Fingermarks, more than just a ridge pattern
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CHAPTER 9

ON THE AUTOFLUORESCENCE OF AGED FINGERMARKS

**ABSTRACT**

One of the challenges in forensic science is accurate estimation of the time at which a trace has been left at a crime scene. To this day, the age estimation of fingermarks is not possible. An important property of fingermarks, currently only used for detection, is the ability to emit fluorescence upon illumination with ultraviolet and visible light. Fingermark autofluorescence changes in time, both spectrally and in total intensity. In this study we have investigated which components in the aged fingermarks cause this change in autofluorescent signal. Thin layer chromatography combined with fluorescence spectroscopy was used to identify fluorescent aging products. Based on our results, tryptophan derivatives, including indoleacetic acid, (nor)harman and xanthurenic acid are indicated as main contributors to the autofluorescence of aged fingermarks. A consistent pattern of eluted fluorescent spots was found in all developed TLC plates with aged fingermarks, indicating that the aging process is not dominated by the inter- and intra-donor variability found in the initial composition of the fingermarks. This knowledge can be used to develop fingermark age estimation methods and to develop older fingermarks by specifically targeting aging components.
1. INTRODUCTION
Accurate estimation of the time at which a trace is deposited at a crime scene is one of the major challenges in forensic research [1-2]. Despite the high forensic value, only a few techniques are available that estimate the age of a trace, such as the time of death [3] and the age of bloodstains [4-5]. However, no methods are available that can accurately estimate the age, and with that, the time of deposition of fingermarks.

Since each fingermark is different in its initial chemical composition [6], the age determination of fingermarks is difficult. The secretion rate of biological materials by the skin, such as proteins and fatty acids, depends on factors like diet, age and gender [6-7]. Environmental factors, such as temperature, humidity and light exposure also influence the degradation rate of the fingermark [6]. As the fingermark is a small and often invisible trace, external contributions such as dirt or cosmetics have a relatively large impact on the total composition of a fingermark.

Several studies on the aging of fingermarks were conducted to find characteristic changes in the chemical and/or morphological properties of aged fingermarks. Morphological changes over time in aged fingermarks were recently described by De Alcaraz-Fossoul et al. and Merkel et al. [8-9]. Their results led to the conclusion that morphological features of fingermarks currently provide limited information on the age estimation of fingermarks. Other research groups have focused on the changes in chemical composition, especially on the changes in lipid content and degradation [7, 10]. An important feature of fingermarks is their ability to display autofluorescence when excited with ultraviolet (UV) and visible light [11-14]. Saitoh et al. observed two characteristic fluorescence emission peaks upon 266 nm illumination of the fingermarks, one peak with an emission maximum at 340 nm and another peak with an emission maximum at 440 nm [14]. After exposing the fingermarks for a longer time to UV light, a change in intensity of the two peaks was observed, resulting in a decrease of the 340 nm peak and an increase in intensity of the 440 nm peak. Menzel et al. also demonstrated a shift in the wavelength range of the fluorescence with time. Fresh fingermarks showed a yellow fluorescence, whereas older fingermarks exhibited a more orange fluorescence [15]. We have proposed protein bound tryptophan to be the main source of autofluorescence in fresh fingermarks and we have demonstrated that protein fluorescence decreases upon fingermark aging [13, 16]. We thus hypothesize that tryptophan and its derivatives play a major role in the fluorescent properties of aged fingermarks. We have recently proposed a fingermark age estimation method based on the oxidation of proteins and lipids and the subsequent changes in fluorescence. We were able to estimate the age of fingermarks, under laboratory conditions, up to three weeks old with an uncertainty of 1.9 days old [16].

The aim of this study is to identify which components in aged fingermark residue cause the change in autofluorescence signal. We will use thin layer chromatography (TLC) to
separate the different fluorophores in fingermarks at different time points after deposition, and subsequently use fluorescence spectroscopy to identify the fluorophores in the fingermark, by comparison with tryptophan and its derivatives.

2. MATERIALS AND METHODS
Materials and instruments used in our experiments are listed in table 1.

Table 1. Overview of type of surfaces used including the supplier’s name.

