Automated analysis of human cardiomyocytes dynamics with holographic image-based tracking for cardiotoxicity screening

Ezat Ahamadzadeh a, Keyvan Jaferzadeh b, Seonghwan Park a, Seungwoo Son a, Inkyu Moon a,∗

a Department of Robotics Engineering, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Hyojeong-eup, Dalseong-gun, Daegu, 42988, South Korea
b Department of Electronics Design, Mid Sweden University, 85170, Sundsvall, Sweden

1. Introduction

Understanding the mechanics of human cardiomyocytes (CMs) is particularly important to study as the main constituent of the heart to pump the blood to the entire body in a synchronized manner (Hu et al., 2018). Biochemical changes in metabolic processes cause a variety of problems including dysfunction and cardiac diseases leading to heart-beat fluctuations is one of the top priorities in cardiovascular-related studies (Fathi et al., 2018). Human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are used for modeling human disease, cardiotoxicity, drug response testing, and therapy in vitro which have high similarity in the formation and spontaneous contraction to in vivo cardiac cells (Abassi et al., 2012; Fleischer et al., 2019; Moon et al., 2018; Rappaz et al., 2015). Digital holographic microscopy (DHM) is a promising tool that achieved a remarkable role in the field of living cell analysis by providing quantitative phase images (QPIs) well-suited for the label-free study of cell behavior (Ahmadzadeh et al., 2020; Anand et al., 2017; Dubois et al., 2006; Javidi et al., 2005, 2018; Krinke et al., 2009; Memmolo et al., 2014, 2015; Merola et al., 2017; Moon et al., 2009, 2019; O’Connor et al., 2020; Rawat et al., 2017). During cardiac cell mechanical beatings activity, dry mass redistribution occurs which can be efficiently monitored with DHM (Rappaz et al., 2009, 2015; Shaked et al., 2010). A major contributor to lengthy and high-cost drug development processes is the lack of fast, label-free, and high throughput screening for in vitro CM characterization. Various efforts have been carried out to characterize hiPS-CM’s for disease modeling and quantify drug response including patch clamping (Brüggemann et al., 2017; Ossola et al., 2015), which requires expensive equipment, calcium imaging (Dempsey et al., 2016; Grespan et al., 2016), which is not label-free and requires fluorescent markers, the electrocardiogram (Krinke et al., 2009; Reppel et al., 2005) which needs costly equipment and expertise in the filed and cell-based biosensors also have limitations including the need for high-cost specific hardware, sensors, and trained personnel in this field or cardiomyocytes to be cultured onto specialized personnel in this field or cardiomyocytes to be cultured onto specialized materials which renders the process challenging (Caluori et al., 2019; Gupta et al., 2019; Wang et al., 2013). In our previous study, we showed that multiple CM’s responses to pharmacological compounds can be characterized by monitoring the dry mass changes (Jaferzadeh et al., 2018).

Keywords:
Cardiomyocyte characterization
Label-free biosensors
Optical imaging
Cardiotoxicity screening
High content screening
Cardiomyocyte motion tracking

