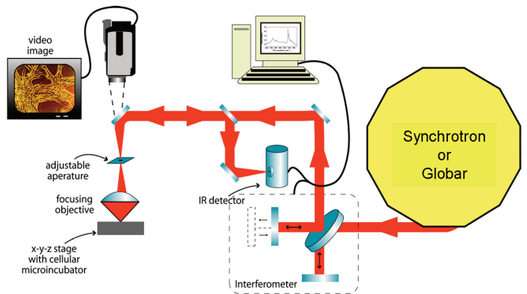
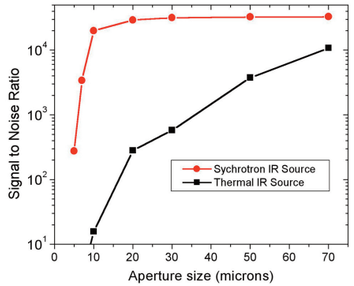
BERKELEY SYNCHROTRON INFRARED
STRUCTUAL BIOLOGY PROGRAM
Real-Time Biochemistry of Living Cells

Advantages of Synchrotron IR Spectromicroscopy

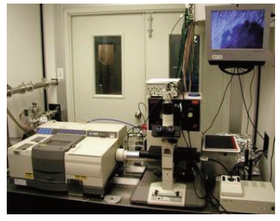
- Diffraction limited spot size (2-10 µm)
- Large signal-to-noise ratios
- High spectral resolution
- Non-invasive (non-ionizing and label free)
- Hyperspectral imaging of living cells

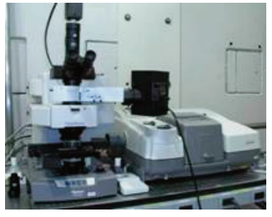


Synchrotron infrared (IR) radiation is 1000 times brighter than a conventional blackbody source. When combined with a microscope, this increased brightness provides diffraction-limited spatial resolution with high signal-to-noise ratios. Because IR is non-ionizing and requires no external labeling, it can be used to chemically image single living cells non-invasively.



Beamline 1.4





SPOT SIZE . . . . . 2-10 µm (diffraction-limited)
SPECTRAL RANGE. . . . . 650 – 10 000 cm-1
SPECTRAL RESOLUTION . . . 0.125 cm-1
ADDITIONAL TECHNIQUES . . Fluorescence, DIC, and polarization capabilities
APPLICATIONS . . . . . Biological and environmental samples, novel compounds, forensic studies, polymers, fibers, particulate contamination, material sciences, and more…

Beamline 5.4 (opening mid 2010)

- Designed to have more flux and wider spectral range than Beamline 1.4
- > 700 square feet for bioprep space and equipment
- 3 endstations, including 2 microscopes and a high resolution (0.001 cm-1) spectrometer
- Plans to develop multi-modal microscopy techniques, including IR, Raman, fluorescence, and near-field imaging

Beamtime is requested at the Advanced Light Source (ALS) by submitting proposals that undergo a peer-review process. This process happens twice a year with deadlines typically in January and July. There are no fees at the ALS for non-proprietary research.

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BERKELEY LAB · ALS ADVANCED LIGHT SOURCE · U.S. DEPARTMENT OF ENERGY

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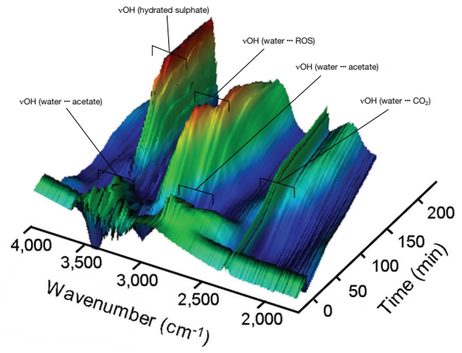
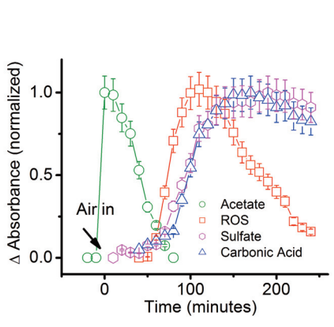
Synchrotron radiation-based Fourier transform infrared (SR-FTIR) microscopy is a label-free non-invasive molecular technique that couples the high brightness of synchrotron radiation with the high throughput and vast analytical capabilities of FTIR spectrometers. With a synchrotron source, FTIR microscopes are capable of diffraction-limited chemical imaging with signal-to-noise-ratios 100-1000 times greater than standard blackbody sources. This enhancement of spatial resolution and signal levels enables investigations of sophisticated microbial biochemistry for a broad range of innovative applications.

