Report Title:

Blood on Black- Enhanced Visualization of Bloodstains on Dark Surfaces

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Authors:

Peter R De Forest, Rebecca Bucht, Frani Kammerman, Brooke Weinger, Lauren Gunderson

Abstract

Accurately visualizing and documenting bloodstains and patterns is an integral part of crime scene investigation and can provide crucial information for both the analysis of evidence in the laboratory and crime scene reconstruction efforts.

Visualization of bloodstains is trivial on white or lightly colored surfaces. However, on darkly colored or black surfaces, this visualization can be extremely difficult. The failure to visualize and thereby recognize blood and bloodstain patterns on darkly colored surfaces has had seriously adverse consequences for important criminal investigations.

There are two aspects to the problem. First, the presence of blood may not be recognized at critical stages in the investigation. Second, where the presence of blood is recognized, the pattern of blood-staining may not be appreciated. Sampling of bloodstains for DNA typing and other analyses must take place with knowledge of the bloodstain patterns. Otherwise important information may be destroyed. In a significant number of cases knowing how the bloodstains were formed is more important than knowing the biological source of the stains. In most cases the two types of information are complementary.

Photography represents a nondestructive method of documenting stains. Traditionally, black and white photography uses color filters to either lighten or darken a stain against the surrounding background to elucidate the forensic information contained on a difficult substrate. This technique, however, provides little benefit with bloodstains on very dark and reflective surfaces. Observing and documenting bloodstains on these surfaces is problematic due to the glare reflected off of the surface as well as the lack of contrast between the stain and substrate.

Previous studies have shown the usefulness of chemical enhancement techniques on bloodstain patterns, with the drawback of potentially compromising DNA analysis and altering the stains. IR photography, performing background corrections on digital images and the combination of digital photographs taken at several wavelengths have also been shown to enhance visualization of blood on some strong colored substrates.

During the course of examining evidence in cases, we observed stunningly dramatic improvement in the contrast between the otherwise subtle bloodstains and the dark or black background by using polarizing filters over the light source and the camera lens. This project has resulted in the determination of the optimum parameters and limitations for the enhancement, a better understanding of the mechanism behind the phenomenon and increased awareness of the technique within the field.

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Introduction

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In the Principal Investigator's experience there are two aspects to the problem. First, the presence of blood may not be recognized at critical stages in the investigation. Second, where the presence of blood is recognized, the pattern of blood-staining may not be appreciated. Sampling of articles bearing bloodstains for DNA typing and other analyses must take place with knowledge of the bloodstain patterns. Otherwise important information may be destroyed. In a significant number of cases knowing how the bloodstains were formed is more important than knowing the biological source of the stains. In most cases the two types of information are complementary.

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Previous studies have shown the usefulness of chemical enhancement techniques on bloodstain patterns, including luminol, amido black, and leucocrystal violet, with the drawback of potentially

compromising DNA analysis and altering the morphology of the stains. Documenting the result of some chemical enhancements also requires specialized photographic techniques.

Performing background corrections on digital images and the combination of digital photographs taken at two or three wavelengths have also been shown to lead to enhanced visualization of blood on some strong colored substrates. With this method there is a significant time lag between capturing the image and being able to visualize the stain.

IR photography has also been used to enhance the contrast between bloodstains and certain substrates, especially those that are multicolored with dyes that do not absorb IR.

During the course of examining evidence, we have done some exploratory work using polarizing filters over the light source and the camera lens. We have observed stunningly dramatic improvement in the contrast between the otherwise subtle or even unrecognized bloodstains and the dark or black background.

Samples and equipment

All stains used were prepared by the project staff using fresh blood. The blood was obtained, in accordance with IRB approved methods, by piercing the tip of a finger with a lancet designed for that purpose. Four different types of stains were produced. ‘Smear’ stains were produced by smearing a thin layer of blood across the surface of the substrate using the pierced finger. ‘Contact’ stains were produced by blotting the surface of the substrate with the pierced finger. ‘µL’ stains were produced by pipetting a given µL amount of freshly bled blood onto the surface of the substrate. ‘Small spatter’ stains were produced by dipping the bristles of a toothbrush into freshly shed blood and creating small spatter by flicking a finger across the bristles while holding the toothbrush over the substrate. All stain making methods were tested on white paper in order to ascertain that the stain making methods resulted in the type of stains we were intending to make. These four stains cover the sizes and thicknesses or blood stains one could expect to find in case scenarios.

The polarized method requires three main components, a white collimated light source with a linear polarizing filter, the surface to be sampled on a dark matte background, and a camera with a linear polarizing filter.

Polarized Light Method Setup & Components

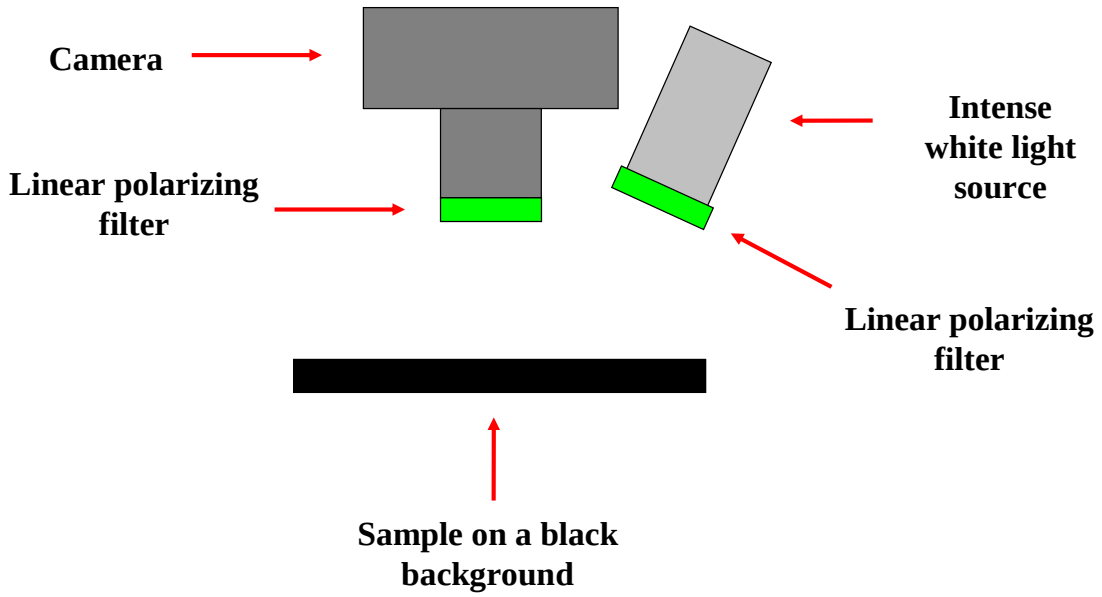


Fig. 1

Optimum parameters

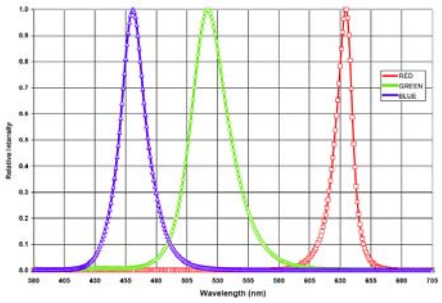
The lack of a suitable light source was the main problem in early experimentation using this enhancement method. The best results were obtained with a fiber optic light, which also caused the most damage to the polarizing filter in front of it. Less intense lighting options also caused considerable damage to the polarizing filter, even if a heat absorbing glass and air flow was present between the light source and the filter. Since experiments with narrow wavelength sources and polarizing filters did not yield particularly good results, we assumed that the entire white light spectrum would be needed. LEDs produce 'white' light without extraneous IR or UV radiation, so we started our search by experimenting with LEDs.

LEDs use one of two technologies to produce white light. The more common method, known as 'white LED', is a blue diode covered with phosphor to convert a portion of the blue light to yellow light. This combined spectrum is perceived as white light. The output spectrums of these LEDs have a specific shape but do vary somewhat depending on the wavelength of blue LED and phosphorescent compound used.

The less common method is known as RGB LED and uses a combination of red, green and blue LEDs to mimic the CIE colorspace of white light. The main variables in these LEDs are the wavelengths of the red, green and blue LEDs as well as the output intensities of those three LEDs.

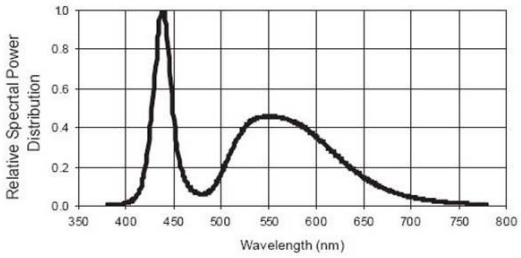
The RGB LED, the Zylight Z90, turned out to be by far the best option. The enhancement produced is comparable to that obtained with traditional white light sources and there has been no noticeable damage to the polarizing filter, even after over one year's heavy use. It is the only commercially available RBG LED portable and maneuverable enough to be used comfortably and efficiently in a lab or crime scene setting.

RGB LED vs White LED



Z90 Spectrum, RGB LED

Blood on leather, Z90



Foster Freeman S80 spectrum, 'white' LED

Blood on leather, S80

Fig.2

The effects of LED intensity, saturation and color temperature were tested. It appeared that the intensity did not significantly affect the enhancement, but a lower intensity requires a longer exposure time which can be more cumbersome in practical applications of the technique. The neutral or zero saturation provided the best results. Though the appearance of the image changed noticeably with the change in color temperature, there was little difference in the enhancement produced. With the higher color temperatures, the substrate background often took on a colder black color which can make distinguishing small or faint spatter easier, particularly on the ribbed cotton.

The Fuji ISPro has a number of setting choices for dynamic range, color saturation, tone, film simulation, white balance, color temperature and exposure compensation. Changing the dynamic range did not appear to impact the enhancement. Color saturation, tone and sharpness did show a visible difference between the high and low extremes of the settings, but did not seem to significantly impact the enhancement or the ability to discern the stain from the substrate.

The most dramatic changes were seen in varying the exposure compensation and film simulation. F2, the Fuji film simulation choice that mimics slide film and is geared towards landscape and nature photography by providing a 'vibrant reproduction of natural colors' produced a discernibly improved enhancement over the other film simulation options. Within the F2 film simulation, one can also vary the color, tone and saturation settings. Decreasing the sharpness to -2 and color to -1 produced the best results.

The best images were produced by a shorter shutter speed than recommended by the built in light meter. This was accomplished by using the exposure compensation function. It was found that a compensation of -2 or -3 produced the best results. Longer exposure times tended to overexpose the image, making small spatter in particular hard to distinguish.

A number of pairs of linear polarizing filters, both low end and high end, were purchased. The polarizing filters with the better extinction ratio, ie which transmitted the least light while crossed, produced the better enhancement. The span across which the filter showed near-zero transmission was a better indicator of its performance than the maximum transmission. Though the True Pol had a lower maximum transmission than the Hoya and Heliopan, the two latter ones showed near zero transmission across more of the 400-700nm range and produced better images. The filters that produced the better results also required the longer exposure time under the same settings.

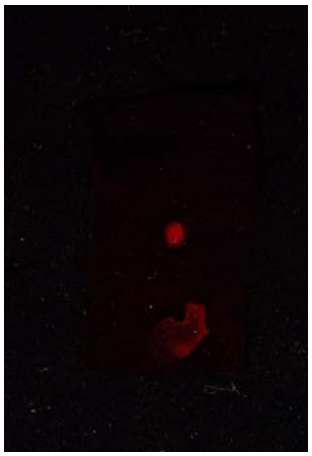
The effect of changing the angle of incidence of the polarized light was explored. Having the incident light at 90 or 45 degrees produced the best overall enhancement, partially because the surface examined was more evenly darkened at those angles. This held true for close up photographs of stains as well.

The effect of the orientation of the plane of incident polarized light was found to be substrate dependent. Some substrates like wool and leather did not react noticeably to the orientation of the plane of incident polarized light. Others such as polyester and silk were strongly influenced.

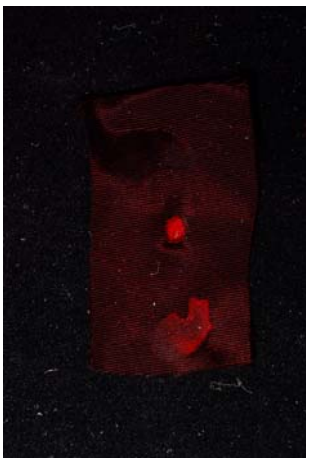
In the case of one polyester sample, rotation of the incident polarized light changed the substrate appearance from red to black. Stains were, naturally, more visible when crossing the polarizers resulted in a dark background. It should be noted that this change from red to black background is not readily apparent through the viewfinder.

Effect of Orientation of Incident Polarized Light on Polyester

Uncrossed



Crossed



Crossed rotated ~20°

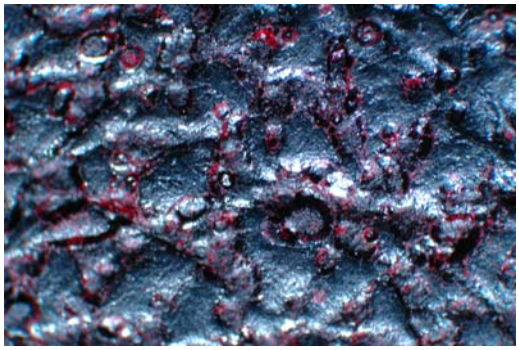
Fig. 3

Mechanism

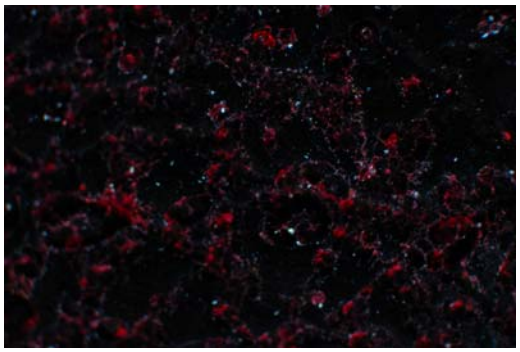
It is readily apparent from the photographs that turning the two polarizing filters to extinction reduces the glare from the surface of the substrate as well as the stain.

These stains were observed with an Olympus BX41 PLM, using the Z90 to produce reflected polarized lighting. Both the difference in color between the thick and thin portions of the stain and the reduction of glare from the stain and substrate are apparent.

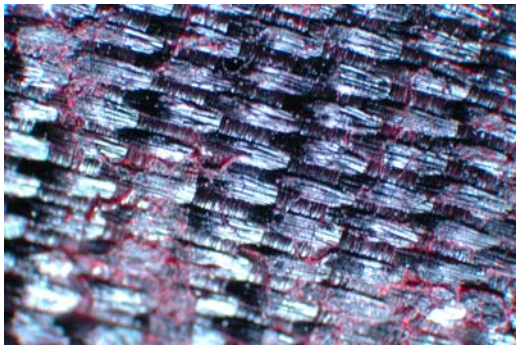
Micrographs



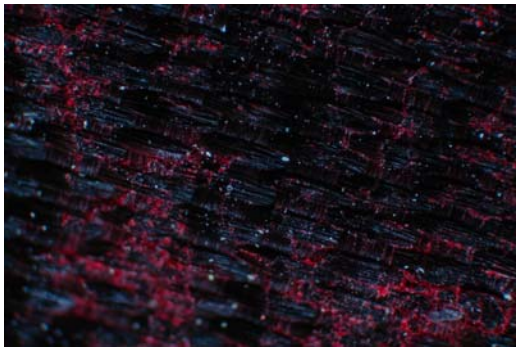
Smear on leather x40 Uncrossed



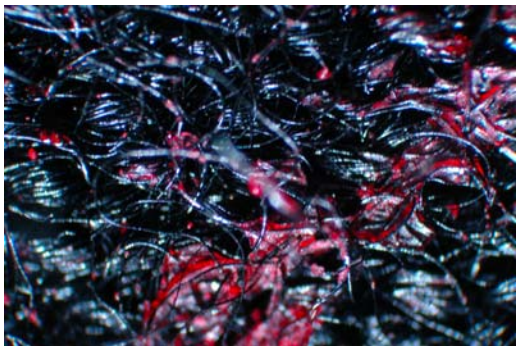
Smear on leather x40 Crossed



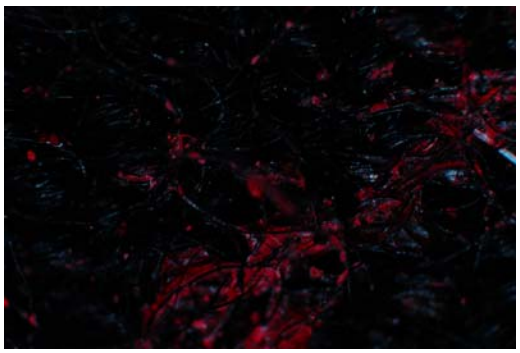
Smear on polyester x40 Uncrossed



Smear on polyester x40 Crossed



Smear on wool x40 Uncrossed



Smear on wool x40 Crossed

Fig.4

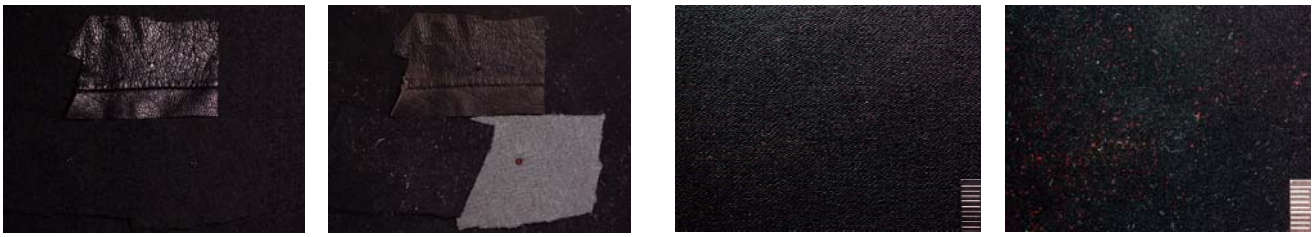
Features & Limitations

Stain size impacted the enhancement through its effects on the absorption and thickness of the stain. Otherwise the limitations of visualization were those posed by zoom, resolution and focus of the photography.

Stains that were absorbed into the substrate as opposed to drying on the surface of the substrate were harder to visualize. The contours of these stains were more prominent. Small spatter did not tend to be absorbed into the substrate which leads to it being particularly successfully enhanced on most dark substrates. Due to its small size, some zooming in on larger surface area images is necessary in order for the smaller spatter to be properly resolved.

Different Stain Types

Contact on wool, leather & cotton **Smear on wool, leather & cotton**



10µL drop on wool, leather & cotton **Small spatter on wool**

Fig.5

Initial experimentation with the five different substrates confirmed that all substrates did not interact with the blood or the polarized light in a uniform manner.

Stains on substrates which contain one or more lighter colored elements show barely any enhancement. The reflection off the lighter colored elements is not diminished by crossing the polarizers, and

the excess reflected light dominates over the reflection from the stain. If the pattern of the substrate allows for it, this excess reflection from the lighter portions can be minimized by covering them with a dark swatch or choosing the field of view so that it contains only the dark portions of the sample. When the sample or object being examined is small, it should be photographed on a dark substrate to minimize the stray light interfering with the enhancement.

Uniformity of the plane of the substrate can also be an issue. If the substrate is uneven, it can be difficult to illuminate sufficient portions of it so that the entire field of view is under crossed polar lighting.

During our stain making process we observed that the stains would not retain their red color while drying. When first deposited on the substrate, the blood would appear very bright. During the process of the stain drying, the bright red would fade away and then reappear as the stain dried, if the stain was a thin stain. Thick stains remained dark. Once the stain was dry, there was no discernible change to the color or enhancement. The stains also maintained this contrast as they aged. Stains were also photographed up to one year after they were made and no visible change in the enhancement was observed.

IR Photography is another bloodstain visualization and documentation method that does not involve any physical or chemical alteration of the surface being examined. Due to the development of digital SLRs with IR sensitivity, IR photography is gaining popularity.

In terms of equipment, crossed polar illumination does not require a specially configured camera. Taking the photograph is also easier because one can observe the subject through the viewfinder. With IR, the lens needs to be focused prior to the addition of the IR filter because the IR filter is not transparent in the visible range.

Six of the plain substrates with all four types of stains on them were photographed using both methods. With respects to stain thickness, IR is complementary to crossed polar illumination as thick stains are visualized better with IR. Certain substrates like silk and polyester that can be more difficult to visualize with crossed polarized light are also better visualized with IR.

In order to compare the visualization of small spatter, we focused in on a few swatches in particular. We took close up photographs of the silk and polyester swatches with the 60mm lens. Silk and polyester were chosen because bloodstains on them were enhanced the best with IR photography. Even though the smears, contact and drop stains are more enhanced with the IR, the small spatter is not discernable at all under IR.

Discussion of findings

This research further confirmed the utility of crossed polar illumination in visualizing and documenting bloodstains on dark surfaces. Though there are limitations to the technique, the instances in which it can be of great value are plentiful.

Micrographs show that even seemingly matte surfaces like wool have a significant amount of white light reflected from them. They also show that the stain surface reflects white light as well. Crossed polar illumination eliminates this reflection from both surfaces, resulting in the two appearing more true to their intrinsic colors. This glare reduction appears to be the key element in the enhancement.

