AXION BIOSYSTEMS

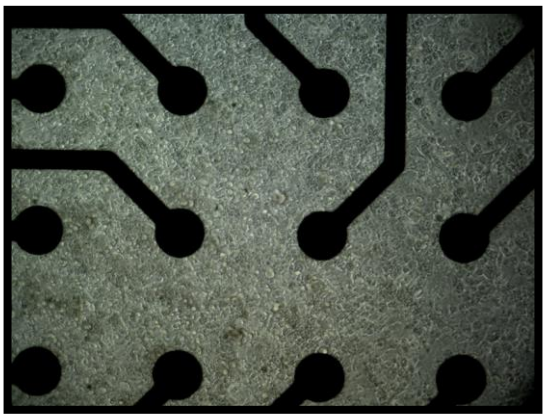
CARDIAC ACTIVITY ASSAY

WHY MEASURE CARDIAC ACTIVITY?

In vitro models are a proven powerful strategy for studying disorders of the human heart. Many of these disorders are the result of subtle changes to cardiomyocyte excitability, contractility, or both. The Maestro platform captures live cell activity from cardiomyocytes in real-time, providing you the functional cell information you have been missing.

WHAT IS MEA?

Axion's microelectrode array (MEA) plates have a grid of tightly spaced electrodes embedded in the culture surface of each well. Electrically active cells, such as cardiomyocytes, can be cultured over the electrodes. Over time, as the cultures become established, cardiomyocytes can form a beating syncytium. The Maestro makes it easy to record spontaneous or evoked electrical activity from each electrode on a microsecond timescale, providing both temporally and spatially precise data.



THE COMPLETE CARDIAC ASSAY

Maestro Pro and Maestro Edge are the next generation of electrophysiology plate readers. Record the four key measures of functional cardiac performance, label-free and in real-time in every well of the multiwell plate using a single system.

Action Potential

Field Potential

Propagation

Contractility

Read on to learn more about each of these assays.

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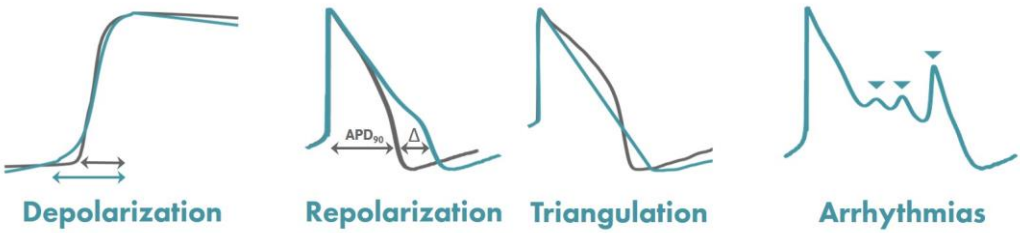
ACTION POTENTIAL

HOW DOES IT WORK?

Axion's patent-pending Local Extracellular Action Potential (LEAP) assay allows you to record extracellular action potential waveforms, which are stable for 10 to 20 minutes or more. The new LEAP assay signal allows quantification of action potential morphology and characterization of complex repolarization irregularities such as early afterdepolarizations (EADs). LEAP is label-free and doesn't disrupt the underlying biology, meaning you can focus on the science and not on dye-drug or dye-biology interactions. The theory behind LEAP is similar to that of the patch clamp technique, where the recorded signal amplitude is proportional to the sealing resistance between the electrode and the cell. The LEAP induction process increases the coupling between the cells and electrode, which enables recording of an action potential signal rather than a field potential signal.

