Computer Analysis of Isolated Cardiomyocyte Contraction Process via Advanced Image Processing Techniques

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Abstract

Isolated cardiomyocytes have been used as valid and useful model in experimental cardiology research for decades. The cell contraction function is usually measured via expensive and complex instruments which can either damage the cell or take much time for setting up. In contrary, recent development of optical microscopy and digital cameras suggests utilization of touch-less cardiomyocyte video acquisition in connection with advanced image processing techniques for evaluation of the cell contraction process. The proposed paper presents an automatic membrane detection method via computer processing of acquired video-sequences by utilization of an active contour model. Evaluation of detected cell area is used for estimation of cardiomyocyte contraction function. The method is evaluated utilizing the comparison with contraction measurement performed via atomic force microscopy technique.

1. Introduction

Isolated cardiomyocytes have been used as valid and useful model in experimental cardiology research for decades. A single cardiomyocyte is considered as a functional unit with electrical, signaling, and mechanical functions of cell excitation-contraction process. The contraction function is usually measured via expensive and complex instruments which can either damage the cell or take much time for setting up, e.g. light diffraction techniques [1], photodiode arrays [2], atomic force microscopy (AFM) [3-4], or scanning ion conductance microscopy [5]. In contrary, recent development of optical microscopy and digital cameras suggests utilization of touch-less cardiomyocyte video acquisition in connection with advanced image processing techniques for automatic and precise evaluation of cardiomyocyte contraction process [6-10]. However, many of these optical methods can face problems with changes of cell geometry, proper alignment of the cell, cell rotation and translation during the acquisition process.

As an addition to the current state-of-the-art, the proposed paper presents an automatic membrane detection method via computer processing of video-sequences acquired by bright field optical microscopy. The method utilizes a dynamic active contour model for segmentation of the cell boundary. Evaluation of detected cell area is used for estimation of cardiomyocyte contraction function. In addition, to reflect the most recent trend, the method is applied on processing of images of cardiac EBs (embryoid bodies) [11].

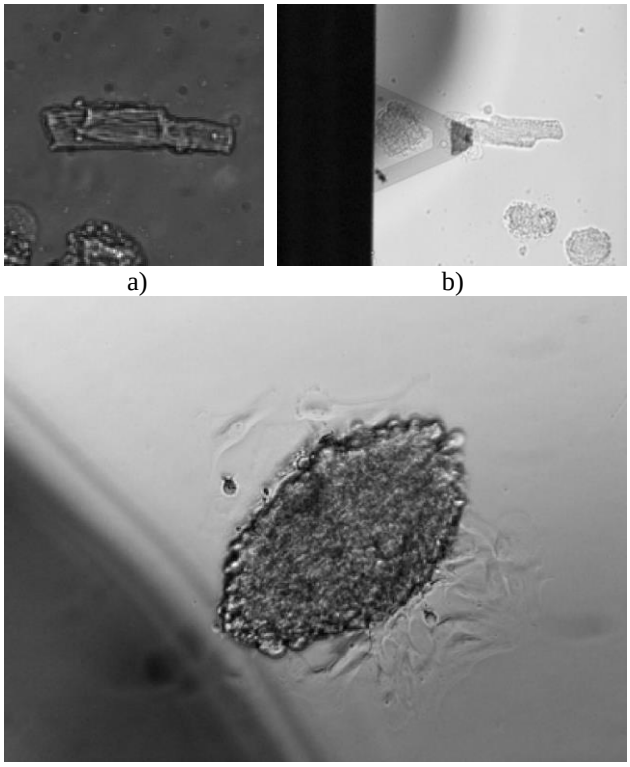
2. Experimental datasets

A typical adult cardiomyocyte is a cylindrically-shaped cell that can be observed as a bright structure in bright-field optical microscopy image (Fig. 1a). A set of eight isolated cardiomyocyte video-sequences was acquired using bright-field optical microscopy equipped with high-speed scientific CMOS camera ANDOR Zyla 5.5 with framerate 50-100 fps and 512×512 pixel resolution (Fig. 1a). The acquisition time of all video-sequences is 5 to 25 s.

To be able to quantitatively evaluate results of the proposed method, a set of three isolated cardiomyocytes video-sequences was acquired via AFM system (Fig. 1b) equipped with OLYMPUS DP73 CCD camera. The AFM cantilever is attached on one side of the cell during the acquisition, as can be seen in Fig. 1b. The dark part of the image represents the area outside the field of view (FOV). The sequences were acquired with 20 fps and 800×600 pixel resolution. The acquisition time is 60 to 120 s.

EBs represent a formation of the three dimensional cardiac cell aggregates that can be observed as a cluster of cells in a microscopy image (Fig. 1c) [11]. A set of two

EBs video-sequences was acquired with the purpose to test the developed method to be used for evaluation of the EBs contractility function. The sequences were acquired by ANDOR Zyla 5.5 camera with 25 fps and 1600×1200 pixel resolution (Fig. 1c). The acquisition time is 11 and 67 s.



a) b)

c)

Figure 1. Examples of one frame from particular experimental datasets of a) isolated cardiomyocytes, b) isolated cardiomyocytes with AFM, and c) cardiac EBs.

