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)

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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

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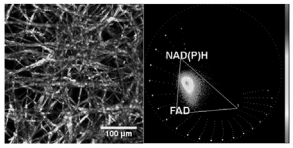
Nonlinear microscopy, which in combination with fluorescence spectroscopy is called nonlinear spectral imaging (NLSI), affords access to specimen morphology and (auto)fluorescence spectra at the same time. Thus, it measures the biochemical composition, while also distinguishing different parts of the 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

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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

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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

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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

are able to resolve relative mole fractions in two-dye mixtures when pure samples are used as standards. In multiple-well experiments the variation in total measured fluorescence is comparable to steady-state instruments, while the precision in lifetime is better than 2%. These features will enable high-throughput TRF experiments to detect changes to structure and dynamics in solution, cells and reconstituted systems. Acknowledgements: Spectroscopy experiments were performed at the Biophysical Spectroscopy Center, University of Minnesota. Excellent computational resources were provided by the Minnesota Supercomputing Institute. This work was funded by NIH grants to DDT (R01 AR32961, P30 AR057220) and KJP (T32 AR007612.)

1038-Pos Board B793

Electronic Transition Moments of 1,3,2-Benzodiazaboroline ('External' Bn Indole) and 'Fused' Bn Indole, Containing the 1,2-Dihydro-1,2-Azaborine Core

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The biological importance of tryptophan as an amino acid, an essential metabolite, and a precursor to biosynthetic pathways cannot be overstated. As such, the photo-physical properties of tryptophan are of great interest for spectroscopic studies to probe the local interactions between bio-molecular residues in proteins and macromolecular complexes. Although tryptophan is intrinsically fluorescent, it is often difficult to isolate its signal from background and scattered excitation light. Here we investigate the electronic structure of a tryptophan analogue with distinct optical properties that can potentially be used as a tryptophan substitute in many biological contexts. Boron-Nitrogen/Carbon=Carbon isosterism of indole-based structures provide a class of boron containing indole derivatives, which exhibit distinct electronic characteristics. We studied the orientations and magnitudes of the electric dipole transition moments (EDTMs) of 1,3,2-benzodiazaboroline ('external' BN indole) and 'fused' BN indole, containing the 1,2-dihydro-1,2-azaborine core. A combination of absorbance spectroscopy, ultraviolet linear dichroism (UV-LD), infrared linear dichroism (IR-LD) and quantum chemical calculations provided the means to assign two EDTMs for the external BN, and two EDTMs for the fused BN indole within the 30,000-70,000 cm-1 spectral range. We constructed an excited state model for each molecule utilizing Density-Functional Theory for the lowest ground state equilibrium geometry with a single point first-excited state, using BL3YP and ωB79X. The theoretical predictions for low-energy transition orientations are in good agreement with our experiment data.

1039-Pos Board B794

Sensitive Time-Correlated Single Photon Counting Enables Efficient Singlet Oxygen Detection

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Single photon counting based data acquisition has proven to yield a major sensitivity increase in the optical evaluation of pharmaceuticals and biotechnology products. We will show for the first time that a state of the art time-correlated single photon counting (TCSPC) based fluorescence lifetime spectrometer is able to quantify singlet oxygen generation and to characterize the singlet oxygen phosphorescence decay. This makes TCSPC based fluorescence lifetime spectrometers a valuable tool for studying photosensitizers widely used for example in photodynamic therapy (PDT). The detection of the faint singlet oxygen phosphorescence signal has been made possible by using a special burst mode for the pulsed laser excitation and a new generation of TCSPC electronics with a significantly reduced dead-time which enables efficient multi-stop photon detection.

Thanks to a recently developed integrating sphere add-on we are also able to measure fluorescence quantum yields with the same instrument. Leveraging the possibility to measure fluorescence lifetime in conjunction with quantum yield, we performed a systematic investigation of the relation between reduced fluorescence emission and different contributions of dynamic and static quenching processes.