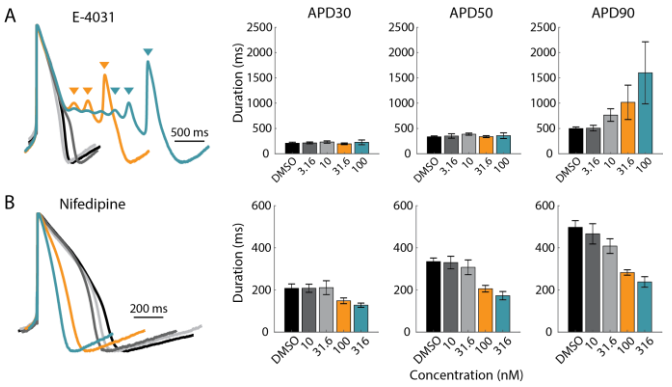
WHAT CAN YOU MEASURE?

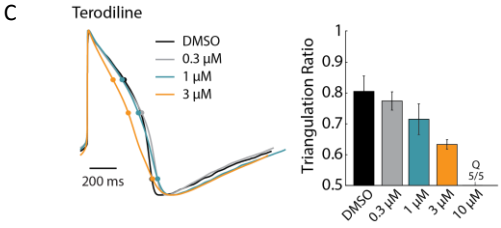


The Maestro system detects key parameters of the cardiomyocyte action potential waveform, including depolarization (rise time), repolarization (APD_{30}, APD_{50}, APD_{90}), triangulation, and irregular beating (arrhythmia).

LEAP CASE STUDIES IN PHARMACOLOGY

LEAP enables fully automated signal morphology analysis and EAD detection. The innovation of LEAP was benchmarked against the Maestro CM-MEA FP assay, establishing reliability and accuracy in tracking the cardiac action potential. Detection of hiPSC-CM action potential triangulation with LEAP is consistent with previous reports using manual patch clamp and alternate *in vitro* models.





A) E-4031 first prolonged repolarization and then generated repolarization irregularities. B) Nifedipine shortened the duration of the LEAP signal in a dose-dependent manner. C) Terodiline induced triangulation at higher concentrations, consistent with patch clamp results.

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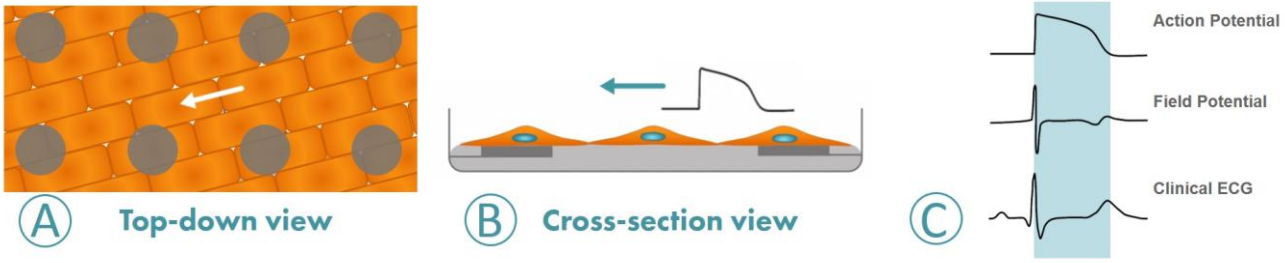
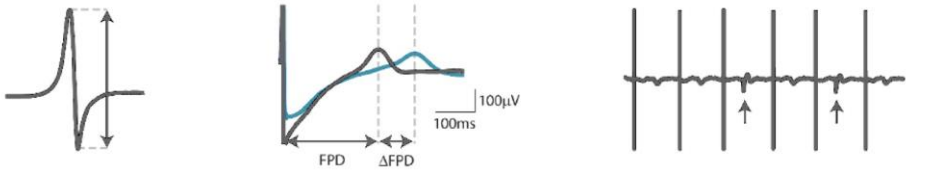
FIELD POTENTIAL

HOW DOES IT WORK?



