Development of operational protocols for the analysis of primary and secondary fingermark lifts by MALDI-MS Imaging

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Development of operational protocols for the analysis of primary and secondary fingermark lifts by MALDI-MS Imaging

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Abstract

Eight years of intensive research have demonstrated that Matrix-Assisted Laser Desorption/Ionisation Mass Spectrometry Profiling and Imaging (MALDI-MSI and MSP) are powerful tools to gather intelligence around a suspect lifestyle, directly from the identifying ridges of a latent fingermark. In the past three years, many efforts have been invested into translating laboratory methodologies to the field; this was undertaken by devising protocols either for (a) enabling initial fingermark visualisation, such as through the Dry-Wet method, recovery and subsequent MALDI MS based analysis, or for (b) rendering the MS methodologies compatible with the prior application of commonly employed fingermark enhancement techniques (FET). In the present work a major point of interest concerned the sample treatment of FET visualised-lifted fingermarks and the subsequent MS performance of primary tape lifted fingermarks ("primary lifts") versus secondary tape lifted fingermarks (recovery from the surface a second time following the initial primary lift). This was necessary since it may not always be possible to obtain primary lifts of marks visualised at crime scenes for remote MALDI-MSP and MSI. The work illustrated here has provided methodological insights into establishing how to best treat a few types of developed marks in preparation for MALDI-MSI when presented as both secondary and primary lifts; it was demonstrated, as expected, that primary lifts generally yield much higher quality chemical/physical information and are therefore crucial to maximise chances of suspect identification and of retrieval of chemical intelligence. When analysing secondary lifted marks that have been initially developed using aluminium or carbon powders, any of the trialled sample preparation methodologies can be employed except the Dry-Wet method. In the case of TiO_2 powder developed marks, the best ridge coverage was achieved by re-enhancing the mark using the initial powder and spray-coating with MALDI matrix. Primary lifts of fingermarks contaminated

with an exogenous substance (used as a reference model) yielded the best ridge detail quality whilst for secondary lifts of natural marks pre-enhanced with aluminium powder, significantly greater intensity of the ion image was observed for the sections subjected to either no further enhancement or re-enhancement using aluminium powder.

Keywords: Fingermarks; MALDI; Imaging; powders; lifts

1.0. Introduction

While successful academic research is paramount for scientific progress, it is its translational feasibility that enables the impact of this research in the real world. With this view in mind, these authors are actively pursuing the development of protocols enabling MALDI-MS based technologies, with particular focus on the imaging modality, to be implemented into the current fingerprinting workflow.

The pioneering development of MALDI-MS methodologies in fingermark analysis has a short but strong track record history of success, demonstrating the opportunity to retrieve additional chemical and physical intelligence from latent marks[1]. In 2011, the Fingermark Research Group (FRG) at Sheffield Hallam University (SHU) embarked in transition "from the lab to the field", greatly assisted by partnerships with the Home Office and West Yorkshire Police (WYP) UK. While the former partner helped in understanding the fundamentals of the conventional fingerprinting workflows, WYP helped the group to understand the requirements for crime scene investigation as well as admissibility of evidence in court.

As a result, protocols were developed to make MALDI-MS based methods compatible with the prior application of many fingermark enhancement techniques (FET)[2-4]. These methods have been directly trialled on crime scene evidence (stains and marks) in collaboration with WYP and some of the results have been published in peer-reviewed journals[3,5]. However, it was clear that for devising an optimal and fully functional and operational protocol, many variables had to be understood and managed including; optimal collection of the specimen (packaging) and transportation of the evidence, storage and maximum storage time prior to deterioration of the evidence. While these authors addressed these questions already and this will not be described in the present paper, an equally crucial issue concerned the optimal preparation of pseudo-operational and operational marks for MALDI-MS based analyses and this required a systematic approach.

At the beginning of the investigations, the authors were only given access to the analysis of "secondary lifts" of crime scene fingermarks. This terminology refers to instances in which the fingermark is enhanced by the crime scene investigators (CSI), using the technique they deem most appropriate in a given scenario, recovered from the surface using forensic tape ("primary lift") leaving a minute residue of the fingermark behind on the surface, which can be recovered by the mass spectrometrist by lifting a second time. Therefore, given that there will be minute amounts of material (which cannot be quantitatively assessed and can differ on every occasion), there remains the question of how to best treat the sample for "optimum results". By "optimum

results" WYP means either/both the ability to provide enhanced ridge clarity/detail[6] and/or providing chemical information on the suspect's lifestyle and activities prior to the accidental deposition of the mark, that are otherwise inaccessible using conventional fingerprinting methodologies. Both of these forensic opportunities are very important as the former boosts the chances for suspect identification while the latter enables a new form of criminal profiling, no longer based on behavioural science but on chemistry ("chemical criminal profiling", CCP). Furthermore the ability to map/profile chemical intelligence directly onto the identifying fingermark ridges enables the link between the biometric information (suspect I.D.) and the *corpus delicti*.

