Infrared Spectroscopy and Microscopy Using Synchrotron Radiation

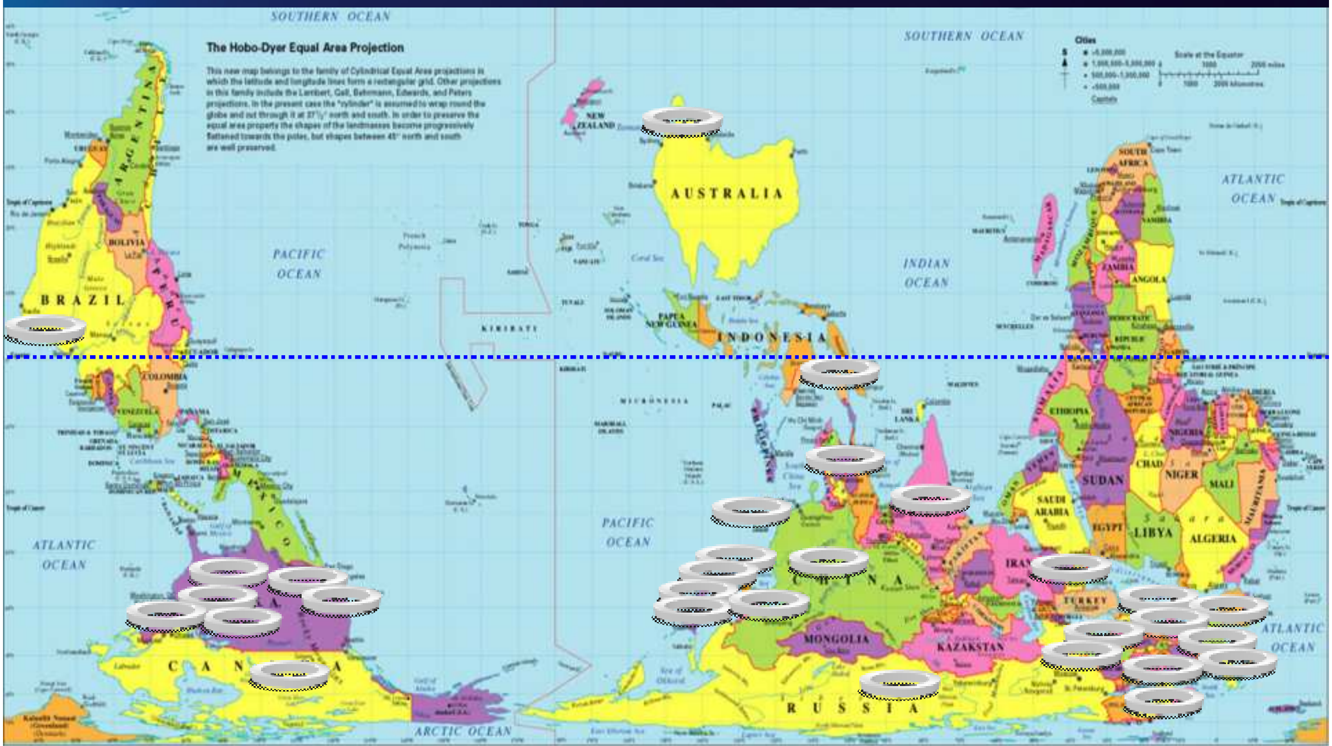
Ljiljana Puskar

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

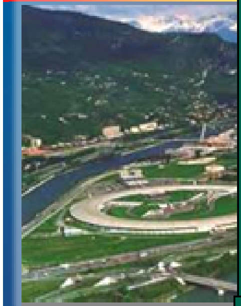
Australian Synchrotron in Clayton

The Synchrotron World Map – as seen from Australia

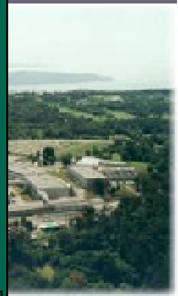


	Number of beamlines	Purpose	Status
America and Canada			
ALS Berkley	1	Microscopy and Far-IR	Operational
CAMD Baton Rouge	1	Microscopy	Planned
CLS Saskatoon	2	1 for microscopy, 1 for Far-IR	Operational
NSLS Brookhaven	6	3 Microscopy, 2 Far-IR, 1 THz	Operational
Surf III Gaithersburg	1	Microscopy	Planned
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
SESAME, Jordan	1	Microscopy	Planned
Europe			
ESRF, Grenoble	1	Microscopy	Operational
Soleil, St. Aubin	2	Microscopy and Far-IR	Operational
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 Lab, Lund	2	Microscopy and Far-IR	Operational
SRS, Daresbury		Microscopy and Far-IR	Closed 4th August 08
DIAMOND, Didcot	1	Microscopy	Users expected Oct 2009!
ALBA, Barcelona	1	Microscopy	Planned for second-phase

Australian Synchrotron



ESRF, Grenoble
Circumference=



, Italy
=259.2 m

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34 IR Beamlines in the world!

Spring-8 , Japan
Circumference=1436 m

Circumference=249.6

Facility
Circumference=345 m

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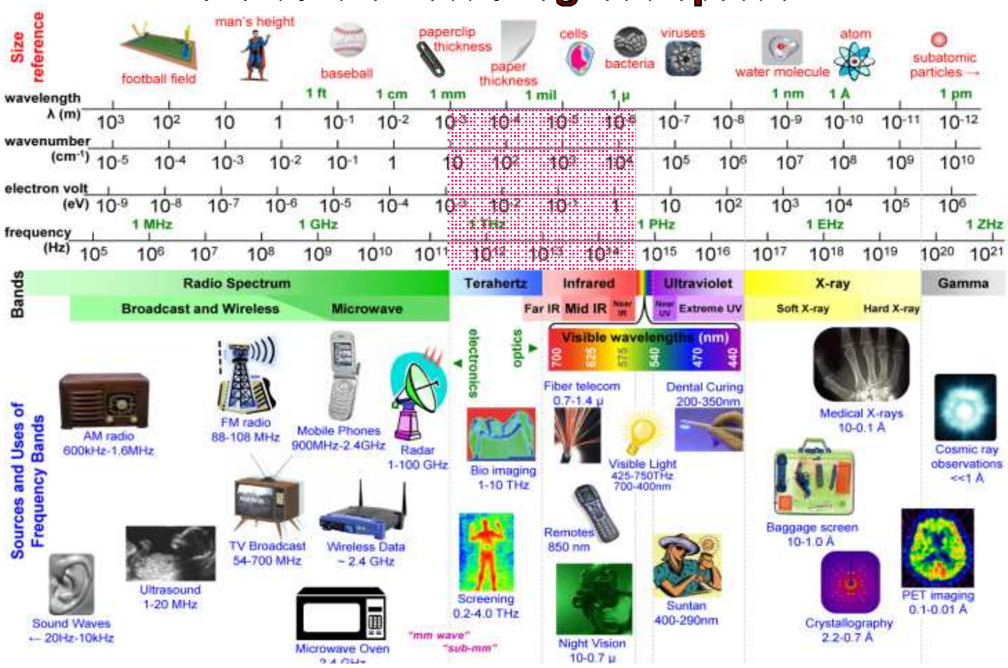
INTRODUCTION TO INFRARED SPECTROSCOPY...

Chart of the Electromagnetic Spectrum



The Infrared part of the EM Spectrum covers ~ 1 meV to 1.7 eV

IR units: wavenumbers (cm^{-1})
Far-IR: 10 – 500 cm^{-1}
Mid-IR: 500 – 4000 cm^{-1}
Near-IR: 4000 – 14000 cm^{-1}

10 micron wavelength = 1000 cm^{-1}
1 eV ~ 8100 cm^{-1}
1 THz ~ 33 cm^{-1}
300 Kelvin ~ 210 cm^{-1}

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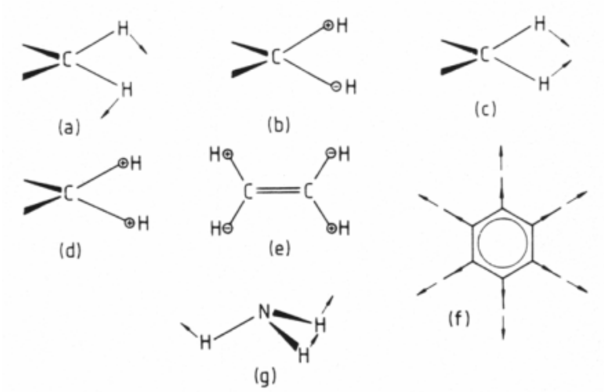
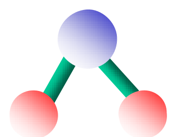
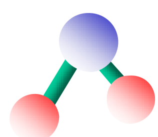
Infrared Spectroscopy

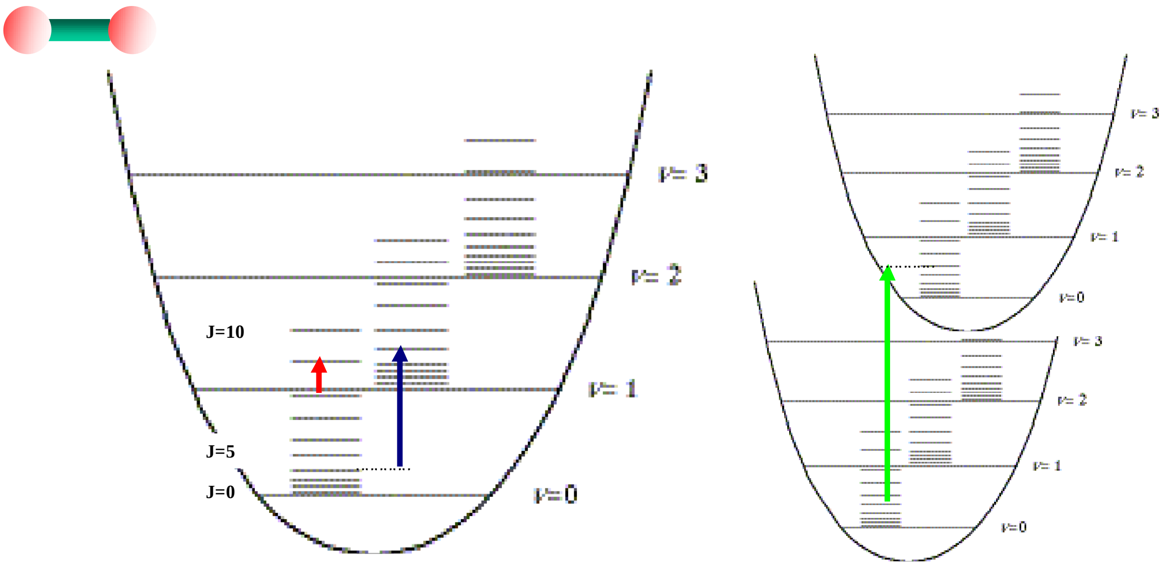
Infrared spectroscopy provides information on molecular vibrations and allows chemical fingerprinting. This is a non-destructive technique which requires only a small amount of sample for analysis; it is widely used for the analysis of both organic and inorganic samples.

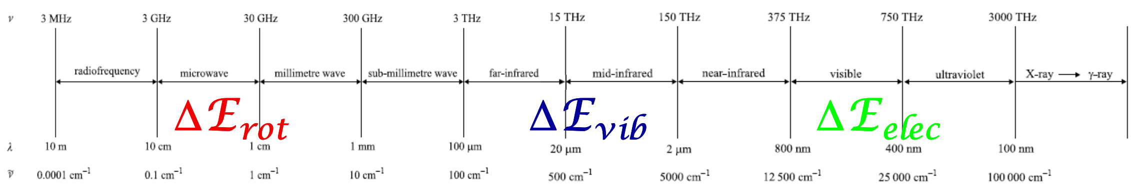






Rotational, Vibrational and Electronic transitions





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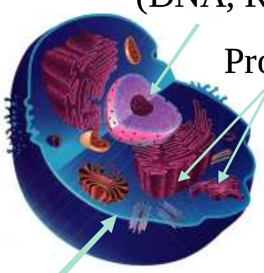
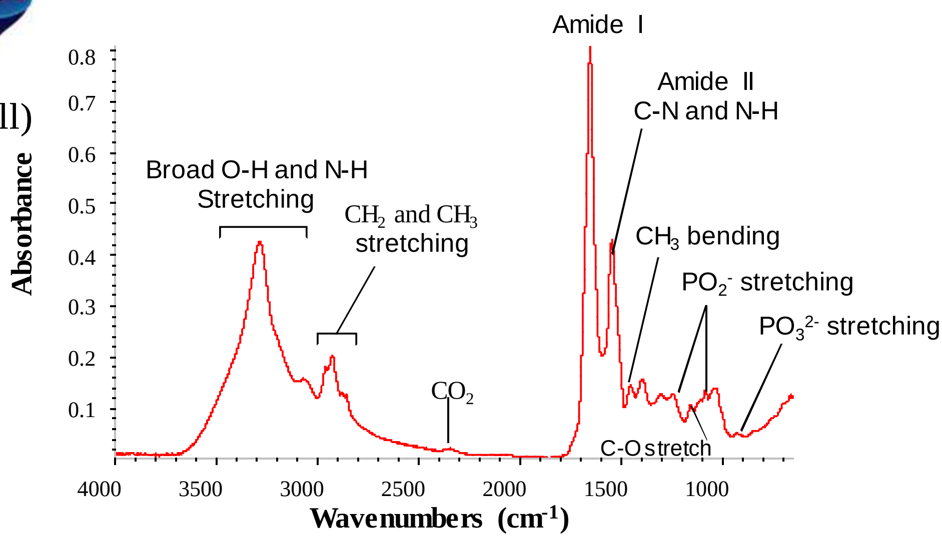
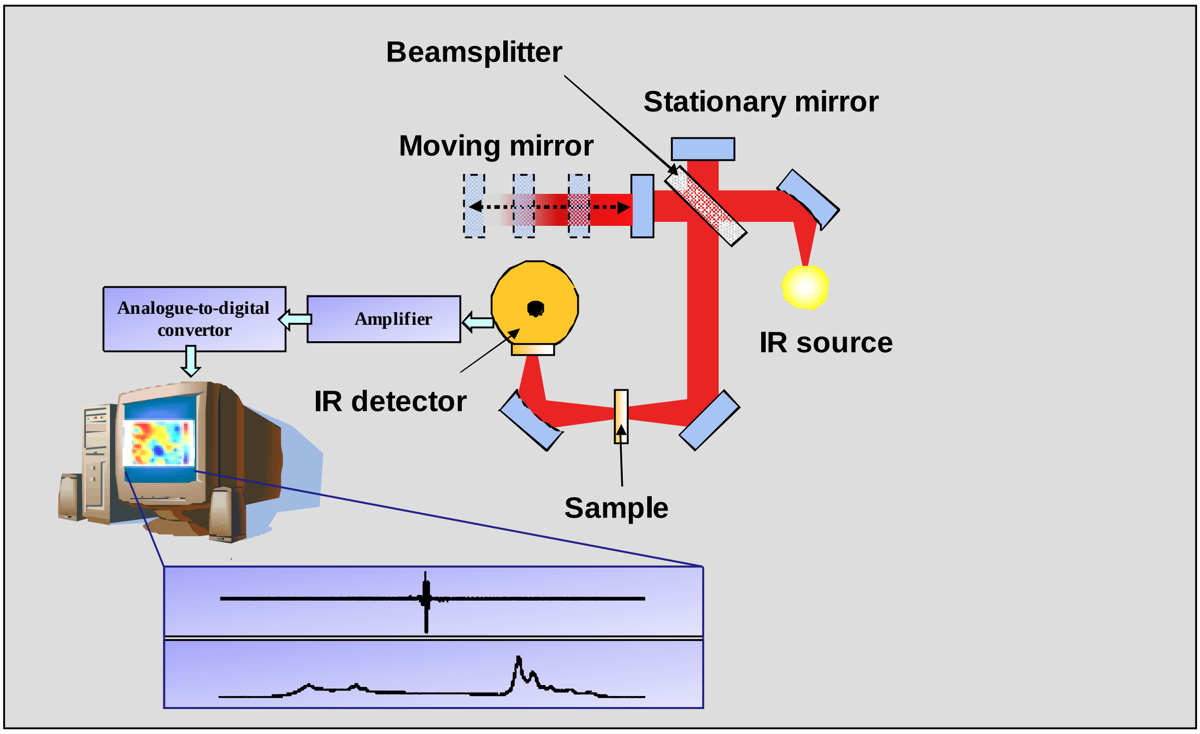
Typical Infrared Spectrum of Biological sample



