Fourier-Transform Infrared Spectroscopy

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- Introduction to infrared and IR spectroscopy
- How an FTIR bench works
- Why we use a synchrotron
- Some examples

Ernest Orlando Lawrence Berkeley National Laboratory

William Herschel

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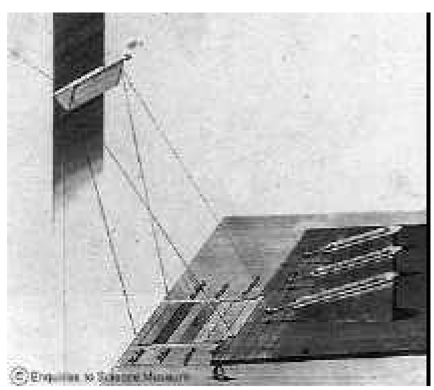
Around 1800, Herschel studied the spectrum of sunlight using a prism. He measured the temperature of each color, and found the highest temperature was just beyond the red, what we now call the 'infrared'.



The Science Museum, UK

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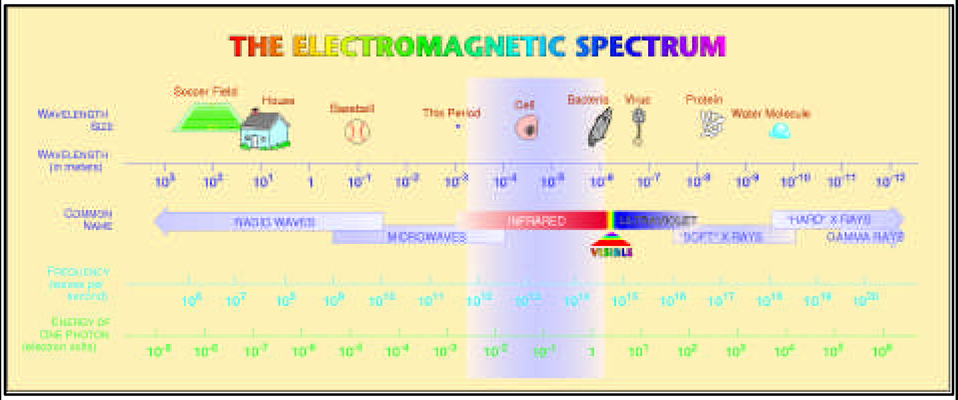
The Infrared Part of the EM Spectrum



IR units: wavenumbers (cm^{-1}),
10 micron wavelength = 1000 cm^{-1}
1 eV ≈ 8100 cm^{-1}
1 THz ≈ 33 cm^{-1}
300 Kelvin ≈ 210 cm^{-1}

Near-IR: 4000 – 14000 cm^{-1}
Mid-IR: 500 – 4000 cm^{-1}
Far-IR: 5 – 500 cm^{-1}

IR covers ~ 1 meV to 1 eV

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What can we learn from IR spectroscopy?

- **Atoms vibrate with frequencies in the IR range**
- **Chemical Analysis:**
  - Match spectra to known databases
    - Identifying an unknown compound, Forensics, etc.
  - Monitor chemical reactions *in-situ*
- **Structural ideas:**
  - Can determine what chemical groups are in a specific compound
- **Electronic Information:**
  - Measure optical conductivity
    - Determine if Metal, Insulator, Superconductor, Semiconductor
    - Band Gaps, Drude model

Contact-less Measurements

- **Much easier to mount & measure samples**
- **Can work with solids, liquids, gases**
- **Is easier to vary other sample properties via**
  - Temperature (cryostats, heaters)
  - Pressure (Diamond Anvil Cells)
  - Magnetic Field

Optical Spectroscopy Equations

- **Things we want to know about a sample:**
  - Index of refraction: $N(\omega) = n + ik$
  - Conductivity: $\sigma(\omega) = \sigma_1 + i\sigma_2$ (All complex functions)
  - Dielectric function: $\varepsilon(\omega) = \varepsilon_1 + i\varepsilon_2$
- These are all related:

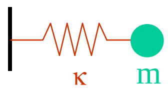
$$\varepsilon(\omega) = 1 + \frac{4\pi i}{\omega}\sigma(\omega) \qquad N(\omega) = \sqrt{\varepsilon(\omega)}$$

