Infrared Spectroscopy and Microscopy Using Synchrotron Radiation

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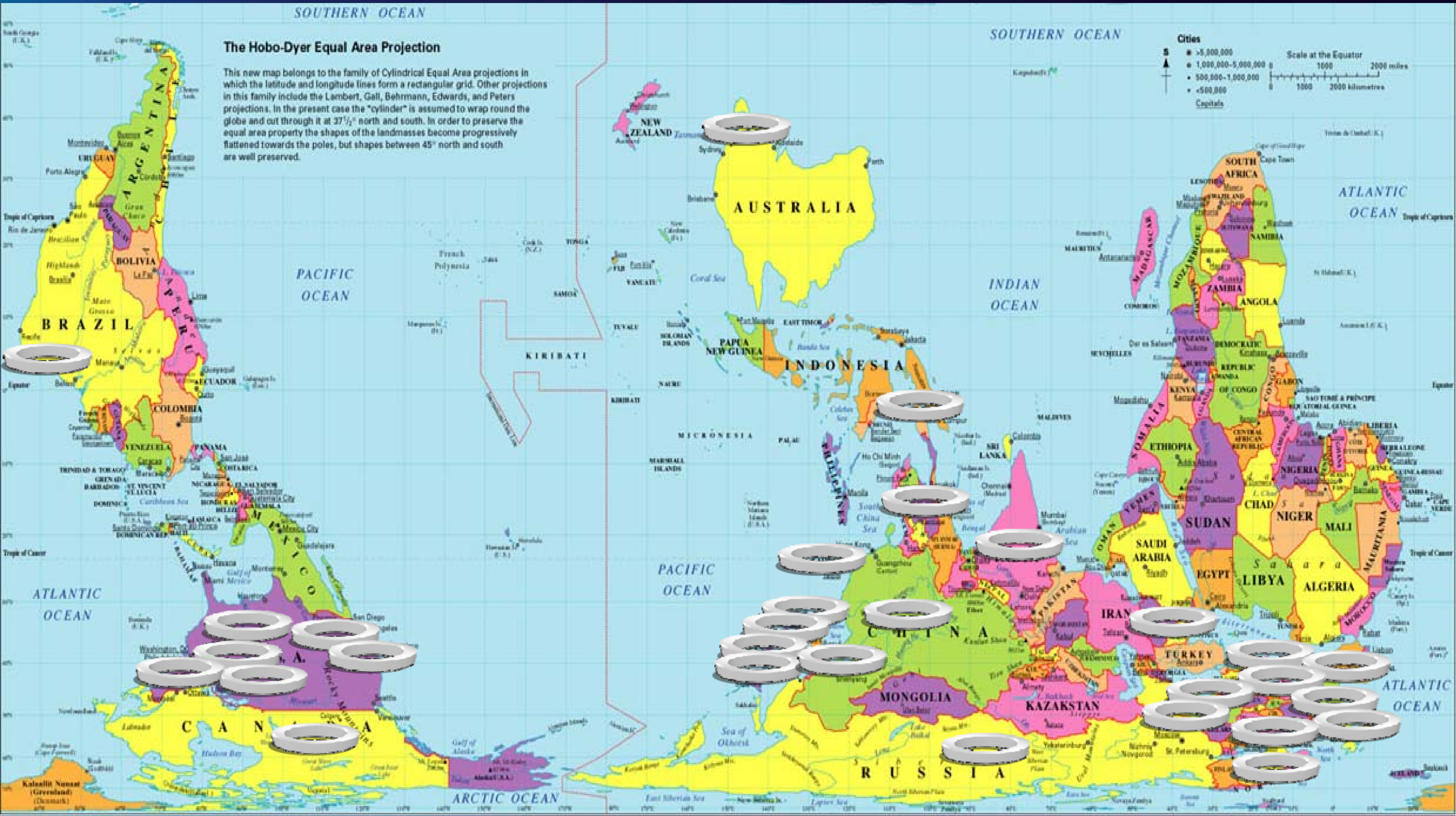
Australian Synchrotron

- 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

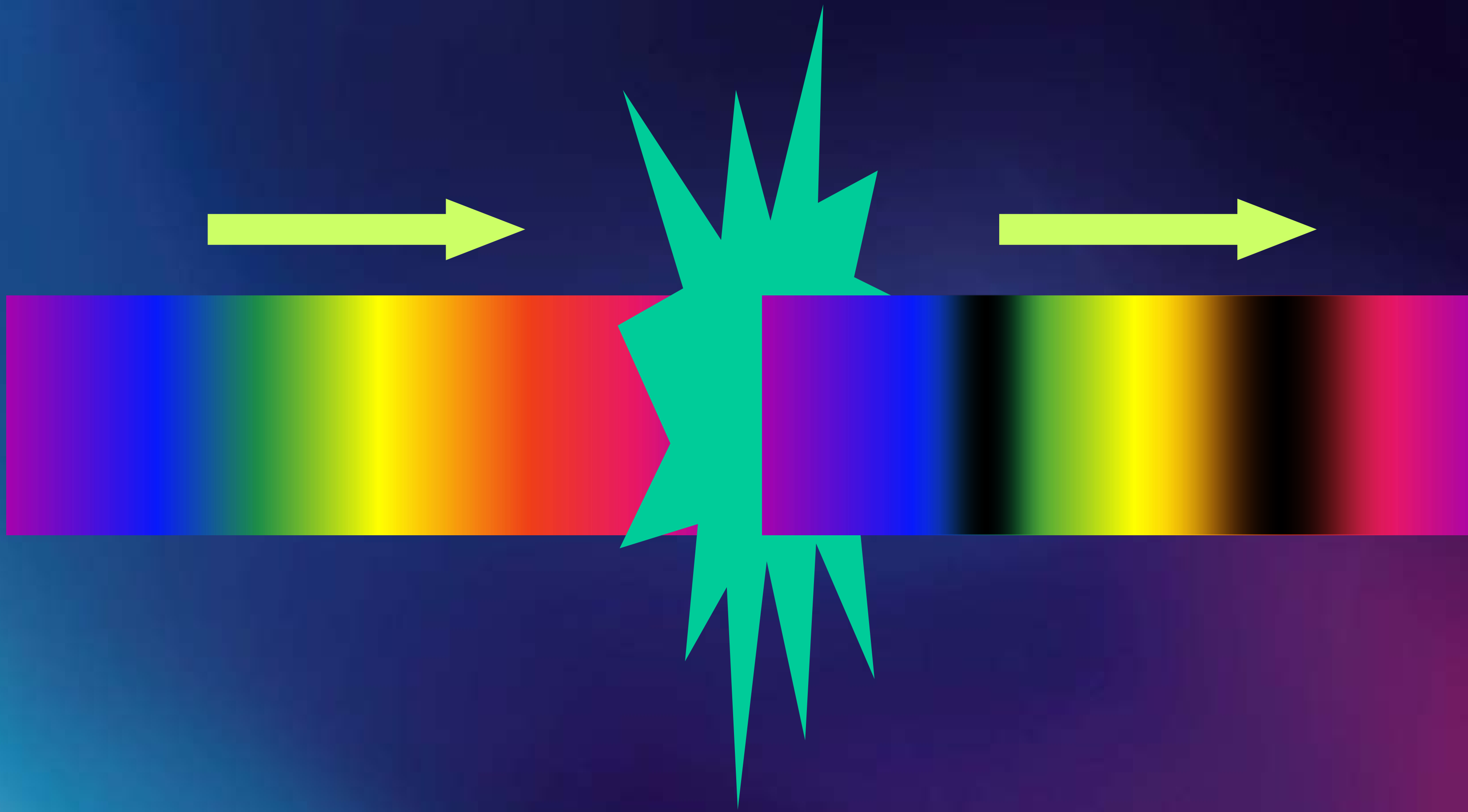


Infrared beamlines worldwide

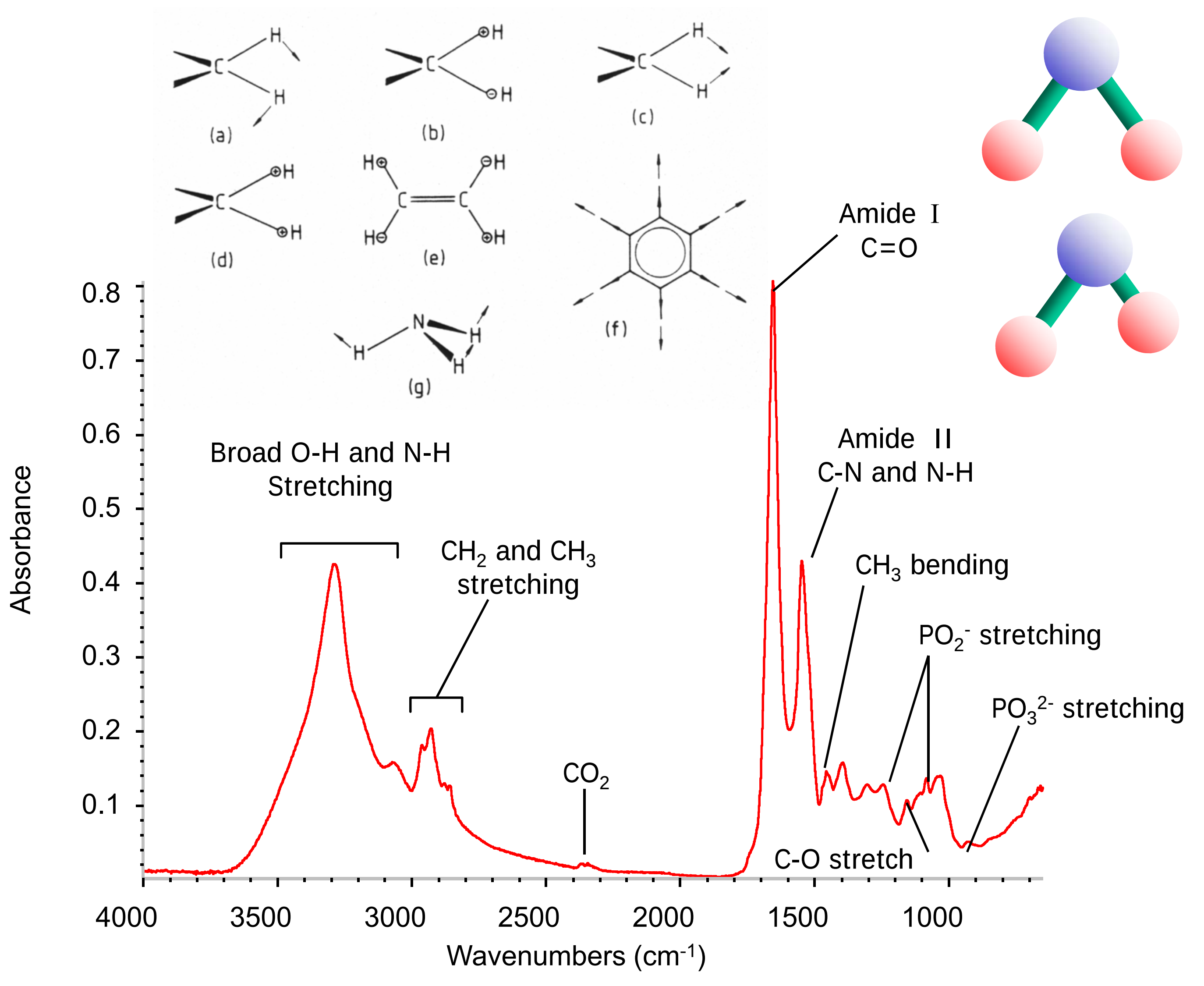
	Number of beamlines	Purpose	Status
America and Canada			
ALS Berkley	1	Microscopy and Far-IR	Operational
CAMD Baton Rouge	1	Microscopy	Planned
CLS Saskatoon	2	1 for microscopy, 1 for Far-IR	Operational
NSLS Brookhaven	6	3 Microscopy, 2 Far-IR, 1 THz	Operational
Surf III Gaithersburg	1	Microscopy	Planned
SRC Madison	1	Microscopy	Operational
Asia and Australia			
Australian Synchrotron	1	Microscopy and Far-IR	Operational
INDUS I, India	1	Microscopy	Planned
Helios II, Singapore	1	Microscopy and Far-IR	Operational
NSRRC, Taiwan	1	Microscopy	Operational
NSRL, Heife	1	Microscopy and Far-IR	Planned
BSRF, Beijing	1	Microscopy	Planned
Spring-8, Himeji	1	Microscopy and Far-IR	Operational
UVSOR, Okazaki	1	Far-IR	Operational
Europe			
ESRF, Grenoble	1	Microscopy	Operational
Soleil, St. Aubin	2	Microscopy and Far-IR	Under Construction
ELETTRA, Trieste	1	Microscopy and Far-IR	Operational
DAPHNE, Frascati	1	Far-IR	Operational
SLS, Villigen	1	Microscopy and Far-IR	Commissioning
ANKA, Karlsruhe	1	Microscopy and Far-IR	Operational
BESSY II, Berlin	1	Microscopy and Far-IR	Operational
DELTA, Dortmund	1	Microscopy	Planned
MAX II, Lund	2	Microscopy and Far-IR	Far-IR operational, microscopy under construction
SRS, Daresbury	2	1 Microscopy, 1 Far-IR	Operational
DIAMOND, Didcot	1	Microscopy	Planned

INTRODUCTION TO INFRARED SPECTROSCOPY

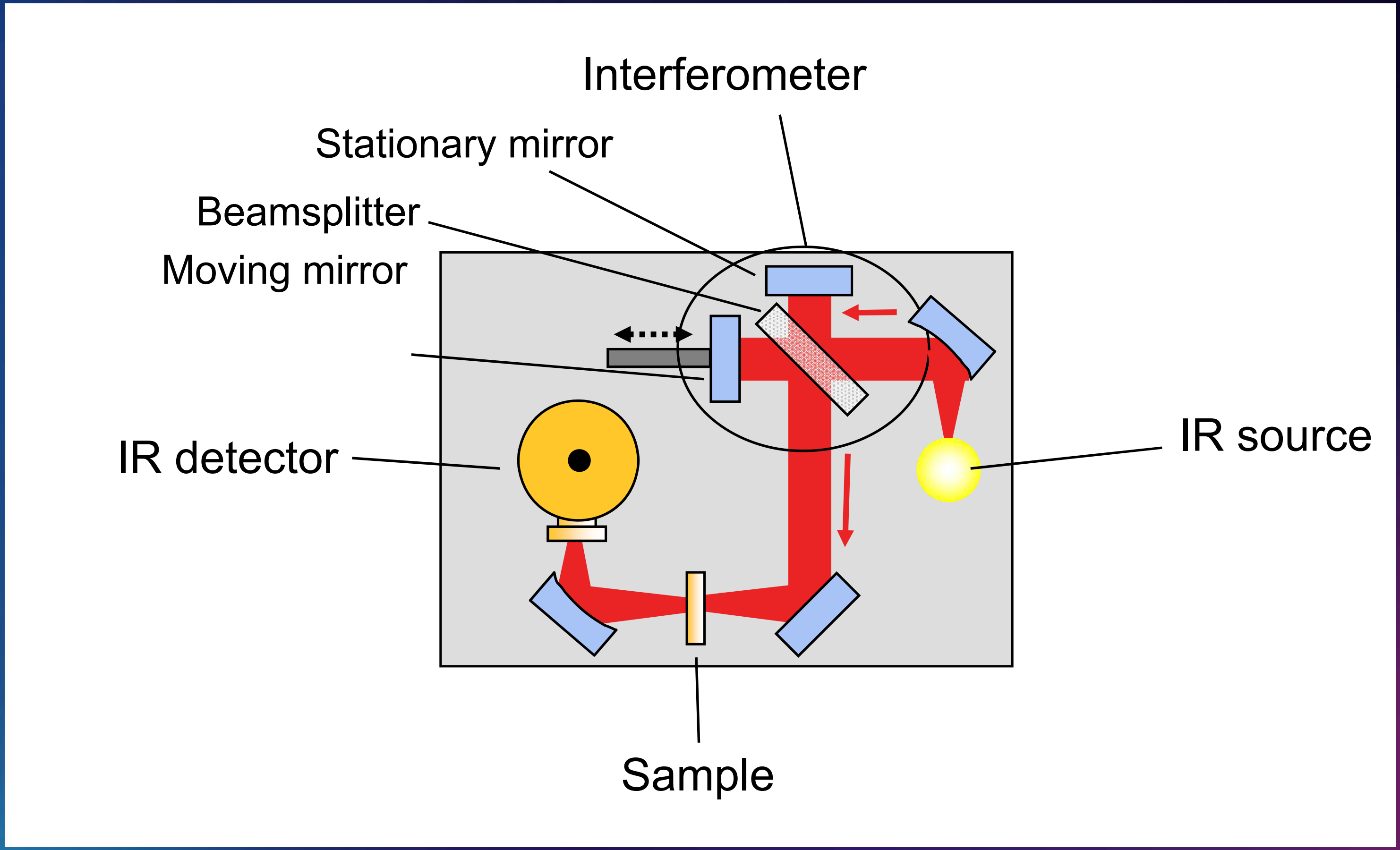
Spectroscopy…



Infrared spectroscopy

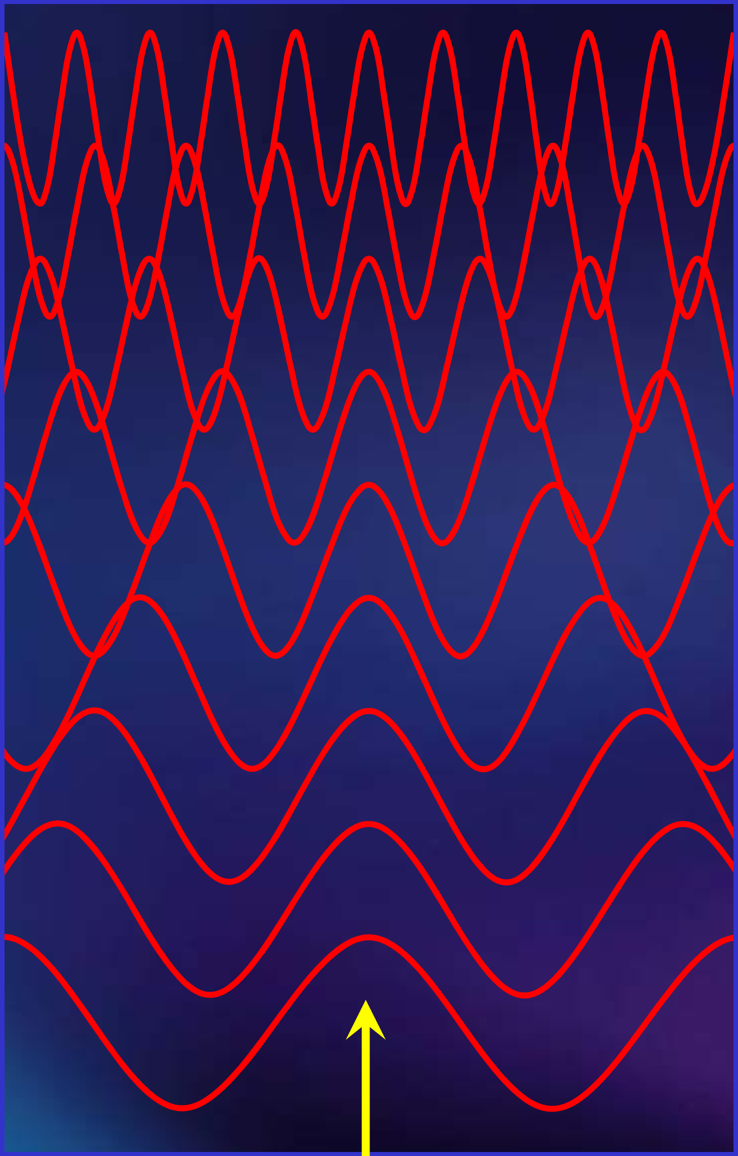


Method of data collection



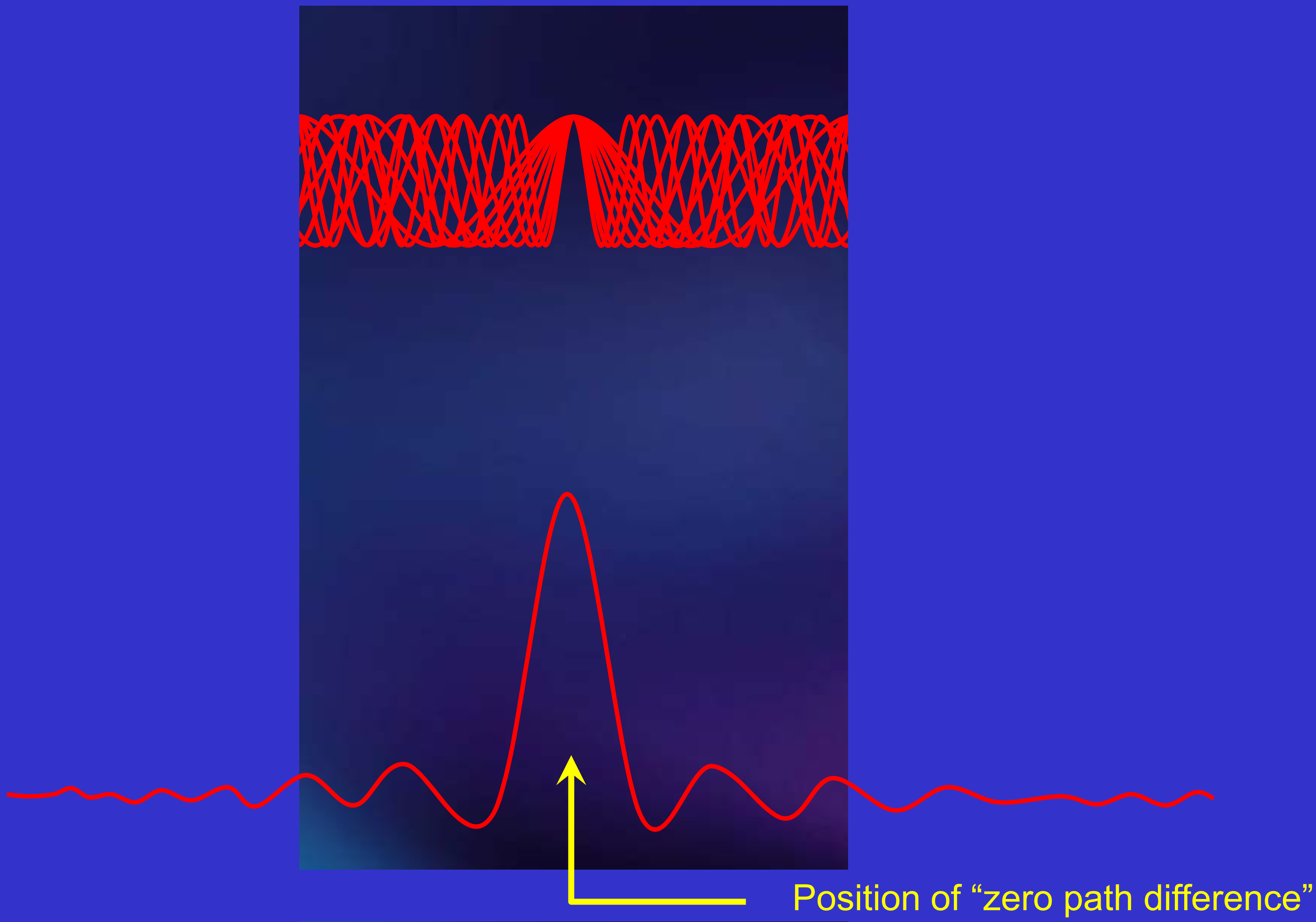
Fourier Transform Infrared is more common, but dispersive has applications, particularly for fast timing with intense beams

