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

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Supporting Information

1. Experimental section

Fingermarks
Prior to the fingermark deposition on cleaned steel surfaces the donor pressed the fingertip (of index finger) against the forehead to increase the amount of substances secreted from the sebaceous glands, e.g. fatty acids, cholesterol, fatty waxes and squalene. Other types of molecules as water, salts, amino acids, proteins, urea and lactic acid are secreted from eccrine glands found all over the body.

Fingermarks were deposited on clean steel substrates. In total fingermarks from five adult donors were studied. In the initial experiments, fingermarks of two male adults were stored in ambient light and spectra were compared between fresh and one month old FM. FMs from two other persons, a male (person A) and a female (person C), were studied in time series more frequently over three weeks with samples stored in ambient light (~12 h light over one day). In a second time series FMs from two individuals, person A and a female (person B), were analyzed over four weeks where the samples were kept in darkness (except during the time period of the measurements including both Raman and fluorescence spectroscopy). The samples were always stored in room temperature in desiccator to keep the humidity constant (44 % RH).

Raman spectroscopy
A commercial confocal Raman microscope (LabRam HR800UV, Horiba Jobin Yvon) was used by letting the laser beam of 514 nm focus through an 100x objective (NA = 0.90) on fingermark residues with a spot size of 1-2 μm in diameter. Subsequently, 180° back scattered photons were collected and linked to a spectrometer of 800 mm focal length equipped with grating of 600 grooves/mm and detected with an Andor Newton thermoelectrically cooled (~−65 °C) EM-CCD camera with the gain set to zero. The instrument was equipped with so-called DuoScan™; two mirrors in the laser path attached to piezo crystals which allow deflection of the laser beam through the objective to obtain an average of the Raman signal over a larger area (macrospot) set to 10 × 10 μm. The molecular fingerprint region 200 – 1800 cm⁻¹ was measured using 10 s integration times with 3 accumulations, but occasionally to achieve extra good S/N ratio also integration times of 30 s with 4 accumulations were applied. Eventual spectral effects induced by the laser illumination itself was carefully checked. It was established using a confocal hole of 500 μm giving a laser power on sample of 5 mW ensured that spectral uniqueness was not affected. In control experiment decay kinetics accumulated from laser irradiated spots were compared to those from non-irradiated ones, and no difference could be seen except for the carotenoid bands that occasionally showed accelerated degradation. Spectral calibration against Si first order phonon band at 520.7 cm⁻¹ was performed routinely and the spectral resolution was typically 4-5 cm⁻¹.

In all Raman experiments the sample spot was viewed with video camera in prior to the data acquisition and it was noted whether the area was of oily or particulate character. Huge variations in fluorescence intensity were observed from particles. In the measurements corresponding to Figures 1 and S5A particles (below) showing only relatively weak fluorescence signals were chosen for analyses. In the series with samples stored in daylight particles were showing large variations in fluorescence intensity, and those measurements that saturated CCD detector were excluded. This data set gave in hand more noisy spectra and corresponding data is not shown. Within the series with samples stored in darkness all particle like spot areas were photobleached 60 s before the Raman measurement was initiated. This probably diminished the carotenoid Raman bands. Extraordinary highly fluorescent particles were here excluded and not analyzed. Five and three replicates were measured in the time
series storing FMs in ambient light and darkness, respectively, on different spots, of oily and particulate character, and afterwards average spectra were calculated.

Straight line with additional concave rubberband baseline correction was performed. Both vector and min-max normalization were tested and give very comparable results, but here only min-max is presented. Over the time period the aging series were run, i.e. 3 and 4 weeks, we are assuming the maximum peak due to CH\textsubscript{2} scissoring mode at 1442 cm\textsuperscript{-1} is constant, meaning that all changes upon aging studied here are in relation to this peak. This approximation seems to be valid as a first approach due to its high peak intensity and spectral stability over the time window that is examined.

**Fluorescence spectroscopy**

A tunable, pulsed (~5 ns, FWHM) laser source was used to induce the fluorescence from the fingermarks. Excitation wavelengths of 280 and 360 nm were used, respectively. The laser light was attenuated to a fluence of about 5 µJ/cm\textsuperscript{2} per pulse for both wavelengths to avoid/reduce any UV light induced degradation. (Kinetic series measurements revealed that almost constant intensity was observed after multiple measurements on the same spot, suggesting that the weak laser light did not affect the fingermarks to a large extent.)

