3434-Pos   Board B589
Bayesian Estimation of Diffusion Constants from Single Particle Tracking Data
Patrick J. Cutler, Peter Relich, Keith A. Lidke.
University of New Mexico, Albuquerque, NM, USA.
Single particle tracking (SPT) is an important tool for investigating the mobility of proteins on cell surfaces. Typically, the analysis of such trajectories involves the calculation of the Mean Squared Displacement (MSD) versus time interval and the resulting curve is fit to extract the diffusion constant. However, MSD analysis cannot easily handle correctly the variability in localization precisions and trajectory intermittency that occurs when tracking intermittent probes such as quantum dots.
We have developed an analysis of SPT trajectories that takes into account the effects of missing data, finite exposure time of the camera, and variable localization precision. We have derived a recursion relation that can be used to calculate the probability of the observed trajectory given diffusion constant and the assumption of free Brownian motion. using the method of Bayesian data analysis, we calculate the posterior distribution of the diffusion constant and use this to provide credible intervals for the estimated parameter. We compare the performance of this Bayesian estimator with that of a traditional MSD analysis. In systems with large localization errors, the Bayesian method provides a more accurate estimate of the diffusion constant. We demonstrate the performance of the estimator using simulated data, experimental data collected with known trajectories created using a piezoelectric nanostage, and data collected from various membrane proteins on live cell membranes.

3435-Pos   Board B590
Three-Dimensional Thermal Noise Imaging of Collagen Networks
Tobias F. Bartsch[1], Martin D. Kochanczyk[1], Janina Lange[2], Ernst-Ludwig Florin[1].
[1]University of Texas at Austin, Austin, TX, USA, [2]University of Erlangen-Nuremberg, Erlangen, Germany.
Extracellular biopolymer networks show interesting mechanical properties that are essential for living organisms. In particular, a highly nonlinear elastic response to strain is seen, which gives biopolymer networks the ability to comply with small stresses but to resist large ones. The macroscopic mechanical properties have their origin in the properties of the individual filaments and the properties of the network that they form, like their cross-linking geometry and pore size distribution. While the macroscopic properties of biopolymer networks have been extensively studied, there has been a lack of experimental techniques that can simultaneously determine mechanical and architectural properties of the network in situ with single filament resolution. Thermal Noise Imaging is a scanning probe technique that utilizes the confined thermal motion of an optically trapped particle as a three-dimensional, noninvasive scanner for soft, biological material. It achieves nanometer precision in probe position detection with MHz bandwidth. Thermal noise imaging visualizes single biopolymer filaments as nanoscale channels and allows for the quantification of their mechanical properties from their transversal fluctuations. using feedback control, we have recorded micrometer scale thermal noise images inside a collagen network for the first time. We extract quantitative information about cross-linking geometry and fiber elasticity from the data. These results pave the way for an investigation of force distributions inside biopolymer networks on the single filament level.