Each cellular component has **distinct IR marker bands.**



Method of data collection
Fourier transform Infrared spectroscopy



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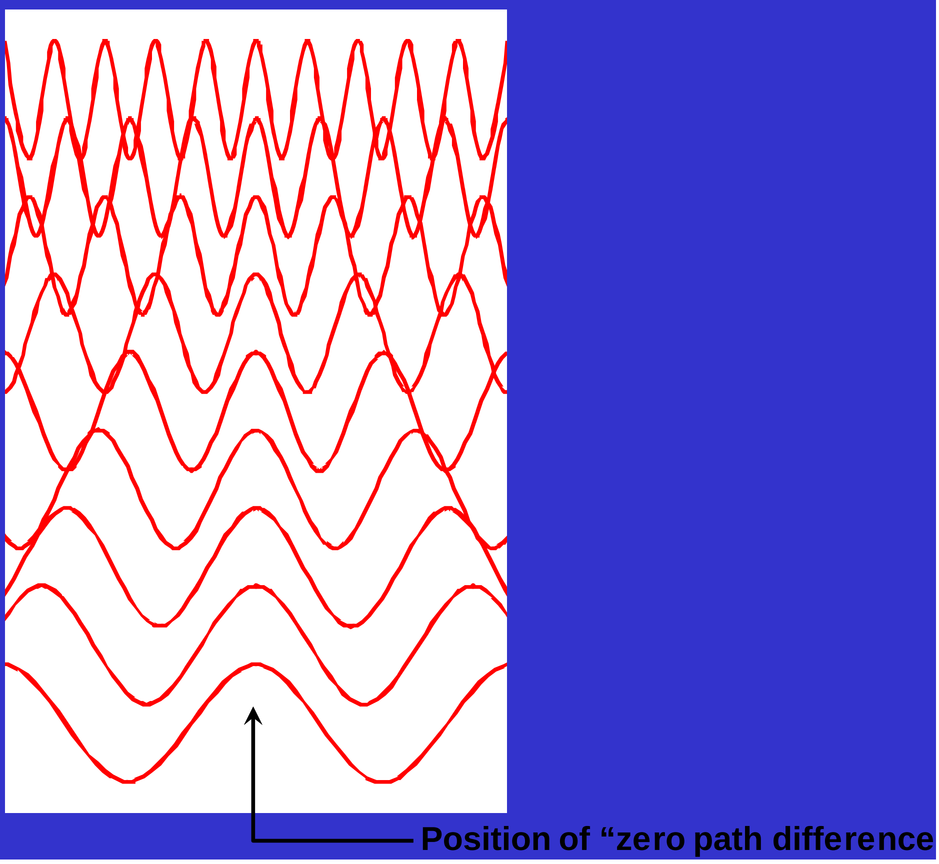
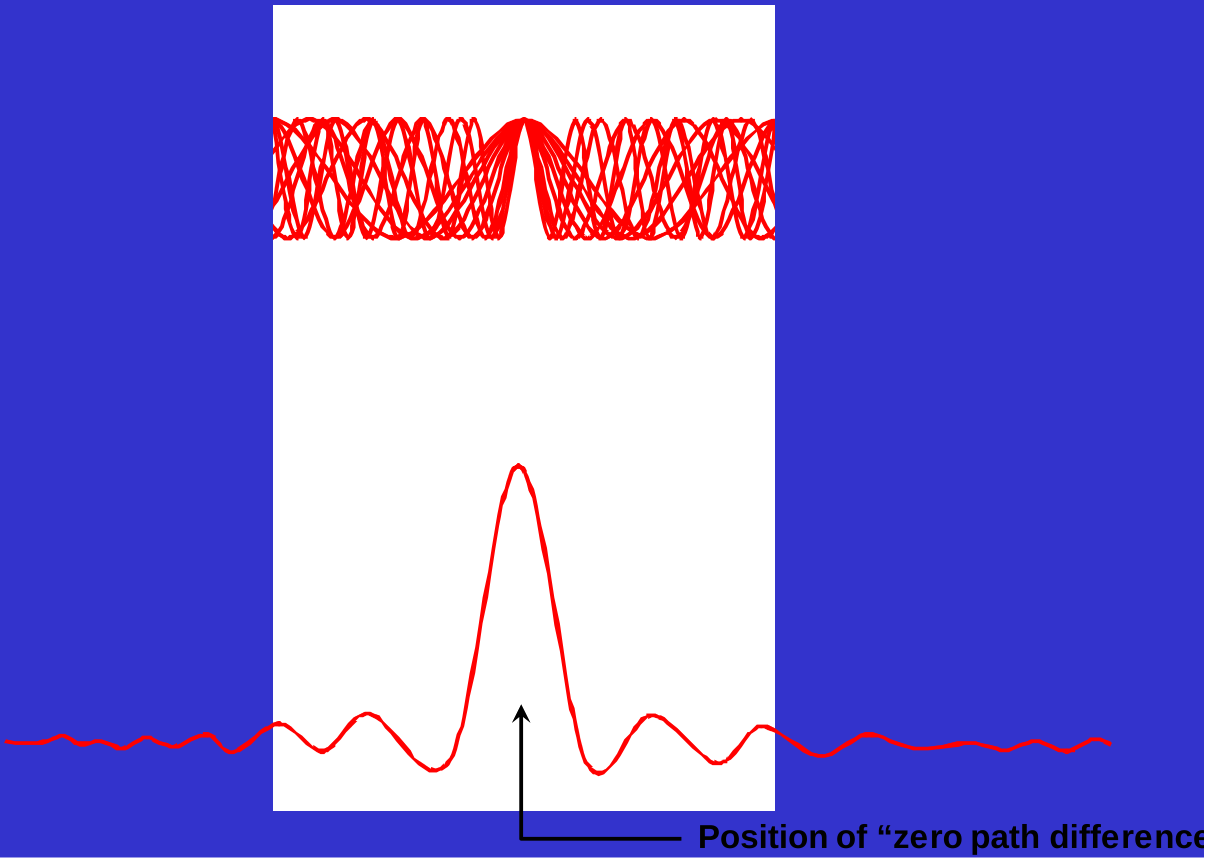
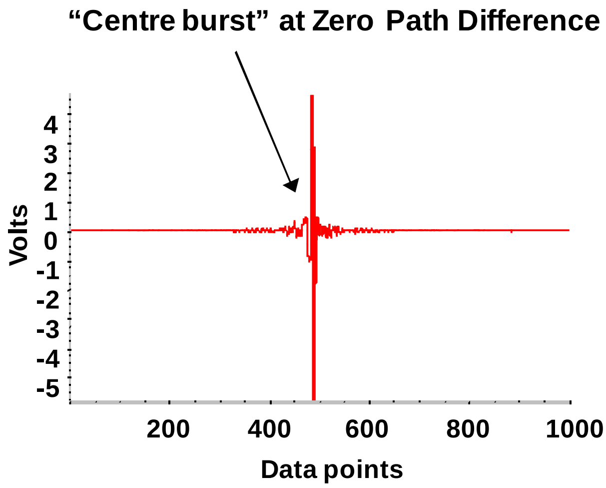
Many frequencies are present in the infrared beam

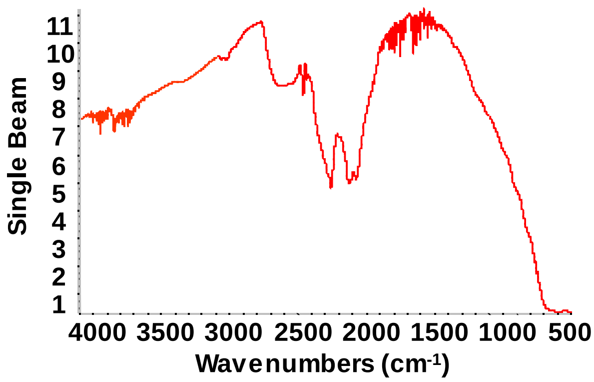


Summing of all frequencies for each position of the mirror



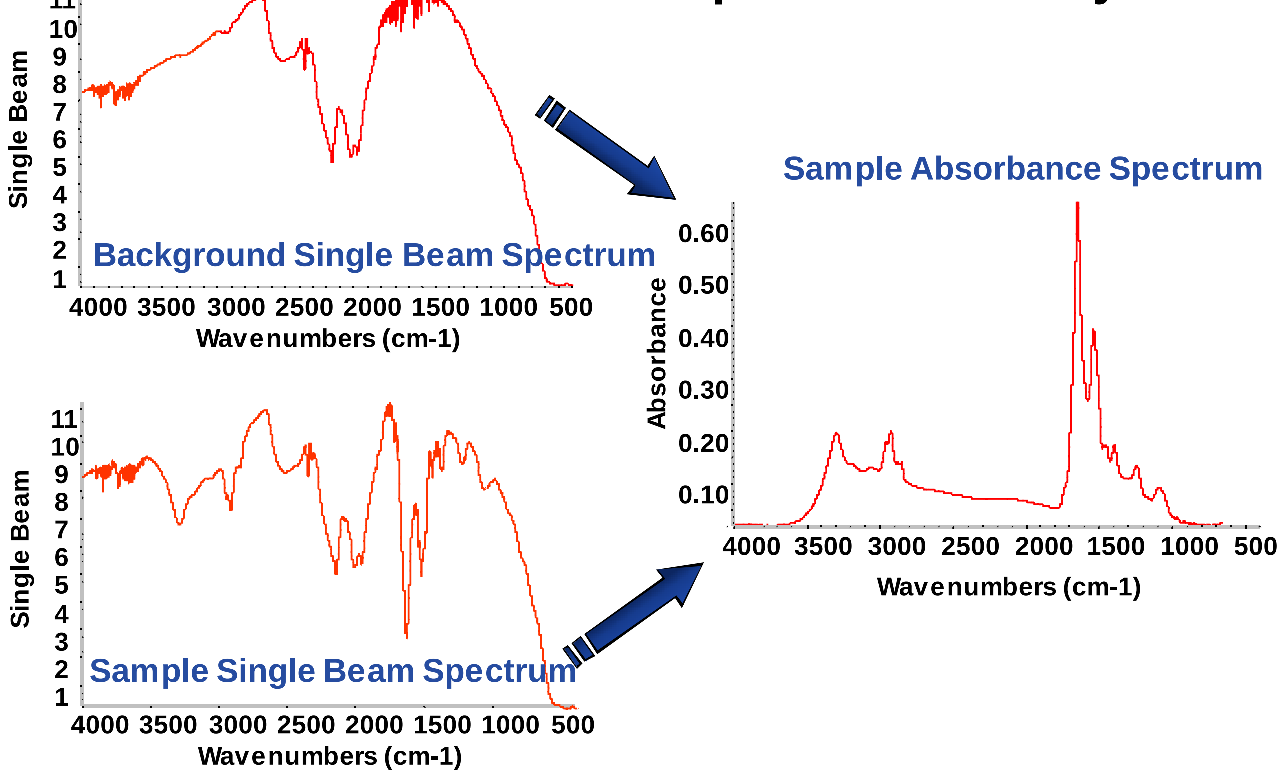
Data output from FTIR system



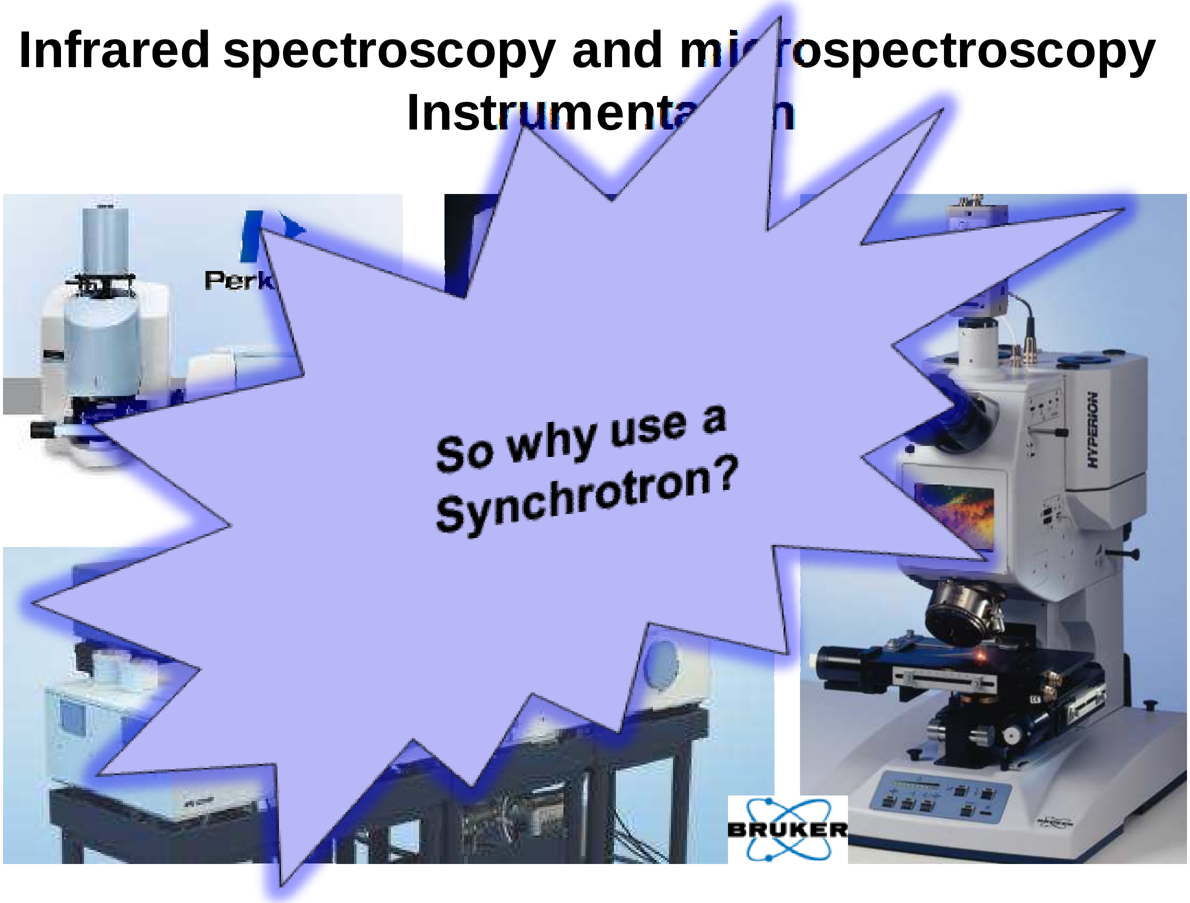


Interferogram → **Fourier Transform** → **Single Beam Spectrum**

Data output from FTIR system

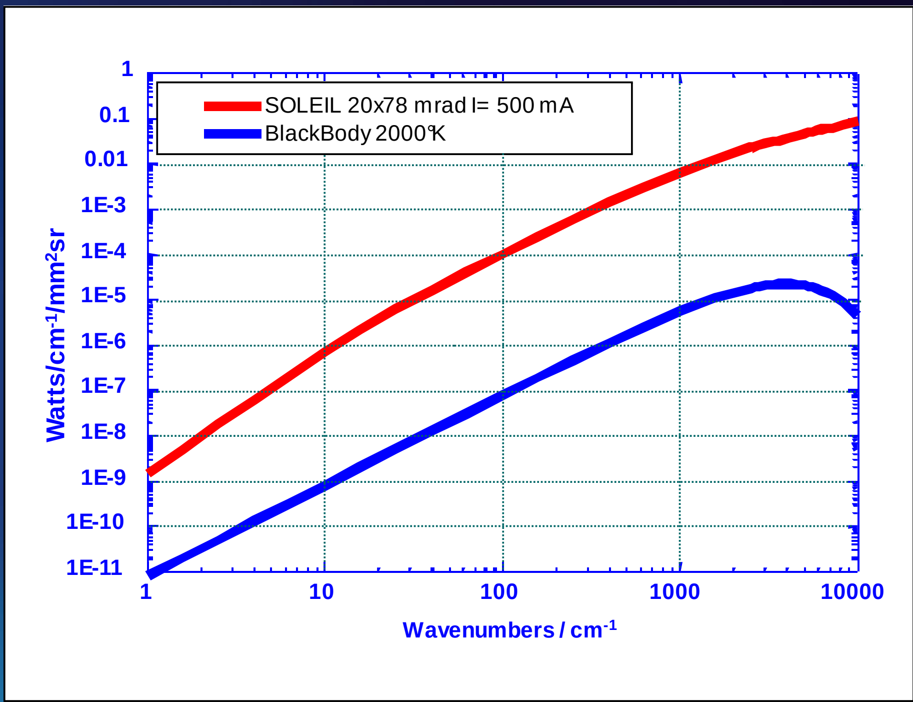


Infrared spectroscopy and microspectroscopy Instrumentation



It's the synchrotron BRIGHTNESS that counts

Is the synchrotron IR beam very intense?



