

FTIR spectral imaging applications in trace evidence

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ABSTRACT

Infrared spectral (or chemical) imaging is emerging as an exciting new tool in forensic analysis. Here we present some of our work in the application of infrared spectral imaging to different types of trace evidence: automotive paint chips, bicomponent fibers and the detection of illicit substances in fingerprints.

INTRODUCTION

Fourier Transform Infrared (FTIR) spectral (or chemical) imaging is emerging as an exciting new tool in forensic analysis. It involves the simultaneous collection of multiple mid-infrared spectra across a given sample. Since the mid-infrared region is capable of discriminating between different molecules, the data collected can be used to visualize chemical differences across the sample. The Digilab 'Stingray'® infrared chemical imaging system used in this study has a 64 × 64 pixel mercury cadmium telluride (MCT) focal plane array (FPA) detector. Using this detector, 4096 infrared spectra can be obtained simultaneously in the form of an image, with one spectrum recorded for each pixel. This is in contrast with conventional infrared microscopy, where only one spectrum can be collected at a time. The image data collected can be thought of as a three-dimensional datacube, which contains vertical and horizontal spatial dimensions, and a spectral frequency dimension.

There are many advantages the technique can bring to forensic analysis including the ability to obtain images which show contrast based on chemical differences, an obvious advantage for imaging fingerprints (1) and questioned document analysis e.g. intersecting lines. This technique has also been applied to forensic trace evidence such as multi-layered paint chips (2) and bicomponent fibers (3). The simultaneous visualization and identification of chemical differences in samples allows for new, more powerful ways to analyze and present trace evidence in a legal context. The large number of spectra which are rapidly obtained from the sample increases the confidence in the results obtained, and multivariate statistics can be used on the large datasets to draw more powerful conclusions from the evidence.

MATERIALS AND METHODS

Sample preparation

A multi-layered automotive paint chip from previous research (2) was set in Fimo (014 Transparent, Eberhard Faber) prior to transverse sectioning using a Leica RM2165 motorized microtome. Paint chip sections of 5-10 μm thickness from the sample were allowed to separate or fall out of the embedding medium, and were then carefully laid onto a bed of spectroscopic grade potassium bromide (KBr, Merck) powder in a dye. The KBr powder and paint chip sections were then pressed into a conventional 13 mm diameter disk, using a press. Individual paint chips were selected for imaging if they remained flat (unfolded) and parallel to the surface of the disk after pressing.

Fiber samples were analyzed in cross section via transmission or with a germanium hemispherical attenuated total reflectance (micro-ATR) crystal (Harrick Scientific SplitPea). For transmission analysis, a single fiber was taped across a window cut out of acetate frames (EXP photocopier transparency sheets), to assist with positioning for sectioning and also to prevent the fibers from crimping. They were then set in Spurr's (ProSciTech) resin. Due to difficulties locating the colorless fiber cross-sections in the colorless resin, methyl violet 6B indicator (Searle Diagnostic) was used to dye the resin purple. Sections of 5-10 μm thickness were microtomed using a LEICA RM2165 motorized microtome. The fiber cross-sections were then placed on KBr plates and imaged in transmission mode as described previously. Background images were obtained from vacant areas of the KBr plates. Samples analyzed via micro-ATR were embedded in the same way and the resin block was microtomed to expose the fiber cross section and ensure a flat surface. The bottom surface of the resin block was also microtomed flat to ensure it was parallel with the upper surface. Fibers were also analyzed intact (side-on) by flattening in a diamond anvil cell (DAC) (High Pressure Diamond Optics, Inc). Background images were obtained through the vacant DAC.

To prepare fingerprint samples, a clean index finger was rubbed over the face/neck and through the hair to ensure sufficient sebaceous secretions were present. This finger was then pressed into a small amount of the substance of interest. Any excess substance was brushed away with the other hand leaving behind nil or minimal visible trace of the substance on the finger. The finger containing the substance of interest was then lightly pressed onto either a metal oxide-coated glass slide (Kevley Technologies) (for reflection experiments) or a silicon window (for transmission experiments).

