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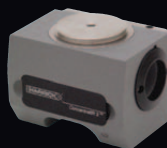
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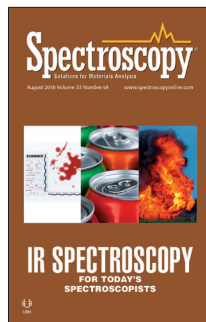
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# FT-IR Spectroscopy for Identification of Biological Stains for Forensic Purposes

Given the large variety of evidence that can be found at a crime scene, a universal method for initial screenings to determine what samples to collect and document for further analysis would be useful. Traces of body fluids are a crucial form of forensic evidence that provides information about potential suspects and victims. It is essential for the screening test to be nondestructive and preserve the evidence for further analysis. Fourier transform infrared (FT-IR) spectroscopy is emerging as the technique of choice for nondestructive analysis of the chemical composition of unknown biological stains. This method is advantageous over most current biochemical tests because of its high specificity, universality, non-consumptive nature, and potential for quick in situ analysis where the stain is examined directly on the substrate. Here, we provide a brief overview of the novel applications of FT-IR spectroscopy for forensic analysis of body fluid traces. In addition to the confirmatory identification of the fluid type, this technique helps estimate the stain's age, as well as discriminate species. We also describe the application of FT-IR spectroscopy to biological stains on various substrates and samples exposed to specific environmental conditions to imitate forensically relevant scenarios.

Ewelina Mistek and Igor K. Lednev

Current forensic practices pay special attention to body fluid traces as the main source of individual DNA evidence. It is extremely important to identify the biological fluids, and develop a potential scenario of how their traces got to the crime scene. Traditionally, forensic investigation of body fluids followed several steps: visual examination, presumptive tests, confirmatory tests, and DNA analysis (1). However, to

save time, law enforcement practitioners proceed directly to DNA analysis these days (2,3). DNA typing is relatively expensive and time consuming, which consequently limits the amount of evidence being processed and ultimately contributes to significant backlogs (2).

Very often, it is challenging for investigators to locate biological stains, depending on the color appearance on the substrate where the stain was depos-



ited. To visualize potential body fluids, alternate light sources are often used (4–6). Some alternate light sources can even discover stains that were cleaned or wiped. Presumptive and confirmatory assays are specific to individual body fluids, since they rely on the reaction of chemical components characteristic to a body fluid with a reagent or antibody. For example, hemoglobin is the most targeted component for blood, amylase for saliva, urea or creatinine for urine, acid phosphatase or prostate-specific antigen for semen, and glycogenated epithelial cells for vaginal fluid (2, 4–6). Both presumptive and confirmatory tests can still provide false positive results for other substances containing these or similar characteristic biochemicals. Because these tests require either chemical reaction or antibody–antigen interaction, they destroy the sample, preventing its further use. This sample loss may be an issue if a limited amount of evidence was available at the scene of the crime. Therefore, there is a great need for a universal, nondestructive, rapid, and in situ method for identification and analysis of body fluid traces.

Fourier transform infrared (FT-IR) spectroscopy is a technique already being used during forensic investigations for analysis of physical evidence (such as controlled substances, fibers, and paint) (1). A significant advantage of this technique is its nondestructive nature and specific signature for different sample types based on their chemical composition.

The advantages of FT-IR spectroscopy, as well as other vibrational spectroscopic techniques for various forensic applications, have been recently overviewed by Muro and colleagues (7). The analysis of body fluids using vibrational spectroscopy

for a wide spectrum of applications has also been reported (8). Novel methods for forensic body fluid identification and characterization, which include vibrational spectroscopy, have also been reviewed (9,10). Aforementioned review articles cover many topics, and prove that research using FT-IR spectroscopy for body fluid examination is rapidly growing. Here, we briefly overview recent studies on the identification and analysis of body fluid traces using FT-IR spectroscopy for forensic purposes.

### **Identification of Body Fluid Traces**

Since the identification of biological traces can still be problematic for forensic investigators, a nondestructive, universal, and ideally portable method is desired. Elkins (11) was the first to show the potential of FT-IR spectroscopy with attenuated total reflection (ATR) accessory for identification of body fluids and materials. This was done by using unique vibrational signatures of different substances. In this work, spectra of the following biological materials were evaluated: blood, cerumen, feces, fingernails, fingerprints, hair, nasal mucus, vaginal mucus, saliva, semen, and urine. The samples were analyzed in their pure form (as liquid or solid samples) as well as deposited on various substrates imitating forensic samples. The amide I peaks ( $1700\text{--}1600\text{ cm}^{-1}$ ) and fingerprint region ( $<1500\text{ cm}^{-1}$ ) were found to be the most informative features for discrimination between different samples.

Orphanou and colleagues (12) investigated the possibility of discrimination between IR signatures of different body fluids. In their work, they used blood, saliva, semen, and vaginal secretions to compare as potential fluids in violent and sexual abuse cases.

Spectral regions for biological samples were discussed because they all show contributions from biomolecules: lipids (3000–2800  $\text{cm}^{-1}$ ), proteins (1700–1600  $\text{cm}^{-1}$  and 1560–1500  $\text{cm}^{-1}$ ), and nucleic acids (1250–1000  $\text{cm}^{-1}$ ). The body fluids studied showed different IR spectra because of their different biochemical composition. The data was differentiated based on spectral patterns, peak combination, and peak frequencies. The reported characteristic peak assignment for the body fluids can be seen in Tables I and II.

Forensic practice rarely involves pure body fluids. Therefore, the focus of research moved toward the analysis of traces deposited on different surfaces. Gregório and coauthors (13) analyzed body fluids that were potentially involved in sexual offences deposited on superabsorbent pads. Semen, vaginal fluid, and urine, as well as mixtures, were studied. Semen and vaginal fluid showed different IR signatures in the region between 1800 and 1480  $\text{cm}^{-1}$ , which corresponds to vibrations of proteins, and therefore is distinguishable from urine. However, since semen and vaginal fluid each contain proteins, it was difficult to discriminate between these body fluids on substrates based on their IR spectra. In their second study, Gregório and colleagues (14) employed statistical analysis to strengthen the identification process for semen. This approach showed potential advantages for the screening of semen deposited on various substrates.

Because of difficulties of discrimination between some samples of similar composition, Zapata and his coauthors (15) also used chemometrics for data obtained from external reflection FT-IR spectroscopy. Stains of body fluids deposited on fabrics and possible

false positive substances were studied. Visual differences in spectra were shown, which were also confirmed by principal component analysis (PCA) and soft independent modeling of class analogy (SIMCA) models by correct classification of unknown stains. None of the possible false positives used here (cosmetics or foodstuff) were misclassified as body fluid. Moreover, different colors of cotton did not interfere with results. These results show that chemometrics and FT-IR spectroscopy can be combined to increase discriminating power.

Takamura and colleagues (16) developed a statistical method based on IR spectra of various body fluids (blood, saliva, semen, urine, and sweat). The combination of partial least squares discriminant analysis (PLSDA), linear discriminant analysis (LDA), and Q statistics was employed. The dichotomous classification tree for the ATR FT-IR spectra of five body fluid types, based on hierarchical clustering analysis, can be seen in Figure 1. The method was successfully applied for fresh body fluid samples, and for non-body fluid materials which could potentially interfere with routine crime scene investigation. Samples subjected to an aging process were more difficult to classify. However, the approach showed overall great performance with potential for practical forensic applications.

