Chemical imaging of latent fingerprints, paint chips, and fibers using µ-FTIR: An experiment for forensic chemistry and instrumental analysis courses

Reba E. A. Chamblee[1], **Kendall M. Wontor[1], James V. Cizdziel***

Department of Chemistry and Biochemistry, University of Mississippi, University, MS 38677
corresponding author: cizdziel@olemiss.edu
[1]Co-first authors.

Abstract: Emerging technology combining spectroscopy with microscopy is advancing the analysis of trace evidence with the potential to revolutionize forensic microscopy and excite a new generation of forensic microscopists. In this laboratory experiment, developed for undergraduate forensic chemistry and instrumental analysis courses, students use Fourier transform infrared (micro)spectroscopy (µ-FTIR) to analyze mock forensic samples commonly encountered at crime scenes, including latent fingerprints (laced with ibuprofen to mimic an illicit drug), vehicle paint chips, and acrylic fibers. Unlike light microscopy, µ-FTIR provides information on the spatial distribution and chemical nature of the sample. The learning objectives were to reinforce key concepts covered in the classroom, including collection and preparation of trace evidence, forensic microscopy, and vibrational spectroscopy, as well as to provide students hands-on experience using a state-of-the-art instrument. Students prepared the fingerprint and fiber samples, whereas the paint chip was previously cross-sectioned to save time. The students collected and processed their own data, including generating chemical distribution maps. Student responses were positive and reports written by the students demonstrated an increased awareness of the capabilities of FTIR microscopy and chemical imaging. Overall, the exercise helped remove the "black box" mentality, where students analyze samples without understanding the fundamentals of the technique, which is important to recognize poor data quality and troubleshoot instruments. This report describes the laboratory exercise and student experience, includes data and chemical images collected by students and aspects of the experiment that could be modified to improve learning outcomes as well as adaptations for use with an ATR-FTIR.

Keywords: FTIR microscopy, chemical imaging, trace evidence, fingerprints, paint chips, fibers

Introduction

Forensic microscopy utilizes microscopy techniques to characterize evidence for use in civil or criminal law. The Locard exchange principle, the basis for much of forensic trace evidence, states that when there is contact between two items a cross-transfer of physical evidence will occur. Microscopy can provide insight into the identity, origin, and history of a trace material, and the route(s) it may have taken between a victim, suspect, and crime scene (1). Today, microscopic trace evidence is mainly used to establish associations that can be presented at trial. However, it is often overlooked as a tool to aid forensic investigations which was not always the case. Early forensic scientists used microscopic trace evidence to aid detectives by providing facts and developing well-reasoned inferences to assist in the search for locations, persons, and vehicles (1). Yet, forensic microscopy is on the decline in many forensic labs due in part to the rise of DNA technology and DNA indexes (e.g., CODIS) which have revolutionized forensic science, garnering much attention and resources in forensic laboratories. The shift away from forensic microscopy has also crept into academia, with forensic microscopy classes being phased out or minimized in many forensic science programs. The decline in forensic microscopy expertise will be exacerbated as senior microscopists retire.

Recently, emerging technology combining spectroscopy with microscopy is advancing the analysis of trace evidence with the potential to revolutionize forensic microscopy and excite a new generation of forensic microscopists (2). Vibrational spectroscopy (IR absorption and Raman scattering) is ideal for trace analyses because it is non-destructive and highly selective, yielding vibrational fingerprints characteristic of the sample (3). Spectral databases have been established that contain FTIR spectra for tens of thousands of compounds. Moreover, recent advances in detectors, sample positioning stages, and

image process software have led to the rise in chemical imaging spectroscopy, where spectroscopic properties of the sample are represented in a false color "chemical image". Such imaging excels at mapping the spatial distribution of chemicals in a sample and can be used to characterize multilayered films (e.g., paints) and microscopic particles. For imaging in the IR region, a focal plane array (FPA) detector is utilized, which is essentially a digital camera where each pixel expresses an entire FTIR spectrum (4). With thousands of spectra collected simultaneously, the image contains a huge amount of chemical information. This wealth of information is invaluable in forensic microscopy. Although the collection and interpretation of chemical image data may seem daunting, it is not. Instruments like the LUMOS II from Bruker Corp., used in this study, have built-in automation and a user-friendly interface so that users of different skill levels can use the advanced technique with minimum effort and time. Further, image processing software is fully integrated and minimizes subjectivity with hit quality indices to compare known and unknown spectra. As a result of this new technology, exciting new chemical imaging applications (often developed in academia) are becoming practical in forensic labs equipped with instruments capable of microspectroscopy. These include the detection of illicit substances in fingermarks, and discrimination between counterfeit banknotes, electrical tapes, and human body fluids (5–8). It should be noted that Optical – Photothermal Infrared (O-PTIR) spectroscopy is another analytical technique recently commercialized that has lower (sub-micron) resolution than both traditional IR and Raman spectroscopy.

