

APPLICATION NOTE

# Assaying cardiotoxicity with ImageXpress high-content screening systems

## Introduction

The discovery and development of novel drugs is a difficult and expensive process due to lack of tools and techniques to accurately reproduce the human physiological response in the laboratory. Off-target cardiotoxicity remains a significant cause of pre- and post-approval safety-based drug attrition because of the disconnect between the behavior of cultured immortalized cells and *in vivo* animal models to accurately simulate human cardiac disease states. Using high-content imaging systems, like the ImageXpress® Micro system, it is possible to assess the cytotoxic effect of compounds on pertinent human cardiac cells derived from induced pluripotent stem cells.

## Analyze gross cytotoxicity using live/dead assay

Membrane-permeable Calcein AM is cleaved by esterases in live cells to produce green fluorescence in the cytoplasm. In dead cells, the cell membrane is compromised, allowing the dye, ethidium homodimer, to enter and stain the nucleus (Figure 1).

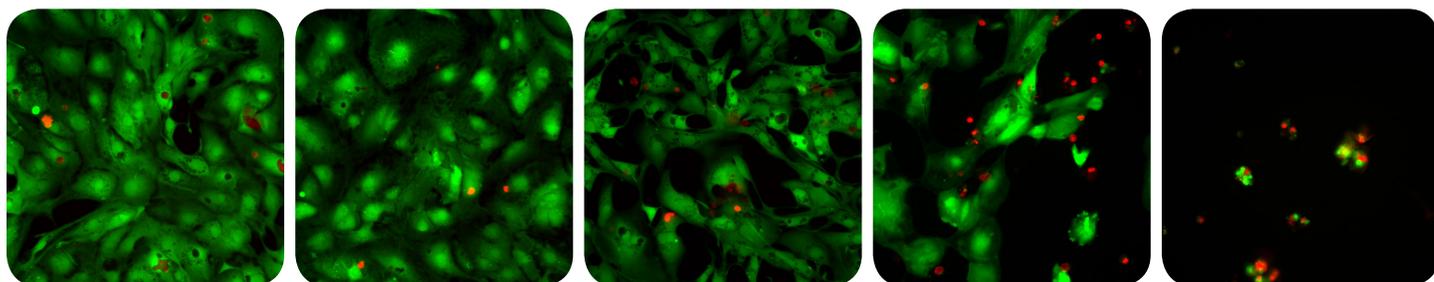
Cardiomyocytes derived from human induced pluripotent stem cells (iPsc, iCell Cardiomyocytes from Cellular Dynamics Intl.) were incubated with varying concentrations of drugs which were analyzed for cardiotoxicity using high-content imaging (Figure 2) with the data plotted normalized to control (Figure 3). Valinomycin (columns 9 and 10) were highly cardiotoxic even at the lowest levels tested.

## Monitor mitochondria membrane depolarization with JC-10

JC-10 aggregates in the mitochondria of healthy cells to produce an orange/red emission at 570 nm. Some compounds cause immediate toxic effects by disrupting the membrane potential of the mitochondria. If the mitochondrial membrane is compromised, the JC-10 monomers disperse in the cytoplasm where they emit at 530 nm. Quantitative detection of the mitochondrial membrane change is possible by monitoring the fluorescence intensity ratio at 570 nm/530 nm or simply by quantitating the amount of orange mitochondria detected per cell.

## Benefits

- Assess cytotoxic effect of compound derived from induced pluripotent stem cells
- Expedite drug discovery process by reducing unforeseen cardiac side effects late in clinical trials
- Implement high-throughput screening for multiplexed, cell-based assays

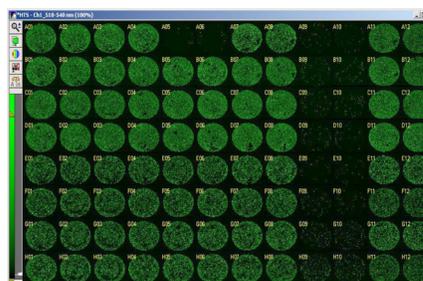


**Figure 1. Cytotoxicity assay.** Cardiomyocytes exposed to increasing levels of a toxic compound (left to right). Live cells exhibit green fluorescence and an increasing incidence of red stained dead cells is evident at higher doses.

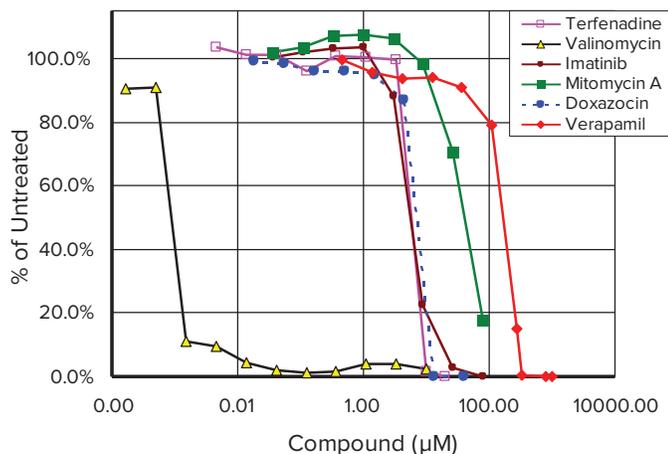
The ImageXpress Micro systems are a fully integrated hardware and software system for automated acquisition and analysis of images for high-throughput cell-based cytotoxicity testing. When configured with optional environmental control, we could monitor living cell responses or kinetic reactions in real-time for several days. In this study, we incubated cardiomyocytes and applied increasing concentrations of Antimycin A. After 90 minutes of incubation, we quantitated the cardiotoxicity (Figure 4). The saved images could be reviewed at any time and further analyzed with one of the MetaXpress® software application modules.

### Summary

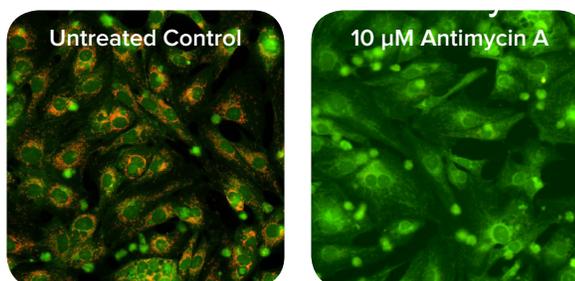
The ImageXpress Micro system is a powerful system for analysis of *in vivo* drug cardiotoxicity using cardiomyocytes derived from human induced pluripotent stem cells in both the whole cell cytotoxicity and mitochondria membrane depolarization assays. The system provides pharmaceutical scientists with critical information on compound cytotoxicity early in the drug discovery process to help reduce unforeseen cardiac side effects late in clinical trials.



**Figure 2. Whole well images of toxicity assay.** Cardiomyocytes screened against an 8 point dose-response of 5 different compounds tested in duplicate. The compound Valinomycin, in columns 9 and 10, was toxic even at the lowest dose tested (row H).



**Figure 3. Toxicity dose response curves.** Normalized results of a live/dead assay run on 6 different compounds after 48 hours of drug treatment.



**Figure 4. JC-10 mitochondrial membrane potential assay.** Increasing concentrations of Antimycin A cause more depolarization of mitochondrial membrane leading to a lower ratio of red-stained mitochondria to green fluorescent cytoplasm. Live cell images were acquired at 20X using ImageXpress Micro System with environmental control.

### Contact Us

Phone: +1-800-635-5577  
 Web: [www.moleculardevices.com](http://www.moleculardevices.com)  
 Email: [info@moldev.com](mailto:info@moldev.com)  
 Check our website for a current listing of worldwide distributors.