

# IN VITRO ASSESSMENT OF DRUG EFFECTS ON HUMAN IPSC-DERIVED CARDIAC SPHEROID CULTURES

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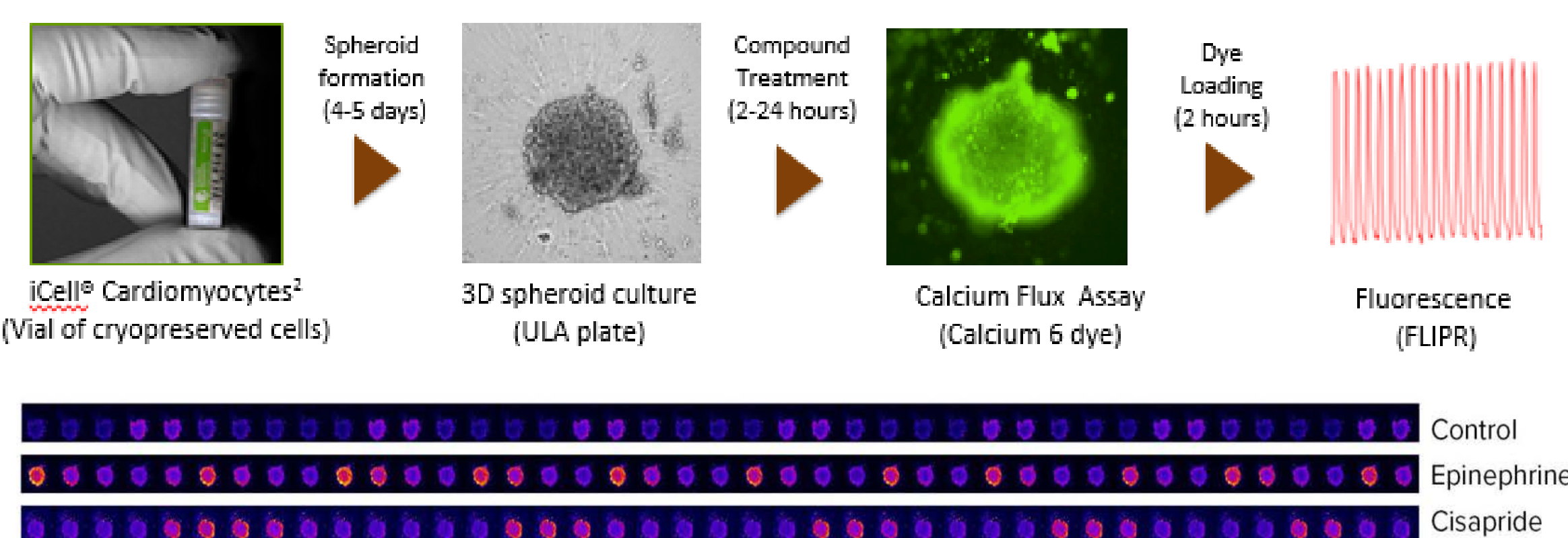
## Introduction

There is increasing interest in exploring the use of three-dimensional (3D) cell culture for modeling developmental and tissue biology with the goal of accelerating translational research. Such 3D models can provide different perspectives from traditional 2D cultures on the responses of cells and tissues to drug treatments. Accordingly, the development of quantitative assays in higher throughput using 3D cultures is an important area of investigation. In this study, we developed methods for the formation of 3D cardiac spheroids using human iPSC-derived cardiomyocytes (iCell Cardiomyocytes<sup>2</sup>). We used high content imaging and fast kinetic fluorescence imaging on the FLIPR<sup>®</sup> Tetra System to measure the impact of various compounds on the beating patterns and rates of cardiac spheroids as monitored by changes in intracellular Ca<sup>2+</sup> levels with calcium-sensitive dyes. We tested a set of known cardioactive and cardiotoxic compounds, including  $\alpha$ - and  $\beta$ - blockers, cardiac glycosides, ion channel blockers, anti-cancer drugs, and compounds with other mechanisms of action. This assay was optimized for HTS in 384-well plates and allows for the characterization of cardiac spheroid beating profiles by using multi-parametric analysis with outputs such as beat rate, peak frequency and width, and waveform irregularities. In addition, the impact of drug treatment on cell viability and mitochondrial integrity was evaluated by high content imaging. To further evaluate the impact of 3D culture on cardiomyocyte responses, we compared the effects (EC<sub>50</sub> or IC<sub>50</sub> values) of different compounds in 3D versus 2D culture formats and demonstrated significant differences in assay sensitivity to compound-induced effects. In conclusion, we demonstrated that 3D cardiac spheroids formed with human iPSC-derived cardiomyocytes can be used for drug development and toxicity assessment.

