Simultaneous multiwell optogenetic stimulation and microelectrode array recording for disease modeling and toxicological assays

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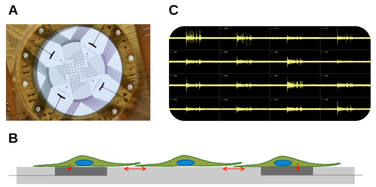
Multiwell MEA Technology

Why use *in vitro* microelectrode arrays?

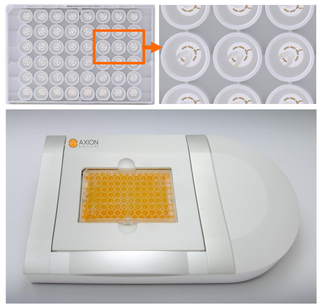
Thorough neural experimentation often requires analysis of both single cell activity and network function. Patch clamp techniques provide detailed single-cell analysis but little insight into how that cell behaves in a population.

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.

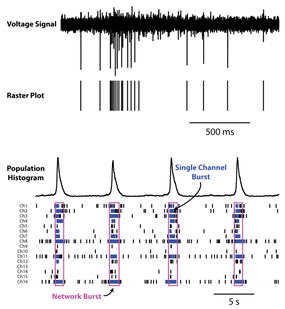
Why use the Maestro?



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

- **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:**
  - **Design disease-in-a-dish models** for phenotypic characterization of patient-derived cells or genetic edits
  - **Assess safety and toxicology** 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

Neural network activity profiles



Neural action potentials are detected as changes in voltage above a user-defined threshold. A simple view of this activity is a raster plot where each detected action potential is represented by a "tick" mark to denote the spike time.

The timing of the spikes contains all of the information required to calculate measures like mean firing rate (activity over time) or bursting (clusters of action potential activity).

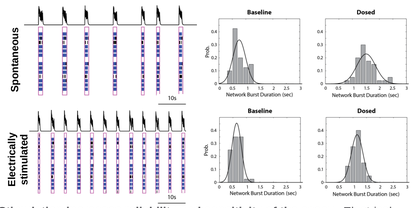
A well-wide raster plot enables visualization of network activity across all electrodes in a well, and is computed automatically in Axion's AxIS™ software.

- 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 rectangle).

Why add stimulation?

While neural cultures are often spontaneously active, stimulation provides control over cellular activity that 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.

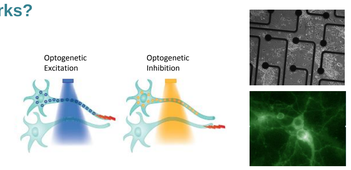


Stimulation increases reliability and sensitivity of the assay. Electrical stimulation was used to "pace" the network bursts across wells, leading to greater consistency across wells in the baseline and dosed (picrotoxin) condition, and increased sensitivity overall.

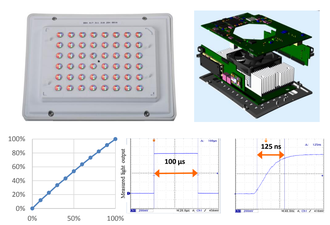
Multiwell optical stimulation for optogenetic control

Why apply optogenetics to cultured neural networks?

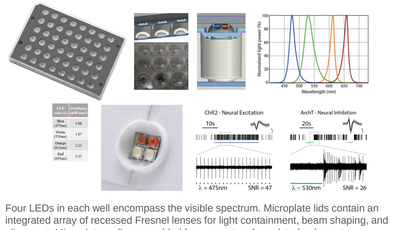
- Cell-specific modulation of neuronal sub-types through genetic targeting
- Bi-directional control of activation and suppression
- Elimination of stimulus artifacts
- Spatially uniform stimulus delivery across cultures
- Control intracellular signaling or gene expression
- Influence differentiating iPS cells



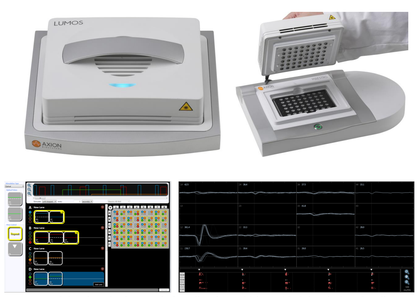
Design and validation of the Lumos system for multiwell optical stimulation



For precise control over intense light delivery, customized instrumentation was designed around an on board, dual-core CPU with tightly integrated FPGA co-processor. This setup provides independent control of each high-intensity LED, at 100µs temporal resolution, with finely graded control over intensity levels for arbitrary irradiation patterns such as intensity ramps.



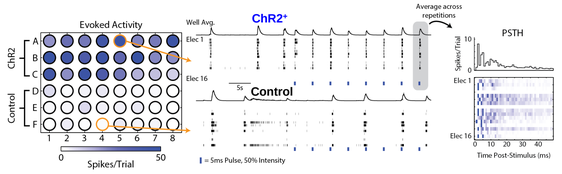
Four LEDs in each well encompass the visible spectrum. Microplate lids contain an integrated array of recessed Fresnel lenses for light containment, beam shaping, and alignment. Microplate walls are molded from a custom formulated polymer to maximize specular reflection and light delivery, for providing evenly diffused light within each MEA well, and minimizing bleed-through of light between adjacent wells.



