A Protocol for Discovery of Latent Bloodstains on Dark and Patterned Clotting

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Abstract: Blood evidence on clothing items taken from victims, suspects or crime scenes has been overlooked because it could not be recognized with the unaided eye. This study sought to identify simple and cost-effective photographic methods that investigative and forensic personnel could use to find blood evidence on clothing while preserving the DNA. Articles of clothing were treated with neat, whole blood. Standard light, infrared (IR), ultraviolet (UV), cross-polar, Alternate Light Source (ALS)-aided, computer enhancement, and fluorescein techniques were used to examine and document the samples. On light colored clothing, standard light examinations proved sufficient for identification of probable blood evidence. On dark clothing, (NIR) and cross-polar examinations proved the most effective for identification of probable blood stains. Hemascein application identified a bloodstain not visualized by the other sources.

Introduction

This project was brought about by real case experience in which key evidence was overlooked due to difficulties locating bloodstains on clothing. In one case, materials were examined in multiple labs with the result being the same: no blood was found during the examinations. The material was a multi-colored, patterned, reflective surface material. Ten years later, fluorescein was applied and revealed the location of blood evidence. The blood was swabbed and analyzed, and provided a full DNA profile. In another case involving a dark colored, highly absorbent, non-reflective surface, Near-infrared (NIR) photography was used to visualize bloodstains at the scene prior to movement of the victim’s body.

DeForest, et al., explored the issue of bloodstains and dark surfaces, and learned that polarizing filters and NIR can aid in identifying bloodstains on dark and reflective surfaces. Connor and associates used 24 different fabrics to test NIR digital photography for visualization and learned that NIR can aid when substrates do not absorb in the same wavelength as blood. Visualization of blood on fabric can be affected by the fabric texture, porosity, absorbing power, impact formation, volume of blood, and impact force. Infrared digital imaging was used with computer enhancement for successful visualization of bloodstains on a black shirt and black cargo pants retrieved from a homicide scene.

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Dr. Austin Richards, Brooks Institute of Photography, explored the use of Ultraviolet (UV) photography for forensic applications. His work cited viable applications for bite marks on human bodies and impressions on light colored surfaces, but not for bloodstains on dark surfaces. From the authors’ experience, forensic investigators often used light in the 400nm, purple light range, 450 nm, blue light range, and the 580 nm, green light range, to detect bloodstains. The technique applied oblique light in the specific wave length to create contrast. Bloodstains appeared darker than the surrounding substrate surface. These techniques were often referred to as UV light applications, and documentation by photography was often called UV photography. Although they are not truly UV photography applications as defined by Dr. Brooks, they have been techniques used by investigators so we included them in our project.

The use of fluorescein solutions has enjoyed success in detecting bloodstains on surfaces. The fluorescein solution interacts with proteins and iron ions in hemoglobin, and will fluoresce when exposed to light in the 420nm to 485 nanometer range. Digital photography can be used to document the reaction location of bloodstains. An orange barrier filter is required for visualization and documentation purposes.

We undertook this project to look at differences in clothing and how this may impact locating latent bloodstains. The primary issue seemed to be with clothing containing patterns or screen prints, and dark, absorbent clothing. The focus of this study was to identify non-destructive, quick, and effective methods of revealing latent bloodstains while maintaining the integrity of any DNA evidence. The end goal of this study was to develop a simple protocol moving from least-involved to the most-involved measures that forensic personnel could follow to maximize the probability of discovering latent bloodstains while preventing the degradation of DNA evidence.

