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

fraction for such *adapted cells* varies from phi = 0.16 at 0.10 Osm to phi = 0.36 at 1.45 Osm. For cells grown at 0.28 Osm, a similar range of is obtained by *plasmolysis* (sudden osmotic upshift) using NaCl as the external osmolyte, after which the cellular response is passive loss of cytoplasmic water. Using fluorescence recovery after photobleaching (FRAP), we measure the effective axial diffusion coefficient D_{GFP} of green fluorescent protein in the cytoplasm of live *E. coli* cells as a function of for both plasmolyzed and adapted cells. For *adapted* cells the median diffusion coefficient $D_{GFP}{}^{m}$ decreases by only a factor of 2.1 as phi increases from 0.16 to 0.36. In sharp contrast, for *plasmolyzed* cells $D_{GFP}{}^{m}$ decreases by a factor of 70 as phi increases from 0.16 to 0.33. Clearly GFP diffusion is not determined by phi alone. By comparison with quantitative models, we show that the plasmolysis data cannot be explained by simple crowding theory in a homogeneous medium. We will also report on measurements of time-resolved fluorescence anisotropy of GFP in the cytoplasm, diffusion of RNA polymerase in the cytoplasm, and diffusion of GFP in the periplasm of *E. coli.* Time lapse measurements monitor the recovery of cell volume and GFP diffusion after plasmolysis, which may be a key determinant of the time scale of the recovery of growth.

156-Pos Board B35

Measuring the Number of LuxR Proteins in a Single Cell of *V. harveyi*

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We have determined the number *N* of LuxR proteins in a single cell of the bacterium *V. harveyi* by measuring the distribution functions of cell volumes and protein-fluorescence intensities during cell division. In quorum sensing, the LuxR protein population, which regulates many (~70) genes, is sensitive to the concentration of auto-inducer molecules (AIs). We utilized a strain that is incapable of producing AIs. The LuxR proteins are tagged by a red fluorescent protein (mCherry). In the absence of AIs, the cells maintain a baseline residual concentration of LuxR that is remarkably constant over 8-10 cycles of cell division. We recorded the growth of a single cell into a large colony by imaging both phase contrast and mCherry intensity every 2 minutes in a 6-hour movie. The phase-contrast image was used to measure the volume of each cell, while the mCherry intensity monitored the LuxR population. At each cell-division event, we determined the fractional partitioning of the cell volume and the LuxR population. From the large number of cell-division events (~300), we obtained the normalized distributions of both the volumes and the mCherry intensities. Our procedure allows an accurate measurement of the width of the volume distribution ($\sigma = 0.031 \pm 0.003$). Significantly, the width of the LuxR distribution was observed to be much broader, presumably because of small-number fluctuations. By deconvoluting the Gaussian distributions, we find that the average LuxR copy number *N* equals 140 ± 10 just before cell division. Repeating the experiment at successively higher levels of applied AI concentration, we confirmed that when *N* is 10 times larger, the 2 distributions converge to the same width σ. This technique may be applied quite generally to other systems.

157-Pos Board B36

Influence Of P-selectin Structure On Its Mobility In The Weibel-Palade Body And Plasma Membranes

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The leukocyte adhesion molecule P-selectin is stored in Weibel-Palade bodies (WPBs), a secretory organelle of endothelial cells. The extracellular domain of P-Selectin comprises 9 consensus repeats (CRs), an EGF domain (E) and a Lectin domain (L) at the N-terminus, forming a rod-like structure approximately 48nm in length. Although truncation of extracellular CRs of P-Selectin impairs leukocyte capture under flow conditions, how such modifications affect the mobility of P-Selectin in the WPB membrane and in the plasma membrane (PM) after exocytosis is not known. Using single WPB FRAP or TIRFM with single fluorophore (SF) detection and tracking the diffusion of P-Selectin-EGFP and N- and C-terminal truncations of P-Selectin-EGFP was investigated in WPB or PM during ionomycin (1 μM) -evoked WPB exocytosis at 37°C.

