

Techniques for Fingermark Analysis Using MALDI MS: A Practical Overview

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Abstract Matrix-assisted laser desorption/ionization (MALDI), mass spectrometry profiling (MSP), and mass spectrometry imaging (MSI) are continuously pushing the boundaries of many applications in which they can be employed. In the field of forensic science, MALDI MS has proven to be a useful technique but reports were sporadic and only in an academic context until 2009. However, in the past 6 years it has been demonstrated that MALDI MSP and MSI can be practically implemented as an analytical forensic tool in the context of fingermark/fingerprint analysis. Previously always regarded ‘simply’ as physical evidence, fingermarks are an invaluable source of chemical information that can be exploited to provide intelligence on an individual’s lifestyle and activities taking place prior to the crime. A solid proof of principle of the versatility and robustness of MALDI MSP and MSI has gained MALDI a place in the newly launched *Fingermark Visualisation Manual* edited by the Home Office of the United Kingdom (UK), whose guidelines are followed in the UK and are highly regarded worldwide. Protocols are currently being developed to be used operationally within primarily UK law enforcement. In this chapter, a practical overview is given for informed method development. Protocols that have been successful in answering specific research questions will also be outlined along with the most significant and interesting applications of MALDI MSP and MSI in fingermark/fingerprint analysis.

1 Introduction

There is little doubt that MALDI-based techniques are very versatile and can assist in the elucidation of a number of different research questions in the life sciences. The year 2009 saw the birth of yet another application of MALDI, namely the chemical mapping of fingermarks.

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For over a hundred years, fingermarks/fingerprints¹ have been seen ‘only’ as a source of physical evidence; the fingermark/fingerprint ridge² pattern, including the ridge flow (level 1 details) and local characteristics of the ridge flow called *minutiae* (level 2 details) have been exploited for suspect identification on the basis that each individual has unique fingerprints. Physical comparison of level 1 and level 2 details between a ridge impression found at a crime scene and fingerprints stored in databases is the process through which fingerprint experts may identify a suspect, following strict country-specific evaluation and cross-examination rules for declaring a match.

Despite the advent of DNA analysis, ‘fingerprinting’ is still the most used and successful form of biometric identification, leading the way in the UK for suspect identification and being responsible for two thirds of all identifications (N. Denison, Head of Identification for the Humber and Yorkshire Region UK, personal communication).

Although hugely successful, ‘fingerprinting’³ has its pitfalls and does not succeed when the quality of the visualized crime scene ridge impression is inadequate; this could be due to its original nature (smudged, partial, empty, overlapped marks) or the inadequacy of the forensic enhancement workflow, the choice of which is heavily dependent on the level of expertise/competence of the CSI,⁴ the surface of deposition, the environmental conditions to which the ridge impression was exposed, and the age of the fingermark. Some of these factors, such as environmental conditions and age, are either very difficult or impossible to correctly evaluate at the crime scene, making the visualization and development of the fingermark not trivial, despite world-leading guidelines, published in the *Fingermark Visualization Manual* edited by the Home Office, UK (Bandey et al. 2014). Additionally, should the suspect not have been previously convicted, their fingerprints will not be present in the National Database, making the quality of the crime scene ridge impression unimportant for database search, comparison, and match.

However, ridge impressions are not ‘simply’ physical evidence but indeed a very valuable source of chemical intelligence. They originate from the transfer of sweat to a surface from an individual’s fingertip upon contact. If this contact is intentional, such as control ridge impressions taken at police stations or airports, we should use the term ‘fingerprints’ as opposed to ‘fingermarks,’ which instead result from the accidental and involuntary contact with surfaces. Sweat carries a plethora of biological substances and consequently latent fingermarks⁵ are a complex biological matrix featuring the presence of inorganic and organic constituents such as alkali metals/salts, amino acids, lipids, and proteins (Knowles 1978; Ramotowski 2001),

¹Fingerprints are obtained intentionally, for instance at police stations or airports, while fingermarks are a result of accidental and involuntary contact with surfaces (more details below).

²In fingerprints, ridges are the ‘lines’ generated by the raised parts of the skin on fingertips.

³In a forensic context ‘fingerprinting’ refers to the process of identifying an individual through their fingerprints. This is different from ‘fingerprinting’ used in proteomics where this term refers to the identification of a protein usually through a bottom-up approach.

⁴Crime Scene Investigator.

⁵The most used definition of latent fingermark is ‘a fingermark invisible to the naked eye.’

which we can define as *endogenous* as they come from within the body. In addition, any substance our fingertips come in contact with can be transferred to a surface and subsequently detected in a fingermark; we can commonly refer to these as *exogenous* substances. Examples of these substances as obvious sources of forensic intelligence may include drugs of abuse, explosives, condom lubricants, make-up, paint, and blood.⁶ Our group also refers to a third category of species as *semi-exogenous* when they are not naturally present in our body but they are, in different ways, introduced and excreted (intact or metabolized) through sweat, such as substances (metabolites) derived from prescription medications or drugs (Bradshaw et al. 2015), including smoke (Benton et al. 2010a, b) and even consumed food and drinks (Bradshaw et al. 2012).

These three categories of chemical species, endogenous, exogenous, and semi-exogenous are an invaluable source of intelligence. For example, lipids and proteins (endogenous species) can often act as biomarkers, a term used in the biomedical field for (biomolecular) species indicating a physiological state of the individual, being pathological (diseases), pharmacological (response induced by medications), or biological (molecular make-up placing the individual into a biological category such as diabetic). It is therefore entirely conceivable to think of the detection of these substances as a forensic opportunity to provide additional intelligence on the suspect, as will be discussed in Sect. 2.

Given the huge potential of fingermarks as a source of ‘enhanced’ intelligence and the profound implications and impact this could have on a more efficient Criminal Justice System (with attached savings to the public purse), it is not surprising then that the analytical science community, with a keen interest in forensic science, has made tremendous efforts in the last few years to develop, either existing or new, analytical tools specifically for the detection, and in some cases, mapping of fingermark residuals. A plethora of different and possibly suitable analytical techniques have been reported in the literature for the analysis of fingermarks but only a number of the authors have shown dedication to this area of research with follow-ups and clear developments. These techniques can be grouped into spectroscopic and mass spectrometric, with the latter category being the strongest in terms of diversity of the ionization techniques used (DESI MS, SALDI MS, DART MS, SIMS, and MALDI MS) and ‘presence’ in the literature. Figure 1 shows a representation of the above analytical efforts in the form of number of publications per technique, starting from 2008 onwards.

Although DESI was the first mass spectrometric technique showing its potential for fingermark profiling and imaging (Ifa et al. 2008), only MALDI MS and SIMS are presently featured in the recently re-edited Home Office *Fingermark Visualization Manual*.⁷ Compared to the other techniques reported in Fig. 1, and despite inevitable

⁶Blood may be present on the surface prior to the deposition of the fingermark (mark in blood) or landing some time after the fingermark deposition (coincidental association), or coming from the fingertip of the owner of the mark (blood mark).

⁷MALDI is reported as a new process having high potential to be implemented in the forensic practice (Cat C) but still under development to reach the required maturity (TLR 3–4).

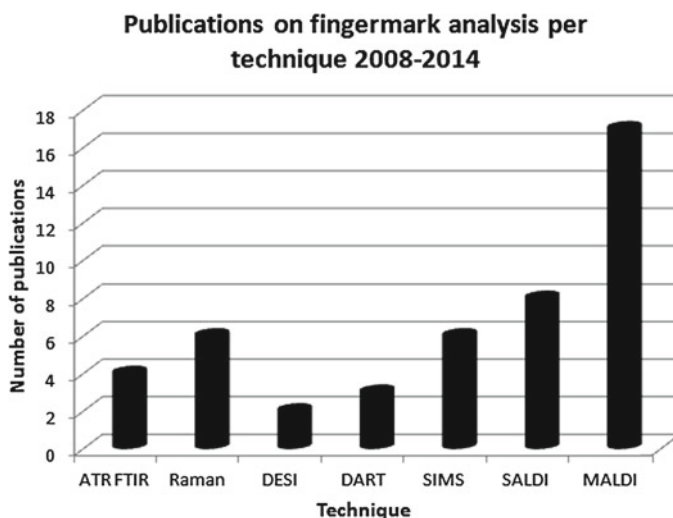


Fig. 1 Histogram showing the number of publications featuring the use of either ATR FTIR, Raman (in its various forms including SERS and CARS), DESI, DART, SIMS, SALDI, or MALDI (in profiling and/or imaging mode) for the analysis of fingerprints. Figures reported refer to peer-reviewed papers in 89% of the cases while the remaining 11% originate from online magazines, technical articles, or application notes. This information has been obtained by searching both Pubmed and ISI Web of Science databases using the keywords fingerprints, fingermarks, MALDI, ATR FTIR, Raman, DESI, DART, SIMS, SALDI, and MALDI

limitations as with any analytical technique, MALDI MS has clearly demonstrated its potential, particularly with respect to versatility, robustness and compatibility with current fingerprint enhancement techniques (FET) in a number of different scenarios, in both profiling and imaging mode (Bradshaw et al. 2013a), and having the highest number of publications to date. In terms of feasibility, a leap forward was made with the invention of the *dry-wet* method (Ferguson et al. 2011). This 2-step method enables visualization of fingerprints on surfaces by: 1) brushing the surface with α -cyano-4-hydroxycinnamic acid (CHCA), a common MALDI matrix, and recovering the evidence through tape-lifting, and 2) homogeneously and rapidly spraying the mark with a solvent mixture, in which both the matrix and the analytes of interest are soluble, making the mark ready for MALDI MSI analysis. Together with its wide compatibility with current FET, this method probably initiated the superiority of MALDI over those analytical techniques that can only be used in academic settings. Therefore, for MALDI, the *dry-wet* method ‘bridges the gap’ by actually making the MS analysis of ‘real’ fingerprint evidence possible. Applications and protocols for this method are discussed in Sects. 2 and 3, respectively.

Though the option is now available to locate, recover, photograph, and analyze the mark with the *dry-wet* method (albeit further development is in progress for wider applicability), it is not expected that this method entirely replaces a century of forensic practice to visualize marks on surfaces. Therefore, in order to integrate MALDI in the armory of techniques for fingerprint analysis, the application of

MALDI has been demonstrated to be compatible with the pre-application of existing chemical and physical developers currently used to localize marks at crime scenes. FET compatibility is strongly desired by the UK Home Office as it would allow new and emerging technologies to provide additional forensic intelligence to both inform investigations and provide court room evidence. With regard to mass spectrometry, two UK groups have demonstrated a certain degree of compatibility of the techniques SIMS and SALDI MS with cyanoacrylate fuming, with SIMS showing compatibility also with powder suspension (Bailey et al. 2013; Sundar and Rowell et al. 2014). Our group has shown much wider compatibility of MALDI (for MSP, MSI or both) as it can be used in tandem with some blood enhancement techniques (BET) (Bradshaw et al. 2014), enhancement powders, vacuum metal deposition, 1,8-diazafluoren-9-one, and ninhydrin (Bradshaw et al. 2013a) on a number of different surfaces and under different environmental conditions in laboratory settings and, in some cases, in pseudo-operational trials in collaboration with West Yorkshire Police (Bradshaw et al. 2014). For operational deployment, these findings are very encouraging and justify further research into the development of sample preparation and analytical protocols for MALDI MSP and MSI of fingermarks in forensics.

Indeed, since the pioneering start in 2008, the rapid evolution in the applicability of MALDI MS-based techniques for fingermark analysis required extensive methodological developments, taking into account the complex and varying chemical nature of fingermarks, the most appropriate corresponding options for their preparation as well as their correct storage. In this chapter, the most notable protocols of MALDI MS in the field of fingermark analysis will be discussed together with the limitations of the technique. ‘Tips’ will be given to assist both keen beginners and experienced MALDI users approaching fingermark analysis for the first time, thus enabling their contribution towards the development of operationally deployable protocols.