Analyzed area was ~1 mm\textsuperscript{2} as imaged 1:1 onto a round-to-slit fiber bundle coupled to the entrance of a f = 500 mm Czerny-Turner type spectrograph equipped with an intensified CCD detector. The detector was gated for 5 µs and opened (triggered) at the laser pulse. A 150/mm grating allowed for a ~250 nm wavelength window to be captured. 100 spectra (pulses) were accumulated at three different spots for each fingermark and aging time. Obtained spectra were not corrected to overall system response.

### 2. Results

**Optical micrographs**

In this section representative optical micrographs (video images) of fingermarks are shown.

*Figure S1. Optical micrographs of fingermark deposited on steel measured with Raman microscope system with 10x (A) and 100x (B) magnification. The white square in A corresponds to whole area in B.*
Raman spectroscopy
Raman spectra of pure chemicals
Raman spectra were acquired for a few abundant fingermark chemicals as powders (purchased from Sigma-Aldrich) and their spectra are shown in Figure S4.
Figure S4. Normalized Raman spectra of few abundant compounds in fingermarks: a) squalene, b) cholesterol, c) tryptophan, and d) urea.

Raman spectra of fingermarks and assignments

Figure S5. With exception for baseline correction raw Raman spectra are shown. Raman spectra of fingermarks deposited on steel. A) Spectra obtained when a less fluorescent particle is measured after few hours (red) and after 1 month (blue) aging (without photobleaching). B) Spectra obtained when an oily area is illuminated after few hours (red) and after one month (blue) aging. Integration times were set to 30 s and the number of accumulations were 4. Representative spectra are shown.
Figure S6. Min-max normalized Raman spectra measured on a fingerprint deposited on steel with laser beam focusing on oily spots of a fresh fingerprint (few hours old) and of a month old (blue). Only fingerprint region is shown and peak positions are denoted. See also Figures S7-9 below. (Corresponding image for particulate deposits are shown in Figure 1.)

Table S1. Peak assignments of Raman bands in measured fingerprints deposited on steel substrates[1].

| Peak (cm⁻¹) | Assignment                     | Intensity, compound | Comment |
|-------------|--------------------------------|---------------------|---------|
| 225         | S(CH₂) def, C-S str            | m                   | stable  |
| 404         |                                 | w                   | stable  |
| 535         | S-S                            | m                   | stable  |
| 570         |                                 | m                   | stable  |
| 621         | ω(C-C) twist, ν(C-S)            | vw, phenylalanine  | stable  |
| 643         | ω(C-C) twist, ν(C-S)            | vw, tyrosine, amide IV | stable |
| 758         | indole ring                    | vw, tryptophan      | stable  |
| 830         | ring breathing                 | w, tyrosine         | stable  |
| 854         | ring breathing                 | w, tyrosine         | stable  |
| Line | Symbol | Description | Notes |
|------|--------|-------------|-------|
| 893  | C-C    | vw, glycine, gluathione, glutamine | stable |
| 928  | N-Cα-C | w, proteins | stable |
| 966  | -------- | vw, carotenoids | decline |
| 970  | Bending C-H aromatic ring breathing | vw, fatty acids | stable |
| 1004 | | s, phenylalanine | stable, distinct particles |
| 1006 | C-CH₃ rocking | s, carotenoids in oil (dark) | decline hours |
| 1008-1015 | | occasionally (possibly urea), NCN symmetric stretching mode observed at 1012 cm⁻¹ | decline hours, broad |
| 1030 | δ(C-C) | w, phenylalanine | stable (particles) |
| 1066 | ν(C-C) skeleton | m, trans conformation | stable |
| 1080 | ν(C-C) skeleton | m, random conformation | stable |
| 1127 | ν(C-C) skeleton, ν(C-N) | m, saturated fatty acids, proteins | stable |
| 1157 | u(C-C) | s, carotenoids | decline hours |
| 1208 | ring breathing | w, tyrosine | stable |
| 1266 | δ(=C-H ) | w, unsaturated fatty acids, amide III | stable, shoulder |
| 1302 | twisting(CH₂) | s, lipids | |
| 1328 | δ(CH₃), δ(CH₂), δ(CH) | vw, squalene | decline |
| 1341 | δ(Cα-H) | vw, Trp | |
| 1368 | -------- | w | increase, visible at longer times |
| 1382 | δ(CH₃), δ(CH), u(C-C) | w, squalene | decline |
| 1417 | δ(CH₂) | m, saturated and Z-configurated unsaturated lipids | stable |
| 1441 | δ(CH₂/CH₃) | vs, lipids | stable |
| 1459 | δ(CH₂/CH₃) | s, lipids | stable shoulder |
| 1522 | u(C=C) | s, carotenoids | decline hours |
| 1556 | indole ring vibration | w, tryptophan | |
| 1605-1620 | | Trp, Tyr, Phe | stable |
| 1630 | C=C | w, protein β-sheet, vs, unsaturated fatty acids / proteins (amide I, α-helix) | decline slowly |
| 1667 | u(C=C) | vs, squalene, | decline, light dependent |
| 1672  | u(C=C) | β-sheet (anti-parallel), cholesterol decline, light dependent |
|-------|--------|-------------------------------------------------------------|
| 1744  | u(C=O) | TAG's (di,tri-, acylglycerol), Cholesterol(ester) stable     |

