CHAPTER 8

THE RELATIONSHIP BETWEEN DNA CONTENT AND AUTOFLUORESCENCE OF FINGERMARKS: A PRELIMINARY STUDY

Unpublished data
ABSTRACT
Obtaining full DNA profiles from latent fingermarks is a difficult task. DNA levels in fingermarks are typically below or close to detection limits and highly variable between fingermarks. Currently, methods which can estimate in situ the DNA content non-destructively are lacking. In order to determine whether the intensity of autofluorescence light can be used to indicate the DNA content in fingermarks, in this pilot study, the relationship between DNA content and autofluorescence of fresh fingermarks is investigated. Thereto, fingermarks were left half on white tiles and half on plastic sheets. The partial marks on white tiles were used to evaluate the intensity of autofluorescence of the fingermarks using a UV crime-lite® torch. The part of the fingermark left on plastic sheets was used for human-specific DNA quantification. Autofluorescence images were digitally recorded and their intensities were off-line determined subjectively by multiple observers. For statistical analysis a Spearman’s rank correlation analysis was performed. In two of the three series a moderate, but significant correlation could be found between the DNA content and the autofluorescence of the fingermarks. However, this correlation is too weak to guide the forensic investigator reliably to the fingermarks with a substantial DNA content.
1. INTRODUCTION

The recovery of complete DNA profiles from fingermarks is challenging. Fingermarks are composed of a variety of endo- and exogenous materials, all present in very low quantities [1]. Despite the improved sensitivity of DNA recovery and analysis techniques from technical improvements in recent years, in most cases the human DNA amounts in fingermarks are below those needed to obtain complete DNA profiles [2]. Reported fingermark DNA contents lie between no detectable DNA to hundreds of picograms and differ between and within individuals [2-6]. Also, DNA recovery from fingermarks is destructive to the fingermark itself and thus fingermark visualization and imaging should occur prior to DNA analysis [7]. Thus, it would be opportune to have methodology to select fingermarks suited for genotyping. However, non-destructive methods to estimate the DNA content of a fingermark prior to DNA extraction are lacking.

A non-destructive method to visualize fingermarks is to excite them with UV and visible light and subsequently detect the intrinsic fluorescent components within a fingermark, also known as autofluorescence [8-12]. The exact origin of this intrinsic fluorescence is largely unknown [12-13]. Several studies have focused on the determination of the biological fluorescence components in fingermarks, but no distinctive outcome was found. Aromatic amino acids, like tryptophan and tyrosine are indicated to play a role in the autofluorescence of fingermarks [12, 14]. Also, skin epithelial particles have autofluorescence properties, when excited with 350 nm, 390 nm and 475 nm [15].

In this study we explored the relation between the intensity of the autofluorescence and the DNA content in fingermarks, which has not been investigated before. The rationale behind this relation is that a fingermark containing higher amounts of biomaterial will display a stronger autofluorescence and is therefore more likely to contain DNA. This knowledge may help in selecting fingermarks for DNA recovery and analysis. Therefore, fresh fingermarks were investigated that were placed half on white tiles and half on plastic sheets, which both are non-porous materials. The halve fingermarks left on tiles were illuminated with a UV Crime-lite® torch and used to determine the intensity of autofluorescence. During criminal investigations fingermarks will be qualified based on the personal view of the forensic investigator. Therefore, we chose to assess the intensity of autofluorescence by scoring the relative evaluations of multiple observers. The halve fingermarks left on plastic sheets were used for human DNA quantification. The plastic sheets facilitate DNA analysis without the necessity of an extra transfer step (for example swabbing). The relation between both datasets was assessed.

2. MATERIAL AND METHODS

2.1 Fingermark collection.

Fingermarks were collected during working hours. Twenty donors were asked to deposit a fingermark. Fingermarks were placed partly on a white tile (Praxis, the Netherlands) and partly on a plastic sheet (high speed copier transparencies, Esselte, the
Netherlands). Fingermark depositions were carried out by moderate pressing during a short period (max. two seconds). No further instructions were given to the donor, which means that fingermark depositions had natural variation. The depositions were performed in triplicate, resulting in three different series of 20 fingermarks. Fingermark depositions left on plastic sheets were secured separately in 6-wells plates (Corning corporate Costar, USA) and sealed with Parafilm\textsuperscript{®} (Parafilm\textsuperscript{®}, USA). Samples were stored in a dark box and transported to the Netherlands Forensic Institute (NFI) for DNA isolation and quantification.