When cardiomyocytes are cultured in MEA plates [A], spontaneous action potentials from a group of cells can be detected as fluctuations in the extracellular field potential at the adjacent recording electrode. The recorded cardiac field potential signal arises from the propagation of the cardiac action potential across the functional syncytium, much in the same way the clinical ECG arises from the propagation of the cardiac action potential across the heart [B]. The field potential signal has clear markers for depolarization and repolarization enabling the quantification of important beating parameters [C].

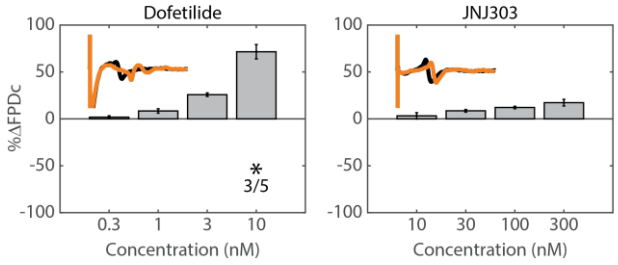
WHAT CAN YOU MEASURE?



The Maestro system detects key parameters of cardiomyocyte activity, including depolarization, repolarization (FPD), beat timing and irregular beating (arrhythmia).

FIELD POTENTIALS ARE GOLD STANDARD FOR *IN VITRO* DRUG SAFETY

The sensitivity of the Maestro hiPSC-CM field potential (FP) assay is the result of years of development alongside CiPA, with a significant focus on accuracy and reliability. The sensitivity extends to chronic assays due to the label-free, noninvasive signal acquisition and precise environmental control. Axion's focus on accuracy and reliability led to the industry-leading performance of the Maestro in the CiPA Phase I and Phase II studies (see link for more details).



The sensitive Maestro MEA platform can detect large increases in FPD in response to Dofetilide as well as subtle prolongation induced by JNJ303.

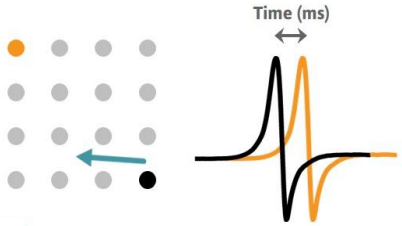
AXION BIOSYSTEMS

PROPAGATION

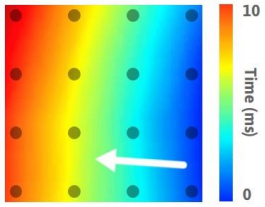
HOW DOES IT WORK?

In the human heart, a coordinated contraction is required for efficient circulation. Slowed or disrupted conduction can lead to irregular heart beats, known as arrhythmia, making conduction a key component of cardiac assessment *in vitro*. When cardiomyocytes are cultured in MEA plates, each beat is initiated in one part of the culture and propagates across the monolayer. The measurement of cardiac beat propagation and conduction velocity is only possible with an array-based approach. Providing up to 64 recording sites in each well, the Maestro MEA platform measures changes in propagation patterns and conduction velocity in response to pharmacological interrogation and during cardiomyocyte differentiation.

WHAT CAN YOU MEASURE?



Conduction velocity

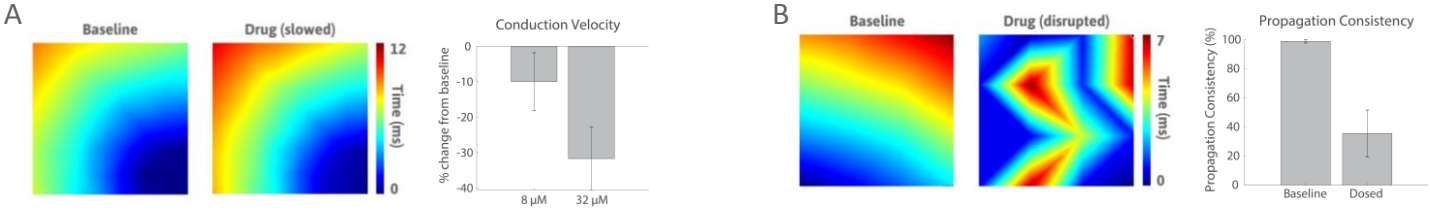


Propagation pattern

The Maestro system detects key parameters of the cardiomyocyte beat propagation, including conduction velocity, and determines the propagation pattern for each beat in order to report metrics describing the consistency in beat propagation.

