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ABSTRACT

The objective of our research funded by NIJ was to demonstrate proof of concept for a rapid and nondestructive tool using infrared spectroscopy for visualization of blood at crime scenes. Current visualization methods for blood are not specific, require dark conditions, and may not be very sensitive. High discriminating power is important at crime scenes so that time and resources of forensic investigators are not wasted on the collection and analysis of false positive samples.

We have designed a prototype camera using mid-infrared (IR) spectroscopy with a thermal imaging detector that has a spectral response tuned by filters of polymer films. We have also devised a lock-in amplifier that constructs the contrast image of the scene pixel-by-pixel basis in real-time using techniques designed to enhance visualization of blood. An infrared source (e.g., a small heating plate, glow-bar, or space heater) is employed to illuminate a scene with IR light. Light reflected from the scene is employed to achieve imaging by chopping the source, and digitally processing each pixel by a lock-in amplifier approach, to produce an output that is proportional to contrast between stain/no-stain regions. The infrared camera response is also sensitized to spectral regions where blood components (e.g., proteins) show absorbance using a combinatorial simulation-driven design process that selects chemical filters to maximize discrimination between blood-stained and unstained surfaces. Further data processing methods develop and display scene images, with regions indicative regions of the target analyte (latent blood) showing contrast from background. This approach has produced acceptably high signal-to-noise ratios and enabled visualization of blood well below 100× dilutions with visible contrast, while providing some discrimination against substances reported to give false-positive response with other techniques. Besides being rapid, IR imaging for bloodstain detection offers advantages: examiners are not exposed to chemicals, the technique can be used indoors or outdoors under ambient light, patterns are not smeared, and stains are not diluted or altered by chemical reagents. The current instrument is installed on a laboratory optical table and has never left that room; future studies may involve design of a portable instrument that can be carried to other locations for real-world testing and evaluation.

We have concurrently conducted fundamental studies to advance the scientific basis of infrared imaging for crime scene visualization. These efforts have included a study of coating effects on the infrared reflectance spectra of fabrics, evaluation of blood discrimination on textile fabrics, determination of achievable detection limits for blood on fabrics, examination of the effects on spectra of blood induced by fabric orientation and coating uniformity, estimation of the age of blood stains up to 9 months old by infrared spectroscopy, and fundamental studies on optical properties of surfaces and a novel investigation extending the Kubelka-Munk model of diffuse reflectance to three dimensions. While providing a firm scientific background for future studies, this research has opened up novel applications of diffuse reflectance imaging in the mid-infrared region of the spectrum which may have valuable future forensic applications to biological materials on surfaces.
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EXECUTIVE SUMMARY

The research objective of this project was to achieve proof-of-concept for the development of a rapid and nondestructive tool for visualization of blood at crime scenes using infrared spectroscopy. Detection, collection, and analysis of blood and/or semen evidence recovered from a crime scene can be critical in a forensic investigation. Latent stains, those invisible to the naked eye, can result from attempts to alter or clean a surface by an individual. Patent stains, those visible to the naked eye, can still be difficult to detect at the crime scene, especially if a lack of contrast exists between the stain and the background surface.

Crime scene investigators often use a high intensity light source to identify stains for further visual inspection. However, this step can be insufficient for detection if only trace amounts of blood are present, if the bloodstained area has been cleaned, and/or if a strong contrast does not exist between the blood and a dark surface. If nothing is observed, but there is reason to believe blood might be present, luminol or another enhancement chemical (such as amido black, fluorescein, or leuco-crystal violet) is often used. However, such presumptive tests suffer from both false positive and false negative results. For example, because fluorescence of luminol is catalyzed by iron in blood hemoglobin, false positive reactions can occur with any materials containing iron, as well as with other common household materials. False negatives, usually the result of a strong reducing agent being present that interferes with the oxidation-reduction reaction, can lead to potentially probative blood samples being missed at the scene. Finally, health concerns exist for crime scene investigators with the use of any of the chemical reagents required for stain enhancement and/or presumptive testing. Confirmatory tests, conducted in the laboratory and to prove presence or absence of blood, include microcrystalline tests such as the Takayama or Teichman tests. That blood is of human origin can be shown with immunological tests such as the precipin test. Following confirmation and human identification, DNA profiling of the bloodstain is used for individualization.

Methods to replace the currently used enhancement reagents and presumptive tests for blood and other biological material have been sought continuously. Recent interest has been heightened by the advent of low copy number DNA techniques which make even the smallest traces of blood forensically relevant. Fourier transform infrared spectroscopy (FTIR) has potential for detection of both blood and semen stains because of the strong absorbing amide bands observed in the infrared spectra of hemoglobin at 1650 cm\(^{-1}\) (amide I) and 1540 cm\(^{-1}\) (amide II). These absorbance features are largely clearly seen against the background of common surfaces and textiles.

