Lumos™: a multiwell optogenetic stimulation device for the precise control of *in vitro* cellular network activity

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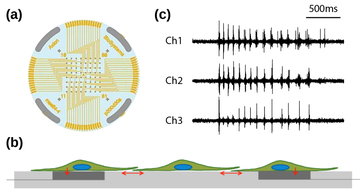
Multiwell MEA Technology

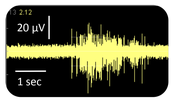
Why use microelectrode arrays?

Microelectrode array technology offers a platform for directly connecting key biological variables, such as gene expression or ion channels, to measures of cellular and network function.

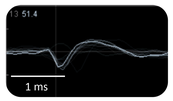
A planar grid of microelectrodes (a) interfaces with electro-active cultured cells (b), modeling complex, human systems in a dish. The electrodes detect changes in raw voltage (c) caused by the electrical activity of nearby neurons or cardiomyocytes.



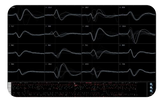
Raw Voltage



Extracellular Action Potentials



Network Activity



Raw voltage signals can be 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 high throughput evaluation of neural and cardiac activity 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

Challenges and Opportunities in MEA Applications

Challenge: Cardiomyocyte cultures may beat at different spontaneous rates across wells, complicating analysis
Opportunity: Reducing variability across wells will significantly improve the reliability and sensitivity of the assay

Challenge: Mixed neural populations incorporate important complexity into the model, but make interpretation difficult
Opportunity: Understanding how components interact in a biological system adds significant impact to a model

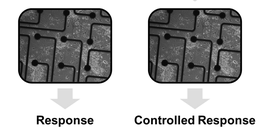
Challenge: Some networks are not very active, requiring long experiments to get sufficient data for analysis
Opportunity: Increasing activity levels in a controlled manner may reduce assay times and simplify analysis

Challenge: The model exhibits a phenotype of interest, but it's buried within noisy biological activity
Opportunity: Methods to target and evoke a specific phenotype will heighten the sensitivity of an assay

Why use stimulation?

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

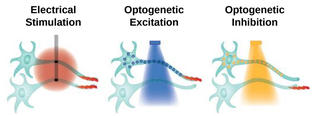
Stimulation can be used to:
- Evaluate measures of evoked activity
- Reduce variability across wells
- Create application specific protocols to assess features of network connectivity
- Reduce assay duration by increasing activity levels.



Lumos: 48-well Optogenetic Stimulation

Why use optogenetics?

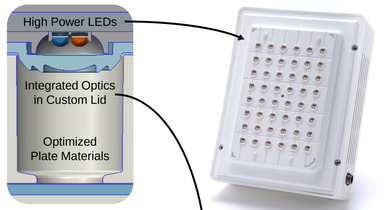
Optogenetics is the integration of fast, light-activated channels (opsins) that allow targeted, precise manipulation of cellular activity. Upon incident light of the correct wavelength, the opsins produce currents that directly hyperpolarize or depolarize the cell.



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

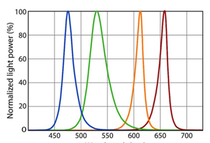
- **Bi-directional control** enables activation and suppression of neural cultures.
- **Genetic targeting** allows cell-type specificity when stimulating complex networks.
- **Control intracellular signaling or gene expression** to enhance development of disease-in-a-dish models.
- **Optical stimulation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

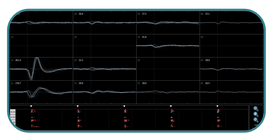
Lumos



Lumos is the first commercial multiwell light delivery device designed for optogenetics. It integrates seamlessly with the Maestro and AxIS, affording an array of features:
- Increased throughput – 192 LEDs across 48 wells
- Maximal intensity – high power LEDs couple with optimized plate materials and custom plate lid optics for unmatched intensity and reliability
- Use any opsin – wavelength options cover 460-670nm, with 4 wavelengths per well, allowing the use of any opsin and multiple opsins per well
- Fully configurable in AxIS Stimulation Studio, with microsecond precision and adjustable intensity for each LED – *independently* and *simultaneously*

