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TECHNICAL NOTE

CRIMINALISTICS

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Preliminary Observations on the Ability of Hyperspectral Imaging to Provide Detection and Visualization of Bloodstain Patterns on Black Fabrics

ABSTRACT: The analysis of bloodstain patterns can assist investigators in understanding the circumstances surrounding a violent crime. Bloodstains are routinely subjected to pattern analysis, which is inherently dependent upon the ability of the examiner to locate and visualize bloodstain patterns on items of evidence. Often, the ability to properly visualize bloodstain patterns is challenging, especially when the stain patterns occur on dark and/or patterned substrates. In this study, preliminary research was performed to better understand how near-infrared reflectance hyperspectral imaging (HSI) could be used to observe bloodstain patterns on commonly encountered black fabrics. The ability of HSI to visualize latent bloodstains on several commonly encountered substrates is demonstrated. The images acquired through HSI are of sufficient quality to allow for differentiation between stains produced from an impact mechanism or a transfer mechanism. This study also serves as a proof of concept in the differentiation of multiple staining materials. Because of its ability to generate spectral data, the data provide a preliminary separation of stains where more than one type of stain existed.

KEYWORDS: forensic science, hyperspectral imaging, bloodstain pattern analysis, near-infrared, spectroscopy

Biological evidence is often the byproduct of a violent crime. Blood and the stain patterns created during these acts of violence are of particular interest to the forensic examiner. Bloodstains are routinely utilized to make critical forensic links between victim(s), suspect(s), physical evidence, and the crime scene. To assess the entire forensic value of a bloodstain, the stain must be located, and the physical characteristics of the individual stains that make up the overall pattern must be visualized in a nondestructive manner. Merely locating bloodstains on a garment is sufficient for DNA testing, but to fully understand the entire value of the stain patterns, there must be an ability to visualize the physical characteristics associated with the stain: stain size, shape, distribution, location, overall physical appearance, as well as the interrelationship between the bloodstain and the substrates on which they are located. A bloodstain pattern analyst will evaluate physical characteristics to determine what type(s) of mechanism(s) could account for a specific pattern. This pattern analysis can then be incorporated with other related forensic findings to reconstruct the events surrounding the blood-shedding event(s).

The detection and visualization of bloodstain evidence can be challenging. Searching for bloodstains on dark, patterned, or otherwise interfering substrates is especially difficult. These substrates unquestionably inhibit the examiner's ability to assess the physical characteristics of patterns. Although blood-searching chemicals such as luminol can successfully locate blood stains on dark substrates by means of chemiluminescence, a completely dark environment is necessary for visualization, and the luminescence must be photographed quickly as it fades in a matter of minutes (1). Film-based infrared (IR) photography has been utilized in forensic casework to document bloodstains on dark surfaces (2,3). Fuji took IR photography a step further when they introduced a UV/IR digital camera, which has since been discontinued. Currently, those desiring single lens reflex (SLR) IR digital cameras must send their SLR cameras out for aftermarket modification of the camera's sensors. Lin et al. (4) had success using an IR light source with a camera containing an IR-sensitive CCD array to visualize and capture bloodstains on dark fabrics. IR photography has illustrated that visualization of bloodstains on some dark substrates can be improved by viewing them in the IR range. IR photography techniques require the examiner to manually change filters to move between wavelengths in the IR spectrum. Another method that has been explored for the visualization of blood stains on black fabrics is the use of polarized light filters over both the camera lens and light sources (5).

Near-infrared (NIR) hyperspectral imaging (HSI) is a versatile technology that has been shown to assist forensic scientists in the characterization and differentiation of various types of evidence (6). NIR HSI combines digital imaging technology with conventional spectroscopy for analysis of samples. It provides high spatial resolution, high image definition, and full spectrum analysis. In operation, digital images of the sample are recorded as a function of wavelength through the use of a liquid crystal tunable filter (LCTF), generating a fully resolved spectrum for each pixel location in the multiframe image (i.e., hypercube) (7,8). The LCTF is computer controlled and may be tuned to any wavelength in the electromagnetic spectrum from 400 to 1100 nm, providing access

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to hundreds of spectral bands. In the resulting hypercube, contrast within the image is based on the varying amount of absorption, reflection, or scattering that the various components of the sample exhibit at different wavelengths. Because each component of the sample has a complete spectrum associated with it, signal(s) associated with background or other interfering components can be effectively removed or minimized, with the remaining signal resulting only from the components of interest. The ability to process an image by using the underlying spectral information produces a significant enhancement in the image contrast and therefore allows for a better characterization of the overall sample. The combined spatial and spectral information, along with image analysis software (ChemImage Xpert™; ChemImage Corporation, Pittsburgh, PA), can reveal subtle features of a material that are often missed using traditional imaging techniques.

In this article, the utilization of NIR HSI for the detection of bloodstain patterns on black fabrics is discussed. HSI technology presents forensic scientists with an analytical technique that provides advanced visualization and spectral characterization of bloodstain patterns without harming the integrity of the evidence.