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SR Advantages over thermal sources

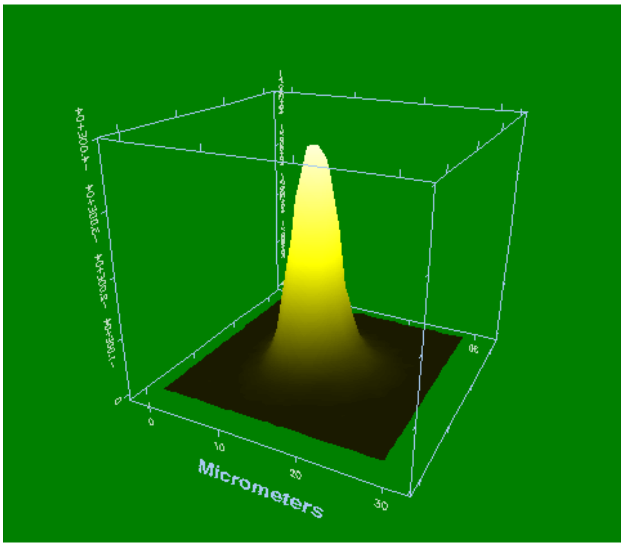
- **Brightness: Better Signal to Noise ratio!**

Brightness = $$B = \frac{P}{\Delta A.\Delta\Omega}$$

Power per unit area per unit solid angle

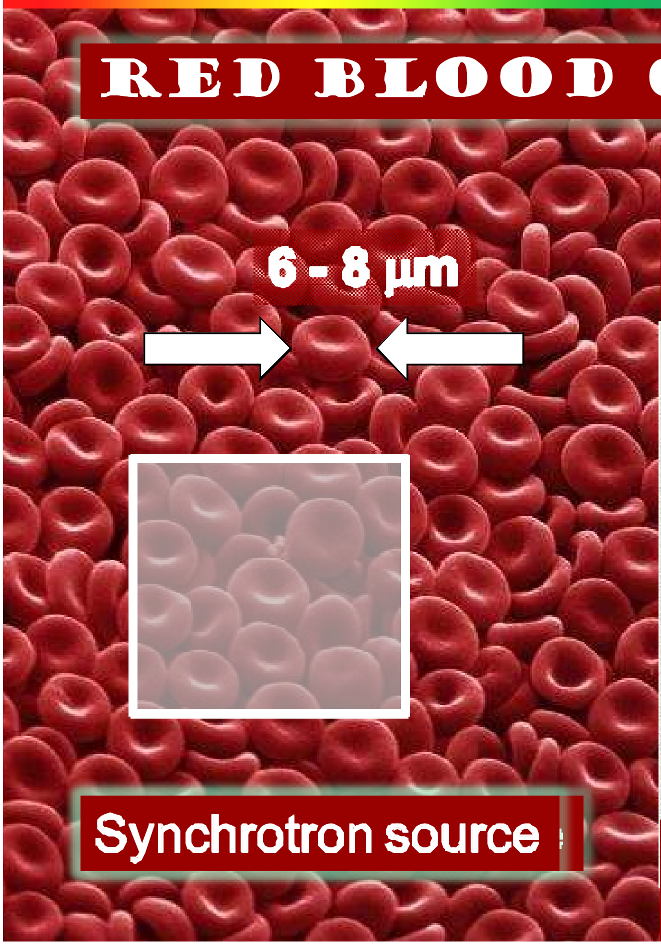
- **Small source: better throughput with small samples**
- **Highly collimated: higher resolution achievable**
- **Polarized: ellispsometry**
- **Pulsed: pump & probe experiments**

Synchrotron infrared beam focused on sample



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

RED BLOOD CELLS

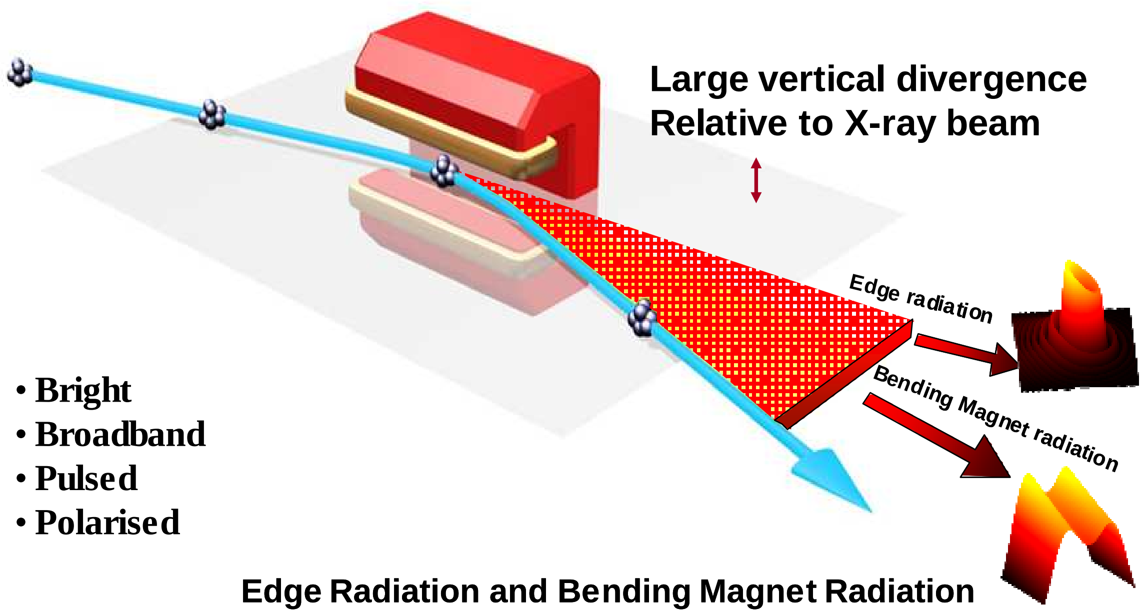


Single malaria infected cells at different stages of the intra - erythrocytic life cycle

Grant Webster, Don McNaughton, Bayden Wood, Monash University, Torsten Frosch (University Jena)

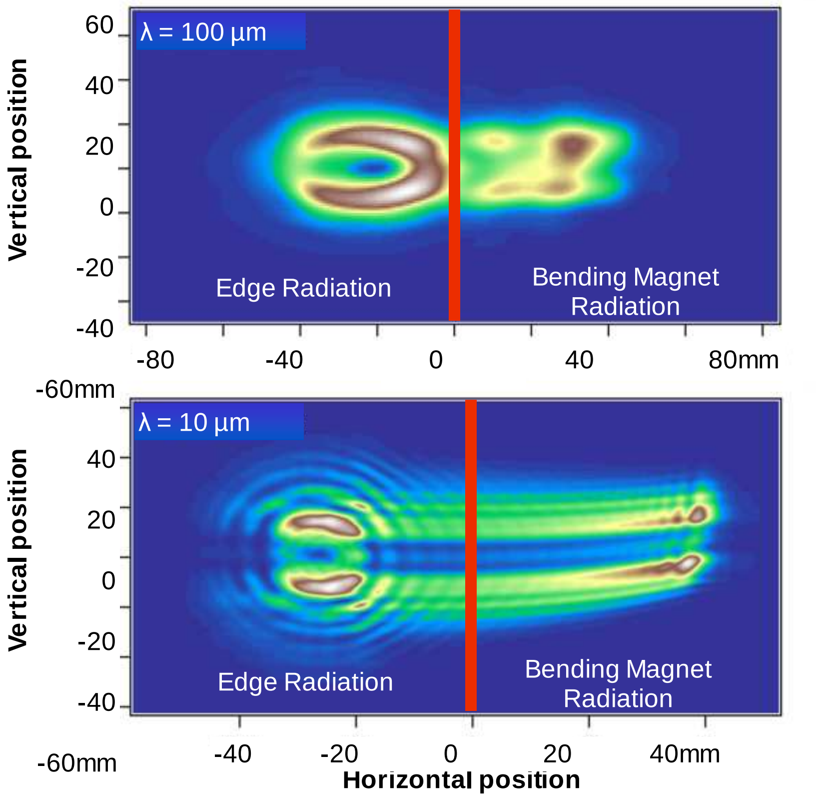
EXTRACTION OF INFRARED LIGHT FROM A SYNCHROTRON

Infrared emission from a synchrotron bending magnet



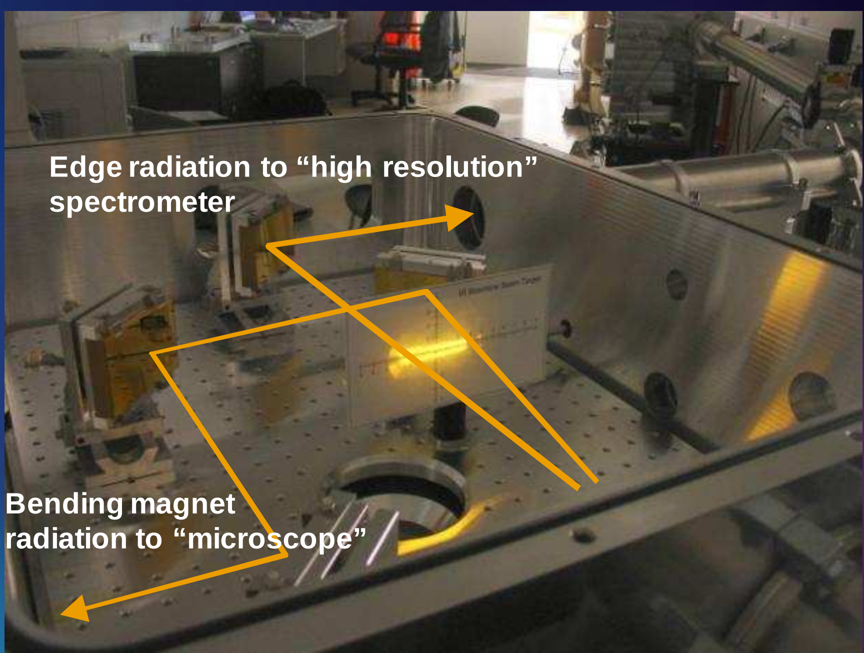
- Bright
- Broadband
- Pulsed
- Polarised

Edge Radiation and Bending Magnet Radiation

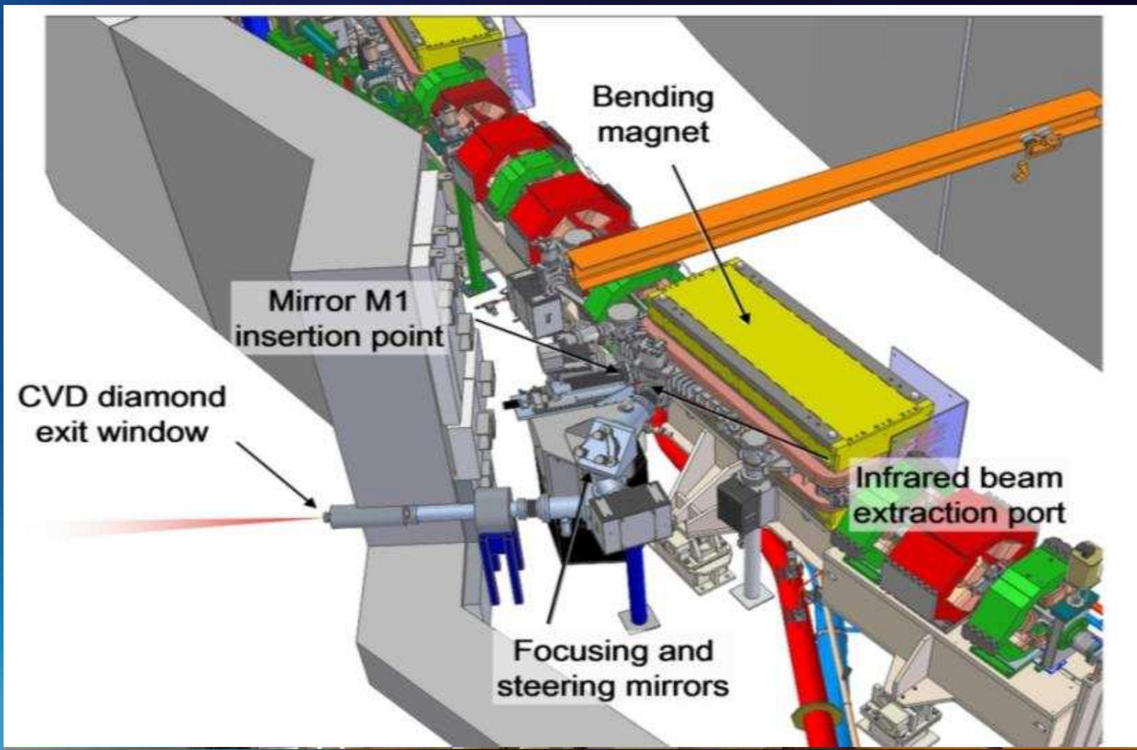


Australian Synchrotron

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

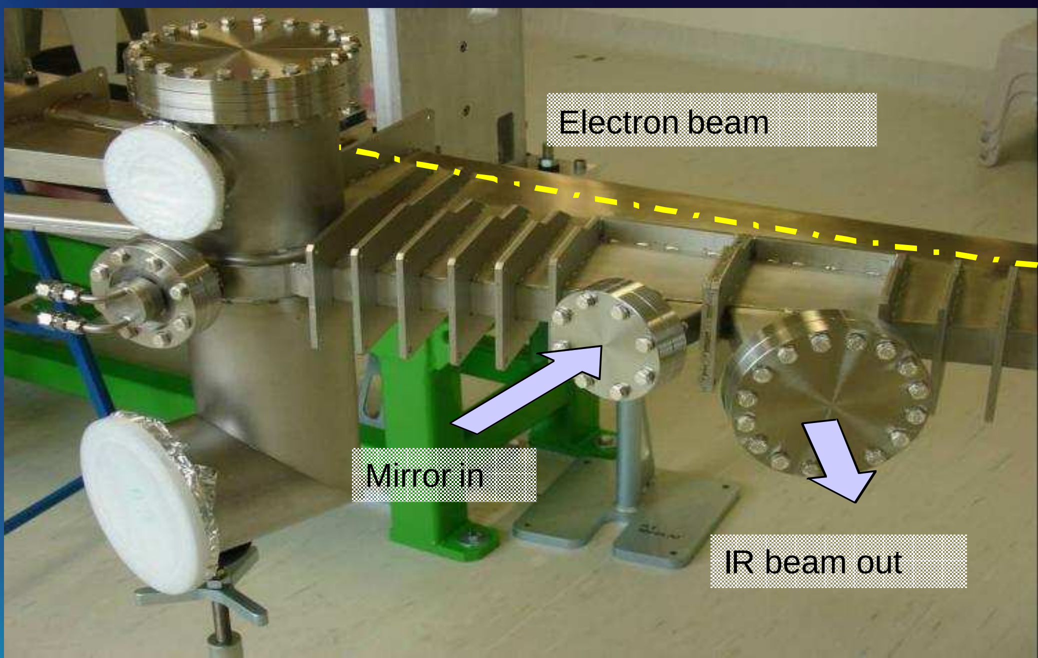


Mirror inserted into dipole chamber from side



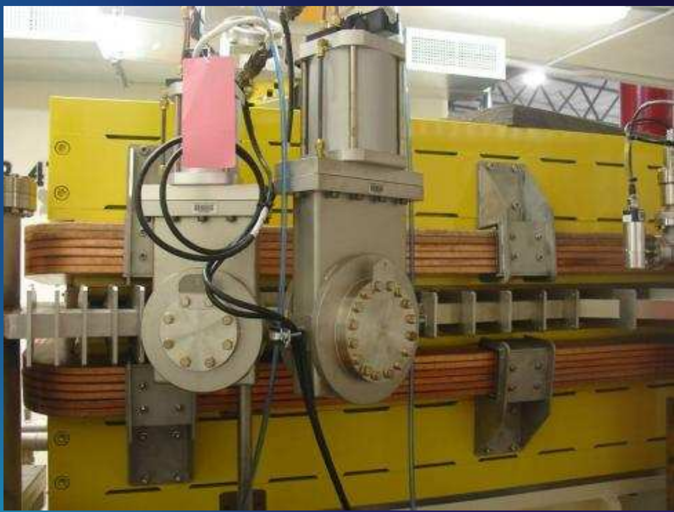
Australian Synchrotron

Mirror inserted into dipole chamber from side



Specially adapted Infrared Dipole Chamber at Australian Synchrotron

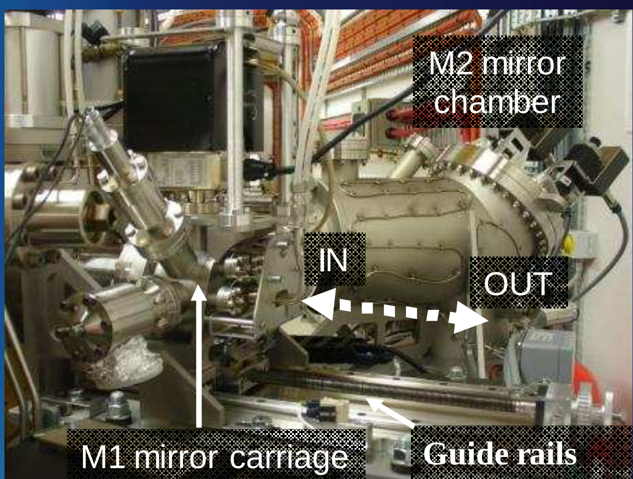
Dipole Chamber in Storage Ring and Mirror M1 prior to Installation

