

# Maturation of Human iPSC-CM Function for Improved Predictivity of *in vitro* Action Potential and Contractility Assays

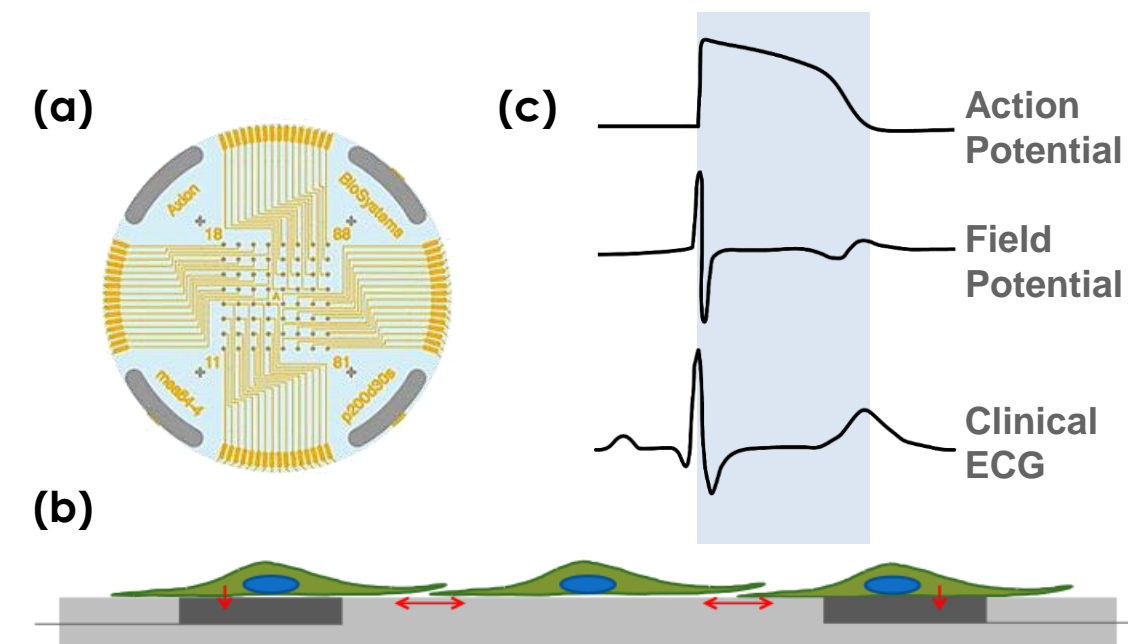
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## Multiwell MEA Technology

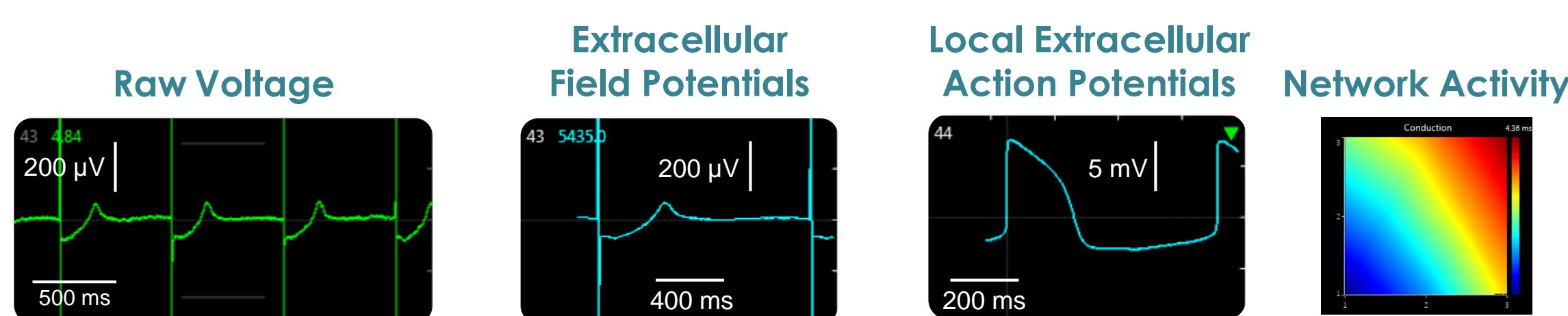
### Microelectrode Array Technology

The flexibility and accessibility of neural and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that provides a functional phenotype. Measurements of electrophysiological activity across a networked population offer a comprehensive characterization beyond standard genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell microelectrode array (MEA) platform provides this comprehensive functional characterization. The Maestro is a non-invasive benchtop system that simply, rapidly, and accurately records functional activity from cellular networks cultured on a dense array of extracellular electrodes in each well.

