Fourier Transform Infrared Microspectroscopy with Synchrotron Light

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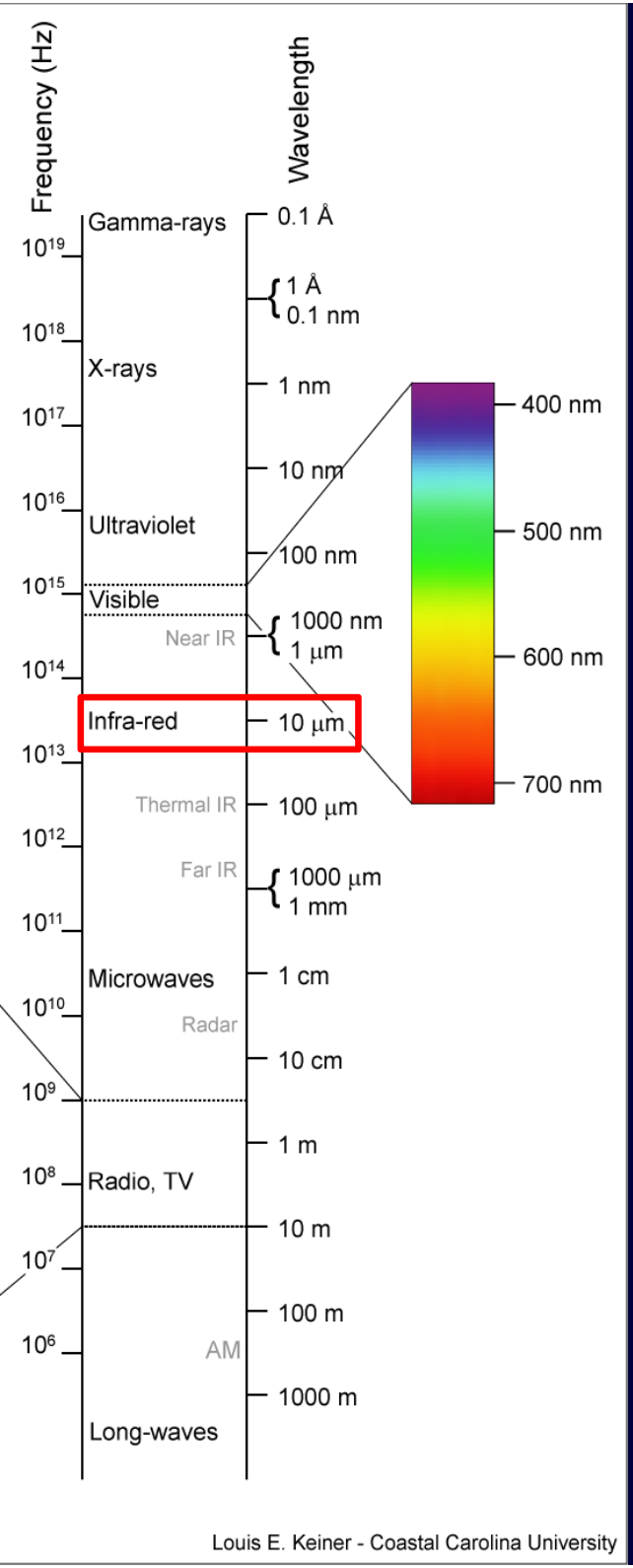
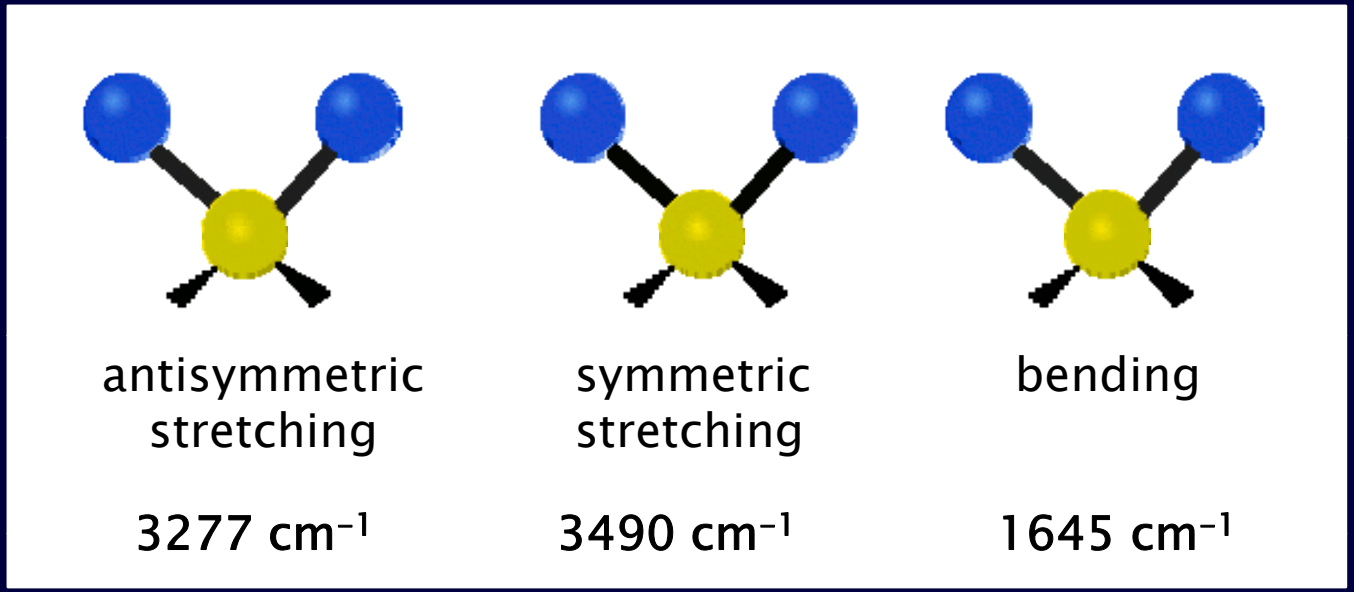
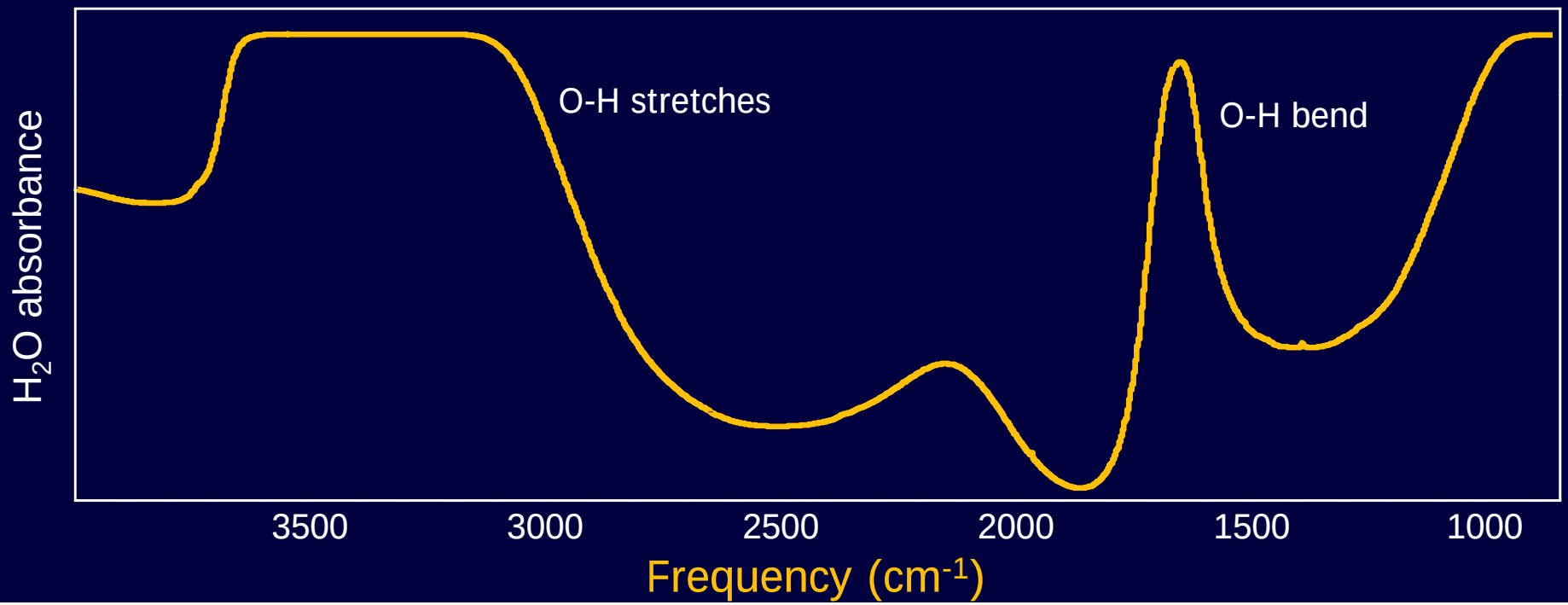


Outline

- Introduction to FTIR spectroscopy
- Infrared microscopes
- Sample preparation and data collection
- Why do we need a synchrotron light source?
- Examples

Vibrating Molecules Absorb Infrared Light

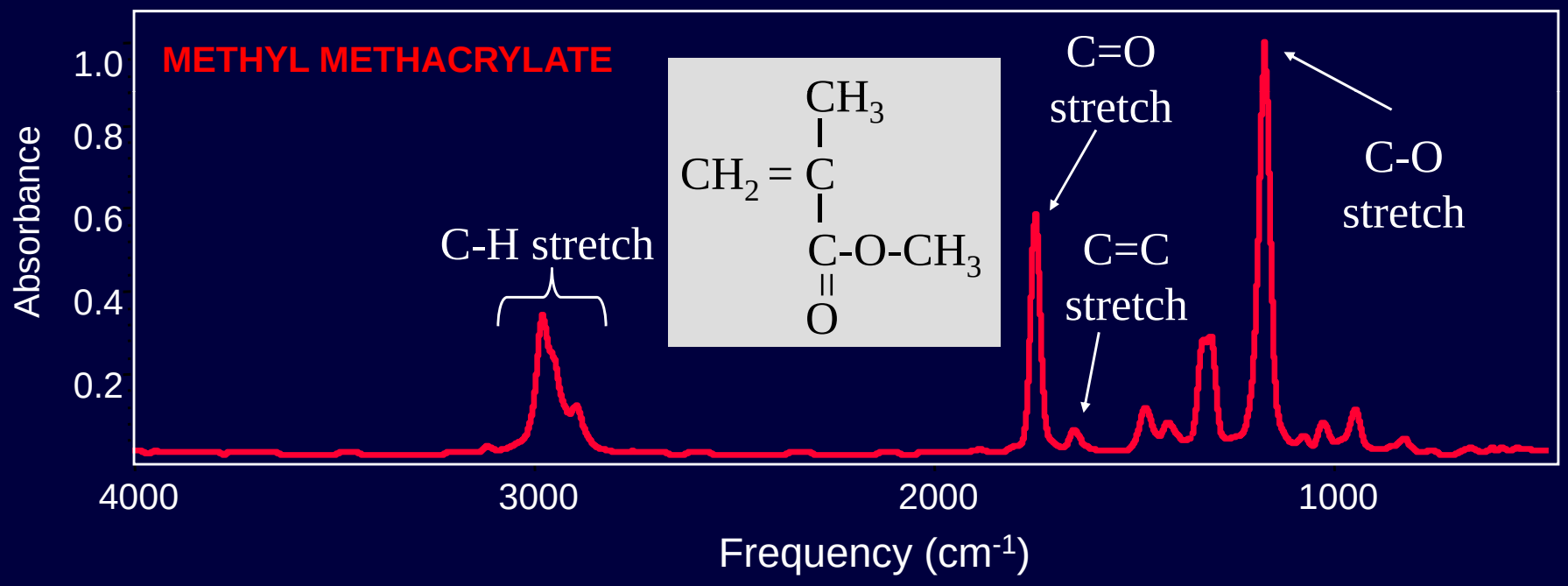




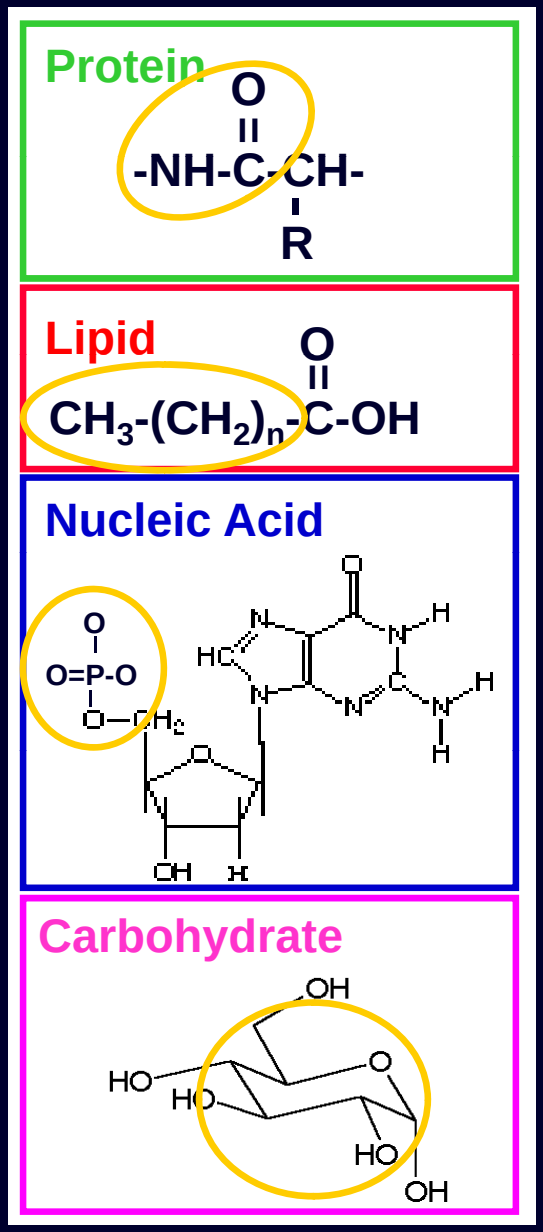
Infrared Microscopy = Molecular Fingerprint

