Multi-disciplinary research and teaching by means of employing FTIR Spectroscopic Imaging System and characterization techniques

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She is currently the director of Alavi FTIR Spectroscopic Imaging Lab (AFISIL) and supervises multiple undergraduate students in their research. Her research interest includes characterization of biological samples by employing FTIR Spectroscopic Imaging techniques and developing novel digital image processing and analysis algorithms to process the collected FTIR-spectro-microscopic data. Additionally, Dr Alavi is a member of IEEE, ASEE and she has been an active member of McLeod Institute of Simulation Science and pursues research in advanced control systems simulation. Dr Alavi also conducts research in promoting electrical engineering undergraduate education and is the recipient of the best paper award in the Electrical and Computer Engineering Division of American Society of Engineering Education.

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Abstract

This paper focuses on discussing the efforts made to engage students in multi-disciplinary research and integrate teaching and research in the areas of FTIR Spectro- microscopy and image processing and analysis. The author (PI) and co-PIs acquired a Fourier Transform Infrared (FTIR) Spectroscopic Imaging equipment through the National Science Foundation- Major Research Instrumentation (NSF- MRI) grant (#1827134). This project aims to use the equipment to conduct undergraduate and graduate research projects and teach undergraduate and graduate classes. The NSF awarded the California State University Chico (CSU Chico) $175,305 to acquire an FTIR spectrometer and microscope, which are important tools for chemical characterization of samples with infrared active molecules. FTIR Spectroscopic Imaging System especially provides accurate chemical images that reveal the variations in images' pixels which are mappings of constituent materials of samples rather than a single visible image with slight variations. By employing this equipment in research and the Image Processing course, students can learn how to collect, process and analyze the imaging data of samples and the corresponding spectral data. The students not only will learn how to process a single chemical image, but also will work with the data cubes to consider the pixel intensities along the IR spectrum, experience working with big data, hone the skills to design experiments, analyze larger data sets, develop pre- and post-image processing techniques, and apply and refine math and programming skills. Image processing course conventionally is based on math, digital signal and systems, and requires programming skills such as Matlab, C++, and Python. along with the mentioned knowledge. Additionally, the research conducted by this equipment promotes collaboration between engineering major students and science major students. In this paper, the author will explain how collecting data through running experiments with the FTIR Spectroscopic Imaging equipment helps students visualize theory and relate it to real world problems. This paper also discusses the results of engaging undergraduate students from various majors in research. Moreover, it will discuss some of the projects that were conducted by undergraduate students and their learning outcomes. The objective of the research projects was material characterization towards contribution to health by employing FTIR Spectroscopic Imaging System.

I) Motivation

This paper explains the NSF-MRI award that the author and co-PIs received, and the current uses of the equipment purchased through this award. The acquired equipment was a Fourier Transform Infrared (FTIR) Spectroscopic Imaging System. The purpose of the paper is to discuss the efforts made to engage students in a multi-disciplinary research and integrate teaching and research in the areas of FTIR Spectro- microscopy and image processing and analysis. The objective of the research projects was material characterization towards contribution to health by employing FTIR Spectroscopic Imaging System.

In the introduction of this paper, the equipment capabilities, applications, its components, and its use in one of the engineering courses will be introduced. Then, the system outputs, and the research topic examples enabled and initiated will be discussed. Finally, the learning opportunities and the outcomes provided by this equipment as well as future directions will be explained.

Fourier Transform Infrared (FTIR) Spectroscopic Imaging System

FTIR spectrometer and microscope are important tools for chemical characterization of samples with infrared active molecules. An infrared spectrum of a sample shows absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material [1]. This system employs an interferometer and uses Fourier Transform process which improves the quality of the IR Spectra and makes the data acquisition much faster. When the absorbance (or transmittance) intensities from all the spectra at a specific wavelength are inserted together in a matrix, the chemical image at that wavelength is formed. FTIR Spectroscopic Imaging can reveal the variations in the intensity of images' pixels which are mappings of constituent materials of samples rather than a single visible image with slight variations [2-3]. The FTIR spectrometer part of this equipment is normally used in science departments of universities without the microscope. Having acquired the FTIR spectrometer and microscope together for an engineering college opens a new avenue for both teaching and research. Several industries use this equipment for various purposes including quality control, research and development, and more. Therefore, by having access to this equipment, students can prepare for future careers that need micro-spectroscopic imaging skills.

Applications of Fourier Transform Infrared (FTIR) Spectroscopic Imaging System

FTIR hyperspectral imaging makes FTIR a powerful tool for identification of key compounds in highly variable heterogeneous biomaterials as well as identification of unknown materials. FTIR spectroscopic imaging is an emerging technique for characterization and structural analysis of biological samples including intact cells and tissues [4]. In biology field, it can be used as a label-free characterization of specific membrane domains within intact cells before and after treatments, as well as variations in membrane polarizability due to cancer [5]. It is a powerful method to determine the identity of organic and inorganic materials in the field of chemistry and biochemistry. Other applications include determining the quality or consistency of a sample in quality control of products in sustainable manufacturing field [6]. Additionally, the spectrum peak intensity determines the amount of components in a mixture, which can be used for quantification of sample constituents.