Here, we describe a laboratory exercise introducing FTIR microspectroscopy (μ-FTIR) to undergraduate forensic chemistry majors in an instrumental analysis class. The goal was to reinforce key concepts in spectroscopy and forensic microscopy covered in class by providing students a hands-on opportunity to use μ-FTIR chemical imaging to analyze fingerprints, automobile paint chips, and fibers, all important categories of forensic trace evidence.

Methods

Course background and laboratory logistics

This laboratory experiment was developed for an undergraduate course at the University of Mississippi titled Advanced Instrumental Analysis (CHEM 512). The class had 21 students with 16 being senior B.S. forensic chemistry majors. Briefly, the course was designed to instruct students on modern chemical analysis using instrumental techniques not typically covered in lower-level courses, and to provide students opportunities to conduct analyses using select instruments. The goal is to provide the students with a thorough understanding of not only the principles of analytical instruments, but also of

their capabilities, limitations, applications, and some practical aspects of sample analyses. Removing the "black box" mentality that students may have about instrumentation is critical for them to recognize instrumental problems, identify data quality, and conduct basic instrument maintenance and troubleshooting. The course has both lecture and laboratory components. Students meet three hours a week for the lecture and discussions, most of which focus on theoretical underpinnings of the instruments, but also includes preparation for experiments and demonstrations.

In preparation for this experiment, students were required to watch 3 short YouTube videos on the features and general operation of the LUMOS II instrument and to provide a brief summary of each as well as to read a mini-review on chemical imaging of latent fingermarks, focusing on the introduction and spectroscopy sections (9). They were also provided links for refreshing their memory on the basics of FTIR analyses, though use of this material was optional. The instructor also discussed the laboratory in class, highlighting the instrumental technique, discussing the samples and logistics of the experiment, and covering expectations and learning objectives.

In the laboratory, groups of 3-4 students met at the designated time with a teaching assistant (TA), a graduate student who was trained on the instrument and who was herself a graduate of the forensic chemistry program. Lab schedules were flexible, but each component was to be finished within a specific time frame. Under the guidance of a TA each student group spent the first lab session (2-3 h) becoming familiar with the instrument and learning the key features of the software (OPUS, Version 8.5.29), including generating chemical maps, data processing (peak picking, baseline correction, and spectral subtraction), and database searching. During this session, students also examined the effect of changing spectral resolution (2 cm^{-1}, 4 cm^{-1} and 16 cm^{-1}) and scan number (4 and 16) by comparing and contrasting chemical images, S/N ratios of spectra, and analysis time. While this lab session may not be necessary to conduct the main laboratory experiment, we found that it gives the students a familiarity with the instrument that allows them to focus on the analyses of their samples in the next lab session. The second lab session (3-4 h) was used to analyze the forensic trace evidence as described below, and a third (optional) lab session was also available, if needed, to retrieve data, ask questions, and revisit and clarify aspects of the experiment. Students were allowed to operate the instrument under TA supervision during both the demonstration and the actual analysis of their forensic samples.

Finally, students were required to submit a single-author written report in journal format (Abstract, Introduction, Methods, Results, Discussion, Conclusions, Literature Cited) with subsections on fingerprints, paint, and fibers. They are provided a grading rubric that includes points for pre-lab assignments. Overall grammar, style, and

appearance are evaluated as written communication is a key element in the work place.

Preparation of Fingerprint Samples

A volunteer from each student group used their fingerprint for analyses. Before fingerprint application, the student washed their hands with soap and water, rinsed them clean, and allowed them to air dry. The index finger was then pressed gently to the forehead or rubbed behind the ears, to insure sufficient sebaceous secretions were added to the fingerprint region. The finger was then gently pressed for a few seconds onto the left portion of a reflective side of a low-e (MirrIR) glass slide (Kevley Technologies) (**FIGURE 1**). Low-e glass slides are composed of two layers of silver sputtered onto float glass, providing ~95% infrared light reflection while simultaneously allowing visible light transmission (10). The previous steps were then repeated, but following the addition of the sebaceous secretions, the finger was pressed lightly into the powder of a crushed over-the-counter ibuprofen drug tablet. The exact identity of the drug was not given to the students. The excess ibuprofen powder was brushed away, and the finger was then pressed onto the right portion of the low-e glass slide for a few seconds.

