

absorption, part of red light escapes from the tissue without causing any effect. In thicker 10mm slabs, where less light escapes, maximum temperature is higher (3.6°C and 3.9°C for 530 and 660 nm, correspondingly). Compared to 3mm slabs, the maximum is reached at a larger depth and takes longer to develop. This research was partially funded by European Social Fund under the Global Grant measure and NIH grant 5R03TW008039.

3446-Pos Board B601

Wanted: Scalable Tracers for Diffusion

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Scalable tracers are potentially a useful tool to understand diffusion mechanisms and to predict diffusion coefficients, particularly for hindered diffusion in complex, heterogeneous, or crowded systems. Scalable tracers are defined as a homologous series of tracers varying in size but with the same shape, structure, surface chemistry, flexibility, and diffusion mechanism. Both chemical homology and constant dynamics are required. Specifically, branching must not vary with size, and there must be no transition between ordinary diffusion and reptation. Ideally the tracers would be uniform, monodisperse, metabolically inert cylinders in 2D or spheres in 3D with continuously variable radius and tunable surface properties. Scalable tracers would facilitate more rigorous diffusion measurements in which two types of measurements would be clearly distinguished: using scalable tracers to find the mean diffusion coefficient as a function of size, and using nonscalable tracers to find the variation due to differences in shape, surface properties, and the like. Candidate scalable tracers are discussed for 2D diffusion in membranes and 3D diffusion in cytoplasm and nucleoplasm. Specific recommendations for 3D measurements include the use of synthetic dendrimers or random hyperbranched polymers instead of dextran, and the use of core-shell quantum dots in which the shell thickness is used to vary the overall diameter. A set of scalable tracers varying in flexibility would also be useful. These would be made by varying the density of crosslinking in a polymer, to make say “reinforced Ficoll” or “reinforced hyperbranched polyglycerol.” (Supported by NIH grant GM038133.)

3447-Pos Board B602

Novel Bio-Imaging Techniques by Harnessing Optical Highlighter Fluorescent Proteins

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Optical highlighters are a remarkable family of fluorescent proteins (FPs) that could change their excitation and emission spectra upon certain wavelength illumination. Optical highlighters have been extensively used in super-resolution imaging, protein dynamics, gene expression and cellular trafficking. Herein we are harnessing these unique proteins in three different imaging applications: genetically-encoded microviscosity sensor, ultra-deep tissue imaging, and light-driven fluorescent timers. First, we report that the photoswitching kinetics of the chromophore inside Dronpa, a FP with the reversible on-off switching capability, is actually slowed down by increasing medium viscosity outside Dronpa. This effect is attributed to protein-flexibility mediated coupling where the chromophore's cis-trans isomerization is accompanied by conformational motion of protein beta-barrel. Based on this effect, we developed a genetically encoded protein-specific micro-viscosity sensor. Secondly, we have demonstrated a spectroscopy concept to extend the fundamental imaging-depth limit of multiphoton microscopy by generating super-nonlinearity of photo-switchable probes. Due to the long-lived nature of these switchable states, the nonlinearity effect could accumulate as the population is being cycled through these states. Conceptually different from conventional multiphoton processes mediated by transient virtual states, our strategy constitutes a new class of super-nonlinearity fluorescence microscopy mediated by real population transfer. Last, we demonstrate a novel class of light-driven fluorescent timer (FT), based on the photo-convertible fluorescent proteins. It's a new method to image the protein “age” in live cells with a simple snap-shot measurement. This tunable light-driven FT will be a valuable tool that does not only provide the spatial location information of an individual protein, but also its temporal dynamics.