Many frequencies are present in the infrared beam

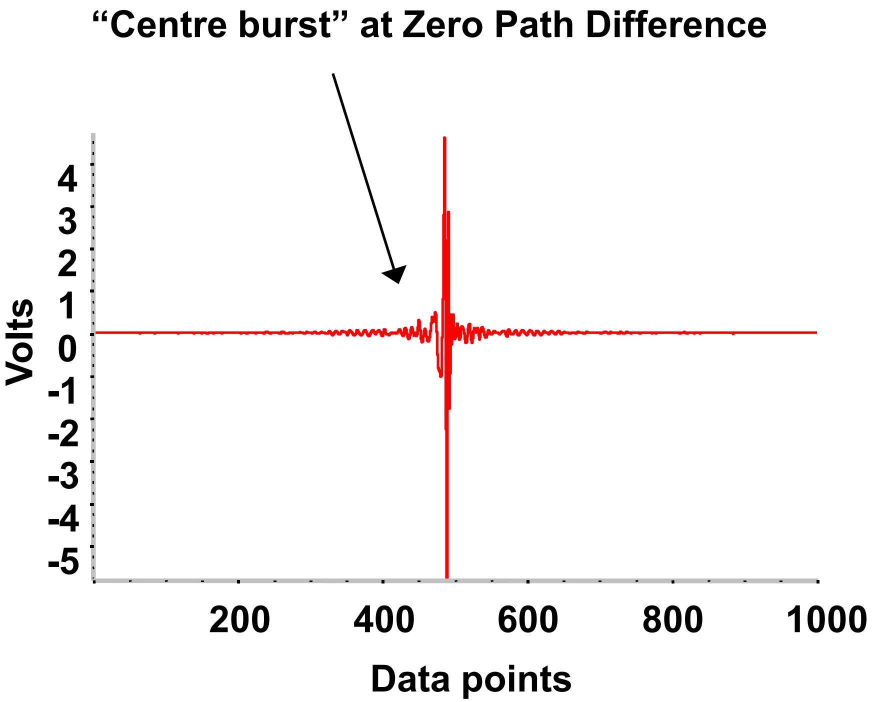


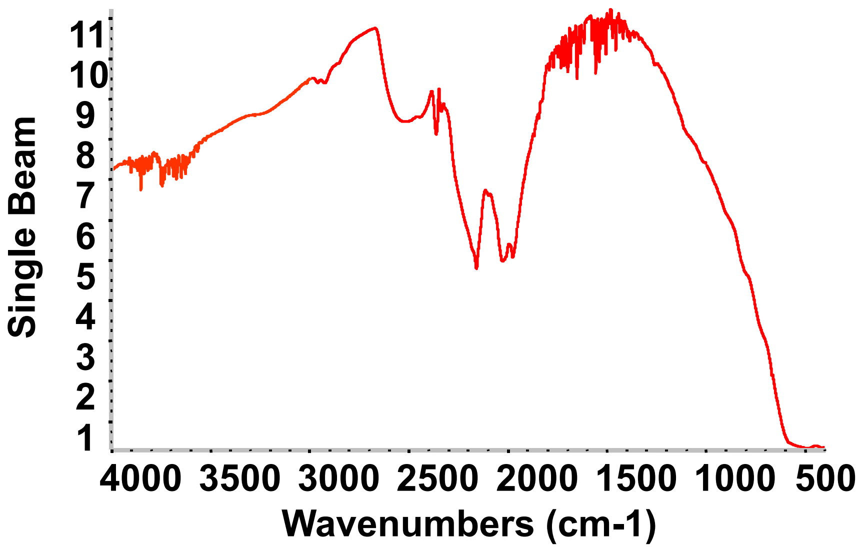
Position of "zero path difference"

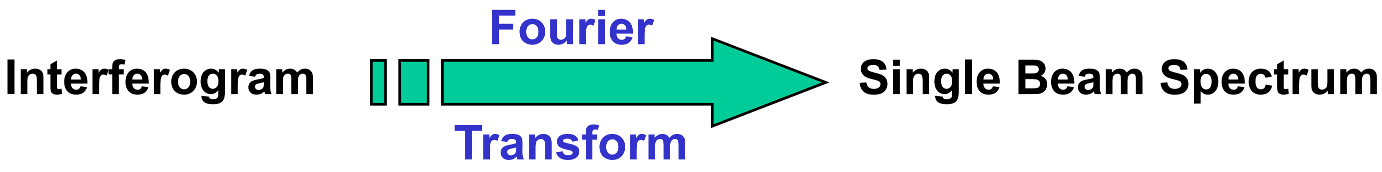
Summing of all frequencies for each position of the mirror



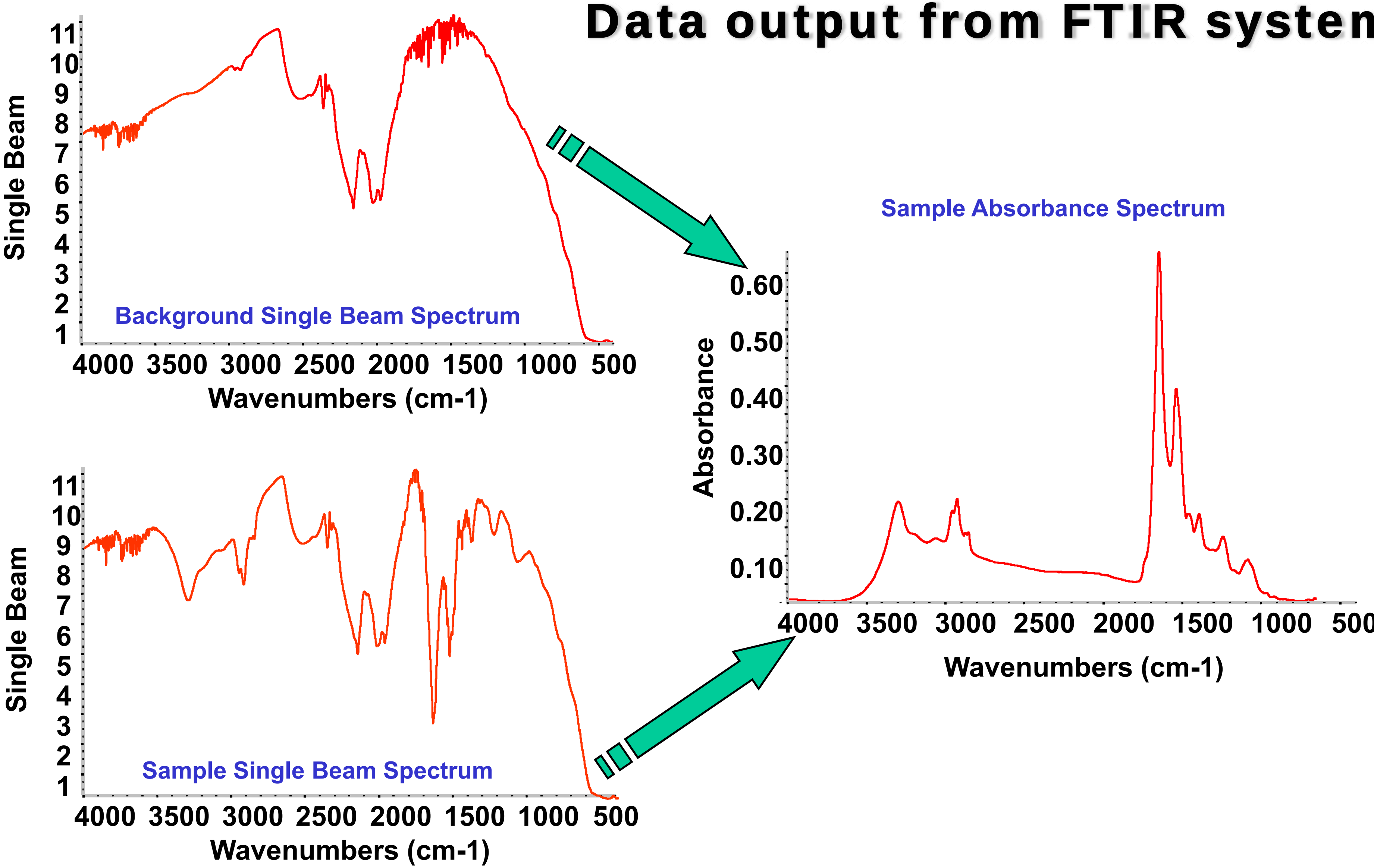
Data output from FTIR system







Data output from FTIR system



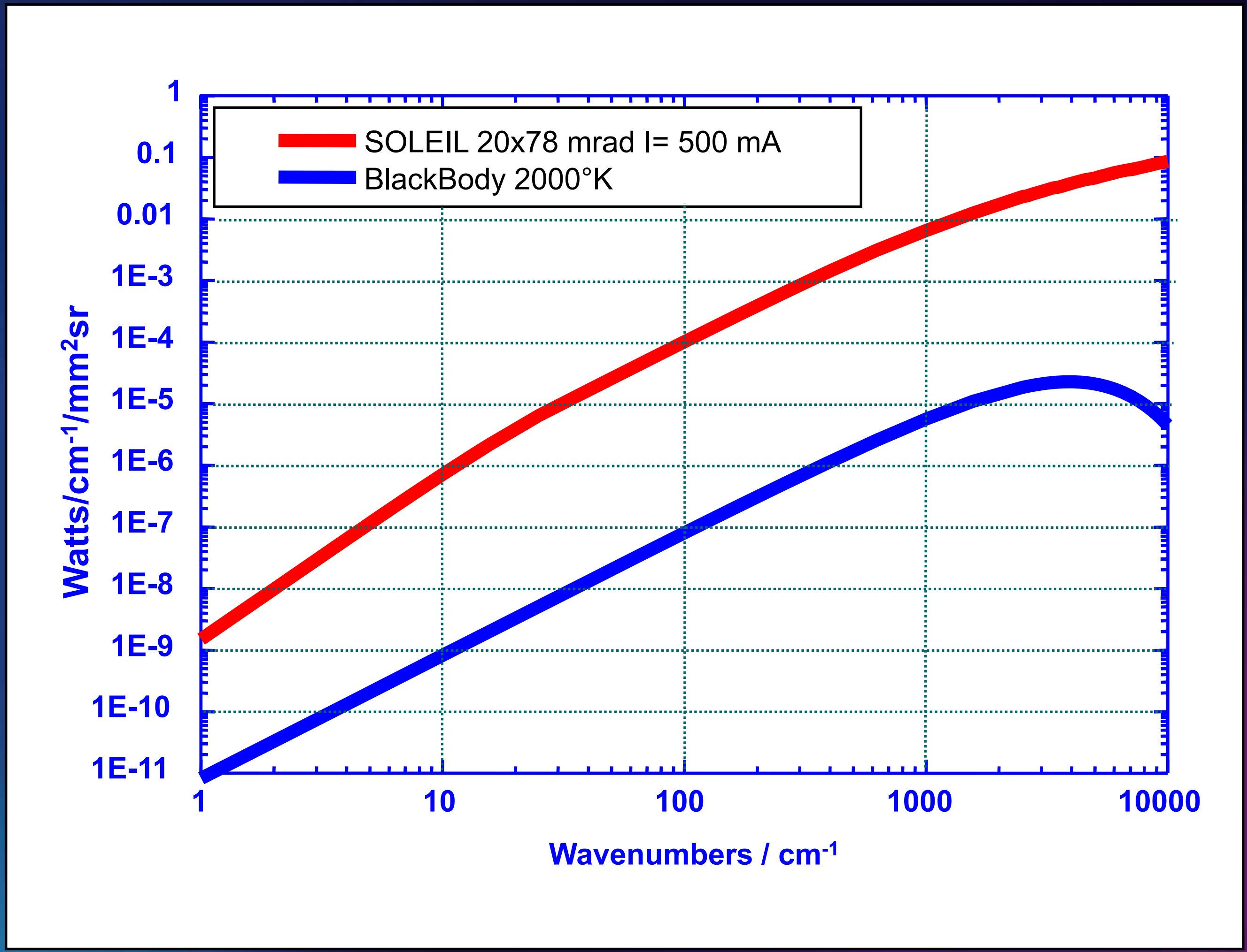
Infrared spectroscopy and microspectroscopy - Instrumentation

Generally designed for benchtop Mid-IR sources

So why use a Synchrotron?

It's the synchrotron **brightness** that counts

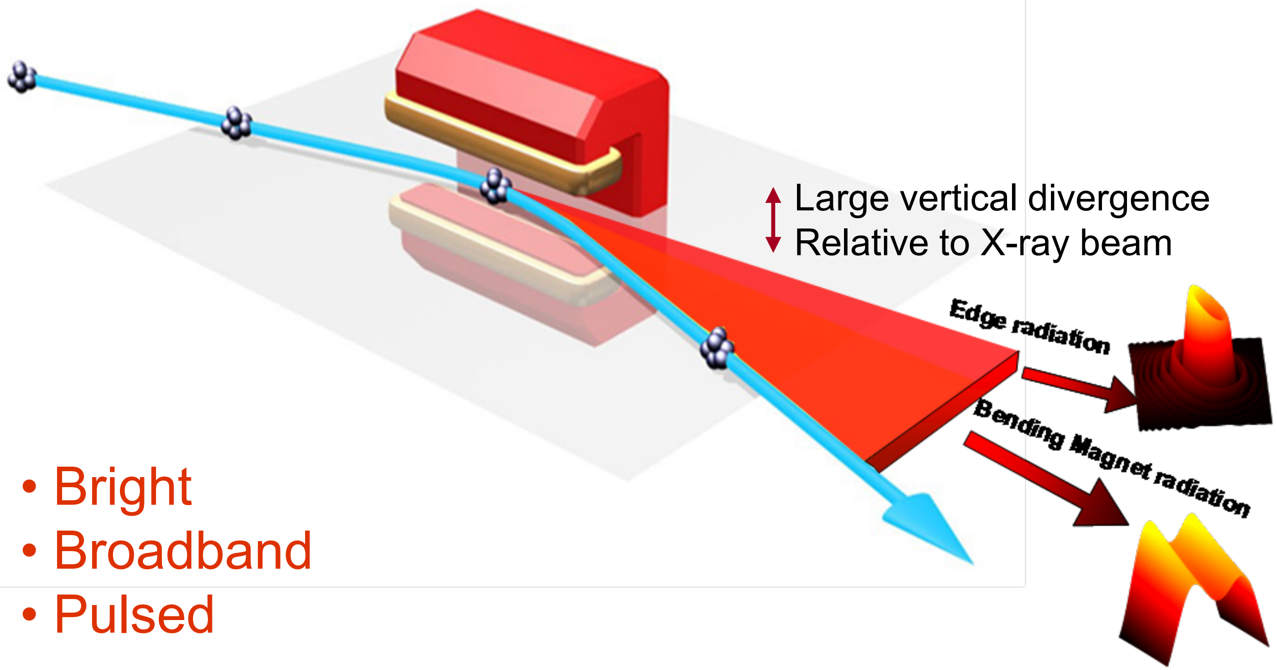
Is the synchrotron IR beam very intense?



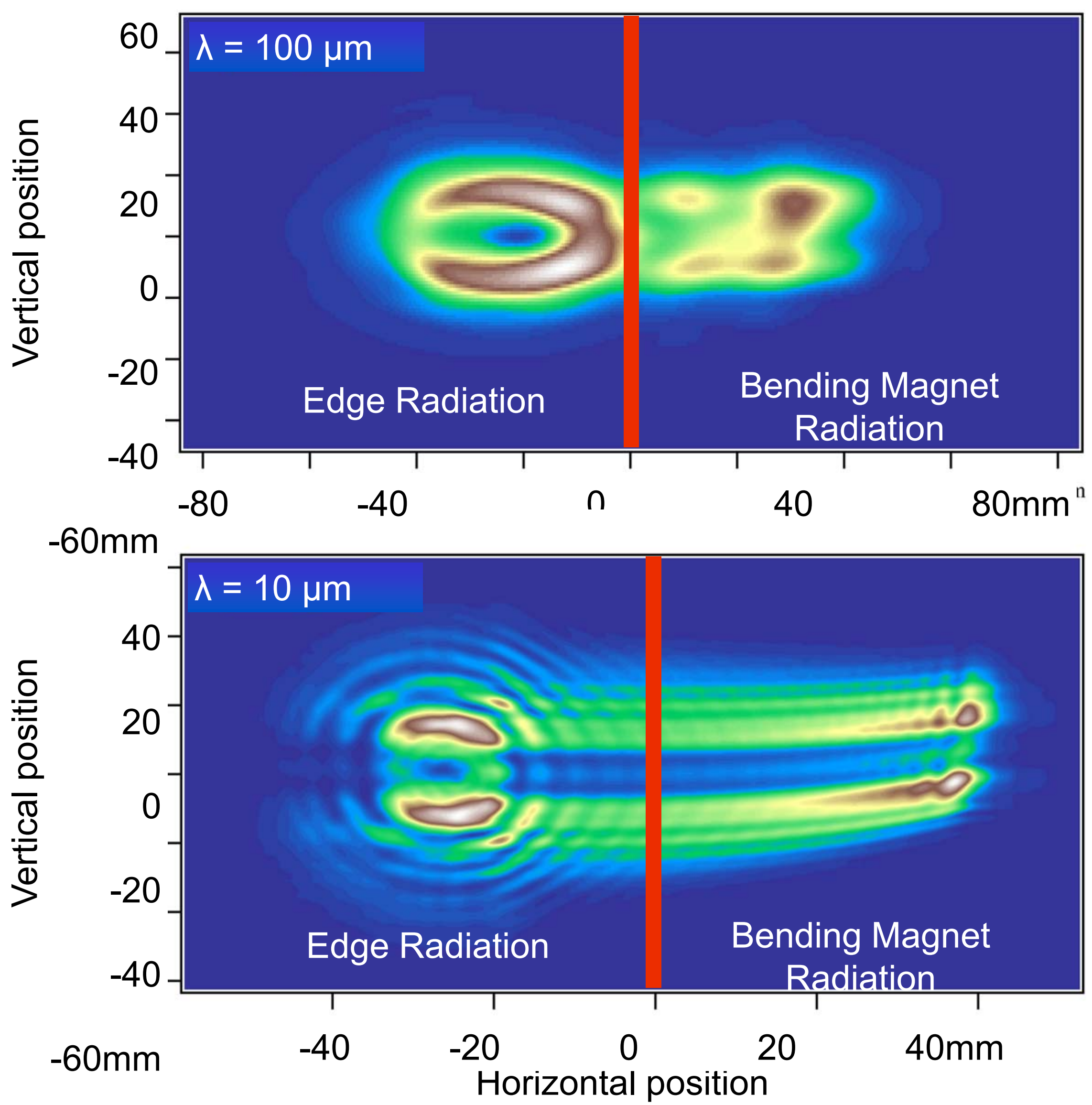
EXTRACTION OF INFRARED LIGHT FROM A SYNCHROTRON

Infrared mission from a synchrotron bending magnet

Edge Radiation and Bending Magnet Radiation

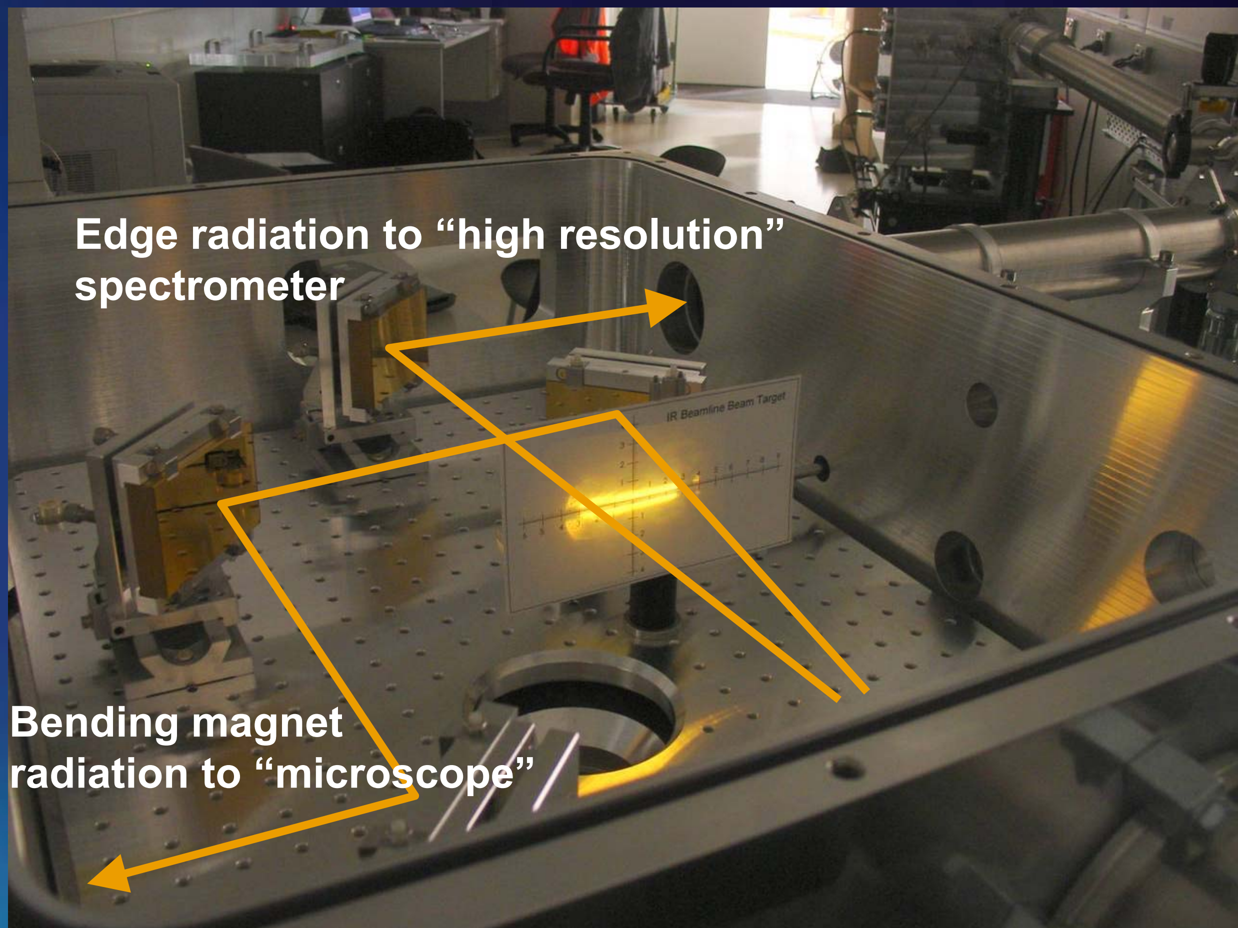


- Bright
- Broadband
- Pulsed



λ = 100 μm
Vertical position
60
40
20
0
-20
-40
-60mm
-80
-40
0
40
80mm
Edge Radiation
Bending Magnet Radiation
λ = 10 μm
Vertical position
40
20
0
-20
-40
-60mm
-40
-20
0
20
40mm
Horizontal position
Edge Radiation
Bending Magnet Radiation

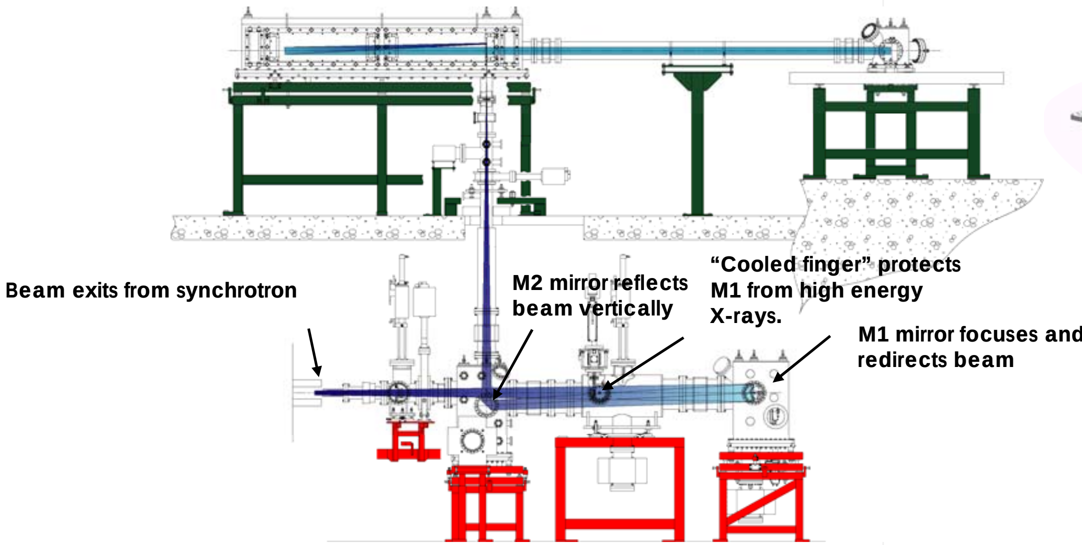
Visible light in the beamsplitter vessel at the Australian Synchrotron Infrared beamline

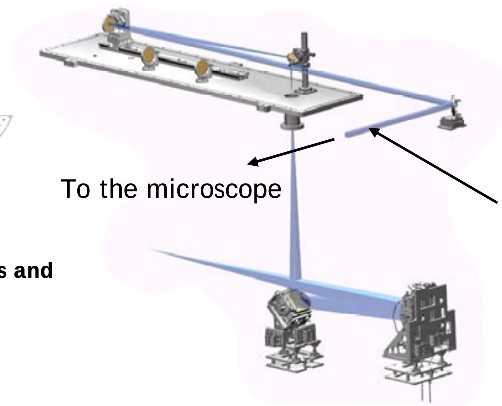


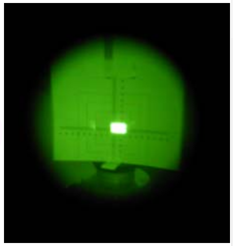
ALTERNATIVE METHODS OF EXTRACTION

1. Large aperture in dipole with M1 external to synchrotron

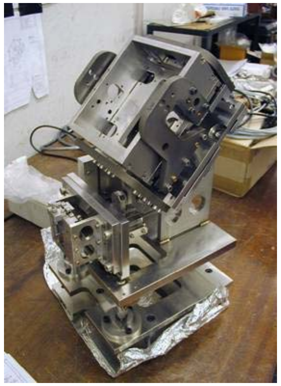
e.g. SRS at Daresbury Laboratory

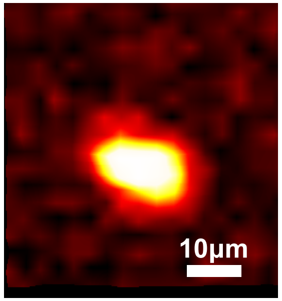






The collimated beam, viewed with an infrared image intensifier.