P-Selectin-EGFP was immobile in the WPB membrane, but its N-terminal truncations rendered it mobile. On exocytosis SFs of P-Selectin-EGFP and its mutations were found to diffuse approximately freely in the PM in the vicinity of WPB fusion sites. The diffusion coefficient ***D*** for P-Selectin-EGFP was 0.14 $\mu m^2/s$, (n=2890 SF). Deletion of 8 of the 9 CRs increased ***D*** to 0.18 $\mu m^2/s$, (n=3907 SF). Removal of the L domain alone increased ***D*** to 0.24 $\mu m^2/s$, (n=1716 SF). Deleting both L and 8CRs increased ***D*** to 0.29 $\mu m^2/s$ (n=1818 SF). Removing E had no effect. C-terminal truncation also altered ***D***. The structure of P-Selectin influences its mobility in the WPB and PM.

158-Pos Board B37

Quantitative Analysis of Spatial Protein-protein Proximity in Fluorescence Confocal Microscopy

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Colocalization between fluorescently-labeled proteins has turned to be a measure of protein-protein interactions and a tool in cell biology. However, its evaluation has inherent caveats. The popular overlay method is qualitative and greatly depends on user setting for threshold values. Quantitative methods are also available, but the results can be unreliable because of the questionable assumption that proteins are uniformly distributed, and of the failure to minimize the influence of nonspecific labeling and random fluorescence noise. In order to quantify colocalization in a more absolute manner, we extended the use of image cross-correlation spectroscopy (ICCS) 1 to minimize the effect of protein distribution, non-specific labeling and random noise. The numerical procedure to separate the fluorescent components is based on the fact that the crosscorrelation and autocorrelation image values as function of x,y pixel shift have a peak at zero pixel shift decaying with sharp and shallow components as a function of x and y pixel shift. The sharp component corresponds to the co-localized proteins while the shallower one corresponds to non-specific labeling. By fitting the sharp and shallow landscapes of the crosscorrelation and autocorrelation functions to the sum of two Gaussian distributions, one can extract the peak amplitude of the specific sharp components to calculate the protein proximity index (PPI) from the ratio between the crosscorrelation and autocorrelation values at x,y=0 pixel shift. In summary, our method extracts the colocalization value from background generating consistent results from both computer simulated images and biological confocal images. Thus, it is a powerful microscopy tool to determine the nature of macromolecular complexes and their dynamic changes in biological processes.

1. Comeau JW, Costantino S, Wiseman PW. A guide to accurate fluorescence microscopy colocalization measurements. Biophys J. 2006;91:4611-22.

159-Pos Board B38

The White Confocal - Controlling Spectral Fluorescence

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Fluorescence has evolved to the most important tool in modern biological research. Specific histological stainings, antibody-based protein-markers and DNA-hybridization were the classical targets. Fluorescent proteins and other advanced stainings allow tracing molecules and structures in living samples - both by classical imaging as well as by modern analytical approaches; e.g. fluorescence correlation and its derivatives.

A very beneficial phenomenon in fluorescence is the fact that it comes in an infinite number of colors - which is at the same moment the most challenging feature. Multiple colors are available simultaneously - but at the price of a very elaborate illumination scheme, tricky beam splitting and efficient but selective detection of the various colors which are used to stain different structural elements in the sample.

All three modules, that are required for incident light fluorescence measurement instruments, are now spectrally tunable: white laser light sources, programmable acousto optical beam splitters and tunable multi-band emission detectors. These tunable elements allow for any spectral combination both on the excitation and the emission side. Reduction of crosstalk, more specificity and new measurements like excitation-emission correlation are some of the benefits of these developments. And as a side-effect: the acousto-optical devices transmit much better as compared to commonly used filters and dichroics.

Here, new approaches to use spectral information of both emission and excitation are presented and examples are given.

160-Pos Board B39

Identifying Components Of Astroglial Autofluorescence Using The Spectral Separability Index, Xijk

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In in multi-color fluorescence, endogenous fluorophores have been considered more of a nuisance than a signal. Many of them co-exist (e.g., mitochondrial NADH and flavins) or, as ceroids and lipofuscins, have intrinsically broad fluorescence excitation and emission spectra. Thus, the presence of autofluorescence, along with cross-excitation and fluorescence bleed-through of one color channel into the neighboring one, bring up the question to which extent different color channels contain truly independent fluorophore information.