Given the huge potential in informing both investigations and judicial debates, as well as the opportunity offered at the time to only work with secondary lifts, there were grounds justifying the undertaking of further developmental work to devise optimal methods for sample treatment of secondary lifts; additionally, while a few other groups have now published reports on the use of MALDI-MS based methods for the chemical mapping of fingermarks[7-11], none of these studies has dealt with secondary lifts. There are indeed different ways to treat such samples: (a) recovering the fingermark residue remaining after the primary lift with no prior attempt to re-enhance the residue, followed by matrix spray-coating for direct MALDI MS-Imaging/Profiling analysis; (b) subjecting the fingermark residue remaining after the primary lift to a secondary lift following re-enhancement using the same FET initially employed, with this lift being subsequently subjected to matrix spray-coating for direct MALDI-MS Imaging/Profiling analysis; (c) prior to MALDI-MS Imaging/Profiling analysis, subjecting the fingermark residue remaining after the primary lift to a secondary lift following re-enhancement by the Dry-Wet method[12,13] with the sample being subsequently either (c-1) spray-coated using a suitable solvent in which both the matrix and analytes can dissolve[12] or (c-2) matrix spray-coated with the matrix re-enhancement using the dry powder as a seeding layer for the co-crystallisation formation[14]. These four methods were trialled, compared and contrasted to provide preliminary recommendations on how to best treat secondary lifts. These recommendations specifically pertain to the prior application of powders as FET, specifically aluminium, TiO_2 (white) and carbon (black) powders, the most commonly used by West Yorkshire Police CSI. Method efficiency was investigated for both endogenous compounds (those naturally present in sweat secretions and therefore in fingermarks) and for an exogenous compound (dimethylbenzylammonium ion, DMA) used as a model due to its high ionisation efficiency[5, 15,16]. Ungroomed fingermarks[17] were preliminarily investigated as a reference and to reduce chemical compositional variability; subsequently the methods were tested on natural

fingermarks (fingermarks generated with no prior enrichment/depletion of the fingertip original content) as eventually these are the type of mark specimens found at crime scenes. Results have provided an indication of the most efficient method to apply depending on the enhancement powder being used.

Despite the optimal protocol for the analysis of secondary lifts being very desirable, given the kind of additional intelligence that could potentially be retrieved, there was an argument to persuade WYP in eventually allowing the analysis of primary lifts whilst keeping the "chain of custody", paramount for the admissibility of the evidence in Court. As, understandably, primary lifts contain a lot more "material" than secondary lifts, the chances for retrieving the desired chemical intelligence are higher; especially for high profile and violent crimes, this opportunity cannot be ignored. Some of the aforementioned studies published in the literature[7-11] had also investigated the use of primary lifts, though no developmental work had been undertaken; Kaplan-Sandquist et al used in one study artificial sweat and contaminants (pharmaceuticals and explosives) to produce fingermarks[8]. However, in only one instance, marks were dusted and tape-lifted and only one fingerprint development powder was used (black powder); only aspirin and ibuprofen were detected though no ridge detail was obtained. In their second published work[10], they were mainly concerned with "touch chemistry" and did not attempt fingermark imaging in primary lifts obtained using conventional fingerprint black powder and conductive tape recovery. Spot images and detection of TNT in tape lifts, by their analytical nature, did not have the purpose of full ridge pattern reconstruction and are reported associated with a broad, overlapping and unconfirmed interested ion signals, whereas procaine detection seems realistic, though again no MS/MS analyses were performed. Finally, Sundar and Rowell, spiked fingermarks with drugs of abuse and therapeutic drugs prior to powdering (a) and apply cyanoacrylate fuming followed by powdering (b)[9]. Powdering was performed with ground DHB matrix when MALDI MSI was employed prior to tape lifting the marks (the study at large concentrated on the use of the SALDI MS technique). However no MALDI MS images or spectra of the primary lifts were reported.