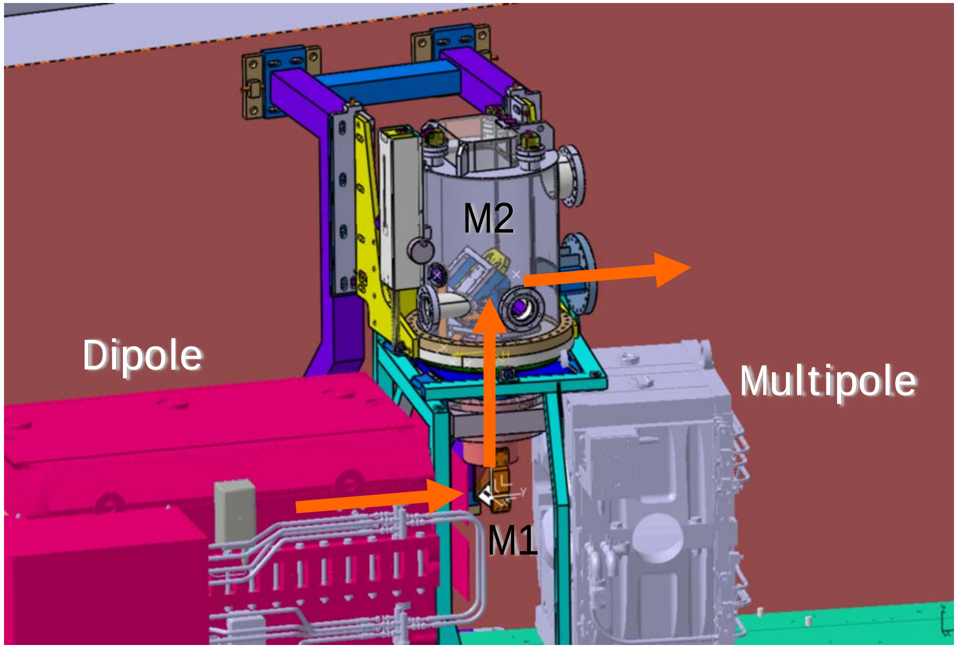


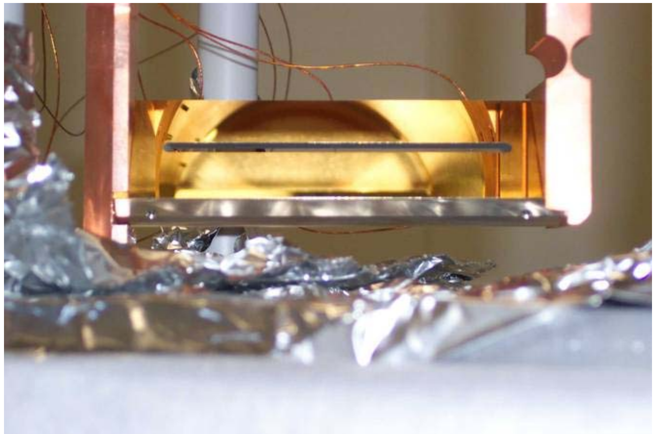


Mid IR beam profile at sample

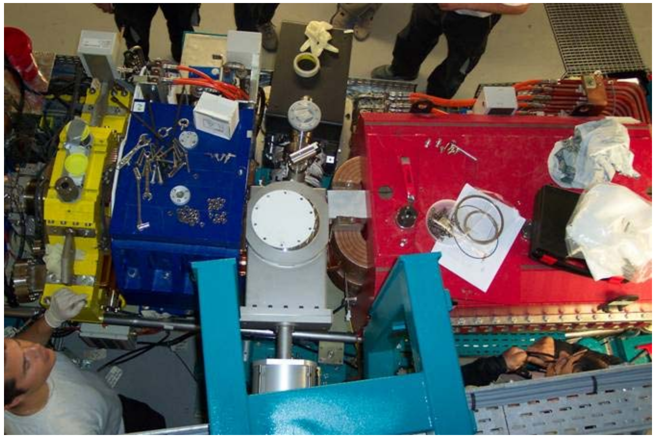
2. Mirror M1 inserted into dipole "crotch" from above or below

e.g. Soleil, ESRF...





M1 Mirror with thermocouple wires

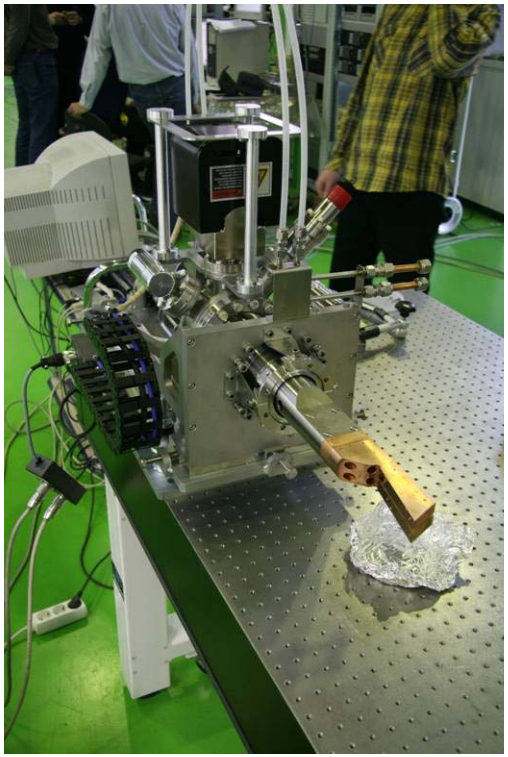


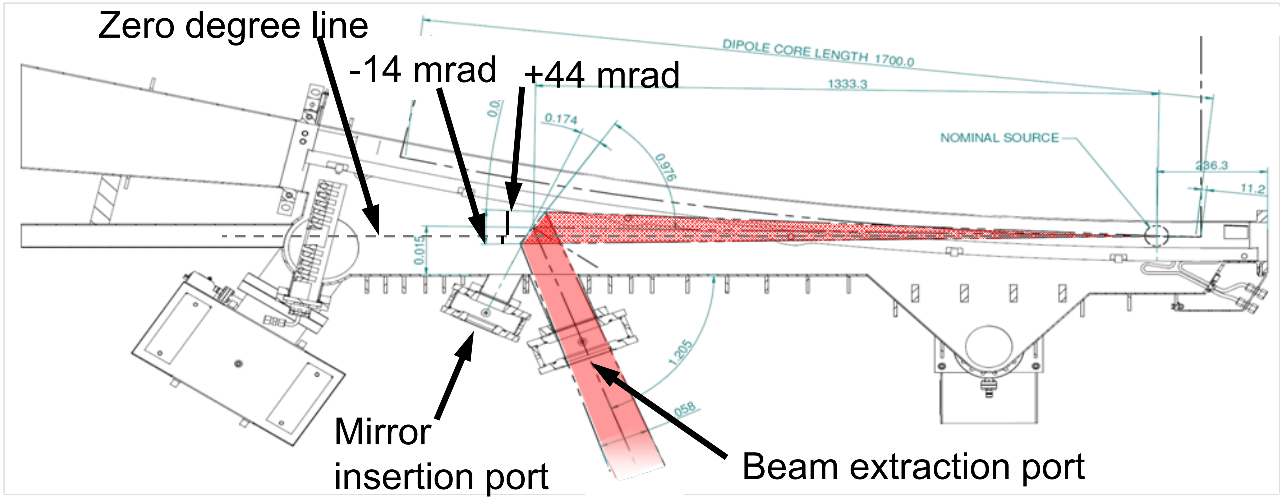
Top view of mirror insertion port

Images courtesy of Paul Dumas, Soleil.

2. Mirror inserted into dipole chamber from side

e.g. Australian Synchrotron

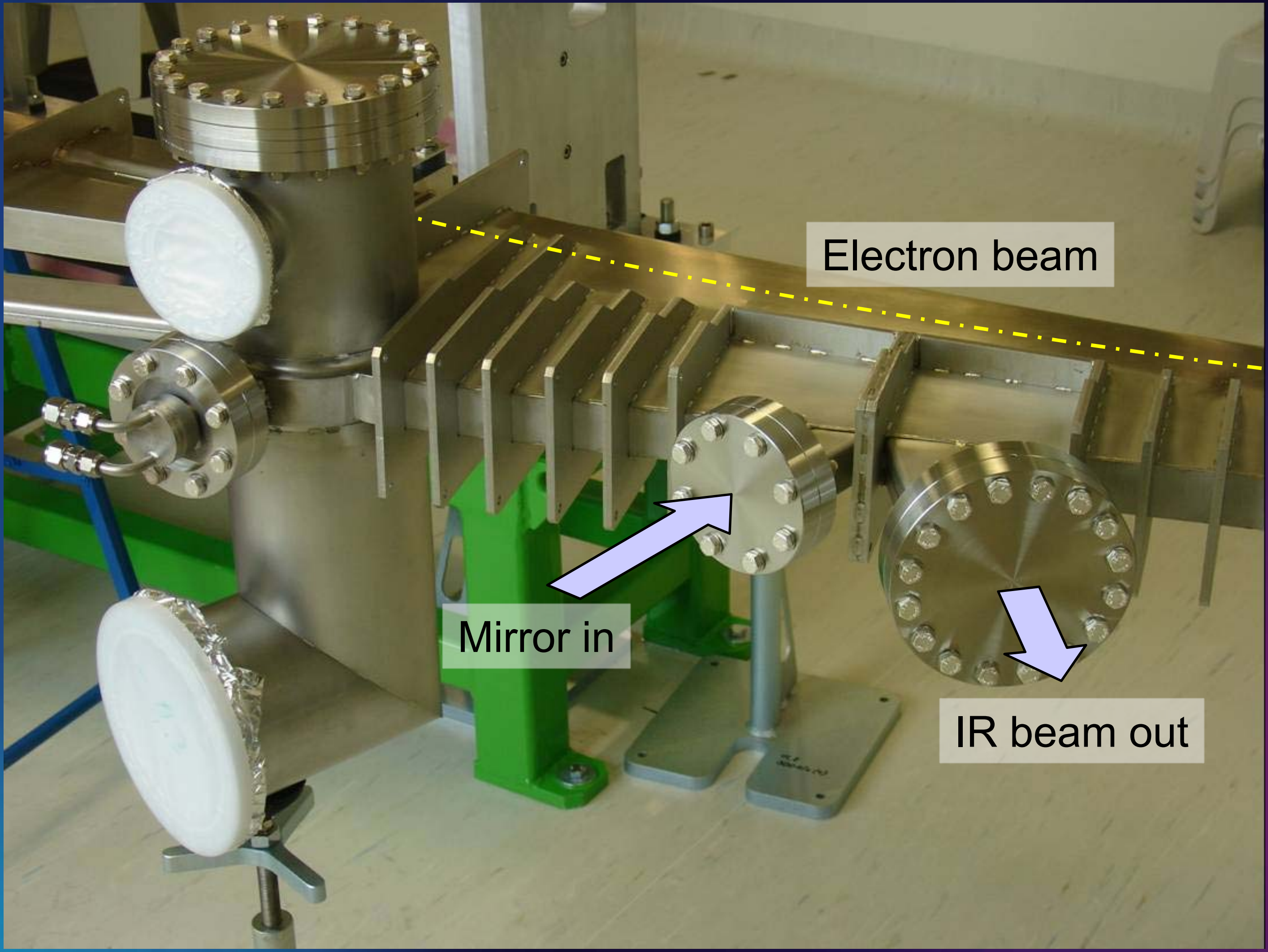




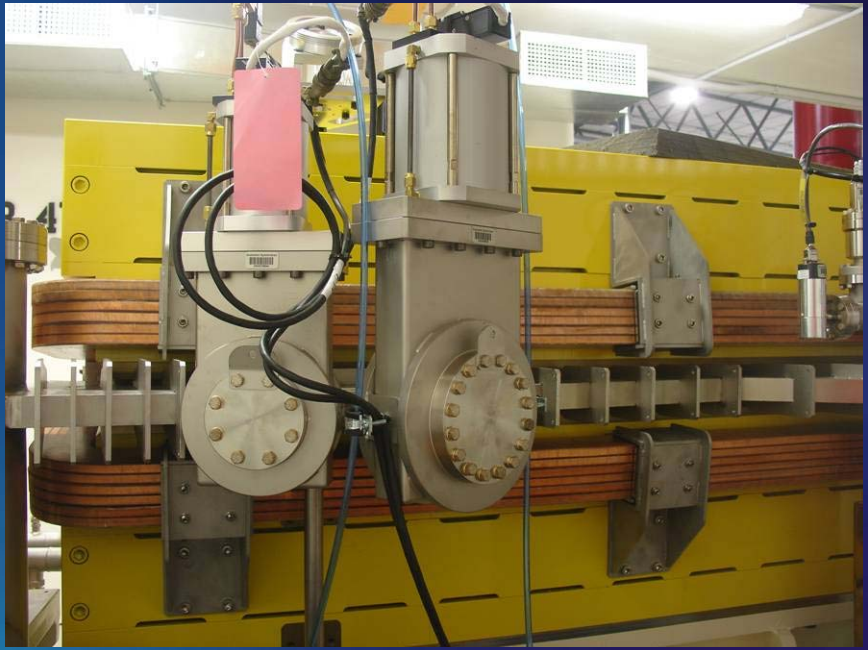
Which brings me to…

TheÅAustralianÅSYNCHROTRONÅ INFRAREDÅBEAMLINEÅ

Adapted Infrared Dipole Chamber at Australian Synchrotron



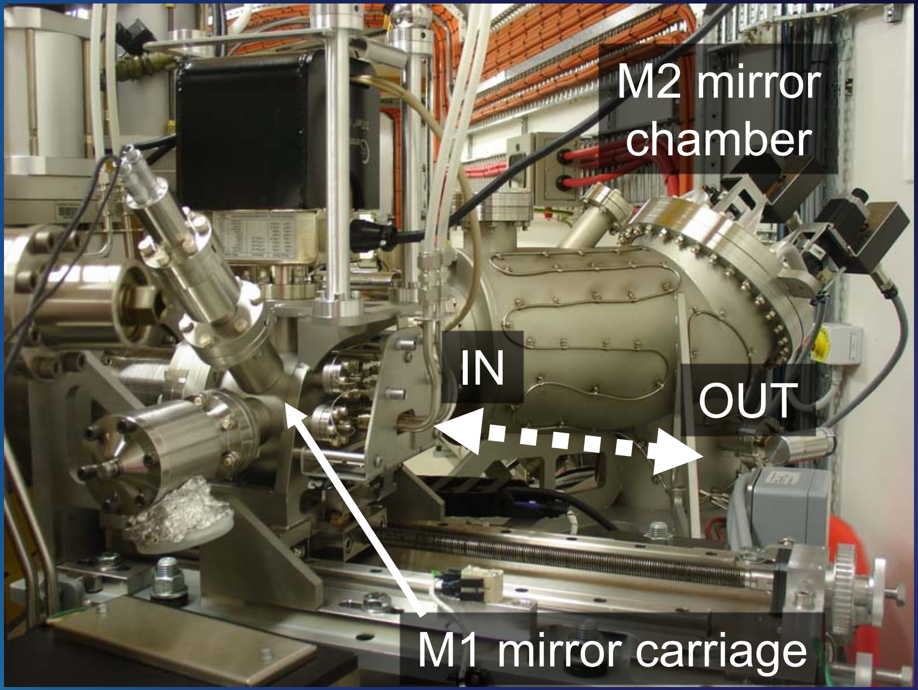
Dipole Chamber in Storage Ring and Mirror M1 prior to Installation

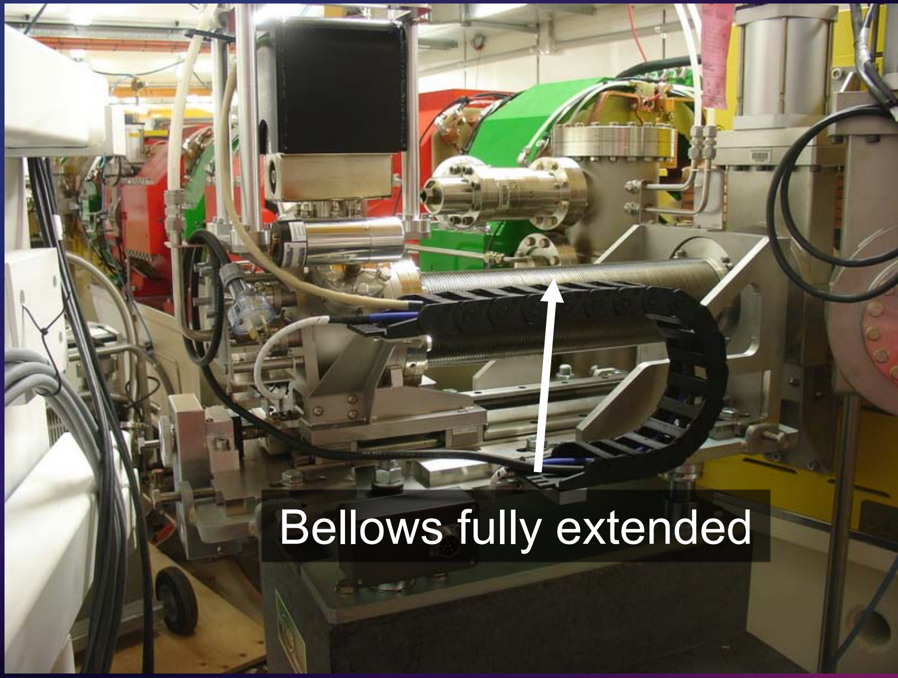


Infrared dipole chamber installed with vacuum isolation gate valves installed

Mirror M1 undergoing vibration testing prior to installation

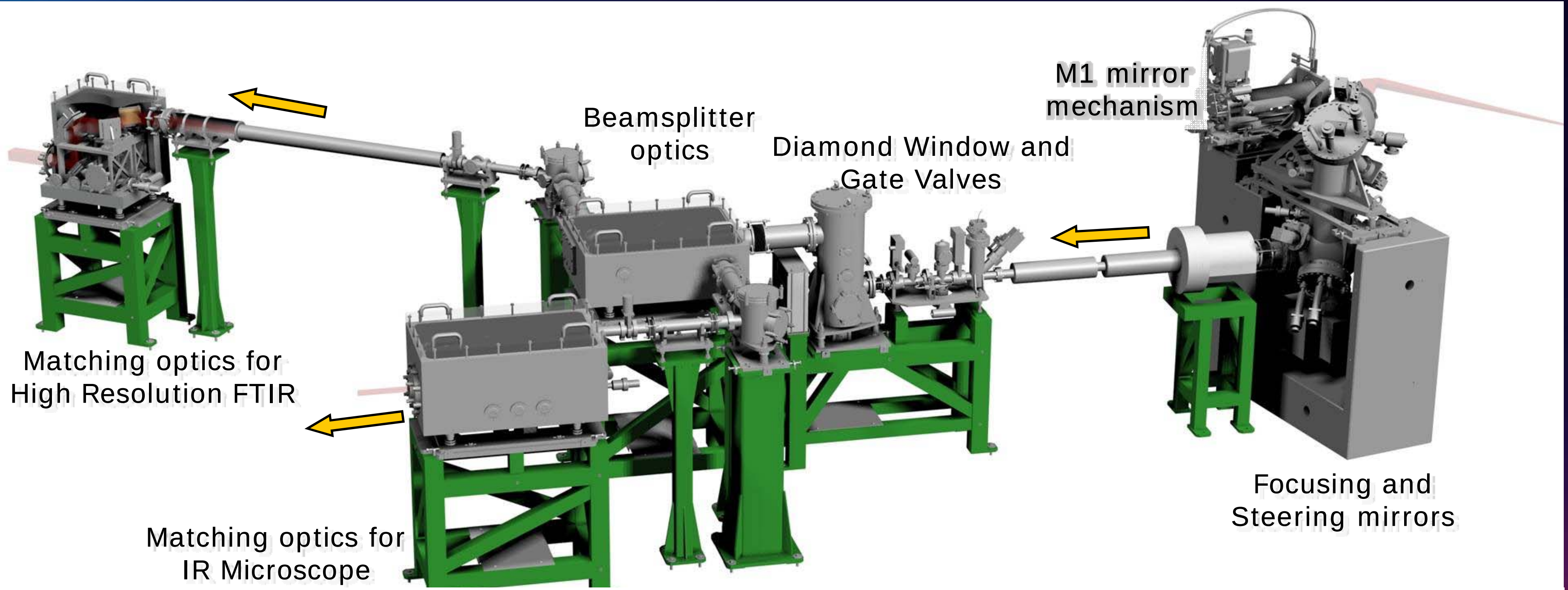
M1 Mirror Inserted (left) and Withdrawn (right)





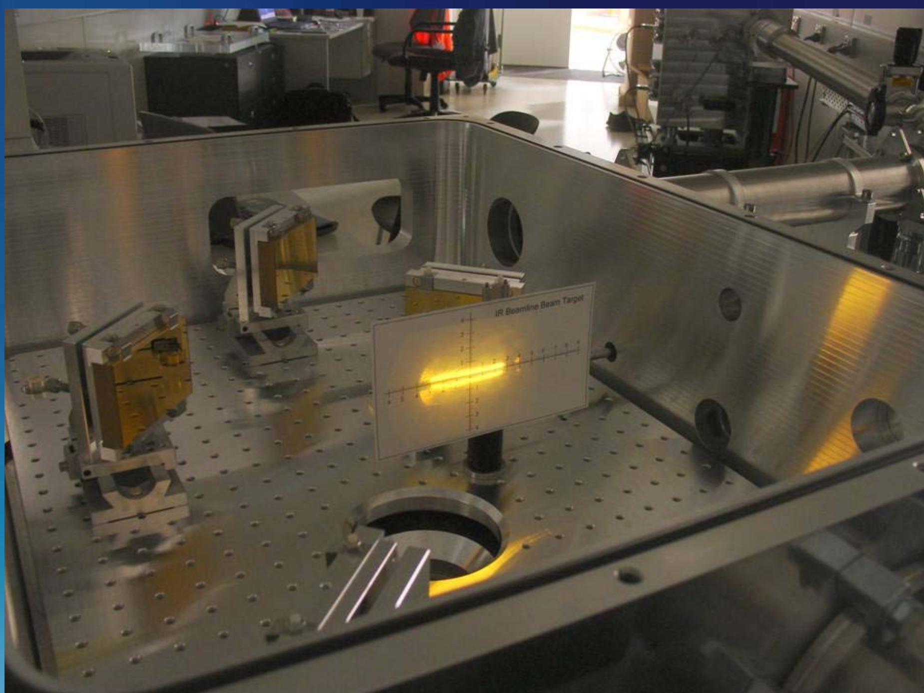
Note: M2 mirror chamber not yet installed in this photo

Australian Synchrotron

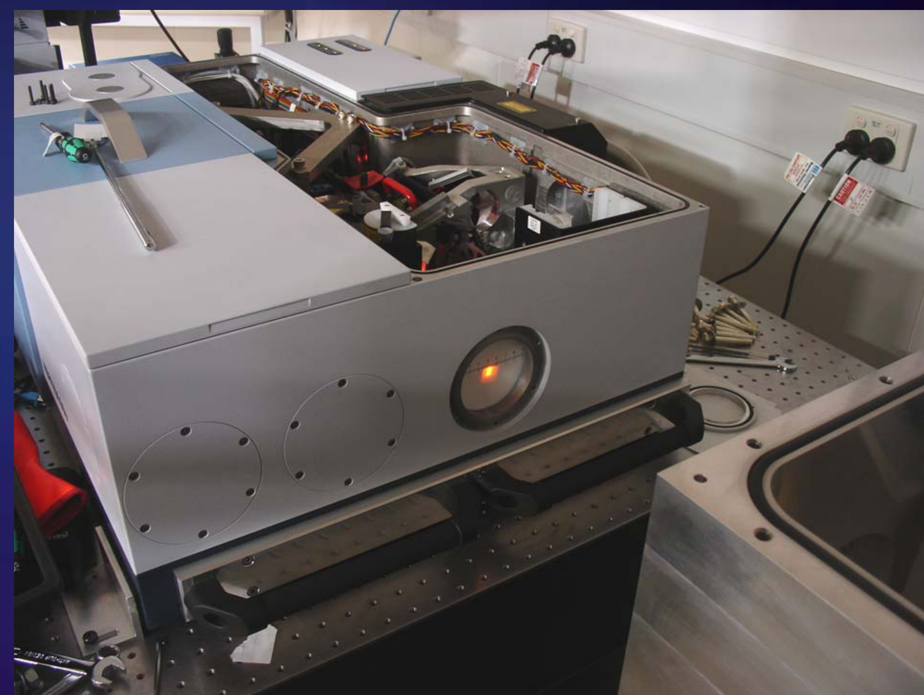


Infrared beamline showing (from right) synchrotron beam entering front end optics (M1, M2, M3, M3a), diamond exit window, beamsplitter optics vessel and matching optics boxes for the two endstation instruments.