FTIR chemical imaging

Infrared chemical imaging was carried out using a Digilab Stingray system, comprised of an FTS 7000 FTIR spectrometer, coupled with a UMA 600 infrared microscope with a Lancer 64×64 focal plane array (FPA) detector. Images and spectra were collected and processed with Digilab Win IR Pro software. Images may be collected in either the "normal field of view", in which

each individual image tile is approximately $350 \times 350 \mu\text{m}$ in size, or using an expanded field of view (EFOV) setting, which produces an image tile of approximately $700 \times 700 \mu\text{m}$ in size.

Paint chip samples were imaged in transmission mode using the normal field of view mode (image size $350 \times 350 \mu\text{m}$). Within each image datacube, absorbance spectra were collected at 8 cm^{-1} resolution, using 64 or 256 co-added scans. The spectral range collected was $900\text{-}4000 \text{ cm}^{-1}$, with the lower value being determined by the limit of the FPA detector. Background image files were collected from vacant areas of the KBr disks/plates.

The infrared analysis of bicomponent fiber samples was performed in normal transmission mode and using a germanium attenuated total reflectance microscope objective (micro-ATR) (Harrick Scientific SplitPea). All fiber samples analyzed via transmission were imaged using the normal field of view (image size $350 \times 350 \mu\text{m}$). The spectral range collected was $900\text{-}4000 \text{ cm}^{-1}$. In a typical data set, the spatial resolution varies depending upon the wavelength chosen to form the image. At best, this will be approximately $5.5 \mu\text{m}$ at higher wavenumber values, determined by the detector (pixel) size, and at worst, it will be approximately $10 \mu\text{m}$, restricted by the wavelengths of light involved at the low wavenumber end of the spectrum. Microtomed embedded fiber samples were placed in contact with the germanium micro-ATR crystal and a contact alert system (SpectraTech) was used to monitor the pressure applied. For ATR analysis, 256 co-added scans were collected at 8 cm^{-1} resolution. This image size was $80 \times 80 \mu\text{m}$.

Fingerprint samples were imaged in reflection mode using the expanded field of view (EFOV) setting, in which each individual image tile is approximately $700 \times 700 \mu\text{m}$ in size. Typically, 256 (16×16) tiles, with a pixel aggregation factor of 16, were collected, giving a total area imaged of $1.12 \times 1.12 \text{ cm}$ at a spatial resolution of about $44 \mu\text{m}$. Image collection times using these parameters were typically of the order of one hour. The infrared spectra within each image were collected at 16 cm^{-1} resolution, using 16 co-added scans. Sample spectra were ratioed against background spectra so as to give $\log(1/\text{reflectance})$ output. The data collected comprised of 65,536 infrared spectra which were used to create a spectral library. Reference spectra of compounds of interest were then used to search against this library[ref] and the spectra ranked according to their similarity to the reference spectrum. An image was then generated which showed the spatial distribution of pixels which exceeded a selected threshold of similarity to the reference spectrum.