ARTICLE INFO

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ABSTRACT

This paper proposes a new non-invasive, low-cost, and fully automated platform to quantitatively analyze dynamics of human-induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) at the single-cell level by holographic image-based tracking for cardiotoxicity screening. A dense Farneback optical flow method and holographic imaging informatics were combined to characterize the contractile motion of a single CM, which obviates the need for costly equipment to monitor a CM’s mechanical beat activity. The reliability of the proposed platform was tested by single-cell motion characterization, synchronization analysis, motion speed measurement of fixed CMs versus live CMs, and noise sensitivity. The applicability of the motion characterization method was tested to determine the pharmacological effects of two cardiovascular drugs, isoprenaline (166 nM) and E–4031 (500 μM). The experiments were done using single CMs and multiple cells, and the results were compared to control conditions. Cardiomyocytes responded to isoprenaline by increasing the action potential (AP) speed and shortening the resting period, thus increasing the beat frequency. In the presence of E–4031, the AP speed was decreased, and the resting period was prolonged, thus decreasing the beat frequency. The findings offer insights into single hiPS-CMs' contractile motion and a deep understanding of their kinetics at the single-cell level for cardiotoxicity screening.
However, there is still essential to developing high throughput, label-free, low cost, and non-destructive screening system for single hiPS-CMs motion characterization in vitro without the need for manually tuning software parameters or biochemical alternation to preserve the sample’s originality. This creates several challenges for efficient single CM motion monitoring to obtain functional signal capable of revealing detailed information regarding the pharmacological effects of compounds. The optical flow-based motion tracking methods are low-cost and high throughput methods capable of monitoring cardiomyocyte contractile activity without physical contact. The Farneback dense optical flow method performs the tracking of moving objects with single-pixel displacement detection (Fortun et al., 2015; Plyer et al., 2016). Furthermore, using the non-invasive property of DHM and optical flow-based analysis, cells remain intact for long-term monitoring. Using motion waveforms generated from the cell’s motion speed measurement, the cell’s dynamic parameters including the contraction period, relaxation period, beating period, and resting period can be automatically quantified.

In this work, a novel platform for single CM’s contractile motion characterization was designed using the Farneback dense optical flow method and holographic imaging informatics for cardiotoxicity screening applications. First, we image cardiac cells with DHM and perform motion tracking at the single-cell level using the Farneback dense optical flow method capable of detecting high-resolution contractile centers. In this way, the CM contractile motion speed was measured which reflects the changes in cell morphology. Afterward, we implemented a computational algorithm, to further characterize CM’s motion waveform, and multiple temporal parameters including the contraction period, relaxation period, beating period, and resting period were measured. Since our assessment method is at the single-cell level, the CMs synchronization can be qualitatively assessed. We demonstrated the application of the proposed method for cardiotoxicity screening by analyzing the pharmacological effects of isoprenaline (166 nM) and E-4031 (500 μM) response in CM motion speed in comparison to the control condition at the single-cell level. In the presence of 500 μM E-4031, the contraction-relaxation motion speed is declined compared to the control condition with prolongation in the resting period. The contraction-relaxation motion speed is increased in response to 166 nM isoprenaline with shortening the resting period. The other critical temporal parameters including contraction period, relaxation period, resting period, and beating period were compared from the statistical point of view. The statistical analysis of results was performed using the unpaired Student t-test. We also validated the proposed platform for whole slide QPI of cardiomyocytes motion characterization by applying several whole slides QPI of multiple cardiomyocytes for motion characterization and the generated motion waveform was quantified. The platform was validated by speed measurements of fixed cardiomyocytes versus live cardiomyocytes, single-CM synchronization testing, and a...
noise sensitivity analysis.

2. Materials and methods

2.1. Digital holographic imaging

To obtain cardiomyocyte images, off-axis DHM was used. Fig. 1(a) shows a schematic of an off-axis DHM. A recorded hologram of the cardiac cell sample is shown in Fig. 1(b). Afterward, three bandwidths of the real image, virtual image, and zero-order noise are obtained using the Fourier transform as shown in Fig. 1(c). A spatial filter to cover only the bandwidth of the real image is used (see Fig. 1(d)). Eventually, the amplitude and the quantitative phase images are obtained after numerical reconstruction and phase unwarping (see Fig. 1(e) and (f)), respectively (Cuche et al., 1999; Marquet et al., 2005). In Supplementary section 1, we show in more detail how the holographic imaging can be obtained.

2.2. Cardiomyocyte preparation and imaging conditions

Human-induced pluripotent stem (iPS) cell-derived CMs obtained from Cellular Dynamics Int. (Madison, WI) were cultured and grown according to the manufacturer’s instructions for 14 days before recording a hologram. Measurements were acquired in a Chamlide WP incubator system with a 96-well plate (LCI, South Korea) at 37 °C with 5% CO₂ and high humidity. Images were recorded by a commercially available DHM T-1001 from LynceeTec SA (Lausanne, Switzerland) equipped with a motorized stage (Märzhäuser Wetzlar GmbH & Co. KG, Wetzlar, Germany, ref. 5429). Images were acquired using a Leica 20×/0.4NA objective (Leica Microsystems GmbH, Wetzlar, Germany, ref. 11566049). The camera resolution was 1920 × 1200 pixels (the hologram size was 1024 × 1024, which is efficient for FFT computation).

