

# A virtual or in-person infrared spectroscopy laboratory activity for the analysis of fibers and understanding of spectral library searches

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**Abstract:** The COVID-19 pandemic caused a rapid pivot of instruction in higher education from traditionally in-person modes to virtual modes with a need to maintain student learning outcomes, especially in laboratory courses. The next pandemic and other natural disasters cannot be predicted, and there are other reasons such as lack of resources that may inhibit courses from being implemented in-person. This paper provides a laboratory activity that can be completed in-person or with virtual instruction. This laboratory activity provides a novel approach to teaching students about fiber analysis using ATR-FTIR and elucidates the process of spectral analysis with databases. Students use Excel to simulate spectral library searches on available data and compare those results to the spectral images of standard samples to identify the composition of an unknown fiber. This allows students to understand how spectral library software comparisons are generated as well as the importance of visual examination to identify fiber makeup. The laboratory activity was also used with student generated data combining a traditional hands-on laboratory experience with further understanding of spectral libraries.

**Keywords:** Laboratory activity, fiber analysis, FTIR, virtual instruction

## Introduction

The COVID-19 pandemic presented a world-wide emergency for educational institutions to rapidly adjust to virtual modes of instruction while maintaining student learning outcomes. Laboratory courses were greatly affected and there will continue to be a need for dual modality laboratory exercises not only in preparation of another global pandemic but other scenarios such as natural disasters (i.e. hurricanes) or lack of equipment. This transition to remote instruction impacted many types of scientific laboratory courses including those in forensic science (1).

The ability to deliver meaningful laboratory experiments is necessary to meet accreditation standards set forth by the Forensic Science Education Programs Accreditation Commission (FEPAC) (2). Other fields of science education have examined the impact of virtual laboratory instruction. In a comparison between virtual and traditional laboratory chemistry learning, it was found that while laboratory learning does have a positive impact on student achievement, virtual laboratories provided a compliment to traditional laboratories by yielding significantly higher achievement for students (3). Other research has found that the transition to remote laboratory activities had no impact on students grades when compared to a fully on-the-ground version of the same course based undergraduate research project (4).

Combined-mode activities have also been shown to be beneficial to students and can have a significant impact on student achievement (5). The presented laboratory activity was created during the COVID-19 pandemic and could be utilized in multiple modes.

The student learning outcome of this laboratory activity is for students to be able to obtain spectra from fibers using Fourier Transform Infrared Spectroscopy (FTIR) with an Attenuated Total Reflectance (ATR) accessory. After obtaining a spectrum, students perform a simulated (or real) spectral library search to better understand how library searches are performed by the computer. A comparison between the library search results and visual examination allows students to understand the importance of human interpretation through direct visual examination of spectra. When using an automated spectral library search, the underlying mechanisms, and algorithms for how the matches are calculated is not readily apparent. Even instruction materials for such software may only vaguely describe how the matches are determined (6). To be a truly knowledgeable technician in applying the technique, having completed such a library search using the underlying methods themselves serves to benefit the examiner with a better understanding of what the software is actually doing. Infrared spectroscopy is a widely employed analytical technique used for the identification of a variety of forensically important samples from drugs

to fibers. While this technique is successful when pure samples are analyzed, forensic samples are not typically pure and shifts in spectra can occur, due mainly to matrix effects. The steps of analysis are defined by the Scientific Working Group of Materials Analysis (SWGMA) (7) and the ASTM E2224-23ae1 Standard Guide for Forensic Analysis of Fibers by Infrared Spectroscopy (8).

