP	A4	80gsm	Everyday inkjet paper
6	WH Smith	A5	–	Writing paper
7	PUKKA	A4	80gsm	Premium quality lined writing paper
8	Woolworths	A4	–	Premium pad lined
9	Tesco value	A4	–	Refill pad lined
10	Tesco	C4	–	White envelopes
11	Tesco value	C4	–	Brown envelopes

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12	Woolworths	50cm x 5m	–	Brown paper
13	*TV Choice*	–	–	TV magazine
14	*Heat*	–	–	Magazine
15	*Sun*	–	–	Newspaper
16	Ryman	A4	–	Silk finish paper laser printers
17	Ryman	C4	130gsm	White envelopes
18	Ryman	A4	90gsm	Ivory parchment paper

Summary of porous surfaces used in comparative studies [15].

3.9 This study consisted of over 180 deposited marks per substrate, per condition examined. For experiments using all 18 substrates, more than 180 x 18 prints were examined. The intensity of the fluorescence for developed marks was measured using a Minolta 100LS spot meter, and marks were graded using a 0 to 4 grading scheme, the grading scheme being described elsewhere [25]. The fluorescence conditions used were illumination with the 473 to 548 nm excitation band of a Quaser 40, viewed through a 549 nm cut-on long-pass filter (Schott glass OG570).

3.10 Initial studies compared the effectiveness of 'optimised' formulations including zinc salts, developed by the Australian Federal Police, CAST in the UK, and the US Secret Service in the USA.

<u>Australia 1,2 indandione-zinc formulation</u>

0.5g 1,2 indandione

15mL dichloromethane

30mL ethyl acetate

5mL acetic acid

0.5mL $ZnCl_2$ stock solution

450mL HFE7100.

<u>UK 1,2 indandione-zinc formulation</u>

0.125g 1,2 indandione

45mL ethyl acetate

5mL acetic acid

0.25mL $ZnCl_2$ stock solution (0.2g $ZnCl_2$ in 5mL absolute ethanol)

500mL HFE7100.

<u>USA 1,2 indandione-zinc formulation</u>

0.5g 1,2 indandione

15mL dichloromethane

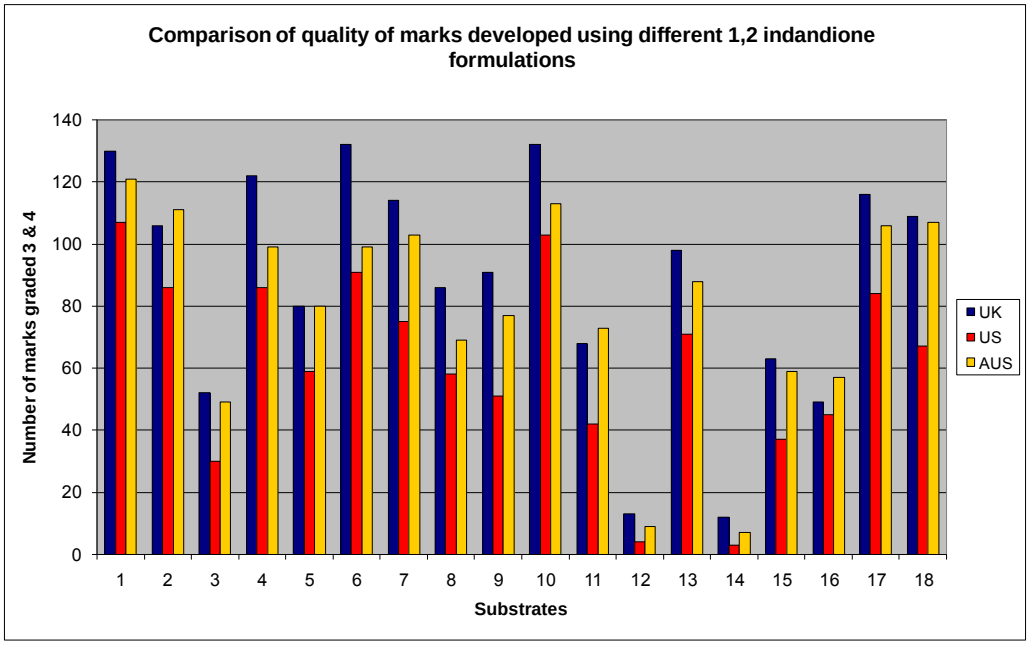
30mL ethyl acetate

5mL acetic acid

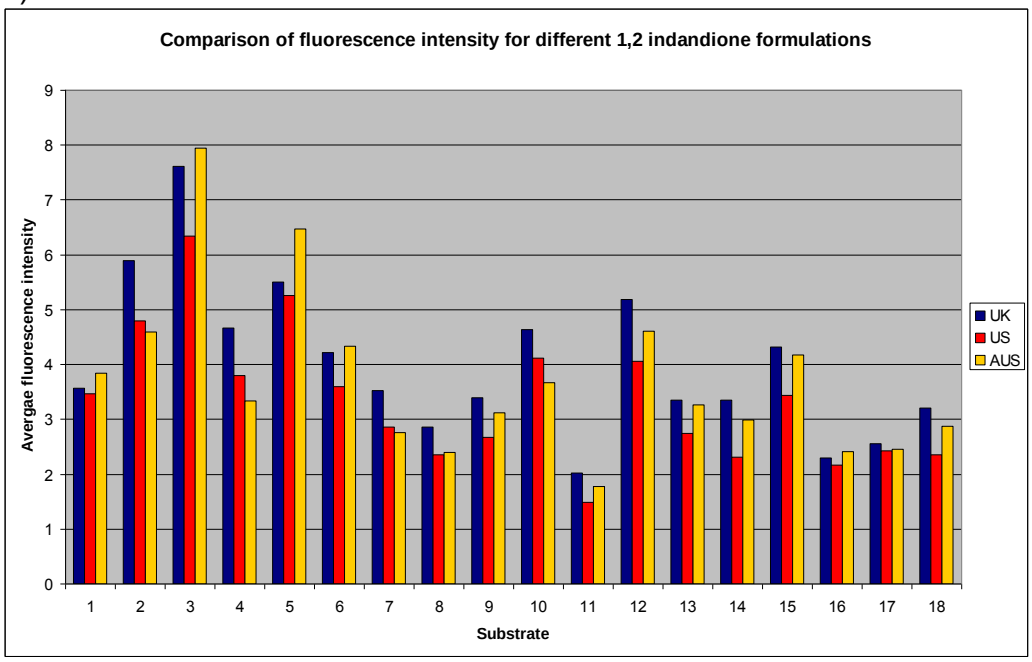
2mL $ZnCl_2$ stock solution

448mL petroleum ether.

3.11 The results of this pre-selection exercise are depicted graphically below.



a)



b)

Results of comparative trials between different 1,2 indandione formulations based on a) *fingermark quality and* b) *intensity of fluorescence [15].*

3.12 From these trials it appeared that, under UK conditions, the CAST formulation gave the best performance in terms of both quality of fingermarks developed and the intensity of fluorescence from the developed mark. The CAST formulation with zinc salt additions was therefore compared with the existing CAST DFO formulation, both formulations being given below.

UK 1,2 indandione-zinc formulation

0.125g 1,2 indandione

45mL ethyl acetate

5mL acetic acid

0.25mL $ZnCl_2$ stock solution

500mL HFE7100.

DFO formulation

0.25g DFO

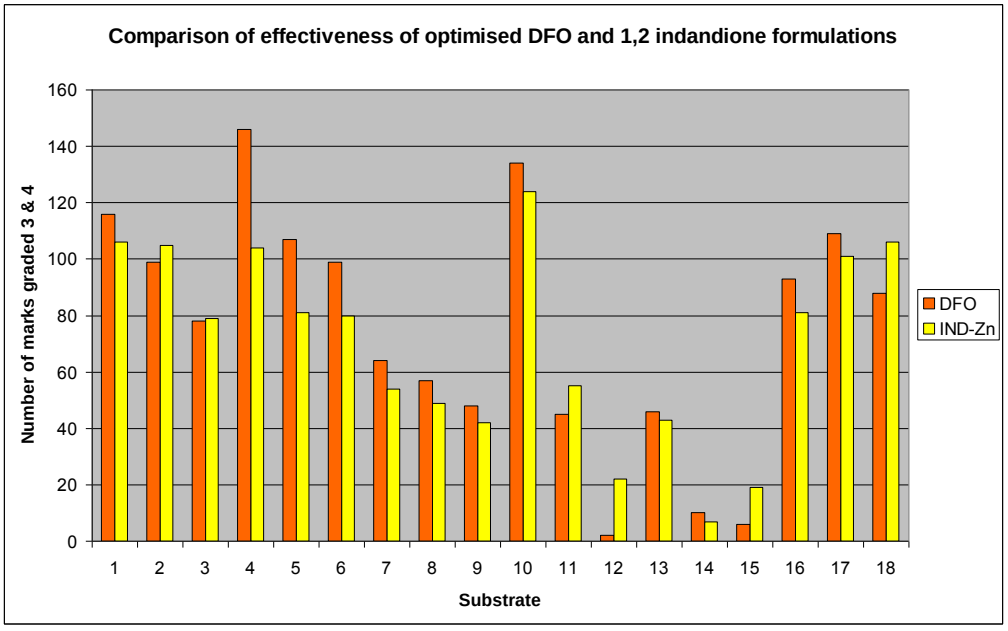
30mL methanol

20mL acetic acid

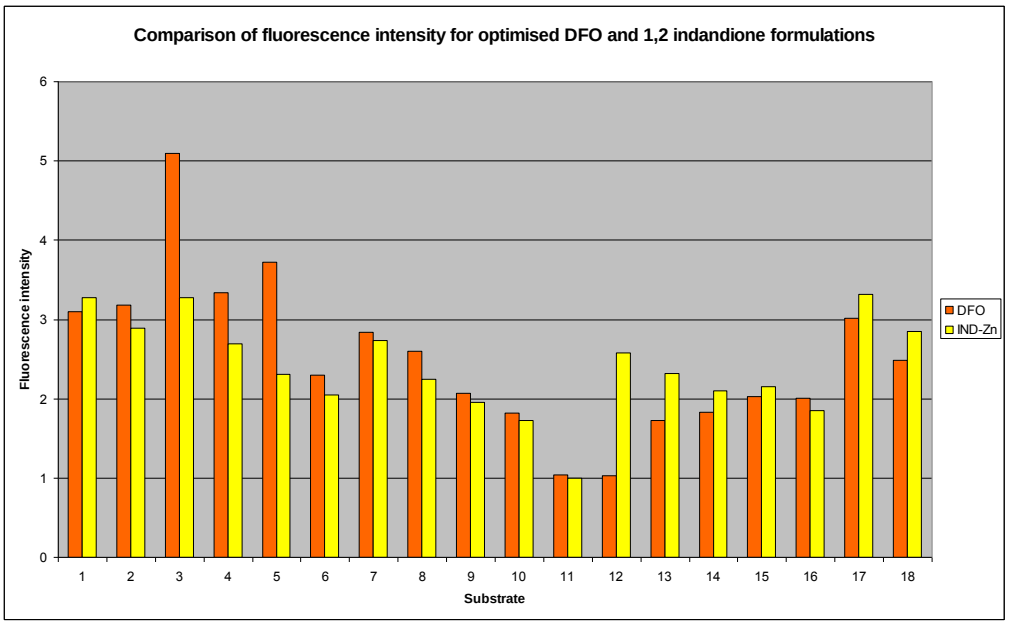
275mL HFE71DE (trans-1,2-dichloroethylene)

725mL HFE7100.

3.13 Once again, comparisons were made between the quality of the developed mark and the intensity of fluorescence. Results from this comparison on two-day-old marks are shown in the graphs below.



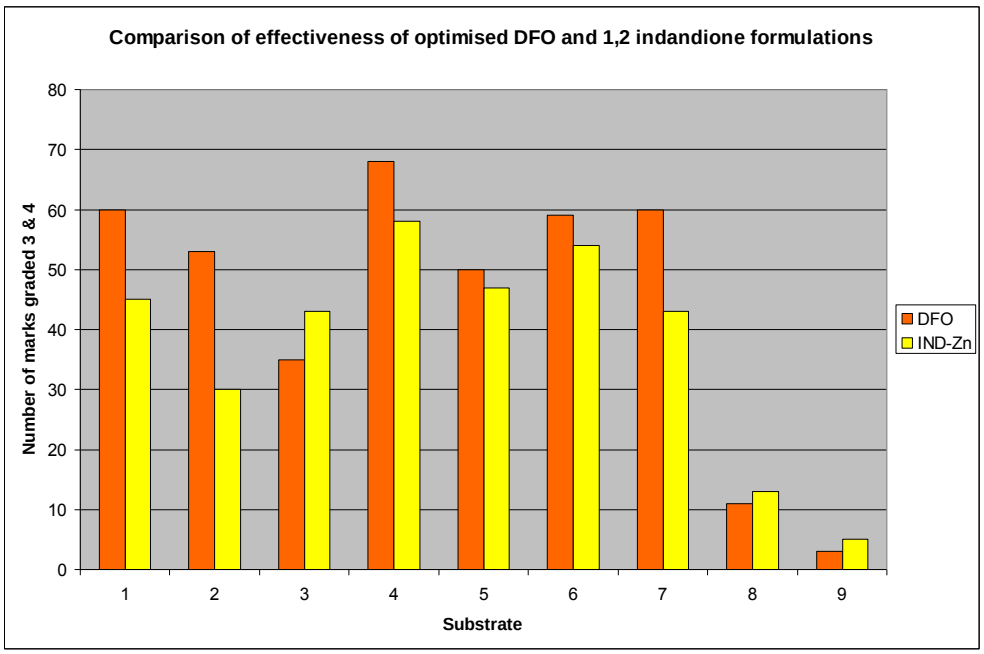
a)



b)

Results of comparative trials between 1,8-diazafluoren-9-one and 1,2 indandione formulations on two-day-old marks based on a) *fingermark quality and* b) *intensity of fluorescence [15].*

3.14 In these trials, the formulations gave closely equivalent performance, with DFO giving marginally better results. An assessment on 14-day-old marks was commenced but it was not possible to complete the study in the time available. However, initial results (illustrated below) suggested a similar trend.

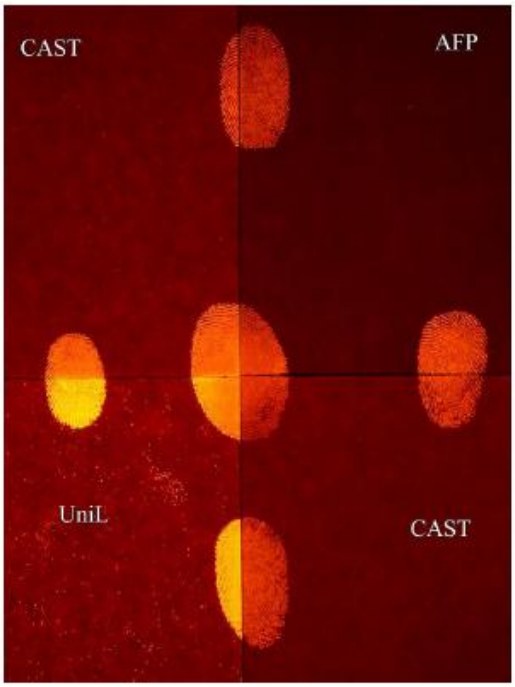


Results of comparative trials between 1,8-diazafluoren-9-one and 1,2 indandione formulations on 14–day-old marks, assessing fingermark quality alone [15].

3.15 The results obtained showed no improvement in performance from 1,2 indandione over DFO. This, combined with reports that ninhydrin develops no additional marks after 1,2 indandione but is known to do so after DFO, resulted in CAST recommending no further evaluation of 1,2 indandione. DFO was therefore retained as the recommended CAST process and the sequential treatment of DFO-ninhydrin-physical developer remained unchanged.

3.16 However, as outlined in the sections above, subsequent studies have been carried out in Australia, which again found that 1,2 indandione-zinc outperformed DFO (in this case the CAST DFO formulation) under Australian conditions. Similarly, the US Secret Service has developed a revised 1,2 indandione-zinc formulation, which appears to give improved performance over the CAST DFO formulation in a limited study under UK conditions.

3.17 The results from a study focused on brown paper and card [17] have already been summarised in Chapter 3, Chemical and Physical Processes, DFO. These indicated that, on this type of substrate, 1,2 indandione gave consistently better results than DFO. These experiments utilised the CAST 1,2 indandione formulation highlighted above. More recent project work at CAST re-evaluated this process, in terms of the formulation, the temperature items are heated to, and the method of delivering heat [20].



Example of a 'quartered fingermark' experiment conducted to compare the fluorescence obtained from three different formulations of 1,2 indandione processed under nominally similar conditions.

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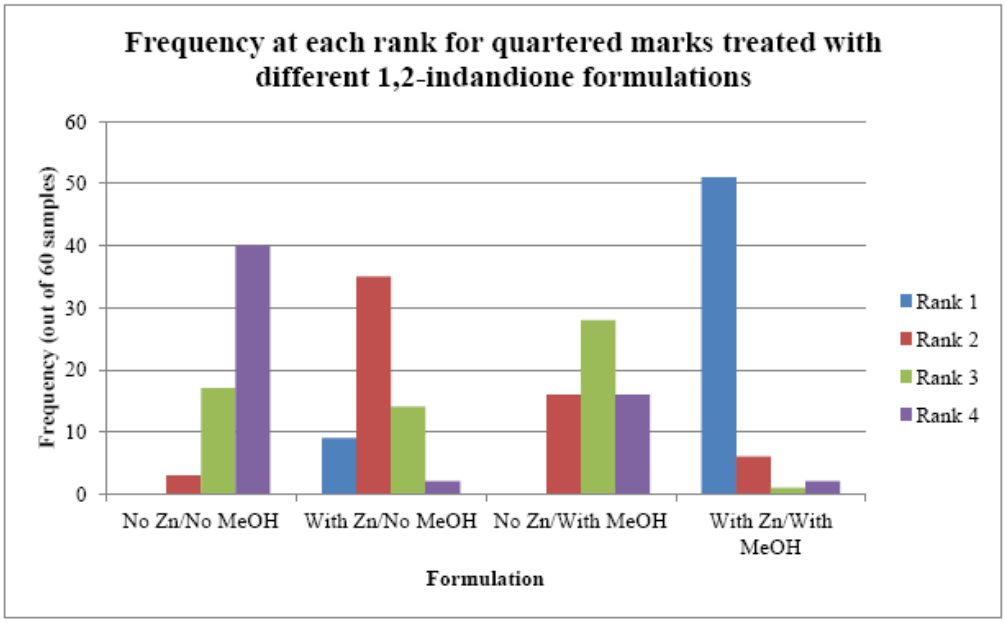
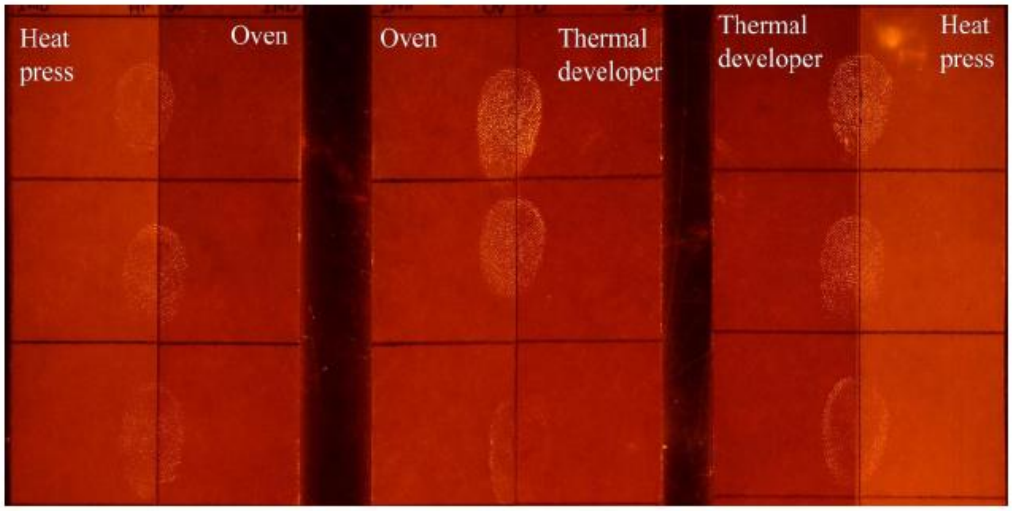


Results obtained from quartered fingermark experiments varying different elements of the formulation (in this case incorporating zinc salts and/or methanol into the formulation) [20].



Results obtained from the same formulation of 1,2 indandione processed at the same nominal temperature using different methods of heating [20].

3.18 The outcome of this study established that the presence of both zinc salts and methanol in the formulation is important in producing the most intensely fluorescent marks. An optimised formulation was produced for a further series of comparative trials with DFO [24].

Chemical	Chemical grade	Quantity
1,2-Indandione	>99%	0.25g
Acetic acid	Analytical ≥99.7%	10mL
Ethyl acetate	Analytical ≥99.7%	45mL
Methanol	Analytical ≥99.7%	45mL

HFE 7100	As supplied	1L
Zinc chloride stock		1mL

Formulation of 1,2 indandione used in 2015-2016 comparative trials [24].

Chemical	**Chemical grade**	**Quantity**
Zinc chloride	Reagent grade ≥98%	0.1g
Acetic acid	Analytical ≥99.7%	1mL
Ethyl acetate	Analytical ≥99.7%	4mL

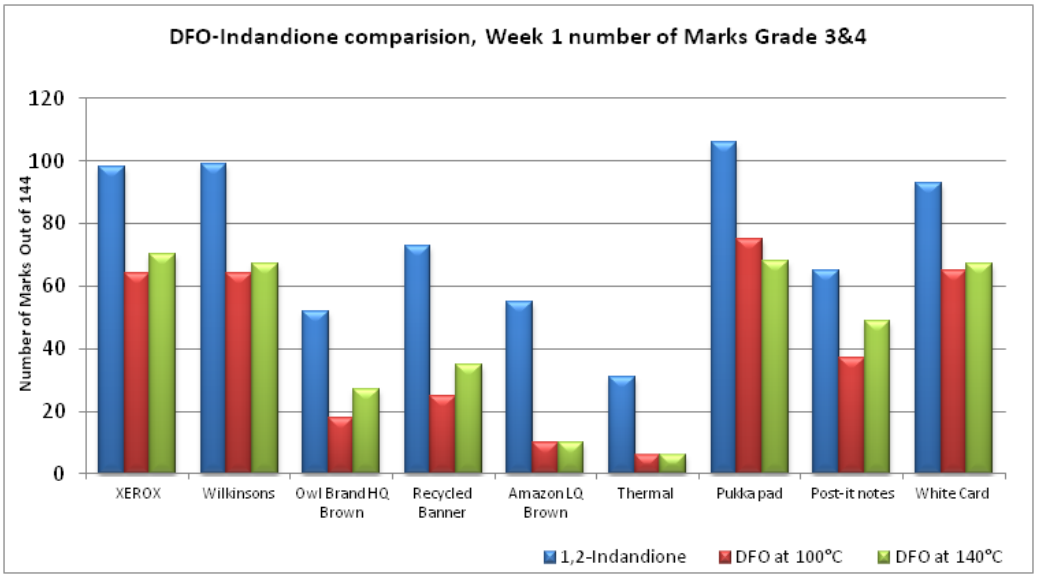
Formulation of zinc chloride stock solution used in 1,2 indandione solution [24].

3.19 The formulation above was evaluated against DFO, which was processed both at the recommended temperature of 100°C and a higher temperature of 140°C for 20 minutes. Articles treated with 1,2 indandione were heated to 100°C for 10 minutes. Split depletions were laid on 9 paper types using 12 donors and 2 aging periods (1 week and 3 weeks) for the fingermarks. In total 7,776 split marks were graded on all paper types; 144 fingermarks were processed on each paper type with each solution. The paper types used were:

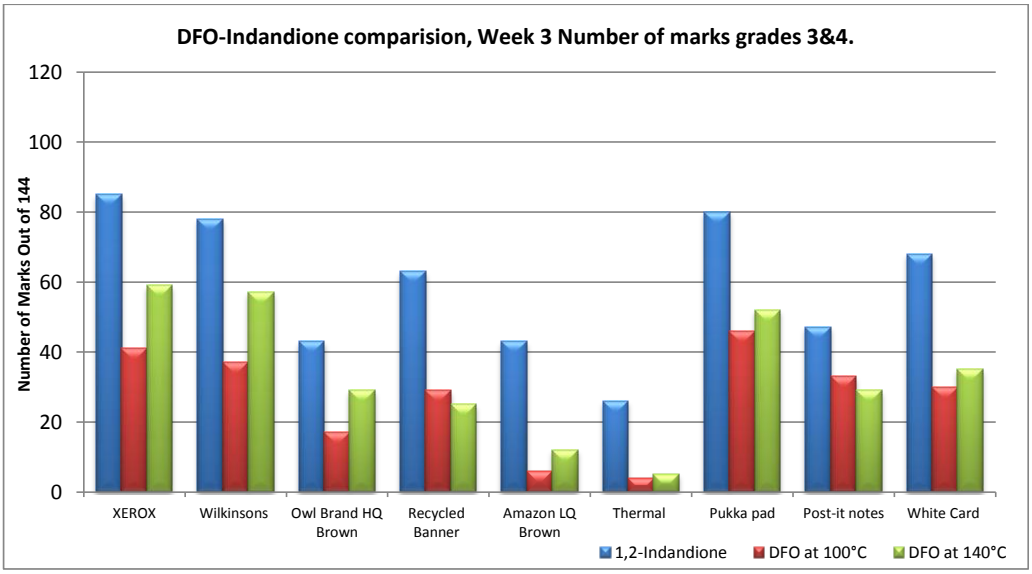
Substrate	**Brand**	**Size**	**Weight**	**Description**
1	Xerox Performer	A4	80gsm	High quality white copier paper
2	Wilkinsons recycled	A4	80gsm	Low quality white copier paper
3	Owl	A4	115gsm	Brown envelope
4	Banner recycled	A4	80gsm	White copier paper
5	Amazon	A4	90gsm	Low quality brown envelope
6	-	-	60gsm	Thermal paper till roll
7	Pukka	A4	80gsm	Premium quality lined writing paper
8	Post-it	-	70gsm	Yellow post-it notes
9	-	-	220gsm	White card

Summary of porous surfaces used in comparative studies [24].

3.20 The results of this study demonstrate that 1,2 indandione consistently develops more high quality fingermarks than DFO, regardless of the processing temperature used or the age of the fingermark.

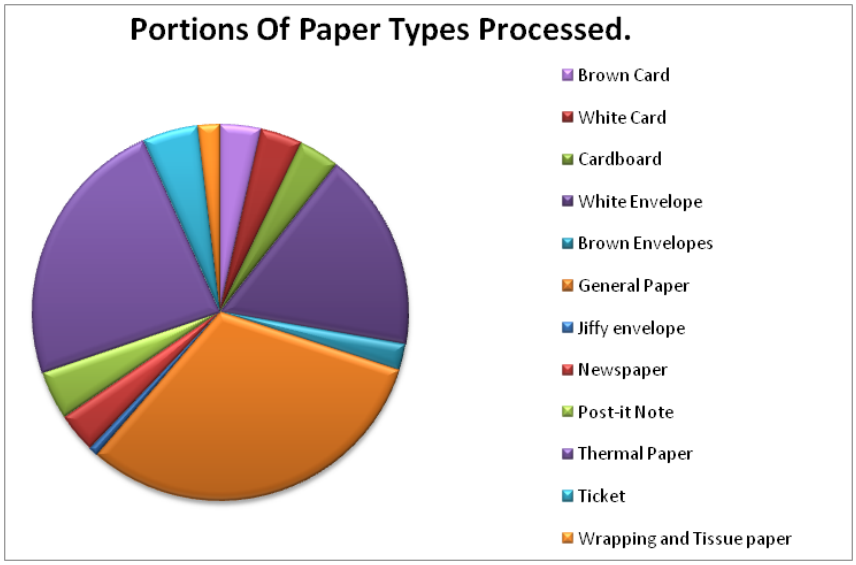


Comparison of the effectiveness of 1,2 indandione, DFO at 140°C and DFO at 100°C on 1 week old fingermarks on a range of paper types [24].



Comparison of the effectiveness of 1,2 indandione, DFO at 140°C and DFO at 100°C on 3 week old fingermarks on a range of paper types [24].

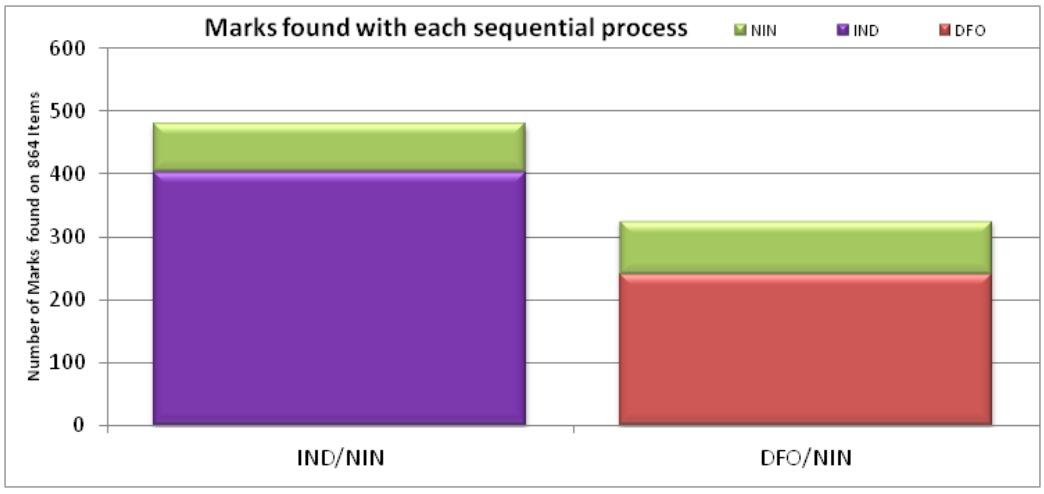
3.21 The results of the split depletion study were followed by a pseudo-operational trial for which 1,000 varying porous items were collected from a range of sources. A summary of the types of item used in the trial is given below.



Relative proportions of the types of porous items treated in the pseudo-operational trial [24].

3.22 The items were separated into two roughly equivalent piles, where batches of smaller items were split equally between the two piles and larger items were halved, with one half placed in each pile. One pile was then treated with 1,2 indandione, the other with DFO, and the number of fingermarks developed were recorded. Both sets of items were then treated with ninhydrin, and the number of additional fingermarks developed was recorded. The results indicated that:

- the 1,2 indandione ninhydrin sequence produces ~30% more marks overall than the DFO ninhydrin sequence; and
- that the number of additional marks developed by ninhydrin is roughly equivalent for each sequence.



Cumulative totals of fingermarks developed by the two sequences used in the pseudo-operational trial [24].

4. References

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Iodine

1. History

1.1 Iodine is one of the earliest chemical processes proposed for the development of latent fingermarks and is still in operational use today. The observation that iodine fumes could be used both to detect handwriting alterations and to develop latent fingermarks was reported by Coulier in 1863 [1]. In a review of early literature relating to fingermark development conducted by Morris [2], references to the use of iodine fuming are made in 1891 and a procedure for its application given in 1912. It was noted that fumes of iodine directed onto paper produced a yellow colour where the iodine was absorbed by the fingermark residues. However, this staining was only transitory, fading in minutes, and further experiments were conducted to identify a method of fixing the mark.

1.2 Iodine fuming was in operational use in the UK by 1931 [3] and a method of transferring and fixing developed marks using moist paper carrying rice starch was proposed in 1935 [2]. An alternative means of 'lifting' developed marks by means of a silver foil was being used by the 1960s [4], the iodine selectively reacting with the surface to form silver iodide, which then darkened when exposed to strong light. A refinement to the starch fixing process was proposed at about the same time [5], the method proposed being to brush the mark with finely ground starch powder, blow to remove the excess and then expose the mark to gentle steam for 1 to 2 seconds. In a summary of methods used to develop fingermarks produced by Scotland Yard in 1970 [6], iodine fuming was among the recommended development techniques, in this case utilising the starch powder fixing method. Iodine fuming was either applied within an enclosure, or could be applied to surfaces using a fuming pipe, however the latter approach is no longer recommended because of health and safety concerns.

1.3 The lifting of fingermarks developed using iodine with silver or tin plates was further investigated as a means of recovering fingermarks from skin [7,8,9]. Experiments demonstrated that marks could be recovered from both live and dead skin using this technique and although marks could be recovered up to 72 hours after deposition on dead skin, the retention time on live skin was significantly shorter. It was also observed that only oil-rich, sebaceous marks were developed in this way, no development being observed for eccrine marks.

1.4 Further work was carried out on iodine fixatives in the 1970s. 'Tetrabase' (4,4-bis(dimethylamino)diphenylmethane) was investigated as a fixing solution and also as an additive in uncured silicone rubber mixes, which could be moulded over a developed mark to fix it without recourse to solvent dipping or spraying [10]. Other researchers proposed α-naphthoflavone (7,8-benzoflavone) [11], with this method of fixing ultimately being favoured in the UK for operational use [12,13]. Simultaneous fuming of iodine and steam was studied as a means of

improving the sensitivity of iodine fuming on paper and also improving the performance of the reagent on older marks [14]. Iodine fuming was also applied to non-porous surfaces, with successful results being claimed on brass [6].

1.5 It was subsequently proposed that the sensitivity of the technique could be improved by applying the iodine in solution, combined with the α-naphthoflavone fixative [15]. This formulation used cyclohexane as the solvent for iodine, which is highly flammable and not considered appropriate for use at scenes of crime. The Metropolitan Police and Home Office Central Research Establishment (HO CRE), Aldermaston, developed a non-flammable, two-part formulation with the objective of treating large areas such as painted and papered walls at scenes [16]. This formulation was based on iodine dissolved in Fluorisol (1,1,2-trichlorotrifluoroethane, also known as CFC113 or Arklone), with the α-naphthoflavone fixative as a separate solution in dichloromethane which was added just prior to use. Comparative trials were carried out with the chlorofluorocarbon (CFC)-based ninhydrin formulation – known as non-flammable ninhydrin (NFN) – then in operational use, recording the marks developed under ambient conditions [16]. It was shown that the iodine solution was more effective in these conditions, although on paper and paper-based wallpaper ninhydrin gave superior performance if it was exposed to elevated temperature and humidity. Iodine solution was introduced into operational use by some organisations with marks being developed at around one-third of scenes treated [16].

1.6 The iodine solution formulation developed by HO CRE was considered for inclusion in the first edition of the *Manual of Fingerprint Development Techniques*, primarily as a process for application to wall surfaces at crime scenes. However, further comparative testing carried out by the Home Office Scientific Research and Development Branch (SRDB) between iodine solution and the CFC-based ninhydrin formulation indicated that ninhydrin was in fact the more effective process and that the sequence of iodine solution and fixative followed by ninhydrin may produce fewer marks than ninhydrin as a single treatment [17]. The principal advantage of iodine solution was that it developed marks instantly, compared with the period of up to ten days required for the development of marks treated with ninhydrin at a scene. Because of the potentially detrimental (albeit slight) effect of iodine solution and fixative on subsequent ninhydrin development, the process was ultimately omitted from the manual. The possibility of applying the reagent as a spray was also investigated [18], and was claimed to be more effective than both ninhydrin and the brush application of iodine solution.

1.7 With the introduction of the Montreal Protocols in 1987 and the banning of the use of CFCs, it became necessary to look at alternative formulations of iodine solution. PSDB initiated a programme of work to revisit the iodine solution formulation and assess alternatives to the solvent, fixing agent and to iodine itself [19]. These studies identified heptane and methylcyclohexane as possible alternative solvents to

CFC113. However, both these solvents are flammable and not suitable for crime scene use without significant precautions. Alternative non-flammable solvents gave inferior performance in the development of fingermarks. Of the range of fixing agents studied, α-naphthoflavone proved to be the most effective in terms of colour and longevity of the fixed mark.

1.8 Australian researchers also studied the use of formulations based on the non-CFC solvents 1-methoxynonafluorobutane (HFE7100) and 2,3-dihydrodecafluoropentane (HFC4310mee) [20]. Although not as effective as the CFC-based formulation, the HFC4310mee formulation was investigated as a spray reagent on a range of surfaces, including treated wood, glass, wallpaper, vinyl, paint, brick and raw wood, and its performance compared with powdering and a ruthenium tetroxide spray reagent. In these trials, iodine was found to be the most effective treatment of those evaluated for vinyl, wallpaper and brick. Other studies using iodine have looked at α-naphthoflavone as a fixative for marks developed on skin using fuming [21] and fuming as a technique for developing marks on adhesive tape [22].

1.9 In 2004 to 2005, CAST included a flammable (heptane-based) iodine solution in comparative studies of techniques for developing marks on contaminated surfaces, where it was compared with solvent black 3 and basic violet 3. On certain surfaces, iodine did appear to give superior results and it will be necessary to explore this in more detail to see if iodine has a place in some sequential treatment charts.

1.10 A further study has been conducted by CAST to compare the flammable, heptane-based iodine solution to ninhydrin on a range of wall coverings representative of those commercially available in 2009 [23]. The materials used to manufacture wallcoverings have changed in the years since the previous studies, and the results demonstrated that the flammable iodine formulation was far more effective than ninhydrin on the surfaces studied, in contrast to previously observed trends. This does raise some operational issues because the flammable formulation should not be applied at scenes and the effectiveness of ninhydrin has evidently declined. Subsequent treatment of these surfaces with powder suspensions has indicated that this process is potentially far more effective than either iodine or ninhydrin and further work is required to optimise advice given for treatment of such surfaces.

1.11 The concerns regarding the scene use of the flammable iodine solution led to its use being discontinued in the UK, but the perceived gap in capability following this decision prompted further work into non-flammable formulations. The starting point for these studies was the HFC4310mee-based formulation proposed by Flynn *et al.* [20]. Walton [24] compared a formulation of iodine in HFC4310mee solvent with ninhydrin on both painted woodchip wallpaper and lining paper for marks aged up to 6 weeks old. It was found that iodine solution consistently gave better results than ninhydrin for marks up to 4 weeks old, whereas,

for marks older than this, ninhydrin began to be more effective. Tsala [25] used a slightly different formulation based on HFC4310mee solvent with 5% vol/vol additions of methanol, and compared this to a flammable iodine solution formulation using methylcyclohexane as the solvent on a range of surfaces including gloss painted wood, PVC, varnished wood and untreated wood. In these tests, the HFC4310mee-based formulation generally gave better results. The results from both these studies indicate that formulations based on HFC4310mee may offer a non-flammable solution that can be used at scenes, although some measurements of flammability will be required, particularly for formulations including additions of methanol.

1.12 Iodine fuming has received little research attention in recent years, although Kelly *et al.* [26] observed that iodine fuming of thermal receipts that had been processed using ethanol to remove the text followed by ninhydrin made the erased text visible again and, in some cases, enhanced the contrast of marks developed using ninhydrin.

2. Theory

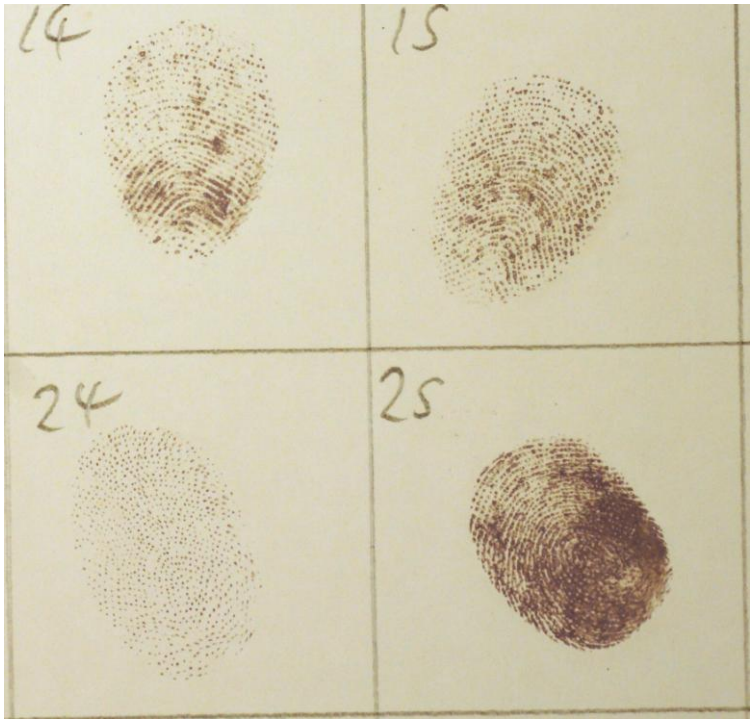
2.1 It has been suggested that the development of fingermarks using iodine occurs by an addition reaction across the carbon double bonds in the unsaturated fatty acid components of the fingermark residue [27], giving a brown colour characteristic of the presence of the triiodide (I_3^-) ion. It has also been observed that iodine interacts particularly strongly with the squalene constituent of fingermarks, which contains several carbon double bonds. The readily reversible nature of this reaction is used to explain the rapid fading of prints developed using iodine.

2.2 However, observations by subsequent researchers indicate that this may not be the sole reaction mechanism [14]. The following reasons for this are cited:

- The addition reaction across the double bonds of unsaturated compounds is known to be slow, whereas the development of prints using iodine is instantaneous and still occurs at sub-zero temperatures.
- The reactions that occur to fix the developed marks would not occur unless free iodine was present; the iodine compounds formed by the saturation reaction would not react in the same way.
- Laboratory trials with chemical compounds representative of other fingermark constituents, including saturated hydrocarbons, amino acids, inorganic salts and water, also gave visible reaction products on exposure to iodine fumes.

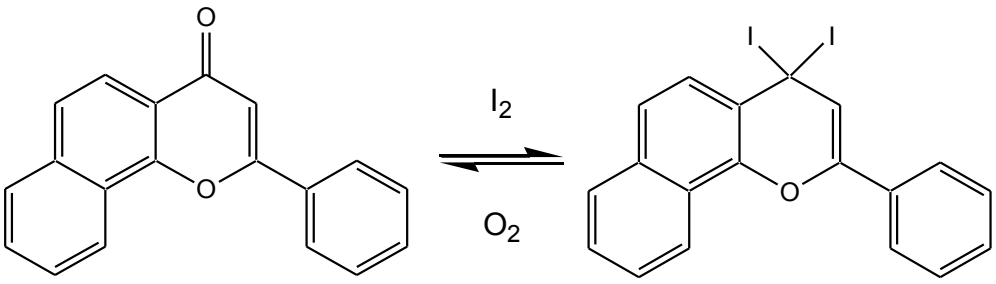
2.3 It was proposed that the principal mechanism binding iodine into the fingermark deposit and causing its yellow/brown colouration is the attractive interaction between the constant dipole of water molecules in the fingermark and a dipole induced on the iodine molecule. It is

proposed that this effect is enhanced by the presence of inorganic salts in the fingermark residue [14]. Because the presence of water is necessary, this would account for the observed poor performance of iodine on older marks where water has evaporated.



Fingermarks from different donors developed on glossy paper by iodine fuming.

2.4 The mechanism of the fixing reaction has not been conclusively identified, but Sears [17] suggests a reversible reaction between iodine and α-naphthoflavone, which would account for the fading of the fixed marks with time.

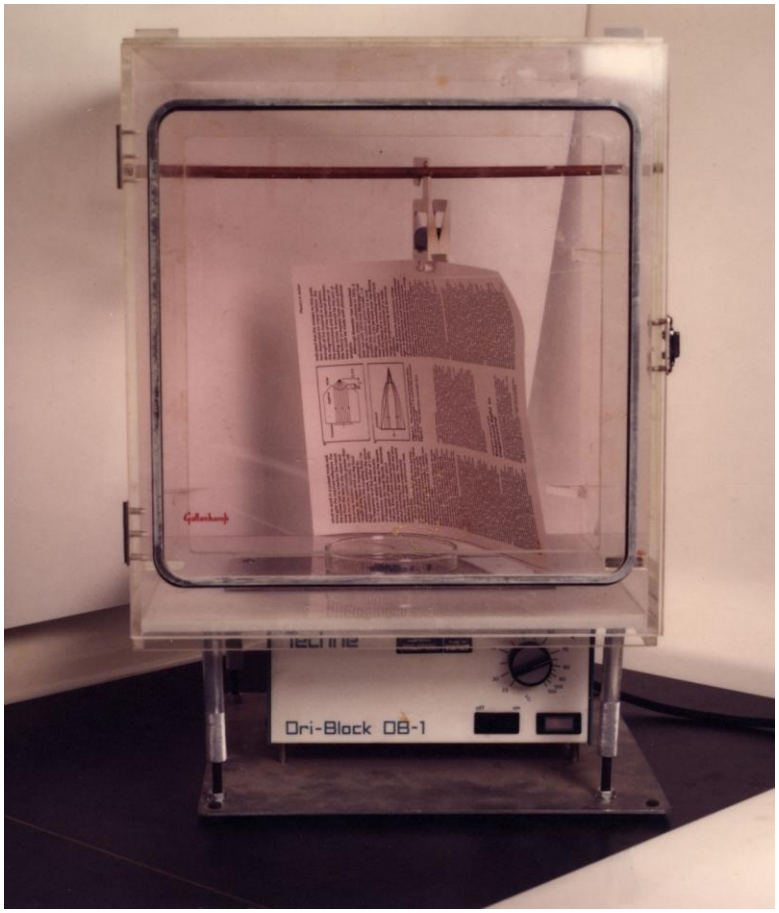


Proposed fixing mechanism for iodine using α-naphthoflavone.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Iodine fuming and iodine solution are both included as Category B processes in the *Fingermark Visualisation Manual* [28], because it is recognised that there are niche applications for both processes. Iodine fuming is recommended for use in situations where it is not possible to use solution dipping (e.g. documents are fragile or valuable), and iodine solution for use on walls at crime scenes where there are time constraints and marks may need to be developed more rapidly than is achievable with ninhydrin, or where the performance of iodine has been demonstrated to be superior to ninhydrin for a particular substrate type.

3.2 Process instructions are given for iodine fuming because the process is established; however, because there are multiple options for iodine solution, only an outline of the process is provided. In the fuming process, the article to be treated is supported or suspended within a small chamber, 1 g of iodine placed onto a glass dish at the base of the chamber, and the chamber sealed. The iodine is then allowed to sublime (or can be gently heated to 50ºC), producing a violet/brown vapour.



Article being treated by iodine fuming.

3.2 Development of fingermarks is monitored and when the maximum contrast is reached between ridges and the background, the excess

iodine vapour is removed from the chamber and the article removed and photographed.

3.3 Fingermarks may then be fixed using a solution based on α-naphthoflavone, but the formulation formerly given in the 2nd edition of the *Manual of Fingerprint Development Techniques* [13] requires review because it was based on CFC113.

4. Critical issues

4.1 Iodine fumes are corrosive and harmful, and iodine solution based on heptane (or methylcyclohexane) is harmful and flammable. Neither process should be used outside the controlled environment of a laboratory. HFC4310mee-based formulations may be suitable for use at scenes with adequate precautions, but more assessment is required before this can be confirmed.

4.2 Marks developed using iodine fuming may fade rapidly and require fixing to make them more visible and more permanent for subsequent imaging.

4.3 The effectiveness of iodine fuming is reduced on marks more than a few days old, and should not be used if older marks are being targeted.

5. Application

5.1 Suitable surfaces: Iodine fuming is suitable for porous surfaces, in particular paper. Performance is best on glossy paper types. It can be used on non-porous surfaces, but is most suited for those where greasy contamination is present. Iodine solution is suitable for all surfaces where iodine fuming is successful, and is also effective in developing fingermarks on painted wall surfaces. However, it is not recommended for use on painted walls because of the flammability of the solution (unless a non-flammable formulation is employed).

5.2 Iodine fuming is the only chemical treatment that can be used without leaving visible traces on the treated article. There are specialist applications where the lack of a visible, developed mark is important and iodine fuming is an option in these cases. However, because it is not often possible to determine what substances may be present in an article before treatment iodine should be used with caution because some substances may be capable of temporarily fixing the mark and inhibiting the normally rapid fading process.

5.3 Iodine fuming (and iodine solution) are also capable of detecting fingermarks on contaminated surfaces. Because iodine, basic violet 3 and solvent black 3 all develop marks in slightly different ways and may not target the same constituents [29], iodine may develop marks where other processes are ineffective. At least one practical case of this is

known (see section 8 on validation and operational experience, below). Because it has proved difficult to generate consistent 'contaminated' surfaces for laboratory trials, it is not currently (2016) possible to give comprehensive guidance for when (or if) iodine should be considered in either fuming or solution form, or to propose sequences with other reagents for contaminated surfaces.

5.4 In the laboratory, iodine fuming should be carried out in a chamber sited within a fume cupboard. Fuming can also be carried out on larger items or at scenes of crime using portable glass pipes with heated compartments to start iodine fuming, and desiccant crystals to dry the fumes. Because of the toxic and corrosive nature of iodine vapour, this should only be carried out in well ventilated and/or extracted areas by operators with the appropriate protective equipment.

5.5 Iodine solution can also be applied in a laboratory or at a crime scene by brushing or spraying. The solvents used as carriers for iodine are either flammable or capable of displacing air and should therefore be used with appropriate health and safety measures.

6. Alternative formulations and processes

6.1 Several processes have been proposed as alternatives to the fuming technique outlined in the manual [28]. The principal one of these is the cyclohexane-based iodine solution treatment, in regular use up to around 2008 in the UK by the former Forensic Science Service (FSS). CAST does not recommend this process for a variety of reasons, including effectiveness (although this may need to be reassessed), impact on subsequent treatments, health and safety, and scene clean up considerations. However, it is recognised that the technique does have some potential advantages and there are benefits in exploring options for a formulation suitable for scene use.

6.2 Previous assessments of the iodine solution process carried out by CAST in the late 1980s and late 1990s [17,19] have included investigations into alternatives to the solvent, fixing agent and iodine itself.

6.3 In the late 1980s, replacements for cyclohexane as the solvent for iodine were considered [17], with the main consideration being to identify a less or non-flammable formulation. Several candidate systems were rejected on the basis of cost (dichlorocyclohexane, dibromocyclohexane, 1,9-dichlorononane, 1,10-dichlorodecane and 1,7-dibromoheptane). Decahydronaphthalene ('Decalin') was tested as an alternative solvent and found to give fingermark development equivalent to the cyclohexane formulation. However, evaporation time of the solvent from the treated surface increased from seconds to 20 to 40 minutes and this was not deemed practical for operational use. Ultimately, the CFC113-based

formulation developed by HO CRE provided a non-flammable system that could be used at scenes of crime.

6.4 Replacements for dichloromethane in the α-naphthoflavone fixing solution were also investigated. Ethanol, ether, 2-ethoxyethanol, and 1,1,1-trichloroethane did not dissolve α-naphthoflavone and were therefore unsuitable. α-Naphthoflavone did dissolve in acetone and glacial acetic acid but, in both cases, the quantity of solvent required was far greater than the amount of dichloromethane and no change to the existing formulation was made.

6.5 After CFCs were withdrawn from regular use, CAST reassessed several formulations that included CFC113, including iodine solution. The objective was to produce an all-in-one formulation containing iodine and fixing agent. The CFC formulation was compared with a range of different solvent types, including:

- hydrofluorocarbons (HFCs) – 2,3-dihydrodecafluoropentane (HFC4310mee), 1,1,1,3,3-pentafluorobutane (HFC365mfc), 1-methoxynonafluorobutane (HFE7100) and 1-ethoxynonafluorobutane (HFE7200);
- siloxanes –octylmethylcyclotetrasiloxane (Volasil 244), decamethylcyclopentasiloxane (Volasil 245); and
- hydrocarbons – cyclohexane, heptane, methylcyclohexane.

6.6 Of these, iodine had only limited solubility in the hydrofluorocarbons, as did α-naphthoflavone. This resulted in rapid precipitation of the fixative unless excess dichloromethane was added. Solutions based on the siloxane solvents were more stable, but often took in excess of one hour to evaporate from the surface being treated. Solutions based on siloxanes also developed fewer fingermarks. All hydrocarbon solvents produced solutions that were effective in fingermark development but are highly flammable.

6.7 The opportunity was also taken to review alternatives to α-naphthoflavone. The alternatives considered were starch, β-cyclodextrin and the leuco-dyes leucocrystal violet, leucomalachite green, leuco patent blue and leuco berbelin blue.

6.8 The leuco dyes were effective fixing agents but, for a variety of reasons, including the cost of the reagent and background staining, were not proposed as replacements for α-naphthoflavone. Starch was the least effective of the fixing agents examined and while β-cyclodextrin did fix marks, the colour contrast was poor and ninhydrin could not be used sequentially after it.

6.9 The interhalides iodine monobromide and iodine monochloride were considered as replacements for iodine. Solutions formed with these compounds were less stable and the colours of the fixed marks were

less intense. As a consequence, these compounds were not pursued further.

6.10 The most effective all-in-one iodine solution was identified as:

Part A: 0.4 g iodine dissolved in 194 mL of heptane or methylcyclohexane;
Part B: 0.6 g α-naphthoflavone dissolved in 6 mL of dichloromethane.

Part B is added to Part A, then well-mixed. The resultant solution is allowed to stand for a couple of minutes before being filtered and applied with a brush.

6.11 However, there were disadvantages with the formulation (such as flammability) making it difficult to recommend for widespread use, especially at crime scenes. This formulation is used internally by CAST as the standard formulation for comparative laboratory studies of technique effectiveness.

7. Post-treatments

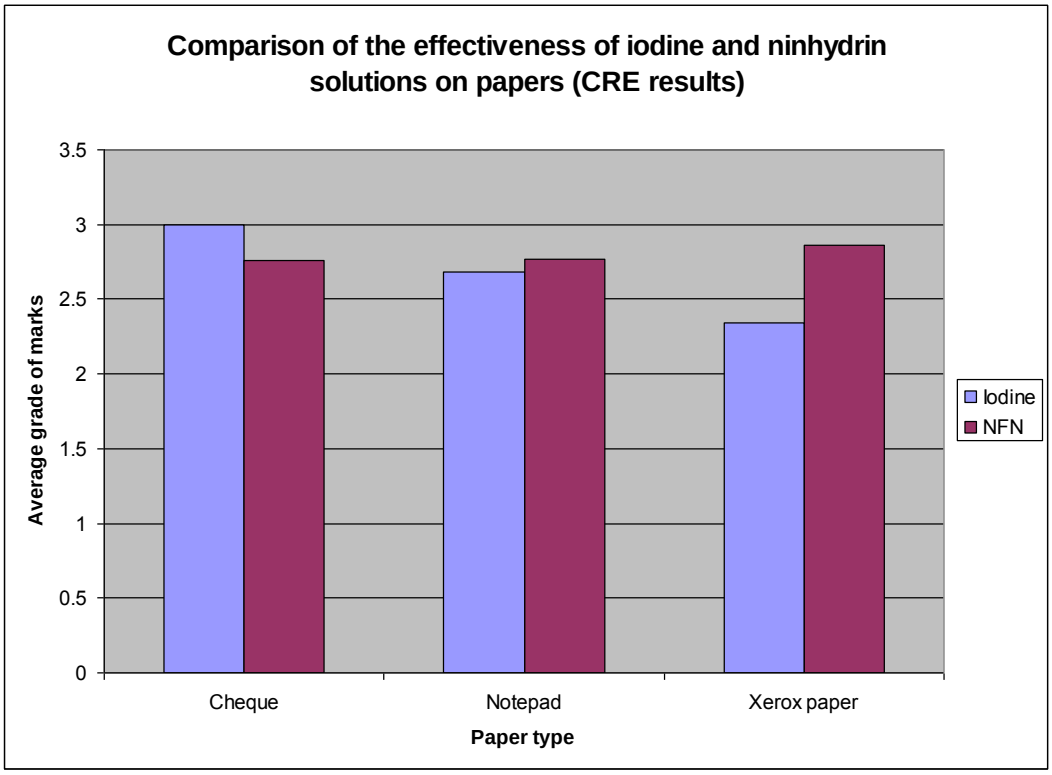
7.1 The principal post-treatment used for iodine fuming is fixing solution. The fixing solution converts the yellow/brown marks into a product with a more highly contrasting colour, and prevents them from rapidly fading. The most commonly used fixing agent for iodine is α-naphthoflavone, which gives a deep blue colouration.

7.2 For fingermarks developed using iodine fuming on skin, lifting using highly-polished tin or silver plates has been proposed, which involves placing the metal plate in contact with the developed mark. The reactive iodine will form a metal iodide on regions of the metal in contact with the fingermark ridges, which can then be darkened by illumination with strong light to reveal the ridges.

8. Validation and operational experience

8.1 <u>Laboratory trials</u>

8.1.1 Iodine solution was proposed as one possible treatment for paper samples in a laboratory during the early/mid-1980s. To explore this potential application, SRDB and HO CRE both carried out laboratory trials on paper exhibits, comparing the effectiveness of iodine solution and the non-flammable ninhydrin formulation then in operational use. The results obtained by HO CRE are tabulated below, being based on the results of developing and assessing single fingermarks deposited by 100 different donors and aged for 2 days.



Comparative results obtained for 2 day old marks processed with iodine and ninhydrin (NFN) on porous surfaces.

8.1.2 The SRDB studies consisted of trials using 40 split marks (2 from each of 20 donors) on white card and cheques. Three comparisons were made: iodine versus ninhydrin; the effect of subsequent ninhydrin treatment after iodine; and the iodine/ninhydrin sequence versus ninhydrin. The results are summarised below.

Grading	Iodine = ninhydrin	Iodine > ninhydrin	Iodine < ninhydrin
Percentage	75	10	15

Grading	Iodine = iodine/NFN	Iodine > iodine/NFN	Iodine < iodine/NFN
Percentage	70	25	5

Grading	Iodine/NFN = ninhydrin	Iodine/NFN > ninhydrin	Iodine/NFN < ninhydrin
Percentage	60	10	30

Results of comparative tests between iodine, ninhydrin and iodine/ninhydrin sequences.

8.1.3 The HO SRDB results were in accordance with the HO CRE results. Iodine solution was, in general, a less effective treatment than ninhydrin for paper samples and the sequential use of ninhydrin after iodine did not yield as many marks as ninhydrin alone. Iodine solution was therefore not recommended for use on paper articles by SRDB.

8.1.4 To assess the effectiveness of iodine solution on wall surfaces, Pounds [16] carried out a series of laboratory trials at HO CRE comparing iodine solution and the CFC-based ninhydrin formulation on surfaces representative of wall coverings. Initial tests looked at sheets of substrate stored in a laboratory, and consistently showed the iodine solution to be more effective when marks were developed under ambient conditions. Subsequent tests utilised actual sections of painted wall and powders were added to the techniques used in comparative studies. The results of these studies are tabulated below.

Storage condition	Development method	Days treated and assessed					
		0	5	12	20	32	53
Wall by window	Iodine solution	3.1	1.5	1.7	1.5	1.5	1.1
	Ninhydrin (CFC)	2.2	–	–	–	–	1.6
Wall in shade	Iodine solution	2.9	1.7	2.1	1.4	1.4	1.6
	Ninhydrin (CFC)	2.0	–	–	–	–	1.9

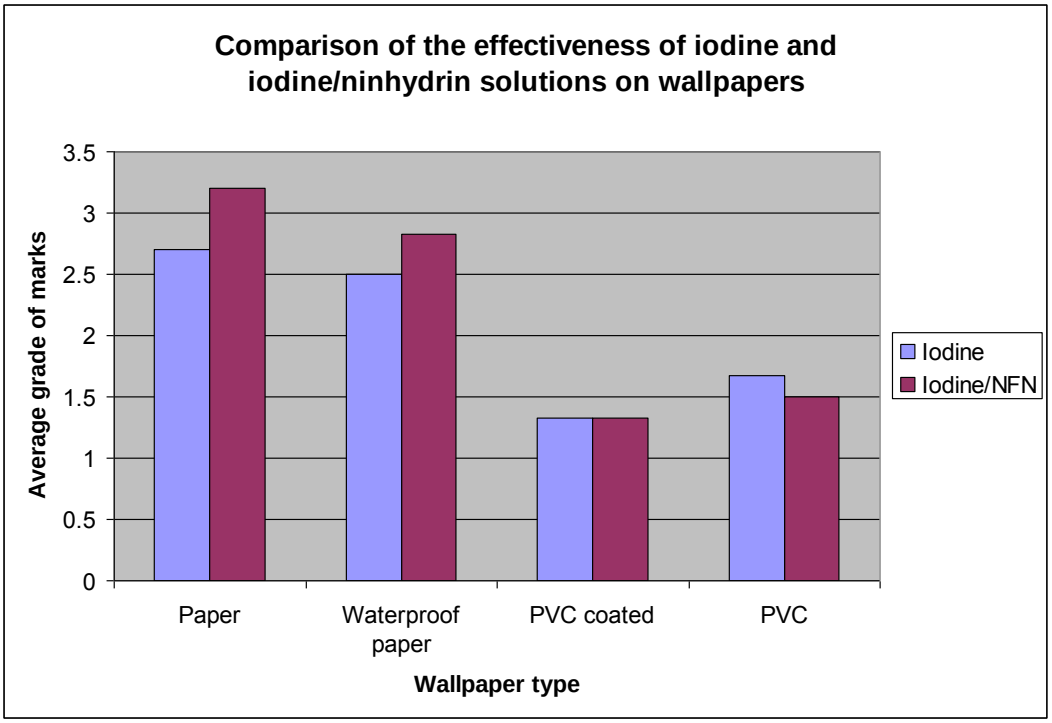
Average quality score for fingermarks developed on emulsion painted wall.

Development method	Days treated and assessed			
	1	5	12	27
Iodine solution	3.9	3.3	2.9	1.9
Ninhydrin (CFC)	1.0	–	–	–
Magnetic powder	2.9	–	–	–
Aluminium powder	1.0	–	–	–

Average quality score for fingermarks developed on emulsion painted wall.

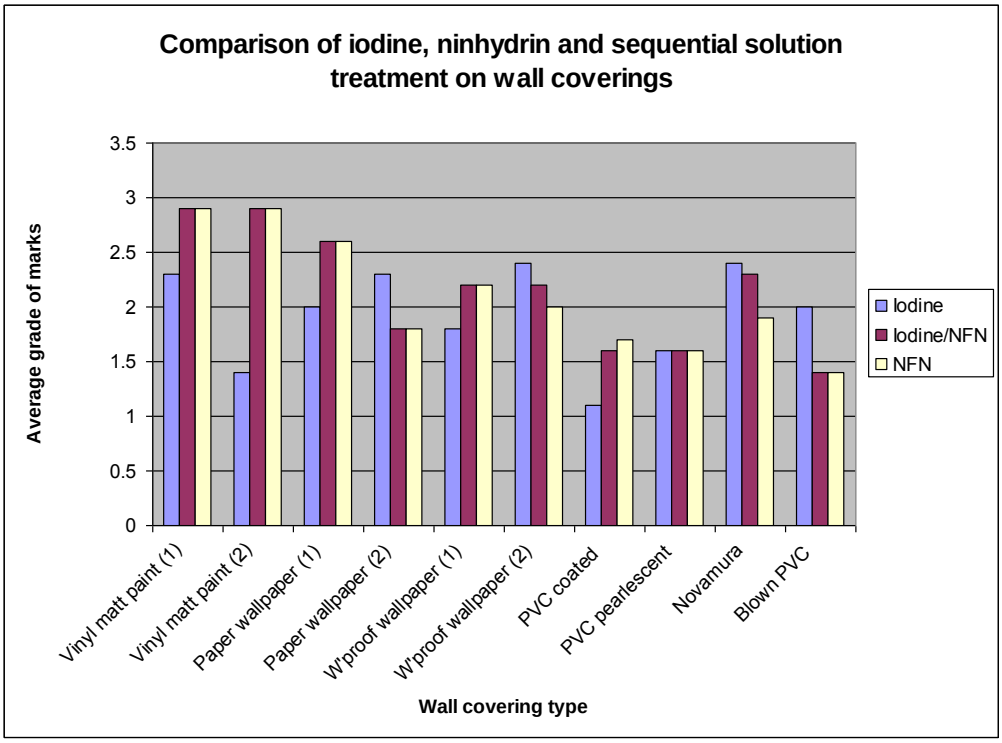
8.1.5 SRDB carried out similar studies [17] looking at a wider, more representative range of wall coverings, including painted walls and different types of wallpaper. Two trials were conducted, both involving the grading of over 200 marks. The first looked at aluminium powder, black granular powder, iodine and iodine followed by ninhydrin on the full range of surfaces. In this trial, aluminium powder was found to out-perform iodine solution on vinyl silk painted walls but, apart from this surface, both powders produced significant clogging on the surface and were not recommended for use. On matt paint, iodine worked well, but additional marks were developed by subsequent ninhydrin treatment. A summary of the results for the wallpaper surfaces is given below.

305 Publication number: 081/17



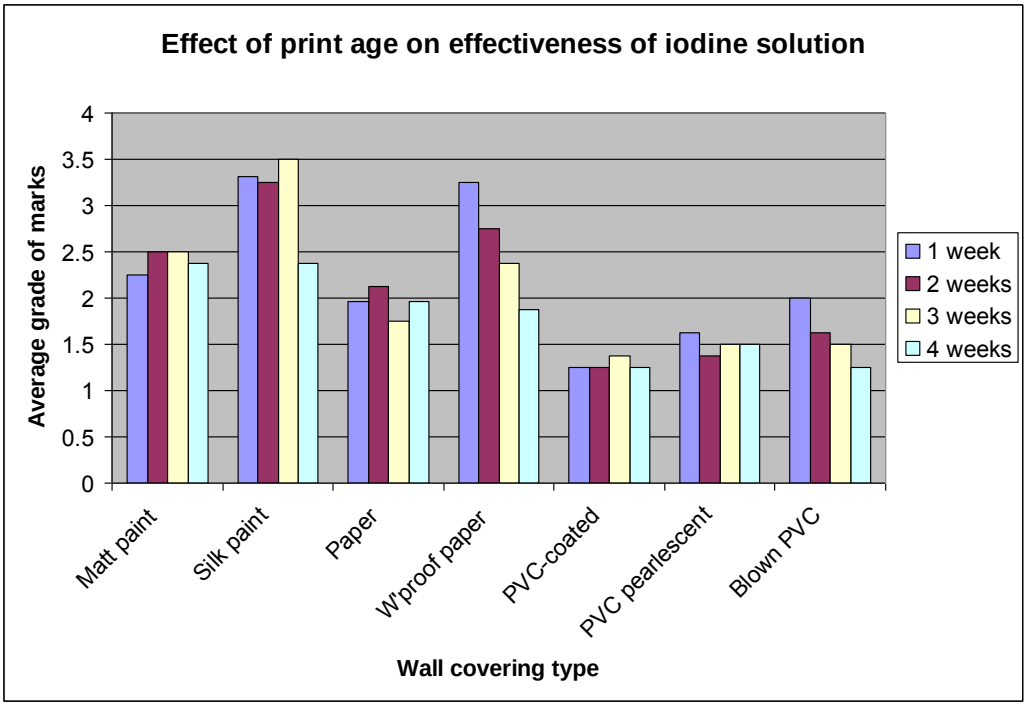
Comparative results obtained for iodine and iodine/ninhydrin on wallpapers.

8.1.6 The results again showed a general trend that ninhydrin developed more marks after iodine solution, but this was not true on every surface examined. This trial was repeated, but now including marks that were treated with ninhydrin alone. The results were similar to those above, showing that, in general, the iodine solution was less effective than the iodine/ninhydrin sequence and ninhydrin alone. However, there were certain surfaces where iodine solution was the single most effective treatment, although it was not always possible to determine which type of surface was present before commencing treatment.



Comparison of effectiveness of different processes and sequences on different wall coverings.

8.1.7 The effect of the age of the mark on development using iodine solution was also studied by SRDB, with 96 marks being deposited and graded for each age (from one to four weeks). Results are summarised below.



Effect of age of mark on effectiveness of iodine solution.

8.1.8 There is again agreement between the SRDB results and those obtained by HO CRE. In general, the effectiveness of iodine solution falls with time but on certain surfaces there is a less obvious fall off.

8.1.9 Based on laboratory trials, HO CRE introduced iodine solution into operational use in the late 1980s. The performance of the Fluorisol (CFC113)-based iodine formulation was found to be equivalent to the cyclohexane-based formulation in laboratory tests, and superseded it in operational use until CFCs were banned by the Montreal Protocols and the formulation reverted to one based on a flammable solvent. Operational performance figures recorded for iodine solution (including results obtained using both formulations) are given below.

Type of case	**Number of scenes**		**Number of iodine marks recorded**
	Examined	**Marks found**	
Murder	71	28	56
Rape	12	3	4
Burglary	9	4	6
Other major crime	11	2	3
Total	**103**	**37**	**69**

Operational results obtained by the use of iodine solution at scenes of crime.

8.1.10 The results presented above were criticised by SRDB at the time in that they did not provide a detailed assessment of the surface types the iodine solution had been applied to (only the crime type), nor did they record the effectiveness of subsequent ninhydrin treatment [30]. In several of these cases, it was known that ninhydrin had developed significant numbers of additional marks. Subsequent development of a spray formulation [18] resulted in further operational trials by HO CRE, the initial results of which are given below.

Surface type	**Number of scenes**		**Number of iodine marks recorded**
	Examined	**Marks found**	
Wallpaper	5	2	15
Emulsion paint	11	6	24
Total	**16**	**8**	**39**

Operational results obtained by the use of iodine spray at scenes of crime.

8.1.11 Subsequent testing of the different application routes for iodine solution by PSDB [19] found that solution dipping was the most effective, followed by brush application, with spray being the least effective. Brush application is the technique that was used at crime scenes up until 2008.

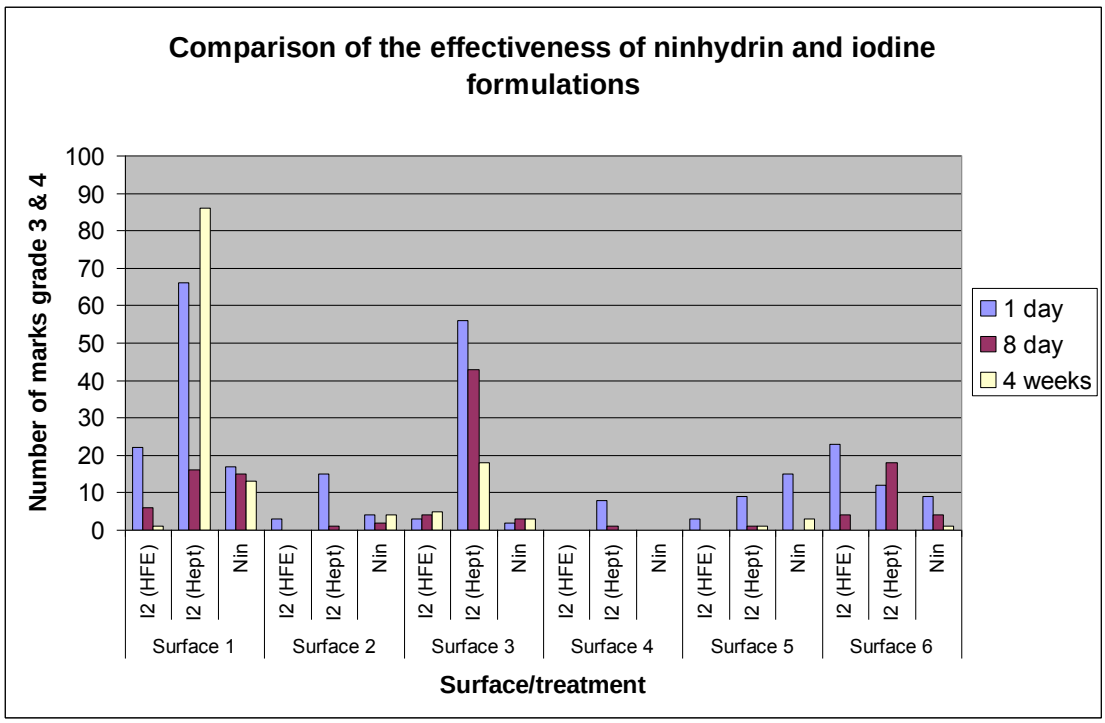
8.1.12 One of the other potential applications of iodine solution (and fuming) is in the development of marks on contaminated surfaces. Comparative laboratory trials were carried out between the CAST iodine solution formulation and solvent black 3. These are more fully reported in Chapter 3, Chemical and Physical Processes, Solvent black 3 and demonstrated that, in general, solvent black 3 was more effective, although there were some surfaces, such as gloss painted wood, where iodine solution gave better results.

8.1.13 In the repeat trials on wallcoverings conducted in 2009 [23], the following surfaces were examined.

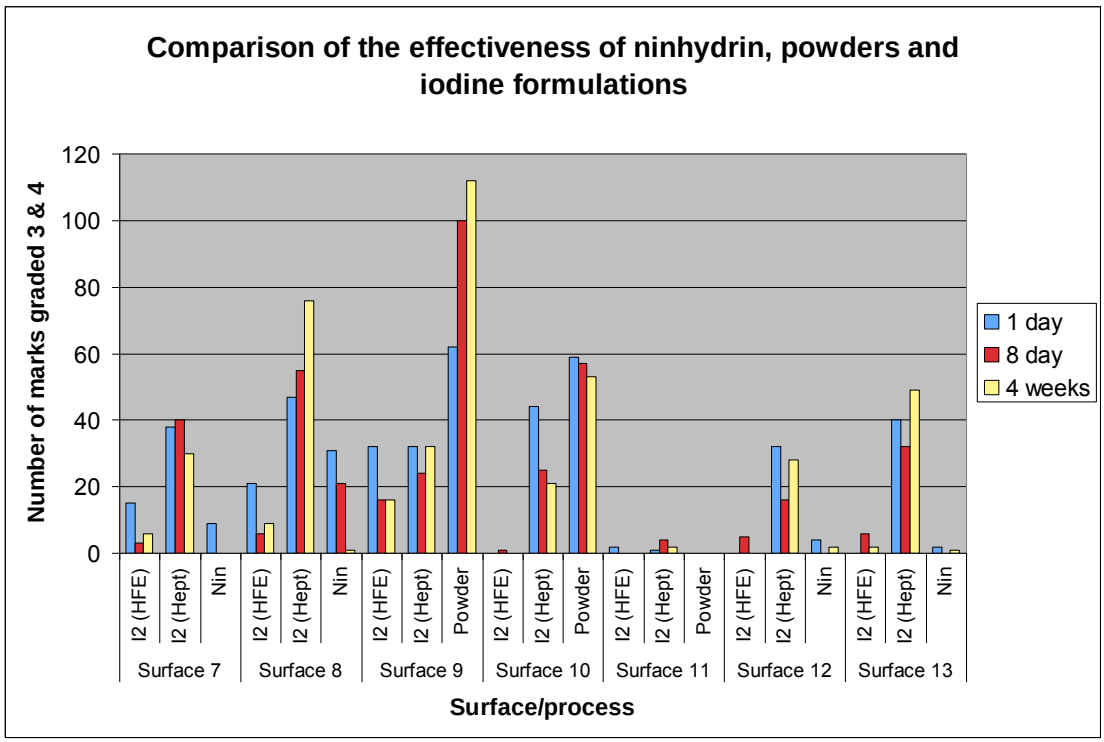
Surface	**Type**	**Porosity**
1	Wickes master washable matt paint	Porous
2	Dulux interior matt paint	Porous
3	Wallpaper – pulp	Porous
4	Wallpaper – vinyl	Non-porous
5	Wickes interior matt emulsion paint	Porous
6	Crown silk emulsion	Non-porous
7	Wallpaper – foamed polyethylene	Semi-porous
8	Wallpaper – washable vinyl coated	Semi-porous
9	Wickes liquid gloss paint	Non-porous
10	Dulux liquid gloss paint	Non-porous
11	Crown non-drip satin paint	Semi-porous
12	Dulux grease and stain resistant, tough matt paint	Porous
13	Wallpaper – vinyl coated	Semi-porous

Description of the surfaces examined in the 2009 study.

8.1.14 For all surfaces except gloss paint, the effectiveness of the heptane-based iodine solution was compared with a low-flammability, HFE71DE-based iodine solution and ninhydrin, whereas on gloss painted surfaces powders were substituted for ninhydrin. Over 4,500 marks were graded in this study.



Results of the 2009 comparative study on surfaces 1 to 6 [23].



Results of the 2009 comparative study on surfaces 7to 13 [23].

8.1.15 It can be seen that the heptane-based iodine solution out-performed the HFE71DE-based iodine solution and ninhydrin on almost all surfaces, except for 1 day old marks on surface 6. The heptane-based solution gave far less background staining than the HFE71DE-based solution, and marks of greater contrast than ninhydrin. On gloss

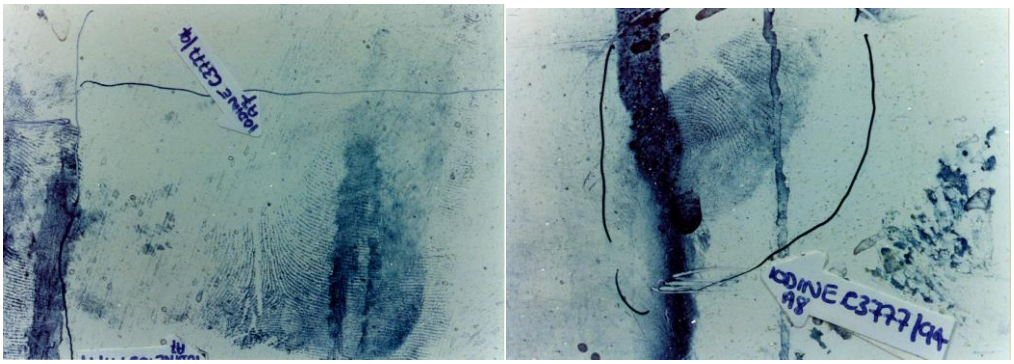
surfaces, powdering gave superior performance to both of the iodine solutions. Very few additional marks were found to be developed by leaving surfaces treated with ninhydrin for a further two-week period. Subsequent treatment of the surfaces with powder suspensions produced a significant improvement in the number and quality of developed marks over and above all of the initial treatments, although the mess associated with the use of powder suspensions at scenes make this an unattractive alternative. The more recent results from external studies using formulations based on HFC4310mee solvent [24,25] suggest that this may offer the potential for a non-flammable iodine solution that is more effective than ninhydrin, at least on recently deposited marks.

8.1.16 There are considerably less comparative studies using iodine fuming. A limited study was conducted by CAST to evaluate the effectiveness of iodine fuming against other non-destructive and low-impact processes on a range of different paper types. In this study, 36 different donors placed a single fingermark on five different paper types, which were aged for 1 day and one week prior to examination using light sources (violet-blue Quaser, green and yellow lasers), short wave ultraviolet reflection, ESDA and iodine fuming. The results from this study are shown in Chapter 3, Chemical and Physical Processes, ESDA and demonstrate that iodine fuming is generally more effective than light sources, UVC reflection and ESDA on most paper types, and is particularly effective on glossy/semi-glossy paper types. The results confirm that the effectiveness of iodine fuming generally decreases as the age of the mark increases.

8.2 Pseudo-operational trials and operational experience

8.2.1 The use of iodine fuming on operational work is rare, because it is only recommended in special circumstances, such as where the surface is contaminated or the treatment should ideally leave no trace on the article being examined. However, there are recorded cases where iodine has produced marks of value and the other processes recommended for contaminated surfaces (basic violet 3, solvent black 3) have not. PSDB was involved in the treatment of a contaminated fridge from a fast food outlet in the 1990s where iodine fuming yielded identifiable marks.

Contaminated fridge treated using iodine fuming followed by fixing with α-naphthoflavone.



Marks developed on contaminated fridge using iodine fuming followed by fixing with α-naphthoflavone.

8.2.2 The operational use of iodine solution has been more contentious, with CAST not recommending the process and the FSS (until 2008) often using it on serious operational cases. One of the reasons CAST has not recommended iodine solution is that previous studies conducted in the late 1980s and again in the late 1990s indicated that it was less effective than ninhydrin across the range of surfaces it was likely to be applied to, and the use of fixative may inhibit subsequent ninhydrin development. However, the most recent studies (2009) have shown that this may no

longer be true, with considerably more marks being found by iodine solution than were developed by ninhydrin. This has been supported by more recent evaluation of an HFC4310mee-based formulation [24] which also found iodine solution to develop a higher number of high quality marks on painted woodchip wallpaper and lining paper. In addition, marks are revealed instantly with iodine solution and may take several days to develop fully with ninhydrin. Some of the background data behind these original recommendations are presented here, although it should be noted that all the early comparisons were between iodine solution and the CFC-based ninhydrin formulation (NFN). The more recently developed HFE7100-based ninhydrin formulation was more effective than the CFC-based formulation on paper (see Chapter 3, Chemical and Physical Processes, Ninhydrin) but, until the recent study [23], no tests had been carried out using this ninhydrin formulation on surfaces representative of wall coverings.

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Miscellaneous amino acid reagents:

Fluorescamine

1. History

1.1 Fluorescamine (4-phenylspiro[furan-2(3H), 1'-phthalan]-3,3'-dione) was developed in the early 1970s as a fluorescent reagent for automated assay of amino acids [1,2]. This was based on earlier work showing that fluorescent products could be obtained when conducting blood phenylalanine assay with ninhydrin. The initial reaction (found to be specific to phenylalanine) produced phenylacetaldehyde that subsequently reacted with additional ninhydrin and any peptides (or primary amines) present to give fluorescent products. It was later found that some other aldehydes were also capable of yielding fluorophors by reaction with ninhydrin and amines. By deducing the structure of these fluorescent products, it was possible to identify a novel reagent (fluorescamine) that would react directly with primary amines to give the same fluorescent reaction products. Subsequent tests demonstrated that fluorescamine was capable of detecting both amino acids and peptides and had a high level of sensitivity.

1.2 Several studies were carried out to compare the sensitivity of fluorescamine, ninhydrin and *o*-phthalaldehyde (another reagent proposed for assay of amino acids). These concluded that for detection of most free amino acids, fluorescamine offered no advantages over ninhydrin. However, for recovery of peptides, fluorescamine did appear to work over a wider range of substances than ninhydrin [3].

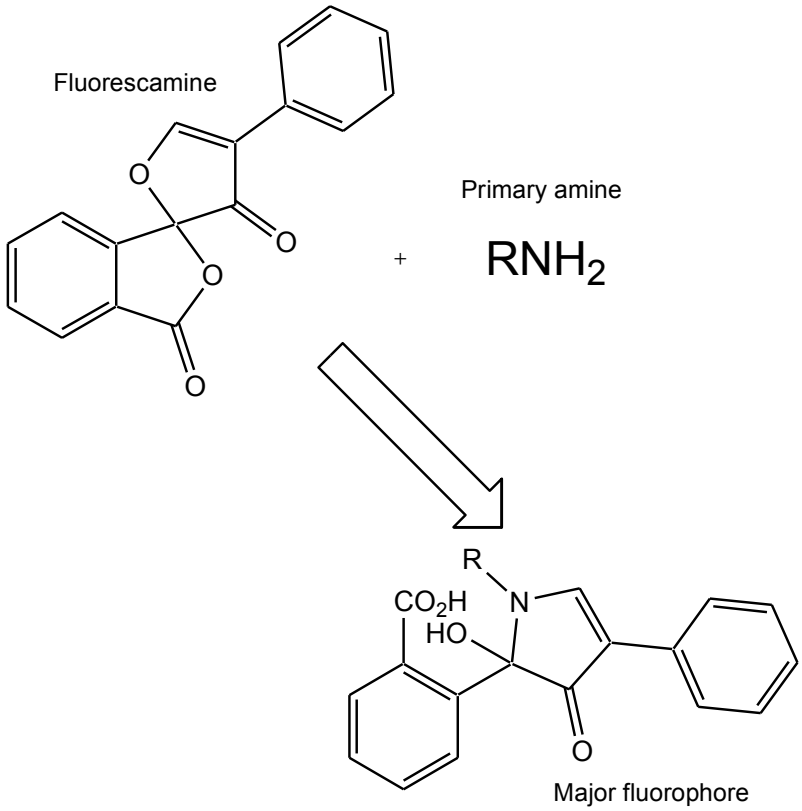
1.3 The reagent also became considered as an alternative to ninhydrin for the development of fingermarks on porous surfaces. However, initial tests indicated that the aqueous buffer required to provide the optimal pH environment washed out the fingermark ridge detail and therefore organic bases were investigated as alternative ways of providing an alkaline environment. A suitable formulation was developed based on fluorescamine dissolved in acetone with addition of triethylamine [4].

1.4 This formulation was then compared with ninhydrin and an optimised formulation of o-phthalaldehyde for the detection of fingermarks deposited on a range of surfaces, all reagents being applied as sprays [5]. These studies indicated that fluorescamine had some advantages over ninhydrin, including greater sensitivity, ability for mark detection on dark and multicoloured surfaces, and the fact that heat is not required for the reaction to occur. However, there were also some disadvantages: the solution does not have long-term stability and water will hydrolyse fluorescamine to a non-fluorescent product; in addition, ultraviolet (UV) radiation is required to visualise developed marks.

1.5 The reagent does not appear to have become widely used for fingermark detection, possibly because of the greater ease in visualising the purple marks produced by ninhydrin and the fact that ninhydrin solution is more stable for long-term storage. The increasing use of optical brighteners in papers also means that many such surfaces now fluoresce a bright blue when illuminated with UV radiation and this will swamp the weaker, pale blue fluorescence of any marks developed using fluorescamine. The technique is therefore no longer appropriate for the types of surfaces that it was originally intended for.

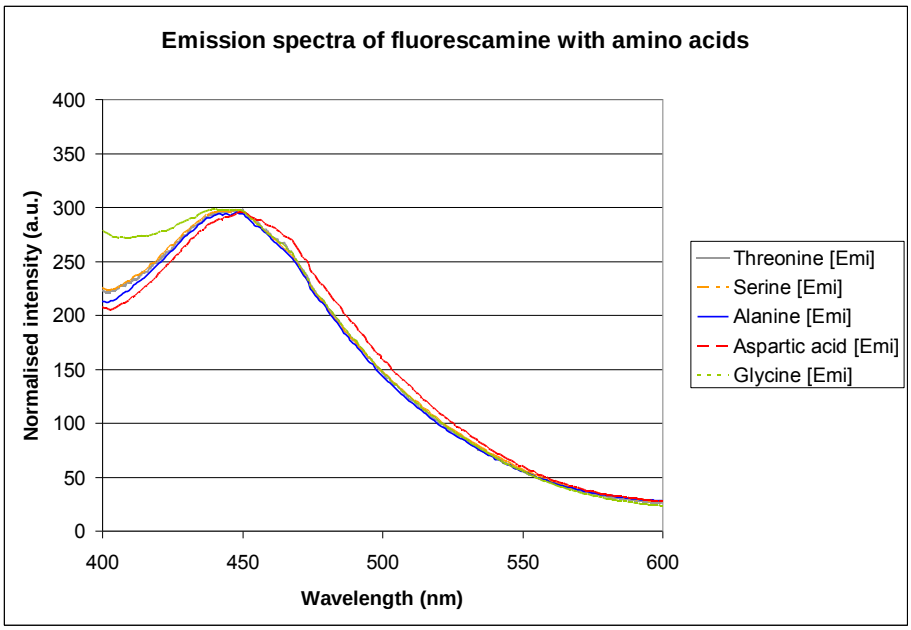
2. Theory

2.1 The way in which fluorescamine works is by a chemical reaction between the fluorescamine molecule and the amine groups present in amino acids and peptides to give a fluorescent reaction product. This is illustrated below:

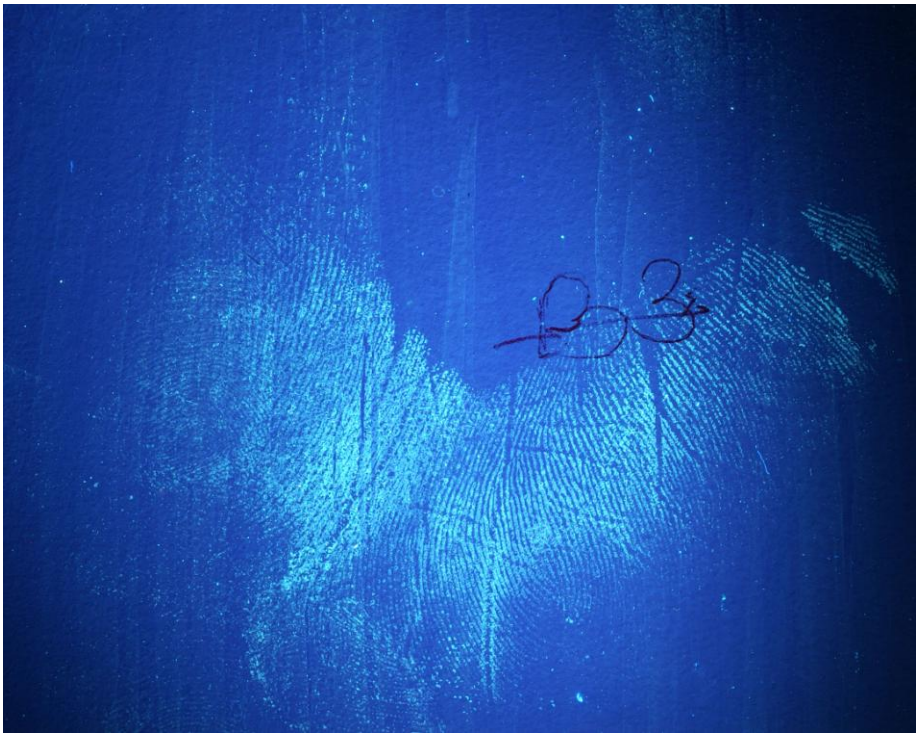


Reaction of fluorescamine with amines to form fluorescent products.

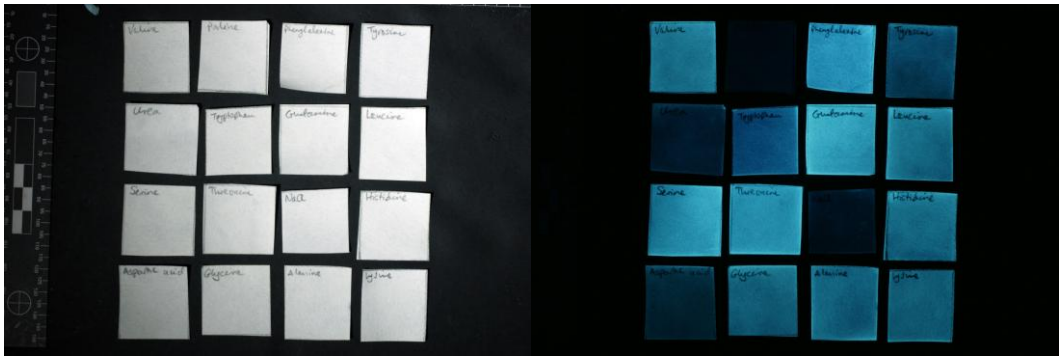
2.2 The major fluorophore produced by this reaction can be best visualised using an excitation wavelength ~390 nm (long-wave UV) that produces a visible emission in the region 475 to 495nm.



Emission spectra of reaction products of fluorescamine with amino acids.



Palm mark developed on painted wall using fluorescamine.



a) b)

Reaction products formed between fluorescamine and 0.1M solutions of amino acids and other fingermark constituents a) *visible and* b) *fluorescence.*

2.3 The formulations proposed for use in the late 1970s utilised acetone as the principal solvent, small additions of triethylamine as an organic base, and fluorescamine. One such formulation is given below [6].

15 mg fluorescamine
100 mL acetone
0.1 mL triethylamine

2.4 These constituents were mixed together and then sprayed using an atomiser onto the surface being treated.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Although the technique was evaluated in the late 1970s, the Home Office Centre for Applied Science and Technology (CAST) has not recently conducted any extensive trials to compare fluorescamine with ninhydrin and/or 1,8-diazafluoren-9-one (DFO) and therefore it is not known whether there are any operational benefits in its use. However, reformulation work would be required to change the base solvent for fluorescamine from acetone to another less flammable substance less likely to cause ink to run and affect any subsequent document analysis, e.g. 1-methoxynonafluorobutane (HFE7100). Some components of the original formulation (such as dichloromethane) also have health and safety issues associated with them and alternatives would need to be identified. The solution is also unstable in contact with water and is more difficult to store than ninhydrin.

3.2 The fact that long-wave UV is required to visualise the developed fingermarks also makes fluorescamine less attractive for operational use. Extended usage of long-wave UV radiation sources does have health and safety implications for the operator and many modern papers also contain optical brighteners that are excited by long-wave UV, making

developed marks more difficult to see against the fluorescing background. As a consequence, fluorescamine is listed among the amino acid reagents with no known operational benefits in the Category E processes in the *Fingermark Visualisation Manual.*

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***O*-phthalaldehyde**

1. History

1.1 *O*-phthalaldehyde is another reagent originally developed for assay of amino acids in the early 1970s. Initially, *o*-phthalaldehyde was not found to be as effective as ninhydrin or fluorescamine for detection of peptides, but by the mid-1970s revised formulations were published that were stated to overcome these issues [1]. The authors suggested that *o*-phthalaldehyde was actually preferable to fluorescamine for fingermark development because it exhibited greater fluorescent quantum yields, was stable in aqueous buffers, and was cheaper.

1.2 Similarly to fluorescamine, work was carried out to adapt the assay formulations for the development of fingermarks. One reported study investigated the use of a Babington nebuliser to provide a means of delivering *o*-phthalaldehyde to large areas without saturating the surface [2]. In this formulation boric acid and potassium hydroxide were used as a buffer solution, with additions of a detergent (Brij 35) and 2-mercaptoethanol.

1.3 *O*-phthalaldehyde was also compared with ninhydrin and fluorescamine in spray reagent form. No single reagent out-performed the others in all respects, with *o*-phthalaldehyde performing well in terms of sensitivity but suffering from a complex formulation and application procedure coupled with lack of stability in air [3].

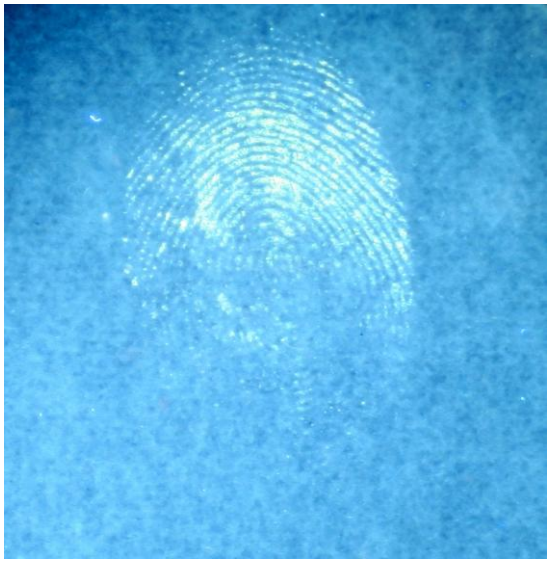
1.4 Alternatives to the boric acid/potassium hydroxide buffer solution were investigated, this being found to cause diffusion of ridge detail. Ohki reported a formulation based on chloroform, triethylamine and 2-mercaptoethanol that overcame this problem [4].

1.5 Subsequently Fischer [5] investigated a simpler and less hazardous formulation that involved dissolving *o*-phthalaldehyde in acetone, dipping the exhibit and then lightly spraying with a 1% nitric acid solution in acetone. The fluorescent products produced in this way were excited with blue/green light rather than ultraviolet (UV).

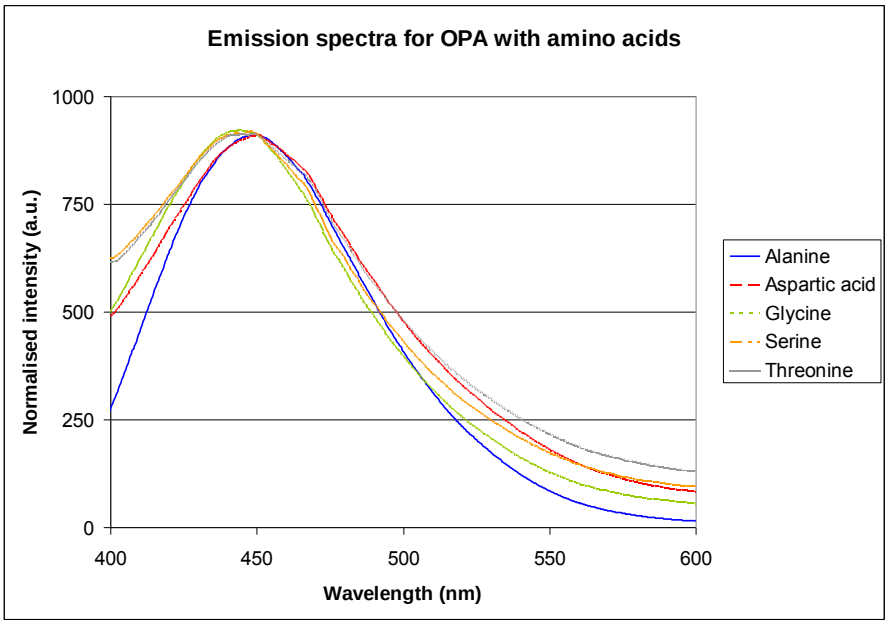
1.6 The reagent does not appear to have become widely used for fingermark detection, possibly because of the greater ease in visualising the purple marks produced by ninhydrin. The increasing use of optical brighteners in papers also mean that many such surfaces now fluoresce a bright blue when illuminated with UV radiation and this will swamp the weaker, pale blue fluorescence of any marks developed using *o*-phthalaldehyde. The technique is therefore no longer appropriate for the types of surfaces for which it was originally intended.

2. Theory

2.1 *O*-phthalaldehyde undergoes a chemical reaction with primary amines that may be present in fingermark deposits to form fluorescent reaction products. The reaction products have an optimum excitation wavelength of ~340 nm and an emission ~455 nm.



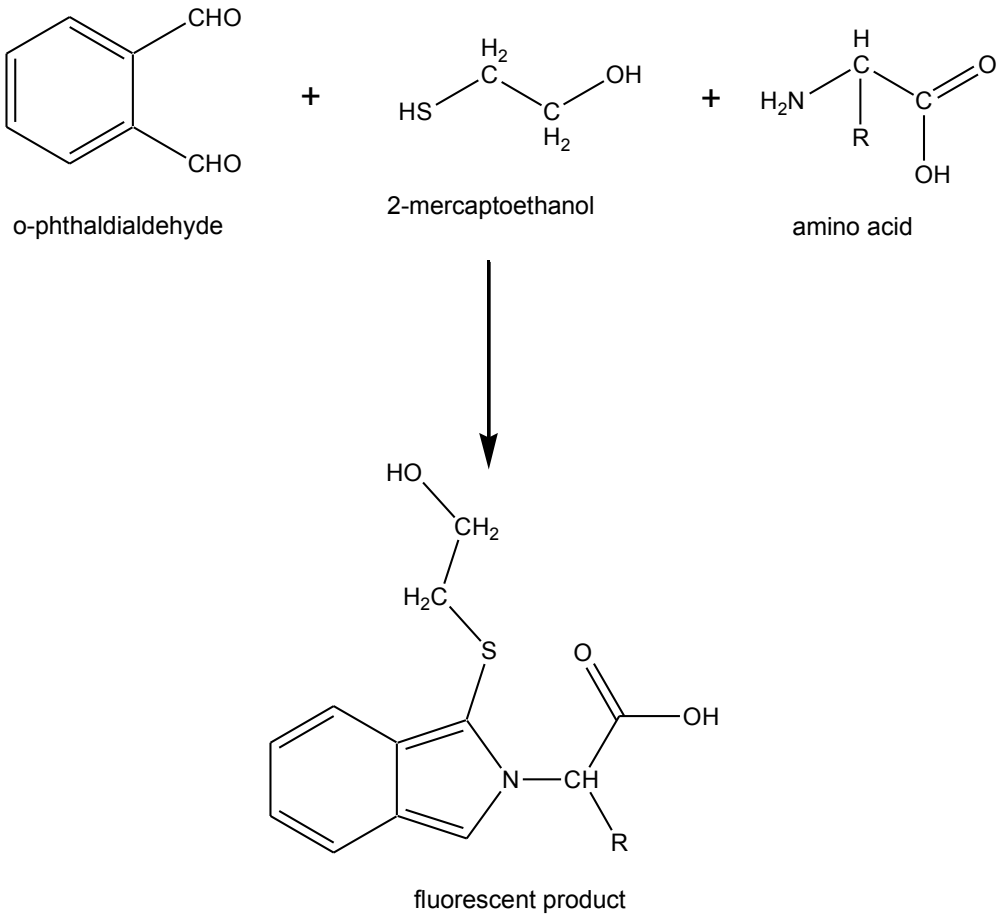
Fingermark developed on filter paper using o-phthalaldehyde.



Emission spectra for reaction products of o-phthalaldehyde (OPA) with amino acids.

2.2 Research has indicated that the fluorescent reaction products are 1-alkylthio-2-alkyl-substituted isoindoles [6,7,8].

2.3 Some of the reactions proposed for *o*-phthalaldehyde are given below.



Proposed reaction between o-phthalaldehyde, 2-mercaptoethanol and α-amino acids [8].

2.4 Lee and Attard [3] proposed a two-part formulation with an aqueous base, where solution A comprised:

2.5 g boric acid
95 mL distilled water
pH adjusted to 10.40 with additions of 6M potassium hydroxide
0.3 mL Brij 35 detergent
0.2 mL 2-mercaptoethanol;

and solution B comprised:

0.5 g *o*-phthalaldehyde
1 mL methanol.

The solutions were mixed together and then sprayed.

2.5 Ohki [4] proposed an alternative, one-part solution with an organic base:

40 mg *o*-phthalaldehyde

1 mL 95% ethanol
50 mL chloroform
0.5 mL triethylamine
0.1 mL 2-mercaptoethanol.

Again, the solution was sprayed onto the surface being treated.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST has not recently (since the late 1970s) conducted any extensive trials to compare *o*-phthalaldehyde with ninhydrin and/or 1,8-diazafluoren-9-one (DFO) and therefore it is not known whether there are any operational benefits in its use. However, most *o*-phthalaldehyde formulations are based on 2-mercaptoethanol, which is toxic, corrosive and dangerous for the environment and therefore it is unlikely that any formulation based on this substance would be recommended for operational use for health and safety reasons. Alternatives are available, but this would require extensive reformulation work for little operational benefit.

3.2 In common with fluorescamine, there is the problem that long-wave UV is required to visualise the developed marks and this brings with it health and safety issues associated with long exposures, and also interference with the developed mark from background paper fluorescence. The solution is also unstable in contact with air and may need to be stored under an inert gas, making it impractical for routine use. As a consequence, o-phthalaldehyde is listed among the amino acid reagents with no known operational benefits in the Category E processes in the *Fingermark Visualisation Manual.*

4. References

1. Benson, J. R. and Hare, P. E. (1975) ‘o-Phthalaldehyde: Fluorogenic Detection of Primary Amines in the Picomole Range. Comparison with Fluorescamine and Ninhydrin’, *Proc. Nat. Acad. Sci*. *USA*, vol. 72 (2), pp 619–622.

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3. Lee, H. C. and Attard, A. E. (1979) ‘Comparison of Fluorescamine, O-phthalaldehyde, and Ninhydrin for the Detection and Visualization of Latent Fingerprints’, *J. Police Sci. & Admin*., vol. 7 (3), pp 333–335.

4. Ohki, H. (1978) ‘A New Detection Method for Latent Fingerprints with O-Phthalaldehyde’, *Rep. Nat. Res. Inst. Police Sci.*, vol. 31 (4), pp 295–300.

5. Fischer, J. F. (1990) 'A Modified o-Phthalaldehyde Technique Utilizing Blue-Green Light Excitation for Developing Luminescent Latent Prints', *J. Forens. Ident*., vol. 40 (6), pp 327–333.

6. Simons, S. S. and Johnson, D. F. (1976) 'The Structure of the Fluorescent Adduct Formed in the Reaction of Ortho-Phthalaldehyde and Thiols with Amines', *J. Am. Chem. Soc*., vol. 98, pp 7098–7099.

7. Svedas, V. J. K., Galaev, I. J., Borisov, I. L. and Berezin, I. V. (1980) 'The Interaction of Amino Acids with O-Phthaldialdehyde; A Kinetic Study and Spectrophotometric Assay of the Reaction Product', *Anal. Biochem*., vol. 101, pp 188–195.

8. Lee, K. S. and Drescher, D. G. (1978) 'Fluorometric Amino-Acid Analysis with o-Phthadialdehyde (OPA)', *Int. J. Biochem*., vol. 9, pp 457–467.

Genipin and lawsone

1. History

1.1 Genipin is a natural product that can be extracted from the fruit of Gardenia jasminoides. Since the 1960s it has been recognised that genipin brought into contact with skin produces an indelible blue-violet colour and that the same reaction readily occurs with amino acids [1]. However, the potential of such systems for the development of latent fingermarks was only explored in the mid-2000s when Almog *et. al*. [1] demonstrated that genipin could be mixed into solution with solvents such as 1-methoxynonafluorobutane (HFE7100) and petroleum ether, and the resultant formulations used to develop fingermarks on porous surfaces. It was also noted that in addition to the colorimetric reaction giving developed fingermarks a blue/black colour, the reaction products were also fluorescent with maximum emission at the red end of the spectrum.

