3878-Pos
In Vivo Chondrocyte Mechanics
Ziad Abusara, Andre Leumann, Ruth Seerattan, Walter Herzog.
University of Calgary, Calgary, AB, Canada.
Mechanical loading of joints deforms articular cartilage cells, and that these cells provide the signals for maintaining cartilage health. Excessive loading of cartilage and cells leads to cartilage degeneration and osteoarthritis.
Recently, we developed a novel *in vivo* testing system that allows for quantification of the mechano-biology of chondrocytes in the intact knee of live mice. Mice are fixed in a custom-built jig on the stage of a dual photon microscope (Zeiss LSM 510 META NLO). Controlled forces of the knee extensor muscles are achieved through direct muscle stimulation. Imaging of the chondrocytes is performed using a Zeiss 40x 0.95 NA water-immersion objective coupled with a Coherent Chameleon IR laser tuned at 780 nm for two-photon excitation. Dynamic deformations of chondrocytes were successfully measured for the first time in the intact, loaded knee. Chondrocytes deformed 25% during sub-maximal muscular loading. Cell deformations occurred within seconds of loading, but required minutes to recover following load removal (Fig. 1). Chondrocyte volumes in the load bearing area were 2-3 times smaller and were aligned and structured compared to chondrocytes in the non-load-bearing area of the tibial plateau.

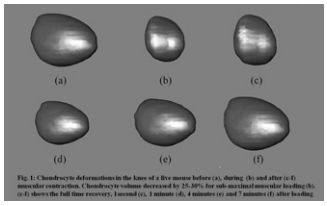
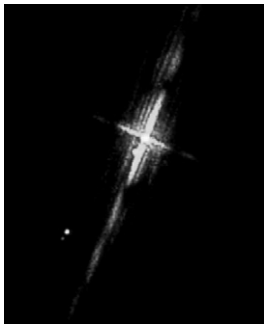


Fig. 1: Chondrocyte deformations in the knee of a live mouse before (a), during (b) and after (c-f) muscular contraction. Chondrocyte volume decreased by 25-30% for sub-maximal muscular loading (b). (c-f) shows the full time recovery, 1second (c), 1 minute (d), 4 minutes (e) and 7 minutes (f) after loading.

3879-Pos
Diffraction Patterns of Live C. Elegans: Acquisition and Analysis
Jenny Magnes, Kathleen M. Raley-Susman, Rahul Khakurel, Alicia Sampson, Margo Kinneberg, Rebecca Eels.
Vassar College, Poughkeepsie, NY, USA.
Ideal studies of any type of biological systems are conducted in the subject's natural environment. Microscopy has made huge strides during recent years. Images of life systems are not only available but phenomenal. Nevertheless, powerful microscopic systems can be costly with images tied to focal planes. Sometimes there are other hard to control parameters such as wavelength, intensities, and phosphorescence. Here we present an alternative technique to observe physical and biological parameters of live C.elegans using diffraction. Physical parameters such as worm growth can be observed and quantified over extended time periods using video analysis. Three-dimensional organismal activity, such as locomotion, behavioral response to crowding, egg-laying or physiological processes such as defecation, can also be observed in real-time and quantified. Experimental setup and limiting parameters of diffraction experiments are explicitly discussed. The commentary will elaborate on the hardware for this type of diffraction experiment.