This project has produced proof-of-concept development of a prototype camera requiring minimal operator technical knowledge that is capable of rapid and selective identification of blood stains in ambient lighting without the use of enhancement reagents. Our prototype camera uses mid-infrared (IR) diffuse reflectance spectroscopy based on the unique absorbance of blood proteins in the infrared spectrum. The current paradigm for instrument operation involves an infrared source (a glow-bar or space heater) to illuminate a scene with IR light. The thermal light source is combined with a conventional thermal infrared camera. Imaging is achieved by chopping the source and digitally processing each pixel by a lock-in amplifier approach to produce an output that shows visual contrast between stain/no-stain regions. We demonstrate that
digital lock-in amplifier techniques can increase the chemical contrast in an active thermal infrared image using both reflectance and thermal re-emission. We show this method is useful for visualizing thin coatings on fabrics that are invisible to the eye. We also take advantage of a “like-detects-like” chemical filtering approach to chemical selectivity for the purpose of chemical identification using a broadband thermal detector. The response of the detector was optimized by a combinatorial simulation-driven design process to select chemical filters that maximize the discrimination between blood and unstained surfaces. There are many factors involved in optimizing discrimination by using optical filtering aids, including, but not limited to, the detector response, optical throughput of the system, optical properties of the samples, and optical properties of the materials for sensitizing films/filters. There are nearly infinite possible setups for the system, which means it is neither cost- nor time-efficient to physically test each one. In lieu of this, we developed approaches to simulate the camera output, per pixel, given specific conditions. Beginning with measured spectra of calibration samples or standards, a figure of merit (in our case, the discrimination between stained and non-stained regions) was employed to predict performance for large numbers of combinations of chemical films as filters. This approach has produced acceptably high signal-to-noise ratios and enabled visualization of blood well below 100× dilutions with visible contrast, while providing some discrimination against substances reported to give false-positive response with other techniques. We have also demonstrated that this method can be used to discriminate between a blood stain and four common interferents to other blood detection methods: bleach, rust, cherry soda, and coffee. These results indicate that this system could be useful for crime scene investigations by focusing non-destructive attention on areas more likely to be suitable for further confirmatory analysis.

Concurrent with research in instrument development, we have conducted fundamental studies to advance the scientific basis, and our understanding of, infrared imaging for crime scene visualization. Knowledge and understanding of the nature of diffuse reflectance on surfaces coated with chemical stains may have further implications for the interpretation and uses of the infrared reflectance of many types of coated materials. Ultimately, the fundamental relationships observed could lead to the design of an improved system for the measurement of surface coatings of forensic relevance. Specific research conducted includes a study of coating effects on the infrared reflectance spectra of fabrics, evaluation of blood discrimination on textile fabrics, determination of achievable detection limits for blood on fabrics, examination of the effects on spectra of blood induced by fabric orientation and coating uniformity, estimation of the age of blood stains 3-9 months old by infrared spectroscopy, and a theoretical investigation of the fundamental Kubelka-Munk model of diffuse reflectance and an extension of that model to three dimensions.

The sections of the main body of the technical report document the accomplishments, methodology, and results of our project. The appendix to this technical report contains papers that have been accepted or submitted for publication and other manuscripts that are in revision prior to submission for publication.
I. INTRODUCTION

1. Statement of the problem.

Crime scenes involve a wide range of materials of potential probative value. However, these items may not be arranged in an orderly manner; more often, a crime scene is chaotic. The initial task of a forensic investigator is to recognize items that might have evidentiary value and to collect samples for further study. Biological evidence, such as blood, is important because of potential extraction and amplification of DNA, as well for spatter pattern analysis. However, biological fluids or their dried stains may be hard to detect. Latent stains, those invisible to the naked eye, may result if only trace amounts of blood are present, or if an attempt has been made to modify or clean a surface. Even patent stains, those visible to the naked eye, can still be difficult to detect if a lack of contrast exists between the stain and the background surface. If nothing is observed, but there is reason to believe blood might be present, a presumptive test such as luminol or another enhancement chemical (such as amido black, fluorescein, leuco-crystal violet, phenolphthalein, leucomalachite green, and benzidine) is often used.\(^1\)\(^-\)\(^5\) Methods to replace the currently used enhancement reagents and presumptive tests for blood and other biological material have been sought continuously. A major issue is that the crime scene can be contaminated thoroughly by such treatment. An approach to visualization of blood at crime scenes that is rapid, non-invasive, and not adversely affected by potential interferents would be ideal. Recent interest has been heightened by the advent of low copy number DNA techniques which make even the smallest traces of blood forensically relevant.

2. Literature citations and review

Crime scene investigators often employ high intensity light sources to highlight stains for further visual inspection. However, this step can be insufficient for detection if only trace amounts of blood are present, if the bloodstained area has been cleaned, and/or if a strong contrast does not exist between the blood and a dark surface. If nothing is observed, but there is reason to believe blood might be present, chemical enhancement reagents (e.g., luminol, amido black, fluorescein, leuco-crystal violet, phenolphthalein, leucomalachite green, and amido black) are used for visualization and presumptive testing for blood and bloodstains.\(^1\)\(^-\)\(^7\) A presumptive test is a test, which is used to screen for the presence of a substance, typically performed when there is doubt as to whether an object at a crime scene should be processed and collected.

The major components of blood are plasma and red blood cells.\(^2\) Plasma consists of soluble proteins, the two most prevalent being albumin (70%) and immunoglobulin G (10%). Red blood cells are 90% hemoglobin, which is also a soluble protein. The heme moiety is usually the part of the hemoglobin that interacts with a chemical enhancement reagent for detection of blood. For example, the phenolphthalein and leucomalachite green tests are based on an oxidation-reduction reaction with heme, causing conversion of the colorless reagents to colored by-products after oxidation. The luminol test is based on the peroxidase activity of the heme moiety, with a positive result indicated by chemiluminescence over the first minute or two after exposure of blood to luminol. The chemiluminescence, including splatter patterns, can then be photographed for documentation. In many cases, luminol is used for dual purposes, both to visualize patterns and as a presumptive test for blood.\(^1\)\(^-\)\(^4\),\(^6\)\(^-\)\(^10\) Although crime scene investigators at the South
Carolina State Law Enforcement Division often use amido black or crystal violet at crime scenes, contrast issues can occur with dark colored surfaces because of the dark blue and dark purple respective colors produced by these chemicals.