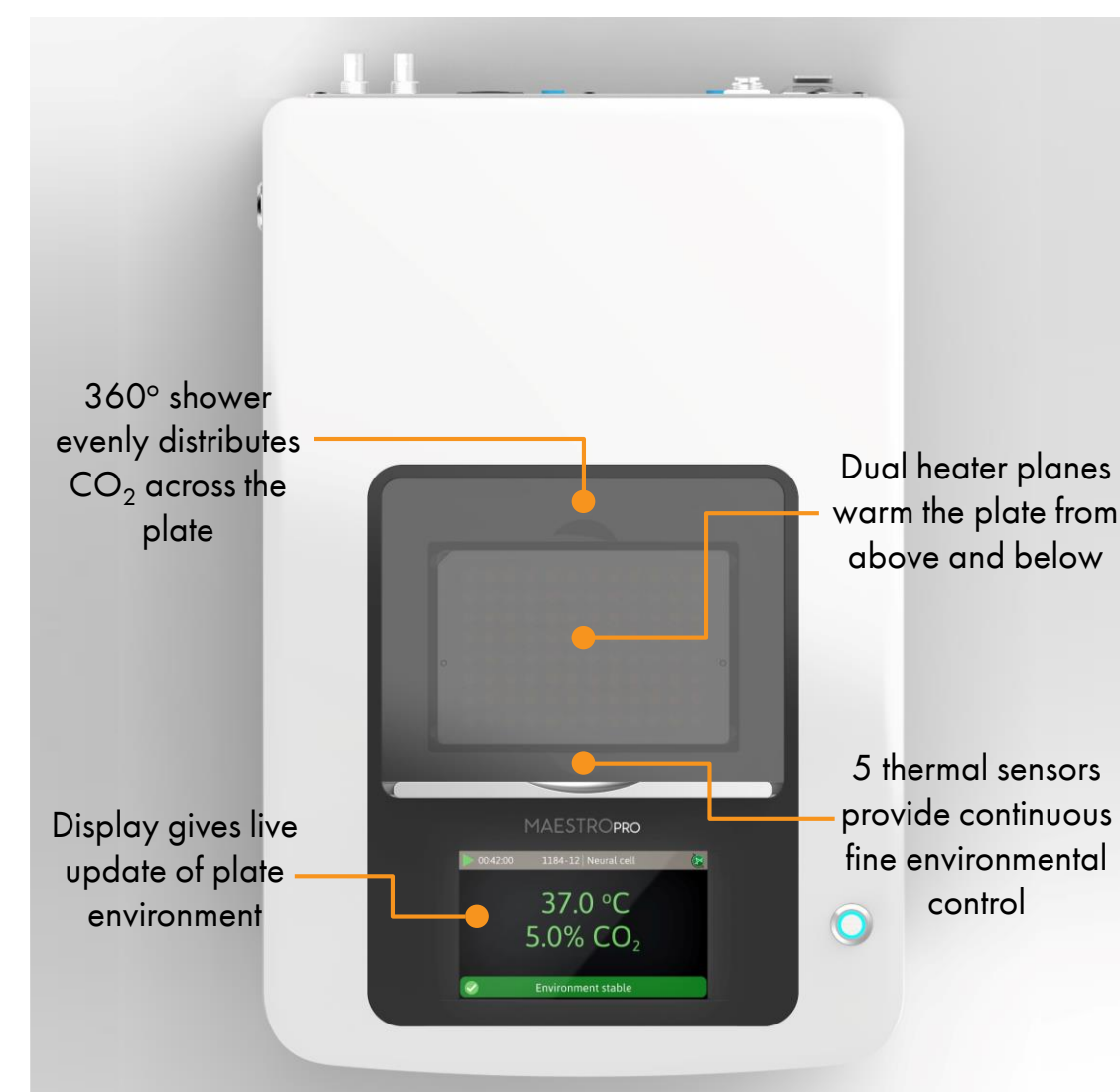


A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), to model complex, human systems. Electrodes detect changes in raw voltage (c) and record extracellular field potentials.