The light source and polarizing filters are the most imperative components of this method. LEDs produce intense white light with the least heat damage to the filter in front of the source and RGB LED is the preferred LED technology. The poor performance and blue green tint of the bloodstain produced using the 'white' LED technology may be partially due to its output in the 400-450nm area being stronger than that across the remainder of the visual spectrum and the crossed polarizing filters not being able to block the 400-5000nm range as effectively as the 450-700nm wavelengths.

The difference between the performances of most polarizing filters is less drastic than that of the different LED types. In terms of the transmission curve of two crossed polarizers across the visual range, the wavelengths across which there was virtually zero transmission was more indicative of better performance than the maximum intensity of transmitted light. For filters with similar ranges of near-total extinction, the ones with a lower maximum transmission were better. Given the dependence of the enhancement on the effective blockage of glare, this is to be expected.

Though it may seem counterintuitive, seeing better enhancement with a negative exposure compensation, which produces a darker photograph, is in line with Weber's law for light and sound perception and the theories behind visual adaptation. The less brightness there is from the background, the more contrast is produced by even a small luminance difference between the stain and the background. Other camera settings which improve the enhancement are hard tone settings and slide film simulation.

Another essential tool for this method is a tripod, copy stand or other camera support structure. Despite the better enhancement with underexposed images, the exposure times were always well beyond the limits of handheld photography. Attempting to overcome this handicap by pushing the ISO setting of the camera requires an ISO number in the thousands. Such a high ISO setting or an unstable camera compromises the resolution when zooming in on the image, which affects the ability to detect small spatter.

The stains that are enhanced the best with this technique are thinner stains and small spatter. Thick stains are harder to locate using this method unless they are surrounded by thinner stained areas that are enhanced. In practice this does not constitute a problem, as thick stains tend to be easily visualized using oblique lighting or IR photography.

Substrate features have the potential to interfere quite a lot with the enhancement. Materials that absorb well leave little residue on the surface to be enhanced. Reflection off of any light colored components of the substrate is not as effectively quenched under crossed polar illumination, which limits the enhancement possible. Some dyes and fiber types are affected by the orientation of the incident polarized light and may require some experimentation with different filter rotations to achieve the best darkening of the background. Uneven surfaces are harder to work with, as the area which can be successfully brought under extinction is smaller. Any manual flattening out of surfaces should be done carefully in order to minimize potential dislodgement of stains or other materials.

The area which can be covered by each frame is limited not only by the lens and working distance, but also because thin, faint stains and small spatter require close up imaging to be detected. It should be noted that these stains are also rarely distinguishable though the viewfinder or the LCD display of the camera and require the photograph to be viewed on a larger screen, such as that of a portable computer.

The testing of false positive, false negatives and dilutions suggest that as long as a stain retains a red hue, this redness will be enhanced. The darker appearance of thicker bloodstains on white and light colored substrates may explain why they are not as well enhanced as the thinner stains that appear red.

Fibers and many other small particles were also enhanced so this method may also be useful for finding and documenting other types of evidence.

Implications for policy and practice

Virtually every law enforcement agency in this country has occasion to investigate crimes against the person in which patterns of bloodstains are encountered and require photographic documentation for subsequent bloodstain pattern analysis and crime scene reconstruction. There are three main aspects of bloodstain analysis that this visualization and documentation contribute to.

The first one of these is the detection of blood in the early stages of the investigation. This allows for proper measures to be taken for documentation and preservation of the blood evidence. The presence, location and morphology of blood stains are often of great importance in any investigation, and the earlier this information is available, the better. This technique provides a method for early screening of surfaces that is temporally and logistically convenient.

The second point to be made is that of intelligence driven sampling. Stains are commonly analyzed in order to confirm that they are blood, and often further analyzed to determine their origin. Being able to visualize the stains allows for selective processing of the surface. The morphology of the stain provides information about how it was deposited on the surface, which allows for selection of the more pertinent stains to sample. In cases where the surface examined is large, fewer samples need to be taken as the sampling can be focused on specific areas. Being able to focus on more heavily stained areas also ensures the collection of ample sample for further analyses. Where there are multiple sources of blood, the occurrence of mixed profiles in consequent DNA analysis can be minimized by sampling stains individually. More focused sampling also lessens the likelihood of disturbing or contaminating other potential evidence on the surface.

The third area where this visualization and documentation is important is that of the interpretation of the evidence. The location and morphology of the stains are key elements not only in the investigation, but

also in any event reconstruction efforts. The ability to assign a DNA profile to a particular stain as opposed to a surface or collection of stains is important both in cases with multiple sources of blood or DNA but also where there is a single source of blood or DNA.

Thorough testing and documentation of the enhancement technique has not only determined the optimum conditions and limitations of the technique, it can also serve to satisfy the scientific testing required of forensic methods presented in the courts of law.

Final Technical Report

I. Introduction

Statement of the problem

Accurately visualizing and documenting bloodstains and patterns is an integral part of crime scene investigation and provides crucial information for both the analysis of evidence in the laboratory and crime scene reconstruction efforts.

Visualization of bloodstains is trivial on white or lightly colored surfaces. However, on darkly colored or black surfaces, it can be extremely difficult. The failure to visualize and thereby recognize blood and bloodstain patterns on darkly colored surfaces has had seriously adverse consequences for important criminal investigations.

In the Principal Investigator's experience there are two aspects to the problem. First, the presence of blood may not be recognized at critical stages in the investigation. Second, where the presence of blood is recognized, the pattern of blood-staining may not be appreciated. Sampling of articles bearing bloodstains for DNA typing and other analyses must take place with knowledge of the bloodstain patterns. Otherwise important information may be destroyed. In a significant number of cases knowing how the bloodstains were formed is more important than knowing the biological source of the stains. In most cases the two types of information are complementary.

In the O.J. Simpson case the defendant's black nylon dress socks were inspected and manipulated by defense pathologists before bloodstains later identified as having come from Nicole Simpson were visualized and documented by the Los Angeles Police Department Laboratory. The defense pathologists later claimed that they had seen no blood. This led to a series of problems that adversely affected the prosecution. Despite the flaking off of blood due to the unnecessary handling, blood was later visualized on the critical sock. It would have been far better if this had been done earlier. In the Carolyn Warmus or "Fatal Attraction" homicide case, bloodstains on black cashmere gloves were not recognized initially. This lack of recognition led to serious problems for the prosecution.

Photography represents a nondestructive method of documenting stains. Traditionally, photography uses color filters to either lighten or darken a stain against the surrounding background to elucidate the forensic information contained on a difficult substrate. This technique, however, provides little benefit with bloodstains on very dark and reflective surfaces. Observing and documenting bloodstains on these surfaces is problematic due to the glare reflected off of the surface as well as the lack of contrast between the stain and substrate.

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Performing background corrections on digital images and the combination of digital photographs taken at two or three wavelengths have also been shown to lead to enhanced visualization of blood on some strong colored substrates. With this method there is a significant time lag between capturing the image and being able to visualize the stain.

IR photography has also been used to enhance the contrast between bloodstains and certain substrates, especially those that are multicolored with dyes that do not absorb IR.

During the course of examining evidence in less well-known cases, we did some exploratory work using polarizing filters over the light source and the camera lens. We observed stunningly dramatic improvement in the contrast between the otherwise subtle or even unrecognized bloodstains and the dark or black background.

In one bludgeoning case spatter-type bloodstains on the black nylon jacket of the defendant were easily visualized by the technique we developed. This was important evidence in the prosecution of the case. More recently, the full extent of bloodstains on a black leather jacket was missed by DNA analysts in a large state laboratory. The case involved a father accused of stabbing his seven year-old daughter multiple times. The defense offered the explanation that the victim had frequent nosebleeds. In such a situation DNA typing would have limited value. Our technique of visualization via polarized light photography revealed

bloodstains produced by airborne droplets and projected blood, as well as those produced by contact on significant areas of the jacket. The type and totality of the stains on the jacket proved crucial in the prosecution of the case.

Review of relevant literature

Visualizing and documenting the presence of bloodstains on substrates has long been recognized as an integral part of crime scene investigation. Blood evidence can be associated with many types of crimes and can be analyzed in terms of reconstruction as well as being associated with particular individuals as possible sources (7). For instance, Pizzola, Roth and DeForest (1986) discussed the dynamics of blood droplets that make them useful for reconstruction efforts (19,20). The first step in recognizing the presence of bloodstains is to visualize them on the substrates on which they are found. Often, dark surfaces pose problems with visualization efforts (23).

Chemical methods of identification are numerous. Grodsky, Wright and Kirk (1951) discussed and compared early methods of presumptive blood testing (12). Higaki and Philp (1976) further studied sensitivity, stability and specificity of phenolphthalein as a presumptive blood test (13). Garner, Cano, Peimer, and Yeshion (1976) evaluated tetramethylbenzidine as a presumptive test for blood (8). Bodziak (1996) proved the usefulness of leucocrystal violet to enhance shoeprints in blood (2). Theeuwen, van Barneveld, Drok, Keereweer, Limborgh, Naber, and Velders (1998) further classified the usefulness of chemical methods on footwear impressions in blood including in their study Amido Black, Coomassie Blue, Crowle's staining solution, DAB, DFO, Fuchsin acid, LCV, Merbromin, Ninhydrin, *o*-Tolidine, and TMB (25). Lee, Gaensslen, Pagliaro, Guman, Berka, Keith, and Phipps (1989), however, showed that many presumptive test reagents including fingerprint enhancing chemicals can have a significant destructive effect on subsequent serological testing of the bloodstain (15).

In addition to chemical methods of visualization, Lytle and Hedgecock (1977) discussed chemiluminescence methods to aid in the visualization of bloodstains (16). Gimeno and Rini (1989) presented a full flash photo luminescence method of photographing luminol bloodstain patterns (7). Laux (1991), however, studied the effect of using a chemiluminescent method of visualizing bloodstains

establishing that, while luminol did not affect some subsequent confirmatory and ABO typing tests, it did affect genetic marker analysis (14).

Visualizing bloodstains can also be done via methods of light source enhancement. Anderson and Bramble (1997) discussed using argon ion lasers, Polilight V, Polilight green, Superlite and shortwave UV for enhancement of bloodstains, but also found that shortwave UV for more than 30seconds precluded further PCR analysis of the stain (1). Platt (1982), however, showed that the extended use of an argon laser to visualize bloodstains resulted in little or no reaction using presumptive reagents afterwards (21). Shipp, Roelofs, Togneri, Wright, Atkinson, and Henry (1993), using white cloth substrates and limited exposure times and wavelengths, found no destructive effects of argon laser light, ALS and cyanoacrylate fuming on subsequent RFLP analysis (24). Hyperspectral Imaging is a liquid crystal-based imaging technique that combines standard digital imaging with common spectroscopic methods, such as Near-IR, Colorimetric and Fluorescence imaging and has been used to visualize and document bloodstains on a variety of otherwise difficult substrates (4).

Infrared photography has also been used in documenting bloodstains. Raymond and Hall (1986) presented an early article regarding the application of this technique to bloodstains, showing that the success rate is dependent on the condition of the blood, the substrate on which it is deposited and the region of the infrared spectrum being used (22). Perkins (2005) applied traditional (non-digital) infrared photography methods to bloodstains found on clothing, but had problems using this method on fabric samples that showed glare (18). Miskelly and Wagner (2005) discussed using spectral information in forensic imaging, specifically digital photography (17).

The use of digital photography in forensic science is an excellent way of documenting evidence. Goldthorpe and McConnell (2000) discussed the use of digital photography and video recording as a successful method of recording clinical forensic evidence for court presentations (10). Dalrymple, Shaw and Woods (2002) presented optimized digital recording protocol for crime scene impressions (6). Bullard and Birge (1996) established an application for a digital darkroom in a forensic science laboratory (3). The use of digital photography in forensic science often elicits some concern regarding evidence alteration. However, Grady (2001) showed that Adobe Photoshop's channel mixer served as a viable method for evidence enhancement (11). Crispino, Touron and Elkader (2001) worked to define strict guidelines for forensic scientists regarding digital equipment and the use of Adobe Photoshop software (5). More recently and

applicable to our study, Wagner and Miskelly (2003) offered background correction options for digital photography of blood evidence (26,27).

Rationale for the research

Our work prior to this project had been preliminary. There was much more to be done to elucidate the mechanism of the dramatic enhancement we had observed, determine the optimum equipment and parameters for the enhancement, and to thoroughly document it so that it can be shared with other forensic scientists.

This project has developed and introduced an improved method of photographing these dark, bloodstained substrates without the use of chemical enhancement techniques, specialized film needs or post-exposure manipulation of digital images.

II. Methods & Results

The methods and results of this study are described below along with descriptions of the samples and equipment used. The methodology has been broken down into the three main objectives of the research, finding the optimum parameters, determining the mechanism behind the enhancement and investigation of the limitations of the method.

Samples and equipment

Setup

Unless otherwise specified, all images were taken with the camera and sample on a Polaroid MP4 copy stand. Ambient light was minimized by the lack of windows, closing the door and turning off overhead lights. None of the images were enhanced or treated with Photoshop© or other image manipulation software.

Polarized Light Method Setup & Components

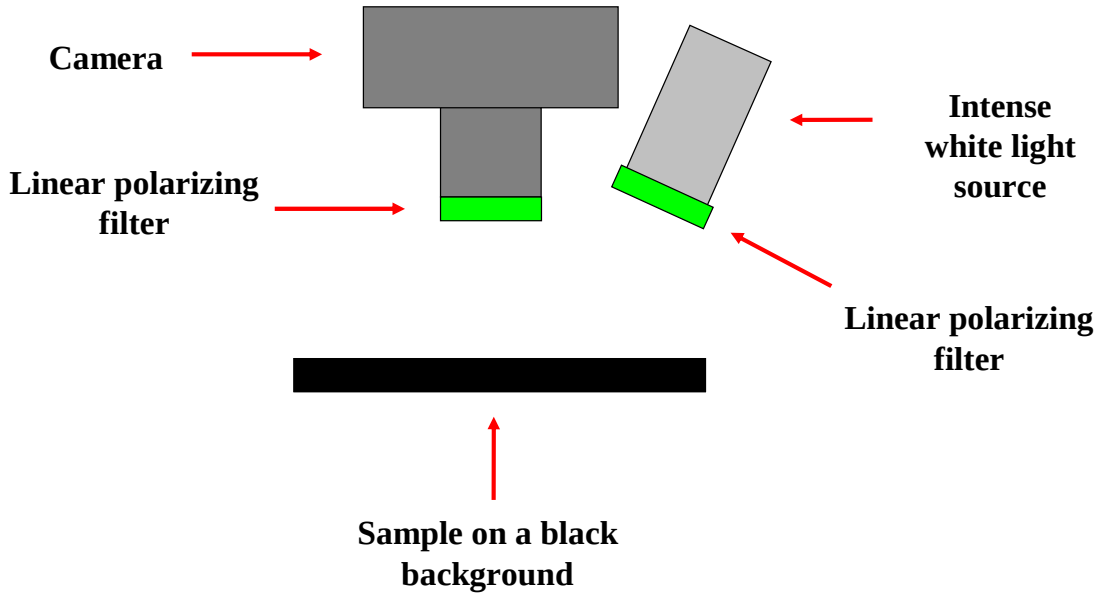


Fig. 1

Camera equipment

The camera used for this project was the Fuji ISPRo. It was chosen mainly for its IR capabilities as we wanted to compare the polarized light enhancement with that obtainable by IR photography. The lenses used were the Nikon 60mm f/2.8 macro lens and the Nikon 50 mm f/2 lens. The Peca 916 visible pass filter, which blocks most of the UV and IR that the ISPro CCD is sensitive to, was used for all regular and polarized light photography in order to have the IS Pro mimic the performance of a regular digital SLR.

Micrographs were taken using a Nikon D70 and an Olympus BX41 PLM.

Substrates

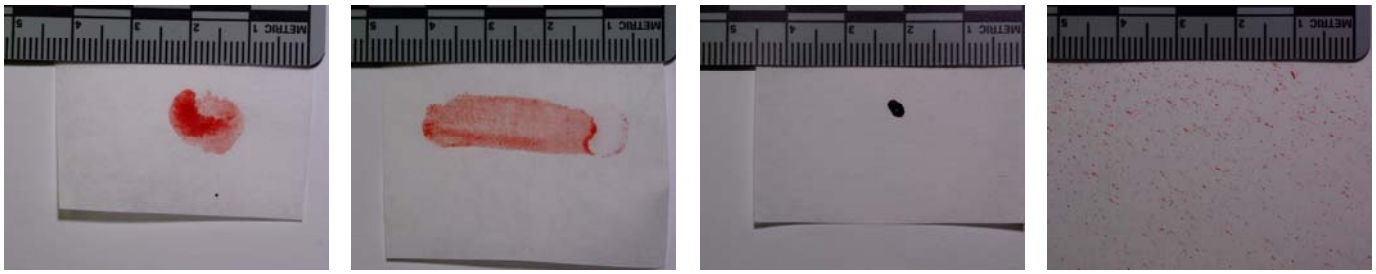
Plain black cotton, leather, wool, silk and polyester purchased from a fabric store were used for the majority of the experiments. These represent common synthetic and natural clothing materials. Use of other substrate types and materials was limited to the 'substrate characteristics' portion of the project.

Making bloodstained samples

All stains used were prepared by the project staff using fresh blood. The blood was obtained, in accordance with IRB approved methods, by piercing the tip of a finger with a lancet designed for that purpose. Four different types of stains were produced. 'Smear' stains were produced by smearing a thin layer of blood across the surface of the substrate using the pierced finger. 'Contact' stains were produced by blotting the surface of the substrate with the pierced finger. 'µL' stains were produced by pipetting a given µL amount of freshly bled blood onto the surface of the substrate. 'Small spatter' stains were produced by dipping the bristles of a toothbrush into fresh blood and creating small spatter by flicking a finger across the bristles while holding the toothbrush over the substrate. All stain making methods were tested on white paper in order to ascertain that the stain making methods resulted in the type of stains we were intending to make. These four stains cover most of the sizes and thicknesses of blood stains one could expect to find in case scenarios.

A full listing of the stains made can be found in appendices 1 and 2 (all the substrates listed in appendix 2 were stained with all 4 types of stains).

Stain Types on White Paper



Contact **Smear** **10µL drop** **Spatter**

Fig. 2

Image Analysis

The histograms of a selection of images from the 'optimum parameters' were analyzed in order to attempt to obtain a more quantitative measure of the enhancement than visual evaluation. A stained area and a background area were selected from each image and the values of luminosity, red, green and blue of those areas entered into an excel table for processing. White and black areas from the reference scale in one of the images were sampled to provide an estimate of maximum contrast for comparison purposes. A number of calculations were performed using these values and their suitability to the task at hand evaluated by comparing the results between photographs where the difference in enhancement was obvious. This excel spreadsheet is attached as Appendix 3.

The World Wide Web Consortium has developed equations for brightness difference and color difference in order to provide guidance in selecting appropriate background and foreground color combinations for web pages. To determine the brightness difference one first converts the RGB values into YIQ values before performing the subtraction. The equation for the RGB to YIQ conversion is ((Red x 299) + (Green x 587) + (Blue x 114)) / 1000. The maximum color difference is 255, between white and black, and the suggested minimum value by the W3C is 125. The equation for color difference is (maximum Red value - minimum Red value) + (maximum Green value - minimum Green value) + (maximum Blue value - minimum Blue value). The maximum color difference is 765 and the suggested minimum value by the W3C is 500. The brightness difference between the white and black reference areas was 232.20 and the color difference 715.84.

Weber contrast, Michelson contrast, Lightness difference and Luminance ratio values were also calculated. These four equations focus on the luminance of colors as opposed to their red, green and blue components. Luminance is a value designed to account for the fact that lights of equal power but different wavelengths do not appear equally bright. Luminosity is a relative measure of luminance and was substituted directly for luminance in our calculations.

These calculations take into consideration two features, the difference between the luminances in question and visual adaptation. Visual adaptation is the response of vision to a temporal or spatial change in the physical power of a stimulus. The eyes react strongly to temporal changes in the applied power of the

stimulus but respond less to, or adapt to continued steady application of the same power. As a result of this, a luminance difference that produces a large brightness difference on a dim background will produce a smaller lightness difference on a brighter background.

The Weber contrast is designed for use in instances of small features on a large even background where the background luminance is approximately equal to the average luminance. The equation is $C_W= L_S-L_B/L_B$, where L_S is the luminance of the symbol or features and L_B is the luminance of the background. Where the background is lighter than the features, C_W has a negative value between 0 and 1 and where the background is darker than the features, C_W has a positive value. The C_W value for the black and white reference areas was 10.88.

The Michelson contrast is for where the background is not a large area of uniform luminance that dictates the observer's brightness adaptation. The equation is $C_M= L_{MAX}-L_{MIN}/L_{MAX}+L_{MIN}$, where L_{MAX} is the maximum luminance and L_{MIN} the minimum luminance The C_M for the black and white reference areas was 0.84.

