the trap stiffness which was determined from Boltzmann distribution and power spectrum was measured, respectively. When using the ative method, the trap-stiffness which was analyzed through the data of the amplitude and phase information of the tradpped particle by using an lock-in amplifier was measured. The trap stiffness values obtained from two different methods were compared.

900-Pos Board B686

Tracking the Location and Short-Time Dynamics of the Origin of Chromosomal Replication in *E. coli*

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The location of the origin of chromosomal replication (*oriC*) in *E. coli* has been previously studied along the long axis of the cell, but little is known about its radial position. It is thought to interact with the cell membrane in a manner which helps regulate the initiation of replication. *oriC* was labeled with GFP using a ParB-*parS* system which was a gift from the Gourse lab at UW Madison. For cells taken from a stationary phase culture, the *oriC* radial distribution peaks ~250 nm from the center of the cell and not at the inner membrane, which is ~400 nm from the center. Cells in other stages of growth will be examined to determine the position of *oriC* throughout the growth cycle and in various media.

The dynamics of *oriC* on the several second timescale were also investigated. *oriC* was tracked at 10 Hz for 15 seconds. Mean squared displacement versus time along the long and short axes of the cell were fit with a confined diffusion model including a term for diffusion of the confinement domain. Diffusion analysis shows that short-term diffusion (1-2 s) along the long axis (5.2×10^{-3} $\mu m^2 s^{-1}$) is slightly faster than along the short axis (4.0×10^{-3} $\mu m^2 s^{-1}$); the confinement domain is slightly larger along the long axis as well. We suggest that the highly compacted DNA is stiffer along the short axis than along the long axis, as befits a large polymer confined within a spherocylindrical geometry. The diffusion constant on the 2-5 s time scale is 3.3×10^{-4} $\mu m^2 s^{-1}$ in both dimensions, representing movement of the confinement domain.

901-Pos Board B687

Self-Assembly of a DNA-Based Switchable Linker for Single-Molecule Force Spectroscopy

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The ability to manipulate and observe single biological molecules has led to both fundamental scientific discoveries and new methods in nanoscale engineering. A common challenge in many single-molecule experiments is reliably linking molecules to surfaces, and identifying their interactions. We have met this challenge by nanoengineering a novel DNA-based linker that behaves as a force-activated switch, providing a molecular signature that can eliminate errant data arising from non-specific and multiple interactions. By integrating a receptor and ligand into a single piece of DNA using DNA self-assembly, a single tether can be positively identified by force-extension behavior, and receptor-ligand unbinding easily identified by a sudden increase in tether length. Additionally, under proper conditions the exact same pair of molecules can be repeatedly bound and unbound. Our approach is simple, versatile and modular, and can be easily implemented using standard commercial reagents and laboratory equipment. In addition to improving the reliability and accuracy of force measurements, this single-molecule mechanical switch paves the way for high-throughput serial measurements, single-molecule on-rate studies, and investigations of population heterogeneity.

902-Pos Board B688

High Bandwidth Resonant Radio Frequency Circuit for Lipid Bilayer Detection

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Research on ion channels are traditionally performed in the DC regime. Only a few attempts having been made to conduct RF measurements for probing ion channel properties. We present here simulations and subsequent measurements of an integrated coplanar waveguide and microfluidic tank circuit detecting bilayer formation. We observe a 5 MHz shift in resonant frequency and a 1.4 dB shift in amplitude when a lipid bilayer is formed. These measurements allow for in-vitro radio frequency recordings with nanosecond readout times. The designed structure also enables the incorporation of simultaneous RF and DC recordings from lipid bilayer embedded proteins such as ion channels.

903-Pos Board B689

Complementation Activated Light Microscopy for Nanometer Accuracy Single-Molecule Targeting and Tracking in Cells and Living Animals

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Detecting single biomolecules in their native environment is challenging, in particular in living tissues. In animals, variable protein expression levels and inadequate intravital probe delivery strongly complicate the specific detection of individual biomolecules. In addition, the complex and generally highly autofluorescent environments of tissues often hinder measurements. To circumvent these limitations, we recently developed a methodology named Complementation Activated Light Microscopy (CALM), in which proteins of interest are fused to dark split-GFP which are stochastically activated into bright GFP by irreversible complementation with exogenous synthetic peptides. We describe how we use CALM to specifically target, image and track proteins with nanometer accuracy in cells and in living C. elegans nematodes, independently of protein expression levels and at micromolar probe concentrations.

904-Pos Board B690

Quantification of Protein Concentration using Single Molecule Western Blot

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As a major protein detection system, western blot has commonly used to probe changes in amount of total or activated proteins in the cell. In this technique, antibodies that are specific to target proteins are recruited as to measure protein amount in the cell extract. Nevertheless, as the detection level of the western blot changes depending on antibody efficacy and differences in individual systems, protein amount has been obtained only in qualitative manner. In this study, we introduce Single Molecule Western Blot(SMW) assay that incorporates conventional western blot system into single-molecule fluorescence microscopy, providing expression levels of proteins using whole cell extract. Our results demonstrate the use of this technique into a cell signaling system suggesting its potency for future biomedical research and diagnostics.

905-Pos Board B691

Non-Gaussianity in Single Particle Tracking: Use of Kurtosis to Learn the Characteristics of a Cage-Type Potential

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Nonlinear interaction of membrane proteins with cytoskeleton and membrane leads to non-Gaussian structure of their displacement probability distribution.

We propose a statistical analysis technique for learning the characteristics of the nonlinear potential from the time dependence of cumulants of the displacement distribution.

The efficiency of the approach is demonstrated on the analysis of kurtosis of the displacement distribution of the particle traveling on a membrane in a cage-type potential.

Results of numerical simulations are supported by analytical predictions. We show that the approach allows robust identification of the potential for the much lower temporal resolution compare with the mean square displacement analysis.

906-Pos Board B692

Single-Image Axial Localization Precision Analysis for Individual Fluorophores

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Bio-mechanism investigations demand single particle tracking with high spatial and temporal resolutions which require single fluorophore 3D localization measurements with matching precision and speed. Although the precision for lateral-localization measurements is well described by an analytical expression, for the axial direction, it is often obtained by repeating location measurements or by estimating a lower bound. Here, we report a precision expression for an axial-localization method that analyzes the standard deviations of single fluorophores' intensity profiles. Like the lateral-localization precision, this expression includes all relevant experimental effects measurable from a Gaussian intensity profile of the fluorophore. This expression completes the precision analysis for single-image 3D localization of individual fluorophores and lifts the temporal resolution to the typical exposure timescales of milliseconds.