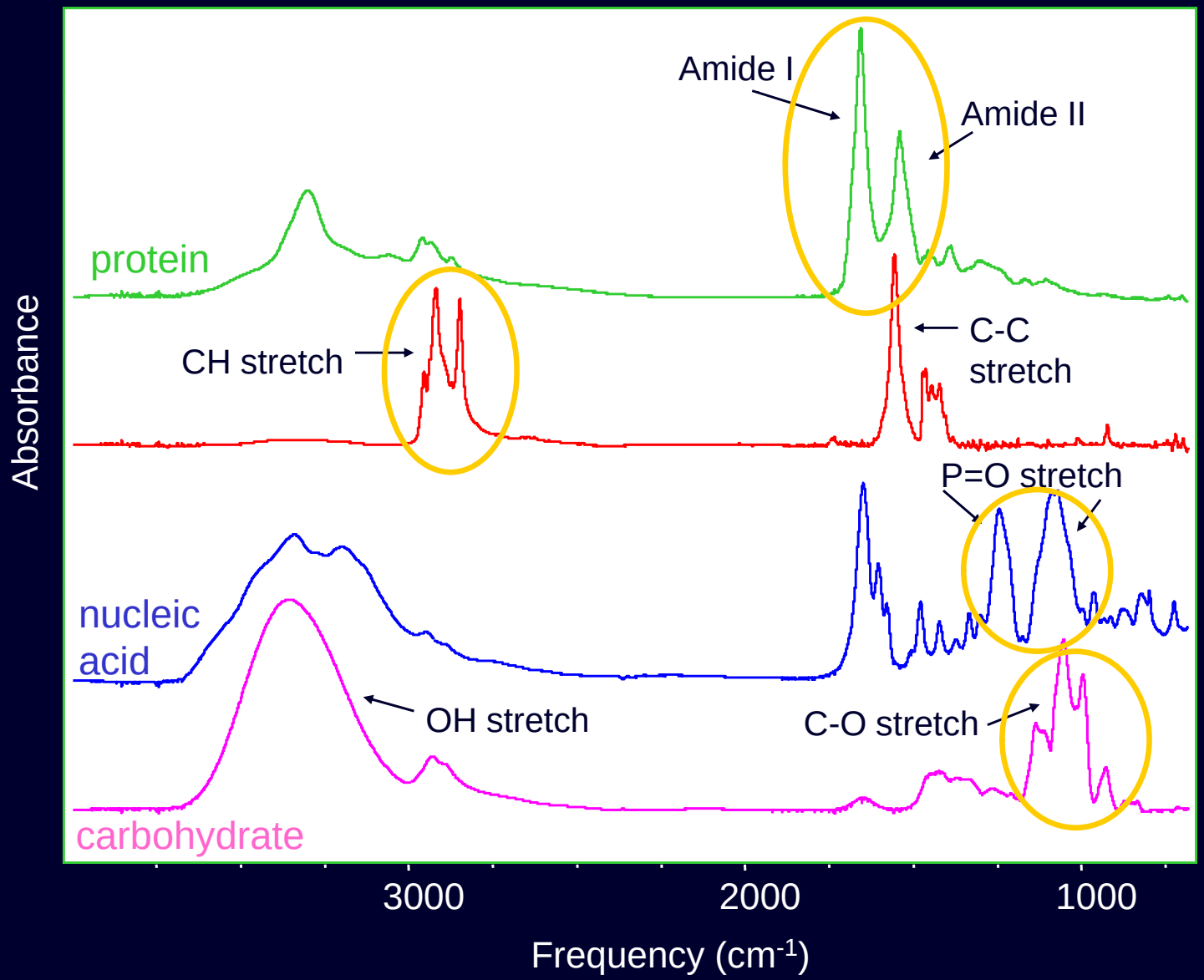
–C–O	1050–1150 cm^{-1}
–C=O	1640–1750 cm^{-1}
–C–Cl	600–800 cm^{-1}
–C–Br	500–600 cm^{-1}

atom type, size, and bond strength influence vibrational frequencies

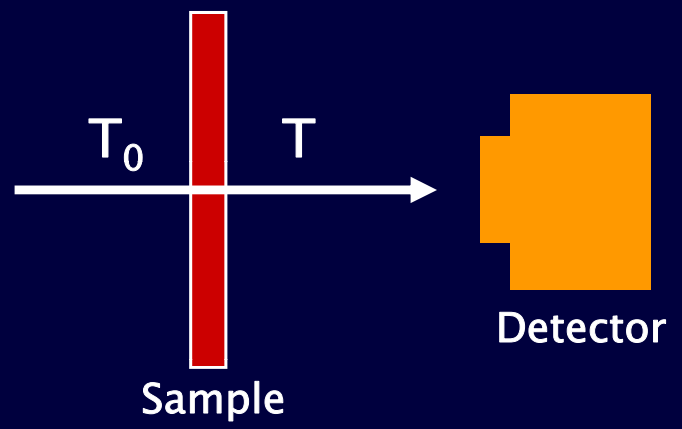


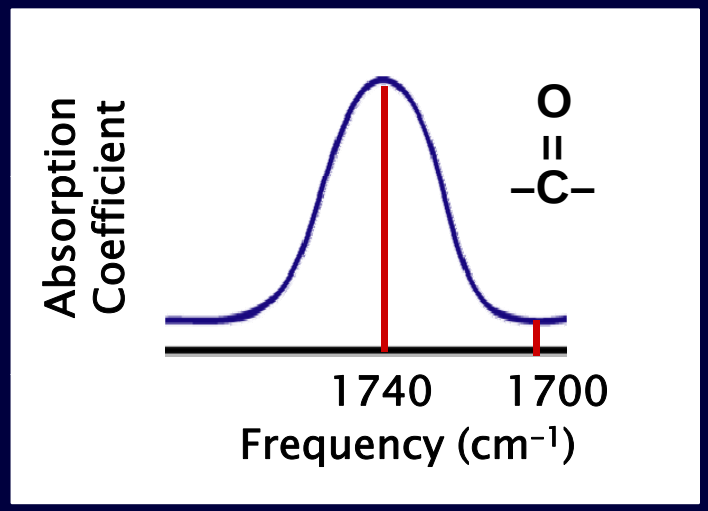
Chemical Features of Biological Components





Quantification and the Beer–Lambert Law





$-\log(T/T_0) =$ Absorbance

Beer–Lambert Law: sample absorbance is directly proportional to concentration

$$A = \varepsilon b c$$

Absorbance
- units: none

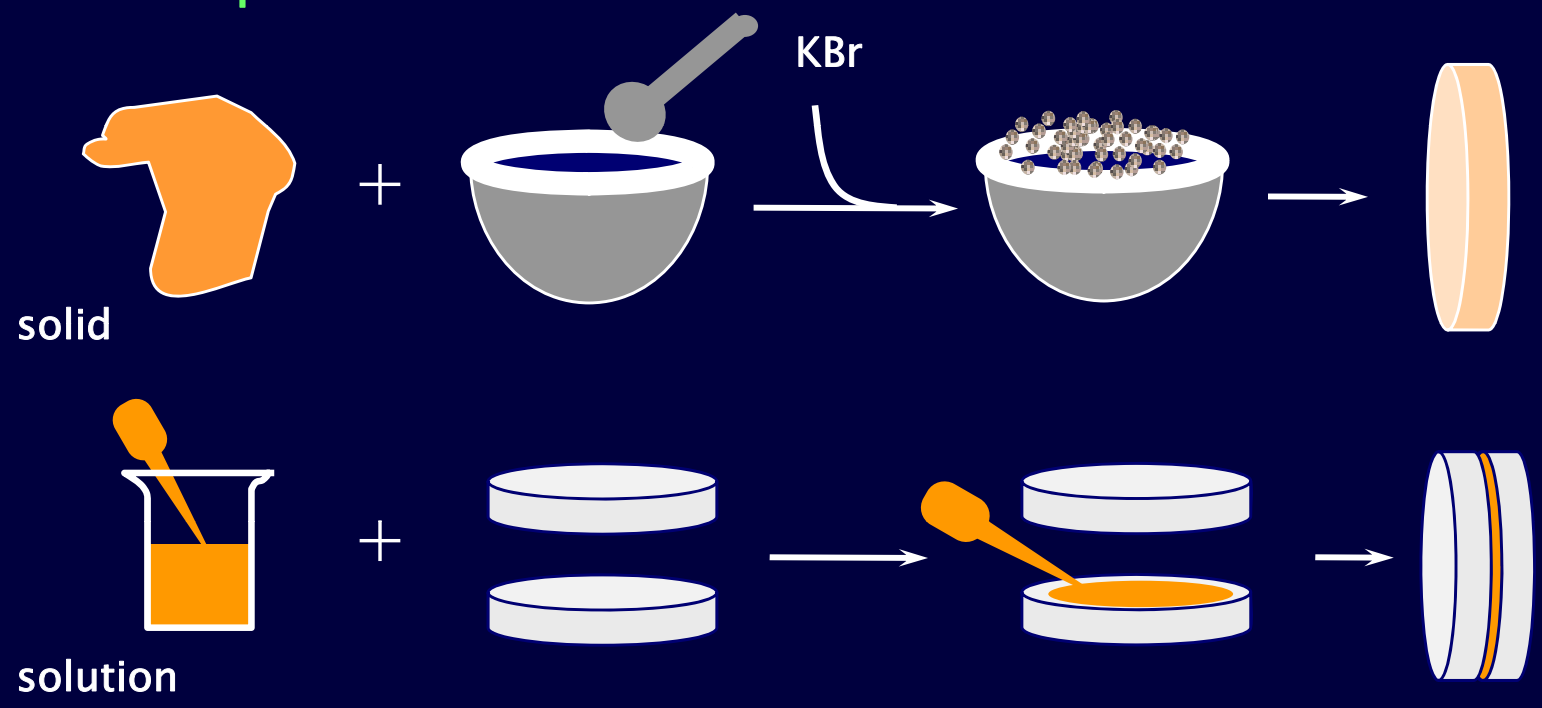
Absorption coefficient
- specific to sample
- specific to frequency
- units: mm^{-1} mM^{-1}

Thickness
- units: mm

Concentration
- units: mM

IR Spectroscopy vs. Micro-Spectroscopy

Bulk samples



Spatial Resolution:
~1-3 mm

Microscopic Heterogeneity

Spatial Resolution:
~ 25 - 30 μm (globar)
~ 3 - 10 μm (synchrotron)

2 Types of Infrared Microscopes

Beamline U2B

Infrared "micro-spectroscopy"

- Dual aperture
- Single point detector
- Raster-scanned sample through beam
- Slow, highest resolution
- All synchrotron microscopes today

Beamline U10A+B

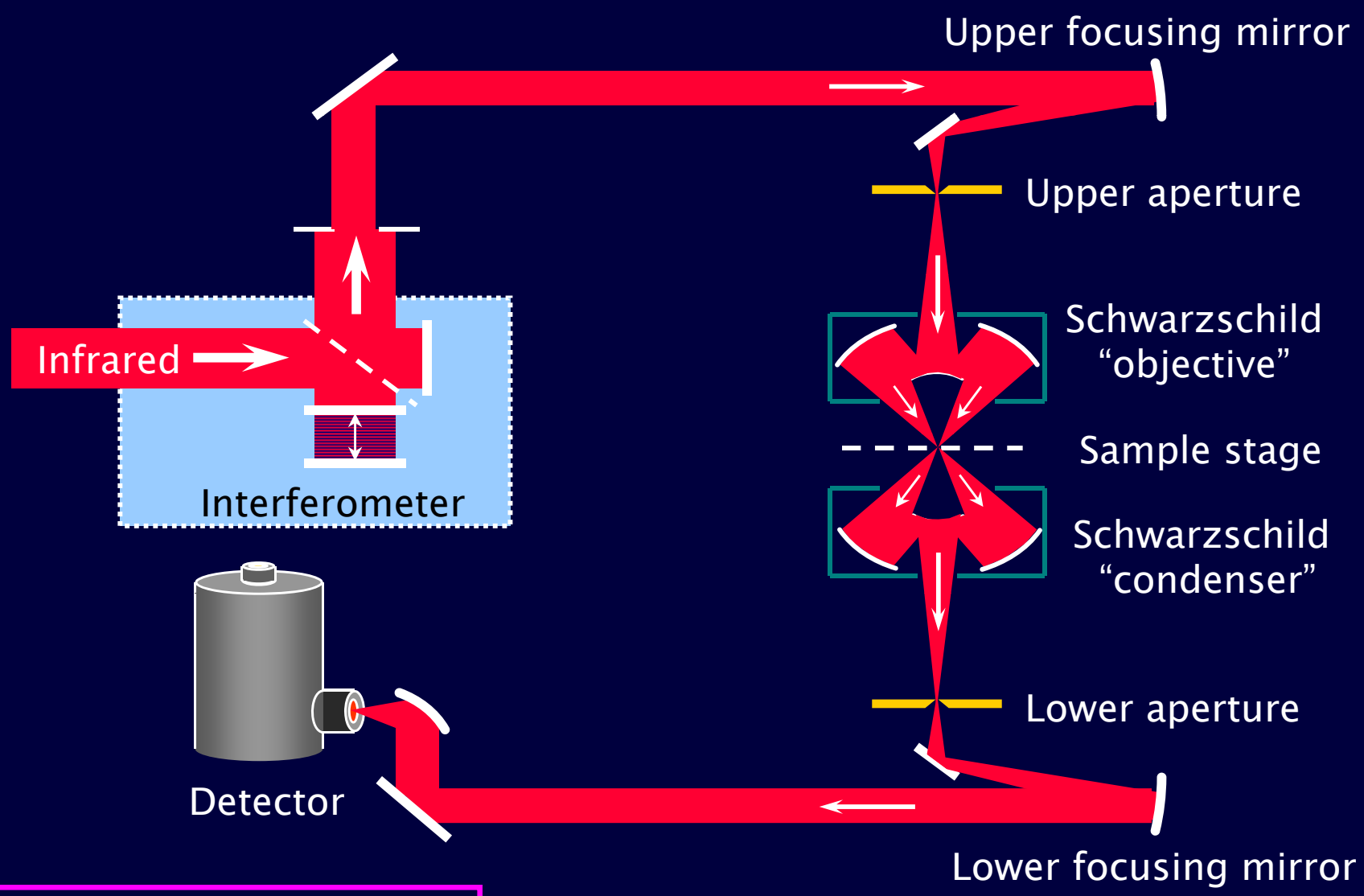
Infrared "imaging"

