compartments. Such a mechanism has another advantage by providing flexibility. If telomere binding is switched off (e.g., by shortening the binding time), this will allow the nucleus to undergo architectural changes.

[1] I. Bronstein, Y. Israel, E. Kepten, S. Mai, Y. Shav-Tal, E. Barkai and Y. Garini, Transient anomalous diffusion of telomeres in the nucleus of mammalian cells. Physical Review Letters **103**, 018102 (2009).

**1114-Plat**

**Mapping Neuronal Connectivity Using Stochastic Optical Reconstruction Microscopy (Storm): The Brainstorm Project**

**Melike Lakadamyali**, Mark Bates, Hazen Babcock, Jeff Lichtman, Xiaowei Zhuang.
Harvard University, Cambridge, MA, USA.

The human brain is a highly sophisticated circuit consisting of hundreds of billions of neurons that are interconnected by an even larger number of synapses. This dense network of neurons and their connections holds key information to understanding normal brain function and perhaps what underlies its disorders. Obtaining a physical map of the brain's connectivity, however, is highly challenging due to the small size and high density of neuronal processes within a given volume. Therefore, in order to generate a map of neuronal connectivity a technique that can provide high spatial resolution and molecular specificity is needed.

We are using 3D multi-color stochastic optical reconstruction microscopy (STORM) in order to trace neuronal networks in culture at high spatial resolution. In order to capture an entire network of connections, we are using an automated, motorized piezo stage to image large areas in x-y ($\geq$ 120x120 μm) as well as in z ($\geq$ 2 μm). With this technique we can not only outline neuronal morphology at 30 μm lateral and 50 μm axial resolution, but we can also image synaptic content with high molecular specificity and identify synaptic connections. These techniques will be greatly useful for generating connectional maps of neurons in the mammalian brain and help obtain a physical understanding behind brain function.

**1115-Plat**

**Monitoring the [ATP]/[ADP] Ratio in Beta-Cells During Glucose Stimulated Insulin Secretion Using the Genetically Encoded Fluorescent Reporter Perceval**

**Gert-Jan Kremers**, Amicia D. Elliott, W. Steven Head, David W. Piston.
Vanderbilt University Medical Center, Nashville, TN, USA.

Pancreatic beta-cells secrete insulin in response to elevated blood glucose levels. Glucose stimulated insulin secretion depends on glucose metabolism that produces ATP. The resulting increase in [ATP]/[ADP] ratio closes ATP-sensitive potassium (KATP) channels, which leads to membrane depolarization and opening of voltage-dependent Ca2+ channels. This causes an elevation of intracellular free Ca2+ and insulin exocytosis. Insulin is secreted in a pulsatile manner, which is thought to be regulated in part by oscillations in glucose metabolism. Such metabolic oscillations would also lead to oscillations in the [ATP]/[ADP] ratio and hence regulate KATP channel activity.

Oscillations in [ATP]/[ADP] ratio have been demonstrated using biochemical and luciferase assays, but neither approach allows measurements of such oscillations in single cells. Perceval is a recently developed fluorescent protein biosensor for [ATP]/[ADP] ratio, and it permits direct measurement of [ATP]/[ADP] ratios inside living cells. We use Perceval in combination with quantitative confocal and two-photon excitation microscopy for direct measurement of the [ATP]/[ADP] ratio in beta-cells during glucose stimulated insulin secretion. For this purpose we have developed an adenoviral vector to express Perceval specifically in the beta-cells of intact mouse islets. Dynamic changes in [ATP]/[ADP] ratio can be correlated with glucose metabolism (by simultaneous imaging of Perceval fluorescence and NAD(P)H autofluorescence) and with intracellular free Ca2+ levels (by simultaneous imaging of Perceval fluorescence and the calcium sensor, FuraRed). This data allows us to test hypotheses regarding the role of localized subcellular signaling complexes and putative microdomains of glucose metabolism, [ATP]/[ADP] ratio, and Ca2+ dynamics in the regulation of glucose stimulated insulin secretion.