Studies presented here show great potential of FT-IR spectroscopic techniques to be utilized for body fluid identification with promise for analyzing samples on various substrates. Additionally, the method can be paired with chemometrics to add statistical confidence to findings. It would be extremely beneficial for fo-

**Table I: Peak assignment in ATR FT-IR spectra of blood and saliva. Adapted with permission from reference 12.**

Body Fluid	Peak Frequency (cm <sup>-1</sup> )	Peak Assignment	Vibrational Mode
Blood	3292	Amide A	Symmetric N-H stretching
	2956	Methyl stretches of lipids in plasma	CH <sub>3</sub> stretching
	1651	Amide I ( $\alpha$ -helix)	C=O stretching
	1540	Amide II	N-H bending coupled to C-N stretching
	1456	Methyl bending of amino acid side chains, lipids, and proteins	Asymmetric CH <sub>3</sub> bending
	1395	Fibrinogen/methyl bending of amino acid side chains, lipids, and proteins	Symmetric CH <sub>3</sub> bending
	1350-1220	Amide III	C-N stretching
	1250-925	Carbohydrates (glucose)	Symmetric C-O stretching
Saliva	3282	Amide A	Symmetric N-H stretching
	2926, 2850	Methylene stretches of lipids in oral mucosa	Asymmetric and symmetric CH <sub>2</sub> stretching
	2059	Thiocyanate anions (SCN <sup>-</sup> )	C-N stretching
	1645	Amide I ( $\alpha$ -helix)	C=O stretching
	1544	Amide II	N-H bending coupled to C-N stretching
	1452, 1393	Methylene bending of amino acid side chains, lipids, and proteins	Asymmetric and symmetric CH <sub>2</sub> bending
	1239	Amide III/phospholipids	Asymmetric C-N stretching, PO <sub>2</sub> <sup>-</sup> stretching
	1080-950	Sugar moieties (glycosylated proteins)	CH <sub>2</sub> OH groups, C-O stretching and COH groups bending, symmetric PO <sub>2</sub> <sup>-</sup> stretching

rensic practical applications to investigate the performance of portable, or even handheld, instruments which are already commercially available. This would also require investigating the subtraction of substrate (like polymers or fibers) to analyze the sample with minimal manipulation by leaving it in place.

## Blood Analysis

Since blood is a biological fluid often found at the scene of a violent crime, its analysis was the main focus for many research groups. One of the largest opportunities for adding forensic intelligence is the potential to determine the age of a bloodstain (2). Aging bloodstains can add tremendous value to investiga-

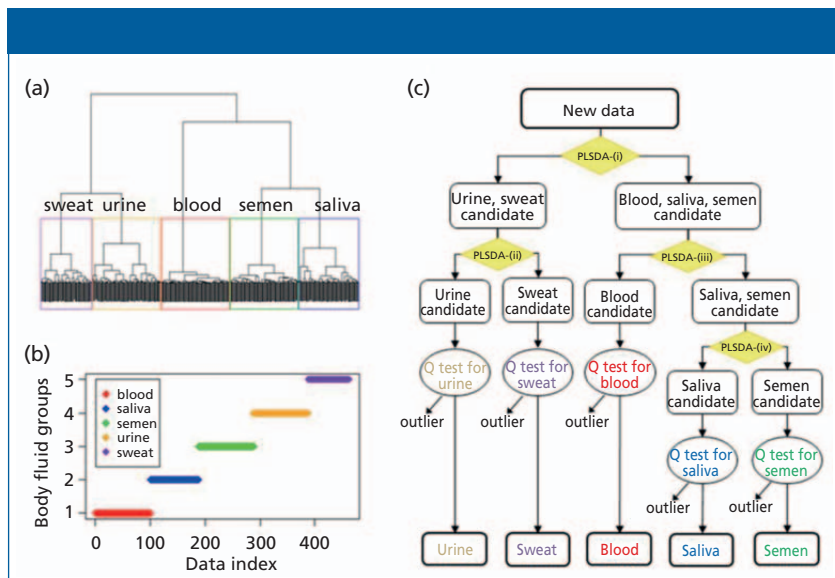
**Table II: Peak assignment in ATR FT-IR spectra of semen and vaginal secretions. Adapted with permission from reference 12.**

Body Fluid	Peak Frequency (cm <sup>-1</sup> )	Peak Assignment	Vibrational Mode
Semen	3268	Amide A	Symmetric N-H stretching
	2950	Methyl stretches of lipids	CH <sub>3</sub> stretching
	1625	Amide I (β-sheets)	C=O stretching
	1540	Amide II	N-H bending coupled to C-N stretching
	1450, 1393	Methyl bending of amino acid side chains, lipids, and proteins	Asymmetric and symmetric CH <sub>3</sub> bending
	1240	Nucleic acid phosphate	Asymmetric PO <sub>2</sub> <sup>-</sup> stretching
	1059	Prostate specific antigen (PSA)/fructose/nucleic acids	CH <sub>2</sub> OH groups, C-O stretching and COH groups bending, symmetric PO <sub>2</sub> <sup>-</sup> stretching
	980	PSA/fructose	Unknown
Vaginal secretions	3274	Amide A	Symmetric N-H stretching
	2923	Methylene stretches of lipids in vaginal mucosa	CH <sub>2</sub> stretching
	1641	Amide I (unordered)	C=O stretching
	1542	Amide II	N-H bending coupled to C-N stretching
	1454, 1406	Methyl bending of amino acid side chains, lipids, and proteins	Asymmetric and symmetric CH <sub>3</sub> bending
	1223	Nucleic acid phosphate (PO <sub>2</sub> <sup>-</sup> )	Asymmetric PO <sub>2</sub> <sup>-</sup> stretching
	1127–1030	Glycogen from sloughed cervical epithelia	CH <sub>2</sub> OH groups, C-O stretching and COH groups bending, symmetric PO <sub>2</sub> <sup>-</sup> stretching

tions by targeting valuable biological material from previously deposited material, which will assist in screening and interpretation (2). Edelman and colleagues (17), in addition to visible-near infrared (NIR) spectroscopic analysis of blood, studied the stain's age using Fourier transform NIR spectroscopy. The results showed changes of spectra within the first days of aging. During the first 10 min, when the stain was still

fresh, the spectra were dominated by the water contribution. As time progressed, the spectra showed more visible peaks coming from biomolecules.

The sample's age was also studied by Zou and coauthors (18), where the combination of vibrational spectroscopic techniques (that is, ATR FT-IR and Raman spectroscopy) was used. The analysis of blood and semen samples was targeted. The authors were able to



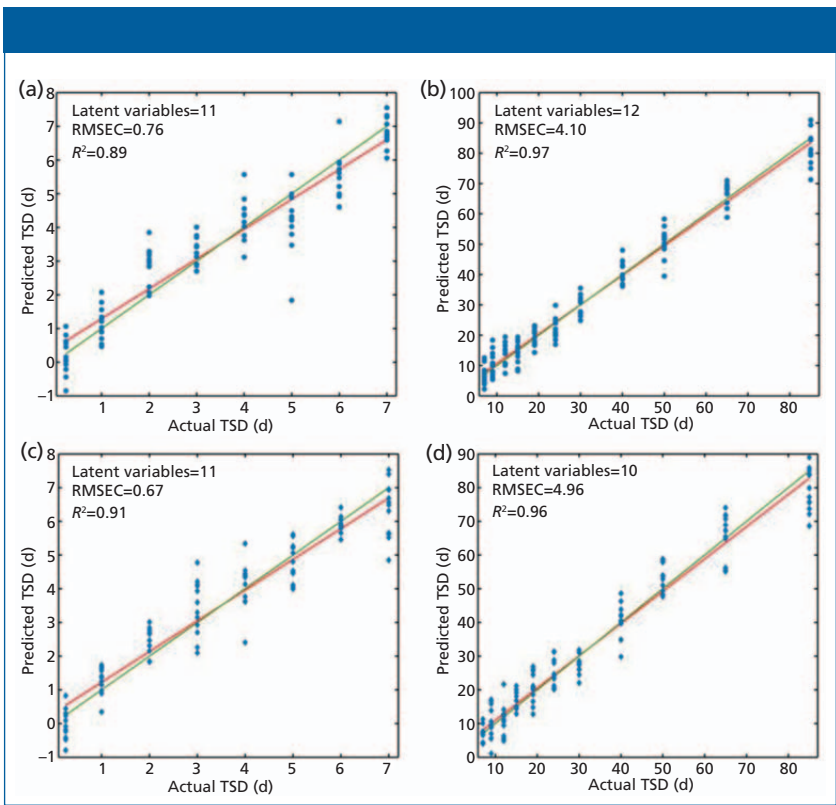
**Figure 1:** Development of a dichotomous classification tree for the ATR FT-IR spectra of five body fluid (BF) types based on hierarchical clustering analysis: (a) A dendrogram established for the PLSDA scores of 1-day BF spectra predicted by the 1day-5class PLS-LDA-Q model. Borders drawn in the dendrogram indicate clusters of data from each BF group, peripheral blood (red), saliva (blue), semen (green), urine (orange), and sweat (purple). (b) Assignment of the data from each spectrum into the respective BF group. (c) A discrimination scheme using a dichotomous classification tree followed by Q-tests for each type of BF. The dichotomous classification tree was built according to the dendrogram. Four 2-class PLSDA models for the corresponding BF groups were set at each node of the tree: (i) blood-saliva-semen versus urine-sweat using four latent variables (LVs), (ii) urine versus sweat using four LVs, (iii) blood versus saliva-semen using two LVs, and (iv) saliva versus semen using three LVs. Adapted with permission from reference 16.

select peaks that are characteristic for either blood or semen, and therefore distinguish between these two. For the stain age discrimination, the proposed method was based on the ratio of two specific peaks for each biological fluid: 1531 and 1635  $\text{cm}^{-1}$  for blood, and 1240 and 1633  $\text{cm}^{-1}$  for semen.

