CiPA Phase 2 Study: A case study in development and validation of an automated microelectrode array (MEA) assay of hiPSC-derived cardiomyocyte electrophysiology for cardiac safety evaluation

C. Peritore, D.C. Millard, A.M. Nicolini, S.A. Chvatal, H.B. Hayes, M. Clements, J.D. Ross

Axion BioSystems, Atlanta, GA

Maestro™ multiwell MEA

Abstract

The need for simple, reliable and predictive pre-clinical assays for cardiac safety has motivated initiatives world-wide including the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) and Japan iPS Cardiac Safety Assessment (JiCSA). Towards this end, the Maestro MEA platform enables assessment of functional *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 of the MEA plate. 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.

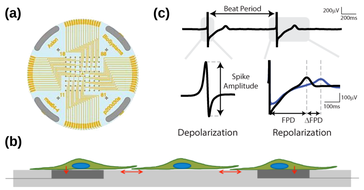
Consistent with other cell-based assays, preparation and maintenance of cultured cells on MEA plates can be tedious, error prone, and time consuming when performed manually. Maestro APEX, the industry's first MEA workstation, fully automates MEA plate preparation, maintenance, and Maestro assay execution. All of APEX's components are seamlessly integrated into a sterile compact workstation, which includes a robotic liquid handler, 44-plate capacity incubator, environmental controller, and HEPA filtration system.

Here, we present the validation of automated MEA plate preparation, maintenance, and drug toxicity evaluation using Maestro APEX and iCell® Cardiomyocytes^2. Cardiomyocytes seeded using the automated plating procedure exhibited extensive coverage across the MEA plates. Electrophysiological responses were reliably and accurately detected across replicates in an automated dosing procedure using positive control compounds. These results demonstrate automation of the cardiomyocyte-MEA assay will significantly improve reliability and throughput of cardiac risk assessment *in vitro*.

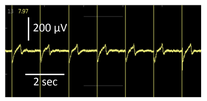
Why use microelectrode arrays?

Microelectrode array technology offers a platform for directly connecting key biological variables, such as gene expression or ion channels, to measures of cellular and network function.

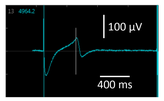
A planar grid of microelectrodes (a) interfaces with electro-active cultured cells (b), to model complex, human systems in a dish. The electrodes detect changes in raw voltage (c) caused by the electrical activity of cardiomyocytes, analogous to the ECG *in vivo*.



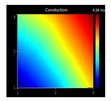
Raw Voltage



Extracellular Action Potentials



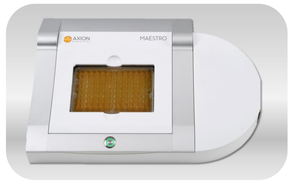
Network Activity



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

- **Label-free and non-invasive recording** of extracellular voltage from cultured cells on Axion MEA plates
- **Environmental control** provides a stable benchtop environment for short- and long-term toxicity studies
- **Fast data collection rate (12.5 KHz)** accurately quantifies the magnitude of depolarization events
- **Sensitive voltage resolution** detects subtle extracellular action potential events
- **Industry-leading array density** provides high quality data through high-integrity information from multiple locations in the culture
- **Scalable format (12-, 48- and 96-well plates)** meets all throughput needs on a single system

Why use the Maestro?

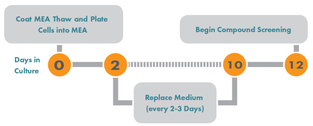


Axion's Maestro multiwell microelectrode array (MEA) platform enables high throughput evaluation of neural and cardiac activity on the benchtop, with an industry leading 768-electrodes across all plate formats.

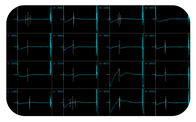
Typical Workflow

Plate Preparation

Maintenance and Experiments



Data Analysis



Maestro and AxIS™ software have made significant gains in experimental throughput and analysis ease. However, reliability and speed of plate preparation are two critical assay factors that have limited MEA screening applications. Understanding that scientists require a complete solution to automate all facets of MEA preparation and experimentation, Axion developed Maestro APEX.

