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Authors: Eliza C. Moule, Taryn M. Guinan, O.Johan R. Gustafsson, Hilton Kobus, K.Paul Kirkbride, Nicolas H.Voelcker

PII: S1387-3806(17)30138-0
DOI: http://dx.doi.org/10.1016/j.ijms.2017.08.001
Reference: MASPEC 15844

To appear in: *International Journal of Mass Spectrometry*

Received date: 28-3-2017
Revised date: 1-8-2017
Accepted date: 2-8-2017

Please cite this article as: Eliza C.Moule, Taryn M.Guinan, O.Johan R.Gustafsson, Hilton Kobus, K.Paul Kirkbride, Nicolas H.Voelcker, Silver-assisted development and imaging of fingermarks on non-porous and porous surfaces, International Journal of Mass Spectrometryhttp://dx.doi.org/10.1016/j.ijms.2017.08.001

Silver-assisted development and imaging of fingermarks on non-porous and porous surfaces

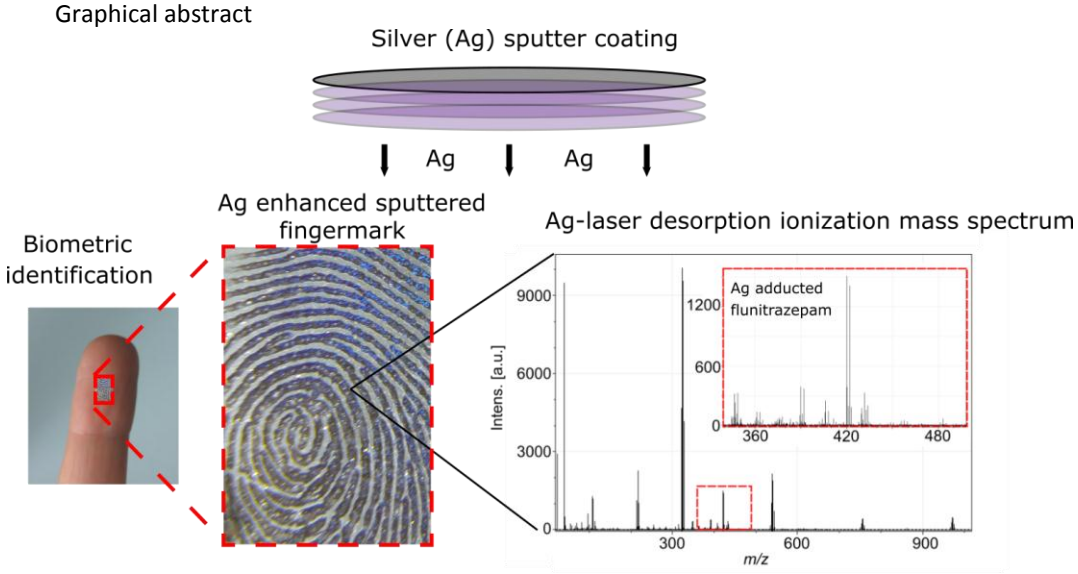
Eliza C. Moule[a±] eliza.moule@flinders.edu.au
Taryn M. Guinan[b, c, 1±] taryn.guinan@monash.edu.au
O. Johan R. Gustafsson[b,c] johan.gustafsson@unisa.edu.au
Hilton Kobus[a], Hilton.kobus@flinders.edu.au
K.Paul Kirkbride[a] paul.kirkbride@flinders.edu.au
Nicolas H.Voelcker [b,c, 1*] nicolas.voelcker@monash.edu.au

a. *Flinders University South Australia, Sturt Rd, Bedford Park; SA, Australia*
b. *Future Industries Institute University of South Australia, Mawson Lakes Blvd, Mawson Lakes; SA, Australia*
c. *ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Mawson Institute, Mawson Lakes Blvd, Mawson Lakes; SA, Australia*

[±]*These authors contributed equally to this work*

[*]*Corresponding author*

Graphical abstract



Highlights

- One-step method to enhance latent fingermarks and facilitate mass spectral imaging
- Fingermark imaging based on mapping of small endogenous compounds on ridges
- Differentiation between compounds on surface and ridges through bisecting k-means
- Identification and mapping of illicit substances on the ridges of fingermark

