133-Pos Board B12
FCS and Sub-diffraction Resolution Fluorescence Imaging of Membrane Receptors in Living Organelles
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Fluorescence microscopy is a standard tool in molecular biophysics, but even the best resolution obtained by diffraction-limited conventional optical techniques misses the molecular level by two orders of magnitude. In order to overcome the classical diffraction limit, several sub-diffraction resolution imaging methods have been introduced so far.
Direct stochastic optical reconstruction microscopy (dSTORM) (1) and PAINT (points accumulation for imaging in nanoscale topography) (2) have the potential to shed light on the intracellular organization of cells with near-molecular resolution.
These techniques will be used to localize labelled peptides binding to receptors located in the membrane of protoplasts of the flowering plant *Arabidopsis* (a model organism for plant research). Binding dynamics will be studied by fluorescence correlation spectroscopy (FCS).
References:
(1) M. Heilemann et al., Angew. Chem. Int. Ed. 2008 **47** p. 6172.
(2) M. Hochstrasser et al., PNAS **103** p. 18911 (2006).

134-Pos Board B13
Single molecule image deconvolution. I. Standard deviation analysis of immobile fluorescent molecules
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Single molecule fluorescence imaging has been a powerful technique in studying individual processes not accessible by bulk, ensemble-averaged measurements [1]. Improvements in image analysis are required for high temporal and spatial precision in the localization of single fluorescent molecules. We present the first thorough standard deviation analysis for point spread functions (PSFs) of single immobile fluorescent molecules. Using this new single molecule image deconvolution (SMID) method, we show that 3D localization of individual molecules with sub-nanometer precision can be achieved. We have derived an expression estimating the standard error of the PSF's standard deviation, incorporating experimental effects of the number of collected photons, finite pixel size, and background noise. The localization precision obtained via this expression is approximately 1.5 times better than the current available methods. The use of SMID to extract subexposure dynamics of mobile molecules will also be discussed.
[1]. Wang, Y. M, R. H, Austin, & Cox, E. C. 2006 *Physical Review Letters* **97**, 048302(1-4).

135-Pos Board B14
EstimationTool And FandPLimittool - User-friendly Software Packages For Single Molecule Localization/Resolution And Accuracy Calculations.
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The 2D/3D localization and resolution of single molecules from their images has the potential to provide much biological insight. However, they are complex and challenging tasks that involve fitting various model profiles to single molecule images using various parameter estimation algorithms. We developed the EstimationTool (1) to facilitate this process. Through a user-friendly graphical user interface, the tool allows a user to select from a variety of localization/resolution tasks, select from a variety of image models (Gaussian, Airy, Born-Wolf), noise models (Poisson, Gaussian), and estimation algorithms (maximum likelihood, least squares), and customize every aspect of the associated calculations. The accuracy of results from the various localization/resolution tasks varies based on the estimation algorithm, noise model, and image profile used. Therefore it is important to know the best possible accuracy with which the location of a single molecule or the distance between closely-spaced single molecules can be estimated. Various localization/resolution measures have been developed for this purpose (2,3,4,5). The FandPLimitTool (Fundamental and Practical Limit Tool) is a software package that calculates these localization/resolution measures for various estimation scenarios (6). Together, the EstimationTool and FandPLimitTool provide significant assistance in the quantitative analysis of single molecule data.
1. http://www4.utsouthwestern.edu/wardlab/EstimationTool
2. Ober, R.J., Ram, S., and Ward, E.S. (2004) Biophys. J. 86, 1185-1200.
3. Ram, S., Ward, E.S, and Ober, R.J. (2005) Proc. of SPIE 5699, 426-435.
4. Ram, S., Ward, E.S., and Ober, R.J. (2006) PNAS 103, 4457-4462.
5. Ram, S., Abraham, A.V., Ward, E.S., and Ober, R.J. (2007) Proc. of SPIE 6444, D1-D9.
6. http://www4.utsouthwestern.edu/wardlab/FandPLimitTool

136-Pos Board B15
Maximum-Likelihood Position Sensing of Single Molecules in a Confocal Microscope
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In wide-field microscopy, the location of a single molecule can be measured to within tens of nanometers by imaging the point spread function over a number of camera pixels and finding the center of the image. However, for many single-molecule applications, confocal microscopy is preferable to wide-field imaging, as it provides improved signal-to-noise ratio due to the very small detection volume; it is necessary for two-photon excitation, which offers potential advantages for intracellular studies; and it facilitates monitoring of sub-millisecond dynamics and fluorescence lifetimes by use of a single-photon avalanche diode (SPAD) detector for time-resolved single-photon counting. Here, we report studies of the capabilities for sub-diffraction, single-nanoparticle position determination in a confocal two-photon microscope. To measure the position, the beam from a femtosecond laser is split and recombined at beam splitters to produce four beams that are focused to slightly offset spatial positions centered at the vertices of a tetrahedron, and with pulses that are temporally offset so as to yield pulse-interleaved excitation at the four overlapping focal volumes. Two-photon-excited fluorescence is collected from the entire four-beam excitation volume onto just one SPAD detector. Time-gated photon detection provides information on the volume from which each photon was most likely emitted and hence, the most likely position of the single particle. As this form of position sensing requires only a single SPAD, it is scalable to multiple detectors for multi-color observations, and can thus be used to find the separations of differently colored molecules over a distance range that is complementary to that achievable by FRET.