- IR beam is not apertured
- Array detector (up to 256 x 256 pixels)
- Whole image collected simultaneously
- Very fast, lower resolution
- Not implemented permanently on a synchrotron source yet (many ongoing efforts)

Confocal FTIR Microspectroscopy

Thermo Continuum located at Beamline U2B

Confocal FTIR Microspectroscopy

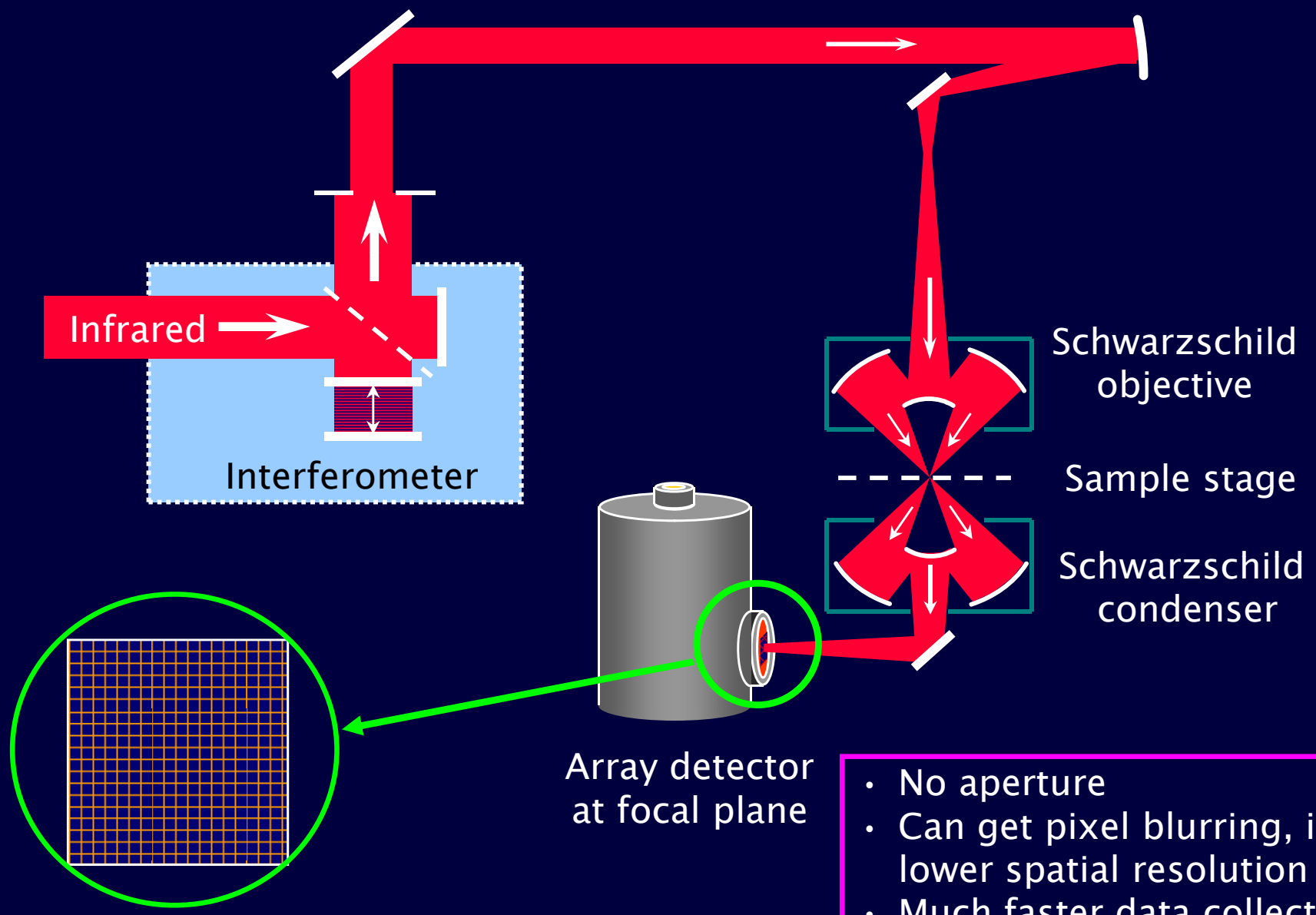


- Dual aperture
- Maximum spatial resolution
- Single-pixel data collection

FTIR Imaging with a Focal Plane Array Detector

Bruker Hyperion 3000 located at Beamline U10A+B

FTIR Imaging with a Focal Plane Array Detector



- No aperture
- Can get pixel blurring, i.e. lower spatial resolution
- Much faster data collection

Advantages of a Synchrotron IR Source

BRIGHTNESS (1000x)

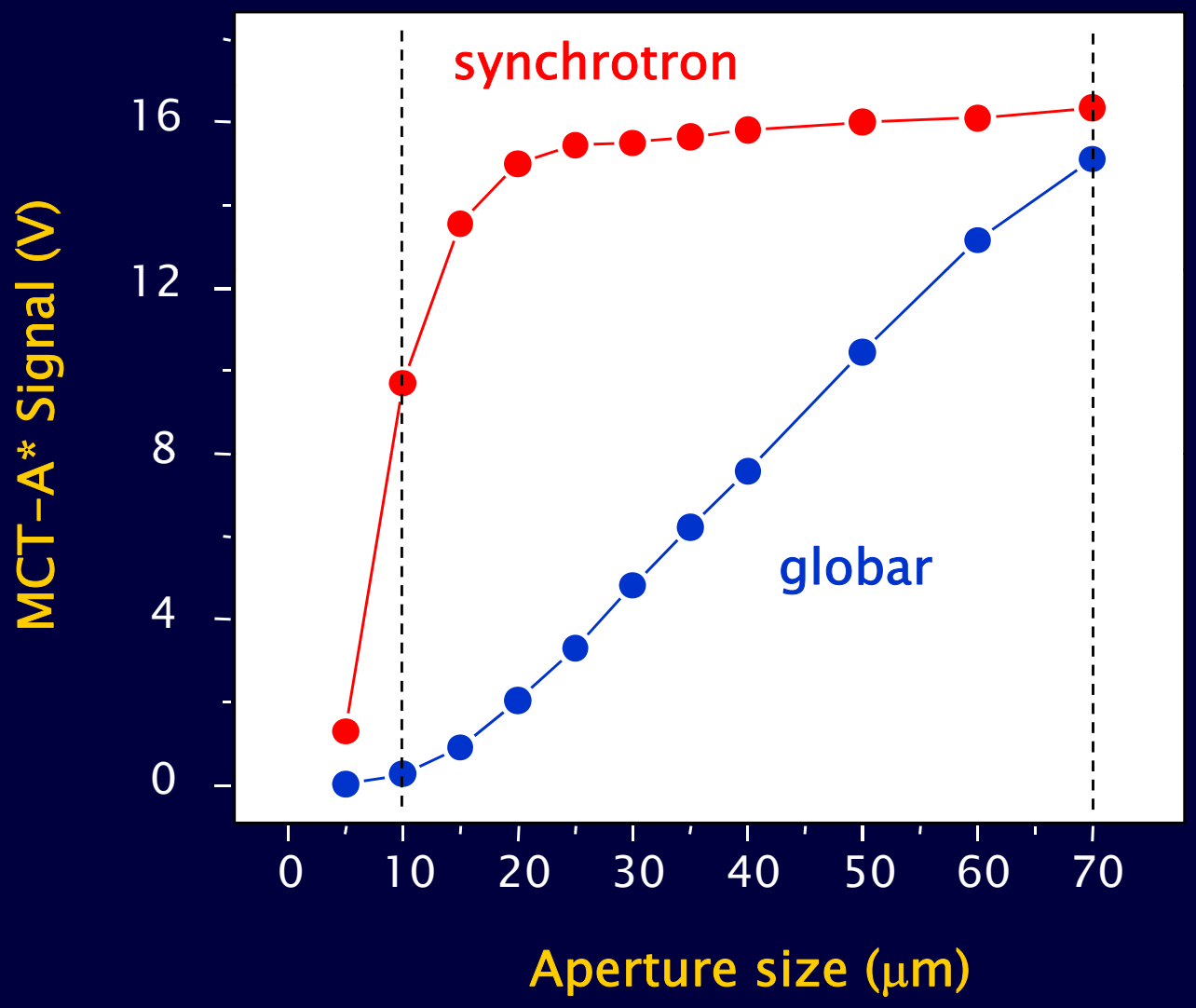
- Higher Spatial Resolution
- Better Signal-to-Noise
- Faster Data Collection

+

BROADBAND

- Spectroscopy (near-, mid-, far-IR)

1000x Brighter

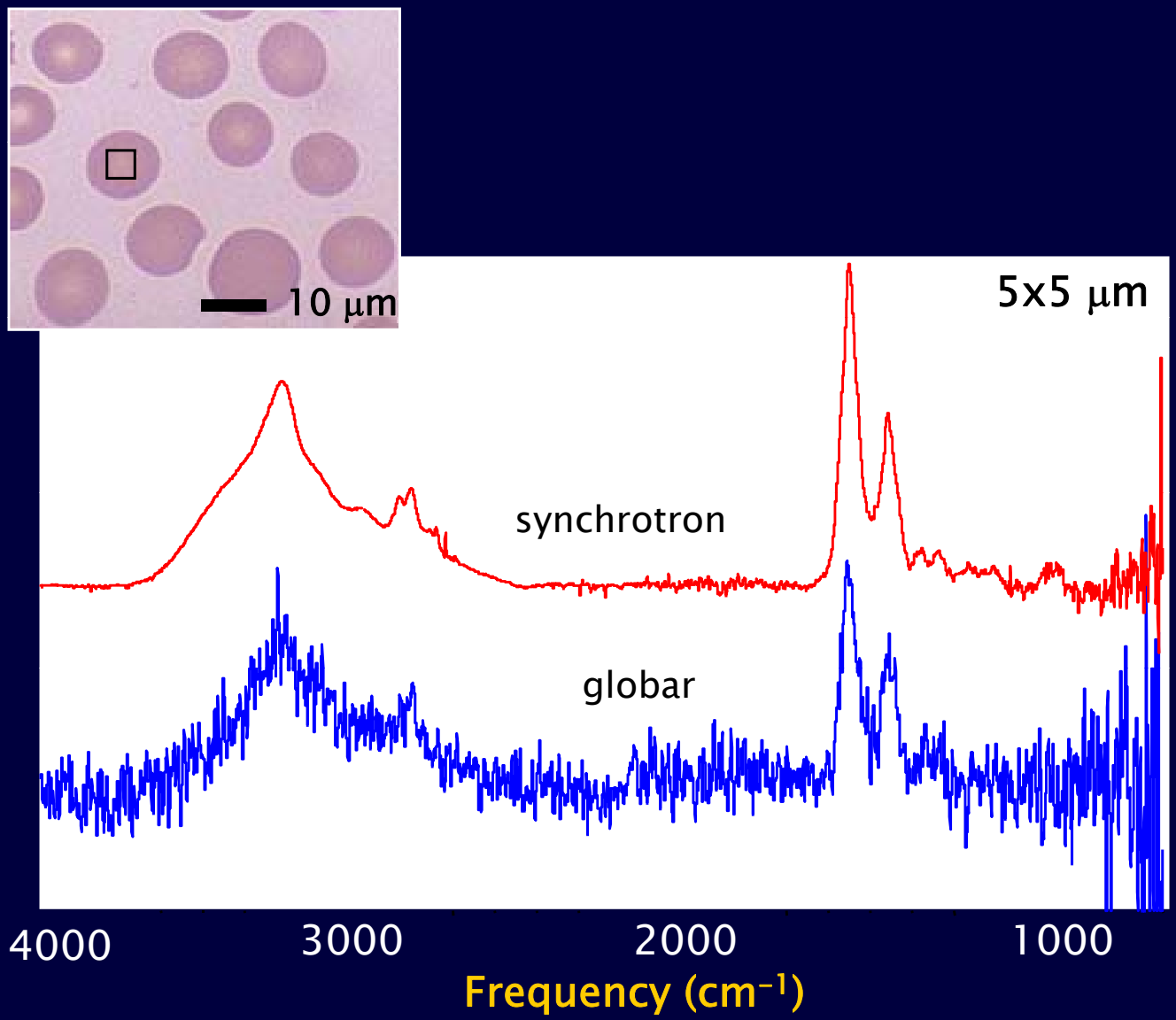


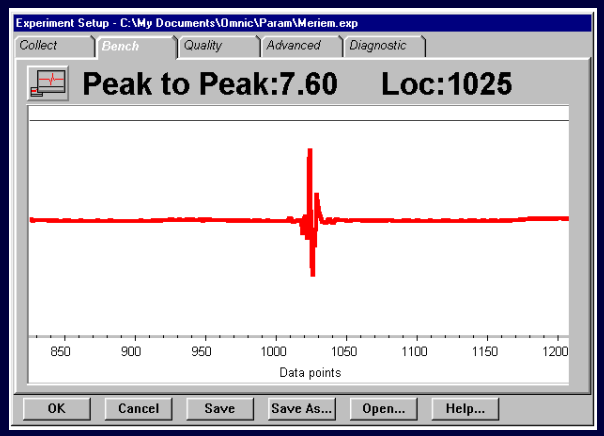
1. 1000x brighter that conventional thermal (globar) source
2. Confocal (dual aperture) arrangement of microscope

