>> Characterization of an integrated platform using sensory neurons as bio-digital sensors for PNS applications

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AXION BIOSYSTEMS
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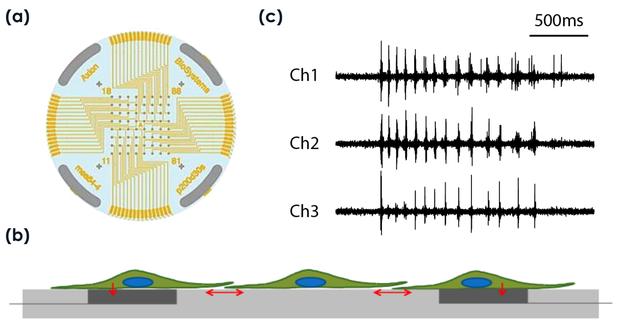
Multiwell MEA Technology

Microelectrode Array Technology

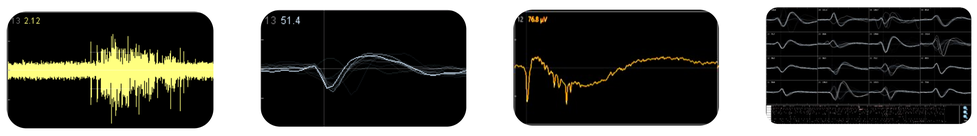
The flexibility and accessibility of induced pluripotent stem cell (iPSC) technology has allowed complex human biology to be reproduced in vitro at previously unimaginable scales. Accurate characterization of stem cell-derived neurons requires an assay to provide a functional phenotype. Measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling.

Axion BioSystems' Maestro™ multiwell 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 in each well.



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.

MEA Neural Signals



Raw Voltage | *Extracellular Action Potentials* | *Local Field 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 models, and stem cell characterization

The Maestro MEA Product Family

Features	*Maestro Pro*	*Maestro Edge*	*Maestro Volt**
Throughput (well format)	6, 24, 48, 96, 384**	6, 24, 96**	6
MEA Mode	✓	✓	✓
MEA Viability	✓	✓	
Impedance Mode	✓	✓	
Environmental Control	✓	✓	✓
Automation API	✓	✓	
Stimulation	Electrical & Optical	Electrical & Optical	Electrical
Omni Compatible	✓	✓	✓

The Maestro Pro™ (left), Maestro Edge™ (middle), and Maestro Volt™ (right) offer the latest MEA technology for optimal neural and cardiac data acquisition.

The NeuroFluidics™ MEA Platform

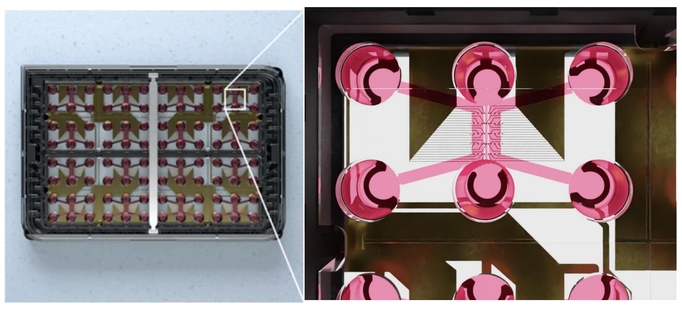
In this study, we utilized the NeoBento™ HTS format outfitted with 16 DuaLink MEA chips, that combine NETRI's microfluidic architectures with an Axion Biosystems' bespoke multi-electrode array (MEA) surface.

This platform allows for simultaneous recording from 16 DuaLink MEA chips containing 42 electrodes each, distributed among compartmentalized chambers and microchannels.

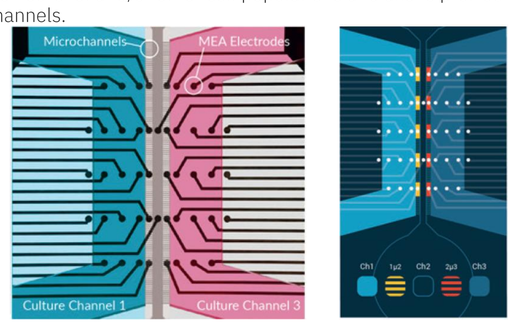
Neurons as Bio-digital Sensors

DuaLink™ MEA

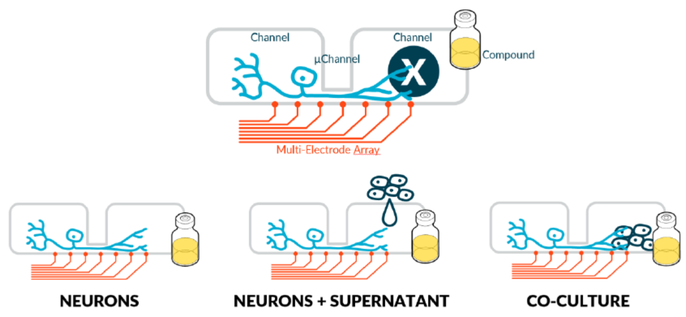
DuaLink MEA plates consist of 16 microfluidic chips, each chip containing three compartments connected via microchannel tunnels. Microchannels enable axonal connectivity between compartments while maintaining fluidic isolation.



