Reflective lens-free imaging on high-density silicon microelectrode arrays for monitoring and evaluation of in vitro cardiac contractility

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Abstract: The high rate of drug attrition caused by cardiotoxicity is a major challenge for drug development. Here, we developed a reflective lens-free imaging (RLFI) approach to non-invasively record in vitro cell deformation in cardiac monolayers with high temporal (169 fps) and non-reconstructed spatial resolution (352 µm) over a field-of-view of maximally 57 mm². The method is compatible with opaque surfaces and silicon-based devices. Further, we demonstrated that the system can detect the impairment of both contractility and fast excitation waves in cardiac monolayers. Additionally, the RLFI device was implemented on a CMOS-based microelectrode array to retrieve multi-parametric information of cardiac cells, thereby offering more in-depth analysis of drug-induced (cardiomyopathic) effects for preclinical cardiotoxicity screening applications.

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1. Introduction

Monitoring of the contractility of cardiac cells in vitro is crucial for the preclinical assessment of drug-induced cardiotoxicity, which accounts for up to 45% of market withdrawal [1]. Today’s preclinical toxicity assessment focuses on measuring the delays in ventricular repolarization (also known as QT prolongation), as longer QT intervals increase the risk of potentially fatal Torsades de Pointes arrhythmia [2]. Food and Drug Administration guidelines recommend assessment of drug effects on hERG (Kv11.1) ion channels, but hERG blockage screening assays are not fully predictive [3] and necessarily imply cardiotoxic side effects [4]. Thus, preclinical cardiotoxicity screenings require a functional evaluation of cardiotoxic drug effects on in vitro cell cultures through electrophysiological and contractile properties.

Cardiomyocyte functionality is primarily analyzed by electrophysiological characterization. Planar patch clamp is part of the standard electrophysiological toolbox for the determination of in vitro cardiotoxicity [5]. Although these systems can register intracellular action potentials of dissociated cells [6], the method is invasive and thus only short measurements are feasible. A non-invasive alternative to record electrical activity are microelectrode arrays (MEAs). These system address the limitations by enabling the recording of extracellular and intracellular [7–9] electrical activity from cellular monolayers for extended time periods [10]. Moreover, CMOS-based MEAs greatly enhance the throughput, spatial resolution and quality of these measurements compared to more simple glass-based MEAs [7,11–14]. Another approach involves measuring the fluorescence of...
cardiac cells genetically altered to produce voltage sensitive dyes, but fluorescence is susceptible to photobleaching and phototoxicity [15].

An alternative strategy to evaluate cardiac function is analyzing the physical beating of cardiac cells. The contractility of a cell depends on the interplay of various ion channels and proteins. Traditionally, fluorescent labels are used to visualize the intracellular Ca\textsuperscript{2+} concentration as an indirect marker for contractility [15,16]. Unfortunately, these are invasive, end-point measurements and can affect cell function [17,18]. Contractility can also be monitored non-invasively by electrochemical impedance spectroscopy (EIS) using interdigitated microelectrodes [19,20]. However, their spatial resolution is low and the interpretation of the data is not straightforward. Various optical-based, label-free strategies have been developed to directly image the shortening of cardiomyocytes through light microscopy. These systems evaluate beating parameters of groups of cells by recording light microscopy movies at high spatiotemporal resolution. Contractile characteristics are extracted from videos through algorithms based on pixel intensity variations [21], block matching algorithms [22], digital image correlation [23] or optical flow techniques [24], which are then analyzed for the effect of compounds on the contraction dynamics. Additionally, the phase shift of images of cardiac cultures has been used to quantify cell beating characteristics and morphology [25,26]. Another approach involves visualizing the shortening of sarcomeres [27], which are the protein complexes that comprise the basic unit of contraction. Nevertheless, these methods still require bulky optical setups, which limits their scalability in high-throughput applications. Furthermore, lens-based systems typically have a limited field-of-view (FOV), which hampers monitoring of excitation wave propagation in cardiac monolayers.