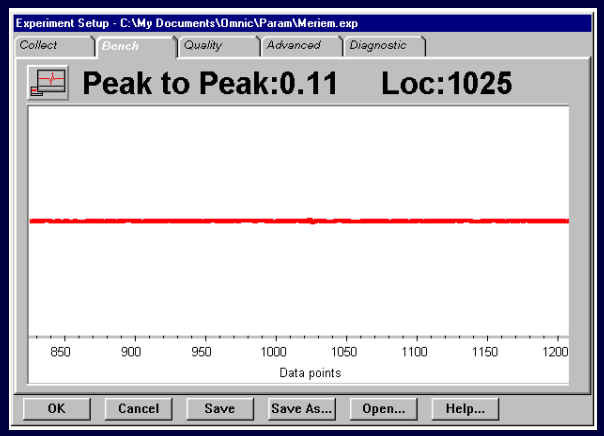
= Unsurpassed Spatial Resolution for FTIRM

L.M. Miller &, R.J. Smith. *Vibr. Spectr.*, 38, 237-40 *(2005).*

1000x Brighter: Improved S/N

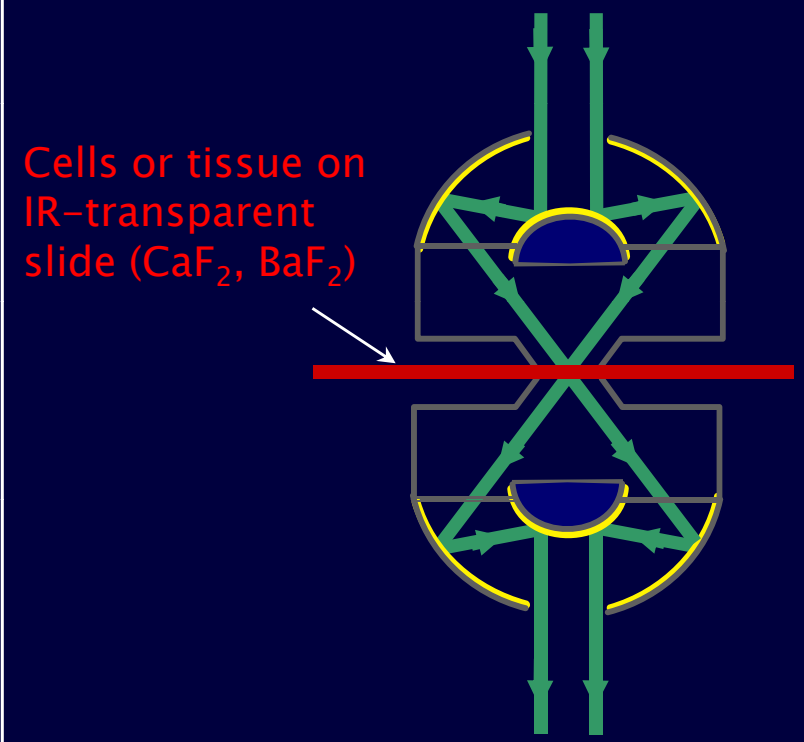




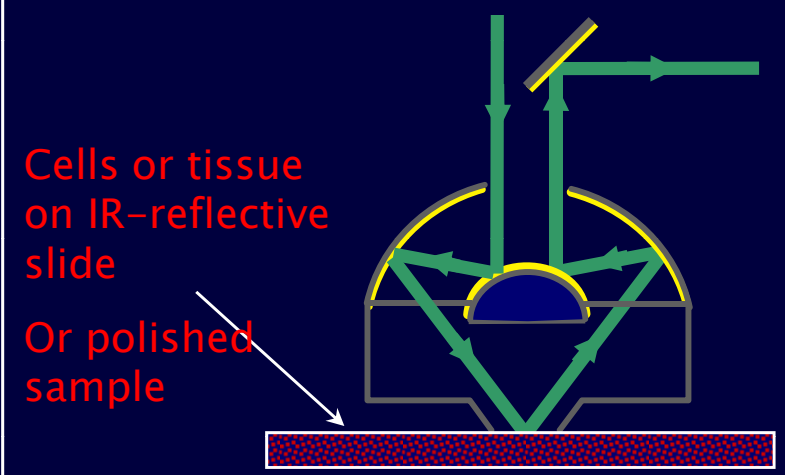


Modes of Data Collection

Transmission

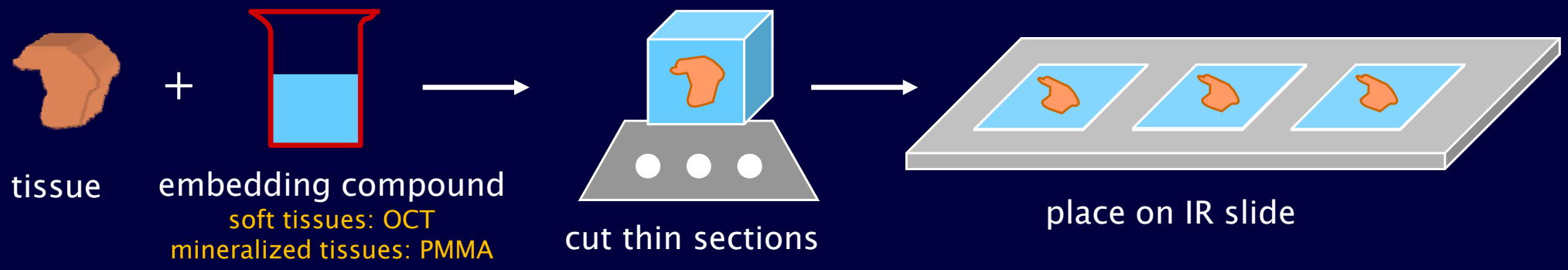


Reflection

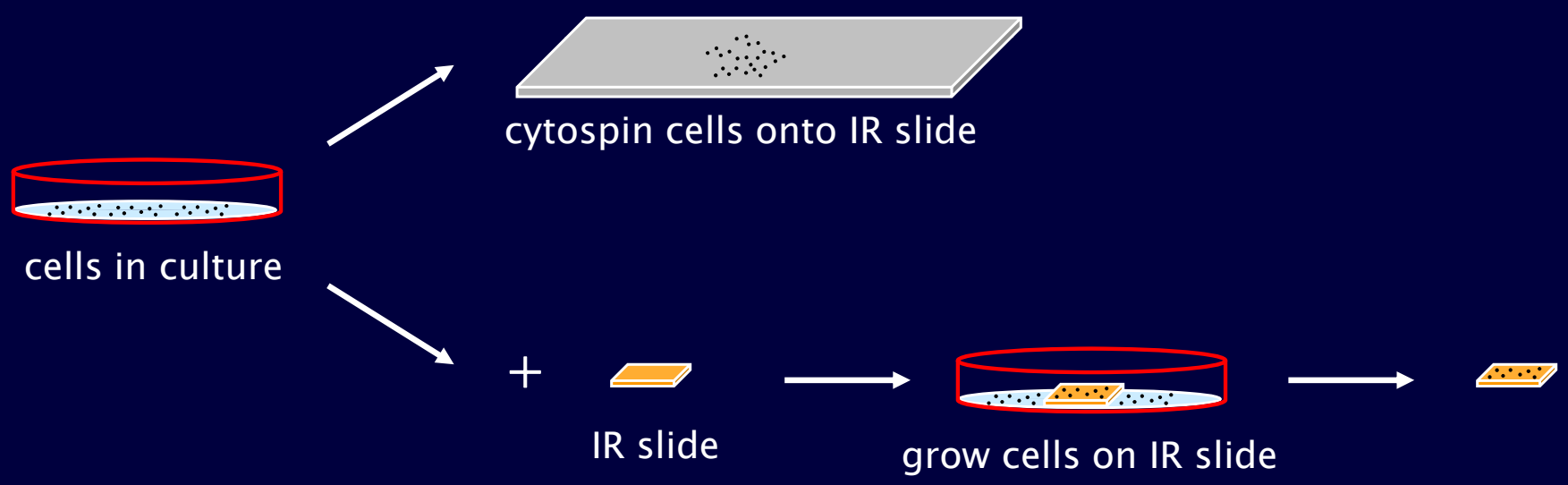


Sample Preparation

Tissues



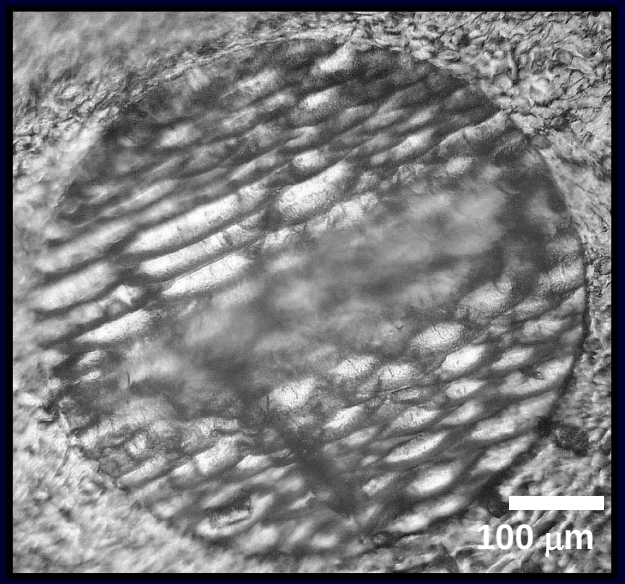
Cells

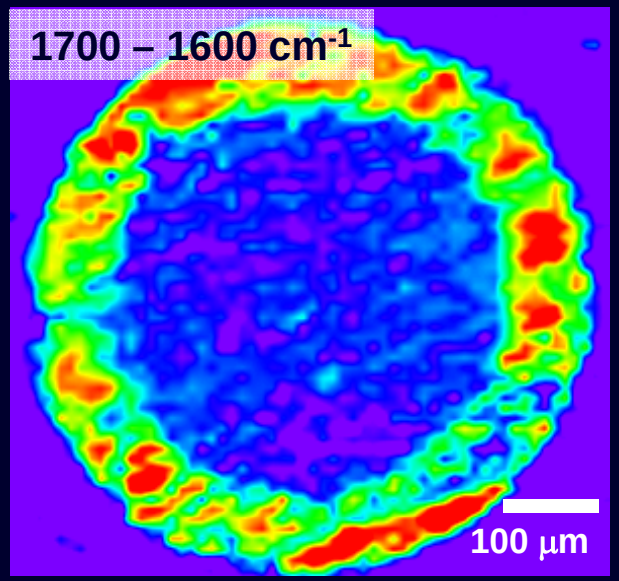


Examples

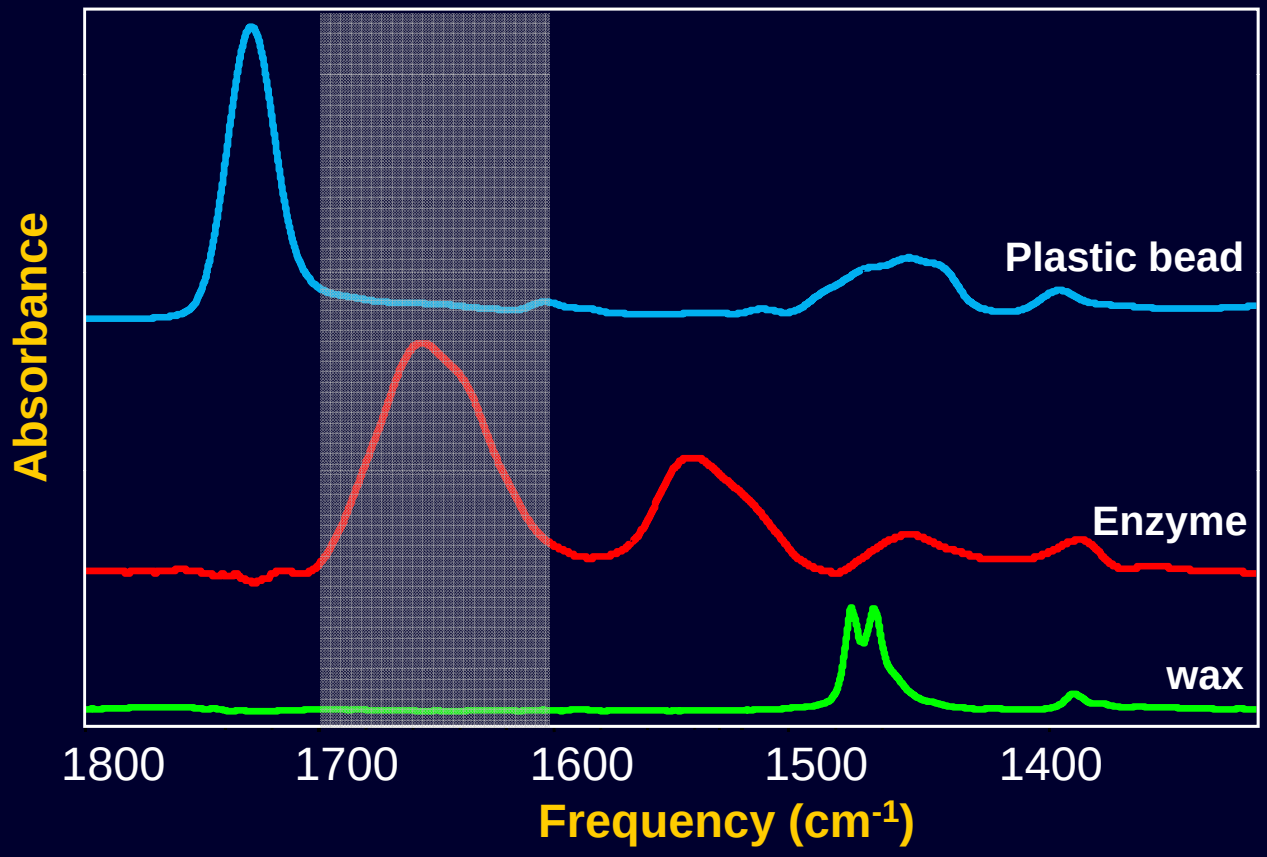
- Polymers
- Fingerprints
- Hair
- Plants
- Soil
- Bones, Fossils
- Biological Cells

