150-Pos Board B29
Investigating the Efficacy of Peptide-based Inhibitors Against the Earliest Oligomers of Amyloid-β Peptide
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Alzheimer's disease (AD) is linked to the self-association of amyloid-β peptide (Aβ), a protein of 39-43 amino acids that is normally soluble in the plasma and cerebrospinal fluid. Although large, fibrillar aggregates were long thought to be the pathogenic agents, recent evidence indicates that soluble Aβ oligomers are more closely linked to disease progression. In fact, negative effects have been observed from oligomers as small as dimers and trimers. A number of compounds have been found to inhibit the large-scale aggregation of Aβ in bulk solution, typically by manipulating the β-sheet structure characteristic of these assemblies, but little is known regarding inhibition of the earliest association steps. We have used single-molecule fluorescence spectroscopy to characterize the efficacy of four known peptide-based inhibitors toward preventing or reversing association in the earliest Aβ oligomers (n = 2-5). Fluorescein- and biotin-labeled Aβ(1-40) is tethered to functionalized cover slips (pM concentrations) through biotin-streptavidin binding. Spatially resolved monomers and oligomers are examined, one at a time; the number of associated peptides in each species is determined based on quantized photobleaching of the individual dye molecules. Distributions of Aβ monomers and oligomers are determined through examination of dozens of individual peptide species, and permit comparison of the different inhibitor compounds. Results will be presented for inhibition under neutral versus acidic conditions (pH 7.4 versus 5.8). Collectively, these studies will provide new insight into the potential for reversing or preventing Aβ association in its earliest stages.

Imaging & Optical Microscopy I

151-Pos Board B30
BioTIFF: A New BigTIFF File Structure For Organizing Large Image Datasets And Their Associated Metadata
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The Tagged Image File Format data structure (TIFF) is a well known and widely used digital image format. The standard TIFF uses 32 bit offsets giving a 4G maximum size for a multi-page TIFF. However, recently a Big TIFF project has been initiated (http://www.awaresystems.be/imaging/tiff/bigtiff.html) allowing for 64bit offsets so that one file can contain 16 ExaBytes (16 Million TeraBytes). Although that size may at first glance appear to be essentially infinite, mega TeraByte data sets are not inconceivable when using high performance/resolution colour cameras to explore biophysical phenomena in multiple equivalent samples under multiple conditions before averaging. Cloud computing and storage services allows for ubiquitous ad hoc access to large data dataset processing environments opening up the possibility of comprehensive and distributed ''whole dataset'' analysis of the fit between theory and experiment. The BioTIFF format enables a non-relational presentation of large datasets for such distributed analysis. BioTIFF 1.0 is being released as a free and opens source TIFF writer reader that can annotate and locate large image-based datasets (see www.biotiff.org). It includes encryption tags as well as means for remotely accessioning, curating and reusing original data and interpretations, assuring their provenance and enabling distributed usage. The potential of using the BioTIFF for parallel high-throughput high-content screening will be illustrated. A BioTIFF reader-writer running under the LTS 64 bit Ubuntu 8.0 operating system will demonstrated.

152-Pos Board B31
Virtual FRAP - an Experiment-Oriented Simulation Tool
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The aim of this work is to create data- and experiment-driven simulation software that leverages kinetic models of intracellular processes. There is a need for specific tools that combine descriptions of mechanistic hypotheses of cellular physiology with detailed descriptions of experimental protocols and manipulations. We used components and technologies from the Virtual Cell platform (VCell; http:/vcell.org/), which was designed to provide a separation of layers representing biological models, physical mechanisms, geometry, mathematical models and numerical methods. We are developing standalone software packages for kinetic model-assisted simulation, analysis, and interpretation of common experimental protocols. The initial focus is on fluorescence microscopy - the Virtual Microscopy suite. The first prototype is the ''Virtual FRAP'' tool. This is a mostly data-centric experiment analysis framework that allows incorporating fluorescence microscopy (instrumentation, indicators, labels) into existing models of cellular physiology. The microscopy data is formally represented, and it is used to derive cellular geometry, distributions of molecules, and to perform quantitative comparisons with simulated model predictions. This overcomes the limitations of ''traditional'' approaches that use simplified parameterized ''models'' with closed-form analytical solutions. Such a customized environment combines powerful experiment description and analysis features with the flexibility of simulating more general classes of spatial models.

153-Pos Board B32
Live Cell Imaging: Tips and Tools
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Imaging of living cells and tissue is now common in many fields of the life sciences and the physical sciences as well. It is critical when performing such experiments that cell viability is at the forefront of any measurement to ensure the physiological/biological processes under investigation are not altered in any way. The main cause of cyto-toxicity in living cells is due to the reaction of free radical species, generated during the excitation of fluorescent proteins, or dye molecules, with surrounding molecules. The amount of photo-toxicity is directly related to the amount of light energy put into the system, thus, it is critical to minimize light exposure as much as possible. This commentary discusses how to set up a suitable environment on the microscope stage to maintain living cells. While the main focus, is on general and imaging platform specific ways to minimize light exposure during live-cell imaging. Reducing the power of excitation light, maximizing efficiency of the optical path, and optimizing detector settings are all ways light exposure can be minimized. Brief suggestions for useful microscope accessories as well as available fluorescence tools are also presented. Finally, a flow chart is offered to assist readers in choosing the appropriate imaging platform for their experimental system.

154-Pos Board B33
Chondrocytes Deformation In The Live Mouse Knee
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It is well established that cells in musculoskeletal tissues such as bone, cartilage, ligament and tendon deform when the tissue is loaded and that these deformations activate biological signal pathways responsible for adaptive or degenerative changes of the tissue. However, much of the work relating mechanical states of cells and their biosynthetic response are based on isolated cells, or cells in explant samples removed from their natural in situ environment. Neither the mechanics nor the associated biological responses of chondrocytes (the active cells in articular cartilage) have been studied in intact cartilage attached to its native bone or in the intact joint.
Recently, we developed a novel in vivo testing system designed on the stage of a dual photon excitation microscope for direct observations of chondrocytes in the knee of live mice. The mouse is anesthetised and the knee joint secured in a stereo-taxic frame built onto the stage of a dissecting microscope. A medial incision exposes the joint. The meniscus is excised to allow for a full view of the articulating surfaces between tibia and femur. The mouse is then positioned in a custom-built jig on the stage of the dual photon microscope (Zeiss LSM 510 META NLO) coupled with a Coherent Chameleon IR laser tuned at 740 nm for two-photon excitation. Physiologically relevant loads are applied using controlled electrical stimulation of the knee extensor muscles.
Chondrocyte and nuclei deform in excess of 20% for sub-maximal muscular loading of the knee. Deformation of cells occurs within seconds in the loading phase, but full shape recovery requires 5-10 minutes. We conclude that articular cartilage chondrocytes experience significant strain for low level physiological loading of the knee in the live mouse.

155-Pos Board B34
Protein Diffusion in the E. coli Cytoplasm and Periplasm under Osmotic Stress
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The *E. coli* cytoplasm contains the nucleoid, ribosomes, mRNA and tRNA, and numerous globular proteins. Much of the water is associated with biopolymer surfaces. Facile diffusion of globular proteins within this complex medium is essential to normal cellular biochemical activity and growth. Remarkably, *E. coli* grows in minimal medium over a wide range of external osmolalities (0.03-1.8 Osm). The mean cytoplasmic biopolymer volume