The use of the FTIR Spectroscopic Imaging system can enable a variety of projects in various courses. Currently, the Electrical and Computer Engineering Department, Mechanical and Mechatronic Engineering and Sustainable Manufacturing Department, and Chemistry and Biochemistry Departments at CSU Chico are using this equipment in several courses such as Digital Image Processing, Material Science and Engineering, Material Science and Engineering Laboratory, Organic Chemistry Laboratory, Integrated Laboratory and Special Problems.

FTIR Spectroscopic Imaging System Components

Figure 1 shows the FTIR spectroscopic imaging system. An FTIR spectrometer, an FTIR microscope, Data Acquisition system, an optical table and a liquid nitrogen generator were purchased through this grant. Liquid nitrogen is used to cool the microscope detector.

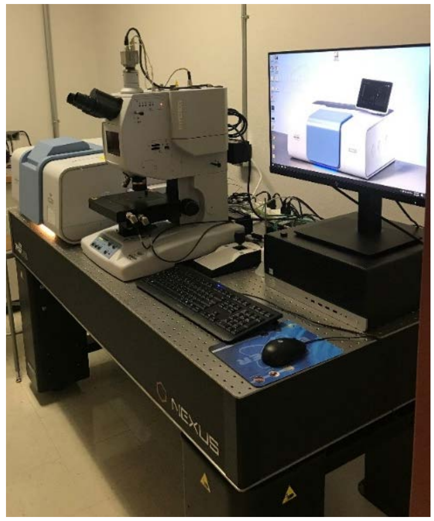

Figure 1. The FTIR Spectroscopic Imaging System

The Research and Development Bruker Invenio-R FTIR Spectrometer shown in Figure 2a enables us to collect an average IR spectrum of a sample and characterize it accordingly. Additionally, an Attenuated Total Reflection (ATR) unit was purchased to eliminate sample preparation steps when using the spectrometer. ATR is a sampling technique used in IR spectroscopy which enables collecting spectra of samples in the solid or liquid state without significant preparation. This unit is mounted in the sampling compartment of the spectrometer as shown in Figure 2b.

The Bruker Hyperion 2000 FTIR microscope shown in Figure 3 features fully automated infrared chemical imaging, sample viewing, a 15x IR and visible objective and a 4x visible objective. This microscope enables efficient chemical microanalysis. The spectral range of this microscope is mid to near IR and the visible range up to 25,000 cm^{-1}. While the ATR unit of the spectrometer provides us with the IR spectrum of the sample which is a footprint of the functional groups of the sample, it does not allow us to choose a specific spot on the sample to obtain the spectrum. This becomes important if the sample is not homogeneous. The FTIR microscope resolves this issue and allow us to view the sample two-dimensional image and choose the pixels at which we need to evaluate the spectra. The OPUS software controls the FTIR system and allow us to choose the pixels on a line, single data points or a grid of pixels (chemical image). The resolution of the IR image depends on the selected grid size.

Figure 2. a) The FTIR Invenio-R research and development spectrometer. b) The ATR unit of the spectrometer.

Figure 3. The Hyperion 2000 FTIR microscope

Use of the equipment in Digital Image Processing course

Digital Image Processing is one of the engineering courses that can use the acquired equipment and can be taught in several majors since it has applications in almost all fields. Normally, it is taught in the Computer Science Departments, Electrical and Computer Engineering Departments and Biomedical Engineering Departments. The Electrical and Computer Engineering students have a solid background to learn this course in their senior year since they pass Signals and Systems during their junior year. During the course, students learn how the image acquisition takes place; but mostly learn how to present and process the images obtained from various imaging modalities. The common topics covered in this course are digital image fundamentals, intensity transformations and spatial filtering, filtering in the frequency domain, image restoration and reconstruction, color image processing, image compression and watermarking, morphological image processing, image segmentation, edge detection, thresholding, and region detection, feature extraction, image pattern classification by employing techniques such as Deep Learning (DL) and Artificial Neural Network (ANN). As can be seen from the topics, the course is math intensive, and students are expected to learn substantial amounts of theory. Therefore, hands-on activities and working with an imaging equipment is crucial to engage students. The imaging equipment that is currently used in the course is Fourier Transform Infrared (FTIR) Microscope.