<table>
<thead>
<tr>
<th>Material</th>
<th>Supplier</th>
<th>Address information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroform, L-tryptophan, L-kynurenine,</td>
<td>Sigma Aldrich</td>
<td>Zwijndrecht, the Netherlands</td>
</tr>
<tr>
<td>xanthurenic acid, 4-aminoacetophenone,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kynurenic acid, indole acetic acid, nor-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>harman, harman, tryptophan, anthranilic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acid, hydroxyl quinaldic acid, hydroxyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>anthranilic acid, hydroxykunurenine, Ehrlich</td>
<td></td>
<td></td>
</tr>
<tr>
<td>reagent and bovine serum albumin (BSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol and TLC Silicagel 60 aluminium sheets</td>
<td>Merck KGaA</td>
<td>Darmstadt, Germany</td>
</tr>
<tr>
<td>N-formylkynurenine (purified using RP-HPLC)</td>
<td>Synthesized in the laboratory of Prof Dr. Hoffmann [17]</td>
<td>Institute of Bioanalytical Chemistry, Leipzig University, Germany</td>
</tr>
<tr>
<td>Sterile Medical Wire MWSCS Taper-Tip swabs</td>
<td>WA products</td>
<td>Essex, UK</td>
</tr>
<tr>
<td>Crime-lite® 2 torches: UV (365 nm, 10% band width 350-380 nm), Blue (445 nm, 10% band width, 420-470 nm)</td>
<td>Foster and Freeman</td>
<td>Worcestershire, UK</td>
</tr>
<tr>
<td>Plastic goggle: Clear (D-21000, optical density 190-400 nm 5+), and orange (I-505600, optical density: 190-534 nm 3+)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camera filters: Yellow (GG495, 1% nom 476 nm, long pass filter) and a polarizer filter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nikon D40X digital camera</td>
<td>Nikon</td>
<td>Tokyo, Japan</td>
</tr>
<tr>
<td>LS 55 Luminescence spectrometer</td>
<td>Perkin Elmer</td>
<td>Groningen, the Netherlands</td>
</tr>
<tr>
<td>Fiber optic accessory</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.1 Fingermark preparation
Volunteers were asked to wash their hands with water and soap, rinse well, and dry them with a paper towel. After drying, fingertips were rinsed with 70% ethanol and air-dried by waving the hands through the air. Afterwards, volunteers were asked to wipe their fingertips across the forehead and from the bridge of the nose to the cheeks. Volunteers wearing facial cream were asked to wipe behind the ears instead of wiping the fingertips on facial regions. After that volunteers were to deposit their fingermarks on the requested surface.
2.2 Fingermark deposition and storage on a TLC-plate
Fingermarks from six volunteers were deposited directly upon an activated thin layer chromatography (TLC) plate, for activation of TLC plate, see method section ‘2.4 Thin layer chromatography’. To obtain a stronger fluorescent signal five fingermarks (from five different fingers of one hand) were deposited on top of each other. The fingermarks were aged for 0, 1, 2 and 3 weeks under two different conditions: under office conditions (office room with TL-lighting during working hours) or in a dark room (closed closet). Upon aging, TLC plates were developed, as described in the method section ‘Thin layer chromatography’. Also, twelve volunteers were asked to each deposit 10 fingermarks on top of each other on the TLC plates to obtain an even stronger fluorescent signal. One TLC-plate was stored for three weeks under office conditions; the other TLC-plate was stored for three weeks in a dark room, before analysis.

2.3 Fingermark deposition and storage on a white tile
Fingermarks were collected during a period of seven weeks. Each week, five volunteers respectively deposited ten fingermarks on top of each other on a white tile and fingermarks were aged for 0, 1, 2, 3, 4, 5, 6, and 7 weeks in a dark room before analysis. To transfer the fingermark depositions from the tile to the TLC plate, fingermarks were swabbed with sterile Medical Wire MWSCS Taper-Tip swabs, pre-wetted with the mobile phase solution (see next section). All swabbed fingermarks (collected from the five different donors) were spotted on top of each other on a TLC plate. As control, an empty tile was swabbed with sterile swabs and spotted on the activated TLC plate.