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



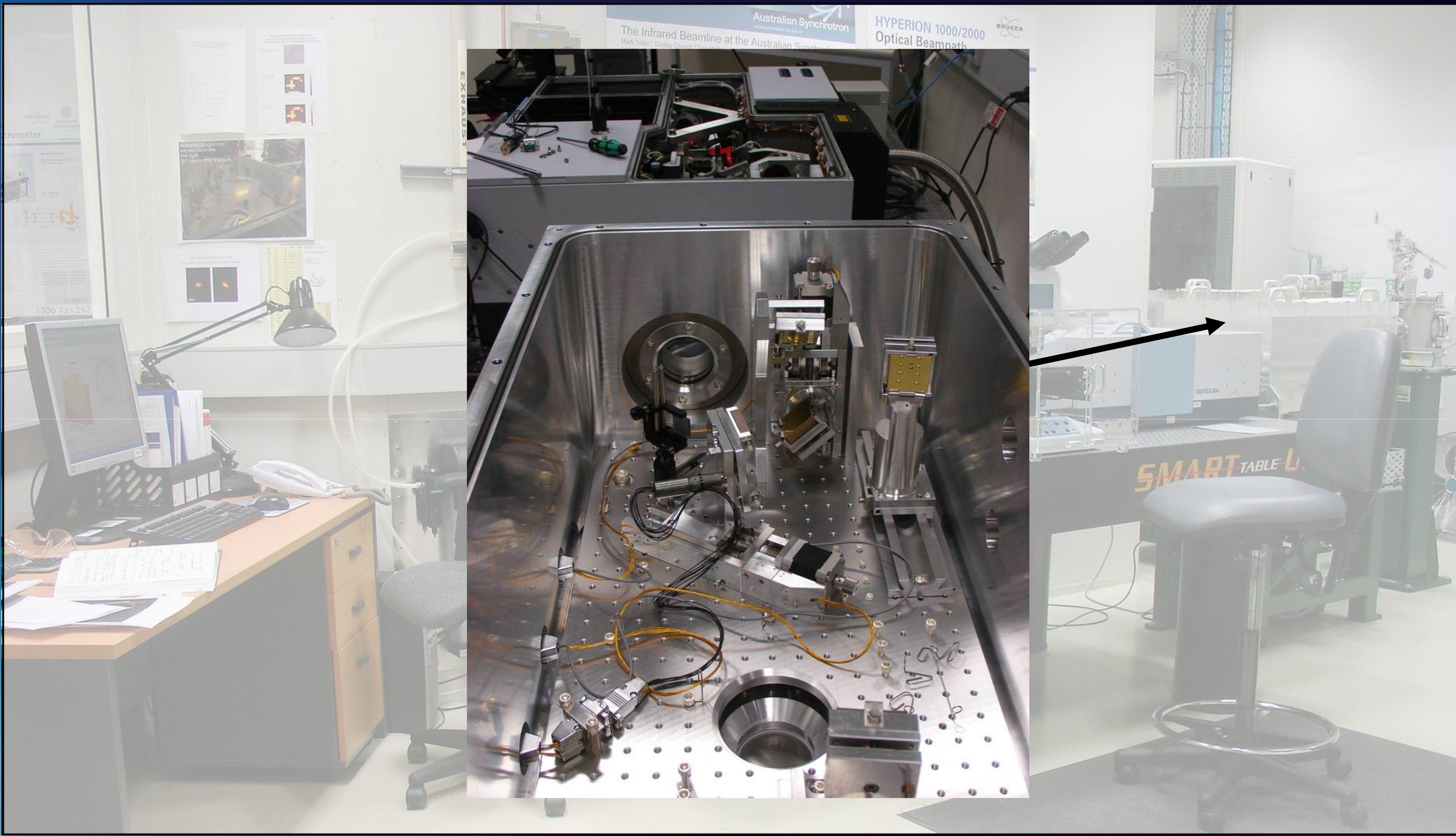
Visible beam profile in Beamsplitter Vessel



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

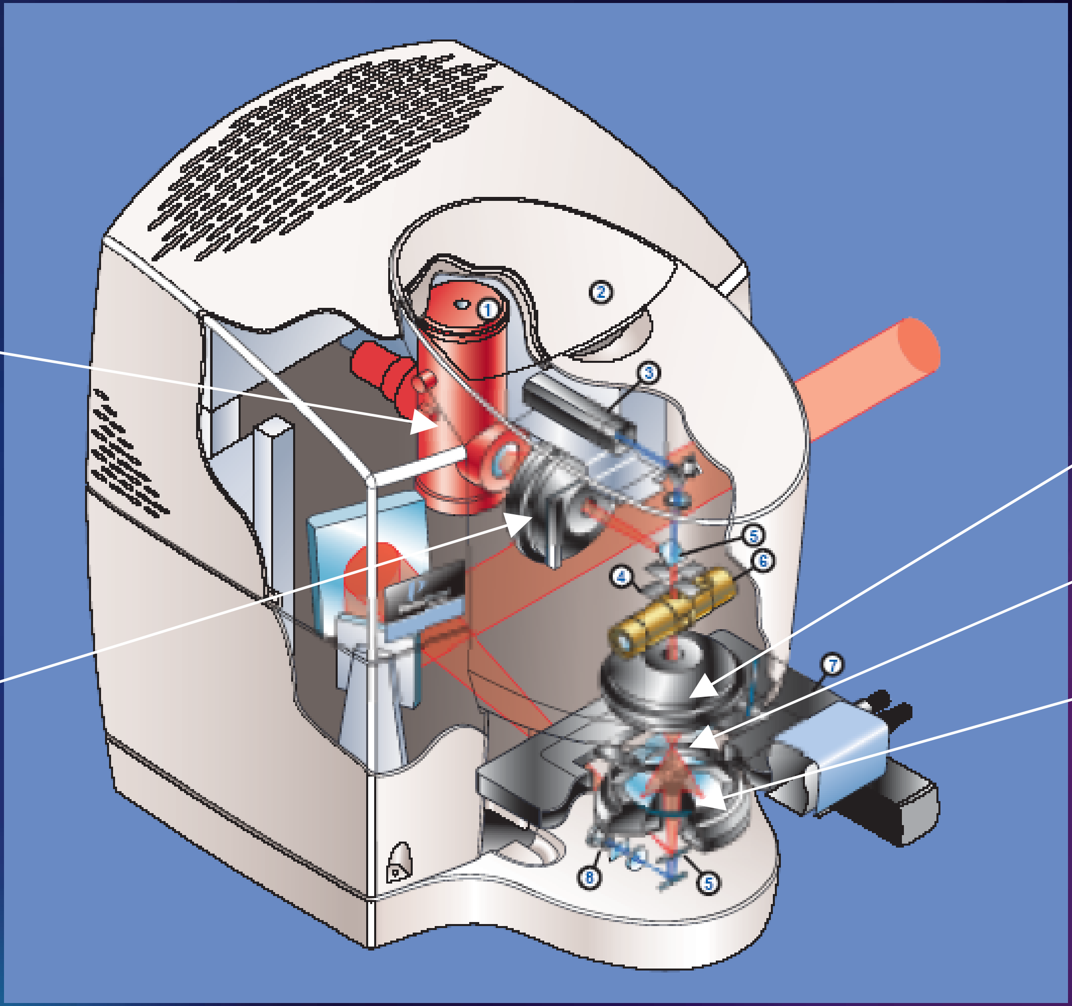
INFRARED BEAMLINE INSTRUMENTATION

Confocal point scanning - current technology

Narrow-Band MCT
50x50 micron

Wide-Band MCT
250x250 micron

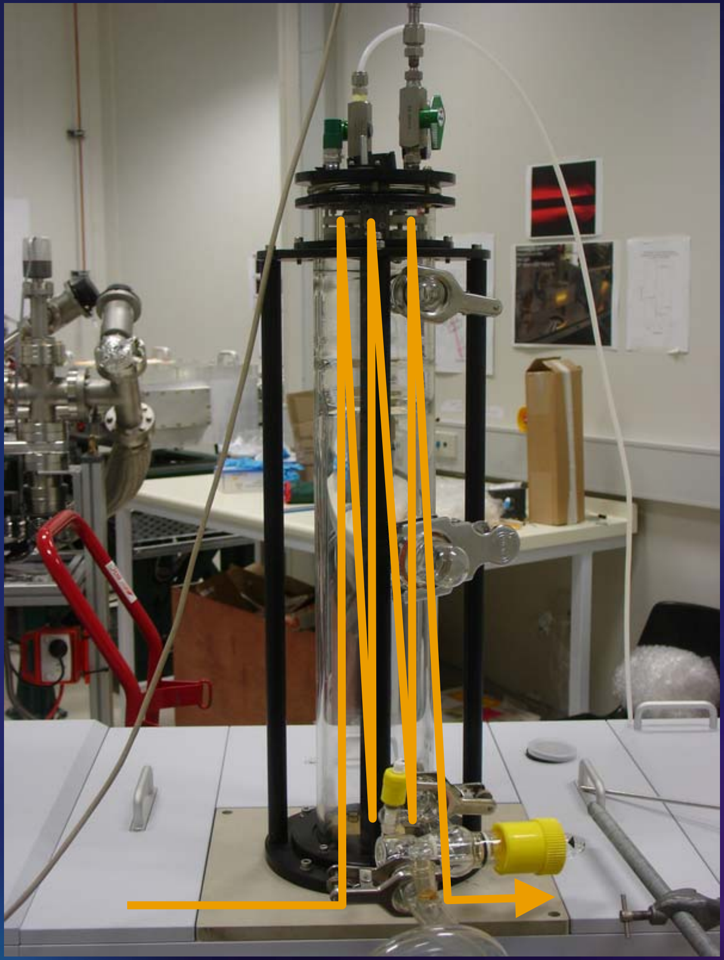
Focal plane imaging - Example



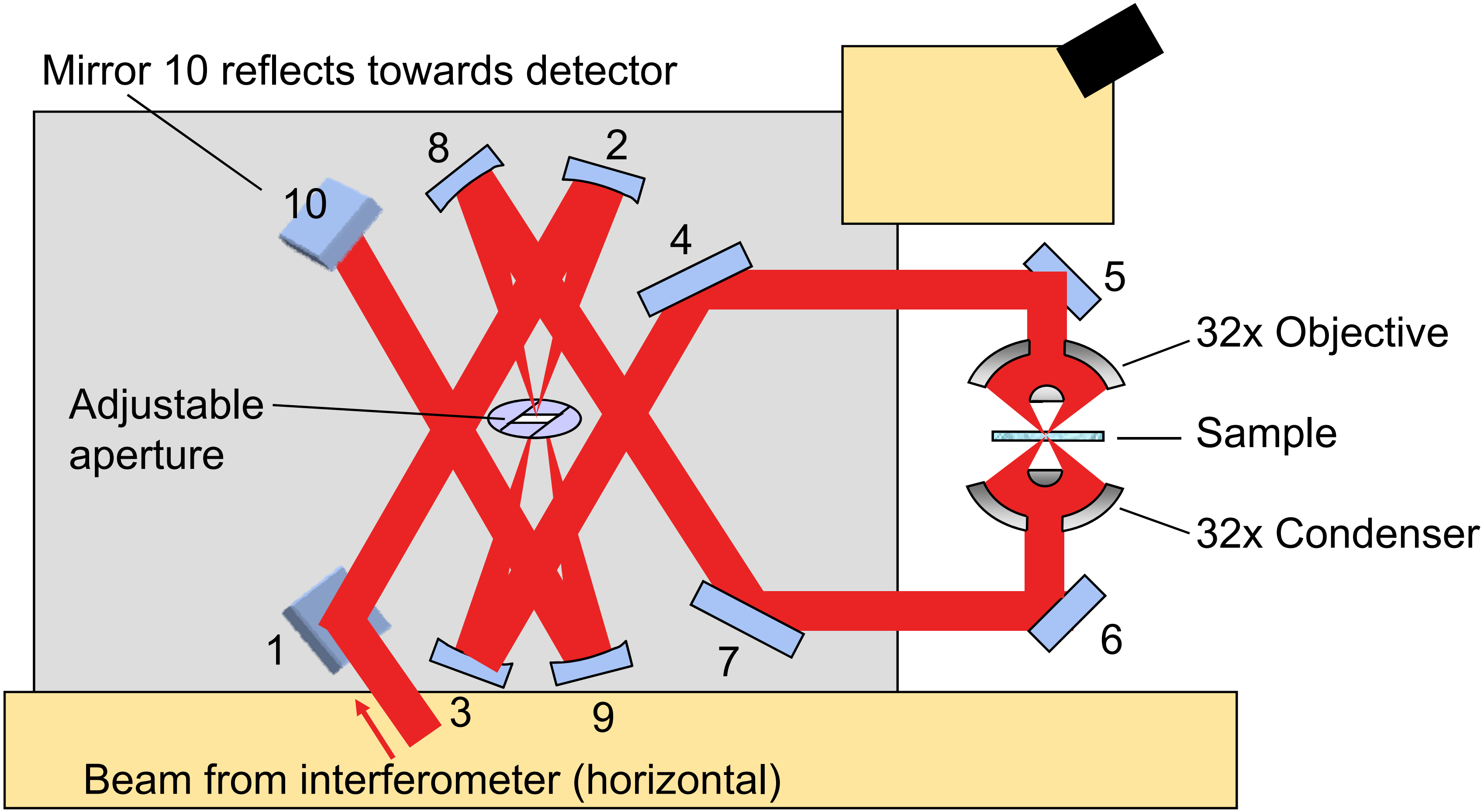
Infrared Beamline at the Australian Synchrotron
High Resolution brtanch

Bruker IFS 125HR High Resolution FTIR Spectrometer

Multipass gas cell for high resolution spectroscopy



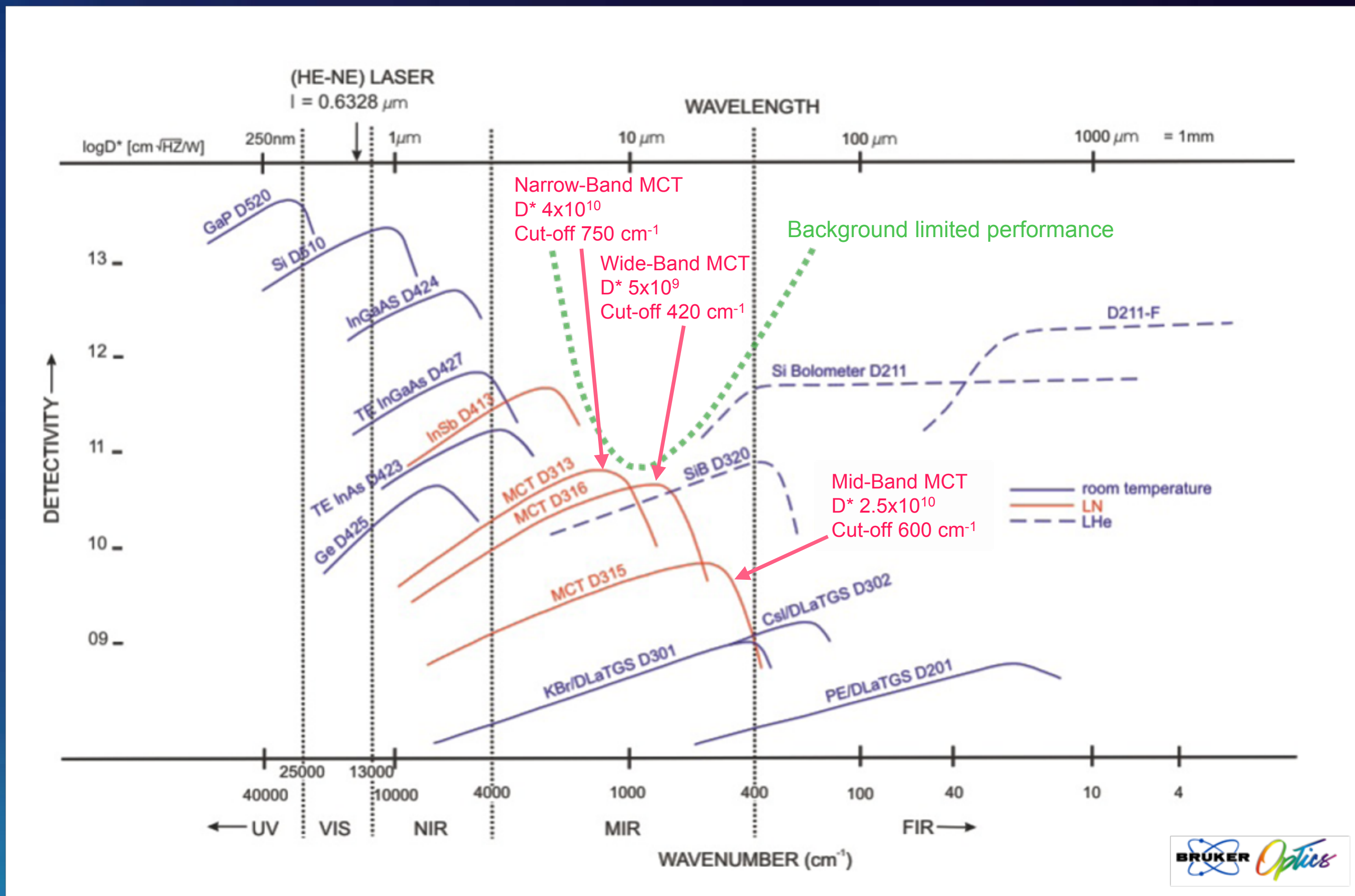
Typical confocal point scanning IR microscope



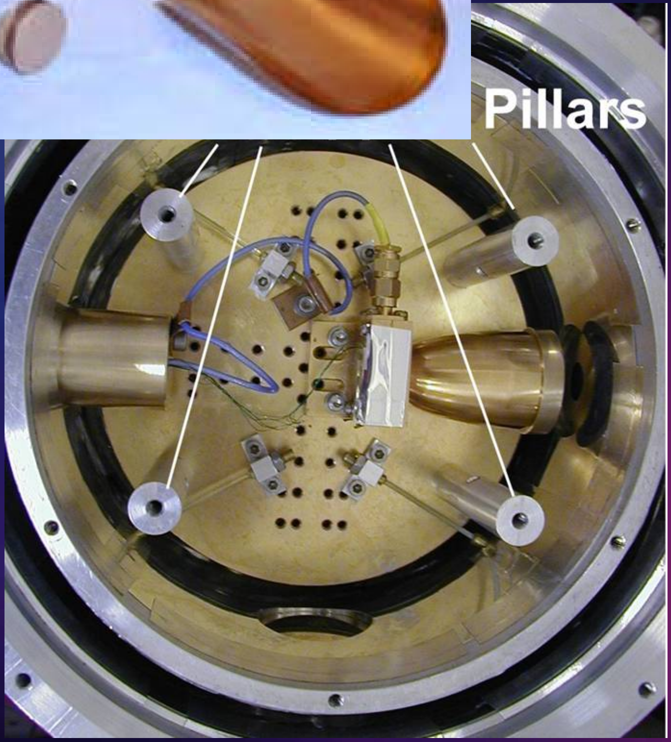
Side view of beam path through microscope after interferometer

