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Towards Fingermark Dating: A Raman Spectroscopy Proof-of-Concept Study

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Fingermarks have, for a long time, been vital in the forensic community for the identification of individuals, and a possibility to non-destructively date the fingermarks would of course be beneficial. Raman spectroscopy is, herein, evaluated for the purpose of estimating the age of fingermarks deposits. Well-resolved spectra were non-destructively acquired to reveal spectral uniqueness, resembling those of epidermis, and several molecular markers were identified that showed different decay kinetics: carotenoids > squalene > unsaturated fatty acids > proteins. The degradation rates were accelerated, less pronounced for proteins, when samples were stored under ambient light conditions, likely owing to photo-oxidation. It is hypothesized that fibrous proteins are present and that oxidation of amino acid side chains can be observed both through Raman and fluorescence spectroscopy. Clearly, Raman spectroscopy is a useful technique to non-destructively study the aging processes of fingermarks.

A number of techniques are currently available for the routine characterization/imaging of fingermarks (FMs). In parallel, new and ongoing advances in forensic analysis have been realized, which provide additional information to the forensic investigator,[1] for example chemical identification of contaminant particles,[2] age estimation of FMs[3] and blood stains,[1c] and gender determination based on saliva[4] and blood.[5] Currently, no reliable fingermark dating methodology exists for crime scene forensic applications. Such a technique, especially if non-destructive, would give considerable impact to the forensic community and strengthen the value of collected evidence. Large challenges are expected for the development of such a FM age determination method, mainly owing to the large amount of variables influencing the kinetics involved in FM degradation.[6] However, promising advances have recently been reported by using light spectroscopy and widely accessible technologies.[1a,7] Spectroscopic techniques such as infrared (IR), Raman, and fluorescence can be used in non-destructive and non-contact mode, leaving the FM unspoiled for further forensic analysis. Moreover, they often encompass hyperspectral imaging, that is, the image contrast is governed by spectral information from each pixel. Thus, by using vibrational spectroscopy (Raman and IR), it is from spectral uniqueness, or the "chemical fingerprint", possible to identify foreign compounds in the FM.[1b,2,8] In this respect, IR hyperspectral imaging has been used to study FMs contaminated with residues from cosmetics and drugs,[8c] as well as chemical degradation (aging) of latent fingerprint residues in a controlled environment.[9] Additionally, new concepts have been developed utilizing Raman hyperspectral imaging on contaminated FM.[2,8a,10] In contrast to IR[6a,11] and fluorescence[1c,12] spectroscopies, no studies related to age determination of FMs using Raman spectroscopy (RS) have been found in the literature. In the present proof-of-concept study, RS has been evaluated to non-destructively probe the chemical composition of latent fingerprints and to gain insights into the FM aging process. In parallel, similar fluorescence experiments to those performed by van Dam et al.[12c] were also performed.

Optical microscopy images revealed, in accordance to others, two distinct different features (oily and solid) in the FMs[8b,13] (Figures S1–S3). Likely, the chemical composition of the oily regions is represented by sebaceous secretions, whereas the solid particulates mainly originate from the epidermis.[8b] The latter are of higher protein content[13] and generally give rise to higher fluorescence signals compared to the oily deposits. Not seldom were the two features, at least partly, superimposed and mixed (Figure S1 B).

Raman spectra acquired on a fresh and one month old FM showed obvious spectral discrepancies (see Figures 1 and S5–S6). High spectral richness was found with major bands around 1660, 1440, and 1300 cm^{-1}, in agreement with the literature.[8b] Biomolecules such as lipids,[14] fatty acids,[14] and proteins[15] exhibit many (partly overlapping) vibrational bands in these regions. Most of the Raman peaks agree well with those of skin,[16] as has been recognized by others,[8b] especially when studying particulates in FMs (peak assignments can be found in Table S1).