- Kramers-Kronig relations hold:

$$\sigma_2(\omega) = -\frac{2\omega}{\pi}\int_0^\infty \frac{\sigma_1(\omega')d\omega'}{\omega'^2 - \omega^2}$$

- **Optical measurements:**
  - Reflectivity: $R(\omega) = \left|\frac{\sqrt{\varepsilon} - 1}{\sqrt{\varepsilon} + 1}\right|^2 = \frac{(1-n)^2 + k^2}{(1+n)^2 + k^2}$
  - Transmission: $T(\omega) = |t(\omega)|^2 = \left|\frac{4N(\omega)}{(1+N(\omega))e^{-2\pi i d N\omega} - (1-N(\omega))e^{2\pi i d N\omega}}\right|^2$

A Simple Oscillator



Imagine the mass is an atom, carrying a charge. This will then couple to the electric field in light.

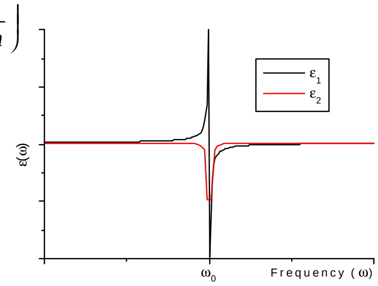
$$Ideal: m\ddot{x} = -\kappa x, \omega_0 = \sqrt{\frac{\kappa}{m}}$$

$$General: m\ddot{x} = -\kappa x - \gamma\dot{x} + E(t), \gamma = damping, E(t) = qE_0 e^{-i\omega t}$$

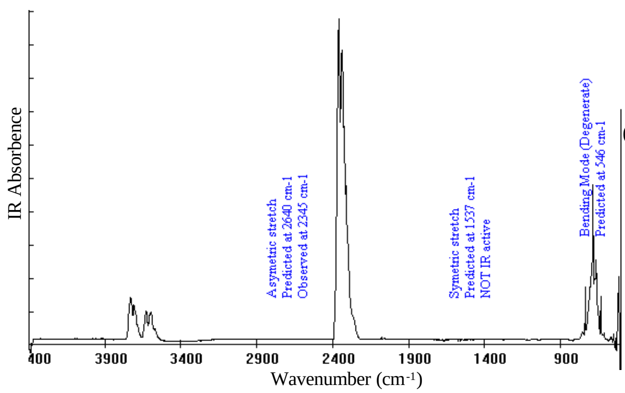
$$Solution: x = \left(\frac{qE_0}{m\omega^2 - \kappa + i\omega\gamma}\right)e^{-i\omega t} = \left(\frac{qE_0/m}{\omega^2 - \omega_0^2 + i\omega\frac{\gamma}{m}}\right)e^{-i\omega t}$$

$$\omega_p = \sqrt{\frac{4\pi q^2}{m}}, \chi(\omega) = \frac{x}{E(t)}, \varepsilon(\omega) = 1 + 4\pi\chi(\omega)$$

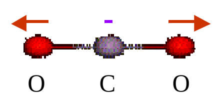
$$\varepsilon(\omega) = 1 + \frac{-\omega_p^2}{\omega^2 - \omega_0^2 + i\omega\frac{\gamma}{m}}$$



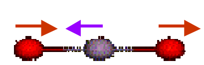
An Example: CO_2



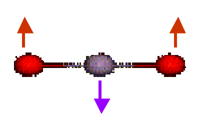
A Dipole Moment = charge imbalance in the molecule



Symmetric Stretch
(Dipole moment = 0 so **not** IR active)

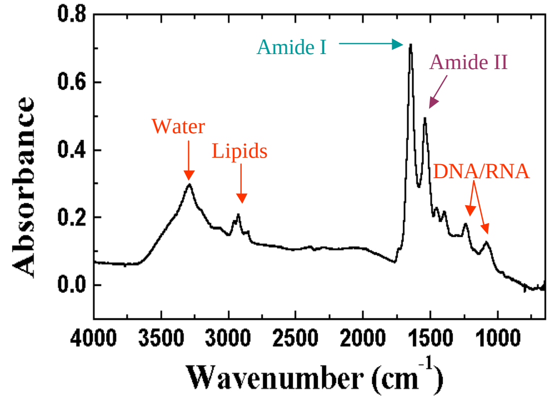


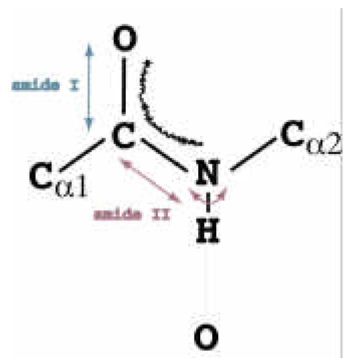
Asymmetric Stretch
(Has dipole moment so IR active)



