It is known that bone and tendon are piezoelectric, and it is believed that the piezoelectric charges produced under mechanical deformation in these tissues have a potential role in mechanoelectric transduction leading to their growth and remodeling. With high resolution PFM we probe piezoelectric properties in bone and show that single collagen fibrils are responsible for piezoelectric behavior of bone and behave predominantly as shear piezoelectric materials. Furthermore, we show that there is an intrinsic electromechanical heterogeneity in axial direction of individual fibrils that holds even for the collagen fibrils embedded in bone matrix. Such heterogeneity may have implications in regulating the ionic environment in bone responsible for bone remodeling.

3953-Plat
SAXS: Structure Verification of an S-Layer Protein using a Fractal Mean Force Potential
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Using a combination of SAXS and molecular dynamics simulations we get an integrated picture of the structure of S-layer proteins, for which no crystallized structure is currently available. S-layers are the most commonly observed cell surface structure of prokaryotic organisms and they are made up of identical protein subunits. One of the most striking properties of S-layers is that they are able to self-assemble into crystalline lattices in suspension and on various solid substrates. The resulting ordered molecular layer provides a matrix for the binding of various biomolecules and nanoparticles. Due to the complex biochemical properties of these proteins, classical techniques such as NMR or X-ray crystallography have not been able to provide an atomistic structural model for S-layer proteins. Motivated by the results obtained through the use of a fractal concept for the analysis of SAXS data in cluster physics, here we employ such fractal concept for the investigation of the structure of S-layer proteins. We fit the SAXS intensity as a function of the scattering angle using both a fractal form factor and a fractal structure factor. We compute the form factor by a Fourier transform of an average fractal delta function of characteristic units, thereby allowing us to investigate the presence of local, rather than global, electron densities in the structure of S-layer protein monomers. The structure factor is calculated by a Fourier transform of a fractal potential of mean force. Using this fitting function, we calculate the shape of the monomers, which is in good agreement with the shape obtained using molecular dynamics simulations. Our approach reveals itself as a novel means to get a detailed insight into the structure of proteins that adopt a fractal self-assembly and that lack of crystallized structure.

3954-Plat
Is Theory Leading Neutron Diffraction in Macromolecular Solvent Networks?
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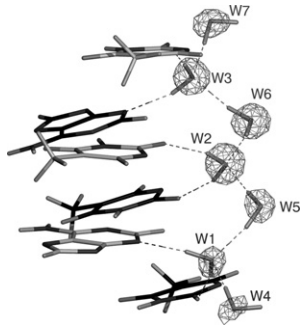
Standard crystallographic practice models electron scattering as a spherically symmetric phenomenon about atoms in the absence of any external effects. We have recently developed a polarizable atomic multipole refinement method for macromolecular crystallography that presents a significant improvement to the resultant information contained in an atomic model. We apply this method to high resolution lysozyme and trypsin data sets and validate its utility for precisely describing biomolecular electron density as indicated by a decrease in 5-6% in the R and Rfree values relative to the deposited values. The resultant models also illustrate the ability of force field electrostatics to orient water networks and catalytically relevant hydrogens that can be used to make predictions regarding active site function and activity. Finally, a DNA model generates the zig-zag spine pattern of hydrogen bonding in the minor groove without manual intervention. Comparison of the solvent networks with macromolecular neutron models suggest the hydrogen bonding patterns and distances generated by our protocol are more consistent with condensed phase measures and more likely to yield energetically favorable hydrogen bonds. The refinement technique proposed should be useful in applications to enzymology, drug design, and protein folding.



3955-Plat
Identification of Functional Surfaces of Proteins from Sequences
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The identification of protein functional surfaces is important for understanding enzyme mechanism, protein function prediction, compound-protein docking, and drug design. As the speed of rapid accumulation of protein sequence information far exceeds that of structures, it is important to construct accurate models of protein functional surfaces and identify key residues on these surfaces. A promising approach is to build comparative models from sequences using known structural templates. We assess how well this approach works by building three-dimensional comparative models of proteins using standard tools and determine how well functional surfaces can be accurately reproduced. We use the pocket algorithm based on alpha shapes computed for the modeled protein structures and characterize potential binding surfaces on these structures. Based on a large scale study, we give general criteria on when such comparative models can give accurate information on functional surfaces. We also provide assessment on the applicability of this approach to the universe of currently known protein sequences. We further point out methods for improved models of protein functional surfaces.