## Goals of the Study

- In this study, we describe the methods for the formation of 3D cardiac spheroids using human iPSC-derived cardiomyocytes and illustrate how they can be used in phenotypic assays for cardiac toxicity assessment.

## 3D Spheroid Model Workflow



**Figure 1.** Schematic diagram of 3D cardiac spheroid formation and the Ca<sup>2+</sup> flux imaging assay workflow (top). Time-lapse image series of contracting iPSC-derived cardiomyocyte spheroids loaded with a Ca<sup>2+</sup> sensitive dye. Each image was taken at 100 millisecond intervals. Pink with yellow at the edges in the false color scale indicates a high Ca<sup>2+</sup> concentration and a state of contraction.

## Materials & Methods

### Human iPSC-derived CMs: iCell® Cardiomyocytes<sup>2</sup>

Cryopreserved cells from Cellular Dynamics International (CDI) were used. Cells were thawed and plated at 20,000/well (96-well format) or 10,000/well (384-well format) into ultra-low attachment (ULA) plates (Corning) and incubated for 4 days in maintenance media to form spheroid cultures. The presence of strong synchronous contractions in the 3D cultures was confirmed visually prior to running experiments.

### High-Content Imaging

Image acquisition was done on an ImageXpress<sup>®</sup> Micro XL-C System to obtain time-lapse images using these parameters: 10 reads/second, 5-10 second reads or longer, and 20x or 10x magnification with FITC excitation and emission filters. Cells were kept under environmental control (37 °C, 5% CO<sub>2</sub>).

### Ca<sup>2+</sup> Flux Assay on the FLIPR Tetra System

FLIPR<sup>®</sup> Calcium 6 Dye (Molecular Devices, LLC) was used to monitor changes in Ca<sup>2+</sup> fluxes synchronous with cell beating. Reagent (2X concentration) was added to the plates and incubated for 2 hours at 37 °C, 5% CO<sub>2</sub>. A pre-drug read was acquired at ~8 frames per second using a FLIPR Tetra System using 485 nm excitation and 530 nm emission settings. Additional reads were acquired during and after compound addition at prescribed times (~ 2 min read times).

