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Fingermark Detection on Satin Fabrics

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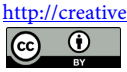

Abstract

Diamond™ Nucleic Acid Dye is a fluorescent reagent that can be used to detect the presence of DNA-containing cells on surfaces. The purpose of this research was to observe Diamond™ Nucleic Acid Dye, on different colors of satin polyester fabrics, using two fingermark types, sweaty and oily. Each sample was photographed and analyzed for the number of DNA-containing cells. Most colors of satin polyester fabric with the use of Diamond™ Nucleic Acid Dye, had visible cells with visualization being better for some color satin fabrics than others. There was no statistically significant difference in DNA-containing cells between the oily and sweaty fingermarks. Substrate color affected the ability to visualize the nucleic acid molecules; contrast and resolution were improved by camera filters, focused UV light, magnification and storage at −20˚C.

Keywords

Diamond™ Nucleic Acid Dye, DNA, Fingermarks, Satin Fabric

1. Introduction

Touch DNA or touch deposits are important biological evidence for many types of crimes including criminal possession of weapons, robbery, and property crime as well as significant violent crimes against people. Although there is scientific research on touch DNA, there is still a paucity of literature regarding touch DNA deposition, adherence, and DNA recovery from fabrics [1]-[7]. This article examines the visualization process of DNA deposits on those surfaces by using a fluorescent dye called Diamond™ Nucleic Acid Dye that binds to the DNA and RNA molecules in the fingermark. Diamond™ Nucleic Acid Dye has an excitation maximum of 495 nm and a yellow-green emission maximum of 558 nm.

Touch DNA was first mentioned in 1997 by van Oorschot who noted the pos-

sibility of touch as a means of deposit and transfer of DNA to surfaces [8]. Originally, transfer of DNA and cellular material was thought to be a rare occurrence but as technology and sensitivity of detection of DNA improved, the phenomenon of DNA transfer is now considered a common place example of Locard's Exchange Principle. DNA transfer can occur between objects and surfaces and people and therefore DNA deposits from fingermarks are often a mixture of the donor DNA and transfer DNA that has accumulated on the fingertips over time. Imaging DNA and cellular material for evidence collection is not common on fabrics, and most DNA is collected intuitively based on what scientists infer about how an object is used, worn or handled. This article examines a fluorescent nucleic acid binding dye, Diamond™ Nucleic Acid Dye, that can be useful as an enhancement reagent to visualize locations of DNA on fabric evidence, to maximize the recovery of the biological sample for DNA analysis and sourcing.

First used as a DNA detection reagent for visualizing DNA in electrophoretic gels [9] and quantitative PCR [10], the use of Diamond™ Nucleic Acid Dye was further explored as a dye to visualize DNA from hair roots [11] and follicles [12]. Additional studies used this dye detection system for latent DNA from fingermarks [4]-[6] [13]-[16]. Diamond™ Nucleic Acid Dye is not compatible with red and black magnetic powders for latent fingermark enhancement; however, no effect was observed for the STR profiling results [17]. White and aluminum powders do not affect the visualization of Diamond™ Nucleic Acid Dye when co-applied to latent fingermarks and do not affect the downstream DNA processing [17]. Cyanoacrylate fuming and Diamond™ Nucleic Acid Dye did not exhibit positive results after co-application for STR profiling [17]. To optimize the application of Diamond™ Nucleic Acid Dye to test surfaces, a spray device with fine mist was determined to improve latent fingermark enhancement compared with a micropipette application [13]. Quenching of the fluorescence of the dye was also noted for some dark or black surfaces [13]. To date, Diamond™ Nucleic Acid Dye has been used on tape, wood, glass, plastic, and metal surfaces effectively [18]-[27].