On the contrary, lens-free imaging (LFI) techniques are compact systems where the optical components, such as the lens, are omitted. The FOV is only limited by the size of the image sensor. For digital in-line holography systems, samples are illuminated with coherent light. Low-cost and high-performance image sensors record the interference fringes created by the light scattered off the sample and the un-scattered reference light. The holograms are then reconstructed in post-processing to obtain the object image [28,29]. LFI systems have shown wide potential in biological applications [30–33]. However, reconstruction is not always necessary. The unprocessed interference patterns already offer insight into dynamic processes, such as the beating rate of cardiac samples [34]. Optical flow analysis of these interference patterns have also led to insight into contraction and relaxation dynamics of cardiac monolayers, as well as monitoring of excitation wave propagation over large FOV [35].

Combined contractile and electrophysiological monitoring offers a unique and more thorough insight on drug-induced side-effects [36]. Contractility monitoring on MEAs has been demonstrated by simultaneous bright field microscopy [37] or electrical impedance measurements [20]. Unfortunately, these techniques cope with important disadvantages such as a limited field of view, low spatial resolution, need for labels [15,18,38], poor signal quality or limited number of readout sites (electrodes). In the case of LFI, only biological samples grown on transparent substrates have been analyzed. Even though microfabricated silicon chips are used increasingly in biological applications to improve resolution, sensitivity and throughput in cell analysis [39–41], compatibility with silicon-based devices is not yet achieved. This problem can be circumvented by reflection lens-free imaging (RLFI), which uses off-axis holography based systems [42]. However, this approach was only applied to optically dense or spatially connected specimens (e.g. tissue slices) on transparent glass slides [43]. Additionally, the low temporal resolution, limited FOV and long analysis times impede contractility monitoring. As a result, there is a need for label-free, fast and high-quality imaging on opaque active silicon chips.

We developed a non-invasive, label-free method to monitor deformation of \textit{in vitro} grown ventricular cardiomyocytes on silicon-based substrates through an RLFI implementation. To
avoid the limitations of off-axis lensless digital holography, our reflective LFI devices was based on in-line digital holography. Unprocessed interference patterns of spontaneously beating cells were recorded at high frame rates and analyzed by optical flow algorithms to obtain the relative cellular deformation (RCD) and relative cellular deformation rate (RCDr). By implementing the RLFI device onto a fluorescence microscope, the biological relevance of the RCD measurement was shown. Additionally, as RCD was measured over a wide FOV, the system was used to monitor excitation wave propagation over tens of thousands of cardiac cells. Further, we used the system to detect both the weakening of RCD by the drug blebbistatin, an inhibitor of the contractile apparatus, and also conduction disturbances caused by the drug 1-octanol, a gap junction uncoupler. Finally, we implemented the RLFI technique onto a CMOS-based microelectrode array to simultaneous monitor intracellular electrical activity and contractility, and evaluated the uncoupling of the electrical and contractile activity after the addition of blebbistatin.

2. Methods

2.1 Reflection lens-free imaging system

The developed RLFI technique is based on in-line digital holography system. A schematic diagram of the principle and photographs of the RLFI system is shown in Fig. 1. The illumination source of this system consists of a fibre optic cable connected to a laser source (Thorlabs MCLS1, class 3B, λ = 638 nm at 15 mW) and placed at 0.3 m from the object of interest at an angle of ~35° from vertical. For these experiments, the sample consisted of glass wells of a height of 0.6 mm, glued on opaque surfaces (silicon and PCB). The top of the well was closed off with a cover slip to prevent the formation of a meniscus and noise caused by movement over the water surface. Finally, a Ximea xiQ camera was placed directly over the sample at a height of 1 cm over and parallel to the surface of interest. The reflection interference fringes were recorded by a CMOS CMV2000 sensor (CMOSIS, 2048 x 1088 pixel array with 5.5 µm pitch). Movies of 15 seconds were taken at maximally 169 frames per second and transmitted by USB 3.0 connection to a laptop and then further processed. All measurements were taken inside of a fanless direct heat incubator (Galaxy 170 S) at 37°C placed on an antivibration table to remove ambient vibrations. The resulting system was small (6000 cm³ excluding breadboard), modular and compatible with cell culture conditions.