RESULTS

Note: Color images available in online version

Multilayered paint chips

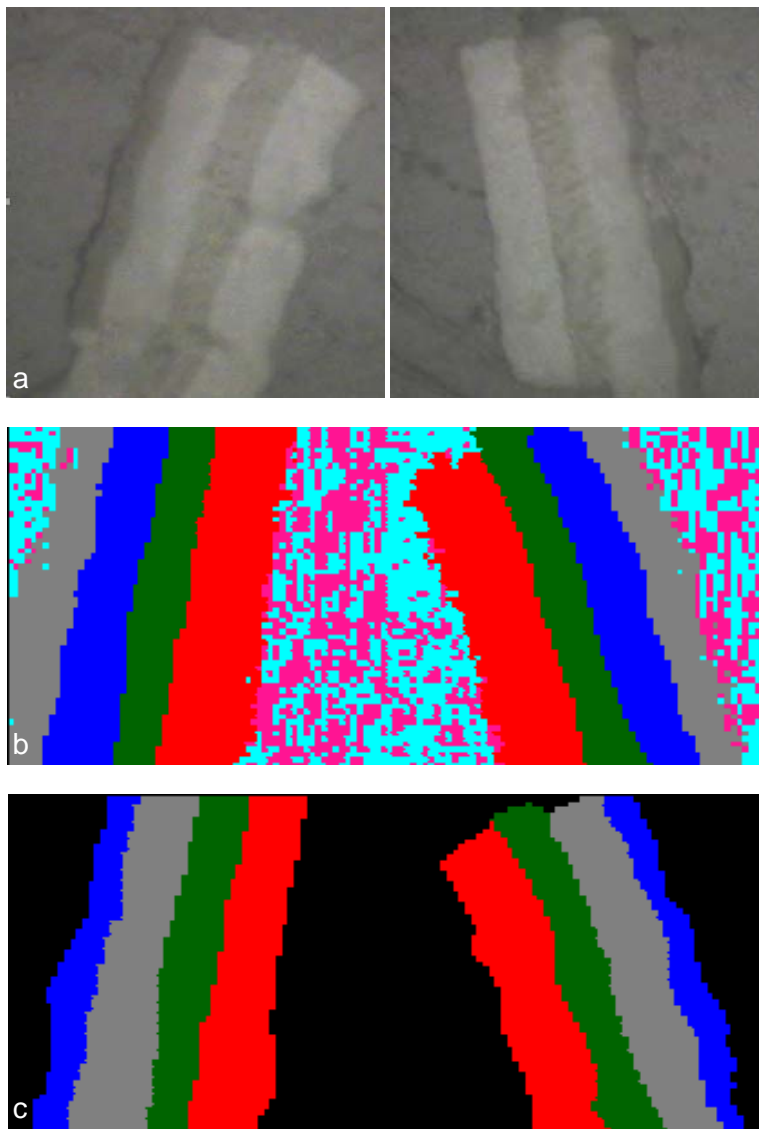


Figure 1: a) Visible light image of two paint chips mosaicked together (# 442) b) Cluster image resulting from hierarchical cluster analysis (HCA) of paint chips with six clusters c) Cluster image resulting from hierarchical cluster analysis (HCA) of paint chips (with KBr spectra removed) with four clusters.

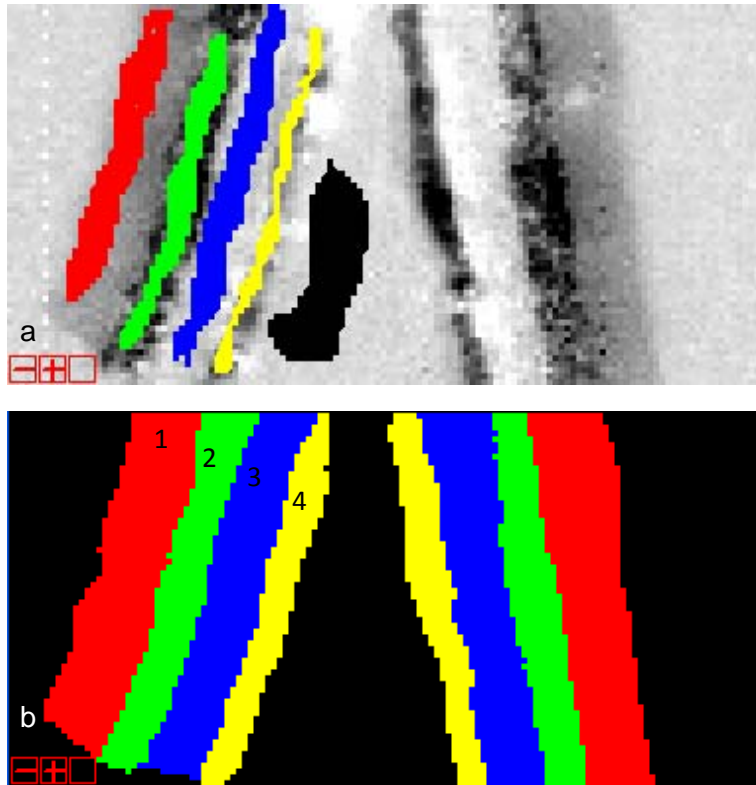


Figure 2: a) FTIR chemical image (using intensity at 987 cm^{-1}) of two paint chips showing selected 'regions of interest' b) results of Mahalanobis distance classification in ENVI