Platform BI: Imaging & Optical Microscopy II

3956-Plat
Self-Organization of the Escherichia Coli Chemotaxis Network Imaged with Super-Resolution Light Microscopy
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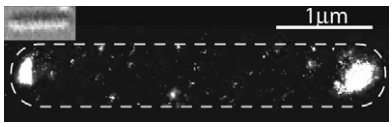
The Escherichia coli chemotaxis network is a model system for signal transduction and processing. Chemotaxis receptors assemble into large clusters containing tens of thousands of proteins which have been observed at cell poles and future division sites. Despite extensive study, it remains unclear how chemotaxis clusters form, what controls cluster size and density, and how the cellular location of clusters is robustly maintained in growing and dividing cells. Here, we use a super-resolution optical technique called photoactivated localization microscopy (PALM) to map the cellular locations of three proteins central to bacterial chemotaxis (the Tar receptor, CheY, and CheW) with a precision of 15 nm. We find that cluster sizes are approximately exponentially distributed, with no characteristic cluster size. One-third of receptors are part of smaller lateral clusters that have not been previously observed. Analysis of the relative cellular locations of 1.1 million individual proteins (from 326 cells) suggests that clusters form via stochastic self-assembly. The super-resolution PALM maps of E. coli receptors support a growing collection of evidence that stochastic self-assembly can create and maintain periodic structures in biological membranes, without direct cytoskeletal involvement or active transport.



3957-Plat
K-Space Image Correlation Spectroscopy of Quantum dot Labeled T Cell Receptors Characterizes their Nanoscale Clustering in Living Cells
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Changes in distribution of membrane receptor organization are used by cells to modulate the dynamic range of their responses to environmental cues. Consequently, it is important to develop experimental methods that can accurately measure receptor transport and aggregation. We used quantum dot (QD) labeling of T cell receptors (TCR) and a recently developed technique, k-space image correlation spectroscopy (kICS) to characterize TCR state as a function of cell differentiation. We developed kICS to measure transport coefficients of fluorescently labeled membrane proteins while taking into account nanoparticle emission blinking. We use kICS to measure T cell receptor (TCR) aggregation in live cells by characterizing quantum dot (QD) blinking and distribution on the cell surface. 2C TCR transgenic cells in culture were observed from the naïve state to 12 days after activation by antigen. Cells were labeled

with biotin-MHC/peptide to bind the TCR with relatively high avidity, followed by streptavidin-quantum dots. They were then imaged on an emCCD-equipped microscope and kICS analysis was applied. Spatial intensity fluctuations in an image measured the clustering of receptors on the 100s of nm length scale. Changes in the intensity correlation function of the blinking QDs characterized clustering on the 10s of nm length scale. We also used kICS to measure changes in TCR diffusive transport. When T cells exhibited maximum activity 3-4 days after exposure to antigen, the degree of their TCR aggregation on both length scales was significantly higher than that of naïve cells, while TCR diffusion was a minimum. This new technology has powerful applications as it can be applied to just a few cells and we will show that it is able to detect changes in receptor organization of cells in vivo.

3958-Plat
The Pair-Correlation Approach to FCS
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Molecular diffusion and transport processes are fundamental in physical, chemical, biochemical and biological systems. Current approaches to measure molecular transport in cells and tissues based on perturbation methods like fluorescence recovery after photobleaching are invasive, fluctuation correlation methods are local and single particle tracking requires the observation of isolated particles for relatively long periods of time. We propose to detect molecular transport by measuring the time cross-correlation of fluctuations at a pair of locations in the sample. When the points are further than two times the size of the point spread function, the maximum of the correlation is proportional to the average time a molecule takes to move from a specific location to another. We demonstrate the method with simulations, using beads in solution and by measuring the diffusion of molecules in cellular membranes. The spatial pair cross-correlation method detects barriers to diffusion and heterogeneity of diffusion because the time of the correlation maximum is delayed in the presence of diffusion barriers. This non-invasive sensitive technique follows the same molecule over a large area producing a map of molecular flow and does not require isolated molecules thereby many molecules can be labeled at the same time and within the point spread function. Work supported in part by U54 GM064346 Cell Migration Consortium (MD and EG), NIH-P41 P41-RRO3155 (EG) and P50-GM076516 (EG).