## References

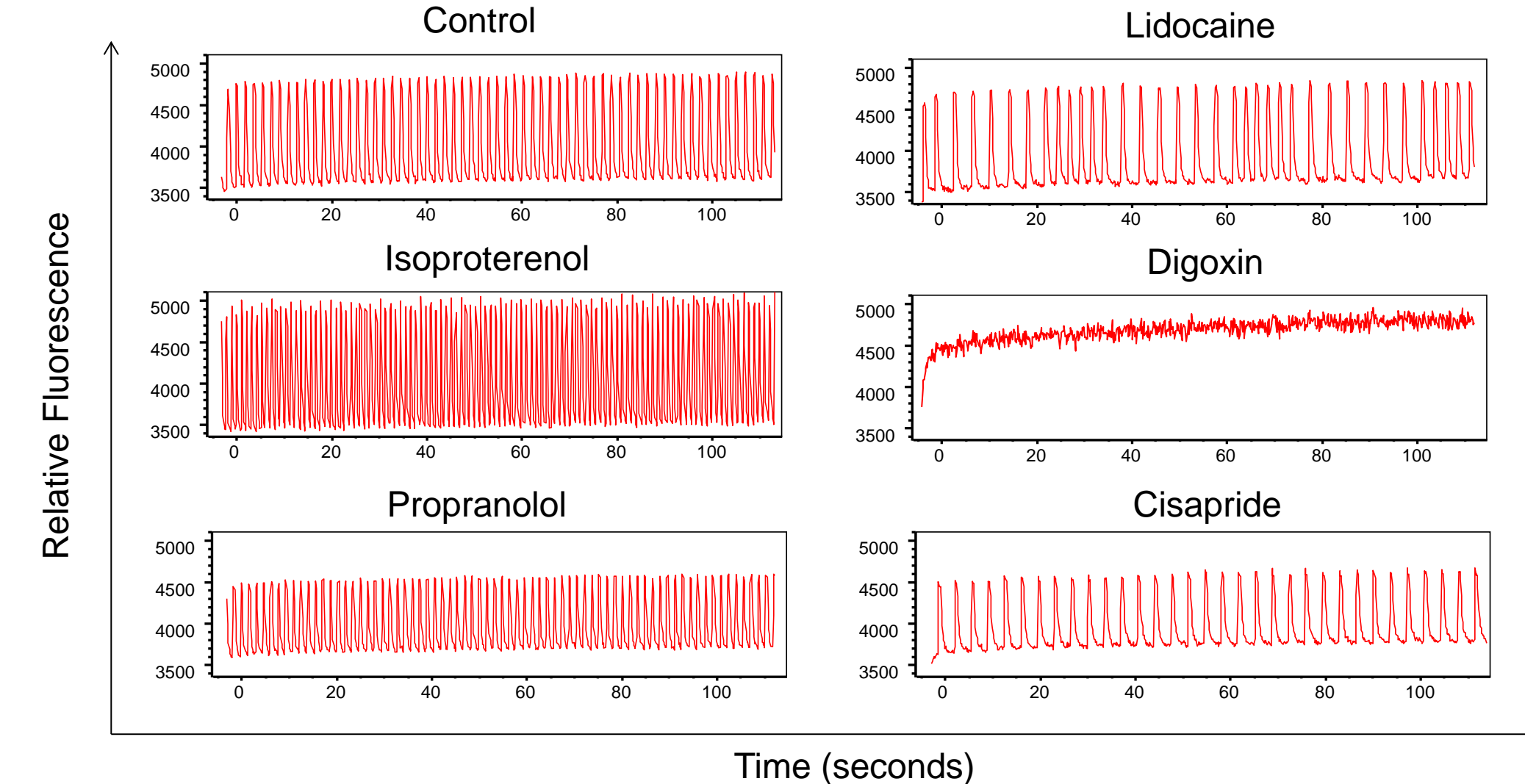
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## Results