Mid-Infrared Imaging





plastic bead soaked in enzyme, embedded in wax, and cut to thin section



Y. Mei, et al., *Biomacromolecules*, 4 (1): 70-74 (2003).

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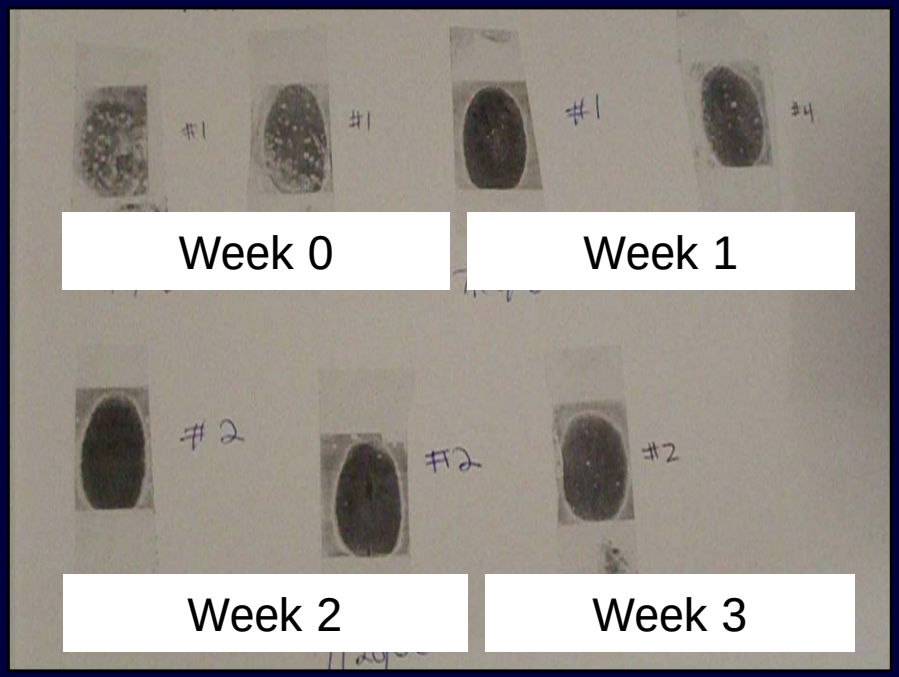
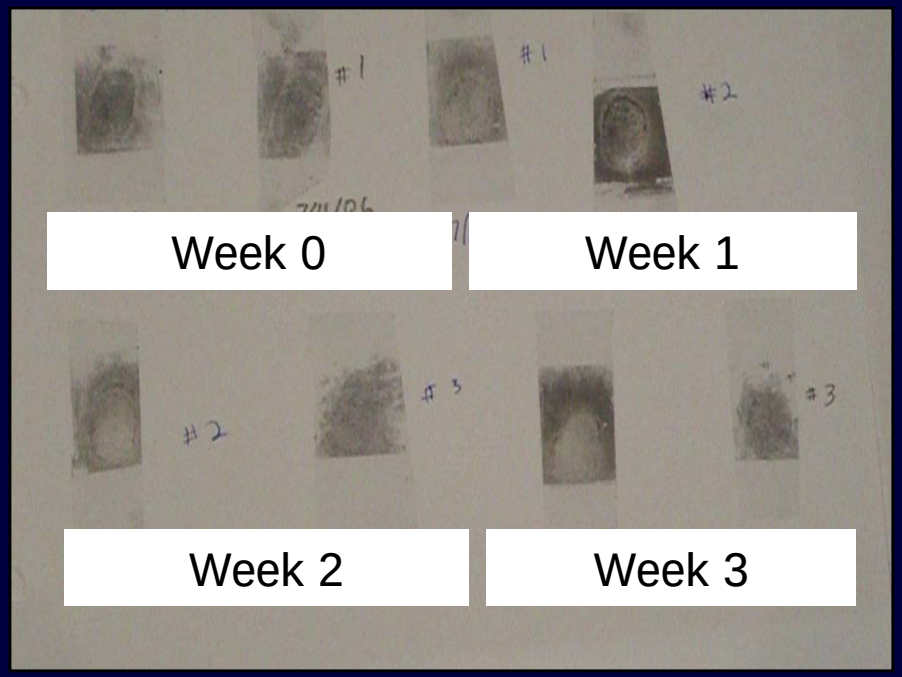
Children's vs. Adults' Fingerprints

Fathers

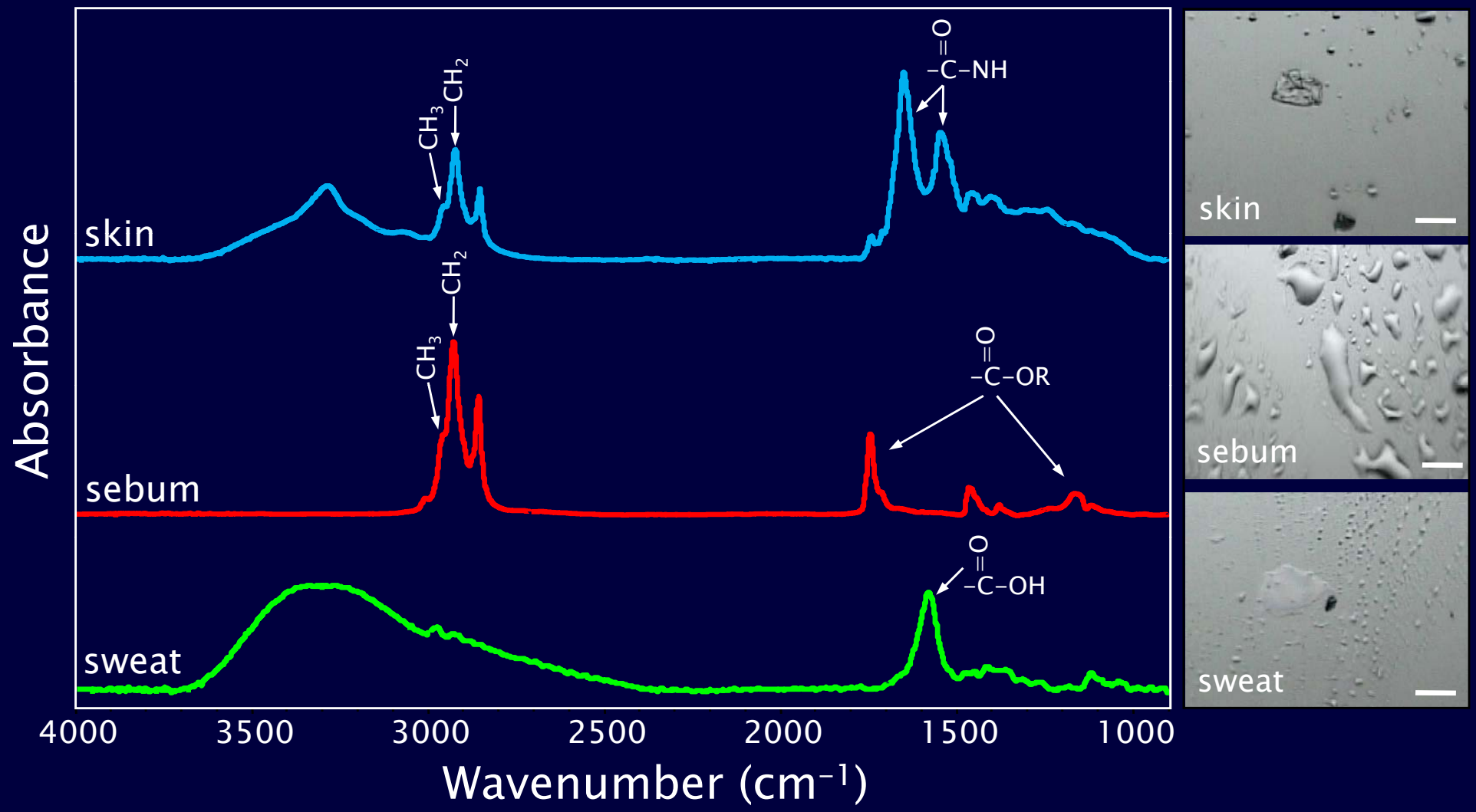


Sons



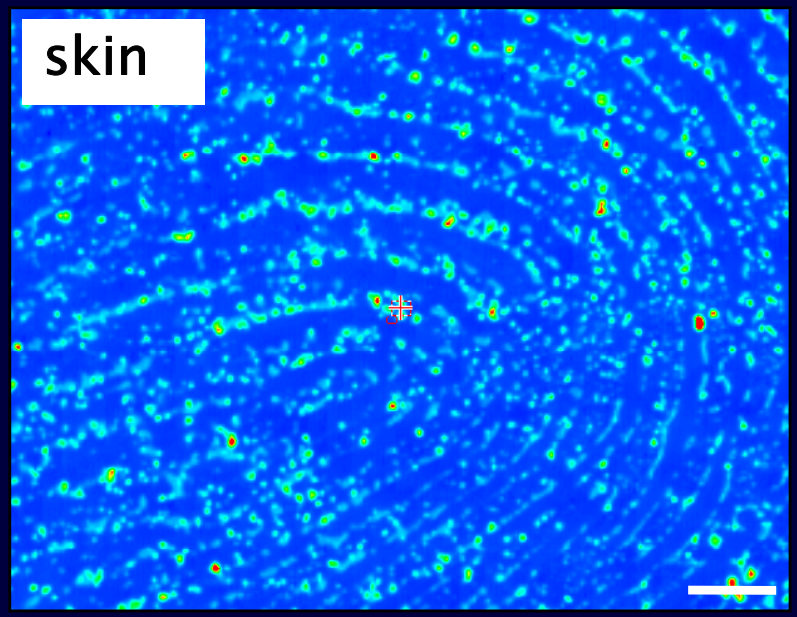
Why do children's fingerprints disappear faster than adults?

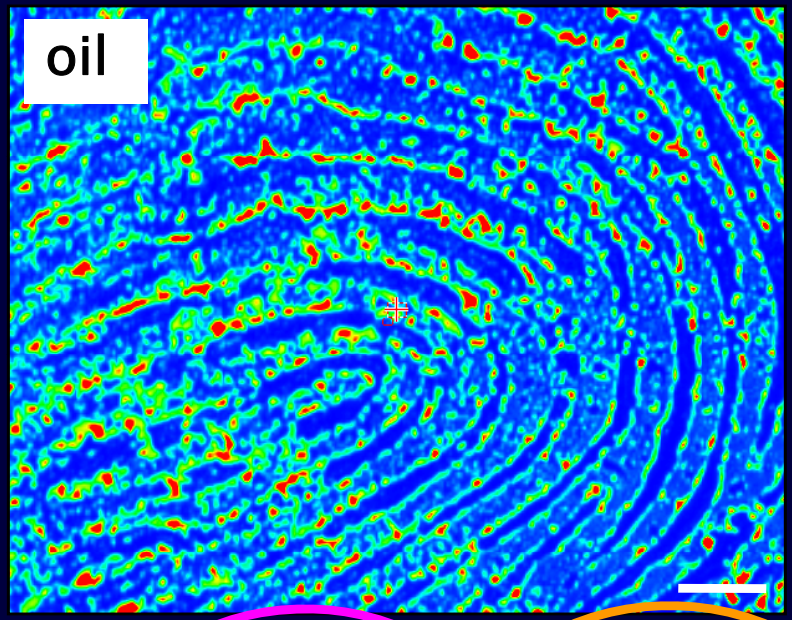
Children's vs. Adults' Fingerprints

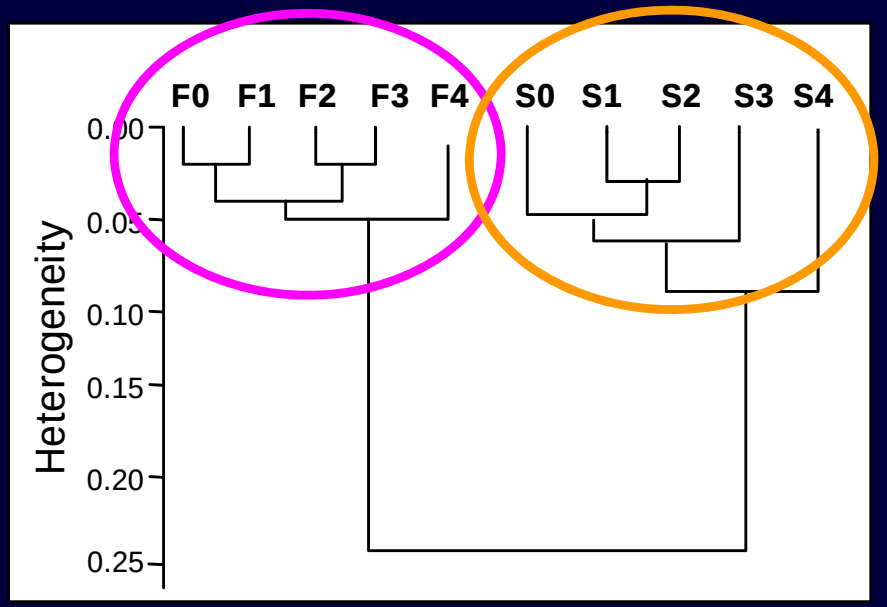


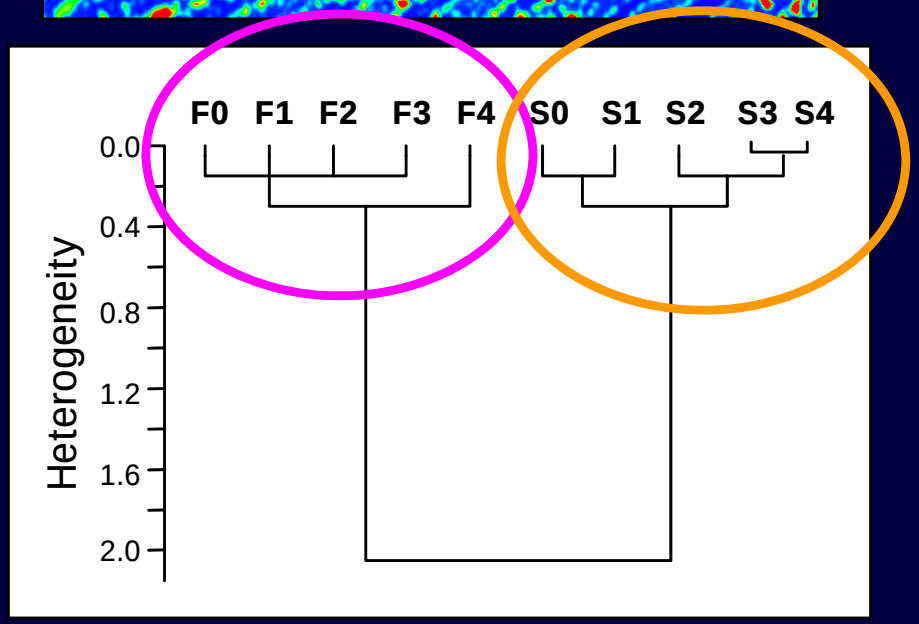
Antoine, et al., *J. Forensic Science*, 2010

