Monitoring the chemical changes in fingermark residue over time using synchrotron infrared spectroscopy

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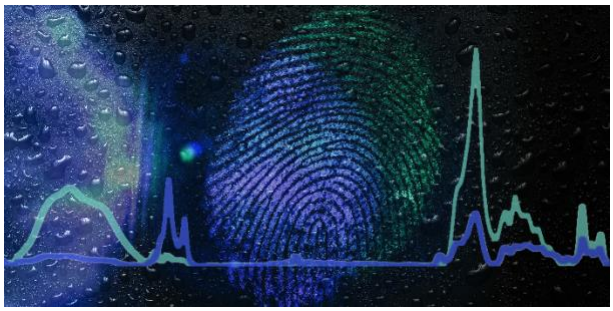

Abstract

Degradation of fingermark residue has a major impact on the successful forensic detection of latent fingermarks. The time course of degradation has been previously explored with bulk chemical analyses, but little is known about chemical alterations at the micron-scale. Here we report the use of synchrotron-sourced attenuated total reflection-Fourier transform infrared (ATR-FTIR) microscopy to provide spatio-temporal resolution of chemical changes within fingermark droplets, as a function of time since deposition. Eccrine and sebaceous material within natural fingermark droplets were imaged on the micron scales at hourly intervals for the first 6 – 12 hours after deposition, revealing that substantial dehydration occurred within the first 8 hours. Changes to lipid material was more varied, with samples exhibiting an increase or decrease in lipid concentration due to the degradation and redistribution of this material. Across 12 donors, it was noticeable that the initial chemical composition and morphology of the droplet varied greatly, which appeared to influence on the rate of change of the droplet over time. Further, this study attempted to quantify the total water content within fingermark samples. The wide-spread nature and strength of the absorption of Terahertz/Far-infrared (THz/Far-IR) radiation by water vapour molecules were exploited for this purpose, using THz/Far-IR spectroscopy. Upon heating, water confined in natural fingermarks was evaporated and expanded in a vacuum chamber equipped with multipass optics. The amount of water vapour was then quantified by high-spectral resolution analysis, and fingermarks were observed to lose approximately 14 – 20 µg of water. The combination of both ATR-FTIR and Far-IR highlight important implications for experimental design in fingermark research, and operational practices used by law enforcement agencies.

Introduction

Fingermark ridge patterns are characteristic to an individual, with the marks left following contact between the fingertip and a surface frequently utilised for identification purposes in forensic science.[1] The residue left on contact is often invisible to the naked eye, requiring chemical or physical treatment to enable detection. Despite ongoing research to improve fingermark visualisation procedures, there remains significant detection challenges due to the chemical variability of a fingermark.[2] Following deposition there are changes in morphology and the chemistry of fingermark residue due to interaction with the surface, the sample environment, and the fingermark residue itself.[3] The above factors can impact fingermark chemistry at variable rates, hindering successful fingermark development.[3] Whilst the surface and sample environment are conditions which can be controlled and monitored, the chemistry of fingermark residue is multifaceted and more variable and therefore, is an area of interest for fingermark researchers.

Fingermark residue is made primarily of a mixture of secretions from the eccrine and sebaceous glands, and is likely to be contaminated with constituents picked up through contact during daily activity.[4-6] Once deposited, this residue is not static with a potential to move across a surface, and undergo chemical and physical changes. Sebaceous material is an oily mixture of lipids, which are known to degrade over time, studies have attempted to correlate this rate of degradation to time since fingermark deposition.[7-9] However, as the lipids may originate from different sources, such as sebaceous glands or from physical contact with certain parts of the body, there can be large variation in the initial lipid content and composition, which makes age prediction of fingermarks challenging.[10, 11] The eccrine substituents are an aqueous mixture containing amino acids, salts and proteins. The water content undergoes evaporation as one of the first signs of degradation, which can be a main driver towards altering the physical and chemical properties of the remaining residue.[12, 13]

The water content of fingermarks is known to decrease as a function of time since deposition, yet, fundamental knowledge, such as the exact amount of water contained in a fingermark is not well known. The water content is known to have a direct impact on fingermark detection, with dried, aged fingermarks showing weaker performance when detected with some operational methods.[14-17] There are inherent challenges when attempting to measure the quantities of material present within a fingermark due to the small quantities and variability of material present. The water fraction of a fingermark is expected to make up a significant portion by mass; however, there have been many conflicting reports on how much water is within a fingermark and its rate of evaporation.[12, 18, 19] Current methods have used microbalances to measure the mass of this material and its change over time with great disparity in the findings.[12, 18] Keisar *et al*. calculated the mass of a fingermark to be between 2 –

9 μg, based on the weight of a smaller subsection of the mark, whilst Bleay *et al*. reported a mass range of 0.33 – 29 μg.[6, 18]

Several studies have explored fingermark degradation, by visually comparing how fingermark development methods are affected by evaporation and time since deposition.[14, 17, 20, 21] An alternative approach has been to explore the chemical alteration within fingermark residue, by directly measuring the chemical constituents present at different time points since deposition, using techniques such as gas chromatography (GC) and liquid chromatography – mass spectroscopy (LC-MS).[7-10, 22-24] Whilst each of these methods can provide valuable bulk chemical information on the degradation process, spatially resolved chemical information is not provided. A method or methods, which can determine both chemical specificity and spatial information can provide a better overall picture of fingermark residue as it ages.[25]