### Calcium Flux Assay in 3D Spheroids

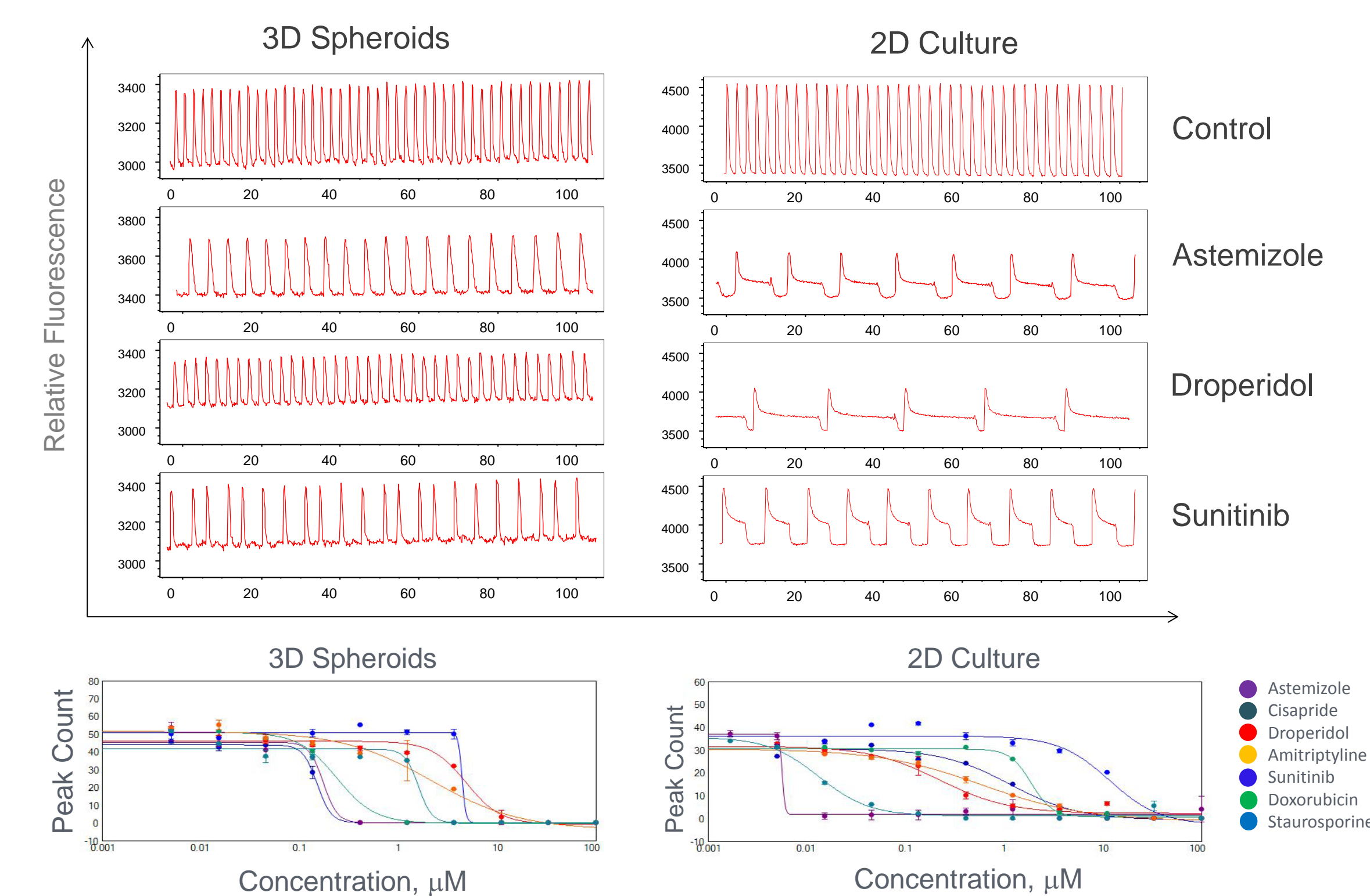
Cardiac spheroids were efficiently formed from iPSC-derived cardiomyocytes and started contracting spontaneously after 3-4 days in culture. These 3D cell models could then be used for cardiotoxicity assessment by staining them with a Ca<sup>2+</sup> sensitive dye. Changes in the fluorescent intensities in response to Ca<sup>2+</sup> flux were used as a surrogate marker for spheroid contraction. Significant alterations to the control beating pattern were observed in response to cardioactive and cardiotoxic compounds.

Effects on cardiophysiology were assessed using the FLIPR Tetra System. Cells were loaded with FLIPR Calcium 6 dye and incubated for 2 hours. Spheroids were exposed to compounds for 1 hour, 24 hours, or 5 days. Automatic data analysis was performed using the FLIPR PeakPro software.



