tivity and cooperative dependence on ER [$Ca^{2+}$], suggesting that the accumulation and activation of CRAC channels at the junctions are driven entirely by STIM1 accumulation. To multimerize STIM1 independently of store depletion, we replaced the lumenal domain of STIM1 with FRB or FKBP. In cells coexpressing these two chimeric proteins, addition of a rapamycin analog caused STIM1 multimerization and redistribution to ER-PM junctions, as well as the activation of CRAC channels even though the ER was replete with $Ca^{2+}$. These results demonstrate that the multimerization of STIM1 is both necessary and sufficient to activate CRAC channels and therefore constitutes the critical event that couples store depletion to the activation of $Ca^{2+}$ entry.

**Platform AS: Single Molecule Biophysics II**

## 1845-Plat Observing Individual Protein Conformation In Live Cells With Single Molecule FRET

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The unique capabilities of the single molecule approach are likely to provide insights into a wide variety of cellular signaling pathways. Single particle tracking experiments in live cells are beginning to realize this potential. Here we present progress in our efforts to combine single particle tracking with single molecule spectroscopy to allow fluorescence resonance energy transfer (FRET) to report the real time conformational dynamics of individual protein molecules inside live cells. Recombinantly expressed proteins are dye-labeled and microinjected into cells for in vivo tracking using total internal reflection microscopy. Simultaneous spectroscopy allows the degree of FRET to be determined. We present initial results detecting single molecule FRET in live cells.

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## 1846-Plat Single Molecule Imaging with Self-Assembled Colloidal Lenses

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Single molecule spectroscopy has grown into a powerful technique that allows for the study of the behavior of individual molecules in complex local environments, effectively removing the ensemble averaging of bulk measurements. Wide-field single molecule imaging techniques such as epifluorescence and total internal reflection microscopy often employ high numerical aperture (NA) objective lenses to maximize their photon collection ability. These objectives are challenging to use because they are expensive and have small fields of view, short working distances, and require fluidic contact via an index-matching liquid. The principle of colloidal lensing overcomes these limitations by incorporating a focusing element in immediate proximity to the emitting molecule or nanoparticle. The colloid acts as a lens and dramatically improves the photon collection efficiency of the optical system. By self-assembling a high index of refraction colloid in close proximity to a fluorophore, we have shown that it is possible to image single DNA molecules using an inexpensive objective with a large field of view, long working distance, and low light collection ability (20x 0.5 NA air).

## 1847-Plat Differential Traveling Wave Technique with Ångstrom Resolution for Tracking Fast Bio-molecular Processes

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We have developed a new experimental optical technique that provides extremely high temporal (microseconds) and spatial resolution (with less than 0.1 nm noise at 10kHz) that are key to studying bio-molecular processes and Brownian dynamics at unprecedented resolution.

In this work I will present the new optical experimental technique that is based on a differential measurement of a traveling wave which is highly sensitive and minimally invasive. This technique allows the study of real time dynamics of molecular motors in the single-molecule limit. I will present our results of the application of the technique to the study of kinesin-microtubule systems and Brownian dynamics in confined dimensions

## 1848-Plat Multicolor Single Quantum Dot-DNA Dimers As A Nanometer Ruler

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Single Quantum Dot-dsDNA dimers (QD-dimers) have been constructed for use as an optical ruler in ultrahigh-resolution colocalization experiments. By varying the length of the dsDNA, the length of the ruler can be precisely controlled and multiple distance measurements can be accomplished. This technique makes use of an advantageous property of quantum dots (QD's) which allows QD's of different emission wavelengths to be excited by a single laser. A closed loop piezo scanning confocal microscope was used which allows for the simultaneous imaging of multiple colored QD's, free of chromatic aberrations. This is achieved by scanning the sample pixel by pixel using the piezo scanner and collecting the emission wavelengths and intensities of each pixel with two avalanche photodiodes. The QD images are then fit to the excitation point-spread function of the laser to precisely locate their respective (x, y) 2-dimensional positions. Using this method, we demonstrate distance measurements with sub-nanometer resolution.

