HIGH SPEED IMAGING OF CALCIUM DYNAMICS IN CARDIOMYOCYTES WITH A FLEXIBLE LIGHT SHEET FLUORESCENCE MICROSCOPE

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The high prevalence and poor prognosis of heart failure are two key scientific drivers behind research into the electrophysiology of healthy and damaged cardiac tissue. Dyssynchronous calcium release and disorganization in the t-tubule structure within individual cardiomyocytes (CM) has been linked to poor contractile function and arrhythmia. Correlative imaging of the calcium dynamics of CM and their microstructure calls for the use of imaging techniques capable of high-speed 2D or real-time video-rate 3D microscopy of cardiac tissue at subcellular resolution with low cell phototoxicity and photodamage. This work implements a custom-developed dual-channel inverted light-sheet fluorescence microscope [1] for high-speed 2D imaging of calcium dynamics in CM and 3D imaging of calcium dynamics in human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CM). Volumetric acquisition for 40 planes at 1 μm spacing at 8 Hz is achieved using folded remote refocusing [2] of the detection focal plane synchronously with the Gaussian light sheet swept along the detection axis, without mechanical disturbance of the sample.

The spatial and temporal resolution achieved allows multidimensional characterization of calcium dynamics across single CM in correlation with t-tubule microstructure. We present two-dimensional optically sectioned time-lapse imaging of calcium dynamics in isolated live rat CM at 400 fps and 0.5 μm lateral resolution. Electrical pacing is used to stimulate calcium transients with decoupled contraction. The fluorescence intensity time-trace of calcium indicator Fluo-4 is used to characterize the spatial variation in calcium transient rise time across the cell, and detected calcium sparks are mapped onto the transverse tubule structure (Fig. 1). Calcium transient spatial dyssynchrony and spark spatio-temporal properties and correlation to the cell microstructure are compared for left and right ventricle CM.

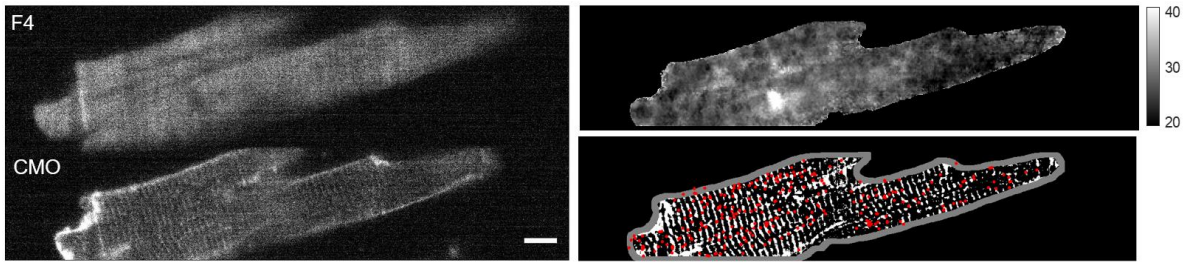

Fig. 1 Correlative calcium dynamics characterization in live cardiomyocytes. Left: spatially separated calcium indicator Fluo-4 (F4) and membrane stain Cell Mask Orange (CMO) emission acquired at the peak of a Ca^{2+} transient. Top right: spatial variation of the time to half maximum (ms) across the cell. Bottom right: Spark centre of mass coordinates (red) overlaid with the intracellular tubule structure, with cell membrane outline indicated in gray. Scalebar: 10 μm

[1] H Sparks et al (2020) *J. Biophotonics* **13** (6)
[2] E.J. Botcherby et al (2007) *Optics Letters*, **32** (14), 2007-2009a