2 Applications

Valuable intelligence from latent fingermarks can be obtained using MALDI in profiling and/or imaging mode. In profiling mode (using MS and MS/MS), MALDI can be used for the quick detection or confirmation (in minutes) of the presence of forensically interesting substances. Speed in the acquisition of the intelligence is very desirable and the profiling mode can be employed on fingermarks previously visualized using a FET, which already yielded a suitable image of the ridge impression for suspect identification purposes. Like some other analytical techniques, MALDI can also be used for chemical imaging; this feature enables multiple images of the same mark to be obtained in under 90 min from sample preparation to the end of acquisition (depending on the mass spectrometer and laser frequency), potentially succeeding where FET failed or further ‘improving’ the quality of the mark image. Multiple images may be stitched together (Fig. 2a) or superimposed (Fig. 2b) to improve ridge continuity, to increase completeness of the mark image, or even to separate

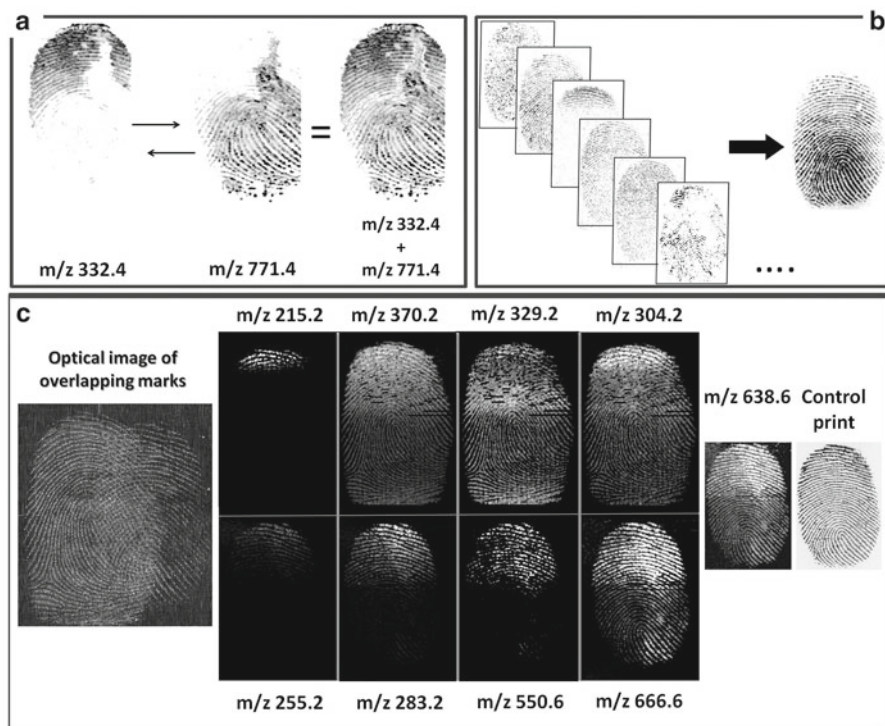


Fig. 2 Improving the quality of fingermark images by MALDI MSI: (a) stitching m/z images together and (b) superimposing multiple m/z images of the same mark to improve ridge continuity; (c) separation of overlapping marks through interrogation of multiple m/z ions (Francese et al. 2013b, *Analyst*, **138**, 4215–4228, adapted and reproduced by permission of The Royal Society of Chemistry)

overlapping ridge impressions as previously shown by our group and illustrated in Fig. 2c (Francese et al. 2013b; Bradshaw et al. 2012). This in turn improves the quality of the database search and increases the chance of suspect identification.

When providing intelligence to inform investigations or court cases, mapping forensically interesting chemicals in fingermarks is also very important to make robust associative evidence. The use of MSI shows whether the chemical is, in fact, present on the ridges, rather than on the surface of the deposition where the mark lies. In the latter case, the detection of this chemical is just a contamination and this changes the forensic situation significantly.

For example, in a hypothetical rape case where the perpetrator had worn a condom, the ability to determine whether the condom lubricants are present on the ridges (i.e., condom lubricants-contaminated fingertip, indicating that the accused had handled the condom) or on both the ridges and the furrows⁸ (i.e., mark made in

⁸In fingerprints/fingermarks, furrows, otherwise termed valleys, are the ‘voids’ in between ridges and are generated by the lower parts of the skin on fingertips.

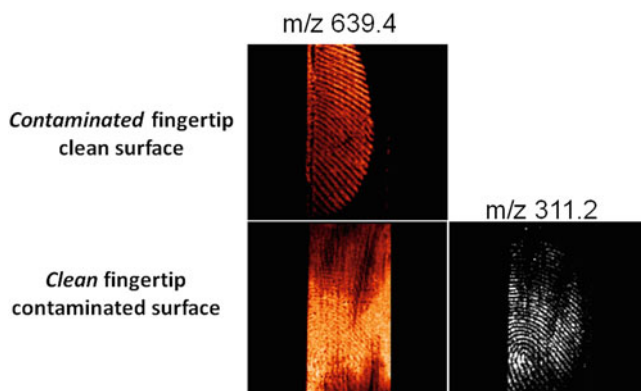


Fig. 3 MALDI MSI of *Trojan Enz*-contaminated fingerprints. The ion at m/z 639.5 (9-mer-nonoxynol 9) is solely distributed on the ridges of a mark if the fingertip came in contact with the condom lubricant prior to touching a surface. The same ion is distributed both on the ridges and the furrows if the mark was generated by a clean fingertip upon contact with a contaminated surface and conclusions can be corroborated by imaging another ion such as an endogenous fatty acid (m/z 311.2), the sole distribution of which on the ridges proves that the nonoxynol-9 image is not an artifact (Bradshaw et al. 2013b, *Analyst*, **138**, 2546-2557, adapted and reproduced by permission of The Royal Society of Chemistry)

condom lubricants, pointing at sexual activity prior to the alleged crime) might make the difference between an acquittal or a conviction. The opportunity to discriminate between these two scenarios has been illustrated by our group (Bradshaw et al. 2013b) and is shown in Fig. 3.

Indeed, the detection of condom lubricants may provide important associative evidence, which becomes probative when the lubricants profile detected in a mark matches that of an unopened condom packet found in possession of the alleged offender or within the victim's vaginal swab (if this is taken soon after the crime). It has also been demonstrated that 'condom discrimination' at a brand level (and in some cases at a type level) is achievable (Bradshaw et al. 2013b).

In the above work, a MALDI MSP sample preparation protocol was also suggested to obtain comprehensive information on the condom lubricant composition; polydimethylsiloxane (PDMS) was never detected using MALDI MSP and MSI in fresh contaminated marks. However, a set of experiments performed with the purpose of evaluating robustness of the methodology for aged condom-contaminated marks revealed that it was only possible to detect PDMS in aged marks, that is, marks kept at 25 °C and 60% relative humidity for a month. It can be speculated that the ageing treatment caused a decrease in the presence of species that are either more volatile or more prone to degradation than PDMS (under those conditions) and that these species may act as PDMS suppressants in fresh (non-aged) fingerprints. Detection of PDMS and ethoxylate-based polymers will be briefly discussed in Sect. 3.5.

In addition to lubricant detection and mapping by MALDI MSI, a protocol was devised to make this information more robust and retrievable if this technology was used in conjunction with other spectroscopic ('non-destructive') techniques such as Raman and ATR-FTIR spectroscopy (Sect. 3.5). This protocol provided complementarity of information on the condom lubricant composition together with the detection and mapping of the fingermarks' endogenous species as illustrated in Fig. 4.

Although much has been done to allow the detection of exogenous substances, valuable intelligence can also originate from the exploitation of the endogenous content of fingermarks. Lipids are certainly the easiest class of molecules to ionize as demonstrated by the first publication in the literature showing MALDI MSI analysis of endogenous lipids in ungroomed⁹ marks (Wolstenholme et al. 2009). They can be readily detected using CHCA as matrix in positive ionization mode. Although we found that this matrix was the most efficient overall, certain classes of fatty acids (nonpolar such as squalene) cannot be efficiently ionized, and doping the matrix with cations such as Li⁺ is certainly an option. Matrix-spotting and spraying techniques as well as the patented *dry-wet* method (see below in this section) work well to ionize lipids. In terms of spraying, our group has resorted to the SunCollect auto-sprayer (SunChrom, Friedrichsdorf, Germany) employing pneumatic atomization and being capable to spray an entire fingermark within 30 min for the matrix solution and within 10 min just for the solvent.

Recently, another automated matrix sprayer was trialed, the TM sprayer (HTX Technologies, NC, USA), and a very efficient protocol, allowing clear and detailed ridge visualization in under 1 min, was devised for the interrogation of lipids, which will be illustrated in Sect. 3.2. Lipids can be used for improving the quality of the ridge detail (Fig. 2) but they can also be an indicator of pathological states (biomarkers). Therefore, their detection and mapping may have both forensic and biomedical (prognostic) applications.

Another interesting class of biomolecules in fingermarks are peptides and proteins. Our group has exploited the detection of these species from the fingermarks of 80 donors to 'biologically categorize' and distinguish between fingermarks belonging to men and those belonging to women (Fig. 5), with an 85% prediction accuracy to date (Francese et al. 2011; Ferguson et al. 2012). This advance has unleashed our imagination and that of other researchers with respect to the achievable information on the individual's lifestyle and physiological state, using what could be termed 'offender chemical profiling.' Further work is in progress in order to increase the accuracy of prediction and robustness of the method by using a larger number of donors with no participation exclusion criteria, which were applied to the previous study.

Fingermark proteins originating from either the victim or the offender can also be detected and mapped. This has been the case when applying MALDI MSP and MSI to the specific detection of blood in fingermarks (or in bloodstains). A reliable

⁹Ungroomed fingermarks are 'lipid-depleted' marks from fingers that have been washed and have subsequently not touched any body parts or contaminants (to test the sensitivity of the technology). For a more comprehensive description, see Sect. 3.1.

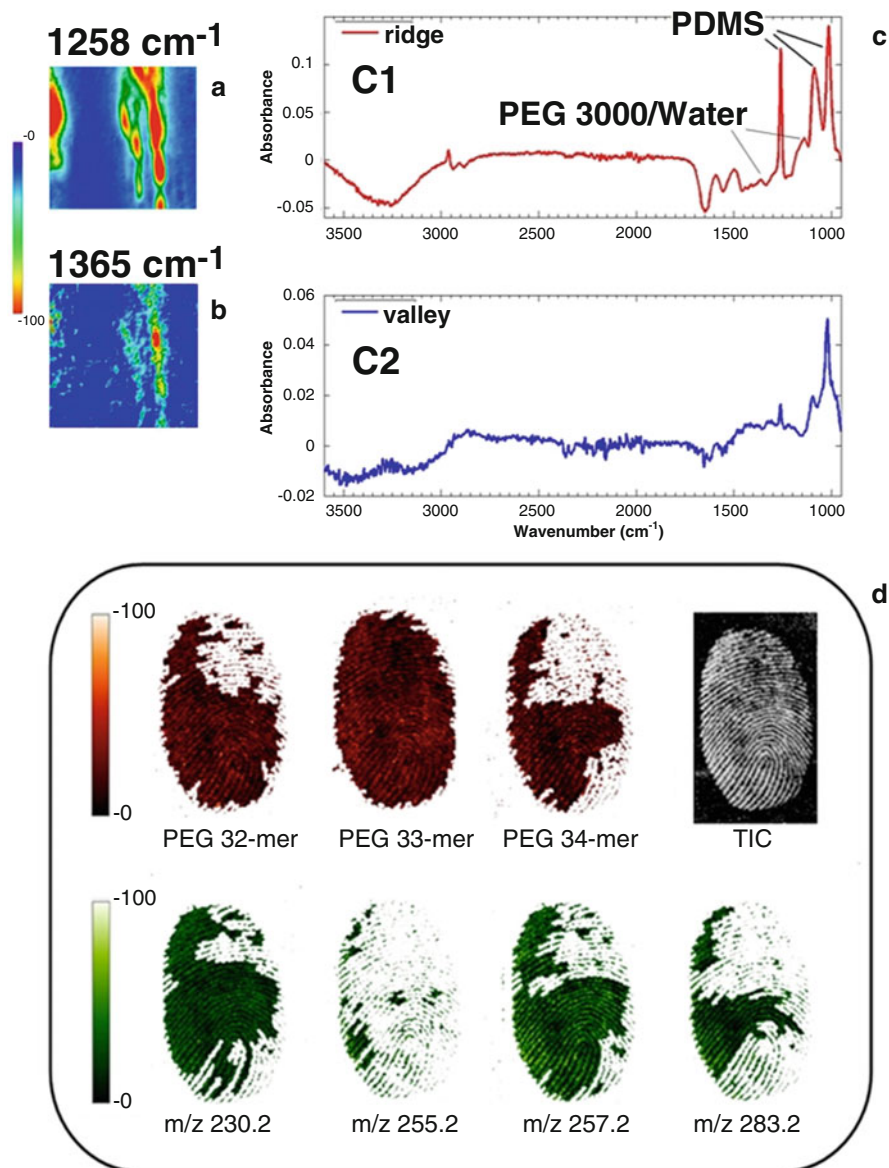


Fig. 4 Combined ATR-FTIR imaging/MALDI MSI analysis of a *Condomi Max Love*-contaminated fingermark. Panels A and B show the ATR-FTIR image of PDMS at 1258 cm⁻¹ and the ATR-FTIR image of PEG at 1365 cm⁻¹ present on the same ridges of a *Condomi Max Love*-contaminated fingermark selected region. Panel C shows two ATR-FTIR spectra from the ridge (C1) and the valley (C2) (high and low concentration of the two polymers respectively). Panel D displays the MALDI MS images of the PEG 32-mer, 33-mer, and 34-mer ion signals, the complete ridge pattern provided by the image of the total ion current (TIC), as well as a small sample set of the many fatty acids detected (Bradshaw et al. 2013b, *Analyst*, **138**, 2546-2557, adapted and reproduced by permission of The Royal Society of Chemistry)

