# Enhanced light delivery to multiwell microplates for high-throughput optical control of activity and cellular processes

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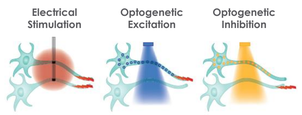
## Multiwell optical stimulation for control of complex *in vitro* systems

### Optical stimulation to control complex biology

- Optical stimulation techniques enable non-invasive control over cultured cells, tissues, and small organisms with specificity and precision.
- For example, optogenetics techniques enable
  - Activation or inhibition of cell activity
  - Genetic targeting for cell type specificity
  - Control of gene expression and intracellular signaling
  - Influence over developing cell cultures
  - Control over intracellular processes such as protein localization
- Optical stimulation also provides control through a range of other techniques, including release of optically-caged compounds, photobiomodulation, phototoxicity assays, and small organism assays.

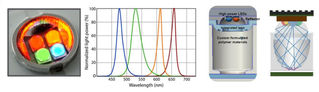



Optogenetics involves the integration of light-activated ion channels (opsins) to enable targeted manipulation of cell activity or intracellular signaling. Optogenetic stimulation enables selective, bi-directional modulation of cell activity

### The Lumos system for multiwell optical delivery



- **High-throughput** with up to 192 LEDs over 24, 48, or 96 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 and materials
- **Precise control** with microsecond precision and finely adjustable intensity for each LED
- **Flexible control** as each LED can be controlled independently and simultaneously
- **Compatible design** with a top-side light delivery format that enables simultaneous pairing with other technologies, such as electrophysiology or imaging




Four individually controllable wavelengths per well for activation of multiple targets

Swappable head units for 24-, 48-, and 96-well plates

OptiClear plates have optical specializations to eliminate light bleed-through and a highly transparent bottom. 63x view of a neural culture (scale=50µm).


AxIS Software provides intuitive stimulus design with drag and drop blocks to create light delivery patterns and easy selection of target wells

The Lumos ECbase enables environmental control during experiments involving extended light delivery.

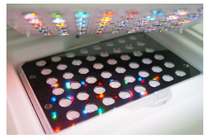
## Multiwell optical delivery example application: Modulation of growth/regeneration in cultured neurons

(Data courtesy Claire McGregor and Arthur English, Emory University)

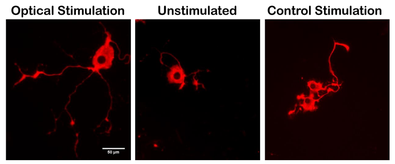
**Background/Methods:**

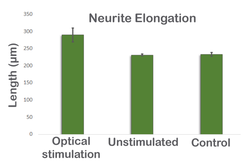
Over 33% of the population express the BDNFMet gene mutation, resulting in decreased activity-dependent release of BDNF. It was hypothesized that in BDNFMet+ dorsal root ganglion (DRG) neurons, neurite outgrowth would be reduced, especially when treatments were applied that are known to induce neuronal BDNF expression.

DRGs were harvested from BDNFMet+ and control adult mice, both from a Thy1ChR2 population. After 48 hours in culture, cultures were stimulated with blue light (475nm) for 1h (5ms pulses @ 20 Hz) to induce neuronal BDNF expression. Control stimulation was performed with 655nm red light. Cultures were fixed 24h later, and immunostained to quantify neurite lengths.



Dissociated DRGs were cultured on OptiClear 48 plates and stimulated with the Lumos device. The Lumos ECbase was used to maintain environmental control during stimulation.



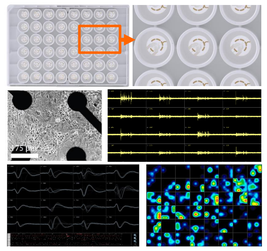




**See the full poster Wednesday 3-4pm :** 741.11 / B18 - Neurite elongation is enhanced in cells heterozygous for BDNF Val66Met polymorphism *C. MCGREGOR[1], A. W. ENGLISH[2]; [1]Cell Biol., Emory Univ., Decatur, GA; [2]Dept Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA

## Multiwell MEA Technology

### 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 and connectivity. MEAs provide a powerful approach for modeling *in vivo* neural behavior and can be applied to disease modeling, stem cell characterization and phenotyping, neurotoxicity, and safety.



An array of microelectrodes in each well interfaces with cultured cells. Activity across the cell network is captured and analyzed.

### Why use the Maestro Pro™?

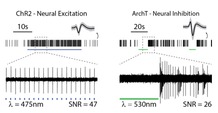
Axion's Maestro Pro™ multiwell MEA platform enables functional cellular analysis on the benchtop with an industry-leading 768 electrodes across all plate formats.

- **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

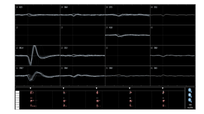
## Multiwell MEA recording with simultaneous optogenetic stimulation

### System design and validation

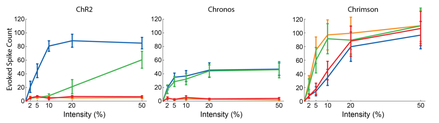

Optogenetic stimulation provides an artifact-free, targeted, and precise means of perturbing cell and network behavior during electrical activity recordings.




Sample electrode recording



Stimulus responses are visualized in real time for easy interpretation.




Light intensity and wavelength were varied in 5ms pulses. ChR2 and Chronos were activated preferentially with blue light, with a larger evoked response from ChR2+ neurons, due to slower opsin kinetics. Chrimson's red-shifted excitation spectrum resulted in maximum excitation with green and orange light.

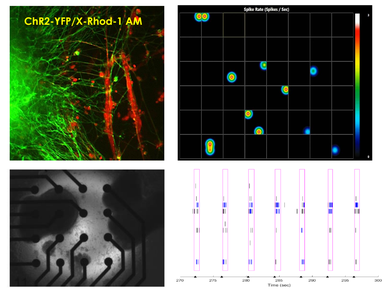
### Multiwell Optogenetics + MEA example application: Cell-type specific stimulation in a neuromuscular co-culture

(Data courtesy Elliot Swartz and the Coppola lab, UCLA)

**Background/Methods:**

Understanding of the pathogenesis in neuromuscular disease that leads to the collapse of the neuromuscular junction (NMJ) is poor. A human iPSC derived co-culture system comprised of skeletal myotubes and spinal motor neurons was developed to recapitulate the physiology of the NMJ in diseases like ALS.

As part of this work, optogenetics was used to selectively activate motor neurons during simultaneous MEA recordings of muscle activity. This setup enables characterization of potential electrophysiological phenotypes, breakdown of signaling, and developmental features relevant to the NMJ.




**From Sunday AM nanosymposium presentation:** 104.09 - Establishment of a human induced pluripotent stem cell derived neuromuscular co-culture platform for disease *E. SWARTZ[1], G. SHINTANI[2], J. WAN[2], S. WANG[2], M. PRIBADI[2], Z. YANG[2], L. HAVTON2, G. COPPOLA[2]; [1]Neurosci., [2]Neurol. and Psychiatry, UCLA, Los Angeles, CA

## Conclusions

- Optical stimulation provides a powerful and precise means of influencing cellular systems.
- The Lumos system enables highly controllable and flexible optical stimulation at the multiwell level for advanced and high-throughput experimentation.
- The top-side light delivery format of the Lumos enables simultaneous pairing with other technologies, such as bottom-side imaging or electrophysiology.
- Optogenetic stimulation supplements MEA assays by enabling cell-type specific modulation of activity, real-time tuning of network state, and reduction in well-to-well variability, for enhanced disease modeling and dissection of neural circuitry.