• 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

The Hobo-Dyer Equal Area Projection

This new map belongs to the family of Cylindrical Equal Area projections in which the latitude and longitude lines form a rectangular grid. Other projections in this family include the Lambert, Carl Bätteners, Edwardes, and Peters projections. In the present case the "cylinder" is assumed to wrap round the globe and cut through it at 45° north and south. In order to preserve the equal area property the shapes of the landmasses become progressively flattened towards the poles, but shapes between 45° north and south are well preserved.
## Infrared beamlines worldwide

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of beamlines</th>
<th>Purpose</th>
<th>Status</th>
</tr>
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<tbody>
<tr>
<td><strong>America and Canada</strong></td>
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<tr>
<td>ALS Berkeley</td>
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<td>Microscopy and Far-IR</td>
<td>Operational</td>
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<td>CLS Saskatoon</td>
<td>2</td>
<td>1 for microscopy, 1 for Far-IR</td>
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<td>NSLS Brookhaven</td>
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<td>3 Microscopy, 2 Far-IR, 1 THz</td>
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<td>SRC Madison</td>
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<td>Microscopy</td>
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<td><strong>Asia and Australia</strong></td>
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<td>Operational</td>
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<td>INDUS I, India</td>
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<td>Helios II, Singapore</td>
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<td>Operational</td>
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<td>Spring-8, Himeji</td>
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<td>UVSOR, Okazaki</td>
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<td>Far-IR</td>
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<td><strong>Europe</strong></td>
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<td>Soleil, St. Aubin</td>
<td>2</td>
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<td>Under Construction</td>
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<td>ELETTRA, Trieste</td>
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<td>2</td>
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<td>Far-IR operational, microscopy under construction</td>
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<tr>
<td>SRS, Daresbury</td>
<td>2</td>
<td>1 Microscopy, 1 Far-IR</td>
<td>Operational</td>
</tr>
<tr>
<td>DIAMOND, Didcot</td>
<td>1</td>
<td>Microscopy</td>
<td>Planned</td>
</tr>
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INTRODUCTION TO INFRARED SPECTROSCOPY
Spectroscopy...
Infrared spectroscopy

- Absorbance
- PO₃²⁻ stretching
- PO₂⁻ stretching
- C-O stretch
- CH₃ bending
- Broad O-H and N-H stretching
- CH₂ and CH₃ stretching
- C=O
- Amide I
- C-N and N-H
- Amide II
Method of data collection

Fourier Transform Infrared is more common, but dispersive has applications, particularly for fast timing with intense beams.
Many frequencies are present in the infrared beam
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**
Data output from FTIR system

Background Single Beam Spectrum

Sample Single Beam Spectrum

Sample Absorbance Spectrum

Absorbance

Wavenumbers (cm⁻¹)
Infrared spectroscopy and microspectroscopy - Instrumentation

Generally designed for benchtop Mid-IR sources

So why use a Synchrotron?
Is the synchrotron IR beam very intense?

It's the synchrotron brightness that counts.
EXTRACTION OF INFRARED LIGHT FROM A SYNCHROTRON
Infrared mission from a synchrotron bending magnet

Edge Radiation and Bending Magnet Radiation

- Bright
- Broadband
- Pulsed

Large vertical divergence Relative to X-ray beam
Visible light in the beamsplitter vessel at the Australian Synchrotron Infrared beamline

Edge radiation to “high resolution” spectrometer

Bending magnet radiation to “microscope”
ALTERNATIVE METHODS OF EXTRACTION
1. Large aperture in dipole with M1 external to synchrotron

Beams exits from synchrotron
M2 mirror reflects beam vertically
"Cooled finger" protects M1 from high energy X-rays.
M1 mirror focuses and redirects beam

To the microscope

The collimated beam, viewed with an infrared image intensifier.

Mid IR beam profile at sample

e.g. SRS at Daresbury Laboratory
2. **Mirror M1 inserted into dipole “crotch” from above or below**

e.g. Soleil, ESRF...

Images courtesy of Paul Dumas, Soleil.
2. Mirror inserted into dipole chamber from side

e.g. Australian Synchrotron

Which brings me to...
The Australian Synchrotron Infrared Beamline
Adapted Infrared Dipole Chamber at Australian Synchrotron
Dipole Chamber in Storage Ring and Mirror M1 prior to Installation

Infrared dipole chamber installed with vacuum isolation gate valves installed

Mirror M1 undergoing vibration testing prior to installation
M1 Mirror Inserted (left) and Withdrawn (right)

M1 mirror carriage

M2 mirror chamber

IN

OUT

Bellows fully extended

Note: M2 mirror chamber not yet installed in this photo
Infrared beamline showing (from right) synchrotron beam entering front end optics (M1, M2, M3, M3a), diamond exit window, beamsplitter optics vessel and matching optics boxes for the two endstation instruments.
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
Infrared Beamline at the Australian Synchrotron
Microscope Branch

Bruker V80v with Hyperion 2000 microscope
INFRARED BEAM LINE INSTRUMENTATION
Confocal point scanning - current technology

