Optimization of optogenetic transduction of stem cell derived cardiomyocytes with adeno-associated virus for optically-paced cardiac electrophysiology assays

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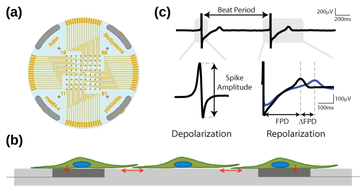
Multiwell MEA Technology

Microelectrode Array Technology

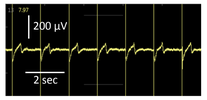
The flexibility and accessibility of neural and cardiac *in vitro* models, particularly induced pluripotent stem cell (iPSC) technology, has allowed complex human biology to be reproduced *in vitro* at unimaginable scales. Accurate characterization of neurons and cardiomyocytes requires an assay that provides a functional phenotype. Measurements of electrophysiological activity across a networked population offer a comprehensive characterization beyond standard genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell microelectrode array (MEA) platform provides this comprehensive functional characterization. The Maestro is a non-invasive benchtop system that simply, rapidly, and accurately records functional activity from cellular networks cultured on a dense array of extracellular electrodes in each well.

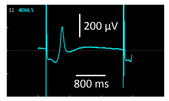


A planar grid of microelectrodes **(a)** interfaces with cultured neurons or cardiomyocytes **(b)**, to model complex, human systems. Electrodes detect changes in raw voltage **(c)** and record extracellular field potentials.

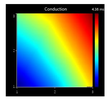
Raw Voltage



Extracellular Action Potentials

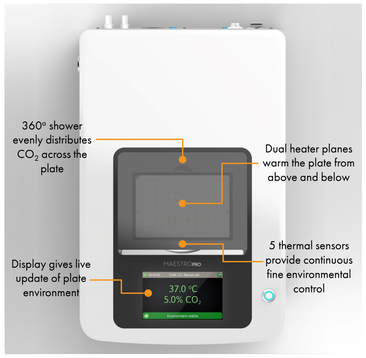


Network Activity



Raw voltage signals are processed in real-time to obtain extracellular field potentials from across the network, providing a valuable electrophysiological phenotype for applications in drug discovery, toxicological and safety screening, disease modeling, and stem cell characterization.

Introducing the Maestro Pro™ and Maestro Edge™



- **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

The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

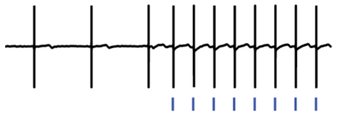
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	24-Well	12-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	No	Yes

Optimization of Optogenetic Pacing

The Lumos Multiwell Optical Stimulator Enables Cardiac Pacing

With optogenetics, light can be used to control and pace cardiomyocytes without artifact. Pacing cardiomyocytes offers many advantages:

- Specify beat rate at 1Hz for enhanced physiological relevance
- Establish well-to-well and plate-to-plate consistency with matched beat rates in all wells
- Detect use-dependent drug effects for superior safety screening



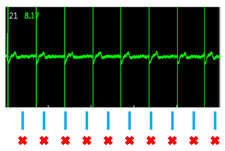
The Lumos™ is the first commercial multiwell light delivery device designed for *in vitro* optogenetics. The Lumos provides precise control over cardiomyocyte beat rate or neural activity.

The Lumos Advantage

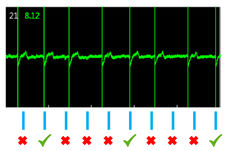
- **Artifact free stimulation and pacing**
- **High throughput** with 192 LEDs over 48 wells
- **Compatible with any opsin** with 4 wavelengths encompassing the visual spectrum (460-670 nm)
- **Maximal intensity** with high power LEDs and optimized plate and lid optics on the Lumos MEA
- **Precise control** with microsecond precision and finely adjustable intensity for each LED
- **Flexible control** as each LED can be controlled independently and simultaneously