Infrared Detectors

Some currently available IR detectors



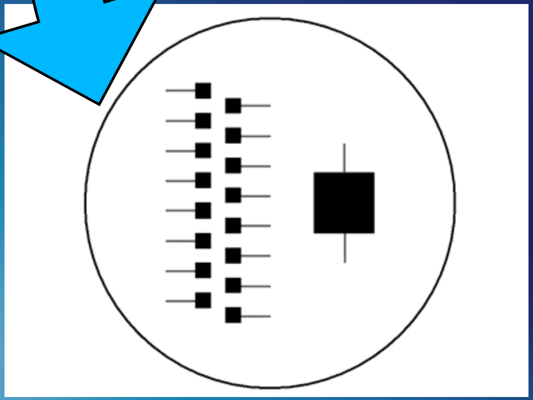
Typical far-IR bolometer with cryostat

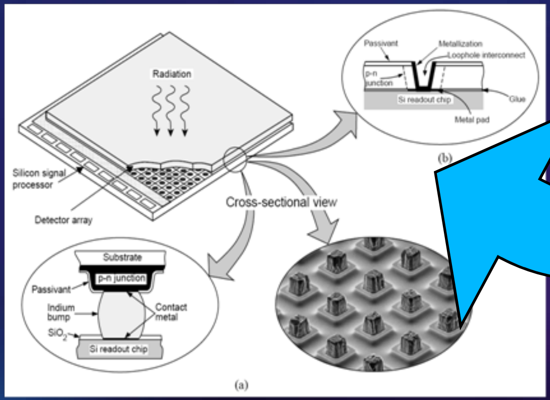


Two formats of focal plane array imaging systems

2x8 photoconductive MCT array

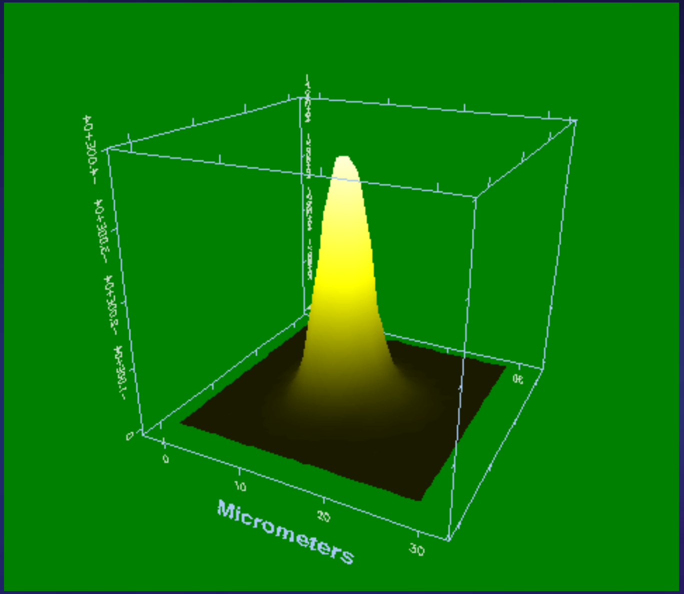
64x64 photovoltaic MCT array





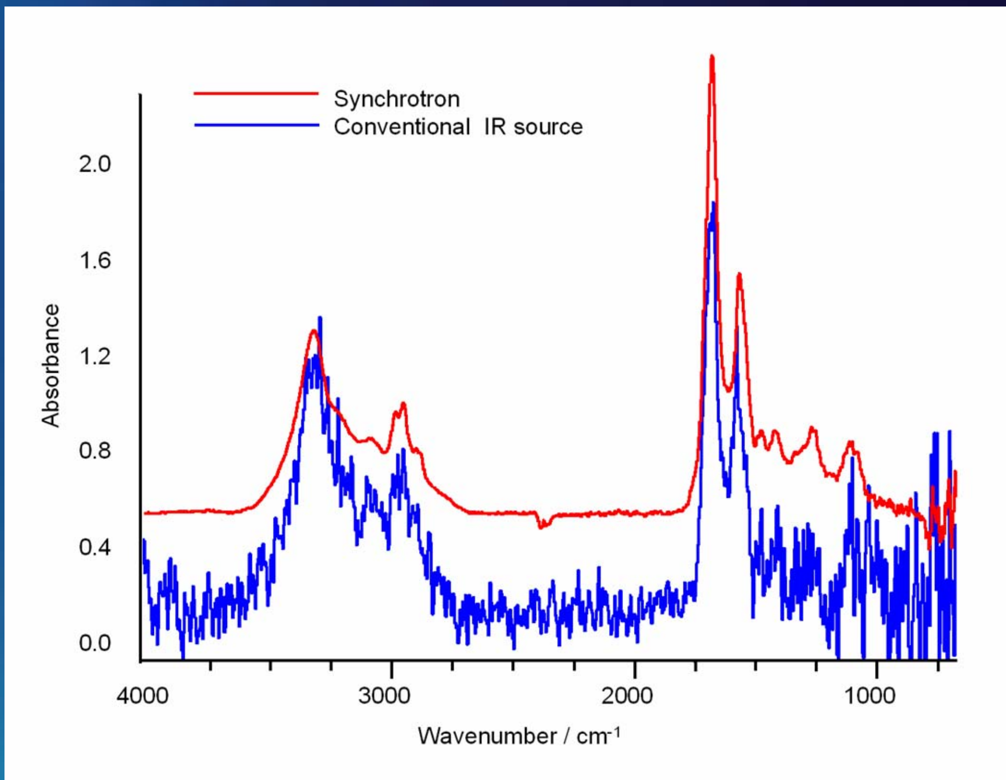
Assessing beamline performance

Synchrotron infrared beam focused on sample



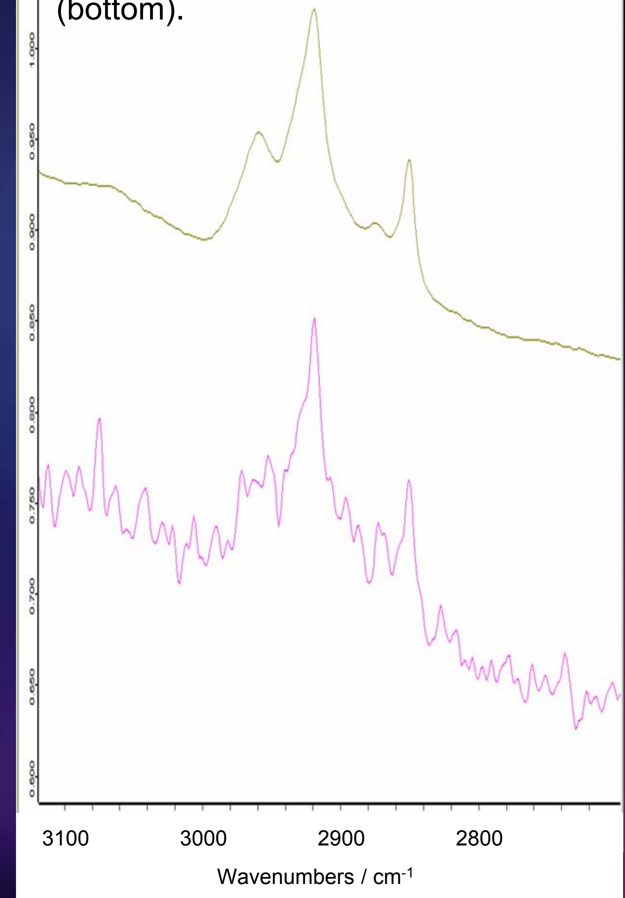
Beamline 11 at SRS - unapertured beam profile at sample stage.
Area mapped = 30x30 μm. Beam halfwidth = 8x8 μm.

Advantage of using a synchrotron seen in spectra…



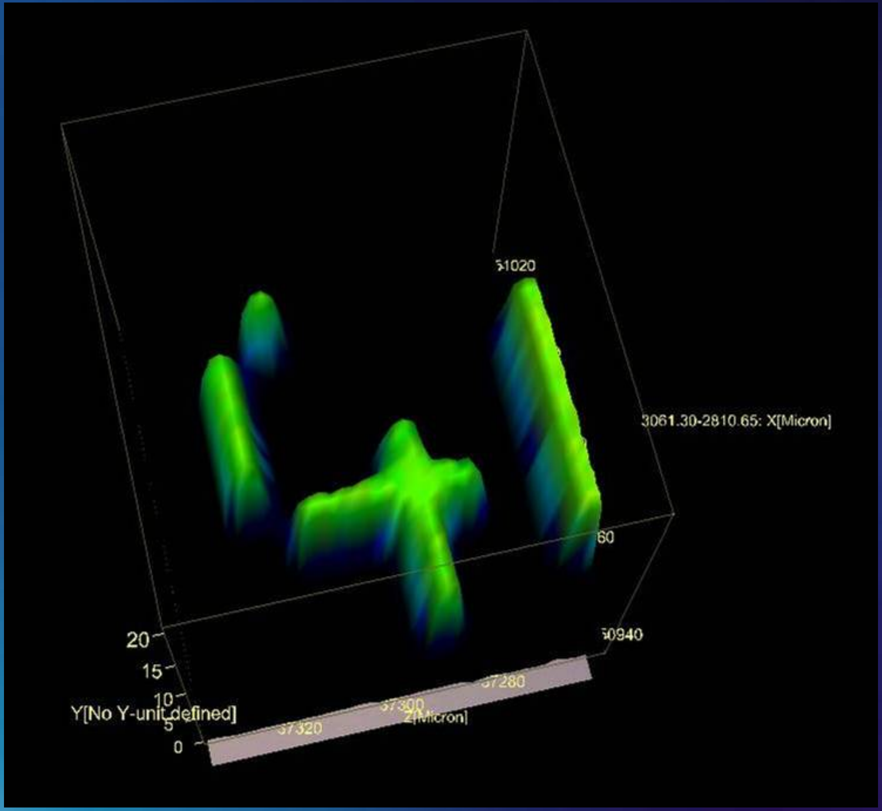
Absorbance spectra of tissue sample recorded at 10 µm spatial resolution under identical collection conditions using a Globar™ infrared source and synchrotron radiation.

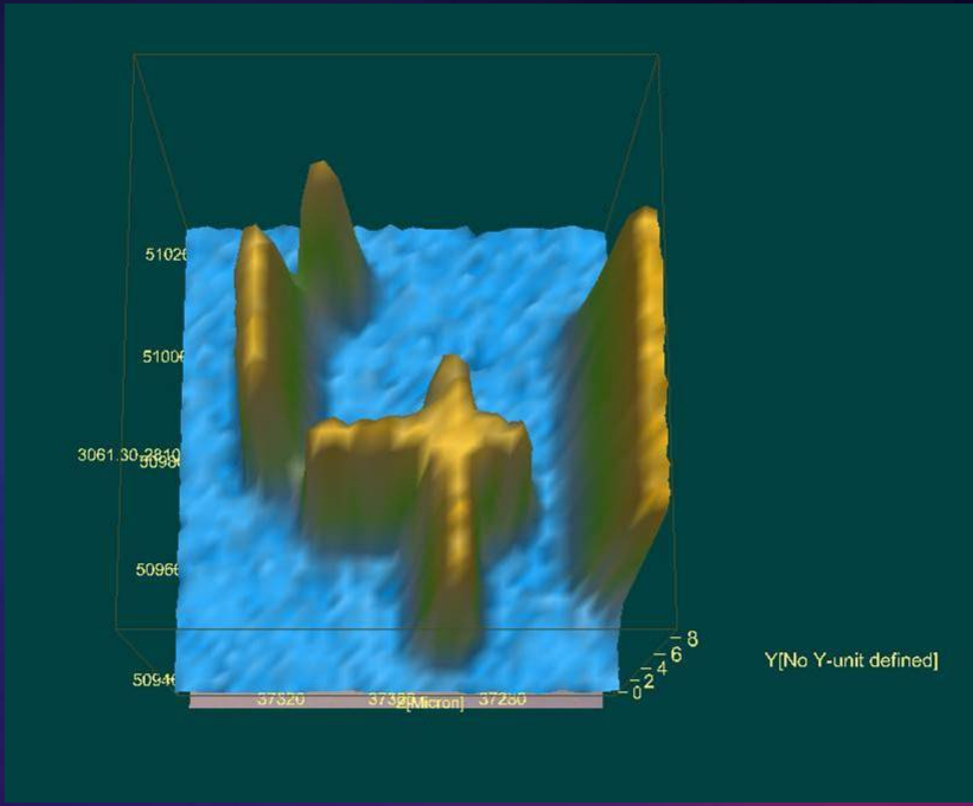
CH stretch absorption bands from 5 micron spot in tissue sample. Equivalent spectra recorded using synchrotron (top) and Globar (bottom).



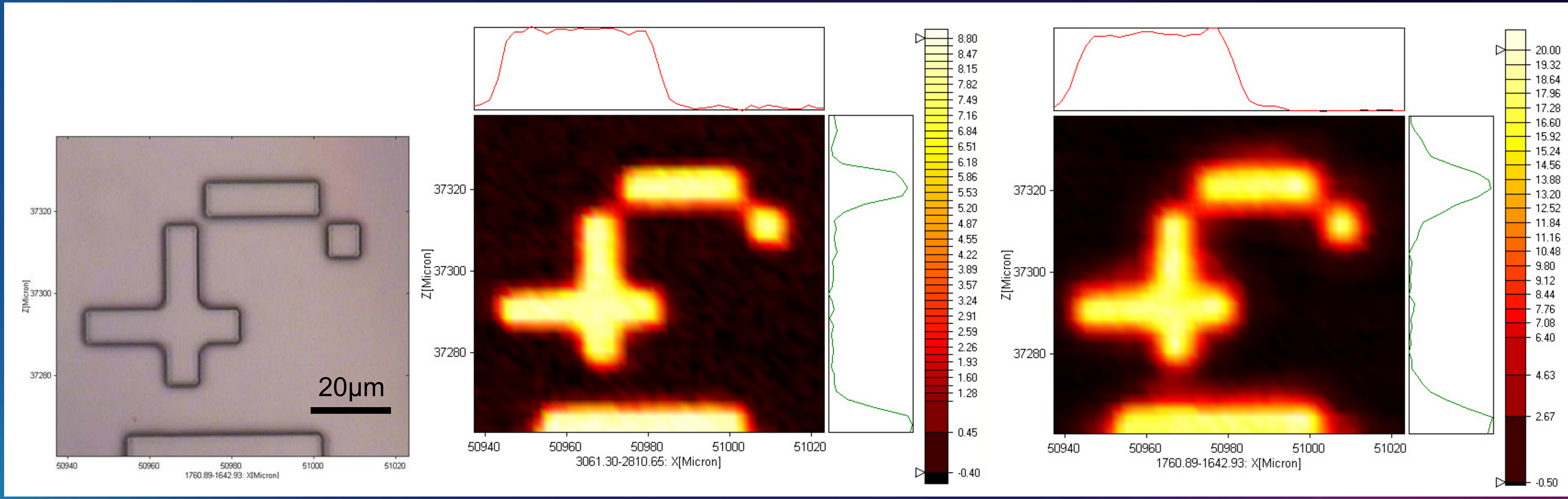
Australian Synchrotron

Testing the IR Beamline Performance with Custom Resolution targets





WAVELENGTH DEPENDENCE OF MICROSCOPE SPATIAL RESOLUTION DEMONSTRATED AT INFRARED BEAMLINE



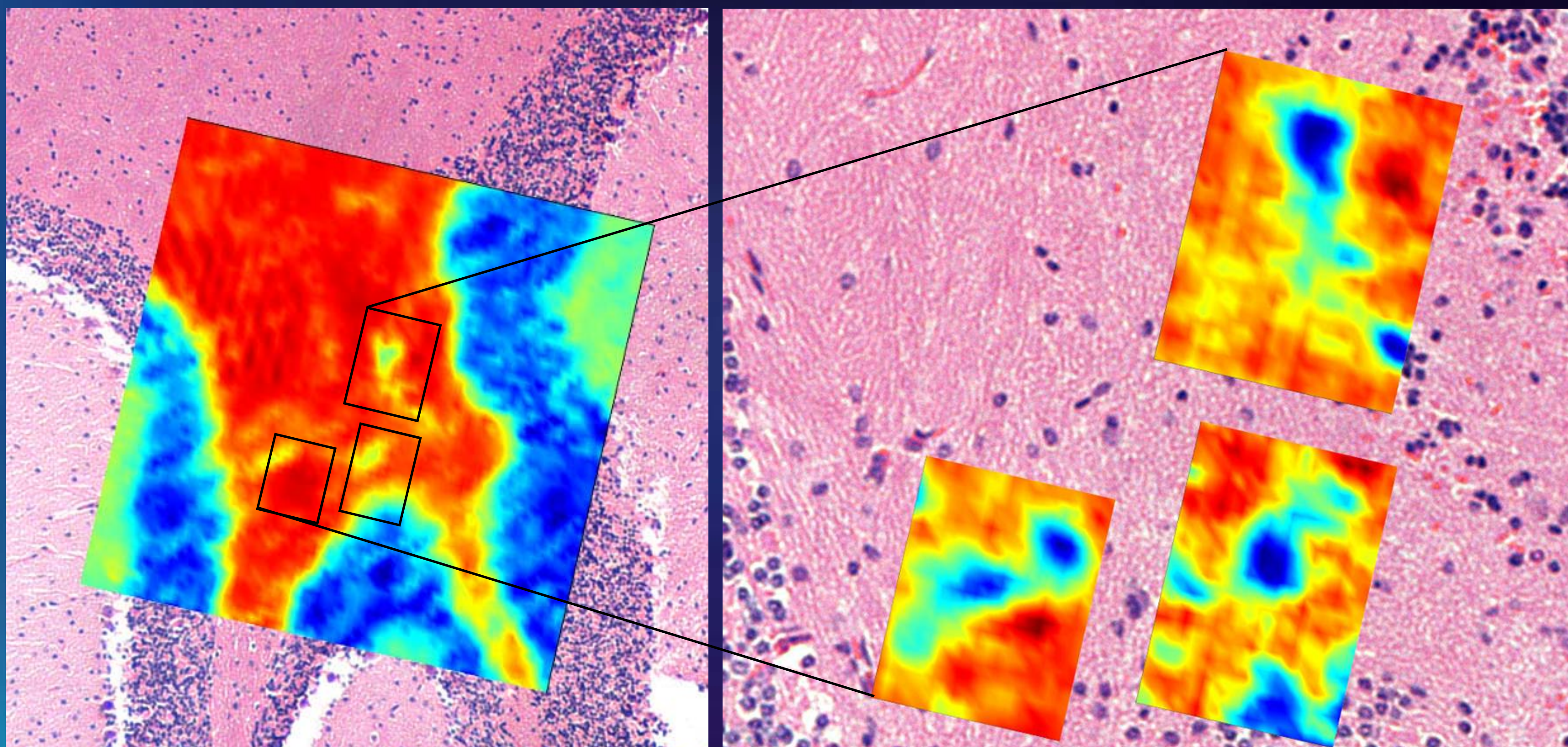
Polymer pattern on CaF_2 produced by photolithography

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.

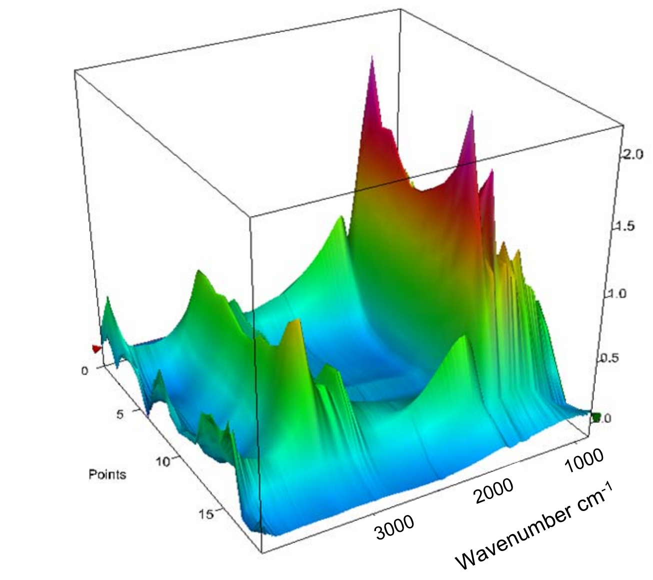
Bayden Wood, Don McNaughton, Alice Brandli, Cassie Jean
Monash University

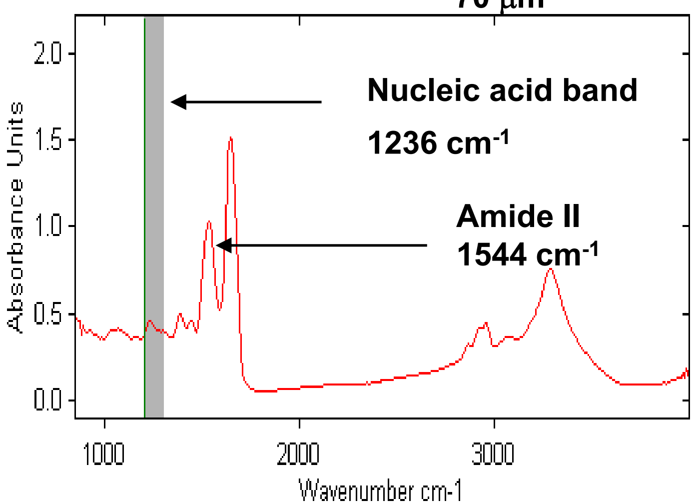
5 µm x 5 µm aperture
97 mA current
16 scans 6cm^{-1}

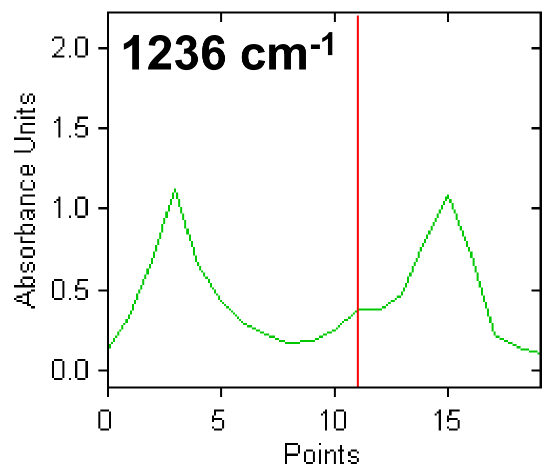
Germinal Vesicle Oocyte

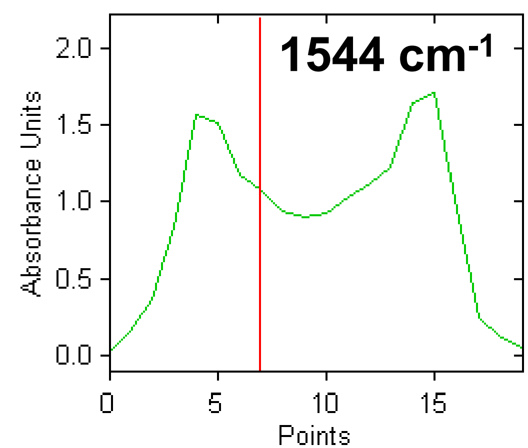
(a)

(b)

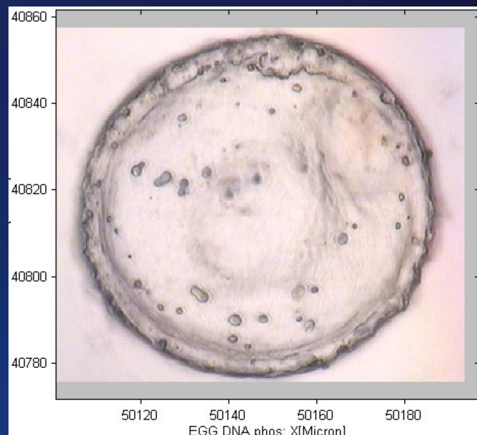


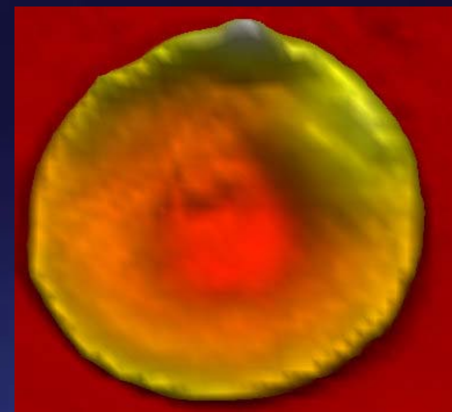




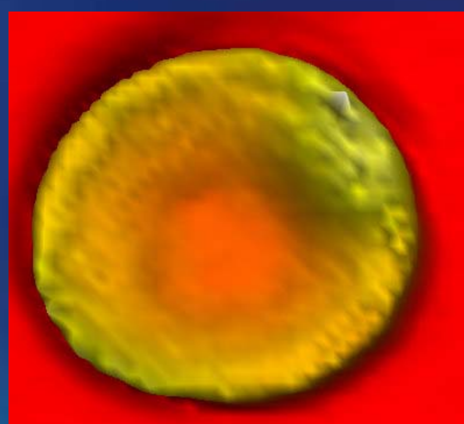


FTIR synchrotron maps of a GV oocyte

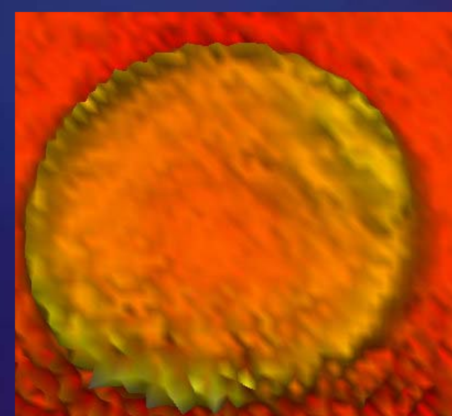




3000-2832 Lipid



1700-1600- protein



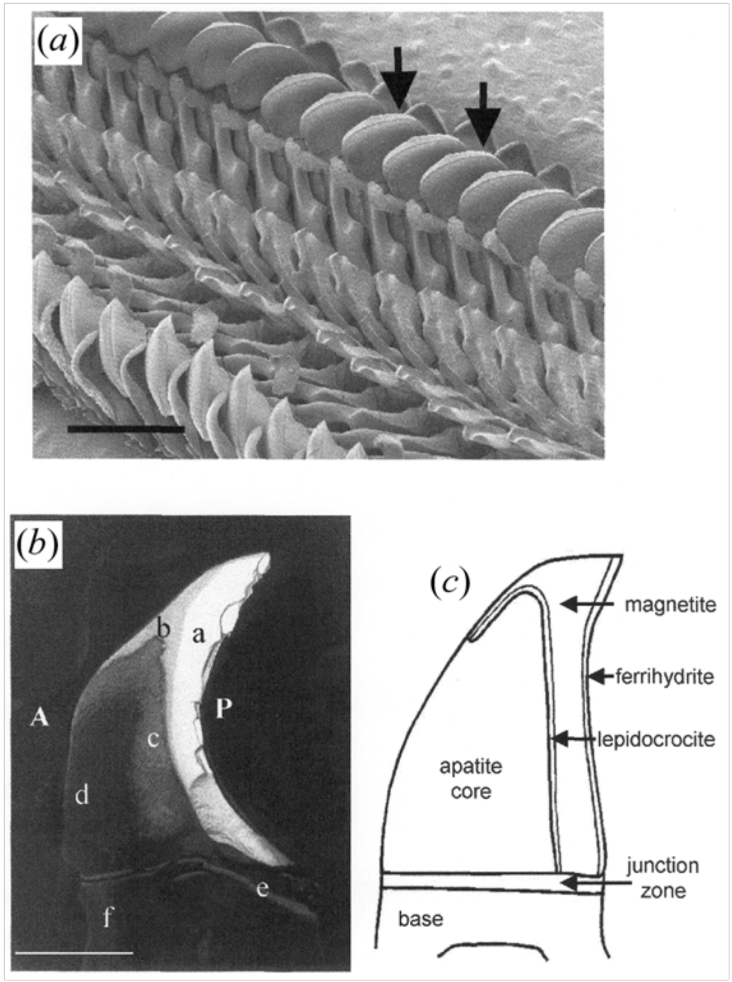
1260-1230- Nucleic acid

5 × 5 μm aperture

2 μm step size

16 scans, 6 cm^{-1}

Biomineralisation in Chiton Teeth



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).

