High Throughput Cardiotoxicity (Proarrythmia + Cardiomyopathy) Screening in iPSC-Derived Cardiomyocytes using Kinetic Image Cytometry

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Abstract:

Recently reviewed emerging evidence strongly suggests that the use of iPSC-derived cardiomyocytes (iPSC-CMs) with physiologically relevant cardiac measurements in vitro improves the detection of structural cardiotoxicity (cardiomyopathy), which, when added to use of iPSC-CMs as an integral component of the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative, could create comprehensive in vitro cardiosafety screening (Yang and Papoian, J Appl Toxicol, 2018). The kinetic phenotypic readout of cardiomyocyte contractility, a key physiologically relevant cardiac measurement reviewed, is compelling because it has the potential to detect increased or decreased structural function independent of mechanism. Here we report progress on single assays combining calcium-and-contractility and voltage-and-contractility utilizing fluorescent indicators for calcium and voltage, respectively. A key challenge was overcoming the artifacts in contractility measurements produced by changes in fluorescence intensity. Vala previously assayed action potential (voltage) activity for proarrhythmia for the CiPA Phase II study of 28 compounds using iPSC-CMs from both Ncardia and Cellular Dynamics. By inspection, we observed more pronounced movement (contraction) with Ncardia iPSC-CMs, and thus reanalyzed those data with the new voltage-and-contractility algorithm. Of the 28 CiPA Phase II compounds, the negative inotropes are Bepridil, Disopyramide, Quinidine, Diltiazem, Nifedipine, Verapamil; and the positive inotropes are d,l-Sotalol and Dofetilide, although the latter is a reverse use (rate) dependent positive inotrope, and alterations in contractility may be difficult to detect. Here we report the inotropic analyses of these compounds derived from recomputing contractility on the CiPA Phase II screen. Contraction artifacts produced by intensity changes were removed. Our data demonstrate repeatable measurements of contractility using fluorescent physiological indicators and thus validate the ability to combine proarrhythmia and cardiomyopathy into the same high throughput screen.

Introduction

Cardiosafety is a key issue as even subtle changes in cardiac performance brought about by drugs can have harmful, even fatal, effects. In the 1980's and 90's several FDA-approved drugs were linked to unexpected deaths; these drugs prolong the QT interval of the electrocardiogram and increase the incidence of of Torsade de Pointes (TdP), a ventricular tachyarrhythmia that leads to Sudden Cardiac Death (SCD). In addition to drug safety, effective therapeutics for heart failure are desperately needed as this affliction, which arises from high blood pressure, heart attacks, heart valve disorders, and certain arrhythmias, is a leading cause of death and current medications have limited effects. iPSC-CMs are emerging as an important preclinical model system for cardiosafety/drug efficacy testing. Since this cell type represents humans, readouts from these cells are likely to be more predictive vs. data obtained from in vitro model systems using cardiomyocytes from animals. Vala Sciences Inc, has developed methods to simultaneously quantify effects of compounds on the cardiac action potential and contractile motion (cell displacement) of iPSC-CMs, using a fluorescent indicator of voltage (e.g., FluoVolt™) and Vala's IC200 Kinetic Image Cytometer® (which acquires images from cells plated on 96- or 384-well dishes at rates up to 1500 frames per second) providing an unprecendented platform for preclinical cardiosafety and drug efficacy testing. We report here data we obtained from iPSC-CMs exposed to compounds from the recent CiPA Phase II study, which features compounds with established clinical effects on AP-duration and contractile performance.

IC200-KIC™

Kinetic Image Cytometry (KIC™)	
Acquisition Frequency	- Time-series acquisition at 0.1-100 full frames per second. - Time-lapse acquisition for repeated sequence of an imaging protocol.
Stimulation Options	- Electrical Stimulation: available with the automated electrode option. - Optogenetics: Optical stimulation using any of the available excitation lines.
Environmental Control	- Temperature (30-40° C) and CO2 (5-10%) available. - Hypoxia (5%, 10% or 15%) available.
Cameras	Up to two 5.53MP Scientific CMOS cameras
Objectives	Plan APO 4x NA 0.20 (1.63 µm/Pixel) Plan APO 10x NA 0.45 (0.64 µm/Pixel) Plan APO 20X NA 0.75 (0.32 µm/Pixel) Plan APO 40X NA 0.95 (0.16 µm/Pixel) Plan APO 60X NA 0.95 (0.11 µm/Pixel)
Well Plates Supported	Supports 1536, 384, 96, 48, 24, 12 and 6 well plates. Ability to define custom formats.
Standard Filter Sets	- DAPI, FITC, TRITC, Cy5 and Cy7 standard - Other filter sets available.
Dimensions	21" x 21" x 22" (53cm x 53cm x 56cm) WxDxH including light engine

