Lumos™: A multiwell optical stimulation device for precise control of cell activity

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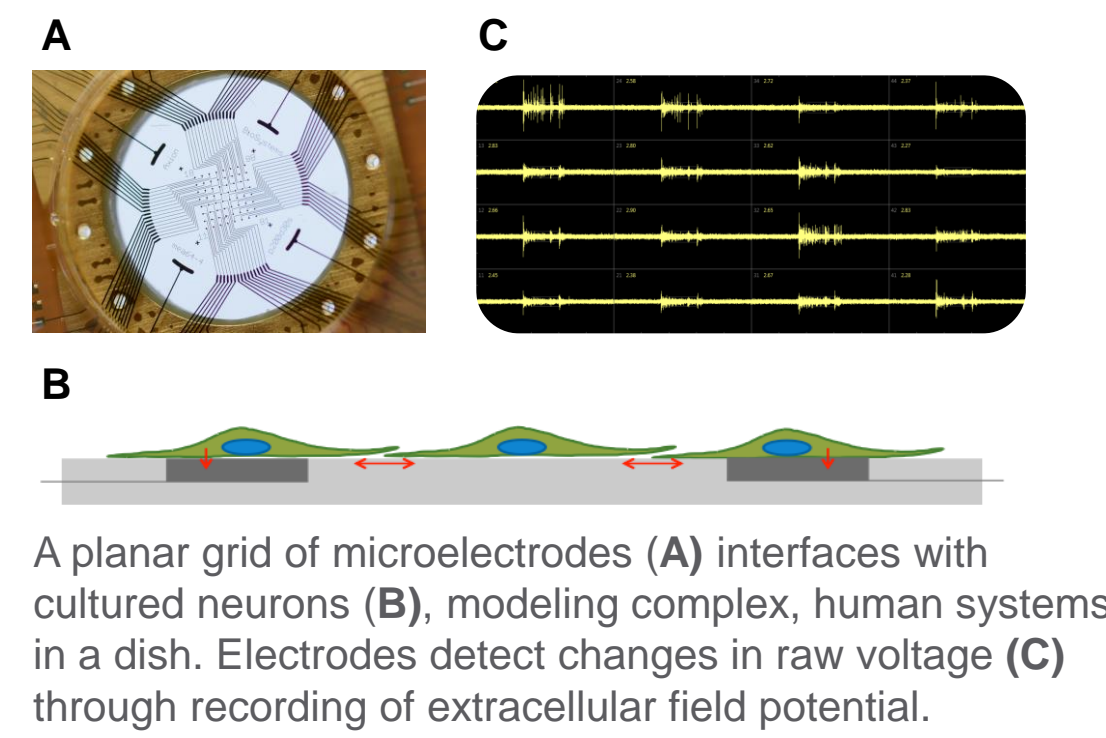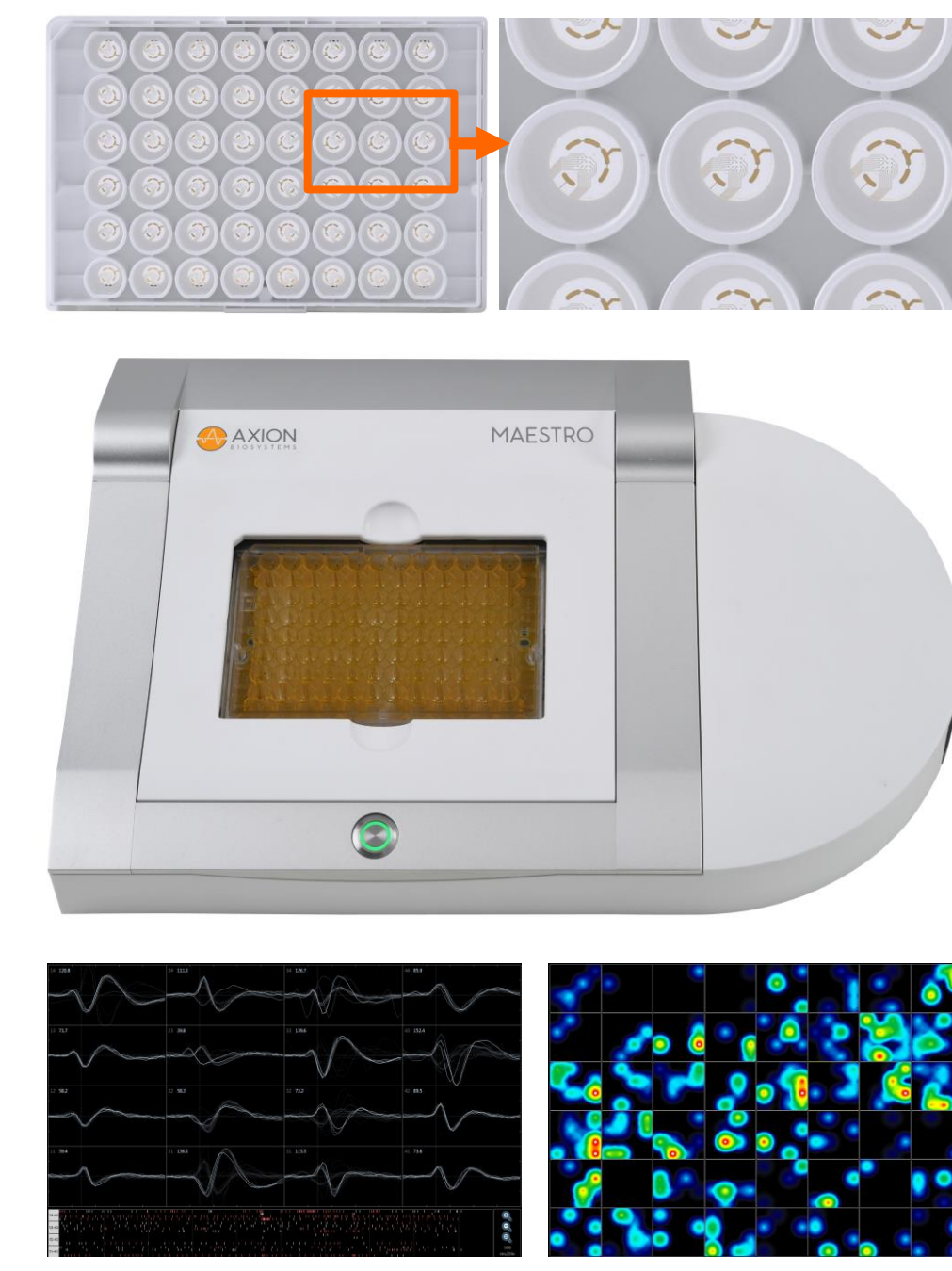
Axion BioSystems, Atlanta, GA

