Infrared Spectroscopy and Microscopy Using Synchrotron Radiation

Ljiljana Puskar
IR Beamline Scientist
Australian Synchrotron

- Infrared Spectroscopy and Microscopy
- IR Spectroscopy Using a Synchrotron
- The Infrared Beamline at the Australian Synchrotron
- Applications of Synchrotron Infrared Microscopy
- Future Developments
Australian Synchrotron in Clayton

The Synchrotron World Map – as seen from Australia
### Synchrotron Facilities

<table>
<thead>
<tr>
<th>Facility</th>
<th>Circumference</th>
<th>Country</th>
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<tbody>
<tr>
<td>ESRF, Grenoble, France</td>
<td>844 m</td>
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<td>ELLETRA, Italy</td>
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<tr>
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<tr>
<td>Spring-8, Japan</td>
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<tr>
<td>ALBA, Barcelona, Spain</td>
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### Table: Number of Beamlines and Facilities

<table>
<thead>
<tr>
<th>Region</th>
<th>Facility</th>
<th>Number of Beamlines</th>
<th>Purpose</th>
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<tbody>
<tr>
<td>America and Canada</td>
<td>ALS Berkeley</td>
<td>1</td>
<td>Microscopy and Far-IR</td>
<td>Operational</td>
</tr>
<tr>
<td></td>
<td>CAMD Baton Rouge</td>
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<td>Microscopy</td>
<td>Planned</td>
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<tr>
<td></td>
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<td>2</td>
<td>1 for microscopy, 1 for Far-IR</td>
<td>Operational</td>
</tr>
<tr>
<td></td>
<td>NSLS Brookhaven</td>
<td>6</td>
<td>3 Microscopy, 2 Far-IR, 1 THz</td>
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<tr>
<td></td>
<td>Surf III Gathersburg</td>
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<td>Microscopy</td>
<td>Planned</td>
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<td></td>
<td>SRC Madison</td>
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<td>SESAME, Jordan</td>
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**Europe**

<table>
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<td>ELETTRA, Trieste</td>
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<td>Far-IR</td>
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<tr>
<td>SLS, Villigen</td>
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<td>Microscopy and Far-IR</td>
<td>Commissioning</td>
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<td>ANKA, Karlsruhe</td>
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<td>BESSY II, Berlin</td>
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<td>Microscopy</td>
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<tr>
<td>SRS, Daresbury</td>
<td>1</td>
<td>Microscopy and Far-IR</td>
<td>Closed 4th August 08</td>
</tr>
<tr>
<td>DIAMOND, Didcot</td>
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<td>Microscopy</td>
<td>Users expected Oct 2009</td>
</tr>
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<td>ALBA, Barcelona</td>
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**Notes:**
- **Closed 4th August 08**: Facility was closed on that date.
- **Users expected Oct 2009!**: Facility expected to open for users in October 2009.
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**34 IR Beamlines in the world!**

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**Introduction to Infrared Spectroscopy...**
Infrared Spectroscopy

Infrared spectroscopy provides information on molecular vibrations and allows chemical fingerprinting. This is a non-destructive technique which requires only a small amount of sample for analysis; it is widely used for the analysis of both organic and inorganic samples.
Rotational, Vibrational and Electronic transitions

Typical Infrared Spectrum of Biological sample

Each cellular component has distinct IR marker bands.
Method of data collection
Fourier transform Infrared spectroscopy

Many frequencies are present in the infrared beam

Position of “zero path difference”
Summing of all frequencies for each position of the mirror

Data output from FTIR system

“Centre burst” at Zero Path Difference

Interferogram  Fourier Transform  Single Beam Spectrum
Infrared spectroscopy and microspectroscopy

Wavenumbers (cm⁻¹)

4000 3500 3000 2500 2000 1500 1000 500

Absorbance

0.60 0.50 0.40 0.30 0.20 0.10

Data output from FTIR system

So why use a Synchrotron?
It’s the synchrotron BRIGHTNESS that counts.

- Small source: better throughput with small samples
- Highly collimated: higher resolution achievable
- Polarized: ellipsometry
- Pulsed: pump & probe experiments

SR Advantages over thermal sources

Brightness: Better Signal to Noise ratio!

\[
B = \frac{P}{\Delta A \cdot \Delta \Omega}
\]

Power per unit area per unit solid angle
Synchrotron infrared beam focused on sample

Microscope Beamline at SRS - unapertured beam profile at sample stage. Area mapped = 30x30 µm. Beam halfwidth = 8x8 µm.