Raw voltage signals are processed in real-time to obtain extracellular field potentials from across the network, providing a valuable electrophysiological phenotype for applications in drug discovery, toxicological and safety screening, disease models, and stem cell characterization

### Introducing the Maestro Pro™ and Maestro Edge™



360° shower evenly distributes CO<sub>2</sub> across the plate  
Dual heater planes warm the plate from above and below  
5 thermal sensors provide continuous fine environmental control  
Display gives live update of plate environment

- **Label-free, non-invasive recording** of extracellular voltage from cultured electro-active cells
- **Integrated environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the depolarization waveform
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data from across the entire culture
- **Scalable format (6-, 24-, 48- and 96-well plates)** meets all throughput needs on a single system
- **State-of-the-art electrode processing chip (BioCore v4)** offers stronger signals, ultra-low frequency content, and enhanced flexibility



Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	6-, 24-Well	6-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	Yes	Yes

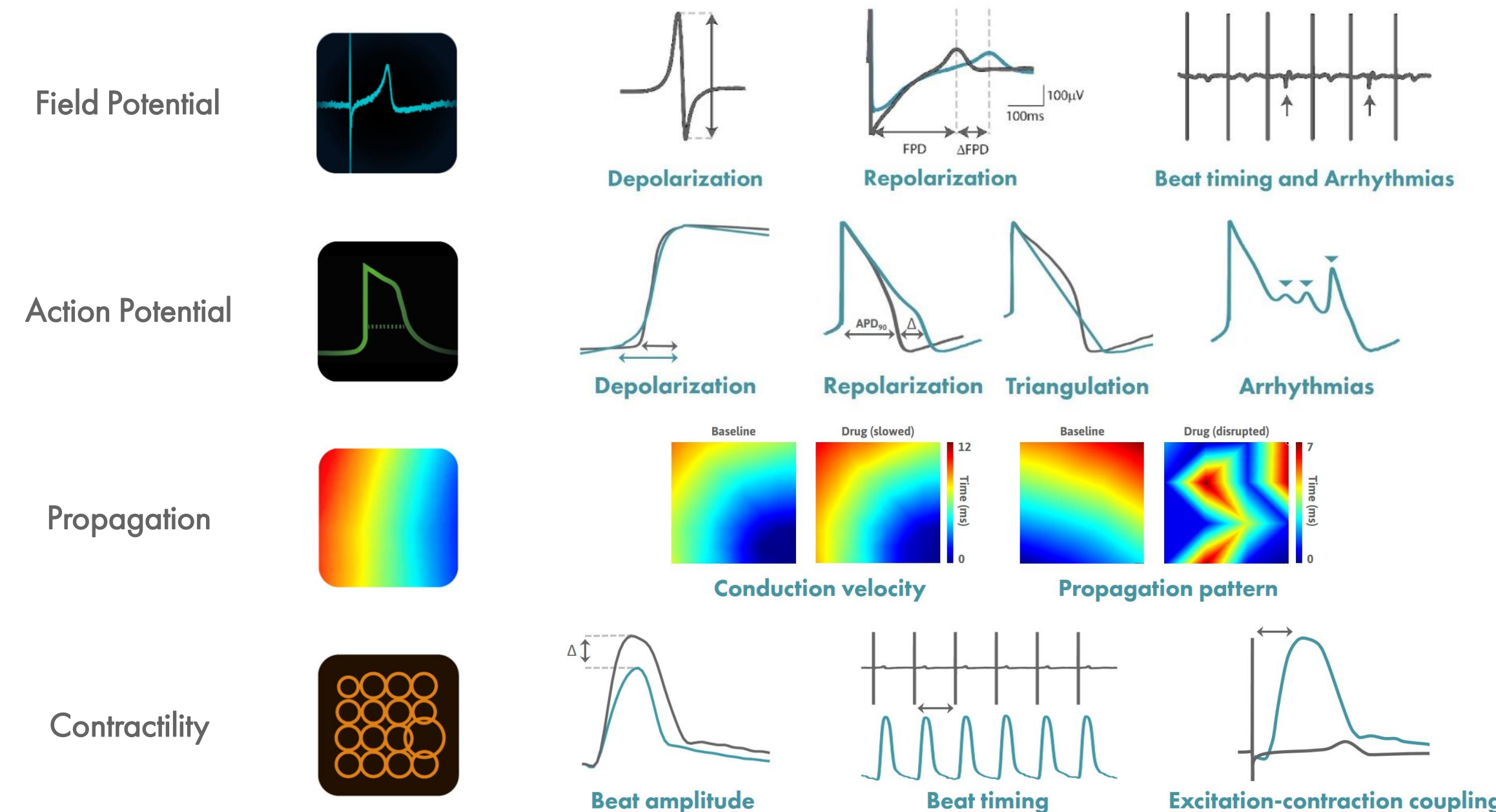
The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