1.2 Further experiments were carried out to establish optimum processing conditions for genipin, to compare its sensitivity with both ninhydrin and 1,8-diazafluoren-9-one (DFO) and to look at the reaction products formed between genipin and a range of amino acids [2]. The studies of these exercises identified a formulation based on genipin dissolved in ethanol/ethyl acetate and diluted using HFE7100, which could be used on documents without causing inks to run. It was found that genipin was slightly less sensitive than ninhydrin when considering the coloured reaction product, and less sensitive than DFO when considering the fluorescent reaction product. However, unlike DFO the emission spectra obtained from reaction products with a range of amino acids differed slightly from each other. On some types of paper genipin gave advantages over DFO because the longer emission wavelength of the

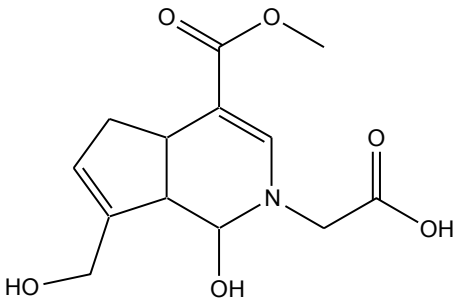
fluorescent product resulted in reduced interference from background fluorescence of the paper and/or inks.

1.3 More rigorous comparative testing against other reagents with dual colorimetric and fluorescent reaction products, e.g. ninhydrin combined with metal salts [3] confirmed that genipin was, on the whole, less sensitive than such reagents, but that this longer wavelength fluorescence could make genipin the reagent of choice on brown paper articles where background fluorescence may cause problems in imaging developed marks.

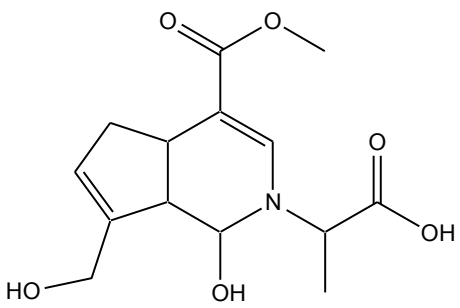
1.4 The research into genipin has since prompted research into other naturally occurring products that could be used as fingermark development reagents, and information has recently been published on lawsone (2-hydroxy-1,4-napthoquinone), a component of henna [4]. This gives purple-brown marks with a red fluorescence when reacting with the amino acids in fingermarks. Isatin (1*H*-indole-2,3-dione), an oxidation product of the naturally occurring indigo dye, has also been studied as a fingermark reagent [5] although the more toxic solvents and higher processing temperatures required make it less attractive for use than the existing reagents. Further developments based on naturally occurring products are anticipated.

2. Theory

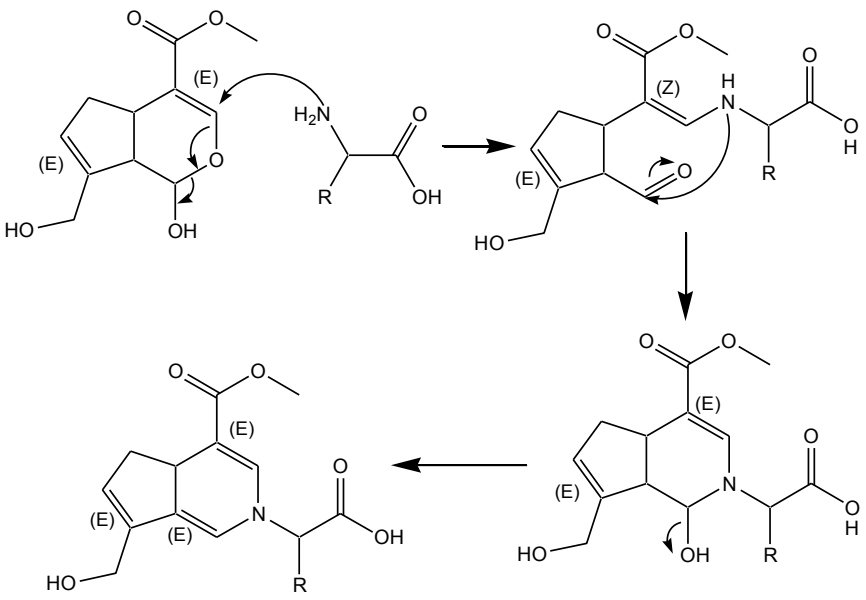
2.1 The mechanism of the reaction between genipin and amino acids and the nature of the coloured and fluorescent reaction products has not yet been established. The studies above [2] have established that slightly different reactions will occur between genipin and the different amino acids present in the fingermark. Some of the blue reaction products produced with amino acids have been identified and the proposed reaction and structures are shown below.



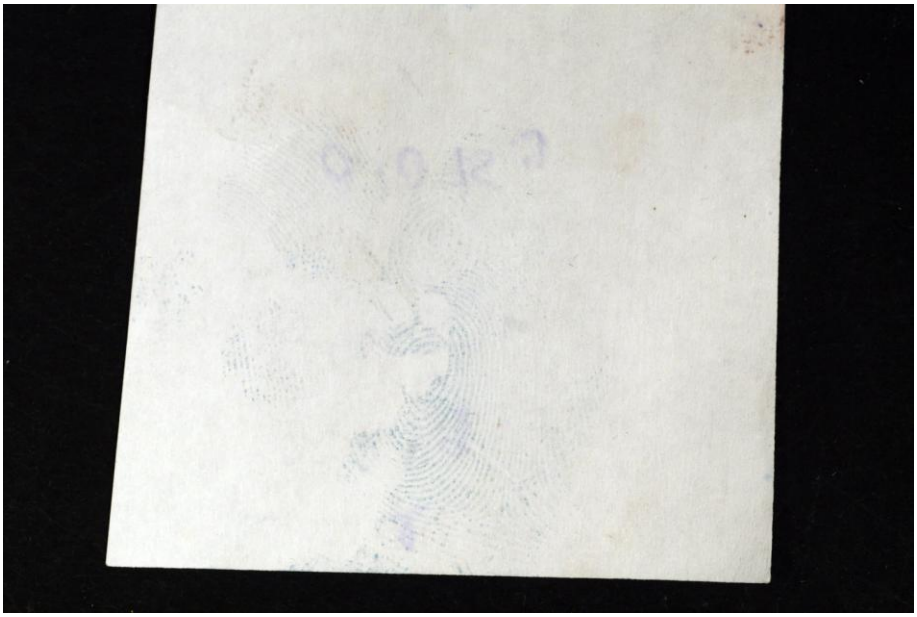
Genipin/Glycine



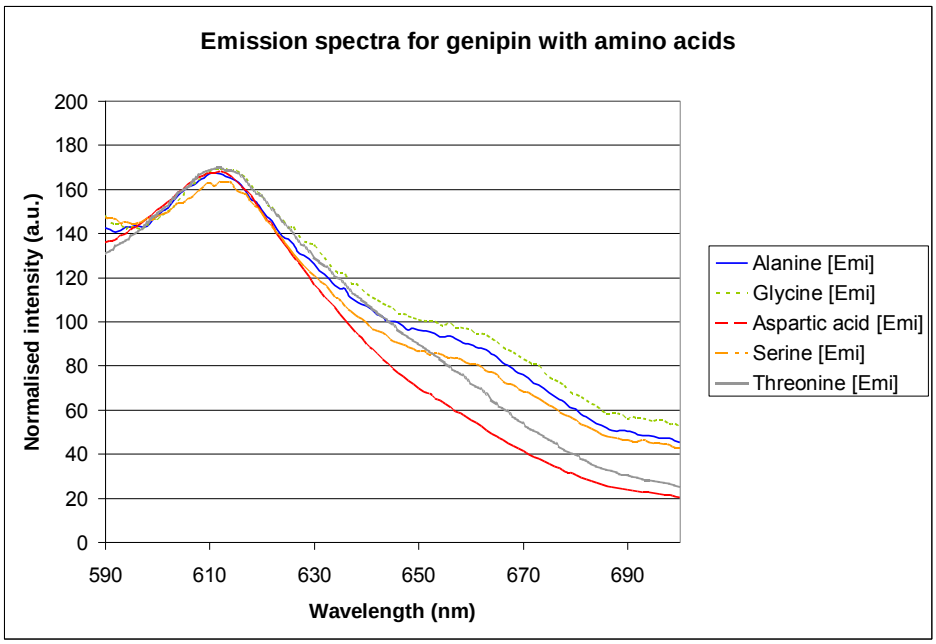
Genipine/L-Alanine



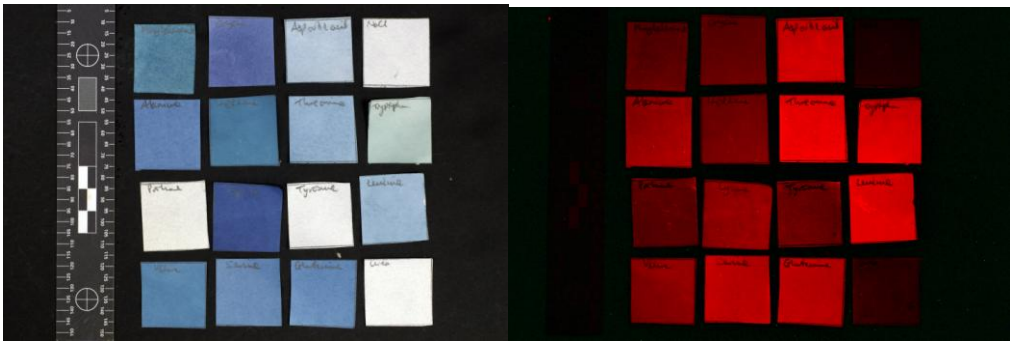
Proposed reaction mechanism for genipin with amino acids and structures of some reaction products [6].



Blue reaction product obtained from genipin (in this case very faint).



Emission spectra for reaction products of genipin with amino acids.



a) b)

Reaction products formed between genipin and 0.1M solutions of amino acids and other fingermark constituents a) *visible and* b) *fluorescence.*

2.2 The formulation proposed for use by the Israeli National Police (and used in the comparative studies below) comprises:

1.7 g genipin
57 mL absolute ethanol
86 mL ethyl acetate
857 mL HFE7100.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Genipin is not recommended for routine operational use because it is not as effective as ninhydrin in colorimetric mode, and not as effective as DFO in fluorescence mode.

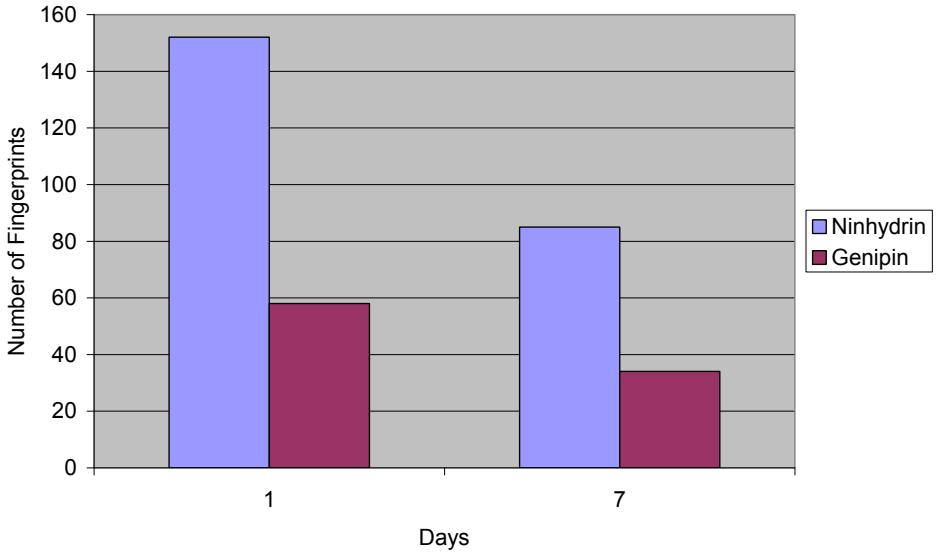
3.2 On certain paper types genipin may give better results than ninhydrin or DFO, but unless a detailed analysis of paper type is carried out prior to chemical treatment it will not be possible to identify when genipin should be used. This is clearly not practical for routine operational work.

3.3 These observations are based on the results of a short study of the effectiveness of genipin conducted by CAST in 2005 [7]. These studies utilised six donors leaving depletion series of six fingermarks on ten different types of paper found in the UK, namely:

business paper (wove);
parchment paper;
photocopier paper;
writing paper;
white envelope;
brown envelope;
yellow card;
laser printer paper;
newspaper;
magazine.

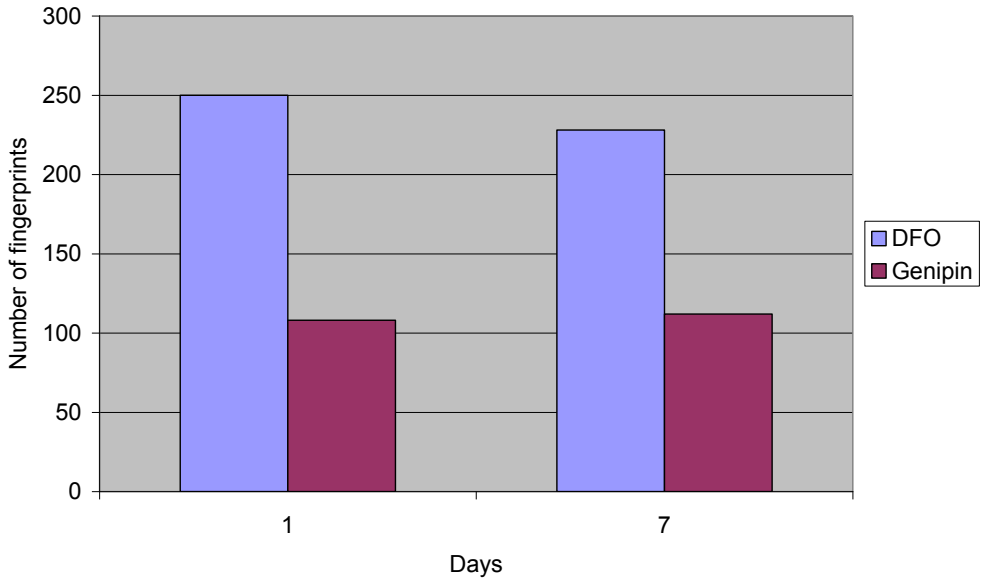
3.4 The depletion series were split down the middle, one-half being treated with genipin and the other with ninhydrin or DFO. Marks were aged for one day and seven days before processing. The results are illustrated below.

A graph to show fingerprint development using visual examination.



a)

A graph to show total fingerprint development using fluorescence.

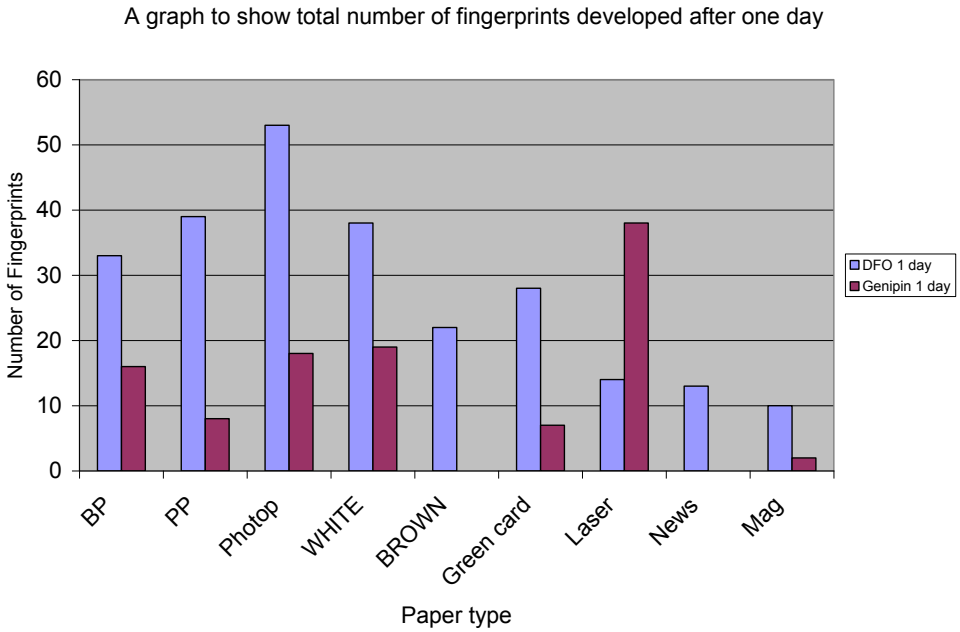


b)

Comparison of the effectiveness of genipin with existing techniques for porous surfaces a) *with ninhydrin in colorimetric mode and* b) *with DFO in fluorescence mode.*

3.5 It can be seen that when overall numbers of marks developed on all types of paper are considered, genipin is clearly not as effective as either DFO or ninhydrin. However, a more detailed breakdown by paper type (an example is given below) actually showed that on laser printer paper genipin was the most effective reagent. However, this observation is of no operational benefit unless it is positively known that a particular

exhibit is laser printer paper and genipin can be recommended as an alternative treatment.



More detailed comparison of results obtained comparing genipin to DFO on individual paper types.

3.6 More recently, studies in Taiwan [8] have indicated that genipin may be the single most effective process for developing marks on the 'red pockets' traditionally used to present money gifts in China and other far East countries. As a consequence, genipin has been included in the *Fingermark Visualisation Manual* as a Category C process for this particular niche application. Small scale studies on lawsone by CAST did not show any benefits over existing processes and the longer processing times and higher processing temperatures than DFO make it less attractive for use. As a consequence, lawsone is listed among the amino acid reagents with no known operational benefits in the Category E processes in the *Fingermark Visualisation Manual.*

4. References

1. Almog, J., Cohen, Y., Azoury, M. and Hahn, T. - R. (2004) 'Genipin – A Novel Fingerprint Reagent with Colorimetric and Fluorogenic Activity', *J. Forens. Sci.*, vol. 49 (2), pp 255–257.

2. Levinton-Shamuilov, G., Cohen, Y., Azoury, M., Chaikovsky, A. and Almog, J. (2005) 'Genipin, a Novel Fingerprint Reagent With Colorimetric and Fluorogenic Activity, Part II: Optimization, Scope and Limitations', *J. Forens. Sci.*, vol. 50 (6), pp 1367–1371.

3. Almog, J., Levinton-Shamuilov, G., Cohen, Y. and Azoury, M. (2007) 'Fingerprint Reagents with Dual Action: Color and Fluorescence', *J. Forens. Sci.*, vol. 52 (2), pp 330–334.

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7. Fitzgerald, L. (2005) *Feasibility Report on Genipin*, Internal HOSDB Report, October. London: Home Office.

8. Cheng, S-G., Chen, C-M., Liu, C-L., Liu, S-Q and Lim, S. K. (2013) 'Evaluation of Fingerprint Development Techniques Applied in Red Pockets and Its Subsequent Process' *Presentation at International Fingerprint Research Group*, 9-14 June, 2013. Jerusalem: Israeli National Police

Alloxan

1. History

1.1 Reactions of the substance alloxan with amino acids had been observed in the 1860s, and the formation of red reaction products between alloxan and the amino acid glycine were noted at the beginning of the 20th century. After his synthesis of ninhydrin in 1910, Ruhemann conducted a series of experiments that demonstrated that alloxan and ninhydrin must be closely related compounds [1] because of the similar nature of their reactions.

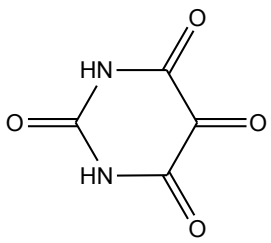
1.2 Alloxan was not investigated as a fingermark reagent until the discovery that ninhydrin could develop fingermarks on paper in 1954. This led to the re-evaluation of several related compounds in the same role and alloxan formulations for fingermark development were reported in Japan in the late 1950s [2], the fingermarks thus developed being orange-yellow in colour. However, it was noted that for the majority of surfaces studied ninhydrin gave superior performance.

1.3 The use of alloxan for fingermark development was mentioned in the 1970s [3], although it was still regarded as inferior to ninhydrin, developing fewer fingermarks with lower contrast and higher levels of background staining. The most recent comparative study of alloxan was carried out by Almog [4] in 1987, in an assessment of the reactivity and

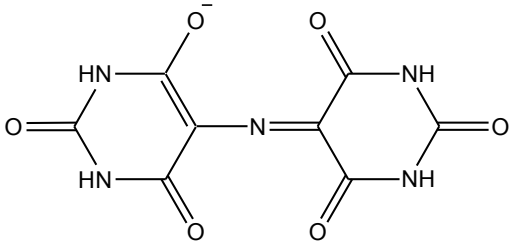
colour intensity of a range on ninhydrin analogues. It was concluded that alloxan was inferior to ninhydrin as a fingermark development reagent in all respects.

2. Theory

2.1 The reaction between alloxan and amino acids is directly analogous to that with ninhydrin, and a Ruhemann's purple analogue is formed as a result. The structure of alloxan and the corresponding coloured product is shown below.



Alloxan



Ruhemann's purple analogue

Structures of alloxan and the corresponding Ruhemann's purple analogue.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not recommend alloxan because it is significantly less sensitive than the currently (2016) available 1,8-diazafluoren-9-one (DFO) and ninhydrin processes. As a consequence, alloxan is listed among the amino acid reagents with no known operational benefits in the Category E processes in the *Fingermark Visualisation Manual.*

4. References

1. Ruhemann, S. (1911) 'Triketohydrindene Hydrate. Part III. Its Relation to Alloxan', *J. Chem. Soc*., vol. 23, pp 792–800.

2. Morris, J. R. (1974) *An Examination of the Chemical Literature on Fingerprint Technology for the Period 1890 to August 1974*, AWRE SSCD Memorandum 359, October. Aldermaston: Atomic Weapons Research Establishment.

3. Caton, H. E. (1974) 'Physical and Chemical Aspects of Latent Print Development', *Proceedings of the Conference on the Science of Fingerprints*, 24–25 September 1974, London, UK.

4. Almog, J. (1987) 'Reagents for Chemical Development of Latent Fingerprints: Vicinal Triketones – Their Reaction with Amino Acids and with Latent Fingerprints on Paper', *J. Forens. Sci*, vol. 32 (6), pp 1565–1573.

4-Chloro-7-nitrobenzofurazan (NBD chloride)

1. History

1.1 In the late 1960s and early 1970s, a series of chemicals were developed that gave fluorescent reaction products with amino acids. The primary application of these compounds was in amino acid detection in thin layer chromatography, although it was soon recognised that these could also be applied to fingermark detection in the same way as ninhydrin. 4-Chloro-7-nitrobenzofurazan (NBD chloride) was one such compound, introduced in the late 1960s with investigations of its effectiveness in amino acid detection under way in the early 1970s [1].

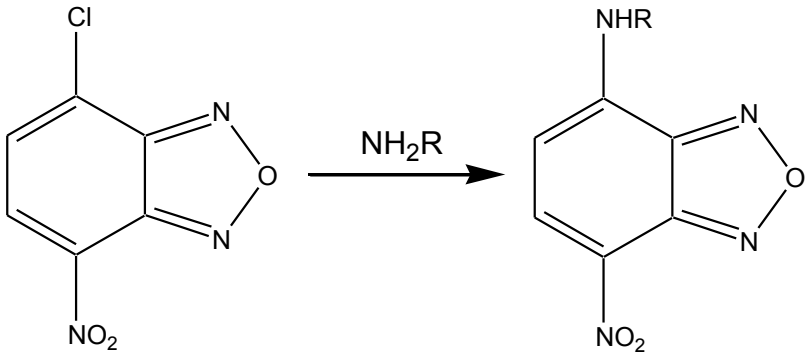
1.2 Initial studies into the use of NBD chloride as a fingermark reagent were conducted in the late 1970s [2], the results of which suggested that the technique may give improved sensitivity over ninhydrin when developed marks were excited using a laser.

1.3 By the early 1980s, NBD chloride was still being evaluated as a fingermark reagent for the development of fingermarks on porous surfaces, using blue light (~475 nm) from a filtered xenon arc lamp to promote fluorescence in the developed marks [3]. A further, more extensive comparative study with ninhydrin demonstrated similar sensitivity between the two techniques [4]. In some cases the background fluorescence of the paper caused issues and it was recommended that an area of paper be tested to assess the level of background fluorescence prior to treatment of the entire exhibit.

1.4 The process was introduced into operational use in several police forces, including the Metropolitan Police [5] where it was used as part of a sequential treatment routine in serious cases. However, by the late 1980s, concerns were being raised about the fact that NBD chloride was a potential mutagen and its use began to decline. Almog *et al.* investigated the synthesis and properties of a range of NBD chloride derivatives [6] and identified several with potential for further study, but with the introduction of 1,8-diazafluoren-9-one (DFO) this class of compounds does not appear to have been developed further.

2. Theory

2.1 NBD chloride is a non-fluorescent compound that reacts with amino acids to produce a fluorescent reaction product, shown in outline below.



NBD chloride

Fluorescent product

Fluorescent product formed by reaction between 4-chloro-7-nitrobenzofurazan and amino acids.

2.2 Published NBD chloride formulations utilised chlorofluorocarbon (CFC) 1,1,2-trifluorotrichloroethane (CFC113) as the carrier solvent and either ethanol or acetonitrile as the principal solvent. The formulation used by Salares [2] consisted of:

20 mg NBD chloride
2 mL absolute ethanol
20 mL CFC113.

2.3 The resultant solution was sprayed, the treated article allowed to dry and then heated for 10 minutes at 90°C. Other researchers [3] used the solution as a dip bath, and suggested heating for the same time at the slightly higher temperature of 110°C.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not recommend the use of NBD chloride because it is not as effective as DFO, and there are concerns about it being a suspected mutagenic compound. As a consequence, NBD chloride is listed among the amino acid reagents with no known operational benefits in the Category E processes in the *Fingermark Visualisation Manual.*

4. References

1. Fager, R. S., Kutina, C. B. and Abrahamson, E. W. (1973) 'The Use of NBD Chloride (4-chloro-7-nitrobenzo-2-oxa-1,3-diazole) in Detecting Amino Acids and as an N-Terminal Reagent', *Anal. Biochem*., vol. 53, pp 290–294.

2. Salares, V., Eves, C. and Carey, P. (1979) 'On the Detection of Fingerprints by Laser Excited Luminescence', *Forens. Sci. Int*., vol. 14, pp 229–238.

3. Warrener, R. N., Kobus, H. J. and Stoilovic, M. (1983) 'An Evaluation of the Reagent NBD Chloride for the Production of Luminescent Fingerprints on Paper: I. Support for a Xenon Arc Lamp Being a Cheaper and Valuable Alternative to an Argon Ion Laser as an Excitation Source', *Forens. Sci. Int*., vol. 23, pp 179–188.

4. Stoilovic, M. Warrener, R. N. and Kobus, H. J. (1984) 'An Evaluation of the Reagent NBD Chloride for the Production of Luminescent Fingerprints on Paper: II. A Comparison with Ninhydrin', *Forens. Sci. Int*., vol. 24, pp 279–284.

5. Creer, K. E. and Brennan, J. S. (1987) 'The work of the serious crime unit', *Proceedings of the International Forensic Symposium on Latent Prints,* 7–10 July 1987, pp 91–99. Virginia USA: FBI Academy, Quantico.

6. Almog, J., Zeichner, A., Shifrina, S. and Scharf, G. (1987) 'Nitrofurazanyl Ethers – A New Series of Fluorigenic Fingerprint Reagents', *J. Forens. Sci*, vol. 32 (3), pp 585–596.

Dansyl chloride

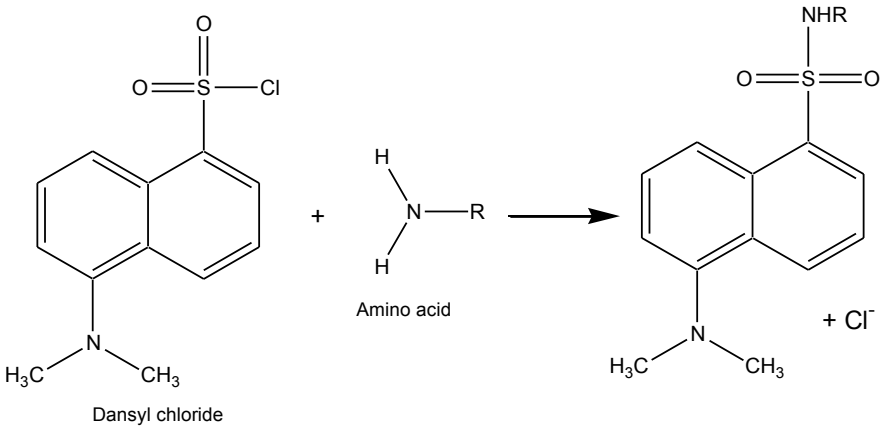
1. History

1.1 Dansyl chloride is another reagent originally developed for the analysis of amino acids [1-3], producing a fluorescent reaction product that is excited by ultraviolet (UV) radiation. In common with other amino acid detection compounds, it has been investigated as a fingermark development reagent [4]. In tests where ninhydrin and dansyl chloride were used as spray reagents on brown paper and cardboard, dansyl chloride appeared to give higher sensitivity on weaker marks. However, the process has not been extensively pursued as a practical technique since the mid-1980s.

2. Theory

2.1 The dansylation reaction of amino acids is described in detail elsewhere [1]. The reaction product formed by the reaction of dansyl chloride with

fingermark residues has been shown to absorb at 360 nm (UV) and an emission maximum at around 475 nm.



Reaction between dansyl chloride and amino acids.

2.2 A formulation given for dansyl chloride is:

0.2 g dansyl chloride
100 mL acetone
adjust pH to 10 using additions of 8M potassium hydroxide.

The resultant solution was applied by spraying.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not recommend the process because no extensive comparative studies have been carried out on its effectiveness. Dansyl chloride is also corrosive and potentially explosive under certain conditions and is therefore not recommended for health and safety reasons. As a consequence, dansyl chloride is listed among the amino acid reagents with no known operational benefits in the Category E processes in the *Fingermark Visualisation Manual.*

4. References

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Miscellaneous lipid specific reagents

Ruthenium tetroxide

1. History

1.1 The use of ruthenium (and osmium) tetroxide for fingermark development has been reported since the 1920s [1]. In its early application the process was extremely dangerous to use, requiring ruthenium crystals to be heated in a water bath at temperatures not exceeding 50ºC. Explosions could occur if heating was too rapid or the temperature exceeded 50ºC, making the technique unsuitable for use in most laboratories [2].

1.2 The risk of explosion while fuming ruthenium tetroxide (RTX) was overcome by the discovery of a chemical method for producing fumes by Mashiko *et al.*[3]. In this technique a solution consisting of 0.1 g of ruthenium chloride (III) hydrate in 100 mL of water was added to a second solution containing 11.3 g of ammonium cerium (IV) nitrate in 100 mL of water. The fumes generated in this reaction were circulated with a development chamber using a fan, and the authors demonstrated that sebaceous fingermarks could be developed on both porous and non-porous surfaces. Some work on sequential treatment was carried out, showing that ruthenium tetroxide must be used before ninhydrin and 1,8-diazafluoren-9-one (DFO), but cannot be used in sequence with physical developer. Some interference with superglue and Gentian Violet (basic violet 3) processing was also observed.

1.3 However, it was difficult to generate sufficient quantities of fumes by the chemical reaction process and Mashiko and Miyamoto [4] later proposed a solution consisting of 0.25 g per 100 mL of tetradecafluorohexane (C_6F_{14}), which was applied to articles via spraying directly from a glass bottle through a nozzle. Solution dipping was also proposed for exhibits such as adhesive tapes. Wilkinson *et al.* [5] investigated the use of ruthenium tetroxide solution for the development of fingermarks on skin and although the process was found capable of developing marks, these appeared to be of lower contrast than marks produced using other techniques, and could not be lifted.

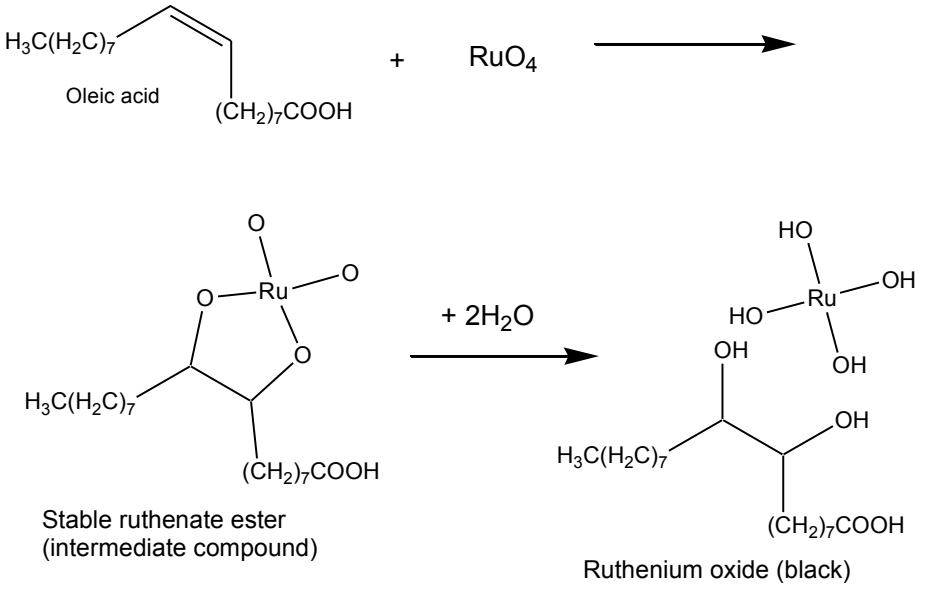
1.4 Mashiko later developed ruthenium tetroxide as a commercial product and has advertised its use in fingermark journals, [6] although there has been ongoing debate about the safety of the process [7,8].

1.5 In the one published comparative study carried out to date that incorporated ruthenium tetroxide, Mashiko's commercial product was not used for cost reasons. The researchers attempted to prepare solutions by dissolving ruthenium tetroxide fumes in carrier solvents of 1-methoxynonafluorobutane (HFE7100) or 2,3-dihydrodecafluoropentane (HFC4310mee). The best results were obtained from HFE7100, which gave a solution of equivalent effectiveness to the commercial

formulation. Ruthenium tetroxide solution was then spray applied and the results obtained compared with those obtained from the spray application of iodine solution and powdering. In these trials ruthenium tetroxide was only found to be the best process for very fresh marks on wallpaper and paint. For marks over one day old, performance decreased significantly. Ruthenium tetroxide could not be used in sequence with powders, and inhibited the take-up of fluorescent dye in marks developed using superglue.

2. Theory

2.1 Ruthenium tetroxide (and the closely related process osmium tetroxide) develops fingermarks by reacting across the carbon double bonds present in unsaturated fatty acids in fingermark residues. The reaction product is a black hydrous oxide that allows the fingermark to be visualised.



Reduction of ruthenium tetroxide by reaction with unsaturated fatty acids, adapted from equivalent reaction for osmium tetroxide (Olsen, 1975)[2].

2.2 The same reaction will occur whether ruthenium tetroxide is applied by fuming or as a solution.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Home Office Centre for Applied Science and Technology (CAST) does not recommend the use of ruthenium tetroxide because it is not as effective as other available processes and there are health and safety concerns about its use. As a consequence, it appears in the processes listed in Category F (not recommended for health and safety reasons) in the *Fingermark Visualisation Manual*. In contrast to the other processes listed, the toxicity of ruthenium tetroxide is stated as being unknown rather than a known hazard.

3.2 CAST has not carried out any comparative studies on ruthenium tetroxide because of health and safety concerns raised by other researchers. In the only published comparative study involving ruthenium tetroxide published to date [9], the reagent was found to be less effective than both powders and iodine-benzoflavone spray for most of the scenarios studied.

3.3 With regard to the health and safety aspects, there has been published debate about whether ruthenium tetroxide is toxic or not. There is also confusion as to whether the material safety data sheet (MSDS) data referred to in information supplied with the commercial product are for ruthenium dioxide or ruthenium tetroxide. CAST has reviewed the chemical literature available on the toxicity of ruthenium tetroxide and at best the substance has not been fully evaluated. Until this has been satisfactorily resolved CAST does not intend to carry out comparative trials or to recommend the process for operational use.

4. References

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Osmium tetroxide

1. History

1.1 Osmium tetroxide was already being proposed as a reagent for developing fingermarks on paper in the early 1900s. Mitchell (1920)[1] was able to describe two application techniques, namely:

- 'osmic acid', a 1% aqueous solution of osmium tetroxide brushed onto a document; and
- osmium tetroxide fuming, where the paper exhibit was held over a dish of the boiling 1% aqueous solution.

The 'osmic acid' solution treatment was stated to produce black marks if the surface was kept moist whilst exposed to sunlight, whereas the prints produced in the fuming process were grey. A further fuming process was later proposed, involving placing osmium tetroxide crystals in a small, shallow glass dish within a fuming cabinet and adding ethyl ether or carbon tetrachloride [2]. It was essential not to apply heat in this process because of the risk of an explosion.

1.2 Later researchers used pre-prepared ampoules of osmium tetroxide within a fuming cabinet, and used a sensitising chemical (5-norbornene-2-carbonyl chloride) in vapour form as a pre-treatment to produce additional linkages to react with the osmium tetroxide [3].

1.3 Bones [4] carried out a detailed assessment of the osmium tetroxide fuming process, looking at:

- different environments for the fuming process (air, argon);

- different development conditions (light, dark, vacuum); and
- the effects of ageing and humidity on the quality of prints developed.

Bones concluded that the process was equivalent to ninhydrin in sensitivity, and that the optimum processing conditions were in an air environment and in darkness. It was also shown that osmium tetroxide could develop handprints on fabrics, although there was negligible ridge detail visible.

1.4 Smith Jr [5] later proposed the osmium tetroxide fuming technique for the development of fingermarks on adhesive tapes, including medical tapes and strapping tapes. The exhibits were processed in air and stored in the dark; progressive darkening of the substrate was observed if exhibits were exposed to the light, and this could obscure marks.

1.5 In the early 1980s the Home Office Scientific Research and Development Branch (SRDB) included osmium tetroxide in a comparative study of techniques for development of fingermarks on fabrics [6], which included vacuum metal deposition and radioactive sulphur dioxide. Of these techniques osmium tetroxide, both as a fuming process and in solution, proved significantly less effective than radioactive sulphur dioxide and vacuum metal deposition, and no further work was carried out on this reagent.

2. Theory

2.1 The theory associated with osmium tetroxide is identical to that described for ruthenium tetroxide above. Osmium tetroxide reacts across the carbon double bonds in the unsaturated fatty acids within fingermark deposits to form intermediate osmate ester compounds that finally produce the black osmium dioxide compound [7].

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The Centre for Applied Science and Technology (CAST) does not recommend the osmium tetroxide process because of the highly toxic nature of the substance. As a consequence, it appears in the processes listed in Category F (not recommended for health and safety reasons) in the *Fingermark Visualisation Manual*. In comparative studies that have been carried out it has not proved to be any more effective than any other process currently (2016) in use.

4. References

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Europium chelate

1. History

1.1 The use of lanthanide series elements in fingermark detection has been considered in a range of techniques. The attraction of these elements is that they can form fluorescent complexes with large Stokes shifts, meaning that they can be illuminated in the ultraviolet region of the spectrum and emit in the red/infrared region. The decay time during fluorescence is also longer than many other fluorescent species, making them useful in time-resolved imaging applications and for visualising fingermarks on fluorescing backgrounds.

1.2 Initial studies into the potential of these elements for fingermark detection utilised europium salts as complexing agents for the post-treatment of marks developed using ninhydrin [1]. However, it was recognised that europium complexes also had the potential for use as a superglue dye, especially in circumstances where background fluorescence caused problems and a large Stokes shift was desirable [2,3,4]. The dye was successfully applied to superglue marks developed on multicoloured surfaces and on skin. Dyes were dissolved in methyl ethyl ketone [2,3] or petroleum ether [4].

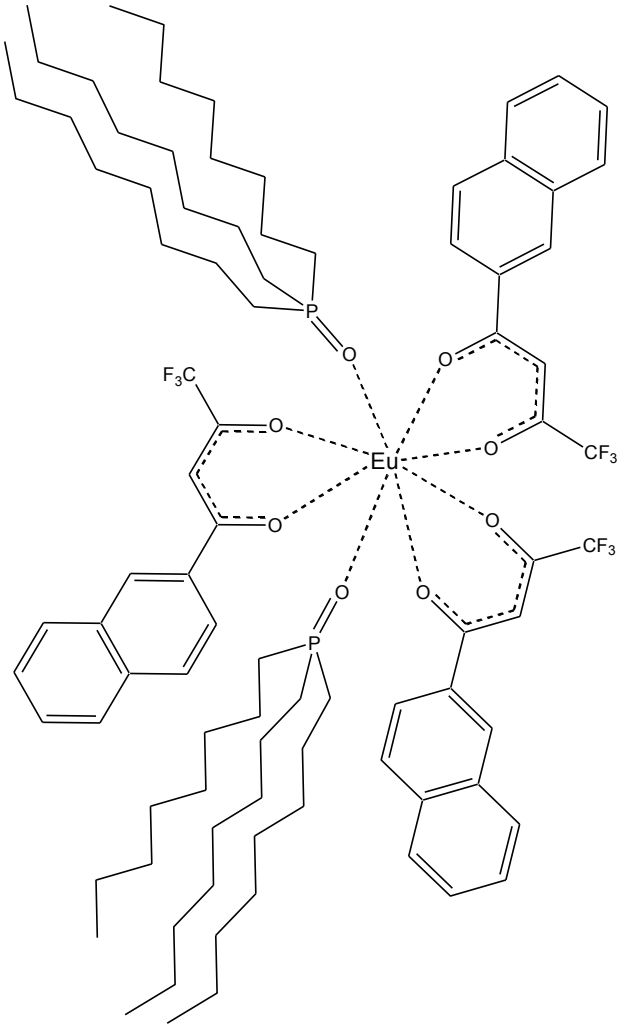
1.3 Later researchers have considered europium chelates as a fingermark development reagent in their own right, producing a range of formulations that can either be applied by spraying or as a solution that exhibits can be dipped into [5,6,7,8,9]. Bright, fluorescent marks were successfully developed on both porous and non-porous items in

laboratory trials, although these were not replicated when the technique was applied to casework.

1.4 More recently, a revised formulation of europium chelate was included in studies comparing methods for enhancing grease contaminated marks on dark non-porous surfaces [10], where it was shown to give equivalent performance to alternative reagents being considered (natural yellow 3) for certain types of greasy contaminant.

2. Theory

2.1 The theory associated with the europium chelate reagent is that the europium complex is in some way attracted by the lipid components of the fingermark deposit and absorbed into it from solution. Wilkinson [7] suggests that the presence of methanol may aid the transfer process from solution into the fingermark. Methanol partially dissolves in the lipids of the fingermark residue, and because the europium complex is water insoluble and prefers the hydrophobic environment of the fingermark lipids, some of the complex is transferred with the methanol. Once absorbed by the lipids, the water molecules attached to the europium complex are displaced and replaced by various lipid-based ligands. The resultant structure is a fluorophore and will fluoresce when illuminated with light of an appropriate wavelength.



Structure of a biological fluorophore (Wilkinson, 1999)[7].

2.2 The bulky fluorophore structure protects the europium from the aqueous environment of the biological medium (in this case the water present in the fingermark residue). A detergent is added to isolate further the europium ion from the water molecules.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

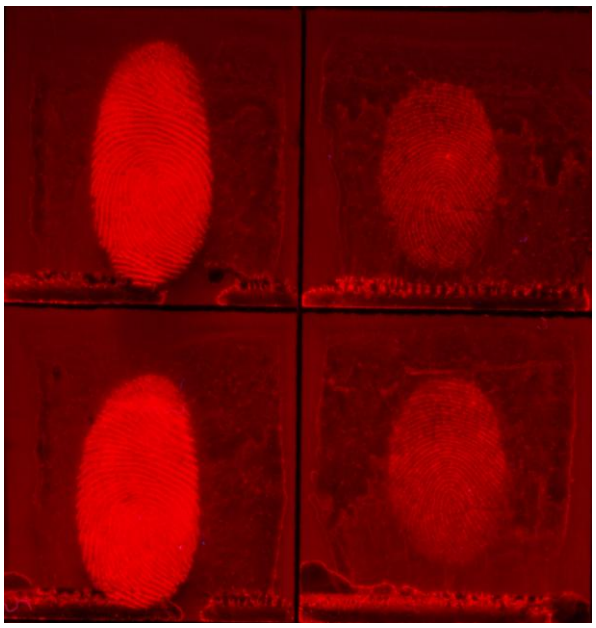
3.1 Europium chelate is currently (2016) classified as a Category B process in the *Fingermark Visualisation Manual*, with niche applications for enhancing grease contaminated fingermarks on dark surfaces where marks developed using solvent black 3 and basic violet 3 would not be visible. It may be comparable to the other two dyes in effectiveness, and could be used in sequence with them, but has not yet been sufficiently evaluated against them or the alternative dye natural yellow 3 to justify placing it in Category A. Work that has been conducted by the Centre for Applied Science and Technology (CAST) is outlined below.

3.2 The formula proposed by Wilkinson [7] was used as the basis of the formulation used in comparative studies. This is made up as a two- part system and is as follows:

Solution A – 23 mg europium chloride hexahydrate;
300 mL distilled water;
2 mL Tergitol 7.

Solution B – 42 mg thenoyltrifluoroacetone;
50 mg trioctyl phosphine oxide;
700 mL methanol.

3.3 The two solutions are then mixed together for 30 minutes, and articles to be treated are immersed in the resultant solution for 5 seconds then washed in water and allowed to dry.



Sebaceous marks deposited on a ceramic tile and developed using europium chelate.

3.2 The most recent study conducted by Gaskell [10] modified this formulation, using dioctyl sulfosuccinate, sodium salt (DOSS) in place of Tergitol 7 (the same replacement made for the reformulation of small particle reagent), and ethanol in place of the more toxic methanol. The formulation used was therefore:

Solution A
6 mg europium (III) chloride hexahydrate
75 mL distilled water
0.5 mL DOSS stock detergent

mixed with

Solution B
10 mg thenoyl trifluoroacetone
12 mg trioctyl phosphine oxide
175 mL ethanol

Solutions A and B being mixed in the proportion 30:70

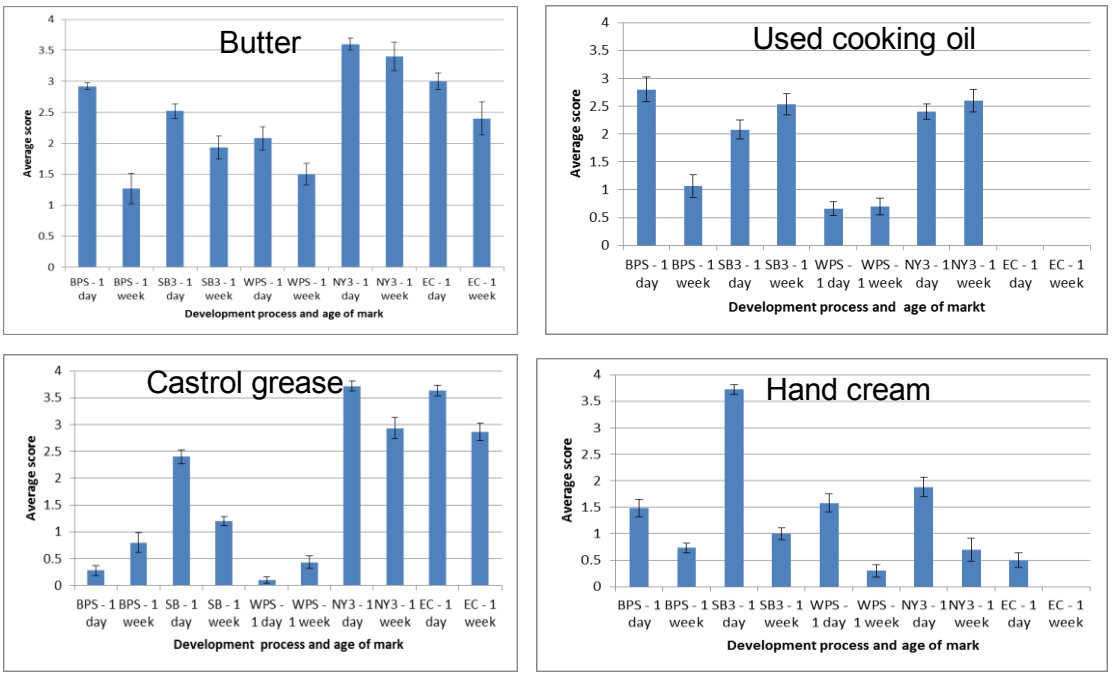
3.3 Fingermarks developed using europium chelate were examined and photographed under high intensity illumination using the Quaser 2000 (Foster & Freeman, Evesham, UK) with the following settings:

- excitation filter: 340–413 nm;
- viewing filter: Schott glass GG435.

3.4 A preliminary study investigated the ability of reagents to visualise traces of 35 different contaminants representative of 'kitchen', 'bathroom' and 'garage' environments. Further experiments focused on four of these contaminants (butter, used cooking oil, hand cream and engine grease) in scenarios where contaminated marks were deposited onto clean surfaces, and where 'natural' fingermarks were deposited over and underneath a layer of contamination.

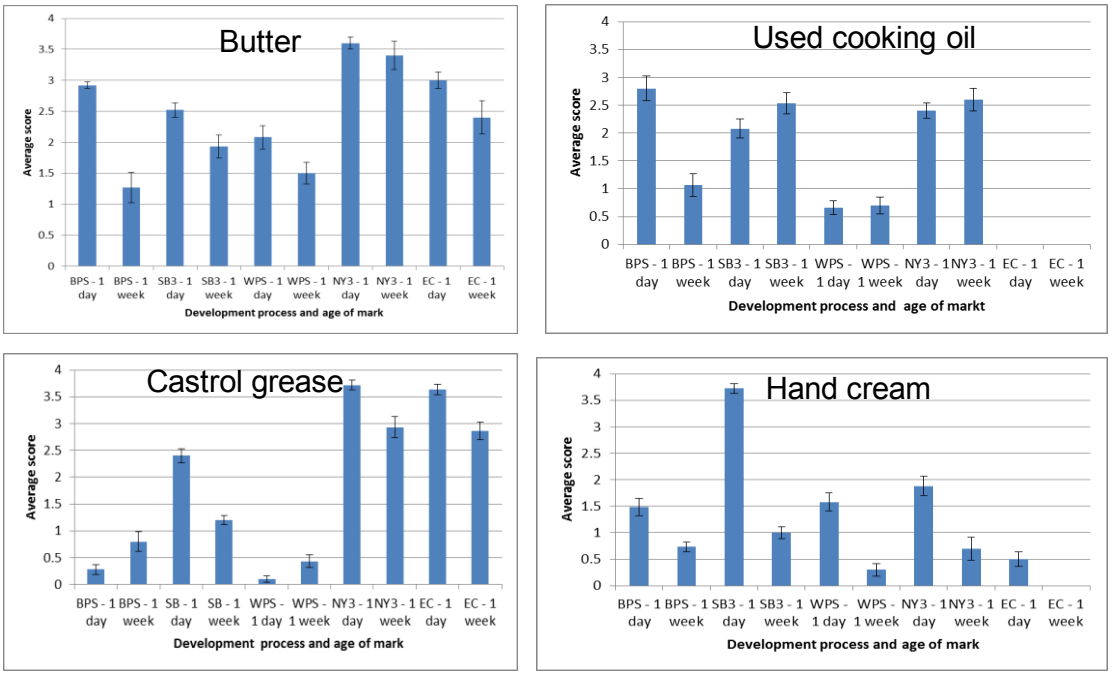
3.5 In the preliminary trial, europium chelate worked well for visualising certain types of contaminants, particularly those based on animal and vegetable fats. In some cases europium chelate appeared to work better on marks developed one week after deposition than one-day-old marks (possibly because the marks had dried out).

3.6 For contaminated marks on clean surfaces, europium chelate worked well in detecting marks in butter and used cooking oil, and gave fair results in detecting marks contaminated with hand cream. Good results were obtained for engine grease, but this was primarily due to the grease itself fluorescing under the long ultraviolet wavelengths used for examination rather than any preferential staining of the grease by europium chelate.



Results of comparative tests between reagents for visualisation of marks in different contaminants deposited on clean ceramic tiles (BPS = black powder suspension, SB3 = solvent black 3, WPS = white powder suspension, NY3 = natural yellow 3, EC = europium chelate)[10]

3.7 In scenarios where marks were deposited over and underneath layers of contaminant the performance of europium chelate was erratic. Some contaminants (e.g. used cooking oil) were not stained at all and others (Castrol grease) produced artificially good results because of the grease being naturally fluorescent under the long wave ultraviolet examination conditions. Marks that were visible were primarily due to the disturbance in the layer of grease. Europium chelate was not capable of detecting natural fingermarks deposited underneath a layer of greasy contaminant.



Results of comparative tests between reagents for visualisation of marks deposited into layers of different contaminants (BPS = black powder suspension, SB3 = solvent black 3, WPS = white powder suspension, NY3 = natural yellow 3, EC = europium chelate)[10]

A fingermark deposited in a layer of butter and subsequently visualised using europium chelate.

4. References

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Basic violet 2

1. History

1.1 In common with many other synthetic dyes, basic violet 2 (new fuchsin) was first synthesised in the 1850s. It is one of the four principal constituents of the dye mixture Magenta, but can be extracted as an individual dye. From the early 1900s basic violet 2 began to be considered for staining biological tissues in addition to its main use as a textile dye. In biological staining (e.g. for elastic tissue or bacteria cell

walls) it may be used either singly or in combination with other dye components [1,2].

1.2 The use of basic violet 2 as a fingermark reagent was first considered by Sutcliffe [3] in a study into lipid dyes capable of selectively staining fingermarks. It was then studied in greater detail by Miller [4] with the specific objective of finding an alternative to basic violet 3 for developing fingermarks on adhesive tapes. The reason for this was that basic violet 3 is a known carcinogen and basic violet 2 was then a possible safer alternative. However concerns have subsequently been raised about basic violet 2 also being a suspect carcinogen and there may be little benefit in selecting the dye on this basis.

1.3 Garrett and Bleay [5] conducted a re-evaluation of basic violet 2, looking at some of its fundamental interactions with lipid constituents of fingermarks and also investigating the quality of developed marks in both visible and fluorescence modes. Previous studies had either found no fluorescence [3] or considered only the visible mark [4]. These studies indicated that basic violet 2 may actually interact with a wider range of fingermark constituents than basic violet 3 and the fluorescent marks produced are easier to detect than those produced by basic violet 3 (although there is less colour intensity of visible marks).

2. Theory

2.1 Basic violet 2 is a basic dye, and the mechanism by which basic violet 2 stains fingermarks is the same as that described for basic violet 3 in Chapter 3, Chemical and Physical Processes, Basic violet 3. This mechanism is chemical binding interactions occurring between the amine (NH_2) groups of the dye molecule and acid groups present on the fingermark constituents.

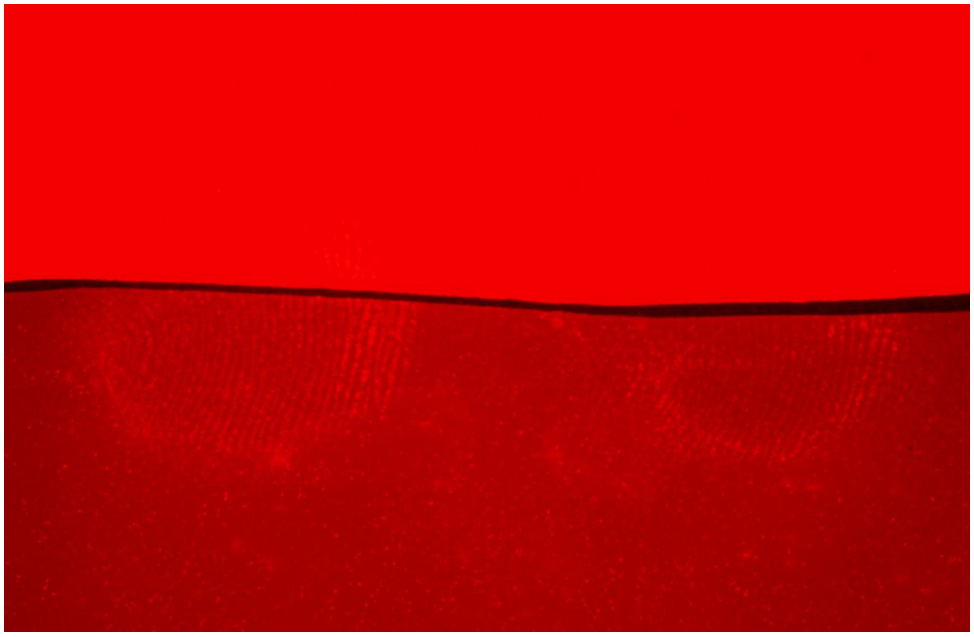
3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Basic violet 2 is included as a Category C process in the *Fingermark Visualisation Manual,* as a possible alternative to basic violet 3 under certain circumstances where either a different visible colour or fluorescence wavelength is desirable. It is of low maturity because only limited comparative studies have been conducted; these are summarised in Chapter 3, Chemical and Physical Processes, Basic violet 3.

3.2 The formulation proposed by Miller [4] and further investigated by Garrett and Bleay [5] consisted of a stock solution that is diluted with water to form a working solution prior to use.

3.3 The stock solution consists of 1 g basic violet 2, 6.46 g benzoic acid and 50 mL of ethanol. This is diluted in the ratio 1 part stock solution to 30 parts of water to form the working solution, and items are processed in the same way as for basic violet 3.

3.4 Fluorescence is obtained by illuminating with light sources in the green region of the spectrum, and viewing through orange filters.



Comparison of marks on adhesive tape stained with basic violet 3 (top) *and basic violet 2* (bottom) *and viewed using fluorescence examination with a green light source and orange viewing filter.*

4. References

1. Chance, H. L. (1953) 'A bacterial cell wall stain', *Stain Technol.,* vol. 28 (4), pp 205–207.

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Natural yellow 3 (curcumin)

1. History

1.1 Curcumin (natural yellow 3) is a naturally occurring substance that is the principal active constituent of the spice turmeric, itself an extract of the root of the *Curcuma Longa* plant. It has been used as a natural textile dye and food colourant for many years, but has more recently been used for scientific applications. Stockert *et al.* (1989)[1] proposed the use of curcumin as a stain for tissue sections, the stained tissues being fluorescent.

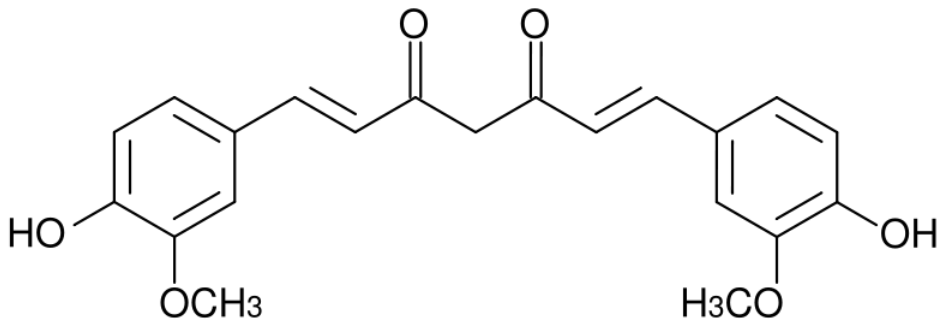
1.2 The fluorescent nature of curcumin meant that it was evaluated by the Home Office Centre for Applied Science and Technology (CAST) as a dye for marks developed by superglue fuming in the early-mid 1980s. However, ultimately basic yellow 40 was selected for this application and work on curcumin was not progressed further.

1.3 Garg *et al*. (2011) proposed the natural spice turmeric (which contains curcumin as its principal active ingredient) for the development of fingermarks by a powdering process. During an evaluation of naturally occurring substances with the potential to develop fingermarks by CAST, curcumin was reassessed, initially as a superglue dye but also in solution form for enhancing grease contaminated marks on dark non-porous surfaces [3,4]. An initial formulation closely based on the ethanol-based solvent black 3 solution was used for comparative trials, in which curcumin showed promise. Subsequent work has been conducted to develop an optimised formulation with increased sensitivity to latent fingermarks and reduced background staining [5,6]. This has continued to indicate the promise of the process and further comparative testing is anticipated.

1.4 The potential application of natural yellow 3 as a matrix for enhancing the compositional analysis of fingermarks when using matrix assisted laser desorption/ionisation (MALDI) has also been noted [7]. In this case the curcumin was applied to the mark as a powder, but there may be the potential to use natural yellow 3 as a multipurpose reagent, for both developing fingermarks and enabling subsequent chemical analysis of them.

2. Theory

2.1 Natural yellow 3 is believed to stain fingermarks by a similar mechanism to solvent black 3 and Oil Red O, both being covered in separate sections to this chapter, with the curcumin molecule preferentially migrating into the fatty constituents of fingermarks from the staining solution in which it is sparingly soluble.

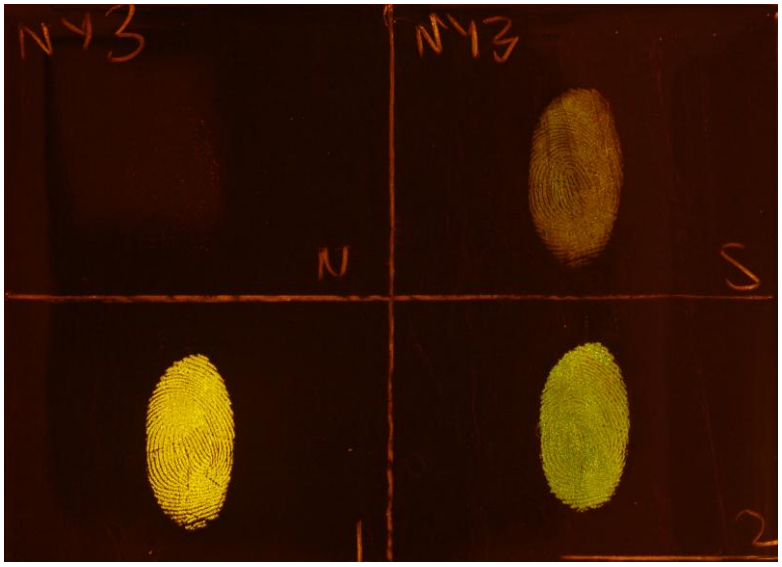


The chemical structure of natural yellow 3.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Natural yellow 3 is currently (2016) classified as a Category B process in the *Fingermark Visualisation Manual*, with niche applications for enhancing grease contaminated fingermarks on dark surfaces where marks developed using solvent black 3 and basic violet 3 would not be visible. It may be comparable to the other two dyes in effectiveness, and could be used in sequence with them, but has not yet been sufficiently evaluated to justify placing it in Category A.

3.2 The formulation reported by Gaskell *et al*. [3] and used in the comparative work reported in the section on europium chelate above consisted of 0.9 g of natural yellow 3 dissolved in a sovent mixture of 100 mL ethanol and 50 mL water. These studies indicated that natural yellow 3 worked well for visualising certain types of contaminants, particularly those based on animal and vegetable fats, and in some cases appeared to work better on marks developed one week after deposition than one-day- old marks (possibly because marks had dried out).



Fluorescent marks produced by staining different types of fingermark with natural yellow 3 (from top left): *natural, sebaceous, butter, vegetable spread).*

3.3 The optimum fluorescence conditions identified for natural yellow 3 are illumination using a blue or blue-green light source and viewing through a yellow-orange filter. The colour of the fluorescence produced can be modified by the pH of the environment that the molecule is in, and can range from green-yellow to orange-red in colour.

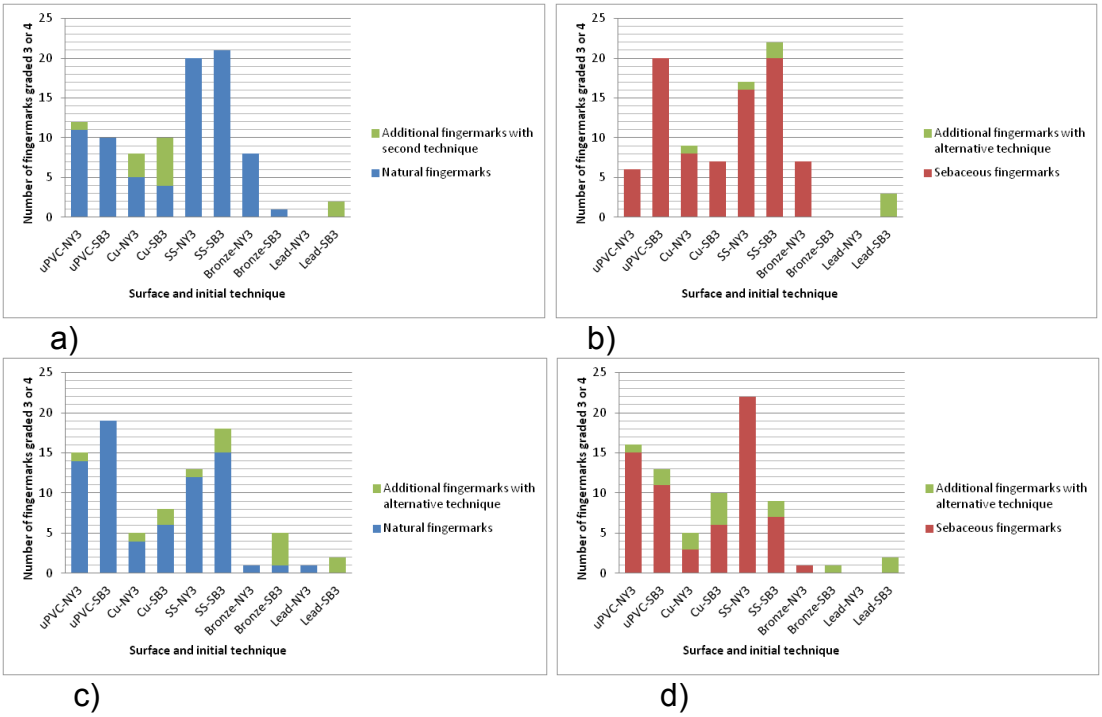
3.4 Subsequent work to optimise formulations has been conducted by Perry [5,6], who also included some comparative studies on weathered metallic surfaces in her evaluation. At the time of the study (2013) these were surfaces of operational interest because of the rising levels of metal theft being experienced. The formulation used in this work was:

5.00 g (approx 70%) curcumin (natural yellow 3)
500 mL propylene glycol methyl ether (PGME)
250 mL 1% sodium chloride solution in water
250 mL 1% DOSS solution in water

3.5 Natural yellow 3 was selected for use on these surfaces because it targets the water insoluble constituents that are most likely to remain after exposure to wetting in outside environments where weathered metals are generally encountered.

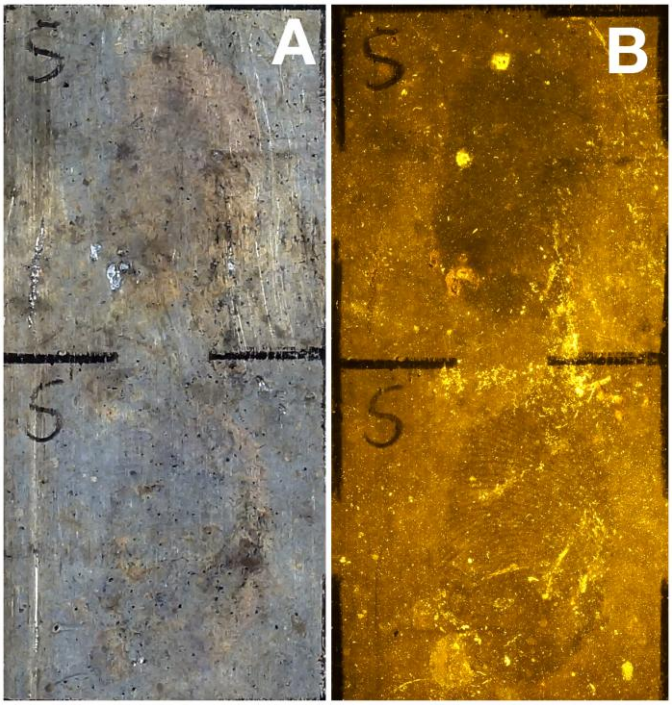
3.6 Depletion series of natural and sebaceous marks were collected from nine different donors on samples of uPVC, stainless steel, bronze, lead and copper and aged for between two and seven days before being treated with natural yellow 3 and solvent black 3. Following the grading of the marks developed, the samples were then treated with the alternative dye, so that the samples originally treated with natural yellow 3 were subsequently treated with solvent black 3 and vice versa. The

number of additional marks of high quality developed by the application of the second reagent was recorded.



The number of fingermarks graded 3 or 4 after development with natural yellow 3 (NY3) and solvent black 3 (SB3) and the additional fingermarks developed when samples were treated with alternative dye. a) *two day old natural fingermarks,* b) *two day old sebaceous fingermarks,* c) *seven day old natural fingermarks and* d) *seven day old sebaceous fingermarks [5]*

3.7 It can be seen that, in general, the number of fingermarks graded 3 or 4 for natural yellow 3 is similar to those developed by solvent black 3 for both two- and seven-day-old fingermarks across all the surfaces tested. The results for using the dyes in sequence show that additional fingermarks are developed by both sequences. However, it appears to be generally more beneficial to use natural yellow 3 after solvent black 3, especially on lead surfaces where the slight background fluorescence adds contrast with the darker marks.



Photograph of two depleted fingermarks on lead a) *after treatment with solvent black 3 and* b) *following subsequent treatment with natural yellow 3.*

3.8 The comparative study indicated that, in addition to the enhancement of grease contaminated marks, natural yellow 3 had the potential to enhance latent marks on a range of metal and plastic surfaces recovered from outside crime scenes. It may be especially useful on dark surfaces where fingermarks developed with solvent black 3 are difficult to see by eye and may be missed. Natural yellow 3 can be effective when used in sequence after solvent black 3, and could be added to the end of the existing sequence for non-porous surfaces.

4. References

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3. Gaskell, C., Bleay, S. M. and Ramadani, J. (2013) 'Natural Yellow 3: A Novel Fluorescent Reagent for Use on Grease-Contaminated Fingermarks on Nonporous Dark Surfaces', *J. Forens. Ident.,* vol. 63 (3), pp 274–285.

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Nile Red

1. History

1.1 In common with many other lipid specific reagents described in this chapter, Nile Red was first reported as a biological stain before it was considered as a fingermark reagent. In its biological staining application Nile Red was proposed as a fluorescent biological stain for intracellular lipids [1], the dye not becoming fluorescent until it migrated into the lipidic environment.

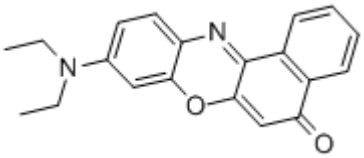
1.2 The first application proposed for Nile Red in fingermark enhancement was in 1996, as a stain for marks enhanced using cyanoacrylate fuming [2], although more recently research has focused on developing formulations capable of enhancing fingermarks on wetted papers [3], thus providing an alternative to Oil Red O and physical developer, both covered in separate sections of this chapter. Nile Red has recently been compared to physical developer, Oil Red O and solvent black 3 in a preliminary study on wetted papers [4], and found to give reasonable results. The attraction of Nile Red is that fluorescence generally only occurs where the dye has stained the fingermark, and dye retained in the paper remains non-fluorescing, overcoming issues with background staining where dyes are being viewed in the visible mode only.

1.3 One issue with Nile Red is that the dye is costly. To overcome this Frick *et al* [5] have proposed an alternative using an aqueous solution of the much cheaper dye Nile Blue A, which converts to Nile Red during the

staining process in the fingermark and produces the same fluorescent product.

2. Theory

2.1 Nile Red stains fingermarks because the molecule migrates into the fats present in the fingermark due to preferential solubility. Nile Red is not generally fluorescent in the polar solvent system, but once in a lipid rich environment it can become intensely fluorescent.



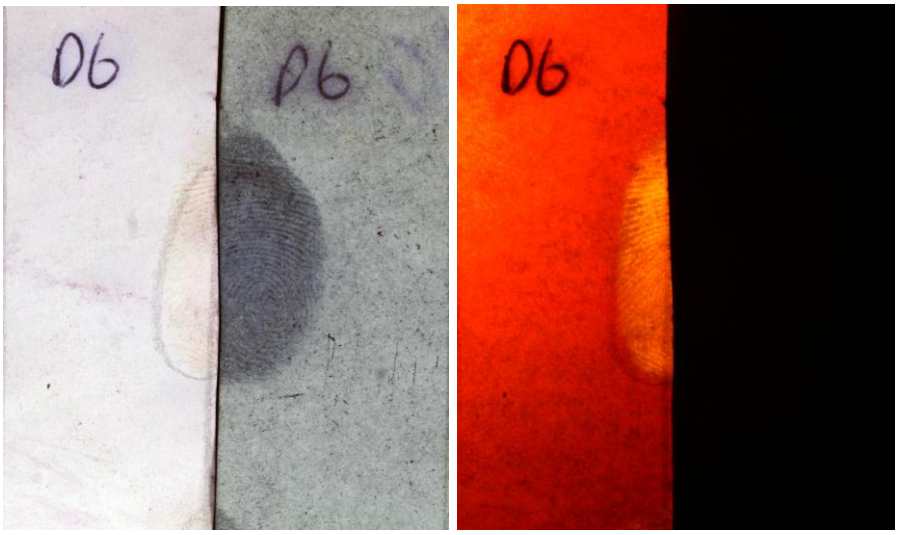
The chemical structure of Nile Red.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Nile Red is included as a Category C process in the *Fingermark Visualisation Manual,* as a possible alternative to physical developer under certain circumstances where paper is known to have been wetted but physical developer cannot be used. It is of low maturity because only limited comparative studies have been conducted by the Centre for Applied Science and Technology (CAST).

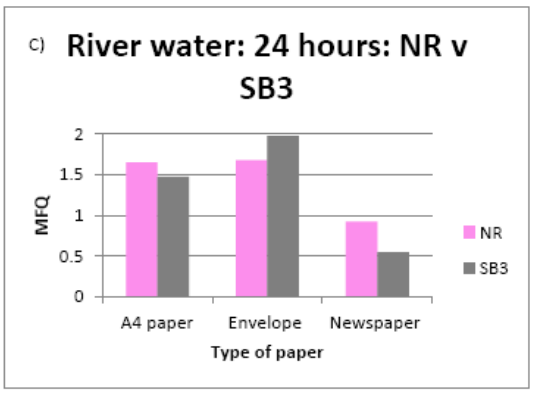
3.2 The formulation that has been proposed by Braasch *et al.* [3] and used in comparative studies consists of a Nile Red stock solution and a sodium hydroxide solution that are mixed together immediately before use to give a working solution. The Nile Red stock solution consists of 0.1 g of Nile Red in 1 L of methanol, and the sodium hydroxide solution is made up from 0.1 g sodium hydroxide in 1 L deionised water. The working solution is made from mixing 250 mL of Nile Red stock solution with 250 mL of sodium hydroxide solution.

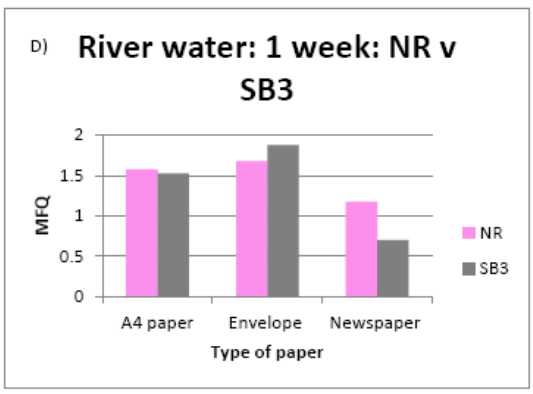
3.3 Items are immersed in a water bath for five minutes then immersed in the working solution for up to an hour (or until marks become visible as faint red ridges), then rinsed again before being allowed to dry and examined using fluorescence examination. Fluorescence is obtained by illuminating with light sources in the green region of the spectrum, and viewing through orange filters.

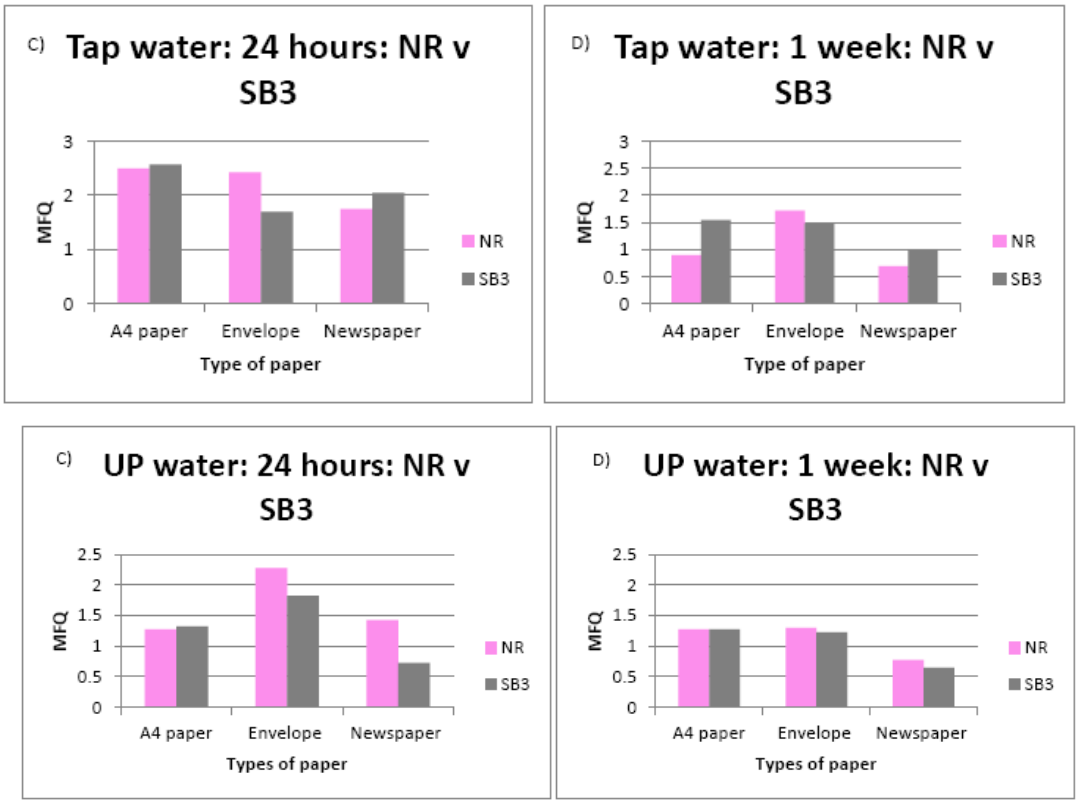


A split fingermark on wetted paper treated using Nile Red (left hand side) *and solvent black 3* (right hand side)*, viewed under white light and fluorescence examination.*

3.4 In the limited comparative trials conducted by Dhakal [4], Nile Red was compared with the original solvent black 3 formulation proposed for use on wetted paper [6]. An equivalent evaluation was conducted between Oil Red O and physical developer, these results being reported in Chapter 3, Chemical and Physical Processes, Oil Red O. The results indicated that Nile Red and solvent black 3 were similar in performance, although both marginally less effective than Oil Red O. There are situations where having a fluorescent reagent for wetting paper will be advantageous and therefore it is anticipated that Nile Red will be researched further.







Average scores for graded marks deposited on different porous substrates, aged for 1 day and 1 week, immersed in tap, river and ultrapure water, then developed using Nile Red (NR) and solvent black 3 (SB3)[4].

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Multimetal deposition and its derivations – single metal deposition

1. History

1.1 The multimetal deposition (MMD) system for developing fingermarks was first proposed by Saunders [1] in the late 1980s. The system incorporated principles of both small particle reagent and physical developer and provided a developing agent capable of producing marks on porous, semi-porous and non-porous surfaces. For this reason, it was originally called the *"Universal Process (for fingerprint detection)"* [2].

1.2 To carry out the MMD process, items were immersed in colloidal gold solution for 30 to 120 minutes (porous items were required to be first immersed in distilled water for 20 to 30 minutes), rinsed in distilled water (for up to 15 minutes in the case of porous items) and then immersed in a modified silver physical developer solution for 5 to 15 minutes (although more recent instructions reduce this to 1 to 2 minutes). After a final rinse in distilled water items were air dried and photographed.

1.3 After the publication of this technique, researchers in the UK and elsewhere began to investigate the capabilities of MMD. In the UK, the Central Research and Support Establishment (CRSE) of the Home Office Forensic Science Service (FSS) carried out a trial comparing MMD with superglue fuming and vacuum metal deposition (VMD) on a range of surfaces known to be difficult to treat. These included cling film, plastic shotgun cartridges, masking tape and expanded polystyrene [3]. These results suggested that for some of these surfaces MMD did produce superior results, but it did not appear that the process could be used sequentially after superglue (although this observation has since been contradicted by more recent work, see section 8.2.11).

1.4 The Police Scientific Development Branch (PSDB) also carried out an assessment of the process and confirmed that it worked on a wide range of substrates, including polythene bags, metal, fabric tape, coated cardboard, masking tape, wax candles, leather and cling film [4]. Tests were carried out on paper, but no results were obtained because the paper blackened. Development of fingermarks on fabrics was also attempted, as was subsequent radioactive toning of any marks developed. Faint ridges were seen during drying but these were not visible when fully dry, although some detail could be seen after radioactive toning and autoradiography. The microstructure of the marks developed was also studied by scanning electron microscopy. The Centre for Applied Science and Technology (CAST) concluded that MMD was a versatile technique, but gave no better results for any given surface than other techniques already available, and therefore it was not pursued further.

1.5 The process was later extensively re-evaluated by Schnetz and Margot [5]; they proposed an improved formulation offering increased reactivity,

improved resolution and greater amplification selectivity (and therefore reduced background interference). Important elements in the revised formulation were:

- the use of an alternative means of producing colloidal gold, giving smaller particle sizes; and
- the replacement of the silver nitrate/iron(II), iron(III) redox system in the physical developer stage with silver acetate/hydroquinone.

1.6 Jones [6] used the revised MMD formulation in an extensive study of processes for developing fingermarks on semi-porous surfaces. It was found that although not particularly effective on the polymer banknotes used in Australia, MMD did have potential applications for other semi-porous surfaces, including expanded polystyrene, latex and nitrile gloves, and waxed paper.

1.7 Developments were also carried out by Bécue *et al.*[7,8] to investigate the possibility of functionalising the gold nanoparticles with colorimetric or fluorescent tags. These studies may yield further revised formulations in future.

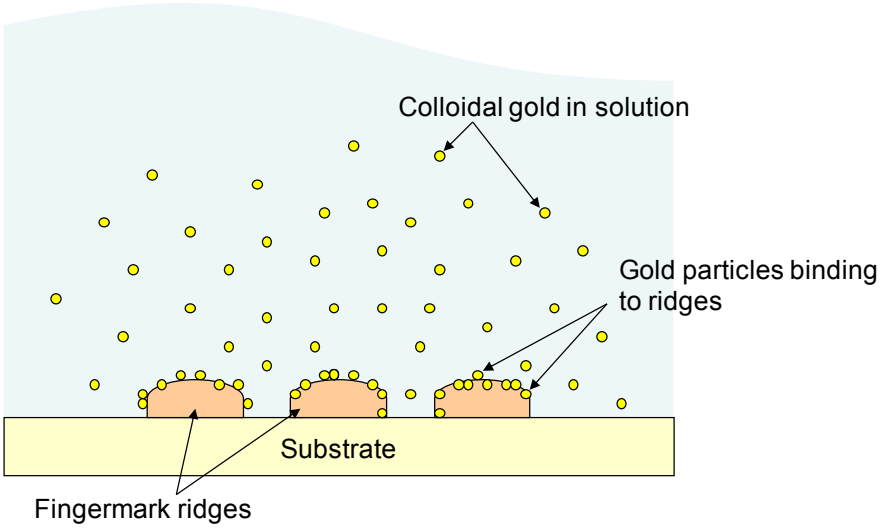
1.8 One of the latest refinements of the original MMD technique have included the development of the so-called 'single metal deposition' (SMD) process [9,10] where the silver deposition (post-colloidal gold) has been replaced by a gold deposition mechanism. This is claimed to have the advantages of reducing the number of different reagents and associated costs, and utilising reagents with a longer shelf life.

1.9 In 2010 CAST started a reassessment of the MMD process with a view to establishing whether it had applications in enhancing fingermarks on a range of surfaces known to be problematic. These included masking tape, leather, plasticised PVC, and cling film [11,12]. Initial studies looked at a range of formulations including MMD I, MMD II and SMD I on a range of different substrates. The results indicated that MMD I showed particular promise for developing marks on cling film and further work was carried out focusing on cling film, both clean and after exposure to operationally realistic environments, e.g. drugs contamination and water immersion [13,14]. The results of these tests confirmed MMD I to be significantly more effective than any other process available for treatment of cling film and as a consequence MMD was incorporated into the *Fingermark Visualisation Manual* [15].

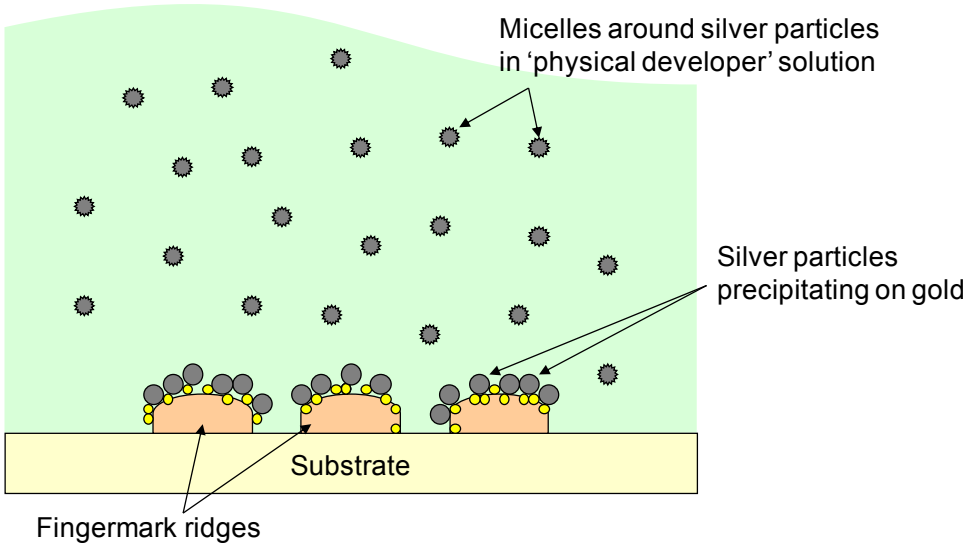
1.10 Subsequent to completion of these studies, further refinements were reported to the SMD process by Moret *et al* [16,17], with the objective of increasing effectiveness and simplifying the processing steps, leading to the SMD II protocol. This evolution has not yet been incorporated into any comparative assessments conducted by CAST.

2. Theory

2.1 MMD is essentially a two-phase development process, illustrated schematically in the diagrams below. The exhibit to be processed is immersed in an acidified solution containing colloidal gold particles, which bind preferentially to the amino acid, protein and peptide constituents incorporated in the non-hydrosoluble fraction of the fingermark. This stage alone generally gives poor contrast of the ridges and therefore an amplification stage is used. This involves the use of a modified physical developer solution for MMD, or gold reduction for SMD, resulting in growth on the gold nanoparticles present on the ridges through metal deposition. As a consequence of this enhancement step, ridges become dark grey to black in colour for MMD, and purple to light grey for SMD.



a)



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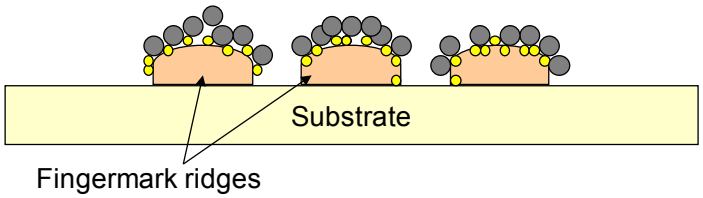


c)

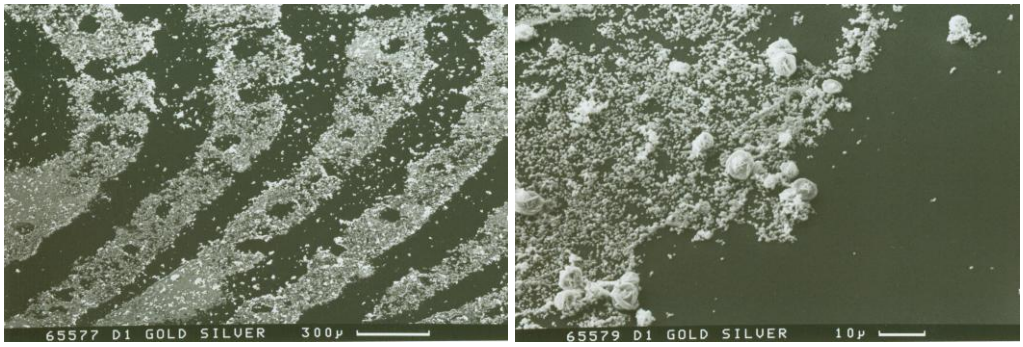
Schematic diagrams illustrating the stages in the multimetal deposition process a) *colloidal gold binding to ridges* b) *preferential deposition of silver particles on pre-existing gold and* c) *dried mark with contrast provided by silver particles.*

2.2 The reason that gold nanoparticles (in the case of MMD/SMD formed by the chemical reduction of tetrachloroauric acid by sodium citrate) are used is that they are extremely stable in solution and show an affinity for secretion residues in specific experimental conditions. Binding between organic compounds and colloidal gold particles can occur by both electrostatic interactions and chemical reactions. The dominant binding mechanism varies with acidity (pH), the maximum detection efficiency being observed at low pH. Schnetz and Margot [5] have suggested that it is the electrostatic interactions that are responsible for the reaction with fingermark deposits, and the pH of the treatment solution is kept low (pH 2.5 to 3) to facilitate this. Mildly acidic compounds such as amino acids, fatty acids and proteins carry a positive charge under these conditions and attract and bind to gold particles from the solution. Recently, Moret showed that chemical bonds can be formed between the carboxylic acids (present on the gold colloids) and the amines (present in the fingermark residue), and this could partly explain the development mechanism [17].

2.3 The size of the gold particles is also regarded as important, with smaller particles claimed to result in higher specificity. A size range of 10 to 30 nm has been observed for all MMD/SMD formulations.

2.4 The amplification stage in MMD is a modified physical developer system based on that used to develop fingermarks on paper. This contains silver ions in the presence of a reducing system, the solution being stabilised by surfactants. The silver ions are reduced to silver metal and the gold particles bound to the ridges act as a nucleation site for this to occur. The gold particles also act to catalyse the reduction of the silver.

2.5 Scanning electron micrographs of a fingermark developed using MMD are shown below [4].



a) b)

Scanning electron micrographs of marks developed using multimetal deposition a) *low magnification showing fingermark ridges and* b) *higher magnification showing precipitated particles (PSDB, 1992)[4].*

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014). The purpose of this publication (the *Fingerprint Source Book*) is to report the history, theory and validation work associated with the process. The MMD process recommended by CAST is that reported by Fairley *et al.* [11,12], which is essentially the original formulation proposed by Saunders [1] with minor modifications to the pH of the colloidal gold solution.

3.2 The principal solutions used in the process are a colloidal gold working solution, and a silver-based physical developer solution. These are complex formulations produced by preparing several other solutions and mixing them together.

3.3. The colloidal gold working solution utilises a gold chloride stock solution (1 g tetrachloroauric[III] acid trihydrate [$HAuCl_4.3H_2O$] dissolved in 10 mL distilled water), a sodium citrate solution (0.1 g sodium citrate tribasic dihydrate dissolved in 10 mL distilled water) and a 0.5 M citric acid solution.

3.4 To prepare the solutions 1 mL of the gold chloride stock solution is mixed with 1 litre of distilled water and brought to the boil. Once the solution is boiling, 10 mL of sodium citrate solution is added. Boiling continues for 10 minutes, during which time the solution should first turn black and then become a deep red/purple colour. Then 5 mL of Tween® 20 detergent is added and mixed, and the solution is taken off the heat and allowed to cool. Additions of 0.5 M citric acid are then made until the solution pH is in the range 2.5 to 2.8 (approximately 18

mL of citric acid is usually sufficient). The volume lost by boiling is then restored by adding distilled water to make the total volume 2 litres.

3.5 In this working solution:

- gold (III) chloride hydrate provides the colloidal gold particles;
- sodium citrate provides citrate ions that surround the gold particles and stabilise them;
- Tween® 20 is a detergent that also assists in stabilising the colloidal gold particles; and
- citric acid reduces the pH of the working solution to the desired range.

3.6 The modified physical developer working solution is very similar to that used in the physical developer process, but has a reduced stability that results in the more rapid deposition of silver from solution. It is made by producing a redox solution and a silver nitrate solution and mixing them together immediately before use.

3.7 The redox solution is produced by adding 33 g of iron (II) nitrate nonahydrate, 89 g of ammonium iron (II) sulphate hexahydrate and 22 g of citric acid into 1 litre of distilled water and mixing until a clear solution is produced. Then 1 mL of Tween® 20 detergent is added and mixed in.

3.8 The silver nitrate solution is produced by mixing 2 g of silver nitrate into 10 mL distilled water. The role of the constituents in the formulation are as described in Chapter 3, Chemical and Physical Processes, Physical developer. In this case Tween® 20 provides less stability to the solution than the detergent combination used in physical developer, and the silver precipitates more rapidly from solution.

3.9 The process is conducted by immersing the item in a series of solutions contained in non-metallic, scratch-free dishes:

- dish 1 – rinse the item thoroughly in 2 changes of distilled water;
- dish 2 –immerse the item in colloidal gold working solution for 45 minutes;
- dish 3 –rinse the item thoroughly in 2 changes of distilled water;
- dish 4 – immerse the item in physical developer solution (mixed immediately before use) for approximately 10 minutes (or until development is seen);
- dish 5 – rinse the item thoroughly in 2 changes of distilled water;
- the items are then hung, allowed to air dry and then photographed.

4. Critical issues

4.1 The MMD process should be conducted out of direct sunlight and preferably under subdued lighting conditions, otherwise the silver precipitates from the physical developer solution too rapidly for fingermark development to occur.

4.2 The pH of the colloidal gold working solution should be kept in the range 2.5 to 2.8, otherwise non-optimal development occurs.

5. Application

5.1 MMD is capable of developing marks on a wide range of non-porous, semi-porous and in some cases porous surfaces. In initial tests carried out by the PSDB in 1992 there was no single surface on which MMD gave better results than any other recommended process. However, more recent studies have shown that MMD is the most effective process available for enhancing marks on cling film, even after wetting and exposure to a range of drug contaminants.

5.2 MMD is also highly effective on other flexible polymers such as plasticised vinyl and 'leatherette'. The complex nature of the process means that it is unlikely to be routinely used, but in high priority cases MMD may provide the only means of enhancing marks on certain types of surface.

6. Alternative formulations and processes

6.1 Apart from the MMD formulation originally proposed by Saunders [1], now generally referred to as MMD I, there are two formulations that have also been widely reported and investigated, these being MMD II and SMD. The essential features of these formulations are described below.

6.2 MMD II
MMD II also uses a colloidal gold working solution and a silver-based physical developer, the principal difference between this and MMD I being the formulations used for each of these solutions. MMD II was claimed to give improved effectiveness over MMD I, but some of the requirements for solution manufacture make it potentially more time consuming to apply.

6.3 The colloidal gold working solution utilises the same solutions of gold (III) chloride and sodium citrate that are used in MMD I. MMD II also uses citric acid, but a 0.1 M solution as opposed to the 0.5 M solution used in MMD I. An additional 1% solution of EM grade tannic acid is used. The working solution is outlined below.

6.4 To make the colloidal gold working solution 0.5 mL of gold (III) chloride solution is added to 400 mL of distilled water. A separate solution is

then made by mixing 75 mL of distilled water, 20 mL of sodium citrate solution and 0.1 mL of tannic acid solution. Both these solutions are heated to 60°C and then mixed together; the temperature of the solution is then raised to its boiling point. The solution should eventually turn ruby red in colour through yellow, to colourless, to purple and then finally red. The solution is then allowed to cool to room temperature, and can be stored refrigerated for several months. Immediately before use, the solution is returned to room temperature and 0.5 mL of Tween® 20 added, followed by additions of 0.1 M citric acid to bring the pH into the range pH 2.5 to 2.8. It should be noted that this stage is highly labour intensive, and this has tended to preclude the widespread use of MMD II.

6.5 The silver-based physical developer is different in composition from that used in MMD I. A silver acetate solution is first produced by dissolving 200 mg of silver acetate in 100 mL distilled water. A separate solution is produced by mixing 1 g of hydroquinone with 200 mL of a pH 3.8 buffer solution (24 parts citric acid solution (2.55 g/L), 22 parts sodium citrate solution (235 g/L), 50 parts distilled water).

6.6 Processing is carried out in a similar way to MMD I, by immersing the item in a series of solutions contained in non-metallic, scratch free dishes:

- dish 1 – rinse the item thoroughly in distilled water for 2 to 3 minutes;
- dish 2 – immerse the item in colloidal gold working solution for 15 minutes;
- dish 3 – rinse the item thoroughly in distilled water for 2 to 3 minutes;
- dish 4 – immerse the item in a solution produced from 100 mL of hydroquinone solution mixed with 100 mL distilled water for approximately 2 to 5 minutes;
- dish 5 – immerse the item in a solution produced from 100 mL of hydroquinone solution mixed with 100 mL silver acetate solution for approximately 15 minutes;
- dish 6 – rinse the item thoroughly in distilled water for 2 to 3 minutes;
- dish 7 – soak the developed item for 2 to 5 minutes with photographic fixing solution (1:9 dilutions with water);
- rinse with tap water.

6.7 SMD I uses the same colloidal gold working solution as MMD II, the difference being that a gold-based physical developer is used (hence only a single metal is deposited on the fingermark). The gold-based physical developer is produced by mixing 0.2 mL of the gold chloride stock solution described above with 200 mL of distilled water, followed by the addition of 1 mL of an hydroxylamine hydrochloride solution (695 mg mixed with 100 mL of distilled water).

6.8 Processing is carried out in a series of solutions contained in non-metallic, scratch free dishes:

- dish 1 – rinse the item thoroughly in distilled water for 2 to 3 minutes;
- dish 2 – immerse the item in colloidal gold working solution for 15 minutes;
- dish 3 –rinse the item thoroughly in distilled water for 2 to 3 minutes;
- dish 4 – immerse the item in the 'gold – hydroxylamine' enhancement solution for approximately 10 to 15 minutes;
- dish 5 – rinse the item thoroughly in distilled water for 2 to 3 minutes.

6.9 The SMD II formulation [16,17] offers further refinements to the colloidal gold solution used in SMD I, and involves the initial preparation of several solutions.

- Solution A: 10% (w/v) tetrachloroauric acid trihydrate in reverse osmosis/deionised (RO/DI) water. This solution must be kept refrigerated and is stable for several months.

- Solution B: 2% (w/v) trisodium citrate dihydrate in RO/DI water. This solution is stable at room temperature for several months.

- Solution C: 0.12 g sodium hydroxide and 0.38 g of L-aspartic acid in 25 mL RO/DI water. L-aspartic acid is difficult to dissolve. The solution must be prepared under intense stirring. This solution is stable at room temperature for several months.

- Solution D: 1 M citric acid monohydrate in RO/DI water. This solution is stable at room temperature for several months.

- Solution E: 1 g hydroxylamine hydrochloride in 50 mL RO/DI water. This solution is stable at room temperature for several months.

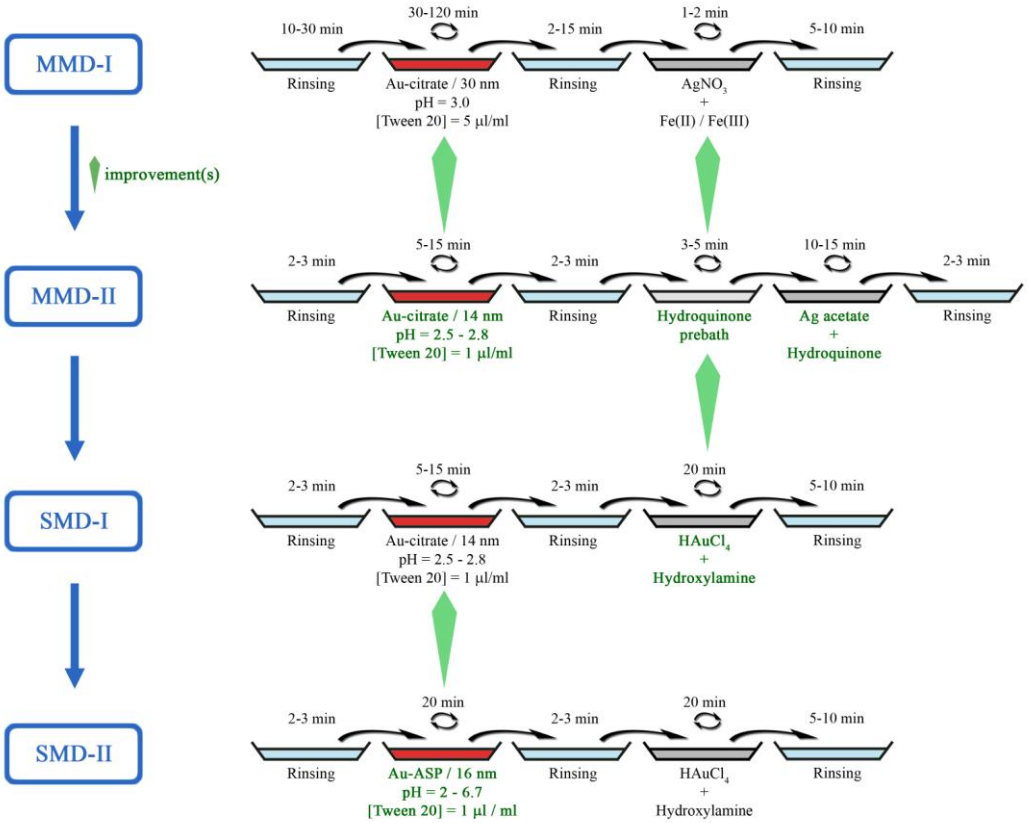
6.10 The colloidal gold solution used in SMD II is prepared as follows: 1 mL of solution A is added to 460 mL of RO/DI water and heated to boiling point under constant stirring. In a separate beaker 42 mL of solution B is mixed with 420 µL of solution C. When the first solution reaches its boiling point, quickly pour all of the second solution into it and keep heating the solution stirring intensely until the solution turns deep ruby red. Dilute with RO/DI water to reach the final volume of 2.5 L and add 2.5 mL of Tween 20 (preferentially using a positive displacement pipette) under stirring. The solution should be ruby red; purple tones at this stage may result from insufficient boiling time and could seriously hamper the efficiency of the solution. The resultant colloidal gold stock

solution is stable for several months in the fridge, when stored in polypropylene containers.

6.11 Processing is carried out in a series of solutions contained in non-metallic, scratch-free dishes:

- dish 1 – rinse the item thoroughly in deionised water for 2 to 3 minutes;
- dish 2 – immerse the item in colloidal gold working solution (which has been allowed to warm to room temperature and adjusted for pH by adding 3 mL of solution D per 100 mL of colloidal gold stock solution under constant stirring) for 20 minutes under gentle orbital shaking;
- dish 3 –rinse the item thoroughly in deionised water for 2 to 3 minutes;
- dish 4 –produce a solution by intensely stirring together 200 µL of solution A with 200 mL of RO/DI water and 200 µL of solution E, and immerse item for approximately 20 minutes (longer if necessary) under moderate orbital shaking;
- dish 5 – rinse the item thoroughly in deionised water for 2 to 3 minutes.

6.12 A summary of the stages in MMD I, MMD II, SMD I and SMD II is shown schematically below.



Schematic diagram showing the stages in the multimetal deposition and single metal deposition processes, and their durations [17].

6.13 Fairley [11] conducted a comparison of MMD I, MMD II and SMD I on a range of substrates including cling film and plasticised vinyl. He also investigated alternative combinations of colloidal gold solutions and physical developers (e.g. the colloidal gold solution from MMD I with the physical developers from MMD II and SMD I).

6.14 These comparative tests confirmed that the MMD II process was the most effective. However, it was felt that this was impractical for routine use and the MMD I technique, with the pH of the colloidal gold solution reduced to pH 2.5 to 2.8, was chosen as the preferred method for comparative trials.

6.15 Subsequent to this study, the SMD II formulation has been proposed [16] giving improved stability and effectiveness. These have not yet been included in comparative tests.

