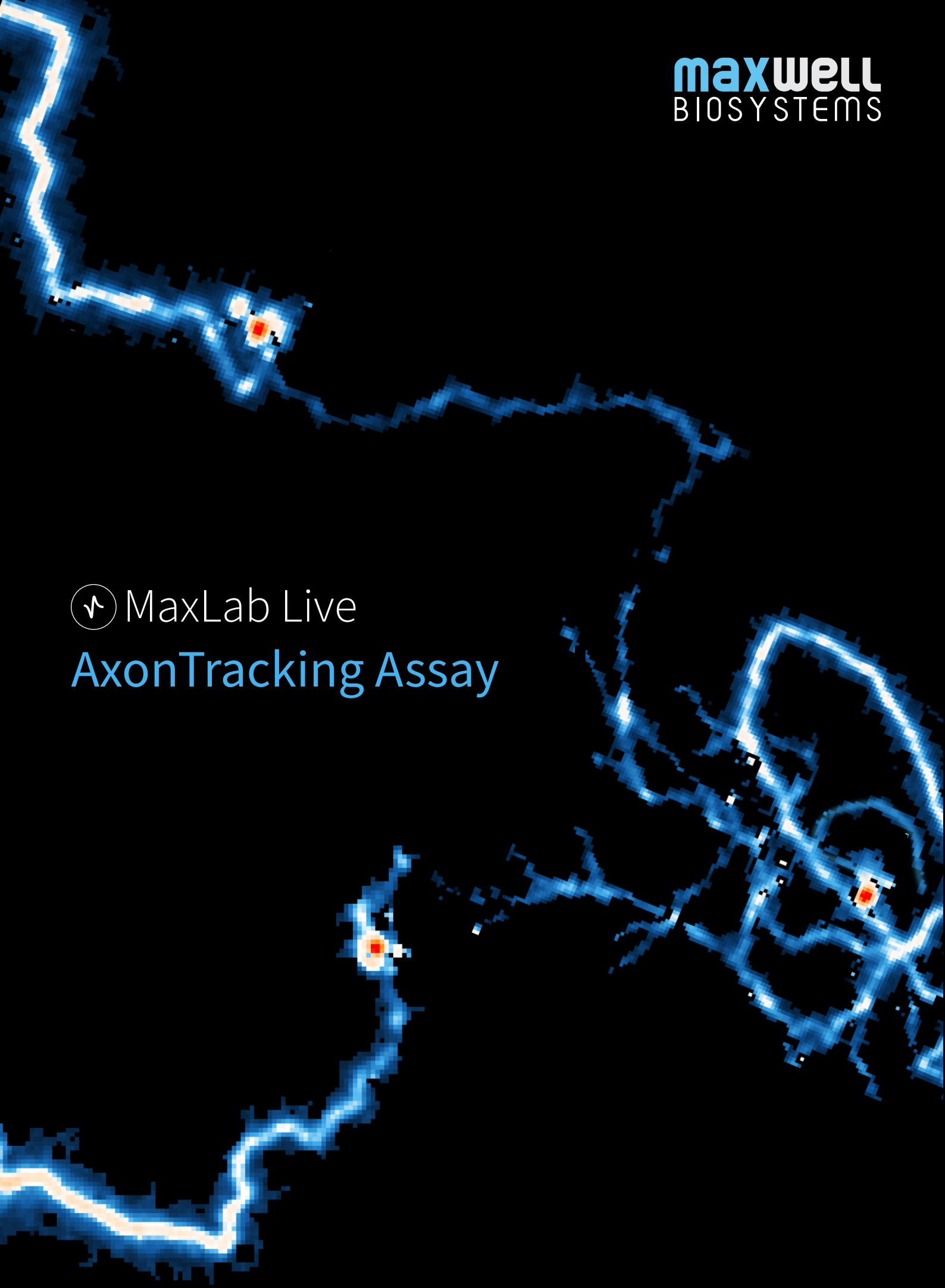
maxwell BIOSYSTEMS



MaxLab Live

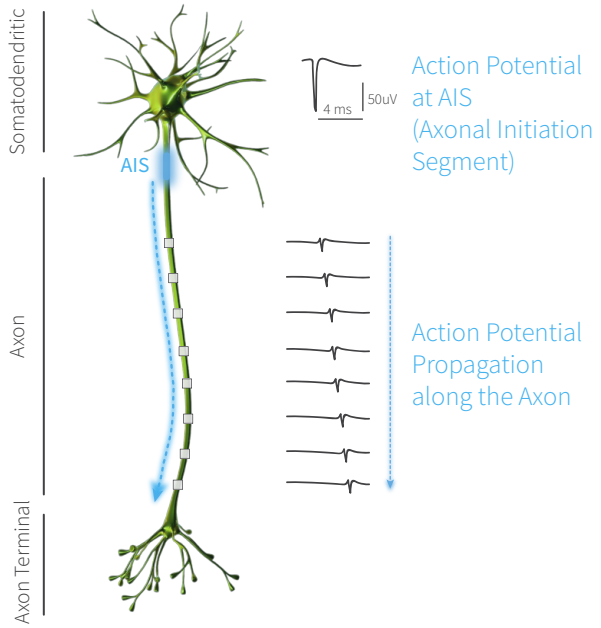
AxonTracking Assay

MaxLab Live
AxonTracking Assay

A Novel Approach

Neurons communicate within a network via **action potentials (APs)** propagating along axons. The capability to access axonal physiology is crucial for studying information processing among neurons in healthy and diseased states. However, axonal signals are difficult to measure at a large-scale. Therefore, the combination of reliability, ease of use, throughput, long-term and non-invasive measurement are necessary to monitor and understand neuronal function at a scale that was previously not possible.