## MEA Assay with Cardiomyocytes

### Cardiomyocyte Electrophysiology Phenotypes

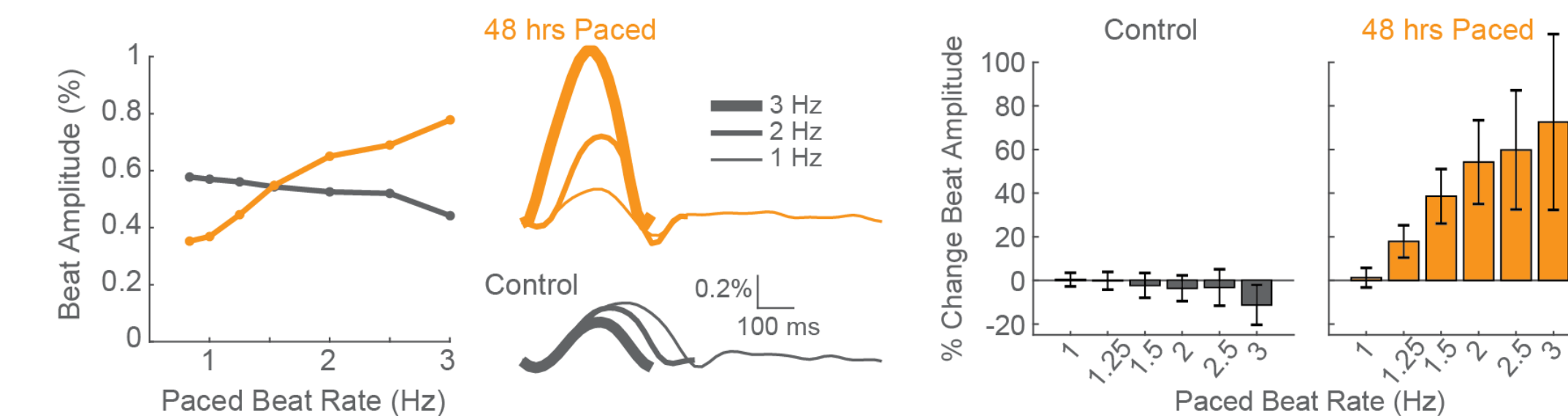
The need for simple, reliable, and predictive pre-clinical assays for cardiac safety has motivated initiatives world-wide, including the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) and Japan iPS Cardiac Safety Assessment (JiCSA). The Maestro MEA platform enables assessment of functional *in vitro* cardiomyocyte activity with an easy-to-use benchtop system. The Maestro detects and records signals from cells cultured directly onto an array of planar electrodes in each well of the MEA plate, with each of the following four modes providing critical information for *in vitro* assessment:

- **Field Potential** – “gold standard” measurement for multiwell cardiac electrophysiology.
- **LEAP** – first truly scalable technique for acquiring action potential signals from intact cardiac monolayers.
- **Conduction** – detect speed and direction of action potential propagation.
- **Contractility** – assess the contractility of cardiac monolayers adhered to the 2D array.

