Imaging & Optical Microscopy III

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Imaging the Glucagon-Secreting Alpha Cells

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Pancreatic 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, Zn^{2+}, GABA.

Recently, a fragment of a rat glucagon promoter has been successfully used to specifically drive the expression of fluorescent proteins in mouse alpha-cells. Thus, it is now possible to easily identify glucagon-secreting cells within isolated islets or to flow sort a purified population of alpha-cells. Here, we report the use of dynamic fluorescence imaging techniques on these transgenic islets and cells. In particular, we investigated how glucose affects 1) alpha-cell metabolism via measurement of NAD(P)H autofluorescence by two-photon excitation microscopy; 2) alpha-cell intracellular calcium concentrations, via the loading of alpha-cells expressing red fluorescent proteins (tdRFP) with the calcium indicator dye Fluo4-AM; and 3) alpha-cell membrane potential, via application of the fluorescent probe $DiSBAC_2(3)$. The results of these biophysical measurements will also be compared to parallel glucagon secretion in response to glucose from both intact islets and sorted alpha-cells.

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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 nucleofiliment, it unwinds dsDNA and changes the dsDNA structure. We measured with two complementary techniques, namely 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 polystyrenes 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 33.6pN and allowed to interact with RecA (by injecting a solution containing RecA protein and ATPγS) and subsequently to dissociate with RecA (by injecting de-ionized distilled water into the chamber to wash off the ATPγS). The association rate increased with increasing concentration of RecA.

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Extending The Resolution In Total Internal Reflection Fluorescence (TIRF) Microscopy

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Total internal reflection fluorescence (TIRF) microscopy is the method of choice to image structures close to the cell-substrate interface. Illuminating the specimen with evanescent light decaying exponentially in axial direction results in essentially two-dimensional images without interference from regions deeper within the specimen. However, the lateral resolution of TIRF microscopy remains diffraction-limited to about 240 nm for green emission. Lateral resolution can be increased by up to a factor of 2.5 when illuminating the sample with evanescent standing waves [1].

Here we report a compact and versatile illumination set-up employing two beam splitters and two piezo-actuated mirrors featuring two angular degrees of freedom. The piezo-actuated mirrors provide full control over the orientation and penetration depth of the evanescent standing wave. In contrast to grating-projection set-ups [1], beam steering via mirrors facilitates simultaneous excitation at multiple wavelengths since the angle of incidence is set by reflection off the mirrors and does not vary for different wavelengths. To obtain a two-dimensional image with extended resolution, the specimen is illuminated with at least two differently oriented standing wave patterns. More directions lead to higher isotropy of the extended optical transfer function (OTF). In practice, however, we found that the cloverleaf-shaped OTF resulting from two illumination directions yields images of comparable morphology as the more isotropic OTF resulting from three illumination directions. Using only two directions increases imaging speed, whereas three illumination directions lead to more spectral overlap within the extended OTF, which facilitates image reconstruction. We further demonstrate the potential of this technique for biological imaging on examples including HeLa cells expressing GFP-actin.

[1] Beck, M., Aschwanden, M. & Stemmer, A. (2008) Sub-100-nanometre resolution in total internal reflection fluorescence microscopy. J. Microsc., 232, 99-105.

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Multicolor Three-dimensional Whole-cell Imaging With Nanometer Scale Resolution

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The ability to directly visualize nanoscopic cellular structures and their spatial relationship in all three dimensions will greatly enhance our understanding of molecular 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 photoswitchable 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 a spatial resolution of 20 - 30 nm and 60 - 70 nm in the lateral and axial dimensions, respectively. Using this approach, we imaged the entire mitochondrial network in mammalian cells and studied the spatial relationship between mitochondria and the microtubule cytoskeleton. The 3D STORM images clearly resolved the hollow mitochondria outer membrane structures obscured in conventional fluorescence images. Distinct mitochondrial morphologies were observed, ranging from thin elongated tubes to globular compartments. Interestingly, 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. Super-resolution optical microscopy techniques such as STORM promise to significantly expand the understanding of biological structures and their interactions on a molecular level.

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3D-Resolution In FPALM/PALM/STORM

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Light microscopy is one of the most widespread imaging techniques used by biologists, but even the best traditional microscopes are limited by diffraction to a resolution of about 250nm, leaving many sub-cellular structures in an unresolved blur. Over the last few years, several groups have pioneered localization techniques to surpass the resolution limit[1-4]; however those techniques were limited to two dimensions. Recently, two techniques have been employed that extend localization into the third, axial dimension[5,6]. Biplane fluorescent photoactivatable localization microscopy[6] (BP-FPALM) splits the detected fluorescence into two paths[7], one slightly longer than the other to image two