**Figure 2.** Representative Ca<sup>2+</sup> oscillation traces (by FLIPR Tetra system) for 3D cardiac spheroids in response to cardio-active compounds. Traces presented are from assaying 1  $\mu$ M test compound concentrations, with the exception of lidocaine (10  $\mu$ M), after 60 minutes of exposure. Phenotypic changes observed included: positive or negative chronotropic and inotropic effects with isoproterenol and propranolol, blocking of beating and Ca<sup>2+</sup> flux with digoxin, prolongation of repolarization with cisapride, and beating irregularity with lidocaine.

### Comparison of Compound Effects Between 3D Spheroid and 2D Assays



**Figure 3.** Comparison of the Ca<sup>2+</sup> flux measurements between 3D spheroid and 2D cultures of cardiomyocytes treated with select compounds. Top: traces presented are from assaying 1  $\mu$ M concentrations of compounds (staurosporine 0.3  $\mu$ M) after 24 hours of exposure. Bottom: dose response curves for select compounds after 24 hours exposure are based on changes in peak count. EC<sub>50</sub> values were shifted toward greater concentrations in 3D.

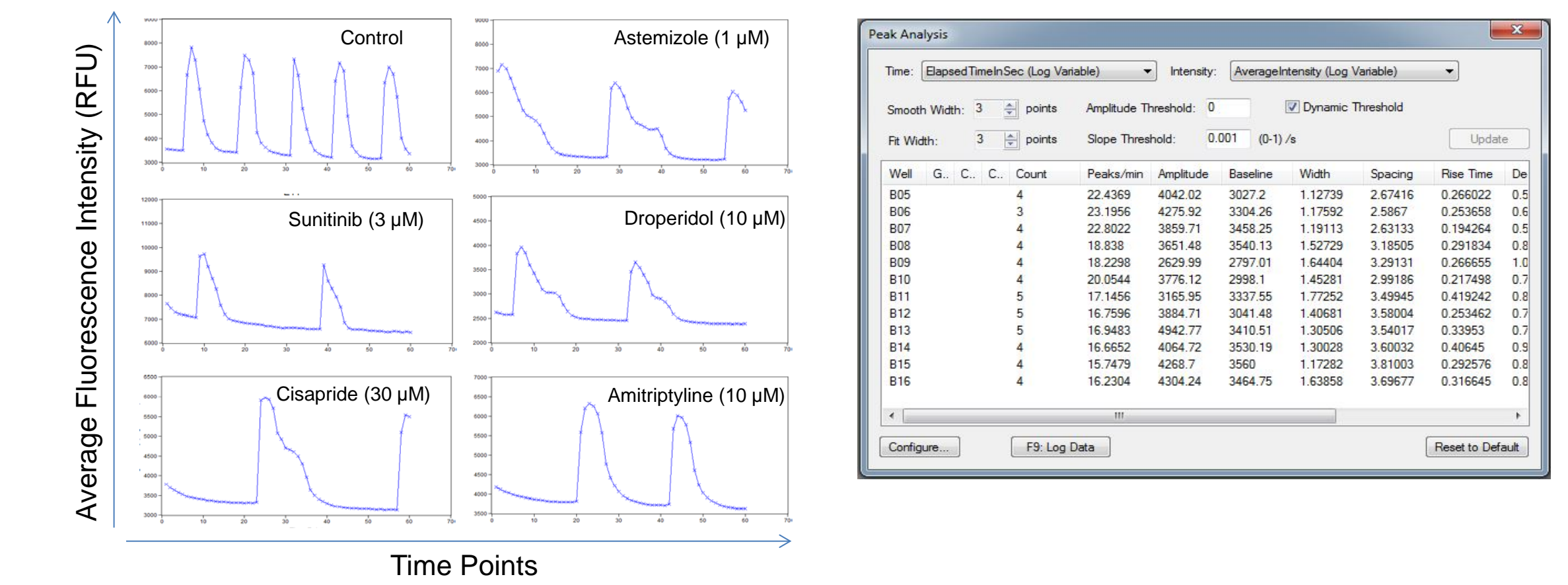
**Table:** Comparison between 3D spheroid and 2D cultures. IC<sub>50</sub> values (in  $\mu$ M) measured for tested compounds using peak count as a read-out.