For drug-treated CMs, a sequence was recorded before treatment for the control conditions. Then the drug was added, and after 15 min of incubation, the images were recorded again. The reconstruction process of the cardiac cells’ phase image was conducted using a standard computer at a rate of several images per second. The image of cardiomyocytes was acquired at three sampling frequencies of 10 Hz, 25 Hz, and 50 Hz.

2.3. Cardiac cell fixation

Cardiomyocytes were fixed using a 4% formalin solution (Sigma-Aldrich) and incubated for 15 min at room temperature (RT). Subsequently, cells were washed three times with phosphate-buffered saline (PBS) for 10 min at RT. 1500 images were acquired at a sampling frequency of 50 Hz before and after cell fixation.

2.4. Statistical analysis

For better evaluation, data were compared to control conditions with an unpaired student’s t-test for statistical analysis using GraphPad Prism. Data are expressed as the means ± standard deviation (SD). Statistical differences between the two groups with p-value < 0.05 were considered statistically significant.

2.5. Single CM motion tracking with Farneback optical flow

The steps of single cardiac cell motion tracking using the Farneback optical flow method and motion waveform generation are shown in Fig. 2(a). In the first step, the algorithm generates a hierarchy of resolution levels from the original image using Gaussian pyramids, where each level has a lower resolution than the previous level (see Fig. 2(b)). Fig. 2(c) shows the optical flow method for pixel displacement estimation in two successive image frames where \( I(x, y, t) \) denotes the pixel position in the reference frame and \( I(x + dx, y + dy, t + dt) \) refers to the

![Fig. 2](image-url). Overview of the workflow for motion tracking of single cardiac cells and beating profile quantification. (a) Quick steps of single cardiac cell motion tracking using the Farneback optical flow method and motion waveform generation. (b) Multi-resolution leveling of an image with three levels. The image resolution at each level was downsized. (c) Diagram of the optical flow for pixel displacement estimation. (d) Close-up QPI of a single CM with superimposed motion vectors on the cell’s image for contraction. (e) Relaxation state, (f) resting state (first row), and corresponding heat map generated from absolute motion (second row) with encircled contractile centers. The contractile centers refer to a region in which contractions are maximized. (g) Quantification description is explained in Section 2.6. (h) Beating activity profile of a single cell.
pixel displacement in the subsequent frame (current frame). The Farneback algorithm is a dense optical flow method that performs motion tracking in multi-resolution levels (Plyer et al., 2016). In the first step, the algorithm generates a hierarchy of resolution levels from the original image using Gaussian pyramids, where each level has a lower resolution compared to the previous level (see Fig. 2(b)). The tracking process starts with the lowest resolution and continues to the highest resolution. Consequently, the displacement of two local patches in consecutive image frames is calculated by approximating the neighborhood of each pixel with a quadratic polynomial (Farneback, 2003) as in the following equation:

\[ I_1(x) = X^T A x + b^T x + c. \]

(1)

where \( A \) stands for a symmetric matrix, \( b \) is a vector, and \( c \) is a scalar. The vector \( x \) is a 1 \times 2 vector containing running variables \( x_1 \) and \( x_2 \). \( A \) is a symmetric matrix 2 \times 2 of unknowns that capture information about the even parts of the signal, and \( c \) is an unknown scalar. The first neighborhood is approximated by the following equation:

\[ I_1(x) = X^T A_1 x + b_1^T x + c. \]

(2)

