

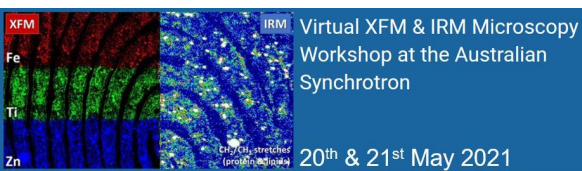
CAPABILITIES OF THE INFRARED MICROSCOPY BEAMLINE

Mark Tobin

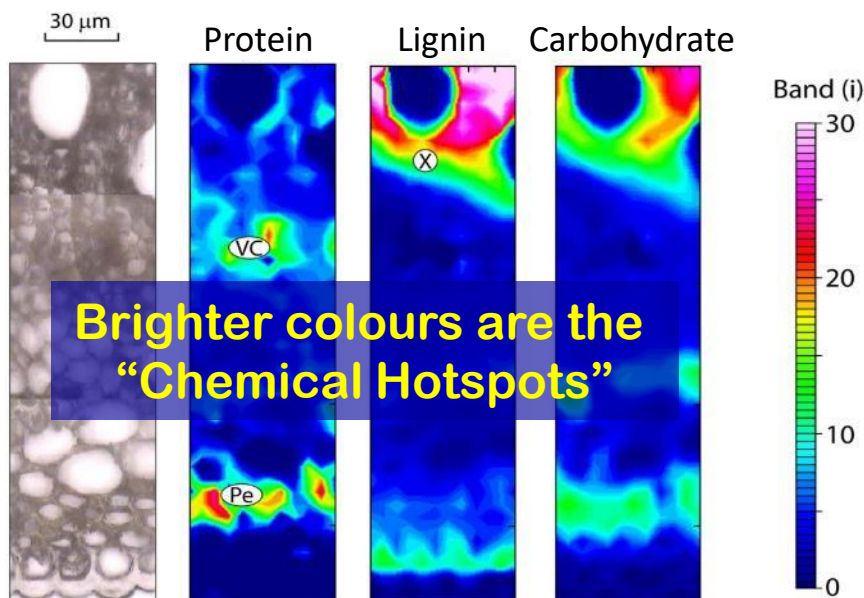
Australian Synchrotron

OVERVIEW

- Introduction to Infrared Microspectroscopy
- Infrared Microspectroscopy with a Synchrotron
- Sampling methods at the IRM beamline
- Examples from the IRM Beamline



WHY INFRARED MICROSCOPY ?



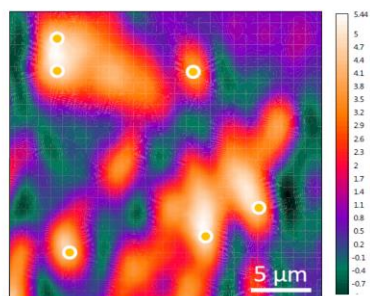
- FTIR provides information on both organic and inorganic components (e.g. proteins, lipids, and polymers, pigments, minerals) in a single analysis.
- Raster scanning of the sample generates 2D functional group maps.
- Ideal for the study of fine layers and microscopic particles



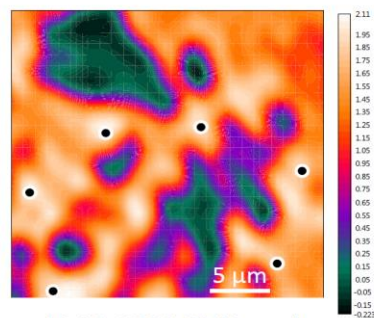
Example - Chemical Images
of the inside of a decaying gumleaf

WHY INFRARED MICROSCOPY ?

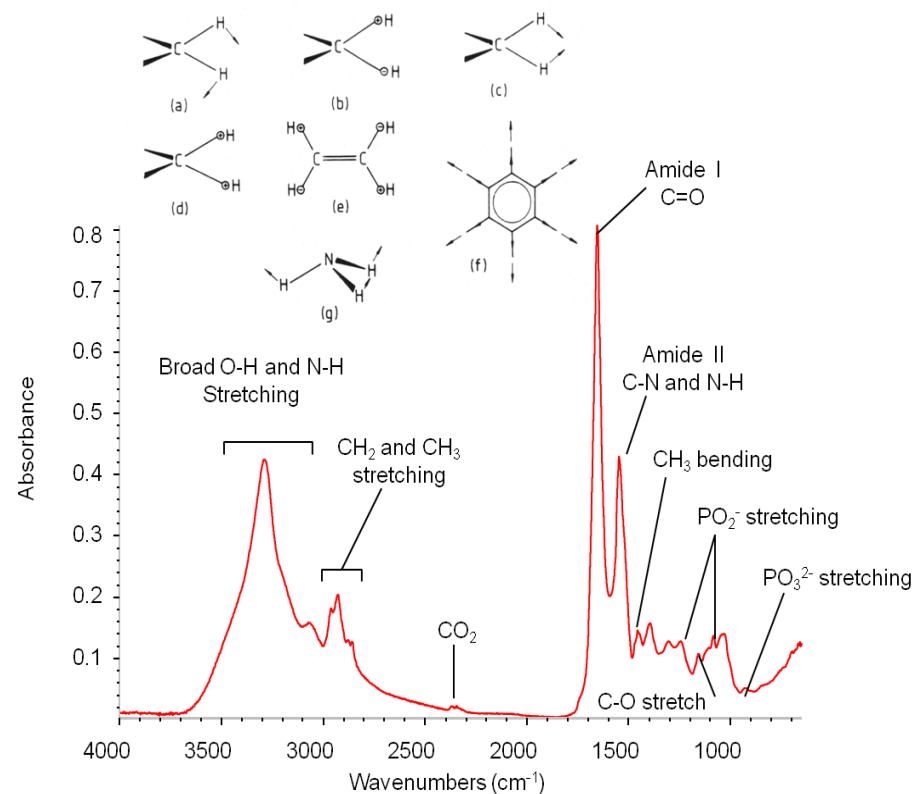
- Used for characterisation and identification of materials
- Peak shapes and positions are sensitive to molecular environment
- Applied to solids, liquids and gases
- Spectroscopic mapping and imaging
 - Visualise the distribution of chemical components
 - Maps/images are generated using unique spectral features
- Non-destructive



