Frontiers of infrared spectroscopy: Infrared Beamlines and Applications in Biology, Geology and Environmental Remediation

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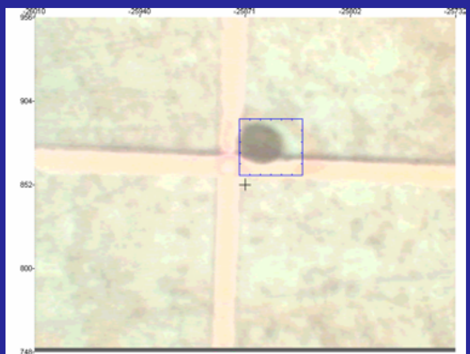
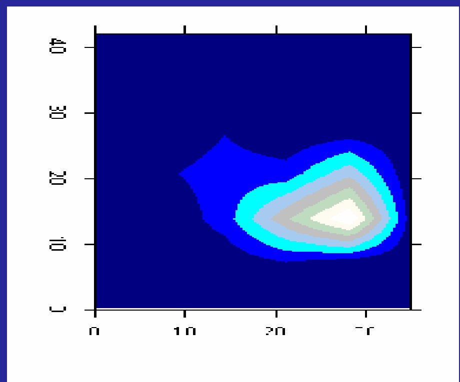
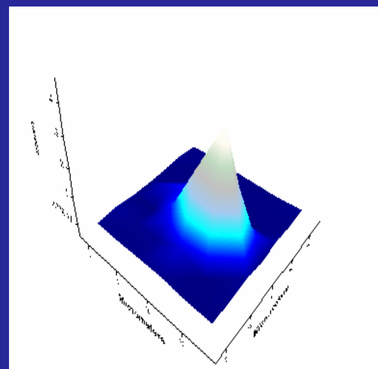
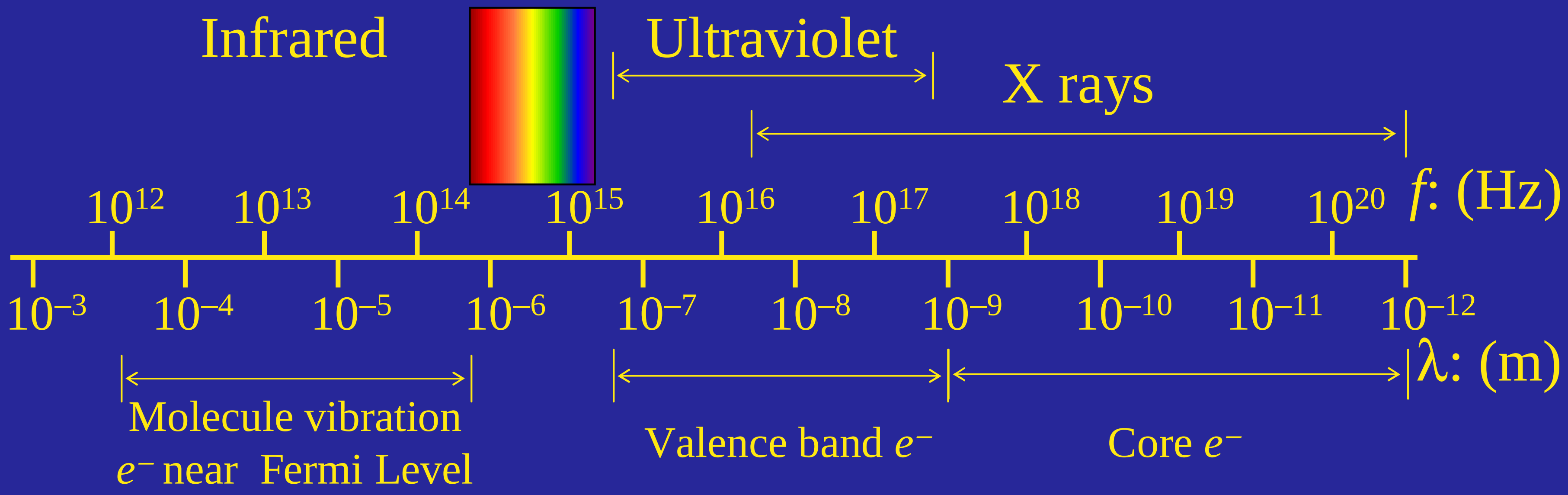




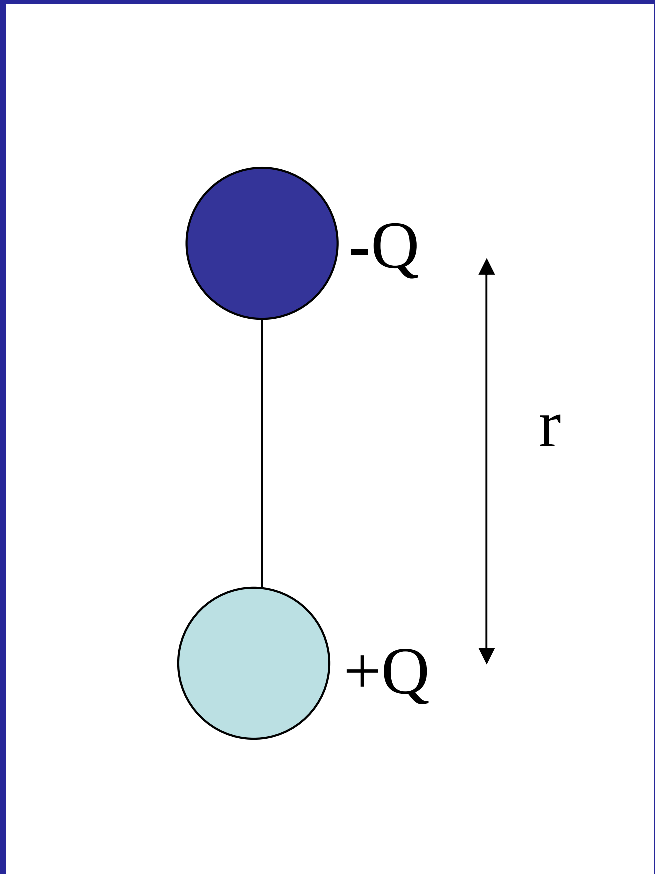


What is Infrared Spectroscopy?

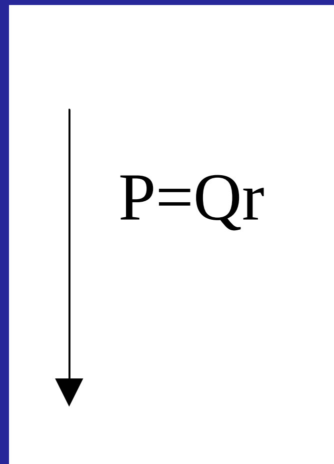


Absorption of Low Energy (IR) Photons causes:
Displacement of atoms (Vibrations)
Functional groups (CH, OH, etc.) within larger molecules exhibit similar absorption energies

What is a Static Dipole



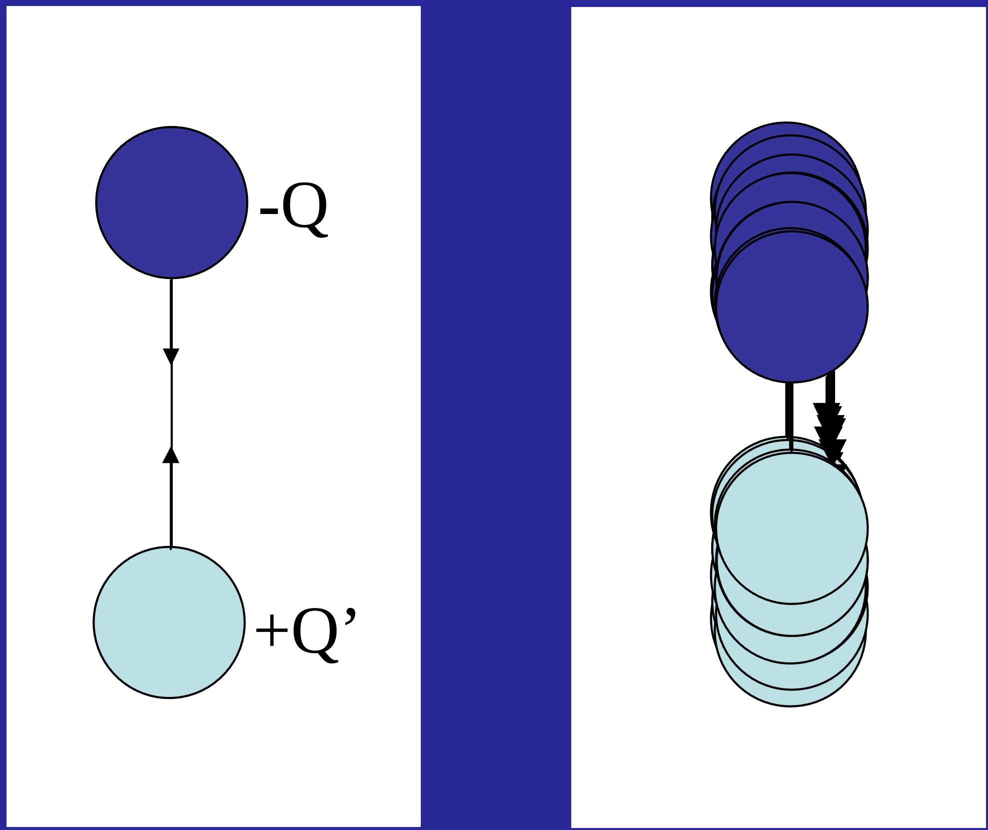
Q is charge
r is distance between the charges
P is dipole



Which of the following molecules are dipoles:

O_2, CO, N_2, NO?

What is a Dynamic Dipole



Separated charge distribution that is moving

Which of the following would be Dynamic Dipoles:

Vibrating N_2

Vibrating CO

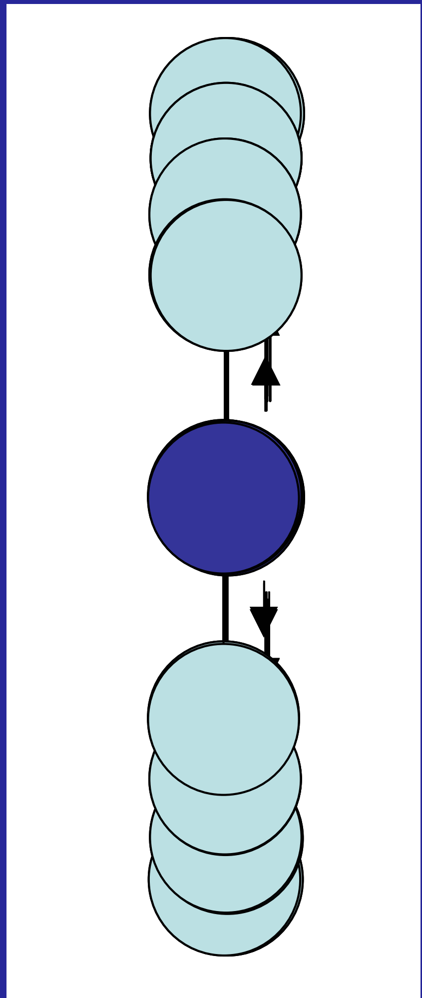
Vibrating O_2

Vibrating NO

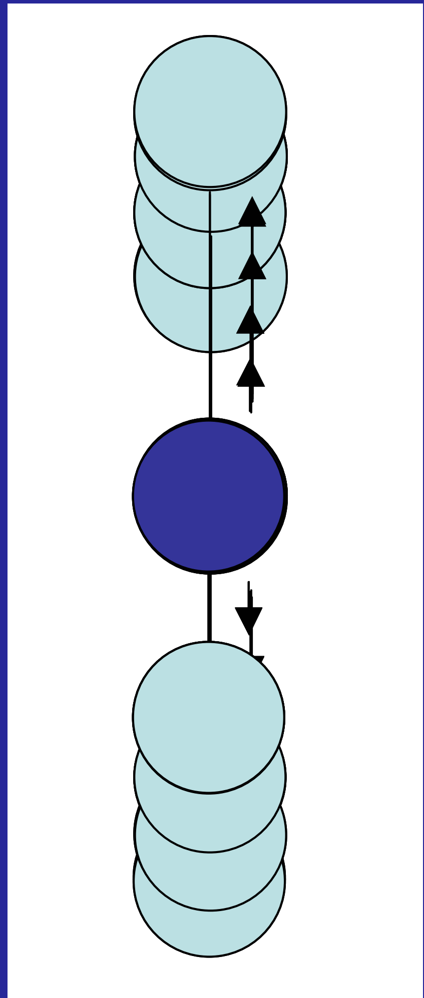
Vibrating CO_2????

Ionic bonds in molecules can have infrared active vibrations,
Covalent bonds in molecules are not infrared active

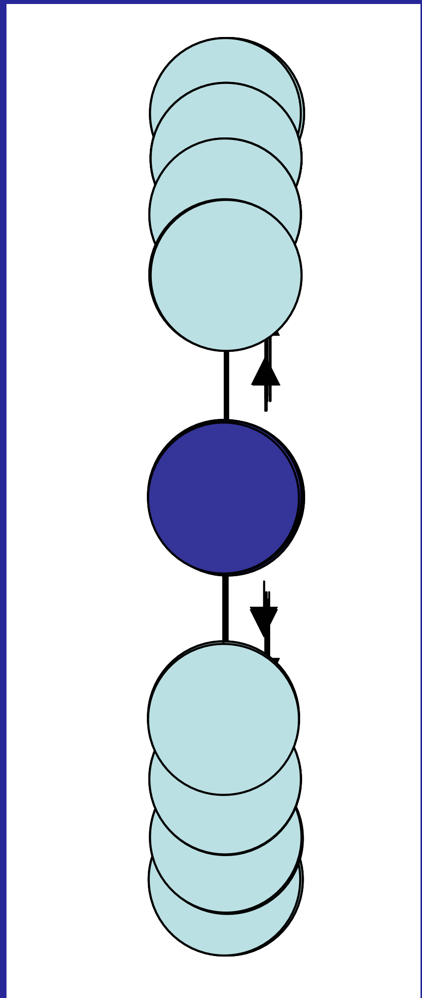
CO_2 Vibrations: Stretching Modes



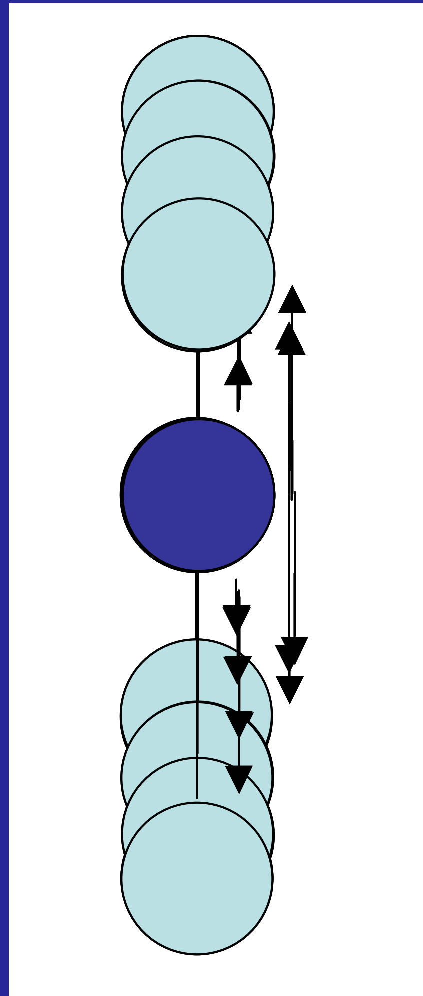
CO_2 Vibrations: Stretching Modes



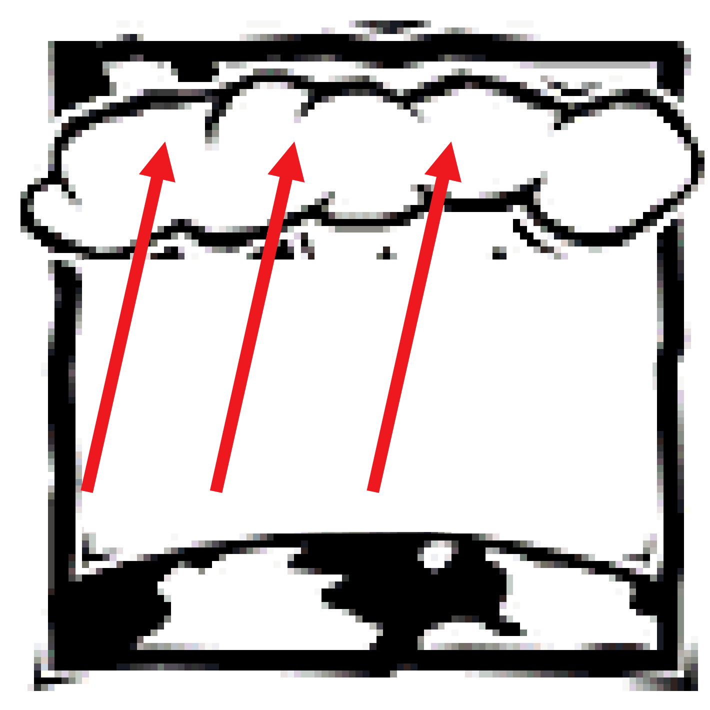
CO_2 Vibrations: Net Dipoles



CO_2 Vibrations: Net Dipoles



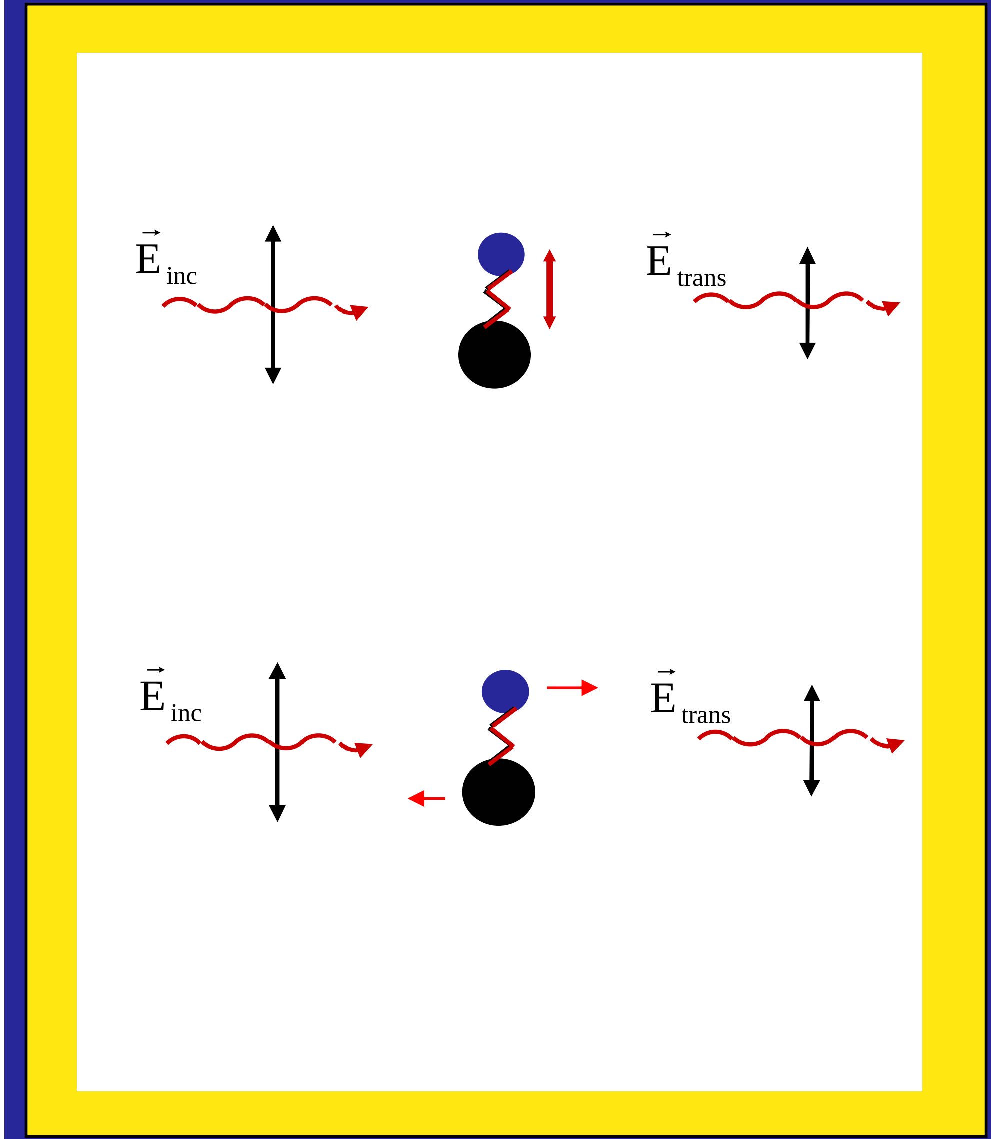
What are greenhouse gases?



