Spatiotemporal Quantification of In Vitro Cardiomyocyte Contraction Dynamics Using Video Microscopy-based Software Tool

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Abstract

Stem cell derived cardiomyocytes provide a platform for a variety of studies. The typically performed electrophysiological measurements do not describe the primary function of these cells, contraction and its biomechanics. Video microscopy-based analysis of motion has become a feasible option for these studies.

Here, we demonstrate methods for spatiotemporal quantification of stem cell derived cardiomyocytes, implemented in an in-house developed MATLAB-based software tool. The tool is capable of characterizing cardiomyocyte contraction with minimal user bias.

The results show that automatic segmentation using a power spectral density -based measure enables segmentation based on contractile function. Further, based on segmented boundaries, we introduce automatically calculated parameters for quantification the contractile function and its propagation through the cell culture based on timings of different contraction phases.

The methods presented here form a basis for quantifying and understanding the contraction dynamics and the propagation of contraction in cultures involving cardiomyocytes.

1. Introduction

Human induced pluripotent stem cell (hiPSC) derived cardiomyocytes (hiPS-CMs) provide a basis for in vitro studies, enabling research ranging from genetic cardiac disease to pharmaceuticals. Typically, these models are analyzed by measuring their electrophysiological activity with methods such as patch clamp, microelectrode arrays and calcium imaging. Measuring their biomechanical function, however, has only recently been in focus. Video microscopy-based analysis of contractility using optical flow methods has become widely used due to its non-toxic and label-free nature [1]–[3]. We have previously shown the characterization of biomechanical phenotypes of cells derived from long-QT syndrome patients [4], simultaneous measurement with calcium reporters [5].

The currently available tools each have their own use cases, but the methods typically focus on the temporal aspects and magnitude of the contraction. In addition, the studied cell cultures vary – dissociated cells, monolayers, small aggregates and larger clusters have their own analysis needs – not to mention their different stages of maturity. For these reasons, developing functions capable of automatic analysis is not straightforward. Further, the spatiotemporal quantification of contraction and its propagation have not been typically included in these tools. Here, we describe the methods that incorporate this facet of biomechanical analysis to our in-housed developed MATLAB-based analysis tool CellVisus [1]. We introduce novel spatiotemporal segmentation and demonstrate these features on a hiPSC-CM monolayer. The methods presented here provide a basis for studying the connections between the cellular structure and contractile function.

2. Materials and Methods

2.1. Cell culture used in demonstration

UTA.04602.WT hiPSC line [6] was used in the demonstration of the software and methods. Embryoid body differentiation was used to differentiate the hiPSCs into cardiomyocytes. Cardiomyocytes were dissociated to single cell level followed by magnetic activated sorting for enrichment. The hiPSC-CMs were suspended to EB medium and seeded to 48-well plates coated with gelatin-gellan-gum hydrogels [7] as cell sheets (density ~ 98,000 cells/cm2).

2.2. Video processing

Videos at 59 frames per second (FPS), recorded from beating hiPSC-CMs are processed by calculating minimum quadratic difference (MQD) based optical flow between subsequent video frames, and as described previously [1], resulting in velocity vector fields $\bar{V}(x,y,t) = \bar{u}_{(x,y,t)} + \bar{v}_{(x,y,t)}$. The frame indicating the maximum contraction velocity is determined from all obtained velocity vector fields $\bar{V}$. Within this frame, $\nabla \cdot \bar{V} < 0$ is determined and local minima are defined, indicating the contraction centers of the cells. Based on

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these contraction centers, the directionality of contraction and relaxation associated motion is determined, defining contraction as positive and relaxation as negative. The mean of dot products of each velocity vector and its location vector, with respect to the nearest contraction center, is calculated to derive a signal characterizing the contraction motion.

2.3. Vector field segmentation

In existing applications, the region of interest area in the cell culture for segmenting $\bar{V}$ is done either manually, or based on beating intensity [2], [3]. However, this segmentation approach may miss weak beating regions in the presence of stronger beating regions, or include regions with motion unrelated to contraction, introducing noise and artefacts to the measurement. To overcome this, we propose to use power spectral density (PSD) -based segmentation, where spatial segmentation is performed by estimating power spectra using Welch's method at each vector location (x,y), throughout the video. Then, a ratio describing the spectra is calculated by integrating over the frequency spectrum ω as described below.

$$PSD_{ratio}(x,y) = \frac{\int_0^{1/6} PSD_{(x,y)}(\omega)d\omega}{\int_{1/6}^{1} PSD_{(x,y)}(\omega)\,d\omega}$$

The resulting map PSD_{ratio} is then thresholded by the mean of PSD_{ratio} to create a segmentation map for regions with most prominent relative low frequency content.