The new neighborhood affected by displacement \( d \) is calculated as follows:

\[ I_2(x) = I_1(x-d) = (x-d)^T A_1 (x-d) + b_1^T (x-d) + c_1 \]

\[ = x^T A x + (b_1 - 2 A_1 d)^T x + d^T A_1 d - b_1^T d + c_1 \]

\[ = x^T A x + b_2^T x + c_2. \]

(3)

Using the different coefficients from the two polynomials \( I_2(x) \) and \( I_1(x-d) \) gives \( A_2 = A_1, b_2 = b_1 - 2 A_1 d, \) and \( c_2 = d^T A_1 d - b_1^T d + c_1 \). The distance \( d \) is used to approximate the optical flow and is obtained by the following equation:

\[ d = - \frac{1}{2} A^{-1}_2 (b_2 - b_1). \]

(4)

The tracking is refined at each resolution level by starting from the lowest resolution level and moving to the highest resolution. The detected tracking points at each level are the base points for the next level, and large displacement can be detected.

2.6. Workflow for beating signal quantification

Ten single CMs were manually extracted from different parts of the cardiomyocytes’ QPI (see Fig. 2(a)). The extracted cell area mostly includes the nucleus section assuming the nucleus section is the center of the motion (Ahmadzadeh et al., 2020). During the cardiac cell’s beating activity, an array of motion vectors is generated by repeating the optical flow procedure shown in Fig. 2(a) which demonstrates the CM’s motion direction, and the action potential (AP) speed is calculated. The CM’s motion waveform is generated by AP speed calculation using the following equation:

\[ \text{Speed}_{\text{AP}} = \frac{\text{Displacement}}{\text{Time}} = \sqrt{\left(\frac{dx}{\text{Time}}\right)^2 + \left(\frac{dy}{\text{Time}}\right)^2} . \]

(5)

where \( dx \) and \( dy \) are displacements in \( x \) and \( y \) directions estimated by Farneback algorithm representing CM motion in the \( x \) and \( y \) direction, and time is considered as the time between two consecutive frames. Once the motion waveform is generated, one can find the contraction and relaxation peaks obtained by the automated peak identification method along with multiple auxiliary points for CM’s physiological behavior quantification. A single cardiac cell with superimposed motion vectors on the image referring to the motion directions for different beating statuses shown in Fig. 2(d), (e), and (f) corresponding to contraction, relaxation, and resting beating status respectively. It can readily be seen that during contraction and relaxation, motion vectors are indicating opposite directions. In contrast, vectors indicate no motion (displacement) in the resting status. The corresponding heat map generated from absolute motion for each beating status is also shown (see the second row in Fig. 2(d), (e), and (f)). Heat map represents the specific regions of the cells which is a center of contraction. While the cell’s contraction status, more contractile centers are observed on the heat map image compared to relaxation status. In contrast, the heat map shows almost no contractile centers in resting status which leads to nearly zero value for motion speed. Temporal motion speed monitoring can reveal details of the beating activity of the cardiac sample (see Fig. 2(g)).

An accurate peak detection (see Fig. 2(h)) and averaging period selection allowed the reconstruction of important temporal parameters namely, contraction period, relaxation period, resting period, and beating period. Fig. 2(g) shows details of the obtainable averaged temporal parameters related to the cell’s mechanical events. To calculate the characteristics of the cardiac sample activity from the motion speed signal, single beating profiles are extracted. It requires two main peaks of contraction and relaxation and three auxiliary points 1) Start-of-Contraction, 2) End-of-Contraction and 3) End-of-Relaxation. The representation of these points is shown in Fig. 2(g). Details of accurate peak detection along with auxiliary points are described in our previous study (Ahmadzadeh et al., 2020). The description of dynamic parameters measured for each extracted cardiac cell is as follows: 1) Maximum contraction speed (see #1 in Fig. 2(g)): the average amplitude of contraction peaks; 2) Beating period (see #2 in Fig. 2(g)): the time between two adjacent contraction peaks; 3) Contraction period (see #3 in Fig. 2(g)): the average time between the start of contraction and end of contraction points; 4) Relaxation period (see #4 in Fig. 2(g)): the average time between the start of relaxation to the end of relaxation points; 5) Maximum relaxation speed (see #5 in Fig. 2(g)): the average amplitude of relaxation peaks; 6) Resting period (see #6 in Fig. 2(g)): the average time between the end of relaxation to the next start of contraction points.

