Pancrætic islets secrete insulin and glucagon, two critical hormones for the blood glucose homeostasis. Islets are composed mainly of insulin-secreting beta-cells (~80%) and glucagon-secreting alpha-cells (10-15%). Typically, insulin is secreted after a meal to reduce blood glucose levels, whereas glucagon is released in times of starvation. In order for insulin and glucagon to exert their opposite effects, glucose must also have an inverse effect on the secretory function of the two cell types. While the mechanisms of glucose-stimulated insulin secretion by beta-cells are fairly well understood, the study of the mechanisms underlying glucagon secretion has been hindered by the lack of reliable methods to distinguish between alpha- and beta-cells. Thus, it is not clear how glucose suppresses glucagon secretion. There is still little agreement as to whether this inhibition is a direct effect on alpha-cells or mediated by a paracrine inhibition from beta-cells via secretion of molecules such as insulin, Zn²⁺, and GABA. Recently, a fragment of a rat glucagon promoter has been successfully used to image structures close to the cell-substrate interface. Illuminating the glucagon-secreting alpha cells with discrete attachment sites, while such an interaction mode was completely unresolvable with conventional fluorescence. Super-resolution optical microscopy, such as STORM promise to significantly expand the understanding of biological processes in cells. In this work, we have developed multicolor three-dimensional (3D) stochastic optical reconstruction microscopy (STORM) as a tool to probe molecular structures and their interactions on sub-diffraction length scales. STORM achieves sub-diffraction limit image resolution by using photoactivatable fluorescent probes to separate the spatially overlapping images of individual probes in time. Only a small subset of probes was activated at any given time, allowing us to resolve individual activated probes and determine their positions with high precision. A super-resolution image was then constructed by plotting the measured probe positions accumulated over time. With this we have generated 3D whole cell images, several micrometers thick, with sub-diffraction resolution on the nanometer scale. Distinct mitochondrial morphologies clearly resolved the hollow mitochondria outer membrane structures obscured in conventional fluorescence images. Distinct mitochondrial morphologies were observed, ranging from thin elongated tubules to globular compartments. Integrin-integrin interactions, while globular mitochondria are relatively dispersed in size, from 200 nm to 1500 nm, the tubular structures are more uniform in diameter, taking a narrow distribution around 200 nm. The images also displayed several distinct interaction modes between mitochondria and microtubules. Notably, elongated mitochondria were observed to “inchworm” along microtubules with discrete attachment sites, while such an interaction mode was completely unresolvable with conventional fluorescence.

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*Probing the Interaction of RecA and a dsDNA Segment via Optical Tweezers*

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RecA plays an important role in homologous recombination of DNA. When RecA combines with dsDNA to form RecA-dsDNA nucleofilament, it unwinds dsDNA and changes the dsDNA structure. We measured with two complementarity stationary optical tweezers and oscillatory optical tweezers, the force-extension relation and the elastic constant of a segment (4μm) of dsDNA as a function of the stretching force before and after its interaction with RecA. The dsDNA sample was attached to two polystyrene particles, one at each end; the smaller particle (diameter = 2μm) at one end was trapped by optical tweezers while the larger particle (diameter = 20μm) at the other end was fixed to the cover glass of the sample chamber which was filled with an appropriate buffer solution and was mounted on a PZT-driven translational stage. In consistent with the description of the worm-like chain (WLC) model, the elastic constant increased monotonically from approximately 8.6pN/μm to 35.9pN/μm when stretching force varied from 1.8pN to 17.0pN, and reached a constant value of approximately 41pN/μm for stretching force in the range of 20.0pN and 33.6pN (the enthalpic regime in the WLC model). After fully interacted with RecA, the elastic constant of the resulting RecA-dsDNA filament was determined to be approximately 47.3pN/μm in the enthalpic regime.

We also studied the dynamics of the interaction of dsDNA with RecA protein in terms of the elastic constant as function of time while the DNA was stretched at a constant stretching force of 33.0pN and allowed to interact with RecA (by injecting a solution containing RecA protein and ATP[S]) and subsequently to dissociate with RecA (by injecting de-ionized distilled water into the chamber to wash off the ATP[S]). The association rate increased with increasing concentration of RecA.
planes at different depths in the sample. The other method introduces a cylindrical lens to the detection path, which causes astigmatism in the detected fluorescence. This results in a stretch along one of the two lateral axes depending on the axial position of the fluorescent particle. This work determines the best optical parameters for each method in order to localize over the largest axial range with best possible uniformity in localization accuracy.


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**Imaging Actin Filaments in Synaptic Spines Beyond the Diffraction Limit of Light**

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The development of novel physical tools to image biological samples at a resolution determined by the wavelength of the light limited the detailed analysis of the spatiotemporal organization and compartmentalization of the actin cytoskeleton. Here, we present high-resolution imaging of actin filaments in synaptic spines using photoactivatable tdEos tagged actin-binding peptides APB-tdEos in hippocampal neurons, and indirectly determine the structure of the cytoskeleton in spines, without interfering with the F-actin structure itself. A low density of tdEos molecules were photoactivated, imaged and bleached continuously, followed by image reconstruction, resulting in actin images with subdiffraction resolution.