Protein: 1588-1704 cm^{-1}



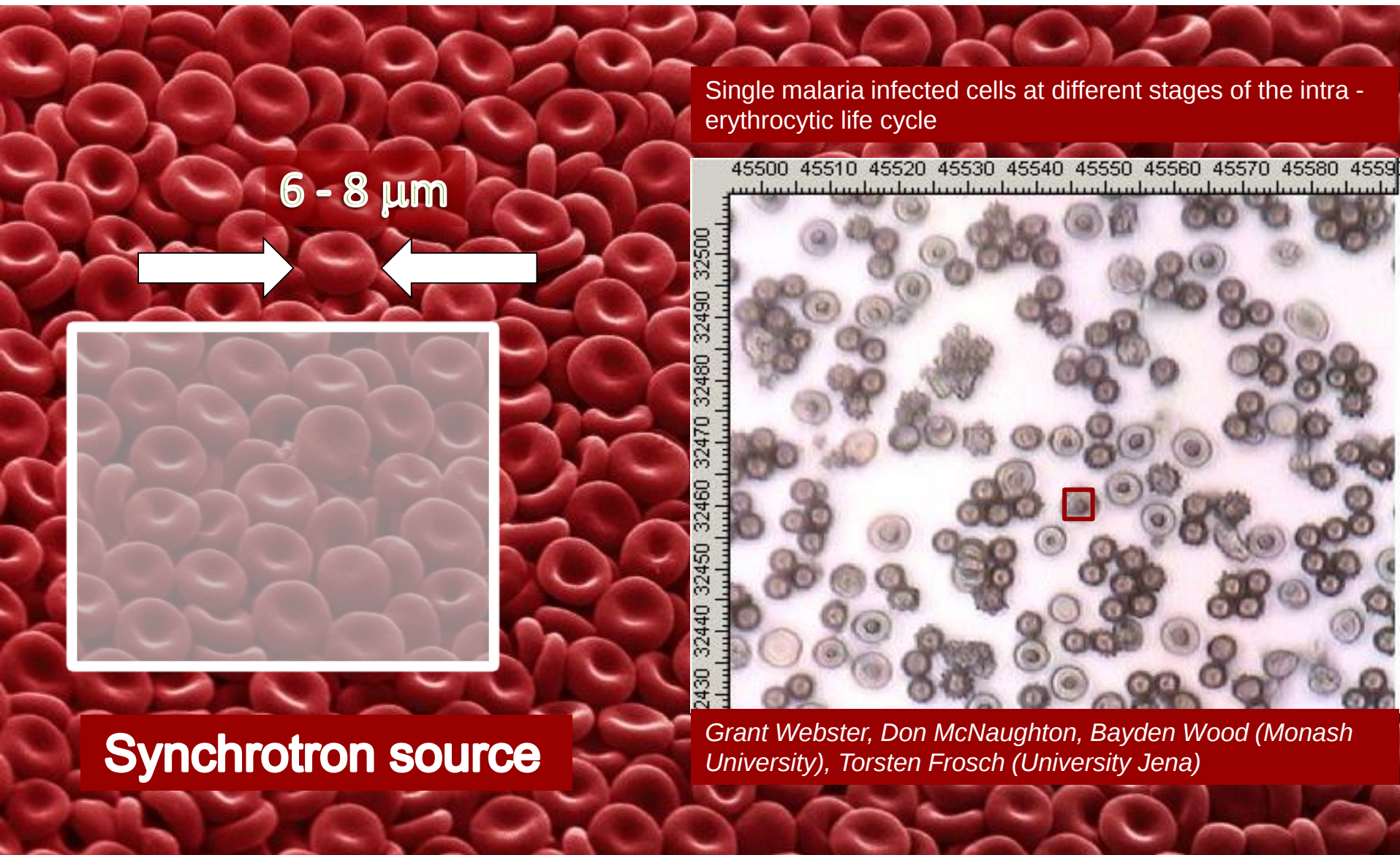
Lipid: 2881-2946 cm^{-1}



WHY INFRARED MICROSCOPY ?

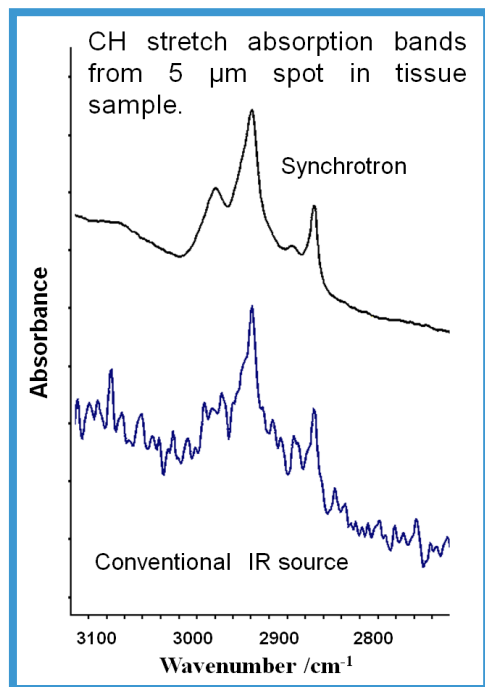


INFRARED MICRO SPECTROSCOPY WITH SYNCHROTRON LIGHT

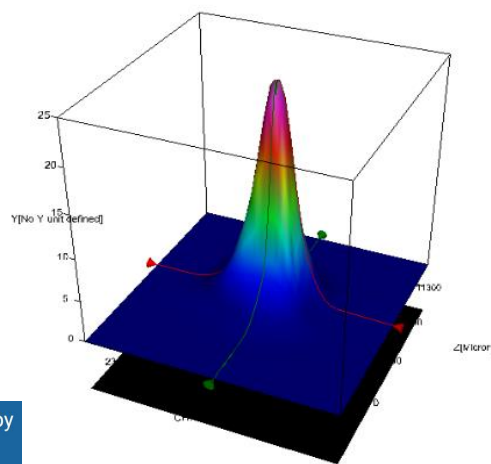


INFRARED MICRO SPECTROSCOPY WITH SYNCHROTRON LIGHT

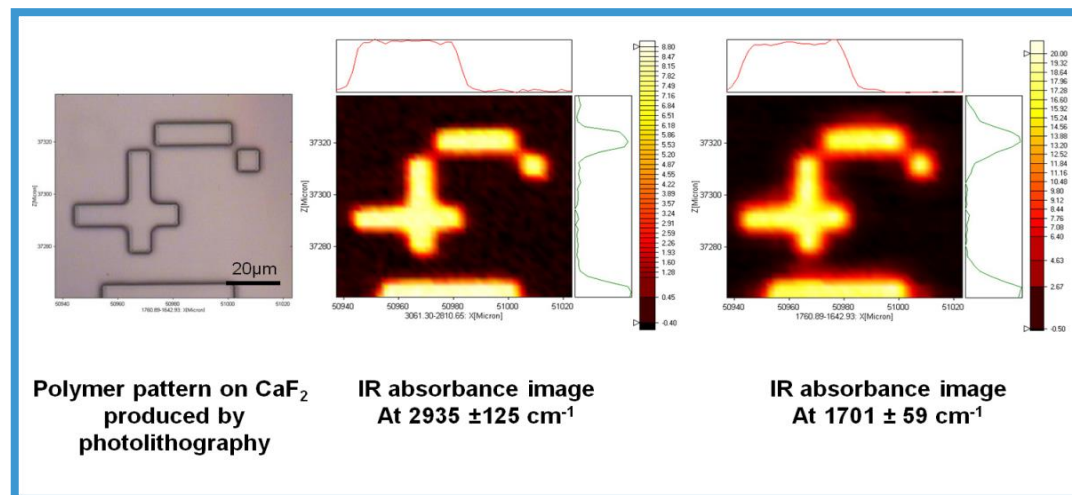
SIGNAL-TO-NOISE



Profile of “High Brightness”
Beam on sample



SPATIAL RESOLUTION

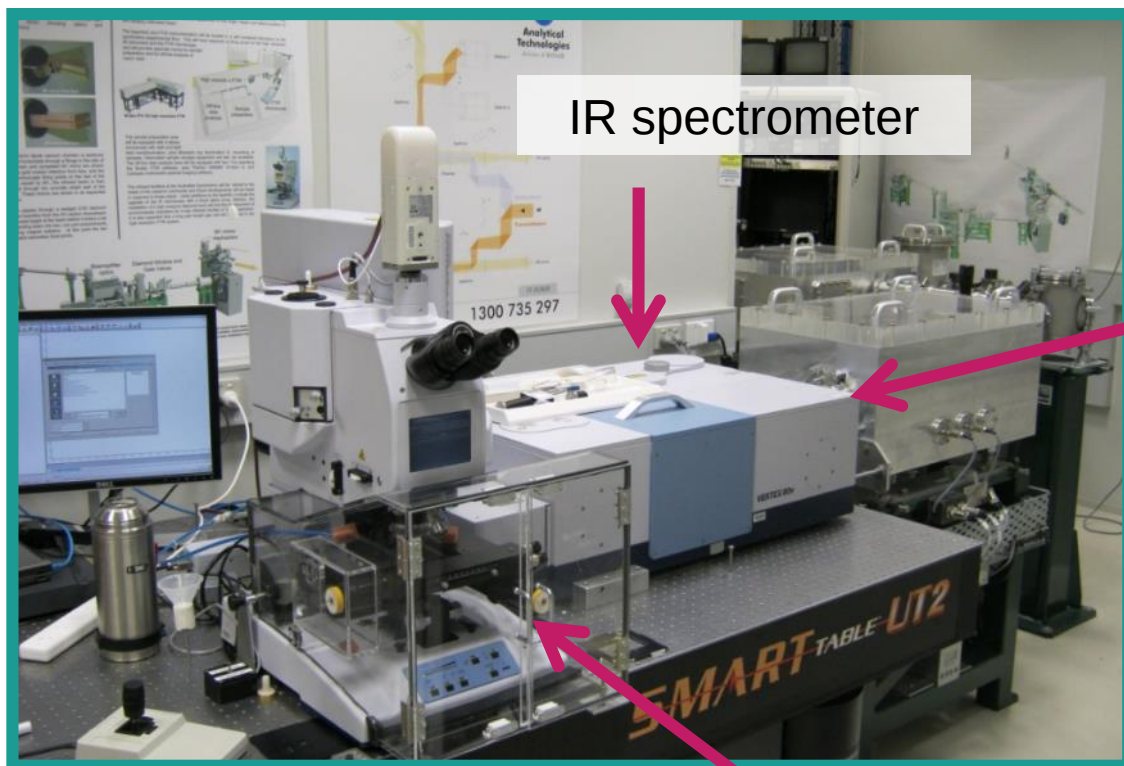


- Fast data acquisition
- Superior signal to noise
- Diffraction limited lateral resolution

Mapped area = 40 x 40 microns.