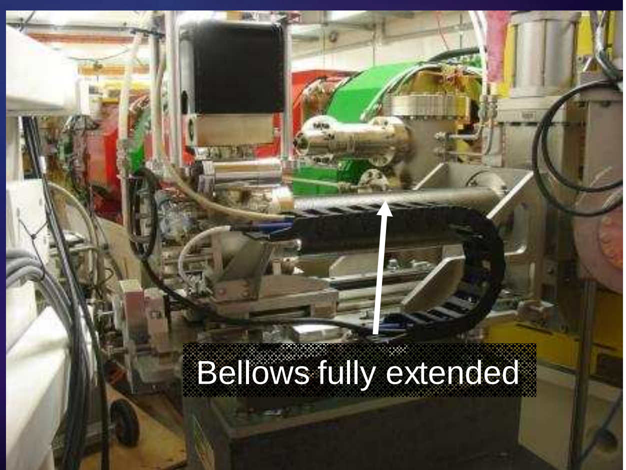


Infrared dipole chamber with vacuum isolation gate valves installed

Mirror M1 undergoing vibration testing prior to installation

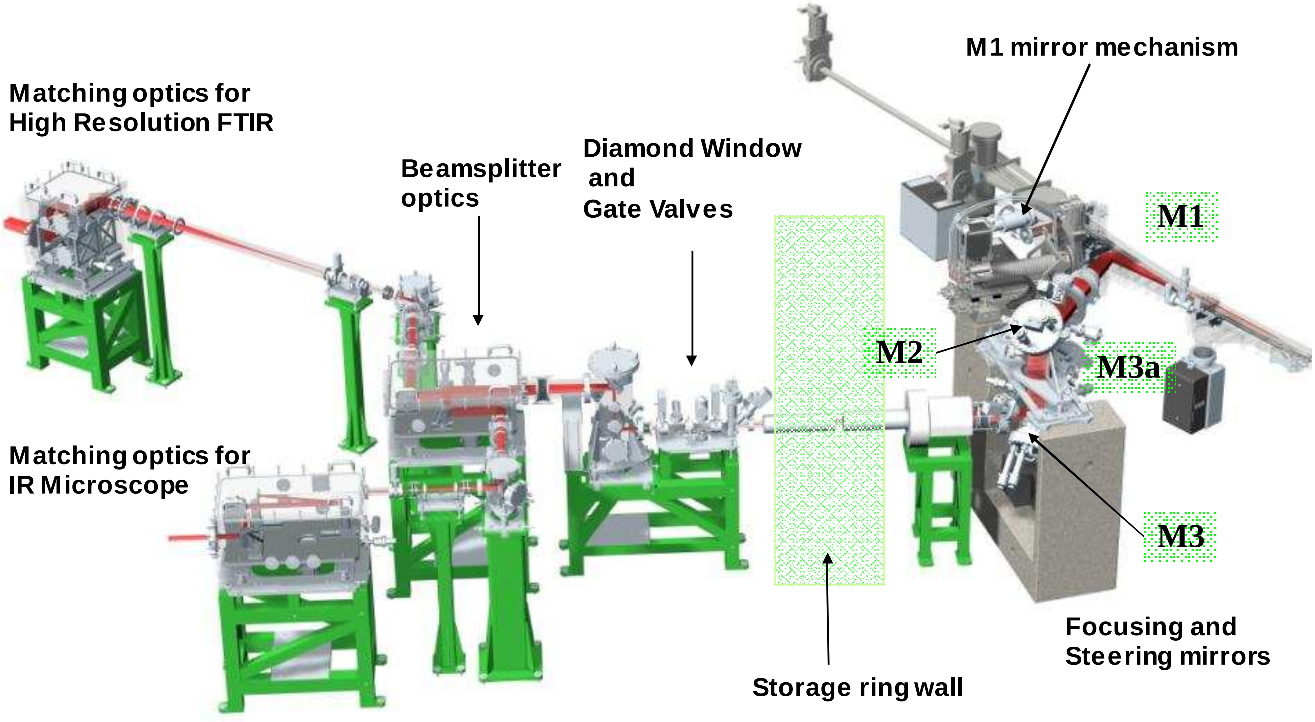
M1 Mirror Inserted (left) and Withdrawn (right)



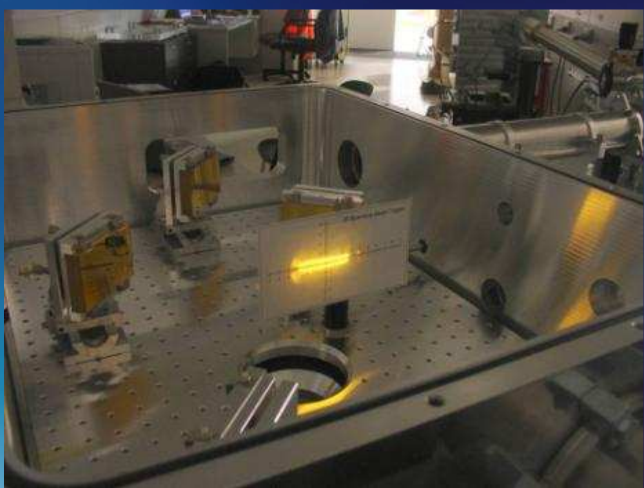


Note: M2 mirror chamber not yet installed in this photo

Schematic of IR beamline



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



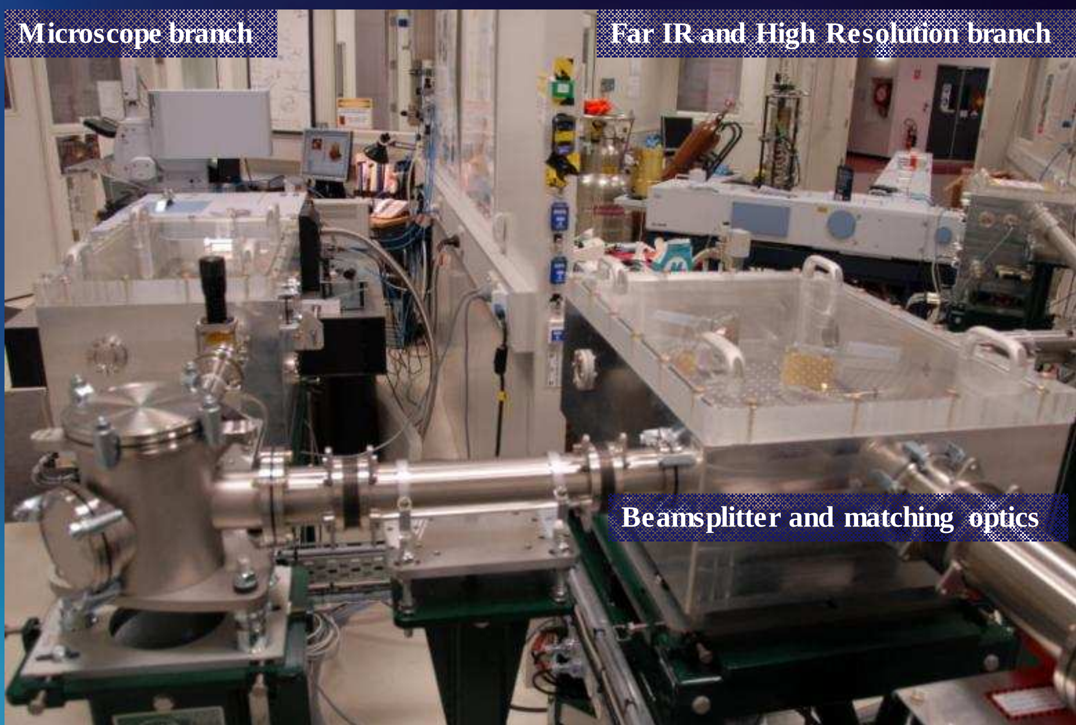
Visible beam profile in Beamsplitter Vessel

Collimated beam at entrance to FTIR spectrometer

IR beam profile – comparison with SRW

Australian Synchrotron

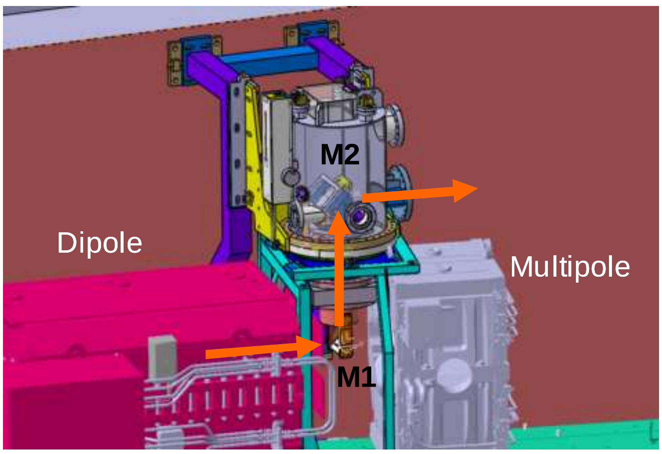
Photo of the Australian Synchrotron Infrared beamline



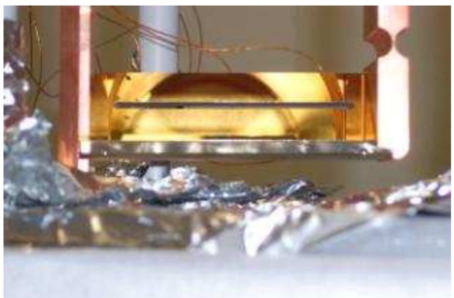
ALTERNATIVE METHODS OF EXTRACTION

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

e.g. Soleil, ESRF…



Images courtesy of Paul Dumas, Soleil.

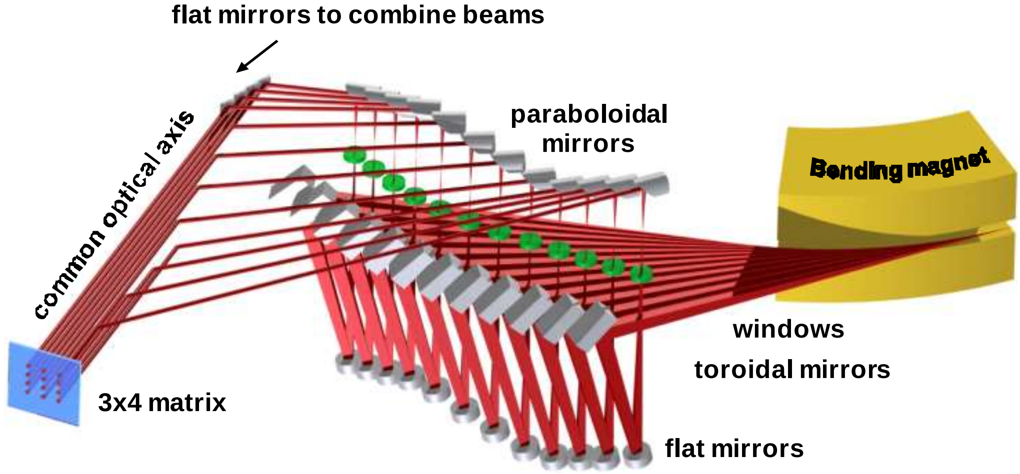


M1 Mirror with thermocouple wires

Top view of mirror insertion port

Multiple beam extraction from the bending magnet

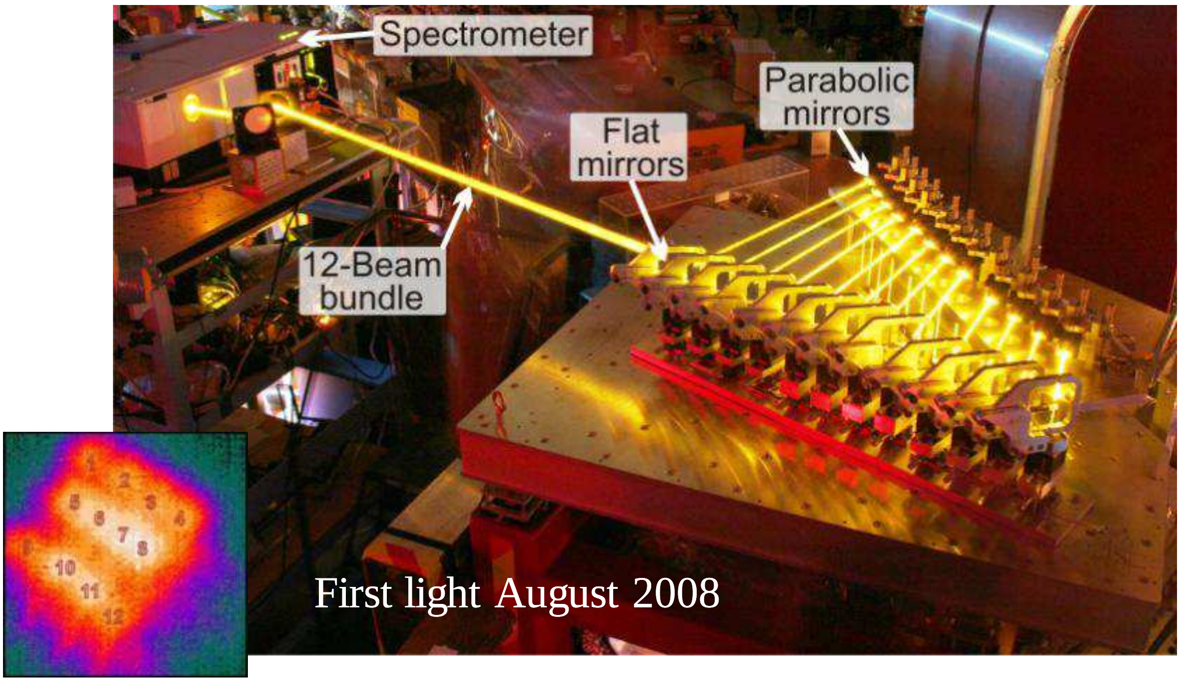
Infrared Environmental Imaging (IRENI) at the Synchrotron Radiation Center, UW-Madison



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

M. J. Nasse, R. Reininger, S. Janowski, T. Kubala, E. Mattson and C. Hirschmugl, The University of Wisconsin-Milwaukee and SRC.

Multiple beam extraction from the bending magnet



M. J. Nasse, R. Reininger, S. Janowski, T. Kubala, E. Mattson and C. Hirschmugl, The University of Wisconsin-Milwaukee and SRC.