Abstract

In order to deal with the range of surfaces encountered in crime scenes and items associated with crimes, forensic fingermark examiners must have access to a range of latent mark enhancement techniques, each compatible with a particular type of surface. Consequently, the development of techniques with universal or even wide-ranging surface compatibility would be valuable to law enforcement.
Herein, we describe a one-step silver sputtering method for the enhancement of latent fingermarks on plastic, glass, paper and metal substrates. Whilst this method allows for the ridge pattern to be captured for human identification purposes, the majority of this article relates to downstream mass spectrometric imaging of the fingermark in order to display the spatial distribution of common endogenous and exogenous substances such as illicit drugs

Key Words: Silver, identification, mass spectrometry imaging, fingermark development, drugs.

1. Introduction

Fingermarks detected at crime scenes are often crucial to an investigation as they provide identifying information regarding the donor. Furthermore, with appropriate chemical analysis, compounds within fingermarks can be identified, which offers the potential to reveal circumstantial, non-biometric evidence in the form of substances that the donor has touched or ingested prior to deposition of the fingermark. Analytical techniques based on mass spectrometry (MS) are valuable in this application. These range from non-imaging approaches (e.g., swabbing the mark followed by gas chromatography-mass spectrometry [1]) to techniques involving mass spectrometry imaging (MSI) with spatial resolution sufficient to demonstrate fingermark ridge pattern detail (e.g., time of flight-secondary ion MS [1], desorption electrospray ionization MS [2], matrix assisted desorption electrospray ionization MS [1, 3, 4] and laser desorption ionization techniques [5]). Substances detected have ranged from environmental contaminants, such as quaternary ammonium compounds and siloxanes, to illicit drugs and their metabolites and many endogenous compounds [2, 3, 4, 5].
Substrates onto which fingermarks are typically deposited vary in composition, and are divided into three categories – porous (e.g. paper), semi-porous (e.g. polymer banknotes) and non-porous (e.g. glass or metal). A range of particular protocols for enhancing or developing latent fingermarks (i.e., marks not visible to the naked eye) on each of these surfaces is available to the forensic fingermark examiner [6]. Unfortunately there is no one universal technique that can be applied to visualize latent fingermarks on all surfaces.
Techniques for the detection of fingermarks by metal deposition are available to the fingermark examiner, such as multi metal deposition (MMD) [7] and vacuum metal deposition (VMD) [8]. These are relatively complex techniques or involve multiple preparation steps. For example, MMD requires the immersion of substrates in colloidal gold (Au) and subsequent enhancement using a physical developer [7]. For this preparation the Au particle size, homogeneity and concentration as well as the pH and temperature of development are critical [7]. Vacuum metal deposition

requires the placement of a latent mark into a high vacuum chamber in which a metal (or metals) is vaporized at high temperature for condensation onto the fingermark [8]. We report here a variant of metal-assisted enhancement of latent fingermarks that involves simple sputter-coating of silver onto the fingermark and its substrate. In metal sputter-coating, a metal target is bombarded with heavy gas ions, such as those in an argon (Ar) plasma. Sputtering occurs when the collisional energy transferred to the metal target exceeds surface binding energy and metal atoms are ejected from the target [9]. Traditionally, sputter-coating of substrates with Au, silver (Ag) and platinum (Pt) is used prevent sample charging and increase thermal conduction in electron microscopy, although there are reports of sputtered coatings of gold, copper, platinum and zinc being used to enhance latent fingermarks [10-11]. More recently, sputter coating of these metals has found application in mass spectrometry imaging (MSI) [12-14], where mass spectra are acquired in a rastered grid across samples that range from tissue sections to fingermarks. MSI is an emerging technique of interest for forensic applications as it allows the spatial mapping of chemicals trapped in fingermarks, including drugs, explosives, wax esters (WE), triacylglycerols (TAGs), and fatty acids (FAs) [12, 13]. In addition to aiding molecular ionization, Ag-assisted MSI has several advantageous features, including the introduction of Ag clusters at regular mass intervals, allowing internal recalibration of MSI data, and the formation of Ag adducts with FAs, WEs and sterols [12-14]. During MSI experiments in our laboratories, it was observed that Ag sputter coatings create significant visible contrast between the substrate and deposited fingermark material. This was observed previously by Lauzon *et al*., specifically for the MSI analysis of paper and glass samples [12]; the present technical note is an exploratory extension of Lauzon's work. Here, we show that sputter coating with Ag coats the ridges (unlike VMD that coats the valleys) allowing for MSI analysis of ridge contents in fingermarks. Our method does not require the addition of matrix (which can degrade spatial resolution), uses Ag coatings for accurate mass identification and allows high fidelity optical imaging of ridge patterns.

