Fingermarks, more than just a ridge pattern
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CHAPTER 12

DISCUSSION AND CONCLUDING REMARKS
1. INTRODUCTION
The aim of this thesis was to develop techniques that aid in the detection, analysis and identification of components present in fingermarks using immunolabeling and fluorescence spectroscopy. The main findings are that immunolabeling has been proven to be an excellent technique, first of all, for the specific detection of components of interest, which might provide donor profiling information, and secondly for the (re)development of fingermarks. Furthermore, fluorescence imaging can help in the visualization of fingermarks and fluorescence spectroscopy for the age estimation of time since deposition of fingermarks. Thin layer chromatography combined with fluorescence spectroscopy provides additional information about the fluorescent components present in the fingermark residue. This combined method can be used to indicate fingermark fluorophores in fresh and old fingermarks. We indicated tryptophan (protein-bound) and its derivatives as major contributors to the fluorescent signal of fresh and aged fingermarks.

2. LIMITATIONS AND FUTURE APPLICATIONS OF IMMUNOLABELING
Immunolabeling is a promising technique that offers great opportunities when implemented in the forensic field. As presented in chapter 3-6, the immunolabeling technique is matured to a level that it can be integrated into the forensic investigation for the (re)development of fingermarks, especially on those that lack visibility. Because specific components can be detected with immunolabeling, more detailed information can be obtained from immunolabeled fingermarks even after initial development with traditional techniques. Still, immunolabeling aided donor profiling needs more investigation, specifically validation studies, and therefore cannot yet be applied on fingermarks found at crime scenes. Like most of the existing techniques, also immunolabeling has its limitations.

One drawback is that immunolabeling cannot directly be applied on items found at the crime scene, but that items have to be transferred to a laboratory. A solution would be to directly label the fingermarks that have been lifted from the crime scene and which are used for the identification process. However, immunolabeling of lifted fingermarks has not been investigated yet and more research is needed. A portable method, that can directly be applied to fingermarks and other traces found at the crime scene may be an even more optimal solution. However, since several washing steps are involved in the immunolabeling method, it is doubtful whether a portable immunolabeling method will be feasible.

Another limitation is the processing time. Depending on the surface, immunolabeling may take 4 hours (porous surfaces) to one day (non-porous surfaces), mainly due to the incubation time of the antibodies with the fingermarks. The actual hands-on laboratory work is however limited and takes, using our protocol, less than one hour. The whole processing time can be shortened more, for instance by increasing the incubation temperature. In our work, all experiments were performed under room temperature. A
temperature increase to 37˚C may speed up the reaction between antibodies and antigens. Another step to decrease the processing time, is the use of direct labeling instead of the indirect labeling method, whereby a visual enhancer is conjugated directly to primary antibodies, as described in chapter 3.

Fingermarks, but also background surfaces have the ability to emit autofluorescence upon illumination with UV/blue light. The intensity of this autofluorescent signal is different for each fingermark and may interfere with the fluorescent tags conjugated to primary and/or secondary antibodies. To overcome the problem of this background fluorescence, other luminescent particles can be used, specifically upconversion nanoparticles (UCNPs). These particles can directly be conjugated to the primary antibodies, but also to the secondary antibodies. The UCNPs have unique luminescence characteristics. Instead of the stoke-shift luminescence observed in biological fluorophores, UCNPs use anti-stoke conversion luminescence: excitation with a longer wavelength (near-infrared) generates luminescence of a shorter wavelength. The components present in fingermarks and background material do not possess this specific luminescence property, which results in a strong optical contrast between the labeled components in the fingermarks and the fingerprint and background surface [1].

A remark, concerning donor profiling of fingermarks should be made: most of the studies that described the detection of certain components in fingermarks, such as drugs, explosives and caffeine, used spiked fingermarks. Consequently, the fingermarks were doped with the substance of interest, resulting in probably a higher amount of the substance present in the fingermark residue than expected in fingermarks found at crime related objects. In future experiments we therefore suggest to include natural fingermarks, from donors that have been in contact with the components in a more natural way. Also, for the determining whether an individual has consumed drugs, we suggest to work with fingermarks of donors that have been ingested the drugs, instead of investigating fingermarks that have been spiked with the metabolites of interest.

The future potentials of immunolabeling on fingermarks is broad. Currently, detection of drug metabolites is possible in fingermarks. However, the detection of drug metabolites with the immunolabeling method needs to be further investigated, since most of the studies found in literature on the detection of drug metabolites in fingermarks using antibodies do not show proper control experiments. Therefore, in these cases no conclusions can be made on the specificity of the antibodies to their antigen. Another remark is that these studies do not perform blocking steps and the applied washing steps are minimal, which may lead to non-specific binding of the antibodies to fingermark components and/or the substrate [2-6].

