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White Paper

# Hyperspectral Imaging Provides Detection and High Contrast Imaging of Biological Stains

Keywords: Forensic Science, forensic biology, biological fluids, hyperspectral imaging, nondestructive analysis

## ABSTRACT

The analysis of biological fluids at a crime scene or on an article of physical evidence can assist investigators in understanding the circumstances surrounding a violent crime as well as provide a link between a suspect, the victim and the crime scene through DNA analysis. Initial screening for biological fluid evidence typically involves scanning an area that is untreated, or has been treated with fluid contrast enhancement reagents, with a specific wavelength of light while wearing colored goggles. Any contrast that is observed could indicate biological evidence. Once evidence is located, chemical tests are often performed to presumptively identify the unknown material. Both optical searching and chemical test techniques are reasonably reliable but present a unique set of challenges. These challenges include the need to work in a completely dark environment and the potential for sample adulteration/destruction due to the use of contrast enhancement reagents. These challenges, along with other difficulties associated with forensic casework (e.g. inhomogeneous samples located on non-ideal substrates, evidence recovered under non-ideal environmental conditions, the inability to appropriately obtain evidence at a crime scene, etc.) emphasize the necessity to modify existing techniques and instrumentation to better locate, capture, and document evidence. In this study, ChemImage's HSI Examiner™ 1000 (Examiner), employing visible and near-infrared (NIR) reflectance hyperspectral imaging (HSI), was used to observe saliva, sweat, urine, blood and semen stains on a variety of natural and semi fabrics. The Examiner's capability for visualizing and presumptively locating biological stains on a variety of challenging substrates is demonstrated.

## INTRODUCTION

Biological evidence is often the by-product of a violent crime. Blood, semen, saliva, urine, and sweat, and their corresponding stain patterns deposited during acts of violence are of particular interest to the forensic examiner. This type of evidence is routinely utilized to make critical forensic links between victim(s), suspects, physical evidence, the crime scene, and the nature of the crime. In order to assess the entire forensic value of a stain, it must be located and the physical characteristics of the individual stains which make up the overall pattern must be visualized and documented in a nondestructive manner. Merely locating stains on a garment may be sufficient for DNA testing but in order to fully understand the forensic value of the stain, the stain must be visualized so that physical characteristics such as type, size, shape, distribution, location, and overall physical appearance can be determined. This pattern analysis can then be incorporated with other related forensic findings to reconstruct the events surrounding a crime.

The detection and visualization of biological stain evidence on dark, patterned or otherwise interfering

substrates can be challenging. These substrates often inhibit the examiner's ability to assess the physical characteristics of patterns (5,17). Chemical treatments may aid in locating fluids on difficult substrates. Chemical treatments for presumptive screening include acid phosphatase (AP), Phadebas®, and Luminol, for semen, saliva, and blood stains, respectively (16). In operation, chemical treatments such as Luminol can successfully visualize blood stains on dark substrates by means of chemiluminescence, but a completely dark environment is necessary for visualization and the luminescence must be photographed quickly as it fades in a matter of minutes (1). Additionally, the repeated application of Luminol may diffuse a bloodstain pattern on non-porous surfaces (16).

The reflectance/absorbance of individual substrates is one factor that influences visualization of stains 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 (11).

In addition to the reflectance or fluorescence properties of a substrate, the texture of the substrate can also affect the ability to view a stain pattern. Not only can light reflect off the weave in varying directions, possibly interfering with the light being reflected from or absorbed by the stains, but the weave of the fabric can also distort the stain itself. A fabric that is highly absorbent with a course weave is found to distort a stain more so than a fabric that has been treated or that exhibits a tighter weave (12).