Single malaria infected cells at different stages of the intra-erythrocytic life cycle

Grant Webster, Don McNaughton, Bayden Wood, Monash University, Torsten Frosch (University Jena)
Infrared emission from a synchrotron bending magnet

- Bright
- Broadband
- Pulsed
- Polarised

Edge Radiation and Bending Magnet Radiation
Visible light in the beamsplitter vessel at the Australian Synchrotron Infrared beamline

Visible light in the beamsplitter vessel at the Australian Synchrotron Infrared beamline

Visible light in the beamsplitter vessel at the Australian Synchrotron Infrared beamline
Mirror inserted into dipole chamber from side

Specially adapted Infrared Dipole Chamber at Australian Synchrotron
Dipole Chamber in Storage Ring and Mirror M1 prior to Installation

Infrared dipole chamber with vacuum isolation gate valves installed

Mirror M1 undergoing vibration testing prior to installation

M1 Mirror Inserted (left) and Withdrawn (right)

Bellows fully extended

Note: M2 mirror chamber not yet installed in this photo
Matching optics for High Resolution FTIR

Matching optics for IR Microscope

Beamsplitter optics

Diamond Window and Gate Valves

Storage ring wall

M1 mirror mechanism

Focusing and Steering mirrors

M1

M2

M3

M3a

M1 mirror mechanism

Visible Beam Profile in Beamsplitter Vessel and at Entrance to V80v Spectrometer

Visible beam profile in Beamsplitter Vessel

Collimated beam at entrance to FTIR spectrometer
IR beam profile – comparison with SRW

Photo of the Australian Synchrotron Infrared beamline
Mirror M1 inserted into dipole “crotch” from above or below

e.g. Soleil, ESRF...

Images courtesy of Paul Dumas, Soleil.
Multiple beam extraction from the bending magnet

Infrared Environmental Imaging (IRENI) at the Synchrotron Radiation Center, UW-Madison

The light from a bending magnet is separated into 12 collimated Synchrotron beams rearranged back into a 3x4 matrix and sent into an IR microscope and spectrometer (48 mirrors in total).


First light August 2008

Infrared Beamline at the Australian Synchrotron: Microscope branch

Bruker V80v with Hyperion 2000 microscope
Confocal point scanning - current technology

Narrow-Band MCT
50x50 micron

Wide-Band MCT
250x250 micron

Infrared Detectors
Some currently available IR detectors
Focal Plane Array Detectors

- Developed for the US military
- Multi-element IR detector

Bruker Hyperion 3000: Focal Plane Array Detector

Solid state array with 64 x 64 pixels elements so that 4,096 spectra can be acquired simultaneously.

FPA imaging vs SR-FTIR single point mapping

Early stages of Experimental Autoimmune Encephalitis (model for MS) detected in animals before onset of clinical symptoms

Map showing ester carbonyl absorbance (1740 cm$^{-1}$)

Phil Heraud, Claude Bernard, Vivienne Juan, Sally Caine, Monash Immunology and Stem Cell Laboratories
Far IR and High Resolution branch

Beamsplitters
- Multi/Mylar: 30 – 630 & 12 – 35 cm\(^{-1}\)
- Ge/KBr: 450 – 4 800 cm\(^{-1}\)

IR Detectors
- Si bolometer: 10 – 370 cm\(^{-1}\)
- Si:B bolometer: 300 – 1850 cm\(^{-1}\)
- DTGS: 100 – 3000 cm\(^{-1}\)
- MCT\(_S\): 700 – 5 000 cm\(^{-1}\)
- MCT\(_M\): 600 – 5 000 cm\(^{-1}\)

Sources
- Synchrotron: \(mw \rightarrow vis\)
- Hg-Arc lamp: 5 – 1 000 cm\(^{-1}\)
- Globar: 10 – 13 000 cm\(^{-1}\)
- Tungsten lamp: 1 000 – 25 000 cm\(^{-1}\)

Optical Filters
- series of narrow band pass IR filters

Apertures
- 0.5 – 12.5 mm

Bruker IFS 125HR FTIR Spectrometer
Spectral resolution > 0.001 cm\(^{-1}\)
Far and Mid-IR capability.

Multipass gas cell for room temperature samples.

Enclosive Flow Cooling cell for cryogenic temperatures.

Small quantity of sample to minimize Pressure broadening effects.
Portion of the Far-IR spectrum of Formamide at 0.00096 cm\(^{-1}\) resolution

Far-IR Gas phase applications
- Atmospheric: CFC’s, HFC’s, HCFC’s
- Chemical dynamics: Radicals
- Astrophysics: Hydrocarbons, Radicals

Far-IR condensed phase
- Geological: clay samples
- Metal oxides
- Biomolecules
- Protein structures
- Clusters
Advantage of using a synchrotron seen in spectra…

Absorbance spectra of tissue sample recorded at 10 µm spatial resolution under identical collection conditions using a Globar™ infrared source and synchrotron radiation.
Wavelength dependence of microscope spatial resolution demonstrated at Infrared beamline