When an unknown sample, like a fiber, is analyzed using infrared spectroscopy, the spectra is often sent to a spectral library for comparison and a "match" is produced. These computer systems make the match based on one of several algorithms that compare the peaks present in a known library standard and the unknown sample (9). For example, the software OMNIC Spectra allows users to choose between 5 different algorithms, which are optimized for different situations (6). Although, many more methods exist for matching spectra against a library (10), the simplest method is a direct correlation of the absorbances of peaks between the unknown and spectra in the library, in which the coefficient of determination ( $R^2$ ) is calculated. The  $R^2$  gives an indication of the match, for example, an  $R^2 = 0.87$  fundamentally being indicative of an 87% match. This correlation method is the most reliable across various situations and is the default method in OMNIC Spectra (6,10). An alternative method is to determine the significance of the correlation with a t-test, but if multiple spectra return a significant correlation the results are not necessarily helpful (9).

While these computer systems have the ability to generate a match outcome, one must consider if a visual comparison is also required. ASTM E2224-23ae1 (8.4.1) (8) states that "Spectral overlay is an approach for comparing data where the presence or absence of peaks, peak shapes, and relative intensities are all considered in the evaluation as to whether exclusionary differences exist between compared samples." This involves more than just the presence or absence of peaks. Many library searches are looking just at the positions of the absorption bands and are only as good as the number of reference samples in the library (9). The goal of this activity is for students to understand why the examiner is important in the analysis of any type of evidence that is using FTIR to determine classification.

## Methods

### *Course Background and Laboratory Logistics*

This laboratory activity was designed for an undergraduate course at Lynn University, Forensic Chemistry (FOR 430). The student population consists of senior level biology majors who are obtaining an optional forensic science concentration. Each year the number of students can range from 1, in a directed study version of the course, to 5 students, in a lecture version of the course. The course content covers basic instrumental

analysis concepts as well as their application in the analysis of various types of forensic chemical evidence. The goal of the laboratory activity is to provide students with a better understanding of how infrared spectroscopy software performs library searches and why analyst interpretation remains an important part of identification of a forensic sample such as paint binders or fibers. In this activity, we specifically examined fibers. The course meets twice a week for 2.5 hours per meeting over the course of an 8-week term. This activity was developed during the COVID-19 pandemic, where students were remote and faculty had limited access to instrumentation. Thus, two different modalities of preparation and delivery of the experiment are presented: fully virtual with no direct ATR-FTIR sample preparations from students or faculty and in-person where students have the ability to prepare their own samples for analysis. While the virtual version does not substitute for a hands-on laboratory activity, it is a valuable exercise that can be incorporated into a lecture course or during times where a hands-on activity is not possible, such as in cases of natural disasters. Prior to performing the experiment, students were instructed in the lecture on the use of ATR-FTIR and how it is used to distinguish fiber classes. Both iterations of the laboratory activity were presented to students during a single 2.5 hour lecture meeting where laboratory activities also take place.

### *Preparation of Fiber Samples (virtual or in-person)*

Depending on the availability of ATR-FTIR instrumentation, the fiber sample spectra varied. We present two different methods for obtaining the infrared spectra.

#### *Virtual*

Due to the COVID-19 pandemic and lack of access to an instrument, the initial ATR-FTIR spectra were obtained from the Database of ATR-FTIR Spectra of Various Materials (11). This database contains spectral images as well as labeled spectral peaks from 4000 – 225  $\text{cm}^{-1}$ . The measurements in the database were obtained using a Thermo Scientific Nicolet 6700 FTIR spectrometer with a "Smart Orbit" micro-ATR accessory (11). The spectra of single component fibers made of cotton, linen, polyacrylic, nylon, polyamide and a two-component fiber made of 70% cotton-30% polyamide were obtained from the database (9) and combined into pdf documents for students to access through our learning management system.

#### *In-Person*

White fibers were obtained from Michaels® Stores with the following compositions: cotton, nylon, polyester,

rayon and 25% cotton-75% polyester. Spectra were obtained from 4000 - 225  $\text{cm}^{-1}$  for 16 scans and 4  $\text{cm}^{-1}$  resolution using a Thermo Scientific Nicolet iS20 FTIR spectrometer with a Smart iTX ATR accessory. These samples were analyzed by the students or prepared by the instructor. When fiber samples are analyzed directly by the students using the ATR-FTIR, this laboratory activity provides the hands-on skills for spectral analysis and instrumentation use.