Materials and Methods

Collection

Samples that contained *transfer stains* and *spatter stains* were examined (9). The blood on the analyzed samples was fresh human blood, containing no preservatives or anticoagulants. Prior to examination with HSI, the bloodstains were not chemically treated or prepared in any additional way. Figure 1 depicts the spatter apparatus utilized to create the spatter stains; this device was made in-house. The upper jaw is powered with a spring-loaded door hinge, which when released, impacts a known quantity of blood on the lower jaw. This device allows the researchers to control the quantity of blood being impacted, the magnitude of force impacting the blood, and the distance between the fabric samples and the impact site. By controlling these variables, the researchers are able to produce impact patterns with reproducible distributions and size range of spatters on the black fabric samples. Initially, the spatter apparatus was utilized to spatter blood onto white cardboard to assure that the apparatus was producing spatter of a uniform size and distribution (10). The transfer stains were created by swiping a bloody finger across the surface of the fabric. One transfer stain and one spatter stain were examined for each substrate: 100% cotton T-shirt (black), 100% cotton denim (black), and 50/50 polyester/cotton blend T-shirt (black). The size of the substrate fabric swatches was approximately 4×4 inches. As a proof of concept, only three materials were chosen to demonstrate the basic abilities of HSI to visualize bloodstains on commonly encountered fabrics. Black fabrics were chosen as sample substrates because of their known difficulty for stain visualization (11).

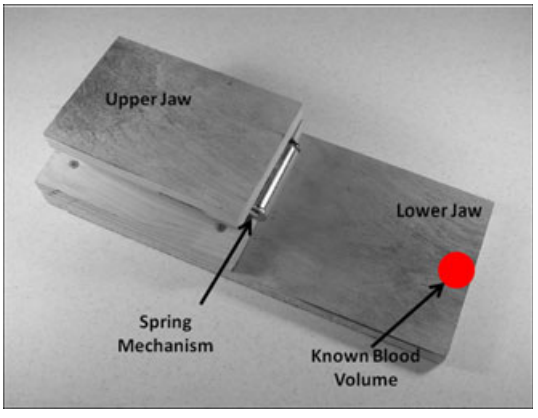

FIG. 1—*Spatter apparatus utilized to create the spatter stains. A blood spatter pattern is created when the spring loaded upper jaw is released and subsequently impacts a known volume of blood located on the front of the lower jaw.*

A sample containing stains from three different materials (blood, lotion [Vaseline® Intensive Care®; Unilever, Trumbull, CT], and lipstick Bodyography™ Super Naturals™, Oasis; Santa Monica, CA]) was also prepared. The purpose of this sample was to show the spectral and visual difference between blood stains and stains of other materials. Each substance was deposited on the 100% cotton T-shirt material in the form of a transfer stain. In addition to samples noted previously, two samples, both on black, 100% cotton T-shirt material, were analyzed as "search samples" where the preparer deposited the blood on the samples in locations blinded to the analyst. For these particular samples, the analyst, unaware of the bloodstain location or stain pattern type, used HSI to scan the fabric for possible bloodstain deposits.

Hyperspectral Imaging—Data Collection and Processing

HSI in the NIR region (650–1100 nm) was performed using a Visible/NIR CONDOR™ Hyperspectral Imaging System (ChemImage Corporation). A 50W Quartz Tungsten Halogen (QTH) white light source was used for sample illumination. ChemImage Xpert™ software was used for the control of the instrumentation during data collection. Because of the strongly absorbing nature of the denim fabric, a longer exposure time was required to achieve a suitable signal, as compared with the T-shirt samples. Because the bloodstain pattern on the denim sample could not be resolved using the NIR region of the spectrum, a short-wave infrared (SWIR) CONDOR™ Hyperspectral Imaging System (960–1650 nm) was also used to collect data on the denim fabric samples, in the hopes that the extended NIR range would yield greater contrast. As with the NIR CONDOR™, a QTH white light lamp was utilized for sample illumination. The experiment parameters for both NIR and SWIR data collection are listed in Table 1.

Varying levels of magnification were used throughout the experimentation process to collect images of both the overall stain pattern and individual droplets. For each zoom setting, an image was taken on a 99% reflectance standard for the purpose of flat-field correction. A flat-field correction was performed to account for instrument and illumination response in the data.

Once the images were collected and flat-field corrected, ChemImage Xpert™ software was used to further process the images. To view the stains, the images were processed to increase the blood to background contrast. Because each pixel has an associated spectrum, spectral processing, noise-reducing filters, and chemometric tools such as principal component analysis (PCA) were applied to images in order to enhance spectral differences among various chemical species to increase visualization. Flat-fielding, background division, and spectral normalization will be referred to as "basic processing steps," as these steps were applied consistently to all images before any additional steps such as PCA were applied. Background division is a spectral processing technique that minimizes the spectral influence of the substrate from the spectra of areas of interest. Background division is performed by manually selecting an area of pixels that is representative of only the substrate. The spectrum of the selected pixels is then divided out from the image.