Hyperspectral Imaging (HSI) is a proven, versatile technology that assists examiners in locating and visualizing biological stains, especially when these stains occur on dark, patterned or otherwise interfering substrates (6). HSI combines digital imaging technology with optical spectroscopy for analysis of samples. It provides high spatial resolution, high image definition and full spectrum analysis. In systems developed by ChemImage Corporation and in use by Forensic Examiners around the world, digital images of the sample are recorded as a function of wavelength modulated by liquid crystal tunable filters (LCTF), generating a fully resolved spectrum for each pixel location in the multi-frame image (also known as a hypercube) (7,8). The LCTF is computer controlled and may be tuned on demand to any wavelength in the electromagnetic spectrum within the free spectral range of that LCTF. In practice, this provides forensic users access to many hundreds of unique spectral bands throughout the visible and near-infrared spectrum when employed in combination with a silicon focal plane array detector. In the resulting hypercube, contrast within the image is based on the varying amount of absorption, reflection, or scattering that the individual sample components 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 minimized or even removed. The remaining signal, therefore, is a result of only 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 (HSI Examiner software v. 2.3.0.12, ChemImage Corporation, Pittsburgh, PA), can reveal subtle features of a material that are often missed using traditional imaging techniques. (6,7,15)

In this White Paper, the utilization of Visible and Near-Infrared HSI for the detection of biological stains on a variety of natural and semi-synthetic fabrics is discussed. As shown here, HSI technology provides forensic scientists with an analytical technique that offers advanced visualization of biological stains, in a non-destructive manner, thus preserving evidence integrity.

## MATERIALS AND METHODS

*Stain Preparation*

All biological fluid samples were procured from Lee Biosolutions, Saint Louis, MO and arrived frozen. The samples were defrosted and prepared immediately upon receipt

*Biofluids on Variable Substrates Feasibility Study*

20 μL of human saliva, sweat, seminal fluid, urine, and blood were individually spotted in different locations onto different fabrics. **Figure 1** depicts the pattern in which each of the five pure fluids were prepared on each of nine fabrics for the feasibility study.

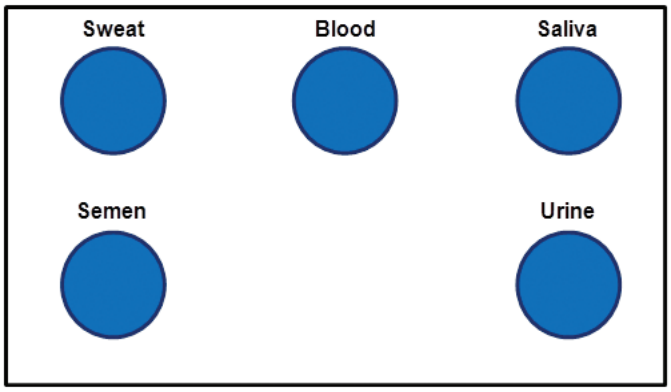



*Figure 1. Pattern of fluid spots on fabric.*

| Fabric Sample | Substrate Material |
|---|---|
| White | 100% Cotton |
| White | 60% Cotton, 40% Polyester |
| Black | 100% Cotton |
| Black | 60% Cotton, 40% Polyester |
| Blue Denim | 100% Cotton |
| Dark Gray Denim | 100% Cotton |
| Dark Gray Denim | 99% Cotton, 1% Spandex |
| Green Floral Print | 100% Cotton |
| Dark/Red Multicolored Print | 70% Cotton, 28% Polyester, 2% Spandex |

Table 1: Types of fabric utilized in the feasibility study.

*Mock Crime Samples*

5 pairs of Women's 100% Cotton, Hanes™ Bikini Briefs (White, Black, Purple, Blue/Gray Floral on White, and Black pattern on White) were used as the Mock Crime Substrates. 1000 μL of blood and 100 μL of semen were spotted on the crotch area of the underwear, overlapping the spots to some degree. 100 μL of saliva, 100 μL of sweat, and 100 μL of urine were spotted on the middle area of the underwear between the waistband and the crotch, overlapping the spots to some degree. **Figure 2** illustrates the spotting pattern.