Maestro APEX™

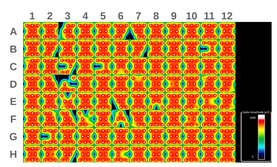
Why incorporate automation?

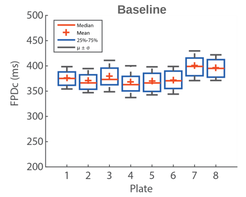
Maestro APEX features a 4-chennel robotic liquid handler, on-board gas mixer, dedicated Maestro deck position, and an integrated cell culture incubator.

- **Automated cell culture improves consistency and reliability of cultures.**
- **Significant walk-away time frees the user for other tasks, increasing efficiency.**
- **Preconfigured routines for cell spotting, media change, and dosing carry the user through the entire experiment.**
- **Redesigned environmental control provides continuous delivery of CO_2 at all times.**
- **Incorporated incubator with 44 plate capacity supports many simultaneous studies.**
- **Integrated HEPA filter and UV illumination ensures sterile operation.**

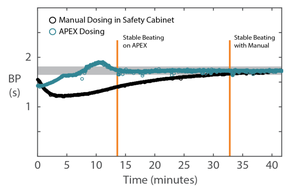
Cell Plating Reliability

Precision automation ensures reliability of cell spotting across wells (left) and across plates (right). Left, a heat map showing spontaneous cardiomyocyte signal spike amplitude across all 768-electrodes in a 96-well plate. Functional cardiac cultures developed in 99.4% of wells across eight 96-well plates, with corrected field potential duration (FPDc) consistently in the expected range (right).



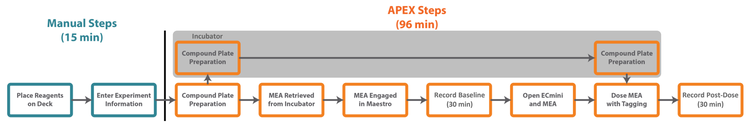


Biological Stability



It is commonly known that small perturbations to cardiomyocyte cultures can result in unstable assay performance. APEX minimizes changes to the local MEA plate environment via:
1) a sterile plate deck that enables dosing directly on the Maestro, and
2) an on-board gas mixer that provides CO_2 concentration compensation during dosing to facilitate a rapid return to stable beating patterns (left).

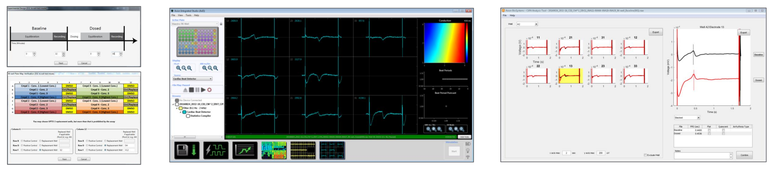
Efficiency and Throughput



APEX offers significant efficiency gains, as user time is minimal for completing important tasks. As an example, to execute the CiPA dosing procedure, the user simply supplies the compounds at a stock concentration and enters experiment information. APEX then completes compound plate preparation, MEA plate dosing, and data acquisition, freeing the user to engage in other activities.

Procedure	Upfront manual interaction-time	APEX work-rate
Half Media Change	5 min	12 min /plate
Full Media Change	5 min	30 min /plate
Surface Coating	5 min	28 min /plate
Cell Spotting w/ Media Addition	25 min	36 min /plate
CiPA Compound Prep. and Dosing	15 min	96 min /plate

Turn-Key Execution

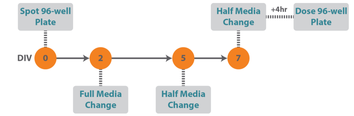


Axion software provides a comprehensive suite of tools to guide a user through all phases of experimentation and analysis. A custom APEX user interface provides access to pre-written protocols and configuration options (left); AxIS software enables real-time data visualization including conduction velocity maps and arrhythmia detection plots (center); the CiPA Analysis Tool complements the cardiac analysis performed within AxIS by providing semi-automated algorithms for fast and accurate data processing (right).

