Pulse Application Note

Evaluation of Drug-Induced Cardiotoxicity Through Contractility Analysis

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1. Introduction

Recent advances in stem cell technologies have enabled routine analysis of patient-derived cardiomyocytes, opening up new opportunities for drug safety and efficacy testing. Numerous studies have demonstrated that induced pluripotent stem-cell-derived cardiomyocytes (iPSC-CMs) display physiologically relevant characteristics and recapitulate aspects of patient cardiac pathology/phenotypes in vitro [1-4]. Improved cell-culturing technologies now allow production of well-characterized cardiomyocytes at scale, providing a reliable source for routine screening applications. Therefore, accurate and reliable characterization of these cells, and their response to different chemical compounds, plays a critical role in their successful utilization in drug development and safety testing.

Pulse applies patented computer vision algorithms [5-7] to measure contraction of cells from videos of cardiomyocytes. Contraction velocity and displacement are measured over time using optical flow and correlation analysis. Noisy signals are rejected using a deep learning model that has been trained on many signals across a large number of experiments. Signal processing techniques are then used to generate robust and automated measurements of beating parameters such as beat rate, contraction displacement, contraction velocity, duration of contraction and relaxation, and more.

In this application note, we present a case study for using Pulse in high throughput to measure contractility parameters for an experiment testing a panel of drugs with known cardiotoxicity profiles.

2. Materials and Method

iPSC-derived cardiomyocytes from Cellular Dynamics International (CDI) were cultured on standard 384-well plates at Tenaya Therapeutics. Multiple doses of 10 different compounds (Aspirin, Bortezomib, Doxorubicin, Erlotinib, Quinidine, SAHA, Sorafenib, Terfenadine, Verapamil, and a test drug) were applied, in addition to DMSO only (**Figure 1**). Brightfield videos at each well were captured by an SI8000 Cell Motion Imaging System with duration of 8-seconds per video, at 3 different timepoints: at baseline, and after 24 hours and 48 hours of drug exposure. Videos of beating cardiomyocytes were automatically uploaded using the Pulse desktop application software and were analyzed using Pulse for measuring contractility parameters.

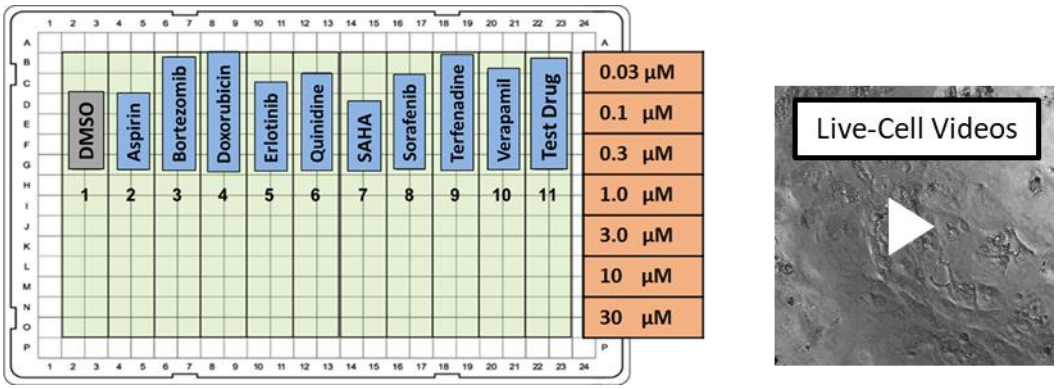

Figure 1 Experimental layout. Multiple doses of drugs were applied to iPSC-CMs and live cell videos were captured at baseline, and after 24 hours and 48 hours of drug exposure. Videos were analyzed with Pulse.

3. Results

Figures 2 shows an example of signals estimated for each video. The top signal is the contraction velocity (pixels/second), where typically the contraction and relaxation show distinct peaks in every beat. The signal on the bottom is the contraction displacement (pixels), which is the basis for automated measurements. (The displacement can easily be converted from pixels to microns by applying a calibration factor from the microscope.) **Figures 3** and **4** show the heatmaps of parameters measured by Pulse at baseline and after 24hrs and 48hrs of exposure.

