reductions in LV torsion were also observed in heterozygous cMyBP-C null mice (cMyBP-C+/-). The time course of peak fiber torsion and strain appeared to be accelerated in cMyBP-C-/- mice, while peak strain development was slowed in MLP-/- mice. The lack of MyBP-C and MLP in the cardiac myocyte led to hypertrophic and dilated cardiomyopathy, respectively, and resulted in severe deficits in the overall contractile function of the heart. However, the pattern and time course of LV torsion and principal strain development appear to differ in these two models of heart failure, reflecting a contrast in the functional roles of cMyBP-C and MLP in the myocyte.

1723-Pos Board B633

Kinetic Imaging Cytometry of Stem Cell-Derived Cardiomyocytes Paced by Electrical Field Stimulation

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Human stem cell-derived cardiomyocytes provide a powerful model system for basic research studies, drug discovery and toxicology screening. Due to the electrical nature of their activity, cardiomyocytes are routinely studied using electrophysiological techniques. However, in addition to being very expensive and time-consuming, electrophysiology-based testing employs ''one-cell-at-a-time'' approach, which significantly limits the amount of the data collected during an experiment. Imaging methods, on the other hand, allow acquiring the enormous amount of information from many cells simultaneously, although they generally lack the ability to trigger the cellular activity in a physiological manner.

To address this problem, our group has introduced an automated optical Calcium Transient Image Cytometer (CTIC) that is capable to physiologically activate cells via electrical field stimulation and to simultaneously monitor kinetic fluorescent signals from many cells in real time with the sub-cellular resolution.

For proper assessment of the cardiomyocyte activity, cardiomyocytes have to be activated multiple times using the specific stimulation pattern. The need to preserve the viability of cardiomyocytes through the complicated multi-parameter stimulation experiments is imposing more stringent requirements on the cell-friendliness of the electrical stimulation. To achieve this challenging goal, we have developed a novel electrical stimulation module for the CTIC. By varying the configuration of electrodes and assay conditions we were able to achieve a highly reproducible cell-sparing electrical stimulation while decreasing the voltage required to initiate the response by ~4 fold.

These innovations in the design of the electrical stimulation module allowed us to perform high content screening of stem-cell-derived cardiomyocytes at the different stages of their maturation as well as to carry out the pharmacological studies of use-dependence mechanisms of different pharmacological agents.

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Photon Diffusion Attenuation Length in Tyrode and Blood-Perfused Myocardial Tissue

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Photon attenuation length (δ) in biological tissues determines interrogation depth, spatial resolution, and amplitude of fluorescence signal in various types of optical imaging, including imaging of cardiac excitation using voltage-sensitive dyes. We assessed δ in human and pig myocardium at excitation/emission wavelengths of commonly used and recently developed near-infrared voltage-sensitive dyes. We also compare δ in Tyrode vs blood-perfused tissues and simulate respective voltage-sensitive fluorescent signals in the context of potential clinical applications (in vivo optical mapping of heart electrical activity). Experiments were conducted in isolated slabs of ventricular myocardium. Light decay inside the tissue was measured via a 600μm diameter optrode at 520, 650, and 715nm. The δ was determined by fitting data to a theoretical formula for light attenuation [Mitrea et al. 2009].

For wavelengths tested, (see table) the δ in porcine and human myocardium are similar, which makes porcine myocardium a good model for development of clinical imaging applications. Blood perfusion reduces δ, particularly at 520nm. However, our simulations show that, the resulting reduction of optical action potential is <16% (for NIR dyes). which would not be a major impediment for in vivo imaging of cardiac excitation.