Specificity can also be an issue if the chemical employed also reacts with other protein-based biological fluids. While many of the false positive reactions can be identified during the presumptive testing procedure, problems can arise if the examiner does not exactly follow the prescribed procedure within the allotted time frame and/or has not conducted extensive testing on known standards so as to have a full understanding of a positive reaction process due to blood. Furthermore, false negatives can occur with these tests, causing blood samples to be left at the scene. This is usually the result of a strong reducing agent being present that interferes with the oxidation-reduction reaction, preventing or delaying color formation.

Due to the possibility of false positives and false negatives with presumptive testing, confirmatory testing is ultimately required in the laboratory to prove the presence or absence of a substance. Confirmatory methods for blood include microcrystalline tests such as the Takayama or Teichman tests in which chemical reagents are added to blood causing the formation of distinctive hemoglobin derivative crystals. To verify that blood is of human origin, an immunological test (e.g., the precipin test) uses anti-human hemoglobin serum to react with human hemoglobin. Following confirmation and human identification, DNA profiling of the bloodstain is used for individualization.

Although presumptive tests for blood can be easy to perform and are effective with both fresh and aged bloodstains, drawbacks exist. While luminol is more sensitive than the phenolphthalein and leucomalachite green presumptive tests, the reaction must be observed in the dark. This makes subsequent manipulation by examiners difficult and may limit its use at outdoor crime scenes. The reaction typically only lasts a few seconds; additional treatment may be needed to maintain a positive reaction for long-exposure photography to document the stain. Luminol does not appear to have a detrimental effect on short tandem repeat DNA results, but treatments with enhancement sprays can dilute trace bloodstains and have “dire consequences” when insufficient biological material is retained for genetic marker analysis. Using water-based reagents on non-absorbent surfaces can also cause bloodstain splatters to smear and may obliterate patterns. Further, like the phenolphthalein and leucomalachite green presumptive tests, luminol is not very specific for blood. It can give false reactions with any biological material with heme or heme-like structures. Interference can also occur with peroxidases (found in pulps of certain root vegetables), copper metal, some metal salts, some enamel and spray paints, furniture polish, certain disinfectants, and hypochlorite-based cleaning agents. Even vehicle parts can cause catalytic interference with the luminol test. Finally, health concerns exist for crime scene investigators with the use of any of the chemical reagents required for stain enhancement and/or presumptive testing. These drawbacks have also fostered research efforts with alternative chemical detection strategies.

Methods to replace the enhancement reagents and presumptive tests for blood are sought continuously. Trombka, et al. explored the use of X-ray fluorescence (XRF) for portable presumptive testing for blood by detecting iron from the hemoglobin of blood. Thorogate, et al. used immunofluorescence to detect and visualize trace amounts of human blood; the method was...
human-specific, and relatively quick (under 45 min), but required a washing procedure to remove the unbound fluorescent antibody for analysis. One of the simplest tests used by crime scene investigators is an alternate light source (ALS) to illuminate a suspect area with a high intensity light of controllable wavelength. Typically, an ultraviolet light source is employed to detect bloodstains by the reflected light, or by the emitted fluorescence. Stoilovic used the Polilight®, an adaptable light source for blood detection based on a strong narrow absorption band near 415 nm. This device was reported to be able to detect biological fluids on different fabric types even though it performed poorly at distinguishing between the types of fluid. It has been reported that alternate light sources should be used with caution due to the fact that some ultraviolet wavelengths can damage DNA evidence.

3. Statement of hypothesis or rationale for the research

The unique infrared spectral signature of blood proteins (Figure 1) has been used for detecting stains on fabrics and surfaces. Amide I and amide II bands from proteins manifest in the mid-infrared fingerprint range at 1650 cm\(^{-1}\) and 1540 cm\(^{-1}\) (6.1 and 6.5 μm), and the amide III band is present around 900 cm\(^{-1}\) (11.1 μ). Strong peaks in the N-H stretch region at the amide A band near 3300 cm\(^{-1}\) (3 μm) and in the C-H stretch region at the amide B band near 2900 cm\(^{-1}\) (3.4 μm) produce additional regions in which analysis can be performed. Absorbances within these regions are strong enough that sub-micrometer-thick films, invisible to the eye, give rise to measurable spectral effects. In clinical chemical applications, Wang, et al. found IR bands from 1700 cm\(^{-1}\) to 1500 cm\(^{-1}\) to be indicative of blood proteins, but stated that bands near 3000 cm\(^{-1}\) could be obscured by peaks from water in moist samples. Van Dalen showed that Fourier transform infrared spectroscopy (FTIR) with attenuated total reflection (ATR) can measure hemoglobin in bloodstained cloth. The amide I and II bands were unhampered by bands at < 1500 cm\(^{-1}\) from cotton. Botonjic et al. also found bands in the near and mid-IR spectra to be diagnostic of bloodstains. A few researchers have also employed IR light sources with coupled with photography or video imaging to detect and record latent blood.