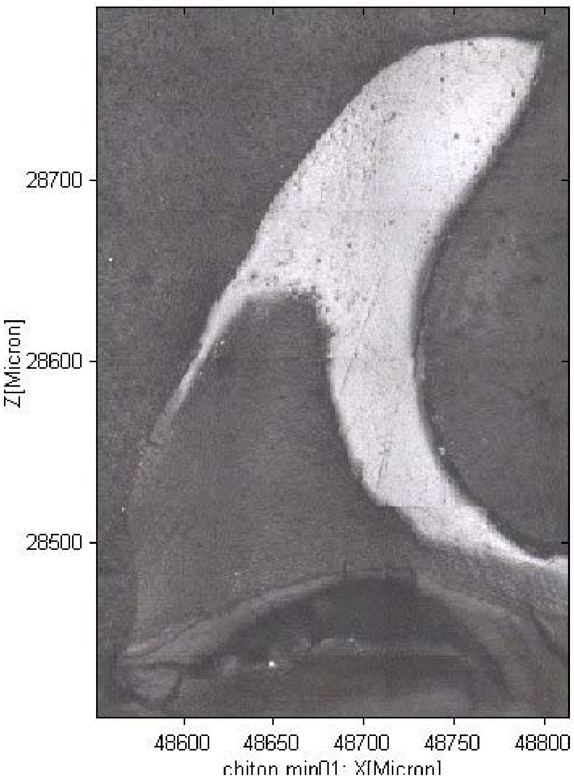
(*b*) Back-scattered electron micrograph of a ground and polished major lateral tooth in longitudinal section, depicting the six major regions of mineralization:

- **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.

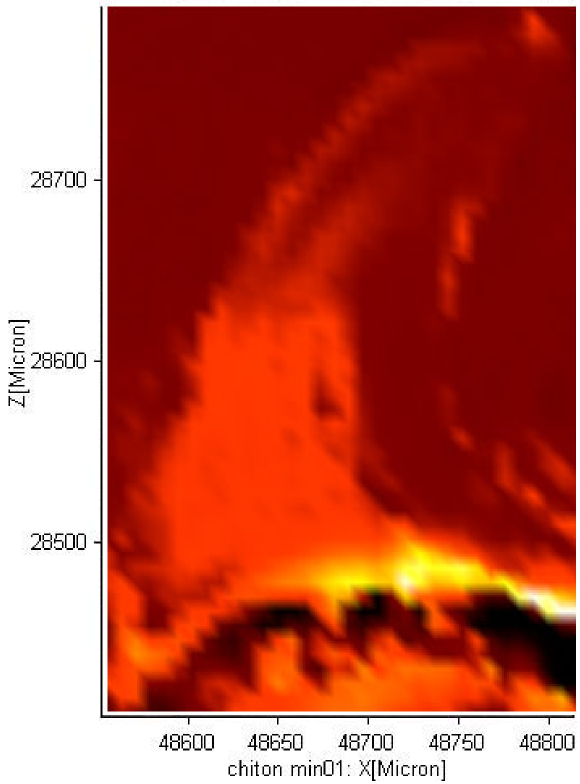
(*c*) Diagrammatic representation of a major lateral tooth depicting the various regions found in a fully mineralized major lateral tooth.

Bill van Bronswijk
Curtin University, Perth

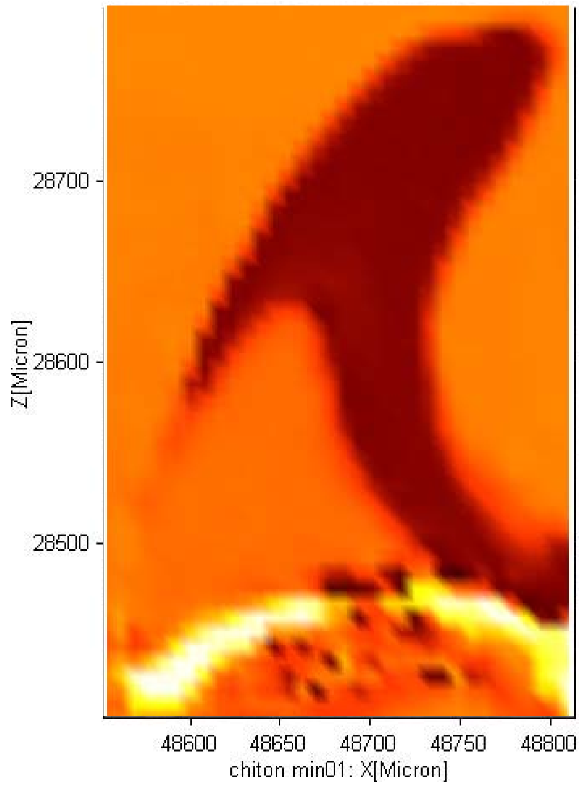
Infrared absorbance maps of single chiton tooth collected using IR microscope at Australian Synchrotron operating in reflectance mode.



Reflected light visible image of polished tooth section

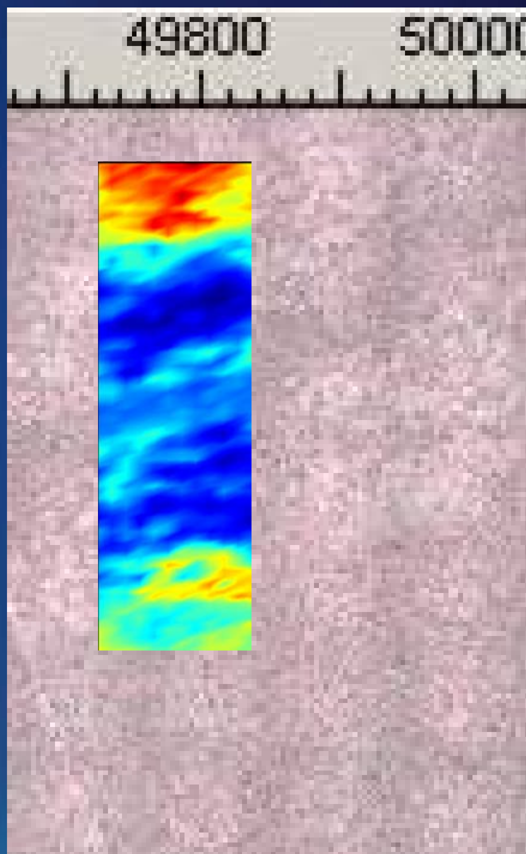


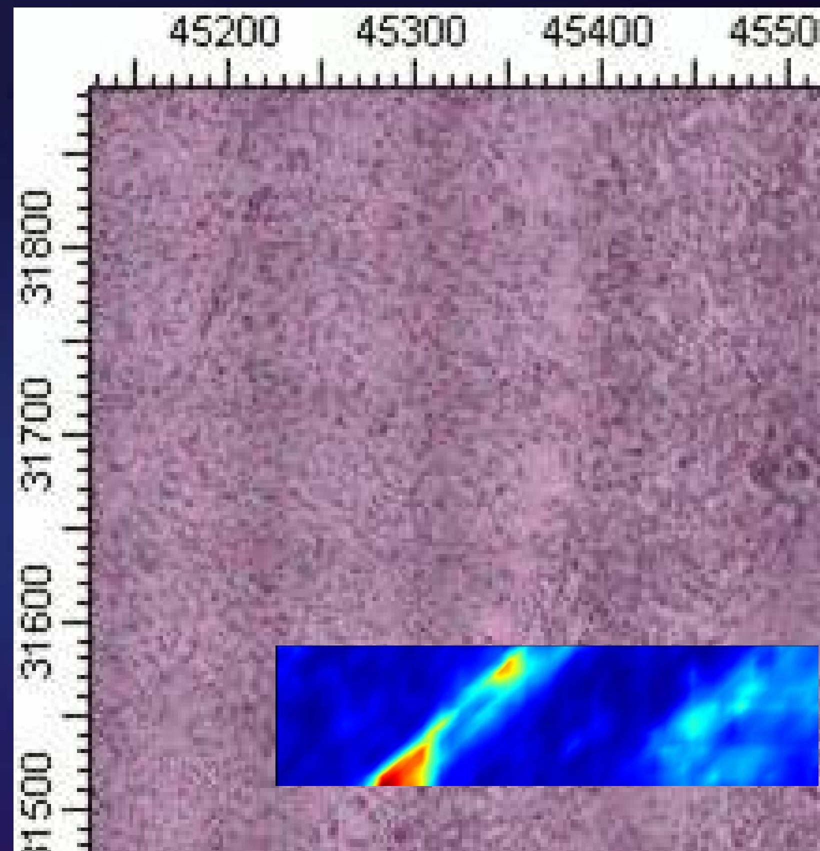
1030-1153 cm^{-1}
Probably apatite



1020-1190 cm^{-1}
Possible strong carbonyl stretch

IR mapping of cerebellum tissue infected with cerebral malaria





Control

Cerebral malaria

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

Liz Carter, Mark Hackett
University of Sydney

SR-FTIR Analysis of Cardiomyocytes

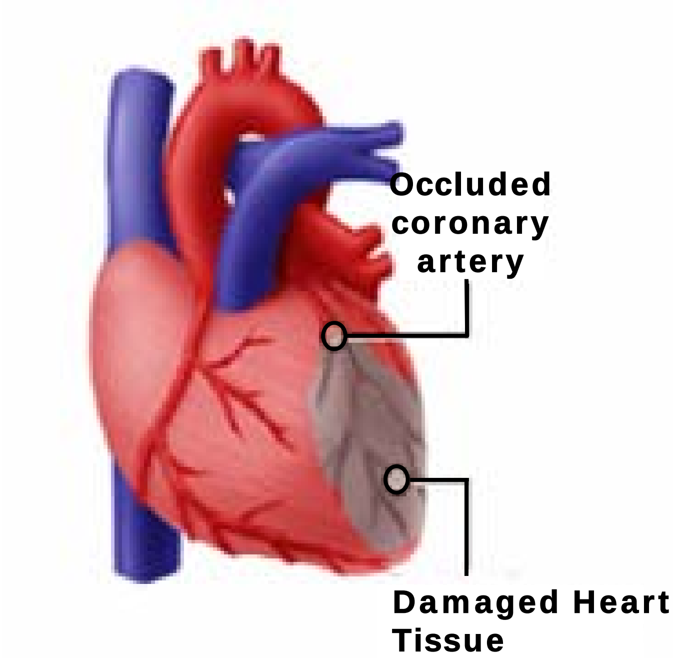
Ben Rayner, Paul Witting, Vascular Biology Lab, Anzac Research Institute, Concord, NSW.
Liz Carter, Peter Lay, Vibrational Spectroscopy Facility, University of Sydney, NSW

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

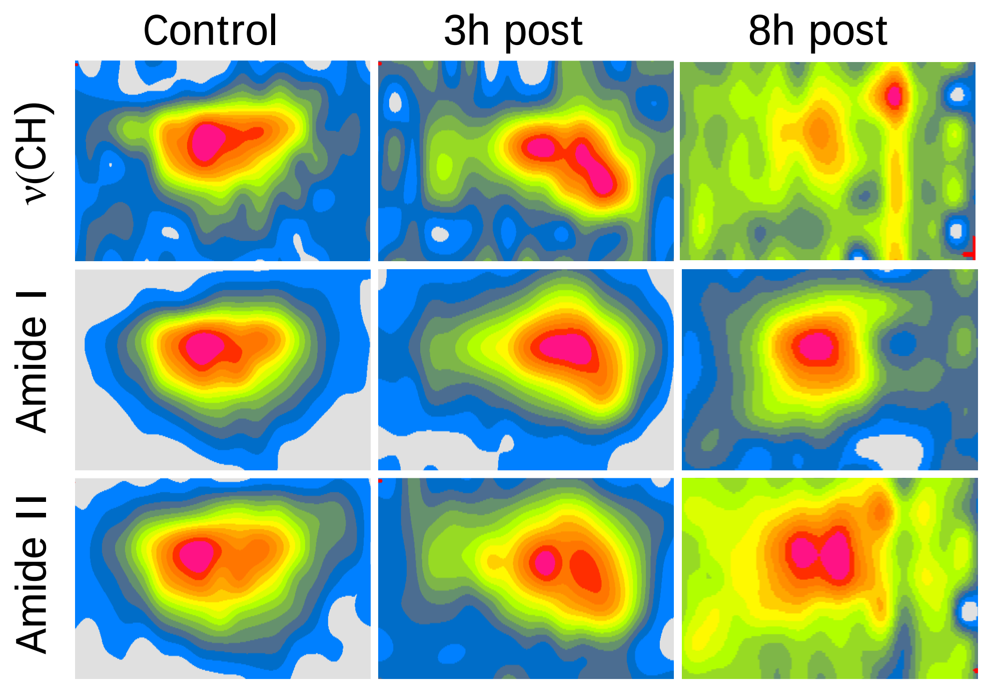


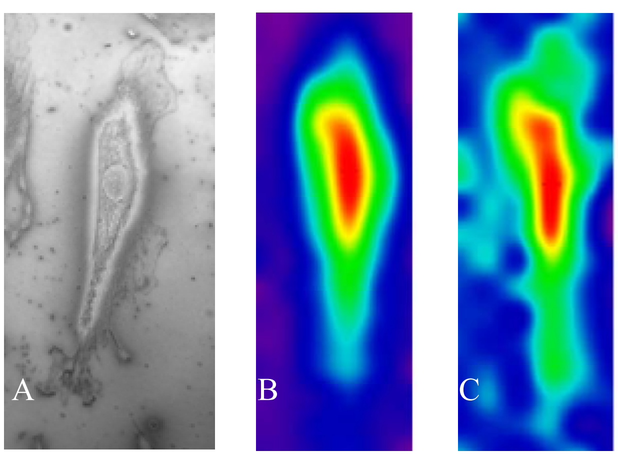
B. S. Rayner, E. A. Carter, Y.–C. Lee , C.–I. Chen, P. A. Lay, P. K. Witting. *Assessment of protein and lipid changes within an in vitro model of cardiac ischemia reperfusion injury*. Manuscript in Preparation.
B. S. Rayner, H. H. Harris, S. Vogt, Z. Cai, B. Lai, P. A. Lay, P. K. Witting. *Elemental ion flux in cultured cardiomyocytes subjected to hypoxia re-oxygenation*. Manuscript in Preparation.

Functional Group Maps of Cardiomyocytes

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

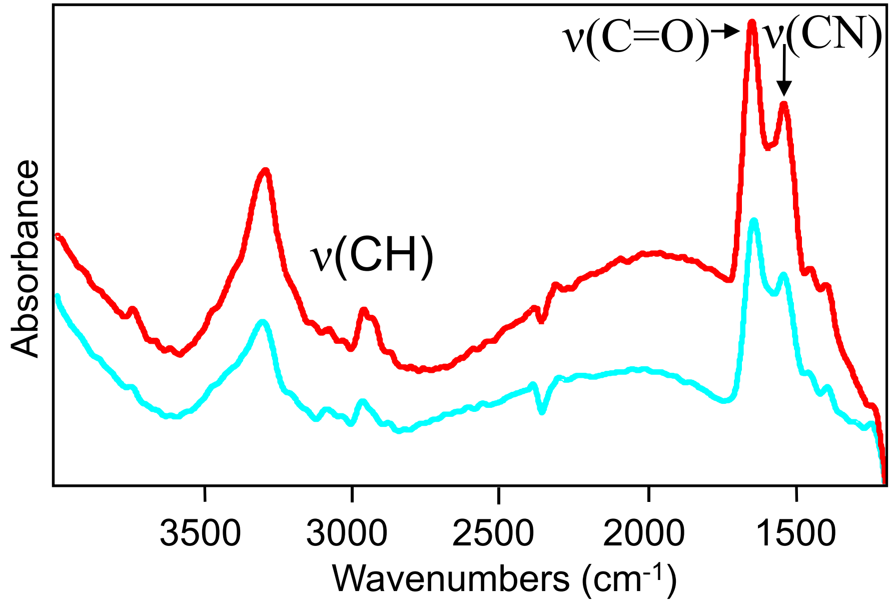
- Increase in level of mitochondrial dysfunction
- Increase in level of apoptosis and necrosis
- IR functional group maps visualise the loss of lipid and protein structure.
- Particular evident in nuclear region of cell.





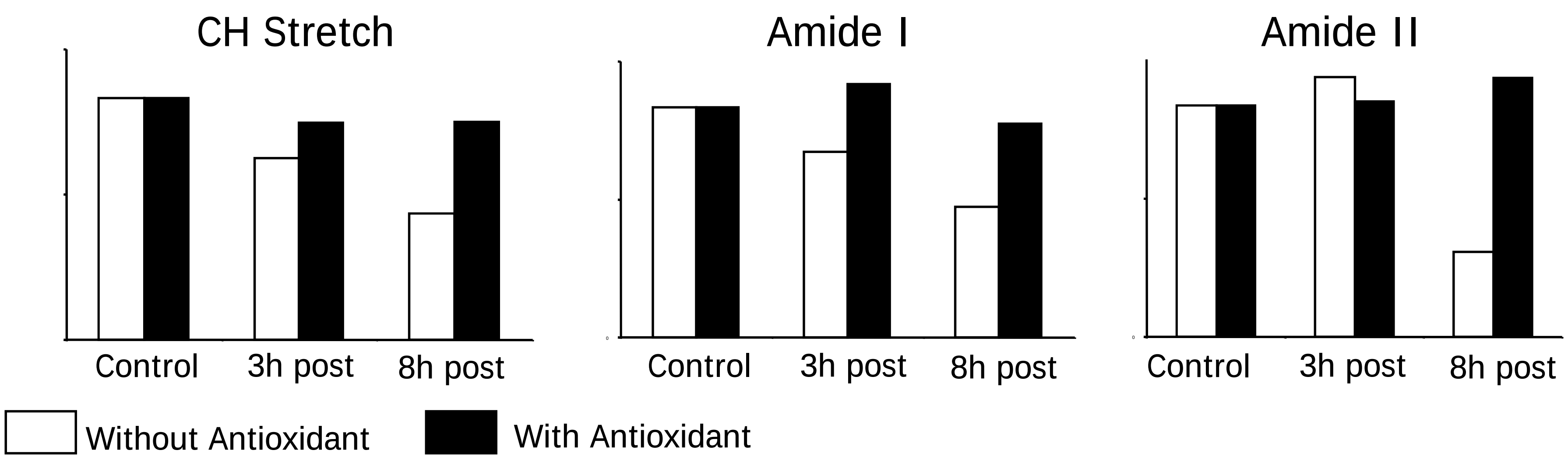
A) White light image of cardiac myocyte
B) Amide I (1771–1587 cm–1)
C) CH region (3000–2842 cm–1)

Anti-Oxidant Effectiveness

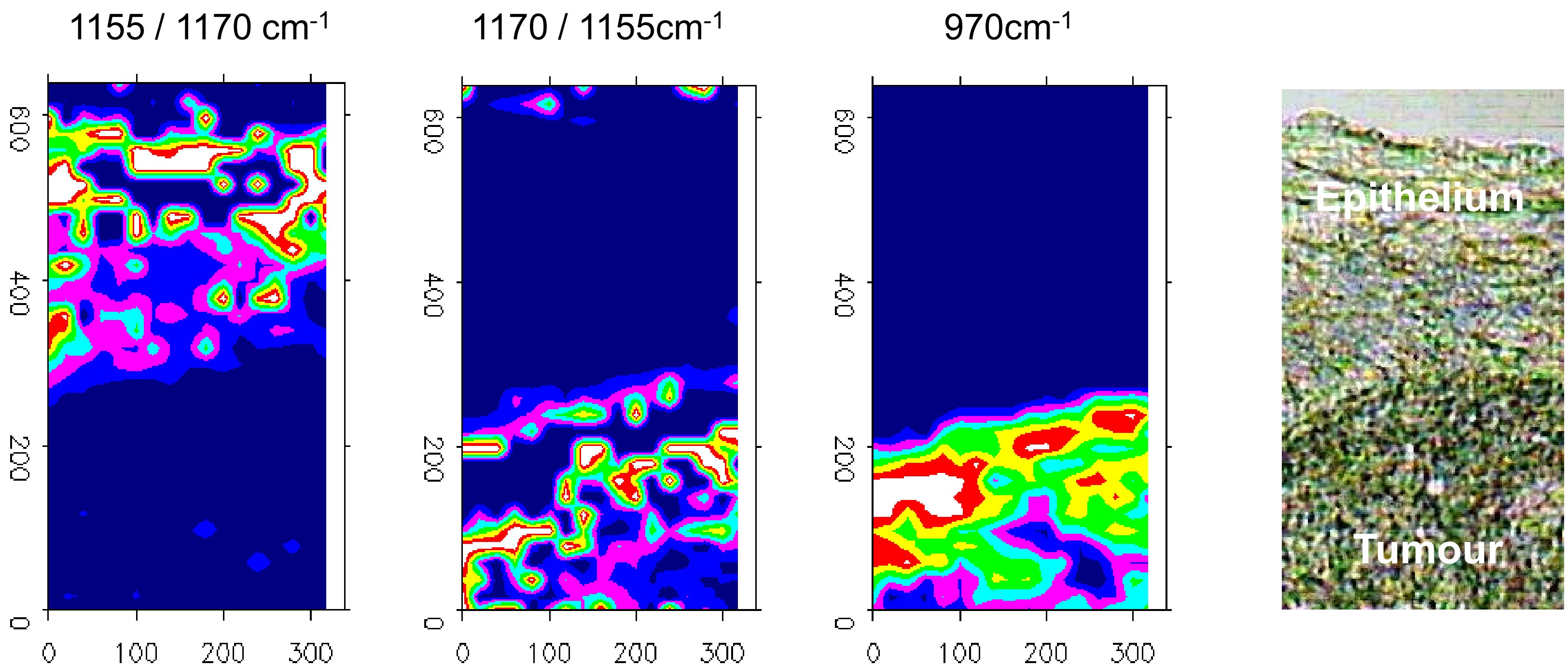


Aim:

- To use IR spectroscopy to test the effectiveness of an antioxidant in ameliorating damage caused by H/R injury



FTIR Microspectroscopy of Diseased Tissue



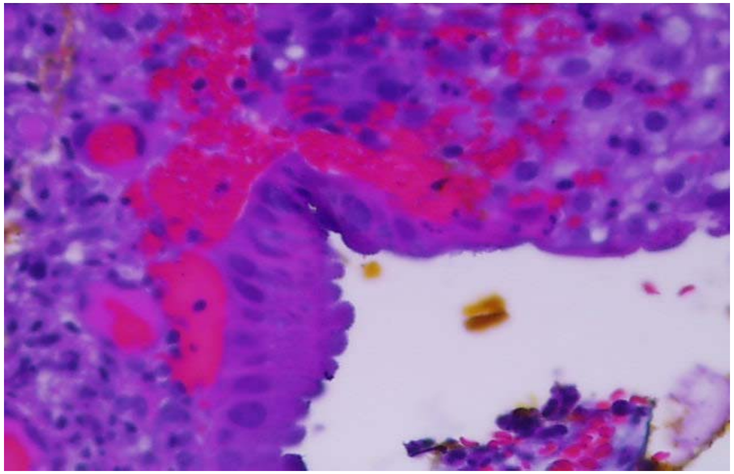
Bright points show non-malignant IR profile