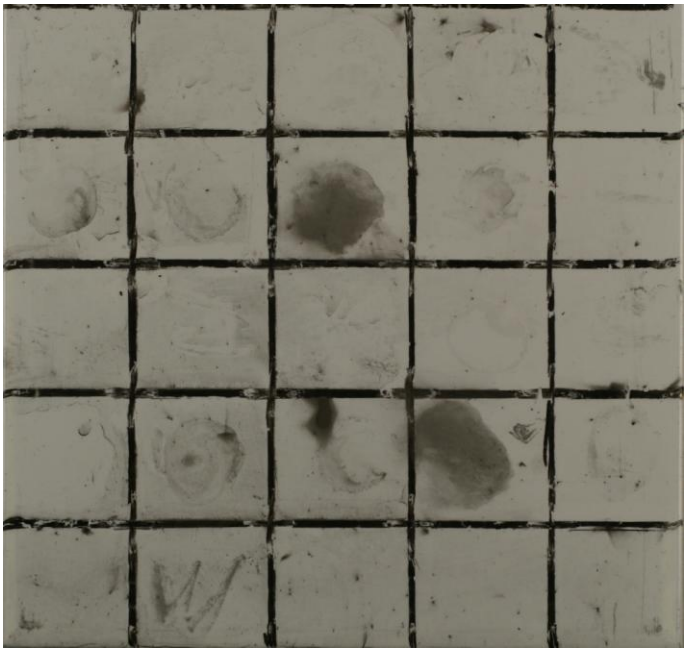
7. Post-treatments

7.1 There are no currently recommended post-treatments for MMD, but it is possible that processes used for enhancing marks developed with physical developer could also be applied

8. Validation and operational experience

8.1 Fundamental studies

8.1.1 Charlton [13] conducted some spot tests using spots of eccrine and sebaceous fingermarks deposited on a white ceramic tile and processed using the modified MMD I formulation proposed by Fairley [11]. Of the constituents investigated, only the spots of the amino acid serine showed strong development, with weaker development observed for urea and alanine as shown below.



CHOLESTEROL	CHOLESTROL ACETATE	GLYCEROL TRISTEARATE	GLYCEROL TRIPALMITATE	SQUALENE
UREA	ALANINE	SERINE	SODIUM CHLORIDE	PALMITIC ACID
OCTADECANOIC AICD	CHOLESTEROL	CHOLESTROL ACETATE	GLYCEROL TRISTEARATE	GLYCEROL TRIPALMITATE
SQUALENE	UREA	ALANINE	SERINE	SODIUM CHLORIDE
PALMITIC ACID	OCTADECANOIC AICD	------	------	------

Spot tests conducted on a white ceramic tile, and a plan showing the constituent deposited in each position [13].

8.1.2 The results indicate that MMD interacts with compounds containing amine groups, consistent with the original function of such formulations in labelling proteins. This observation is consistent with Moret's conclusions about the creation of an amide bond between carboxylic acids and amines [18].

8.1.3 Charlton [13] also conducted an evaluation of the effect of ageing the stock and working colloidal gold solutions on the quality of development. In this investigation of the practical use of MMD the stock (gold [III] chloride) and working solutions were aged for up to one month to see if fingermark development quality diminished. It was shown that the contrast and number of fresh fingermarks developed successfully were not affected by the age of any of the solutions within the range of ages studied.

8.2 Laboratory trials

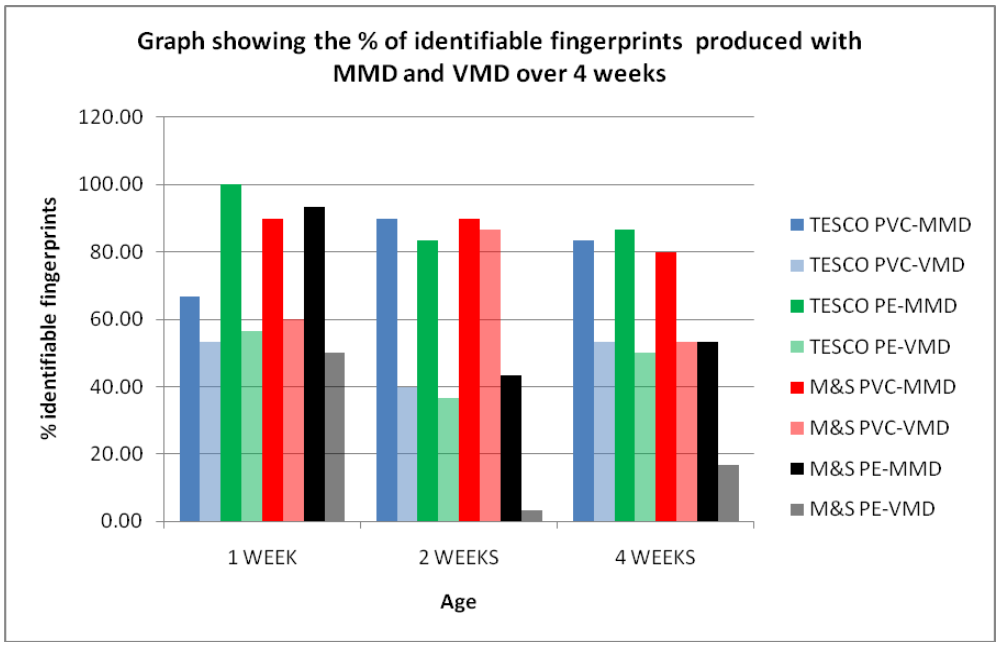
8.2.1 Fairley [11] conducted a comparison between MMD I and a range of processes that were then (2011) recommended by CAST for surfaces known to give low rates of fingermark recovery, as summarised below

.

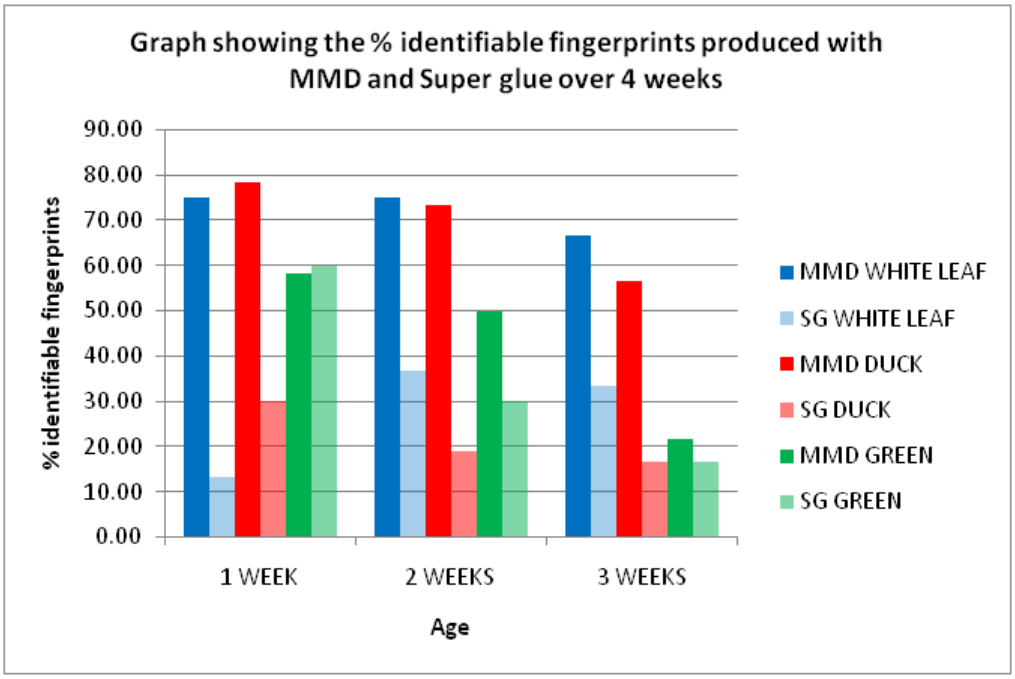
Surface	Recommended process at time of study (2011)
Cling film – PVC/PE-based	Silver vacuum metal deposition (VMD)
Shower curtains – vinyl-based	VMD and cyanoacrylate fuming
Leatherette – PVC-based	Powder suspensions – Wet Powder Black/White™
Leather	Powder suspensions – Wet Powder Black/White™

Surfaces for which comparative experiments were carried out, and the processes used in the comparisons. Wet Powder Black is a commercial carbon-based powder suspension, Wet Powder White a commercial titanium dioxide – based powder suspension

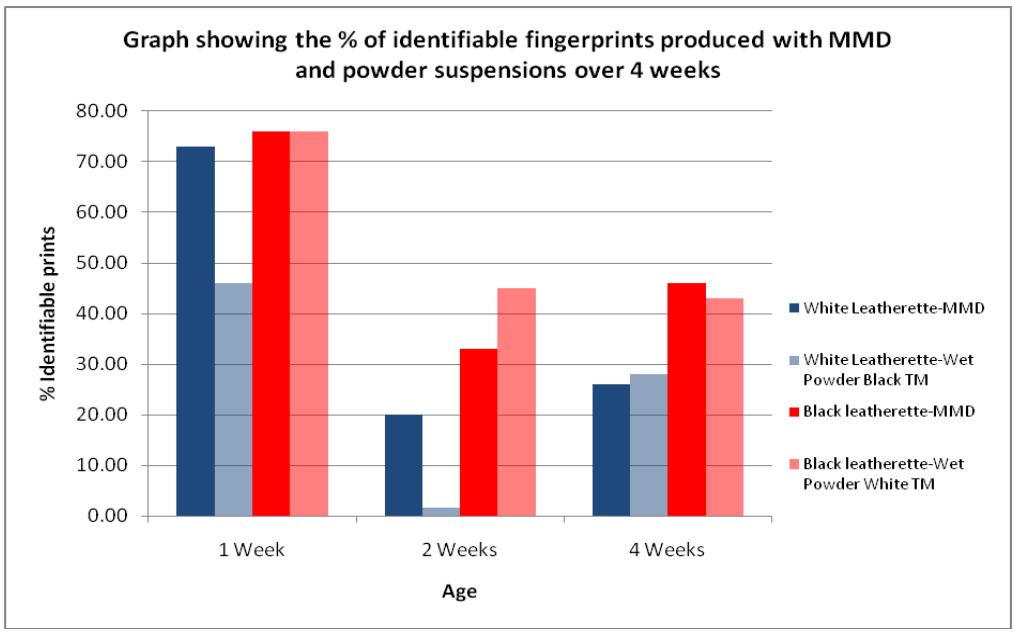
8.2.2 The results of these comparisons are summarised in the series of graphs and tables below.



Graph showing the proportion of potentially identifiable (i.e. graded 3 or 4) fingermarks developed on clingfilm over four weeks [11].



Graph showing the proportion of potentially identifiable (i.e. graded 3 or 4) fingermarks developed on shower curtains over four weeks [11].



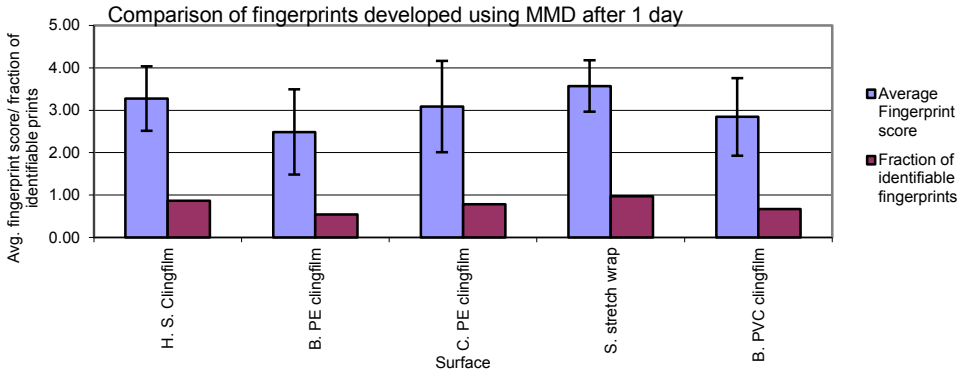
Graph showing the proportion of potentially identifiable (i.e. graded 3 or 4) fingermarks developed on leatherette over four weeks [11].

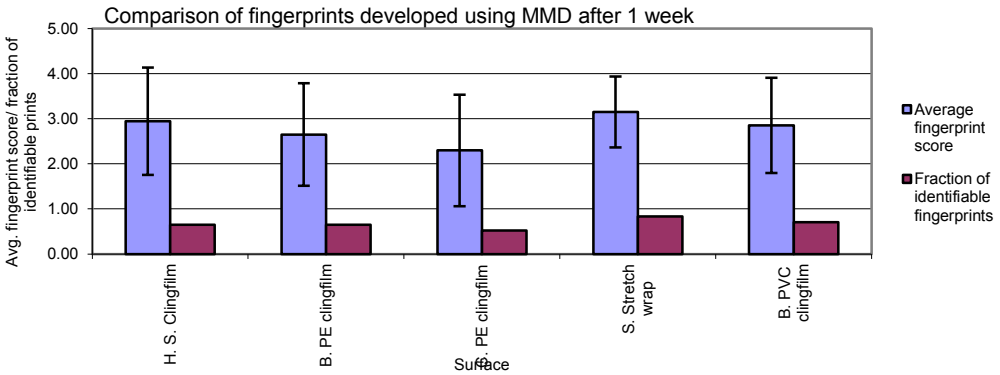
White textured leather		
	MMD I	**Wet Powder Black™**
% Identifiable marks	5.00	18.00
Average score	0.57	0.97
Standard deviation	0.89	1.37

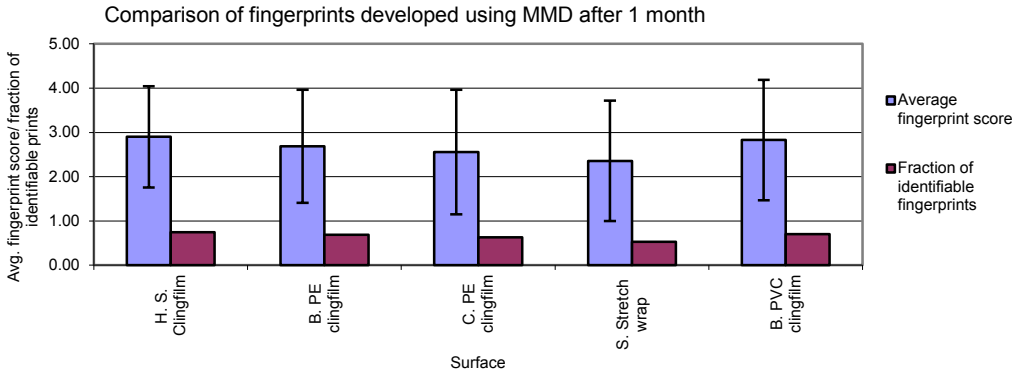
The average score, proportion of identifiable marks and standard deviation for marks developed using multimetal deposition and powder suspension on white leather – whole marks [11].

8.2.3 It was shown that MMD I gave improved performance over existing techniques for vinyl-based polymer surfaces in general, and on cling film in particular. These results suggested there could be operational merit in using MMD on such surfaces. As a consequence, further research was conducted to see if the process could be incorporated into processing sequences.

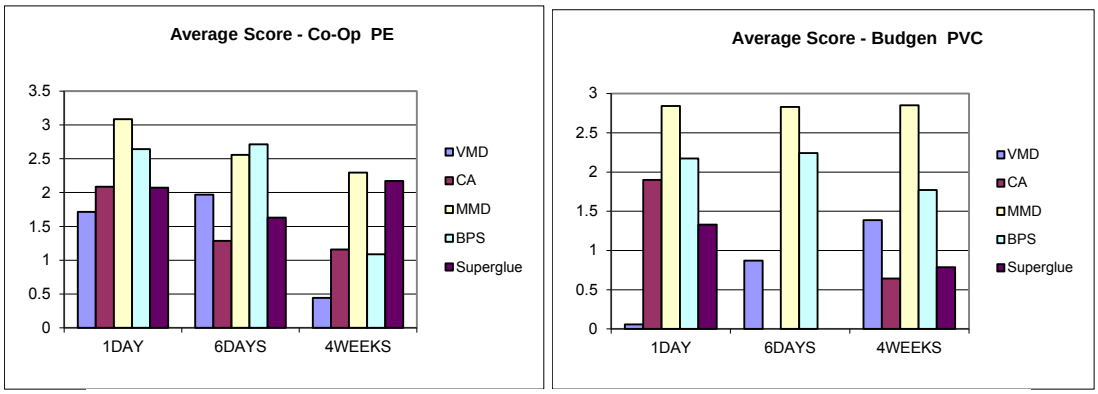
8.2.4 In these studies by Charlton [13], initial work focused on confirming Fairley's results [11] on cling film. Five different cling film and stretch wrap surfaces (three polyethylene based, two polyvinyl chloride-based) were used to collect a series of nine depletions from six donors. Surfaces were then aged for either one day, six days or four weeks. The results for MMD alone are shown for the five different surfaces, together with the results in comparison with other processes (silver vacuum metal deposition, superglue fuming followed by dyeing with basic yellow 40, iron oxide-based black powder suspension) that have previously been considered for the development of fingermarks on cling film. Charlton used iron-based powder suspension in these studies instead of the carbon-based system used by Fairley, because iron-based powder suspension was going to be recommended by CAST for use on plastic bags.

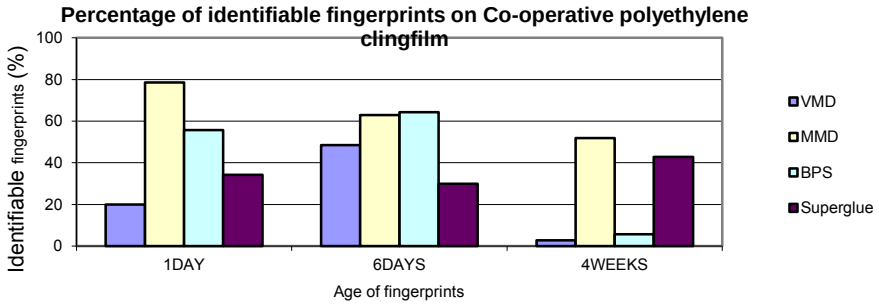


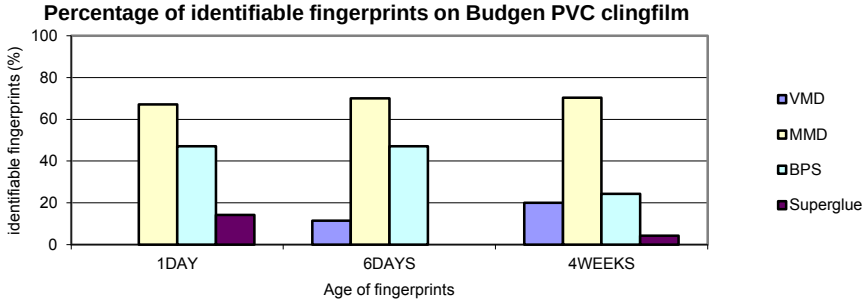




Results obtained for marks developed using MMD I on a range of different types of cling film after ageing for different periods of time [13].







Results obtained in comparative trials between MMD I, VMD, superglue/BY40 and iron oxide-black powder suspension on PE and PVC-based clingfilm[13].

8.2.5 It can be seen that on cling film the performance of MMD I was little affected by the age of the fingermark, especially on PVC-based cling film. In comparison to VMD, black powder suspension (BPS) and superglue/basic yellow 40 (BY40), MMD I consistently developed the most marks of high quality, and its performance was least affected by the age of the mark. VMD often gave empty development and when fingermarks were developed they were faint with low contrast to the background. BPS initially showed comparable results to MMD I on polyethylene after one day and six days, but after four weeks high background staining occurred.

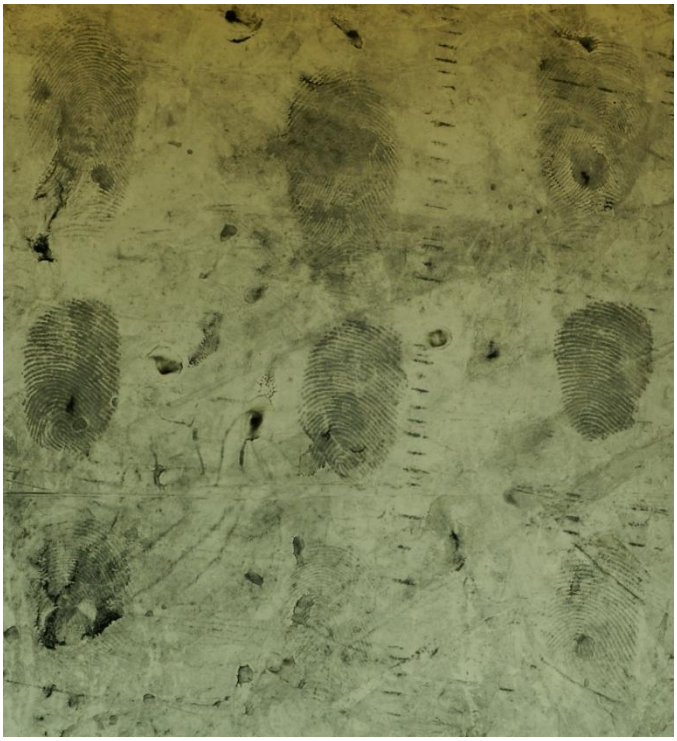
8.2.6 In addition to ageing under ambient conditions, fingermarks on cling film surfaces were also exposed to more extreme, operationally representative conditions. Depletions series from 5 different donors were deposited, aged for 1 day and then subjected to water immersion for periods of 1, 2, 6, 24 and 50 hours. Control series were deposited at the same time and stored under ambient conditions, then all sets of marks were developed using MMD I and compared. Results obtained after 50 hours immersion are illustrated below. It was observed that water immersion could cause some dissolution and redistribution of fingermark constituents, but not sufficient to prevent ridge detail being observed. The conclusion was that MMD was suitable for use on samples that had been wetted.

a) b) c) d)

Strips of cling film exposed to differing degrees of water immersion prior to enhancement with MMD a) *PVC cling film, immersed 50 hours in water,* b) *PVC cling film, kept dry for 50 hours,* c) *PE cling film, immersed 50 hours in water,* d) *PE cling film, kept dry for 50 hours [14].*

8.2.7 The effect of exposing fingermarks to drugs contamination was also assessed. Depletion series from three different donors were deposited on polyethylene-based stretch wrap. After deposition the stretch wrap was wrapped around the drug/cutting agent so that the fingermarks were in direct contact with the contaminant, and left in place for one week prior to development with MMD I. Three surfaces were not exposed to drugs and used as controls. The other six surfaces were exposed to either cocaine (43%,59% or 65%) or common cutting agents, caffeine, benzocaine, or D-mannitol. Cocaine was obtained from samples seized by the UK Border Agency and held at the Home Office for experimentation. Further tests looked at other seized drugs as contaminants, including heroin, MDMA (3,4-Methylenedioxymethamphetamine, commonly known as ecstasy), mephedrone, and cannabis.

8.2.8 MMD I was shown to be capable of developing fingermarks on cling film samples that had been contaminated with cocaine and common cutting agents after deposition, an example being illustrated below.

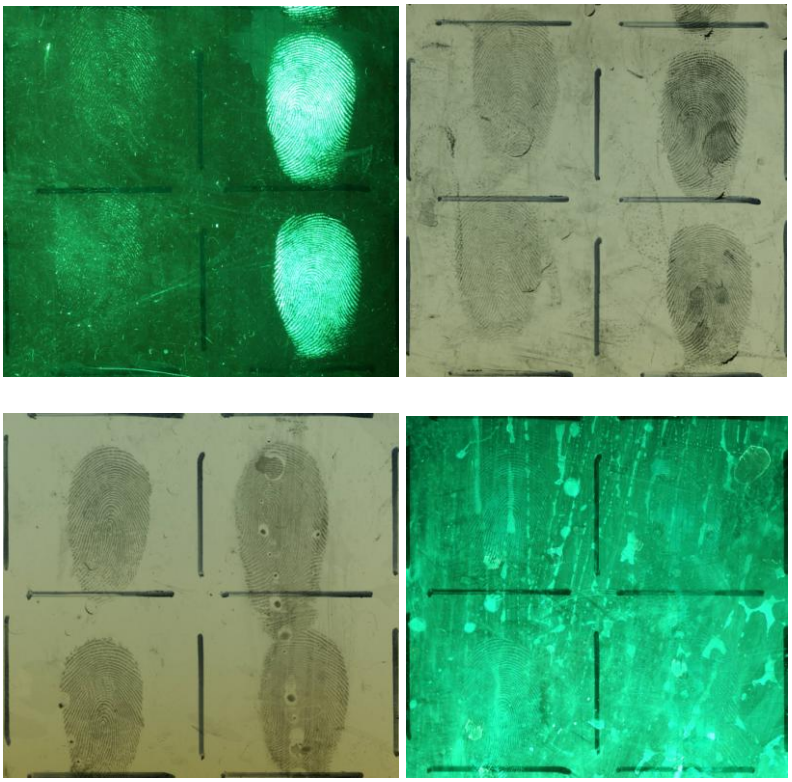


Marks developed on a stretch wrap surface after being wrapped around a sample of cocaine (65%).

8.2.9 With all contaminated samples a higher background was seen compared with the control sample. In some cases development was hindered by the contamination, which was due to either not being water soluble or adherence to the cling film was not entirely removed from the surface by the initial washing stage. The cutting agents appeared to have a greater detrimental effect on development than the cocaine samples. Benzocaine seems to have the greatest effect and caused large amounts of silver to deposit randomly. Cannabis and mephedrone also caused detrimental amounts of background development, but it was concluded that MMD I was still capable of developing marks in the presence of drug contamination.

8.2.10 Charlton [13] also investigated the use of MMD I in sequence with VMD, BPS and superglue to assess whether there could be any benefit in incorporating MMD I in processing sequences. Depletion series from six donors were collected on clear stretch wrap. After one day six of the surfaces were treated by MMD I, dried and photographed, and then treated with BPS, superglue or VMD. The other six surfaces were treated with VMD, BPS or superglue, then dried and photographed before all being treated by MMD I. All surfaces were then dried and photographed again.

8.2.11 It was found that when MMD I was used in sequence before VMD and BPS, there was little benefit in applying the subsequent technique. When MMD I treatment was carried out after BPS, little advantage was seen. When used after VMD, MMD I gave a slight improvement in contrast although there was little effect on ridge detail. If MMD I is used first superglue is detrimentally affected, as shown below. This can be explained by the detrimental effect on superglue of prolonged immersions of the items in water baths.



Sequences of MMD I with superglue/BY40: (top) *superglue/BY40 followed by MMD I,* (bottom) *MMD I followed by superglue/BY40.*

8.2.12 Overall, the results do not show any benefit in using MMD I in sequence with other techniques as no new marks or ridge detail were detected by any of the sequences studied.

8.3 <u>Pseudo-operational trials and operational experience</u>

8.3.1 Charlton (*ibid.*)[13] conducted a small-scale pseudo-operational trial using cling film samples with unknown histories that were collected after normal use as sandwich or food wrappings. After one week a section of each piece of cling film was taped to plastic frames allowing both sides to be treated simultaneously. The cling film was then treated with MMD I, allowed to dry and photographed. These samples were intended to be more operationally realistic with no control over the contaminants present, the way the samples were handled or what cling film was used. Partial fingermarks were developed on many of the

samples. These results confirmed the potential of MMD I for operational use.

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Ninhydrin

1. History

1.1 Ninhydrin was first synthesised by Ruhemann in 1910, and soon after this the development of a purple ('dark blue') reaction product was observed between the new compound and amino acids and proteins [1]. This reaction was further investigated by Abderhalden and Schmidt [2]; they tested a large number of compounds, both singly and in combination, in terms of the reaction products formed with ninhydrin. The purple reaction product was observed to form with proteins and polypeptides. Abderhalden and Schmidt also investigated the reactions with amino acids and different types of body fluid, noting that purple reaction products could be formed with sweat [3], and this could contaminate analyses unless it was ensured that reaction vessels, stirrers, etc. were clean.

1.2 The sensitivity of ninhydrin for proteins and amino acids resulted in its use for detection of amino acids by chromatography techniques and for quantitative measurements of amino acid contents. The first published suggestion that ninhydrin could be used for fingermark detection was made by Oden and von Hofsten in 1954 [4] based on observations of fingermarks accidentally developed on paper items. They proposed a solution of ninhydrin dissolved in acetone and tested it on fingermarks deposited on a range of different types of paper. Oden later patented a refined formulation [5] that also included acetic acid and this soon became adopted worldwide as an alternative to the iodine and silver nitrate techniques then in use for detection of fingermarks on paper.

1.3 In 1969 Crown [6] proposed an alternative ninhydrin formulation based on petroleum ether solvent in place of acetone, with minor additions of methanol. Diethyl ether was also investigated as a solvent, but this was regarded as too volatile for spraying on documents because of the flammable atmospheres created. The reason for using these non-polar solvents was to minimise ink run on the documents being treated with ninhydrin, thus preserving evidence for subsequent document examination. Crown observed that the reaction could be accelerated by heating, but did not recommend temperatures in excess of 100ºC because this caused unwanted background reactions that could obscure prints. Crown also reported improved results when placing bowls of water in treatment ovens to produce more humid atmospheres.

1.4 Lesk [7] reported the use of both acetone and petroleum ether-based formulations in combination, with petroleum ether being used in most cases to minimise ink running. However, it was also observed that occasionally additional marks could be developed by retreatment of the article in the acetone-based formulation.

1.5 In the early 1970s the Police Scientific Development Branch (PSDB) contracted researchers at the Atomic Weapons Research Establishment

(AWRE) at Aldermaston to investigate improvements to chemical reagents then in use for fingermark development. Ninhydrin was investigated as part of this contract. An initial observation was that the formulation developed by Crown [6] could be improved in sensitivity by the addition of acetic acid. In 1971/2 a police officer from the Kent constabulary contacted PSDB and asked whether one of the chlorofluorocarbon (CFC) solvents such as 1,1,2-trifluorotrichloroethane (CFC113) could be used as a safe solvent for ninhydrin. His idea was passed on to the AWRE team and as a result the so-called non-flammable, or new formulation, ninhydrin (NFN) was developed by Morris and Goode [8]. This solvent had the additional benefits that it minimised ink running when used to treat documents [8].

1.6 At about the same time Linde [9] observed that processing exhibits treated with ninhydrin in a high humidity oven at 60ºC gave superior results to dry treatment at 100ºC. At first this was not universally accepted. In comparisons of oven processing and treatment with a steam iron, Morris and Gray [10] noted that oven treatment was superior and specifically stated that the steam setting of the iron should not be used during processing. Despite this advice, and although not approved of by PSDB, a number of police forces, in particular Avon and Somerset, regularly used steam irons to speed up the ninhydrin reaction. The recommended procedure at the time was to put treated articles in a brown envelope and wait for three weeks, and the use of a steam iron gave results in a significantly shorter time. Jones and Pounds [11] reinforced the earlier work of Linde, presenting the beneficial effects of steaming exhibits for 10 to15 seconds prior to heating in an oven at 80ºC for 3 minutes. Subsequent work by PSDB confirmed the importance of humidity for the optimum development of marks and found the optimum to be around 65% relative humidity [12]. PSDB worked with Gallenkamp around 1987 to 1989 to modify one of their production humidity cabinets to provide rapid humidification for the ninhydrin process. The resulting modified humidity oven was trialled on operational work in August 1989 at Essex Police. The trialled showed the humidity oven gave more marks more rapidly than using a dry oven [13]. Subsequently these were installed in all UK police forces.

1.7 Often the contrast between the Ruhmann's purple and the background was poor and ways of increasing this were considered. It had been found that the contrast between the developed ninhydrin mark and the background could be improved by using coloured filters, and green and green/yellow filters to enhance the purple mark were in common use by police photographers in the 1970s. Contrast between the fingermark and the background could also be improved by other means, and Morris found that post-treatment of the purple reaction product with different metal salts resulted in the formation of complexes with different colours, including blue, red, pink and orange [14]. The best results were obtained with the salts of zinc, cadmium and lead.

1.8 It was also found that marks developed using ninhydrin could be enhanced by illuminating the exhibit using light of a wavelength where the Ruhemann's purple product absorbed and the background fluoresced [15]. This was followed by the discovery that some of the coloured reaction products produced by treating purple ninhydrin marks using metal salts were fluorescent [16] and could be revealed using an argon ion laser [16] or an appropriately filtered xenon arc lamp [17]. It was also shown that the intensity of this fluorescence could be increased by cooling the exhibit to low temperatures using liquid nitrogen [17]. Subsequent researchers also investigated a wider range of metal compounds for complexation [18,19] and concluded that zinc and cadmium gave products with the optimum fluorescence. It was also suggested that moisture and elevated temperature during processing were necessary to achieve the optimum fluorescence from the reaction products [20,21]. Rare earth elements were also proposed for metal complexation with ninhydrin, the long fluorescence decay time for toning elements such as Europium offering potential for use with techniques such as time-resolved imaging to reduce background fluorescence [22].

1.9 Researchers also began to synthesise analogues of ninhydrin, either to change the colour of the principal reaction product with amino acids, e.g. benzo(f)ninhydrin [11,23], or to give reaction products that gave greater fluorescence intensity when treated with metal salts, e.g. 5-methoxyninhydrin. Some of these analogues are covered in greater detail in Chapter 3, Chemical and Physical Processes, Ninhydrin analogues, but at the present time (2016) none have displaced ninhydrin in regular operational use.

1.10 The principal driver for further changes to the ninhydrin formulation arose as a consequence of the Montreal Protocols in 1987 banning the use of ozone depleting solvents, including CFCs. Researchers worldwide began investigating alternatives to the non-flammable ninhydrin formulation. In 1992 Jungbluth [24] proposed the use of a mixture of hydrochlorofluorocarbon (HCFC) and hydrochlorocarbon (HCC) solvents as a substitute to CFC113 in both ninhydrin and 1,8-diazafluoren-9-one (DFO) formulations. Lennard and Mazella [25] proposed reverting to a formulation based on petroleum ether with additions of methanol, acetic acid and ethyl acetate and reported that it gave superior performance to the CFC113 formulation. Watling and Smith [26] suggested using heptane as the primary solvent. However, both formulations presented the issue of solvent flammability and ideally a non-flammable formulation with equivalent (or better) performance to the CFC113-based system was required.

1.11 PSDB alerted the UK police forces to the potential issues that would be caused by phasing out CFCs, and began a comprehensive programme to identify replacement solvent systems. PSDB also investigated a range of alternative, solvent-less carrier systems including supercritical carbon dioxide (CO_2) [27]. The extensive CFC solvent replacement programme was conducted over 5 years and evaluated approximately 300

formulations to an initial stage, with two formulations taken through substantial operational trials. This programme originally considered the previously published formulations based on heptane and HCFC solvents, refining these formulations and comparing them with CFC113 [28]. A heptane-based formulation giving good results for fingermark development was produced but was not considered safe to use because of the flammable atmospheres generated around articles that were apparently dry, and the large quantities of solvent that would need to be evaporated from ninhydrin-treated articles. Adoption of the heptane-based formulation would have required specially adapted humidity ovens and laboratories for safe working, and this was considered impractical primarily due to the very high cost of either adapting or building new laboratories and manufacturing blast proof ovens. HCFC-based formulations were not considered further because of health and environmental questions and they also caused excessive ink running.

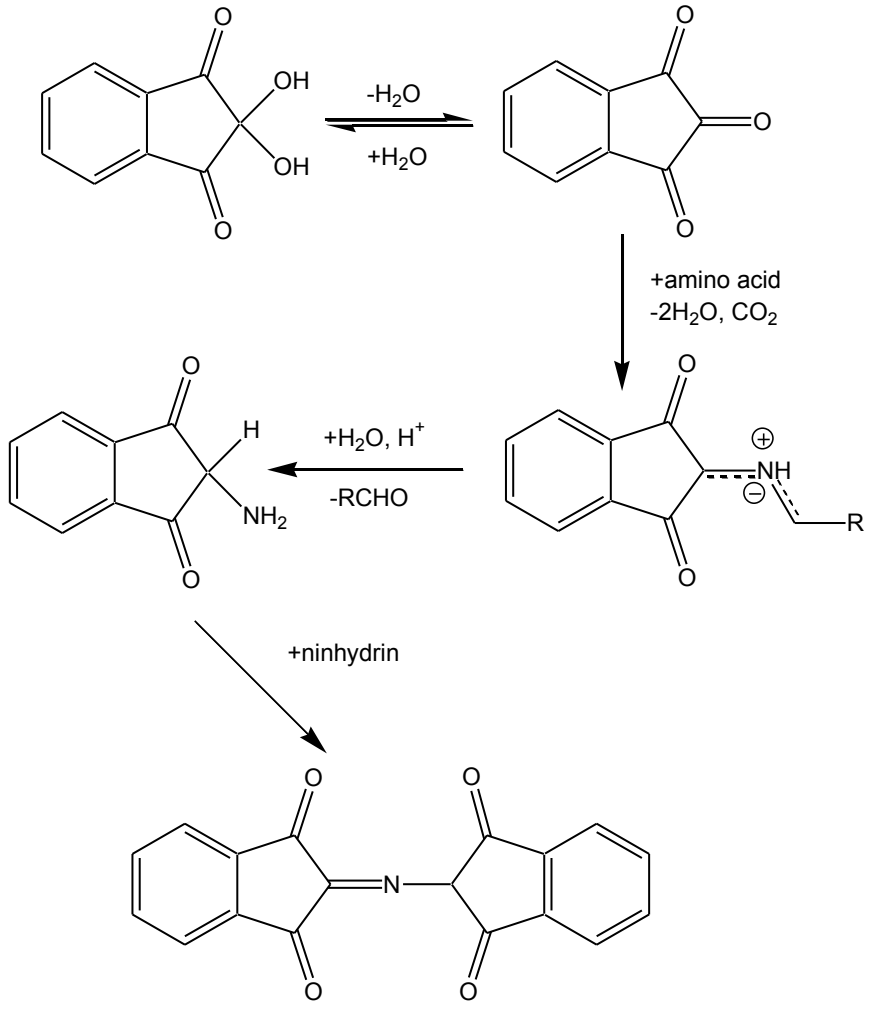
1.12 The next classes of solvents investigated were hydrofluorocarbons (HFCs) and hydrofluoroethers (HFEs) and it was found that excellent results could be obtained from formulations based on two solvents, 2,3-dihydrodecafluoropentane (HFC4310mee) and 1-methoxynonafluorobutane (HFE7100) [29]. These out-performed the CFC113-based formulation in laboratory trials and therefore the evaluation proceeded to a full operational trial of all three formulations [30]. The results of this two-month study indicated that the HFE7100-based formulation gave the best results overall and this was recommended for operational use in the UK. Petruncio [31] independently reported results of a comparative study between HFC4310mee-, HFE7100- and petroleum ether-based formulations and found the HFC- and HFE-based systems gave better results in terms of the number of marks developed and reductions in ink run damage caused to treated documents.

1.13 As a consequence of this development work, the HFE7100-based formulation is currently (2016) the only one recommended for operational use by CAST. However work to investigate possible alternative solvents and other methods of application continues because of on-going concerns over fluorinated greenhouse gases which include the HFEs and HFCs.

2. Theory

2.1 Many comprehensive studies of the reaction mechanisms, colour formation and kinetics of reaction have been carried out and published for the formation of Ruhemann's purple by reactions of ninhydrin with amino acids. These include the studies by McCaldin in 1960 [32]; Friedman and Sigel in 1966; Friedman and Williams (1974 [33,34]; Yuferov in 1971; [35] and most recently by Joullie *et al*.in 1991 [36]. Some of these papers propose detailed reaction mechanisms for ninhydrin with individual amino acids under different conditions, and seek

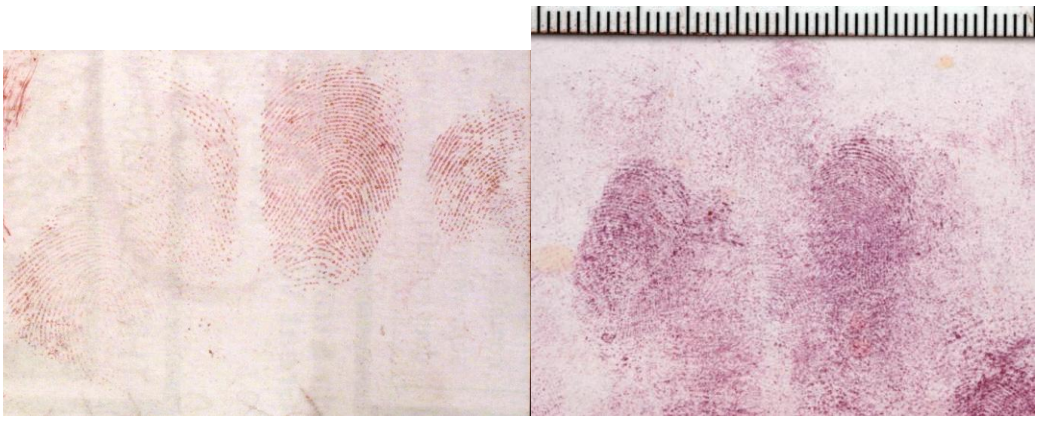
to identify all intermediate forms that arise during the reaction. The reaction mechanism outlined below is typical of the generally accepted reaction pathway between ninhydrin and amino acids. For amino acids it is the amine group that ninhydrin is reacting with to form Ruhemann's purple, whereas the anomalous reactions that occur with other compounds do not proceed all the way to the formation of the purple product.



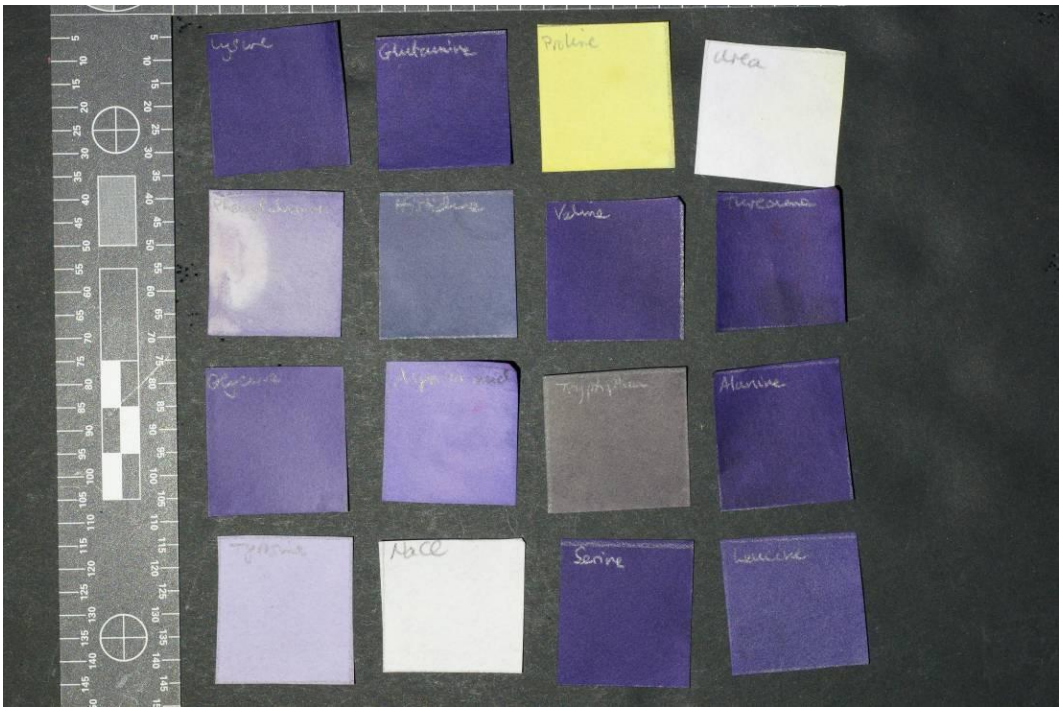
Generally accepted reaction pathway between ninhydrin and amino acids to form Ruhemann's purple.

2.2 The reaction products formed between ninhydrin and different amino acids are not all purple and the colour of the developed fingermark can vary from nearly red to deep violet, depending on the composition of the fingermark. Some examples are shown below. Another contributing factor to this difference in colour may be that the reaction above may not have proceeded to completion. There is a coloured intermediate (an imine or an aldimine) in the full ninhydrin reaction scheme that is also

coloured, and the reaction may stop at this point if the acidity (pH) is not high enough. A pH of less than five is required for the reaction to proceed past the intermediate product, although if the pH is less than two the reaction proceeds to formation of the colourless hydrindantin product instead of to Ruhemann's purple. The colour of the intermediate imine compound is dependent on the R groups attached to the active species.

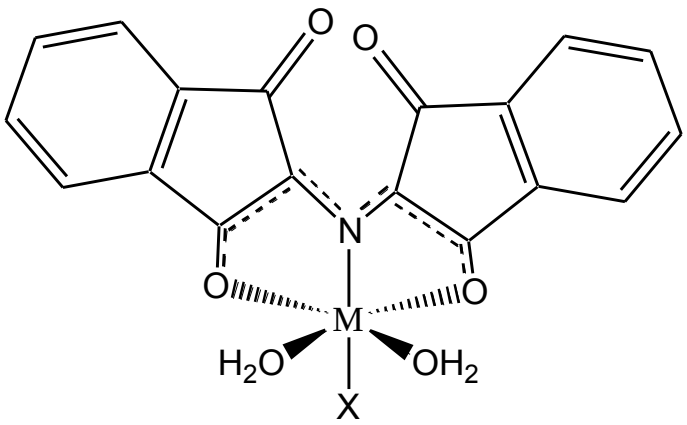


Fingermarks of differing colours developed by treatment using ninhydrin.



Reaction products formed between ninhydrin and 0.1M solutions of amino acids and other fingermark constituents.

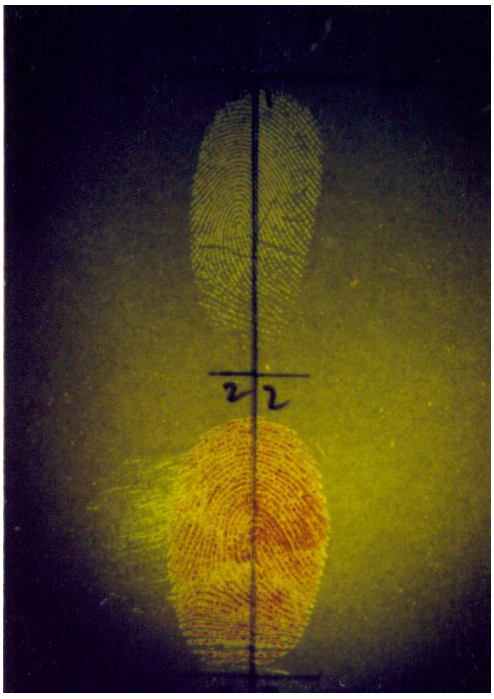
2.3 Studies have also shown that the subsequent complexation reaction with metal salts gives a complex of the generic structure below.



Complex formed between metal salt and Ruhemann's purple, in some cases giving rise to colour changes and fluorescence.

2.4 In the case of zinc and cadmium, the complexes formed are fluorescent in nature. The pictures below illustrate the visible appearance of marks treated with zinc chloride toning solution and the same marks when viewed using fluorescence examination. Studies by Australian researchers [21] identified two different coloured zinc/Ruhemann's purple complexes, one appearing orange and the other magenta/pink. The magenta/pink complex was found to be more fluorescent than the orange one, the differences being attributed to the amount of water bound into the complex. The importance of water in the formation of the more fluorescent complex makes humidification an important stage in the toning process.

a)



b)

Ninhydrin marks toned with zinc chloride solution a) *viewed under room lighting, and* b) *viewed using fluorescence examination.*

2.5 It should also be noted that ninhydrin does not only react with amino acids. A wide range of coloured reaction products can also be obtained from different amine-containing substances. Dent [37] carried out an extensive study in 1947 of 60 different compounds that reacted with ninhydrin, recording both colour of the reaction product and their natural occurrence. Although these substances react with ninhydrin the reactions cannot proceed to the Ruhemann's purple product because they do not have the structure to react beyond the coloured intermediate compounds. Cashman *et al.* [38] and Dutt and Poh [39] also report the use of ninhydrin for the detection of phenethylamines and other basic drugs, and some of these substances or their metabolites may occur in fingermark residues. As a consequence, the reaction mechanism given above may not be the only one operating and ninhydrin may detect additional fingermarks that do not contain amino acids.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process(es) used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The Category A ninhydrin process currently (2016) recommended by CAST in the *Fingermark Visualisation Manual* [45] involves the initial preparation of a concentrated solution, followed by the preparation of a working solution which should last at least one year.

3.2 The concentrated solution is produced by weighing 25 g of ninhydrin and stirring 225 mL of absolute ethanol into it to form a slurry. To this should be added 10 mL of ethyl acetate and 25 mL of acetic acid to form a clear yellow concentrated solution, which should be stored in a cupboard.

3.3 To produce the working solution, 52 mL of concentrated solution should be measured out and 1 L of HFE7100 added to it. This solution is then poured into a shallow tray and exhibits either pulled through it with forceps or immersed for a maximum of five seconds. The working solution only has a limited stability in air before precipitation of ninhydrin occurs. Treated articles are then allowed to dry on a sheet of cardboard before being placed into a humidity-controlled oven at 80 $\pm$ 2ºC and 62 $\pm$ 5 % relative humidity for a time that will depend on the recovery time of the oven to 76^{o}C and 58 %RH and will typically be between four and seven minutes. Developed fingermarks can be photographed immediately but further marks will continue for up to 2 weeks (although additional marks have still been observed to develop after 13 weeks in some cases) during which exhibits should ideally be kept in the dark. The time marks take to develop is dependent on the surface and may be

related to the pH because more acidic papers, such as cheques, generally develop more marks.

3.4 The role of the constituents in the CAST formulation can be identified as follows.

3.5 Ninhydrin is the principal active component and reveals fingermarks by means of the (primarily) purple product formed in its reactions with amino acids and proteins. It has limited solubility in the main carrier solvent and is present in as high a concentration as possible without making the working solution rapidly unstable.

3.6 Ethanol is required to ensure solubility of ninhydrin in the carrier solvent.

3.7 Ethyl acetate is added as a co-solvent to inhibit the esterification reaction by shifting its equilibrium towards formation of ethanol and acetic acid, thus preventing water droplet production during processing, which may diffuse fingermark ridges.

3.8 Acetic acid and a tiny amount of water are required to catalyse the reaction of ninhydrin with amino acids, the water being supplied in a controlled manner by the humidity oven. The acetic acid content is kept as low as possible to minimise any ink diffusion on documents being treated, but there is also a balance to be achieved in having sufficient acid present to ensure the reaction proceeds to the formation of Ruhemann's purple. This is of particular relevance for alkaline paper types, such as magazine pages, which have high filler contents and may remove the hydrogen ions provided by the acetic acid [28].

3.9 HFE7100 is the main carrier solvent for ninhydrin and meets the criteria of being non-toxic, non-flammable and causing minimal damage to documentary evidence. It is, however, expensive and the use of specially designed shallow dipping trays is recommended to minimise the volumes of solution required.

3.10 Heating accelerates the reaction and the development of fingermarks, but temperatures much in excess of 100ºC may cause unwanted background reactions as may exposure to 80^{o}C when simultaneously exposed to high levels of humidity.

3.11 PSDB carried out studies into the effect of humidity on processing in the late 1980s/early 1990s [12] which indicated that settings producing 65% relative humidity in the treatment areas of the oven gave the best results. These results are summarised in section 8 below. 62 %RH is recommended in practice to reduce over-humidification during recovery of the humidity oven to set values after items have been loaded, as exposure to more than 80 %RH for more than a few seconds at 80^{o}C can cause unwanted background reactions.

3.12 Ninhydrin enhancement by zinc toning is currently included in the *Fingermark Visualisation Manual* [45] as a Category D process, primarily intended for use in circumstances where corrective action is required. In cases where ninhydrin marks require enhancement by converting them to a fluorescent product, better results could generally have been obtained by using DFO in the first place. However, it is recognised that in situations where additional marks have been developed using ninhydrin after DFO, further marks may be made visible by ninhydrin enhancement using zinc toning. If toning is to be carried out after development of marks, CAST recommends the use of a zinc chloride-based toning solution, produced by mixing 50 mL of ethanol, 10 mL of propan-2-ol, 10 mL of acetic acid and then stirring in 6 g of zinc chloride. To this is added 200 mL of HFE7100 (used as a direct replacement for the CFC113 in the original formulation), stirring to produce a clear solution. This solution is then sprayed very lightly over the marks (with care not to over-spray as this will diffuse and possibly wash away marks) and they are retreated in the humidity oven at 80ºC and 62% relative humidity.

Zinc chloride solution being applied to an exhibit.

3.13 PSDB also carried out studies into the effectiveness of zinc toning [40] and confirmed the observation that humidity was required to accelerate the complexation reaction with the metal salt [21]. The approximate times for the formation of the orange complex are given below.

Relative humidity	42	47	56	57	60	78	83
Development time	> 1 hr	> 1 hr	< 5 mins	< 5 mins	< 5 mins	< 1 min	< 1 min

Approximate development times for different treatment temperatures.

3.14 Zinc chloride is preferred over cadmium salts for producing fluorescent marks because of the toxicity issues associated with the use of cadmium.

3.15 The ThermaNin process is also included as a Category C process in the *Fingermark Visualisation Manual* [45] for the niche application of developing fingermarks on thermal papers where it is considered necessary to retain printed text. This process is described in greater detail in section 6.2 of this chapter.

4. Critical issues

4.1 In common with many of the chemical development processes there are several important issues to consider when using ninhydrin.

4.2 The reaction will proceed under conditions of room temperature and humidity, but will be considerably accelerated and enhanced by the use of elevated temperature (80°C) and humidity (around 65%). Treatment at temperatures over 100°C, or 80°C when simultaneously exposed to humidity, may cause excessive background development. Design of the humidity oven is critical. Since the exposure time required is relatively brief, 4 to 7 minutes, it is essential that the humidification system provides a substantial quantity of water vapour immediately after the door is closed otherwise the true exposure time will be unknown. Many humidity cabinet designs are constructed for long term environmental testing and do not have this required rapid humidity and temperature recovery time.

4.3 Marks developed using ninhydrin may fade and should be imaged as soon as possible after development. Conversely, marks may continue to develop on items treated using ninhydrin for several days afterwards, and items should be re-examined after two weeks.

4.4 Cloudy solutions or solutions that have separated, having oily droplets on the surface, should not be used to treat articles and must be discarded.

4.5 Ninhydrin will not develop marks on articles known to have been wetted because the amino acids targeted by the reagent will have been washed away.

5. Application

5.1 Suitable surfaces: Ninhydrin is suitable for use on all porous surfaces including paper, cardboard, raw wood and matt painted walls.

5.2 Ninhydrin is the most widely used process around the world for the development of fingermarks on porous surfaces. This is not because it is

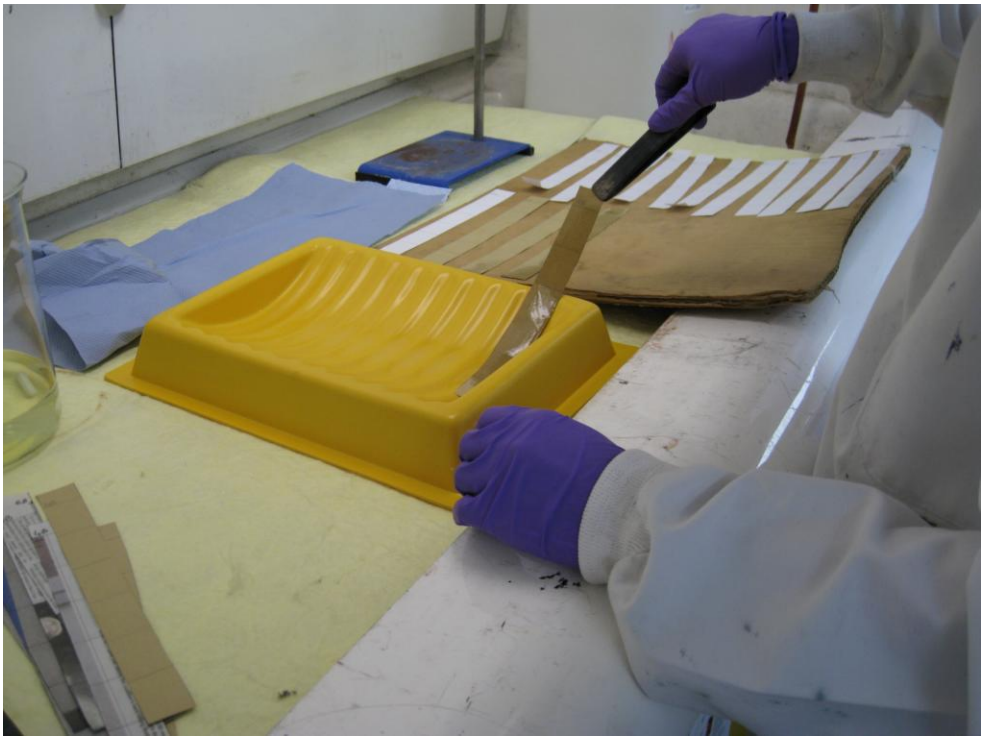
the most effective process – DFO and 1,2 indandione will develop higher numbers of marks overall [41,42]. Ninhydrin is so widely used is because it develops visible marks that can be quickly and easily captured using non-specialist equipment (e.g. cameras, scanners, photocopiers). It is thus well suited to applications in volume crime, where it is necessary to process large numbers of exhibits rapidly and it is considered that DFO treatment and subsequent fluorescence examination is too time-consuming. However, caution should be exercised if ninhydrin is to be used in this way because a) it is less effective than other processes and b) marks continue to develop up to two weeks after treatment. Potentially identifiable marks will missed for these reasons if ninhydrin is used as the sole process.

5.3 Ninhydrin is best suited to be used as part of a sequential processing regime for porous exhibits. Although it is not as effective as DFO, it will regularly develop additional marks if used sequentially after it because the DFO reaction with amino acids does not proceed to completion and some residue will be left to react with ninhydrin. In addition, ninhydrin may react with some non-amino acid compounds that may be present in fingermarks, which are not targeted by DFO. Use of ninhydrin does not preclude subsequent treatment of the exhibit with physical developer.

5.4 Ninhydrin will also react with proteins and can be used for the enhancement of marks in blood on porous surfaces. It will not be possible to determine whether a mark is actually in blood by this method alone, but ninhydrin can be used as a sensitive enhancement reagent if blood is known to be present. The application of ninhydrin for the enhancement of marks in blood has not been found to be detrimental to the subsequent recovery of DNA [43,44].

5.5 If ninhydrin has been applied, it is not possible to go back and retreat an exhibit using DFO so if a mark has been developed on a surface where it may subsequently benefit by converting the mark to a fluorescent product the zinc toning process can be applied. Examples where this may be relevant are paper banknotes treated by ninhydrin, where parts of the developed mark are obscured by patterned backgrounds. However, previous work by CAST [40] indicates that zinc toning is only truly effective on predominantly white backgrounds that do not fluoresce. Cooling the marks to liquid nitrogen temperatures was sometimes also required to optimise the fluorescence viewed.

5.6 Ninhydrin is a versatile process and can be applied both in a laboratory and at scenes of crime. In a laboratory thin paper exhibits can be drawn through a shallow tray and allowed to dry before processing in a humidity-controlled oven. A recommended specification for a humidity oven suitable for developing marks on articles treated with ninhydrin is given in the *Fingermark Visualisation Manual* [45].



Use of a shallow dipping tray for treatment of paper items with ninhydrin.

5.7 Small paper items should be placed into the oven and treated on sheets of cardboard. This minimises the time taken to load the oven and also avoids direct contact with any condensation that may have formed on the shelves. Treatment time for exhibits will vary according to the time taken for the oven to recover the temperature and humidity levels once the door is opened to insert exhibits and then closed. This can be recorded for a particular oven, and the treatment time used will be the recovery time plus two minutes. This typically results in a treatment time of between four and seven minutes. It is recommended that the oven parameters are regularly (annually) checked to ensure that the temperature and humidity values are being displayed accurately, and, where applicable, that the wick in the oven is checked on a daily basis when the oven is required for use to ensure that it is moist.

5.8 For larger articles that can be fitted into the humidity oven but cannot be drawn through the dip bath, the ninhydrin solution can be applied with a soft brush and the exhibit allowed to dry before treating it in the oven. If articles are particularly dense (e.g. thick card, wood or plasterboard), they should be heated before being placed in the humidity oven to ensure that the entire exhibit reaches the required reaction temperature and to prevent a thin layer of condensation forming on the surface. The formation of such a layer may have the detrimental effect of diffusing the amino acids in the latent fingermarks. Corrugated cardboard is generally not dense enough to require this unless the card between the corrugations is particularly thick. A pre-heating stage in a dry oven at 80°C is recommended for a period long enough to bring the surface of

the article up to 80°C. This generally should not exceed 1 hour as this should be long enough for the vast majority of articles.

5.9 Ninhydrin solution can be used at scenes, again using a soft brush to apply it to the surface being treated. The marks produced in this way may require time (up to two weeks) to develop. Development rate can be increased by raising the temperature in the room and increasing humidity if possible without allowing any condensation on surfaces. Ninhydrin should never be spray applied at scenes; spray application is less effective and the solvent, although not toxic or flammable, may rapidly displace breathable air in the scene if used in this way.

5.10 Ninhydrin solution will keep for 12 months if stored at room temperature, although any solution appearing cloudy or having a separate 'oily' layer or droplets should be discarded. Precipitation of ninhydrin from the working solution will occur with time after exposure to air. This is attributed to the fact that as the HFE7100 evaporates it lowers the temperature of the solution to a point where ninhydrin precipitates. As a consequence, the solution should only be poured out immediately before treating the articles and limit the amount of solution to that required immediately. The ninhydrin working solution should be discarded after use.

5.11 Articles to be treated with ninhydrin should not be stored in high humidity environments (e.g. in non-porous bags with other damp articles) as this will cause diffusion of amino acids. After treatment articles should be kept in the dark because developed marks may fade on exposure to light. For this reason developed marks should be photographed as soon as possible after treatment, but because additional marks may continue to develop the article should be re-examined after two weeks.

6. Alternative formulations and processes

6.1 Formulations for standard papers

6.1.1 Many ninhydrin formulations have been proposed since its first reported use for fingermark development in 1954. The formulation first used by Oden and von Hofsten [4] in1954 consisted of a 0.4% solution of ninhydrin in acetone. Oden [5] patented a revised formulation consisting of 0.2% ninhydrin and 4% acetic acid in acetone or diethyl ether. These formulations would not be recommended by CAST because of the ink running that would potentially be caused by the solvents, combined with their high flammability.

6.1.2 The formulation proposed by Crown in 1969 [6] consisted of 7.5 g ninhydrin in 40 mL methanol, then the addition of 960 mL of petroleum ether. The main purpose of this formulation was to reduce the damage caused to documents by the acetone solvent. The formulation satisfied these criteria, but again is based on highly flammable solvents and would

not be recommended by CAST. In addition the toxicity of methanol would make this formulation unsuitable and the absence of acidity in the formulation would reduce effectiveness.

6.1.3 The non-flammable ninhydrin (NFN) formulation developed by AWRE under contract to PSDB in the early 1970s consisted of 25 g ninhydrin, 50 mL acetic acid, and 100 mL ethanol mixed to form a stock solution. Subsequently, 30 mL of stock solution was added to 1 L of CFC113 to give a working solution. This equates to 5 g ninhydrin, 10 mL acetic acid, 20 mL ethanol, and 1 L CFC113 in the working solution. This was the formulation published in the first edition of the *Manual of Fingerprint Development Techniques* [46] and continued to be recommended until 2002, when it was replaced by the HFE7100 formulation. It could not now be used because the use of CFC113 was banned under the Montreal Protocol for substances that deplete the ozone layer and is no longer manufactured for commercial applications.

6.1.4 Studies by PSDB indicated that supercritical CO_2 could be used as a solvent for ninhydrin [27], with the advantage that the solvent caused minimal damage to the document being treated. However, specially built reactors were required to produce the supercritical CO_2 and it was considered unlikely that these would prove cost effective for the processing of large numbers of exhibits.

6.1.5 Another formulation that is in operational use is the formulation based on petroleum ether solvent proposed by Lennard and Mazella [25]. This is formulated by dissolving 4 g of ninhydrin in 20 mL methanol, adding 10 mL of acetic acid and 70 mL of ethyl acetate, then adding 900 mL of petroleum ether. CAST would not recommend this formulation because of the high flammability of the solvent, but other researchers have indicated that similar ninhydrin formulations based on petroleum ether also give inferior results in terms of fingermarks developed and detrimentally affect the documents being treated.

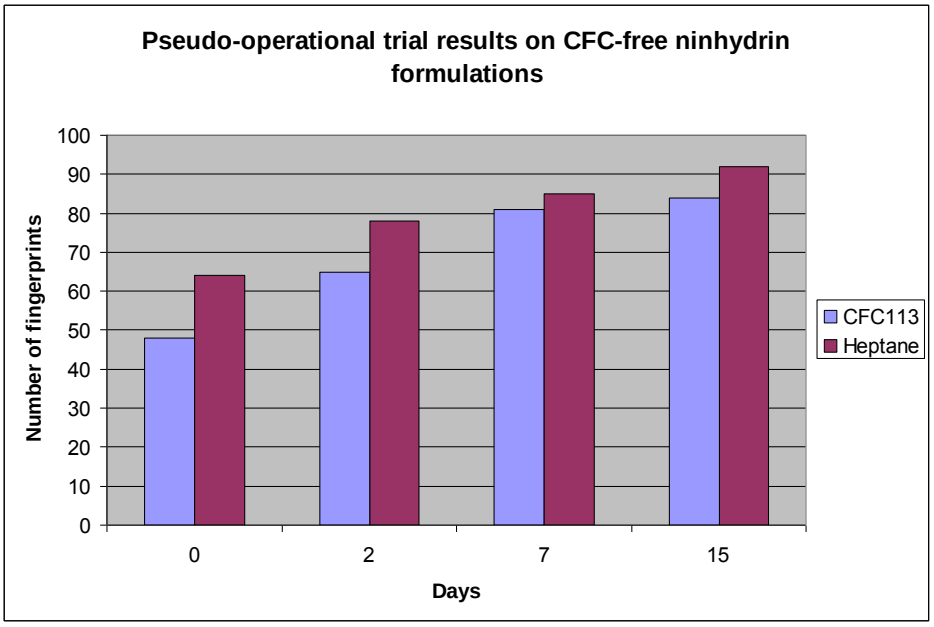
6.1.6 In the comparative study carried out by Petruncio [31], a ninhydrin formulation consisting of 5 g ninhydrin, 20 mL methanol, 10 mL acetic acid and 1 L petroleum ether was trialled against the PSDB formulations based on HFE7100 and HFC4310mee. The study compared ink run and contrast and clarity of latent prints, and produced the results below.

	HFE7100 better	**Equal**	**Pet. ether better**
Latent print quality	47.8%	45.6%	6.7%
Ink run	33.3%	66.7%	0%

	HFC4310 better	**Equal**	**Pet. ether better**
Latent print quality	48.9%	45.6%	5.6%
Ink run	41.7%	58.3%	0%

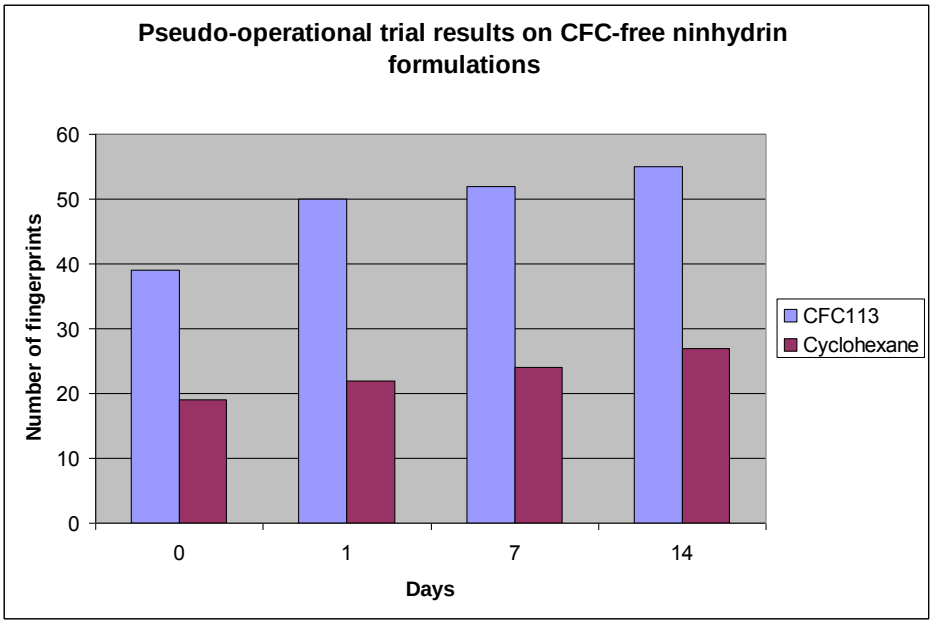
Comparison of the effectiveness of HFE, HFC and petroleum ether-based ninhydrin formulations.

6.1.7 Other comparative trials conducted by PSDB in the search for a CFC113 replacement used the heptane-based formulation proposed by Watling [26] as a starting point for an optimised heptane system [28]. This comprised 5 g ninhydrin, 75 mL ethanol, 25 mL ethyl acetate, 3 mL acetic acid and 1 L heptane, and performed well against the CFC113 formulation. However, at the time it was not recommended by PSDB because of its high flammability.



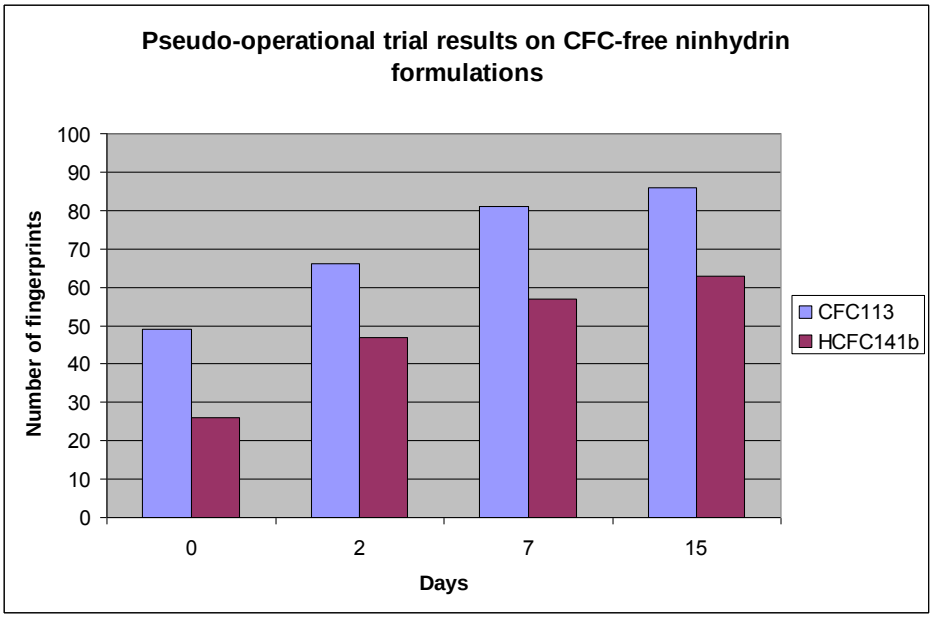
Comparative test results on batches of 75 cheques for different ninhydrin formulations.

6.1.8 A cyclohexane-based solution was also developed, containing 5 g ninhydrin, 20 mL ethanol, 10 mL propan-2-ol, 10 mL acetic acid and 1 L cyclohexane. In trials, this was significantly less effective than the CFC113 formulation and was not recommended.



Comparative test results on batches of 75 cheques for different ninhydrin formulations.

6.1.9 The final class of solvents assessed in the initial phase of solvent replacement studies were the HCFCs [28] and the following formulation was identified for trial: 5 g ninhydrin, 15 mL ethanol, 5 mL ethyl acetate, 10 mL acetic acid, 1 L of HCFC141b. In comparative trials this did not perform as well as the CFC113 system, caused more ink running and there were concerns at the time that HCFCs would also ultimately be banned (which has subsequently come to pass). As a result, the formulation was not pursued further.



Comparative test results on batches of 75 cheques for different ninhydrin formulations.

6.1.10 Although effective, the cost of the HFE7100 solvent makes the volume use of ninhydrin expensive and if cheaper, similarly effective, alternatives were to be identified this would assist police forces in cost savings. CAST has recently been assisting in research conducted by police laboratory staff to evaluate novel, cheaper solvent systems and further work is anticipated. The system showing most promise to date is Asahiklin AE-3000, produced by the Asahi Glass Company in Japan [47]. This was originally projected to be priced around 30% less than HFE7100, but more recent estimates by the supplier indicate that there will be little, if any, cost savings. AE-3000 along with HFE7100 are fluorinated greenhouse gases and may soon be subjected to the restrictions on use that have already been imposed on the HFCs such as HFC4310mee (Vertrel). HFCs are seeing a year on year reduction of the quantity being placed on the market and will be phased out by 2030.

6.2 Formulations for thermal papers

6.2.1 A modified formulation has been proposed by CAST for the treatment of thermal receipts [48]. When thermal receipts are treated with ninhydrin they blacken due to reaction between acetic acid and the thermal ink layer. Blackening also occurs due to the heat applied to the exhibit in the oven used to develop marks. To counteract this, CAST carried out trials and devised a formulation with an additional 45 mL of ethanol added per litre. This dissolves away the thermal ink layer and significantly reduces subsequent blackening. The thermal paper is retained in the dip bath until all the black deposit is removed from the surface of the paper, then placed into the oven. In practice, this did reduce the problems associated with blackening of thermal receipts but as ink compositions changed it did not prove possible to remove all of the ink layer easily in this way. Pre-dipping the receipt in ethanol until all the text is removed and then allowing it to dry prior to dipping in a solution of the standard formulation has proved more effective [49]. A study carried out at HOSDB in 2011 showed that a pre-wash of acetone be used to remove the thermal before treating with DFO or ninhydrin [50].

6.2.2 Two other ninhydrin formulations have been proposed for the development of fingermarks on thermal papers. In the 'Nin-Dry' process proposed by McMahon [51], 30 to 50 g of ninhydrin is dissolved in 1.5 L of acetone and this solution is used to impregnate sheets of paper by soaking the paper and then letting it dry in a vented fume cupboard. The document to be treated is placed between two impregnated sheets of paper in a sealed plastic bag and left for three to seven days. If faster development is required, the sandwich of document and impregnated paper sheets can be covered in a moist towel and an iron used to apply gentle heat and humidity.

6.2.3 The final process is the use of hemiketals of ninhydrin, generally used as a means of developing fingermarks on thermal paper without darkening the background. The use of isononyl ninhydrin was first proposed by Japanese researchers [52] and further investigated and

adopted for operational use by the German Bundeskriminalamt. A similar compound is the commercially available 'ThermaNin' product marketed by BVDA, which consists of a hemiketal of ninhydrin with the water molecule exchanged for an alcohol. On contact with the water present in paper (or in the atmosphere) ThermaNin converts to ninhydrin and the original alcohol molecule. The combination of alcohol and the ninhydrin then becomes available for reaction with the fingermark residues. Other constituents of the formulation have the purpose of preventing darkening of the thermal paper background. The working solution suggested by BVDA consists of 4–5 g ThermaNin, 5 mL propan-2-ol, 15 mL ethyl acetate and 980 mL of HFE7100 (petroleum ether or heptane may be used as alternatives). Fingermarks are developed by dipping the exhibit in the solution and leaving the exhibit overnight at elevated humidity (~80% relative humidity), at room temperature in the dark. Thermal papers treated in this way retain all printed text while developing the characteristic purple fingermarks. CAST has initiated a comparative trial between ThermaNin and other techniques capable of developing fingermarks and leaving printed text intact [53]. The results of this exercise are summarised in Chapter 3, Chemical and Physical Processes, 4-Dimethylaminocinnamaldehyde (DMAC), but ThermaNin performed well, giving results closely equivalent to physical developer. No direct comparison has been conducted between ThermaNin and the standard ninhydrin formulation.

7. Post-treatments

7.1 Post-treatments for ninhydrin can be divided into two main categories: optical techniques that increase the contrast between the ninhydrin mark; and the background and chemical treatments that change the colour and/or fluorescence properties of the mark.

7.2 Marks developed using ninhydrin are non-fluorescent over broad regions of the visible spectrum, and this can be used to make the marks appear dark against a light background where appropriate light sources are used to produce background fluorescence [15].

7.3 The spectral reflectance curve of ninhydrin exhibits two minima in the region of 410 nm and 535 nm, which may be utilised to enhance the contrast of the mark. By either illuminating the marks with monochromatic light of these wavelengths [54] or using narrow bandpass filters passing these wavelengths in front of the imaging system significantly enhanced the contrast of the ridges that can be obtained. A green (~535 nm) bandpass camera filter is most commonly used for the capture of marks developed using ninhydrin. Alternatively, modern digital imaging systems and processing tools allow digital filtering of the red, blue and green channels to achieve a similar end product.

7.4 The use of metal salt spray treatments to form metal complexes with Ruhemann's purple has been described in sections 2.3, 2.4, 3.12 and

3.13. Originally this was investigated as a means of producing a colour change in the mark although in most cases the colour change was only slight, typically from purple to red, orange or pink, and insufficient to significantly enhance the mark. However, the observation that some of these complexes are also fluorescent has proved more useful as a post-treatment, with the complexes produced using zinc and cadmium giving the most intense fluorescence. For safe, practical purposes, zinc toning is the only chemical post-treatment for ninhydrin recommended by CAST. Cooling of the exhibit enhances the intensity of fluorescence produced and ideally zinc toning should be combined with fluorescence examination with the exhibit cooled to liquid nitrogen temperatures. Fluorescence examination should be carried out using an excitation around the 470 to 525 nm range with an appropriate safe long-pass viewing filter. A 488 nm laser may also be used.

8. Validation and operational experience

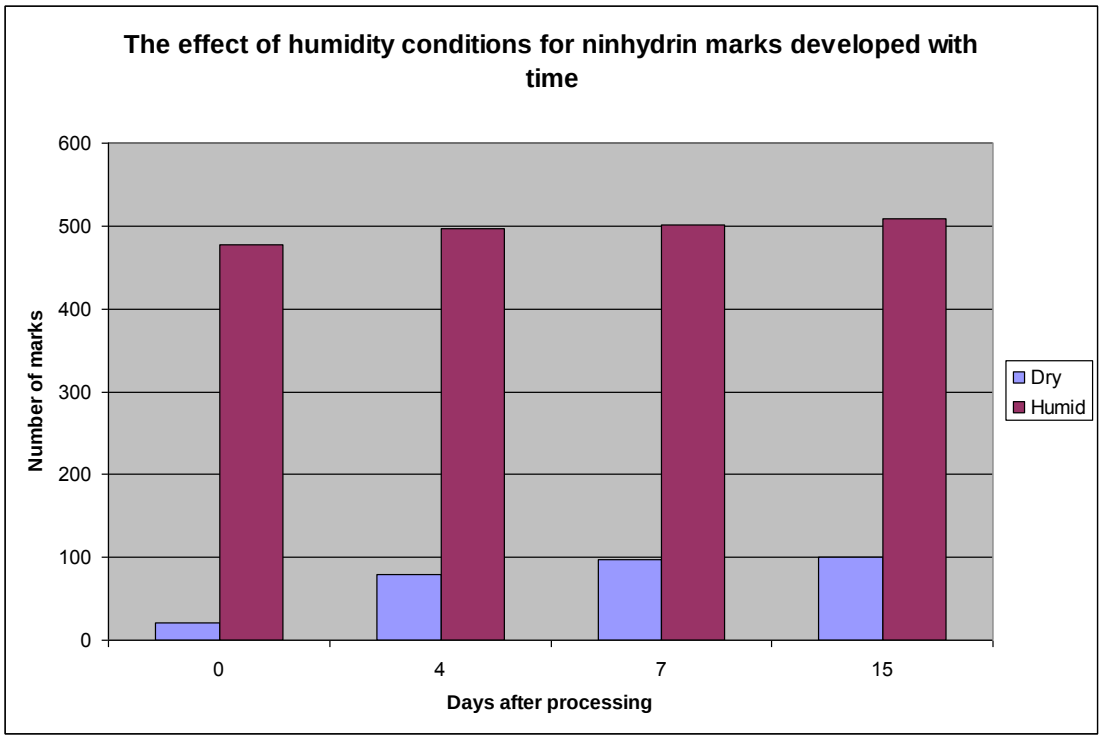
8.1 Laboratory trials

8.1.1 Although laboratory trials were conducted during the initial development of ninhydrin formulations in the mid-1970s, these results are no longer available. It has been found from experience that planted prints rarely give operationally representative results in such trials, typically performing worse than seen on casework [55]. This is possibly because perpetrators of crimes may be under increased stress and sweat more, giving more eccrine prints than those deposited in the laboratory. As a consequence, development of revised formulations at CAST is usually carried out using small-scale comparative tests until best performing formulations are identified, after which testing proceeds to pseudo-operational trials using realistic items such as bundles of cheques, as can be seen in many of the results reported in this section.

8.1.2 A recent exception to this is precursor work carried out to evaluate possible alternative solvents to HFE7100, which carried out tests on split depletion series deposited on a range of different paper substrates [56]. The two solvents investigated in this study were Asahiklin AE-3000 (1,1,2,2-tetrafluoroethyl-2[2,2-trifluoroethyl ether]) and Lenium (75% 1,1,1,3,3-Pentafluorobutane + 25% 1,1,1,2,2,3,4,5,5,5-Decafluoropentane). The results showed no significant difference between the performance of the three solvents when used for fingermark development, although the Lenium solvent did cause more ink running on treated documents. Lenium ultimately became unavailable and subsequent pseudo-operational trials focused on the AE-3000 solvent [47].

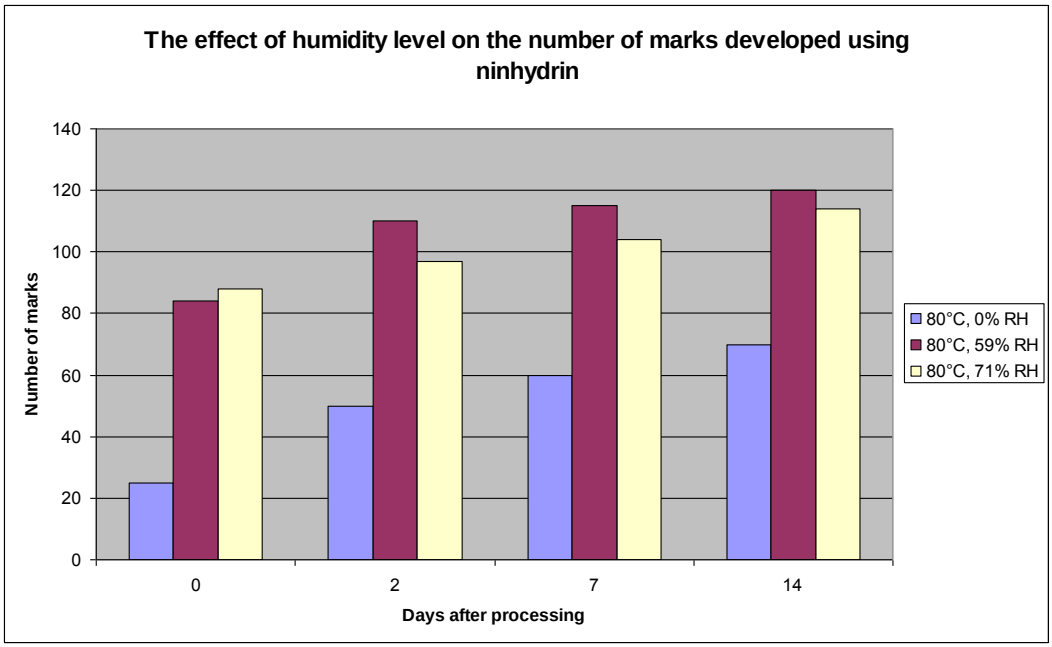
8.2 Pseudo-operational trials and operational experience

8.2.1 An important element in optimising the ninhydrin process was to establish the role of humidity in fingermark development. Work to investigate this was conducted by SRDB, later PSDB in the late-1980s/early-1990s [12]. Initial trials carried out by counting fingermarks developed on 250 cheques representing 77 separate cases clearly demonstrated that humid processing conditions produced up to 5 times more marks, and that these marks developed more quickly.



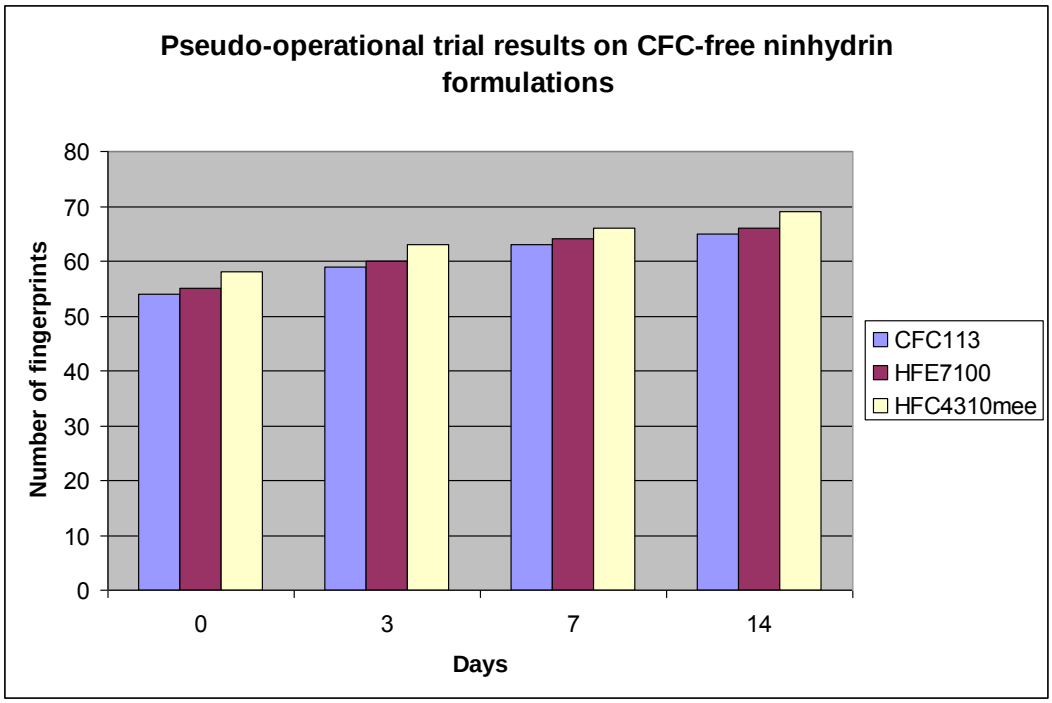
Results of trials carried out on cheques to establish the effect of humidifying exhibits treated with ninhydrin during processing [12].

8.2.2 Later trials in 1992 refined the humidity conditions required and tests on batches of 100 cheques, 25 from each of 4 banks, indicated that an oven humidity setting of 59% relative humidity gave the best results. This setting actually equates to a higher humidity (around 65%) in the region where the exhibits are treated, but means that the oven should be set to 59% relative humidity to achieve optimum development, which avoids issues associated with 'overshoot' in the humidification system. This is described in the *Fingermark Visualisation Manual* [45].



Results of trial to refine the optimum humidity level required for development of marks using ninhydrin

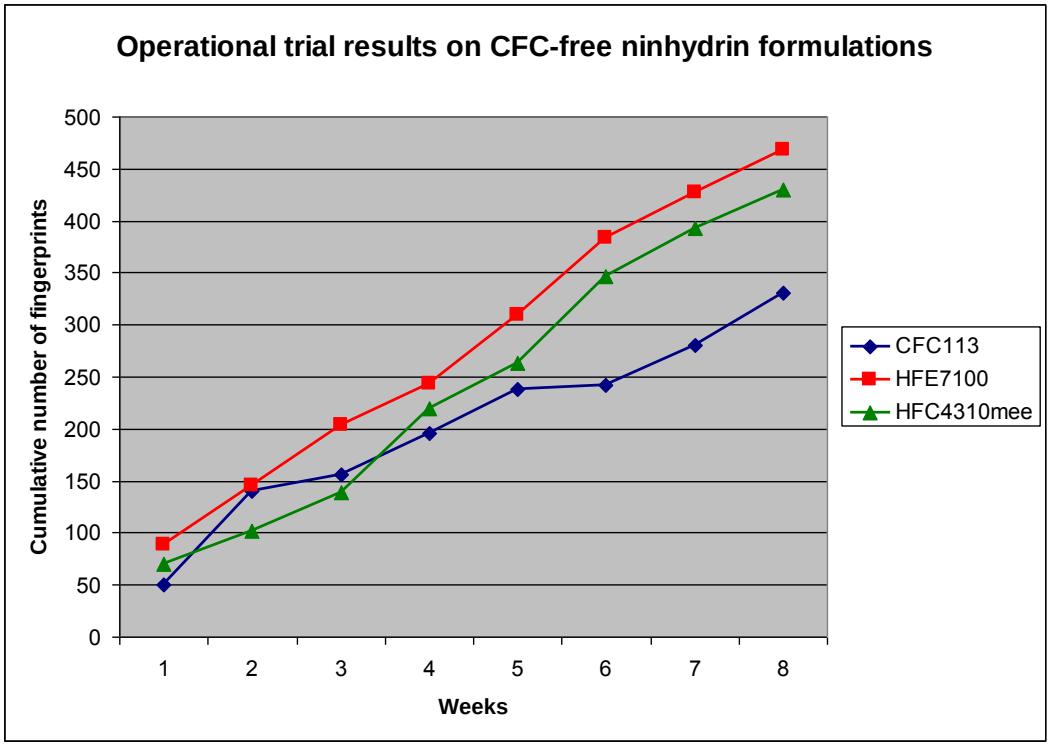
8.2.3 The HFE7100-based formulation now recommended in the CAST *Fingermark Visualisation Manual* [45] has been trialled under UK conditions and found to be superior in performance to the CFC113-based formulation previously used. As part of the programme to find a suitable CFC-free ninhydrin formulation, Hewlett and Sears first tested a number of CFC-free formulations against the CFC113 formulation then in use. Some of these early studies are reported under section 6 'Alternative formulations and processes' above. A pseudo-operational trial, counting numbers of fingermarks with >8 minutiae developed using each technique on batches of 75 fraudulently passed cheques, gave the following result for the most promising HFC and HFE compounds.



Pseudo-operational trial results obtained on batches of fraudulently passed cheques.

8.2.4 These results indicated that both formulations had the potential to give equivalent, if not better, performance compared with the CFC113 formulation and fingermarks were developed over a similar timescale. As a consequence, both formulations were carried forward to a full operational trial carried out over a period of eight weeks at Essex Police. Articles suitable for ninhydrin treatment were separated into three batches, one treated with the CFC113 formulation, one based on HFE7100 solvent and the other based on HFC4310mee solvent. The number of fingermarks with > 8 minutiae was recorded, with exhibits being examined for fingermarks after 2 days and again after 2 weeks. Over the 8 weeks, 110 cases were treated by each process with an equivalent number of articles treated by each process overall. The results are tabulated and displayed graphically below.

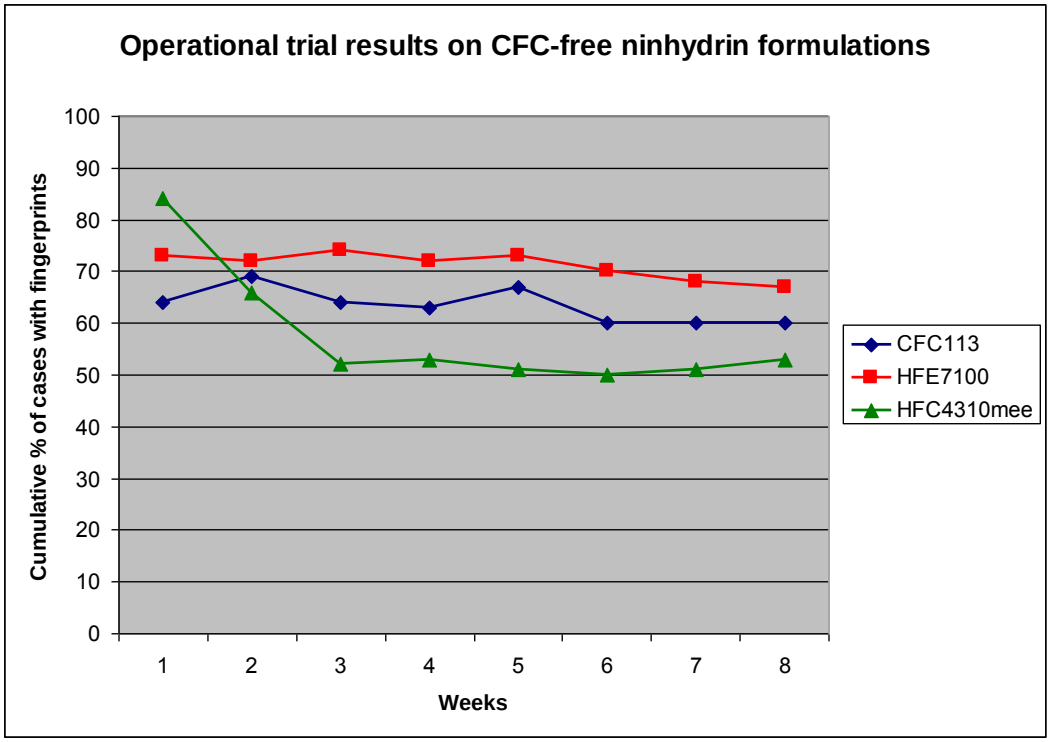
Week	**Cumulative figures**					
	CFC113		**HFE7100**		**HFC4310mee**	
	Cases	F'marks	Cases	F'marks	Cases	F'marks
1	14	50	15	89	12	70
2	29	140	32	146	29	102
3	39	156	42	204	42	139
4	54	196	54	243	55	220
5	69	238	66	309	68	263
6	80	242	83	384	79	347
7	102	280	102	427	102	393
8	110	331	110	468	110	430



Number of fingermarks developed in operational trial on chlorofluorocarbon-free ninhydrin formulations.

8.2.5 Although this analysis shows HFE7100 and HFC4310mee to perform better than CFC113 it was considered that these results may be misleading because single cases could yield disproportionate numbers of fingermarks; one-sixth of all fingermarks developed using HFC4310mee coming from a single case. It is statistically good practice to remove 'outliers' (i.e. the largest and smallest figures) from such analyses for the reason given above. The data were therefore also analysed in terms of the proportion of cases where fingermarks were developed, and these results are given below.

Week	**Cumulative figures**					
	CFC113		**HFE7100**		**HFC4310mee**	
	Cases	% with f'marks	Cases	% with f'marks	Cases	% with f'marks
1	14	64	15	73	12	84
2	29	69	32	72	29	66
3	39	64	42	74	42	52
4	54	63	54	72	55	53
5	69	67	66	73	68	51
6	80	60	83	70	79	50
7	102	60	102	68	102	51
8	110	60	110	67	110	53



Proportion of cases yielding fingermarks in operational trial on chlorofluorocarbon-free ninhydrin formulations.

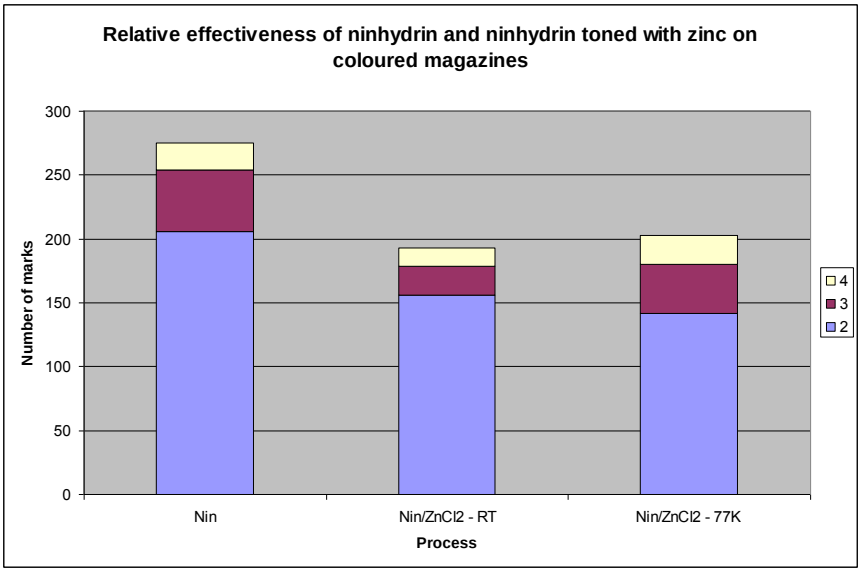
8.2.6 Under this analysis it appeared that the HFE7100-based formulation was the most effective, having the dual advantage of being non-ozone depleting and more effective than CFC113-based ninhydrin on operational work. This formulation was therefore recommended for operational use. It was also observed that the HFC-based formulation became less effective as the solution used became older, indicating that there may have been additional interaction between the HFC4310mee solvent and other constituents. The reasons for this were not explored further.

8.2.7 This study only refers to the use of ninhydrin as a single treatment, where in practice it may be used in sequence after DFO. Studies reported in Chapter 3, Chemical and Physical Processes, 1,8-Diazafluoren-9-one (DFO) demonstrate that as a single process ninhydrin is less effective than DFO, but if used sequentially after DFO, ninhydrin will develop additional marks.

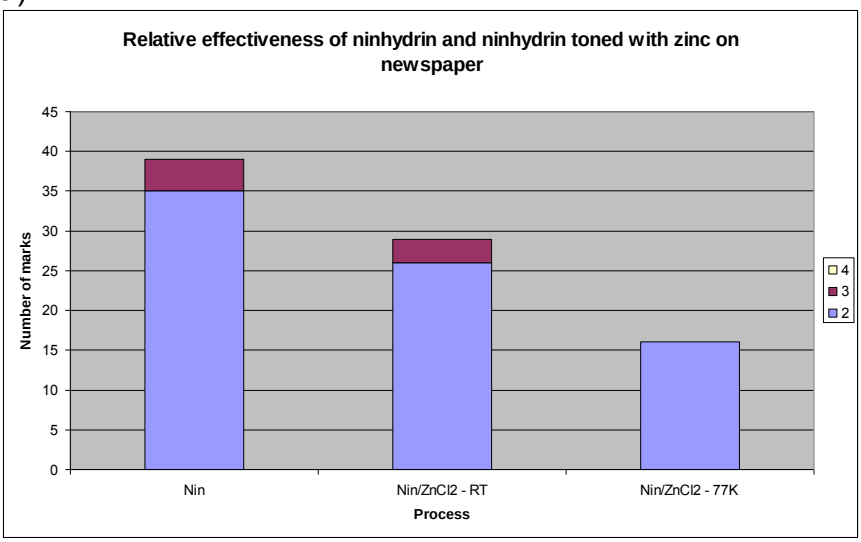
8.2.8 Results reported in Chapter 3, Chemical and Physical Processes, Physical developer also indicate that the application of ninhydrin is not detrimental to subsequent physical developer treatment and that physical developer can develop additional marks after ninhydrin. The recommended sequence of DFO-ninhydrin-physical developer for porous exhibits continues to be used successfully in the UK.

8.2.9 The effectiveness of zinc toning was also investigated in a pseudo-operational trial in the late-1980s, looking at marks deposited on

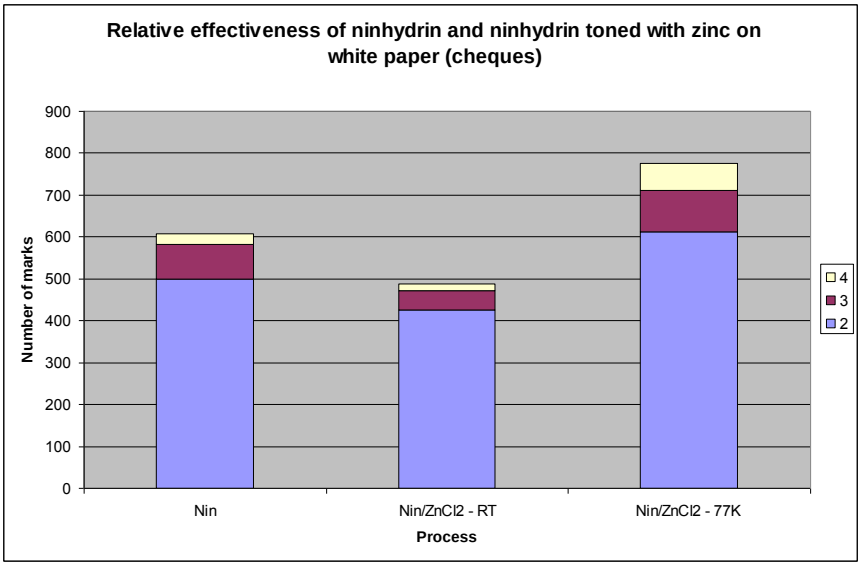
cheques, coloured magazines and newspaper. The results of this trial indicated that results were poor on newspaper and obscured by background fluorescence on coloured magazines, but on cheques (based on white, non-fluorescing paper) zinc toning and fluorescence examination increased the number of marks recovered if the paper was chilled to liquid nitrogen temperature.



a)



b)



c)

Psuedo-operational trial results (marks graded 2, 3 and 4) on naturally handled items treated with ninhydrin and subsequently toned with zinc, a) *coloured magazine substrates,* b) *newspaper substrates, and* c) *white paper (cheque) substrates.*

8.2.10 The most recent assessment of ninhydrin has been a pseudo-operational trial to compare the effectiveness of the HFE7100-based formulation with a revised formulation based on the alternative solvent AE-3000 [47, 56]. This trial utilised items representative of casework, including envelopes, receipts from retail shops, newspapers and letters. The items were divided into 8 experimental batches of 50 exhibits and 4 control batches of 10 exhibits, the types of exhibits being evenly distributed among the groups. These exhibits were then processed using the standard ninhydrin conditions, comparing the effectiveness of the two formulations and gathering additional information about long-term stability. The results were analysed in several different ways: using the basic CAST grading scheme; using a grading scheme taking into account additional factors, such as ridge continuity and background development developed at Staffordshire University; and also by running the developed marks on an Automated Fingerprint Identification System (AFIS) system. The results were analysed statistically using several different models, and all results indicated that there was no statistical difference between the effectiveness of the HFE7100 and AE-3000-based formulations.

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Ninhydrin analogues

1. History

1.1 Many ninhydrin analogues have been synthesised, but the first concerted synthesis of such analogues for assessment as fingermark development reagents was carried out by Almog *et al.*[1] in the early 1980s. These studies identified benzo[f]ninhydrin as a reagent with potential for operational use, the reaction product being a dark green in colour.

1.2 Benzo[f]ninhydrin was first assessed in the UK by Jones and Pounds [2], who conducted a comparison of the new reagent with ninhydrin. These studies found that there was little difference in sensitivity between the two reagents, but benzo[f]ninhydrin was less soluble and the increased solvent levels required in the formulation caused ink to run. However, it was noted that benzo[f]ninhydrin may allow better distinction of marks on coloured backgrounds, because of the darker colour of the developed marks.

1.3 It was later found that benzo[f]ninhydrin could be treated with metal salts in a similar manner to ninhydrin to produce a fluorescent reaction product. An examination of zinc chloride ($ZnCl_2$)-toned benzo[f]ninhydrin marks was conducted using a neodymium:yttrium aluminium garnet (Nd:YAG) laser (green, 532 nm), and these were found to be well-matched to the absorption spectrum of the toned mark [3].

1.4 In the mid-1980s, a wider range of ninhydrin analogues were synthesised including 5-methoxyninhydrin. These studies included an extensive investigation of the reactions between these analogues and metal salts and the fluorescence characteristics of the reaction products [4], on the basis that such treatments were already known to give fluorescent reaction products with ninhydrin. The same researchers carried out further studies of the fluorescence produced from metal toning [5] and found that in this respect benzo[f]ninhydrin and 5-methoxyninhydrin were particularly useful. Both these compounds gave reaction products with more intense fluorescence than ninhydrin and fluorescence occurred at longer wavelengths, thus reducing problems associated with background fluorescence. Another potential advantage of the analogues over the ninhydrin parent was that the fluorescence obtained after treatment with $ZnCl_2$ could be readily observed at room temperature.

1.5 A further investigation into reactions of both ninhydrin analogues and related compounds with amino acids was carried out by Almog [6]. It was observed in these studies that only cyclic triketones gave coloured reaction products with amino acids, whereas open chained triketones did not.

1.6 The intense fluorescence from metal-toned 5-methoxyninhydrin was investigated using a copper-vapour laser [7,8]. Marks developed using

this reagent had the same visible appearance as ninhydrin but were considerably more fluorescent when illuminated with the copper-vapour laser at 510.6 nm. The laser was found to be the most appropriate light source for excitation of this fluorescence. Early operational successes were achieved using these analogues. In 1987, several years before DFO was introduced, 5-methoxyninhydrin was successfully used in the fluorescence mode to develop marks identified to the culprit on an extortion letter in a high profile case [7].

1.7 An extensive review of ninhydrin, its analogues and reactions was published by Joullie *et al.*[9]. However, the interest in developing ninhydrin analogues for the fluorescent properties of their reaction products did fall off with the introduction of 1,8-diazafluoren-9-one (DFO), which did not require a post-treatment to produce fluorescent marks. One final class of ninhydrin analogues that were investigated were the thioninhydrins [10], which were found to give the most intense fluorescence from marks after metal toning than any other ninhydrin analogue.

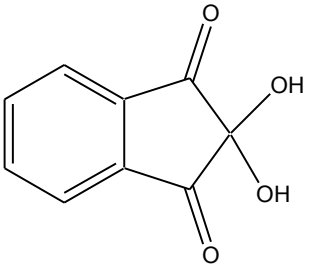
1.8 The Police Scientific Development Branch (PSDB) carried out limited evaluations on some ninhydrin analogues, including 5-methoxyninhydrin and 5-(2-thienyl) ninhydrin. The most comprehensive study was carried out on benzo[f]ninhydrin in collaboration with the Israeli National Police, comparing the effectiveness of the two reagents on bundles of cheques in a pseudo-operational trial [11]. It was found that ninhydrin gave significantly better results and therefore benzo[f]ninhydrin was not recommended for operational use in the UK.