FTIR Microscopy of Fingerprints









Fathers' prints cluster separately from the sons' prints

Examples

- Polymers
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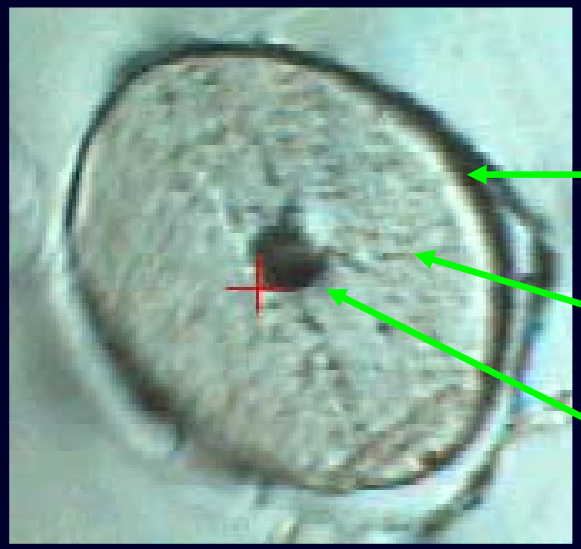
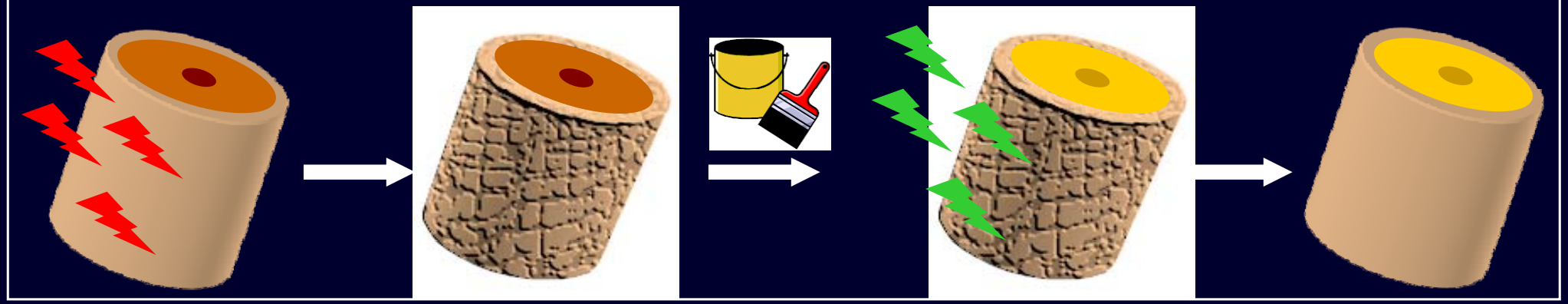
- Hair
- Plants
- Soil
- Bones, Fossils
- Biological Cells

Chemical Imaging of Hair

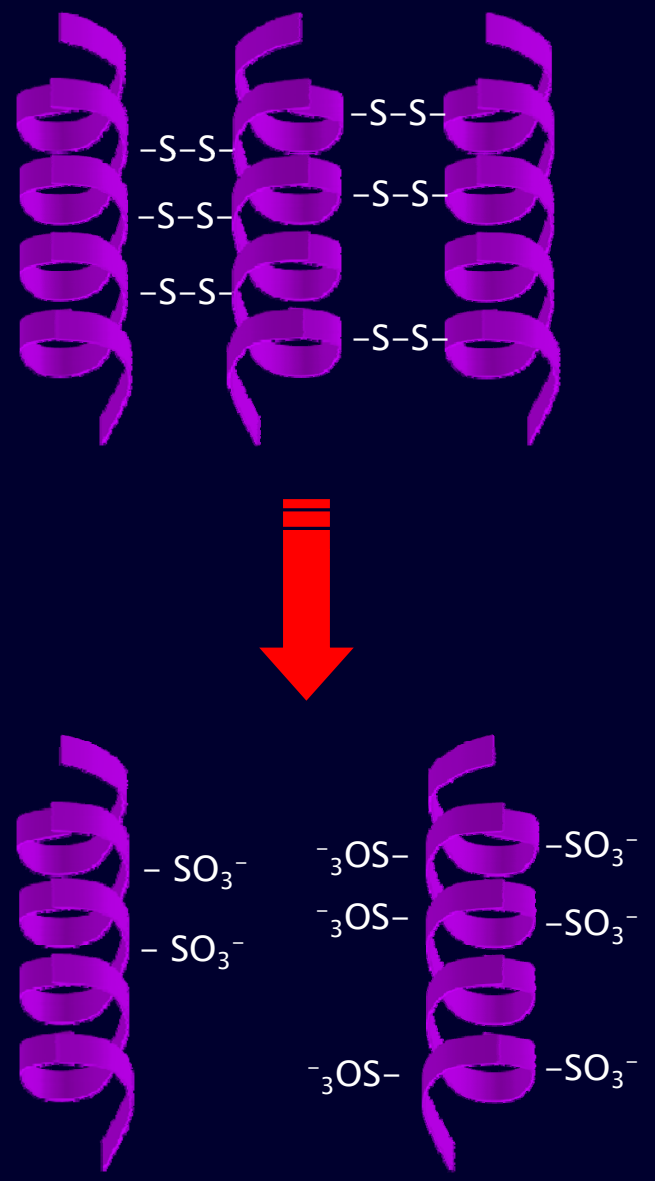
Jean Louis Bantignes and Paul Dumas, *LURE*



Chemical treatments of hair: Bleaching, Coloring

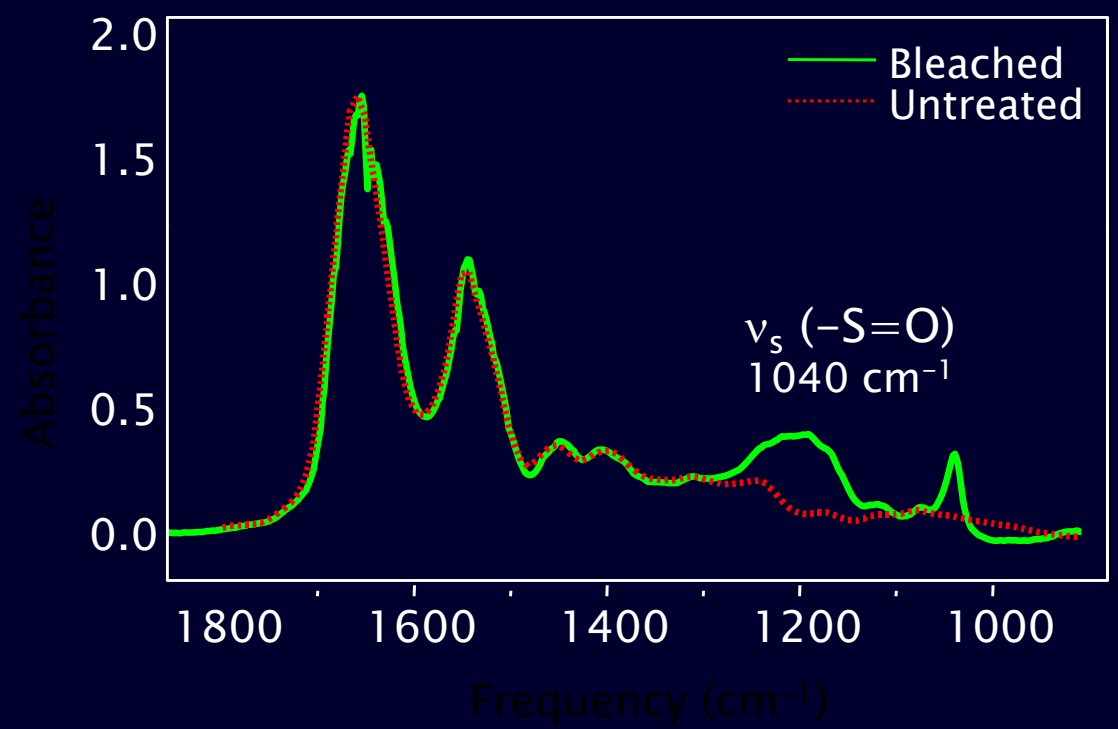


Bleaching Causes Keratin Oxidation

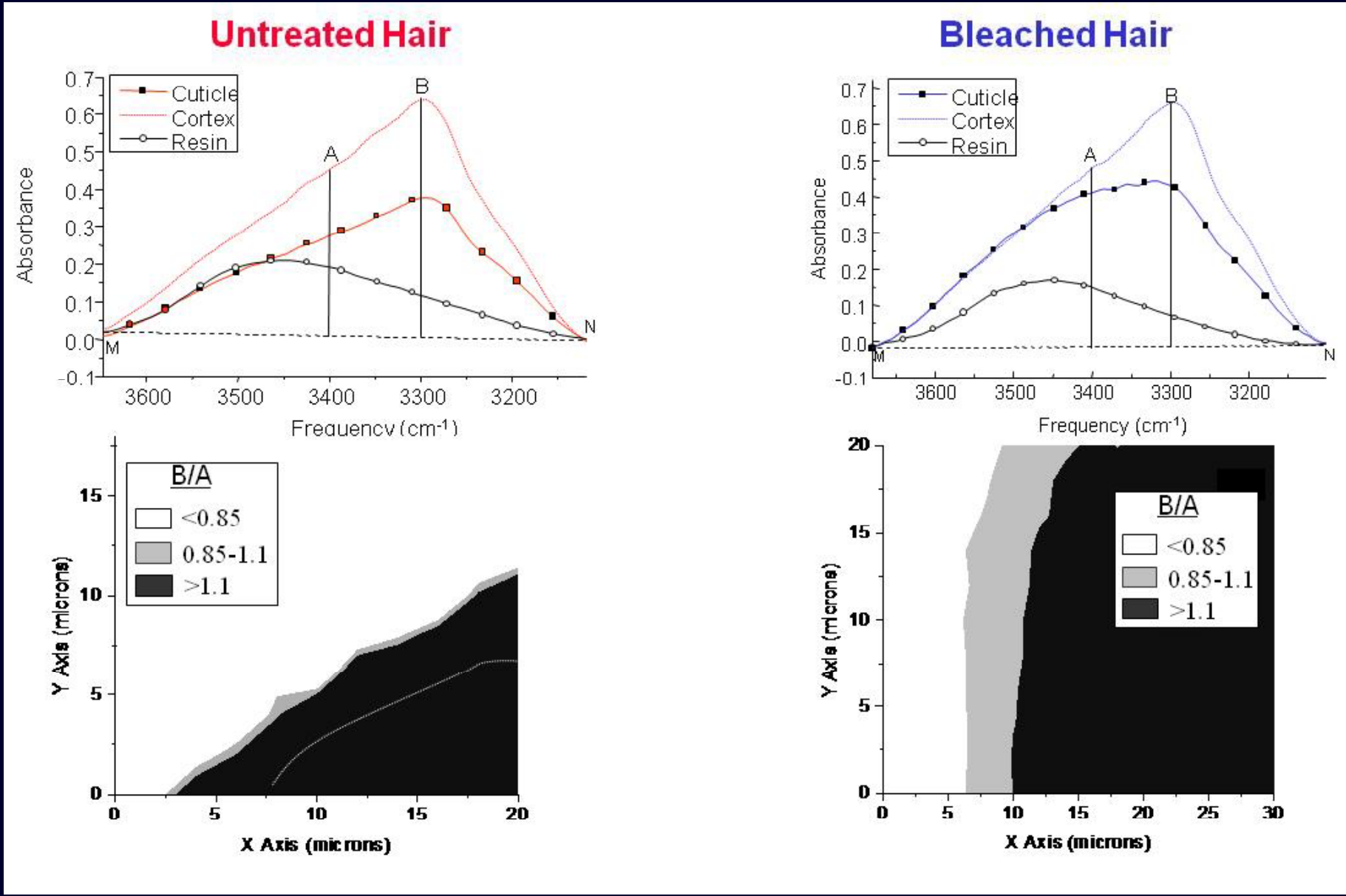


- keratin helices are cross-linked with disulfide bonds
- bleaching disrupts cross-linking as cysteine is oxidized