Bending Mode
(Has dipole moment so IR active)

Example infrared spectrum of a biological system





Typical IR absorbance positions:

Protein Amide I:	1690-1600
Protein Amide II:	1575-1480
Lipid $=CH_2$:	3100-3000
Lipid $-CH_2$, $-CH_3$:	3000-2850
Nucleic Acid $-PO_2^-$:	1225, 1084

The peak positions of Amide I and II are sensitive to the protein secondary structure (α-helix, β-sheet, random coils, etc.)

A good reference: Mantsch and Chapman, *Infrared spectroscopy of biomolecules*. 1996, New York: Wiley-Liss.

Drude Model of a Metal

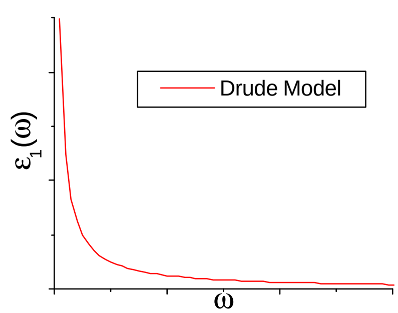
Electrons in a metal are free, but collide at an average time τ.

$$\sigma = \frac{ne^2\tau}{m}$$

Or in optical terms:

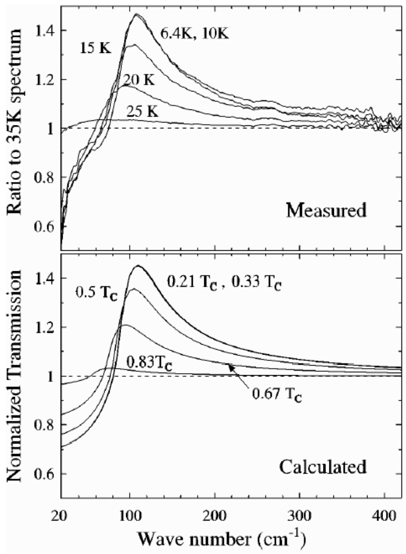
$$\varepsilon(\omega) = \frac{-\omega_p^2}{\omega^2 + i\frac{\omega}{\tau}}$$

This is exactly an oscillator at $\omega_0 = 0$.



An Example: Superconducting Rb_3C_{60}

A superconducting gap opens up below T_C. From these measurements we determined that $2\Delta = 4.1\ k_BT_C$.

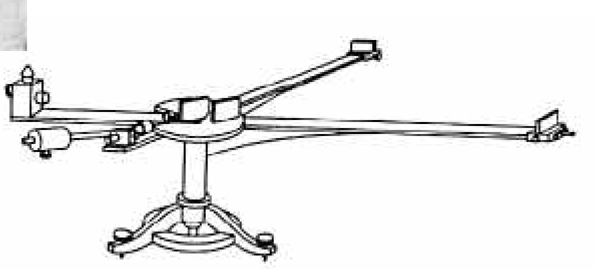


Koller, Martin, Mihaly, Mihaly, Oszlanyi, Baumgartner and Forro. Phys. Rev. Lett. **77**, 4082 (1996).

Albert Michelson (1852-1931)

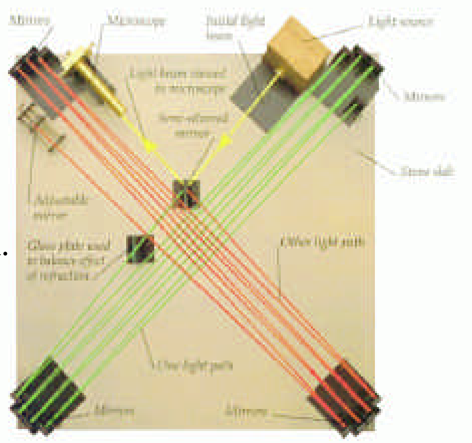
Michelson wanted to measure the speed the the earth moves through the ether (the medium in which light travels). By measuring the interference between light paths at right angles, one could find the direction & speed of the ether.

