AUTOMATIC CHARACTERIZATION OF IN VITRO CARDIOMYOCYTE MOTION

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ABSTRACT

Optical methods can be applied to estimate biologically relevant measurements of a cardiomyocyte population. The proposed method holds the promise of monitoring the differentiation of cardiomyocyte populations. More importantly this approach could potentially be applied to quantify the reaction of a cardiomyocyte population to non-cardio-vascular pharmaceuticals in a high-throughput fashion.

I. INTRODUCTION

The ability of monitoring cardiac action potentials by using simple and efficient methods plays an important role in drug toxicity screening. The cardiac action potential has some unique properties that are necessary for the function of the electrical conduction system of the heart. A number of non-cardio-vascular pharmaceuticals have been removed from the market in the United States and elsewhere because of their propensity to prolong th QT interval [1]. Although a direct link between QT interval prolongation and arrhythmogenesis is still unclear, QT prolongation is now the subject of increased regulatory review and is viewed a risk factor to human health. As a consequence of related regulatory guidelines early identification of QT prolongation has become and important issue for drug development.

Cell assays hold the promise of studying drug-induced QT interval prolongation during preclinical assessment. Cell assays are commonly used for high-throughput screening in the pharmaceutical industry. In contrast to expensive in-vivo studies in animals or even humans they offer a cost-effective way to study the effects of drugs. In certain conditions cardiomyocytes self-organize into contractile cardiac tissue, which is similar to cardiac muscle in terms of cell-to-cell connectivity and contractility. The resulting tissue constructs can be used to study the effects of gene therapy or drug constructs. Zhang *et al.* [2] evaluated the electrophysiological properties of cardiomyocytes derived from embryonic stem cells and embryonic carcinoma cells and determined the extent to which they might show arrhythmic proclivity. Now electrophysiological assays are available to study the drug response to cardiomyocytes. Cellular Dynamics International Inc. started to offer a Repolarization Assay, which allows drug candidates to be tested for cardiac toxicity using cardiomyocytes derived from human embryonic stem cells. The company Multi Channel Systems GmbH offers a similar system. Hescheler and colleagues [3], [4], were able to show that simultaneous recordings of action potentials (with intracellular electrodes) and field potentials (with extracellular electrodes) have a correlation between the rise time of the cardiac action potential (AP) and field potential (FP) as well as between AP and FP duration. The correlation between the waveform components and the ion channel activities was shown by using ion channel blockers or by depleting the medium of the respective ions. This approach, however, is difficult and only offers low throughput. Here we propose an optical method to study the motion of cardiomyocytes in assays that can be used in a high-throughput microscope.

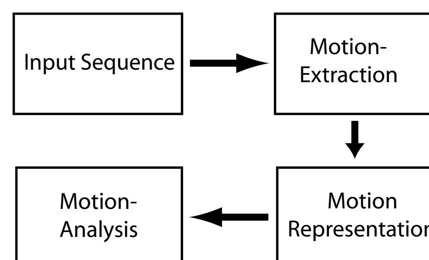

Fig. 1. **Approach.** This figure give a broad overview of the approach. Given an input video sequence, we extract the motion field from every frame to capture the change in apparent motion. The sequence of motion fields is then projected onto a low dimensional space. The resulting time-series data can be analyzed using existing methods.

The focus of this work is to analyze the apparent motion of a population (see Figure 4) of cardiomyocytes using video microscopy. As opposed to analyzing single cells we aim to estimate the motion of entire population. Consecutive frames of the video sequences are used to estimate the motion field. As a result of this processing step the motion information is captured in form of a sequence of vector fields

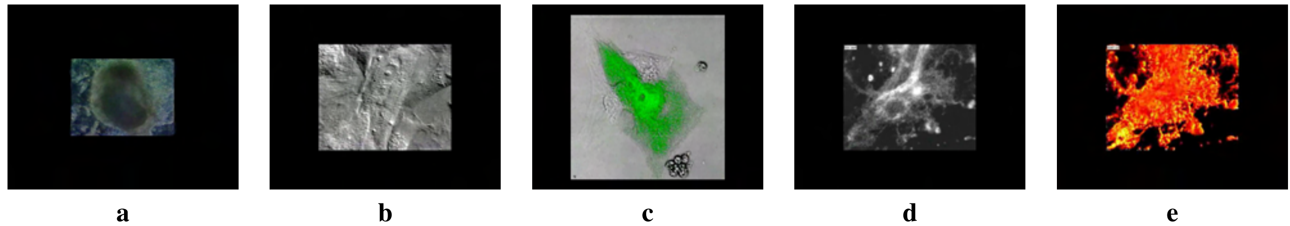

Fig. 2. **Appearance Variation.** *Shown are various examples of assays that make use of cardiomyocytes. Images b and c are acquired using phase contrast microscopy. A GFP label is used in c. Voltage sensitive dyes are used in examples d and c. Notice an in all those examples motion information would be useful to provide additional information. (Images are courtesy of C. Mummary, Hubrecht Laboratory, Netherlands Institute of Developmental Biology)*