Preparation of Car Paint Samples

The paint chips that we used for this laboratory were provided by the Royal Canadian Mounted Police (RCMP) for a separate research project involving their Paint Data Query (PDQ) program. However, almost all modern automotive paint chips will contain the paint layer system that is necessary for this experiment. Instructors interested in including this laboratory in their own courses may be able to source paint chips from local automotive shops. Shops that offer sunroof installation may be an especially good place to start, as the panel that is cut out to form the sunroof is generally regarded as waste and will also provide an abundance of material to generate paint chips from. Instructors that can only source a single paint chip can focus their lesson on the difference in chemical composition as seen below. For those that are able to obtain multiple paint chips, however, the experiment can be broadened to include the compositional differences between the same paint layer from two different manufacturers. Doing so would help students understand the scientific underpinnings of the PDQ database as well as driving home key points about the strengths and limits of class evidence.

Paint chips were prepared by the TA in advance of the laboratory because it would be too time consuming to have the students do so themselves. However, the procedure was described to the students. Samples were prepared by placing the chip tightly within a micro-vice holder and using a scalpel to slice off the paint layer system as thinly

as possible. This allows for the removal of the majority of the substrate while still ensuring all layers of the chip will be analyzed. The thin paint layer slice was then placed into an embedding mold which was promptly filled with the Tissue-Tek O.C.T. (Optimum Cutting Temperature) compound and placed on dry ice for 15-20 minutes until frozen. Following removal from the mold, the sample was placed in a Cryostat and sliced into 5μm thick cross sections. At this stage, the cross-section is still embedded in Tissue-Tek O.C.T., so the sample was then placed in a small dish under the stereomicroscope and washed with water to remove any of the Tissue-Tek O.C.T. Lastly, the sample was dried in an oven overnight at 40ºC to remove any remaining moisture. Following removal from the oven, samples were stored in a desiccator until analysis.

To prepare the paint chips for analysis with the FTIR microscope, the dried sample was placed on the diamond compression cell and viewed under the stereomicroscope (**FIGURE 1**). Once all layers were in focus, the top of the compression cell apparatus was placed over the sample cell and screwed into place. The screws and top of the cell were then removed, and the sample was inspected under the stereomicroscope to confirm that the sample was appropriately flattened. At this point, students were encouraged to observe the sample underneath the stereomicroscope and attempt to identify layers in the paint system visually. The bottom half of the compression cell and the flattened paint chip were then carefully placed on the FTIR microscope sample stage for analysis.

Preparation of Fiber Samples

This section of the experiment was adapted from practical exercises found in the Scientific Working Group for Materials Analysis (SWGMAT) Forensic Fiber Examiner Training Program (11). Using the field standard guidelines provided by SWGMAT ensured that the lab would be similar to the training students would receive in the trace evidence section of a forensic laboratory. A single fiber pulled from a piece of acrylic (polyacrylonitrile) yarn (Red Heart© Super Saver) was secured tightly across the frosted end of a glass microscope slide using small pieces of adhesive tape. While viewing it with the stereomicroscope, gentle pressure was applied using a glass stir rod as a sample roller to flatten the fiber. Students were instructed to keep the rod level while rolling in order to flatten the fiber to a uniform thickness. A portion of the flattened fiber was then cut with a scalpel and mounted across the cell window of a diamond compression cell, with care taken to avoid twisting the fiber (**FIGURE 1**). The apparatus was inspected under the stereomicroscope to confirm that the sample was flat and placed correctly on the compression cell window. It should be noted that the compression cell was not used to flatten the sample further, but merely to provide an IR transparent mounting surface. As such, the top of the compression cell was not used. The

fiber mounted on the cell apparatus was then carefully moved to the FTIR microscope sample stage.
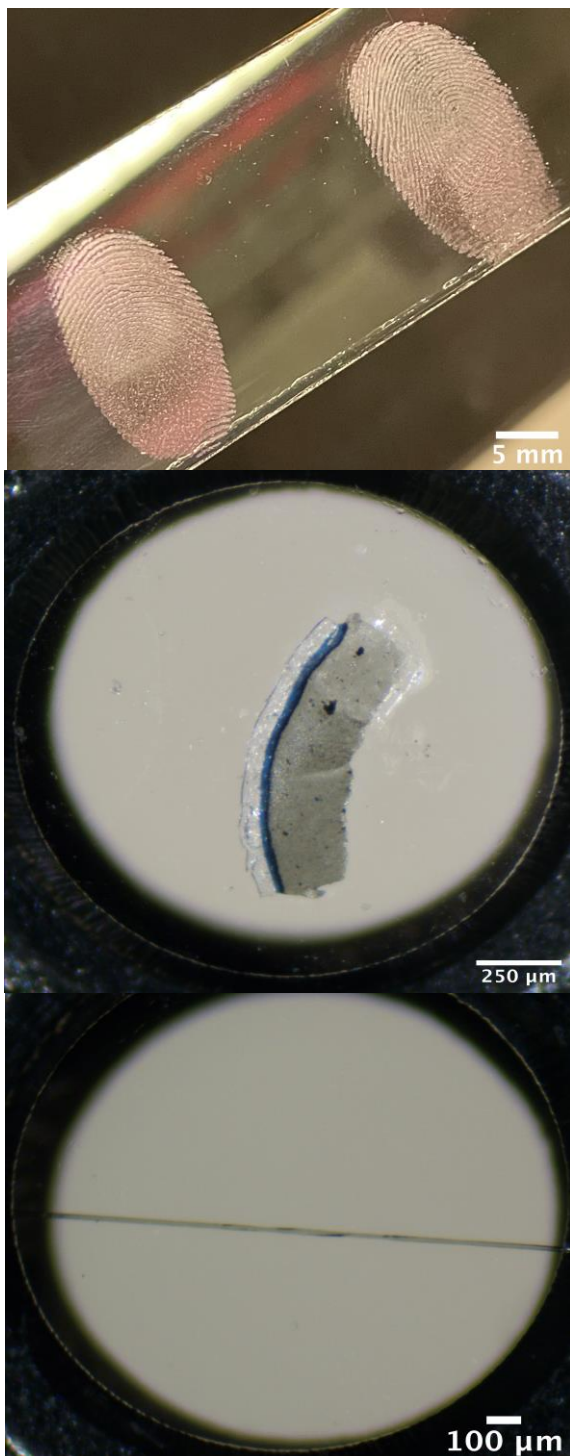