Air:
N_2,O_2, CO_2, CH_4, H_2O

Which are absorbing the infrared radiation reflecting from the earth?

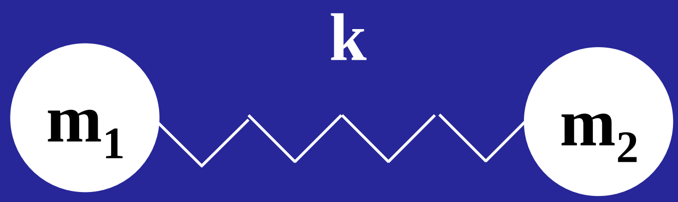
Vibrational Strengths



High Frequency Vibrations: Stretching Modes

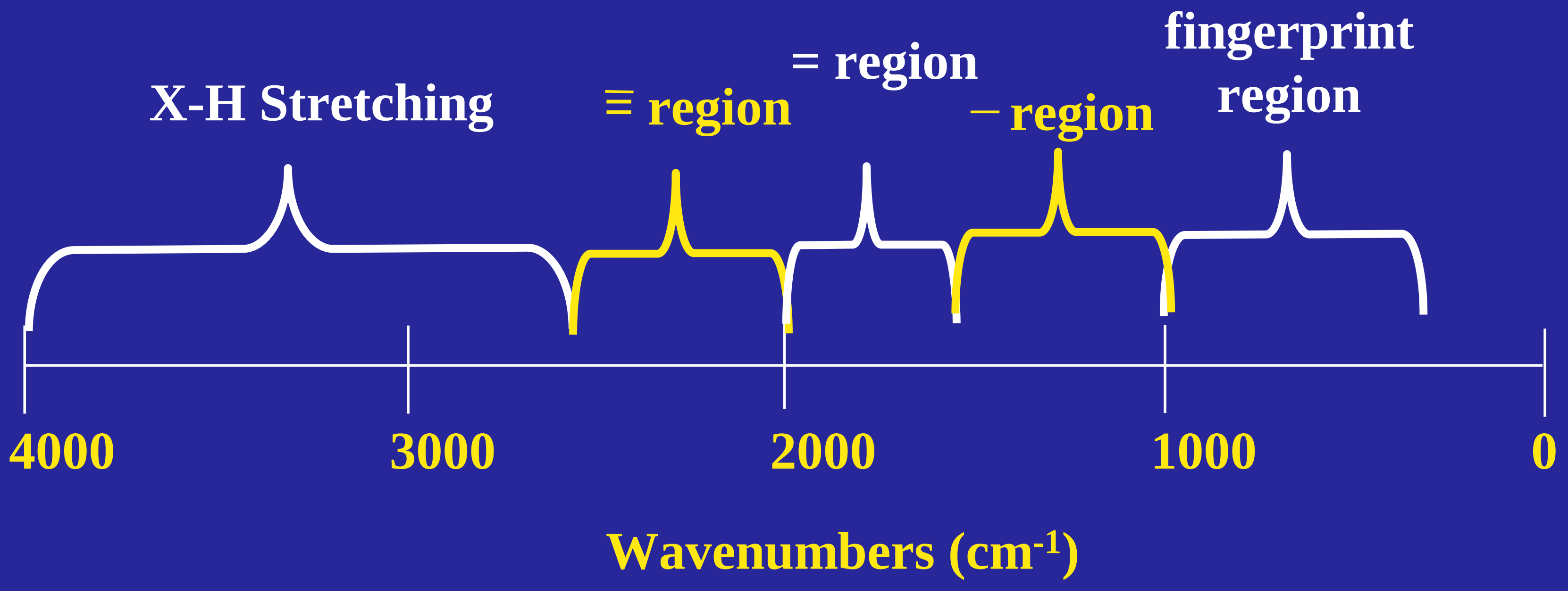
Lower Frequency Vibrations: Rotational Modes (bending and wagging modes)

Vibrational Strengths

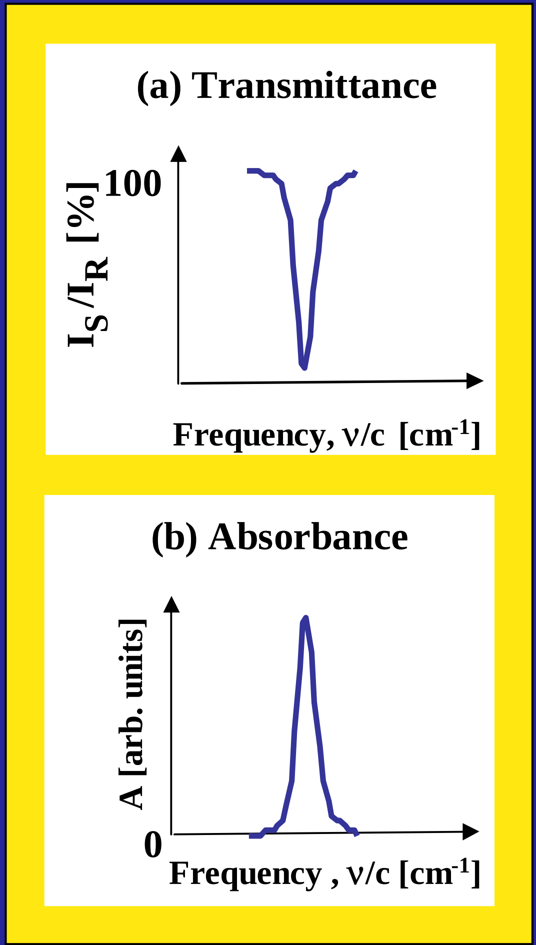


$$\nu = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

Atoms in Typical Functional Groups include
C, N, O, H, P, S, Si, Al



Signatures in Infrared Spectroscopy?



The energy for each vibration is dependent upon the mass and spring constant, IR absorption spectroscopy is chemically specific.

Sources of Infrared Light

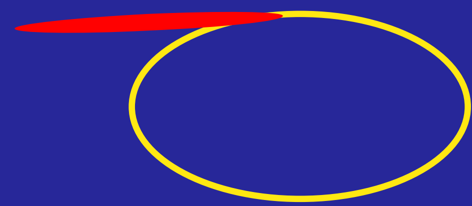
Blackbody Radiation

Hot filament or rod
(Globar or Nernst Glowar)

Flux radiated in all directions

Flux ∝ Temperature

Storage Ring Radiation

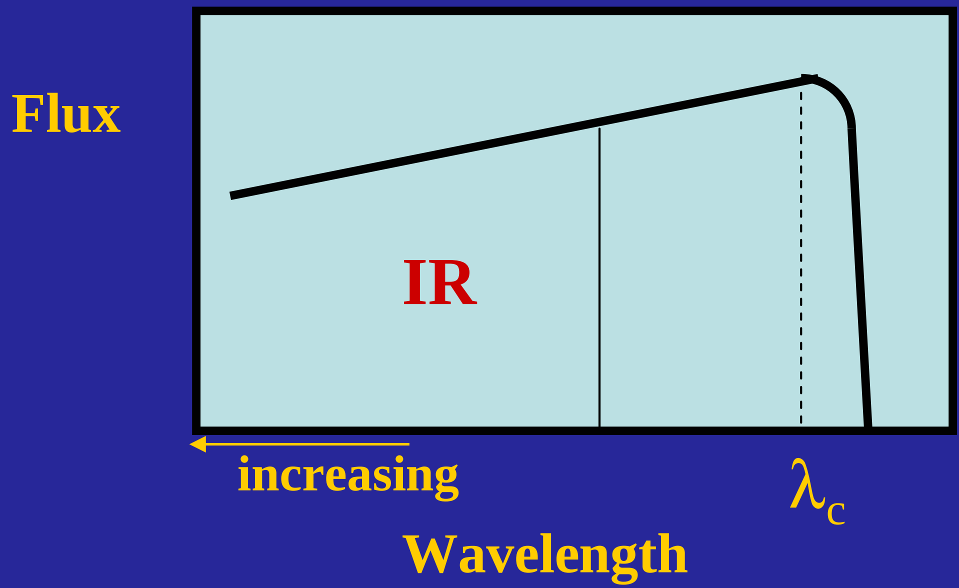


From relativistically accelerated charged particles

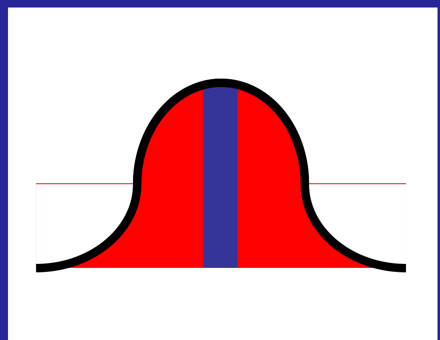
Flux radiated in a tangential, well defined cone

Flux ∝ Beam current

Synchrotron Characteristics: IRSR



IR Flux ∝ Beam Current

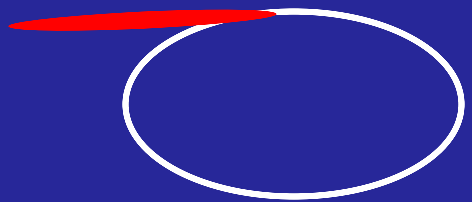


Beam Profile

IR Source: wide beam profile
Large opening angle
Mixed polarization

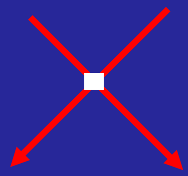
Why use a storage ring source?

Storage Ring



Small source size
Small solid angle

Spectromicroscopy



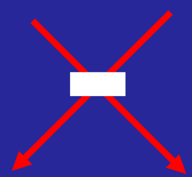
Very small sample
1 μm x 1 μm

Grazing incidence reflection-absorption spectroscopy

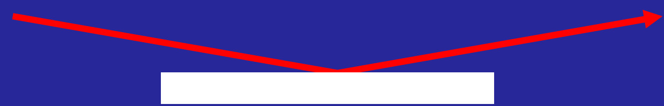
Very small
angular acceptance
< 5°

Globar

Large source
Large solid angle



Small sample
30 μm x 30 μm



Small angular acceptance
> 10°

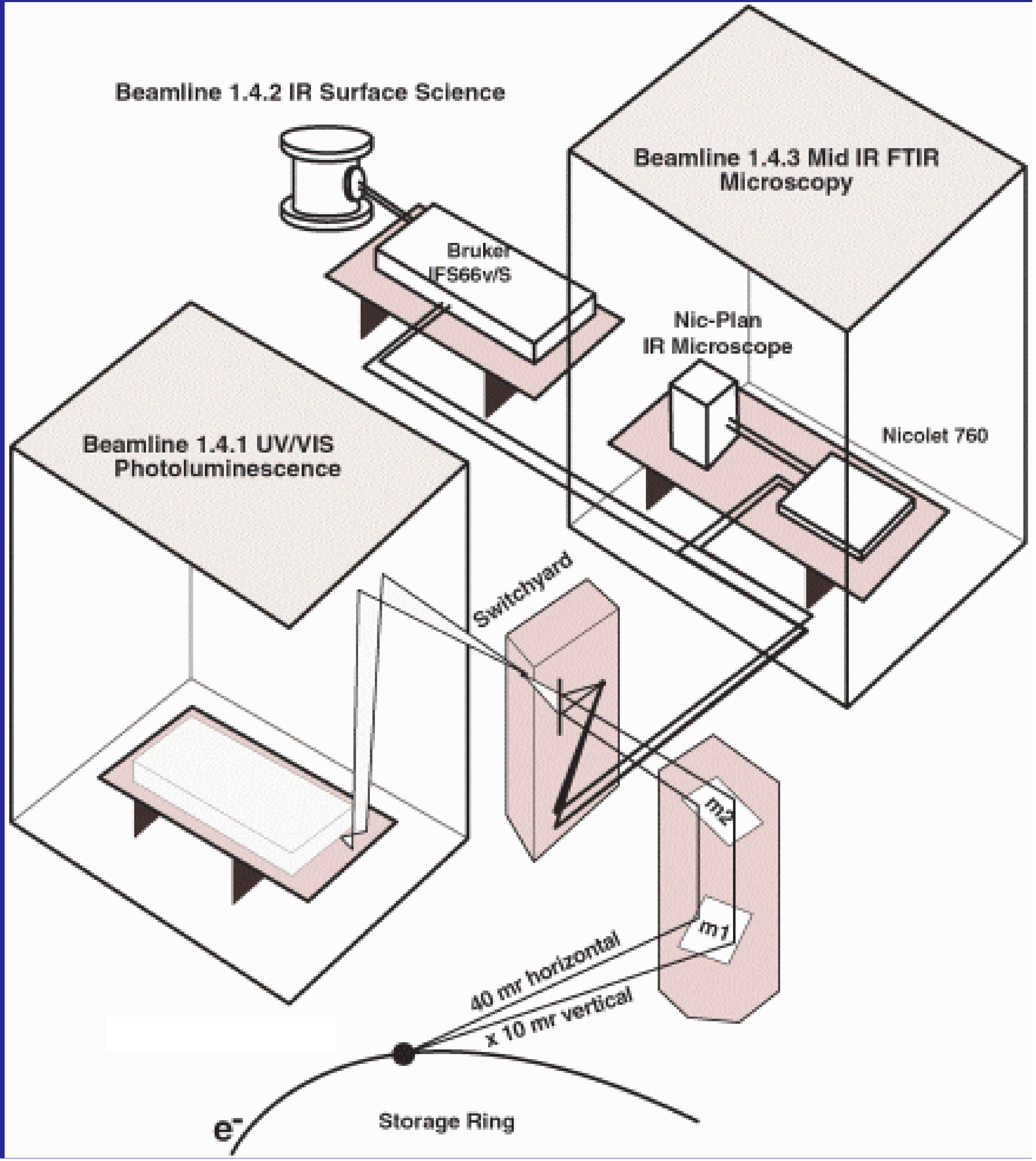
Advanced Light Source Infrared Facility

Necessary Beamline Components:

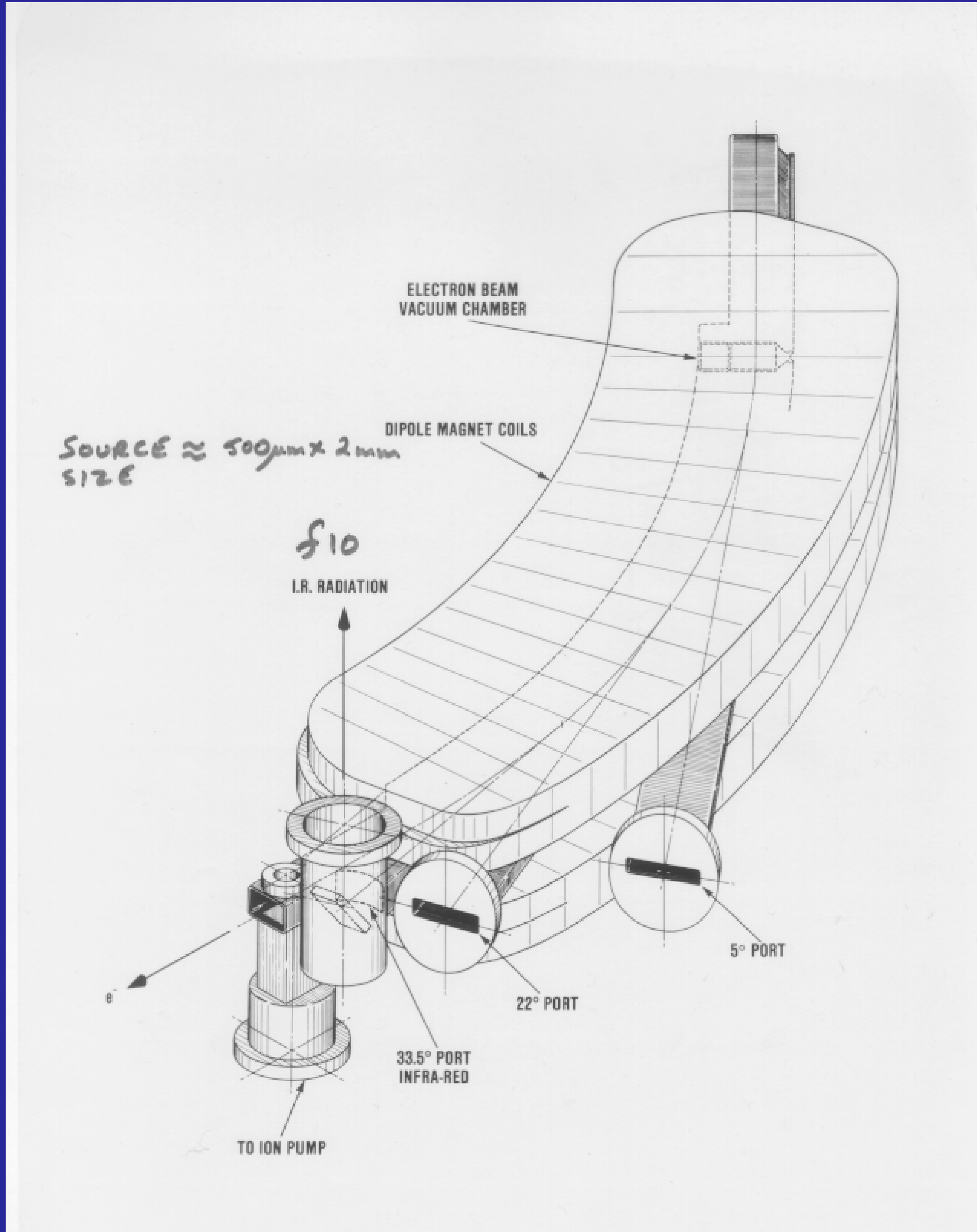
Large extraction port
Large Water Cooled Flat Mirror
Off-Axis Parabolic Mirror
with long focal-lengths
Wedged Diamond Window at
focal point
Collimating Optics
Spectrometer: FTIR (Vacuum or
Nitrogen Purge) w/ LN2 or
LHe cooled detectors