Lightness difference describes contrast in terms of the lightness of the features and background relative to the maximum possible luminance, or white point. The equation is $\Delta L^*=116[(L_S-L_B)/L_N]^{1/3}$, where L_S is the luminance of the subject or feature, L_B is the luminance of the background and L_N is the maximum luminance. The maximum luminance was taken to be the luminance of the white standard, 253, in order to account for the fact that the software luminosity being substituted for luminance in these calculations is a relative measurement of true luminance. The lightness difference of the black and white reference areas was 112.74

Luminance ratio is simply the ratio between the luminance of the feature or subject to that of the background, $C_R=L_S/L_B$, where L_S is the luminance of the feature or subject and L_B is the luminance of the background. Like the Weber contrast it assumes that the background luminance describes the visual adaptation, but it does not take into account the difference in the luminances. The luminance ratio for the black and white reference areas was 11.88.

Optimum parameters

Light source

The lack of a suitable light source was the main problem in early experimentation using this enhancement method. The best results were obtained with a fiber optic light, which also caused the most damage to the polarizing filter in front of it. Less intense lighting options also caused considerable damage to the polarizing filter, even if a heat absorbing glass and air flow was present between the light source and the filter. Since experiments with narrow wavelength sources and polarizing filters did not yield particularly good results, we assumed that the entire white light spectrum would be needed. LEDs produce 'white' light without extraneous IR or UV radiation, so we started our search by experimenting with LEDs.

LEDs use one of two technologies to produce white light. The more common method, known as 'white LED', is a blue diode covered with a phosphorescent material that emits light across the remainder of the visible spectrum. This combined spectrum is perceived as white light. The output spectrums of these LEDs have a characteristic shape but do vary somewhat depending on the wavelength of blue LED and phosphorescent compound used.

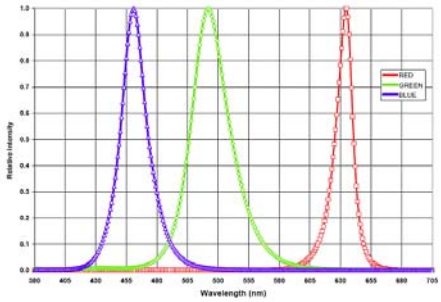
The two white LEDs tested did not perform well at all, producing a green tinted bloodstain and significantly less contrast between the stain and substrate. It is worth nothing that all current forensic white light LEDs use this white LED technology.

The less common method is known as RGB LED and uses a combination of red, green and blue LEDs to mimic the CIE colorspace of white light. The main variables in these LEDs are the wavelengths of the red, green and blue LEDs as well as the output intensities of those three LEDs.

The RGB LED tested, the Zylight Z90, turned out to be by far the best option. The enhancement produced is comparable to that obtained with traditional white light sources and there was no noticeable damage to the polarizing filter, even after over one year's heavy use. It is the only commercially available RBG LED portable and maneuverable enough to be used comfortably and efficiently in a lab or crime scene setting.

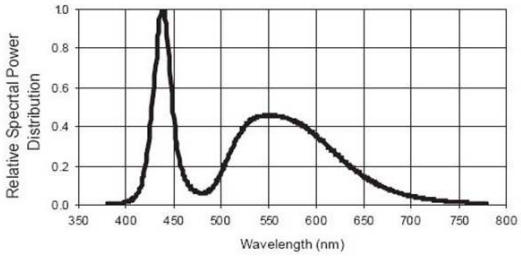
It should be noted that even the inferior results obtained with the 'white' LEDs and crossed polarizing filters were still an improvement over the contrast seen without the crossed polarizing filters.

RGB LED vs White LED



Z90 Spectrum, RGB LED

Blood on leather, Z90



Foster Freeman S80 spectrum, 'white' LED

Blood on leather, S80

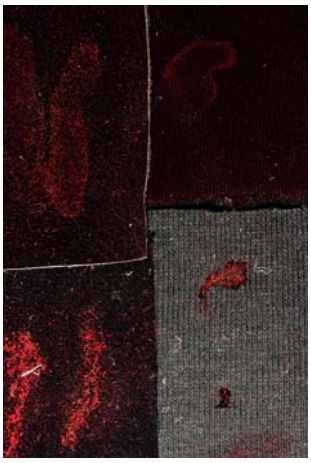
Fig.3

Light source settings

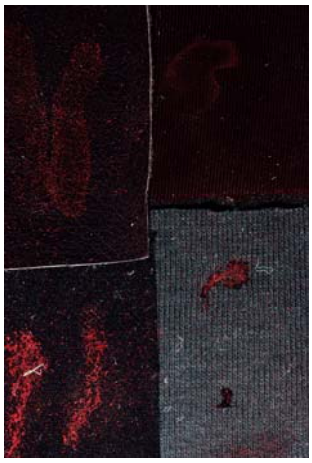
The Zylight Z90 allows the user to choose the output intensity as well as which color temperature and saturation of white light it produces. Changing the saturation setting on the Zylight shifts the white light towards green for the positive number settings and red for the negative number settings. The color temperature range that the Z90 is set for ranges from 2500-9000K and can be changed by 50K increments. The effects of intensity, saturation and color temperature were tested by photographing the same stain, keeping all other conditions constant while varying one of them at a time. For the rest of the photographs,

the camera was set to F2 film simulation and -2 exposure compensation but all other camera settings, including white balance, were kept at the standard or automatic settings. The photos were recorded as jpeg files with the highest resolution/lowest compression ratio and largest number of recording pixels available.

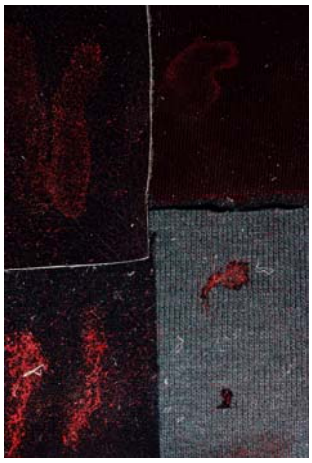
Changing Z90 Color Temperatures



2500K



5500K



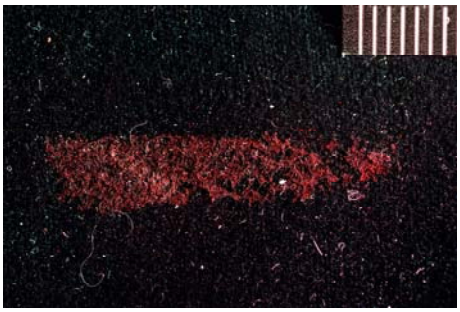
8500K

Substrates clockwise from top left: leather, polyester, cotton, wool

Fig.4

Changing Z90 Intensity

Minimum Intensity, shutters speed 18sec



Maximum Intensity, shutter speed 1.5 sec

Stain: smear on wool

Fig. 5

It appeared that the intensity did not significantly affect the enhancement, but a lower intensity requires a longer exposure time which can be more cumbersome in practical applications of the technique. The neutral or zero saturation provided the best results. Though the appearance of the image changed noticeably with the change in color temperature, there was little difference in the enhancement produced. With the higher color temperatures, the substrate background often took on a colder black color which can make distinguishing small or faint spatter easier, particularly on the ribbed cotton.

Camera settings

The Fuji ISPro has a number of setting choices for dynamic range, color saturation, tone, film simulation, white balance, color temperature and exposure compensation. The same stain, the 'smear' on wool substrate, was photographed while varying these settings, one at a time.

Changing the dynamic range did not appear to impact the enhancement. Color saturation, tone and sharpness did show a visible difference between the high and low extremes of the settings, but did not seem to significantly impact the enhancement or the ability to discern the stain from the substrate.

The most dramatic changes were seen in varying the exposure compensation and film simulation. F2, the Fuji film simulation choice that mimics slide film and is geared towards landscape and nature photography by providing a 'vibrant reproduction of natural colors' produced a discernibly improved enhancement over the other film simulation options. Within the F2 film simulation, one can also vary the color, tone and saturation settings. Decreasing the sharpness to -2 and color to -1 produced the best results.

The best images were produced by a shorter shutter speed than recommended by the built in light meter. This was accomplished by using the exposure compensation function. It was found that a compensation of -2 or -3 produced the best results. Longer exposure times tended to overexpose the image, making small spatter in particular hard to distinguish.

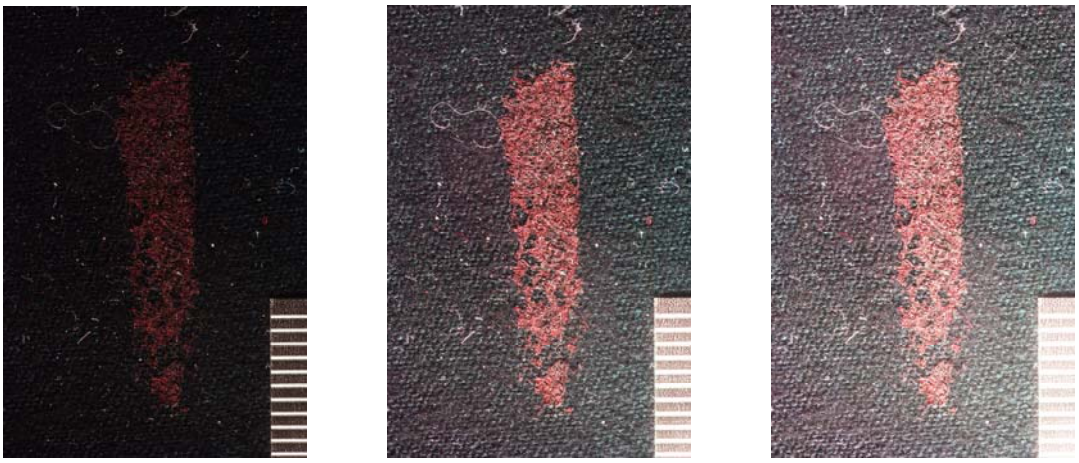
Changing Film Simulation

Uncrossed **F2** **F2 adjusted**

Substrates clockwise from top left: leather, polyester, wool and cotton

Fig.6

Changing Exposure Compensation



+/- -3 **+/- 0** **+/- +1**

Stain: smear on wool

Fig.7

Quantitative contrast measurement

Image analysis was performed to compare dynamic range, color saturation, saturation, tone, film simulation, white balance, color temperature, exposure compensation, Z90 intensity, Z90 saturation, and Z90

color temperature. The stain used for this was the smear on wool. Film simulation and exposure compensation were used to evaluate the different contrast calculations since choosing F2 film simulation and -2 or -3 exposure compensation produced a markedly superior enhancement than the other film simulation and exposure compensation options. Weber contrast, Michelson contrast and luminance ratio were the only calculations that produced results consistent with the observations of the film simulation and exposure compensation differences. These three measurements were used to evaluate the impacts of the remaining camera settings.

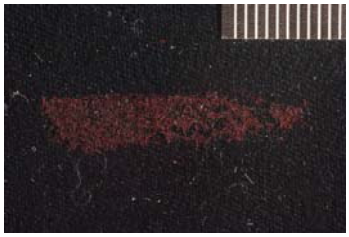
Summary of Contrast Calculation Results

Feature	Michelson Contrast				Weber Contrast				Luminance Ratio			
	mean	max	min	range	mean	max	min	range	mean	max	min	range
D-range	0.42	0.42	0.41	0.01	1.44	1.48	1.43	0.05	2.44	2.48	2.43	0.05
Color saturation	0.42	0.42	0.41	0.01	1.42	1.44	1.41	0.03	2.42	2.43	2.41	0.02
Tone	0.41	0.52	0.30	0.22	1.45	2.18	0.86	1.32	2.45	3.18	1.86	1.32
Film simulation	0.52	0.65	0.41	0.24	2.32	3.69	1.41	2.28	3.32	4.69	2.41	2.28
White balance	0.40	0.41	0.37	0.04	1.34	1.41	1.30	0.11	2.34	2.40	2.17	0.23
Color temperature	0.39	0.41	0.37	0.04	1.31	1.39	1.18	0.21	2.31	2.39	2.18	0.21
Exposure compensation	0.33	0.39	0.23	0.16	1.02	1.29	0.61	0.68	2.02	2.29	1.61	0.68
Z90 Intensity	0.57	0.62	0.57	0.05	2.71	3.25	2.41	0.84	3.71	4.25	3.41	0.84
Z90 Saturation	0.42	0.44	0.40	0.04	1.48	1.59	1.33	0.26	2.48	2.59	2.33	0.26
Z90 Color temperature	0.63	0.67	0.62	0.05	3.16	4.05	3.87	0.18	4.48	5.05	3.87	1.18

Fig. 8

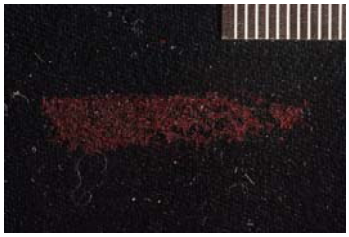
The three highest mean contrast figures and highest single values were the same with all calculations: 9000K Z90 color temperature with automatic white balance metering, lowest intensity Z90 illumination and F2 film simulation. The range, which would indicate having a large impact on the contrast, was highest for film simulation and tone with all three methods, with the third being exposure compensation with the Michelson contrast, Z90 intensity with the Weber contrast, and Z90 color temperature with the luminance ratio. The tone differences are shown below.

Changing Tone Setting



Org (C_W 0.86,C_M 0.30,C_R 1.86)

Hard (C_W 2.18,C_M 0.52,C_R 3.18)



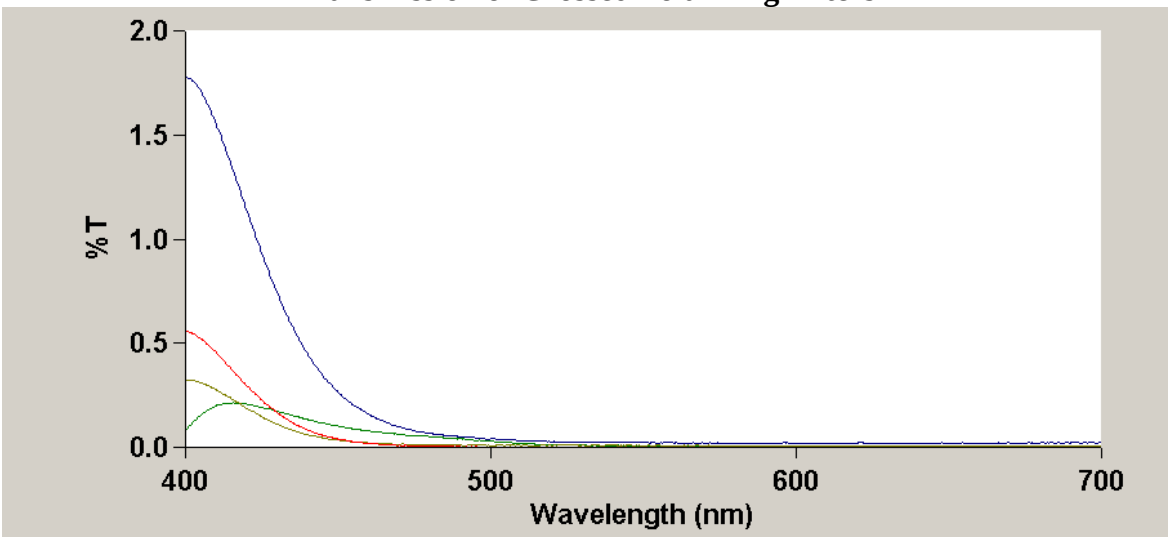
Standard (C_W 1.44,C_M 0.42,C_R2.44)

Fig.9

Polarizing filters

A number of pairs of linear polarizing filters, both low end and high end, were purchased. The extinction ratio or polarizing efficiency of the filters was measured by crossing them and measuring the transmittance of light from 400-700nm. This measurement was made with a Cary 100 UV/VIS spectrophotometer, with a scan rate of 200nm/min and data intervals of 0.333nm.

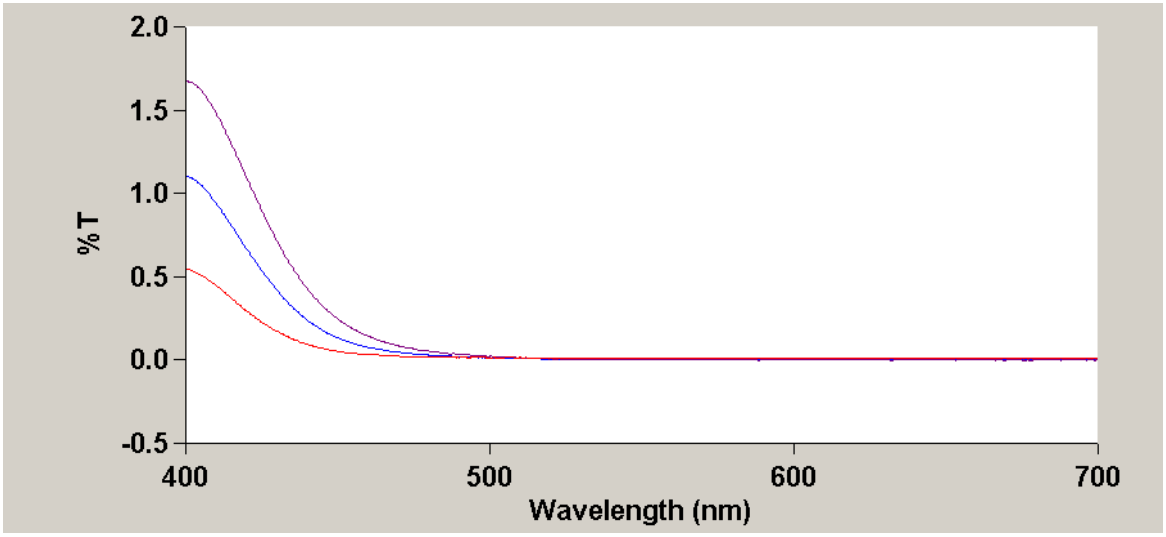
Transmission of Crossed Polarizing Filters



Blue = B&W, Red = Heliopan, Khaki = Hoya, Green = True Pol

Fig.10

The difference between crossing two of the same make and efficiency polarizing filter and crossing two of different makes was also looked at.



Red = Heliopan, Purple = B&W, Blue = B&W and Heliopan

Fig. 11

Photographs were taken with the polarizing filters in order to compare the transmittance profiles with the enhancement produced. The stains used for this included small spatter and contact on leather, smear and drop on wool and contact on cotton.

Stains Photographed with Different Polarizing Filters

Uncrossed (SS 1/6)

Hoya (SS 7.1)

B&W (SS 4)

Heliopan (SS 7.1)

True Pol (SS 4)

B&W and Heliopan (SS 4.5)

Substrates clockwise from top left: cotton, leather (contact), leather (spatter) and wool

Fig.12

The polarizing filters with the better extinction ratio, ie which transmitted the least light while crossed, produced the better enhancement. The span across which the filter showed near-zero transmission was a better indicator of its performance than the maximum transmission. Though the True Pol had a lower maximum transmission than the Hoya and Heliopan, the two latter ones showed near zero transmission across more of the 400-700nm range and produced better images. The filters that produced the better results also required the longer exposure time under the same settings.

Combining a lower grade filter with a higher grade filter, the resulting transmission curve and enhancement was better than the lower grade filter but inferior to the higher grade filter.

As was the case with the LEDs, the enhancement produced with the inferior polarizing filters was still markedly better than that seen without any polarizers.

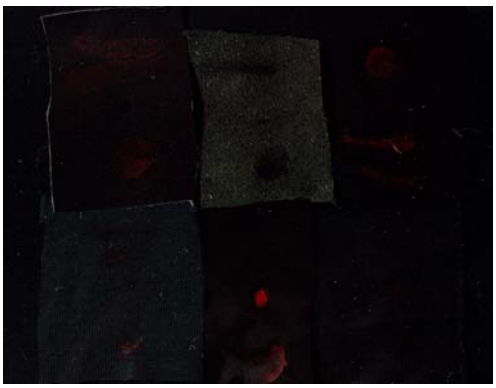
Light angle

The effect of changing the angle of incidence of the polarized light was also explored. Substrates containing four types of stains, smear, contact, small spatter and 5µL were photographed with different incident light angles. The Z90 was kept at a constant distance of 15-20 cm from the stains in order to ensure the most even lighting across the substrate. The angles were measured by keeping a Sears craftsman protractor flush on top of the Z90. The angles used were 90°, 45°, 20° and 0°, measured relative to the plane of the stained substrate.

Having the incident light at 90 or 45 degrees produced the best overall enhancement, partially because the surface examined was more evenly darkened at those angles. This held true for close up photographs of stains as well.

Changing the Angle of Incident Light

90°



45°

20°

0°

Substrates clockwise from top left: leather, suede, wool, silk, polyester, cotton

Fig. 13

Orientation of the incident polarized light

The effect of the orientation of the plane of incident polarized light was found to be substrate dependent. Some substrates like wool and leather did not react noticeably to the orientation of the plane of incident polarized light. Others such as polyester and silk were strongly influenced.

In the case of one polyester sample, rotation of the incident polarized light changed the substrate appearance from red to black. Stains were more visible when crossing the polarizers resulted in a darker background. It should be noted that this change between red and black of the substrate is not readily apparent through the viewfinder.

Effect of Orientation of Incident Polarized Light on Polyester

Uncrossed **Crossed** **Crossed rotated ~20°**

Fig. 14

In the case of silk, rotation of the incident polarized light affected the darkness of the background under crossed polar illumination. When the incident polarized light was rotated to produce the darkest background, the small spatter and drop stain were at their most enhanced. Rotating it to where the background was at its lightest, the redness of the small spatter and drop is less prominent, but a darkening of the substrate can be observed where the smear and contact stains are.