3. Results and discussion

The single-CM motion characterization, beating profile quantification results, and synchronization analyses are demonstrated in Fig. 3. Heat map analysis of the absolute motion was used to monitor the contractile centers (see Fig. 3(a), (b), (c), second row). Four single CMs are shown as examples, and the complete visualization of a CM’s beating activity is shown in Movies 1-4.

The beating profile of cells #5 to #10 is shown in Supplementary Figure S1. The maximum contraction speed is larger than the maximum relaxation speed, also the contraction period is shorter than the relaxation period (see Fig. 3(d)) due to the presence of different ion channels and transporters expressed in cardiomyocytes membrane and the mechanisms by which their activities are sequentially orchestrated during cell contraction and relaxations. The motion vectors indicate opposite directions to each other during CM contraction-relaxation beating activity while during the CM resting status, the motion vectors are too weak which specifies the cell is almost immobile (Fig. 3(a), (b), (c) first row). The quantification results demonstrated the average beating rate and average beating period are similar for all cells and the beating activity occurred at regular intervals (see Fig. 3(e)). Note that there is a cell-to-cell variation in the magnitude of speed value, whereas the CM’s beating rate and other characteristics related to the physiological aspects of the sample (contraction and relaxation period, etc.) are almost the same. As it can rapidly be seen from Fig. 3(d), the signal shape was compromised by the biological processes happening inside the cell membrane and flows through ion channels. This implies that we obtained high-quality signals.

Since our analyses are at the single-cell level, they can provide reliable synchronization analysis. Fig. 3(f), (g) show the synchronization investigation for all extracted single CMs. As shown in Fig. 3(f), the temporal activity (contraction-relaxation) in different periods beat with
the same frequency. As shown in Fig. 3(g), the cross-correlation evaluation between the beating activity signals of cardiac cells, the maximal cross-correlation value is on the time lag zero. It shows that CM signals are perfectly synchronized in time (see Fig. 3(g)).

3.1 Verification of the proposed motion tracking method

3.1.1 Whole slide image motion characterization

To demonstrate the robustness of the proposed CM motion characterization method, we analyzed five different whole slide QPI samples of multiple CMs that were imaged at different sampling frequencies (10 Hz, 25 Hz, 50 Hz). Fig. 4(a) shows sample #1 with superimposed motion vectors for contraction beating status and the corresponding heat map is shown in Fig. 4(b). The beating activity profile with detected contraction-relaxation peaks and auxiliary points is shown in Fig. 4(c). Samples #2-#5 are shown in Supplementary Figure S2 and visualization is provided by Supplementary Movie 5. It is worth mentioning that the average contraction-relaxation motion speed of the whole image is affected by the motionless areas which mostly there are no cells grown. This results in declining average speeds comparing to the single-cell level analysis.

3.1.2 Speed measurement of fixed cardiomyocytes versus live cardiomyocytes

The method was verified by measuring the speed of fixed cardiomyocytes versus live cardiomyocytes, as shown in Fig. 4(d). The amplitude of the speed of fixed cells fluctuates around zero. The amplitude is much smaller than both contraction-relaxation peaks and similar to the amplitude of the resting state. Visualization is provided by Supplementary Movie 6.

3.1.3 Noise sensitivity analysis

In general, noise may be caused by several factors. To demonstrate the robustness of the proposed method for CM motion characterization in noisy images, we artificially applied Gaussian noise ranging from 5% to 20% on the single CM #1 phase image (see Fig. 4(e)) and the motion...
waveform generated by the proposed method are quantified. The cell’s beating profile regularity remained constant for all different noise levels and quantification results show that dynamic beating parameters also remained almost constant. (see Supplementary Figure S4).