TABLE 1—*Parameter outline for data collection on various substrates.*

Experiment	Fabric	Wavelength Range of Data Collection (nm)	Step Size for Data Collection (nm)	Exposure Time	Frame Averages per Data Collection	Total Experimental Time (approximately)
NIR	100% cotton, 50/50 polyester/cotton	650–1100	10	0.5 sec	1	30 sec
	100% cotton denim	650–1100	10	10.0 sec	1	7.5 min
SWIR	100% cotton denim	960–1650	10	16.38 msec	30	1 min

Spectral normalization is a scaling technique that adjusts the image contrast to compensate for sample topography. Normalization was consistently applied to the images in order to improve sample contrast between the blood droplets and the substrate. The software employs an auto-contrast function that visually portrays the image reflectance relatively within all pixels within the image. After the data sets are normalized, some of the figures show bloodstains that appear white on a dark background at some wavelengths, while they appear dark on a white background at other wavelengths. The stains appear this way because the data are auto-contrasted based on spectral response. If the substrate reflects less intensely (i.e., absorbs more light) than the bloodstained areas, the substrate will appear dark, and the bloodstain will appear light. Conversely, if the substrate reflects more intensely (i.e., absorbs less light) than the bloodstained areas, the substrate will appear light, and the bloodstain will appear dark.

Results

The CONDOR™ Hyperspectral Imaging system provides the methods necessary to visualize bloodstains on dark fabrics that are otherwise imperceptible to the naked eye, without damaging the sample. Using NIR HSI, bloodstains were visualized on all of the substrates tested. Both transfer stains and spatter stains were visible using HSI. The level of overall stain visualization was affected only by the underlying substrate. By adjusting the zoom lens to its highest magnification setting, an examiner can easily observe a single droplet of blood. Figure 2 shows a digital image of the bloodstain sample in which the bloodstains are not visible to the unaided eye and also a hyperspectral image single-frame extract at 810 nm of the blood droplets visualized on the 100% cotton sample. Also shown in Fig. 2 is a magnified HSI image extract at 810 nm showing an individual spatter stain on the sample. In the magnified image, the interrelationship between the spatters and the weave of the fabric is clearly observable.

Shown in Fig. 3, bloodstains on the 50/50 polyester/cotton T-shirt fabric were not visible to the naked eye under white light, but were easily visualized at 810 nm of the HSI image without applying any data processing steps. The contrast was further improved with the application of only basic flat-fielding and normalization processing steps. Analysis of the transfer stain on 100% cotton yielded similar results. However, the denim material samples proved to be more challenging, requiring advanced image processing steps for partial visualization of the bloodstains.

Even after the advanced processing, the bloodstains on the denim material did not provide as high of contrast between the blood and the background as did the T-shirt materials. Figure 4*b* shows the SWIR hyperspectral image extract at 1600 nm with only the basic processing steps (flat-fielding, background division, and normalization) applied, while Fig. 4*c* demonstrates the appearance of the image after convolution filtering has been applied. Figure 4*d* shows the extract after PCA was applied to the data set. PCA is a data reduction technique designed to describe variance, where the first principal component (PC) describes the maximum amount of variance (12). That variance is subtracted from the entire image, and the second PC describes the maximum amount of variance that is remaining, with the process continuing until all variance within the image has been described. Figure 4*d* corresponds to PC 2.

HSI was able to visualize stains that were present on Search Sample A. Two stains were captured in a single-camera field of view (Fig. 5), with a third stain located in a second field of view. The locations of the stains were determined by switching the instrument to "live" mode and visually scanning the sample for stains. Live mode provides a real-time live image of the sample at one chosen wavelength (810 nm). Once the locations of the stains were determined, hyperspectral images were collected of the two fields of view to compare, at multiple wavelengths, the stains on Search Sample A to known bloodstains. The hyperspectral image of Search Sample A was concatenated with the hyperspectral image of the sample containing impact spatter on 100% cotton T-shirt material (see Fig. 2). Figure 6*a*,*b* show the concatenated NIR hyperspectral image extracts at 810 and 1080 nm, respectively. The consistent appearance of the stains in both 810 nm extract images and in both 1080 nm extract images shows that the contrast in Search Sample A is similar to the contrast observed in known bloodstains. To further compare Search Sample A stains to known bloodstains, reflectance spectra were generated for the stains that were visible in both samples. Figure 6*c* displays the spectra correlating the stains on Search Sample A (solid line) and the stains on the known sample (dotted line), after background division had been applied to the spectra. The spectral and visual comparisons of the stains on these samples show that the stains reflect similarly throughout the range of data collection. The entire surface area of Search Sample B was analyzed in the same manner as Search Sample A, with no stain location upon completion. After reporting the results, the preparer confirmed that blood was not deposited on Search Sample B and that this sample was used as a negative control.