To monitor the effects on Raman spectra over time, two experimental time series were set up on duplicate samples: one

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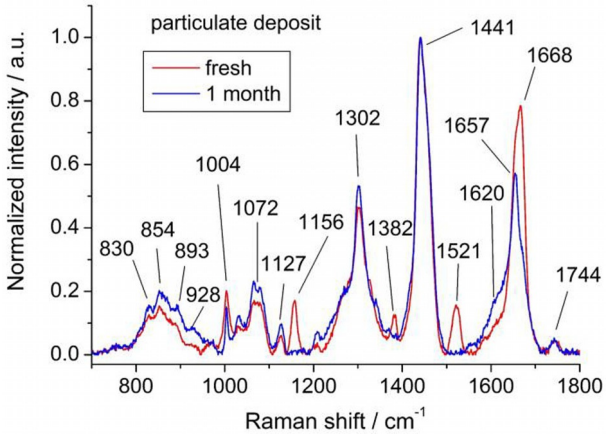

Figure 1. Raman spectra from a freshly deposited FM (red) and after one month of aging (blue). Measurements obtained from a particulate deposit.

with FMs stored in darkness for 4 weeks and examined at different times with both RS and fluorescence spectroscopy, and another time series with FMs kept under ambient light and analyzed with RS over 3 weeks. A general observation was that the surrounding light influences the decay rates of certain Raman bands attributed to carotenoids, squalene, and unsaturated fatty acids, whereas bands assigned to proteins, for example bands of aromatic side chains and amide I, are more stable (see the Supporting Information).

The carotenoid bands (for the dark series) found at 1521, 1157, and 1007 cm^{-1},[17] attributed to C=C, C–C stretching, and C–CH_3 rocking modes, respectively,[18] rapidly decline synchronously (Figure S7), likely owing to oxidation.[19] When considering oily spots, the carotenoid bands vanished after approximately 8 h, whereas only minor peaks are observed initially for particles, probably owing to accelerated decay rates induced by the photobleaching. Recently, resonance RS was non-invasively applied in vivo to detect carotenoid antioxidant levels in human skin, which was related to food intake and used as a health indicator.[17] However, our results suggest that these compounds can be considered to be chemically unstable in vitro[20] and, consequently, in a forensic perspective, too short-lived and sensitive to environmental parameters (e.g. light). Interestingly, as carotenoids are more stable in oxygen-free environments, they have been identified as potential biomarkers when searching for signs of life on planet Mars using RS.[21]

The peaks at 1668 and 1382 cm^{-1}, thought to be dominated by squalene,[22] are decaying slower than the carotenoid bands. In addition, a more rapid decay is observed in ambient light conditions (Figure 2 A), which has also been observed by other techniques.[3] For the samples in darkness, a 1668 cm^{-1} peak could still be observed after 700 h; whereas, when exposed to light, the peak vanished in the background noise after approximately 140 h, interpreted as squalene decomposing in a matter of days, also in good agreement with other studies.[23] For the samples kept in darkness, this band slightly increased and shifted to 1672 before returning to 1668 cm^{-1} and finally declined after about 50–70 h, indicating different reaction pathways.