For both iterations, images of the single component fiber spectra with labeled peaks were provided to students and labeled with the fiber composition in a single document as positive control samples. A second document contained an image of the two component fiber spectra with labeled peaks and was labeled as an “unknown” composition. An Excel (Microsoft 365) document (Supplemental Document) was prepared for students listing the names of the single component fibers as well as the “unknown” two component fiber and spectral peak numbers from 1400 – 600  $\text{cm}^{-1}$  representing the fingerprint region. For student generated samples, spectral peaks in the fingerprint region were obtained by setting the threshold to the baseline after the spectra were normalized.

### *Analysis of Spectra*

The analysis of spectra follows the most basic and common method for determining matches of unknowns to library spectra: a correlation of peak absorbances via  $R^2$ . This method was chosen as it is the most common method for calculating matches (6) and demonstrates the general idea behind how matching software operates. As an alternative method, and to demonstrate that different techniques can be employed in determining a match, the significance of each correlation was also demonstrated (as suggested in 9). These methods are particularly appropriate for this class as a statistics course is a prerequisite for this course which includes activities in correlation, scatter plots, and hypothesis testing (including p-values).

For determination of the  $R^2$ , students were instructed to make a scatter plot of the absorbances of the unknown samples and controls by inserting a blank scatter plot. The data was then added to the plot by right clicking and selecting “select data”. A new menu would appear that allowed students to then select the control spectra for the y-values and unknown spectra for the x-values. Once the plot was made, students were instructed to right click on a data point to bring up a menu and then for them to select “Add Trendline” and click “display  $R^2$  on chart” from the associated menu. The higher the  $R^2$  the better the correlation and likelihood that the sample was comprised of that specific material.

For determination of the significance of the correlation, students were instructed to use the Data

Analysis Toolpak under the Data Tab by selecting “Data Analysis”. From the new menu, students were instructed to select “Regression”. To be consistent with the scatter plots, the y-values were selected to be the control spectra and the x-values to be the unknown spectra. In the output, the p-value of X Variable 1 represents the significance, with a lower p-value indicating a more significant correlation between the two spectra.

These steps were repeated for each unknown/control pairing. More advanced alternatives exist such as the following. First, all correlations can be plotted on the same scatter plot, reducing the number of plots, but increasing the number of steps in creating each plot. Plotting all correlations together allows for students to compare them visually in addition to just the  $R^2$ . Second, the regression analysis using the Data Analysis Toolpak can be completed as a multi-variate regression in which all of the control spectra are selected in the x-data box. This multi-variate regression option reduces the output to just one table. However, the output in this method is more complicated and may be more difficult for students to interpret.

### *Hazards and Safety Precautions*

There are no hazards or safety precautions in the virtual version of this laboratory activity. Students must be properly trained in the use of the infrared spectrometer to provide minimal risk in the in-person version.