FIGURE 1 Photos of samples analyzed. Fingerprints on low-e glass slide (top); Paint chip cross-section (center) showing its layers including the bluish base coat sandwiched between the whitish top coat and greyish plastic substrate (note that the primer is not visible in this

image); and fiber (bottom). For scale, the diameter of the compression cell (white area surrounding the paint chip and fiber) is 1.6 mm.

FTIR Microscopy Analysis

All samples were analyzed using the Bruker LUMOS-II FTIR microscope with the liquid nitrogen cooled 32x32 FPA detector. Visible images were collected for each of the samples before FTIR analysis. The backgrounds were acquired over the 4000-700 cm^{-1} range on a blank (clean) portion of the sample space. Background scan parameters were set at 16 co-added scans, 100 co-added scans, and 64 co-added scans at 4 cm^{-1} spectral resolution for the print, paint chip, and fiber samples, respectively. The sampling regions for IR imaging were selected via the visible image and are indicated by a superimposed red square grid. For the IR images, fingerprint samples were analyzed via reflection-absorption mode while both the paint chips and fibers were analyzed using transmission mode.

All data analysis was completed using the Bruker OPUS Software v8.5. The main purpose of the experiments was to create chemical images for each of the samples. This was completed via two paths: integration of specific peaks within the spectra (**TABLE 1**) and using the Factorize 3D function, which creates a chemical image based on spectral differences calculated by the program. Following the creation of a chemical image, spectra were extracted for baseline correction and peak labeling. Lastly, these spectra were searched against the databases currently set up within the program to potentially confirm the identity of the unknowns. Searches were completed via the standard search method, which looked for one main compound. Data analyses were completed by the students with help from the TA (**FIGURE 2**).
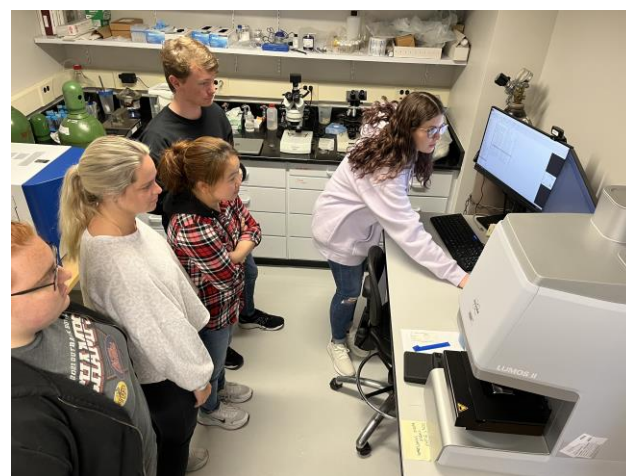

FIGURE 2 Graduate student teaching assistant demonstrating aspects of the Bruker Lumos II μ-FTIR (bottom right) to forensic chemistry students. The under-graduate students then took turns "driving" the instrument to

collect data and chemical images. Note that the FPA detector is cooled with liquid nitrogen, which is supplied through a port on the top of the instrument. See text for details.

