Optically integrated multiwell microelectrode arrays: High-throughput disease modeling and drug discovery with optogenetics

I. P. CLEMENTS, D. C. MILLARD, A. M. NICOLINI, M. W. BROCK, A. J. PREYER, J. D. ROSS

Axion BioSystems, Inc., 1819 Peachtree Road, Suite 350, Atlanta, GA, 30309

TT80
271.05

Introduction

Microelectrode Arrays (MEAs) reveal neuronal network interactions by monitoring and manipulating electrical activity at both the single neuron and network scale. Applications include:

- Screening of drugs, gene edits, or toxins for influence on single cell and network activity
- "Disease-in-a-dish" models capturing phenotypic components of specific clinical conditions
- Induced pluripotent stem cells (iPSCs) enable human cell-based models of neural diseases

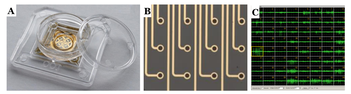


Fig. 1: (A) Single MEA culture well (B) A grid of 64 microelectrodes on the well bottom monitors overlying cell activity (C) Sample voltage recording traces from sixty-four electrode sites

***Multiwell* MEAs enable high-throughput experimentation at reduced experimental time and cost.**

- Multiwell MEAs consist of multiple distinct culture wells on a single microplate, allowing for parallel experimentation with many experimental conditions, control cultures, and high 'N' counts.
- Axion's Maestro® system accommodates 12, 48, or 96 well plates, each with 768 microelectrodes distributed through the wells.

Fig. 2: (A, B) A 48-well MEA plate docks within (C) the Maestro system. (D) Spike-rate activity across 768 channels and 48 wells

Optically stimulating multiwell plates allows for enhanced control and exploration of neuronal culture networks

- Existing electrical stimulation capabilities allow neural cultures to be perturbed during recordings for a more complete characterization of underlying network states.
- The capability to optically stimulate individual MEA wells augments existing electrical stimulation capabilities for exploring cultured neural networks and characterizing disease states.
  - Cell-specific activation or inhibition of neuronal sub-types
  - Minimal stimulus artifacts during electrical recordings
  - Spatially uniform stimulus delivery across cultures
  - Tuning of activity levels and network states
  - Modulation of protein interactions

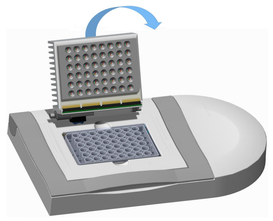
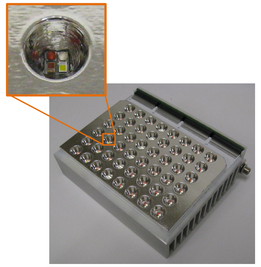
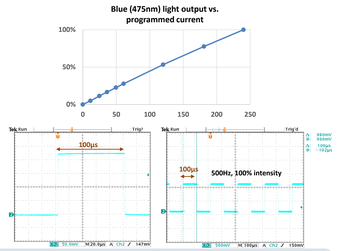


Fig. 3: Multiwell light delivery device design

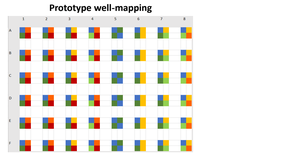
Characterization of optical delivery



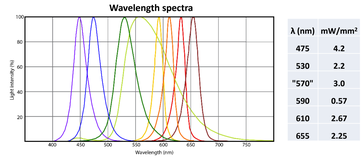
Each LED bank was surrounded by a parabolic metallized reflector to concentrate and isolate light delivery to the underlying target MEA culture well.



Light delivery vs. programmed control current was linear over the full output range. Light patterns were controllable with microsecond precision up to 500Hz (100µs pulses) and precise and stable control of intensity.



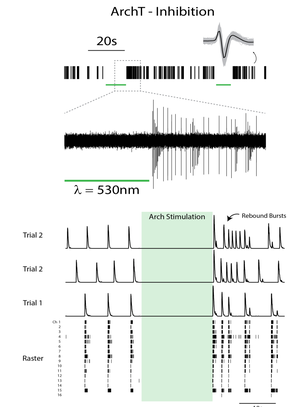
Different combinations of LED wavelengths were laid out on the prototype device for testing. A "classical" case (columns 1, 2) included 475nm (*e.g.* for ChR2), 530nm (*e.g.* Arch), 610nm (*e.g.* Halo), 655nm (*e.g.* Chrimson). In other columns, alternative wavelengths were paired within the same wells (e.g. 590nm & 610nm; 530nm & broad "570"nm) for parallel comparison of efficacy. Finally, other LED banks featured multiples of the same wavelength to provide the option for increasing the achievable intensity from a given wavelength.