Optional Beamline Components: (Based on experiment)

Infrared Microscope
Surface Science Chamber
Beam position feedback system



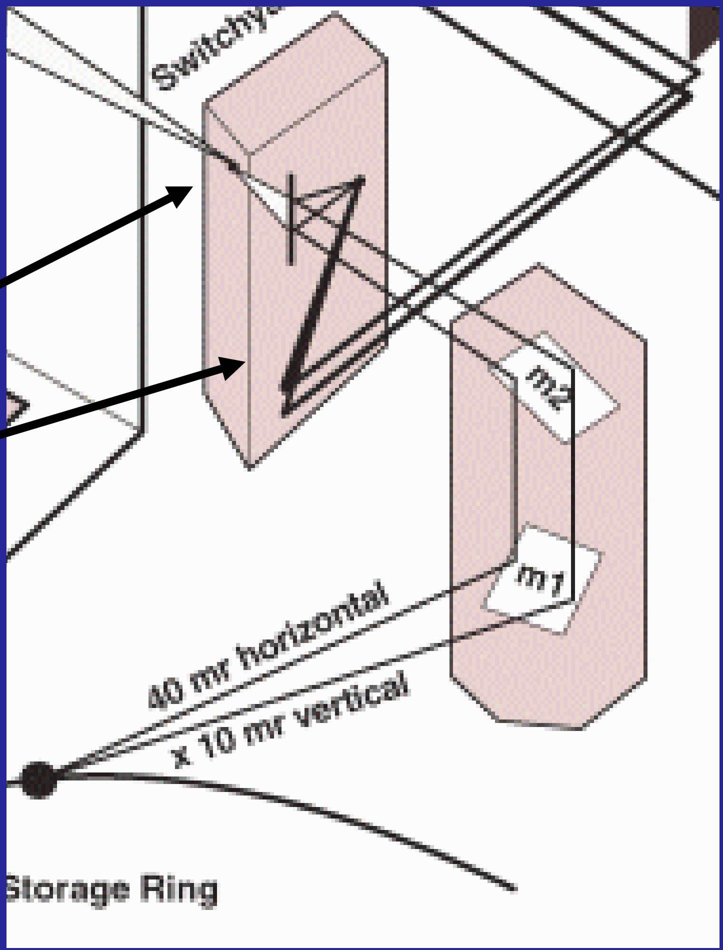
NSLS, BNL Vacuum Tank Schematic



NSLS, BNL
Vacuum
Tank
IR and UV

Advanced Light Source Infrared Facility

m1: Large Water Cooled Flat Mirror
m2: Off-Axis Parabolic Mirror with long focal-lengths
Wedged Diamond Window at focal point
Collimating Optics



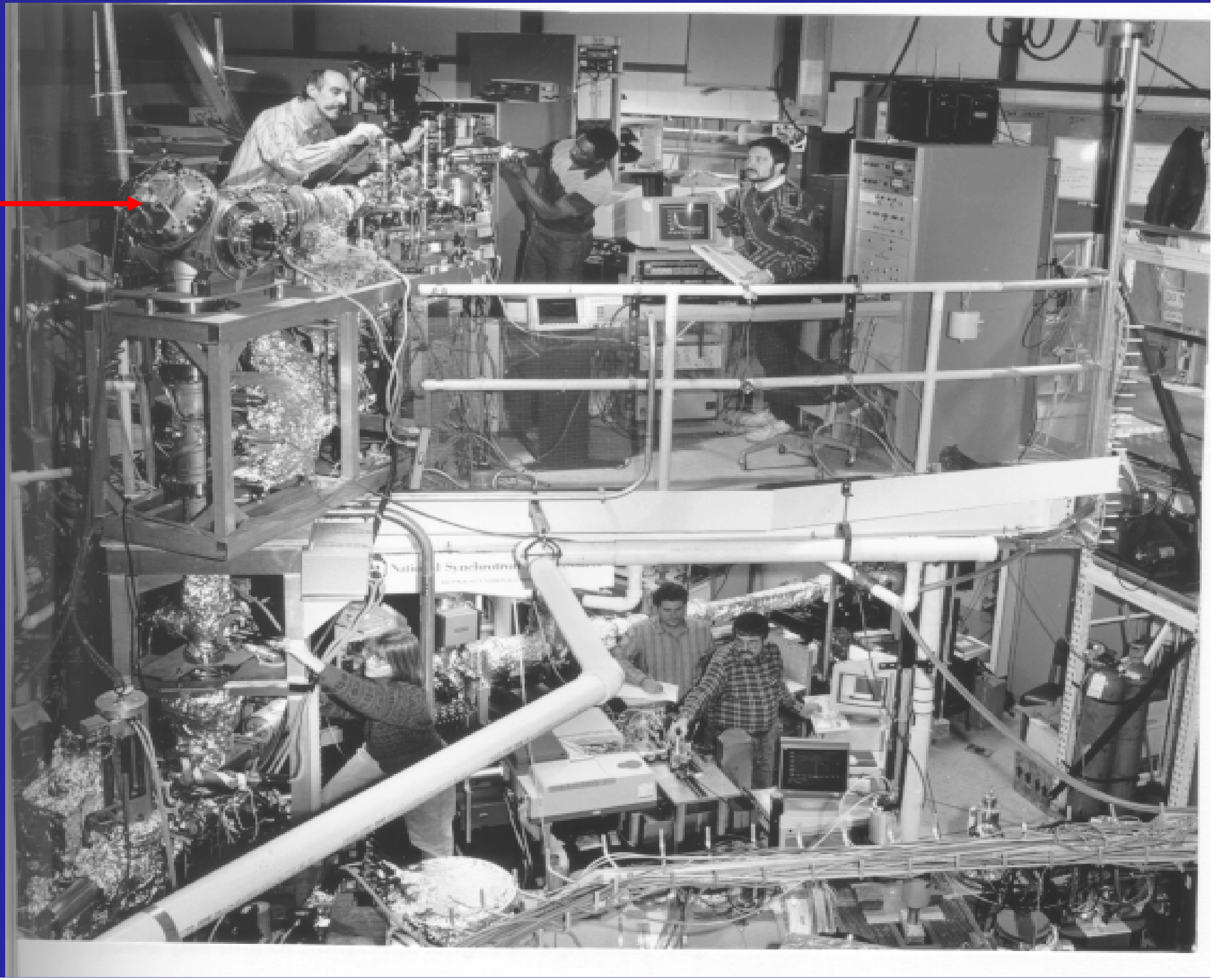
Water Cooled Plane Mirror and Off Axis Paraboloid for ALS IR Beamline

Off Axis Paraboloid

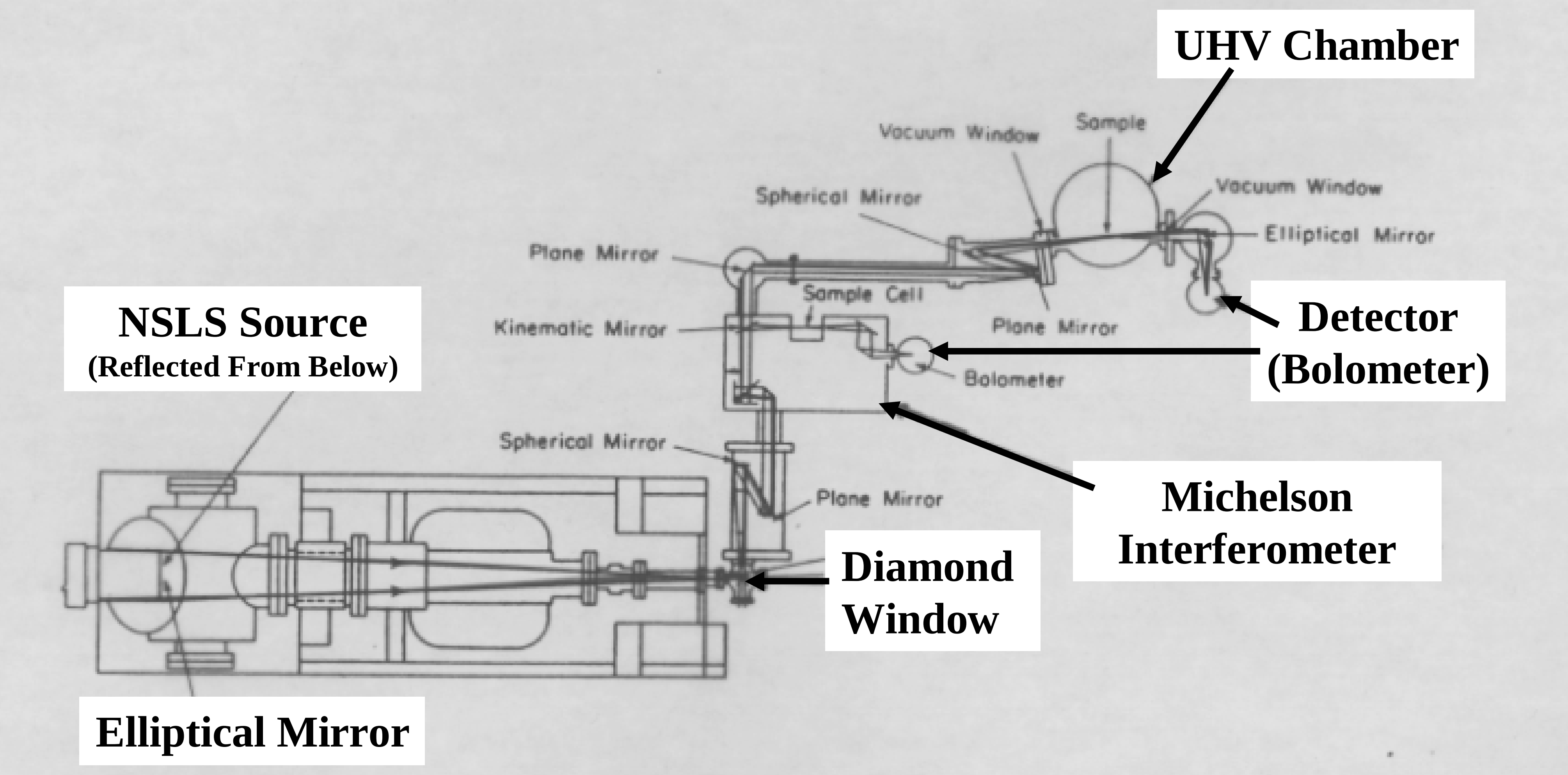
Plane Mirror

NSLS U4IR and U2B Infrared Beamlines

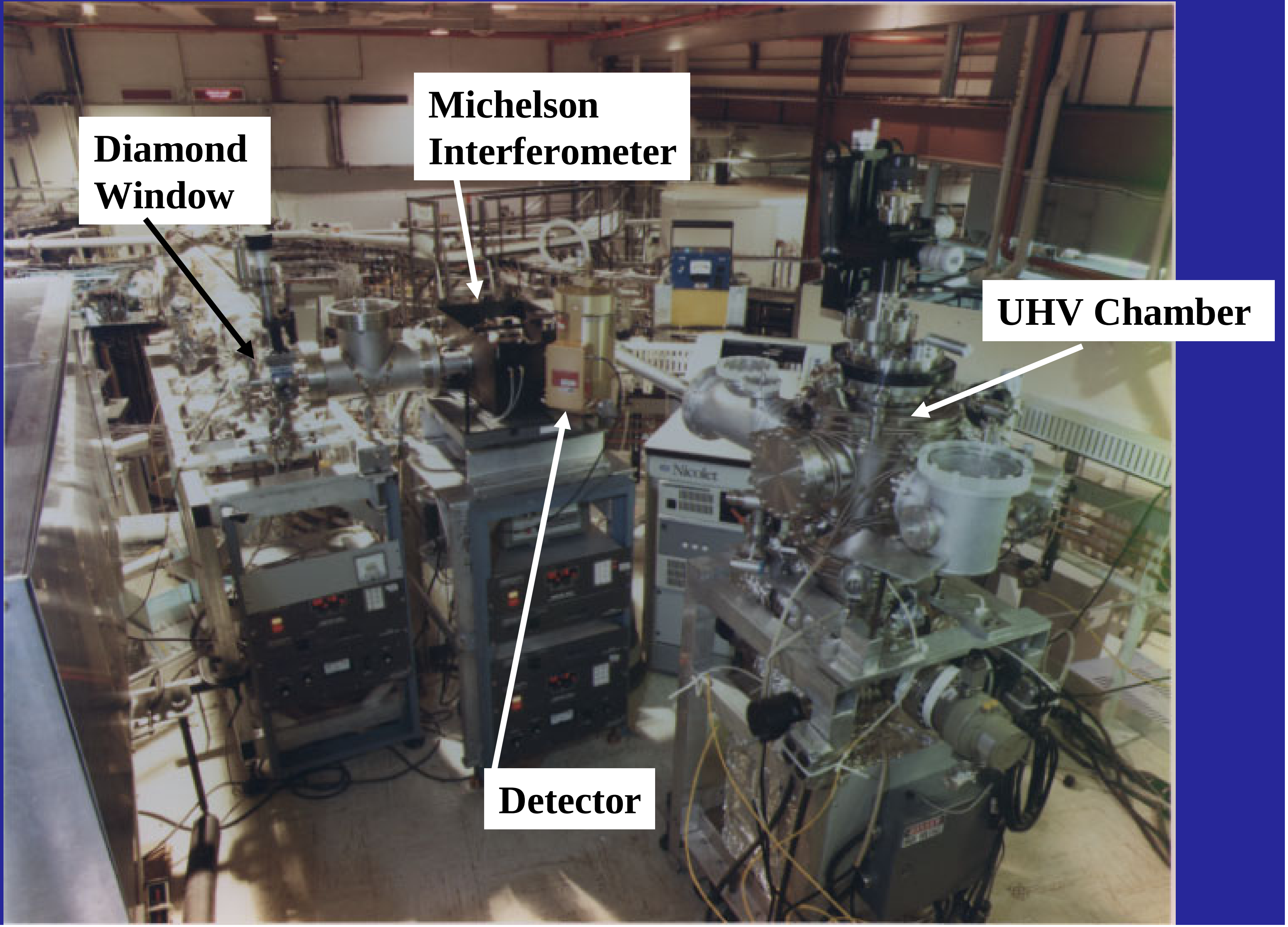
Off axis
Paraboloid



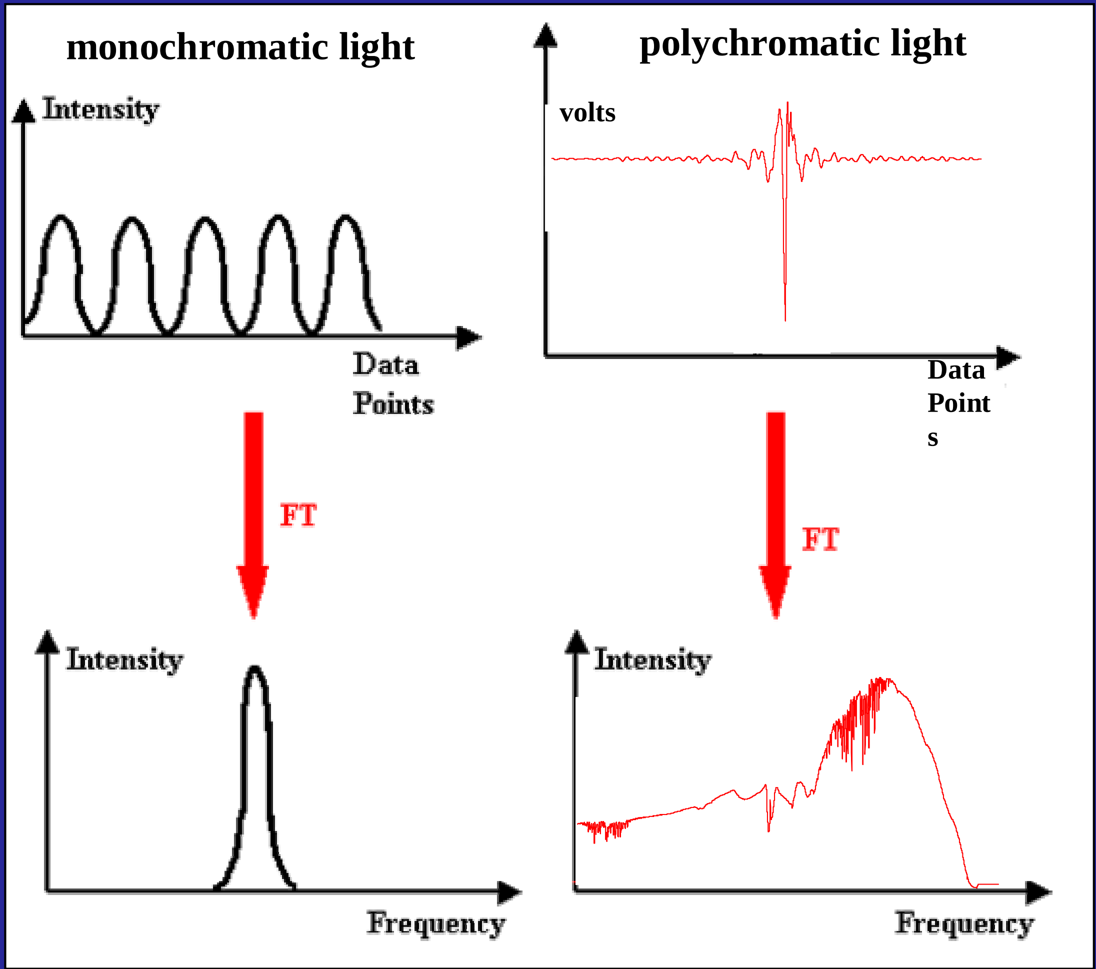
Infrared Beamline Overview:
NSLS Beamline U4IR

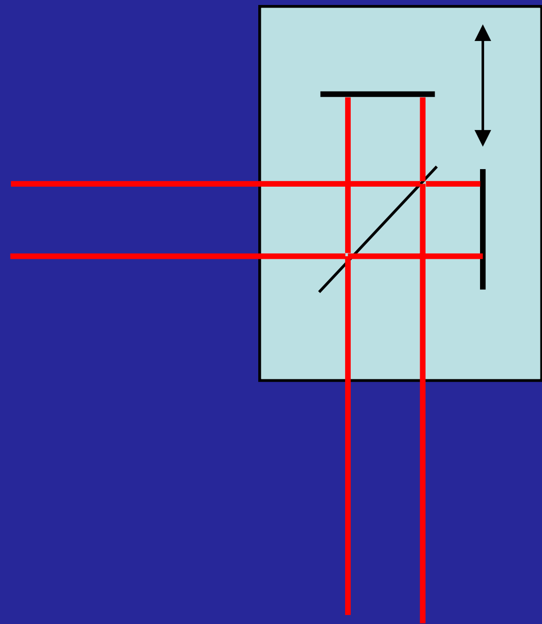


NSLS Beamline U4IR



FTIR Michelson Interferometer





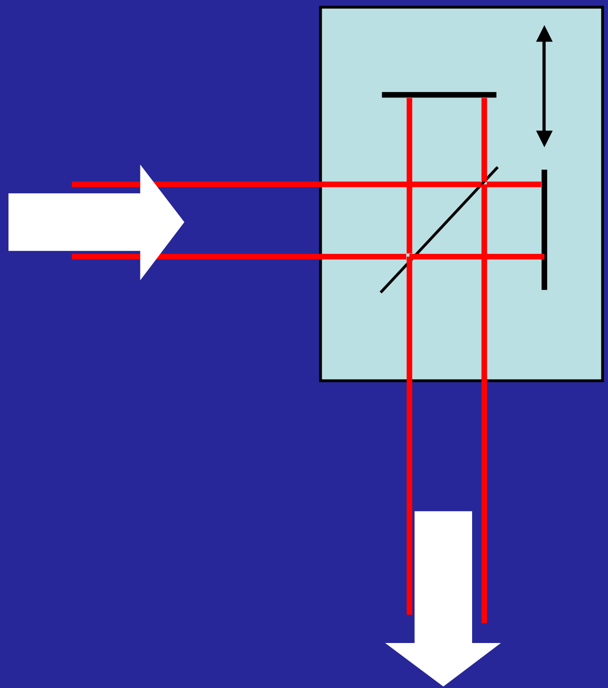
Rapid Scan Michelson Interferometer

Broadband IR from Storage Ring
(1 cm^{-1} - 40,000 cm^{-1})

+

"Noise" from Electron beam motion (e.g. 100 Hz)

Examples include:
RF Side bands (700-3000 Hz)
Ripple on Power Supplies (multiples of 60 hz)
etc.