II) System outputs

The output of the FTIR microscope is not a single image. It is a cube of data with multiple chemical images that is shown in Figure 4. The number of images depends on the spectral range and spectral resolution. The common spectral range is 600cm^{-1}- 4000cm^{-1}, and the spectral resolution is 4cm^{-1} which yields 850 images. Each 2D image in the x-y coordinate is a representation of the absorbance intensities of the sample at a specific wavenumber. Grouping these images together in the cube similar to the one shown in Figure 4, one can obtain the spectrum of each pixel. Therefore, after the FTIR spetro-microscopic experiments we will have as many as spectra as the number of the pixels of the image.

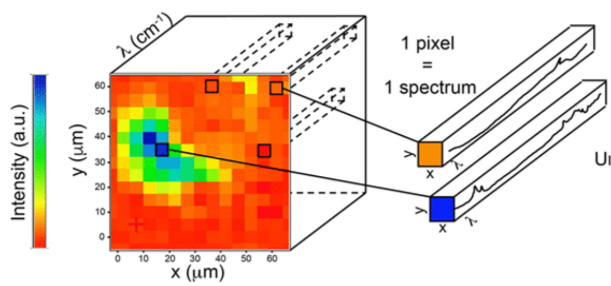

Figure 4. Each 2D image collected by the FTIR microscope is a cube of data [7].

The output of the ATR unit of the spectrometer is a single spectrum which is generated by averaging the single spectra obtained from several scans. An example of a stack of spectra is demonstrated in Figure 5. This is a stack of the spectra of a Gala Apple. The spectra are corresponding to the different depths of the apple tissue. The purpose of this experiment was to investigate the peak intensity changes when samples from different depths of Gala Apple were inserted into the ATR component of the spectrometer. The red spectrum was obtained from under the apple skin. The blue, pink and green spectra show the spectra at approximately 0.66cm, 1.33cm, and 2cm deep from the apple skin respectively. As can be seen, as we get deeper into the apple, the intensity of the peak at 3292cm^{-1} decreases which could be an indication of more constituent with this functional group. Further analysis is needed to precisely comment on the spectra, which is out of scope of this paper.

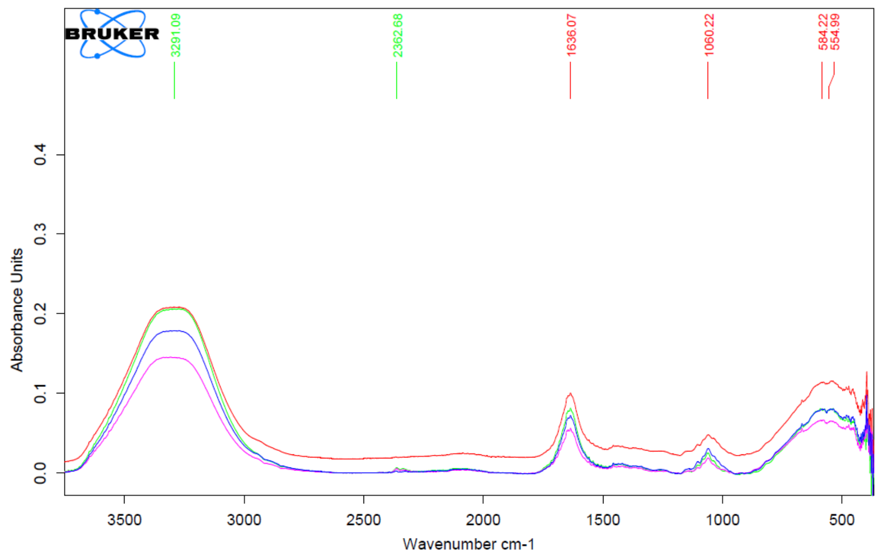

Figure 5. Example of spectra of the Gala apple tissues at different depths.

III) Examples of the enabled activities and projects and the learning outcomes

Some of the projects that were enabled by this equipment are listed below:

1) Employing FTIR Hyperspectral imaging techniques towards investigating microbial interactions with plant roots,
2) Employing FTIR Hyperspectral imaging techniques towards detection and analysis of wax artifacts in fruit tissues,
3) Employing FTIR spectroscopic imaging techniques for evaluation of quality changes of fresh fruits and vegetables when processed for retailed baby food.
4) Developing a Deep Learning algorithm for FTIR Spectroscopic Imaging data and creating its Graphical User Interface (GUI).

In the above three projects, the students from Biochemistry and Chemistry Departments, Mechanical and Mechatronic Engineering and Sustainable Manufacturing Department, Electrical and Computer Engineering Department collaborated, learned new techniques, obtained new skills and experienced a new area of research.

