Bright lights

New developments in image analysis and correction are boosting the IR microspectroscopy capabilities at the Australian Synchrotron.

MICROSCOPY HAS CERTAINLY come a long way since the mid-1600s, when Robert Hooke and Anton van Leeuwenhoek marvelled at the new biological world revealed through their microscopes filled with carefully polished glass. In 2012, the choice in microscopy technology is somewhat more varied. One can still peer through a light microscope similar in kind to those made by hand 400 years ago and observe the wondrous morphology of insects and basic cells.

Another choice is travelling to the Australian Synchrotron in Melbourne, sitting down not far from a 216-metre circular beam of tightly corralled electrons hurtling at near the speed of light, and using some of the radiation scattered from that beam to wonder at all manner of samples.

A synchrotron is effectively a circular particle accelerator, which propels charged particles, such as electrons, around a 432 metre diameter ring circuit at over 99.99 per cent of the speed of light. The electrons are steered by incredibly powerful dipole magnets, which keep them focused into a tight beam.

Such particle accelerators were once the domain of particle physicists studying subatomic collisions. However, it soon became apparent that electrons magnetically coerced to travel in a high-energy circle release their frustration in the form of radiation, or so-called 'synchrotron light.' This scattered energy could then be harnessed using specialised magnets and mirrors into selected wavelengths, or 'beamlines,' for use in a wide range of high-resolution imaging and analytical experiments.

The 28-magnet arrangement in the storage ring of the Australian Synchrotron can potentially generate that many separate beamlines, allowing a wide range of experiments or measurements to be carried out at any one time. All bar one of the current beamlines emit X-ray light, with the final one producing light in the infrared (IR) spectrum, allowing IR spectroscopy/microscopy and far-red gas phase analysis to be carried out.

Synchrotron light greatly enhances the potential of infrared techniques, especially in microspectroscopy, which is used across many fields including biological and environmental science, materials science and geology, as well as in forensic and cultural artefact studies.

"The unique properties of synchrotron light mean that experimental results are far superior in accuracy, clarity, specificity and timeliness to those obtained using conventional laboratory equipment," says Dr Mark Tobin, the Senior Scientist on the IR beamline.

"Synchrotron techniques can generate images plus elemental, structural and chemical information from diverse sample types ranging from biological to industrial materials. The broad range of available wavelengths allow scientists to look at the size and shape of macromolecules and voids in bulk materials, peer into the biochemistry of single cells and delve all the way down to the bonds between atoms."

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By Fiona Wylie

POWER OF THE RING

According to Tobin, the scientific heavy lifting is done by the equipment plugged onto the side of the synchrotron ring to catch the scattered radiation. These units select the desired wavelengths of light, focus it using X-ray or IR mirrors, and direct it onto the experimental sample.

"The synchrotron light is directed through the wall via evacuated tubes and transported to the relevant analytical equipment housed in laboratory-equivalent 'hutches' where the users come to do their experiments," he says.

"In our case, the hutch contains an IR spectrometer and IR microscope. Our users have often already done some IR analysis back in their own labs, but may need the higher intensity IR energy provided by the synchrotron to examine smaller features or to get better quality spectra off their samples."

Users of the synchrotron might be interested in basic chemical identification of materials in their sample, finding out about spatial heterogeneity with regard to the chemical make-up or looking for changes in a material, such as degradation, aging or a reactiown based on changed atmosphere or temperature.

"Sample types include basically anything you can fit under the microscope and find a way of putting the beam through," says Tobin. Roughly 40 per cent of the IR beamline users are looking at biological and biomedical applications, at least in terms of hours used, he says.

"You generally get the best quality data if you can get your sample in the form of a thin section for beam transmission, although we can also work in other modes such as reflectance." Another of the IR beamline scientists, Dr Ljiljana Puskar, has helped many of these users and is happy to talk about some of the more interesting projects that have drawn on the power of the ring.

PARING BACK THE LAYERS

An interesting ongoing application of the IR beamline at the Australian Synchrotron is in the area of art conservation and trying to identify components that need restoring, such as paint layers that have either degraded or been added to, or both, over many years. A specific example that Puskar worked on was from an ancient piece of religious art from Serbia that was overpainted in the 19th century.