2.4 Thin layer chromatography.
The TLC method was used as introduced by Bramble et al. and modified by Jones et al. Silica coated TLC plates were pre-washed in methanol and activated for 30 min at 120°C. Chloroform/methanol (1:4) was used as mobile phase. Stock solutions of reference compounds were freshly prepared in 1% methanol in milliQ. Concentrations of solutions are described in the supporting information. The following reference compounds were tested: tryptophan, aminoacetophenone [18], anthranilic acid [18-19], N-formylkynurenine [20], 3-hydroxyanthranilic acid [18-19], 3-hydroxykynurenine [18-19], 8-hydroxy quinaldic acid [21], indoleacetic acid [22-23], kynurenic acid [19, 24], kynurenine [13, 18], norharman [25], harman [25-26] and xanthurenic acid [13, 19]. Samples of 2 µl were applied to the TLC plate. For analysis dried plates were studied with UV and blue Crime-lite®2 torches in combination with the clear and yellow goggles. Next, excitation and emission spectra of the observed spots were obtained using the fiber optic accessory of the fluorimeter. Finally the plates were sprayed with Ehrlich’s reagent. 20 µl aliquots of 50 mg ml-1 BSA were pipetted onto a TLC plate to serve as fluorescence reference for fingermark residues.

2.5 Visualization of fingermarks
UV (365 nm, 10% band width 350-380 nm) and blue (445 nm, 10% band width 420-
470 nm) Crime-lite® 2 torches were used to visualize the spots on the developed TLC plates. Digital images were taken using the polarizer filter (to reduce direct reflection) and the yellow (GG495) filter supplied with the blue Crime-lite® 2 torches. The digital camera and lamp were placed approximately at a distance of 40 cm of the samples and the following settings were employed for developed fingerprints directly left on TLC plate: aperture f/5 and iso-800. The amount of fluorescence originating from the background and fingerprints depended on the Crime-lite® 2 torches used, resulting in a different exposures time used for different light sources: UV: 25 s; blue: 8 s.

2.6 Aged reference compounds
Fresh tryptophan and 3-indoleacetic acid were aged in the same manner as the stacked fingerprints. Tryptophan and indoleacetic acid were aged on TLC-plates for one, two and three weeks under office conditions or in a dark room. Also, fresh tryptophan and indoleacetic acid were developed on a TLC-plate.

2.7 In- and exclusion criteria fingerprints spot and reference compounds
Developed fingerprints and reference compounds were examined with an UV and blue Crime-lite® 2 torch, as described previously [13]. The fluorescent spots obtained from the fingerprints and reference compounds, after running the TLC-plates with chloroform/methanol (1:4), were screened by: 1) the color of the fluorescent spot, which was determined by visual examination; and categorized in a purple, blue, green, yellow or white color; 2) the Rf-value of the fluorescent compounds, meaning the distance traveled by the compound divided by the distance traveled by the mobile phase; 3) fluorescent excitation and emission spectra of the spot; 4) color reaction with Ehrlich’s reagent. Reference samples were in- or excluded of being a possible source of the fluorescent fingerprint spots based on the fluorescent color, Rf-value, excitation and emission spectra and reaction with Ehrlich’s reaction (color and Rf-values of spots of reference compounds can be found in the supplemental data).

3. RESULTS
After running the aged fingerprints on the TLC plates, and visualizing the plates using the Crime-lite® 2 torches, a consistent pattern was found in the fluorescent spots observed at the developed TLC-plates. Fingermarks deposited by different volunteers and aged for one, two or three weeks, showed an identical pattern of fluorescent spots observed between volunteers in all developed fingerprint as depicted in figure 1 and figure 2. The clarity of this pattern increased with age. Fingermarks aged under office conditions and stored for a longer time showed an increase in the amount of fluorescent spots compared to fresh fingerprint. After three weeks of aging, six characteristic spots (B,D,E, F/G and H) (fig. 1) could be observed in all developed fingerprints of the different donors, whereas in the fresh developed fingerprints a few weak fluorescent spots could be detected. Inter-donor variability in the initial composition of fingerprints from donors was observed, because fresh fingerprints displayed fluorescent spots of diffe-
Figure 1. Aging of fingermarks (5x) stored under office conditions on TLC plates and developed using chloroform/methanol (1:4) as mobile phase. Differences in fluorescence intensity and amount of fluorescent spots can be observed over time. Images were obtained using the UV Crime-lite® 2 torch and a polarizer filter.

