A bright source for infrared microspectroscopy: synchrotron radiation

Paul Dumas and Mark J. Tobin

A particle, such as an electron, emits synchrotron radiation when accelerated or (decelerated). The radiation is emitted along the direction of motion of the particles. A number of synchrotron accelerator facilities exist around the world specifically to provide an intense source of radiation. They have been exploited, for spectroscopy, in the VUV, far-UV and soft X-ray regions. Synchrotron radiation has been used successfully in both the mid-IR and far-IR regions for about ten years, and has an equivalent blackbody temperature in excess of 10,000 K! It is particularly attractive for IR microspectroscopy, as exemplified in this article.

IR microspectroscopy

Synchrotron radiation: a bright source of infrared photons

Electron storage rings (or synchrotrons) use magnetic fields to bend electrons into a closed orbit. Synchrotron radiation is produced at each of the bending magnets. Infrared radiation is generated by electrons travelling at relativistic velocities, either inside a curved path through a constant magnetic field (bending magnet radiation) or by longitudinal acceleration or deceleration when leaving or entering a magnetic section (edge radiation). The emitted radiation spans an extremely broad spectral range, extending from the X-ray to the very far-infrared region.

The effective synchrotron source size is quite small, and can be on the order of ~100 µm. Furthermore, the light is emitted into a narrow range of angles.

The intensity distribution, for a particular wavelength, is dependent on the mode of emission, and has to be considered carefully, in order to optimise the extraction geometry and to couple efficiently all collected photons into the microscope.

This can be illustrated by considering the intensity profile of the beam at two characteristic wavelengths, 10 and 100 µm, and for a storage ring operating at an energy of 2.75 GeV, see Figure 1(a, b). In Figure 1, two extraction geometries have been considered...
Clearly, the intensity of the emitted source is not distributed uniformly. For edge radiation, rings of maximum emission are produced, with increasing size for longer wavelengths. At 2.75 GeV (recent third generation synchrotron facilities have energy in the 2–3 GeV range), a 10 × 10 mrad aperture can be considered for the bending magnet, while a 20 × 20 mrad aperture is appropriate for a bending magnet. The synchrotron beam can be efficiently collimated and is directed into the interferometer, which modulates the infrared light, and then is directed towards the IR microscope. Since the synchrotron source produces high throughput at small aperture sizes, the diffraction limit is achieved when the microscope’s apertures define a region with dimensions equal to the wavelength of interest. However, the use of a confocal optical arrangement leads to ~30% improvement in the spatial resolution, in agreement with diffraction theory.

### Some applications

Several synchrotron infrared microscopy beamlines are operating around the world, and several others are under construction or planned. Applications are multidisciplinary, and the number continues to increase as these beamlines become accessible to an increasing number of users. High spatial resolution, excellent spectral quality (signal-to-noise), and fast data acquisition are the essential features of the technique.

### Polymer films studies

It is well known that vibrational microscopy is a powerful tool for characterising polymeric materials, providing both compositional and structural information. Most new polymeric materials are multiphase systems. They include homopolymers and copoly-
morphism is described elsewhere.\textsuperscript{7} The crystallisation temperature. Optical images, within the blue square, the mapped area, with marked steps of 3 µm. From the cluster analysis of the spectra recorded, fuzzy-C means-clustering images have been generated, and each of the image clusters corresponds to different crystalline morphologies. The characteristic spectra of each cluster are also displayed, and subtle differences in spectra can be seen.

**Human tissues investigations**

IR spectroscopy is being employed increasingly in the study of biomedical conditions, where it has been shown to be capable of detecting subtle biochemical changes within tissues. The coupling of a microscope to a Fourier transform infrared spectrometer, complemented by the use of a synchrotron source has brought the potential to examine tissues at cellular and subcellular resolution. The applicability of micro-spectroscopy, and hence imaging, in particular to biological and pathological problems relies on the information being obtained at high lateral spatial resolution. Spectroscopic imaging provides diagnostic information in a visual form, a prospect appealing to physicians and biologists. Image methods can provide potentially far more information to non-specialists than their non-imaging counterparts. However, the analysis and diagnostic potential of IR imaging strongly depends on the quality of the spectra acquired. Clearly, a parameter such as the signal-to-noise ratio, strongly affects the image quality.