Therefore, given the absence of studies on secondary lifts optimisation and of informative studies on primary lifts, the aims of this initial study were: 1) to investigate on the most efficient method to recover and treat secondary lifts; 2) to demonstrate that primary lifts are more efficient than secondary lifts in providing the desired intelligence; 3) establish the most appropriate technique/combination to yield the most chemical/visual information amongst the different sample preparation techniques for MALDI analysis, combined or not with FET for both secondary and primary lifts. With regards to point 1), it is important to note that proof of this fact was

required so that Police Forces may recognise (on the basis of scientific facts and not assumptions) the necessity of devising ways, compatible with the chain of custody and admissibility to Court, in which primary lifts are directly accessed by the mass spectrometrist. This study was in particular a direct response to West Yorkshire Police (UK) request for researching into these areas demonstrating that primary lifts outperform secondary lifts, if suitably prepared, and are paramount to help criminal investigations.

In order to demonstrate a real need for analysing preferably primary lifts, experiments were undertaken to compare and contrast the quality of the mass spectrometric information between the MALDI MS images of primary and secondary lifts. The overall analytical strategy did not significantly change; the same three enhancing powders were investigated, using the same surface of deposition as a model (aluminium slides) for both ungroomed and natural fingermarks. Both endogenous compounds and the exogenous substance, DMA, were used as molecular references. The method that provided the poorest performance in the initial set of experiments (when investigating secondary lifts only) was not carried forward in later experiments when comparing primary and secondary lifts. Results show, as expected, that overall, primary lifts yielded significantly higher quality fingermark MALDI MS images ("higher grade according to the grading scheme suggested by Bandey et al[18]) and as a result, the current ongoing collaboration with WYP has granted the FRG at SHU the opportunity to analyse real crime scene primary lifts, a topic that will be presented and discussed in a future publication.

2.0. Materials and Methods

2.1. Materials

Trifluoroacetic acid (TFA), α-cyano-4-hydroxycinnamic acid (α -CHCA), benzalkonium chloride and ALUGRAM1 SIL G/UV254 pre-coated aluminium slides were purchased from Sigma Aldrich (Poole, UK). Acetonitrile (ACN) and acetone were obtained from Fisher Scientific (Loughborough, UK). MALDI target OPTI TOF spotless inserts were purchased from Applied Biosystems (Foster City, CA, USA). Double-sided conductive carbon tape was purchased from TAAB (Aldermaston, UK). Klenair Air Dusters were obtained from Klenro Ltd (Swindon, UK). Fingerprint brushes were purchased from Tetra Scene of Crime Ltd (Essex, UK). All fingermark development powders were provided by the West Yorkshire Police Forensic Laboratory (Wakefield, UK).

2.2. Instrumentation

All mass spectrometric analyses were conducted using a modified Applied Biosystems API "Q-Star" Pulsar i hybrid quadrupole time-of-flight (QTOF) instrument (Concord, Ontario, Canada). The orthogonal MALDI source has been modified to incorporate a SPOT 10 kHz Nd:YVO_4 solid-state laser (Elforlight Ltd., Daventry, UK), having a wavelength of 355 nm and a pulse duration of 1.5 ns[19]. Images were acquired at a spatial resolution of 150 x 150 µm in raster imaging mode, using 'oMALDI Server 5.1' software supplied by MDS Sciex (Concord, Ontario, Canada). MALDI MSI acquisition was performed in positive ion mode in the mass range between m/z 50 and 1000. The declustering potential 2 was set at 25 arbitrary units, with an accumulation time of 0.117 min. Each split fingermark image was acquired in around 120 min run time.

2.3. Data processing

For uncontaminated marks, MALDI MS images were processed using Biomap software (Novartis, Basel) by normalising against the total ion count (TIC) and visualising the selected *m/z* values with the optimal contrast and intensity to visualise the fingermark ridges. For DMA contaminated fingermark samples, following normalisation the selected m/z values were set to the same contrast and intensity to directly compare the efficiency of the MALDI-MS analyses in relation to the initial development powder employed. Mass spectra were processed using Analyst MDS Sciex (Concord, Ontario, Canada) and the open source multifunctional mass spectrometry software mMass[20]. Assessment of the image quality (clarity and ridge pattern continuity) was carried out by the authors (and subsequently confirmed in a blind assessment by a Fingerprint Expert from West Yorkshire Police) according to the grading scheme published by Bandey et al[18] from the Home Office, UK. According to this scheme, grade zero describes a mark yielding no evidence; evidence of contact but no ridge detail is described as a grade 1 mark; a grade 2 and 3 marks are associated to marks with about 1/3 of ridge detail, that probably cannot be used for identification, and to an identifiable mark (between 1/3 and 2/3 of ridge detail) respectively. Grade 4 is assigned to an identifiable mark with full ridge detail.