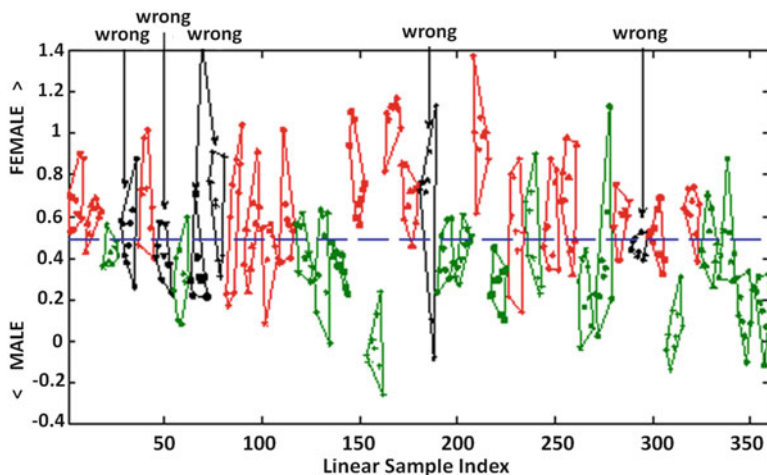


Fig. 5 Sex classification results and validation using an independent test set within partial least square discriminant analysis (PLSDA). Multiple spectra from each donor are grouped together and can be found within the area defined by a line connecting the peripheral data points. Prediction above the *blue threshold line* results in a female and below the threshold in a male sex assignment. Donors plotted in *red* are correctly predicted as female, *green* represents correct male prediction, and donors plotted in *black* and labeled with 'wrong' have more than half of their spectra assigned to the opposite sex (Ferguson et al. 2012, Analyst, 137, 4686-4692, adapted and reproduced by permission of The Royal Society of Chemistry)

identification of this fluid at the scene of a violent crime bears considerable forensic value. A few forensic tests are currently applied to the detection of blood in both bloodstains and fingermarks. However, these tests are presumptive as they can only indicate the presence of blood rather than confirming it (Bradshaw et al. 2014). This is due to the lack of specificity as the molecular triggers of the color change may come from a different source than blood. It is common knowledge that MALDI MS is a versatile technique in terms of the m/z range of the species that can be detected and is a particularly suitable technique for large molecules. Reports already showed the ability to detect both hemoglobin and heme in different fields of application such as microbiology, plant biology, and biomedicine (Yang et al. 2013; McComb et al. 1998). Encouraged by these preliminary reports, our group developed a method to detect both heme and hemoglobin from fingermarks and bloodstains. When devising a methodology with operational deployment in mind, one should try to minimize the number of protocols to apply. Therefore, after testing a range of conditions, the optimization of one CHCA matrix system was achieved (Sect. 3). Although MALDI MS has a suitable dynamic range for detecting both heme and hemoglobin simultaneously, the calibration of the MALDI instrument that was employed (Voyager DE-STR) did not cope with the large mass range, and the mass accuracy for the heme species was severely compromised. However, the method developed, using one matrix system for both molecules, allowed imaging analysis to map heme and a subsequent profiling analysis to detect hemoglobin in a 7-day-old mark

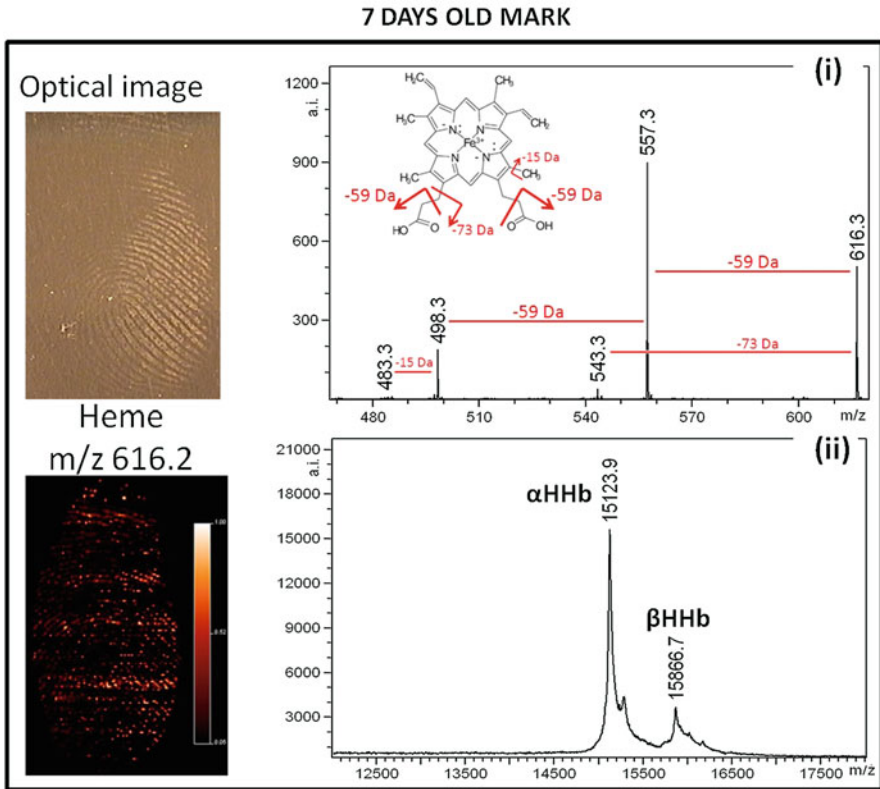


Fig. 6 MALDI MSI of a 7-day-aged blood mark. Heme (m/z 616.2) was successfully imaged and confirmed by the product ions at m/z 557.3 and 498.3 in the MS/MS spectrum of the parent ion at m/z 616.3 taken from a small area of the mark (i). Presence of human hemoglobin was ascertained by MALDI MSP analysis on the same mark in the higher mass range (ii) in a subsequent MALDI MS acquisition. Reprinted from Bradshaw et al. 2014, Direct detection of blood in fingerprints by MALDI MS profiling and imaging, *Sci. Justice*, **54**, 110-117, with permission from Elsevier

(Fig. 6), using two different calibration mixtures, one more appropriate to a narrower and lower mass range (phosphorous red for heme analysis) and the other more suitable for higher molecular weight species (protein standards mixture of ubiquitin, cytochrome C, hemoglobin, and apomyoglobin for hemoglobin analysis). This strategy also allowed us to confirm the presence of heme by MS/MS.

The devised protocol proved to be applicable to real crime scene samples as tested during a site visit alongside officers from the West Yorkshire CSI, UK. On that occasion a bloodstain, recovered and testing positive for blood by using the conventional Kastle Meyer Test, was also analyzed by MALDI MS, leading to the detection of the hemoglobin in addition to the heme ion signal (Bradshaw et al. 2014).

A significant part of the fingerprint analysis work using MALDI is also dedicated to make this application compatible with BET. In this context, MALDI MSI can be compatibly used with amino acid reagents (ninhydrin) and Acid Black 1 (protein

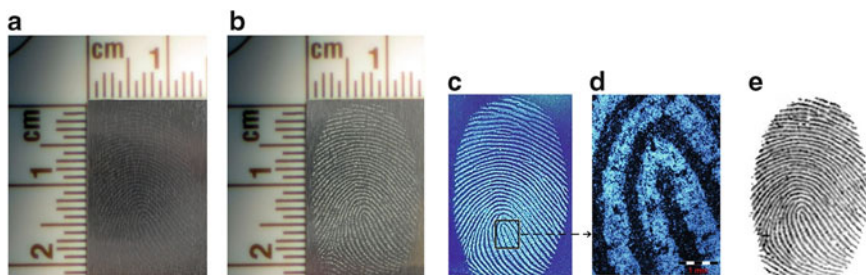


Fig. 7 Potential forensic fingerprint analysis workflow using the *dry-wet* method. The latent fingerprint (a) can be enhanced by brushing a MALDI matrix over the mark and photographed (b). The dusted fingerprint image can be captured using UV light at 365 nm for a more accurate database comparison (c). The dusted fingerprint can also be subsequently inspected by using fluorescence microscopy for accurate visualization of the minutiae: (d) illustrates a high magnification image of the fingerprint loop highlighted by the box in Panel (c). The fingerprint can finally undergo MALDI MSI analysis for obtaining chemical information: (e) shows the mass image of the m/z species at m/z 304 previously identified as dimethylbenzylammonium ion. Reprinted with permission from Ferguson et al. 2011, *Anal. Chem.*, **83**, 5585–5591. Copyright 2011 American Chemical Society

staining) in the case of fresh marks. However, a general decrease in the ion signal intensity for heme and hemoglobin is observed, and quantification of the loss of these signals compared to undeveloped marks remains a challenge. Though blood has been successfully and reliably detected in 7-day-old marks, work is in progress to determine the robustness of the methodology with older marks and for pre-developed marks (using other BET). A proteomic approach is currently being applied to make the techniques robust against possible hemoglobin degradation and loss of amino acid residues due to time and environmental conditions.

In many of the applications referenced above, the *dry-wet* method was successfully employed. As explained earlier, this novel method is considered a significant step forward towards implementing MALDI into forensic casework. In our workflows, marks are visualized by brushing the surfaces with the MALDI matrix (Fig. 7a), the visualized mark can then be photographed (Fig. 7b) or directly removed through tape-lifting. As the matrix is an UV-absorbing substance, UV image capture can also be utilized (Fig. 7c) as well as fluorescent microscopy (Fig. 7d) to reveal fine details of the ridge pattern, including pores. Photos or digital scans can be retained as evidence and, provided that the chain of custody is preserved, the same mark can be subjected to MALDI MSI (if additional images are needed) or MALDI MSP for additional intelligence as shown in Fig. 7e.

This workflow has been successfully trialed on a large range of deposition surfaces (including glass, different types of metal, wood, plastic, leather, cardboard, and ceramic tiles) of which Fig. 8 shows some examples.