TABLE 1 *FTIR peaks selected for chemical mapping of compounds or classes of compounds. See Results for discussion on peak selection.*

Compound	Band Assignment	Wavenumber (cm^{-1})
Carbohydrates	C-O stretches	1120-990
Protein	Amide I and II	1700-1480
Lipids	Ester C=O stretch	1770-1710
Collagen	Side chain vibrations	1305-1160
Sebum	Methyl C-H stretch	~2854
Ibuprofen	C-O stretch	~1123
Polyacrylonitrile	Nitrile	~2246

Hazards and Safety Precautions

While there are few hazards associated with this lab, it is important to note that FTIR microscopes require liquid nitrogen to cool their FPA detector. Liquid nitrogen poses both an extreme cold hazard and an asphyxiation risk to users due to its ability to displace oxygen. All users should therefore undergo safety training on the use of liquid nitrogen before handling it. The LUMOS II's FPA detector needs to be cooled once every ~6 hours, so the instructor or TA may choose to do this themselves before the students arrive. Other models of FTIR microscopes or those from other manufacturers may have different cooling needs or timeframes, so instructors using such instruments may need to adjust the cooling protocols accordingly.

Additionally, if the instructor chooses to have the students prepare the car paint sample(s) during class, they should be aware that doing so will require the use of dry ice and a cryostat or microtome. Instructors should review the proper safety procedures for these instruments with their students before use to reduce the risk of injury. It may be preferable to have the instructor or TA be the one to cut the samples, either before or during the lab session to avoid the risk to students. Finally, students should also be reminded to keep their hands clear of the sampling stage on the FTIR microscope to avoid injuring their fingers.

Adaptations for use with an ATR-FTIR spectrometer

While this paper is focused on the use of an FPA-FTIR microscope, which is suited for chemical imaging and microscopic analyses of fibers and paint chips, not every institution will have access to this instrument due to its high cost. The following modifications are provided for instructors seeking to run this experiment (minus the chemical imaging) using an FTIR spectrometer with an ATR attachment. Such instruments are more widely used and should be available to most instructors in an accredited forensic science program at the university level. When testing these adaptations, all spectra were collected using the macro ATR unit of the LUMOS II with 64 co-added sample and background scans, a wavenumber range of 4000-600 cm^{-1}, and a resolution of 4 cm^{-1}. All spectra were baseline corrected with a rubber band correction using 100 points and smoothed with the Savitzky-Golay method using 13 points.

Although it is not possible to chemically image fingerprints using an ATR, it is still possible to identify drugs found within them using this method. To do so, samples should be prepared as previously described. However, they do not need to be places on a low e$^-$ glass slide when using the ATR method. Students should analyze a fingerprint without the drug before preparing the fingerprint with drug residue to serve as a standard for fingerprint oil. Before analyzing the drug laced print, students will need to visually identify where the drug particles are present in the print. A stereomicroscope will make this significantly easier. Students can then press the area of the print containing the drug particles onto the ATR crystal. Once a spectrum of the drug laced print has been collected, the fingerprint oil should be subtracted from it. This results in a spectrum of the drug that can be searched against a library database. Note, the fingerprint on the slide needs to make direct contact with the ATR crystal, so instruments with crystals depressed in plates (sample wells) won't work unless the plate is removed or the configuration is changed. Also, some ATR crystals are relatively soft (e.g., ZnSe) and can crack if too much pressure is applied.

For the automotive paint chips, each sample will need to be cut into individual layers instead of in cross-section before they can be analyzed. These can be sliced with a cryostat or microtome but can also be separated manually with a stereomicroscope, a scalpel, tweezers, and/or a micro-vise. If separating manually, the clearcoat layer can be analyzed before cutting for simplicity. It can then be scraped off to reveal the basecoat underneath, which can be analyzed and then scraped off to reveal the primer. Additionally, instructors may wish to use paint systems on metal substrates if they are having students manually separate the samples, as their thicker layers make the sample preparation easier. Once the layers have been separated, they can be placed directly on the ATR crystal for individual analysis. Alternatively, if students are able to cleanly separate the paint layers, instructors may wish to have students analyze their samples according to the PDQ's standard operating procedure, wherein the manually separated layers are analyzed in transmission mode using a diamond compression cell and 100 co-added scans.

Fibers can be stretch across the ATR crystal and analyzed directly with no sample preparation. For best

results, instructors should choose fibers with a single component. Fibers that are made of multiple materials twisted together may not yield optimum results. Alternatively, instructors may wish to intentionally include such samples and use them to illustrate the limits of the ATR method compared to the chemical imaging method.

Results

All student groups were able to create a chemical image of their respective samples and accomplish the following: identified ibuprofen and illustrated its distribution within the latent fingerprint, distinguished three different layers within the paint chip, and identified the acrylic fiber.