References:

Yang, B., Papoian, T. 2019. Preclinical approaches to assess potential kinase inhibitor-induced cardiac toxicity: Past, present and future. J. Appl. Toxicol. 38(6): 790-800

Contact

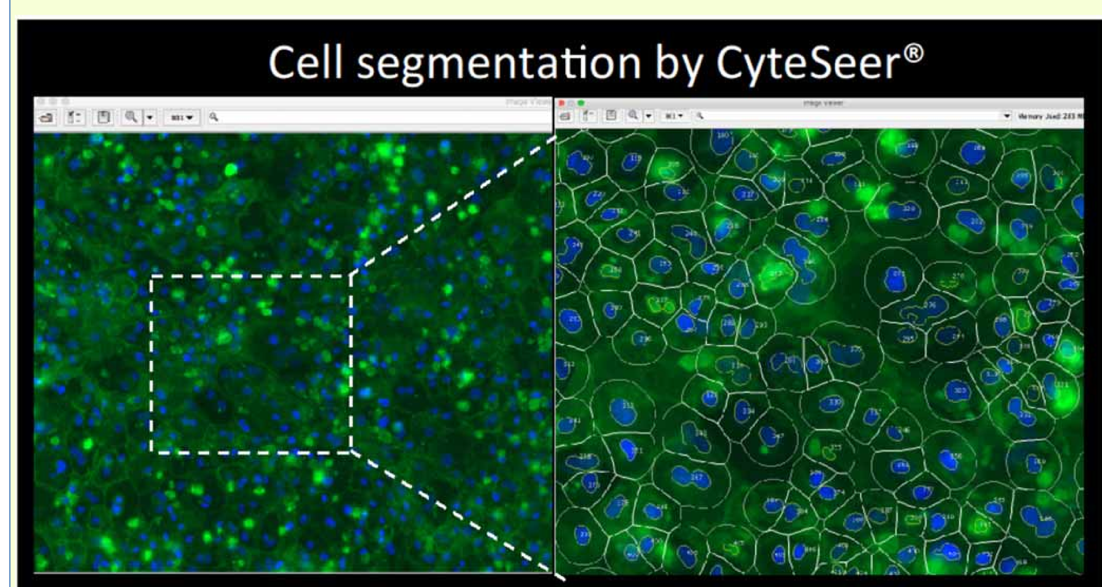
For more information regarding the IC200, CyteSeer, or high content screening services, please visit: www.valasciences.com or contact us directly at: info@valasciences.com or call us toll-free at (858) 742-8252