3.2. Monitoring the pharmacological effects of compounds on CMs’ AP waveform

3.2.1. Whole slide QPI analysis of drug-treated cardiomyocytes

We next applied the platform to test the response to pharmacological compounds on single CMs’ motion activity. We analyzed the effects of an hERG channel blocker (E-4031) and an adrenergic receptor agonist (isoprenaline) on cardiomyocytes’ contractile speed using the proposed platform. We treated multiple cardiomyocytes with 166 nM of E-4031 and 500 μM of E-4031 and compared the beating activity parameters to those obtained in the control conditions (see Figs. S5(a) and 6(a)). The quantification results are shown in Supplementary Figs. S4 and S6. The cardiomyocytes responded to the E-4031 drug by decreasing their contractile speed with the prolongation of the resting period, thus decreasing their beating frequency compared to the control conditions. This is in agreement with the drug’s mode of action. Isoprenaline raised the AP speed and shortened the beating frequency compared to the control conditions. This is also in line with previous findings (Hayakawa et al., 2014; Treat et al., 2019).

3.2.2. Single CM motion characterization of isoprenaline-treated cardiomyocytes

Fig. 5(a) shows the contractile speed of the whole-slide QPI of multiple CMs in control and drug-treated conditions in response to 166 nM of isoprenaline. Fig. 5(b) demonstrates an example of single beat profiles of a whole-slide QPI with a comparison between the control and drug-treated conditions. The contractile motion activity during the contraction speed, but it was not significantly increased compared to the control condition. The resting period was significantly shortened and caused a significant increase in the beating frequency. The bars represent the mean and SD for each quantification parameter. These findings are in line with previous reports (Isobe et al., 2018; Luo et al., 2017).

3.2.3. Single CM motion characterization of E-4031-treated cardiomyocytes

It was previously reported that the treatment of cardiac cells with E4031 significantly slowed the heart rate of the loss of intracellular K⁺ in cardiac cells (Dempsey et al., 2016; Luo et al., 2017). Fig. 6(a) shows the contractile motion of the whole-slide QPI of multiple CMs in control and drug-treated conditions in response to 500 μM of E–4031. After drug treatment, a reduction in the contraction-relaxation speed was observed with prolongation in the resting period, thus decreasing the beating frequency compared to the control conditions. The quantification of the beating parameters exhibited a prolongation in the relaxation period (Supplementary Fig. S6). Fig. 6(b) demonstrates an example of a single beat profile comparison.

The effects of E-4031 on AP were tested at both the whole-slide QPI and single-cell levels. The single CM beating patterns for cells #1 to #4 are given in Fig. 6(c) and (e). A comparison of the beating profile quantification parameter was performed at the single-cell level as shown in Fig. 6(d). We observed a contractile response of a CM to the E–4031 drug, which demonstrated that the average AP of all single CMs decreased compared to the control conditions. The average resting period for all extracted single CMs in drug-treated conditions demonstrated almost the same value, which shows significant prolongation compared to the control conditions. Thus, there was a significant decrease in the beating period. Some single CMs’ motion profiles exhibited irregular beating patterns after drug treatment. The average results of each quantification parameter for all extracted single cells are shown in Fig. 6(f). These findings are in agreement with previous findings (Hayakawa et al., 2014). The bars represent the mean and SD for each quantification parameter of all extracted single cells.

We have proposed a motion characterization platform for single hiPS-CMs using the optical flow method combined with digital holographic imaging for cardiotoxicity application which obviates the need for costly equipment to monitor a CM’s mechanical beat activity. We obtained detailed information about CM functionality from generating
the CM beating activity profile based on the cell’s speed calculation. Quantification of the beating profile was performed for every extracted single cell using our automated peak identification method. Validation of the proposed CM characterization method was performed by measuring the contractile speed of fixed cardiomyocytes versus live cardiomyocytes and a noise sensitivity analysis. Based on experimental results, it was shown that the proposed method and quantification have the potential to be used in high-throughput analysis of hiPS-CMs in compound cardiotoxicity screening. Our results revealed quantification features of the beating profile, including the contraction period, relaxation period, and resting period. Also, we investigated the beating synchronization of the single CMs using the proposed method.

