Characterization of electrical and optical pacing of hiPSC-derived cardiomyocytes in vitro using multiwell multielectrode array technology

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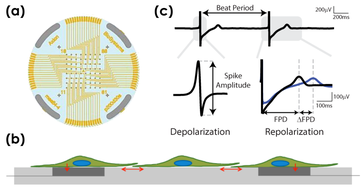
Multiwell MEA Technology

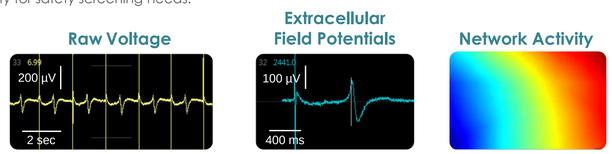
Why use microelectrode arrays?

The need for simple, reliable and predictive pre-clinical assays for cardiac safety has motivated initiatives world-wide such as the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) and Japan's iPS Cardiac Safety Assessment (JiCSA).

Axion BioSystems' Maestro™ multiwell MEA platform enables assessment of *in vitro* cardiomyocyte activity with an easy-to-use benchtop system. The Maestro detects and records electrical signals from cells cultured directly onto an array of planar electrodes in each well. Multiple electrodes in each well provide mechanistic electrophysiological data, and enable analysis of conduction across the cardiomyocyte syncytium. With plate capacity up to 96 wells, the Maestro offers high throughput capacity for safety screening needs.



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



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 modelling, and stem cell characterization.

Why use Maestro™?

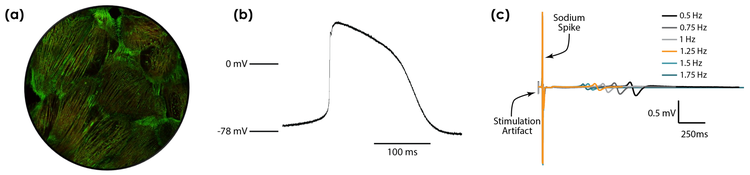
Axion's Maestro Pro™ multiwell microelectrode array (MEA) platform enables functional cellular analysis on the benchtop with an industry leading 768 electrodes on all plate formats.

- **Label-free, non-invasive recording** of extracellular voltage from cultured cardiomyocytes
- **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-, 48- and 96-well plates)** meets all throughput needs on a single system
- **State-of-the-art processing chip** offers stronger signals, ultra-low frequency content, and enhanced flexibility

BioCore v4

Pluricyte® Cardiomyocytes by Pluriomics

Pluricyte® Cardiomyocytes are functional human induced pluripotent stem cell-derived ventricular cardiomyocytes (hiPSC-CM). The relatively low spontaneous beat rate of Pluricyte® Cardiomyocytes (< 0.5 Hz) make them particularly suitable for electrical or optical pacing across a wide range of beat rates for exploring beat rate dependent drug effects. Here, Pluricyte® Cardiomyocytes were used in combination with the Maestro MEA platform, E-Stim+ MEA plates, and Lumos multiwell optical stimulator to evaluate the advantages of pacing cardiomyocytes for enhanced cardiac safety assays.

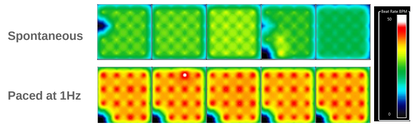


Pluricyte® Cardiomyocytes have a high degree of ultra-structural sarcomeric organization (**a**, green – alpha actinin, red – MHCβ) and a low resting membrane potential (**b**, manual patch clamp). Their beat rate is readily controlled with electrical stimulation on the Maestro (**c**, field potential with electrical pacing from 0.5 to 1.75 Hz). The field potential duration (FPD) adapts to each pacing rate.

Pacing Cardiomyocytes

Why pace cardiomyocytes?

- 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

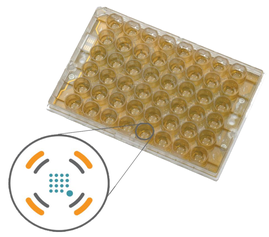


Multiwell Electrical Pacing

Why use E-Stim+?

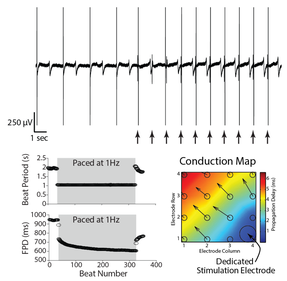
Axion BioSystems' E-Stim+ Classic MEA™ 48 plate delivers high-quality MEA results with superior stimulation capacity.

