1034-Plat
Mapping the Evolution of Molecular Flow Fields in Migrating Cells with Time-Resolved STICCS
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Cell migration is a complex process that involves an intricate choreography between cytoskeleton and adhesion molecules with precise regulation of their transport and interactions in space and time in living cells. Although there have been many advances recently in improving spatial resolution of optical microscopy methods, temporal resolution remains a bottleneck for many methods. Previously we have reported spatio-temporal image cross-correlation spectroscopy (STICCS) in combination with TIRF microscopy to provide snap shots of flow fields of adhesion proteins and the cyctoskeleton in migrating cells. However, the temporal resolution of the STICCS transport maps was low compared to the frame time resolution of the TIRF imaging. Here we report the extension of STICCS to its absolute temporal resolution limit as set by the imaging frame rate by applying a short correlation window of 10 or fewer frames with the analysis window iterated sequentially by single frame steps. Although computationally more intensive, this enables us to capture the time evolution of the flow transport and interactions of adhesion components in living cells with a STICCS vector map for every frame of the TIRF movie. We illustrate the method with measurements of time evolving transport maps of the adhesion related macromolecules alpha5, alpha6 and alphaL integrins with paxillin, and actin within, or associated with the basal membrane in adherent U2OS and CHO.B2 cells plated on extracellular matrix components fibronectin, laminin, or ICAM-1. The time resolved cross-correlation vector maps clearly show that the dynamic interactions between alpha6 or alphaL integrins with paxillin evolve in space and time only at active adhesions in protruding and retracting regions of the cells.

1035-Plat
Spatiotemporal Relationship between Ca^{2+} Release and Action Potential in Cardiomyocytes Probed by Random Access Microscopy
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Action potential, via the transverse axial tubular system (TATS), synchronously triggers uniform Ca^{2+} release throughout the cardiomyocyte. Cardiac diseases associated with TATS structural remodeling preclude a uniform Ca^{2+} release across the myocyte, contributing to contractile dysfunction. A simultaneous recording of intracellular local Ca^{2+} release and action potential in tubular network can be useful to unravel the link between TATS abnormality and dysfunctional EC coupling. Here we combine the advantage of an ultrafast random access multi-photon (RAMP) microscope with a double staining approach to optically record AP in several TATS elements and, simultaneously, the corresponding local Ca^{2+} transient. Isolated rat cardiomyocytes were labeled with a novel voltage sensitive dye (VSD) and a calcium indicator. RAMP microscope rapidly scans between lines drawn across the TATS of the cardiomyocyte to perform a multiplexed measurement of the two fluorescence signals. Although the calcium and voltage indicators can be excited at the same wavelength, the large Stokes shift of the VSD emission allows us to use spectral unmixing to resolve the voltage and calcium responses. In healthy cardiomyocytes, we found uniform AP propagation within the TATS and homogeneous Ca^{2+} release throughout the whole cell. The capability of our technique in probing spatiotemporal relationship between Ca^{2+} and electrical activity was then explored in a model of acute detubulation in which failure to conduct AP in disconnected TATS may cause local delay of Ca^{2+} transient rise leading to non-homogenous Ca^{2+} release. The research leading to these results received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under Grant Agreement 284464.

1036-Plat
Dynamic Three-Dimensional Imaging of Cellular Shape Changes and Protein Expression in the Developing Zebrafish Heart
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We present our results in dynamic three-dimensional (3D) imaging and quantification of the cellular shape changes and gene expressions of the developing zebrafish heart, in the effort to understand the mechanisms of the embryonic construction of this critical organ. The vertebrate heart is built up through a series of steps taking two flat layers of cells to a hollow heart tube to a multi-layered, multi-chambered, chirally twisted structure of the mature organ. Additionally, the heart is the first organ in the developing embryo to function, through its beating and pumping of the blood, shortly after the formation of the heart tube. Despite this intrinsic dynamic 3D nature of the developing heart, previous works documenting its development consist of largely 2D and/or static imaging (utilizing pharmacological means to stop the beating of the heart), due to the challenges in achieving fast, high 3D-resolution with conventional imaging modalities. To overcome these challenges, we employ 2-photon light sheet microscopy and a wavelet-based synchronization and registration method to achieve the required spatial and temporal resolution to capture the 3D motion of the heart. The high speed 3D imaging and analysis is carried out on several transgenic zebrafish lines that have been recently generated in our lab where proteins important for heart development are fluorescently tagged at their endogenous loci. We thus document not only cellular morphology but also critical genes' expression, with sub-cellular resolution, of the developing heart, over its beating cycle and at different development times. These results provide the necessary groundwork to start deciphering the process where the dynamic changes in cellular shapes, gene expressions, and cellular physical properties participate, in concert with the genetic program, in the development of the vertebrate heart.

Platform: Excitation-Contractions Coupling

1037-Plat
N-Acetylcysteine, a Potent Anti-Oxidant, Rescues the Malignant Hyperthermia and Environmental Heat Stroke Phenotype of Calsequestrin-1 Knockout Mice
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Calsequestrin type 1 (CASQ1) is both the major Ca^{2+} binding protein in the sarcoplasmic reticulum (SR) in skeletal muscle and a direct modulator of type 1 ryanodine receptor (RYR1) Ca^{2+}-release channel activity. Mice lacking CASQ1 (CASQ1-null) exhibit increased susceptibility for Malignant Hyperthermia (MH) and Environmental Heat Stroke (EHS), life-threatening disorders triggered by volatile anaesthetics and high temperature, respectively. Excessive oxidative stress during MH/EHS crises are proposed to result in increased RYR1 nitrosylation, and subsequently, enhanced SR Ca^{2+} leak that leads to hypercontractures and rhabdomyolysis. Here, we treated CASQ1-null mice for 2 months (from 2-to-4 months of age) with N-acetylcysteine (NAC, a potent anti-oxidant) provided *ad libitum* in their drinking water (1% w/v). NAC treatment significantly protected CASQ1-null mice from lethal episodes induced by both halothane (2%, 1h at 32°C) and heat challenge (41°C, 1h) with the rate of mortality being 79 *vs* 25% and 86 *vs* 30% in control *vs* NAC-treated mice, respectively. This protection resulted from several factors including: a) *reduced oxidative stress* measured as both GSH/GSSG ratio and the frequency of mitochondrial superoxide flash activity ($P<0.05$); b) *decreased maximum core temperature* during heat challenge (from 42.1 to 40.8°C); and c) *reduced number of fibers undergoingrhabdomyolysis* (from 37.6 to 11.6 %). Furthermore, in-vitro contracture test (IVCT) showed that the threshold for caffeine-induced contracture is back to normal values. These results support the hypothesis that excessive production of reactive oxygen species plays a critical role in the onset of MH/EHS crises. Therefore, anti-oxidants may be useful for the treatment and prevention of oxidative stress related skeletal muscle disorders.

1038-Plat
Sarcolemmal Calcium Influx in Malignant Hyperthermia Susceptible Muscle
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The most common cause of malignant hyperthermia (MH) susceptibility is a mutation in RyR1 (ryanodine receptor type 1), the skeletal muscle sarcoplasmic reticulum Ca^{2+} release channel. Muscles expressing MH-RyR1s have an increased cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) at rest which is exacerbated by volatile anesthetics. The Ca^{2+} overload induced by anesthetics is fatal unless treated with dantrolene. Here we show that the sarcolemmal Ca^{2+} influx and $[Ca^{2+}]_i$ are greater at rest in MH-RyR1^{R163C} myotubes than in WT cells.