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

Carol Hirschmugl
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

\[ P = Qr \]

- \( Q \) is charge
- \( r \) is distance between the charges
- \( P \) is dipole

Which of the following molecules are dipoles:

\( \text{O}_2, \text{CO}, \text{N}_2, \text{NO} \)?
What is a Dynamic Dipole

Separated charge distribution that is moving
Which of the following would be Dynamic Dipoles:

- Vibrating $\text{N}_2$
- Vibrating $\text{CO}$
- Vibrating $\text{O}_2$
- Vibrating $\text{NO}$
- Vibrating $\text{CO}_2$?

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:
N₂, O₂, CO₂, CH₄, H₂O

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

**X-H Stretching**

Wavenumbers (cm\(^{-1}\))

- 4000
- 3000
- 2000
- 1000
- 0

fingerprint region
The energy for each vibration is dependent upon the mass and spring constant, IR absorption spectroscopy is chemically specific.
## Sources of Infrared Light

<table>
<thead>
<tr>
<th>Blackbody Radiation</th>
<th>Storage Ring Radiation</th>
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<tbody>
<tr>
<td>Hot filament or rod (Globar or Nernst Glowar)</td>
<td>From relativistically accelerated charged particles</td>
</tr>
<tr>
<td>Flux radiated in all directions</td>
<td>Flux radiated in a tangential, well defined cone</td>
</tr>
<tr>
<td>Flux $\propto$ Temperature</td>
<td>Flux $\propto$ Beam current</td>
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Synchrotron Characteristics: IRSR

Flux

IR Flux $\propto$ Beam Current

IR Source: wide beam profile
Large opening angle
Mixed polarization

Beam Profile
Why use a storage ring source?

**Storage Ring**
- Small source size
- Small solid angle

**Spectromicroscopy**
- Very small sample
  - $1 \, \mu m \times 1 \, \mu m$

**Grazing incidence reflection-absorption spectroscopy**
- Very small angular acceptance
  - $< 5^\circ$

**Globar**
- Large source
- Large solid angle

**Small sample**
- $30 \, \mu m \times 30 \, \mu m$

**Small angular acceptance**
- $> 10^\circ$
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
IR and UV
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
NSLS U4IR and U2B Infrared Beamlines

Off axis Paraboloid
Infrared Beamline Overview:
NSLS Beamline U4IR

- NSLS Source (Reflected From Below)
- Elliptical Mirror
- Diamond Window
- Michelson Interferometer
- Detector (Bolometer)
- UHV Chamber
FTIR Michelson Interferometer
Rapid Scan Michelson Interferometer

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

+ “Noise” from Electron beam motion
  \((\text{e.g. 100 Hz})\)

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

Moving mirror velocity = 1 cm/s

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

+ Signal Frequency “Noise”
  \((100 \text{ cm}^{-1} \text{ spike})\)
Thermo Nicolet Infrared Microscope at ALS

Collimated beam from Storage Ring

FTIR Spectrometer

Infrared Microscope
Infrared Microscope

- Synchrotron Radiation
- Michelson Interferometer
- Schwarzchild Objectives
- Sample
- x-y motorized stage
- Detector
Infrared Beamlines U12, U10A and U10B at the NSLS
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
Particles collected from the MIR shuttle

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)
Chemical Fingerprint of Ink

Wilkinson et al., Physics World, March 2002, 43
Infrared Microscopy: IR Imaging of Living Cells

*Euglena gracilis*

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WATER INSTITUTE, University of Wisconsin Milwaukee

and

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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

Drying algae

Gold plated slide

Measured in reflection geometry, Infrared light is absorbed by the algae twice.
Chemical Mapping and Chemical Image:

Frequency (cm⁻¹) X,Y Position

Infrared Spectra
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

Euglena gracilis and IR spectra

Spectrum a

Spectrum b

Spectrum c

Spectrum d
Lipids

a. C=O functional group

\[ \text{C=O Stretch vibration at } 1742 \text{ cm}^{-1} \]

b. CH\(_2\) functional group

\[ \text{Asymmetric Stretch} \]
\[ \text{Symmetric Stretch} \]

\[ \text{symmetric CH}_2 \text{ stretch vibration at } 2852 \text{ cm}^{-1} \]
Amides I

$\text{CH}_3\text{CH}_2\text{CH}_2-\text{C}\\\text{H}\\\text{H}\\\text{N}\\\text{H}$

- 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

$\text{CH}_3\\\text{N}\\\text{H}$

- Amides II peak at 1545 cm\(^{-1}\) represents 60% N-H bending and 40% C-N stretching vibration

a. NH\(_2\) functional group

- Asymmetric Stretch
- Symmetric Stretch
- Scissors Mode
Euglena gracilis and Chemical Map: Proteins

**AMIDES I**
Water
Peak 1650 cm$^{-1}$

$CH_3CH_2-NH_2$

**AMIDES II**
Peak 1545 cm$^{-1}$

$CH_3CH_2-NH$
Chlorophyll Spectrum

Summary of Findings

(a) Protein  (b) Lipid
(c) Chlorophyll  (d) Paramylon

6 µm x 6 µm spectrum at chlorophyll maximum

Algae 1

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\(^{-1}\)**

**CO\(_2\) vibrational band**

**Aliphatic CH stretch**

**Distance (in µm)**

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

Si-O Stretch Region at 10 µm

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

Amorphous silicates

Absorption Spectrum  
A: GEMS rich IDP

Emission Spectra

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

Absorption Spectrum  
A: GEMS

Emission Spectra

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

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.
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 \( \Rightarrow 2800-3000 \text{ cm}^{-1} \)

Aromatic HCs: cyclic hydrocarbons
C-H stretched \( \Rightarrow \) above 3000 cm\(^{-1}\)
Conclusions:

Silica
Aliphatic HCs are sorbed in patches
Polyaromatic are sorbed in coexistence with Aliphatic HCs

Coal and Wood
Aliphatic are uniformly distributed throughout
Polyaromatic are concentrated at surfaces, with higher concentration than on silica

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.)
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 ($\text{PO}_4^{3-}$) between 500-650 cm$^{-1}$:
603/563 (ratio of absorption strengths) $\Rightarrow$ Crystallite content
538/563 (ratio of absorption strengths) $\Rightarrow$ Acid Phosphate content

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

CH Aliphatic
Lipids

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

Chemical Imaging of Hair and Skin

Paul Dumas et al., Physics World, May 2001, 2223
Collaborators and Funding

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