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### 3349-Pos Board B454

#### Analyzing A $\beta$ Aggregates with High Resolution Microscopy

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In a world where more people grow older aging-related neurodegeneration like Alzheimer's disease (AD) affects more and more people.

Today, AD can be diagnosed with certainty only post mortem, detecting insoluble  $\beta$ -amyloid peptide (A $\beta$ ) aggregates and neurofibrillary tangles in the patient's brain tissue. Aggregates consisting of A $\beta$  are a fundamental pathologic feature of AD. Today in many studies, concentrations of monomeric A $\beta$  in body fluids are investigated, especially for diagnostic purposes. Nevertheless, for the detection, quantitation and qualification of aggregated pathologic A $\beta$  forms, also in the course of aging, a highly sensitive detection assay system for aggregated A $\beta$  species is necessary.

We developed an ultra-sensitive assay for the detection of aggregated protein species out of body fluids. This highly specific and sensitive assay uses confocal fluorescence spectroscopy methods and is sensitive enough to detect single aggregates. For the procedure, pathologic aggregates out of body fluids are immobilized on a glass chip, subsequently fluorescence labeled and detected via confocal spectroscopy.

Actually, we are optimizing the assay in concerns of instrumentation (imaging) and microscopy high-resolution and even super-resolution methods. We are developing methods to analyze aggregates via super-resolution microscopy. Setups like PAINT (Point Accumulation for Imaging in Nanoscale Topography) or STORM (Stochastic Optical Reconstruction Microscopy) allow resolutions in nanometer-range. PAINT is based on replacing the point-spread-function (PSF) of a fluorophore by a point in the middle of a 2D gaussian fit. First measurements show resolutions of 30 nm. STORM is based on high-accuracy localization of photoswitchable fluorophores. During one imaging cycle, only a small part of the fluorophores is turned on. This allows a high accuracy in determining the fluorophore position by replacing the PSF. The fluorophore positions obtained from a series of imaging cycles can be used to reconstruct the whole image.

### 3350-Pos Board B455

#### Real-Time Hyperspectral Imaging of Multiple Biosensors in Pancreatic Beta Cells

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Spectral imaging techniques are important for many biological experiments, particularly live-cell quantitative imaging of multiple fluorescence probes. Existing hyperspectral imaging systems require sequential techniques, limiting the data acquisition rate. A newly-developed snapshot device, the Image Mapping Spectrometer (IMS), acquires full spectral information simultaneously from every pixel in the field with image acquisition rates up to 10 frames/second. The IMS maps adjacent pixels from the object to create space between them in the image, and then uses a grating to spread wavelength content from each pixel into this space. Direct image re-mapping provides the final 3D (x, y, lambda) data cube.

Fluorescent protein (FP)-based biosensors are increasingly valuable tools for identifying subcellular dynamic processes in live cells. Many biosensors are based on FP-FRET, and measurements of the resultant small changes in FRET require high quality data. Tracking intracellular free Ca<sup>2+</sup> levels is also crucial to elucidating signaling events, but the best Ca<sup>2+</sup> indicator dyes overlap in the spectral emission range of the common FP-FRET biosensors. We have used the IMS system to simultaneously image multicolored FPs (i.e., CFP, GFP, YFP) in combination with extrinsic indicator dyes, such as Fluo-4.

To demonstrate the speed and resolution of the IMS approach, we monitored both intracellular Ca<sup>2+</sup> oscillations and caspase-3 activity during hydrogen peroxide-induced apoptosis. Ca<sup>2+</sup> activity was measured with Fluo-4 (emission peak at 517 nm) and caspase-3 activity was measured with SCAT3.1, a FRET biosensor based on the ECFP (emission peak at 484 nm) and EYFP (emission peak at 527 nm) pair. Using the IMS, the three fluorophores were imaged with sub-second temporal resolution and spectrally unmixed in real-time. This permits direct correlation of Ca<sup>2+</sup> activity with other apoptotic signaling events and demonstrates the power of the IMS for measuring dynamic physiological processes.