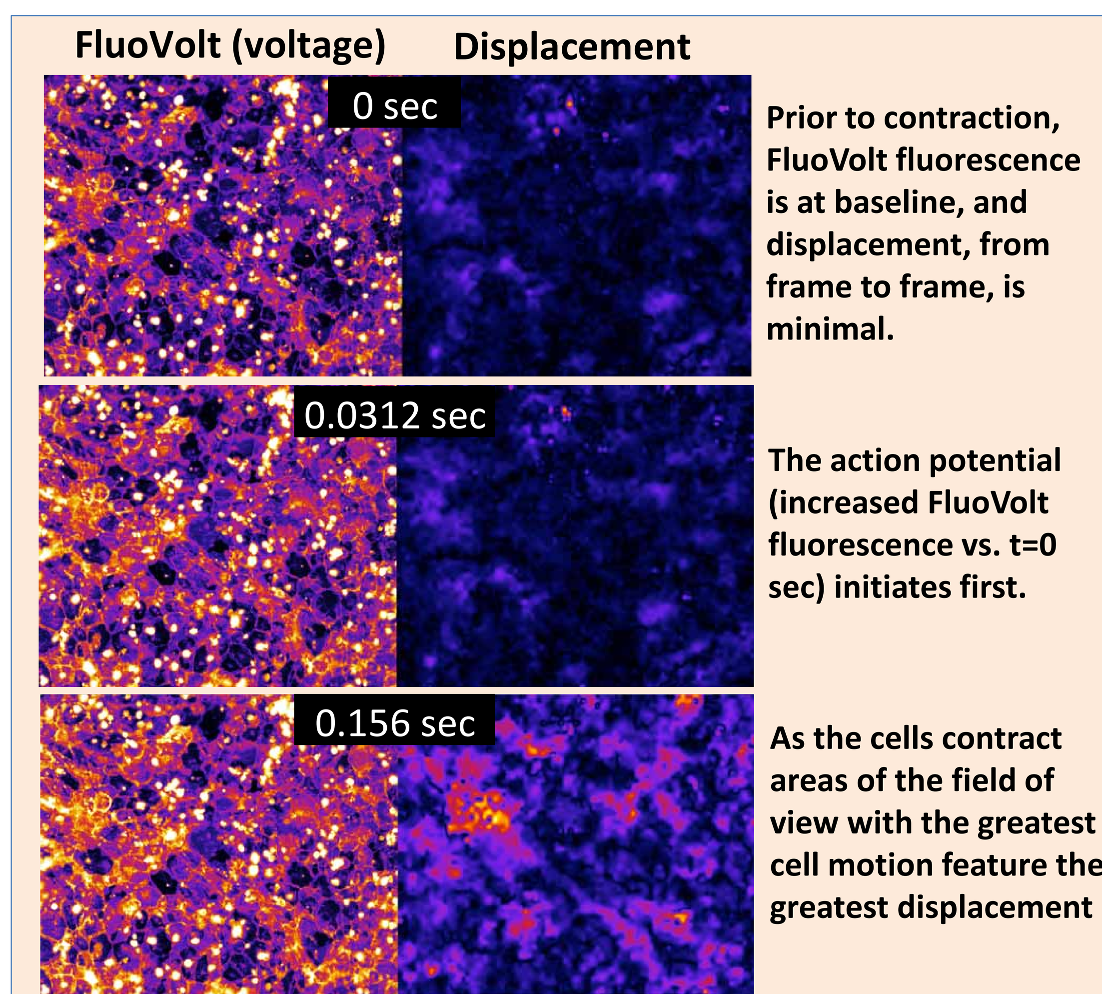
Methods

Cell plating and plate map: iPSC-CMs (NCardia, Cor.4U) were plated in 96-well imaging dishes at 1.40×105/cm² on fibronectin (10µg/mL), in plating media at 37°C and 5% CO2. After 3 hr, cells were switched to the maintenance medium (from NCardia), which replaced after 24 hr and every 48 hr till assay (six days of culture). iPSC-CMs were loaded with FluoVolt™ per manufacturer instructions, in a loading buffer of 20mM HEPES in HBSS and 200ng/mL Hoechst 33342. Cells were loaded for 30 minutes at 37°C. Stock solutions of each blinded compound dose were prepared in DMSO at 1000-fold the test concentration, aliquoted and stored at -80°C. Following dye loading, the cells were washed with 37°C Tyrode's solution. The compounds or vehicle control, diluted to their final test concentrations, were then 100% exchanged with solutions in the appropriate wells. Each compound was tested at 4 doses, with n=5 wells/dose. Each plate featured 10 wells treated with DMSO, only (controls), and 5 wells with 3 nM dofetilide (see plate map). Compounds were tested in a blinded manner for the CiPA project. Images were acquired with a 20X objective at 32 frames per second.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B	A-DMSO	A-DMSO	A-DMSO	A-DMSO	E-C1D4	E-C1D4	E-C1D4	E-C1D4	E-C1D4	D-C1D3	D-C1D3	D-C1D3
C	B-C1 D1	B-C1 D1	B-C1 D1	B-C1 D1	B-C1 D1	C_C1D2	C_C1D2	C_C1D2	C_C1D2	C_C1D2	D-C1D3	D-C1D3
D	A-DMSO	A-DMSO	A-DMSO	A-DMSO	I-C2D4	I-C2D4	I-C2D4	I-C2D4	I-C2D4	H-C2D3	H-C2D3	H-C2D3
E	F-C2D1	F-C2D1	F-C2D1	F-C2D1	F-C2D1	G-C2 D2	G-C2 D2	G-C2 D2	G-C2 D2	G-C2 D2	H-C2D3	H-C2D3
F	A-DMSO	A-DMSO	J-3 nM Dof	J-3 nM Dof	J-3 nM Dof	J-3 nM Dof	J-3 nM Dof	J-3 nM Dof				
G												