**1116-Plat**

**Multiple Components Mapping of Live Tissue by Phasor Analysis of Fluorescence Lifetime Imaging**

**Chiara Stringari**, Michelle Digman, Peter Donovan, Enrico Gratton.
University California Irvine, Irvine, CA, USA.

In fluorescence lifetime microscopy (FLIM) of live tissues a major issue is the assignment of autofluorescence to specific molecular components and their interactions within the physiological context. Here we use the phasor approach to fluorescence lifetime imaging to analyze complex decays in a live tissue. The tissues used were seminiferous tubules from the testes of wild type mice or mice expressing GFP from an Oct4 transgene. Lifetime images were acquired in the time domain and analytically transformed in the phasor representation. By examination of the clustering of the phasors we identified different molecular components: auto fluorescence, GFP, collagen and retinol. Each chemical species was identified and categorized by its specific location in the phasor plot. This phasor fingerprint reduces the importance of knowing the exact lifetime distribution of the fluorophores and emphasizes the contribution of the species to the signal. To better identify specific tissue components we also used spectral imaging and second harmonic generation microscopy. Linear combinations in the same pixel of molecular species were recognized and their relative fraction was calculated and mapped. The analysis of the fluorescence decay with higher harmonics of the phasor plot separates different molecular components that have the same location in the phasor plot at one harmonic but arise from different lifetime distributions. The phasor approach to lifetime imaging in live tissue provides a unique and straightforward method for interpreting complex decays in terms of molecular features by identifying fluorophores and obtaining functional maps of their relative concentration. This method has the potential to become a non invasive tool to characterize the local microenvironment and monitor differentiation and diseases in label-free live tissues. Work supported by NIH-P41 P41-RRO3155 and P50-GM076516, NIH RO1 HD49488, NIH PO1 HD47675, CIRM RC1-00110 PD.

**1117-Plat**

**Ultra-High Resolution Imaging of the Dynamic Nature of Post-Synaptic Molecules**

Deepak Nair, **Jean-Baptiste Sibarita**, Daniel Choquet.
Institut François Magendie - Université Bordeaux 2, Bordeaux, France.

The spatial and temporal regulation in the composition of the postsynaptic membrane of synapses participates in the different forms of synaptic plasticity that trigger the cellular processes of memory formation, consolidation, and retrieval. Neurotransmitter receptors move rapidly in and out of synapses by lateral diffusion. This mobility is crucial to control the number of receptors present at a given synapse. Thus, the equilibrium between the synaptic and extra synaptic AMPA receptor number is crucial in controlling basal transmission and synaptic plasticity. This balance is regulated by the subunit composition of these receptors and by the interaction of intracellular scaffold proteins. However, how the trafficking of receptors and the scaffolding molecules in and out synapse is controlled remains unknown. Here we attempt to determine the relative distribution and trafficking properties of AMPA receptors and various scaffold proteins at unprecedented spatial ($<$ 40nm) and temporal resolution ($>$50 Hz) using a variety of novel ultra-high resolution fluorescence imaging approaches. We combine Single Particle Tracking (SPT) and Photo Activation Localization Microscopy (PALM) to map trajectories at the level of individual molecules. Here we describe the implementation of a multimodal microscope along with the development of a new dedicated analysis for single molecule segmentation and tracking. Furthermore we will discuss the application of SPT-PALM experiments on living neurons. With this novel approach, we expect to comprehend the motilities of receptors or scaffolding proteins when they traffic between the submicron sized molecular zones of dendritic spines. The combination of this type of detection and analysis will provide the information from thousands of discrete trajectories from a single cell with which it would be possible to appreciate finer details of versatile molecular mechanisms pertinent in the functioning of an excitatory synapse.

**1118-Plat**

**Optical Recording of Electrical Activity of Cortical Layer 2/3 Pyramidal Neurons Using A Genetically-Encoded Voltage Probe**

**Walther Akemann**, Hiroki Mutoh, Reiko Yoshida, Tomomi Shimogori, Thomas Knopfel.
RIKEN Brain Science Institute, Wako City, Japan.