![Fig. 1.](image)

**Fig. 1.** (a) Principle behind the reflective lens-free imaging based on digital in-line holography. The sample is illuminated by coherent light which consists of parallel wave fronts, and the light is reflected off the sample surface. The image sensor records the interference pattern created by the light scattered from the sample and the un-scattered reference beam. (b) Photograph of the RLFI system (c) the glass well on a blank SiO₂ wafer placed directly under the camera.

2.2 Cellular deformation analysis

After recording the beating of cardiac cells by RLFI, the sequence of reflected interference fringes was analyzed to extract relative cellular deformation (RCD), rate of cellular deformation (RCDr) and contractility parameters. These analyses were carried out by image processing scripts that were adapted from previous transmission lens-free imaging systems.
In this script, the full interference pattern was split into regions of interest (ROI) of 64x64 pixels (approximately 0.12 mm²) and each ROI was separately analyzed. First, the total pixel intensity variation over the ROI was calculated as follows:

\[ I_{\text{var}}(t) = \sum_{i=x_o}^{x_f} \sum_{j=y_o}^{y_f} \left| I(i,j,t) - I_{\text{ave}}(i,j) \right| \]  

where \( I_{\text{var}}(t) \) is the pixel intensity variation over the full ROI, \( I(x,y,t) \) the intensity measured at each pixel, and \( I_{\text{ave}}(x,y) \) is the average intensity of each pixel, which is summed over all pixels in the region encompassed by \( x_o \) to \( x_f \) and \( y_o \) to \( y_f \). The moments of contraction were determined by detecting maxima in pixel intensity variation. Then, the relative cellular deformation was determined by calculating the motion vectors between each frame and a ‘relaxed’ reference frame (Dense Optical Flow implementation of the Farneback algorithm from OpenCV). The ‘relaxed’ reference frame was calculated by averaging out frames wherein cardiomyocytes were fully relaxed (frames halfway between contractions). The RCD was calculated as follows:

\[ RCD(t) = \sum_{i=x_o}^{x_f} \sum_{j=y_o}^{y_f} \left( v_x(i,j,t)^2 + v_y(i,j,t)^2 \right) \]  

where \( v_x(x,y,t) \) and \( v_y(x,y,t) \) are the obtained motion vectors in the x and y direction respectively, which is summed over all pixels in region encompassed by \( x_o \) to \( x_f \) and \( y_o \) to \( y_f \). These profiles were further smoothed (3rd order Savitzky-Golay filter over an interval of 18 ms) and temporally interpolated (10x). The RCDr was obtained from the first derivative of the RCD and smoothed. Both RCD and RCDr were normalized by either its maximal value (for single measurements) or the maximal contraction of baseline measurements in the ROI (for series of measurements). The procedure was repeated over the full interference pattern.

The excitation wave propagation was analyzed through the temporal delays in contraction between the initiation of contraction in each ROIs. In this analysis, the start of contraction is defined as the time points at which the smoothed second derivative of the RCD reaches a maximal value. The delay in contraction initiation is then calculated over all ROI and averaged over multiple contractions. The propagation velocity was calculated by dividing the distance between the ROI where contraction is initially determined and the ROIs furthest in the direction of the excitation wave propagation by their respective delay and averaged.

