

# MULTIPLEXED AUTOMATED IMAGING ASSAYS FOR COMPOUND TESTING USING INDUCED PLURIPOTENT STEM CELL-DERIVED CELLS

Oksana Sirenko, Felix Spira, Ryan Gordon\*, Grischa Chandy  
Molecular Devices LLC, San Jose, California, USA ; \*StemoniX Inc., Maple Grove, MN

## INTRODUCTION

There is an increased need for expanding variety and complexity of cell-based assays for biologic research and drug discovery. Stem cell-derived cells and tissues become an increasingly attractive alternative to traditional *in vitro* and *in vivo* testing in pharmaceutical drug development and toxicological safety assessment. In this study, we used human iPSC-derived cardiomyocyte and neuronal cell models to develop functional and morphological readouts for testing effects of different compounds in a multi-parametric assay format.

We used automated cell imaging and analysis with the ImageXpress® Pico Automated Cell Imaging System to simultaneously determine calcium oscillation frequency, cell viability, cytoskeletal integrity, apoptosis, and mitochondrial function. Effects on cardiomyocyte beating frequency were characterized by measurements of calcium oscillations. For neuro-spheroids, automated imaging was used to evaluate the number of live cells and monitor calcium oscillations using time-lapse imaging. Multiplexed assessment of different readouts provides additional insight of the mechanisms of action of various compounds. The methods were characterized using a set of cardio-active drugs, neurotransmitters, and selected neurotoxic or cardiotoxic compounds.

Our results demonstrate how a variety of assays can be utilized for quantitative screening of chemical effects in iPSC-derived cardiomyocytes and neuronal models and enable rapid and cost-efficient multidimensional biological profiling.

## INSTRUMENT

Cell-based assays were performed using the ImageXpress Pico system in combination with CellReporterXpress® Image Acquisition and Analysis Software. The imager provides 4 fluorescence channels plus transmitted light time-lapse capacity to enable automatic monitoring of cell proliferation, differentiation, compound toxicity, and a variety of other cell-based assays.

- **ImageXpress Pico** Automated Cell Imaging system
- Equipped with 5 fluorescence channels plus transmitted light; 4x-63x objectives; EC chamber and Z-stack capabilities
- **CellReporterXpress** Image Acquisition and Analysis Software
- Includes 20+ preconfigured assay protocols



## METHODS

### Environmental Control and Time-lapse monitoring:

ImageXpress Pico system equipped with environmental control chamber enables the control and monitoring of temperature, CO<sub>2</sub> and O<sub>2</sub> content, as well as, humidity. In combination with time-lapse imaging, it provides an efficient tool to perform live experiments, including hypoxia conditions.

**Cell Culture:** Human iPSC-derived cardiomyocytes or hepatocytes and the appropriate media were purchased from Cellular Dynamics International, Fujifilm Co. Cells were plated into 384-well black clear bottom plates at a density of 10,000 cells per well and cultured as recommended by protocols from CDI. Treatment with compounds was performed for 1h or 24h, or as indicated in the figures. Neurospheroids were obtained from StemoniX Inc (Maple Grove, MN).

**Cell Staining:** To visualize Ca<sup>2+</sup> oscillations, cells were loaded with the Calcium 6 dye (Molecular Devices). To assess phenotypic changes, live cells were stained using a mixture of three dyes: viability dye Calcein AM (1 μM), mitochondria potential dye MitoTracker Orange (0.2 μM), and nuclear dye Hoechst (2 μM) (all from Life Technologies). For visualizing the actin cytoskeleton, cells were fixed with 4% formaldehyde (Sigma) and stained with AlexaFluor 488 (AF488) labeled phalloidin stain. Neurons were stained with anti-TuJ-1 antibodies (BD Biosciences).