<table>
<thead>
<tr>
<th>Fresh</th>
<th>1 week old</th>
<th>2 weeks old</th>
<th>3 weeks old</th>
</tr>
</thead>
</table>

|  }H     |  }F/G      |  }E         |  }D         |

|  }B     |            |             |             |

Figure 2. Aging of fingermarks stored in a dark room on TLC plates and developed using chloroform/methanol (1:4) as mobile phase. Differences in fluorescence intensity and amount of fluorescent spots can be observed over time. Images were obtained using the UV Crime-lite® 2 torch and a polarizer filter.

<table>
<thead>
<tr>
<th>Fresh</th>
<th>1 week old</th>
<th>2 weeks old</th>
<th>3 weeks old</th>
</tr>
</thead>
</table>

|  }H     |  }F/G      |  }E         |  }D         |

|  }C     |            |             |             |

|  }A     |            |             |             |
rent colors and Rf-values, which is in accordance to previous findings [13].

Fingermarks developed on the TLC-plates stored in a dark office room showed an almost identical pattern of eluted fluorescent spots as the fingermarks stored under office conditions (fig. 2). However, the fluorescence intensity of the spots aged in a dark room was brighter. Spot A was exclusively observed on plates from fingermarks stored under dark conditions (fig. 2). Thus, a total of seven characteristic fluorescent spots (A, C, D, E, F/G and H) resulted from fingermarks aged for three weeks under dark conditions. Spot B (office conditions) and spot C (dark conditions) have slightly different Rf-values (B=0.50; C=0.55), however, the fluorescence color and characteristic V-shape of the spots was identical (fig. 1 and 2).

3.1 Ehrlich’s reagent
Development of the TLC plates with Ehrlich’s reagent, resulted in the coloration of specific spots. A purple and pink coloration could be observed in respectively, spot F/G and spot H in the fingermarks stored under office conditions and dark room (fig. 3). Ehrlich’s reagent is able to react with tryptophan and indole-groups, resulting in a specific

<table>
<thead>
<tr>
<th>Stored under office conditions</th>
<th>Stored in dark</th>
<th>3 weeks old</th>
<th>3 weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td>H{F/G} E{D}</td>
<td>H{F/G}</td>
<td>C{A}</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Fingermarks (10x) aged for three weeks on a TLC plate. After development with chloroform/methanol 1:4, the plates were developed with Ehrlich’s reagent.
color depending on the structure of the compound and is therefore an indicative test for unknown compounds.

3.2 Reference compounds

**Tryptophan** is a fluorescent amino acid and indicated as one of the major contributors of the autofluorescence of fingermarks [13, 16, 27]. Results of the developed TLC plates and spectroscopy of fresh and aged tryptophan are shown in figure 4. Fresh tryptophan did not result in any eluted fluorescent spots. However, leaving the fresh developed tryptophan for one hour in office light resulted in one fluorescent spot, with a large tail (not shown). Upon one to three weeks aging of tryptophan on the TLC-plate and subsequent development, six fluorescent spots were distinguished, as shown in figure 4 (1*, 2* and D-G). The elongated spot 1* and spot 2* of the aged tryptophan could not be observed in the spots of the developed aged fingermarks. The Rf-values of spots D-G of the aged tryptophan (fig.4-A) were in correspondence with the fluorescent color and Rf-values of spots found in the aged fingermarks (spot D-G, stored office conditions and dark room). Spectroscopic analysis of the tryptophan derivative spot D and the eluted spot D obtained from the fingermarks resulted in a match. Spot D (fig. 2 and 3) of the aged fingermarks yielded excitation and emission spectra comparable to those of aged tryptophan spot D (fig.4-B and C). Spot D did not react with Ehrlich's reagent in both aged fingermarks as aged tryptophan. These results imply that tryptophan derivatives play a major role in the degradation of fingermarks.