INFRARED BEAMLINE INSTRUMENTATION

Australian Synchrotron

Infrared Beamline at the Australian Synchrotron: Microscope branch

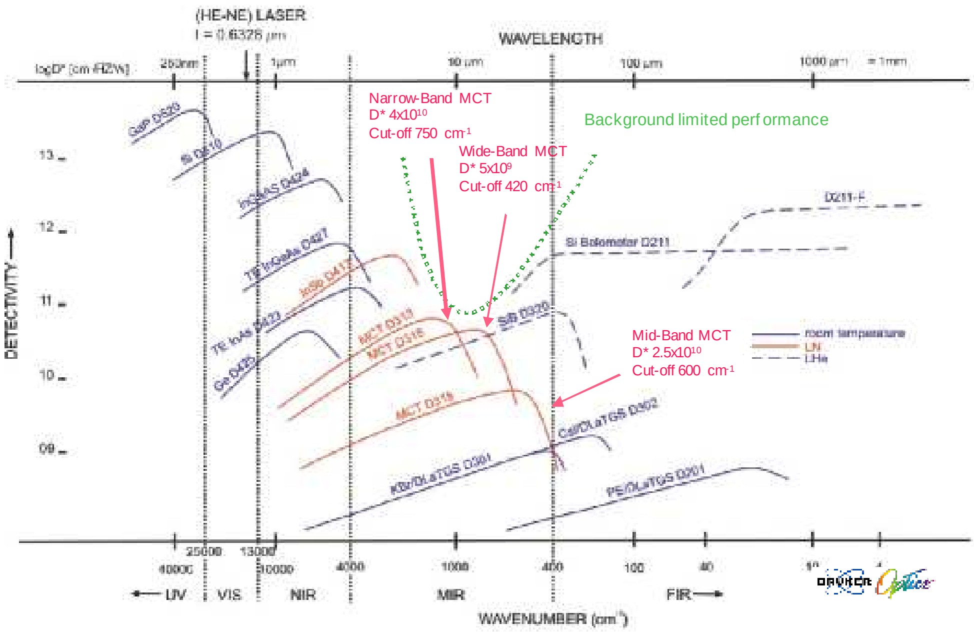
Bruker V80v with Hyperion 2000 microscope

Confocal point scanning - current technology

Narrow-Band MCT
50x50 micron

Wide-Band MCT
250x250 micron

Infrared Detectors
Some currently available IR detectors

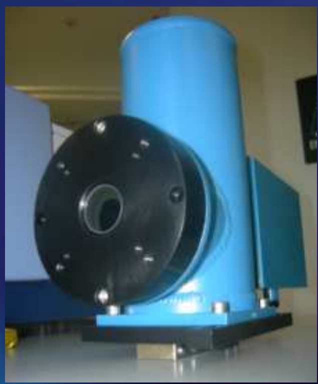


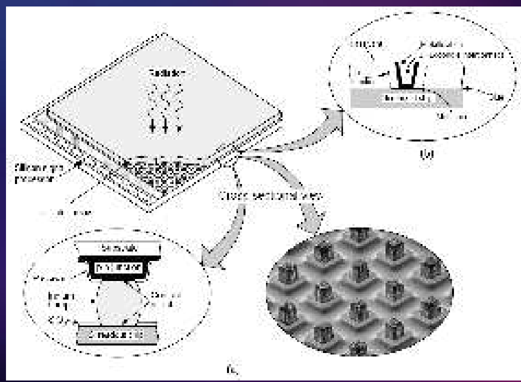
Focal Plane Array Detectors

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

Bruker Hyperion 3000: Focal Plane Array Detector

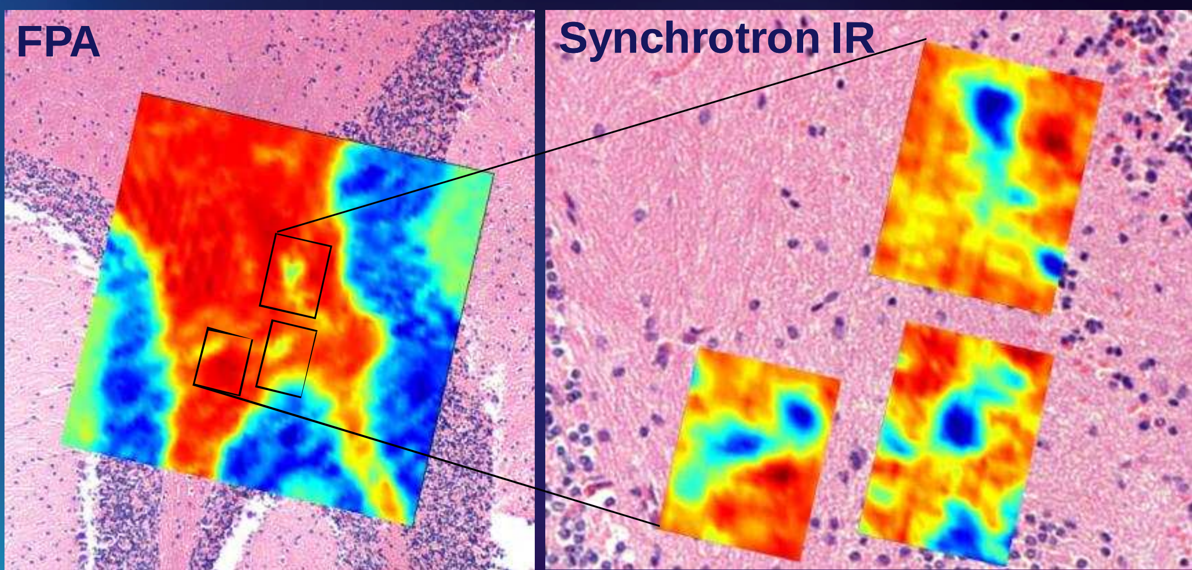
Solid state array with 64 x 64 pixels elements
so that 4,096 spectra can be acquired simultaneously !





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

Australian Synchrotron

Far IR and High Resolution branch

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

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_N	700 – 5 000 cm^{-1}
• MCT_M	600 – 5 000 cm^{-1}

Sources

• Synchrotron	*mw – 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

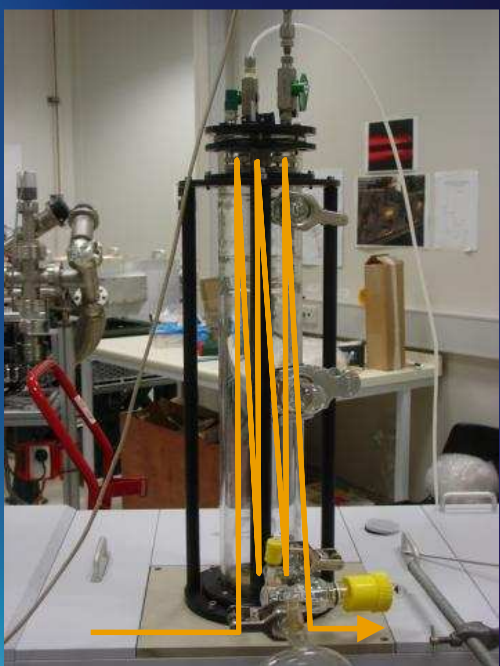
• series of narrow band pass IR filters

Apertures

• 0.5 – 12.5 mm

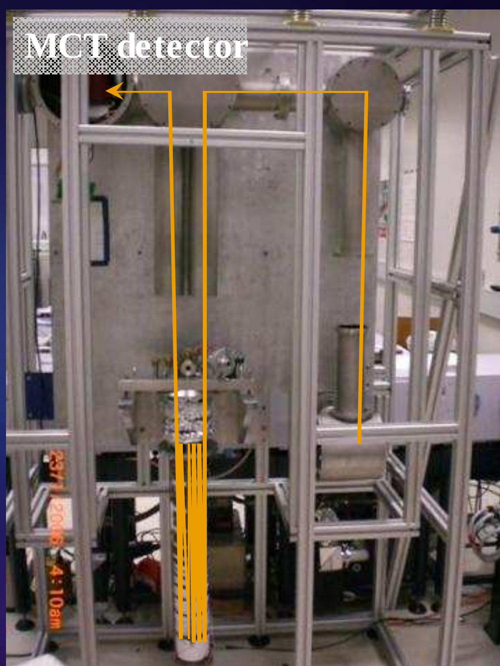
Australian Synchrotron

Multipass gas cell for room temperature samples.

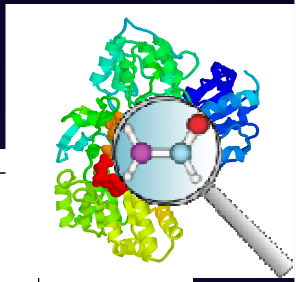


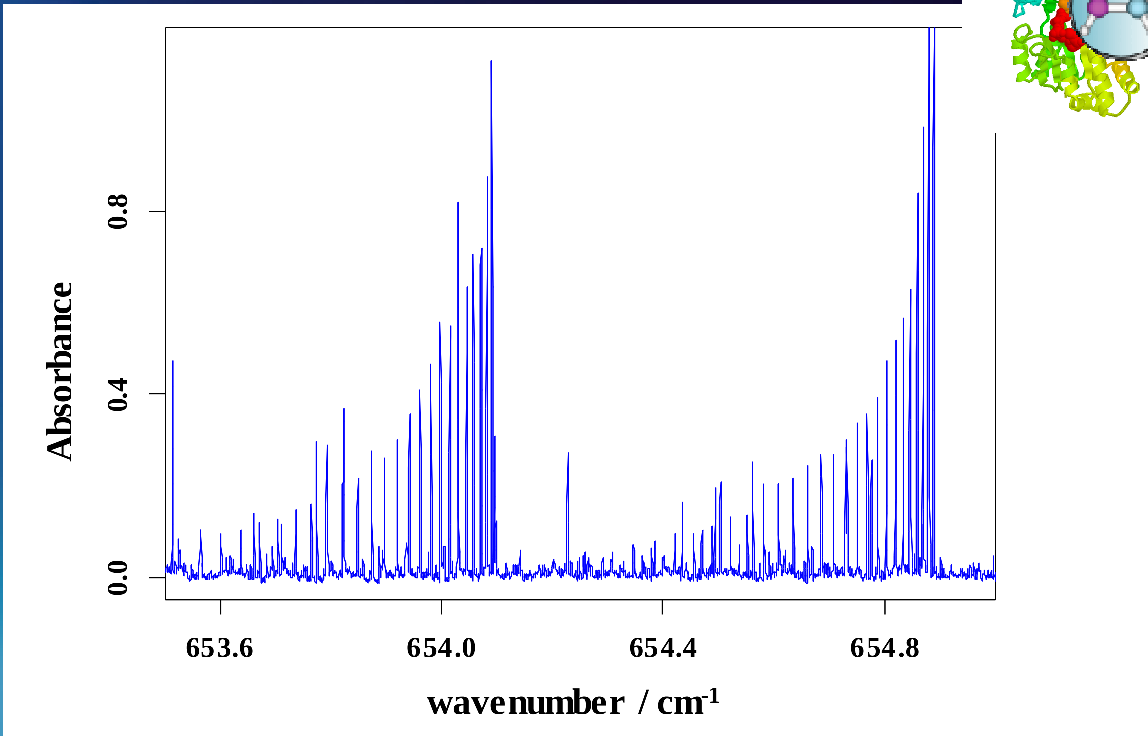
Small quantity of sample to minimize Pressure broadening effects.

Enclosive Flow Cooling cell for cryogenic temperatures.

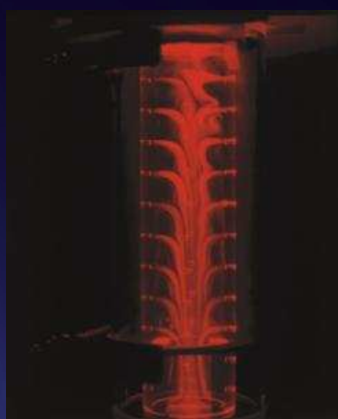


Portion of the Far-IR spectrum of Formamide at 0.00096 cm^{-1} resolution





Far-IR Gas phase applications



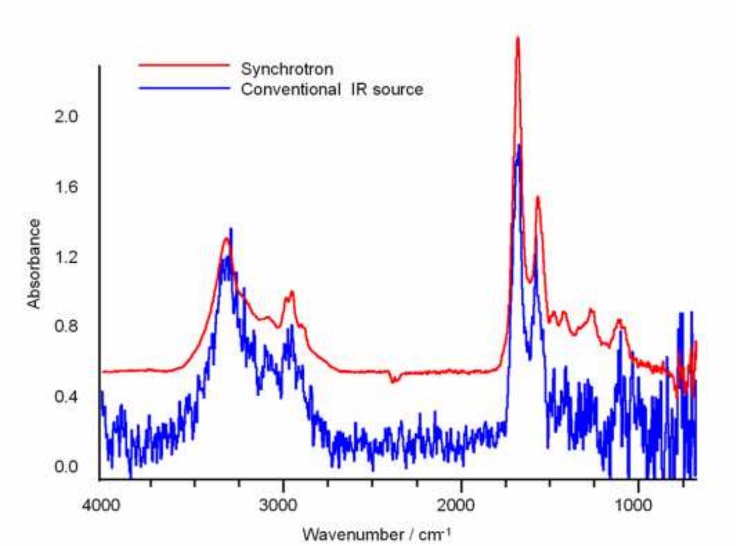
Atmospheric: CFC's, HFC's, HCFC's
Chemical dynamics: Radicals
Astrophysics: Hydrocarbons, Radicals

Far-IR condensed phase

Geological: clay samples
Metal oxides
Biomolecules
Protein structures
Clusters

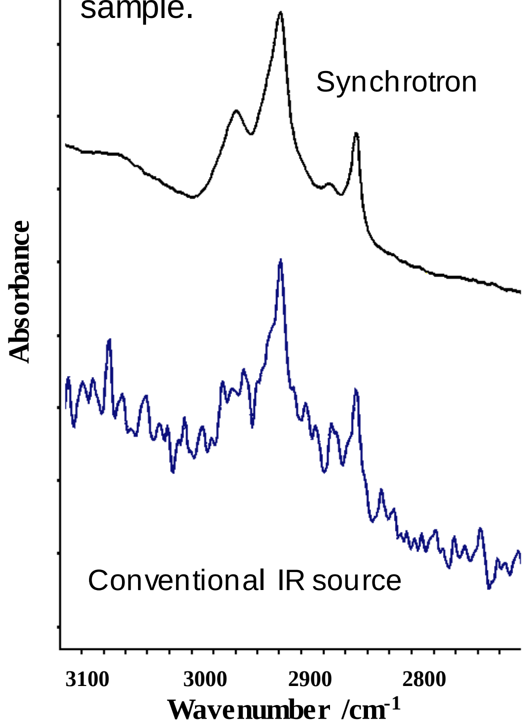
ASSESSING BEAMLINE PERFORMANCE

Advantage of using a synchrotron seen in spectra…

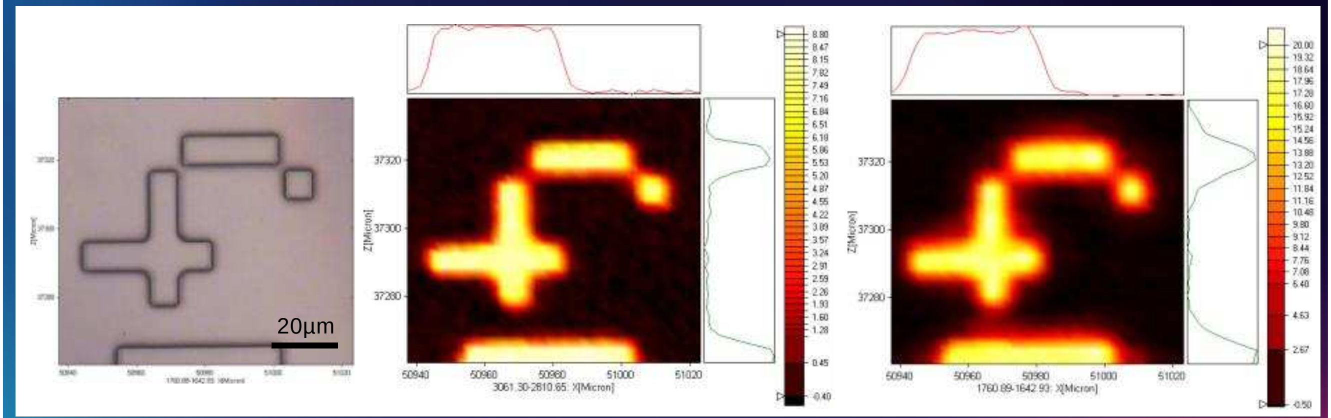


Absorbance spectra of tissue sample recorded at 10 µm spatial resolution under identical collection conditions using a Globar™ infrared source and synchrotron radiation.

CH stretch absorption bands from 5 µm spot in tissue sample.