The subjects that they learned during this research included:

- Learning the theory behind FTIR spectroscopy and FTIR spectroscopic imaging technique.
- Preparing samples for the FTIR experiments.
    - Running experiments using microscope: These samples need to be 5-15µm thick. Otherwise, the spectra of the pixels will be noisy and due to the low signal to noise ratio (SNR), the smaller peaks of the spectra that are used for characterizing the sample will not be recognized. Based on the sample, the student will take the steps to make the samples thin enough. The common technique is to microtome

the sample using a microtome equipment. Many organic samples need to be freeze dried before being microtomed. This can be done by an engineering student or a science major student.
  - Running experiments by the ATR unit of the spectrometer: The advantage of using this accessory of the spectrometer is that the need for sample preparation is minimized.

- Hands-on experiences:
  - The students learn how to operate the equipment by the OPUS software to run the experiments.
  - They learn what parameters affect the results of the FTIR spectroscopic imaging experiments and how to set the correct parameters to run the experiments.
  - The students learn how to find the field of view and focus on the sample when using the microscope, and how to switch between different objectives and modes of the microscope for viewing the sample and collecting FTIR images.

- Learning how to validate and analyze the spectra
  This can be either analyzing the spectrum obtained from the ATR unit of the spectrometer, or the multiple spectra obtained from the microscope. After running the experiments, the quality of the spectra is evaluated, and the peak positions are associated with a functional group. The OPUS Software that runs the experiments has a library that can assist with finding the peaks. However, a Chemistry major student contribution is recommended in this part to confirm the results. If the sample is not homogeneous, by looking at the spectra at multiple locations of the sample and comparing the spectra, the student can characterize the various components of the sample. Occasionally, other equipment such as HPLC is required to complete the analysis.

- Learning data structure and image processing techniques
  Students need to understand the layout of the imaging data before being able to analyze or process the data. The data is collected in a binary format (OPUS) with other options such as Matlab files. Since the image is not a single 2D image and it will be a 3D data cube, when taking the Matlab data set from OPUS into Matlab Software to process and visualize them, the student needs to examine the image layout saved to make sure it is consistent in both OPUS and Matlab Software.
  One of the helpful tools in the area of image processing which greatly overlaps with machine learning is image segmentation. This can be utilizing a clustering or classification algorithm to find specific features in an image or alternatively in an image dataset clusters. The image dataset provided by this equipment are great candidates for being used in the image processing field. Specifically, of the interest is finding the pixels that have the same functional groups and associated peaks in heterogeneous samples. The features that present each pixel to the machine learning algorithm are the spectra of each pixel. The algorithms that students have used in research and class have been Thresholding, Kmeans Clustering, and Deep Learning techniques. During a research

project, an Electrical Engineering undergraduate student was able to develop a Deep Learning classification tool for the FTIR Spectroscopic Imaging data and create a GUI for the tool by employing PyCharm, Matlab and integration of the two software capabilities.

The purpose of this paper is not to discuss the scientific results of these projects but rather to discuss the learning and research opportunities the equipment provided to the students at multiple colleges of CSU Chico.

IV) Outcomes and future directions of using the equipment for research and instruction

The FTIR Spectrometer and microscope have enabled interdisciplinary research collaborations between the Colleges of Engineering, Computer Science and Construction Management, Natural Sciences and Agriculture. The collaborations have been established between students and faculty of both colleges. This has effectively contributed to how the students look at science and engineering and has prepared them for problem solving in a broader area. Six graduate and undergraduate Chemistry/Biochemistry, biology and engineering students have collaborated in the research projects to date. The equipment has enabled multiple experiments in the engineering courses such as digital image processing, and Materials science and engineering as well as science courses such as Organic Chemistry and Organic Chemistry Laboratory. Approximately 650 students from the departments within the mentioned colleges use the FTIR spectroscopic imaging system and/or analyze data collected by this system per academic year. CSU Chico students who include significant numbers of students who are first-in-family to enter college, veterans, Hispanic, and are from other nontraditional and underserved populations, gain exposure to a state-of-the-art characterization system and hone the skills to design experiments, analyze large data sets, develop pre- and post-image processing techniques, and apply and refine math and programming skills.

The future plans of this project include connecting with more industries such as pharmaceutical and solid state electronics companies, and exploring additional real world problems that could be solved by using the FTIR Spectroscopic Imaging technique. Undergraduate and graduate students will be involved in hands-on research activities that will boost their knowledge and skills.

V) Acknowledgement

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VI) Conclusion

The FTIR Spectroscopic Imaging system has enabled establishing a new lab facility at the College of Engineering, Computer Science and Construction Management of CSU Chico. The lab equipment serves as an important role in both research and instruction. Multi-disciplinary research between faculty and students that initiated by using this equipment will lead to peer review publications. Additionally, the equipment provides hands-on opportunities in imaging for the students who take Digital Image Processing course. The subjects that students learn when engaged in a research project or a course project that utilize this equipment were discussed. This equipment has already served several students who are first-in-family to enter college, Hispanic, and are from other nontraditional and underserved populations, and will continue serving CSU Chico students and faculty in research and teaching.

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