### 2.3 Simultaneous calcium imaging and cellular deformation monitoring

The RLFI setup was built around a fluorescence microscope (Zeiss Axio Examiner Z1) to simultaneously measure RCD and relative intracellular Ca²⁺ concentration ([Ca²⁺]) of a monolayer of cardiomyocytes. Due to geometric constraints, the camera and laser were placed at angles of 60° from the horizontal, which resulted in skewed interference patterns. These were flattened during post-processing. The RCD was determined over a region of 0.3 mm² around the area analyzed by calcium imaging. The [Ca²⁺], of a monolayer of cells located in a region of 0.011 mm² was recorded at 59.6 fps through a Hamamatsu ImagEM X2 EM-CCD camera (C9100-23B, Hamamatsu) with 10x dry objective. Both devices were synchronized by a PCB which read out the output pulses from the Ximea I/O, and activated the fluorescent microscope through a separate I/O pulse. After signal synchronization in post-processing, RCD and [Ca²⁺] were averaged over 10 s.

### 2.4 Combined reflective lens-free imaging and microelectrode array setup

Simultaneous electrophysiological and RCD measurements were carried out on two different systems. RCD was calculated in a region of 0.124 mm² around the electrodes of interest. In the first system, simultaneous intracellular action potential measurements were obtained by
combining the RLFI setup with a CMOS-based microelectrode array with 16,384 individually addressable electrodes as previously described [7,39]. Briefly, intracellular access was achieved by electroporating the cell membrane locally through sub-cellular sized electrodes, achieving intracellular-like (‘open-cell’) recordings. The output from the camera I/O was recorded by the same setup as which recorded the electrical activity on the MEA, and signals were synchronized in post-processing. Measurements were carried out inside an incubator and repeated over the same electrodes. In the second system, the RLFI setup was combined with a so-called passive microelectrode array system in which extracellular field potentials were simultaneously measured over multiple electrodes. The passive MEA system [44] contained rows of 12 electrodes (area of 225 µm²) spread out over a length of 4.4 mm and signals were amplified by a MEA 1060-Up-BC amplifier (Multichannel Systems). Both the camera and passive MEA system were synchronized by sending I/O pulses to a separate PCB, which allowed for signal synchronization in post processing. An integrated heating element was used to maintain the temperature of the sample at 37 °C.

2.5 Cardiomyocyte cell culture

Primary neonatal rat ventricular cardiomyocytes were extracted from 2 day old Wistar rats and cultured in glass wells on both blank SiO₂ and microelectrode array chips, as described previously [7,35]. Cardiac culture medium (Ham F10 containing 5% FCS, 1% PenStrep, Hepes, 0.5% ITS, 0.1 mM Norepinephrine, and 2 µg/ml vitamin B-12) was refreshed on the first, third and fifth day. Experiments were carried out on day 5. All measurements were carried out on cells in cardiac medium, unless stated otherwise. For measurements involving calcium imaging, 50 µg fluo-4 AM was dissolved in DMSO containing 20% Pluronic and diluted to 10 µl/ml in cardiac medium. Cardiomyocytes were incubated for 30 minutes in fluo-4 AM solution at 37°C and washed with cardiac medium. Additionally, cell cultures were exposed to cardiac medium, 0.015% DMSO, and an increasing concentration of blebbistatin (1 nM, 10 nM, 100 nM, 500 nM, 1 µM and 5 µM). A second drug-dose response experiment was carried out by exposing cells to 0 µM, 1 µM, 10 µM, 100 µM, 400 µM, and 1000 µM 1-octanol in cardiac medium. These experiments were also carried out at 37 °C and 15 second recordings were taken after 45 minutes of incubation. Finally, for the combined MEA-RLFI system, cells were exposed to cardiac medium, 0.015% DMSO and 5 µM blebbistatin.

