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Imaging & Optical Microscopy I

1721-Pos Board B613

Simultaneous Detection of Bio-Molecular Interactions and Surface Topography using Photonic Force Microscopy

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Photonic force microscopy (PFM) is an optical tweezers-based scanning probe microscopy, which measures the forces in the range of fN to pN. The low stiffness leads proper to measure single molecular interaction.

We introduce a novel photonic force microscopy to stably map various chemical properties as well as topographic information, utilizing weak molecular bond between probe and object's surface.

First, we constructed stable optical tweezers instrument minimized instrumental noise, where an IR laser with 1064 nm wavelength was used as trapping source to reduce damage to biological sample. To manipulate trapped bead two-axis Galvano mirror were used for x, y directional probe scanning and a piezo stage was used for z directional probe scanning. For resolution test probe scans with vertical direction repeatedly at the same lateral position, where the vertical resolution is ~25 nm. To obtain the topology of surface of etched glass, trapped bead scans with hopping mode and measures the contact position in every cycle.

To obtain the chemical mapping, wedesign the DNA oligonucleotide pairs combining as a zipping structure, where one is attached at the surface of bead and other is fixed on surface. We measured the rupture force of molecular bond to investigate chemical property on the surface with various loading rate. We expect this system can realize a high-resolution multi-functional imaging technique able to acquire topographic map of objects and to distinguish chemical difference between these objects simultaneously.

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Deep Imaging of Biological Tissue by Ultra-Efficient Photon Collection

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We present an upright two-photon fluorescence microscope that is capable of imaging in turbid media up to 3mm depth with micron resolution. The system utilizes a high power Ti:Sa Mai Tai laser with a group velocity dispersion compensator (DeepSee) for two-photon fluorescence excitation. The especially designed fluorescence detector, which is a key feature of the system, is capable of collecting fluorescence photons from the wide surface area (25mm diameter) of the specimen. This novel detection scheme has proven to be extremely efficient in the collection of fluorescence photons scattered by turbid media and allows about 6 fold increase in imaging depth when compared with conventional two-photon microscopes. The system is also equipped with a second fluorescence detector that allows its use as a conventional two-photon microscope and the comparison of the data acquired by both detection methods. In addition, the presented microscope is coupled to the FLIMbox (ISS, Inc.) and it has in depth FLIM imaging capabilities. The detection scheme captures fluorescence light in a transmission configuration, which was proven extremely efficient for the detection of SHG signals, due to their intrinsically forward propagating nature. We are also presenting in depth imaging experiments of tissue phantoms and in vivo and ex vivo biological tissue, including murine colon, small intestine, xenograft tumors, and skin vasculature.

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Two-Photon Imaging of Electrical Activity in Mouse Cortex using a Genetically-Encoded Voltage Indicator

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Genetically-encoded voltage indicators (GEVIs) of the VSFP (voltage-sensitive fluorescent protein) family based on the Ci-VSP (*Ciona intestinalis* voltage-sensitive phosphatase) voltage sensor domain were demonstrated to report membrane voltage fluctuations of genetically-targeted cells in a number of *in-vitro* and *in-vivo* preparations using single photon (1P) fluorescence excitation and wide field image detection [1, 2]. However, *in-vivo* 1P epifluorescence imaging captures optical signals only from superficial layers and does not optically resolve single neurons. Two-photon (2P) excitation imaging, on the other hand, has not yet been convincingly applied to GEVI experiments. Here, we show GEVI two-photon imaging in mouse brain slices and mouse cortex *in-vivo* expressing VSFP Butterfly 1.2 in cortical layer2/3 pyramidal cells. We show that 2P excitation at 920-940 nm of the mCitrine/mKate2 FRET reporter in Butterfly 1.2 yields an optical voltage signal from neuron membranes in brain slices in response to field stimulation with a time course identically revealed by 1P excitation and with a 2-3 larger $\Delta R/R$ value. Two-photon imaging of mouse cortex *in-vivo* achieved cellular resolution throughout layer 2/3 of Butterfly 1.2-expressing pyramidal cells. In somatosensory barrel cortex we recorded sensory responses to single whisker deflections in anesthetized mice at 30 frames per second in full frame view. The 2P-excited voltage signal was localized within the functional map established by 1P VSFP imaging in the same preparation. Our results demonstrate the feasibility of GEVI-based functional 2P imaging of sensory-evoked electrical activity in mouse cortex.