Recent advances in scientific capabilities have utilised methods such as atomic force microscopy (AFM), x-ray fluorescence microscopy (XFM) and attenuated total reflection-Fourier transform infrared (ATR-FTIR) microscopy, to bring together spatial and chemical specific information on fingermark residue.[26-28] There are a number of advantages to these methods, particularly the capability to image fingermarks without any chemical alteration, treatments, or solvent extractions prior to measurement. In-situ label-free measurements, where possible, can give a more realistic representation of the chemical species naturally present in fingermark residue. The spatial resolution of these techniques is on the micron scale, and therefore allows investigation of individual droplets in fingermark residue. As an example, synchrotron-sourced ATR-FTIR microspectroscopy was recently used to identify the distribution of eccrine and sebaceous components present within a single droplet.[28] The application of these methods provides the opportunity to further understand the degradation of fingermark residue, by directly imaging the chemical and morphological changes of the eccrine and sebaceous material within a natural fingermark as a function of time since deposition.

In this study, we exploit the unique characteristic of the highly intense synchrotron-IR beam that allows shorter data collection time, while still maintaining high spectral quality, and the advantage of enhanced spatial resolution (down to 2-3 μm) offered by the synchrotron ATR-FTIR microspectroscopic technique, to measure the chemical changes within a fingermark droplet as a function of time since deposition.[29] Further, the Terahertz/Far-infrared (THz/Far-IR) region offers sensitivity to pure-rotational water vapour transitions and provides an intriguing opportunity to measure the total water content in fingermark residues by heating the sample and evaporating the water into the gas phase for high spectral resolution analysis. Taken together, the results from synchrotron-sourced ATR-FTIR microspectroscopy and THz/Far-IR spectroscopy in the gas phase

provide new insights into the chemical changes that occurred within fingermarks as a function of time since deposition, as well as an estimate of the total initial water content contained within a fingermark.

Experimental

Synchrotron ATR-FTIR Microspectroscopy

Fingermark Deposition

Fingermarks were deposited directly onto to the ATR germanium (Ge) hemispherical crystal by lightly pressing the centre of the fingertip on the centre of the crystal for 5 seconds (See Figure 1C). Natural fingermarks were collected from 12 donors, donor information is provided in Table 1.

Table 1: Fingermark Donor Information

Donor	Age	Gender	Cosmetic Use
1	78	Male	None reported
2	27	Female	None reported
3	22	Male	None reported
4	24	Male	None reported
5	41	Male	None reported
6	24	Female	Yes
7	55	Male	Yes
8	48	Male	None reported
9	26	Female	Yes
10	41	Female	Yes
11	-	Female	None reported
12	23	Female	None reported

Instrumentation and Data Collection

Synchrotron ATR-FTIR experiments were conducted on the Infrared Microspectroscopy (IRM) beamline at the ANSTO – Australian Synchrotron (Clayton, Victoria). The FTIR instrument on the IRM beamline consists of a Bruker Hyperion3000 microscope coupled to a Bruker Vertex V80v FTIR spectrometer, with a liquid nitrogen cooled mercury cadmium telluride (MCT) narrow band detector (Bruker Optik GmbH, Ettlingen, Germany). The ATR-FTIR accessory was equipped with a germanium (Ge) hemispherical crystal (n_{Ge} = 4.0), which has a 1 mm diameter active sensing area. Spectral maps were acquired using a 20× objective (NA = 0.60; Bruker Optik GmbH, Ettlingen, Germany), with an effective spot size of 1.88 µm on the sample surface and a step interval of 1 µm between the measurement points. A background spectrum of the clean Ge-ATR crystal was measured using 256 co-added scans and 8-cm^{-1} spectral resolution. Each sample spectrum was recorded using 4 co-added scans and 8-cm^{-1} spectral resolution. The experiment was performed under ambient room conditions without nitrogen purge and with opened purge box, to ensure a natural evaporation of water in the fingermarks throughout the measurement. Data acquisition and analysis were conducted using OPUS v8.0 software suite (Bruker).

Data Analysis

The raw FTIR spectral maps were first pre-processed for the ATR correction to take account of the refractive index of the Ge ATR crystal, and atmospheric compensation before further analysis using the OPUS v8.0 (Bruker) and CytoSpec 2.00.03 software (Cytospec Inc., Boston, MA, USA). The ATR-FTIR chemical images were further processed with ImageJ 1.50i software.

Synchrotron THz/Far-IR Spectroscopy

Sample Preparation

In order to study the water content in fingermarks, a standard 200 mm long 5 mm in diameter chemistry glass rod was used to deposit the fingermarks of donors 7 and 10 (see Table 1 for donor information). The glass rod was first cleaned with isopropanol and left to warm to room temperature prior to sample deposition. Then, the fingermarks were deposited by rolling the fingertips across a glass rod back and forth for 5 seconds. Three types of fingermarks were collected, and are outlined in Table 2; these types were decided based on the varying amounts of water that could be yielded.

