FINAL TECHNICAL REPORT

Federal Agency and Organization Element to Which Report is Submitted:

U. S. Department of Justice, National Institute of Justice

Federal Grant or Other Identifying Number Assigned by Agency:

NIJ award number 2007-DN-BX-K199

Project Title:

Rapid Visualization of Biological Fluids at Crime Scenes using Optical Spectroscopy

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Submission Date:

21 March 2011

DUNS and EIN Numbers:

DUNS number	11-131-0249
EIN number	57-0967350

Recipient Organization (Name and Address):

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Project/Grant Period (Start Date, End Date):

1 October 2007-30 September 2010

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 bloods 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