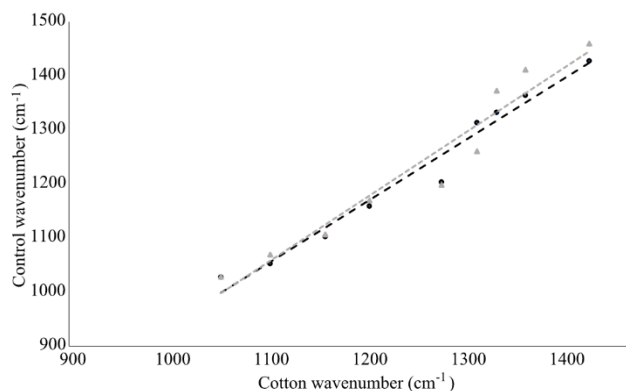
## **Results**

### *Controls*

Students were provided with the prepared Excel file of wavenumbers for the fibers (either the virtually produced data or in-person self-generated data). The instructions were split between the controls (single composition fibers) and unknown. Using the control data, students first created correlation curves comparing the other control fiber compositions to the cotton fiber using the provided wavenumbers reporting the  $R^2$  value. Additionally, for each curve, students used Excel to produce the significance of the slope (Regression Data Analysis Tool) and reported the p-value for each comparison. Students used the  $R^2$  and p-value outputs for each comparison to determine which fiber was the most significantly correlated to the cotton fiber and explained why. Following this numerical comparison, mimicking a library search, students were then instructed to open the document containing the labeled images of the control fiber spectra to determine if they can observe a difference between the most significantly correlated fiber they determined and cotton by using a visual comparison. They were then asked to describe the differences (if any) they observed. Students were also asked to reflect on the

visual difference between cotton and the other control fibers.

Using the virtual data set, the correlation curves produced for the selected control fibers are in **FIGURE 1**. Examining the  $R^2$  values, the value closest to 1, 0.9729, was produced in the comparison between cotton and linen. This comparison also produced the lowest p-value. Students were able to determine that the cotton and linen fiber were the most significantly correlated, reasoning that this was due to their shared major chemical component being cellulose. When students moved to examine the spectral images, they observed very minor differences, such as a difference in wavenumber by 1 or 2  $\text{cm}^{-1}$  or in the intensity of a single peak. This is an expected observation as they contain the same major component. Students were also able to reflect that even though the other correlation curve comparisons all produced  $R^2$  values greater than 0.9, they observed major visual differences in the fingerprint region of the infrared spectra of the controls due to polymer differences.



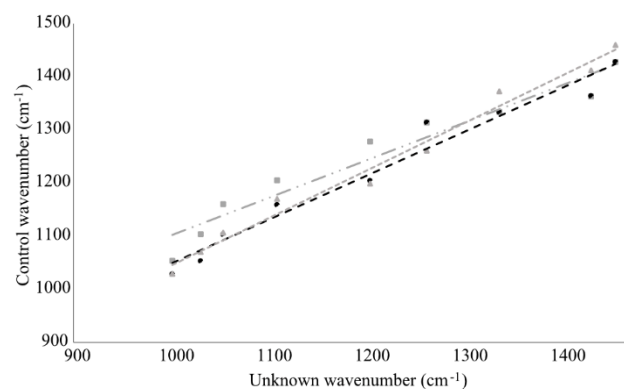
**FIGURE 1** Correlation curve example comparing cotton fiber spectra to other control fibers. Linen fiber (black dashed lines, circle marker) produced an  $R^2 = 0.9729$  and the lowest p-value from ANOVA demonstrating the strongest correlation. Polyamide fiber (grey dotted line, triangle marker) produced an  $R^2 = 0.9375$  and the highest p-value from ANOVA demonstrating the strongest correlation.

#### Unknown

Students then repeated this process of generating correlation curves and determining the significance of the slope by comparing the wavenumbers of the “unknown” fiber, which is a two-component fiber, to each of the control fibers. Students used the  $R^2$  and p-value outputs for each comparison to determine which fiber was the most significantly correlated to the “unknown” fiber. Following this numerical comparison, mimicking a library search, students then were instructed to open the document containing the labeled image of the “unknown” fiber spectra and perform a visual comparison of this

spectra to the images of the control fiber spectra. Students were then asked if their visual comparison provided the same conclusions as the numerical analysis or if they could make different conclusions.