Materials and Methods

Substrates and Blood

Seven articles of clothing were purchased at an area secondhand store. Whole, neat blood was drawn from a sixty-eight-year-old male donor with no known issues that would affect standard properties of blood. The genetic profile had been previously established for this donor. The drawn blood was immediately and randomly dropped onto the articles of clothing using an eye dropper. The blood had been drawn into a red top tube without any anti-coagulant additive. Hence, it was necessary to drop the blood before it started to coagulate. The blood was allowed to dry overnight in the university laboratory open spaces. The bloodstained articles of clothing were left uncovered and not in any protective package nor storage cabinet.
<table>
<thead>
<tr>
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<th>Type Material</th>
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<td>Black</td>
<td>Shirt</td>
<td>Plain/Logo</td>
</tr>
<tr>
<td>B</td>
<td>100 % Cotton</td>
<td>Gray</td>
<td>Shirt</td>
<td>Plain/Logo</td>
</tr>
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<td>White</td>
<td>Shirt</td>
<td>Plain/Screen Print</td>
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<tr>
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<td>100 % Acrylic</td>
<td>White</td>
<td>Vest</td>
<td>Palin/Logo</td>
</tr>
<tr>
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<td>Blue/Purple</td>
<td>Blouse</td>
<td>Paisley Patterned</td>
</tr>
<tr>
<td>F</td>
<td>96 % Polyester, 4 % Spandex</td>
<td>Red</td>
<td>Blouse</td>
<td>Patterned</td>
</tr>
<tr>
<td>G</td>
<td>Unknown</td>
<td>Red/Green</td>
<td>Top</td>
<td>Floral Patterned</td>
</tr>
</tbody>
</table>

Table 1: Articles of Clothing

Figure 1: Standard photographs of blood-spattered articles of clothing,
Camera, Light Sources, and Filters

A Fujifilm IS-1 digital camera was used for all photography applications. The camera was purchased from RC Forensic as an infrared-ready digital camera. The kit included a 58mm Tiffin standard hot mirror filter and an F-PRO B+W 58mm 093 IR filter.

A 58 mm Marumi, YA2 filter was used for fluorescein applications, and as an orange barrier filter for alternate light source (ALS) applications. A yellow filter 58 mm, Bower, Y2 was also used for ALS light applications.

Polarization was obtained with a 58 mm CPL circular polarizing filter, and a sheet of linear polarized material attached over the lens of a Ryobi Xenon Hi-Beam hand held spot light. The polarized film was a sheet of PF006-Linear Polarizer purchased from AFlash Photonics, http://www.polarization.com/polarshop/.

The authors were aware that DeForest, et al. used linear polarizing material for the camera filter and the light source filter. Baldwin used a linear polarizing material on a camera flash light source, and a circular polarizing filter on the camera. Robinson noted that circular polarizing filters work best on digital cameras with auto focus features. We elected to use circular polarizing filter for the camera based on cost and ease of use of a polarizer. A Horiba Jobin Yvon, model HS-100-12F, Handscope was used as the alternate light source (ALS). This light source allows multiple wavelength settings, and is not unlike those used by many crime scene investigation units. Fluorescein was used and applied via a standard Hemascein kit from Abacus Diagnostics: http://www.abacusdiagnostics.com/hemascein.htm. Infrared light was provided using a handheld FoxFury Scout Series, Model 300-117 Forensic IR light, and a standard silver dome with an EIKO, Photoflood, BBA light bulb. We named this unit the DOME/EIKO NIR system.

Ultraviolet light was provided using the FoxFury Scout Series, Model 300-111 Forensic UV, and the ring light, UV light, from RC Forensics.
Methods

Once the blood had dried, standard photographs were taken of each article of clothing using autofocus and without the use of flash. The Fujifilm IS-1 required placement of the hot mirror filter on the camera for standard color photographs.

Infrared photographs were taken using an F-PRO B+W IR filter with manual focus and the aid of flash. The Fujifilm IS-1 required removal of the hot mirror filter prior to use of the IR filter. Infrared photographs were taken using the FoxFury light source and the DOME/EIKO light source. This provided three methods of IR light.

The on-camera flash with the IS-1 was part of the camera and did not entail additional cost. The FoxFury handheld source starts at about $100.00, and the Dome/EIKO bulb costs about twenty dollars. The FoxFury and the Dome allows hand adjustments of the light source. The on-camera flash is a fixed position source.

