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Trace DNA Detection Using Diamond Dye: A Recovery Technique to Yield More DNA

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TRACE DNA DETECTION USING DIAMOND DYE:

A RECOVERY TECHNIQUE TO YIELD MORE DNA

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TRACE DNA DETECTION USING DIAMOND DYE: A RECOVERY

TECHNIQUE TO YIELD MORE DNA

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ABSTRACT

This study aspires to find a new screening approach to trace DNA recovery techniques to yield a higher quantity of trace DNA from larger items of evidence. It takes the path of visualizing trace DNA on items of evidence with potential DNA so analysts can swab a more localized area rather than attempting to recover trace DNA through the general swabbing technique currently used for trace DNA recovery. The first and second parts consisted of observing trace DNA interaction with Diamond Dye on porous and non-porous surfaces.

The third part involved applying the Diamond Dye solution by spraying it onto brand new and laundered brassieres that had trace DNA placed by donors on the cup and clasp areas. The stained brassieres were then visually analyzed using a Canon T8i camera and EF-S 60 mm macro lens under 455nm alternate light and a 550nm emission filter to locate areas that fluoresced, meaning that trace DNA is present, and images were captured for record. The final part of the study consisted of swabbing laundered brassieres that donors deposited trace DNA onto using the blind double swabbing and Diamond Dye-localized double swabbing techniques on the cup and clasp areas.

The swabs were put through DNA extraction via the Qiagen's QIAamp Investigator kit and quantification via Thermo Fisher's Quantifiler Trio then analyzed for the quantity of DNA present. The data was separated into swab techniques and the data was compared using an independent t-test at 95% confidence. The one-tail analysis determined a p-value of 0.0883 with the goal being a p-value of less than 0.05. Statistically, the results show that there is not a significant difference in the amount of trace DNA retained based on whether the DNA is visualized before proceeding with double swabbing.

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INTRODUCTION

One of the most solidifying pieces of evidence when it comes to a crime, especially if it goes to trial, is deoxyribonucleic acid or DNA. The presence of someone's DNA within a scene of a crime scientifically shows that the individual, at some point in time, was present or associated at the scene where a crime occurred. This evidence aids tremendously in an investigation whether it be finding a witness who may have vital information to help the case or even finding the perpetrator responsible for the crime. While having blood, semen, and saliva stains as evidence are the most common ways to obtain DNA as they are in macroscopic measures, there are other sources where DNA can be found microscopically like trace and transfer DNA (Cook, Mitchell, & Henry, 2021) Trace DNA, or touch DNA as referred to in some literature, refers to microscopic amounts of DNA that are left behind by an individual when they come in contact with an object or person. The source of the DNA comes from skin cells that have been shed and perspiration from an individual's hands (Williamson, 2012). The quantity of DNA that is found within trace DNA is not nearly as much as one would find with common sources of DNA, but trace DNA has led to DNA profiles even if the contact with an object is brief (Kanokwongnuwut, Kirkbride, & Linacre, 2018).

One thing that must be considered when it comes to recovering DNA from a trace sample is the possibility that there is transfer DNA within a sample. Transfer DNA is like trace DNA in the way that it is skin cells that have been shed and perspiration, but different because it is DNA of a person that is not the one who made the contact being investigated. This phenomenon can occur due to many reasons from skin-to-skin contact like shaking hands to a person touching an object that another person touched and taking DNA off the object and onto their skin (Meakin & Jamieson, 2012). This approach to microscopic traces of DNA makes trace DNA more

complicated as one who transfers someone else's DNA may only leave behind the DNA of another or leave behind both the transfer DNA and their own, but there will be more of the transfer DNA present in any given sample (Sessa, et al., 2019).

A great benefit to trace DNA is the fact that it is everywhere. Any object that a person places their hands, face, or skin on leaves behind trace DNA. This is significant in the fact that trace DNA can be implemented into forensic investigation techniques in many fashions. There comes an issue, though, with trace DNA currently: how can we find it on such a macroscopic level? Trace DNA is not visible to the naked eye, so the technique would need to involve an aiding material or equipment to locate what is being searched for. Research in trace DNA is still developing to this day and one of the most looked upon methods for trace DNA detection involves a reagent called Diamond Nucleic Acid Dye, or DD. DD, when applied to a surface or object with trace DNA present, binds to the backbone of human DNA which allows for specific locating of cells via fluorescence. Fluorescence refers to the emission of visible or invisible radiation from certain substances as a result of incident radiation of a shorter wavelength (Wilson, 2017). The DD "external groove-binding molecule" is also significant to DNA detection as it is not compatible with bacterial DNA based on its coiled structure and has been seen to have negligible effect on DNA when pursued for DNA profiling (Kanokwongnuwut, Kirkbride, & Linacre, 2018).

Initial research with DD has been performed within the last few years to map out the identification of trace DNA on primarily non-porous materials. While this is of importance, there are two major concerns with the work that has been done thus far: the application of DD and the variety of surfaces tested upon. One of the first successful uses of DD in an experiment was when the DD was applied via pipetting onto the testing surface (Haines, Kanokwongnuwut,

Harbison, Cocerton, & Linacre, 2019). Pipetting is useful in the sense that an area that is being tested is small, but that is not realistic in the sense of most evidence found at a crime. A larger dispersal method of the dye would be necessary to advance this technique into a functioning forensics routine to be efficient with evidence recovered from a crime. Another issue at hand is the type of surfaces and materials that research has mainly been conducted with DD are non-porous surfaces (Meakin, Butcher, & van Oorschot, 2017). Non-porous surfaces are relevant in the sense that they are present at scenes and could give trace DNA for example on a soda can or a person's keys, but not all objects at a scene are non-porous. Some could have the ability to potentially absorb or collect the DNA within the object which one encountered. Research has attempted to bring absorbent materials into the field of DD detection, but higher success was seen in the non-porous objects that were used within the experiment. This aspect displayed that DD works on non-absorbent surfaces, but objects that can absorb material, like clothing and bedding, still have a long path ahead to determine what forensics can do to better find and visualize trace DNA (Champion, Kanokwongnuwut, van Oorschot, Taylor, & Linacre, 2021).

The importance of finding trace DNA within evidence creates the opportunity to recover more DNA from the scene of a crime or even find DNA of a person that wasn't present on a larger scale (van Oorschot, et al., 2003). Prior recovery techniques when it came to trace DNA was general swabbing of evidence for the DNA. While it could be effective, there is a much higher risk of missing the area that DNA is present in or not obtaining enough DNA because one is unable to locate an exact area with microscopic biological material. Having a way to efficiently display the location of trace DNA within common items of evidence, like clothing, can bring in more biological material from a scene. Testing out DD on mock crime scene evidence, like clothing from a sexual assault, could help to further understand and develop the

technique used to find trace DNA and could allow for better recovery of DNA to lead to a more complete DNA profile (Kanokwongnuwut, Martin, Taylor, Kirkbride, & Linacre, 2020).

LITERATURE REVIEW

While DNA itself is a very in-depth field, visualization technique(s) for trace DNA doesn't have as much knowledge behind it. Scientists are aware of these gaps in the forensics world and are working to produce useful experiments which will advance trace DNA in the right direction. Many aspects have been laid out in an overview to discuss topics like the fact that trace DNA can transfer, a general understanding of how touch DNA has been seen in demonstration, factors that could impact the recovery of DNA, and the ability to further use the DNA recovered to perform testing and profiling of the biological material (Meakin & Jamieson, 2012). Other overviews have also touched on the limitations of the quantity of DNA recovered, mixed samples, contamination, and difficulty with amplification. Having ideas of what to pursue and potentially how to pursue those ideas and concerns helps to build up the world's approach on touch DNA specifically in the application of forensic science (van Oorschot, Ballantyne, & Mitchell, 2010).

To better understand what could be found within such samples, testing of touch DNA began. The first research produced on the topic was published in 1997 and was the first to associate DNA with objects that have been touched with bare skin. Testing showed that accurate genetic profiles could be recovered from various objects including leather briefcase handles, a glass, the inside of a worn condom with no ejaculation, polypropylene tubes, and more (van Oorschot & Jones, 1997). The study showed the potential for a new technique in DNA identification but was reviewed and critiqued for limitations that were present like the amount of DNA being recovered versus how much is needed to create a profile and the use of only one STR locus. Further discussion mentioned that profiles could be and were obtained using single cells and an already-existing STR profiling system. Not every sample could provide a full profile, but

partial profiles that were found could be compared to known profiles for exclusion purposes. Even though the review brought forth issues with the first experimentation, it came to the same conclusion that DNA could be recovered from areas that could have skin cells like a smudged fingerprint or flakes of dandruff (Findlay, Taylor, Quirke, Frazier, & Urquhart, 1997).

Along with the transfer of DNA, it needed to be known whose DNA was being transferred. An initial study approached the ability of DNA transfer to occur based on the supposed shedding status of an individual that has contacted plastic tubes. Results came to show that quantifying or categorizing the shedding status in relation to DNA recovery quality (Phipps & Petricevic, 2006). A later study showed that samples with multiple profiles can come from a single contact and samples could display more DNA of an individual who only had contact with an object of clothing once. This was specifically seen when brassieres that were worn by female subjects for at least 12 hours showed higher DNA profiles of male subjects that came in contact with areas of the brassieres for varying amounts of time from two seconds to 60 seconds (Sessa, et al., 2019). The process of being able to roughly identify the transfer path was successful. Another experiment was conducted with a similar purpose for examining transfer DNA recovery when it came to regularly used knives. Handlers would be handled for certain lengths of time by donors then the donors would participate in various lengths of handshakes. The handshakes would allow for the transfer of the hand shaker's DNA onto the donor who would then handle the knives. DNA recovery would be performed on the handles to show the ability to recover DNA profiles from the primary donors and the secondary handshaking volunteers (Meakin, Butcher, & van Oorschot, 2017).

A variable that had a major impact on the success of DNA recovery, though, was that people knew where to swab. At a crime scene, prior knowledge of the location of biological

materials on evidence before entering for the first time is very limited. This issue was approached through the works of Diamond Dye which fluoresces when contacting any human DNA. Initial experimentation started the foundation with analyzing glass slides with fingermarks partially stained using 5μL of a 20x Diamond Dye water-diluted solution under a handheld digital Dino-Lite microscope at 50x magnification (Haines, Kanokwongnuwut, Harbison, Cocerton, & Linacre, 2019). Future projects took the process and applied it to scenarios like DNA transfer from a primary source to a secondary source. Even with the second transfer, Diamond Dye was able to illuminate where the DNA was located on the last substrate that encountered biological material (Champion, van Oorschot, & Linacre, 2019).

The involvement of Diamond Dye, while successful, is still short of the goals for the forensic world. Widening the substrate type made the studies tie into forensic science as touch DNA is found on almost anything that people encounter like credit cards, bullet cases, and other evidence. Having visual confirmation of touch DNA on everyday non-absorbent objects opened the doors for further testing, especially when it was determined that Diamond Dye does not illuminate when in contact with prevalent bacteria DNA (Kanokwongnuwut, Kirkbride, & Linacre, Detection of latent DNA, 2018). Unfortunately, not all evidence that could be found at the scene of a crime is going to be non-porous. This aspect was attempted to be understood through more research with different materials like paper, cotton fabric, and cigarettes. The non-porous material used displayed cells successfully, but many complications of background reflectance on the porous materials contributed to the inability to interpret most of the visible data (Champion, Kanokwongnuwut, van Oorschot, Taylor, & Linacre, 2021).