- Narrow-Band MCT 50x50 micron
- Wide-Band MCT 250x250 micron
Focal plane imaging - Example

- 2x8 FPA
- Objective
- Sample
- "Condenser"
- Condenser
Infrared Beamline at the Australian Synchrotron
High Resolution branch

Bruker IFS 125HR High Resolution FTIR Spectrometer
Multipass gas cell for high resolution spectroscopy
Side view of beam path through microscope after interferometer

Adjustable aperture

Mirror 10 reflects towards detector

Beam from interferometer (horizontal)

32x Objective

Sample

32x Condenser

Typical confocal point scanning IR microscope
Infrared Detectors
Some currently available IR detectors

Narrow-Band MCT
D* 4x10^10
Cut-off 750 cm⁻¹

Wide-Band MCT
D* 5x10^9
Cut-off 420 cm⁻¹

Mid-Band MCT
D* 2.5x10^10
Cut-off 600 cm⁻¹

Background limited performance
Typical far-IR bolometer with cryostat
Two formats of focal plane array imaging systems

64x64 photovoltaic MCT array

2x8 photoconductive MCT array
Assessing beamline performance
Beamline 11 at SRS - unapertured beam profile at sample stage.
Area mapped = 30x30 µm. Beam halfwidth = 8x8 µm.
Absorbance spectra of tissue sample recorded at 10 µm spatial resolution under identical collection conditions using a Globar™ infrared source and synchrotron radiation.

Advantage of using a synchrotron seen in spectra…

CH stretch absorption bands from 5 micron spot in tissue sample. Equivalent spectra recorded using synchrotron (top) and Globar (bottom).
Testing the IR Beamline Performance with Custom Resolution targets
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$^{-1}$
APPLICATIONS OF SYNCHROTRON INFRARED LIGHT
Early stages of Experimental Autoimmune Encephalitis detected in animals before onset of clinical symptoms by FPA and Synchrotron IR

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

Phil Heraud, Claude Bernard, Vivienne Juan, Sally Caine
Monash Immunology and Stem Cell Laboratories
Oocyte *in vitro* maturation

- At present oocyte maturation *in vitro* is not efficient enough for routine clinical application.

- Although oocyte maturation has been achieved, it currently results in reduced development potential.

- There is no method to measure completion of cytoplasmic maturation, other than successful fertilization and embryonic and foetal development.

Bayden Wood, Don McNaughton, Alice Brandli, Cassie Jean
Monash University
Germinal Vesicle Oocyte

5 μm x 5 μm aperture
97 mA current
16 scans 6cm⁻¹

1236 cm⁻¹
Amide II
1544 cm⁻¹

Nucleic acid band
1236 cm⁻¹
FTIR synchrotron maps of a GV oocyte

5 × 5 μm aperture
2 μm step size
16 scans, 6 cm⁻¹

3000-2832 Lipid

1700-1600- protein

1260-1230- Nucleic acid
Mineralization in the major lateral teeth of the chiton *Acanthopleura echinata*.

(a) SEM of a representative section of the radula. Arrows indicate the major lateral teeth (scale bar = 1 mm).

(b) Back-scattered electron micrograph of a ground and polished major lateral tooth in longitudinal section, depicting the six major regions of mineralization:
- **a**, the magnetite region that comprises the posterior cutting surface;
- **b**, the lepidocrocite region;
- **c**, the anterior apatite region;
- **d**, the centro-posterior apatite region;
- **e**, the junction between the tooth cusp and its base;
- **f**, the tooth base (scale bar = 100 μm);

**A** and **P** refer to the anterior and posterior surfaces, respectively.

(c) Diagrammatic representation of a major lateral tooth depicting the various regions found in a fully mineralized major lateral tooth.

Biomineralisation in Chiton Teeth

Bill van Bronswijk
Curtin University, Perth
Infrared absorbance maps of single chiton tooth collected using IR microscope at Australian Synchrotron operating in reflectance mode.

Reflected light visible image of polished tooth section

1030-1153 cm\(^{-1}\)
Probably apatite

1020-1190 cm\(^{-1}\)
Possible strong carbonyl stretch
IR mapping of cerebellum tissue infected with cerebral malaria

Lipid to protein ratio indicated in IR map (high = red, low = blue)

Liz Carter, Mark Hackett
University of Sydney
Heart Attack
- Blockage of a major artery producing a hypoxic environment i.e. low oxygen
- Treatment removes the blockage but also provides a ‘burst’ of oxygen that leads to generation of free radical species
- Ischemic reperfusion injury (IRI)

Current research
- Antioxidant development
- *In vitro* model of IRI used to investigate intracellular changes


Functional Group Maps of Cardiomyocytes

Cardiomyocytes subjected to hypoxia/re-oxygenation (H/R) injury

- Increase in level of mitochondrial dysfunction
- Increase in level of apoptosis and necrosis
- IR functional group maps visualise the loss of lipid and protein structure.
- Particular evident in nuclear region of cell.