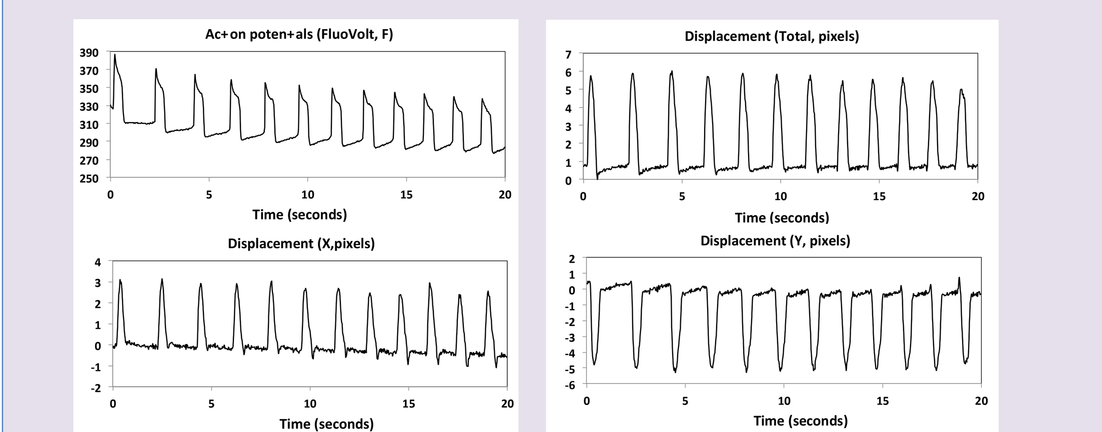
Cell segmentation and analysis of voltage and contractile motion: CyteSeer® identifies each nucleus in the field of view. Next, it identifies th cytoplasm region of each cell. For voltage, Average Pixel Intensity for the cytoplasm is identified for each frame. To quantify motion (displacement), images are subjected to a proprietary analysis method that returns the overall displacement distance as well as x- and y- vector components.

