Simultaneous multiwell optogenetic stimulation and microelectrode array recording for neural electrophysiology assays

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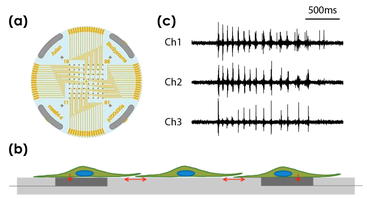
Multiwell MEA Technology

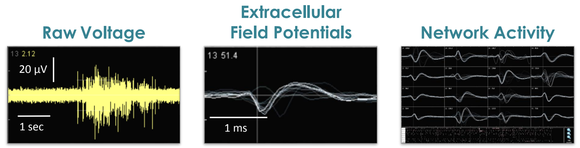
Why use microelectrode arrays?

Microelectrode arrays (MEAs) monitor and manipulate cultured cell activity *in vitro*, providing insight into neural networks to inform disease-in-a-dish models, stem cell characterization, and drug development. Axion BioSystems' Maestro™ multiwell MEA platforms enable high-throughput assessment of neural networks at reduced time and cost.

Optogenetics can further enhance neural assays by providing artifact-free, precise, and targeted stimulation. Here, we evaluate the application of the Lumos, a commercial multiwell optical stimulation system, and next generation opsins for *in vitro* neural assays.

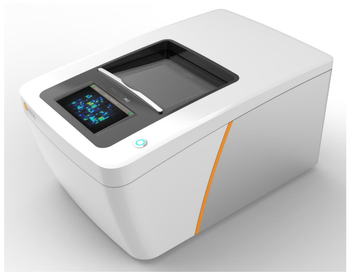


A planar grid of microelectrodes **(a)** interfaces with cultured neurons or cardiomyocytes **(b)**, to model complex, human systems. Electrodes detect changes in raw voltage **(c)** and record extracellular field potentials.



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™?



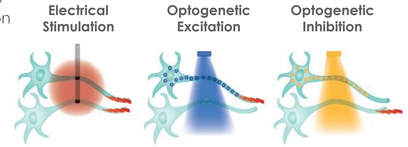
Axion's Maestro Pro™ multiwell microelectrode array (MEA) platform enables functional cellular analysis on the benchtop with an industry leading 768 electrodes across all plate formats.

- **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

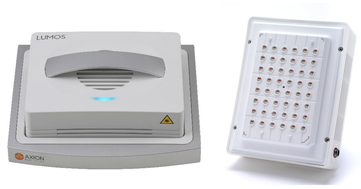
Optogenetics is the integration of fast, light-activated ion channels (opsins) to enable targeted manipulation of cell activity or intracellular signaling. Optogenetic techniques enable:

- 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



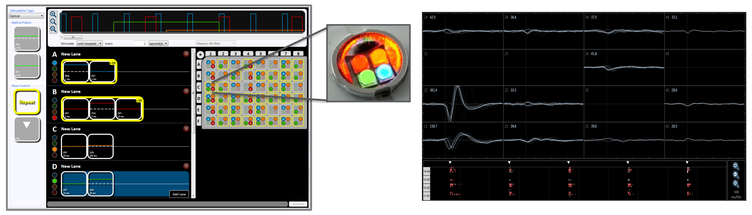
Multiwell Optical Control

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.

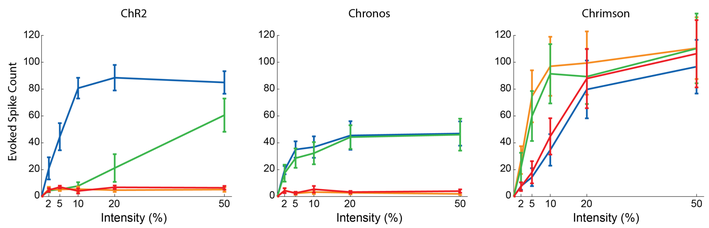
- **Artifact free stimulation and pacing**
- **High throughput** with 192 LEDs over 48 wells
- **Compatible with any opsin** with 4 wavelengths encompassing the visual spectrum (460-670 nm)
- **Maximal intensity** with high power LEDs and optimized plate and lid optics on the Lumos MEA
- **Precise control**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



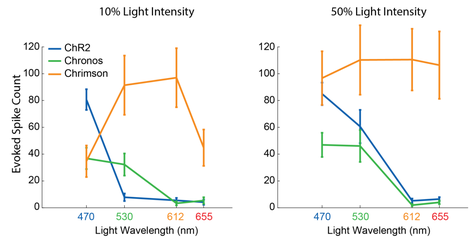
AxIS Stimulation Studio offers intuitive stimulus design with drag and drop blocks to create light delivery patterns and clickable selection of target wells. Stimulus responses are visualized in real time for easy interpretation.