Voltage-Sensitive Fluorescent Protein 2.3, VSFP2.3, is a genetically-encoded probe of membrane voltage using fluorescence resonance energy transfer (FRET) between a pair of cyan (CFP) and yellow (YFP) fluorescent proteins to convert voltage-activated motions of a voltage sensor domain from *Ciona intestinalis* voltage-sensitive phosphatase (Ci-VSP) into a differential voltage dependent fluorescence signal. To evaluate the utility of VSFP2.3 as a probe of electrical activity of neurons in intact brain tissue, we performed targeted whole cell current clamp and simultaneous optical recordings from L2/3 pyramidal

cells of the mouse cerebral cortex in acute brain slices. VSFP2.3 expression in neocortical cells was achieved by *in-utero* electroporation into the cortical ventricular zone at embryonic age 15.5 of a plasmid vector containing VSFP2.3 under the CAG hybrid promoter. This procedure resulted in strong VSFP2.3 fluorescence at postnatal age (up to day 30 tested) from a restricted cortical area, mostly within somato-sensory cortex, with the fluorescence originating from a clustered population of pyramidal neurons with cell bodies in layer 2/3. Electric current injection into VSFP2.3-positive cells (postnatal day 16-22) revealed an optical response signal to sub-threshold slow depolarization of the somatic membrane that could be resolved in single trials. While the optical signal in response to fast action potentials was noisy in single trials, S/N above two was obtained by event-triggered averaging over a few (5-10) action potentials. We also tested for the optical response to synaptically evoked EPSPs which were reliably detected at near threshold amplitudes in single trials. Our results provide the first demonstration of an optical readout of neuronal activity at cellular resolution using a genetically-targetable voltage probe in intact brain tissue *in-vitro*.

**1119-Plat**
**Functional and Structural Characterization of A New Monomeric Far-Red Fluorescent Protein**
**Michael Z. Lin**[1], Michael R. McKeown[2], Ho Leung Ng[3], Tom Alber[3], Roger Y. Tsien[2].
[1]Stanford University, Palo Alto, CA, USA, [2]University of California, San Diego, La Jolla, CA, USA, [3]University of California, Berkeley, Berkeley, CA, USA.
Fluorescent proteins have become valuable tools for biomedical research as protein tags, reporters of gene expression, biosensor components, and cell lineage tracers. However, applications of fluorescent proteins for deep tissue imaging have been constrained by the opacity of tissues to excitation light below 600 nm, due to absorbance by hemoglobin. Fluorescent proteins that excite efficiently in the "optical window" above 600 nm are therefore highly desirable. We report here the evolution of a far-red fluorescent protein with peak excitation at 600 nm and peak emission at 650 nm. This, Neptune, performs well in imaging deep tissues in living mice. The crystal structure of Neptune reveals novel mechanisms for red-shifting, including the acquisition of a new hydrogen bond with the chromophore. Neptune may serve as the basis for fluorescent indicators or FRET reporters that are more compatible with deep tissue imaging.

**1120-Plat**
**Adaptive Phase Modulation for Multiphoton Microscopy**
**Rebecca M. Williams**, Warren R. Zipfel.
Cornell University, Ithaca, NY, USA.
Tissue structures present index mismatches at a variety of spatial scales that can aberrate the focal volume and thus blur cellularly resolved multiphoton images acquired within biological tissues and live animals. We are investigating the extent to which adaptive phase modulation can be used to reconstruct the point-spread-function (PSF) and enable deeper and clearer multiphoton imaging into biological tissues. To do this, a Ti:Sapphire beam is reflected off of a reflective spatial light modulator conjugate to the objective pupil plane. The excitation PSF is directly imaged with a separate objective mounted perpendicular to the optic axis. We find that the fluorescence signal increases with increasing size of the scattering structures. Resolution degradation, however, reaches a maximum with scatterer spatial frequencies at one tenth of the maximal frequency allowed by the focusing objective NA. PSF aberrations from tissue structures can be somewhat compensated by modulating the phase at the back aperture using Zernike polynomials as a basis set for increasing overall image brightness. In this scheme two fitness measurements are required for each Zernike order. Initial results through tissue sections show that spherical aberration is a problem, but not the only problem. (Research supported by NIH/NCI R01 CA116583.)

