Characterization of Local Field Potential Activity in a Cell-based Neuronal Assay for Neurotoxicity and Disease Modeling

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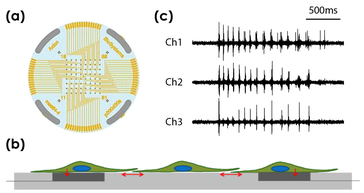
Multiwell MEA Technology

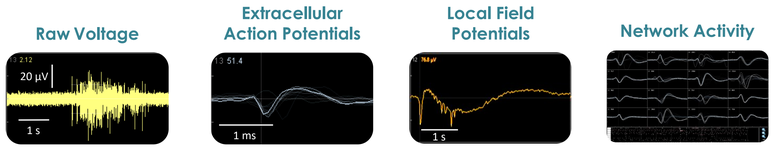
Microelectrode Array Technology

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.

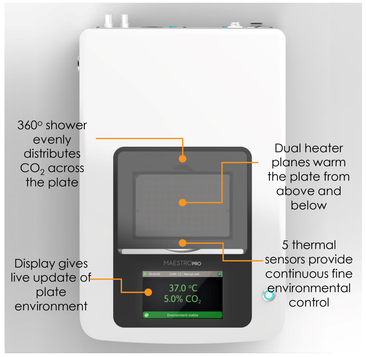


A planar grid of microelectrodes (a) interfaces with cultured neurons (b), modeling complex, human systems over an electrode array. Electrodes detect changes in raw voltage (c) through recording of extracellular field potential.



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

Introducing the Maestro Pro™ and Maestro Edge™



The Maestro Pro™ (left) and Maestro Edge™ (right) offer the latest MEA technology for optimal data

- **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

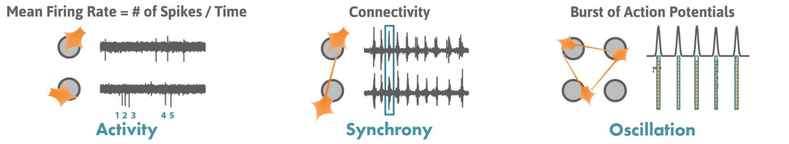
BioCore v4

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

MEA Assay with Neurons

Functional Neuronal Phenotypes

AxIS Navigator analysis software provides straightforward reporting of multiple measures of cell culture maturity.

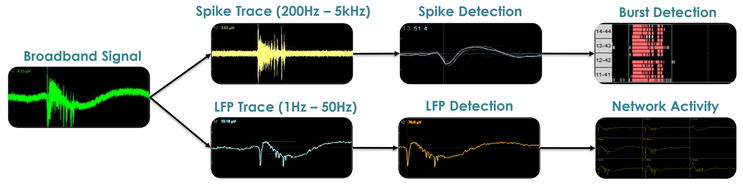


Are my neurons functional?
Action potentials are the defining feature of neuron function. High values indicate neurons are firing action potentials frequently. Low values indicate neurons may have impaired electrophysiological function.

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.

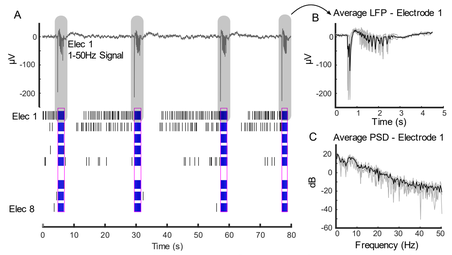
Is my network functional?
Neuronal oscillations, or alternating periods of high and low activity, are a hallmark of functional networks with balanced excitatory and inhibitory neurons. Oscillation is a measure of how the network activity is organized in time.

Neural Broadband Signals



Electrodes measure broadband (1-5000 Hz) local field potentials, which can be filtered into high frequency spike traces and low frequency LFP traces for feature detection via thresholding and statistical algorithms. Spikes, bursts, and LFPs can be detected and analyzed using AxIS Navigator and the Neural Metric Tool.

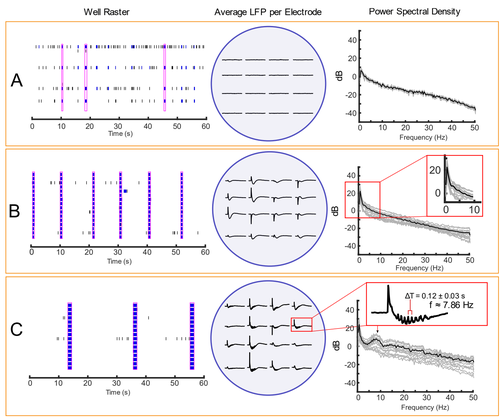
Network Burst Triggered Collection of Local Field Potentials



In mature, well-connected neuronal cultures, the burst structure is often organized into time periods of silence punctuated by strong network-wide activity called network bursts. The Neural Metric Tool can isolate low frequency activity near these bursts for analysis in the time or frequency domains.