Moving mirror velocity = 1 cm/s

Broadband IR Signal
(1 cm^{-1} - 40,000 cm^{-1})

+

Signal Frequency "Noise"
(100 cm^{-1} spike)

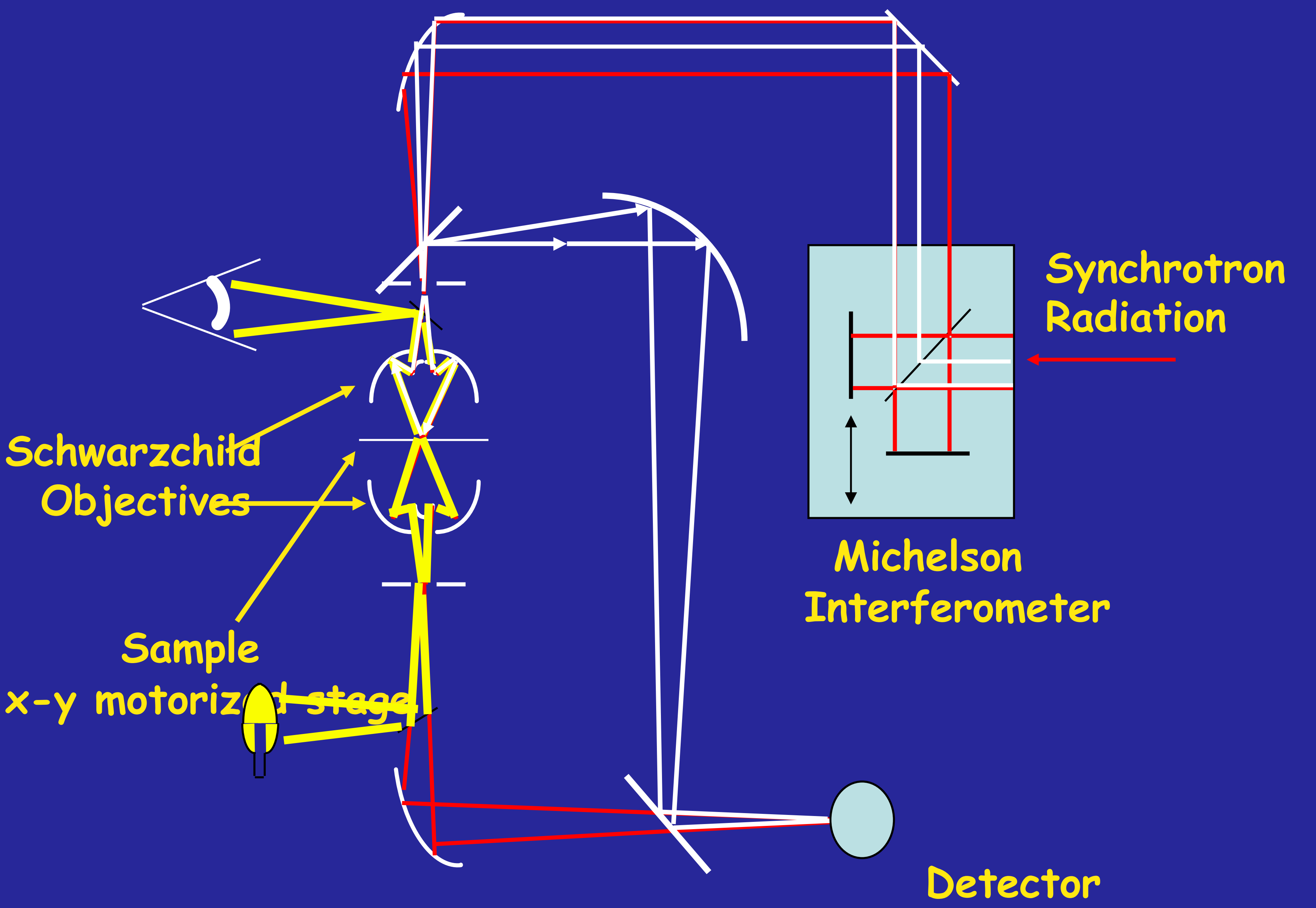
Thermo Nicolet Infrared Microscope at ALS

Collimated beam from Storage Ring

FTIR Spectrometer

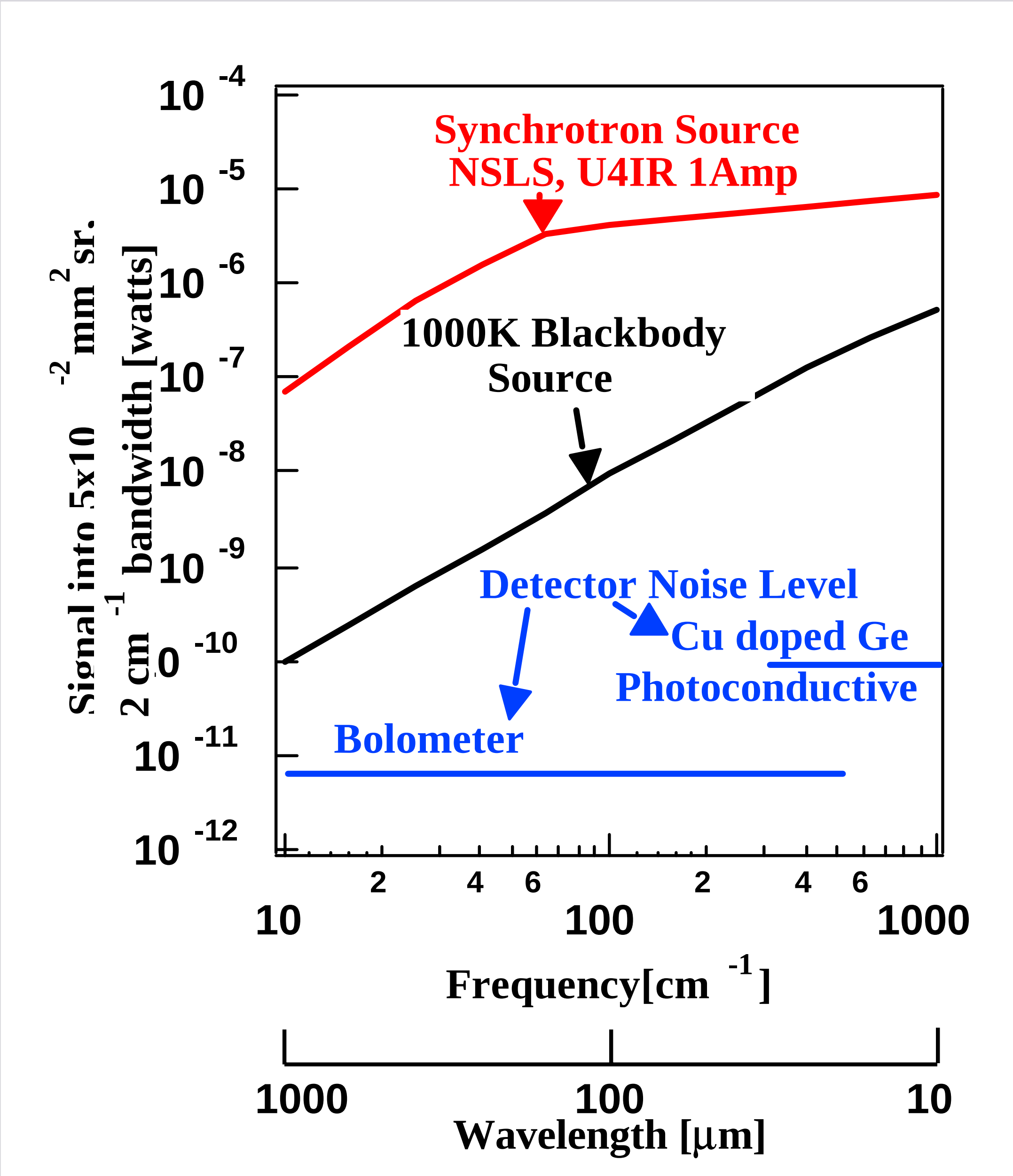
Infrared Microscope

Infrared Microscope



Infrared Beamlines U12, U10A and U10B at the NSLS

How Much Signal?



Signal to Noise Ratio:

is 100-1000 times larger from a synchrotron source than from a globar through a limited aperture

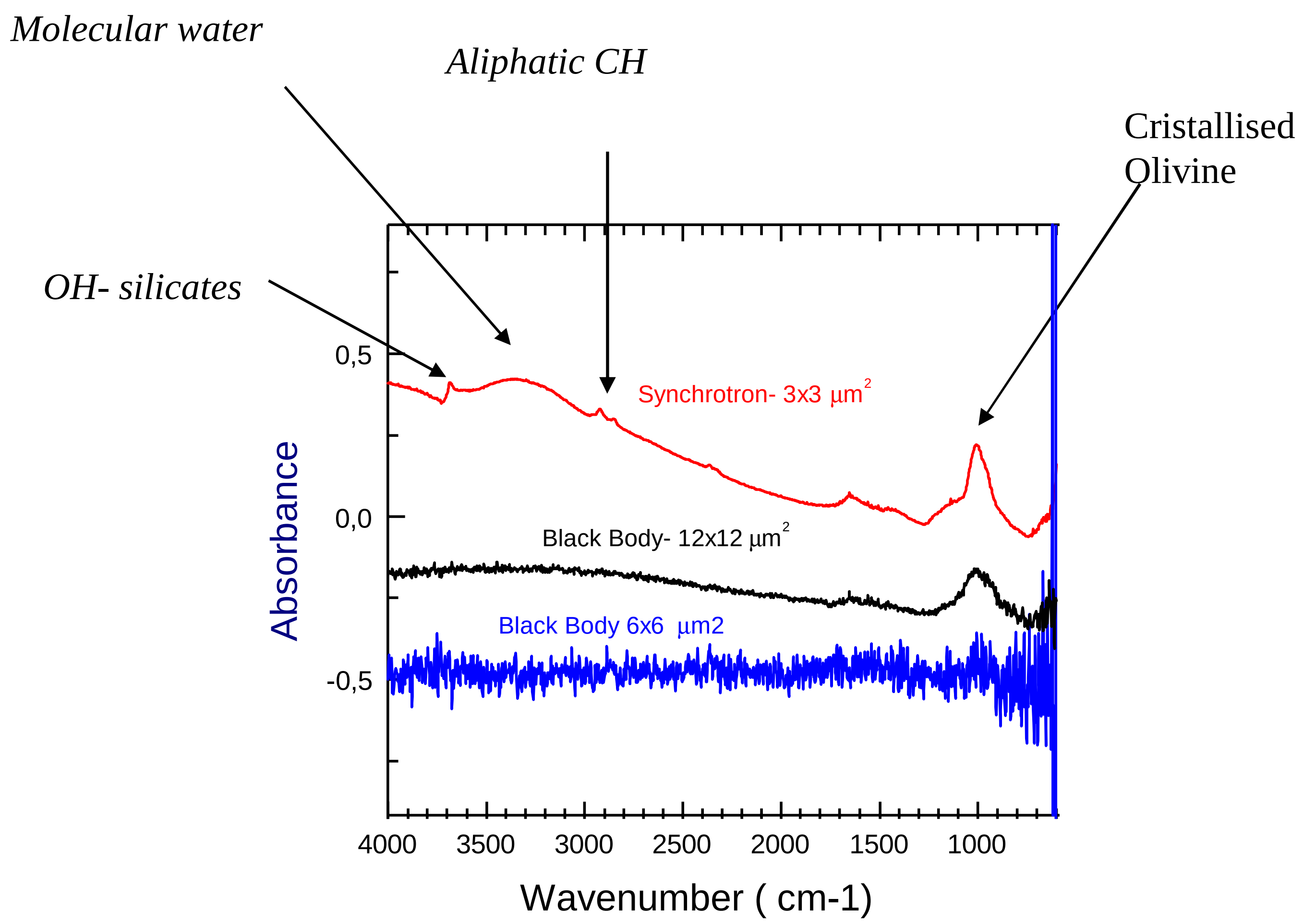
Liquid Helium Cooled detectors to reduce background noise

Interstellar micron size dust analysis

comparison SRS-Black body:

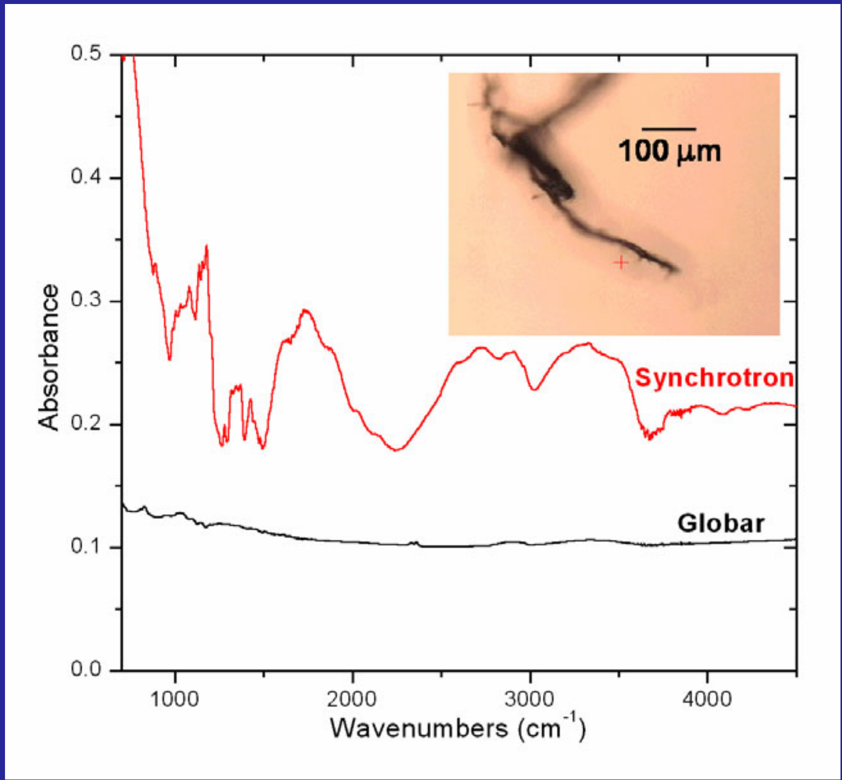
3 micron Particles from asteroid "Orgueil"

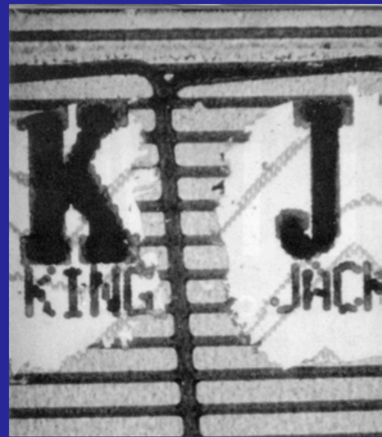
From:
G.Quitte
J.P.Bibring,
L.D'Hendecourt
(IAS),
P.Dumas (LURE),
G.L.Carr,
G.P. Williams
(NSLS)

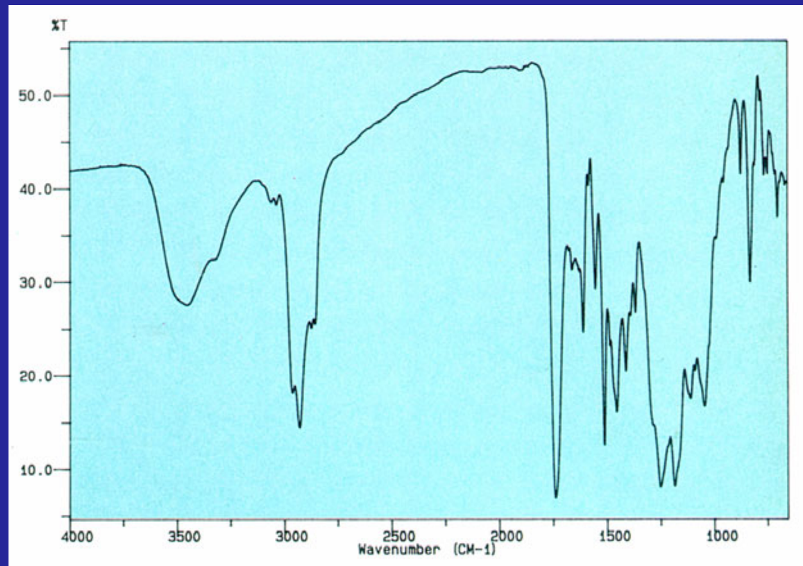


Particles collected from the MIR shuttle

Chemical Fingerprint of Ink







Wilkinson et al., Physics World, March 2002, 43

Infrared Microscopy: IR Imaging of Living Cells
Euglena gracilis

Carol Hirschmugl, Maria Bunta, Justin Holt
Physics Department, University of Wisconsin-Milwaukee