We hypothesised that Ag sputter coating may i) be a viable addition to the range of metal deposition-based fingermark development protocols currently used in forensic science, ii) be amenable to a broad range of surfaces, and iii) facilitate the MS and MSI of endogenous and xenobiotic molecules in fingermarks.

The present work demonstrates that this one-step technique does potentially offer benefits, both as an alternative to the complex development procedures currently used to visualize fingermark ridge pattern and as a second tier of data that can be overlaid with fingermark morphology to provide mapping of small molecules of interest. We present the use of ≈15 nm sputter-coated Ag layers to develop and image fingermarks on porous (paper, cardboard and Parafilm^{TM}), semi-porous (polystyrene, PS and polypropylene, PP based plastic objects) and non-porous (glass, aluminium and silicon wafers) substrates. Subsequent interrogation of fingermarks on porous (card) and non-porous surfaces (silicon piece) by MSI was used to map fingermark-specific compounds and, for the first time, successfully detect flunitrazepam in latent fingermarks on glass.

2. Materials and Methods

2.1 Fingermark deposition

Fingermarks from a female donor were deposited as a depletion series onto various substrates. The index finger was wiped once across the forehead and then consecutively deposited on various substrates (n=8) in no particular order. Marks were sputter coated within 10 min of deposition. Prints were photographed after Ag deposition and then subjected to analysis by MSI.

2.2 Ag sputter coating

Substrates were coated with an approximately 15 nm thickness Ag layer using a Q300T-D sputter coater equipped with a quartz crystal microbalance (QCM, Quorum Technologies, United Kingdom). Ag of 99.9999% purity was used.

2.3 Flunitrazepam-spiked fingermark

The spiked fingermark was prepared by rubbing a male donor's finger across the back of the donor's neck and then rubbing the finger in a residue prepared by applying 50 µL of flunitrazepam solution in acetonitrile (200 µg/mL) to a microscope slide and then allowing it to dry. The fingermark was subsequently deposited on a glass slide and sputter-coated with Ag prior to MSI analysis.

2.4 Scanning fingermarks

After fingermark deposition and sputtering, substrates were scanned at 4800 dpi resolution using an Epson Perfection V600 flatbed scanner (Officeworks, Australia). After scanning, the substrates were attached to a pre-spotted anchor chip adaptor plate (Bruker Daltonics) with double sided carbon tape and MSI measurements were performed.

2.5 MSI analysis

Mass spectra were collected using an ultrafleXtreme MALDI-TOF/TOF mass spectrometer, equipped with a 2 kHz pulsed Nd:YAG laser, in reflectron positive mode in the mass range *m/z* 20-1500 (Bruker Daltonics, Bremen, Germany). Data acquisition used flexControl 3.4.78 software and data analyses were performed using flexImaging 3.4 and SCiLS Lab (SCiLS GmbH, Bremen, Germany), resolution of the MSI was 60µm. Laser power was user-optimised, as required, for each Ag-coated substrate investigated. Ag isotopomers were used as internal calibrant points, as in Guinan *et al.* [13].