Chemical Imaging of Latent Fingerprints

For mapping sebum deposits, students used the optical image to select a point on the slide where the sebum was present and displayed the resulting spectrum. Based on this spectrum, they selected a peak to integrate to create the chemical distribution map. For the representative image shown in this article, students used the 2854 cm^{-1} peak, a methyl C-H stretch prominent in sebaceous oils. The chemical image for the ibuprofen was created similarly. Students used the optical image of the print to find and select unknown particles. After displaying the particle's IR spectra, they then created a distribution map by integrating the peak found at 1123 cm^{-1}. This C-O stretching peak was specifically chosen because it is found at a different location in the ibuprofen spectra than in the sebaceous oil spectra. Representative chemical images illustrate the distribution of the signal for these selected peaks within the area analyzed (**FIGURE 3**).

While this lab focused on the use of single peak integration to create chemical images, it should be noted that the OPUS software does contain a chemical mapping function that creates images based on multiple peaks. If using a single peak, as done in this experiment, it is crucial that the peak chosen for the drug does not overlap with a peak in the sebum spectrum. If overlap does occur, the resulting map will show the distribution of both the drug and fingermark residues. This is because the chemical image is actually depicting the distribution of the selected peak rather than the compound associated with that peak. Taking **FIGURE 3** for example, just because a pixel is highlighted in red does not mean that ibuprofen is present at that location. In order to conclusively identify that particle, the analyst must extract the spectrum from that location and compare it to a reference spectrum. Using the single peak integration method made this point easy to demonstrate to students by first creating a distribution map using an overlapping peak. When students then inspected the spectra from the highlighted areas, they could clearly see the drastic differences that indicated that two distinct compounds were present.

When searching the spectrum for the unknown powder against the databases within the OPUS program, the best match was ibuprofen, with a hit quality score of 309 (with a perfect match being a score of 1000). This low score is likely a result of both the low number of co-added scans used in the experiment as well as the use of a commercial ibuprofen tablet. These tablets contain inactive ingredients used as binders, such as cornstarch, that can introduce changes into the tablet's FTIR spectrum compared to the reference spectrum of the pure compound. Using pure ibuprofen might have resulted in a higher match score, but having the students use the commercial tablets better approximated the challenges involved with real-world samples. While the students did successfully identify the unknown drug as ibuprofen, the low quality hit score allowed them to think critically about their confidence in their results and theorize about what they could have done to improve them.

A variety of methods can be used to improve hit quality score values. Instructors may choose to have students increase the number of co-added scans and binning area, which will increase spectral quality at the expense of time. Additionally, selecting larger particles to analyze will also often lead to better quality spectra. Post-analysis corrections to the spectra, including baseline corrections and smoothing, can also help increase the hit quality score during the search process. Instructors can also have students create a library standard from the drug tablet they are using to account for the effect of inactive ingredients and additives. For example, when adding our own standard ibuprofen tablet to the library and then completing a search on the same extracted spectra, the hit quality score improved to 509 from 309.
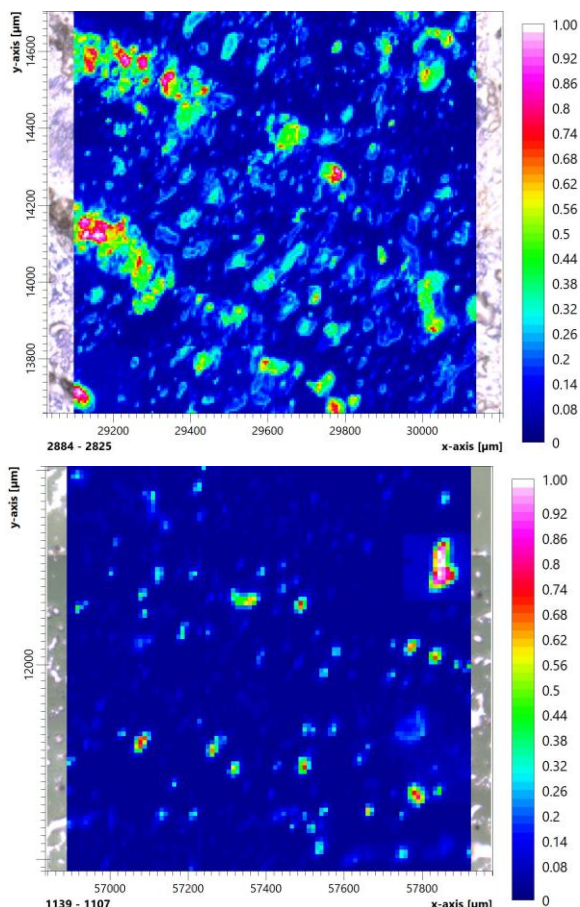
FIGURE 3 Chemical images of a fingerprint depicting the distribution of the 2854 cm-1 peak, which is associated with natural fingerprint secretions with two ridge lines curving downward (top) and the sporadic distribution of the 1123 cm -1 peak, which here corresponds to ibuprofen (bottom).