As illustrated in Fig. 7, HSI is able to provide preliminary stain differentiation on a sample containing more than one type of staining material. The differentiation is based on the contrast provided at several wavelengths, demonstrating that the staining materials display varying reflectance and absorbance properties. Spectral data assist in the verification of the chemical contrast by providing chemical profiles to further illustrate the variations in reflectance and absorbance properties at various wavelengths. Three extracts from the hyperspectral image are shown in Fig. 7*a–c*, displaying the varying absorbance and reflectance that occur from the different components at various wavelengths (650, 810 and 1060 nm, respectively). The reflectance spectra associated with the components of this sample are shown in Fig. 7*d*. Notice that each of the components (lipstick, lotion, and blood) has a reflectance spectrum associated with it; to assist with stain differentiation, the spectral influence of the background was divided out, so that the spectra of the components would be less influenced by absorption and reflectance of the black dyes and more representative of the substances

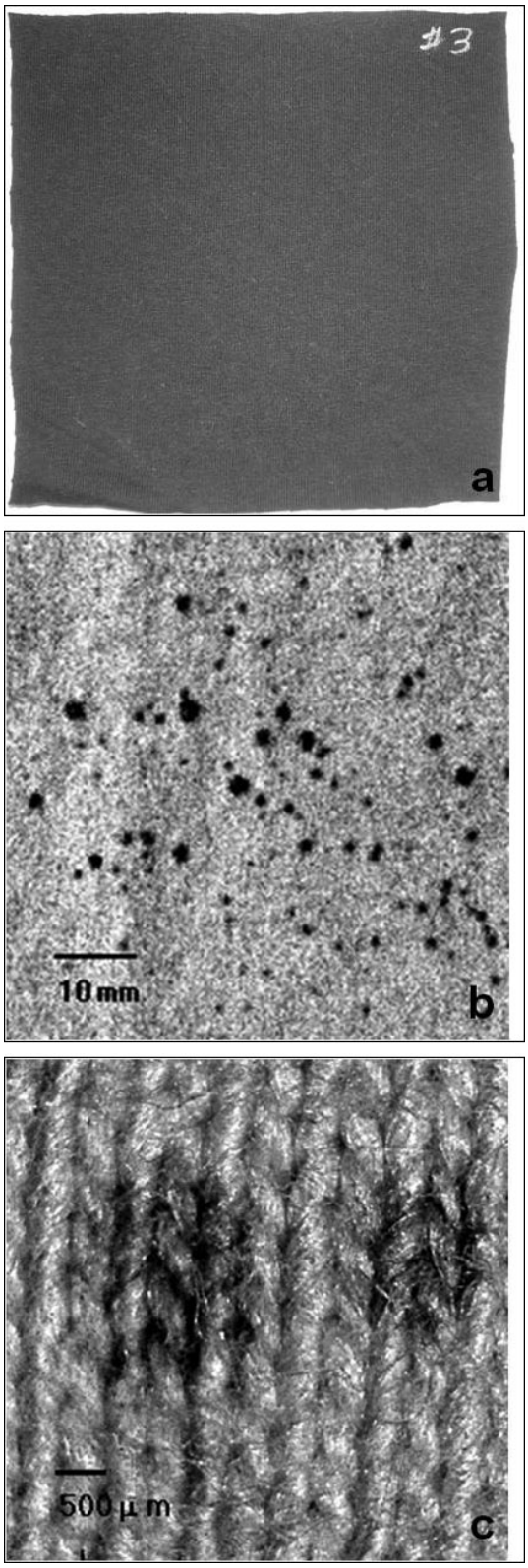

FIG. 2—*(a) Digital image of black 100% cotton fabric with spatter stains. (b) Hyperspectral image at 810 nm of spatter stains on black 100% cotton fabric. (c) Magnified image of individual blood spatters within the spatter pattern on the black fabric.*

of interest. Therefore, without using any other test or chemical treatment, determining the presence of multiple stains caused by a variety of substances is possible.

