Journal of Biomedical Optics

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Abstract. An optical lensless configuration for a remote noncontact measuring of mechanical contractions of a vast number of cardiac myocytes is proposed. All the myocytes were taken from rats, and the measurements were done in an *in vitro* mode. The optical method is based on temporal analysis of secondary reflected speckle patterns generated in lensless microscope configuration. The processing involves analyzing the movement and the change in the statistics of the secondary speckle patterns that are created on top of the cell culture when it is illuminated by a spot of laser beam. The main advantage of the proposed system is the ability to measure many cells simultaneously (~1000 cells) and to extract the statistical data of their movement at once. The presented experimental results also include investigation of the effect of isoproteranol on cell contraction process. [DOI: 10.1117/1.JBO.18.10.101310]

Keywords: myocyte cells; speckle; lensless imaging.

Paper 130233SSR received Apr. 11, 2013; revised manuscript received May 31, 2013; accepted for publication Jun. 12, 2013; published online Jul. 15, 2013.

1 Introduction

Cardiovascular diseases were ranked as America's No. 1 killer, claiming the lives of >41.4% of more than 2.4 million Americans who die each year.[1] In addition, 61.8 million Americans have some form of cardiovascular disease. This includes diseases of the heart, stroke, high blood pressure, congestive heart failure, congenital heart defects, hardening of the arteries, and other diseases of the circulatory system. Early detection of abnormality is thus the key in treating cardiovascular disease early and reducing the enormous death toll. The diagnosis of myocardial ischemia is often difficult to establish in its early stages when treatment is most effective.

There are many methods for trying to diagnose cardiac ischemia as early as possible. Some of those methods include measuring the electrical signals of the heart, and some use ultrasound waves in order to measure the heart contractions.[2–7] Although plenty of work has been done on researching the heart as a complete organ, in this paper we aim to measure and understand the contraction behavior of cardiac myocytes in their most simple form—in a culture.

The clinical motivation for this research could be divided into two. (1) As described in the text below, our work proposes a simple and robust way for measuring and testing the influence (or the lack of it) of certain substances on contractions (rate and magnitude) of cardiac myocyte cells. (2) This work could be used as ground base study for enabling the capability of precisely (within nanometer accuracy) measuring the propagation of the electronic stimulation waves within the culture. Such capability could be very useful in order to measure and "capture" the phenomena called re-entrant arrhythmias—when the electrical signal travels in a circle instead of propagating in a way more resembling "from point A to point B." Such capability could be translated to an investigation of the whole heart since these re-entry arrhythmias could also be seen on the pericardium of the heart. One possibility for such an *in vivo* investigation could be done by using a miniature special endoscope, as described in Ref. 8, that could be inserted into the body and that allows sensing the movements of the tissue positioned close to its tip.

It is also important to clearly state the two biological assumptions of this study. (1) When cardiac cells are electrically stimulated by a periodic signal (within the cells' sensitivity range), after a few minutes, cells' contraction rate aligns with the rate of the stimulus. There will be no spontaneous contractions in the culture. Spontaneous contraction, which is not the topic of this paper, was thoroughly investigated in Ref. 9, where the measurements were done via the secondary speckle patterns technique and they were compared with high-precision reference measurement instrumentation. (2) Statistical data of the cell culture (in a sense of measuring many cells at once) could be an indicator for some vital physiological conditions (specifically as those mentioned above, when the propagating wave measurement is performed).

As is well known, the signal propagating in the heart (and thus also within myocyte cultures) is doing this in a coupled manner: electrically and mechanically.[10–14] Meaning, if one

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0091-3286/2013/$25.00 © 2013 SPIE

wants to obtain the contractions of the myocytes, it could be done by measuring the electrical signal or the mechanical one.

In this paper we propose a very simple configuration to measure the statistics of cell contractions by measuring their physical contraction-expansion process. The measurement utilizes an optical method, as explained in Sec. 2, and three different features are being experimentally demonstrated: spatial distribution, influence of addition of ingredients, and simultaneous cell contractions. The experimental setup and results are summarized in Sec. 3, while the paper is concluded in Sec. 4.