Chemical Imaging of Paint Chips

For the paint chip, a chemical image was created using the Factorize 3D function to characterize the differences between each layer (clear coat, base coat/pigment, primer, and substrate) (**FIGURE 4**). The Factorize 3D function condenses the spectral data from each pixel and groups them by common factors. It then uses the eigenvalues generated to produce series of chemical images that apply a color scale to identify similar spectra. While there were distinct spectral differences between two of the layers (the clear coat and the substrate), the pigment layer and primer were harder to discriminate, especially because the primer layer is extremely thin (~5 µm). The third spectra selected, which optically fell within the base and primer layers, seems to be closely associated with that of the clear coat, with only one peak being similar to the substrate layer. A spectrum from each of the layers was also searched against the database. The substrate spectrum brought up a match to polypropylene

with a hit quality of 795, a high match, while the clear coat gave Toso CSM 530 (chlorosulfonated polyethylene) as a result with a hit quality of 519. For the basecoat, the search resulted in a hit quality of 592 to Proviplast PLS Green 8, or 2-thylhexyl epoxy soyate. All components from the search are compounds that would be associated with use in vehicle components. However, it is important to note that the current databases loaded into OPUS are not all-encompassing. Therefore, it is possible that the search results merely represent the closest match in the database as opposed to a definitive identification. This issue was introduced to students, who were then allowed to theorize about possible solutions. As part of this discussion, the TA brought up the use of a more thorough search involving many databases (e.g., KnowItAll Spectral Library from Wiley) to improve the accuracy of the search results.
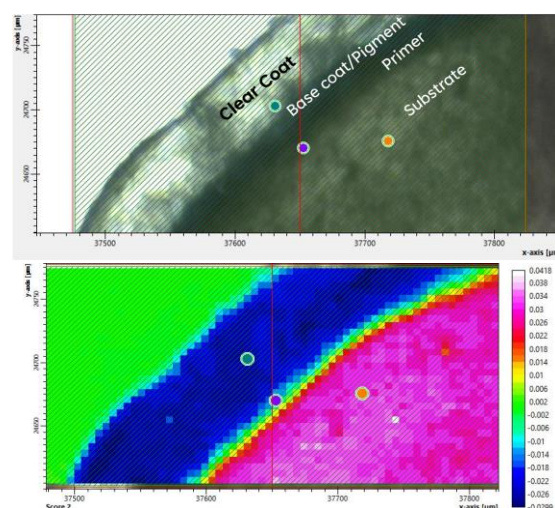

FIGURE 4 Visible (optical) image of the paint chip with each of the layers labeled (top). Chemical image obtained via Factorize 3D function illustrating the different layers of a paint chip (bottom).

Chemical Imaging of Fibers

For the fiber, only one type of material was present as all points selected throughout the analysis area gave the same spectrum. The nitrile peak from this spectrum (2246 cm-1) was selected to create a chemical image (**FIGURE 5**). The fiber spectrum was searched through the libraries and produced a hit to polyacrylonitrile, with a score of 363. The dyes present in the fiber likely accounts for the relatively low hit quality score. Polyacrylonitrile fibers are generally known as acrylic fibers which are used to make "artificial wool" type clothing. Though unknown to the students, like the powder for the fingerprints, the fiber sample was indeed a polyacrylonitrile fiber, indicating another successful match from the analyses. As with the latent prints, this experiment illustrated the differences between reference and evidentiary samples for the students. Doing so also provides them with an opportunity to think critically about how they would

present such findings in court and whether they feel additional testing is necessary to be confident in their results.
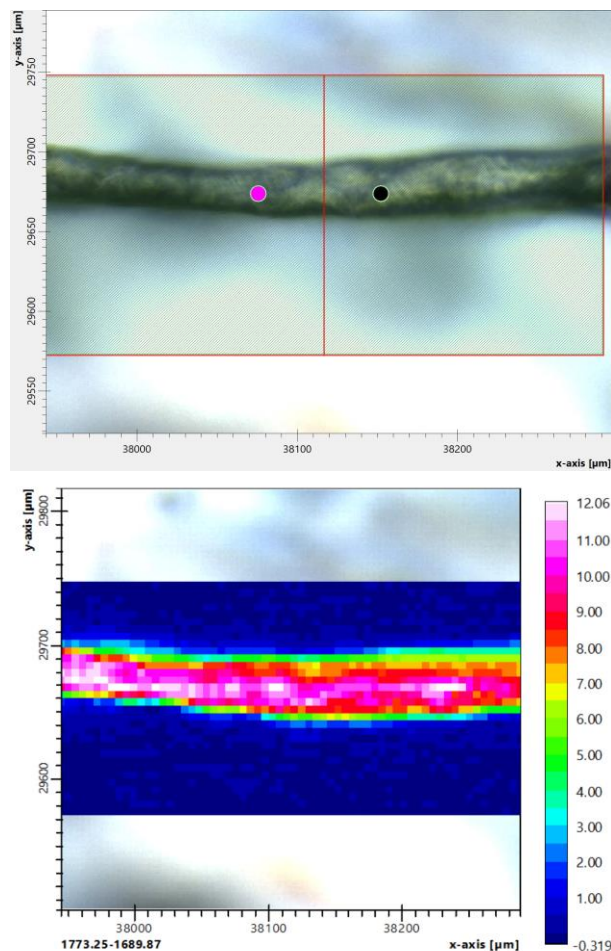

