Technical Note

Sequencing of a Modified Oil Red O Development Technique for the Detection of Latent Fingermarks on Paper Surfaces

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Abstract: A modified detection sequence is presented for the recovery of latent fingermarks on porous substrates. 1,2-Indanedione, Oil Red O (ORO) in propylene glycol, and physical developer (PD) were successfully used to develop recently deposited latent fingermarks when applied in the order given. The incorporation of ORO into the detection sequence increased the number of latent fingermarks that were detected compared to using the standard sequence of 1,2-indanedione followed by PD only.

Introduction

In order to detect latent fingermarks on a variety of surface types, many physical and chemical development methods have been devised. A surface that is being examined for latent fingermarks is typically subjected to a range of these techniques, targeting different components of latent fingermark residue, to maximize the number of latent fingermarks that may be recovered [1, 2]. The development methods that are used in such detection sequences are determined by substrate type–broadly categorized into porous, semiporous, and nonporous, and further

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divided into adhesive, dry, or wet (or has been wetted) [3]. The order in which these methods are applied is selected so that the success of each technique is not hindered by a preceding technique.

Porous substrates, such as paper, are typically treated with one or several amino acid-sensitive reagents such as ninhydrin, 1,8-diazafluoren-9-one (DFO), or 1,2-indanedione, followed by subsequent treatment with physical developer (PD) [2, 3]. The application of PD is usually only necessary should the former methods produce no satisfactory detail, such as in instances when the substrate has been wetted (thereby dissolving the amino acids from the latent fingermark) or when the latent fingermark residue contains a low concentration of amino acids because of natural donor variation [4]. PD interacts with the water-insoluble fraction of latent fingermark residue, making it one of the few routine development techniques that can be successfully utilized on porous surfaces that have been exposed to water or high humidity [3, 5].

A novel Oil Red O (ORO) reagent, based on a propylene glycol histological stain, has been reported [6]. This solution is able to detect lipid-rich latent fingermarks on porous substrates and is comparable in performance to Beaudoin's methanolic ORO formulation [7], while having the advantage of using fewer, nontoxic components. However, to be truly useful to forensic investigations, it needs to be determined whether ORO in propylene glycol is compatible for use with other latent fingermark development methods. Similar investigations have already been carried out using the methanolic ORO reagent, with the conclusion that the insertion of ORO between amino acid-sensitive reagents and PD increases the number of latent fingermarks that are able to be detected [8, 9].

This paper reports the incorporation of the propylene glycol-based ORO reagent into a development sequence with 1,2-indanedione and PD for porous substrates. This work forms the basis of an on-going, large-scale research project into possible correlations between donor traits and impression development quality, as a reflection of latent fingermark composition. It also provides an overview of the considerations that must be made when attempting to use several development methods in conjunction with each other.

Materials and Method

Chemicals

1,2-Indanedione (CASALI/Optimum Technology, Australia), anhydrous zinc chloride (BDH, Radnor, PA), dichloromethane (Mallinckrodt Chemicals, Hazelwood, MO), ethyl acetate (Univar Analytical, Australia), glacial acetic acid (CSR Chemicals, Australia), absolute ethanol (CSR Chemicals, Australia), HFE-7100 (1-methoxynonafluorobutane, 3M Novec, Australia), petroleum spirits 60–80 °C (APS Chemicals, Australia), Oil Red O (Sigma-Aldrich, USA), propylene glycol (Sigma-Aldrich, USA), maleic acid (Sigma-Aldrich, USA), silver nitrate (Chem-Supply, Australia), ferric nitrate nonahydrate (Chem-Supply, Australia), ferrous ammonium sulphate hexahydrate (Sigma-Aldrich, USA), citric acid (Ajax Finechem, Australia), Tween 20 (Sigma-Aldrich, Australia), and n-dodecylamine acetate (Optimum Technology, Australia) were all used as received and were of analytical reagent grade unless otherwise stated.