The experimental setup used in this research consists of electrical shock stimulator, inverted microscope, cell culture, laser at 532 nm, and a CMOS camera. Reference data were recorded from cultures of cells without applying any electrical stimuli. A differentiation is made between synchronized cells contraction, when stimulus is applied, versus nonsynchronized cells contraction, when stimulus is not applied. All the data (videos of 5 s, recorded with 30 frames per second sampling rate) were recorded and then processed with unique Matlab algorithm.

2 Theoretical Explanation

The method used in this paper, for measuring the mechanical properties of the cells, is based on analyzing secondary back-reflected speckle patterns. Coherent light reflected from a rough surface would create speckle patterns on the screen where it is being detected. Those speckle patterns are actually randomly distributed regions of destructive and constructive interference of the beam reflected from different regions on the rough surface. A static surface would cause a static speckle pattern, while a dynamic surface would result in a dynamic time-varying interference pattern. By doing a precise reverse-engineering process, one can obtain the movements in time of the surface from the temporal movements of the speckle patterns.

The resolution or the size of the speckle pattern that could be obtained on a plane located at a distance Z from the back-reflected surface and imaged to the sensor plane is given by

$$\delta x = \frac{\lambda Z}{D} \cdot M, \tag{1}$$

where λ is the optical wavelength, D is the spot size falling on the surface, and M is the imaging system's magnification. In this study we used the following values; $\lambda = 532$ nm; $Z \approx 3$ to 4 cm (depending on the experiment); $D \approx 1.2$ mm; $M = 10/20$ (depending on the experiment).

The conversion between the tilting angle α of the reflecting surface to the displacement d of the speckle pattern on the camera could be calculated by the following equation:

$$d = Z \cdot \alpha \cdot M. \tag{2}$$

This relation is valid when the imaging lens is highly defocused or, as in our case, when the sensing configuration is lensless.

It is important to note that the contraction amplitude figured in this paper is not the cell movements, as expected, but rather the reflection of such movement on the movement of the generated speckle pattern. In order to convert it to a geometrical cells movement and displacement, one must perform a fine calibration of the test system. The method and its mathematical background are widely explained in Refs. 15 and 16.

3 Materials and Methods

The electrical signal was generated with a signal generator, model type: Grass SD9 stimulator (Natus Neurology Incorporated, USA). The signal properties were as follows: frequency of 1 Hz; pulse width of 10 ms; amplitude of 50 V. The laser used in the experiment was Photop DPGL-2050F (Photop, China), with a typical wavelength of 532 nm and optical output power of 50 mW.

The cells were grown and prepared in the following manner: Sprague-Dawley rat hearts (2 to 3 days old) were removed under sterile conditions and washed three times in phosphate-buffered saline (PBS) to remove excess blood cells. The hearts were minced and then gently agitated in RDB (Biological Institute, Ness-Ziona, Israel), a solution of proteolytic enzymes prepared from fig-tree extract. RDB was diluted 1:100 in Ca^{2+}- and Mg^{2+}-free PBS at 25°C and incubated with the heart fragments for several cycles of 10 min each as previously described in Refs. 17, 18, and 19. Dulbecco's modified Eagle's medium, supplemented with 10% inactivated horse serum (Biological Industries, Kibbutz Beit Haemek, Israel) and 0.5% chick embryo extract, was added to the supernatant containing a suspension of dissociated cells. The mixture was centrifuged at $300g$ for 5 min. The supernatant was discarded and the cells were resuspended. The cell suspension was diluted to 10^6 cells/mL, and 1.5 mL suspension was placed in 35-mm plastic culture dishes coated with collagen/gelatin. The cultures were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. A confluent monolayer exhibiting spontaneous contractions developed within 2 days. The experiments were performed on 4- to 6-day-old cardiomyocyte cultures kept in PBS at room temperature.

For test A, the following imaging equipment and parameters were used: PL-B741 PixeLink camera (PixeLink, Canada) with pixel size of 6.7×6.7 μm. A window of 400×400 pixels was chosen, and the microscope magnification (Olympus, USA) was set to 10×. The sampling frame rate was set to 130 frames per second. The screen was divided into 10×10 equal squares, and a threshold of 20% was chosen (meaning that only squares that are at the top 20% of the sensed movement magnitude in each frame are counted; the rest are forced to be zero). In order to graphically illustrate it without any noise, all the squares that have been cut off were forced to be zero (blue square on the color map scale).