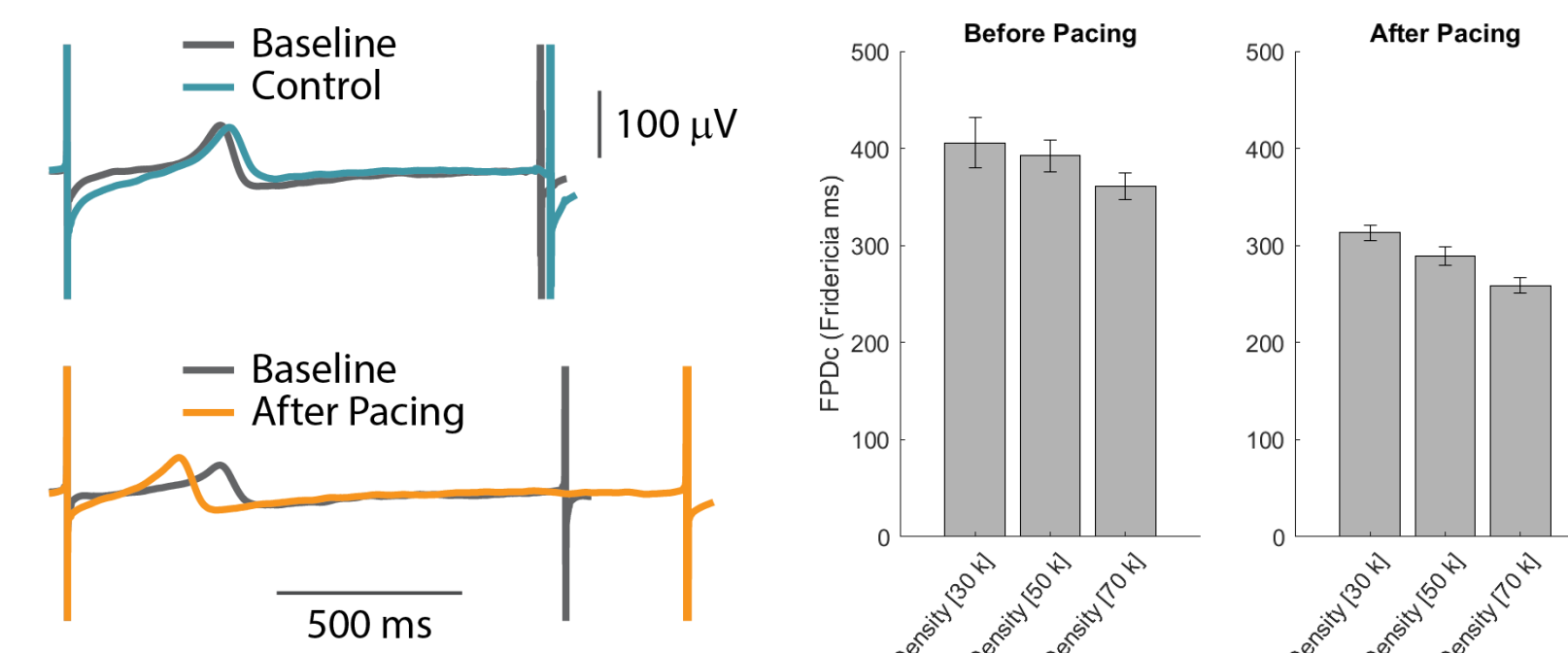


### Chronic Pacing Induces Functional Maturation after 48 hours

hiPSC-CMs display aspects of functional immaturity, including immature calcium handling and contractile function. Physical conditioning of hiPSC-CMs via electrical or mechanical stimulation facilitates maturation as measured by a positive force-frequency relationship. Current *in vitro* protocols require 2-4 weeks of chronic pacing. Using array-based contractility and local electrical stimulation, we detected functionally mature phenotypes in hiPSC-CMs after only 48 hours of chronic pacing.



CDI iCell CM<sup>2</sup> cardiomyocytes were chronically paced at 2 Hz for 48 hours using a CytoView MEA 24. After pacing, the cells were paced again at 0.8 up to 3 Hz to assess the force-frequency relationship. Paced wells (orange) exhibited a positive force-frequency relationship after only 48 hours, while control wells (gray) showed no relationship. Inset shows representative paced and unpaced control contractility traces at 1 Hz, 2 Hz, and 3 Hz.

