Multiwell optogenetics for enhanced control of human iPSC-derived cells

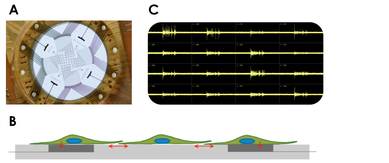
Peritore C.S., Clements M., Swift O, Millard D.C., Nicolini A.M., Clements I.C., Ross J.D.
Axion BioSystems, Atlanta, GA

AXION BIOSYSTEMS explore life's circuitry™

Maestro: Multiwell MEA system for analysis of cell network activity

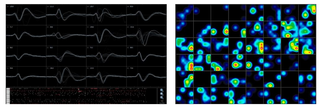
Why use microelectrode arrays?

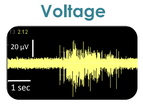
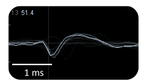
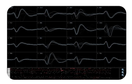
Microelectrode arrays (MEAs) provide a high-throughput, benchtop method for evaluating the activity of cultured neurons. MEAs collect data simultaneously from many discrete locations in a cultured neural population, delivering information on both activity and connectivity. MEAs provide a powerful approach to modeling *in vivo* neural behavior and can be applied to disease modeling, stem cell characterization and phenotyping, neurotoxicity, and safety.



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.

Why use the Maestro?



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

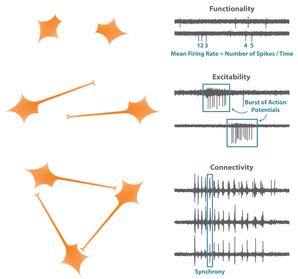
- **Label-free and non-invasive recording** of extracellular voltage from cultured neurons
- **Environmental control** provides a stable benchtop environment for short- and long-term studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the magnitude of depolarization events
- **Sensitive 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

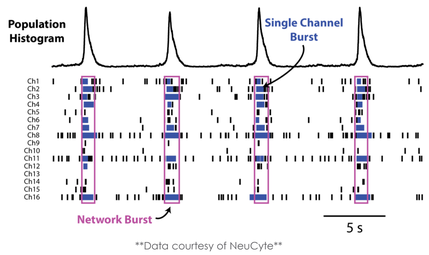
Axion's Maestro multiwell microelectrode array (MEA) platform enables functional cellular analysis on the benchtop with 768 electrodes across all plate formats.

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.





Data courtesy of NeuCyte

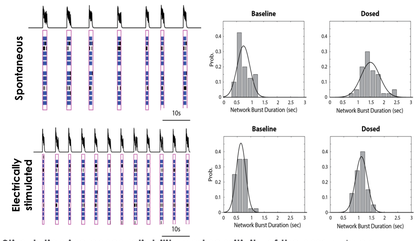
A well-wide raster plot enables the visualization of network activity across all electrodes in a well, and is computed automatically by AxIS and the Neural Metric Tool.
- Each "tick" mark represents a detected action potential.
- Each row illustrates a single electrode in the well.
- Multiple spikes occurring in a short time span defines a burst (blue).
- Coordinated bursting across a well is characterized as a network burst (pink).

Lumos: Multiwell optical stimulation for control of cell activity

Why use stimulation?

While neural or cardiac cultures are often spontaneously active, stimulation allows the user to control the input to the cells.

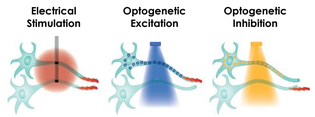
Stimulation can be used to:
- Evaluate measures of evoked activity
- Reduce variability across wells
- Create application specific protocols to assess features of network connectivity
- Reduce assay duration by increasing activity levels



Stimulation increases reliability and sensitivity of the assay. In an epilepsy-in-a-dish model, electrical stimulation can "pace" network bursting activity, leading to greater consistency across wells and increased sensitivity overall.

Why use optogenetics?

Optogenetics is the integration of fast, light-activated ion channels (opsins) that allow targeted, precise manipulation of cellular activity. Upon incident light of the correct wavelength, the opsins produce currents that directly hyperpolarize or depolarize the cell.



Over 30 opsins have been engineered and described, spanning a variety of excitation wavelengths (colors) and distinct functionality.

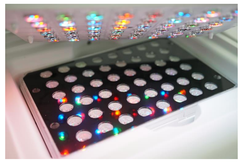
- **Bi-directional control** enables activation and suppression of neural cultures.
- **Genetic targeting** allows cell-type specificity when stimulating complex networks.
- **Control intracellular signaling or gene expression** to enhance development of disease-in-a-dish models.
- **Optical stimulation eliminates artifacts**, simplifying the analysis process.
- **Establish well-to-well consistency** for more reliable results.

Why use Lumos?