![Figure 4](image)

**Figure 4.** A: Tryptophan aged for 0, 1, 2 and 3 weeks on TLC plates stored in under office conditions or in a dark room. After aging, plates were developed using chloroform/methanol (1:4). Images were obtained using the UV Crime-lite® 2 torch and a polarizer filter. B: Excitation spectra of fluorescent spot D and aged tryptophan spot D (3 weeks) eluted with MP, excitation wavelength 365 nm. C: Emission spectra of fluorescent spot D and aged tryptophan (3 weeks) spot D, emission wavelength 440 nm. Rf-value spot D tryptophan 0.82-0.85.
Next, tryptophan derivatives were included as potential contributors to the autofluorescence of fingermarks. Aminoacetophenone [18], anthranilic acid [18-19], N-formylkynurenine [20], 3-hydroxyanthranilic acid [18-19], 3-hydroxykynurenine [18-19], 8-hydroxy quinaldic acid [21], indoleacetic acid [22-23], kynurenic acid [19, 24], kynurenine [13, 18], norharman [25], harman [25-26] and xanthurenic acid [13, 19] were spotted on the TLC-plates and developed.

Based on their fluorescent color, Rf-value, excitation and emission spectra and reaction with Ehrlich’s reagent, four reference samples gave a very strong indication of being present in aged fingermark: 3-indoleacetic acid, norharman, harman and xanthurenic acid. Detailed information on the results obtained for the reference compounds that did not match all our inclusion criteria can be found in the supporting information.

**3-Indoleacetic acid** is a fluorescent metabolite of tryptophan which can be formed during bacterial breakdown of tryptophan [22-23]. Fresh indoleacetic acid displays a blue fluorescence (Rf-value 0.98). Indoleacetic acid showed similarities with spot H of aged fingermarks (fig. 1 and fig. 2). Indoleacetic acid gave a pink/purple color reaction when treated with Ehrlich. The color of the reaction product showed similarities with the Ehrlich reaction of spot F-H of the aged fingermarks, as depicted in figure 3. Like tryptophan, 3-indoleacetic acid was also aged for one to three weeks. The aging of 3-indoleacetic acid resulted in two/three yellow fluorescent degradation products (Rf-values ranging from 0.84 to 0.97). Excitation and emission spectra obtained from the indoleacetic acid spot with an Rf-value of 0.97 were very similar to those of spot H of fingermarks aged for three weeks in a dark room, as depicted in figure 5. We have thus a strong indication that indoleacetic acid is one of the major players to the autofluorescence of aged fingermarks.

**Norharman** also called beta-carboline is a strong blue fluorescent compound resulting from tryptophan degradation [25]. The obtained Rf-value of norharman is 0.84. The fluorescent color, Rf-value, and the shape of the fluorescence spectra were similar to that of spot D of the aged fingermarks (fig. 1, 2 and 6). No reaction with Ehrlich reagent could be observed. This all indicates that norharman, which can be formed out of tryptophan, is one of the contributors of autofluorescence in aged fingermarks left on TLC plates.

**Harman** belongs also to the class of beta-carbolines and has similar properties to norharman. The molecular difference with norharman is that harman contains a methyl-group [25-26]. The eluted harman resulted in a spot with an Rf-value of 0.79. The fluorescent color, Rf-value and the shape of the fluorescence spectra were similar to that of spot D of the aged fingermarks (fig. 1, 2 and 6). Also, the excitation and emission spectra obtained from harman is similar to spectra obtained from spot D, as depicted in figure 6. Norharman and harman have similar characteristics, based on the fluorescent
Figure 5. Excitation (emission wavelength at 440 nm) and emission spectra (excitation wavelength at 365 nm) of yellow fluorescent spot (spot F/G, black line) and 1 week aged indoleacetic acid with Rf-value of 0.97 (office conditions, dotted line).

Figure 6. Excitation (emission 440 nm) and emission spectra (excitation 365 nm) of blue fluorescent spot (spot D, black line), norharman, Rf-value 0.84 (striped-dotted line) and harman, Rf-value 0.79 (dotted line).