1.9 Recently Israeli researchers have revisited the toning of ninhydrin analogues with metal salts, most notably by incorporating the metal salts into the formulation and eliminating the need for a post-treatment stage [12]. The analogues used in this study were 5-methoxyninhydrin (5-MN) and 5-methylthioninhydrin (5-MTN). It was reported that these ‘dual action’ reagents gave a more intense colorimetric reaction than ninhydrin, and the zinc toned marks of 5-MTN produced a fluorescent product of intensity equivalent to DFO.

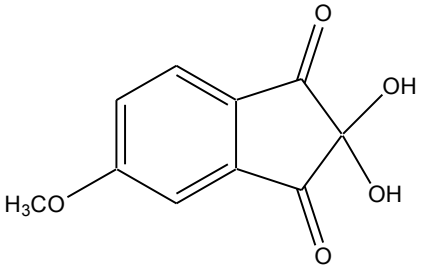
2. Theory

2.1 All ninhydrin analogues essentially follow a similar reaction path with amino acids to ninhydrin itself, and for those analogues that do form complexes with metal salts, the structures of these complexes are similar to those observed for ninhydrin.

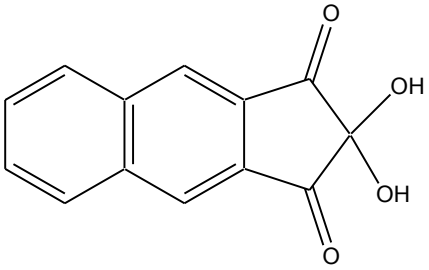
2.2 The structures of ninhydrin and the principal analogues that have been considered for fingermark development are illustrated below.



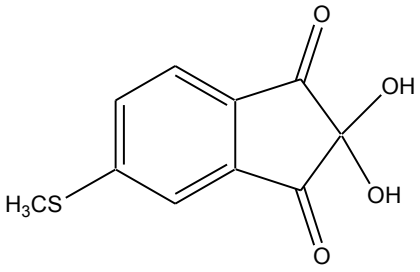
Ninhydrin



5-methoxyninhydrin



Benzo[f]ninhydrin



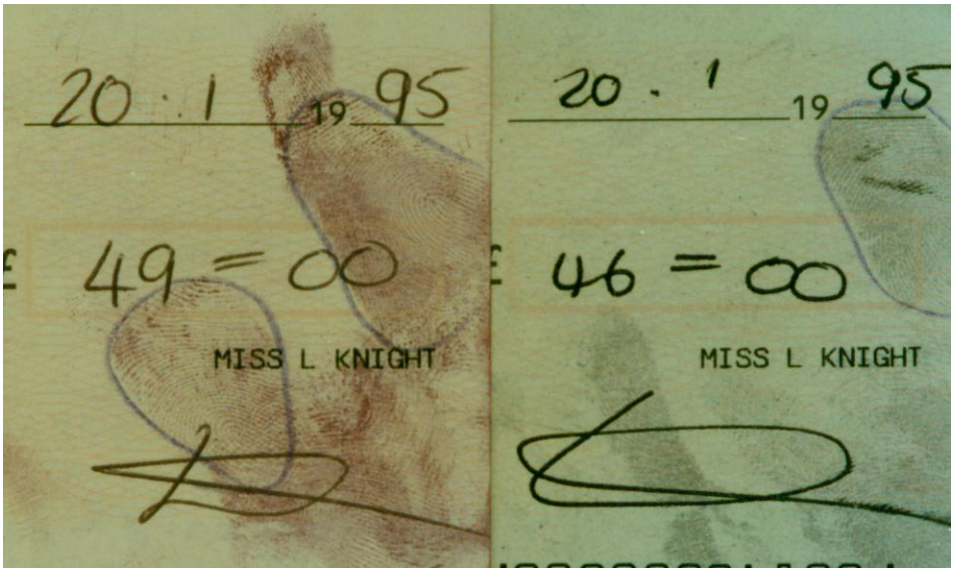
5-methylthioninhydrin

Structures of some of the principal ninhydrin analogues.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST does not currently (2016) recommend the use of ninhydrin analogues because those studied to date offer no performance benefits over ninhydrin itself. Those that have been studied and reported are listed among the Category E processes in the *Fingermark Visualisation Manual* [12]. The two analogues that have generated the most interest, benzo[f]ninhydrin and 5-MTN, may have niche applications (for example where a different coloured reaction product gives better contrast), but in routine use are no more effective than ninhydrin in colorimetric mode or 1,2 indandione or DFO in fluorescence mode..

3.2 Benzo[f]ninhydrin has been of interest because it produces a grey-green reaction product, which may be easier to distinguish on coloured papers than the purple colour produced by ninhydrin. It also fluoresces at a longer wavelength after metal toning than ninhydrin, which again may be useful in distinguishing developed marks against background fluorescence.



a) b)

Comparison of reaction products produced with a) ninhydrin and b) benzo[f]ninhydrin.

3.3 However, in comparative trials between ninhydrin and benzo[f]ninhydrin, ninhydrin was found to be significantly more effective in terms of the numbers of fingermarks developed on batches of cheques from different banks [11]. A brief summary of this trial is given below.

3.4 The formulations used were as follows:

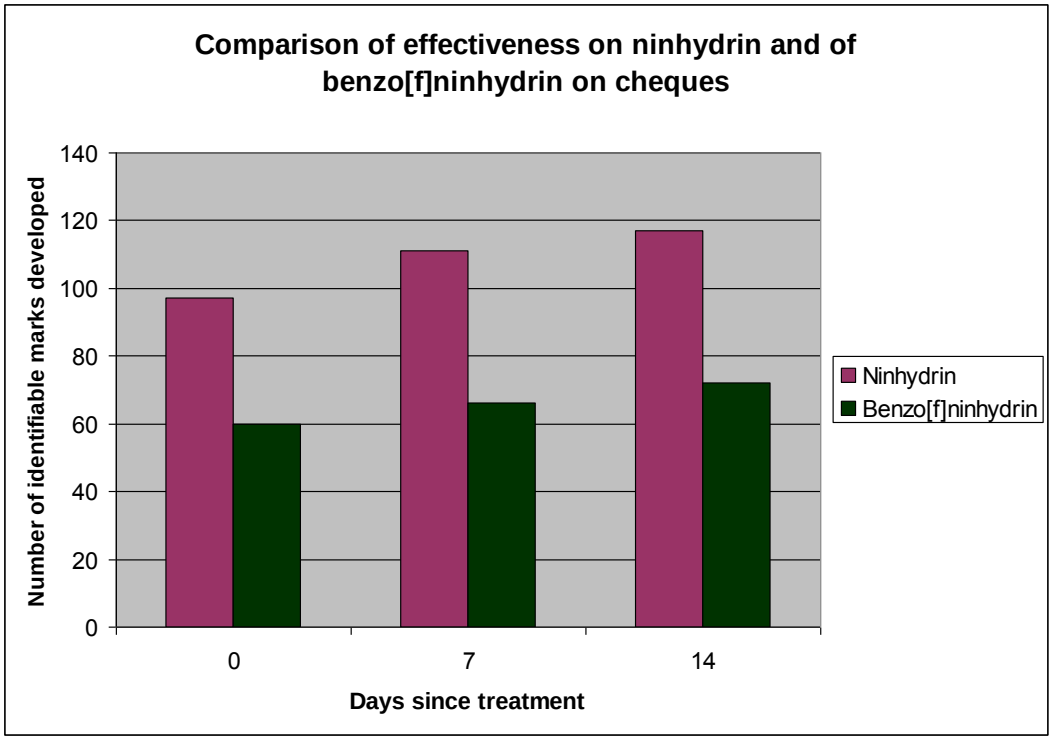
ninhydrin: 5 g ninhydrin, 45 mL ethanol, 5 mL acetic acid, 2 mL ethyl acetate, 1 L 1-methoxynonafluorobutane (HFE7100);

benzo[f]ninhydrin: 6 g benzo[f]ninhydrin, 60 mL methanol, 30 mL acetic acid, 60 mL methyl acetate, 850 mL 1,1,2-trifluorotrichloroethane (CFC113).

3.5 The numbers of fingermarks containing more than eight points developed using each process is recorded in the table below, and also shown graphically.

Days since treatment	Number of fingermarks							
	Ninhydrin (HFE7100)				Benzo[f]ninhydrin (CFC113)			
	B	M	N	Total	B	M	N	Total
0	22	41	34	97	12	21	27	60
7	28	46	37	111	13	23	30	66
14	30	50	37	117	15	26	31	72

Number of fingermarks developed on bundles of fraudulently passed cheques (B = Barclays, M = Midland, N = Natwest).



Total number of fingermarks developed on bundles of 75 fraudulently passed cheques.

3.6 As can be seen, the results do not justify the operational use of benzo[f]ninhydrin on grounds of effectiveness, although there may still be niche applications, such as the development of marks on coloured surfaces.

3.7 The more recent development of 5-MN and 5-MTN formulations incorporating metal salts [12] offered processes with the potential for use as 'dual action' reagents with both colour and fluorescence. Both 5-MN and 5-MTN are of interest because they produce a more intensely fluorescent reaction product than ninhydrin when post-treated with metal salts. However, they are no more sensitive than ninhydrin and the visible reaction product is almost identical in colour. The requirement for intense fluorescence after metal toning reduced significantly with the introduction of reagents producing fluorescent products such as DFO and therefore it

was not considered necessary to change from the currently recommended ninhydrin formulation. The recent observation that metal salts can be incorporated into 5-MN and 5-MTN formulations rather than being used as an additional, post-treatment step revived interest in these compounds and CAST conducted a comparison between 5-MTN-zinc, ninhydrin, DFO and 1,2 indandione-zinc [14].

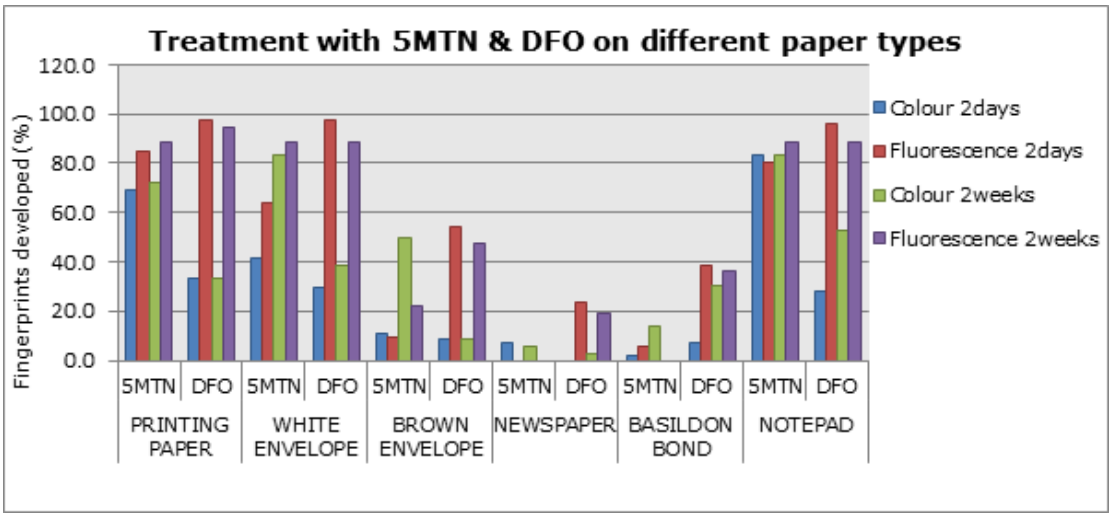
3.8 Split depletion series were used carried this study. Six donors (two good, two medium, and two poor amino acid secreters) deposited a series of six fingermarks on the sheet of paper. Marks were aged for periods of 2 days and 2 weeks prior to developing the marks. The experiments were conducted using six paper types:

- Xerox A4 printing paper,
- Ryman A4-size white envelopes,
- Ryman A4-size brown envelopes,
- Basildon Bond Champagne writing paper,
- Ryman spiral bound notepad (all purchased from Ryman);
- several copies of Metro newspaper were collected on 23rd June 2010 and the same page from the different copies was used for all the experiments.

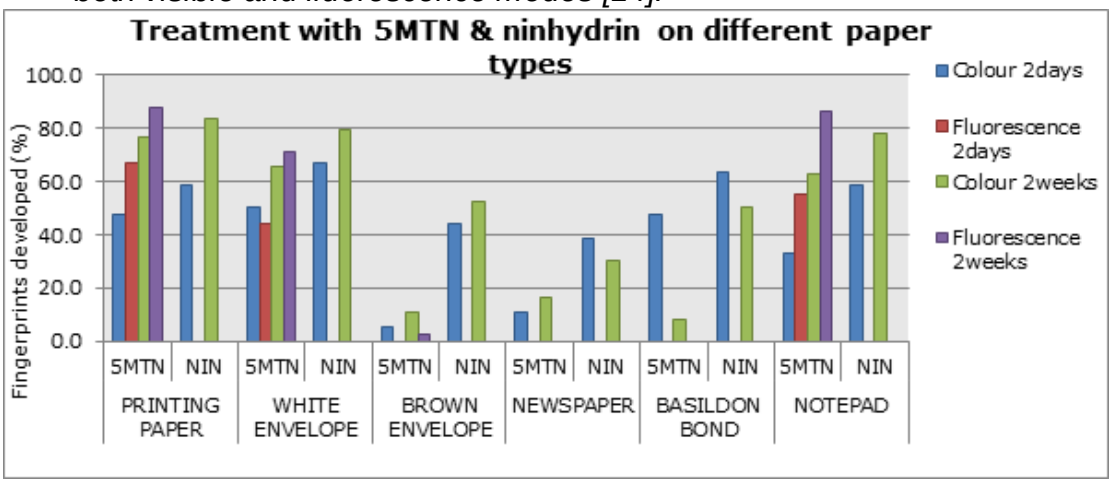
3.9 Ninhydrin and DFO working solutions were prepared according to the *Fingermark Visualisation Manual* [12]. 1,2 IND/$ZnCl_2$ solution was prepared following the UK protocol developed for a previous study [15]. 5-MTN formulation and processing parameters were determined by initial optimisation experiments, and were based on the solvent mixture of the existing ninhydrin formulation with the following quantities of ninhydrin analogue and zinc chloride:

- 0.03% w/v of 5MTN
- 0.01% w/v of $ZnCl_2$.

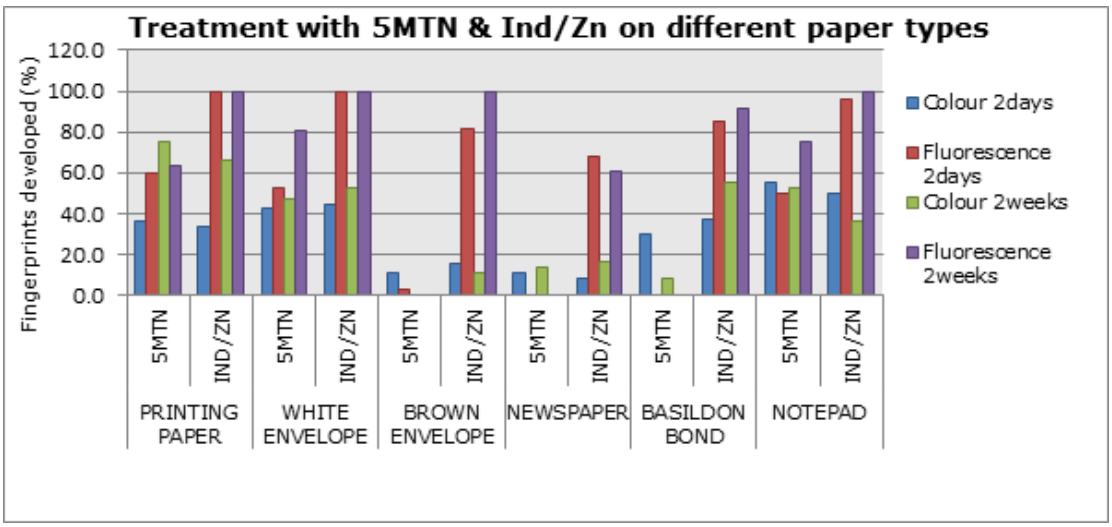
Marks treated using 5-MTN-zinc were processed in a ninhydrin oven using the same processing parameters as for ninhydrin. Preliminary experiments showed that processing in the ninhydrin oven developed ~70% more marks than treatment using the DFO oven and processing conditions. Results were assessed both in terms of proportion of marks developed overall, and also percentage of marks developed to a high quality suitable for identification (grades 3 and 4).



Proportion of fingermarks developed using 5MTN and DFO, evaluated in both visible and fluorescence modes [14].



Proportion of fingermarks developed using 5MTN and ninhydrin, evaluated in both visible and fluorescence modes [14].



Proportion of fingermarks developed using 5MTN and 1,2 indandione-zinc, evaluated in both visible and fluorescence modes [14].

	5MTN/$ZnCl_2$–DFO comparison		**5MTN/$ZnCl_2$–1,2 IND/$ZnCl_2$ comparison**		**5MTN/$ZnCl_2$–Ninhydrin comparison**	
	5MTN	**DFO**	**5MTN**	**IND**	**5MTN**	**NIN**
Colour mode	4.9%	0.7%	5.8%	4.9%	2.1%	16.0%
Fluorescence mode	16.7%	20.8%	4.4%	31.5%	10.4%	0%

Percentages of fingermarks that were graded as '3s' & '4 s' after development with each reagent. 432 fingerprints were collected for each comparative study.

3.10 5MTN/$ZnCl_2$ is more effective than DFO and similar to 1,2 indandione-zinc in the colour mode, however both DFO and 1,2 indandione-zinc produce significantly more fluorescent marks than 5-MTN-zinc.Ninhydrin revealed significantly more marks than 5MTN-zinc in the colour mode, but marks developed using ninhydrin did not exhibit any fluorescence (as expected). The trends observed in the number of identifiable marks (grades 3 and 4) are similar to those in the number of marks developed overall, Because DFO and 1,2 indandione both outperform 5-MTN-zinc in fluorescence mode, and ninhydrin outperforms it in colour mode, it was not decided to proceed with further evaluation of this reagent.

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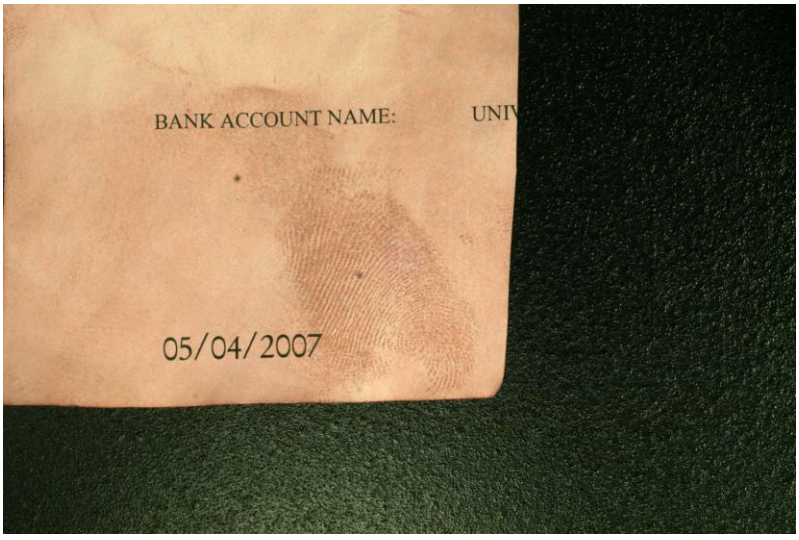
Oil Red O

1. History

1.1 Oil Red O (also known by its Colour Index name solvent red 27) is a superlipophilic diazo dye and is closely chemically related to solvent black 3. It has been used as a fat stain for biological samples and also industrially as a colorant for oils, fats and waxes. As the name suggests, the dye is red in colour and selectively stains lipid components. The Police Scientific Development Branch (PSDB) initially investigated Oil Red O, amongst other lipid dyes, as an alternative to solvent black 3 on non-porous surfaces [1]. These studies indicated that solvent black 3 was a superior dye for the particular range of surfaces being investigated (i.e. non-porous surfaces) and no further work was carried out on Oil Red O at this time.

1.2 The next reported forensic application of Oil Red O was for the development of lip prints [2], with a range of similar dyes including Oil Red O, solvent black 3, solvent red 23 (Sudan III) and solvent red 24 (Sudan IV) being applied, both in powder form and in solution for the staining of lip prints deposited on tissue paper.

1.3 In 2004, Beaudoin [3] reported an Oil Red O formulation for the development of fingermarks on wetted papers. The work was carried out to identify alternatives to the complex and time-consuming physical developer process, and resulted in a two-stage method consisting of a dip bath of Oil Red O in a methanol/sodium hydroxide solvent, followed by immersion of the exhibit in a sodium carbonate/nitric acid buffer solution. Initial tests on wetted surfaces ranging from porous to non-porous in nature indicated that Oil Red O was effective on porous and semi-porous surfaces, but that developed marks were difficult to visualise on non-porous surfaces.



Photograph of fingermark developed on paper using Oil Red O.

1.4 This was followed by a comparative study looking at the relative effectiveness of Oil Red O and physical developer on thermal papers, white printer paper and brown Kraft paper [4]. In these studies sebum-rich fingermarks were deposited on paper that was wetted, divided in two and then treated using the selected process. For the range of surfaces examined, Oil Red O gave superior results on both thermal papers and white printer paper. On the brown Kraft paper, average scores were similar, but physical developer gave more marks of high quality.

1.5 A further study was conducted to look at the insertion of Oil Red O into sequential treatments on porous surfaces [5]. Again sebum-rich fingermarks were used, and comparisons made between the quality of fingermarks developed in the sequences including Oil Red O and those omitting it. Both wetted and dry papers were considered in these studies. For white paper, results indicated that improved fingermark quality could be achieved by inserting Oil Red O into standard sequential treatments as the stage before physical developer. For brown papers Oil Red O was found to be detrimental, primarily because of the pink background staining caused by Oil Red O making marks subsequently developed using physical developer more difficult to visualise.

1.6 The promise of these studies has resulted in more detailed studies being carried out in several countries, including Australia, the UK and the USA [6]. These studies have generally used ‘standard’ fingermarks rather than deliberately sebum-rich marks and have tended to indicate that the effectiveness of Oil Red O begins to fall with the increasing age of the mark, and for marks much older than four weeks, the marks are very diffuse with little ridge detail being developed. The same effect is observed for longer immersion times in water. In both these cases, physical developer continues to develop marks with good clarity of ridge detail.

1.7 Further studies have been carried out at universities within the UK [8,9]. These again demonstrated that on groomed, sebum-rich prints Oil Red O gave superior performance to physical developer, but when normally deposited marks were used, the performance was closely equivalent. It was shown that exposing porous surfaces to accelerant was detrimental for both processes, no marks being developed by Oil Red O or physical developer after exposure.

1.8 Refinements have been made to the original formulation published in 2004. In 2011 Beaudoin published an alternative formulation for the buffer solution, based on sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate in distilled water [10]. Frick et al [11] subsequently proposed a simpler formulation of Oil Red O based on propylene glycol, which negated the need for a separate buffer solution. The item was simply exposed to the staining solution for approximately 15 minutes, then removed and rinsed with water. This was shown to be capable of developing additional marks when used in

sequence with physical developer [12]. More research needs to be conducted in order to establish the relative effectiveness and performance on aged marks of the two Oil Red O formulations (methanol and propylene glycol-based).

1.9 Other studies have focused on the impact of water immersion on the effectiveness of Oil Red O, generally in comparison with physical developer but also with other lipid specific reagents. Wood and James [8] and Dhakal [13] deposited deliberately sebaceous marks on a range of porous surfaces and immersed them in water prior to development. Both these studies indicated that Oil Red O was more effective than physical developer, but again this is unsurprising taking into account that sebaceous, as opposed to 'natural' marks had been used. Dhakal also considered the potential effect of water quality on mark development, but did not observe significant differences between river water, tap water and ultra-pure water. Simmons et al. [14] investigated natural marks aged for different periods of time prior to immersion in water, and in this study found Oil Red O to be significantly less effective than physical developer, in particular where marks had been aged for longer time (28 days) prior to immersion and development.

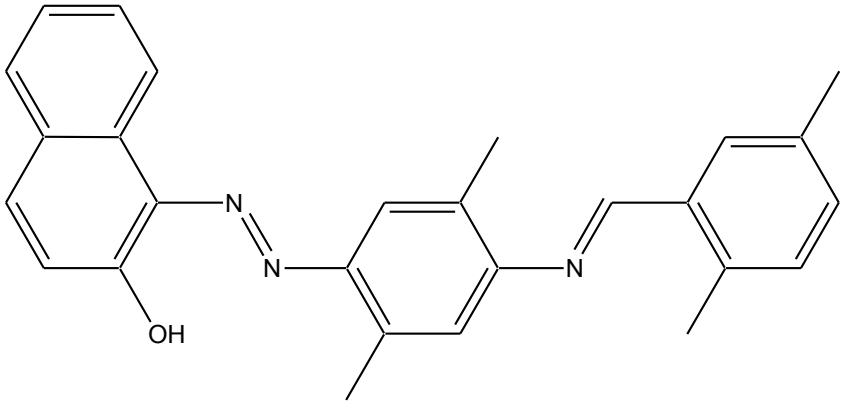
2. Theory

2.1 Oil Red O is a lysochrome, more simply described as a fat stain. Most lysochromes are azo dyes that, because of their structure, have undergone molecular rearrangement making them incapable of ionising.

2.2 The basis for these dyes colouring fats is that they dissolve into it. From another perspective, the fat is the solvent for the dye. Lysochromes are mostly insoluble in strongly polar solvents, such as water, and somewhat more so in less polar solvents, such as ethanol. They are quite strongly soluble in non-polar solvents, such as xylene. Triglycerides, being non-polar compounds, dissolve them quite well. Other lipids, having fatty components, may also dissolve them.

2.3 Lysochromes such as Oil Red O are applied from solvents in which they are sparingly soluble. As they come into contact with materials in which they are strongly soluble (e.g. fats), they prefer to transfer into them to a significant extent, often colouring the fat more strongly than the original solvent. This process is known as preferential solubility.

2.4 Oil Red O is more strongly hydrophobic than some earlier dyes used for staining lipids, and it is thought that this makes it more effective in staining applications [7]. The structure of Oil Red O is shown below.



Structure of Oil Red O (solvent red 27).

2.5 The formulation proposed by Beaudoin [3] consists of three separate baths, a staining bath to stain the lipid components of the fingermark, a buffer solution to neutralise the base side of the staining solution and stabilise the developed marks, and finally a water wash. The formulations used are as follows:

stain bath – dissolve 1.54 g Oil Red O in 770 mL methanol;
dissolve 9.2 g of sodium hydroxide (NaOH) in 230 mL water;
add the two solutions, mix together, filter and store in a brown bottle.

buffer solution – add 26.5 g of sodium carbonate ($Na_2CO_{3)}$ to 2 L of water and stir to dissolve;
add 18.3 mL of concentrated nitric acid (HNO_3);
increase volume of solution to 2.5 L with water.

2.6 Articles to be treated are immersed in the stain bath for up to 90 minutes, then removed, drained and placed in the buffer solution. Finally the articles are rinsed in distilled water and allowed to dry.

2.7 The more recent formulation proposed for the pH 7 buffer solution [10] is as follows:

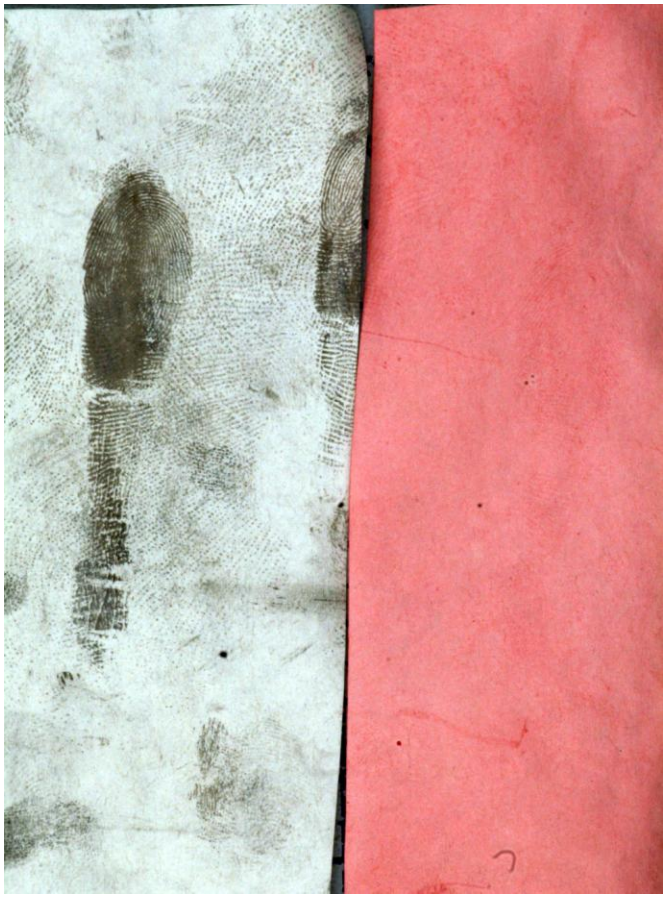
Add 101.5 g of sodium phosphate monobasic monohydrate (NaH_2PO_4 •H_2O) to 1 L of distilled water and shake until it is dissolved.
Add 339 g of sodium phosphate dibasic heptahydrate (Na_2HPO_4 •$7H_2O$) to 1 L of distilled water and shake until it is dissolved.
Mix the two solutions.
Add enough distilled water to increase volume to 4 L.

3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The principal reason that Oil Red O is not included as a Category A process in the CAST *Fingermark Visualisation Manual* [15] is because it is not as effective as physical developer. Although papers referenced above [4,5,8,13] indicate the reverse to be true, these experiments have been performed using sebum-rich marks, which are not truly representative of what may be encountered on real exhibits. Subsequent experiments using 'natural' fingermarks and depletion series of marks [6,14] are in general accord that:

- Oil Red O is not effective on marks older than four weeks;
- Oil Red O is not effective on marks exposed to prolonged immersion in water;
- some solvents used in ninhydrin and 1,8-diazafluoren-9-one (DFO) formulations outside the UK (e.g. petroleum ether) may dissolve the constituents targeted by Oil Red O and therefore it cannot be used in sequence after these processes.

3.2 A small-scale study carried out by CAST on marks known to be one year old confirmed that physical developer was a far more effective reagent and that Oil Red O developed very few marks on articles of this age.

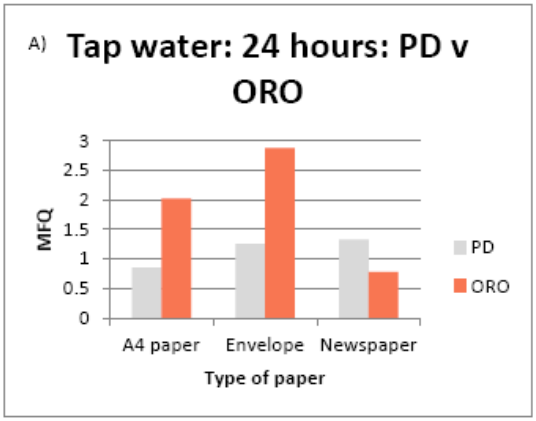


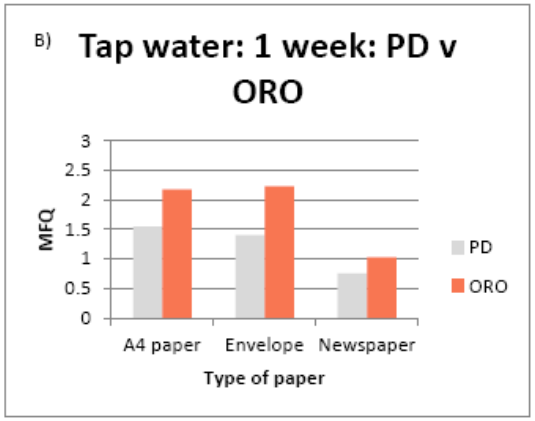
a) b)

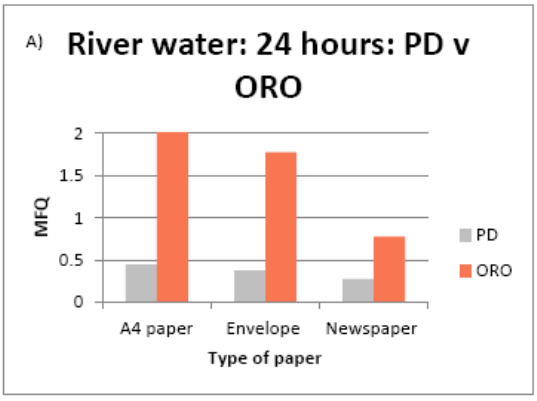
Palm mark approximately one year old a) *treated with physical developer* b) *treated with Oil Red O.*

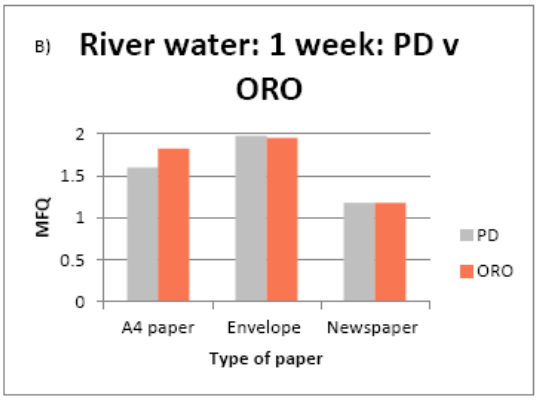
3.3 In addition to this, although Oil Red O involves fewer processing steps overall than physical developer, with the original formulation proposed by Beaudoin [3] it may actually take up to 90 minutes for marks to develop and therefore the overall process may actually be slower than physical developer in many cases.

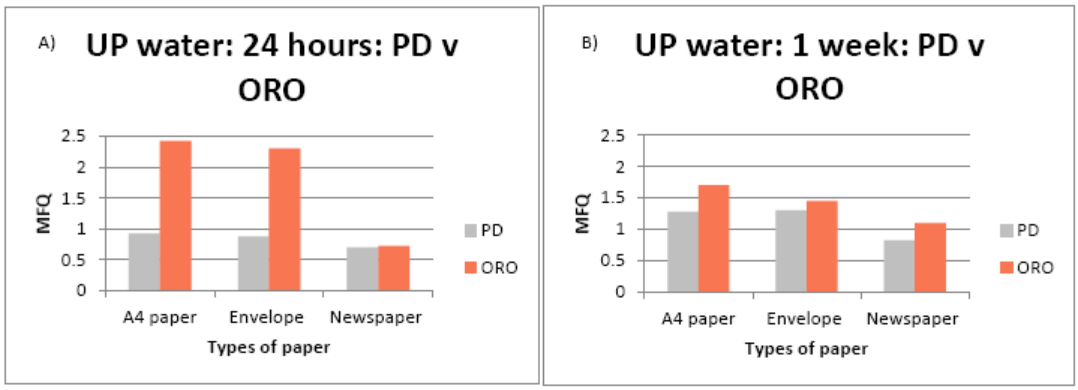
3.4 Despite these limitations, Oil Red O is one of a limited number of processes capable of developing marks on wetted paper, and in circumstances where fingermarks are either grease contaminated or rich in sebaceous content it may actually be the best processing option available. The benefits of Oil Red O over physical developer in these circumstances can be seen in the results obtained by Dhakal [13]. A further potential benefit of Oil Red O is that it is not necessary to monitor the substrate during staining to prevent overdevelopment [16].











Average scores for graded marks deposited on different porous substrates, aged for 1 day and 1 week, immersed in tap, river and ultrapure water, then developed using Oil Red O and physical developer.

3.5 Oil Red O is included in the *Fingermark Visualisation Manual* [15] as a Category B process, with potential niche applications. It has been observed that some papers with high recycled contents can become extremely fragile and disintegrate when treated with physical developer, and others may give high background deposition. If the paper is known to have been wetted and cannot be treated with physical developer for one or both of the reasons above, Oil Red O is a viable processing option. It may also be possible to use Oil Red O in sequence after physical developer on wetted papers as means of developing additional marks.

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Physical developer

1. History

1.1 Physical developer solutions had been in use for many years in the photographic industry for the development of film. These worked with exposure to light causing silver bromide or silver iodide crystals in the film to reduce to specks of silver, these specks becoming sites for the subsequent deposition of silver from solution. In 1969, Jonker *et al.* at the Philips Research Laboratory in Eindhoven published a series of papers on physical developers when investigating methods for making printed circuit boards. These began with a review of classic physical developer solutions [1] but most importantly also included the description of a stabilised physical developer formulation [2,3] with the addition of surfactants and ferrous ions to suppress spontaneous deposition of silver from solution.

1.2 In common with many processes, the potential for fingermark development was recognised when fingermarks were accidentally developed during the processing of photographic plates. Work to evaluate the technique as a fingermark development process began at the Atomic Weapons Research Establishment (AWRE) Aldermaston in the early 1970s [4,5], with Morris and Goode recognising that although the process could develop marks on both non-porous and porous surfaces, it was most effective on porous items. The process was assessed against ninhydrin and osmium tetroxide on paper, both in the dry condition and after wetting. It was found that although the performance was not as good as ninhydrin or osmium tetroxide on dry paper, physical developer was the only process to develop marks on wetted paper and it was concluded that further trials should be conducted by the Police Scientific Development Branch (PSDB).

1.3 These studies included background research on the electrochemical characteristics of the formulation, together with investigations of alternative metals to silver [6]. This work primarily focused on taking the existing formulation towards operational use. Laboratory trials were conducted across a range of different paper types, both fully wetted and exposed to high humidity environments [7]. Rigorous testing, leaving paper samples in cages in the river Thames, indicated that fingermark ridge detail was still developed on paper samples which were close to physical disintegration.

1.4 The use of a radioactive toner based on ^{35}S for the revelation of developed marks on patterned backgrounds was also proposed, using autoradiography of the radioactive toned item to separate the mark from the background. The recommendation of the original study was to proceed to a one-year operational trial for both the basic process and the toning technique. This commenced at Sussex Police and the Metropolitan Police in 1976, with PSDB staff processing the exhibits in police laboratories [8]. The operational trial confirmed the laboratory

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observations; additional marks were developed using physical developer after ninhydrin treatment in 41 out of 175 cases, marks were developed on items known to have been wetted, and the radioactive toning process was successfully used to reveal marks on patterned backgrounds. The trial was continued without PSDB involvement, using trained police staff to process exhibits [9] and it was shown that similar results could be achieved. However, during the early stages of this phase of the work it was observed that the physical developer solutions were unstable, resulting in rapid 'fogging' of the entire exhibit. Work was carried out by PSDB to establish the reason for this [10], which concluded that the principal cause was excessive exposure of the solution to light. With elimination of this factor, results improved significantly. It was also discovered that water quality was crucial for the production of stable solutions, so there was a move to use only distilled, not deionised water. After further testing, PSDB progressed with the operational implementation of the process across the UK at the beginning of the 1980s [11].

1.5 Although the technique had been introduced into operational use, the fingermark constituents responsible for influencing development were still not firmly established. Early work by Morris [5] had suggested that cholesterol esters, hydrocarbons or triglycerides may trigger deposition but in later tests by Gray [12] using a range of model compounds it was not possible to identify clearly which were actively promoting deposition and it may be that combinations of substances are responsible rather than any constituent in isolation.

1.6 A problem sometimes observed during operational use of physical developer was that background interference could occur. In some cases this was seen as light greying, which did not affect visualisation of the developed mark, but in other cases dark grey/black patching occurred, which could obscure marks. Investigations at PSDB established that this was caused by the calcium carbonate filler present in many papers, which made them alkaline in nature. The acidic nature of the physical developer solution results in a reaction with the carbonate filler that liberates bubbles of carbon dioxide. This in turn provides sites for silver deposition over the entire surface of the paper, which turns uniformly brown/black. The proposed solution was to neutralise the paper before the application of physical developer and a range of acids were tested in this role, with maleic acid ultimately being selected by PSDB (dilute nitric acid being recommended as an alternative by the Home Office (HO) Forensic Science Service (FSS) laboratory at Aldermaston and used operationally by the Serious Crime Unit at the Metropolitan Police Forensic Laboratory). Another refinement to the formulation made by PSDB (by then renamed SRDB) in the mid-1980s was the reduction in the concentration of surfactants used, made possible by the availability of higher purity surfactant grades. The quality of the water used has also been found be an important factor in optimising formulations, with revisions to quantities of surfactants also being required when the grade

of water used by PSDB was changed from distilled to reverse osmosis/deionised.

1.7 The technique began to be used worldwide, with published papers promoting the benefits of the technique and giving case studies where success had been obtained on wetted items [13, 14] and on items over 30 years old [14]. The importance of using an acid pre-wash to neutralise alkali fillers in most commercial papers was emphasised [15]. As discussed above, without this pre-wash a reaction occurred that caused the paper to darken, obscuring developed marks and inhibiting more widespread use of the technique. A range of commercially produced, pre-mixed physical developers were evaluated by the same researcher [16], none of which supplied, or commented on, the need for a pre-wash. Almost all were capable of giving reasonable performance if a pre-wash was used, but the researcher expressed concern that the lack of this advice may cast doubt on the effectiveness of the process. Few commercially produced packs explicitly state the constituents used, and CAST encourages UK police forces to make their own solutions for operational work to optimise performance.

1.8 The basic formulation recommended for operational use by CAST is little modified from that originally described by Jonker *et al.* in 1969 and has continued in use to the present day. Research into alternative formulations has been predominantly carried out in the USA, with the objectives of reducing cost, simplifying the process, reducing the time taken to process exhibits and to improve visualisation of the developed marks. Saunders experimented with a range of different physical developer solutions at different dilutions [17], starting to process exhibits with dilute solutions and if development did not occur silver nitrate was progressively added until success was obtained.

1.9 Other adaptations investigated included:

- copper-based physical developers [18]
- toning of marks to make them fluorescent [18]
- bleaching of marks to make them more visible on darker backgrounds [19].

1.10 A revised formulation was published by the US Secret Service in 2003, incorporating malic (as opposed to maleic) acid in the neutralising solution and reductions in the amount of silver, surfactants, ferrous salt and citric acid [20, 21]. Split comparisons with the established process suggested that the revised formulation gave equivalent, if not better, development. A comprehensive review of the physical developer process and alternative formulations investigated has been produced by Cantu [22].

1.11 More recently, acid-free formulations have been suggested for the development of marks on porous and non-porous surfaces [23] but other researchers have not been able to recreate these results. There has also been concern about the continued availability of Synperonic N, one of

the surfactants used in the formulation, and work has been carried out both in the USA and by CAST in the UK [24] to assess possible alternatives. None of those investigated has yet proved to be as fully effective as Synperonic N, although formulations based on Tween 20 give closely equivalent performance and give a good alternative when Synperonic N becomes unavailable.

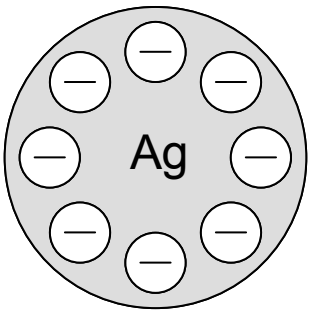
1.12 Physical developer remains an important reagent for fingermark development on porous surfaces. It appears to target different fingermark constituents to the amino acid reagents 1,8-diazafluoren-9-one (DFO) and ninhydrin, and on some types of porous substrate will regularly develop additional marks if used sequentially after them (although it should be noted that there are also substrates for which success rates are uniformly low). It has also been shown to develop marks on exhibits exposed to some of the harshest environments, including long periods of water immersion, charring [25], gamma ray irradiation [26] and on paper nearly 60 years old [27].

1.13 More recently there have been published papers demonstrating that Oil Red O can also develop fingermarks on wetted surfaces and in some situations may be more effective than physical developer. This debate is more fully addressed in Chapter 3, Chemical and Physical Processes, Oil Red O, but the CAST position is that physical developer remains more effective under typical operational conditions and should continue to be the technique of choice for use on wetted paper. Many of the studies on Oil Red O have used freshly deposited, ‘groomed’ marks and this is not representative of marks encountered operationally.

1.14 A number of workers overseas have indicated that they have problems implementing the physical developer process, in particular the development of high backgrounds. A team from PSDB carried out trials during a visit to Israel in the late 1990s and concluded that local differences in paper manufacture, possibly including the nature of the inorganic fillers used, can affect the levels of background development. Similar problems have been reported in China and in Taiwan. Some of these issues may arise from differences in the balance of water quality and grades of detergents but others may result from differences in paper manufacture and additives used.

2. Theory

2.1 In conventional physical developer solutions, spontaneous, homogeneous nucleation of silver nuclei occurs by reduction of silver ions. These nuclei carry a negative charge, and grow by progressive silver deposition from solution, the negative charge being maintained throughout their growth.



Negatively charged silver nuclei.

2.2 In stabilised physical developer solutions, several other chemicals are added to suppress the reduction of silver ions to elemental silver unless a suitable initiation site is present. In the case of the physical developer solution used for fingermark development, the initiation sites are the fingermark ridges (although as mentioned above it is not fully clear which constituents actually initiate deposition).

2.3 The process associated with silver deposition has been described in detail elsewhere [28], a summary is given below. The physical developer solution contains both ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions, setting up a ferrous/ferric couple reaction that acts as a reducing agent for the silver ions. The reversible reaction below is set up:

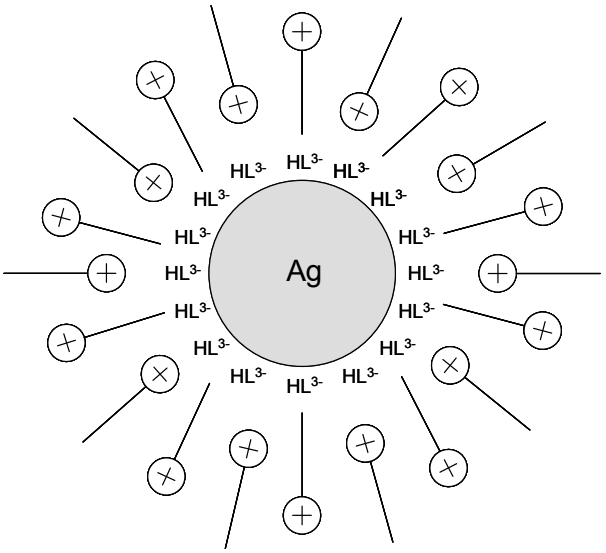
$$Ag^{+} + Fe^{2+} \leftrightarrow Ag + Fe^{3+}$$

2.4 Addition of citric acid reduces the ferric ion concentration by the formation of ferric citrate, which releases three protons and essentially drives the overall reaction in the direction of suppressing elemental silver deposition.

$$Ag^{+} + Fe^{2+} + H_3Cit \leftrightarrow Ag + FeCit + 3H^{+}$$

2.5 By adjusting the relative concentrations of each component, the reduction reaction can be balanced so that it only occurs on fingermark ridges (or other sites where initiators are present) rather than in solution. However, once a silver nucleus has formed, it acts as a site for further silver deposition and this will result in depletion of silver ions from the solution unless the initiation capability of the nucleus is suppressed.

2.6 Surfactants are added to the formulation in order to inhibit the growth of the colloidal silver particles. As stated above, the silver nuclei formed in solution are negatively charged, attributed to the adsorption of the negatively charged citrate anions on the surface. A cationic surfactant is therefore added to suppress particle growth, with the molecules of the surfactant arranging around the silver particle in a staggered fashion to form a micelle.

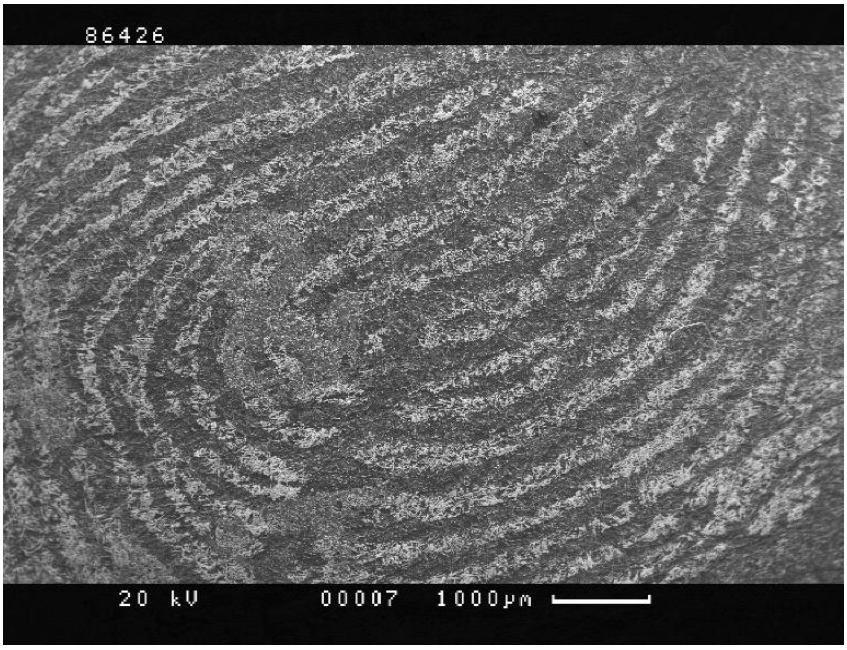


Micelle formed around silver particle by cationic surfactant molecules interacting with citrate anions (HL^{3-}).

2.7 A further non-ionic surfactant is added to prevent the cationic surfactant being precipitated out of solution.

2.8 Despite several studies failing to identify conclusively individual fingermark constituents responsible for triggering nucleation, it is thought that the essential element in the selective deposition of silver on fingermark ridges is that the fingermark residue becomes positively charged when exposed to the acidic (pH < 3) conditions of the physical developer solution. This may be due to protonation of the amine groups of proteins held within the emulsion of the fingermark deposit, or by olefins in the residue acquiring a positive charge. A brief study by Wright [24] showed that physical developer gave weak positive development with an amino acid mixture, a strong development with a lipid mixture and the strongest reaction with a mixture of lipids and amino acids. The mixed chemical environment within the fingermark residue may create a better environment for protonation and subsequent deposition to occur.

2.9 As described above, any silver nuclei formed in the solution will be negatively charged. It is likely to be enveloped by the cationic surfactant molecules, but close to the fingermark ridges there is competition from the positively charged components of the residue. In this environment the micelle may be destabilised and the silver nucleus deposited on the ridge, where it becomes neutralised and can begin to grow. Once a metallic silver particle has formed it can grow autocatalytically, resulting in a series of silver particles 10–40 μm in diameter deposited along the length of the fingermark ridge.



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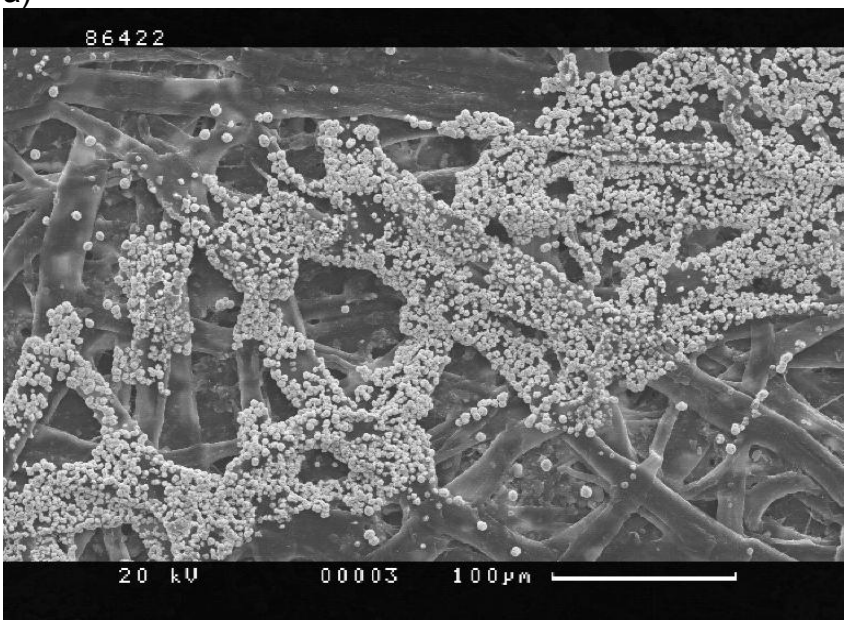

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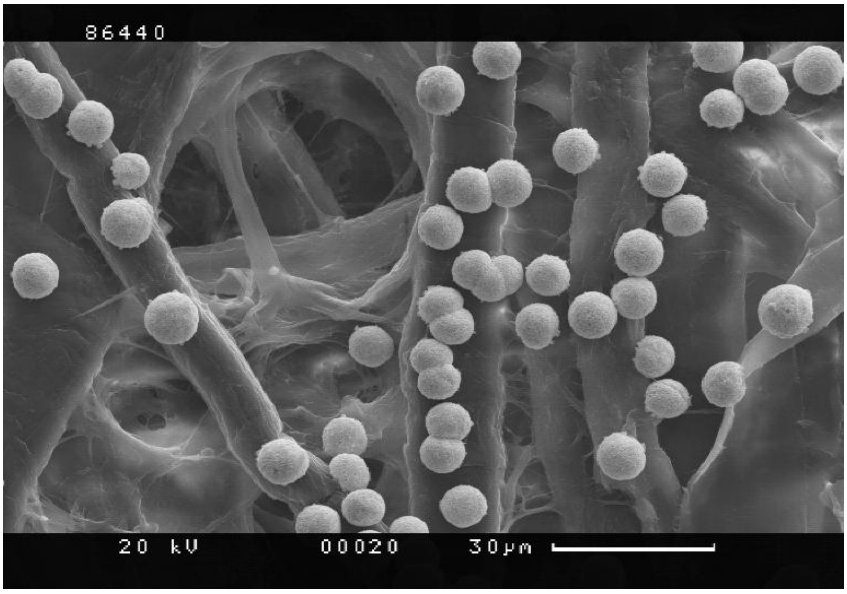


c)

Scanning electron micrographs of a fingermark treated with physical developer a) *low magnification, showing fingermark structure* b) *medium magnification showing fingermark ridge and* c) *high magnification showing individual particles.*

3. CAST processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The process recommended by CAST consists of three stages. In the first stage, the exhibit is exposed to an acid pre-wash to ensure that the substrate is neutralised and that darkening of the background will not occur. In the second stage the exhibit is placed in the physical developer working solution and agitated until it is considered that optimum development has occurred. In the final stage, the exhibit is taken through a series of water wash baths, removing all traces of the physical developer solution and stopping the reaction. It is recommended that the glassware used for all these treatment baths is kept scrupulously clean to prevent silver depositing on residual impurities such as dust particles. In addition, any metallic items such as staples or paperclips should be removed from the items prior to processing.

3.2 The acid solution used for the pre-wash is a 2.5% w/v solution of maleic acid, prepared by dissolving 25 g of maleic acid in 1 L of de-ionised water. The role of the maleic acid is to neutralise the calcium carbonate filler found in many papers. Maleic acid reacts with calcium carbonate to form calcium maleate, releasing bubbles of carbon dioxide. The reaction is generally considered to be complete when bubbles are no longer seen forming on the surface of the paper although some paper types may take

longer to neutralise and testing may be required to identify optimum times.

3.3 The physical developer working solution is produced by adding a pre-mixed stock detergent solution and a pre-mixed silver nitrate solution to a further solution containing the ferrous and ferric ions and citric acid.

3.4 The stock detergent solution is produced by adding 2.8 g of n-dodecylamine acetate to 1 L of distilled water then stirring. Once it has dissolved, 2.8 g of Synperonic N is added and stirred for 24 hours, with the container being covered in clingfilm to prevent ingress of foreign particles that may destabilise physical developer solutions the detergent is subsequently added to. The role of n-dodecylamine acetate is to act as the cationic surfactant, forming micelles around any silver nuclei forming in the physical developer working solution. Synperonic N is the non-ionic surfactant, primarily added to prevent precipitation of the cationic surfactant from solution although it is thought that it may have other functions in the development reactions. It is known that without the non-ionic surfactant being present, physical developer solutions do not work. It is essential that the resultant working solution is clear at this stage for optimum performance, cloudy solutions giving poor results. Cloudy solutions may arise if the temperature in the laboratory is too low (<17°C) [24] or from contamination in one of the components; both causes should be investigated if this issue begins to arise.

3.5 Silver nitrate solution is produced by dissolving 10 g of silver nitrate in 50 mL of distilled water, then storing it in a dark cupboard until required. Silver nitrate is the source of the silver ions (Ag^{+}) in the redox reaction leading to silver deposition.

3.6 To prepare the working solution, a redox solution is first produced. 900 mL of distilled water is measured out and then the following chemicals are stirred into solution: 30 g iron (III) nitrate, 80 g ammonium iron (II) sulphate, 20 g citric acid. To this redox solution are then added 40 mL of the stock detergent solution and all of the silver nitrate solution. The iron (III) nitrate is the source of the ferric (Fe^{3+}) ions for the redox reaction, and ammonium iron (II) sulphate provides the ferrous (Fe^{2+}) ions. Citric acid acts as a buffer for the reaction, reducing pH to below three and suppressing formation of elemental silver. The addition of all of these chemicals reduces the temperature of the solution by means of an endothermic reaction. If the temperature of the solution is already low (<17°C), unwanted precipitation may occur at this stage. In previous editions of the Manual, the order of addition was stipulated. Some researchers still believe this is important, whereas other recent studies [29] indicate that it has little effect on the final product.

3.7 The concentrations of each component have been selected such that the redox reaction is balanced in favour of silver deposition on initiation sites among the fingermark residue, and not in solution.

3.8 It is possible to reduce the time taken for the washing stage of physical developer by introducing a fixing bath that removes all unreacted silver salts after treatment with physical developer [30]. A commercial photographic fixing agent including chemicals such as sodium thiosulphate can be used for this purpose, following the manufacturer's instructions. This has the advantage of reducing the overall treatment time but means that it will not be possible to retreat the exhibit with physical developer if faint marks are present that could have benefited from a longer development time.

4. Critical issues

4.1 There are several critical issues relating to the successful implementation of the physical developer process.

4.2 An acid pre-wash is essential for paper items so that the alkali fillers present in most papers are neutralised. If this stage is omitted heavy background development may occur, which obscures marks.

4.3 The glassware used to carry out the process must be kept scrupulously clean because scratches and impurities may act as preferential nucleation sites and cause silver to precipitate out of the solution.

4.4 The water used should be at least as pure as distilled and without particles. Changing the grade of water used may require subsequent work to re-optimise the detergent concentrations.

4.5 The presence of a non-ionic surfactant in the formulation is essential for development to occur. The process is critically dependant on the surfactants used and their purity. The early work conducted at AWRE used a stock of dodecylamine acetate which was subsequently found to be of low purity but produced excellent results. A subsequent purchase of dodecylamine acetate, believed to be of higher purity, produced acceptable results but the concentration recommended in the formulation was revised downwards from 4 g to 2.8 g per litre for this batch. There are still some questions over the performance of current sources of dodecylamine acetate and no definitive comparisons have been reported.

4.6 The process should be carried out at temperatures above 17°C to avoid the formation of cloudy solutions, which are less effective in developing marks.

5. Application

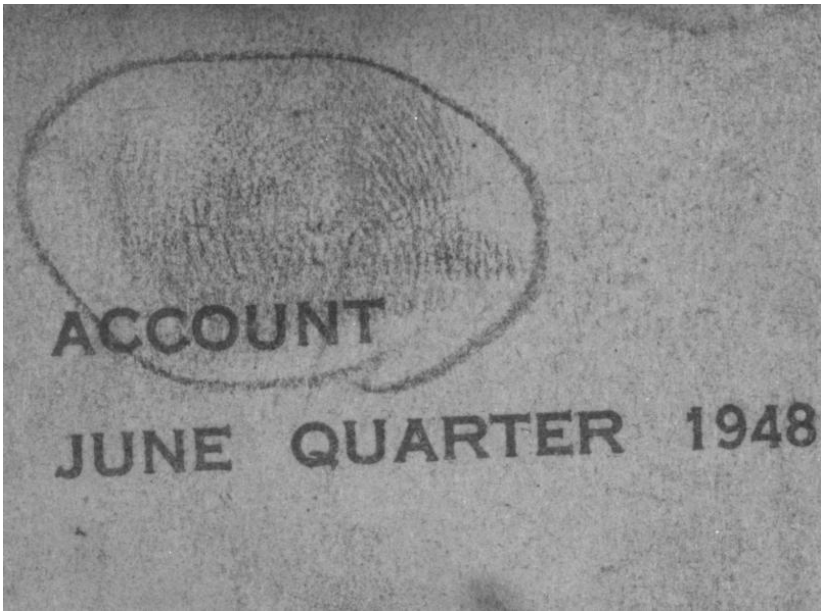
5.1 Suitable surfaces: Physical developer is suitable for use on all porous surfaces, including paper, cardboard and raw wood.

5.2 The principal application of physical developer is the final stage in any sequential treatment process for porous items. It has been repeatedly demonstrated that physical developer targets different components within fingermark deposits than DFO and ninhydrin, and will frequently develop additional marks if used sequentially after them. It should not be used before DFO and ninhydrin in a sequence because on its own it is less effective than either of the reagents and the aqueous solutions used will dissolve the amino acids targeted by these processes.

5.3 Physical developer is also the reagent of choice when it is known a porous item has been wetted. Because it targets insoluble components of the fingermark residue (or soluble components retained within an emulsion of insoluble components) it is capable of developing fingermarks after long periods of immersion in water. Operationally, fingermarks have been developed on exhibits immersed for over three months [13].

5.4 Physical developer has also been shown to develop marks on exhibits exposed to temperatures in excess of 200ºC [25], providing evidence that the components targeted by the process are resilient to adverse conditions. Supporting this are other results obtained during treatment of articles known to be nearly 60 years old [27], where physical developer produced several identifiable marks. It is therefore a process that can be applied when it is known that an exhibit has been exposed to extreme conditions.

a)



b)

Photographs of marks developed on articles exposed to extreme conditions using physical developer a) *marks on charred paper and* b) *mark on a bill nearly 60 years old.*

5.5 In a laboratory, physical developer is applied to articles by processing them through a series of shallow dishes. The paper article is first placed into a dish containing the acid pre-wash, agitating the dish gently at least until bubbles are no longer formed on the surface (potentially longer if testing shows it is required to fully neutralise additives). It is then transferred to a dish containing the physical developer working solution, which is rocked gently until optimum development of the marks has been observed. This typically takes 10 to 15 minutes, but may take longer. Finally, the paper is removed to a series of water wash baths before being allowed to dry in air on an absorbent surface. Once the article is dry and developed marks have been examined, a decision can be made about whether a retreatment with physical developer is required or a post-treatment should be used to improve contrast. It is important to control the temperature during processing, with temperatures below 17ºC inhibiting successful development by destabilising the developer solution [24].

5.6 Physical developer is not a technique suited to application at scenes of crime, although there are occasions where improvisations are thought to have been made, such as half-fish tanks pressed against walls and successively filled with each treatment solution in turn.

6. Alternative formulations and processes

6.1 Alternatives have been considered to all elements of the physical developer formulation.

6.2 Several different acids were considered for the pre-wash before maleic acid was selected. More recently nitric acid and malic acid have been studied as possible alternatives [19], but none of them have given noticeably better performance over maleic acid.

6.3 With regard to the metal component of the formulation, early studies by Fuller and Thomas [6] indicated that solutions based on palladium, rhodium and gold were investigated and although these deposited metal on the surface as expected they did not appear to develop fingermarks. Ramatowski and Cantu [18] reported research into a copper-based physical developer using copper sulphate in place of silver nitrate. Although development was obtained via this route, it has not proved as sensitive as the silver-based system and is not recommended as a replacement for it.

6.4 As part of a drive to reduce the cost of the large quantities of physical developer used by the US Secret Service, revisions to the CAST formula were investigated. These resulted in a revised formulation incorporating malic acid [20,21], with reductions in the concentrations of ammonium iron (II) sulphate, citric acid, both surfactants and silver nitrate.

6.5 The results presented suggested that the above formulation was as effective as the CAST formulation, if not more so. However, no comprehensive trial has yet been reported that compares the two formulations either in laboratory tests or on operationally representative exhibits, and other researchers have also found the methods reported to be difficult to replicate. As a consequence, it is not yet possible to state whether a revision to current (2016) UK practice is required. An adaptation using Tween 20 in place of Synperonic N was developed by the US Secret Service, as a precaution against the possibility of Synperonic N becoming unavailable. This formulation was tested against the CAST formulation in trials of Synperonic N-free systems, and although it gave better results on one-day-old prints it was poorer on prints that were two weeks old. However, the lifetime of the working solution of the Tween 20 – based formulation has been found by the US Secret Service to be longer (~2 months) that the Synperonic N-based system (a few days). Further details of the comparative trial are given below.

6.6 Another revised formulation omitting the maleic acid pre-wash was issued by Yapping and Yue [23], but attempts by other researchers to reproduce this formulation and the results claimed for it were unsuccessful and at present it is discounted.

6.7 Concerns have been expressed about the environmental issues associated with compounds closely related to Synperonic N. These are becoming banned because they closely mimic oestrogen in structure and as these compounds enter the environment they may lead to reduced sperm count and increases in testicular cancer. Recent research in both the USA and the UK has therefore investigated alternative surfactants to

Synperonic N. In the UK study [24], the following alternative compounds were considered.

Surfactant	General description
Tween 20	Polyoxyethylene sorbitan (fatty acid ester)
Tween 80	Polyoxyethylene sorbitan (fatty acid ester)
Synperonic 91/5	Fatty alcohol ethoxylate
Synperonic 91/6	Fatty alcohol ethoxylate
Synperonic 13/6.5	Fatty alcohol ethoxylate
Synperonic 13/8	Fatty alcohol ethoxylate
Caflon-N	Fatty alcohol ethoxylate

Surfactants used in the comparative study.

6.8 The solutions containing the different surfactants were compared with the Synperonic N-based solution and applied to split depletions of several thousands of marks deposited on a range of paper types. The results showed that none of the proposed replacements for Synperonic N gave equivalent performance, the nearest being the formulation based on Tween 20 recommended by the US Secret Service from their own internal research. The long-term availability of Synperonic N remains a concern and therefore it is likely that this study will have to be revisited at some point in the future. One observation that has been made subsequently by the US Secret Service is that the Tween 20 formulation benefits from being used after ageing for several days, and that the solution used in the CAST comparative studies may have been 'too fresh'. This is another factor that requires further investigation.

7. Post-treatments

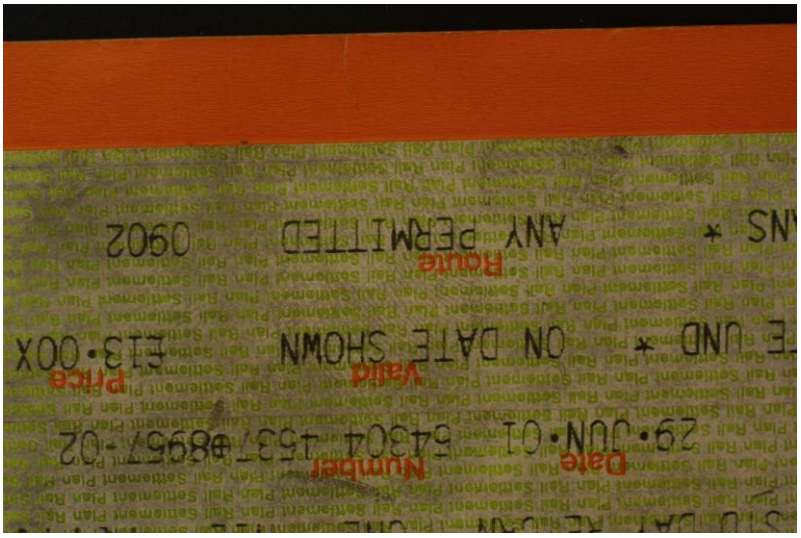
7.1 A range of post-treatments have been proposed for enhancing marks. One of the earliest treatments proposed [6], and now identified as a process in its own right in the CAST *Fingermark Visualisation Manual* [31] is the sulphide toning of developed marks. Sulphide toning is a two stage process that utilises a ferricyanide-bromide 'bleach' solution that reacts with the silver in the mark and converts it to silver bromide. The formulation proposed consists of 5 g potassium bromide and 15 g potassium ferricyanide dissolved in 500 mL distilled water, and the item is immersed in this solution for 5-10 minutes followed by a water wash. This is followed by a second treatment with an alkaline solution of thiourea which converts the silver bromide to the drak grey/black silver sulphide. The toning solution is produced from 25 mL of a thiourea stock solution mixed with 500 mL distilled water, with the stock solution consisting of 5 g thiourea and 20 g sodium hydroxide dissolved in 50 mL distilled water. The item is immersed in this solution for 15-30 seconds prior to a water wash and drying. This process has also been used in conjunction with a radioactive toner, followed by autoradiography to

capture the marks. The principal application of this technique was to reveal developed marks that would otherwise be obscured by highly coloured or patterned backgrounds. In the radioactive toning process, the exhibit is treated with radioactively labelled thiourea. This converts the silver particles to silver sulphide, resulting in the radioactive sulphur being bound into the fingermark ridges. The treated exhibit is then sandwiched between sheets of film for several days, during which radiation emitted from the sulphur causes the film to darken in regions where it is present. On development the film will show all the regions of the film that have become radioactive. The fingermarks will only be more useful if the underlying background, ink or contamination, has not taken up the radioactive sulphur. The radioactive variant of the technique has not been used for many years for reasons of health and safety and practicality, but details are reproduced above for historical interest.

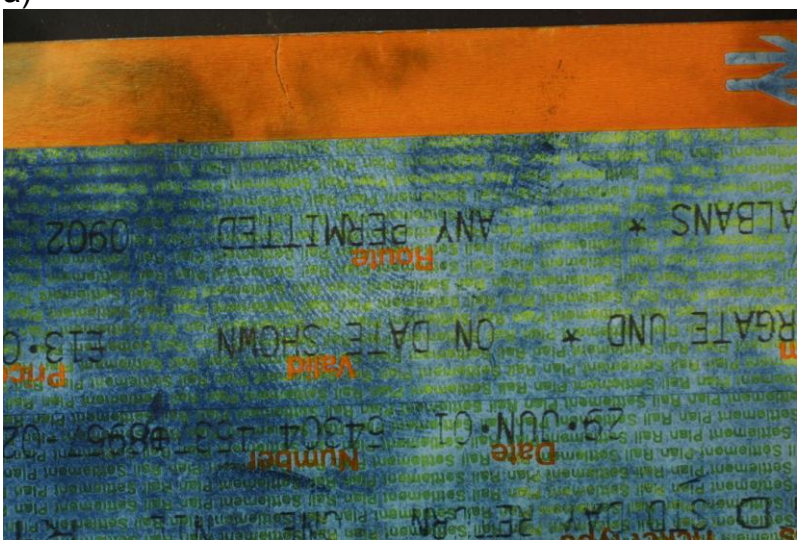
7.2 Bleaching has also been proposed as a technique for both revealing fingermarks on dark papers or as an initial step in the coloured toning of developed marks [19,22,32]. Several techniques have been proposed for treating developed marks, the most widely used technique being iodide toning, proposed by Saunders [28]. This is a two stage process that first uses a hypochlorite 'bleach' solution to oxidise the silver to Ag_2O, which is darker in colour than elemental silver. The bleach also lightens the background and this step alone may improve the contrast of the mark with the background. A second step is then carried out, using a solution containing potassium iodide to converting Ag_2O to the pale coloured silver halide. This is particularly useful on dark coloured papers where the grey silver marks may be difficult to see. The tri-iodide ions in the solution may also react with starch present in paper to darken the background, increasing the contrast with pale white-yellow silver iodide. To carry out the process, a bleach solution is prepared from 3 g sodium hypochlorite dissolved in 1 L of water. The item is immersed in this solution for 3 to 5 minutes, then washed in distilled water for 5 to10 minutes. The toning solution is produced by first preparing a stock solution of 20 g potassium iodide in 100 mL of distilled water, and one part of this solution is then added to 19 parts of the standard physical developer redox solution. The item to be treated is then immersed in the solution until the paper background becomes blue-black (this is thought to be due to the starch in the paper reacting with iodine) and good, light coloured ridge detail is observed. This may take between 15 minutes and 2 hours. The item is then washed again in water before being allowed to dry.

7.3 Alternatively, developed marks can be treated with standard photographic colour toning solutions, e.g. Fotospeed Blue Toner (BT20), using the manufacturer's instructions to change the colour of the mark and enhance its contrast [33]. Any silver deposited on the background will also be toned in this way. Commercially available photographic blue toners generally consist of a combination of chemicals that undergo a series of reactions that convert the grey silver to a blue coloured iron compound. A typical blue toner formulation utilises potassium

ferricyanide, which first reacts with silver to produce silver ferrocyanide (an almost colourless compound). This in turn reacts with ferric ammonium citrate in the toner solution to form ferric ferrocyanide (Prussian Blue).



a)



b)

Marks on a rail ticket obtained by using physical developer a) *as developed and* b) *after bleaching and treatment with blue photographic toner.*

7.4 A final post-treatment that can be applied to articles treated using physical developer is infra-red (IR) imaging, described in detail in Chapter 2, Optical Processes, Infrared imaging. The principle used is that the marks produced using physical developer remain visible in the near IR and some printing inks use organic pigments that are IR transparent. If a camera sensitive in the near IR is used in combination with an appropriate light source and a long-pass filter blocking the visible region of the spectrum, it may be possible to suppress the background pattern and reveal the features of the mark.

8. Validation and operational experience

8.1 There have been a limited number of extensive trials carried out on physical developer, primarily because of its position within sequential processing regimes. Physical developer is only going to be used as the first process on items that are known to have been wetted, where until recently it was the only process that could be considered for this role. It is accepted that physical developer is less effective than DFO, 1,2 indandione-zinc and ninhydrin, but because it develops additional marks when used after them rather than being considered in place of them, large-scale validation using tens of thousands of marks and monitored operational trials (essential when replacing a treatment such as ninhydrin that is used on a daily basis) has been considered unnecessary. Instead, the demonstrated and recorded ability of physical developer to continue to develop marks not found by other processes when used sequentially after them justifies its inclusion in sequences, even though it generally develops fewer marks if used as a single process.

8.2 Laboratory trials

8.2.1 The first reported comparative studies of the effectiveness of physical developer were carried out at AWRE Aldermaston in 1975, where it was compared with the non-flammable ninhydrin formulation being developed by the same research group, and to osmium tetroxide [5].

8.2.2 In this study, single fingermarks from two separate fingers from the same donor were used and these were aged for different periods of time. One set of exhibits was then wetted, and the paper processed using the three processes being compared. A basic, non-numeric grading system was used where:

none = no trace of fingermark;
very poor = traces of fingermark only;
poor = just sufficient for general classification;
good = sufficient detail for identification;
very good = easily identifiable;
excellent = all ridge detail developed.

The results are summarised below.

Age of mark	Number of prints	Dry			Wet		
		Ninhydrin	OsO_4	PD	Ninhydrin	OsO_4	PD
1 day	1	Excellent	Excellent	V. good	None	None	V. good
	2	Excellent	Excellent	V. good	None	None	V. good
5 months	1	Good	Good	Good	None	None	V. poor
	2	Fair	Good	Poor	None	None	Poor
6 months	1	V. good	V. good	Good	None	None	Poor
	2	V. good	V. good	Good	None	None	Good
7 months	1	V. good	V. good	Good	None	None	Poor
	2	V. good	V. good	Good	None	None	Poor

8 months	1	Good	V. good	Good	None	None	Good
	2	Good	V. good	Good	None	None	Excellent
10 months	1	Good	Good	Good	None	None	Poor
	2	Good	Good	Good	None	None	None

Results of early comparative trials on both dry and wetted paper articles.

8.2.3 It was evident that physical developer was not as effective as ninhydrin or osmium tetroxide for marks on dry surfaces, but was the only process to develop marks on paper soaked for 24 hours.

8.2.4 Continuation of this work was carried out at PSDB in 1975 and 1976 and focused on evaluating the effectiveness of physical developer against ninhydrin on paper kept in conditions where the surface became wet [7]. Two trials were conducted, both using split palm prints where one-half were kept indoors under dry conditions and the remainder were exposed to the wet environment.

8.2.5 The first trial exposed palm prints on paper kept exposed to the atmosphere in an outside test rig at PSDB Sandridge over the period November 1975 to January 1976. The grading system below was used.

1 = no reaction;
2 = reaction, no useful ridge structure;
3 = useful, poor contrast;
4 = useful, good contrast;
5 = useful, very good contrast;
6 = excellent.

Week	**£5 Banknote**		**Kraft paper**		**Glazed paper**		**Bond paper**	
	Control	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
1	2+	2	3	4	5	4	3+	3
2	5	3+	5	4	4	4	3	4
4	4	5	3	3+	5	4	5	5
9	-	-	3	4	-	-	5+	4+

Ninhydrin comparison

Week	**£5 Banknote**		**Kraft paper**		**Glazed paper**		**Bond paper**	
	Control	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
1	3+	1	3+	1	4	1	4	1

Results of early trials on paper items exposed to outside environments.

8.2.6 A follow-on test was carried out holding samples in a water immersion rig in the River Thames. This gave the following results.

Day	**£5 Banknote**		**Kraft paper**		**Glazed paper**		**Bond paper**	
	Control	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
1	1	3	3	2	3	4	2	3

2	3	2	3+	4	3	4	3	1
3	4	3	1	1	3	4	5	1
4	3	1	5	3	5	1	5	3

Ninhydrin comparison

Week	**£5 Banknote**		**Kraft paper**		**Glazed paper**		**Bond paper**	
	Control	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
1	3+	1	3+	1	3+	1	3+	1

Results of early trials on paper items immersed in the River Thames.

8.2.7 It was evident that for wetted surfaces ninhydrin gave no reaction, and that physical developer should be the development technique of choice.

8.2.8 The most recent laboratory trials conducted by CAST focused on comparisons of the existing physical developer formulation with those based on the alternative surfactants identified in the 'alternative formulations and processes' section above. The solutions containing the different surfactants were applied to split depletions of several thousands of marks, deposited on a range of paper types consisting of:

- brown envelope;
- white envelope;
- parchment paper;
- magazine;
- newspaper;
- printer paper;
- green card;
- silk finish paper;
- wove paper.

8.2.9 The number of marks scoring three and four (equating to clearly identifiable marks) were recorded for each process on test strips aged for one day and two weeks. The differential between the number of marks graded three and four between the two techniques is recorded below, with negative scores indicating that the surfactant performed worse than Synperonic N.

	One-day differential	**Two-week differential**	**Average differential**
Tween 20 (US Secret Service formulation)	11	-15	-2
Synperonic 91/6	-3	-7	-5
Tween 80	-6	-6	-6
Caflon-N	-7	-9	-8
Synperonic 91/5	-5	-18	-11.5
Synperonic 13/6.5	-22	-18	-20

Tween 20	-33	-40	-36.5

Performance of different surfactants in physical developer solution relative to Synperonic N.

8.2.10 The results show that none of the proposed replacements for Synperonic N gave equivalent performance, but the formulation based on Tween 20 recommended by the US Secret Service from their own internal research gave improved performance on fresher marks and at present would be the formulation recommended once Synperonic N becomes unavailable. Production of Synperonic N is being discontinued so it is necessary to implement a revision to the surfactant used, and further refinements to formulation are likely to be required to ensure that there is minimal detriment to the performance of physical developer for fingermark development unless a more suitable replacement is identified.

8.2.11 CAST conducted a further study in 2010-2011 to explore optimum sequences for treatment of brown papers and cardboard [34]. This study included a comparison between physical developer and silver nitrate as the final treatment in a sequence following amino acid reagents, and also considered the use of the two processes in sequence. These results are described in detail in Chapter 3, Chemical and Physical Processes, Silver Nitrate, but concluded that physical developer was more effective than silver nitrate across a wider range of surfaces.

8.2.12 Laboratory and simulated field trials that CAST have initiated [35,36] have also indicated that as paper is exposed to increasingly high temperatures (above 150°C), the effectiveness of the amino acid reagents DFO and ninhydrin decreases but physical developer continues to develop marks, even on paper that has begun to char.

8.3 Pseudo-operational trials and operational experience

8.3.1 The results of the operational trials conducted in 1977 prior to implementation of physical developer throughout the UK [9] are summarised below.

	Number of marks			%		
	New Scotland Yard	Sussex (1–29)	Sussex (30–90)	New Scotland Yard	Sussex (1–29)	Sussex (30–90)
Neither process +ve	11	0	6	28.9	0	10
Ninhydrin +ve PD -ve	6	13	8	15.8	46.4	13.3
Ninhydrin -ve PD +ve	11	0	8	28.9	0	13.3
Both processes +ve	10	15	38	26.4	53.6	63.4
Total cases	38	30	60	-	-	-
Total articles	433	69	234	-	-	-

Operational casework results obtained by applying physical developer after ninhydrin on a range of porous articles.

8.3.2 There were differences between the results obtained at different sites and between different phases of the work at the same site, but in general it was observed that physical developer consistently developed additional marks when used after ninhydrin. In the first phase of the work at Sussex the physical developer solution was degraded by exposure to light and results were poor, in the work at New Scotland Yard exhibits were selected because they were less likely to give positive results using ninhydrin, hence the results obtained in the second phase of the work at Sussex (where all exhibits passing through the laboratory were processed) were probably most representative. In the course of the trial the value of the technique in developing marks on wetted items was confirmed, as was the usefulness of the radioactive toning post-treatment for revealing marks on patterned backgrounds. The subsequent operational recommendations that physical developer should be used sequentially after ninhydrin and as a treatment for wetted paper items were supported by the results above.

8.3.3 Further evidence on the capability for physical developer to develop additional marks as the final treatment in sequential processing and to develop marks on older documents is provided by the work conducted by Fitzgerald in 2003 [27]. In this study, batches of fraudulently passed cheques were used. Twenty five cheques were collected from each of four banks, and a maximum of four cheques taken from any single case.

Name of Bank	**Year(s) of Cheque(s)**	**Oldest age of Cheque(s)**	**Number of cases**
Natwest	91- 95	12yrs	12
Co-op	92, 96 +97	11yrs	11
Midland	87	16yrs	12
Barclays	86	17yrs	12

Summary of the age range of cheques used in the 2003 study [27].

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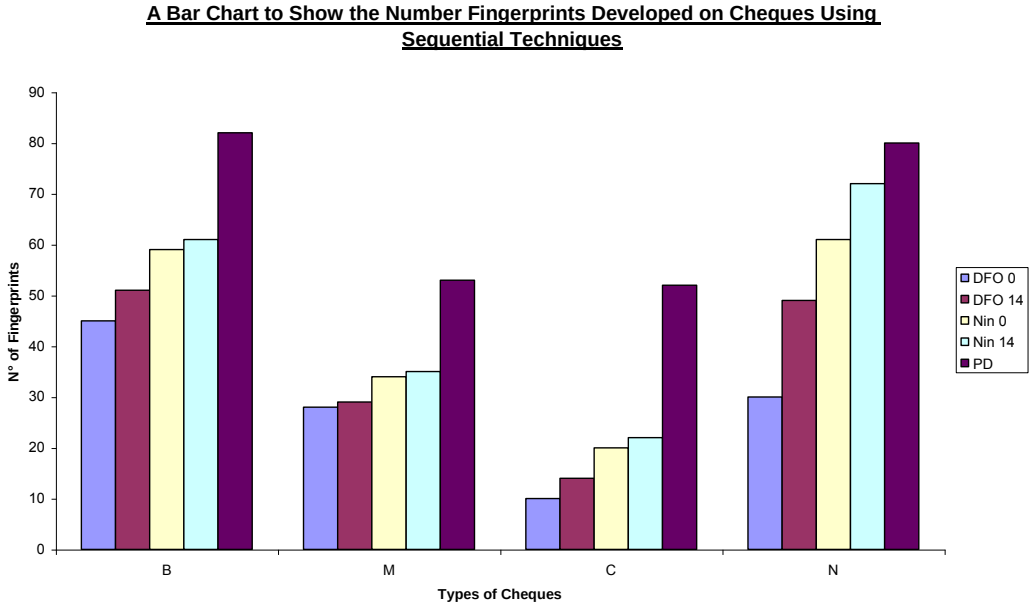
8.3.4 All 100 cheques were treated with DFO, marked up, then placed into a drawer for a period of two weeks to see if further development of ridge detail with DFO would occur. The cheques were then sequentially treated with ninhydrin, and also marked up immediately after treatment and again after 2 weeks in a dark environment. The cheques were then treated with physical developer before being marked up a final time. The results for the cumulative number of marks obtained from this sequential processing experiment are shown below.



Results of sequential processing of 100 cheques, showing the cumulative number of marks developed after each processing step.

8.3.5 It is apparent that physical developer is capable of developing a significant proportion of additional marks when used at the end of processing sequences, and also remains effective on paper items over 10 years old.

8.3.6 The original recommendations for operational implementation are still supported by operational results where physical developer continues to develop additional marks as the final stage of sequential treatments and as the sole treatment for wetted items. In some cases the items treated have been over 25 years old and have been immersed in water.

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Powders

1. History

1.1 The use of powders is one of the oldest reported techniques for development of latent fingermarks. Faulds, in his publication 'Dactyloscopy, or the study of fingerprints' of 1912 [1], refers to the experiments conducted by Forgeot in the late 19th century as the first studies into the powdering technique, and also comments on subsequent experiments of his own [2]. By 1912 Faulds [1] was able to describe formulations and application techniques for both black and white powders, and by 1920 many more types of powders had been reported for development of fingermarks, including mercury-chalk (hydrargyrum–cum–creta), graphite, lamp black, ferric oxide, magnesium carbonate, aniline dye stuffs, lycopodium powder-Sudan Red mixture, red lead oxide, lead carbonate, lead iodide and lead acetate [3]. By the end of the decade a further selection of fingermark development powders had been reported, including the first references to the use of aluminium powder. The purpose of many of these materials was to provide investigators with a range of different coloured powders that could be used to both develop a crime scene mark and provide contrast with coloured backgrounds. Some of these early powders persisted in use for many years. Mercury-chalk was still in use in the UK in the 1970s, and carbon black-based powders remain in use worldwide to the current day (2016).

1.2 Another technique for providing contrast between the developed mark and the substrate and considered relatively early in the history of fingermark development was fluorescence. Zinc sulphide and anthracene were proposed as fluorescent dusting powders in the 1930s [4], with the developed marks being illuminated with long-wave ultraviolet (UV) radiation to promote phosphorescence and luminescence respectively. Variants of these powders were still being recommended for development of latent fingermarks on multi-coloured surfaces in 1954 [5].

1.3 The range of powders that have been formulated and marketed for fingermark development in the intervening years far exceeds the number of chemical development techniques, and more enter the market every year. Some examples of powder 'recipes' that have been used by police forces in the past [6,7] but that are now predominantly obsolete, are given in the table below.

Colour of powder	Constituents	Wt% of constituent
Black	Lamp black Graphite Gum acacia	70 20 10
	Black magnetic ferric oxide Rosin Lamp black	50 25 25

White	Titanium dioxide Kaolin French chalk	67 16.5 16.5
	Titanium dioxide Basic lead carbonate Gum arabic	33.3 33.3 33.3
Grey	Mercury Chalk Aluminium powder	25 50 25
	Basic lead carbonate Gum arabic Aluminium powder Lamp black	87.5 12.5 trace trace to give colour
Red/orange	Red lead oxide Rosin	33 67
	Lycopodium Sudan Red III	90 10
Fluorescent	Anthracene White tempera	50 50

Published formulations for various types of early fingermark powders.

1.4 These early powder formulations do not appear to have been devised by any standardised testing system, nor were any recorded comparative trials carried out to establish which formulations were most effective. Their use was often according to the personal preferences of the person treating the marks at the crime scene rather than any scientific assessment of which powder was most appropriate for a particular type of surface. As a consequence, no single type of powder predominated and many local variations in practice arose worldwide.

1.5 Some of the constituents used in early fingermark powder formulations were toxic or carcinogenic and their prolonged use could cause health problems. The best documented of these problems is the occurrence of mercury poisoning among officers in UK police forces [8,9], initially reported in the late 1940s and caused by the use of mercury-chalk powder. Although most of these powder formulations have since been withdrawn, it is still recommended that users consult material safety data sheets before employing any new type of powder.

1.6 Many powders used for fingermark development in the first half of the 20th century were also granular in nature, typically applied with animal hair brushes. Photography of the marks developed by powdering was almost exclusively carried out in situ. Developments in the 1960s meant that alternative types of powders began to become more widely used. The first of these developments was the ‘Magna brush’ in the early 1960s [10], consisting of a retractable bar magnet within a non-magnetic cover material. When dipped into a pot of magnetic powder, a brush-like head of powder became attracted to the magnet, which could then be drawn across the surface like a hairbrush. A range of magnetic powders

were soon developed for use with this brush. The second development was the increasing recognition that aluminium flake powder, already in operational use in the 1950s, had a combination of properties that made it ideally suited for use with lifting media, thus overcoming the need for photography in situ and enabling the separation of the developed mark from backgrounds that may have made photography difficult.

1.7 PSDB has carried out several unpublished surveys of the types of powders in use in police forces around the UK, showing the progressive change in the types of powders used. The use of aluminium flake powder and subsequent lifting of the mark was adopted by the Metropolitan Police around 1971 after observing the practice in the USA. The principal objective of this change in practice was to avoid the transport of exhibits for photography; although it was argued that results were as good as or better than previous procedures. It was widely adopted around the UK over the next 5 to 10 years, although two forces were still almost exclusively using white and black powders into the late 1980s.

1.8 Since then, aluminium flake and magnetic powders have been increasingly used in place of granular powders and the types of powder currently (2016) in widespread use can be grouped into four main classes, namely:

- metal flake powder (e.g. aluminium and bronze);
- granular powder (black and white);
- magnetic powders;
- fluorescent powders.

1.9 The categories above represent a general classification, the actual number of powder formulations that are available on the world market can be numbered in the hundreds and some formulations actually fall into more than one category. Each of these different powder types have particular types of surface to which they are most suited – there is no one powder that will consistently develop marks of optimal quality on all surfaces. However, despite this recognised performance variation there is very little reported evidence of large-scale comparative studies to demonstrate the relative effectiveness of powders other than the experiments carried out by the Home Office Scientific Development Branch (HOSDB) [11,12,13]. These comparisons were limited to a small number of powders identified as being representative of the general categories by a survey of police force scene of crime units and by preliminary evaluations. By tracing the commercial powders back to source it was established that many differently labelled products were in effect the same powder, and some other less-used powders performed poorly in early trials and were therefore eliminated from subsequent studies. This enabled the large-scale trials to focus on powders that were effective, and/or widely used. A methodology is presented [11] that allows researchers to carry out similar comparative assessments for any new powder system.

1.10 Powdered marks probably account for the largest number of fingerprint identifications worldwide, in the UK alone approximately 50% of the approximately 50,000 fingerprint identifications per annum arise from marks developed using this process. It is therefore evident that even the small proportional improvements that can be achieved by the selection of the optimum powder and brush combination for a particular surface have the potential to provide significant operational benefits, and further study of this area will be required.

2. Theory

2.1 The development of fingermarks by powdering occurs by preferential adhesion of powder particles to the ridges, with the background material having less affinity for the particles. This means that powders should not be used where surfaces are sticky or heavily contaminated because the particles will generally not be able to discriminate between the constituents in the fingermark residues and the contaminant, and will adhere across the entire surface.

2.2 The factors that are thought to play a role in promoting powder particles to adhere to fingermark ridges are:

- particle shape;
- surface chemistry of the powder particle;
- electrostatic charge on the particle;
- adhesion to grease or liquid;
- low(er) adhesion to the substrate.

The overall adhesive effect of a particle to a fingermark ridge is likely to be a combination of all these factors and therefore no one dominant mechanism can easily be identified.

2.3 In terms of particle shape, it has been suggested that flake powders are more sensitive than granular powders because their shape gives them a higher surface area and hence better contact with the fingermark deposits.

2.4 With regard to surface chemistry, it is known that the adhesion of a powder particle to a solid surface in air or a gaseous medium is partly due to molecular forces [14]. It is therefore anticipated that changing the molecules on the surface of the powder particle will have an effect on the interaction between that particle and the medium it adheres to. It has been demonstrated that surface coatings do play a role in the effectiveness of metallic flake powders for fingermark development. Experiments conducted by James *et al.* [15,16] demonstrated that flake powders without stearic acid coatings were poor for fingermark development, irrespective of flake diameter. Further investigation of stearic acid coating thickness showed that optimum results were obtained for a coating thickness of 70 nm.

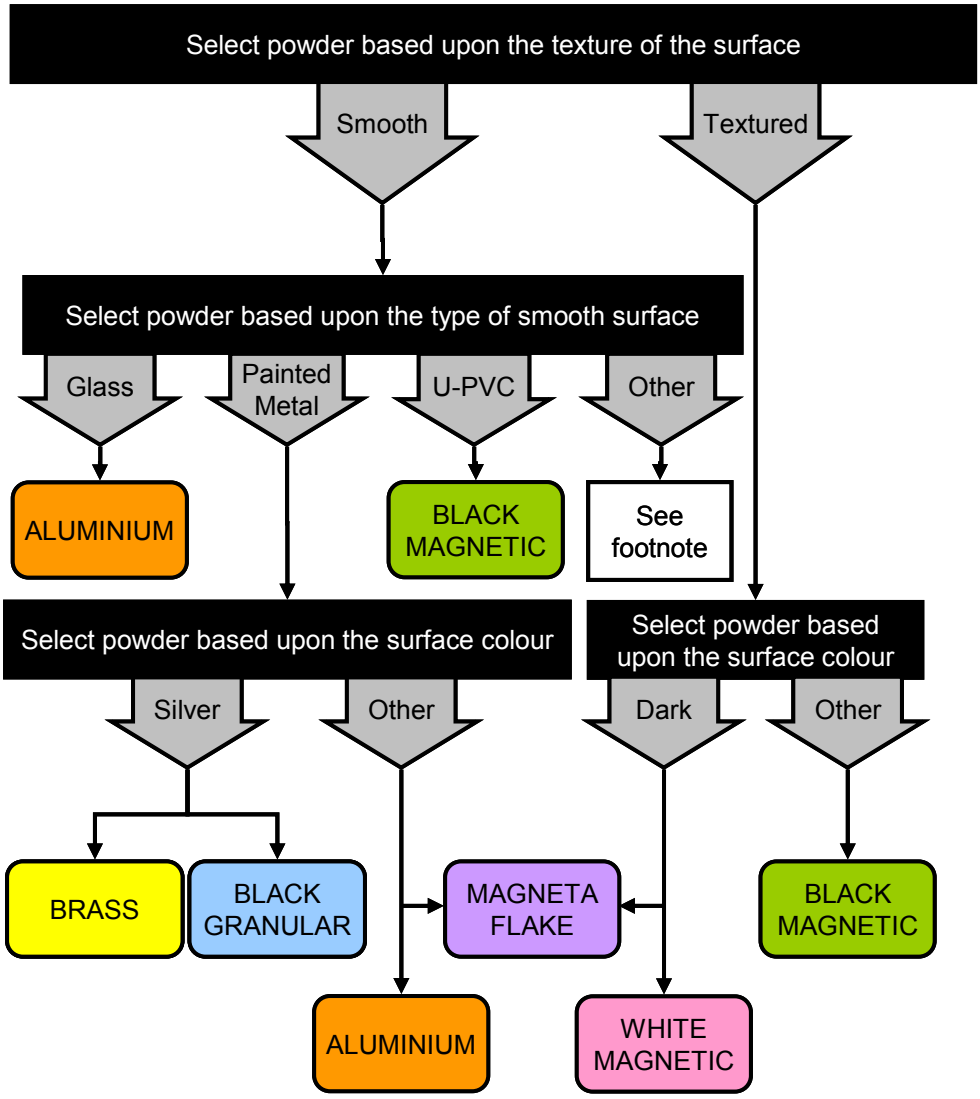
2.5 Electrostatic charge can potentially make large contributions to adhesion. It has been stated [14] that if particles are highly charged, the value of the attractive Coulomb forces exceeds that of other contributions to adhesion. Researchers have investigated various ways of utilising this effect for enhancing fingermark development using powders, but it is not the major mechanism used in any of the types of powder widely used at crime scenes.

2.6 The presence of liquid or grease in a fingermark deposit will promote adhesion of the particle to it for two principal reasons. The first is that the liquid is able to wet the surfaces, thus giving a greater contact area for the powder particles. The second is the capillary force of the liquid caused by surface tension. In atmospheres of relative humidity in excess of 70% the increase observed in the adhesion of microscopic particles is due to capillary forces. It has been suggested that in dry climates or for fingermarks that have dried out, 'huffing' (blowing warm, humid air or breath over the mark) or rehumidification prior to powdering may improve the quality of the developed mark [17].

2.7 Once the initial layer of powder particles have adhered to the fingermark ridge, the process of auto-adhesion (the interaction between individual powder particles) becomes important. In the case of aluminium powders it is suggested that repeated passes of the brush are used to 'build up' the mark, indicating that strong auto-adhesive bonds do exist between aluminium powder particles. For powdering with magnetic flake powders, a single sweep of the applicator is suggested, with further passes thought to 'fill in' or reduce the quality of the fingermark. This indicates that auto-adhesive forces between magnetic flake particles are weak, and there is a possibility that the magnetised particles may repel each other. The shape of the particles may also play a part in how the marks build up during powdering. For example, flake powders may be considered more like sheets of paper; the 'sheets' can be piled on top of one another up to a point where the pile collapses or merges with another pile (which may be the case in over-powdering); whereas granular powders are more like spheres and when 'spheres' are placed on top of one another there becomes a limit where a conical shape is achieved and no more can be added.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. CAST recommendations suggest the use of several different generic types of powder, the advice regarding selection being dependent on the type of surface being treated. The current (2016) recommendations are as follows [18]:



Home Office Centre for Applied Science and Technology flowchart for the selection of powders.

3.2 Aluminium flake powder is the most effective powder on glass, but shows similar performance to several alternative powders on other smooth, non-porous surfaces. For these surfaces aluminium may still be the powder of choice as it is easy to apply and develops good contrast marks on most smooth surfaces. The most effective applicator for use with aluminium powder is the Zephyr-style glass fibre brush. Although monitored trials established that exposure to aluminium dust in normal usage is an order of magnitude lower than allowable exposure limits, dust masks should be used with this powder when used in confined environments.

3.3 Brass (copper/zinc alloys commonly referred to as ‘bronze’ or ‘gold’ due to their colour) flake powders perform similarly to aluminium flake powder, but should only be used on smooth, silver coloured surfaces where aluminium would give low contrast. An appropriate dust mask must be worn when using this type of powder because the exposure

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limits for this type of powder are lower than those for aluminium flake and can be exceeded during normal use.

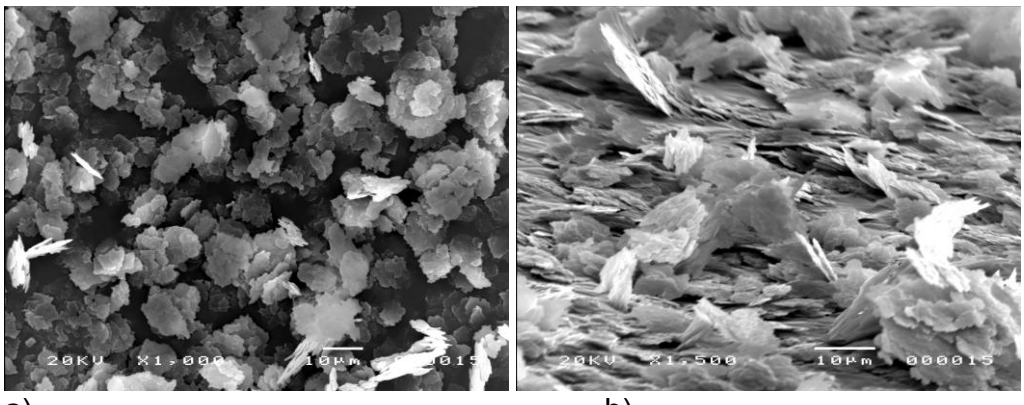
3.4 Black granular powder may be used on some smooth surfaces only and can be considered as an alternative to brass flake powder on silver coloured surfaces. Dust masks should be worn when using this powder.

3.5 Black magnetic powder is the most effective powder on textured surfaces and unplasticised polyvinylchloride (uPVC). Similar results were obtained with 'jet black' magnetic powder, but others (grey, silver, etc.) were found to be considerably less sensitive. White magnetic powder, although less sensitive, may be used on dark, textured surfaces when contrast between the developed mark and the surface is an issue.

3.6 Magneta Flake powder is slightly less sensitive than black magnetic powder on textured surfaces, but may offer an alternative on dark textured surfaces. It may also be used on most smooth surfaces although application can be difficult and inconsistent.

3.7 Further information on each type of powder is given below.

3.8 <u>Aluminium powder</u>
The aluminium powder that was widely used throughout the UK was either 'Aluminium Super 8000' or 'Offset 901', both supplied by Wolstenholme International Ltd. Wolstenholme has recently been taken over and a closely equivalent powder is now supplied by the new parent company (Eckart Effect Pigments). Small-scale tests and microscopy indicate no significant differences in morphology or performance. They are metal flake powders, with smooth surfaces and jagged edges. The diameter of the particles falls within the range 1 to 12 μm and the thickness is approximately 0.5 μm. The flakes are coated with stearic acid during the milling process to prevent clumping.



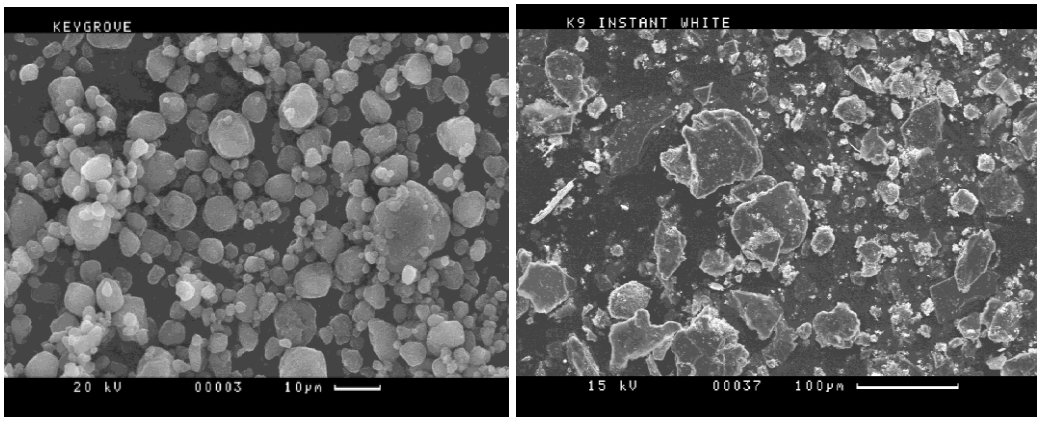
a) b)

Scanning electron micrographs of Wolstenholme Super 8000 aluminium powder showing a) *flakes viewed from above and* b) *flakes viewed from the side.*

3.9 Granular powders

Most black granular powders are carbon-based. The main carbon supplier in the UK is Cabot Ltd, which supplies most forensic providers with the Elftex 415 grade of carbon powder. This is an amorphous, elemental carbon with a particle size in the range 5 to 10 μm and a textured, irregular (but smooth) shape.

3.10 White powders may contain more than one particle type. The example shown below consists of large flakes of magnesium silicate (20 to 100 μm in size) with small granules of titanium dioxide (mostly smaller than 1 μm). The small granules coat the surface of the flakes, suggesting that the flakes act as the carrier for the titanium dioxide granules.



a) b)

Scanning electron micrographs of typical a) *black and* b) *white granular powder.*

3.11 Magnetic powder

There are two distinct types of magnetic powder used in the UK, Magneta Flake and black magnetic powder (traditionally called Magna powder). Magneta Flake was developed as part of a joint project between the Home Office and the University of Swansea in the early 1990s [15,16] and is now manufactured and distributed by CSI Equipment Ltd. It is produced by milling spherical carbonyl iron with 3 to 5% stearic acid in an appropriate solvent to produce a smooth edged flake with particle sizes in the range 10 to 60 μm. Other types of magnetic flake powder are now available from other suppliers.

3.12 Black magnetic powder has a substantially different microstructure, consisting of large magnetic carrier particles of elemental iron (20 to 200 μm) and smaller non-magnetic particles of iron oxide (Fe_3O_4) with a particle size in the range 3 to 12 μm. The larger particles act as a carrier medium for the smaller particles, which adhere to the fingermark ridges and develop the mark.