TABLE . Photon diffusion attenuation length (mm)

Preparation \ Wavelength	520 nm	650 nm	715 nm
Blood-perfused, pig (n=6)	0.46 ± 0.01	1.83 ± 0.02	2.31 ± 0.05
Blood-perfused, human (n=4)	0.48 ± 0.02	1.98 ± 0.07	2.74 ± 0.13
Tyrode-perfused, pig (n=6)	0.61 ± 0.03	2.15 ± 0.05	2.63 ± 0.07

1725-Pos Board B635

Single-Camera Multi-Parametric Optical Mapping During Local Excitation of Isolated Rat Heart

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Simultaneous optical mapping of multiple electrophysiologically relevant parameters in living tissue is desirable for integrative exploration of mechanisms underlying organ function in norm and pathology. Current multi-parametric methods are technically challenging, usually involving several sensors, complex optical configurations/alignment and moving parts (e.g. mechanical shutters and filter wheels). This increases logistic and economical thresholds, preventing turn-key solutions and broader application. Therefore we developed a simple, affordable and effective method for single-sensor multi-parametric optical mapping.

The developed system can measure membrane potential (V_m) and intracellular free calcium concentration ($[Ca^{2+}]_i$) using the following functional dyes: Di-4-ANBDQPQ (V_m), Fura-2 ($[Ca^{2+}]_i$). Four parameter (ratiometric V_m and ratiometric $[Ca^{2+}]_i$) configuration has been designed using light-emitting-diode (LED) excitation sources ranging from UV to red wavelengths. High-speed coordination between the LEDs and a 128x128, 16-bit electron-multiplied charge-coupled device (EMCCD) camera system (Cascade 128+; Photometrics) was achieved with inexpensive and versatile microcontroller-based electronics. Emission fluorescence from dyes was passed through off-the-shelf and custom multiband filters, a technology not widely explored in the context of optical mapping.

Here, we present simultaneous ratiometric V_m and ratiometric $[Ca^{2+}]_i$ imaging with Di-4-ANBDQPQ and Fura-2 in the rat whole-heart. For proof-of-principle application, we integrated this imaging technology with timed local electrical and mechanical stimulation to study mechano-electric coupling at the organ level.

Combining an affordable off-the-shelf camera with optical filters and LEDs, single-sensor multi-parametric optical mapping can be practically implemented and applied to heart research. The moderate system complexity and component cost (less than 5% of camera cost) may lower the threshold to broader application of functional imaging and ease implementation of more complex optical mapping approaches such as panoramic multi-parametric optical mapping.

1726-Pos Board B636

Myonuclear Domain Size and 3d Myonuclear Organization in Single Muscle Fibers from Myostatin Deficient Or IGF1 Overexpressing Mice

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Myostatin deprived or IGF-1 over-expressing mice are characterized by a 2-3 fold increase in muscle size compared to controls. Despite the hypertrophy these mice show significant difference in force generating capacity, i.e., maximum force normalized to muscle fiber cross-sectional area or specific force. That is, specific tension in IGF1 overexpressing transgenic mice is similar to controls while significantly lower in the myostatin knock out mice. The mechanism underlying this compromised muscle function is unknown. In an attempt to explore this mechanism we have investigated the size of cytoplasmic volume (myonuclear domain MND) supported by individual myonuclei in single muscle fiber segments from myostatin deficient, IGF1 over-expressing and control mice, using a novel algorithm to measure the MND in 3D. Single skinned muscle fiber segments were mounted at fixed sarcomere length corresponding to optimum filament overlap for force generation and stained with DAPI (myonuclei) and rhodamine (actin). Our image analysis algorithm was highly effective in determining the spatial organization of myonuclei and distribution of MNDs along the length of the fiber. Early results point towards an inverse relationship between MND and specific force. This implies that hypertrophy is primarily due to expansion of existing MNDs in myostatin knock-outs, and addition of more myonuclei in IGF1 over-expressing mice. This is suggested to have significant effects on transcriptional control of protein synthesis/degradation, turnover rates and/or posttranslational modifications of contractile proteins. We conclude that a maintained MND size is a prerequisite for force generation capacity in hypertrophied muscle fibers.

1727-Pos Board B637

Dipole Emitter Lateral Point Spread Function Communicates Orientation and Axial Position

Thomas P. Burghardt.

Photoactivatable fluorescent probes developed specifically for single molecule detection extend advantages of single molecule imaging to high probe density regions of cells and tissues. They perform in the native biomolecule environment and have been used to detect both probe position and orientation. Native, high density, single molecule detection may have added significance if