Biomedical Application of Infrared Spectroscopic Microscopies in Obtaining Structural Information from Complex Systems

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Synchrotron-based FTIR spectra were recorded at the infrared beamline (BL14A) at the National Synchrotron Radiation Research Center (NSRRC), Taiwan using a Nicolet Magna 860 FTIR spectrometer equipped with a Continuum IR microscope (Spectra Tech), mapping stage controller, 32× objective (0.65 numerical aperture) and a MCT detector. The bench was configured with a collimated synchrotron light, which served as an external input to the spectrometer. The modulated light was directed into the infrared microscope for spectroscopy. The beam current was 250 mA (now operating at 300 mA) and the synchrotron was running in top-up mode.

The spectra were collected in the mid-IR range of 4000–700 cm^{-1} using a spectral resolution of 4 cm^{-1}, with the co-addition of between 64 and 128 scans, aperture of either 10 µm×10 µm or 12 µm×12 µm, and step size of between 8 and 10 µm. The optics were purged using dry N_2 and the automatic atmospheric suppression function in the OMNIC™ software was used to minimise infrared absorption by CO_2 and water vapour in the ambient air. A single-beam background spectrum was collected from an area free of sample. Stage control and data collection were performed using OMNIC™ (OMNIC, 2001). The IR data were collected using the OMNIC software coupled with a gating system for eliminating the noise during electron injection of 60 s periods for synchrotron top-up operation. Optical images were obtained using a camera linked to the Continuum microscope.

SR-FTIR functional group maps were produced using OMNIC™ Atlµs™ (2005). In order to produce the maps, data were first truncated then baseline corrected and finally the area under the band of interest was measured. The two diagnostic regions chosen for analysis were the CH stretching region from 3000–2800 cm^1 and the amide I band from 1713–1603 cm^1. FTIR mapping and imaging provides information on the total biochemical content of, and distribution within cells and how these change with disease conditions and drug treatment. Figure 1 shows an FTIR map of an untreated A549 cell. Again the biochemical information can be related to the morphology shown in the optical image, where the highest concentrations of protein are within the nucleus due to the tightly packed protein in chromatin. While the lipid also has a high density around the nucleus because of the contributions from the outer membrane, the nuclear membrane and some underlying C–H stretches from other biomolecules, it illustrates protrusions from the cell that have a relatively low protein:lipid ratio and are not evident in the protein map. By the integration and deconvolution of signals in regions of the spectra (e.g., amide I region), considerable information can be obtained on changes in ratios of various biochemicals and changes in conformations of proteins as a function of disease conditions and/or treatments with drugs and toxins such as Cr(VI). These types of studies are currently underway.

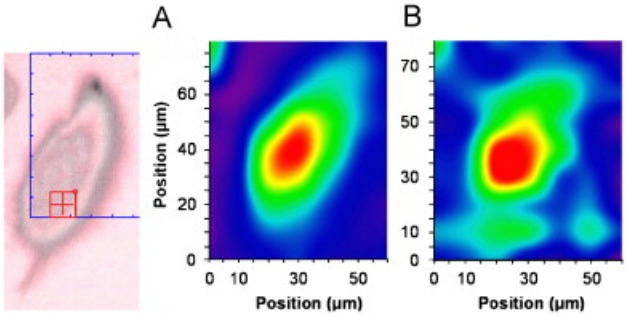

Fig. 1: SR-FTIR functional group maps of the: (A) the amide I region (1713–1603 cm^1); and (B) the CH stretching region (3000–2800 cm^1) (64 scans, 12×12 $µm^2$, 10 µm step size) of an untreated A549 cell.

References

[1] E. A. Carter, C. P. Marshall, M. H. M. Ali, R. Ganendren, T. C. Sorrell, L. Wright, Y.-C. Lee, C.-I. Chen, and P. A. Lay, ACS Symp. Ser. **963**, 64 (2007).
[2] L. M. Miller and P. Dumas, Biochim. Biophys. Acta Biomembranes **1758**, 846 (2006).