Capture Threshold Assay to Evaluate Pacing Efficacy

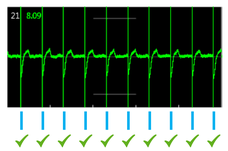
No Capture



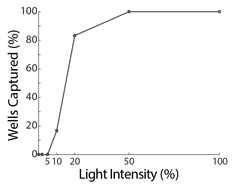
Partial Capture



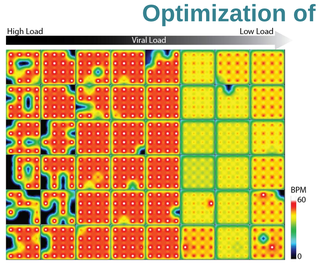
Full Capture

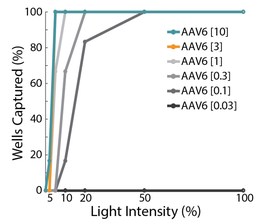


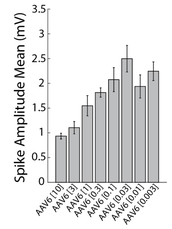
For low light intensity, or ineffective optogenetic transduction, none of the light pulses will elicit or "capture" a cardiac beat (left). For higher levels of light, some beats will "capture", whereas other stimuli will fail to elicit a beat, resulting in partial capture (middle). With sufficient light intensity, full capture can be achieved at a variety of pacing rates (right). With efficient optogenetic transduction, full capture is typically achieved at ~20-50% light intensity across all wells (bottom).



Optimization of Optogenetic Transduction





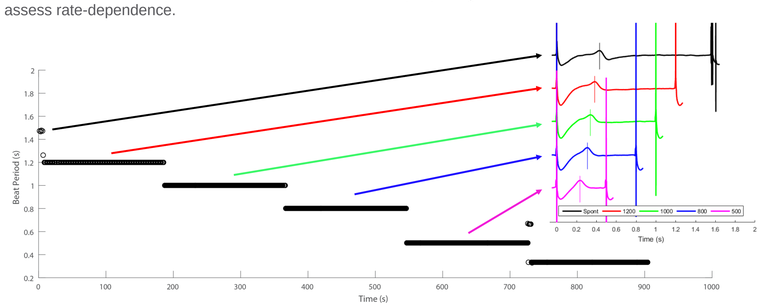


The amount of AAV6 added to transduce the cardiomyocytes was varied column-wise across the plate. Higher viral load lead to significantly lower light intensity thresholds for achieving full capture in all wells. However, the cardiomyocytes exhibited a dose-dependent reduction in spike amplitude with increasing viral load, possibly due to increased leak currents. The optimized transduction protocol used 0.3uL of virus added per column of cardiomyocytes on a 48-well plate.

Paced hiPSC-CM Electrophysiology

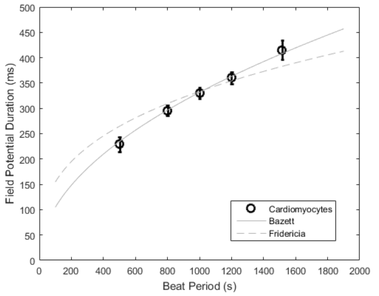
"Chirp" Protocol to Assess Repolarization and Beat Frequency

Repolarization timing is intrinsically linked to the beating rate of cardiomyocyte cultures. Pacing enables changes in repolarization to be isolated from changes in beating rates by forcing a known beating rate. A "chirp" protocol enables repolarization to be evaluated at multiple pacing rates to define rate correction criteria or assess rate-dependence.

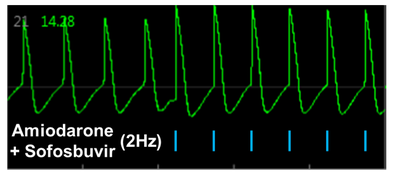


The iCell Cardiomyocyte[2] was paced at multiple beat periods (0.5, 0.8, 1, 1.2 seconds) and repolarization timing measured as the field potential duration. The relationship between field potential duration and beat period was most closely modeled by the Bazett correction factor (solid line).

The cells were paced for 3 minutes at each beat period to allow repolarization to stabilize. The beat period was reduced in successive steps to enable the cells to track faster beat rates.



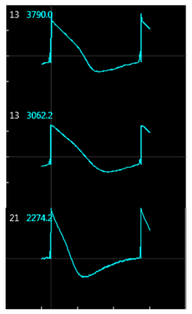
Optogenetic Pacing with LEAP



DMSO

Amiodarone

Amiodarone + Sofosbuvir



With the new BioCore4, the Maestro Pro can now induce and record long-lasting, stable, extracellular action potential-like signal shapes, known as local extracellular action potentials (**LEAP**), on MEAs. Optogenetic pacing and LEAP were used to confirm previous results regarding the drug-drug interaction between Amiodarone and Sofosbuvir. Pacing at 2Hz across all conditions revealed that the combination of Amiodarone and Sofosbuvir causes a significant shortening of the cardiac action potential that is independent of changes in beating rate.

Conclusions

Optogenetics is a powerful tool. When combined with MEA assays, optogenetics can enhance your neural or cardiac assays by reducing well-to-well variability, detecting rate and activity-dependent drug effects, and systemically controlling cell activity for better sensitivity and specificity.