Normally, the matrix of choice for MALDI MS fingerprint analysis is CHCA as it has so far proven to be the most efficient and versatile. However, a novel matrix has been recently reported by our group to work in some cases more effectively than

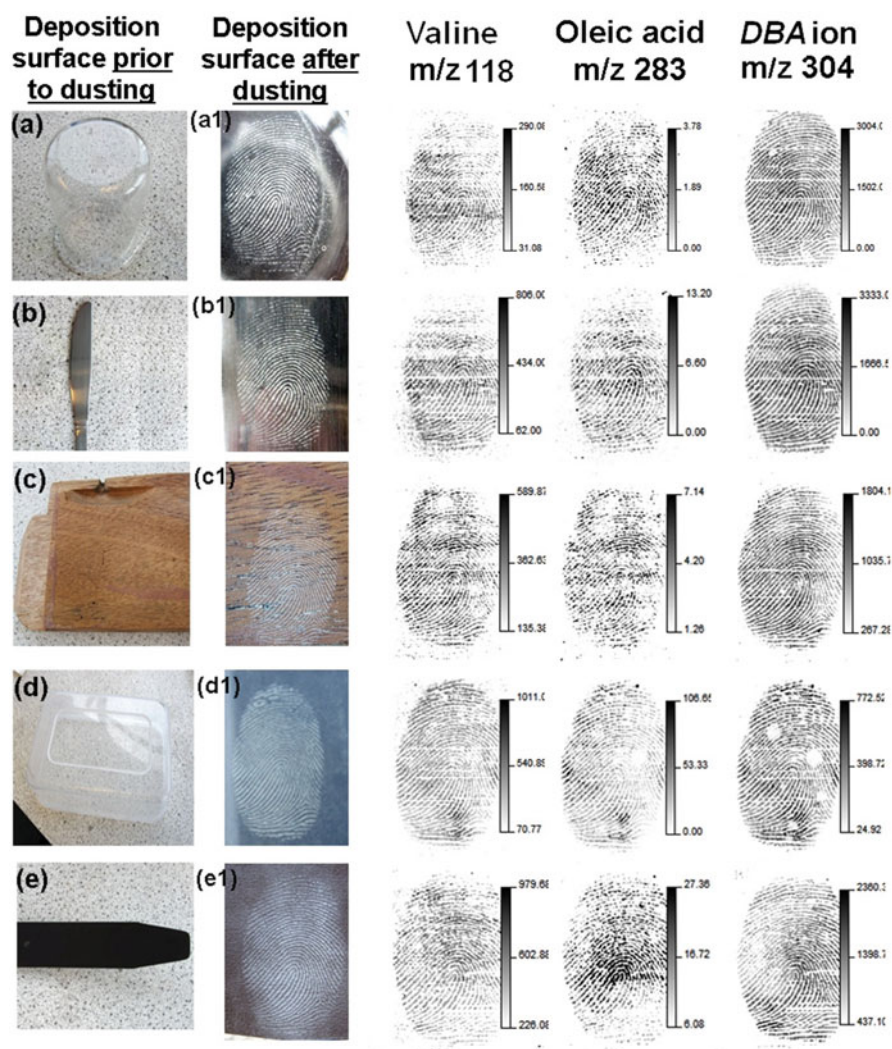


Fig. 8 Recovery and MALDI MSI analysis of ungroomed fingermarks lifted from different surfaces (panels a–e) using the *dry-wet* method. Representative MS images of an endogenous amino acid, an endogenous fatty acid, and an exogenous compound (dimethylbenzylammonium ion, DBA, m/z 304) are shown for each deposition surface. Reprinted with permission from Ferguson et al. 2011, *Anal. Chem.*, **83**, 5585–5591. Copyright 2011 American Chemical Society

CHCA (Francese et al. 2013a) as shown in Fig. 9. This novel matrix compound is curcumin, which is a polyphenolic compound naturally present in the *Curcuma longa* plant and also known as turmeric, displaying an extended structure of conjugated double bonds and making it a very efficient UV-absorbing molecule. Curcumin has proven to be versatile as it assists with the detection of pharmaceuti-

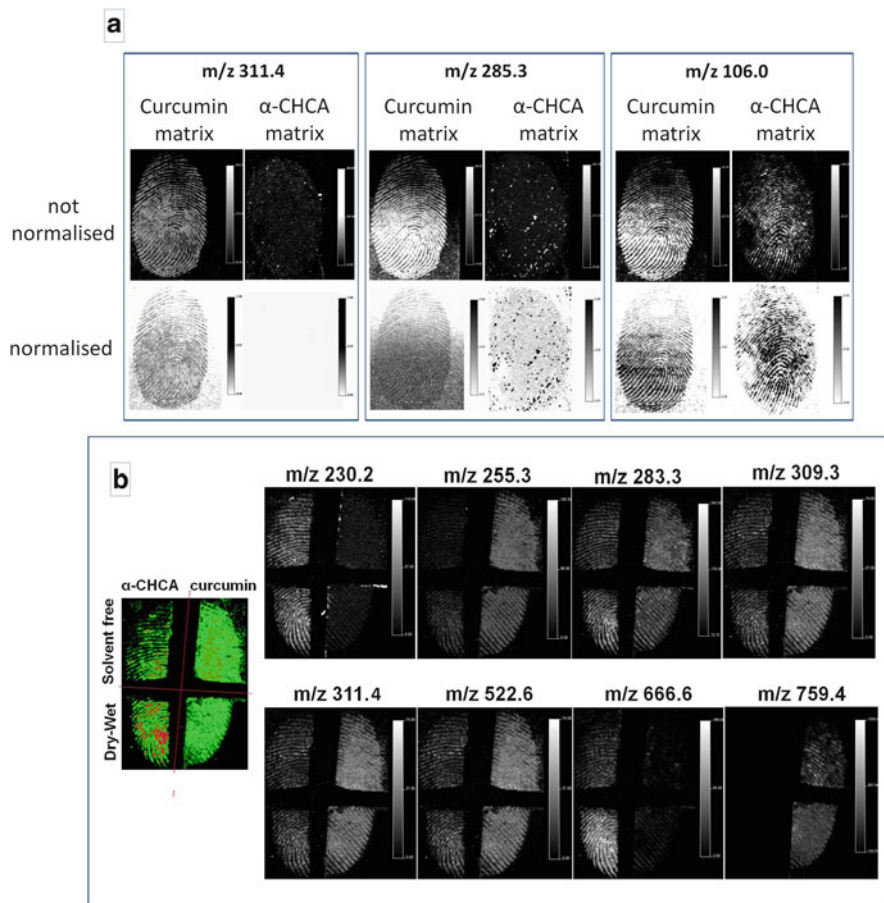


Fig. 9 Comparative MALDI MSI of ungroomed fingermarks using either synthetic curcumin or CHCA as MALDI matrix. Panel A shows the molecular maps of three endogenous species imaged either by using curcumin or CHCA and the *dry-wet* method. In a separate experiment (Panel B), an ungroomed mark was split in quarters which were each treated with one of the following methods: (*upper left*) solvent-free using CHCA, (*lower left*) *dry-wet* method using CHCA, (*upper right*) solvent-free using curcumin, and (*lower right*) *dry-wet* method using curcumin. Lipid molecular maps were reported for a comparative evaluation of the four methods. Reprinted with permission from Francese et al. 2013a, *Anal. Chem.*, **85**, 5240–5248. Copyright 2013 American Chemical Society

icals, lipids, peptides, and proteins. It is fluorescent and therefore fingermark images can be captured using commercially available crime lights. In pseudo-operational trials with the West Yorkshire Police, it was shown that curcumin can be used on a vast range of colored surfaces and that only a very small amount of curcumin is needed to visualize marks as it has a strong adherence to surfaces. The latter is not surprising as one of its uses is in the coloring industry. However, this characteristic

could pose some limitation to the operational application of curcumin as it has proven to be extremely difficult to wash it off the surface with possible liability in terms of damage to the property.

All of the referenced applications have benefited from an extremely careful approach to sample preparation and the development of optimized protocols. Some of these protocols as well as suggestions derived from our experience in fingermark preparation are shared in the following sections.

3 Materials and Protocols

3.1 How to Prepare Fingermarks for Method Development

For all samples and specimens to be subjected to MALDI MSP and MSI, preparation is crucial and is a strong determinant for the success of such analyses. Fingermarks are no exception and care must be taken in the selection of the sample preparation conditions. There are fundamentally four types of fingermarks that can be prepared and investigated:

- (a) *Eccrine*: produced by (1) thoroughly washing hands¹⁰; (2) drying them with tissue paper; (3) resting them in a closed plastic bag for 10 min to produce excess sweat; (4) the deposition of the mark on the surface of interest.
- (b) *Groomed*: produced by (1) thoroughly washing hands; (2) drying them with tissue paper; (3) rubbing the fingertips against the base of the neck or forehead¹¹; (4) the deposition of the mark on the surface of interest.
- (c) *Ungroomed*: produced by (1) thoroughly washing hands; (2) drying them with tissue paper; (3) carrying out 15 min of activities such as typing on a keyboard¹² whilst avoiding touching any part of the body or coming into contact with other contaminants; (4) the deposition of the mark on the surface of interest.
- (d) *Natural*: produced by depositing a mark on the surface of interest without any prior treatment.

Knowledge of the (bio)chemistry associated with the different types of fingermarks is paramount for designing the experiment and understanding its outcome.

Eccrine marks directly relate to the production of eccrine sweat. In general terms, this is generated by the two to four million eccrine sweat glands (located in the dermis) present throughout the body, with the highest density on the palms of the hands and the soles of the feet. Eccrine sweat is largely constituted of water (98%) though many

¹⁰Common protocols include the use of soap and plenty of water or a 50/50 solution of ethanol/water.

¹¹Grooming fingertips by touching the face is also viable, though one needs to be aware of possible contamination deriving from make-up or moisturizers, which may cause ion suppression.

¹²Previously cleaned with a 50/50 solution of ethanol/water.

inorganic and organic species are present (Ramotowski 2001). These are the types of marks containing the least abundance of lipids. Amino acids are the most abundant species of the organic content with their amounts being reported to be several times higher in sweat than in plasma (Miklaszewska 1968). Peptides and proteins are also present in eccrine sweat, although the epidermis is another source of these species as a result of the desquamation process (Drapel et al. 2009; Girod et al. 2012) as well as of the defense against microorganisms (antimicrobial peptides and proteins). Inorganic constituents include ammonia, chloride, bromide, fluoride, phosphate, sulfate, sodium, and potassium amongst others. These species can exert considerable ion suppression in MALDI. Therefore, sample preparation methods are needed to counteract the effect of their presence and enable ionization of the species of interest.

Because of the way they are generated, groomed marks are rich in sebum originating from the contact with parts of the body containing sebaceous glands. Sebaceous glands are the second major class of secretory glands and are present throughout the body except for the palms and the dorsum of the feet (Strauss et al. 1991) with the highest prevalence around the face and scalp. Together with traces of organic materials including aldehydes, amines, alcohols, phospholipids, and sulfides amongst others, sebaceous sweat mainly contains triglycerides, free fatty acids,¹³ wax esters, squalene, cholesterol, and cholesterol esters.

Groomed marks are valuable for studying lipids but may need additional sample preparation when investigating other substances of both endogenous and exogenous nature as lipids will in fact exert ion suppression. Also, due to the diversity and abundance of lipids, they may be isobaric with forensically interesting substances (e.g., drugs, medications, explosives), thus masking their presence. A third type of gland, named apocrine, is located predominantly in the genital and axillary regions of the dermis, and therefore it is believed that fingermarks mainly comprise the species present in the eccrine and sebaceous glands.

Ungroomed marks contain species predominantly arising from the epidermis material and the eccrine glands, and therefore all of the above described inorganic and organic constituents can be found in addition to lipids, which are still detectable (Wolstenholme et al. 2009). Here, the potential ion suppression exerted by the salts is not observed, likely due to their much lower abundance. Because of the way they are prepared, these marks are considered 'depleted specimens,' i.e., containing the minimum amount of biological material, though this has not been quantified. This type of marks should only contain endogenous or semi-exogenous material and no external contaminants. However, this is not always the case as the presence of exogenous species depends on their persistence.

Though eccrine, groomed and ungroomed marks do not have a fixed chemical composition, as this varies according to the individual and their physiological state and age, *natural* marks have the most variable chemistry as fingertips may have been in contact with any body part as well as with surfaces and external contaminants. Therefore the resulting fingermarks may contain a wide range of endogenous, semi-endogenous, and exogenous substances.

¹³Saturated, mono- and polyunsaturated.