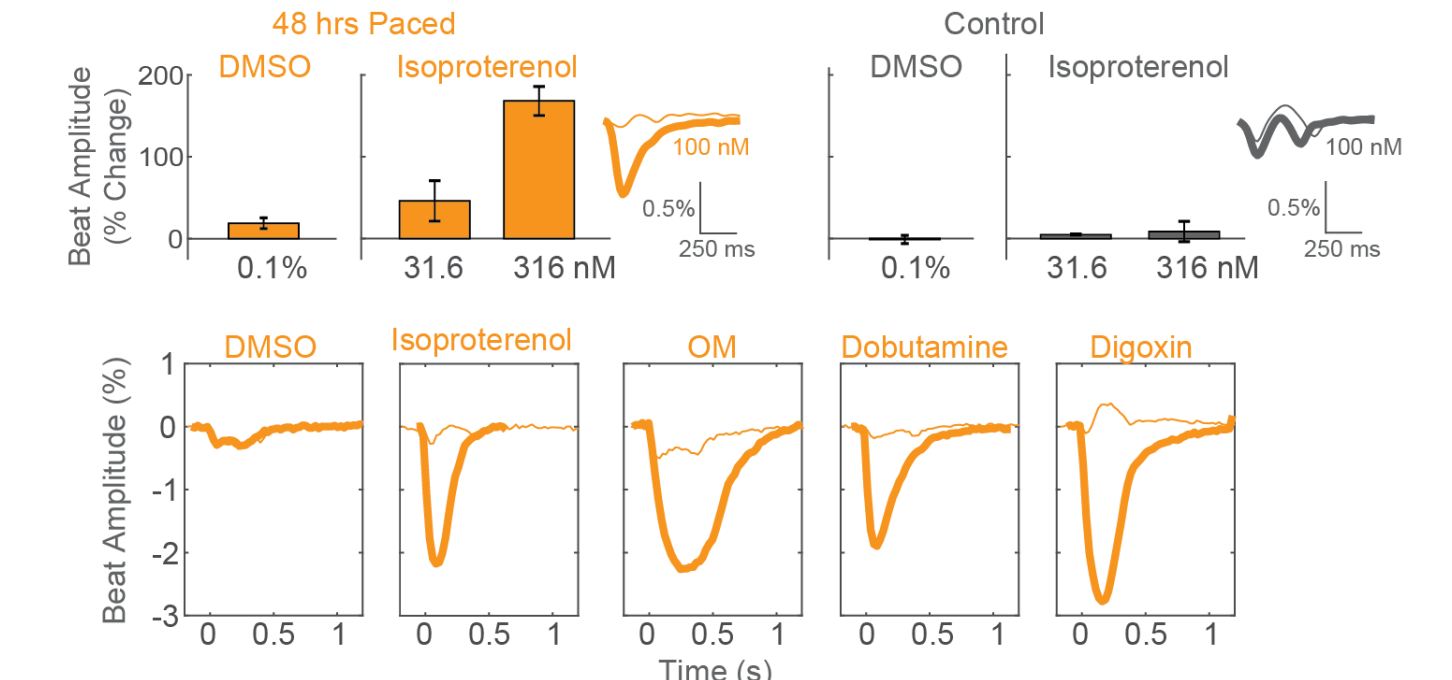


CDI iCell CM<sup>2</sup> cardiomyocytes seeded at varying densities were chronically paced at 2 Hz for 48 hours using a CytoView MEA 24. Cells plated a higher density displayed slightly shorter FPDC. After chronic pacing, the CMs at all cell densities exhibited earlier repolarization, as measured by FPDC.

## Functional Maturation of hiPSC-CMs

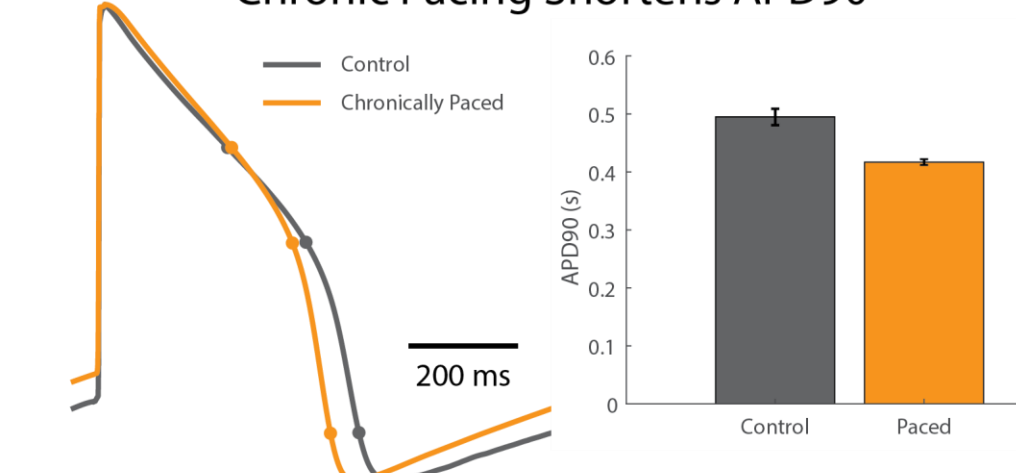
### Detection of Positive Inotropes after Chronic Pacing for 48 hours

After chronic pacing at 2 Hz for 48 hours, CDI iCell CM<sup>2</sup> cardiomyocytes were dosed with positive inotropes, such as isoproterenol. Chronically paced wells (orange) showed a dose-dependent increase in beat amplitude in response to isoproterenol, while unpaced control wells (gray) showed no response. Similarly, chronically paced wells also successfully detected a variety of other positive inotropes.