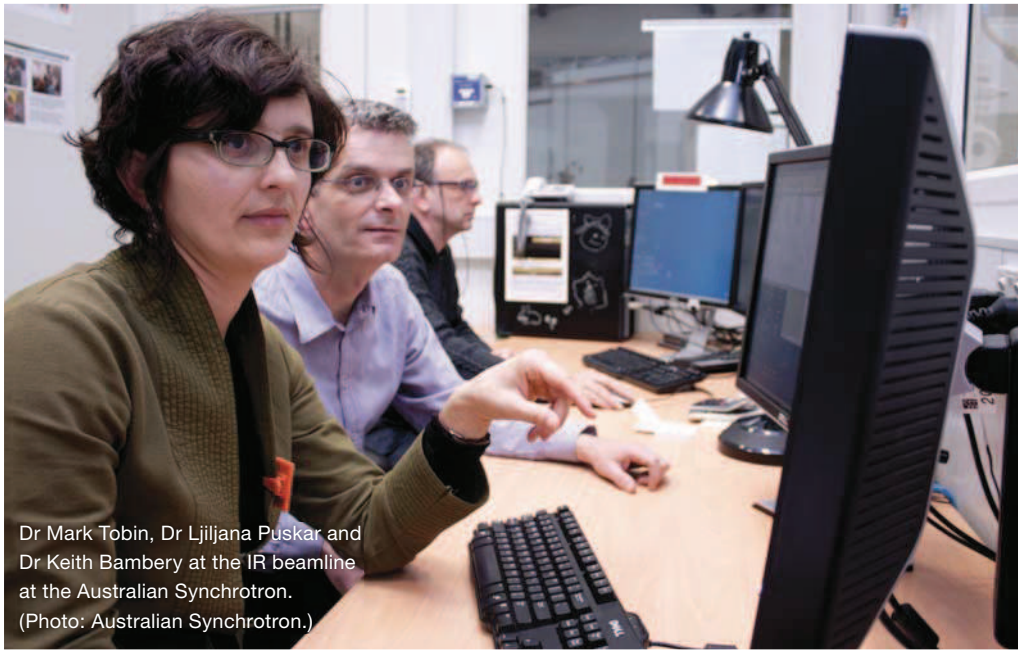
"We needed to identify the original layers, which are sometimes covered by several thin layers of 30-100 microns in thickness to work through," she says. "The synchrotron is very important for such work because quite often we receive only a small amount of pigment from the artwork to analyse and you need the high-energy beam sensitivity to characterise each layer individually and distinguish them from the mix of layers. It is difficult to get that information from a conventional technique or microscope."

Occasionally the projects that Tobin and Psukar collaborate on at the IR beamline come with a few tricky issues. "For instance, we have one project with a group at the University of Adelaide that is half way between materials science and biology, looking at what the researchers term biolubricants," says Tobin.

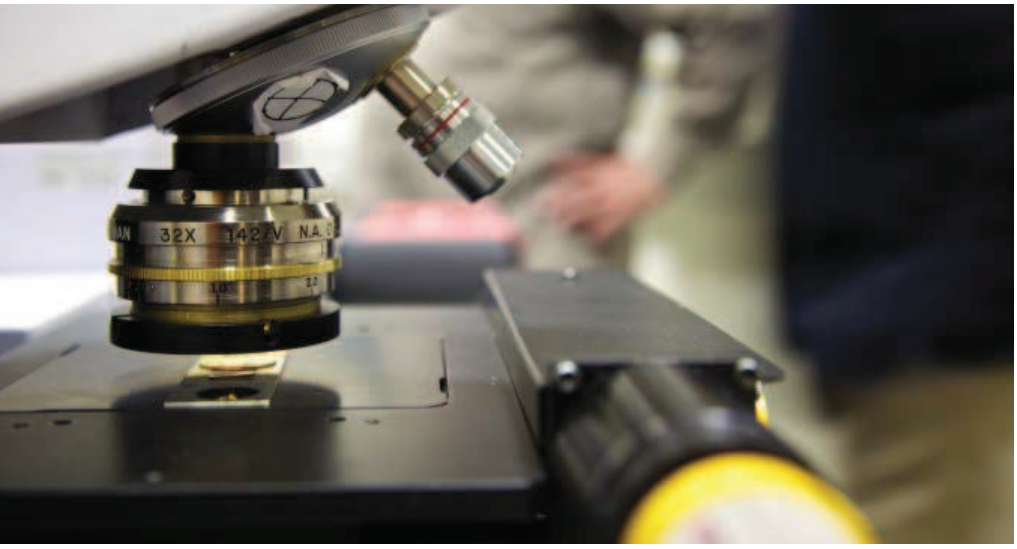
"These are layered molecules that mimic the natural biolubricants in joints etc. and around tissues in the body, and we want to know how these materials respond to the different stresses present in a body or biological system."