The IR filter was then removed, the cut filter replaced, and the orange filter (Marumi 58 mm YA2) was added. Photographs were taken of the dark and patterned clothing with the orange filter and using a Handscope Horiba Jobin Yvon model HS-100-12F as the ALS light source. The Handscope was set to CSS, 615 nm, and 415 nanometers (nm). Photographs were taken at all wavelengths. The orange filter was then removed while the cut filter remained in place, and a 58mm, Bower, Y2 filter was added. Photographs were again taken with the Handscope set to CSS, 615, and 415 nanometers. Only samples A and G were photographed before it was realized that no bloodstains were enhanced with these techniques.

A circularly polarized light filter (58 mm CPL filter) was attached on top of the cut filter to the camera, and a sheet of linearly polarized light filter was cut and attached to a Ryobi Xenon Hi-Beam light source. Photographs were taken of the garments with and without the linearly polarized light source. The circularly-polarized light filter and cut filter remained on the camera throughout the testing of this technique. This allowed visualization with just the circularly-polarized filter, and visualization with the combination of the circularly-polarized filter and linearly-polarized filter. White garments, samples C and D, were not photographed because the bloodstains were visible to the naked eye without the need for further enhancement.

The efficacy of fluorescein as an aid for discovering bloodstain evidence on clothing was tested. A Hemascein kit from Abacus Diagnostics was used for this technique. The application technique recommended by Abacus Diagnostics as presented in the literature with the kit was followed for the application of the fluorescein product. Swabs of bloodstains from each garment were taken prior to the addition of Hemascein for DNA analysis purposes. The Hemascein material and five percent hydrogen peroxide (H₂O₂) were lightly misted on each garment and the reaction was allowed to proceed for approximately five minutes. When no visible reaction was observed, a heavier application of Hemascein was used and allowed to react. The Handscope set to the CSS setting for the ALS, and the orange filter was used on the
camera. We used orange plastic viewing sheets to aid us personally in viewing the reaction. We learned, serendipitously, that after dousing the garments with Hemascein and hydrogen peroxide, reactions were visible to the unaided eye. This provided the opportunity to take photographs using only the standard color photography mode. Bloodstains on each saturated garment were swabbed again for DNA analysis purposes.

Results

This study revealed that IR photography worked the best of the all of the tested techniques for discovery and photographic documentation of bloodstains on dark clothing. The only drawback to this seemed to be availability of an IR ready camera. Connor\textsuperscript{9} and Richards\textsuperscript{10} noted that materials that absorb NIR light nearly the same as blood will not show photographic contrast. Figure 4 shows that bloodstains were indicated on all materials using NIR photography with the Fujifilm IS-1 on-camera flash. Although some images presented low contrast, the authors have experienced increased contrast using computer enhancement methods.

The on-camera flash required using the camera auto focus for ease of operation. This led to some blurred focus of images. Images were taken using the FoxFury and the DOME/EIKO as the light sources. Item D, the white vest, was not included since NIR added nothing to discovery of bloodstains visible with the unaided eye. Use of the additional light sources indicated NIR photography captured the contrast between the substrate and the bloodstains.
Unlike Connor, et al. we captured contrast on black cotton material. Contrast was also captured on patterned, synthetic, materials. The use of a tripod and manual focus added to the clarity of the images.

Figure 6: NIR photography with FUJI IS-1, FoxFury light source, tripod, and manual focus.

Figure 7: NIR photography with FUJI IS-1, Dome/EIKO light source, tripod, and manual focus.
We later discovered that there was a small bloodstain in the right eye area of the sunburst image in Item C, the white t-shirt with screened image. We discovered this bloodstain after application of Hemascein and illumination of the substrate with an RC Forensics handheld UV ring light. The bloodstain had not presented in the NIR applications. Hemascein reactions can be viewed just starting around the bloodstains to the right of the screened image. See the image inside the blue circles on the below photograph. The upper and lower right eye showed a reaction that was not as visually prominent as the other bloodstains. This area bounded by the red circle in Figure 8 was determined to be the location of a bloodstain. The areas bounded by the blue circle corresponded to initial Hemascein reactions on higher volume bloodstains. The area bounded by the green circle corresponded to a bloodstain of higher volume not illuminated with the UV light source.