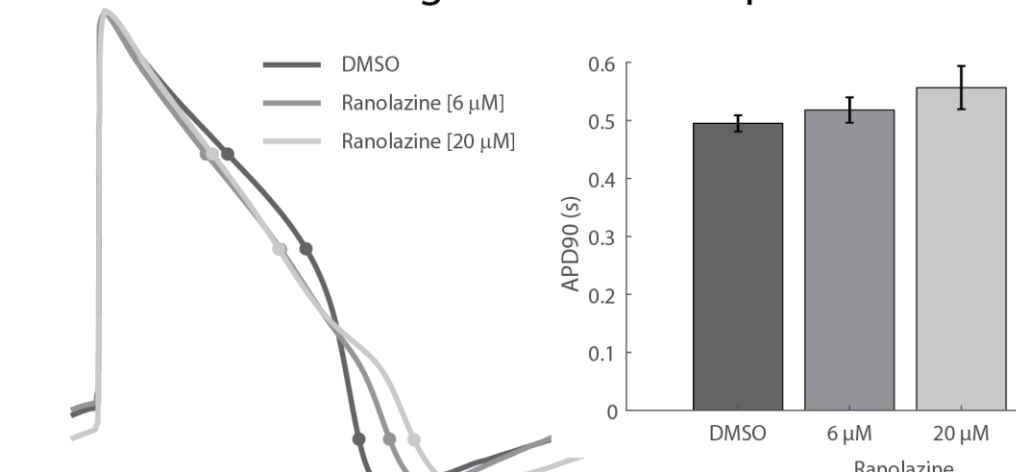
### Maturation of hiPSC-CM Functional Responses with Chronic Pacing

#### Chronic Pacing Shortens APD90

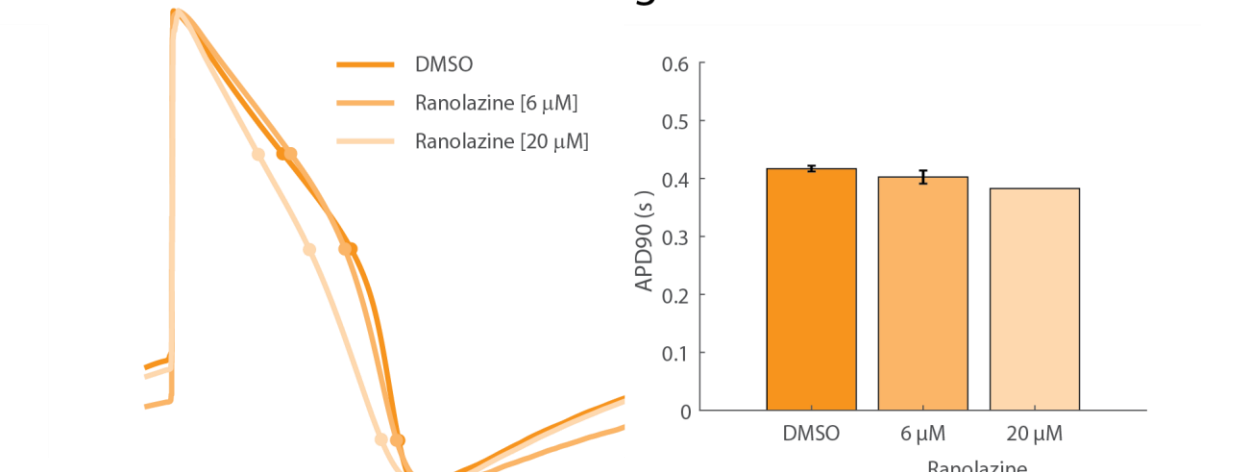


After chronic pacing at 2 Hz for 48 hours, CDI iCell CM<sup>2</sup> cardiomyocytes exhibited a shorter action potential duration (APD), as compared to unpaced control wells, when measured using LEAP technology. The APD90 was reduced by ~100 ms in “matured” wells that underwent chronic pacing, comparable to the reduction in FPD observed in this and other experiments.