Preparation of Reagent Solutions

"Wet contact" 1,2-indanedione reagent was prepared as recommended by the Australian Federal Police (AFP) [10]. "Dry contact" 1,2-indanedione reagent was prepared as described by Patton et al. using two formulations, one containing HFE-7100 and another containing petroleum spirits [11]. Treatment papers for the dry contact method were prepared by dipping A4 white copy paper in the working solution and allowing the sheets to air-dry before storing in a sealed ziplock plastic bag. The preparation of stock and working solutions for both 1,2-indanedione formulations is summarized in Table 1.

The ORO formulation used was that described by Frick et al. [6] ORO reagent was prepared by dissolving 0.05 g ORO in 100 mL propylene glycol at 95 °C with constant stirring. The solution was left to cool before undissolved ORO was removed using vacuum filtration. The solution was left to stand until completely cooled before use. The stain solution was stored at room temperature in Schott bottles wrapped in aluminum foil.

The PD stock and working solutions used in this study (Table 2) were prepared as described by the AFP [10] with the following modification: Tween 20 was substituted for Synperonic N [12], using the formulation described by Sauzier et al. [13] The PD working solution was prepared fresh as needed and was used twice before discarding.

Solution	Reagent Preparation
Standard 1,2-Indanedione Reagents	
1,2-Indanedione Stock Solution	4 g 1,2-indanedione dissolved in 450 mL ethyl acetate and 50 mL glacial acetic acid
Zinc Chloride Stock Solution	8 g zinc chloride dissolved in 200 mL absolute ethanol
Working Solution	2 mL zinc chloride stock solution and 50 mL stock solution added to 450 mL HFE-7100 solvent
Dry Contact 1,2-Indanedione Reagents	
1,2-Indanedione Stock Solution	0.75 g 1,2-indanedione and 20 mg zinc chloride dissolved in 0.5 mL ethanol, 15 mL dichloromethane, and 35 mL ethyl acetate
Working Solution	5 mL 1,2-indanedione stock solution added to 45 mL HFE-7100 or 45 mL petroleum spirits

Table 1

Preparation of 1,2-indanedione stock solutions and working solutions.

Solution	Reagent Preparation
Detergent-Surfactant Solution	0.5 g n-dodecylamine acetate and 0.5 g Tween 20 dissolved in 125 mL deionized water
Redox Stock Solution	7.5 g ferric nitrate nonahydrate, 20 g ferrous ammonium sulphate hexahydrate, 5 g citric acid, and 10 mL detergent-surfactant solution dissolved in 225 mL deionized water in order given
Silver Nitrate Solution	10 g silver nitrate dissolved in 50 mL deionized water
Maleic Acid Prewash	6.25 g maleic acid dissolved in 250 mL deionized water
Working Solution	7.5 mL silver nitrate stock solution added to 142.5 mL redox stock solution

Table 2

Preparation of PD stock solutions and working solution.

Collection of Latent Fingermarks

Latent fingermarks were collected on white copy paper (Fuji Xerox Professional) from 11 donors. Donors had not consumed food or handled chemicals for at least 30 minutes before providing samples. Both charged latent fingermarks, prepared by having donors rub their fingers on their face or hair immediately prior to deposition, and uncharged latent fingermarks, requiring no preparation, were collected. Donors were instructed to gently place fingertips onto the substrate and not to remove their hands until fingers had been outlined in graphite pencil (less than 10 seconds deposition time). Samples were treated within 24 to 36 hours following deposition. At least 10 samples from 4 to 5 donors were collected for each experiment, except for the lipid reagents versus sequence study, which used 5 samples from donors.

Development of Latent Fingermarks Using 1,2-Indanedione Methods

The wet contact 1,2-indanedione treatment was carried out as described by the AFP [10]. Samples were developed by dipping them briefly in the working solution and allowing them to air-dry before heat-treating them for 10 seconds with an Elna laundry press (set at 160 °C).

To determine whether the 1,2-indanedione solvents dissolved any lipids that may have been present in the latent fingermark, additional samples were developed without heat treatment to prevent the pink 1,2-indanedione reaction product from obscuring any subsequent ORO treatment. These samples were dipped briefly in the working solution and left to air-dry.

