Calcium Activity in a Myocardium-on-chip Model Analysed with Computer Vision Techniques for the Assessment of Tissue Contractile Properties

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Abstract

Models of cardiac tissue accurately simulating the structure and function of myocardium might allow to better understand and treat cardiac disorders such as arrhythmia. One of indicators of contraction, a key feature of cardiac tissue, is calcium (Ca) activity, which can be detected and recorded with fluorescence microscopy imaging. Here we use a range of computer vision techniques to analyse Ca activity in a myocardium-on-chip model, in tissues of GCaMP6-infected myocytes, grown in a microphysiological environment. We analyse and quantify various aspects of Ca activity both local and global properties alongside temporal dynamics, considering aspects such as instantaneous and average occurrence rate of Ca waves, size of areas covered by individual waves, or a degree of regularity of Ca activity. Simple summary statistics computed allow for a comparison of Ca activity for tissues recorded in different experimental conditions.

Keywords: Calcium imaging, cardiomyocytes, image processing

1. Introduction

Nowadays cardiac disorders are one of the most observed causes of death. Plenty of them can be avoided if these illnesses are better understood. One of the potential solutions for this problem is building models of cardiac tissues with simulation of the structure and function of myocardium alongside analysis of its properties (measurement of diversified parameters).

In this work, the authors would like to present their own methodology connected with the topic. We introduced image processing and analysis methods for determination of Calcium (Ca) activity in artificially grown cardiac tissue. It needs to be pointed out that this indicator is one of the most important when it comes to determination of contraction. Calcium ejection occurs before the heart cell contraction (however, it is not deterministic – Ca ejection can be observed but contraction may not occur). Our experiments were performed with the videos collected by the Team of prof. Oscar Castano Linares from University of Barcelona. The dataset consists of more than 40 videos. In each of them, one can observe myocardium-on-chip model with tissues of GCaMP6-infected myocytes. Our Computer Vision-based approach allows to calculate diversified parameters related to Ca ejection (as velocity of the wave).

In the literature, one can find the approaches that consume Computer Vision techniques for analysis of cellular behavior and tissue properties. The significant example is [1]. In other works, the Authors proposed techniques that enable the automated extraction of relevant features from microscopic images, allowing for comprehensive assessment of tissue contractive properties and calcium activity [2, 5]. One can also observe the works connected with analysis of the heart (Ca waves) in the course of diversified diseases (they are related to early detection too [3]. Machine learning methods are also playing an important role here [4].

2. Experimental Setup

HL-1 cardiac cells, derived from mouse heart atrial tissue, were transfected with a Ca indicator GCaMP6. Tissues were grown in a micro physiological environment, with coating concentration 50 μl/ml, and in some cases placed on polylactic acid electrospun (PLA) fibers. Three different experimental conditions were considered, that is, either no PLA fiber or PLA fibers either aligned uniformly in one direction or non-aligned with random orientations. For each condition, there were tissues grown on multiple microchips. All tissues were recorded with fluorescent microscopy for Ca activity imaging, with each chip recorded multiple times, and each recording being ca. 1 minute in duration.

3. Approach and Results

To detect tissue areas covered by Ca activity and describe quantitatively various aspects of its dynamics, we analyzed light intensity in the fluorescent microscopic videos, with video frames stored in grayscale format.

First, we detect time moments of occurrence of large-scale Ca activity (Ca waves), by finding peaks of light intensity in the monochromatic recordings, with intensity averaged over the entire area of each frame. To remove a trend apparent in the initial part of some of the recordings (being an artifact due to a microscope adaptation), we calculate a moving average of light intensity over 10 preceding frames and remove such baseline level from each frame. Based on the peak detection results, we measure the rate of Ca waves' occurrence (which may be related to rate of tissue contraction), both instantaneous, as a time series, and one average rate value for a given recording. Result of this analysis for example recording is presented on Figure 1.