Polymer pattern on CaF$_2$ produced by photolithography

IR absorbance image
At 2935 ±125 cm$^{-1}$

IR absorbance image
At 1701 ± 59 cm$^{-1}$

FIR Synchrotron performance < 400 cm$^{-1}$

Exp. Conditions: Si Bolo detector, 4 mm apt., 6 μm Mylar beamsplitter

Acquisition Time
SR(164mA) 480 mins (1 shift)
Hg <14 mins

Ratio of Intensities

% Transmission

Wavenumber/cm$^{-1}$
Mounting samples on the IR microscope

Infrared objective

Motorised sample stage
Mounting samples on the IR microscope

1. Special resolution patterns on mirror slides, for testing the beamline performance.
2. Transmitting disks such as zinc selenide (yellow), CaF₂, BaF₂ for thin sections of samples.
3. Gold or aluminium mirrors for materials such as fine powders and biological cells.
4. Very hard samples can be embedded in plastic and polished.
5. Biological samples such as tissue sections mounted on specially coated glass microscope slides.
6. Silicon Nitride membranes (also suited for X-ray microscpectroscopy).
Types of measurements for IR microscope

- Transmission
- Reflectance
- ‘Transreflectance’
- Grazing angle Reflectance measurements
- Attenuated Total Reflectance

Transmission measurements

- Samples should be 10 microns or thinner, either freestanding or supported on an IR transmitting material such as KBr, BaF$_2$, ZnSe or CaF$_2$ windows or the silicon nitride membrane.
- Flow through liquid cells or compression cells are also used.
Transmission measurements:
Study of polymer laminates – simple identification of layers
Industrial and forensic applications

Sample preparation

- Polymer laminates from three packaging materials
- Samples microtomed and mounted between two diamonds
- Focused IR beam used to identify polymer layers

Reflectance measurements

- Ideally requires a polished flat surface
- Spectra require additional correction procedures due to dispersion artefacts (Kramers-Kronig-Transformation).
**Reflectance measurements**

Conservation of culturally important materials

**Sample preparation**

Paint chip embedded in resin. Surface of the sample must be very well polished.

*Stephen Best, Caroline Kyi, Robyn Sloggett (Melbourne University and Centre for Cultural Materials Conservation)*

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**“Transreflectance” measurements**

Biological samples on specially coated glass microscope slides

Absorption/reflection (Ag undercoat/SnO₂ overcoat), visible transmission. Cheaper than IR transmitting windows but not re-usable. Sample should be between 5 and 10 microns thick depending on material. Problem: dispersion artifacts in thin parts of tissue.
Grazing angle Reflectance measurements

The grazing angle objective provides IR radiation at grazing incidence (65 to 85 degrees) for the analysis of ultra-thin (sub-micron) coatings on metallic substrates.

Poly ethylene glycol monolayer gradient on reflective surfaces (eg. ITO glass) can be studied. Understand the mechanism of protein repellent properties of PEG coatings.

Donna Menzies, Thomas Gengenbach, Celesta Fong, John Forsythe, Ben Muir – CSIRO / Monash University

Grazing angle Reflectance measurements

Spatial resolution

The resolution target (metal pattern on glass window) was used to test spatial resolution measurements.

The resulting IR images are shifted with respect to the visible image (~60 μm horizontal (right) shift and ~20 μm vertical shift (up).
Attenuated Total Reflection (ATR)

IR absorbance spectrum is generated when the IR beam reflects inside a crystal that is in contact with the sample.

Attenuated Total Reflection (ATR)

Forensic application: examination of paper documents

Conserving National Heritage: 19th century parchment Sample

Alana Treasure, Dudley Creagh (Uni Canberra) / Simon Lewis, Bill van Bronswijk (Curtin University) Kenneth Paul Kirkbride, Vincent Otieno-Alego (AFP)
APPLICATIONS OF SYNCHROTRON INFRARED LIGHT
Use of the IR Microscope at the Australian Synchrotron by research area-2008

Understanding the autoimmune disease: Rheumatoid Arthritis

Complete mouse paw image taken using Vis 4x objective.

Looking at the changes that occur in the cartilage in the tips of the paws of mice with arthritis.

Light microscope image after staining the cartilage. The colour change at the surface shows cartilage damage.

The region of the cartilage examined. Spectra were obtained from the cells marked in red. 5x5 µm aperture size was used.

Average spectra from single cells in the cartilage showing the large differences between control mice (blue) and mice with arthritis (purple).

Mice with arthritis
Control mice

Allyson Croxford and Merrill Joy Rowley (Monash Uni)
Infrared analysis of fingerprints
Study of in-situ chemistry of novel revealing agents

Treated and untreated latent fingerprints on aluminium backed cellulose TLC plates were analysed.

Comparison shows slight variations between samples.