Table 2: THz/Far-IR Fingermark Sample Types

Sample Type	Sample Preparation
Natural	Donor was instructed to go about their daily activities for 30 minutes, no washing of the hands in this time.
Air	Donor was instructed not to touch anything for 30 minutes, hands were open to atmosphere
Gloves	Donor was instructed not to touch anything for 20 minutes then wore nitrile gloves for 10 minutes

In order to quantify the amount of water detected in the donor fingermarks, a calibration curve was generated from carefully measured volumes of deionised water (0.25 µL, 0.5 µL, 0.875 µL, 1.25 µL, and 1.5 µL). The water was deposited in the middle of the glass rod using a 0.1 – 2.5 µL pipette.

Instrumentation

In this study, we used a furnace to heat a 300 mm long Qz tube with a 20 mm inner diameter coupled to 10 L glass gas-cell via the gas-manifold located on a mobile gas-handling vacuum (MGHV) system. The gas-cell itself was placed on the sample compartment of a Bruker IFS125/HR FT spectrometer. The quartz tube was heated to 70 (+/-0.5)°C using the furnace while the gas cell was heated to 70 (+/-5)°C using heating tapes.

For these measurements, the optical configuration of the spectrometer consisted of a 12.5 mm aperture, a 6 um multilayer Mylar beamsplitter, and a 4.2 K Si bolometer from Infrared Laboratories equipped with a PE window and a 13 um thick stretched PE film cold filter (< 800 cm^{-1}). The spectra were recorded at 0.01 cm^{-1} spectral resolution, at a scanner speed of 2.5316 cm/s (40 kHz),

and the pre-amplifier gain on the Si bolometer was set to 200. Sets of 10 scans were accumulated yielding a signal-to-noise (S/N) ratio of approximately 194.

The gas-cell is made of borosilicate glass, and with variable path-length gold-coated optics, M35V, from Infrared Analysis; the cell has a nominal single path-length of 625 mm, and the number of times the light can be bounced off its mirrors can be varied from 4 up to 56 times in multiples of 4, yielding an optical path-length from 2.5 m to 35 m. For this spectral region, the gas-cell was equipped PE wedged windows, and the path-length set to 2.5 m.

Data Collection

The complete system consisting of the furnace, MGHV system and gas-cell was first evacuated to its base pressure ($< 1 \times 10^{-3}$ mbar). Prior to inserting a sample, the furnace was isolated from the vacuum and was brought up to atmospheric pressure and purged with dry N_2(g) from the pressurised boil-off of the N_2(ℓ) tanks. The flow of the purged N_2(g) was halted during sample insertion to prevent any H_2O in the fingermarks from being sublimed; then, the furnace was sealed and isolated after sample insertion while the sample line was evacuated to base-pressure. After the system (excluding the furnace) was brought back to base pressure, a measured amount (~ 5 mbar) of the warm gas mixture consisting of H_2O(g) mainly from the rod, fingerprints and the purged N_2(g) was allowed to flow from the furnace into the gas cell for analysis.

Data Analysis

All raw FTIR spectra were measured and analysed using Bruker OPUS v8.0. A background spectrum was first recorded, and was used to calculate the absorbance spectrum of each sample. For the background spectra, a cleaned glass rod was inserted in the quartz tube, and the warm gas mixture consisting mainly of H_2O(g) from the rod and the dry N_2(g) was allowed to flow from the furnace into the gas cell for analysis the gas-cell was filled with 5.01 mbar of N_2(g).

The absorbance spectrum of each sample was first normalised to the pressure when the background spectrum was recorded (5.01 mbar) before the background absorbance was subtracted from the sample absorbance to yield the true absorbance of H_2O coming from the fingermark samples only (sample = H_2O droplets and fingerprints).

Results and Discussion

Study 1 – The chemical and morphological changes in eccrine and lipid material within natural latent fingermarks in the first 12 hours following deposition.

In this study, the chemical and morphological changes of individual droplets within natural fingermarks were measured in the first 6 - 12 hours following sample deposition using synchrotron ATR-FTIR technique. Specifically, spectroscopic marker bands of eccrine and sebaceous material were monitored (eccrine marker: O-H stretching (3000-3500 cm^{-1}), sebaceous marker: C-H stretching of lipids (2800-3000 cm^{-1})), as per our previous studies.[26, 28]

Twelve donors provided fingermark samples in which droplets containing eccrine and lipid material could be identified. Across each sample, it was noticeable that the fingermarks deposited by different donors showed diverse morphology and varied composition of lipid and eccrine material, which were consistent with our past work.[26, 28] Figure 1 shows a representative sample of a fingermark droplet provided by donor 1. This particular sample appears to be a central eccrine droplet surrounded with lipid material. Across a period of 7 hours, there is a noticeable change in lipid and eccrine concentration, based on the C-H stretching (2800-3000 cm^{-1}) and O-H stretching (3000-3500 cm^{-1}) bands, previously reported to be indicative of these substituents.[28] This sample shows an exponential decrease in the signal attributed to the eccrine material, based on the average intensity of the O-H stretching peak integrated across the droplet area. This trend can be further observed when looking directly at the spectra extracted from the images (Figure 2), where the peak intensity of the O-H stretching peak (3000-3500 cm^{-1}), and the O-H bending peak (1540-1700 cm^{-1}) can be clearly seen to decrease as the time since deposition increases. In contrast, the opposite trend is seen for sebaceous material, with a clear increase in peak intensity for the C-H stretching (2800-3000 cm^{-1}) band (Figure 2).