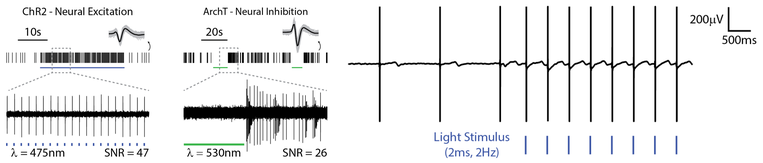
AxIS 2.2





Stimulus Design and Visualization

Optogenetic Stimulation



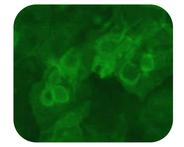
Each opsin has been designed for a specific functionality. For example, channelrhodopsin-2 (ChR2) can be used to activate neurons or cardiomyocytes *in vitro* in response to blue light, whereas ArchT suppresses neural activity upon incident green light. These techniques provide unprecedented control over electrophysiological activity, with negligible artifacts.

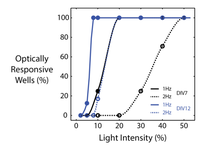
Applications in Screening

Getting Started

The following simple steps may be used achieve optically-evoked activity *in vitro*

1. Obtain viral vector encoding desired opsin
2. Add viral vector directly to thawed vial of cryopreserved cells, or to a vial of freshly harvested cells.
3. Wait 7-12 days for expression
4. Evaluate optically-driven functional activity

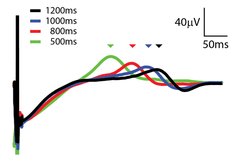


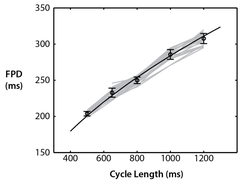


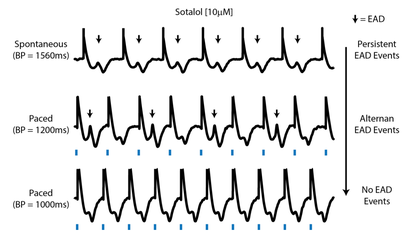
Opsin expression may be verified using fluorescence microscopy or through evaluation of optically-evoked functional activity endpoints.

Control Cardiac Beating

Cardiac repolarization is intrinsically linked to the beating frequency, both of which are sensitive to pharmacological manipulation. Optogenetic stimulation can be used to control the beating frequency and remove its influence on the physiology, resulting in increased reliability and sensitivity of the repolarization measurement.



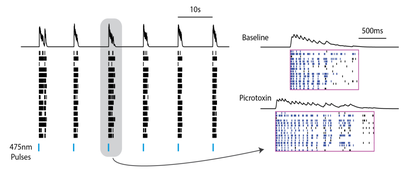


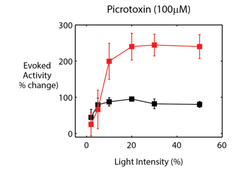


Quantify Arrhythmic Risk

Arrhythmic indicators, like EADs (arrows), are also sensitive to the beating frequency of cardiomyocyte networks. Optogenetic stimulation can be used to control for emergent arrhythmic events or more precisely quantify cardiac arrhythmias.

Induce or Suppress "Seizures-in-a-dish"





Optogenetic stimuli may be used to induce or suppress specific activity phenotypes, such as seizure-like activity. Induction of seizure-like activity may be used to screen for proconvulsant liability or the efficacy of anti-epileptic drugs, while suppression of seizure-like activity may be used to tune activity states in "disease-in-a-dish" models of epilepsy.

Conclusions

- The Maestro multiwell MEA platform connects key biological variables to cellular and network function by extracting information from complex biological systems *in vitro*.
- Optogenetics enables cells to be controlled by light, offering the opportunity to precisely control or manipulate complex, *in vitro* cell models.
- Lumos, the first commercial optogenetic stimulation device, enables high throughput optogenetics with unprecedented control over light delivery in a easy-to-use format.
- Together, the Lumos and Maestro improve the reliability and sensitivity of existing assay screens, while simultaneously enabling new directions in high throughput network electrophysiology.