δ = deformation (scissoring, wagging, rocking)
ν = stretching

**Raman spectra of fingermarks deposited on steel without time series**

Well resolved spectra are displayed in Figure S5-6 of fresh (red) and one month old (blue) FM when particulate (Figure S5A) and oily (Figure S5B) area are probed. In Figure S6 a shorter spectral window is viewed for the oily region with peak positions given, corresponding spectra for particulates are shown in Figure 1.

Peaks found at 621, 1004, 1032 and 1174 cm\(^{-1}\) are ascribed to phenylalanine, 643, 830, 854, 1174 and 1208 cm\(^{-1}\) to tyrosine and very weak bands at 758 and 1556 cm\(^{-1}\)\(^{[1b]}\) to tryptophan. Other contributing vibrational bands of proteins are the amide I (1600-1690 cm\(^{-1}\))\(^{[1c, 2]}\) and amide III (1230-1300 cm\(^{-1}\))\(^{[1c, 2]}\), both known to be dependent on the secondary protein structure. Aromatic amino acid side chains are responsible for peaks in the area of 1605-1620 cm\(^{-1}\)\(^{[2]}\). Unlike the very weakly fluorescent carotenoids\(^{[3]}\) these amino acids are considerably fluorescent upon UV excitation, which has been utilized in a study to estimate age of FMs\(^{[4]}\). Other evident spectral features in Figure 1 and S6 is the 1442 and 1302 cm\(^{-1}\) peaks ascribed to scissoring modes of CH\(_2\) and CH\(_3\) likely largely dominated by lipids, e.g. (un)saturated fatty acids, and the 1302 cm\(^{-1}\) band corresponding to twisting modes of CH\(_2\)\(^{[1d]}\). Unsaturated fatty acids display significant Raman band about 1657 cm\(^{-1}\) manifested by C=C stretching mode whereas corresponding mode of cholesterol derivatives usually is centered around 1670 cm\(^{-1}\)\(^{[1d]}\). The minor peak about 1740 cm\(^{-1}\) in Figure 1 and S6 is attributed to carbonyl group, e.g. in lipids.

Interestingly with respect to FM aging is the peak located at 1630 cm\(^{-1}\) that here is apparent, which also is seen in Figures below.
Raman spectra of fingermarks deposited on steel monitored in time series

Oily spots

Figure S7. Min-max normalized average Raman spectra measured on fingermark spots of oily character. A-B) FM stored in darkness. C-D) FM stored in ambient light (~12 h light per day).
Figure S8. Same spectra as in Figure S7 but a shorter wavelength range is displayed here.