2.4. Latent Fingermark Deposition

All fingermarks were prepared fresh (no aging) and analysed following sample preparation after deposition. Both latent "ungroomed" and "natural" fingermarks from one male donor were deposited onto aluminium slides, used as the only reference surface, at a pressure between 400-

1000 g as previously described[21] A total of 18 full fingermarks have been imaged throughout these experiments, amounting to a total of 72 quarter samples prepared using different methodologies. Fingermarks spiked with dimethylbenzylammonium ion (DMA) were prepared using a contact-transfer methodology as previously reported[4]; briefly, a total of 100 µL of a 10 µg/mL MeOH solution of the compound was deposited onto glass slides. The solvent was allowed to evaporate and either a natural or ungroomed fingertip was rubbed across the entirety of the surface in order to ensure equal distribution of the exogenous species on the fingertip, before depositing a fingermark onto an aluminium slide.

2.5. Fingermark development and preparation

Fingermarks deposited onto aluminium slides were developed using either; aluminium powder, TiO_2 (white) powder or carbon (black) powder before being recovered from the surface using Sirchie lifting tape. In order to avoid cross contamination between fingermark samples, different brushes were employed for uncontaminated/contaminated marks. Three different experiments were performed, employing either; ***Set of experiments (A)*** - comparison of secondary lifts (Figure 1 A) or ***Set of experiments (B)*** - comparison of primary and secondary lifts (Figure 1 B).

In ***Set of experiments (A)***, a fingermark was deposited onto an aluminium sheet and developed using one of the three conventional powders. The mark was then recovered from the surface using forensic lifting tape. The aluminium sheet was then cut into 4 sections and subjected to either (i) no further enhancement, (ii) re-enhancement using the same powder employed for the initial development or (iii) and (iv) re-enhancement with α-CHCA (dry powder). A "secondary lift" (additional recovery from the aluminium slide using forensic lifting tape) was then performed and each of the sections was stuck onto a MALDI plate in the initial orientation in order to re-form the full fingermark. Sections (i, ii and iv) were then sprayed with a MALDI matrix solution whereas section (iii) was sprayed with solvent to induce co-crystallisation of the dry matrix powder with the remaining fingermark residue as previously reported[13].

In ***Set of experiments (B)***, fingermarks deposited onto aluminium slides were developed using one of the three conventional powders. The developed mark was then recovered as a primary lift and half was reserved for analysis. One half of the remaining remnants of the mark were split into three sections and treated using the optimised methodologies determined by ***Set of experiments (A)***. Once recovered from the surface, each of the sections was stuck onto a MALDI plate in the

initial orientation in order to re-form a full fingermark and sprayed with the MALDI matrix prior to MALDI MSI analysis.

These experimental layouts have been illustrated in figure 1.

In an additional experiment, a natural fingermark was deposited onto an aluminium slide and was developed using carbon black powder before being recovered from the surface using forensic lifting tape. One half only of the fingermark was sprayed with the MALDI matrix. Both halves were then stuck onto a MALDI plate to reform a full fingermark prior to MALDI-MSI analysis.

2.6. Matrix deposition

Latent fingermarks recovered using forensic lifting tape were either spray-coated with α-CHCA MALDI matrix or solvent following the protocol outlined in the 'Dry-Wet' method[13]. Both of these methodologies employed the use of a 'SunCollect' autosprayer (SunchromGmbH, Friedrichsdorf, Germany). For the sections spray-coated with the MALDI matrix, a solution of 5 mg/mL α-CHCA in 70:30 ACN: 0.5% TFA was sprayed onto the sample surface for a total of 4 layers at a rate of 2 µL/min, using a ''slow'' raster setting. Fingermark sections subjected to the conventional Dry-Wet method were dusted with the MALDI matrix (α-CHCA) powder before being sprayed with a solvent solution of 70:30 ACN: 0.5% TFA for a total of 5 layers at a rate of 5 µL/min, using the 'medium' raster setting.