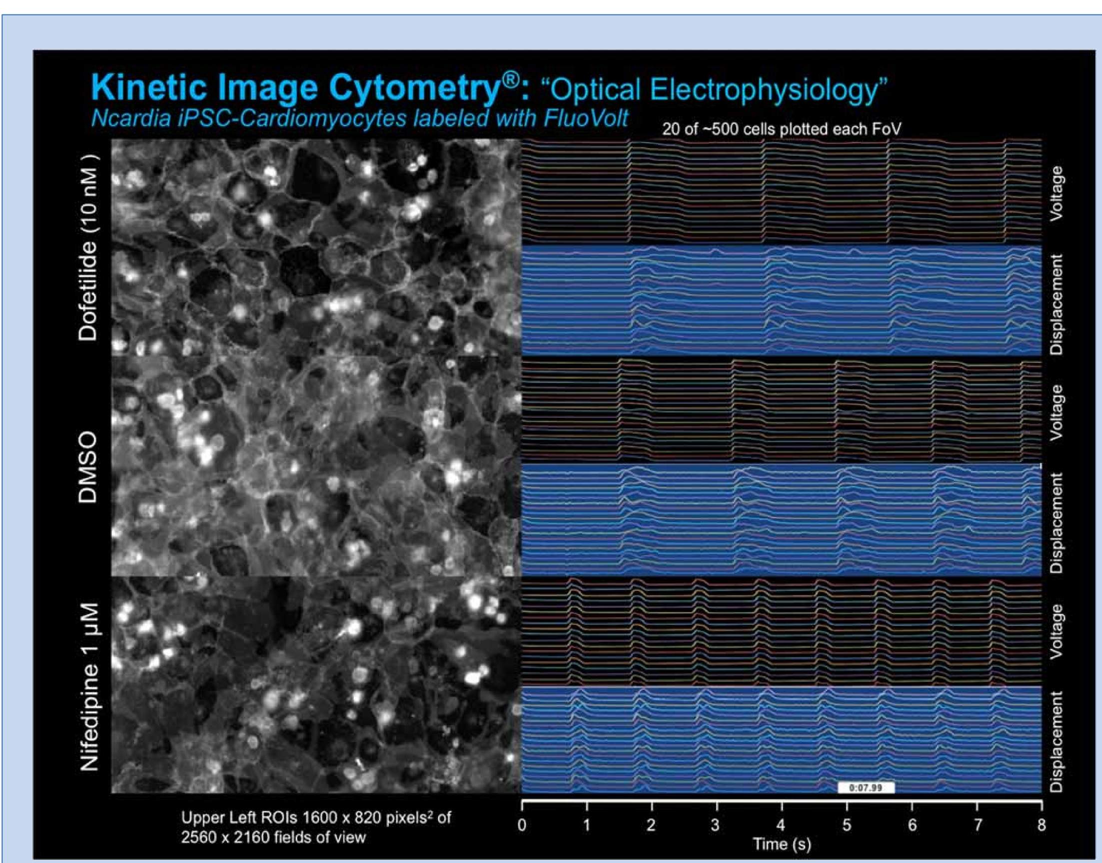




To quantify motion (displacement), images are subjected to a proprietary analysis method that returns the overall displacement distance as well as the x- and y- vector components.

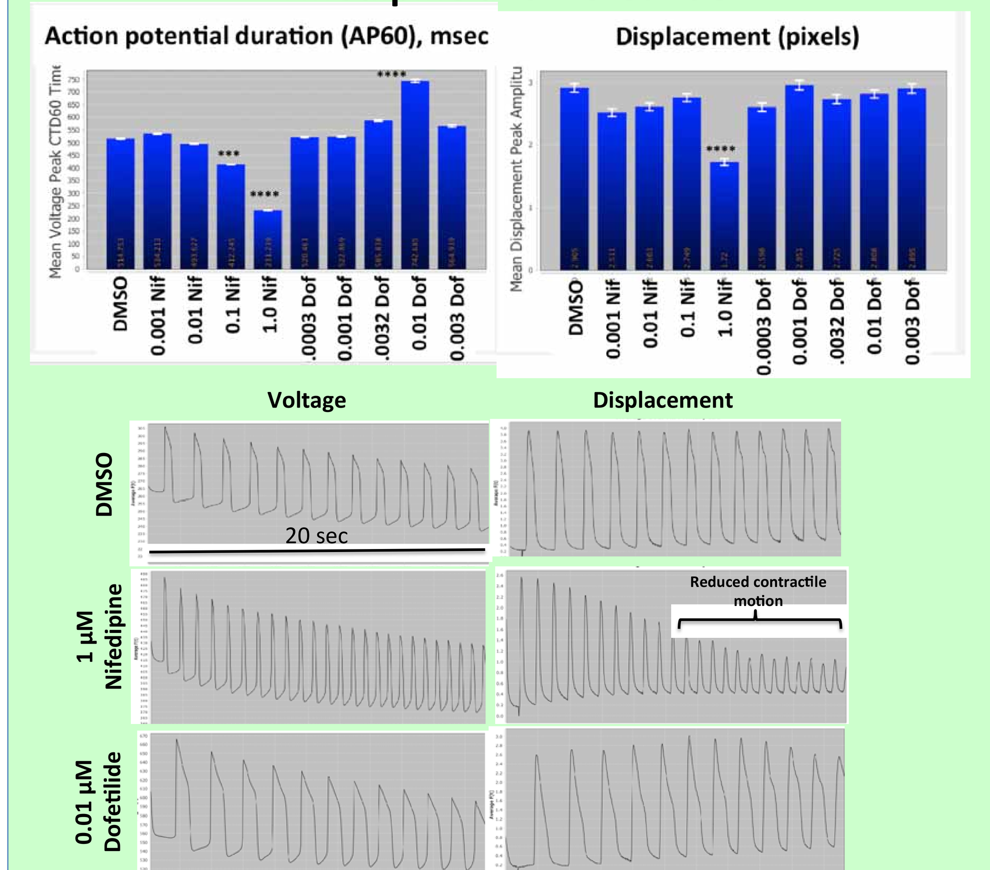
Voltage vs. Displacement traces for well B1 (DMSO), cell 324