Zhang and colleagues (19) examined the changes in blood spectra as the stain dried out. Researchers measured peaks at 3308  $\text{cm}^{-1}$  and 1541  $\text{cm}^{-1}$ , and prepared correlation analyses. Human and rat blood samples were subjected for investigation under specific environmental conditions: temperature, humidity, and

sample concentration. Logistic regression analysis showed a correlation between absorbance at 3308  $\text{cm}^{-1}$  and the sample's age.

Lin and colleagues (20) brought the estimation of the age of bloodstains to the next level. They analyzed samples applied to indoor and outdoor crime scene conditions. The examined stains ages were between 0.25 and 107 days. For validation purposes, the partial least squares regression (PLSR) models were built to estimate the age of a sample (Figure 2). Furthermore, PLSDA models were built to distinguish between fresh (up to one day old) and older



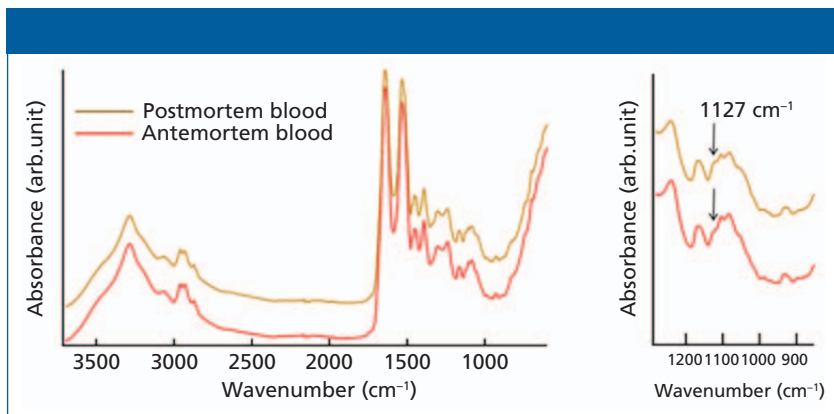
**Figure 2:** PLSR plots for indoor bloodstain samples in the (a) 0.25–7 d and (b) 7–85 d periods and outdoor bloodstain samples in the (c) 0.25–7 d and (d) 7–85 d periods showing the calibrated age predictions versus the actual age. Adapted with permission from reference 20.

samples. For both indoor and outdoor conditions, the PLSDA models showed accuracy of 0.99 for external validation.

Very frequently, there is a good chance that the size of a sample recovered from a crime scene may be limited, which means establishing the limit of detection of a potential technique is very important. Nonconsumptive in situ testing preserves a sample so maximum biological material is available for subsequent testing such as DNA analysis. Lu and coauthors (21) examined the limit of detection for blood on fabrics using ATR FT-IR spectroscopy. To calibrate spectra of blood dilutions, PLSR

was used. The limit of detection for blood on fabrics was found to be 0.0010  $\mu\text{g}$  for cotton, 0.0077  $\mu\text{g}$  for nylon, 0.011  $\mu\text{g}$  for acrylic, and 0.0066  $\mu\text{g}$  for polyester. The same group reported a better limit of detection for blood deposited on the last two (acrylic and polyester) using infrared diffuse reflection spectroscopy (22). However, it was noted that ATR FT-IR spectroscopy provides extreme surface sensitivity, which leads to the improved limit of detection.

Currently, the discrimination between menstrual and peripheral blood on site is challenging for forensic investigators. Menstrual blood is a mixture of

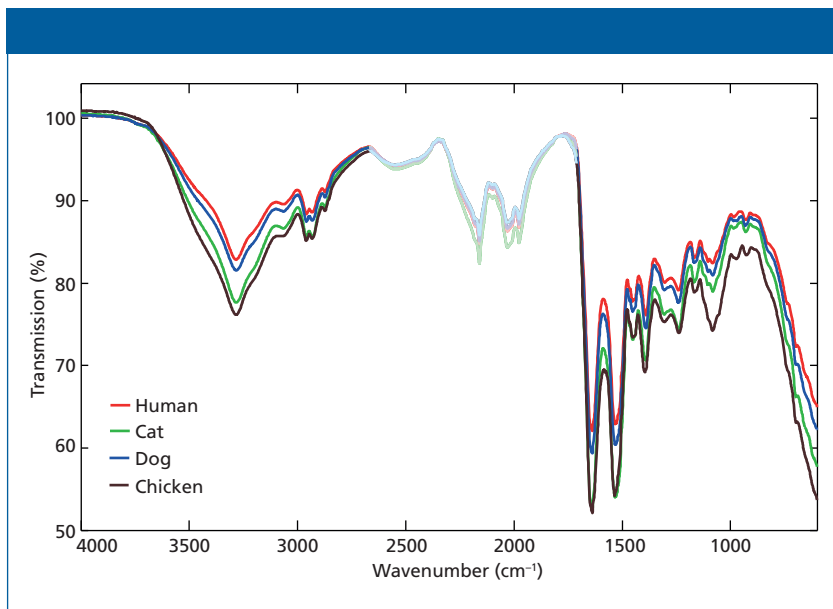


**Figure 3:** Discrimination of ATR FT-IR of pure postmortem and antemortem blood. Average ATR FT-IR spectra of pure postmortem blood (camel) and antemortem blood (red) (left), and a region representing a distinctive difference between them (right). The 1711–2669  $\text{cm}^{-1}$  region was excluded to avoid interference from the ATR crystal. Adapted with permission from reference 24.

peripheral blood and vaginal discharge, and there is no reliable test that can distinguish these two blood types in current forensic practices (2). It would be extremely beneficial for cases involving violence and sexual assault to distinguish those two body fluids. Quinn and Elkins (23) examined the capability of ATR FT-IR spectroscopy for simultaneous discrimination between several body fluids, with emphasis of differentiation between menstrual and peripheral blood. The study confirmed that tested body fluids (menstrual blood, venous blood, semen, saliva, and breastmilk) have their own characteristic IR signatures. When IR spectra of neat samples of peripheral blood, semen, saliva, and breastmilk from two different donors were compared, they showed minimal to small variation in each body fluid. When menstrual blood spectrum was compared to peripheral blood spectrum, it illustrated an enhanced peak at 1039  $\text{cm}^{-1}$  being assigned to phosphoric acid characteristic for menstrual discharge. However, no fluctuations between men-

strual blood spectra from more donors were studied here. The testing of samples deposited on various materials showed the fluids could still potentially be detected, but to a lesser degree than pure samples. This is very dependent on the porosity and weave of the substrate.

Another very important aspect of forensic practice is discrimination between postmortem (after death) and antemortem (before death) blood. Takamura and colleagues (24) introduced a novel multivariate spectral processing for discrimination between these two blood classes. The classification model was prepared based on pure samples of blood, spectra of which can be seen in Figure 3. Then blood samples were prepared on various materials (such as polyester, cotton, and denim). After the signal of blood was extracted from the signal of an entire sample (blood and substrate), the blood signal was used for classification, using a model built on pure samples. The discrimination accuracy was improved by using weighted least squares regression. Overall, the method showed high accu-



**Figure 4:** Raw mean IR spectra of human (red line), cat (green line), dog (blue line), and chicken (brown line) blood. The region of 1711–2669  $\text{cm}^{-1}$  was excluded to avoid interference from the diamond ATR crystal. Adapted with permission from reference 26.

racy for discrimination between post-mortem and antemortem blood in the presence of substrate interference.

