Automated Microscopy-based Characterization of Stem-Cell Derived Cardiomyocytes

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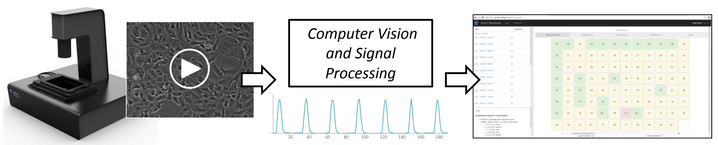
Introduction

- Stem cell-derived cardiomyocytes hold tremendous potential for drug development and safety testing related to cardiovascular health.
- The characterization of cardiomyocytes is most commonly performed using electrophysiological systems, which are expensive, laborious to use, and may induce undesirable cellular response.
- We present a new method for label-free, contact-free characterization of cardiomyocytes using video microscopy and image analysis. Our approach captures beating patterns and arrhythmias across a wide range of plating densities. We demonstrate the strengths of our algorithm by characterizing the effects of two commercial drugs known to modulate beating frequency and irregularity.
- Our results provide, to our knowledge, the first clinically-relevant demonstration of a fully-automated and non-invasive imaging-based beating assay for characterization of stem cell-derived cardiomyocytes.

Overview

We use Cellogy's Pulse imaging system to acquire phase-contrast videos of cardiomyocyte beating. A system overview is shown below:

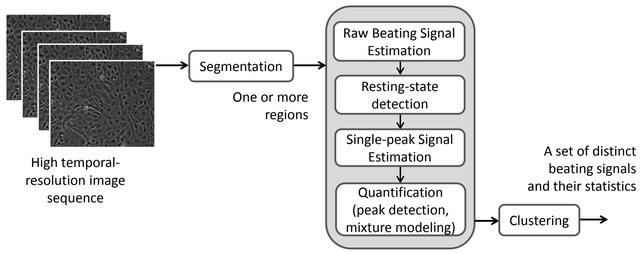


Video of cardiomyocyte beating | *Automated characterization* | *Cloud-based data management with website user interface*

- Stem cell-derived cardiomyocytes are cultured in standard multi-well plates (6 well through 96 well), and are placed in the stage-top incubator on the imaging system.
- After acquiring a high-temporal resolution image sequence, our automated image analysis software estimates the beating signals and derive measurements such as frequency, duration of contraction and relaxation, and beat variation over time.
- The results of our non-invasive analysis are accessible through our website application.

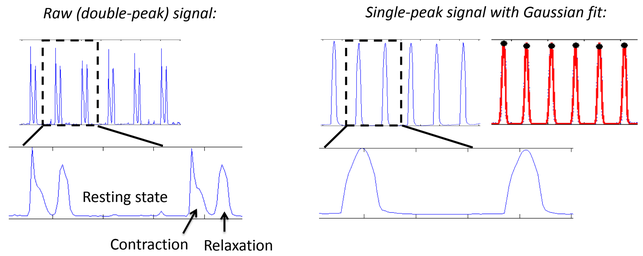
Image Analysis Flowchart

Images are segmented into a set of distinct beating regions. Signals are extracted for each region, quantified, and then clustered in order to join regions with similar beating characteristics. The output is a set of distinct beating signals and their measurements.

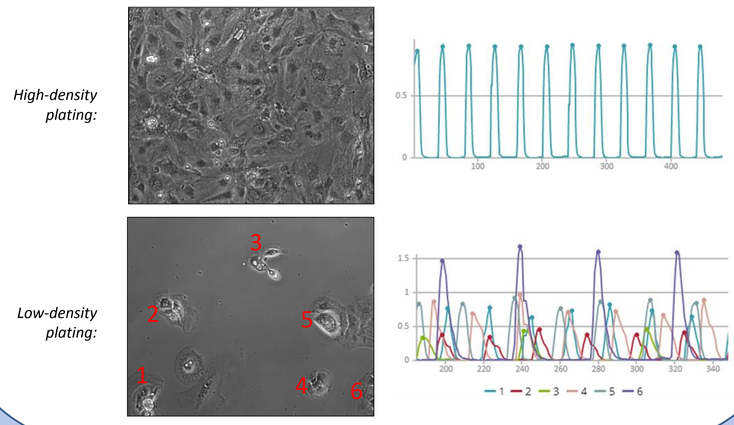


Beating Signal Analysis

The figures below shows two beating signals obtained for a cardiomyocyte dataset. The raw double-peak signal typically exhibits three states: a resting-state, a contraction state, and a relaxation state. Although the beating pattern and frequency can be measured from this signal, automatic identification of beating intervals is challenging due to the presence of double peaks and the lack of prior knowledge on their relative magnitude or distances. We therefore generate a single-peak signal, followed by Gaussian mixture modeling, to measure the shape and peaks.

