Motion Analysis Method for Determining Cardiomyocyte Beating Properties Based on Digital Image Correlation and Templates

Antti Ahola[1§], Paruthi Pradhapan[1§], Eeva Laurila[2], Katriina Aalto-Setälä[2,3], Jari Hyttinen[1]

[1]Computational Biophysics and Imaging Group, Department of Electronics and Communications Engineering, and BioMediTech, Tampere University of Technology, Tampere, Finland
[2]Heart Group, BioMediTech, University of Tampere, Tampere, Finland
[3]Heart Hospital, Tampere University Hospital, Tampere, Finland
[§]Authors with equal contribution

Abstract.

Video-based analysis of cardiomyocytes provides a non-invasive and label-free method of analyzing their beating characteristics. Here, we aim to demonstrate that defining averaged signal templates can improve the determination of cardiomyocyte beating characteristics.

Video recordings of human iPSC derived cardiomyocytes were performed. Beating patterns from different sectors of the cell were calculated from displacement vector fields using our in-house developed digital image correlation based video analysis method. A cross-correlation template based average waveform was computed for individual cell sectors, representing their beating characteristics. We also studied the effect of video sampling frequency and video duration on template formation to optimize the recording process.

By comparing the average waveforms from different sectors, we observed the fusiform nature of iPSC derived cardiomyocytes. Our results indicate that using templates allows minimizing measurement time. However, then the sampling frequency should be at least 60 Hz, for high quality single cell dynamics.

To conclude, the sector approach is beneficial for analysis of iPSC derived cardiomyocytes. Also, the presented methods improve the parameterization of the signal.

1. Introduction

The development of induced pluripotent stem cells (iPSC) has opened new avenues for in-vitro modelling of disease conditions and drug screening. With the advent of these new applications, there is an increased need for novel analysis methods. We have shown before [1] that video-based microscopy measurements provide a feasible, non-invasive and label-free method for measuring beating dynamics of human iPSC derived cardiomyocytes (CMs). As the sarcomeric structure of iPSC derived CMs is not fully organized [2], the displacement of cell is far from the uniform beating of the mature CM and thus traditional methods are not well suited for them.

Analyzing the mechanical functionality of single CMs has traditionally been a laborious and time consuming task. Atomic force microscopy and cellular electric impedance methods can be used to quantify the mechanical properties of the cells, but they are not well suited for long term measurements with high spatial resolution. Video-based methods provide a non-invasive way of analyzing CM beating dynamics. We have previously presented a digital image correlation (DIC) method [1] for the analysis of single CMs.

The aim of this study is to demonstrate the use of template matching in signals obtained by video analysis of CMs. We have shown that cross-correlation based template matching accurately and consistently determines differences in beating properties of CMs [3]. In this study, our aim is to establish inter-sector differences in the beating dynamics by comparing average waveforms from video-based displacement data of various sectors. Further, the optimal sampling frequency parameters and measurement time for video recording are studied. While the method by itself is suitable for long term measurements, in laboratory use CMs cannot be kept out of an incubator for extended periods of time. Therefore it is important to minimize the recording time so that the cell condition remains the same throughout the measurement, while still obtaining as much information on the beating as possible.

In this article, we intend to demonstrate that the combination of our previously reported sector approach of video analysis with template matching algorithm can provide additional information on the beating dynamics of iPSC derived CMs.

2. Materials and methods

2.1. Ethics statement

This study was approved by the ethical committee of Pirkanmaa Hospital District (R07080).

2.2. Cell culture

All iPSC lines used in this study were prepared as described by Lahti et al. and Takahashi et al. [4, 5]. The CM differentiation and dissociation were performed by using the previously described protocols [1, 6]. Briefly, the iPS cells were co-cultured with END-2 cells to induce cardiac differentiation, and after a minimum of 14 days, the spontaneously beating colonies were mechanically excised and treated with collagenase A (Roche Diagnostics, Mannheim, Germany). The dissociated CMs were plated and on 2 cm^2 wells on standard cell culture multi-well plates and cultured for 2-14 days for video recording.

2.3. Video recording

CM beating was recorded using a Nikon (Nikon, Tokyo, Japan) Eclipse TS100 inverted microscope with an attached heating plate, and an IMPERX (Boca Raton, FL, USA) B1620 camera. A single, dissociated beating cell was visually selected and recorded at 60 frames per second (fps) or 120 fps. In total, six videos were recorded and further analyzed.

2.4. Digital image correlation analysis

The recorded videos were processed applying DIC to provide beating signals, as described by Ahola et al. [1]. Velocity vector fields were obtained by calculating the displacement between each subsequent video frame. The cell area was divided to 8 sectors, with the center being the visually approximated beating focus point. In contrast to the previously published method [1], the radial and tangential components for each velocity vector were calculated based on their location with respect to the beating focus point. The cell area was segmented semi-automatically, using segmentation based on thresholding and manual selection, to reduce analysis of areas with little to no movement.

