>> Functional Analysis of iPSC-derived Three-Dimensional Models on Traditional and Novel Microelectrode Arrays

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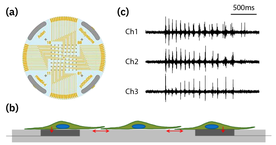
Live Cell Analysis

Microelectrode Array Technology

The complexity and difficulty of measuring signals from the brain make the *in vivo* study of neurological disorders, as well as toxicological drug screening, laborious and costly processes. *In vitro* neural cultures provide an alternative to *in vivo* studies and allow for high throughput analysis of neurological disease states and neurotoxic and seizurogenic assessment of drugs. 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.

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
**Matrix 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*

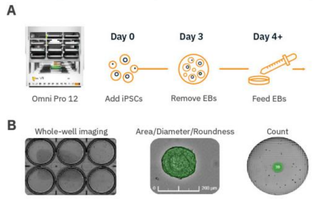
The Omni Product Family

- ***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.*
- ***Get started quickly –*** *With an easy-to-install, maintenance-free device that does not require calibration, a short training is all it takes to start using the Omni.*

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

MEA Assay for 3D Neural Models

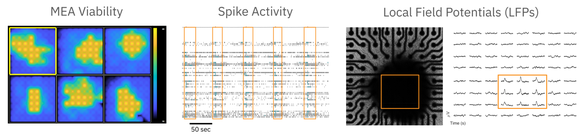
Organoid Analysis Monitors Embryoid Body Number and Size



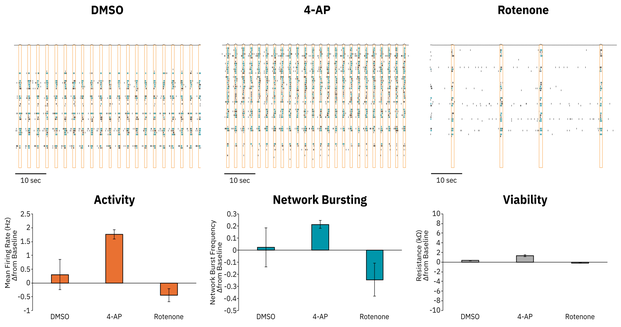
A. EBs were formed via forced centrifugation in Aggrewell™ 800 plates and monitored over several days via the Omni platform.
B. Example whole-well brightfield images of EBs and the metrics provided by the Organoid Analysis module.

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.

Characterization of Neural Organoids using the Maestro



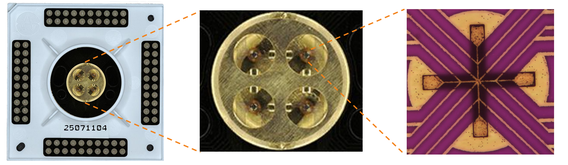
Neural organoids are three-dimensional *in vitro* cell cultures that recapitulate aspects of human brain physiology, structure, and developmental processes. The Maestro MEA platform can be used to characterize the activity of iPSC-derived neural organoids in real-time by measuring important neural metrics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



We dosed pre-made midbrain organoids (STEMCELL Technologies, Cat. # 200-0793) at 125 days post differentiation with 4-AP and rotenone. 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, led to marked decreases in mean firing rate and network bursting. Viability remained constant throughout the experiment, illustrating that changes in activity were due to the compounds' effects on the organoid electrophysiology and not overall cell health.

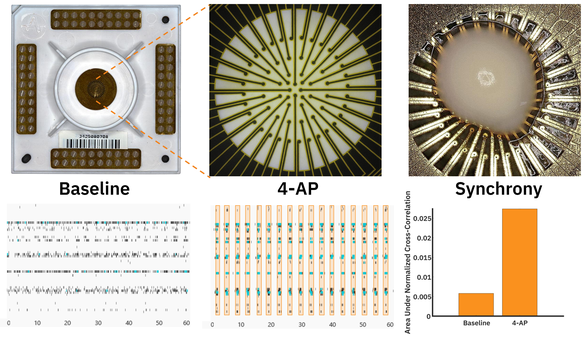
SpheroHD and 3DMap™ MEA – Solutions for Organoids

SpheroHD MEA



The SpheroHD MEA is comprised of an Ibidi 4 Well Fultrac micro-insert adhered to a microelectrode array chip, with each microwell positioned over an array of 16 high-density (50 µm spacing) electrodes. The chip's design leads to improved plating accuracy, ensuring the capture of neural activity on several electrodes even when measuring from small spheroids.

3DMap™ MEA



The 3DMap™ MEA uses flexible cantilevers with 64 electrodes that bend to conform to plated neural organoids, enabling 3D electrophysiological recording. Activity was measured from a pre-made midbrain organoid (STEMCELL Technologies, Cat. # 200-0793) at 111 days post differentiation. The organoid was also dosed with 4-AP, which led to marked increases in network bursting and synchrony.

Conclusions

- The Maestro MEA and Omni platforms work together to provide valuable morphological and electrophysiological characterization data during neural organoid differentiations.
- The SpheroHD MEA chip contains arrays of high-density electrodes and utilizes a specialized insert to provide precise, easy-to-use plating and robust measurement from neural spheroids.
- The 3DMap™ MEA chip's flexible electrodes enable three-dimensional recordings across multiple Z-planes, allowing high-resolution characterization of neural organoid responses to neuroactive compounds such as 4-AP.

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