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.)

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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.

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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.

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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.

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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.