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ABSTRACT: The first analytical intercomparison of fingerprint residue using equivalent samples of latent fingerprint residue and characterized by a suite of relevant techniques is presented. This work has never been undertaken, presumably due to the perishable nature of fingerprint residue, the lack of fingerprint standards, and the intradonor variability, which impacts sample reproducibility. For the first time, time-of-flight secondary ion mass spectrometry, high-energy secondary ion mass spectrometry, and X-ray photoelectron spectroscopy are used to target endogenous compounds in fingerprints and a method is presented for establishing their relative abundance in fingerprint residue. Comparison of the newer techniques with the more established gas chromatography/mass spectrometry and attenuated total reflection Fourier transform infrared spectroscopic imaging shows good agreement between the methods, with each method detecting repeatable differences between the donors, with the exception of matrix-assisted laser desorption ionization, for which quantitative analysis has not yet been established. We further comment on the sensitivity, selectivity, and practicability of each of the methods for use in future police casework or academic research.

Characterization of the chemical composition of latent fingerprints is of interest in many disciplines. In police casework, if a fingerprint is smudged or if the donor is not listed on a fingerprint database, it can be impossible to make an identification. However, it is known that the endogenous composition of a fingerprint varies from donor to donor,1–3 and it is thought that this information could be used to give intelligence on a donor’s age, gender, ethnicity, medical history, or drug habits.4–6 Knowledge of the endogenous composition of fingerprints deposited on various surfaces is, additionally, of importance for the optimization of fingerprint development reagents7–10 which are used to enhance ridge detail. The relative abundance of endogenous compounds in a fingerprint is known to change as a function of aging11–13 and so is pertinent to understanding the aging processes of fingerprints exposed to different environments, which are not yet well understood. This knowledge will help to optimize the

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performance of fingerprint reagents and perhaps assist with the determination of the age of a latent fingerprint, which would significantly aid police investigations.\textsuperscript{14–16} Similarly, measurement of exogenous compounds in fingerprints may be used to link a suspect to a particular substance.\textsuperscript{14–17}

A number of analytical techniques have been used to characterize the endogenous composition of fingerprints, mainly mass spectrometry or spectroscopic techniques.\textsuperscript{16} Each method has different capabilities in terms of sensitivity, selectivity, reproducibility, and ultimately practicability either for use in routine police work or for future academic research. Due to the lack of fingerprint standards, the perishable nature of fingerprint residue, and the difficulty in obtaining a reproducible sample, no attempt has hitherto been made to compare the techniques available for the chemical characterization of fingerprints. We aim to provide a comparative analysis of fingerprint residue based on a standardized protocol and using a suite of relevant analytical techniques to aid the selection of appropriate techniques for future targeted studies. For the applications envisaged, it will be necessary to identify a range of compounds in the fingerprint and to quantify (in either relative or absolute terms) the concentration of each compound so that differences between different fingerprints can be reliably measured.

Much of the prior research into the endogenous composition of fingerprints has been carried out using gas chromatography/mass spectrometry (GC/MS).\textsuperscript{1,2,8,18} Previous studies have also found that matrix-assisted laser desorption ionization (MALDI)\textsuperscript{1,2,1,17,46,48} and attenuated total reflection Fourier transform infrared spectroscopic imaging (ATR-FT-IR)\textsuperscript{16,23–26} are sensitive to endogenous compounds in fingerprints. Time-of-flight secondary ion mass spectrometry (ToF-SIMS),\textsuperscript{19–21} its high-energy variant, MeV-SIMS,\textsuperscript{22} and X-ray photoelectron spectroscopy (XPS)\textsuperscript{27} have been found to be sensitive to exogenous compounds in fingerprints, but no attempt has hitherto been made to detect endogenous compounds or quantify them. Each technique offers a potentially useful feature for the analysis of fingerprint chemistry, as summarized in the Supporting Information. Recent studies have used fluorescent nanoparticles with immunochemical tags to simultaneously detect target compounds in fingerprints, but these methods are at present limited to a small number of preselected compounds.\textsuperscript{47}