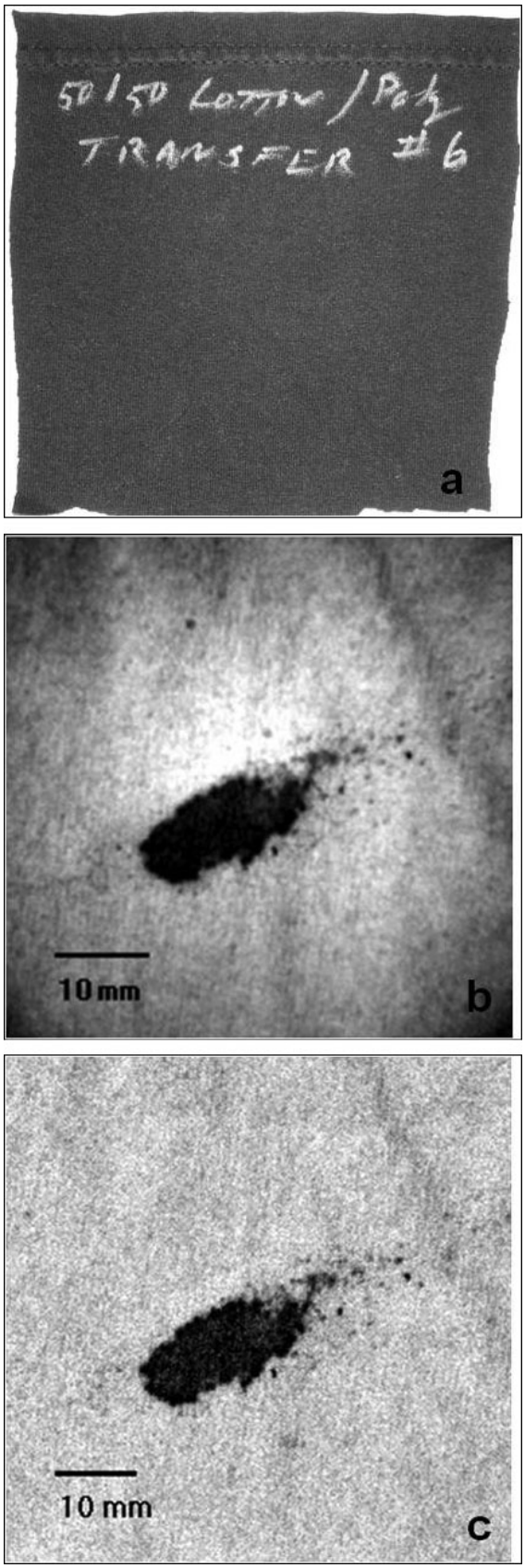

FIG. 3—*(a) Digital image of black 50/50 polyester/cotton fabric with a transfer stain. (b) Unprocessed hyperspectral image at 810 nm of a transfer stain on black 50/50 polyester/cotton fabric. (c) Hyperspectral image at 810 nm of the transfer stain on black 50/50 polyester/cotton fabric after basic processing steps had been applied.*

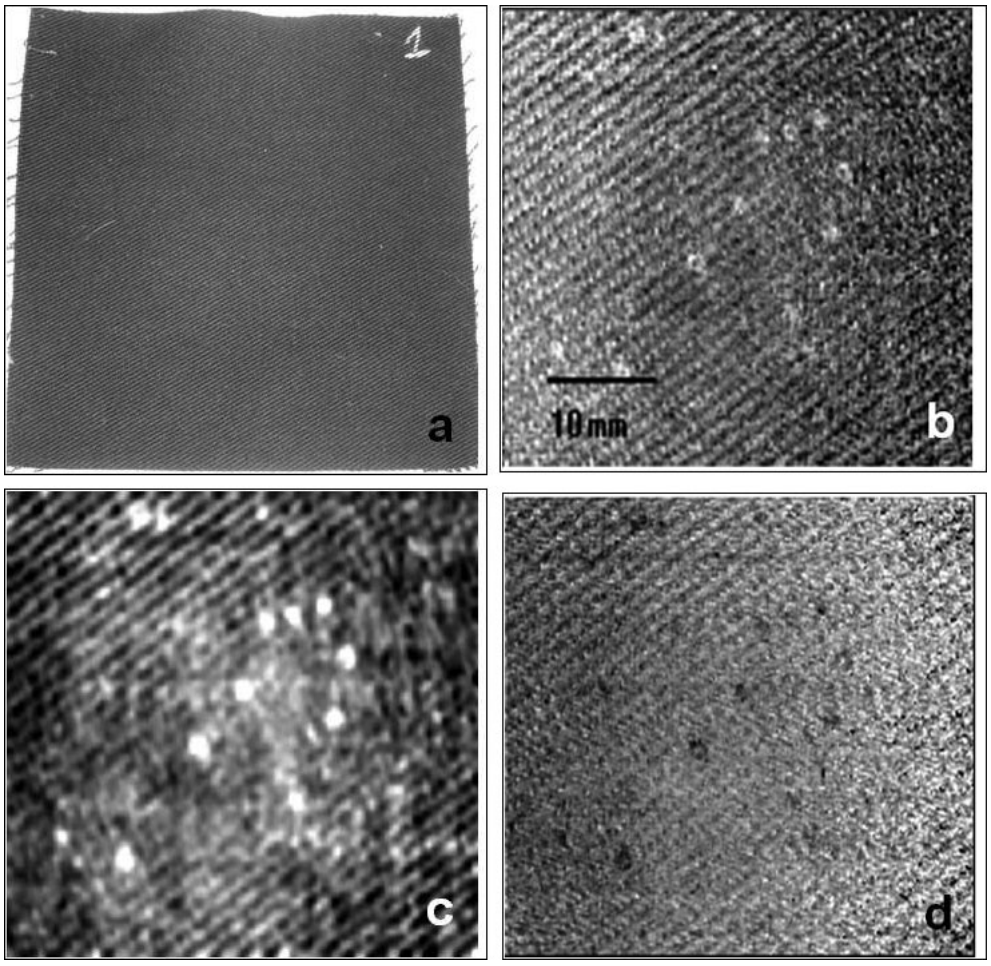

FIG. 4—*(a) Digital image of spatter stains on black 100% cotton denim fabric. (b) Hyperspectral image at 1600 nm of spatter stains on black 100% cotton denim fabric after basic processing steps had been applied. The scale on image (b) is applicable to the following (c, d) images. (c) Hyperspectral image at 1600 nm of impact spatter on black 100% cotton denim fabric after convolution filtering had been applied. (d) Principal component image of spatter stains on black 100% cotton denim after principal component analysis had been applied.*

Discussion

The samples analyzed in this study are representative of substrates and types of bloodstain patterns commonly encountered in forensic casework. HSI with NIR and SWIR reflectance provides a useful tool for visualizing bloodstains on these substrates. HSI is a noncontact, nondestructive technique for attaining images of spatter stains and transfer stains. Because of the noncontact and nondestructive nature of HSI, the integrity of the sample is maintained to permit possible future analyses, such as DNA testing. The acquisition time for scanning the sample, collecting the data sets, and processing the bloodstain images is relatively fast, often less than 5 min. However, this acquisition time is largely dependent on the size and properties of the substrate. The main limitation to acquiring data is with large samples (shirts, pants, bed sheets, etc.). The maximum imaging field of view the instrument provides is 10×10 cm, while the entire sample enclosure is only about 30.5×38.1 cm. The current size of the enclosure makes the analysis of large garments time consuming.

