the ToxR/TcpP/*toxT* protein-DNA complex important in early pathogenesis. In addition to elucidating the regulatory pathway of *V. cholerae*, the impact of this work will be to further provide a general model for outer-membrane-bound transcription control in bacteria and nuclear-membrane-bound transcription in eukaryotic cells.

Advances in UV-VIS-IR Spectroscopy

1033-Pos Board B788

Chemical Analysis Below the Diffraction Limit using Infrared-Coupled Atomic Force Microscopy (AFM-IR)

Sara Heedy[1], Michael Lo[1], **Eoghan Dillon**[1], Qichi Hu[1], Craig Prater[1], Roshan Shetty[1], Kevin Kjoller[1], Curtis Marcott[2], Alexandre Dazzi[3], Christopher Yip[4].
[1]Anasys Instruments Corp., Santa Barbara, CA, USA, [2]LightLight Solutions LLC, Athens, GA, USA, [3]Université Paris-Sud XI - Campus d'Orsay, Paris, France, [4]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada.

High resolution infrared spectroscopy is a popular technique for investigating biological structures. It is relatively simple to use, and in some cases considered to be a non-destructive technique. By combining atomic force microscopy and infrared spectroscopy (AFM-IR) into a single bench-top instrument, it is possible to resolve chemical differences on the scale of *ca.* 100 to 200 nm, which often reveals information that could not have been obtained with conventional infrared microspectroscopy. The AFM-IR technique is based on observing the rapid thermal expansion and contraction of material due to the absorption of nanoseconds-long IR radiation pulses, which is collectively known as the photothermal induced resonance (PTIR) phenomenon. This rapid movement is captured by an AFM cantilever equipped with a sharp tip that is in direct contact with the sample material. The resulting amplitude of the ringdown response is directly related to the absorption characteristics of the material across a given range of wavenumbers. Therefore, AFM-IR spectral band shapes are similar to the bulk IR measurement and the spectra are searchable against existing databases. By further modulating the pulse frequency of the infrared laser radiation to coincide with the contact resonance of the AFM cantilever, sensitivity is enhanced, enabling the detection of ~ 20 nm-thick organic materials. In this presentation, we will examine several biological systems using this AFM-IR technique. Spectral changes in the IR spectra can be seen through the whole or cross-sections of proteinaceous materials. Functional group IR images acquired using the AFM-IR technique also reveal the spatial distribution of chemical species in the form of absorption characteristics can be achieved at below the diffraction limit.

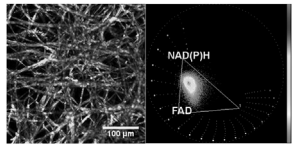
1034-Pos Board B789

Nonlinear Spectral Imaging of Fungal Metabolism

Helene Knaus[1,2], **Gerhard A. Blab**[1], Hans C. Gerritsen[1], Han A.B. Wösten[2].
[1]Debye Institute, Utrecht University, Utrecht, Netherlands, [2]Department of Microbiology and Kluyver Centre for Genomics of Industrial Fermentation, Utrecht University, Utrecht, Netherlands.

Nonlinear microscopy, which in combination with fluorescence spectroscopy is called nonlinear spectral imaging (NLSI), affords access to specimen morphology and (auto)fluorescence spectra at the same time. Thus, it measures the biochemical composition, while also distinguishing different parts of the tissue. Data interpretation is simplified by a new analysis method, the spectral phasor [1], which results in a robust, quick, and semi-blind spectral unmixing of fluorescent species.

NLSI and the spectral phasor are a very user-friendly technique and have the potential to address a broad range of microbiological questions. We introduce them as a novel minimum-invasive technique to monitor the state of "fungal cells" (hyphae). Fungi, both used as consumables and organisms to produce industrial and pharmaceutical compounds, require stringent quality control during their growth. To this end, and as one possible application of NLSI and the spectral phasor, we present their use in monitoring the quality and freshness of white button mushrooms.



RGB-representation of a non-linear spectral image of the white button mushroom (*A. bisporus*, left) and the corresponding phasor.

[1] F. Fereidouni, A.N. Bader, and H.C. Gerritsen, *Optics Express*, **20**,12729-12741 (2012).

1035-Pos Board B790

A Micro-Perfusion System for the Fluorescence-Based Monitoring of Physiological Responses to High Hydrostatic Pressures

Jeff Maltas, Zac Long, Alison Huff, Paul Urayama.
Physics, Miami University, Oxford, OH, USA.