INFRARED MICRO SPECTROSCOPY WITH SYNCHROTRON LIGHT

A Bruker V80v FTIR spectrometer and Hyperion 3000 microscope are coupled to the synchrotron beam



IR spectrometer

IR microscope

Synchrotron Beam



- SR source, single element detector
- **High spatial resolution, down to a few microns**
- Mid-IR range, $750\text{--}3850\text{ cm}^{-1}$
- Extended range possible to 260 cm^{-1}

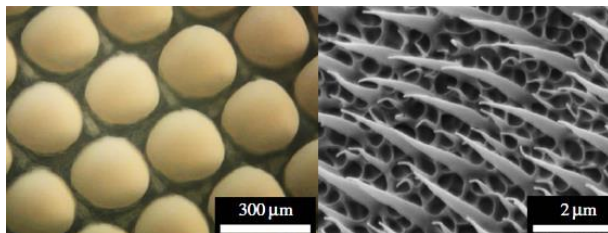
THE IRM BEAMLINE HAS APPLICATIONS IN MANY FIELDS

Materials

Understanding the properties of natural and manufactured surfaces



Anti-bacterial



Self-cleaning



Health

Studying the chemical changes associated with disease

Normal bone



Osteoporosis



Industry

Improving manufacturing of advanced materials



Carbon fibre production



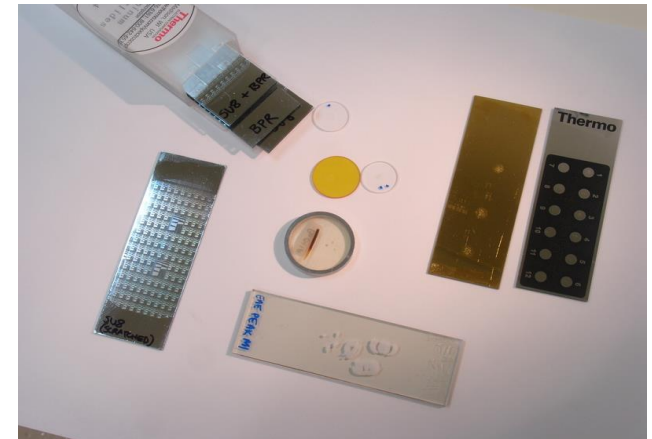
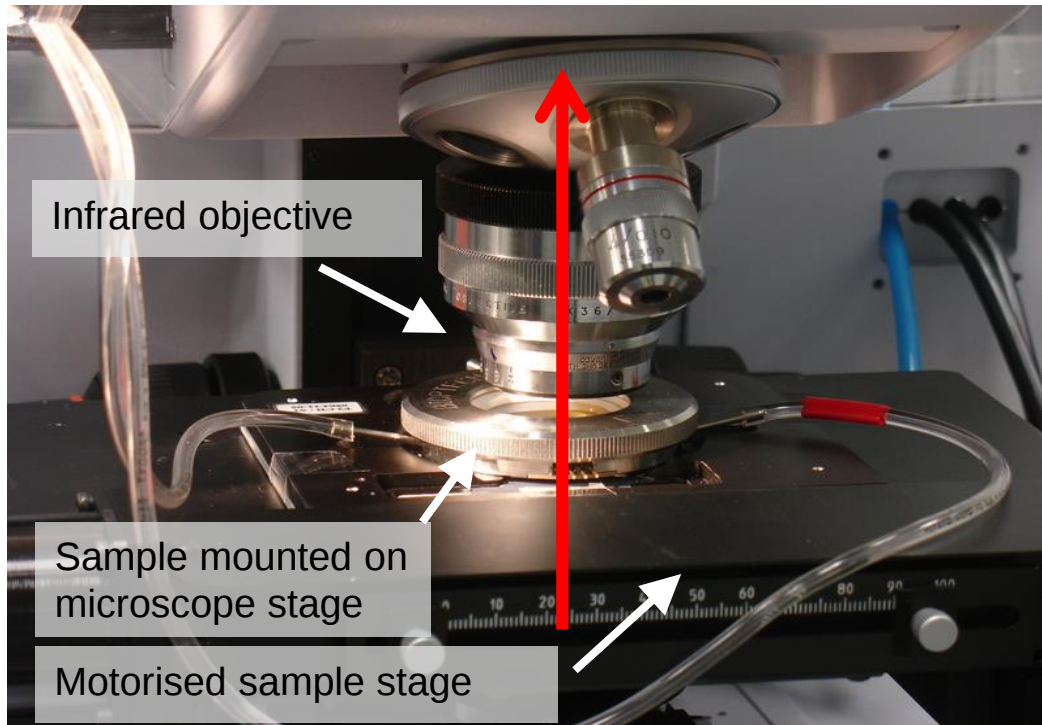
SAMPLING METHODS AT THE IRM BEAMLINE

The physical nature of your samples, and the questions you want to ask about their chemical properties, together determine the most appropriate choice of sampling method

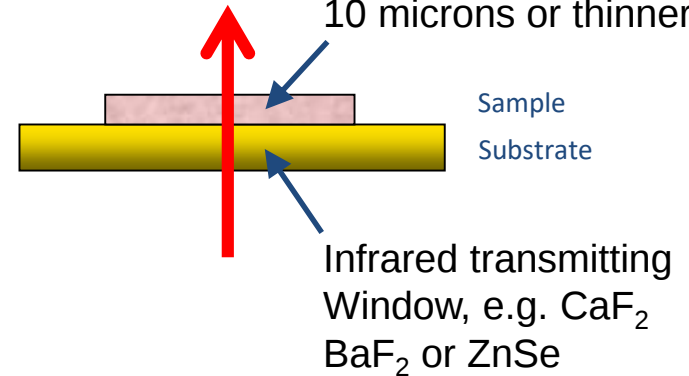


TRANSMISSION MICROSPECTROSCOPY

SAMPLE PREPARATION



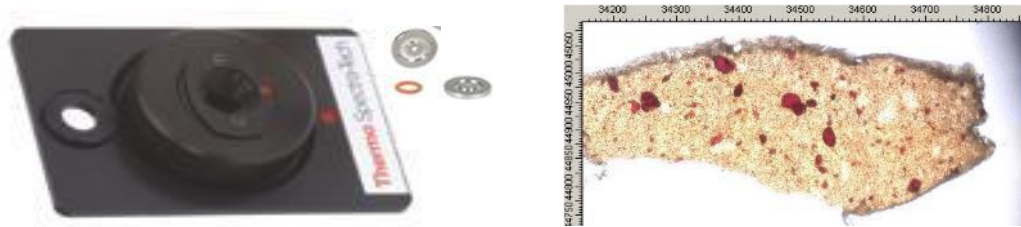
Most samples must be 10 microns or thinner



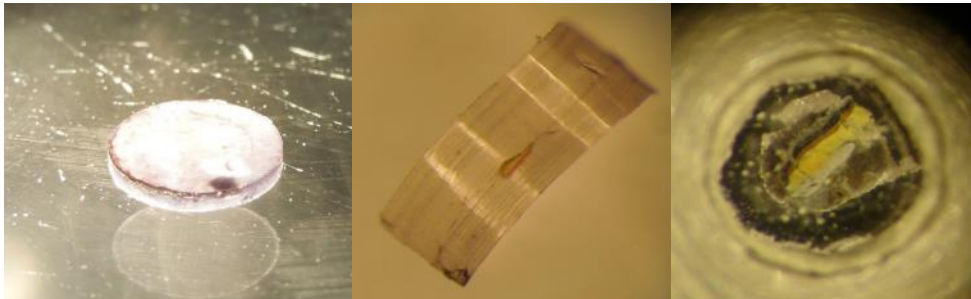
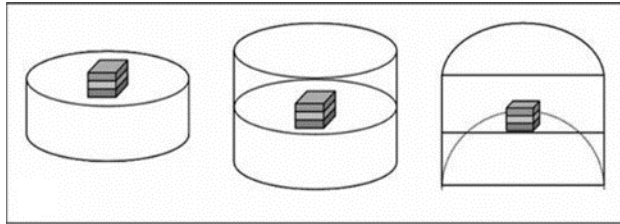
TRANSMISSION MICROSPECTROSCOPY