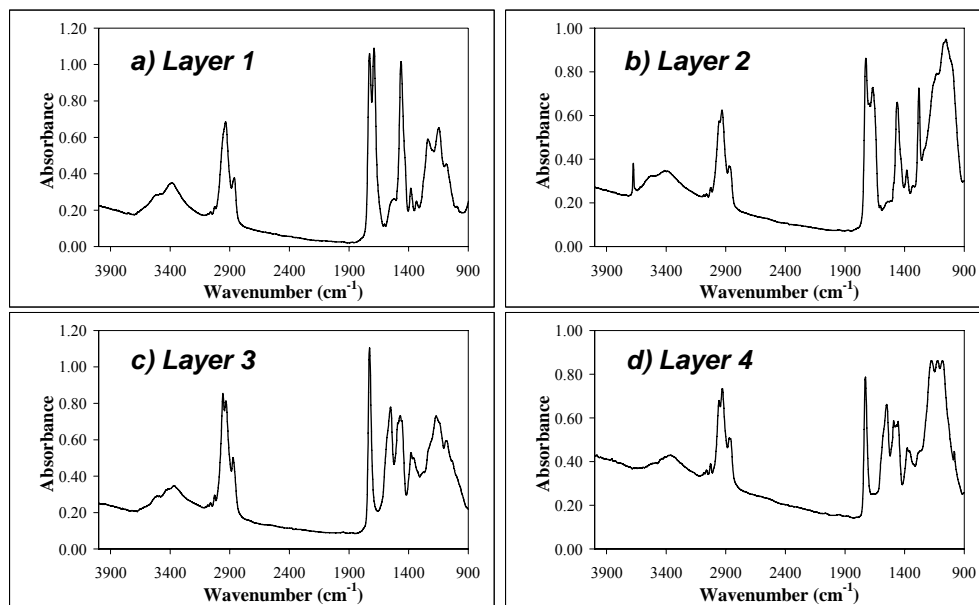


Figure 3: Infrared spectra of: a) Layer 1 – Identified as Acrylic-urethane b) Layer 2 – Identified as Acrylic-alkyd with talc pigment extender c) Layer 3 – Identified as Acrylic-melamine d) Layer 4 – Identified as Acrylic-melamine with barium sulfate extender.

Bicomponent fibers

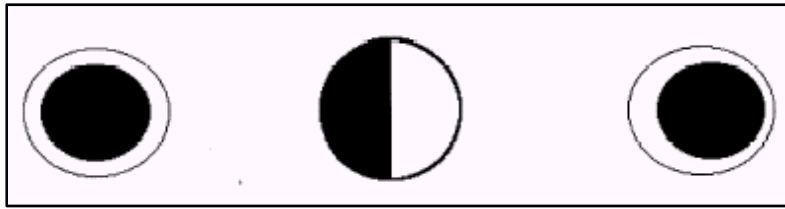


Figure 4: Types of bicomponent fibers from left to right – core sheath, side-by-side and eccentric core-sheath.