3. Results and Discussion

Figure 1 displays manually grey-scaled optical scans of fingermarks deposited onto example substrates and sputter-coated with Ag (≈ 15 nm). In each example, a clear contrast was observed between the substrate and the fingermark. On the semi-porous (polypropylene plastic bottle cap, Figure 1A) and non-porous (aluminium, Figure 1B and polished silicon, 1D) substrates analysed in this sample set, clear ridge and pore detail was observed. For example, microscope images at both 0.75 and 2x magnification, respectively, clearly showed visible pores and minutiae for Ag-coated semi-porous PS based coffee lids (Figure S1A-B). In contrast, for porous materials such as paper (Figure 1C), cardboard (Figure 1E) and Parafilm (Figure 1F), clear ridge detail

is shown but the pores become less visible: these fingermarks were fresh (10 min old) and as such it is expected that older fingermarks may be more difficult to image in this way, due to continual absorption of the deposit into the paper matrix[15]. The absence of clear pore structure is further demonstrated by the Parafilm fingermarks where pores were not clearly visible (Figure S2A-B). However, the ridge detail itself (yellow in colour) was evident with respect to minutiae. A Ag-coated polypropylene bottle cap lid (Figure S3A) and aluminium foil (Figure S3B) are also shown. Fingermarks deposited onto aluminium foil (Figure 1B and Figure S3B) show clear contrast and the presence of both minutiae and pore patterns. Finally, contrast between the fingermark and substrate with good ridge detail was observed for sputtered Ag on both non-porous rough and polished silicon wafers (Figure S4).
These key findings suggest that Ag-sputtering may make the enhancement of fingermarks on porous substrates more straightforward than current metal-based techniques on these difficult substrates. This versatile, one-step technique for latent print enhancement can be applied to a broad range of substrates, as demonstrated by this work, which has significant forensic applications to visualise latent prints on objects found at a crime scene.

To demonstrate that the Ag-coating method allows for MSI of fingermarks on these varied substrates, laser desorption ionization (LDI)-MSI experiments were conducted on a non-porous (rough silicon) and porous sample (paper) [Figure S4/S5]. Figure 2 highlights that, for both paper and rough silicon MSI, common fingermark constituents were observed: this includes potassium (*m/z* 38.963), Ag-adducted stearic acid (*m/z* 391.176), Ag-adducted WE 36:1 (*m/z* 641.142) and TAG 48:1 (*m/z* 827.710) [Ag-calibrated spectra in Figure S6A-B and S7A-B]. Interestingly, pores that were not visible in the scanned image of the paper substrate (Figure S5) were detected and mapped in the MSI analysis. Morphological similarities in the Ag-MSI data revealed that “pore” like structures (≈100-150 µm in diameter) could be correlated to the same features in the fingermark optical scans (Figure S8A-B), however further imaging experiments on multiple donors would need to be carried out to confirm this. Furthermore, not only can fingermarks be developed for visual inspection and identification, but MSI can augment this visual aspect to provide molecular composition information for a given donor fingermark. From a forensic standpoint, the Ag sputtering of evidentiary items is of interest for latent prints from a crime scene. The application of MSI to fingermarks on NIMS substrates [13] identifies similar characteristics, however has less forensic crime scene value.

To further demonstrate the utility of combining fingermark development with targeted MSI, fingermarks were spiked with flunitrazepam and deposited onto a glass slide (optical scan provided in Figure S9). A high concentration of flunitrazepam was utilised in order to compensate for loss of drug during collection of the drug from the glass slide and subsequent print deposition, however limits of detection for this technique were determined to be 10 ng/mL (Figure S10). Ag-MSI demonstrated that Ag-adducted flunitrazepam could be mapped (Figure 3A) with the ion distribution closely following the fingermark ridge pattern. A full mass spectrum recovered from the ridges of the fingermark displaying the detection of flunitrazepam is provided in the supplementary information (Figure S11) along with the chemical structure (Figure

S12). A full mass spectrum recovered from the ridges of an un-sputtered fingermark on glass is also provided in the supplementary information (Figure S13), for comparison with the sputtered spectra (Figures S6 & S11). Figure 3B shows a single Ag-MSI spectrum for flunitrazepam detection (3B, black trace) from the Ag-MSI data in Figure 3A, as compared to an un-spiked control fingermark spectrum where these peaks are absent (3B, blue trace). The measured isotopic distribution for Ag-adducted flunitrazepam overlaid well with the expected theoretical isotopic distribution, further supporting the assignment of this peak cluster as flunitrazepam (red trace, isotope abundances are provided in Table S1). Silver ion clusters produced from the sputtered layer during ionization allowed for internal mass calibration. This straightforward approach allowed for a match error between measured and theoretical *m/z* of less than 5 ppm for this drug (values provided in Table S2).