In this study, we assess the sensitivity of GC/MS, MALDI, ToF-SIMS, MeV-SIMS, XPS, and ATR-FT-IR spectroscopic imaging to a selection of endogenous compounds in fingerprints that were deposited using a standardized protocol. A noteworthy exception is desorption ionization mass spectrometry (DESI),\textsuperscript{4} which could not be included in this study due to technical problems. We assess, for the first time, the ability of the less established techniques (ToF-SIMS, MeV-SIMS, MALDI, XPS) to detect endogenous compounds in fingerprints and to detect reproducible differences between the donors. We corroborate these results between each technique and with the more established GC/MS and ATR-FT-IR spectroscopic imaging. We then comment on the selectivity and the appropriateness of each of the techniques for future academic research or police casework.

\section*{MATERIALS AND METHODS}

The production of a standard set of fingerprint samples is not trivial, since fingerprint composition varies between and within donors and due to age and storage conditions.\textsuperscript{1,2,11} To provide each analyst with a comparable set of samples, a protocol for fingerprint deposition and analysis was developed. All fingerprints used in the study were deposited during an interval of less than 3 h by three staff members (two male (donors A and C), one female (donor B)) of the Home Office. The donors were allowed to drink water but not consume food during the depositions. The fingerprints were deposited according to Home Office internal guidelines: hands were washed using soap and water, and the soap residue was washed away before the hands were placed in clean polythene bags for 20 min followed by one light contact to the forehead prior to the palms of the hands and fingertips being rubbed together. Depositions were then made from the index, middle, and ring fingers of each hand using touch pressure, with two successive fingerprints being deposited per active finger before the entire process was repeated. Analysts selected substrates of dimensions and composition to suit their analytical technique.

Each analyst was allocated three fingerprints per donor, except for GC/MS and MALDI. GC/MS required eight fingerprints per analysis for sensitivity reasons. MALDI used three fingerprints per analysis for both imaging and profiling (point analyses). Samples were boxed immediately, covered in aluminum foil to keep out light, placed on dry ice, and shipped to the analyzing laboratory. Upon receipt, each analyst stored the samples at $-20 \degree C$ or below. Samples were allowed to reach room temperature prior to analysis. The samples were all analyzed 1 week after the deposition date and within 4 days of each other, except for the samples analyzed by MALDI and MeV-SIMS, which, due to technical difficulties, were analyzed within 3 months of the deposition date.

\textbf{XPS.} Fingerprints were deposited on silicon wafers cut into $3 \times 3$ cm squares and then cleaned. XPS measurements were made on a VG Escalab 210 photoelectron spectrometer, with a nonmonochromated Al Ka X-ray source (1486.6 eV), operated with an X-ray emission current of 20 mA, an anode acceleration voltage of 12 kV, and a takeoff angle of 90° relative to the sample plane, with an acquisition area of $\sim 5 \times 2$ mm. Each analysis consisted of a wide survey scan (pass energy 50 eV, 1.0 eV step size) and high-resolution scans (pass energy 50 eV, 0.05 eV step size). The binding energy scale was calibrated using the Au 4f\textsubscript{5/2} (83.9 eV), Cu 2p\textsubscript{3/2} (932.7 eV), and Ag3d\textsubscript{3/2} (368.27 eV) lines of cleaned gold, copper, and silver standards from the National Physical Laboratory (NPL), United Kingdom.

\textbf{CasaXPS 2.3.15}\textsuperscript{28} was used to fit the XPS spectra, and the theoretical Scofield relative sensitivity factor library was used to generate quantitative data. The carbon peak (C1s) was fitted with four components: carbon bound only to carbon and hydrogen, C–(C,H), at $\sim 285.0$ eV, carbon singly bound to oxygen from ethers, alcohols, amines, and/or amides, C–O, at $\sim 286.0$ eV, carbon doubly bonded to oxygen or singly bonded to two oxygen atoms from amides, carboxyls, carboxylates, esters, acetals, and/or hemiacetals, C=O and O–C–O, at $\sim 288.0$ eV, and carbon attributable to ester carboxylic functions, COOR, at $\sim 289.5$ eV.\textsuperscript{29,30} To account for differences in the ridge width and density between different fingerprints, the intensity of the elements detected was normalized to a correction factor using the Si 2p intensity over the analyzed area, where the Si was assumed to have come from the substrate only.