The dry contact 1,2-indanedione treatment was carried out as described by Patton et al. [11] Samples were sandwiched between two treatment papers and were stored in a ziplock plastic bag for 24 to 36 hours in the dark. No heat treatment was applied to dry contact-treated samples.

Development of Latent Fingermarks Using Oil Red O

Sample treatment with ORO was carried out as described by Frick et al. [6] Samples were placed in a glass tray and immersed in ORO reagent for 15 minutes, with manual agitation provided by gently rocking the tray for 30 seconds at the beginning of treatment. After development, ORO-treated samples were rinsed twice in a deionized water bath under running water and were air-dried on paper towels at room temperature.

Development of Latent Fingermarks Using Physical Developer

Latent fingermark development with PD was carried out as described by the AFP [10] with one minor modification: the maleic acid pretreatment step was increased from 5 minutes to 30 minutes, as recommended by Salama et al. [8] Each step was carried out in a separate glass tray. Samples were rinsed twice in deionized water for 10 minutes, immersed in maleic acid for 30 minutes, then rinsed again in deionized water for 10 minutes. Samples were then immersed in the working solution for up to 20 minutes. After development, samples were rinsed several times in deionized water and were air-dried on paper towels at room temperature, away from direct light.

Photography of Samples

Samples were photographed using a Nikon D300 camera, equipped with an AF-S Micro-Nikkor lens, mounted on a Firenze Mini Repro tripod, and connected to a computer using Nikon Camera Control Pro Version 2.0.0. 1,2-Indanedione-treated samples were photographed in luminescence mode; ORO- and PD-treated samples were photographed in absorbance mode (Table 3). Illumination in luminescence mode was achieved using a Rofin Polilight PL500 (Rofin, Australia), with an excitation wavelength of 505 nm and an orange camera filter attachment (550 nm barrier filter). Illumination in absorbance mode was achieved using incandescent light bulbs with no camera filter attachments.

	Absorbance Mode	**Luminescence Mode**
Focal Length/mm	60	60
Exposure Mode	Manual	Manual
White Balance	Auto	Auto
Shutter Speed/s	1/20	2
Aperture	f/11	f/11
Sensitivity	ISO 200	ISO 200

Table 3

Photographic conditions for absorbance and luminescence mode photographs.

Visual Analysis of Developed Latent Fingermarks

Developed impressions were graded using a 5-point system based on that used by the United Kingdom Home Office Police Scientific Development Branch (HOPSDB) (Table 4) [14]. Images were adjusted for brightness and contrast using Adobe Photoshop CS5 Version 12.0. Adjustment of photographed samples (equally for each "half print") was performed only for clarity of the figures in this article. Evaluation of development was carried out on the raw images.

Grade		Description
0	No development	No visible ridge detail
1	Weak development	Signs of contact, but less than 1/3 of fingermark visible as continuous ridges
2	Medium development	1/3–2/3 of fingermark visible as continuous ridges
3	Strong development	More than 2/3 of fingermark visible as continuous ridges, but not quite a "perfect" fingermark
4	Full development	Whole fingermark visible as continuous ridges

Table 4

Grading system for developed latent fingermarks.

Results and Discussion

Comparisons Between Wet and Dry Contact 1,2-Indanedione

When designing a detection sequence for latent fingermarks, the order in which these reagents are applied should be such that the success of a reagent is not affected by the application of a preceding method [2]. Because the aqueous immersion baths and rinses included in the ORO and PD methods would cause dissolution of amino acids that are present in latent fingermarks, 1,2-indanedione treatments were placed first in the detection sequence.

To determine the effect of the 1,2-indanedione reagent on the integrity of the lipid fraction of latent fingermarks, halved fingermarks were treated with either 1,2-indanedione followed by ORO or ORO only. Preliminary comparisons suggested that prolonged immersion in the 1,2-indanedione working solution (~5 seconds) caused subsequent ORO treatment to appear fainter, with less clearly defined detail (median grade of 1) compared to samples treated with ORO only (median grade of 2.5) (Figure 1).