Cortex before and after bleaching



Bleaching Causes Hydration of the Cuticle



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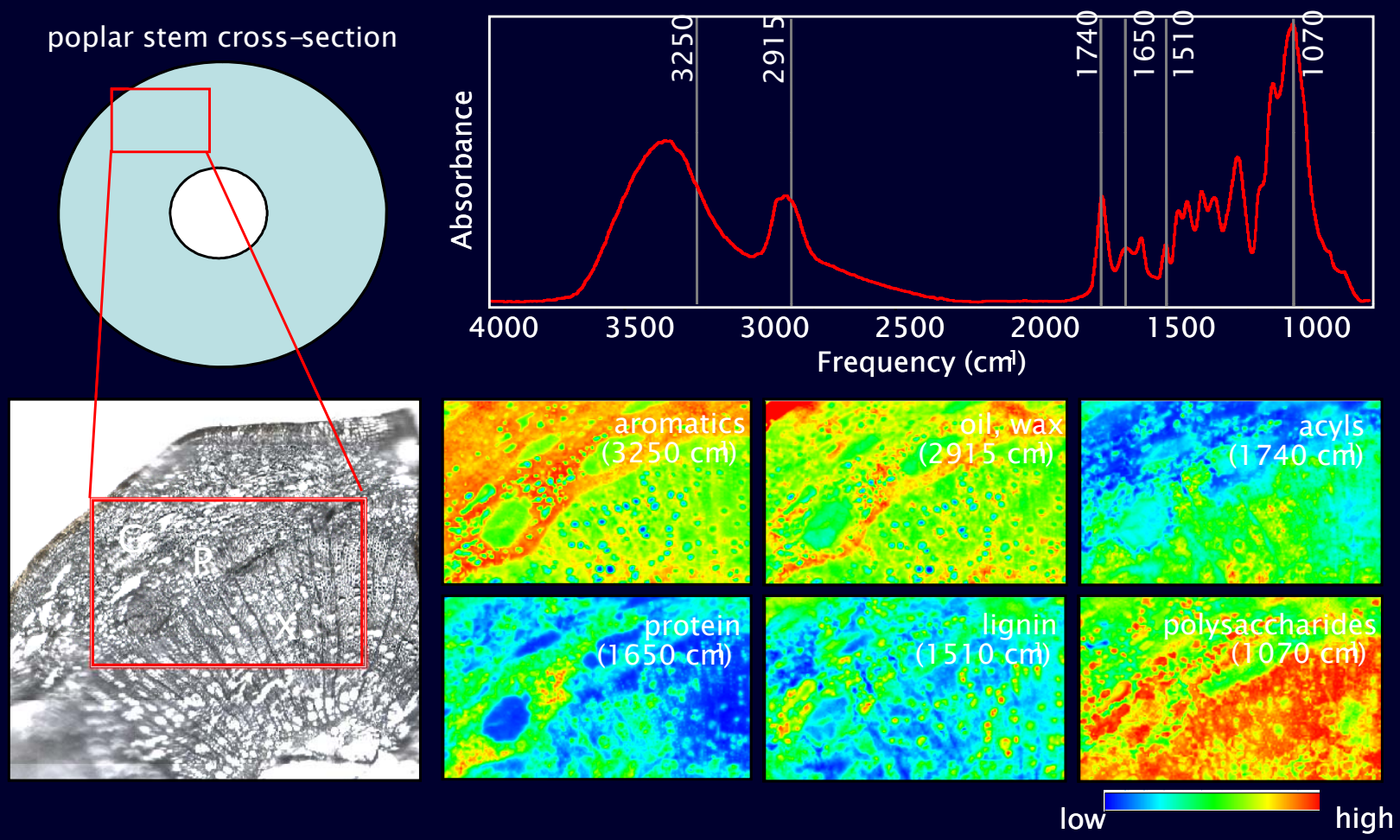
IR Imaging of Plant Composition



- In order to convert cellulose to ethanol, degradation of plant cell walls is necessary
- To make efficient biofuels, cell wall degradation needs to be improved
- Understanding plant composition can improve biofuel efficiency

J. Gou, X.H. Yu, S. Park, L.M. Miller, C.J. Liu, *Planta*, 2009.

Examples

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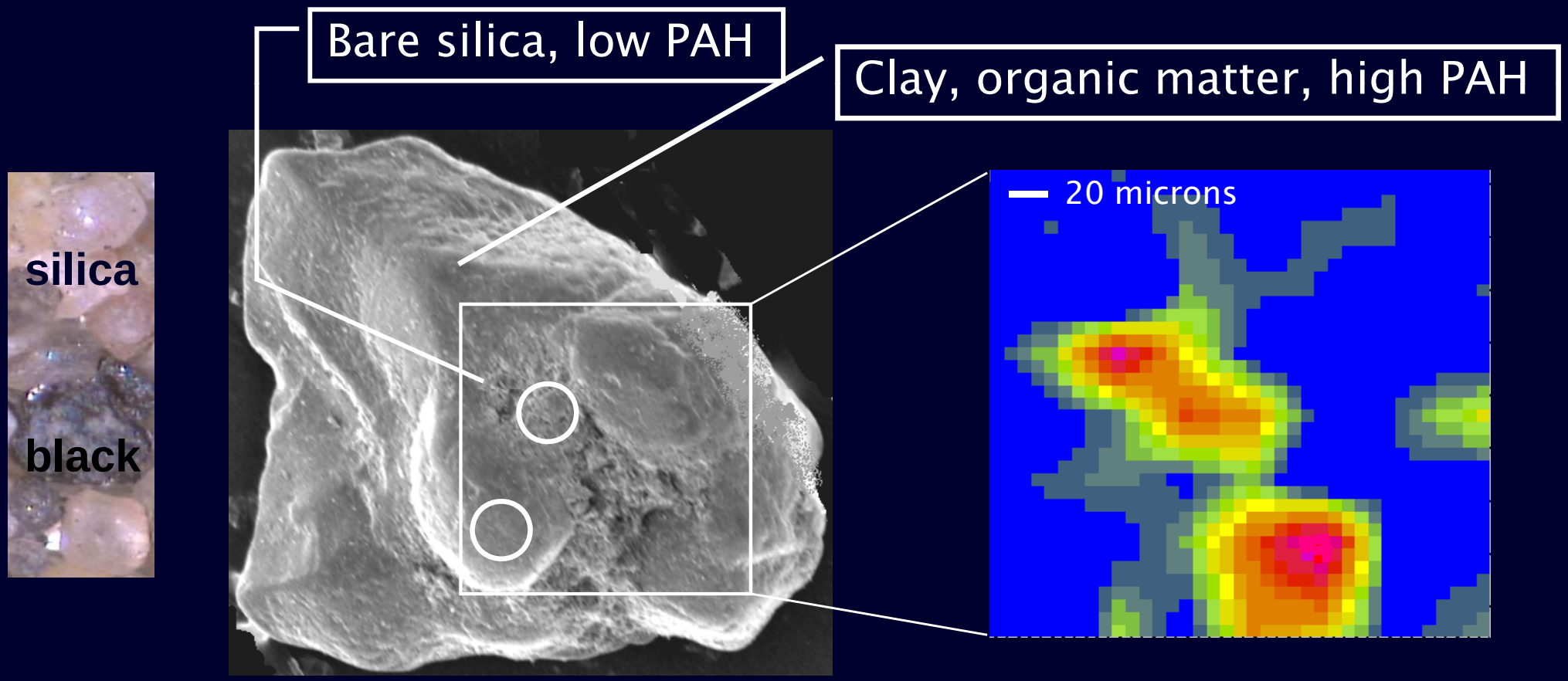
Polycyclic Aromatic Hydrocarbons (PAHs) in Sediments

U. Ghosh and R.G. Luthy, *Carnegie Mellon University*

How to stabilize PAHs in dredged soil?

Association of PAHs with Silica Particles



PAHs present on silica particles were found associated with organic carbon locations. These regions containing PAHs are colonized by bacteria during biodegradation studies.

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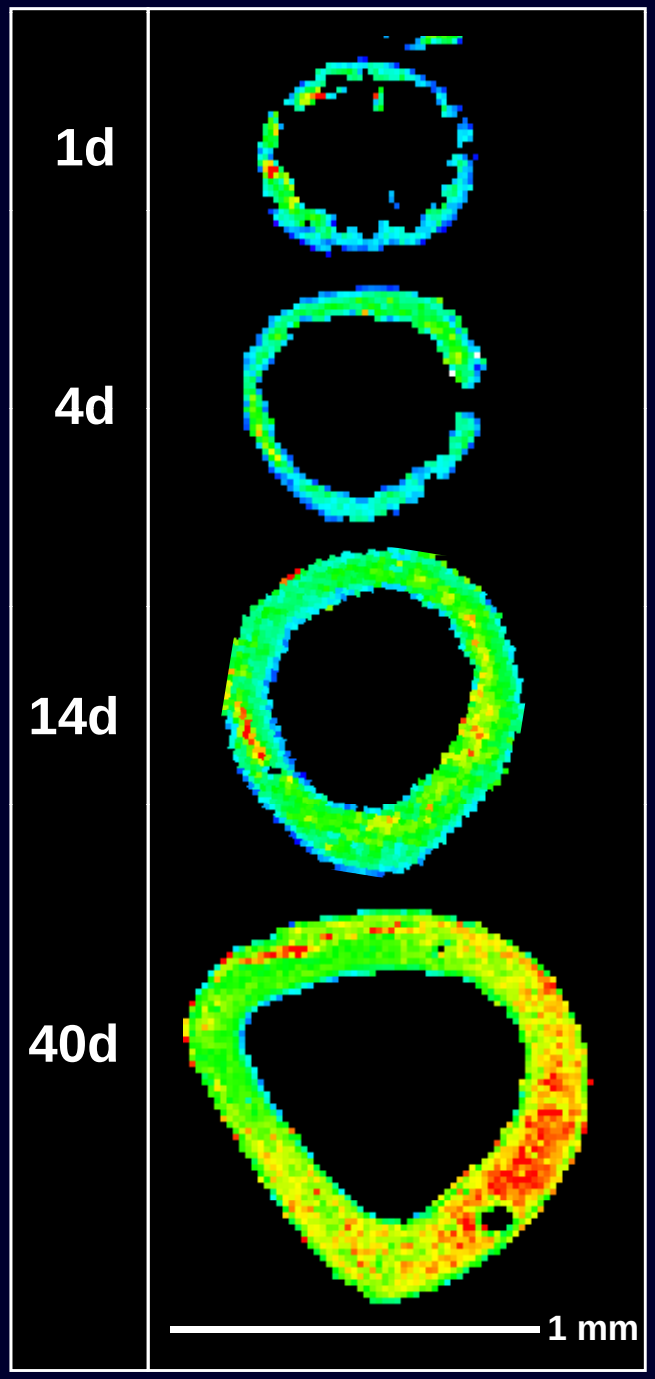
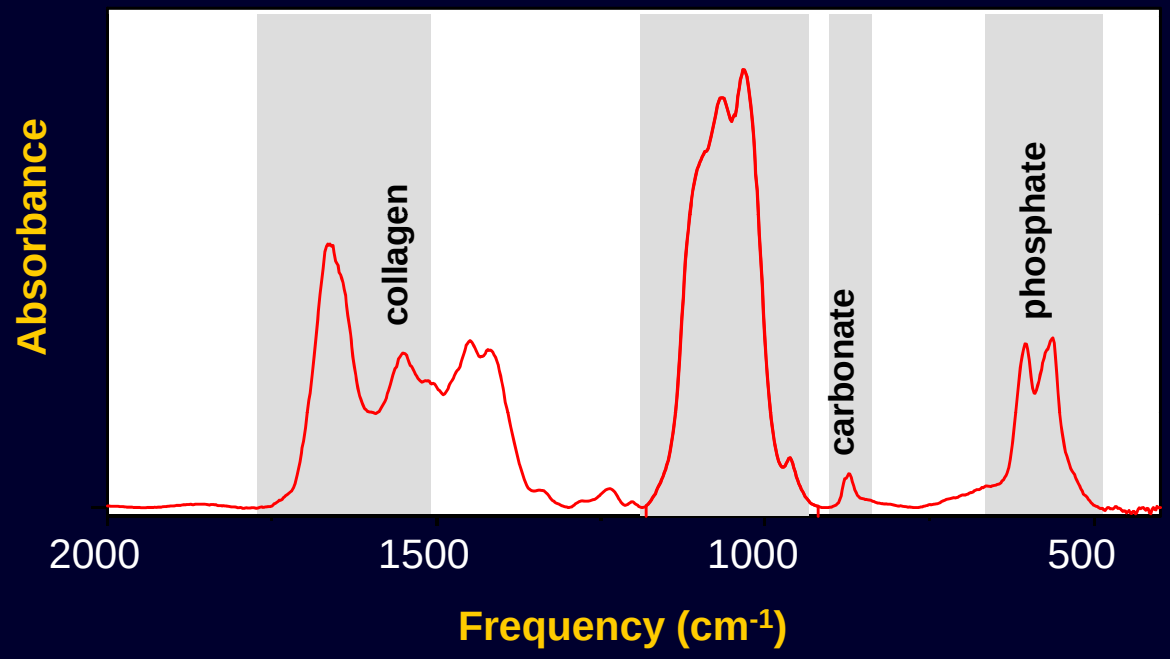
Bone Composition in Osteoporosis



Alvin Acerbo, Lisa Miller, *BNL*
Stefan Judex, *Stony Brook University*



- Bone is composed of protein (collagen) and mineral (hydroxyapatite)
- Bone mineralization increases with age.
- Bone quantity is decreased in osteoporosis, but is the mineralization affected?