Wavelength dependence of microscope spatial resolution demonstrated at Infrared beamline



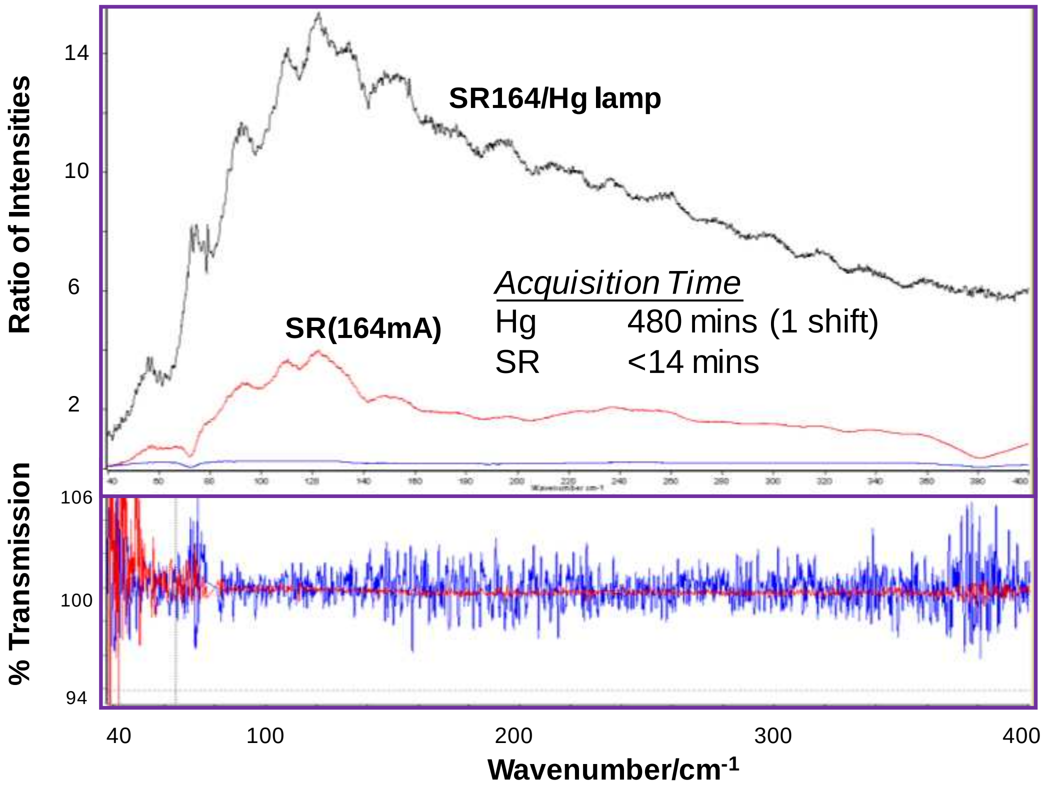
Polymer pattern on CaF_2 produced by photolithography

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**



Sample preparation / types of measurements for IR microscope

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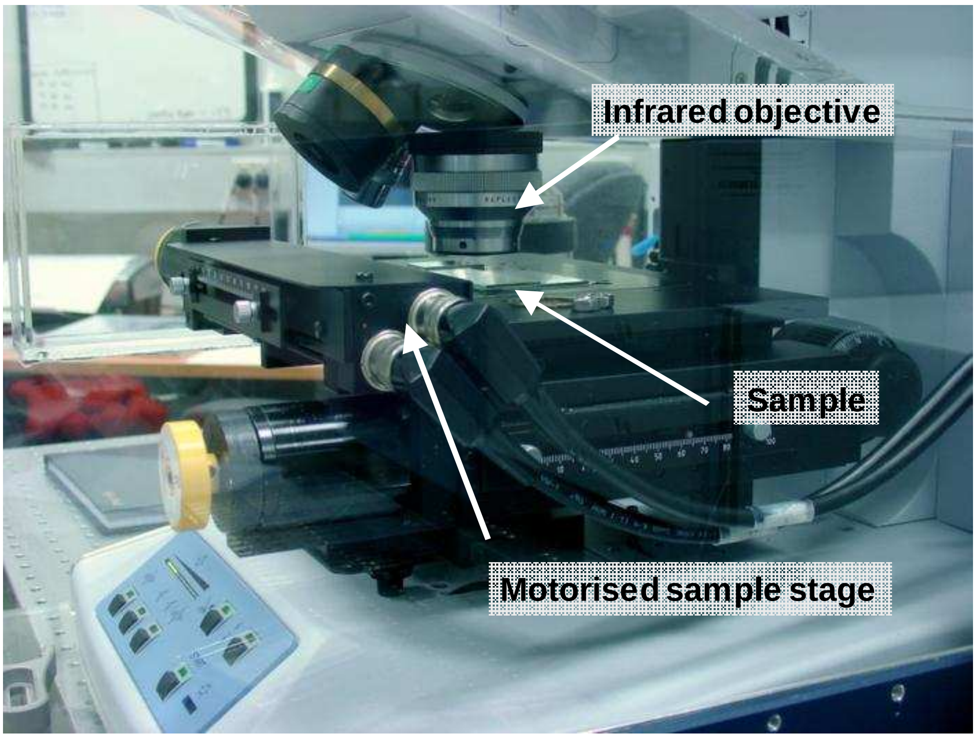
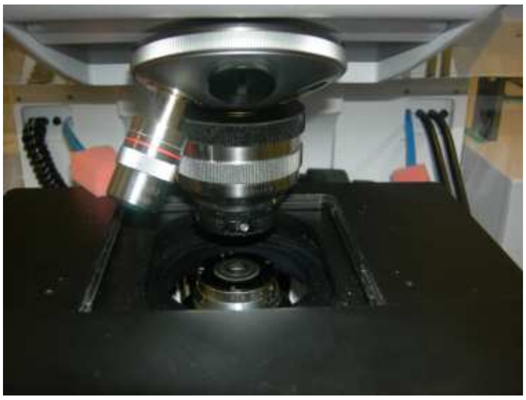
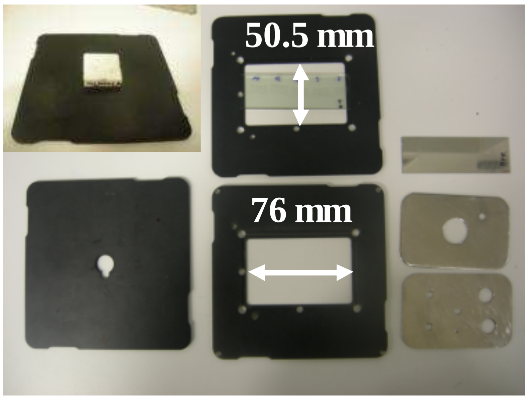
Mounting samples on the IR microscope

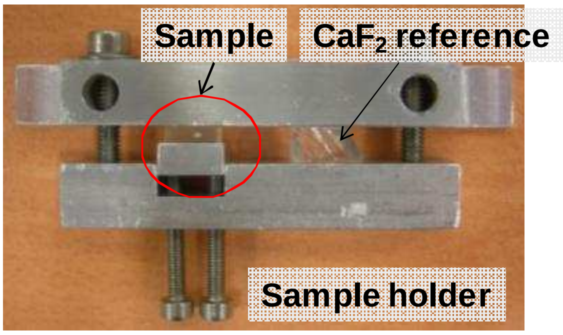


Mounting samples on the IR microscope





Diamond compression cell



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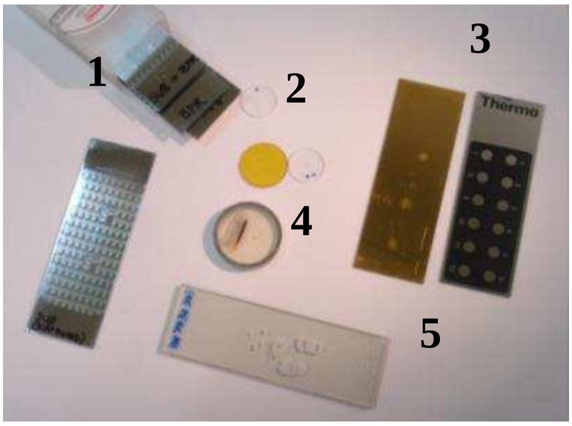
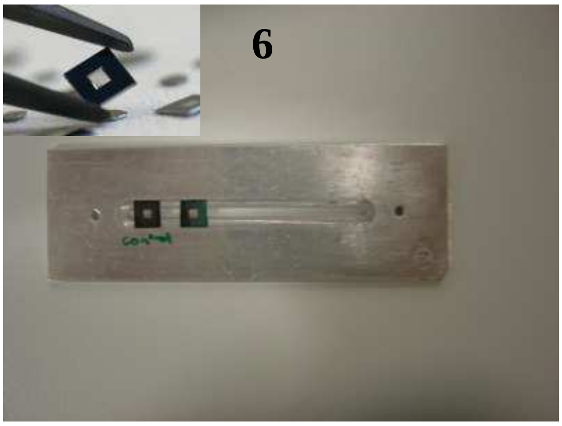
Mounting samples on the IR microscope



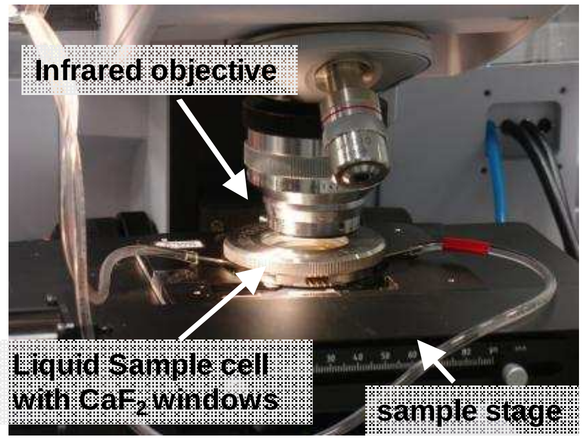


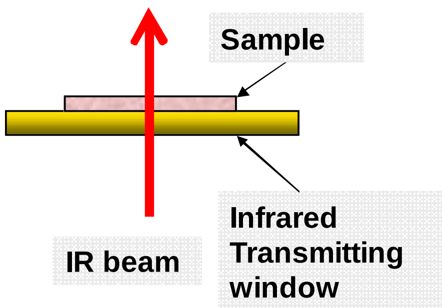
1. Special resolution patterns on mirror slides, for testing the beamline performance.
2. Transmitting disks such as zinc selenide (yellow), CaF_2, BaF_2 for thin sections of samples.
3. Gold or aluminium mirrors for materials such as fine powders and biological cells.
4. Very hard samples can be embedded 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





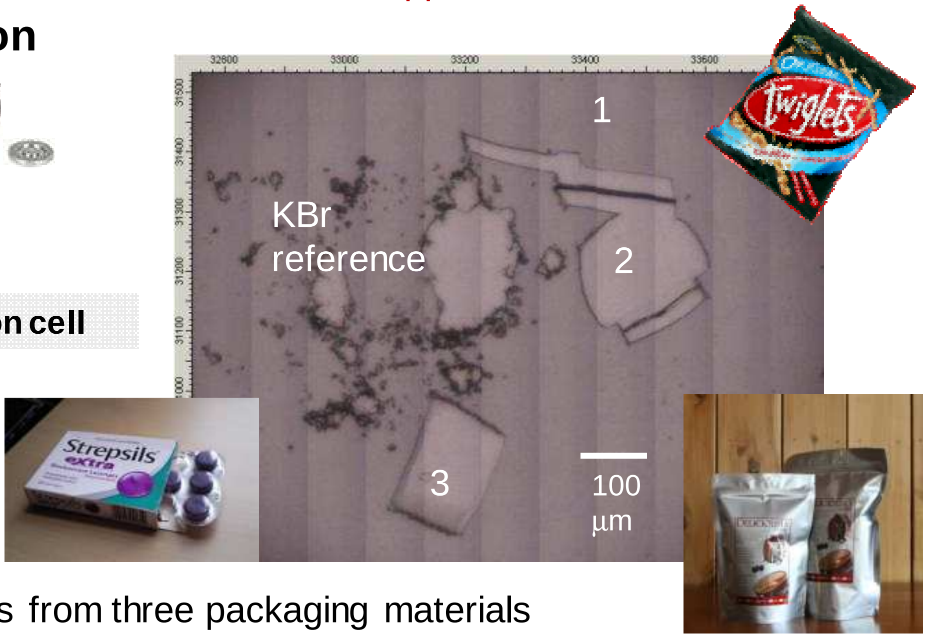
- Samples should be 10 microns or thinner, either freestanding or supported on an IR transmitting material such as KBr, BaF_2, ZnSe or CaF_2 windows or the silicon nitride membrane.
- Flow through liquid cells or compression cells are also used.

Transmission measurements:

Study of polymer laminates – simple identification of layers
Industrial and forensic applications

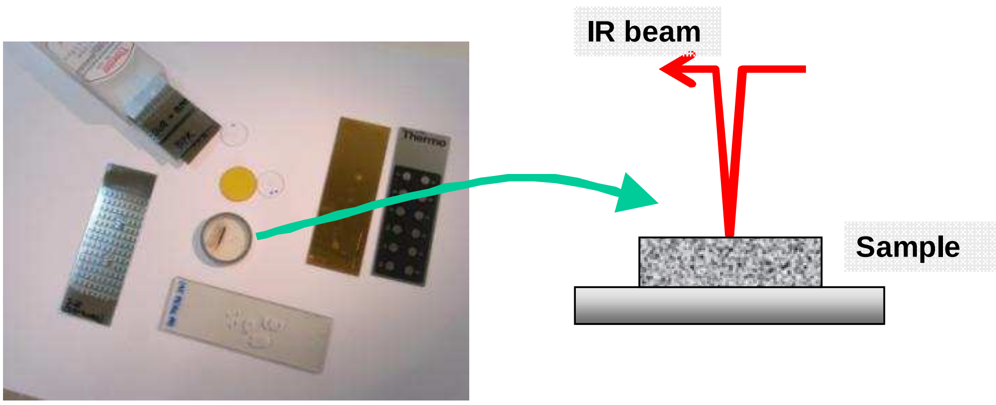
Sample preparation

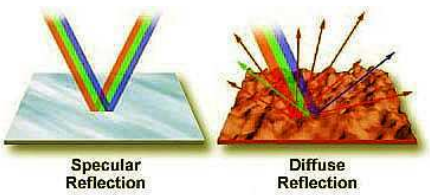
Diamond compression cell



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

Reflectance measurements



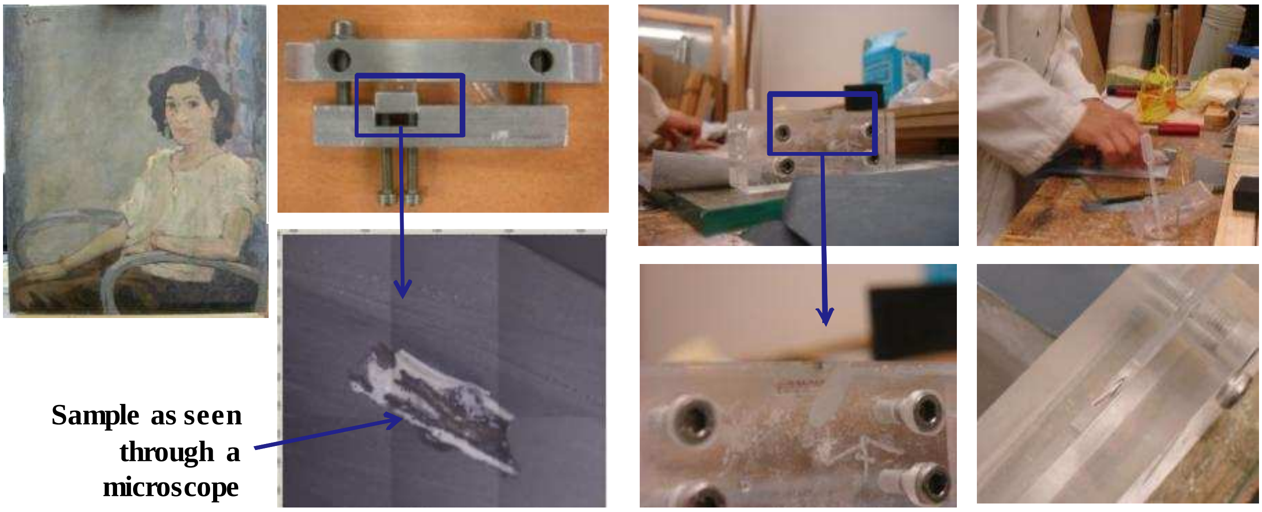


- Ideally requires a polished flat surface
- Spectra require additional correction procedures due to dispersion artefacts (Kramers-Kronig-Transformation).