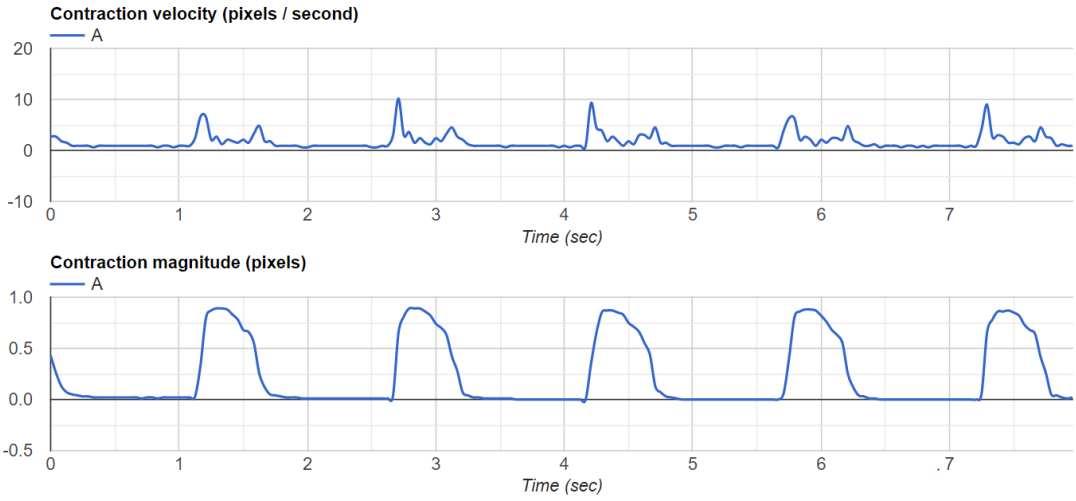

Figure 2 Example signals generated for each video. Top: contraction velocity in pixels/sec, bottom: contraction magnitude in pixels.

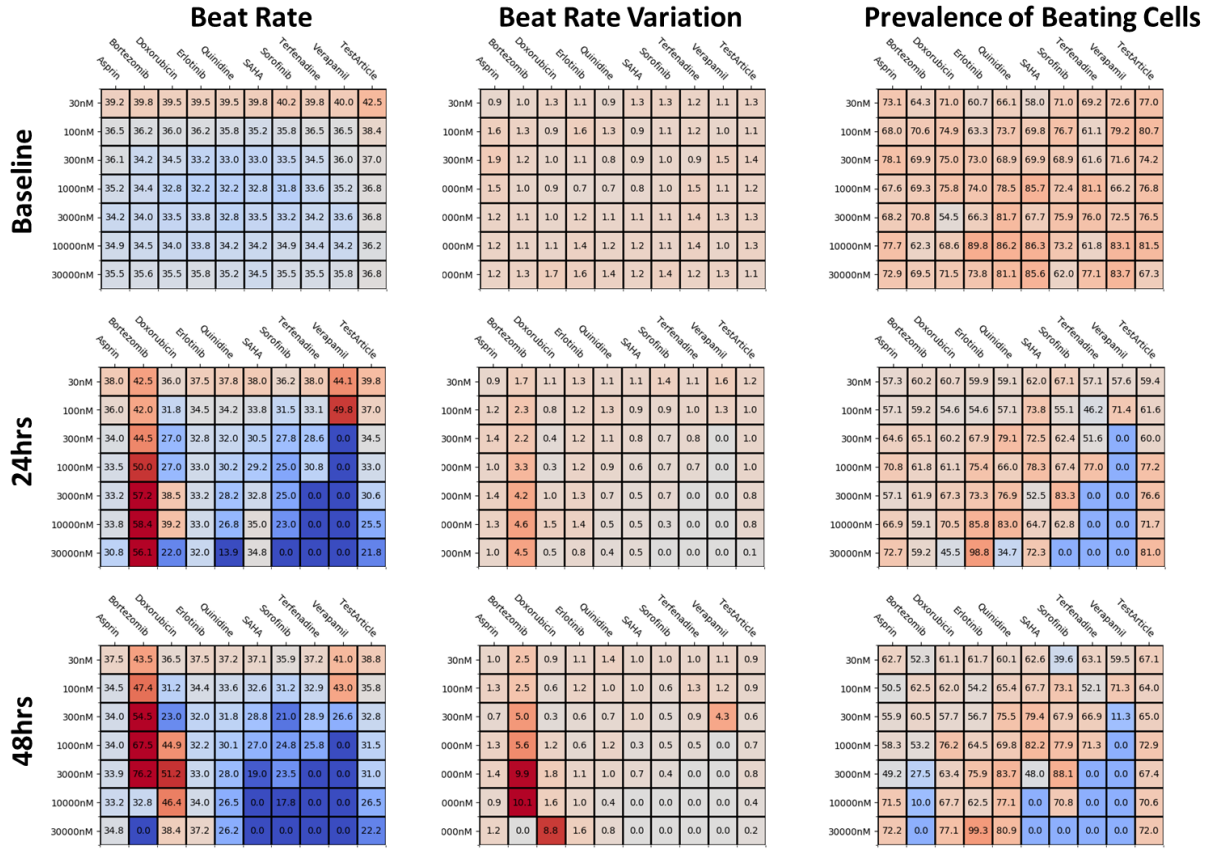

Figure 3 Parameters measured by Pulse at baseline, and after 24 hours and 48 hours of drug exposure. Shown are beat rate (beats/minute), beat rate variation (a measure of arrhythmia), and prevalence of beating cells (area of beating cells to the total field of view). Blue indicates decrease in value, and red indicates increase in value.

As shown in **Figures 3** and **4**, the baseline Pulse measurements for all parameters are consistent across the plates, with small variations at the border wells. For Aspirin, no significant changes to any beating parameters were observed at either 24 or 48 hours of exposure. For Bortezomib, an anticancer therapeutic with known cardiotoxicity, Pulse shows an increase in beat rate and beat rate variation, and a decrease in contraction, duration, and prevalence of beating cells at both 24 and 48 hours of exposure. For Verapamil, a calcium channel blocker, Pulse shows a decrease in beat duration and displacement at lower doses while higher doses cause inhibition of contraction and cell death. Conversely, for Quinidine, a sodium channel blocker and hERG inhibitor, Pulse shows a significant increase in total beat duration, which is more pronounced in the duration of relaxation. Finally, the test drug shows a slight decrease in beat rate but otherwise unaffected contractility compared to the panel of drugs.