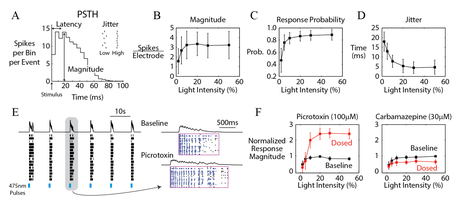
Stimulus design and visualization within AxIS Stimulation Studio

- **Lumos** is the first commercial multiwell light delivery device designed to promote the advancement of *in vitro* optogenetic assays. It 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 4 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.

Enhanced phenotypic characterization of network activity



Representative neural response data from Maestro MEA assay, demonstrating simultaneous multiwell stimulation. **Left**: Plate map showing levels of evoked activity. **Middle**: Raster plots from representative wells. **Right**: Peri-stimulus time histograms, averaged across stimulus repeats from a sample well.

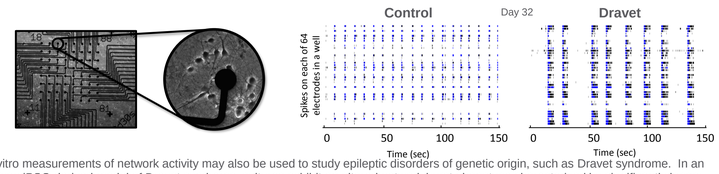


Quantification of optically-evoked neural activity for screening applications. **A-D)** Metrics of evoked neural activity are extracted from the peri-stimulus time histogram (PSTH) and peri-event raster plot. These neural response metrics are directly modulated by the strength of the light stimulus. **E)** ChR2⁺ neural cultures dosed with picrotoxin exhibited prolonged network bursts following stimulation. **F)** Picrotoxin significantly increased the magnitude of optically-evoked neural activity.

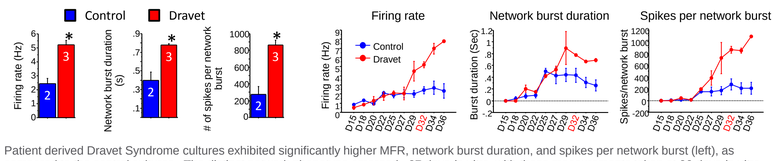
MEA / optogenetic assay for disease-in-a-dish modeling

Disease-in-a-Dish Models – Dravet Syndrome

Data courtesy of Dina Simkin and Evangelos Kiskinis, Northwestern University; 689.22 / K13 (Wed. 9-10am)

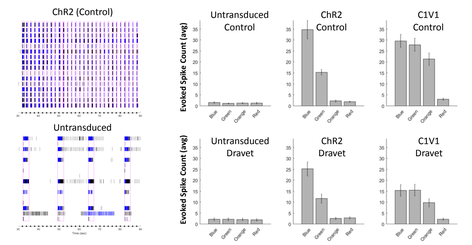


In vitro measurements of network activity may also be used to study epileptic disorders of genetic origin, such as Dravet syndrome. In an human iPSC-derived model of Dravet syndrome, cultures exhibit an altered network burst phenotype characterized by significantly longer bursts after the cultures have matured.



Patient derived Dravet Syndrome cultures exhibited significantly higher MFR, network burst duration, and spikes per network burst (left), as compared to the control cultures. The distinct network phenotype emerged ~27 days *in vitro*, with these measurements taken at 32 days *in vitro*.

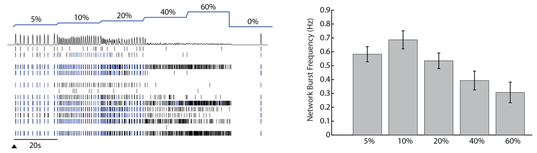
Optically-evoked network activity differs between diseased and healthy networks



Cultures were transduced with ChR2 or C1V1 opsins to enable optogenetic modulation of network activity. Light pulses of 5ms duration were applied at various frequencies during MEA-based recordings of network activity. (Untransduced cultures were unaffected by optical stimulation.) Response to the four light wavelengths on the Lumos were tested in each case. As expected, ChR2⁺ cultures responded maximally to blue light, whereas C1V1⁺ were also responsive to red-shifted light.

Using optogenetics to modify network states

Increasing light intensity depolarized the cultured networks, causing a change in network bursting phenotype. Synchronized network bursts became less frequent and were eventually eliminated. Controlling the network state can switch between normal and seizurogenic activity patterns, which may increase assay sensitivity to pronconvulsant compounds and anti-epileptic drugs, respectively.



Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity and connectivity, and the Lumos optical stimulation system enables precision optogenetic modulation. Both systems are provided in a flexible, easy-to-use, benchtop format.
- Viral vectors, concentrations, and delivery techniques can be efficiently optimized in parallel, using the Maestro and Lumos systems
- Human-derived iPSC networks were effectively modulated by multiple opsins
- Optogenetic stimulation provides enhanced metrics for evaluating drug response and mutation phenotypes in models of neural disease
- These findings demonstrate the potential of optically-integrated multiwell MEA systems to enable high-throughput drug screening and phenotypic modeling of neurological diseases

689.22 / K13 - Wed (9-10am) Pathogenic aspects of SCN1A haploinsufficiency in human ipsc derived cortical neurons. (Wed 11-12am).
691.12 / N1 - Quantification of seizurogenic activity with multiwell microelectrode array technology for proconvulsant risk assessment and disease-in-a-dish epilepsy models