- Large dedicated stimulation electrode (teal) ensures reliable stimulation capture
- AccuSpot on-plate spotting guides (orange) yield consistent cell placement and electrode coverage
- Seamless integration with AxIS makes stimulation simple yet customizable
- Optimized artifact elimination and automated feature detection makes analysis easy and reproducible



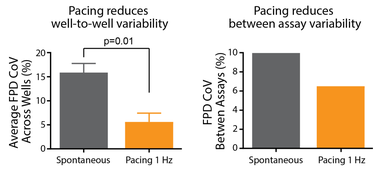
Electrical pacing reduces assay variability

Pacing Pluricte® Cardiomyocytes at 1 Hz



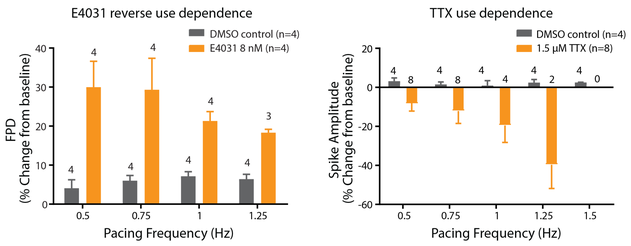
Pacing stimuli set beat rate at 1Hz (top, arrows). Beat period and FPD quickly adapted (bottom left). Conduction consistently started from the dedicated stimulation electrode (bottom, right).

Pacing at 1 Hz reduced both well-to-well and plate-to-plate variability



Pluricyte® Cardiomyocytes were recorded at spontaneous beat rates and then paced at 1Hz on an E-Stim+ Classic MEA 48 plate. Variability was measured as the coefficient of variability (CoV) across wells (left) and plates (right). Pacing at 1 Hz significantly reduced well-to-well variability in field potential duration (FPD, p = 0.01). Pacing also reduced variability between plates.

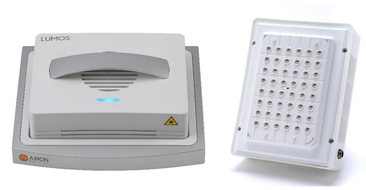
Electrical pacing reveals use dependent drug effects



Many compounds exhibit use-dependent effects. Reverse use dependence, in which a compound causes greater effects at slower beat rates, is an important indicator of proarrythmic risk. Here, Pluricyte® Cardiomyocytes were dosed with E-4031, a hERG potassium channel blocker, and TTX, a sodium channel blocker. Pacing at multiple beat rates revealed reverse use dependence of E4031 and use dependence of TTX.

Multiwell Optical Pacing

Why use Lumos™?



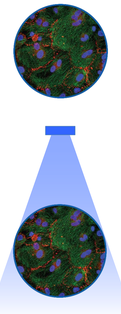
The Lumos™ is the first commercial multiwell light delivery device designed for *in vitro* optogenetics. The Lumos provides precise control over cardiomyocyte beat rate.

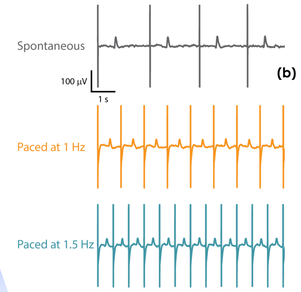
- **Artifact free pacing**
- **High throughput** with 192 LEDs over 48 wells
- **Compatible with any opsin** with 4 wavelengths encompassing the visual spectrum (460-670 nm) that are controlled independently and simultaneously
- **Maximal intensity** with high power LEDs and optimized plate and lid optics on the Lumos MEA
- **Uniform delivery** across the whole eliminates conduction pattern variation
- **Precise control** with microsecond precision and finely adjustable intensity for each LED

(a)

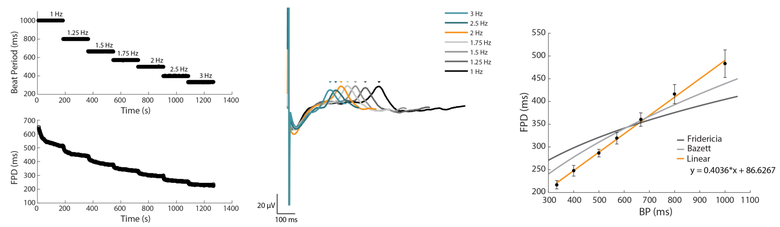
Optical artifact-free pacing of Pluricyte® Cardiomyocytes

Pluricyte ® Cardiomyocytes were transfected with mRNA-ChR2. Cardiomyocytes were paced at multiple beat rates using the Lumos™, while simultaneously recording on the Maestro™. Changes in beat rate and field potential duration (FPD) were analyzed using AxIS and Axion's offline analysis tools.





Optical pacing reveals cell specific FPD and BP relationship



A "chirp" assay was used to sequentially increase the beat rate of Pluricyte® Cardiomyocytes. The field potential duration (FPD) adapted with each sequential beat rate increase up to 3 Hz (left, middle). Typical clinical correction formulas, the Fridericia and Bazett corrections, did not accurately predict the FPD (right). However, pacing with the Lumos revealed the cell-specific beat rate correction relationship.

Conclusions

- The Maestro™ MEA platform in combination with Pluricyte ® Cardiomyocytes provide a robust *in vitro* model for advanced cardiac safety screening and drug development
- The ability to specify beat rate enhances physiological relevance and reduces well-to-well, assay-to-assay, and batch-to-batch variability
- The ability to precisely and systemically vary beat rate enables detection of use-dependent drug effects, which can be important predictors of proarrhythmic risk
- Pacing can also be used to derive cell-specific rate correction relationships