Bright points show malignant IR profile

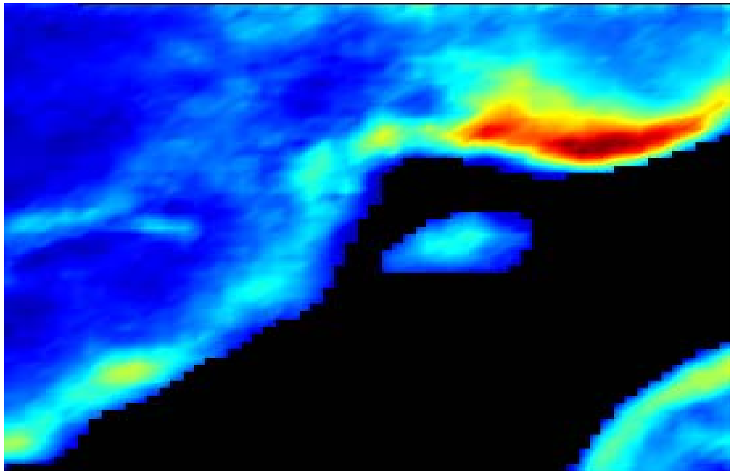
Bright points show malignant IR profile

FTIR Mapping of the Cervical Transformation Zone

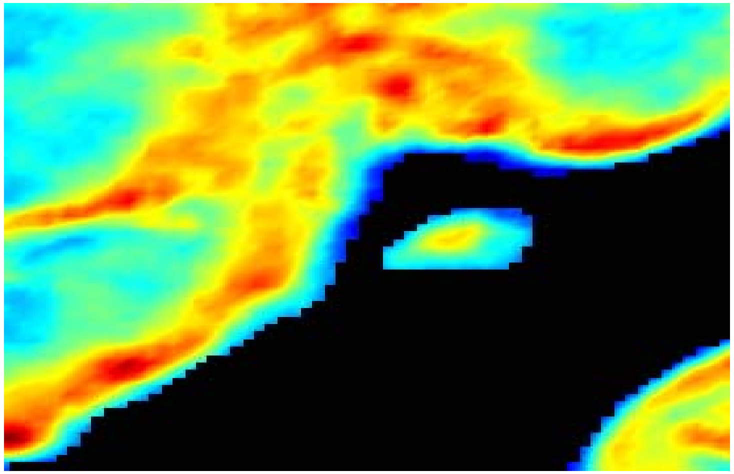
Bayden Wood, Monash Centre for Biospectroscopy
Michael Quinn, Royal Melbourne Hospital



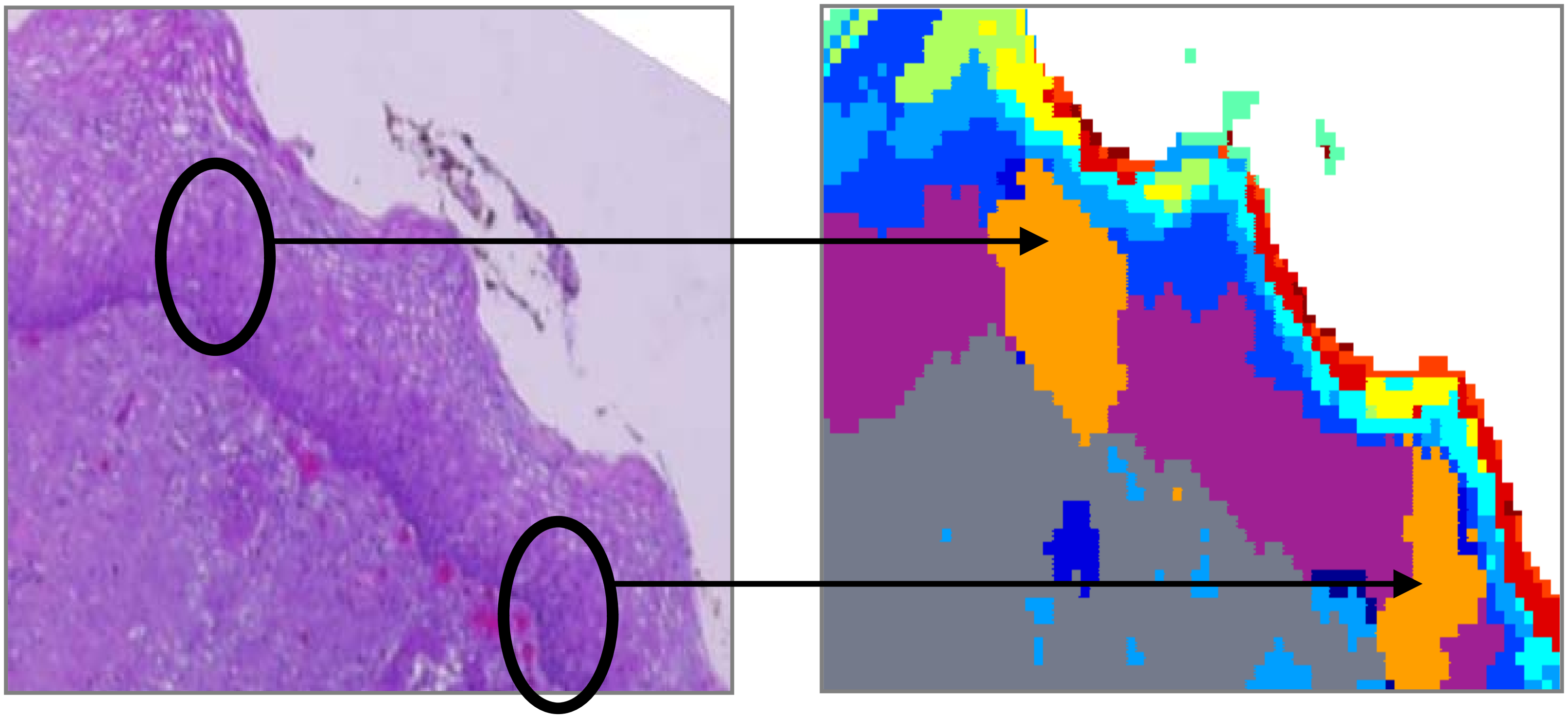
1024 cm^{-1} glycogen distribution



1544 cm^{-1} protein distribution



The tenth cluster (orange) highlights two potential foci of dysplasia (pre-malignant cells)

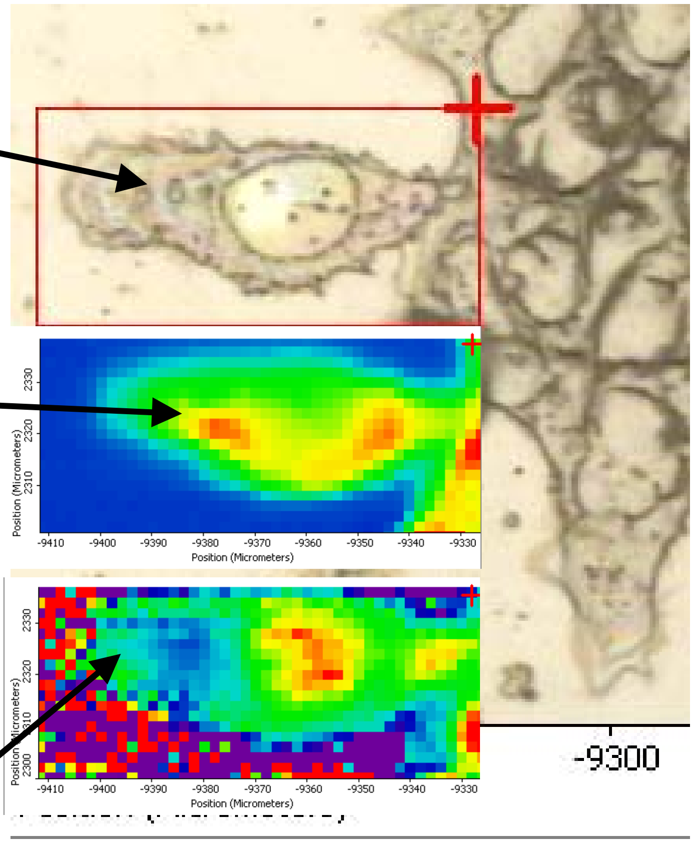


High resolution FTIR imaging of membrane organisation in single cells

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.

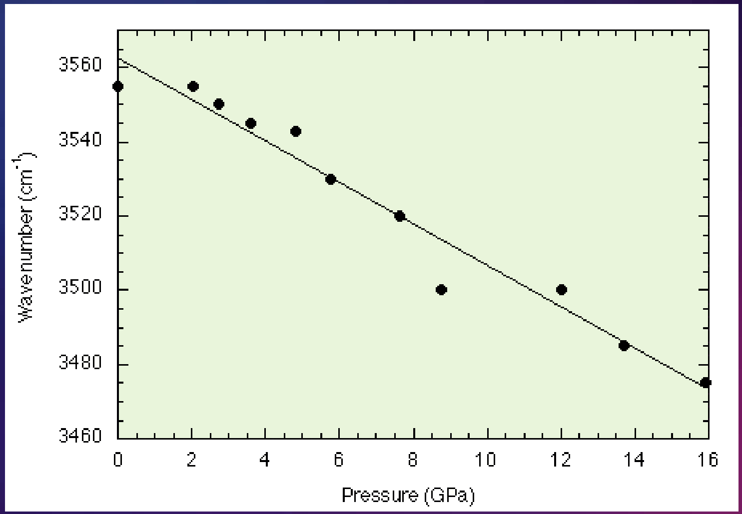


Green area = possible membrane reorganisation at leading edge of cell

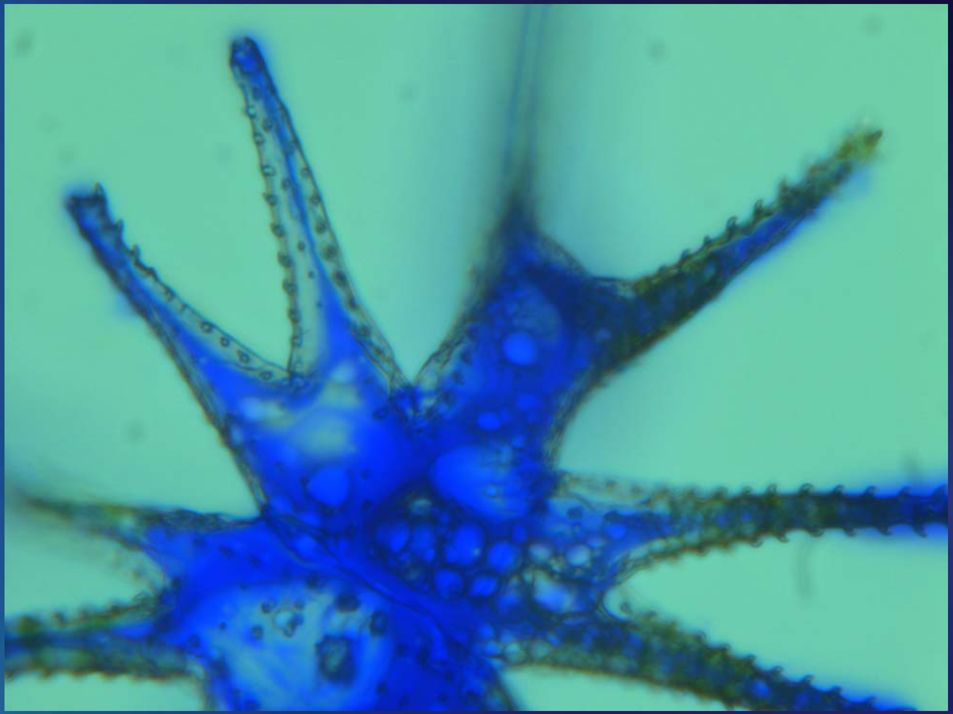
High pressure studies of minerals

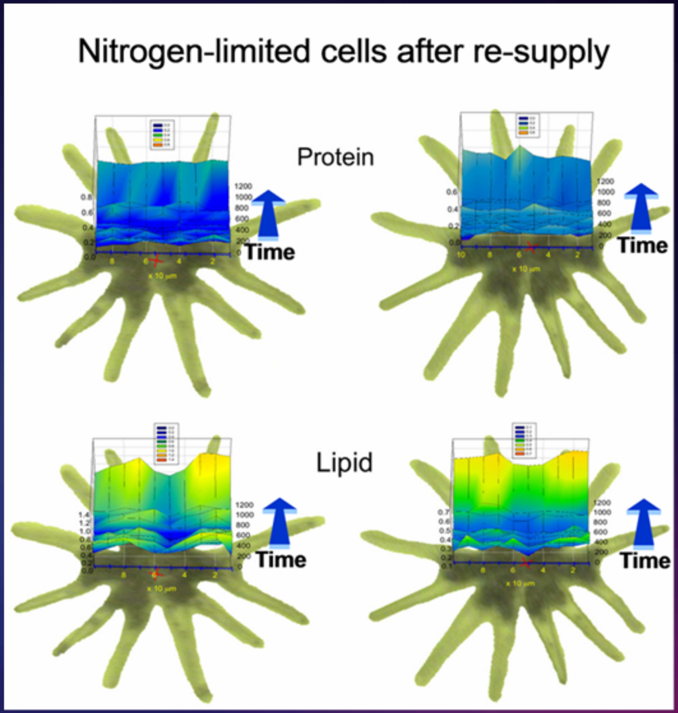
Lawsonite is an important reservoir of water within the Earth's mantle

- It is stable at very high pressures
- It contains 11% water
- O-H bonds are being used in studies of phase changes



Environmental Science applications

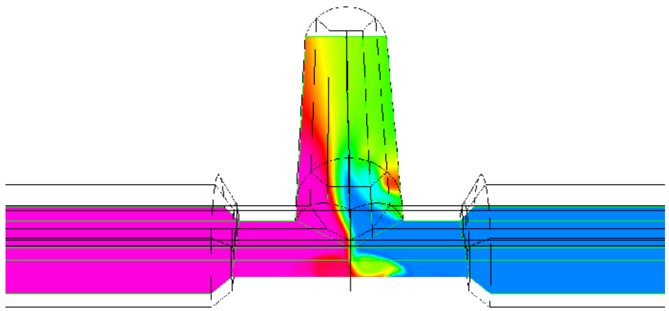




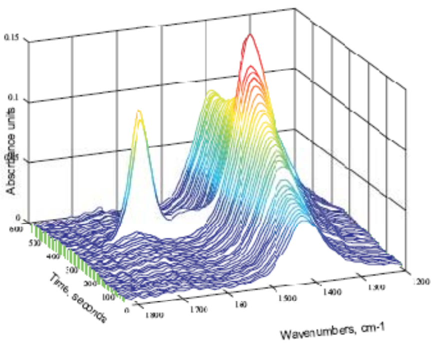
IR synchrotron microspectroscopy reveals microscale biochemical changes occurring in living plant cells. This allows researchers to better understand how plants cells respond to changes in the environment. Image (left) and FTIR maps (right) of freshwater alga *Micrasterias hardyi.*

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

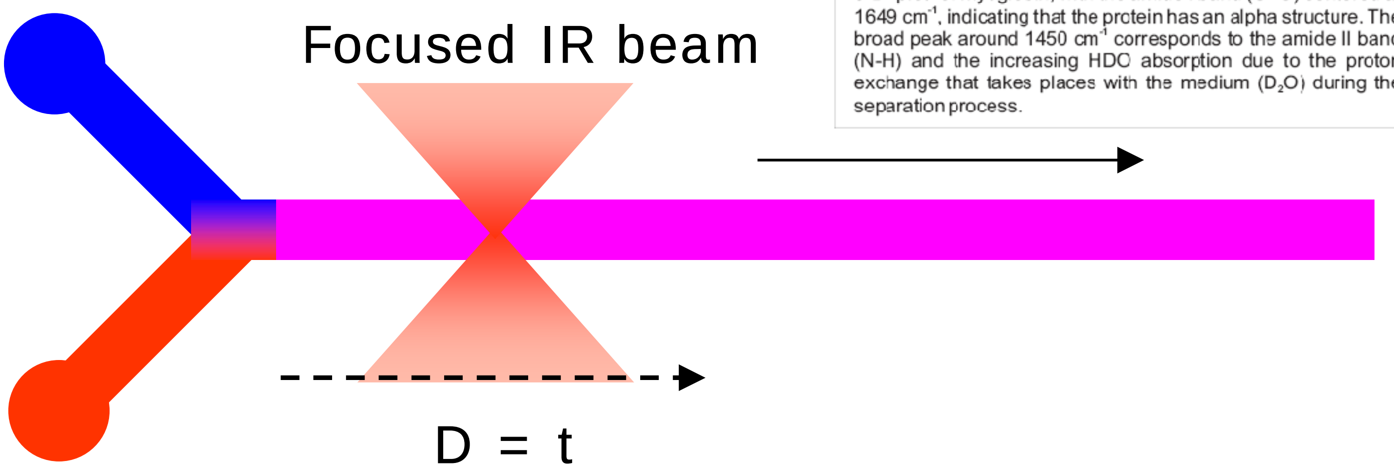
Microfluidics for time resolved protein folding studies



Protein mobility in a CaF_2 CE chip

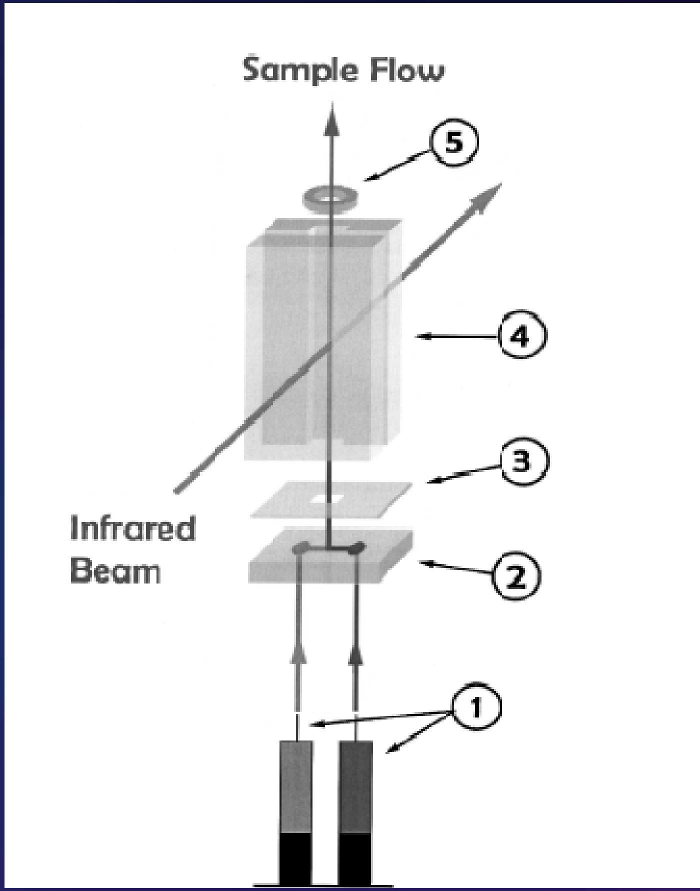


3-D plot of myoglobin, with the amide I band (C=O) centered at 1649 cm^{-1}, indicating that the protein has an alpha structure. The broad peak around 1450 cm^{-1} corresponds to the amide II band (N-H) and the increasing HDO absorption due to the proton exchange that takes places with the medium (D_2O) during the separation process.



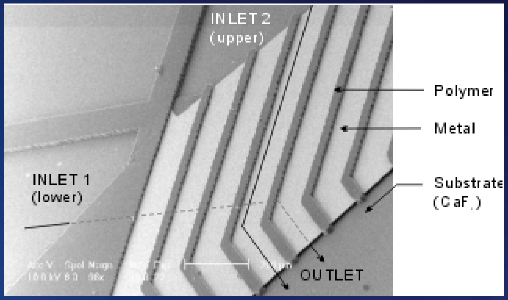
Time resolved FTIR - complementary to CD, and benefits from highly focused SR-IR beam

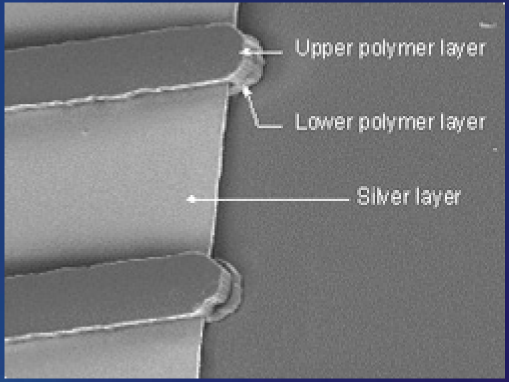
Simple Brookhaven flow cell

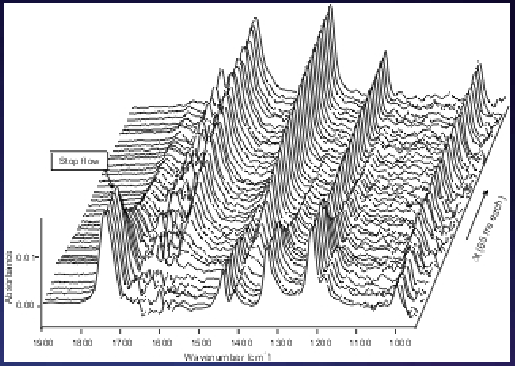


50μm path length (so D_2O required)
ZnSe windows
~ms time resolution claimed
High flow rate (1 ml min^{-1})
2% path length change under pressure

Microfluidics for time resolved protein folding studies







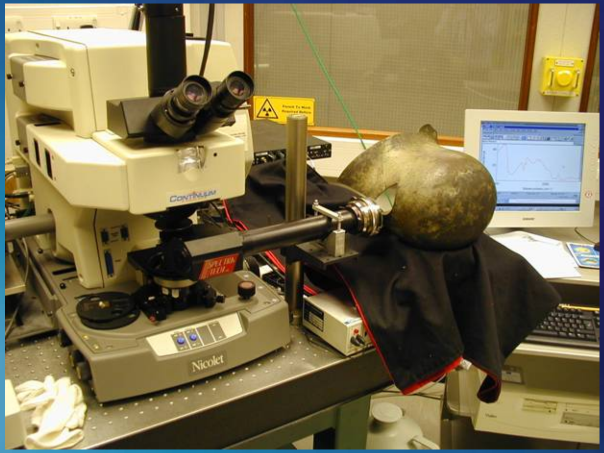
Main requirement - very high S/N in ~10x10┌m (5x10^{-5} A.U.)
Can integrate for seconds

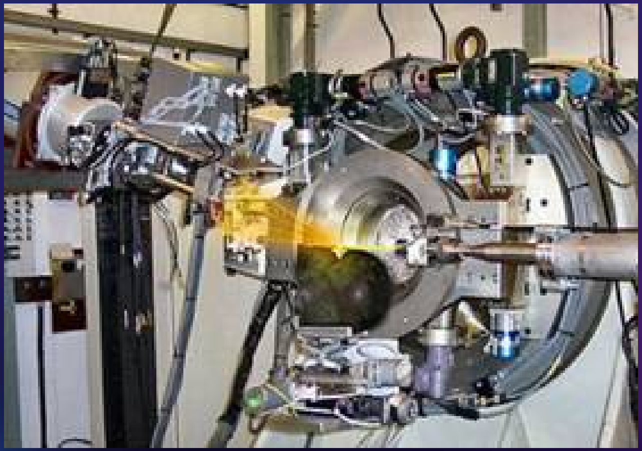
Images and spectra courtesy of N. Kaun and B. Lendl, T.U. Vienna

Cultural Heritage applications

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.





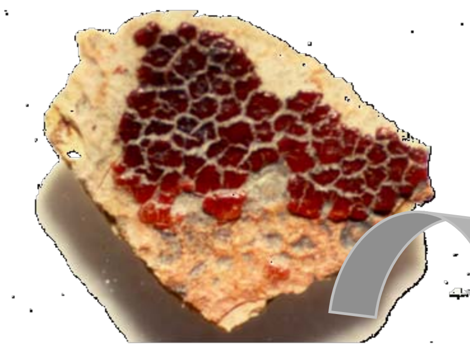
Cultural Heritage applications

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

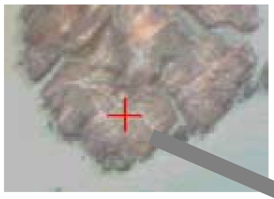
Catalan gothic altarpiece (Retaule del Conestable) by Jaume Huguet, 15th century, one of the most important artists of the period.

Cultural Heritage applications

Evidence for presence of carminic acid in an egg albumin binding matrix.