Conventional IR: spectra obtained are dominated by cellulose (background) to a degree where the sample can not be distinguished. Synchrotron ATR allows in-situ analysis of Lawsone (2-Hydroxy-1,4-naphthoquinone).

Studying naturally occurring revealing agents – Lawsone


Renee Jelly, Emma Patton, Simon W. Lewis, Keiran Lim, Bill van Bronswijk

Conservation of culturally important materials

• Study cross-sections of paint chips from the Provincial Hotel in Fitzroy imbedded in polymer.

• Obtain information on pigment, binder and filler distribution.

Stephen Best, Caroline Kyi, Robyn Sloggett (Melbourne University)
IR research in High Pressure

[A] Nicolet IR microscope and Nicolet Magna-IR 560 Spectrometer with optics matching boxes integrating the microscope to the IR beam.

[B] Pressure control and pressure calibration (Ruby fluorescence) set up.

The Diamond Anvil Cell

Custom made DAC with a membrane system for pressure application
(Institut de Minéralogie et Physique des Milieux Condensés, Université Pierre et Marie Curie, Paris).

Type II diamonds with 500 microns culets diameter.
High pressure phase change of carbonate minerals to understand the behaviour of carbon in the mantle.

<table>
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<th>Band</th>
<th>Assignment</th>
<th>Ba(CO$_3^-$) / cm$^{-1}$</th>
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<td>$\nu_2$</td>
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<td>$\nu_4$</td>
<td>In plane bending of the carbonate group</td>
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Environmental studies: Nutrient response in a model photosynthetic microorganism

IR maps (series of line maps across the centre of the cell taken every 30 min over 24 h period) describing the effect the nutrient conditions change has on cell.

Image (left) of freshwater alga *Micrasterias hardyi*. Experiment set-up on IR beamline (right)

*Phil Heraud, Anthony Eden, Don McNaughton, Bayden Wood* Monash University
Environmental Science applications

IR synchrotron microspectroscopy reveals microscale biochemical changes occurring in living plant cells.

This allows researchers to better understand how plant cells respond to changes in the environment.

FTIR maps (right) of freshwater alga *Micrasterias hardyi*.

Phil Heraud, Anthony Eden, Don McNaughton, Bayden Wood, Monash University

Monitoring the biological effect of chemotherapeutic drugs

Study single live Leukaemia Cells using in fabricated CaF₂ liquid cell.

Carolyn Dillon, Kristie Munro (University of Wollongong), Keith Bambery, Bayden Wood (Monash University)
An AFM-based thermal probe is used to map the Si$_3$N$_4$ sources in contact with the substrate bond pads.

- These probes can measure:
  - Force
  - Temperature
- They can act as highly localised heat sources

Hubert Pollock, University of Lancaster UK, Alexandre Dazzi, Université Paris Sud, Mike Reading, UEA, UK
Coherent Synchrotron Radiation

Use of coherent enhancement for Far-IR and THz studies

Summary

- Synchrotrons provide intense beams at long wavelengths into the Far-IR.
- IR spectroscopy provide information on the chemical composition of materials based on the vibration of the bonds present.
- Synchrotron IR allows rapid measurements of diffraction limited SPATIAL resolution (a few microns using IR microscope), or at low concentration (and high SPECTRAL resolution).
- Synchrotron IR applications in a diverse range of research areas.
- Future developments will allow imaging below the diffraction limit and the use of intense Far-IR and Terahertz beams.
IR beamline staff at the Australian Synchrotron

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• Don McNaughton – Monash University
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• Emma Patton – Curtin University
• Simon Lewis – Curtin University
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• Dudley Creagh – Canberra University
• Alana Treasure – Canberra University
• Allyson Croxford – Monash University
• Merrill Joy Rowley – Monash University
• Donna Mensies – Monash University
• Thomas Gengenbach – CSIRO
• Celesta Fong – CSIRO
• John Forsythe – Monash University
• Ben Muir – Monash University
• Mark Hackett – University of Sydney
• Liz Carter – University of Sydney
• Peter Lay – University of Sydney

• Stephen Best – Melbourne University
• Robyn Sloggett – Melbourne University
• Caroline Kyi – Melbourne University
• Mark Tobin – Australian Synchrotron
• Dom Appadoo – Australian Synchrotron
• Danielle Martin – Australian Synchrotron
• Carol Hirschmugl – University Wisconsin
• Michael Nasse – University Wisconsin
• Paul Dumas – Soleil
• Jean-Paul Ilbé – Université Pierre et Marie Curie
• Larry Car – Brookhaven
• Phil Heraud – MSCL
• Sally Gaine – MSCL
• Janice Williams – La Trobe University
• Ewen Silvester – La Trobe University
• Carolyn Dillon – University of Wollongong
• Kristie Munro – University of Wollongong
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