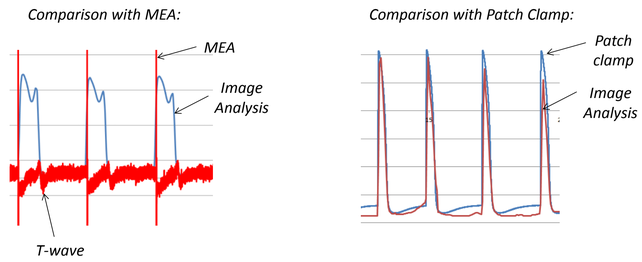


Below are two examples of beating signal analysis for high-density (monolayer) and low-density (single-cell) plating.. Our technique automatically recognizes and analyzes each distinct beating region.



Comparison with Electrophysiology

As an initial step towards validating our non-invasive analysis, we compared the results generated by our algorithm to data collected on different electrophysiology systems. In the first example below, we compare our analysis to a micro-electrode array (MEA) system (Axion Biosystems), where imaging was performed by Pulse directly on an MEA dish. In the second example below, we compare to manual patch clamp, where a video camera was installed on the patch clamp rig (ChanTest Corporation and Ionic Transport Assay).



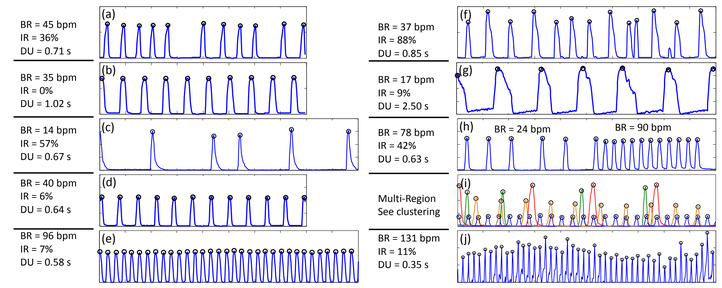
Cell Culture and Experiments

Cell Culture: To assess the performance of our method, we performed a series of experiments using iPSC-derived cardiomyocytes obtained from commercial vendors, including Axiogenesis and Cellular Dynamics. Cardiomyocytes were cultured in multi-well plates following standard culture protocols.

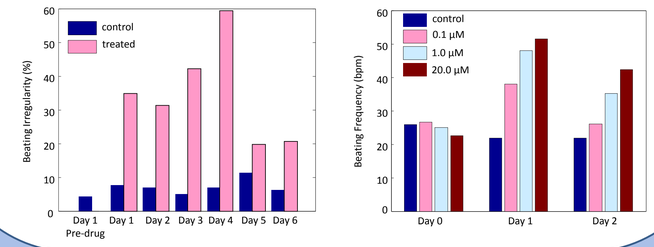
Experiments: Videos of beating cardiomyocytes were acquired on Cellogy's Pulse system at a rate of 24 frames/second for a duration of 15-30 seconds. We collected and analyzed more than 500 videos of cardiomyocyte cultures from different sources, plated with varied cell culture densities. We observed variation of beating characteristics over time, after media changes, and with addition of chemical compounds.

Experimental Results

The following figure demonstrates the ability to capture beating profiles for a wide range of cellular behavior. Effective beat rate (BR), a measure of beating irregularity (IR), and the average beat duration (DU) are automatically measured for each sequence. (a,h): Diseased lines, (b,d): controls, (f): after addition of a compound, (c,g,i): single-cell plating, (e,j) after media change.



We also performed a preliminary set of compound experiments using Cisapride and Norepinephrine applied to high-confluency cell cultures. As shown below, we measured an average 3-fold increase of beating irregularity with Cisapride-treated cells compared with the controls, observed over 6 days, as well as a dose-dependent increase of beating frequency for Norepinephrine-treated cells, observed over 2 days.



Conclusion

- We presented a new method for non-invasive characterization of stem cell-derived cardiomyocytes using video microscopy and image analysis.
- These results provide the first clinically-relevant demonstration of a fully-automated and non-invasive imaging-based beating assay for characterization of cardiomyocytes.
- Our future work is focused on performing validation studies and measuring additional parameters of interest such as contraction strength and patterns.