SAMPLE PREPARATION

Micro compression cell with diamond windows



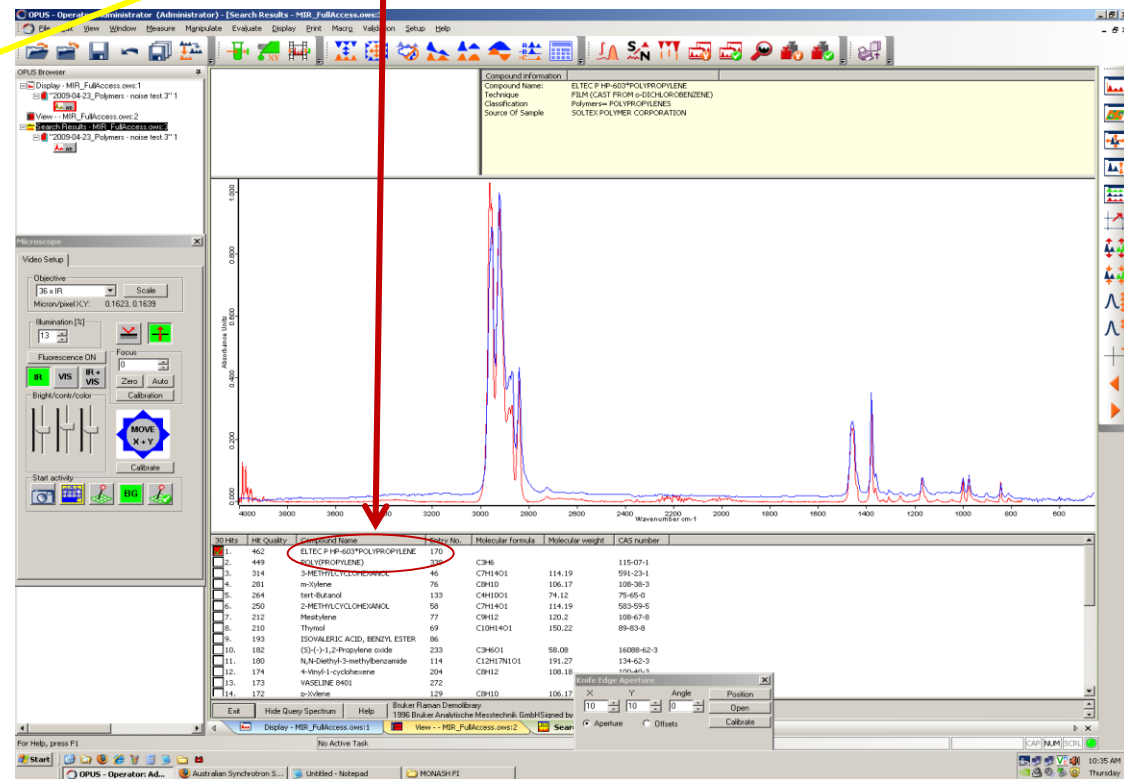
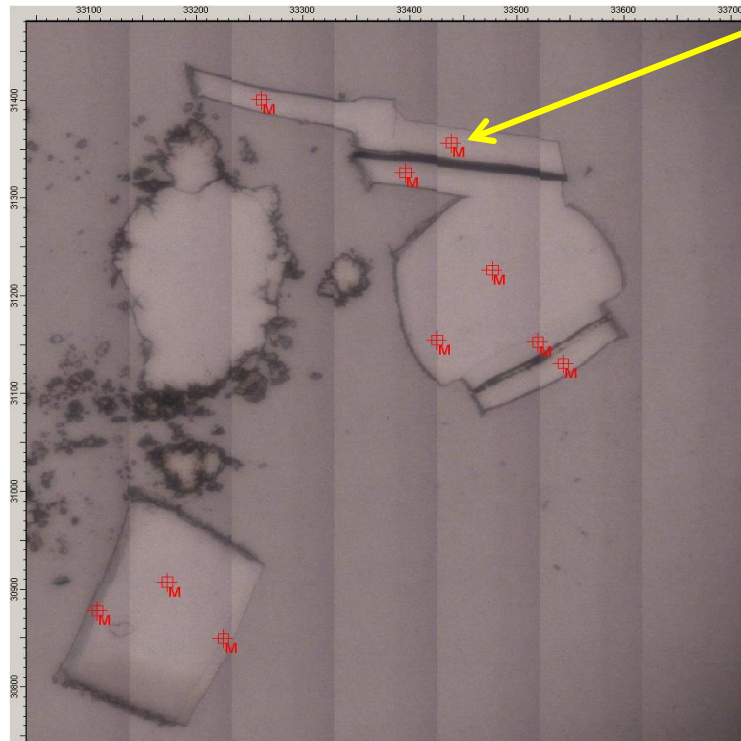
Embedding of sample for thin sectioning



Sample fragments embedded in AgCl are microtomed and compressed between diamond windows of a compression cell.

TRANSMISSION MICROSPECTROSCOPY FOR MATERIALS IDENTIFICATION

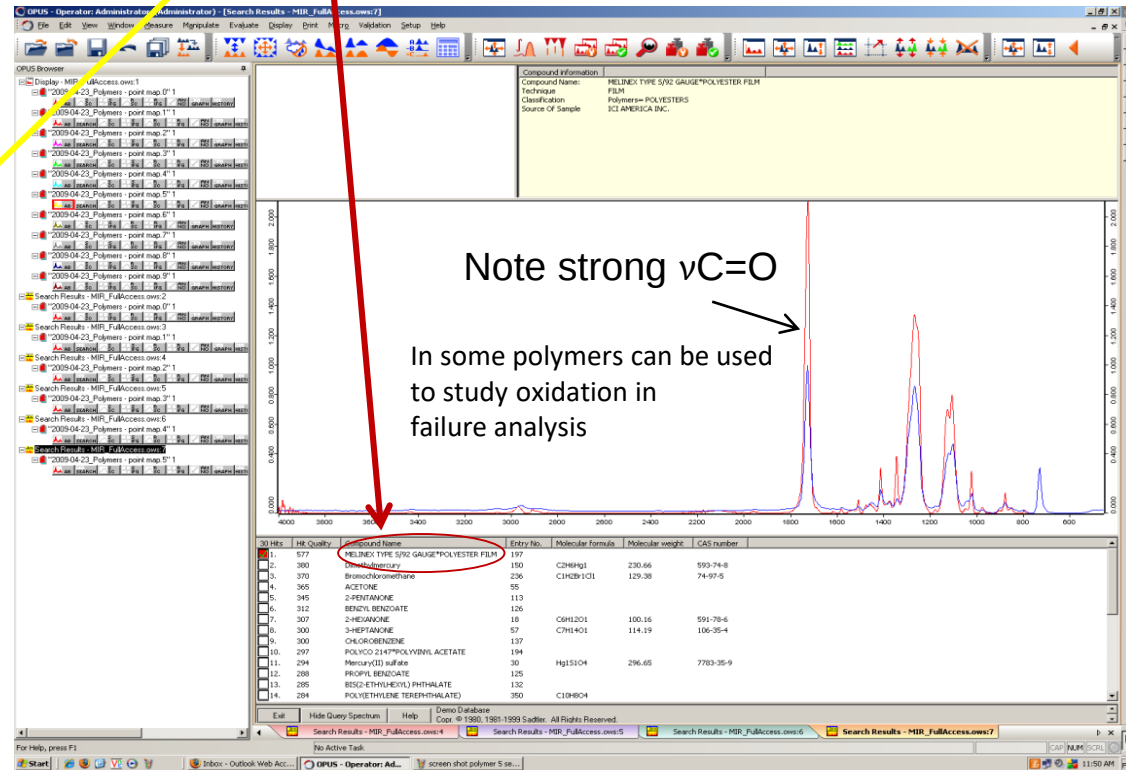
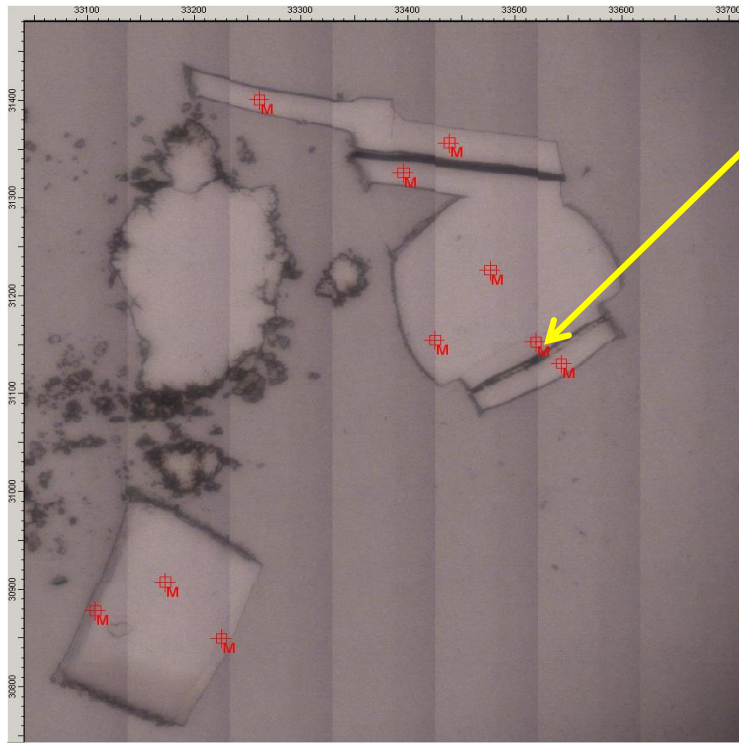
POLYPROPYLENE