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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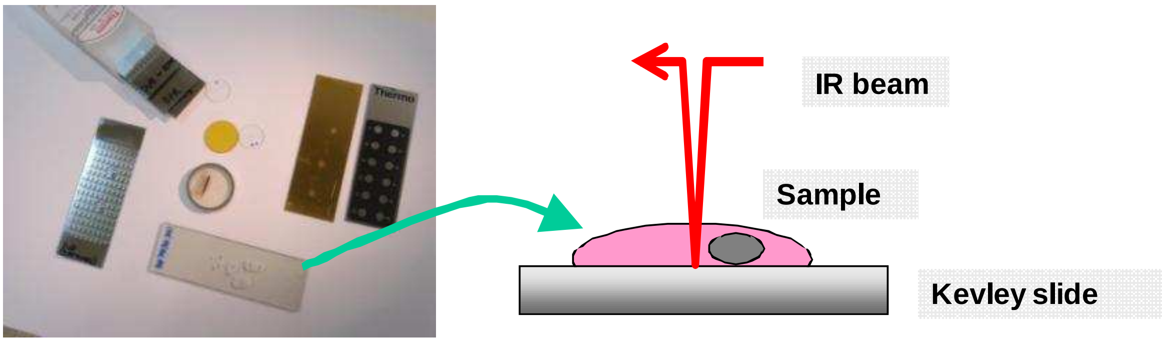
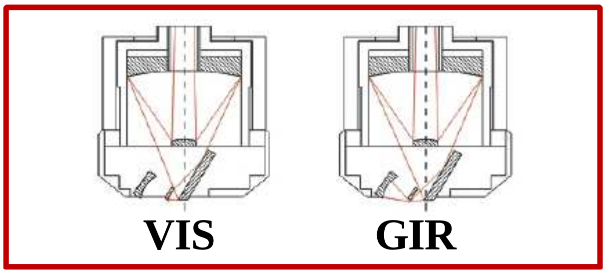
"Transreflectance" measurements

Biological samples on specially coated glass microscope slides

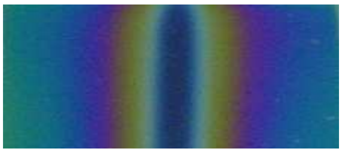


Absorption/reflection (Ag undercoat/SnO_2 overcoat), visible transmission.
Cheaper than IR transmitting windows but not re-usable.
Sample should be between 5 and 10 microns thick depending on material.
Problem: dispersion artifacts in thin parts of tissue.

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.

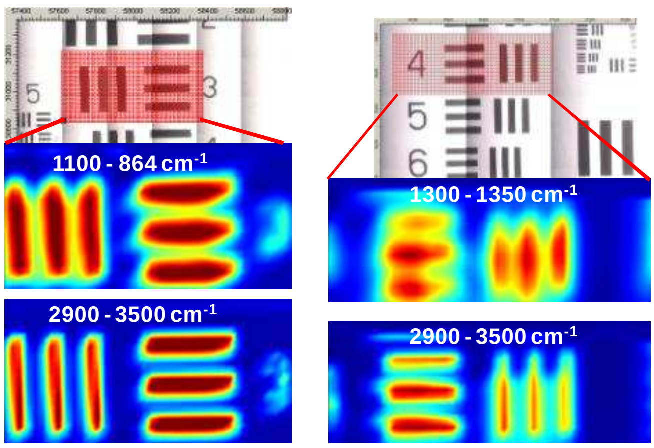
Donna Menzies, Thomas Gengenbach, Celesta Fong, John Forsythe, Ben Muir – CSIRO / Monash University

Grazing angle Reflectance measurements

Spatial resolution

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

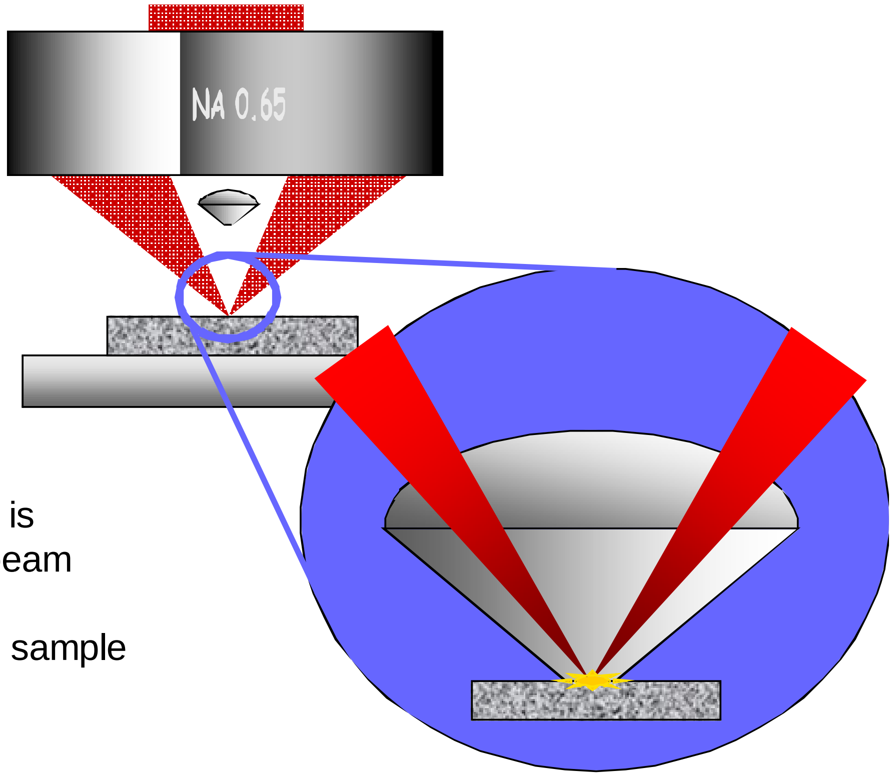
The resulting IR images are shifted with respect to the visible image (~60 μm horizontal (right) shift and ~20 μm vertical shift (up).



20x20 μm aperture
50 μm features and
20 μm scan steps.

10x10 μm aperture
20 μm features and
10 μm scan steps.

Attenuated Total Reflection (ATR)



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

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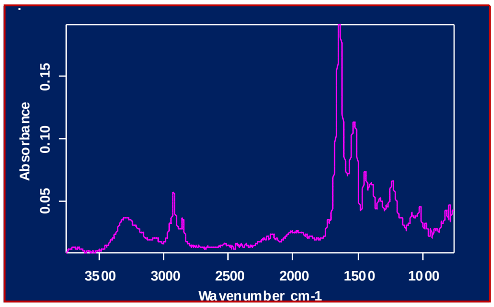
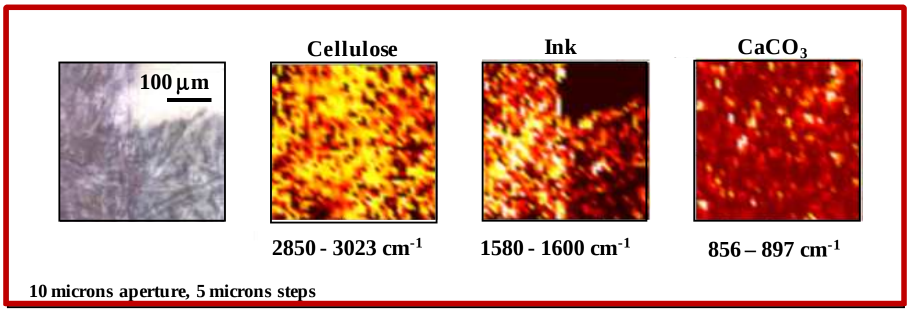
Attenuated Total Reflection (ATR)

Conserving National Heritage:
19th century parchment Sample

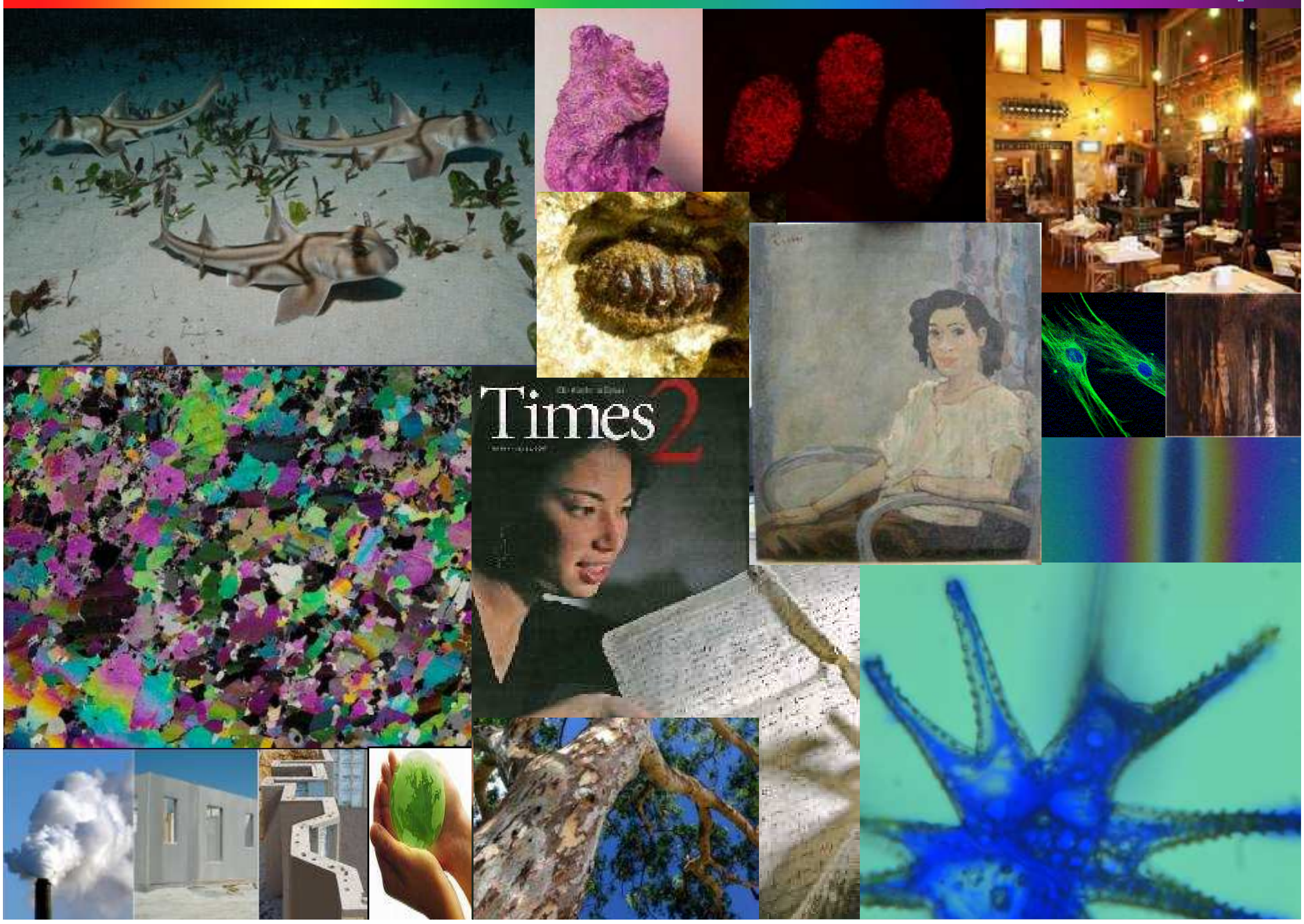


Forensic application:
examination of paper documents

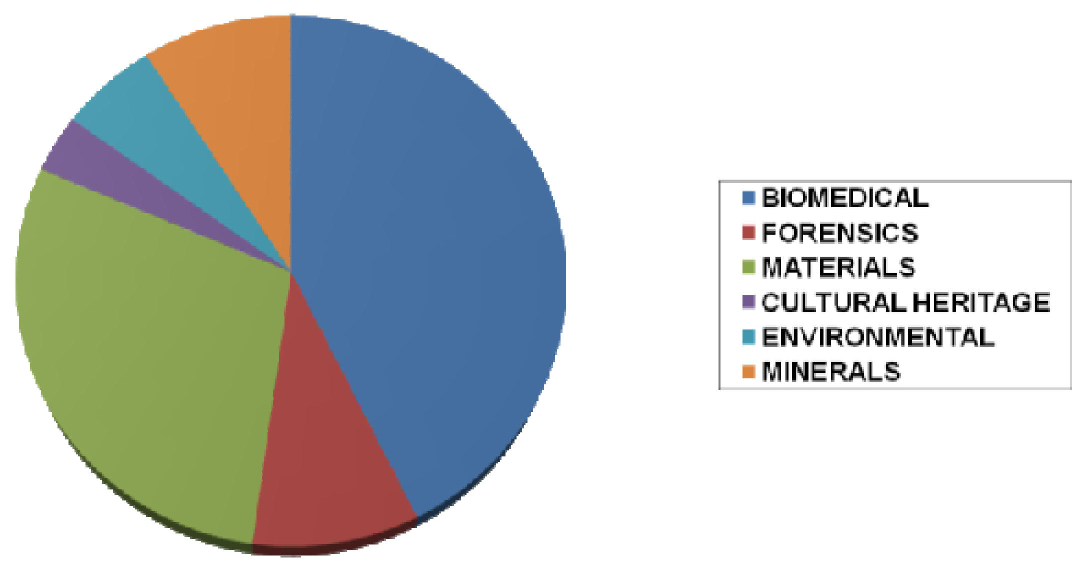


Alana Treasure, Dudley Creagh (Uni Canberra) / Simon Lewis, Bill van Bronswijk (Curtin University) Kenneth Paul Kirkbride, Vincent Otieno-Alego (AFP)

Applications of Synchrotron Infrared Light

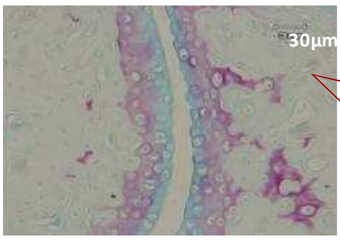


Use of the IR Microscope at the Australian Synchrotron by research area-2008



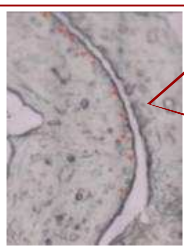
Understanding the autoimmune disease: Rheumatoid Arthritis

Complete mouse paw image taken using Vis 4x objective.



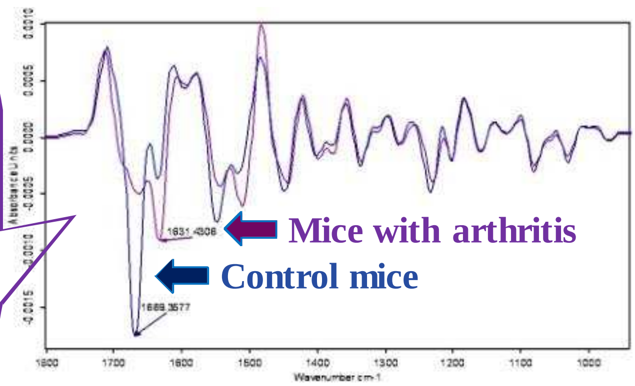
Light microscope image after staining the cartilage. The colour change at the surface shows cartilage damage.

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



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

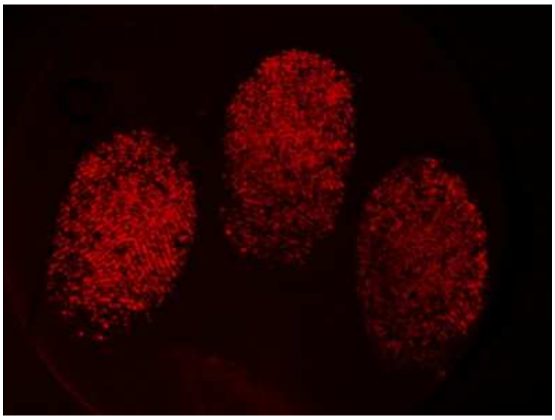
Average spectra from single cells in the cartilage showing the large differences between control mice (blue) and mice with arthritis (purple).



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 fingermarks 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).