Effect of Plane of Incident Polarized Light on Silk

Uncrossed **Crossed** **Crossed rotated ~20°**

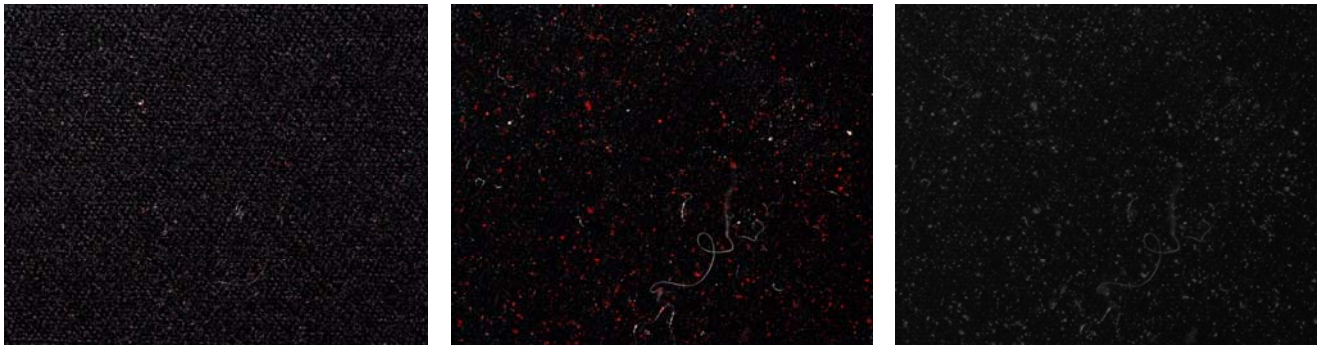
Fig. 15

Additional filters

When this method has been used in conjunction with black and white photography, it was recommended that a red filter be used in conjunction with the polarizing filter in order to enhance the contrast by lightening the grey produced by the red blood. We added a red 29 filter to the camera lens and photographed stains using both the color and the black and white mode of the camera.

When comparing the crossed polar image with the black and white image, both with and without the red filter, it becomes clear that the image in color without the 29 filter is better for distinguishing between bloodstains and other debris. In particular, light colored fibers and flecks of dust are indistinguishable from bloodstained fibers and smaller bloodstains when using the additional red filter or black and white mode, making it appear that there is more staining present than is actually the case.

Small Spatter on Wool-Filter Effects



Uncrossed **Crossed polars** **+ filter 29 in BW mode**

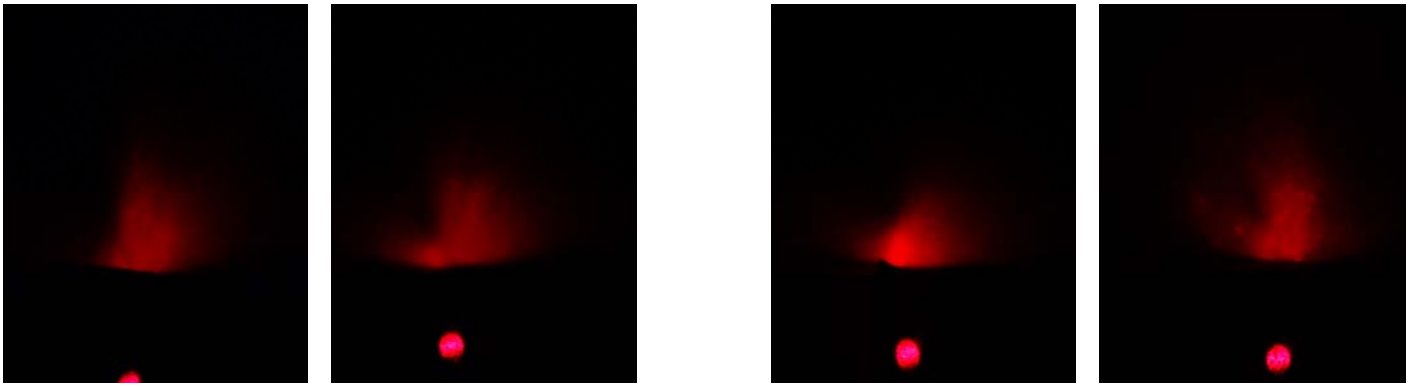
Fig.16

Mechanism

Scatter

Scattering of light by the stain was tested by observing the reflectance of a red laser pointer from the stain. The scattering of the stain surface relative to the substrate was tested by first shining the light at an unstained portion of the substrate and then shining it on a stained portion. There was little difference between the scattering from the leather and that of the 10μL stain. With the contact stain on leather a small but discernable difference was observed between the two. This suggests that the contact stain surface scatters light differently than the surface of the drop stain.

Surface Scatter of Red Laser



Leather **10 µL** **Leather** **Smear**

Note that red dot is where the laser hits the surface, not the stain

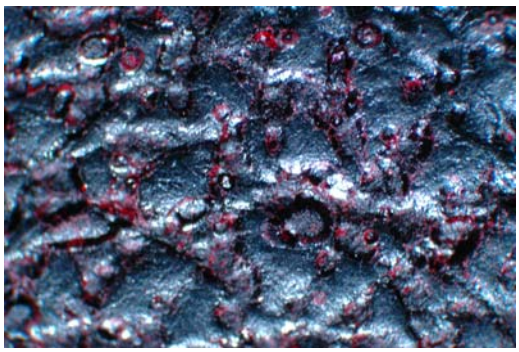
Fig.17

Glare reduction

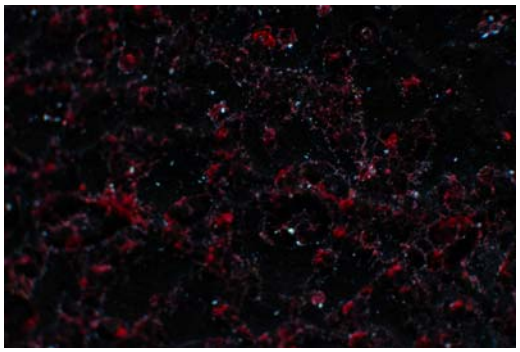
It is readily apparent from the photographs that turning the two polarizing filters to extinction reduces the glare from the surface of the substrate as well as the stain. This phenomenon was further explored by observing the stains under higher magnification.

These stains were observed with an Olympus BX41 Polarized Light Microscope (PLM), using the Z90 to produce reflected polarized lighting. Both the difference in color between the thick and thin portions of the stain and the reduction of glare from the stain and substrate are apparent. As is most evident in the micrograph of the smeared bloodstain on wool, some portions of the bloodstained area appeared equally red both with and without the incident light being polarized or viewing through crossed polarizing filters, suggesting that there is no specific interaction between plane polarized light and blood contributing to the red color being visualized.

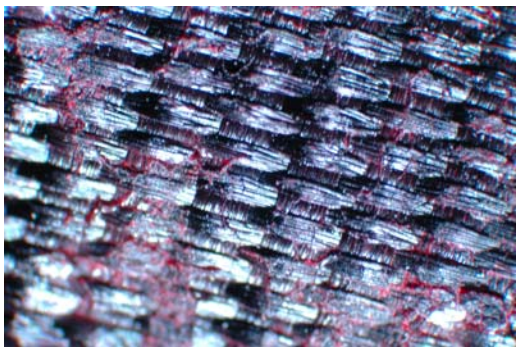
Micrographs



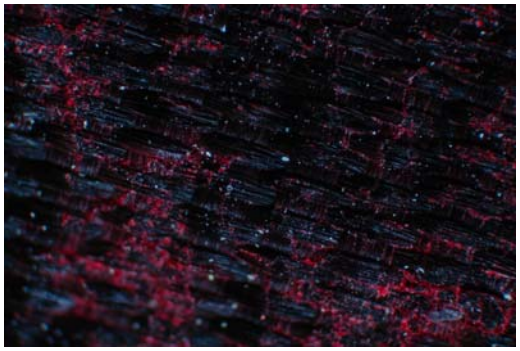
Smear on leather x40 Uncrossed



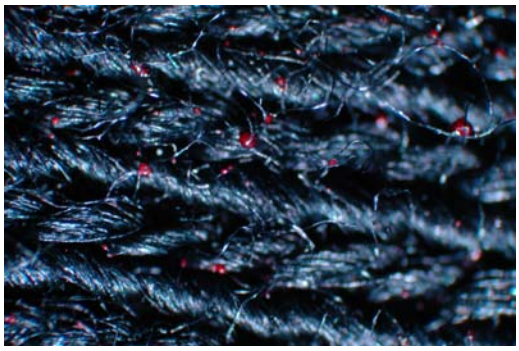
Smear on leather x40 Crossed



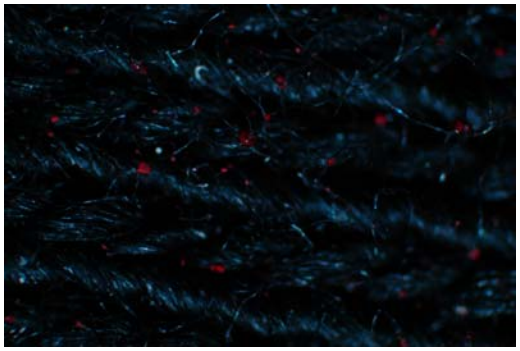
Smear on polyester x40 Uncrossed



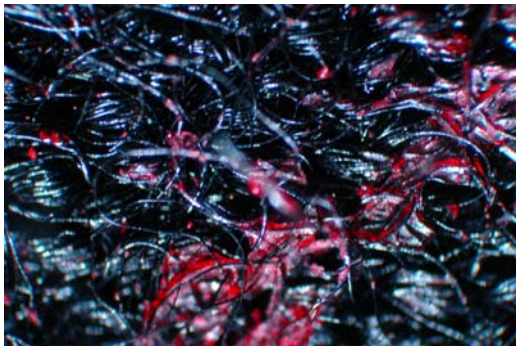
Smear on polyester x40 Crossed



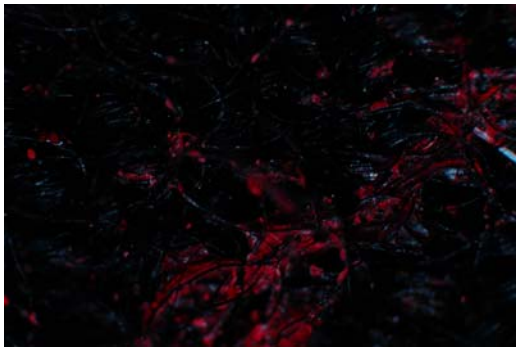
Spatter on cotton x40 Uncrossed



Spatter on cotton x40 Crossed



Smear on wool x40 Uncrossed



Smear on wool x40 Crossed

Fig.19

Features & Limitations

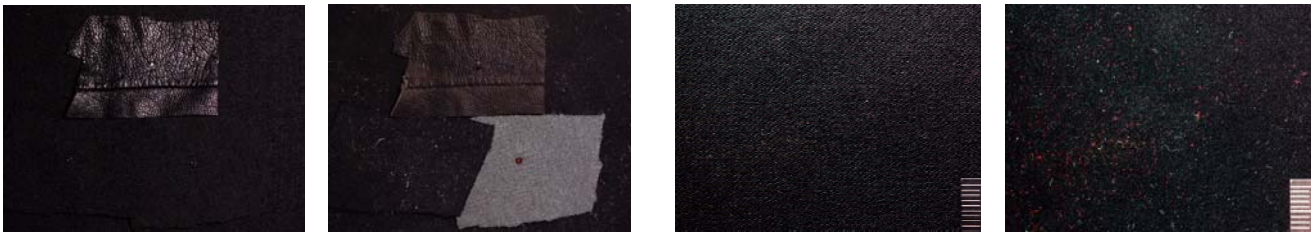
Stain characteristics

Stains that were absorbed into the substrate and did not leave a thin film on the surface of the substrate were harder to visualize. Often the contours of these stains were more prominent. Blood that formed thick stains on the surface of the substrate were not enhanced but were easily detected with the use of oblique lighting.

Small spatter tended to be deposited in small spheres on the surface of the substrates and was particularly successfully enhanced on most dark substrates. Due to its small size, some zooming in on larger surface area images is necessary in order for the smaller spatter to be properly resolved. It should be noted that this small spatter was very seldom apparent when looking through the viewfinder.

Different Stain Types

Contact on wool, leather & cotton **Smear on wool, leather & cotton**



10µL drop on wool, leather & cotton **Small spatter on wool**

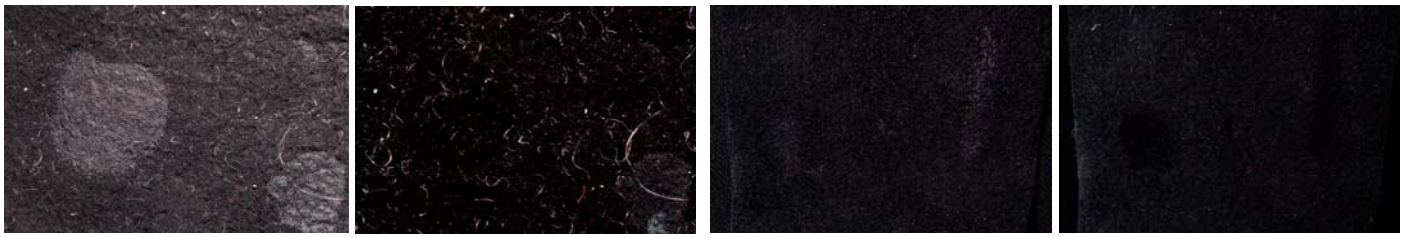
Fig.19

Substrate characteristics

Initial experimentation with the five different substrates confirmed that all substrates did not interact with the blood or the polarized light in a uniform manner.

With some materials, crossed polar illumination can diminish glare and shadow effects which can hinder the ability to locate stains by observing a change in the surface of the substrate. In the case of suede, some blood stains can be more distinguishable, albeit not red, with regular lighting as it results in a localized matting of the surface. With crossed polar illumination, this subtle matting can be less apparent and it can be more difficult to locate the stain. Two of the suede materials we tested behaved differently, with one the stains were easier to distinguish; with the other it was virtually impossible to visualize them. In both cases, there was no red color apparent, other than in the small spatter on the surface of suede #2.

Stains on Suede

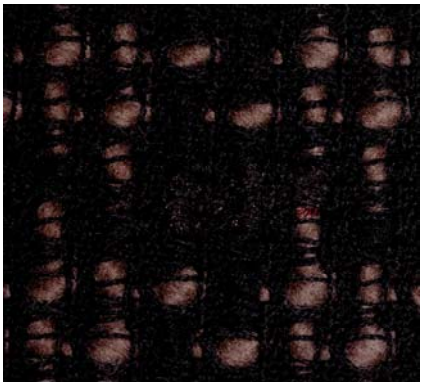


Uncrossed suede #1 **Crossed suede #1** **Uncrossed suede #2** **Crossed suede #2**

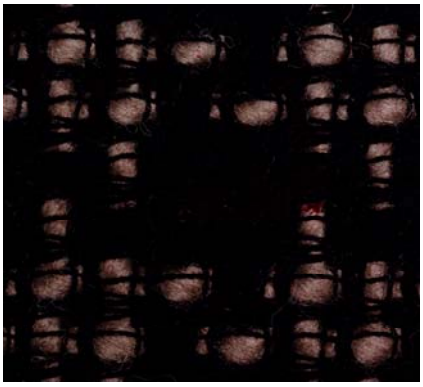
Fig. 20

Stains on substrates which contain one or more lighter colored elements show barely any enhancement. The lighter colored elements reflect a large amount of light even with crossed polarizers, significantly shortening the shutter speed and dominating over the red from the stain. If the pattern of the substrate allows for it, this reflection from the lighter portions can be minimized by covering them with a dark swatch or choosing the field of view so that it contains only the dark portions of the sample. When the sample or object being examined is small, it should be photographed on a dark matte surface to minimize any stray light interfering with the enhancement.

Black/White Upholstery Fabric with Smear and Spatter



Uncrossed



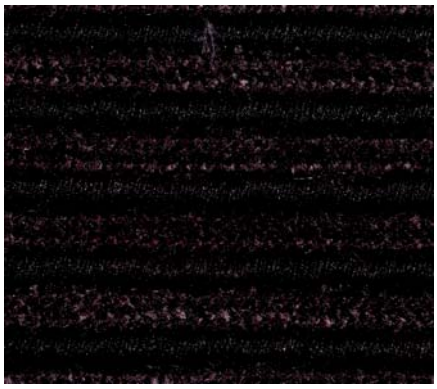
Crossed

Fig. 21

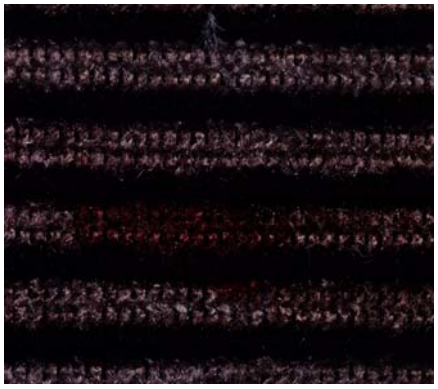
Uniformity of the plane of the substrate can also be an issue. If the substrate is uneven, it can be difficult to illuminate sufficient portions of it so that the entire field of view is under crossed polar lighting simultaneously.

A selection of different materials used for upholstery, wallpapering, carpeting and clothing was obtained. A swatch of each of them was stained with small spatter, a smear and a 5µL drop.

Grey Black Striped Carpeting with Smear and Spatter



Uncrossed



Crossed

Fig. 22

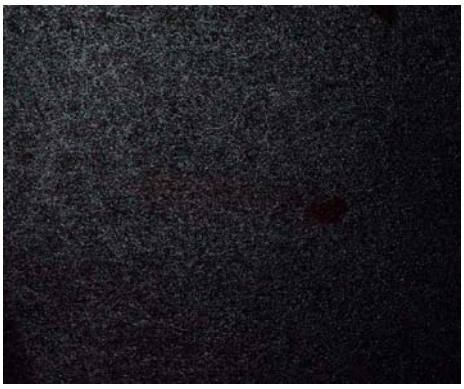
Leather Upholstery Fabric with Smear and Spatter

Uncrossed

Crossed

Fig. 23

Dark Grey Wallpaper with Smear and Spatter



Uncrossed

Crossed

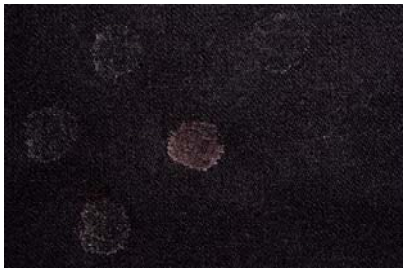
Fig. 24

Effects of Dilution

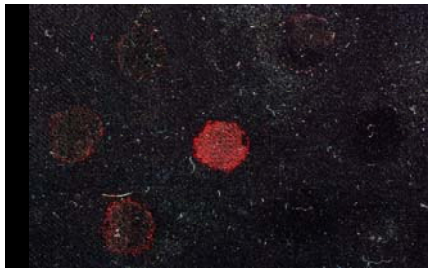
Successive dilutions of blood with distilled water were made to test the approximate concentration of blood required for the stains to be visually enhanced when photographed with crossed polarized light.

Approximately 50µL of each were pipetted onto the substrate and spread out into a 1 cm diameter round stain. The substrates used were leather, wool, cotton and polyester. Stains made on white butcher paper with the same technique and dilutions were used as a control.

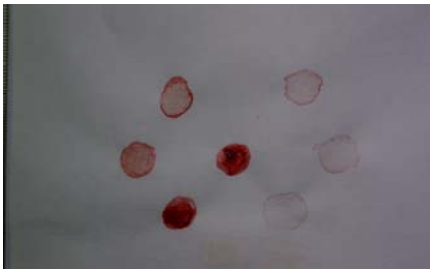
Effects of Dilution



Uncrossed on Wool



Crossed on Wool



On paper

Whole blood in center

Clockwise from top left: 1:5, 1:25, 1:50, 1:100, 1:1, 1:2,

Fig.25

Diluting the blood had an effect on how well it was absorbed by the substrates which contributed to a significant change in the appearance of the stain. On the wool substrate, dilutions up to 1 in 25 were visually enhanced. For the leather and cotton substrates, dilutions up to 1 of 10 were enhanced and for polyester the limit was 1 of 2, mainly due to the spreading out of more dilute stains.

False Positives

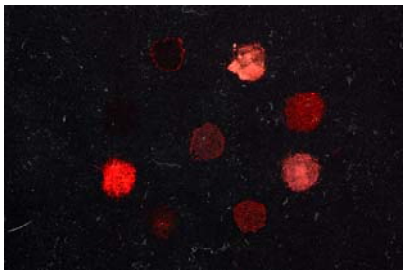
A number of commonly found red and brown materials were collected in order to determine to what extent the enhancement observed was unique to bloodstains. The following materials were tested: red Sharpie permanent marker ink, red Pentel Rolling Writer pen ink, red nail polish by Milani, Benjamin Moore paint in Spanish red, Rust-oleum gloss regal red paint, Rust-oleum rusty metal primer paint, red M.A.C. lipstick, red and brown Rose Art Crayons, FD & C Red #4 dry powder and water solution, 0.1% congo red, 'vampire blood' from a vampire make-up kit, Heinz tomato ketchup, Smucker's red raspberry jam, red wine

(Shiraz by Woop Woop), red Crayola modeling clay, red recorder ink from Ralph Gerbrands Company, and Ward's simulated blood.