2.2 Autofluorescence of fingermarks.
Fluorescent images of fingermarks on tiles were obtained using a UV Crime-lite\textsuperscript{®} 2 torch (365 nm, 10% bandwidth 350-380 nm, Foster and Freeman, UK) to visualize fingermarks. Digital images were taken through a linear polarizer filter supplied with the Crime-lite\textsuperscript{®} 2 torches using a Nikon D40X digital camera (Nikon, Japan) (distance camera to tile 10.5 cm, distance light source to tile 22 cm). A linear polarizer filter was used to increase the contrast and to filter out light reflections. All images within each series were collected under identical circumstances and camera settings (focal length: 32 mm, f-number: F/6.3, exposure time: 5 seconds, ISO400).

2.3 Subjective assessment.
Fluorescent images obtained from the three different series of 20 fingermarks left on white tiles (total 60 fingermarks, triplicate deposition) were presented to ten volunteers. Each volunteer was asked to sort the images based on intensity of fluorescence. Fingermarks were given a number between one and twenty, with one corresponding to the image with the lowest and 20 to the image with the highest autofluorescence intensity. A score was formed by averaging the outcomes of the ten volunteers. Scores were rescaled, whereby fingermarks with the lowest intensity obtained a score of zero and fingermarks with the highest intensity obtained a score of 100.

2.4 DNA extraction, isolation and Alu quantification.
White light digital images of fingermarks on sheets were obtained with a Nikon D40X camera to visualize the area of the fingermark. The surface of the whole fingermark, the area encompassed by the fingermarks, was calculated by determining the number of pixels covering the fingermark and recalculating the original size in cm\textsuperscript{2} using Image J. The area encompassed by the fingermark was used to calculate DNA yield per cm\textsuperscript{2}.

Prior to DNA extraction, the plastic sheets with fingermarks were cut into smaller fragments to fit the 1.5 ml tube used for extraction. DNA was extracted using the QIAamp DNA mini Kit (QIAGEN Benelux B.V., The Netherlands). Cells were lysed in 360 µl ATL buffer, 40 µl Proteinase K (20 mg/ml, QIAamp DNA mini Kit, Qiagen) and incubated for 2 h at 56°C. To separate sheet fragments and lysate, both were transferred to a QIAshredder column (QIAGEN Benelux B.V., The Netherlands) and centrifuged at 11,000 rpm.
Next, 400 µl AL buffer with 0.5 µg/µl carrier RNA were added and incubated for 10 min at 70°C. Subsequently, 420 µl absolute ethanol (Merck KGaA, Germany) was added and the total volume was transferred to a spin column, which was centrifuged for 1 min at 8,000 rpm. The DNA column was washed once with 500 µl AW1 buffer, and washed twice with AW2 buffer and centrifuged for 3 min at 13,200 rpm. To dry the column, a final spin for 1 min at 13,200 rpm was performed. Finally, the DNA was eluted twice in 50 µl 25% AE solution which was pre-heated to 70°C.

Human-specific DNA concentrations were obtained for all samples using a protocol based on Nicklas and Buel [16] with minor adjustments for the primer concentrations as 200 nM Alu forward primer and DYZ5 reverse primer were used. The standard curve was prepared by diluting the Human Genomic DNA Male stock (Promega Benelux BV, The Netherlands) to a range of 64 ng/µl to 4 pg/µl in TE buffer (10 mM Tris (Merck Millipore, Germany), 0,1 mM EDTA (Gibco by Life Technologies, The Netherlands). RT-PCR was performed in a total volume of 20 µl. The reaction contained 2 µl DNA, 10 µl 2x Absolute qPCR mix ROX (Thermo Scientific, UK), 2 µl non-acetylated BSA (1,6 µg/µl, Sigma Aldrich, UK), 2 µl Alu primerset (4 µM each, Applied Biosystems (AB), UK) and 1 µl 5'-labeled VIC® Alu probe (4 µM, AB), 2 µl DYZ5 primerset (4 µM each, AB) and 1 µl 5'-labeled 6-FAMTM DYZ5 probe (4 µM, AB). All samples were transferred to an optical 96-Well reaction plate and covered with an optical adhesive film (AB). After a short spin the plate was loaded into a 7900HT Fast RT-PCR System (AB) and underwent an initial incubation step of 15 min at 95 ℃, followed by 40 cycles of denaturing for 30 sec at 95 ℃ and combined annealing/extension for 60 sec at 60 ℃. DNA quantities were calculated using SDS software version 2.4 (AB), with the manual ct threshold set at 0.1 and the manual baseline set at automatic.