While there is a handful of literature here that has been presented, there is more to discover in the sense of touch DNA, specifically in the realm of porous or absorbent sources.

Clothes, blankets, and other fabrics tend to be vital pieces of evidence within a crime, so being able to find one more source of DNA could be the extra step needed to close a case.

MATERIALS AND METHODS

Before applying the approach of trace DNA to this concept, the ability of a Diamond Dye solution to work on non-porous materials, like a glass slide, and porous materials, like fabric, should be investigated. The first part of this research investigated the interaction of Diamond Dye with trace DNA cells on a clean glass slide. A fingerprint was placed on the middle of the slide and a 20x Diamond Dye solution diluted with sterile water that was applied using a continuous spray bottle from 15 cm away. Once the dye solution was applied, the slide was examined under multiple filters of a Mini Crimescope MCS-400 and an orange emission filter to see which of the alternate light wavelengths worked best in visualizing the cells as well as photographing them using a Canon T8i camera with an EF-S 60mm macro lens and a Tiffen Orange 21 filter attached. This camera and alternate light set-up would be used for all other parts of the research. All photos captured using this set up were taken in a room with no ambient light present as Diamond Dye cannot be exposed to ambient light for optimal use.

The second part of the research involved observing trace DNA interaction with the applied Diamond Dye solution on porous materials. Different materials like cotton, polyester, nylon, and polyester/spandex blend pieces were collected for testing. These selections reflect the most common porous materials that are used to make clothing like brassieres, shirts, and pants. The fabrics were UV crosslinked for 600 seconds (about 10 minutes) in order to eliminate outside sources of DNA that could cause misinterpretation of the effectiveness of the Diamond Dye solution. Once crosslinked, trace DNA was placed on known areas of the fabrics. 20x Diamond Dye solution diluted with sterile water was applied to the fabrics which were then viewed and photographed with the camera and alternate light set-up in order to cause the areas of DNA to fluoresce. This helped to determine what the stained cells would look like on various

porous fabrics as well as the dye's interaction with the different fabrics. Materials for analysis were prepared prior to beginning the experimental procedures as Diamond Dye needed to be thawed for dilution. The solution used was a diluted 20x Diamond Dye and water solution. Water was chosen as the dilutant because the materials being tested were porous and a prior study showed more success with a water-based solution of Diamond Dye compared to an ethanol-based solution (Champion, Kanokwongnuwut, van Oorschot, Taylor, & Linacre, 2021). Once the solution was made, it could be stored at room temperature without exposure to any ambient light for 3 days before it was no longer effective.

Parts 3 and 4 of the research involved mock evidence of brassieres. The top crime for DNA testing at the time of design was sexual assault, so the intimate clothing was supposed to replicate evidence potentially recovered from a scene. Part 3 of the experiment was conducted on twelve store-bought women's brassieres. The brassieres were then separated into two groups: control substrates and test objects. Two brassieres were set aside and used as substrate controls, while the remaining ten were used as test substrates. The brassieres used within the experiment as test objects were then separated into two subgroups: brand new and laundered. If a brassiere was placed in the brand-new subgroup, it was handled and tested as it is from the store without being washed, while the laundered subgroup was put through a normal cottons washer cycle with the "colors" setting for temperature (20º - 40ºC) and Gain laundry detergent as well as a 60-minute dry cycle before experimentation begins. All brassieres, brand new, laundered, or substrate controls, were UV crosslinked for 600 seconds each, in order to eliminate trace DNA that was not contributed by the research to avoid contamination. Five donors participated by first washing their hands for 20 seconds then allowing for 30 minutes after hand washing for cell regeneration. Then, donors placed trace DNA onto the cup areas and clasp areas on one

randomly assigned new brassiere and on one laundered brassiere. After DNA deposition, the brassieres were placed into paper evidence bags and sealed with evidence tape before being stored at 4ºC for 72 hours (about 3 days) to replicate a storage period before examination. After 72 hours, the brassieres were individually unpackaged and placed onto clean sheets of butcher paper and 20x Diamond Dye solution diluted with water was applied via spray 6 inches above the brassiere. All brassieres were then visualized, and the cup and clasp areas were photographed using the camera and alternate light set-up.

Part 4 of the research was conducted only using laundered brassieres to best replicate the condition of clothing that would be taken from a scene as most people wash clothes before wearing them. Eight laundered test brassieres and two substrate controls, one positive and one negative, were all UV crosslinked for 600 seconds before being used for research to get rid of outside trace DNA that could misrepresent the data collected from this part. The handwashing, cell regeneration, random assignment, deposition of trace DNA by 4 donors, and 72-hour storage process remained the same as that listed in part 3. After the storage of the brassieres, two brassieres were randomly assigned to blind swabbing while two other brassieres were randomly assigned to Diamond Dye-localized swabbing. The blind swabbed brassieres were removed from their packaging and swabbed in the cup and clasp areas, respectively, using a wet-dry double swabbing technique with the wet swab being wiped and rotated in three 7.5 cm lines followed by the dry swab with the same technique over the same lines as the wet swabs. The swabs were stored in sterile tubes and kept refrigerated until they were analyzed through DNA extraction and quantification. The extraction process was carried out using the QIAGEN QIAamp Investigator kit and the DNA extracts were quantified using the Quantifiler Trio quantification kit and was processed on the real-time QuantStudio5 instrument.

After the samples were quantified, the amounts were put into various charts categorizing the data based on the swabbing technique used to collect the trace DNA, the condition of the swab used, and the location of where the swabs were taken from. Two independent t-tests assuming equal variances were conducted on the quantities when they were categorized by swabbing technique as well as by the location of where the swabs were taken from. Both independent t-tests were performed at 95% confidence. The t-test for the swabbing technique was observed using a one-tail analysis while the t-test for the location of the swab collections were observed using a two-tail analysis. Only the focus for the quantities of the location swabbing would be different which allows for both greater and less than hypotheses to be possible. The hypothesis of Diamond Dye localization before swabbing will increase the amount of trace DNA compared to blind swabbing creates the one-sided analysis represented as a one-tail analysis.

RESULTS

Part 1 was implemented into this project to act as an initial positive control for the observation of trace DNA using Diamond Dye on a non-porous surface. This part was a qualitative measure to test what worked best for visualization in terms of alternate light wavelengths and emission filters. A clean finger was placed onto the glass slide and held for approximately 5 seconds. Removing the finger showed little evidence of the fingerprint on the slide. Once sprayed with the Diamond Dye solution, orange, fluorescent marks were visible under the Crimescope "CSS" light setting at 455nm with the aid of an orange emission filter at 550nm. Since the location of the print was known, it was assumed that all fluorescence present on the slide under the respective lighting, as seen in Figure 1, was trace DNA given that the initial slide was cleaned.

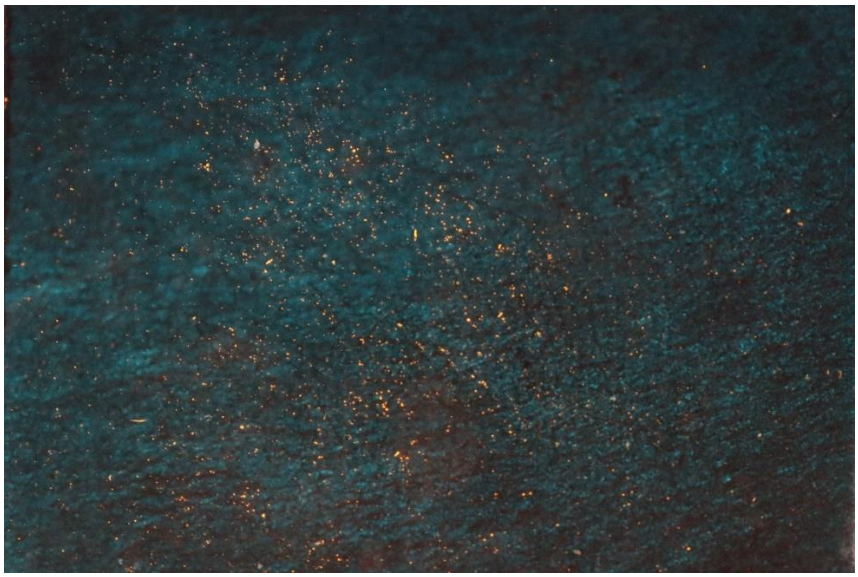

Figure 1 – Macro image of glass slide with a fingerprint with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550nm).

Multiple wavelengths on the Mini Crimescope were examined ranging from UV 365nm to 535nm under an orange 550nm emission filter with the best visibility being with the Crime

Scene Setting (CSS) of 455nm. The visibility of the trace DNA in this research appeared different from the literature due to the differing alternate light settings used, but the light settings in the literature were not the best option during this project. These fluorescent marks were intense enough to be captured as various contrasts and settings using the Canon T8i camera with an EF-S 60 mm macro lens and orange filter attachment. The fluorescence was present after 24 hours when the slide was stored in a slide case undisturbed at room temperature as seen in the Appendix.

Part 2 involved the observation of the Diamond Dye solution, but this time DD was sprayed onto four different porous fabrics: 100% cotton, 100% nylon, 100% polyester, and a 90% polyester/10% spandex blend as seen in Figure 2.

Figure 2: Image of four fabric swatches used in Part 2. Swatches 1-4 are 100% cotton, 100% nylon, 100% polyester, and a 90% Polyester / 10% Spandex blend, respectively.

The choices of fabric represented the top fabrics used to make brassieres which are the mock evidence items in parts 3 and 4. The swatches had DNA deposited on with hands, after swatches were crosslinked, to best ensure the trace DNA being viewed was deposited and not pre-existent. Each was observed under the CSS wavelength lighting of the Crimescope with orange filters and each fabric displayed different patterns of staining and fluorescence as seen in Figures 3-9. The cotton swatch showed areas within the fabric that had orange, fluorescent spots present that were distinctive from other markings or debris on the fabric. It displayed the best fluorescence with minimal background bleaching or "splotching" from the Diamond Dye as seen in Figure 3.