For tests B and C, the following imaging equipment and parameters were used: the imaging optics, when used only for reference purposes, was an inverted Olympus microscope (Olympus) with a magnification of 20×. The camera was PixeLink PL-E531 (PixeLink) with a CMOS detector, with 5.2×5.2 μm pixel size. A Matlab script was used for analyzing the recorded data. The analysis involved performing correlation between sequential frames and extracting the relative movement of the speckle patterns generated between those frames. The experimental setup can be seen in Fig. 1.

All three experiments (tests A, B, and C) were repeated ~10 times.

4 Experimental Results

Several different features have been measured during the experiments. All of them have a common data flow as depicted in the block diagram presented in Fig. 2.

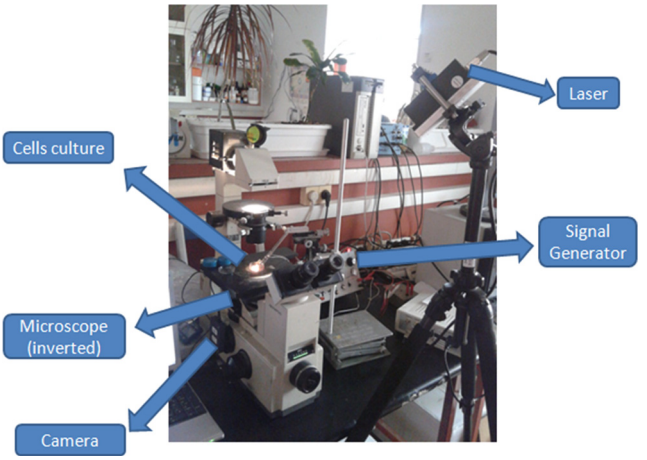

Fig. 1 Experimental test setup.

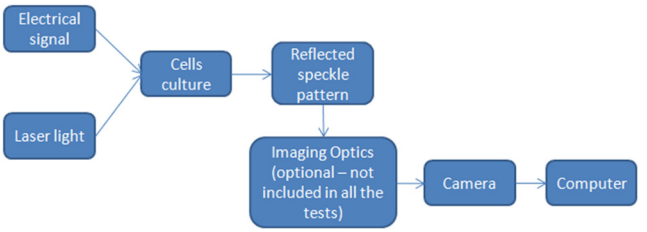

Fig. 2 Experimental data flow.

4.1 *Test A—Spatial Distribution of Cells within the Culture*

In test A we show how one can get a rough estimation of the spatial distribution of the cells within the culture. The pictures of Figs. 3 and 4 were calculated after analyzing the recorded clips via Matlab.

Figure 3 shows the total movement (sum of movement in all squares) as a function of frame number. It can be seen that the "distance" between every peak frame is exactly 130 frames, which equals 1 s, thus matching the 1-Hz stimulus rate.

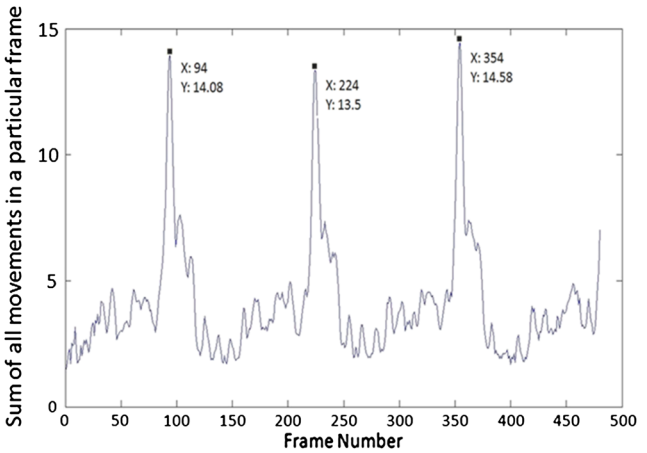

Fig. 3 Total movement (sum of movement in all squares) as a function of frame number.

Also, one can see that for frames (94, 224, and 354) representing an occurrence of electric pulse, the amplitude (Y value) is much higher than that for the in-between frames. The movement was extracted by computing the shift in the correlation peak computed after correlating adjacent frames with the time-varying speckle patterns.