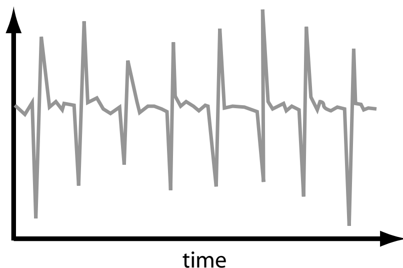

Fig. 3. **One Dimensional Motion Signal.** The goal of our approach is to extract a one dimensional signal which correlates to the actual electrical activity of the cardiac tissue. Notice the regular spacing of the peaks. See text for a more detailed discussion of this result.

(see Figure 4). The resulting high dimensional signal is then decomposed into different components. Our results demonstrate the promise of this method to extract one-dimensional signals that could potentially be used for estimating action potentials.

II. APPROACH

The frequency of the apparent motion of the cardiomyocytes is an indication of their biological function. Hence, any divergence from the normal beating frequency is biologically relevant, and needs to be detected as such. We estimate the apparent motion and use it to characterize the electrical activity of the cardiac tissue. A high-level overview of the algorithm is given in Figure 1. A motion extraction step, described in section IV is applied to capture the apparent motion of the cardiomyocyte population. As a result of this processing step motion is captured in form of a sequence of vector fields (see Figure 4). Similar to electrocardiography we then extract a one dimensional signal (see section V) which allows to characterizes the electrical potentials of the tissue. Advanced statistical methods can them be applied to analyze short and long term motion parameters.

III. PROCESSING REQUIREMENTS

The beating frequency of cardiomyocytes [5] can vary between 30 and 100 beats per minute. Hence, cardiomyocytes should be imaged at least 5 Hz so that the motion parameters can be accurately estimated. The MetaVue (www.moleculardevices.com) imaging system was used in a preliminary experiment to capture an image sequence of multiple minutes. These results indicate that this experimental setup allows to capture time-lapse data with a constant frame-rate. The frame-rate itself depends on the size of the image region that is being captured. Using a standard PC, areas up to 400x500 pixels can be imaged with a frame-rate of greater than 5Hz. Because the frame-rate is variable it is necessary to save the acquired image sequence in the Stack file format.

IV. MOTION FIELD ESTIMATION

Producing stem cell derived cardiomyocytes is still a challenge. The structure, cell density and size of the cardiomyocytes can vary dramatically. See figure 2 for examples. In addition, dyes such as voltage sensitive dyes can change the appearance of the cell populations. Given the complex structure of the imagery basic gradient based approaches such as optical flow [6] cannot be applied. Instead a modified version of a deformable registration algorithm [7], [8] is applied. Deformable registration methods are mainly developed in the context of medical imaging in order to bring images of biological strictures, as for example human neuroanatomies, in alignment. These methods specifically address the challenges that relate to comparing complex anatomical pattern. Only small parameter adjustments are necessary to extract the motion information from different tissue samples.

One could argue that for this application temporal continuity could be used when estimating the deformation fields as time series data is available. However, since we need to be able to detect sudden changes in contractile activity, we avoid using temporal information and aim to compute the most accurate deformation field possible. Given a sequence

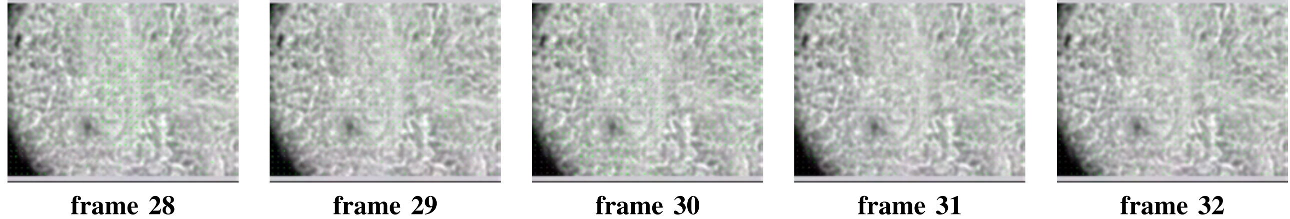

Fig. 4. **Cardiomyocyte Motion.** Shown are five consecutive frames of a video sequence. The estimated motion fields M_t are superimposed. It can be seen that the motion field changes very suddenly. An accurate estimate of the motion fields is necessary to detect subtle changes in motion patterns.

of images $\{I_t\}_t$ all motion fields M_t are calculated on the basis of two consecutive frames I_t and I_{t-1}.