- Software used to mark positions for analysis
- Spectral library used to assist identification

TRANSMISSION MICROSPECTROSCOPY FOR MATERIALS IDENTIFICATION

POLYESTER



- Software used to mark positions for analysis
- Spectral library used to assist identification

CHARACTERISATION OF DART POISONS

ROSEMARY GOODALL, MUSEUMS VICTORIA

Is any residual poison present on these Malaysian Darts?
What is the poison on the tips?

(a) & (b) Malaysia Darts and Bamboo quiver

(c) Sarawak Malaysia Darts and Bamboo quiver



CHARACTERISATION OF DART POISONS

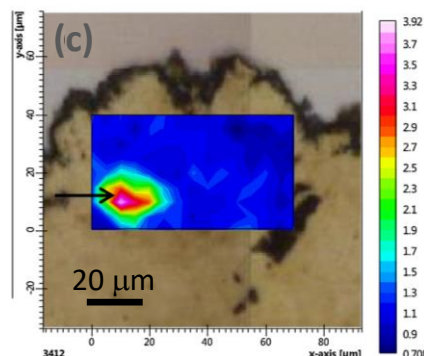
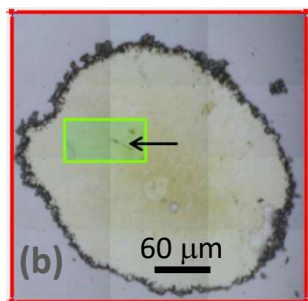
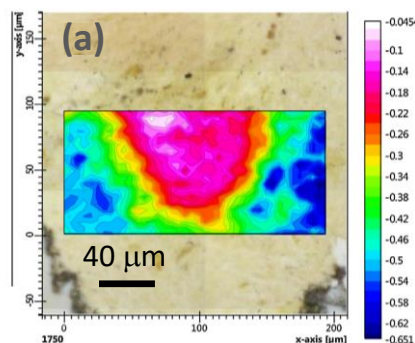
ROSEMARY GOODALL, MUSEUMS VICTORIA



www.natgeocreative.com/photography/1771099



A. toxicaria latex cardiac-glycoside poison, mixed with poison from *Amorphophallus* species (Arum lilies)



Steroidal toxic compounds of the strychnine type (*Strychnos* genus) mixed with other plant fibres

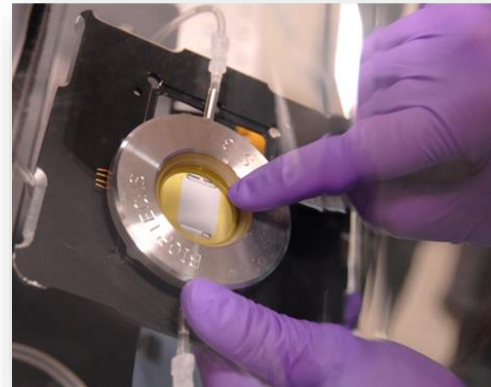
opendata.keystone-foundation.org/strychnos-potatorum-l-f/strychnos-potatorum

TRANSMISSION MICROSPECTROSCOPY OF LIVE BIOLOGICAL SAMPLES

Micro-fabricated liquid cell
holders on CaF_2 windows
For static live cell measurements



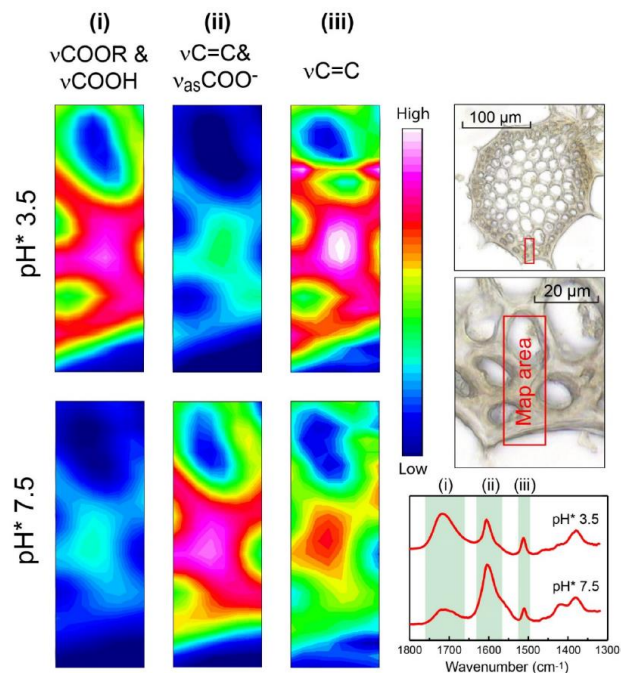
Modified Biopetechs FCS3 flow cell
Allows nutrient medium exchange



TRANSMISSION MICROSPECTROSCOPY OF LIVE BIOLOGICAL SAMPLES

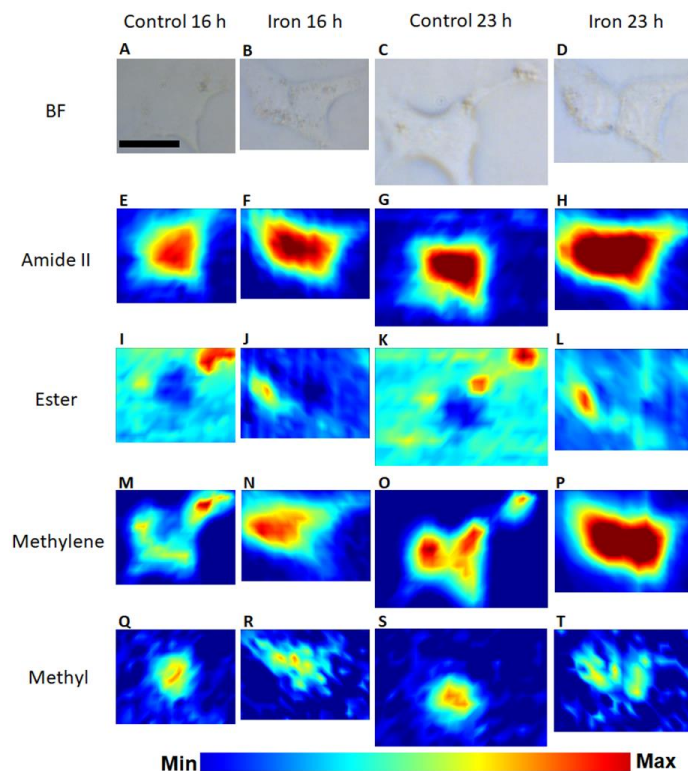
Response of *Sphagnum* cell wall material
to its aqueous chemical environment