3.0. Results

In an initial set of experiments, both ungroomed and natural uncontaminated fingermarks were developed using one of three conventional fingermark development powders; aluminium, TiO_2 (white) or carbon (black). The developed marks were then treated according to **Set of Experiments A** (comparison of secondary lifts) (Figure 1 A) or **Set of Experiments B** (comparison of primary and secondary lifts) (Figure 1 B). For each fingermark sample, MALDI MS images of the three most abundant ions (normalised against the total ion current and set to the optimal contrast and intensity to accentuate the fingermark ridges) were generated and the effectiveness of the protocols were evaluated based on the ridge pattern clarity according to the Bandey et al grading scheme[18] (relatively to the ion signal yielding, the best ridge clarity within the set of three ions selected). All fingermark grading have been adjudicated and confirmed by a Fingerprint Identification Expert from West Yorkshire Police, UK.

For ***Set of experiments A***, the initial observation from the MALDI MS images was that the conventional Dry-Wet method (the bottom left quartile) performed less efficiently than all the other three trialled methods (Fig 2); grade 0 images were produced for all fingermark samples, with the exception of *m/z* 666.6 in the aluminium powder developed ungroomed and natural fingermarks whereby faint ridge characteristics were observed.

Aluminium powder developed ungroomed and natural marks (Grade 3) and the carbon powder developed natural mark (Grade 1) showed similar ridge detail quality in each of the three remaining sections. The TiO_2 and carbon powder developed ungroomed marks provided most ridge clarity in the section re-enhanced using the initial powder (Grade 2), whereas for the TiO_2 developed natural mark the best results were obtained from the sections re-enhanced using TiO_2 and α-CHCA powders respectively (Grade 2).

Using ***Set of experiments B***, fingermarks were developed in the same manner as described previously, however an entire half of the primary lift was reserved for direct MALDI MSI analysis and compared to the three successful secondary lift methodologies determined by ***Set of experiments A*** (Figure 1 B). Results are illustrated in Fig 3. The aluminium powder developed ungroomed mark, TiO_2 powder natural developed mark and carbon powder developed ungroomed and natural marks showed significantly better ridge detail within the primary lifted portion of the samples (Grades 2, 2, 1 and 2, respectively) with faint ridges only being observed in the secondary lifts of the aluminium powder developed ungroomed mark (Grade 1). On the contrary, for the aluminium powder developed natural mark and TiO_2 powder developed ungroomed mark, a similar ridge pattern quality was observed in all the sections except that re-enhanced using the α- CHCA matrix (Grades 2 and 1, respectively).

In a final experiment, primary and secondary lifts of fingermarks obtained by spiking the fingertips with DMA, an exogenous compound often found in antibacterial products, were analysed by MALDI MSI following the application of ***Set of Experiments B***. Following image normalisation, the MALDI MS images of the parent ion (*m/z* 304.2) and the in-source fragment of DMA (*m/z* 212.2) were set to the same contrast and intensity to allow direct comparison of the efficiency of the conventional powder-MALDI MSI workflow when employing each of the different methodologies. For each of the fingermarks, post-image MALDI MS/MS analyses were conducted to confirm the identity of this species within the fingermark ridges. A typical MALDI-MS and MS/MS spectrum obtained from the analysis of DMA have been included in Figures 4C and 4D, respectively. In most

instances, the primary lifted portion of the fingermark image showed a greater amount of ridge detail clarity for both DMA and its in-source fragment. For carbon black powder developed marks, Grade 4 images were obtained for the primary lifts, whereas Grade 2-3 ridge quality was observed in each of the secondary lifted sections. For aluminium powder developed ungroomed and TiO_2 powder developed natural and ungroomed marks, Grade 3 images were observed for DMA within the primary lifted sections in comparison to Grade 1-2 within the secondary lifts. A reduction in fingermark ridge quality was observed through molecular mapping of the DMA in-source fragment, with Grade 1 images in the primary lifts compared to Grade 0 images within the secondary lifted portions of the aforementioned fingermark samples. One exception occurred, however, for the aluminium powder developed natural fingermarks whereby ridge detail clarity was the same overall (Grade 3 for the parent ion and Grade 1 for the in-source fragment, though a significantly greater intensity of the ion image was observed in the secondary lifts for the sections subjected to either no further enhancement or re-enhancement using aluminium powder.