The demons registration algorithm in the ITK toolkit [9] is used to compute the sequence of motion fields M_t. In this approach, each image is viewed as a set of iso-intensity contours, and a rectangular grid of forces corresponding to pixel centers is used to deform one of the images such that it matches the other. The orientation and magnitude of the displacement at each pixel are estimated based on optical flow as follows

$$\vec{d} = \frac{(I_t - I_{t-1})\nabla\vec{I_{t-1}}}{\parallel \nabla\vec{I_{t-1}}) \parallel^2 + (I_t - I_{t-1})^2}. \quad (1)$$

Here the second term in the denominator is used to handle the instabilities when $\parallel \nabla\vec{I_{t-1}}) \parallel^2$ gets too small [8]. The robust and stable implementation of the demons registration method allows us to produce very accurate deformation fields as shown in 4.

V. SIGNAL EXTRACTION

As opposed to monitoring individual cells our objective is to monitor contractile tissue derived from cardiomyocytes. Goal of the video analysis is the estimation of the underlying signal. Similar to an electrocardiogram we aim to extract a one-dimensional signal. There is reason to believe that this signal will have a high correlation with the electrical activity of the cardiac tissue. In order to extract this one-dimensional signal we view the sequence of deformation fields $\{M_t\}$ as a dynamic texture which is controlled by an input signal. The goal of this analysis is to estimate this signal. In consequence we are not interested in analyzing the motion pattern at every pixel location in the image. Instead, the motion pattern in a given image region is being analyzed. This concept is closely related to the idea of *dynamic textures*. A number of computer vision researchers have proposed low dimensional representations [10], [11]. The motion fields M_t capture the apparent motion and represent it in a very high dimensional space. Projecting the individual motion fields onto its principal components [12] is one way to achieve this. N motion fields M_t are being used to form a data matrix X. By taking the singular value decomposition of the data matrix we obtain

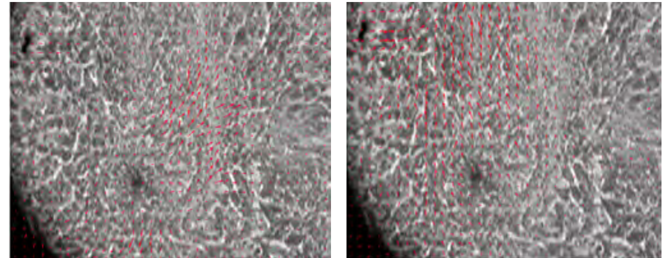

Fig. 5. **Principal Components of the Motion Fields.** *Shown are the first two principal components of the motion fields M_t. These two principal have comparable eigenvalues. They seem to capture different directions of the motion.*

$$X = U\Lambda V^T \ , \quad (2)$$

where Λ is a diagonal matrix of non-negative entries λ_i. The columns XV are the principal components of X. We are interested in the first columns of XV since they are the linear projection of X onto the dimensions of th largest variance. The i-th column of the matrix XV will be denoted by Y_i. We are not in the position of computing one-dimensional time series by projecting the data onto the different principle components, i.e.

$$\omega_i(t) = Y_i M_t \ . \quad (3)$$

For completeness we also compute a second and third time series, ω_2 and ω_3 by projecting the motion data M_t onto the second and third principal component. The resulting graphs are shown in figure 6. Traditional linear systems theory [13] and the underlying theory of detecting a abrupt changes in time series [14] is then applied to detect abrupt changes in frequency of the apparent motion.

VI. EXPERIMENTS

The algorithm was tested on two different data sets. In all cases the video data was captured such that it satisfies the