![Figure 1](image)

**Figure 1.** Average mid-IR spectrum (10 replicates, 64 scans, 4 cm\(^{-1}\)) of whole rat blood, collected using an ATR accessory.
The objective of this work was to achieve proof-of-concept for thermal infrared imaging of blood at crime scenes by development of a prototype IR imaging system that is capable of rapid and selective visualization of blood at crime scenes. Our successful prototype design uses mid-infrared (IR) diffuse reflectance spectroscopy to locate blood on common surfaces. The current paradigm for instrument operation involves an infrared source (a hot plate) to illuminate a scene with IR light. Imaging is achieved by chopping the source and digitally processing each pixel by a lock-in amplifier approach to produce an output that shows visual contrast between stain/no-stain regions. The response of the detector was optimized by a combinatorial simulation-driven design process to select chemical filters that maximize the discrimination between blood and unstained surfaces. This approach has produced acceptably high signal-to-noise ratios and enabled visualization of blood well below 100× dilutions with visible contrast, while providing some discrimination against substances reported to give false-positive response with other techniques. Besides being rapid, IR imaging for bloodstain detection offers advantages: the crime scene can be visualized from a distance (currently 1-2 m) without touching or contaminating the area, examiners are not exposed to chemicals, the technique can be used indoors or outdoors under ambient light, patterns are not smeared, and stains are not diluted or altered by chemical reagents. The eventual goal of further development might be a camera system, operated by a laptop and requiring minimal operator technical knowledge.

Concurrent with research in instrument development, we have conducted fundamental studies to advance the scientific basis, and our understanding of, infrared imaging for crime scene visualization. These efforts have included a study of coating effects on the infrared reflectance spectra of fabrics, evaluation of blood discrimination on textile fabrics, determination of achievable detection limits for blood on fabrics, examination of the effects on spectra of blood induced by fabric orientation and coating uniformity, estimation of the age of blood stains up to 9 months by infrared spectroscopy, and a theoretical investigation of the fundamental Kubelka-Munk model of diffuse reflectance and an extension of that model to three dimensions.

This research has to date created five publications in print and one patent award. Another three preliminary patents have been filed, and at least two additional research papers are being edited for publication.

II. Methods and Results

1. Demonstration of mid-IR thermal imaging of blood.

Our papers34-36 describing the design of the camera system and results from imaging blood stains compared to some substances reported to give false positive responses to luminol were published in Analytical Chemistry in October 2010. We have designed a prototype portable camera using mid-infrared (IR) spectroscopy with a thermal imaging detector for visualization of blood at crime scenes (Figure 2). An infrared source (e.g., a small heating plate, glow-bar, or space heater) is employed to illuminate a scene with IR light. Light reflected from the scene is employed to achieve imaging by chopping the source, and digitally processing each pixel by a lock-in amplifier approach in real time, to produce an output that is proportional to contrast between stain/no-stain regions. The infrared camera response is also sensitized to spectral regions where blood components (e.g., proteins) show absorbance using a combinatorial
simulation-driven design process that selects chemical filters to maximize discrimination between blood-stained and unstained surfaces. The selectivity of our system is the result of a secondary response mode in which the spectral response is tuned by filters of polymer films to produce a 'like detects like' effect. A filter of a blood-like material is placed in the path to the IR camera; if the image contrast is diminished (due to absorption of light at wavelengths related to thermal emission of blood proteins), then the confidence that image contrast is due to the presence of blood is increased. Further data processing methods develop and display scene images, with regions indicative regions of the target analyte (latent blood) showing contrast from background. This approach has produced acceptably high signal-to-noise ratios and enabled visualization of blood well below 100×dilutions with visible contrast, while providing some discrimination against substances reported to give false-positive response with other techniques. Besides being rapid, IR imaging for bloodstain detection offers advantages: examiners are not exposed to chemicals, the technique can be used indoors or outdoors under ambient light, patterns are not smeared, and stains are not diluted or altered by chemical reagents.

While further development and validation is necessary for realization of the ultimate forensic goals, the present research has opened novel and intriguing imaging applications for diffuse reflectance in the mid-infrared region spectrum for forensic applications.

![Photograph of the multimode imaging system](image)

**Figure 2.** Photograph of the multimode imaging system, with all key components labeled. The light source is a hot plate. The chopper was based on a modified fan bearing with a geared motor drive; the standard was used to synchronize data analysis. The filter was made from a KBr salt window coated with a film by dip coating. The camera is an 8-13 \( \mu \text{m} \) microbolometer-based thermal imager with 12-bit digitization, driven by LabView software written in-house.

### 2. Detection limits for blood on fabrics presently near a dilution factor of 200.

By controlling the uniformity of coating and orientation of calibration samples, reproducible blood-doped standard calibration samples at varying dilution levels have provided an estimate of the achievable detection limit for blood using a benchtop mid-infrared diffuse-reflectance...
instrument. Figure 3(left) shows 95% confidence ellipsoids in principal component space for 20 spectra from neat nylon fabric versus nylon doped with 25× diluted blood; Figure 3(right) shows the projections of spectra into the (single) linear discriminant space for the same data. At this level of dilution, spectra of blood stained fabric are clearly distinguished from spectra of neat samples, and this distinction continues down to the lowest dilutions tested. Other experiments\textsuperscript{37,38} show the effect of a chemical coating (blood) on the infrared reflectance spectra of fabrics is nonlinear, with low concentrations of doping producing a spectrum of greater intensity than might be expected, while high concentrations tend to reach a plateau in response. This effect is partly a consequence of the blood flowing into crevices between and around fibers, and thus roughening the fiber surface in a non-uniform manner. Estimates of the detection limits of blood on nylon, acrylic, cotton, and polyester fabric are at ca. 100× to 200× diluted blood, which corresponds to a mass percentage on fabric of about 0.01%. The spectra on which these estimates are based were taken on a benchtop diffuse reflectance instrument, rather than our more selective camera system, where we have seen signal-to-noise ratios for blood approaching 1000:1.