Hereafter, we illustrate how high spectral quality obtained at diffraction-limited spot size sheds new light into the biochemical composition and protein secondary structure of human tissues: in this case, hair.

Human hair sections have been studied intensively using synchrotron infrared microspectroscopy.\textsuperscript{8} They are 50–100 µm in diameter and their cross-section reveals three major identifiable regions. The medulla is the centre-most portion of the hair. It is 5–10 µm in thickness and composed of loosely packed, keratinised cells that distribute moisture and nutrients to the hair strand. The medulla can be either continuous or discontinuous along the hair length, and often it is completely absent. The cortex makes up the bulk of a hair fibre and determines the strength of a hair. It is 45–90 µm in diameter and composed of long embedded cortical cells. It also contains the hair pigment, melanin. The outermost layer of a hair strand is the cuticle, which is less than 5 µm in thickness. It is a dense layer of flat, keratinised cells, which protects the hair fibre. Infrared spectra were recorded across hair fibres with an aperture size of 3 × 3 µm\textsuperscript{2}.

The chemical images of the lipids (height of peak at 2920 cm\textsuperscript{-1}), displayed in Figure 3(a), show that lipids are predominantly located inside the outer cuticle layer, as well as inside the medulla (orange and red regions). The high quality of the recorded spectra has allowed subtle difference in the peak position and lineshape of the stretch modes of CH\textsubscript{2} to be observed between lipids located in the medulla or in the cuticle. Fuzzy-C means-clustering images have been generated in the 2800–3000 cm\textsuperscript{-1} region, and associated images of the two main classes of CH\textsubscript{2} stretch modes are shown in Figures 3(b) and 3(c), which reveal the different nature of the lipids in this two regions. These differences can be related to the relative chain lengths (CH\textsubscript{2}/CH\textsubscript{3} ratio) of the prevalent lipids in each region.

IR spectroscopy can also reveal the different composition of the protein secondary structures. For hair sections, the Amide I band contour is different between spectra recorded from inside the cortex and from inside the cuticle, see Figure 3(c). The red spectrum has been recorded inside the cuticle, with an aperture of 3 × 3 µm\textsuperscript{2}. Clearly, the peak position exhibits a downward wave-number shift, which indicates a higher relative concentration of β sheet to α
helix inside the cuticle. This relative concentration is shown in Figure 3(d).

Synchrotron infrared microspectroscopy appears to be extremely well suited to the study of hair biochemical composition, and its variation with age, ethnic origin, sex etc., as well as for studying the interaction of external agents (for example, cosmetics) on hair composition and structure, and is the subject of several current investigations.

Single cell studies

Using synchrotron infrared microscopy, Jamin et al. were the first to demonstrate that the chemical components of single living cells, e.g. proteins, lipids, and nucleic acids, can be imaged with high signal-to-noise at a diffraction-limited spatial resolution. This work was pursued further by the extended study of programmed cell death, i.e. apoptosis, using a combination of fluorescence microscopy and synchrotron infrared microscopy.10

Detailing the bio-composition and protein secondary structures inside one single cell is a subject of paramount importance, in order to understand the changes of the cell cycle, as well as any abnormality that would ultimately lead to a disease.

To illustrate the sub-cellular capability studies of synchrotron infrared microscopy, we have studied the behaviour of single HL60 cells during differentiation. HL60 cells are often studied as an attractive model for differentiation, and are used specifically for human myeloid cell differentiation. Among several agents used for differentiation, phorbol myristate acetate (PMA) induces HL60 cells to differentiate into mature monocytes/macrophages in vitro.