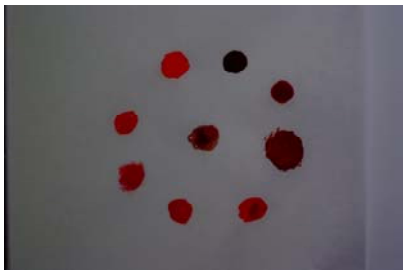
Approximately 50µL of each test substance were pipetted onto the substrate and spread out into a 1cm diameter round stain. For the pens and crayons, the stains were made by direct contact with the substrate to make a 1cm diameter round spot. The substrates tested were silk, leather, wool, cotton and polyester. Stains made on white butcher paper with the same technique and dilutions were used as a control.

False Positives

Uncrossed on Wool



Crossed on Wool



On paper

Blood in center
Clockwise from top left:
FD+C red #4 in dH_2O, Rustoleum primer, Rustoleum gloss
Benjamin Moore paint, Ward's simulated blood, Red nail polish,
Mac red lipstick, Red Sharpie

Fig. 26

There were a number of substances tested that produced stains similar in color and appearance to that of a bloodstain, others, despite being equally enhanced, did not produce stains that looked like blood on the dark or white background. The red nail polish, Rust-oleum regal red paint, red lipstick, and Ward's simulated blood were among those which most closely resembled bloodstains. The 'vampire blood', ketchup, Smucker's red raspberry jam, red wine, brown crayon, and red modeling clay were easily distinguished from bloodstains.

Just as is the case with stains on lighter substrates, one should be aware of the potential for a number of common household substances to produce stains with an appearance similar to blood.

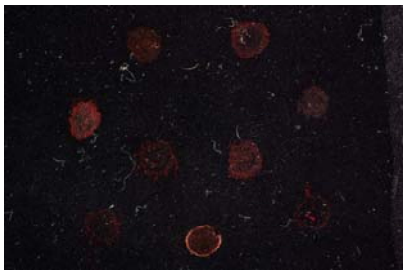
False Negatives/Interferences

In order to test for possible interferences with the enhancement, blood was mixed in a 1 to 1 ratio with a variety of materials. The following liquids were tested: water, saliva, semen, Chlorox bleach, Windex, Lysol, saline, 4M and 0.4M sodium hydroxide, 3% hydrogen peroxide, concentrated hydrochloric acid, and glacial acetic acid.

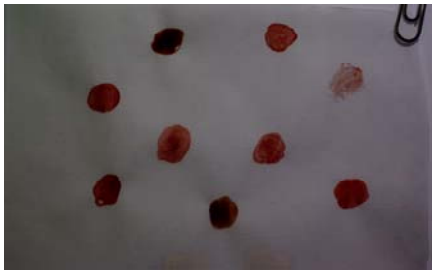
Approximately 50µL of each mixture were pipetted onto the substrate and spread out into a 1 cm diameter round stain. The substrates used were polyester, wool, leather and cotton. Stains made on white butcher paper with the same technique and dilutions were used as a control.

False Negatives/Interferences

Uncrossed on Wool



Crossed on Wool



On paper

Blood with dH_2O center left, blood with saline center right

Clockwise from top left:

Conc HCl, bleach, glacial acetic acid

Windex, 4M NaOH, 3% H_2O_2, Lysol

Fig.27

Though the color hue of some of the stains was different than that of blood diluted with water, the majority of the stains remained distinguishable from the substrate and retained their red color and visual

enhancement with crossed polarized light photography. Just as is the case with stains on lighter substrates, one should be aware of the potential for a number of common household substances to affect the appearance of bloodstains when the two are mixed.

Working distance & field of view

The further away from the surface that the camera is, the larger the area covered by a single frame. The effects of varying the distance between the lens and the substrate was tested by moving the camera further away from the substrate. Most of the images from this project were captured with a lens-substrate distance of 10-20cm. With the camera moved up to ~50 cm, the area covered by the field of view was approximately 20cm x 30cm. The crossed polar enhancement was still visible at this distance, but the exposure time was greatly increased due to the fact that the light also had to be moved further away from the substrate in order to ensure even lighting across the field of view. Also, the ability to resolve small spatter stains was compromised by the loss of resolution when zooming in on the photo to find them.

Larger Field of View-Smear, Contact, 10µL and Spatter

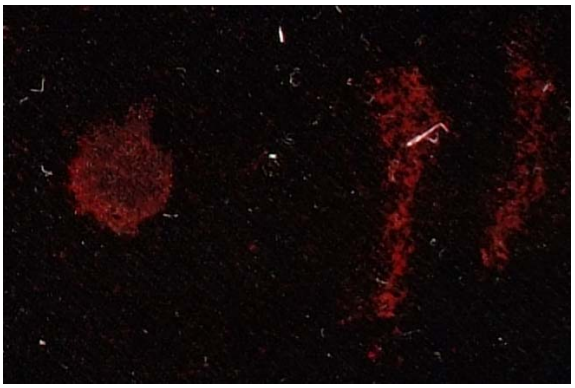
Uncrossed

Crossed

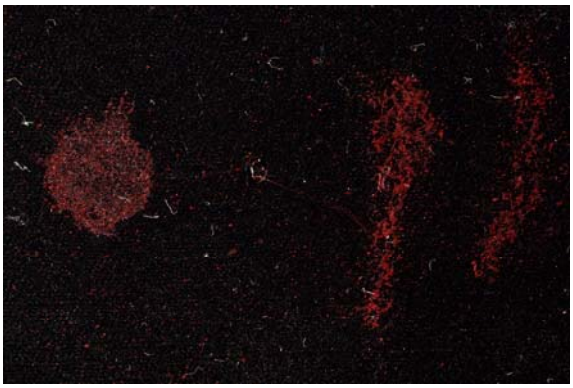
Clockwise from top left: wool, polyester, leather, cotton

Fig. 28

Wool-Digital Zoom vs Optical Zoom



Digital Zoom(from above picture)



Optical Zoom(closer working distance)

Fig. 29

Drying process

During our stain making process we observed that the stains would not retain their red color while drying. When first deposited on the substrate, the blood would appear very bright. During the process of the stain drying, the bright red would fade away and then reappear as the stain dried, if the stain was a thin stain. Thick stains remained dark. Once the stain was dry, there was no discernible change to the color or enhancement.

Drying of a Smear on Wool

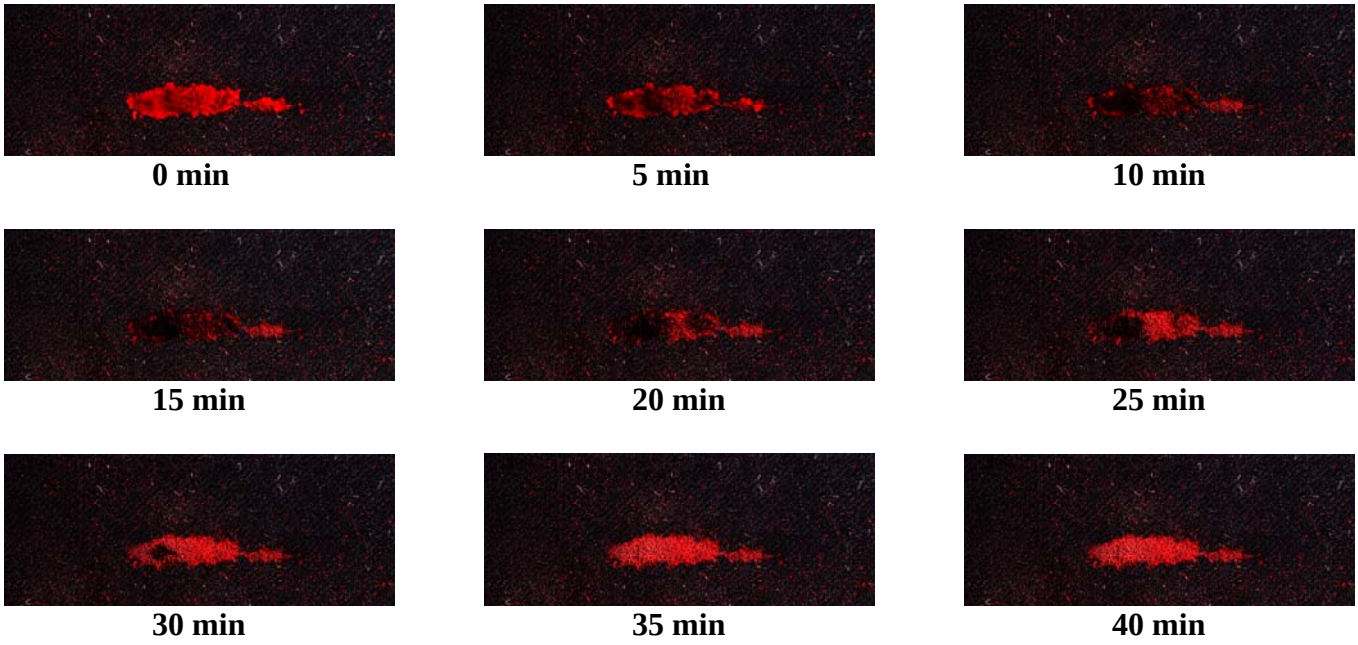
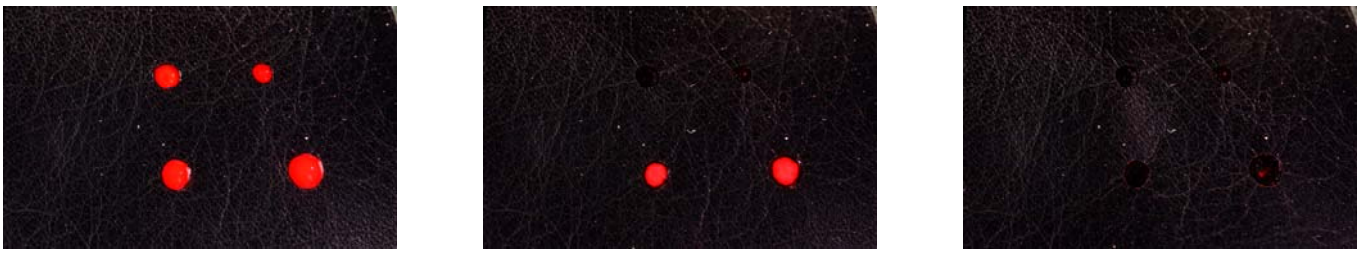


Fig.30

Drying of Drops on Leather



Drop size from top left: 2 µL, 1 µL, 5 µL, 10 µL

Fig. 31

Aging & Heat exposure

Contact and smear stains on wool were heated in an oven at 72C between 30mins and 6hrs. No change in the enhancement was observed. Stains were also photographed up to one year after they were made and no visible change in the enhancement was observed.

Comparability to IR Photography

IR Photography is another bloodstain visualization and documentation method that does not involve any chemical or physical alteration of the surface being examined. Due to the development of digital cameras with IR sensitivity, IR photography has gained popularity.

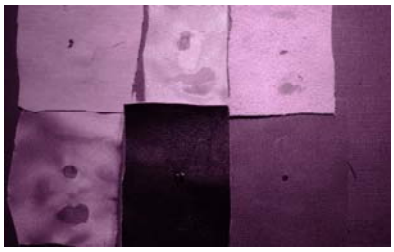
In terms of equipment, crossed polar illumination does not require a specially configured camera. Taking the photograph is also easier because one can observe the subject through the viewfinder. With IR, the lens needs to be focused prior to the addition of the IR filter because the filter blocks out light in the visible range. The live view function available on some cameras can be used to guide the aim of external IR sources and shutter speed selection, but does not provide sufficient resolution for focusing the lens.

Six of the plain substrates with all four types of stains on them were photographed using both methods. A Foxfury 850nm handheld IR light was used for the IR photographs. With respects to stain thickness, IR is complementary to crossed polar illumination as thick stains are visualized well with IR. Certain substrates like silk and polyester that can be more difficult to visualize with crossed polarized light are better visualized with IR.

Crossed Polars vs IR

Uncrossed

Crossed



IR

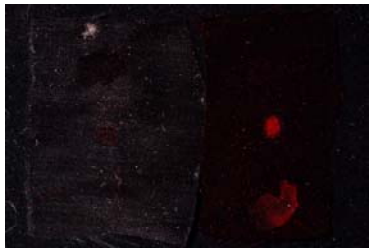
Substrates from top left: cotton, polyester, suede, silk, leather, wool

Fig.32

In order to compare the visualization of small spatter, we focused in on a few swatches in particular. We took close up photographs of the silk and polyester swatches with the 60mm lens. Silk and polyester were chosen because bloodstains on them were enhanced the best with IR photography. Even though the smears, contact and drop stains are more enhanced with the IR, the small spatter is not discernable at all under IR.

Silk and Polyester, Crossed Polars vs IR

Uncrossed

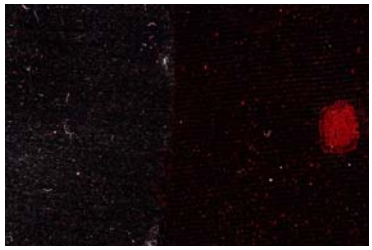


Crossed

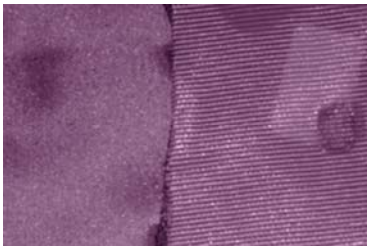
IR

Close up of Small Spatter

Uncrossed



Crossed



IR

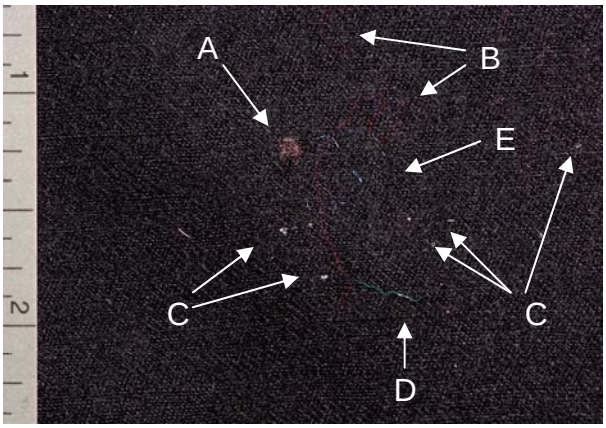
Left: silk, Right: polyester

Fig. 33

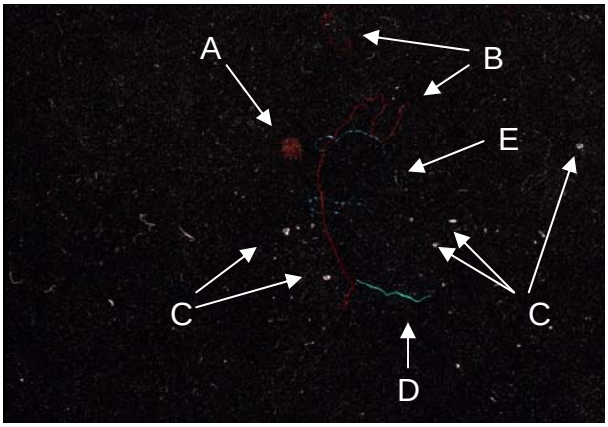
Other Materials

Through our work, particularly in testing for false positives, we observed that cross polarized illumination also enhances the visibility of other materials on dark surfaces. We photographed a selection of fibers and glass shreds on wool and observed an increase in contrast where the fibers were of a lighter color than the substrate. Glass shreds were better visualized with an exposure compensation of -3 as opposed to the -2 that is recommended for stains and fibers.

Fibers, Glass and Bloodstain on Wool



Uncrossed



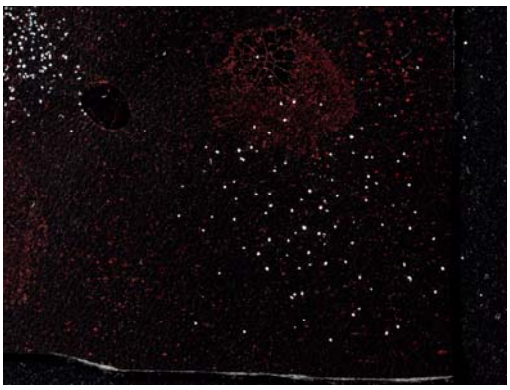
Crossed (+/- -3)

Bloodstain (A), Red Acrylic fiber (B) Glass shards (C)
Green Olefin fiber (D), Blue Rayon fiber (E)

Fig. 34 N.B. Scale in inches

Blood and Glass on Leather

Uncrossed



Crossed

Fig. 35

III. Conclusions

Discussion of findings

This research further confirmed the utility of crossed polar illumination in visualizing and documenting bloodstains on dark surfaces. Though there are limitations to the technique, the instances in which it can be of great value are plentiful.

Micrographs show that even seemingly matte surfaces like wool have a significant amount of white light reflected from them. They also show that the stain surface reflects white light as well. Crossed polar illumination eliminates this reflection from both surfaces, resulting in the two appearing more true to their intrinsic colors. This glare reduction appears to be the key element in the enhancement.

The light source and polarizing filters are the most imperative components of this method. LEDs produce intense white light with the least heat damage to the filter in front of the source and RGB LED is the preferred LED technology. The poor performance and blue green tint of the bloodstain produced using the 'white' LED technology may be partially due to its output in the 400-450nm area being stronger than that across the remainder of the visual spectrum and the crossed polarizing filters not being able to block the 400-5000nm range as effectively as the 450-700nm wavelengths.

The difference between the performances of most polarizing filters is less drastic than that of the different LED types. In terms of the transmission curve of two crossed polarizers across the visual range, the wavelengths across which there was virtually zero transmission was more indicative of better performance than the maximum intensity of transmitted light. For filters with similar ranges of near-total extinction, the ones with a lower maximum transmission were better. Given the dependence of the enhancement on the effective blockage of glare, this is to be expected.

Even when using the worst performing illumination and polarizing filters the bloodstains were far easier to visualize and document than with regular lighting.

Though it may seem counterintuitive, seeing better enhancement with a negative exposure compensation, which produces a darker photograph, is in line with Weber's law for light and sound

perception and the theories behind visual adaptation. The less brightness there is from the background, the more contrast is produced by even a small luminance difference between the stain and the background. Other camera settings which improve the enhancement are hard tone settings and slide film simulation.

Another essential tool for this method is a tripod, copy stand or other camera support structure. Despite the better enhancement with underexposed images, the exposure times were always well beyond the limits of handheld photography. Even with the ISO simulation on the digital camera pushed to 3200, the maximum of the camera we used, the shutter speed required was 1/15 or 1/30 which is too long for successful handheld photography. Such a high ISO setting and an unstable camera compromise the resolution when zooming in on the image, which affects the ability to detect small spatter. Besides being used with a tripod or with a copy stand, this illumination method can also be adapted to a stereoscope or rolling scope for more detailed examination and screening or surfaces. Small spatter can in some instances be visualized without photography with the increased magnification provided by the stereoscope but the attachment of a camera to the stereoscope and photography of the surface s is still recommended.

The stains that are enhanced the best with this technique are medium thickness stains such as smears and contact stains and small spatter. Thick stains are harder to locate using this method unless they are surrounded by thinner stained areas that are enhanced. In practice this does not constitute a serious problem, as thick stains tend to be easily visualized using oblique lighting or IR photography.

Substrate features have the potential to interfere quite a lot with the enhancement. Materials that absorb blood well leave little residue on the surface to be enhanced. Reflection off of any light colored components of the substrate is not as effectively quenched under crossed polar illumination, which limits the enhancement possible. Some dyes and fiber types are affected by the orientation of the incident polarized light and may require some experimentation with different filter rotations to achieve the best darkening of the background. Uneven surfaces are harder to work with, as the area which can be successfully brought under extinction simultaneously is smaller and the glare from any areas not under extinction interferes with the enhancement. Any manual flattening out of surfaces should be done carefully in order to minimize potential dislodgement of stains or other materials.

The area which can be covered by each frame is limited not only by the lens, working distance and illumination, but also because thin, faint stains and small spatter require close up imaging to be detected. It should be noted that unlike larger, medium thickness stains, these stains are also rarely distinguishable though the viewfinder or the LCD display of the camera and require the photograph to be viewed on a larger screen, such as that of a portable computer.

The testing of false positive, false negatives and dilutions suggest that as long as a stain retains a red hue, this redness will be enhanced. The darker appearance of thicker bloodstains on white substrates may explain why they are not as well enhanced as the thinner stains that appear red when on white substrates.

During this research, ambient light was minimized by closing the door and turning off overhead lights. The best results are produced when the vast majority of the light hitting the target surface is from the polarized source. A computer screen was adjacent to the copy stand but having it turned on or off did not seem to affect the enhancement so a complete darkroom is not necessary. Where the minimization of ambient light is difficult, having a shorter working distance and the polarized light closer to the surface and using screens to block off ambient light improves the enhancement produced.