[1] Akemann W, Mutoh H, Perron A, Rossier J, Knöpfel T, Nat Methods 7, 2010
[2] Akemann W, Mutoh H, Perron A, Park KY, Iwamoto Y, Knöpfel T, J Neurophysiol, 2012 Jul 18

1724-Pos Board B616

Dopamine Receptor D3 Signaling in the Pancreatic β-Cell

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Glucose homeostasis is maintained by small clusters of hormone secreting cells in the pancreas: the pancreatic islets. Insulin secreting β-cells make up ~ 80% of the mouse islet and secrete insulin in a tightly regulated manner. Understanding the mechanisms that regulate insulin secretion is a key factor in developing drugs and therapies for type-2 diabetes and metabolic syndrome.

The pancreatic β-cells synthesize dopamine from the circulating precursor L-dopa. We have shown that during glucose stimulated insulin secretion, co-secreted dopamine acts as an autocrine negative regulator of insulin secretion. It does so by activating the dopamine receptor D3 (DRD3) – a member of the G-protein coupled receptor family. DRD3 are present on β-cell plasma membrane, and upon dopamine binding, they attenuate intracellular Ca^{2+} dynamics. In fact, the frequency of the $[Ca^{2+}]_i$ oscillations triggered by 8 mM glucose is diminished by dopamine; these effects are blocked when a selective DRD3 antagonist is added [Ustione and Piston, Mol. Endo. 2012].

We investigate the molecular mechanism downstream of DRD3 activation that leads to the changes in intracellular Ca^{2+} dynamics and insulin secretion. We propose that dopamine activation of DRD3 directly affects β-cell calcium influx via $G_{\beta\gamma}$ complex interaction with the L-type Ca^{2+} channel ($Ca_V1.2$). We test this hypothesis using FRET and two photon excitation FCCS experiments on βTC-3 cells with fluorescent protein labeled versions of the $G_{\beta\gamma}$ complex, and of the $Ca_V1.2$ subunit α_{1C}. The goal is to detect the dopamine triggered interaction between the $G_{\beta\gamma}$ complex and the $Ca_V1.2$. We can modulate the FRET signal by using the photo-switchable version of the red fluorescent protein rsTagRFP, therefore we can measure FRET from the small percentage of interacting proteins, even in the presence of significant background signal arising from the majority of labeled but noninteracting proteins.

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Interstitial-Matrix' Fluid-Fluxes *In Vivo*: Subatmospheric Pressure Reverses Hydration-Potential in Heat-Denatured Dermis

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Interstitial flows modulate (a) the interchange of nutrients and metabolites between blood and parenchyma; (b) paracrine and endocrine signals; and (c) fibroblast mechanical responses. Previously, we found that hydration potential (HP), a measure of the local forces that drive interstitial flows, increases in dermis heated *ex vivo*. Here, we measured HP in dermis heated *in vivo* to probe its effectinintact animals. Subatmospheric pressure, *125mmHg*, was applied *in vivo* for 3 hours to heated *(15 sec, 100°C)* and control dermis obtained as by-products from unrelated studies as approved by the IRB. Differential scanning calorimetry was used to determine the extent of collagen unfolding, and ^1H magnetic resonance imaging to determine apparent diffusivity and anisotropy. HP was measured by osmotic stress, *3-219 mmHg*, and influx/efflux trajectories were followed for *~20 h* to near equilibrium. The extent of unfolded collagen *in vivo* and *ex vivo* was 33 $\pm$ 3 and 34 $\pm$ 2 % of controls, respectively; apparent diffusion coefficients increased (10.1 $\pm$ 2 and 11.3 $\pm$ 1.5 versus 6.3 $\pm$ 3.7 $x10^{-4}$ mm^2/s), while fractional anisotropy decreased (0.289 $\pm$ 0.15 and 0.174 $\pm$ 0.07 versus 0.689 $\pm$ 0.27). These results suggest similar structural changes in both type-specimens. Mean HP of *in-vivo* denatured dermis decreased relative to the control and *ex-vivo* denatured explants: -15.2 ± 12.6 versus 30.9 $\pm$ 4.8 and 98 $\pm$ 7.4 *mmHg,* respectively *(P-value < 0.05)*. After subatmospheric pressure, mean HP (48.1 $\pm$ 4.6 *mm Hg*) in *in-vivo* denatured dermis returned to baseline, while diffusivity decreased, and anisotropy increased. Lower HP values indicate excess fluid accumulation, consistent with suction forces developing *in vivo* upon denaturation; emerging convection-enhanced interstitial flows could explain the local edema (HP decrease) and its reversal (HP increase) by externally applied subatmospheric pressure.