EWEN SILVESTER, KERRY WHITWORTH & ANNALEISE KLEIN
LA TROBE UNIVERSITY AND ANSTO



Tracking biochemical changes induced by
iron loading in AML12 hepatocytes

ROSS GRAHAM AND CLINTON KIDMAN
CURTIN UNIVERSITY



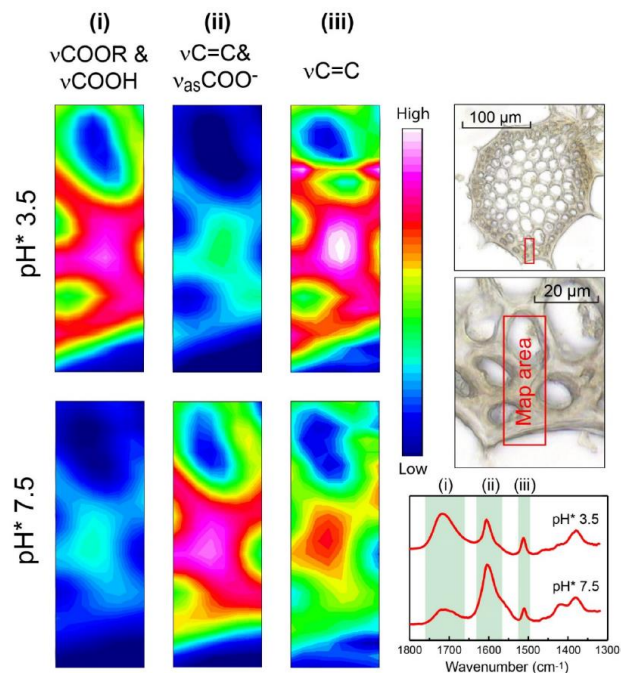
Ewen Silvester, Annaleise R. Klein, Kerry L. Whitworth, Ljiljana Puskar, Mark J. Tobin.
Environmental Chemistry **15**, 513 (2018)

Clinton J. Kidman, Cyril D.S. Mamotte, M. Adrien Eynaud, Juliane Reinhardt, Jitraporn Vongsivut,
Mark J. Tobin, Mark J. Hackett, Ross M. Graham. *Biochemical Journal* **478**, 1227-1239 (2021)

TRANSMISSION MICROSPECTROSCOPY OF LIVE BIOLOGICAL SAMPLES

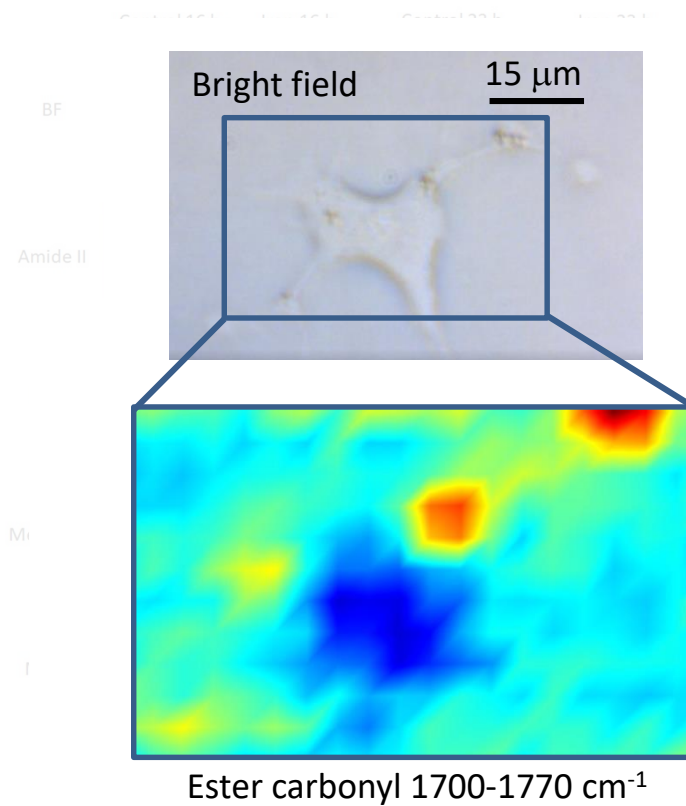
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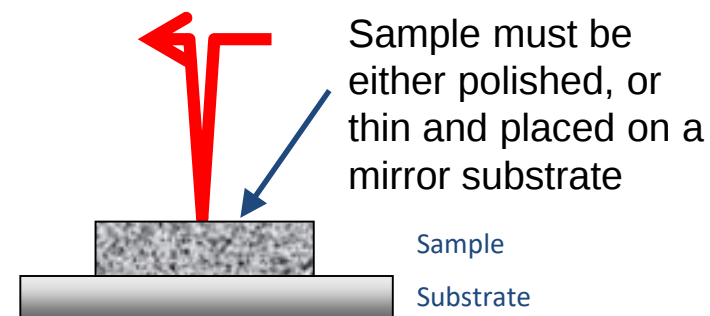
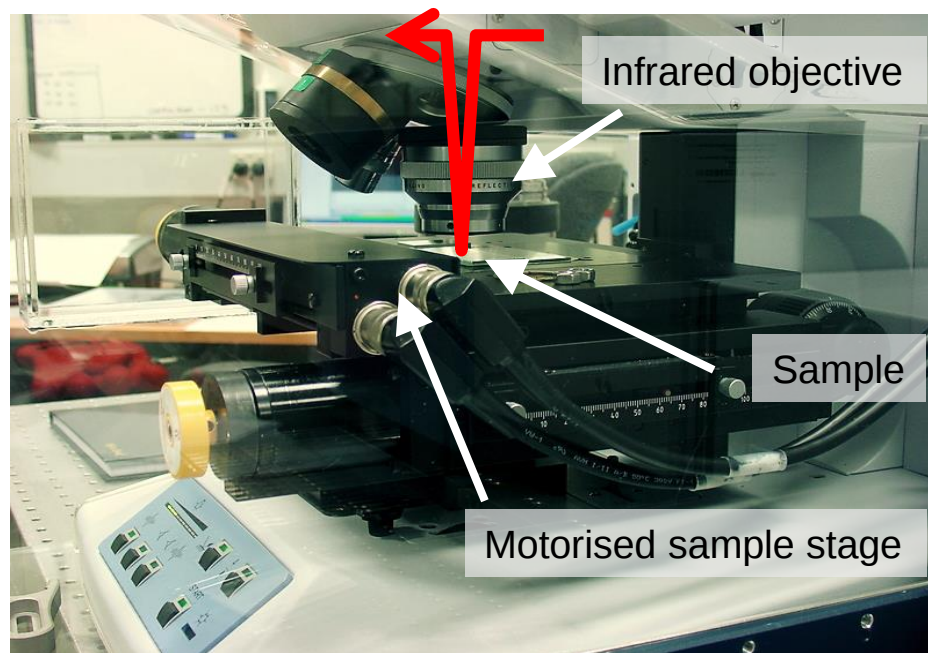
Ester carbonyl 1700-1770 cm⁻¹

Changes in cell morphology and
distribution over 8 hours

Ewen Silvester, Annaleise R. Klein, Kerry L. Whitworth, Ljiljana Puskar, Mark J. Tobin.
Environmental Chemistry **15**, 513 (2018)