AXION BIOSYSTEMS
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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 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?

- **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
- **Example applications:**
  - **Assess safety and toxicity** through functional evaluation of human biology *in vitro*
  - **Optimize stem cell differentiation** and culturing protocols by assessing network development with functional endpoints
  - **Perform phenotypic drug discovery** utilizing functional cell-based models in a high-throughput MEA assay
  - **Design disease-in-a-dish models** for phenotypic characterization of patient-derived cells or genetic edits

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

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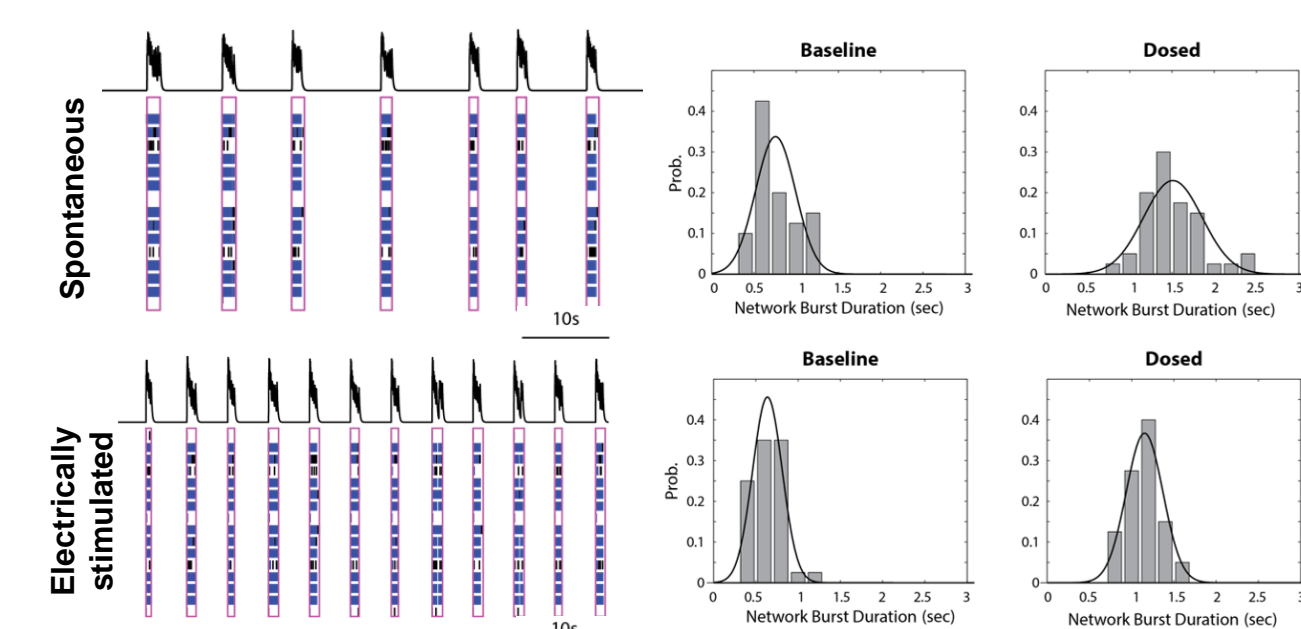
Stimulation increases reliability and sensitivity of the assay. In an epilepsy-in-a-dish model, electrical stimulation can "pace" network bursting activity, leading to greater consistency across wells and increased sensitivity overall.

Lumos: Multiwell optical stimulation for control of cell activity

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.

Electrical Stimulation | Optogenetic Excitation | Optogenetic Inhibition
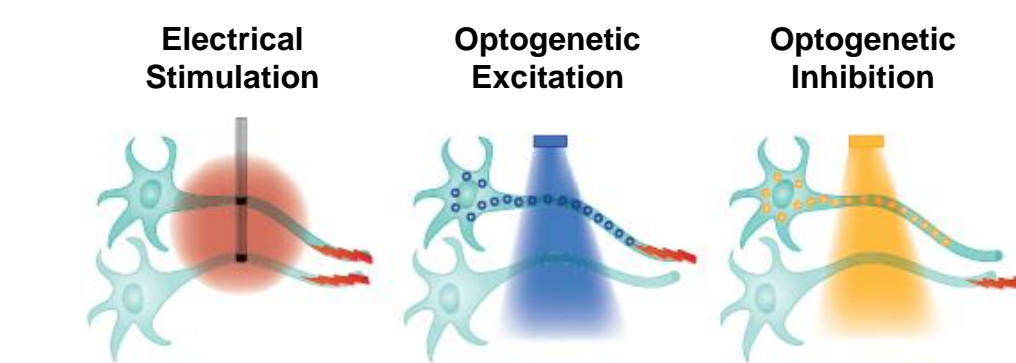
Over 30 opsins have been engineered and described, spanning a variety of excitation wavelengths (colors) and distinct functionality.

- **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™
2016
SLAS 2016 New Product Award

TheScientist TOP 10 INNOVATIONS

- **Lumos** is the first commercial multiwell light delivery device designed to promote the advancement of *in vitro* optogenetic assays. It operates independently and also integrates seamlessly with the Maestro and AxIS, affording an array of features:
- **Increased throughput** – 192 LEDs over 48 wells
- **Maximal intensity** – high power LEDs coupled with optimized plate materials and custom lid optics for robust performance and reliability
- **Use any opsin** – wavelength options cover 460-670nm, with four wavelengths per well, allowing the use of any opsin and multiple opsins per well
- **Precision control is fully user-configurable** through an intuitive interface in AxIS software; microsecond precision and finely adjustable intensity for each LED – independently and simultaneously.