3. Results and discussion

3.1 Neonatal rat ventricular cardiomyocytes interference patterns

To evaluate the performance of the digital in-line holography RLFI system, we first imaged neonatal rat ventricular cardiomyocytes in glass wells glued onto SiO₂ wafers. The illuminating angle was set at 35° from the vertical to minimize the distance from the sensors to the sample. In this way, the reflection from the top glass coverslip was blocked off from the sensor by the casing of the camera and did not interfere with the recording (Fig. 2(a)). At smaller angles than 35°, the camera blocked off the illumination light, and interference from the top angle is detected over part of the sensor surface. Sparsely seeded primary rat ventricular cardiomyocytes were imaged over a FOV of 57 mm² (Fig. 2(b)), where the interference patterns created by individual cardiac cells are detected. Following this, we imaged dense monolayer of cardiac cells (Fig. 2(c)), which resulted in much denser interference patterns. By basing our RLFI system on in-line digital holography, we monitored biological activity over a FOV 6x larger than previously reported [43]. One of the main advantages of the system is that its FOV is not limited by a beam splitter. Removing the beam splitter also reduces the distance from the sample to the object, leading to a lower signal-to-noise ratio. Additionally, lower exposure times led to much higher temporal resolutions of up to 169 fps.
3.2 Detection of cellular deformation by reflective lens-free imaging

The RLFI system monitored spontaneously beating cardiac cells cultured on non-transparent surfaces, such as silicon chips, to assess \textit{in vitro} contractility. Cardiac cells grown in the silicon wells were electrically coupled and had a developed contractile apparatus (Appendix Fig. 9). Contractions were imaged at high speed (169 fps) and captured over a FOV of 57 mm². Obtained interference fringes already showed rhythmic intensity oscillations (see Visualization 1). The interference patterns were further processed by custom developed algorithms based on pixel intensity variation and optical flow to analyse contraction of monolayers of cells (Fig. 3(a)). First, the full image was split into regions of interest (448 regions of 0.124 mm², corresponding to a region of ~100 cells). In each ROI, the obtained motion vectors were summed to represent the relative cellular deformation (RCD) and then further processed to obtain relative cellular deformation rates (RCDr). Figure 3(b) demonstrates a typical trace of the RCD (bottom trace) and RCDr (top trace) of cardiomyocytes measured at day 5 \textit{in vitro}. The RCDr trace contains two peaks: a faster, larger peak representing the contraction rate, and a second slower, smaller peak representing...
the relaxation rate. Although smaller ROIs are possible, the analysis will result in noisier signals. These results resemble previous data reported using phase contrast microscopy [22] and transmission LFI [35]. Additionally, the detected contractions were averaged over multiple contractions, and the frequency, duration at 20% peak value, deformation amplitude, (Fig. 3(c)), relaxation rate amplitude and contraction rate amplitude (Fig. 3(d)) were obtained. These contractility parameters grant non-invasive insight into the cellular dynamics of groups of ensembles of cultured cardiac cells.

3.3 Simultaneous calcium imaging and cellular deformation monitoring

An increase of the intracellular calcium concentration is necessary to activate the contractile proteins of cardiac cells. Calcium imaging is therefore a well-established and important method to assess cardiac contractility [45]. To validate the biological relevance of RCD, we implemented the RLFI onto a fluorescence microscope and performed synchronous fluorescent intracellular calcium imaging and contractility measurements. After pre-loading the cells with Fluo-4AM, measurements were recorded at a rate of 60 fps and 169 fps for calcium imaging and RLFI, respectively. The RCD (Fig. 4(b), blue trace) was calculated over an area of 0.3 mm$^2$ and overlapped with the relative intracellular calcium concentration [Ca$^{2+}$]$_i$ (DF/FO; Fig. 4(b), yellow trace) taken from an area of 0.011 mm$^2$ (Appendix Fig. 10 and Visualization 2). As expected, cellular deformation only initiated after Ca$^{2+}$ influx and terminated once the Ca$^{2+}$ concentrations decreased to resting values. Beat-to-beat intervals for both measurements showed no significant difference (Fig. 4(c)). Direct comparison of both techniques demonstrates the value of RCD measurements as a label-free alternative to calcium imaging for detection of several contractile parameters. Moreover, RLFI is not hampered by dye bleaching and label-induced cytotoxicity. This allows to monitor cells over long periods and in physiological conditions.