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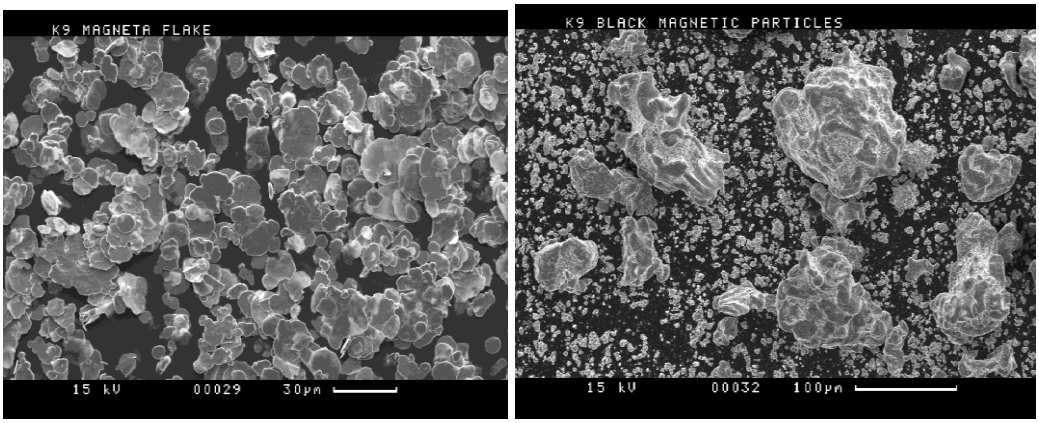


a) b)

Scanning electron micrographs of magnetic powders a) *Magneta Flake and* b) *black magnetic powder.*

4. Critical issues

4.1 There are several critical issues to consider before powdering a surface. Before any powder is applied, a search should be made using a white light source to establish whether any visible marks are present. These should be captured before proceeding because not all marks found in this way will subsequently develop using powders.

4.2 An assessment should be made of the surface itself. If the surface is heavily contaminated, highly textured and/or porous, powdering may not be the best technique to use and alternative processes should be considered.

4.3 The type of powder used should be selected according to the nature of the surface, choosing both a powder type known to work well on that surface and a powder colour that gives a good contrast with the background.

4.4 The means of application should be compatible with the powder selected. Aluminium powders are best applied using a glass fibre Zephyr brush, magnetic powders using a magnetic applicator, and granular powders using a soft mop style of brush.

4.5 The decision on whether to lift the mark or to image in situ must be made according to the type of powder used. Aluminium (and brass) flake powders are well suited to lifting, magnetic and granular powders may be better imaged in situ first. However, regardless of the powder used there is always the possibility of damage during lifting and photography of the mark in situ should always be considered as a first option.

4.6 The sequential use of powders should be considered. It is possible that marks will not be developed by one type of powder, but may be subsequently enhanced by use of a different type.

5. Application

5.1 Suitable surfaces: Powders can be used on all non-porous types of surface including glass, plastics, metals, painted and varnished wood and ceramic, although they may not be the most effective process for that surface. In general, as the surface becomes rougher and more porous, the less effective powdering is likely to be.

5.2 The principal application of powders is the development of fingermarks on smooth non-porous surfaces at crime scenes, although recent research has shown them to be a valuable method for finding marks on textured or semi-porous surfaces such as wallpaper. The brush application method allows large areas such as windows, doors and door frames to be speculatively treated without recourse to more messy or time-consuming chemical treatments. The speed and effectiveness of the technique makes powders well-suited to volume crime applications. The fact that other treatments (such as blood dyes, powder suspensions and superglue) can be used sequentially after powdering also makes it an important first treatment at serious crime scenes.

5.3 In the laboratory, powders can be used on non-porous exhibits where it is suspected there may be a mixture of latent prints and marks in blood. This is because they can develop both types of mark and have no detrimental impact on subsequent treatment with blood dyes (unlike the alternative treatment option, superglue).

5.4 Powders should not be used if it is suspected that a surface is contaminated with any sticky residues (e.g. foodstuffs, oils) because powder will adhere to the entire surface and marks will not be resolved.

5.5 The means by which the powder is applied to the surface can also affect the quality of the mark. It has been recognised [19] that marks may be damaged by poor powdering practice and/or the use of the wrong type of brush. Similarly, brush application may often develop surface texture instead of the mark, and selection of an appropriate applicator may in some cases be more important than selection of the correct powder. HOSDB carried out extensive studies [11,20] to determine the optimum brush for use with aluminium powder and concluded that glass fibre, Zephyr-style brushes gave the optimum combination of ridge detail developed, contrast of the developed mark and minimal brush damage. This is because the glass fibre brush retained the powder well and released it gradually, which is most compatible with the gradual build up of the marks produced with this type of powder. In contrast, squirrel hair, mop style brushes give significantly worse performance in all three respects for aluminium powder, but are the most widely used brush for use with granular powders.

5.6 Magnetic powders (both black magnetic and Magneta Flake) are applied using magnetic wand applicators, where a small magnet in the tip of the wand picks up a 'brush' of powder when dipped into the powder container. This powder 'brush' is then applied to the surface, thus avoiding any direct contact between the applicator and the surface. Although such powders are relatively easy to apply to horizontal surfaces, application to vertical surfaces is less straightforward and powder may drop off. Ease of application to a particular surface should be taken into consideration when selecting the powder to use.

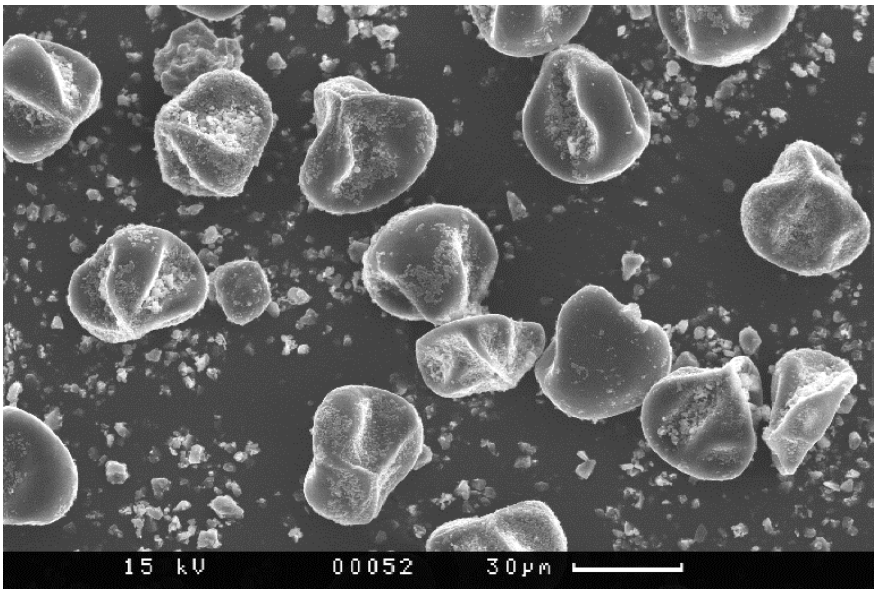
6. Alternative formulations and processes

6.1 There are many different types of powder being sold for fingermark development applications and it is not possible to evaluate every product on the market. As a consequence, the advice given in the powder selection flow chart above refers to generic powder types only and not to a specific manufacturer's products. It is known that several nominally similar products are now available on the market (e.g. 'Magneta Flake' and 'Mag100') and not all of these have been tested by CAST.

6.2 It is possible that some products may give better performance than those covered in the existing CAST guidance. If the use of a product not currently (as of 2016) within the generic powder types outlined above is proposed, it should be extensively evaluated against the existing powder types in laboratory trials on representative surfaces before being used operationally. The guidance given by CAST originates from tests utilising thousands of developed marks, and any trials recommending changes to that guidance should incorporate an equivalent number.

6.3 PSDB funded work in the mid- to late-1970s to develop an electrostatic powder process for developing fingermarks at scenes of crime [21-25]. The perceived advantages of the technique were that it could develop fingermarks without making any contact with the latent mark, and that the developed mark could be enhanced by removing excess powder without damaging the mark. The concept proposed by Roy [22] was the use of a positively charged high voltage electrode introduced above a quantity of powder within an insulating container to attract a powder coating onto the electrode. Holding the electrode over a surface bearing a fingermark resulted in the formation of a powder cloud, with charged powder particles moving between powder and surface and some being retained on the fingermark ridges. Several different powders were studied, the most appropriate for this purpose being found to be the semiconductor calcium tungstate ($CaWO_4$). Work was also carried out to develop a practical apparatus for powder delivery [21] and to explore mechanisms of deposition and cheaper alternative powders to calcium tungstate [23-25]. Ultimately the system did not enter widespread operational use, possibly because of limited benefits over conventional powdering, coupled with the added complexity of the application device compared with brushes.

6.4 Another widely available class of powder is fluorescent powders. These have been proposed for use on multi-coloured and textured surfaces where it may be difficult to visualize marks developed by other powders. Fluorescent powders can also be used to enhance marks developed using other processes, such as cyanoacrylate fuming. Most commercially available fluorescent powder formulations incorporate large organic particles such as corn starch to act as carriers for the fine fluorescent particles, which are produced in a range of colours (e.g. red, green, yellow) giving a range of powders that can be selected to give optimum contrast with the surface.



Scanning electron micrograph of a fluorescent powder, showing large corn starch particles and fine fluorescent dye particles

6.5 Another emerging class of powders are functionalised nanopowders. Different types of functionalised powder have been reported, some tagged with antibodies to give highly selective adhesion to species in fingermarks [26] and others that adhere to fingermarks by conventional means but subsequently provide a means of enhancing the analysis of the fingermark composition [27]. Some of these powders are now becoming commercially available.

7. Post-treatments

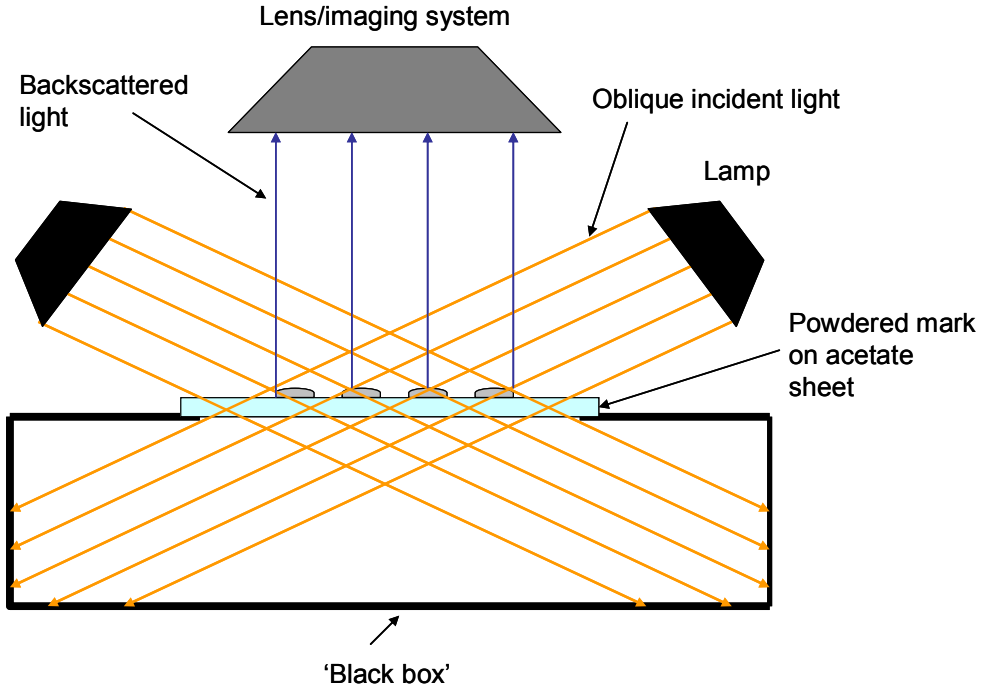
7.1 The main post-treatment for powdered marks is that of lifting. Advantages of this process include the fact that it enables a large number of marks developed using powder to be rapidly collected from a scene, it removes the powdered mark from the background environment it has been developed on and thus makes imaging of the marks in isolation easier, and it removes many issues associated with the level of skill of the crime scene photographer in capturing a good quality image.

7.2 To counter this, some disadvantages are that lifting may remove contextual information about the environment the mark was found in, and the quality of the lifted mark is potentially degraded from the mark developed in situ because some powder remains on the surface while the remainder adheres to the lifting medium. Lifting is most compatible with flake powders, it is less appropriate for granular and magnetic powders and may cause greater degradation to the quality of the lifted mark for these powder types. If it has been decided that the developed mark is to be lifted there are several types of material that can be used as lifting media, including:

- adhesive tapes and sheets;
- gelatine lifts;
- casting compounds.

7.3 In common with powders and brushes, selection of the optimum lifting medium for a particular type of mark may improve the quantity and quality of the marks recovered. However, there are few extensive published studies in this area.

7.4 The lifting process is principally used for aluminium powdered marks, but may be used for marks developed using other types of powder. For marks developed using aluminium flake, clear adhesive tapes are most commonly used as the lifting medium. The lifted mark is stuck to a clear acetate sheet, which is then retained as the exhibit. The contrast between the reflective aluminium powder and the transparent tape and acetate can be utilised to capture images of the lifted mark. Techniques used include contact printing using equipment such as the Camtac (although the advent of digital imaging is leading to this method becoming obsolete), scanning using a glossy black backing sheet, or using a ‘black box’ to enhance the contrast.



'Black box' imaging arrangement used to enhance contrast of aluminium lifts.

7.5 The type of lifting tape used does have an effect on the quality of the mark, and some studies have been carried out to assess this [28]. However, in practice there are few, if any, adhesive tapes produced solely for forensic use and it is difficult to ensure that any particular tape type will perform consistently from roll to roll. For this reason, CAST does not recommend any specific brand of lifting tape. Small-scale tests by CAST indicate that black gelatine lifts may actually be better than adhesive tape in lifting aluminium powdered marks, but this type of lift is more expensive, and more difficult to store and transport than tape lifts and is not routinely used.

7.6 Other recent developments associated with the lifting process include the introduction of wireless transmission of the fingermark image from the crime scene to the fingerprint bureau. One approach [29] uses a flatbed scanner with a gloss black backing paper to scan the aluminium lift and image compression software to reduce the file size to a level that can transmitted over a mobile phone network in around 30 seconds.

8. Validation and operational experience

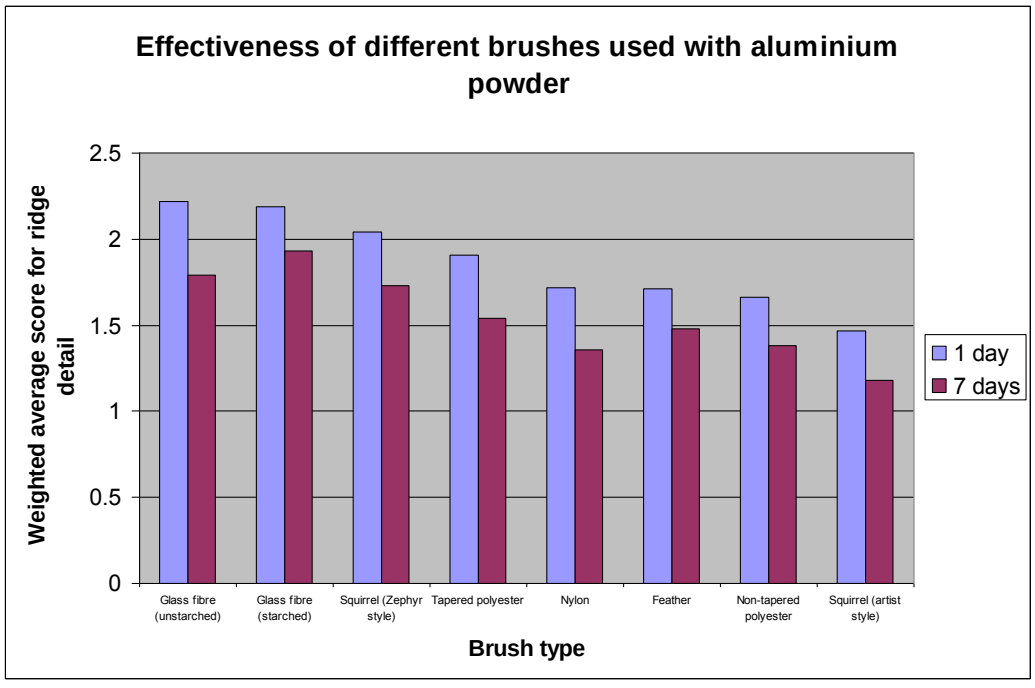
8.1 Powdering is a very important process for fingerprint identification, with approximately 50% of fingerprint identifications (in excess of 25,000 per annum) being obtained from marks developed using this technique. As a consequence, any improvement in the effectiveness of powdering or

guidance associated with its application has the potential to provide a significant number of additional identifications.

8.2 Laboratory trials

8.2.1 CAST has conducted extensive laboratory trials on both powders and the brushes used to apply them. Each study has involved the development and assessment of approximately 10,000 fingermarks.

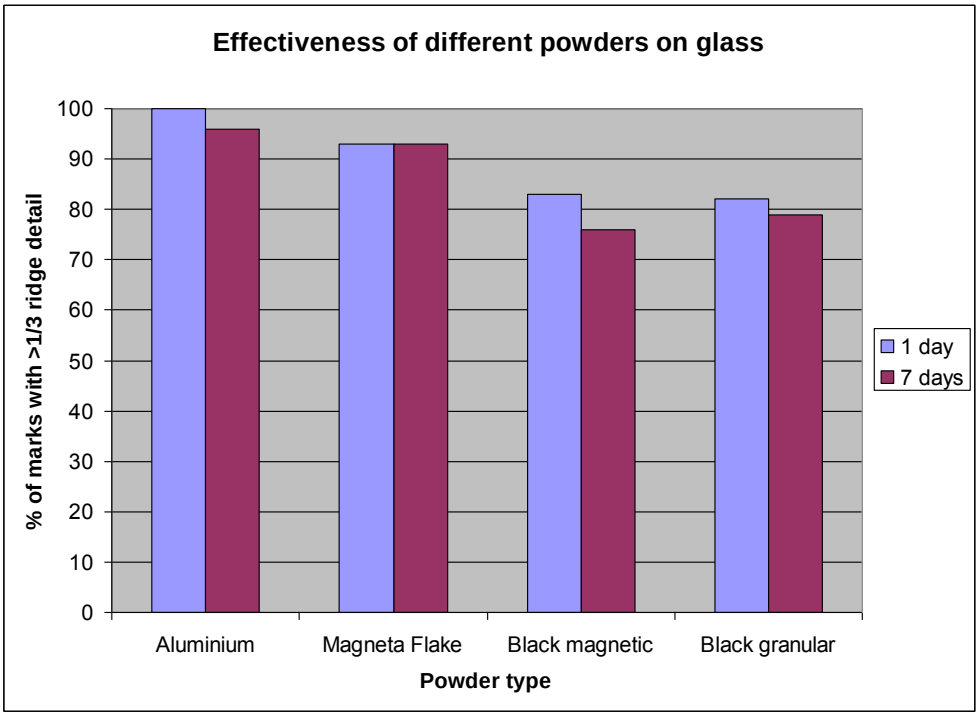
8.2.2 Surveys carried out on powdering practice in the UK confirmed that by far the most widely used powder was aluminium, although many different brushes were being used for its application, including glass and polyester Zephyr, feather, and squirrel hair. The initial study [11,20] looked at the most effective brush type for the application of aluminium powder. Trials were carried out on four surfaces identified by a survey of scene of crime officers (SOCOs) as those most representative of those found at crime scenes, namely glass, uPVC, painted wood and painted metal. In all 12,640 marks were powdered and graded in terms of ridge detail developed, contrast and brush damage. The conclusion of this work was that glass fibre, Zephyr-style brushes gave the best results for this type of powder.



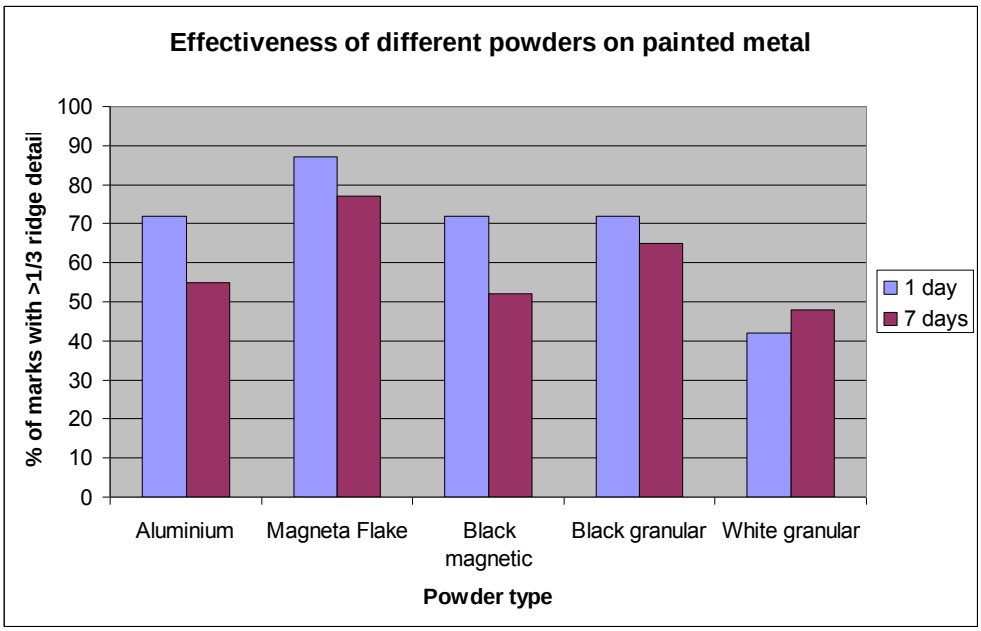
Summary of results obtained comparing the effectiveness of different types of brush used with aluminium powder [11].

8.2.3 It was recognised that although aluminium is routinely applied to all the surfaces used in the trial summarised above, it may not actually be the best powder to use in all cases. The next stage of the work [12] therefore compared the effectiveness of aluminium against other types of regularly used powder on a series of smooth, non-porous surfaces (glass, painted metal, ceramic and gloss painted wood). Approximately 1,500 marks

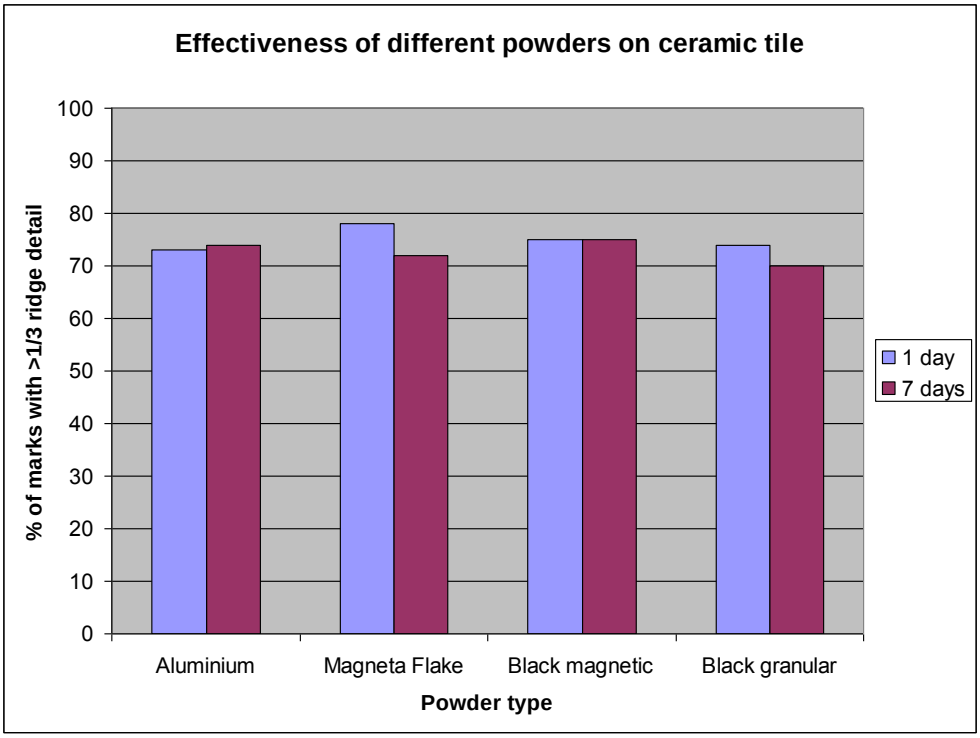
were developed on glass and approximately 2,500 on the other three surfaces. The results are shown below.



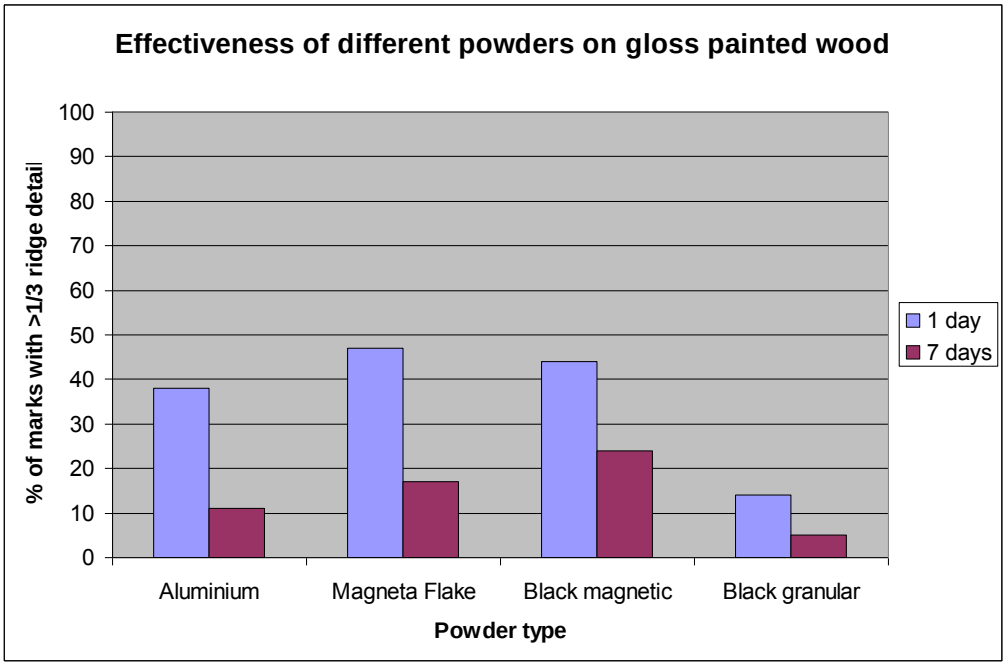
Comparison of different powder types on glass surfaces [12].



Comparison of different powder types on painted metal surfaces [12].



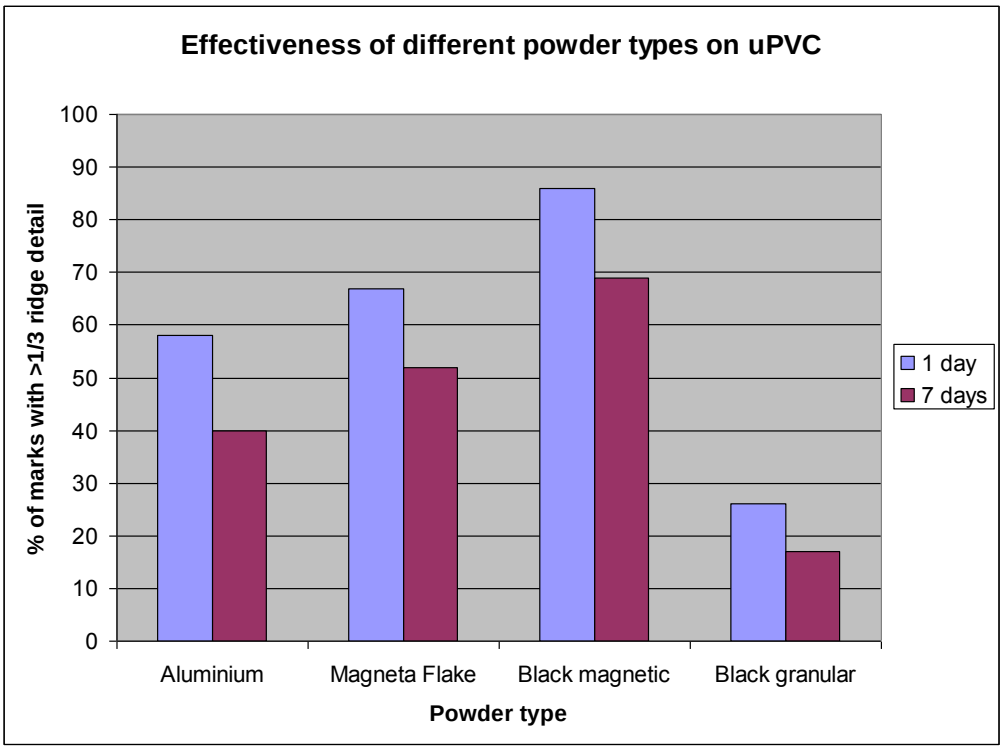
Comparison of different powder types on ceramic tiles [12].



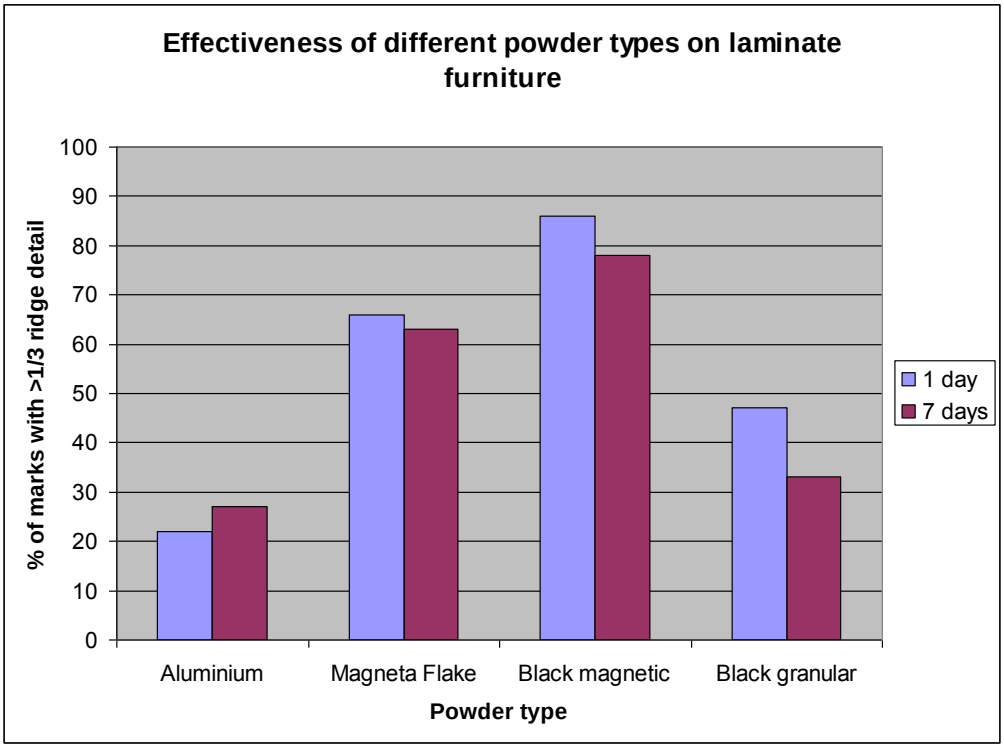
Comparison of different powder types on gloss painted wood [12].

8.2.4 The results indicate that although aluminium powder is the best performing powder on glass, on other smooth surfaces magnetic powders may actually give slightly better performance. As the roughness of the surface increases the effectiveness of aluminium drops off and both types of magnetic powder are more effective. In order to investigate this further, the next trial compared a range of powders on surfaces with

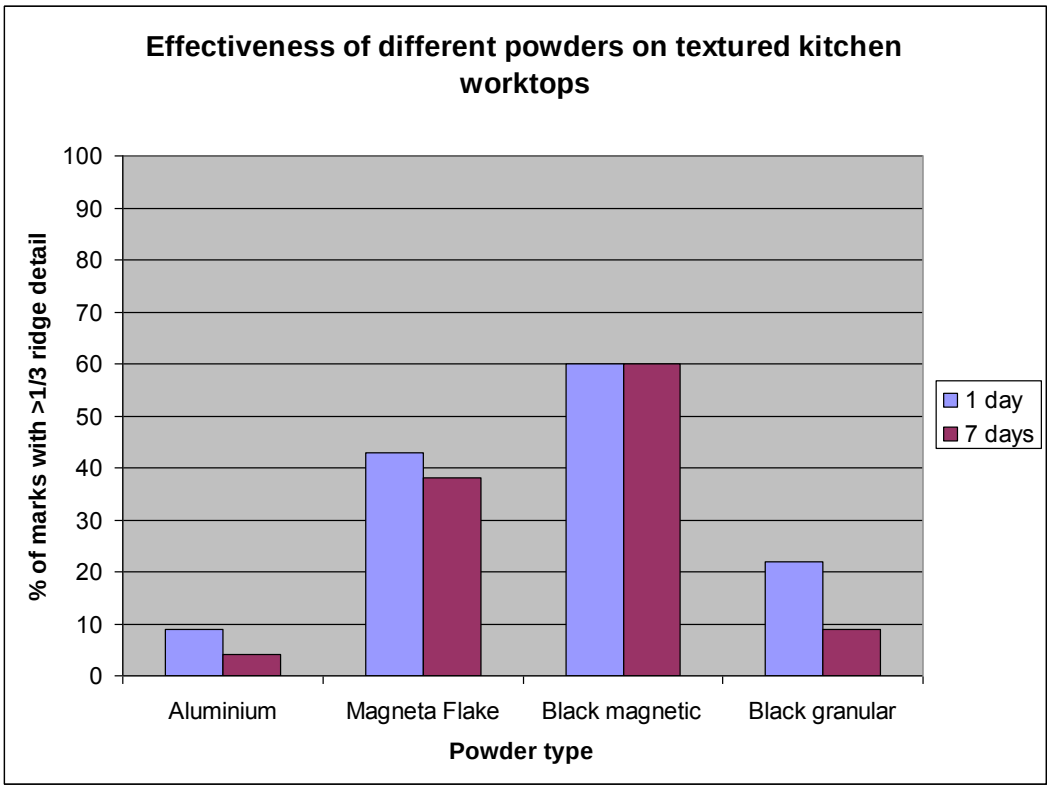
different levels of surface texture including uPVC, laminate furniture, kitchen worktops and wood furniture [13,30]. The graphs below show the results of this study, which developed and graded 9,560 marks.



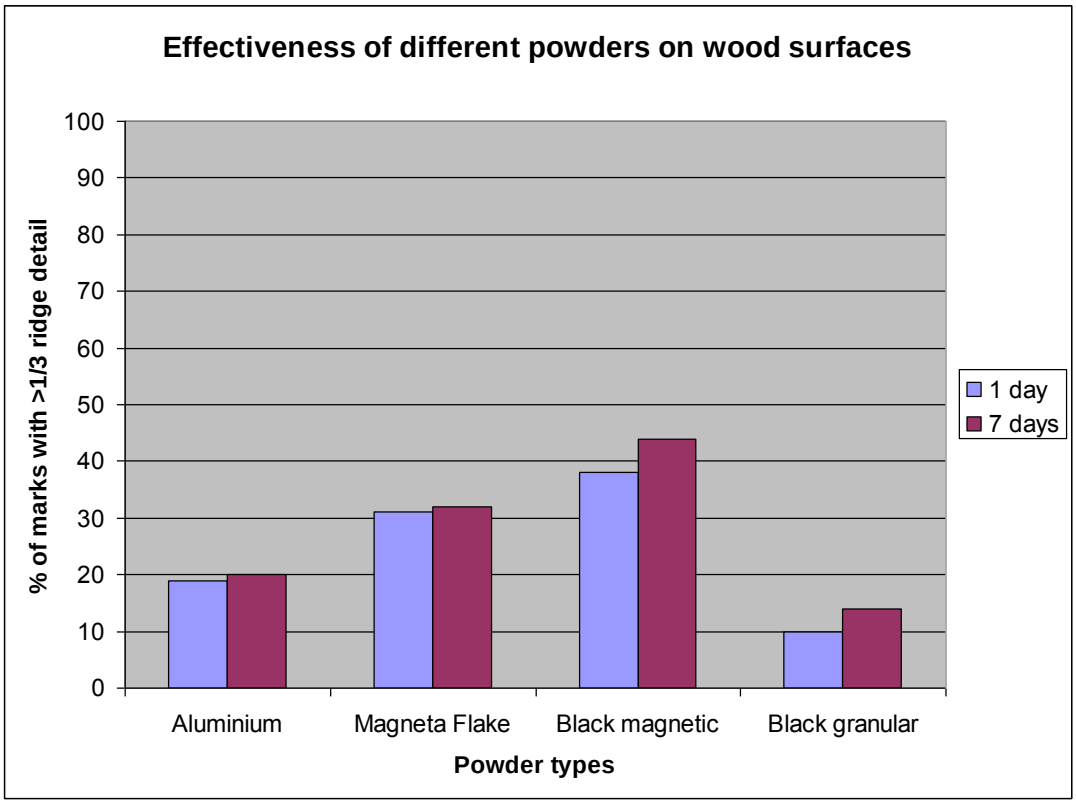
Comparison of different powder types on uPVC [13].



Comparison of different powder types on laminate furniture [13].



Comparison of different powder types on textured kitchen worktop material [13].



Comparison of different powder types on wood furniture [13].

8.2.5 When considering the results obtained from all surfaces examined, it is evident that as the surface becomes more textured, the effectiveness of both aluminium flake and black granular powder decreases significantly. The effectiveness of both types of magnetic powder also decreases as surface texture increases, but the degradation in performance is not as great and these powders are recommended for use on this type of surface.

8.3 Operational experience

8.3.1 Since the issue of the *Fingerprint Powders Guidelines* [18] in 2007, CAST has supplemented this with several training sessions targeting SOCOs at individual police forces. In some cases there have been reported rises in the use of black magnetic powder at the expense of aluminium flake and an increase in marks developed, but at present (up to 2016) it is difficult to assess whether both trends will be sustained in the long term.

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Powder suspensions

1. History

1.1 During the development of the small particle reagent (SPR) in the late 1970s, many other particulates were investigated as constituents in the formulation, including amorphous carbon and graphite and the oxides of the magnetic elements cobalt and iron [1]. All of these gave good results, but none were as consistent as molybdenum disulphide and therefore were not pursued as systems for operational use.

1.2 In a significant development that appears to have been overlooked at the time, Haque and co-workers developed an alternative 'small particle suspension' based on iron oxide (Fe_3O_4) in 1989, and stated that this gave better results than the molybdenum disulphide-based small particle reagent in terms of sensitivity and contrast [2]. The new formulation was also noted to work on wetted surfaces, and to enhance further marks previously developed by powdering. This formulation does not appear to have entered widespread use for non-porous surfaces and was not developed further.

1.3 Although not unrelated to small particle reagent formulations in terms of composition (a powder in a surfactant solution), the development of the modern powder suspension formulation followed from a Japanese technique for visualising fingermarks on the sticky-side of adhesive tape [3]. This was noted by an American police officer on secondment in Japan and, after experimentation with the technique, he contacted the Lightning Powder Company, who commercialised the process as the 'Sticky-Side Powder' product, consisting of a grey/black powder that was blended with Kodak Photoflo surfactant and distilled water before use. The resulting suspension was painted on to the adhesive side of tapes, and then washed off using running water to reveal developed marks.

1.4 The new Sticky-Side Powder system was compared with techniques in general use for adhesive tapes in 1996, primarily gentian violet [4]. The powder suspension formulation was found to perform better than gentian violet, in particular on marks known to be eccrine in nature. Several researchers began to investigate alternative powder suspension formulations, looking at the combination of commercial powders with surfactant/water mixtures. Bratton and Gregus [5,6] looked at Lightning Black Powder with Liquinox surfactant and reported it to give better results than Sticky-Side Powder, noting that the revised formulation reduced the occurrence of background staining that sometimes obscured marks with Sticky-Side Powder. Kimble [7] studied a wider range of powders, including grey and coloured systems, with Photoflo surfactant and water in different ratios. It was concluded that other powders could be used and a formulation incorporating a grey powder was proposed for black adhesive tapes. Other workers also investigated formulations for black adhesive tapes, Parisi [8] testing 'Pink Wop' fluorescent powder and a white fingermark powder with Liquinox and Photoflo, and Martin [9]

looking at an ash grey powder with Photoflo. White powder in Liquinox and ash grey powder in Photoflo both gave suspensions that developed good quality fingermarks. Further testing of these revised powder suspension formulations against gentian violet/basic violet 3 continued to indicate that powder suspensions were the more effective single process for these surfaces [10].

1.5 The Police Scientific Development Branch (PSDB) began experimenting with powder suspensions for development of fingermarks on adhesive tapes in the late 1990s [11]. An initial assessment was carried out on the original Sticky-Side Powder formulation, characterising the base powder by electron microscopy and looking at optimised formulations. It was found that the base powder consisted of fine (approximately 1 μm) particles of iron oxide interspersed with larger (10 to 20 μm diameter) flakes of aluminium. A range of other powder suspension formulations were investigated, with two ultimately being recommended for further research. A black powder suspension based on precipitated, magnetic iron oxide was proposed, together with a white powder suspension based on titanium dioxide powder. Both formulations utilised Photoflo as the surfactant. These formulations were trialled against Sticky-Side Powder, where the black formulation was shown to give superior results.

1.6 The black iron oxide-based formulation was then compared in effectiveness with two other treatments for the adhesive side of tapes, basic violet 3 and superglue followed by dyeing with basic yellow 40 [12]. In these trials powder suspension gave closely equivalent results to superglue, with the contrast of developed marks being slightly better. Basic violet 3 was found less effective than either powder suspension or superglue, in accordance with previous observations.

1.7 Other researchers also concluded that titanium dioxide was the optimum particulate for white powder suspension formulations. Wade [13] used a commercially available white small particle reagent formulation based on titanium dioxide as a starting point, and demonstrated that improved performance was obtained by concentrating the solution and adding Photoflo. Alternative formulations based on different grades of titanium dioxide were investigated and it was observed that better results were obtained using the rutile, rather than anatase, form of titanium dioxide. Williams and Elliot [14] also looked at modifying white small particle reagent with Photoflo and studied different application methods including spraying, immersion, dipping, painting and pouring. It was concluded that the best development could be obtained by immersion and this method also reduced the risk of over-development but was also the most time-consuming.

1.8 Until the mid-2000s, development of fingermarks on adhesive surfaces was the sole application considered for powder suspensions. In 2004, Auld [15] carried out an investigation into the effectiveness of various fingermark development techniques for detecting marks on the exterior surfaces of motor vehicles, including vehicles that had been wetted. He

compared powdering, superglue, small particle reagent and Sticky-Side Powder, and found that Sticky-Side Powder was the most effective treatment for several scenarios, in particular where cars had been wetted at some stage prior to fingermark development.

1.9 Around the same time, Strathclyde Police had begun to investigate the use of black and white powder suspensions for the treatment of articles recovered from arson scenes, the treatment both removing soot deposits and developing marks [16].

1.10 These observations resulted in further experimentation on non-porous surfaces, both by police forces on operational casework and by CAST in laboratory trials [17]. The CAST studies sought to establish the relative effectiveness of the black powder suspension technique on a range of different surfaces and the position of powder suspensions in sequential processing. It was soon apparent that superglue and powder suspensions are mutually exclusive processes and if one is applied the other is relatively ineffective when used afterwards. An equivalent study was subsequently carried out for white powder suspensions on dark, non-porous surfaces, which came to similar conclusions, although white powder suspension was found to be less effective than black powder suspension overall [18]. Operationally, police forces applied powder suspensions either at crime scenes after powdering, or in the laboratory as a replacement for superglue on articles likely to have been wetted or contaminated (cowlings, number plates, drugs packaging). In both cases additional marks were found or recovery rates increased.

1.11 CAST also continued the assessment of powder suspensions for use on adhesive tapes, comparing the formulations developed in earlier work [11] with a range of commercially available powder suspensions. For the white powder suspension [19] it was found that the original CAST formulation gave marginally better results and this was used in a subsequent operational trial. For black powder suspensions it was discovered that commercial formulations based on carbon out-performed the CAST iron oxide-based formulation and work therefore focused on developing a non-proprietary carbon-based formulation [20]. It was not possible to identify a formulation giving equivalent or improved performance over the commercial systems and therefore commercial, carbon-based systems were included in operational trials. The results from these trials showed that carbon-based, black powder suspensions are the most effective process for the adhesive side of light coloured tapes, whereas for dark tapes superglue/basic yellow 40 is more effective, and white powder suspensions are only recommended for this application if it is known that the article has been wetted.

1.12 Subsequent work both at CAST and in operational police laboratories has continued to explore the range of surfaces for which powder suspensions can be used. Recent research has shown that they can be applied to plastic bags [21], semi-porous surfaces [22,23,24] and are one of the most effective processes for surfaces contaminated with drugs

[25]. Based on the results of these studies the powder suspension process was incorporated into the processing charts for both semi-porous and non-porous surfaces in the *Fingermark Visualisation Manual* [26].

1.13 Since the publication of the Fingermark Visualisation Manual (1st edition) [26], the investigation into the use of powder suspensions for the visualisation of fingermarks on modern and challenging materials has continued. The results of feasibility studies found that iron oxide-based powder suspension and superglue fuming were the most effective techniques for fingermark visualisation on leather (although recovery rates were very low [27]). In addition, carbon, titanium dioxide-based, and iron oxide-based powder suspensions all demonstrated reasonable capability on artificial leather in these limited tests [27]. Other recent work from American researchers explored fingermark visualization options for Tyvek Large Pak and Padded Pak shipping envelopes from FedEx [28]. Wetwop black and Sticky-side Powder were found to be promising initially, and through subsequent study, modified and diluted powder suspensions based on Wetwop were indicated to be the most effective powder suspensions for Tyvek Large Pak and Padded Pak envelopes (respectively) [28].

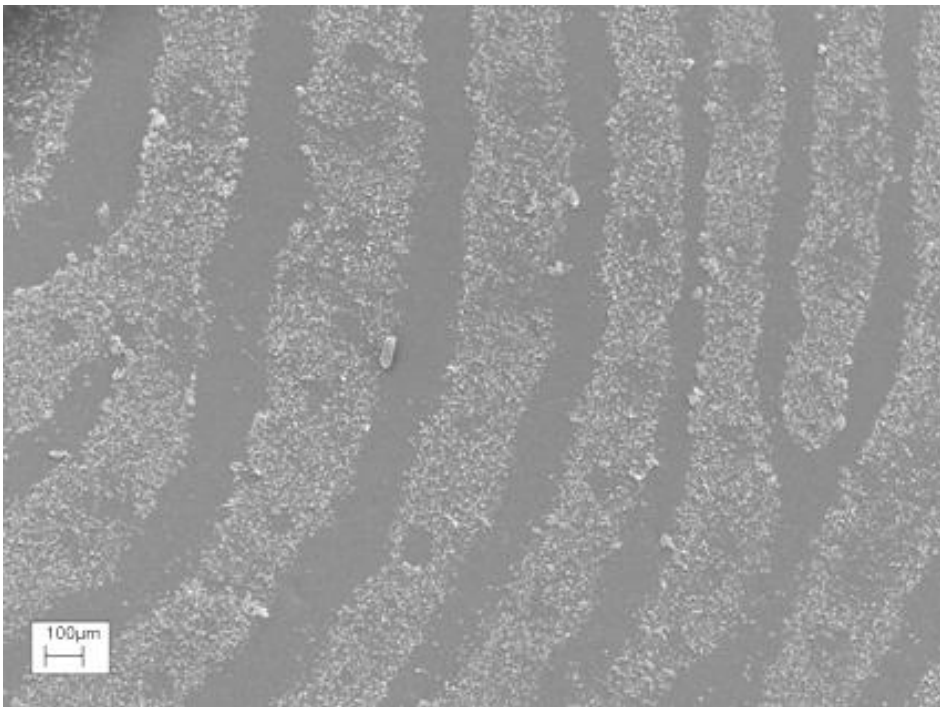
1.14 Recently powder suspensions have also been considered for challenging coastal conditions: Wetwop white and iron oxide-based powder suspension and were found to be more effective than powders and SPRs for the recovery of fingermarks from glass following exposure to sea spray aerosol [29].

2. Theory

2.1 The exact mechanism for fingermark visualisation using powder suspensions is unknown, and studies by CAST and academia to establish which factors are most important are continuing. Recently published work [30] concerning the concentration of Triton™ X-100 surfactant in iron oxide-based powder suspension demonstrated a change in the selectivity of the process towards fingermarks that approximately coincided with the critical micelle concentration (c.m.c.) for Triton™ X-100. Powder suspensions produced with Triton™ X-100 surfactant solutions at or below the c.m.c. caused indiscriminate iron oxide deposition across the surface for most of the treated samples, whereas formulations with Triton™ X-100 above the c.m.c. were selective towards fingermarks [30]. These findings support earlier theories suggesting that surfactant micelles play a role in particle dispersion in powder suspension by inhibiting random surface deposition. An improved understanding of the powder suspension process in general will likely be garnered through detailed investigations into the interaction between the powder particles and the surfactant molecules.

2.2 In contrast to small particle reagent, powder suspensions do not appear to have a strong affinity for sebaceous fingermark constituents and in fact may give stronger development in regions where eccrine constituents are expected to be concentrated. Powder suspension formulations contain far higher concentrations of powder than small particle reagent and this may account for some differences in behaviour noted between the two processes.

Different types of mark from the same donor developed using iron oxide-based powder suspension on a white ceramic tile. From left: 'natural', sebaceous and eccrine marks.



Scanning electron micrograph of fingermark developed using black powder suspensions on clear adhesive tape, showing particles deposited on fingermark ridges but not on background.

2.3 However, it has been shown that powder suspensions continue to develop marks on surfaces that have been wetted [31,32] suggesting that eccrine constituents encapsulated within non-water soluble constituents may be responsible for controlling deposition, because those exposed on the surface are likely to be dissolved and washed away during processing. Powder suspensions have also been observed to give better results when marks are older, or have been exposed to moderate temperatures to accelerate them drying out [33]. It is proposed that as the fingermark deposit dries out, the interaction distance between the encapsulated eccrine constituents and the particles in the suspension decreases, making deposition more likely.

2.4 The proposal that it is an electrical interaction that destabilises the surfactant micelles (or particle-surfactant associations) and results in particle deposition, and that this is proximity dependent, is supported by Bacon *et al.* [34]. These researchers found that titanium dioxide pigment particles blended into a polymer substrate acted as preferential deposition sites for the carbon particles in powder suspension, and this effect was not observed where the pigment particles were more than 30 nm below the surface [34].

2.5 The mechanism proposed above has not been conclusively demonstrated and research is continuing in this area.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* [26], the purpose of this publication being to report the history, theory and validation work associated with the process.

3.2 There are three slightly different powder suspensions recommended for operational use, these being outlined below.

3.3 <u>Black powder suspension for use on the adhesive side of adhesive tapes (carbon-based):</u> Commercially available, pre-mixed carbon-based powder suspensions, either Kjell Carlsson Wet Powder (Black) or Lightning Powder Company WetWop™ (Black).

3.4 <u>Black powder suspension for use on light, non-porous surfaces (iron oxide-based):</u> Weigh 20 g precipitated magnetic iron oxide (Fe_3O_4/Fe_2O_3) into a glass beaker, and to the dry powder add 20 mL of a pre-mixed stock detergent solution consisting of 350 mL ethylene glycol, 250 mL Triton™ X-100 and 400 mL of deionised water and mix thoroughly to form a paste. The formulation first published [36] used a premixed 1:1 solution of Photoflo and deionised water in place of the current Triton™ X-100/ethylene glycol solution. Because of concerns that Photoflo may be discontinued as use of wet photography continued to

decline, this was replaced in 2009 by the closely equivalent detergent mixture made from readily available chemicals.

3.5 White powder suspension for use on dark, non-porous surfaces and wetted dark adhesive tapes (titanium dioxide-based): Commercially available, pre-mixed titanium dioxide-based powder suspensions, either Kjell Carlsson Wet Powder (White) or Lightning Powder Company WetWop™ (White).

3.6 The ratio of powder to surfactant/distilled water mixture recommended in the CAST formulations for application to adhesive tapes have been determined by laboratory tests [11]. If there is excess surfactant/water present, a thinner suspension is produced, which does develop marks although these are significantly fainter than those obtained with optimum formulations. If there is insufficient surfactant/water present, the suspensions do not flow and clumps of powder may be left behind on the tape.

3.7 For use on non-porous surfaces, it has been observed that the powder suspension can be diluted from the thicker paste applied to adhesive tapes and can still give effective results.

3.8 The role of the detergent in the formulation is to stabilise the suspension against indiscriminate precipitation over the entire surface. The 2009 CAST formulation utilises a mixture of Triton™ X-100, ethylene glycol and distilled water, but the commercial formulations may contain other surfactant systems.

4. Critical issues

4.1 Performance of powder suspensions is thought to be strongly influenced by the particle size and the shape of the materials concerned which can vary widely with methods of preparation. Use of other generic sources of what is nominally the same chemical may result in very different results (an example with different iron oxides was published recently [35]) and batch testing is recommended.

5. Application

5.1 Suitable surfaces: The full range of application areas for powder suspensions are still being explored, but they are now recommended for use in the following circumstances.

- On most types of non-porous surfaces as an alternative to superglue fuming
- On the adhesive side of light coloured, polymer backed adhesive tapes with both rubber- and acrylic-based adhesives (carbon-based black powder suspension only).

- On the adhesive side of dark coloured, polymer backed adhesive tapes with both rubber- and acrylic-based adhesives
- On non-porous surfaces where it is thought that the surface has been wetted or exposed to high humidity environments.
- On non-porous surfaces where powder or particulate contamination (e.g. soot or drugs residues) is present on the surface.
- On non-porous surfaces where there is a surface layer of oily contamination present.
- On some 'semi-porous' surfaces, such as cardboard with glossy printed exteriors, and leatherette.
- In a sequential treatment process after powders at a scene of crime and in laboratories.
- As a final treatment after blood dyes on non-porous surfaces.

5.2 Powder suspensions are applied to the surface of interest using a soft squirrel-hair brush, ensuring that the brush is pre-moistened and well loaded with the suspension mixture to avoid damage that could be caused to the fingermark by a dry brush and to avoid 'streakiness' in background development. The suspension should be stirred to achieve a paint-like consistency and painted onto the surface, left in situ for 10 to 15 seconds and then washed off using running water (either from a tap, hose or wash bottle). The temperature of the wash water should be cool, but this is not thought to be critical.

5.3 Prints can become over-developed if the suspension is left on the surface too long because the suspension starts to dry and fills in ridges. Powder suspensions can also be reapplied, if necessary. There is also evidence to suggest that the different types of powder suspension can be applied in sequence and still develop additional marks.

5.4 The process is suited to application both in a laboratory and at scenes of crime (pending consultation with the Environment Agency), however, the process is messy to apply and the implications for cleaning of the scene should be considered before application.

6. Alternative formulations and processes

6.1 The first true 'powder suspension' (as opposed to SPR-like) formulation proposed for adhesive tapes was Sticky-Side Powder, consisting of Sticky-Side Powder (a mixture of iron oxide particles and aluminium flakes) mixed with a 1:1 blend of Photoflo and water, added until a thin paint consistency was achieved. Soon after this an alternative formulation was proposed that used 20 g of Lightning Black Powder as the particulate, mixed with 20 g Liquinox surfactant and 40 mL of distilled water.

6.2 CAST evaluated both of these formulations in comparative trials with many different types of particulate fillers in powder suspensions. In the initial investigation of an optimum formulation for the treatment of adhesive tapes [11], the range of powders below were tested in combination with Photoflo surfactant as candidate black powder

suspensions.

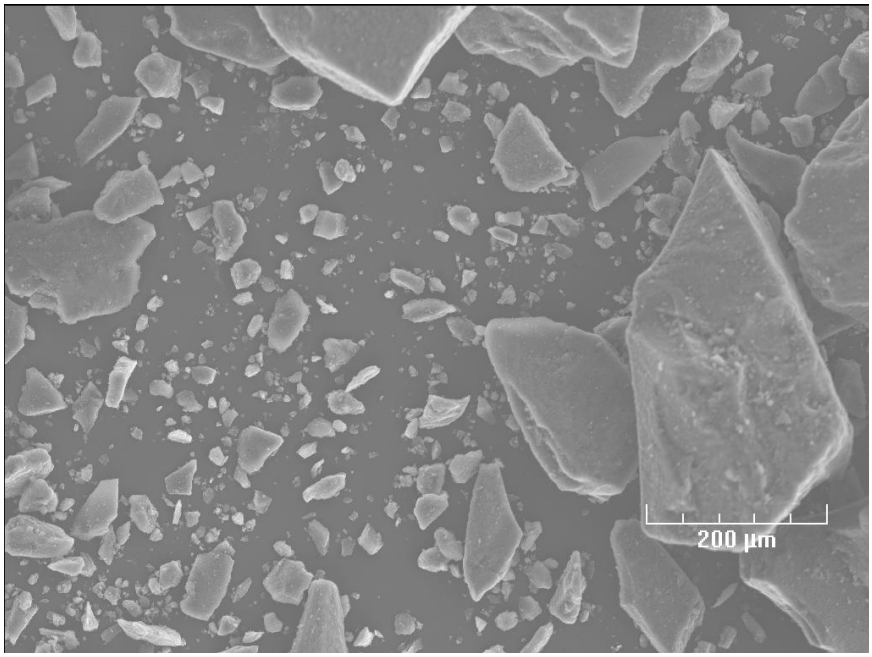
Powder sample	**Specific gravity**	**Particle size**	**Manufacturer/ supplier**
Fe_3O_4	5.18	> 10 μm	BDH Chemicals
Fe_3O_4	5.18	> 5 μm	Sigma – Aldrich
Fe_3O_4 – magnetic/ precipitated	5.18	> 1 μm	Fisher Chemicals Ltd
Fe_2O_3 – red, precipitated	5.24	> 5 μm	BDH Chemicals
Fe powder	-	9 – 110 μm	–
Lightning Black Powder	~1.8	> 1 μm (aggregates up to 40μm)	Lightning Powder Company
Lightning Magnetic Black Powder	-	Range from 1–30 μm	Lightning Powder Company
Cobalt (II, III) oxide	6.11	> 1 μm	Sigma – Aldrich
K9 – Black Fingerprint Powder	1.7–1.9	> 1 μm (aggregates up to 150 μm)	K9 Scene of Crime Ltd
K9 – Black Magnetic Powder	~1.8	> 1 μm (aggregates up to 150 μm)	K9 Scene of Crime Ltd
K9 – Jet Black Magnetic Powder	~5.18	> 1 μm (aggregates up to 150 μm)	K9 Scene of Crime Ltd
K9 – Magneta Flake	7.8	–	K9 Scene of Crime Ltd
K9 – Gold Powder	8.5	–	K9 Scene of Crime Ltd
K9 – Grey Magnetic Powder	~2.7	–	K9 Scene of Crime Ltd
Dactyl Black Fingerprint Powder	~2	> 1–24 μm	Speciform
Copper (II) oxide	6.315	–	Sigma – Aldrich
Activated Charcoal	~2	–	BDH Chemicals
Graphite Powder	~2.09–2.23	–	Sigma – Aldrich
Graphite Powder (synthetic)	~2.09–2.23	–	Sigma – Aldrich
Molybdenum disulphide	4.80	–	-
Manganese disulphide	–	–	Sigma – Aldrich
Vanadium (III) oxide	4.87	–	Sigma – Aldrich

Particulates investigated by the CAST as the basis for black powder suspensions for adhesive tapes.

6.3 Of these, the precipitated magnetic Fe_3O_4 powder proved most effective (out-performing both formulations originally proposed for adhesive tapes in the literature) and was therefore used in the CAST formulation initially

proposed for adhesive tapes. This formulation was subsequently found to give excellent results on non-porous surfaces.

6.4 Commercial, pre-mixed black powder suspensions have become available since 2004, including Wet Powder – Black (Kjell Carlsson Innovation) and WetWop™ – Black (Lightning Powder Company). An initial assessment of these formulations indicated that they were probably based on a powdered graphitic material.



Scanning electron micrograph of particulates from commercial carbon-based powder suspension.

6.5 It was established by comparative trials that carbon-based black powder suspensions were superior to iron oxide-based formulations on all types of adhesive tapes with non-porous backings, and therefore a more in-depth assessment was carried out on carbon particulates. This focused on graphitic powders although several other forms of carbon were also investigated [20], as outlined in the table below.

Powder	Particle size	Manufacturer/supplier
Coke FC800	0.8 mm	TIMREX
Graphite T800	0.71 mm	TIMREX
Graphite	150 μm	Fisher Chemicals Ltd
Activated charcoal	50–150 μm	Sigma – Aldrich
Swedish black powder	95 μm	BVDA
Natural graphite	75 μm	GTC
Synthetic graphite	53 μm	GTC
Graphite powder	50 μm	VWR
Activated charcoal	40 μm	Sigma – Aldrich
KS44	44 μm	TIMREX
HSAG 300 AE-109	32 μm	Timcal
Graphite	20 μm	Sigma – Aldrich
Micronised graphite	10 μm	GTC
Graphite KS6	7 μm	TIMREX
Dispersion LB1300	7 μm	TIMREX
Activated carbon	0.8μm	Sigma – Aldrich
Monarch 280 carbon black	0.41μm	Cabot Carbon
Carbon nanopowder	0.3 μm	–
Vulcan VXC 72R	0.3 μm	Cabot Carbon
Mogul L	0.24 μm	Cabot Carbon

Carbon powders evaluated as constituents for non-proprietary carbon powder suspension formulation [20].

6.6 Several surfactants were also evaluated in this study, including:

- Photoflo;
- Aerosol OT;
- Liquinox.

6.7 For white powder suspensions, white powders with relatively high density and a spherical shape were researched [11]. Of these, initial trials indicated that zirconium oxide and titanium dioxide gave the best results, with titanium dioxide giving marks of higher contrast. Further studies therefore focused on optimising the titanium dioxide formulation.

6.8 A range of commercial white powder suspensions have also become available, including Wet Powder – White (Kjell Carlsson Innovation), WetWop™ – White (Lightning Powder Company) and Adhesive Side Powder – Light (Sirchie Fingerprint Laboratories). These have all been evaluated against the original CAST formulation on adhesive tapes [19] and found to give closely equivalent performance. A comparative trial on non-porous surfaces [18] found that for the limited range of surfaces evaluated there was little significant difference between any of the commercial formulations and the CAST adhesive tapes formulation, and the white powder suspensions can be used interchangeably.

6.9 Various nanopowders were also been evaluated by CAST in 2007 (including aluminium, magnesium, titanium, tin, yttrium, iron, zirconium, copper, neodymium, tungsten, lanthanum, terbium, ytterbium, and bismuth oxides, silicon carbide, and carbon) [20]. Many of these failed to develop fingermarks when used in suspensions, but of those that did the best were found to be iron oxide, titanium dioxide and carbon (the same constituents as used in existing formulations), but none gave better results than the formulations outlined in the CAST processes section above.

6.10 Recent work has presented alternative iron oxide-based powder suspension formulations featuring surfactant solutions with reduced Triton™ X-100 and ethylene glycol concentrations [30], with respect to the formulation recommended by CAST in 2009 and incorporated into the Fingermark Visualisation Manual [26] (see table below). The 10% and 1% surfactant solution powder suspensions demonstrated near equivalence to the 2009 formulation in terms of the quality and quantity of visualised fingermarks in laboratory-based tests on clean surfaces [30].

Powder suspension	**Surfactant solution component details (percentages by volume)**
Iron oxide-based powder suspension (CAST, 2009) [26]	- 25% Triton™ X-100 - 35% ethylene glycol - 40% deionised water

10% surfactant solution iron oxide-based powder suspension	- 10 % of stock containing:
	  ○ 25% Triton™ X-100
	  ○ 35% ethylene glycol
	  ○ 40% deionised water
	- 90 % deionized water

1% surfactant solution iron oxide-based powder suspension	- 1 % of stock containing:
	  ○ 25% Triton™ X-100
	  ○ 35% ethylene glycol
	  ○ 40% deionised water
	- 99 % deionized water

Surfactant solutions for iron oxide-based powder suspensions – 2009 formulation [26], and formulations with reduced Triton™ X-100 and ethylene glycol concentrations.

6.11 These more dilute powder suspension formulations were noted to be easier to rinse off, offering advantages for processing at crime scenes. They are however untested on unclean or contaminated surfaces, and therefore require further evaluation before deploying operationally [30].

Follow on studies at CAST's laboratories have detected a potential compatibility issue with some batches of iron oxide and these more dilute detergent solutions.

7. Post-treatments

7.1 Marks developed using powder suspensions can be lifted once dry in the same way as marks developed using small particle reagent, using either adhesive tape or gelatine lifts.

8. Validation and operational experience

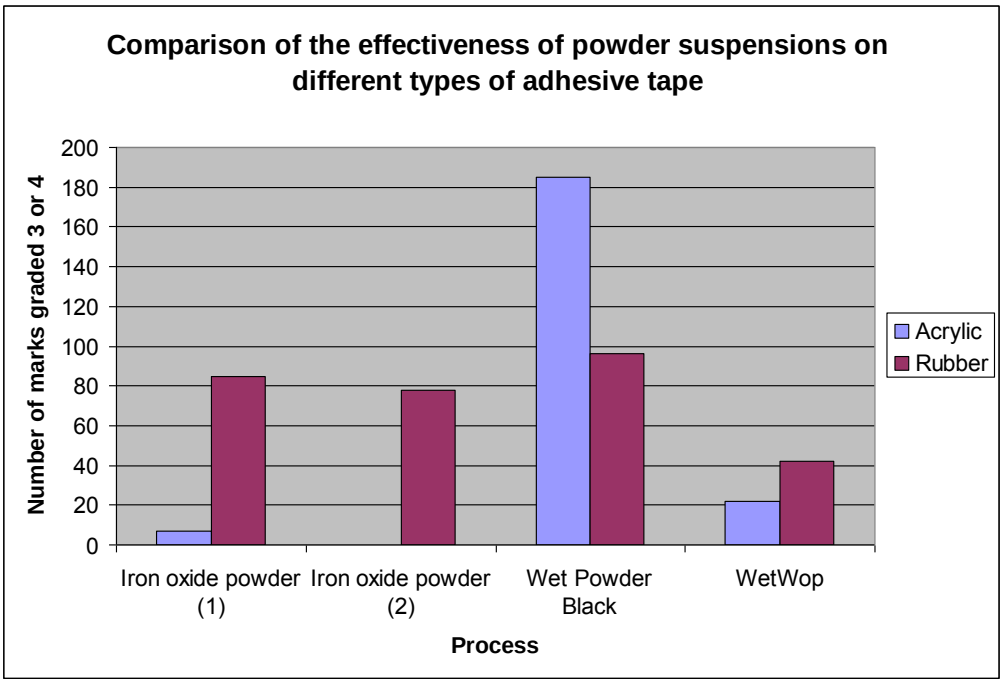
8.1 The operational experience of powder suspensions must take into account two primary applications – their use on adhesive tapes and their use on non-porous surfaces. There is a greater background knowledge regarding the effectiveness of powder suspensions on adhesive tapes, although the application of powder suspensions to other non-porous surfaces is becoming more widespread.

8.2 Laboratory trials

8.2.1 Initial laboratory comparisons on adhesive tapes were carried out at CAST in 2000 between basic violet 3, iron oxide-based black powder suspension and superglue, with over 1,600 fingermarks being evaluated for each process [12]. In these trials superglue and iron oxide-based black powder suspension gave the best results and were very similar in performance, but powder suspension marks had better contrast. An equivalent trial was carried out using titanium oxide-based white powder suspension, superglue and basic violet 3 (imaged via fluorescence and via the transfer technique). The results were closely equivalent to those observed for light tapes, with white powder suspension and superglue being closely equivalent in performance and both more effective than basic violet 3. The powder suspension again showed better contrast for developed marks.

8.2.2 During these trials it was observed that some tapes exhibited extensive background staining when treated with iron oxide-based powder suspensions whereas others did not. It was established by infrared (IR) spectroscopy that tapes using rubber-based adhesives did not background stain while those with acrylic-based adhesives did. This resulted in the initial recommendation that a spot test be carried out to see whether background staining occurred prior to selecting a treatment [11]. However, it was subsequently noted that there were differences between powder suspensions, not all staining the background of acrylic tapes. It was established that the suspensions that did not stain the background contained carbon instead of iron oxide particulate, and a comparison of the relative effective of iron oxide- and carbon-based black powder suspension (WetWop™, Wet Powder Black) was carried

out on both rubber and acrylic adhesive tapes [20]. This trial looked at 300 half marks over a range of acrylic tapes and 480 half marks over a range of rubber tapes [20].



Results of comparative trials using different black powder suspensions on adhesive tapes [20].

8.2.3 These trials demonstrated that Wet Powder – Black gave the best overall performance, with both carbon powder formulations working on acrylic and rubber-based adhesives. Background staining of acrylic-based adhesive tapes by iron oxide powder suspension formulation was again observed.

a) b)

Development of marks on adhesive tapes: a) *acrylic-based adhesive tape showing background staining by iron oxide-based powder suspension applied to left half, and no background staining from carbon-based powder suspension applied to right half,* b) *rubber-based adhesive tape showing no background staining from iron oxide-based powder suspension applied to left half or carbon-based powder suspension applied to right half*

8.2.4 A similar comparison has been conducted for white powder suspensions on dark tapes. An initial investigation [19] compared the CAST formulation against the following commercially available products:

- Wet Powder – White (Kjell Carlsson Innovation);
- WetWop™ – White (Lightning Powder Company);
- Adhesive Side Powder – Light (Sirchie Fingerprint Laboratories).

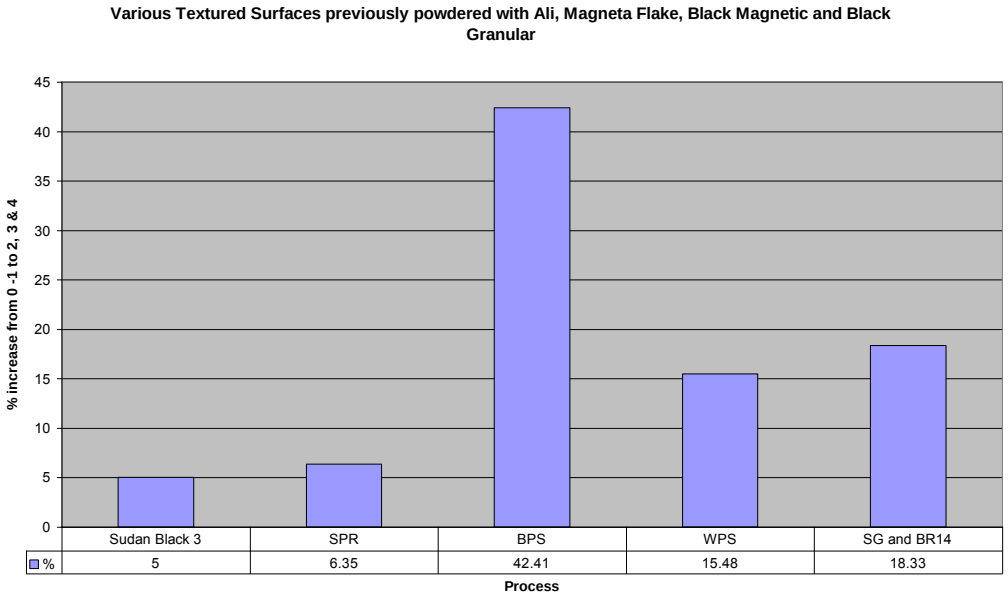
8.2.5 The results of these studies are summarised in the table below, but in general all formulations gave similar results, with the CAST formulation performing marginally better. Some differences were observed between the level of background staining, but in general all marks were clearly visible against the background. Approximately 14,400 marks were examined in this study.

Mark grade	**Sirchie**	**Wet Powder**	**WetWop**	**Stan Chem TiO_2-based(CAST)**
0	2.30%	1.63%	0.95%	1.51%
1	7.86%	6.83%	5.99%	4.09%
2	10.52%	13.63%	13.29%	11.88%
3	17.34%	21.79%	22.22%	20.79%
4	61.98%	56.13%	57.54%	61.73%
3s and 4s	**79.33%**	**77.92%**	**79.76%**	**82.53%**

Results of laboratory comparative trials for different white powder suspensions [19].

Comparison of fingermark and background development using Home Office CAST formulation (left) *and Sirchie Adhesive Side Powder* (right) *on black tapes.*

8.2.6 Studies into the use of powder suspensions for non-porous surfaces was prompted by the work conducted by Auld [15], and commenced with an initial assessment of the number of additional marks developed (or enhanced) on textured surfaces by subsequent chemical processing after powdering. Several different processes were studied, including solvent black 3, small particle reagent, superglue and basic red 14 dye, and both white and black powder suspension formulations as published by CAST [36]. This was conducted by Hardy in 2006 following completion of a study into powdering of textured surfaces [37]. The results of this unpublished exercise are illustrated below and clearly demonstrated that there were potential advantages in applying powder suspensions after powdering.



Results obtained by applying a secondary fingermark development process (SPR = small particle reagent, BPS = iron oxide-based black powder suspension, WPS = white powder suspension, SG and BR14 = superglue dyed with basic red 14) in sequence after powdering [37].

8.2.7 This prompted a further, in-depth study of the application of powder suspensions and alternative processes, both singly and in sequence [17]. It was soon established that carbon-based black powder suspensions were ineffective in comparison to the iron oxide-based formulation on the non-porous substrates considered (a difference from observations on adhesive surfaces) and studies therefore focused on the iron oxide-based black powder suspension formulation instead. (N.B. This was the formulation published by CAST in 2006 [36], and was prepared in these trials using iron (II/III) oxide powder from Fisher Scientific (code I/1100/53) [17]).

a)

b)

Black powder suspensions applied to a smooth, non-porous surface (patterned ceramic tile); a) *iron oxide-based formulations, and* b) *commercial carbon-based formulation.*

8.2.8 The study examined 37,560 marks deposited overall, on 23 different smooth and textured non-porous (and in some cases semi-porous) surfaces representative of those that may be encountered at crime scenes. These included ceramic tiles, laminates, painted metal, and uPVC, as summarised below.

General surface classification	**Specific description**
Smooth, non-porous	Ceramic tile
	Smooth wood effect laminate
	Shiny, striped laminate
	Beige laminate
	White painted metal
	Red painted metal
	Glass
	Perspex
	Polyethylene
	Polypropylene
Rough, non-porous	Textured ceramic tile
	Cream textured laminate
	Wood effect laminate
	Granite effect laminate
	Grey textured laminate
	Beige textured laminate
	Textured painted metal
	Fake leather texture laminated aluminium
	Varnished wood
Other	uPVC
	Silk emulsion painted plasterboard
	Kitchen/bathroom painted plasterboard
	Textured vinyl wallpapered plasterboard

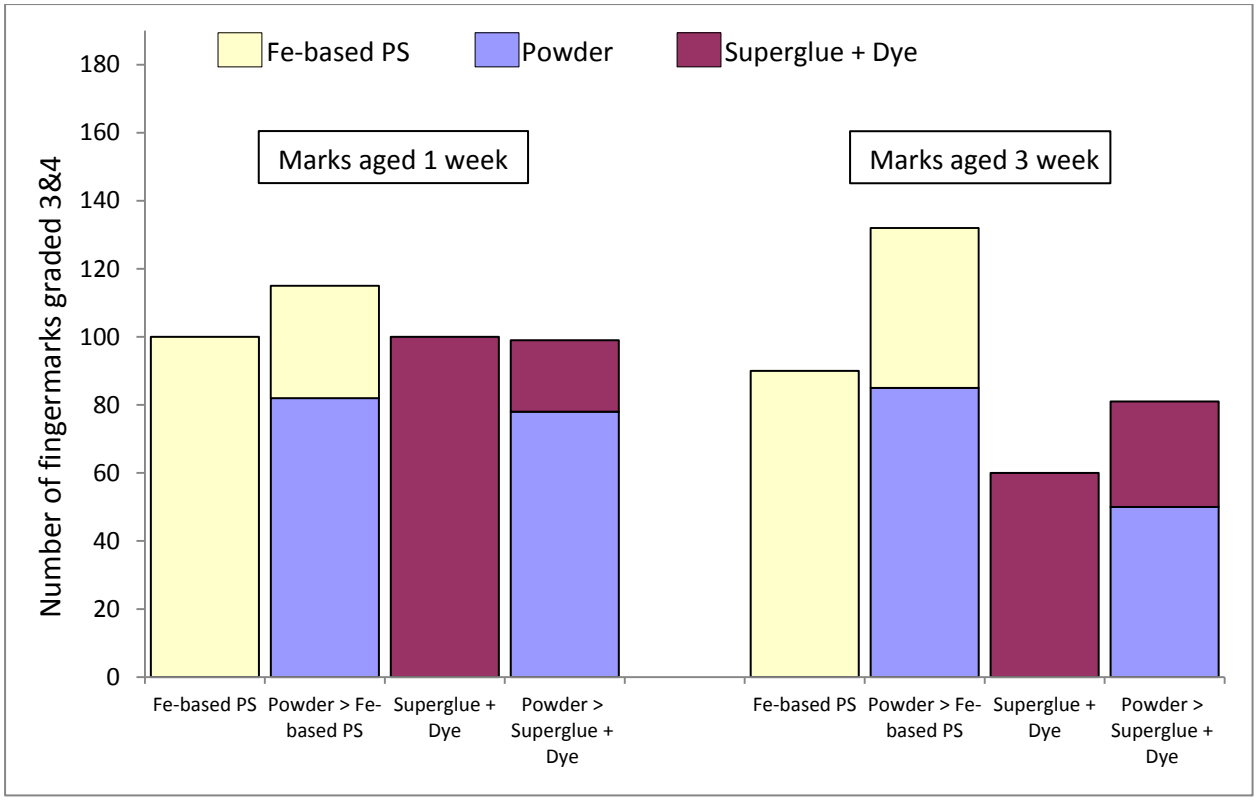
Description of the surfaces used in the comparative study between various sequences of superglue, powders and powder suspensions

8.2.9 The study became predominantly concerned with a principal comparison between the following processes or process combinations [17]:

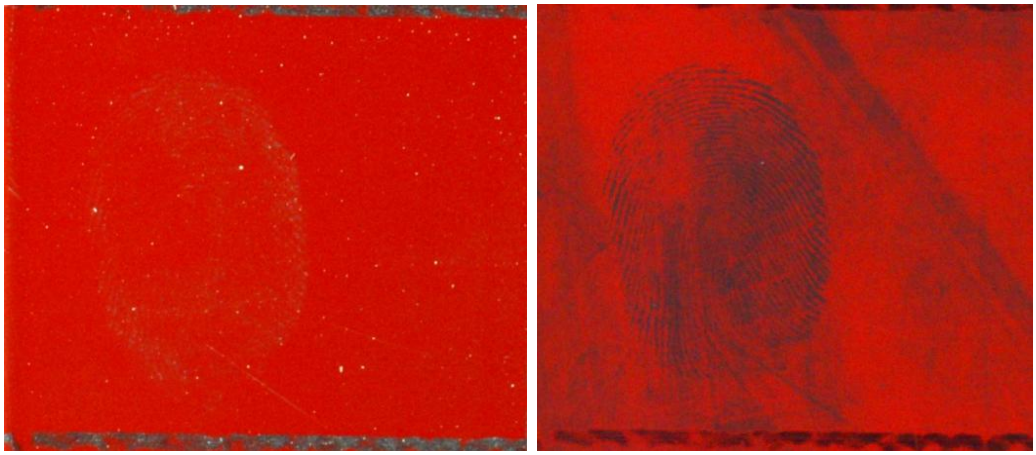
- Powder → iron oxide-based powder suspension (in sequence)
- Powder → superglue and basic yellow 40 dye (in sequence)
- Iron oxide-based powder suspension
- Superglue and basic yellow 40 dye

(N.B. other options were also considered at various stages)

8.2.10 The overall findings indicated that iron oxide-based powder suspension and superglue followed by basic yellow 40 dye staining were approximately equivalent for fingermark visualisation, as were the two sequences beginning with powder. Furthermore, the sequences beginning with powder generally recovered more fingermarks than either iron oxide-based powder suspension or superglue and basic yellow 40 used as singular processes. Typical results from a surface are illustrated below.

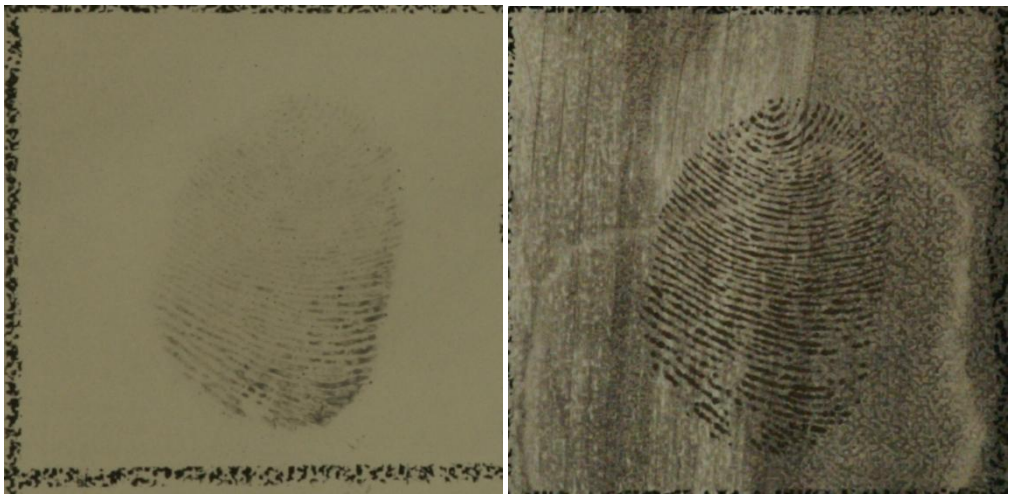


Typical results obtained comparing the effectiveness of iron oxide-based ('Fe-based') powder suspension, and superglue (with basic yellow 40*), both singularly and in sequence following the use of powders (a smooth surface in this example) [17].*



a) b)

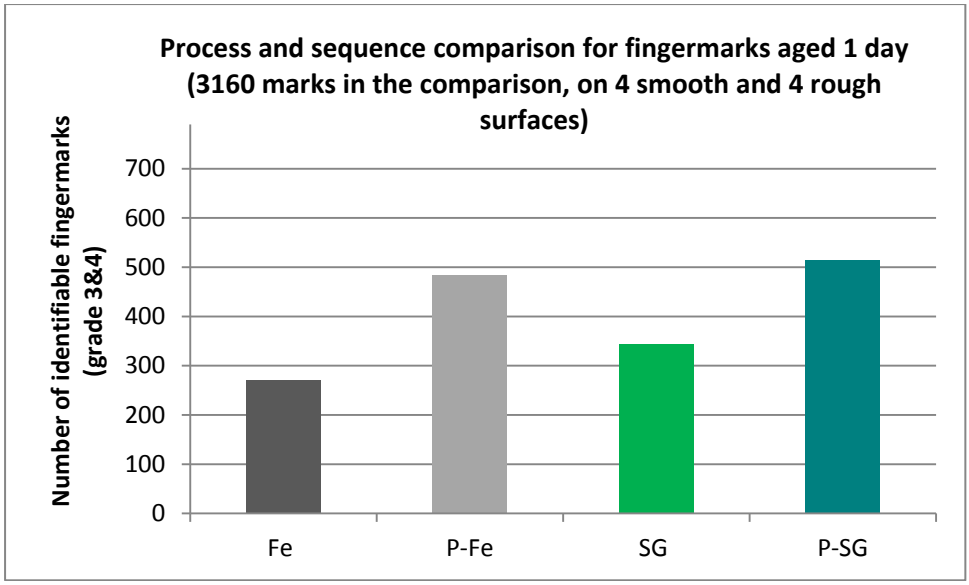
Enhancement of a mark on a smooth painted surface a) *after application of aluminium powder and* b) *improvement obtained by subsequent treatment with iron oxide-based powder suspension.*

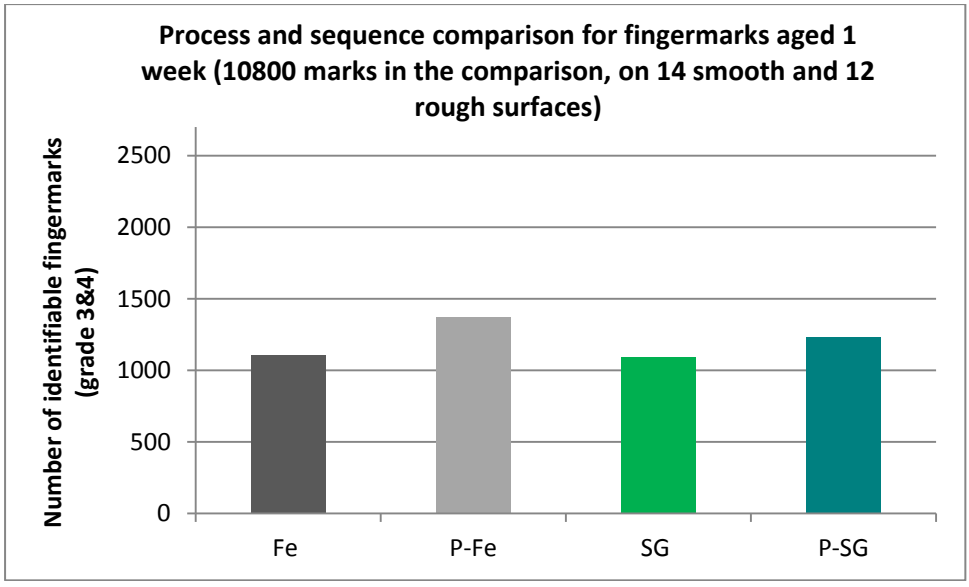


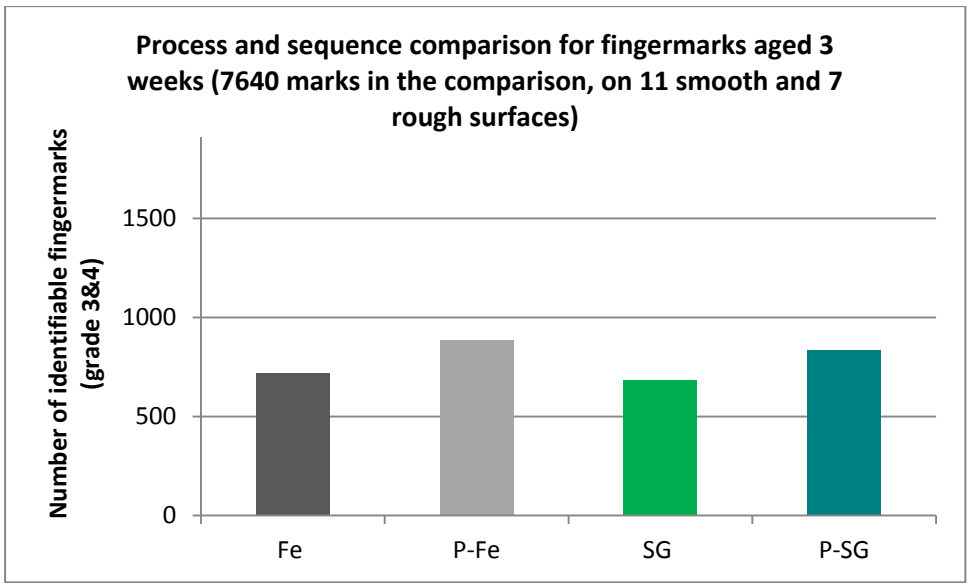
a) b)

Enhancement of a mark on a textured surface (white melamine) a) *after application of black magnetic powder and* b) *improvement obtained by subsequent treatment with iron oxide-based powder suspension.*

8.2.11 The overall trends of this study are summarised in the graphs below.


Process and sequence comparison for fingermarks aged 1 day (3160 marks in the comparison, on 4 smooth and 4 rough surfaces)
Number of identifiable fingermarks (grade 3&4)
700
600
500
400
300
200
100
0
Fe
P-Fe
SG
P-SG


Process and sequence comparison for fingermarks aged 1 week (10800 marks in the comparison, on 14 smooth and 12 rough surfaces)
Number of identifiable fingermarks (grade 3&4)
2500
2000
1500
1000
500
0
Fe
P-Fe
SG
P-SG


Process and sequence comparison for fingermarks aged 3 weeks (7640 marks in the comparison, on 11 smooth and 7 rough surfaces)
Number of identifiable fingermarks (grade 3&4)
1500
1000
500
0
Fe
P-Fe
SG
P-SG

8.2.12 A similar study was conducted with white powder suspensions on dark surfaces to enable firm recommendations to be made [18]. In this study the following surfaces were examined.

General surface classification	**Specific description and designation**
Smooth, non-porous	S1 Grey PVC
	S2 Black polypropylene
	S3 Dark brown wood effect melamine laminate
	S4 Black gloss painted metal
	S5 Dark blue ceramic tile
	S6 Black compressed polystyrene
Rough, non-porous	R1 Mottled grey kitchen worktop melamine
	R2 Black 'fake leather' laminate on aluminium
	R3 Black polythene
	R4 Black matt painted metal
	R5 Black textured compressed polystyrene

Description of the surfaces used in the comparative study between various sequences of superglue, powders and powder suspensions on dark surfaces.

8.2.13 In this study, 21 donors placed depletion series of 10 marks on each of the 11 different surfaces studied. The experiment looked at marks that were 1 week and 3 weeks old, giving a total number of 4,620 graded marks. The purpose of the experiment was to determine the optimum processing sequence, assuming that powders would always be the first process used. White powder suspensions and superglue + basic yellow 40 were compared in terms of their effectiveness as secondary treatments. A summary of the trends observed in the data across all surfaces studied is given below.

Surface	**Best sequence**			
	1-week-old marks		**Older marks (> 1 week)**	
	Powders + superglue/dye	**Powders + powder suspensions**	**Powders + superglue/dye**	**Powders + powder suspensions**
S1		X	X	
S2		X		X
S3	X		X	
S4		X		X
S5		X	X	
S6		X		X
R1	X		X	
R2	X		X	
R3	X		X	

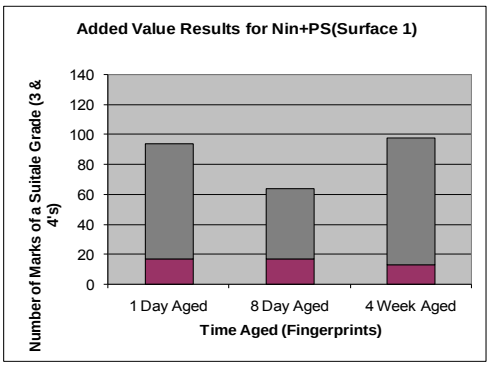
R4		X		X
R5		X		X

Best optimum processing sequence for different ages of marks across all surfaces studied.

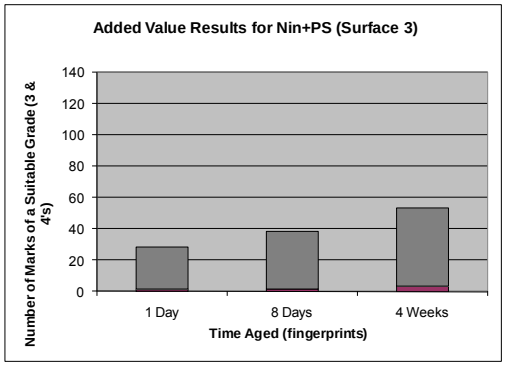
8.2.14 On smooth, dark surfaces, powders followed by white powder suspensions give closely equivalent performance to powders followed by superglue and both sequences can be recommended with equal weighting. On rougher, dark surfaces the sequence of powders followed by superglue gives better results and would be the sequence of choice, unless it is known that the surface has been wetted.

8.2.15 Comparative work has also been carried out to establish the effectiveness of powder suspensions on wetted non-porous surfaces [31,32]. The results indicated that on certain wetted surfaces powder suspensions may be more effective than vacuum metal deposition [32]. Slight differences were also observed between the effectiveness of different formulations of powder suspension [31].

8.2.16 The feasibility of using powder suspensions to develop marks on different types of fixed surface that may be encountered at crime scenes has also been studied by CAST. Fletcher [38] conducted an assessment of the relative effectiveness of iodine solution, ninhydrin and powders on a range of wall coverings including different types of matt paint, gloss paint, vinyl coated wallpaper and paper-based wallpaper. After these surfaces had been treated and graded, Mehmet [39] applied iron oxide-based powder suspension and re-graded the panels, recording the number of additional marks developed by powder suspensions.



a)



b)

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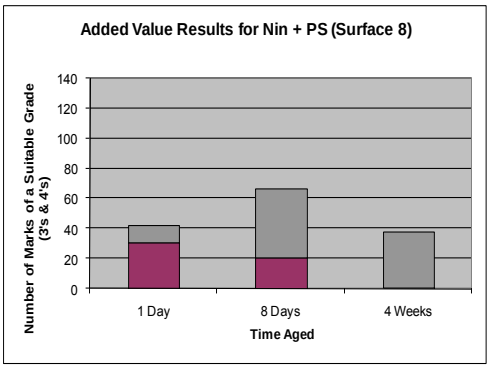
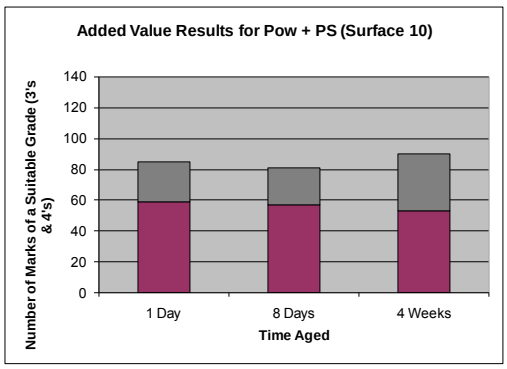


c)



d)

Examples of 'value added' assessments conducted after applying powder suspensions after treatment with ninhydrin or powders. Surfaces shown include a) *matt paint,* b) *paper-based wallpaper,* c) *vinyl coated wallpaper and* d) *gloss paint*

8.2.17 The results clearly show the potential to use powder suspensions after existing processes on wall surfaces, but the process is extremely messy and will cause damage. This may be acceptable in some circumstances, but ideally a less messy means of applying and removing the powder suspension needs to be researched.

8.2.18 Investigations into fingermark visualisation strategies for semi-porous materials were conducted at CAST between 2009 and 2011 [23, 24]. Semi-porous materials were represented in these studies by glossy cards, printed-on cards, magazines (covers and interior pages) and wrapping papers. Initial work by Thatcher [23] explored the use of iron oxide-based powder suspension along side powders, superglue fuming, vacuum metal deposition (VMD), 1,8-diazafluoren-9-one (DFO), ninhydrin, and physical developer. Results indicated that iron oxide-based powder suspension, powder, superglue fuming and vacuum metal deposition (the techniques typically used on non-porous substrates) offered greater overall capability than DFO, ninhydrin and physical developer (the processes typically used on porous items) [23]. Further sequential evaluations demonstrated that the initial use of ninhydrin was detrimental to the effectiveness of powder suspension, superglue fuming or vacuum metal deposition when used subsequently. However, ninhydrin could be used to some effect when applied after vacuum metal deposition or superglue fuming [23].

8.2.19 This led to a comparison of sequential process strategies (listed below) by Kelly [24] on magazine pages and wrapping papers. This was a relatively small-scale study, but the results indicated that all four options offered similar overall capability [24].

- VMD → DFO → Ninhydrin → Physical developer
- Black magnetic powder → DFO → Ninhydrin → Physical developer
- Superglue fuming + black magnetic powder → DFO → Ninhydrin → Physical developer

- Iron oxide-based powder suspension → Physical developer

8.3 Pseudo-operational trials and operational experience

8.3.1 Initial operational trials have been carried out to compare the effectiveness of basic violet 3 with black powder suspensions on the adhesive side of tapes. The results of these trials are summarised in Chapter 3, Chemical and Physical Processes, Basic violet 3, and demonstrate that powder suspensions are the more effective process. However, superglue is also known to be a highly effective treatment for adhesive tapes. A subsequent operational trial commenced comparing iron oxide-based black powder suspensions with the superglue process, looking at marks developed on both adhesive and non-adhesive sides of the tape.

8.3.2 Based on the results obtained using carbon-based black powder suspensions, this operational trial was modified to include a commercial carbon-based formulation in addition to the iron oxide-based formulation. The trial results are recorded below.

Process	**Cases**	**Number of positive results (cases)**				**% positive**
		Non-adhesive	Adhesive	Both	Total	
Superglue/basic yellow 40	59	9	13	1	23	39
Iron oxide powder suspension	45	1	15	1	17	38
Carbon powder suspension	33	1	14	1	16	48

Operational trial results for different processes on light coloured adhesive tapes, combined results from two UK police laboratories between 2006 and 2008.

8.3.3 It can be seen that carbon-based black powder suspensions were found to be the most effective process for the adhesive side of tapes, and were therefore recommended for operational use.

8.3.4 The CAST white powder formulation was then used in an operational trial, comparing results with those obtained using superglue and dyeing. The trial results are summarised below.

Process	**Cases**	**Number of positive results (cases)**				**% positive**
		Non-adhesive	Adhesive	Both	Total	
Superglue/basic yellow 40	33	1	11	1	13	40
White powder suspension	39	1	11	2	14	36

Operational trial results for different processes on dark coloured adhesive tapes, combined results from two UK police laboratories between 2006 and 2008.

8.3.5 Superglue was found to be the more effective process on operational casework and white powder suspensions were not ultimately recommended for use on adhesive tapes, except in circumstances where dark tapes had become wetted.

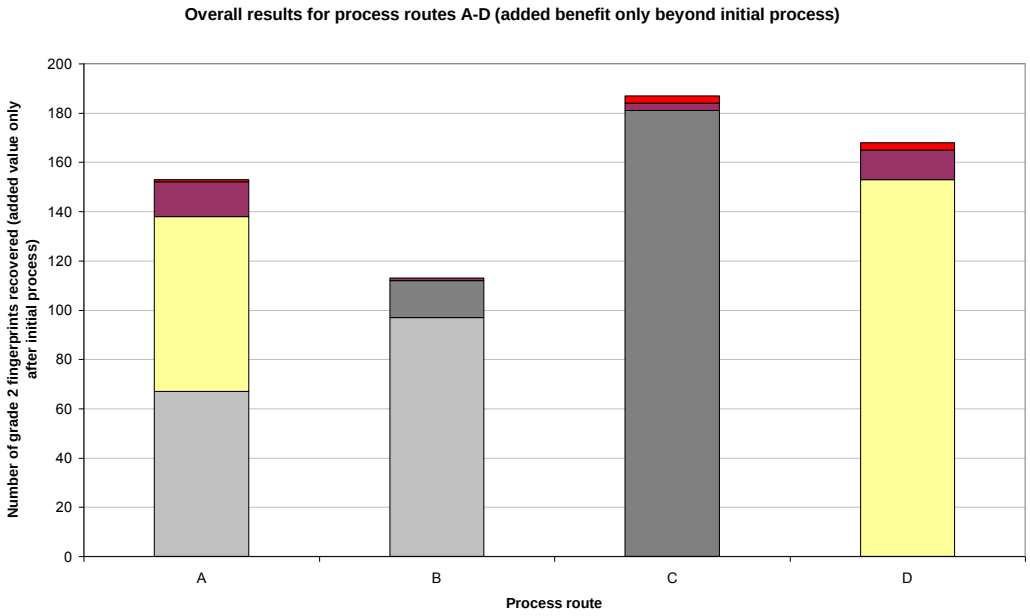
8.3.6 In 2009 CAST conducted a similar investigation to the pseudo-operational trial on plastic bags conducted in 1986. In this more recent trial, 100 bags and plastic packaging materials from different sources (e.g. supermarket carrier bags, 'bags for life', black bin bags, clear magazine wrappings) were collected from as realistic environments as possible [21,40]. Each bag was divided into quarters, with each quarter being examined using a different fluorescence examination regime followed by a separate sequence of chemical treatments [21,40]. The number of marks developed using each process was recorded. The results from the fluorescence examination stage in the trial have already been included in Chapter 2, Optical Processes, Fluorescence examination. Iron oxide-based powder suspension and White Wet Powder™ were included as the powder suspensions in these trials (for lighter and darker items respectively), both as an initial process and as a secondary treatment subsequent to vacuum metal deposition (VMD).

8.3.7 The results of these trials for the first 50 items are summarised below:

- Process route A = VMD –superglue + BY40 – basic violet 3;
- Process route B = VMD –powder suspensions – basic violet 3;
- Process route C = powder suspensions – basic violet 3;
- Process route D = superglue + BY40 – basic violet 3.

Process route	1st process	2nd process	Basic violet 3/ visible	Basic violet 3/ 577nm laser
A	67	71	14	1
B	97	15	1	0
C	181		3	3
D	153		12	3

Summary of the number of marks visualised on flexible plastic packaging at each stage of sequential processing routes (added benefit only beyond 1st process) [21].

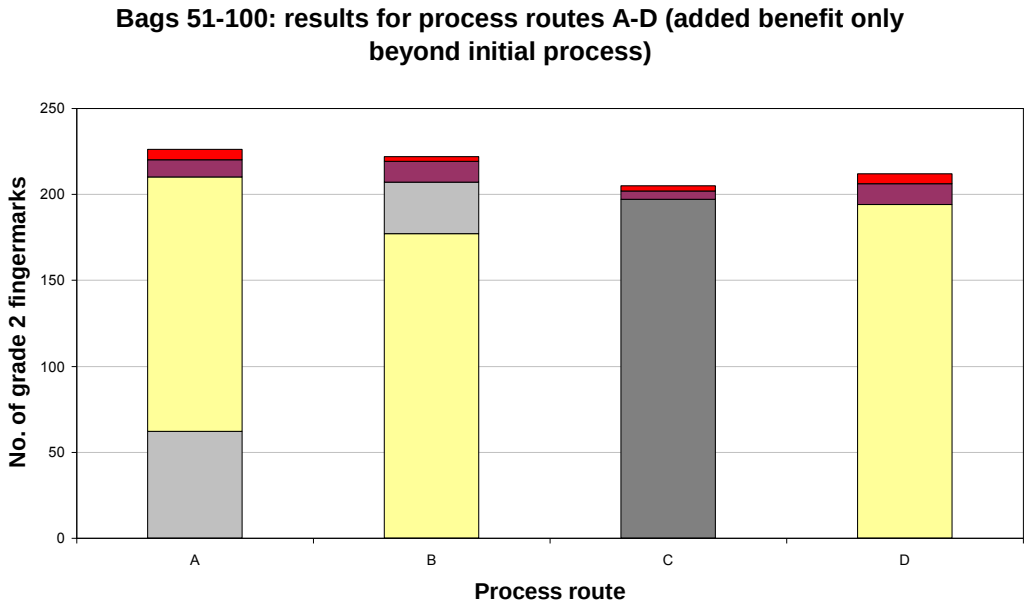


Graphical representation of the data summarised in the table above (light grey = VMD, dark grey = powder suspensions, yellow = superglue + basic yellow 40, purple = basic violet 3 (visible), red = basic violet 3 (fluorescence with 577nm laser) [21].

8.3.8 For the second 50 bags, the poorly performing process route B (VMD – powder suspensions – basic violet 3 sequence) was replaced by superglue + BY40 – VMD – basic violet 3. The results from these studies are summarised below.

Process route	1st process	2nd process	Basic violet 3/ visible	Basic violet 3/ 577nm laser
A	62	148	10	6
B	177	30	12	3
C	197		5	3
D	194		12	6

Summary of the number of marks visualised on flexible plastic packaging at each stage of sequential processing routes (added benefit only beyond 1st process) [21].



Graphical representation of the data summarised in the table above (light grey = VMD, dark grey = powder suspension, yellow = superglue + basic yellow 40, purple = basic violet 3 (visible), red = basic violet 3 (fluorescence with 577nm laser) [21].

8.3.9 Powder suspensions performed well in these trials, giving equivalentperformance to superglue and BY40 dye. However, superglue + dye can be followed by VMD in sequence, and this may therefore present the most effective overall strategy (unless the plastic packaging or bag is known to have been wetted). For wetted items, the powder suspensions process is the main process recommended [21,40].

8.3.10 Farrugia et al [41] conducted a pseudo operational trial comparing the effectiveness of powder suspensions, superglue fuming and BY40 dye, and the one-step Lumicyano™ superglue method for plastic carrier bags. One hundred items were processed, and overall the processes yielded comparable numbers of fingermarks [41].

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8.3.10 Many police forces have now implemented operational use of both black and white powder suspensions, both in a laboratory as a replacement for superglue on items that may have been wetted (cowlings, car number plates) or contaminated (drugs wraps), and at scenes after the application of powders. Significant increases in the number of marks developed have been reported.

8.3.11 Powder suspensions have also been successfully used to develop marks on items recovered from arson scenes by more than one police force, in accordance with observations during CAST studies [42]. Laboratory tests have indicated that it may also be the best treatment for situations where cars have been sprayed with WD40 to destroy fingermarks [43]. This has been reinforced by subsequent studies on grease contaminated surfaces by Gaskell *et al.* [44], who show that in general both black and white powder suspensions have greater affinity for latent fingermark constituents than they do for grease contamination and have the potential for use in sequence before processes that target the grease contamination.

8.3.12 It has also been observed in studies into recovery of fingermarks and footwear marks in blood [45,46] that powder suspensions can be used as an additional treatment after acid dyes to enhance any marks present, and they are recommended for operational use for this purpose. However, powder suspensions are not specific to blood and therefore the presence of blood will need to be confirmed by other means.

8.3.13 Powder suspensions have been shown to be a highly effective alternative to superglue fuming in many applications, and the fact they can still develop marks on wetted surfaces makes them potentially more versatile. They are also particularly effective when used after powders and the process has been incorporated into the non-porous and semi-porous charts in the *Fingermark Visualisation Manual* [26], including the charts where blood and grease contamination are present.