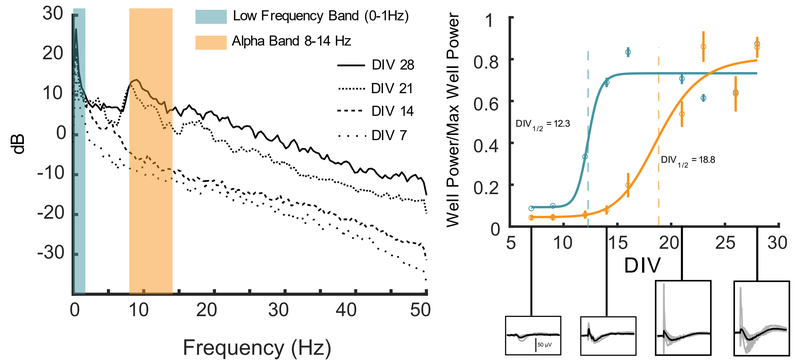
Power Spectral Density as a Sign of Bursting Complexity

The frequency domain can be a useful tool in characterizing LFP signals obtained near network bursts. Since these signals are believed to be the summed effects of post synaptic potentials, they may provide insight into the balance of excitation and inhibition. Neural firing with weak synchrony and poorly organized bursting results in very little deviation in LFP signal, corresponding to a flat power spectrum (A). More organized activity will have large voltage deflections in LFP, producing a peak at low frequencies in the power spectrum (B). More complex network bursting with episodic spiking can produce additional peaks in the power spectrum at relevant rhythms(C).



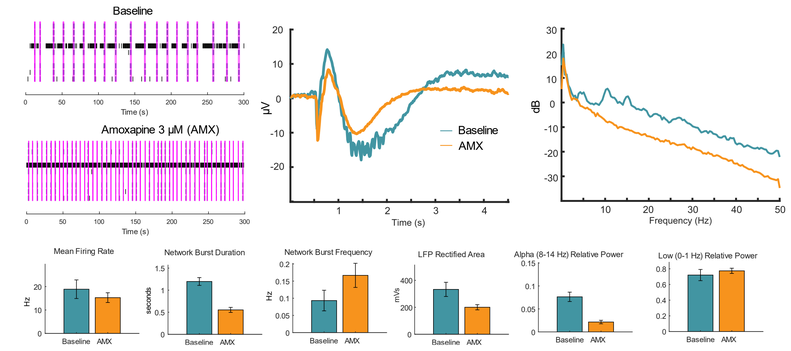
Using LFP Signals

Maturation of Rodent Cortical Neuron Networks



The power spectral density can be used to compute the amount of power present in defined frequency ranges, or bands. Rodent cortical neurons *in vitro* display an increase in power in all bands when maturing over 4 weeks. At DIV 7, LFPs are small deflections in voltage, and have a relatively smooth PSD. Around DIV 12, LFPs are much larger and contain more low frequency content. Around DIV 19, burst complexity begins increasing. There is more variability in LFPs detected between electrodes in the well, and ripples in the LFP begin to manifest. Also, as neural activity increased, the power at higher frequencies increased and peak appeared in the range of frequencies often referred to as the alpha band (8-14Hz) in field potential and EEG studies.

Pharmacological Modulation of LFP Dynamics



Just as pharmacology can affect spiking and bursting patterns, drugs can have drastic effects on the spectral content of network burst detected LFPs. Here the tricyclic antidepressant Amoxapine was applied to DIV 28 rodent cortical neurons in culture. At baseline, complex bursting activity is present with ripples in the average LFP and spectral peaks in the power density. While mean firing rate of the neurons is relatively similar after dosing, network bursts become shorter and more frequent, while LFPs become shorter and smaller. Peaks in the Alpha Band spectrum are also eliminated.

Conclusions

- The Maestro multiwell MEA platform enables functional characterization of neural cell culture activity with a flexible, easy-to-use benchtop system.
- In addition to recording data for spike train and burst analysis, the Maestro can now simultaneously record low frequency signals and detect local field potential events.
- Low frequency signals and power spectrums of local field potentials can be indicators of complex interactions in *in vitro* neural models. As research continues with *in vitro* models of disease, they may begin to recapitulate low frequency phenomena similar to local field potential rhythms measured *in vivo* and in EEG/ECOG studies. The Maestro and Axion software tools provide methods for easily exploring and analyzing low frequency activity in high-throughput, label-free testing environments.