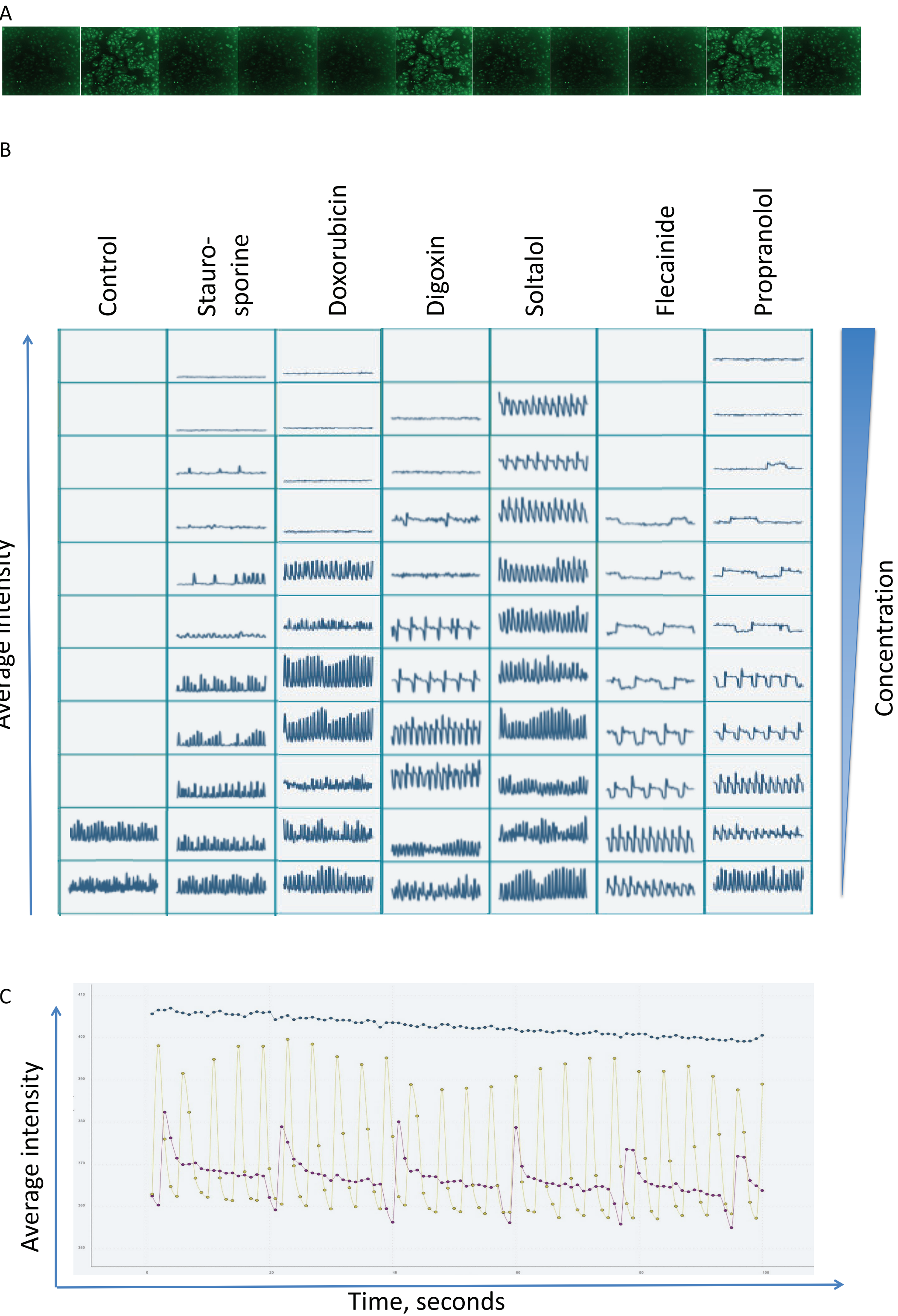
## STEM CELL-DERIVED CELL MODELS

There is an increasing interest in using stem cell-derived cell models in assay development. Stem cell-derived cardiomyocytes, liver cells, and neurons provide very useful models for compound testing and toxicity assessment. Cardiotoxicity and hepatotoxicity remain among two of the main reasons for drug attrition during clinical trials and post-marketing. In addition, a significant percentage of cardiovascular diseases are believed to be due to environmental exposures. Accordingly, an important target area for assay development for *in vitro* screening includes the detection of potential toxic effects.