137-Pos Board B16
Quantitative Study Of Single Molecule Localization Techniques
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Among the various techniques by which the locations of single molecules can be estimated from microscopy images, the question arises as to which produces the most accurate results. The accuracy of an estimation technique is measured by the standard deviation of its estimates. The estimated location of a single molecule can deviate from its true location because of the stochastic nature of the photon emission/detection process, extraneous additive noise, and pixelation. Here we examine the estimates from the maximum likelihood and the non-linear least squares estimators when fitting Airy and Gaussian profiles to single molecule images. We see that on average both estimators recover the true location of the single molecule. Comparing the standard deviations of the estimates from both estimators, the maximum likelihood estimator appears to be generally more accurate. Since the accuracy of estimation techniques varies, it is important to know the best possible accuracy that can be achieved for a given set of imaging conditions. We have previously developed a method by which this can be determined (1). We find that the accuracy of the maximum likelihood estimator is typically close to the best possible accuracy. We also observe that localization accuracy is dependent on the specific image profile used to fit the data. Gaussian profiles are often used to fit single molecule images even though the image of an in-focus point source may be modeled more accurately by an Airy profile in many cases. We explore the effect on localization accuracies of performing estimations with such a model mismatch.
1. Ober, R.J., Ram, S., Ward, E.S. (2004) Biophys. J. 86, 1185-1200.

138-Pos Board B17
Creation and Mixing of Monodisperse Sub-femtoliter Bioreactors
Jianyong Tang[1], Ana M. Jofre[2], Rani B. Kishore[3], Joseph E. Reiner[3], Mark E. Greene[3], Geoffrey M. Lowman[3], John S. Denker[3], Christina Willis[3], Kristian Helmerson[3], **Lori S. Goldner[4].**
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We have developed and describe a method to generate monodisperse optically-trappable aqueous emulsion droplets (''hydrosomes'') on demand by piezoelectric injection. The droplets have been measured to have radii as small as 368 nm $\pm$ 16 nm, corresponding to a volume of 212 aL $\pm$ 27 aL. The hydrosomes are injected into a perfluorocarbon continuous phase. The refractive index of the

perfluorocarbon is lower than that of water, rendering the hydrosomes trappable by a conventional optical tweezer. Using the injector and optical manipulation we demonstrate fusion of two hydrosomes, one containing a calcium-sensitive dye and the other containing calcium chloride, and we measure the mixing times to be on the order of 10 millisecond or less. The monodispersity, repeatability, small size, and fast mixing afforded by this system offer unprecedented opportunities for nanochemistry and single molecule or single nanoparticle studies.

139-Pos Board B18

Simultaneous Measurement Of Ionic Current And Fluorescence From Single Protein Pores

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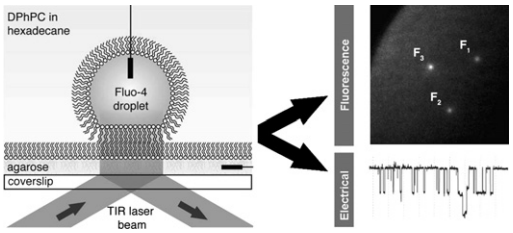
The ability to simultaneously monitor both the ionic current and fluorescence from membrane proteins has the potential to link structural and functional changes in a protein. However, demonstrating the synchronised detection of current and fluorescence at the single molecule level remains a serious challenge. We present a new method for simultaneously measuring single-channel electrical currents and fluorescence from ion channels by using an in vitro water-in-oil droplet bilayer system. We demonstrate fluorescence and electrical detection of stochastic blocking by cyclodextrin in multiple staphylococcal alpha-hemolysin pores. The combined fluorescence signal from individual pores exhibits the same sequence of blocking events as the total current recording, providing unambiguous correlated fluorescence and electrical signals for every protein pore in a bilayer.



140-Pos Board B19

Laser Induced Popcorn-like Conformational Transition of Nano-diamond as a Nanoknife

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Nanodiamond (ND) is surrounded by layers of graphite on its surface. This unique structure feature creates unusual fluorescence spectra, which can be used as an indicator to monitor its surface modification. Meanwhile, the impurity, nitroso (C-N=O) inside the ND can be photolyzed by two-photon absorption, releasing NO to facilitate the formation of a sp^3 diamond structure in the core of ND and transforming it into a sp^2 graphite structure. Such a conformational transition enlarges the size of ND from 8 nm into 90 nm, resulting in a popcorn-like structure. This transition reaction may be useful as nano-knives in biomedical application.