Compared to other specimens, fingermarks probably require extra sample preparation planning as the kind of answers being sought dictate the type of fingermark samples that needs preparing. In order to test new methodologies (or modified methods) and techniques for fingermark development or analysis, recommendations are in place with regard to what kind of fingermarks should be used as test samples. The International Fingermark Research Group, of which the author of this chapter is a member, has provided guidelines for developing a methodology through application and implementation in casework (IFRG 2014). In particular, these guidelines say that eccrine and groomed marks are not suitable for method/technique evaluation as they are ‘unrealistic’ samples, though may be used for initial pilot studies. Generally speaking, ungroomed fingermarks should be employed in method development because prior substance depletion makes them useful to probe sensitivity and feasibility of the methodology, whereas for validation purposes, natural fingermarks should be used to test the robustness of the method/technique being applied. Although these guidelines were more specifically written for techniques used by crime scene investigators to visualize the marks (enhancement or detection), in the view of the author of this chapter, they are also relevant for analytical techniques exploiting the fingermark’s chemical composition to provide additional information including multiple images of the same mark for identification purposes. However, the case is different for controlled fingermark/fingertip smear¹⁴ collection, for example in hospitals, work environments, or drug rehabilitation clinics. Here, methods can be specifically developed for the type of fingermark/fingertip smear that yields the highest abundance of the substances of interest. Depending on the fingermark type and molecular target, protocols have been devised for the detection of lipids, peptides, proteins, metabolites, and exogenous contaminants by MALDI MSP and MSI. The majority of these protocols will be summarized in the next sections together with lessons learnt in the detection and imaging of the above species.

3.2 Define Your Strategic Approach: Profiling or Imaging?

In the classic approach to fingermark preparation for MALDI MS analyses, the matrix solution is either spotted on the mark (manually or robotically) to quickly gain a molecular profile or homogeneously sprayed on the mark (mainly robotically) for imaging purposes (Fig. 10).

At the beginning of any MALDI MSI method development, it is recommended to start with the design of profiling experiments via matrix-spotting and their optimization for two main reasons:

¹⁴Fingertip smears are specimens that do not allow a linkage to the biometric data of the individual. Like fingermarks, it is possible to obtain eccrine, groomed, ungroomed, and natural smears by preparing the fingertips as previously described for fingermarks and pressing the fingertip on a surface sliding it to the side.

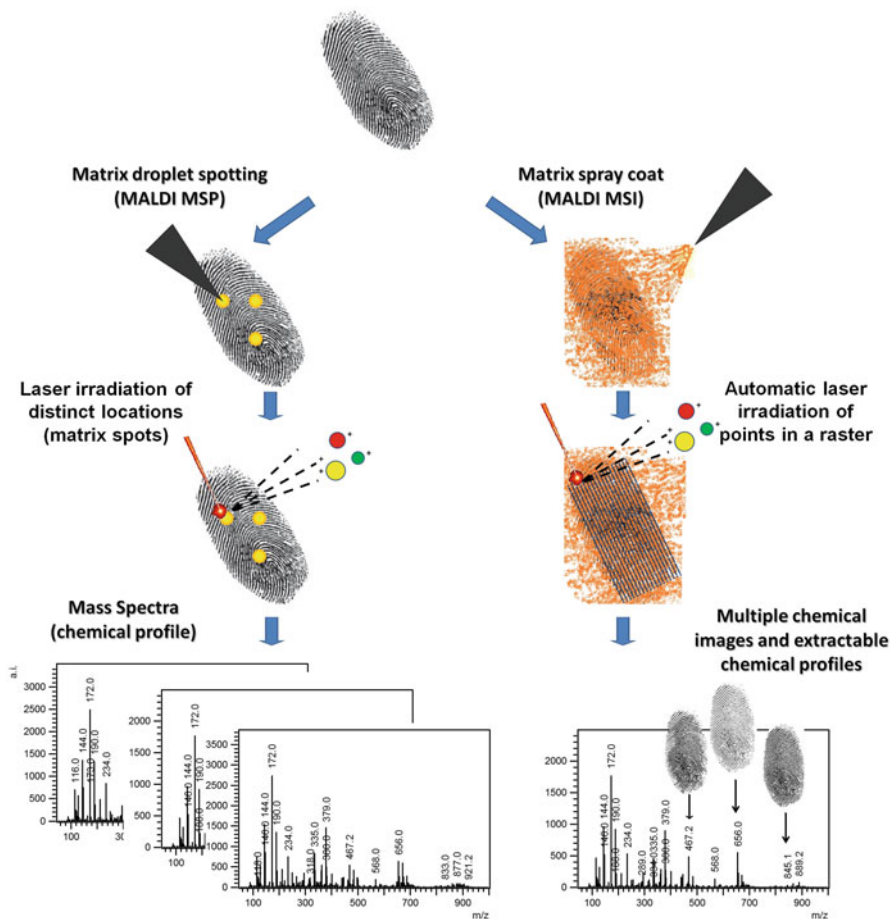


Fig. 10 Classic workflows for MALDI MSP (profiling, *left*) and MALDI MSI (imaging, *right*) applied to fingerprint analysis. In profiling experiments, the MALDI matrix is usually spotted and mass spectral profiles from specific fingerprint locations are obtained. In imaging experiments, the matrix is usually sprayed and spectra are automatically acquired at each x,y fingerprint location in a raster fashion (Francese et al. 2013b, Analyst, **138**, 4215-4228, adapted and reproduced by permission of The Royal Society of Chemistry)

1. Analyte extraction, desorption, and ionization are generally much more efficient with spotting than with spray-coating techniques.
2. If the molecular target of interest has low or no ionization efficiency, imaging experiments are an expensive and time-consuming way to find this out.

Where possible, method development should start with the analysis of the matched standard using conventional MALDI sample preparations such as the dried-droplet method (especially true for exogenous and semi-exogenous substances). Sample prep-

aration conditions (matrix, matrix concentration, solvent mixture) and instrumental parameters for both MS and MS/MS analyses can be optimized allowing a rapid discovery of the molecular targets that can be ionized and the conditions leading to maximum ionization efficiency. Using an iterative exclusion process, a set of optimized conditions can then be found and applied to the reference molecular target/s spotted on the mark at different concentrations. This set of experiments will allow to gain insights into the ion suppression effects exerted by the fingermark molecular make-up as well as the determination of the limit of detection (LOD), which is typically lower than in imaging mode (e.g., when the matrix is applied through spray-coating).

In such experiments, to reduce response variability (e.g., due to the well-known ‘sweet spots’ in MALDI) across the marks tested with different concentrations, the generation of reproducible fingermarks (e.g., through the same pressure, contact time and contact angle) is recommended. Within the author’s group a custom-built device is used for this purpose, termed the Reed-Stanton press rig (Reed et al. 2015 *in preparation*), though another device, named fingerprint sampler, with different features has already been patented and published (Fieldhouse 2011) and is commercially available. Alternatively, the mark can be split into the number of regions required in order to analyze different analyte concentrations (and different matrix deposition methods), thus maintaining the same chemical environment for comparative evaluation. Note that also in this case, replicates are still necessary to reach statistically significant conclusions.

After the matrix spray-coating has been optimized and assessed by using MALDI MSP,¹⁵ the method development for MALDI MSI should be straightforward, provided that the laser pulse repetition rate does not need adjusting to obtain the right compromise between speed and sensitivity.

When MALDI MSP and MSI are fully optimized, the methodology can then be applied to real samples. A workflow summarizing the recommendations for MALDI MSI method development is depicted in Fig. 11.

- **TIP:** *Whether or not a fingermark sampler is used, it is recommended to rub the fingertips against each other and use different fingers for the generation of the replicate marks and NOT the same finger as otherwise biological material depletion will affect the validity of the experiment conclusions.*

3.3 *Matrix Deposition: Spray-Coating and the Dry–Wet Method*

Initially, for many of the applications reported by our group, the classic matrix spray-coating technique was employed for imaging purposes. As described earlier, the SunCollect autosprayer was the device employed for all of the sample preparation method developments. This sprayer is simple to operate and is overall

¹⁵This includes optimization of the instrumental settings.

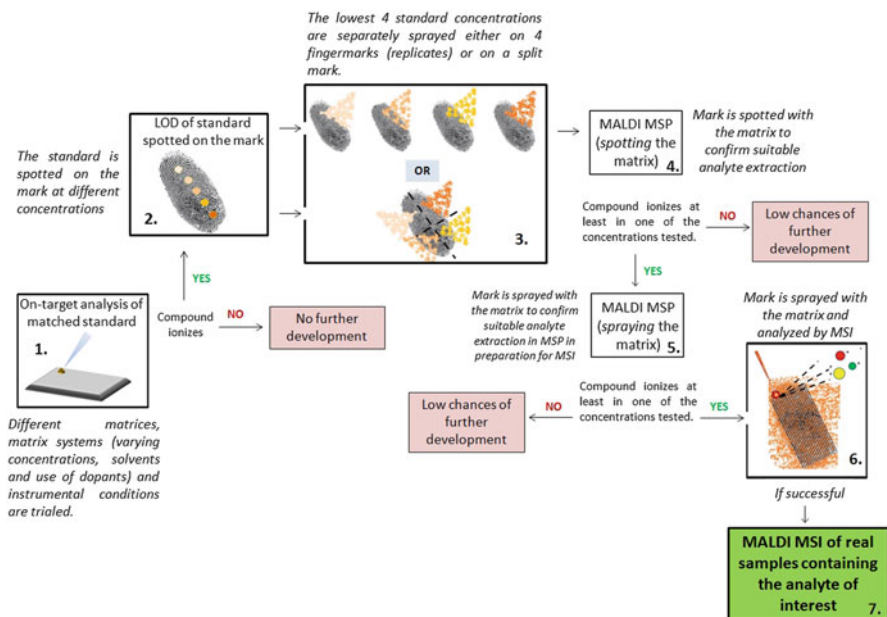


Fig. 11 Recommended workflow to develop a MALDI MSP and a MALDI MSI method for chemical species analysis in fingermarks. (1) Different matrices, matrix systems (including varying concentrations, solvents and use of dopants), and instrumental conditions are trialed; (2) the standard is spotted on a fingermark at different concentrations with the optimal MALDI sample preparation from step 1 and analyzed by MALDI MS; (3) the lowest four standard concentrations are separately sprayed on either four fingermarks (replicates) or four sections of a split mark; (4) matrix is spotted on the standard-containing mark(s) to confirm suitable analyte desorption/ionization conditions. Here instrumental conditions can already be optimized; (5) matrix is now sprayed onto fingermarks with various amounts of spray-coated standard and the mark is analyzed by MSP in preparation for imaging experiments; (6) the standard spray-coated mark is sprayed with the matrix and analyzed by MALDI MSI to confirm suitable analyte extraction and mapping in imaging mode. Here matrix deposition parameters are optimized for the lowest detectable concentration of the spray-coated standard; (7) if step 6 is successful, the method can be transferred to a real fingermark sample naturally containing the analyte

reproducible in the amount of matrix deposited. However, geometrical and physical parameters such as nozzle-to-surface distance, N_2 pressure, number of layers, and flow rate per layer require optimization. A few protocols are illustrated in this section for various applications.

In collaboration with HTX Technologies LLC (Carrboro, NC, USA), our group developed a matrix spray-coating method on a conceptually different automatic depositor, namely the TM sprayer (Bradshaw et al. 2013c). In order to achieve homogenous matrix deposition and maximum analyte extraction with minimal to no delocalization, this device employs a heated and pressurized matrix solution. Different variables such as N_2 pressure, nozzle temperature, number of passes, pump flow rate, velocity, and the track spacing were systematically investigated.