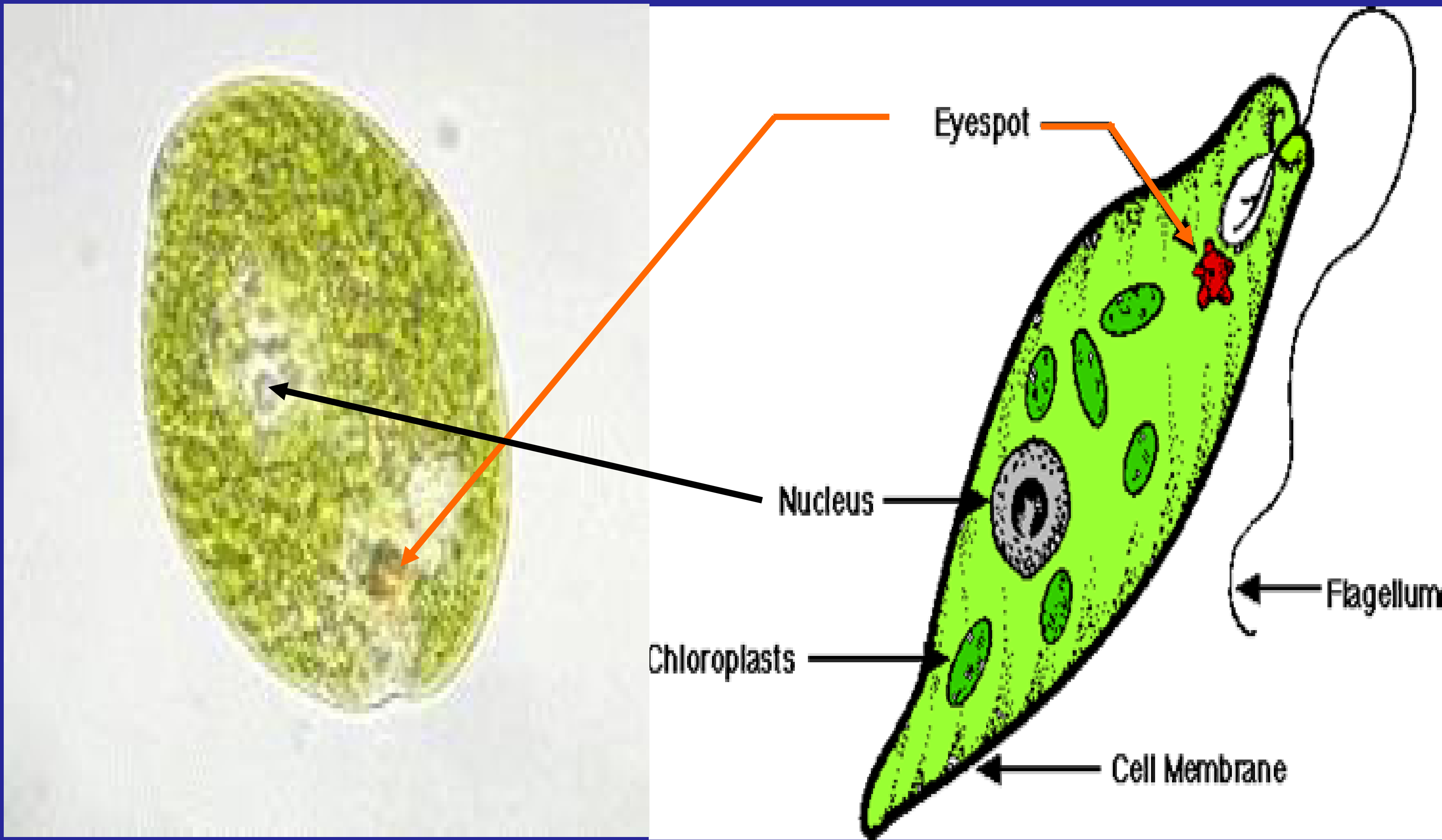
Andrej Skylarov
Advanced Analysis Facility, University of Wisconsin Milwaukee

J Rudi Strickler
WATER INSTITUTE, University of Wisconsin Milwaukee

and
Mario Giordano
Marine Biology, University of Ancona, ITALY

Algae Biology : *Euglena gracilis*

approx 15 x 15 microns

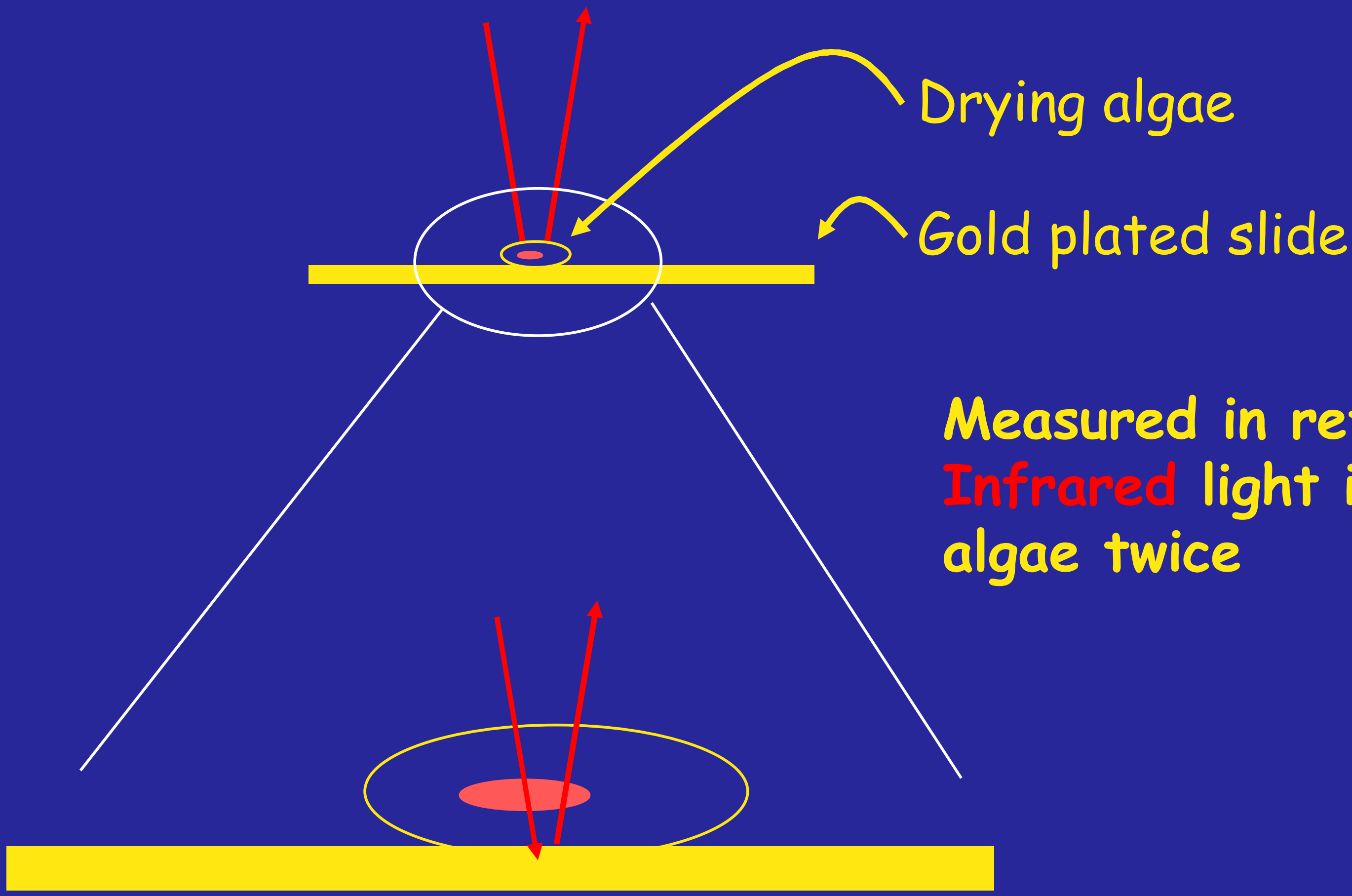


Euglena gracilis under the microscope

Euglena gracilis
swimming

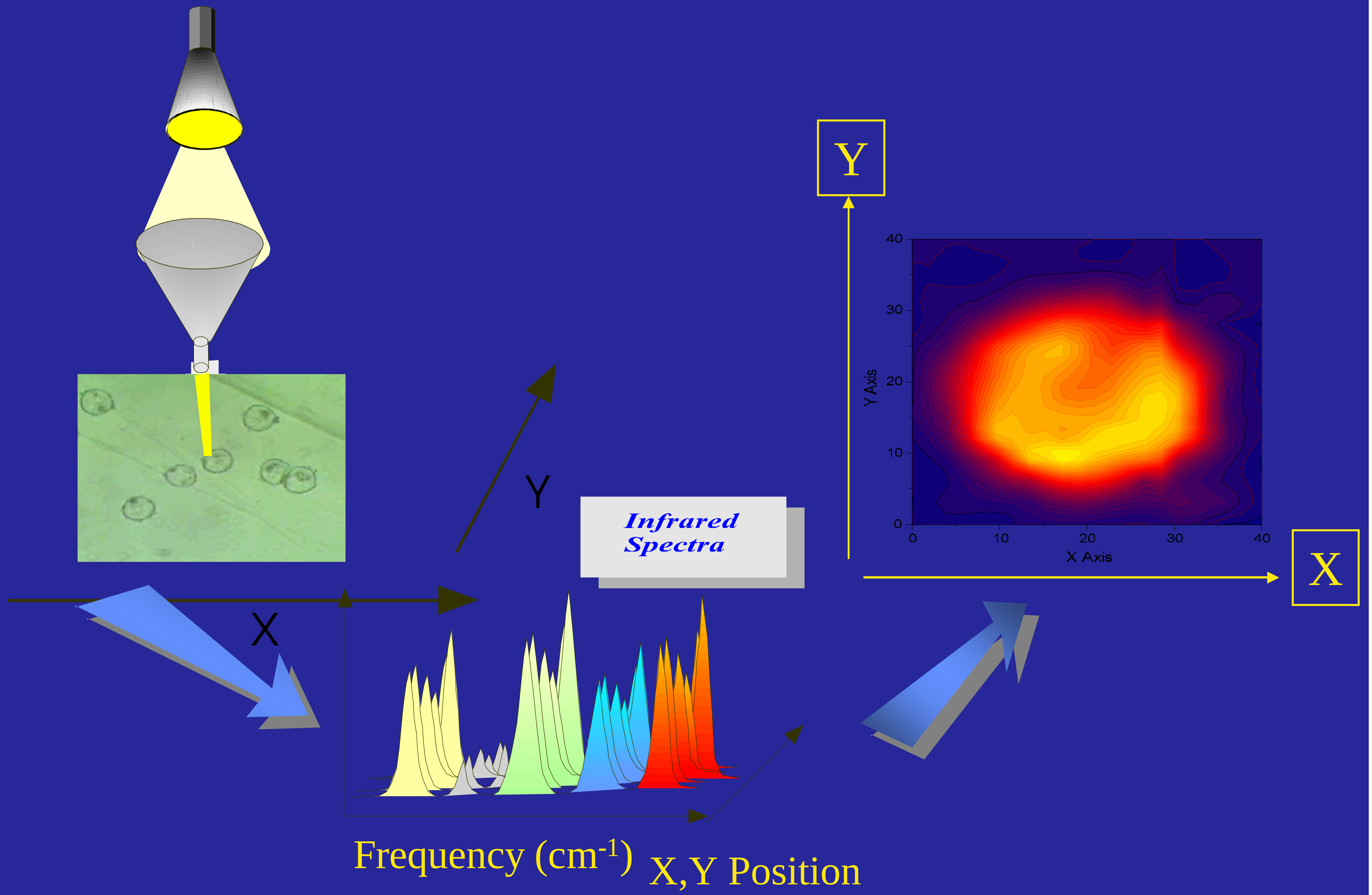
Euglena gracilis
encysted

Experimental Conditions



Measured in reflection geometry, Infrared light is absorbed by the algae twice

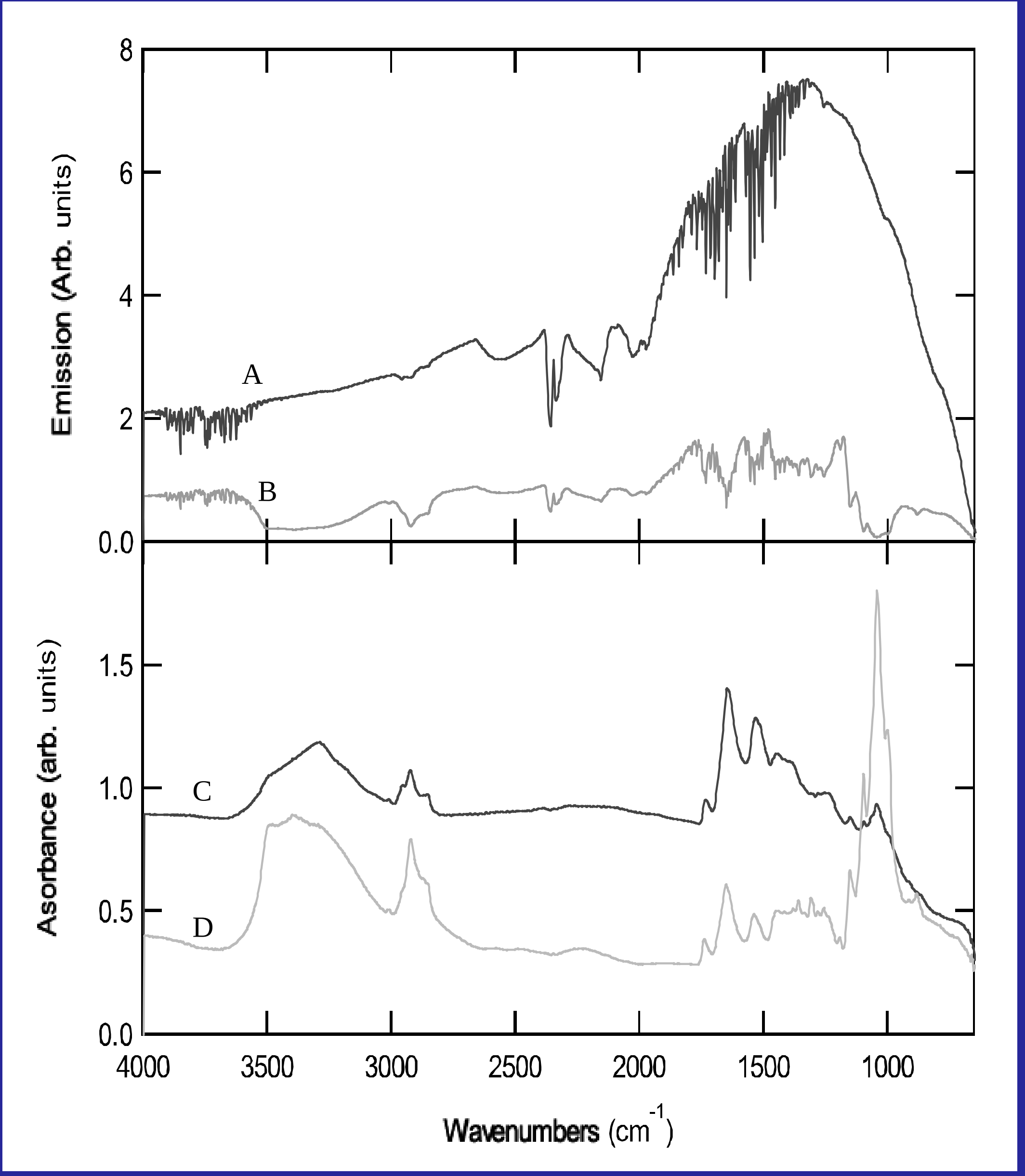
Chemical Mapping and Chemical Image:



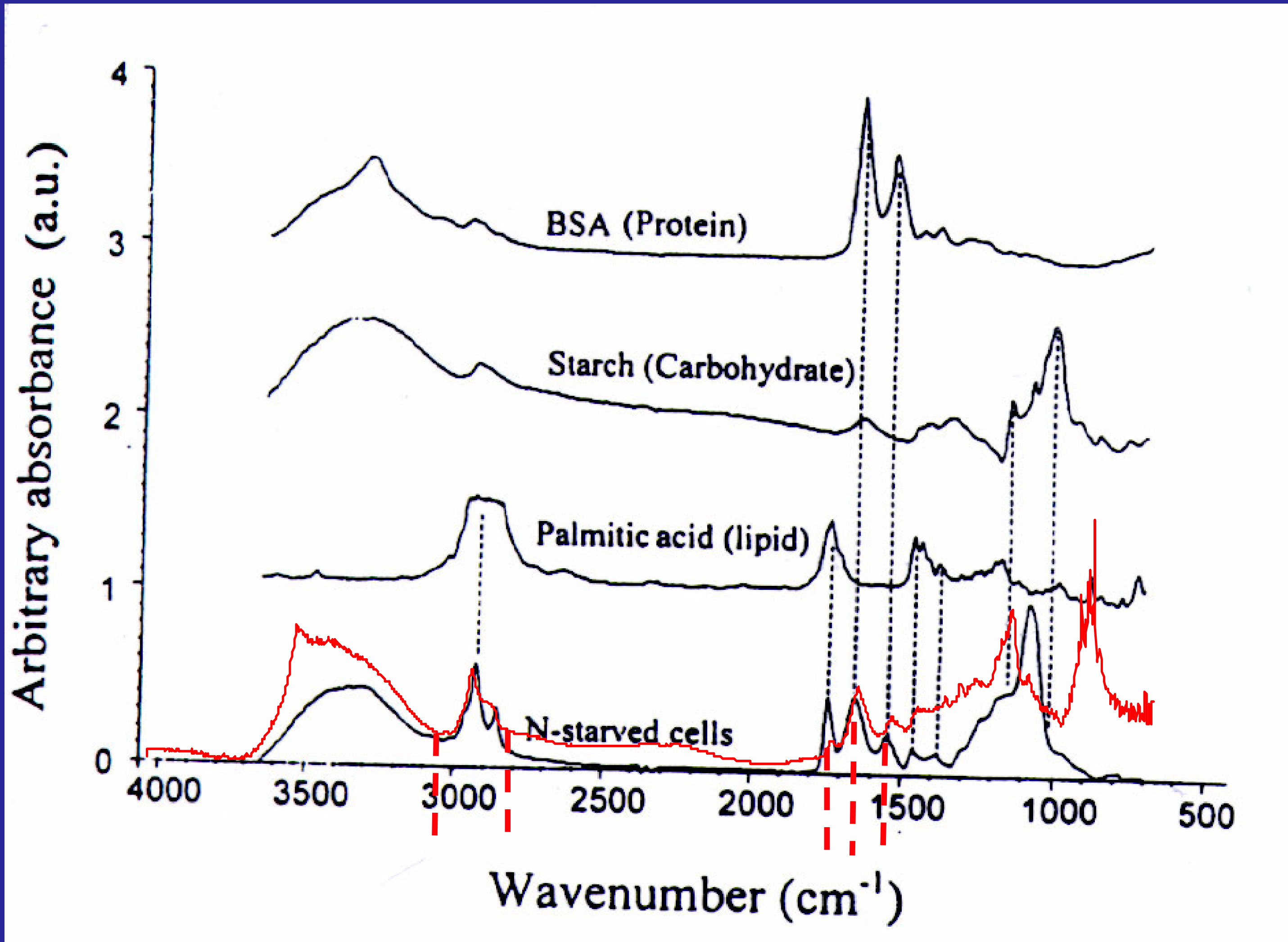
Reflected and Absorbed Intensities From *Euglena gracilis*