It was observed that fibers, dust and other small particles were also enhanced by this method. This suggests that crossed polar illumination is useful for screening surfaces for a number of different types of evidence.

Implications for policy and practice

Virtually every law enforcement agency in this country has occasion to investigate crimes against the person in which patterns of bloodstains are encountered and require photographic documentation for subsequent bloodstain pattern analysis and crime scene reconstruction. There are three main aspects of bloodstain analysis that this visualization and documentation contribute to.

The first one of these is the detection of blood in the early stages of the investigation. This allows for proper measures to be taken for documentation and preservation of the blood evidence. The presence, location and morphology of blood stains are most often of great importance in any investigation, and the

earlier this information is available, the better. This technique provides a method for early screening of surfaces that is temporally and logistically convenient.

The second point to be made is that of knowledge driven sampling. Stains are commonly analyzed in order to confirm that they are blood, and often further analyzed to determine their origin. Being able to visualize the stains allows for selective processing of the surface. The morphology of the stain provides information about how it was deposited on the surface, which allows for selection of the more pertinent stains to sample. In cases where the surface examined is large, fewer samples need to be taken as the sampling can be focused on specific areas. Being able to focus on more heavily stained areas also ensures the collection of ample sample for further analyses. Where there are multiple sources of blood, the occurrence of mixed profiles in consequent DNA analysis can be minimized by sampling stains individually. More focused sampling also lessens the likelihood of disturbing or contaminating other potential evidence on the surface.

The third area where this visualization and documentation is important is that of the interpretation of the evidence. The location and morphology of the stains are key elements not only in the investigation, but also in any event reconstruction efforts. The ability to assign a DNA profile to a particular stain as opposed to a surface or collection of stains is important both in cases with multiple sources of blood but also where there is only a single source of blood.

The described method for enhancing bloodstains has great potential to improve the visualization and documentation of bloodstains not only in a laboratory setting but also at crime scenes. The procedure is non-destructive and produces time and cost efficient results without the use of potentially harmful chemicals or radiation. These chemicals are not only logistically demanding, but also have the potential to interfere with the subsequent recovery and analysis of the stains or other items on the surface.

Even with the use of film based SLRs the image in the viewfinder can be used to inform and direct the sampling of stains during the documentation. Caution is necessary with respect to small airborne droplet stains that may be too small to be seen in the viewfinder. Digital photography has an advantage in this regard if the image is transferred to a computer and enlarged to allow visualization of these airborne stains prior to sampling.

Thorough testing and documentation of the enhancement technique has not only determined the optimum conditions and limitations of the technique, it can also serve to satisfy the scientific testing required of forensic methods presented in the courts of law. There is also the potential for developing an off-the-shelf apparatus to provide the polarized light that the technique requires.

Further research

Our findings could be used to develop off the shelf apparatus to further simplify the application of this method. The most useful would be a RGB LED source to produce the polarized white light required, particularly as the forensic light sources currently available all utilize the inferior 'white' LED technology.

Some form of hood like contraption to block off ambient light from the area being analyzed could be useful where a darkened workspace is not available. A portable viewing screen system could be considered to eliminate the need to download the photographs onto a computer or other device for viewing and analysis. This would most likely require some cooperation with camera manufacturers to ensure the compatibility of the viewing screen device with the cameras.

Work could also be done to adapt the technique to video recording devices for the purposes of scanning a larger area for stains or other evidence. Considering the shutter speeds required with regular digital cameras, even when pushed to an ISO equivalent of 3200, this might require waiting for the development of more sensitive digital imaging detector technologies.

Dissemination of Research Findings

The products of this research were presented at the 2008 American Academy of Forensic Sciences Annual meeting in Washington DC, the 2008 International Association of Forensic Sciences triennial conference in Louisville Kentucky, the 2008 International Association for Identification Educational Conference in New Orleans Louisiana and the Australia New Zealand Forensic Science Society Biennial Meeting 2008 in Melbourne. Poster presentations will also be made at the 2009 FBI Trace Evidence Symposium and, pending acceptance, the 2009 European Academy of Forensic Science.

The results were formatted into an article and will be submitted for publication in Forensic Science International. Technical notes will be submitted for publication or other distribution to the International Association for Identification, the International Association for Bloodstain Pattern Analysis, The International Association of Forensic Sciences, The FBI Bulletin and any other relevant association that we can establish contact with. In addition, a practical, quick guide to the method, seen in appendix 5, has been composed in pdf form and will be distributed to people in the forensic community.

IV. References

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Appendix 1
Stains Made

Stain #	Substrate	Stain Type
01	Polyester	Contact
02	Silk	Contact
03	Wool	Contact
04	Cotton	Contact
05	Leather	Contact
06	Polyester	Smear
07	Silk	Smear
08	Wool	Smear
09	Cotton	Smear
10	Leather	Smear
11	Polyester	5 microliter drop
12	Silk	5 microliter drop
13	Wool	5 microliter drop
14	Cotton	5 microliter drop
15	Leather	5 microliter drop
16	Polyester	10 microliter drop
17	Silk	10 microliter drop
18	Wool	10 microliter drop
19	Cotton	10 microliter drop
20	Leather	10 microliter drop
21	Polyester	Blood "splash"
22	Silk	Blood "splash"
23	Wool	Blood "splash"
24	Cotton	Blood "splash"
25	Leather	Blood "splash"
26	Polyester	spray bottle
27	Silk	spray bottle
28	Wool	spray bottle
29	Cotton	spray bottle
30	Leather	spray bottle
31	Wool	contact
32	Wool	Ward's "fake" blood
33	Wool	Ward's fake blood + cuprous sulfate
34	Wool	Ward's fake blood + ferrous sulfate
35	Polyester	Contact and smear

Appendix 1
Stains Made

Stain #	Substrate	Stain Type
36	Polyester	Contact and smear
37	Silk	Contact and smear
38	Silk	Contact and smear
39	Wool	Contact and smear
40	Wool	Contact and smear
41	Cotton	Contact and smear
42	Cotton	Contact and smear
43	Wool	Fine spatter
44	Wool	Pipetted and spread out
45	Wool	Vampire blood
46	Wool	Contact, Vampire blood + copper metal (rust-colored)
47	Wool	Contact, Vampire blood + nickel metal (light-gray)
48	Wool	Contact, Vampire blood + ferric oxide (rust-colored)
49	Wool	Contact, Vampire blood + ferrous sulfate (light blue)
50	Wool	Contact, Vampire blood + cobalt chloride (purple)
51	Wool	Contact, Vampire blood + Magnesium Acetate (white)
52	Wool	Contact, Vampire blood + silver nitrate (white)
53	Wool	Contact, Vampire blood + lithium hydroxide (white)
54	Black Ceramic Tile	Contact and smears
55	PVC Pipe	Contact and smears
56	Black coated metal	Contact and smears
57	Plastic	Contact and smears
58	Wood laminate	Contact and smears
59	Cotton	Fine spatter
60	Polyester	Fine spatter
61	Leather	Fine spatter
62	Silk	Fine spatter
63	Wool	Smear
64	Wool	Contact
65	Cotton	Contact
66	Leather	Contact
67	Wool	Smear, contact
68	Wool	Smear, contact
69	Wool	Smear, contact
70	Wool	Smear, contact

Appendix 1
Stains Made

Stain #	Substrate	Stain Type
71	Wool	Smear, contact
72	Wool	Smear, contact
73	Wool	Smear, contact
74	Wool	Smear, 10 ul, fine spatter
75	Leather	Smear, 10 ul, fine spatter
76	Leather	15ul, 10ul, 5ul and 2ul
77(a)	Wool	Smear
77(b)	White butcher paper	Smear
78(a)	Wool	Drops
78(b)	White butcher paper	Drops
79(a)	Wool	Drops
79(b)	White butcher paper	Drops
80(a)	Wool	Drops
80(b)	White butcher paper	Drops
81	Denim	Smear
82	Denim	Contact
83	Denim	Fine spatter
84	Denim	Fine spatter
85	Cotton	Fine spatter, drop, smear, contact
86	Wool	Fine spatter, drop, smear, contact
87	Leather	Fine spatter, drop, smear, contact
88	Silk	Fine spatter, drop, smear, contact
89	Polyester	Fine spatter, drop, smear, contact
90	Suede	Fine spatter, drop, smear, contact
91	White butcher paper	Fine spatter
92	White butcher paper	Smear
93	White butcher paper	Contact
94	White butcher paper	10 microliter drop

Appendix 2
Substrates Tested

Sample Number	Composition
1	100% Linen
2	100% Polyester (Stain Repellant)
3	56% Viscose; 33% Cotton; 8% Polyester; 3% Poliamide
4	66% Viscose; 20% Pearl; 14% Cotton (osborne & little)
5	51% Polyurethane; 41% Polyamide; 8% Lycra
6	100% Cotton (Teflon)
7	Paper Backed Vinyl
8	85% pvc; 15% fiberglass (backing: 100% polyester)
9	96% wool; 4% nylon
10	92% wool; 8% nylon
11	100% eco intelligent polyester
12	56% Polycotton; 41% Pearl; 3% Viscose (osborne & little)
13	47% Cotton; 35% Pearl; 18% Viscose (osborne & little)
14	100% non woven backing (elitis - # TP 124 01) - could be vinyl
15	65% Wool; 20% Polyamide; 15% CA (osborne & little - pattern # FJ022-03)
16	44% cotton, 38% polyester, 18% silk
17	european full grain vegetable tanned aniline dyed cowhide
18	100% nylon (Backing: teflon & light acrylic)
19	FACE: 68% Cotton, 22% Silk, 10% Linen; 78% Cotton, 12% Silk, 10% Linen
20	100% Viscose
21	Moore & Giles Leather
22	38% Polyamide; 34% Polyester; 28% Polyurethane
23	50% Polyacrylic; 30% Polyester; 20% Cotton
24	61%Viscose, 25% Cotton, 7% Polyester, 7% Polyamide (osborne & little)
25	67% Viscose; 33% Pearl (osborne & little)

Appendix 2
Substrates Tested

Sample Number	Composition
26	58% Viscose; 23% PI, 19% Cotton (osborne & little - pattern # F1254/04)
27	69% Polyurethane; 31% Polyester
28	67% Viscose; 33% Pearl (osborne & little - pattern # F1452-04)
29	82% Rayon; 18% Silk
30	88% Polyester; 12% Polyurethane
31	100 trevira CS (Bergamo - Cachemire 7948-3)
32	63% Polyester; 37% Cotton
33	59% spun rayon; 34% cotton; 7% polyester
34	70% Mohair, 30% Silk (p); 53% cotton, 33% mohair, 14% silk (A)
35	56% Viscose; 33%; Cotton; 8% Pearl; 3% Polyamide (osborne & little)
36	69% Polyurethane; 31% Polyester
37	51% Polyester; 49% Acrylic
38	100% Cotton
39	Unlabeled
40	100% PL trevira CS = 100% Polyester trevira cs (flame retardant introduced at the molecular level)

Appendix 3
Image Processing

Testing for	Image Number	STAIN															
		Sampled Area				Luminosity			R			G			B		
		top x	top y	bottom x	bottom y	mean	std dev	median	mean	std dev	median	mean	std dev	median	mean	std dev	median
d range	572	1188	1767	1497	2204	40.98	34.85	30	62.74	43.7	51	32.46	32.49	21	32.28	32.2	21
d range	573	1188	1767	1497	2204	41.98	36.51	30	64.69	46.14	51	33.04	33.97	21	32.88	33.65	21
d range	574	1188	1767	1497	2204	41.3	34.93	30	63.33	43.88	51	32.69	32.56	22	32.35	32.19	22
d range	575	1188	1767	1497	2204	40.85	34.55	30	63.22	43.68	51	32.08	32.14	21	31.8	31.79	21
d range	576	1188	1767	1497	2204	40.79	34.14	30	63.62	43.67	51	31.81	31.66	21	31.56	31.28	21
d range	577	1188	1767	1497	2204	41.24	34.44	30	64.29	43.85	52	32.16	31.99	21	32	31.65	21
d range	578	1188	1767	1497	2204	41.89	34.45	31	65.29	43.93	53	32.67	31.99	22	32.46	31.62	22
color sat	579	1173	1772	1482	2209	40.34	34.38	29	63.25	43.92	51	31.37	31.94	20	30.93	31.49	20
color sat	580	1173	1772	1482	2209	44.28	36.18	33	68.63	46.02	56	34.66	33.64	23	34.41	33.23	23
color sat	581	1173	1772	1482	2209	42.71	35.42	31	64.46	44.08	52	34.18	33.08	23	34.04	32.74	23
color sat	582	1173	1772	1482	2209	42.59	35.31	31	63.14	43.44	51	34.6	33.07	24	34.29	32.71	24
color sat	583	1173	1772	1482	2209	42.19	34.89	31	42.19	34.89	31	42.19	34.89	31	42.19	34.89	31
tone	584	1184	1787	1493	2224	33.75	35.64	21	56.14	46.71	41	24.97	32.64	13	24.69	32.33	13
tone	585	1184	1787	1493	2224	38.38	35.59	26	61.28	45.86	48	29.39	32.82	18	29.11	32.51	18
tone	586	1184	1787	1493	2224	48.38	34.13	38	71.09	42.86	60	39.47	31.83	29	39.16	31.44	29
tone	587	1184	1787	1493	2224	51.83	32.01	43	73.19	39.68	64	43.5	30.01	34	43.11	29.63	34
sharpness	588	1180	1748	1489	2185	40.46	39.36	27	62.47	37.19	48	31.82	37.19	19	31.69	36.9	19
sharpness	589	1180	1748	1489	2185	41.51	37.4	29	64.2	46.15	51	32.56	35.1	20	32.52	34.84	21
sharpness	590	1180	1748	1489	2185	40.85	32.05	31	63.31	41.57	53	32.02	29.52	22	31.86	29.17	23
sharpness	591	1180	1748	1489	2185	41.9	30.2	34	65.06	40.36	55	32.76	27.48	25	32.63	27.08	25
film sim	592	1184	1787	1493	2224	42.41	34.77	32	62.6	42.84	51	34.23	32.35	24	35.99	32.71	25
film sim	593	1184	1787	1493	2224	42.17	34.4	31	62.48	42.92	51	34.33	31.92	24	33.71	31.66	24
film sim	594	1184	1787	1493	2224	35.72	36.43	23	56.52	47.51	42	27.77	33.17	15	26.51	32.75	14
film sim	595	1184	1787	1493	2224	32.18	35.96	19	53.36	46.29	38	23.73	32.98	11	24.54	33.11	12
film sim	596	1184	1787	1493	2224	32.76	37.76	19	63.2	51.97	49	20.5	34.39	5	20.51	33.95	5
white balance	605	1108	1991	1417	2428	40.2	33.34	30	50.94	37.76	40	33.85	31.7	23	49.08	35.05	38
white balance	606	1108	1991	1417	2428	46.99	33.87	38	79.53	46.06	70	33.97	30.28	25	33.07	29.58	24
white balance	607	1108	1991	1417	2428	45.03	33.89	35	70.82	43.85	61	34.16	30.76	25	37.7	31.51	28
white balance	608	1108	1991	1417	2428	47.44	34.69	38	77.24	46.5	67	33.77	30.77	24	44.06	33.5	35
white balance	609	1108	1991	1417	2428	45.96	35.11	36	65.4	43.38	54	35.72	32.04	26	51.99	35.99	42
white balance	610	1108	1991	1417	2428	43.63	34.44	33	57.52	40.52	47	35.56	32.14	25	53.12	36.33	43
white balance	611	1108	1991	1417	2428	43.91	33.62	34	70.1	43.99	60	33.6	30.8	24	32.74	30.26	23
color temp	612	1108	1991	1417	2428	47.07	33.57	38	83.78	47.9	74	34.05	30.16	25	22.31	25.84	14
color temp	613	1108	1991	1417	2428	46.96	34.03	37	78.69	46.42	69	34.86	31.04	25	26.18	27.98	17
color temp	614	1108	1991	1417	2428	46.8	34.25	37	75.91	45.61	66	36.07	31.44	26	30.14	29.3	21
color temp	615	1108	1991	1417	2428	41.03	33.88	30	55.99	39.95	45	33.23	31.87	23	46.3	34.74	36
color temp	616	1108	1991	1417	2428	40.35	32.81	30	44.34	34.71	34	35.29	31.75	25	60.19	38.23	49

Appendix 3
Image Processing

Testing for	Image Number	STAIN															
		Sampled Area				Luminosity			R			G			B		
		top x	top y	bottom x	bottom y	mean	std dev	median	mean	std dev	median	mean	std dev	median	mean	std dev	median
exposure comp	626	1118	2075	1427	2512	30.49	25.27	22	50.79	34.47	41	22.59	22.55	15	22.4	22.43	15
exposure comp	627	1118	2075	1427	2512	46.15	34.65	36	76.28	46.39	65	34.06	31.37	24	33.8	31.06	24
exposure comp	628	1118	2075	1427	2512	76.22	48.37	64	116.6	59.57	107	59.97	46.01	47	58.47	44.78	46
exposure comp	629	1118	2075	1427	2512	110.8	56.7	101	159	61.94	157	91.31	57.26	79	88.8	55.38	77
exposure comp	630	1118	2075	1427	2512	153.5	57.77	152	199.4	51.81	209	135.3	63.13	131	131.3	60.74	127
Z90 Intensity	707	1392	2764	1701	3201	58.57	54.57	39	95.14	65.98	81	43.85	52.09	22	42.86	50.61	22
Z90 Intensity	708	1392	2764	1701	3201	64.17	56.82	45	106.7	68.23	94	46.7	54.36	25	46.88	53.27	26
Z90 Intensity	709	1392	2764	1701	3201	65.46	56.92	47	108.8	68.05	97	47.85	54.61	27	47.05	53.21	27
Z90 Intensity	710	1392	2764	1701	3201	42.19	46.66	23	69.5	57.84	52	31.28	43.64	11	31.19	43.02	11
Z90 Sat	719	1392	2764	1701	3201	61.26	56.3	41	102.4	68.21	89	44.45	53.61	21	44.4	52.53	23
Z90 Sat	720	1392	2764	1701	3201	64.17	59.31	44	95.57	68.34	81	51.63	57.5	29	50.8	56.06	29
Z90 Sat	721	1392	2764	1701	3201	60.58	58.86	40	80.94	65.46	63	53.18	57.82	31	49.72	55.56	28
Z90 Sat	722	1392	2764	1701	3201	70.43	56.14	52	132.6	69.65	124	42.41	52.77	19	55.94	56.71	36
Z90 Sat	723	1392	2764	1701	3201	69.58	48.44	53	154.7	66.55	151	28.51	43.15	3	62.19	55.51	44
Z90 Color temp	728	1315	2778	1624	3215	39.27	52.14	15	55.4	56.1	33	32.38	50.71	8	36.79	52.26	13
Z90 Color temp	729	1315	2778	1624	3215	37.59	40.29	22	71.17	53.44	57	23.53	36.71	6	26.37	38.1	9
Z90 Color temp	730	1315	2778	1624	3215	34.41	48.3	12	50.41	52.86	30	27.59	46.55	5	31.97	48.83	10
Z90 Color temp	731	1315	2778	1624	3215	40.65	41.99	25	78.44	56.13	66	24.76	38.22	6	27.78	39.67	10
Z90 Color temp	732	1315	2778	1624	3215	35.8	49.44	13	51.97	53.95	31	28.86	47.72	6	33.51	50.05	10
Z90 Color temp	733	1315	2778	1624	3215	39.35	41.18	24	76.21	55.47	63	23.8	37.31	6	27.21	38.86	10
Z90 Color temp	734	1315	2778	1624	3215	33.34	47.23	12	49.2	51.96	29	26.62	45.39	5	30.75	47.66	9
Z90 Color temp	735	1315	2778	1624	3215	44.09	42.32	29	85.93	56.97	75	26.32	38.35	8	30.28	40.3	13
Z90 Color temp	736	1315	2778	1624	3215	32.91	46.76	12	48.42	51.41	28	26.47	45.04	5	29.76	46.73	8
Z90 Color temp	737	1315	2778	1624	3215	42.36	41.6	27	81.17	55.49	70	26.02	37.88	8	29.14	39.4	12
Z90 Color temp	738	1315	2778	1624	3215	34.69	46.93	14	54.75	53.09	35	26.48	44.76	5	28.77	46.2	7
Z90 Color temp	739	1315	2778	1624	3215	44.15	41.59	29	88.12	56.52	78	25.73	37.66	8	28.14	38.69	11
Z90 Color temp	740	1315	2778	1624	3215	33.91	44.56	15	59.77	53.4	41	23.27	41.52	3	25.32	43.05	5
Z90 Color temp	741	1315	2778	1624	3215	42.87	39.63	29	91.97	56.76	83	22.29	35.09	5	24.45	36.04	8
Z90 Color temp	742	1315	2778	1624	3215	33.87	45.66	14	55.35	53.3	36	23.16	42.4	2	37.07	47.69	17
Z90 Color temp	743	1315	2778	1624	3215	46.17	41.71	32	92.13	57.44	82	23.71	37.06	5	45.58	42.18	31
Z90 Color temp	744	1315	2778	1624	3215	39.56	47.79	19	69.52	56.8	52	28.57	45.87	7	22.03	41.29	0
Z90 Color temp	745	1315	2778	1624	3215	44.84	40.56	31	97.16	58.6	88	24.45	37.01	7	17.06	31.56	0
Z90 Color temp	746	1315	2778	1624	3215	37.47	45.78	18	66.21	54.55	49	27.08	44.02	6	20.14	38.92	0
Z90 Color temp	747	1315	2778	1624	3215	41.63	38.64	28	91.05	56.26	81	22.41	35.18	6	15.48	29.74	0
black/white	727	256	598	268	615	253.6	1.32	254	255	0.07	255	253.6	1.88	254	253.6	1.63	254