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Radioactive sulphur dioxide

1. History

1.1 The potential application of radioactive sulphur dioxide ($^{35}SO_2$) for the development of latent fingermarks was first reported by Grant *et al.* in 1963 [1] during the course of investigations into the resistance of paper to attack by atmospheric pollution. They observed that when developing autoradiographs of paper treated with SO_2, spots could be seen on the paper that on closer examination were identified as fingermarks. A further publication by Grant *et al.* [2] gave more background detail on the method used. Radioactive SO_2 was measured into an evacuated flask and the pressure raised to atmospheric by the addition of air at a controlled humidity of 66%. The paper sample was exposed to the gas mixture for 12 hours, then placed against x-ray film for 1 week. Other experiments carried out by the researchers demonstrated that ageing of the marks reduced the chances of fingermark development. It was also found that alkaline fillers in the paper could give rise to heavy SO_2 take-up by the background and that metal impurities also picked up SO_2.

1.2 The results of further research into the technique were reported by Spedding in 1971 [3]. He suggested that SO_2 was reacting with the lipids present in fingermark deposits and noted that reactions occurred with oleic and linoleic acids. Prints were also developed on paper that had been wetted. Spedding *et al.* also issued a more detailed report [4], providing details of the apparatus used for development of fingermarks. This consisted of a treatment box within which samples could be hung on a rail. Humidity inside the box was raised to 60%, radioactive SO_2 introduced and the samples exposed for 30 minutes before being removed and placed in contact with x-ray film. Trials were also conducted for a range of paper types, comparing the effectiveness of SO_2 for fingermark development with that of ninhydrin and iodine. SO_2 was found to be the most effective technique across the range of paper types investigated. Spedding *et al.* also considered the potential effects of the SO_2 technique on subsequent development techniques, in particular the 60% humidity and SO_2 concentration used. It was considered that the humidity could be detrimental to the subsequent use of silver nitrate, but that other techniques should be unaffected. The report also suggested that radioactive SO_2 could prove a useful technique for the development of fingermarks on fabrics.

1.3 The wider application of the technique to substrates other than paper was reported in late 1970 [5]. Excellent results were reported for PVC sheet and initial results on fabrics were encouraging. Further studies into the optimum humidity for treatment were presented, with humidity levels in excess of 60% giving rise to an increase in the uptake of SO_2 in the substrate compared with that in the fingermark, and therefore being undesirable.

1.4 The initial results obtained for paper exhibits had been encouraging and the technique was used on operational exhibits of types that had previously given poor results with ninhydrin, iodine and silver nitrate. An early operational success was obtained on forged £5 notes [6].

1.5 Research into the technique continued, with the objectives of establishing optimum processing conditions and the range of substrates that radioactive SO_2 could develop marks on. A more detailed study was carried out into fingermarks deposited on paper [7], investigating the effect of storage time (1 to 6 days) and storage humidity (31 to 93% relative humidity) on the quality of fingermarks developed using radioactive SO_2, ninhydrin, iodine, silver nitrate and vacuum metal deposition (VMD), then also in a developmental stage. Across the range of conditions studied VMD gave the best results, followed by ninhydrin. In these trials radioactive SO_2 performed relatively poorly. In contrast, studies conducted on dry paper identified SO_2 as being more effective than ninhydrin, silver nitrate and a sequence of ninhydrin followed by silver nitrate [8]. One advantage of the SO_2 process was that it eliminated much of the printed text that could potentially obscure minutiae. An optimum development sequence of SO_2 > ninhydrin > silver nitrate was proposed for paper exhibits.

1.6 The major area of research for the practical application of radioactive SO_2 was the development of fingermarks on fabrics and a comprehensive report into these studies was issued by Wells in 1975 [9]. The equipment used in these studies consisted of a 150 litre Perspex box into which a mixture of radioactive SO_2 and nitrogen gas (N_2) was introduced. The optimum humidity was identified as 65%, but effectiveness fell rapidly in the range 66 to 75% and 60% was recommended for operational purposes. The addition of ozone into the gas mixture was found to increase SO_2 uptake by the fingermark and thus reduce autoradiography times. An autoradiography guide was developed for a range of substrates including fabrics, plastic wrappings and banknotes, outlining optimum development times. The use of a dark, sealed enclosure containing desiccant was recommended for storage of exhibits prior to treatment. Fingermarks were successfully developed on a wide range of fabrics, although the quality and number of marks were significantly reduced when ageing conditions involving any degree of high humidity were used. Extended exposure to atmospheric, non-radioactive SO_2 was also thought to desensitise the print. Prints on Melinex film were least affected by these conditions, followed by prints on fabrics, with paper being the most affected. Throughout the studies operational work was performed to see if marks could be developed on real fabric exhibits and parallel studies were also performed on fabrics worn for different periods of time, both next to the skin and as outer garments. It was concluded that for operational work on fabrics, exhibits needed to be dry, of fine weave and not worn next to the skin.

1.7 In the mid-1970s an Atomic Weapons Research Establishment (AWRE) system using compressed SO_2 cylinders was used on adhesive tape

from Irish Republican Army (IRA) improvised explosive devices (IEDs) and numerous fingermarks were found. The rise of Republican terrorism and the planting of IEDs on the mainland led to a need for a method of processing adhesive tape from unexploded devices. The Police Scientific Development Branch (PSDB) worked closely with the anti-terrorist unit and eventually trained members of the unit to use the radioactive SO_2 system. A number of identifications from the terrorists fingermarks on adhesive tape were found. The main reason for the use of SO_2 was that most of the tape was black and a non-destructive method was required in order to carry out other forensic examinations for fibres, hair and mechanical fit. The equipment using a pressurised gaseous source of SO_2 was potentially hazardous and PSDB designed and built a metal-free reaction chamber and control system, and a simple Perspex chamber was developed for treatment of exhibits [10]. The source of SO_2 was changed from pressurised gas cylinders to paper impregnated with radioactive thiourea, which was ignited to release radioactive SO_2 gas. This became the standard system introduced in the UK for operational work although only two other systems were built, one for the Metropolitan Police Forensic Science Laboratory Serious Crimes Unit (MPFSL SCU) and one for the Birmingham laboratory of the Forensic Science Service (FSS). PSDB also developed light-tight sachets based on aluminised Melinex for autoradiography of non-flat items in daylight [11] and further research was carried out to investigate methods for developing marks on curved surfaces [12]. The development of the basic violet 3 (Gentian Violet) transfer technique subsequently reduced the need for SO_2 on tapes.

1.8 Initial studies to investigate the relative effectiveness of SO_2 on adhesive tapes established that marks could be detected on both sides of the tape using this technique. Marks were shown to survive for 64 days on the adhesive side, although survival times were shorter on the non-adhesive side and dependent on whether the tape was stored indoors or outdoors [13]. The technique was found to give excellent operational results on adhesive tapes in several terrorism-related cases in the 1970s, and became regarded as an essential treatment for this type of exhibit in serious and terrorist-related cases [14]. A further comparative trial was carried out on fabrics in the early 1980s, investigating the relative effectiveness of several techniques including SO_2, VMD and osmium tetroxide fuming [15,16]. This study looked at ageing fingermarks on a range of fabrics and types of weave. These studies showed that SO_2 was the most effective of the three techniques, developing appreciably more high quality marks than VMD, the next most effective technique.

1.9 A small operational trial was also carried out with some ridge detail being developed in at least two operational cases on fine synthetic outer garments. A small scale evaluation of SO_2 as an enhancement technique for superglue on synthetic substrates was also carried out and an identifiable, policeman's fingermarks were found on one nylon outer garment. With this operational work showing limited success, the technique became mainly limited to use on adhesive tapes. However, the

complexity of equipment required to carry out the processing, and the health and safety issues associated with the use of radioactive isotopes, led to a gradual decline in the operational use of the technique The last operational equipment was decommissioned by the FSS, Lambeth in 2005. The CAST equipment has been transferred to the Radiochemistry laboratories at Loughborough University with a view to restoring it to operational use for experimental, comparative work with other processes but timescales for this have yet to be determined.

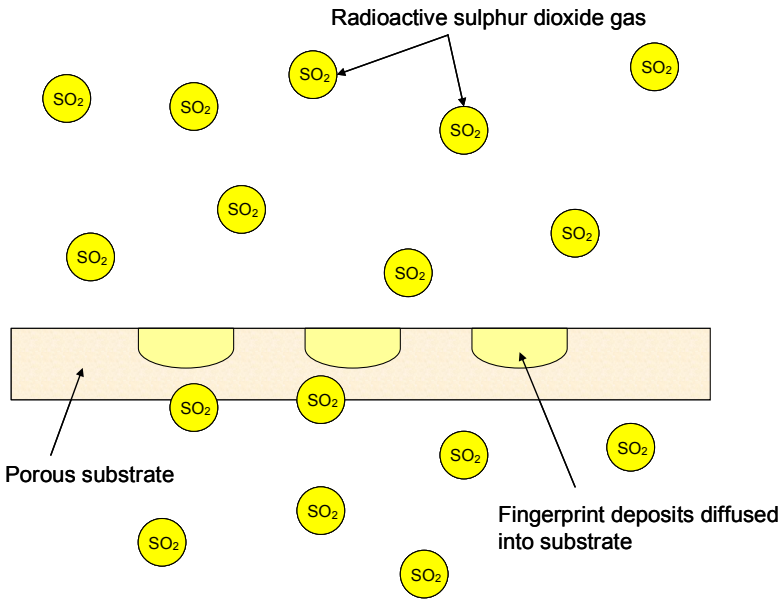
2. Theory

2.1 Wells [9] in his comprehensive report of the radioactive SO_2 process, suggested that several reactions with fingermark deposits were possible and that a complex combination of these contributed to the fingermark development process. The mechanisms processed by Wells included the following.

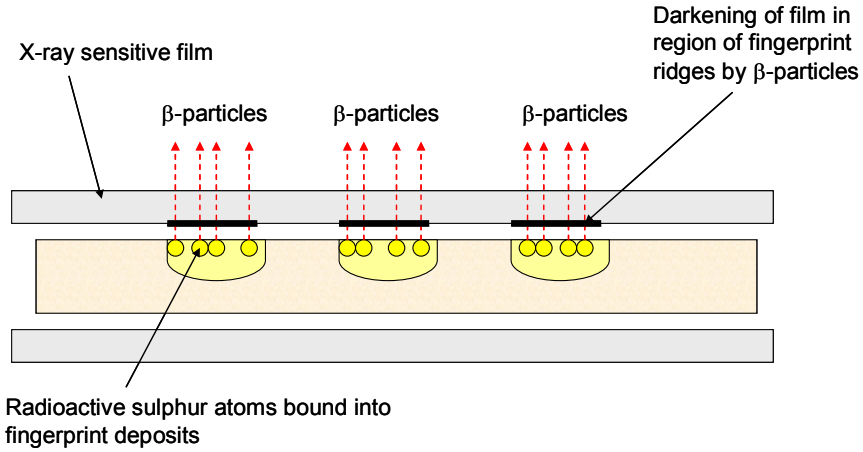
- The fixation of SO_2 as SO_4^{2-} in the water phase associated with sebum and in water adsorbed from the atmosphere due to the hygroscopic nature of the deposit.
- The sensitisation of wettable substrates (e.g. paper, fabric) by adsorbed layers of water molecules directly as a result of contact by fingermark ridges.
- Reaction(s) with lipids, which may involve the double-bonds of unsaturated free fatty acids, etc.

2.2 The strong dependence of the SO_2 reaction on the relative humidity during treatment tends to support the theory that the main reaction occurring is the water phase fixation mechanism.

2.3 The development of fingermarks by the radioactive SO_2 process and subsequent autoradiography is illustrated schematically below.



a)



b)

Schematic diagram illustrating the radioactive sulphur dioxide process a) *sulphur dioxide gas diffusing through porous substrate and* b) *autoradiography of sample with radioactive sulphur bound into fingermark ridges.*

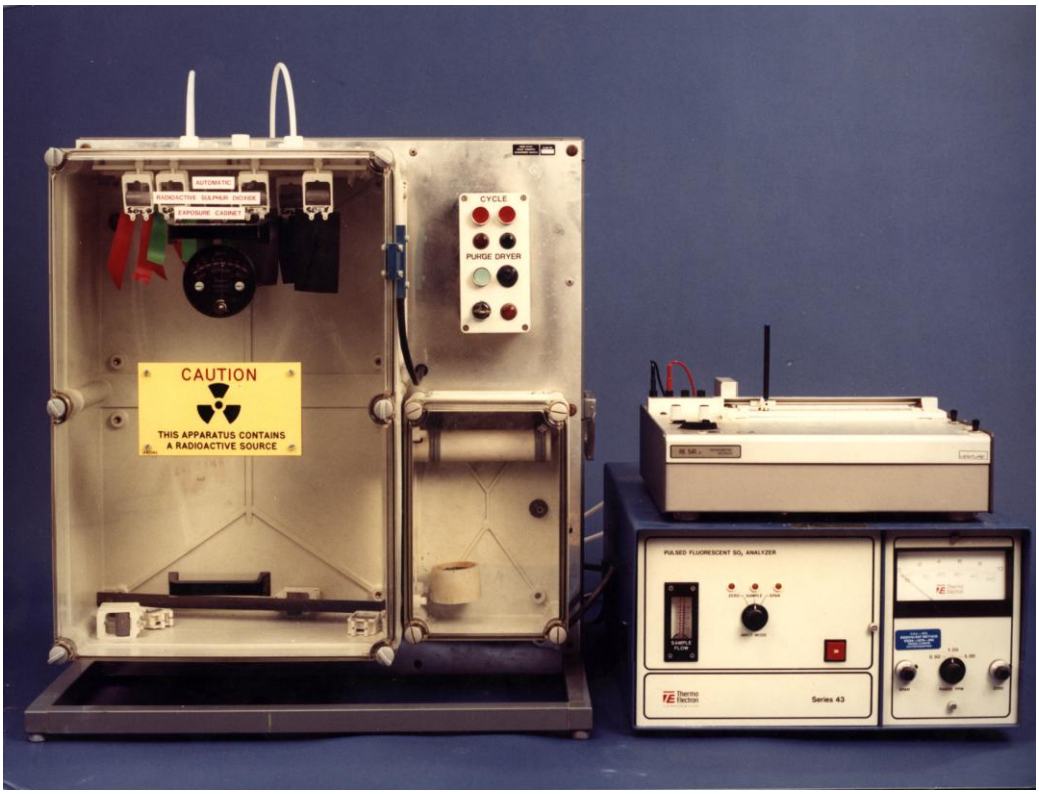
3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 The process is not included as a Category A process in the *Fingermark Visualisation Manual* (published 2014) because it is not currently

available for operational use. It is retained as a Category B process with niche applications, but this is subject to the equipment being restored to working order so that meaningful comparisons can be conducted with other processes. The technique ultimately recommended by the Home Office Centre for Applied Science and Technology (CAST) was the combustion of filter paper impregnated with radioactive thiourea in a humidity-controlled cabinet.

3.2 The SO_2 sources were prepared by dissolving radioactive thiourea in water and decanting small aliquots of solution onto discs of filter paper. It is essential to use readily combustible cellulose-based filter papers for this purpose. The concentration of the solution was adjusted to give a concentration of 1 mCi (milliCurie) per 50 μL, with 5 μL being impregnated into each disc to give a disc content of 0.1 mCi of thiourea.

3.3 The impregnated disc was then loaded into the crucible chamber of the radioactive SO_2 apparatus. The system used activated charcoal to remove the SO_2. After the normal treatment time of 20 minutes, the gas content of the chamber was passed through the charcoal scrubbing system. A separate chamber containing water was used in the initial humidification phase, which was manually controlled.



Photograph of the radioactive sulphur dioxide apparatus.

3.4 Samples were then suspended in the main chamber, which was sealed and brought to a relative humidity of 55%. The impregnated disc was then ignited and allowed to fill the chamber with the pre-determined concentration of radioactive SO_2 released by combustion. Once the cycle

had completed and the SO_2 level had returned to the value before commencing treatment, articles were removed from the chamber, sandwiched between two sheets of x-ray film and then placed in a press. Activity was monitored with a Geiger counter to calculate exposure times, typically seven to ten days.

3.5 The humidity level in the chamber and concentration of radioactive thiourea used in the process were chosen to give the optimum conditions identified in early experimental work. The role of the thiourea in the process was to release SO_2 as a combustion product.

3.6 The process involved constant monitoring of all items of laboratory equipment, clothing and exhibits that came into contact with radioactive material, and the disposal of contaminated articles in an approved fashion.

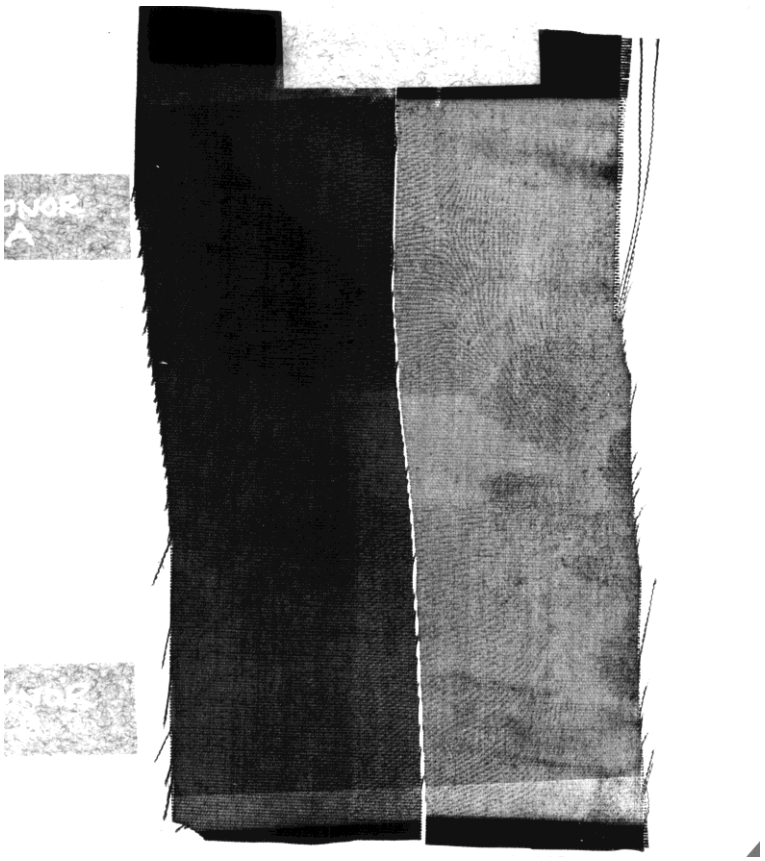
4. Critical issues

4.1 The technique is no longer used operationally and therefore there are no critical issues associated with its use. However, continuous monitoring of radioactivity levels was required when carrying out processing.

5. Application

5.1 Suitable surfaces: Radioactive SO_2 was suitable for use on both sides of adhesive tape and on fabrics. In practice it could be used on both porous and non-porous surfaces, but was restricted to articles small enough to fit inside the reaction chamber.

5.2 The two applications for which radioactive SO_2 was suggested in the *Manual of Fingerprint Development Techniques* [17] are as part of a sequential treatment process for adhesive tapes, and as the principal treatment for fabrics. In theory it was a versatile technique and could be applied to both porous and non-porous surfaces, a potential advantage being that patterned backgrounds that could obscure the developed mark were not visible in the autoradiograph.



Autoradiograph of fabric sample exposed to different environments and treated with radioactive sulphur dioxide.

5.3 When applied to adhesive tapes, the technique was capable of developing marks on both sides of the tape simultaneously, and was also effective on vinyl tapes where techniques such as VMD performed poorly. During the 1970s this type of tape was often found on explosive devices and radioactive SO_2 gave good results, resulting in its continued use on terrorist-related cases until the mid-2000s.

5.4 The technique was shown to be the most effective process for development of marks on fabrics, although in practice no marks with sufficient detail for a positive identification were obtained from operational work.

6. Alternative formulations and processes

6.1 Other vapour phase materials labelled with radioactive isotopes have been considered for the development of fingermarks using autoradiographic methods. Goode *et al*. [10, 18] considered the use of radioactive bromine in two forms, ^{80}Br and ^{82}Br. Bromine was considered for its potential reaction with unsaturated fats in the fingermark deposit and for the fact that this reaction is rapid. Both isotopes also have a shorter half life than radioactive SO_2, which is advantageous. Fingermarks were successfully developed on a range of paper

substrates using radioactive Br_2 and the process shown to be quicker than SO_2 [18]. The quality of the developed prints was shown to be similar to those produced by SO_2, although the contrast of the marks was significantly degraded by exposure to ultraviolet radiation. The technique as originally applied utilised vacuum equipment and this was thought to be clumsy compared with the apparatus used for the more established SO_2 technique. As a consequence, radioactive Br_2 was not pursued further.

6.2 Higgins also reported the use of radioactive iodine (^{128}I) in iodine vapour and in radioactive iodine monochloride (ICl) [19] for the development of fingermarks on paper and again a reduced processing time was achieved compared with SO_2. Although initial trials were successful, the technique was not progressed further.

7. Post-treatments

7.1 No post-treatments are used with the radioactive SO_2 technique other than autoradiography for developing the marks on photographic paper.

8.Validation and operational experience

8.1 Laboratory trials

8.1.1 The largest recorded laboratory trial for radioactive SO_2 was a comparison with VMD on a range of different fabrics representative of over- and undergarments [16]. This trial used six donors, each placing one mark that was split and aged for one day prior to processing. The results are outlined below in terms of individual fabric type, and further summarised in the second table.

Material	**Process**	**Grade of mark**			
		1	**2**	**3**	**4**
Brown, 100% Nylon Warp knit, 1.5 stitches x 3 rows	SO_2	0	2	3	1
	VMD	1	5	0	0
Cream, 100% Silk Standard weave, 3 weft x 3 warp	SO_2	0	3	1	2
	VMD	0	5	1	0
White, 100% Acetate Standard weave, 3 weft x 3.5 warp	SO_2	0	1	4	1
	VMD	4	2	0	0
Grey, 100% Polyester Standard weave, 3.5 weft x 4 warp	SO_2	6	0	0	0
	VMD	5	1	0	0
Cream, 65/35% Polyester/Cotton Standard weave, 3 weft x 4 warp (well worn)	SO_2	6	0	0	0
	VMD	6	0	0	0
White, 65/35% Polyester/Cotton Standard weave, 3 weft x 4 warp	SO_2	3	3	0	0
	VMD	0	4	2	0
White/blue stripe, 65/35% Polyester/ Cotton, Standard weave, 3 weft x 4 warp (well worn)	SO_2	3	3	0	0
	VMD	2	4	0	0
Yellow, 65/35% Polyester/Cotton Standard weave, 3 weft x 4.5 warp (well worn)	SO_2	0	6	0	0
	VMD	2	4	0	0
Red, 80/20% Polyester/Cotton Standard weave, 3 weft x 3.5 warp	SO_2	2	3	1	0
	VMD	3	3	0	0
White, 100% Nylon 'antistat' Warp knit, 1.25 stitches x 2 rows (well worn)	SO_2	0	5	1	0
	VMD	0	6	0	0
White, 100% Nylon Kayser 'antistat', warp knit, 1.5 stitches x 2 rows	SO_2	0	5	1	0
	VMD	0	6	0	0
White, 100% Nylon Fine Fare, Warp knit, 2 stitches x 2 rows	SO_2	0	5	1	0
	VMD	0	6	0	0

White, 100% Nylon 'counterstat', Warp knit, 2 stitches x 2.5 rows	SO_2	1	4	1	0
	VMD	0	4	2	0
White, 100% Nylon Kayser, Warp knit, 2 stitches x 2.5 rows	SO_2	0	6	0	0
	VMD	0	5	1	0
White, 100% Polyester, 4 Float satin weave, 4 weft x 4 warp	SO_2	0	1	2	3
	VMD	0	3	3	0

Material	**Process**	**Grade of mark**			
		1	**2**	**3**	**4**
All fabrics	SO_2	21	47	15	7
	VMD	23	58	9	0

Summary of comparative study carried out between radioactive sulphur dioxide and vacuum metal deposition on fabrics.

8.1.2 The results indicate that that radioactive SO_2 produced about 50% more marks with ridge detail worth initiating a search against (grades 3 and 4) than VMD, and hence radioactive SO_2 was the principal technique recommended for treatment of fabrics. It should be noted that when this trial was conducted in 1984, an optimised superglue technique was not available.

8.1.3 A similar comparison was carried out between radioactive ICl and SO_2, again using six donors, each placing one mark that was split and aged for one day prior to processing. ICl did not develop any marks in this study, but radioactive SO_2 was found to give similar results to the initial trial against VMD.

Material	**Process**	**Grade of mark**			
		1	**2**	**3**	**4**
All fabrics (one-day-old marks)	SO_2	33	34	14	3
	ICl	84	0	0	0
All fabrics (one-week-old marks)	SO_2	45	27	8	4
	ICl	84	0	0	0

Results of further comparative studies between radioactive sulphur dioxide and iodine monochloride on fabrics.

8.1.4 Further comparative trials against osmium tetroxide as a fuming process and as a spray also showed SO_2 to be the most effective process on fabrics.

8.2 Pseudo-operational trials and operational experience

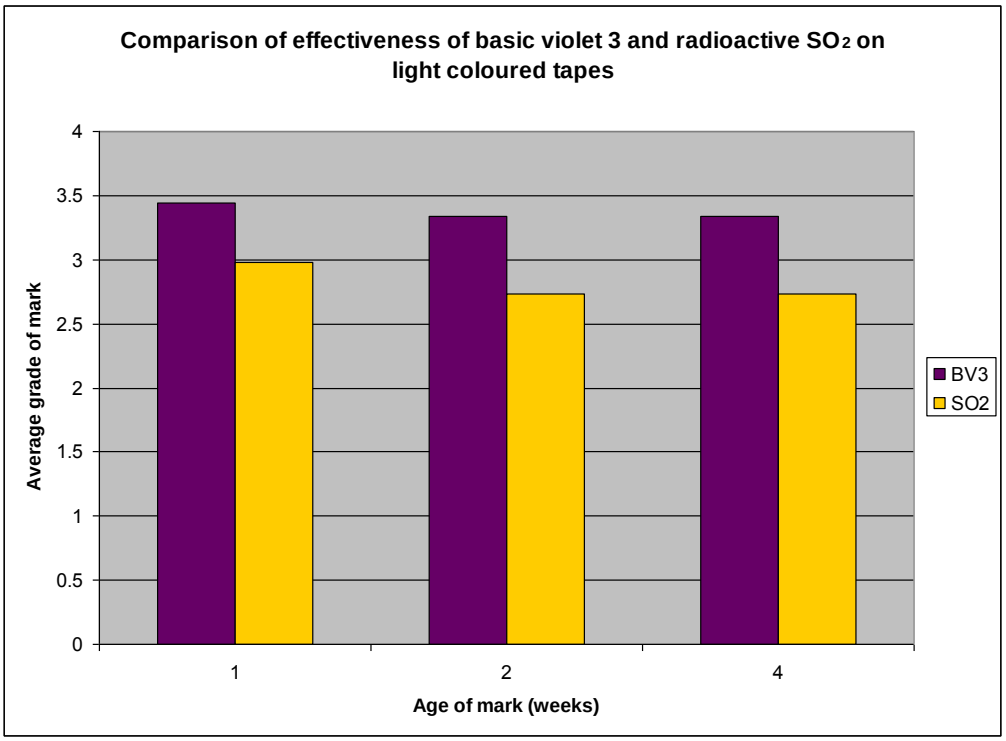
8.2.1 Operational figures for the first years that radioactive SO_2 was used are summarised below.

Period	Number of cases	Number of articles	Marks developed (cases)	Comments
01/01/1975 – 01/05/1975	4	118	4	Mainly PVC tapes
02/05/1975 – 02/05/1976	28	677	12	Mainly tapes and plastic bags
03/05/1976 – 31/03/1977	49	754	9 + 7 with fragmentary marks	Mainly tapes

Results of casework using radioactive sulphur dioxide in the mid-1970s.

8.2.2 The main successes of the technique were on dark and coloured PVC tape, where none of the techniques then available (basic violet 3, VMD) were capable of yielding marks. Many of these successes were in high-profile cases involving explosive devices.

8.2.3 A laboratory comparison was carried out by PSDB in the late 1970s, comparing basic violet 3 and radioactive SO_2 on a range of light coloured adhesive tapes. The results from approximately 300 graded marks are illustrated below.



Comparison of the relative effectiveness of radioactive sulphur dioxide and basic violet 3 on a range of light coloured adhesive tapes.

8.2.4 These results indicate that basic violet 3 is the more effective process, but the processes do not target the same constituents and may be used in sequence. In 1983 SRDB treated a series of tape exhibits using radioactive SO_2 and developed 11 marks, only one of which was subsequently detected by basic violet 3.

8.2.5 A further operational case in 1984 gave a further opportunity to assess the sequential processing of tapes from 18 separate exhibits, using basic violet 3 after radioactive SO_2. Results of this exercise are summarised below.

Side of tape	Both processes negative	Both processes developed same ridge detail	SO_2 developed more ridge detail	Basic violet 3 developed more ridge detail
Adhesive	28	0	2	11
Non-adhesive	18	12	10	1

Results of casework using radioactive sulphur dioxide and basic violet 3 on adhesive tape in 1984.

8.2.6 Again, basic violet 3 appeared the more effective process on the adhesive side but it was apparent that the two processes could be used sequentially.

8.2.7 The laboratory trials conducted by SRDB and reported above indicated that radioactive SO_2 was the most effective technique for development of marks on fabrics, and selected operational exhibits were treated between 1980 and 1984.

Period	Number of cases	Number of articles	Marks developed (cases)	Comments
01/09/1980 – 01/10/1984	12	13	4	No marks with sufficient ridge detail for identification

Results of casework using radioactive sulphur dioxide on fabrics from 1980 to 1984.

8.2.8 The factors affecting the recovery of identifiable marks were the time lapse before receipt of the exhibit (in many cases greater than one week) and the pattern of the fabric warp/weft obscuring ridge detail. At the present time (2016), the number of points of detail required for identification of a fingermark are less than they were in the 1980s (when a minimum 16-point standard was in place), and there are digital filtering techniques that can remove the patterned background from the image (such as fast Fourier transforms). Both these factors may have made the marks developed more operationally significant if developed in the current environment.

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Solvent black 3 (Sudan Black B)

1. History

1.1 Solvent black 3, alternatively known as Sudan Black B, is one of a class of azo dyes. Although some related compounds such as Sudan III (solvent red 23) and Sudan IV (solvent red 24) were available in the late 1800s and early 1900s, solvent black 3 was not introduced until the mid-1930s. Industrially, the dye is used for the coloration of organic solvents, printing inks, lacquers and a range of fats and wax substances [1].

1.2 Soon after its introduction the dye was proposed as a stain for fats and various other microbiological applications and has been successfully utilised in this role to this date (2016). The first published use of solvent black 3 for the development of latent fingermarks was by Mitsui *et al.* in 1980 [2]. They used a solution of solvent black 3 in a mixture of ethylene glycol, ethanol and water to develop marks on water-soaked paper items, the performance of solvent black 3 being shown to be superior to ninhydrin on this type of exhibit. This was soon followed by a further study by Stone and Metzger [3], comparing solvent black 3 with black magnetic powder on wetted porous items. In this comparison magnetic powder was found to give the best results.

1.3 In the early 1980s the Home Office Central Research Establishment (CRE) conducted an evaluation of over 60 biological dyes for their ability to develop latent fingermarks on both paper and polythene surfaces [4]. These studies also identified solvent black 3 as having particular potential for the development of fingermarks, in this case the best results being obtained on polythene. It was decided to proceed with an operational trial comparing the effectiveness of solvent black 3 with the two existing techniques recommended for polythene at the time, vacuum metal deposition (VMD) and small particle reagent (SPR) [5]. An initial phase of the work suggested that solvent black 3 gave superior results to VMD on polythene bags and the study was extended to a full operational trial. In these more detailed studies both VMD and SPR were found to be more effective than solvent black 3 and the reagent was not considered further for these applications.

1.4 The Police Scientific Development Branch (PSDB) subsequently re-evaluated the reagent and found that it had potential for developing fingermarks in cases where surfaces were contaminated and powdering was not possible. Examples of this type of surface included takeaway food containers or carbonated drinks cans. The process was subsequently included in the Home Office Centre for Applied Science and Technology's (CAST's) *Manual of Fingerprint Development Techniques* [6] and recommended for these applications.

1.5 PSDB carried out a re-evaluation of a range of lipid reagents from 1999 to 2000 [7] and investigated several other lysochromes including Oil Red O (solvent red 27) and Sudan III (solvent red 23). These studies

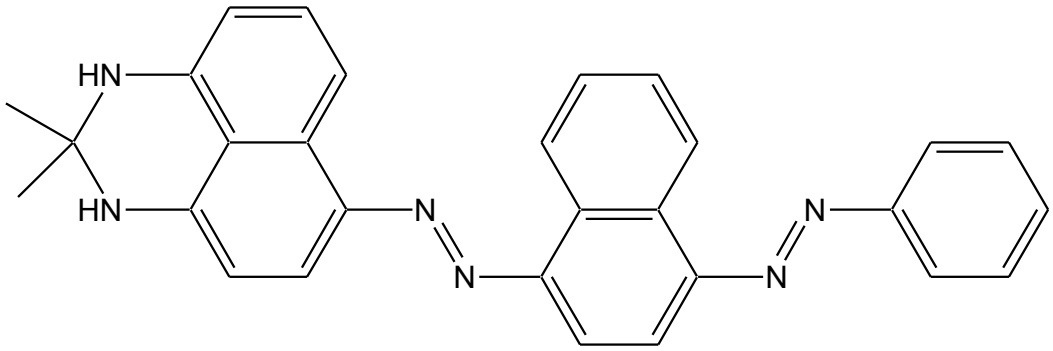
confirmed solvent black 3 to be the best performing of this type of lipid dye and it was not considered worthwhile initiating development of formulations based on other dyes. Instead, research was initiated to develop a formulation based on a less flammable solvent than ethanol that gave potential for the reagent to be used at crime scenes. As a consequence of this research 1-methoxy-2-propanol was identified as a suitable solvent and laboratory trials indicated that there was no discernible difference between this and the ethanol-based formulation. This formulation was subsequently published for operational use [8]. The studies did raise the issue of how best to test reagents for contaminated surfaces, because a method for consistently contaminating test surfaces needs to be devised. Several alternative techniques were investigated during the course of the experiments [9] but none of these were regarded as being truly satisfactory.

1.6 From 2005 to 2011, there was very little published work on the use of solvent black 3. One study assessed the effectiveness of solvent black 3 in both powder and solution form, with the solution treatment found to be more effective. Marks up to 75 days old were successfully detected on porous surfaces using this approach [10]. The authors also recommended the reagent for the development of lipstick marks.

1.7 During the process of collating information to demonstrate validation of the processes within the scope of CAST's ISO 17025 accreditation, it was recognised that some fundamental test data for solvent black 3 were lacking. Therefore a programme of work was put in place to obtain additional supporting information about the reactions of solvent black 3 and its effectiveness. These studies included an assessment of the interactions of solvent black 3 with a range of sebaceous fingermark constituents [11], a focused study on the effectiveness of solvent black 3 in enhancing latent, sebaceous and grease contaminated marks [12,13], and a broad ranging study comparing solvent black 3 with other processes in a variety of scenarios where grease contamination was present [14]. These studies indicated that solvent black 3 remained the most effective process for the enhancement of marks on grease-contaminated, light coloured surfaces and that the 1-methoxy-2-propanol-based formulation appeared marginally more effective than the ethanol-based formulation. The 1-methoxy-2-propanol-based formulation was therefore preferred for operational use.

2. Theory

2.1 Solvent black 3 is a lysochrome, more commonly known as a fat stain. Most lysochromes are azo dyes, which because of their structure have undergone molecular rearrangement making them incapable of ionising.



Structure of solvent black 3.

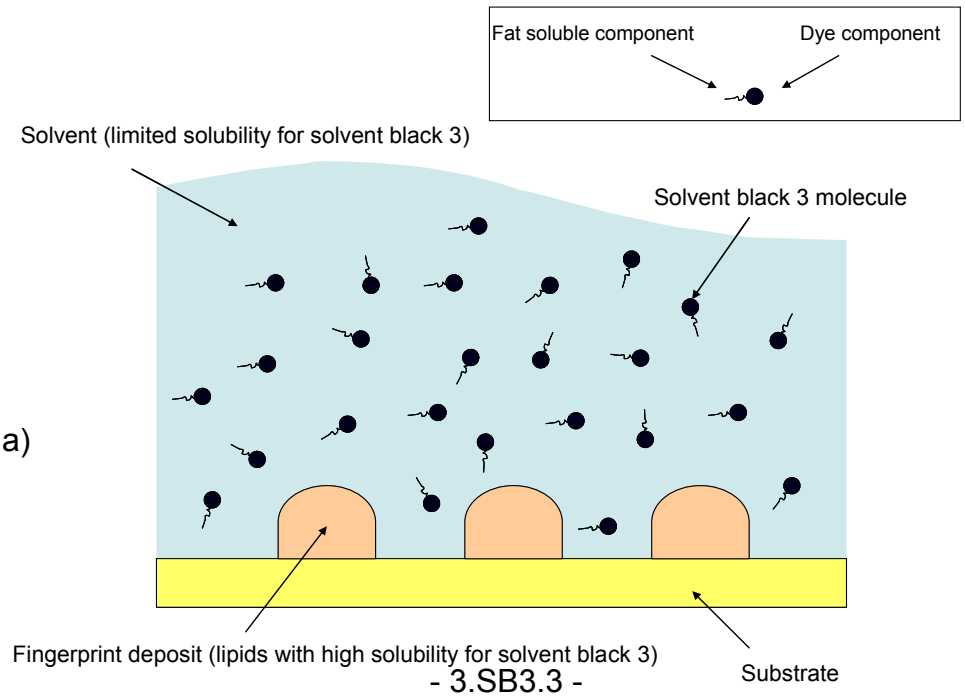
2.2 These dyes essentially colour fats by dissolving into them. From another perspective, the fat is the solvent for the dye. Lysochromes are mostly insoluble in strongly polar solvents, such as water, and somewhat more so in less polar solvents, such as ethanol. They are quite strongly soluble in non-polar solvents, such as xylene. Triglycerides, being non-polar compounds, dissolve them quite well. Other lipids, having fatty components, may also dissolve them.

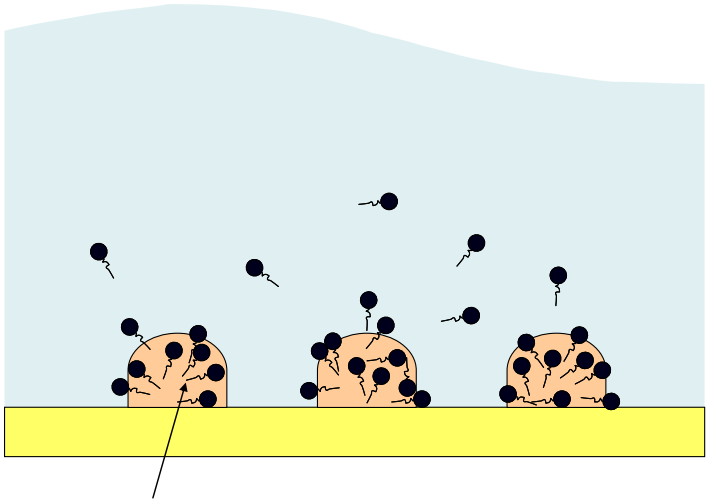
2.3 Lysochromes such as solvent black 3 are applied from solvents in which they are sparingly soluble. As they come into contact with materials (such as fats) in which they are strongly soluble, they migrate into them from the solvent, often colouring the fat more strongly than the original solvent. This process is known as preferential solubility.

2.4 Although the primary action of solvent black 3 is to stain lipids by dissolving in them, it can also stain materials ionically. This may result in some background staining.

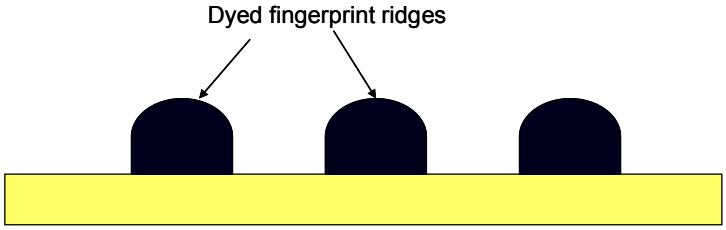
2.5 The dyeing process of solvent black 3 is illustrated schematically below.

a)





b)



c)

Schematic illustration of the solvent black 3 process a) *solvent black 3 molecules in solvent with limited solubility* b) *lipophilic component of solvent black 3 molecule preferentially dissolving into lipids in fingermark ridges and* c) *fingermark after drying, leaving dyed ridges.*

3. Centre for Applied Science and Technology processes

3.1 The most current version of the processes used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014). The purpose of this publication is to report the history, theory and validation work associated with these processes. The solvent black 3 process now (2016) recommended by CAST is the 1-methoxy-2-propanol-based formulation, which differs slightly from that originally proposed by the CRE. The solution consists of 10 g of solvent black 3 dissolved in 500 mL of 1-methoxy-2-propanol, to which is subsequently added 500 mL of distilled water.

3.2 The role of solvent black 3 in the formulation is to act as the dye for the fingermark ridges. The concentration used is such that the limit of solubility in the 1-methoxy-2-propanol/water solvent is almost exceeded, and some precipitation of solvent black 3 is occurring.

3.3 The role of 1-methoxy-2-propanol is to act as the initial solvent for solvent black 3 and it is capable of dissolving the quantity of solvent

black 3 outlined above. It also has a reduced flammability compared to the ethanol solvent used in previous formulations.

3.4 Solvent black 3 is insoluble in water, and the addition of water reduces the solubility of solvent black 3 to the point where precipitation is beginning to occur.

3.5 The solvent black 3 process involves wetting the surface being treated with solvent black 3 for a minimum of ten seconds, and then rinsing the excess dye from the surface using water. For non-porous surfaces longer staining times can be used, but for surfaces that are semi-porous in nature background staining can occur, which ultimately obscures developed marks, so shorter staining times are preferred.

4. Critical issues

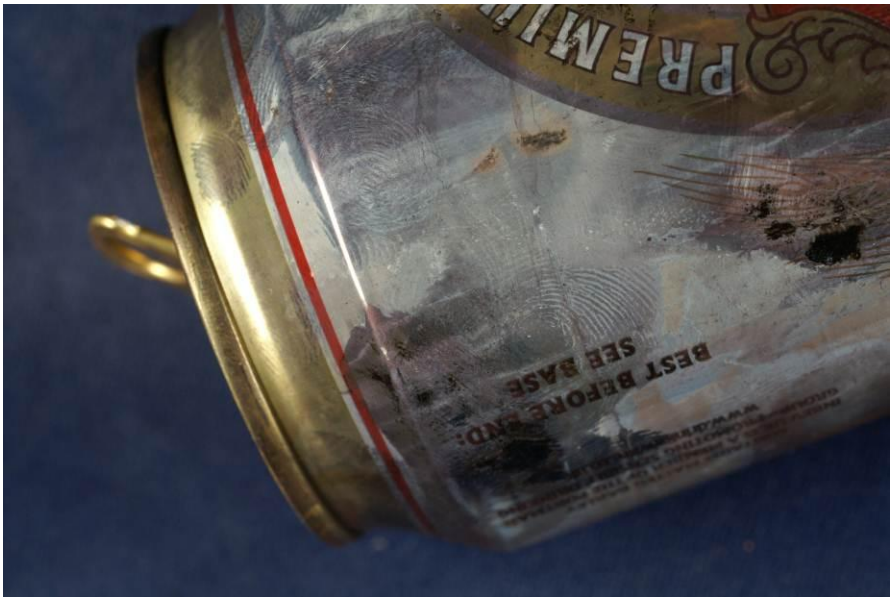
4.1 Any metallic films forming on the surface of the working solution should be removed using tissue or blotting paper prior to use. This is because these films will otherwise cause excessive staining of the background and may obscure marks.

5. Application

5.1 Suitable surfaces: Solvent black 3 is suitable for use on all types of non-porous surface where particular types of contamination are present. The two types of contamination for which solvent black 3 is known to be effective are:

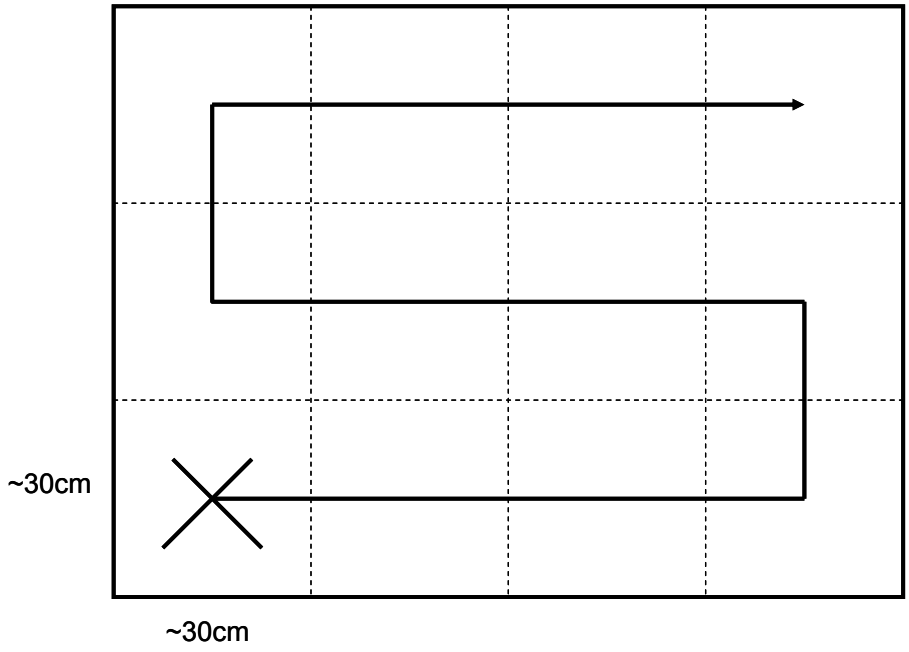
- fatty deposits (similar in nature to sebaceous fingermarks); and
- drinks' residues, where chemical discrimination may be of value.

5.2 Solvent black 3 is not recommended as a primary treatment for any particular surface, but appears in several 'grease specific' processing charts for semi- and non-porous surfaces as a treatment for surfaces that have been contaminated. In these situations, the lipid specific nature of solvent black 3 may enable it to stain fingermark ridges selectively without causing background staining of the contaminant. Basic violet 3 can be considered as an alternative treatment for contaminated surfaces and although laboratory trials indicate that solvent black 3 may be more effective than basic violet 3 on latent prints, the most effective treatment on contaminated surfaces has not been conclusively identified. Examples of the types of exhibit that can be effectively treated with solvent black 3 include fast food containers and drinks cans.



Photograph of beer can treated with solvent black 3, showing developed ridge detail.

5.3 The 1-methoxy-2-propanol-based formulation of solvent black 3 with reduced flammability was originally developed [15] with the potential for use at crime scenes. The types of scenes where this formulation could be used include potentially contaminated areas such as kitchens and bathrooms. Guidelines for application are given [7,15], starting at the bottom of the surface and then working up. This minimises dye running down over unprocessed areas and affecting the subsequent development of fingermarks.



a)

537 Publication number: 081/17

b)

Application of solvent black 3 at scenes of crime a) *suggested application sequence for vertical surfaces, and* b) *solvent black 3 being applied to a cupboard.*

6. Alternative formulations and processes

6.1 The original ethanol-based formulation recommended for operational use consisted of 15 g solvent black 3 dissolved in 1 litre of ethanol with the subsequent addition of 500 mL distilled water. This formulation was used from the mid-1980s until replaced by the 1-methoxy-2-propanol-based formulation in 2014. The main issue with the ethanol-based formulation was the flammability of the solvent, making its use at crime scenes problematic.

6.2 CAST therefore carried out an evaluation of a range of alternative solvents with the objective of providing a less flammable solvent black 3 formulation with the potential for use at scenes of crime. These solvents were tested individually, and in some cases diluted with water or heptane. A summary of the systems evaluated is given in the table below.

Solvent	**Formulations examined**	**Flammability**	**Results**
Dichloromethane/ Heptane	Various	Not studied	Only faint staining of prints

Ethyl acetate/ Heptane	Various	Not studied	Only faint staining of prints
Acetone	25, 50, 75, 100%	Similar to ethanol formulation	Similar level of fingermark development with existing ethanol formulation
Propan-2-ol	25, 50, 75, 100%	Slightly lower than ethanol formulation	Similar level of fingermark development with existing ethanol formulation
Propylene carbonate	100%	Not studied	Immiscible with water – poor results
Propylene glycol methyl ether acetate (PGMEA)	100%	Not studied	Immiscible with water – poor results
Dipropylene glycol dimethyl ether (DPGDME)	100%	Not studied	Immiscible with water – poor results
2,2-Dimethoxy Propane (2,2-DMP)	100%	Not studied	Immiscible with water – poor results
Propan-1,2,3-triol	Various	Ethanol had to be added to dissolve solvent black 3, resultant solution similar to ethanol formulation	Solvent black 3 not soluble in glycerol or water/glycerol mix
Propan-1,2-diol	25, 50, 75, 100%	Lower than ethanol formulation	Poor performance in staining marks
Propylene glycol methyl ether (PGME)	Various, including 40, 50, 55, 60, 75%	Much lower than ethanol formulation	Equivalent level of fingermark development to existing ethanol formulation
Dipropylene glycol methyl ether (DPGME)	Various, including 30, 40, 50, 60%	Much lower than ethanol formulation	Equivalent level of fingermark development to existing ethanol formulation

Solvents investigated as alternatives to ethanol in the solvent black 3 formulation [Hart, unpublished PSDB project data, 2002].

6.2 The results indicated that solvent black 3 was soluble in most polar organic solvents and that formulations based on diluted solvents worked

better in the development of fingermarks. Water was found to be essential to give good fingermark development.

6.3 Of the range of solvents investigated, propylene-based glycol ethers were identified as the best performing group in terms of reduced formulation flammability and good fingermark development. Optimised formulations were subsequently developed based on propylene glycol methyl ether (PGME) and dipropylene glycol methyl ether (DPGME). Further detail on both of these solvents is provided below.

6.4 PGME 1-methoxy-2-propanol, dowanol PM
Molecular formula: $C_4H_{10}O_2$
CAS number: 107-98-2
Boiling point: 118–119°C
Flash point: 33.88°C
Lower flammability limit: 1.8%
Upper flammability limit: 16.0%
Purity: 99.5+%
Main contaminant: 2-methoxypropan-2-ol

6.5 DPGME, dowanol DPM
Molecular formula: $C_7H_{16}O_3$
CAS number: 34590-94-8
Boiling point: 90–91°C
Flash point: 74°C
Purity: 97% (mixture of isomers)

6.6 The two best performing systems of those optimised were:

- 10 g solvent black 3, 500 mL PGME (1-methoxy-2-propanol), 500 mL water (50%);
- 10 g solvent black 3, 400 mL DPGME, 600 mL water (40%).

6.7 The flash points of both PGME- and DPGME-based solvent black 3 formulations were also assessed, and found to be:

- PGME = 55ºC;
- DPGME = >87ºC.

6.8 Considering that both these flash points were well in excess of temperatures typically experienced at scenes of crime and that their effectiveness in developing fingermarks was equivalent to the existing ethanol-based formulation, the PGME (1-methoxy-2-propanol-based) formulation was ultimately recommended for operational use both at scenes and in the laboratory.

7. Post-treatments

7.1 No post-treatments are used after solvent black 3.

8. Validation and operational experience

8.1 Fundamental studies

8.1.1 The natural constituents and greasy contaminants targeted by solvent black 3 and the way that it stains fingermarks were investigated in studies carried out in 2011.

8.1.2 Garrett and Bleay (2013)[11] carried out spot tests using substances typical of those found in the sebaceous component of fingermark residue deposited on a white ceramic tile and then enhanced these using both ethanol- and 1-methoxy-2-propanol-based formulations of solvent black 3. The substances used included ten fatty acids with carbon chain lengths ranging from C_6 to C_{24} (hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, myristic acid, palmitic acid, octadecanoic acid, eicosanoic acid, docosanoic acid, and tetracosanoic acid); cholesterol; two cholesterol esters (cholesteryl acetate and cholesteryl benzoate); squalene; and two triglycerides (glyceryl tripalmitate and glyceryl tristearate). Spots of contaminants such as hand cream, butter, vegetable spread and olive oil were also investigated as a preliminary step to a more focused study into the relative effectiveness of the two solvent black 3 formulations [12,13]. Results of these spot tests are summarised in the table below.

Component	Solvent black 3 (ethanol-based)	Solvent black 3 (1-methoxy-2-propanol-based)
Hexanoic acid	**	*
Octanoic acid	*	*
Decanoic acid		**
Dodecanoic acid	*	***
Myristic acid	*	*
Palmitic acid		*
Octadecanoic acid	*	*
Eicosanoic acid	*	***
Docosanoic acid		*
Tetracosanoic acid	*	*
Cholesterol	-	-
Cholesteryl acetate	**	**
Cholesteryl benzoate	*	*
Squalene	*	*
Glyceryl tripalmitate	*	*
Glyceryl tristearate	*	*
Hand cream	**	**
Butter	***	***
Vegetable spread	***	***
Olive oil	**	**

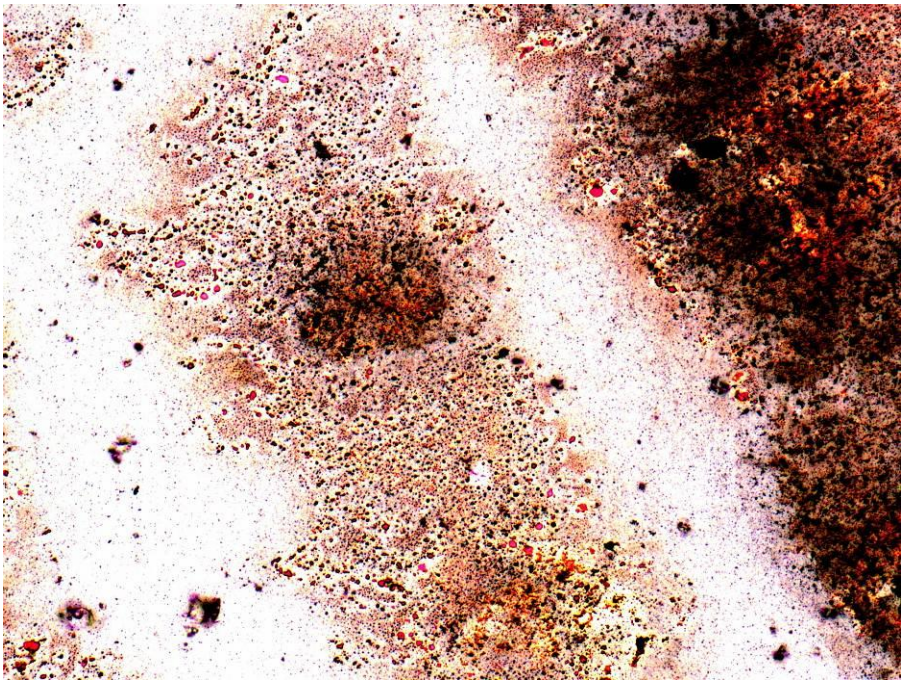
Note: - = no evidence of staining/fluorescence; * = partial or weaker staining/fluorescence seen; ** strong staining/fluorescence seen; *** = very intense staining/fluorescence seen.

Summary of data obtained from spot tests with solvent black 3 formulations [11].

8.1.3 Solvent black 3 gave the most intense staining for the contaminants (hand cream, butter, vegetable spread and olive oil) and to a lesser

extent with cholesterol acetate. It did not appear to interact particularly strongly with most of the fatty acids, squalene or the triglycerides, and not at all with cholesterol. For decanoic, dodecanoic and eicosanoic acids the 1-methoxy-2-propanol-based solvent black 3 formulation appeared to produce more intense staining of the test spots than the ethanol-based formulation.

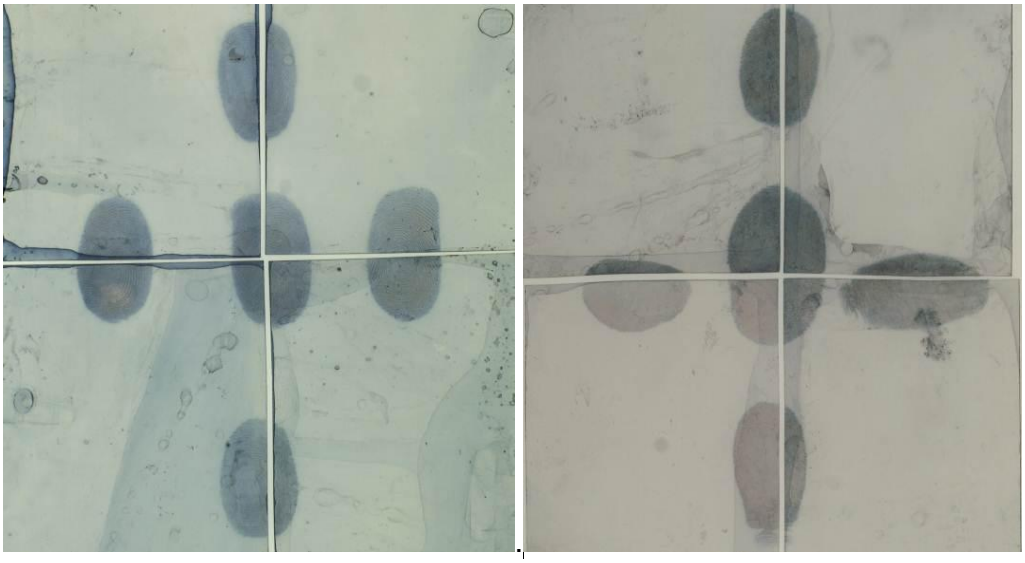
8.1.4 The way in which fingermarks are stained by solvent black 3 was also investigated in the same study. Fingermarks were deposited on clean microscope slides, allowed to age for a selected period of time, and then stained using one of the two solvent black 3 formulations under evaluation. The stained fingermarks were then examined using high magnification optical microscopy. It was shown that the way in which marks were stained was essentially independent of the solvent that the dye was applied from. It could be seen that solvent black 3 was selective and stained discrete droplets of constituents within the fingermark, rather than uniform staining of the entire ridge, as shown in the image below.



High magnification microscopy showing fingermark ridges stained using solvent black 3.

8.1.5 Tests were also conducted to evaluate the sensitivity of the solvent black 3 formulation to gross changes in the dye concentration. These tests used a quartered fingermark technique, dividing a series of deposited fingermarks into four and processing each quarter with a different concentration of dye in the formulation. The four dye concentrations used were 1x, 0.75x, 0.5x, and 0.25x the quantity of dye in the current 1-methoxy-2-propanol-based and ethanol-based formulations. Deliberately sebaceous fingermarks, produced by wiping the finger against the side of the nose and the forehead, were used in this experiment so that there

would be sufficient material in the mark for the dyes to interact with. Typical results are illustrated in the images below.



The effect of varying dye concentration on the effectiveness of (left) *ethanol-based and* (right) *1-methoxy-2-propanol-based solvent black 3 formulations.*

8.1.6 It can be seen that reducing the concentration of dye in the 1-methoxy-2-propanol-based solvent black 3 formulation has a significantly greater effect on the intensity of visible staining than reducing the concentration of solvent black 3 in the ethanol-based formulation. However, it is still possible to reduce the dye concentration by 25% without any visible degradation to the staining effectiveness of the formulation.

8.1.7 A broader investigation of the types of contaminant that can be detected with solvent black 3 was conducted by Gaskell *et al* [14]. A total of 35 different contaminants were used, selected as typical of substances that may be encountered in 'kitchen', 'bathroom' and 'garage' environments, and are listed in the table below.

Environment		
'Kitchen'	**'Bathroom'**	**'Garage'**
Sunflower oil	Garnier hand cream	WD40
Olive oil	Oil of Olay face cream	'3 in 1' drip oil
'Flora light' spread	Nivea sun cream	Hydraulic fluid
Butter	Boots No. 17 tinted moisturiser	Silicone grease
Lard	Ulta foundation	RS anti-seize compound
Coca Cola	Ulta eyeshadow	Shell motor oil
Stella Artois lager	Ulta lipstick	Swarfega
Gaymers cider	Johnson's baby oil	Unleaded petrol
Red wine	Vaseline	Brake pad residue
Orange juice	Buttercup cough syrup	Castrol grease
Tomato ketchup		Used engine oil

Mayonnaise		
Used cooking oil		

Table summarising the initial range of contaminants assessed in terms of their interactions with fingermark enhancement processes [14].

8.1.8 Marks were deposited on a clean ceramic tile by dipping a finger into the contaminant, dabbing it free of any excess, then leaving it for one day or one week prior to enhancement. Although the ethanol-based solvent black 3 formulation was used for this study, from the tests conducted by Garrett and Bleay[11], and Cadd *et al* [12,13], it is anticipated that equivalent results would be obtained for the 1-methoxy-2-propanol-based formulation. Results from these tests are shown in the table below.

Contaminant	**Solvent black 3**	
	1 day	**1 week**
Natural print	3	3
Deliberately sebaceous	4	4
Sunflower oil	2	4
Olive oil	1	2
Flora light	4	4
Butter	3	4
Lard	3	4
Coca Cola	2	0
Stella Artois	3	0
Gaymers cider	1	0
Red wine	3	4
Orange juice	4	3
Ketchup	4	4
Mayonnaise	2	4
Used cooking oil	3	4
Hair wax – Brylcreem	1	1
Garnier hand cream	4	4
Face cream – Oil	2	3

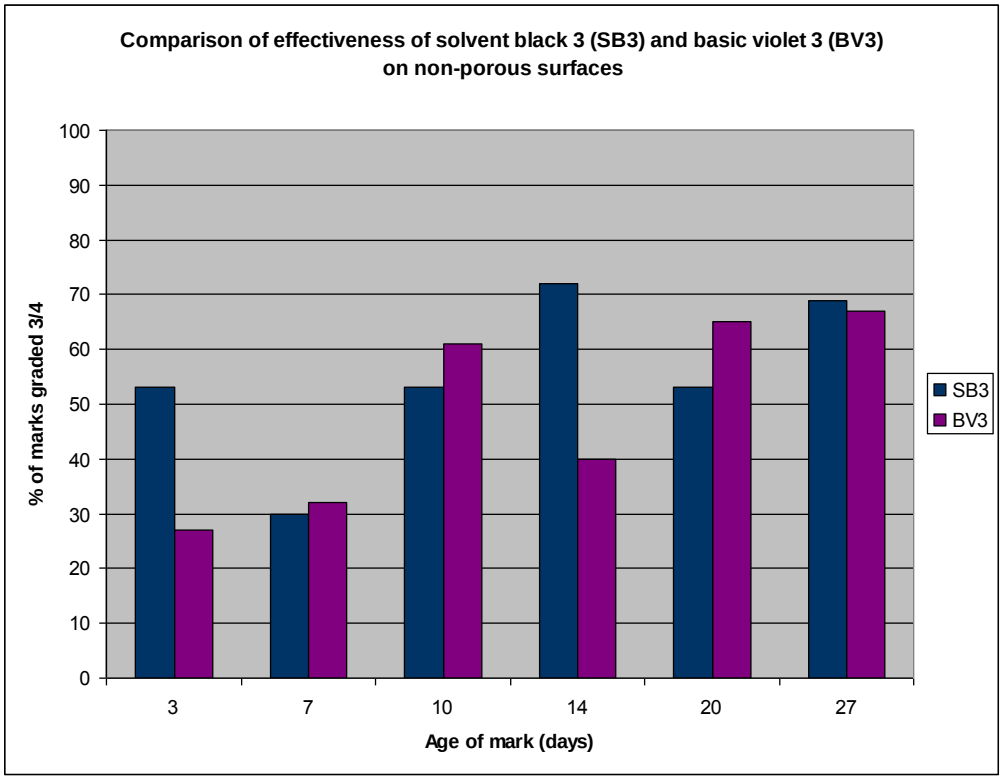
of Olay		
Nivea spf 15 suncream	4	4
Tinted moisturiser	4	3
Powder foundation	1	0
Eyeshadow	1	0
Lipstick	3	3
Baby oil	2	1
Vaseline	2	2
Buttercup cough syrup	4	3
WD40	4	4
3 in 1 multipurpose oil	2	1
Hydraulic fluid	2	3
Silicon grease	1	1
RS anti seize compounds	1	2
Shell motor oil	1	2
Swarfega	4	4
Petrol	1	0
Brake pad residue	0	0
Castrol grease	3	4
Dipstick residue	2	2

Table summarising the effectiveness of solvent black 3 on a range of contaminants, giving grades for marks aged for 1 day and 1 week.

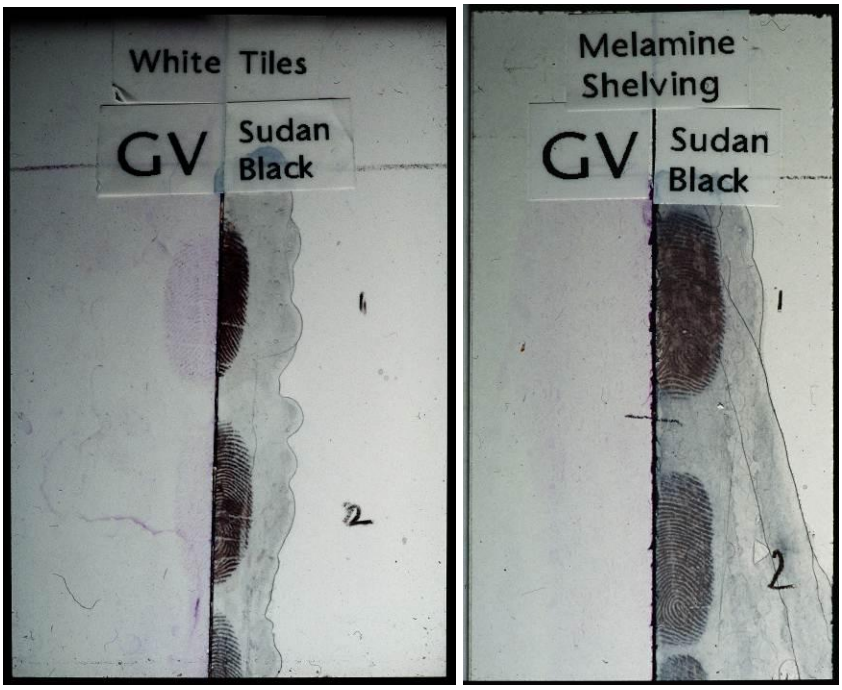
8.1.9 It was shown that solvent black 3 was one of the most effective dyes studied for the enhancement of greasy contaminants, enhancing almost all substances covered to some extent. Other ‘fat stains’ included in the same study (basic violet 3, iodine solution) did not give the same intensity of reaction, nor did they react with the same number of contaminants.

8.2 Laboratory trials

8.2.1 The effectiveness of the ethanol-based solvent black 3 formulation for 'natural' marks on non-porous surfaces was evaluated in a laboratory trial in 2002, comparing it with the other reagent recommended for contaminated surfaces, basic violet 3. The results of this trial, carried out on 2,592 half marks, are illustrated below.



a)



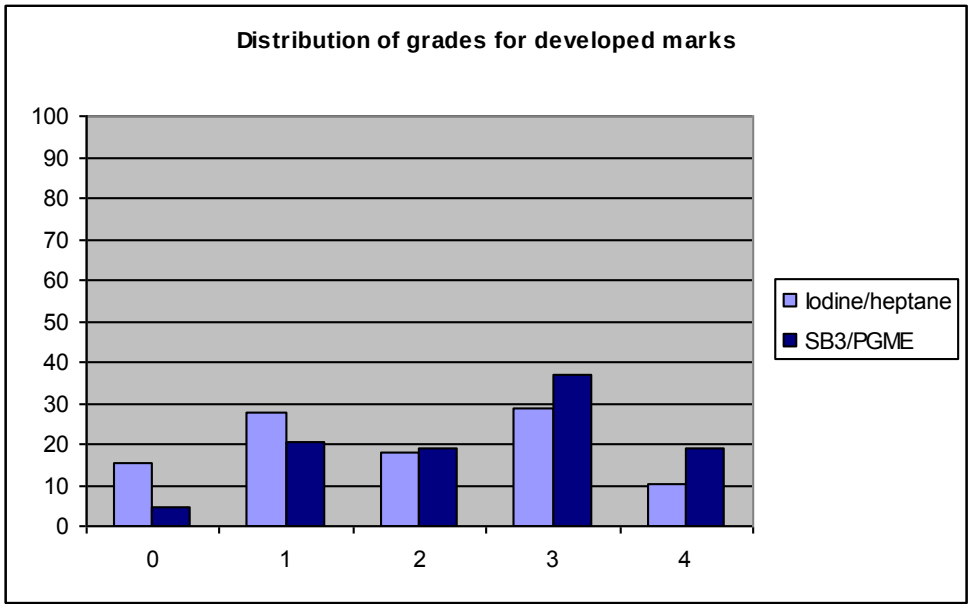
b)

Results from a comparison of the effectiveness of solvent black 3 (Sudan Black) and basic violet 3 (GV) on non-porous surfaces a) results of grading marks of different ages and b) photographs of these marks developed on different surfaces [Hart, unpublished PSDB project data, 2002].

8.2.2 These results indicate that solvent black 3 may be more effective for enhancing natural, uncontaminated fingermarks, but are not conclusive. The trials were conducted on clean non-porous surfaces and are therefore not fully representative of the contaminated surfaces that the techniques are proposed for. However, there are fewer health and safety issues associated with solvent black 3, which may make it preferable to basic violet 3 for operational use on contaminated exhibits.

8.2.3 Comparisons were also carried out between 1-methoxy-2-propanol-based solvent black 3 and a heptane-based iodine solution. This involved grading 2,592 half prints, the results of numbers of fingermarks at each grade being summarised below:

Grade	Technique		
	Iodine/heptane	SB3 after iodine	SB3/PGME
0	297	63	83
1	361	152	304
2	212	95	245
3	341	326	465
4	85	84	199
Total	**1,296**	**720**	**1,296**

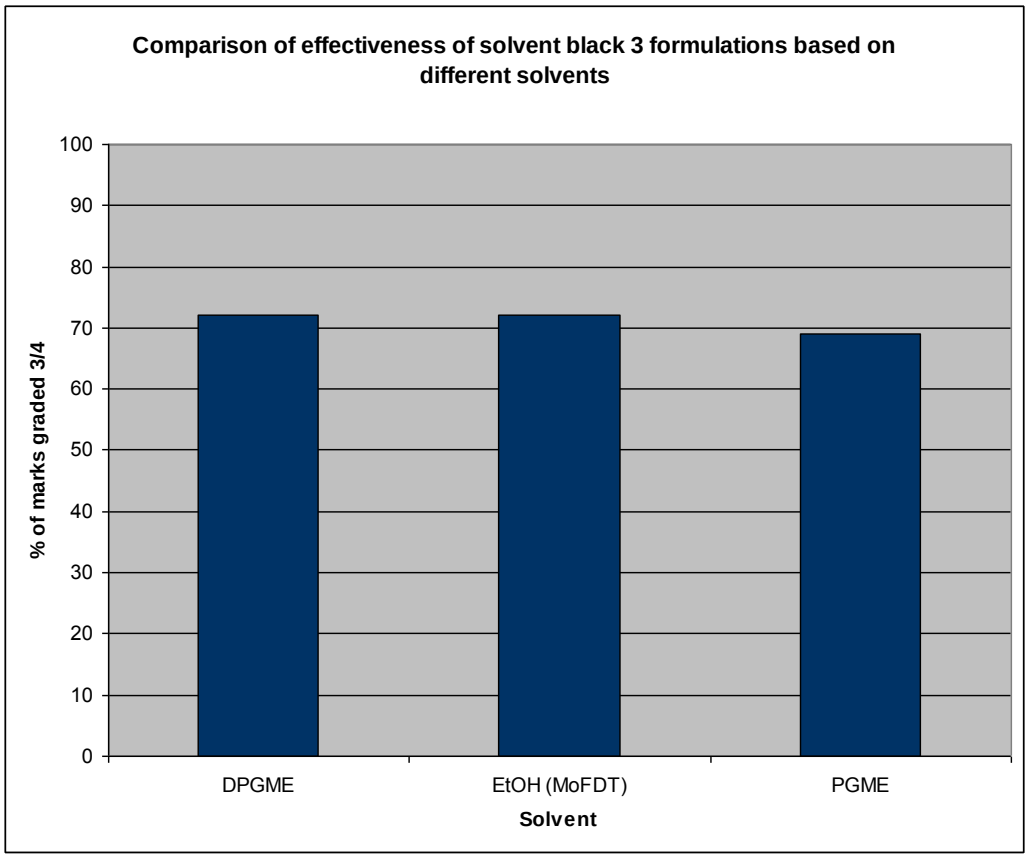


Results of comparative tests between solvent black 3 and iodine solution [Hart, unpublished PSDB project data, 2002].

8.2.4 In general the results show that solvent black 3 is a more effective treatment than iodine for latent fingermarks. However, an in-depth analysis of the results across all the surfaces examined (which included various laminates, uPVC, ceramic tiles and gloss painted wood) showed that there were certain surfaces (e.g. gloss painted wood) where iodine did out-perform solvent black 3. However, the overall better performance of solvent black 3 combined with the flammability issues associated with heptanes-based iodine solutions, meant that solvent black 3 continued to be the technique recommended for operational use.

8.2.5 Prior to the publication of the current reduced flammability solvent black 3 formulation in 2005 [8], a three-way trial was carried out comparing PGME (1-methoxy-2-propanol-based) and DPGME-based formulations with the ethanol-based formulation recommended in the *Manual of Fingerprint Development Techniques* [6].

8.2.6 During the course of this three-way trial, 5,040 half fingermarks were graded. The results of this study are illustrated below.



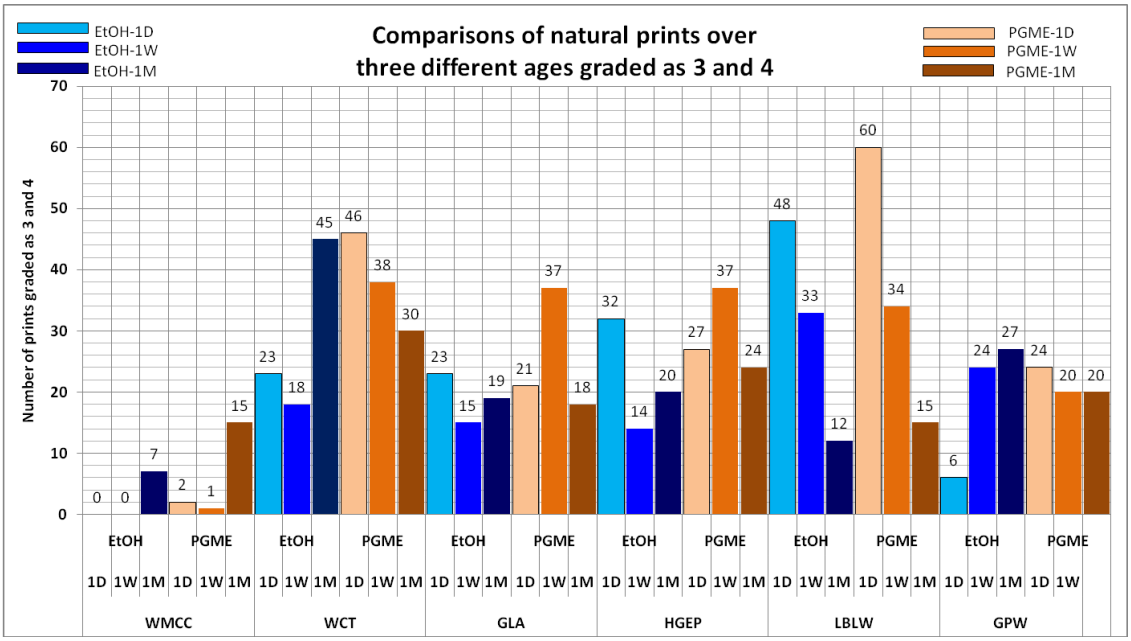
Results of three-way comparison between solvent black 3 formulations based on different solvents [Hart, unpublished PSDB project data, 2002].

8.2.7 The results demonstrate closely equivalent performance between all three formulations, and it was considered that they could be used interchangeably according to circumstances.

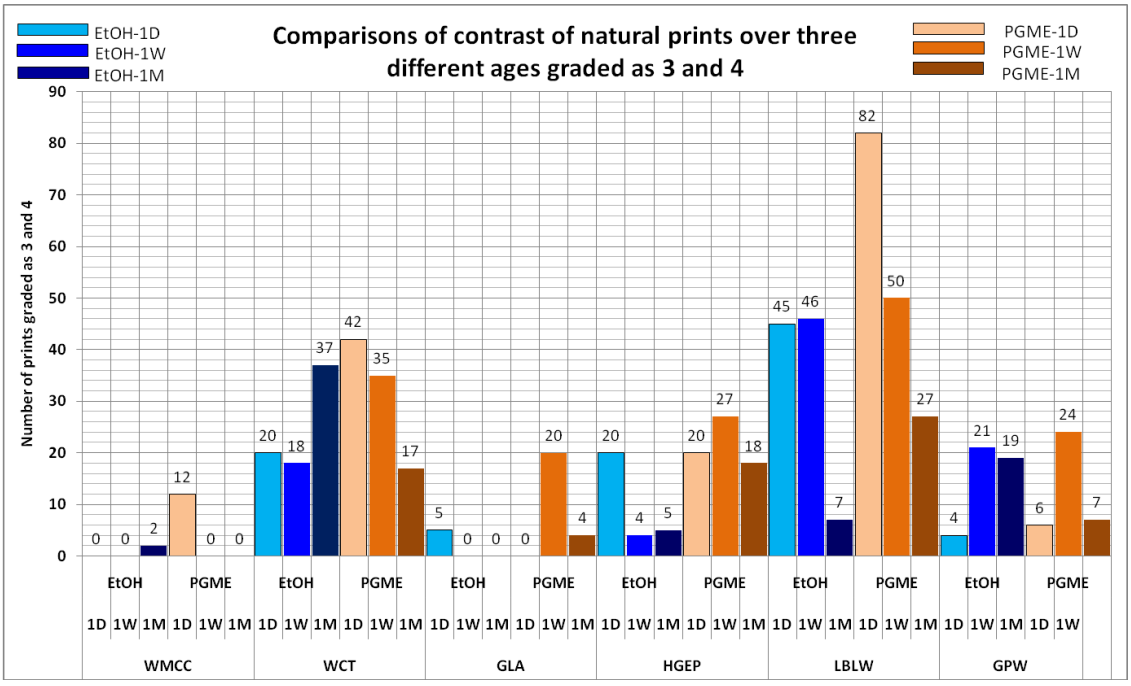
8.2.8 It should be noted that all trials outlined above utilised latent fingermarks, and therefore the fact that marks are developed at all is because of the sebaceous constituents that are present in 'natural' marks. These results are therefore not truly representative of the operational use because solvent black 3 is recommended for use on greasy, contaminated surfaces and fingermarks. However, there are difficulties in producing a model 'contaminant' for such studies in the same way that horse blood is used as a contaminant for studies into blood dyes. This can be addressed by including operationally representative contaminants into the testing phase, as carried out by Cadd *et al* [12,13] in tests carried out in 2011 to establish whether the ethanol-based formulation could be phased out and replaced by the 1-methoxy-2-propanol-based formulation.

8.2.9 This trial considered natural, sebaceous and contaminated marks deposited on six different types of porous and semi-porous substrate (white melamine coated chipboard, white ceramic tile, toughened glass, hard grey engineering plastic, light brown laminated wood, white gloss painted wood), with marks aged for one day, one week and one month.

The contaminants used were representative of generic classes of grease that may be found in a kitchen-type environment, and were olive oil, butter, vegetable spread and hand cream. A summary of the grading results obtained for ridge detail and contrast of natural marks after processing is presented below.



a)



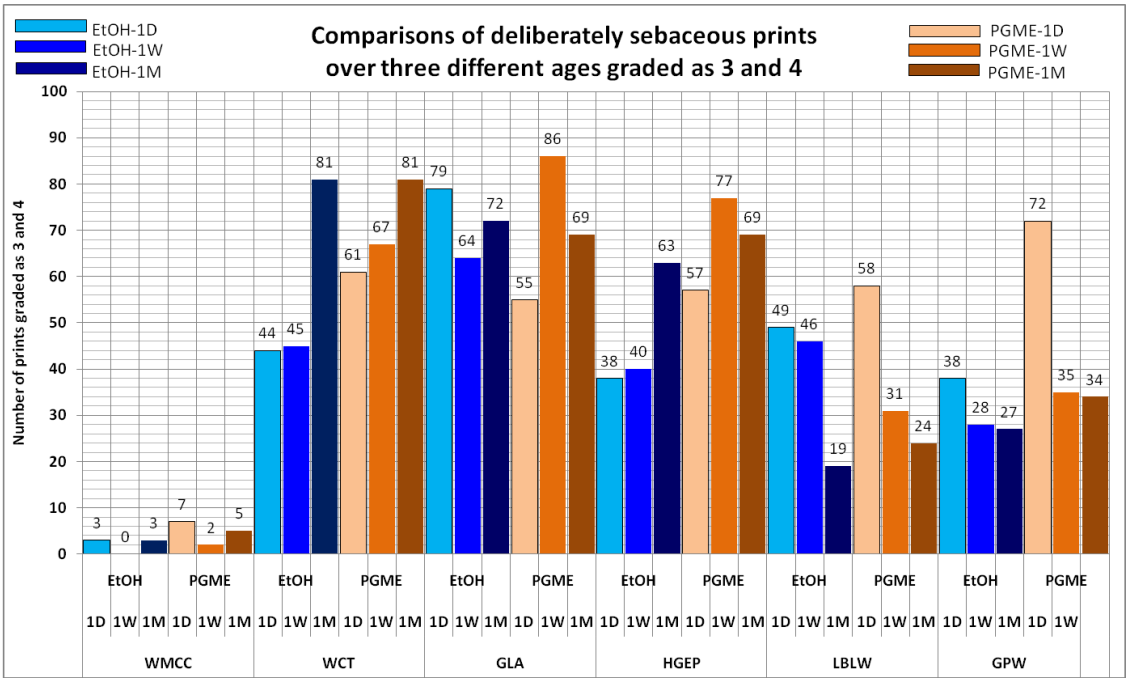
b)

Results of grading natural marks of all ages across all surfaces enhanced with both solvent black 3 formulations in terms of a) ridge detail, and b) contrast. Substrates used were white melamine coated chipboard (WMCC), white ceramic tile (WCT), toughened glass (GLA),

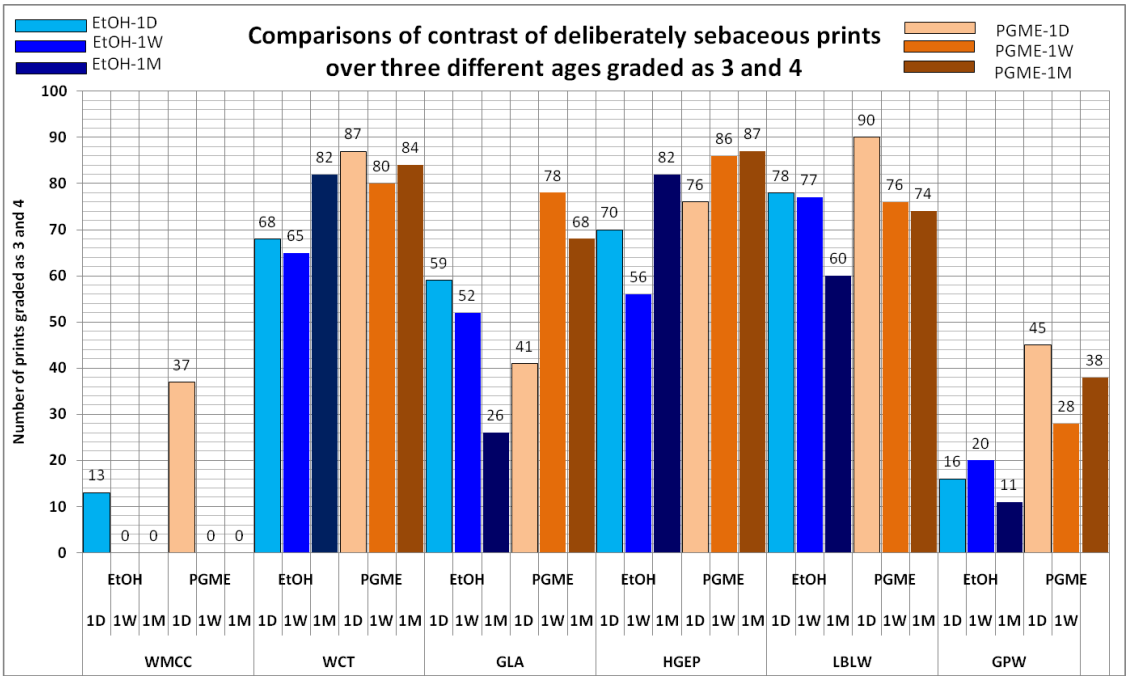
hard grey engineering plastic (HGEP), light brown laminated wood (LBLW), white gloss painted wood (GPW) [12].

8.2.10 The inherent variability of 'natural' marks between donors was observed on the test panels, with marks from some donors not being developed at all and others being developed reasonably strongly. In general, marks developed using the 1-methoxy-2-propanol-based formulation exhibited more ridge detail and stronger contrast with the background than those developed using the ethanol-based formulation. Differences were also observed between the different surfaces used. Non-porous surfaces (e.g. ceramic tiles, wood laminates) produced many marks of high contrast with minimal background staining, although on others (e.g. glass) developed marks could be of low contrast and were sometimes difficult to visualise. Semi-porous surfaces (e.g. gloss painted wood, white melamine coated chipboard) exhibited heavy background staining and many marks that were developed were very difficult to see.

8.2.11 A summary of the results obtained for ridge detail and contrast of sebaceous marks is presented below.



a)



b)

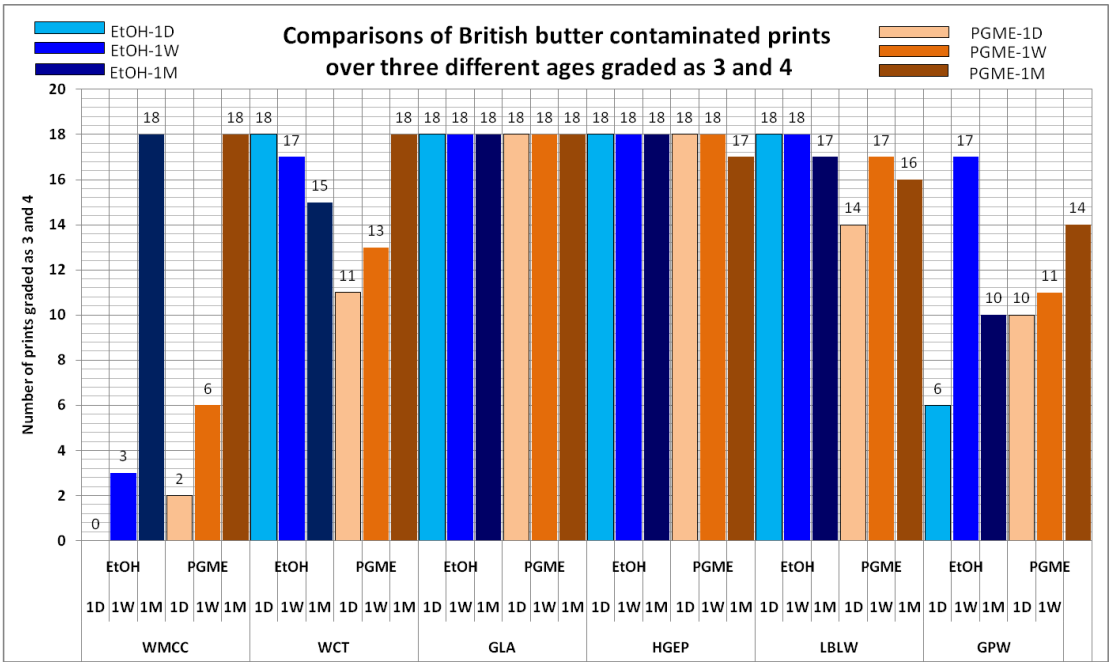
Results of grading sebaceous marks of all ages across all surfaces enhanced with both solvent black 3 formulations in terms of a) ridge detail, and b) contrast. Substrates used were white melamine coated chipboard (WMCC), white ceramic tile (WCT), toughened glass (GLA), hard grey engineering plastic (HGEP), light brown laminated wood (LBLW), white gloss painted wood (GPW) [12].

8.2.12 The trend in the results is similar to that for natural marks, with the 1-methoxy-2-propanol-based formulation generally developing more marks of higher quality and contrast than the ethanol-based formulation, although as expected the number and quality of sebaceous marks enhanced overall is greater than that observed for latent marks.

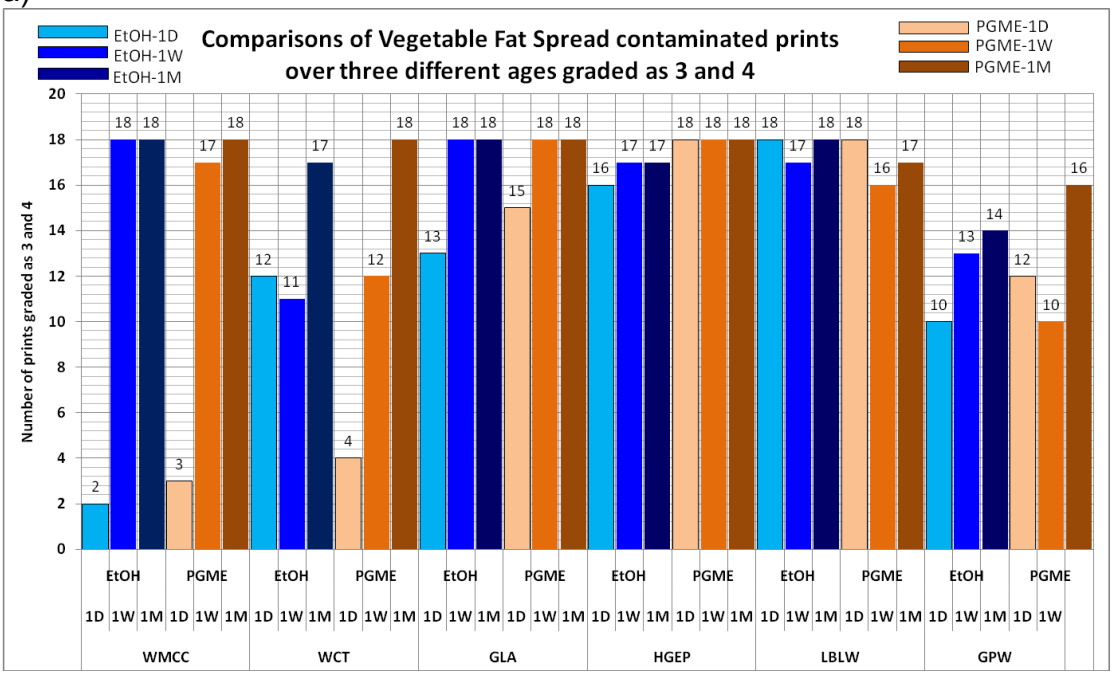
8.2.13 Significant differences were observed between the behaviour of the reagents on each type of contaminant, and this was to some extent modified by the type of surface the marks were deposited on.

8.2.14 The olive oil contaminated marks had generally poor ridge detail due to the fluid nature of the contaminant, which did not fully dry out even after ageing for one month. Because oil and water are immiscible, rinsing the surface with water can progressively lift the oil off the surface and therefore it was recommended that photography of marks be carried out immediately after enhancement.

8.2.15 Butter and vegetable fat spread contaminated marks performed similarly, generally giving good ridge detail because the solid nature of the contaminant both retained ridge detail and hardened over time. The contrast of the marks was good for all of the surfaces and ages of mark investigated, as shown below.



a)

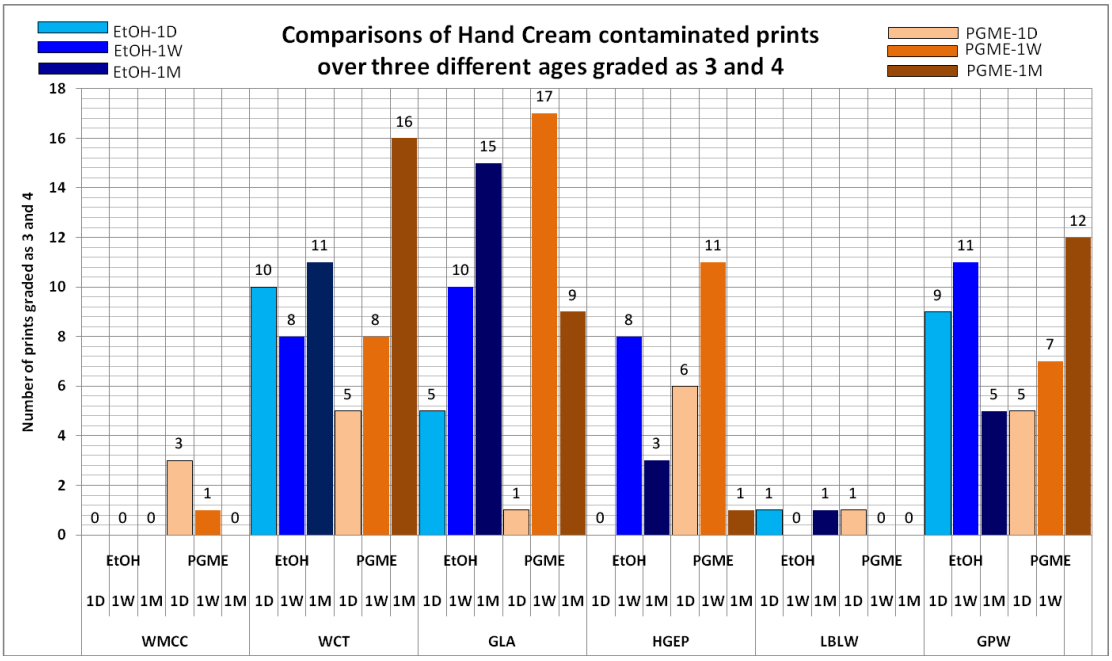


b)

Results of grading detail of contaminant marks of all ages across all surfaces enhanced with both solvent black 3 formulations for a) butter contaminant, and b) vegetable spread. Substrates used were white melamine coated chipboard (WMCC), white ceramic tile (WCT), toughened glass (GLA), hard grey engineering plastic (HGEP), light brown laminated wood (LBLW), white gloss painted wood (GPW) [12].

8.2.16 Although hand cream itself was not stained as well by solvent black 3 as the other contaminants evaluated, it was still possible to obtain good ridge detail from hand cream contaminated marks. On surfaces that exhibit strong background staining, marks in this contaminant were

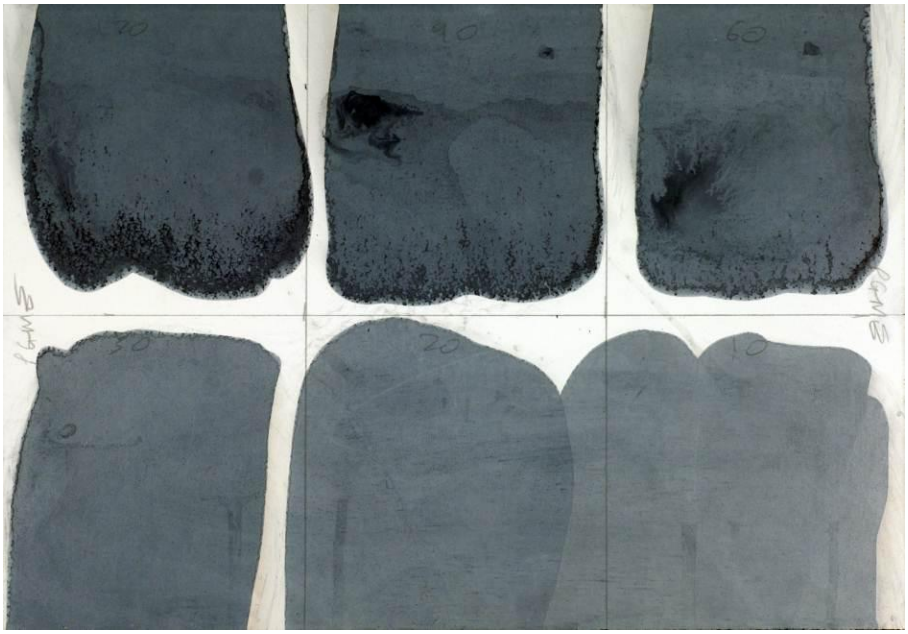
sometimes observed as pale regions of contaminant against a dark background. On surfaces that did not exhibit heavy background staining, the marks were stained to some extent and were seen as darker ridges against a light background. Results are shown below.



Results of grading detail of contaminant marks of all ages across all surfaces enhanced with both solvent black 3 formulations for hand cream contaminant. Substrates used were white melamine coated chipboard (WMCC), white ceramic tile (WCT), toughened glass (GLA), hard grey engineering plastic (HGEP), light brown laminated wood (LBLW), white gloss painted wood (GPW) [12].

8.2.17 For most of the scenarios evaluated, the 1-methoxy-2-propanol-based formulation gave equivalent to or better performance than the ethanol-based formulation. This was seen both in terms of more fingermarks being developed and as a reduced intensity of background staining. There were some scenarios where the ethanol-based formulation did give better performance but if a single formulation were to be recommended the 1-methoxy-2-propanol-based formulation would be preferred.

8.2.18 It was observed during the staining process that fingermarks became visible after less than ten seconds, indicating that the previously recommended staining time of two minutes may be excessive. This was especially true for the semi-porous surfaces such as the white melamine coated chipboard where a reduction in staining time improved the contrast of the marks by reducing background staining, as shown below.

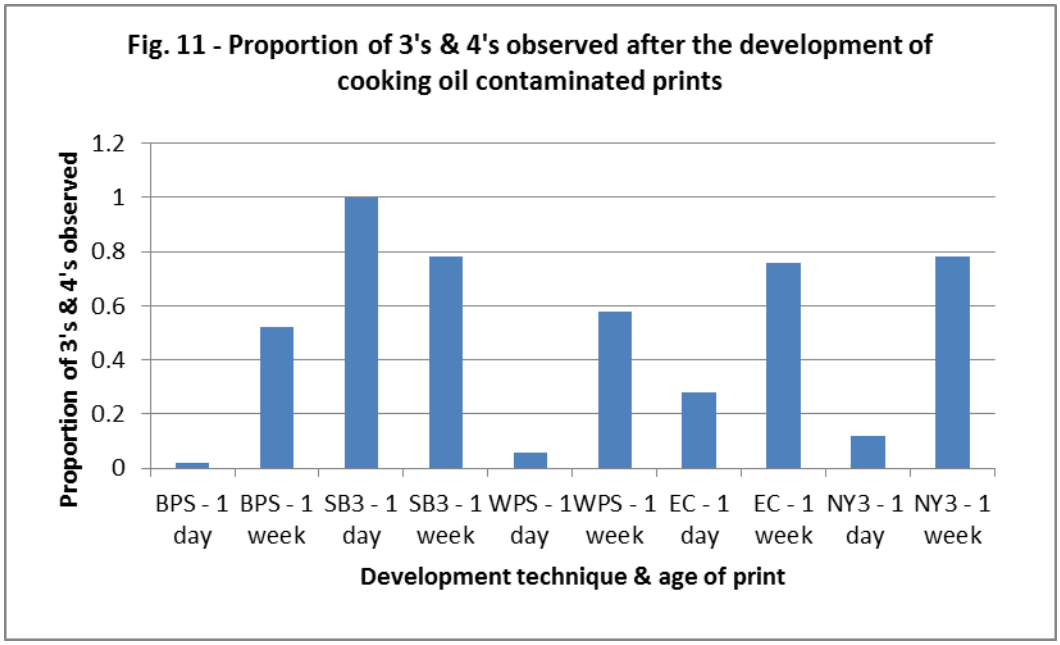


The effect of using staining times of 10, 20, 30, 60, 90 and 120 seconds for PGME-based solvent black 3 on white melamine coated chipboard.

8.2.19 More recent work by Perry (2013)[16], although primarily directed towards formulations of the natural yellow 3 dye, has demonstrated that there is the potential to reduce background staining without detriment to the effectiveness of the dye in enhancing fingermarks. This is done by the addition of other chemicals such as sodium chloride to the formulation. There is the potential to refine the solvent black 3 formulation further to reduce the level of background staining; this is expected to be an area of future study.

8.2.20 The scenario where marks are deposited in contaminant on a clean surface is not the only situation where solvent black 3 may be employed. Gaskell [14] also considered latent marks deposited on top of a layer of pre-existing contaminant, and latent marks subsequently covered with a layer of contaminant. The contaminants used in this study were butter, used cooking oil, engine grease and hand cream.

8.2.21 The results for marks deposited in contaminant on clean surfaces confirmed that solvent black 3 was the best of a range of similar lipid reagents evaluated for this purpose. An example of the results obtained (in this case for used cooking oil) being illustrated below.



Results obtained for development of fingermarks contaminated with used cooking oil on a white ceramic tile with a range of reagents including solvent black 3 (SB3) [14].

8.2.22 In the alternative scenarios where continuous layers of contaminants were present, solvent black 3 stained the continuous layer and fingermarks underneath them were obscured. Where marks had been deposited into the contaminant layer, in some cases the disturbance to the layer caused by the contact of the finger was sufficient for the mark to be detected, as shown below.

Mark deposited in a layer of Castrol grease and developed using solvent black 3.

8.2.23 Other processes (such as black powder suspension) were found to be capable of developing latent fingermarks and causing minimal staining to the contaminant layer. As a consequence of these results it was recommended that in scenarios where grease contamination is suspected to be present, solvent black 3 should be used towards the end of the sequence, after black powder suspension.

8.3 Pseudo-operational trials and operational experience

8.3.1 Initial operational trials were carried out in 1986 to determine the relative effectiveness of the technique in developing fingermarks on polythene bags. In these trials solvent black 3 was compared with VMD and SPR [5]. The results of this comparison are reproduced below.

	Characteristics				**Number of fingermarks**	
	Greater than16		8 to16*			
	SB3	VMD	SB3	VMD	SB3	VMD
Number of cases	11	18	13	6	24	24
Number of fingermarks	56	81	80	102	–	–

** Number of fingermarks of 8 to16 characteristics recorded only when no marks of greater than 16 characteristics were revealed.*

	Characteristics				**Number of fingermarks**	
	Greater than16		8 to16*			
	SB3	SPR	SB3	SPR	SB3	SPR
Number of cases	4	10	5	10	39	28
Number of fingermarks	8	24	21	72	–	–

** Number of fingermarks of 8 to16 characteristics recorded only when no marks of greater than 16 characteristics were revealed.*

Results of comparative trials between solvent black 3, small particle reagent and vacuum metal deposition [5].

8.3.2 These trials indicated that solvent black 3 was not as effective as either VMD or SPR for developing fingermarks on polythene bags and it was not subsequently recommended for this application. However, the potential of the technique to develop marks on greasy, contaminated surfaces was later recognised and the technique was developed for this purpose.

8.3.3 A full operational trial has not been conducted on the use of solvent black 3 on contaminated surfaces, nor was a side-by-side comparison conducted between ethanol and 1-methoxy-2-propanol-based solutions. This is because there are so few cases where the use of solvent black 3 is necessary and to build up statistically meaningful operational data would have taken several years. Because the nature of the contaminant is known, unlike ‘real’ fingermarks that are variable in composition, the performance in operational use will be the same as that in laboratory

tests. In the case of the solvent black 3 formulation, the decision was taken to issue the less flammable formulation because this provided a scene of crime capability where none was previously available. Laboratory results suggest that the two formulations are very similar in performance and there is no reason to assume that this would significantly change when applied at a scene.

9. References

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Superglue (cyanoacrylate fuming)

1. History

1.1 Superglue was developed in the 1950s by researchers trying to produce an acrylic polymer for the aircraft industry. It found commercial use as an adhesive system for non-porous surfaces, a main advantage being the very short cure time. Exposure to superglue vapour was reported as a possible method for the development of latent fingermarks in the late 1970s. It was reported apparently independently in Japan, North America [1] and in a private communication with the UK Home Office from Laurie Wood of Northamptonshire Police [2, 3]. Early research in the UK was carried out by the Home Office Central Research Establishment (HO CRE) investigating the relative effectiveness of a range of different commercial superglues [4] and the development of fingermarks on a range of surfaces including polyethylene, PVC and adhesive tape [5]. The use of powdering to enhance developed ridge detail was also investigated [5] and Bristol Black powder found to be most effective for this purpose.

1.2 The first literature publications detailing the use of the process for fingermark development began to appear in the early 1980s [6]. Initially little was known about the reaction mechanism or the optimum treatment conditions, and fingermark development was often slow and inconsistent, sometimes taking 24 hours to produce a developed mark. Various police forces around the world used (and some still use) the technique in a relatively uncontrolled way by treating exhibits in containers, such as fish tanks, with various proprietary cyanoacrylate adhesives. Some experimented with heating the glue to speed the process [7,8]. Others proposed the use of other accelerating agents, including sodium hydroxide [9] and sodium carbonate [10] and comparative trials were reported between these techniques and a commercial system ‘Hard Evidence’, where vapours were released from a cyanoacrylate impregnated gel exposed to the atmosphere [11]. Commercial superglue fuming chambers began to be manufactured, with systems such as the ‘Visuprint’ [12] being available in 1983.