Finally, as part of a data-driven discovery approach, a bisecting k-means segmentation was applied, with the aim of isolating regions within the MSI data that exhibited similar peak composition for their underlying spectra [16]. Figure 4 presents the results of the segmentation for the paper substrate (scan in Figure 4A). The result indicated the presence of at least five different segments, which differentiate the ridges (light purple, Figure 4B) from the valleys (dark purple, Figure 4B) of the fingermark, and two different areas of the printed ink design on the paper (white, orange and red in Figure 4B). As expected, the spectra on and between the ridges were dissimilar to the spectra from the printed design. For the printed design, the segments spatially matched closely to the visible text of the print design on the paper; the molecular profile underlying the letter from the logo, “E”, could be clearly delineated. The contrast between the printed design and the fingermark mainly arises due to the abundance of potassium in the spectral data. As potassium is present in both the ridge pattern and the printing, the level of contrast achieved is quite remarkable and demonstrates the richness and potential power of the data acquired through MSI.

4. Conclusions

Herein, we have highlighted a straightforward, one-step preparation method for the detection and enhancement of fingermarks using sputter-coated Ag. We demonstrate that this method is useful for enhancing the fingermark ridge pattern on porous, semi-porous and non-porous samples, with the possibility for subsequently mapping and identifying small molecules on these fingermarks using MSI. This is possible in either a targeted approach (flunitrazepam) or in a non-targeted segmentation approach (bisecting k-means) to identify the ridge pattern itself and other underlying changes in molecular composition. Future validation experiments to determine whether comparable sensitivity is achieved across donors and whether fingermark aging has any impact on detection will allow a comprehensive assessment of this technique. It will be important to determine whether limits of detection of drugs in fingermarks approaches the range of 0.001 mg/mL (expressed as drug concentrations in sweat), which is required to detect a typical therapeutic dose of drugs such as flunitrazepam [17]. Additionally, development of methods for detection of drug metabolites is vitally important as the presence of metabolites ensures that proof of drug ingestion is

detected, rather than simply drug handling. Whilst the Ag-sputtering technique is applicable to a range of surface types and topographies, MSI as described in this article is applicable only to flat surfaces. Further development of techniques for the MSI of fingermarks on 'rough terrain', akin to the developments reported for laser ablation electrospray ionization MSI [16] will be valuable so that fingermarks lifted from crime items can be analysed in the manner described herein. This work is ongoing at the Future Industries Institute (Adelaide, South Australia), with the use of Al for a similar method having already been described [4].