The sum of the radial components of each sector was calculated to provide a signal representing the radial displacement in that area of the CM. Tangential displacement was calculated similarly. This process generates in total 16 beating signals from each cell. Each signal was then filtered using a 20-point Gaussian window. For the analysis in this paper, we chose the signals with highest quality. An example of such signal is illustrated in Figure 1a.

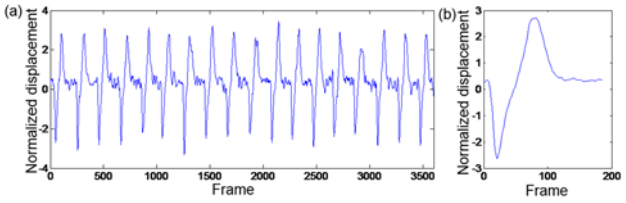

Figure 1: Cardiomyocyte beating signals. a) A beating signal as a function of frames. The magnitude of the beating illustrates the normalized displacement of the sector. The downward peak represents the contraction and the upward peak the relaxation. b) An average waveform template calculated from the signal in a). The template starts from the onset of the contraction. The template baseline after relaxation shows the mean interval between beats.

2.5. Template setup

A template based cross-correlation analysis was implemented to identify the beats that closely resembled the beating pattern in individual sectors. This method has been presented earlier for determining field potential durations from electrical signals of CMs plated on microelectrode arrays in a software that can be found online (http://www.biomeditech.fi/CardioMDA/)[3]. In this study, we applied the template matching method to compute an average waveform for individual sectors of the CM using video recordings. The template was initialized using the beat complex occurring at the half-way point of the total recording. Cross-correlation is then performed for the vector field signal against the selected template. The correlation coefficient measures the degree of similarity between the template and each beat complex identified from the vector field. It is computed as the sum of the products of corresponding pair of points from the two complexes within a specific time window. The cut-off for correlation coefficient was set such that all beat complexes whose resemblance to the template is greater than or equal to 80% were selected for averaging. This average waveform represents the general beat morphology of all the beats in the vector field. Figure 1b shows an example of an averaged waveform, calculated from the signal in Figure 1a.

The average waveforms from different sectors were then compared to determine differences in beating patterns between individual sectors.

2.6. Analysis parameter optimization

A test set for analyzing the effect of sampling frequency on the optimal parameters for template analysis was created. Four 120 fps videos were decimated to 60 fps and 40 fps. The videos were then analyzed using DIC

analysis to create beating signals. From these signals, average waveform templates were calculated for the signals with low noise characteristics. Templates from 120 fps videos were downsampled to match those from low fps videos in order to calculate correlation between the averaged waveforms from original and decimated videos. The templates were shifted temporally so that the contraction happens in both templates at the same time in order to make comparisons more valid.

Additionally, we calculated templates for the 120 fps videos by reducing the number of beats, one beat at a time from the end of the signal. This allowed us to study the effect of decreasing video recording time.

3. Results

3.1. Template matching

The beating signal templates from different sectors of the same cell were compared with each other. The templates were synchronized with the contraction peak.

Figure 2 shows the templates calculated from two cells, recorded with 60 fps, displaying the differences in beating dynamics across the cell. Figure 2a illustrates a case in which contraction and relaxation occur similarly in measured sectors. In Figure 2b, relaxation occurs at a different time in all three sectors. This further illustrates the fusiform nature of the cell.

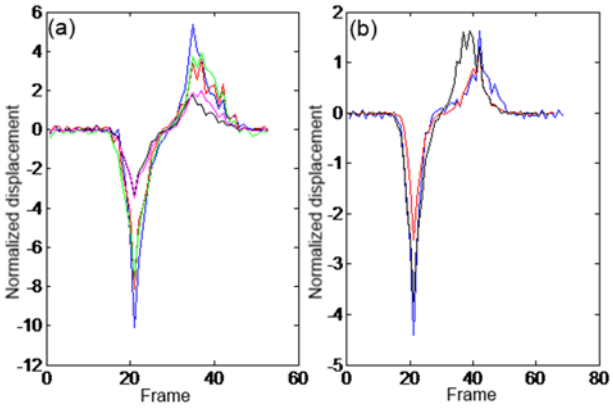

Figure 2: Templates calculated from different sectors of two cells. a) The beating characteristics of the cell are similar in all measured sectors – both contraction and relaxation last a similar time in all sectors. b) The beating characteristics of the cell differ from each other. The relaxation of the cell begins first in the sector marked with black, continues to sector marked with red and finally reaches the sector marked with blue.