A decrease in water content was observed across 10 donors over time, as represented in Figure 3, with the water content appearing to plateau after approximately 8 hours. As eccrine material is an aqueous mixture, the reduction in water content can be attributed to evaporation. This extends on recent work by Keisar *et al*., which explored the evaporation of water within the first few minutes post fingermark deposition.[18] Although demonstration that water evaporates from a fingermark is not particularly surprising, a key observation from these results has been the evidence that water can continue to evaporate from a fingermark sample at room temperature for at least 8 hours.

In addition, the lipid concentration and distribution were observed to be more varied with greater disparity seen across donors compared to the eccrine material. The sample shown in Figure 1 demonstrates an increase in lipid material, this was consistent with 5 donors, whilst the remaining 7 donors decreased in overall lipid concentration. The discrepancies could be justified by the increase in

concentration of the residual lipid content, and also the redistribution of lipid material that led to an increase in contact areas between the lipid material and the Ge crystal as a result of the water evaporation. Interestingly, a distinct pattern of changes in lipid composition was observed. The C-H stretching peaks (2800-3000 cm^{-1}) showed a gradual increase over the 8 hour period, suggesting an increase in lipid concentration. However, the C=O stretching region (1715-1750 cm^{-1}) decreased in the first 4 hours and then increased between 4 to 8 hours. These results could indicate initial degradation of lipid material before the potential oxidation of these compounds.

The oxidation of sebaceous lipids, such as squalene, cholesterol and unsaturated fatty acids, has previously been reported in studies aiming to predict fingermark age.[7, 21, 30] Factors influencing the starting material in fingermark residue have been shown to have a major impact on the rate of degradation of these components, making it impossible to identify universal trends.[5, 11] The findings reported here reinforce the challenges of calculating time since deposition, with significant variation appearing within the morphology and lipid composition of single droplets in fresh natural fingermarks. These studies focused on the first 12 hours since deposition, further work should be done to explore a longer time period to investigate whether further morphological and chemical changes can be observed on the macro scale with this method over longer time periods.

Noticeably, Donor 12 was an anomaly with the O-H peak intensity increasing over the first 8 hours (Figure 3). To investigate further, measurements were repeated with this donor by taking triplicate fingermark samples (Figure 4). The two repeat measurements behaved more consistently with the rest of the data for eccrine material, displaying a time-dependent decrease in the O-H peak, which can be ascribed as water content in the eccrine material (Figure 4). We attribute the variation in response from donor 12, to the high lipid content present in the initial fingermark and the large size of the droplet. The droplet imaged in replicate 1 appeared to cover the entire imaged area, meaning the droplet size was > 80 $\times$ 80 μm^2, whereas replicates 2 and 3 were smaller, fitting within a 75 $\times$ 75 μm^2 imaged area. It is understood that the morphological size of the original drop would appear to change the rate of evaporation of water within the sample. It is possible evaporation occurred outside the immediate imaged area, and thereby is not considered within our results. Amongst the triplicate samples from the single donor it was apparent that the lipid material also did not behave consistently, with two replicate measurements showing an increase and one a decrease in lipid material. These results did not appear to be related to droplet size or distribution adjacent to eccrine material, although previous work has demonstrated an increase in lipid degradation when fingermarks were stored in an aqueous environment.[23] On a longer time scale, it is possible that the sebaceous material distributed amongst the aqueous material within a fingermark could influence the rate of degradation. This emphasises how unpredictable the rate of change of a fingermark is, even within the same donor,

suggesting it near impossible to use the chemistry of the fingermark to predict the time of deposition without knowing the initial chemistry of the residue.

This study is the first report to use chemical imaging techniques to demonstrate how fingermark chemistry changes in the immediate hours following deposition, and has important implications for fingermark research. Specifically, the International Fingerprint Research Group (IFRG) provides guidelines when conducting fingermark research and recommends the use of fingermarks, which are left to age for a minimum of 24 hours before development.[31] The results shown here provide context for why fresh fingermarks should not be used for research into latent fingermark detection techniques, demonstrating the changes in residue chemistry, specifically the evaporation of water content that can occur within the first 8 hours following deposition. By leaving fingermarks for 24 hours, researchers can provide a more realistic representation of what would be encountered in casework as it is unlikely for fingermarks to be collected within the hours immediately following transfer of material. Whilst this study focused primarily on the first 12 hours following deposition, further work should be conducted to see whether additional changes can be seen in the morphology and chemical composition of the droplet for a longer time frame.

