Novel methods for assessing electrophysiological function of cardiac and neural 3D cell cultures with MEA technology

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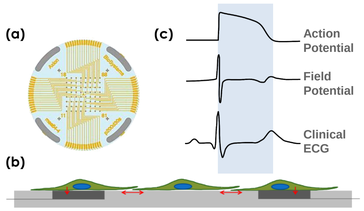
Multiwell MEA Technology

Microelectrode array technology

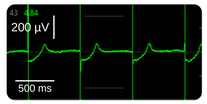
The flexibility and accessibility of neuronal and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that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

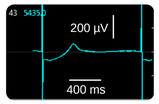


A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), to model complex, human systems. Electrodes detect changes in raw voltage (c) and record extracellular field potentials.

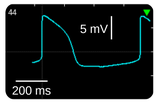
Raw Voltage



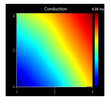
Extracellular Field Potentials



Local Extracellular Action Potentials

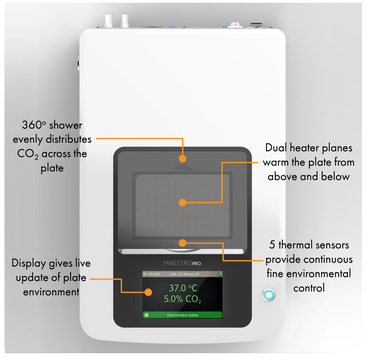


Network Activity



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 (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

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

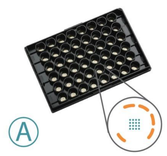
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	6- and 24-Well	6-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	Yes	Yes

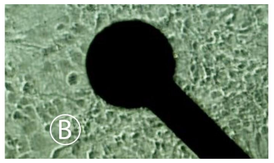
MEA Assay with Cardiomyocytes

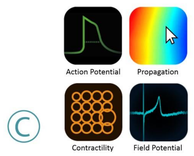
Functional Cardiac Phenotypes

The need for simple, reliable, and predictive pre-clinical assays for drug discovery (e.g. "disease-in-a-dish" models) and safety have motivated the development of 2D and 3D in vitro models with human stem cell-derived cardiomyocytes. 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.
- **Action Potential** – first scalable technique for acquiring action potential signals from intact cardiac models.
- **Propagation** – detect speed and direction of action potential propagation.
- **Contractility** – assess the contractility of cardiac monolayers adhered to the 2D array.

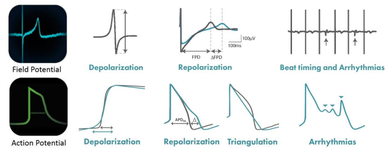


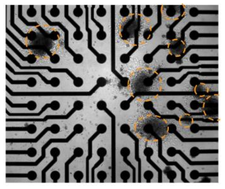


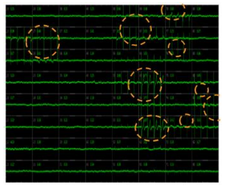


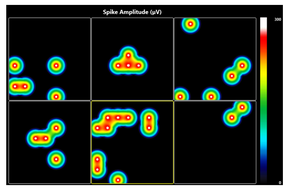
Multiwell Measurements of Functional Cardiac Spheroids

Multiwell MEA plates may be used to assess one or more cardiomyocyte spheroids per well. The Field Potential or Action Potential assays provide information on the cardiomyocyte electrophysiology, specifically effects related to depolarization or repolarization.





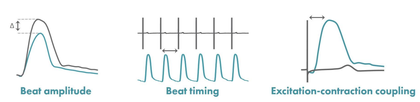


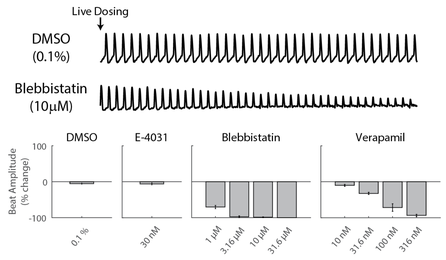


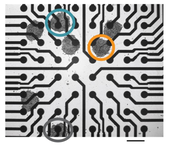
Multiple cardiomyocyte spheroids were deposited into each well of a 6-well CytoView MEA plate. The transparent plate substrate allowed visualization of spheroid attachment (left) to complement the field potential measurements of the cardiac spheroid activity (middle). Functional activity was detected from multiple spheroids in each of the wells on the plate (right).