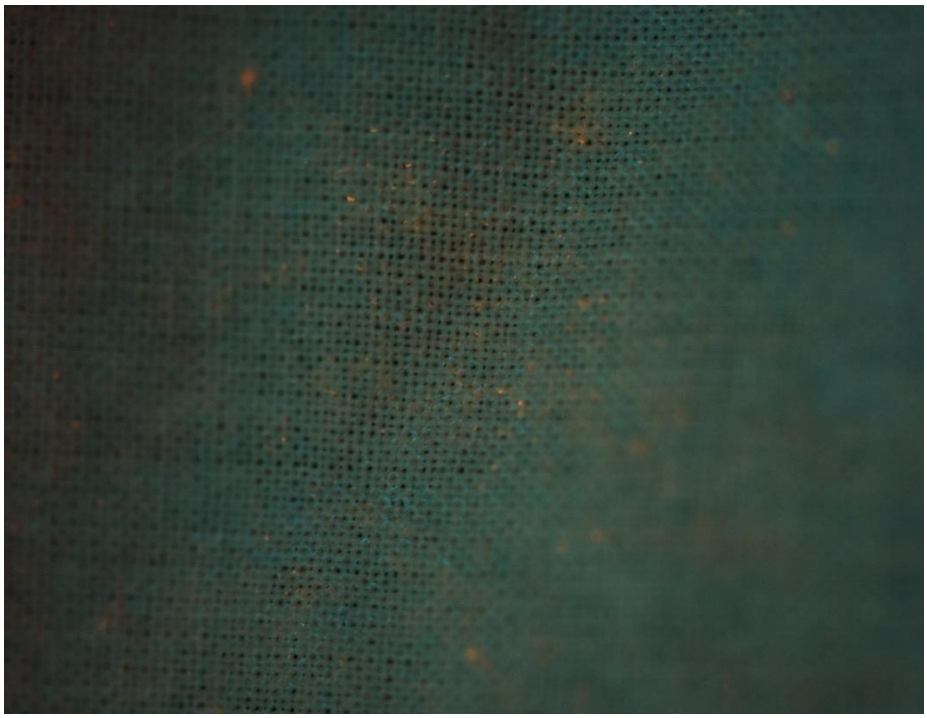

Figure 3 - Macro image of 100% cotton swatch that was rubbed on bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

The nylon swatch also showed some orange, fluorescent spots present, but not in the amount that was witnessed on the cotton. Nylon also contained some areas of oversaturation or "splotching" of the dye as seen in Figures 4 and 5.

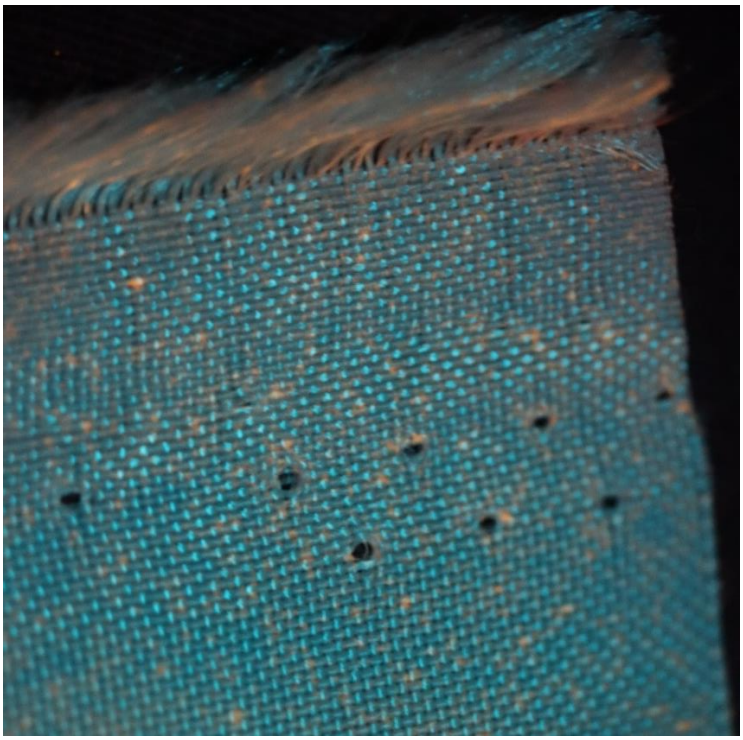

Figure 3- Macro image of 100% nylon swatch that was rubbed on bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

Figure 5 - Macro image of 100% nylon swatch that was rubbed on bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

Additionally, both swatches that contained some polyesters were not easily interpreted due to background fluorescence from the fabric under the light settings. The 100% polyester swatch under the lighting was covered in yellow-orange spots and "splotches" throughout the entire swatch as seen in Figure 6.

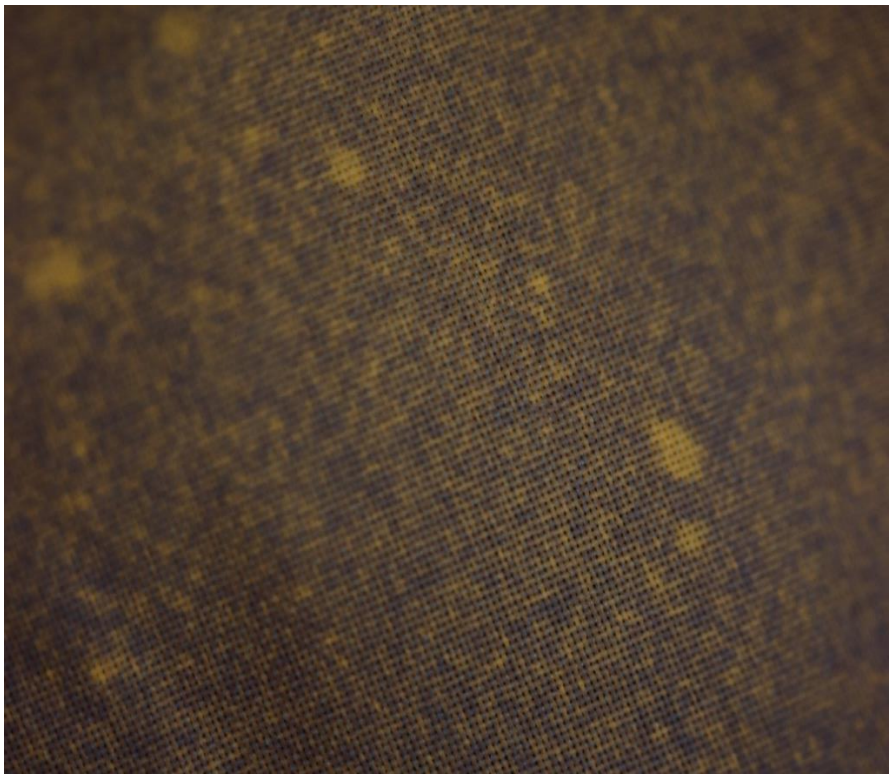

Figure 4 – Macro image of 100% polyester swatch that was rubbed on bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

Consequently, polyester did not display as much, if any, noticeable fluorescence throughout the fabric. Orange fluorescence was only minimally observed on the fraying of the end of the swatch of material displayed in Figure 7. The polyester/spandex fabric mix had a singular spot of orange fluorescence under the lighting that could be noticed within the weave of the fabric as seen in Figure 8. Throughout the rest of the fabric, there were "splotch-like" visible droplet patterns that

could be faintly observed, but not photographed well with the Canon T8i/EF-S 60mm macro lens set up as displayed in Figure 9.

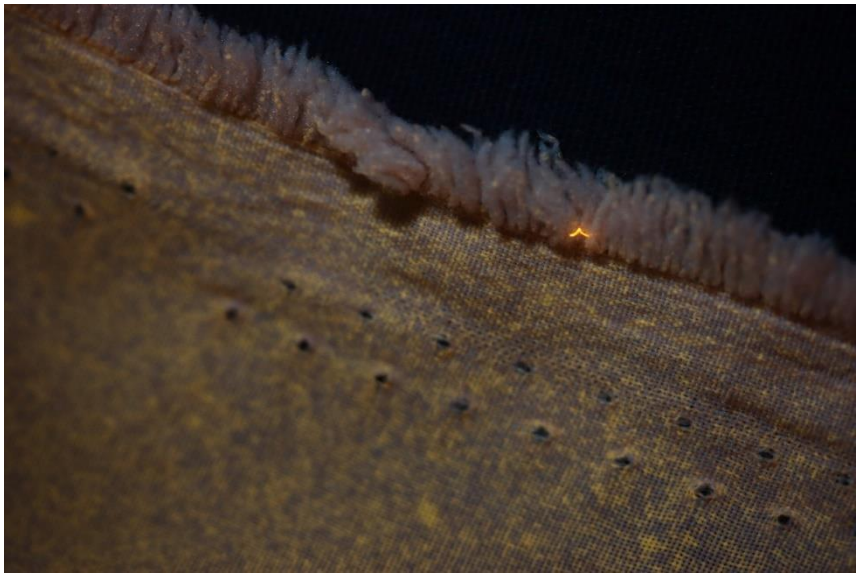

Figure 5 - Macro image of 100% polyester swatch that was rubbed on bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

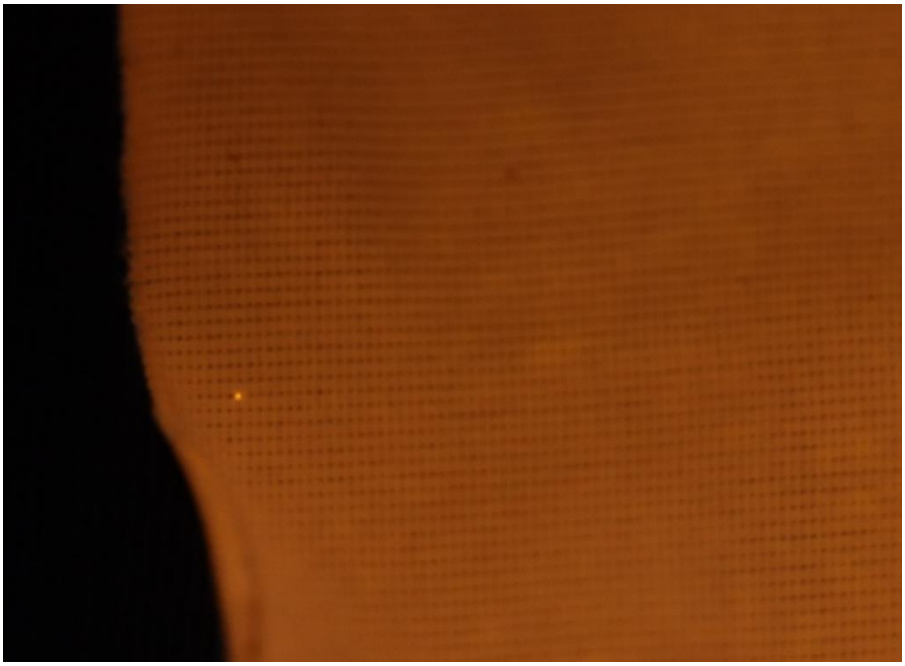

Figure 6 – Macro image of 90% polyester / 10% spandex swatch that was rubbed with bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

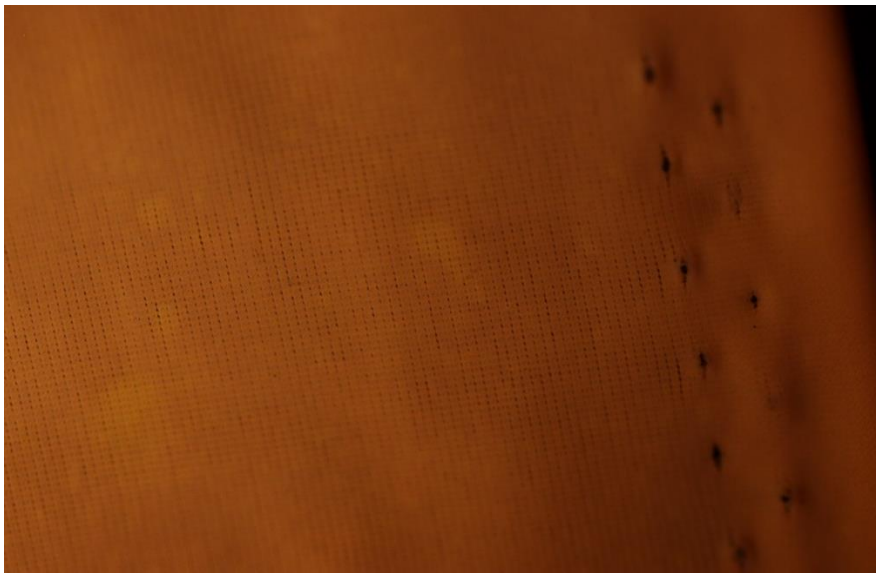

Figure 7 - Macro image of 90% polyester / 10% spandex swatch that was rubbed with bare hands and with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

These observations suggest that the Diamond Dye solution interacted better for its purpose on the natural fiber, cotton, than on the synthetic fibers, nylon, polyester, and the polyester/spandex blend. Further research on this observation would need to be conducted to see its validity or reasoning for all fabrics of those types.

