Multiwell Optogenetics for Enhanced Cell-based Assays

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AXION BIOSYSTEMS
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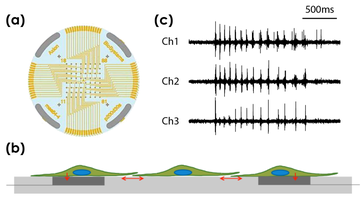
Multiwell MEA Technology

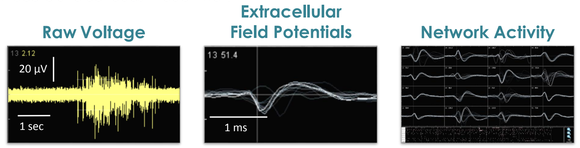
Why use microelectrode arrays?

The flexibility and accessibility of neural and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that provides a functional phenotype. Measurements of electrophysiological activity across a networked population offer a comprehensive characterization beyond standard genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell microelectrode array (MEA) platform provides this comprehensive functional characterization. The Maestro is a non-invasive benchtop system that simply, rapidly, and accurately records functional activity from cellular networks cultured on a dense array of extracellular electrodes in each well.



A planar grid of microelectrodes **(a)** interfaces with cultured neurons or cardiomyocytes **(b)**, to model complex, human systems. Electrodes detect changes in raw voltage **(c)** and record extracellular field potentials.



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

Why use the Maestro Pro™?

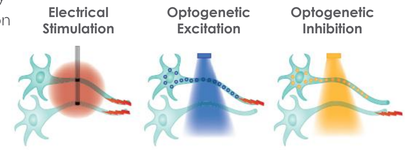
Axion's Maestro Pro™ multiwell microelectrode array (MEA) platform enables functional cellular analysis on the benchtop with an industry leading 768 electrodes across all plate formats.

- **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 (12-, 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

Optogenetics to control complex biology

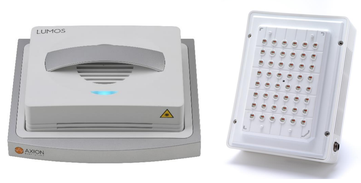
Optogenetics is the integration of fast, light-activated ion channels (opsins) to enable targeted manipulation of cell activity or intracellular signaling. Optogenetic techniques enable:

- Artifact-free stimulation for pacing cardiomyocytes or controlling neural activity
- Bi-directional control of activity via activation or inhibition of cell subtypes
- Genetic targeting for cell type specificity
- Control of gene expression and intracellular signaling for enhanced development of disease-in-a-dish models
- Establishing well-to-well and assay-to-assay consistency for more reliable results



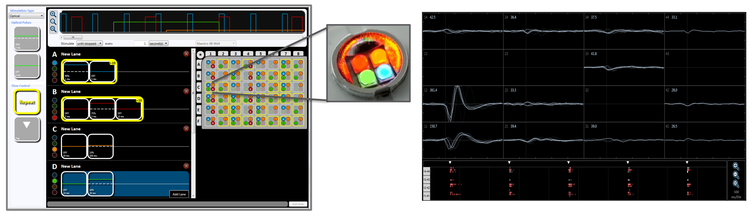
Multiwell Optical Control

Why use the Lumos™?



The Lumos™ is the first commercial multiwell light delivery device designed for *in vitro* optogenetics. The Lumos provides precise control over cardiomyocyte beat rate or neural activity.

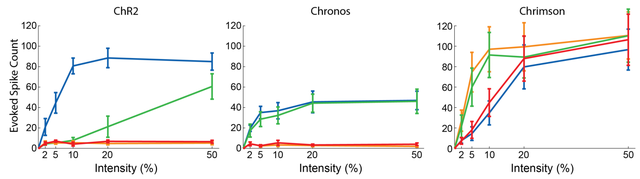
- **Artifact free stimulation and pacing**
- **High throughput** with 192 LEDs over 48 wells
- **Compatible with any opsin** with 4 wavelengths encompassing the visual spectrum (460-670 nm)
- **Maximal intensity** with high power LEDs and optimized plate and lid optics on the Lumos MEA
- **Precise control** with microsecond precision and finely adjustable intensity for each LED
- **Flexible control** as each LED can be controlled independently and simultaneously



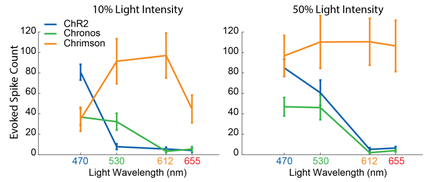
AxIS Stimulation Studio offers intuitive stimulus design with drag and drop blocks to create light delivery patterns and clickable selection of target wells. Stimulus responses are visualized in real time for easy interpretation.

Optogenetics in Neural MEA Assays

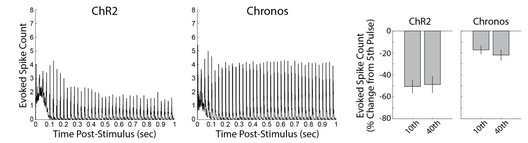
Transduction of neuronal cell populations with opsins allows for precise, artifact free control of neuronal activity. Here, primary rat cortical neurons (QBM Cell Science) were transduced with excitatory opsins. The Lumos applied intensity sweeps across 4 light wavelengths to explore the magnitude and timing of each opsins' response.