To demonstrate our method we compare this method to another cellular function-based segmentation, velocity vector magnitude-based segmentation, calculated as the cumulative sum M(x,y) of all velocity vectors at each vector location (x,y) as shown below, and thresholded at 0.5 times the maximum cumulative value.

$$M(x,y) = \sum_{t=1}^{T} \sqrt{\left(\bar{u}(t)_{(x,y)}\right)^2 + \left(\bar{v}(t)_{(x,y)}\right)^2}$$

2.4. Signal parametrization

After segmentation, the contraction-relaxation signal is calculated from the segmented vector fields. Key points of contraction start, contraction end, relaxation start, and relaxation end are determined initially by determining the contraction and relaxation peaks. Using cumulative integration, peak base is detected for the start and end points. Using these points, durations of contraction and relaxation are calculated, as well as the duration when the cell stays contracted with no motion.

2.5. Contraction quantification

The extent of contraction, displacement, is calculated by calculating MQD for the contraction cycle, using the contraction starting point as the reference frame, resulting in contraction vector field $\bar{C}(x,y,t)$. Here, typically maximum and mean values are calculated. However, these measures are influenced by artefacts and non-beating regions. This effect can be reduced by calculating the mean of the largest 10th percentile of $\bar{C}$, $Mean_{10\%}$.

Here, we also evaluate the propagation of contraction spatially. We define a contraction propagation map by calculating for each vector location the frame with a certain reached threshold. These thresholds may be the set as pixels or as the maximum of each vector location. Based on these maps, data on the initiation of contraction and the contraction propagation can be determined as time from the first region reaching the set threshold till the last region.

Further, this allows the concurrence of the measured region to be evaluated – a value between [0,1] indicating how synchronously the measured region reaches these threshold values, with 1 being simultaneous.

3. Results

The methods presented here are implemented in an in-house developed MATLAB software tool CellVisus, and the results demonstrated here are calculated automatically from an hiPSC-CM monolayer.

3.1. Segmentation

The software was capable of automatically detecting and parametrizing contraction-related motion using the PSD_{ratio}-based region of interest selection. In contrast to magnitude-based selection M, PSD-based masking separated contracting regions clearer than magnitude-based masking. This is shown in Figure 1 below.

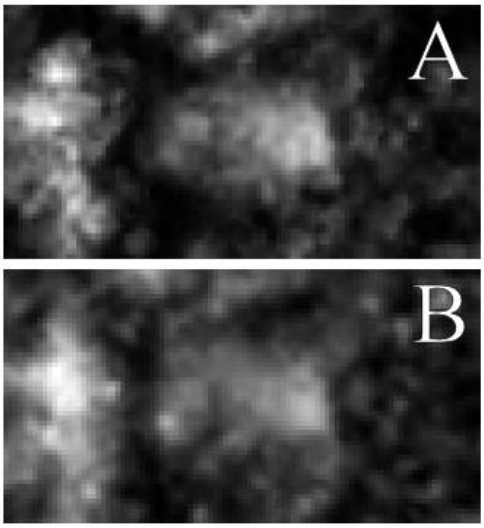

Figure 1: Parameter maps for segmentation. A) Map of calculated with PSD_{ratio} for the cell culture. B) Map of cumulative vector magnitude for the cell culture.