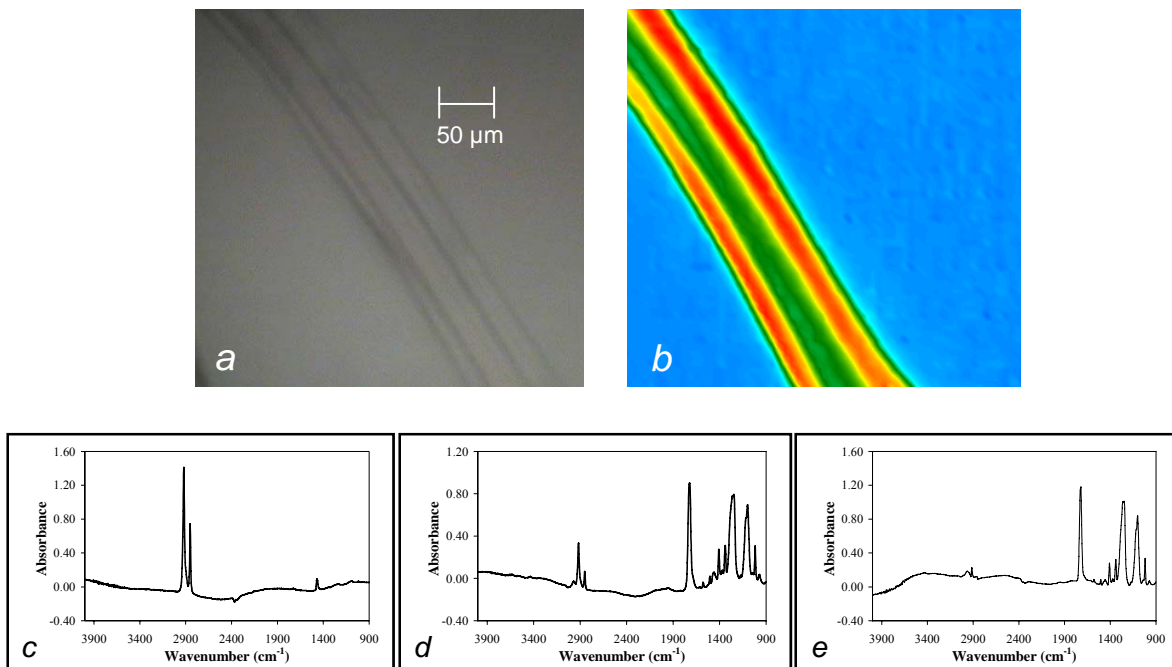


Figure 5: (a) Visible image of Trevira 256 fiber and (b) infrared spectral image of Trevira 256 formed by imaging at 2916 cm^{-1} . Infrared spectra of (c) sheath component, identified as PE (d) combined core and sheath components (PE and PET) and (e) core component, identified as PET, from Trevira 256.

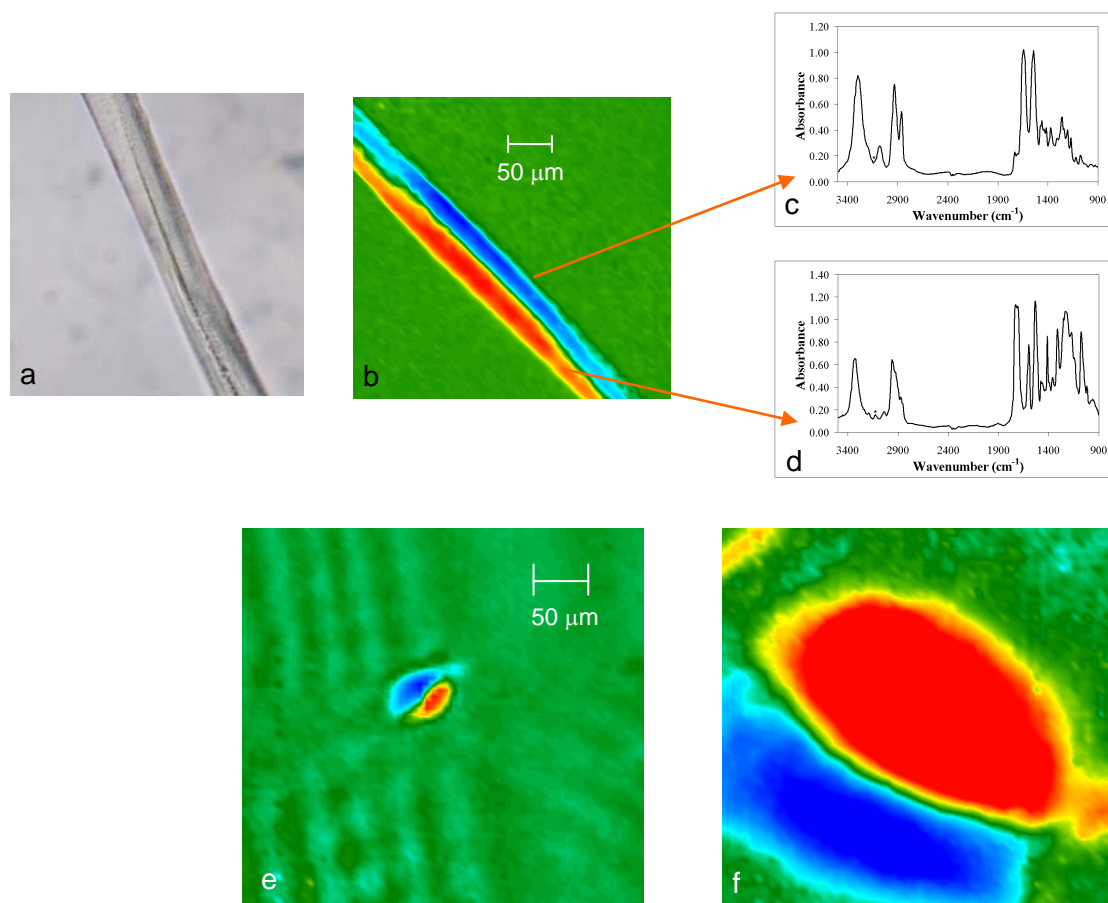


Figure 6: Monvelle (Monsanto) side-by-side bicomponent fiber: a) white light image b) transmission infrared spectral image formed using intensity at 3123 cm^{-1} c) FTIR spectrum of top component identified as nylon d) FTIR spectrum of bottom component identified as polyurethane e) transmission cross-sectional infrared spectral image (1648 cm^{-1}) f) cross sectional ATR infrared spectral image (1648 cm^{-1}) of a Monvelle (Monsanto) fiber cross-section.