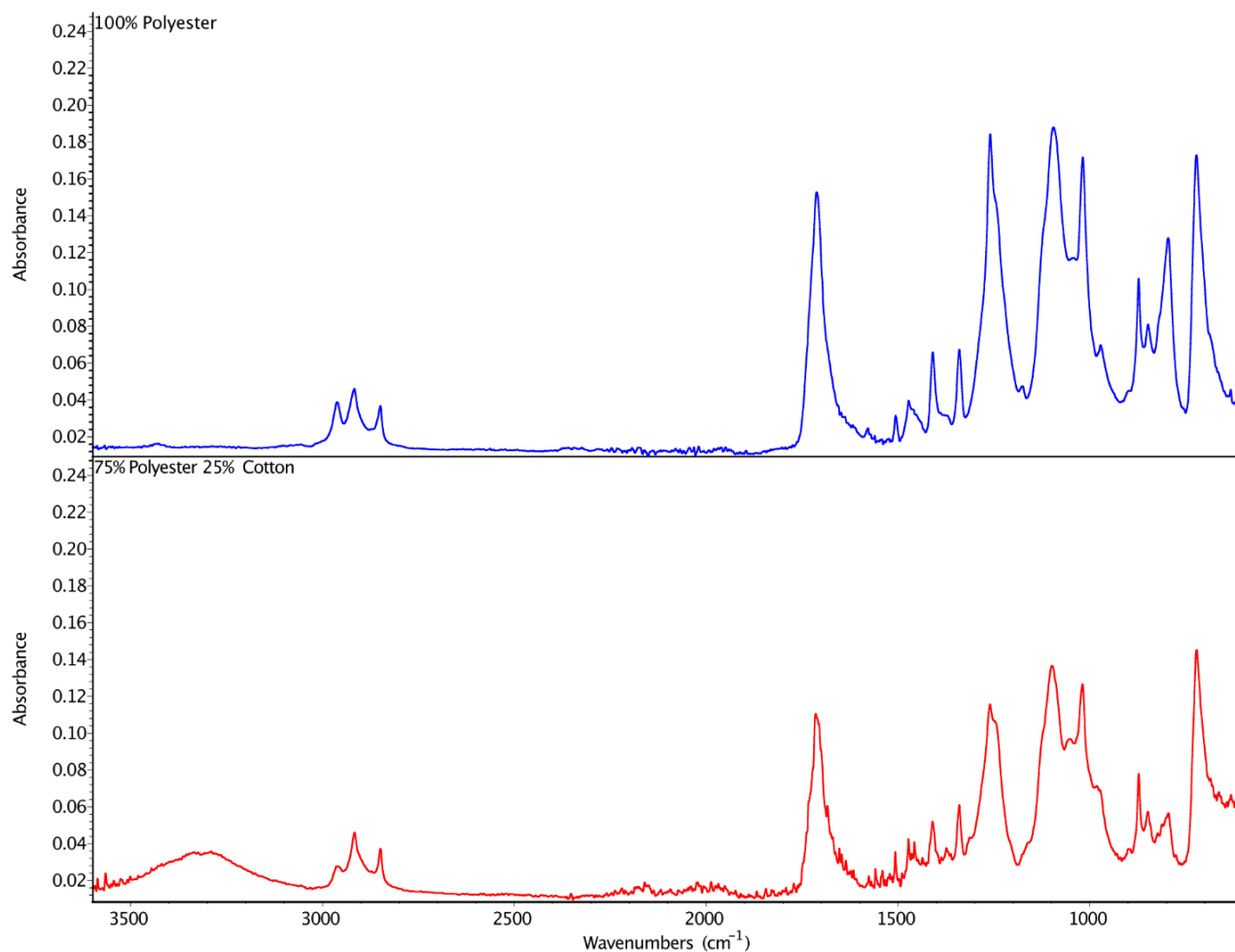
Using the virtual data set, the correlation curves produced for the selected control fibers compared to the “unknown” two component fiber are shown in **FIGURE 2**. Examining the  $R^2$  values, the value closest to 1, 0.9817, was produced in the comparison between the “unknown” and polyamide. This comparison also produced the lowest p-value. Examining just the numerical output, students determined that the “unknown” and polyamide fiber were the most significantly correlated. However, when students moved to examine the spectral images, they reported that they observed very major differences in the fingerprint region. They observed that while there were similarities to the polyamide spectra, there were also many similarities to the spectra of cotton and linen. As the unknown was a two-component fiber made of 70% cotton-30% polyamide, these conclusions were correct.