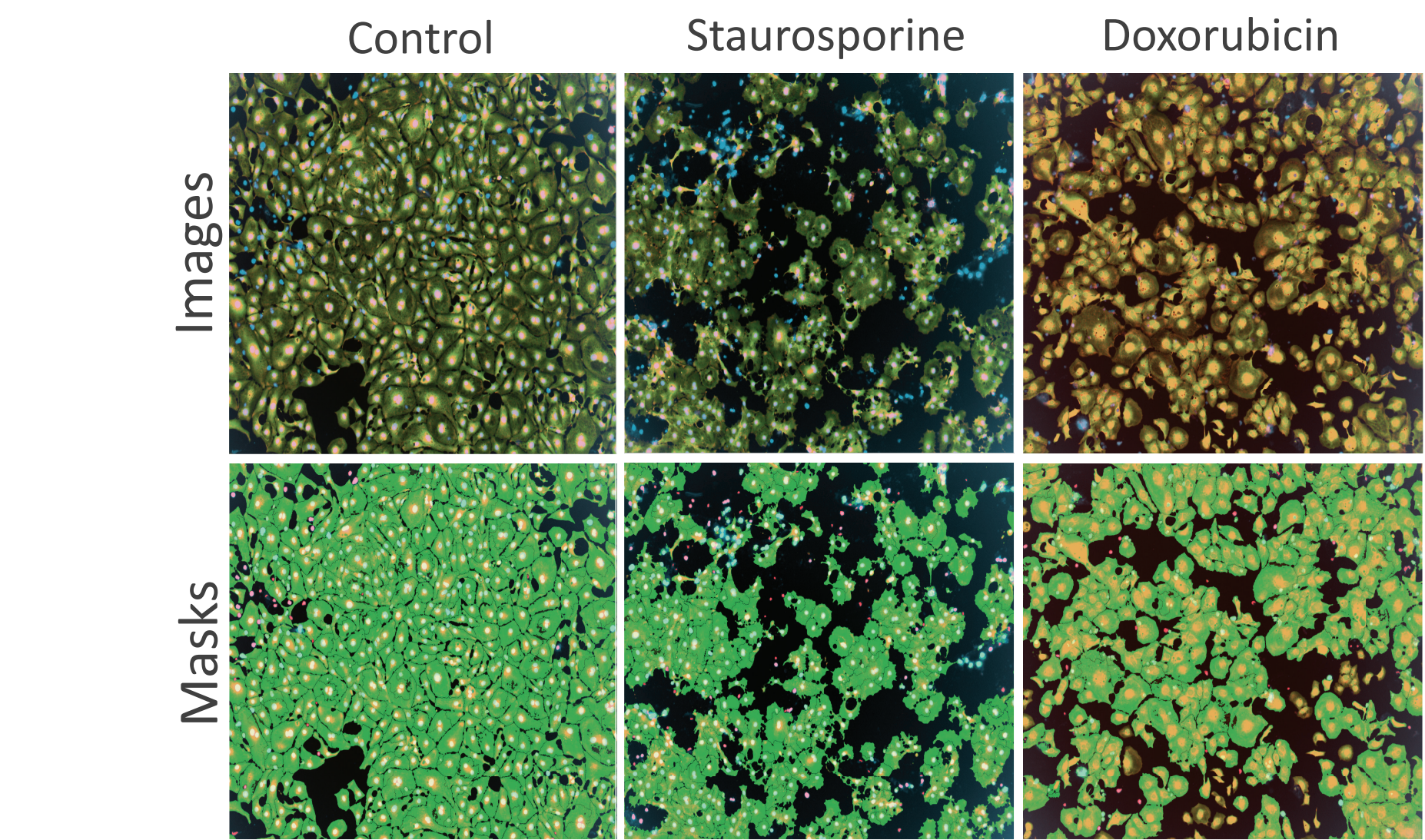
## RESULTS:

