# Characterization of Human iPSC-derived Cardiomyocyte Electrophysiology with the Local Extracellular Action Potential (LEAP) Assay

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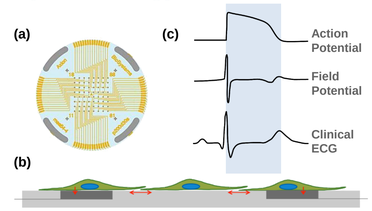
## Multiwell MEA Technology

### Microelectrode array technology

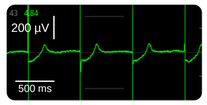
The flexibility and accessibility of neural and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that 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.

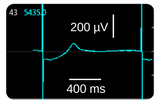



A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), to model complex, human systems. Electrodes detect changes in raw voltage (c) and record extracellular field potentials.

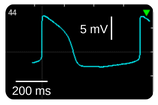
**Raw Voltage**



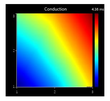

**Extracellular Field Potentials**




**Local Extracellular Action Potentials**

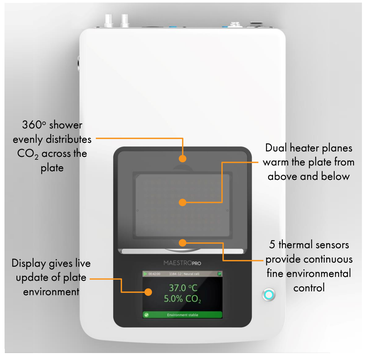



**Network Activity**



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™




- **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 (12-, 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

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

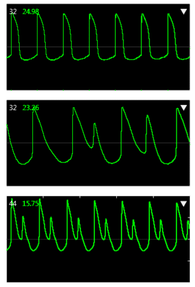
| Feature | Maestro Edge | Maestro Pro |
|---|---|---|
| Recording Electrodes | 384 | 768 |
| BioCore Chip | 6 Chips (v4) | 12 Chips (v4) |
| MEA Plates | 24-Well | 12-, 24-, 48-, 96-Well |
| Integrated Hard Drive | 0.5 TB | 1.0 TB |
| Touchscreen | No | Yes |
| Optical Stimulation | No | Yes |

## Local Extracellular Action Potential

### LEAP Provides Measures of Action Potential Morphology

The LEAP signal reveals action potential morphology phenotypes ranging from normal cardiac repolarization (top) to early after-depolarization (EAD) events (middle) and more severe repolarization instabilities (bottom). Here, we have evaluated the LEAP morphology using the Ncardia Cor4U and Pluricyte CMs.

The LEAP signal provides a new set of measurements for cardiac electrophysiology applications. The duration of the LEAP signal (LPD) can be measured at each point in repolarization (e.g., LPD30 at 30% repolarization or LPD90 at 90% repolarization). The result is a label-free, non-invasive measure of action potential morphology with high signal-to-noise ratio.

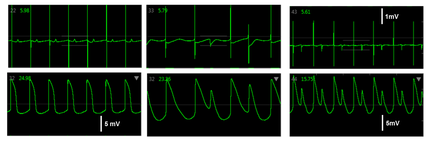


#### The LEAP Advantage

- **Label free and non-invasive** measurement of action potential-like signal shapes
- **High amplitude potential** (5-15 mV) and high signal-to-noise ratio
- **Long-lasting and stable signals** (> 10 min, up to hours)
- **Easy inspection** of potential prolongation and EADs
- **Simple induction and high throughput**

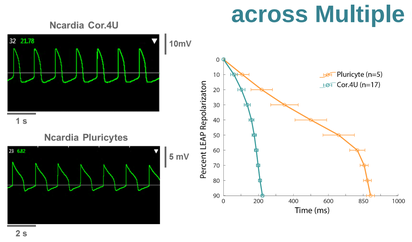
### LEAP Signals Link Field Potential and Action Potential Morphology

The LEAP signal may be induced on a subset of electrodes, allowing simultaneous measurement of field potential and LEAP signals. This facilitates direct comparison of field potential and action potential morphology during the depolarization and repolarization stages of the cardiac action potential.