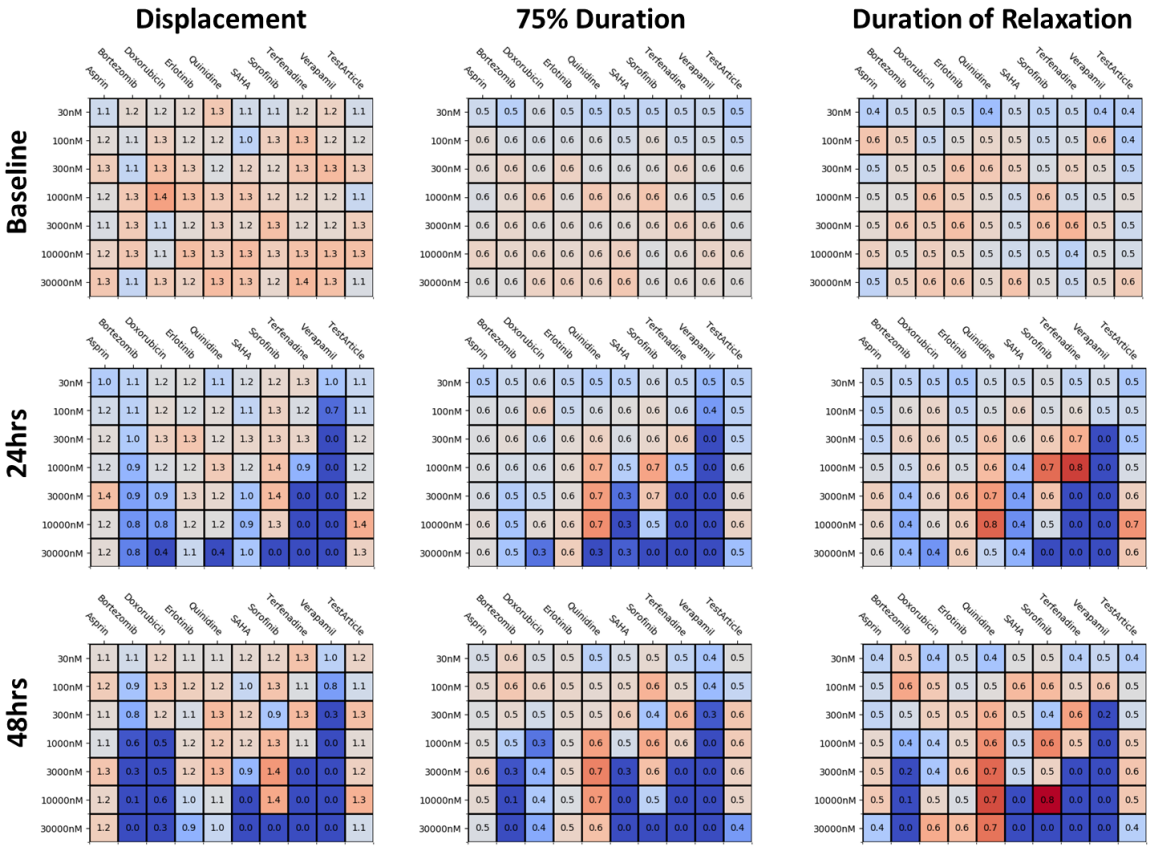

Figure 4 Parameters measured by Pulse at baseline, and after 24 hours and 48 hours of drug exposure. Shown are contraction displacement (pixels), 75% beat duration (seconds), and duration of relaxation (seconds). Blue indicates decrease in value, and red indicates increase in value.

4. Conclusion

Using Pulse, we have demonstrated recapitulation of well-characterized drug effects in iPSC-derived cardiomyocytes. The Pulse contractility assay can be used for high-throughput cardiotoxicity screens and studying physiologically relevant disease phenotypes. Pulse is completely automated, making it easy to use and amenable to high-throughput studies, without the need for extensive post-acquisition data processing. Video-based analysis is non-invasive to the cells, enabling longitudinal and long-term studies on the same cell populations. In addition to contractility analysis with brightfield videos, Pulse can be used to measure calcium signaling with fluorescent videos. In summary, Pulse can be used in conjunction with any imaging system and is the most reliable software currently on the market for measuring contractility and calcium signaling in high throughput.

5. References

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6. Maddah M., Loewke K. Non-Invasive Characterization of Stem Cell-Derived Cardiomyocytes from Phase-Contrast Microscopy. Proceedings of Medical Image Computing and Computer Assisted Intervention (MICCAI), 2014.

7. Maddah M. et al., A Non-invasive Platform for Functional Characterization of Stem-Cell-Derived Cardiomyocytes with Applications in Cardiotoxicity Testing. Stem Cell Reports. 4(4), 621-631, 2015 (Cover Image.)