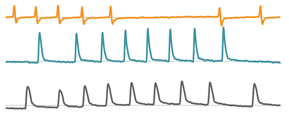
Contractility Adds a New Dimension to Cardiomyocyte Assays

Acquisition of contractility signals from the 2D MEA accurately detects beat amplitude for controls, and dose-dependent trends for Blebbistatin and Verapamil (bottom left). High-resolution contractility is robust for various culture conditions, including advanced structures like spheroids (bottom right).







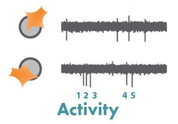


MEA Assay with Neurons

Functional Neuronal Phenotypes

AxIS Navigator analysis software provides straightforward reporting of multiple measures of cell culture maturity.

Mean Firing Rate = # of Spikes / Time



Activity

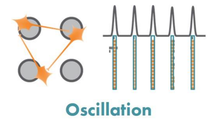
Are my neurons functional?
Action potentials are the defining feature of neuron function. High values indicate neurons are firing action potentials frequently. Low values indicate neurons may have impaired electrophysiological function.

Connectivity

Synchrony

Are my synapses functional?
Synapses are functional connections between neurons, such that an action potential from one neuron affects the likelihood of an action potential from another neuron. Synchrony reflects the strength of synaptic connections.

Burst of Action Potentials



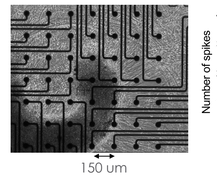
Oscillation

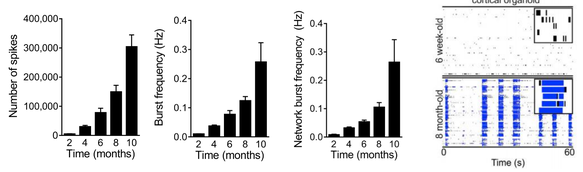
Is my network functional?
Neuronal oscillations, or alternating periods of high and low activity, are a hallmark of functional networks with balanced excitatory and inhibitory neurons. Oscillation is a measure of how the network activity is organized in time.

Cortical organoid activity mimics early human brain development

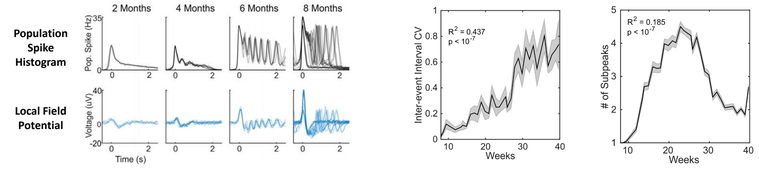
Data courtesy of the Alysson Muotri Lab. Modified from Trujillo et al, Cell Stem Cell, 2019.

Cortical organoids were generated from human iPSCs and plated on the CytoView MEA 6-well plates at 6 weeks. Spontaneous activity, bursting, and network oscillations were monitored on the Maestro Pro using both neural spikes and local field potential recordings, once per week for 10 months. The organoids exhibited increasingly complex activity over time, characterized by increasing mean firing rate, synchrony, single channel burst frequency, and network burst frequency, indicative of an evolving neural network.

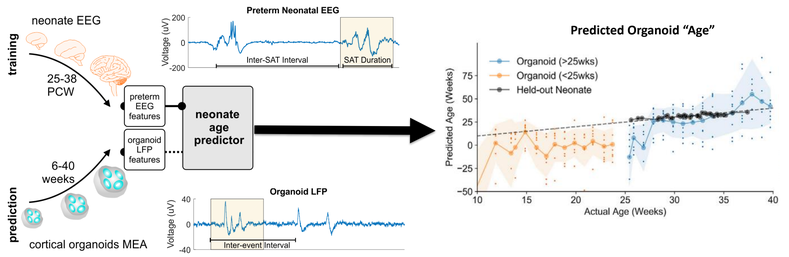




Network events became more frequent over time. After 4 months, secondary nested faster oscillations, or subpeaks (2-3 Hz), emerged inside the larger network events. The events became more variable over time, quantified as a an increase in inter-event interval coefficient of variation (CV).



To determine whether the emergence of complex network oscillations reflect early human neurodevelopment, organoid LFP features were compared to electroencephalograms (EEG) from 39 premature neonates. EEG data was used to train and validate a regularized regression model to predict organoid "age" over time based on 12 LFP features. Predicted organoid "age" increased over weeks in culture, suggesting that cortical organoid network dynamics mimic the evolving network dynamics of early human brain development.



Conclusions

By bringing biology to a dish, stem cell derived neurons and cardiomyocytes may be used to develop advanced, three dimensional models of human biology and disease. The Maestro multiwell MEA platform enables functional characterization of 3D neural and cardiac model in a benchtop system. AxIS Navigator software offers an array of automatically generated metrics and reports.