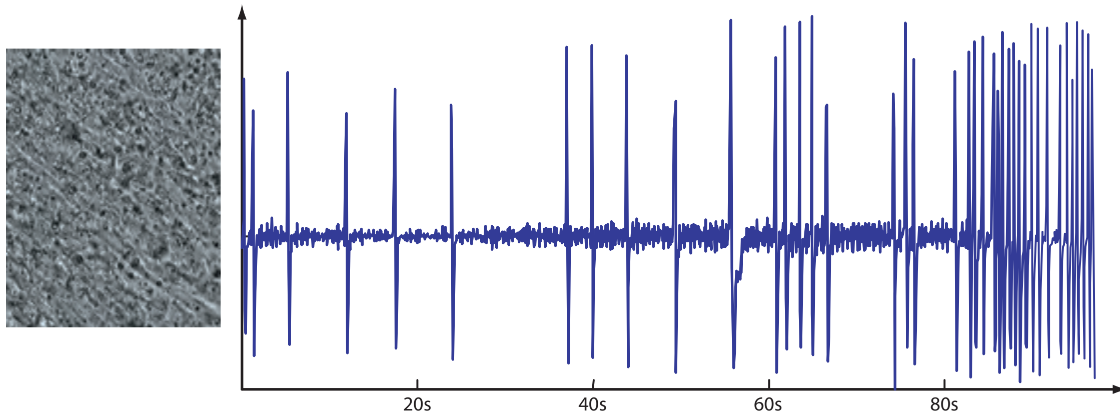

Fig. 7. **Cardiomyocyte Differentiation Signal.** Left: Example frame from human cardiomyocytes data. Right: The result of the analysis shows that the periodicity of the motion pattern is difficult to estimate as only a small sub-population of cells shows signs of contractile motion.

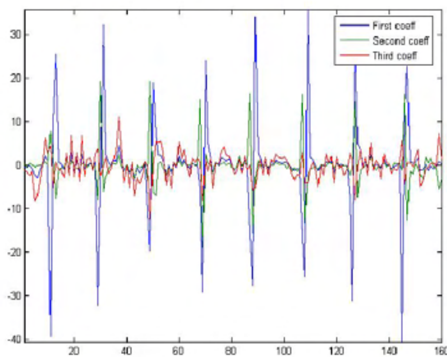

Fig. 6. **Motion signals ω_1, ω_2, and ω_3.** The graphs how the signals that relate to the first, second and third principal components. Although the first and second principal components are quite different (see figure 5). The resulting signals ω_1 and ω_2 contain more or less the same information. This indicates that there is only one underlying stimulus.

requirements outlined in section III. The first set of video sequences was collected form a sample of rat cardiomyocytes. Here all cells in the field of view have formed a nice sheet of contacting cardiac tissue. Simple visual inspection of the video data indicates that there is only one motion stimulus. The result of the automated motion analysis is captured in figure 3.

In the absence of electrophysiological data we validated the result by comparing the signal ω_1 with the video signal. The number of detected beats and overall shape of the signal appear to be correct. It should also be noted that the signals ω_1 and ω_2 are highly correlated. This confirms the hypothesis that there is only one underlying stimulus. Signals that would result from projecting the motion data into other principal components do not reveal any more information.

A second set of experiments was conducted on samples taken from human cardiomyocytes. Here only a small sub-population of cells shows signs of contractile motion. In this case it is also much harder to estimate the periodicity of the motion pattern by visual inspection. The resulting signals, shown in figure 7 confirm this. The regularity of the motion pattern obviously depends on the organization and differentiation state of the cell population. This seems to open the possibility of using the proposed method not only of monitoring drug effects but also monitoring the cell differentiation itself. Here the advantages of a non-invasive and contact free method are even more obvious. Any cardiomyocyte population could contain a number of isolated colonies of spontaneously self-contracting cells. In order to automatically analyze a larger cell population it would be necessary to segment the motion signal into regions of coherent motion.

VII. CONCLUSION AND FUTURE WORK

Our preliminary results indicate that our automated analysis of video microscopy data hold the potential of becoming a valuable tool for both monitoring cardiomyocyte differentiation and studying the effects of drugs on cardiac tissue. While this initial study shows promising results, a number of technical issues need to be addressed before a complete system can be build.

In addition it would be necessary to validate our results. Here two approaches are possible. Firstly one could correlate the results obtained using the proposed method with those generated from a more established methods such as the repolarization assay mentioned in the introduction. Such a study would ensure that the visual motion analysis is in fact

correlated to the electrical action potential. An alternative would be to screen a number of compound that are known to cause a change of the action potentials. If the results obtained by the proposed method show statistically significant differences between treated cell cultures and controls it could be accepted for screening.

In order to provide a complete solution that would enable automated screening of cardiomyocytes it would be necessary to segment the motion fields automatically to identify sub-populations of cells that spontaneously self-contract. Each of those cell clusters would need to be analyzed separately. An additional requirement would be the analysis of the signal ω_1. Detecting very subtle changes reliably is an open challenge. In summary, a well designed screening experiment would be necessary to address these open challenges and validation issues.

VIII. REFERENCES

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