\textbf{ATR-FT-IR Spectroscopic Imaging.} Fingerprints were deposited directly on gel lifters from BVDA International b.v., Postbus 2323, 2002 CH Haarlem, The Netherlands. FT-IR
images of fingerprints deposited directly on Si wafers could be obtained in transmission with greater sensitivity, but gelatin tape was used to lift fingerprints from the surface of Si wafers because of the relevance of this lifting approach to real forensic situations. The tape was then placed on the surface of a ZnSe crystal for ATR measurements. The arrangement of the optics in this ATR approach is described in more detail elsewhere.\textsuperscript{6,24,25} FT-IR chemical images were collected using a Varian system, comprising a 670 FT-IR spectrometer coupled with a 64 × 64 focal plane array (FPA) infrared detector. The imaging area measured using an ATR accessory (VeeMaxII from PIKE) and this particular FPA detector was 4.3 × 5.9 mm\textsuperscript{2}. The infrared spectra within each image were collected at 8 cm\textsuperscript{-1} spectral resolution using 200 coadded scans (giving a total scanning time of approximately 150 s per image).

**GC/MS.** Samples were deposited on a cleaned Mylar 002 polyester film, 0.23 μm thickness (DuPont Teijin Films (UK) Ltd., Middlesbrough, U.K.), 10 × 2 cm, precut in half lengthways. Negative controls were prepared at the same time using 10 × 2 cm strips of Mylar, without the addition of fingerprints. The samples were extracted and derivatized as described in a previous study,\textsuperscript{2} which included the addition of p-chlorophenylalanine and \( d_{35} \)-octadecanoic acid as internal standards. Samples were analyzed on a Perkin-Elmer 600 gas chromatograph coupled to a Perkin-Elmer 600C mass spectrometer. Two microlitr aliquots were injected in split mode (10:1) onto a DB-17 ms fused silica capillary column (30 m × 0.25 mm internal diameter × 0.15 μm film thickness, J & W Scientific, Folsom, CA). The remaining GC/MS parameters were as previously described.\textsuperscript{2} Samples were analyzed in selected ion monitoring (SIM) mode for quantification. The spectrometer was set to monitor three characteristic ions per compound, a quantitative ion (for quantification) and two qualifier ions (to confirm identification). Samples were also analyzed in scan mode (50–500 m/z at one scan per second following a 4.5 min solvent delay) for qualitative purposes. For quantification, calibration samples were prepared by depositing known amounts of amino acids (0.03–5 nmol), fatty acids (0.1–37 nmol), and squalene (0.3–125 nmol) standards on 2 × 2 cm pieces of Mylar film, adding 8 μL of \( p \)-chlorophenylalanine in methanol (0.3125 μmol mL\textsuperscript{-1}) and 8 μL of \( d_{35} \)-octadecanoic acid in hexane (1.875 μmol mL\textsuperscript{-1}). The calibration samples were then extracted and derivatized as described for the fingerprint samples. All fingerprint samples were corrected using appropriate blanks.

**ToF-SIMS.** Fingerprint samples were deposited on polished silicon wafers cut into squares of 2 × 2 cm. Analyses were carried out on an IONTOF GmbH (Münster, Germany) ToF-SIMS 5 spectrometer, employing a 25 keV Bi\textsuperscript{3+} primary ion beam delivering 0.35 pA of current. Images were acquired at 128 × 128 resolution in the MacroRaster mode of operation over a 5 × 5 mm area. Image data were acquired using 256 cycles per pixel point with 1 scan per pixel and a cycle time of 100 μs. The mass calibration was performed by defining peaks with a known mass using a common series of C\textsubscript{n}H\textsubscript{2n+2} peaks between m/z ≥ 1 and m/z ≤ 157 using the instrument’s IonSpec (version 4.1.0) software. For each peak of interest, the maps for that m/z were inspected to see if ridge detail could be observed. If ridge detail was not clearly observed in the maps, or did not correlate inversely with the silicon signal from the substrate, it was concluded that the peak was not in the fingerprint. To account for differences in the ridge width and density between different fingerprints, the detected intensity of each peak was normalized to a correction factor using the Si (m/z = 27.98) intensity over the analyzed area, where the Si was assumed to have come from the substrate only.