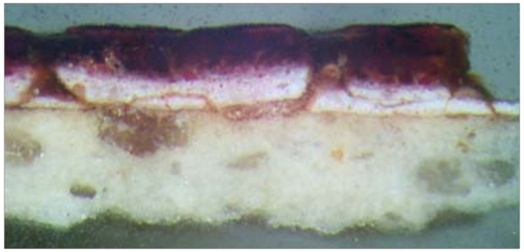


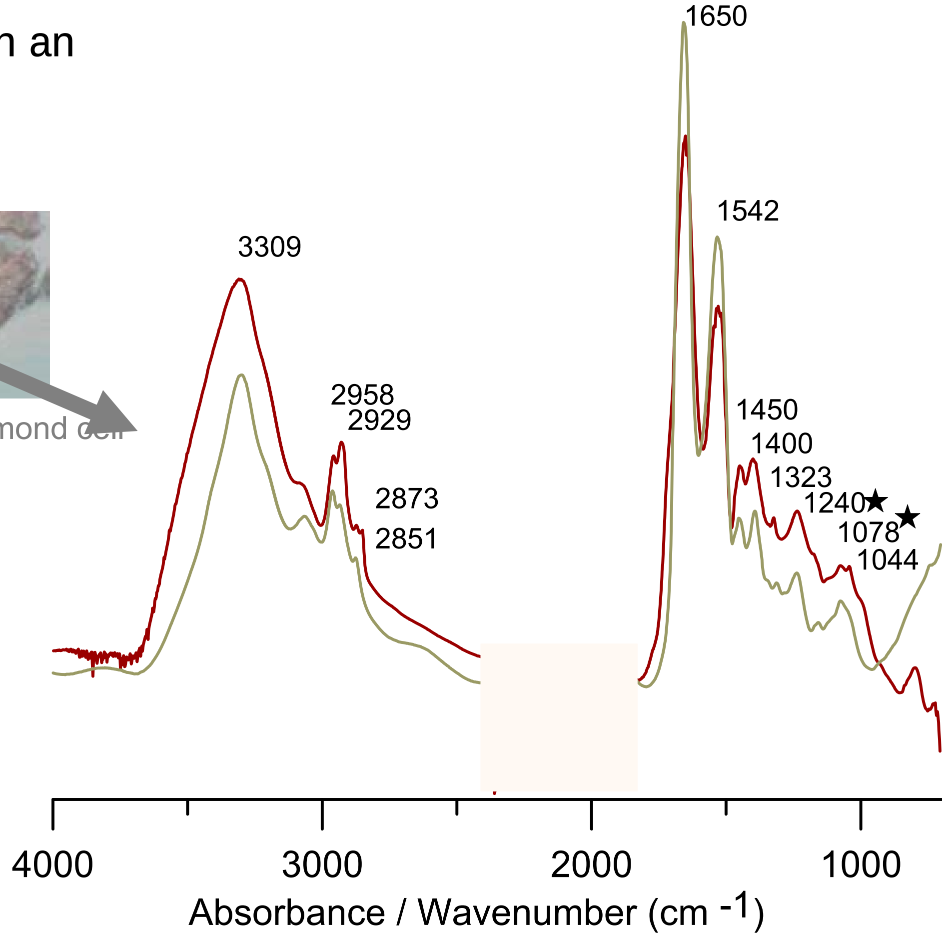
dark red sample



Preparation on diamond cell

Fragment from dark red sample



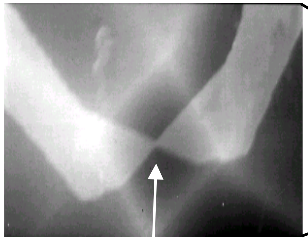


SR-FTIR microspectroscopy spectra (128 scans, 4cm^{-1} resolution, spot size 10x10) from dark red layer (—) and aged egg white (—).

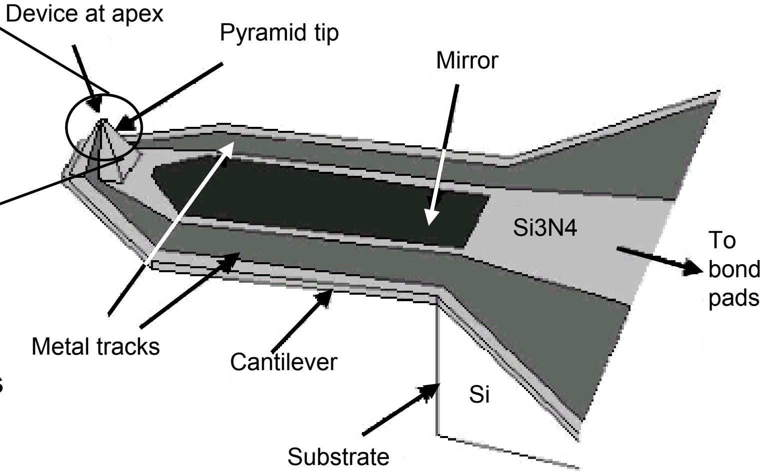
FUTUREÅDEVELOPMENTSÅIN SYNHCROTRONÅIRÅSPECTROSCOPY

Breaking the diffraction limit – developing photothermal microspectroscopy

Micromachined probe (University of Glasgow)

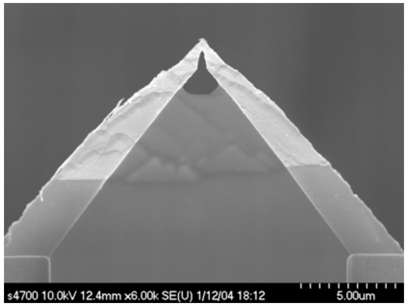


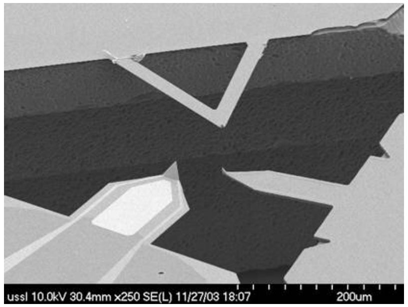
Resistive element:
Palladium
50 nm thick
"bowtie" taper measures 150 nm



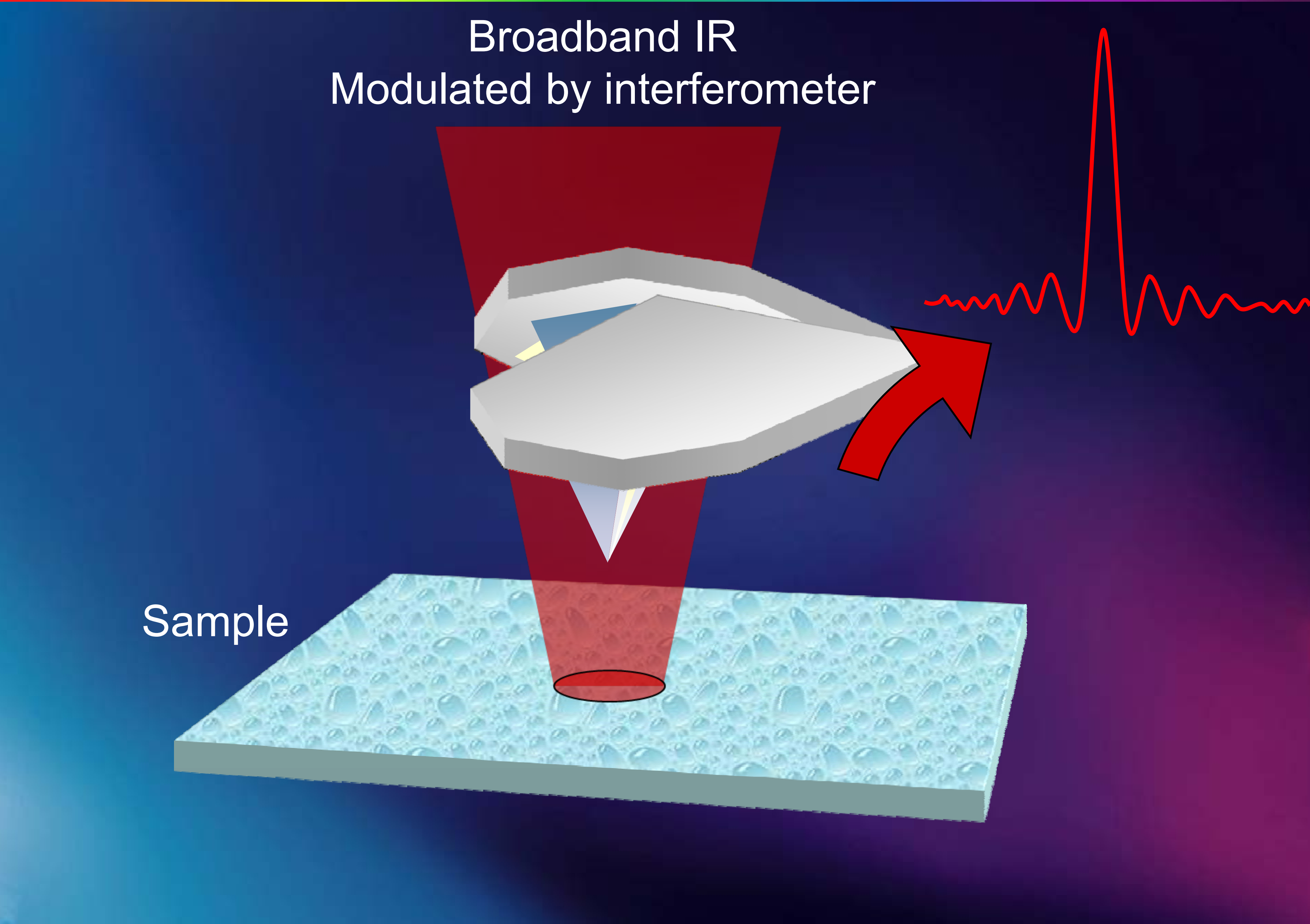
Silicon nitride
Fabrication process involves:
- **Photolithography**
- **Potassium hydroxide etching**
- **Multiple levels of electron-beam lithography**

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

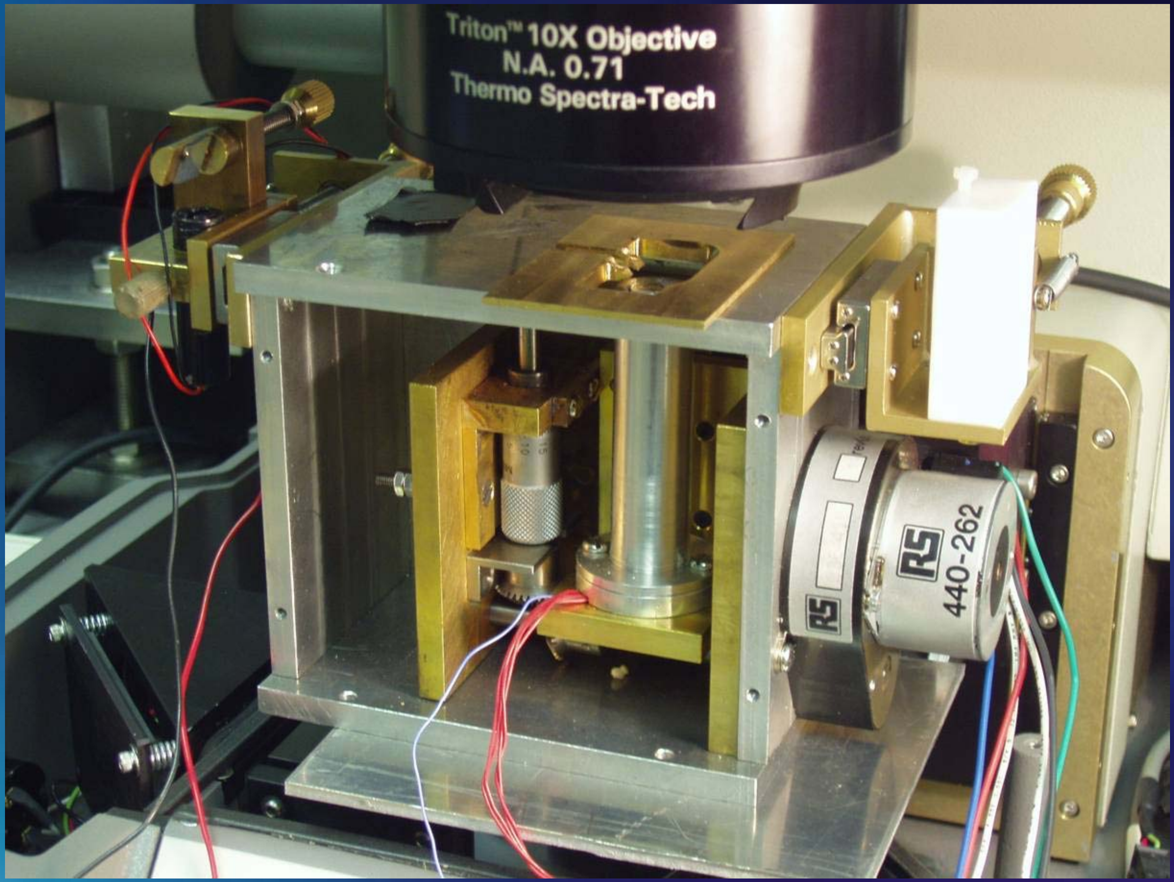


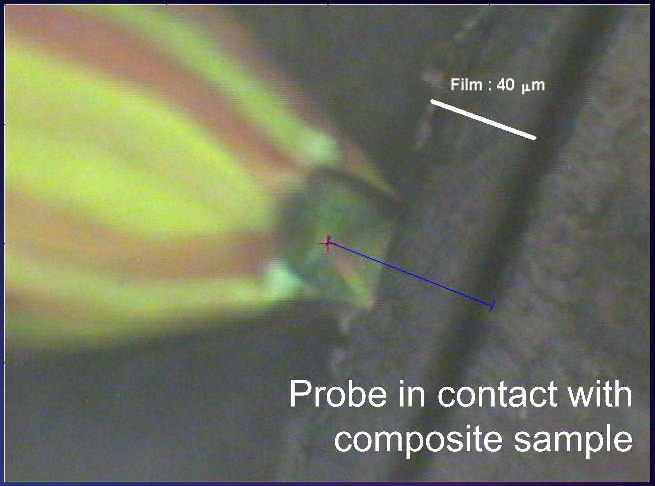


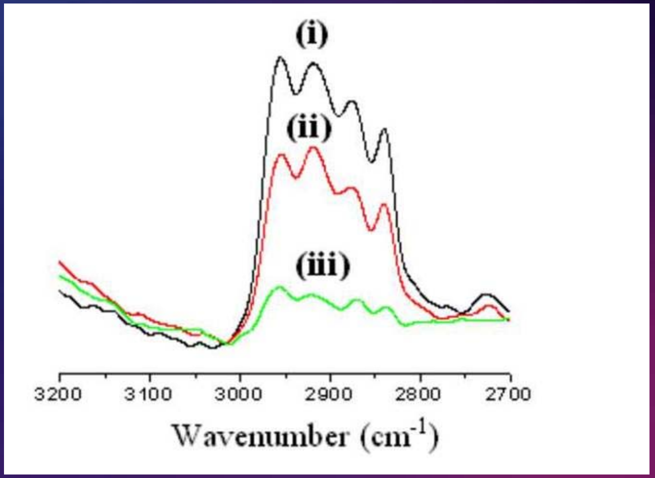
- **These probes can measure:**
  - **Force**
  - **Temperature**
- **They can act as highly localised heat sources**


Broadband IR
Modulated by interferometer
Sample

Breaking the diffraction limit – developing photothermal microspectroscopy





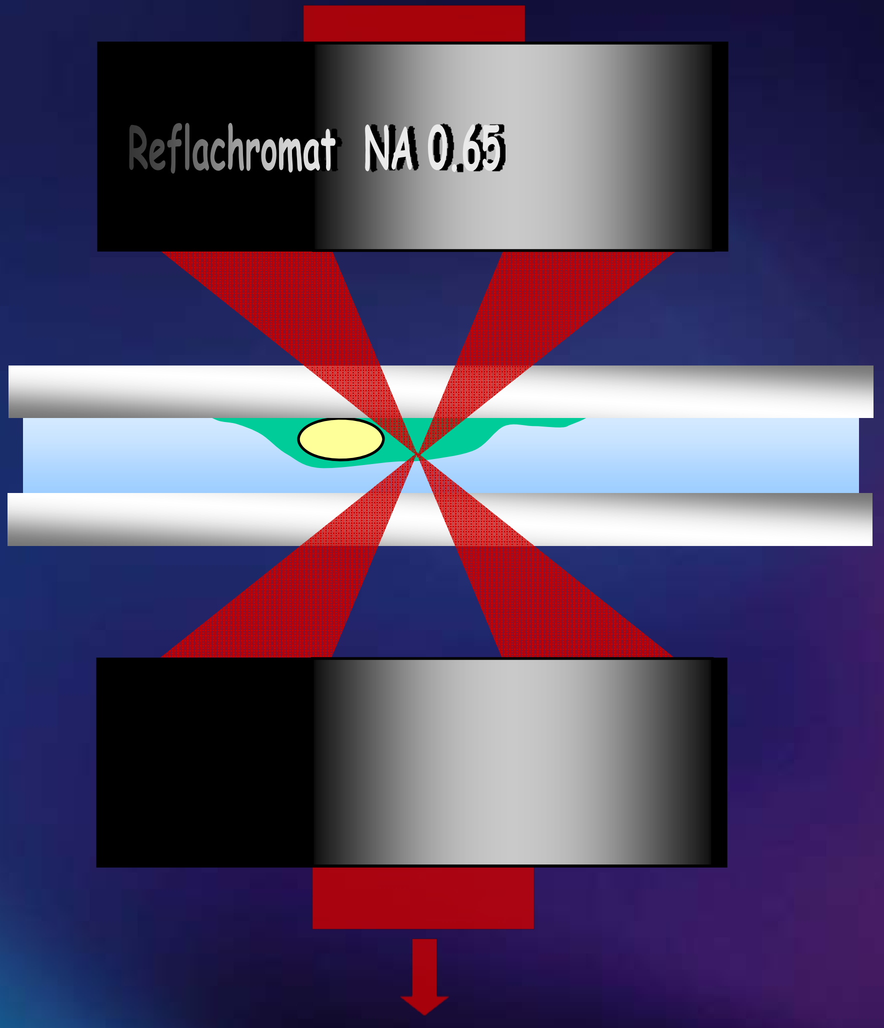


An AFM-based thermal probe is used to map the surface of samples in the SR-IR beam.

Azzedine Hammiche, Hubert Pollock
University of Lancaster UK

Transmission measurement of wet sample

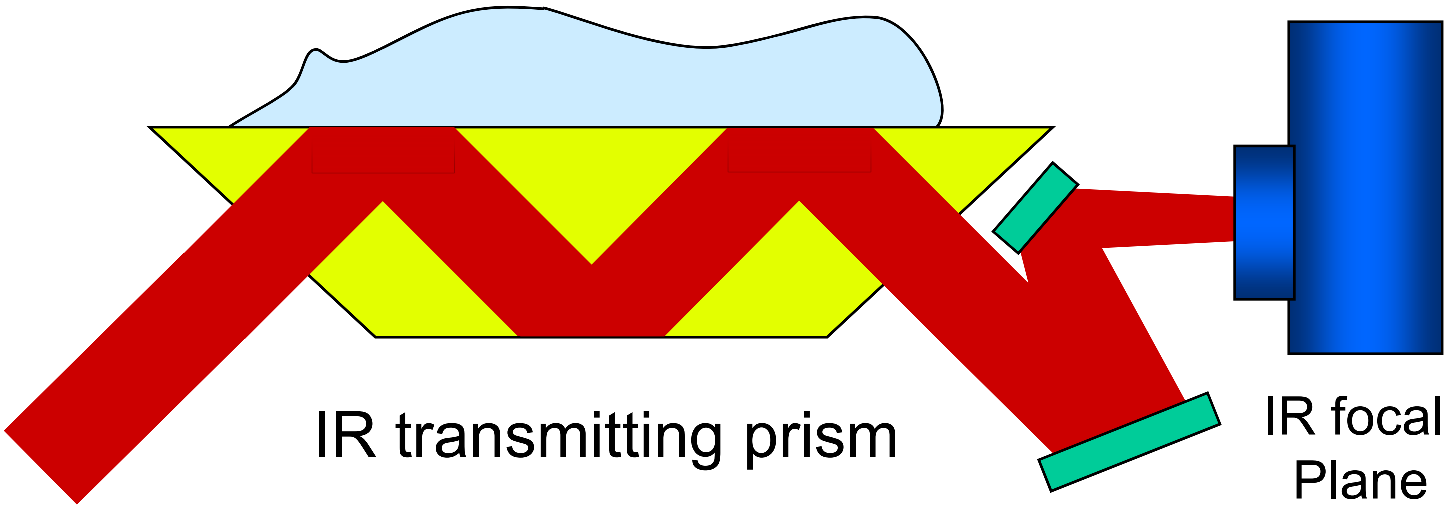
Liquid cell, or flow cell



Max liquid depth ~ 10 µm

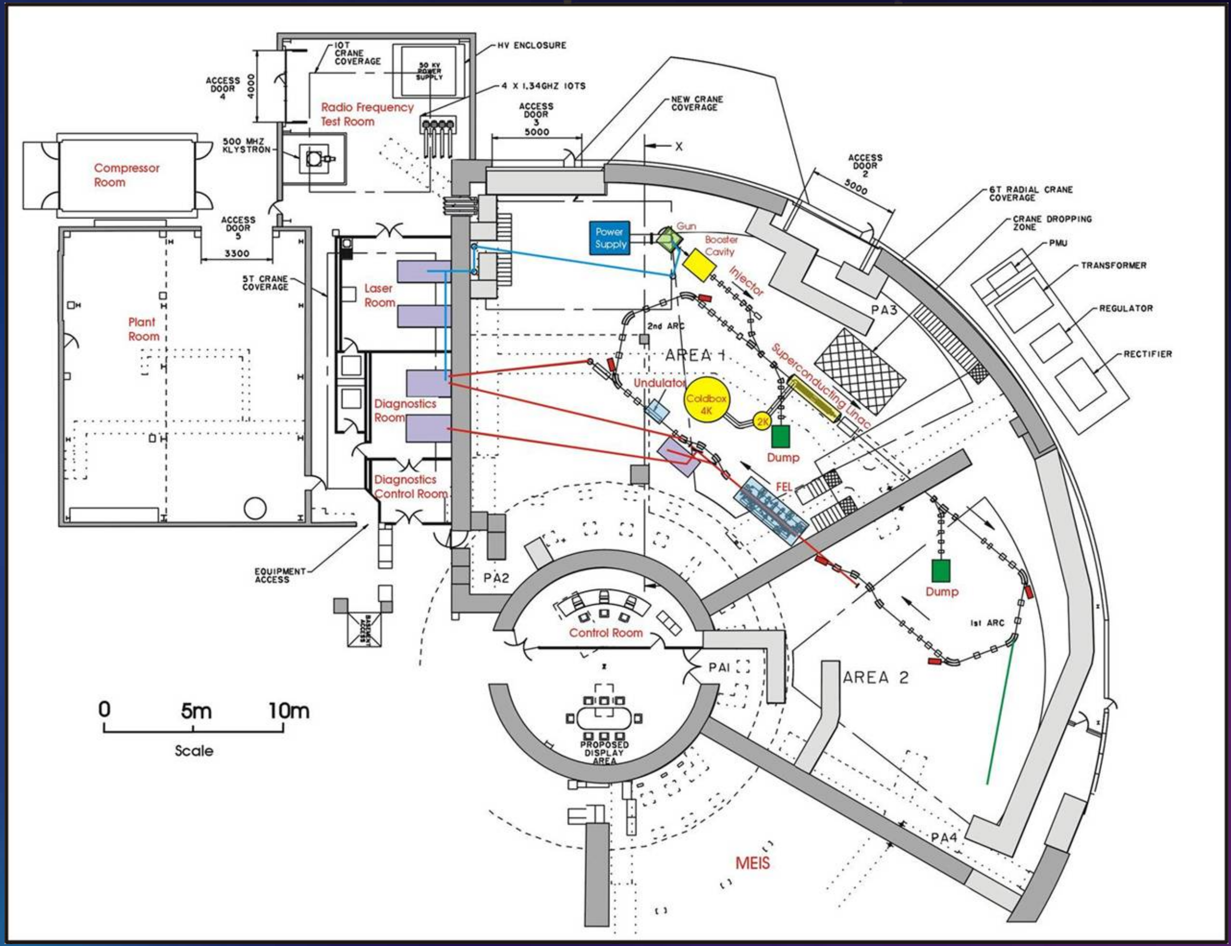
ATR spectroscopy – and imaging

$$d = l / (2\pi\, n_1[\sin^2\theta - (n_2/n_1)^2]^{1/2})$$



For λ = 6 μm   d = 1.6 μm

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

Acknowledgements

- Dudley Creagh – Canberra University
- Don McNaughton – Monash Univerrsity
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- Cassie Jean – Monash University
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- Evan Robertson – Monash University
- Ljiljana Puskar – Monash University
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- Mike Martin – ALS
- Ulli Schade - BESSY II
- David Moss – ANKA
- Yves-Laurant Mathis – ANKA
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- Nati Salvado – University of Barcelona
- Azzedine Hammiche – University of Lancaster
- John Prag – Manchester Museum
- FMB – Berlin
- Biolab/Bruker Instruments

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