Quantification of Sarcomere Length Oscillations in Human Cardiomyocytes

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Introduction: The average human heart beats ~2.8 billion times over a 70 year lifetime. In the failing heart, alterations in the contraction-relaxation cycle of the sarcomere (the basic structural unit of the heart) can be visualized by high-speed time-lapse phase contrast microscopy. Here, we describe the methodology used to quantify changes in sarcomere length over time.

Methods and Results: Isolated bundles of frozen (-196°C) human cardiomyocytes from left ventricles were slowly warmed to room temperature and then induced into an auto-oscillatory state known as SPontaneous Oscillatory Contractions (SPOC). Changes in half-sarcomere length were acquired by high-speed time-lapse phase contrast (Leica SP5 Multiphoton) microscopy. We also used the second harmonic feature of this microscope to identify the myosin A bands that are known to not change length during SPOC. Data analysis requires three steps: (1) detecting changes in half-I bands; (2) monitoring the time course of these changes; and (3) modelling SPOC length changes as a sawtooth waveform.

An appropriate region of interest (ROI) was selected from each 1000-image stack on the basis of frame-by-frame (FF) and difference-from-first (DF) analyses. FF analysis detects low-intensity, high-frequency changes in pixel intensity that correlate with contraction-relaxation phases of sarcomere length changes. The opposite intensity-frequency was detected by DF analysis, and is associated with the SPOC wave. The position of each sarcomere within the ROI, or the X position of the darkest pixels (32-bit gray scale) was traced frame by frame (Fig A). The averaged difference between each dark pixel was fitted by a sawtooth model (Fig. B).B- note the abscissa labels allowing the following five parameters: changes in sarcomere length, period and rate of contraction (shortening), and period and rate of relaxation (lengthening).

Conclusion: We have previously demonstrated that SPOC measurements can sense changes in failing cardiomyocytes that are closely related to the disease phenotype. SPOC measures length changes of ~0.1µm with a temporal acquisition of ~15ms/frame. To our knowledge, no commercial software can track changes with such accuracy. These methods can be applied to other oscillatory/cyclic movements need to be measured.