**MeV-SIMS.** Fingerprint samples were deposited on square 2 × 2 cm polished silicon wafers. The samples were analyzed using a 2 MV tandem accelerator, onto which a target chamber was integrated with a linear ToF mass analyzer to analyze the secondary ions desorbed by the megaelectronvolt primary ions.\textsuperscript{31,32} Primary ions of 3 MeV \( 16\text{O}^{+} \) focused to 3 × 3 μm and with beam currents of 150 pA were pulsed to a width below 30 ns at a frequency of 5 kHz. The ToF device was mounted at a 45° angle to the incident beam and normal to the sample’s surface plane. All measurements were performed in positive polarity mode and over a 2 × 2 mm scan. The data were analyzed using the method described for ToF-SIMS.

**MALDI MS Profiling and Imaging.** Fingerprinted samples were deposited on aluminum sheets (ALUGRAM SIL G/UV254 precoated aluminum sheets from Sigma-Aldrich, Poole, U.K.) from which the silica had been previously removed using acetone.

**MALDI MS Imaging (MALDI MSI).** Fingerprinted samples subjected to MALDI MSI analyses in the lipid range were homogeneously coated in \( \alpha \)-cyano-4-hydroxycinnamic acid (CHCA) matrix using the SunCollect autospraying system (Sunchrom GmbH, Friedrichsdorf, Germany). The matrix was prepared at a concentration of 5 mg mL\textsuperscript{-1} in a 70:30 (v/v) acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA) solution and sprayed in three layers at a rate of 2 μL min\textsuperscript{-1}. Fingerprint samples were then mounted onto a stainless steel MALDI OPTI TOF insert using double-sided conductive tape and then submitted to MALDI MSI analysis on a modified Applied Biosystems API Q-Star Pulsar i hybrid quadrupole time-of-flight (QTOF) instrument.\textsuperscript{46} Image acquisition was performed using OMALDI server 5.1 software supplied by MDS Sciex (Concord, Ontario, Canada) at a resolution of 100 μm × 150 μm at a laser repetition rate of 5 kHz. MS images were processed using Biomap 3.7.5 software (Novartis, Basel, Switzerland). For accurate mass-to-charge observation, individual spectra were viewed in Analyst (MDS Sciex, Concord, Ontario, Canada) prior to being exported into mMass.\textsuperscript{33,34} mMass, where the spectra were smoothed, normalized, and deisotoped. Alternatively, average spectra were extracted by selecting, in Biomap, the whole fingerprint as a region of interest and exporting the data as TXT files for viewing in mMass.

**MALDI MS Profiling.** Fingerprint samples were manually spotted by pipetting 1 μL of CHCA in triplicate. In particular, CHCA (10 mg/mL) in 70:30 (v/v) ACN/TFA (0.1%) was employed to profile lipids in the mass range between m/z 100 and m/z 1000 using a modified Applied Biosystems API Q-Star Pulsar i hybrid QTOF instrument. Declustering potential 2 was set at 15 arbitrary units and the focus potential at 10 arbitrary units, with an accumulation time of 0.117 min. The spectra were internally calibrated using the matrix peaks at m/z 190.05, 212.03, and 361.08. MALDI MSI profiles of peptides and proteins were obtained using an Applied Biosystems MALDI TOF Voyager de-STR in positive linear mode in the mass range between m/z 1000 and m/z 10000. The instrument was calibrated using a peptide/protein mixture containing DCD-1 (4.8 kDa), insulin (5.7 kDa), and cytochrome c (12.4 kDa). The accelerating voltage was set at 20 kV and the delay time at 750 ns. Details on matrix preparation to profile peptides and small proteins will be reported elsewhere.\textsuperscript{38}
RESULTS

Each technique measured significant variation in the composition of the three repeat samples of each donor’s fingerprints, highlighting the difficulty in depositing fingerprints in a reproducible manner. Despite this intrasample variability, most of the techniques detected reproducible differences between the donors.