### Assessing Compound Effects on Calcium Oscillations in Cardiomyocytes

iPSC-derived cardiomyocytes are a very attractive *in vitro* model as they form a synchronously beating monolayer that can be used to reliably reproduce drug-associated cardio-physiologic phenotypes using a fast kinetic fluorescence assay that monitors changes in intracellular Ca<sup>2+</sup> flux (Grimm et al. 2016; Sirenko et al. 2013). In this work, we have adopted a calcium oscillation assay for a new imaging system, the ImageXpress Pico system, that allow live cell assays including time-lapse imaging and environmental control.



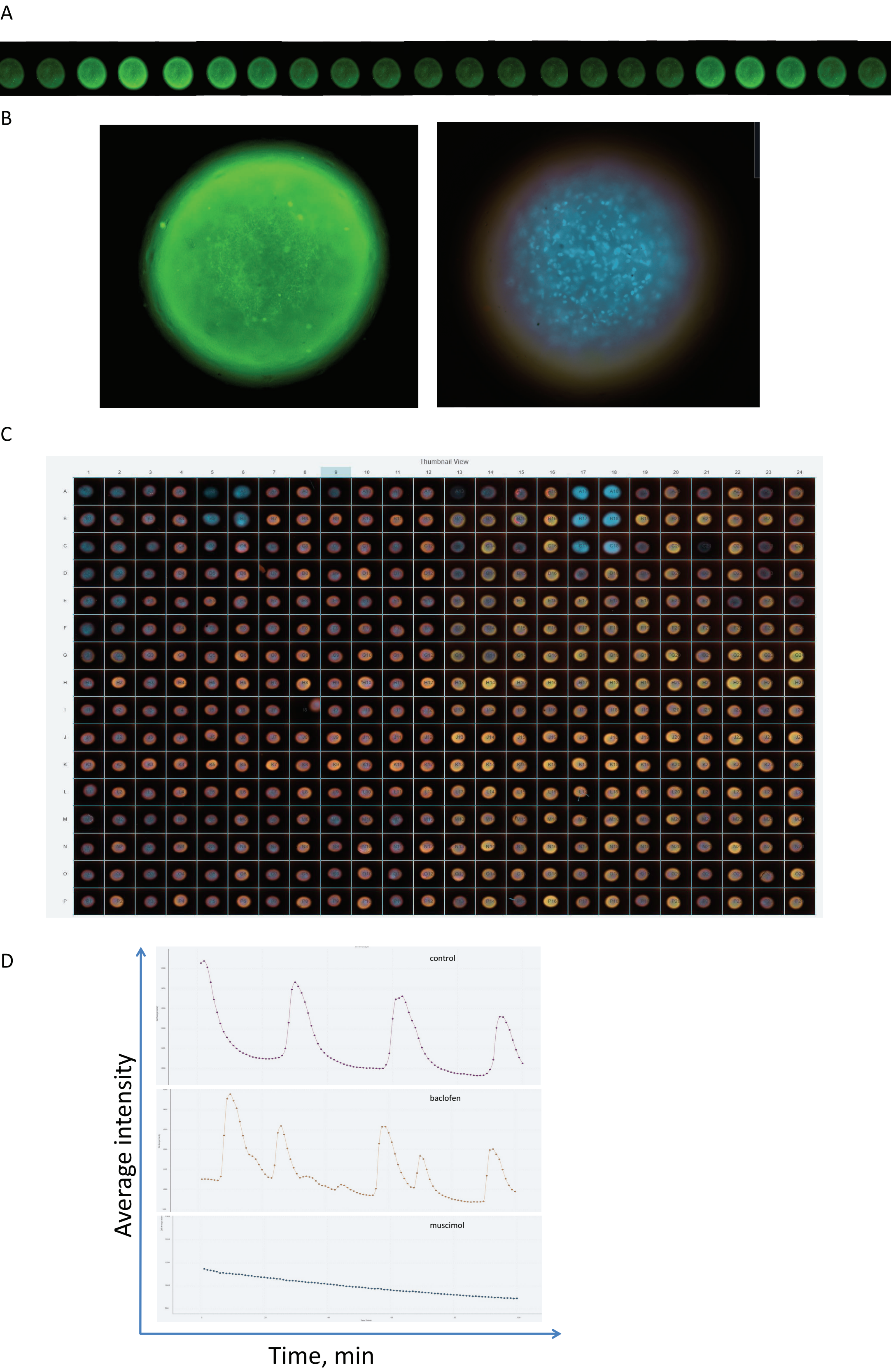
**Figure 1.** To measure Ca oscillations, iPSC cardiomyocytes were loaded with EarlyTox® Calcium dye then imaged using the ImageXpress Pico system in the FITC channel using a time-lapse mode. A. The panel above presents a series of images taken with 0.5 sec interval. B. Traces of calcium oscillations shown for increasing concentrations of indicated compounds. C. Traces compared for control (0.1% DMSO, yellow), doxorubicin (1 μM, blue), or flecainide acetate (1 μM, purple).