Figure 8: Image taken using FUJI IS-1 setup with IR filter removed, Hot Mirror Filter installed, Marumi, YA2 filter installed, and substrate illuminated with RC Forensic UV ring light.

The reaction in the eye may be due to a lower volume of blood. Hemascein seems to work best with lower volumes of blood. Large volumes take longer for a visible reaction as indicated by the stains in the blue circles. The effect of the absorbance value of the substrate and the confusion caused by the pattern of the image along with the volume of the bloodstain may have affected the inability of the NIR and other techniques to visualize this bloodstain. We
noticed this bloodstain in the eye became more visual as the material was saturated with Hemascein.

It has long been a practice among crime scene investigators, in the authors’ experience, to use an alternate light source (ALS) to locate bloodstains. Light wavelengths in the blue light range, approximately 450 nm, and the green light range, approximately 580 nanometers, are common ALS settings. Robinson gives an example in his textbook on enhanced visualization of a bloodstain on multi-colored material using UV techniques. An orange or yellow barrier filter is often used for discovery of other body fluids and fiber trace evidence. We used these techniques since they are commonly employed to determine if there was any value in discovery of latent bloodstains. Our results indicated that there was little value in using orange or yellow filters or an ALS.

Figure 9: Images were taken using the FUJI IS-1 with hot mirror filter and Marumi YA2 orange filter with ALS HANDSCOPE set to CSS, 615, AND 415.
Figure 10: Images taken using ALS set at CSS with yellow camera filter. Only samples A and G were photographed because it was noticed that enhancement was not presented with this technique.

A UV light source, purple light, approximately 390-400 nm, and an orange filter produced some results on the black shirt. As can be seen, green light aided little in visualization of bloodstains on the black shirt.

Figure 11: UV on the left, green ALS on the right

The cross-polar technique using the linear polarization on the light source and circular polarization on the camera presented some value in discovery of bloodstains. Our results indicated that use of the circular polarized filter only on the camera worked equivalently to the cross-polar application. The camera-only filter technique allowed more ease of operation in adjusting the filter. With computer enhancement, the use of polarization could become a more suitable technique. Figure 14 is the enhanced image of the black shirt in Figure 12. This enhancement used Irfanview photo software. Irfanview, a free photo editing software, can be found at http://www.irfanview.com/. The image in Irfanview was enhanced using “Auto adjust colors,” and “Show channel > red channel.”
Figure 12: Images taken with combined polarizers.

Figure 13: Images taken with only circular polarizing filter on camera.
Figure 14: Enhanced Image of black shirt shown in Figure 12.

Figure 15: Images of articles after application of Hemascein.
Hemascein was applied to all of the articles of clothing. We found that given sufficient time, it would provide results that indicated location of bloodstains. In the case of the white t-shirt with the sunburst screen-printed pattern, Figure 8, it manifested a bloodstain that had been missed with the other techniques. This manifestation, however, was after the reaction had been allowed to proceed for several minutes. In one of the author’s experience, it often takes Hemascein about 15 minutes before the reaction can be visualized.

We discovered, serendipitously, that a reaction to the Hemascein technique can visually manifest without application of a light source or use of an orange barrier filter. We had applied Hemascein to the black shirt-shirt and were not seeing much of a reaction. Additional Hemascein and hydrogen peroxide were applied and the shirt was viewed using the Handscope CSS setting and an orange SPEX sheet barrier filter. Like NIR, the Hemascein technique can be affected by the absorption and reflection of light, which can be affected by the nature of the substrate. It can also be affected by the volume of blood and the age of the blood. Hemascien seems to work best on fresh low volumes of blood, and especially low volumes. The attempts to get a Hemascein reaction on the shirt led to a saturation of the shirt. We noticed that it eventually started to “glow” in the known areas of the bloodstains. The reaction could be enhanced by further light spray application of hydrogen peroxide.