*Hyperspectral Imaging - Data Collection and Processing*

Hyperspectral imaging in the visible-NIR region of the spectrum (400-1100 nm) was performed using the HSI Examiner™ 1000 (ChemImage Corporation, Pittsburgh, PA). Three 75 Watt Quartz Tungsten Halogen (QTH) white light lamps with enhanced NIR emission provided sample illumination for white light collection. Additionally, a mini-Crimescope 400 (Spex Forensics) 400 W metal halide arc lamp with multiple excitation bandpass filters was used to excite samples for luminescence data collection. The following table shows the illumination/ excitation wavelengths and corresponding data collection parameters.

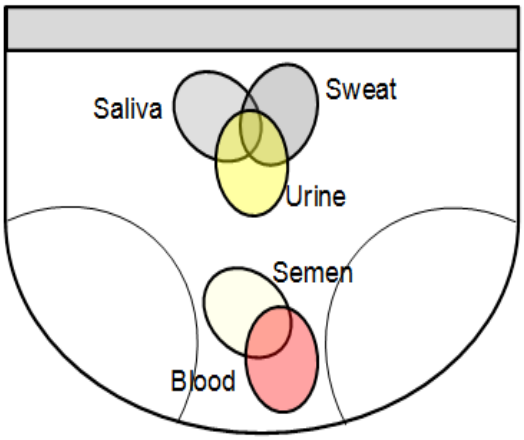



*Figure 2. Spotting pattern of biological fluids on mock crime scene sample.*

| Illumination/Excitation Wavelength | Exposure Time(s) | Wavelength Range (nm) | Wavelength Step Size (nm) | Binning |
|---|---|---|---|---|
| White Light | Variable using LCTF Compensation | 400-1100 | 2 | None |
| 300-400 nm | 20 | 400-720 | 10 | 2x2 |
| 415 nm | 15 | 460-1100 | 10 | 2x2 |
| 455 nm | 10 | 510-1100 | 10 | 2x2 |
| CSS (Crime Scene Search-Short Pass SP540 nm) | 5 | 540-1100 | 10 | 2x2 |
| 495 nm | 2 | 550-1100 | 10 | 2x2 |