The reflectance/absorbance of individual substrates is one factor that may influence the ease of visualization of the bloodstains on that particular substrate. Blood is largely absorbing across the entire light region (UV, Visible, and NIR); therefore, if a substrate is also absorbing in the same region, little to no contrast will be observed, even after extended image processing steps (13). In the case of the samples examined in this study, the T-shirt material fabrics appeared to have dye components that reflected in the NIR region of the spectrum, therefore causing the absorbing bloodstain to be quite visible against the light-reflecting background. The denim material, on the other hand, appeared to contain dye components that absorb light across the entire spectral range, making the visualization of the bloodstain quite difficult. Further studies are necessary to better understand the impact of various dyes on the overall quality of visual contrast provided by HSI. It is likely that with dark-colored materials, the light-absorbing properties of the dye also affect the incident light when staining materials become absorbed into the fabric (14).

In addition to the reflectance properties of a substrate, the texture of the substrate can also affect the ability to view a bloodstain pattern. Not only can light reflect off the weave in varying directions, possibly interfering with the light being reflected from or absorbed by the bloodstain, but the weave of the fabric can also distort the bloodstain itself. A fabric that is highly absorbent with a course weave is found to distort a bloodstain more so than a fabric that has been treated or that exhibits a tighter weave (15).

Determining the mechanism (spatter vs. transfer) by which a stain is deposited onto an article of clothing is not a simple task and requires the analyst to take into consideration a number of

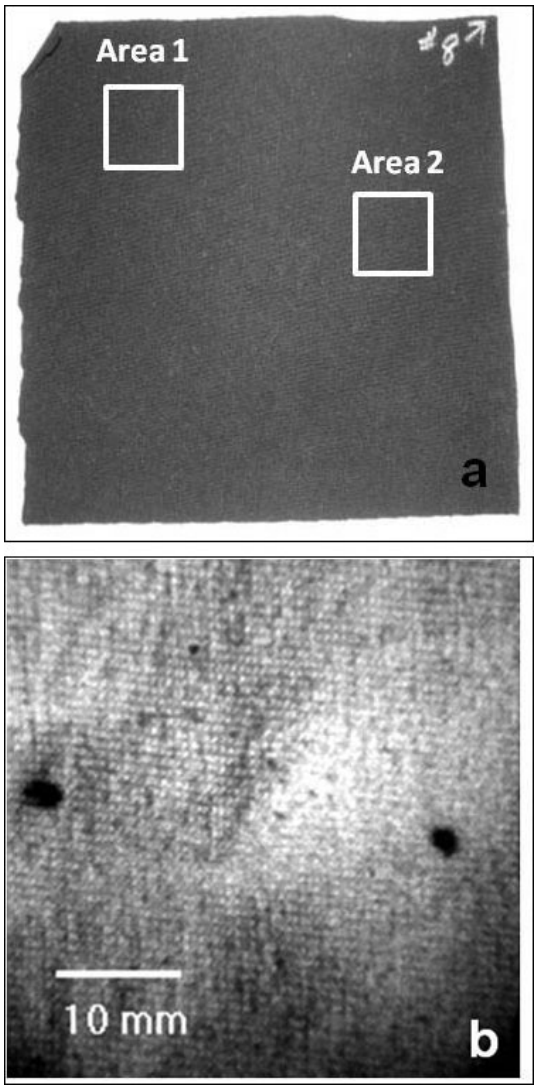

FIG. 5—*(a) Digital image of Search Sample A. The boxes outline the areas where bloodstains were found on the sample. (b) Hyperspectral image at 810 nm of the bloodstains found on the sample in area 1.*