![Figure 3](image)

**Figure 3.** Projection of infrared diffuse reflectance spectra into the space of the first three principal components (left) and the space of the linear discriminant for neat nylon and nylon coated with blood diluted 25× in water (95% confidence ellipsoids are shown in the PCA space).

3. AC-modulated thermal infrared imaging can see dilute bloodstains on fabrics.

Thermal imaging cameras are normally used to detect temperature gradients. However, their sensitivity to mid-infrared light makes them ideal for viewing biological stains on surfaces and fabrics from a distance of a few feet using a diffuse reflectance approach. The reflectance of an infrared light source can be distinguished from the large blackbody radiation background in the image by AC modulation and lock-in-amplifier-type detection.

Figure 4 shows an image of a dark-colored polyester fabric stained with blood using a pipette.\textsuperscript{36} The letter “I” in the image is made using neat blood. The letters “X”, “V”, “L” and “C” were deposited using diluted blood, where the dilution factors are 10, 25, 50 and 100 times.
4. Simulation-designed instrument can discriminate blood from other stains.

We have shown that blood is readily detected by AC-modulated thermal infrared imaging. We have also worked to improve the method’s ability to distinguish blood from interfering compounds by viewing samples through one or more chemical filters composed of polymer films on IR-transparent substrates. We required a means of determining the best filter or filter set to achieve this purpose. We have the capability of creating a wide range of possible filtering elements, and for each filter element we have the capability of producing them in various optical thicknesses. At a minimum, hundreds of thousands of different filter selections are possible, and it is impractical to measure them each experimentally for optimal performance. In such cases, simulation of the instrument performance can provide a computer system with a means of testing large numbers of elements to arrive at optimized designs in relatively short order.

The simulation is based on Equation 1 below, which provides the estimated signal when sample \( i \) is viewed through a filter element \( j \). In the equation, \( T_j \) is the transmission spectrum of the filter, \( R_i \) is the reflectance of the sample, and \( \eta_c \) is the spectral response of the camera system (composed of the irradiance of the light source, \( \Phi_p \), the transmittance of the lens element, \( T_{AR} \), and the response of the detector itself, \( R_d \)):

\[
S_{ij} = \sum_{\lambda_i} R_i(\lambda) \times \eta_c(\lambda) \times T_j(\lambda)
\]

where \( \eta_c(\lambda) = \Phi_p(\lambda) \times T_{AR}(\lambda) \times R_d(\lambda) \)

**Figure 4.** Image of a polyester fabric with lettering made from blood. The letter “I” was made with neat blood, while the letters “X”, “V”, “L” and “C” were made using blood of 10-, 25-, 50- and 100-fold dilutions. This image was made using in-phase detection of an AC-modulated reflectance. The object in the lower right is a reflectance reference for phase detection.\(^36\)
We began our simulation by experimentally determining the reflectance properties of the neat and reproducibly-stained fabrics. We then developed Matlab® programs to calculate the expected camera response for a given setup (e.g., single filter and single calibration sample). The results of simulation were validated using experimental results using the modeled setup. This validated simulation is now available to find the optimal set of filters to distinguish a given analyte on a known substrate (e.g., blood on fabric). The design is optimal in the sense that the best filter is chosen by linear discriminant analysis\(^39\) to be the most discriminating.

The spectra of possible filtering elements is determined by estimating the spectral absorption coefficients of the filtering materials, and then simulating the filters at a range of filter thicknesses. The computer routine is then used to simulate each measurement of the neat and stained fabrics when viewed through the chemical filters. In this simplified simulation, the Fisher ratio between the neat and stained responses is calculated by linear discriminant analysis\(^39\) (LDA), which is used as the figure of merit (FOM) for the predicted contrast in an image.

5. Thermal-reemission sees physical contrast in images.

Although most of our imaging effort went into developing a reflectance measurement, we realized early in the work that the use of an AC-modulated light source offered additional imaging modalities. For example, we observed that images required time to stabilize once the modulated infrared light source was turned on, as illustrated in Figure 5:

![Figure 5](image)

**Figure 5.** Example of the single-pixel raw data for a 15 sec acquisition period. The gold standard shows sharp on/off transitions, while the fabric data show heating and cooling effects throughout the measurement. Note the scale of the gold signal is about 10\(^\times\) greater than the fabric signal because reflectance of fabric is smaller.

The reflectance standard showed no gradual increase in signal, while the reflectance of the fabric samples increased approximately exponentially. Further, the modulation of signal from the fabric samples did not follow the square-wave pattern of the source with fidelity. The reason was...
determined to be heating and cooling of the fabric samples. During the “on” portion of the cycle, blackbody radiation from the sample increased as it warmed, with cooling during the “off” part of the cycle. This thermal modulation was determined to be about 1/3 °C, but its phase is shifted approximately 90 degrees from the excitation. Although small compared to the reflectance, the reflectance portion of the signal follows the source faithfully and can be cancelled out, leaving an image of the thermal properties of the sample as shown in Figure 6.