Reflectivity from
A) Gold
B) Gold + Alga

Absorbance from
C) Alga #1
D) Alga #2

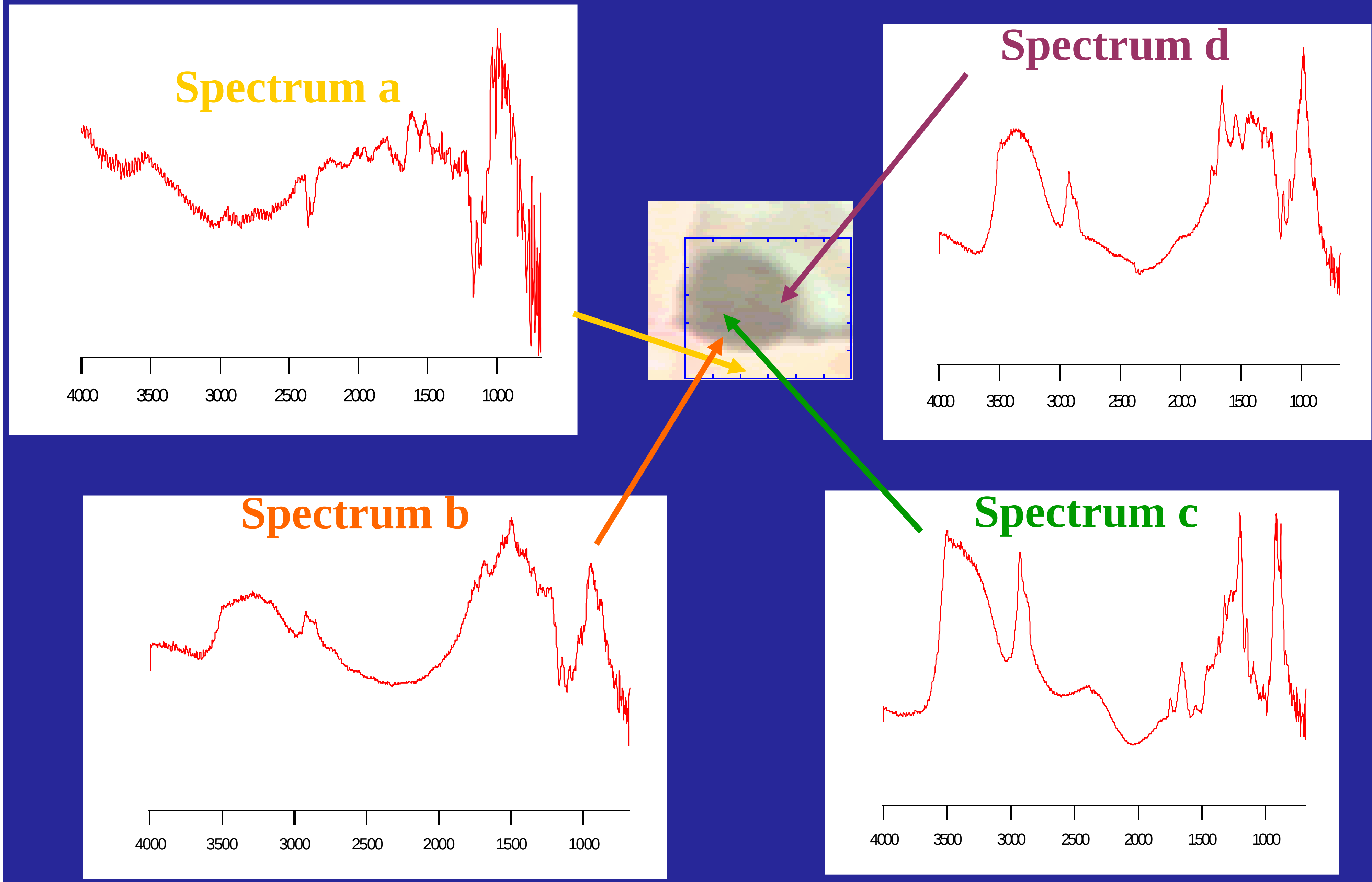


Infrared Spectrum from Alga and Chemical Standards

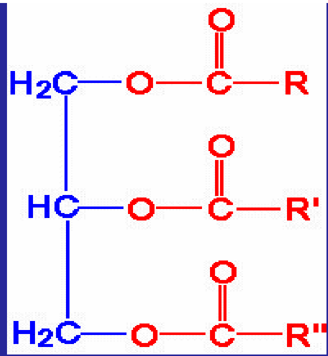


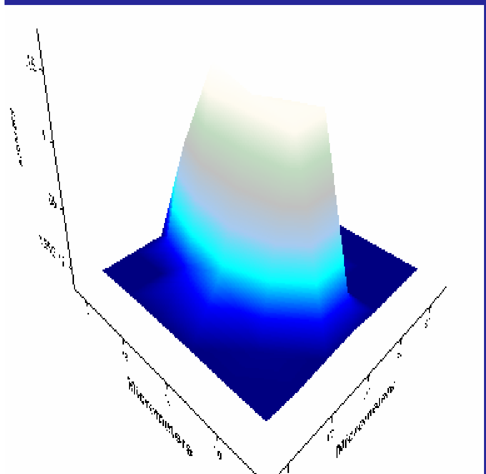
Giordano, et al., J. of Phycology, **37**, 271 (2001)

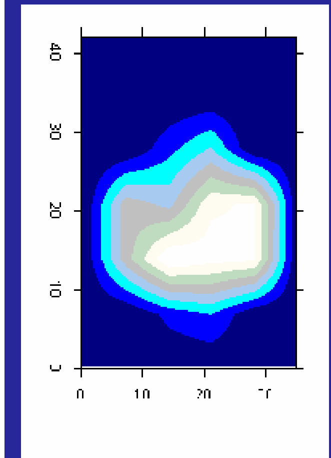
Euglena gracilis and IR spectra

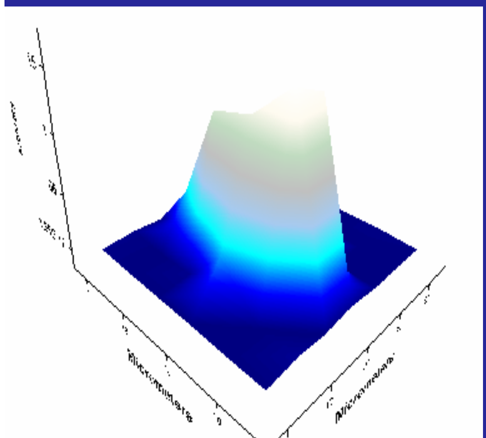


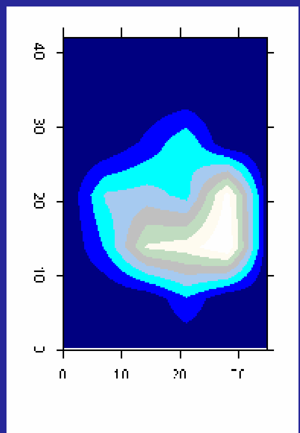
Lipids



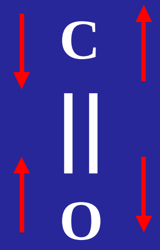






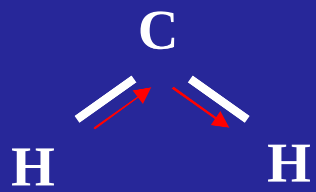


a. C=O functional group

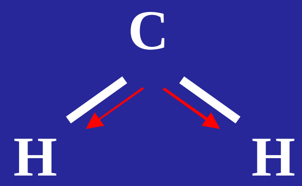


C=O Stretch vibration at 1742 cm^{-1}

b. CH_2 functional group



Asymmetric Stretch

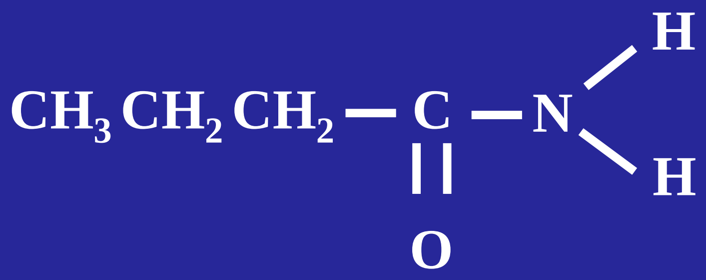


Symmetric Stretch

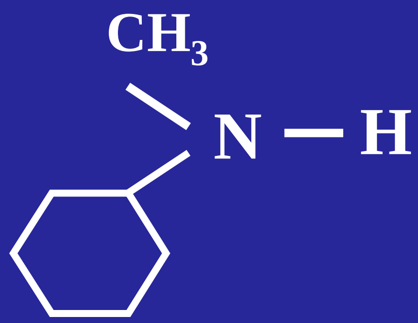
symmetric CH_2 stretch vibration at 2852 cm^{-1}

Proteins

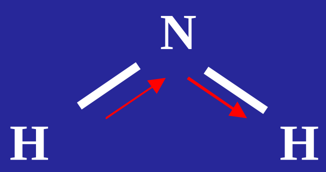
Amides I



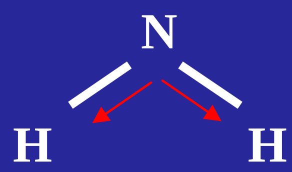
Amides II



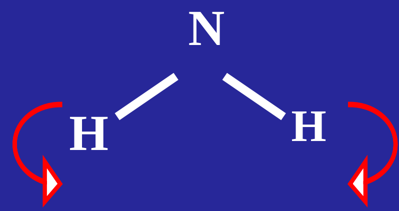
a. NH_2 functional group



Asymmetric Stretch



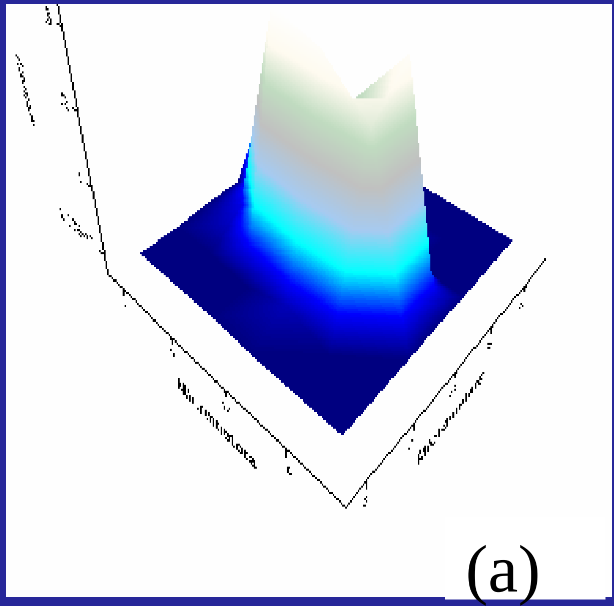
Symmetric Stretch

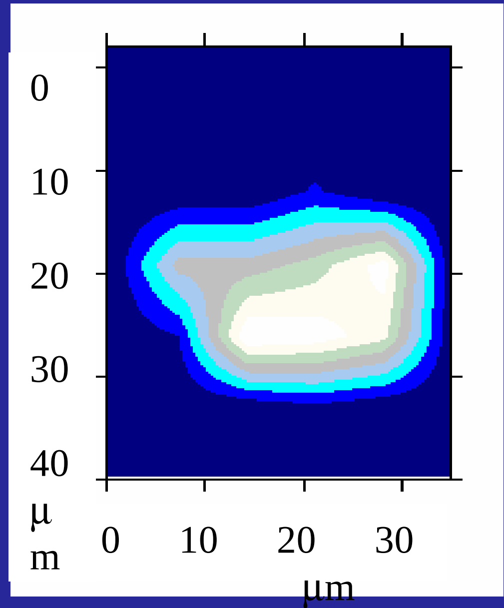


Scissors Mode

- Amides I peak at 1650 cm^{-1} represents 80% C=O stretching vibration, 10% C-N stretching vibration and 10% N-H bending
- Amides II peak at 1545 cm^{-1} represents 60% N-H bending and 40% C-N stretching vibration

Euglena gracilis and Chemical Map: Proteins



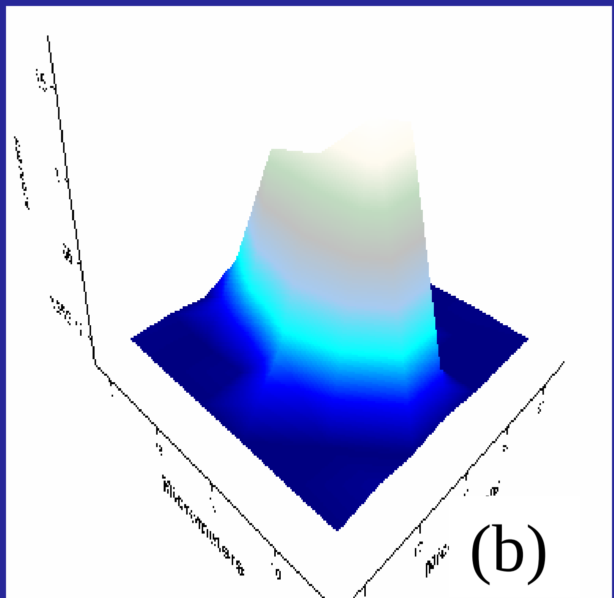


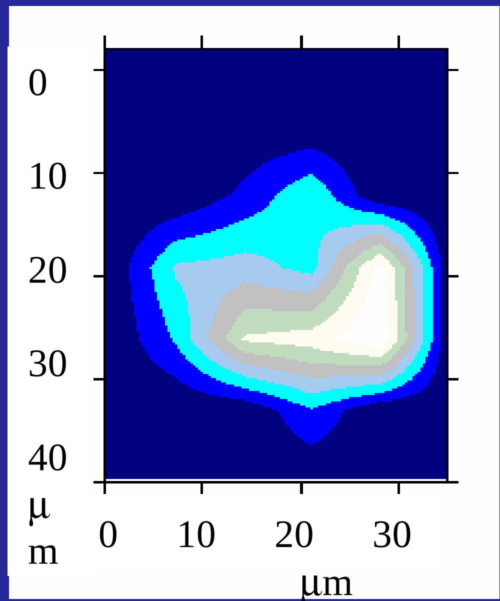
AMIDES I +

Water

Peak 1650cm^{-1}

CH_3CH_2-NH_2



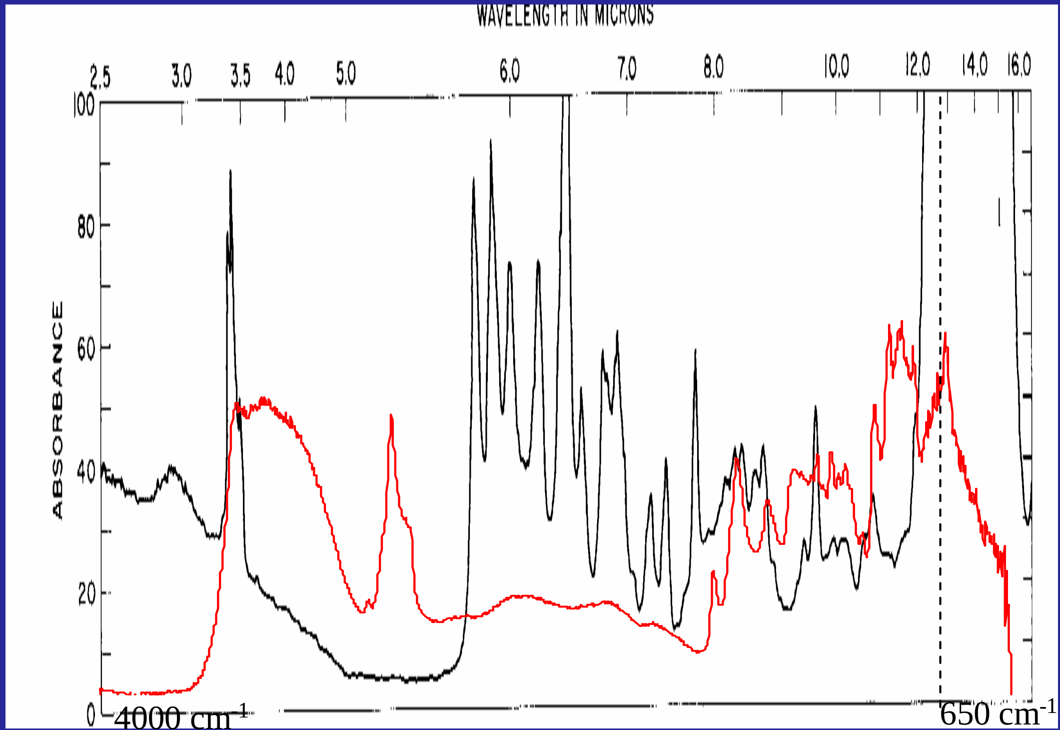


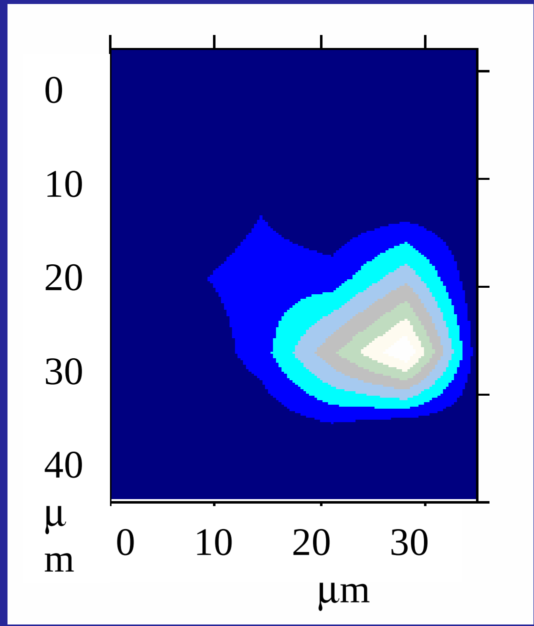
AMIDES II

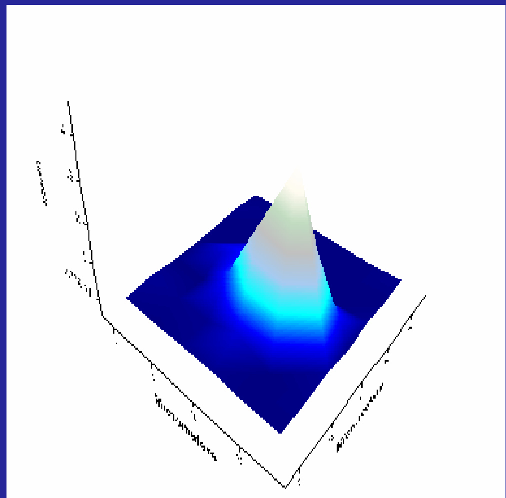
Peak 1545 cm^{-1}

CH_3CH_2-NH

Chlorophyll Spectrum



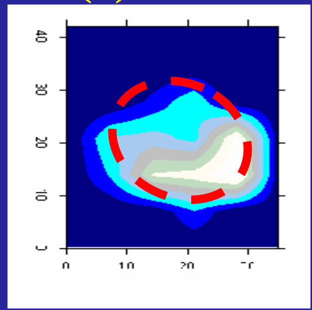




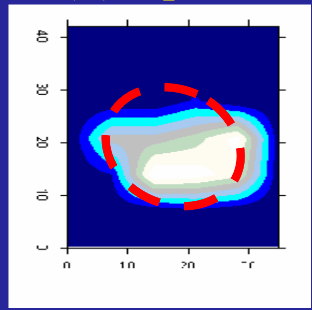
Vernon LP, Seely GR, *The Chlorophylls*, Academic Press, New York, London, 1966, 193.