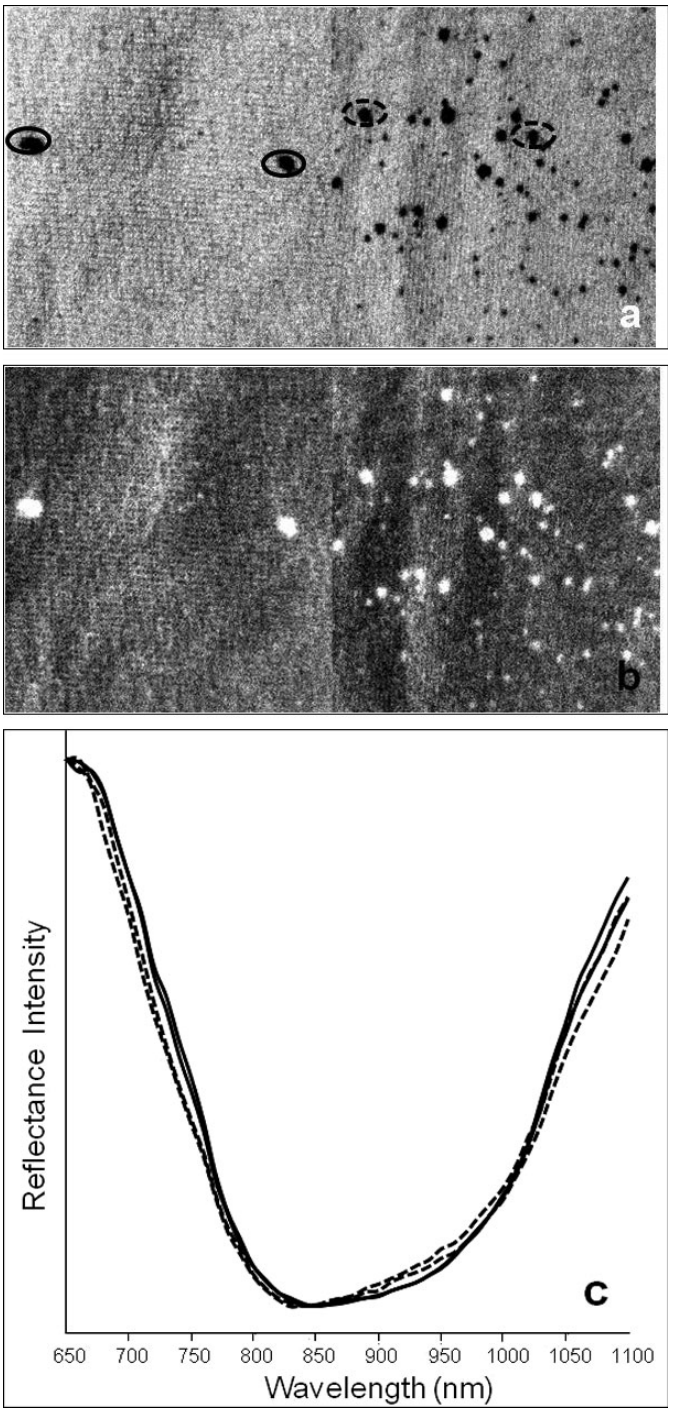

FIG. 6—*(a) Concatenated hyperspectral images at 810 nm of the bloodstains found in area 1 of the search sample and known bloodstains. The blood droplets outlined indicate areas which were selected to produce spectra representative of the stains. (b) Concatenated hyperspectral images at 1080 nm of the search sample stains and known bloodstains. (c) Spectra of the stains found on the search sample (solid lines) and spectra of known bloodstains (dotted lines), after background division had been applied. The spectra appear similar across all wavelengths of data collection indicating that the stains may be of a similar substance.*

factors in regard to the fabric such as its composition, laundry treatments, weave structure, and environmental factors prior to even beginning to ascertain how the stain was deposited (15). One of the more important factors a bloodstain pattern analyst will evaluate is the interrelation between the blood and the actual weave of the stained fabric. This type of observation normally requires low-power magnification, which is made even more complex with dark-colored fabrics. Generally, transfer stains will stay on the upper portion of the weave, whereas spatter stains will penetrate within the weave of the fabric. If the stain is indeed caused by a transfer of blood, rather than a spatter, deeper areas of the fabric weave will not exhibit blood. With a spatter stain, the inner areas of the weave will likely exhibit blood because the blood is projected onto the fabric. Therefore, when evaluating a fabric that contains a bloodstain, the analyst must assess the substrate, the overall pattern, as well as the interaction of the blood within the weave of the fabric (15). As demonstrated in this study, HSI gives the examiner the capability to collect a hyperspectral image of the overall stain pattern and then zoom in on a particular area of interest to collect a hyperspectral image of a significantly smaller area. HSI's capability to magnify the sample while viewing in the NIR portion of the electromagnetic spectrum is of significant value to the bloodstain pattern analyst. The examiner, therefore, has additional information about the bloodstain pattern that cannot be obtained with traditional IR photography.