**Figure 6.** (A) In-phase IR reflectance image of a fabric sample labeled with neat blood (“B”), soda (“S”), bleach (not visible), coffee (not visible) and rust (not visible). (B) Out-of-phase image showing heating/cooling effects of sample, contrast is measurable for the blood stain only. The origin of this contrast is not chemical, but physical due to the larger amount of solids in the blood stain that affect the thermal properties of the fabric.

These thermal property maps might find uses in detecting non-IR active stains, revealing repairs to materials, etc. The wood grain of the plywood on which the fabric is mounted can be seen in these images.

### 6. Contact stains can likely be detected.

Most of the work we have performed to date involves diffuse reflectance, in which light penetrates into the near-surface areas of fabrics and other materials. However, we also worked to understand the behavior of materials when the substrate has a very high absorbance, limiting the penetration depth of light so that only the topmost surface reflection, called the Fresnel diffuse reflectance or the specular reflectance, of the fabric can be observed.  

We observed that the reflectance of fabrics change even in this “high absorbance” region when a coating is applied, presumably due to physical optical effects (like changing surface roughness at the top of the fabric)(Figure 7). The high absorption of the fabric ensures that only the topmost surface of the fabric contributes to this spectral change, and we estimate a detection limit to be in the range of film thicknesses of 40 nm or so, and relatively independent of the nature of the film substance. Consequently, we believe this phenomenon may find use in detecting contact or surface stains, such as fingerprint residues, on fabrics or other surfaces.
III. CONCLUSIONS

1. Discussion of findings

The practical benefits of diffuse reflectance IR approach for imaging for blood at crime scenes include:

(a) IR imaging is stand-off and non-invasive, requiring access only within several feet of an area of potential interest;

(b) the testing does not involve chemical treatment or dilution of blood samples with reagents; although an IR light source is required, heating effects on sample areas are minimal;

(c) the potential for increased specificity for detection via further tuning with ‘like detects like’ filters to enhance sensitivity for blood while decreasing response to potential interferences;

(d) experimental results and theoretical explorations of IR imaging for thin coatings of analyte on a surfaces provide a scientific basis that confirms the ability to detect low concentrations of stains of organic materials;

(e) the approach is not limited to blood stains; additional potential targets include biological materials such as semen, fingerprints, sweat deposits, or other traces of human cells; and,
(f) the growing use of so-called ‘touch DNA,’ or low copy number DNA profiling provides real world forensic relevance to the ability of IR imaging small concentrations of biological materials.

Conventional instrumentation that is intended for general spectroscopic applications could, in principle, make measurements for blood detection at a crime scene. However, the equipment required would be slow, cumbersome, expensive, and difficult to maintain. The prototype system that we have devised suggests the possibility of visualization tools that are faster, smaller, less expensive, and easier to maintain as a result of being tailored to a specific application rather than for general use. A side benefit of tailoring to a specific application is that these instruments can be used by a non-specialist rather than requiring a Ph.D.

Our work on the scientific basis of diffuse reflectance infrared spectroscopy has implications for remote detection of stains on surfaces. Films of varying thicknesses of a model polymer were deposited onto cotton and polyester fabric samples by dip-coating from solution. Scanning electron microscopy (SEM) images of the coated fabric samples were used to evaluate the quality of the polymer coating. The samples were analyzed by infrared diffuse reflectance spectroscopy to determine the relationship between film thickness and the effect of the coating on the spectroscopy of the two fabrics.

We have also evaluated the persistence of our ability to detect blood and the possibility of estimating age of dried bloodstains using attenuated total reflection (ATR) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Spectral regions identified as significant for detection and aging include the distinctive amide I and amide II bands (1650-1540 cm\(^{-1}\)) due to blood proteins such as hemoglobin and albumin. Blood stains on fabrics exposed outdoors yielded age uncertainty estimates (root-mean-square errors of cross validation) ranging from 5 to 6 days for calibrations up to 90 days; other exposure conditions produced higher prediction errors, likely due to the slower rates of degradation.

Our research on coating and uniformity effects in IR spectra, on estimates of limits of detection of blood, and the theoretical paper on the Kulbelka-Munk formula support the chemical and spectroscopic basis for the detection of informative analyte features in light reflected from chemical stains on surfaces. This work is novel, and should be of interest to a wide range of analytical chemists and forensic scientists. Taken as whole, the studies conducted for this project studies provide a firm scientific background to support future studies targeting infrared imaging of organic biological materials on surfaces.

2. Implications for policy and practice

Beginning with *Forensic Science Weighing Bullet Lead Evidence* (2004), and continuing with *Strengthening Forensic Science in the United States: A Path Forward* (2009), the National Academies of Sciences has presented plans for addressing forensic science needs and inadequacies. Many of the relevant scientific issues are viewed in the context of admissibility of evidence in the court room as guided by *Daubert vs. Merrill Dow Pharmaceuticals Inc.* and related cases. The *Daubert* decision established a checklist for assessing the reliability of scientific expert testimony, one that includes whether the rate of error of the technique or theory has been established. The development of improved imaging approaches for rapid non-invasive
visualization and screening for presumptive identification of a blood stain is not as critically affected by Daubert issues as is a confirmatory test for blood, which is more likely to be introduced into probative scientific testimony. However, a visualization tool might be employed for blood stain pattern analysis, for example. As such the technique might be subject to questions of scientific basis and validity in terms of limits of detection, potential interference effects, and reproducibility of results under a variety of environmental conditions.