Study 2 – Measuring the volume of water lost through evaporation of latent fingermarks

The results from study 1 demonstrated that eccrine material in a fingermark showed a substantial change in the first hours since deposition, thus impacting the chemical composition and the morphology of the mark as it ages. Whilst eccrine sweat is made up of 99% water, water is estimated to comprise anywhere between 20-70% of the total fingermark residue, varying significantly between donors based on a range of factors.[18, 19] There are a number of challenges in measuring the volume of material deposited in fingermark residue, with the inherent variability and influence from the surrounding environment, making it difficult to standardise an analytical approach. Recent work has utilised a quartz crystal microbalance (QCM) to determine the amount of water lost through evaporation from a fingermark sample.[6, 18] The results demonstrated that the majority of water content was evaporated in the first few minutes following deposition, however the results shown here in study 1 suggest there is a longer time period of evaporation.[18] In study 2, we used high-spectral resolution gas-phase THz/Far-IR spectroscopy to estimate the total volume of water contained in a fingermark. Fingermarks were heated to 70 degrees, and the water content measured in the gas phase using a gas cell with 2.5 m optical path difference. The water content was determined using a linear calibration, developed from the rotational spectrum of water; a portion of the rotational spectrum void of strong and overlapping peaks was selected, in particular a peak located at 202.69 cm^{-1} was chosen (see Figure 5). A plot of the absorbance intensities with respect to volume of H_2O deposited on the glass rod showed a linear relationship with an R^2 value of 0.9932. This linear relationship was

then used to predict the volume of water in the fingermark sample, outlined in Figure 6. Of the three sets of fingermarks taken the natural fingermarks had the lowest water content, between 14-20 µg (volume of water converted to mass of water, assuming a density of 1 g/mL). Two further samples were taken, fingermarks deposited after the hands were left in the air for 30 minutes, minimising contact with a surface and fingermarks deposited after wearing nitrile gloves for 10 minutes. These samples demonstrated increased water content, 60-79 µg and 77-101 µg respectively. The increase in water content could possibly be attributed to the loss of material on the surface of the hands in natural daily behaviour, by minimising contact with a surface the eccrine material is left to build up on the fingertip, potentially increasing the material deposited in this study. Wearing gloves can increase the amount of eccrine material produced by increasing the perspiration rate from the hands, and therefore the increase in water content calculated from these samples is not unexpected.[3]

The literature contains few reports concerning the determination of the mass of a fingermark, which are often conflicting with respect to the volume of water present in fingermark residue.[6, 12, 18] This is most likely due to the challenge of working on the microscale with material so sensitive to change and influence from the surrounding environment. The measurements recorded in this study are higher than that reported by Keisar *et al.*, with their total fingermark mass calculated to be between 2 – 9 µg.[18] Their approach measured a 0.2 cm^2 area of the fingermark (~ $1/10^{th}$ of the full fingermark surface area) using a QCM, with the entire mass calculated using the Sauerbrey equation, not taking into account the inherent variability across a fingermark.[18] Bleay *et al.* reports studies which measured a fingermark mass across a range of 0.33 and 29.00 µg, with large discrepancies not unexpected as the water content is anticipated to vary significantly between samples.[6] The results shown here for natural fingermarks appear to lie at the upper range of this mass, suggesting the water content could comprise 40 – 70% of the total fingermark mass – a figure which aligns with recently proposed values in literature.[18] Further work should consider the correlative use of high-spectral resolution gas-phase THz/Far-IR spectroscopy alongside microbalance measurements, using an increased number of donors to improve the accuracy of this method and provide complementary data to assist in the determination of the volume of water lost through evaporation of fingermark samples.

Conclusions

This study has reinforced the complexity of fingermark chemistry using synchrotron FTIR spectroscopy in both mid-IR and THz/Far-IR spectral ranges. Single droplets within natural fingermarks from 12 donors were imaged at the micron scales using the synchrotron ATR-FTIR technique. The variation in the initial chemical composition and morphology has demonstrated a significant impact on the rate of change of the droplet over time, highlighting the inherently complex nature of this biological material. Broadly, there is a noticeable change within the fingermark sample in the immediate hours

following deposition, with a decrease in water content seen within the first 8 hours. The lipid material is more multifaceted, with samples shown to both increase and decrease in lipid concentration following deposition due to the degradation and redistribution of lipid material. The results challenge the suggestion that one can simply predict the age since deposition of a fingermark. The variability of interdonor and intradonor fingermark chemistry corroborates the unpredictable nature of the rate of change of fingermark residue, particularly without knowledge of the initial material present. By imaging this change in the first few hours, we have explicitly shown how dynamic fingermark residue is, which must be considered in both operational and research contexts.

To determine the extent of change in water content, high-spectral resolution gas-phase THz/Far-IR spectroscopy was used to quantify the amount of water that could be evaporated from a fingermark sample. The intensity of the rotational transitions of water vapour were measured to determine the approximate loss of water ranging between 14 to 101 µg for natural fingermarks and those which had been groomed for increased water content. The results appear to be within the same range as those recently reported for natural fingermarks;[6] however, given the highly variable nature of fingermark material further work should be conducted with a wider range of donors to provide a more developed understanding of the potential of this method to measure water within fingermark residue.

Acknowledgements

Components of this research were undertaken at the Infrared Microspectroscopy and the THz/Far-IR Spectroscopy beamlines, at the Australian Synchrotron, part of ANSTO (Victoria, Australia). The authors acknowledge the contribution of an Australian Government Research Training Program Scholarship and Australian Institute of Nuclear Science and Engineering Postgraduate Research Award (REB) in supporting this research. The authors thank all the fingermark donors for their co-operation, Wilhelm van Bronswjik for his guidance throughout this study, and to those who contributed their time to assist in data collection. This project has been approved by the Curtin University Human Research Ethics Committee (Approval Number HRE2018-0476).