Figure 7. Excitation (emission 440 nm) and emission spectra (excitation 365 nm) of fluorescent spot (spot F/G, black line), xanthurenic acid, Rf-value 0.93 (striped-dotted line).
color and fluorescence spectra, and it is thus impossible with the applied methods to differentiate between norharman and harman. No reaction with Ehrlich’s reagent could be observed in spot D and the spots obtained from norharman and harman. Based on these findings, we have a strong indication that beta-carbolines are one of the major contributors to the autofluorescence of aged fingermarks.

**Xanthurenic Acid** is known as a blue fluorescent tryptophan metabolite [19]. In previous research we described xanthurenic acid as one of the possible contributors to the fluorescence of fresh fingermarks [13]. Xanthurenic acid is observed, in contrast to the reported blue fluorescence, as a green-yellowish fluorophore upon excitation with the UV and blue Crime-lite® 2 torch. Eluted xanthurenic acid resulted in two different spots (0.93 and 0.98). The excitation and emission spectra obtained from xanthurenic shows similarities with eluted fluorescent spots (F/G) in aged fingermarks (figure 7). No color reaction could be observed after development with Ehrlich’s reagent. Therefore, xanthurenic acid could not be excluded as being a contributor to the autofluorescence of aged fingermarks, based on fluorescent color of the spots, the Rf-value, excitation and emission spectra and the reaction with Ehrlich’s reagent.

After development, fluorescent fingermark residues can still be observed at the location at which the fingermarks have been place initially on the TLC plates, as can be seen in figure 1 and 2. These residues mostly display a blue fluorescent signal and can be observed in all fresh and aged fingermarks. Fluorescence spectra were taken from these residues and their spectra were consistent with the excitation and emission spectra of BSA, as shown in figure 8. We hypothesize that the fluorescence signal in the fingermarks residues is mainly caused by peptides and/or proteins.

To investigate whether the aging of fingermarks is surface dependent, fingermarks were left on a forensic relevant surface, namely white tiles and stored for 0 to 7 weeks in a

![Excitation and Emission Spectra](image)

**Figure 8.** Excitation (emission 340 nm) and emission spectra (excitation 265 nm) of BSA (black line) and three fingermark residues left on a TLC plate aged for three weeks (dotted line).
dark room. After storage, fingermarks were swabbed and transferred to TLC-plates for development and analysis. Again, a pattern of consistency could be observed in the fluorescence spots obtained from the aged fingermarks left on the white tiles, as depicted in figure 7. However, fewer fluorescent spot were detected compared to spots obtained in fingermarks placed and stored on TLC-plates (fig. 2). Four fluorescent spots could be obtained from the developed TLC-plates. The four spots showed similar fluorescent properties and Rf-values as spot D, F/G and H of fingermarks stored in a dark room on TLC-plates. No fluorescence spectra could be obtained for the spots, because the fluorescence intensity of the spots was too low to be measurable.

Figure 9. Fingermarks aged on tiles stored under dark conditions and transferred to a TLC-plate before development. Images were obtained using a UV Crime-lite® 2 torch (365 nm, 10% band width 350-380 nm) and a polarizer filter.

4. DISCUSSION

In this study, we have shown that tryptophan derivatives play a major role in the aging of fingermarks. Indoleacetic acid, beta-carbolines and xanthurenic acid are indicated as major contributors to the fluorescence of fingermarks aged upon three weeks. The reference compounds, kynurenic acid and kynurenine, could not be excluded of being contributors to the fluorescence of aged fingermarks, as presented in the supporting information. We excluded aminoacetophenone, anthranilic acid, N-formylkynurenine, 3-hydroxyanthranilic acid, 3-hydroxykynurenine and 3-hydroxyquinaldic acid as contributors to the autofluorescence of aged fingermarks. TLC in combination with fluorescence spectroscopy and Ehrlich treatment has thus yielded new information on the nature of the fluorescent aging products present in fingermarks.

An important finding was that a consistent pattern was observed in the eluted spots from aged fingermarks. Already after one week of aging a similar pattern in fluorescen-
ce spots can be observed between individuals, but also within individuals.

