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.