FIGURE 5 Visible (optical) image of the fiber with two points selected for spot analyses (top). Chemical image of the fiber showing the distribution of the 1731 cm^{-1} peak, representing a C=O stretch, throughout the fiber (bottom).

Discussion and Conclusion

This laboratory experiment, designed for an undergraduate class in forensic chemistry or instrumental analysis, offers an introduction to the basics of FTIR microscopy and illustrates how the technique can be applied to forensic samples that are important to criminal cases. The students become acquainted not only with the preparation of these types of samples for microscopy, but also with microspectroscopy and chemical imaging, an increasingly important area across scientific fields, including forensics and environmental and life sciences. Introducing forensic chemistry students to spectrochemical analyses of fingerprints, paint chips, and fibers aids in their understanding of concepts in microscopy, spectroscopy, and forensic science.

With forensic microscopy on the decline in many forensic labs due to increased DNA technology, the loss of forensic expertise in this area is of great concern. This hands-on laboratory proactively seeks to re-introduce and revitalize forensic microscopy education using chemical imaging by FTIR microscopy. Through these experiments, forensic students are able to use current research methods to prepare and analyze real examples of forensic evidence which provides them with insight into the forensic practices. Analyzing fingerprints, paint chips, and fibers not only emphasizes the importance of trace evidence in investigations, but also provides a basis for discussions on analyzing other types of evidence (drugs, gunshot residue, etc.) with spectroscopy. The key principles of FTIR spectroscopy and microscopy were also reinforced as the students themselves operated the instrument and performed their own analyses. While the analyses done here are focused on the use of the Bruker LUMOS II FTIR microscope, it is possible to accomplish chemical imaging via other similar instrumentation, meaning the lab can be tailored to the available instrument. While the initial cost of an FTIR microscope is high, operating expenses for this experiment are low as the supplies for the fingerprint and fiber analyses are relatively inexpensive. The low-e glass slides are more expensive than traditional slides, but they can be washed and reused. Further costs and time can be reduced by removing the paint chip portion of the lab. This would also make the lab feasible for a wide variety of curriculums. In all, this lab culminates in an increased awareness of trace evidence analyses and the usefulness of chemical imaging via FTIR spectroscopy.

References

1. Stoney DA, Stoney PL. Critical review of forensic trace evidence analysis and the need for a new approach. Forensic Sci Int 2015;251:159–70.

2. Kammrath BW. Combining spectroscopy with microscopy for advancing the analysis of forensically relevant traces. Spectroscopy 2020;35(7):33–6.

3. Muro CK, Doty KC, Bueno J, Halámková L, Lednev IK. Vibrational spectroscopy: Recent developments to revolutionize forensic science. Anal Chem 2015;87(1):306–27.

4. Dorling KM, Baker MJ. Rapid FTIR chemical imaging: highlighting FPA detectors. Trends Biotechnol 2013;31(8):437–8.

5. Ng PHR, Walker S, Tahtouh M, Reedy B. Detection of illicit substances in fingerprints by infrared spectral imaging. Anal Bioanal Chem 2009;394(8):2039–48.

6. Ajid NFD, Keat How F, Mahat NA, Desa WNSM, Kamaluddin MR, Mohamed Huri MA, et al. Counterfeit one hundred Malaysian ringgit banknotes discrimination using chemical imaging inspection and pattern recognition. Aust J Forensic Sci 2022;54(5):695–709.

7. Nimi C, Chophi R, Singh R. Discrimination of electrical tapes using ATR-FTIR spectroscopy and chemometrics. J Forensic Sci 2022;67(3):911–26.

8. Takamura A, Watanabe K, Akutsu T, Ozawa T. Soft and Robust Identification of Body Fluid Using Fourier Transform Infrared Spectroscopy and Chemometric Strategies for Forensic Analysis. Sci Rep 2018;8(1):8459.

9. Wei Q, Zhang M, Ogorevc B, Zhang X. Recent advances in the chemical imaging of human fingermarks (a review). Analyst 2016;141(22):6172–89.

10. FAQ | Kevley Technologies [Internet] [cited 2022 Nov 8]. Available from: http://kevley.com/FAQ.aspx

11. A Forensic Fiber Examiner Training Program. Scientific Working Group for Materials Analysis Fiber Subgroup, 2004.