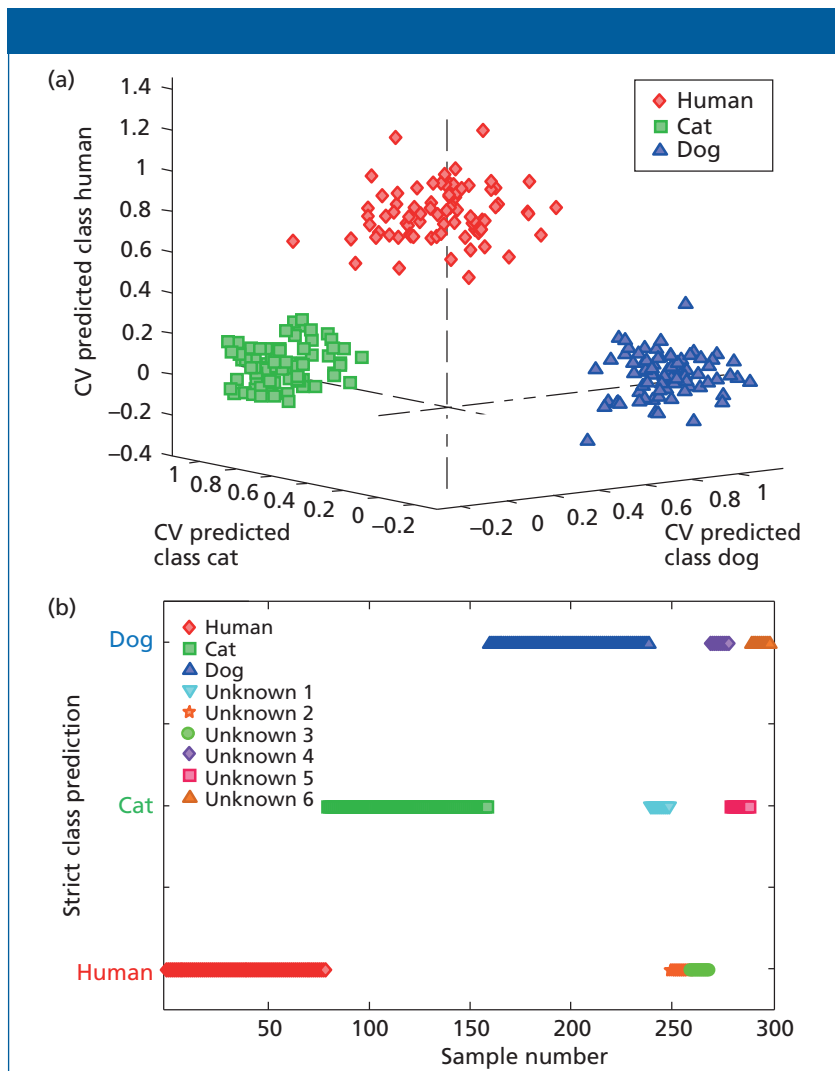
### Species Identification

During a forensic investigation, the identification of the bloodstain origin is very important. Current biochemical tests destroy the sample for further analysis, and still have limitations. In the study performed by De Wael and coauthors (25), a combination of spectroscopic techniques was used to analyze blood in particular, so as to discriminate between species. Together with microspectrophotometry and Raman spectroscopy, ATR FT-IR spectroscopy was used to discriminate between human, cat, and dog blood samples. None of these methods proved successful. Spectra of both Raman and ATR FT-IR spectroscopy for the three species presented were not able to be

differentiated. This lack of differentiation is not surprising, since all the samples consist of the same components with only quantitative variations within them. Therefore, in the study performed by Mistek and Lednev (26), chemometrics was applied to strengthen classification power. Raw mean IR spectra of different species applied in the study (human, cat, dog, and chicken) can be seen in Figure 4. In their work, PLS-DA models were built to discriminate between human and nonhuman blood, and human, cat, and dog. The models allowed for entire separation between classes and external validation of the models performed with unknown samples resulted in 100% prediction accuracy (Figure 5).

Lin and colleagues (27) expanded the study on species identification by adding more animal classes, and performing a study on samples exposed to specific





**Figure 5:** (a) Cross-validated prediction results for the individual species PLSDA model. All three classes are well-separated and grouped together within classes. (b) Strict class prediction results for unknown blood samples (from external experiment) by the species-specific PLSDA model. Each symbol represents a spectrum from human, cat, or dog blood (10 spectra per donor). No unassigned or misclassified spectra are observed. Adapted with permission from reference 26.

environmental conditions. Samples were subjected to indoor and outdoor conditions, as well as an aging process. Two models were built: one to discriminate between human, other mammals, and domestic fowl, and the other to identify

species specifically (human, rat, rabbit, dog, chicken, and duck). Both models illustrated complete separation between classes; no false positive or false negative assignments were observed. For external validation, which was performed using

samples exposed to environmental conditions, the first model showed 100% prediction accuracy, while the species specific model showed 94.2%.

It was shown here that FT-IR spectroscopy was applied for a wide spectrum of studies on bloodstains. Researchers used this technique for predicting the age of a bloodstain, estimating the limit of detection for blood, and discriminating between menstrual and peripheral blood, as well as postmortem and antemortem blood. One of the very important aspects in bloodstain analysis is identification of species. The ATR FT-IR spectroscopic approach showed promise for discriminating between human and nonhuman classes, and even between several different animal groups with statistical confidence. It would be extremely beneficial to expand the study with even more species, as it was successfully shown in study where Raman spectroscopy was used (28).

## Conclusions

The importance of biological traces that can be found at a crime scene attracts researchers from around the world to apply and examine different approaches for nondestructive identification and analysis of body fluids. This review article was intended to emphasize the great potential of FT-IR spectroscopy for the examination of body fluid traces for forensic purposes.

It was proven that FT-IR spectroscopy is suitable to distinguish between body fluids and various substances which could be mistaken as body fluid. Additionally, this technique shows potential for identification of body fluids that were deposited on substrates. This seems to be most successful by using the combination

of FT-IR spectroscopy with statistical data analysis.

Blood is one of the most valuable and frequently found biological fluids on a crime scene. Therefore, its identification and analysis are essential. Many studies focus on the analysis of bloodstains to gather as much information from a sample as possible. A sample's age and limit of detection of blood were investigated using FT-IR spectroscopy. Likewise, discrimination between menstrual and peripheral blood, as well as postmortem and antemortem blood were targeted. ATR FT-IR spectroscopy with chemometrics was successfully applied for discrimination between various species based on blood samples.

Even though FT-IR spectroscopy showed great performance for forensic analysis of body fluids, more investigation needs to be performed before the technique can be used in practical applications. Raman spectroscopy, an alternative vibrational spectroscopic method, showed great potential for human phenotype profiling. The combination of Raman spectroscopy and advanced statistical analysis was applied to identify human sex from bloodstains (29), saliva (30), human race from traces of blood (31), and semen (32), as well as differentiate the age groups of donors (33). It would be important to investigate the capabilities of FT-IR spectroscopy for this application. Our laboratory and others are currently working in this direction. Moreover, portable or handheld instruments should be tested to bring the analysis to a crime scene, saving time and reducing costs of forensic investigation.

This FT-IR spectroscopic technique proved to be advantageous over currently used methods for body fluid analysis. It is particularly beneficial because it is non-

destructive and requires a small sample size, and the entire examination can be performed on scene because of the availability of portable instruments. Most importantly, the method is universal and can be used for main body fluids, such as blood, semen, vaginal fluid, saliva, sweat, and urine. FT-IR spectroscopy could have an extremely important role in forensic practice when the method is fully developed.

### Acknowledgment

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# Standoff, Wide-Area Explosive and Narcotic Detection Using Shortwave Infrared Hyperspectral Imaging

Shortwave infrared (SWIR) hyperspectral imaging (HSI) is a useful technique for soldiers and first responders to combat the ever-changing threat landscape. SWIR HSI provides the ability to rapidly analyze a large, complex scene, and detect small constituents located within. Two key threat scenarios that are currently being addressed by SWIR HSI are the detection of explosive or chemical warfare agent threats before potential harm can occur, and to intercept narcotics entering prisons and jails. Tunable filter-based SWIR HSI sensors can be configured to use either single-bandpass or multiple-bandpass wavelength tuning. These filter types enable hazardous material detection in stationary, moving sensor, or moving target scenarios, while providing near real-time results. Tunable-filter HSI also provides users the ability to add new materials to a target library, allowing the sensors to adapt to changing threats or applications. This article provides an overview of SWIR HSI using liquid crystal tunable filters and provides detection results for key threat applications.