2.5 Statistical analysis.
Statistical analysis was performed using Graphpad Prism 5. Correlations between the DNA content and the intensity of the autofluorescence were estimated by Spearman’s rank correlation coefficient.

2.6 Protein autofluorescence
Different amounts of bovine serum albumin (BSA), 1mg/ml, 10 mg/ml and 100 mg/ml were dissolved in milliQ. Drops of two µl of the solution were pipetted on white tiles. Drops were dried and digital images were taken through a linear polarizer filter supplied with the UV Crime-lite® 2 torches using a Nikon D40X digital camera (Nikon, Japan).

3. RESULTS
All fresh fingermarks left on white tiles were visualized using the UV Crime-lite® 2 torch. The autofluorescence intensity of different fingermarks was highly variable as shown in figure 1 for seven fingermarks left by seven different individuals, ranging from almost
invisible to strong autofluorescence. The distribution of autofluorescence within a fingerprint appears to be more homogenous.

Fingermarks were ordered on their autofluorescence intensities by ten volunteers. Next the rank of each fingerprint was averaged and rescaled in zero (low fluorescence) to 100 (high fluorescence). In figure 1, seven arbitrarily selected fingermarks were arranged according to the average intensity score obtained from the volunteers. The other half of the fingermarks was used for human DNA quantification. DNA yield was recalculated to pg/cm². The numbers in figure 1 correspond to the sequence in which the fingermarks can be ranged based on their DNA content, for which 1 represents a low and 7 represents a high DNA amount. Human DNA content for all fingermarks ranged from non-detectable DNA to 6.6 pg/cm².

Figure 1. Autofluorescence of fingermarks. Fingermarks are arranged by fluorescence intensity determined subjectively. Numbers correspond to the ranking based on the DNA content from 1 (lowest) to 7 (highest).

In figure 2 the relationship between the DNA content and the autofluorescence of fingermarks is displayed. No normal distribution of the DNA content is found in the data-set, as shown in figure 2 and a positive skew is observed in all three series. A Spearman’s rho test was performed on the dataset, which is able to capture nonlinear relationships and can be used as nonparametric test. The outcome of the test resulted in different outcomes for the three different series. In series A (fig. 2), no significant correlation between DNA content and autofluorescence could be found (Spearman’s correlation coefficient = 0.3971, p = 0.0829). In series B (fig. 2) and C (fig. 2) a weak to moderate significant correlation could be observed. (Series B: Spearman’s correlation coefficient = 0.5327, p = 0.0156) (Series C: Spearman’s correlation coefficient = 0.4992, p = 0.0250).

4. DISCUSSION
The goal of this study was to get more insight in the relationship between the DNA content and the autofluorescence intensity of fingermarks. A subjective assessment to determine the intensity of fingerprint autofluorescence was chosen as during criminal investigations fingermarks will be qualified based on the personal view of the forensic investigator.

We hypothesize that more deposited biomaterial as indicated by the autofluorescence using the UV crime-lite® torch will result in a higher DNA content. A relation between the amount of amine-reactive groups and the presence of DNA in swabs taken from
Figure 2. Frequency histograms of series A, B and C, respectively show the total number of fingermarks in a particular range of amount of human DNA yield (pg/cm²). A positive skew is evident in the histograms of all three series. Graphs show the relation between measured human DNA content and autofluorescence of fingermark, which was determined subjectively by ten different volunteers. Subjectively ranked fluorescence intensity is rescaled to a 0 to 100 range and ordered from lower to higher fluorescence intensity as an average score (a.s.) indicated on the horizontal-axis.

handled items was found by Anslinger et al. [17]. Ninhydrin treatment was used as a screening method for the suitability of swabs for DNA analysis. Their results suggest that the presence of amino acids and proteins in biological samples can be related to the presence of DNA [17]. In 76% of the ninhydrin positive samples a DNA profile could be obtained, whereas only 9% of the ninhydrin negative samples yielded a DNA profile.
In this study, a weak to moderate significant correlation between the DNA content and the autofluorescence intensity of fingermarks was found in two out of the three series studies. Thus, the presented data does not strongly support our hypothesis that higher fluorescent fingermarks contain more DNA. The studied fingermarks were natural depositions. Various explanations are plausible for the absence of a good correlation between the autofluorescence and the DNA content in fingermarks. Firstly, biomaterial can comprise human eccrine sweat, skin cells, sebaceous material, saliva and microorganism that associate human skin [1, 18]. Secondly, exogenous material, like dust and fluorescent particles from touched objects, can contribute to the fluorescence of the fingermarks. A third explanation could be found in the presence of microorganisms which are also a source of protein fluorescence, but not a contributor to human DNA [19].