1.3 After finding the technique variable and somewhat unreliable, HO CRE handed the work over to the Police Scientific Development Branch (PSDB) in 1982 for further investigation. It was quickly determined that humidity was playing a crucial role in the speed and sensitivity of the reaction [13]. The humidity was optimised primarily for polythene and other plastics, with a relative humidity (RH) level of around 80% being recommended [14]. This is the point at which solid sodium chloride will take up water from the atmosphere. Lower humidity levels resulted in slower and less effective development and higher humidity levels resulted in high background development. These experiments were repeated by HOSDB in 2009, with the results confirming the recommendations of the original study (and described in section 8). Several prototype treatment cabinets with controlled humidity were built.

A novel electronic humidity control system was developed by a contractor (Nick Hartley) and by 1986 the commercial 'Sandridge' superglue cabinets were being installed in police forces [15]. This cabinet was designed to carry out development under the optimum conditions of humidity, evaporating ethyl cyanoacrylate at 120°C and venting to the atmosphere.

The prototype 'Sandridge' controlled-humidity superglue cabinet developed by the Police Scientific Development Branch.

1.4 The Sandridge cabinet had a capacity of approximately 0.5 m^3 and controlled the humidity by injection of vapour from an ultrasonic humidifier. The humidity was monitored, during the typical 15 to 30 minute treatment time, by wet and dry thermocouples linked to an electronic control system. It was manufactured by the Mason Vactron Company in Acton, London and was installed in most police and forensic service provider fingerprint laboratories in the UK and many across Europe, some of these still being in use today. This cabinet produced much more rapid and consistent results than the ad hoc arrangements

most had been using and provided police forces with an effective and reliable process for the development of fingermarks on many non-porous surfaces. In the late 1990s HOSDB was involved in, and funded in collaboration with the USA, the initial development of a larger cabinet with capacity of approximately 2 m^3 [16]. The prototype was purchased by Thames Valley Police and was in operational use until recently (up to 2016). The design was substantially modified after the original Mason Vactron Company was purchased by Foster and Freeman and is now marketed as the MVC5000. Cabinets of smaller capacities, the MVC3000 and MVC1000 and variants of them, have subsequently been developed and marketed.

1.5 Superglue development is rarely used in isolation. The white deposit of a superglue-developed fingermark can be difficult to see and photograph, especially on light coloured surfaces, although ultraviolet (UV) imaging and several of the processes described for visual examination can enhance ridge contrast. Attempts were made to improve contrast by powdering the developed ridges with coloured powders [5], but the best results were obtained by using fluorescent dyes to dye the deposit. The potential of using fluorescent dyes in this way was recognised soon after the first published papers on superglue fuming appeared. Menzel *et al.* [17] suggested the use of Rhodamine 6G (basic red 1), either by evaporation or solution staining, in combination with an argon ion laser light source. The evaporation of Rhodamine was also reported by Vaughn [18]. Stoilovic and co-workers investigated Coumarin 540 as an alternative solution staining dye [19,20], for use with the filtered xenon lamp-based forensic light sources then under development in Australia. The high cost and limited availability of lasers and alternative forensic light sources in the early 1980s prompted investigations into dyes excited by long-wave UV [21,22], for which cheap radiation sources were readily available. As a consequence of these studies, Ardrox was proposed for use [23] as a UV-excited alternative to Rhodamine 6G.

1.6 From the early 1980s through to the current time (2016), Rhodamine 6G has been one of the most widely-used dyes for developed superglue marks. Many formulations used methanol as the solvent. Methanol is extremely hazardous by skin absorption and Rhodamine remains a suspect carcinogen, although this is still debated and no studies have been conducted on humans. Alternative dye systems to Rhodamine 6G were being investigated by the mid-1980s [24]. In the mid-1980s PSDB set out to find a safer alternative which could be excited in the blue region of the spectrum and that would preferably emit in the green-yellow region. In 1985 the dye basic yellow 40 (BY40) dissolved in ethanol was identified by Sears and this was included in the manual issued in 1986 [14]. BY40 has subsequently proved to be one of the most effective dyes for dyeing marks developed with superglue, combining high fluorescent yield with low toxicity. The absorption of the dye in the violet-blue region of the spectrum and corresponding emission in the green-yellow region are particularly convenient for visualisation of developed marks. The BY40 dye has been shown to enhance the superglue-developed

fingermarks to the extent that twice as many identifiable fingermarks are found on some surfaces after dyeing compared with those seen after superglue treatment alone. The subsequent dye process is therefore an important step in sequential treatment procedures. Attempts have also been made to combine the superglue fuming and subsequent dyeing of the deposit into a single stage by co-volatilising thermal dyes that sublime [25] , and investigations have also been conducted into tagging cyanoacrylates with fluorescent species. Although early attempts were relatively unsuccessful, with the intensity of fluorescence observed being considerably lower than that achieved using dye staining, more recently products have been developed that give closely equivalent fluorescent intensity. These include Lumicyano [26,27, 28], a liquid incorporating a dye that is processed using the same conditions as conventional cyanoacrylate, and Polycyano UV [29,30,31], a solid powder that is fumed from a hot plate at a higher temperature of 230°C. Other researchers have reported independent attempts to formulate one step fluorescent cyanoacrylates [32,33,34] and the use of subliming dyes as a vapour phase stain for marks developed using cyanoacrylate fuming [35].

1.7 A less effective water-based version of BY40 was introduced at a later date, this formulation being intended for use on surfaces where ethanol had detrimental effects (such as varnishes and some surfaces printed with inks), or in areas with poor ventilation. HOSDB subsequently reviewed the water-based BY40 formulation and investigated a further range of alternative water-based dyes [36]. The outcome of this study was the issue of a more effective water-based dye formulation incorporating basic red 14 (BR14), published for operational use in 2004. The recent development of portable, high power green lasers offers the possibility of increasing the number of fingermarks detected after staining with BR14 because the output wavelength of the laser (532 nm) is well matched to the excitation characteristics of the dye.

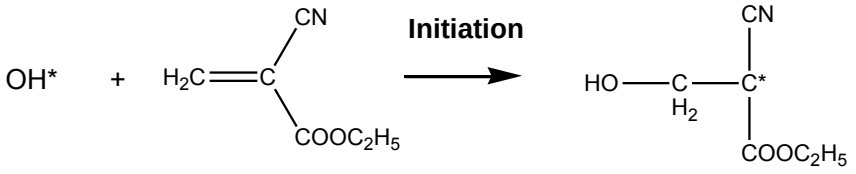
1.8 Vacuum superglue fuming has been proposed as an alternative to the atmospheric, high humidity superglue development process and equipment has been developed and manufactured for this purpose [37]. Several comparative studies have been carried out between the vacuum and high humidity techniques [38,39,40,41,42] which demonstrated that each process has advantages and disadvantages. The high humidity technique develops marks that can be more easily seen without subsequent fluorescent dyeing and absorbs far more dye. It is therefore considered by CAST to be more appropriate to a wider range of exhibits, hence it is the technique recommended for use in the UK. However, studies into the vacuum technique have continued and refinements to the technique and equipment have been proposed [43,44,45].

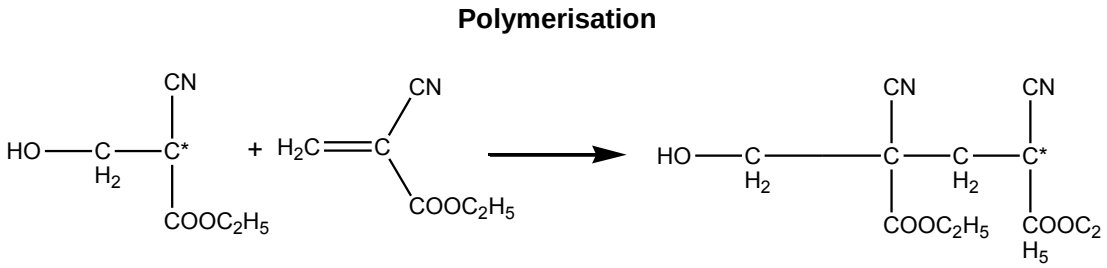
1.9 Superglue fuming, using a range of different systems, has also been used for the development of fingermarks in cars and at scenes for many years [46,47,48,49]. None of these systems have been shown to be as effective as treatment in a controlled-humidity cabinet in a laboratory (the

effect of humidity and temperature is elaborated in a later sections 2.5 – 2.9, but undoubtedly they have a role in the development of fingermarks on surfaces that cannot be powdered or recovered to a laboratory. PSDB conducted an evaluation of the SuperFume system produced by Foster and Freeman [50], which came to similar conclusions to earlier studies. The SuperFume process has been successfully deployed at scenes such as hydroponics factories growing cannabis within the UK, but the suitability of the surfaces for powdering should also be considered prior to use because in many cases this may give better results. A further study comparing SuperFume to aluminium powdering [51] confirmed that the type of surface present is an important consideration in process selection, with SuperFume more effective at developing latent fingermarks on textured and smooth plastic surfaces whereas aluminum powder was more effective on glass, enameled metal paint, and varnished wood.

2. Theory

2.1 Marks developed by superglue become visible because white deposits are preferentially formed on fingermark ridges during treatment. These white deposits are polycyanoacrylate, formed by the polymerisation of the cyanoacrylate monomer. The polymerisation reaction for ethyl cyanoacrylate is shown below; a similar mechanism occurs for methyl cyanoacrylate, which is used as an alternative by some practitioners.

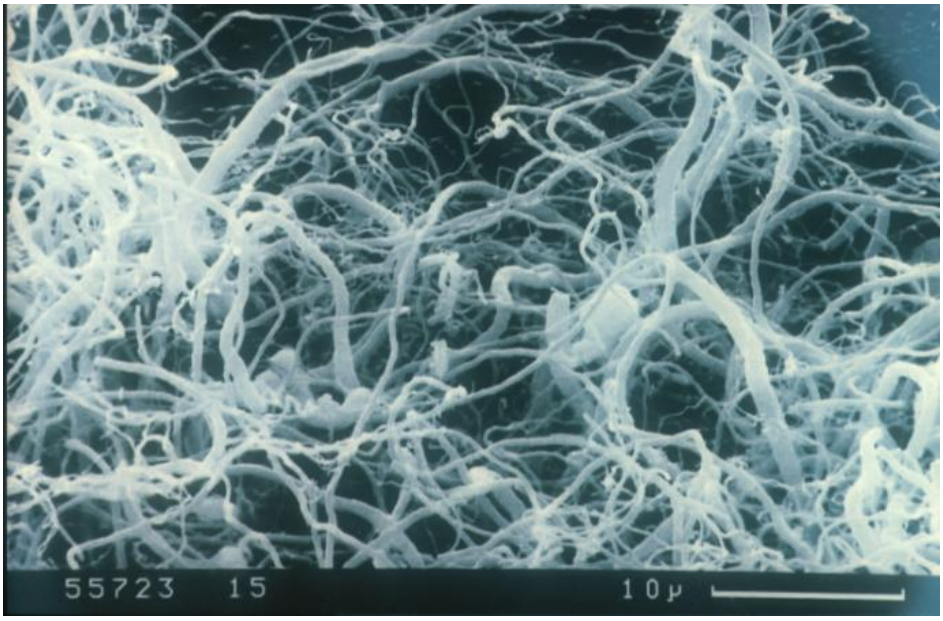




Polymerisation reaction for ethyl cyanoacrylate

2.2 The precise mechanism of the growth of poly-ethyl-cyanoacrylate on fingermark residue is unclear. Electron microscopy studies by PSDB showed the growth of long fibrous deposits when the humidity was elevated (80% RH), these were not present at lower humidity levels

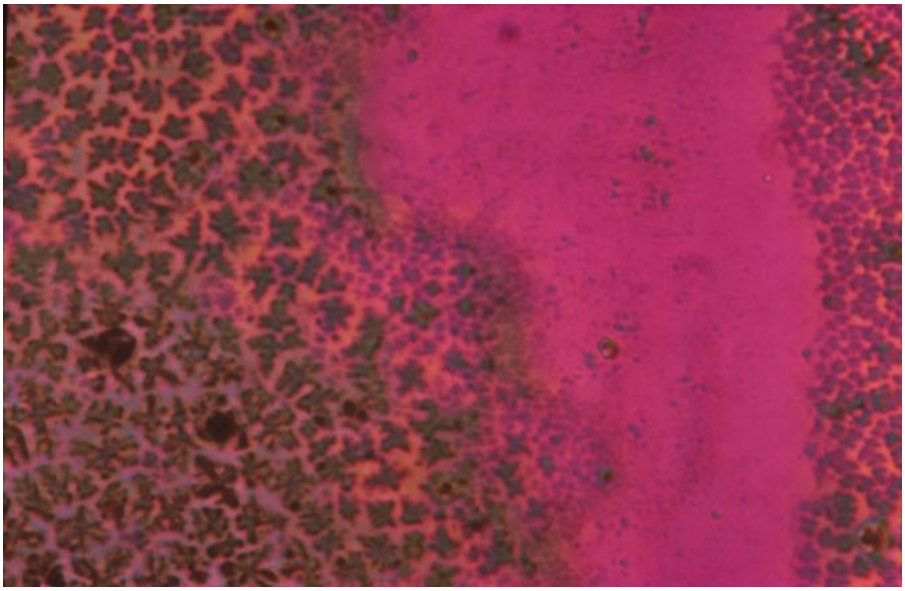
(40% RH). These long fibrous deposits make the developed mark easier to see by eye. A recent study [52] compared various alkyl cyanoacrylates (methyl, ethyl, n-butyl and 2-octyl) and reported that ethyl and butyl polymers formed polymer microstructures that scattered light more effectively than methyl and octyl polymers.



Electron micrograph of the fibrous deposits formed by superglue development at high humidity.

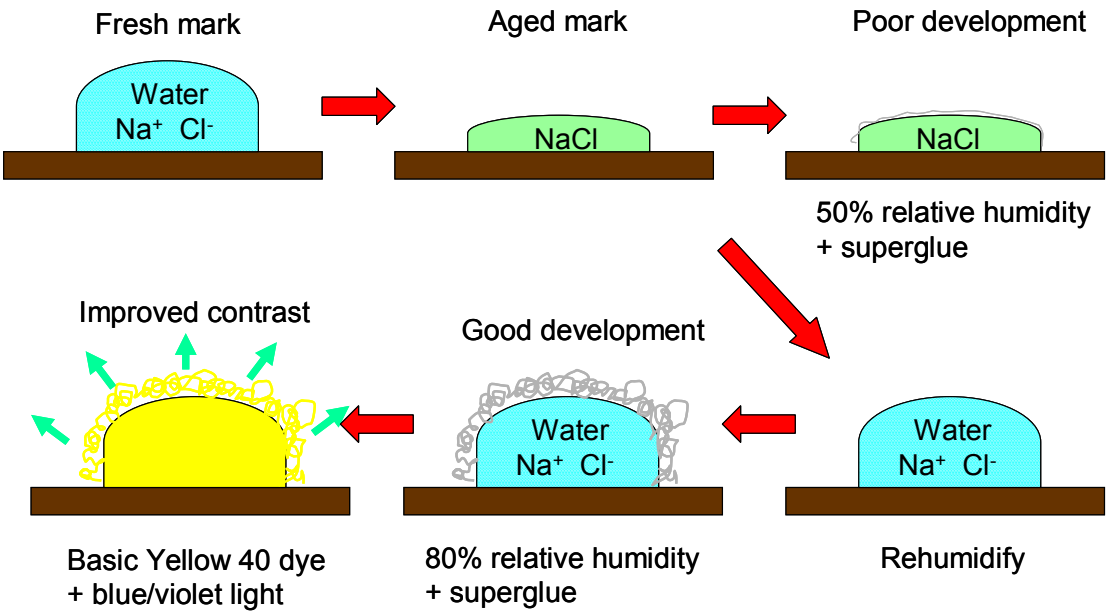
2.3 Cyanoacrylate polymerisation is base-initiated and even weak bases, such as water, will initiate polymer growth. It is believed that elevating the RH to around 80% causes sodium chloride crystals in the latent fingermark to take up water. A saturated solution of sodium chloride (NaCl) with excess solid in a closed volume will create an RH above the solution of 75% at equilibrium. Therefore, at RH values above this NaCl crystals will absorb water from the environment around it. Similarly, any NaCl crystals in fingermarks will absorb moisture from the environment when the cabinet is set to 80% RH.

565 Publication number: 081/17



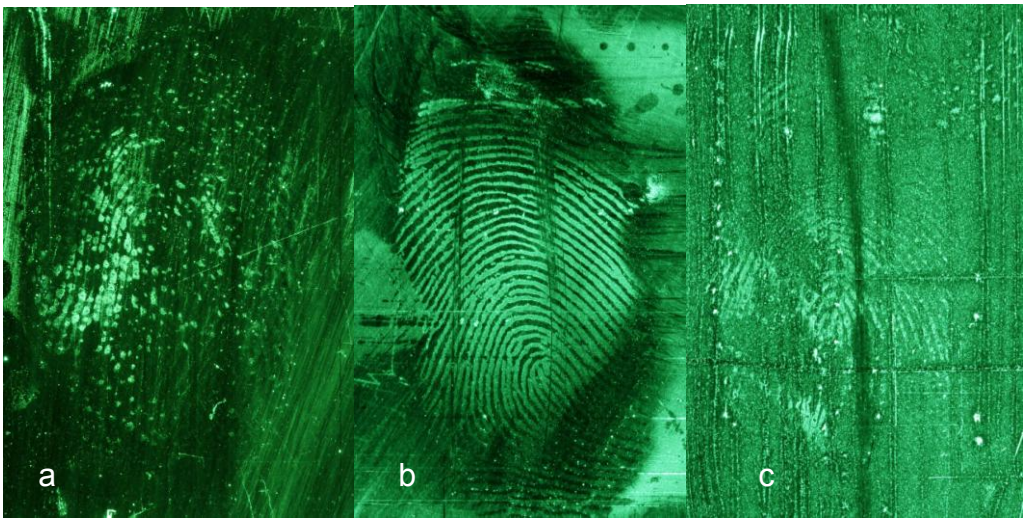
Optical interference micrograph of a dehydrated fingermark ridge showing the formation of sodium or potassium chloride salt crystals.

2.4 This description explains one possible mechanism for polymer growth. There are undoubtedly other bases within fingermark residues and some of these may also initiate polymerisation. Most fingermarks, however, have an initially significant water and chloride content, this is therefore likely to be a significant initiation mechanism. It is also suggested that short chains, oligomers, of cyanoacrylate may be formed due to atmospheric humidity, which may take part in further polymerisation on the fingermark or the substrate. The superglue process is illustrated schematically below.

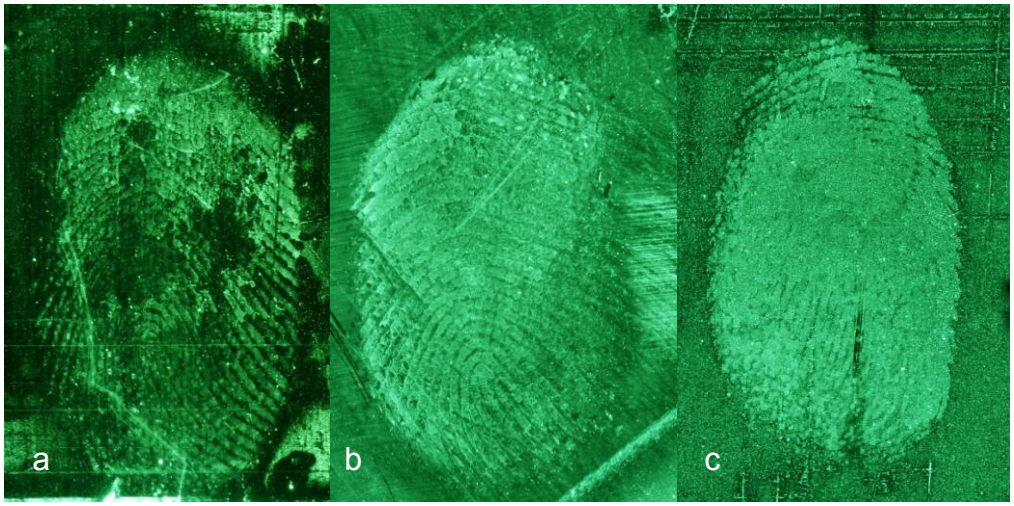


Schematic diagram of the superglue development and dyeing process.

2.5 Humidity levels below 75% RH give underdeveloped marks, humidity levels above 80% RH cause an increased background development and reduced definition of the developed mark. This can be seen in the series of photographs below, obtained for predominantly eccrine and predominantly sebaceous marks [53,54].

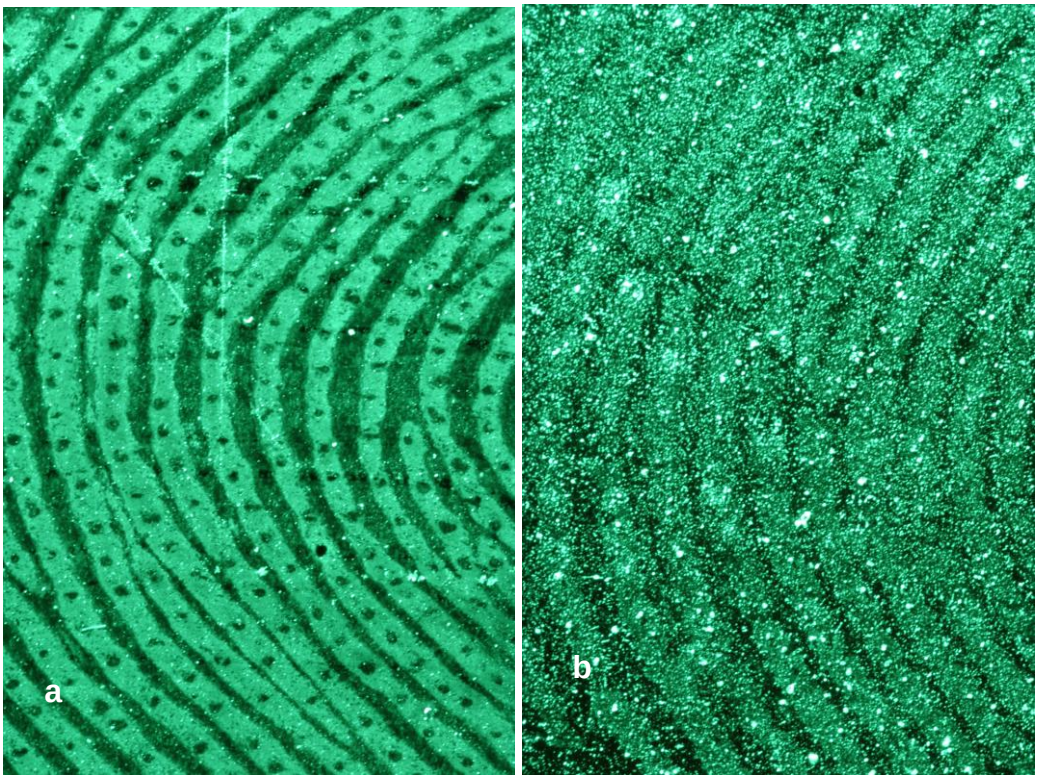


Eccrine fingermarks developed with superglue/BY40 and observed with a violet/blue excitation source (band pass filter 385–469 nm at 1% cut-on and cut-off points respectively) and viewed with a yellow long pass 476 nm filter (1% cut-on point) at a) *60%* b) *80%* c) *100% relative humidity.*



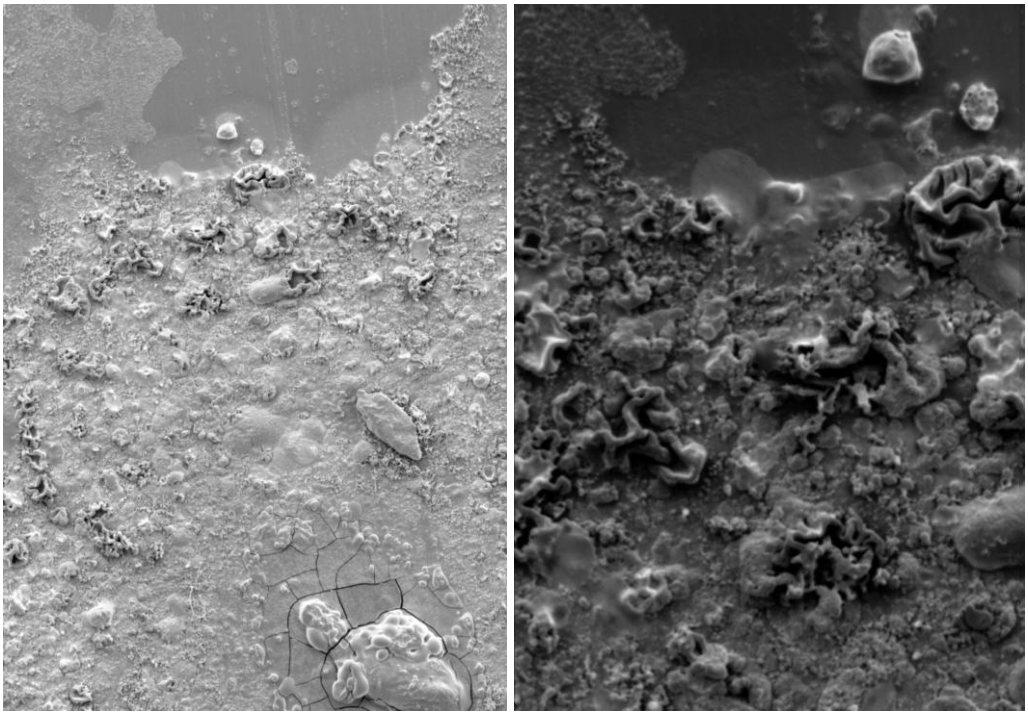
Sebaceous fingermarks developed with superglue/BY40 and observed with a violet/blue excitation source (band pass filter 385–469 nm at 1% cut-on and cut-off points respectively) and viewed with a yellow long pass 476 nm filter (1% cut-on point) at a) *60%* b) *80%* c) *100% relative humidity.*

The overdevelopment at higher humidity can be better seen in the higher magnification images below.

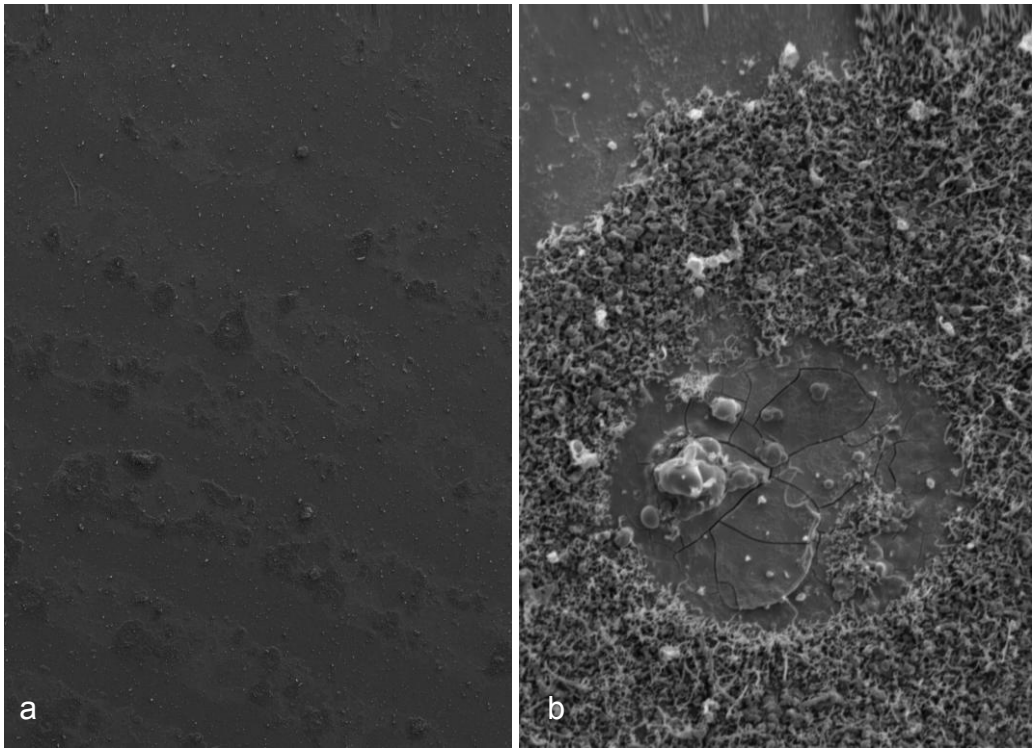


Normal (un-groomed) fingermarks developed with superglue/BY40 and observed with a violet/blue excitation source (band pass filter 385–469 nm at 1% cut-on and cut-off points respectively) and viewed with a yellow long pass 476 nm filter (1% cut-on point) at a) *80% and* b) *100%.*

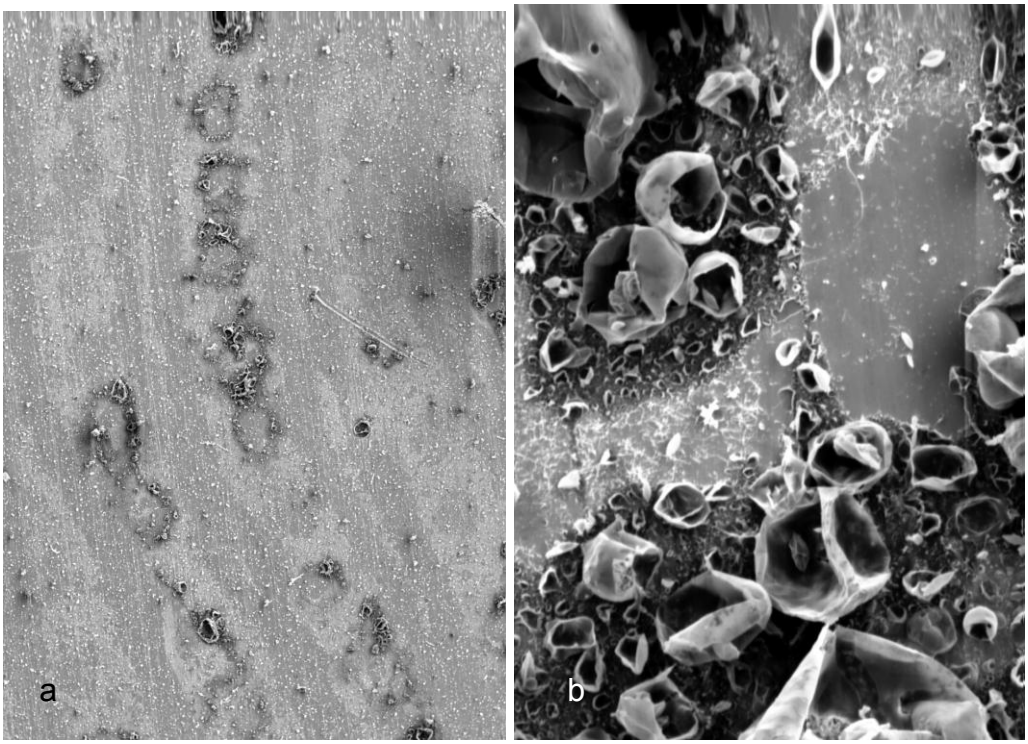
2.6 The different levels of superglue development for fingermarks under different conditions of humidity can be seen to be associated with different microstructures. Scanning electron microscopy has been conducted on samples developed under different humidity conditions [53,54], some of the results being illustrated below. It should be noted that these microscopy results are from a very limited subset of the donors used in the full study, and further investigation is required to see how consistent such observations are across a range of donors.



Fingermark ridge developed at 60% relative humidity a) *x 580 magnification* b) *x 1,700 magnification.*



Fingermark developed at 80% relative humidity a) *x 50 magnification* b) *x 1,100 magnification.*



Fingermark developed at 100% relative humidity a) *x 50 magnification* b) *x 1,000 magnification.*

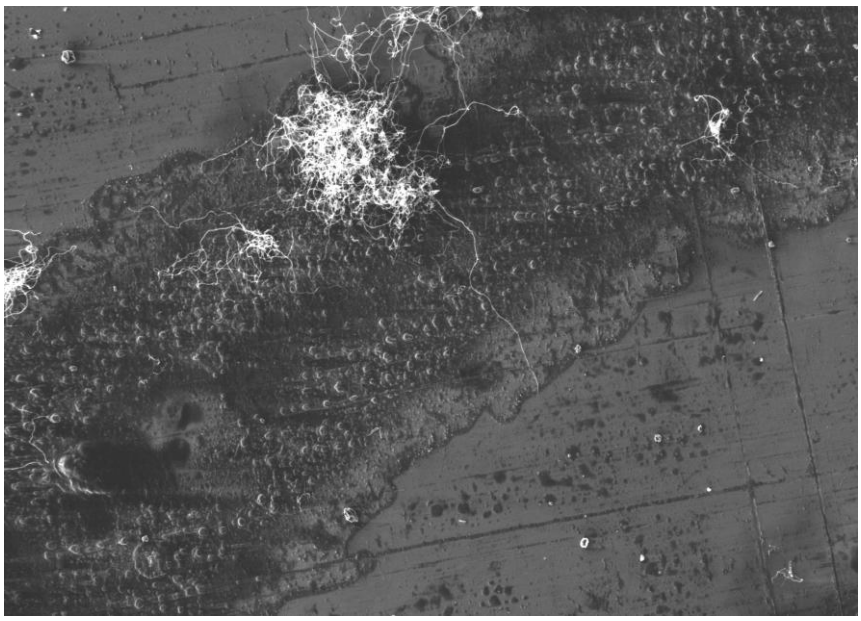
2.7 At 60% RH, the polymer resembled a film that was gathered at some points into a tortellini-like structure. This type of polymer morphology has been observed in fingermarks developed at lower humidity by others [55]. It has been suggested that this polymer film is a result of initiation by a hard anion, leading to rapid initiation and many active centres of polymer growth and hence the polymer grows in many directions, producing a two-dimensional film [56].

2.8 Fingermarks developed at 80% RH had less polymer development between the ridges than those developed at 100%. There was a very high concentration of noodle-type polymer in the ridges, with a particularly high concentration around the pores. This is thought to be because the concentration of eccrine secretion is higher in these areas. It has been suggested that this type of polymer is a result of slower initiation of polymerisation, leading to fewer active centres of polymer growth, and hence growth in a single direction, producing the noodle morphology [56]. It is not clear as to why initiation might be slower at 80% RH than at 60%. If anything, it would make sense for initiation to be faster at 80% because there is a higher concentration of water molecules to initiate polymerisation. It is possible that the presence of the water molecules influences how other constituents of the fingermark initiate polymerisation.

2.9 Fingermarks developed at 100% RH produced an interesting morphology that seems to be unlike any observed in the literature. The polymer resembles collapsed spheres of varying size and was mostly concentrated around the fingermark pores. The structure of the

developed marks is predominantly flat in nature for the 60% and 100% RH samples, with some isolated raised features ('tortellini' for 60% RH, and 'collapsed spheres' for 100% RH). The noodle-like structure developed at 80% RH is most effective for scattering light and in retaining the fluorescent dye molecules used to enhance the marks.

2.10 There has been further investigation into which constituents of fingermark residues may be responsible for initiation of the polymerisation reaction. Lewis *et al.* [57] found that moisture in the print prior to the fuming process was an important factor in the development of fingermarks. Eccrine marks showed a marked drop-off in quality of developed marks with time, attributed to loss of moisture from the mark. In contrast, sebaceous marks showed less age-dependence. It was thought that sebaceous constituents in the print could retain moisture in the residues, but these constituents were not, in themselves, responsible for initiating the polymerisation reaction. In the recent study conducted by CAST and London South Bank University [53,54] to investigate the effects of RH on fingermark development, the microstructure of purely sebaceous marks developed at 80% RH was found to differ considerably from eccrine and 'normal' marks, suggesting a different mode of polymer growth.



Sebaceous fingermark developed at 80% relative humidity (x 250 magnification).

2.11 In sebaceous marks developed using superglue, there is a large amount of spherical polymer throughout the ridge, as well as clumps of noodle-type polymer, presumably where some eccrine material is present on the ridge. The edge of the ridge shows where the oily material has spread outwards. It has been suggested that the capsule-type polymer morphology is a result of emulsion polymerisation, with fatty acids acting as emulsifiers of aqueous and oily phases [57]. The presence of small clumps of noodle-type polymer would seem to suggest that whatever is

initiating noodle-type growth in eccrine fingermarks is present in unevenly distributed, smaller quantities in sebaceous fingermarks.

2.12 More recent research [58] looked at the role of fingermark constituents initiating polymerisation and indicated that some of the components of eccrine sweat (lactate and alanine) were both capable of initiating the polymerisation reaction, in both cases initiation occurring via the carboxylate functional group. There was no evidence that the amine functional group in alanine played any role in polymerisation. However, the fingermark environment is a complex one and it may be that the presence of combinations of constituents is actually more important than individual constituents in the initiation process. Another study [59] also reported that acid moieties reacted with cyanoacrylate vapours; however, the amines in amino acids and the hydroxyl moiety of lactic acid had greater initiating properties for the polymerisation of cyanoacrylate. Based on this research, a number of studies [60,61,62] have investigated pre-treatments (e.g. acetic acid, ammonia and methylamine) to improve the cyanoacrylate polymerisation. Other studies [63,64] investigated the influence of temperature and it was observed that pre-cooling evidential articles prior to fuming increased the rate of polymerisation.

2.13 With regard to the selection of dyes for the enhancement of developed superglue marks, it should be noted that many of the most successful dyes are basic in character. It is believed that basic dyes work better in this application because they form weak Van der Waals bonds with the polycyanoacrylate 'noodles' formed during development, predominantly due to weak binding of the dye cation with the anions associated with cyanide (CN^-) groups in the polymers strands.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014) [65], the purpose of this publication being to report the history, theory and validation work associated with the process. The CAST procedure recommends the use of a controlled-humidity superglue cabinet, four of which are known to comply with the technical specifications devised by CAST. These are the Sandridge cabinet, produced by the original Mason Vactron Company and still in use in some fingerprint laboratories, the MVC5000, MVC3000 and MVC1000 systems produced by Foster and Freeman (the company that purchased Mason Vactron), and the superglue cabinet produced by Labcaire. There are other systems on the market that may meet specifications, but CAST has not carried out an evaluation of them.

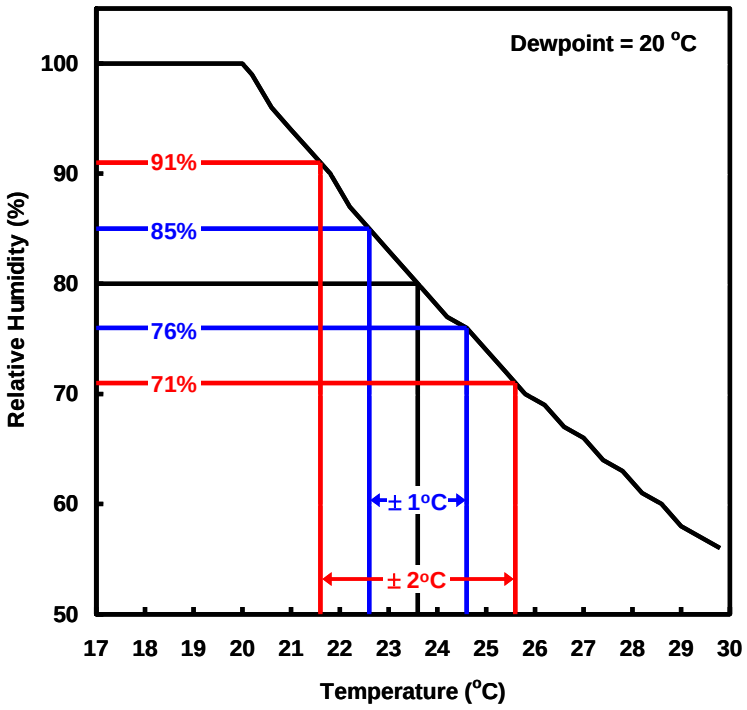
3.2 The process involves first raising the humidity in the treatment chamber to 80% RH. This value has been found by empirical testing to give a visible white deposit on the developed fingermark and minimal

background staining for the typical ambient temperature range experienced in the UK.

3.3 Once the humidity in the chamber has reached the required level, superglue is evaporated from an aluminium pot placed on a heater and heated to ~120ºC to speed evaporation. This temperature provides enough heat to allow the ethyl cyanoacrylate to evaporate gently over a set period of time without vigorous boiling, and is low enough to avoid the risk of the chemical breaking down to produce fumes of hydrogen cyanide [66].

3.4 The amount of superglue used varies between different types of chamber depending on capacity, but is selected to give a sufficient concentration of superglue vapour in the atmosphere to allow the polymerisation reaction to proceed to the extent that marks are visible. The quantity actually used should be optimised for a particular cabinet configuration by observing the quantity of residue left in the aluminium pot and adjusting it to ensure very little excess remains at the end of the cycle.

3.5 Obtaining a constant temperature within the closed treatment chamber is essential in order to maintain a constant RH because of the relationship between the two variables in a situation where air currents and fans carry air around a closed system. A theoretical plot derived from a known mass of water contained in the air at 80% RH and 20°C is illustrated below [67].



Relationship between temperature and humidity in a closed system.

3.5 It can be seen that small fluctuations in temperature can have appreciable effects on the local RH, and it is therefore essential to

ensure that the temperature profile within the treatment chamber is as even as possible. Within the old 'Sandridge' style cabinet, the heat from the light bulbs used in the chamber was observed to cause fluctuations in temperature (and therefore RH), and a change to 'low energy' bulbs was recommended to overcome this [67]. This also suggests that the cabinet in the laboratory should be positioned in a location away from direct sunlight and other heating sources.

3.6 The use of low viscosity (unthickened) ethyl cyanoacrylate is thought to give better results than those including thickeners. Methyl cyanoacrylate seems to give similar results to the ethyl system. A recent study [52] compared various alkyl cyanoacrylates (methyl, ethyl, n-butyl and 2-octyl) and reported that ethyl and butyl polymers formed polymer microstructures that scattered light more effectively than methyl and octyl polymers.

3.7 CAST recommends that initial photography of any visible marks be carried out after superglue treatment and prior to proceeding to treatment with a fluorescent dye stain. This is because some marks may be degraded or destroyed by the dye process and to maximise evidence recovery all marks should be recorded before dyeing.

3.8 The primary fluorescent dye recommended by CAST in the 2nd edition of the *Manual of Fingerprint Development Techniques* [68] and the subsequent Fingermark Visualisation Manual [66] is BY40, dissolved in ethanol. Fingermarks dyed with BY40 are best visualised by illuminating them using the violet/blue (400 to 469 nm) excitation band of a Quaser light source (or equivalent) and viewing the resultant blue/green fluorescence through a Schott glass GG495 filter (which has a 1% 'cut-on' limit at 476 nm). BY40 is selected because trials by CAST in the 1980s (for which original data are no longer available) have shown it to be at least as effective in terms of fluorescence intensity as Rhodamine 6G. BY40 is preferred by CAST because it has been demonstrated not to have any of the issues of suspect carcinogenity associated with Rhodamine derivatives. With the recent development of a blue laser operating at 460 nm, it may be possible to increase the number of marks detected after dyeing with BY40, although no dedicated study has yet been carried out.

3.9 The dye basic red 2 (Safranine O) was also recommended for use by CAST. In comparative studies in the 1980s (for which original data are no longer available) it was found to be slightly less sensitive than BY40 but is excited by the green (473 to 548 nm) excitation band of the Quaser light source and has an orange fluorescence that is viewed through a Schott glass OG570 ('cut-on' 549 nm) filter. This may be a useful alternative dye for situations where the background fluoresces when illuminated by violet/blue light and obscures the developed mark, although BR14 is now preferred for this role.

3.10 The solvent recommended for the dyeing of superglue marks is ethanol. This is selected because it is non-toxic (unlike earlier formulations based on methanol) and has been shown to be effective in delivering the dye into the polymer deposits. However, there are cases where the flammable ethanol-based formulations cannot be used (e.g. in a laboratory that has insufficient extraction, if the dye is being applied at a scene, in cases where ethanol is causing some printed inks to run or there is excessive dye take-up by the substrate) and a water-based BY40 formulation is recommended as an alternative. However, the water-based BY40 formulation is less effective in dyeing the fingermarks and the resultant fluorescence is markedly less intense [36]. More recently CAST has reviewed a number of alternative water-based dyes and have found the most effective of these to be BR14. A formulation for this was issued [69] for operational use, and further improvements in performance may be possible by using this dye in combination with the green (532 nm) laser. However, issues with availability of the Levercet CC carrier material for the water-based formulation resulted in work to identify alternatives [70], and ultimately a formulation containing only water and a stock detergent solution was recommended for operational use [66].

3.11 It is recognised that ethanol may cause some dyes on surfaces to run, and to reduce this effect propanol-based dye formulations have been studied [71], directly replacing the ethanol content of the dye formulation with propanol. These studies indicated that propanol-based dyes were only marginally less effective than the ethanol-based formulation and they were therefore included as a Category B process in the *Fingermark Visualisation Manual* for use in situations where ethanol-based dyes caused damage to the surface and water-based dyes were not sufficiently effective.

4. Critical issues

4.1 There are a number of critical issues associated with the superglue process as recommended by CAST.

4.2 Superglue development should be carried out in a closed, temperature- and controlled-humidity cabinet at an RH of 80%, because these conditions give the optimum development of marks. There is evidence that some cabinets may overshoot and do not provide close control of humidity. Ideally control limits should be determined and specified. Cabinets should be kept clean and maintained regularly.

4.3 Marks developed using superglue should be imaged wherever possible after superglue development and prior to dyeing. There is no guarantee that marks visible after the development process will still be present after dyeing.

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4.4 Superglue should not be used if the surface is suspected of being wetted at any point after fingermark deposition because the fingermark constituents that initiate polymerisation will have been dissolved.

4.5 The nature of the surface needs to be taken into consideration prior to dyeing the developed marks. Some surfaces may be damaged by ethanol and require dyeing with a water-based formulation, some surfaces may be strongly background fluorescent under blue/violet light and require a dye excited in a different part of the spectrum, and some surfaces may strongly absorb dye and require the developed marks to be enhanced with another means such as powders.

4.6 Marks may develop on different surfaces at different rates, observation of the development process is recommended and the process should be halted if over-development begins to occur, or extended if it is felt that further development of faint marks is possible.

5. Application

5.1 Suitable surfaces: Superglue is suited for use on all types of non-porous surface, including glass, plastic bottles and plastic packaging, metals, ceramics and both sides of many adhesive tapes. It is superior to powders in developing marks on surfaces that are more textured. Superglue can also be used on some 'semi-porous' surfaces, but in such situations the dyeing stage is usually omitted to prevent staining of the background.

5.2 The principal application of superglue is for the development of fingermarks on non-porous surfaces and adhesive tapes (although not on the adhesive side of acrylic adhesive-based tapes). It is an effective process on articles such as plastic bags, drinks cans, bottles, cowlings and vehicle number plates. Superglue generally gives better results than powdering on textured surfaces, where powders tend to fill in the texture and clog the surface. However, the polymerisation process is thought to be initiated by water-soluble components of the fingermark and as a consequence the process is not generally suitable for articles that have been wetted because these components are likely to have been washed away [72]. For wetted items the use of an alternative process, such as vacuum metal deposition, small particle reagent or powder suspensions, is recommended instead.

5.3 The technique can be effective on semi-porous items or items with glossy, non-porous coatings on porous backings (e.g. glossy magazines, printed cardboard packaging) but in these situations dyeing the article can lead to severe background staining or uptake in the porous substrate. Marks developed on these surfaces should be imaged under oblique light or UV imaging, or enhanced using a dry process such as powders or vacuum metal deposition.

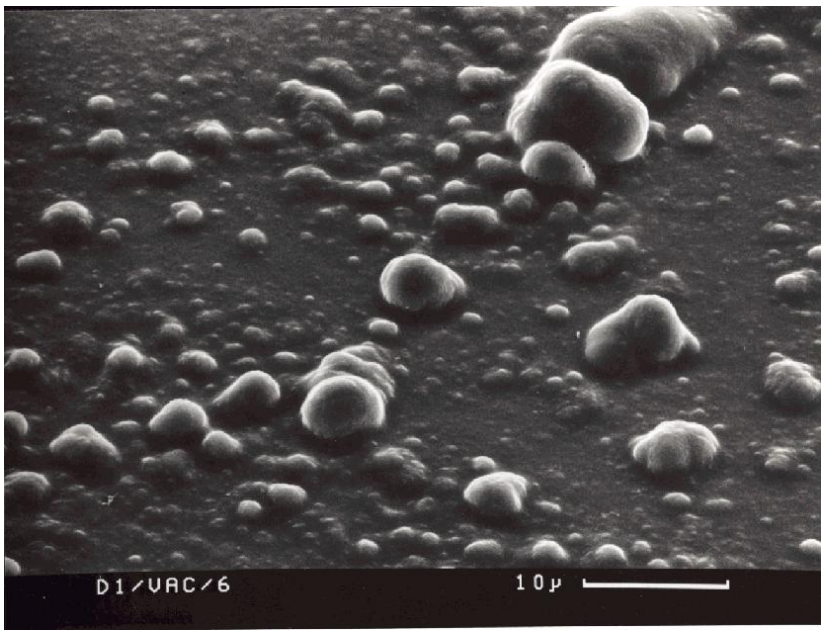
5.4 The use of superglue fuming has been reported for fingermarks deposited on human skin [73] but developed marks are not easy to visualise and require dye staining.

5.5 The method recommended for application of superglue in a laboratory is by the use of controlled-humidity cabinets. The articles to be treated are suspended or placed on shelves within the cabinet, ensuring sufficient space between them for circulation of the vapours and exposure of all surfaces of interest. Ideally, similar items should be treated together in batches. The cabinet is then humidified to the recommended level of 80% RH, and then an appropriate amount of superglue is evaporated from an aluminium foil pot on a heater at approximately 120ºC. The glue cycle can be allowed to run for a set period of time, but it is best practice for the operator to watch development on the samples and halt the cycle if it looks as if overdevelopment of marks is beginning to occur. The cabinet is then placed through a purge cycle to remove fumes of cyanoacrylate vapour before the cabinet is opened and articles are removed. Articles with underdeveloped marks can be replaced into the cabinet and redeveloped. The cabinet allows several items to be treated in a single run unlike some processes, such as vacuum metal deposition, where it may only be possible to treat one item at a time.

5.6 If an article is to be dyed, it is immersed in a tank containing dye solution (either ethanol or water-based), then removed to a second tank containing running water until excess dye has been removed. The dyeing time for the ethanol-based dye is approximately one minute, but longer dyeing times (~ two minutes) may be required when water-based dyes are used. The article is then allowed to dry at room temperature. For larger articles, the fluorescent dye solution may be applied from a wash bottle (but never sprayed), and the dye washed off using a wash bottle, hose, or running tap water.

6. Alternative formulations and processes

6.1 The reaction of cyanoacrylates with fingermarks under low humidity and low pressure is also reported in the literature [37,38,39,40,41,42,43,44,45] and several comparisons have been made to the high humidity technique. In the vacuum superglue technique, the articles to be treated are placed in a chamber with a quantity of superglue, and the chamber is evacuated to a level in the region of 0.3–7 mbar. When most of the air has been pumped out, the chamber is sealed from the vacuum pump and the superglue continues to vaporise to its room temperature vapour pressure. In general, the 'vacuum superglue' reaction does not give rise to the white fibrous deposit, instead it produces small beads of polymer.



Scanning electron micrograph of superglue deposit formed during vacuum superglue fuming.

6.2 The principal advantage of the technique over the high humidity process is that it is less prone to overdevelopment of marks. Another advantage is that vacuum fuming is effective at developing latent marks on areas not directly exposed to the cyanoacrylate fumes (e.g. plastic bags/items sealed in another plastic bag and on CDs/DVDs stacked on top of each other. An operational example may include drugs packaging where the action of unwrapping one layer may damage fingermarks on further layers below. However, the small, granular bead structure can limit the scattering of light making the developed marks less easy to see and require dyeing to aid visualisation. Studies by PSDB [40] have indicated that it is more difficult to obtain dye uptake in the bead-like deposits formed by vacuum superglue and consequently it is the high humidity process that CAST has recommended.

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a)

b)

Photographs showing differences between vacuum superglue development (left-hand side) and high humidity superglue development (right-hand side) a) *showing over-development of high humidity mark and* b) *showing generally fainter appearance of vacuum developed marks*

6.3 Comparative studies carried out by PSDB in the early 1990s [40] involved a pseudo-operational trial, dividing plastic (polyethylene) bags from high street stores into quarters. Two quarters were treated with high humidity superglue and the other two with vacuum superglue. All were then dyed with BY40 and examined using the violet/blue output of a Quaser 100. From this and parallel studies using split depletions deposited on clear polythene substrate, PSDB concluded that vacuum superglue was generally less sensitive or effective in the development of latent fingermarks. However, it should be noted that other researchers reached the opposite conclusion [38, 41] and it is recognised that both techniques have their advantages and disadvantages, vacuum superglue

being preferred where development is not closely observed and the risks of overdevelopment can be mitigated. A 2015 large-scale pseudo-operational trial on plastic bags comparing atmospheric/humidity and vacuum [45] reported the same conclusions as the previous PSDB studies.

6.4 The use of the one-step fluorescent superglue process Lumicyano was also investigated under vacuum [45]. Fingermarks were successfully developed, however, it was less sensitive and effective when compared to the atmospheric/humidity conditions for the one-step process. The use of vacuum fuming with Lumicyano did not adversely affect subsequent Lumicyano fuming under atmospheric/humidity conditions.

6.5 The use of superglue as a fingermark development technique for use at crime scenes has also been investigated and several systems have been developed for the treatment of car interiors, rooms and localised treatment of small areas [46,47,48,49,50,51]. It is difficult to control accurately the humidity conditions during treatment at scenes. Consequently, where comparative assessments have been made between portable fuming equipment and treatment in controlled-humidity cabinets, the laboratory results have been superior. In 2002 PSDB assessed the SuperFume system produced by Foster and Freeman, comparing it with both powdering and superglue treatment in the controlled conditions of the MVC5000 cabinet [50]. Over 6,000 marks were deposited across a range of surfaces in a small room. Marks developed at the scene were dyed with water-based BY40, those developed using the MVC5000 were dyed with the ethanol-based formulation.

6.6 It was concluded that although there were a range of surfaces where superglue gave better performance than powders at a scene of crime, if the surface could be recovered to a laboratory and treated under controlled conditions the number and quality of marks developed was increased. The study concluded that scene portable fuming systems such as SuperFume do have an important role to play in treatment of scenes, in particular on textured surfaces that cannot be recovered to a laboratory. This was supported by the results of a similar comparative study with powdering [51]. However, if articles are portable they should be taken back to a laboratory for treatment unless time and cost considerations indicate in situ treatment is preferable. The use of superglue at a scene will have health and safety implications, both in application and in the subsequent clean up. During application the fumes given off by the superglue must be contained and then safely vented, and after application vapours may still be trapped in porous items such as soft furnishings. The vapours may subsequently be released to the atmosphere, and there is a possibility that superglue deposits on hot surfaces can degrade to form hydrogen cyanide, carbon monoxide and carbon dioxide. It should also be noted that the water-based BY40 dye used in this study has since been superseded by a water-based BR14 formulation and the effectiveness of treatment at scenes should now be

improved because the water-based BR14 dye gives more intense fluorescence than water-based BY40, and will be particularly effective if used with the higher power 532 nm laser.

6.7 With regard to the dye systems used in combination with superglue, several alternatives to BY40 have been proposed in the literature. A summary of these is given to below, together with some comments about why they are not currently (2016) recommended for regular operational use by CAST.

Dye	Excitation band (nm)/colour	Viewing filter cut-on (nm)/ fluorescence colour	Comments
Rhodamine 6G (Basic red 1)	495–540 (green)	549 (orange)	Unconfirmed health and safety concerns. No better than BY40
Safranine O (Basic red 2)	473–548 (green)	549 (orange)	Recommended in CAST manual 2nd edition, but less sensitive than BY40
Ardrox	365 or 435–480 (UV or blue)	476 (blue/green)	Health and safety issue with prolonged use of UV-A
Basic red 28	470–550 (green)	549 (orange)	Not tested by CAST
Liqui-drox	365 (UV)	415 (blue)	Health and safety issue with prolonged use of UV-A
MBD	415–505 (blue/green)	515 (yellow)	Not tested by CAST
Nile Red	450–560 (green)	549 (orange)	Not tested by CAST as superglue dye
Thenoyl europium chelate (TEC)	365 (UV)	593 (red)	Health and safety issue with prolonged use of UV-A. Tested *vs*. BY40 by CAST, found to be inferior
MRM 10 (Rhodamine 6G, BY40, MBD)	430–530 (blue/green)	549 (529) (yellow/orange)	Not tested by CAST
RAM (Rhodamine 6G, MBD, Ardrox)	415–530 (blue/green)	529 (yellow/orange)	Not tested by CAST
RAY (Rhodamine 6G, BY40, Ardrox)	450–550 (green)	549 (orange)	Not tested by CAST

Some fluorescent dyes reported for use with superglue [19,21,22,24,74,75].

6.8 Not all the above dyes are fully soluble in ethanol, and other combinations of solvents may be recommended.

6.9 In order to minimise background fluorescence and improve contrast between ridges and the background, approaches to maximise the shift between excitation band and emission wavelength have been investigated. Successful approaches have included the use of dye mixtures [74], where energy transfer occurs between the excited states of the combined dyes and emission occurs at the longest wavelength from illumination at the shortest excitation band, and of thenoyl europium chelate, which naturally has a large Stokes shift and emits in the deep red/near infrared after excitation in the long-wave UV [75]. These approaches can be used operationally if it is not possible to distinguish any of the recommended dyes against background fluorescence.

6.10 CAST has researched alternatives to the water-based BY40 formulation, the most promising candidate systems being BR14 and Disperse Yellow 82 [36]. Disperse Yellow 82 proved difficult to dissolve into the water/carrier mix and therefore only the BR14 formulation was taken forward. The resultant formulation proved more intensely fluorescent than the water-based BY40 formulation, but not as intense as BY40 in ethanol. The effectiveness of BR14 in ethanol is closely equivalent to BY40 in ethanol and could be substituted for it, especially if used in combination with the new generation of scene-portable 5 W green lasers emitting at 532 nm.

a) b)

Comparison of water-based basic red 14 with a) *water-based basic yellow 40 (right), showing higher intensity of basic red 14 and* b) *ethanol-based basic yellow 40 (left), showing higher intensity of basic yellow 40 (this image should be viewed electronically to see the true intensity levels).*

6.11 Simultaneous processes for fuming and dyeing in a single step have were first reported in the 1990s [25] and in some cases carry-over of the coloured or fluorescent dye into the developed ridges was achieved. However, at the time of the first issue of this Source Book (2011) the resultant fluorescence was not comparable with that obtained in a two-step process. The approaches that have been considered include co-evaporation of a coloured or fluorescent dye with the superglue [25], and tagging of the monomer molecules with fluorescent species [32]. This has been difficult to achieve because it is hard to get the monomer and dye to evaporate at equivalent rates, and tagging the molecules generally increases molecular weight and increases the temperatures required for evaporation. Around 2005, CAST evaluated an experimental product utilising the co-polymerisation of a cyanoacrylate and solvent yellow 43 mixture that was heated to a temperature between 170 and 185^{o}C. Although carry-over of the dye occurred, the resultant fluorescence was weak and subsequent staining with BY40 provided fluorescence that was 5 to 10 times brighter. It should be noted that solvent yellow 43 has subsequently been reported as liganded to cyanoacrylate to form CN-yellow, a one-step fluorescent cyanoacrylate product [76,77].

6.12 Subsequent research has led to the introduction of the commercial products including Lumicyano [26,27,28] and Polycyano UV [29,30,31]. Lumicyano is applied under the same conditions as a conventional cyanoacrylate. The first generation of Lumicyano was supplied as a 1% (weight/weight) pink solution of fluorescent dye in cyanoacrylate. The second generation of Lumicyano separated the cyanoacrylate and dye as Lumicyano solution and Lumicyano powder where the recommended optimum concentration of dye was initially 4% and later revised to 5% and then 8% for stronger fluorescence. Polycyano UV is a fine powder, again incorporating a fluorescent species (about 5% weight/weight dimethylaminobenzaldehyde), which is co-fumed using a higher hot plate temperature of 230°C. Both these products give fluorescence that is initially equivalent to dye staining, although CAST have observed the intensity of the fluorescence to reduce with time (Lumicyano more rapidly than Polycyano UV). Hence, it is recommended to perform fluorescence examination and photography immediately after fuming and when this is not possible, to store treated articles in a cool, dark, and dry place, ideally sealed in a brown paper envelope to prevent air circulation [27]. For Lumicyano, using higher concentrations of the dye results in stronger fluorescence and therefore the fluorescence decay will be slower; however, it is not recommended to go above 8%. It is also possible to restore fluorescence by re-fuming the articles under examination and/or subsequent treatment with a fluorescent stain such as BY40. Such processes may result in additional marks being detected.

6.13 Both Polycyano UV and Lumicyano have fluorescence which is excited using long wave ultraviolet radiation, although Lumicyano can also be excited using blue/green wavelengths, which may be an advantage when trying to view fluorescence on surfaces containing optical brighteners.

Both products have the potential to be used on semi-porous surfaces that could not previously be treated using superglue fuming and dye staining, and research is ongoing to establish where the one-step process could have most benefit. Other one step fluorescent cyanoacrylate products that have entered the market include CN-Yellow, Fuming Orange and PECA Multiband [33,76,77,78]. All of these one-step processes are in powder form and require hot plate temperatures of 230^{o}C to sublime the powder, with the exception of Lumicyano which only requires a hot plate temperature of 120^{o}C to evaporate the solution.

7. Post-treatments

7.1 There are several post-treatments that can be applied to marks developed using superglue in order to improve their visualisation. The application of fluorescent (or indeed coloured) dyes has been discussed above; these are most often applied as solutions but sublimation has also been investigated. The intention is to stain selectively the fingermark ridges to enhance their contrast with the background.

7.2 For marks on surfaces that cannot be solution-dyed, powdering is a possible alternative. Powders may also selectively adhere to developed areas of ridge detail although early trials indicated that not all powders are effective and some trial and error may be required to identify the most appropriate powder to use. Reasonable results have been reported with Bristol Black and black magnetic powder [5], but these are by no means the only powders to use. Powdering may also destroy marks and photography should be carried out before powdering if possible.

7.3 Oblique lighting and UV imaging [79] have also been used to improve the contrast between the ridges and the background. In both cases the scatter of incident light from the rough texture of the developed ridges is used to discriminate the ridges from the smooth background. The advantage of both techniques is that they are non-contact. Further detail on both these techniques is given in Chapter 2, Optical Processes, Ultraviolet imaging and Visual examination respectively.

7.4 Another technique that may be used to separate superglue developed marks from patterned backgrounds is lifting using black gelatine lifters. These pick up a loose surface layer of white superglue deposit that can then be easily visualised against the black glossy background of the gel. Where marks have been dyed there is also a limited amount of dye carry-over and the lifted marks can also be viewed by fluorescence.

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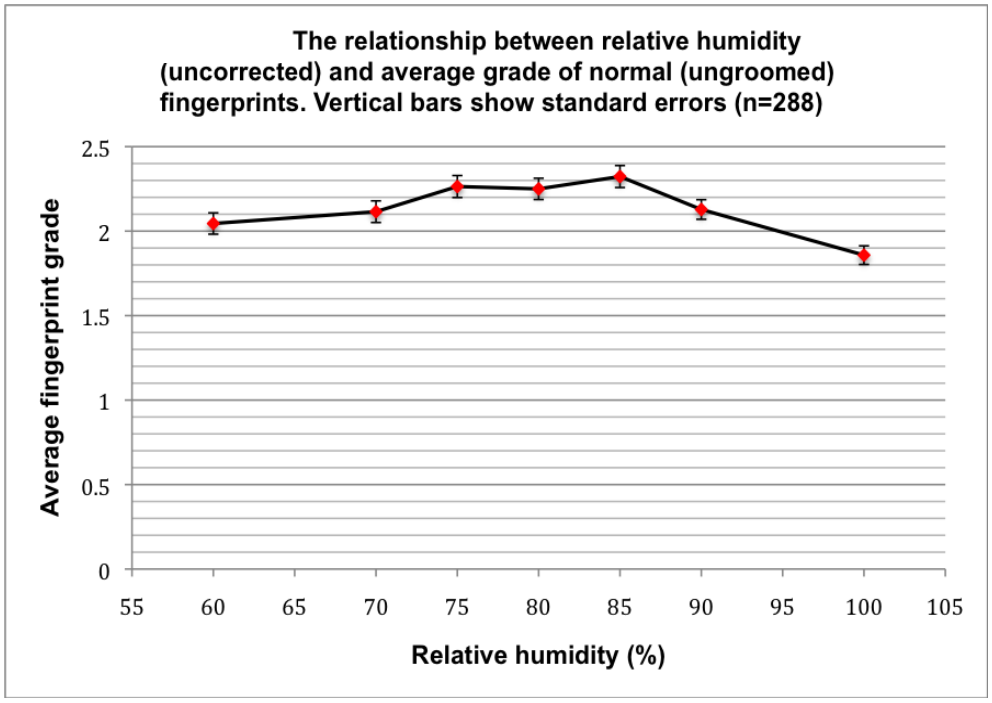
8. Validation and operational experience

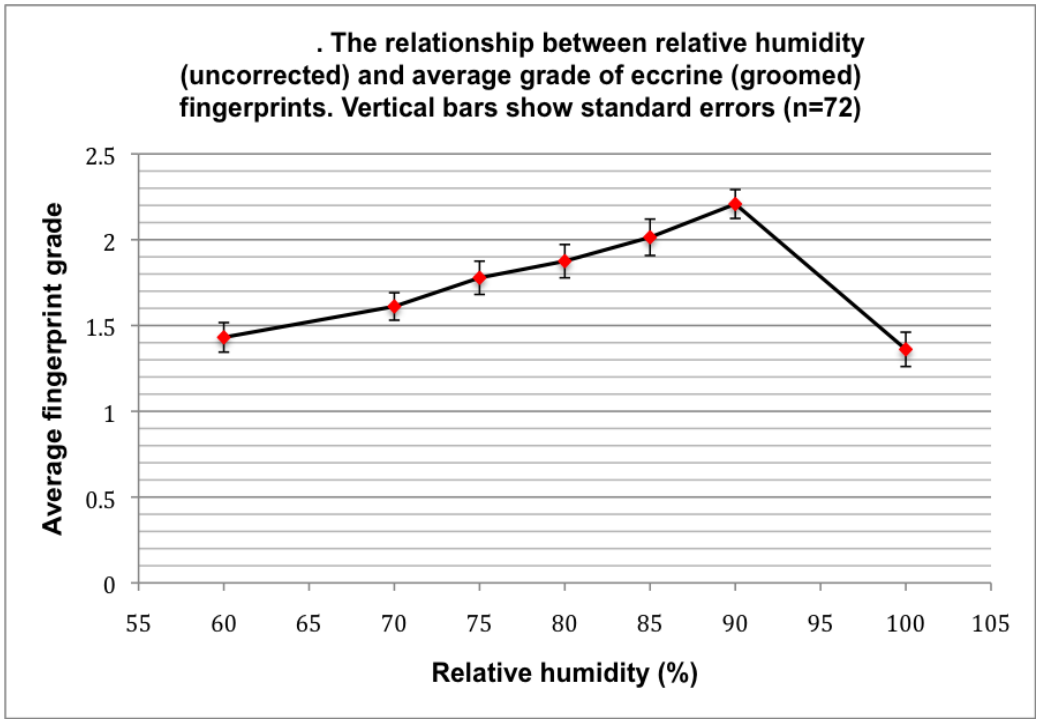
8.1 The effectiveness of superglue has been compared with that of a range of other techniques recommended for use on non-porous surfaces in a series of laboratory and pseudo-operational studies conducted by CAST from the mid-1980s to the present (2016).

8.2 Laboratory trials

8.2.1 Laboratory trials were first conducted by HOSDB in the mid-1980s to establish the optimum conditions of RH for development of fingermarks. Unfortunately, the results of these trials no longer survive for inspection and the work was repeated in 2009 to re-validate the recommendation for 80% RH during processing [53,54]. The results of developing and grading 2,016 'normal' marks, 502 'eccrine' marks and 502 'sebaceous' marks are illustrated below.



a)



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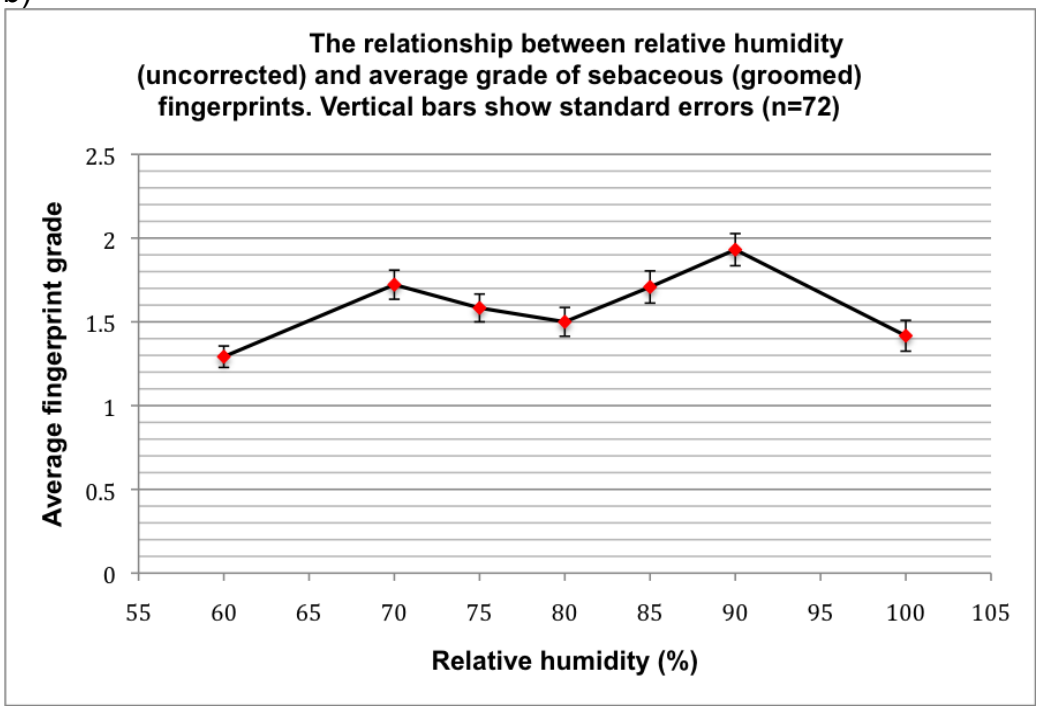


c)

The effect of relative humidity on the quality of marks developed using superglue a) *normal prints* b) *eccrine prints and* c) *sebaceous prints*

8.2.2 It can be seen that the quality of the marks developed at the extremes of RH investigated, 60% and 100%, were inferior to those developed between 70% and 90% with the optimum actually being between 85% and 90%. The lower value of 80% RH is chosen for operational work because it gives some margin of error during processing and is not too close to 100%.

8.2.3 Laboratory trials have also been carried out to compare the effectiveness of superglue with both powders and powder suspensions on a range of substrates [80,81].

8.2.4 Laboratory trials were carried out in 2003–2004 to establish whether any clear recommendations could be made regarding the use of superglue or powders on non-porous surfaces [80]. A two-way trial was conducted on a range of textured surfaces comparing superglue and subsequent BY40 dyeing, and powdering with black magnetic powder. A subsequent three-way trial was performed on smooth surfaces, comparing aluminium powder, black magnetic powder and superglue, and BY40 dyeing. Both of these studies included marks of ages one day, one week and one month. Almost 10,000 marks were deposited and graded during these trials.

8.2.5 In the trial on textured non-porous surfaces 12 different surfaces were studied, including a range of laminates with different effect facings (e.g. marble, wood, granite), stone floor tiles, uPVC, computer casings and kitchen unit material. The summary of grading over 7,500 marks of all ages on all substrates is recorded in the table below.

Grade of fingermark	**Process**	
	Black magnetic powder (%)	**Superglue + BY40 (%)**
0	12.04	16.93
1	29.61	29.19
2	19.09	16.48
3	26.26	21.08
4	13.00	16.32
Total % grade 3 + 4	**39.26**	**37.40**

Results of initial comparative experiments between superglue and powders.

8.2.6 The results obtained by the two techniques are closely equivalent, although when the results were analysed surface by surface, it could be seen that superglue developed more marks of high quality on the rougher surfaces and on older marks. It was therefore concluded that the techniques should be given equal weighting in updates to the sequential processing charts for textured non-porous surfaces in the *Manual of Fingerprint Development Techniques* [68].

8.2.7 In the second trial on smooth surfaces four materials were used, including glass, patterned and white ceramic tiles and smooth plastic-faced chipboard. Over 2,000 marks were graded in this exercise. The results are summarised in the table below.

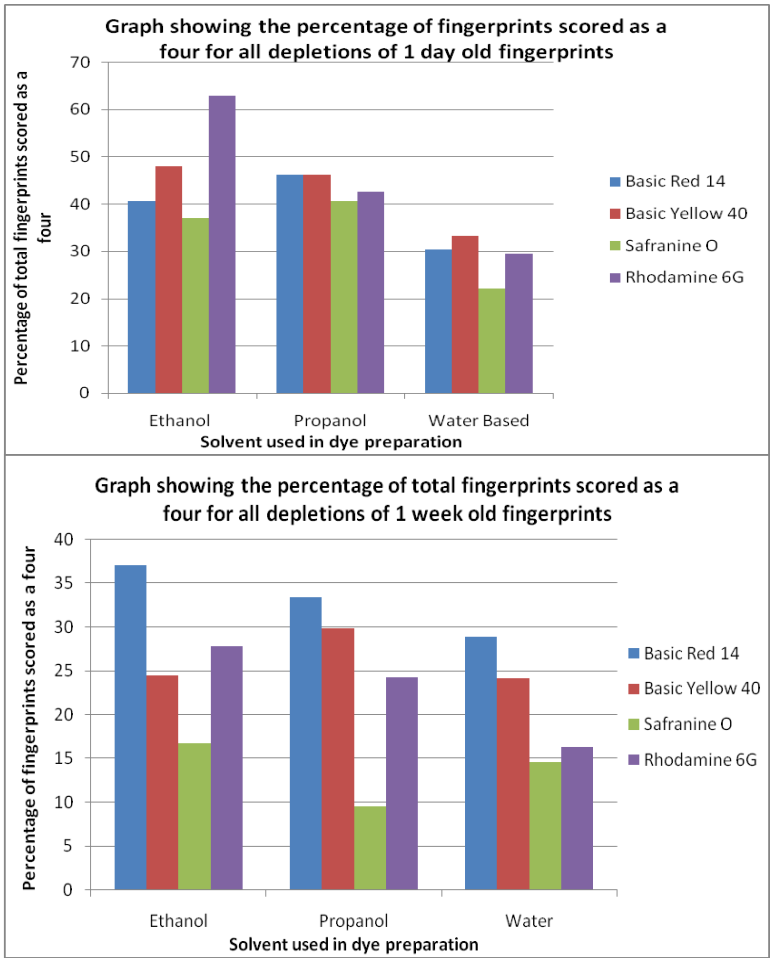
Grade of fingermark	Process		
	Aluminium powder (%)	**Black magnetic powder (%)**	**Superglue + BY40 (%)**
0	4.6	5.7	9.7
1	20.4	20.3	24.6
2	16.5	17.2	11.9
3	22.4	30.0	25.4
4	36.1	26.8	28.3
Total % grade 3 + 4	**58.5**	**56.8**	**53.8**

Results of further comparative experiments between superglue and powders.

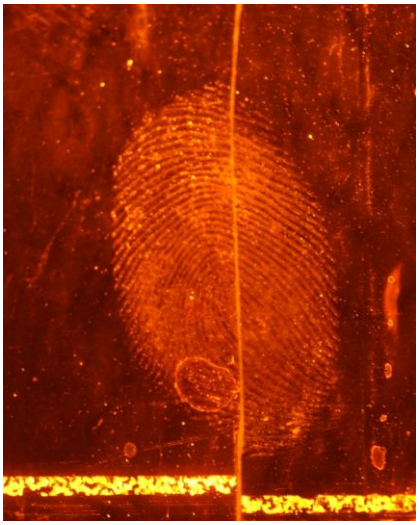
8.2.8 Overall, there was little difference between the three processes, although the observation in previous trials that aluminium powder performed best on smooth surfaces was confirmed. Powdering would marginally be the preferred process on smooth non-porous surfaces, but superglue gave closely equivalent performance.

8.2.9 The comparison with powder suspensions carried out more recently (2007) is more fully reported in Chapter 3, Chemical and Physical Processes, Powder suspensions. This was an extensive study looking at over 37,500 marks over 23 different surface types. There were variations in performance across individual surfaces, but general trends could be seen. These indicate that superglue and powder suspensions are closely equivalent in performance when used to develop fingermarks on non-porous surfaces, but the sequence of powders followed by powder suspensions was found to be more effective than superglue and dyeing overall [81].

8.2.10. Fotheringham [71] conducted an comparison of different fluorescent dye stains (basic yellow 40, basic red 14, Rhodamine 6G and safranine) in different solvents (ethanol, propanol and water). The objective of the study was to establish whether propanol could be substituted for ethanol in circumstances where ethanol caused dyes on the surface to run and also to reduce the cost of the dye stain. It was concluded that although the ethanol-based formulations were generally more effective, propanol-based dyes were very similar in performance and were therefore included as a Category B process in the *Fingermark Visualisation Manual* [65].



Percentage of 1 day and 1 week old fingermarks scored as highest quality after development with different fluorescent dye formulations [71].



1 day old fingermark dyed with Basic Red 14 in ethanol (left) *and propanol* (right).

8.2.11 In addition to the work on one step fluorescent superglue systems conducted by others and referenced in section 1, CAST conducted trials

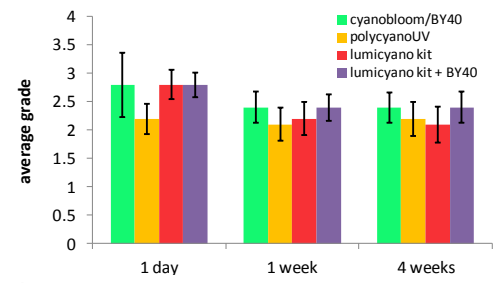
in 2014 [82] to compare the performance of superglue/BY40 with the Polycyano UV product marketed in the UK by Foster & Freeman, and the Lumicyano reagent kit marketed by Global Forensics. For Lumicyano, the powder and solution were mixed to produce a 4% by weight concentration of dye (representing manufacturer's advice at the time).

8.2.12 Fingermarks were deposited by 7 donors onto six substrates,

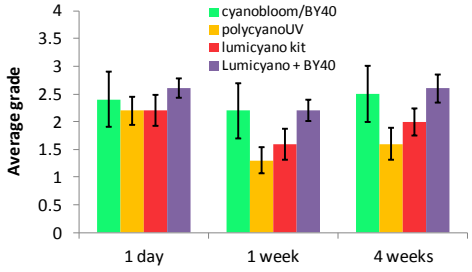
- Black, High density rigid black polyethylene (HDPE)
- Painted steel sheet
- uPVC "soffit board"
- Toughened glass
- Glossy printed corrugated product packaging cardboard
- Biodegradable (HDPE) plastic bag material

The substrates were then aged for one day, one week and four weeks before treatment with one of the three processes under test. For some substrates, the panel treated with Lumicyano was subsequently dyed with BY40 to explore whether this could improve the quality of the marks.

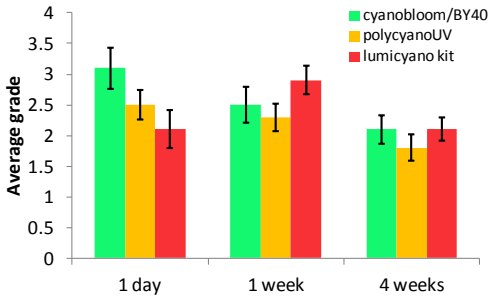
8.2.13 Results for each of the surfaces indicate all three processes performed equally well over all aging periods and surfaces. Only on biodegradable plastic bag material, where superglue/BY40 was more effective than the other methods, was there any significant difference. However, it should be noted that this assessment is based on a limited number of marks and more work would be required to make firm recommendations on where these systems are best deployed. The Lumicyano formulation has changed at least once since these tests were conducted and work would need to be repeated with the most current formulation.



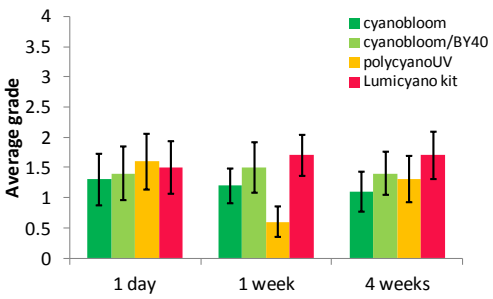
a)



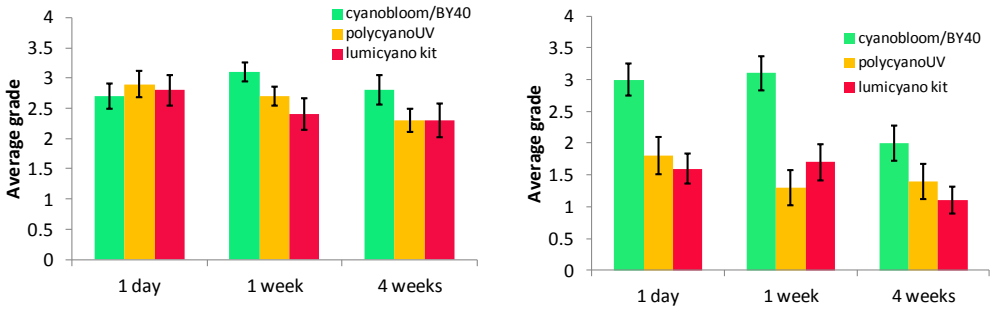
b)



c)



d)

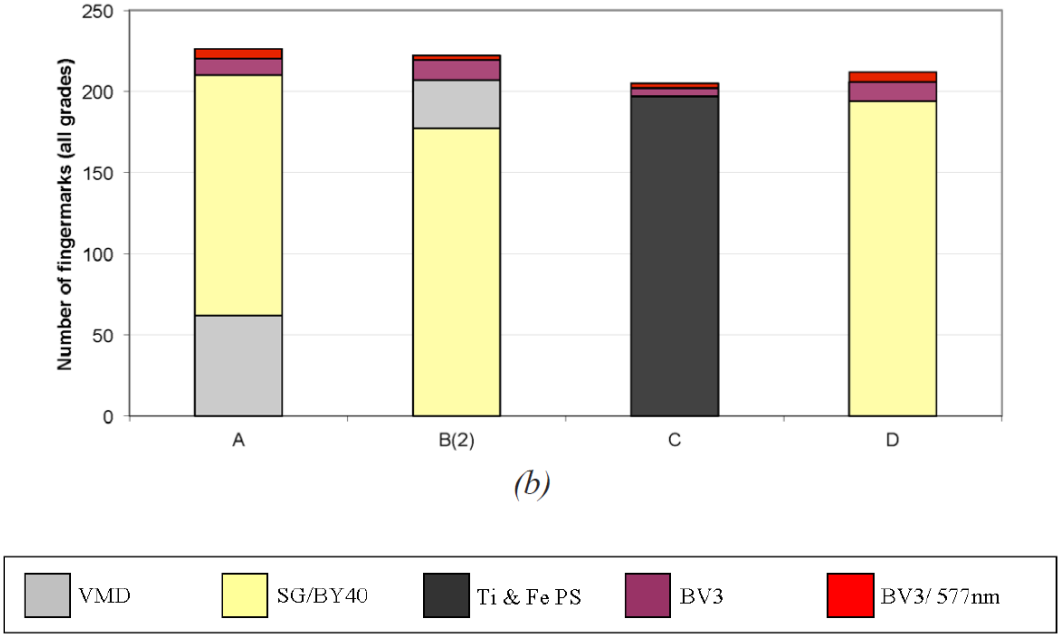


Average grades for developed fingermarks on: a) *HDPE,* b) *painted steel sheet,* c) *glass sheet,* d) *corrugated cardboard,* e) *uPVC,* f) *biodegradable plastic bag. Error bars indicate the 95% confidence interval of the mean [82].*

8.3 Pseudo-operational trials and operational experience

8.3.1 PSDB carried out a pseudo-operational trial on 200 plastic bags in 1986, and demonstrated that superglue combined with dyeing and subsequent fluorescence examination was a highly effective process for this type of surface, although not as effective as vacuum metal deposition. The results of this study are described in greater detail in Chapter 3, Chemical and Physical Processes, Vacuum metal deposition. Because not all police forces had vacuum metal deposition equipment, superglue and dyeing was considered an effective alternative.

8.3.2 A follow up study by CAST in 2009 using 100 plastic packaging items revealed that the effectiveness of VMD has diminished relative to that of superglue and BY40 [83]. This may be due to changes in the chemistry of plastic material. Furthermore, the use of iron- or titanium-based powder suspension (this development process was not available in 1986) was equivalent in effectiveness to superglue and BY40 staining. The 2009 trial confirmed that VMD is no longer the most effective process for plastic bags, but instead should be used after superglue in a sequential processing route.



Results of pseudo-operational trial carried out on plastic packaging material in 2009 [83].

8.3.3 A comparison between vacuum metal deposition and vacuum superglue was also carried out by Misner [84], who found that vacuum metal deposition developed 180/229 (79%) deposited marks to an identifiable standard compared with 141/229 (62%) marks developed by superglue, a similar margin to that in the PSDB study. Taroni *et al.* [85] conducted a similar study but concluded that superglue and vacuum metal deposition were of a similar sensitivity. However, a further study by Masters and DeHaan [86] again concluded that vacuum metal deposition was more sensitive than superglue on older marks (> three years) although equivalent on fresher marks (< two months).

8.3.4 PSDB conducted a comparative pseudo-operational trial between high humidity and vacuum superglue in the early 1990s [40], dividing ten plastic (polyethylene) bags obtained from high street stores and donated after use into quarters. Two quarters were treated with high humidity superglue and the other two with vacuum superglue. All were then dyed with BY40 and examined using the violet/blue output of a Quaser 100. The number of fingermarks and scraps of ridge detail developed were recorded, the results being summarised below.

High humidity superglue		**Vacuum superglue**	
Fingermarks	Ridge detail areas	Fingermarks	Ridge detail scraps
32	47	16	29

Results of comparative experiments between vacuum and high humidity superglue techniques.

8.3.5 From this and parallel studies using split depletions deposited on clear polythene substrate, PSDB concluded that vacuum superglue was generally less sensitive or effective in the development of latent fingermarks. PSDB found that there were problems with low dye take-up by marks developed using vacuum superglue, resulting in less developed marks being detected. However, other researchers carrying out similar studies reached the opposite conclusion [38,41] and it is recognised that both techniques have their advantages and disadvantages. However, high humidity superglue continued to be the primary process recommended for operational use in the UK. Another pseudo-operational trial in 2015 on 100 polyethylene bags [45] also reported the superiority of the atmospheric and humidity process.

8.3.6 An analysis of the effectiveness of the superglue process on operational work in Essex Police Laboratory was conducted by Taylor [87] over a period of three months in 1995. Results from processing 430 items are summarised below. At the time of the work a 16 point standard was in place for fingermark identification in the UK, and the marks were assessed by a fingerprint expert in terms of the number of 'second-level detail' features present.

Article type	**>16 points**	**8–15 points**	**Items overall**
Adhesive tape	3	6	18
Cash bag	0	4	46
Cowling	3	57	145
Credit cards	0	3	22
Latex gloves	0	0	16
Numberplates	2	4	42
Screwdrivers	0	0	13
Store cards	0	0	21
Sweet wrappers	0	0	11
Bullets/pellets	0	0	8
Crisp packets	0	1	7
Polythene bags	0	0	7
Miscellaneous	5	31	74

Results of operational work using superglue.

8.3.7 The miscellaneous items where the superglue process had most success included bin liners, a photograph and a telephone. The majority (91%) of items for which marks containing >16 points (the minimum fingerprint quality standard then in place) were recovered were plastics, suggesting that superglue is effective for this type of article. The effect of time before treatment on the number of marks developed was also assessed, as was the effect of any contamination present on the surface on subsequent development. Results indicated that superglue became less effective as the age of the mark increased. Development was still observed on articles contaminated by chemicals, blood or oil, but the

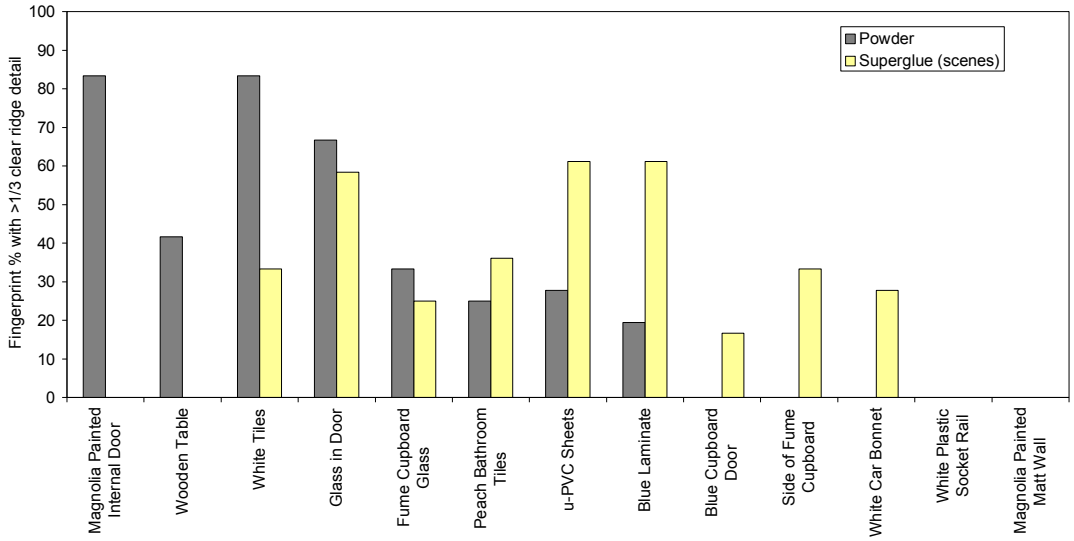
presence of drugs residue or powder inhibited development and no marks were found on articles known to have been wetted. At this time, fluorescent dyeing was not being used as a secondary treatment; had this been the case, the number of recorded marks would have increased.

8.3.8 Subsequent operational experience has shown that superglue continues to be highly effective on plastic, non-porous items and the technique is extensively used on plastic bags and cowlings. However, some police forces had found that powders give equally good results on the inside of cowlings and it has recently been observed that powder suspensions may be more effective still. Two police forces recording data and changing from superglue to powder suspensions as a development technique on cowlings have observed increases in the number of marks developed. This is thought to be partly attributed to the fact that such items may be exposed to moist or wet environments, which are less detrimental to the powder suspension process.

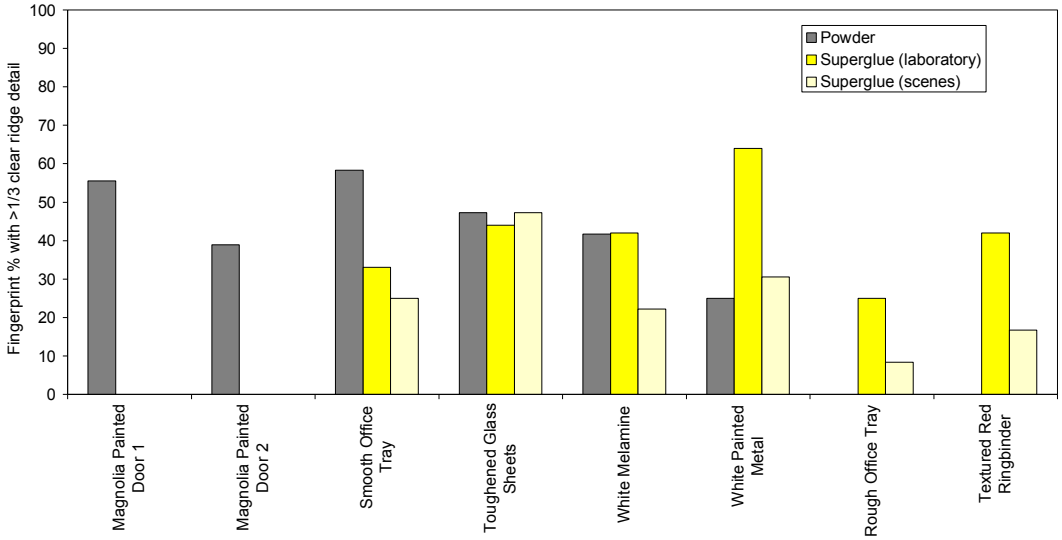
8.3.9 The operational work outlined above also shows that superglue is an effective process for developing fingermarks on adhesive tapes. Recently concluded research by CAST [88,89,90] indicates that superglue is the most effective process for developing fingermarks on the non-adhesive side of tapes, and is closely equivalent in performance to powder suspensions on the adhesive side of tapes with rubber-based adhesives. For treatment of such tapes superglue has the advantage that it develops marks on both sides of the tape simultaneously. However, on tapes with acrylic-based adhesives superglue does not develop marks on the adhesive side and carbon-based powder suspensions should be used instead. An operational trial to compare the effectiveness of superglue and carbon-based powder suspensions was carried out and indicated that carbon-based powder suspensions were superior in this application, this being reported in Chapter 3, Chemical and Physical Processes, Powder suspensions.

8.3.10 Pseudo-operational trials were also conducted to establish the relative effectiveness of superglue carried out at scenes using a SuperFume unit, and those developed under laboratory conditions in an MVC5000 unit [50]. In this study over 6,000 marks were deposited across a range of surfaces in a small room. Marks developed at the scene were dyed with water-based BY40, those developed using the MVC5000 were dyed with the ethanol-based formulation. This was representative of what would be carried out at scenes and in most laboratories. The results of this study are summarised below.

595 Publication number: 081/17



Comparison of the effectiveness of superglue and powders on surfaces treated at a simulated scene [50].



Comparison of the effectiveness of superglue and powders on surfaces treated at a simulated scene, and surfaces treated with superglue in a laboratory [50].

8.3.11 It could be seen that SuperFume gave better results than powdering on several surfaces, although better results still could be obtained where a controlled-humidity superglue cabinet was used. The ultimate choice of technique should take into account effectiveness and time and cost considerations. It should also be taken into account that aluminium powder was used on all surfaces, whereas more recent guidelines may dictate use of an alternative powder. The results obtained from powdering in this trial are therefore less than optimum, and further work may be required to clarify this.

8.3.12 The most recent pseudo-operational trial that has been carried out by CAST including superglue has been the reassessment of the optimum processing sequences for plastic bags and packaging material [83]. Once again, the results are more fully reported in Chapter 3, Chemical and Physical Processes, Powder suspensions, but indicate that superglue followed by vacuum metal deposition may be the best processing sequence for this type of surface.

8.3.13 CAST has not to date conducted any pseudo-operational trials including single step fluorescent formulations such as Lumicyano and Polycyano UV, although studies of this type have been reported by others [27,28] Observations from those trials included that the original Lumicyano 1% formulation, two-step cyanoacrylate/BY40 and iron-oxide powder suspension produced a similar number of marks; however, the use of BY40 after 1% Lumicyano visualised an additional 15% of marks. The sequential process of Lumicyano fuming at atmospheric/humidity conditions followed by an additional Lumicyano fuming cycle at the same conditions was also investigated. The second fuming cycle resulted in the detection of marks that were not observed during the first cycle.

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Silver nitrate

1. History

1.1 The use of silver nitrate for the study of eccrine sweat constituents (and hence latent fingermarks) on porous surfaces was supposedly first noted by Aubert towards the end of the 19th century [1], and together with iodine offered the only effective techniques for this type of surface until the use of ninhydrin was proposed in 1954. In the process silver nitrate reacts with the chlorides in the fingermark to give silver chloride, which is converted to metallic silver on exposure to light.

1.2 Various formulations had been reported, utilising both water and alcohol as solvents. The concentration of silver nitrate in these formulations typically varied from 3 to 10%, often with small additions of nitric acid to the aqueous solutions [1]. In 1969 Cuthbertson published the results of an extensive investigation of fingermark chemistry and utilised the silver nitrate reaction to determine chloride contents in fingermark deposits [2] As a consequence of these studies Cuthbertson proposed that the optimum silver nitrate concentration was 1%. Below this level there was insufficient reagent to react with the chloride available in the fingermark and above 10% the background coloration began to become excessive [1]. It was also noted by Cuthbertson that under conditions of high humidity the chlorides in the fingermark migrated and ultimately the mark became diffuse and undetectable. The operational implications of this study were published by Godsell [3] who recommended that UK police forces adopt the 1% silver nitrate formulation for operational use and ensure that exhibits for treatment were stored in low humidity environments.

1.3 The principal issue with the use of silver nitrate as a fingermark development reagent was the progressive darkening of the background after treatment and research was carried out in the late 1960s and early 1970s in an effort to overcome this. Green [4] investigated the use of alternative silver salts with greater stability to light, and also explored the use of a sodium thiosulphate-acetic acid solution as a fixing process. Morris and Goode [5,6] developed a modified silver nitrate process to overcome both the background darkening and the lack of control over the photochemical development step. The preferred method ultimately proposed by Morris and Goode was to convert the silver chloride to silver sulphide using thiourea, giving a more stable final product. A complexing agent, disodiumethylenediaminetracetic acid (Na_2EDTA), was used in the silver nitrate solution to form complexes with unreacted silver so that it could be washed from the surface more easily. This was found to significantly reduce background darkening [1].

1.4 During the assessment of experimental techniques in the UK in the early 1970s, silver nitrate was used in comparative trials with other processes, including iodine, ninhydrin, radioactive sulphur dioxide and vacuum metal deposition. These trials showed that silver nitrate was the process most

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adversely affected by storage conditions of high humidity or exposure to moisture [7]. However, if dry storage conditions were used silver nitrate developed a higher proportion of marks than ninhydrin, although not as many as radioactive sulphur dioxide. However, it must be noted that these experiments were conducted before the heat and humidification protocols were introduced for ninhydrin. Using silver nitrate after ninhydrin was found to produce marginally more marks than either process alone (56% for the sequence compared to 52% for ninhydrin and 55% for silver nitrate), and to improve the visual quality of 20% of the marks initially developed using ninhydrin [8]. These results were also confirmed by Caton in 1974, who reported the results of an assessment on over 6,000 paper and cardboard items; 1,617 marks were developed by ninhydrin, with a further 170 developed by subsequent silver nitrate treatment [9].

1.5 Although development of marks was typically carried out using light (ultraviolet or photoflood lighting being recommended), chemical developers could also be used [10]. Products typically used for photographic development were suggested, although the use of an additional immersion stage was not considered desirable because of the potential damage to some types of paper.

1.6 Silver nitrate was also considered as a technique for the intensification of faint ninhydrin marks, using a modified formulation using ethanol instead of water as the solvent [11]. This prevented the diffusion of the amino acids that occurred when the water-based formulation was used and meant that any marks developed using silver nitrate enhanced the existing ninhydrin marks and did not degrade any ridge detail already present. Other researchers have also considered non-aqueous alternatives to silver nitrate, one published formulation consisting of 3% silver perchlorate in toluene [12].

1.7 Other approaches to make the silver nitrate technique more practical were considered, including the use of ‘stopping solutions’ (i.e. solutions that neutralise the active chemical and halt the reaction) based on methanol, acetic acid, glycerol and water [13]. This slowed the background darkening effect and negated some of the need for immediate photography and storage of exhibits in the dark. However, despite these refinements the technique was rarely used on paper after the mid-1970s, and although recommended as a reagent for raw wood its use in the UK declined after it was withdrawn from the second edition of the *Manual of Fingerprint Development Techniques* [14]. No further developments have been reported since 1998.

2. Theory

2.1 The theory of the silver nitrate process is that the silver nitrate in solution reacts with the chloride constituents of fingermark deposits to produce insoluble silver chloride.

$AgNO_3$ *(aq) + NaCl (aq)* → *AgCl (s) + $NaNO_3$ (aq)*

2.2 Silver chloride is light sensitive and when exposed to ultraviolet light darkens rapidly as metallic silver is formed.

AgCl (s) + hv → *Ag (s) + ½Cl_2 (g)*

2.3 The treated exhibit is therefore exposed to ultraviolet radiation (or white light) to promote development although the optimum exposure time will vary from surface to surface and is not always easy to establish because both the print and the background progressively darken with time. In the case of the background this occurs due to gradual breakdown of unreacted silver nitrate in the porous substrate, and treated exhibits should be stored in the dark to reduce the speed at which this occurs.

2.4 The formulation formerly published by the Home Office Scientific Research and Development Branch (SRDB) for operational use on raw wood [15] was as follows.

2.5 Mix 10 g of silver nitrate with 500 mL of methanol. Immerse article in solution for a maximum of 5 seconds and allow to dry in the dark. Illuminate article and continue exposure until the background starts to darken.

SILVER NITRATE developed fingerprint. Inset: illuminating to develop fingerprints

Development of fingermarks using silver nitrate.

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3. Reasons the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 CAST did recommend and issue the silver nitrate process in the first edition of the *Manual of Fingerprint Development Techniques* [15], primarily as a process for the development of fingermarks on light coloured, raw wood. It was withdrawn from the manual in subsequent editions [14] because it was considered that physical developer was equally as effective in this application and had no issues associated with progressive darkening of the background on exposure to light.

3.2 On paper items, silver nitrate can develop additional marks if used sequentially after ninhydrin because it is targeting different constituents in the fingermark deposits. However, chlorides are more affected by moisture and high humidity conditions than many other fingermark constituents and silver nitrate cannot be used on items that have been wetted. For this reason, physical developer is the preferred method for sequential treatment after ninhydrin because it targets different constituents and can be used on wetted items.

3.3 Silver nitrate has subsequently been reinstated in the *Fingermark Visualisation Manual* [16] as a Category B process, and a formulation and processing instructions are provided. It is recognised that there are possible niche applications for silver nitrate, in particular in the treatment of large areas of raw wood or cardboard which would be too difficult and/or time consuming to process with physical developer.

3.4 The reinstatement is based on work conducted by Mayse [17], comparing the effectiveness of silver nitrate and physical developer on a range of brown papers, cardboard and Kraft paper. Whole fingermarks from 26 donors were collected on 4 pages of each substrate. Split depletions were not used so as to prevent preferential silver deposition on the cut edges, which may leave a depleted solution for fingermark development. The fingermarks were stored in the dark for 4 days and 9 days respectively prior to treatment.

3.5 The range of substrates used in the experiment were:

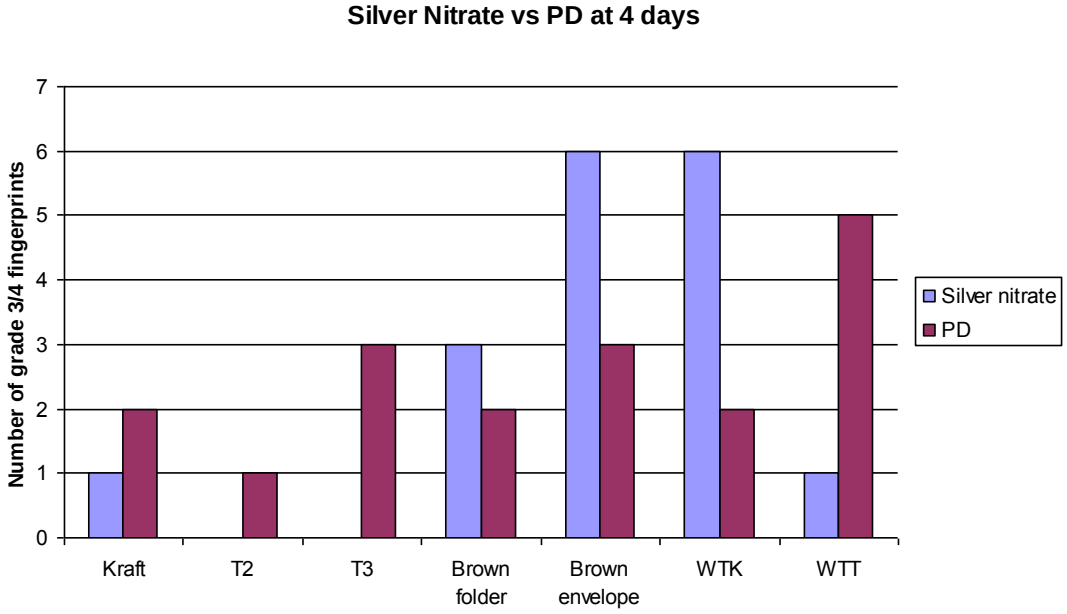
110 g/m^2 Kraft (brown, mostly virgin cellulose fibres)
120 g/m^2 Test 2 (high content of recycled fibres)
115 g/m^2 Test 3 (enitrely recycled fibres, recycled multiple times)
115 g/m^2 White Top Kraft (WTK)
125 g/m^2 White Top Test (WTT).
Brown envelope (kraft)
Brown envelope (manilla).

3.6 Physical developer was applied using the standard CAST method. The silver nitrate was applied as outlined in chapter section 2.5 and exposed to long wave ultraviolet radiation in 1 minute cycles until maximum

contrast was achieved between the fingerprint and background. The fingerprints were then graded immediately in white light.

3.7 The samples that were aged for 9 days prior to treatment were then stored for a further 7 weeks after being graded. The physical developer samples were then processed with silver nitrate, allowed to dry and exposed to long wave ultraviolet radiation for 1 minute and then natural light for a further 2 days before being graded under white light. The silver nitrate samples were then processed with physical developer, left to dry and then graded under white light.