Infrared spectromicroscopy for linking microbial stress-adaptive responses to genomic properties

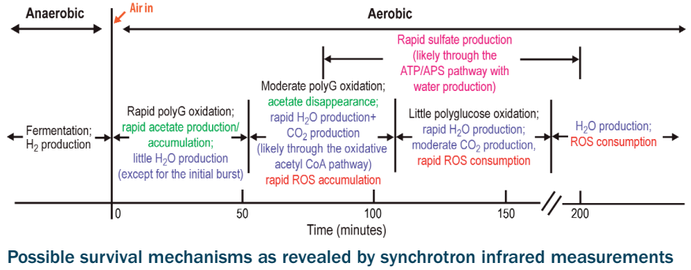
Determining transient chemical properties of the cellular environment can elucidate the paths through which a microbial system adapts to changes in its hostile environment. Understanding such paths will enable us to better utilize sophistications of microbes for a broad range of biotechnology applications. We have investigated how obligate anaerobe *Desulfovibrio vulgaris* cells survive short-term exposure to air by using SR-FTIR to monitor hydrogen bonding changes in cellular water.



A 3-D frequency-time-intensity plot in the hydride-OH dominated stretch region. Positive values arise from νOH of water molecules forming H-bonding with various small molecules including reactive oxygen species (ROS).



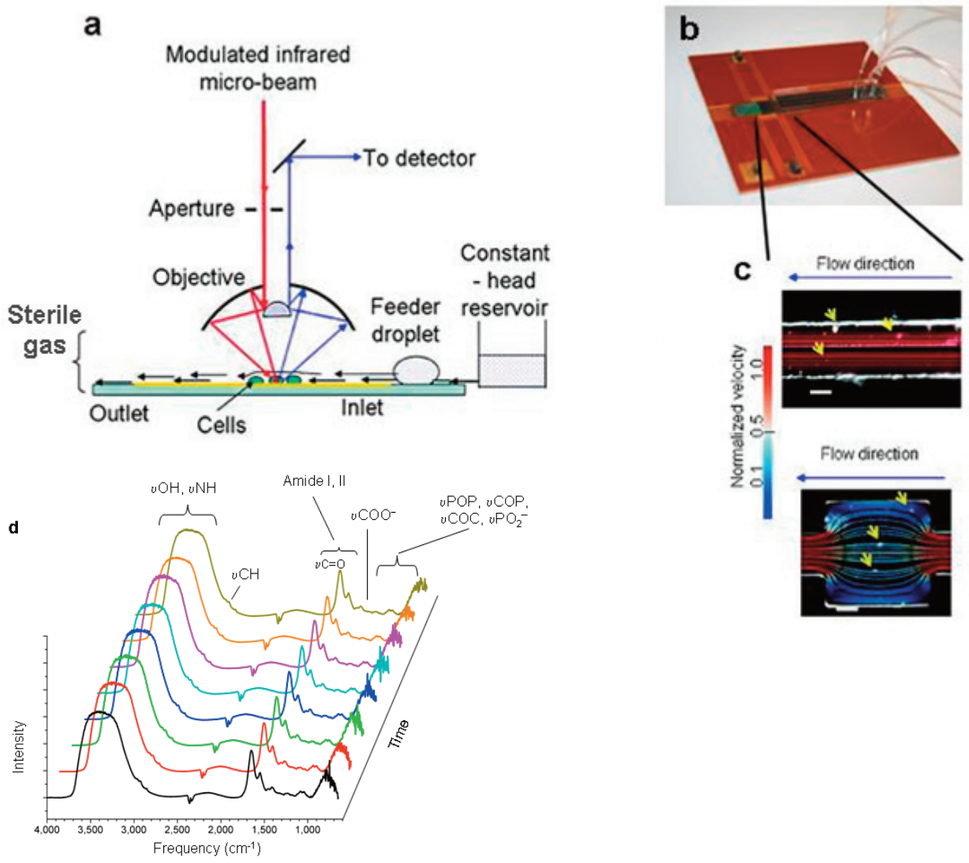
Transient chemistry as seen by the time course of the difference νOH intensity.



Possible survival mechanisms as revealed by synchrotron infrared measurements and analyses. (polyglucose is labeled as PolyG.) Holman et al., *PNAS*, **106**(31), 12599, 2009.

Circumventing water absorption barrier for studying microbes in aqueous environments

Microbes often form structured dynamic communities of aggregated cells enclosed in a self-produced polymeric matrix that adheres to both inert and living surfaces in aqueous environments. Aqueous environments hinder SR-FTIR's sensitivity of bacterial activity. The recent development of *in situ* open-channel microfluidic culturing systems (team with the Microfluidics Systems Group at Lawrence Livermore National Laboratory) circumvents this water-absorption barrier, enables real-time chemical imaging of bacterial activities in biofilms, and offers opportunities to facilitate comprehensive understanding of the structural and functional dynamics in a wide range of microbial systems.



