Characterization of an in vitro synaptic propagation assay using iPSC-derived neurons and multiwell microelectrode array technology

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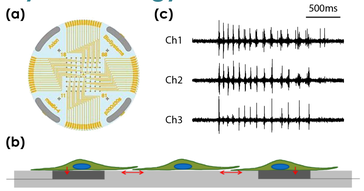
Poster #777

Multiwell MEA Technology

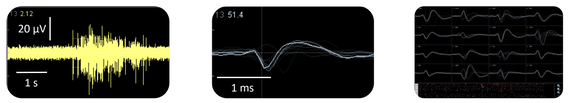
Microelectrode Array Technology

The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced *in vitro* at previously unimaginable scales. Accurate characterization of stem cell-derived neurons requires an assay to provide a functional phenotype. Measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling. The Maestro™ microelectrode array (MEA) platform offers such a solution by providing a label-free, non-invasive bench-top system to simply, rapidly, and accurately record functional activity from a population of cells cultured on an array of extracellular electrodes.



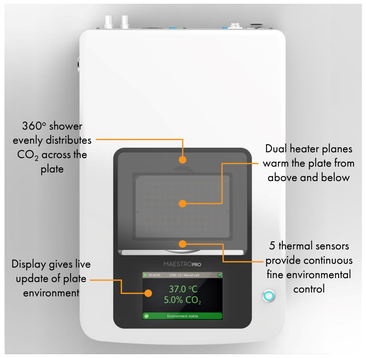
A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.

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.

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 (6-, 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

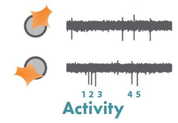
Feature	Maestro Edge	Maestro Pro
Recording Electrodes	384	768
BioCore Chip	6 Chips (v4)	12 Chips (v4)
MEA Plates	6-, 24-Well	6-, 24-, 48-, 96-Well
Integrated Hard Drive	0.5 TB	1.0 TB
Touchscreen	No	Yes
Optical Stimulation	Yes	Yes

Evoked Assay of Synaptic Propagation

Neural Electrophysiology Phenotypes

AxIS Navigator™ control and analysis software provides straightforward reporting of multiple measures of the cell culture maturity:

Mean Firing Rate = # of Spikes / Time

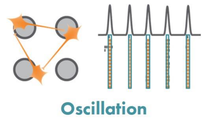


Are my neurons functional?
Action potentials are the defining feature of neuron function. High values indicate the neurons are firing action potentials frequently. Low values indicate the neurons may have impaired electrophysiological function.

Connectivity

Are my synapses functional?
Synapses are functional connections between neurons, such that an action potential from one neuron affects the likelihood of an action potential from another neuron. Synchrony reflects the strength of synaptic connections.

Burst of Action Potentials

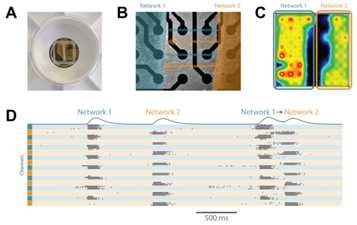


Is my network functional?
Neural oscillations, defined by alternating periods of high and low activity, are a hallmark of functional networks with excitatory and inhibitory neurons. Oscillation is a measure of how the network activity is organized in time.

Compartmentalized Model

Synaptic connections are a fundamental building block of neuronal function, enabling neuronal circuits to process and relay information downstream via action potential propagation. However, traditional in vitro "disease-in-a-dish" neuronal models comprise only a single neuronal circuit, whereas animal models are too costly and complicated to facilitate a screen on compounds or genetic edits that affect synaptic propagation. Previously, we have described the development and characterization of a simple in vitro assay of synaptic propagation between two distinct neural circuits with rodent cortical neurons (see below).

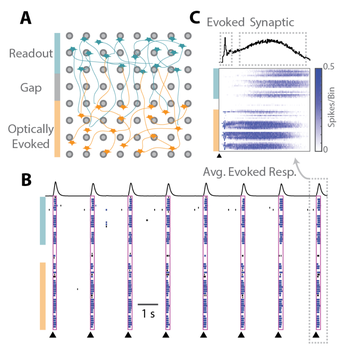
Silicone inserts with two compartments were added to each well of a CytoView MEA 6-well plate. Cortical neurons were seeded into each of the compartments and cultured for 2 days. At 2 days in vitro, the insert was removed, such that axonal projections could cross the cell-free gap and establish functional connections between the two distinct cortical networks as the cells were cultured over time.