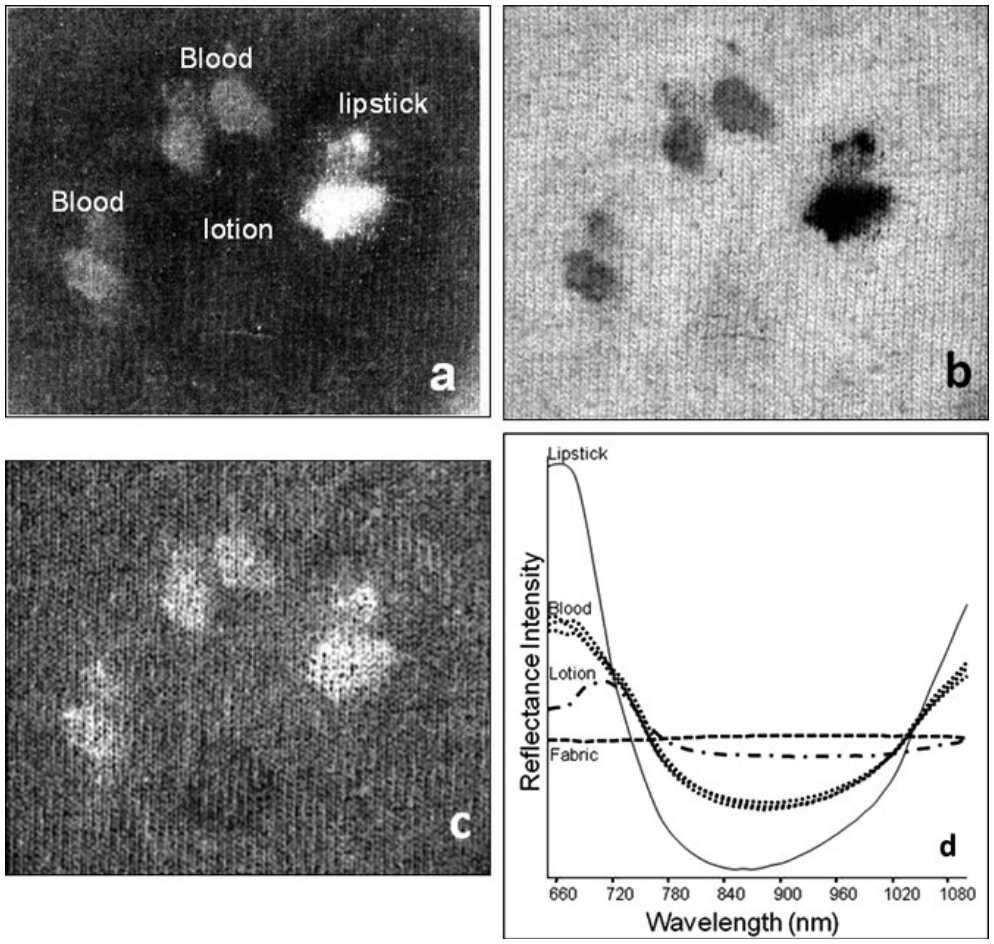

FIG. 7—*(a) Hyperspectral image of blood, lipstick, and lotion stains on black fabric at 650 nm. The lipstick and lotion stains can be visually differentiated from the bloodstains, and each other, by viewing the images at several wavelengths. (b) Hyperspectral image of blood, lipstick, and lotion stains on black fabric at 810 nm. (c) Hyperspectral image of blood, lipstick, and lotion stains on black fabric at 1060 nm. (d) The spectra corresponding to the blood, lipstick, lotion, and black fabric substrate after background division had been applied. Dissimilarities can be seen between the spectra of different stains, indicating that the stains are not all from the same origin.*

Another main advantage of HSI is the ability to generate a full spectrum for every pixel in the hypercube. Having this ability is especially important in instances where one or more components of the sample must be differentiated from others. Presumptive chemical tests can help the examiner to distinguish bloodstains from stains of foreign materials. However, the examiner must take care not to consume the entire spot when performing presumptive tests, so further testing (confirmation of blood, DNA testing) can be performed on the sample (personal communication, Kish P, 2009). Another drawback to presumptive chemical testing is that other nonblood substances can give a false-positive result ([10]; personal communication, Kish P, 2009). The HSI method provides preliminary discrimination between stains caused by different substances occurring on the same substrate without applying any chemical treatment and without consuming or destroying any portion of the sample ([15]; personal communication, Kish P, 2009). These substances may appear visually similar upon an initial inspection; however, after collecting a hyperspectral image, a spectral characterization is generated for each of the substances. Within the images of the hypercube, the substances will appear dark or light depending on the reflectance/absorbance properties at a particular wavelength, and therefore, the substances can be discriminated visually. The visual contrast is directly related to the chemical properties of the sample, thereby providing chemical characterization. Also, because each substance will exhibit a characteristic reflectance/absorbance profile, the substances can also be discriminated spectrally.

Conclusions

The HSI methods described in this article successfully provided images with clearly visible bloodstain patterns. The capability of HSI to acquire images in a nondestructive manner is especially important to examiners who must use the evidence for further testing, such as DNA. This proof-of-concept study is only the beginning of what HSI can do for bloodstain pattern analysts. Although further studies are necessary to demonstrate the overall capability of HSI as a means of stain differentiation, the images acquired through HSI are of sufficient quality to assist a bloodstain pattern examiner in differentiating between stains produced from a spatter mechanism or a transfer mechanism. In addition, while the generation of spectra is not necessary in every case, having that ability in cases where more than one type of stain may exist will help the examiner separate the stains based on the visual contrast that is directly related to the chemical components of the materials. By using HSI in conjunction with technologies such as alternate light sources, the potential of locating and visualizing bloodstains is increased. HSI also has the added benefit of providing preliminary

information that can be used for stain differentiation based upon the reflectance and absorbance properties of various staining materials. Further research is required to optimize and validate the above techniques for a larger variety of substrates and staining materials, including commonly encountered body fluids such as semen, saliva, and urine. Future studies will be necessary to better understand not only the visualization capabilities of bloodstains in comparison to other body fluids on dark substrates but also body fluid stain spectral differentiation.

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