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Studying naturally occurring revealing agents – Lawsone

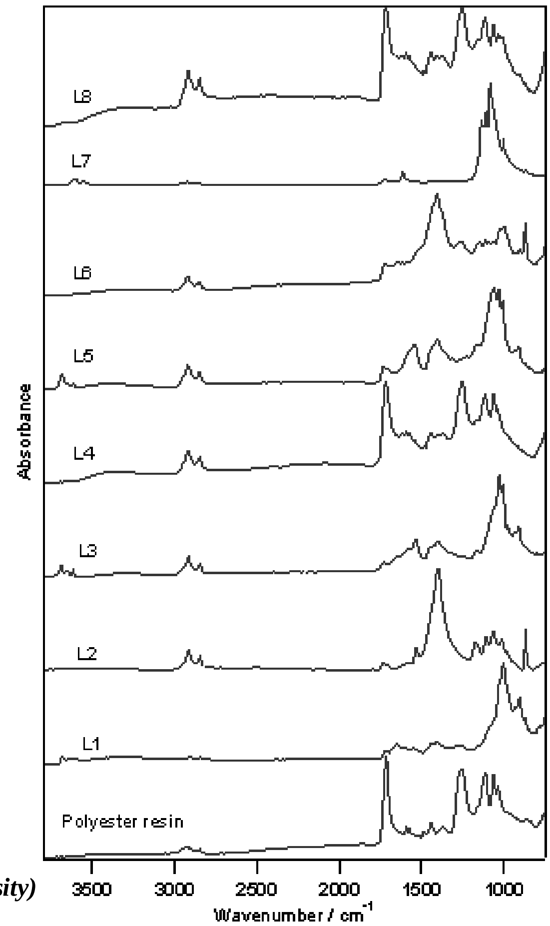
R. Jelly, S.W. Lewis, C. Lennard, K.F. Lim and J. Almog, *Lawsone: A novel reagent for the detection of latent fingermarks on paper surfaces*, Chemical Communications, 2008, 3513 - 3515

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

Curtin University of Technology

Australian Synchrotron

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)

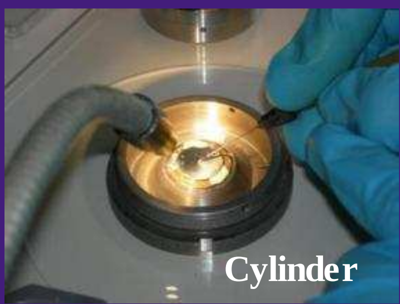
IR research in High Pressure

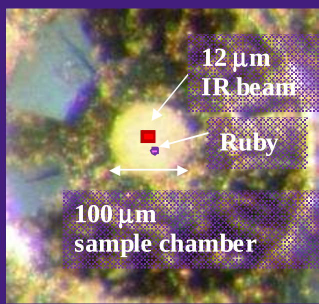
SMIS beamline at SOLEIL Synchrotron

[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

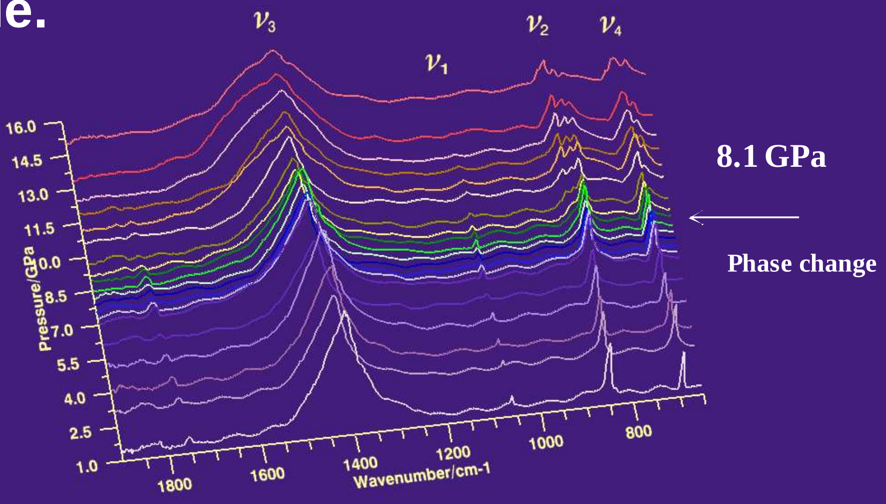




Custom made DAC with a membrane system for pressure application
(Institut de Minéralogie et Physique des Milieux Condensés, Université Pierre et Marie Curie, Paris).

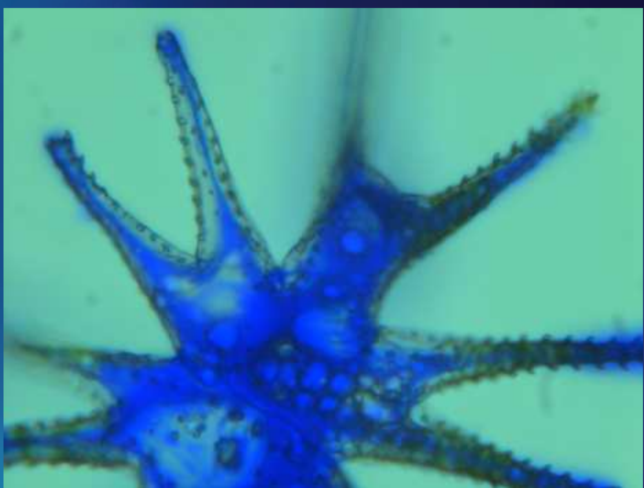
Type II diamonds with 500 microns culets diameter.

High pressure phase change of carbonate minerals to understand the behaviour of carbon in the mantle.



Band	Assignment	$Ba(CO_3)$ / cm^{-1}
ν_3	Asymmetric stretching vibration of the CO_3^{2-}	1449
ν_1	Symmetric stretching vibration of the CO_3^{2-}	1060
ν_2	Out of plane bending of the carbonate group	859
ν_4	In plane bending of the carbonate group	694

Environmental studies: Nutrient response in a model photosynthetic microorganism

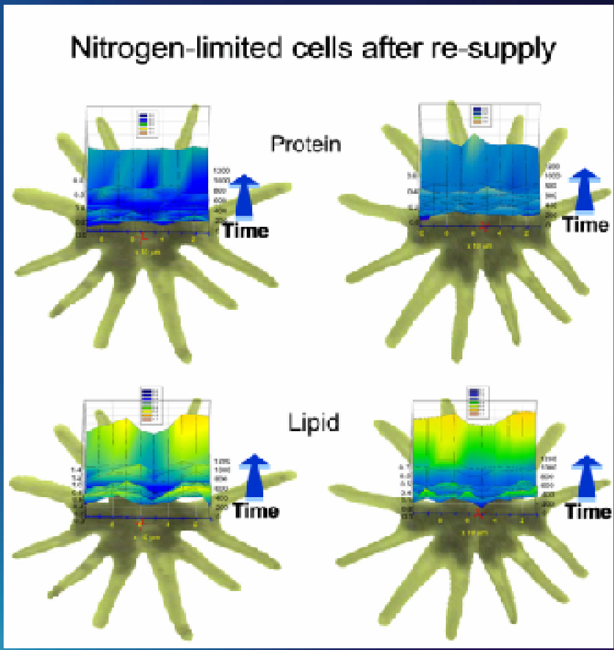


IR maps (series of line maps across the centre of the cell taken every 30 min over 24 h period) describing the effect the nutrient conditions change has on cell.

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

Phil Heraud, Anthony Eden, Don McNaughton, Bayden WoodMonash University

Environmental Science applications



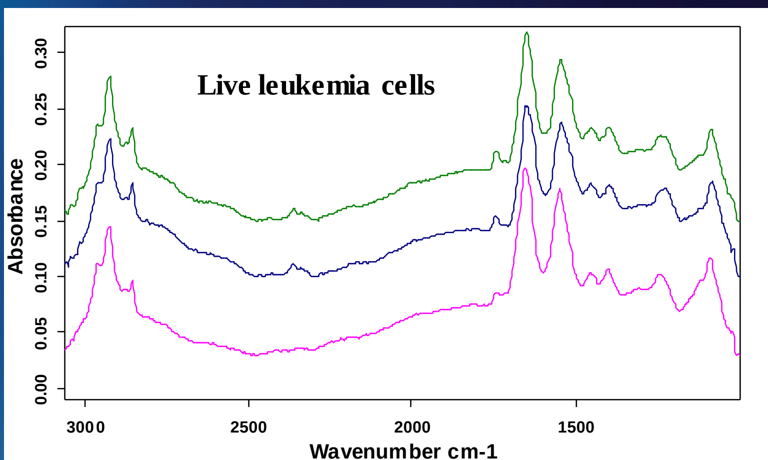
IR synchrotron microspectroscopy reveals microscale biochemical changes occurring in living plant cells.

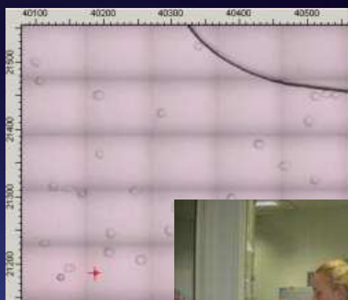
This allows researchers to better understand how plants cells respond to changes in the environment.

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

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

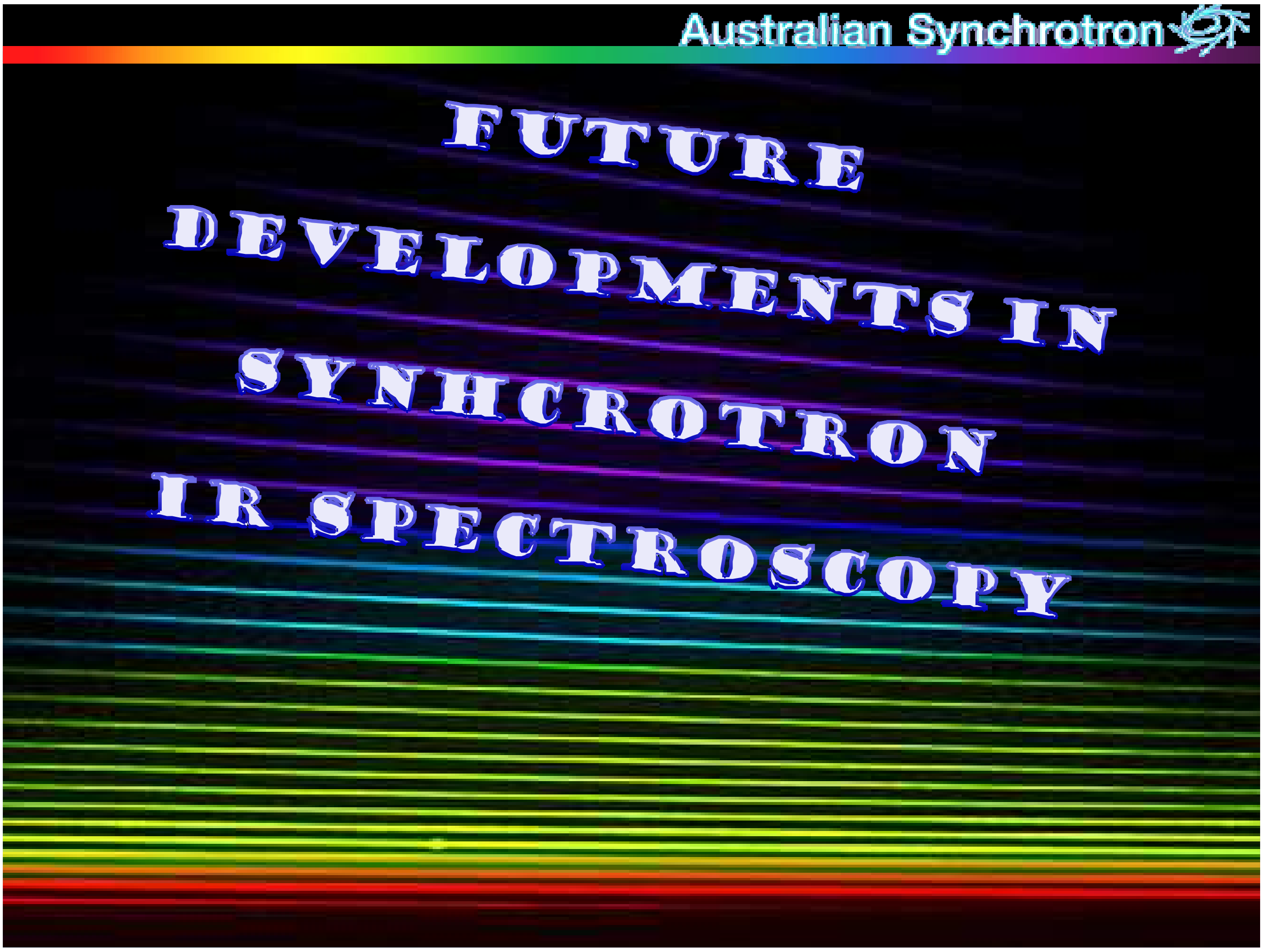
Monitoring the biological effect of chemotherapeutic drags



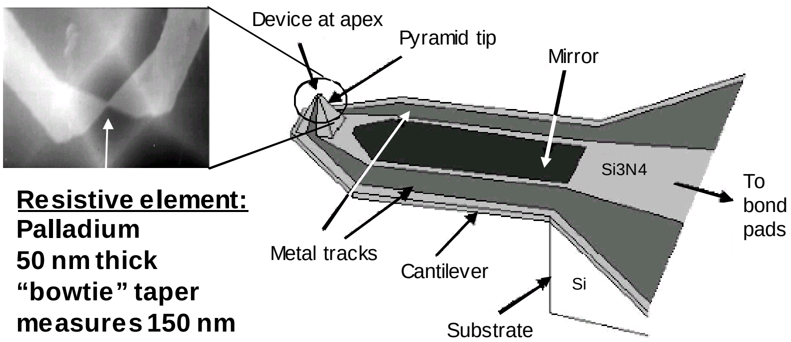


Study single live Leukaemia Cells using in fabricated CaF_2 liquid cell.

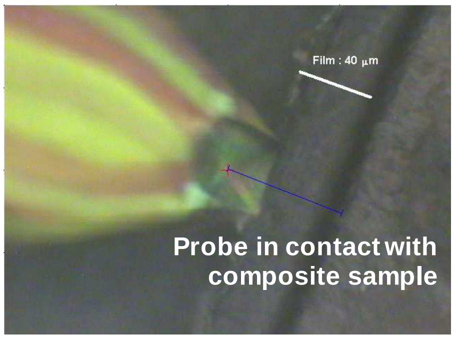
Carolyn Dillon, Kristie Munro (University of Wollongong), Keith Bambery, Bayden Wood (Monash University)

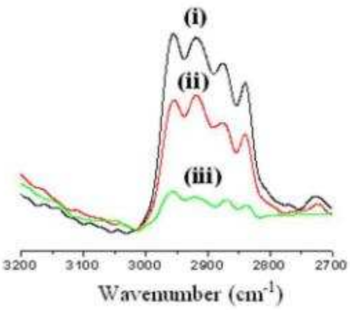


Breaking the diffraction limit – developing photothermal microspectroscopy



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





- **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

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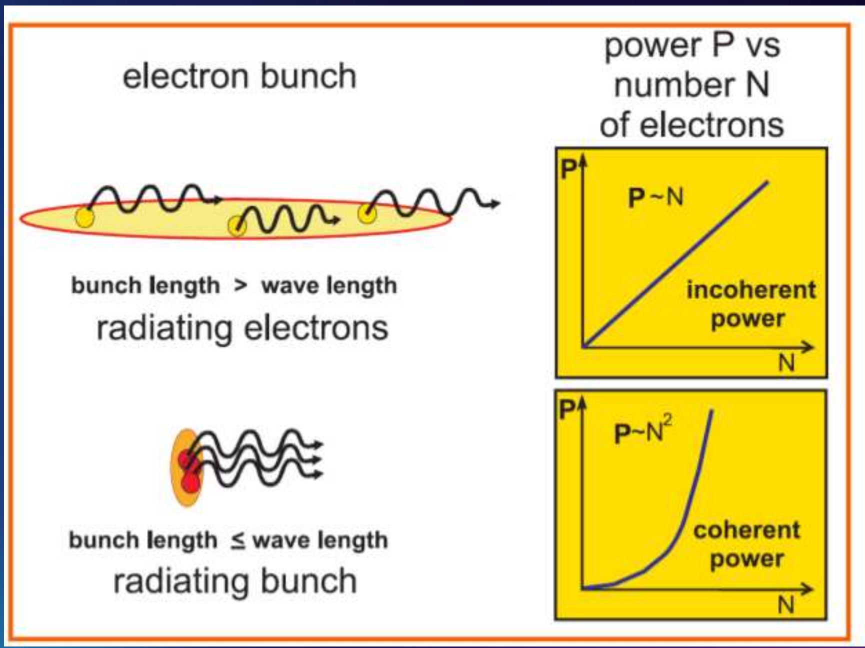
Coherent Synchrotron Radiation



BESSY website

Use of coherent enhancement for Far-IR and THz studies

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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 micoscope), 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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Acknowledgements:

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- Finlay Shanks – Monash University

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- Emma Patton – Curtin University
- Simon Lewis – Curtin University
- Kieran Lim – Deakin University

- Dudley Creagh – Canberra University
- Alana Treasure – Canberra University

- Allyson Croxford – Monash University
- Merrill Joy Rowley – Monash University

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

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- Liz Carter – University of Sydney
- Peter Lay – University of Sydney

- Stephen Best – Melbourne University
- Robyn Sloggett – Melbourne University
- Caroline Kyi – Melbourne University

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- Dom Appadoo – Australian Synchrotron
- Danielle Martin – Australian Synchrotron

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- Michael Nasse – University Wisconsin
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- Jean-Paul Itié – Université Pierre et Marie Curie
- Larry Car – Brookhaven

- Phil Heraud – MISCL
- Sally Caine – MISCL

- Janice Williams – La Trobe University
- Ewen Silvester – La Trobe University

- Carolyn Dillon – University of Wollongong
- Kristie Munro – University of Wollongong

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