Part 3 implemented the mock evidence brassieres into the research. The new and laundered brassieres were randomly assigned to five donors for DNA deposition. All brassieres were the same color and style to ensure consistency of observations. The brassieres were split into brand new and laundered treatments to see if there would be a variation in the fluorescence of material that had been loosened by the process of being laundered. Each brassiere, regardless of condition, was cross linked in order to eliminate extraneous DNA. Five donors washed their hands to get rid of extraneous DNA then waited for cell regeneration on their hands for 30 minutes before placing trace DNA on the cup and clasp areas. Each donor was given one brand new and one laundered brassiere for equal opportunities for both conditions. All brassieres were stored in their own paper

evidence bag and sealed with evidence tape when stored in the refrigerator for 72 hours for the storage of evidence and not being examined right after DNA deposition. After the storage period, each brassiere was placed on their own piece of butcher paper and had Diamond Dye applied with the spray bottle over the whole item. Each was observed under the CSS light setting with an orange emission filter. A positive and negative control were also viewed under the alternate light and filter set up. The positive control showed the fluorescence of trace DNA clearly against the dark coloring of the cup and clasp areas under the alternate light set up as seen in Figures 13-16.

Figure 8 - Macro image of a brassiere's eye closures with Diamond Dye solution applied and trace DNA deposited (positive control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

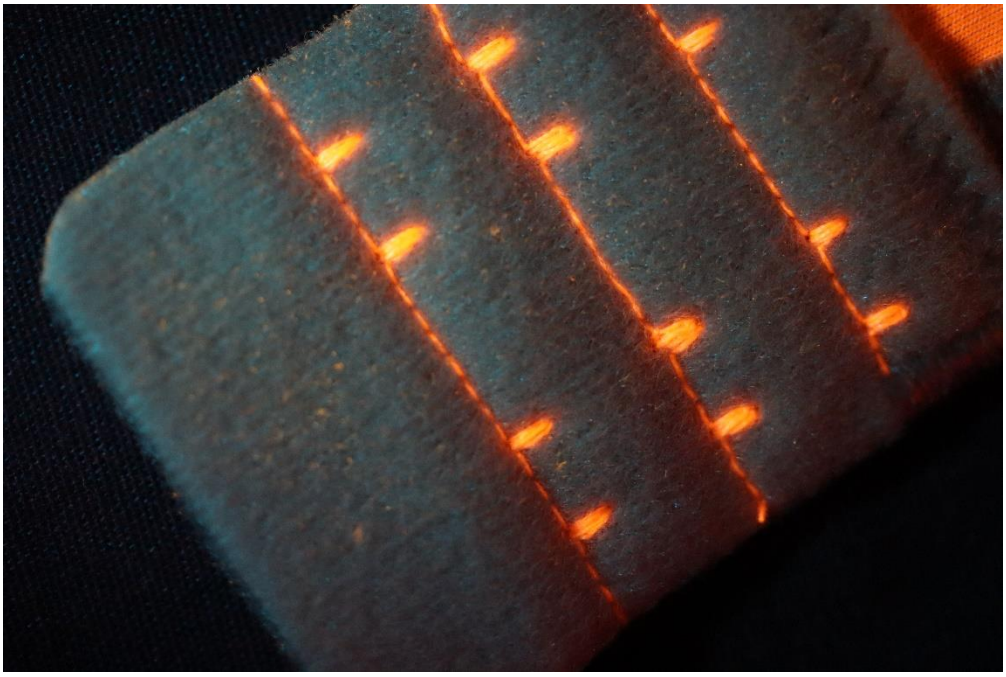

Figure 9 - Macro image of the back of a brassiere's eye closures with Diamond Dye solution applied and trace DNA deposited (positive control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

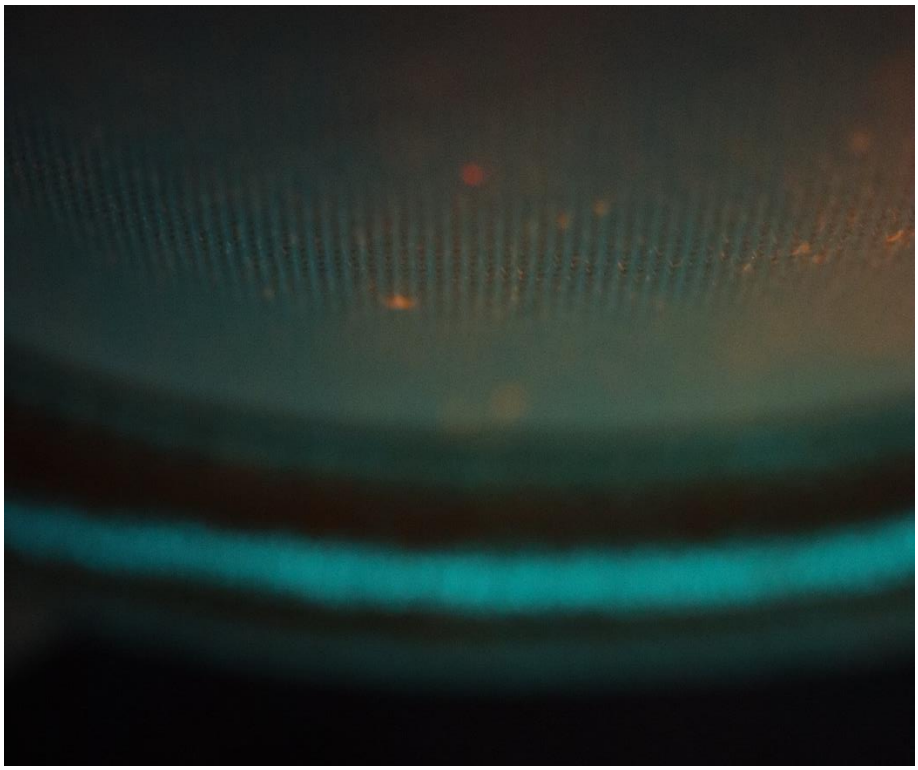

Figure 10 - Macro image of a brassiere cup with Diamond Dye solution applied and trace DNA deposited (positive control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

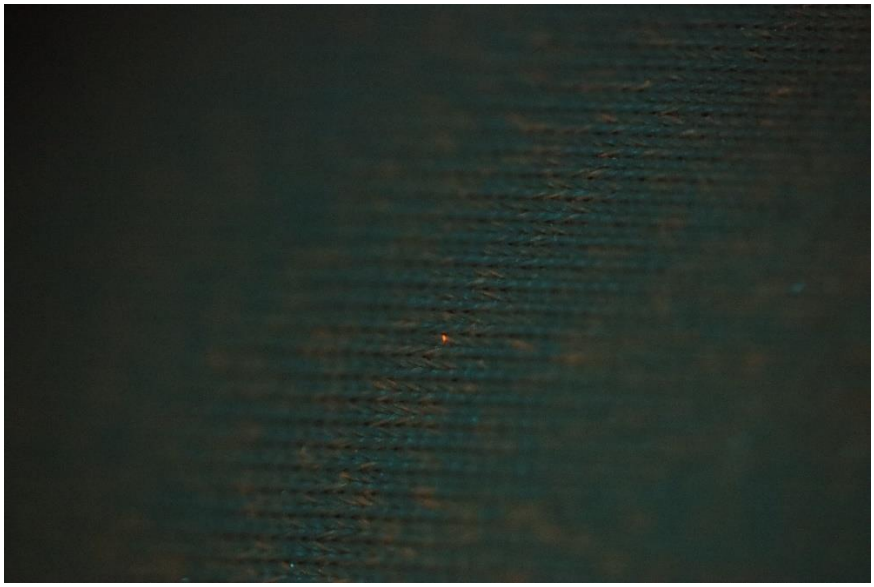

Figure 11- Macro image of a brassiere cup with Diamond Dye solution applied and trace DNA deposited (positive control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

The negative control displayed no signs of fluorescence in the coloring that was observed on the positive control, or the test brassieres as seen in Figures 10-12.

Figure 12 - Macro image of a brassiere's eye closure with Diamond Dye solution applied and no trace DNA deposited (negative control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

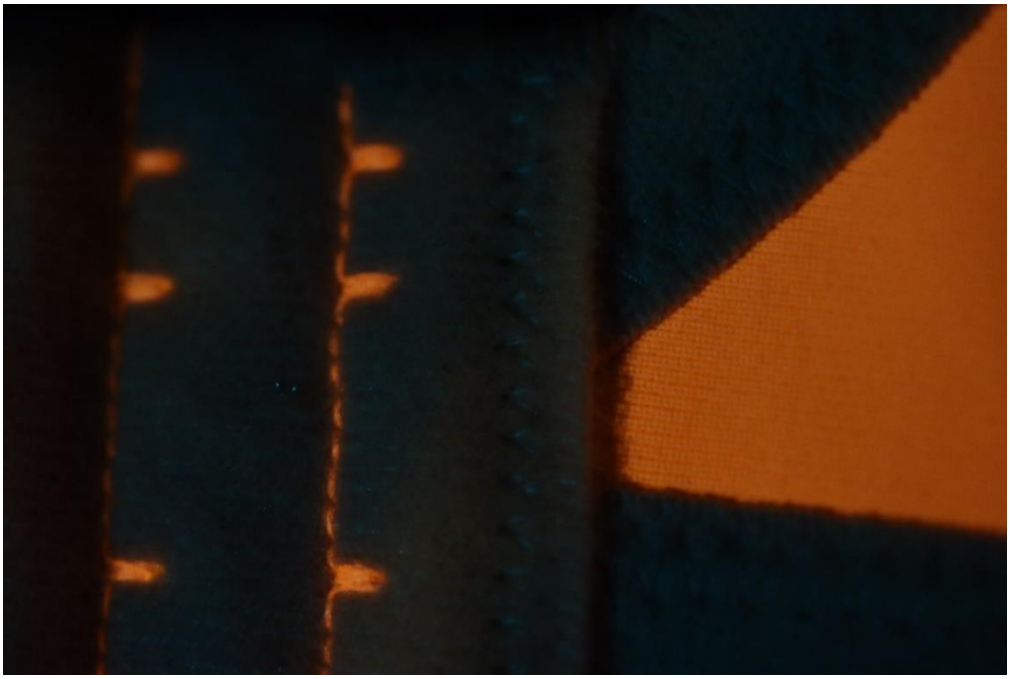

Figure 13 - Macro image of the back of a brassiere's eye closure with Diamond Dye solution applied and no trace DNA deposited (negative control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