Nathaniel Gomer, Shawna Tazik, Jeffrey Beckstead, Charles Gardner, and Matthew Nelson

**E**xplosive and narcotic materials are a potential danger to human life. The risk of improvised explosive devices and the rise in deaths related to narcotic materials have created a need for improved sensing capabilities that can target these materials at safe distances, before the threat can be introduced to the general population (1,2). In addition, the threats them-

selves can change over time. These changes include variations in material type, composition, or method of introduction. With a dynamic threat landscape, a sensing approach must be adaptable to interdict threats before they cause harm.

Unfortunately, the current generation of spectroscopic detection approaches has limitations. Commonly,



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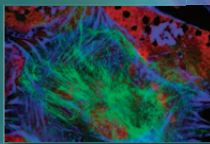


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these include close-to-target proximal requirements, reduced area search rate, eye safety concerns from focused excitation (such as Raman spectroscopy), and long analysis times that have hindered the ability of spectroscopic instrumentation to fully combat this problem and be adopted by first responders (3–6).

One approach that has the potential to address these problems is short-wave infrared (SWIR) hyperspectral imaging (HSI) (7–10). SWIR HSI is beneficial for detecting small constituents located within complex scenes, where the target concentration is commonly low. SWIR HSI typically uses the wavelength range 1000–1700 nm and incorporates indium gallium arsenide (InGaAs) focal plane arrays (FPAs) as detectors. InGaAs FPAs can be uncooled, which reduces system size, weight, and power constraints, and makes a SWIR HSI sensor more portable.

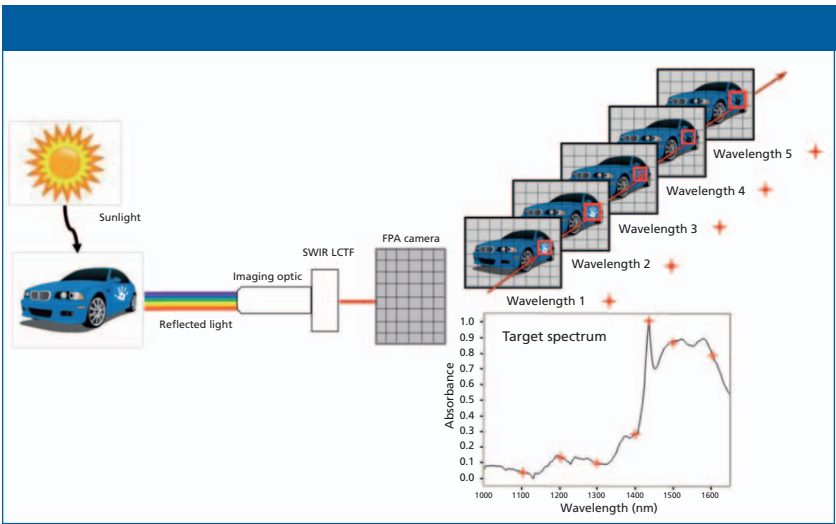
SWIR region absorption bands are derived from overtones and combination bands of O-H, N-H, C-H, and S-H bend and stretch vibrations. C-H bond vibrations are the most prevalent in explosive and narcotic materials, with each containing numerous bonds typically found within  $-\text{CH}_2$  or  $-\text{CH}_3$  functional groups. These bands are typically broad, leading to complex spectra for which it can be difficult to assign specific chemical components to specific spectral features. The use of multivariate analysis techniques may be used to extract this information.

In addition, the SWIR region is insensitive to target area temperature interference (such as heat emitted from a target does not interfere with analysis), which can be a problem for

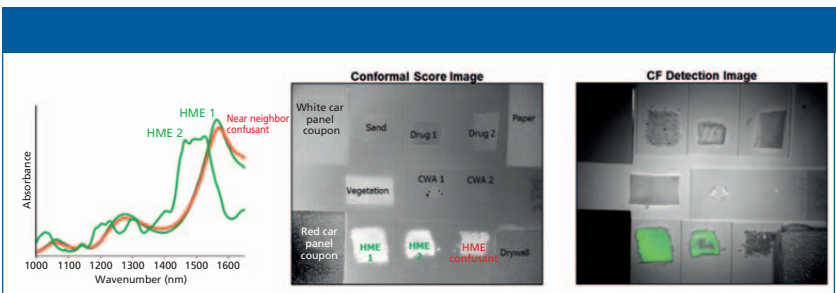
midwave-length infrared (MWIR) and long-wavelength infrared (LWIR) sensors. The response in the region is only to photons that are absorbed or reflected by a target. SWIR sensors are capable of utilizing solar illumination for outdoor target excitation. For indoor applications, low-cost broadband illumination or SWIR light-emitting diodes (LEDs) may be integrated onto a sensing platform.

Although there are many configurations for HSI instrumentation (such as pushbroom and snapshot [11]), full-frame imaging using a tunable filter has certain advantages that can address current sensing shortcomings, such as higher imaging fidelity and target library expansion. One filtering approach utilizes liquid crystal tunable filters (LCTFs), where a specific wavelength or passband is output through the filter. An LCTF is composed of multiple stages (up to four) where the output of each stage is voltage modulated so the overall bandpass of the filter is the desired wavelength or passband.

There are two filtering modes for LCTFs: discrete band filtering and conformal filtering (CF). Discrete band tuning is when the filter is set to pass a single wavelength. Switching between wavelengths occurs at speeds under 100 ms and a full hypercube (a sequential scan of wavelength specific images) may be collected in typically under 1 min, depending on the filter tuning increment. Each hypercube contains a fully resolved, unique spectrum for each pixel on the FPA, which provides this approach the capability to detect particles on a single-pixel level. After the spectral information about the target is known, a condensed subset of imagery can be collected at wavelengths related



**Figure 1:** Overview of SWIR HSI using a liquid crystal tunable filter (LCTF) with broadband irradiance.



**Figure 2:** Overview of conformal filter breadboard testing, with the sensor trained to identify two HMEs amongst a complex target set, including a near neighbor confusant.

to the absorption bands of the target to reduce the sensor's time to detect.

Figure 1 shows the concept of SWIR HSI using a discrete-band LCTF. In the figure, a vehicle with a threat deposition (handprint) on the door is illuminated with solar irradiance. The light reflected off the vehicle is collected by the sensor and images can be taken at each wavelength in the SWIR region, allowing measurements of absorbance for all materials in the field of view. Each material in the field of view (FOV) is spatially separated on

the FPA, which provides the ability to resolve small constituents in an expansive scene.

The second filtering approach, CF, is designed to pass multiple optical passbands, not a single discrete wavelength, where the desired passband is optimized to discriminate a desired target or target class from the background. The passband is calculated based on the concepts of compressive sensing and multivariate optical computing (MOC) (12–14), where a regression vector is coded into the transmission

of the filter. The regression vector is aligned with the absorption properties of the target material, which improves target discrimination in the presence of a complex background. CF can also be used for real-time motion-sensitive applications, like drone detection or people screening, since it does not require sequential wavelength tuning.

Figure 2 shows the concept of conformal filtering and how CF can identify two homemade explosive precursors against complex background materials and a near-neighbor confusant. The target board scene contains a mixture of target materials (homemade explosive [HME] 1 and 2), substrates (white and red car coupons, paper, and drywall), environmental clutter (sand and vegetation), and confusant materials (near neighbor HME confusant, drug 1 and 2, and chemical warfare agent simulant 1 and 2). The score image shows the response of the filter, with brighter pixels having a higher score value and being more likely to be considered a detection. The spectral traces show the SWIR absorption spectra for the two HMEs and the near neighbor confusant material. The final image is the detection image, with materials overlaid in green being considered a detection, which indicates that the two HMEs are being detected while the near neighbor confusant and all other scene constituents are not.

## Experimental

ChemImage LCTF-based SWIR HSI sensors have been constructed in two application configurations: benchtop and standoff. The benchtop configuration has been designed to screen articles of mail in either transmittance or reflectance modes. The sensor is

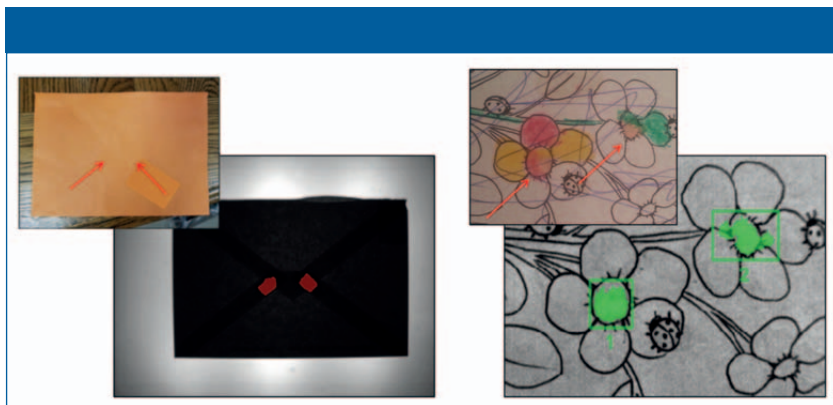
mounted onto a tabletop assembly with transmittance and reflectance oriented halogen lights for target illumination. A piece of mail is placed on a glass plate, above the transmittance lighting, and the sensor is positioned above the target facing toward the glass. The field of view of the sensor is set to image the full glass plate area (a rectangular area approximately 8.5 in. x 11 in.).