To do this, Tobin and his colleagues designed a small liquid cell that goes in the microscope and allows them to vary the pressure applied to the sample and measure how the molecular conformation of these lubricants respond in terms of their structure and water content. "This project has in part shown that we can get a range of measurements from one sample that is just not possible to gain any other way."

In the biomedical arena, the Synchrotron users examine tissues, as well as live and fixed cells, and the team at the Synchrotron has developed specialised equipment to meet specific user experimental goals. A recent example of this ingenuity is a flow cell, which keeps cells alive while allowing the flow of specific factors such as nutrients or drugs



Dr Mark Tobin, Dr Ljiljana Puskar and Dr Keith Bambery at the IR beamline at the Australian Synchrotron. (Photo: Australian Synchrotron.)



through the cellular environment. The effects of such components can then be assessed by measuring various chemical and constitutional changes over time.

"Spectral changes in the cellular DNA or protein can determine whether the drug, or whatever is being tested, is directly affecting the DNA in the nucleus, or whether it is affecting other protein pathways in the cell, such as the activation of receptors," explains Tobin. "These sorts of changes are not detectable by other conventional means of analysing protein and DNA, especially in a live cell."

SCATTERING ISSUES

One major barrier to the advancement of IR microspectroscopy in biological research has always been an aberration called resonant Mie scattering (RMieS), and the IR beamline team recently held a workshop on new advances in correcting for this problem.

Named after the first person to describe this type of scattering, Mie scattering can easily ruin a perfectly good IR analysis, as Dr Keith Bambery from the team explains. "When measuring samples in air, as is the case with many biological samples, you get a lot of light scattering from the sample due to the large difference in refractive index. The IR spectra reflecting off the different molecules and molecular structures in these samples gets confounded or spoiled on many occasion by the contributions to the spectra coming from this scattering of light," says Bambery.

A group from Manchester has just completed an extensive theoretical analysis of this scattered light over the last few years and worked out a way to remove the scattering effects, leaving just the pure sample-derived spectral data. The correction involves computationally modelling the likely scattering in the sample and then subtracting it away.

However, the correction process is very computationally intensive, says Bambery. "To do one spectrum is fairly quick and easy, but we are often taking maps of areas of the samples and these may generate thousands to tens of thousands of spectra per area of tissue or culture of cells. And when you get into those sorts of numbers that you want to correct for the scattering, it can take an awfully long time – days on an ordinary computer."

When Bambery joined the IR beamline team at the Australian Synchrotron at the beginning of this year, and found out that there was a high-performance computing cluster on site called MASSIVE (Multi-modal Australian Sciences Imaging and Visualisation Environment), he immediately saw the potential to speed up the new Mie scattering correction program. And that is what he achieved

Bambery developed an interface, a workflow and special software that allows users to submit their large sets of map data for Mie scattering correction using MASSIVE and get the results back around 100 times faster than possible using the fastest personal computers today. So now such data goes directly from the user to the high-performance computer system at the Synchrotron and then the results are fed back to the user, either at the Synchrotron's workstation or in the comfort of their own lab.

"One major advantage of this is users can be acquiring their data on the IR microscope and then move to the room next door and do the computing analysis in a similar time to what it takes to acquire the data. This means they can look at their results straight away and alter the experimental conditions as necessary before the next acquisition or experiment during their expensive beam time." ALS

In focus

In June of this year, Puskar organised the third in a series of IR data analysis workshops at the Australian Synchrotron. This one was specifically on the recently developed resonant Mie scattering (RMieS) technique to remove distortions in IR spectra that are particularly prevalent in biological samples.

"The workshop was designed to inform and train all interested users in this new correction method at the same time," says Puskar. "So users doing all sorts of different research could get together and discuss their common issues, both in using the IR beamline and in getting the best chemical information possible from their sample using the correction algorithm."

Workshop participants had some interesting IR projects of their own, including phytoplankton and sea ice, regeneration of corals and fingerprint analysis for forensic purposes. Workship attendees were also introduced to the MASSIVE high performance computing cluster and its new role in reducing the computation time for RMieS correction by up to 100 times.