Appendix 3
Image Processing

Testing for	Image Number	BACKGROUND															
		Sampled Area				Luminosity			R			G			B		
		top x	top y	bottom x	bottom y	mean	std dev	median	mean	std dev	median	mean	std dev	median	mean	std dev	median
d range	572	0	3183	309	3630	16.77	14.81	12	17.25	14.57	13	16.91	14.99	12	19.01	14.99	14
d range	573	0	3183	309	3630	16.95	15.3	12	17.29	15.08	13	17.15	15.46	12	19.21	15.5	14
d range	574	0	3183	309	3630	16.88	14.87	12	17.36	14.64	13	17.02	15.04	12	19.04	15	14
d range	575	0	3183	309	3630	16.71	14.74	12	17.28	14.47	13	16.83	14.92	12	18.85	14.85	14
d range	576	0	3183	309	3630	16.77	14.38	12	17.38	14.14	13	16.86	14.56	12	19.01	14.54	15
d range	577	0	3183	309	3630	16.88	14.75	12	17.46	14.49	13	16.99	14.93	12	19.12	14.89	14
d range	578	0	3183	309	3630	17.26	14.86	13	17.92	14.61	13	17.35	15.04	13	19.46	15.02	15
color sat	579	0	3183	309	3630	16.6	14.87	12	16.99	14.63	13	16.75	15.05	12	19.15	15.07	15
color sat	580	0	3183	309	3630	18.31	16	13	18.7	15.76	14	18.49	16.17	13	20.68	16.18	16
color sat	581	0	3183	309	3630	17.56	15.46	13	17.92	15.24	13	17.76	15.62	13	19.81	15.59	15
color sat	582	0	3183	309	3630	17.48	15.41	13	18.14	15.2	14	17.55	15.55	13	19.69	15.5	15
color sat	583	0	3183	309	3630	17.54	15.09	13	17.54	15.09	13	17.54	15.09	13	17.54	15.09	13
tone	584	0	3183	309	3630	10.6	13.59	7	11.47	13.48	8	10.59	13.69	7	12.66	13.66	9
tone	585	0	3183	309	3630	14.27	14.49	10	15.04	14.33	11	14.3	14.63	10	16.33	14.57	12
tone	586	0	3183	309	3630	23.32	15.52	19	23.71	15.29	19	23.52	15.68	19	25.5	15.64	21
tone	587	0	3183	309	3630	27.83	15.11	24	28.17	14.88	24	28.05	15.28	24	30.07	15.23	26
sharpness	588	24	1760	333	2197	20.36	16.27	16	20.79	16.06	16	20.59	16.44	16	22.24	16.41	18
sharpness	589	24	1760	333	2197	20.95	15.5	17	21.37	15.29	17	21.18	15.67	17	22.96	15.65	19
sharpness	590	24	1760	333	2197	20.56	13.07	17	21.03	12.85	18	20.77	13.27	17	22.46	13.23	19
sharpness	591	24	1760	333	2197	21.08	12.39	18	21.72	12.18	19	21.23	12.58	18	22.9	12.56	20
film sim	592	0	3183	309	3630	17.6	15.2	13	18.55	15.06	14	17.56	15.31	13	19.77	15.25	15
film sim	593	0	3183	309	3630	17.14	14.76	13	17.9	14.58	13	17.16	14.89	13	19.43	14.83	15
film sim	594	0	3183	309	3630	10.95	15.31	6	11.25	15.41	6	11.14	15.34	6	11.61	15.65	6
film sim	595	0	3183	309	3630	8.54	13.85	4	9.7	13.73	6	8.45	13.95	4	10.46	13.84	6
film sim	596	0	3183	309	3630	6.99	14.89	2	8.16	14.83	4	6.8	14.93	2	9.54	15.25	5
white balance	605	0	3183	309	3630	18.54	16.95	13	14.78	15.73	10	19.37	17.24	14	28.56	19.32	22
white balance	606	0	3183	309	3630	19.55	17.81	14	23.36	18.68	17	18.65	17.53	13	18.07	17.55	12
white balance	607	0	3183	309	3630	18.69	17.84	13	20.58	17.97	15	18.13	17.77	12	21.44	18.1	15
white balance	608	0	3183	309	3630	19.87	18.33	14	21.44	18.48	15	18.95	18.19	13	24.63	19.26	18
white balance	609	0	3183	309	3630	19.3	17.83	13	17.1	17.2	11	19.39	17.82	13	28.82	20.21	22
white balance	610	0	3183	309	3630	18.98	17.41	13	14.5	16.18	9	19.92	17.69	14	30.18	20.2	24
white balance	611	0	3183	309	3630	19.02	17.83	13	20.09	17.68	14	19.04	17.97	13	20.4	17.87	14
color temp	612	0	3183	309	3630	19.66	17.85	14	23.93	18.43	18	19.33	17.52	14	14.85	15.95	10
color temp	613	0	3183	309	3630	19.72	18.14	14	22.88	18.48	17	19.77	18.19	14	16.25	17.17	11
color temp	614	0	3183	309	3630	20.4	18.28	14	22.16	18.38	16	20.69	18.42	14	18.47	17.83	12
color temp	615	0	3183	309	3630	17.95	16.97	12	15.07	16.09	10	18.67	17.12	13	26.41	19.02	20
color temp	616	0	3183	309	3630	18.49	15.5	13	11.97	13.5	8	19.65	15.85	14	34.04	20.35	28

Appendix 3
Image Processing

Testing for	Image Number	BACKGROUND															
		Sampled Area				Luminosity			R			G			B		
		top x	top y	bottom x	bottom y	mean	std dev	median	mean	std dev	median	mean	std dev	median	mean	std dev	median
exposure comp	626	0	3183	309	3630	13.32	12.56	10	14.67	12.49	11	13.15	12.64	9	15.27	12.61	12
exposure comp	627	0	3183	309	3630	20.7	18.91	15	21.8	18.7	16	20.61	19.04	14	22.7	19.15	17
exposure comp	628	0	3183	309	3630	36.58	29.02	27	35.72	28.15	27	37.42	29.62	28	38.86	29.36	29
exposure comp	629	0	3183	309	3630	59.06	40.08	46	57.31	38.87	45	60.28	40.95	47	61.69	40.65	49
exposure comp	630	0	3183	309	3630	95.23	50.66	81	92.84	49.13	79	96.74	51.8	83	98.03	51.31	84
Z90 Intensity	707	49	2306	358	2743	15.92	25.05	7	12.21	22.45	4	17.84	26.68	8	20.06	25.57	11
Z90 Intensity	708	49	2306	358	2743	18.3	27.79	8	14.06	25.06	5	20.43	29.48	9	22.71	28.4	12
Z90 Intensity	709	49	2306	358	2743	19.2	28.17	9	15.47	25.53	6	21.06	29.85	10	23.73	28.78	13
Z90 Intensity	710	49	2306	358	2743	9.93	19.65	4	7.42	17.8	2	11.37	20.84	5	13.48	19.97	7
Z90 Sat	719	49	2306	358	2743	23.65	32.76	10	18.93	29.91	6	26.03	34.53	11	28.11	33.55	14
Z90 Sat	720	49	2306	358	2743	26.75	34.35	12	17.1	29.11	4	31.42	37.38	16	32.29	35.85	17
Z90 Sat	721	49	2306	358	2743	26.03	32.85	12	13.38	25.84	2	32.36	36.92	17	30.95	34.44	16
Z90 Sat	722	49	2306	358	2743	28.09	35.6	13	32.03	36.39	17	24.47	34.95	9	40.7	39.34	25
Z90 Sat	723	49	2306	358	2743	27.27	31.29	15	46.43	39.71	32	13.76	26.34	1	50.83	41.33	36
Z90 Color temp	728	49	2306	358	2743	27.14	44.34	6	25.04	42.17	5	28.37	45.52	6	30.57	45.45	9
Z90 Color temp	729	49	2306	358	2743	7.45	19.49	2	7.79	19.4	3	7.56	19.62	2	10.02	19.47	5
Z90 Color temp	730	49	2305	358	2743	22.62	40.69	3	22.71	39.87	4	22.85	41.13	4	25.64	41.37	6
Z90 Color temp	731	49	2306	358	2743	10.5	22.16	3	11.59	22.26	4	10.36	22.2	3	12.83	22.09	6
Z90 Color temp	732	49	2306	358	2743	23.81	41.71	4	23.37	40.63	4	24.26	42.29	4	27.02	42.54	7
Z90 Color temp	733	49	2306	358	2743	8.73	20.85	2	9.6	20.85	4	8.63	20.91	2	11.43	20.92	5
Z90 Color temp	734	49	2306	358	2743	22.1	39.88	4	22.24	39.04	5	22.41	40.4	4	24.55	40.27	6
Z90 Color temp	735	49	2306	358	2743	10.5	22.2	3	9.69	21.36	3	11.16	22.76	4	13.39	22.3	6
Z90 Color temp	736	49	2306	358	2743	22.18	40.92	3	22.64	40.5	4	22.44	41.27	3	23.82	40.9	5
Z90 Color temp	737	49	2306	358	2743	9.68	20.8	4	8.49	19.79	3	10.65	21.47	4	12.2	20.69	6
Z90 Color temp	738	49	2306	358	2743	23.16	41.81	4	25.78	42.37	6	22.7	41.78	3	22.91	41.12	4
Z90 Color temp	739	49	2306	358	2743	9.82	21.26	3	9.65	20.71	4	10.38	21.74	4	11.27	20.96	5
Z90 Color temp	740	49	2306	358	2743	21.27	39.01	3	27.46	41.81	9	19.28	38.02	2	19.57	37.67	3
Z90 Color temp	741	49	2306	358	2743	9.23	19.94	3	11.24	20.11	6	8.92	20.04	3	9.75	19.52	4
Z90 Color temp	742	49	2306	358	2743	22.35	40.54	4	26.33	42.16	7	20.23	39.63	2	27.12	41.75	8
Z90 Color temp	743	49	2306	358	2743	10.03	21.57	4	10.07	21.15	4	9.79	21.75	3	15.8	22.35	9
Z90 Color temp	744	49	2306	358	2743	25.26	42.74	5	30.78	44.92	10	24.85	43.15	4	17.41	36.38	0
Z90 Color temp	745	49	2306	358	2743	9.85	21.6	3	13.4	22.6	7	9.62	21.7	3	6.3	19.07	0
Z90 Color temp	746	49	2306	358	2743	23.27	40.15	5	29.29	42.66	10	22.54	40.32	4	15.79	33.94	0
Z90 Color temp	747	49	2306	358	2743	9.3	20.74	3	12.9	21.74	6	8.94	20.74	3	6.17	18.58	0
black/white	727	426	560	438	577	21.35	10.87	17	30.78	12.24	26	18.48	10.37	14	15.59	10.19	12

Appendix 3
Image Processing

		W3C				Luminance Contrasts			
Testing for	Image Number	Stain Brightness	Bkgr Brightness	Brightness Difference	Color Difference	Michelson Contrast	Weber Contrast	Luminance Ratio	Lightness Difference
d range	572	41.49	17.25	24.24	74.07	0.42	1.44	2.44	53.06
d range	573	42.49	17.43	25.06	77.09	0.42	1.48	2.48	53.65
d range	574	41.81	17.35	24.46	75.14	0.42	1.45	2.45	53.21
d range	575	41.36	17.19	24.16	74.11	0.42	1.44	2.44	53.01
d range	576	41.29	17.26	24.03	73.61	0.42	1.43	2.43	52.92
d range	577	41.75	17.37	24.38	74.52	0.42	1.44	2.44	53.17
d range	578	42.40	17.76	24.64	76.29	0.42	1.43	2.43	53.36
color sat	579	40.85	17.10	23.76	70.92	0.42	1.43	2.43	52.71
color sat	580	44.79	18.80	25.99	80.56	0.41	1.42	2.42	54.31
color sat	581	43.22	18.04	25.18	77.4	0.42	1.43	2.43	53.74
color sat	582	43.10	17.97	25.13	76.66	0.42	1.44	2.44	53.71
color sat	583	42.19	17.54	24.65	80.9	0.41	1.41	2.41	53.38
tone	584	34.26	11.09	23.17	67.37	0.52	2.18	3.18	52.27
tone	585	38.89	14.75	24.14	64.89	0.46	1.69	2.69	52.98
tone	586	48.89	23.80	25.09	72.46	0.35	1.07	2.07	53.67
tone	587	52.33	28.32	24.02	84	0.30	0.86	1.86	52.90
sharpness	588	40.97	20.84	20.13	67.28	0.33	0.99	1.99	49.87
sharpness	589	42.02	21.44	20.58	71.68	0.33	0.98	1.98	50.24
sharpness	590	41.36	21.04	20.32	71.12	0.33	0.99	1.99	50.02
sharpness	591	42.40	21.57	20.84	75.46	0.33	0.99	1.99	50.46
film sim	592	42.91	18.11	24.81	77.34	0.41	1.41	2.41	53.49
film sim	593	42.68	17.64	25.04	82.05	0.42	1.46	2.46	53.65
film sim	594	36.22	11.23	25.00	79.49	0.53	2.26	3.26	53.46
film sim	595	32.68	9.05	23.63	74.67	0.58	2.77	3.77	52.64
film sim	596	33.27	7.52	25.75	67.14	0.65	3.69	4.69	54.17
white balance	605	40.70	19.05	21.65	71.88	0.37	1.17	2.17	51.13
white balance	606	47.49	19.99	27.50	87.01	0.41	1.40	2.40	55.32
white balance	607	45.52	19.24	26.29	81.71	0.41	1.41	2.41	54.57
white balance	608	47.94	20.34	27.60	89.61	0.41	1.39	2.39	55.41
white balance	609	46.45	19.78	26.67	87.27	0.41	1.38	2.38	54.79
white balance	610	44.13	19.47	24.66	82.48	0.39	1.30	2.30	53.38
white balance	611	44.42	19.51	24.91	76.62	0.40	1.31	2.31	53.55
color temp	612	47.58	20.19	27.39	81.59	0.41	1.39	2.39	55.30
color temp	613	46.98	20.30	26.68	79.91	0.41	1.38	2.38	55.18
color temp	614	47.31	20.88	26.43	82.82	0.39	1.29	2.29	54.61
color temp	615	41.53	18.48	23.05	74.39	0.39	1.29	2.29	52.22
color temp	616	40.83	18.99	21.84	80.66	0.37	1.18	2.18	51.28

Appendix 3
Image Processing

		W3C				Luminance Contrasts			
Testing for	Image Number	Stain Brightness	Bkgr Brightness	Brightness Difference	Color Difference	Michelson Contrast	Weber Contrast	Luminance Ratio	Lightness Difference
exposure comp	626	31.00	13.85	17.15	45.23	0.39	1.29	2.29	47.32
exposure comp	627	46.65	21.20	25.45	62.22	0.38	1.23	2.23	53.95
exposure comp	628	76.73	37.08	39.65	100.17	0.35	1.08	2.08	62.54
exposure comp	629	111.25	59.55	51.70	123.34	0.30	0.88	1.88	68.32
exposure comp	630	153.98	95.72	58.26	257.22	0.23	0.61	1.61	71.09
Z90 Intensity	707	59.07	16.41	42.66	129.15	0.57	2.68	3.68	64.08
Z90 Intensity	708	64.67	18.79	45.89	142.49	0.56	2.51	3.51	65.65
Z90 Intensity	709	65.97	19.69	46.27	153.08	0.55	2.41	3.41	65.84
Z90 Intensity	710	42.70	10.43	32.27	85.04	0.62	3.25	4.25	58.39
Z90 Sat	719	61.77	24.14	37.62	112.78	0.44	1.59	2.59	61.45
Z90 Sat	720	64.67	27.24	37.44	116.25	0.41	1.40	2.40	61.35
Z90 Sat	721	61.09	26.52	34.56	115.04	0.40	1.33	2.33	59.74
Z90 Sat	722	70.93	28.58	42.35	144.49	0.43	1.51	2.51	63.92
Z90 Sat	723	70.07	27.75	42.32	119.75	0.44	1.55	2.55	63.91
Z90 Color temp	728	39.77	27.63	12.14	61.4	0.18	0.45	1.45	42.14
Z90 Color temp	729	38.10	7.91	30.19	80.41	0.67	4.05	5.05	57.08
Z90 Color temp	730	34.91	23.13	11.79	51.26	0.21	0.52	1.52	41.74
Z90 Color temp	731	41.15	11.01	30.15	82.3	0.59	2.87	3.87	57.08
Z90 Color temp	732	36.30	24.31	11.99	55.32	0.20	0.50	1.50	41.98
Z90 Color temp	733	39.86	9.24	30.62	83.78	0.64	3.51	4.51	57.38
Z90 Color temp	734	33.84	22.60	11.24	48.62	0.20	0.51	1.51	41.08
Z90 Color temp	735	44.59	10.97	33.62	97.01	0.62	3.20	4.20	59.18
Z90 Color temp	736	33.41	22.66	10.75	47.54	0.19	0.48	1.48	40.45
Z90 Color temp	737	42.87	10.18	32.68	92.94	0.63	3.38	4.38	58.64
Z90 Color temp	738	35.19	23.64	11.55	50.93	0.20	0.50	1.50	41.43
Z90 Color temp	739	44.66	10.26	34.40	101.79	0.64	3.50	4.50	59.61
Z90 Color temp	740	34.42	21.76	12.66	52.41	0.23	0.59	1.59	42.72
Z90 Color temp	741	43.37	9.71	33.66	97.49	0.65	3.64	4.64	59.21
Z90 Color temp	742	34.37	22.84	11.53	52.34	0.20	0.52	1.52	41.42
Z90 Color temp	743	46.66	10.56	36.10	110.7	0.64	3.60	4.60	60.64
Z90 Color temp	744	40.07	25.77	14.29	62.31	0.22	0.57	1.57	44.52
Z90 Color temp	745	45.35	10.37	34.98	96.43	0.64	3.55	4.55	59.99
Z90 Color temp	746	37.99	23.79	14.20	59.41	0.23	0.61	1.61	44.41
Z90 Color temp	747	42.14	9.81	32.34	91.39	0.63	3.48	4.48	58.43
black/white	727	254.02	21.83	232.20	699.4	0.84	10.88	11.88	112.74

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Visualization of Bloodstains on Dark Surfaces using Polarized Light

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For further information, contact rbucht@gc.cuny.edu

Why Visualization is Important

- Accurately visualizing and documenting bloodstains and patterns is an integral part of crime scene investigation and provides crucial information for both the analysis of evidence in the laboratory and crime scene reconstruction efforts.

- Visualization of bloodstains is trivial on white or lightly colored surfaces. However, on darkly colored or black surfaces, it can be extremely difficult.

- There are three main aspects of bloodstain analysis that visualization and documentation contribute to:

1: The presence of blood may not be recognized at critical stages in the investigation:
   - The presence, location and morphology of blood stains are often of great importance in any investigation, and the earlier this information is available, the better.
   - Where the presence of blood is not recognized, handling of the evidence may disrupt and compromise the bloodstain evidence.

Why Visualization is Important

2: Intelligence driven sampling-being able to visualize the stains allows for more selective processing of the surface:

- Stains are commonly analyzed in order to confirm that they are blood, and often further analyzed to determine their origin.
- In cases where the surface examined is large, fewer samples need to be taken as the sampling can be focused on specific areas.
- Where there are multiple sources of blood, the occurrence of mixed profiles in consequent DNA analysis can be minimized by sampling stains individually.

3: Interpretation of the evidence:

- The location and morphology of the stains are key elements not only in the investigation, but also in any event reconstruction efforts.
- In a significant number of cases knowing how the bloodstains were formed is more important than knowing the biological source of the stains. In most cases the two types of information are complementary.
- The ability to assign a DNA profile to a particular stain as opposed to a surface or collection of stains is important both in cases with multiple sources of blood or DNA but also where there is a single source of blood or DNA.