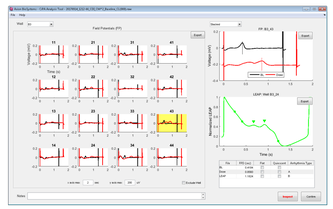


FP and LEAP Signals from the Same Wells, 5x Zoom on the FP

### LEAP Facilitates Comparison of AP Morphology across Multiple CM Lines

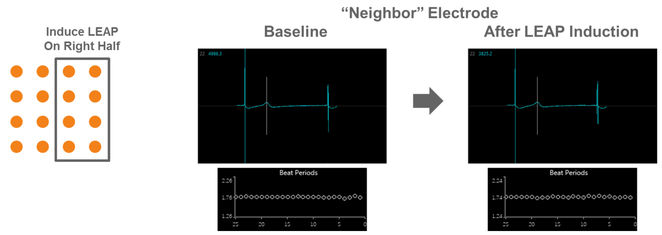



The Cor4U and Pluricyte CM lines exhibit distinct action potential morphology as revealed by the LEAP signals, such that each might tailor towards specific applications.



The CiPA Analysis Tool provides automated analysis of action potential morphology and EAD detection for LEAP signals.

### LEAP Does Not Disrupt the Underlying Biology

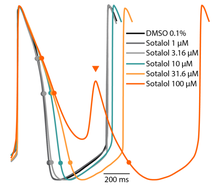



The induction of LEAP does not affect the underlying electrophysiological properties of the cardiomyocyte syncytium. In the example above, the beat period and field potential shape remain constant immediately before and after induction of LEAP on neighboring electrodes in the well.

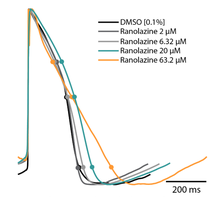
## LEAP Pharmacology with hiPSC-CMs

### LEAP Reveals Changes in Electrophysiology for Multichannel Block

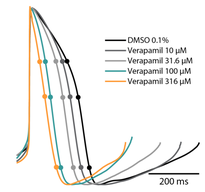
**Sotalol (hERG Block)**




**Ranolazine (hERG + Late Sodium Block)**




**Verapamil (hERG + Calcium Block)**

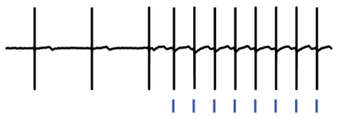


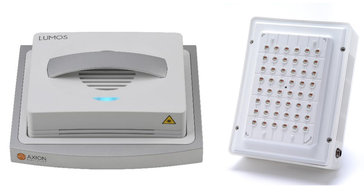

The LEAP signal measures the change in CM electrophysiology in response to block of multiple cardiac ion channels. Cor4U CMs exhibited prolongation and EADs with increasing concentration of sotalol (hERG block, left), whereas compensatory block of late sodium current (Ranolazine, middle) and calcium current (Verapamil, right) produced minimal prolongation or shortening of repolarization, respectively, compared to pure hERG block.

### The Lumos Multiwell Optical Stimulator Enables Cardiac Pacing

With optogenetics, light can be used to control and pace cardiomyocytes without artifact. Pacing cardiomyocytes offers many advantages:

- Specify beat rate at 1Hz for enhanced physiological relevance
- Establish well-to-well and plate-to-plate consistency with matched beat rates in all wells
- Detect use-dependent drug effects for superior safety screening



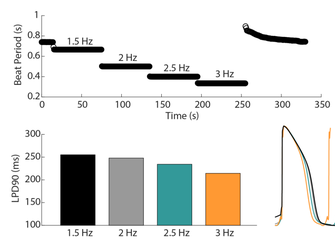


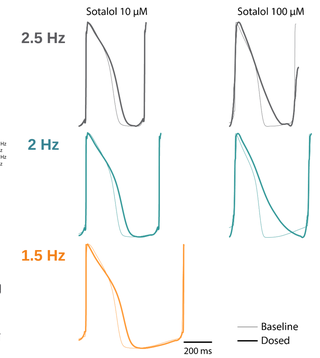

The Lumos™ is the first commercial multiwell light delivery device designed for *in vitro* optogenetics. The Lumos provides precise control over cardiomyocyte beat rate or neural activity.

#### The Lumos Advantage

- **Artifact free stimulation and pacing**
- **High throughput** with 192 LEDs over 48 wells
- **Compatible with any opsin** with 4 wavelengths encompassing the visual spectrum (460-670 nm)
- **Maximal intensity** with high power LEDs and optimized plate and lid optics on the Lumos MEA
- **Precise control** with microsecond precision and finely adjustable intensity for each LED
- **Flexible control** as each LED can be controlled independently and simultaneously

### Combining Optogenetic Pacing with the LEAP Assay




Channelrhodopsin (ChR2), a light sensitive ion channel, was introduced into Cor4U CMs with the Xpress4U reagent allowing optogenetic pacing of the CM beat rate. The CMs were paced at successively increasing rates, such that the rate-dependent repolarization timing was measured with LEAP before and after compound addition.