Touch DNA is comprised of oils, proteins and shed epithelial cells [28]. DNA recovery from touch deposits is expected to be a complex mixture of degraded and fragmented DNA from shed corneocytes in the process of apoptosis as well as intact DNA from keratinocytes. Touch DNA refers to DNA deposits that remain behind on a surface after finger contact. Trace amounts of DNA that have been deposited by touch are likely a combination of DNA found in corneocytes, cell-free DNA, and other epithelial cells that are exuded with perspiration. Humans shed hundreds of thousands or more of these cells every day making this valued evidence in forensic science. DNA deposited by touch can be latent, meaning that one is unable to see it with the naked or unaided eye. In forensic science laboratories, analysts will swab intuitively hoping to collect DNA from the evidence item in an area where they think it might have been touched or held; this is known as blind swabbing. DNA collected using the blind swabbing method is not always 100% recovered as the DNA is invisible and cannot be observed during collection.

The development of DNA binding dyes and their application to latent fingermarks makes visualizing DNA much easier to collect for further testing [9] [10] [13]. Diamond™ Nucleic Acid Dye is a sensitive nucleic acid binding dye, binding only to the external groove of both single and double stranded DNA as well as RNA. Due to this biological specificity, analysts do not have to worry about dye binding to proteins, lipids or carbohydrates and can collect it for use in downstream processes such as PCR amplification or short tandem repeat (STR) analysis.

Diamond™ Nucleic Acid Dye has been studied on non-porous materials, such as glass, but has fewer studies for porous surfaces [21]. Some examples of porous surfaces include paper and fabrics. Different fabrics have different levels of porosity. Satin is very smooth and often described as having a silky texture. Since this fabric is so smooth, it tends to have a lower porosity compared to other fabrics. Since Diamond™ Nucleic Acid Dye was proven successful on non-porous surfaces, satin was chosen for its low porosity and low absorbance to test. Not only do porosity and absorption affect the detection obtained from the use of Diamond™ Nucleic Acid Dye, but color can affect it as well [3] [22] [23]. Different colors may cause the need for different fluorescence and contrasting filters to be used with photography to identify the detection of latent DNA. Satin fabric is characterized by the weave which consists of several floating warp threads over multiple weft threads leading to a glossy smooth surface with a dull back surface (**Figure 1**). It is not of any particular composition but could be made of silk, polyester, rayon or nylon. In this study, the satin was made of polyester threads. Due to the color of Diamond™ Nucleic Acid Dye, it may be easier to see fluorescent cells that have absorbed the Diamond™ Nucleic Acid Dye on darker colors as compared to lighter colors.

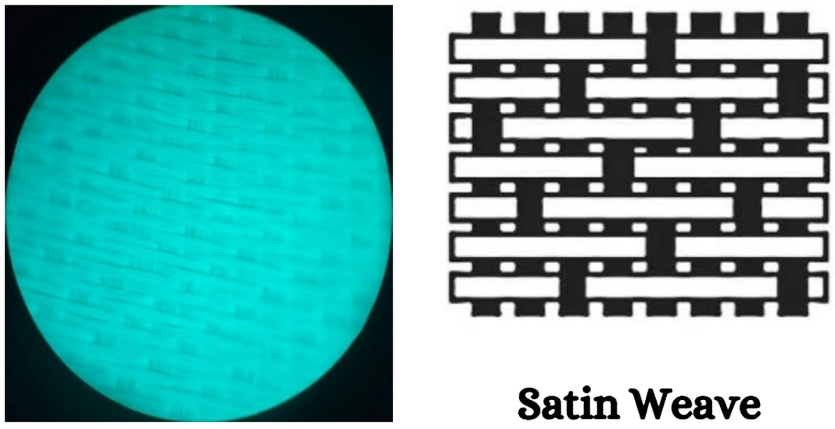

Figure 1. Satin refers to the weave characteristics of this polyester fabric sample shown in green at 400× magnification. Satin fabric is characterized by the weave which consists of several floating warp threads over multiple weft threads leading to a glossy smooth surface with a dull back surface.

2. Materials and Methods

This study's satin fabrics, constructed of 100% polyester of a variety of colors, in-

cluded the colors white, black, silver, gold, red, navy blue, pink, green, purple, brown, and orange. Polyester is the most widely produced synthetic fiber in the world. It was discovered by DuPont chemist Wallace Carothers in the 1930's by mixing alcohol with carboxyl acids. In 1941, two British chemists John Rex Whinfield and James Tennant Dickson developed polyethylene terephthalate (PET) which is a foundational material for modern polyester (terylene). DuPont purchased the rights and in 1951 began manufacturing a similar fiber called Dacron. In the 1980's polyester blends became popular and now they are used in the manufacturing of athletic wear, upholstery, clothing and medical textiles.