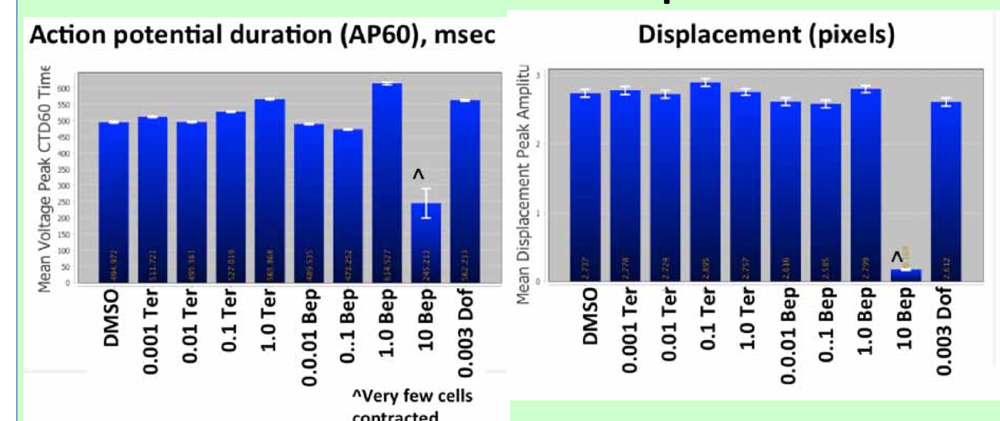
Results

Nifedipine and Dofetilide

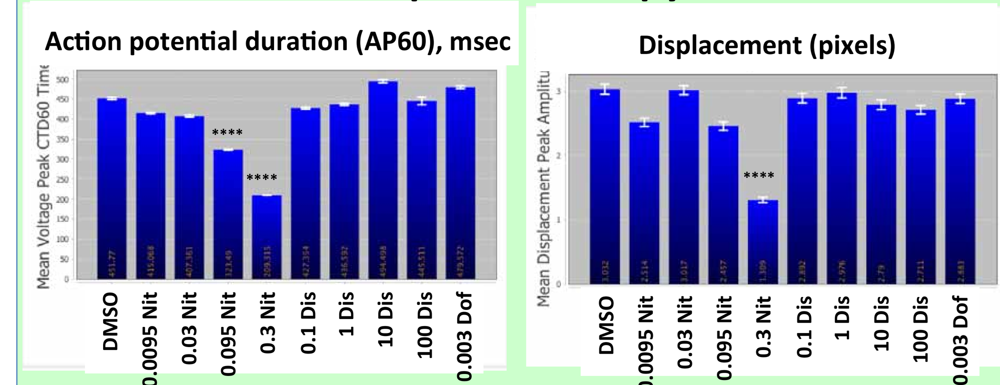


Nifedipine shortened the AP and reduced contractility at 1 µM (this effect increased with time). Dofetilide increased the AP duration at 10 nM. Bars are mean +/- SE on a per cell basis (3000 to 4000 cells/condition). Traces represent a single well (averaged across all cells in the well)