Michelson's first interferometer (1881)



Michelson-Morley Experiment

Michelson-Morley interferometer (1887)



Still no fringes → No ether.
The speed of light is constant.
A new physics of light was needed.

"My honored Dr. Michelson, it was you who led the physicists into new paths, and through your marvelous experimental work paved the way for the development of the theory of relativity." – Albert Einstein, 1931.

More about Michelson

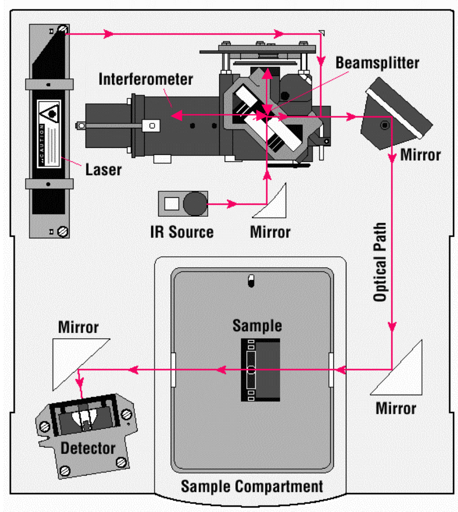
Michelson became the first American to win the Nobel Prize in physics in 1907.

He continued pioneering optical measurements:

- The speed of light
- The size of stars
- Using a particular wavelength of light as a distance standard

How an FTIR Spectrometer Works

A Simple Spectrometer Layout



Pathlength difference = x

The intensity detected of two plane waves:

$$I = |\vec{E}|^2 = |E_1|^2 + |E_2|^2 + 2\vec{E}_1 \bullet \vec{E}_2 \cos(\theta)$$

Normal incidence, θ = kx, can simplify to:

$$I(x) = 2[1 + \cos(kx)]$$

For non-monochromatic light:

$$I(x) = \int_0^\infty [1 + \cos(kx)] G(k) dk$$

$$= \int_0^\infty G(k) dk + \int_0^\infty G(k) \frac{e^{ikx} + e^{-ikx}}{2} dk$$

$$= \frac{1}{2} I(0) + \frac{1}{2} \int_{-\infty}^{\infty} G(k) e^{ikx} dk$$

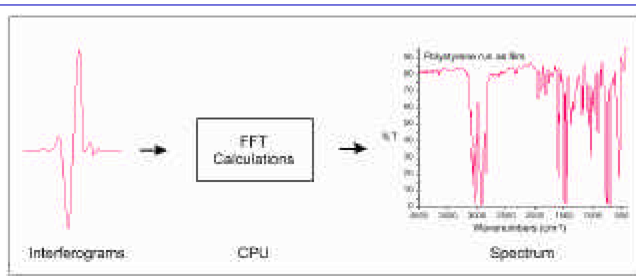
FTIR Math Continued

We can rewrite this to something more familiar:

$$W(x) \equiv \frac{2I(x) - I(0)}{\sqrt{2\pi}} = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{\infty} G(k) e^{ikx} dk$$

A Fourier Transform!

The detected intensity as a function of moving mirror position, I(x), can therefore be converted into G(k), the intensity spectrum as a function of frequency by a simple Fourier transform.



FTIR Spectrometers

In practice one cannot measure from -∞ to ∞. The resolution of a measurement is simply given by how far in x you measure.