When 1,2-indanedione dipping time was reduced to 1 to 2 seconds, ORO development was not significantly worse than ORO only treated halves (median grade of 2 compared to 3, where $n = 4$). There did not appear to be much dispersal of lipid material by the solvent mixture, although 1,2-indanedione treatment resulted in some loss of contrast with ORO treatment, similar to ORO treatment on wetted substrates [6]. Treatment with either 1,2-indanedione method did not appear to have any significant detrimental effect on the PD development processes (Figure 2).

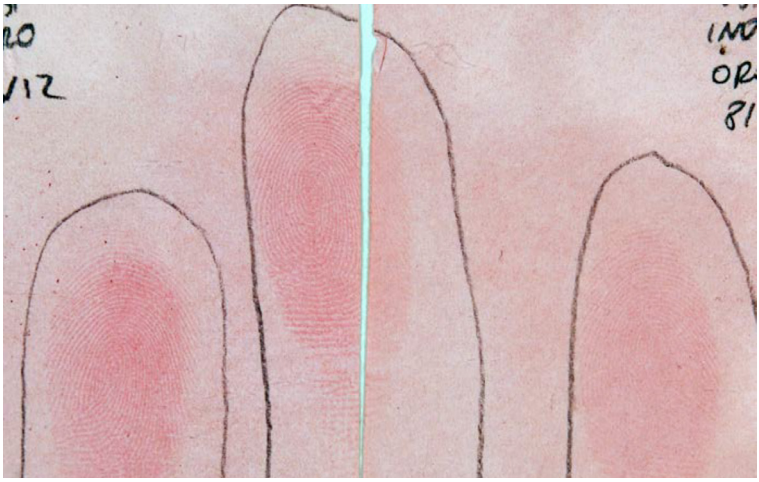

Figure 1

ORO-treated latent fingermark (left) vs 1,2-indanedione → ORO-treated latent fingermark with a dipping time >5 secs (right). Photographs taken with a Nikon D300 camera in absorbance mode; focal length: 60 mm, shutter speed: 1/20 second, and aperture: f/11.

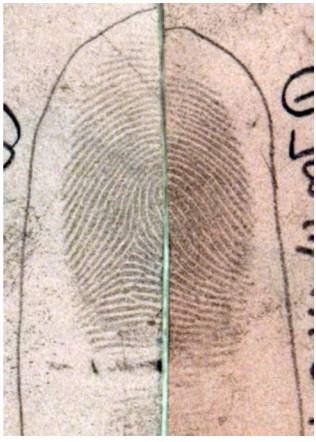

Figure 2

Dry (left) vs wet (right) 1,2-indanedione-treated latent fingermark in sequence with ORO → PD. Photographs taken with a Nikon D300 camera in absorbance mode; focal length: 60 mm, shutter speed: 1/20 second, and aperture: f/11.

Because the HFE-7100 solvent was found to remove some of the ORO-targeted lipids from latent fingermark residue unless used carefully, comparisons were conducted between the standard 1,2-indanedione method and a novel solvent-free treatment, in which samples are sandwiched between two treatment papers prepared by dipping them into a 1,2-indanedione reagent and drying prior to use [11]. It was thought that the solvent-free (and potentially less destructive) dry contact method could be used as an alternative to the wet contact method.

Sample halves were treated with either wet or dry contact 1,2-indanedione followed by ORO, both with and without heating the samples in an Elna press.

The dry contact method has several advantages in that it can be used to develop latent fingermarks on thermal paper, as well as very fragile papers. However, although potentially less destructive, the dry contact method consistently failed to offer the same degree of sensitivity and development compared to the wet method (Figure 3). The difference in performance between the two methods is most likely due to the way in which 1,2-indanedione is delivered to the latent fingermark residue. The wet method uses a solvent as the carrier for 1,2-indanedione, ensuring penetration of the substrate, and therefore a complete reaction with the amino acids, compared to the dry method, which relies on reagent sublimation to develop the latent fingermark, which would only reach the amino acids near the surface of the substrate.