Figure S9. Initial (red) and final (blue) spectra shown of the time series depicted in Figures S7-8. Carotenoid band located at 1522 cm⁻¹ is well resolved for the FM kept in darkness. Notice that the slower degradation samples kept in complete darkness results in relative higher absorption at longer times around 1650-1670 cm⁻¹ than for those kept in ambient light.
**Carotenoids**

Notably, the carotenoid bands are remarkably intense in Figure S7A-B and S9A-B compare to Figure 1. However, these measurements were started immediately after FM deposition in contrast to those highlighted in Figure 1, which shall have impact on the intensity of the carotenoid bands due to its rapid degradation rates. After 12 h aging no carotenoid signals were visible. Another fact worth mentioning is that these experiments were performed in summertime instead of wintertime as those of Figure 1 when food intake of carotenoids usually are higher and subsequently more naturally are secreted from eccrine and sebaceous glands which is reflected in the FM composition\(^5\). Noticeably, lycopene is a common carotenoid in our bodies whose absorption band is more red-shifted compare to others, resulting in resonance Raman signals which are orders of higher intensity than from other carotenoids\(^1h, 6\) when using 514 nm excitation light. Thus the observed carotenoid degradation is probably mostly influenced by lycopenes (Figures S7A-B).

Another note is that the carotenoid band at 1522 cm\(^{-1}\) is continuously shifted towards higher wavenumbers to reach ~1528 cm\(^{-1}\) after 8h, indicating a shorter conjugated system of the carotenoid backbone as a consequence of expected (photo)oxidation breakdown reactions\(^7\). The vibrational band frequencies of C-C and C=C stretching modes of carotenoids are known to be related to the number of \(\pi\)-conjugated electrons, currently in accordance to carotenes possessing 11 conjugated double bonds\(^1f, 1g\) as for example \(\beta\)-carotene and lycopene.

Recently resonance Raman spectroscopy was non-invasive applied to detect in vivo carotenoid antioxidant levels in human skin, and it was concluded that since all tissue carotenoids originate from the diet the obtained levels could be related to fruit and vegetables intake, and therefore be used as a health indicator\(^1h\). I general such type of information would also be valuable in the donor profiling process.

**Squalene**

The strongest bands of squalene in the fingerprint region has earlier been assigned to C=C stretching at 1668 cm\(^{-1}\) and to \(\delta(CH_3)\), \(\omega(CH)\) and \(\nu(C-C)\) at 1382 cm\(^{-1}\)\(^1e\). Observed peaks at these wavenumbers are therefore assumed to be dominated by squalene which are decaying synchronously (slower than carotenoids) with rate constants dependent on ambient light conditions. It is documented that this substance degrades due to oxidation\(^8\) on a timescale of days depending on the situation\(^9\) (see below also). It has been shown that squalene is photo-oxidized with UV-A irradiation on skin surface to produce squalene monohydroperoxide isomers\(^10\) (marker for oxidative stress of skin).

**Fatty acids**

Here we only focus attention on unsaturated fatty acids associated with C=C stretching frequency centered at 1657 cm\(^{-1}\). It has been reported by Archer et al. that the total amount of fatty acids in fingerprints first increases followed by a later decrease and it was explained by the decomposition of wax esters or triglycerides to fatty acids initially\(^11\).

Cholesterol interferes with squalene 1668 cm\(^{-1}\) with its maximum at 1672 cm\(^{-1}\), but probably its lower Raman cross section due to less number of conjugated double bonds makes it only weakly affecting decay rates observed at 1668 cm\(^{-1}\), which is ascribed to mostly be influenced by squalene.
Particulate spots

**Figure S10.** Min-max normalized average Raman spectra measured on fingermark spots of particulate character. FM are stored in darkness and are photobleached in prior to the measurements.

**Figure S11.** A shorter spectral range displayed for spectra in Figure S10.

**Figure S12.** Initial (red) and final (blue) spectra are shown of the time series depicted in Figures S10-11. (Carotenoid band located at 1522 cm⁻¹ obtained for oily spots is not observable here due to photobleaching.) The broad band centered at 1605 cm⁻¹ is ascribed to fibrous proteins, as for example keratins, while the increase around 1630 cm⁻¹ is hypothesized to be a consequence of oxidation of aromatic amino acid side chains and/or protein conformational changes.
Fluorescence

Figure S13. Representative fluorescence emission spectra measured on fresh fingermarks deposited on steel using two different excitation wavelengths (blue line = 280 nm and red line = 360 nm). Double headed arrows represent the wavelength windows used for intensity integration.

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