Compounds/EC50s	60min		24h		Compound description
	3D Spheroids	2D Culture	3D Spheroids	2D Culture	
Amiripryline	0.446±0.04	0.153	0.24±0.032	1.901±0.76	Serotonin-Norepinephrine Reuptake Inhibitor
Astemizole	1.52± 0.26	0.011±0.002	0.176±0.086	0.006±0.008	Antihistamine/ H1 Receptor Antagonist
Droperidol	1.55±1.21	1.46±0.097	4.92±0.946	0.223±0.061	Antipsychotic/ Antagonist of Dopamine receptors
Haloperidol	4.24±3.6	0.731±0.155	8.3	1.04±0.21	Antipsychotic/ Antagonist of Dopamine receptors
Cisapride	4.27±0.73	0.013±0.005	1.61±1.8	0.013±0.002	5-HT4 Receptor Agonist
Sunitinib	1.342	1.082±0.18	2.18±0.68	0.605±0.094	Antineoplastic/ Tyrosine protein kinases inhibitor
Imatinib	10.74	11.73	26.38	3.29±0.566	Antineoplastic/ Tyrosine protein kinases inhibitor
Staurosporine	3.7±1.94	0.109	0.152±0.02	1.16±0.28	Antineoplastic/ Kinase Inhibitor
Doxorubicin	no effects	no effect	10.92	11.83±3.81	Antineoplastic/ DNA Intercalator
Paclitaxel	>100	>100	>100	26.39	Antineoplastic/ Microtubule Polymer Stabilizer
Hexylresorcinol	no effect	no effect	no effect	no effect	Antiseptic
Busulfan	no effect	no effect	no effect	no effect	Antineoplastic/ Alkylating Agents
Etoposide	no effect	no effect	no effect	no effect	Antineoplastic/ Other mechanisms
Leucine	no effect	no effect	no effects	no effect	Cardio safe control
Sorbitol	no effect	no effect	no effects	no effect	Cardio safe control

