173-Plat
Disease Phenotyping with Sub-Resolution Precision by Single Molecule Tracking in Live Animals
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Single molecule (SM) fluorescence microscopy provides non-invasive means to localize biomolecules and characterize their diffusion in cells with a sub-resolution precision. Extending SM imaging techniques to live animals is an exciting, yet challenging endeavor that can potentially reveal how pathological processes affect the nanoscale mobility and the function of biomolecules in their native three-dimensional tissue environment. Here we used Complementation Activated Light Microscopy (CALM) to target, image and track individual voltage-dependent Ca2+ channels (VDCC) with a precision of 30 nm on muscle cells and within neuromuscular synapses of normal and dystrophin-mutant Caenorhabditis elegans worm models of Duchenne muscular dystrophy. Through diffusion and spatial pattern analyses, we show that dystrophin is a load-bearing apparatus and a tension transducer that modulates the confinement of VDCC within sarcolemmal membrane nanodomains in response to varying muscle tonus. SM imaging by CALM opens new avenues to explore the basic principles of homeostatic controls and the molecular basis of diseases at the nanometer scale in intact living animals.

174-Plat
Super Resolution Fluorescence Microscopy by Cathodoluminescence-Activated Excitation
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The nanoscale dynamical processes of organization, complexing, and aggregation of biomolecules lie at the foundation of many critical processes in metabolism, signaling, and disease. Our understanding of these processes, particularly those occurring in crowded environments, remains limited thus far by our inability to probe small aqueous volumes optically. To generate a nanoscale optical probe for visualizing nanoscale biological dynamics, we have developed and demonstrated a new approach that combines the advantages of electron and fluorescence microscopies, namely the nanoscale focusing and fast scanning capabilities of electron microscopy and the chemical specificity and non-invasiveness of fluorescence microscopy. Our new super resolution optical imaging platform consists of a high-brightness, rapidly scannable, 20-nm optical spot in cathodoluminescent (CL) thin film generated by a low energy, tightly focused electron beam from a scanning electron microscope (SEM). Because the CL film is only 10-20 nm thick, optical excitations activated by the electron beam in the CL film can be non-radiatively transferred to adjacent fluorescently labeled molecules in an encapsulated sample volume via Förster resonance energy transfer (FRET). By correlating the position of the electron beam with fluorescence from the sample, we can generate images with nanoscale resolution, high optical contrast, and fast acquisition rates. Using this approach, we have successfully imaged plasmonic metal nanoparticles with 46 nm-resolution and demonstrated high-resolution CL-activated FRET with luminescent polymer blends. By encapsulating a aqueous biological sample adjacent to the film, we anticipate imaging processes, such as DNA repair, protein aggregation, and diffusion of protein complexes on lipid membranes. We aim to achieve a spectrally-specific scanning optical microscopy with at least 20 nm lateral resolution and 10 nm axial resolution with a fast acquisition rate.

175-Plat
3D Superresolution Microscopy by Supercritical Angle Detection
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Currently, two general approaches allow for three-dimensional resolution in localization microscopy. On one hand, the z position of single emitters can be determined from the shape of the point spread function (PSF) either by imaging two focal planes (bi-plane) or after PSF engineering (e.g. astigmatic PSF, double helix PSF or self-bending PSF). Nevertheless, the axial resolution is usually worse than the lateral one, which itself is compromised by the modification of the PSF. On the other hand, the z position can be extracted from relative intensities of single-molecule images when employing an interferometric detection scheme (iPALM, 4Pi-SMS). Unfortunately, such interferometric microscopes are very difficult to built and cumbersome to use.
Here we present a fundamentally different way of achieving 3D resolution in localization microscopy, based on the principle of surface-generated fluorescence. This near-field fluorescence occurs when a fluorophore is in the vicinity of a water-glass interface. It is emitted into large angles above the critical angle and its intensity strongly depends on the distance of a fluorophore from the interface. By splitting high and low emission angles and imaging them simultaneously we can determine the precise axial position of single molecules from their relative intensities in the two channels. A theoretical analysis shows that isotropic resolution on the nanometer scale can be expected in a range of a few hundred nanometers above the coverslip, without compromising the lateral resolution.
We developed a simplified setup that we calibrated with fluorescent beads and provided proof-of-principle by imaging DNA-origami tetraedra. We present first biological data on three-dimensional imaging of clathrin-coated pits and microtubules and discuss ideas for approaching the theoretical resolution limit.

176-Plat
Super Resolution Imaging and Tracking of Protein-Protein Interactions in Sub Diffraction Cellular Space
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Imaging localization and dynamics of individual interacting protein pairs is essential but often difficult due to the fluorescent background from other paired and non-paired molecules, particularly in the sub-diffraction cellular space. Here we develop a new method combining Bimolecular Fluorescence Complementation (BiFC) and Photoactivated Localization Microscopy (PALM) for super-resolution imaging and single molecule tracking of specific protein-protein interactions. The method is used to study the interaction of two abundant proteins, MreB and EF-Tu, in *Escherichia coli* cells. The super-resolution imaging shows interesting distribution and domain sizes of interacting MreB-EF-Tu pairs as a subpopulation of total EF-Tu. The single molecule tracking of MreB, EF-Tu, and MreB-EF-Tu pairs reveals intriguing localization-dependent heterogonous dynamics and provides valuable insights to understanding the roles of MreB-EF-Tu interactions.

Platform: TRP Channels

177-Plat
Surface Characterization and Membrane Interaction of Double-Knot Toxin, an Activator of TRPV1 Channels
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Double-knot toxin (DkTx) is a novel tarantula toxin that activates TRPV1 channels by binding to the extracellular pore domain of the channel, and is composed of two lobes named knot1(K1) and knot2(K2). Previous studies have shown that both lobes can be synthesized separately and activate the channel with different affinities. Recently, near atomic resolution structures of TRPV1 in distinct states (apo, capsaicin bound and DkTx&RTx bound) were reported using electron cryo-microscopy. These structures show that TRPV1 adopts a structure that is similar to Kv channels, and that the pore domain undergoes distributed conformational change upon activation in response to binding of DkTx and RTx. Although these structures show where DkTx binds, they do not have sufficient resolution to reveal the structural basis of the toxin-channel interaction. Here we solved the solution NMR structure of DkTx and dock that structure into the DkTx/RTx bound electron density maps using the Xplor-NIH program. Our results show that the toxin binds to a perimeter of the pore domain at the interface between the pore helix and S6 of neighboring subunit of the channel, and demonstrate that the toxin-channel interface is dominated by hydrophobic interactions. Interestingly, when bound to the channel, several residues on the toxin extend over the edge of the pore domain where they would be expected in interact with the surrounding membrane. To explore this possibility we tested whether DkTx can interact with membranes using a tryptophan fluorescence assay (each lobe contains a single conserved tryptophan). Indeed, each lobe of DkTx interacts with membranes, and the interaction is energetically more favorable in the bivalent toxin.