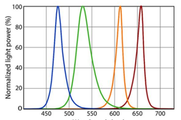
SLAS NPA DESIGNATION™ — **SLAS 2016 New Product Award**

2016 The Scientist TOP 10 INNOVATIONS

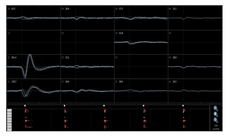
- **High-throughput** - Simultaneous control of light delivery to each well of a standard SLAS-compliant 48-well microplate
- **Fully configurable** - Powerful on-board electronics enable each of the 192 LEDs to be independently controlled with finely graded intensities that are dynamically adjustable on a microsecond scale
- **Complete wavelength coverage** - Four wavelengths per well span the visible spectrum for selective stimulation of all common opsins
- **Environmental control compatibility** - Options for regulation of temperature, CO_2, and humidity enable extended benchtop work
- **User friendly software** - Intuitive "drag-and-drop" style blocks enable quick design of light delivery patterns and selection of target wells
- **Topside light delivery** - Delivery of light from above each well enables even light distribution and leaves the bottom of the microplate open for simultaneous electrophysiology, imaging, or other interfacing modalities
- **Industry-leading light intensities** - Large dynamic range with minimal cross-talk between wells



Lumos also operates independently on standard format microplates, enabling chronic light delivery experiments for influencing cellular processes such as gene, cell growth, or differentiation.



Four wavelengths per well encompass the visible spectrum

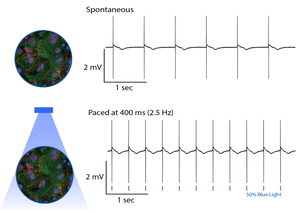


Stimulus design and visualization within AxIS Stimulation Studio

Applications in iPSC characterization

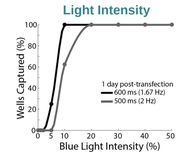
Pacing cardiac beating enhances cell characterization

- Cardiac repolarization timing is intrinsically linked to the beating frequency, which can naturally vary over time and between wells
- Beating frequency and repolarization timing are both sensitive to iPSC characteristics and drug effects.
- Optogenetic stimulation can control beating frequency and remove its influence on cardiac repolarization
- Normalizing beating frequency increases reliability and sensitivity of the repolarization measurement.

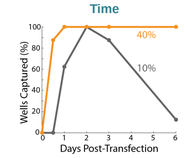


Case study: mRNA mediated opsin delivery for rapid, enhanced characterization assays

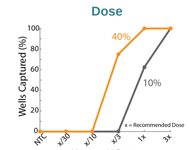
- Axiogenesis Cor4U™ cardiomyocytes were cultured on 48-well MEA plates.
- Cells were transfected with Xpress.4U ChR2 mRNA for artifact-free optical pacing with the Lumos
- Reagent concentration was varied across the plate during transfection, and light intensity and pacing rate were systematically varied 1-6 days post-transfection.
- The multiwell format of the Maestro and Lumos enabled rapid optimization of the Xpress.4U protocol.
- Robust pacing was stable beginning 1 day after transfection.
- Pacing required very little light and was stable from 1-6 days post-transfection.



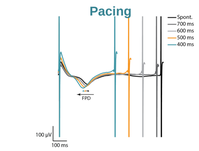
Robust pacing with low light levels



Pacing was stable from 1-3 days

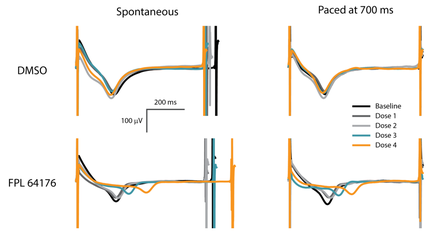


Opsin delivery was efficiently optimized



Cells were reliably paced over a range of beat rates

Isolate Repolarization Effects with Pacing

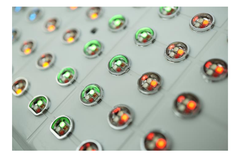


(Left) Application of FPL 64176 induced a significant prolongation of BP and FPD relative to the vehicle control (DMSO).

(Right) Pacing at 700 ms BP controls for the influence of beating rate on repolarization. Under paced conditions, FPL- 64176 produced a dose dependent prolongation of FPD that was independent of beat period.

Future applications: Optical control over developing iPSC cultures

- Using the Lumos system, deliver light over extended periods of time to influence cell culture development
- Alter iPSC maturation by controlling activity levels (*e.g.* Lam *et al.* 2017, Ono *et al.* 2017)
- Influence iPSC differentiation through control over gene expression, protein function, and other intracellular processes (*e.g.* Fischer *et al.* 2016, Ono *et al.* 2017)
- These techniques could be optimized rapidly and efficiently, using the multiwell format of the Lumos system



Conclusions

- Lumos, the first commercial multiwell optogenetic stimulation device, enables high throughput optogenetics with precise control over light delivery in an easy-to-use format.
- The Maestro MEA platform connects key biological variables to cellular and network function by extracting information from complex biological systems *in vitro*.
- Together, Lumos and Maestro improve the reliability and sensitivity of existing assay screens and enable new directions in high throughput network electrophysiology.
- Lumos also operates independently, enabling chronic light delivery experiments for influencing cellular activity and intracellular processes such as gene expression, cell growth, maturation, and differentiation.