Crossed Polarized Light Visualization: Advantages and Disadvantages

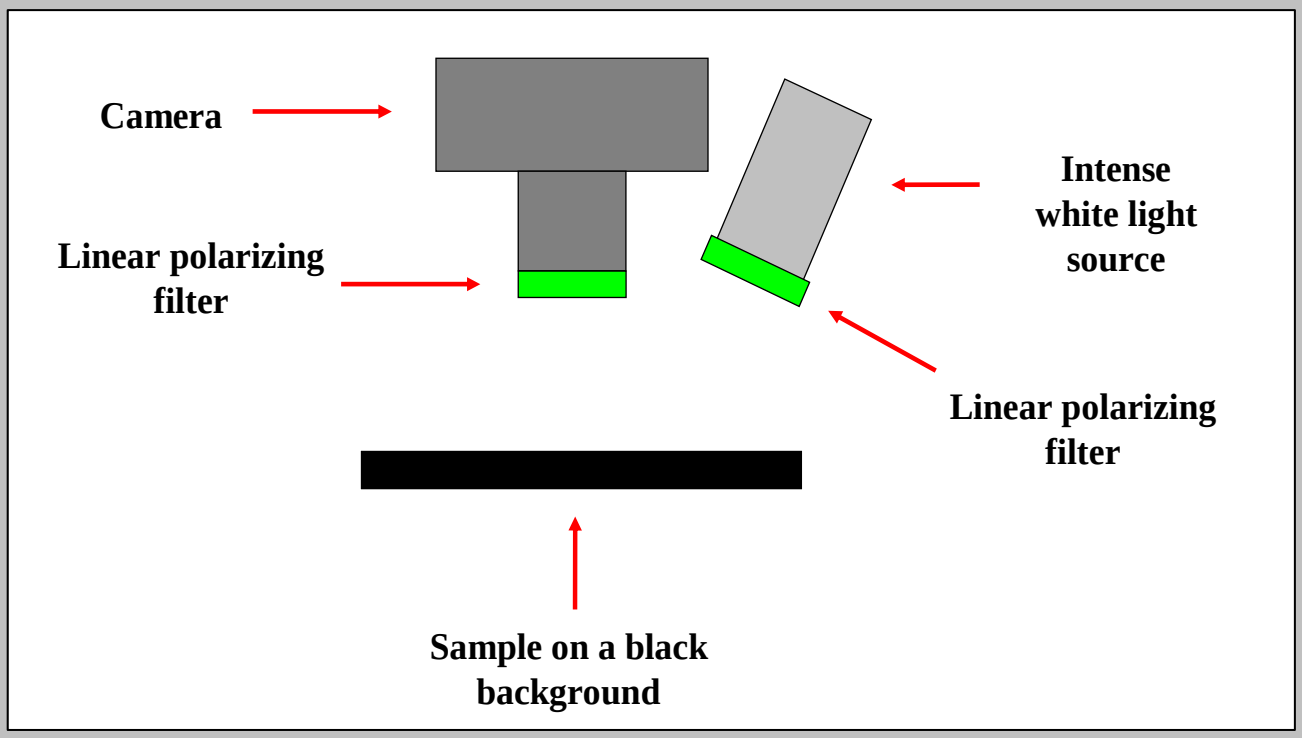
- No contact with the stain or substrate
- Easily adaptable to regular cameras
- Quick and simple procedure
- Can also be used with stereoscopes

- Thick bloodstains are not enhanced
- Uneven surfaces and some types of substrates can be difficult to process
- Screen larger than camera LCD display needed for best results

BUT…

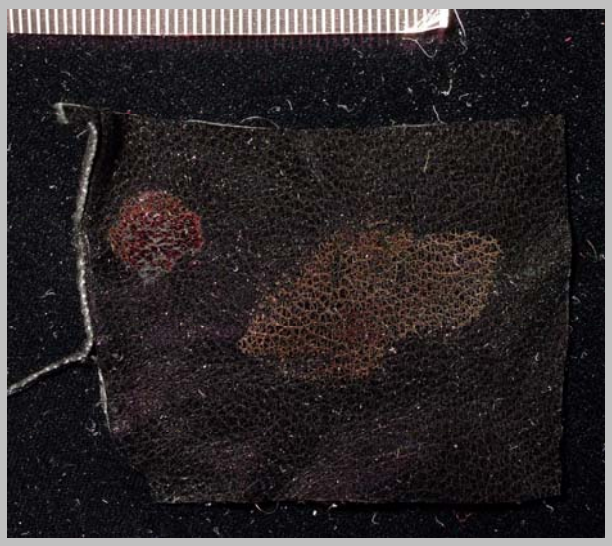
- Even under less than ideal circumstances, polarized light visualization produces a dramatic improvement in the contrast between the otherwise subtle bloodstains and the dark or black background.

Polarized Light Method Setup



Blood on Leather

Uncrossed

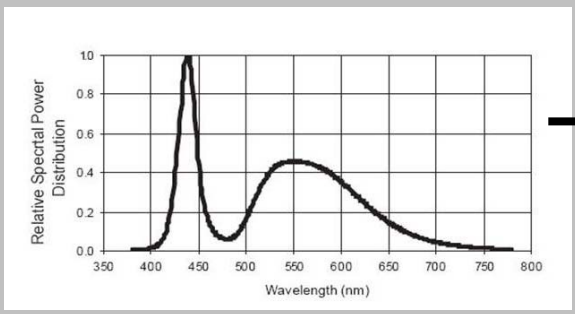


Crossed

Polarized Light Method Components: Light Source

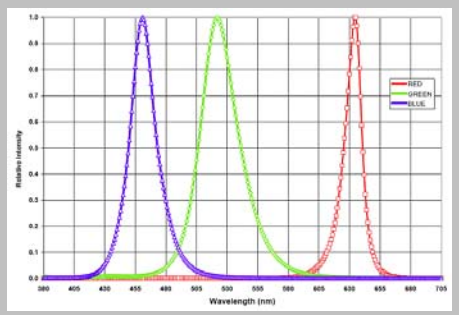
- Full spectrum of white light needed
- Fiber Optics and Xenon lights work well but cause significant heat damage to the polarizing filter in a short amount of time
- LEDs do not cause heat damage to the polarizers
- Not all LEDs output a suitable spectrum, ‘white’ LED’s performance is significantly inferior to that of RGB LEDs
- Zylight Z90 RGB LED chosen for our research

White vs RGB LED Technology



Spectrum of ‘white’ LED

Blood on leather



Spectrum of RGB LED

Blood on leather

Polarized Light Method Components: Polarizing Filters

- Linear polarizing filters, not circular, are required
- The Hoya filter performed the best out of the ones tested

Performance of a selection of Polarizing Filters

Hoya

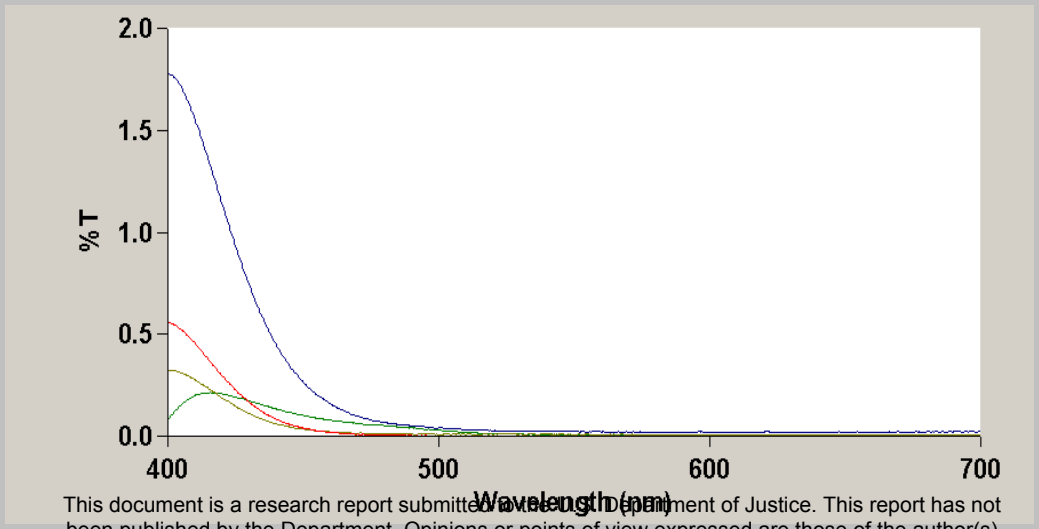
Schneider

Heliopan

Uncrossed

B&W

Transmission of Crossed Polarizing Filters

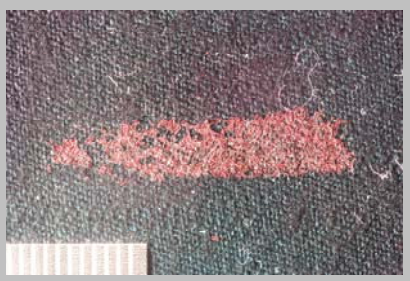


Blue = B&W, Red = Heliopan, Khaki = Hoya, Green = True Pol

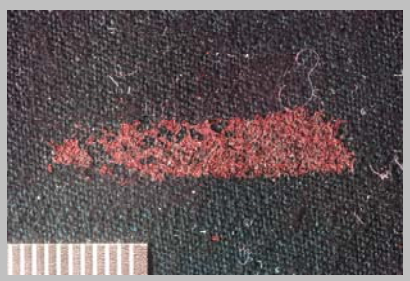
Polarized Light Method Components: Camera Settings

- Long exposure times required, camera must be on tripod or copy stand
- Exposure compensation between -1.5 to -3.0 recommended
- On digital cameras, hard tone and slide film equivalent film simulation settings produce the best contrast

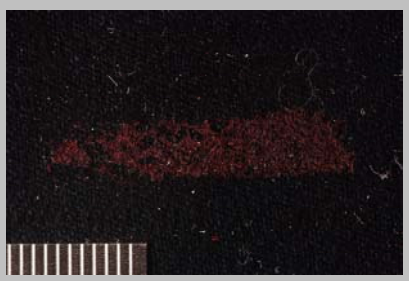
Exposure compensation



+/- +1



+/- 0



+/- -3

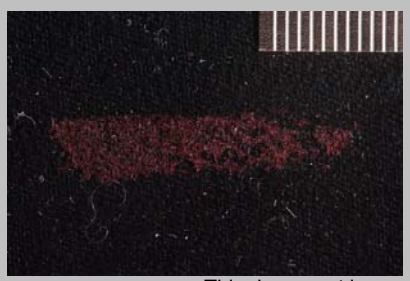
Tone

Org

Standard

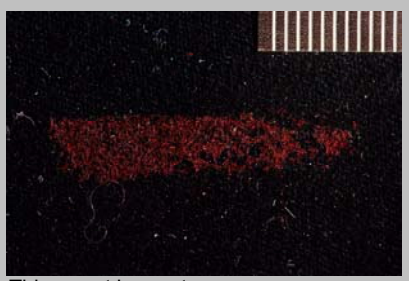
Hard

Film simulation



F1

Standard



F2

Features & Limitations:
Stain Types

- Stains that were absorbed into the substrate and did not leave a thin film on the surface of the substrate were harder to visualize
- Thick stains are not enhanced, but can be visualized using oblique light

Smear stain on leather, wool and cotton

Uncrossed

Crossed

10µL drop on leather, wool and cotton

Uncrossed

Crossed

Features & Limitations:
Stain Types

- Small spatter was particularly successfully enhanced
- Small spatter was very seldom apparent when looking through the viewfinder

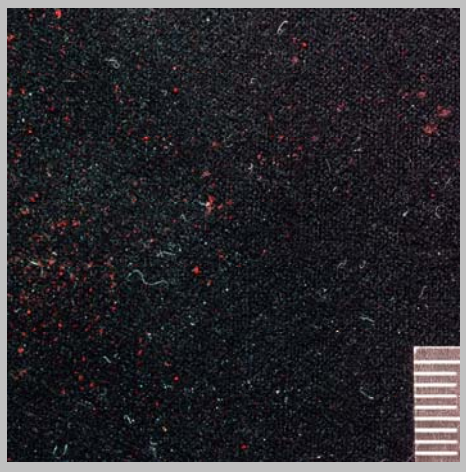
Contact stain on leather, wool and cotton

Uncrossed

Crossed

Small spatter on wool

Uncrossed

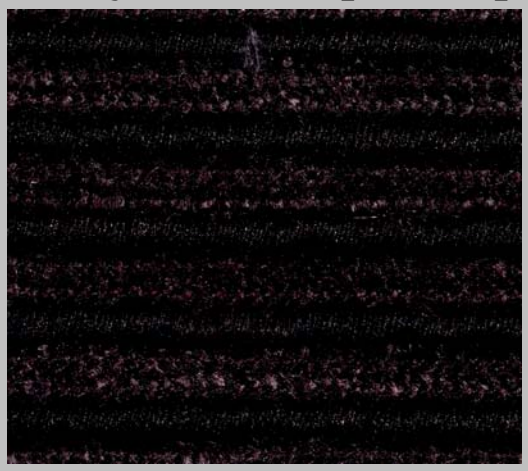


Crossed

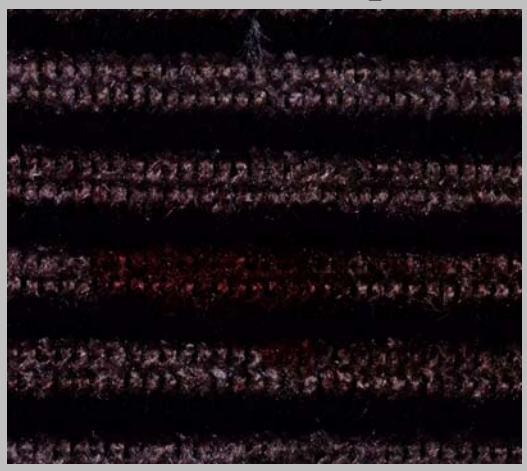
Features & Limitations: Substrate Types

- Substrates did not interact with the blood or the polarized light in a uniform manner
- If the substrate is uneven, it can be difficult to illuminate it so that the entire field of view is under crossed polar lighting simultaneously.

Grey/black striped carpeting with smear and spatter

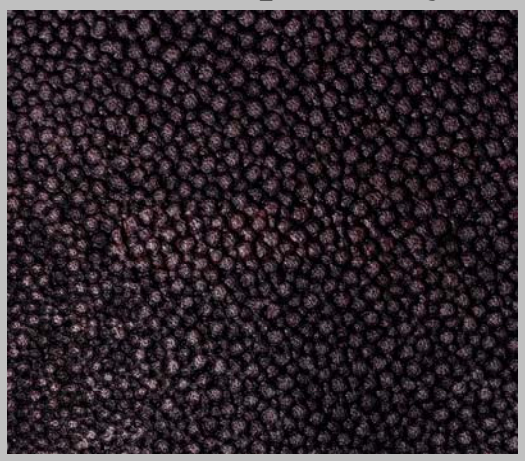


Uncrossed



Crossed

Leather upholstery fabric with smear and spatter



Uncrossed

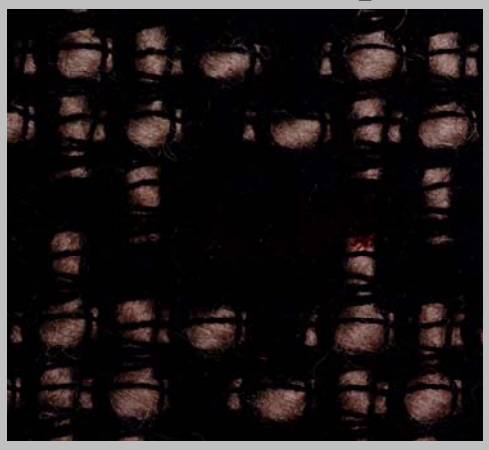
Crossed

Features & Limitations: Substrate Types

- Stains on substrates which contain one or more lighter colored elements show barely any enhancement
- On suede, stains can be visualized with regular lighting by the localized matting of the surface. This subtle matting can be less apparent with crossed polarized illumination, making the stains more difficult to visualize

Black/white upholstery fabric with smear and spatter

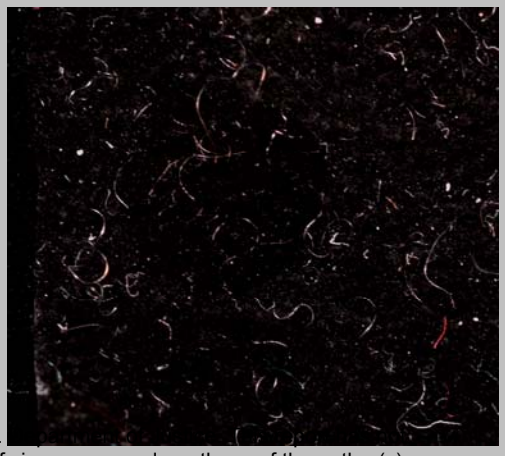
Uncrossed



Crossed

Suede with contact stain

Uncrossed



Crossed

Features and Limitations: Orientation of Incident Light

- The effect of the orientation of the incident polarized light is substrate dependent
- This change in substrate appearance is not readily apparent through the viewfinder
- The orientation of polarized light can affect the general color of the substrate or its darkness.

Polyester

Uncrossed

Crossed

Crossed rotated ~20°

Silk

Uncrossed

Crossed

Crossed rotated ~20°

Features & Limitations:
False Positives, Negatives and Dilutions

- This enhancement method is not unique to blood
- Several red substances produced stains similar in appearance to bloodstains
- While mixing the blood with certain chemicals changed its appearance, the stains remained visible
- Dilutions up to 1:10 and 1:25 could be visualized on less absorptive substrates
- As is the case with blood on lighter substrates, one should be aware of the possibility of false positives, false negatives and the effects of dilution.

Dilutions

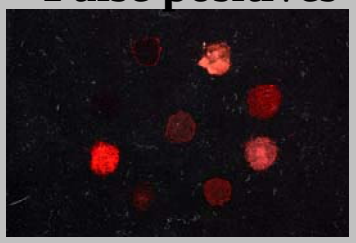
Uncrossed on Wool

Crossed on Wool

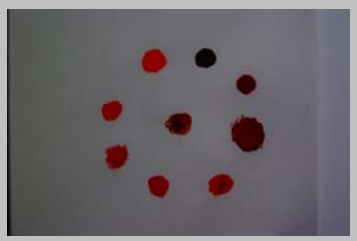
On Paper

False positives

Uncrossed on Wool



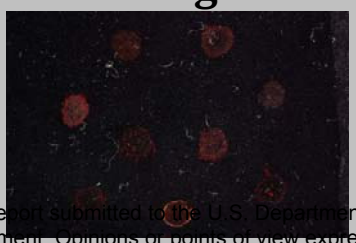
Crossed on Wool



On Paper

False negatives

Uncrossed on Wool



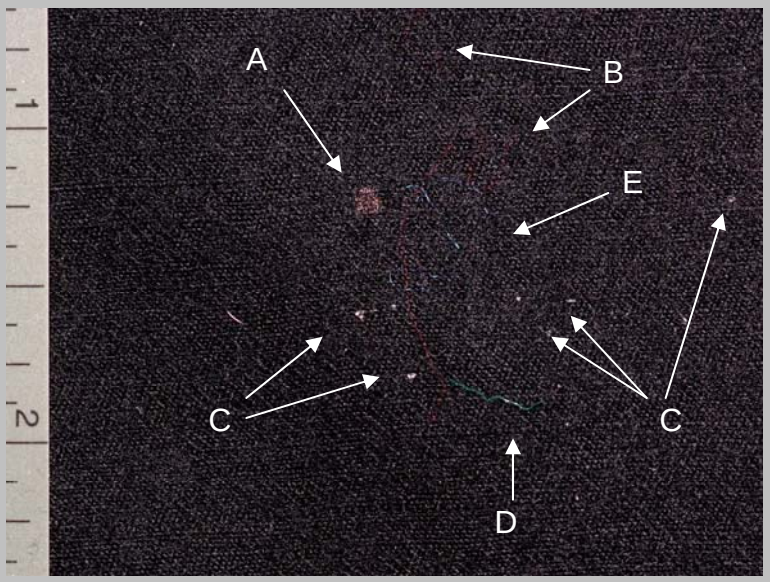
Crossed on Wool

On Paper

Features & Limitations:
Materials other than Blood

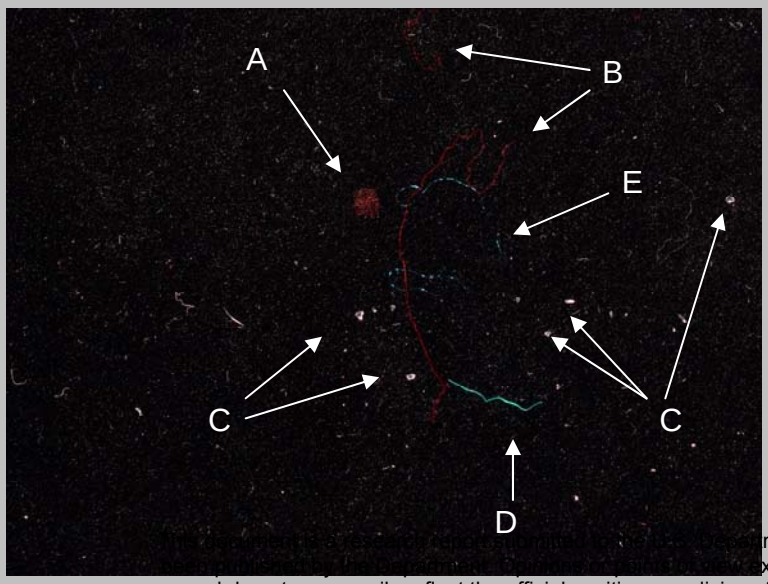
- As was apparent from the false positive testing, this enhancement is not unique to blood
- Other brightly colored items are also enhanced
- This is particularly useful for visualizing fibers and small glass fragments

Blood, Fibers and Glass on Wool



-Bloodstain (A)
-Red Acrylic fiber (B)
-Glass shards (C)
-Green Olefin fiber (D)
-Blue Rayon fiber (E)

N.B. Scale in inches



-Bloodstain (A)
-Red Acrylic fiber (B)
-Glass shards (C)
-Green Olefin fiber (D)
-Blue Rayon fiber (E)