An additional experiment was conducted comparing the MS spectra and the MS images of two halves of the same natural mark, both treated with carbon black though only one half was subsequently sprayed using the MALDI matrix. As illustrated in Fig S1 A, carbon black does not work as an efficient matrix as, contrary to the mark half treated with carbon black and subsequently sprayed with MALDI matrix yielding a grade 3 image, it did not yield any ridge detail within the half mark left matrix-unsprayed (grade 1 image). The mapping of endogenous species, eicosanoic acid (*m/z* 311.2) was selected as an example to illustrate the carbon black performance as a potential matrix. To further confirm this observation, average spectra were extracted from the two regions of interest (ROI) drown over the two halves of the mark, showing a rich MS spectrum from the matrix sprayed mark and no ion signal from the unsprayed half (Fig S1 B and C). The ROI from where the spectra were extracted have also been included in the figure (Fig S1 B(i) and C(i)).

4.0. Discussion

This paper reports on research aimed at providing an indication as to the sample preparation method yielding the most ridge detail in secondary lifts of latent fingermarks as well as demonstrating that primary lifts are paramount to maximise chance of suspect identification through a higher quality ridge detail. In order to avoid repetitions and overlapping with the previous section, conclusions as to the effectiveness of the methods will be discussed without

reporting again on the corresponding technical assessment (grading) for which the reader is reminded to the Results section.

In the comparison of secondary lifts, in terms of ridge pattern image reconstruction, the conventional Dry-Wet method produced significantly poorer results than the other methods adopted within ***Set of Experiments A*** (Fig. 2). However, in previous studies that have employed the Dry-Wet method, fingermark samples have never been subjected to prior conventional FET[13,14 22]. This additional and prior fingermark development step appears to have diminished the effectiveness of the methodology, providing inferior results, in terms of ridge clarity, to the other three sections that were sprayed eventually using a conventional α-CHCA solution. Additionally, the aforementioned studies were also performed on marks that were either un-lifted or obtained as a primary lift. Therefore it is speculated that the results obtained here originate from a sub-optimal matrix-to-analyte ratio (considering the minute amount of the fingermark residue remaining on the surface after the initial lift) therefore negatively impacting upon the efficient co-crystallisation of the matrix with the analytes on the ridges. Based on these results, the conventional Dry-Wet method (application of the dry powder followed by solvent spray only) following FET treatment was removed from subsequent experiments and only the three MALDI matrix spray coated sections will be discussed further.

Throughout the experiments performed using ***Set of experiments A*** and ***Set of experiments B***, the most abundant species observed in the fingermarks included known endogenous lipids; 13-aminotridecanoic acid (*m/z* 230.2), oleic acid (*m/z* 283.2), eicosenoic acid (*m/z* 311.2) and a triglyceride (*m/z* 638.6) (Figure 2 A) and unidentified species (e.g. *m/z* 610.4) were used for easy inter-comparison of the methods' performances. It is important to note, that the identity of any of the species selected within the unspiked marks is irrelevant as the main purpose of these initial experiments was to assess the quality of image reconstruction for each individual fingermark.

For aluminium powder developed ungroomed and natural marks and carbon powder developed natural marks, similar ridge pattern clarity was achieved within each of the sections, suggesting that any of the three sample preparation methodologies could be successfully employed prior to performing a secondary lift. For TiO_2 powder developed marks, the best results were achieved from the sections subjected to re-enhancement using the initial powder. This was an unexpected observation as, although TiO_2 nanoparticles can be used as a MALDI matrix due to their UV absorbing properties[23], previous results have shown that TiO_2 based fingermark enhancement powders cause a reduction in signal in comparison to when using α-CHCA alone[2]. Therefore, the

authors speculate that the application of additional TiO_2 powder in these instances resulted in additional particle adherence to the fingermark ridges, subsequently enabling the recovery of more fingermark material during the secondary lift. A similar explanation can be provided for the results obtained for the carbon powder developed ungroomed fingermark, which also showed better ridge clarity in the re-enhanced portion of the sample.

Although these initial results varied depending on the type of mark and powder being employed, these experiments provided a positive indication that through the application of specific methodologies, if primary lifts are unavailable, secondary lifts could be a viable option to enable additional intelligence to be obtained from fingermarks recovered from crime scenes. Results also provide operational insights on how to best treat the fingermark residue after the first lift for optimal MALDI MSI analysis according to the type of powder employed. One key and expected observation, however, was that in many of the MALDI MS images shown here, the quality of ridge reconstruction was noticeably poorer than what has previously been observed by this group for conventional powder-MALDI MSI workflows[2]. In fact, only the aluminium powder developed marks provided fingermark reconstruction considered as grade 3 according to the Bandey et al grading scale[18]. This is of course due to the fact that in the aforementioned study, powder developed marks were lifted once (primary lifts) and directly analysed by MALDI MSI. Therefore, at least an additional set of experiments was devised whereby secondary and primary lifts were compared to determine the extent of loss in fingermark quality when employing these extended sample preparation methodologies.