We aim to analyze various properties of the detected waves' propagation through the tissue (e.g., direction, velocity, and dependence between these parameters). To obtain these values, we calculate a map of times of Ca activation in different regions relative to a wave onset, and present it as an isochronal activation map, with arrows overlaid on the map indicating local gradients of activation times. Then, we compute an angular histogram showing individual waves' average propagation directions, in polar coordinates. Based on it, we compute a degree of uniformity (randomness) of different waves' propagation directions, which might indicate whether there is one or more distinguished directions in which Ca waves tend to propagate in each tissue.

We compute also waves propagation velocity, as a ratio of distance between two regions of earliest and latest activation, respectively, to the time interval between these activations, presenting these results as a histogram. We combine the information about propagation direction and velocity, calculating a dependence of velocity on propagation direction, to assess whether propagation in some directions occurs more swiftly than in others.

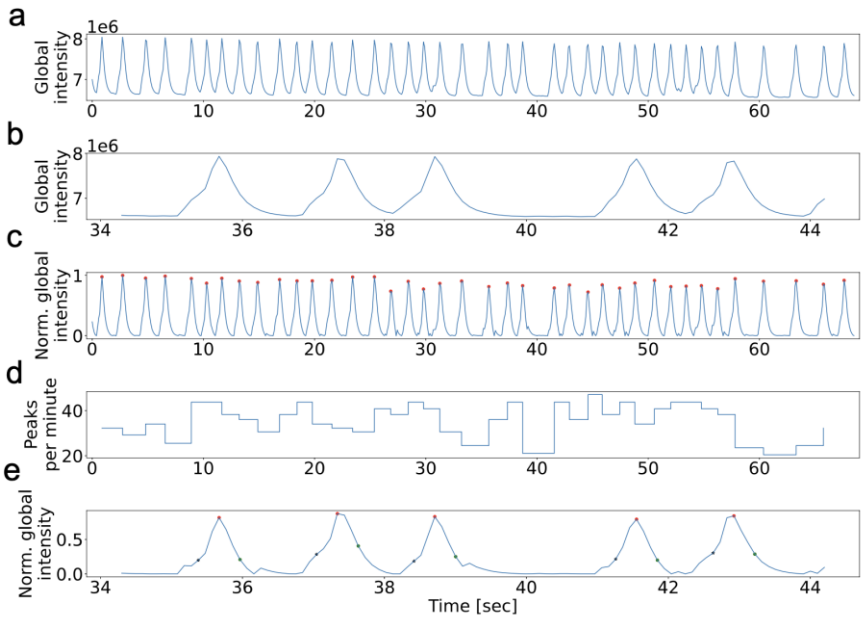

Fig. 1. Temporal dynamics and occurrence rate of large-scale Ca activity. (a) Global light intensity over the course of an entire, raw recording, for one example video. (b) 10 sec-long fragments selected from the trace in (a). (c) Trace from (a) with trend removed and values normalized, with peaks of light intensity (indicating Ca waves) detected marked with red dots, and waves' onsets indicated with green dots. (d) Instantaneous rate of Ca waves occurrence. (e) 10 sec-long trace from (b) with trend removed and values normalized.

Moreover, to assess involvement of different regions of the tissue in Ca activity, we detect and quantify areas covered by local Ca activity. To this end, first we spatially smooth pixel values with a Gaussian blur, apply a mask selecting the pixels with values above a certain threshold cut-off level, and then use a contour detection method to determine the areas of significant Ca activity.

Using results of the above-described analyses we calculate a set of properties summarizing different aspects of Ca activity in different tissues, which allow us to compare the tissues on different chips, with bar plots and a similarity matrix. As the properties, we take: (1) average size of area covered by a Ca activity; (2) average rate of Ca waves occurrence; (3) uniformity of the distribution of waves' propagation directions; (4) std of the distribution of areas covered by Ca activity.