One of the reasons HFE-7100 is the recommended solvent for amino acid-sensitive reagents by the AFP is that it dissolves any lipids that are present in the latent fingermark to a lesser extent than solvents such as hexane or petroleum spirits, and so has less, if any, detrimental impact on subsequent lipid-specific development techniques [8, 15]. Thus, a petroleum spirits-based wet contact formulation was not pursued as a possible development reagent in this study.

1,2-Indanedione → ORO-treated samples viewed under the Polilight at 505 nm showed little fluorescence, indicating that much of the pink, luminescent reaction product formed from 1,2-indanedione and amino acids was washed away by ORO treatment. Another possible scenario was that the presence of ORO had a quenching effect on luminescence, either by the ORO absorbing illumination from the Polilight, or the fluorescence produced by the 1,2-indanedione reaction product. Immersion of 1,2-indanedione-treated samples in propylene glycol for 15 minutes showed the same reduction in fluorescence, indicating that the reaction product was dissolved. This serves as a

reminder that fingermarks should be photographed following the application of every development method in a detection sequence, in the event that the application of a reagent destroys any development produced by a preceding technique.

Determination of Application Order for ORO and PD

Despite its advantages over PD, it is recognized that ORO cannot completely replace the latter technique. The color of the substrate, and especially the presence of text and patterns, affects the contrast of ORO-treated latent fingermarks to a greater extent than PD. PD also produces superior results to ORO on more porous paper types, as well as on latent fingermarks that are over 30 days old. For these reasons, it has been suggested that Beaudoin's ORO and PD be used together in sequence, following the application of amino acid-sensitive reagents [8].

Because it had already been established that 1,2-indanedione would be the first step of the development sequence, investigations were carried out to determine the order in which proylene glycol-based ORO and PD should be applied. Latent fingermarks were halved and treated with the sequences ORO → PD and PD → ORO. Neither sequence appeared to outperform the other, with a median grade of 2 obtained for both. This was not surprising because both reagents target water-insoluble compounds, which would not be affected by the polar solutions used in both methods. It was noted, however, that PD → ORO seemed to produce slightly better contrast (Figure 4). Similar results have been reported for Beaudoin's ORO formulation, with the conclusion that PD → ORO was the superior sequence [8].

It has previously been reported that ORO treatment prior to PD treatment may cause greater destruction to the substrate during the maleic acid pretreatment stage [8]. This effect was not observed in this study; however, latent fingermarks were collected on only one paper type. Other substrates may be more reactive when immersed in maleic acid.

Though better quality development may be obtained using the reversed sequence of PD → ORO, there is a risk that PD may indelibly mar the surface of some paper types, preventing further treatment [8]. The PD working solution is highly reactive with some contaminants and can blacken large portions of the substrate if sample pretreatment is not conducted properly. Because of the destructive potential of this method, PD should be performed last in any detection sequence for the detection of latent fingermarks on porous substrates, thereby following a logical application order of least destructive to most destructive methods [16].

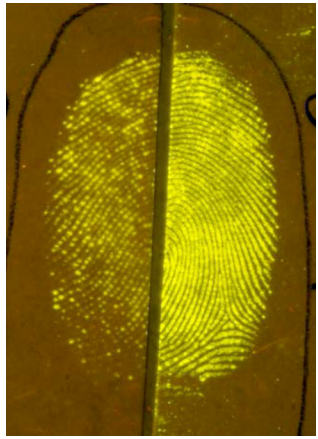

Figure 3

Dry (left) vs wet (right) 1,2-indanedione-treated latent fingermark. Photographs taken with a Nikon D300 camera in luminescence mode; focal length: 60 mm, shutter speed: 2 seconds, and aperture: f/11.