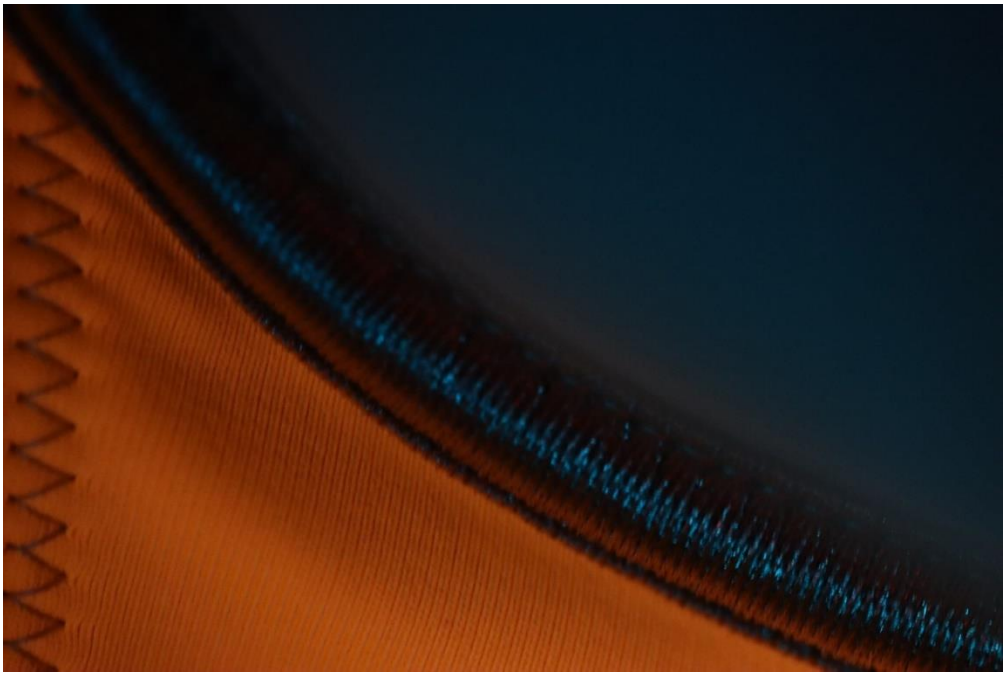

Figure 14 - Macro image of a brassiere cup with Diamond Dye solution applied and no trace DNA deposited (negative control) under Mini Crimescope CSS lighting (455 nm) and an orange filter (550 nm).

The test brassieres were then individually viewed macroscopically as well as photographed which showed that there were areas where fluorescence was clearly observed on the clasp area, as seen in Figures 17 and 18, as well as on the cup area, as seen in Figure 19.

Figure 15: Macro image of the front of a brassiere clasp with Diamond Dye solution applied and trace DNA deposited under Mini Crimescope CSS lighting (455nm) and an orange filter (550 nm).

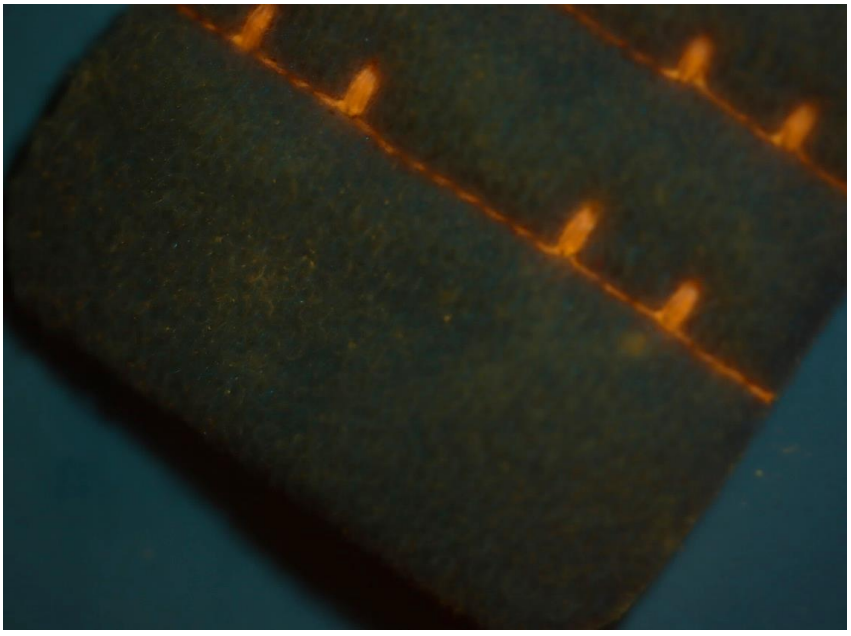

Figure 16: Macro image of the back of a brassiere clasp with Diamond Dye solution applied and trace DNA deposited under Mini Crimescope CSS lighting (455nm) and an orange filter (550 nm).

Figure 17: Macro image of the back of a brassiere cup with Diamond Dye solution applied and trace DNA deposited under Mini Crimescope CSS lighting (455nm) and an orange filter (550 nm).

Although there were some areas where fluorescence was easily observed, there were also areas within both the cup and clasp areas that appeared to have bleaching or reflectance in a droplet or spray pattern. These areas made it more difficult to see fluorescence as well due to the reflectance being an orange coloring as seen in Figures 17 and 19. This could potentially be associated due to the materials used to make the selected brassieres. The cup areas were made with 78% nylon and 22% spandex while the back containing the clasp area was made with 80% nylon and 20% spandex which are both synthetic materials. Photographing of the multiple areas on the test brassieres also proved to be challenging on the cup area due to the curvature of the material and the limited focal area of the macro lens used to capture the images. Another variation in the images came from the power source of the Mini Crimescope used as alternate light. The fan within the instrument, at times, provided dimmer light than usual potentially due to the age of the instrument and its self-protection from overheating as seen in the differences between Figures A5 and A6.

Part 4 also used the test brassieres, but this part only used laundered brassieres as that is most applicable to how they are worn in real life. This section, however, compared quantitative amounts of trace DNA that were recovered via two different swabbing approaches: blind swabbing and Diamond Dye-localized swabbing. The same cross linking, cell regeneration, donor deposit, and storage that was performed in part 3 was performed here for method consistency. After the storage period, the brassieres were separated randomly into blind swabbing and Diamond Dye-localized swabbing. The blind swab brassieres after being removed from the packaging were laid on butcher paper and swabbed in the cup and clasp area using the double swab technique. The wet swab was used in wiping motions while being rotated to ensure as much retention as possible then the head was broken off into a sterile tube. The dry swab followed directly after wiping over the same area the wet swab did and placed into its own tube. Normally, trace DNA wet-dry swabs are used, stored, and tested together, but they were kept separated in this research study. The swabs, once collected, were stored in the refrigerator between collection and extraction. Normally biological evidence is stored cool, dry and at room temperature. However, sexual assault kits are often stored under refrigeration, and many have underwear collected with the kit. The extraction and qPCR processes went according to the manufacturer's protocol. Both blind swabbing and Diamond Dye-localized swabbing recovered trace DNA as there were amounts recorded during quantification of all swabs. Unfortunately, one sample was lost due to the tube accidentally displacing from the tube rack and spilling within the PCR hood workspace. This sample is noted as "ND**" within all data tables.

Once all the swabs were run, it was organized into multiple tables for organization. The first table, Table 1, was organized into columns based on the type of swab technique as well as

the condition of the swab and the rows were categorized to show location of where the swabs were taken.

Table 1: Display of quantified trace DNA swabs, in ng/µL, categorized into type of swabbing technique and condition of the swab.

	Blind		**Dyed**	
	Wet	*Dry*	*Wet*	*Dry*
Clasp	0.0038	0.0023	0.0071	0.005
	0.0018	0.0006	0.0043	0.0013
	0.0034	0.002	0.0034	0.0037
	*ND***	0.004	0.0319	ND
Cup	0.0013	0.0012	0.0097	0.0055
	ND	0.0024	0.0008	0.0015
	0.0031	0.0168	ND	0.0368
	0.0017	0.0059	0.0012	0.0319
Average	0.0022	0.0044	0.0083	0.0122

The next two tables, Tables 2 and 3, separated the blind swab and dye-localized swab techniques into their own tables with the swab condition and location remaining the same. Additional columns were added to both tables with one column totaling all trace DNA recovered by the pair of swabs and the other column giving an estimated number of cells needed to produce the amount of DNA detected.

Table 2: Display of quantified blind swabbing trace DNA values, in ng/µL, categorized into condition of the swab, location of the collection, total DNA collected in the pair, and the estimated number of cells needed to create the total DNA based on one diploid cell = 0.006 ng.

	Blind		**Total DNA**	**# of Cells**
	Wet	*Dry*		
Clasp	0.0038	0.0023	0.0061	1.0167
	0.0018	0.0006	0.0024	0.4
	0.0034	0.0020	0.0054	0.9
	ND**	0.0040	0.0040	0.6667
Cup	0.0013	0.0012	0.0025	0.4167
	ND	0.0024	0.0024	0.4
	0.0031	0.0168	0.0199	3.3167
	0.0017	0.0059	0.0076	1.2667
Average	0.0022	0.0044	0.0063	1.0479

Table 3: Display of quantified dye-localized swabbing trace DNA values, in ng/µL, categorized into condition of the swab, location of the collection, total DNA collected in the pair, and the estimated number of cells needed to create the total DNA based on one diploid cell = 0.006 ng.

	Dyed		**Total DNA**	**# of Cells**
	Wet	*Dry*		
Clasp	0.0071	0.005	0.0121	2.0167
	0.0043	0.0013	0.0056	0.9333
	0.0034	0.0037	0.0071	1.1833
	0.0319	ND	0.0319	5.3167
Cup	0.0097	0.0055	0.0152	2.5333
	0.0008	0.0015	0.0023	0.3833
	ND	0.0368	0.0368	6.1333
	0.0012	0.0319	0.0331	5.5167
Average	0.0083	0.0122	0.0180	3.0021

The cell estimation calculations were based on one diploid cell, which is what most trace DNA is made from, containing 6 picograms or 0.006 nanograms of DNA. Table 4 is all of the data that was collected, but only categorized by the swab technique.

Table 4: Display of quantified trace DNA swabs, in ng/µL, categorized into type of swabbing technique.

	Blind	**Dyed**
	0.0038	0.0043
	0.0023	0.0013
	0.0006	0.0012
	0.0013	0.0008
	0.0012	0.0015
	0.0031	0.0012
	0.0024	0.0319
	0.004	0.0037
	0.0168	0.0097
	0.0018	0.0055
	0.002	0.0071
	0.0017	0.005
	0.0059	0.0368
	0.0034	0.0034
	ND	ND
	ND**	ND
Average	0.0036	0.0081

This table was used to conduct an independent t-test to statistically compare the quantities collected with the printout displayed as Table 6.

Table 5: Display of the results of the independent t-test conducted comparing the quantities found from blind swabbing and dye-localized swabbing at confidence = 0.95, α = 0.05.