## 1849-Plat High-resolution Magnetic Tweezers For Single-molecule Measurements

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The magnetic tweezer is a single-molecule instrument based on applying magnetic forces to a bead that is tethered to a glass surface with a DNA molecule. Traditionally, bead height (and thus DNA length) is measured by imaging the bead with transmitted light, and analyzing its diffraction pattern[1]. This works well in the low-force limit, where Brownian motion dominates the experimental noise; however, in the high-force limit, the magnetic tweezers' resolution is limited by the relatively small variation with bead height of the diffraction pattern. To overcome this, we have adapted Reflection Interference Contrast Microscopy (RICM) for high-resolution bead-height measurement in a magnetic tweezer[2]. RICM is based on objective-side illumination, which creates interference between rays reflecting from the glass and bead surfaces; this interference is more sensitive to changes in bead height than diffraction. We have optimized the intensity and contrast of the RICM interferogram by fabricating thin-films on the bead and glass; these films increase the reflectance of each interface while retaining bio-functionality. Finally, we show that effects of thermal drift can be nearly completely removed from the system by implementing image-analysis-based feedback control of the focal position through piezo-driven motion of the objective. All told, our approach is capable of 0.12 nm resolution at 60 Hz, and is stable over tens of minutes. We present results demonstrating the resolution, calibration, and stability of our apparatus.

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## 1850-Plat Determining Structure and Conformational Dynamics of Proteins and DNA by Single Molecule ALEX-FRET Experiments

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Single-pair Fluorescence Resonance Energy Transfer coupled to Alternating Laser EXcitation (sp-ALEX-FRET) is widely used to measure distance distributions of freely diffusing macromolecules. Moreover, when conformational fluctuations occur faster than the molecule's transit time through the confocal spot, the sp-FRET histogram is altered and hence contains information about the molecule's dynamics. Here we introduce (i) a mathematical model which explains the shape of sp-FRET histograms resulting from various energy landscapes scenarios allowing the determination of the structure and dynamics of the studied molecule and (ii) fluorophore excited-state lifetime analysis based method (Nanosecound-ALEX) allowing the determination of Donor-Acceptor (D-A) distance distributions of fast fluctuating molecules. We applied these newly developed spectroscopic methods to study a variety of biomolecules:

We observed that short ds-DNA are not as rigid as previously believed
We measured the polymer properties (e.g. persistence length) of ss-DNA
We measured the structure and dynamics of two-states DNA hairpins
We measured the average D-A distance in a collapsed state of unfolded protein-L, demonstrating the lack of residual native-like tertiary structure
We measure the D-A distance distribution of unfolded Protein-L and Chymotrypsin Inhibitor-2 (CI2), demonstrating two possible scenarios for protein folding
We measured the change in the conformational dynamics of Lac-Y sugar channel membrane protein upon sugar binding

## 1851-Plat Resolving Single Molecules In 3D With Multifocal Plane Microscopy: How Far Can We Go Beyond The Classical 3D Resolution Limit?

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The distance separating two biomolecules in close proximity inside a 3D cellular environment is important for characterizing the nature of their interaction. Recently, we presented an information-theoretic 3D resolution measure for optical microscopy which shows that separation distances well below the 1 micron limitation imposed by the classical 3D resolution criterion can be resolved [1]. Contrary to common belief, important biomolecular interactions which take place at distances of less than 200 nm can in fact be studied using an optical microscope.

We recently developed a novel imaging modality called multifocal plane microscopy (MUM) which enables the simultaneous imaging of several focal planes within the sample [2, 3]. In the context of the 3D resolution problem, image data captured at the multiple focal planes provide additional information pertaining to the distance between molecules. Based on the statistical theory concerning the Fisher information matrix, we expect that with MUM it is possible to resolve distances well below the classical 3D resolution limit.

In the present work, we present an analytical expression for the 3D resolution measure for a MUM setup. The resolution measure is expressed in terms of quantities such as the photon detection rate of the single molecules, the acquisition time, and the numerical aperture of the objective lens. Specific emphasis is placed on studying the effects that deteriorating experimental factors such as detector pixelation and extraneous noise sources have on the 3D resolution measure. Additionally, comparisons are made with the resolution measure for a conventional optical microscope.