Using ***Set of Experiments B*** (Fig. 1 B), both ungroomed and natural fingermarks were developed using the same three powders and MALDI MSI was employed to compare the ridge detail retrievable from primary and secondary lifts. For both the TiO_2 and carbon powder developed marks and the aluminium powder developed ungroomed mark, the primary lifted sections provided better ridge detail quality in comparison to that obtained from the secondary lifts. These results were expected and confirmed that the primary lift of a developed mark recovers the majority of the fingermark material, thus providing a greater signal in the subsequent MALDI-MS images.

Interesting observations, however, were obtained for the aluminium powder developed natural mark, which provided similar fingermark quality across each of the four sections. Natural marks generally contain more endogenous and exogenous species than the ungroomed mark counterparts due to the method used to prepare the latter[18,20] meaning that more fingermark

residue will be available for the adherence of powders during the enhancement process. It has previously been shown that aluminium powder does lower the ion intensity of species contained within fingermark residue when being analysed by MALDI-MSI[2]. Therefore, it is hypothesised that although the majority of fingermark material is recovered by the primary lift, an excess of aluminium powder deposited onto the natural mark appears to have caused suppression of the MALDI-MS signal and resulted in a similar quality of fingermark ridge reconstruction from each of the sections.

Although the molecular mapping of endogenous species can be useful in providing fingermark image reconstruction suitable for suspect identification, the detection and identification of exogenous species within the fingermark residue could potentially provide additional intelligence about an individual, including their lifestyle and activities prior to committing a crime. Therefore, in a final experiment, fingermarks were contaminated with DMA and were treated according to the sample preparation methods outlined for **Set of Experiments B.** DMA is an exogenous compound often found in antibacterial products, a common contaminant within latent fingermark samples and has high ionisation efficiency, making it an ideal model compound for investigation within this study.

Similarly to the results obtained in the comparison of primary and secondary lifts of uncontaminated marks, all fingermark samples except the natural aluminium powder developed fingermark showed superior ridge detail clarity within the primary lifted sections of the marks. This, once again, can be attributed to the removal of an abundance of the DMA contaminant during the initial primary lift and the suppressing effects caused by aluminium powder which reduced the signal obtained in the primary lifted aluminium powder developed section of the natural mark.

Interestingly, comparison of the results obtained from the different powders, through normalisation of the images, indicated that the most efficient development technique for the subsequent detection of DMA was carbon powder. The application of this powder had previously provided some of the poorest fingermark ridge quality for the uncontaminated fingermark samples (figures 2 and 3) indicating that the performance of this powder in terms of the compatibility with MALDI MSI is not as good when fatty acids are imaged. In future experiments, it will be interesting to employ the same methodology for fingermarks contaminated with a range of different exogenous species to observe if the results are reproducible depending on the contaminant employed. However, overall, these experiments do highlight the preference for

primary lifts analysis to maximise the opportunity to recover the highest ridge detail clarity and ion intensity.

The successful application of post-image MALDI MS/MS analysis to confirm the presence of DMA in each of the fingermark sections (both primary and secondary regions) highlights the opportunity for an extended workflow which can be employed to maximise and provide robust intelligence from a latent fingermark.

Even when a primary lift of a crime scene sample may not be available, it would be possible to use a more sensitive profiling approach on secondary lifts to obtain additional chemical information on minute traces of material. One potential application that has proven to be successful for the detection of trace amounts of exogenous compounds is MALDI ion mobility separation tandem mass spectrometry (MALDI-IMS-MS/MS) which can add confidence to the identification of any detectable species and has been successfully employed previously for the detection of excreted drug metabolites in latent fingermark samples[24].

Although the mechanistic aspects relating to forensic powder compositions affecting ionisation are out of the scope of this paper, a point of interest concerns carbon black powder. Carbon based matrices can be effectively employed in MALDI MS experiments[25]; therefore, it may be speculated that carbon black fingermark enhancement powder could contribute to the ionisation of species contained within the fingermark residue. In order to exclude this possibility , an additional experiment was conducted comparing the MS spectra and the MS images of two halves of the same natural mark, both treated with carbon black though only one half was subsequently sprayed using the MALDI matrix. As described the Results section, carbon black powder did not in any significant way contribute to the ionisation of the molecules contained in the mark (Fig S1). It may be argued that this could be due the chemical composition of the powder which is not exclusively made by carbon black, though the full composition is unknown.

