Label-free Functional Analysis for the Characterization of iPSC-derived Neural Organoid Development and Maturation

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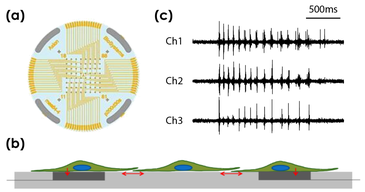
Multiwell MEA Technology

Microelectrode Array Technology

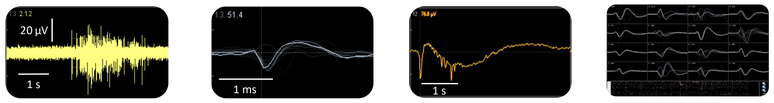
The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced *in vitro* at previously unimaginable scales. Accurate characterization of stem cell-derived neurons requires an assay to provide a functional phenotype. Measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell 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 in each well.



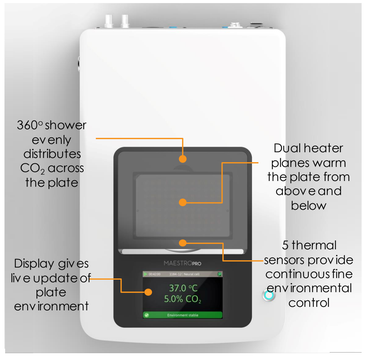
A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

Raw Voltage | **Extracellular Action Potentials** | **Local Field 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™



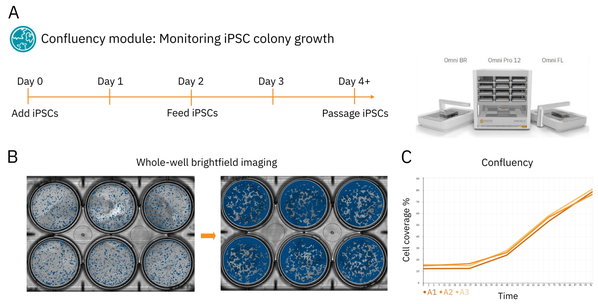
- **Label-free, non-invasive recording** of extracellular voltage from cultured electro-active cells
- **Integrated environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the depolarization 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

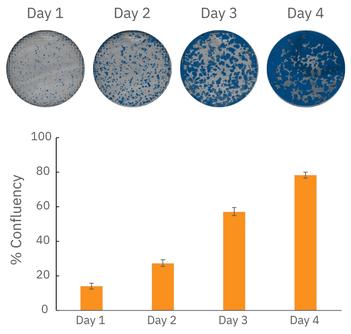
Real-time Monitoring for iPSC Expansion

Confluency Algorithm Tracks iPSC Growth



The Omni is a live-cell analysis platform capable of continuous multi-well imaging directly from the incubator. (A) Human iPSCs were plated and monitored over four days via the Omni Pro 12 platform. (B) Example whole-well brightfield images of iPSC colonies acquired by the Omni at low confluency and high confluency in a 6-well plate with the confluency map overlay. (C) The % cell coverage of iPSCs over time as exported from the Omni Cloud.

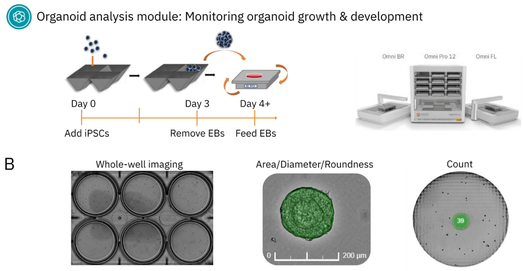
Whole Vessel Imaging Provides Unbiased Data



Manual inspection and estimation of culture confluence can be a time consuming and inaccurate process. Only a small fraction of a given well may be seen at once with a 2x objective, requiring the scientist to navigate across the well and mentally combine confluence estimates. The Omni full-vessel scan eliminates tedious scrolling on the microscope and provides an accurate measure of confluence.