XPS. XPS detected for the first time a range of elements coming from endogenous compounds in the fingerprint samples, including C, O, N, Na, Ca, K, and Cl. A selection of these results is presented in Figure 1, together with the proportion of C bound either as COOR or as C−H/C−C in each fingerprint (note that the C−H and C−C y scale has been reduced by a factor of 10 to fit the plot). Fingerprints are denoted A1, A2, etc., where A, B, or C is the donor and 1, 2, or 3 is the replicate of the fingerprint from that donor.

ATR-FT-IR Spectroscopic Imaging. ATR-FT-IR spectroscopic imaging analysis revealed two bands above the considerable background of the gel-lift substrate, located at 2850 and 1745 cm⁻¹. The band at 2850 cm⁻¹ can be assigned to glycerides, ceramides, phospholipids, glycolipids, wax esters, or fatty acids and the band at 1745 cm⁻¹ to triglycerides and/or phospholipids.²⁴,²⁵ The images from the 1760–1730 cm⁻¹ band for each replicate of each donor’s fingerprints are shown in Figure 2. Similar images were obtained for the band at 2850 cm⁻¹. The highest intensity of these bands is found in the fingerprints of donor A, with some ridge detail also being detected in the maps of these bands for donor C. These bands were not detected for donor B.

GC/MS. The GC/MS results for fatty acids are displayed in Figure 3, showing reproducible differences between the donors. Generally, a higher mass of each fatty acid was detected in donor A and C samples compared to donor B samples, which is consistent with the ATR-FT-IR spectroscopic imaging analyses. Hexadecanoic acid (C16) was the most abundant fatty acid in all samples, with the exception of donor C, where decanoic acid (C10) was, which may be a consequence of a contaminant picked up from touching the face. Octadecanoic (C18) acid, cis-9-octadecenoic (C18:1) acid, and tetradecanoic (C14) acids were the next most abundant in varying orders for each donor. This is partly consistent with previously published data.¹−³,¹¹

Figure 1. Concentration of Cl, N, Na, and K detected by XPS and normalized to a correction factor derived from the Si substrate signal, plotted together with the percentage of C bound either as COOR or as C−H/C−C in each fingerprint (note that the C−H and C−C y scale has been reduced by a factor of 10 to fit the plot). Fingerprints are denoted A1, A2, etc., where A, B, or C is the donor and 1, 2, or 3 is the replicate of the fingerprint from that donor.

Figure 2. Images of fingerprints deposited on gel-lift tape by ATR-FT-IR spectroscopic imaging based on the distribution of the intergrated absorbance of the band at 1760–1730 cm⁻¹.
Homogeneity between samples was good for each donor and all compounds with the exception of C16 and C18 acids in donor B’s samples. Squalene was found to be present at much lower levels relative to other fatty acids than previously reported values of 7–11 μg per fingerprint. This may be a consequence of the sampling protocol used in each of the studies. The full range of detected compounds is tabulated in Table 1.

Twelve amino acids were detected by GC/MS, as documented in Table 1 and the Supporting Information. These were not detected by any of the other techniques, but the compounds detected are consistent with previous GC/MS studies. No systematic differences between the level of any of the detected amino acids in donor A’s and donor B’s fingerprints was seen, but donor C had reproducibly lower levels of serine and asparagine compared with the other two donors.