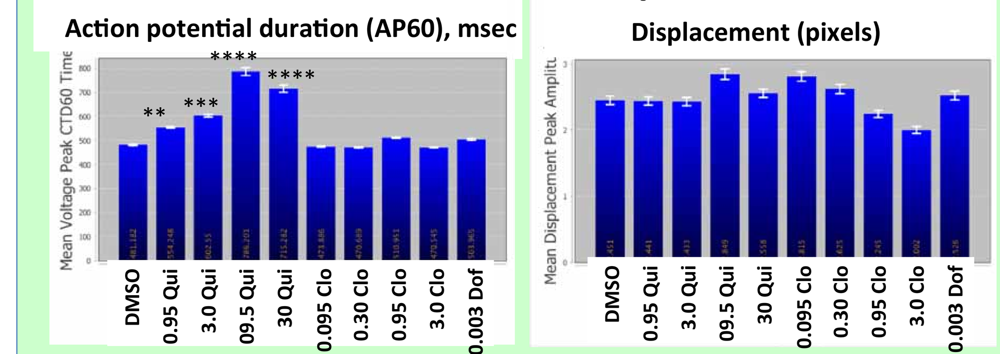
Terfenadine and Bepridil



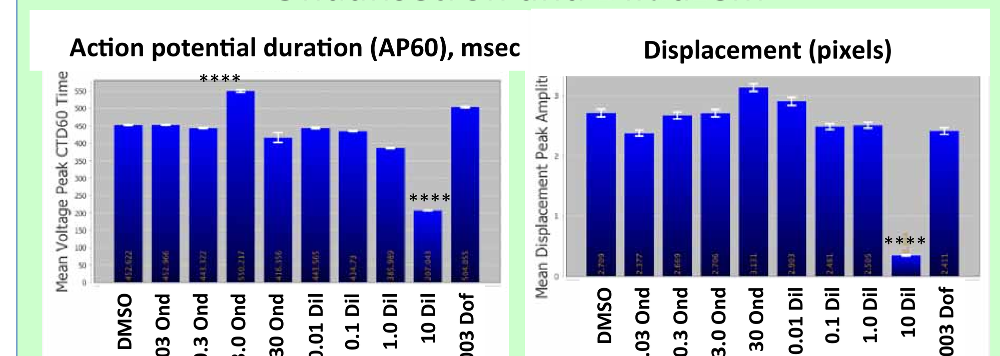
Nitrendipine and Dispyramide



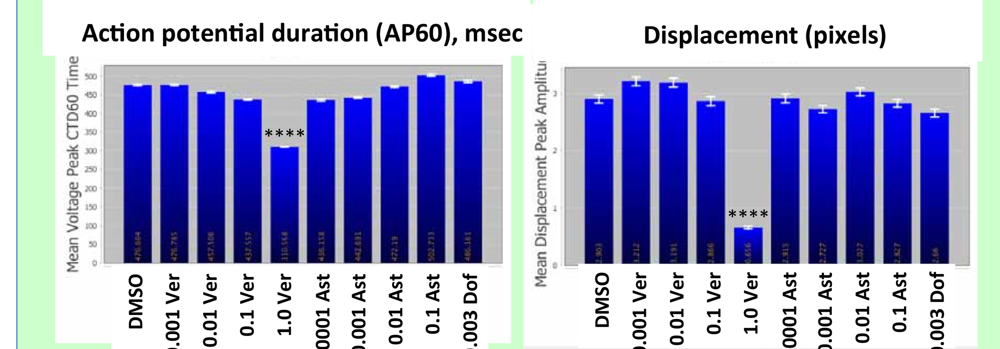
Quinidine and Clozapine



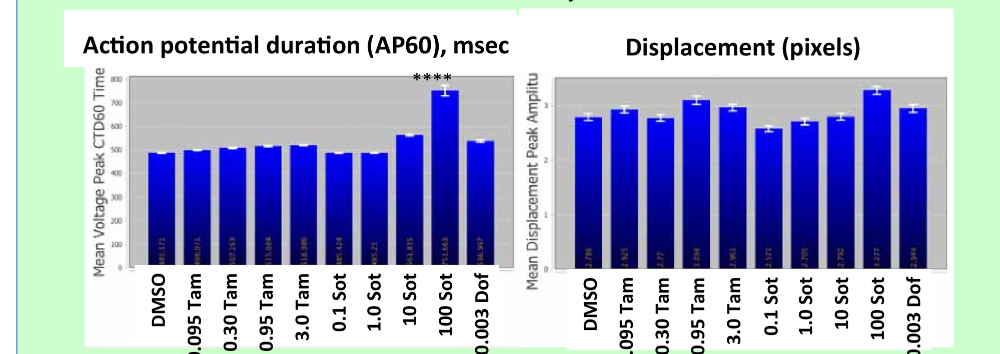
Ondansetron and Diltiazem



Verapamil and Astemizole



Tamoxifen and D,l-Sotalol



Summary:

We have developed Kinetic Image Cytometry® methods for simultaneous quantification of action potential kinetics and contractile motion (displacement) in human stem cell-derived cardiomyocytes. These methods will have applications in preclinical cardiosafety determinations, and in drug-discovery relevant to the treatment of heart failure and cardiomyopathies. Furthermore, this technology will be extended, in the future, to include cardiomyocytes differentiated to ventricular, atrial, or nodal phenotypes, and to include cells representing human with genotypes that may increase their suceptibility to arrhythmias and other cardiac afflictions.