Every set and subset of experiments were undertaken by using one fingermark split in quarters; each quarter was subjected to treatment with a different method. The undertaking of repeats is always desirable. However, these types of studies are extremely time consuming and this is an initial study, unique in its kind thus far. The authors propose this as a suitable method for preliminary investigations because, especially for the use of ungroomed fingermarks, since the fingertips are rubbed against each other, the chemical material will be equally distributed in the mark; therefore, all the quarters should provide the same ridge clarity, if the different methods applied were working equally efficiently. Though a single mark was used, the application of the

four methods on each of the four quarters provides a direct (intra)comparison of the methods themselves.

In future endeavours however, beyond this initial study, in order to make a definitive and exhaustive assessment on the best method, the use of multiple repeats will have to be undertaken, as and where appropriate. Furthermore, in order to avoid analytical bias, additional experiments could be designed in which the order of the methods applied to each of the quarters is randomized. Finally the use of multiple donors, different surfaces and fingermarks of different age exposed to a range of environmental conditions will also yield more exhaustive insights as to the effectiveness of the method proposed relatively to the forensic powders being investigated. This is because all the factors above affect the recovery of the mark due to differential chemical composition. These additional studies are also necessary to pass the scrutiny of the Forensic Regulator which would then enable the Home Office to make the final recommendations as to the use of the method proposed.

Conclusion

MALDI-MSI is an advanced analytical tool that can offer a breadth of information from latent fingermark samples, both in terms of fingermark ridge reconstruction and chemical information about the donor. Conventionally, marks developed at crime scenes are recovered using forensic lifting tape, sealed into acetate and scanned into a National Police fingerprint database for comparison and match. Therefore, only the remaining residue on a surface may be available for MALDI-MSI following a "secondary" lift in order to provide additional information without interfering in the conventional processes. Within this manuscript, it was shown that additional chemical and physical information can still be retrieved from "secondary" lifts though primary lifts generally yield a much better performance. When investigating both endogenous compounds and the exogenous species DMA, the fingermark ridge pattern clarity within primary lifts was of a greater intensity than the secondary lifts for both TiO_2 and carbon powder developed ungroomed/natural marks and also aluminium powder developed ungroomed marks. In both instance, natural marks developed using aluminium powder provided a similar quality of fingermarks across both the primary and secondary lifts which has been attributed to suppression effects caused by an excess of aluminium powder on the fingermark ridges.

Ultimately, the reality of crime scene samples is that as the composition of (natural) fingermarks, in terms of chemical abundance and compounds recovered, will always be unknown. The authors

emphasise that in order to maximize the chances of recovering additional intelligence from a crime scene mark, it is imperative that a primary lift is obtained for subsequent MALDI-MSI analysis.

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Figure legends

Figure 1. Methods employed for the preparation of fingermark samples followed by MALDI MSI analysis, including; ***Set of experiments A*** for the comparison of secondary lifts and ***Set of experiments B*** for the comparison of primary and secondary lifts.

Figure 2. The application of ***Set of experiments A*** for the comparison of MALDI MS images of "secondary" lifts obtained from (A) ungroomed and (B) natural latent fingermarks which had been pre-developed using conventional fingerprint powders.

Figure 3. The application of ***Set of experiments B*** for the comparison of MALDI MS images of "primary" and "secondary" lifts of (A) ungroomed and (B) natural latent fingermarks which had been pre-developed using conventional fingerprint powders.

Figure 4. The application of ***Set of experiments B*** for the comparison of MALDI MS images of "primary" and "secondary" lifts of (A) ungroomed and (B) natural latent fingermarks which had been previously spiked with the exogenous species, dimethylbenzylammonium ion (DMA) followed by development using conventional fingerprint powders. A typical MALDI MS and MS/MS spectrum of DMA have been included in (C) and (D), respectively.

Figure S1. The MALDI MS Imaging analysis of a carbon black powder developed and "primary" lifted split natural fingermark; (A) MALDI-MS image of the endogenous species eicosanoic acid (m/z 311.2), (B) the spectrum extracted from the half of the fingermark treated with carbon black and sprayed with matrix (ROI shown in (i)) and (C) the spectrum extracted from the half of the fingermark only treated with carbon black (ROI shown in (i)). Both the MS image and MS spectra show no contribution of carbon black as a potential matrix to the ionisation of the molecules contained in the fingermark