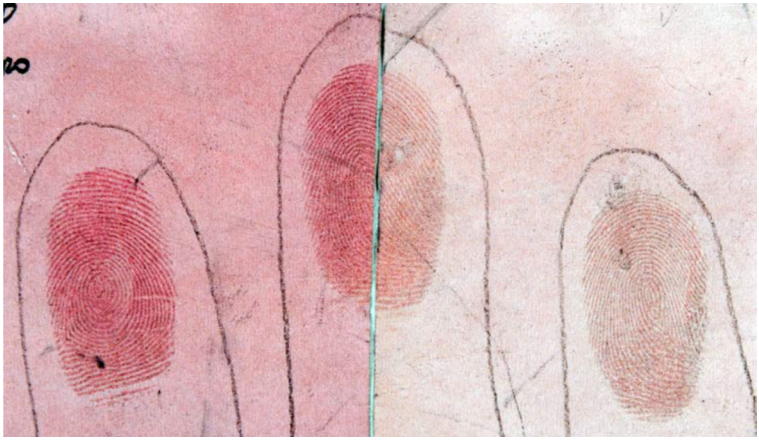

Figure 4

PD → ORO sequence (left) vs ORO → PD sequence (right). Photographs taken with a Nikon D300 camera in absorbance mode; focal length: 60 mm, shutter speed: 1/20 second, and aperture: f/11.

Comparisons Between Detection Sequence and Individual Development Methods

It is recognized that latent fingermark composition can vary greatly between individuals [17]. Donors can be categorized into good or poor amino acid or lipid donors. Wetted substrates also often contain very little amino acid. Because this information is not known prior to attempts at latent fingermark development, the application of a full detection sequence that targets a range of compounds present in latent fingermarks offers a better chance of detection than an individual method that targets only a select fraction.

Investigations were conducted to determine whether the proposed detection sequence that was developed was more effective than ORO or PD alone. Five charged latent fingermark samples were halved and were then treated with ORO followed by PD, or ORO only. Additional treatment with PD did not significantly improve development quality, with latent fingermarks treated with both reagents receiving a median grade of 4 (Figure 5). It was noted, however, that latent fingermarks, or portions of a latent fingermark, that were only faintly developed by ORO (grade 0–1) became more clearly developed when treated with PD (grade 2). PD also improved the contrast between ridges and substrate across all samples. Salama et al. have proposed that ORO might act as a nucleation site for silver deposition [8]. Alternatively, it may simply be that the coloration provided by ORO enhances the appearance of the developed impression on subsequent PD treatment.

Latent fingermark halves were developed either with PD only or the full detection sequence to determine whether the preceding development reagents had any detrimental effect on the performance of PD, which is reputed to be sensitive to contaminants. There did not appear to be any significant difference in the degree of silver deposition with either treatment; however, latent fingermark halves that were subjected to the full detection sequence exhibited enhanced contrast and overall development quality (median grade 2.5 compared to 2) (Figure 6). Although PD is often described as a lipid-sensitive reagent, some charged latent fingermark halves were only able to be detected when also using 1,2-indanedione and ORO with the full detection sequence, whereas latent fingermark halves that were treated with PD only showed little or no development.

It has been hypothesized that the lipids present in latent fingermarks can be divided into two fractions, which goes some

way to explaining the differences in performance between PD and ORO [8]. The relatively short-lived "fragile fraction" is thought to be the target group of ORO. These lipids are more vulnerable to degradation processes and may be dissolved by solvents used in preceding development techniques. PD, on the other hand, is thought to react with the more stable and long-lived "robust fraction", hence its ability to develop latent fingermarks up to several years after deposition in some cases. This may explain why ORO development quality is dependent upon immersion time in 1,2-indanedione, whereas PD appears to remain unaffected.

The steps of the detection sequence are presented in Figure 7. Charged latent fingermarks were deposited and treated with the full sequence (1,2-indanedione, ORO, PD).

The full sequence worked better than any individual reagent, and in the set order presented here, there was no interference between techniques, because each method targeted a different component of latent fingermarks.

Conclusion

We present a latent fingermark detection sequence for the detection of latent fingermarks on porous substrates, consisting of 1,2-indanedione, a novel ORO reagent, and a modified PD reagent containing Tween 20 in place of Synperonic N. The optimum detection sequence was determined to be 1,2-indanedione, followed by ORO and lastly PD, thereby forming a detection sequence for porous substrates that targets three separate groups of fingermark components: amino acids, fragile lipids, and robust insoluble substances.