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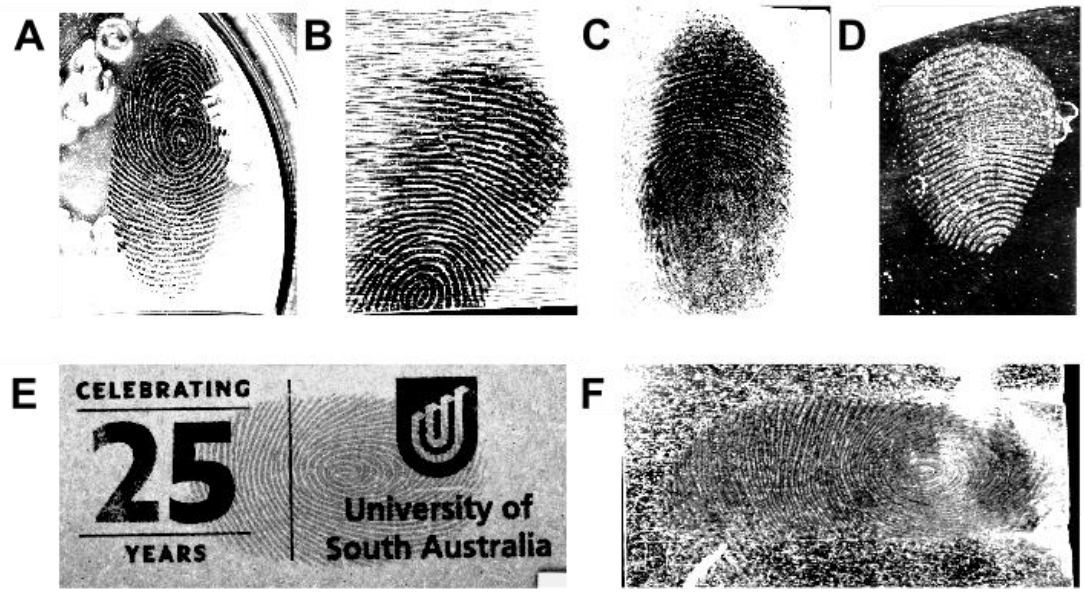

Figure 1. Scan images of fingermarks deposited on A) plastic coffee lid, B) aluminium foil, C) paper, D) polished silicon, E) cardboard business card and F) Parafilm.

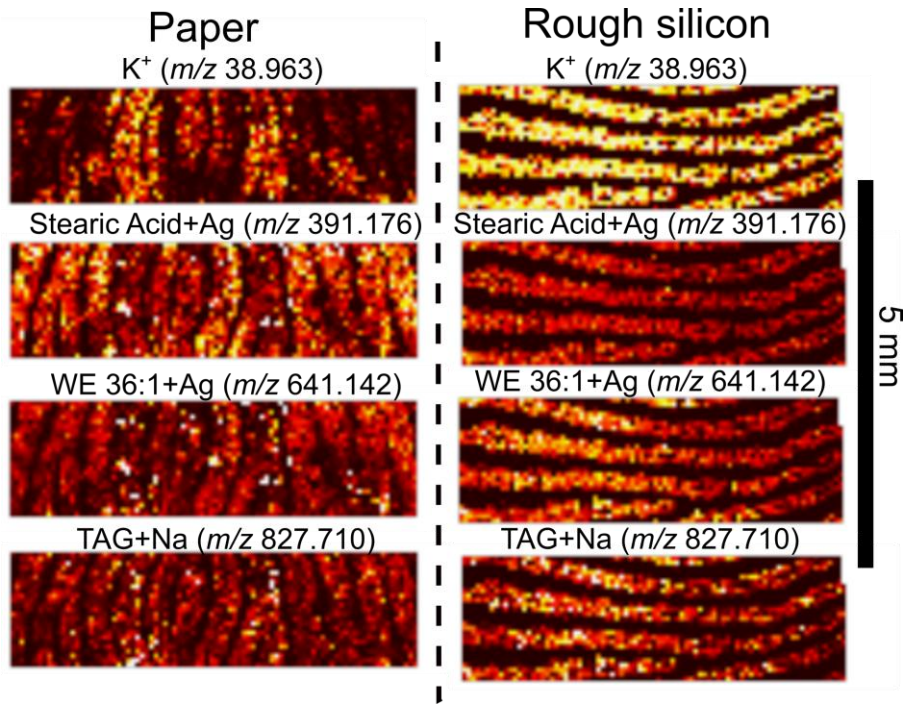

Figure 2. Representative Ag MSI images for K^+ (ion of *m/z* 38.963), Ag-adducted stearic acid (ion of *m/z* 391.176), Ag-adducted WE 36:1 (ion of *m/z* 641.142) and Na-adducted TAG 48:1 (ion of *m/z* 827.710) from paper and rough silicon, respectively. Scale bar is 5 mm.