**Figure 2.** Images of cardiomyocytes (20X magnification) stained with viability dye Calcein AM, Mitotracker Orange dye for detection of mitochondria with intact membrane potential, and Hoechst nuclear dye (all from Thermo Fisher Scientific, 0.5 μM, 0.2 μM, and 0.5 μM respectively).

### Assessing Compound Effects on Neuro-spheroids

To evaluate cytotoxicity effects, microBrain® 3D cells from StemoniX Inc were treated with various neurotoxic compounds for 24 hours. For evaluation of calcium oscillations, cells were loaded with calcium 6 dye. Calcium oscillations were visualized using time-lapse imaging, then images were analyzed with Cell Count analysis using entire spheroid as a “cell” object. Average intensities were then plotted against time that allowed to visualize the patterns of calcium oscillations. For cytotoxicity assessment, cells were stained with Hoechst nuclear stain, MitoTracker Orange dye, and Calcein AM for detection of cell viability.



**Figure 3.** A. Time-lapse string of images shown for neurospheroids. To measure Ca oscillations, iPSC-derived neurospheroids (StemoniX) were loaded with Calcium 6 dye then imaged using ImageXpress Pico system in the FITC channel using a time-lapse mode. Images shown were taken with 1 sec interval. B. Images shown for spheroid stained with Calcium 6 dye (Molecular Devices, stained for 2h, left); or the combination of 3 viability dyes: Hoechst, Calcein AM, and MitoTracker Orange as indicated below (right). C. For cytotoxicity assessment, spheroids were stained for 2h with a combination of Hoechst (2 μM), MitoTracker Orange (0.2 μM), and Calcein AM (2 μM, all from Thermo Fisher Scientific). Images were taken with the ImageXpress Pico system, using a 10X Plan Fluor objective, and the DAPI, TRITC, and FITC channels. Images were processed using the Cell Scoring analysis algorithm. Decreases of Calcein AM or MitoTracker stains were observed for neurospheroids treated with various compounds. D. Traces of calcium oscillations shown for control (0.1% DMSO), baclofen (1 μM), and muscimol (1 μM). E. EC<sub>50</sub> values for indicated measurements defined from concentration –dependence curves are presented in the table.

## SUMMARY

- We presented methods for complex multi-parametric assays utilizing iPSC-derived human cardiomyocytes and neurospheroids
- Multiple readouts allow characterization of phenotypic effects of different compounds and also assessment of effective concentrations
- The study demonstrates the breadth of imaging assays possible using the ImageXpress Pico system and CellReporterXpress software for evaluation of various biological effects