L. Miller, et al., *JBMR*, 2007.

Examples

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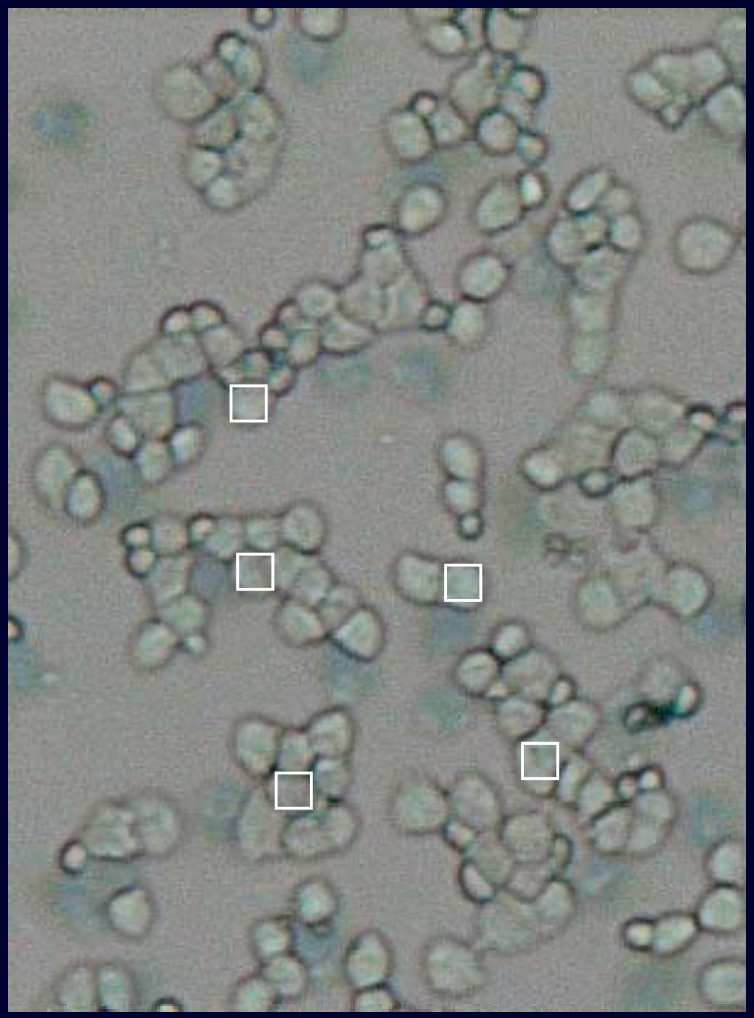
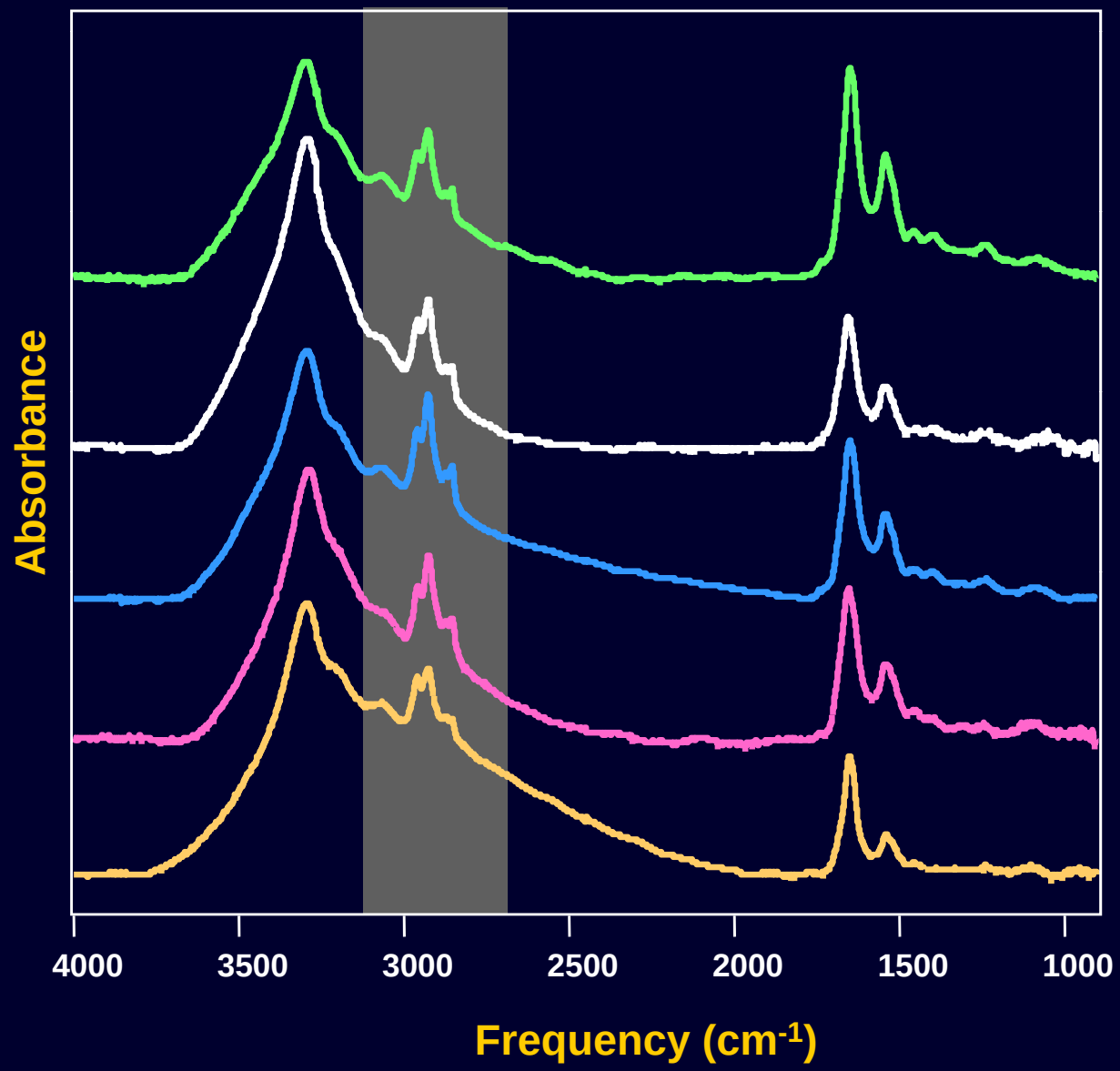
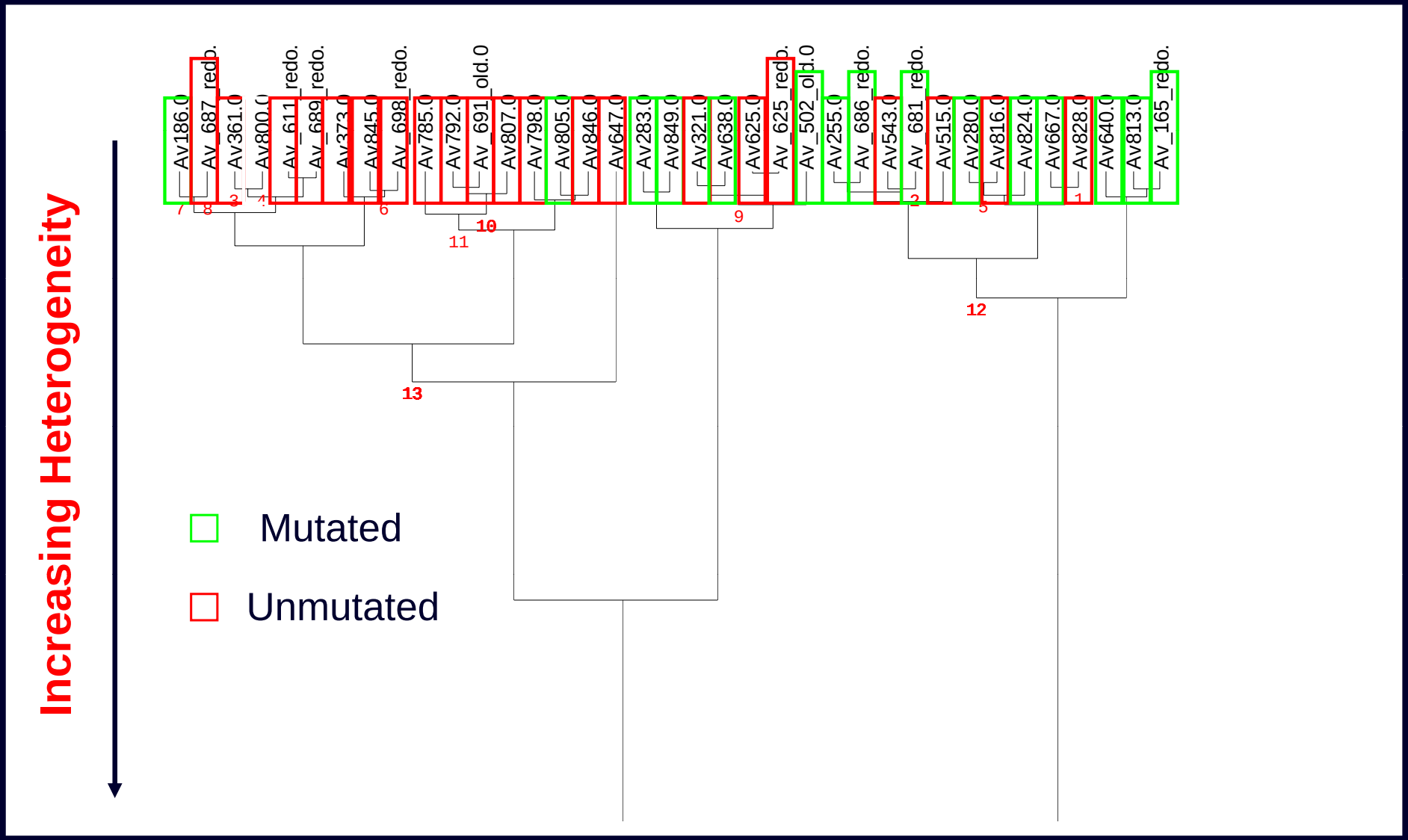
Differentiation of Cell Types

Nick Chiorazzi, *North Shore – LIJ Hospital*





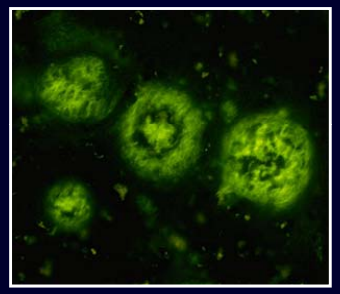
Cluster Analysis of Lipid Region



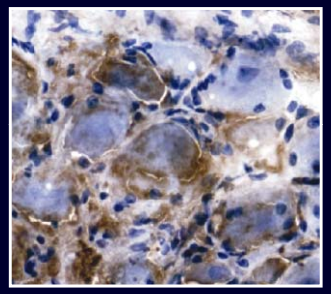
Infrared imaging of lipid region shows separate clusters for progressive vs. benign forms of disease

Other Examples

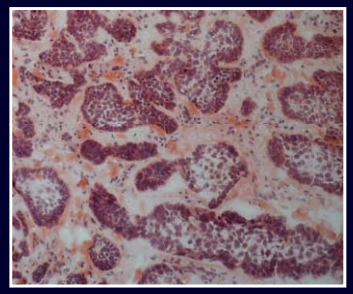
Bone disease



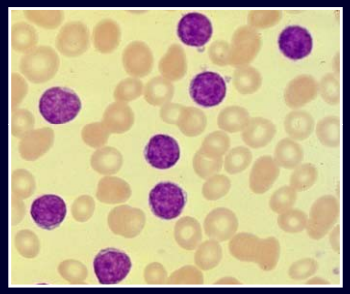
Alzheimer's disease



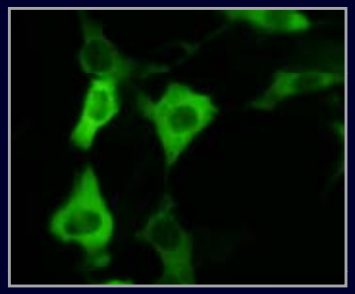
Prion diseases



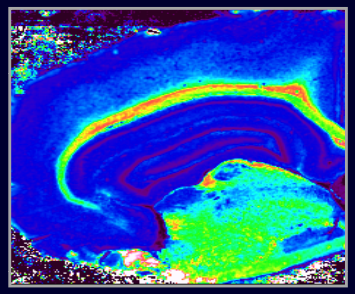
Cancer



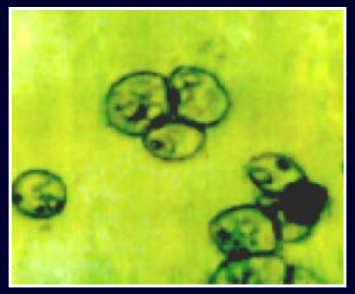
Leukemia



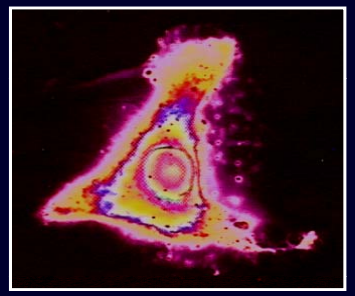
Lou Gehrig's disease



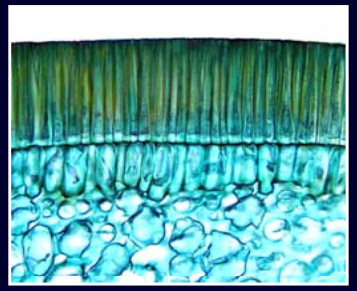
Radiation therapy



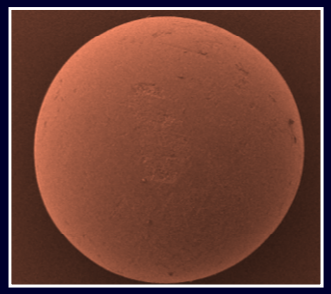
Apoptosis



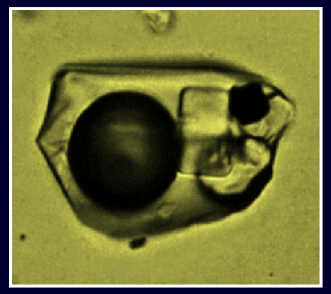
Melanoma



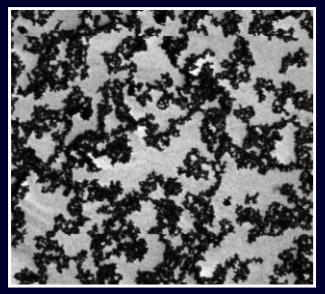
Biofuels



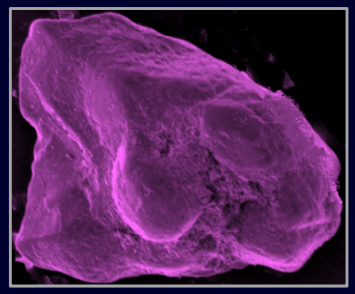
Biopolymerization



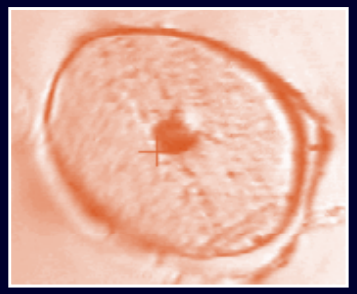
Fluid inclusions



Oil extraction



Remediation



Cosmetics