The color of satin fabric was chosen and laid on clean paper. Three small 5 × 5 mm square cuttings of the fabric were cut with sterile scissors and placed on the paper. The three cuttings were UV irradiated to sterilize with a Stratalinker (Agilent Technologies, Inc., Santa Clara, CA). These samples were then taken to a fume hood. Prior to placing the DNA on the samples, a dilution of Diamond™ Nucleic Acid Dye (Promega Corp, Madison, WI) was made using 500 µL of dye and 10 mL of molecular biology grade sterile water (Fisher Scientific, Waltham, MA). This solution was then transferred to a clean, spray bottle. Each cutting was placed on a clean Kimwipe (Fisher Scientific, Waltham, MA). One cutting was left to the side and untouched to be the negative sample. The other two cuttings had fingermarks from the right thumb placed on them by the researcher. The fingermarks were deposited as single samples using a single donor for each color fabric to reduce genetic variability. Hands were washed and air dried for 15 min. Each fingermark was pressed for 10s flush with the surface using moderate pressure and then lifted off to transfer the fingermark to the fabric. One sample was a sweaty fingermark and the other was an oily print, meaning that the finger was run across the oily parts of the face prior to being placed on the fabric. The fingermark deposits were made at 20 min. intervals between hand cleanings. All three samples were sprayed with Diamond™ Nucleic Acid Dye before being analyzed for each color of fabric. Once each sample was sprayed with the Diamond™ Nucleic Acid Dye, all samples were analyzed under a Leica stereomicroscope at 400× magnification with the use of a UV light and yellow barrier goggles. Photographs of each sample were taken with a Samsung Galaxy S22 camera through the microscope eyepiece. For data analysis, all photographs were overlayed with a 3 × 3 grid. Each grid (9 total) was analyzed and the number of cells counted and recorded, up to 50 dyed cells per grid. These censored counts were totaled and used for the calculation of cell averages for each sample type (oily vs. sweaty prints) and analyzed for statistical significance with a two tailed Student's t-test to determine if there was a significant difference between the mean values. Cell counts were visualized under the microscope by the researcher to verify fluorescence was from the cell nucleus and had characteristic dye color and nuclear morphology to eliminate any stray fluorescence from fibers in direct comparison to photographs. Stray fluorescence was recorded in the negative controls but reflects fiber fluorescence with no cell morphology.

3. Results

On every sample color, except for green, DNA bonded with Diamond™ Nucleic Acid Dye and fluorescent cells were visualized with UV light and yellow barrier goggles. The black satin fabric shown in Figure 2 reflects light but the dye containing cells fluoresces as a pale green. Under high magnification (400×), cells are easily visualized for the counting process. Although there is some inherent variability in the fingermark deposition process, the color contrast may also be a significant factor in the ability of the cell fluorescence to be effectively visualized.

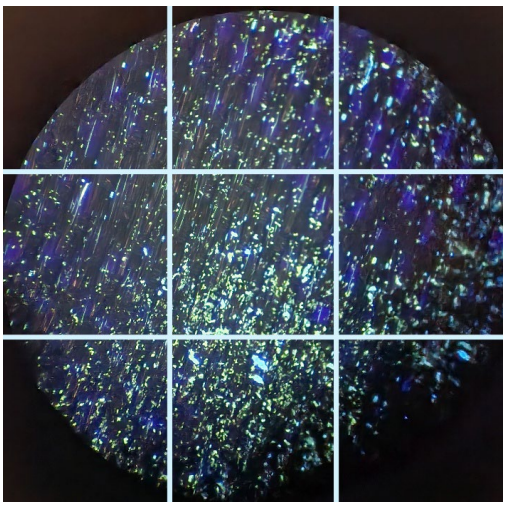

Figure 2. Photo with grid overlay of black satin sweaty print. From left to right, starting at the top. the number of cells present in each grid was counted and used to find the estimated number of cells on each sample.