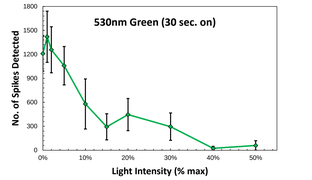
λ (nm)	mW/mm²
475	4.2
530	2.2
"570"	3.0
590	0.57
610	2.67
655	2.25

Maximum irradiance of the MEA surface by individual LEDs was measured. Actual irradiance experienced by cells are likely greater, due to reflections from the MEA bottom (*e.g.* a transparent MEA plate over a reflective surface.) Modifications to microplate material and geometry could also increase practical irradiance. The current material was slightly translucent, as indicated by a slight bleed-through of light between wells. Irradiance can be increased up to 4x with multiple LEDs of the same wavelength per well.

ArchT mediated inhibitory response

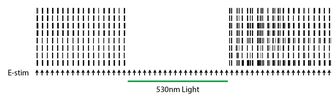


Top: Continuous delivery of green light (530nm) silenced spiking activity in ArchT⁺ cortical cultures
Bottom: Spontaneous rhythmic bursting was reliably silenced by delivery of 530nm light. Light delivery offset was characteristically followed by a series of rebound bursts before cultures resumed baseline activity.



Simultaneous assessment of threshold light intensity across wells. Continuous pulses of green (530nm) light of varying intensity were used to explore the relationship between ArchT⁺ mediated inhibition and spontaneous network activity across wells.

Optical silencing of electrically paced activity



Bi-directional control of network activity. Synchronized bursting evoked through electrical stimulation (1V biphasic pulses) was silenced by optical stimulation of ArchT⁺ channels (30 sec pulse, max intensity). The multi-modal approach enables dissection of network components through the cell-type specificity afforded by optogenetic stimulation.

Methods and device design

Optical design and testing

An optical stimulation device was designed to independently illuminate each well of a standard 48-well microplate. Toward this end, a custom metal core printed circuit board (**Fig. 4A**) was designed with four LED slots per well, to accommodate a total of 192 independently addressable LEDs with a variety of center wavelengths (450nm, 475nm, 530nm, 570nm, 590nm, 610nm, 625nm, 655nm.) A custom array of parabolic reflectors was designed to concentrate and deliver light from each LED bank to the bottom surface of each MEA well (**Fig. 4B**). An aluminum heatsink was manufactured to enhance thermal dissipation (1.5 K/W) (**Fig. 4C**). A custom 192 channel LED controller allows each LED to be driven with 7-bit (128 step) intensity resolution and updated every 100µs (10 kHz) (**Fig. 4D**). A prototype software control GUI was designed in MATLAB to allow generation of illumination patterns and delivery to selected wells (**Fig. 4E**). MEA plates were manufactured with white opaque polystyrene walls for high specular reflection and diffusion of light within each MEA well (**Fig. 4F**). Light delivery to the MEA surface was quantified using a silicon photodiode (FDS1010, ThorLabs) during 1ms test pulses at controlled currents. EMI noise and optoelectric artifact were investigated using Axion's AxIS software analysis system.

Cell culture and viral transduction

Rat cortical neurons (QBM Cell Science) were cultured on 48-well MEAs. Neurons were transduced using one of two methods to express either Channelrhodopsin-2 (ChR2) or Archaerhodopsin (ArchT) along with a green fluorescent protein (UNC Vector Core) via AAV-9 viral vector and CAG promoter.

Transduction in the Tube:
ChR2 = rAAV9/CAG-ChR2-GFP viral vector ($3.9x10^{12}$ viral molecules/mL) (Lot#AV4148C)
Rat cortical neurons (QBM Cell Science) were thawed, centrifuged, re-suspended in 37µL of Neurobasal media , and mixed with 3µL of ChR2 virus, yielding ~$9.6*10^{9}$ viral particles in contact with $1.28x10^{6}$ neurons. Neurons were then plated at 160,000 cells per well (5µL drop size) into 8 wells of a white-walled 48-well MEA plate (Axion BioSystems).

Transduction in the Plate:
ArchT = rAAV9/CAG-ArchT-GFP viral vector ($3x10^{12}$ viral molecules/mL) (Lot#AV6221)
Rat cortical neurons were plated at 160,000 cells per well (5µL drop size) into 8 wells of a white-walled 48-well MEA plate. On day *in vitro* 14, the media was removed from all wells and replaced with 50µL each of serum free Neurobasal media containing 3µL of ArchT virus. The cells were incubated with the virus for 4 hours and then the media was replaced with complete Neurobasal media with B-27.