We later tested the volume of reagents needed to saturate the material to get a reaction that could be viewed without a light source or filter. The results indicated that 20 sprays from the Abacus Diagnostics sprayer included in the kit would provide sufficient saturation. Testing of the sprayer indicated that twenty sprays equated with approximately 20 milliliters of Hemascein solution.

Figure 16: Black tee shirt saturated before and after reaction visualized without light source or barrier filter.

Photo editing software has been used to invert digital images for purposes of visualization of crime scenes. It is a technique that has been testified to in a murder jury trial.
The following images are the inverted images of the articles of this project. Irfanview was the software used for the inverting of the images. To invert an image using Irfanview, open the image, and then select Image > Negative (invert image) > all channels. It can be seen that some images showed a clear contrast, which indicated the presence of a bloodstain. Bloodstains showed a bluish coloration in the invert state. The technique worked best with lighter-colored materials.

![Figure 17. Invert images of articles of clothing](image)

At the time of this report, data is not available on the integrity of the DNA swabs from the bloodstains. We expect, due to previous professional experiences, that there will not be damage to DNA. With the discovery of the effect of Hemascein saturation on bloodstains, establishing the integrity of the saturated bloodstains took priority. Analysis of the bloodstains for DNA is in progress at the time of this report.

**Discussion**

The NIR application enhanced bloodstains on dark or darkly-patterned clothing very well. It also worked well to enhance bloodstains on white garments, as long as no patterns were included. Infrared photography did not enhance bloodstains on gray clothing very well, nor on stains on white shirts when the stains are within a screen-printed design. Volume of blood, nature of the material, force, and light source can have effects on NIR techniques. Materials that absorb NIR at about the same level as blood provided less contrast. This appeared to be possible to overcome with to some degree with computer enhancement. Unlike the research by Connor, et al.\(^\text{12}\), we had success with bloodstains on dark cotton clothing. NIR worked very well with bloodstains on darker patterned clothing. However, we
only tested bloodstains formed from dropping blood. We had a higher volume of blood than stains often made by transfer methods or diluted stains. Based on the authors' experiences, bloodstains cannot be enhanced on black rubber vehicle tires, plastic purses, faux leather coats, and synthetic materials shoes. Due to previous professional experience, we did not feel that testing these materials were necessary for this project based on the focus of the project. It is noted that many variables affect NIR digital photography.

NIR photography required a light source. Sunlight can provide an adequate source. It can be blocked but cannot be moved around. The camera flash on the FUJI IS-1 provided adequate NIR light. The use of the auto camera flash and auto focus system on the camera produced slightly blurred images. In many cases for pure discovery this was an adequate technique. When clear images would be needed it would be best to use an off-camera light source with the camera stabilized on a tripod. This would allow for manual focus, multiple images at the same settings and position, and bracketing target images.

It was possible to have high intensity of IR light from the FoxFury and the Dome/EIKO system. IR images could be burned out by the source. Thus, the ability to move the light source to adjust its intensity is an aid in getting clear and shaper images. In many cases bouncing the IR light off the ceiling or wall was the best method for adding NIR to the process. Connor, et al. found this a useful technique. Light sources add an extra cost to the NIR technique. The FoxFury source provided more than adequate NIR light. Costs start at about $100 for such a handheld source. The DOME/EIKO bulb system costs about $20. One must make sure that the DOME receptacle is sufficiently rated for the EIKO bulb. A 350-watt rating would be sufficient for this bulb. The dome system costs about $15 at most hardware stores. The EIKO bulb costs about $3 at most photography supply stores.