Diamond™ Nucleic Acid Dye was used to enhance two different types of fingermarks, sweaty and oily, placed on a variety of colored satin fabrics. Satin fabric, although porous, was observed to produce good visualization of Diamond™ Nucleic Acid Dye enhanced cells (Table 1). On most fabric colors, fluorescent cells were able to be easily observed with the use of a UV light. On lighter colors, it was more difficult to visualize and count the fluorescent cells. Only one fabric color, the green satin, showed no fluorescent cells. It is noted, however, that Diamond™ Nucleic Acid Dye fluoresces a yellow-green color, meaning that it would have been objectively more difficult to see on a green surface where color light emission would have masked the fluorescence. The authors note that on some surfaces, storage at −20˚C can reduce the background blue fluorescence of the dye and enhance cell imaging.

Using Table 1 data, the average number of cells for sweaty fingermarks versus oily fingermarks was calculated using an Excel spreadsheet, then a paired two-tailed Student t-test was performed to compare cell counts. Each 9-grid fingermark sample was scored up to 450 counts, meaning some samples may contain more than that total number of cells. A Student t-test showed no significant difference ($p = 0.05$) between the two types of fingermarks. For all colors of satin

fabric, dye associated cells were observed except for the green fabric which is the similar color as the dye emission. Very reflective fabrics such as silver also showed a low cell count. The estimated range of DNA recovery (6 pg/cell) from the cell counts is 0 - 2.7 ng from fingermarks on satin fabrics.

Table 1. Total number of dyed cells observed for each satin fabric sample.

Satin Sample Color	Negative Control[a]	Sweaty Fingermark	Oily Fingermark
Black	0	350	92
Navy Blue	64	193	164
Pink	1	95	8
Red	8	296	330
Purple	10	450	350
Green	0	0	0
White	2	74	84
Silver	1	2	12
Brown	5	339	370
Orange	10	405	251
Gold	6	262	37
Total	107	2466	1698

[a]Background fluorescence due to fibers.

4. Discussion

The use of Diamond™ Nucleic Acid Dye to enhance DNA containing cell imaging on fabric and other surfaces has been shown to be effective [21]-[23]. In this study, sweaty and oily fingermarks were imaged from a variety of satin fabric surfaces and compared for cell counts from thumbprint deposition. The relevant transfer factors for effective imaging were identified in the literature as the initial quantity of cells adhering during deposition which may be affected by fabric composition and weave pattern as well as donor considerations of oil, moisturizers, perspiration, pressure applied, contact time and donor genetics [24]-[29]. The successful imaging of a fingermark on a fabric surface was enhanced by magnification, storage at −20˚C, use of contrasting barrier filters, and the use of fabrics with high color contrast compared to the yellow-green, fluorescent Diamond™ Nucleic Acid Dye.

In this experiment, satin weave polyester fabrics were used to observe DNA containing cells from fingermarks with the use of Diamond™ Nucleic Acid Dye on these porous fabric surfaces. Along with this observation, different colors and two different fingermark types were analyzed to observe how well Diamond™ Nucleic Acid Dye enhanced the fingermarks. Different fabric colors were used to

determine if there were any limitations to the use of Diamond™ Nucleic Acid Dye enhancement. Only one sample color produced no visual results, that being green satin due to shared color emission with the dye. Lighter colors were noted to be more challenging to visualize cells on due to background fiber light reflection and fluorescence; however, some cells were still able to be visualized. Yellow and orange barrier goggles were useful when observing cells on lighter colors, primarily on white satin and pink satin to increase visual contrast. In terms of fingermark type, no statistical significance was found between the mean cell counts for all sweaty fingermark samples compared to oily fingermark samples. The average amount of DNA estimated from cell counts was in the range of 0 - 2.7 ng which often may be sufficient to generate a DNA profile (0.5ng required for STR analysis). Some limitations of this study include the use of a single donor, nonreplicated color samples per fingermark type, and censored cell count data. Future research studies would include a larger uncensored data set with more replicates and additional donors as an enhanced fingermark study for satin fabrics.

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Author Contributions

The co-authors contributed equally to the manuscript. M. Young performed the research and wrote portions of the text. H. Miller Coyle wrote portions of the text, reviewed the data, and edited the manuscript for publication.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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