![Fig. 4. (a) Schematic of reflective lens free imaging in combination with a fluorescent microscope for synchronous measurements of the relative cellular deformation and relative intracellular Ca$^{2+}$ concentration. (b) Averaged relative cellular deformation (blue trace, area of 0.3 mm$^2$) of cardiomyocytes calculated over 9 contractions show simultaneous relative intracellular Ca$^{2+}$ concentration (DF/FO; yellow trace; area of 0.11 mm$^2$) and (c) no significant variations in beat-to-beat duration (p = 0.55, Wilcoxon matched-pairs signed rank test, n = 8 contractions).](image)

3.4 Evaluation of the inotropic effects of blebbistatin

Contractility is a major pre-clinical and clinical parameter to assess normal and deficient contractile performance of the heart [46]. The RLFI system was thus evaluated as a platform to detect drug-induced effects on contractility. Cardiac cultures were monitored while subjected to increasing concentrations of blebbistatin, a highly specific myosin II inhibitor that weakens cellular contraction. We observed both a steady decline in the RCD amplitude and duration (Fig. 5(a)) and in RCDr during contraction and relaxation (Fig. 5(b)). Figure 5(c) shows the change in RCD as function of the concentration of blebbistatin. The RCD amplitude decreased to 50%, 17% and 1.7% when exposed to concentrations of 500 nM, 1 µM and 5 µM, respectively (IC$^{50}$ was 451 nM). Furthermore, the reduction in RCDr was more prominent during the contraction phase than during the relaxation phase (IC$^{50}$ were 381 nM and 612 nM, respectively). This demonstrates that lens-free-based contractility
measurements can detect and quantify the blockage of the cellular contractile apparatus. Furthermore, the deformation of cell monolayers is analyzed in small regions over the whole chip surface and is not limited by electrode geometry and dimensions, such as with EIS [19].

Fig. 5. Reduction of amplitude and duration of (a) averaged relative cellular deformation curves and (b) averaged rates of relative cellular deformation upon increasing concentrations of blebbistatin. (c) Relative cellular deformation amplitude in function of blebbistatin concentrations (averaged over 12 regions). An IC50 value of 451 nM was obtained by sigmoidal dose-response curve fitting. (d) Rate of relative cellular deformation during contraction (full line) and relaxation (dashed line) in function of blebbistatin concentration (averaged over 12 regions). A lower IC50 value was obtained for the contraction rate amplitude than the relaxation rate amplitude (381 nM vs 612 nM).

3.5 Simultaneous electrophysiological and contractile measurements

Fig. 6. (a) Schematic of reflective lens free imaging system combined with the CMOS-based microelectrode array. (b) Simultaneous detection of intracellular action potentials by MEA (red trace) and relative cellular deformation by RLFI (blue trace; area of 0.124 mm2). (c) Averaged MEA and RLFI signals measured by the same electrode under conditions of (I) cell medium (n = 10 contractions), (II) cell medium containing 0.015% DMSO (n = 12), and (III) 5 µM blebbistatin (n = 6).

The heart’s ability to pump blood through the body is driven by electrical impulses that are generated by pacemaker cells and propagate through the cardiac tissue. Therefore, both electrical and contractile parameters are necessary to obtain more detailed insight into cardiotoxicity evaluation. Thus, we combined the RLFI system’s ability to measure contraction on opaque surfaces with an in-house developed CMOS-based microelectrode array (MEA) system (Fig. 6(a)). The system offers a high density pattern of microelectrodes [39]. Using this system, electrodes were individually selected to locally electroporate individual cardiomyocytes and obtain single-cell intracellular recordings. The RCD was simultaneously measured in a region of 0.124 mm2 around the electrode of interest and both signal traces were synchronized in post processing. As shown in Fig. 6(b), the combined setup detected that the RCD signal (blue trace) followed the intracellular action potential (AP, red trace), albeit with a small delay, and terminated before the repolarization phase of the action potential (Appendix Fig. 11 and Visualization 3). After blebbistatin exposure, AP and cellular deformation were detected in both cardiac medium and DMSO containing medium (0.015%) (Fig. 6(c), panel I and II). However, once incubated in 5 µM blebbistatin, deformation was inhibited while APs remained present (Fig. 6(c), panel III). The combined
system thus allows for high-quality and non-invasive evaluation of the relationship between contraction and intracellular electrical activity, and impairment of the excitation-contraction coupling.