141-Pos Board B20

High throughput Single-Molecule Spectroscopy Using Nanoporous Membranes

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We describe a novel approach for optically detecting DNA translocation events through an array of solid-state nanopores that potentially allows for ultra high-throughput, parallel detection at the single-molecule level. The approach functions by electrokinetically driving DNA strands through sub micrometer-sized holes on an aluminum/silicon nitride membrane. During the translocation process, the molecules are confined to the walls of the nanofluidic channels, allowing 100% detection efficiency. Importantly, the opaque aluminum layer acts as an optical barrier between the illuminated region and the analyte reservoir. In these conditions, high-contrast imaging of single-molecule events can be performed. To demonstrate the efficiency of the approach, a 10 pM fluorescently labeled lambda DNA solution was used as a model system to detect simultaneous translocation events using electron multiplying CCD imaging. Single-pore translocation events are also successfully detected using single-point confocal spectroscopy.

142-Pos Board B21

Using Nanomaterials to Probe Rotation of Individual Cell Surface Receptors

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Rotation of membrane proteins is a sensitive measure of their aggregation state and their interactions with other membrane species. We have explored the use of nanomaterials, including both gold nanorods (GNR) and asymmetric quantum dots (QD), as non-bleaching imaging probes of the rotation of individual cell surface proteins. GNR exhibit resonant light scattering between 700-800 nm which is highly polarized along the rod axis. Orthogonally-polarized, dark-field images of GNR immobilized on glass contain polarized spots suggesting randomly-oriented individual rods. However, imaging individual antibody-conjugated GNR on cell surfaces has been problematic. QD can also provide orientation-dependent optical signals: one commercial product, rod-like in shape, exhibits an initial fluorescence anisotropy >0.2. These QD, conjugated to anti-insulin receptor β-chain antibody, are easily visualized bound to 2H3 cell insulin receptors (IR) as well as immobilized on glass. Blinking of spots in orthogonally-polarized fluorescence images demonstrates imaging of individual QD. The time-autocorrelation function for fluorescence anisotropy from individual cell-bound QD exhibits a slow component decaying on the 100-200 ms timescale. Whether this represents intrinsically-hindered rotation of individual IR or results from crosslinking of multiple receptors by single QD remains to be determined. Supported in part by NIH grant RR023156 and NSF grant CHE-0628260.

143-Pos Board B22

Manipulating The Environment Of a Single Lipase Molecule

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Lipase are interfacial enzymes with attractive applications. Their activity is greatly enhanced in the presence of a hydrophobic surface, a process called interfacial activation. However, the kinetics of this behavior is not yet fully understood. We measured this kinetics of a lipase from Thermomyces Lanuginose at single enzyme level. We utilized single vesicle arrays as a novel biocompatible scaffold to immobilize enzymes and as an interface to study the effect of the enzyme's binding to the membrane on the observed activity. We used organic polymers to vary the accessibility of the enzyme molecule to the bilayer. By this kind of direct control of the microenviroment of the enzyme, we quantified the activity of enzyme at different degrees of accessibility to the bilayer. Therefore, we gained a clear insight of interfacial activation of lipase and we are currently using this platform to quantify the influence of additional parameters, such as size of vesicles and lipid composition, on enzyme's catalytic behavior.

144-Pos Board B23

Single Molecule Studies of the Interactions between β-Amyloid 1-40 and Supported Planar Lipid Membrane

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Recent evidence supports the hypothesis that the early oligomers formed by the β-Amyloid peptide are cytotoxic and may feature in Alzheimer's disease (AD). While the mechanism of this cytotoxicity remains unclear, interactions of these oligomers with neuronal membrane are believed to be involved. Identifying the cytotoxic species is challenging because the β-Amyloid oligomers are extremely heterogeneous, metastable, and form at very low physiological concentrations (nM). In our study, we use single molecule spectroscopy (SMS) to study the interactions between β-Amyloid 1-40 and supported planar lipid membranes. The evolution of β-Amyloid species on lipid membranes were monitored for up to a few days. The results indicate that the interactions between β-Amyloid 1-40 and planar lipid membranes follow three stages. First, a very small fraction of β-Amyloid peptide binds to the membranes with high affinity (Kd < 470pM), covering the membrane surface uniformly and also diffusing within the lipid molecules. In the second stage, β-Amyloid peptides assemble to form oligomers in the membrane. We observed at least two different pathways of oligomer formation, depending on the aqueous β-Amyloid peptide concentration. In the final stage, after prolonged incubation with the lipid membranes, β-Amyloid peptides start forming mesh-like deposits. With the high sensitivity and spatial and temporal resolution of single molecule spectroscopy, we successfully traced the interactions between β-Amyloid 1-40 peptide and planar lipid membranes at the molecular level. Our results are also in agreement with the molecular model of pore-forming peptides suggested by H.W. Huang et al. (1).

1. Huang, H.W., F.Y. Chen, and M.T. Lee. 2004. Molecular Mechanism of Peptide-Induced Pores in Membranes. Physical Review Letters. 92:198304.