3959-Plat
Spatially Resolved Fluorescence Fluctuation Spectroscopy (FFS) in Living Cells
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Fluorescence (cross-)correlation spectroscopy (FCS/FCCS) and generally fluorescence fluctuation spectroscopy (FFS) are confocal microscopy-based methods that allow to assess diffusion and transport properties as well as interactions of molecules (proteins, nucleic acids, compounds) in vitro and in vivo. Commercially available instrumentation enables routine measurements at one or few specific points inside living cells.
However, conventional FCS/FCCS experiments remain challenging because point measurements in a living cell are associated with large error caused by the heterogeneous environment of the cellular interior. Moreover, biological noise due to cell-to-cell variations of physical and biological parameters (e.g. intracellular viscosity, protein expression levels) induces further variations, which are difficult to separate from the measurement error. Currently, these problems are partially addressed by performing statistical data analysis of measurements from many different cells. However, it is desirable to obtain more reliable and robust data from single cells with spatial resolution. This requires a new approach allowing to perform simultaneous measurements and to circumvent the problems associated with confocal FFS: photobleaching, out-of-focus illumination and loss of spatial definition due to cell movements.
Here, we present a novel microscope that allows spatially resolved FFS measurements in 2D optical sections across cells. The setup is based on a single plane illumination microscope in which a thin diffraction-limited light sheet is used to illuminate a cross-section of the cell. The use of an electron-multiplying charge-coupled device (EM-CCD), placed perpendicular to the light sheet, with hundreds of single pixel detectors instead of an avalanche photodiode (a single pixel detector) enables to record on each pixel the incoming photons with single photon sensitivity and sub-millisecond time resolution. This is predicted to significantly reduce the error associated with single point measurements. It should also provide access to spatially resolved measurements of concentrations, interactions and mobilities.

3960-Plat
Multiplexed Measurement of Molecular Interactions using Hyper-Spectral Imaging and Multi-Parametric Detection
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[1]The Medical Research Council Cancer Cell Unit - Hutchison/MRC Research Centre, Cambridge, United Kingdom, [2]Department of Chemical Engineering and Biotechnology, University of Cambridge, Cambridge, United Kingdom, [3]Department of Molecular Biophysics, Utrecht University, Utrecht, Netherlands, [4]Fondazione Bruno Kessler, Povo, Italy, [5]Department of Oncology, University of Cambridge, Cambridge, United Kingdom, [6]School of Advanced Optical Technologies, Max-Planck-Research Group, Division III, University of Erlangen-Nuremberg, Erlangen, Germany.
A large number of molecules cooperate in an intricate network of interactions for the maintenance of the structural integrity, the metabolism and the function of the living cell. A challenge for engineering and physics in optical microscopy is to provide tools that could offer the highest spatio-temporal resolution with the capability to decode complex networks of molecular interactions by the development of technologies and methods that, at the same time, may provide cost-effective and user-friendly instruments.
We present our latest development of a novel architecture for a spectrograph that permits to characterize fluorescence emission (excitation and emission spectra, fluorescence anisotropy and fluorescence lifetime) in a quantitative and efficient manner. The novel system offers parallel acquisition with a single detector and, by the use of a novel solid-state detector (time-gated single-photon avalanche photodiodes) and a supercontinuum light source, it provides excellent versatility of use at comparatively low costs. We envisage that by the exploitation of Foerster resonance energy transfer between a number of fluorophores, this microscopy platform will be capable to probe multi-molecular interactions and to multiplex a variety of fluorescent biosensors.
Novel biophysical imaging techniques are fundamental for our research activities in cancer research: to probe the key molecular processes underlying genomic stability and for a better understanding of the molecular aspects of cancer.

3961-Plat
Analysis of Cdc-42 Mobility and Dimerization In Vivo by Higher Order Fluorescence Correlation Cumulants
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We demonstrate a new method for measurements of mobility dependent protein oligomerization using higher order fluorescence correlation cumulants. Fluorescence intensity distribution methods including fluorescence cumulant and moment analysis have been successfully used in recent years to analyze oligomerization phenomena. The extension of such methods to treat analysis at different binning times allows for the analysis of mobility dependent oligomerization. Nevertheless, the analysis of time binned distributions is mathematically complex and depends strongly on detector characteristics such as afterpulsing and dead time. Here we develop an equivalent method treating traditional correlation functions as bivariate fluorescence cumulants. Doing so brings the power of the cumulant analysis to bear on the measurement of mobility dependent oligomerization while maintaining the mathematical simplicity of the correlation functions that are the standard within fluorescence correlation spectroscopy. We use this technique to show that the low mobility pool of intracellular EGFP-cdc-42 in living yeast cells is on average a dimer while the high mobility pool is monomeric. Examination of mutant yeast strains suggests that the low mobility pool is associated with recycling vesicles, providing an explanation for both the slow diffusion as well as the oligomeric state of this species. This technique could be easily extended to other proteins in the yeast genome that demonstrate heterogeneous mobility.

3962-Plat
H33D Gen II: A New Photon Counting Camera for Single-Molecule Imaging and Spectroscopy
Xavier Michalet, Ryan Colyer, Oswald Siegmund, Anton Tremsin, John Vallerga, Shimon Weiss.
UCLA, Los Angeles, CA, USA.
We have developed a new generation of photon-counting camera consisting of a large area microchannel plate-based photomultiplier tube associated with a position-sensing anode. Our detector can record local count rates of approx 30 kHz and sustain global count rates of several MHz. We illustrate its capabilities by tracking single quantum dots in live cells with nanometer spatial resolution and sub-ms temporal resolutions over large areas and long durations.