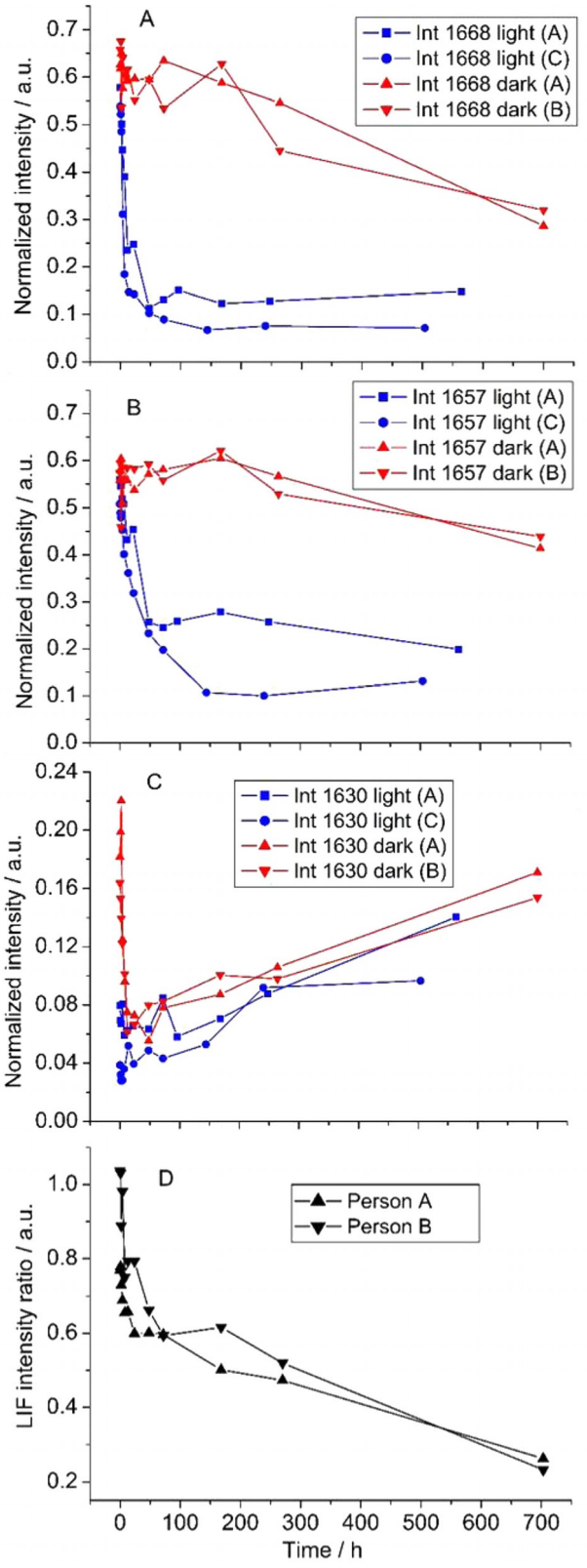

Figure 2. Intensity as a function of time for certain Raman peaks: A) 1668, B) 1657, and C) 1630 cm^{-1}. D) Ratio of fluorescence obtained at the two different excitation wavelengths: 280 and 360 nm.

Unsaturated fatty acids, such as palmitoleic and oleic acids, are expected to contribute considerable to the 1657 cm^{-1}

Raman intensity due to the C=C stretching vibrational mode.[14] Upon oxidation, the double bond breaks and the decay at 1657 cm^{-1} will thus reflect this oxidation process. This peak level is stable over the first 12 days for FMs kept in darkness; whereas, in light, it decreases rapidly (Figure 2B). However, the observed kinetic of this band is slower compared to the peak of squalene.

Proteins are known to be chemically stable residues in fingermarks[6c] and have been utilized as targets for chemical enhancement using ninhydrin since 1950.[24] In the present study, many protein bands were still seen after 4 weeks of aging under ambient light (Figure 1) with peaks located at 830, 854, 1004, 1032, 1174, 1208, 1556,[4] and 1605–1620 cm^{-1},[15] owing to aromatic side chains. Other characteristic protein bands are the amide I (1600–1700 cm^{-1})[5,15] and amide III (1230–1300 cm^{-1})[5,15] bands, both dependent on the secondary protein structure. These bands are more evident in spectra from particles, owing to their higher protein content. Upon FM aging, the relative protein contribution in the 1600–1700 cm^{-1} band increases as other species degrade faster. The outermost part of the skin contains a lot of keratin fibrous proteins (as much as 50–70 wt%[25]) and these are likely present in the FM.[26] Raman bands of keratin have been carefully analyzed by others with the purpose to build models for gender classification of fingernails[27] and to study the effects of humidity on Stratum Corneum structure,[28] and both studies show spectral resemblance to those attained from the aged FM, associated with a maximum at 1654 cm^{-1} and two shoulders at 1675 and 1620 cm^{-1}. The former two bands were assigned to α-helix and β-sheet, respectively, whereas the 1620 cm^{-1} shoulder was attributed to Phe, Tyr, and Trp amino acids. Recently, when keratin from hair with high glycine–tyrosine content was studied,[29] a distinct peak at 1614 cm^{-1} was ascribed to aromatic amino acids. In our study (for particles), a second peak separated from the amide I band was observed in this region centered at 1605 cm^{-1} (Figures 3A and S10–S12). Thus, it is reasonable to assume that keratins or related fibrous proteins of high aromatic amino acid content are present in FMs. It also appears to be heterogeneously distributed, as the intensity of corresponding bands varied from spot to spot, often accompanied with fluorescence of shifting magnitudes. When oily areas of aged (> 100 h) FMs were analyzed (Figures 2C, 3B, and S7–S9), a slight increase in (relative) Raman intensity was seen at approximately 1630 cm^{-1}, regardless of light condition. To speculate, this effect may reflect the oxidation of protein aromatic amino acid side chains or protein conformational changes (or both). On longer time scales, intermolecular interactions in the complex FM environment is likely altered, for example owing to the oxidation of biomolecules (proteins, lipids, DNA),[30] which may impact protein secondary structures. For example, it is known that side chains of protein amino acids are oxidized, by varying mechanisms, to undergo protein carbonylation accompanied with structural changes to cause oxidative stress and diseases.[30–31] Thus, the elevated peak at approximately 1630 cm^{-1} (Figures 2C and 3B) might be an effect of increased (parallel) β-sheet structure[29] and/or of oxidative forms of amino acid side chains in aged FMs. Laser-induced fluorescence (LIF) measurements similar to the van Dam et al. study[12c] were also performed on the FMs kept in darkness. Excitation wavelengths (λ_{exc}) of 280 and 360 nm were used and the ratio of observed fluorescence as a function of aging time is shown in Figure 2D, where the displayed decay kinetics agree well with those of van Dam et al.[12c] Two different decay rates, one fast during the first day(s) and one slower for longer times (similar to RS) can be seen. For λ_{exc} = 280 nm, it is plausible that the observed fluorescence mainly originates from Trp and, as protein-bound Trp is supposed to be reactive and readily oxidizes,[32] it is likely that the initial, rapid decrease is connected to such processes.[31a] At longer times, when most of the Trp has oxidized, the slower decay in ratio may be related to other degradation reactions, for example those that result in protein carbonyl groups. In support of that is the recent discovery of carbonyl-based intrinsic protein fluorescence accompanied by absorbance at 360 nm and emission spectra centered at 430–450 nm (depending on the protein identity).[33]

