Multiwell optogenetics for enhanced control of human iPSC-derived cells
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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 single multiwell plate, collecting robust, real-time recording of extracellular and intracellular activity. This approach provides a more detailed understanding of the intricate interactions within and between individual neurons, as well as the cellular and network-level activity of cultured neurons.

Why use MEA arrays?
Axion’s MEA products enable simple analysis of multiple measures on a single system.

- **High-throughput, simultaneous measurement** of extracellular activity at hundreds of sites, with the ability to control the light stimulus at each site
- **Wide dynamic range** with minimal light intensity fluctuations
- **Epigenetic and genetic control** of cell activity
- **Environmental and time-dependent control compatibility**
- **Robust pacing was stable beginning 1 day after transfection**

Why use the Maestro?
Axion’s multiwell microelectrode array (MEA) platform enables functional cellular analysis to the bench with 748 electrodes across all plate formats.

Lumos: Multiwell optical stimulation for control of cell activity

Why use luminescence?
Luminescence enables activation and suppression of neural cultures.

- **High-throughput** simultaneous control of light delivery to each well of a standard 96-well plate
- **Focal delivery** - deliver light to any specific manipulated region
- **Complete wavelength coverage** - Four wavelengths per well span the visible spectrum for selective stimulation of all common opsins
- **Environmentally compatible** - Options for regulation of temperature, CO₂, and humidity enable extended benchtop work
- **User-friendly software** - Intuitive “drag-and-drop” style enables quick design of light delivery patterns and selection of target wells

Why use optogenetic?
Optogenetics is the integration of fast, light-activated ion channels (opsins) that allow targeted, precise manipulation of cellular activity. Upon activation of the correct wavelength, the opsins produce currents that directly hyperpolarize or depolarize the cell.

Why use Lumos?
Lumos also operates independently on standard 96-well plates, enabling chronic light delivery experiments for influencing cellular activity and intracellular processes such as gene expression, cell growth, maturation, and differentiation.

Applications in iPSC characterization

- **Pacing cardiac beating enhances cell characterization**
  - Cardiac repolarization timing is intrinsically linked to the beating frequency, which can naturally vary over time and between wells.
  - Beating frequency and repolarization timing are both sensitive to iPSC differentiation, drug, and disease effects.
- **Optogenetic stimulation can control beating frequency** and remove influence of cardiomyocyte electrophysiology.
- **Normalizing beating frequency increases reliability and sensitivity of the repolarization measurement**

Case study: mRNAs mediated opsin delivery for rapid, enhanced characterization assays

- **Axogen Cre/Lox** cardiomyocytes were cultured on 96-well MEA plates.
- Cells were transfected with Fos-opsinCre mRNA for orbital-free optical pacing with the Lumos.
- Resistant concentration was varied across the plate during transfection, and light intensity and pacing rate were systematically varied 1-4 days post-transfection.
- The multiwell format of the Maestro and Lumos enabled rapid optimization of the Fos-opsinCre protocol.
- Robust pacing was stable beginning 1 day after transfection.
- Pacing required very little light and was stable from 1-4 days post-transfection.

Isolate Repolarization Effects with Pacing
- **NAPA** Application of PFL 4417A induced a significant prolongation of BP and FPD relative to the vehicle control (DMSO).
- **Pacing at 750 ms BP control** for the influence of beating rate on repolarization. Under paced conditions, PFL 4417A produced a dose-dependent prolongation of FPD that was independent of beat period.

Future applications: Optical control over developing iPSC cultures

- **Using the Lumos system, deliver light over extended periods of time to influence cell culture development**
- **Alter iPSC maturation by controlling activity levels** (e.g., Lam et al. 2017, Oda et al. 2017)
- **Influence iPSC differentiation through control over gene expression, protein function, and other intracellular processes** (e.g., Fischer et al. 2016, Oda et al. 2017)
- **Robust pacing could be optimized rapidly and efficiently using the multiwell format of the Lumos system**

Conclusions
- **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 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 activity and intracellular processes such as gene expression, cell growth, maturation, and differentiation.**