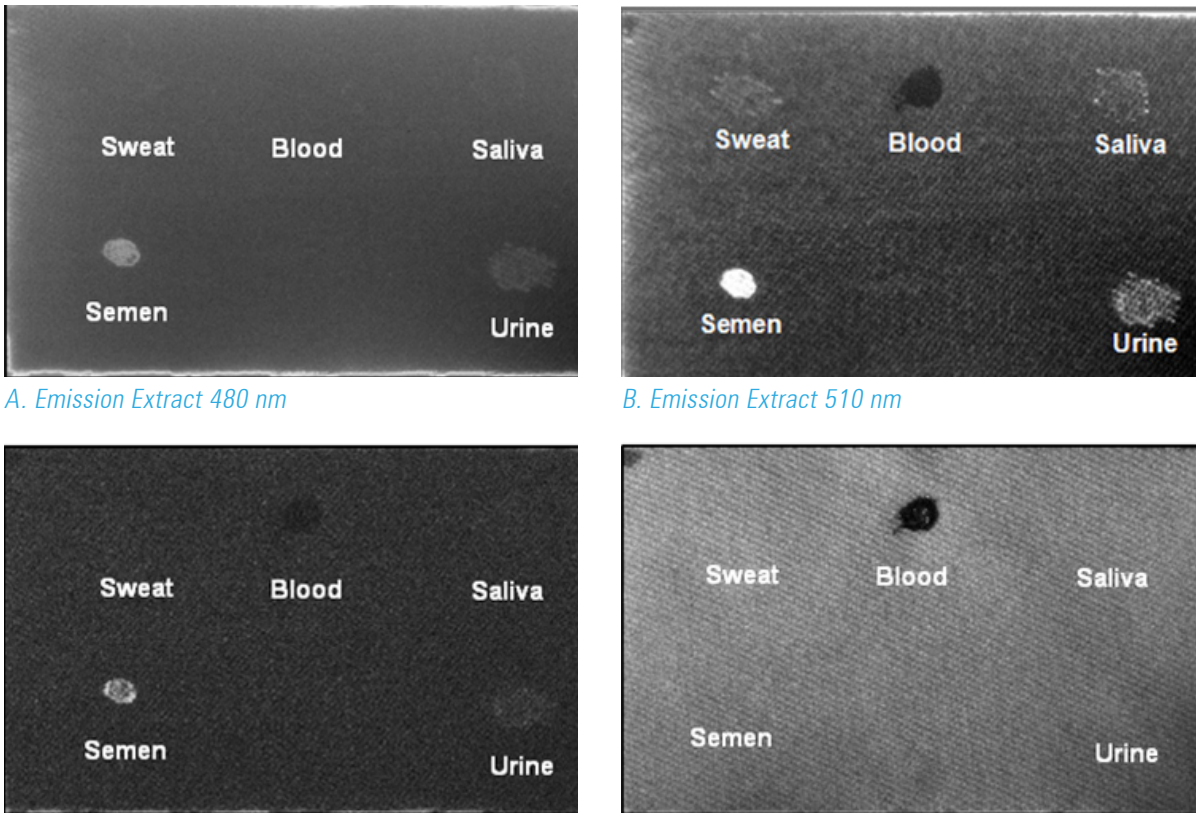
Table 2: Data Collection Parameters

The HSI Examiner Software (v. 2.3.0.12) was used for the control of the instrumentation during data collection.

For optimal observation and discrimination of the stains, the images were processed to increase the stain to background contrast. The first processing step was flat field correction. A flat field correction removes or minimizes the instrument lighting response (including uneven illumination), within the image. For the white light reflectance samples, flat field correction was performed on a wavelength by wavelength method using a white 99% reflectance standard. Each wavelength of the sample hyperspectral image was corrected by the corresponding wavelength image of the reflectance standard. For the luminescence samples, the sample hyperspectral image was divided by a single frame within that same image (typically, the last frame).

The next processing step was spectral vector normalization, a scaling technique, which adjusts the image contrast to compensate for sample topography. Normalization was consistently applied to the images in order to improve sample contrast between the stains and the substrate.

Principal Component Analysis (PCA) is a data reduction technique designed to describe variance, where




*A. Emission Extract 480 nm*

*B. Emission Extract 510 nm*

*C. Emission Extract 700 nm*

*D. Emission Extract 820 nm*

*Figure 3. Visualization of Blue Denim substrate through a 415 nm excitation filter. The various emission wavelengths show contrast between the five different biological stains.*

the first principal component (PC) describes the maximum amount of variance (10). 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.

## RESULTS

The HSI Examiner 1000 provides a nondestructive method to visualize biological stains on fabrics. **Figure 3** shows hyperspectral image extracts of the 100% cotton blue denim. **Figure 4** shows a hyperspectral image extracts of the 100% white cotton. Both the blue denim and the white cotton exhibit differences in contrast between stains throughout the spectrum.

As illustrated by **Figures 3 and 4**, HSI is able to differentiate stains on a sample containing more than one type of staining material. The reflectance spectra associated with the components of this sample are shown in **Figure 5**. Each of the components has a unique reflectance spectrum associated with it. 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 background dyes and more representative of the fluids themselves. Therefore, without using any other test or chemical treatment it is possible to