$$resolution \propto \frac{1}{2\pi x_{max}}$$

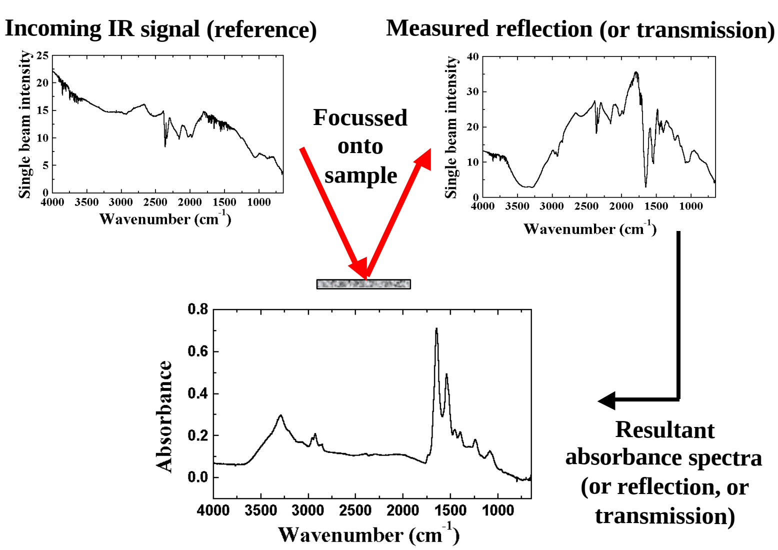
Rapid-Scan measurements:

- Sweep mirror quickly, average many interferrograms
  - Very fast & easy
  - Not high resolution
  - Not for quickly changing signals or very low signal

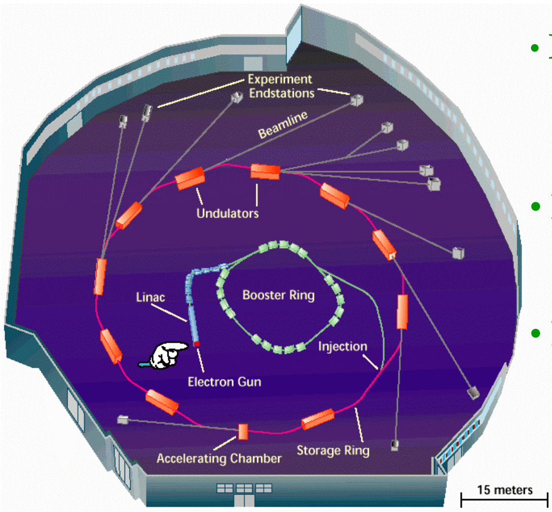
Step-Scan measurements:

- Step to each x position, then measure (long average, or triggered time series). Can have very long path length.
  - Excellent for fast time resolution, low signals (lock-in)
  - Harder to run stably.

Infrared Spectroscopy Measurements

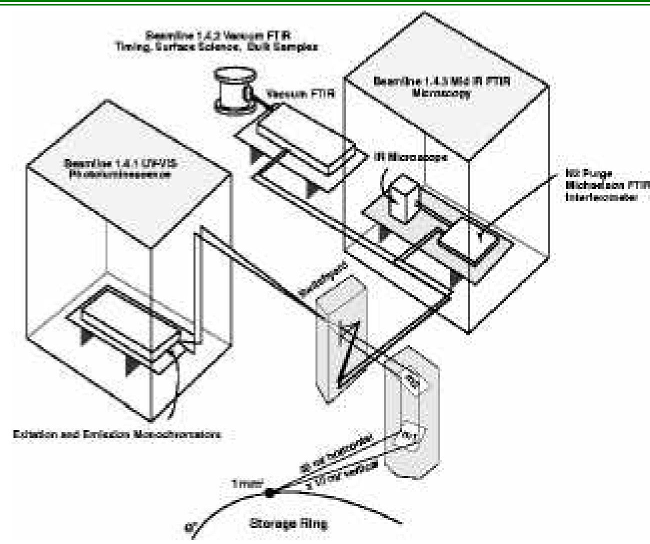


Why use a synchrotron for IR?



- **High brightness**
  - Essentially a point source
  - Can focus light to a diffraction-limited size: Microscopy
- **More far-IR flux**
  - Smaller samples
  - Better signal-to-noise
- **Pulsed source**
  - Light is from electron bunches
  - Fast timing measurements (nsec)

ALS Beamlines 1.4.x Experimental Endstations



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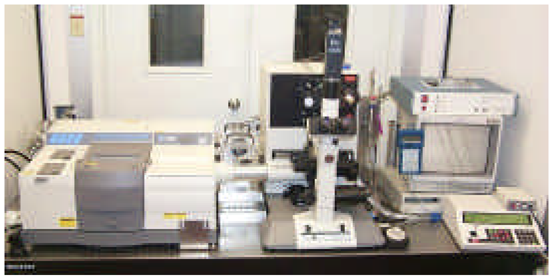
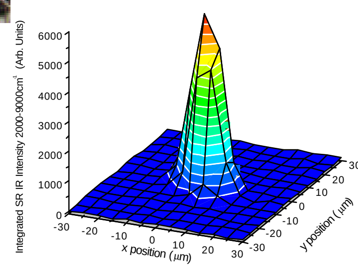
ALS Beamline 1.4.3 FTIR Spectromicroscopy