3.6 Monitoring of excitation wave propagation by RLFI

The excitation wave in a cardiac monolayer can serve as a model for the in vivo situation in the heart, as well as for conditions where conduction is blocked or impaired [47]. However, it is not straightforward to investigate this phenomenon by imaging methods: the wave is typically very fast (>300 mm/s) [48] and lens-based systems are limited by their FOV or resolution [49]. As RLFI can measure deformation of cardiac cells over a large FOV with high temporal resolution, the RLFI system was used to evaluate excitation propagation velocity (EPV) and directionality. Interference patterns of beating cardiac monolayers were recorded over a region of 31.8 mm², and the FOV was divided into 256 areas of 0.124 mm². The delays between the initiation of contraction were determined over all the ROI and averaged over multiple contractions. The excitation propagation profile was plotted as a color-coded map (Fig. 7(a)). The average EPV was of 351 ± 7 mm/s. The RCD measurement had, in this case, a non-reconstructed resolution of 352 µm. If necessary, the resolution can be increased by lowering the ROI size. This would result in lower signal-to-noise ratios.

To validate the RCD measurements of the excitation wave propagation with electrophysiological measurements, the RLFI system was implemented onto a passive MEA system. The extracellular field potential and RCD were simultaneously measured over 12 electrodes spread over a length of 4.4 mm (red and blue traces in Fig. 7(b), respectively). Both techniques allowed to calculate the signal delay over the sample (Fig. 7(c), RCD, blue box plots; electrical field potentials, red box plots) and no significant difference in EPV was detected (370 ± 1 mm/s and 345 ± 32 mm/s, respectively).

3.7 Detecting propagation disturbances caused by 1-octanol

To evaluate the potential of RLFI to detect conduction disturbances, cardiac cultures were subjected to varying concentrations of 1-octanol (Fig. 8(a), Appendix Fig. 12 and Visualization 4, Visualization 5, Visualization 6, and Visualization 7). This organic
compound reduces EPV by uncoupling the gap junctions responsible for electrical connections between cells [50]. No significant changes in EPV were detected at low concentrations (Dunn’s multiple comparison test). At higher concentrations (≥ 100 µM), the EPV decreased significantly (Fig. 8 (b)). As expected, uncoupling of gap junctions occurs at these concentrations which leads to both higher resistance for electrical signal propagation and impaired cellular deformation. At a concentration of 1 mM, conduction blocks prevented the excitation wave propagating through the monolayer and excitation waves branched off into secondary slower conductive areas (Visualization 7). No cellular deformation was detected at higher concentrations (≥ 4 mM). Therefore, RLFI is a promising technique to study fast cellular processes such as excitation waves and impairment of signal transduction in cardiac monolayers.

Fig. 8. (a) Color coded map of excitation wave propagation delay in the cardiac monolayer under following conditions: cell medium (I, n = 14 contractions), 1 µM octanol (II, n = 14) and 400 µM octanol (III, n = 6). (b) Propagation velocity reduction in the presence of increasing concentrations of 1-octanol (IC50 was 45 µM).