Indeed, variation in the color of the autofluorescence was observed, varying from purple to blue fluorescence (example of two different fingermarks: Red-Green-Blue (RGB)-values: 58, 43, 74 to RGB-values: 64, 84, 88). These differences in color can be caused by a difference in composition of the fingermark, but the chemical identity of the fluorophores is not known. In and around the fingermarks bright fluorescence spots can be noticed, which likely originate from dust particles. Contamination of dust is hard to avoid in rooms with a normal airflow. Special equipment is necessary to overcome this problem, but in this study this is not relevant since a simulation of a relevant forensic situation was aspired.

The aromatic amino acids, tryptophan, phenylalanine and tyrosine are responsible for protein autofluorescence [12, 14, 20] Tryptophan is the main contributor to protein autofluorescence [20-22]. Also, DNA itself has intrinsic fluorescent properties when excited with 260 nm, but the quantum yield of this fluorescence is low [23]. The optimal excitation wavelength for phenylalanine, tryptophan and tyrosine are 258 nm and 276 nm, respectively, which is different from the excitation wavelength used in this study (365 nm, 10% bandwidth 350-380 nm,) [14, 21]. Although the wavelengths emitted by the UV Crime-lite® torch are not optimal for the detection of proteins, they still can be used to visualize proteins (figure 3). In this figure, the autofluorescence of a protein, bovine serum albumin (BSA) (dissolved in MilliQ) was visualized using the UV Crime-lite® torch.

Instead of using the UV Crime-lite® torch a more optimal excitation wavelength can be chosen, that specifically excites tryptophan (around 276 nm). Though, using a shorter wavelength is more likely to be destructive to the DNA and is more hazardous than longer UV wavelengths and thus less likely to be used at crime scenes [24]. According to the outcome of our study we suggest that when confronted with the selection between two fingermarks, the one that yields the strongest fluorescence may be used for DNA analysis. However, this study is a preliminary study to indirectly estimate the DNA content and more research is necessary to give a more defined recommendation.
In a follow-up study, we recommend to work with whole fingermarks instead of halve fingermarks. The intensity of the autofluorescence of fingermarks is not equally distributed in the whole fingermark. Also, we expect that the skin cells and other DNA containing cells are heterogeneously spread in a fingermark. Since, we worked with halve fingermark, differences could be found in the two separated halves, leading to an error in our findings. For practical reasons we worked in this study with half fingermarks. Fingermarks were easily to detect on white tiles and fingermarks left on plastic sheets were most favorable for DNA extraction.

Another recommendation we want to make is to use a clear filter in front of the camera instead of the linear polarizer we used in this study. The linear polarizer gave the best contrast between the surface and fingermarks, but this filter is not able to block all the reflected UV, which makes it hard to define whether the observed fluorescence was true fluorescence or also background reflection. The clear filter is able to block all the UV-light, including the UV reflections. In addition, also other Crime-lites than the UV Crime-lite can be investigated. In this study we worked with the UV Crime-lite® torch, since it gave the best contrast between the fingermark and the white tile. The surface at which fingermarks are left on is of influence of a good detection of fingermarks. And therefore, we suggest to test for different surfaces the most applicable wavelength to excite the fingermarks and to reduce background fluorescence.

In this study, we worked with fresh fingermarks left on clean surfaces. In a future experiment aged fingermarks on a range of different surfaces with different levels of cleanliness can be included to help in determining the level of association between fluorescence and DNA retrievability. A subjective assessment of the fluorescence images was applied in this study. In a future study, an objective assessment can be included, using fixed settings, such as camera settings, including background measurements and power of the light source.

**5. CONCLUSION**

In this study, the relation between the autofluorescence and DNA content of a fingermark was investigated. Both the autofluorescence and the human DNA yield were found
to be highly variable for different fingermarks. The findings infer that a weak to moderate significant correlation occurs between the DNA content and autofluorescence intensity of fingermarks, but this correlation is too weak to guide the forensic investigator reliably to fingermarks with a substantial DNA content.

REFERENCES