Author Contributions

Rhiannon E. Boseley: Conceptualization; methodology; investigation; formal analysis; visualization; writing - original draft; writing - review and editing. **Jitraporn Vongsvivut:** Conceptualization; methodology; investigation; supervision; writing - review and editing. **Dominique Appadoo:** Conceptualization; methodology; investigation; writing - review and editing. **Mark J. Hackett:** Conceptualization; supervision; writing - review and editing. **Simon W. Lewis:** Conceptualization; project administration; supervision; writing - review and editing

Figures

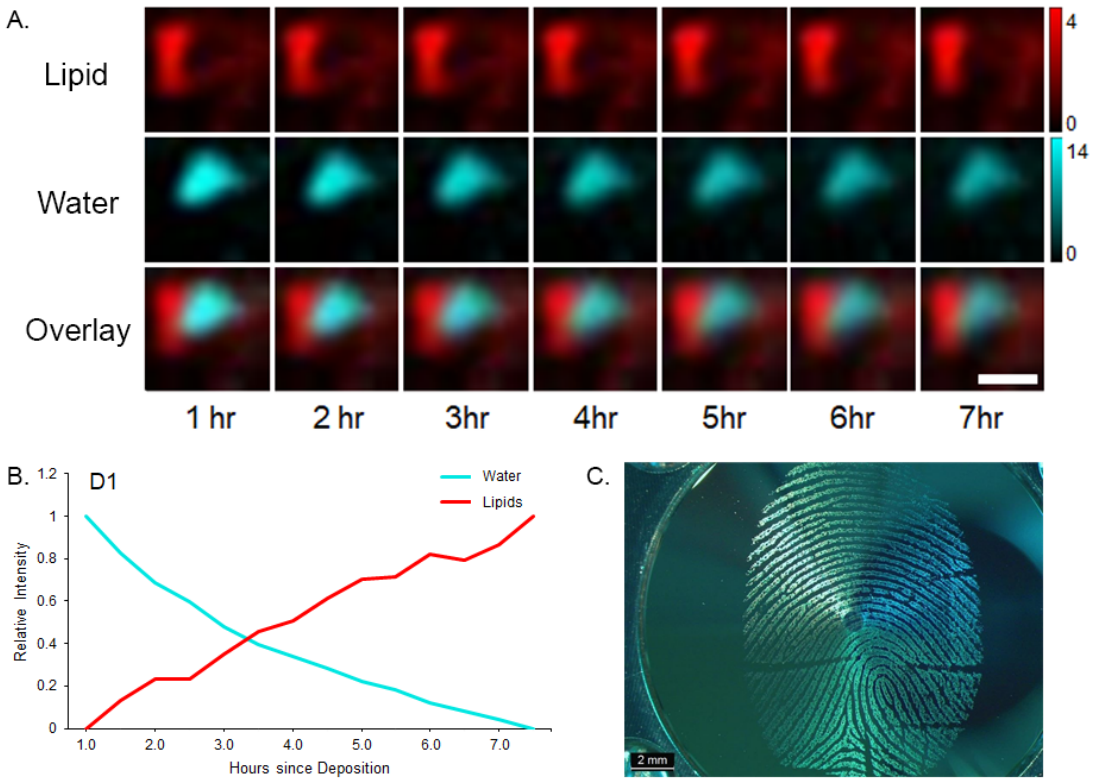

Figure 1: (**A**) Representative example of time-course (7 hour) changes in lipid and H_2O content during air-drying of a natural fingermark, as revealed by ATR-FTIR mapping approach. False colour ATR-FTIR maps were generated by integrating over the ν(C-H) stretching bands (2800-3000 cm^{-1}) as a marker of lipid material (top row) and ν(O-H) stretching bands of H_2O (3000-3500 cm^{-1}) as a marker for eccrine material (middle row). Overlay images are displayed in the bottom row. Scale bar 20 µm. (**B**) The relative changes in lipid and H_2O content as a function of air-drying time can also be visualised as the normalised average band area calculated across the entire droplet. Representative sample corresponds to Donor 1 (D1). (**C**) representative optical image of a natural fingermark deposited on the Ge ATR crystal for the analyses undertaken in this study.