Cell Culture:
Neurons were cultured in Neurobasal media supplemented with B-27 for 30 days with 50% media change every 3 days. All experiments were conducted after 14 days *in vitro* to allow networks to develop and spontaneous synchronized bursting events to emerge. Optogenetic tests were conducted at least 14 days post transduction to allow full phenotypic expression.

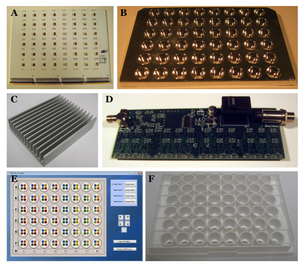


Fig. 4: Light delivery hardware device development

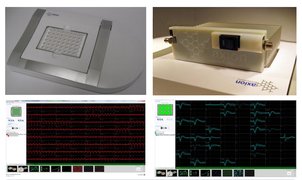
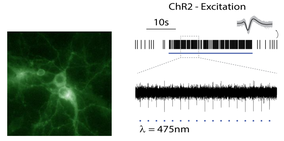


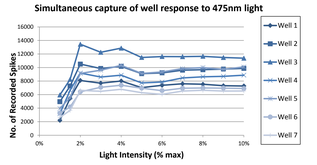
Fig. 5: 48-well plate light delivery experiments, using the Axion Maestro system and AXiS software

Channelrhodopsin-2 mediated excitatory response

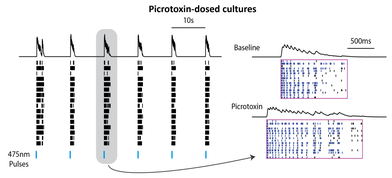
Activation of optically sensitive neurons at fast timescales. Dissociated rat cortical cultures were established *in vitro* and transduced to express ChR2. Pulses of blue light (475nm, 10ms, 5Hz) reliably elicited action potentials from individual neurons within the network. No activity was generated in control cultures, verifying that artifacts from the pulsed light were minimal.



Simultaneous calibration of light intensity across wells. Ten millisecond long pulses of 475nm light were delivered at 0.5Hz to ChR2⁺ cortical cultures to explore the relationship between light intensity and network response. Network bursts were fully entrained at 2% of the maximal light intensity from a single LED. The threshold light intensity was consistent across wells, suggesting an even level of opsin expression. Variations in the saturated response are likely due to differences in the intrinsic excitability across the wells.



Pro-convulsant risk assessment mediated by optical stimulation. Neuronal cultures offer a useful model for evaluating the pro-convulsant nature of candidate compounds. Stimulation enhances the reliability of the assay by standardizing the burst frequency and allowing independent investigation of the network burst duration. As expected, picrotoxin prolonged the duration of the network bursts.



Conclusion and future directions

Conclusions

- Viral vectors, concentrations, and delivery techniques were efficiently optimized in parallel using the Maestro multiwell MEA platform and multiwell light delivery system.
- ChR2 and ArchT activation reliably excited and inhibited, respectively, the network activity of neuronal cultures in vitro
- Scalable optogenetic stimulation has applications towards high-throughput pro-convulsant risk assessment and dissection of network-level neural activity.
- These findings demonstrate the potential of optically-integrated multiwell MEA systems to enable high-throughput drug screening and phenotypic modeling of neurological diseases.

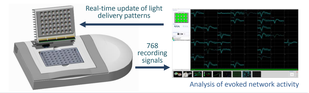
Future directions

- Modification of microplate materials and geometry, forming an "optical cavity" with maximized reflections for high efficiency.
- 96 well devices and spatial constraint of light delivery
- Algorithms for automation and closed-loop control to facilitate high well count experimentation
- Application specific toolsets
- Other *in vitro* models, *e.g.* cultured cardiac cells, protein expression

Microplate / lid modification

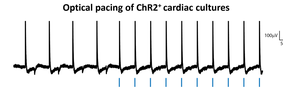
Geometry/materials of microplate/lid/MEA surface can be modified to maximize light reflection to greatly increase practical irradiance of cell cultures.

Closed-loop control of network states



Hardware and algorithms development to remove the operator from the "loop", enabling automated high-throughput experiments and closed-loop control of neural activity.

Optical pacing of ChR2⁺ cardiac cultures



In preliminary experiments, human iPSC cardiac cells (CDI iCell Cardiomyocytes) were transfected with ChR2 or ArchT (AAV-9, CAG promoter). ChR2⁺Light sensitive cultures were uniformly paced with high reliability at frequencies up to 3Hz. ArchT⁺ cultures were reliably silenced.

Acknowledgements

Dr. Tony Krantis - QBM Cell Science / Dr. Blake Anson - Cellular Dynamics Incorproated (CDI) / Roger Olivares (CREE)