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## 1852-Plat Objective, Model-independent Detection of Steps in Noisy Data

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Biophysical techniques, such as single molecule fluorescence microscopy and FRET, single ion-channel patch clamping, and optical tweezers often yield data consisting of noisy dwells separated by discrete steps. When an underlying kinetic model can be assumed, steps may be located in a rigorous fashion using methods derived from the theory of hidden Markov models. In the absence of a kinetic model, such steps are often located by eye or with the assistance of heuristic fitting or filtering techniques. We present an objective, rigorous, model-independent method enabling identification of non-uniform steps present in such noisy data. Our method is based on the Schwarz Information Criterion, a test statistic enabling meaningful comparison between fits with differing numbers of parameters, and does not require the assumption of any underlying kinetic or state models. It is hence particularly useful for analysis of novel and poorly understood systems.

**Platform AT: Membrane Proteins - II**

## 1853-Plat The Outer Membrane Translocon for Nuclease Colicins: Higher Resolution Structure of the OmpF Porin

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The *E. coli* OM translocon for colicins E2/E3 consists of the high affinity receptor, BtuB [1, 2] and receptor-translocators, the OmpF or OmpC porin [2]. A complex containing colicin E3/BtuB/OmpF was cross-linked *in situ*. Channel activity of OmpF can be occluded by colicin E3, or its 83 residue N-terminal disordered translocation domain, T83. Occlusion is negated by the mutations, Asp5Ala and Arg7Ala, implying an electrostatic interaction with one or more critical carboxylate or basic residues in the constriction zone [3]. T83 was shown to bind/insert to/into OmpF at low, but not high ionic strength. A 1.6 Å crystal structure of OmpF was obtained by crystallization of OmpF in the presence of 1 M $MgCl_2$ and T83. T83 was not seen in the electron density. However, a hexa-aquo $Mg^{2+}$ ion is seen that bridges between the two carboxylate residues, Asp113 and Glu117 in the L3 loop of the porin constriction zone, and is the major change in the OmpF electron density compared to that of the OmpF structures obtained at somewhat lower (2.2 Å) resolution [4]. The higher resolution results in the detection of more than 300 crystallographic $H_2O$ molecules with a minimum in the water density at the position of the constriction filter.

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## 1854-Plat Real-time Visualization of the Assembly of Aquaporin-4 Isoforms into Orthogonal Arrays Revealed by Single Particle Tracking (SPT)

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Aquaporins (AQPs) are a family of integral membrane proteins whose function is to facilitate osmotically driven water transport across cell plasma membranes. AQP4 and AQP1 are the primary water channels expressed in the eye and central nervous system. AQP4 naturally exists in two isoforms, a full-length form (M1), and a shorter form (M23), which is truncated by 22 residues at the N-terminus, but otherwise identical to M1. Freeze-fracture electron microscopy has shown that M23, but not M1, forms large orthogonal arrays of particles (OAPs) in the plasma membrane of primary astrocytes and transfected cell lines. We tracked the membrane diffusion of AQP4 isoforms M1 and M23, and AQP1 labeled with quantum dots at an engineered external epitope at frame rates up to 91 Hz and over times up to 6 min (Crane and Verkman. *Biophys. J.* In Press). SPT in transfected primary astrocytes and COS-7 cells showed that >85% of M23 was highly restricted or immobile, with range <50 nm at 1s and diffusion coefficient $D$~$7\times10^{-11}$ $cm^2/s$, consistent with its expected confinement within OAPs. Under identical conditions, >70% of M1 exhibited Brownian diffusion, covering >400 nm in 1s, with $D$~$5\times10^{-10}$ $cm^2/s$. Likewise, >75% of AQP1 showed Brownian diffusion, with $D$~$9\times10^{-10}$ $cm^2/s$. Deletion of a C-terminal PDZ-binding domain in AQP4, as well as disruption of the actin skeleton had no effect on M23 diffusion, indicating that array formation is independent of cytoskeletal interactions. The diffusion of N-terminal deletion mutants of AQP4 were also investigated, as well as AQP4 isoforms following cellular treatments that modulate phosphorylation signaling pathways. SPT permits real-time visualization of AQP4 within OAPs in live cells. Our results indicate that an N-terminal motif in M1 is responsible for the disruption of OAPs.

## 1855-Plat A Novel Experimental Approach To Characterize Membrane Protein Interactions

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We have revisited the technique of fluorescence recovery to probe interactions between membrane proteins embedded within the same or in different membranes.

For this purpose we use a medium of model membranes with tunable intermembrane distance.

When the membranes are far apart, the only possible interactions occur between proteins embedded within the same bilayer, whereas when membranes get closer to each other, interactions between proteins embedded in opposite membranes may occur as well.