3436-Pos   Board B591
Surface Plasmon Resonance Imaging for Cell Biology: Direct Measurement of the Evanescent Wave Penetration Depth
Alexander W. Peterson, Michael Halter, Alessandro Tona, Anne L. Plant.
National Institute of Standards and Technology, Gaithersburg, MD, USA.
Surface plasmon resonance imaging (SPRI) is a powerful label-free technique that has been known to provide sensitive biochemical surface measurements, but has only recently been applied to the field of cell biology. High resolution label-free imaging of cell-substrate contacts can be performed using a high numerical aperture objective lens. The SPRI signal is a result of the mass of material within the evanescent field, the refractive index of the material, and the distance between the material and the substrate. Cell focal contacts and cellular organelles can contribute to the SPRI signal. Unambiguous interpretation of the SPRI data is therefore complicated by uncertainties regarding the penetration depth of the plasmon wave. To gain a better theoretical understanding of the signal present in the image we used micron scale beads of polymers with a refractive index similar to cells as reference materials to calibrate SPRI reflectivity as a function of distance-to-substrate. Multi-wavelength measurements of these beads show that it is possible to tailor the effective depth of penetration of the evanescent wave into the cellular environment. The application of multiple wavelengths will assist the interpretation of the SPRI image.
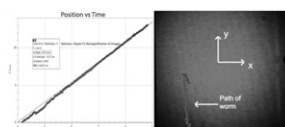
3437-Pos   Board B592
Gravity Studies of C. elegans
Jenny Magnes, Kathleen M. Raley-Susman, Alicia Jago, Karl Spuhler, Dylan Wine, Tewa Kpulun.
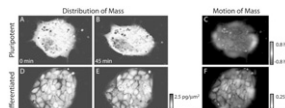Vassar College, Poughkeepsie, NY, USA.
Caenorhabditis elegans is an important model organism in many fields of research. This microscopic worm possesses a primitive nervous system composed of just 302 neurons that shares many features across various species. Despite the simplicity of its nervous system, *C. elegans* exhibits many sensory modalities and behaviors, allowing researchers to perform relatively simple yet highly insightful experiments that probe both the evolution and function of the nervous system. An important unresolved question is whether or not *C. elegans* detects or responds to gravity. The detection of gravity is a poorly understood phenomenon despite its ubiquity. By analyzing gravitational field detection in C. elegans, we will improve our understanding of how more complex nervous systems are capable of detecting and responding to the force of gravity.
Comparing the velocities of living and dead *C. elegans*, it is apparent that living samples were actively swimming in the direction of gravity. their average downward velocity was twice that of our dead samples, suggesting that the *C. elegans* nervous system is capable of detecting gravity.

3438-Pos   Board B593
Biophysical Characterization of Pluripotent Stem Cell Mass Accumulation Rate and Intracolony Motion
Thomas A. Zangle[1], Jennifer Chun[1], Jin Zhang[1,2], Jason Reed[1,3], Michael A. Teitell[1].
[1]University of California, Los Angeles, Los Angeles, CA, USA, [2]Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA, [3]Virginia Commonwealth University, Richmond, VA, USA.
Despite the potential high impact of human pluripotent stem cell (hPSC) research in developmental biology, cancer biology, and regenerative medicine, surprisingly little is known about how hPSCs grow, divide, and respond to their environment. In this talk, we will introduce live cell interferometry (LCI) as a new, biophysical measurement approach for precisely quantifying hPSC colony mass distributions and growth rates (Figure 1A,B,D,F). LCI is a quantitative phase microscopy technique in which the phase shift of light as it passes through and interacts with matter inside a cell is measured. Our measurements with LCI show that retinoic acid-induced differentiation minimally slows the rate of mass accumulation, a surprising result considering the large metabolic and proliferative changes associated with the transition away from the pluripotent state. We also present methods to quantify the rate and coordination of intracolony motion from colony mass distribution measurements (Figure 1 C,G). Differentiated colonies exhibit a significantly slower rate of mass motion and significantly less coordination of motion, a previously unknown behavior that may provide new information on the health and differentiation potential of available hPSC lines.

3439-Pos   Board B594
Optimization of FRET Microscopy for Live-Cell Imaging of Multiple Protein-Protein Interactions
Brandon L. Scott, Adam D. Hoppe.
South Dakota State University, Brookings, SD, USA.
Recent advances in fluorescence resonance energy transfer (FRET) microscopy have made it possible to measure simultaneously, multiple protein-protein interactions inside living cells. To further these methods, we integrated an improved multi-spectral imaging analysis method (N-way FRET) with optimized fluorophores for live-cell FRET microscopy. For 3-way FRET we determined the best cyan, yellow, and red (C-Y-R) FRET-trio by comparing the acceptor photobleaching FRET efficiencies of structurally similar linked fluorescent protein constructs. The pool of molecules examined was constrained to the available C, Y, R molecules with the best photophysical properties (CyPet/TFP1, mCitrine/YPet and TagRFP/mCherry, respectively). From this set, we determined that TFP, YPet or mCitrine and mCherry were the optimal FRET trio. For increasingly complex pathways, more fluorescent probes may be required. To extend this method to the analysis of 4-way FRET we examined fluorophore pairs with highly overlapping excitation and emission spectra (e.g. TFP and GFP or Citrine and GFP). These improvements should enhance the spatiotemporal resolution, the number of possible interacting probes and the reliability of FRET experiments leading to improved monitoring of multiple protein-protein interactions in complex pathways.