LIVE-CELL IMAGING OF CALCIUM DYNAMICS AND CONTRACTILITY IN CULTURED 3D CARDIOSPHERES BY LSFM

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KEYWORDS: Cardiospheres, live cell, calcium imaging, light sheet, organoids, dynamics

The generation of 3D tissue aggregates from human pluripotent stem cells (hPSCs) presents a highly promising tool, both as an *in vitro* model for recapitulating the complexities of cell behavior within intact tissues and for the therapeutic potential in regenerative medicine. Due to the large size (typically >100 um), cellular density and complex architecture, volumetric and dynamic (4D) imaging of these structures is challenging [1]. The increased light penetration and fast and gentle acquisition provided by light sheet fluorescence microscopy (LSFM) make this method an ideal approach for studying organoid structures [2]. In this study, 3D aggregates of hPSC-derived cardiomyocytes (cardiospheres) were generated using microscale technology [3]. Regular calcium fluctuations were recorded in cultured cardiospheres expressing GCaMP6 using 4D LSFM at acquisition rates of 20 fps (Figure 1). Observed beat frequencies, around 500 ms, were indicative of physiologically relevant calcium impulses. The spatial resolution (300 nm) provided by the light sheet gives us the ability to record both coordinated aggregate-wide contractile beating events and intra-aggregate heterogeneity in the firing rates. Thus, we established that LSFM provides a powerful technique for assessing the cardiac microenvironments, phenotypic dynamics and functionalization of cardiospheres.

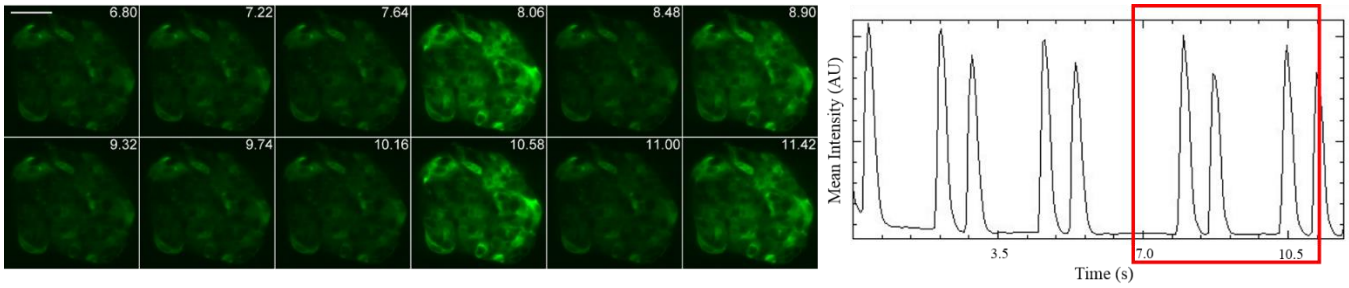

Figure 1: LSFM-generated time series of calcium fluctuations in a GCamp6-expressing cardiosphere over two beating cycles. Scale bar = 50 μm (left). Plot showing dynamic GCamp6 mean fluorescence intensity vs. time (right). The region of the plot corresponding to the time series is indicated by the red box.

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