PROPAGATION QUANTIFIES CELL-TO-CELL COUPLING

In an hiPSC-CM syncytium, beating is generally initiated in one portion of the culture (pacer region) and propagates like a wave through the tissue. Pharmacological agents and cardiac disorders can affect conduction by 1) modifying the excitability of the cells, or 2) altering the gap junction coupling between cells. Paced assays are able to resolve subtle changes in conduction velocity, whereas gross conduction changes appear as inconsistencies in the propagation patterns.



A) When lidocaine, a Na^+ channel blocker, was added to a hiPSC-CM network, a decrease in conduction velocity was observed. B) Addition of an anti-cancer drug treatment to hiPSC-CMs caused a disruption in propagation pattern, and thus a decrease in propagation consistency from beat to beat.

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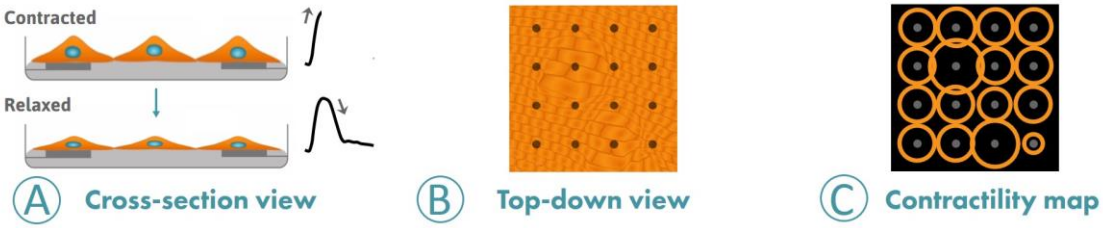
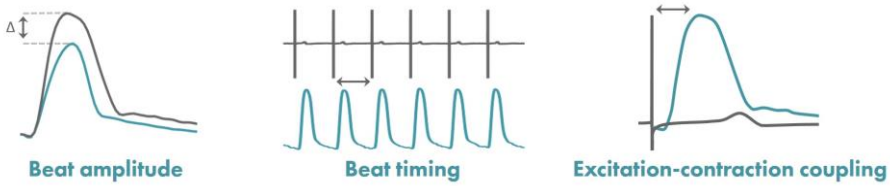
CONTRACTILITY

HOW DOES IT WORK?



Cardiac excitation-contraction coupling describes the series of events from the electrical impulse (action potential) to the contraction of the heart. When cardiomyocytes are cultured on MEAs, they form a spontaneously beating syncytium. As the cardiomyocytes mechanically contract and relax over an electrode, the shape change is detected as an increase and decrease in impedance (grey arrows) [A]. The array of electrodes can detect regions that are contracting while other regions are being stretched [B]. This pattern can be represented as a contractility map, where the relative size of each orange circle indicates whether the local cells are contracting or being stretched [C].

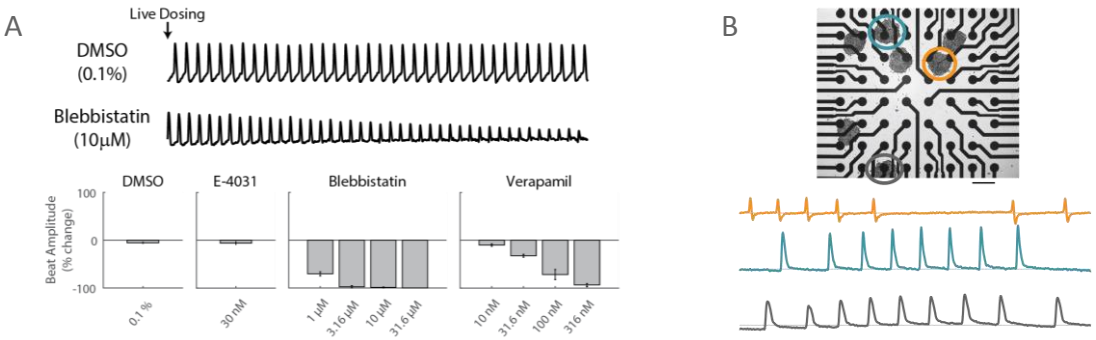
WHAT CAN YOU MEASURE?