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.0036	0.0081
Variance	1.6355E-05	0.0001
Observations	14	14
Pooled Variance	7.3726E-05	
Hypothesized Mean Difference	0	
df	26	
t Stat	-1.3888	
P(T<=t) one-tail	0.0883	
t Critical one-tail	1.7056	
P(T<=t) two-tail	0.1767	
t Critical two-tail	2.0555	

This categorization was also displayed into box-and-whisker plots as seen in Figures 20 and 21. Figure 20 displays all the values from the chart while Figure 21 displays a closer version of the box-and-whisker plot, but without the outlier data points. The outliers displayed in Figure 21 could be associated with the cell regeneration period as what each donor was done in that time was not specified. The donor genetic variability within that time may have promoted the development of skin cells differently in addition to each donor's individual shedder status being different.

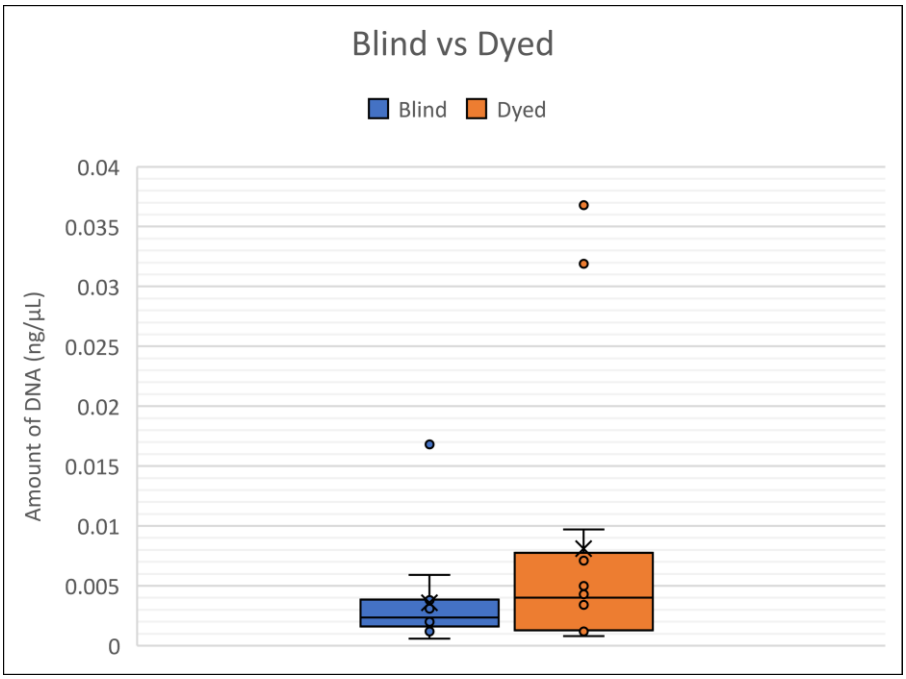

Figure 18: Box-and-whisker plots of the quantified trace DNA swabs, in ng/µL, based on swab technique.

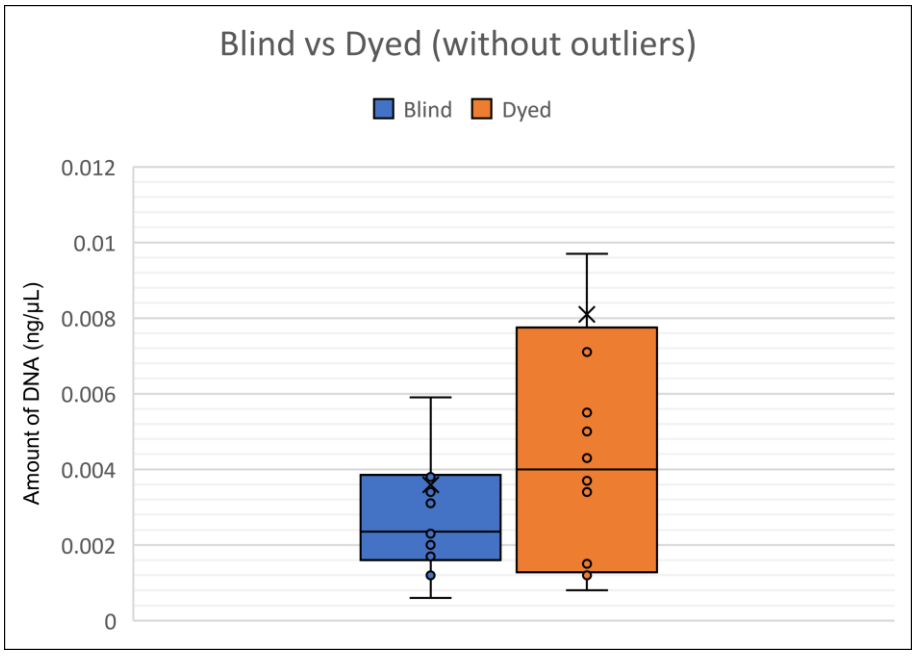

Figure 19: Box-and-whisker plots of the quantified trace DNA swabs, in ng/µL, based on swabbing technique and excluding outliers.

An additional table, Table 5, was created to compare the quantities based on the location of where the swab was taken from with the rows organized by the swab technique. The table also displayed the degradation indexes of the quantities displayed which is a measurement of how degraded a sample is.

Table 6: Display of quantified trace DNA swatches and corresponding degradation indexes, in ng/µL, categorized into location of the swabs and swabbing technique used.

	Cup	**DI (cup)**	**Clasp**	**DI (clasp)**
Blind	0.0013	1.1818	0.0038	1.6522
	0.0031	1.1071	0.0018	3
	0.0017	2.4286	0.0034	2
	0.0012	ND	0.0006	1.5
	0.0024	8	0.0040	1.3333
	0.0168	0.7149	0.0023	0.451
	ND	ND	0.0020	1.25
	0.0059	3.6875	0.0071	1.1639
Dyed	0.0319	0.7419	0.0043	0.8776
	0.0097	3.0313	0.0034	1.0968
	0.0008	1	0.0012	1
	0.0055	0.8954	0.0050	1.9231
	0.0015	1.3636	0.0013	0.5
	0.0368	1.0888	0.0037	1.85
	0.0012	0.7500	ND	ND
	ND	ND	ND**	ND
Average	0.007488	1.9993	0.002927	1.6545643

This is measured by dividing the small autosomal target, which are the quantities displayed in the previous charts, over the large autosomal target identified by the QuantStudio5 instrument and Quantifiler Trio kit. Some values are noted as ND, meaning that a degradation index was unable to be determined. This may have been due to a missing small autosomal target, a large autosomal target, or both. This could have been due to no trace DNA being present or that the DNA was too degraded to be detected.

The cup and clasp columns were used to conduct an independent t-test comparing the quantities by location of where the swabs were collected from which created the print-out displayed in Table 7. Similar box-and-whisker plots as before were created to display the data as it was categorized by location of where the swab was collected. Figure 22 displays all of the quantities while Figure 23 displays a closer version of the plots excluding the outlier points.

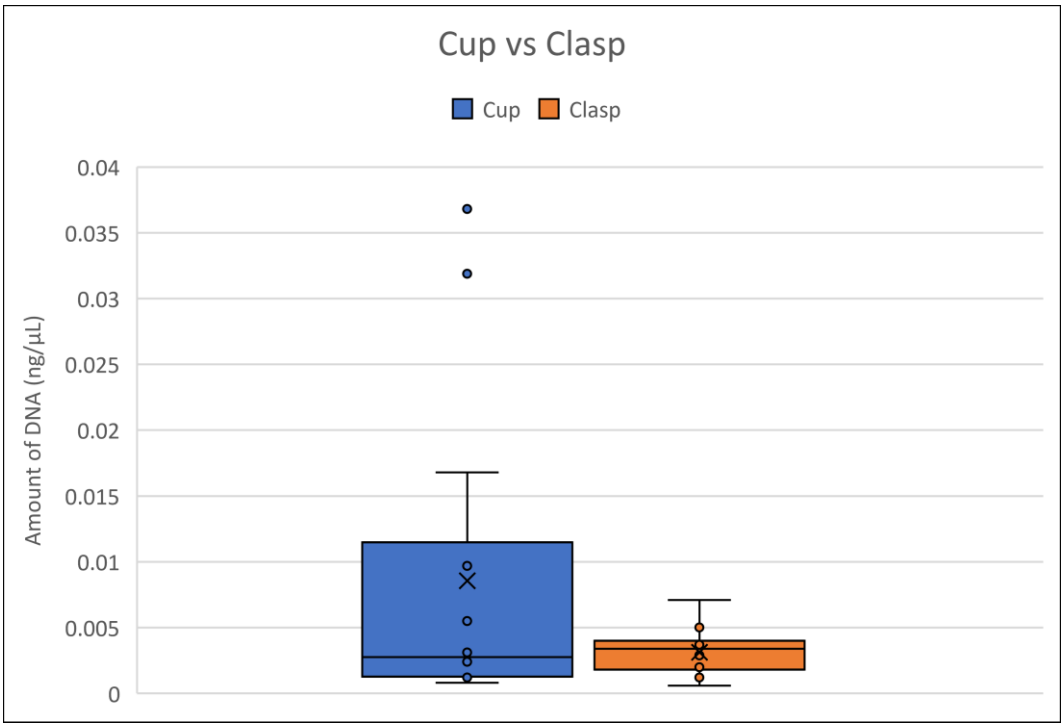

Figure 20: Box-and-whisker plots of the quantified trace DNA swabs, in ng/µL, categorized by location (cup or clasp).

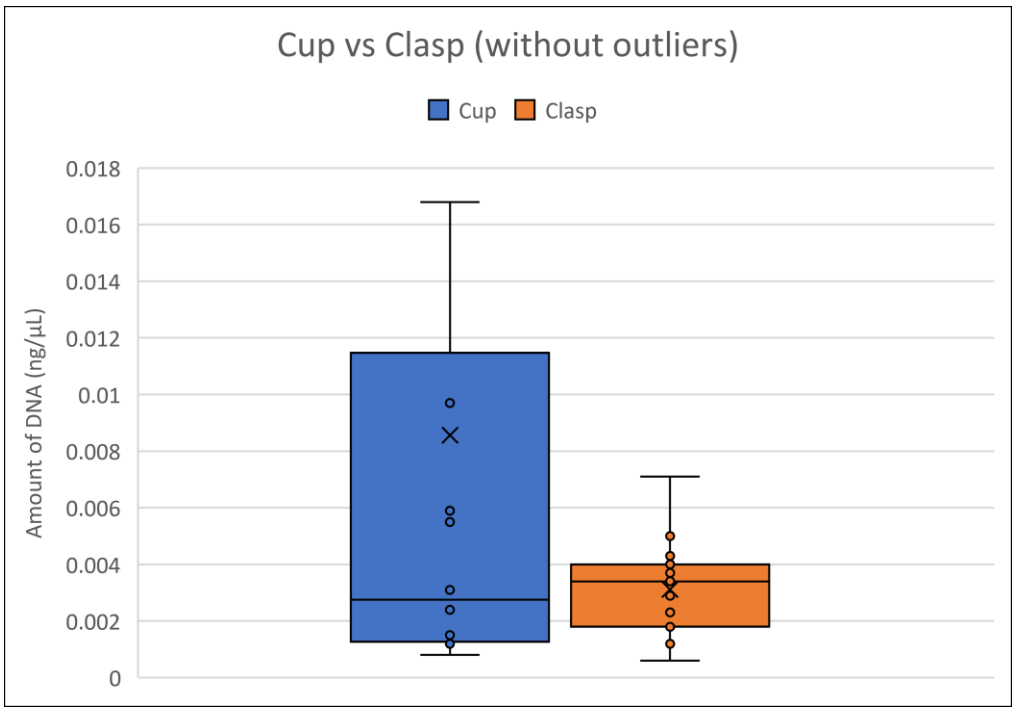

Figure 21: Box-and-whisker plots of the quantified trace DNA swabs, in ng/µL, based on location (cup and clasp) and excluding outliers.