Lumos also operates independently on standard format microplates, enabling chronic light delivery experiments for influencing cellular processes such as gene, cell growth, or differentiation.
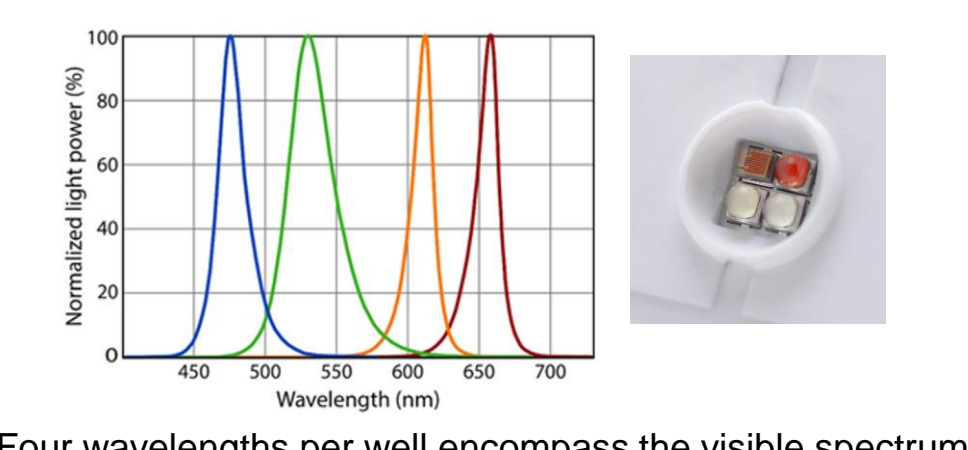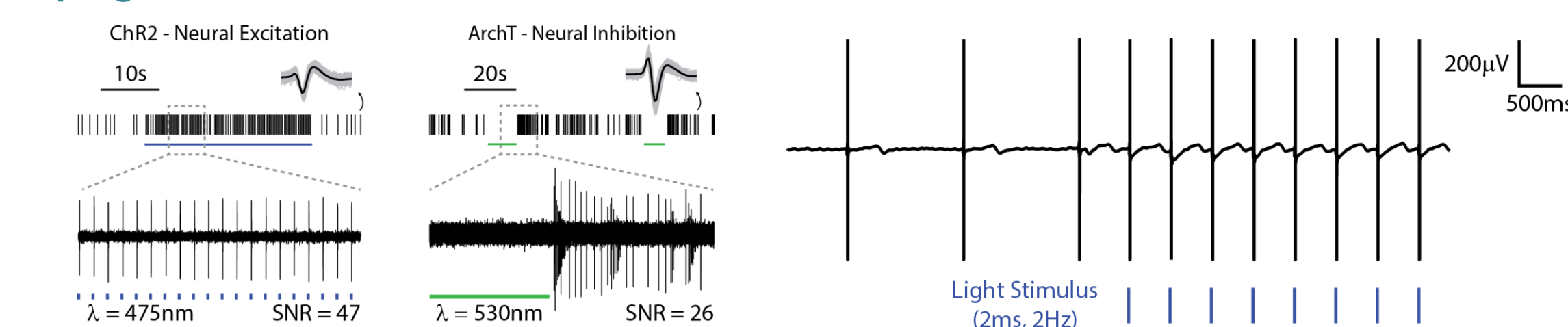
Four wavelengths per well encompass the visible spectrum
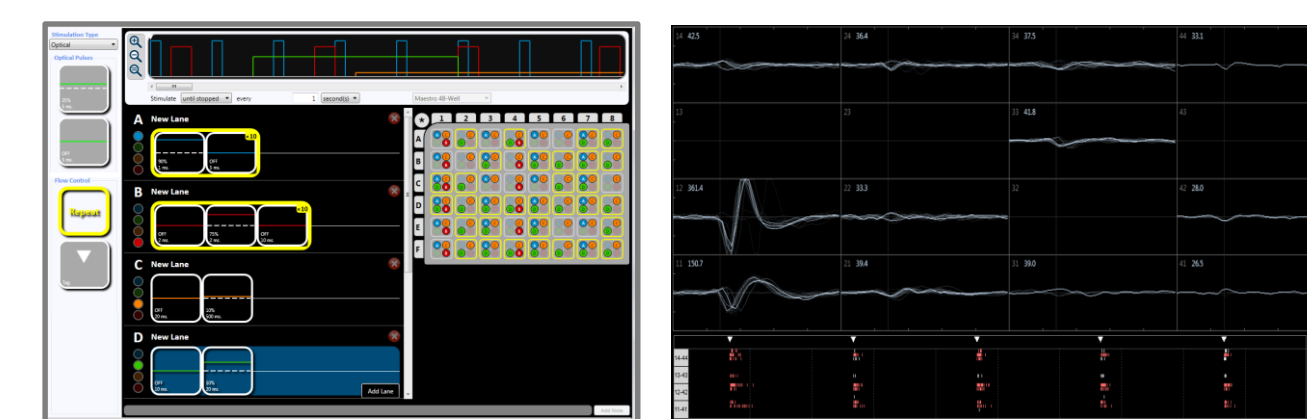
Stimulus design and visualization within AxIS Stimulation Studio