Table 1 Protocol parameters for the spray-coating method using the TM Sprayer (HTX Technologies) and CHCA as matrix^a

Preparation of ungrooved fingermarks using the TM Sprayer (HTX Technologies, NC, USA)						
Fingermark quarter	N ₂ pressure (psi)	Nozzle T (°C)	Number of passes	Pump flow rate (mL/min)	Velocity (mm/min)	Track spacing (mm)
1	10	75	1	0.08	1300	1.5

^aIn the author's lab, this set of parameters provides the best results overall in terms of fingermark ridge detail and speed of preparation

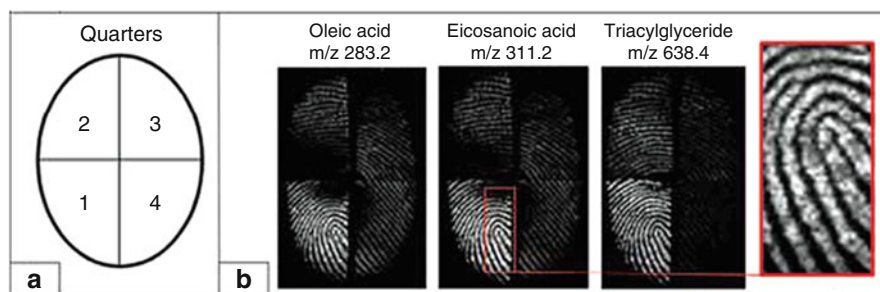


Fig. 12 Optimization of matrix deposition using a TM Sprayer (HTX Technologies, LLC (USA)). (a) Fingermark divided in quarters and numbered according to the number of passes performed. (b) Corresponding MALDI molecular images of three endogenous species; oleic acid (m/z 283.2), eicosanoic acid (m/z 311.2), and a triacylglyceride (m/z 638.4), with a magnified region for eicosanoic acid. Keeping all the other parameters constant, one layer of matrix generated the most satisfying result (see also Bradshaw et al. 2013)

Eventually, all of these parameters were optimized and then kept constant, apart from the number of spray-coating passes which was changed from 1 to 4 and individually applied to quarters of a fingermark. The best performing method used just one layer of matrix (Table 1) and allowed the highest possible quality of ridge detail images of lipids (Fig. 12) (analyzed in positive ion mode) in less than 1 min in total (*one-layer* method).

However efficient, both the classic dried-droplet and spray-coating methods cannot be applied to crime scene marks, if these are not somehow previously enhanced and removed from surfaces (unless, in the latter case, the mark is present on a flat and suitably thin surface). As previously described, the *dry-wet* method represents a significant step here for the implementation of MALDI MSI into the operational fingerprint analysis workflows.

While our group has been devising protocols to enable MALDI MSP and MSI analysis on FET-enhanced marks, the *dry-wet* method is also under further development to establish exactly on which surfaces and under which environmental circumstances, the MALDI matrix can efficiently act as a dual agent, i.e., in addition to its conventional MALDI matrix role also as a fingermark enhancer on surfaces.

During method development studies, our group has found that there are mainly three factors affecting the efficiency of the *dry-wet* method, namely the type of brush, matrix powder excess, and matrix particle size. There are different types of brushes that CSIs use according to the powder to be deployed and the surface. They mainly differ in the chemical nature of the material, the cross-section of the heads, and stiffness of the hairs.

- **TIP:** *Amongst the types of brushes we have tried, the zephyr brushes are the most efficient to deploy the MALDI matrix. It is important that the shaft of the brush is rotated between thumb and fingers while dusting for marks to avoid smudging, merging, or removal of the ridges.*

The second and the third factor should not be surprising for experienced MALDI users and with regard to the existing literature (Sugiura et al. 2006; Puolitaival et al. 2008). Large matrix excess may lead to suboptimal or completely out of range matrix:analyte ratios (often reported to be ideal around a 10,000:1 ratio), leading to the absence of signal (or very low signal intensity).

- **TIP:** *To remove excess matrix, use a quick blast of compressed air (e.g., from a can when at crime scenes) using a distance of about 10 cm.*

As the matrix is dispensed ‘dry’ in the first step of the method, the particle size affects the size of the matrix-analyte co-crystals upon evaporation of the solvent that is sprayed on the matrix/fingerprint as the second step of the process. Therefore, it is very important to use a matrix particle size as small as possible. In recent work, a remarkable difference in the ion intensity and ridge continuity was shown with the quality of the results gradually improving from using commercially available unground matrix, manually ground matrix, mechanically ground matrix sieved through a 38 μm sieve to ground matrix with particle sizes of $<10 \mu\text{m}$ (Fig. 13) (Ferguson et al. 2013b). Once dusted with the matrix, the mark is typically lifted by forensic tape and mounted face up on a MALDI target plate using double-sided conductive tape for subsequent solvent spraying. A working protocol that our group has optimized for the *dry-wet* method preparation using milled matrix is illustrated in Table 2 together with the instrumental parameters for a modified API Q-Star Pulsar *i* hybrid quadrupole time-of-flight (qTOF) instrument (Applied Biosystems), the instrument on which the bulk of our 6 years of research on latent fingerprint analysis has been undertaken.

As discussed in the introduction, the novel matrix curcumin was trialed with the *dry-wet* method and the ionization efficiency was compared with that obtained using both the conventional spray-coating method and by leaving the curcumin-dusted fingerprint unsprayed. The optimized protocol for the application of cur-

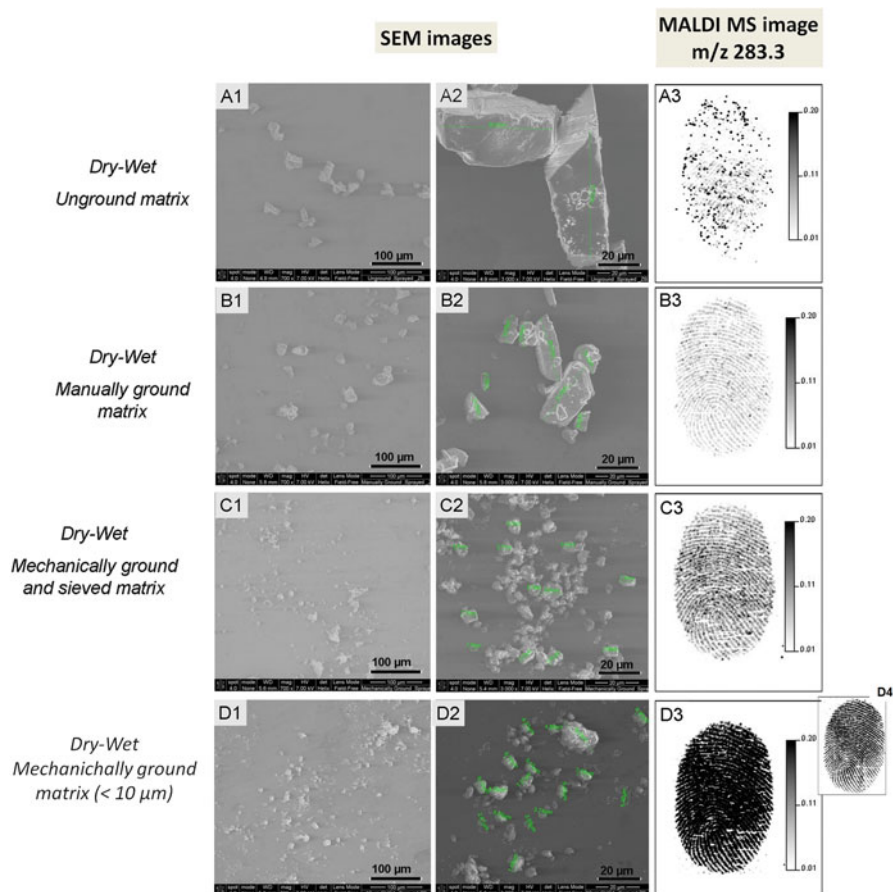


Fig. 13 Comparative analysis of ungrounded fingermark image quality prepared with the *dry-wet* method. Panels A1–D1 show a 700 \times magnification SEM image of the dusted fingermarks using different matrix particle sizes. Panels A2–D2 show a 3000 \times magnification. Panels A3–D3 show the corresponding MALDI MS images of the ion signal at m/z 283.3. Panel D4 displays the normalized image of the same mark shown in panel D3, which has not undergone normalization (Ferguson et al. 2013b, *J. Mass Spectrom.* 2013, **48**, 677–684, adapted and reproduced with permission from John Wiley and Sons)

cumin onto fingermarks for the *dry-wet* method was the same as for CHCA with respect to solvent spraying and instrumental parameters as shown in Table 2 (except for the declustering potential 2 and focusing potential set at 10 and 20 a.u., respectively), and permitted the analysis of lipids.¹⁶

¹⁶The *dry-wet* method employing curcumin has not been trialed on other classes of molecules.

Table 2 Protocol parameters for the application of fine CHCA matrix particles using the *dry-wet* method with a zephyr brush (cf. Ferguson et al. 2011)

Preparation of fingerprints using the <i>dry-wet</i> method for MALDI MSI analysis		Mass spectrometer and instrumental parameters	
<i>Dry-wet</i> method application			
Matrix	α -cyano-4-hydroxycinnamic acid (CHCA)	Mass spectrometer	Q-Star Pulsar <i>i</i> (Applied Biosystems)
Milling instrument	Bench top, single grinding station PM100 Planetary Ball Mill (Retsch)	Mode/laser repetition rate	Continuous raster imaging in positive ion mode/5 KHz
Milling conditions/final particle size	450 rpm for 30 min in agate jar with 15 × 20 mm grinding balls < 10 μ m	Declustering potential 2/Focusing potential	15/10
Autosprayer for solvent spraying	SunCollect (SunChrom)	Accumulation time	0.117 s
Solvent spray conditions	5 layers of 70:30 of an ACN/0.5% TFA solution at a rate of 5 μ L/min	Spatial resolution	100 μ m × 150 μ m

Having very similar protocols is advantageous for method implementation in the forensic practice as, generally speaking, the lower the number of protocols (or variability in the protocols) the quicker they are accepted and integrated in practice.

3.4 Detection and Mapping of Peptides and Proteins

The detection of fingerprint peptides and proteins may have profound implications for forensic practice as well as in the biomedical field. In the former case, our group has demonstrated the ability to detect the sex of individuals through their fingerprints by exploiting the peptide/protein profiles (Ferguson et al. 2012).

- **TIP 1:** Use a *dedicated* zephyr brush to dust curcumin, thus avoiding cross-contamination and ‘unexpected’ results.
- **TIP 2:** Use only a small amount as the ‘staining’ and the adherence to fingerprints are greater with this matrix than with CHCA.
- **TIP 3:** Use synthesized curcumin as opposed to plant-derived commercially available curcumin for greater ionization efficiency and less interfering background signals.
- **TIP 4:** If you do not use the *dry-wet* method but the classic spray-coating method on the SunCollect autosprayer, use a dedicated capillary for each matrix to avoid cross-contamination as matrices take a very long time to clean off the capillary line.

This forensic analysis provides additional intelligence, especially important if DNA cannot be recovered. Implications in the wider biomedical context relate to the exploitation of peptides/proteins for noninvasive screening of disease biomarkers. It is therefore important to devise protocols for their optimal detection. However, to date, the detection of peptides and proteins from fingermarks has been by far the most challenging piece of research and further method development is still in progress. The challenge is mainly due to their low abundance (the total protein concentration in sweat has been reported to be between 15 and 25 mg/dL) and their main origin from the eccrine glands (Ramotowski 2001).¹⁷

The challenges in peptide/protein detection are often fingermark-specific. Eccrine marks should contain most of the proteins but the electrolyte concentration here is also the highest amongst the different types of marks listed in Sect. 3.1. As a result, there is a strong ion suppression phenomenon preventing the detection of peptides and proteins (Ferguson et al. 2012). Groomed marks exhibit the presence of peptides/proteins but the high presence of lipids (as a consequence of grooming) also exerts ion suppression, greatly decreasing the peptide/protein ion population as well as the corresponding MS signal intensity. Ungroomed marks are somewhere in between these two types though proteins are less abundant than in eccrine marks. Natural marks may be problematic as their variable composition (and possible presence of external contaminants) may affect peptide/protein ionization through similar suppression phenomena. We have previously shown that, similarly to the treatment of other specimens subjected to MALDI MSP and MSI, washing steps can greatly improve the detection of peptides/proteins. For example, groomed fingermarks yield a higher peptide/protein ion population and signal intensity when washings are performed to selectively eliminate lipids. Denatured ethanol was the most efficient solvent out of the three trialed which also included chloroform and acetone (Ferguson et al. 2012). These washings were performed by pipetting 750 μ L of the solvent over the fingermark deposited on a suitable support which was held at an angle on a flat surface.

If marks are collected for biomarker discovery studies or in anticipation of their use for medical diagnostics, eccrine marks may be good specimens to use as they can be produced in a controlled way and contain peptides/proteins in higher concentration. However, the ion suppression phenomenon needs to be counteracted. As the challenge here is the presence of electrolytes, we have taken up one of the MALDI MSI sample clean-up strategies applied to tissue samples, employing low concentration of high MALDI-tolerant salts, such as ammonium acetate, to preliminarily treat the mark (Wang et al. 2011). In this preparation, the ammonium and acetate ions will advantageously engage the endogenous chloride and the sodium/potassium ions, respectively. The type of application of ammonium acetate (submersion or buffer pipetting), the volume, the concentration, and the contact time with this buffer all play a role. Our current (so far unpublished) protocol is depicted in Table 3.