ToF-SIMS and MeV-SIMS. A number of the fatty acids detected by GC/MS were also detected by ToF-SIMS for the first time in latent fingerprints, as listed in Table 1. Figure 4 shows the normalized intensity of peaks at m/z 257.24 (assigned to C16) and 411.33 (assigned to squalene), together with the mass per fingerprint of C16 and squalene detected by GC/MS for each replicate of each donor’s fingerprint. There is good general agreement between the two techniques: both techniques show reproducible differences in donor B’s fingerprints compared with those of donors A and C. GC/MS detects a lower level of C16 in donor C’s fingerprints compared with those of donor A, whereas this difference is not clear by ToF-SIMS because the standard deviation of the measurements is higher. The higher standard deviation of the ToF-SIMS measurements is probably due to the fact that only one fingerprint was analyzed for each ToF-SIMS analysis, whereas each GC/MS analysis used eight fingerprints to gain the desired sensitivity. ToF-SIMS did not detect either compound in donor B’s fingerprints, in contrast to GC/MS, presumably because they were below the ToF-SIMS detection limit.

Figure 5 shows the normalized intensity of additional compounds detected for the first time in latent fingerprints by ToF-SIMS that were either not detected or not quantifiable by GC/MS: m/z 313.28 (assigned to C20, arachidic acid), m/z 327.24 (assigned to C21, heneicosanoic acid), m/z 341.29 (assigned to C22, docosanoic acid), and m/z 550.55 (assigned to dimethylidoctadecylammonium). Several of these ions were also detected by MALDI, as shown in Table 1. The ToF-SIMS measurements show clear differences between donors A, B, and C, as shown in Figure 5.

The mass resolution of the MeV-SIMS instrument at the time of analysis was not sufficient to detect fatty acids. This is because the technique is new and under development. However, MeV-SIMS provided maps, showing for the first time that MeV-SIMS can detect ridge detail of latent fingerprints and corroborated the results of ToF-SIMS and XPS that the inorganic content of the fingerprint was highest for donor B (see the Supporting Information).

MALDI. MALDI analyses revealed peaks corresponding to many of the compounds detected by GC/MS and ToF-SIMS. MALDI analysis revealed, for the first time, and as will be shown in more detail elsewhere, the presence of several peptides that were not detected by the other techniques (Table 1 and Supporting Information). These peptides are tentatively identified on the basis of their m/z and in consideration of the assignments previously made by others from the examination of sweat. Some of these putative peptides are reported to be skin natural antimicrobial agents.

Similarly to ToF-SIMS, MeV-SIMS, and ATR-FT-IR, spatial maps of molecules within the fingerprint ridges were produced using MALDI MSI, allowing the verification that a particular compound is located within the fingerprint. This is illustrated in Figure 6, where images of m/z 550.66 (assigned to dimethylidoctadecylammonium) are shown for each replicate fingerprint of donor A. As the signal is only present on the ridges of the mark and not in the valley, it can be inferred that this species comes from the fingerprint and not the substrate. The images from m/z 550.55 (also assigned to dimethylidoctadecylammonium) detected in fingerprints for the first time by ToF-SIMS are also presented in Figure 6. The spatial distribution of the molecule correlates with the distribution of K and is the inverse of the Si distribution (from the substrate), thereby confirming, in agreement with MALDI, that the signal comes from the fingerprint and not the substrate.


**DISCUSSION**

For future studies, the requirements for analytical techniques will depend on whether samples originate from academic studies or police casework. Requirements common to both types of analysis are (a) sensitivity to a range of compounds and (b) the ability to detect reproducible differences between donors. Both these factors contribute to the selectivity of the method.

The techniques had different strengths in terms of sensitivity. In addition to fatty acids, MALDI was also sensitive to large molecules (peptides) in fingerprints that were not detected by the other techniques. The GC/MS method employed here was sensitive to amino acids which were not detected by the other techniques and an extensive range of fatty acids, as well as squalene. The current GC/MS method is biased toward analysis of amino acids (which are present in lower abundance...
than other components) by the nature of the aqueous extraction solvent used. The use of eight fingerprints ensures that there is sufficient material for their detection, which is only useful for academic studies and not practical for crime scene use. Other GC/MS studies have focused on the analysis of lipid material and shown that relatively large lipid compounds can be analyzed by this technique and that single fingerprint analysis is possible if a component is present in sufficient quantity. Amino acids have been detected in fingerprints analyzed by MALDI using the “dry−wet” method of sample preparation, but this was not available at the time of analysis.