3880-Pos
Detection of Anti-Oxidant Marker in Normal Subjects and Patients with Neurodegenerative Disorders using *in Vivo* Magnetic Resonance Spectroscopy
Ishima Badhwar, **Pravat K. Mandal**.
National Brain Research Center, Gurgaon, India.
Alzheimer's disease (AD) is a progressive neurodegenerative disorder which presents as dementia and cognitive decline in the elderly. The precise etiology of this disorder is not known, however, free radical accumulation and consequent membrane lipid peroxidation have been implicated in the disease pathology. Oxidative stress has been considered a major risk factor for AD and also for other neurodegenerative disorders such as Parkinson's disease (PD). Glutathione (GSH), a potent anti-oxidant, is considered to be the molecular marker of the oxidative status. Hence, the measurement of glutathione levels in normal subjects and diseased individuals offers an exciting prospect of developing a molecular marker for neurodegenerative disorders. Magnetic Resonance Spectroscopy (MRS) is an extremely useful technique for measuring different brain neurometabolites including glutathione. MEGA-PRESS pulse sequence is designed for identification of metabolites which are spin coupled systems and available in a low concentration range. In the case of glutathione, chemical shift cysteine H_α is specifically inverted, as a result spin coupled H_β peak intensity gets enhanced.The final MEGA-PRESS spectrum yields only the H_β peak of cysteine residue of glutathione molecule and quantification of glutathione molecule becomes unambiguous. We have generated a concentration calibration curve using MEGAPRESS pulse sequence on phantoms containing glutathione molecule with concentration ranging from 0.5 to 10 mM. The same experiment was successfully performed on healthy volunteers and is now being extended to patients with neurodegenerative disorders (AD and PD). We have hypothesized that the glutathione content is directly co-related to the clinical status of those patients.
* Corresponding Author Dr. Pravat K Mandal

3881-Pos
Freeze-Fracture TEM on Domains in Lipid Mono- and Bilayer and Promoted by Antimicrobial Peptides as a New Generation of Antibiotics
Brigitte Papahadjopoulos-Sternberg[1], Raquel F. Epand[2], Richard M. Epand[2].
[1]NanoAnalytical Laboratory, San Francisco, CA, USA, [2]Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada.
Lateral chemical and physical inhomogeneities of biological membranes such as domains/rafts seem to play an important role in signal transduction, membrane traffic, and diseases. Therefore they are targeted for pharmacological approaches in cure and prevention of such diseases. Freeze-fracture transmission electron microscopy (ff-TEM) as a cryofixation replica technique is a powerful tool to explore small, highly dynamic domains in a probe-free mode. At a 2nm resolution limit we are able to study lipid-, peptide-, protein-, toxin-, as well as drug domains on a nano-resolution scale. Since beam-damage resistant replica can be produced from micro-meter size objects also, ff-TEM allows us to study nano-scale events in micro-scale biological and artificial assemblies. Moreover, the fact that the fracture plane follows the area of weakest forces allows insides into the hydrophobic center of lipid bilayer[1-3] as well as into the lipid/gas interface of lipid monolayer stabilizing gas bubbles to explore domain formation in these otherwise inaccessible areas[4].
Examples will be given for domains detected in lipid mono- as well as bilayer made of drugs, proteins, and toxins[5]. Furthermore, we explored lipid domain formation intensified by arginine-rich antimicrobial peptides as hot candidates for new-class antibiotics.
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3882-Pos
Biometallic Nano-Structures: A Thousand-Fold Fluorescence Enhancement with Nanopetals
Michelle A. Digman[1], Chi-Cheng Fu[2], Giulia Ossato[1], Luke P. Lee[2], Enrico Gratton[1], Michelle Khine[1].
[1]University of California, Irvine, Irvine, CA, USA, [2]University of California, Berkeley, Berkeley, CA, USA.
Although, metal nanoparticles have been shown to increase emission fluorescence of molecules due to the propagation of electron density waves, i.e. surface plasmons, at the interface between the metal and substrate, biometallic nanostructures yield a higher emission enhancement and can be suitable for measuring cell surfaces at the nano scale. We have developed a method to attain bimetallic structures on the surface of memory polymers in order to achieve sharp bi-layered uniaxial and biaxial nanopetals. The sharp edges of the nanopetals exhibit remarkable increase of emission intensity of fluorescent molecules. We observe several thousand fold increase in intensity at the edges or ''hotspots'' of both uniaxial and biaxial nanopetals. The fluorescence intensities observed at the hotspots are brief bursts of intensity as the molecules diffuse through the structures. These bursts are below the resolution limit of our optics and possibly be due to single molecular emission. The intensity of the bursts increases non-linearly with increase laser intensity suggesting that the events may be attributable to stimulated emission, excited-state absorption, or saturation intensity dependent 2-photon emission cross-section. We also show a decrease in the excited-state lifetime of the fluorescence particles, fluorescein, revealing strong plasmonic interactions. Our findings reveal an ultra-sensitive and novel technique using bimetallic nanopetals to enhance fluorescence detection.