#### Ranolazine Prolongs APD90 for Unpaced Controls

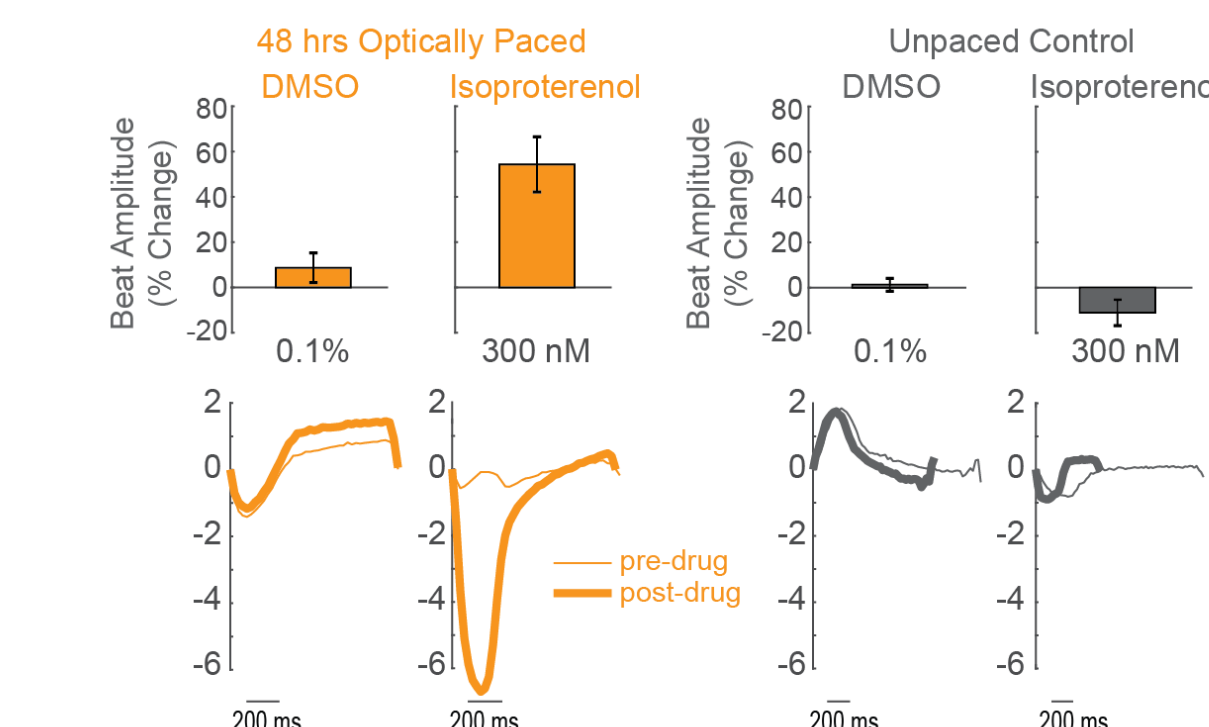


#### Ranolazine Does Not Prolong APD90 in “Matured” Wells



After chronic pacing at 2 Hz for 48 hours, CDI iCell CM<sup>2</sup> cardiomyocytes were dosed with ranolazine to evaluate the effects of “maturation” on the functional electrophysiological response. Unpaced control wells exhibited prolongation of APD with increasing doses of ranolazine, relative to the vehicle control. By comparison, “matured” wells did not prolong APD90 following addition of ranolazine. Also, at 20uM ranolazine, three of the four “matured” replicates became quiescent.

### Light-based Chronic Pacing with Optogenetics for Maturation Studies



CDI iCell CM<sup>2</sup> cardiomyocytes were transduced with AAV4-CAG-ChR2 upon cell seeding. After 10 days in culture, the plate was paced with 5 ms blue light pulses at 2 Hz for 48 hours. Untransduced control wells were exposed to the same blue light, but did not pace in response. The cardiomyocytes were then dosed with isoproterenol, a positive inotrope, or vehicle control. Chronically paced wells (orange) showed an increase in beat amplitude in response to isoproterenol, while unpaced control wells (gray) showed no change in beat amplitude.

### Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural and cardiac cell culture activity with a flexible, easy-to-use benchtop system.
- AxIS Navigator software makes analysis and reporting of functional data simple and hassle-free with an array of automatically generated metrics and advanced analysis tools.
- hiPSC-derived cardiomyocytes exhibit maturation of electrophysiological and contractile function following only 48 hours of chronic pacing, as measured using turn-key assays for action potential and contractility measurements.