## Platform T: Muscle: Fiber & Molecular Mechanics & Structure II

**1121-Plat**
**Relay Loop Stabilizes the Force-Generating Region in Myosin**
**Yuri E. Nesmelov**[1], Roman V. Agafonov[2], Igor V. Negrashov[2], Sarah Blakely[2], Margaret A. Titus[2], David D. Thomas[2].
[1]University of North Carolina, Charlotte, NC, USA, [2]University of Minnesota, Minneapolis, MN, USA.
We have used transient time-resolved FRET ($TR^2FRET$) to monitor the conformation of the relay helix in a myosin II functional mutant during the recovery stroke in real time. Myosin was perturbed with the F506A mutation (*Dictyostelium discoideum* sequence), located within the relay loop in the force-generating region. F506 is a highly conserved residue in myosin II and is a hypertrophic cardiomyopathy mutation site. Previous studies [Tsiavaliaris, EMBO Rep, 2002, 3(11), 1099] showed a significant effect of the F506A mutation on myosin function. Actin affinity in the presence of ATP was increased, and the mutant did not move actin filaments in *in vitro* motility assays. A small decrease in intrinsic fluorescence was observed upon addition of excess ATP, but ATP binding and hydrolysis were not affected by the mutation. It was proposed that the F506A disrupts the communication between the active site and the lever arm. We engineered a double-Cys myosin mutant (A639C:K498C) in the Cys-less background with the F506A functional mutation, and labeled the mutant with optical probes. We used TR-FRET to determine the interprobe distance, and $TR^2FRET$ measurements after rapid mixing with ATP revealed changes in the relay helix conformation during the recovery stroke in real time. The mutation induced significant disorder of the relay helix in the force-generating region, but myosin still produces a recovery stroke, changing the relay helix conformation from straight to bent. We conclude that (a) the relay helix is disordered in myosin functional mutant F506A, which demonstrates the importance of the relay loop - relay helix interaction in the relay helix stabilization, and (b) the relay helix is the major structural element in the force-generating region of myosin, responsible for communication from the active site to the converter domain and the lever arm.

**1122-Plat**
**Converter Domain Residue R759 Interaction with Relay Loop Residue N509 in Drosophila Muscle Myosin is Critical for Motor Function, Myofibril Stability and Flight Ability**
**Girish C. Melkani**, William A. Kronert, Anju Melkani, Sanford I. Bernstein.
Department of Biology, Molecular Biology and SDSU Heart Institute, San Diego State University, San Diego, CA, USA.
We used an integrative approach to probe the significance of the interaction between the relay loop and converter domain of Drosophila melanogaster skeletal muscle myosin. We generated a transgenic line expressing myosin with a mutation in the converter domain (R759E) at the relay loop interaction site. The mutation depresses calcium, basal or actin-activated MgATPase values (Vmax) by ~60% and actin sliding velocity ~35% compared to wild-type myosin. Ultrastructure of two-day-old adult fibers shows cracking and frayed myofibrils with some disruption of the myofilament lattice which becomes more severe in one-week-old adults. Flight ability is reduced in two-day-old flies compared to controls and is absent in 1-week-old adults. Thus appropriate interaction between the relay loop and converter domain is essential for normal motor function, myofibril stability and locomotion. To examine the specificity of this interaction, we used a compensatory mutational approach to attempt to restore the function of the R759E mutant myosin. Our modeling indicates that relay loop residues N509 and D511 interact with converter domain residue R759. To verify our model, we generated two transgenic lines that express R759E and either the N509K or D511K mutations. Interestingly, calcium, basal, and actin stimulated ATPase values are restored to 70% and actin sliding velocity is restored to 90% in N509K/R759E but not in D511K/R759E. Structurally fibers from 2-day or one-week -old adults appear morphologically normal in N509K/R759E and their flight ability is like wild type. However, D511K/R759E myofibrils do not show any improvement compared to R759E and flight ability is worse than R759E. Overall, our results reveal the critical interaction between the converter domain with relay loop residues and their role in myosin motor function and myofibril assembly/stability.

**1123-Plat**
**Familial Hypertrophic Cardiomyopathy Mutations of the Myosin Regulatory Light Chain Remove Myosin Load Sensitivity**
**Michael J. Greenberg**[1], Katarzyna Kazmierczak[2], Danuta Szczesna-Cordary[2], Jeffrey R. Moore[1].
[1]Boston University, Boston, MA, USA, [2]University of Miami Miller School of Medicine, Miami, FL, USA.
The myosin head domain consists of a globular head and an elongated alpha-helical neck region, the "lever arm", which undergoes large conformational changes during the ATPase cycle. This lever arm has been proposed to be part of the communication pathway transmitting external loads to the active site. Since the regulatory light chain (RLC) supports and imparts stiffness to the myosin lever arm, we hypothesized that alterations in the structure of the myosin heavy chain-RLC interaction could alter myosin load-dependent biochemistry.