Nicolet 760 FTIR Bench   Nic-Plan IR Microscope

- $\leq$ 10μm spot size
- Microcooler stage (70 - 740K)
- Grazing incidence objective
- Autofocus capabilities



Applications:
- Single living cells, toxic contaminants, protein microcrystals, rhizoids, water jets, forensic evidence, corroded metals ...

http://infrared.als.lbl.gov/

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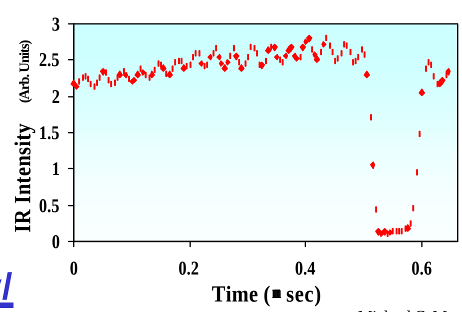
ALS Beamline 1.4.2 FTIR Spectroscopy

Bruker *IFS* 66v/S

- 20 - 25,000 cm^{-1} range
- LHe cryostat (1.4 - 475K)
- 5ns fast timing capabilities
- Grazing incidence UHV chamber



Applications:
- Strongly correlated electron systems, surface chemistry, pump-probe dynamics, corroded metals, ATR cell, ...

http://infrared.als.lbl.gov/

Michael C. Martin

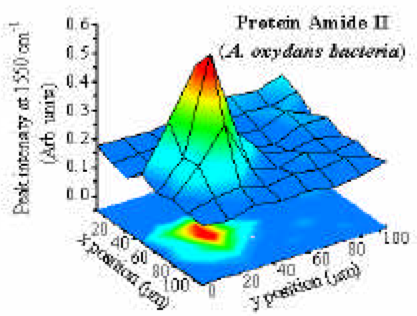
Presently Active User Groups at the ALS IR Beamlines

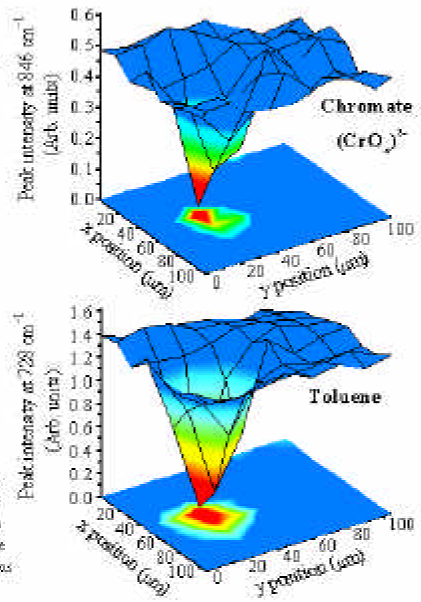
•Arps, Peggy	UC Irvine	Corrosion in metal pipes
•Benning, Lianne	Univ. of Leeds	Cyanobacteria & silification
•Breunig, Thomas	UCSF	Dental research
•Brudler, Ronald	Scripps Institute	Photocycle of PYP
•Chesko, James	Chiron Corp.	Cells & correlation with genomics
•Doner, Harvey	UCB Earth Sciences	Soil sciences
•Erramilli, Shyamsunder	Boston Univ.	Near-field IR microscopy
•Ghosh, Upal	Stanford Univ.	Soil sciences
•Glaeser, Robert	UCB Biochemistry	Bacteriorhodopsin
•Haller, Eugene	UCB Mat. Science	GaN systems
•Heske, Clemens	U. Wuertzburg	Novel Solar Cells
•Holman, Hoi-Ying	LBNL ESD	Microbial transformations
•Huie, Phil	Stanford Pathology Dept.	Single cell metabolism
•Jeanloz, Raymond	UCB Earth Sci.	Water transport in Earth's mantle
•Kauffman, Mary	Idaho National Lab	Bacterial attachment to basalt
•Myneni, Satish	Princeton	Soil chemistry
•Orenstein, Joseph	UCB Physics	Strongly correlated materials
•Raab, Ted	U. Colorado, Boulder	Rhizosphere plants
•Ross, Phil	LBNL, MSD	Electrode surfaces
•Rubinsky, Boris	UCB Engineering	Radiative properties of bio surfaces
•Perry, Dale	LBNL, ESD	Forensic samples
•Saiz, Eduardo	LBNL, MSD	Bioactive glass coatings
•Saykally, Richard	UCB Chemistry	Liquid microjets & near-field
•Simms, Ronald	Utah State Univ.	Water management
•Sigee, David	Univ. of Manchester	Biodiversity in phytoplankton
•Zhang, Miqin	U. Washington	Bio-implants