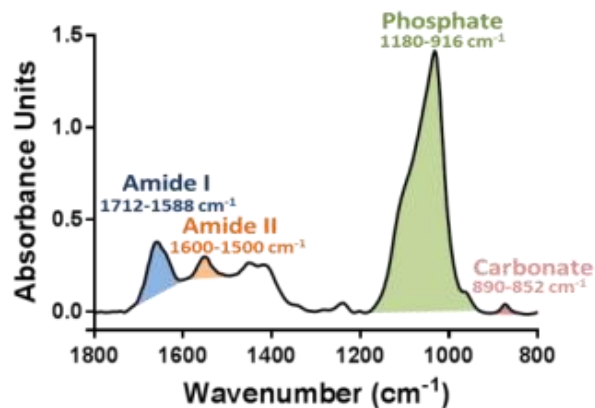
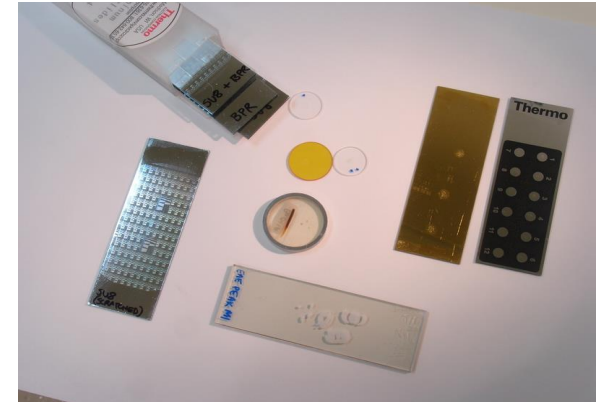
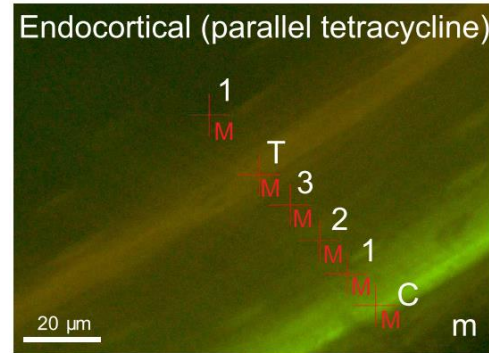
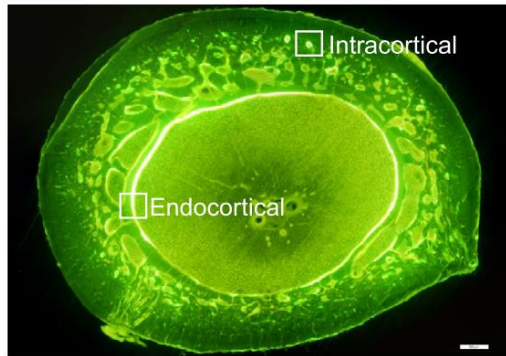
Clinton J. Kidman, Cyril D.S. Mamotte, M. Adrien Eynaud, Juliane Reinhardt, Jitraporn Vongsivut,
Mark J. Tobin, Mark J. Hackett, Ross M. Graham. *Biochemical Journal* **478**, 1227-1239 (2021)

SAMPLING MODES - REFLECTANCE

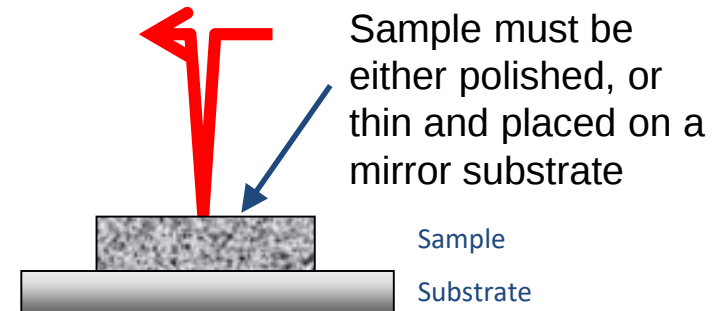


EFFECTS OF PHARMACOLOGICAL AGENTS ON BONE RE-MODELLING IN RABBIT BONE

NATALIE SIMS – ST VINCENT’S INSTITUTE OF MEDICAL RESEARCH
CHRISTINA VRAHNAS – UNIVERSITY OF DUNDEE



- Amide I & Amide II
 - Organic matrix
 - Amide I:Amide II ratio
 - = collagen orientation
- Phosphate
 - Mineral amount, crystal size and distribution
- Carbonate
 - Component of the hydroxyapatite crystal

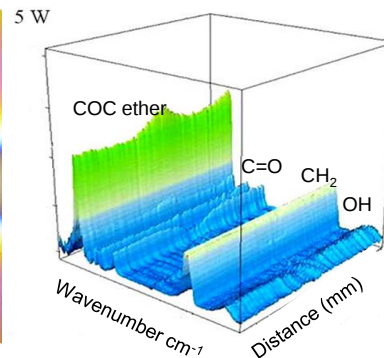
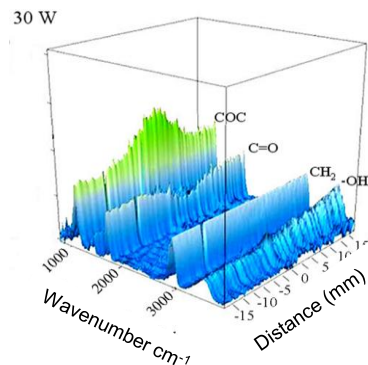
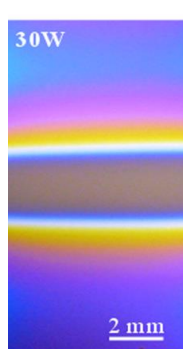
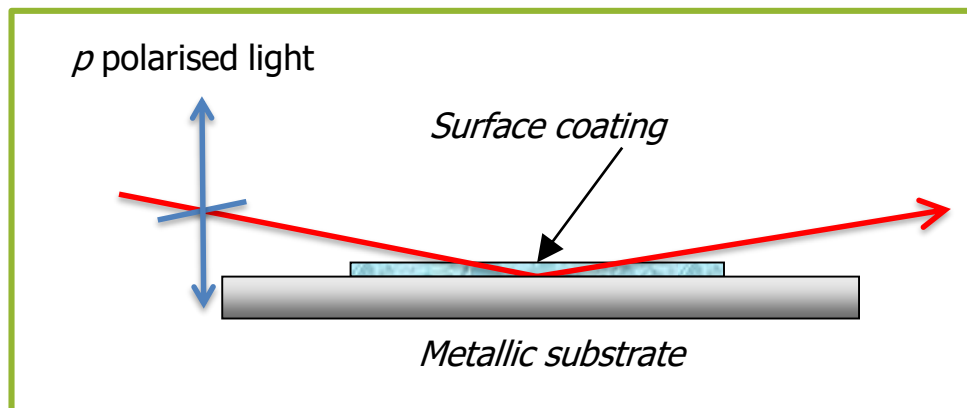


IR absorbance spectra of mouse bone collected in transmission mode

SAMPLING MODES – GRAZING INCIDENCE

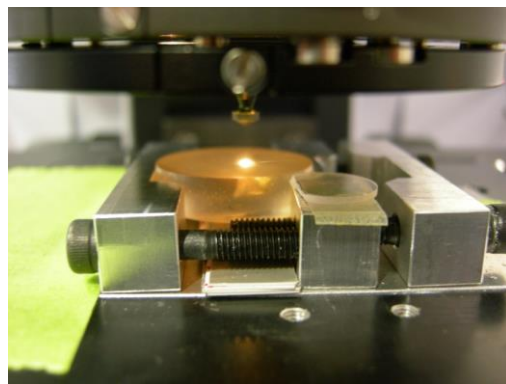
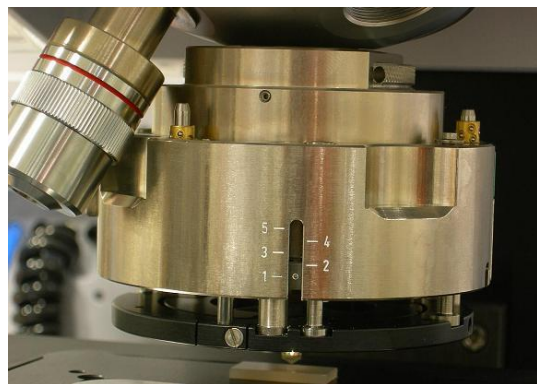


Thin layers of sample down to monolayers on reflective metallic surfaces (e.g. gold, steel, ITO glass)

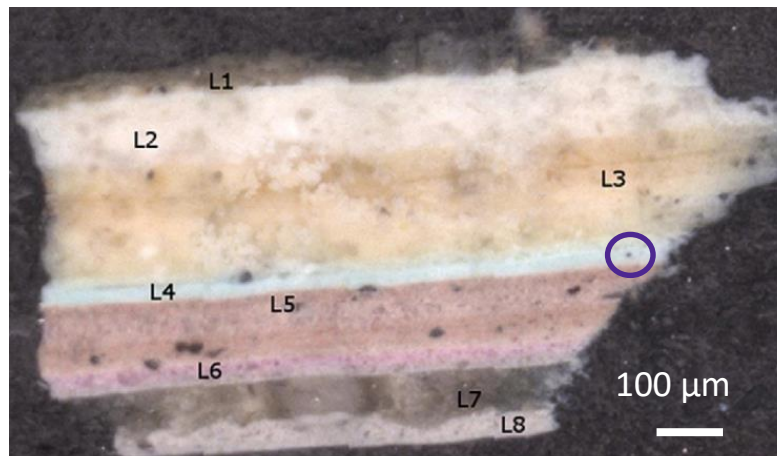


e.g. Protein resistant plasma polymer thin films. Mapping the compositional change along a plasma gradient.