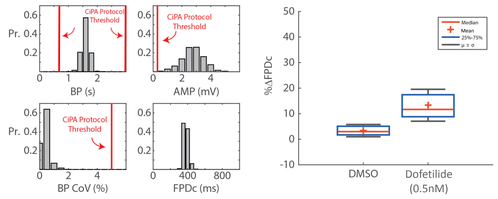
CiPA Phase II

Assay Workflow

The CiPA Phase II study was completed in 12 calendar days using the APEX for all spotting, maintenance, and dosing of the eight 96-well plates. The study required less than 6 hours of human interaction with APEX and 34 hours of unattended, automated routines. Data analysis was performed in parallel while APEX executed other tasks.



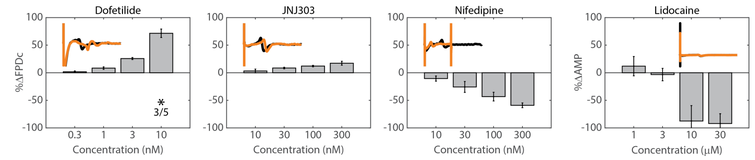
Human Time	5 hr 52 min
Prepare Media	20 min
Cell Thawing	3 hr 12 min
Supply Reagents	1 hr 40 min
Enter Experimental Information	40 min
APEX Time	**34 hr 08 min**
Half Media Change	4 hr 12 min
Full Media Change	4 hr 00 min
Surface Coating	4 hr 00 min
Cell Spotting w/ Media Addition	5 hr 26 min
CiPA Compound Prep. and Dosing	16 hr 30 min
Analysis Time (in parallel)	**8 hr (100 wells/ hr)**



Reliability

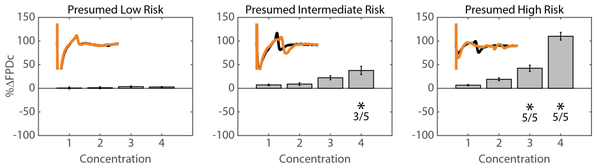
Automated cell plating contributed to highly reproducible endpoints across all eight plates, significantly exceeding CiPA protocol requirements. The stable dosing environment enabled consistent baseline activity and provided high assay sensitivity to resolve the positive control (dofetilide [0.5nM]).

Positive Controls



The positive control compounds demonstrated the expected responses for blockade of potassium, calcium, and sodium currents. Dofetilide, at higher concentrations caused significant prolongation of FPDc and arrhythmia incidence, whereas nifedipine reduced FPDc. JNJ303 produced a subtle, but detectable prolongation of FPDc. Lidocaine had little effect on repolarization, but elicited a significant reduction in amplitude. Asterisk (*) indicates the proportion of wells that showed an incidence of early afterdepolarizations (EADs).

Example Dose Response



Although the compounds remain blinded, the phenotypic response allows for prediction of low, intermediate, and high risk compounds. The presumed low risk compound (left) caused no change in repolarization, whereas the presumed high risk compound (right) induced significant FPDc prolongation and numerous EADs. By comparison, the presumed intermediate risk compound exhibited moderate FPDc prolongation and few EADs. Asterisk (*) indicates the proportion of wells that showed an incidence of EADs.

Conclusions

Maestro APEX, the first automated MEA workstation, provides significant advancements in research productivity and assay reliability.

- **More plates, more data, more discoveries** – automated protocols take care of every aspect of MEA plate preparation, maintenance, and assay execution.
- **More time** – with minimal user interaction, APEX does all the work. For example, the recent completion of the CiPA Phase II study involved less than 6 hours of user time.
- **High-quality results** – automated plate preparation and advancements in local environmental control ensures quality cell cultures and robust data.
- **Ease of use** – from intuitive APEX control interfaces to semi-automated analysis with the CiPA Analysis Tool, Axion makes high-throughput cardiac safety screening simple from start to finish.