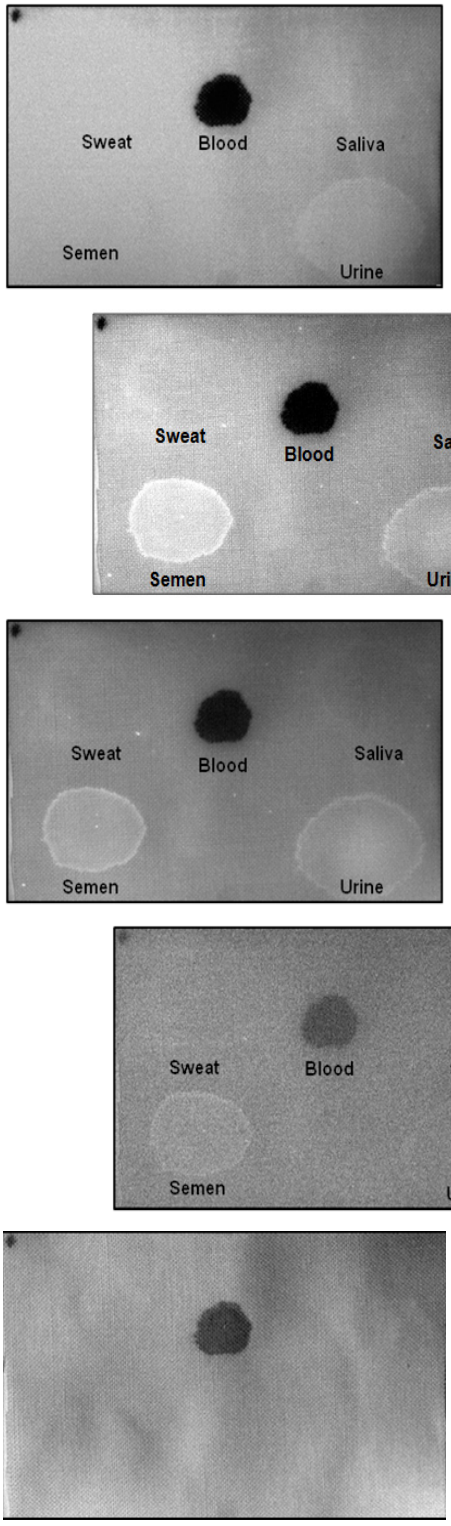
detect and visualize multiple biological stains with a high degree of effectiveness. Figure 6 provides an example of the mock crime scene sample and the conventional photography image. **6A** shows the RGB image, **6B** shows a PC of the hyperspectral image, and **6C** shows the photograph using 455 nm excitation and an orange barrier filter. The conventional photography method with excitation light/barrier filter does not indicate the three stains as clearly as the hyperspectral image.

## DISCUSSION

The samples analyzed in this study are a small representation of substrates and types of biological stain patterns that may be encountered in forensic casework. Vis-NIR HSI provides a useful tool for visualizing stains on these substrates. HSI is a non-contact, nondestructive technique for detecting and visualizing biological stains. Because of the non-contact and nondestructive nature of HSI, the sample is not damaged and its integrity is preserved for possible future analysis, such as for DNA. The acquisition time for scanning the sample, collecting the datasets and processing the images is relatively quick, often less than 5 minutes, but can take as long as 20 minutes. Acquisition time is largely dependent on the size and properties of the substrate, the camera integration time, and the HSI mode used (reflectance vs. fluorescence). The main limitation to acquiring data is with large samples (shirts, pants, bed sheets etc). The maximum imaging field of view that the instrument provides is 231 mm x 310 mm, while the entire sample enclosure is about 40 cm x 80 cm.

The fully resolved spectrum that is available for each pixel within a hyperspectral image acquisition allows for spectral processing that enhances biological fluid detection, while minimizing the reflectance and fluorescence properties of the substrate.

Presumptive chemical tests can help the examiner to distinguish between stains of various biological origins. However, the examiner must take care not to consume the entire spot when performing presumptive tests so




*A. Emission Extract 560 nm*

*B. (Below) Emision Extract 600 nm*

*C. Emission Extract 690 nm*

*D. (Below) Emission Extract 730 nm*

*E. Emission Extract 820 nm*

*Figure 4. Visualization of 100% White Cotton substrate through a 415 nm excitation filter. The various emission wavelengths show contrast between the five biological stains.*