Besides drug metabolites, other possible targets for donor profiling can be food metabolites, gender specific biomarkers or age related biomarkers. Using multiple labeling,
e.g. the AB-blood group type can be determined in single fingermarks. In figure 1, the simultaneous detection of antigen A and B is shown in fingermarks of a donor with blood group type AB. Positive detection of antigen A, red channel and positive detection of antigen B was possible. Since the intensity of the fluorophores is limited, detection of the primary antibodies tagged with a fluorophore could only be achieved with fluorescence microscopy with a magnification of 10 times or higher. The use of UCNPs instead of fluorophores can probably solve this problem. One limitation of blood group typing in fingermarks is that the amount of proteins present in the fingermark should be enough to determine the blood group type. If a donor leaves hardly any proteins, detection of antigen A and B will be tough or even impossible. Therefore, caution should be taken with determining the blood group type. If no detection of antibodies is possible, the blood group type cannot be typed as 0, but should be typed as 0 or no proteins present to determine the blood group type.

A first step is already taken by our group to use fingermarks as non-invasive method to test for allergies. Allergic reactions are caused by an overreaction of the immune system to normally common substances, allergens, present in the environment. To determine whether someone has developed a specific allergy, patients need to undergo a questionnaire performed by a medical doctor, followed by a skin prick test or blood test. Both, skin prick test and blood test are invasive tests that can be recorded as painful and irritating to the patient. It is known that antibodies specific to allergies an allergen can be found in eccrine sweat [7-10].

Figure 1. Simultaneous blood group typing of antigen A and B in a single fingermark of a donor typed with blood group AB.

Besides the use of immunolabeling for forensic purposes, immunolabeling can also be used to obtain medical relevant information. We hypothesize that important information on the health status of the donor is present in the fingermark residue or more broadly in the sweat of an individual.

We hypothesize that antibodies are also present in secretions left by the fingertips. In a first test, we have tried to detect antibodies specific for cow milk allergy in fingermarks of a donor who has developed a cow milk allergy. Fingermarks were incubated with a cow-milk specific protein, casein, conjugated to a Cy3 fluorophore, as depicted in figure 2. As control, fingermarks from a donor who has not developed a cow-milk allergy were
used. A positive staining was observed in the fingermarks of the donor who had developed a cow milk allergy, whereas no staining was visible in the fingermarks of the control donor, as shown in figure 3. This first result indicates that fingermarks contain specific information that can be used for the diagnosis of allergies. If fingermarks can be used as a non-invasive method to aid in the diagnosis of allergies, this will have a major impact in the diagnostics of allergies.

Immunolabeling is not limited to fingermarks only. Also from other human biological traces donor profiling can be obtained using the immunolabeling method. In the case of traces that cannot be used for identification purposes, immunolabeling may aid in

Figure 2. Schematic overview of the detection of allergy specific antibodies in fingermarks

Figure 3. Detection of cow-milk specific antibodies. A: Control, fingermark from donor who has not developed an allergy for cow milk. B: Fingermark from donor who developed a cow-milk allergy. Positive staining is visible at the pore sites of the fingermarks. Images were taken with a fluorescence microscope, 10 x magnification.
donor profiling. Also, instead of antibodies, aptamers can be used to target specific components in fingermarks. For each application the antibodies and aptamers need to be studied thoroughly to limit the occurrence of false-positive results.

3. LIMITATIONS AND FUTURE APPLICATIONS OF FLUORESCENCE SPECTROSCOPY

In this thesis we have used TLC in combination with fluorescence spectroscopy to obtain a better understanding of which components are responsible for the fluorescence of fresh and aged fingermarks, as presented in chapter 7 and 9. Although strong indication of the most important contributors were found, no direct identification of proteins, peptides or components was possible with this technique. Therefore, in future studies it is worthwhile to use an additional method, for instance high pressure liquid chromatography coupled mass spectrometry (HPLC-MS) and mass spectrometry (MS) imaging, to identify the different fluorescent components or mixtures. As shown in chapter 9, the surface at which the fingermark has been left on influences the formation of degradation products in the fingermark. Fingermarks were aged on two different surfaces, directly placed on a TLC-plate or on a white tile. In these experiments only two different conditions were tested, fingermarks stored under office conditions and fingermarks stored in a dark room. Other conditions that will definitely influence the degradation of fingermarks and therefore the formation of fluorescent products include: light and UV exposure, humidity, temperature, air flow and other environmental influences [11]. In future work these conditions need to be tested to find out whether similar degradation products are formed.