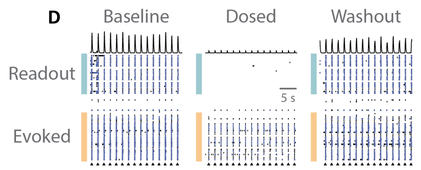
A) Two compartment silicone insert (ibidi). B) Axonal projections cross the cell-free gap within 10 days of removing the insert. C) Activity map illustrating synchronous activity between the two spatially separated networks. D) Raster plot at 17 days *in vitro* illustrating independent network activity from network 1 and network 2, followed by a whole-well network event with network 1 driving the activity in network 2.

Validation of Evoked Synaptic Assay

Following maturation of the functional connection between the two networks, an evoked assay was used to specifically assay the propagation of network activity. In this assay, the "evoked" network (labeled orange below) was either electrically or optogenetically stimulated to trigger the network propagation. So, the activity was initially stimulated in the "evoked" network within each well, and then the activity propagated to the "readout" network (labeled blue below) within each well.



Connection between two rodent cortical networks formed after the well divider was removed. (A) The "evoked" network was stimulated to initiate network activity, and synaptic propagation of electrophysiological activity was observed across the gap to the "readout" network. (B) Raster plot indicates synchronous activity after optical stimulation and (C) the average evoked response denotes an early evoked response followed by a flurry of synaptically-mediated activity. (D) The propagation of network activity was blocked with a CNQX and APV. Washout restored synaptic communication within the "evoked" network and between both cortical networks.

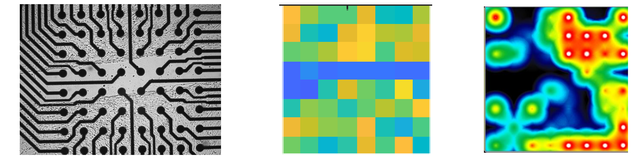


hiPSC-Derived Compartmental Model

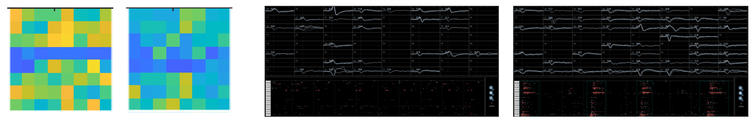
Assay Development with hiPSC-Derived Neurons

Here, we adapted the synaptic propagation model to hiPSC-derived neurons (SynFire MEA Kit, NeuCyte Labs). Excitatory and inhibitory neurons were seeded with astrocytes in each compartment of the silicone insert to produce independent cortical network culture models. The inserts were removed 48 hours after seeding the cells, and the cultures were monitored with hybrid assay measurements: quantifying network function via electrophysiology and culture viability with impedance technology.

Visual Inspection | **Impedance** | **Functional Activity**



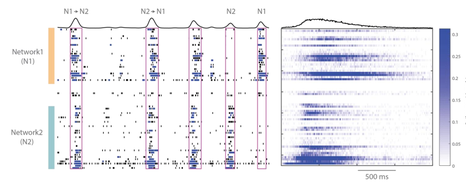
DIV4 | **DIV13** | **DIV21** | **DIV40**

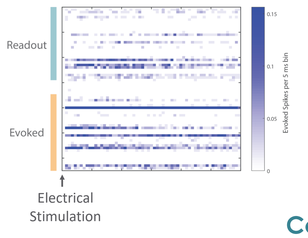


The impedance measurements illustrate how neurons and axons have migrated into the "gap" over time in culture.

Functional activity developed over time in culture, with action potentials and bursts developing first on individual electrodes (DIV21). Eventually network activity developed independently in each network, followed by a functional connection between the networks (DIV40).

By 40 days in culture, the hiPSC-derived neurons had developed significant network activity within and across the individual networks (see right). Networks events occurred individually (N1 or N2) or propagated from one network to another (N1→N2 or N2→N1). The raster plot (far right) of the average network burst indicates that N2 was more dominant in driving propagated network activity.





Electrical stimulation was used to evoke network activity in the "Evoked" network. Functional network activity then occurred reliably, after a propagation delay, in the "Readout" network within the well.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system.
- The Maestro MEA coupled with the ibidi two-compartment insert provides a versatile assay platform for interrogating the connection between two neural networks *in vitro*. The cell types seeded on the MEA, timing of divider removal, and the configuration of the analysis offer flexibility to address a variety of research questions.
- The synaptic assay framework presented here with the Maestro provides a new technique for studying neuro-muscular junctions, developing regenerative therapies aimed at restoring synaptic connections, and modeling neurodegenerative diseases characterized by aberrant or disjointed synaptic activity.