Infrared Spectromicroscopy Observing Bacterial Remediation of Environmental Contaminants

ALS Beamline 1.4.3

Hoi-Ying N. Holman, Dale L. Perry, Michael C. Martin
Wayne R. McKinney, and Jennie C. Hunter-Cevera
Lawrence Berkeley National Laboratory





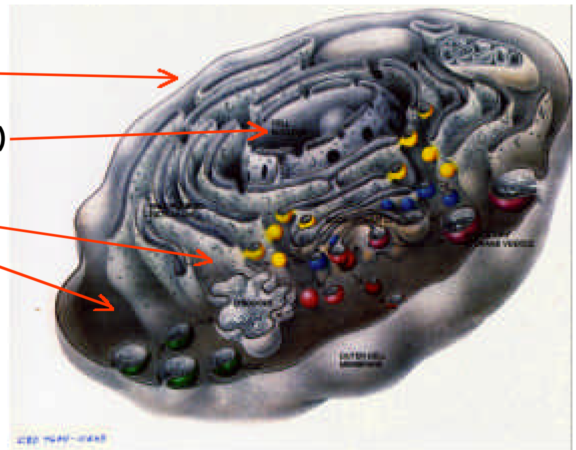
Arthrobacter oxydans bacteria, isolated from a contaminated DOE site in Idaho, attach themselves to magnetite mineral surfaces. We locate the bacteria via their spectral signature (above). We observe a depletion of chromate and toluene (right) by the bacteria after five days of exposure. In this study we will learn how to help *A. oxydans* perform this bioremediation.

Holman et al., Geomicrobiology J., **16**, 307-324 (1999).

Infrared Spectromicroscopy of Individual Living Cells

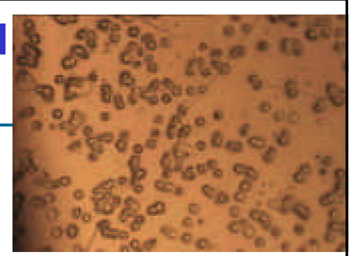
Infrared spectra are obtained from individual living cells.

- **Lipids (cell walls)**
- **Nucleic acids (DNA, RNA)**
- **Proteins**

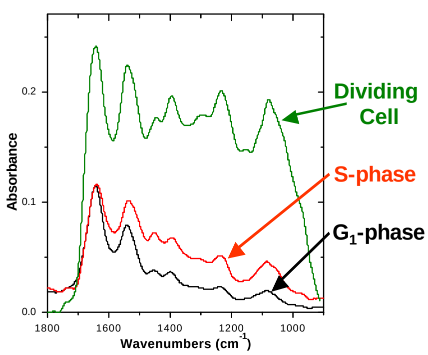


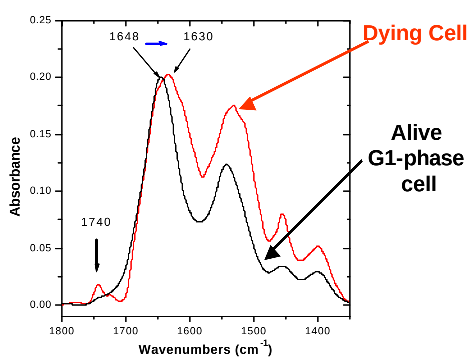
- **Each of these major classes of cellular components have distinct IR markers**