3.8 The results suggest that physical developer was the most effective technique on four of the seven surfaces whilst silver nitrate was the most effective on the remaining three surfaces. For the 4 old fingermarks, only physical developer produced visible fingermarks on the T2 and T3 papers as the colour of the silver nitrate product was brown. This meant that there was little colour contrast between the developed fingermark and the background.



Results of comparative testing between silver nitrate and physical developer for 4 day old fingermarks on a range of brown and Kraft papers and cards [17].

3.9 For the aged 9 day old fingermarks the physical developer was the only technique to develop fingermarks on three of the brown paper surfaces (kraft, T2, T3), although silver nitrate developed a higher number of grade 3/4 fingermarks than physical developer on the brown envelope, WTK and WTT papers.

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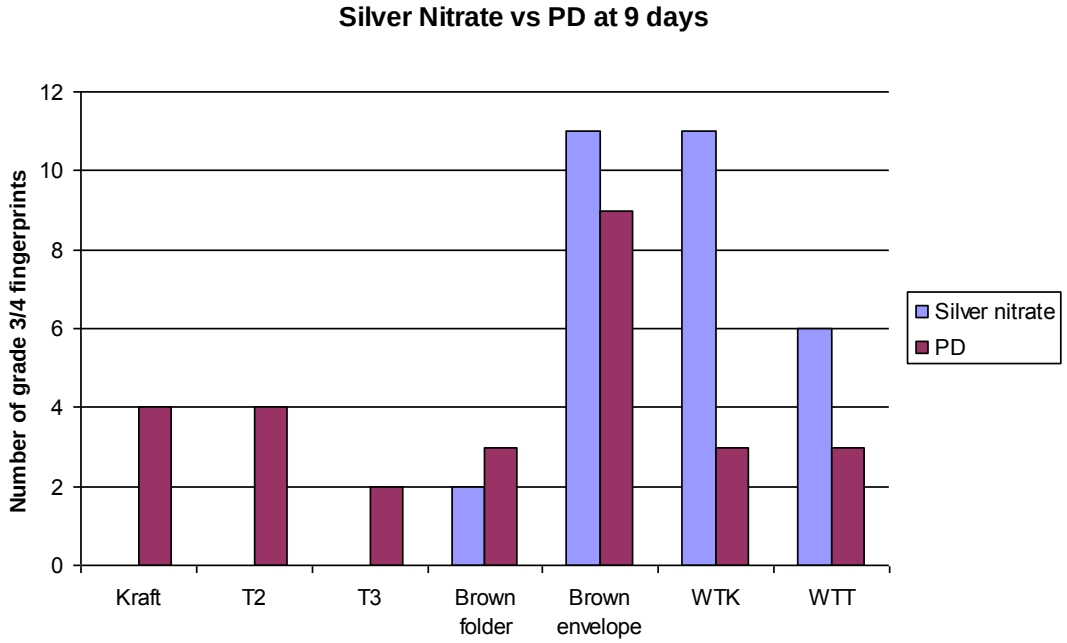

Results of comparative testing between silver nitrate and physical developer for 9 day old fingermarks on a range of brown and Kraft papers and cards [17].

3.10 No additional fingermarks were found with using either of sequences with silver nitrate and physical developer. However with the sequence of physical developer followed by silver nitrate the marks developed with physical developer were occasionally enhanced by silver nitrate producing clearer ridge detail on some fingermarks, with no detrimental effects on the background. Using physical developer after silver nitrate produces a uniform development of the background across the substrate, obscuring marks.

3.11 Although there is potential for the use of silver nitrate on certain types of papers used in the construction of cardboard boxes, and also on some brown envelopes, in general physical developer remains more the effective process across a wider range of substrates. However, silver nitrate could be more practical to use operationally if large areas require treatment and it is not practical to cut items into many smaller pieces that would fit in the physical developer processing baths.

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Small particle reagent

1. History

1.1 Small particle reagent (SPR) was first formulated in the mid-1970s by researchers at the Atomic Weapons Research Establishment (AWRE), Aldermaston, under a Police Scientific Development Branch (PSDB) contract. The objective of the contract was to devise a cheaper alternative to what was then termed surfactant 'stabilised physical developer (SPD)' (now known simply as physical developer) [1,2]. At the time, SPD was being investigated for the development of latent fingermarks on a range of surfaces, including plastics and paper, although it was recognised that the technique worked best on paper samples.

1.2 The SPD system was found to work by the deposition of silver particles, in the presence of a cationic surfactant, onto the surface being processed. Studies into this system showed that finely divided silver particles could also be used to develop latent fingermarks when prepared as a suspension, and that this behaviour was not exhibited when the suspension was dispersed in water alone, prompting studies into fine particle suspensions. This work indicated that the presence of the surfactant was essential if fingermarks were to be developed, and subsequent studies investigated a range of formulations incorporating different powders and surfactants. It was found that formulations containing powders with small particles of about 1 μm suspended in a fluid at concentrations of between 1 and 10 gL^{-1} were effective [2,3,4,5]. The generic name given to these systems was 'surfactant controlled SPR' and a provisional patent application covering such reagents was filed by Morris and Wells in 1976. A more comprehensive study of powders, surfactants and methods of application then followed [2,5].

1.3 Initial experiments showed that good results could be obtained using dish development with molybdenum disulphide (MoS_2) particulate and this was used as a control against which formulations based on alternative powders could be assessed [2,7]. These experiments identified the best performing powders as cobalt oxide(Co_2O_3), lead oxide (PbO_2), molybdenum disulphide (MoS_2), graphite and the pigment Monastral Blue (copper phthalocyanine), although for some of these there was a large batch-to-batch and supplier-to-supplier variation. In this respect MoS_2 was found to be the most consistent in performance across all batches tested. It was also found that all 'ionic' types of surfactant evaluated gave good results, but that poor results were obtained when the surfactant molecule had a 'tail' of fewer than eight carbon atoms (C_8) [2]. On the basis of these studies, a combination of Tergitol 7 (3,9 diethyl-6-tridecanol hydrogen sulphate sodium salt) and choline chloride was selected as the surfactant solution, although it was subsequently found that the latter constituent was unnecessary and it was omitted from the SPR formulation initially recommended for operational use. Subsequently, Tergitol 7 became unavailable because

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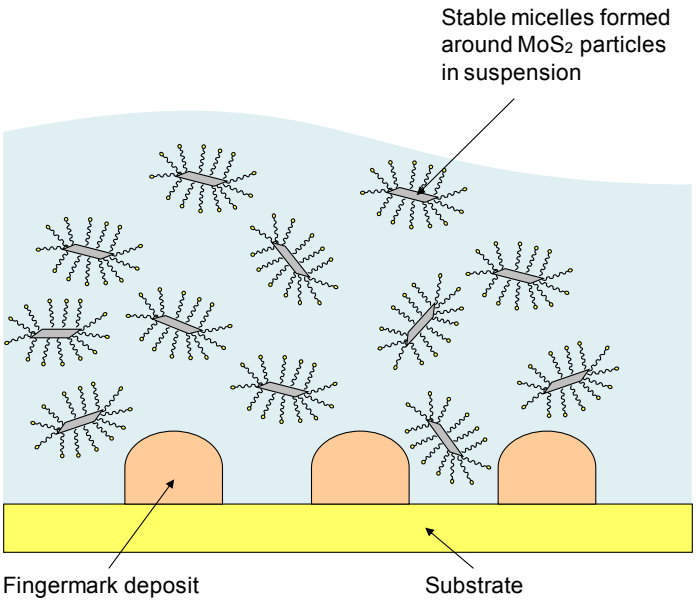
of the harmful impact it could have on the environment, and a revised formulation was developed by the Home Office Scientific Research and Development Branch (SRDB) based on Aerosol OT (AOT) surfactant. It is this formulation that is recommended for use in the UK to the present day (2016).

1.4 SPR dish development was trialled operationally against vacuum metal deposition (VMD) for the development of marks on polythene bags [8]. This trial indicated that although SPR was not as effective as VMD for this type of surface, it was far more effective than powdering and the technique was recommended for operational use on non-porous surfaces and wetted items because it was recognised that few police forces had access to VMD.

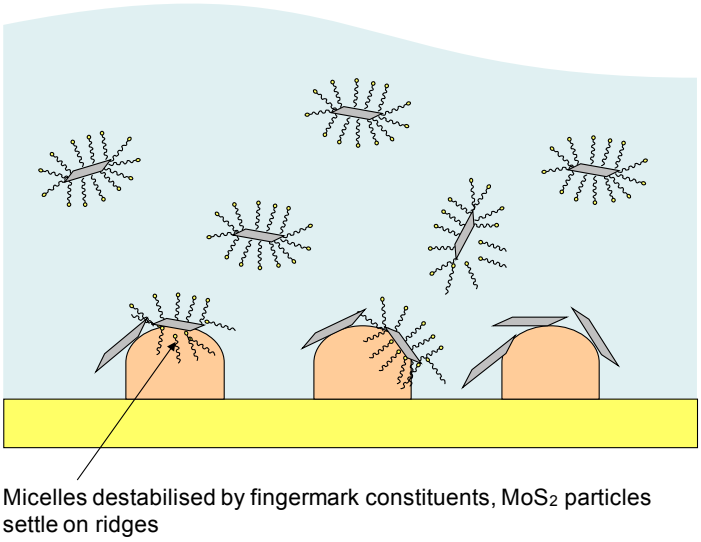
1.5 Work by the Home Office Central Research Establishment (HO CRE) in the early 1980 suggested that spraying of SPR was an effective method for cars which were wet and could not be readily dried (e.g. at scenes where it was still raining), and for other exterior wetted surfaces such as windows and window frames. SPR was found to be capable of developing marks on surfaces exposed to the outside environment for prolonged periods of time, e.g. window glass. SPR was found to detect marks that had not been developed during aluminium powdering [9], although the presence of excess quantities of aluminium powder on the surface were found to inhibit SPR [10]. Operational trials using SPR alone on wetted surfaces, and surfaces that were still wet, demonstrated that the technique was effective in such circumstances [10] and it was subsequently recommended for operational use. However, it is recognised that spray application of SPR is less effective than dish development, and that use at crime scenes should be restricted to surfaces that cannot be recovered to a laboratory. SPR remained the principal treatment for fixed outdoor surfaces that are known to have been wetted until the recent development of powder suspensions in 2009.

2. Theory

2.1 The mechanism by which SPR is thought to develop fingermarks is shown schematically in the illustrations below.



a)



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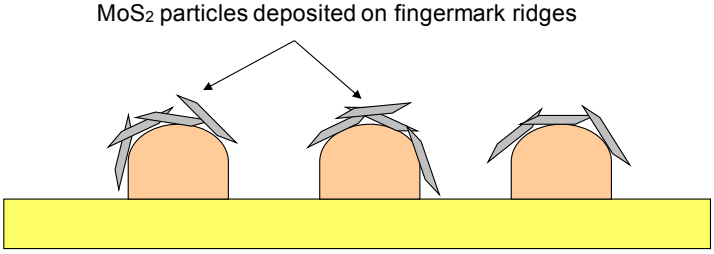


c)

Schematic illustration of the small particle reagent process a) *stable micelles formed around particles of molybdenum disulphide* b) *destabilisation of micelles by fingermark constituents leading to particles settling on ridges and* c) *dried mark, leaving particles adhering to ridges.*

2.2 The fine MoS_2 particles detect high molecular weight constituents and so preferentially adhere to the oily and fatty components of latent fingermarks by interaction between the fatty components present and the hydrophobic tails of the surfactant forming micelles around the particles. The marks are visualised by the dark grey particles preferentially binding to the ridges.

3. Centre for Applied Science and Technology processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The process recommended by CAST is first to prepare a concentrated solution by mixing 7.5 mL of 10% AOT (also known by its chemical name dioctyl sulfosuccinate, sodium salt, (DOSS)) solution with 500 mL of tap water, then add 50 g of MoS_2 powder. It may be difficult practically to prepare a 10% solution of DOSS, and therefore the 10% solution should be attempted as the starting point and small quantities of water added until all solids are dissolved. This concentrated solution is then further diluted according to the development process being used. If the dish development SPR process is required, 4.5 L of water are added to the concentrate and if the SPR is to be used for spray development 3 L of water should be added.

3.2 The role of the DOSS surfactant is to control the deposition of suspended particles onto fingermark ridges in preference to the background surface. The surfactant will form micelles around the suspended particles and although the nature (anionic, cationic, non-ionic) of the surfactant is not critical there are properties that were found to be favourable in surfactant selection:

- it must be suitably soluble to achieve the optimum working concentration;
- the 'tail' of the surfactant should have an open carbon atom chain with no fewer than C_8, with the optimum number of carbon atoms in the chain being between12 and 17.

DOSS meets both these criteria.

3.3 The concentration of DOSS used is again not critical but must be controlled to be below the critical micelle concentration (CMC), the optimum being between one-third and one times of the CMC. The concentration used in both CAST formulations falls within these limits. If DOSS concentration is below this limit, deposition of MoS_2 on the background surface increases and the definition of ridge detail is reduced, while at higher concentrations the clarity of the mark diminishes and at best only a very faint outline of the mark is observed. At these high concentrations little general deposition takes place, signifying that

micelle formation blocks the process of deposition, perhaps by providing a more attractive species for adsorption on the fingermark deposit.

3.4 The role of the MoS_2 is to deposit preferentially on the fingermark ridges and aid the visualisation of the mark. Several different materials can be used in this role, but in general the best results were obtained with materials with a density of ~4 gcm^{-3} and a layer lattice structure, both of which apply to MoS_2. There must be a sufficient quantity of MoS_2 in suspension for the particles to adhere to the fingermark ridges and give a clear mark. However, if the quantity is too great the powder also adheres to the background, giving background staining and development of smudged ridges. The quantity used in the CAST formulation is sufficient to give good development without background staining.

3.5 Uniform wetting of the powder by the DOSS surfactant is difficult to achieve if the powder is directly added to the working concentration solution of surfactant, so the MoS_2 should be added to a concentration greater than the CMC and after dispersion, diluted to the working concentration.

4. Critical issues

4.1 There are no critical issues relating to the application of SPR. The formulation is tolerant of changes in water content and made up solutions will keep indefinitely. In very cold weather additions of ethanol may be required for the spray application method to work effectively.

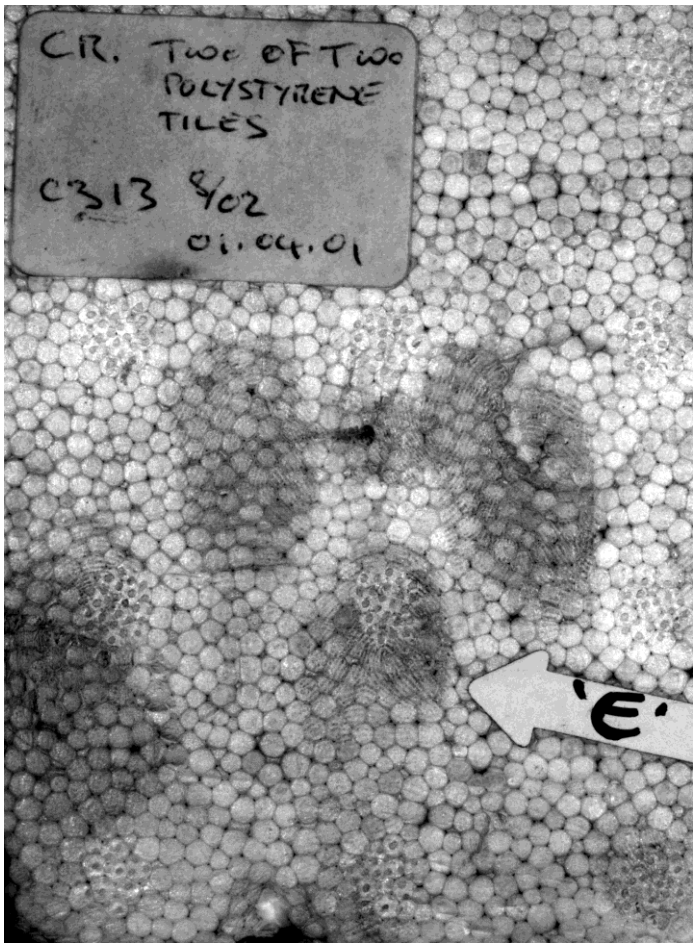
5. Application

5.1 Suitable surfaces: SPR is suitable for use on non-porous surfaces, such as plastic bags, glass bottles, waxed paper and other waxy items, such as candles. It can be used on expanded polystyrene items such as drinking cups. It will still develop marks on surfaces that have been wet, but is not suitable for surfaces that are heavily contaminated with grease, or blood .

5.2 SPR is a process recommended for use on non-porous articles that have been wetted. Because the process targets the insoluble lipid components of fingermark residues, immersion in water or exposure to rain will in many cases leave sufficient deposits for SPR to continue to develop marks. It is not as sensitive as VMD for this type of exhibit, but for the majority of police forces that do not have VMD equipment, SPR was until 2009 the only option for non-porous articles known to have been wetted. Tests have indicated that SPR may still develop additional marks if used in sequence before powder suspensions on wetted non-porous surfaces.

5.3 The two application techniques recommended for operational use are dish development and spray application. The dish development

technique can be applied to non-porous surfaces, such as plastic bags and packaging materials, waxed and plastic-coated paper, small gloss painted or glass articles and expanded polystyrene articles, such as drinking cups and ceiling tiles. Such items are difficult to treat with superglue, where uptake of the fluorescent dye by the expanded polymer makes any marks developed very difficult to visualise.



Fingermarks developed on expanded polystyrene tile using small particle reagent.

5.4 A tray or tank of sufficient size for the article being processed should be filled with sufficient working solution to enable the article to be submerged approximately 50 mm below the surface (giving a reasonable volume of suspension above the article for the particles to settle from). The working solution is then stirred to ensure all powder is in suspension before submerging the article with the surface of interest facing upwards. The article is then kept submerged and stationary for 30 seconds while the MoS_2 particles come out of suspension and settle evenly over the object. For small, complex shaped articles the article may be placed in a dish and the working solution poured over it from a beaker. The article is then removed carefully from the dish and the uniform grey deposit carefully washed off by placing the surface of interest face downwards into a second dish of water and agitating it gently. The article should then be dried at room temperature. The dish development technique limits the

size of the article that can be treated in the laboratory, but for use at scenes a formulation for spray application has been developed.

5.5 Spray application may be carried out on all non-porous surfaces, but it is recommended for objects that are outside, awkwardly shaped, large or immovable. Although wet or damp articles can be processed, when treating articles outside, the area being treated needs to be sheltered from direct rainfall.

5.6 For spray application, a simple, commercially available garden spray unit is used. The nozzle of the unit should be set to give a conical, fine spray and the filter unit removed to prevent it clogging. The working solution should be shaken to give an even particulate distribution and the area to be processed should be sprayed liberally, starting at the top edge and working down towards the bottom. As the liquid runs down the surface fingermarks may begin to become visible and spraying should be continued just above the relevant area until there is no more build up of the grey deposit. A second spray unit filled with water is then sprayed above the developed fingermarks before they have dried, allowing the flowing water to carry away excess particles. Prints should not be directly sprayed with water as this may damage them. In cold weather, 200 mL of ethanol may be added per 1 L of suspension to prevent freezing on the surface.

Spray application of small particle reagent to a car.

5.7 The spray formulation is much less effective than the dish formulation and should only be used where dish development is not possible.

5.8 Studies have shown that SPR has potential for developing fingermarks in specialist applications, such as on wetted firearms [11] and on incendiary bottles soaked in accelerant [12].

6. Alternative formulations and processes

6.1 Several other particles have been investigated as the basis of SPR. Some of those investigated in early studies [2] are summarised in the table below.

Material type	Compound	SPR performance
Metals	Silver powder	Good
	Zinc powder	Fair
	Aluminium powder	Fair
	Aluminium fingerprint powder	Fair
	Lead powder	Poor
	Copper powder	Poor
	Iron powder	Poor
	Manganese powder	Poor
Metal oxides	Iron (Fe_2O_3)	Good
	Cobalt (Co_2O_3)	Excellent
	Chromium (Cr_2O_3)	Good
	Uranium (UO_2)	Good
	Lead (Pb_3O_4)	Poor
	Lead (PbO_2)	Excellent
	Manganese (MnO_2)	Good
	Silver (Ag_2O)	Fair
	Copper (CuO)	Good
Metal sulphides	Zinc (ZnS) Batch 1	Excellent
	Zinc (ZnS) Batch 2	Poor
	Molybdenum (MoS_2) Batch 1	Excellent
	Molybdenum (MoS_2) Batch 2	Good
Other	Tungsten carbide (WC)	Good
	Silicon carbide (SiC)	Good
	Titanium boride (TiB_2)	Poor
	Carbon (amorphous)	Good
	Carbon (graphite)	Excellent
	Monastral Blue	Good

Summary of compounds investigated as the basis of small particle reagent.

6.2 As described above, MoS_2 was ultimately selected because it gave good performance and was more consistent in performance across different batches and different manufacturers. A further series of powders

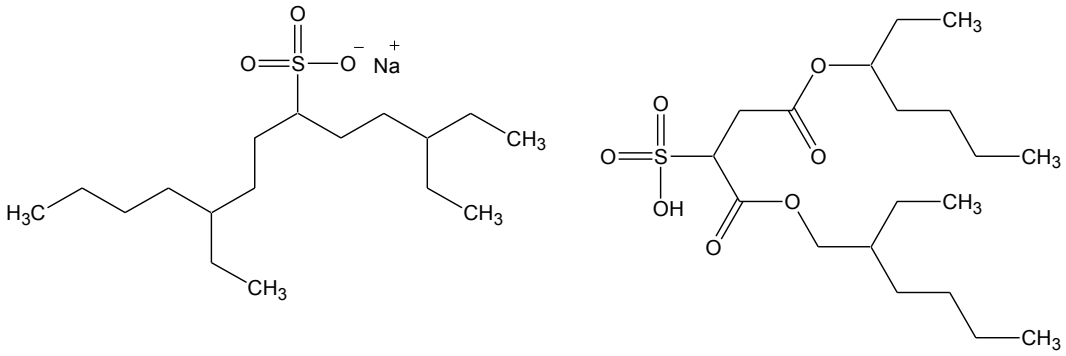
including boron nitride, cadmium sulphide, cadmium selenide, kaolin, molybdenum carbide, silicon nitride and tungsten sulphide were subsequently investigated [13] but none were found to give better performance.

6.3 In addition to the particulate component, a range of different surfactants were investigated [2]. These are also summarised below.

Surfactant			Performance
Name	**Chemical name**	**Ionic type**	
Teepol 610	Sodium lauryl sulphate	Anionic	Good
Teepol 514	Sodium lauryl sulphate	Anionic	Good
–	Sodium lauryl sulphate	Anionic	Good
Teepol Green	Sodium lauryl sulphate	Anionic	Good
Tergitol	Heptadecyl sulphate	Anionic	Excellent
Manoxal 1B	Dibutyl sodium sulfosuccinic acid	Anionic	Very poor
Manoxal OT	Diacetyl sodium sulfosuccinic acid	Anionic	Good
Armac 12D	Lauramine acetate	Cationic	Fair
-	Lauramine acetate	Cationic	Fair
Choline citrate	Trimethyl 2 hydroxy ethyl amine citrate	Cationic	Poor
Choline chloride	Trimethyl 2 hydroxy ethyl amine chloride	Cationic	Poor
Hyamine 2389	Methyl, dodecyl benzyl trimethyl amine chloride	Cationic	Good
Hyamine 1622	Di isobutyl phenoxy ethoxy benzyl amine chloride monohydrate	Cationic	Excellent
Brij 35	Phenoxy ethylated lauryl alcohol	Non-ionic	Excellent
Lissapol NDB			Fair
Lissapol D	Sodium acetorley sulphate		Fair
Lissapol LS	Sodium N octyl amino sulphonic acid		Fair
Flow 7X	Unknown		Good
Photoflo	Unknown		Good

Summary of surfactant systems considered for use in small particle reagent.

6.4 A further range of surfactants were subsequently studied [13] including Nonidet P40, Triton GR-5, Triton X405, and the series of Tween surfactants 85, 80, 40, 20. Manoxol OT (another trade name for DOSS) gave the best performance and ultimately replaced Tergitol 7 in the operational formulation when the latter surfactant became unavailable.



The structures of Tergitol 7 ($C_{17}H_{35}NaO_3S$) and Aerosol OT ($C_{20}H_{37}NaO_7S$).

6.5 More recently, there has been much interest in the use of powder suspensions for the development of fingermarks on the adhesive side of tapes. These have similarities to some of the formulations evaluated for SPR, albeit with far higher solids content, and are available in black (with carbon or iron oxide particulates) and white (with titanium dioxide particulates) forms. It was found that some of these formulations worked very well on non-porous surfaces and results led to them superseding SPR in this application on most types of surface. A detailed description of these formulations is given in Chapter 3, Chemical and Physical Processes, Powder suspensions.

6.6 SPRs based on other particulates have been reported, including light coloured zinc carbonate [14] and fluorescent particles [15]. Commercial, pre-mixed formulations are also available in various colours. The relative effectiveness of these formulations has not been tested by CAST against the recommended process, and in the case of the commercial pre-mixed products the nature of the filler particles is not known.

7. Post-treatments

7.1 Once entirely dry, marks developed using SPR are essentially the same as a mark developed by a regular powdering technique and can therefore be lifted in the same way by low-tack, clear adhesive tapes [3,11]. On occasions the lifting tape may not adhere to the surface very well, so care must be taken not to let the tape slip when lifting the developed mark. Lifting fingermarks is especially useful when dealing with highly patterned and/or coloured surfaces, however damage may be caused to the mark during lifting and the priority should be to photograph the mark in situ first.

8. Validation and operational experience

8.1 Laboratory trials

8.1.1 CAST has carried out few laboratory trials of SPR because the formulation was developed by the Atomic Weapons Research Establishment AWRE and HO CRE, and until recently there has been no other process for treating fixed, outdoor surfaces that have been wetted, to carry out a comparison with. A small-scale study using split depletion series was carried out in 1992 when the surfactant was changed from the discontinued Tergitol 7 to DOSS [16]. This test used five different donors, each depositing five marks in a depletion series on three different plastics. These results, and the grading scheme used, are summarised below.

1 = no obvious development
2 = print area visible but poorly defined ridge structure
3 = some clear ridge structure
4 = useful mark

Grade	**John Lewis white plastic bag**		**Sainsbury's white plastic bag**		**Clear plastic**	
	Tergitol 7	DOSS	Tergitol 7	DOSS	Tergitol 7	DOSS
1	4	4	2	9	0	2
2	5	4	7	2	2	7
3	9	12	7	5	8	6
4	7	5	9	9	15	10

Results of comparative studies on plastic bags using different small particle reagent formulations (numbers represent number of developed marks assessed as being of that grade).

8.1.2 It can be seen that the DOSS formulation is slightly less effective than the Tergitol 7-based formulation, but a range of equivalent tests carried out using different surfactants showed that DOSS was the best performing Tergitol 7 replacement and it was therefore incorporated into the revised formulation for operational use.

8.1.3 During preparation of the *Fingermark Visualisation Manual*, the effectiveness of SPR was compared to a range of other processes for the enhancement of fingermarks on expanded polystyrene [17] and on waxed surfaces [18], these being surfaces where SPR was still thought to offer advantages over powder suspensions.

8.1.4 Taylor [17] used marks deposited on three different types of expanded polystyrene materials: bulky packaging polystyrene commonly used to protect large household items such as fridges and washing machines, thin polystyrene veneer used to reduce condensation and provide additional insulation to walls, and the outside surface of a takeaway food

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carton. In this study, 6 different donors deposited depletion series of 10 natural marks, which were then aged for 2, 8, 14 and 28 days.

8.1.5. A preliminary study was conducted in order to downselect processes for use in the main comparison. These included superglue fuming followed by a range of different enhancement methods, and different formulations of powder suspension.

Technique	Abbreviation
Small Particle Reagent	SPR
Iron Based Powder Suspension (Home Office)	HOSDB PS
Iron Based Powder Suspension (Scottish Police formulation	SPSA PS
Carbon Based Powder Suspension	Carbon Black
Superglue (followed by Basic Yellow 40 dye – ethanol based)	SG + BYE
Superglue (followed by Basic Yellow 40 dye – water based)	SG + BYW
Superglue (followed by Basic Red 14 dye)	SG + BR
Superglue (followed by magnetic powder)	SG + MP
Magnetic Powder	-
Sudan Black B (solvent black 3)	Sudan Black

Processes used in the preliminary investigation on expanded polystyrene [17]

8.1.6 Six processes (superglue fuming followed by enhancement with either basic yellow 40 or magnetic powder, two formulations of iron oxide-based powder suspension, magnetic powder and SPR) were brought forward into the main trial and were used to develop fingermarks which had been aged for 14 and 28 days. The developed marks were graded and the effectiveness of the process assessed in several ways, the most operationally relevant being the number of marks given a grade that would be compatible with easy identification.

Rank	Technique	No. Grades 3 or 4
1	SG + BYE	108
2	SG + MP	95
3	HOSDB PS	4
4	SPSA PS	3
5	Magnetic powder	2
6	SPR	0

The effectiveness of a range of processes on bulky expanded polystyrene surfaces.

Rank	Technique	No. of Grades 3 or 4
1	SG + MP	153
2	SG + BYE	85
3	SPSA PS	27
4	Magnetic Powder	11
5	HOSDB PS	4
6	SPR	0

The effectiveness of a range of processes on thin expanded polystyrene veneer surfaces.

Rank	Technique	No. of Grades 3 or 4
1	SG + BYE	281
2	HOSDB PS	185
3	SG + MP	182
4	SPSA PS	135
5	Magnetic powder	21
6	SPR	20

The effectiveness of a range of processes on takeaway carton surfaces

8.1.7 The results indicate that superglue fuming followed by an appropriate method of enhancement is the most effective process for expanded polystyrene, and SPR performs relatively poorly. However, during the parallel study on waxed surfaces [18] it was observed that results with SPR were significantly improved by leaving the particles to settle from suspension for 2 minutes rather than 30 seconds and this study will need to be revisited. The decision was made to leave SPR in the processing chart for expanded polystyrene, in sequence with powder suspensions (which is known to target different constituents) until this repeat study has been conducted.

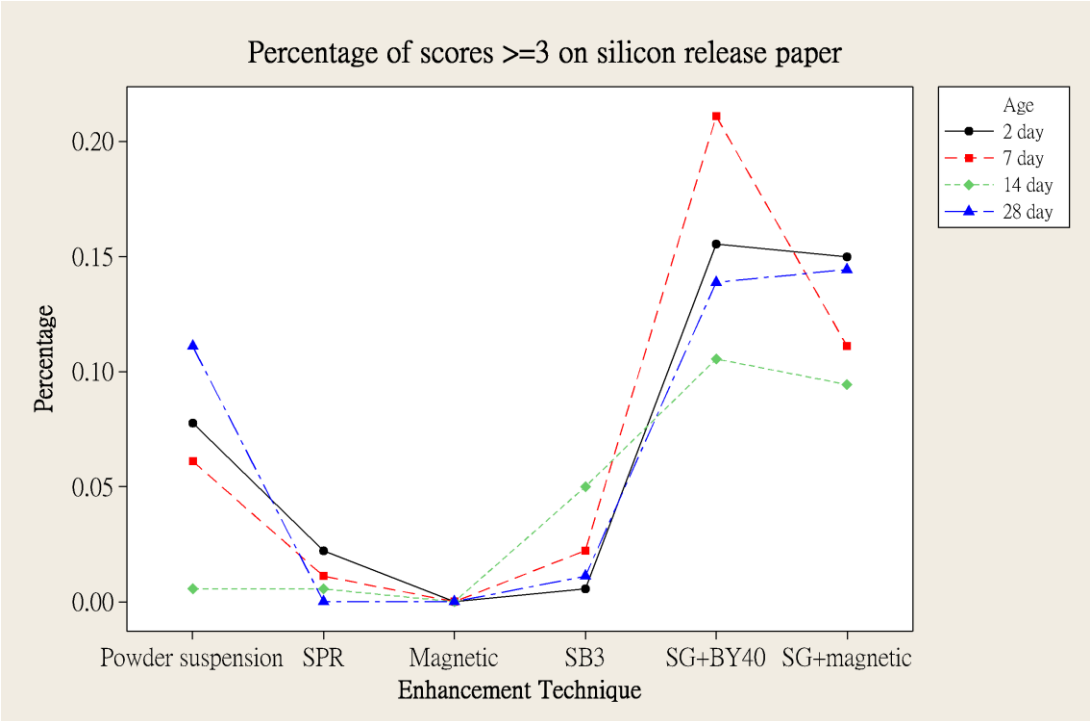
8.1.8 Pong [18] conducted a similar study using both waxed surfaces and alternatives such as papers with polymer coatings that have gradually replaced waxed papers in many applications. The following surfaces were tested: Silicone release paper, waxed paper, fruit juice cartons, candle wax (cast into flat plaques for fingermark deposition). Six donors were used to deposit 10 mark depletion series on each of the surface types, which were aged for 2, 7, 14 and 28 days before enhancement.

8.1.9 According to the *Manual of Fingerprint Development Techniques, 2nd edition,* [19] used as reference in the studies, the development time for SPR should be 30 seconds, and the surface should be rinsed after development. However, it was noticed in a preliminary experiment that 30 seconds did not appear to be enough time for development, and that rinsing caused developed fingermarks to be washed off. Thus, in the full experiment, the development time was increased to 2 minutes, and the surfaces were not rinsed after development.

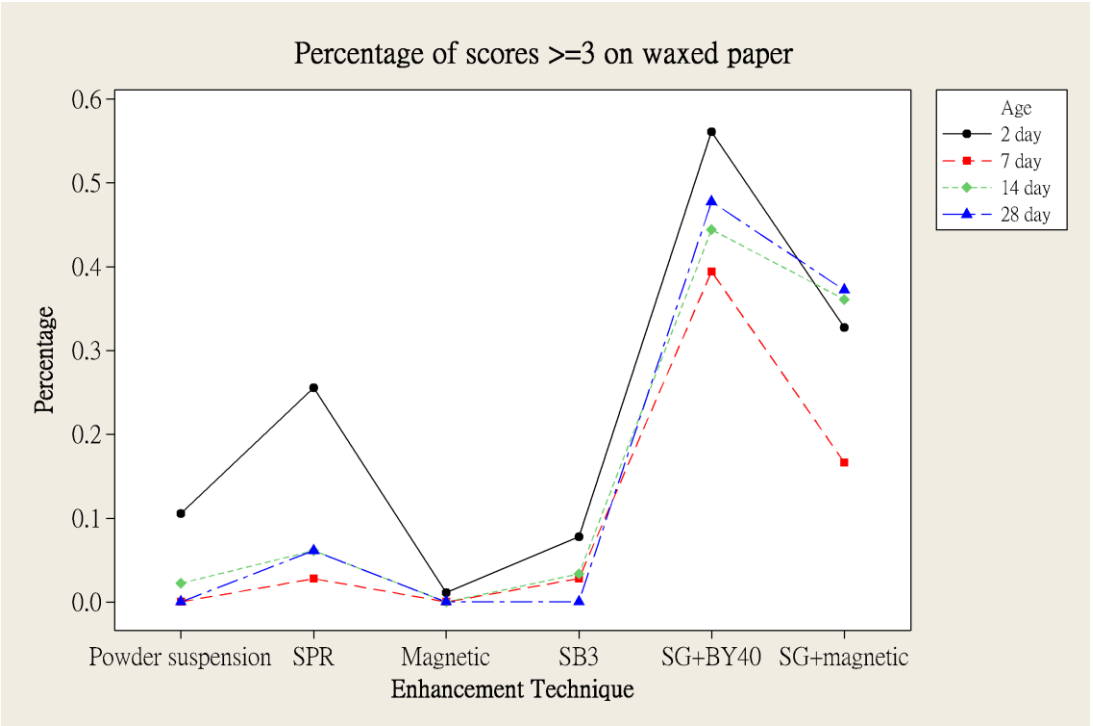
8.1.10 Six processes were used in the full experiment:

- CAST formulation powder suspension
- Small Particle Reagent (SPR)
- Magnetic powder
- Solvent Black 3 (SB3)
- Superglue fuming enhanced with ethanol based (waxed papers and candles) or water based (juice cartons only), BY40 (SG+BY40)
- Superglue fuming enhanced with magnetic powder (SG+Magnetic)

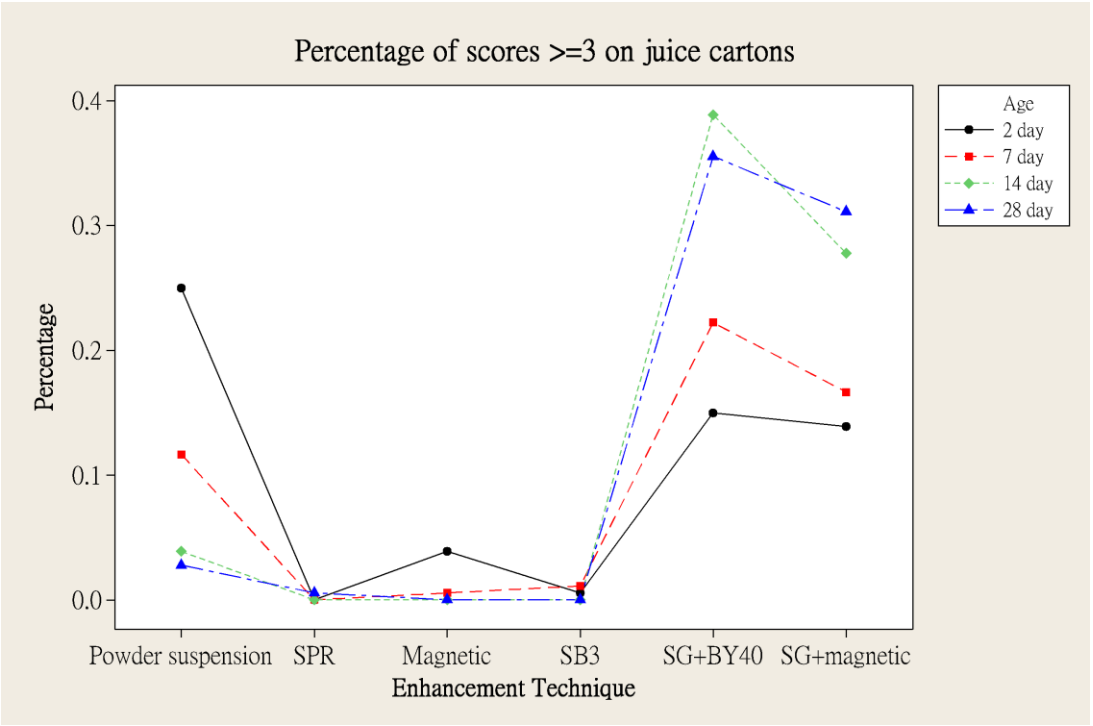
The developed marks were graded and the effectiveness of the process assessed in several ways, the most operationally relevant being the number of marks given a grade that would be compatible with easy identification.



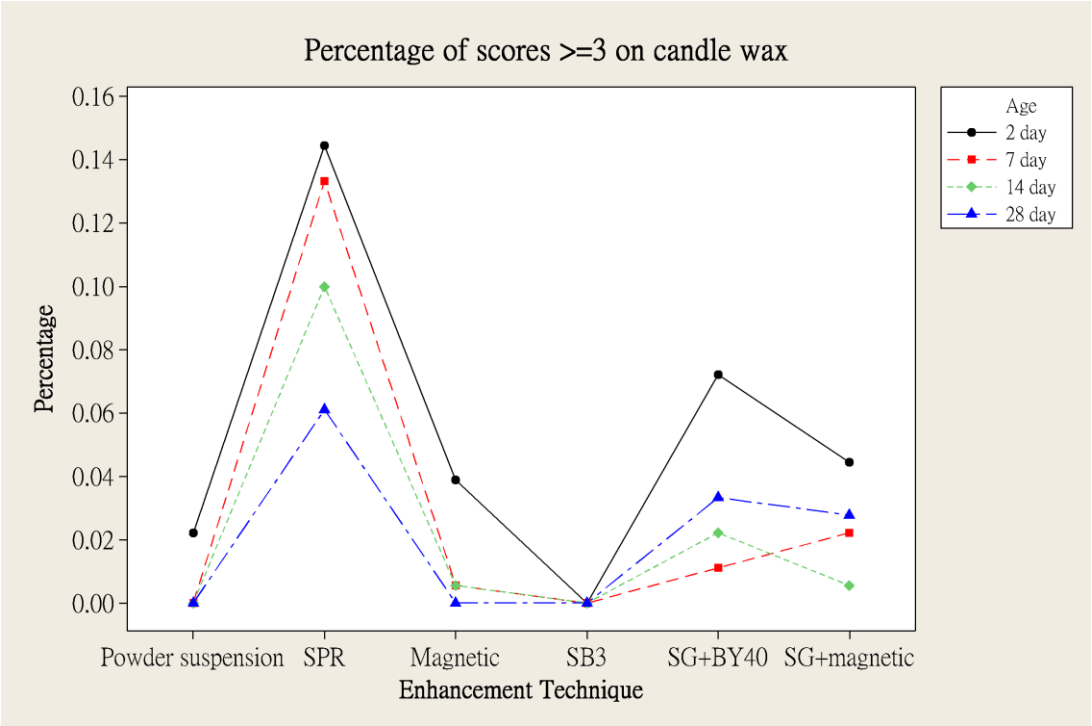
Proportion of developed marks graded 3 or 4 for all processes and ages of mark on silicone release paper (polymer coated).



Proportion of developed marks graded 3 or 4 for all processes and ages of mark on waxed paper (wax coated).



Proportion of developed marks graded 3 or 4 for all processes and ages of mark on fruit juice cartons (polymer coated).



Proportion of developed marks graded 3 or 4 for all processes and ages of mark on candle wax.

8.1.11 The results show that for all polymer-coated surfaces, superglue fuming followed by enhancement with basic yellow 40 or magnetic powder gives the best results. If the surface is truly wax-based, SPR gives good results and would be the process of choice on wax surfaces if they are known to have been wetted. It is observed that the effectiveness of SPR drops off rapidly with the age of the mark and this would need to be taken into account when selecting the most appropriate process.

8.2 Pseudo-operational trials and operational experience

8.2.1 HO CRE carried out several trials before implementing SPR. In the initial investigation, SPR was compared with powders and VMD on paper, polythene and window glass surfaces [9]. On paper SPR gave reasonable results, but it affected subsequent ninhydrin treatment and therefore could not be used in sequence. On polythene, SPR was shown to be capable of developing marks on polythene that had been exposed to the environment (including rain), but VMD gave better results. This observation was confirmed in a full operational trial [8], the results of which are summarised in Chapter 3, Chemical and Physical Processes, Vacuum metal deposition. On window glass SPR gave similar performance to aluminium powder on the inside surface. However, on the outside, which had been exposed to autumnal weather conditions for two weeks, SPR gave significantly improved performance and could be used in sequence after powders.

8.2.2 An operational trial was then conducted over two winter months using three police forces, spraying SPR after powdering [10]. These initial trials gave poor results, which were attributed to excessive application of aluminium powder inhibiting SPR, and therefore sequential processing was not recommended at scenes. A second phase of the operational trial was carried out over two months using four police forces, spraying SPR to surfaces that had not been previously powdered. During this trial 106 outside surfaces were examined and 55 useful marks recovered from 24 of the surfaces. Of these surfaces, five were examined while still wet (something not possible with powders) and seven useful marks were recovered. SPR was therefore recommended for use on wet or damp surfaces and at scenes of crime on articles where powdering is not feasible.

8.2.3 With the development of the superglue process (see Chapter 3, Chemical and Physical Processes, Superglue), the effectiveness of SPR was compared with that of superglue and VMD in a pseudo-operational trial on polythene bags. The results are reported in detail in Chapter 3, Chemical and Physical Processes, Vacuum metal deposition and indicated that SPR was less effective than superglue followed by dyeing and VMD on this type of surface, in accordance with earlier studies.

8.2.4 However, until reasonably recently (2009) SPR remained the process of choice where non-porous exhibits had been wetted and were either not portable or could not be treated with VMD. In the last few years it has become apparent that powder suspensions give superior performance to SPR on most surfaces studied, and advice in the *Fingermark Visualisation Manual* has been updated to reflect this change in recommendations. The only surfaces for which SPR is included in the processing chart are expanded polystyrene (where further work may lead to it ultimately being removed), and waxed surfaces, where it is possibly the most effective process.

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Thermal development

1. History

1.1 Thermal development of fingermarks is a phenomena that was first observed as an fortuitous consequence of the exposure of paper items to heat [1,2], with the paper selectively darkening in the region where fingermark ridges were present. This effect was most commonly observed close to the charred edges of burnt paper, where the mark had been exposed to high temperature but had not become highly discoloured [2]. It was also observed that even in situations where the paper had become charred over its entirety, fingermarks could still be detected by infrared photography [1].

1.2 The first researchers to consider controlling the application of heat to visualise fingermarks were Almog and Marmur [3]. Again, fingermarks were visualised by the application of heat (greater than 100°C) because they darkened more rapidly than the surrounding paper. Later researchers observed that before fingermarks darkened sufficiently to become visible, they became fluorescent and could be visualised by means of fluorescence examination [4,5,6] using illumination in the blue-green to green region of the spectrum. Exposure to heat for longer periods of time resulted in the dark, visible marks observed by previous researchers.

Fingermarks on lined notepaper visualised by thermal development, (left) *mark heated until it darkens more than the paper substrate,* (right) *mark treated to the point that it begins to fluoresce.*

1.3 More recently it has been observed that more gentle heating (less than 80°C) can be used to visualise fingermarks on thermal papers. In this application, the fingermark ridges selectively darken more rapidly than the thermal paper background to visualise the mark [7,8]. In this form of thermal development no fluorescence is observed.

1.4 Commercial equipment, the Foster & Freeman TFD-2 and the Consolite Forensics Hot Print System (HPS) has subsequently been manufactured so

that both types of thermal development process can be easily applied in laboratories.

2. Theory

2.1 The theory associated with thermal development on conventional paper substrates has not been conclusively established. Song *et al.* [6] attribute thermal development to the presence of the fingermark locally changing the thermal properties of the paper, resulting in these regions heating more rapidly than the paper substrate and thus discolouring more rapidly by thermal degradation. An alternative theory is proposed by Dominick *et al.* [5], who ascribe the fluorescence and subsequent visual discolouration observed to the degradation of the amino acid constituents of the fingermarks. The thermal degradation of amino acids in fingermarks has been studied [9], but the formation of fluorescent degradation products under the conditions used for thermal development has not been conclusively proven. It is known that amino acids can bind to the cellulose molecules in paper; it may be the degradation products of these more complex structures that are ultimately responsible for the fluorescence observed. More research is required in this area.

2.2 The visualisation of marks on thermal papers is attributable to the darkening of the active layer in the thermal paper. Whether this darkening is due to a heat-initiated chemical reaction between the fingermark constituents and the chemicals in the active layer, or whether it is due to the presence of the fingermark concentrating the heat into the regions of the fingermark ridges and darkening the thermal layer, has not yet been established.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Thermal development is not currently (2016) recommended as a Category A process in the *Fingermark Visualisation Manual* because none of the commercially available systems have yet been sufficiently evaluated by the Centre for Applied Science and Technology (CAST) to give definitive advice about their operational use. Initial testing of the two commercially available systems (the Foster & Freeman TFD-2 and the Consolite Forensics HPS) indicates that, in general, as a single process they are less effective than the alternative chemical treatments that are available for paper/thermal paper. However, both processes can be conducted without the need to apply chemicals to the substrates. In the case of the development process for thermal papers, there is minimal impact on subsequent processes and it may be capable of visualising sebaceous marks not found by amino acid reagents. The short studies conducted on both systems are summarised below. Based on initial results, it is considered that there are niche applications for both types of commercial system and thermal development is included as a Category C process in the Manual.

3.2 The Foster & Freeman TFD-2 was evaluated by CAST in 2012. The TFD-2 consists of a flat bed that holds the paper, this is then 'scanned' underneath an infrared heating element. The user can control both scan speed and the temperature of operation, thus varying the level of heat and the time that the item being treated is exposed to the heat.

The Foster & Freeman TFD-2 system for the thermal development of fingermarks on flat paper items.

3.3 A series of short experiments were conducted to give an indication of the relative effectiveness of the TFD-2 system compared to amino acid reagents, and how the TFD-2 could fit into existing processing sequences for porous surfaces. These consisted of the following.

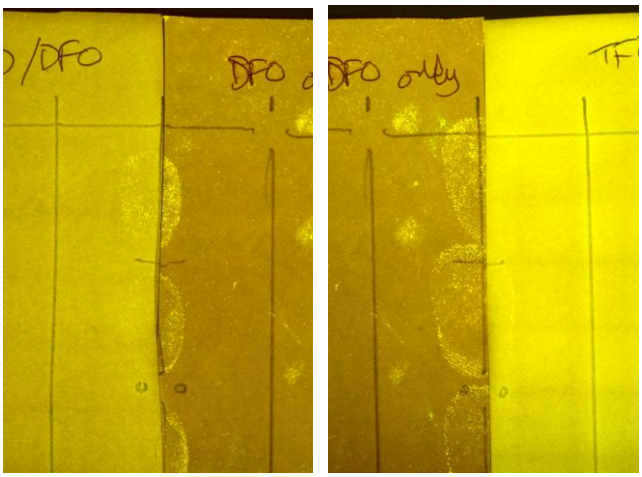
- A multiple donor study utilising single fingermarks deposited on different porous surfaces (white envelope, brown envelope, white printer paper, newspaper) by 30 different donors, producing 2 equivalent sheets for each surface type. One sheet of each pair was processed using a sequence of thermal development (TFD)-1,8-diazafluoren-9-one(DFO)-ninhydrin-physical developer, the other using the conventional DFO-ninhydrin-physical developer sequence.

- A split depletion experiment using a series of six fingermarks deposited on white printer paper, white envelope, brown envelope, newspaper and aged for one day prior to processing. A three-way comparison was conducted between TFD, DFO and the TFD-DFO sequence.

- A split depletion experiment with the processing speed progressively increased to investigate the effect of heating time on fingermark development.

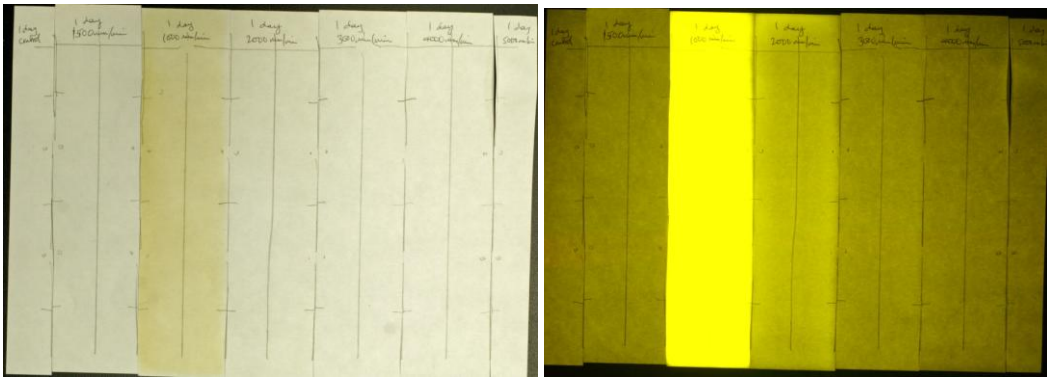
3.4 Observations made during these experiments were that multiple passes were required before a reasonable number of fingermarks were developed using TFD, and that marks were ultimately developed for approximately one third of donors. Significantly more marks were developed during the subsequent treatment with DFO (and ninhydrin), where marks were developed for nearly all

donors, indicating that the amino acid reagents are more effective than TFD if used as a single process. When used in sequences, the initial use of TFD resulted in reduced fluorescence from marks that were subsequently developed using DFO, and reduced colour intensity of marks subsequently developed using ninhydrin. However, initial results indicated that prior application of TFD may increase the contrast (and number) of marks subsequently developed by physical developer.



The results of a three-way comparison showing marks developed using (left) *the TFD-DFO sequence,* (centre) *DFO as a single process, and* (right) *TFD as a single process.*

3.5 It was also observed that increasing processing time resulted in greater visual discolouration of the substrate and also in an increase in its background fluorescence. This was observed for all porous substrates tested, and is noteworthy because there will come a point where the increased background fluorescence will begin to swamp the fluorescence produced by the thermally developed mark.



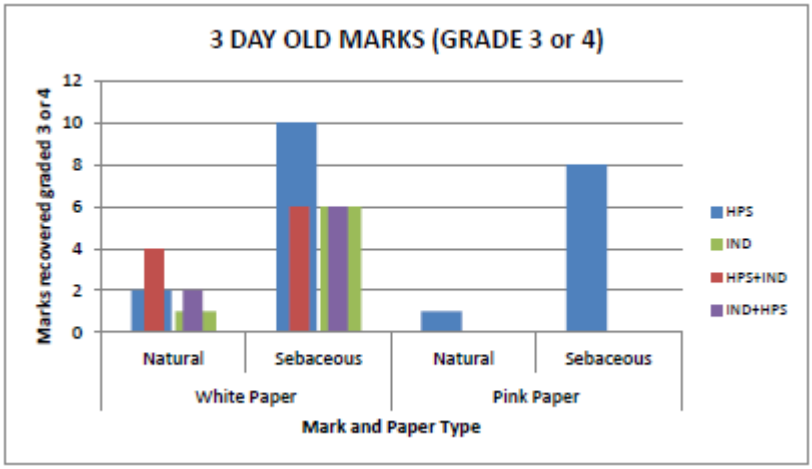
White printer paper exposed to different heating times (scanning speeds) in the TFD-2, (left) *under white light, and* (right) *under fluorescence examination.*

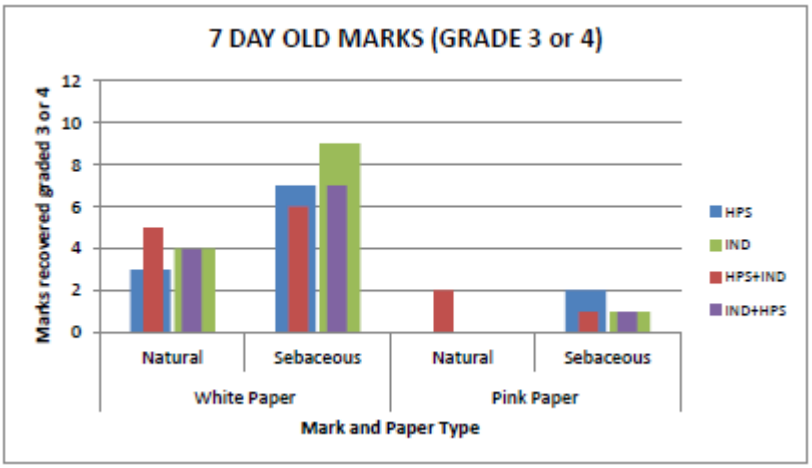
3.6 It was considered that the TFD-2 process may have niche applications, e.g. in situations where chemicals could not be used on paper items. However, more work would be required before firm recommendations can be made.

3.7 CAST conducted an evaluation of the Consolite Forensics HPS for the development of marks on thermal papers in 2014. Five donors deposited a depletion series of three natural marks and three sebaceous marks on white thermal paper and pink thermal paper (both supplied by Epson). Marks were left for three days before processing. One-half of each depletion was heated using the Consolite Forensics HPS while the other half was treated with a CAST indandione-zinc formulation, after removing the thermal layer with acetone. Papers were run a minimum of twice through the HPS before development was considered complete. Sequential processing was then investigated by treating the half processed using HPS with 1,2 indandione-zinc, and vice versa. Any visible marks were then re-graded. The experiment was then repeated with a different set of donors, with the samples being aged for seven days before processing.

White thermal paper with sebaceous marks processed with the HPS (left half) *and with 1,2 indandione-zinc* (right half) *and viewed under* (left) *white light, and* (right) *fluorescence examination with a green 532 nm laser.*

3.8 The number and grade of marks developed after each stage of the sequence for both natural and sebaceous marks was recorded.





Results obtained from experiments developing natural and sebaceous marks on thermal papers for the HPS system (HPS), 1,2 indandione (IND) and the two processes used in sequence [unpublished CAST project work].

3.9 Both experiments show that HPS is effective in developing sebaceous marks deposited on thermal papers, especially on the pink thermal paper used. The results of the relative effectiveness of HPS compared to 1,2 indandione-zinc are different in each experiment. This may be due to differences between the donors used in each experiment, but reinforces the fact that fingermarks vary significantly between donors and a more extensive study would be required to identify trends. Sequential processing of HPS followed by 1,2 indandione-zinc has shown to recover additional marks on white thermal paper, especially on natural marks, whereas the indandione-HPS sequence did not show any significant advantages. As for the TFD-2 system reported above, more work would be required before firm recommendations can be made regarding the operational use of HPS.

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Tagged nanoparticles

1. History

1.1 Because the term 'tagged nanoparticle' is a generic one that encompasses a range of different technologies, providing a definitive history is difficult. The types of nanoparticles described in this section are either functionalised to bind selectively to particular constituents of the fingermark, or adhere to it by conventional means. Once bound to the fingermark the nanoparticles provide a means of enhancing subsequent analytical techniques. A comprehensive review of nanoparticle technologies used for fingermark detection has been conducted by Bécue and Cantu [1].

1.2 Most of the functionalised nanoparticles that have been reported for fingermark detection utilise antibodies as the taggant, enabling highly specific binding to occur to fingermark constituents as opposed to the background. The use of antibodies, lectins and enzymes to target constituents of fingermarks was first reported by Pounds and Hussain [2] of the Forensic Science Service in the 1980s. Although successful in enhancing fingermarks during initial experiments, the process was not ultimately adopted for operational use. With the growing interest in nanoscale technologies and the production of a wide range of nanopowders in the 1990s and 2000s, the possibility of functionalising nanoscale powders with antibodies has consequently been considered by several research groups. Once functionalised, nanoparticles can be delivered to the fingermark by conventional means such as dusting, or by immersion in a liquid suspension. Nanoparticles have been reported for specific binding to fingermarks containing drug metabolites [3], proteins present in blood plasma and skin [4,5], amino acids [6,7] and body fluids [8]. Recently, work has been conducted towards the use of aptamers (i.e. oligonucleotides with binding properties similar to antibodies) as recognition probes to be attached to nanoparticles for the detection of fingermarks. DNA aptamer-nanoparticle conjugates have been successfully applied to fingermark imaging on several surfaces [9] as well as to the detection of drugs present in fingermarks [10].

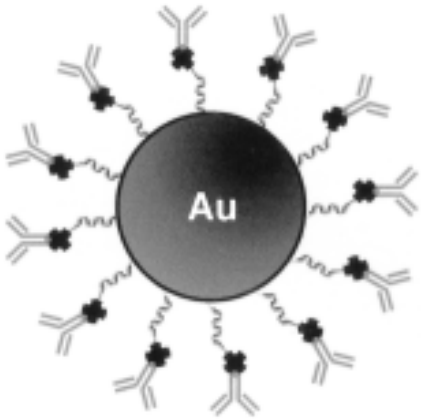
1.3 Other research groups have considered the use of nanoscale powders in place of conventional fingermark powdering methods. The potential advantage is that the nanometre scale of the powders provides better definition of the fingermark ridges than conventional powders, which have particles 10s or 100s of microns in size. By optimising the chemistry of the nanopowder, the particles adhering to the ridges can also play a role in enhancing analytical techniques subsequently used to obtain compositional information from the mark. This approach has been successfully used to identify drugs and drug metabolites in fingermarks developed by a nanopowder [11]. In other approaches, nanohybrid quantum dots have also been used as a functionalised fingermark powder [12]. Reseachers have formulated a powder that changes from fluorescing green when irradiated with ultraviolet under normal conditions to fluorescing red in the presence of TNT, thus giving the capability for the simultaneous identification of a person and information about what they may have been handling.

2. Theory

2.1 As outlined above, nanoparticles can be used for both fingermark enhancement and to obtain additional contextual information from fingermarks once they have been enhanced.

2.2 Surface modification of nanoparticles for fingermark detection involves the attachment of biomolecules, organic chain ligands or functional groups through different coupling strategies. Functionalisation is usually aimed at modifying the properties of the nanoparticles to improve their properties (e.g. stability, solubility, target specificity). Functionalised nanoparticles typically incorporate antibodies that give highly specific binding to chemical species present in the fingermark. The antibodies can be selected to bind to substances present in the fingermark, which may be substances that are naturally occurring but not generally targeted by other chemical processes, (e.g. dermcidin and albumin) or contaminants of operational interest (e.g. drugs and their metabolites).

2.3 The core of the functionalised nanoparticle is a nanometre scale particle of an inorganic material such as gold, iron oxide, titanium dioxide and silica. Antibodies (or any other biomolecule used as a detection moiety) will not bind directly to the nanoparticles, so the surface of the nanoparticle is first modified with linking molecules, and the antibodies subsequently attached to the linking molecule. Other 'building block' molecules can be added to the conjugates to impart other characteristics to the tagged nanoparticle, e.g. fluorescent or colourimetric properties, thus making it easier to detect once it has bound to the surface. The resultant nanoparticle can be described as being analogous to a sticky plant burr, but with the ends of the 'hooks' (i.e. the antibodies) only being able to attach to the parts of the fingermark containing the target analyte. Where these chemical analytes are not present, no binding occurs and the nanoparticles are either washed or brushed from the surface leaving it free of background staining. Because of this high specificity, it may be possible to design functionalised nanoparticles of this type to target substances of interest (e.g. to bind to human blood but not to surrounding regions of animal blood), thus giving greater discrimination than any other chemical technique.



Schematic diagram of a functionalised gold (Au) nanoparticle.

2.4 Where nanoparticles are initially used as a fingermark enhancing powder (or powder suspension) they bind to the fingermark through mechanisms similar to those outlined for conventional powders and powder suspensions, without the specificity achievable with functionalised nanoparticles. However, developing the mark is not the sole function of the powder; it can also be used as an enhancing medium for subsequent analytical techniques such as surface assisted laser desorption/ionisation (SALDI) and matrix assisted laser desorption/ionisation (MALDI). In these cases the composition of the nanoparticle is selected so that it strongly absorbs the incident laser energy and concentrates it into the fingermark. This increases the subsequent ionisation yield from the fingermark and improves the effectiveness of the analytical technique.

3. Reasons why the technique is not designated a Category A process by the Centre for Applied Science and Technology

3.1 Tagged nanoparticles are not recommended as a Category A process in the *Fingermark Visualisation Manual* because, although there are several different types of nanoparticles under development, many are at the early research stages and are not yet available in sufficient quantities for comprehensive evaluation. Some products have begun to reach the market, but they have not yet been evaluated by the Centre for Applied Science and Technology (CAST) in comparison to conventional powders. However, it is recognised that tagged nanoparticles do have potential advantages in terms of increased specificity, filling in ridge detail by targeting constituents not developed by conventional reagents, and/or by providing additional contextual information about the donor of the mark. As a consequence, it is considered that there are niche applications and therefore tagged nanoparticles are included as a Category C process in the Manual.

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Vacuum metal deposition

1. History

1.1 Vacuum metal deposition (VMD) is a long-established industrial technique for the application of metal coatings to components such as glass mirrors. In 1964, Professor S. Tolansky, working on the manufacture of interference filters at the Royal Holloway College of the University of London, noted that the deposition of silver in a vacuum system developed accidentally deposited latent fingermarks on glass optical components. An investigation into the process as a fingermark development technique was proposed to the Home Office by Professor Tolansky. However, this was not pursued at the time by the Home Office because other techniques for fingermark detection on glass were considered cheaper, easier to use, and sufficiently effective.

1.2 In 1968, it was reported by French workers [1] that VMD from a mixture of zinc, antimony and copper powder was capable of developing latent prints on paper. As a consequence of this paper, interest in the technique was revived in the UK and Tolansky initiated a research programme to investigate the optimum conditions and the potential applications for VMD. One of the early objectives of the research was to establish why the French combination of metals was effective. Closer examination of metal coatings deposited by the French laboratory indicated that the coating was almost entirely zinc, the presence of antimony and copper not being necessary to develop prints [2].

1.3 The research programme initiated by Tolansky [2] investigated the deposition characteristics of a range of metals on paper substrates, identifying single metals and metal combinations giving the optimum mark development. Research was also carried out into the ability of the technique to detect latent marks on fabrics. These experiments showed that although some mark development was obtained by the use of single metals, in general the best results were obtained by the use of a combination of metals, typically gold or silver followed by cadmium or zinc. The gold/zinc combination is currently (2014) used operationally.

1.4 The potential of VMD to develop fingermarks on fabrics was further explored by the Atomic Weapons Research Establishment (AWRE) under contract to the Home Office [3,4,5]. The work looked at identifying the best metal combinations for developing marks [3], transfer of both latent and developed marks onto photographic paper [3] , and the effect of humidity [3,4]. The researchers considered the effect of different washing and wearing conditions on mark survival [4] and expanded the study to look at synthetic fabrics [5]. It was considered that the chances of fingermarks surviving on washed and worn fabrics under ‘field’ conditions was small, but finite.

1.5 In the mid-1970s the increasing occurrences of low density polyethylene (LDPE) carrier bags in a variety of crimes, in particular Irish Republican

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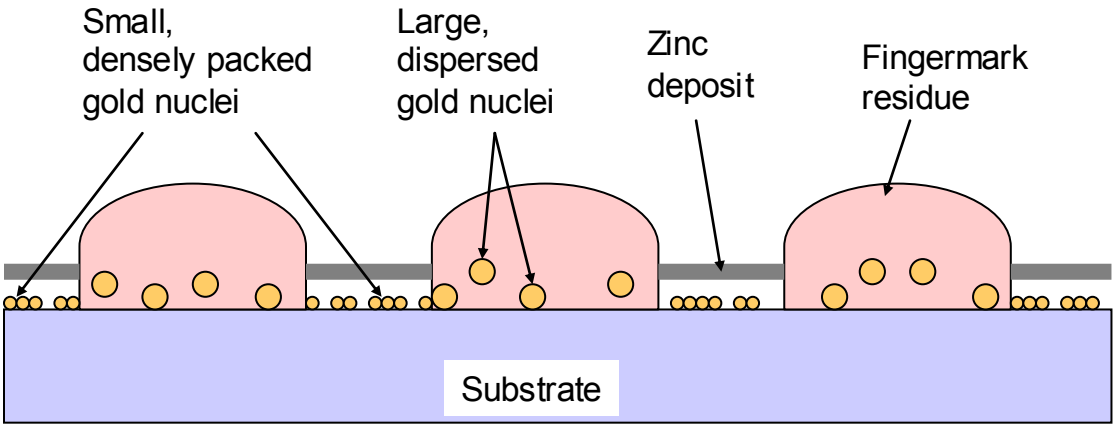
Army (IRA) improvised explosive devices (IEDs), led the Police Scientific Development Branch (PSDB) to look for better ways of developing fingermarks on polyethylene. A programme of work evaluating the various metal combinations on a variety of plastics and rubbers was set up and considerable success achieved using gold followed by cadmium on polythene and most plastics with the notable exception of plasticized PVC. This and all the early experimental work was carried out on small-scale equipment with 30 cm bell jar coaters. Silver-cadmium combinations gave slightly poorer results than gold-cadmium, with copper-cadmium less good. To make the system more viable for operational casework modification of horizontal 60 cm coaters was investigated and a system purchased by PSDB in 1976. This proved very successful during operational trials with police forces and over the next decade around 20 similar machines were installed [6]. Monitoring of the cadmium levels in the vicinity of the chamber and during cleaning operations from 1977 to 1978 indicated that figures approaching 10% of the maximum exposure level were being generated and around this time there was also a proposal to reduce the permitted exposure levels for cadmium (which was known to be toxic). Gold followed by zinc deposition was known to give similar results to gold-cadmium although deposition of zinc is slower and more difficult. The decision was made to switch to gold-zinc for all operational police systems as the maximum exposure limits for zinc were many times higher and there was no likelihood of these being exceeded in operational use.

1.6 From the late-1970s until the late-1990s PSDB worked with manufacturers and introduced a number of improvements including larger chambers, liquid nitrogen cold fingers and semi-automated sample loading systems. Over this time most suppliers had moved from manually operated valve systems to automated, or semi-automated, control systems [7]. Several trials were carried out by PSDB between VMD and other techniques for developing fingermarks on polyethylene including small particle reagent, superglue and fluorescence examination [8,9,10] and it was found to out-perform all of these techniques. In particular VMD was shown experimentally and operationally to develop fingermarks that had been exposed to extended water immersion, something that no other technique at the time (late-1970s and early-1980s) could cope with.

2. Theory

2.1 There is general agreement on the theory associated with normal development of prints by the VMD method. The reason that the metal combinations are postulated to work well is due to the condensation characteristics of zinc (and cadmium). These metals will not condense on grease, such as that found in fingermark residues, even when these substances are only present as a monolayer. However, zinc will deposit on small nuclei of metal, and this is the reason that gold or silver deposition is carried out first. Gold and silver can be deposited over the

entire surface, and begin to form nuclei, the morphology of which depends on the nature of the surface (surface energy, chemical species present) they are being deposited on. The resultant gold coating is very thin (several nanometres only) and discontinuous. However, in the regions coated with the fatty residues of the latent fingermark, the gold diffuses into fat and hence there are no gold nuclei close to the surface. As a consequence, when zinc is subsequently deposited, it will condense on the regions of gold nuclei (i.e. the background substrate), but not on the regions of the fatty deposit (i.e. the fingermark ridges). This theory of nucleation was discussed in more detail by Stroud [11,12]. It should, however, be noted that there is no conclusive evidence of nuclei diffusion and it is possible that the effect observed may be solely attributable to zinc growing on regions of different nuclei size at different rates. The normal development process based on nuclei diffusion is depicted in the schematic diagram and photographs below.



Schematic diagram of normal development, showing zinc depositing where gold nuclei are available on the surface.

Photograph of a normally developed mark on a polyethylene bag.

2.2 Tests carried out to determine which components of the latent mark were most likely to be responsible for inhibiting metal deposition identified several substances, including stearic acid, palmitic acid, cholesterol oleate, glycerol trioleate and amino acids L-arginine monohydrochloride, L-leucine, and DL-threonine. Most of these substances are non-water soluble or long chain fats or acids with low vapour pressure, which determines their stability and non-migration over the surface during the VMD process. These findings were in accordance with the observation that VMD was capable of developing marks on substrates exposed to wet environments, many of these substances being insoluble in water. In the late 1970s PSDB funded work at Imperial College, London using electron spectroscopy for chemical analysis (ESCA) and ion beam etching in order to establish the depth profile of gold on the surface. This was done to determine whether loss of gold nuclei into the fats of a fingermark would account for the 'normal' development of light ridges. Experiments to study the diffusion of gold into thin films of stearic acid [13] indicated that 60% of the gold penetrated the stearic acid to a depth greater than the detection depth of the ESCA surface analysis technique, and hence would probably not be sufficiently close to the surface for zinc to nucleate on it. The work was however regarded as inconclusive as although a slight increase in gold was detected during etching down

through the surface layers it was felt that this might have been due to 'knock on' effects of the heavy ion etching.

2.3 Transmission electron microscopy has also been used to confirm that the size and distribution of gold nuclei formed during the deposition process varied greatly according to the substrate and the chemical species present [14]. Transmission electron micrographs of gold films on carbon support grids which had some deposited LDPE crystals showed few large nuclei on the LDPE compared with large numbers of small nuclei on the carbon. This confirmed that a variation in nuclei size could be produced in areas with different binding energies. Rayleigh scattering from the gold films also showed changes in colour indicating variations in nuclei size. It was this difference in nuclei size and distribution, coupled with diffusion of gold into the fatty deposits that was believed to contribute to the subsequent delineation of the print during VMD.

2.4 In practice, many prints developed using VMD may be 'reverse developed', i.e. zinc preferentially deposits on the fingermark ridges rather than the background. There are differences in opinion as to why this arises, the main theories being outlined below.

2.5 Kent *et al*. [15] attribute reverse development to absorption of mobile species of the fingermark residue into the substrate, leaving a solid, primarily inorganic residue that acts as a preferential nucleation site for the zinc. More gold diffuses into the polymer substrate than into the solid residue, hence zinc deposits on the ridges first. Smith, as quoted by Jones *et. al.* [16,17,18]) proposed that zinc deposits on the ridges because it is able to align crystallographically with some of the crystalline constituents in the deposit (e.g. sodium chloride) and undergo epitaxial growth. Most recently, Jones *et al*. [16,17,18] have proposed an alternative theory related to the types of gold nuclei formed. The gold nuclei in the ridges form at a different rate to those growing on the substrate and in the furrows, hence a regime exists where the gold film on the background has reached a state where zinc cannot nucleate, but on the ridges the nuclei are a suitable size for zinc to deposit. This theory closely relates the type of print developed to the amount of gold deposited initially.

2.6 Current (2016) CAST thinking is that the reverse development is due to the fingermark deposits becoming dried out, either by air drying or by the preferential absorption mechanism outlined by Kent, or being contaminated, thus inhibiting diffusion of the gold nuclei into the fingermark residue. The dried ridge is likely to have a higher surface energy than the background and therefore larger gold nuclei will form in these regions. These larger gold nuclei will sit on the surface of the ridge because their diffusion is inhibited and because the gold nuclei in the region of the ridges are larger, zinc deposition occurs at a faster rate. This is illustrated schematically below.

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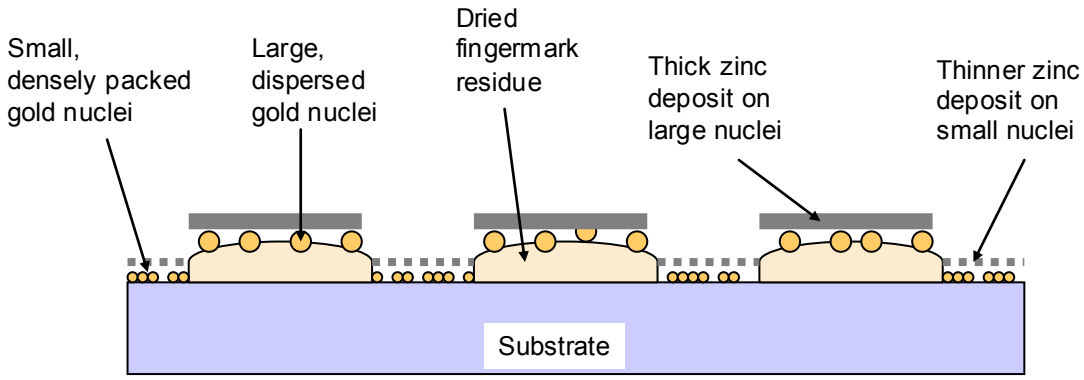
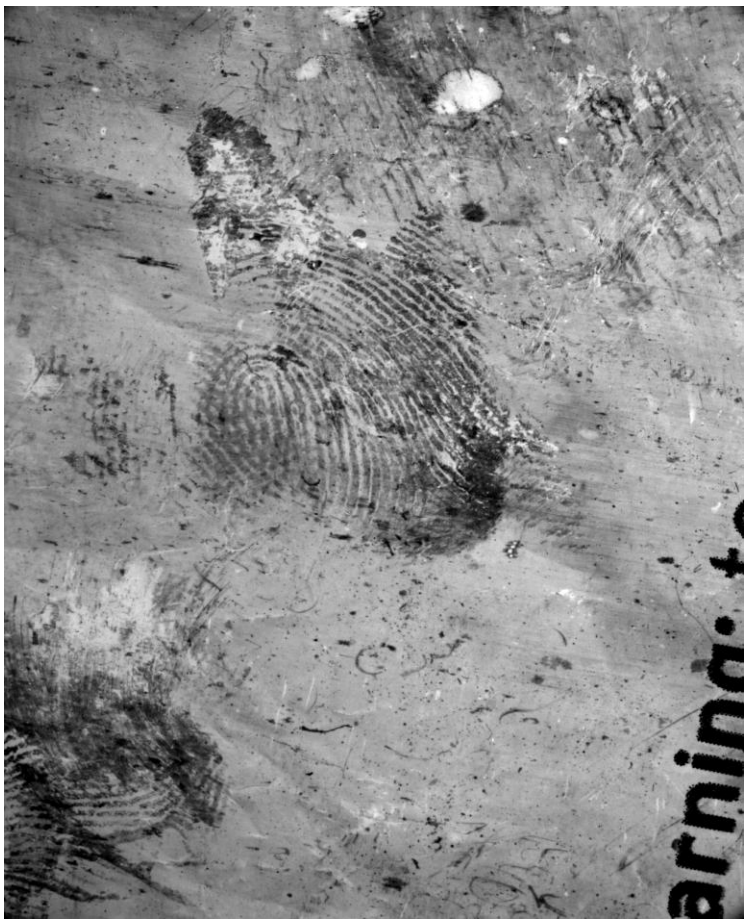


Schematic diagram of reverse development, showing different rates of zinc deposition according to size of gold nuclei available on the surface.

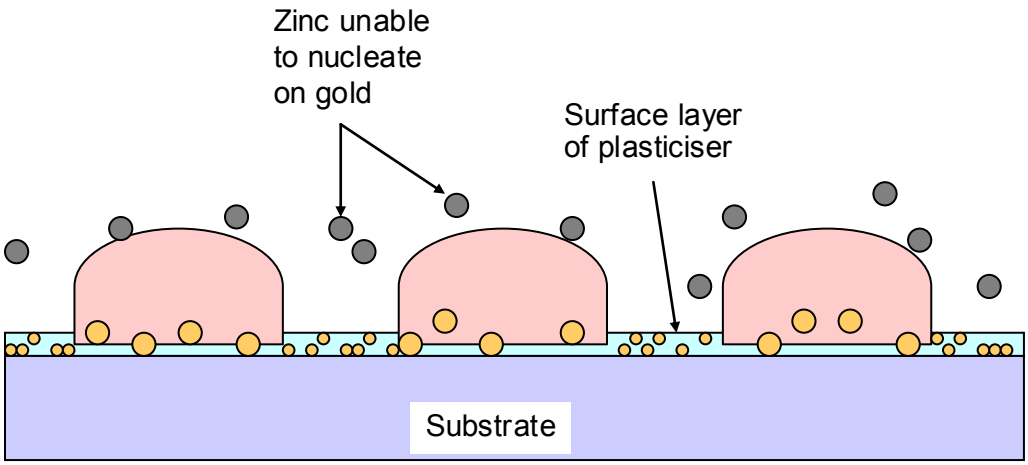
2.7 None of the theories above have been categorically proven, and in some cases reverse and normal development may be observed on the same substrate, although it is stated that this is most common for (if not exclusive to) LDPE substrates. The photograph below shows a 'reverse developed' mark on a polyethylene bag.



Photograph of a reverse developed mark on a polyethylene bag.

2.8 It is recognised that the gold/zinc VMD process does not work well (or at all) on substrates that are heavily plasticised (e.g. clingfilm, plasticised

PVC) or have surface release films or contamination. This is attributed to the fact that gold nuclei diffuse into the surface layer on the substrate as well as the fingermark deposits, with the result that there are no nuclei on the surface of zinc to deposit on, as is illustrated schematically below.



Schematic diagram of no development, showing zinc unable to find gold nuclei on surface.

3. CAST processes

3.1 The most current version of the process used by CAST can be found in the *Fingermark Visualisation Manual* (published January 2014), the purpose of this publication being to report the history, theory and validation work associated with the process. The process outlined in the *Fingermark Visualisation Manual* [19] is essentially as follows:

- evaporate ~2 mg of gold at a pressure of 3 x 10^{-4} mbar or lower;
- evaporate zinc at a pressure between 3–5 x 10^{-4} mbar until a suitable coating is formed.

3.2 These steps can be repeated until the desired level of coating and fingermark development has been obtained.

3.3 The reason for choosing these particular materials and conditions can be expanded as follows.

3.4 The role of gold in the VMD process is to act as the 'primer' for subsequent zinc deposition. Gold is not selective in that it will deposit across the entire surface of the exhibit, but the size and dispersion of the gold nuclei formed will be determined by the nature of the surface (chemistry, roughness, etc.). As outlined above, there is usually a sufficient difference between the nuclei formed in the regions of the fingermark ridges and the background for the print to be delineated during subsequent zinc deposition. Gold is also used as the initial deposition metal because it is inert and does not react with fingermark

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residues or atmospheric pollutants. The low deposition pressure is used so that gold can be deposited directly onto the surface without colliding with a significant number of molecules in the chamber, giving an even coating.

3.5 The role of zinc in the process is to delineate the fingermark, primarily by the difference between the growth rate of zinc on the fingermark ridges and the growth rate on the background. Zinc is highly effective for this purpose because it easily re-evaporates from the surface unless there is a suitable nucleation site present, thus the gold nuclei formed control the way in which zinc layers subsequently form. The sections above outline the different mechanisms by which differences in zinc growth rate can reveal fingermarks. The evaporation pressure used for zinc is higher than that for gold, and this is to allow the user more control over the zinc deposition process. Allowing more air into the chamber makes the deposition of zinc more uniform across the area of the exhibit. It was thought that the additional air molecules present in the chamber would reduce the kinetic energy of zinc atoms as they reach the surface and could increase development rate, but this has never been proven.

4. Critical issues

4.1 Sealed containers such as aerosol cans, sealed drink cans and bottles, batteries and items with sealed air pockets must not be treated using VMD because the expanding gases may cause the item to explode.

4.2 Articles to be treated by VMD must be dry and free of other residual liquids and solids.

4.3 During the zinc deposition stage of the gold/zinc VMD process it is essential for the operator to observe the development of the marks and to stop the process before any over-development occurs. The filament temperature and deposition time required to coat articles will vary according to the type of material and condition of the surface. For this reason multiple exhibits of different types should not be treated together.

4.4 Multiple deposition runs can be used to build up a coating if the initial run fails to develop any marks.

5. Application

5.1 Suitable surfaces: VMD has traditionally been recommended as the primary process for development of fingermarks on plastic bags and wrappings. Although still effective in this role it is no longer as effective as alternative processes, such as superglue and powder suspensions. The silver VMD process is one of the few techniques suitable for clingfilm. VMD is suitable for use on all types of non-porous surface, and is one of the more effective techniques on ‘semi-porous’ surfaces such

as glossy magazines and wrapping paper, and the best process for the non-adhesive side of masking tapes.

5.2 The equipment used for VMD may vary according to manufacturer, but the essential elements of the system are the same. The equipment consists of a vacuum chamber capable of being pumped down to high levels of vacuum (<3 x 10^{-4} mbar), filaments for deposition of gold and zinc, and a viewing window so that the deposition of zinc can be monitored. The chamber may also contain a 'cold finger', chilled to low temperature to aid condensation of contaminants and to reduce pump down times, and a rotary arm allowing the treatment of the entire outer surfaces of cylindrical items. Articles to be coated are attached to the perimeter of the vacuum chamber, above the coating filaments. Various means of attachment may be used, one of the simplest and most effective being small, moveable magnets that are attracted to the metal sample holder. A typical system is illustrated below.

Typical vacuum metal deposition equipment.

5.3 The filaments used for deposition of gold and zinc are typically formed from thin sheets of molybdenum. The gold filament usually consists of a shallow dimple in a thin strip of molybdenum. This is because the quantity of gold used is very small (approximately 2 to 3 mg), and it is important that all the gold reaches the substrate. If deeper containers are used, ‘shadowing’ may occur and not all regions of the article may be

coated. Gold deposition takes place when the chamber has reached a pressure of 3 x 10^{-4} mbar or lower, and the current to the filament is increased until the filament reaches a yellow/white heat. Deposition of gold should be complete within ten seconds, but if any residue is observed on the filament as the current is reduced, the temperature should be increased again until all the gold has been evaporated.

5.4 Once gold deposition is completed, the pressure in the chamber is increased to around 5 x 10^{-4} mbar and the current to the zinc deposition filament(s) turned on. The reason for increasing the pressure in the chamber is to increase the uniformity of the coating produced. The zinc deposition filaments are larger and significantly deeper than the gold filament, and the quantity of zinc added is larger, typically 1 g per run. The zinc used is in the form of foil, shot or powder. For zinc deposition, the current is increased until the filament glows a cherry red/dull orange colour. Once this occurs, the operator should observe the deposition process through the viewing window, ceasing deposition as soon as marks become visible on the substrate. After zinc deposition, the gold filament should be briefly heated to yellow/white heat to burn off any zinc contamination. The process is described in more detail elsewhere [19].

5.5 There is a great variability in the speed at which different substrates coat, and it may take over ten minutes to obtain a suitable coating on some types of material. In some cases it may be necessary to carry out multiple deposition runs in order to obtain satisfactory results, or to develop all the marks present. The presence of surface contamination, release agents or plasticisers may mean that it is not possible to obtain a zinc coating at all and in these circumstances the deposition of approximately 30 mg of silver using the same deposition conditions for gold may yield additional marks.

5.6 The VMD technique was initially adopted as an operational technique for the detection of latent prints on thin polyethylene items such as carrier bags and wrappings, and was shown to be superior to other processes developed subsequent to the initial comparison trials. Although the technique had originally been developed with the intention of being used to detect prints on fabrics, no identifiable prints were successfully obtained in operational trials and VMD is not currently (2016) recommended for operational use on this substrate.

5.7 VMD has now been used operationally for many years, and has been shown to be an effective technique for a wider range of materials than polyethylene. Recent results showing VMD to produce results on a range of substrates include a ticket coated with ferromagnetic ink, and on expanded polystyrene [20]. The use of the technique has also begun to increase in North America, and successful results have obtained from plastic bags, in some cases several years old and exposed to moisture [21].

5.8 The range of exhibits that have been successfully treated using VMD is extensive, and includes:

- plastic bags and packaging;
- glass and plastic bottles;
- firearms;
- glossy card, photographic paper and magazine covers;
- clean leather items (including handbags and shoes);
- adhesive tapes (non-sticky side).

5.9 It is evident that there is much overlap between the types of article that can be treated with VMD and those that are treated using cyanoacrylate fuming. In many cases, the deciding factor as to which technique is to be used is whether the article has been wetted, because VMD remains effective on wetted items whereas cyanoacrylate fuming does not. In practice it is possible to use the two processes in sequence, and more marks may be detected in this way because the two processes work on different fingermark constituents. Powder suspensions is now regarded as an effective alternative to VMD on wetted substrates.

6. Alternative formulations and processes

6.1 Several other materials have been investigated in the VMD process, including metal combinations, single metals, and organic materials. A summary of some of these is outlined below.

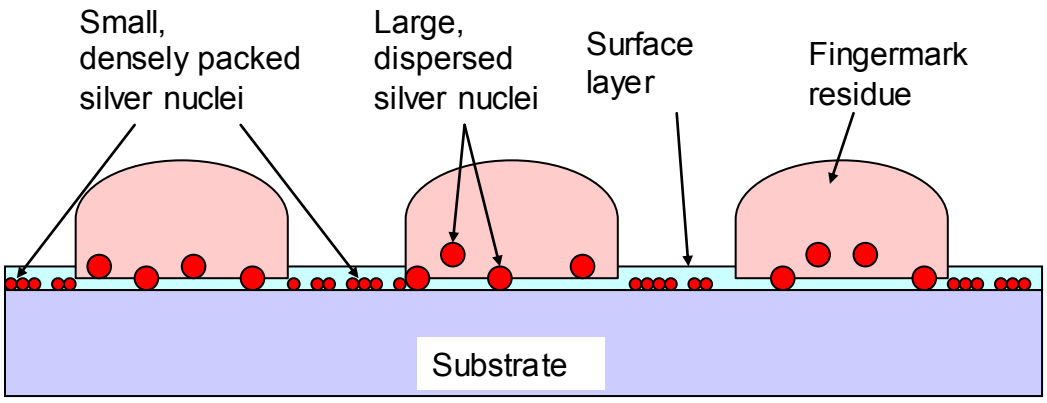
Metal 1	Metal 2	Comments
Gold	Cadmium	Initially, the gold/cadmium combination was selected as the optimum process, with cadmium giving better results than zinc when used as the second metal. It is also easier to produce coatings using cadmium. However, cadmium is very toxic and its use is no longer recommended on health and safety grounds.
Silver	Zinc	Silver can be used in place of gold as the initial deposition metal and limited evidence suggests that it this would have little effect on the effectiveness of the process. However, silver is more likely to interact with fingermark constituents or atmospheric contaminants, and for this reason the more inert gold is preferred.
Silver	Cadmium	See comments for silver and cadmium above.
Copper	Zinc	Copper is potentially more reactive than silver or gold, and hence gold is preferred.
Copper	Cadmium	See comments for copper and cadmium above.

Lead	–	Of all the single metals investigated for fingermark development in early studies (Hambley, 1972) [2], lead gave the best performance. However, lead is very toxic and its use is no longer recommended on health and safety grounds.
Zinc	–	Zinc is capable of developing fingermarks if used as a single metal, but re-evaporates easily from many surfaces and is best used in combinations.
Gold	–	Gold can be used as a single metal, and gives a blue background coloration with pink ridges. However, it has been found to be less sensitive than silver and copper and the gold/zinc combination when used this way.
Magnesium	–	Gives a silvery background, but less sensitive than most other single metals.
Copper	–	Gives a green/grey background coloration with pale yellow ridges. Effective on PVC-based clingfilm but less effective than silver on all other surfaces studied (Philipson and Bleay, 2007) [22]
Indium	–	Gives a pale brown background coloration with pale yellow ridges. Less effective than silver and marks difficult to see.
Tin	–	Gives a pale yellow background coloration with paler yellow/white ridges. Less effective than silver and marks very difficult to see.
Aluminium	–	Gives a silvery coating. Recently proposed as a more effective technique than gold/zinc on black plastic bags (Guraratne *et al.,* 2007) [23]. Ongoing research by CAST suggests no benefit over existing processes.
Silver	–	Identified as an alternative process to gold/zinc for plasticised materials (e.g. clingfilm) and materials with surface layers of contaminant (Philipson and Bleay, 2007) [22], now recommended for operational use by CAST. Can also be used sequentially after gold/zinc to fill in areas where zinc has deposited poorly. Further detail on the silver process is given below.

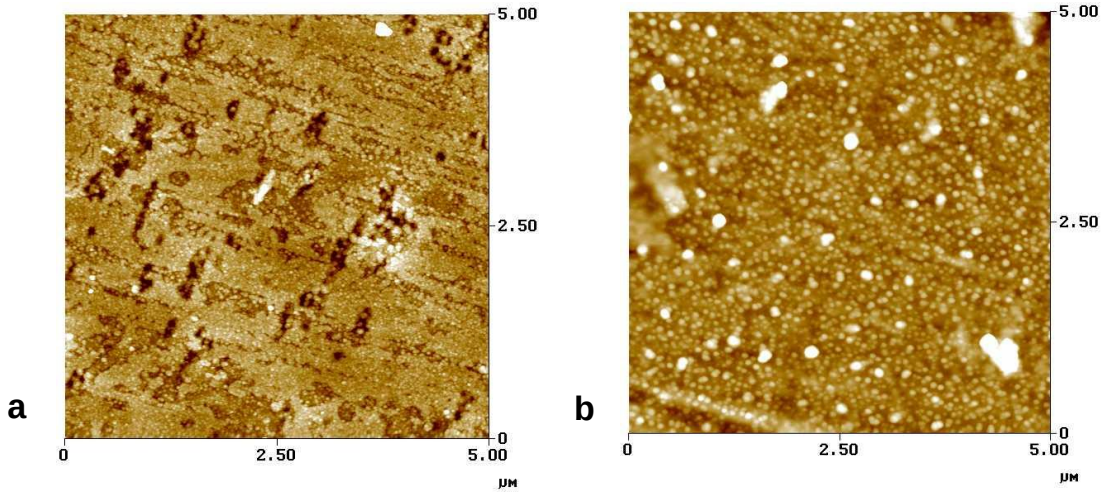
Summary table of alternative vacuum metal deposition processes.

6.2 The silver VMD technique is thought to work because silver, like gold, deposits uniformly across the surface. The nuclei formed vary in size and distribution between the fingermark ridges and the background, giving a difference in colour between the two regions. This is shown

schematically and as viewed by an atomic force microscope in the figures below.



Schematic diagram of silver vacuum metal deposition on a plasticised surface, showing different sizes of silver nuclei in ridges and on surface.



Atomic force microscopy images of polyethylene bag after vacuum metal deposition, showing differences in silver nuclei size and density a) *atomic force microscopy image of polyethylene surface,* b) *atomic force microscopy image of ridge region. Silver nuclei appear as light dots, and are smaller and very tightly packed on the polyethylene surface, and larger and more widely spaced in the fingermark ridge.*

6.3 The silver is deposited using the same conditions as for gold in the gold/zinc combination. The optimum amount of silver to use for most surfaces has been identified as 30 mg. If less silver is used, development is too faint – if too much silver is used, ridges start to become filled in and detail can be lost, as seen in the sequence of images below.

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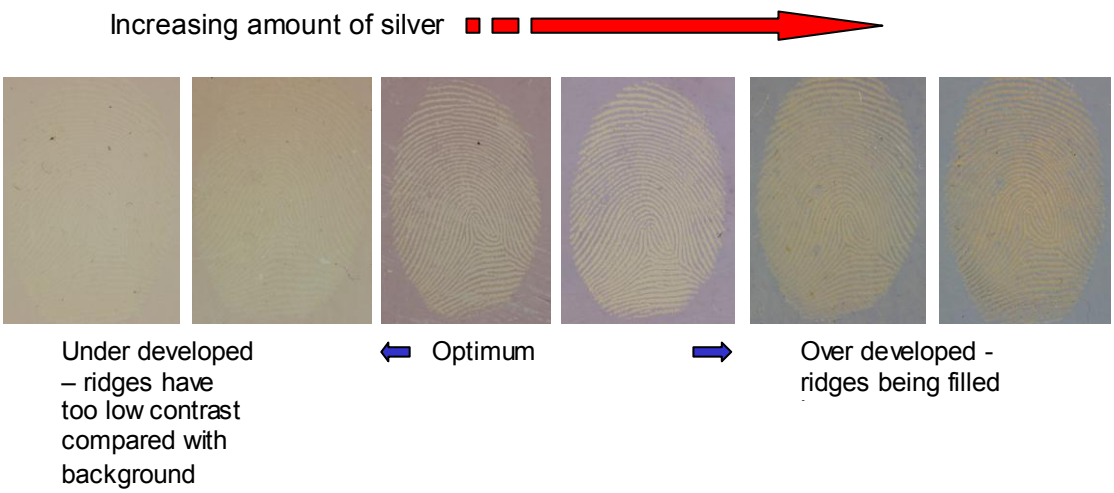


Progression of colours developed using increasing amounts of silver in a single metal vacuum metal deposition process on polyethylene.

6.4 It is thought that copper works in a similar way, but the resultant colour of the film formed is different.

6.5 There has been recent interest in the aluminium deposition process [23], but trials at CAST have been unable to replicate the results in the literature. Most marks developed by this process are not easily visible and consequently are difficult to image. Comparisons of carrier bags cut in half and processed using aluminium and gold/zinc indicate that aluminium finds no marks from natural handling, only deliberately placed, 'groomed' marks.

6.6 Fluorescent, organic materials have also been deposited using the VMD process, most notably anthracene. Anthracene is less sensitive than most of the single metals and metal combinations outlined above and there are health and safety concerns regarding its use in this way. More recently, deposition of Rhodamine 6G in combination with an organic precursor has been investigated as a possible alternative to superglue fuming and dyeing [24]. The process was shown to develop fluorescent marks on surfaces, including metal, glass, plastic and thermal paper, but has not yet been developed further.

7. Post-treatments

7.1 A limited amount of research was carried out in the late-1970s on physical developer enhancement of VMD deposits on banknotes and metal images from banknotes transferred onto gelatine emulsions.

8. Validation and operational experience

8.1 The comparative effectiveness of VMD with other fingermark development processes for the development of fingermarks on plastic (principally polyethylene) bags has been assessed in pseudo-operational and operational trials conducted by HOSDB. The principal results of these trials are reported below.

8.2 Laboratory trials

8.2.1 An initial laboratory trial conducted in 1978 [9] demonstrated that VMD typically developed between 23 and 27% useful marks on polythene bags compared with 7 to 10% for aluminium powdering, concluding that VMD was a superior process for this type of exhibit. This trial utilised planted marks deposited on plastic bags, results being obtained from over 1,000 deposited marks. The subsequent successful introduction of the technique into operational use meant that few other laboratory trials were conducted.

8.2.2 Laboratory trials were carried out when research was being conducted into deposition of alternative metals for development of marks on clingfilm [22]. These investigations compared the effectiveness of depositing silver and copper as single metals, on both polyvinylchloride (PVC) and polyethylene (PE)-based clingfilms. Conventional gold/zinc VMD gave virtually no marks on both these types of clingfilm and was therefore omitted from the trial. Results for one-day-old and one-month-old marks are tabulated below. In the one-day-old experiment, 200 marks were analysed and in the one-month-old experiment, 240 were analysed.

Grade	Silver		Copper	
	PE	PVC	PE	PVC
3–4	10	20	0	24
2	4	10	0	11
1	36	10	10	10
0	0	10	40	5

a)

Grade	Silver		Copper	
	PE	PVC	PE	PVC
3–4	14	2	0	37*
2	28	24	3	5
1	1	12	40	0
0	17	22	16	18

* Many marks faint and difficult to image.

b)

Summary of comparative trials carried out on clingfilm using different vacuum metal deposition processes, a) *results for marks aged for one day,* b) *results for marks aged for one month.*

8.2.3 The results indicated that copper VMD was ineffective on PE-based clingfilm, but gave better results on PVC-based clingfilm than silver. Copper was only recommended for use if it was certain that the clingfilm found was PVC-based.

8.2.4 Comparative tests were also carried out between gold/zinc and silver VMD on two 'non-standard' clear packaging films, polyester terephthalate (PET) and cellophane, where gold/zinc VMD occasionally had problems with 'empty' prints or rapid fading of developed marks.

Grade	Gold/zinc		Silver	
	PET	Cellophane	PET	Cellophane
3–4	9	28	22	31
2	22	13	18	21
1	29*	19	10	8
0	0	0	0	0

* Many empty prints developed.

Summary of comparative trials carried out on marks aged for one day on alternative clear packaging materials using different vacuum metal deposition processes.

8.2.5 It was shown that silver VMD offered an improvement over gold/zinc for development of marks on PET, and could also fill in ridge detail in regions where 'empty' prints developed. Although silver VMD performed well on cellophane, the developed marks faded very rapidly and there was no operational benefit in using the technique.

8.2.6 Finally, investigations were carried out into the use of gold and silver in combination, as opposed to silver as a single metal [25]. These showed no benefit in the use of gold-silver as opposed to silver and were not pursued further.

8.2.7 It should also be noted that with the withdrawal of the radioactive sulphur dioxide process from operational use in the mid-2000s, there has been renewed interest and research into the use of VMD to develop fingermarks on fabric. A comparative study in the early 1980s [26] had indicated that although VMD was less effective than radioactive sulphur dioxide, it was the best of the other techniques investigated at that time. The work conducted since the 1980s has investigated both gold/zinc and silver VMD, and also included some comparisons with cyanoacrylate fuming [27,28,29]. These studies show that VMD remains capable of developing marks on fabrics, but the quality of these is very dependent on the donor and fabric type. However, even where ridge detail is not developed, it is suggested that flexion creases may give useful evidence, the shape of the impression may provide context about the nature of the contact, and the areas developed could be used to target DNA swabbing.

8.3 Pseudo-operational trials and operational experience

8.3.1 With the advent of the small particle reagent (SPR) process in 1976, an operational trial was conducted at Essex Police on plastic bag exhibits submitted to the fingermark laboratory [30]. Each bag was cut in half, one-half being treated with VMD, the other with SPR. The results are summarised below.

Trial overview	
Number of polythene articles received	204
Number of cases received	57
Total number of fingermarks developed using VMD	117
Total number of fingermarks developed using SPR	61
Number of articles where VMD developed marks	36
Number of articles where SPR developed marks	20

Process effectiveness comparison		
	Number of cases	Percentage from total number of cases
Cases with fingermarks only developed by VMD	13	23
Cases with fingermarks only developed by SPR	3	5
Cases where both VMD and SPR developed fingermarks	13	23
Cases where VMD developed fingermarks	26	46
Cases where SPR developed fingermarks	16	28
Total number of cases where VMD and SPR developed fingermarks	29	51

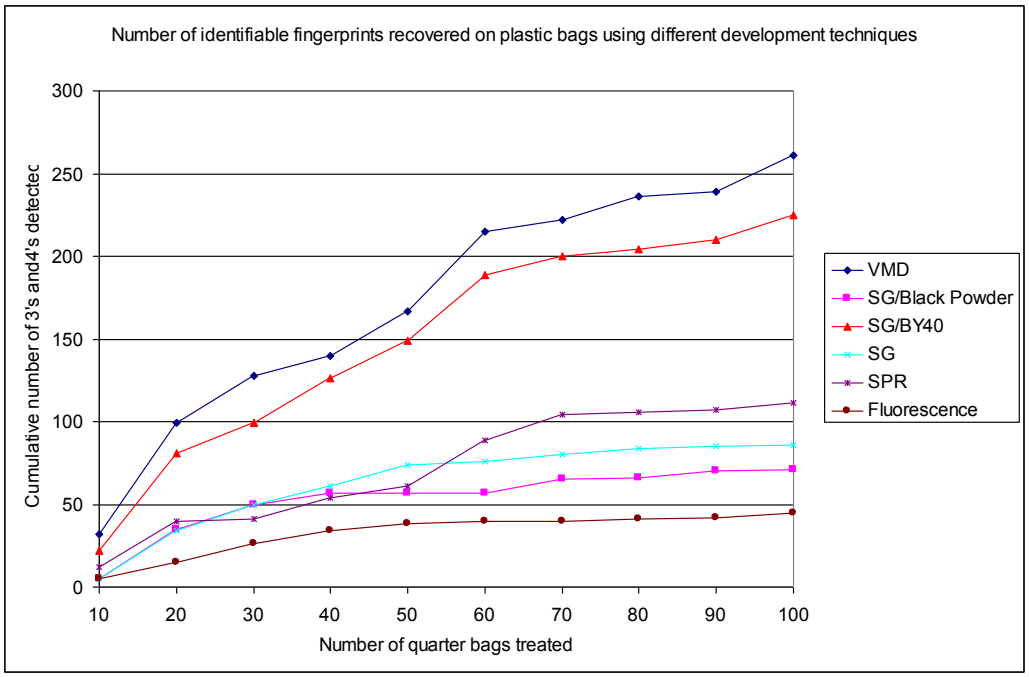
Early operational trial results comparing small particle reagent and vacuum metal deposition.

8.3.2 It was found that VMD was almost twice as effective as SPR on this type of exhibit. It was also observed that SPR could be used sequentially after VMD, but this was expected to be of only limited benefit.

8.3.3 The subject was revisited when an optimised superglue process became available in the mid-1980s, with a pseudo-operational trial being conducted between VMD, SPR, superglue and superglue followed by dyeing with basic yellow 40 and fluorescence examination. The trial was conducted by HOSDB on a large number of plastic bags using the same methodology as the study above, but not using operational casework.

8.3.4 The VMD process produced the largest number of identifiable fingermarks, producing approximately 12% more fingermarks than a combination of superglue, dyeing and fluorescence, and twice as many

fingermarks as fluorescence alone [31]. Results of this exercise are shown below.



Results of pseudo-operational trial carried out on plastic packaging material in 1986.

8.3.5 More recently the composition of plastic bags has changed significantly, typically including more recycled material and observations from police forces using VMD on operational work indicated that the effectiveness had dropped off on this type of exhibit. As a consequence, the pseudo-operational trial above was repeated in 2009, comparing VMD with superglue and powder suspensions in a range of sequential processing scenarios [32]. These studies are more fully reported in Chapter 3, Chemical and Physical Processes, Powder suspensions, and confirmed that VMD is no longer the most effective process for plastic bags, but instead should be used after superglue in a sequential processing route.

8.3.6 Operational trials involving silver have been more limited in extent because the process is only recommended as a secondary treatment after gold/zinc VMD. A small-scale study on clear cigarette wrappings (thought to be polypropylene) is summarised below, showing the number of wrappings yielding particular levels of ridge detail.

Result	Technique		
	Gold/zinc VMD	**Silver VMD**	**Superglue fuming**
Full print	1 (12.5%)	1 (5.6%)	1(12.5%)
Usable fragment	2(25%)	7(38.9%)	1(12.5%)
Unusable fragment	2(25%)	4(22.2%)	2(25%)
No print	3(37.5%)	6(33.3%)	4(50%)

Results of a short pseudo-operational trial on cigarette wrappers.

8.3.7 Silver VMD gives comparable results to gold/zinc VMD in this study, and better results than superglue. However, due to the limited sample size it is not possible to draw strong conclusions.

8.3.8 The process was trialled by some police forces on operational exhibits, using it after gold/zinc VMD where no development or patchy development was found. In these small-scale trials silver VMD was found to develop additional ridge detail in approximately 10% of cases.

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Fingerprint Source Book v2.0, Publication Number 081/17 - Errata

1. Chapter 3: Chemical and Physical Processes, page 287 (3.IND.14), 3.18, Table: '*Formulation of 1,2 indandione used in 2015-2016 comparative trials [24]*' – Methanol quantity is 45mL instead of the stated 1mL.
   [Note: the error also appears in reference 24 from where the information was taken. However the authors can confirm, via laboratory notebook checks, that 45mL was used during trials and the error occurred at the report writing stage.]

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