Detection of illicit substances within fingerprints

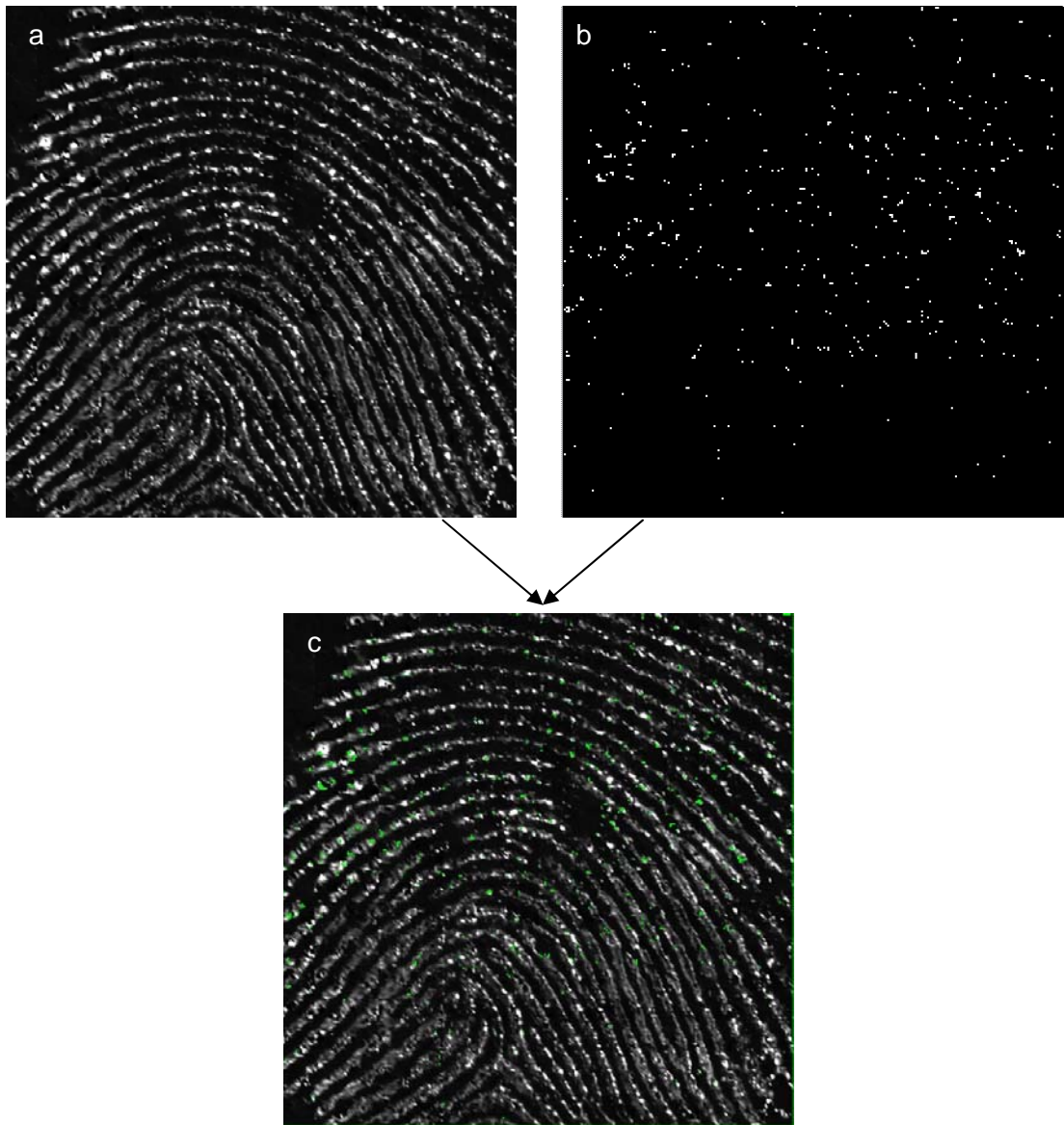


Figure 7: *Fingerprint contaminated with PETN explosive: a) Infrared spectral image showing fingerprint residue b) image showing location of pixels which have been library matched to a reference PETN spectrum c) overlay of Fig. 7 a and b showing location of PETN particles (colored green)*