4. Conclusion

We developed an in-line digital holography based RLFI system to monitor biological activity on Si chips directly in an incubator. Analysis of interference patterns of spontaneously beating cardiac cells cultured on opaque surfaces through optical flow algorithms resulted in detailed cellular parameters such as relative cellular deformation and relative cellular deformation rate. The relevance of RCD as a non-invasive sensor for contractility was shown through simultaneous measurements with fluorescent Ca²⁺ imaging. Additionally, this system shows potential in evaluating fast cellular processes such as excitation waves and impairment of signal transduction in cardiac monolayers. The system detected drug-induced cardiotoxic effects of both the inotropic effects of blebbistatin and conduction disturbances due to 1-octanol. Furthermore, the RLFI system is compact and compatible with other readout systems and offers the resolution needed to study cellular processes such as contractility in detail. By combining the system with high-density CMOS-based MEAs, we developed a high-resolution, label-free to detect excitation-contraction coupling events in cellular monolayers. Finally, we envisage that the combined RLFI-MEA platform will benefit preclinical drug screening by granting unique, multi-parametric insight into drug-induced cardiotoxicity in a non-invasive manner.
5. Appendix

Appendix figures

Fig. 9. Confocal images of cardiac cells monolayers. (a) The contractile apparatus was well developed and sarcomeres were clearly visible (nucleus: Hoechst, blue, α-actinin: anti-sarcomeric primary -α-actinin antibody (Ab) and Alexa Fluor goat anti-mouse Ab, cyan). (b) Cardiac cells were electrically coupled over the monolayer (nucleus: Hoechst, blue, gap junction: anti-Cx43 Ab, Alexa Fluor goat anti-rabbit Ab, red). Scale bar: 50 µm.

Fig. 10. Simultaneous monitoring of cardiac contraction through calcium imaging (green box, 0.11 mm²) and RLFI (grayscale box, 0.3 mm²) in a combined RLFI-fluorescence microscope setup (see Visualization 2). The full sequence of images was processed to obtain the relative intracellular calcium concentration ([Ca²⁺], green trace) and relative cellular deformation (RCD, blue trace). Higher [Ca²⁺] and RCD values were obtained during contraction compared to in between beats. Video stills are shown at (a) 2.89 s and at (b) 6.70 s, and video recordings are shown at actual frame rate.

Fig. 11. Simultaneous electrophysiological and contractile monitoring through a combined CMOS-based MEA and RLFI setup (see Visualization 3). Interference patterns were recorded by RLFI over the full MEA surface (15.7 mm²) and further processed. Intracellular action potential (AP) of single cells were synchronized with the relative cellular deformation (RCD) of a cluster of cells located in a region around the electrode of interest (white square, 0.3 mm²). Video stills are shown at (a) 2.02 s and at (b) 3.04 s, and video recordings are shown at 0.5x actual frame rate.
Fig. 12. Detection of conduction disturbances in excitation wave propagation by increased concentration of 1-octanol: (a): 0 µM, Visualization 4; (b): 1 µM, Visualization 5; 10 µM and 100 µM not shown; (c): 400 µM, Visualization 6; and (d): 1 mM, Visualization 7; 0.1x actual velocity; scale bar: 2 mm). To visualize the excitation wave propagation, sequences of interference fringes obtained by RLFI were processed pixel intensity variation scripts. Briefly, pixel intensity variations were smoothened and locally normalized over regions of 0.05 mm². (a) The propagation of the excitation wave over the cellular monolayer was recorded in cardiac medium and (I) cells were initially relaxed. (II) Contraction is first detected in the left side of the monolayer and (III) the excitation wave propagated to the right over the monolayer leading to over a large area of the monolayer. Then, (IV) contraction is detected over the full monolayer until (V) the cells relax. (VI) Cells were again fully relaxed between contractions. From RCD analysis, an excitation propagation velocity (EPV) of 238 ± 8 mm/s and a constant directionality were detected (white arrow). (b) At 1 µM, the directionality of the excitation wave remained unchanged, while EPV reduced to 219 ± 16 mm/s. (c) At 400 µM, EPV reduced to 83.8 ± 1.7 mm/s, while the direction of the excitation wave remained similar. (d) At 1 mM, prominent conduction disturbances became apparent. The excitation wave broke off into multiple slower branches (white arrows in panels II, III, and IV), while the EPV of the main branch slowed to 14 ± 2 mm/s.

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Disclosure

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