Each chip contains 42 electrodes distributed across all three compartments and connecting microchannels. This arrangement allows for simultaneous recording of separate neuronal and/or other cell populations and axons protruding through microchannels.



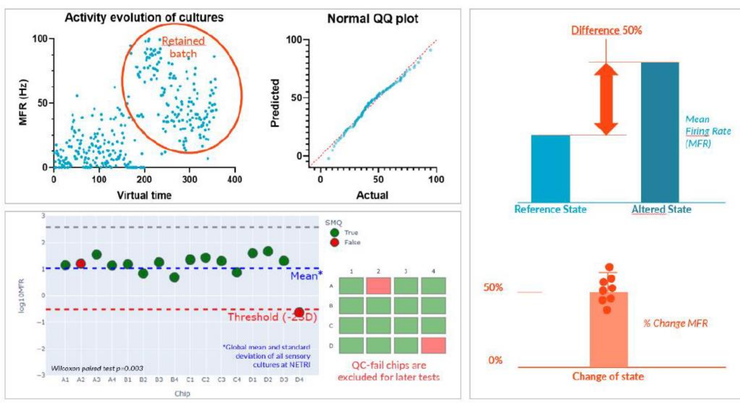
Neurons as a sensor approach



Neurons process their inputs through firing or not firing, which can be equated to producing zeros and ones. Since every single organ in the body is innervated, the peripheral nervous system (PNS) can essentially by seen as a distributed network of bio-digital sensors. By cultivating and fluidically isolating human induced pluripotent stem cell-derived (hiPSC) sensory neuron somas from their endings and stimulating the latter with reference compounds, we can repeatably record electrophysiological responses in a geometrical configuration that models human physiology. This can be used to screen novel compounds in wide ranging PNS applications ranging from dermato-cosmetics to pain management. The platform also allows for compartmentalized cultures of neuronal subtypes and other tissues, which can be assessed independently via electrodes in individual channels. Finally, multi-dimensional metric analysis can pave the way for compound fingerprinting through digital signatures and the emergence of digital libraries for advanced screening based on functional outcomes.

Sensor Repeatability

Reliability and reproducibility is critical to the development of a good sensor. First, we performed supplier batch selection and characterization with axoCells™ Sensory Neurons. We then adapted a single standard culture protocol for DuaLink™ MEA chips to be used across all experimentations. Sensory neurons were seeded only in Channel 1 (Ch1) of the coated DuaLink™MEA (PDL + Surebond-XF) and cultured over 20 days. A standardized quality control (QC) method was applied to each culture based on a nominal basal MEA recording to exclude outliers (based on Negri et al. ENEURO.0080-19.2019 paper). Finally, since MEA recording is non-invasive, this protocol measures the relative change within a single chip, between a reference state (basal activity) and an altered state (added compound). This self-referencing approach mitigates potential culture variability remaining after QC and allows statistical analysis.