The discrete band LCTF standoff system was designed for indoor and outdoor use, for applications such as vehicle screening or room clearing. The sensor is mounted on an FLIR PTU/tripod, and uses eight on-sensor 50-W halogen bulbs, 4x 100-W halogen flood lamps, and solar illumination depending on use scenario and standoff distance. In addition, a conformal LCTF breadboard was used for moving-target applications.

For each configuration, the collection optics pass light through the LCTF and onto the FPA (FLIR Tau 640x512 InGaAs, uncooled). The main optical variation between the two configurations is that the standoff configuration uses an adjustable lens array to manually adjust the focal range for standoff distances between 1 and 20 m. The benchtop configuration uses two fixed lens settings, which allows a selection between high or low zoom, with a sensor to target distances less than 0.5 m. Each sensor applies the same image corrections to ensure maximum performance: background (dark) subtraction, bad pixel correction, and flatfielding.

Imagery analysis and detection results are generated using a real-time SWIR automated detection algorithm (SWIR-ADA). SWIR-ADA provides autonomous analysis of imagery, uti-





**Figure 3:** Detection of Suboxone concealed in an envelope (left) and in a crayon drawing (right) using the benchtop SWIR HSI sensor.

lizing the spectral responses of threat materials in comparison to environmental clutter to establish target selection criteria. The result of a SWIR-ADA scene analysis is an image of the scene with false color overlays in areas where a threat material is located. To achieve at or near real-time results, detection mode uses a spectral library of known threat materials, identifies a subset of wavelengths that isolate the dominant absorption bands for each material or class of material, and then collects imagery for that subset of wavelengths.

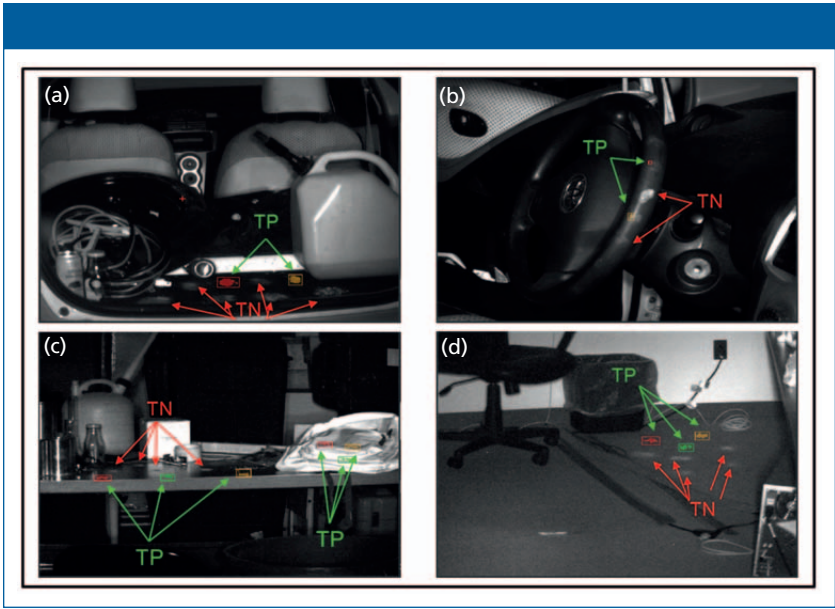
## Results and Discussion

Using the benchtop system to scan articles of mail for narcotics or threat materials is an application of high interest to correctional institutes. Some narcotics of interest include heroin and methamphetamine as well as Suboxone, a prescribed medication that combats opioid addiction. Suboxone is commonly found as an orange sublingual strip and can be easily placed into letters and covered with markers or crayons, or hidden in glue seams in envelopes, masking the

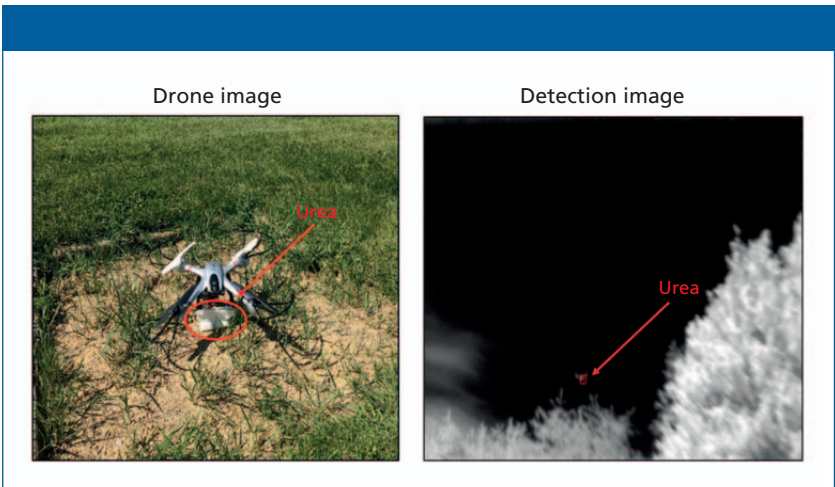
drug to the naked eye. These tainted letters are then potentially mailed into the prison populace and consumed.

Figure 3 (left) shows an example of Suboxone (buprenorphine and naloxone) detection in a concealed sample. A strip of Suboxone (approximate mass of 10 mg) was halved and placed into the seam of an orange envelope and then resealed. The tainted envelope was then placed onto the sensor and scanned for approximately 7 s. During this scan time, the sensor tuned to and acquired images at wavelengths that corresponded to specific absorbing and nonabsorbing bands of Suboxone. The resulting image shows a color overlay on the envelope seam where the Suboxone strip was located. Figure 3 (right) shows a sample where Suboxone had been mixed with an orange crayon, which was used to color two areas on a page. The detection image shows a color overlay on the two spots where the Suboxone was deposited.

The standoff system is designed for an expanded array of applications, with vehicle residue screening and room clearing being two primary focuses. For



**Figure 4:** Detection images from four application scenarios using the standoff SWIR HSI sensor: (a) vehicle trunk clearing, (b) residue detection on steering wheels, (c) clandestine lab investigation, and (d) room clearing.



**Figure 5:** Moving target detection of a drone carrying a bulk bag of urea using the conformal filter breadboard.

testing purposes, materials of interest are used in these scenarios, and their presence is indicated by a specific color overlay: ammonium nitrate (red), urea

(orange), and aspirin (green). Ammonium nitrate and urea are homemade explosive precursors, and aspirin is used as a narcotic simulant.

Figure 4 shows four detection scenarios for standoff SWIR HSI. Each scene contains target material, confusants (NaCl), and realistic environmental clutter: vehicle trunk clearing, residue detection on steering wheels, clandestine lab investigation, and room clearing. In Figure 4a, materials are presented as bulk spills in the trunk of a car. Figure 4b shows residue transfers onto a steering wheel. Figure 4c shows a simulated clandestine lab, where unknown bulk white powders are found on a table and fingerprint residue transfers are on the backpack. Figure 4d shows room clearing, where bulk spills are on the floor. For each example, the true positives (TP) and true negatives (TN) are labeled.

In Figure 5, the conformal breadboard was used for real-time moving target detection of a drone carrying a bulk amount of urea. Conducting real-time measurements is possible because of its dual polarization optical design, where the collected light is split by a polarized beamsplitter, and sent through two conformal filters aligned to the beamsplitter's polarization output. Each filter's output was imaged onto two FPAs, and provides the ability to analyze the target area at the FPA frame rate. The drone was at variable standoff distances during the experiment, but typically was between 20 and 50 m. The prototype sensor was able to track and detect the urea while the drone was in motion.