A) White light image of cardiac myocyte
B) Amide I (1771–1587 cm⁻¹)
C) CH region (3000–2842 cm⁻¹)
Anti-Oxidant Effectiveness

Aim:
- To use IR spectroscopy to test the effectiveness of an antioxidant in ameliorating damage caused by H/R injury
FTIR Microspectroscopy of Diseased Tissue

1155 / 1170 cm\(^{-1}\)

Bright points show non-malignant IR profile

1170 / 1155 cm\(^{-1}\)

Bright points show malignant IR profile

970 cm\(^{-1}\)

Bright points show malignant IR profile

Epithelium

Tumour
FTIR Mapping of the Cervical Transformation Zone
Bayden Wood, Monash Centre for Biospectroscopy
Michael Quinn, Royal Melbourne Hospital

1024 cm\(^{-1}\) glycogen distribution  1544 cm\(^{-1}\) protein distribution
The tenth cluster (orange) highlights two potential foci of dysplasia (pre-malignant cells)
High resolution FTIR imaging of membrane organisation in single cells

A431 cell showing lamellapodia during migration

FTIR map showing lipid distribution. High (red) areas probably due to location of Golgi membranes and associated vesicles.

FTIR peak ratio map showing areas in cell of high (red/green) and low (blue) CH$_2$/CH$_3$ stretch absorption.

Green area = possible membrane reorganisation at leading edge of cell
High pressure studies of minerals

Lawsonite is an important reservoir of water within the Earth’s mantle
- It is stable at very high pressures
- It contains 11% water
- O-H bonds are being used in studies of phase changes
IR synchrotron microspectroscopy reveals microscale biochemical changes occurring in living plant cells. This allows researchers to better understand how plants cells respond to changes in the environment. Image (left) and FTIR maps (right) of freshwater alga *Micrasterias hardyi.*

Phil Heraud, Anthony Eden, Don McNaughton, Bayden Wood
Monash University
Microfluidics for time resolved protein folding studies

Time resolved FTIR - complementary to CD, and benefits from highly focused SR-IR beam
Simple Brookhaven flow cell

- 50µm path length (so D₂O required)
- ZnSe windows
- ~ms time resolution claimed
- High flow rate (1 ml min⁻¹)
- 2% path length change under pressure
Microfluidics for time resolved protein folding studies

Main requirement - very high S/N in $\sim 10 \times 10^{-5}$ A.U. (5x10^{-5} A.U.)
Can integrate for seconds

Images and spectra courtesy of N. Kaun and B. Lendl, T.U. Vienna
Scientists used the SRS at Daresbury, UK to investigate a 27 centuries old Corinthian helmet and confirmed that the noseguard of the helmet was replaced in the 19th century.

They also identified corrosion products and measured the alloy metals used in its manufacture.
Cultural Heritage applications

The characterization of paint microscopic fragments gives information on binding materials, ageing products and the technology of the production of the pigments.

Catalan gothic altarpiece (Retaule del Conestable) by Jaume Huguet, 15th century, one of the most important artists of the period.
Evidence for presence of carminic acid in an egg albumin binding matrix.

SR-FTIR microspectroscopy spectra (128 scans, 4cm\(^{-1}\) resolution, spot size 10x10) from dark red layer (—) and aged egg white (—).
FUTURE DEVELOPMENTS IN SYNCHROTRON SPECTROSCOPY
Micromachined probe (University of Glasgow)

Silicon nitride
Fabrication process involves:
• Photolithography
• Potassium hydroxide etching
• Multiple levels of electron-beam lithography

Department of Electronics and Electrical Engineering, University of Glasgow
(John Weaver & Gordon Mills)

These probes can measure:
Force
Temperature
They can act as highly localised heat sources
Broadband IR
Modulated by interferometer

Sample
An AFM-based thermal probe is used to map the surface of samples in the SR-IR beam.

Azzedine Hammiche, Hubert Pollock
University of Lancaster UK
Transmission measurement of wet sample

Liquid cell, or flow cell

Max liquid depth ~ 10 µm
IR transmitting prism

\[ d = \frac{1}{2\pi n_1 \sqrt{\sin^2 \theta - (n_2/n_1)^2}} \]

For \( \lambda = 6 \, \mu m \) \( d = 1.6 \, \mu m \)

ATR spectroscopy – and imaging
Energy Recovering Linac at Daresbury Laboratory

New sources, including “Fourth Generation” sources and the use of coherent enhancement for Far-IR and THz studies
Summary

• Synchrotrons provide intense beams at long wavelengths into the Far-IR

• IR spectroscopy is used to provide information on the chemical composition of materials based on the vibration of the bonds present.

• Synchrotron IR allows these measurements to be made rapidly at a few microns dimension (micoscope), or at low concentration (and high SPECTRAL resolution).

• Synchrotron IR has applications in a diverse range of research areas.

• Future developments in the field will allow imaging below the diffraction limit and the use of intense Far-IR and Terahertz beams
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