ToF-SIMS analysis detected for the first time many of the lipids detected by GC/MS and MALDI, but was also able to detect inorganic components. XPS was the only technique to give information on the carbon configuration, which discriminated donor B’s samples from those of the other two donors.

The MeV-SIMS analysis was sensitive to the inorganic components Na and K, and the results obtained corroborated the results from ToF-SIMS and XPS and showed repeatable differences between donor B and the other two donors. However, as MeV-SIMS is a new technique, at the time of measurement the mass resolution of the spectrometer was particularly poor, and therefore, organic species were not identified. Significant improvements by the group of Webb have since been made in the mass resolution available by MeV-SIMS, and the technique is expected to give results similar to those of ToF-SIMS. The additional advantage of MeV-SIMS is that, as with ATR-FT-IR spectroscopic imaging and some MALDI systems, the analysis can now be carried out under ambient pressure, making analysis quicker and less intrusive and offering the opportunity of analyzing fingerprints on large substrates without sampling.

It should also be noted that, for each of the mass spectrometry techniques employed, only compounds listed in Table 1 were searched for. Another interesting observation is the untargeted detection by ToF-SIMS and MALDI of dimethyldioctadecylammonium, an exogenous compound. While the purpose of this study was to identify endogenous compounds, this result confirms that additional intelligence can be gained from fingerprints using these techniques.

Figure 4. Relative intensity of $m/z$ 257.24 (assigned to C16) and 411.33 (assigned to squalene) detected by ToF-SIMS and the mass per fingerprint of C16 and squalene detected by GC/MS for each replicate of each donor’s fingerprint. Fingerprints are denoted A1, A2, etc., where A, B, or C is the donor and 1, 2, or 3 is the replicate of the fingerprint from that donor.

Figure 5. Additional compounds detected by ToF-SIMS in each fingerprint: $m/z$ 230.23 (13-aminotridecanoic acid), 282.46 (oleamide), 299.26 (C19, nonadecanoic acid), 313.28 (C20, arachidic acid), 327.24 (C21, heneicosanoic acid), 341.29 (C22, docosanoic acid), and 550.55 (dimethyldioctadecylammonium). Fingerprints are denoted A1, A2, etc., where A, B, or C is the donor and 1, 2, or 3 is the replicate of the fingerprint from that donor.
techniques for this application. In terms of accuracy, GC/MS in this work used appropriate standards and therefore gave quantitative results. However, ToF-SIMS, MeV-SIMS, and MALDI require similar (matrix-matched) standards, which at present do not exist. Instead, the relative concentration (normalized to the signal from the substrate) of a specific element or molecule detected within a donor’s fingerprint was evaluated. In this case, GC/MS, ToF-SIMS, XPS, and MeV-SIMS were in broad agreement with each other, as shown in Figure 4 and in the Supporting Information.

As expected, the attempt to correlate ion intensities to relative concentration measurements using MALDI was not always in agreement with the other techniques. One explanation for this is the 3 month delay in making the MALDI measurements. Another factor is the presence of “sweet spots” generated by the inhomogeneous matrix–analyte cocrysalization. In the case of the MALDI images shown in Figure 6, the three replicates do not show reproducible images, with the second and third replicates being of far superior quality compared to the first one. This could be due to differences in the intradonor deposition (variable angle/contact/pressure/time), but this possibility could not be evaluated through the comparison with ToF-SIMS as, in this case, images were produced for only partial and different locations within the marks. However, clogging issues while autospraying the matrix solution, which were resolved by the time the third replicate was sprayed, have more likely impacted the reproducibility. Issues connected to the matrix deposition have been addressed in subsequent work published by Francese’s group, where the dry–wet method of matrix deposition has been shown to produce reproducible images.