## Conclusions

Hyperspectral imaging is a valuable tool to detect and identify threat materials, such as explosives or narcotics.

In these applications, SWIR HSI has the potential to detect small constituents in scenes with a greater amount of environmental clutter. These sensors have been implemented in benchtop and standoff configurations, using an LCTF to provide high fidelity imagery with at or near real-time detection speeds.

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# External Reflection Analysis of Surface Coatings

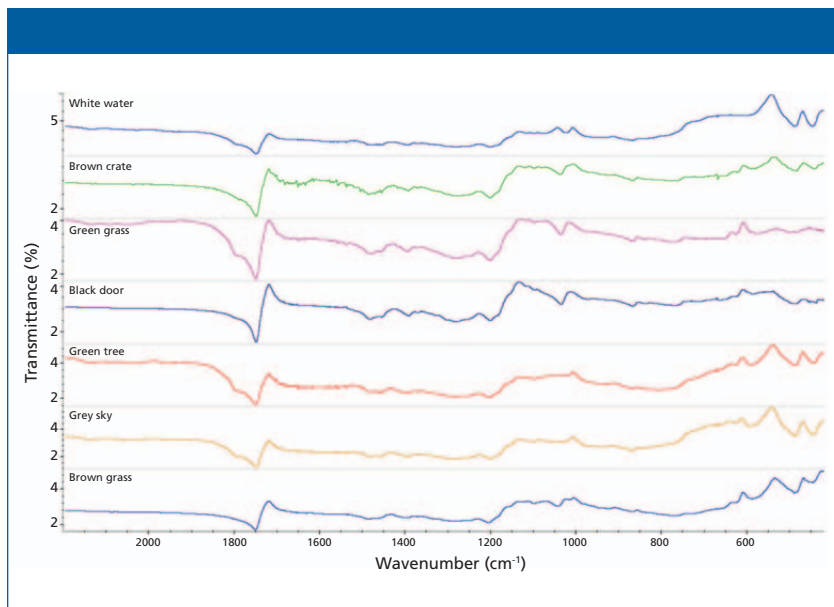
The study of coatings on surfaces affects materials as diverse as soda cans and masterpieces of art. Fourier transform infrared (FT-IR) spectroscopy is ideal for this purpose, because many of those coatings, such as oil-based paints and polymeric films, are organic in origin, and FT-IR is inherently nondestructive. Additionally, the analysis can be completed without making contact with the surface, thus protecting a masterpiece painting or preserving the integrity of the films. We examine here a variety of materials, including polymeric coatings on metal and paint on canvas, using external reflectance. Although the technique was specifically designed for art conservation, we will show it to have utility in a variety of similar applications.

## Michael Bradley

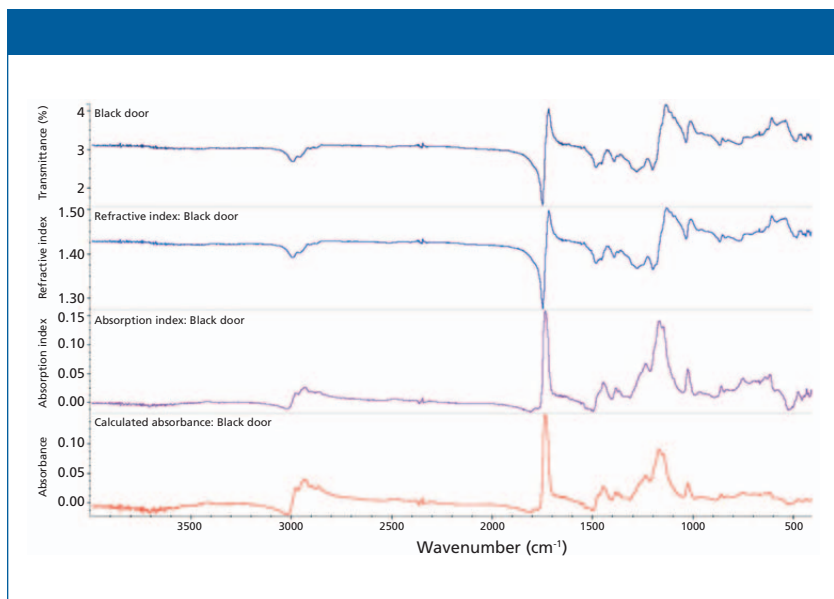
**T**he need for noncontact analysis of surfaces extends over a wide range of applications. For instance, coatings such as paints or polymers are heavily used, performing cosmetic, artistic, or protective functions. Many of these materials have significant organic content, such as the polymeric coatings in soda cans or the oil-based materials used by painters. The analysis of these coatings provides insights into their integrity, robustness, or provenance, the latter being critical in cases of art preservation and fraud detection (1). Being able to nondestructively determine composition quickly and economically provides useful information to quality assurance (QA) and quality control (QC) laboratories, art conservators, and production facilities.

Fourier transform infrared (FT-IR) spectroscopy is an excellent investigative tool for this purpose. First, the sensitivity to organics is very high, especially with polymeric materials. In addition, FT-IR has low energy and low power, which make the probe itself nondestructive. The key is sample presentation. Many methods require sample chips to be removed for contact with an attenuated total reflectance (ATR) crystal. This process may damage a masterpiece painting or scratch a metal surface, exposing it to corrosive environments.

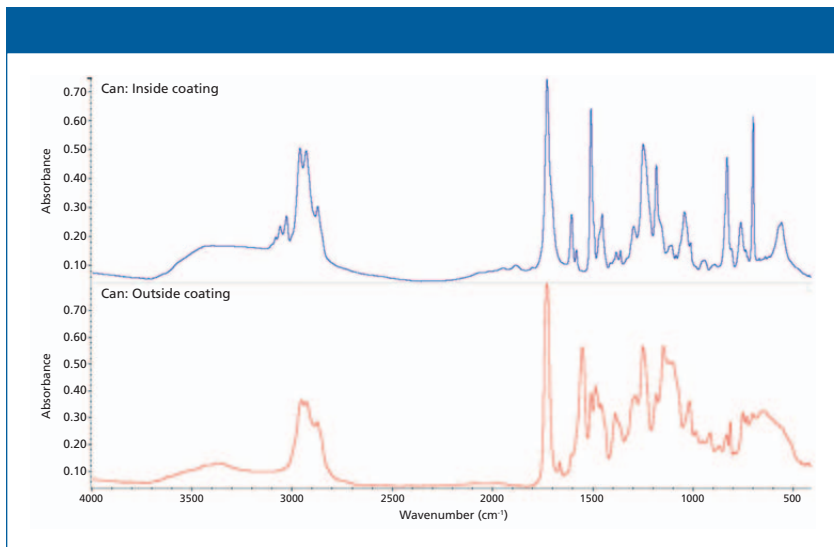
FT-IR reflectance has been successfully used in this setting for some time. For instance, a substantial amount of work was done using FT-IR to study the large frescoes seen in Pompeii (2). However, this required an FT-IR spectrometer or FT-IR microscope to



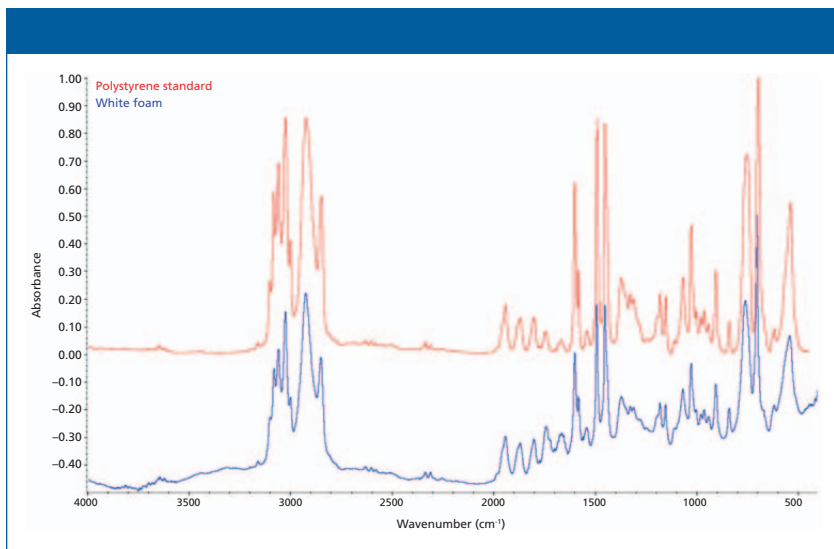
**Figure 1:** Spectra acquired from external reflection off a painting. The signature indicated a date in the early 1900s while the spectra indicate a later date.