3.2. Effect of frame rate

The effect of video frame rate on the quality of the template formation was studied using decimated videos. The correlations between the original template and the template calculated from decimated videos were calculated, and the results are shown in Table 1. Downsampling to 40 fps reduced the quality of the template drastically for two of the cells beating at higher frequencies, rendering the templates unusable.

Table 1. Correlations of the templates calculated from fps decimated videos with the templates from 120 fps videos. Forming a template for 40 fps decimated videos was not possible for cells 2 and 3, due to their higher beating rate.

Cell	bpm	Sector	60 fps	40 fps
1	10	1	0.972	0.939
		2	0.979	0.950
		3	0.986	0.958
2	19.5	1	0.968	N/A
		2	0.960	N/A
		3	0.971	N/A
3	12.5	1	0.970	N/A
		2	0.951	N/A
		3	0.960	N/A
4	9	1	0.984	0.962
		2	0.965	0.935
		3	0.975	0.953

3.3. Effect of the number of beats in template

The effect of the number of beats on the template formation was studied by repeatedly removing the last beat from the calculated video signal. The degradation of the template can be seen in Figure 3.

The beating was recorded at 120 fps for 120 seconds. During this time, the cell beat 20 times. The calculated templates show marginal reduction in quality at 50%, and a significant reduction at 30% of the number of beats. To obtain a functional template, at least 12 beats should be included in the signal.

4. Discussion

We demonstrated the use of averaged waveforms as a basis of determining CM beating behaviour. While the signals obtained using the method previously presented by Ahola et al [1] present well the beating of the cell, a more concise form of presentation is necessary for recognizing beating characteristics with one glance. In this study, we presented that applying the template matching method makes it easier to compare the results obtained both from different cells, and from different areas of the same cell.

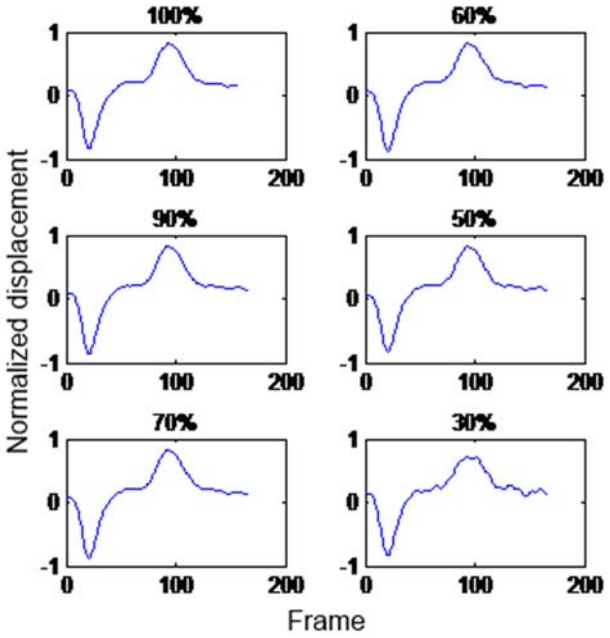

Figure 3: Templates calculated with reduced beat count. The quality of the template remains similar until 60%, after which is starts degrading.

Using the template based video analysis can further help understand the mechanobiological functions of the CMs. In the case of iPS cell derived CMs, the beating behaviour is not as uniform as it is with native, adult CMs. The sectorial approach along with templates will help understand the fusiform nature of the beating.

Our results on the optimization of the analysis parameters provide insight into setting guidelines for the recording process of the video analysis. The results on the frame rate indicate that 60 fps provides a reasonable sampling rate, while the results with 40 fps are often unusable with templates. We suggest that with this method, the sampling rate should be prioritized. At least 60 fps should be used. The sampling rate should be higher with cells beating at high frequencies. Further, the signal should contain at least 12 beats to provide templates that represent best the whole beating signal.

While the test set provided results in the range of 9-19.5 bpm, a larger test set with would be required to draw conclusions about recording protocols for cells beating with higher frequencies.

For this study, the cells were selected to have diverse backgrounds. The cells used in the study were from different individuals and as a result, different cell lines. Additionally, the cell age was different when measured from both differentiation and dissociation. Because of this, our cell material has a more comprehensive interpretation on iPSC derived CMs. As the results were consistent, we ascertain that our method does not depend on the cell culture technique.

Our motion analysis method, therefore, has potential to provide valuable information for different disease models and drug development studies.

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Address for correspondence.

Antti Ahola
Department of Electronics and Communications Engineering
Tampere University of Technology
Finn-Medi 1 L 4, Biokatu 6
FI-33520 Tampere, Finland
antti.l.ahola@tut.fi