To create the similarity matrix, for each pair of chips *i* and *j* we compute the similarity between them taken as in (1)

$$sim^{(i,j)} = \exp\left(-\left|\left|p_i - p_j\right|\right|\right) \tag{1}$$

where p's are centered and normalized vectors of the respective chips' properties. The summary and comparison of tissues' properties is presented in Figure 2.

Using this set of properties, we do not observe any significant dependence of Ca activity on the PLA fibers' alignment, which suggests that this aspect of the experimental setup is not a factor that would significantly affect the tissue development as assessed based on Ca activity in the tissue.

4. Conclusions

The main aim of that work was to propose a pipeline based on Computer Vision algorithms to determine parameters of Ca waves activity in artificially grown cardiomyocytes. Set of diversified values enabled us to observe its dependency (or clearly speaking - no dependency) of PLA fibers' alignment. In further experiments we would like to introduce Machine Learning methods for evaluation of other possible metrics and their impact on cardiomyocytes.

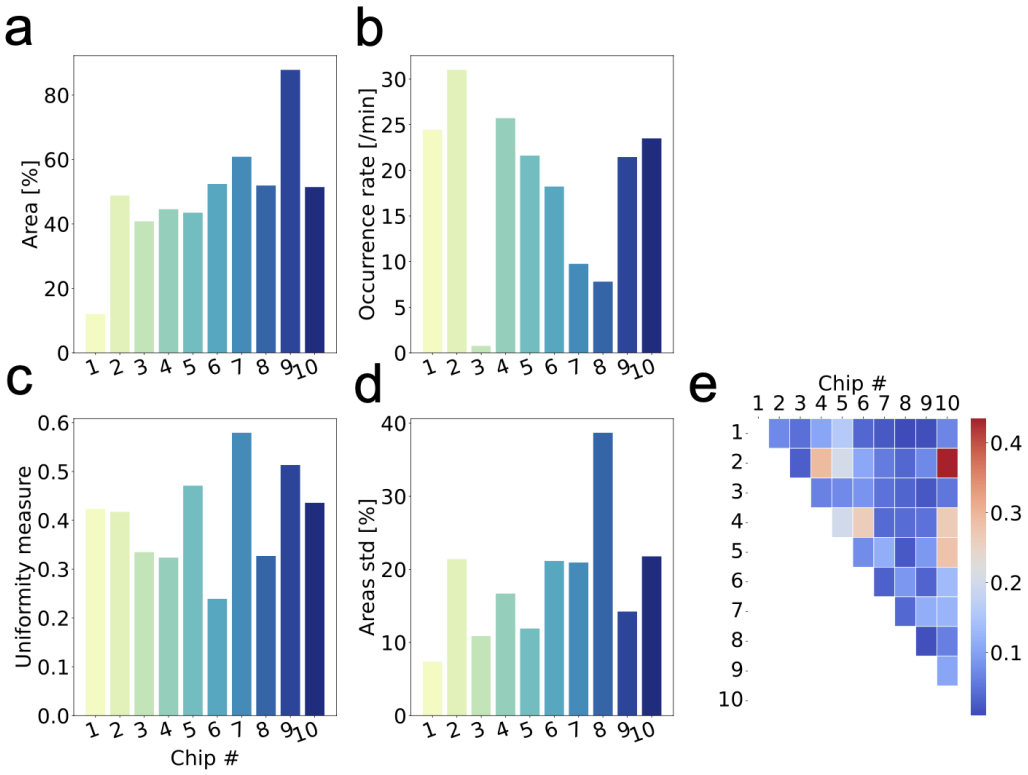

Fig. 2. Tissues' properties summary and comparison. (a) Average area covered by transient Ca2+ activity, expressed as a percentage of the frame total area. (b) Average occurrence rate of large-scale Ca2+ activity, expressed as a number of events per minute. (c) Uniformity of the distribution of directions of Ca2+ activity propagation. (d) Standard deviation of the distribution of peak areas covered by Ca2+ activity. (e) Similarity matrix, with pair-wise comparisons of Ca2+ activity in different tissues (with red color denoting a high degree of similarity).

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