Multiwell Microelectrode Array Technology for the Evaluation of Human iPSC-Derived Neuron and Cardiomyocyte Development and Maturation

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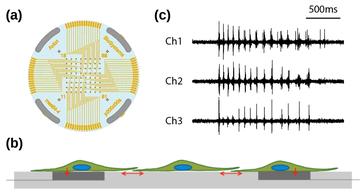
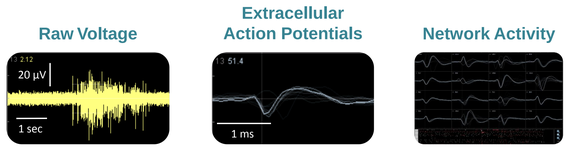
Multiwell MEA Technology

Why use microelectrode arrays?

The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced *in vitro* at previously unimaginable scales. Accurate characterization of stem cell-derived neurons and cardiomyocytes requires an assay to provide a functional phenotype. For these electro-active cells, measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling. The Maestro™ microelectrode array (MEA) platform offers such a solution by providing a label-free, non-invasive bench-top system to simply, rapidly, and accurately record functional activity from a population of cells cultured on an array of extracellular electrodes.

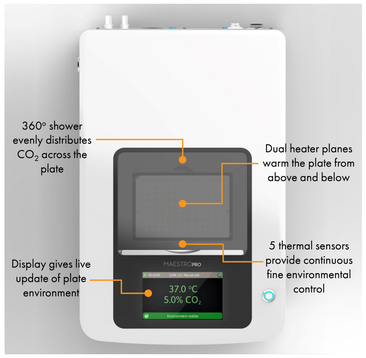


A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.



Raw voltage signals are processed in real-time to obtain extracellular action 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

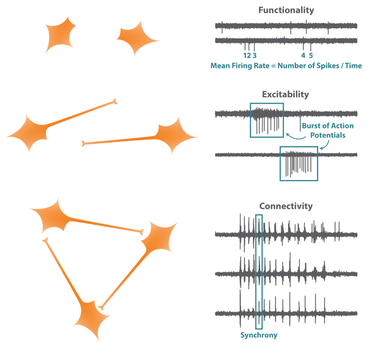
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	24-Well	12-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	No	Yes

MEA Assay with Neurons

Neural Electrophysiology Phenotypes

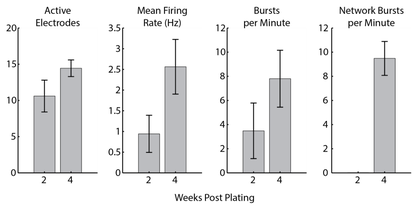
AxIS™ control and analysis software provides straightforward reporting of multiple measures on the maturity of the cell culture:

- **Functionality** – Neurons within the population produce spontaneous action potentials. The mean firing rate (MFR) counts action potentials over time to quantify individual neuron functionality.
- **Excitability** – Neurons may fire multiple action potentials within a short time period, called a burst. Established algorithms detect and quantify burst behavior.
- **Connectivity** – Synaptic connections between neurons in a population may lead to coincident action potentials. Network burst and synchrony measurements quantify connectivity.

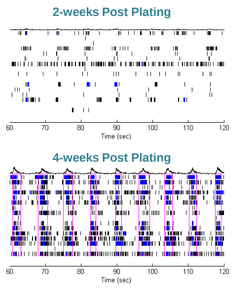


iPSC-Neuron Maturation

The Maestro's high electrode count and label-free recording provides the perfect platform for long-term evaluation of neural network formation from plated iPSC neurons. Maturation of the culture can be confirmed through the evolution of network electrophysiology metrics such as mean firing rate (MFR), bursting, and synchronous network bursts.