Optogenetic Stimulation

ChR2 - Neural Excitation | ArchT - Neural Inhibition

10s | 20s

λ = 475nm SNR = 47 | λ = 530nm SNR = 26

Light Stimulus (2ms, 2Hz)

200μV / 500ms

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

Optically Responsive Wells (%)

Light Intensity (%)
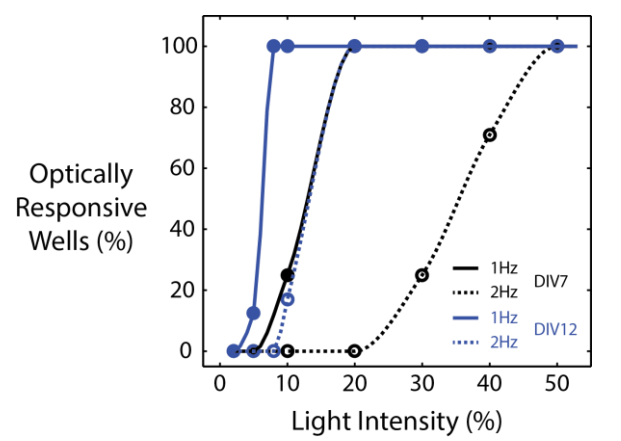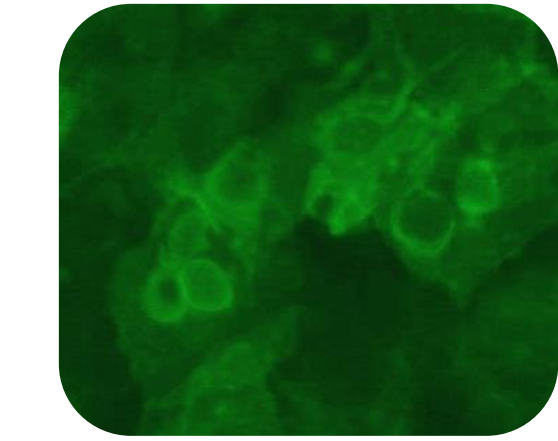
Opsin expression may be verified using fluorescence microscopy or through evaluation of optically-evoked functional activity endpoints.
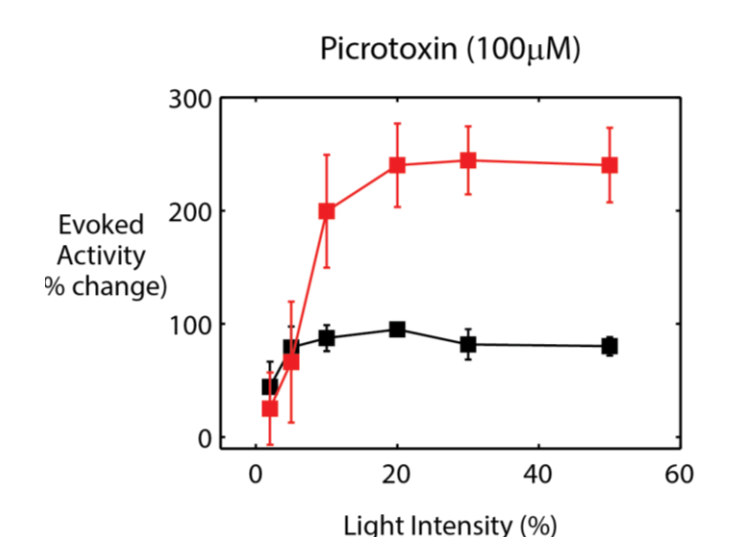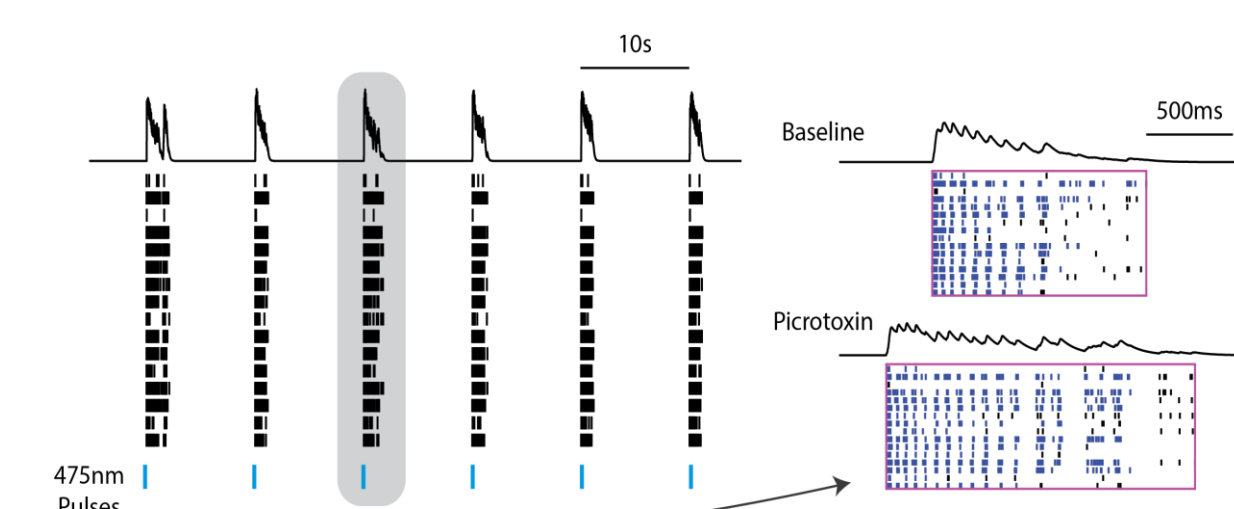
Control Cardiac Beating