Clockwise: (a) Microfluidic SR-FTIR microscopy platform design and setup. (b) Plane view depicts an example chip with several parallel etched microstructures for multiple simultaneous experiments. (c) Flow maps of the microstructures, simulated from experimentally measured path-lines of near-neutral density polystyrene beads (yellow arrows), are superimposed on a snapshot image of the flow visualization experiment: top, flow in a microchannel; bottom, flow in a microwell. Scale bars = 10 micrometer. Velocity = ~ 60 µm/s. (d) Real-time "raw" SR-FTIR spectra of anaerobic microbial micro-colonies [modified from Holman, et al., *Analytical Chemistry,* 2009].

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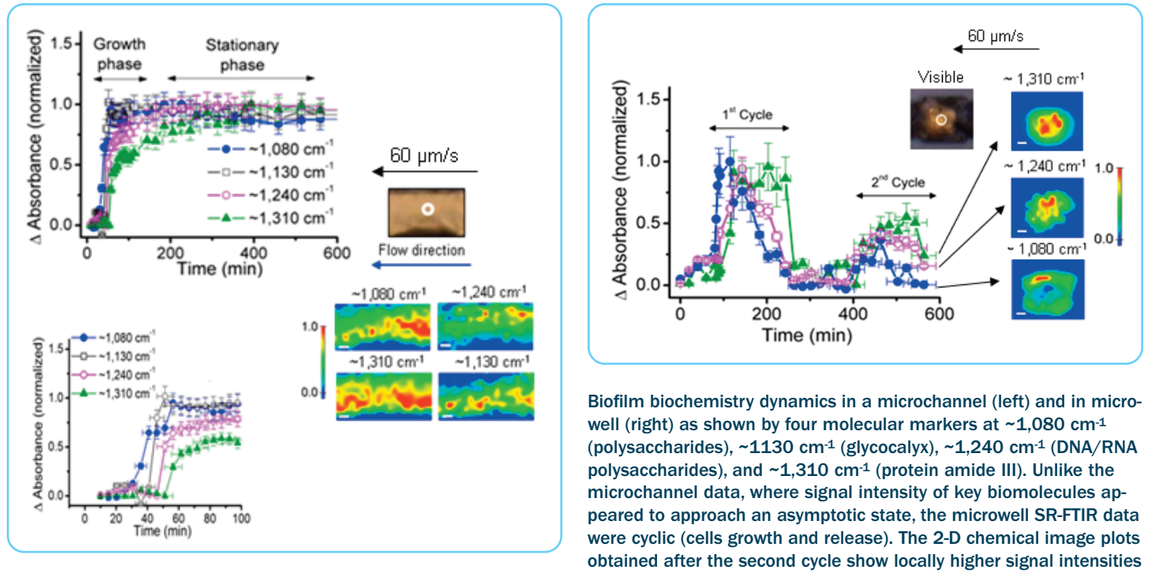
Open-channel microfluidics and SR-FTIR for studying biofilm dynamics

Bacterial biofilms are structured dynamic communities of aggregated cells enclosed in a self-produced polymeric matrix that adheres to both inert and living surfaces in aqueous environments. Many biofilm processes important in pathogenesis and ecology are initiated in confined microscopic spaces. Here we used our microfluidic SR-FTIR microscopy platform to compare the dynamics of biofilm formation in microchannels (higher nutrient supplies/mass exchange) with microwells (lower nutrient supplies/waste removal). We first monitored the SR-FTIR signal at a fixed location (o) over a 9-hour period, then used the mapping mode to obtain chemical images of biofilms in each microstructure by collecting full SR-FTIR spectra at each position.



Biofilm biochemistry dynamics in a microchannel (left) and in microwell (right) as shown by four molecular markers at ~1,080 cm^{-1} (polysaccharides), ~1130 cm^{-1} (glycocalyx), ~1,240 cm^{-1} (DNA/RNA polysaccharides), and ~1,310 cm^{-1} (protein amide III). Unlike the microchannel data, where signal intensity of key biomolecules appeared to approach an asymptotic state, the microwell SR-FTIR data were cyclic (cells growth and release). The 2-D chemical image plots obtained after the second cycle show locally higher signal intensities of protein amide III (~1,310 cm^{-1}) and DNA/RNA polysaccharides (at ~1,240 cm^{-1}) near the microwell center whereas the polysaccharide matrix accumulated near the microwell edge. There is little spectroscopic evidence of glycocalyx (~1,130 cm^{-1}) to facilitate strong adherence to the microwell substrate. Scale bars = 10 micrometers. Holman, et al., *Analytical Chemistry,* 2009.

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