¹⁷Protein-producing apocrine glands are rarely a source of proteins in fingermarks.

Table 3 Parameters for the preparation and analysis of peptides and proteins in eccrine marks

Preparation of the eccrine mark and analysis			
Hands are washed with 50% EtOH _{aq} and kept for 15 min in sealed bag; fingertips are then rubbed together to spread sweat evenly			
Buffer used	Ammonium acetate (AA)	Mass spectrometer	Voyager DE-STR
Concentration	150 mM	Accelerating voltage	25 kV
Application mode	Soak in AA for 5 min	Grid voltage	93%
Buffer volume	500 mL	Delay time	150 ns

Benefits of this method are shown in Fig. 14, where the MALDI spectrum of an unwashed eccrine mark is compared with that of a washed one using the protocol of Table 3.

Sometimes, even successfully optimized MALDI MSP protocols using the dried-droplet method cannot be successfully developed in imaging methods employing matrix spray-coating. In our laboratories, we have trialed a number of different sample preparation protocols to image proteins with samples being analyzed on a Voyager DE-STR MALDI-TOF instrument (Applied Biosystems) equipped with a solid state laser operating at 60 Hz. However, results as reported by Ferguson (2013) in her Ph.D. thesis were disappointing. Generally, with all the sample preparation methods trialed, the intensity of peptide/protein ion signals in imaging mode (with matrix spray-coating) was either too low or signals were not detected at all. Actual presence of peptides and proteins was verified using the classic dried-droplet method, thus indicating that the protein abundance and extraction efficiency in MALDI MSI were insufficient. However, since detection proved to be satisfactory when the dried-droplet method of matrix deposition was applied, the lack of peptide/protein ion signals using the automatic acoustic ejector spotter for sample preparation is somewhat surprising. Amongst the methods trialed, only the one spraying the matrix at 5 mg/mL with 25:25:50 acetonitrile/ethanol/0.5% TFA by the SunCollect autospraying system yielded a couple of signals previously detected with the dried-droplet method (Fig. 15). It was also possible to obtain an image for the ion signal at m/z 4819.5, which was putatively identified as dermicidin (Ferguson et al. 2012) but highly speckled and bearing no hint of ridge detail (Fig. 15c). Though the lack of peptide and protein detection may be a limitation of MALDI MSI for the analysis of latent fingerprints, these experiments are not yet conclusive. Many more conditions can be trialed and our experiments (unpublished data) suggest that fingerprint peptides/proteins are more hydrophobic than previously thought. Furthermore, their low abundance is most likely another decisive factor.

Experiments performed on an Ultraflex III MALDI-TOF/TOF mass spectrometer (Bruker Daltonik, Germany) equipped with a SMART beam laser operating at 200 Hz gave more encouraging results. Here, the *dry-wet* method was applied where an ungroomed mark was dusted with CHCA and sprayed with 3 layers of

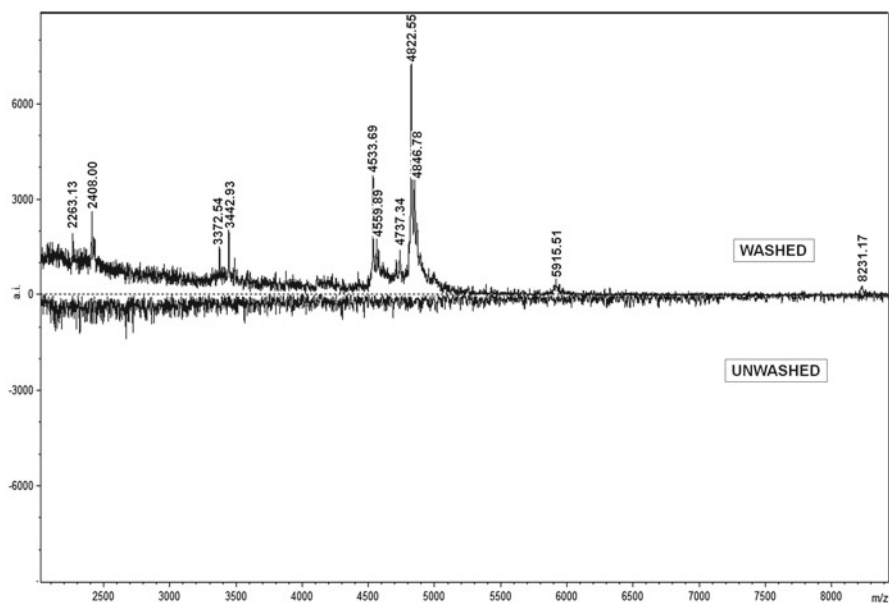


Fig. 14 MALDI MS spectra of peptides and small proteins in eccrine fingermarks. *Top panel:* eccrine fingermark washed using the method reported in Table 3; *bottom panel:* unwashed fingermark

70:30 ACN/0.5% TFA (at a rate of 2 $\mu\text{L}/\text{min}$ and a capillary distance of 31.75 mm) prior to the analysis by MALDI MSI at a spatial resolution of 200 $\mu\text{m} \times 200 \mu\text{m}$. As Fig. 16 shows, despite the mark being only partial and highly speckled, there is a 'hint' of ridge detail.

These initial results indicate that perhaps using a different mass spectrometer with higher sensitivity and spatial resolution in conjunction with the most promising sample preparation methods trialed (and further tweaking of the preparation conditions) may, in a not too distant future, yield protein/peptide images. This work is in progress in our laboratory.

Abundant proteins from an external source (external to the fingermarks) have not been such a challenge. The specific detection and mapping of blood through detecting the hemoglobin protein (α and β chains), in addition to the heme group, was achieved through the application of a standard MALDI MS protein analysis protocol. Although sinapinic acid (SA) was trialed as one of the best matrices for protein analysis, CHCA was the most efficient matrix to detect hemoglobin both in simulated laboratory conditions and for crime scene evidence, though the evidence recovered and analyzed from the latter were bloodstains rather than blood marks. The possibility to use the same matrix for molecules so different in size enables both

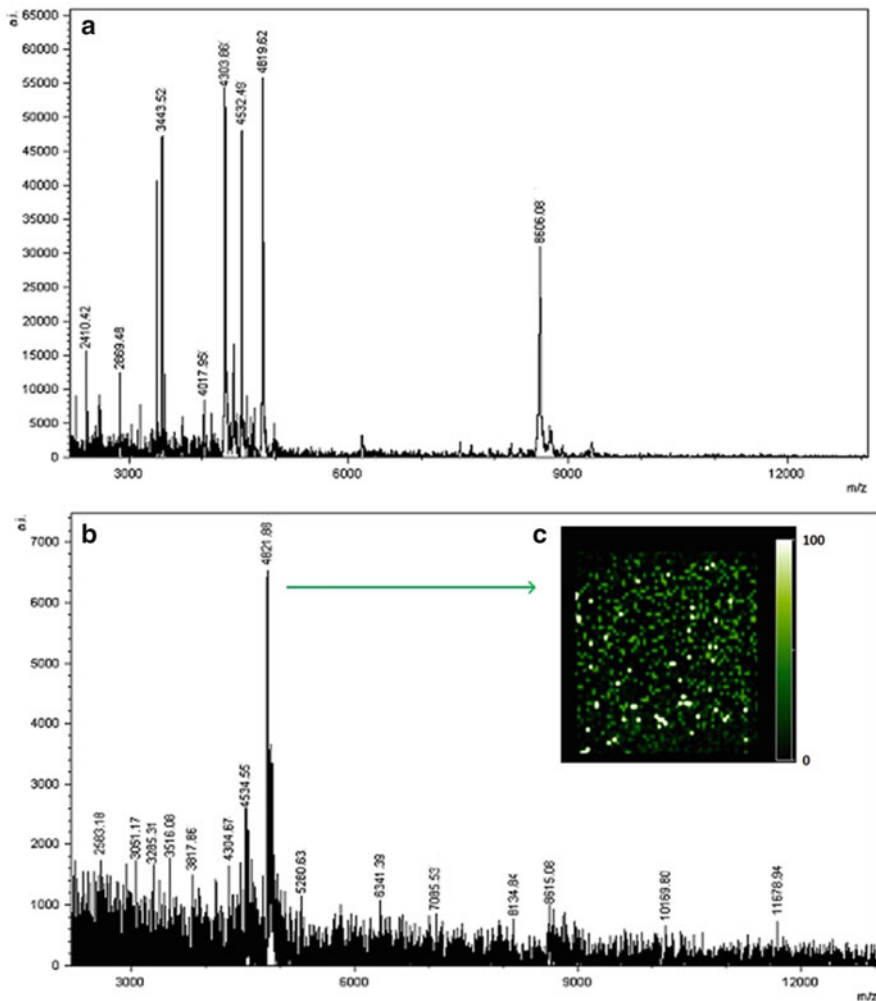
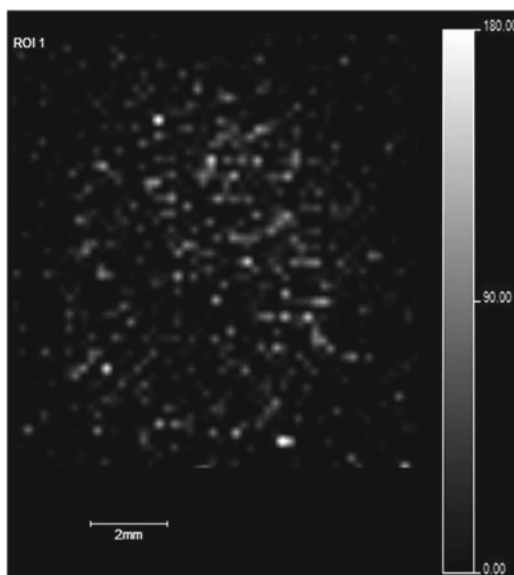


Fig. 15 MALDI MSP and MSI of a split groomed mark. One half was prepared using the dried-droplet method spotting 5 mg/mL CHCA in 25:25:50 acetonitrile/ethanol/0.5% TFA (a) while the other half was prepared using the same matrix solution but sprayed using the SunCollect auto-spraying system (b). Fingerprint of the MS spectrum shown in (b) was then imaged and panel (c) shows the image of the peak at m/z 4821.9, corresponding to the putatively identified dermicidin (the recorded and expected m/z values differ by 2 units). No ridge details could be observed. Analyses were performed on a Voyager DE-STR MALDI-TOF mass spectrometer (Ferguson 2013; permission granted by the author)

the analysis of heme and that of hemoglobin on the same mark in two subsequent acquisitions (instrumental parameters are different in the two cases), without washing off and changing the matrix. The detection of two blood-specific molecules makes blood analysis much more reliable. The protocol adopted for both hemoglobin

Fig. 16 MALDI MSI of an ungroomed fingermark at a spatial resolution of $200\ \mu\text{m} \times 200\ \mu\text{m}$. The mark was prepared using the *dry-wet* method by dusting with CHCA and spraying a 70:30 ACN/0.5% TFA solution using a SunCollect autosprayer. MALDI MSI was performed on an Ultraflex III MALDI-TOF/TOF mass spectrometer



profiling and imaging is depicted in Table 4.¹⁸ This protocol is also compatible with the prior application of the currently used ninhydrin and Acid Black 1 enhancement protocols for the presumptive detection of blood. Despite the fact that the instrument adopted for these analyses is relatively old and does not have a high sensitivity, preliminary experiments on the hemoglobin standard have shown that we can detect this protein at a concentration 1000 times lower than the physiological concentration ($\sim 13\text{--}18\ \text{g/dL}$ for healthy adult males and $\sim 11.5\text{--}16.5\ \text{g/dL}$ for healthy adult females).