Finally, we validated the applicability of the proposed platform for cardiotoxicity screening at the single-cell level by monitoring the effects of E4031 (500 μM) and isoprenaline (166 nM) compounds on multiple single cardiomyocytes’ beating activity-related parameters in comparison to control conditions. The cardiomyocytes responded to the E4031 by decreasing their contractile speed and prolonging their resting period, thus decreasing their beating frequency. The isoprenaline caused an increase in AP speed with a decrease in the resting period, thus increasing the beating frequency (Jaferzadeh et al., 2020; Hayakawa et al., 2014). Finally, we averaged each quantification result for all extracted single cells in control (blue bars) versus drug-treated conditions (red bars). All statistical comparisons were carried using an unpaired student t-test. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
efficiently and accurately reveal detailed quantification results about the pharmacological effects of the drug on single cardiac cells for cardiotoxicity screening and predictive toxicology.

4. Conclusions

We have demonstrated a new, label-free, low-cost in vitro platform for human cardiomyocytes’ motion characterization at a single-cell level by holographic image-based tracking for cardiotoxicity application. The method enabled us to obtain detailed information about a single cardiac cell’s AP parameters. The proposed method demonstrated that it can efficiently and accurately reveal detailed quantification results about the pharmacological effects of a drug on single cardiac cells for cardiotoxicity screening and predictive toxicology. In addition, the results demonstrate the prospects of using the proposed method as a reliable tool for beating synchronization analysis from the cells motion waveform during beating activity.

The current limitations of the holographic image-based tracking for cardiotoxicity screening using the optical flow method are regarding extra noisy images. An extra noisy image can negatively affect the optical flow-based cell tracking method for accurate cell motion speed measurements. As part of our future study we plan on incorporating additional noise information into our cardiac cells motion tracking algorithms. Another interesting future work plan is to investigate whether our proposed optical flow method can provide a fairly good estimate for tracking of cardiac cells with the time-lapse Gabor holography, which allows to automatically quantify their beating activity at a much lower cost.

Supporting information

Supporting document is provided for this work. Fig. S1: The beating activity profile derived from single CMs #5–#10 is shown. Fig. S2: Whole-slide QPI of multiple-cardiomyocyte motion characterization.
Fig. S3: Quantification results of single CM beating profiles supplied with different levels of noise ranging from 5% to 20%. Fig. S4: (a) Whole-slide QPI of cardiomyocytes beating activity in control condition versus drug-treated conditions in the presence of 166 nM of isoprenaline, (b) Beating activity quantification results of drug-treated versus control conditions in presence of 166 nM of isoprenaline. Fig. S5: (a) Single cardiomyocytes’ beating activity of cells #5–#10 in drug-treated conditions in presence of E. Ahamadzadeh et al. (2021). (b) Whole-slide QPI of cardiomyocytes beating activity in control condition versus drug-treated conditions in the presence of E—4031 500 μM, (b) beating activity quantification results in control versus drug-treated conditions. Fig. S7: (a) Single cardiomyocytes’ beating activity of cells #5–#10 in the control conditions, (b) single cardiomyocytes’ beating activity of cells #5–#10 of drug-treated conditions in presence of E—4031 500 μM.

CRedit authorship contribution statement

Ezat Ahamadzadeh: Methodology, Experiments, Validation, Keyvan Jaferzadeh: Experiments, Validation, Seongwoon Son: Experiments. Inkyu Moon: Conceptualization, Validation, Supervision, All authors participated in the manuscript preparation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Dr. Benjamin Rappaz and Prof. Gerardo Turcatti (BSF in EPFL, Lausanne, Switzerland) for helpful discussion and providing the datasets. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2015K1A1A2029224, No. 2020R1A2C3006234). This work was supported by the DGIST R&D Program of the Ministry of Science and ICT (21-Co-E-BT-02).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2021.113570.

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