**References:**

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**Paxillin focal adhesions, localization and implication: insight from Photo-Activated Localization Microscopy**

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Photo-Activated Localization Microscopy (PALM) as described by E. Betzig (2006) optically resolves selected subsets of photo-activatable fluorescent probes within cells at mean separations of less than 25 nanometers through several photo-activation and subsequent photobleaching of numerous sparse subsets of photo-activated fluorescent protein molecules. The position information from all subsets is then assembled into a super-resolution image, in which individual fluorescent molecules are isolated at high molecular densities. In this work COS-7 and ST14A tdEos-Paxillin transfected cells were used. We observed some features that limited the quality of PALM, both in this setup and in its present version. It takes actually hours to go through the cycles of photo-activation and image acquisition, to collect all of the data needed and to generate a single high-resolution image limiting the use to fixed specimens which precludes PALM’s use for imaging of live cells. More important is the loss of data. Depending on the spatial concentration of the PA-FFPs, most of the information about the position of molecules is lost during the photo-activation photobleaching phase, especially during the first cycles of data collection. From the biological point of view, we observe small paxillin clusters along the focal adhesions. Supported by US4 GM064346 CMC (MD, EG), NIH-P41-R03155 (EG, FC), P50-GM076516 (EG).

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**Super-resolution Imaging Of Ca**²⁺ **Flux Through IP3Rs With Millisecond Temporal Resolution and Nanometer Spatial Resolution**

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Advanced imaging techniques such as PALM and STORM have broken the diffraction limit of conventional optical microscopy through their ability to turn fluorescent molecules on and off at low enough densities such that the positions of single molecules can be determined, one at a time, with a precision of ~10 nm (Gustafsson, 2008). However, these techniques involve the use of fluorescently tagged proteins or antibodies, which may alter protein properties and provide only positional, not functional information. Thus, we have developed a technique termed Single Channel Ca²⁺ Nanoscale Resolution (SCCaNR), based on similar principles except that it generates a super-resolution image by using Ca²⁺ sensitive fluorescent dyes to image the stochastic openings and closings of Ca²⁺ permeable ion channels. Subsequently, the point spread function resulting from the diffusion of calcium bound to the indicator dye can be fit to a 2-D Gaussian function, allowing the position of functional calcium channels to be localized with much higher precision (~40 nm) than previously possible. The inositol triphosphate receptor (IP₃R₅) is an ER Ca²⁺ channel that is both facilitated and inhibited by Ca²⁺ itself. This property enables a functional couple of fluorescent dyes and nanoparticles for their suitability and applied them to known as puffs (Yao, et al, 1995). This same property makes IP₃Rs highly dependent on their spatial proximity to one another. Using our SCCaNR technique, we have found that the concerted opening of 4-10 IP₃R channels likely underlies the generation of Ca²⁺ puffs in SH-SY5Y neuroblasta cells. These puffs arise from clusters of IP₃Rs approximately 300 nm in diameter, a dimension below the resolution limit of conventional optical microscopy.

**3286-Pos Board B333**

**Overcoming the Nyquist limit with intensity modulation spectral analysis**

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Power spectral density measurements of any sampled signal are typically restricted by both acquisition rate and frequency response limitations of instrumentation. We present a new method called Intensity Modulation Spectral Analysis (IMSA) that circumvents these limitations, extending the effective bandwidth of potentially any measurement device. We demonstrate this for the specific case of video imaging, where oscillating an LED illumination source allows us to quantify fluctuations of an optically-trapped microsphere at frequencies over 10 times higher than the Nyquist limit.

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**Optimizing Fluorophores For Super-resolution Fluorescence STED Microscopy**

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Far-field fluorescence nanoscopy is an emerging field, surpassing the diffraction barrier of conventional far-field microscopy and visualizing biological specimens in three dimensions, in principle, with molecular resolution. Stimulation of the emission depletion (STED) microscopy is a well-established nanoscopy technique, which can be applied to conventional organic fluorophores and fluorescent proteins. A major bottleneck of fluorescence microscopy including STED microscopy is the photobleaching of fluorophores which limits both brightness and observation time. Therefore, we have assessed several photostable fluorophores and nanoparticles for their suitability and applied them to STED microscopy. Imaging with continuous wave laser as well as with high repetition rates of 80 MHz offers sub-diffraction resolution with strongly improved photostabilities.

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**Ultra Resolution Direct Imaging Optical Microscope**

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The size of the smallest detail visible in conventional microscopy is determined by the wavelength of the light used to image a specimen. For state-of-the-art optical imaging, this diffraction limit is 200-300 nm, leaving a considerable ‘blind spot’ between the angstrom-scale molecular details visible by X-ray crystallography and the those accessible by visible light microscopy. Recently, a number of developments have been reported that allow fluorescence imaging of samples with resolutions of an order of magnitude below the diffraction limit.