Spectrochemical Analysis of Biological Samples with IRENI

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Vibrational spectroscopy has held an important place in the physical sciences for decades. FTIR and Raman instruments are standard equipment in most laboratories; spectra of multitudes of pure compounds are readily available in data libraries; however, complex biosystems create complex analysis issues. Our goal is to characterize the chemistry and biochemistry of systems at biologically relevant length and time scales in an effort to bring FTIR, Raman and SERS spectrochemical imaging into mainstream biological practice.

Fourier transform infrared microspectroscopy is already an excellent tool for biomolecular imaging in situ. Large area images (>3 mm^2) are obtained with our thermal source Focal Plane Array (FPA) imaging system at U. Manitoba. The mid-IR beamline IRENI (InfraRed ENvironmental Imaging, Synchrotron Radiation Center, University of Wisconsin-Madison) is the first, and currently only, system for infrared wide-field imaging. Here, twelve brilliant synchrotron beams homogeneously illuminate a sample, to create spectrochemical images with a pixel resolution of 0.54×0.54 μm^2, two orders of magnitude greater than current systems. Spatial resolution reaches a wavelength-dependent and diffraction-limited fundamental limit, approximately $\lambda/2$, or ~1 to 5 μm. Spatial image restoration techniques can be applied to IRENI data (spatially oversampled for all mid-IR wavelengths) enabling the highest possible spatially-resolved data in a wide field IR imaging microscope.

With IRENI's capabilities, it is possible to study changes in individual cells in situ, and to characterize their surroundings, using only the biochemical signatures of naturally-occurring components in unstained, unfixed tissue. Through comparisons among spectra from live cells and freshly acquired or stored tissues, we have shown that certain restrictions in tissue preparation and storage are critical for preservation of some chemically unstable biomarkers. We are also developing live cell imaging tools for several mammalian and plant cell types.

This presentation will focus on studies undertaken in the last 12 months, mainly on brain tissue from Alzheimer brain and transgenic AD mouse models. Challenges in image analysis include the sheer volume of data that is generated, necessitating new approaches to extract relevant information, mine for unusual, unexpected information, and evaluate time-dependent changes. Further improvements and the development of medium-cost instruments will create the opportunity for novel experiments in many disciplines and bring this tool into mainstream diagnostic laboratories.