Fingermarks, more than just a ridge pattern
van Dam, Annemieke

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Fingermarks are one of the most important pieces of physical evidence that can be found during a crime scene investigation. Since each fingermark is unique per individual, the pattern that is left behind when leaving a fingermark can be used for individualization. Therefore, fingermarks can be used to locate, identify or exclude possible donors.

But fingermarks contain more information than the complicated pattern of ridges and furrows. The chemical composition of a fingermark, comprised of endogenous and exogenous components, holds an enormous amount of undisclosed information on the donor of the mark, including the gender, age, blood group type and personal habits. This additional information can be used to increase the value of evidence, but also to use for fingermarks that are distorted, smudged and/or useless for the identification process.

Therefore, the aim of this thesis is to develop techniques that aid in the detection, analysis and identification of components present in fingermarks using immunolabeling and fluorescence spectroscopy.

The first chapter of this thesis describes, in short, the use of the fingermarks and their ridge pattern for forensic purposes, followed by a short overview of the chemical components present in the fingermark residue. Important components in the fingermark that can be used to retrieve donor information originate from intrinsic components secreted via the pores present in the skin, such as proteins, peptides, amino acids and fatty acids. Besides these endogenous components, also environmental contaminants, like food, cosmetics and drugs, but also endogenous body material, like saliva, can affect the composition of a fingermark. In this thesis, two different techniques are used to detect, analyze and identify chemical components in fingermarks, immunolabeling and fluorescence spectroscopy. In the first part of chapter 2 the basics of the immunolabeling technique are discussed. Immunolabeling is based on the specific binding of antibodies to antigens. A remarkable feature of antibodies is that they can be produced against almost any kind of macromolecule, including proteins, hormones and drug metabolites. Therefore, antibodies can be used to detect specific components in fingermarks. In fingermarks, immunolabeling can serve two purposes: i) to obtain additional information from the donor of the fingermark, and ii) to (re)develop fingermarks. For the detection of specific components it is important that the technique is reproducible and that at least more than one component can be detected at once.

In chapter 3 a reproducible immunolabeling method is described that allows the detection of multiple components simultaneously in fingermark residues. To demonstrate the multiple detection, two general components were selected as antigens of interest, dermcidin and human serum albumin, both secreted via the pores present in the skin.
Conjugation of both antibodies to two different synthetic fluorophores, followed by simultaneous incubation of both conjugated antibodies, resulted in successful multiple immunolabeling of fingermarks left on a porous surface, nitrocellulose membrane, and on a non-porous surface, glass slides. Careful blocking and washing steps were found to be crucial to minimize the amount of false positives results.

Another important condition to make immunolabeling applicable to the forensic field, is the compatibility of the technique with fingerprint development techniques. Most of the fingermarks found at the crime scene are invisible and need to be developed before they can be detected. Therefore, in chapter 4 and 5, the compatibility of immunolabeling with different development techniques was tested. Immunolabeling was successfully performed on fingermarks that have been developed with ninhydrin staining, powder dusting, indanedione-zinc treatment, physical development and different types of cyanoacrylate fuming methods. The compatibility of immunolabeling with standard development techniques makes it possible, that fingermarks first can be visualized, and recorded for identification purposes and subsequently can be used for donor profiling using immunolabeling.

Fingermarks can be found on a large variety of surfaces. In chapter 3 to 5 immunolabeling on fingermarks was tested on the porous surface, nitrocellulose membrane and the non-porous surface, glass. However, in casework all conceivable surfaces are included, including non-porous, porous, colored and structured surfaces. In chapter 6, immunolabeling was tested on fingermarks that have been left on forensic relevant surfaces. Aluminium foil, stainless steel, plastic sheets, different colored garbage bags, sandwich bags and Ziploc bags, white tiles, laminated chipboards, thermal and copy paper were included to investigate the applicability of immunolabeling. Successful detection of dermcidin was possible in fingermarks that have been left on all tested surfaces, except for laminated chipboards and copy paper. Besides specific detection that can be used for donor profiling, also high quality images could be obtained from the fingermarks that have been treated with immunolabeling. The detection of three general fingermark components simultaneously, dermcidin, albumin and keratins, can be used for a good development of fingermarks left on thermal paper.

