Characterization of the local extracellular action potential (LEAP) signal for use in cardiac safety evaluation

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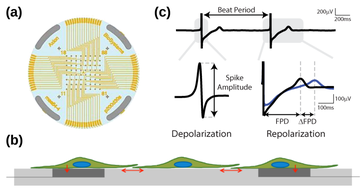
AXION BIOSYSTEMS
explore life's circuitry™

Multiwell MEA Technology

Microelectrode array technology

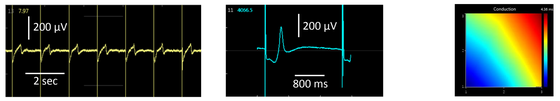
The flexibility and accessibility of neural and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that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



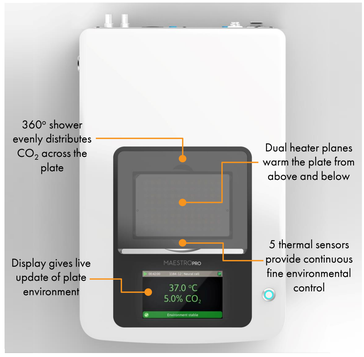
A planar grid of microelectrodes **(a)** interfaces with cultured neurons or cardiomyocytes **(b)**, to model complex, human systems. Electrodes detect changes in raw voltage **(c)** and record extracellular field potentials.

Raw Voltage | **Extracellular 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 modeling, 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

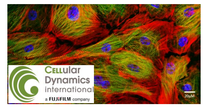
The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

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

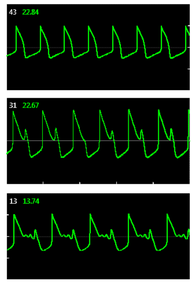
Local Extracellular Action Potential

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 (e.g., "rolling EADs", bottom). Here, we have evaluated the LEAP morphology using the iCell Cardiomyocyte[2].



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).

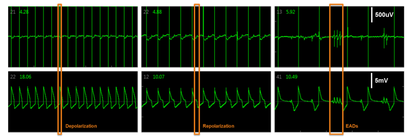


The LEAP Advantage

- **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**

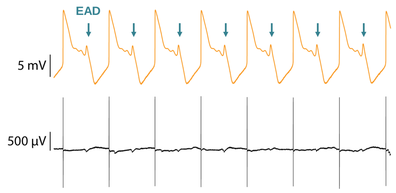
LEAP Signals Link Field Potential and Action Potential Morphology

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.

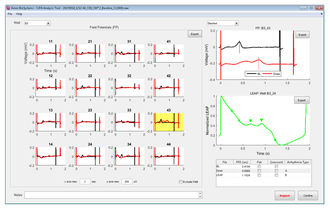


FP and LEAP Signals from the Same Wells, 10x Zoom on the FP

LEAP Facilitates Automated EAD Detection

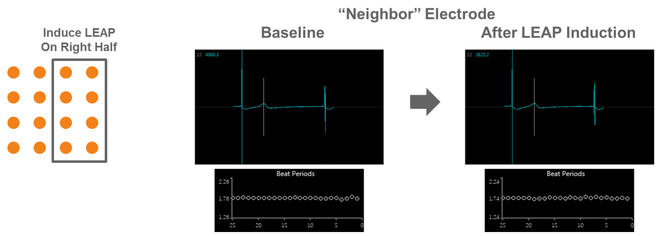


The LEAP signal dramatically increases the signal strength of EADs, which enables automated identification of EADs.



The updated CiPA Analysis Tool provides automated EAD detection for LEAP signals, as well as other LEAP endpoints.

LEAP Does Not Disrupt the Underlying Biology



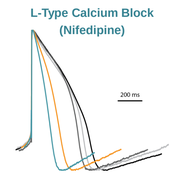
The induction of LEAP does not affect the underlying electrophysiological properties of the cardiomyocyte syncytium. In the example above, the beat period and field potential shape remain constant immediately before and after induction of LEAP on neighboring electrodes in the well.

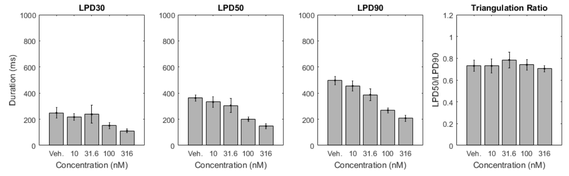
LEAP Pharmacology with hiPSC-CMs

LEAP Captures Expected Changes in Action Potential Morphology

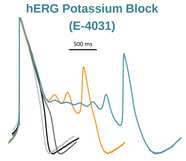
The LEAP signal was used to characterize changes in action potential morphology with common positive control compounds, including Nifedipine (L-type calcium channel block), E-4031 (hERG potassium channel block), and Verapamil (combined calcium and potassium block) using the iCell CM[2]. Expected responses were observed, with shortening of repolarization for Nifedipine and prolongation of repolarization with E-4031. Verapamil displayed shortened repolarization as well as triangulation of the action potential.

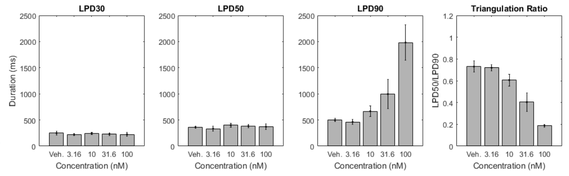
L-Type Calcium Block (Nifedipine)



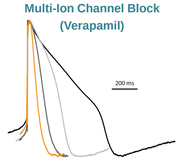


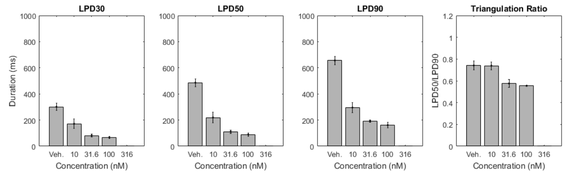
hERG Potassium Block (E-4031)





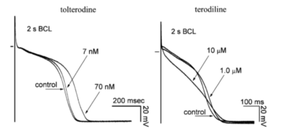
Multi-Ion Channel Block (Verapamil)





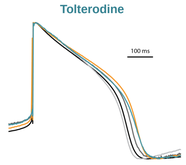
LEAP Detects Drug-Induced Changes in AP Triangulation

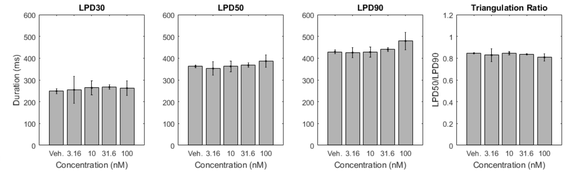
In addition to repolarization delay, the triangulation of the cardiac action potential may be predictive of repolarization instabilities and proarrhythmic risk. A case study with tolterodine and terodiline was used to evaluate the ability of the LEAP signal to quantify triangulation. Tolterodine, a potent hERG blocker, has previously been found to prolong the action potential without inducing triangulation, consistent with a clean clinical profile. Terodiline, however, has been removed from the market due to proarrhythmic risk and is associated with action potential triangulation.



Martin et al (2006) used dog purkinje fiber experiments to identify action potential triangulation associated with terodiline, but not tolterodine.

Tolterodine





Terodiline