An interesting outcome of this study is the corroboration of the results for each of the techniques. It was necessary to test ToF-SIMS, MeV-SIMS, XPS, and MALDI, which have never been used to compare levels of endogenous compounds in fingerprints, with GC/MS and ATR-FT-IR imaging, for which established procedures already exist. For example, GC/MS shows that donor B’s fingerprints contained the lowest quantity of fatty acids. This result corroborates the ToF-SIMS results and the ATR-FT-IR spectroscopic imaging, which detected a systematic reduction in the peak corresponding to fatty acids and wax esters for donor B, compared with donor A or C. Additionally XPS, MeV-SIMS, and ToF-SIMS all showed systematically higher levels of K and Na in donor B’s fingerprints, presumably from salts deposited in the residue.

It could also be observed that, for donor B, the ATR-FT-IR spectra did not show significant bands at 1745 cm$^{-1}$, which are usually attributed to ester carboxylic functional groups arising from triglycerides. However, the XPS spectra showed that some of the carbon atoms were bound as esters (COOR moieties).

An important factor to consider when comparing XPS and ATR-FT-IR spectroscopic imaging data is the difference in analysis depth between the two techniques. The effective penetration depth of the ATR technique is likely to be a few micrometers for biological samples. In XPS, the information depth is 5–10 nm. As a consequence, a very faint fingerprint of $\sim$100 nm thickness would appear the same in XPS as a $\sim$1 $\mu$m thick deposit, but would have a significantly reduced intensity in FT-IR. It is also probable that the chemistry of the deposits is not homogeneous in depth, allowing the ATR-FT-IR spectroscopic imaging to detect a wider variety of compounds across the deposit thickness, whereas the XPS spectra reflect the composition of the top monolayers of the fingerprint.

In terms of selectivity, GC/MS could distinguish between donors A, B, and C on the basis of fatty acids; for example, the mass per fingerprint of C18:1 and C18:2 was systematically different for donors A, B, and C. Similarly, ToF-SIMS could distinguish between all three donors on the basis of fatty acids; for example, the mass per fingerprint of C18:1 and C18:2 was systematically different for donors A, B, and C. Additionally XPS, MeV-SIMS, and ToF-SIMS all showed systematically higher levels of K and Na in donor B’s fingerprints, presumably from salts deposited in the residue.

Another aspect of selectivity is spatial selectivity. ToF-SIMS, MeV-SIMS, MALDI, and ATR-FT-IR spectroscopic imaging were all able to obtain images of the detected compounds within the fingerprints, showing ridge detail. This is an important result as it confirms that the presence of a compound of interest is associated with a fingerprint and not the substrate.
This is not possible using GC/MS or using the XPS system used in this study.

## CONCLUSIONS

A protocol for the comparable analysis of fingerprint residue using a suite of relevant analytical techniques has been developed. We have shown for the first time that it is possible to detect endogenous compounds in fingerprints using ToF-SIMS, XPS, and MeV-SIMS and that the relative quantification methods presented here corroborate the better established methods, GC/MS and ATR-FT-IR imaging, for this application. Of the newer techniques, ToF-SIMS was the most selective, showing reproducible differences between all three of the donors by imaging a fingerprint in situ. MALDI also has imaging capability, but relative quantification using MALDI is yet to be developed. ToF-SIMS is however one of the most time-consuming and expensive techniques, and therefore, academic studies that can sacrifice a large number of fingerprints and are not concerned with spatial resolution might favor the use of GC/MS for its low cost. The choice of technique will also depend on which compounds are of interest: in this study GC/MS was found to be the most sensitive to amino acids, MALDI to lipids and peptides, and XPS to the carbon configuration and inorganics.

Finally, in terms of practicality for future use, XPS, MeV-SIMS, ToF-SIMS, and ATR-FT-IR spectroscopic imaging have the advantage of no sample preparation. MALDI requires application of a matrix prior to analysis, and GC/MS requires the sample to be dissolved and derivatized. ATR-FT-IR imaging is the quickest analysis procedure, with a scan taking only a few seconds. The ToF-SIMS and GC/MS methods are the slowest, with a sample taking up to 3 h to analyze. MALDI, GC/MS, and ATR-FT-IR imaging are cheaper methods than XPS, ToF-SIMS, and MeV-SIMS, which require specialized instrumentation.

## ASSOCIATED CONTENT

1. Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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