A common thread in the recent discussion of forensic techniques by the National Academies of Sciences is the need for continuing research to establish limits and measures of performance for forensic analyses: to clarify the applicability and reliability of techniques for various purposes. We consider our present work as first steps in evaluation of the performance of infrared imaging for crime scene investigations. Another relevant topic is that of subjective interpretation and observer bias in forensic diagnostic testing and the lack of such evaluations in many forensic methods. We have endeavored in the present research to take advantage of accepted and established statistical techniques to insure good decision making processes concerning the practical usefulness and validity of IR imaging for blood stain detection.

Additional instrument development and validation research is necessary for realization of the ultimate forensic goals of the present research. Such topics include making the instrument portable and easy to use by non-technical personnel, and assessing performance measures including rates of false positive and false negatives with a realistic study of potential interferences that might occur at the crime scene. However, our initial research has opened up novel and intriguing applications of imaging that may have valuable forensic applications.

3. Implications for further research

All rapid forensic imaging tools face questions regarding their selectivity: Can a prospective blood (or other) stain be positively identified? Can evidence gathered in this way be presented in a court of law as confirmation of the nature of a stain? To these questions we believe the answer is an unequivocal “No”.

The most selective chemical imaging tools (omitting hyphenated tools combining biochemical assays with chemical imaging) are hyperspectral, and these tools rely on libraries of calibration data for interpretation of their spectra. However, real forensic environments are chaotic by nature and unpredictable—they encompass surfaces and materials of a wider variety than can ever be included in a calibration set, and with a history of chemical and physical environmental effects far more complex than any that can be successfully emulated in a laboratory. Thus, even hyperspectral imaging result will have biases and uncertainties greater than that potentially provided by wet chemistry, biochemical assays, mass spectrometry, or other such tools.

In the opinion of the authors, the proper roles for imaging are to:

(a) reveal the presence and shape of a stain or feature;

(b) provide contrast for presumptive identification of target analytes and reduce false positives and negatives as much as possible; and,
(c) enable sampling for dependable testing. Different imaging tools compete in the areas of detection limits and the ability to distinguish target from non-target features, with size, cost and speed being possible trade-offs.

Many of the tasks required to bring IR imaging to practical forensic applications are obvious. Further validation tests, both under laboratory conditions and with real world crime scenes scenarios, are needed. Direct collaboration with local forensic laboratories can offer guidance and some focus for forensic applicability of such studies. Although the images recorded by our present infrared camera using AC methods provide degrees of chemical contrast, they do not provide detailed information on the actual chemistry or spectroscopy giving rise to the contrast. It is not, for instance, often possible to say that the coating of the fabric visible in basic AC images is consistent with, or inconsistent with, any particular chemical nature—only that an area of potential interest has been identified. For this tool to be useful in the detection, classification or quantitation of chemical factors in a crime scene, it is critical that methods to reduce common false and negatives positives be developed, with the understanding that additional sampling and laboratory testing would be required for confirmation.

Selectivity in hyperspectral imaging is obtained by fully breaking down the image into its component wavelengths to provide a field for chemometric data analysis, but this benefit is obtained at a cost in terms of time, funds, and complexity. The power of such instrumentation is very great, but whether such an instrument will be practical in the near future for common in situ forensic studies by, for instance, state and local law enforcement agencies, is an open question. Our continuing research plans will seek methods that capture as much as possible of the selectivity of the hyperspectral approach without actually demanding conventional hyperspectroscopy.

The main approach suggested by present work, although less powerful than full hyperspectroscopy but much simpler and more cost-effective to implement, is to record images through a set of optical filters. This approach, referred to as molecular factor computing, has been used previously to visualize simple vapors via IR imaging. Simple vapors have relatively few bands and require only a single or a few conventional filtering elements. On the other hand, organic films and background materials such as fabrics have more complicated spectral features that span much of the usual mid-infrared-active spectral region. In this part of the spectrum, absorption bands may be relatively narrow yet still yield in significant absorption in the reflectance of neat powders and fibers, spectral overlap is fairly significant due to high spectral density caused by the presence of observable fundamental, overtone and combination bands.

The ideal optical filters for affecting the contrast observable in a film-on-fabric sample would either pass or block all the significant bands that identify one of the materials. Assuming that the coating material in question exhibits absorption bands in the reflectance IR spectrum of the fabric/film sample, a simple way to approximate such a filter is to deposit a film of the same substance on an IR-transparent substrate. If successful, the absorption bands of the filter will reduce the effects of the same absorption bands in the coated fabric, reducing the observed chemical contrast. This approach is overly simplistic in assuming that absorption bands of the coating are apparent in the spectrum. The strength of the IR transitions in the film/fabric sample is such that often this is not the case—this aspect will also be the subject on proposed future
research in our laboratory. In this general case, the present work (see appendixes B and C) already demonstrated simulation as a more effective means of choosing one or more filter media to aid in classifying the sources of chemical contrast in an image.