## High-Content Imaging

### Characterization of Beating Patterns by ImageXpress Micro 4 System

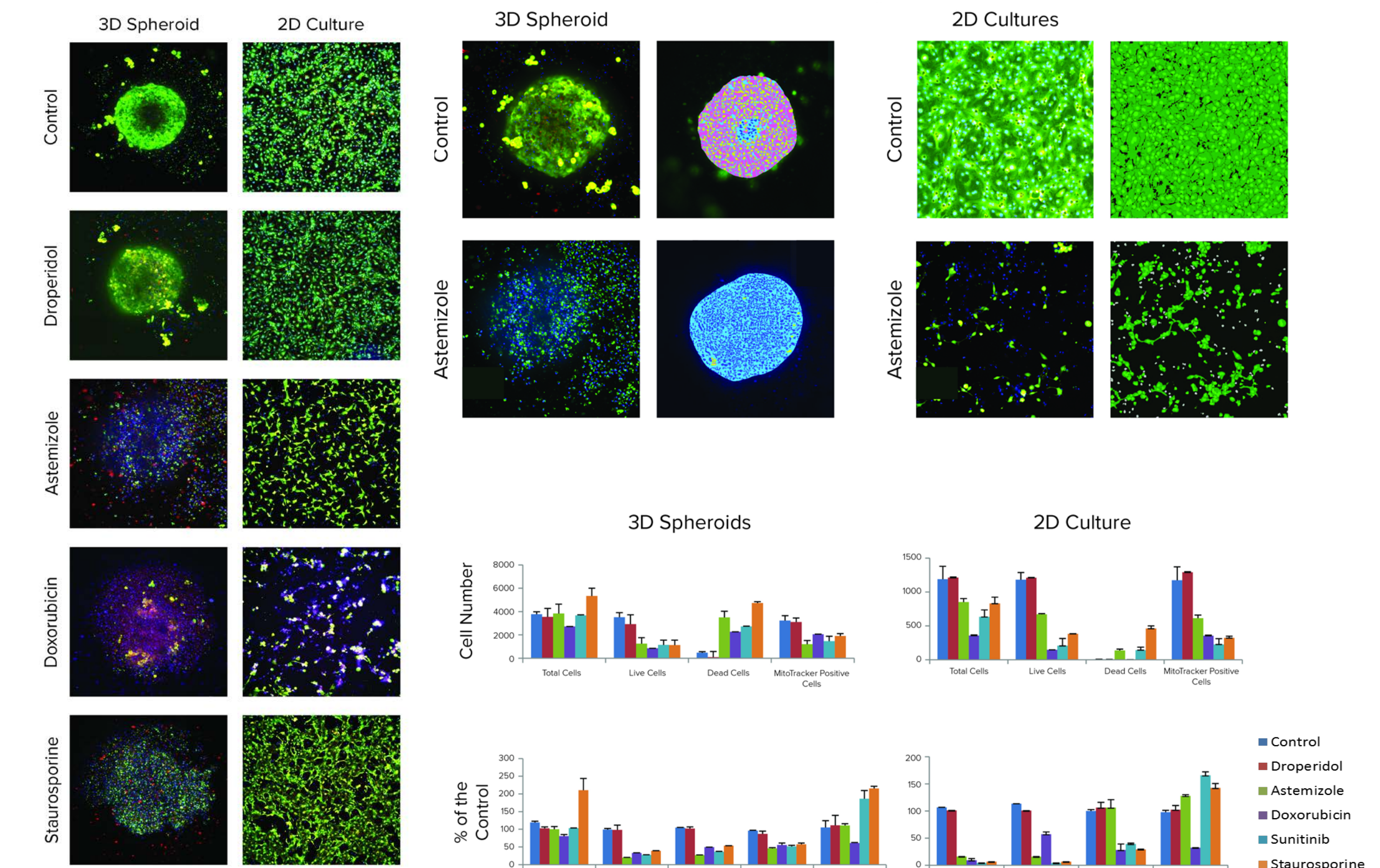
Effects on cardiophysiology were assessed using the ImageXpress Micro 4 System (widefield). Cells were loaded with FLIPR Calcium 6 Dye and incubated for 2 hours. Automatic data analysis was performed using the MetaXpress software.



**Figure 4.** Left panel: Examples of Ca<sup>2+</sup> flux patterns measured from iPSC-derived 3D cultures treated with select compounds. Measurements done using ImageXpress Micro 4 System. Right panel: The screenshot shows the user interface for analysis set-up and calculated read-outs

## Cytotoxicity Assays by High Content Imaging

Cytotoxicity was assessed after compound treatment by staining spheroids with a combination of viability dyes (Calcein AM, Hoechst, Ethidium homodimer) and then imaged using an ImageXpress Micro Confocal system. Images were analyzed via MetaXpress software, using 2D or 3D analysis options.



**Figure 5.** High content imaging examples of compound-induced cytotoxicity effects on 3D cardiac spheroids versus 2D cultures. Quantitation of drug treatment phenotypic responses for 3D cardiac spheroids versus 2D cultures was evaluated based on high content imaging measurements. Cells were treated for 24 hours with indicated compounds. Error bars represent standard deviations (n=3).

## Conclusions

- 3D cardiac spheroid models were generated using human iPSC-derived cardiomyocytes. 3D cardiac models enable the testing of compounds for potential cardiotoxicity via Ca<sup>2+</sup> flux detection on an ImageXpress Micro Confocal System or FLIPR Tetra System.
- We demonstrated the responsiveness of the 3D culture Ca<sup>2+</sup> flux assay to a panel of known cardioactive and cardiotoxic chemicals.
- A comparison of 3D versus 2D assay formats indicated altered pattern for Ca<sup>2+</sup> flux waveforms, in particular for potassium channel blockers. In addition, a significant right-shift was observed towards higher effective compound concentrations in 3D spheroid models.