CONCLUSION/DISCUSSION

Multilayered paint chips

The infrared spectral imaging of multilayered automotive paint chips provides an ideal illustration of the potential of this technique. Infrared spectra from every part of the chip are obtained simultaneously from all parts of the chip, allowing every layer to be identified from one experiment. Multivariate statistics can be used to compare all of the spectra in two paint chips and demonstrate visually the chemical differences or similarities between corresponding layers.

Fig 1 a shows a visible light image of two multilayered paint chips. Figs 1 b and c show the results of infrared spectral imaging and subsequent hierarchical cluster analysis (HCA) of the same paint chips together in a combined image. In HCA, the “distance” in multivariate space (a measure of similarity/dissimilarity) between each pair of spectra in an image is first calculated. Next, the two most similar spectra in the dataset are linked and placed into a group, or cluster, together. The distances between this group and each of the other spectra in the image are then calculated, and the linkage process is repeated, resulting in another spectrum being placed in a group (or cluster), but at a lower level of similarity. This overall procedure is repeated until all of the spectra are in one group (2). Fig. 1 b is an image of the first six clusters; this shows (as expected) one cluster (color) for each of the four layers in the two paint chips, while the background material (KBr) has split into two groups. Imaging of higher numbers of clusters divides the paint layers up (based on spectral noise and baselines, etc) into physically meaningless shapes. Fig 1 c shows the results for the same paint chips if the background material (KBr) spectra are removed prior to HCA. In both cases, the fully classified image allows multiple chemical comparisons to be made rapidly at one glance, in a way that is very easy to understand.

In Fig 2, the same two paint chips are classified together using the ENVI supervised Mahalanobis distance classification routine. Fig 2 a shows the chemical image of the chips at 987 cm^{-1} with pixels selected by the user in each of the five regions of interest in the left hand image only (the four layers apparent in visible light and infrared images, plus the surrounding KBr mounting material). Fig 2 b is the resulting image when the software is allowed to classify all of the pixels in the combined image of the two paint chips. The supervised classification procedure described here can be performed with a prescribed maximum spectral distance threshold that would specify mathematically the greatest variation allowed between two spectra that are classified as representing the same chemical substance. This would confine the input from the operator to the selection of the regions of interest in the sample.

In both methods, the matching pattern of colors for the four layers in the two chips indicates the high spectral and hence chemical similarity of the corresponding layers, and thus dramatically reduces the chances of error when conclusions are being drawn from a comparison. Of course,

individual spectra from the layers can be examined in order to identify their chemical compositions (Fig 3).

Bicomponent fibers

Bicomponent fibers are a special class of fibers that comprise two polymers of different chemical and/or physical properties within the same filament. Infrared spectroscopy is a useful technique for identifying the polymer types present in conventional synthetic fibers, but a drawback of previous studies using IR spectroscopy to analyze bicomponent fibers is that the spatial configuration of a bicomponent fiber, e.g. sheath-core or side-by-side (see Fig 4), needs to be known prior to analysis. This information is unlikely to be known in a forensic case scenario unless it is microscopically obvious. In this work, infrared chemical imaging is investigated as a more suitable and informative technique to analyze bicomponent fibers than conventional infrared spectroscopy and other optical microscopy techniques. It is almost unique in being able to simultaneously identify the bicomponent nature of a fiber and the chemical composition of the components.