Figure 4(a) through 4(c) shows the two-dimensional plots of three peak frames: fr94, fr224, and fr354. One can argue that approximately the same area, i.e., the same squares, are responsible for the top 20% movements. Assuming that on average one square represents the movement of one myocyte cell, one can conclude that a large cluster of myocyte cells were located in the colored area (mainly in the two to three top rows of the imaged culture).

Figure 4 presents a matrix of 100 squares (10×10). We sorted all the values from lowest to highest, and chose to show only the top 20%, forcing the other 80% to be zero (the blue squares around). The colors represent the values of those remaining 20% after doing the sorting. The axes units do not have any physical property; they are simply a representation of the spatial distribution of the cells (movements) in the frame (culture). The values of the colors are also meaningless in a sense that they are not equal to the exact physical movement of the cells, but rather related to the shift of the correlation peak after doing correlation between two adjacent frames (calculated separately for each square, meaning that our window of measured data was divided into matrix of $N \times N$ squares, trying to create a situation where one square represents one cardiac myocyte cell, or as few cells as possible).

4.2 *Test B—Influence of Isoproteranol on Cell Contraction*

In test B we show the influence of adding isoproteranol to the contracting cells. The Y value represents the mean value of the movements in all the squares. It could be seen that according to Fig. 5(a), in cells with isoproteranol, the ratio of average maxima to average minima is approximately 6. That ratio, as could be calculated from Fig. 5(b), showing cells without isoproteranol is also 6. While isoproteranol has an impact on cell spontaneous contractions' rate and amplitude, we have demonstrated that when the culture is being electrically stimulated (i.e., all spontaneous contractions are eliminated) the isoproteranol is no longer affecting the contractions' parameters. We have demonstrated that one could use this method for evaluating the effect of different new drugs added to the culture (isoproteranol in our case). In this work we showed that isoproteranol does not influence either the rate or the magnitude of contractions. This could be expected since the culture was electrically stimulated.

4.3 *Test C—Measuring Simultaneous Cell Contractions without Imaging Lenses*

In test C we demonstrate the ability to measure multiple cell contractions without the use of any imaging elements such as microscopes and/or lenses. The same setup as in test B was constructed, only the reflected speckles from the culture were obtained directly by the camera, without the microscope in the optical pathway. Figure 6 shows an example of the measured results. Again, one can clearly see the 30 frames gap between two adjacent peaks, indicating the 1-Hz stimulus and a 30-frames-per-second sampling rate. The novelty of the presented result is that a simple and lensless configuration can be used for

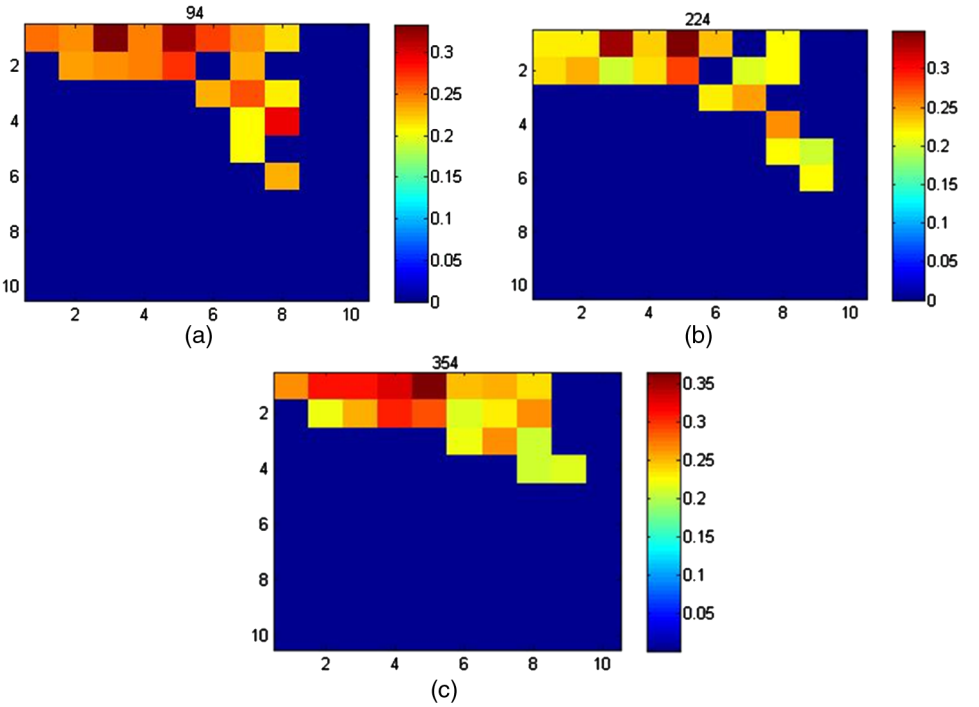

Fig. 4 Two-dimensional plots of three peak frames: fr94 (a), fr224 (b), and fr354 (c).