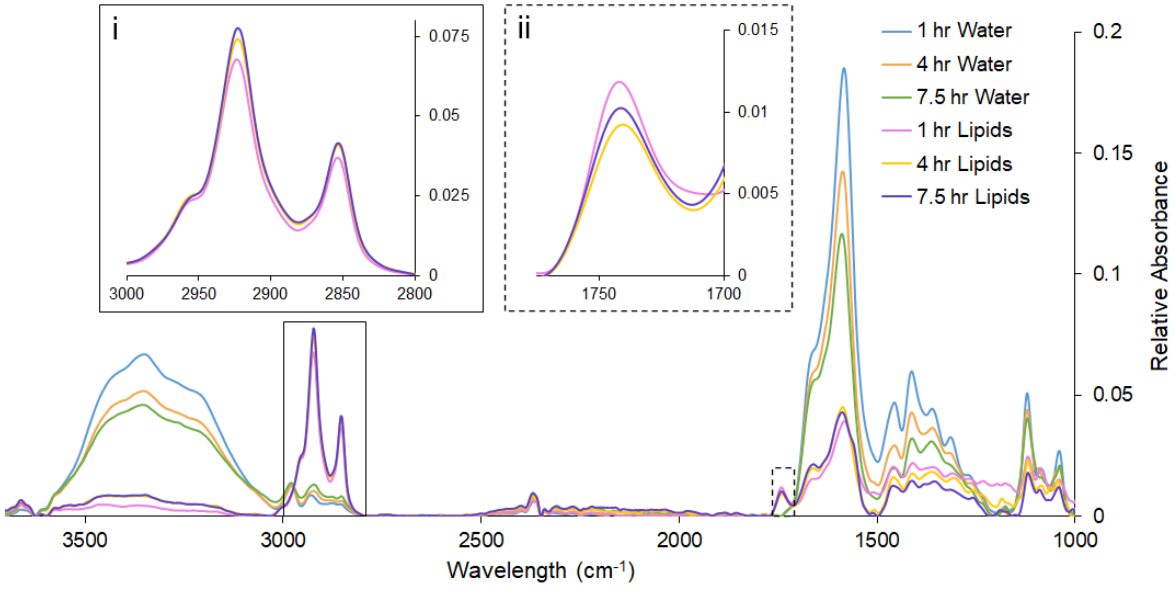

Figure 2: Representative examples of ATR-FTIR spectra collected from a natural fingermark after 1 hour, 4 hours, and 7.5 hours of air-drying period (from the same dataset presented in Figure 1, donor 1). Representative spectra are presented from image regions enriched in lipid material or water (using H_2O content as a marker of eccrine components). The most pronounced changes in lipid concentration are highlighted in (i) ν(C-H) stretching band (2800-3000 cm^{-1}) and (ii) ν(C=O) stretching band (1715-1750 cm^{-1}).

Figure 3: Time-dependent changes in H_2O content (as a marker of eccrine material) and lipid content during air-drying of natural fingermarks was measured for 12 individual fingermarks (donors 1 – 12), using the same ATR-FTIR approach. As expected, a strong time-dependent decrease in H_2O content was observed in donors 1-10 (cyan), with exceptions observed in donors 11 and 12. The H_2O content was measured as the integrated area across ν(O-H) absorbance bands (3000 – 3500 cm^{-1}), and lipid content (red) was measured across ν(C-H) absorbance bands (2800 – 3000 cm^{-1}). The average band area was calculated across the entire fingermark droplet.

