Multiwell microelectrode array (MEA) technology for the quantification of neuronal, synaptic, and network function for in vitro stem cell derived neuronal models

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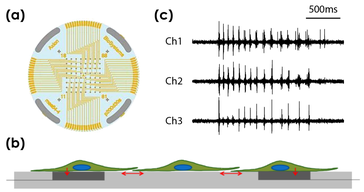
Multiwell MEA Technology

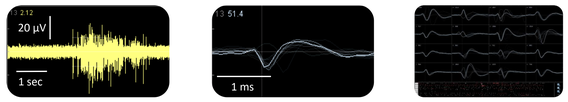
Why use microelectrode arrays?

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.



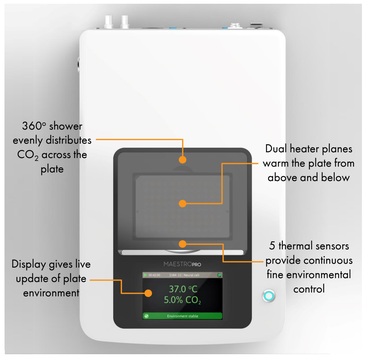
A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

Raw Voltage | **Extracellular Action Potentials** | **Network Activity**



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

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

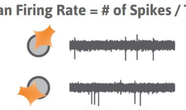
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	6-, 24-Well	6-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	Yes	Yes

MEA Assay with Neurons

Neural Electrophysiology Phenotypes

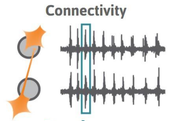
AxIS Navigator™ control and analysis software provides straightforward reporting of multiple measures on the maturity of the cell culture:

Mean Firing Rate = # of Spikes / Time



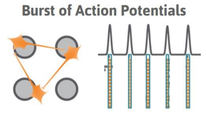
Are my neurons functional?
Action potentials are the defining feature of neuron function. High values indicate the neurons are firing action potentials frequently. Low values indicate the neurons may have impaired electrophysiological function.

Connectivity



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.

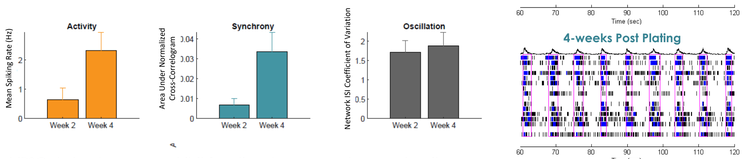
Burst of Action Potentials



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

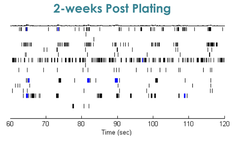
Maturation of hiPSC-derived neural networks

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



hiPSC-derived neurons exhibit functional coverage two weeks after plating with emerging activity (MFR). By week four, the same culture exhibits increased synchrony and consistent oscillatory network bursts, indicative of established synapses and networks.

2-weeks Post Plating



4-weeks Post Plating

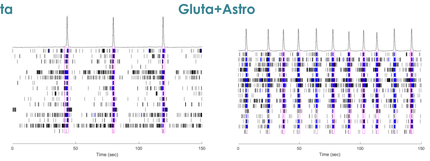
The networks are spontaneously active by week 2, with a network burst phenotype emerging at week 4 in culture.

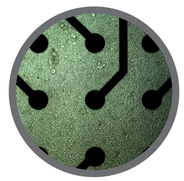
Data courtesy of Steven Biesmans and Anne Bang, SBP

Characterizing hiPSC-derived neurons and compound effects

The Maestro Pro and Maestro Edge are compatible with a wide array of MEA plate types and throughput scales that are ideal for optimizing stem cell development, plating conditions, and exploring compound effects. Here, we used the Maestro Pro to optimize iCell® GlutaNeuron culturing and to evaluate the effects of picrotoxin, a common seizurogenic compound. Network burst phenotypes were compared between iCell GlutaNeurons cultured alone or co-cultured with astrocytes. The CytoView MEA 48 plate allowed for cell and network visualization in parallel with electrophysiological measurements.