Summary of Findings

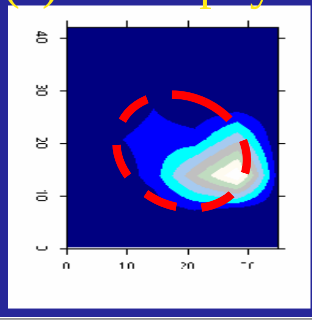
(a)Protein



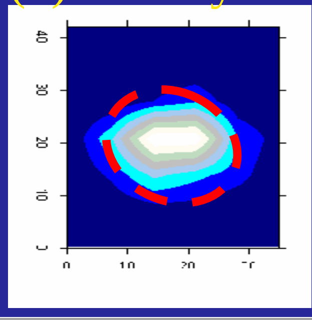
(b)Lipid



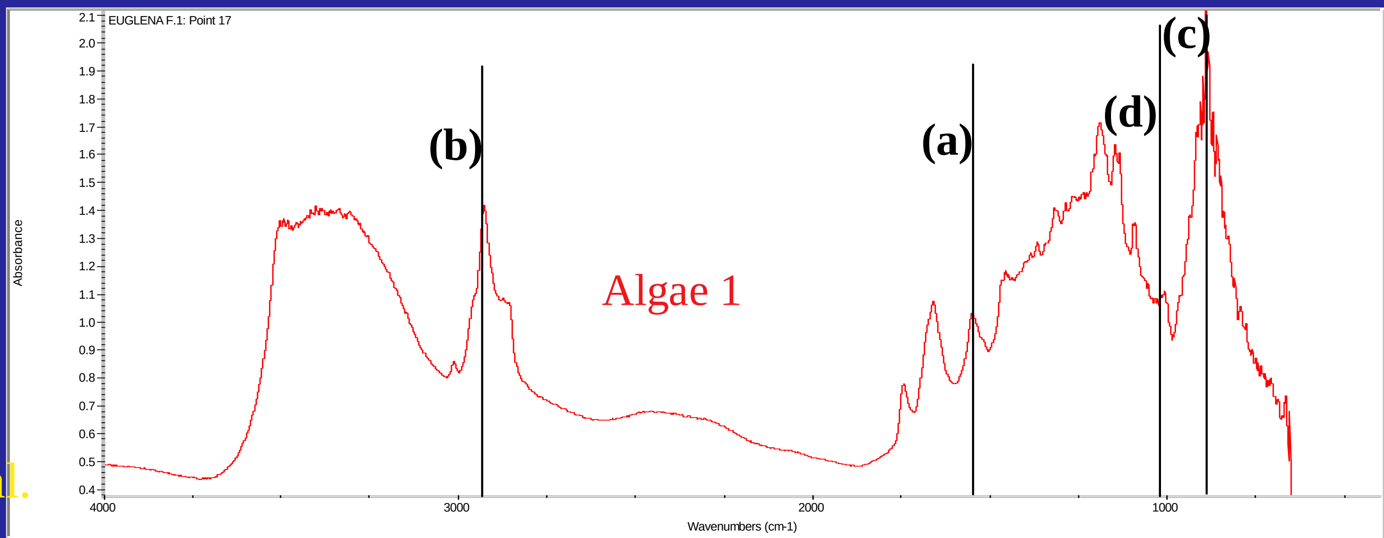
(c)Chlorophyll



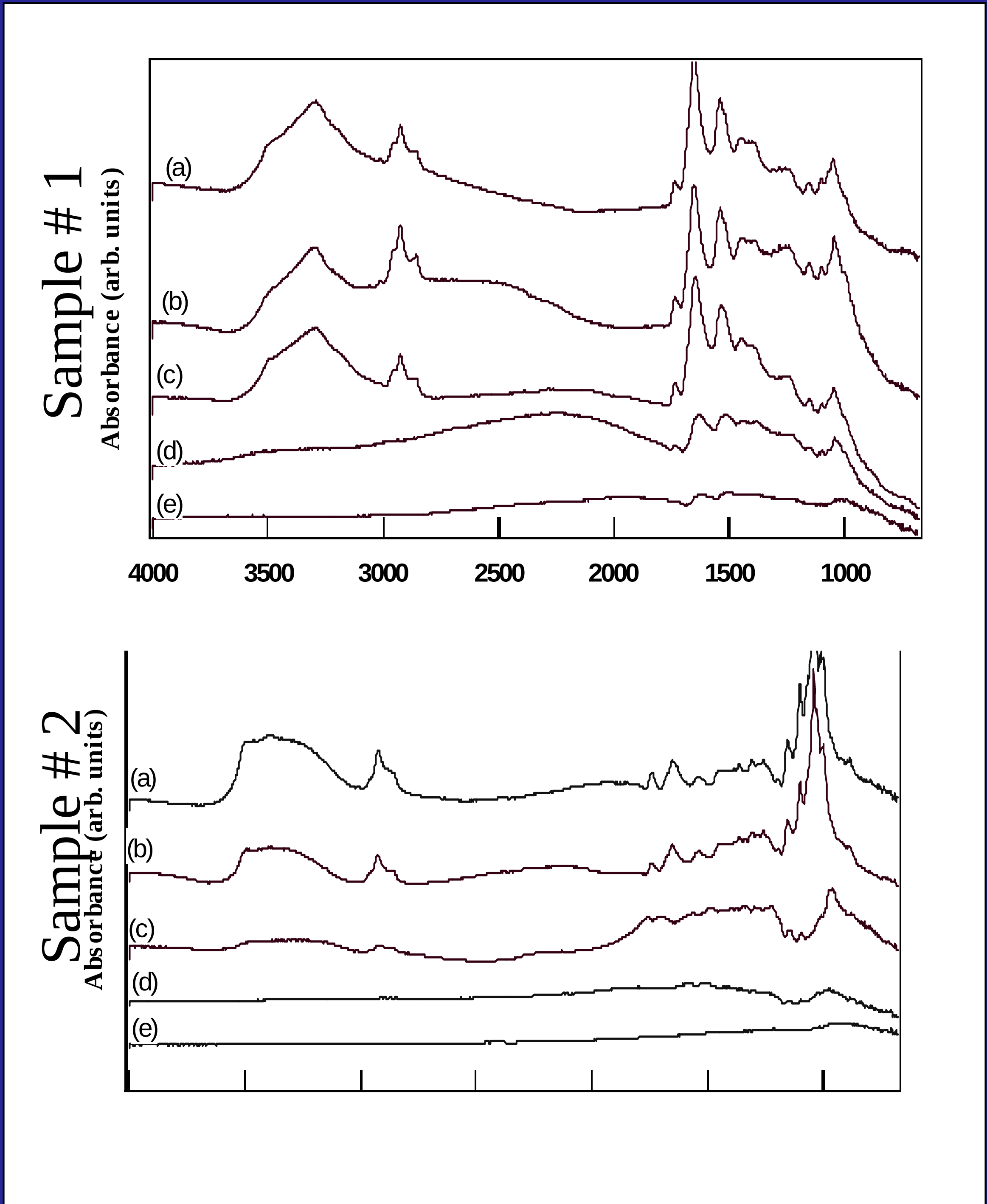
(d) Paramylon



6 µm x 6 µm spectrum at chlorophyll maximum



Hirschmugl et. al.



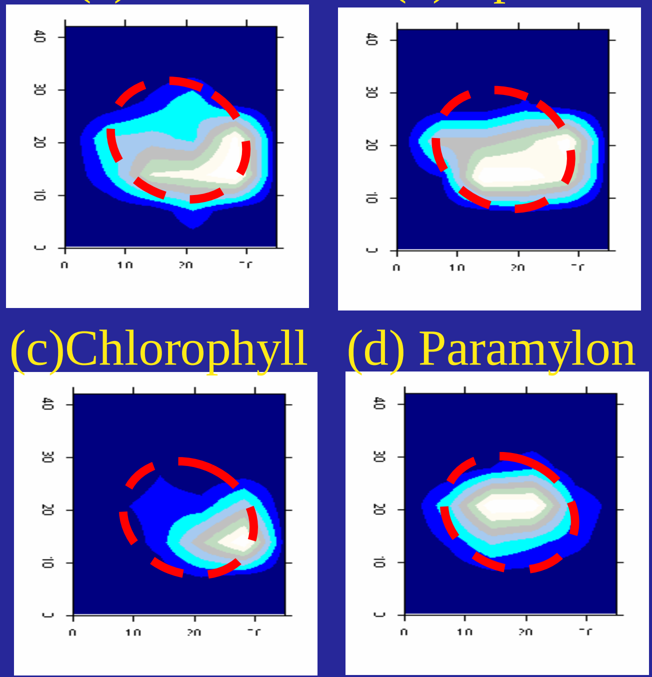
- the protein to carbohydrates ratio :
- sample # 1 alga is 1:0.2
- sample # 2 alga is 1:10
- the relative strength of the lipid to protein band dramatically different
- Sample #2 is nutrient starved
- Conclude: sample # 2 is nitrogen starved

Summary for 2 Samples: *Euglena Gracilis*

Alga #1

(a)Protein (b)Lipid

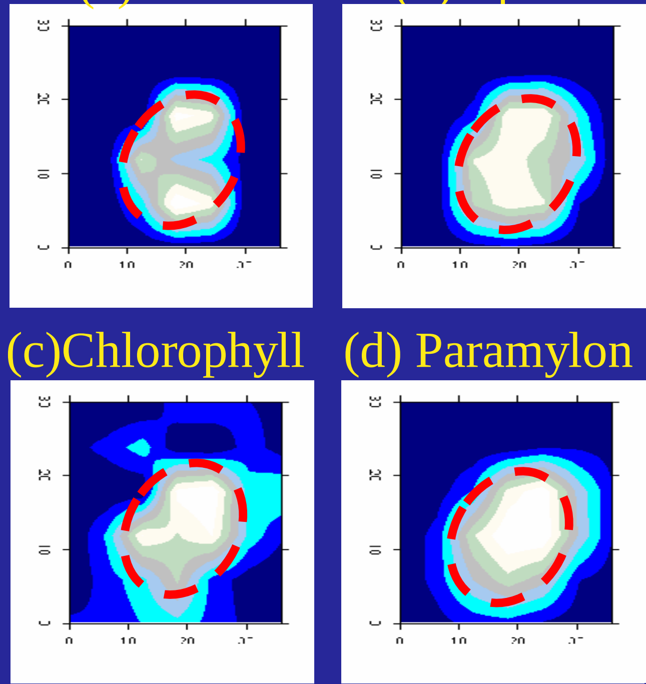
(c)Chlorophyll (d) Paramylon



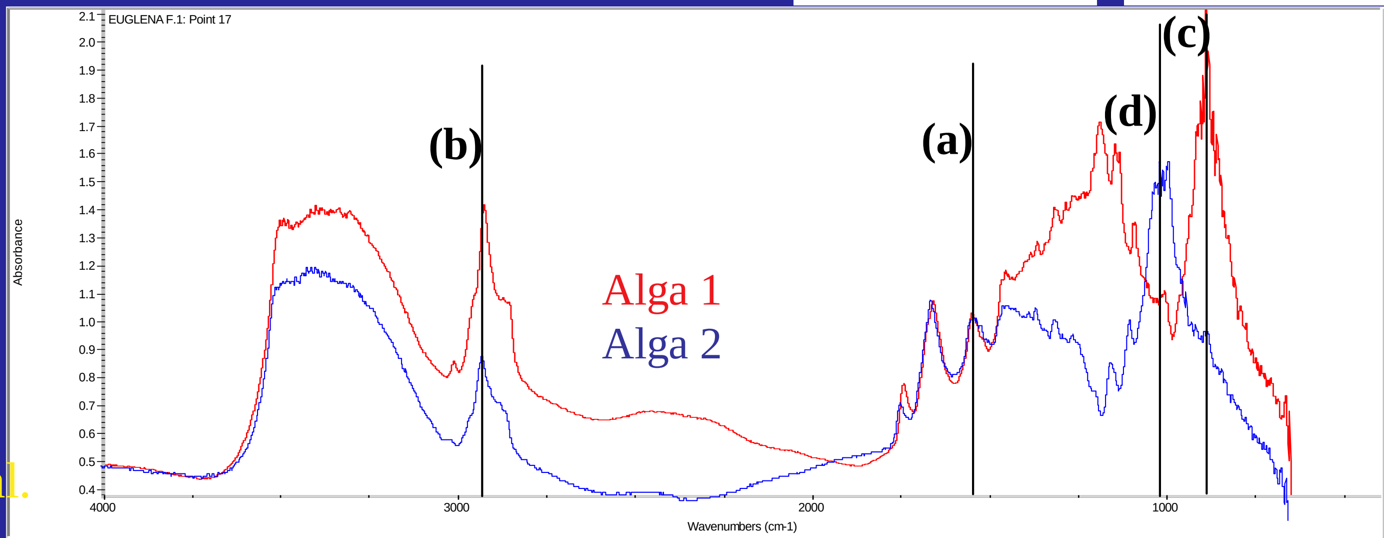
Alga #2

(a)Protein (b)Lipid

(c)Chlorophyll (d) Paramylon



6 μm x 6 μm spectra at chlorophyll maxima



Hirschmugl et. al.

Summary: Algae

IR absorption contour maps of individual alga obtained

Contour maps of individual spectral features
show similar structure as single cell

Chemical Features for two separate alga agree with
Nitrogen Starvation Model

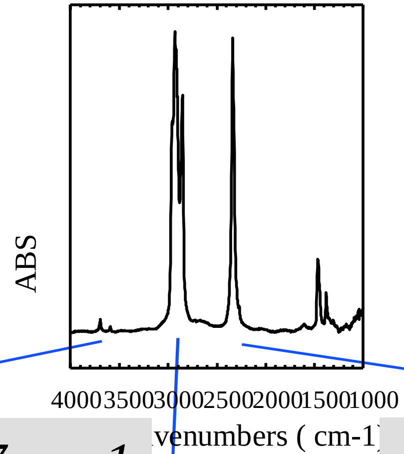
Future

Identify the origin of other ir features

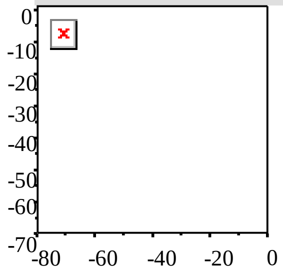
Measure different algae species under
controlled nitrogen environments

Chemical Nature: Inclusions in Geological Specimen

(Guilhaumou et al.)

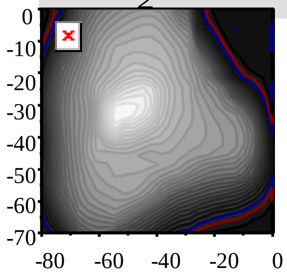


Peak OH at 3697 cm-1



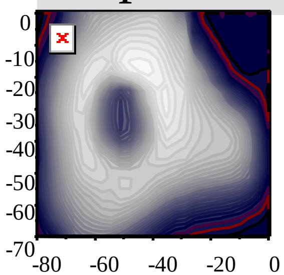
Distance (in µm)

CO_2 vibrational band



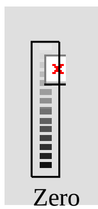
Distance (in µm)

Aliphatic CH stretch



Distance (in µm)

Maximum



Guilhaumou N, Dumas P, Carr GL, and Williams GP, (1998), Appl. Spectroscopy 52 :1029-1034

High Pressure Gas Phase CO_2

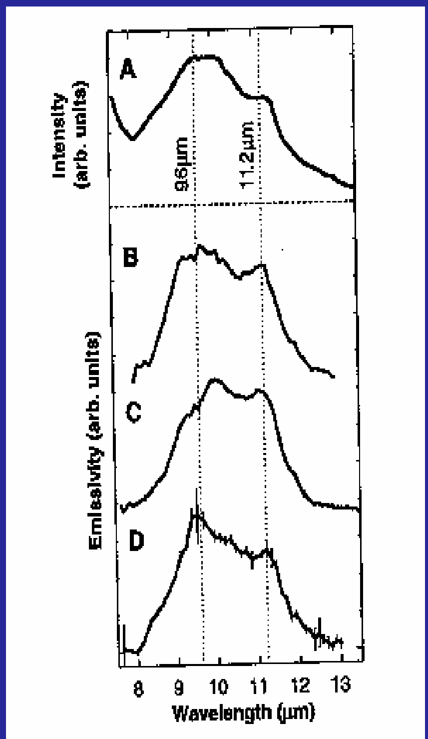
Aliphatic Hydrocarbon (Chains) Liquid Phase Terminated with Hydroxyl Groups

Interplanetary Dust Particles (IDPS)

(Bradley et al.)