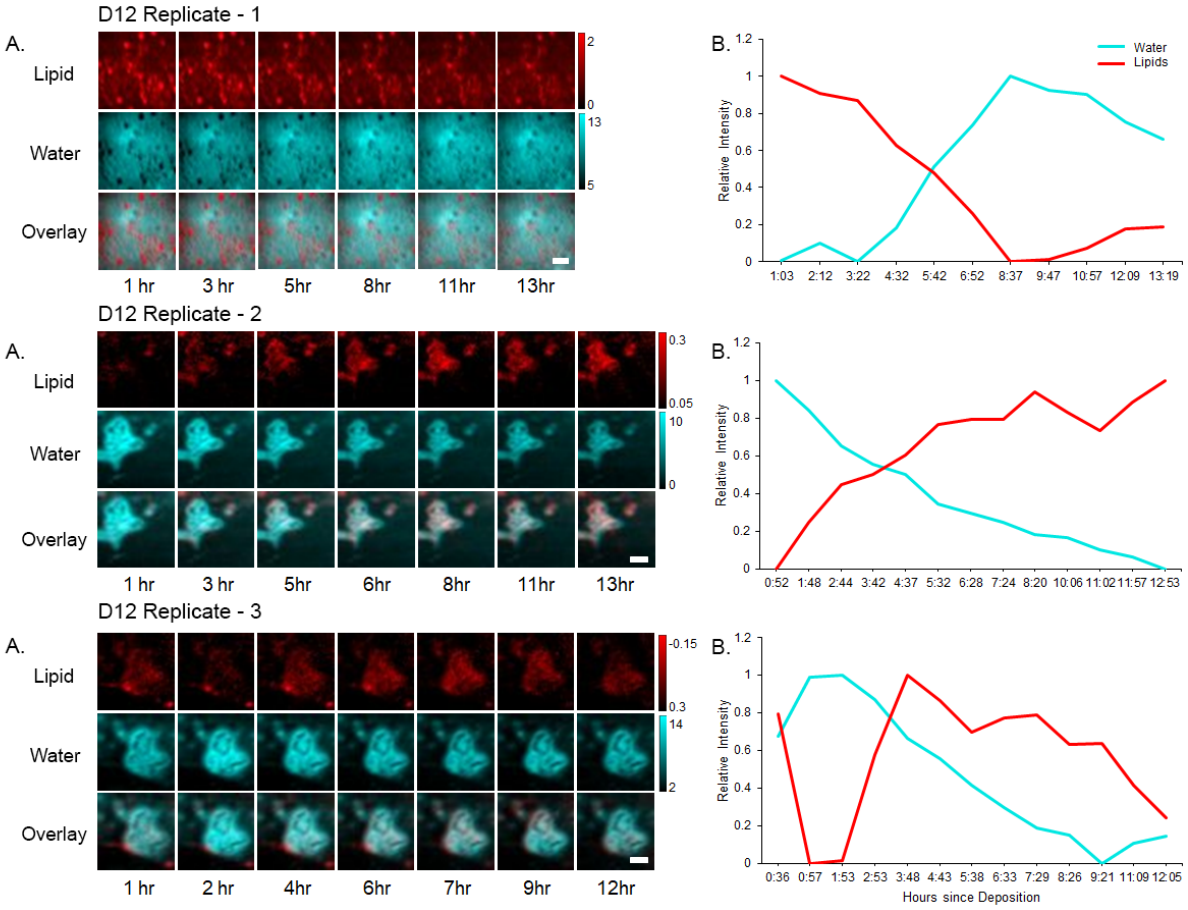

Figure 4: Analysis of intra-donor variance in triplicate fingermarks from donor 12. (**A**) False colour ATR-FTIR maps were generated by integrating over the ν(C-H) stretching bands (2800-3000 cm^{-1}) as a marker of lipid material (top row) and ν(O-H) stretching bands of H_2O (3000-3500 cm^{-1}) as a marker for eccrine material (middle row). Overlay images are displayed in the bottom row. (**B**) The relative changes in lipid and H_2O contents as a function of air-drying time can also be visualised as the normalised average band area calculated across the entire droplet. Scale bar 20 µm.

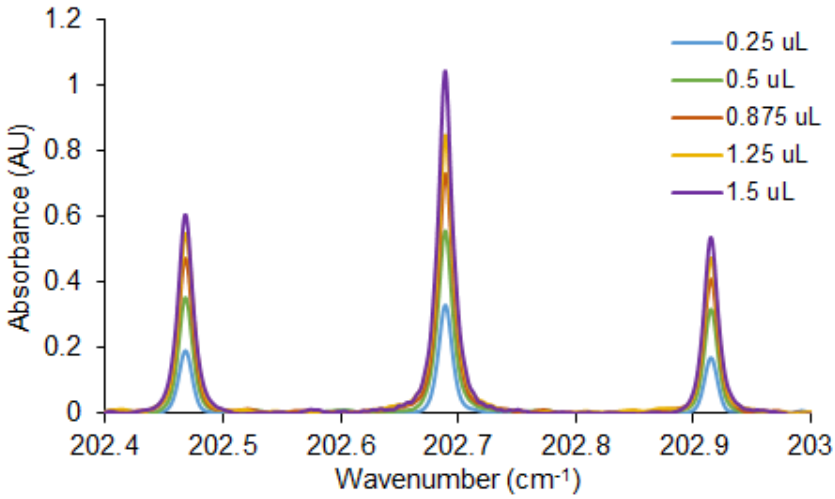

Figure 5: THz/Far-IR gas phase spectra showing H_2O pure-rotational spectral lines for calibration samples, highlighting the linear response between band intensity and volume of water from which the gas phase sample was generated (0.25 µL, 0.5 µL, 0.875 µL, 1.25 µL, and 1.5 µL). The selected spectral region shows the rotational transitions of H_2O at 202.47cm^{-1}, 202.69cm^{-1} and 202.92cm^{-1}.

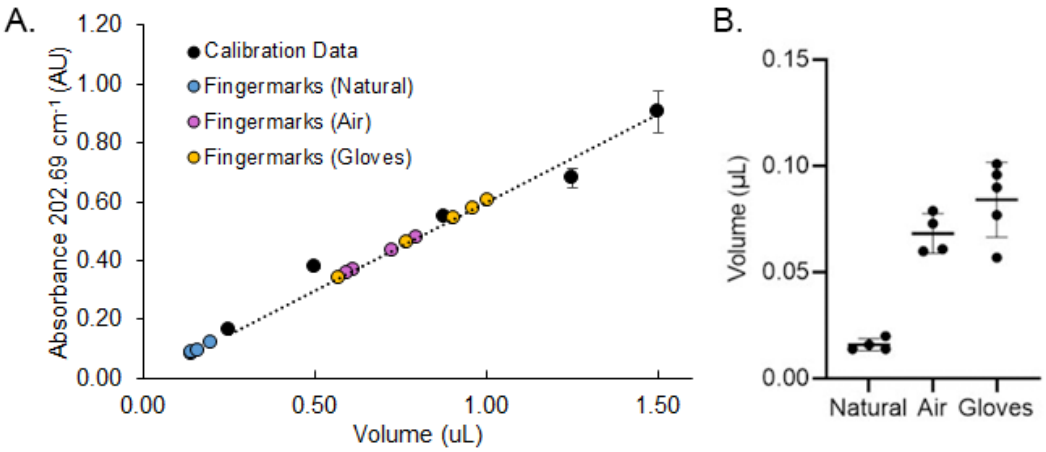

Figure 6: (**A**) The volume of water contained in a latent fingermark was quantified from the calibration curve (black dots, R^2 = 0.9932) using the rovibrational transition of H_2O at 202.69 cm^{-1}. (**B**) The volume of water was calculated for natural fingermarks (n = 4), fingermarks with hands left in the air for 30 minutes (n = 4), and fingermarks after wearing gloves for 10 minutes (n = 5). Volume shown for each data point in panel A, is for a set of 10 fingermarks deposited onto a sample substrate. The volume of water for each sample was therefore, divided by 10, to give an average water volume per fingermark (as shown in panel B). Error bars in A, and B are equal to 2σ.

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