3. Method

Active contours (so-called *snakes*) have become a very popular approach for segmentation of individual objects in the image and have been widely used in many image processing applications [12].

The proposed method uses a dynamic active contour model for cell's membrane detection in acquired video-sequences [12-13]. Since the cell to be segmented is usually placed in the center of the microscope's FOV, an initial contour is automatically selected as a rectangle outside the object of interest. The initial contour is then iteratively evolved to enclose the cell in each video frame.

In fact, active contours can be expressed as a process of energy minimization. Formally, final contour is obtained as a minimum of energy functional defined as [13]:

$$E_{snake} = \int_{s=0}^{1} E_{\text{int}}(v(s)) + E_{image}(v(s)) + E_{con}(v(s))ds\,.$$

The functional E_{snake} includes properties that control the way the contour (snake) can stretch and curve. In this equation, the internal energy E_{int} controls the natural behavior of the contour and the arrangement of the contour points (stretching, bending) due to internal contour forces, E_{image} represents the image energy which controls attraction of the contour to low-level image features (such as lines, edges, or corners) due to image forces, and the constraint energy E_{con} allows higher level information to control the contour evolution by user; E_{con} is not used in our method. Individual energies are functions of the set of points which belong to the contour ($v(s)$), i.e. the set of x and y co-ordinates of the contour points. Variable $s \in [0, 1]$ represents the normalized length of the contour. The energy functional is thus expressed in terms of contour's functions. These functions contribute to the contour energy according to values chosen for respective weighting coefficients. Particular values were identified in heuristic manner in respect to desired application. For minimization of the energy functional E_{snake}, a standard greedy algorithm, which works in an iterative manner, was used [14]. The process starts with an initial contour and then evolves the contour in an iterative manner searching the local neighborhood around contour points to select new ones which result in lower snake energy.

For detailed mathematical description regarding rational of particular energies, parameter settings, optimization process, and implementation, please refer to [12-14].

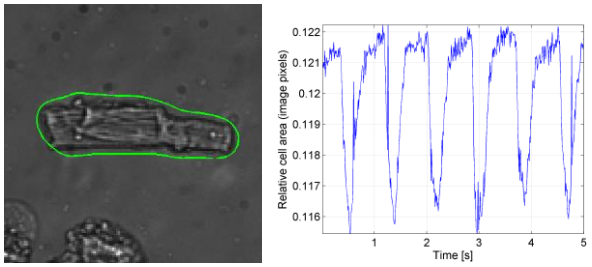
4. Results and discussion

The method was tested on acquired experimental datasets of video-sequences. The cell contour was segmented in each frame of the sequence in order to capture contractile changes of the cell in time. Consequently, a relative pixel area of the segmented cell (normalized to the area of the entire image) was computed and plotted along the time (frames) to get the contraction function.

4.1. Evaluation on images of isolated cardiomyocytes

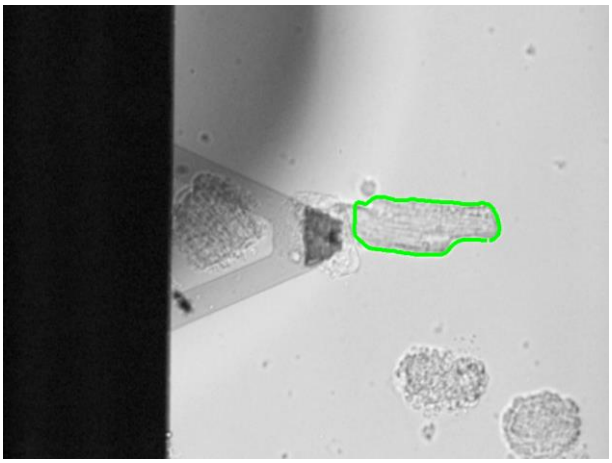
First, a dataset of isolated cardiomyocytes video-sequences was used for testing. Fig. 2 shows one frame in a video-sequence with segmented cell's contour and corresponding contraction function. It can be observed that the function reflects well the contractile changes of the cardiomyocyte. This suggests a possible application of the proposed method for evaluation of the cardiomyocyte beating parameters, e.g. their changes after the drug exposition [15]. In order to evaluate the proposed method quantitatively, a testing on the dataset of isolated cardiomyocytes acquired via the AFM system was

performed. Since the cardiomyocyte is attached to the AFM system, the contour extraction was restricted only to the area outside the cantilever (Fig 3a).