NIR photography requires an IR ready camera. Most digital cameras of the bridge or DSLR model can be converted for NIR applications. LifePixel, http://www.lifepixel.com, is a business used by many for this service. The authors have used http://perfectimagecamera.com owned by Wes Kaufaman for NIR conversion. In addition to the cost of the camera a conversion could cost a substantial amount. The authors' have had cameras converted for $500 with an additional $500 for an IR lens. A good hot mirror lens to fix the camera for standard photography costs about $100. The total cost, including a $750 DSLR digital camera, ran about $1500 for the NIR technique.

Hemascein kits can be order at https://www.abacusdiagnostics.com/orderform.php#G. Hydrogen peroxide, in concentrations of 3-5%, can be purchased at any drug store for $10 or less. A light source, ALS, could cost $5000 or more depending on features of the unit. However, one could use a FoxFury UV light that costs less money.

Polarization worked on some materials. It was not as effective as NIR on dark materials, but with computer enhancement it offered an alternative technique at a much lower cost. A polarized filter can be purchased for under twenty-five dollars. On light materials, it did not add much to visualization since bloodstains are most often readily visual on white materials.
Ultraviolet (UV) and ALS techniques did not add to what could be visualized with NIR, polarization, or inversion of standard color images. However, the UV light, purple light, had some value in discovery of bloodstains, and they are a less expensive and time consuming method than NIR or Hemascein techniques. It seems that a UV light source in the 390 range works best for visualization of bloodstains. Short UV wave lengths present a health hazard, may not work with all digital cameras, and seem to work best on light materials. A handheld UV light in the 400nm range can be purchased for about $50.00 from RC Forensics, Rob Cheeseman. FoxFury makes a handheld UV light, around $100.00, that provides adequate intensity for discovery of bloodstains. It is a technique that offers an alternative to NIR processes.

Hemascein added a dimension most likely left for last-ditch measures. For example, we only noticed the bloodstain in the screen pattern when Hemascein was applied, and the bloodstains on the dark t-shirt became readily visible after saturation of the material.

Based on our research we suggest the following protocol for visualization of potential bloodstains on dark colored, patterned, and screened image clothing.

<table>
<thead>
<tr>
<th>Step</th>
<th>Name</th>
<th>Technique</th>
<th>Equipment</th>
<th>Rating</th>
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<tbody>
<tr>
<td>1</td>
<td>Standard View</td>
<td>View with the unaided eye/white light. Bloodstains on light colored materials are obvious without further technique</td>
<td>Visible light wavelength range</td>
<td>7</td>
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<tr>
<td>2</td>
<td>Computer</td>
<td>Invert standard images, adjust saturation/gamma/contrast, change channels</td>
<td>Computer, photo software</td>
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<td>Polarization</td>
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<td>7</td>
<td>Douse Hemascein</td>
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Conclusion

One should always use standard visualization techniques first. If you can see something with the unaided eye that you can swab and test, that should be the first method used. Swab and test using presumptive tests if appropriate to the event.

The steps in the above chart are based on the most effective technique involving the least amount of time and preparation. However, the most effective method is not always the most efficient method. If one can see bloodstains prior to NIR, such as with use of a UV light source or a polarized camera lens, it makes sense to use those steps.
If one cannot see bloodstains and wants to ensure the best chance to discover the bloodstains through visualization, we recommend following the rating of the methods. NIR, as an example, proved the most effective and efficient at discovering bloodstains on dark and patterned clothing. It has some limitations, as discussed in the literature and in our research.

One may elect to apply Hemascein. It is a rather last ditch effort technique. It has been proven in case work and in our research that it has the capacity to aid discovery of bloodstains not visualized through other methods. We would not have discovered the bloodstain in the eye of the screened pattern without application of Hemascein. In exceptional cases one might want to douse clothing material with Hemascein. We do not know if this technique will affect DNA. Testing is taking place at the time of this report.

Our research was limited by the number and kind of materials, the nature of the bloodstains, the light sources we used, the materials we used, and the cameras we used. Other light sources, filters, and cameras could have different results. We submit, however, that the techniques we presented are viable techniques for the discovery of bloodstains on dark clothing considering there are exceptions to any rule.

Acknowledgement

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References