Si-O Stretch Region at 10 μm

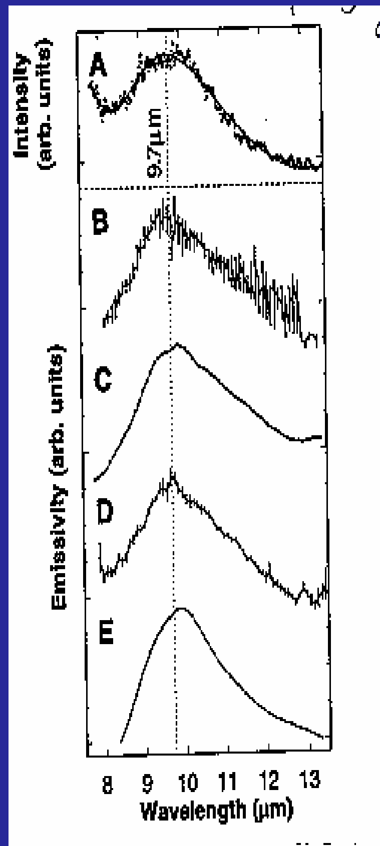
Enstatite 9.6 μm + Fosterite 11.2μm + glassy silicates (broad)



Absorption Spectrum
A: GEMS rich IDP

Emission Spectra
B: Comet Halley
C: Comet Hale-Bopp
D: Late Stage Herbig star

Amorphous silicates



Absorption Spectrum
A: GEMS

Emission Spectra
B: Elias Molecular Cloud
C: Trapezium Molecular Cloud
D: T Tauri Young stellar object DI Celphi
E: M type super gienat μ-Cephei

Bradley JP, Keller LP, Snow TP, Hanner MS, Flynn GJ, Gezo JC, Clemett SJ, Brownlee DE, Bowey JE, (1999) Science 285: 1716-1718

Interplanetary Dust Particles (IDPS)
(Bradley et al.)

Conclusions:

Presolar Interstellar Molecular Cloud could consist of GEMS

Presume solar system formed from intermolecular cloud.

Long sought building block of Solar System.

Environmental Remediation of Soil

(Ghosh et al.)

Goal:

Examine Aliphatic and Polyaromatic Hydrocarbons (PAH) sorbed onto different components in sediment e.g. silica, coal wood

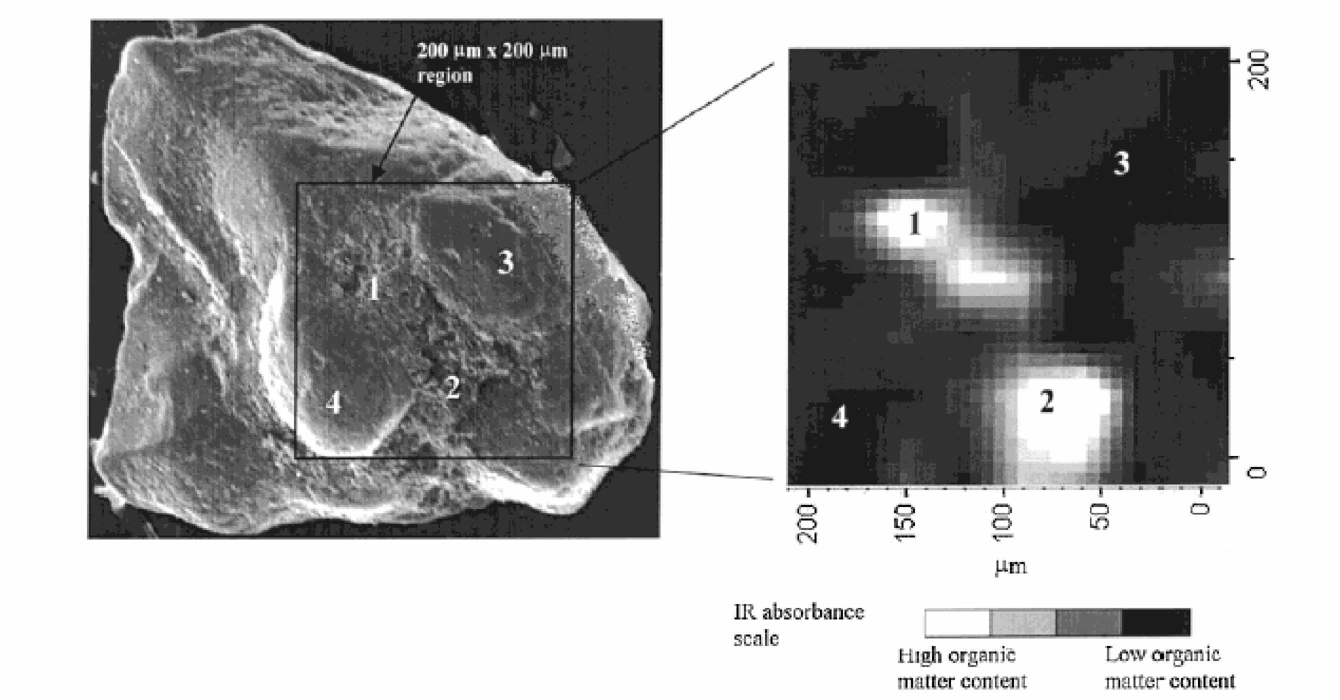
Experimental methods (combination of techniques):

IR microspectroscopy
Scanning Electron Microscopy
Laser desorption/laser ionization mass spectrometry

Aliphatic HCs: linear chains of hydrocarbons
C-H stretches $\Rightarrow$ 2800-3000 cm^{-1}
Aromatic HCs: cyclic hydrocarbons
C-H stretched $\Rightarrow$ above 3000 cm^{-1}

Ghosh U, Gillette JS, Luthy RG, and Zare R, (2000), Environ. Sci. Technol, 34: 1729-1736

Environmental Remediation of Soil



Scanning Electron Microscope (SEM) image of a silica particle having patches of organic matter as indicated by the white regions in the IR mapping of the C-H stretching absorbance (2800 – 3000 cm^{-1}) shown in the right panel. (Ghosh et al.)

Conclusions:

- Silica
  - Aliphatic HCs are sorbed in patches
  - Polyaromatic are sorbed in coexistance with Aliphatic HCs
- Coal and Wood
  - Aliphatic are uniformly distributed throughout
  - Polyaromatic are concentrated at surfaces, with higher concentration than on silica

Bone Composition: Phosphate Bands
(Miller et al.)

Goal:

Determine acid phosphate content and mineral crystallite perfection next to an osteon in bone.

Experimental methods (combination of techniques):

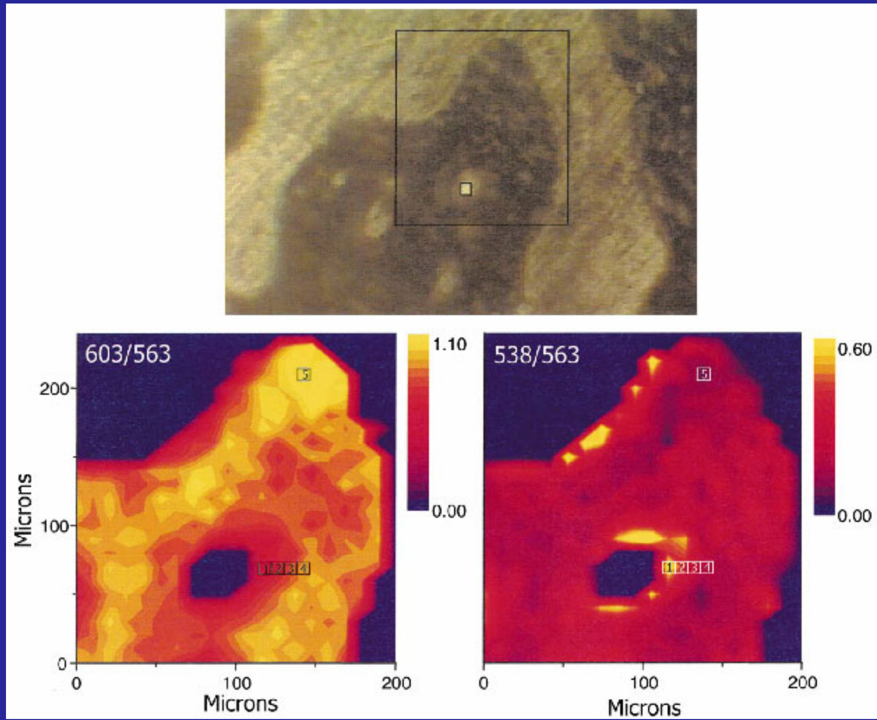
IR microspectroscopy
X-Ray powder diffraction
Correlation between synthetic hydroxyapatite crystals and natural bone powders of various species and ages.

Phosphate bands (PO_4^{3-}) between 500-650 cm^{-1}:

603/563 (ratio of absorption strengths) ⇒ Crystallite content
538/563 (ratio of absorption strengths) ⇒ Acid Phosphate content

Miller LM, Vairavamurthy V., Chance M., Mendelsohn R.,Paschalis EP, Betts F, Boskey AL, (2001) Biochimica at Biophysica Acta 1527 (2001) 11-19.

Bone Composition: Phosphate Bands
(Miller et al.)



Optical and infrared images of human, osteopetrotic, trabecular bone. Data were collected at Beamline U4IR at the NSLS using a Spectra Tech Irμs microscope and a Cu doped Ge detector (Infrared Laboratories). Apertures were sent at 12 by 12 μm and 128 scans were collected at 4 cm^{-1} resolution. Infrared images were generated by plotting peak height ratios of (left) 603/563 cm^{-1} and (right) 538/563 cm^{-1}. Miller et al.

Conclusions:

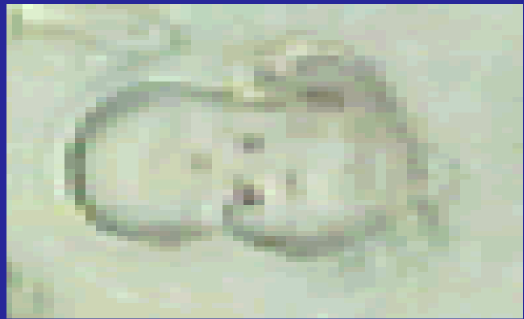
As bone matures average crystallite size increases
Acid Phosphate is found in new bone and decreases as bone ages
Thus, the acid phosphate content and the crystallinity are inversely related.

The most dramatic changes in osteon composition occur within 30 microns of the site of the osteon center.

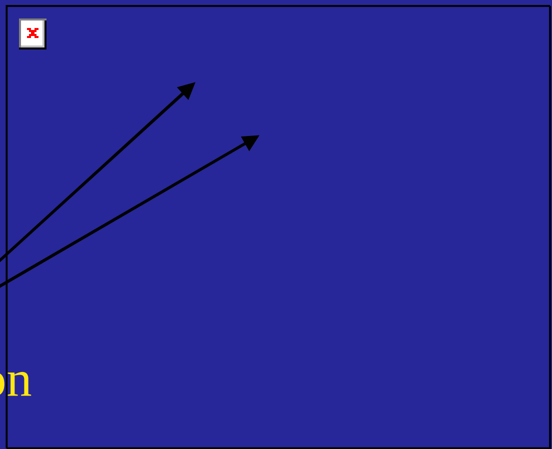
Chemical Imaging of Living Cells: Dividing Cell

(Jamin et al.)

Optical Image

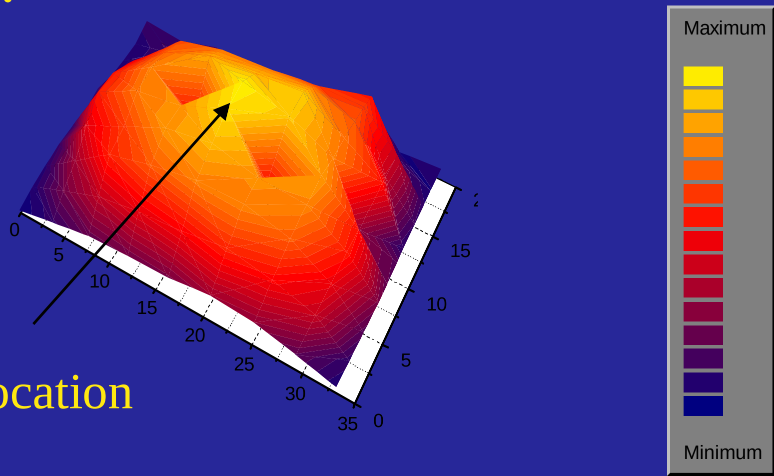


Amide II
Nucleus



Nucleus location

CH Aliphatic
Lipids

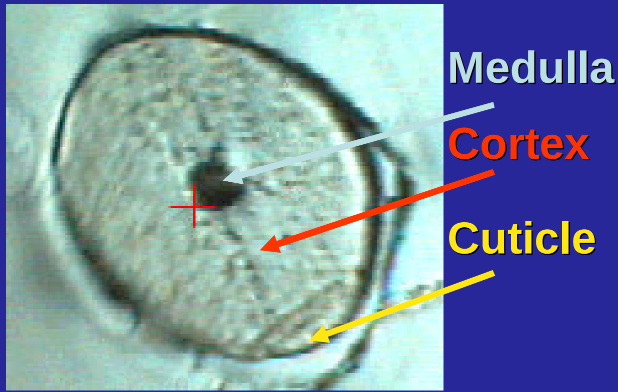


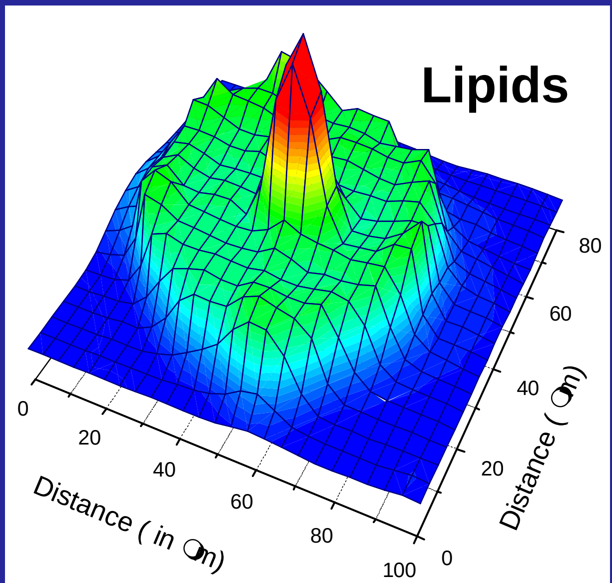
Lipids location

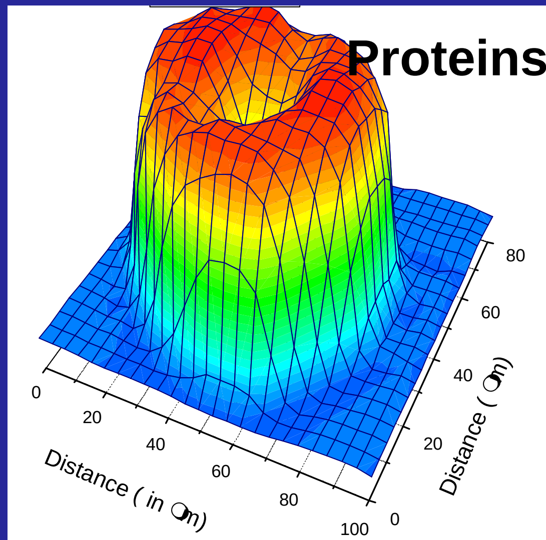
Lipids concentrated at the contractil ring, where the cleavage furrow is located

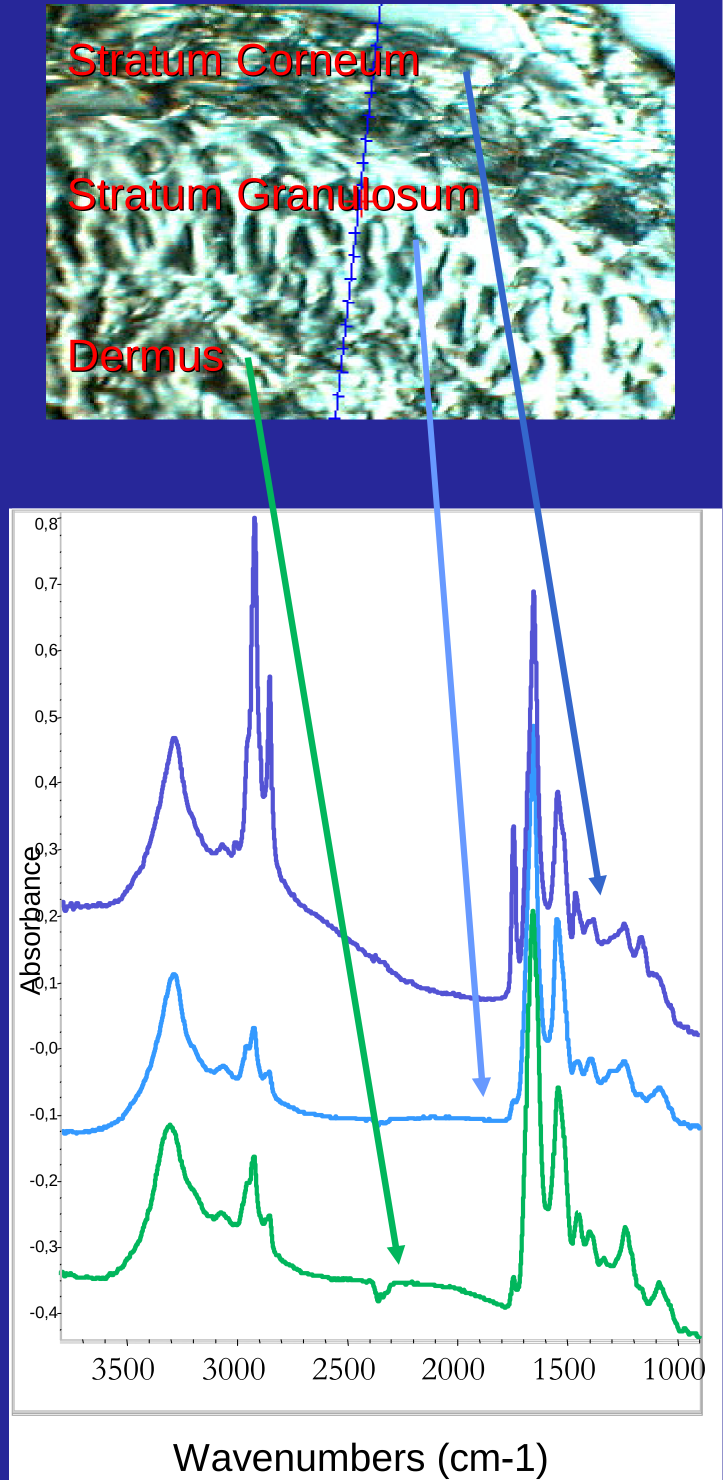
Jamin N, Dumas P, Moncuit J, Fridman W-H, Teillaud J-L, Carr GL, Williams GP, (1998) Proc. Natl. Acad. Sci. 95:4837-4840

Chemical Imaging of Hair and Skin









Paul Dumas et al., Physics World, May 2001 , 2223

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