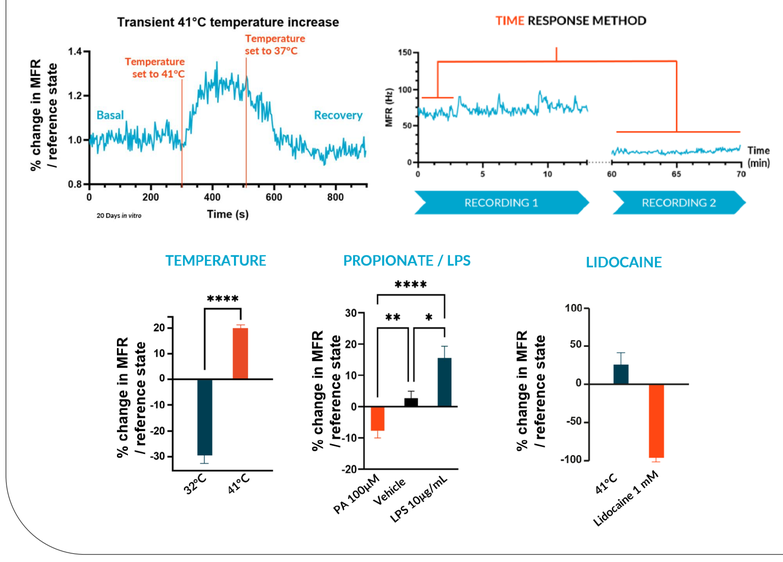


Sensor Sensitivity

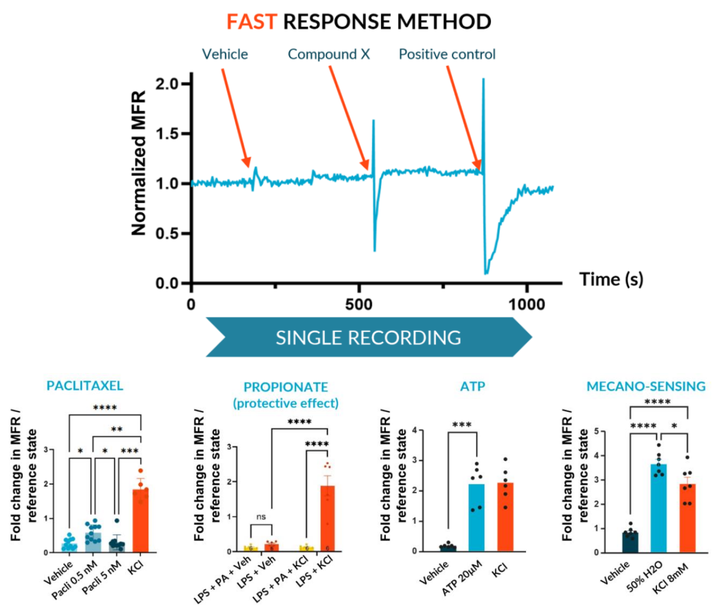
Time Response

Sensor sensitivity is key to assess the ability of the sensor to react different stimuli, and to do so gradually without cut-off thresholds (within a range). We have assessed the sensitivity of our neuron sensors through a variety of reference compounds, using two different methods: Time Response and Fast Response. The choice of method is dictated by each context of use.

The Time Response method, which is suitable when looking at a chronic, or at least long lasting, effect, records activity over two consecutive periods: reference state period and a subsequent altered state period where a compound was added. We subjected our cultures to standard stimuli in Channel 3 (Ch3) that span a range of applications and showed a statistically significant response in each case.



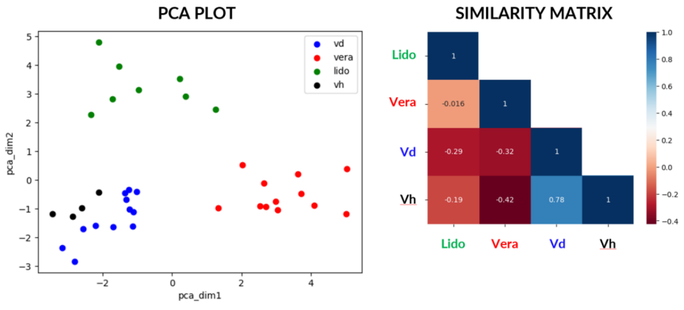
Fast Response



The Fast Response method, suitable for transient effects, records real-time activity for a single period for altered state (a compound) and reference states baselines (vehicle and positive control). We subjected our cultures to standard stimuli in Channel 3 that span a range of applications and showed a statistically significant response in each case. Sensor sensitivity is particularly evident for sensory neurons by recording activity in the microchannels that connect cell/tissue compartments.

Sensor Specificity

Sensitivity is limited in its ability to discriminate between altered states. We can therefore leverage the full suite of metrics that Maestro MEA offers to distinguish seemingly alike signals from each other and confer specificity to our sensors. We conducted a preliminary test using two reference compounds, veratridine and lidocaine, and their corresponding vehicles, DMSO (Vd) and H2O (Vh), added to neurites in Ch3 only. Of all MEA metrics, we isolated the ones that have a Pearson correlation score M<0.8, among which we select the ones that have the highest Information gain for a similarity matrix or ran a PCA for plotting. We also isolated the microchannels and managed to discern meaningful differences between all the states, including vehicles.



Conclusions

- The Maestro MEA platform combined with NeuroFluidics MEA represents a powerful platform to evaluate complex, fluidically isolated neural cultures.
- Neurons can be used in this platform as sensors for a variety of applications, such as drug development and toxicity assessment.
- Sensitivity and specificity measurements can be useful context-dependent readouts for various applications, with multiparametric analysis providing deep insight into compound effects.