**FIGURE 2** Correlation curve example comparing an unknown to student fiber composition spectra to other control fibers. Linen fiber (black dashed lines, circle marker) produced an  $R^2 = 0.9663$ , polyamide fiber (grey dotted line, triangle marker) produced an  $R^2 = 0.9817$ , and cotton fiber (grey dot-dash line, square marker) produced an  $R^2 = 0.9404$ . While the strong correlation indicated that the unknown fiber was polyamide, when students examined the spectra images they observed spectral peaks corresponding to cotton and linen.

After completion of the activity students were then asked to reflect on the use of library searches versus the need for examiner knowledge when it comes to fiber comparison through a laboratory notebook submission. Students determined that there was a need to have both a computer search as well as the observations of an examiner as the computer was not able to determine the mixed fiber composition with the provided input while the student was able to see and identify the differences. This aligns with the guidance produced by ASTM (8).

Similar results were produced when we conducted the same laboratory activity with the in-person generated data set. Students were able to prepare their own fiber samples for analysis and create their own control data. They were previously taught how to use the software to obtain the absorbances (in wavenumbers) for their spectra and generated their own Excel file. Example spectra produced by students from their own fiber samples can be seen in



**FIGURE 3** Stacked spectra of 100% polyester fiber (top) and 75% polyester-25% cotton fiber (bottom). Note differences between 3500-3000 cm<sup>-1</sup> and in the fingerprint region (1800 – 600 cm<sup>-1</sup>).

**FIGURE 3.** Students compared their unknown fiber sample, made of 75% polyester and 25% cotton, to the 100% polyester fiber with a visual examination noting differences in the spectra.

Additionally, by using the in-person data set, this allowed an additional comparison in which students were able to compare their correlations with those produced by the instrument software. Software library searches were performed and acted as a comparison to their regression analyses. As an example, students used the Thermo

Scientific Nicolet iS20 FTIR spectrometer with a Smart iTX ATR accessory to generate a spectrum for a 100% cotton fiber. After performing a spectral library search in the provided OMNIC software, it reported an 88.64% match with 100% linen, and 86.76% match with 100% cotton. The linen and cotton results from the software were comparable with the Excel data which showed the best correlation of cotton with linen. Using the comparison of the spectra images, students were able to

correctly identify those spectral differences and that the fiber was cotton rather than linen.

While students were able to easily complete the analysis there are recommendations for success. Students should already have a fundamental knowledge of the use of Excel in the generation of correlation curves. If not, it is recommended to provide students a tutorial to successfully complete the generation of the graphs. Instruction on how to use the Regression Data Analysis Tool in Excel may also need to be provided to students. When the activity is used in the in-person modality where student generate their own ATR-FTIR spectra, prior instruction on the use of the specific instrument would need to be provided to students.

### Discussion and Conclusion

This laboratory activity successfully demonstrated to students the value of examiner knowledge in the assessment of analytical data as well as to provide a simple understanding into how spectral software makes a match. The virtual modality of this laboratory activity presents an alternative assignment where students still participate in the interpretation of the data even if they do not physically perform the chemical analysis. While the virtual activity is not a replacement for hands-on use of instrumentation, it can be useful in scenarios where a hands-on laboratory activity is not possible. This activity was first developed during the COVID-19 pandemic, but there are many other scenarios such as lack of resources, natural disasters, and distance learning where this can be a practical solution to provide students with the understanding of spectral interpretation and spectral library comparisons.

The in-person modality, where students generate their own ATR-FTIR spectra from fibers using instrumentation, provides students not only with a traditional hands-on laboratory activity but further experience in how spectral library comparisons work, even comparing commercial software results to their own regression analysis.

Both modalities of this activity could be adapted for other types of evidence, such as paints, where spectral analysis of the main component is standard practice.

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