From the beginning of our work, we have kept in mind that the purpose of NIJ’s funding was to benefit the criminal justice community. The work performed during this project shows immediate promise as a tool for crime scene investigation, and we are in discussion with a major manufacturer of alternate light source (ALS) systems for a commercialization review. We have also filed a series of patents covering (1) the detection of surface/contact stains, (2) AC lock-in detection of reflectance and thermal re-emission from fabrics using thermal IR cameras, (3) computer-designed selectivity-enhancement filters for forensic imaging and (4) modified thermal detectors using coated detector arrays for enhanced selectivity.

An important issue is system cost. Related instruments (lacking an active light source and data handling system) are already commercially available to fire departments at a cost between $20,000 and $30,000 and the at least one existing ALS instrument costs $10,000-$20,000). Similar infrared cameras are also available commercially for law enforcement surveillance activities. IR imaging systems of the kind developed by this project could be highly cost-competitive with these existing systems.

IV. REFERENCES

11. Jeffrey Crooks, Crime Scene Investigator, South Carolina State Law Enforcement Division, Forensic Division, Columbia, SC; personal communication.


V. DISSEMINATION OF RESEARCH FINDINGS

1. Progress reports

Six-monthly progress reports and the cumulative final progress report to the National Institute of Justice have been submitted and approved.

2. Patents

One patent has been filed as a result of this NIJ funding: Michael L. Myrick Heather Brooke Stephen L. Morgan. Patent application title: Chemically-Selective Detector and Methods Relating Thereto, Patent application number: 20090250613 (URL: http://www.faqs.org/patents/app/20090250613). Three additional provisional patents have been filed through the USC Research Foundation, Intellectual Property Management Office.
3. Scientific meeting presentations

The following oral presentations have been made at scientific meetings and workshops during this current project, for which NIJ was acknowledged for full support. Abstracts in meeting proceedings were published for all of these presentations.


4. **Publications in print**


Papers B-D describe the design of the camera system and results from imaging blood stains compared to some substances reported to give false positive responses to luminol. These papers were published in *Analytical Chemistry* in October 2010. *Analytical Chemistry* is ranked first in total citations (82,246) in 2009 out of 70 journals in the analytical chemistry category. The journal had a high ISI Impact Factor of 5.214 as reported by the 2009 *Journal Citation Report®* by Thomson Reuters. For our papers in Analytical Chemistry, the American Chemical Society issued a press release at URL: [http://portal.acs.org/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=223&content_id=CNBP_026046&use_sec=true&sec_url_var=region1&__uuid=e23dbfb6-547b-4ae-9ae-99ae-c3c5f4c3b043](http://portal.acs.org/portal/acs/corg/content?_nfpb=true&_pageLabel=PP_ARTICLEMAIN&node_id=223&content_id=CNBP_026046&use_sec=true&sec_url_var=region1&__uuid=e23dbfb6-547b-4ae-99ae-c3c5f4c3b043).

5. Publications accepted or submitted for publication


6. Manuscripts in preparation for publication


7. Graduate students supported by this grant

Four graduate students have been supported by this project. Three of these graduate students have completed Ph.D. degrees in Chemistry as a result of working on this project:


Current position; Postdoctoral researcher at U. S. Naval Research Laboratory, Washington, D. C.

The fourth graduate student, Megan R. Baranowski, is in 4th year of graduate school and planning to graduate by August 2011.

8. NEWS RELEASES AND PUBLICITY

The following news items are posted on the internet URLs cited and can be accessed at http://www.chem.sc.edu/faculty/morgan/news.html if the links below are not operative.


“Chemistry professors developing camera for crime-scene blood detection,” article about Morgan and Myrick research to develop IR detection of blood stains, USC Times, 19 June 2008.

The University of South Carolina released a news item on the Innovista web site on 18 June 2008, “Chemistry professors developing camera for crime scene blood detection.”

Three papers describing the forensic blood imaging project by the Morgan and Myrick groups were recently published by Analytical Chemistry. New Scientist has published a news item on the research. A USC news release with a video interview is also available, as well as another article from SC Radio Network. From there, other news organizations picked up the story.

Dr. Morgan was interviewed concerning the Myrick and Morgan project for infrared blood stain detection for the Carolina Minute radio program on 29 November 2010. Three one-minute interviews [1, 2, 3] were broadcast by the local NPR Affiliate, SC Public Radio.

Dr. Morgan was also interviewed about his and Dr. Myrick's research on IR imaging of bloodstains live on the CNN PM Newsroom show on 22 November 2010.

The local CBS-affiliate Columbia SC TV station, WLTX, did a video interview with Dr. Morgan and graduate student Megan Baranowski Pearl in Dr. Myrick's laboratory in front of our prototype instrument. The interview was broadcast on the WLTX Evening News on Monday, 29 November 2010. The local NBC-affiliate Columbia SC TV station, WIS, also did an interview on 12 December 2010.

Dr. Morgan was interviewed about his and Dr. Myrick's research on IR imaging of bloodstains by Ira Flatow on the National Public Radio show "Science Daily" on 28 January 2011. The audio and transcript of the interview is at http://www.npr.org/2011/01/28/133306349/Blood-Spotting-Made-Easier.
In January 2011, Dr. Morgan and Megan Baranowski were interviewed on video by the Fox News TV Station in Greenville, SC, for a 15-minute science-related news broadcast that is to air within the next two months.


One pending item involves a news article on our blood imaging research to be published in Popular Science magazine and on their web site. We have also had recent contact with producers from the History Channel and the CBS Interactive website www.SmartPlanet.com in the form of requests for interviews.