Furthermore, since quenching normally does not affect the radiative rate constant, this combined set-up allows to verify the accuracy of the extracted lifetime. Especially for very short lifetimes in the range of the instrument response function the presented method allows to assess whether the proper fluorescence lifetime was extracted.

1040-Pos Board B795

Monitoring the Conformation and Concentration of DNA in Live Cells using Fourier Transform Infrared Spectroscopy

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The ability to accurately detect DNA both quantitatively and qualitatively inside cells using Fourier transform infrared (FTIR) spectroscopy has been disputed. Recently, we have demonstrated that the variability of DNA absorptions is due to the dehydrated nature of biological samples prepared for FTIR spectroscopic measurement [1]. We have further demonstrated that in the dehydrated and fixed state DNA in cells assumes an A-DNA conformation instead of the native B-DNA form. Importantly, as well as being detected in eukaryotes which were invariably destroyed during dehydration, this B-A DNA transition has also been observed in desiccation-resistant, dormant bacteria and the native B- conformation has been detected upon rehydration of these cells. This previously undetected reversible transition raises several interesting questions about the role of A-DNA as a defense mechanism and its role in the evolution of nucleic acids.

The B-A conformational transition has also been identified as causing changes to the molar extinction coefficients of several DNA bands explaining previous observations of unexpectedly low DNA absorption intensities. The Beer-Lambert nature of these absorptions was demonstrated by infrared spectroscopy of avian erythrocytes and extracted nuclei in conjunction with Partial Least Squares regression analysis to quantify cellular DNA [2]. Furthermore, spectra of hydrated single cells throughout interphase were also used to investigate the quantitative and qualitative biochemical changes involved in the G_1, S and G_2 phases of the cell cycle [3]. Using Principal Component Analysis cells only two hours apart were successfully clustered based on changes to the concentration and conformation of lipid, protein and DNA.

1. D. R. Whelan, *et. al.*, *Nucleic Acids Research*, *39*, 5439-5448 (2011).
2. D. R. Whelan, *et. al.*,*Journal of Biophotonics*, *10*, 775-784 (2013).
3. D. R. Whelan, *et al.*, *Analyst, 138*, 3891-3899 (2013).

1041-Pos Board B796

Ionizing Radiation Induced Biological Effect on Human Cell HCT116($P53^{+/+}$, $P53^{-/-}$) Observed through Synchrotron-FTIR Microspectroscopy and Imaging

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Ionizing radiations exist ubiquitously and can induce cell apoptosis and necrosis. It is important to investigate the involved physico-chemical processes and mechanisms. Many new tools and methods are developed, among which synchrotron-FTIR microspectroscopy also gains concern for its advantage of non-invasive measurement of cellular chemical compositions with spatial resolution. Recently, we employed s-FTIR microspectroscopy to study the biological effect on *Rhizopus oryzae* induced by energetic-particle-irradiation, and observed the ROS-involved DNA damages, lipid peroxidation and protein denaturation with spatial resolution [Liu et al. ***J Mol Struct,*** 2013, 1031:1-8.]. In the present work, we continued to apply s-FTIR microspectroscopy to investigate the ionizing radiation induced biological effect on the human colon cancer cells HCT116 ($p53^{+/+}$, $p53^{-/-}$). The p53 gene is regarded as an anti-oncogene [Hollstein et al. ***Science,*** 1991, 253:49-53]. It was also reported that X-ray radiation could induce the cycle arrest of cells [Michael et al. ***Cancer Res***2003, 63:7176-7184]. In our study, we were able to make use of s-FTIR microspectroscopy to observe and distinguish the cells in G1, S and G2 phases. We employed different forms of ionizing radiations including particle radiation, gamma and X-ray radiations to irradiation the cells. Our preliminary results showed that the radiations indeed induced the DNA damages and protein denaturation but with different extents of effect for different radiations and different ($p53^{+/+}$, $p53^{-/-}$) cells. The FTIR measurements also definitely revealed radiation-induced cell cycle arrest and the p53 dependent radiation sensitivity. This research demonstrated that s-FTIR microspectroscopy is a useful tool for observing and understanding the biological effect on cells under ionizing radiations.