The intrinsic fluorescence of fingermarks can be used for multiple purposes e.g. for fast and non-invasive detection of fingermarks and other fluorescent traces using forensic light sources. Information on the chemical components present in fresh and aged fingermarks can be used to develop new methods for the development of fingermarks. Most fingermark developing methods are developed to visualize fresh fingermarks. Problems have been observed when developing aged fingermarks. We assume that when targeting specific components present in fresh and aged fingermarks, more detailed information will be obtained from the ridge pattern. Antibodies or aptamers that are able to detect aging products, like norharman, harman and indoleacetic acid may result in an improved enhancement of ridge details in aged fingermarks.

In chapter 8, we have made the first step in the investigation of the relation between the intensity of the autofluorescence signal with the amount of DNA present in the fingermark. We have found that the intensity of the autofluorescence is weakly to moderately correlated with the DNA amount. Preliminary knowledge on the amount of DNA can be helpful in guiding the forensic investigator to useful traces for DNA analysis. However, to get a more reliable estimation of the relation between the intensity of autofluores-
cence and amount of DNA present in fingermarks several adaptations to the protocol described in chapter 8 should be made. Firstly, besides a subjective assessment of the fluorescent intensity of the fingermarks, an objective assessment may provide a more reliable outcome. Both, the subjective scoring and the objective analysis of the intensity of the fluorescent images can then be compared. Next, instead of the Crime-lite® torch described in our study with excitation wavelength of 365 nm (10% bandwidth 350-380 nm), another excitation source can be used that better suits the excitation wavelength of DNA and proteins. The optimal excitation wavelength to excite DNA and proteins is around 260 and 276 nm [12-14]. Therefore, we suggest to work with a shorter excitation wavelength than the one used in our experiments. Additionally, a filter should be included in front of the camera that specifically blocks the excitation light originating from the light source and surface. By applying these adaptations to the described protocol a more defined statement can be made about the relation between the intensity of autofluorescent signal of fingermarks and DNA amount. However, quantification of a fluorescent signal is a difficult, maybe even an impossible task, since the amount of emitted fluorescent light is influenced by a lot of factors, including the intensity of the excitation light at the fingermarks, exposure time, bleaching caused by light exposure, and chemical interactions with other molecules in the sample.

In this thesis we have demonstrated a new method to estimate the age of fingermarks since deposition up to two weeks. We are now able to estimate fingermarks left on TLC plates and aged in a dark room using the aging rate of the fingermarks. However, many factors can influence the aging rate of fingermark residues. Therefore, additional research is required to investigate the influence of environmental factors and the influence of substrate characteristics on the aging process of fingermarks. If more is known about the circumstances at which a trace has been found, corrections can be made to obtain a more accurate age estimation. Fluorescence spectroscopy will not be an adequate technique to estimate the age of fingermarks left on strongly fluorescent backgrounds, thus other techniques should be investigated. The time of deposition is extremely important information for the forensic investigator to determine whether traces are relevant for the committed crime and/or to support or refute victim’s and perpetrator’s statements. In chapter 10, the first step in age determination was presented using the intrinsic fluorescence of fingermarks and the changes that occur over time. We were able to estimate the time of deposition of 55% of the fingermarks from all male donors up to three weeks old with an uncertainty of 1.9 days. This is the first time that the age of fingermarks could be determined within a period of three weeks after deposition. Our method is able to tackle the inter- and intradonor variation, measuring the aging rate of each fingermark. The aging method is not only restricted to fingermarks, but can be used for all traces that can be approached as protein-lipid mixtures, like sperm and vaginal fluids.
4. CONCLUDING REMARKS

In this thesis we have presented various proof of principles that demonstrate that fingerprint marks are much more than only a ridge pattern. Fingermarks are composed of a wide variety of information than cannot only provide information about the donor of the mark, but also contains relevant information about the mark itself. Immunolabeling has been shown to be able to detect specific chemical components in fingerprint marks that can be used for donor profiling, but also for the improvement of ridge details. Besides immunolabeling, more techniques are available for analyzing the chemical composition of fingerprint marks. Combining different techniques, including techniques that have focused on obtaining donor profiling information from ridge details, with analytical methods, may provide relevant information on the donor of the fingerprint mark. Fluorescent components causing the intrinsic fluorescence of fingerprint marks are useful components that can be used for developing aging methods to estimate the time of deposition of fingerprint marks. In the nearby future, we expect that fingerprint marks, but also other traces found at crime scenes provide a lot more useful information that may aid in the forensic investigation.

REFERENCES