Quantification of functional network electrophysiology from stem cell derived neurons with multiwell microelectrode array technology

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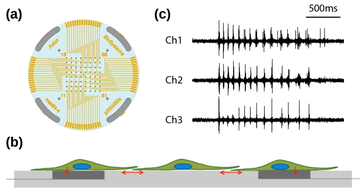
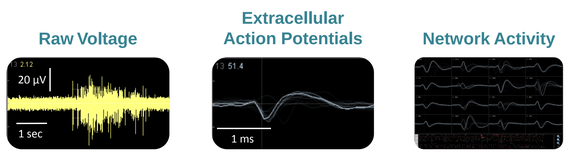
Multiwell MEA Technology

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.

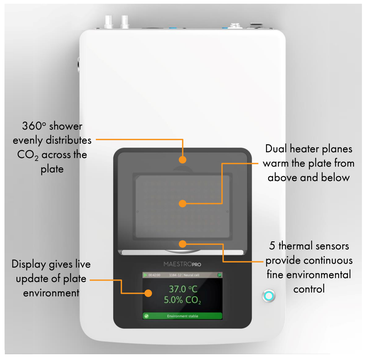


A planar grid of microelectrodes (a) interfaces with cultured neurons or cardiomyocytes (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.



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

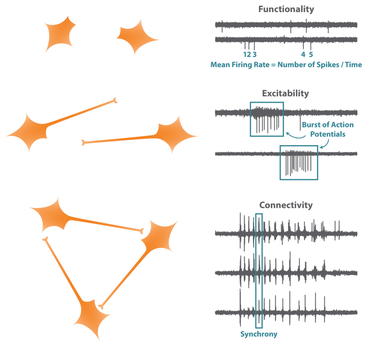
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	24-Well	12-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	No	Yes

MEA Assay with Neurons

Neural Electrophysiology Phenotypes

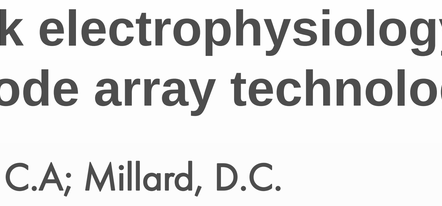
AxIS™ control and analysis software provides straightforward reporting of multiple measures on the maturity of the cell culture:

- **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.

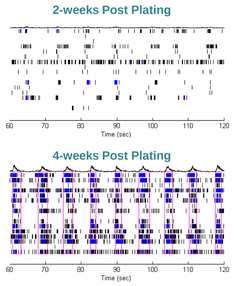


iPSC-Neuron Maturation

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.

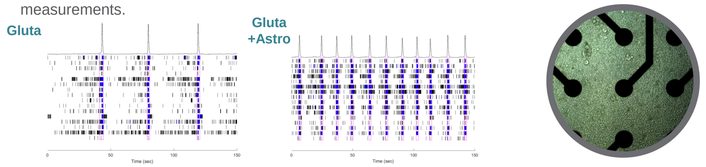


The networks have become spontaneously active by week 2, with a network burst phenotype emerging at week 4 of culture.

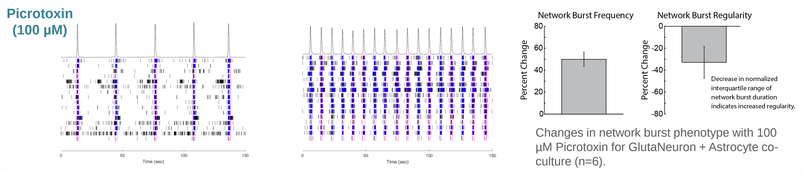
Data courtesy of Steven Biesmans and Anne Bang, SBP

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 on a Classic MEA 48 and CytoView MEA 48. The CytoView MEA 48 plate allowed for cell and network visualization in parallel with electrophysiological measurements.



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



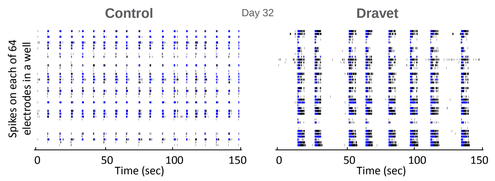
Changes in network burst phenotype with 100 µM Picrotoxin for GlutaNeuron + Astrocyte co-culture (n=6).

Picrotoxin caused an increase in network burst frequency and burst regularity, indicating seizurogenic properties.

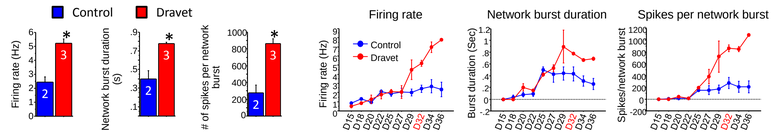
iPSC-derived Models of Neural Disease

Dravet Syndrome

Data courtesy of Dina Simkin and Evangelos Kiskinis, Northwestern University



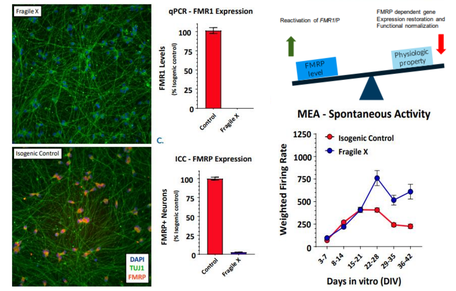
In vitro measurements of network activity may also be used to study epileptic disorders of genetic origin, such as Dravet syndrome. In an iPSC-derived model of Dravet syndrome, the cultures exhibit an enhanced network burst phenotype characterized by significantly longer bursts in mature cultures.



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*.

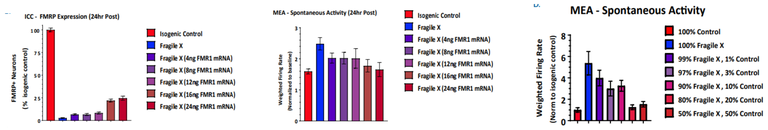
Fragile X

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 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 FMR1 expression in the network.



FMR1 expression was re-introduced to the Fragile X model through addition of FMR1 mRNA. With increasing addition of FMR1 mRNA, the FMR1 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 FMR1 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…