Blue, green, orange, and red light were applied at varying intensities for 5ms each. ChR2 and Chronos responded most strongly to blue light, with ChR2 showing a larger evoked response due to slower opening and closing kinetics. Chrimson's excitation spectrum is red-shifted, yielding maximum excitation with green and orange light.



At 10 and 50% intensity, only Chrimson is significantly activated by orange and red light. Spectral separation of all three opsins was greater at 10%, with ChR2 most strongly activated by blue light, Chronos by green light, and Chrimson by orange and red light. The Lumos' ability to finely tune intensity across a large dynamic range and light wavelengths enables independent activation of multiple opsins and their respective transduced populations.

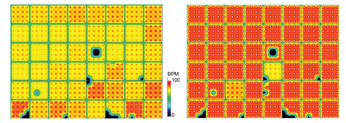


When high frequency blue light stimulation was applied (5ms pulses at 40Hz for 1s), ChR2+ cells showed adaptation with reduced responses to later pulses in the train. In contrast, Chronos' faster kinetics allowed a consistent response to each pulse in the high frequency train.

Optogenetics in Cardiac MEA Assays

With optogenetics, light can be used to control and pace cardiomyocytes without artifact. Pacing cardiomyocytes offers many advantages:

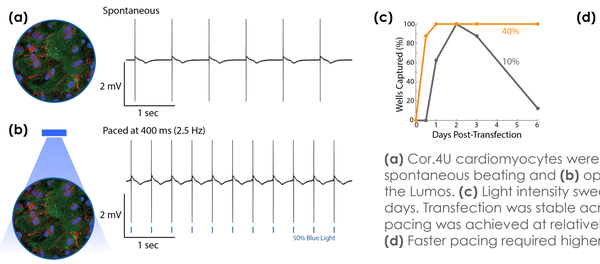
- Specify beat rate at 1Hz for enhanced physiological relevance
- Establish well-to-well and plate-to-plate consistency with matched beat rates in all wells
- Detect use-dependent drug effects for superior safety screening



Cor.4U cardiomyocytes spontaneously beating (left) and paced at 1.6 Hz (right) with reduced well-to-well variability.

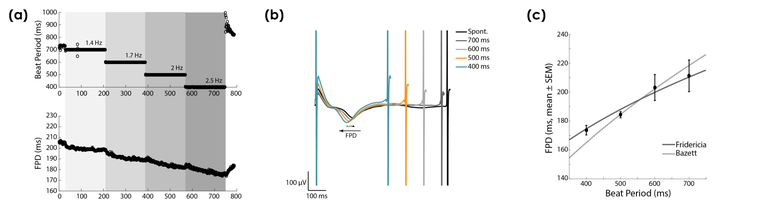
Optical pacing of cardiomyocytes with mRNA-based transfection

Here, Axiogenesis Cor.4U cardiomyocytes were transfected with channelrhodopsin-2 (ChR2) using Xpress.4U ChR2, a transient mRNA-based delivery system. Daily optical pacing and recordings were used to optimize the assay time window and light delivery protocol.



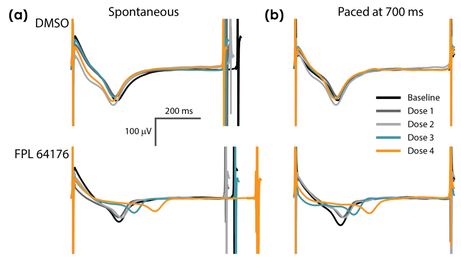
(a) Cor.4U cardiomyocytes were recorded during spontaneous beating and **(b)** optical pacing at 2.5Hz using the Lumos. **(c)** Light intensity sweeps were applied across 6 days. Transfection was stable across multiple days and pacing was achieved at relatively low levels of light (40%). **(d)** Faster pacing required higher light intensities.

Optical pacing reveals FPD and BP relationship



(a) A chirp assay was used to sequentially increase the beat rate of Xpress.4U transfected Cor.4U cardiomyocytes. **(a, b)** The field potential duration (FPD) adapted with beat rate increases up to 2.5 Hz. **(c)** Pacing with the Lumos revealed the cell-specific beat rate correction relationship, which differed from typical clinical correction formulas.

Optical pacing distinguishes drug effects on repolarization and BP



Xpress.4U transfected Cor.4U cardiomyocytes were dosed with FPL 64176, an L-type calcium channel activator, or the vehicle control DMSO. **(a)** During spontaneous beating, FPL 64176 increases beat period and FPD, making it difficult to isolate its effects on repolarization. **(b)** Optical pacing was used to control beat period at 700ms across baseline and dosing. Pacing revealed that FPL-induced FPD prolongation is independent of increases in beat period. Pacing also reduced vehicle control variability across doses.

Conclusions

Optogenetics is a powerful tool. When combined with MEA assays, optogenetics can enhance your neural or cardiac assays by reducing well-to-well variability, detecting rate and activity-dependent drug effects, and systemically controlling cell activity for better sensitivity and specificity.