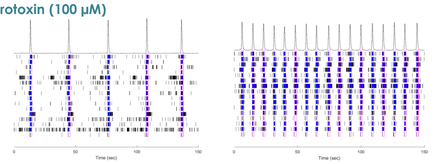
Gluta | **Gluta+Astro**

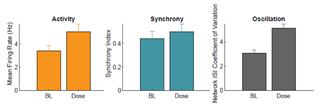




Astrocytes significantly altered the network burst phenotype of GlutaNeurons (data from a CytoView MEA 48).

Picrotoxin (100 µM)





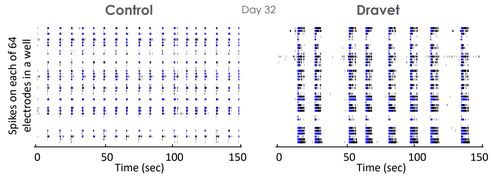
Effects of Picrotoxin (100 µM) on key neural metrics in GlutaNeuron + Astrocyte co-cultures (n=6).

Picrotoxin (100 µM) caused an increase in activity and network burst frequency and regularity, indicating seizurogenic properties.

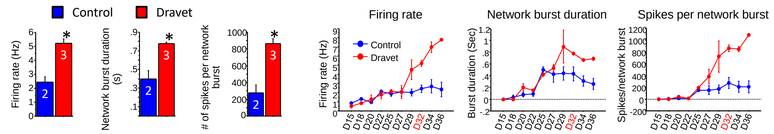
iPSC-derived Models of Neural Disease

Dravet Syndrome

Data courtesy of Dina Simkin and Evangelos Kiskinis, Northwestern University



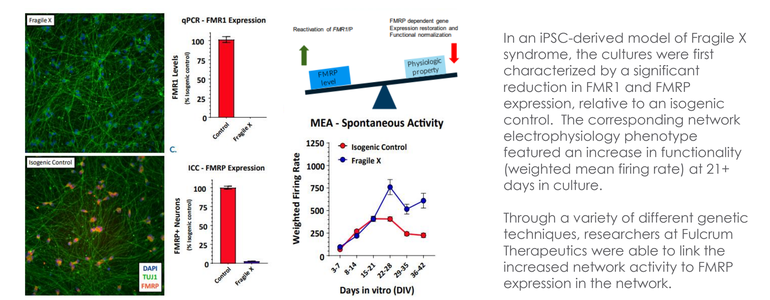
In vitro measurements of network activity may also be used to study epileptic disorders of genetic origin, such as Dravet syndrome. In an iPSC-derived model of Dravet syndrome, the cultures exhibit an enhanced network burst phenotype characterized by significantly longer bursts in mature cultures.



The Dravet Syndrome cultures exhibited significantly higher MFR, network burst duration, and spikes per network burst (left), as compared to the control cultures. The distinct network phenotype emerged ~27 days *in vitro*, with these measurements taken at 32 days *in vitro*.

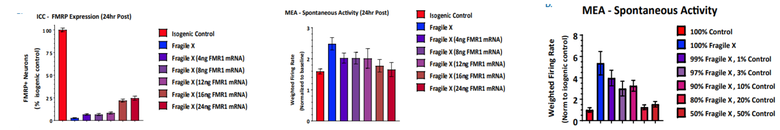
Fragile X

Data courtesy of John Graef, Fulcrum Therapeutics



In an iPSC-derived model of Fragile X syndrome, the cultures were first characterized by a significant reduction in FMR1 and FMRP expression, relative to an isogenic control. The corresponding network electrophysiology phenotype featured an increase in functionality (weighted mean firing rate) at 21+ days in culture.

Through a variety of different genetic techniques, researchers at Fulcrum Therapeutics were able to link the increased network activity to FMRP expression in the network.



FMRP expression was re-introduced to the Fragile X model through addition of FMR1 mRNA. With increasing addition of FMR1 mRNA, the FMRP expression increased, as expected, and the spontaneous network activity decreased to levels matching the control cultures. In addition, the proportion of Fragile X neurons co-cultured with Control neurons was titrated to determine the number of Control neurons, and thus FMRP expression, required for the Control phenotype.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural 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 deliver biologically-relevant data to allow for disease-in-a-dish modeling. The Maestro has been used to publish results with the following models of neural disease:
  - Fragile X, Autism, Epilepsy, Huntington's, Parkinson's, Williams Syndrome, Cockayne Syndrome, ALS, Alzheimer's, and others