**Figure 2:** Kramer-Kronig correction applied to spectrum 16 (black door) from the painting. Top: Raw spectrum. Second: Refractive index. Note steep change around the ester region ( $1750\text{ cm}^{-1}$ ), which is what causes the reststrahlen bands. Third: Absorption index. Bottom: Calculated “transmission equivalent” spectrum. Search result against this spectrum yields close match to an acrylic resin used in printing and packaging.



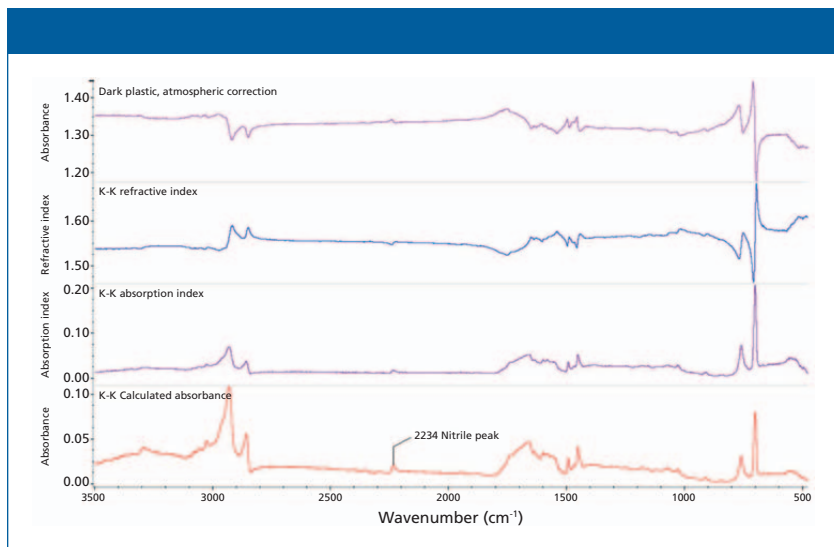
**Figure 3:** Spectra from the inside (top spectrum) and outside (bottom spectrum) of a soda can, acquired using specular reflection.



**Figure 4:** Spectrum of a white packing foam. The sample was handheld at the collection port of the accessory.

be positioned near the fresco, or alternately that a piece of the fresco be supplied in a laboratory setting. We show here results obtained using a portable, noncontact spectrometer and acces-

sory combination that grew out of a collaboration involving the art conservation community, with their specific and strict requirements. We start with the analysis of a painting, but we will



**Figure 5:** Spectrum from a dark plastic piece. Atmospheric correction was applied first, then the Kramer-Kronig correction. The small nitrile band at 2234  $\text{cm}^{-1}$  is clearly seen, helping prove the sample is ABS plastic.

then demonstrate the general utility of the external reflectance method.

## Experimental

The data were collected using a ConservaIR FT-IR external reflection accessory (Thermo Scientific) in the sample compartment of a Nicolet iS5 (Thermo Scientific) FT-IR spectrometer. Since the system can be operated via battery, the entire system is portable and can be used in field locations such as archaeological sites or aircraft hangers. The accessory has an adjustable angle head that enables the analysis of ceilings, wall-mounted paintings, or engine surfaces.

The infrared beam is directed out of the sampling head to a focus about 1 cm away from the sampling head, with a spot size about 1.25 mm in diameter. In the work done here, both diffuse and specular re-

flectance data were collected. Visual images of the surface being analyzed were also collected using the built-in camera, providing the link between the target and the data.

A few of the spectra were processed using an atmospheric correction and the Kramers-Kronig (K-K) correction, as noted in the text. Backgrounds were collected from either clean surfaces (for the metallic materials) or a roughened white ceramic surface for the diffuse reflectance.

## Results and Discussion

The requirements for these measurements were driven by a collaboration with art conservators. A typical question would be whether the formulation of the paints on a canvas are consistent with those in use by an artist or are indicative of a later date. In Figure 1, spectra collected from an oil painting at a few loca-



tions over the surface are shown. Oil paintings are not highly reflective in the infrared range, so the amount of light reaching the detector is low. Further, many of the bands appear with a "first derivative" shape because of the strong reststrahlen effect (3); note especially the ester band at  $1750\text{ cm}^{-1}$ . The refractive index changes sharply near a strong absorption band such as that of the ester, which prevents the IR light from being able to propagate through the heavy oil paints. The resulting "first derivative" bands can be corrected using the K-K correction (Figure 2). Note the sharp change in refractive index (second plot) around the strong peaks. The final spectrum now appears normal and the signal-to-noise ratio is quite good.

The system was not purged for this application. The time between the background data collection and the sample collection was several minutes, and we did not apply atmospheric correction for this data set. Even so, the presence of the atmospheric interferences is weak. This lack of interference shows the system is stable, an important consideration when implementing external reflection through the large spectral sampling opening.

The painter indicated by the signature on the painting worked in the early 1900s. Searching spectrum 16 (black door) against a set of commercial spectral libraries (industrial coatings, paints, inks) yields a strong match ( $>90$ ) to a latex acrylic emulsion. This information was sufficient to rule out this painting being an original, because those materials did not become available until at least the 1930s. The re-

maining spectra show similar provenance—far too modern.

Next, we examine a more general application of coatings on metals that provide aesthetic and protective qualities. A good example involving both characteristics is a soda can. The inner wall requires corrosion resistance because of the acidity of the beverage, and the outside of the can provides a surface for colorful advertising and information. Figure 3 shows spectra collected from both the inside and outside surfaces of a soda can. These spectra were collected in specular reflection mode, and the signals are very intense in both cases with a very high signal to noise. Atmospheric interferences again are extremely minimal here, so no further processing is required.

The bottom spectrum in Figure 3 comes from the outside of the can, where the printing is present. Searching this spectrum against commercial libraries reveals a close match to an isophthalic polyester coating (note the large, intense band near  $1750\text{ cm}^{-1}$ ). This type of resin has a very high resistance to moisture, which clearly is an advantage in this specific application.

The spectrum of the coating from the inside (top spectrum in Figure 3) is quite different. Again, the specular reflection spectral peaks are intense and the spectrum is very clean. The best search result is an epoxy resin with tung and castor oils. This coating would be in keeping with the movement of manufacturers toward more biofriendly materials (4).

External reflection provides an easy-to-implement probe for industrial processes, such as examination

of materials flowing into a recycling facility. In a very simple case, Figure 4 shows the spectrum from a piece of white packing foam. This spectrum is immediately identifiable as that of polystyrene.

In contrast, external reflectance spectra taken from a dark plastic material exhibit reststrahlen bands as seen previously. The spectrum at the top of Figure 5 has undergone atmospheric correction to remove water and carbon dioxide bands. After K-K correction, the final absorbance spectrum is shown at the bottom. The final spectrum matches that of acrylonitrile-butadiene-styrene (ABS) in a spectral search. The telling peak in this analysis is the tiny, but clearly visible, band at  $2234\text{ cm}^{-1}$  that results from the nitrile group ( $\text{C}\equiv\text{N}$ ), a unique absorption band that could be obscured by the carbon dioxide or noise. The observable presence of this band illustrates the responsiveness of external reflection. This quality was exploited recently in an industrial implementation of external reflectance in an automotive production plant QA/QC setting, where the need for a metal surface to be scrupulously clean before sealing is tested by external reflection off the metallic surface. Tiny traces of oils or metal treatment were detectable, leading to a significant improvement in throughput and reliability of the engine parts.

## Conclusion

External reflection is shown to be effective for examining paintings, polymer-coated metals, and even bulk polymers. The responsiveness

depends upon the surface. Shiny, metallic surfaces provide a strong specular reflection spectrum that matches standard absorbance spectra very well. Less-reflective surfaces, like dark plastics, may require additional software processing, such as atmospheric correction (which would likely need to be done in a harsh manufacturing environment) and the K-K transform. The advent of efficient and flexible external reflection analysis solutions on portable platforms will simplify data collection and add to their utility.

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Fourier Transform Infrared Spectrophotometer

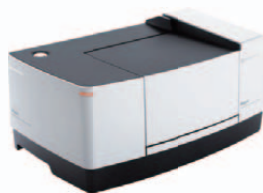
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