Figure 4 illustrates the main observations. Figure 4(a) shows the optical image of one HL60 cell before inducing the differentiation process. Chemical imaging of the lipid profile has been generated using the peak height at 2920 cm⁻¹ [Figure 4(b)]. They are clearly distributed around the nucleus. After 48 hours of differentiation induction time, a clear change in the Amide I lineshape is observed [Figure 4(c)]. It is suggested that following PMA treatment, the α-helix content, in differentiated cell membrane protein, increases. These changes in the membrane protein secondary structures are probably related to the mode of interaction with the inducing agent. By using a statistical analysis in the Amide I and Amide II band frequency region (1485–1710 cm⁻¹) of the high S/N spectra obtained with a 3 × 3 μm² aperture, it is possible to separate out the different relative composition of the protein secondary structure, inside the nucleus and in the cytoplasm. Figure 4(d) shows the two cluster images and the average spectrum of each.

Only the use of a synchrotron source can produce such high contrast chemical images inside individual cells.

Future and perspectives

It is clear that synchrotron radiation provides a high brightness infrared source, which is being exploited successfully in microspectroscopy for high signal-to-noise spectra and for fast data acquisition, because of the readily achievable diffraction-limited spatial resolution. Several infrared microscopes are now implemented at synchrotron facilities around the world, although many applications are still in their infancy. An attractive feature of the synchro-
tron environment is the capability of achieving complementary characterisation using other available synchrotron-based microanalytical tools. Among them, X-ray microscopy is one of the best-suited complementary approaches to infrared microscopy.

Several improvements are now being proposed for synchrotron infrared microscopy. Nowadays, focal plane arrays detectors are being implemented in microscopes that use a globar source, and the performance of these detectors has not yet been exploited with a synchrotron source. Clearly, the size of the detector array has to be adapted to match the projected size of the synchrotron beam, in order to keep the brightness advantage of this source. These small detector arrays might well be available soon, and will allow faster acquisition data, and a slightly improved lateral resolution using point-spread function (PSF) deconvolution.

Interesting new attempts have been initiated with a synchrotron infrared source in order to extend measurements to below the diffraction limit by employing PhotoThermal IR Micro-Spectroscopy (PTMS), see Figure 5. In this technique, an AFM-based thermal probe is used as the infrared absorption sensor, and promising results have already been obtained.

All together, the bright infrared source provided by synchrotron radiation has a tremendous future, in microscopy and near-field microscopy, and such facilities are available to experimenters who are invited to contact the various synchrotron infrared beamline representatives. There are currently four operating beamlines in Europe (Daresbury, UK; ANKA, Germany; BessyII, Germany and LURE, France) and several others are under construction and will be available soon (Maxlab, Sweden; SLS, Switzerland; Elettra, Italy; ESRF, France; SOLEIL, France; Diamond, UK).

It is important to note that applications of synchrotron infrared spectroscopy are not restricted to microscopy but also exhibit a tremendous prospect in other fields, such as far-infrared spectroscopy, time-resolved spectroscopy and two-colour experiments.

Acknowledgements

The authors wish to thank G.P. Williams, G.L. Carr, L.M. Miller, G.A. Ellis, H. Pollock, A. Hammiche and M.A. Chesters for their long-term collaboration and support, as well as for their very fruitful discussions.

References


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Figure 5. (a) PhotoThermal IR Micro-Spectroscopy. A microthermal probe, based on an atomic force microscope (AFM) cantilever tip is placed under the infrared microscope objective at the focus of the synchrotron light. Though the infrared light covers an area defined by the diffraction limit, the probe can potentially sense thermal absorptions from much smaller regions. By modulating the light, the output from the sensor can be used to generate an absorbance spectrum. (b) The scanning probe instrument is coupled to the infrared beamline at the SRS, Daresbury, using a side-port accessory.