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.

40μV / 50ms

1200ms / 1000ms / 800ms / 500ms

FPD (ms) / Cycle Length (ms)
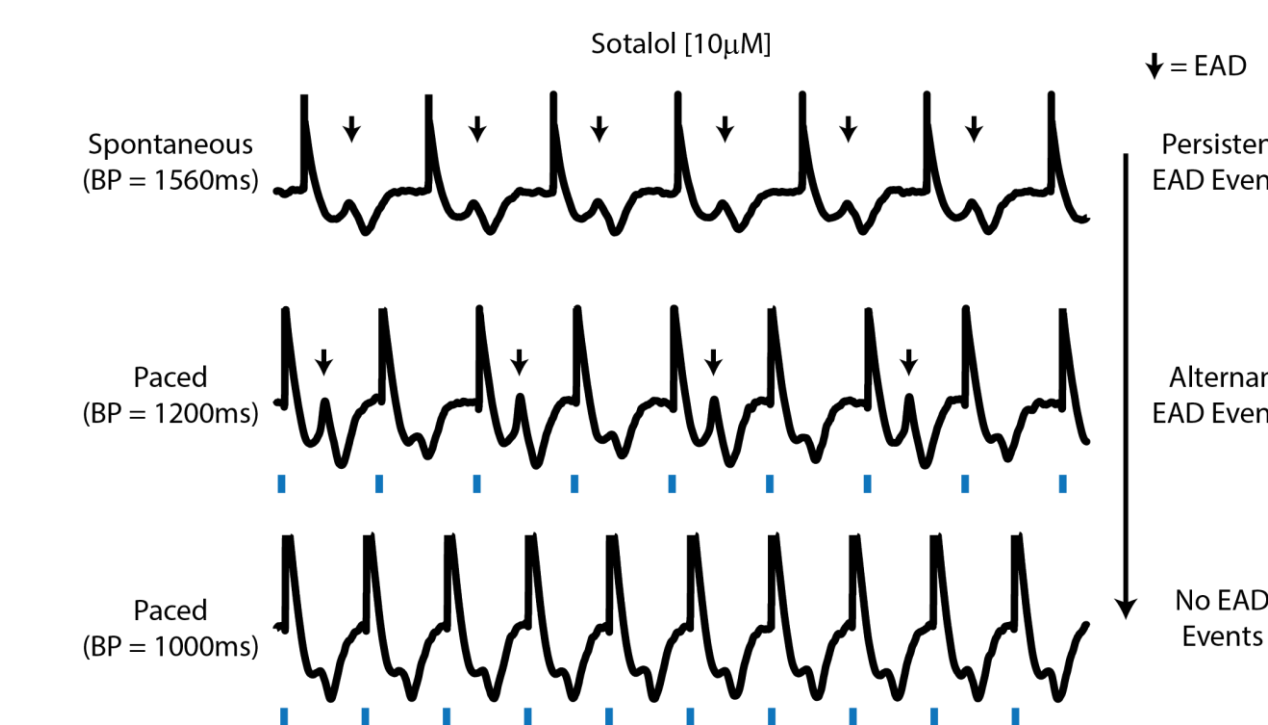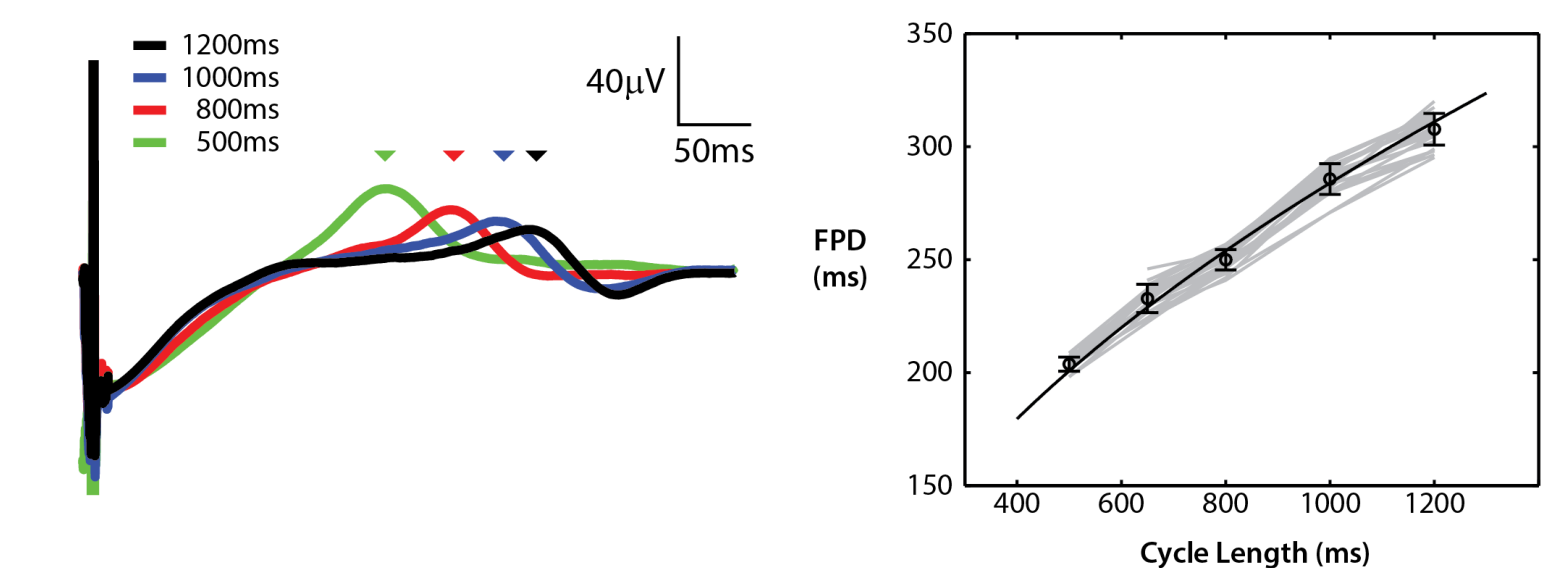
Quantify Arrhythmic Risk

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.

Sotalol [10μM] | = EAD

Spontaneous (BP = 1560ms) — Persistent EAD Events
Paced (BP = 1200ms) — Alternan EAD Events
Paced (BP = 1000ms) — No EAD Events

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

Picrotoxin (100μM)

10s

Baseline / Picrotoxin

500ms

475nm Pulses

Evoked Activity % change / Light Intensity (%)

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

- 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 multiwell optogenetic stimulation device, enables high throughput optogenetics with precise control over light delivery in an easy-to-use format.
- The Maestro multiwell 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 processes such as gene expression, cell growth, and differentiation.