The final table, Table 8, displays the quantities that were recovered from the substrate controls performed for part 4.

Table 7: Display of quantified trace DNA swatches, in ng/µL collected from the positive and negative substrate controls during Part 4.

Substrate Control Sample	Positive	Negative
DRY DYED CLASP	0.0014	0.0077
WET DYED CLASP	0	0.0019
DRY BLIND CLASP	0.0054	0.0023
WET BLIND CLASP	0.0056	0
DRY DYED CUP	0.0021	0.0019
WET DYED CUP	0.0040	0.0029
DRY BLIND CUP	0.0067	0.0020
WET BLIND CUP	0.0124	0.0030

The positive control displayed trace DNA that was present, as expected, but so did the negative control which was not expected from a substrate control after UV sterilization. Laundering and cross linking of both the positive and negative substrate brassieres were performed, so the presence of trace DNA on the negative control was unexpected. This observation led to the idea that cross linking of porous items may not be as effective as it is on non-porous items. Since the swabs involved pressure into the fabric weave, this may have recovered trace DNA that was not eliminated by the UV cross linking if the DNA was further in the weave than the surface of the material. Further research should include carrying out testing the swabs to develop profiles to ensure if the trace DNA originated within the experiment or was extraneous to the research as well as combining the wet and dry swabs for the most accurate quantification based on standard trace DNA testing methods. This will increase the number of samples that can be processed for more representative statistical testing.

The first statistical analysis performed based on the swabbing technique was analyzed as a one-tail t-test analysis. The original hypothesis of the research was phrased such that the Diamond Dye-localized swabs would contain more trace DNA than the swabs that were performed blindly meaning that only one side of the statistical analysis was desired. At 95% confidence, the p value calculated by the t-test was 0.0883 as displayed in Table 6. A statistical significance is observed when a p value at this confidence is less than 0.05 meaning that there is no statistical significance or, in layman's terms, no statistically notable difference between the quantities recovered by the two techniques. However, the average quantity that was collected by blind swabbing was almost three times less than the average quantity recovered using dye-localized swabbing. The second statistical test performed based on the location of the swabs was analyzed as a two-tail analysis. Since the focus of this portion of the data was to see if there was

a difference between the two locations with no preference of greater or less than, results from either end could be observed. The p value at a 95% confidence for this test was 0.1013 which shows no statistical significance or notable difference between the quantities recovered from the cup or the clasp areas as displayed in Table 7.

Table 8: Display of the results of the independent t-test conducted comparing the quantities found from swabs taken from the cup area and swabs taken from the clasp area at confidence = 0.95, α = 0.05.

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	Variable 2
Mean	0.008567143	0.003135714
Variance	0.000139524	3.0394E-06
Observations	14	14
Pooled Variance	7.1282E-05	
Hypothesized Mean Difference	0	
df	26	
t Stat	1.6989	
P(T<=t) one-tail	0.0506	
t Critical one-tail	1.7056	
P(T<=t) two-tail	0.1013	
t Critical two-tail	2.0555	

DISCUSSION

The original hypothesis of this study was that Diamond Dye imaging would enhance the recovery of trace DNA from porous surfaces. A non-porous section was implemented solely for the purpose of interpreting what the dye looked like with the available instrumentation. It was expected, based on the literature referenced, that the fluorescence of DD would appear to be a green color. However, no settings of the alternate light from the Mini Crimescope combined with the orange emission filter created clear displays of the cells in similar coloring. Given that the observations in this study displayed orange and yellow coloring, it is seen that DD, when fluorescing, can be observed efficiently under more than one wavelength depending on what is available to the end user. This also may be due to the coloring of the materials that the cells were present on (the substrate). Technically, the DD solution was challenging to use because of reflectance on white or light backgrounds making it challenging to visualize without a camera filter. Further studies involving DD should be conducted to see if there are optimal alternate light and emission filter set-ups based on the type of material that trace DNA is deposited on and for the color of that material.

When the visualization of cells was possible on the various fabrics, the analysis of those areas was done in a subjective manner. Information observed like the intensity or presence of fluorescence was based on personal observations and understanding of the dye through parts 1 and 2. Fluorescence intensity as well as presence can be quantified through the use of fluorescence microscopy and a corresponding computer program to create more objective and quantifiable analyses. This could include the hues of the colors being detected, measuring of the intensity of the fluorescence, and determining the number of cells present so results can be reproduced and compared in a universal way. Additional training of the eye to recognize the DD

fluorescence was required to become proficient enough for the study to be successful. Documenting fluorescence strictly on personal observation leaves too much room for interpretation as individuals may not witness the same intensities which was a major limitation to the interpretation aspect of this study. Also, the magnification using only a camera and its macro lens is not as high quality or consistent as with microscopy. Reproducibility based on the camera setup alone also made this project subjective as the operation of the camera setup was based on personal experience with cameras. While camera training could be done in a universal fashion, the observations and documentation using the setup can vary making it difficult to accurately reproduce. The implementation of fluorescent microscopy would create objective guidelines for analysts so that the results can be more easily replicated and compared.

Based on the data collected, the average amount of DNA recovered using the dye-localized technique was almost 3 times higher than the amount recovered from blind swabbing which, in general, was expected. Although the averages showed an increase in recovery, the differences between all the quantities in the data were not found to be statistically significant. This determination goes against the hypothesis that there is a difference between the two approaches. It is possible the DNA absorbs and is retained in the fabrics since they are porous. Therefore, the DNA may have been deposited but it was not recovered in the swabbing technique due to the potential imbedding of the cells. Not much is known about cell adherence to fabrics on touch and the amounts that were visualized and recovered were in the range of just a few cells. This lack of understanding could also contribute to the results collected from the substrate controls. If the deposited cells sunk into the fabric weave, it would make cells more difficult to recover using a surface technique like swabbing or to eliminate extraneous DNA for research purposes. Other methods of trace DNA collection that could cover more surface area and

potential depth into an object, like sterile taping or vacuuming, would need to be investigated and compared to understand the best approach for trace DNA retention.

One of the main questions that remains based on the results of this study is whether all the DNA that was quantified originated from within the experiment with the donors or if there was some that would be attributed to outside sources or the person conducting the testing. The research performed in this study proves that trace DNA recovery is possible and could be aided with visualization as well as the level of degradation of the DNA samples, but it did not go as far as understanding where the DNA came from. The substrate controls showed that DNA elimination was not performed as expected, so there is a potential for outside DNA contributing to the quantities that were tested. This question could only be answered if the swabs recovered were carried out through full DNA analysis to get profiles from mock evidence which can be compared back to the donors' known profiles. Having the full analysis process would put major significance behind the quantities that were recovered and may determine not only the effectiveness of the dye in identifying cells, but also that the DNA recovered is relevant to the study. In a forensic setting, understanding this may help when collecting and interpreting DNA from evidence as well as the profiles that are produced if there are extraneous profiles regarding a particular investigation based on the known individuals and suspects associated with the crime.

CONCLUSION

The amount of trace DNA that was recovered from mock evidence brassieres when using Diamond Dye-localized swabbing compared to blind swabbing was proved to be statistically insignificant. The interpretation of this study means that the approach of visualizing trace DNA before swabbing would not recover a notably larger amount of DNA compared to the current technique of blind swabbing. In conducting this study, questions of the effectiveness of Diamond Dye on synthetic fabrics were visible due to the high reflectance of the fabrics used, creating difficulty visualizing fluorescing cells. Also, it was determined that most of the methodology of this study was based on subjective observations, setups, and analyses of the fluorescence of trace DNA. Future studies would need to implement methodologies that can be replicated and interpreted based on objective approaches. This could include the use of fluorescent microscopy at stated magnifications, programs that can detect fluorescence based on quantity and intensity, and a consistent source of alternative light for visualization.

Furthermore, this study should be advanced past the quantitation stage to analyze the trace DNA found. Having the profiles from the swabs recovered will answer the majority of the questions developed throughout the quantification, especially of who the DNA belongs to and if it can be attributed to a potential crime. Overall, the use of Diamond Dye as a spray mechanism can be used as an effective visualization technique for trace DNA but played no significant role in DNA recovery. Other uses of Diamond Dye should be investigated in the future to find a potentially useful application in the field of forensics or crime scene investigation.

APPENDIX A

Figure A1: Macro image of glass slide with a fingerprint with Diamond Dye solution applied under Mini Crimescope CSS lighting (455 nm) and an orange filter (550nm).

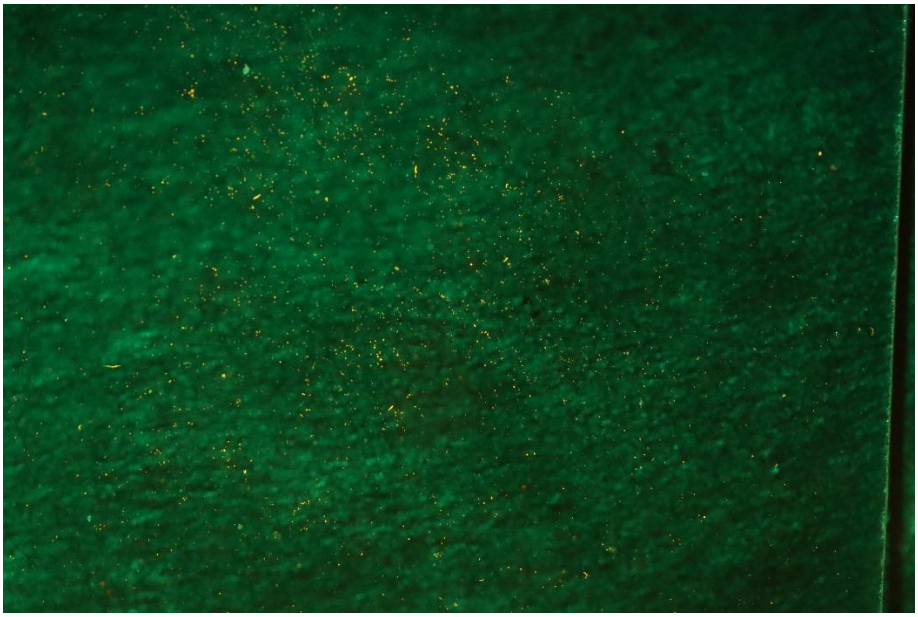

Figure A2: Macro image of glass slide with a fingerprint with Diamond Dye solution applied under Mini Crimescope lighting (515 nm) and an orange filter (550nm).