Detect key parameters of contractility, including beat amplitude, beat timing, and excitation-contraction delay.

EXAMINE PHYSICAL CONTRACTION *IN VITRO*

Contractility provides important information about cardiac beating. For example, blebbistatin, a myosin inhibitor, reduces the array-based cardiomyocyte contractility signal (A). At high concentrations, the cells are no longer contracting. Furthermore, array-based contractility enables advanced applications, such as recording from 3D cultures and robust contractility measurements despite variations in cell coverage across the well (B).



A) After dosing, the contractility signal for blebbistatin rapidly decayed relative to the stable vehicle control. After 30-minutes, blebbistatin and verapamil contractility amplitude showed clear dose-dependent trends, while vehicle control and E-4031 showed no change. B) Seven cardiomyocyte spheroids were deposited in a well. Using array-based contractility, the mechanical beating was measured from these three independently-beating spheroid groups on the array (circles).

MAESTRO MEA SYSTEMS

HOW DOES IT WORK?

Getting started with Maestro couldn't be easier. Culture your cardiomyocytes in an Axion multiwell MEA plate [A]. Load this MEA plate into the Maestro MEA system and allow the environmental chamber to automatically equilibrate [B]. Analyze the activity of the cardiomyocytes in the MEA plate label-free and in real-time with AxIS Navigator software [C].

Culture your cells

A

Record with Maestro

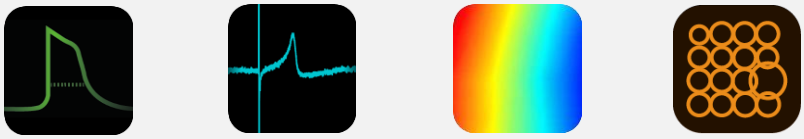
B

Analyze with AxIS Navigator

C

THE MAESTRO ADVANTAGE

- **1 system, 4 assays** – record the four key measures of functional cardiac performance, label-free and in real-time in every well of the multiwell plate: [1] Action Potential; [2] Field Potential; [3] Propagation; and [4] Contractility.



- **Measure what matters** – indirect measures are regularly used to infer cardiac activity. But, for example, calcium imaging is unable to capture important but subtle changes to Na^+ channel functionality, and expression levels of protein markers often poorly correlate with cell model performance. Maestro tracks cardiac activity in real-time, allowing you to answer the questions that matter.
- **Analyze cell activity label-free** – Maestro performs noninvasive electrical measurements from the cultured cardiac population, circumventing the use of dyes/reporters that can perturb your cell model and confound results. Track activity over hours, weeks, or months from the same population of cells.
- **Probe cell models in the same plate they were cultured in** – other higher throughput platforms (e.g. automated patch clamp, flow cytometry) often require cell samples to be transferred into a single-cell suspension before testing. This is far from ideal since [1] the heart exists as a functional network of inter-linked cells, and [2] the cell harvesting process requires numerous handling steps. Maestro captures cardiomyocyte functionality while preserving the morphological complexity of your cardiac cell model.
- **It's easy** – you don't have to be an electrophysiologist to use Maestro. Just culture your cardiomyocytes in an MEA plate, load your plate into the Maestro system, and record your cardiac data. Axion's data analysis tools will then help generate the publication-ready graphs you need.