Investigating Individual Living Cells



Hoi-Ying N. Holman, *Center for Environmental Biotechnology*
Eleanor A. Blakely, Kathy Bjornstad *Life Sciences Division*
Regine Goth-Goldstein, Marion L. Russell *Environmental Energy Tech. Division*
Michael C. Martin, Wayne R. McKinney, *Advanced Light Source Division*

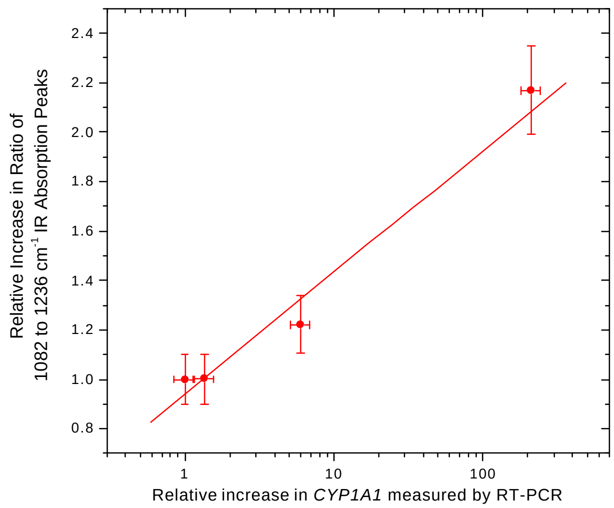




Lung Fibroblast cells (IMR-90)

ALS Beamline 1.4.3

Comparison of TCDD response measured by SR-FTIR and RT-PCR

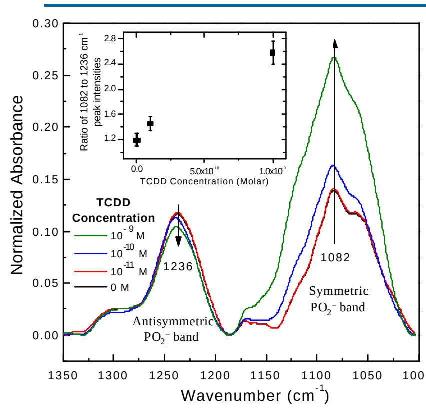


Cells from the same treatments were analyzed for *CYP1A1* expression by an RT-PCR technique.

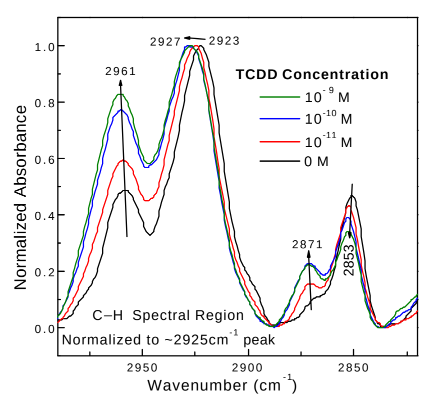
The relative increase in *CYP1A1* expression correlates with the observed PO_2^- infrared bands' intensi changes.

Cellular Responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)

Trypsinized HepG2 cells on gold (37ºC)



Nucleic acid spectral region:
Ratio of symmetric to anti-symmetric band increases with increasing TCDD,
but no shift in peak positions



C–H stretch region:
Relative increase in number of methyl to methylene groups with increasing TCDD.

Hoi-Ying N. Holman, *Center for Environmental Biotechnology,* Eleanor A. Blakely, Kathy Bjornstad *Life Sciences Division*
Regine Goth-Goldstein, Marion L. Russell *Environmental Energy Tech. Division*
Michael C. Martin, Wayne R. McKinney, *Advanced Light Source Division*

ALS Beamline 1.4.3

Ernest Orlando Lawrence Berkeley National Laboratory

Developing SR-FTIR Spectromicroscopy for biomedical research

"Development of Synchrotron Infrared Spectromicroscopy of Individual Living Cells for Biomedical Research Applications"
PI's: Hoi-Ying N. Holman, Michael C. Martin, Wayne R. McKinney
Collaborators: UCSF, Stanford, LBNL
FY01: $124K personnel, $120K equipment (coming soon!) new microscope

Overall goal: Develop equipment and define procedures for medical and biotechnology researchers to best use SR-FTIR.
Specific objectives:

- Build a microscope stage incubator.
- Determine if the SR-IR beam does not alter cell physiology.
- Automatically position SR-IR beam to within 1 μm.
- Software for automated cell location, focus, & measurement.