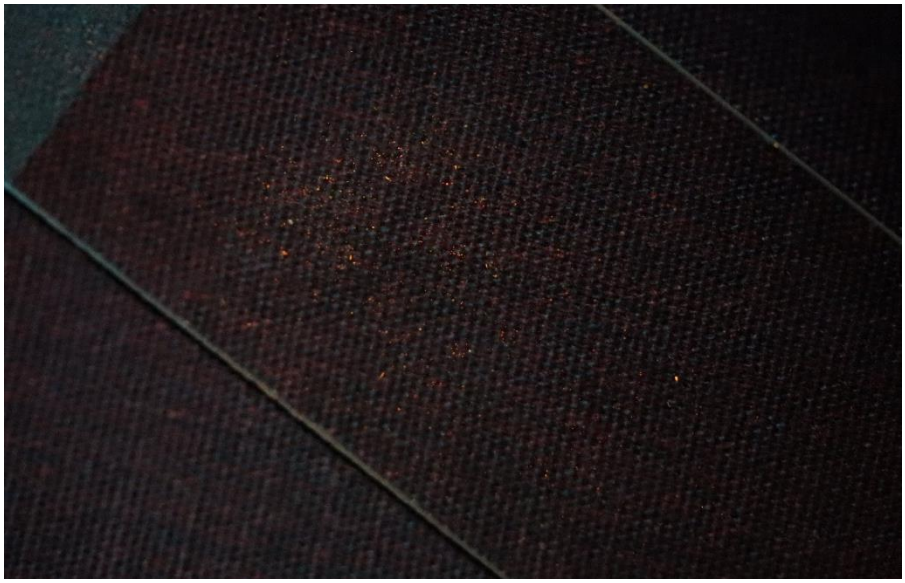

Figure A3: Macro image of the glass slide from part 1 after one day of storage in a slide case at room temperature deposited under Mini Crimescope CSS lighting (455 nm) and an orange filter.

Figure A4: Macro image of the front of the brassiere hook section with Diamond Dye solution applied and no trace DNA deposited (negative control) deposited under Mini Crimescope CSS lighting (455 nm) and an orange filter.

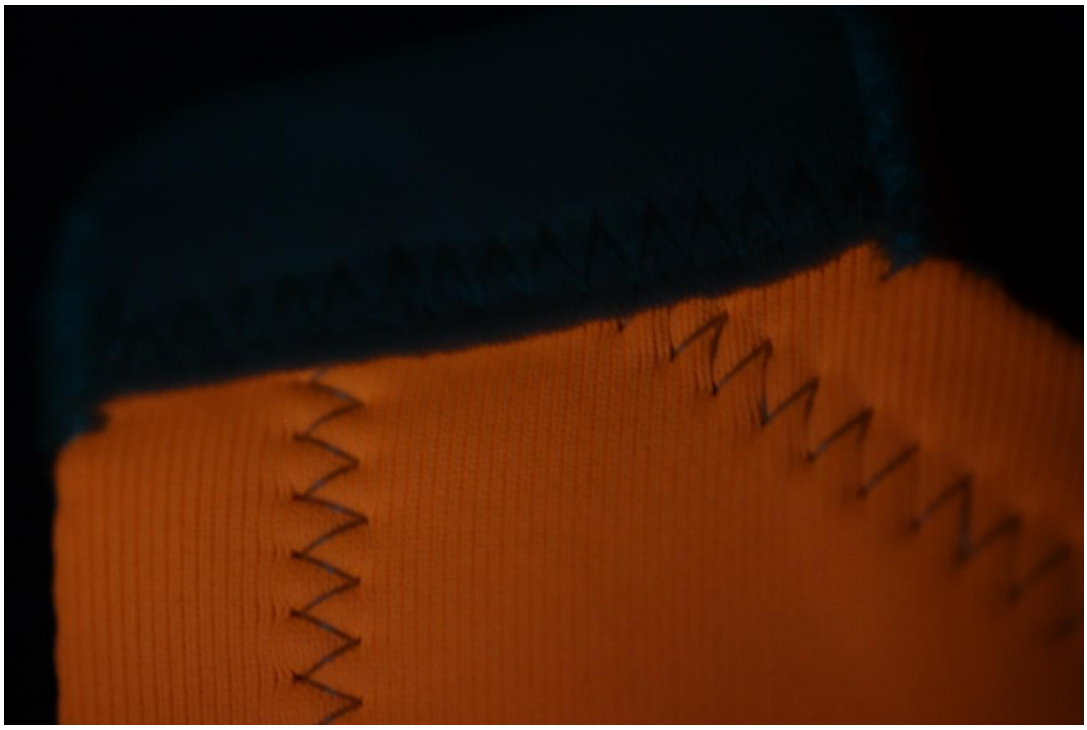

Figure A5: Macro image of the back of the brassiere hook section with Diamond Dye solution applied and no trace DNA deposited (negative control) deposited under Mini Crimescope CSS lighting (455 nm) and an orange filter.

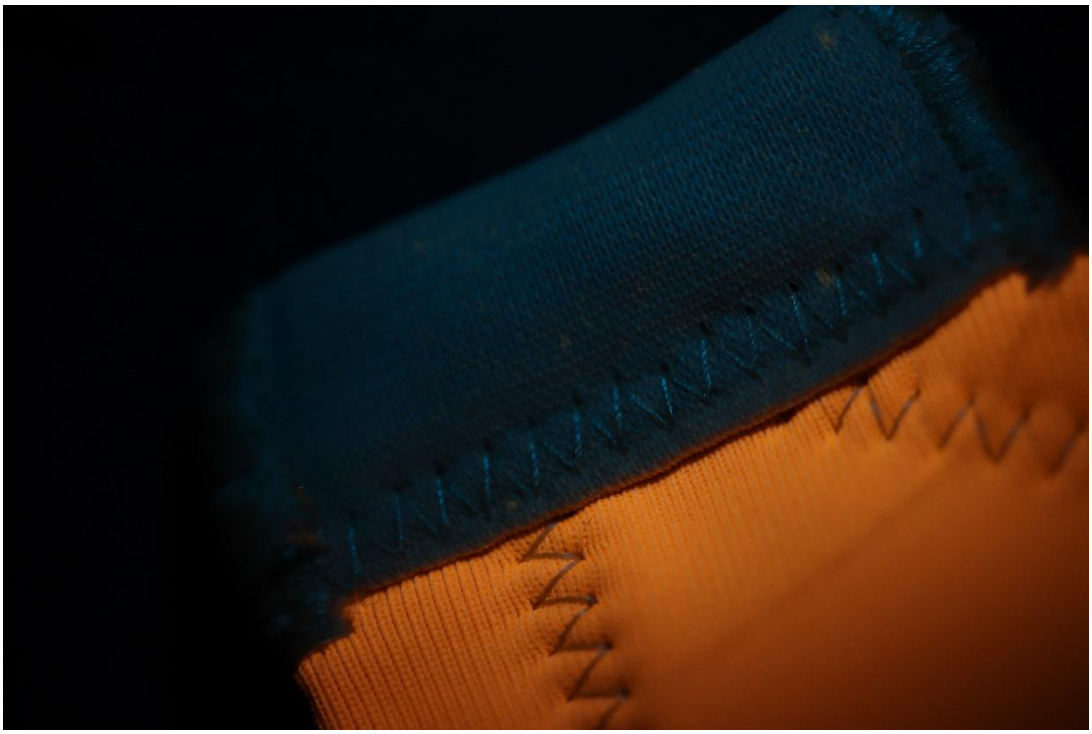

Figure A6: Macro image of the back of the brassiere hook section with Diamond Dye solution applied and trace DNA deposited under Mini Crimescope CSS lighting (455 nm) and an orange filter.

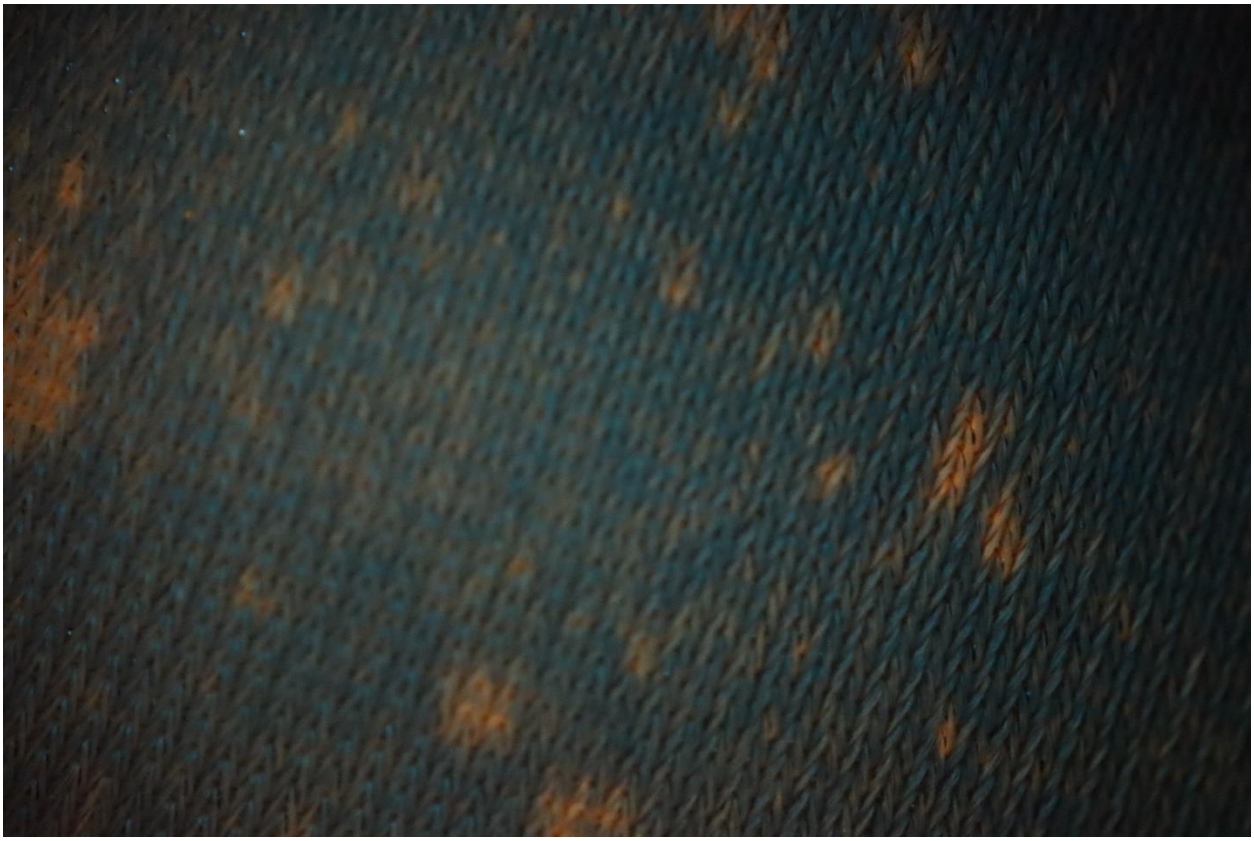

Figure A7: Macro image of the brassiere cup with Diamond Dye solution applied and trace DNA deposited under Mini Crimescope CSS lighting (455 nm) and an orange filter.

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