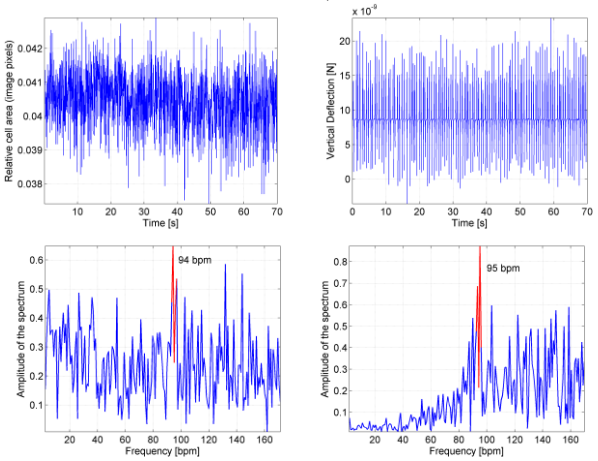


a) b)

Figure 2. a) One frame from the video-sequence of isolated cardiomyocyte and b) extracted contraction function.



a)



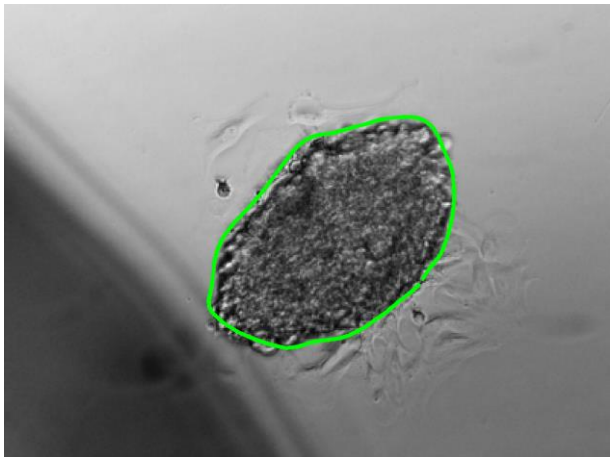
b) c)

Figure 3. a) One frame from the video-sequence of isolated cardiomyocyte, b) extracted video-based contraction function (top) along with corresponding DFT spectrum (bottom), and c) contraction function obtained via the AFM system (top) along with corresponding DFT spectrum (bottom). The red color demarcates the highest spectral line, which represents estimated beating frequency of the cardiomyocyte in bpm (beats per minute).

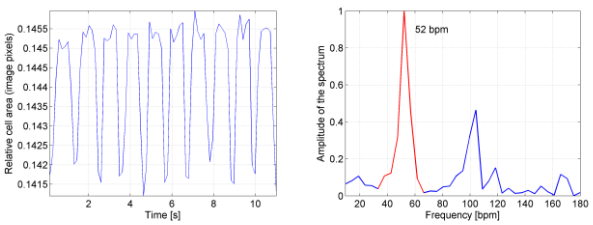
The discrete Fourier transform (DFT) was used for computation of spectra of individual contraction functions in order to estimate the beating frequency (Fig. 3 b-c). The results revealed that the proposed method is able to estimate the beating frequency with rather high precision. However, the small dataset of isolated cardiomyocyte video-sequences acquired simultaneously with AFM measurement limits so far the evaluation. The evaluation is also limited due to the cardiomyocyte attachment which can result in its unexpected behavior during the contraction.

4.2. Evaluation on cardiac EBs

Finally, the proposed method was tested on a dataset of cardiac EBs video-sequences (Fig. 4). It can be seen that the proposed method can satisfactorily capture the contraction function of the EB cluster as well. Thanks to better image quality of the acquired sequences (compared to sequences of isolated cardiomyocytes), the estimated contraction function (Fig. 4b) and corresponding frequency spectrum (Fig. 4c) are considerably less noisy. This allows an assessment of the cell contraction process with much higher accuracy.



a)



b) c)

Figure 4. a) One frame of the EB video-sequence segmented via the proposed method, b) contraction function extracted from the EB video-sequence, and c) corresponding DFT spectrum with demarcated spectral line which corresponds to beating frequency of the whole EB cluster (in bpm – beats per minute).

5. Conclusions

The proposed paper presents the method for segmentation of the cardiac cell contour in experimental video-sequences acquired with bright-field microscopy in order to extract the respective contraction function. The method can be used universally, as it was tested on a dataset of isolated cardiomyocytes as well as a dataset of cardiac EBs. The results signalize that the proposed methodology can be used for evaluation of cardiac cell contraction processes, which can be further useful e.g. in drug screening. The method utilizing an active contour model is also robust against the cell rotation and translation during contraction as well as in case of low-intensity imaging, which allows its utilization for fluorescence applications.

One disadvantage of the method is the need of initial contour definition. We have chosen the straightforward approach when the cell is usually located in the centre of the sequence so the initial contour can be selected as a rectangle around the cell. However, to make the method more robust, some pre-segmentation approaches could be considered for initial contour selection. Another problem raised when applied the method on video-sequences with low framerate. This can result in distorted estimation of the contraction function.

In the future, we intend to test the method on a larger dataset of isolated cardiomyocytes as well as EB sequences. It is also planned to incorporate the extraction of contraction parameters and test the ability of the method to capture changes of these parameters during drug exposition. For extraction of beating parameters, frequency filtration could be considered as a post processing step to eliminate fast non-physiological changes in estimated contraction function.

Acknowledgements

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