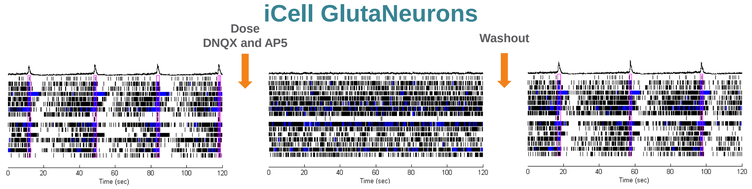
iPSC-derived neurons exhibit functional coverage two weeks after plating with emerging excitability (MFR). By week four, the same culture exhibits a consistent and reliable network burst phenotype indicative of established synapses and *in vivo*-like activity.



The networks have become spontaneously active by week 2, with a network burst phenotype emerging at week 4 of culture.

Data courtesy of Steven Biesmans and Anne Bang, SBP

iCell GlutaNeurons



The iCell GlutaNeurons demonstrate a regular, network bursting phenotype in the baseline condition.

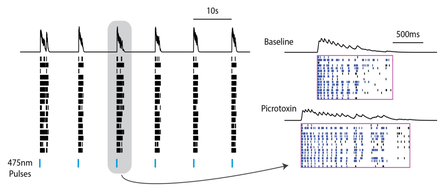
Dosing with DNQX and AP5 eliminates glutamatergic transmission and the network bursting phenotype.

Washout of the compounds restores the regular, network bursting phenotype from the baseline condition indicating the phenotype is mediated by glutamatergic neurons..

Data courtesy of Cellular Dynamics

Evoked Neural Activity for Seizurogenic Screening

Stimulation enables the computation of evoked activity measures. For each electrode, and each well, key parameters of the stimulus-evoked response can be calculated and used to inform assessment of seizurogenic activity. Even illumination ensures reliable results across wells, improving assay sensitivity. The addition of a seizurogenic compound (Picrotoxin, right) significantly prolongs the duration of the evoked network bursts.

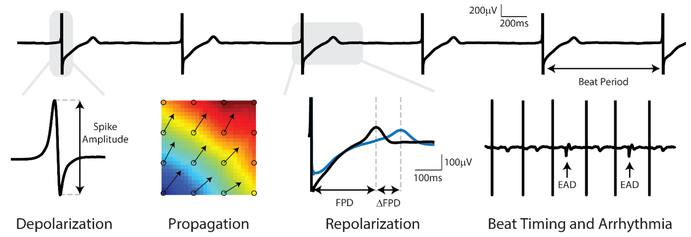


MEA Assay with Cardiomyocytes

Cardiac 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 electrical signals from cells cultured directly onto an array of planar electrodes in each well of the MEA plate. Multiple electrodes in each well provide mechanistic electrophysiological data reflecting the following important variables:

- **Depolarization** – Cardiomyocyte depolarization is detected by the amplitude of the field potential spike (AMP).
- **Repolarization** – The duration of the action potential is signified by the field potential duration (FPD).
- **Propagation** – The field potential (FP) is detected at each electrode as the activity propagates across the syncytium.
- **Arrhythmia** – arrhythmic indicators, such as early afterdepolarizations (EADs), are readily detected in the FP.

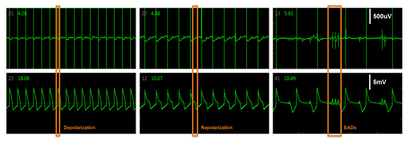


Positive Controls

The positive control compounds demonstrate the expected responses for blockade of potassium, calcium, and sodium currents, respectively. Dofetilide at higher concentrations causes significant prolongation of FPDc and arrhythmia incidence, whereas nifedipine reduces FPDc. JNJ303 produces a subtle, but detectable, prolongation of FPDc. Lidocaine has little effect on repolarization but elicits a significant reduction in amplitude. Asterisk (*) indicates the proportion of wells that show an incidence of early afterdepolarizations (EADs).

LEAP Provides Measures of 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

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 software makes analysis and reporting of functional data simple and hassle-free with an array of automatically generated metrics and advanced analysis tools.
- By bringing human biology to a dish, hiPSC-derived neurons and cardiomyocytes deliver biologically-relevant data to safety and toxicology, disease-in-a-dish modeling, and drug discovery for more accurate and predictive results.