### 3351-Pos Board B456

#### In Vivo Fluorescence Imaging of Blood Flow in Mouse Pancreatic Islets

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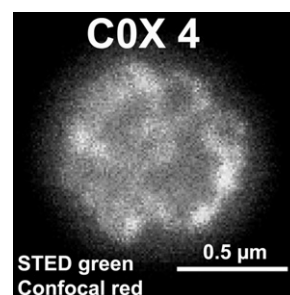
Diabetes is a disease resulting from changes in pancreatic islets, which are insulin secreting micro-organs within the pancreas. With increased blood glucose, insulin is secreted from beta cells in the islets in a coordinated pulsatile manner. At the same time alpha cell glucagon secretion is inhibited. Mechanisms controlling these processes at the intercellular and at the inter-islet level remain unclear. We suggest that the three-dimensional organization of islet cells and the dynamics of islet blood flow have a role in regulating insulin and glucagon secretion. This is suggested by observation that the density of blood vessels within islets is much greater than in surrounding pancreatic tissue, and that most individual islet cells are adjacent to a blood vessel. As an initial test of our hypothesis, we have developed a high-speed in vivo fluorescence imaging method to track pancreatic blood flow in a living mouse. We are also developing methods necessary to analyze the large amounts of data generated. Using high speed line scan confocal microscopy the method has full frame sub-micron spatial and less than 10 ms temporal resolution. Islets are located within the pancreas by using mice with GFP-labeled beta cells. Blood plasma is labeled with a fluorescent dextran, allowing mapping of vascular dimensions and pathway. Individual blood cells are fluorescently labeled by osmotic shock loading with an Alexa dye, which allows tracking of the blood flow. We present current results for blood flow under different levels of blood glucose in clamping experiments. Our previous qualitative results have suggested that there are differences in blood flow parameters at different glucose levels. Here, a more quantitative analysis of blood flow velocity, any observed changes in vessel dimensions, and changes in blood flow coverage inside and outside islets is presented.

### 3352-Pos Board B457

#### Superresolution Optical Microscopy of Isolated Cardiac Mitochondrial Proteins

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To study the structural organization of mitochondrial proteins, we applied Stimulated Emission Depletion (STED) microscopy in isolated mitochondria. In STED microscopy, two laser beams are used: one for excitation of fluorophores and the other, with doughnut shape, to deplete them in order to allow fluorescence emission only from the excited volume located at the doughnut's center. With STED a lateral resolution of ~45 nm was achieved in images of isolated mitochondria. We investigated the localization pattern and distribution of MaxiK $\alpha$ , COX4 and VDAC1. After a combined analysis of classical confocal and STED images, we found distinct distributions for VDAC1, MaxiK $\alpha$  and COX4. COX4 distribution was consistent with localization in the cristae. We established that there are 7-15 clusters of MaxiK $\alpha$ , 10-15 clusters of VDAC1, and 15-20 clusters of COX4 per mitochondria. We have demonstrated that protein clusters in the mitochondria can be resolved with a separation power of ~45 nm, and that it is possible to retrieve quantitative information about the number of clusters and density of proteins in mitochondria. This approach can be extended to eins in mitochondria and subcellular organelles. Supported by NIH.



### 3353-Pos Board B458

#### Zero-Mode Waveguides: A Powerful Tool for Single-Molecule Optical Studies

**Zhuangxiong Huang**, Serge Donkers, Nynke H. Dekker.

Single-molecule fluorescence studies of enzymes that incorporate fluorescently labeled substrate nucleotides typically operate at substrate concentrations well below their  $K_m$  values. While this is inevitable in conventional fluorescence microscopy, the biological relevance of the insights gained into enzyme mechanism may be compromised. Zero-mode waveguides (ZMWs) provide an excellent solution to this problem by greatly reducing the observation volume. We report the nanofabrication of ZMWs, the surface treatment for controlled immobilization of biomolecules and the reduction of background noise. We also present the development of an assay to monitor in real time the incorporation of fluorescently-labeled nucleotides, which paves the way for the studies of nucleic acid polymerizing enzymes, e.g. DNA/RNA polymerase, reverse transcriptase, telomerase, etc.