Besides the use of immunolabeling, we also investigated the use of fluorescence spectroscopy for the analysis of components present in fingermarks. One important feature of the chemical components present in fingermarks is their ability to display an autofluorescent signal when excited with the proper wavelength. In the second part of chapter 2, detection of the autofluorescent signal is described using fluorescence spectroscopy. In chapter 7 to 10 fluorescence spectroscopy was used to obtain more information from the chemical components present in the fingermark, but also to use this intrinsic fluoresce for the aging of fingermarks.
Since it is hardly known which components are responsible for the autofluorescence of fingermarks, a first attempt was made to discover which components contribute to the autofluorescence of fresh fingermarks. In chapter 7, thin layer chromatography, which is a simple method to separate different components in mixtures, was used in combination with fluorescence spectroscopy to identify autofluorescent components in fresh fingermarks. From the results, protein-bound tryptophan was indicated as a major contributor to the fluorescence of fingermarks. Next to protein-bound tryptophan, a kynurenine derivative was responsible for the autofluorescence. A metabolite of chlorophyll, which is a plant pigment, pheophorbide A was inferred as a red fluorescent fingermark component. The presence of plant pigments in fingermark residues may imply that dietary information may be retrieved from fingermark residues using the autofluorescent signal.

In chapter 8, a first step is taken to investigate the relation between the autofluorescent signal and the amount of DNA present in fingermarks. The amount of DNA in fingermarks is typically below or close to detection limits, which makes it hard to obtain full DNA-profiles from fingermarks. Therefore, DNA analysis is seldom performed on fingermarks. In forensic case work, it might be helpful if a non-destructive method is able to estimate the amount of DNA before analysis. In this chapter we hypothesize that the amount of DNA can be estimated by the intensity of the autofluorescent signal of the fingermark. The intensity of the autofluorescent signal was scored subjectively by multiple observers off-line based on digital images. The performance of Spearman's rank correlation analysis resulted in the outcome that two of the three tested series showed a moderate, but significant correlation between the DNA content and the autofluorescence of the fingermarks. However, this correlation is too weak to guide the forensic investigator reliably to fingermarks with a substantial DNA content. A more extensive study should be performed to make a more defined statement on the relation between the DNA amount and the intensity of the autofluorescence of fingermarks.

Accurate estimation of the time at which a trace has been left at a crime scene is one of the major challenges in forensic science. The age estimation of fingermarks is not yet possible. In chapter 9 and 10 an important step is taken to the age estimation of fingermarks by introducing a new method based on fluorescence spectroscopy. We observed that the autofluorescent signal of aged fingermarks was different than that of fresh fingermarks. To identify the components that contribute to this change in fluorescent signal, TLC in combination with fluorescence spectroscopy was used, described in chapter 9. In aged fingermarks, a consistent pattern was found in the formed aging products, in which tryptophan and its derivatives were indicated as the major players of the fluorescent signal. This consistent pattern indicates that the aging of fingermarks is not dominated by the inter- and intra-donor variability found in the initial composition of the fingermarks. We found that indoleacetic acid, (nor)harman and xanthurenic acid are the main contributors to the autofluorescence of aged fingermarks. This knowledge
may help in developing new fingermark development techniques for older fingermarks or in developing an aging method by specific targeting aging components.

In **chapter 10** a new method is introduced, that is able to estimate the age since deposition of fingermarks using its autofluorescent properties. Fingermarks were approached as mixtures containing proteins and lipids and based on the expected protein lipid oxidations reactions an age estimation method was designed. The method is a non-contact method using fluorescence spectroscopy. For the age estimation of fingermarks two measures of oxidation should be present, firstly, a relative amount of fluorescent oxidation products and secondly, the rate at which these products are formed. The age of 55 % of the fingermarks of the male donors could be estimated up to three weeks old with an uncertainty of 1.9 days. In the forensic field, the age estimation of fingermarks could be useful to discriminate relevant fingermarks from irrelevant fingermarks.

In **chapter 11** provides an overview of the possibilities and limitations of donor profiling from fingermarks and lists the different techniques that can be used to obtain this information. In **chapter 12**, all topics described in this thesis are discussed, describing the limitations, possibilities and future applications. The methods presented in this thesis are not limited to fingermarks only, but can also be applied on other traces, such as semen, vaginal fluid or even tears.

In conclusion, this thesis shows that the chemical composition of fingermarks holds a wealth of information, which can be used for donor profiling, developing new fingermark development techniques and the age estimation of fingermarks. The new insights presented in this thesis will lead to new opportunities, which makes it highly valuable for the forensic field.