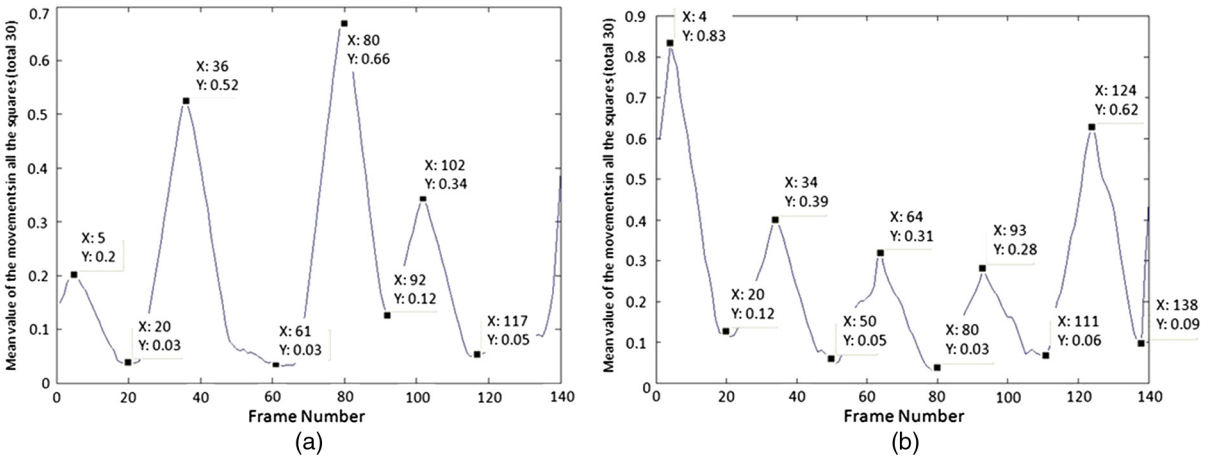

Fig. 5 (a) Mean contraction with isoproteranol. (b) Mean contraction without isoproteranol.

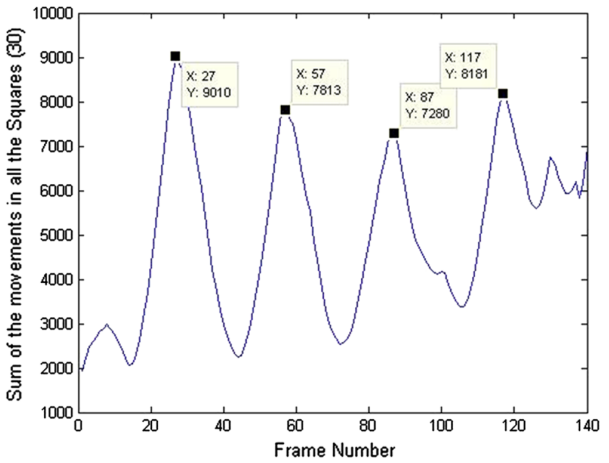

Fig. 6 Example of measuring simultaneous contractions without an imaging element.

simultaneously obtaining statistically averaged information about the contraction of large number of cells (all the cells that are illuminated by the laser beam, which in our case was ~1000 cells).

5 Conclusions

In this paper we have presented a simple approach for measuring the contractions of cardiac myocytes in a culture. The main advantage of the proposed system is that it provides an "instant snapshot" of the culture, thus allowing the measurement of many cells (~1000 in the above-mentioned experiments) instantaneously while obtaining the statistics regarding their contraction process at once.

We have shown that good results could be achieved without the use of any imaging lens and thus have proposed a very simple and low-price configuration to extract the statistics of cells'

contraction. Also it is possible to get a rough estimation of the spatial distribution of the cells within the inspected culture.

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