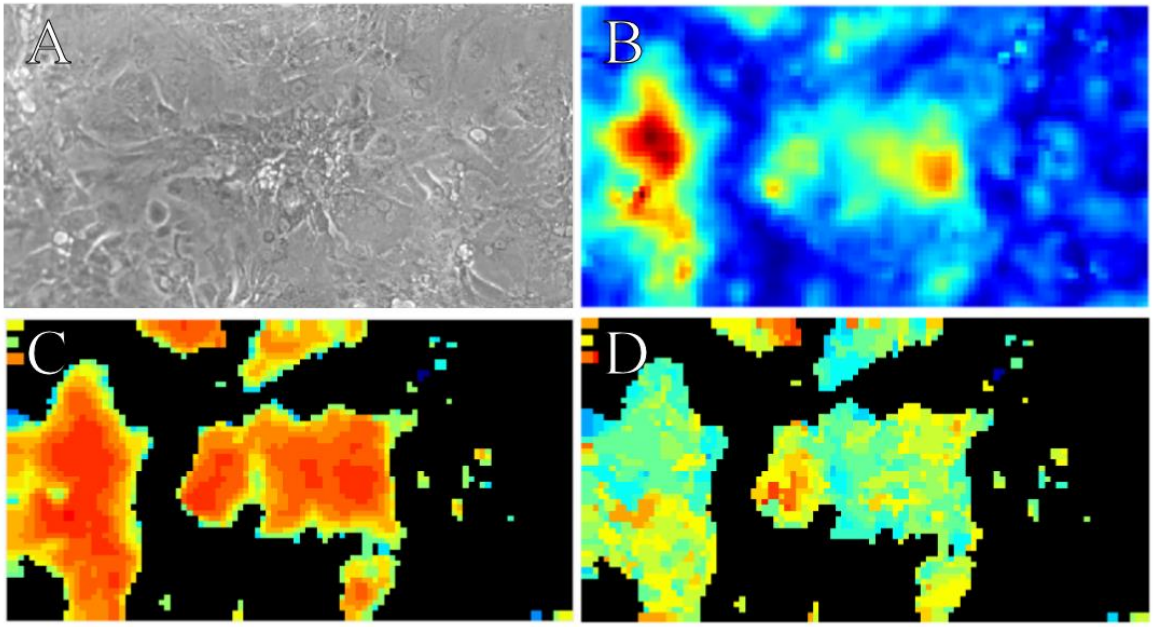

Figure 2: Visual maps of contractility. A) A brightfield image of the culture. B) Map of contraction magnitudes, with high magnitudes shown with red. C) Map of contraction propagation, indicating the order of regions reaching a 3 px displacement. Earliest values shown in red. D) Map of contraction propagation, indicating the order of regions reaching their maximum displacement. Earliest values shown in red.

3.2. Signal parametrization

Parameters characterizing the culture were calculated using three segmentations: PSD-based segmentation, magnitude-based segmentation and whole field of view (FOV). The displacement parameters were calculated for reaching maximum value at each (x,y) location, with a 3 px threshold, shown in Table 1.

Table 1. Parameters characterizing the cell culture calculated using three segmentations.

Parameter	PSD	Magnitude	Whole FOV
Contraction (ms)	180	190	190
Contracted (ms)	40	70	40
Relaxation (ms)	230	190	220
Max displ. (px)	10.4	10.4	10.4
Mean displ. (px)	5.6	5.8	2.9
$Mean_{10\%}$ (px)	9.4	9.6	6.8
Propagation (ms)	130	130	270
Concurrence	0.33	0.30	0.22

PSD-based segmentation and whole FOV produce similar temporal parameters, whereas the displacements calculated using PSD segmentation and magnitude segmentation are similar.

3.3. Contraction quantification

The extent and propagation of contraction in the monolayer, shown in Figure 2A, were calculated. As shown in Table 1 and indicated in Figure 2B, the high magnitudes of contraction are localized in two regions, with vast areas of the culture showing limited contractility. Figures 2B, 2C and 2D together with PSD-based segmentation enable the estimation of primary contraction propagation directions and origin points in the monolayer. While the monolayer reaches the 3 px displacement in a uniform fashion, there is variation in the time at when they reach their peak.

4. Discussion

Video microscopy-based measurement of biomechanics is a developing field, with applications in studies of genetic cardiac disease, pharmaceutical compounds and cellular biomechanics. Here, we introduced robust methods that aid in high-throughput analysis of video microscopy data. The methods shown here and demonstrated on an hiPSC-CM monolayer are implemented in an in-house MATLAB tool CellVisus. The tool is currently being evaluated for releasing in open source.

While automatic analysis methods of hiPSC-CM cultures exist, the field of cardiomyocyte analysis uses single dissociated cells, aggregates, monolayers and clusters in studies. They each have their own specific

characteristics, but the available analysis software tools can be used for all culture types, which may induce errors if the tool has not been specified for that culture type. Thus, it is important to develop these methods further by improving the resilience of these tools, for instance against measurement artefacts, both in parametrization and segmentation as well as possible user bias in semiautomatic analysis.

The characterization of the culture was compared using different schemes of segmentation. The temporal parameters provided by PSD-based segmentation are in line with previously reported values with contraction being shorter in time compared to relaxation [1]–[3], whereas the magnitude-based segmentation had the same durations for both. PSD-based segmentation and magnitude-based segmentation provided similar displacement magnitudes, as well as in propagation duration and concurrence. This type of segmentation is resilient against signal artefacts originating from floating debris or flow from perfusion. Further, as was shown in Figure 1, the boundaries of beating regions are sharper than in magnitude-based segmentation, enabling more definite separation of contractile regions.

Thanks to the segmentation, the propagation of contraction within a hiPSC-CM culture was observed using video microscopy even at 59 FPS. Figures 2C and 2D shows marked differences between the propagation of contraction in different directions, and that these regions do not directly match the magnitude map of 2B. With the data on propagation of contraction, correlations with the cell culture structures could be made in future studies using high speed cameras, enabling greater understanding of cellular connections in the monolayer cellular syncytium.

5. Conclusions

The methods demonstrated here, implemented in a MATLAB software tool, provide a basis for studying the propagation of contraction in cell cultures. In contrast to cell outline based segmentation, PSD-based segmentation provides a cell function-based segmentation method, aimed at detecting regions with contraction-related movement.

With the segmentation, other spatial information can be provided by the tool, including the quantification of contraction propagation, which has applications in studying the connection between the contractile function and the cellular structure.

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