3.5 MALDI MSI of Condom Lubricants in Fingermarks

Two years of research were spent on devising and optimizing protocols for imaging condom-contaminated fingermarks with the ultimate aim to provide information for suspect identification (ridge pattern images) as well as the identification of the condom lubricant chemicals (associative/probative evidence). In some cases, condom brand/type identification was achieved. Condom-contaminated fingermarks/fingerprints can

¹⁸The protocol (instrumental conditions) for the detection, the mapping, and the MS/MS of heme can be retrieved in (Bradshaw et al. 2014).

Table 4 Parameters for the preparation and MSP/MSI analysis of blood fingermarks

Preparation of blood marks for MALDI MSP and MSI analysis			
<i>MALDI MSP analysis</i>			
Matrix (spotted)	20 mg/mL CHCA in 70:30 ACN: 0.5% TFA _{aq} (0.5 µL per spot)	Mass spectrometer	Voyager DE-STR
Application mode	Spotted	Accelerating voltage	25 kV
		Grid voltage	90%
		Delay time	300 ns
		Number of shots	150
<i>MALDI MSI analysis</i>			
Matrix (sprayed)	5 mg/mL CHCA in 70:30 ACN: 0.5% TFA _{aq}	Mass Spectrometer	Voyager DE-STR
Application mode	Sprayed	Accelerating voltage	25 kV
Spraying conditions	SunCollect (SunChrom) 5 layers at a rate of 2 µL/min	Spatial resolution	150 µm × 150 µm (spot-to-spot classic method)
		Grid voltage	90%
		Delay time	300 ns
		Number of shots	100

be easily prepared by washing hands with a 50:50 ethanol:water solution, rubbing fingertips against the condom for a few seconds and subsequently touching the deposition surface of choice.

- **TIP 1:** If you want to preserve the ridge details and avoid smudging due to the oily nature of the lubricants, lightly touch the surface three times in three different areas and analyze the third mark.
- **TIP 2:** Condom lubricants (polymers) are very persistent substances. Therefore, to avoid carry-over effects, use different fingers to deposit replicate marks or marks containing different condom lubricants. Wash hands thoroughly and ensure that no polymer is left, which could interfere with other analyses when still using your fingertips to generate other fingermarks/fingerprints.
- **TIP 3:** Make sure that the sample is completely dry as otherwise pumping down the MALDI mass spectrometer could become challenging.

In general, as well as in condom lubricant analyses, ‘hot spots’ are very much a problem when the matrix is spotted rather than sprayed, which is in agreement with the findings and observations by others (Hensel et al. 1997; Hanton 2001). ‘Hot spots’ are mainly due to the differential incorporation of the analyte(s) into the matrix crystals, depending on the analyte, matrix and crystallization conditions, and thus

Table 5 Parameters for the preparation and MALDI MSI analysis of condom-contaminated marks

Preparation of condom-contaminated fingerprints for MALDI MSI analysis			
Matrix and standards application		Instrumental parameters	
Matrix	10 mg/mL dithranol in 45:40:15 acetonitrile: dichloromethane: tetrahydrofuran	Mass spectrometer	Q-star Pulsar <i>i</i> (Applied Biosystems)
Application mode	Spray-coating using manual sprayer Eclipse HP-CS (Iwata Medea Inc.)	Mode/laser repetition rate	Continuous raster imaging/positive ion mode
Total volume delivered	20 mL	Declustering potential 2/focusing potential	10/30 a.u.
		Accumulation time	0.117 s
		Spatial resolution	150 μm \times 150 μm

leading to significant variability in the ion signal intensity and mass shifts from spot to spot. For polymers, this has been previously described as ‘polymer segregation’ (Gründling et al. 2010), preventing homogenous polymer-matrix co-crystallization.

In our studies, employing MALDI MSI and a matrix spray-coating technique, a dithranol-based matrix application was optimized, enabling the detection and mapping of ethoxylate-based polymers and polydimethylsiloxanes (PDMS). The main method used to perform MALDI MSI of condom-contaminated marks is detailed in Table 5.

Here the SunCollect autosprayer could not be used as the solvent mixture can damage the capillary and the tubing of the device. A manual sprayer (airbrush) was therefore used instead, which is an adequate alternative in the hands of an experienced user. With this device, the distance between the nozzle and the surface as well as the number of passes and the time interval between each pass are all very important parameters to prevent analyte delocalization whilst achieving maximum analyte extraction and optimal co-crystallization with the matrix. Although the protocol shown in Table 5 enables the detection and mapping of ethoxylate-based polymers as well as PDMS, the latter was only accidentally detected after condom-contaminated marks aged at 25 °C and 60% relative humidity for a month (cf. Sect. 1 Introduction). To speed up sample preparation and to achieve completeness of information about the condom lubricant profile, an alternative protocol step was devised, whereby the contaminated mark was kept at 37 °C for a minimum of 10 min. Whether this will be required operationally, given that the majority of sexual assaults are reported weeks, sometimes months after the crime, remains to be seen.

To exemplify the integration of this technology in a workflow where less destructive techniques are used first, MALDI MSI was employed in tandem with ATR-FTIR analysis (Bradshaw et al. 2013b). The development of this workflow required careful planning on the application of these two techniques knowing that ATR FTIR

is to be applied first. The feasibility and efficiency of this new type of workflow is somewhat limited by both the materials used and the high-vacuum environment of conventional MALDI sources. In particular, the gelatin lifters employed to remove the mark from the surface to be subsequently submitted to ATR FTIR are an unsuitable sample support for MALDI MS due to the difficulty in achieving the required high vacuum of the MS instrument. This constraint prevents the same mark to be analyzed sequentially by ATR FTIR and MALDI MSI and dictates a first fingerprint lift using gelatin tape to be analyzed by ATR FTIR and a second lift of the remaining fingerprint material with conventional tape to be submitted to MALDI MSI analysis. However, as shown in Fig. 4, MALDI MSI is sensitive enough to detect the relevant chemicals (polymers and endogenous substances) even in a secondary lift, albeit with lower ion intensity and ridge coverage.

3.6 Further Strategy to Improve Information Reliability of MALDI MSI When Imaging Small Molecules

Whilst molecular detection is achieved in the majority of the cases through optimized protocols, reliable molecular identity is not trivial. Depending on the sample, the presence of isobaric species, including matrix clusters, may often lead to questionable molecular identification if solely based on the m/z value. Therefore, the application of tandem mass spectrometry (MS/MS) is often a requirement. However, even the application of MS/MS may incur the same problem with no reliable identification. For instance, this is the case if the selection mass window of the precursor ion is not narrow enough, thus allowing ions with similar m/z to be fragmented, generating an overpopulated and very complicated MS/MS spectrum. Certainly both high resolution mass spectrometry (HRMS) and Ion Mobility MS (IMS) are two excellent ways to overcome the problem of isobaric ions, although they do come with significant financial implications.

The inclusion of standards combined with imaging tandem mass spectrometry capabilities is a useful strategy, which may help to alleviate the problem of isobaric species and the lack of HRMS and IMS instrumentation. Taking stock from a recently published work from Clench's group (Cole et al. 2013), we have adapted this strategy to latent fingerprints and recently reported the results in a Spectroscopy Europe issue (Bradshaw and Francese 2014). Here, as an example, two species having the same nominal mass, namely protonated cocaine ions (monoisotopic m/z 304.1548) and dimethylbenzylammonium ions (DBA), which can be found in many toiletry products (monoisotopic m/z 304.3004). It is clear that erroneously claiming the presence of cocaine in the defendant's fingerprints (whilst in fact the species in question is just a common bactericide found in many hygiene products) may have profound and unacceptable consequences in a court case. Thus, the claim must be sound and based on solid evidence. In our study, the sample preparation included

Table 6 Parameters for the preparation and MSI analysis of DBA- and cocaine-containing marks

Preparation of cocaine- and DBA-contaminated marks for MALDI MSI analysis			
Matrix and standards application		Instrumental parameters	
Matrix	CHCA solution in 70:30 ACN: 0.5% TFA	Mass spectrometer	Q-star Pulsar <i>i</i> (Applied Biosystems)
Application mode	Spray-coating using SunCollect autosprayer	Mode/laser repetition rate	Continuous raster imaging/positive ion mode
Number of layers and speed	5 at 2 $\mu\text{L}/\text{min}$	Declustering potential 2/focusing potential	40/15 a.u.
Standards	Each standard separately mixed 1:1 with the matrix and 0.5 μL spotted in two distinct locations at the side of each contaminated mark	Collision energy	25
		Accumulation time	0.5 s
		Spatial resolution	150 $\mu\text{m} \times 150 \mu\text{m}$

‘controlled’ contamination of fingertips and the use of *natural* fingerprints to mimic real conditions as far as possible (Bradshaw and Francese 2014). Fingerprint contamination with the two substances was achieved by (a) rubbing a fingertip against a glass slide containing a known dry residue amount of cocaine (5 μg) and (b) wiping a fingertip using Dettol[®] antibacterial surface wipes containing DBA, before depositing separate marks onto aluminum sheets. Preparation and analysis of the marks were undertaken using the method illustrated in Table 6.

As seen in Fig. 17, simple MS data acquisition cannot help in the discrimination of the isobaric species.¹⁹ However, if MS/MS images are interrogated specifically for cocaine product ions (m/z 182.1, 150.1, 82.1), only the cocaine standard spot appears (Fig. 17a). Similarly, DBA-specific product ions (m/z 212.2, 91.0, 58.0) generate images of both the fingerprint and the standard DBA spot with the standard cocaine spot no longer visible (Fig. 17b). In the latter case, a highly informative and visually impactful image was also obtained by superimposing the images from the DBA product ion at m/z 212.2 and the cocaine product ion at m/z 182.1, demonstrating that cocaine is absent in the mark and only present in the standard. In conclusion, this strategy may be adopted for molecular confirmation and does not require HRMS or IMS using reference standards.

¹⁹Note that another limiting factor can be the bin size of the imaging software used (e.g., BioMap; http://www.maldi-msi.org/index.php?option=com_content&view=article&id=14&Itemid=32). Even if the data were acquired in high resolution, software often cannot cope with a narrow bin sizes, thus generating (depending on the decimal places) the same uncertainties in discriminating between two species with the same nominal mass.

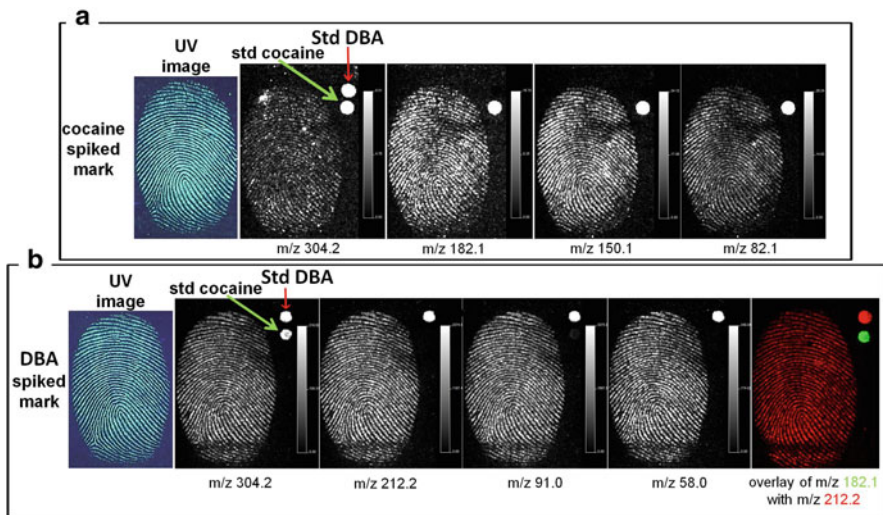


Fig. 17 MALDI MS and MS/MS imaging using reference standards for the discrimination of the isobaric species cocaine and DBA. (a) UV and MS image (m/z 304.2) for a cocaine-spiked fingerprint mark and the corresponding MS/MS images of the cocaine product ions. As only the cocaine standard spot appears in the MS/MS images, it is concluded that cocaine and not DBA is the species present in the mark. (b) UV and MS image (m/z 304.2) for a DBA-spiked mark and the corresponding MS/MS images of the DBA product ions. As only the DBA standard spot appears in the MS/MS images, it is concluded that DBA and not cocaine is present in the mark (Bradshaw and Francese, *Spectroscopy Europe* 2014, 26, 6-8, reproduced with permission from John Wiley and Sons)

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