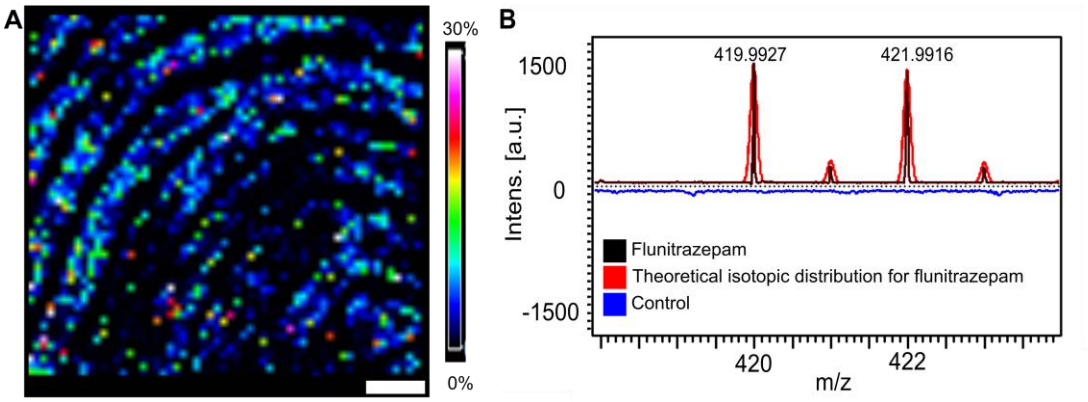

Figure 3. A) Ag-MSI ion intensity map for flunitrazepam (*m/z* 419.9908) as measured from a spiked fingermark on a normal glass slide. Scale bar is 500 µm. B) Overlaid Ag-LDI spectra for a spiked fingermark (black trace) showing the detection of flunitrazepam and an un-spiked control (blue trace) showing no ions at *m/z* 419.9927 or 421.9916. The theoretical isotopic distribution for Ag-adducted flunitrazepam is shown in the red trace (FWHM= 0.1).

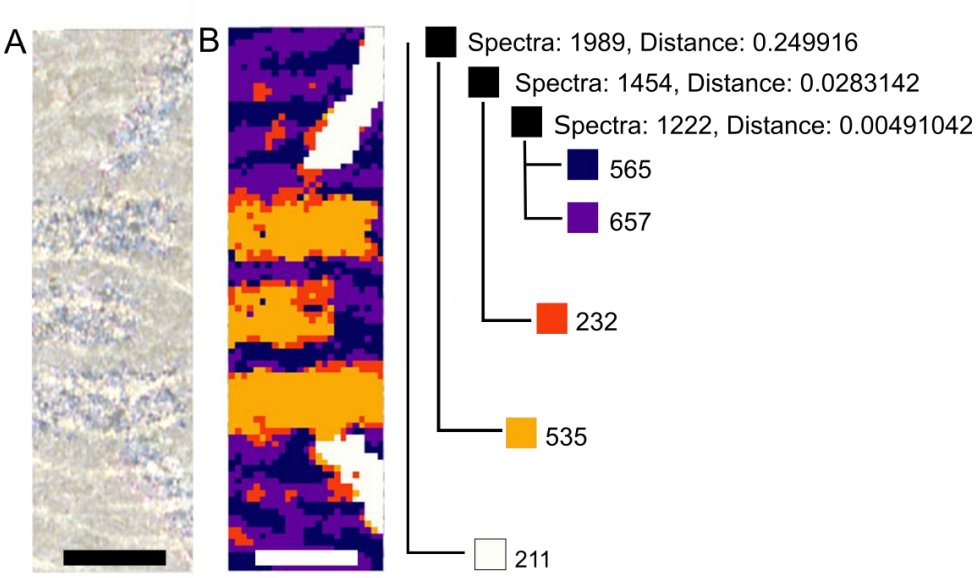

Figure 4. A) Scanned image of the fingermark after Ag sputter coating on paper and B) bisecting k-means results overlaid onto the scan alongside the dendrogram of five identified clusters, showing the relative similarities of the spectra and the corresponding false colours given to the clusters in the fingermark MSI data. Scale bar on both A and B is 1 mm.