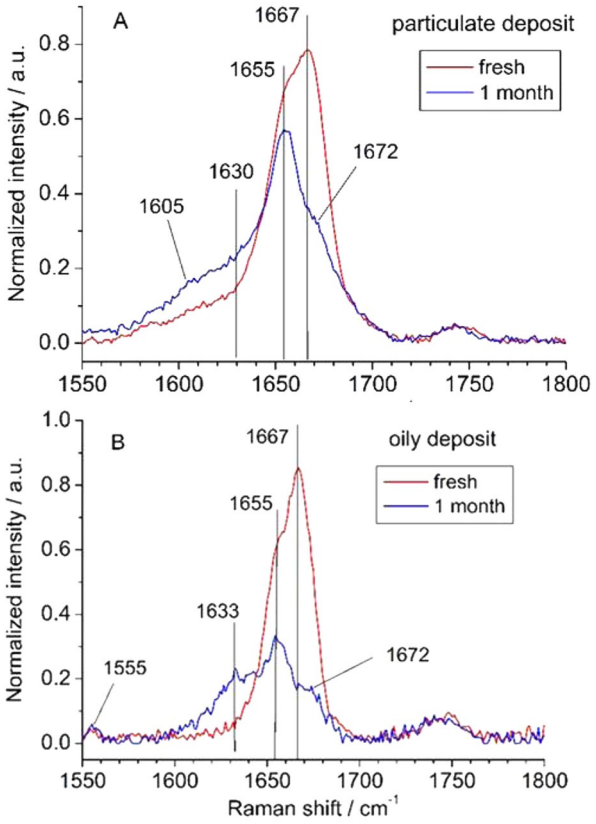

Figure 3. Part of the normalized Raman spectra measured at different times on A) particulate and B) oily spots of a FM deposited on a steel substrate.

This proof-of-concept study illustrates some possibilities of RS to analyze fingermarks to acquire molecular information and different kinetics related to FM aging. It opens up the opportunity for future, more careful investigations accumulating larger data sets sufficient for statistical analysis in order to correlate any discrepancies of FM chemical compositions between groups of individuals, for example those related to gender and/or age, and also to better understand how external parameters such as temperature, humidity, light, and molecular

interactions between endogenous and exogenous compounds affect the degradation rates. Clearly, more data will be needed to gain further insight into the different decay mechanisms. Several markers have, herein, been highlighted appropriate for Raman detection, such as carotenoids, squalene, unsaturated fatty acids, and amino acid moieties in proteins. Decay kinetics were interpreted from changes of Raman bands over time, which was found to be sensitive to light conditions. Furthermore, by combining different, non-destructive spectroscopy techniques (IR, Raman, and fluorescence spectroscopies) with each other and/or with analytical techniques such as mass spectrometry, for example, based on time-of-flight secondary ionization,[34] additional insights in FM aging processes can be expected and may result in a future FM dating technology suitable for on-site forensic analysis.

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Conflict of Interest

The authors declare no conflict of interest.

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