Passaging iPSC colonies at the ideal timepoint is critical to maintaining pluripotent and healthy colonies. iPSC colony growth and coverage was monitored every day on the Omni platform as colonies grew in size from Day 1 to Day 4 of culture. The Confluency module was used to calculate iPSC confluency at each timepoint. The full vessel scan provides a comprehensive view of confluence and colony size.

Organoid Analysis Monitors Embryoid Body Number and Size



(A) EBs were formed via forced centrifugation in Aggrewell™ 800 plates and monitored over several days via the Omni platform.

(B) Example whole-well brightfield images of EBs and the metrics provided by the Organoid Analysis module.

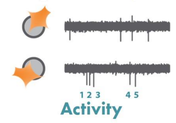
Organoid analysis uses whole-well bright field imaging to accurately analyze iPSC-derived embryoid body populations for area, diameter, roundness, and count. In contrast to current methods that rely on manually acquiring images from a standard microscope, the Omni allows for easy, automated characterization of embryoid bodies prior to the initiation of differentiation. By allowing for upstream quality control, the Omni analysis software greatly reduces the time and efforts the user must spend in optimizing long-term differentiation protocols and can provide guidance in identifying key morphological features that are needed for successful differentiation, improving the final yield and reducing overall culture costs

MEA Assay with Neural Organoids

Functional Neuronal Phenotypes

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

Mean Firing Rate = # of Spikes / Time



Activity

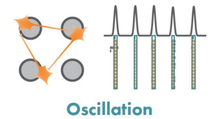
Are my neurons functional?
Action potentials are the defining feature of neuron function. High values indicate neurons are firing action potentials frequently. Low values indicate neurons may have impaired electrophysiological function.

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.

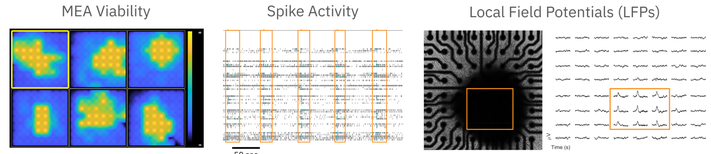
Burst of Action Potentials



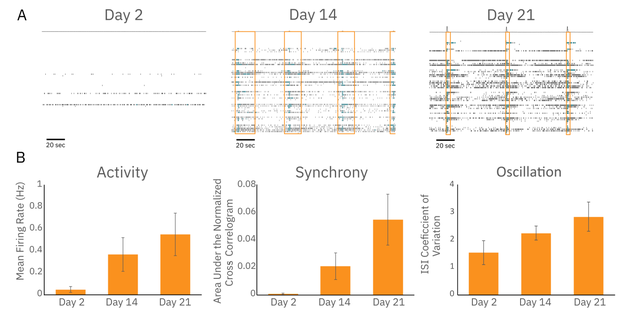
Oscillation

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.

Real-time Functional Analysis of iPSC-Neural Organoids



Neural organoids are three-dimensional *in vitro* cell cultures that recapitulate aspects of human brain physiology, structure, and developmental processes. The Maestro MEA platform can be used to characterize the activity of iPSC-derived neural organoids in real-time by measuring important neural metrics such as viability, neural spike activity, and local field potentials (LFP). Furthermore, the Maestro MEA system detects key parameters of neural network function, including activity, synchrony, and oscillation.



iPSC-derived dorsal forebrain neural organoids (aged Day 50) were plated on a 6-well CytoView MEA plate and maintained in culture for 3 weeks. (A) Raster plots and (B) bar plots demonstrate an increase in neural activity, synchrony, and oscillation over time, indicative of neural organoid maturation and development.

Conclusions

- iPSC expansion and maintenance requires vigilant culture inspection to evaluate colony size and overall confluence. Indeed, if colonies become too large, spontaneous differentiation can occur. The Omni brightfield scan provides an automated, and quantitative, assessment of iPSC cultures.
- The organoid analysis algorithm was used to demonstrate an automated approach for EB monitoring that can streamline the process by tracking EB size, shape, and count, in order to yield consistent and high-quality cultures for downstream differentiation protocols.
- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system and can be used to track neural network development and maturation of iPSC-derived neural organoids.