An example of a core-sheath fiber is the Trevira 256 (Trevira) fiber, which is known to contain polyethylene (PE) and polyethylene terephthalate (PET) components. Fig 5 a shows the visible light image of the fiber sample flattened in the diamond anvil cell, in which it appears division lines between the core and sheath components can be seen, indicating that the sample is a sheath-core bicomponent fiber. The infrared spectral image, formed by imaging the integrated spectral intensity under the peak centered near 2916 cm^{-1} (C-H stretch in polyethylene), clearly shows this sample is bicomponent, and reveals its spatial configuration to be that of a sheath-core bicomponent fiber (Fig 5 b). The infrared spectral image shows the outer sheath component as red (i.e. high intensity of the infrared band 2916 cm^{-1}) and the middle of the fiber as green (i.e. medium intensity, as both the core and sheath components are contributing to the spectra obtained). The two components are clearly distinguished, and through spectral subtraction, the infrared spectra for both the core and sheath components can be easily obtained. By selecting a pixel from the edge of the fiber, an infrared spectrum of the polyethylene sheath is obtained (Fig 5 c). The infrared spectrum obtained from the centre of the fiber image is a combined spectrum of both the sheath (PE) and core (PET) components (Fig 5 d). Through spectral subtraction, a spectrum of the PET core component can be obtained (Fig 5 e).

An example of the side-by-side configuration is the Monvelle (Monsanto) fiber, which is identified in the Microtrace Fiber Reference Collection as containing polyamide and polyurethane components. Fig 6 a shows the visible light image of the sample, in which only a faint division line can be seen, possibly indicating that the sample is a bicomponent fiber. However, an infrared chemical image, formed by imaging the integrated spectral intensity under

the polyurethane band at 3123 cm^{-1} , clearly reveals not only that this sample is bicomponent, but also shows its spatial configuration to be that of a side-by-side bicomponent fiber (Fig 6 b). The two components are clearly distinguished, and the infrared spectra for each component can be easily obtained, simply by selecting a pixel from each side. The infrared spectra for each component are shown in Figs 6 c and d, where the peak used to form the chemical image is highlighted.

Cross-sectional analysis of the fibers in transmission mode was fairly tedious and time-consuming, but dyeing the mounting resin purple made it easier to locate the fiber cross-sections for imaging. Excellent results were obtained for Monvelle (Monsanto), with the two side-by-side components clearly visible in the infrared chemical image of the fiber cross section (Fig 6 e). The difficulty in sectioning fibers for transmission analysis led to an attempt to use micro-ATR imaging as an alternative for visualizing the component configuration in bicomponent fiber cross-sections. The advantages of this approach were that the mounted fiber could be examined in a resin block without sectioning, and much greater spatial resolution could be obtained. This is seen in Fig 6 f, which shows a micro-ATR image of the Monvelle fiber. In this case, the field of view is about $80 \times 80\ \mu\text{m}$, compared with the $350 \times 350\ \mu\text{m}$ field of view in Fig 6 e.

Cross sectional analysis of possible bicomponent fibers using infrared chemical imaging would be the ideal approach where possible and convenient, as it would remove the ambiguities inherent to side-on analysis, particularly for sheath-core fibers and would overcome the occasional problems caused by the flattening of side-by-side fibers [Ref 3]. Micro-ATR imaging would enable the visualization of smaller configurational features in the fiber cross sections.

Detection of illicit substances within fingerprints

As well as its ability to image fingerprints on difficult surfaces, there has been interest in using infrared spectral imaging to find and identify illicit substances present as contaminants in latent fingerprints (4; 5). Fig 7 shows one result from a study which has encompassed the detection of explosive, pharmaceutical and illicit drug contaminants in fingerprints. Fig 7 a is an infrared spectral image formed using the C-H stretching region and hence shows the location of sebaceous fingerprint residue. Fig 7 b shows the location of pixels identified (by searching the 65,536 image spectra as a library against a reference spectrum) as containing the explosive PETN. Fig 7 c is an overlay of the two images, with the PETN pixels colored green (web version). Further results from this study (on pharmaceuticals, drugs and other explosives) will be published elsewhere.

LITERATURE CITATIONS

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- 3) Flynn, K.; O'Leary, R.; Roux, C. and Reedy, B. J. (2006). *Forensic analysis of bicomponent fibers using infrared chemical imaging*. Journal of Forensic Sciences **51**(3): 586-96.
- 4) Bartick, E. G.; Williams, D. K.; Peters, H. L.; Schwartz, R. L.; Crane, N. J.; Bhargava, R.; Fernandez, D.; Huffman, S. W. and Levin, I. (2005). Spectrochemical analysis and spectral imaging of latent fingerprints and trace evidence included within the prints. 37th Middle Atlantic Regional Meeting of the American Chemical Society, New Brunswick, NJ, United States.
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