Hydrostatic pressures of 10^2 to 10^3 atm affect a range of cellular processes, including motility, cell division, nutrient uptake, fermentation, translation and transcription, protein synthesis, and ultimately viability. In order to perform the real time monitoring of pressure effects, we present a micro-perfusion system designed for spectroscopic measurements on cellular systems under high pressure. The system consists of an optically-compatible pressure chamber and interchangeable fluid reservoirs. Perfusion is achieved using a dual pressure-generator configuration, where one positive-displacement generator is compressed while the other is retracted, thus maintaining a pressurized volume while achieving fluid flow. Control over perfusion rates (typically in the 10 µl/s range) and the ability to change between fluid reservoirs while under pressure (up to 600 atm) are demonstrated. Next, the system is used for the time-gated, spectral monitoring of endogenous NADH fluorescence under pressure. Spectrofluorimetric measurements utilize a nitrogen-discharge laser for sub-nanosecond pulse width, 337-nm wavelength excitation, and an intensified CCD coupled to a spectrograph for nanosecond-gated spectral detection. Because emission from NADH is routinely used at ambient pressure for monitoring mitochondrial function, we validate the system by observing the pressurized response of *Saccharomyces cerevisiae* (baker's yeast) to mitochondrial functional modifiers (e.g., cyanide). Because the system is compatible with both spectroscopy and subcellular resolution microscopy imaging, the system represents a robust tool for investigating the biophysical effects of pressure on cellular systems.

1036-Pos Board B791

Fine-Grained Spatial and Temporal Resolution of Water and Protein Contributions to Ultra-Fast and Slower Fluorescence Shifts from MD + QM Simulations

Pedro L. Muíño[1], J Nathan Scott[1], Patrik R. Callis[2].
[1]Chemistry, Saint Francis University, Loretto, PA, USA, [2]Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA.

Vibrant discussion persists as to the origin of the time dependent fluorescence spectral (Stokes) shift (TDFSS) in the range 100 fs to 100 ps for a number of tryptophan (Trp)-containing proteins. TDFSS reports on the dynamic reorganization of the local environment around the large dipole of Trp following excitation to the 1La state. Much of the discussion centers on the ubiquitous "slow" (10 ps-5 ns) TDFSS component found only in proteins. Details of what determines the fast (< 2ps) component in proteins are also of interest. Two questions of interest are: (1) the relative contributions of protein and water; and (2) what length scales characterize these contributions. To help answer these ongoing questions we have performed molecular dynamics simulations in conjunction with semiempirical quantum mechanics (MD + QM) for the proteins STNase, GB1, and monellin, each of which has been the subject of ultrafast experiments. We have examined the spatial contributions to shifts at 1pm intervals of distance, which has revealed that only 5-10 waters less than 0.8nm from Trp contribute to the TDFSS, while ~100 waters out to 1.5 nm often contribute blue shifts that are the same in the ground and excited state. These simulations raise a further question as to the mechanism of fast anti-correlated fluctuations of protein and water contributions to the fluorescence shift.

1037-Pos Board B792

High throughput Time Resolved Fluorescence in a Microplate Reader

Karl J. Petersen[1], Joseph M. Muretta[1], Sutton E. Higgins[2], Kurt C. Peterson[1], Gregory D. Gillispie[1], David D. Thomas[1].
[1]Biochemistry, Molecular Biology & Biophysics, University of Minnesota, Minneapolis, MN, USA, [2]Dartmouth College, Hanover, NH, USA.

We describe a high-throughput time-resolved fluorescence (TRF) spectrometer, able to detect multiple fluorescence lifetimes across 384 wells with short (< 5 min.) read times using direct waveform recording. The instrument combines high-energy pulsed laser sources (5-10 kHz repetition rate, 1-3 ns pulse width) with a photomultiplier and high-speed digitizer (1 GHz, effectively 5 GHz with interleaving) to record a complete fluorescence decay waveform after each pulse. Single-well measurements of dyes with 200-fold signal averaging (0.1 s acq. time) yield lifetimes comparable in accuracy and precision to single photon counting (SPC.) Integrated software enables immediate analysis by fitting exponential decays or by calculating a model-independent truncated first moment. In a 384-well format changes in quencher concentration are readily seen, with the first moment calculation providing resolution comparable to exponential decay models. Further, we