Microelectrode Array: in vitro, Functional Characterization of Stem Cell-derived Neurons

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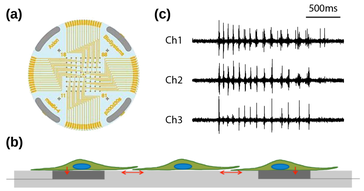
Multiwell MEA Technology

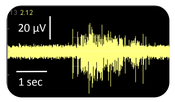
Why use microelectrode arrays?

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 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.

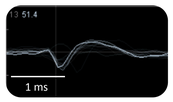


A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems in a dish. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

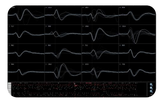
Raw Voltage



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

Why use the Maestro?

Axion's Maestro multiwell microelectrode array (MEA) platform enables functional cellular analysis on the benchtop with an industry leading 768 electrodes across all plate formats.

- **Label-free and non-invasive recording** of extracellular voltage from cultured neurons on Axion MEA plates
- **Environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the magnitude of depolarization events
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data through the integration of information from multiple locations in the culture
- **Scalable format (12-, 48- and 96-well plates)** meets all throughput needs on a single system

Typical Assay Workflow

Plate Preparation

Maintenance and Maturation

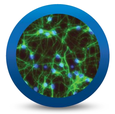
Experiment

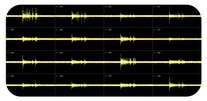
- Maestro experiments involve seeding cells onto the MEA plate and allowing the neural network to mature over a period of days to weeks.
- MEA technology is label-free and non-invasive, such that the maturation process can be monitored through repeated recordings over that time frame.
- The network electrophysiology phenotype provides a functional measure in response to perturbations of key biological variables, such as pharmacology or gene expression.

MEA Assay with iPSC-Neurons

Why measure network electrophysiology?

Neurons within a functional network form connections, called synapses, that enable the transmission of excitation or inhibition from one cell to the next. MEAs record activity from many cells in a population to provide measures of network electrophysiology. The resultant network electrophysiology provides important information on the maturity of the cells in a network, and can be used as a functional measure for a variety of assays types.



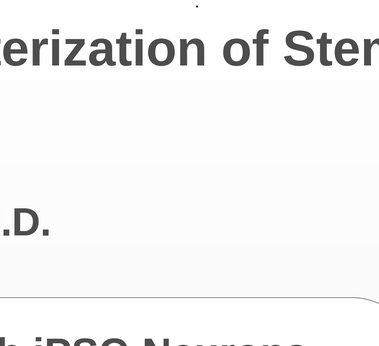


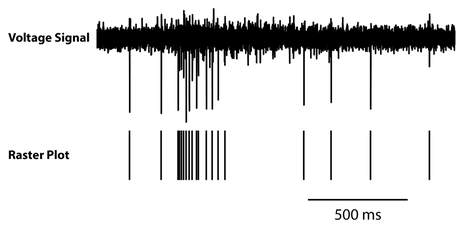
- **Reduce variability** across wells and plates by quantifying the integrated activity of many cells in a network.
- **Assess safety and toxicology** through functional evaluation of human biology *in vitro*.
- **Design disease-in-a-dish models** that characterize patient-specific cell lines or genetic edits.
- **Perform phenotypic drug discovery** by utilizing functional cell-based models in a high-throughput MEA assay.
- **Optimize stem cell differentiation** and culturing protocols by assessing network development with functional endpoints.

Network Electrophysiology Phenotypes

AxIS software enables simple analysis of multiple measures on the maturity of the cell culture:

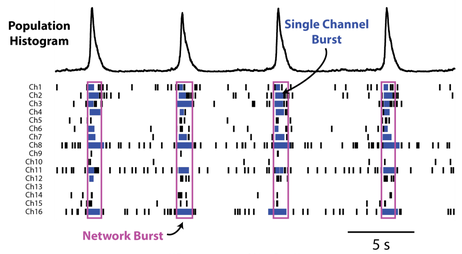
- **Functionality** – Neurons within the population produce spontaneous action potentials. The mean firing rate (MFR) counts action potentials over time to quantify functionality.
- **Excitability** – Neurons may fire multiple action potentials within a short time period, called a burst. Established 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.





Neural action potentials are detected as changes in voltage above a user-defined threshold. A simple view of this activity is a raster plot where each detected action potential is represented by a "tick" mark to denote the spike time.

The timing of the spikes contains all of the information required to calculate measures like mean firing rate (activity over time) or bursting (clusters of action potential activity).



Data courtesy of NeuCyte

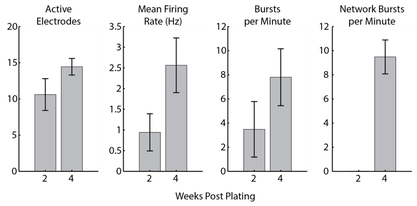
A well-wide raster plot enables the visualization of network activity across all electrodes in a well, and is computed automatically by AxIS and the Neural Metric Tool.

- Each "tick" mark represents a detected action potential.
- Each row illustrates a single electrode in the well.
- Multiple spikes occurring in a short time span defines a burst (blue).
- Coordinated bursting across a well is characterized as a network burst (pink).

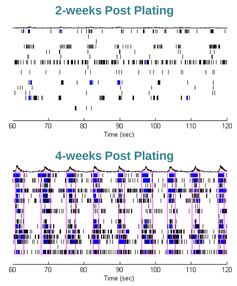
Applications

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.



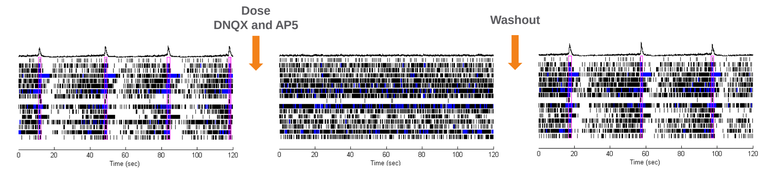
iPSC-derived neurons exhibit functional coverage two weeks after plating with emerging excitability (MFR). By week four, the same culture exhibits a consistent and reliable network burst phenotype indicative of an established synapses and *in vivo*-like activity.



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

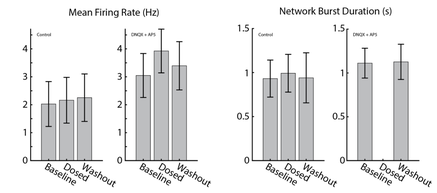
iCell GlutaNeurons



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.



Blocking glutamatergic transmission with DNQX and AP5 eliminates the network bursting phenotype, without affecting the mean firing rate of the cultures.

Thus, in this cell model, the network burst phenotype is likely mediated by high proportion of glutamatergic neurons in the population.

Data courtesy of Cellular Dynamics

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity and connectivity 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 neurobiology to a dish, hiPSC-derived neurons deliver biologically-relevant data to safety and toxicology, disease-in-a-dish modelling, and drug discovery for more accurate and predictive results.