Investigations were also conducted to determine whether standard wet contact or a novel dry contact 1,2-indanedione method held any particular advantage. As long as care is taken not to immerse samples in the 1,2-indanedione reagent for too long (greater than 5 seconds), there was no significant difference in impact of 1,2-indanedione treatment on the subsequent application of the lipid-sensitive methods. The wet contact method is preferred in the sequence because it offers more sensitivity than the dry contact method.

This sequence will be used in further studies into latent fingermark composition as a reflection of development by each of these three reagents and the influence of donor traits.

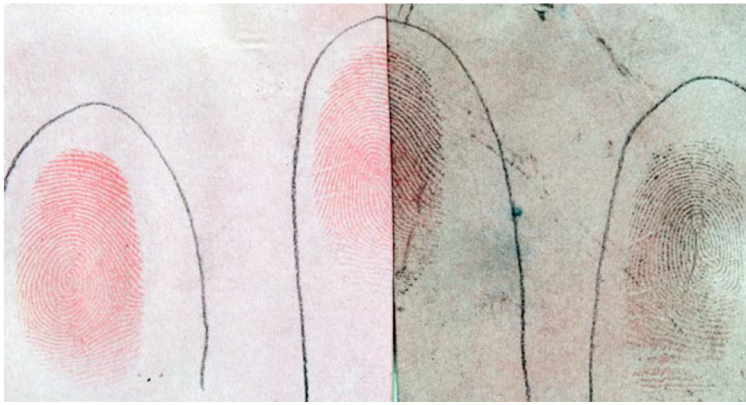

Figure 5

Latent fingermark treated with ORO (left) vs ORO in sequence with PD (right). Photographs taken with a Nikon D300 camera in absorbance mode; focal length: 60 mm, shutter speed: 1/20 second, and aperture: f/11.

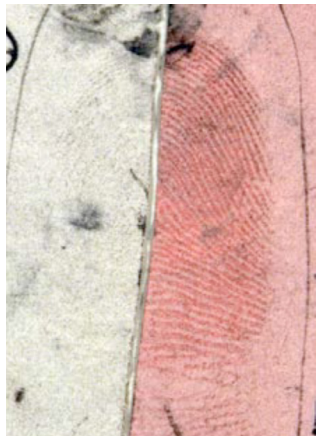

Figure 6

Latent fingermark treated with PD (left) vs full detection sequence (right). Photographs taken with a Nikon D300 camera in absorbance mode; focal length: 60 mm, shutter speed: 1/20 second, and aperture: f/11.

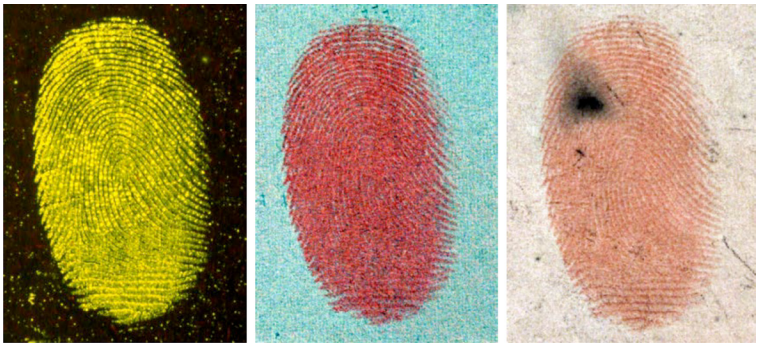

Figure 7

Latent fingermark treated with the full detection sequence: IND, ORO, and PD (from left to right). Photographs taken with a Nikon D300 camera in luminescence mode (IND); focal length: 60 mm, shutter speed: 2 seconds, and aperture: f/11; and in absorbance mode (ORO and PD); focal length: 60 mm, shutter speed: 1/20 second, and aperture: f/11.

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