3448-Pos Board B603

Molecular Imaging Reveals a Novel Yeast Centromere Structural Transition in Anaphase

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We use *in vivo* fluorescence correlation spectroscopy (FCS) and high sensitivity quantitative confocal imaging to demonstrate a dramatic structural rearrangement of the yeast centromere in early anaphase. Using FCS, we show that the yeast centromeric histone, Cse4, is present in the early kinetochore at a stoichiometry of one protein per chromosome and doubles to two copies during the structural change. This transition reconciles the controversy between

previous results observing both forms *in vitro*. We have confirmed this result by quantifying nuclear pore complex stoichiometries which are well known. Fluorescence recovery after photobleaching (FRAP) experiments further confirm our result, demonstrating centromeric protein addition specifically during anaphase. Fluorescence resonance energy transfer (FRET) experiments also confirm the result, demonstrating homotypic interactions only in late anaphase. The yeast centromere is an important model complex whose functions are central to many important biological processes. These results have revolutionized our understanding of these basic processes and provide fertile ground for future studies of the kinetochore. Our method demonstrates a unique modality for FCS and quantitative imaging and will likely provide much needed insight into biologically important structures in the future.

3449-Pos Board B604

Phase Separation in a DMPC/DCHOL Mixed Langmuir Film: A Combined Brewster Angle, Fluorescence and Light Scattering Microscopy Study

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Fluorescence microscopy [FM] is one of the most direct imaging techniques for *in situ* observation of morphology and phase-separation at the macroscopic scale [1] in lipid mono- or bi-layers. However, the presence of fluorescent dye-molecules can affect the system. In Brewster Angle Microscopy (BAM), one can image monomolecular Langmuir films without probes. Here, using a composite set-up of BAM, FM and Light Scattering Microscopy (LSM), we present a comparative study of the three techniques on a binary lipid mixture in the presence of two different probes. In most cases, all three techniques show precisely the same domains. However, depending on conditions, some domain types were more evident in one technique than the others. This established, we can directly test the influence of probe on the domain structure. The appearance of collapsed cholesterol-rich crystallites with film compression, and the influence of probe on this collapse, is also discussed.

3450-Pos Board B605

Micro-Absorption Spectroscopy at the Single Cell Level from the Ultraviolet to the Near-Infrared

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Micro-absorption spectroscopy provides a label-free nondestructive probe of biomolecules and their interaction at the single cell level. Our setup employs a confocal detection system to probe and spectrally resolve the attenuation of a white light beam in the axial direction. The method can be used to study cells in their native environment with a spatial resolution of two micron. We present measurements in the UV spectral range (220 - 350 nm) where aromatic amino acids and bases in nucleic acid absorb. To establish the accuracy of the method, we have measured the concentration dependence of the optical absorption of tryptophan, phenylalanine and DNA bases in a microcapillary with 50 micron pathlength and a volume of a few hundred nanoliters. The absorption in the micro setup is found to vary linearly with concentration. We explore applications to protein and DNA solution in nanoliter quantities and single cells.

3451-Pos Board B606

Label Free Fluorescence Lifetime FRET Imaging

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Multiphoton microscopy with endogenous contrasts in biological tissues have primarily focused on detecting signals from the reduced nicotinamide adenine dinucleotide (NADH), its dinucleotide phosphate (NADPH), riboflavins, and tryptophan. All these fluorophores have emission in the wavelength range of 400-600 nm. According to an earlier studies on the autofluorescence spectroscopy of *ex vivo* leukocyte samples under linear (one photon) excitation conditions, signals from the tryptophan (TRP) moieties in cellular proteins should be much stronger than signals from NAD(P)H on the per cell basis. In recent years, considerable interest has been developed in the effect of various diseases upon the metabolic pathway of L-tryptophan and its derivatives. We describe a new method for imaging tumorigenic and non-tumorigenic cell lines by 3-photon exciting of the endogenous protein fluorescence in the ultraviolet (UV) spectral region, where tryptophan is the major fluorophore. Through systematic analysis of FLIM data from live cells, a statistically significant decrease in the fluorescence lifetime of TRP was observed in response to the increase in protein-bound NADH as cells were treated with glucose. Addition of glycolytic substrates significantly quenches tryptophan lifetime. The results demonstrate the potential use of 3P-FLIM-FRET as a tool for label free screening of the change in metabolic flux occurring in human diseases or other clinical conditions.