>> Label-free Functional Analysis for the Screening of iPSC-derived Neural Organoid Response to Neuroactive Compounds

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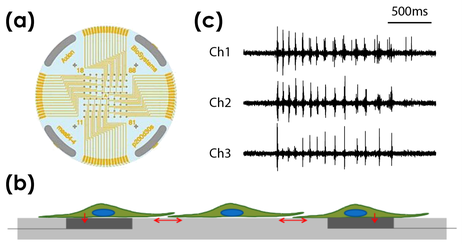
Abstract #4637

Live-Cell Analysis

Microelectrode Array (MEA) 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. 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.



The Maestro MEA Product Family

Features	*Maestro Pro*	*Maestro Edge*	*Maestro Volt**
Throughput (well format)	6, 24, 48, 96, 384**	6, 24, 96**	6
MEA Mode	✓	✓	✓
MEA Viability	✓	✓	
Impedance Mode	✓	✓	
Environmental Control	✓	✓	✓
Automation API	✓	✓	
Stimulation	Electrical & Optical	Electrical & Optical	Electrical
Omni Compatible	✓	✓	✓

*Maestro Volt only available in Europe and Asia
**Well format available in impedance only

- ***Label-free, non-invasive tracking*** *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*

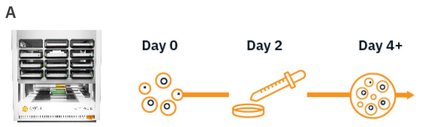
The Omni Product Family

- ***Assay your cells in brightfield and fluorescence –*** *From label-free cell monitoring to fluorescence-based assays, the Omni adds dynamic visual results to any experiment.*
- ***Track every moment, straight from your incubator –*** *The Omni operates within an incubator, automatically capturing images as your cells grow in their optimal environment.*
- ***See every cell –*** *The Omni moves the camera, not the cells, capturing detailed brightfield images of the entire culture without disturbing the cells.*
- ***Monitor and analyze your cells remotely –*** *The software allows you to monitor your cells and perform data analysis from your desktop.*

Features	*Lux3*	*Omni Pro 12*	*Omni*
Whole-well/Plate Brightfield		✓	✓
Automated Acquisition	✓	✓	✓
Fluorescence	✓	✓	✓
Plate Handling	Manual	Automated	Manual
Number of Plates	1	12	1
Incubator Compatible	✓	✓	✓
Dimensions & Weight	166 x 140 x 135 mm 1,3 kg	460 x 417 x 439 mm 40,2 kg	345 x 396 x 171 mm 9,7 kg

Real-time Monitoring for iPSC Expansion

iPSC Module Tracks iPSC Growth

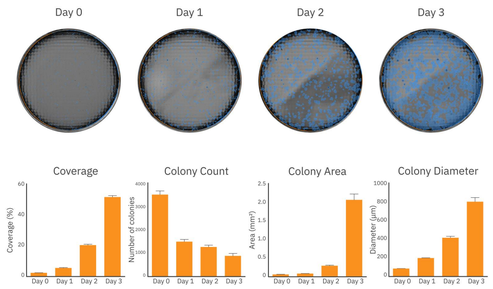


The Omni is a live-cell analysis platform is capable of continuous multi-well imaging directly from the incubator.

(A) Human iPSCs were plated and monitored over four days via the Omni platform.

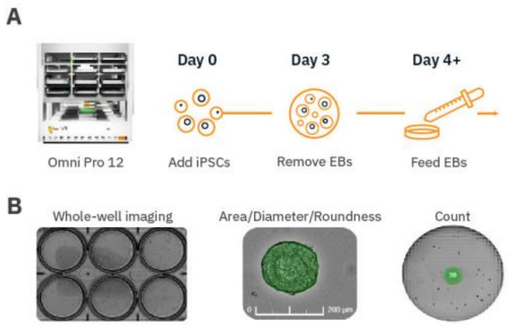
(B) Example whole-well brightfield images of iPSC colonies acquired by the Omni in a 6-well plate and a close up of a single colony with the metrics provided by the iPSC Module including area, diameter, and roundness.

Whole Vessel Imaging Provides Unbiased Data



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

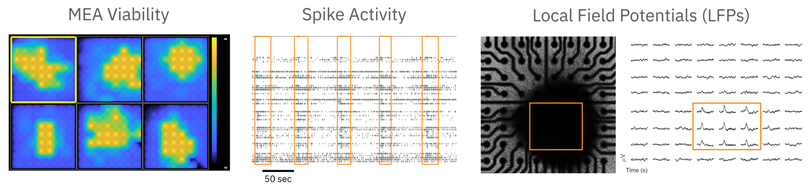


(A) EBs were formed via forced centrifugation in Aggrewell™ 800 plates and monitored over several days via the Omni platform.

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

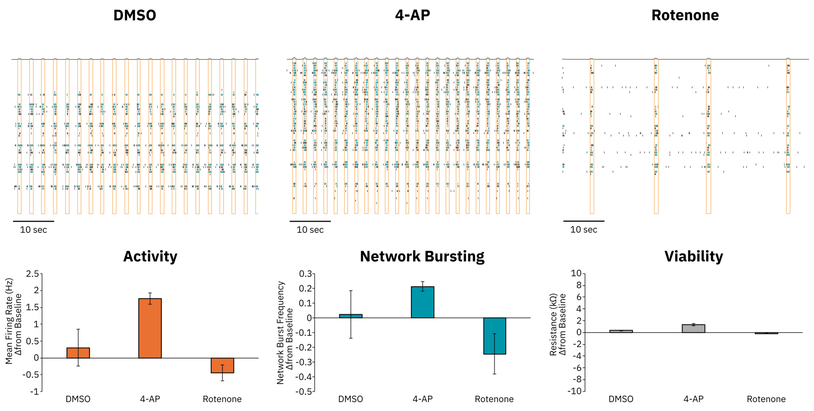
MEA Assay with Neural Organoids

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.

Midbrain Organoid Response to Neuroactive Compounds



Due to their ability to better recapitulate *in vivo* phenotypes, neural organoids are a promising model for screening potentially neurotoxic compounds. Therefore, we dosed pre-made midbrain organoids (STEMCELL Technologies, Cat. # 200-0793) at 125 days post differentiation with 4-aminopyridine (4-AP) and rotenone. At baseline and at one hour post dose, recordings were taken on the Maestro Pro to assess the changes in organoid electrophysiology in response to these compounds. 4-AP, a potassium channel blocker, led to an increase in mean firing rate and network burst frequency. In contrast, rotenone, a pesticide that interferes with complex I of the mitochondria and the electron transport chain, led to marked decreases in mean firing rate and network bursting. Importantly, viability of the organoids remained consistent before and after compound dosing, highlighting that these effects were not due to cell death.

Conclusions

- The Omni brightfield scan provides an automated, and quantitative, assessment of iPSC cultures and organoid differentiation.
- The Maestro multiwell MEA platform enables functional characterization of neural organoids with a flexible, easy-to-use benchtop system.
- Electrophysiological changes in organoids caused by compound dosing can be measured on the Maestro system, allowing for more physiologically relevant neurotoxicity screening.