In previous studies, protein-bound tryptophan was posed to be the major source of the intrinsic fluorescence of fresh fingermarks [13]. Upon aging, fingermarks are exposed to different influences, such as light exposure, temperature and bacterial degradation, which will cause the degradation of fingermarks and thus the degradation of proteins and tryptophan. We assume that the oxidized tryptophan gets detached from the proteins upon aging as the derivatives did elute during TLC. An explanation for the differences found in the fingermarks stored under office conditions and in a dark room, is that light can accelerate the degradation of fingermarks by photo-oxidation [20]. Bacterial breakdown will also affect the degradation of the fingermark deposit. Various bacterial communities live on the human skin and these communities are fairly stable over time. After placing a fingermark, bacteria present on the skin are transferred to the substrate [28]. Bacteria are able to convert tryptophan to different derivatives. Specific bacteria, called the indoleacetic acid producing bacteria, have the ability to convert tryptophan in indoleacetic acid [29-30]. Indoleacetic acid was found to be one of the major contributors of the fluorescence of aged fingermarks and gave a pink/purple color when Ehrlich's reagent was applied. Spot F/G and H (figure 3) in the aged fingermarks reacted with Ehrlich's reagent, indicating the presence of indoles [21]. In aged tryptophan, the fluorescents spots with a comparable Rf-value as indoleacetic acid did not result in a color reaction. The TLC-plates used in our experiments were clean plates, therefore it can be expected that no bacteria were present. Tryptophan used in our experiments was diluted in sterile tubes. Fingermarks were directly deposited on the TLC plates and the deposited fingermarks should contain a high variability of different bacteria normally present on the skin, which explains the formation of indoleacetic acid in the aged fingermarks and not in the aged tryptophan.

Besides environmental factors, such as light exposure and bacterial breakdown, the substrate at which the fingermark has been left on can influence the aging [2]. Therefore, we included not only fingermarks directly left on TLC plates, but also on tiles and these fingermarks were aged for different time intervals (0 to 7 weeks) in a dark room. Upon aging, fingermarks left on the tiles were swabbed and spotted on the TLC-plate. Also, in these eluted fingermarks a pattern of consistency could be found in the observed fluorescent spots. However, a smaller number of fluorescent spots was retrieved compared to those obtained from fingermarks directly placed on the TLC plates. This could indicate a substrate depending difference in aging of fingermarks. Also, the transfer of the fingermarks from the tile to the TLC plate could have affected the fingermark components. Based on the similarity in color of the fluorescent spots and the Rf-values of these spots found on the developed TLC plates from fingermarks aged on the TLC plate and fingermarks aged on tiles and transferred to the TLC plate, we conclude that the fluorescent products that were formed during the aging of fingermarks on tile were similar to the aging products of fingermarks directly placed on the TLC plate, including
indoleacetic acid and xanthurenic acid.

After development of fresh and aged fingermarks, fluorescent residues could be observed at the location at which the fingermarks have been placed. The excitation and emission spectra of these residues were almost identical to those of BSA. This finding indicates that the fluorescent residue consist mainly of peptides or proteins, which has previously been described by Lambrechts et al. [13].

Currently, age estimation of fingermarks in practice is not possible. The main problem is the high inter- and intra-person variability in the initial composition of fingermarks [10]. In this study we have shown that despite this variation, the aging process shows an increasingly consistent pattern of observable fluorescent components. This pattern can be observed in fingermarks stored under office conditions, and also in fingermarks stored in a dark room. Developed fingermarks stored in a dark room express more and brighter fluorescent spots than eluted fingermarks stored in under office conditions. From these findings we can state that the aging process in fingermarks is reproducible, when looking at fluorescent components, even if the initial composition is different.

Finally, knowledge of the specific fluorescent components that are formed during the aging process of fingermarks can also be used for the development of a new technique to develop old fingermarks. By specific targeting aging products, such as carbolines or indoleacetic acid, more information about the ridge pattern can be obtained from aged fingermarks using for example immunolabeling techniques [31].

5. CONCLUSION
A pattern of consistency was found in fluorescent components originating from aged fingermarks, indicating that the aging process of fingermarks from various donors is similar under identical circumstances. The tryptophan derivatives including beta-carbolines, indoleacetic acid and xanthurenic acid were indicated as important fluorophores in aged fingermarks.

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