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.

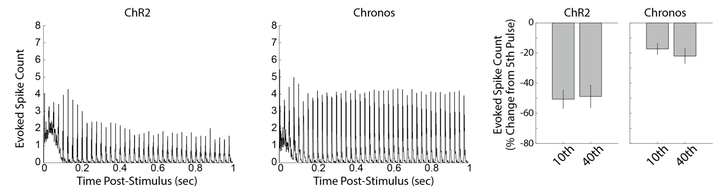


At 10% and 50% intensity, only Chrimson is significantly activated by orange and red light. Spectral separation of all three opsins was greater at 10%, with ChR2 most strongly activated by blue light, Chronos by green light, and Chrimson by orange and red light. The Lumos' ability to finely tune intensity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

High Frequency Optical Stimulation

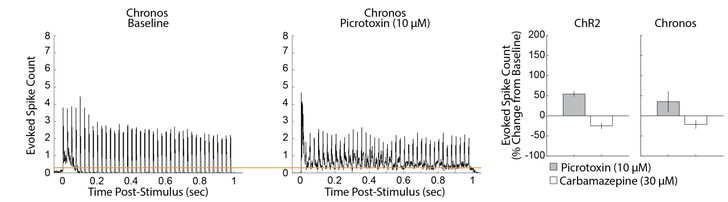
Chronos enables high frequency responses without adaptation

High frequency blue light stimulation (2ms pulses at 40Hz for 1s) was applied with the Lumos to ChR2+ primary rat neurons. The evoked neural response showed adaptation with reduced responses to later pulses in the train. In contrast, Chronos' faster kinetics enabled a consistent response to each pulse in the high frequency train.



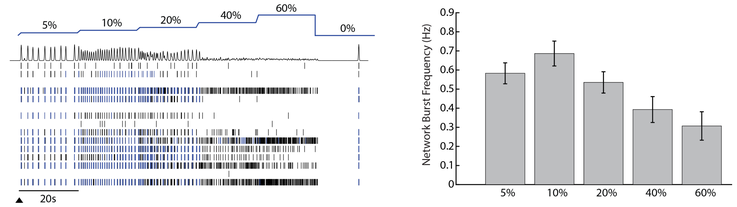
High frequency stimulation to probe excitatory-inhibitory balance

ChR2+ and Chronos+ neurons were dosed with either a proconvulsant (Picrotoxin, a $GABA_AR$ antagonist,10µM), antiepileptic (Carbamazepine, a sodium channel blocker, 30µM), or vehicle control (DMSO). Neural networks dosed with Picrotoxin showed a sustained response to high frequency blue light stimulation (2ms pulses at 40Hz for 1s) in which firing did not return to zero between each pulse, resulting in a higher cumulative spike count across the 40 pulses (right). In contrast, carbamazepine reduced the response, resulting in a lower cumulative spike count.



Modulating Network State with Optical Stimulation

Neural network activity can be quite variable over time, resulting in changes in phenotype and high inter-well variability. Thus, modulating network state is a valuable tool for controlled neural assays. Continuous optical stimulation with the Lumos can be used to alter the state of neural networks. Here, increasing intensities of blue light from the Lumos were used to sequentially depolarize ChR2+ networks, resulting in a change in network bursting phenotype. As light increased, synchronized network bursts became less frequent and were eventually eliminated. Controlling network state with optogenetics can switch between normal and seizurogenic activity phenotypes, which may increase assay sensitivity to proconvulsant and anti-epileptic compounds, respectively.



Conclusions

When combined with MEA assays, optogenetics can enhance your neural assays by reducing well-to-well variability, detecting activity-dependent drug effects, and systemically controlling neural activity for better assay sensitivity and specificity.