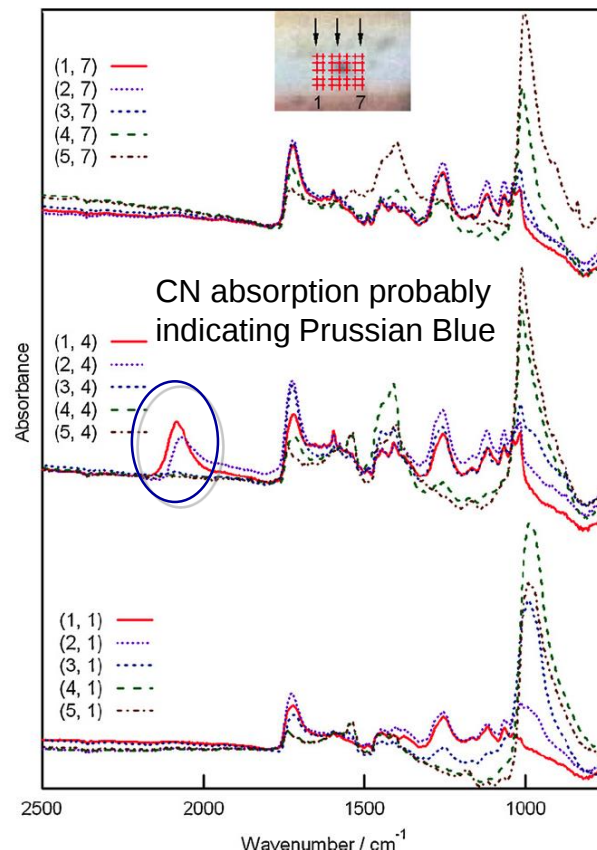
MICRO ATTENUATED TOTAL INTERNAL REFLECTION (ATR)



Bruker ATR 20x objective
Ge-crystal, 100 or 250 micron tip
variable pressure selection.



Multilayer paint fragment from exterior of Provincial Hotel, Fitzroy
Sample was not suitable for thin sectioning.



DATA ANALYSIS FOR IR MICROSPECTROSCOPY

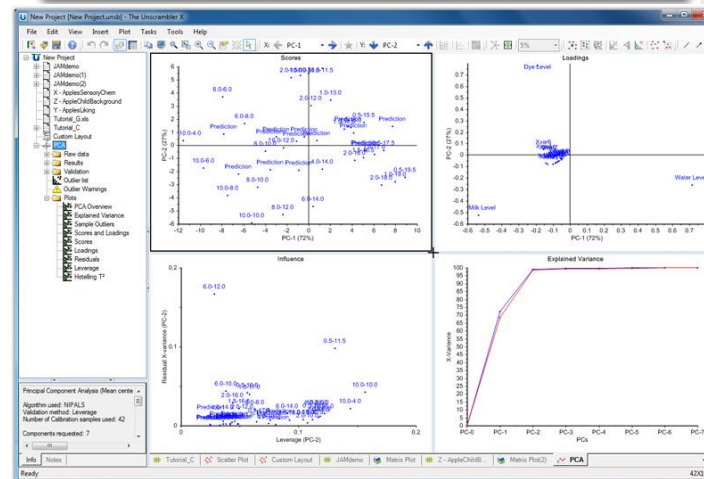
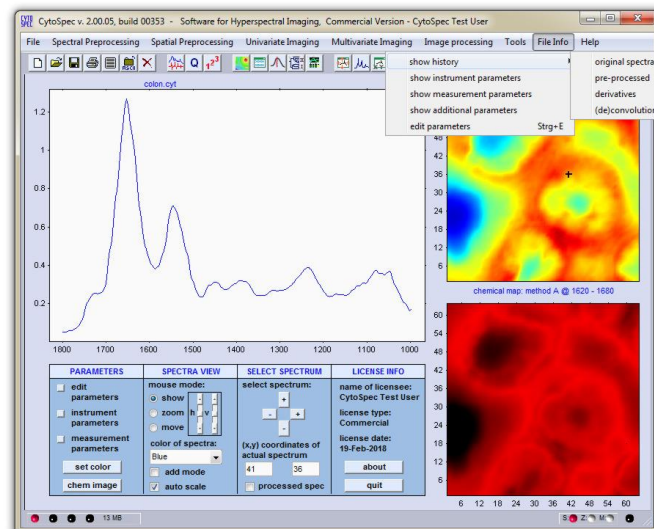
Copy provided to IRM users



Available for use while
at the beamline

CYTOSPEC

The Unscrambler® X



DATA ANALYSIS FOR IR MICROSPECTROSCOPY

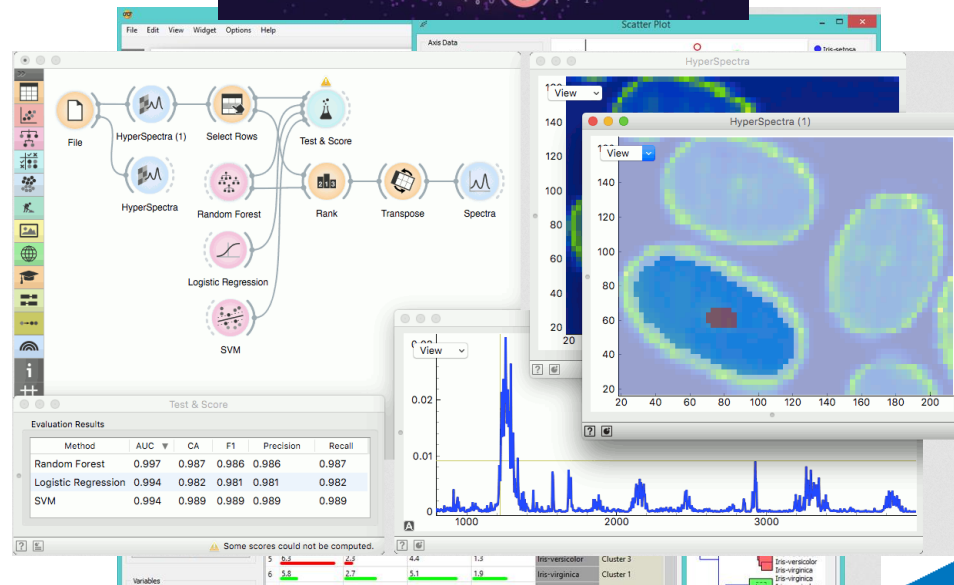
Copy provided to IRM users



Available for use while
at the beamline

CYTOSPEC

The Unscrambler[®] X



REMOTE MAIL-IN EXPERIMENTS ON IRM



7:00 pm · 2 Jul 2020 · Buffer

CONTACT THE BEAMLINE TEAM WITH YOUR ENQUIRIES!



- Advice on sample preparation
- Preparing your experiment proposal
- Design and build of custom accessories
- Help with set up of your accessories
- Assistance with data analysis
- Assistance with manuscript preparation

CONTACT THE BEAMLINE TEAM WITH YOUR ENQUIRIES!



Mark Tobin
Principal Scientist – IRM



Jitraporn (Pimm) Vongsvivut
Senior Beamline Scientist – IRM



Annaleise Klein
Beamline Scientist – IRM



Keith Bambery
Microscopy Group Manager

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ACKNOWLEDGEMENTS

- Rosemary Goodall – Museums Victoria.
- Sally Gras and Lydia Ong – University of Melbourne.
- Bayden Wood – Monash University.
- Ewen Silvester – La Trobe University.
- Ross Graham and Clinton Kidman – Curtin University.
- Thomas Gengenbach – CSIRO.
- David Thurrowgood – Queen Victoria Museum and Art Gallery, Launceston.
- Natalie Sims – St Vincent's Institute Melbourne.
- Christina Vrahnas – University of Dundee.
- Robyn Sloggett, Stephen Best, Caroline Kyi and Nicole Tse – University of Melbourne.
- Ljiljana Puskar – Helmholtz-Zentrum, Berlin.

Beamline Team

Pimm Vongsvivut

Annaleise Klein

Keith Bambery

Andraz Pozar and Simin Chen
(controls)

Mechanical Tech team

Alan Easdon

Rob Rostan

Thank you

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tobinm@ansto.gov.au