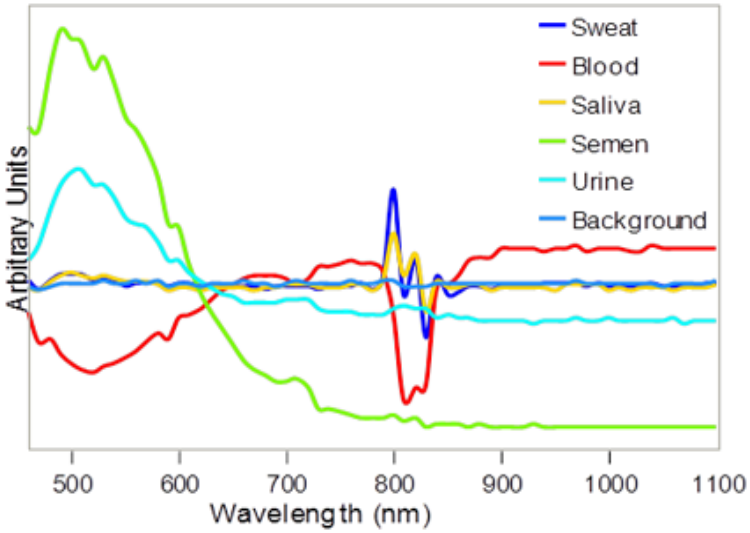



*Figure 5: Reflectance spectra associated with samples used in this study.*

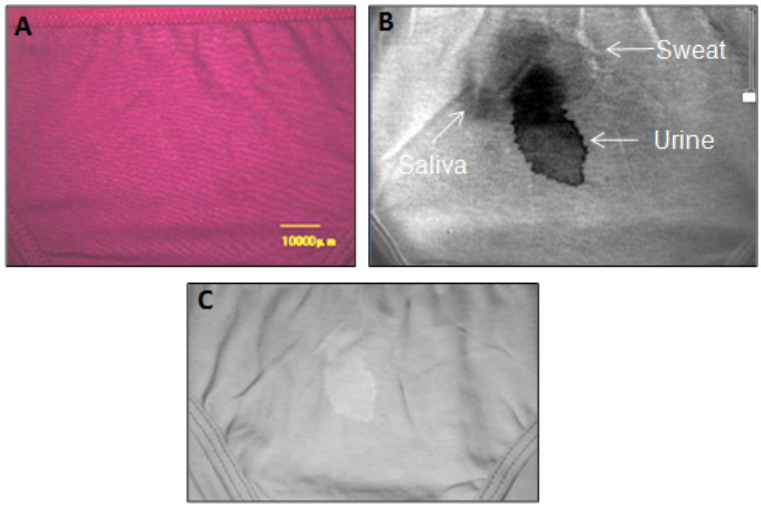



*Figure 6: An example of the mock crime scene sample and the conventional photography image. 6A shows the RGB image, 6B shows a PC of the hyperspectral image, and 6C shows the photograph using 455 nm excitation and an orange barrier filter. The conventional photography method with excitation light/barrier filter does not indicate the three stains as clearly as the hyperspectral image.*

further testing (confirmation of blood, DNA testing) can be performed on the sample (13). Another drawback to presumptive chemical testing is that other substances can give a false positive result, such as a non-blood sample containing iron giving a false positive for blood result (13, 9). The HSI method allows the user to discriminate between biological fluid stains occurring on the same substrate without applying any chemical treatment and without consuming or destroying any portion of the sample (12, 13). These substances may appear visually similar upon an initial inspection; however, after collecting a hyperspectral image, a unique spectrum 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/emission properties at a particular wavelength, therefore the substances can be discriminated visually. Because each substance will exhibit a characteristic reflectance/absorbance/luminescence profile, the substances can also be discriminated spectrally.

HSI often provides an advantage over conventional photography techniques. First, HSI eliminates the need to switch out barrier filters or goggles while using a specific excitation wavelength. Additionally, conventional photography may detect stains, but it cannot discriminate stains with similar responses. By analyzing the spectral responses, two or more stains can be differentiated based on differing spectral responses.

## CONCLUSION

Hyperspectral imaging nondestructively demonstrated sufficient quality images that could assist a bloodstain examiner in setting apart biological fluids from a substrate. Spectral images supply the examiner with an alternate analysis method to separate a bloodstain from stains caused by other materials. This proof-of-concept study indicates the potential of hyperspectral imaging as a solution that addresses the challenges that exist in biological stain analysis. Compared to conventional methods, hyperspectral imaging provides an improved methodology for detecting and visualizing biological fluid stains on difficult backgrounds.

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