High-density microelectrode array (HD-MEA) measurements at unprecedented resolution and high signal quality, using **MaxOne** and **MaxTwo** systems, allow to detect the AP propagation from the initiation site down to distal axonal branches. With the AxonTracking Assay, the identification of the axonal paths is fully automated at the micrometer scale. This live-cell recording and analysis provides novel functional and structural readouts applicable for phenotypic characterization, disease modeling, and drug screening studies.



Automated

The fully automated platform is easy to use and allows for simultaneous recordings of multiple neurons and axonal branches in multiple wells.

Long-Term

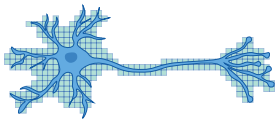
Characterize neuronal maturation, development or treatment effects by recording from your culture over multiple days and weeks.

Label-Free

The electrical recordings are non-invasive and label-free, which avoids introducing side effects from dyes etc.

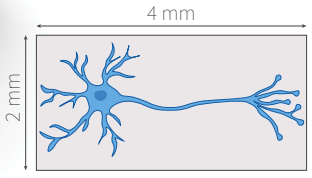
HD-MEA Technology for Recording Axonal Signals

Powered by MaxOne and MaxTwo. Key advantages:



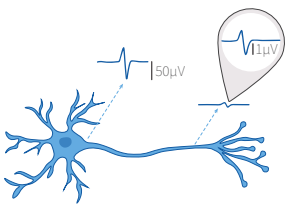
High Spatio-Temporal Resolution

Reconstruct axonal paths by tracking AP propagation at thousands of sites, thanks to the densely packed microelectrode array.



Large Sensor Area

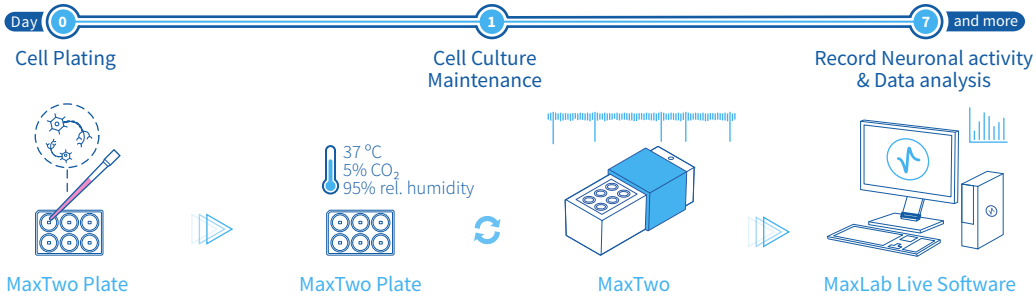
Detect long axonal branches of multiple neurons at the same time with a large sensor area, applicable for 2D and 3D samples.



High Signal Quality

Catch the smallest signals propagating along axons, down to single microvolts-range, with low-noise recording channels.

Experimental Workflow

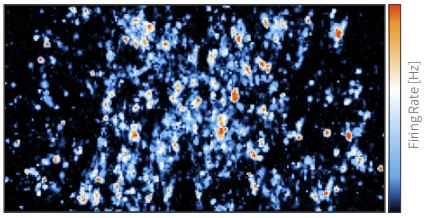


Methodology

Recording

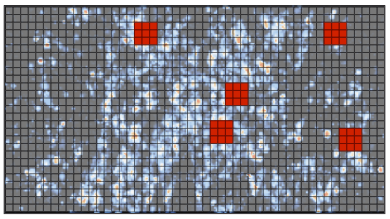
1 ActivityScan

Start with an ActivityScan Assay to locate the positions of the active neurons on the electrode array.



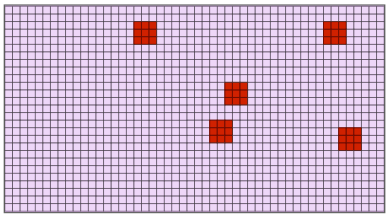
2 Target Units

Select target neuronal units to be measured and reconstructed.



3 AxonTracking Recording

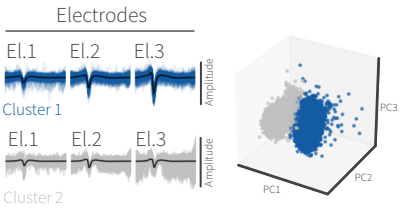
Scan the entire area of interest to resolve the axons of target neuronal units.



Analysis

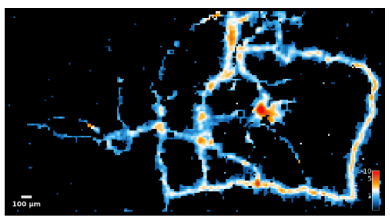
4 Spike Sorting

Classify detected spikes by the waveforms to identify spikes originating from the same neuronal unit.



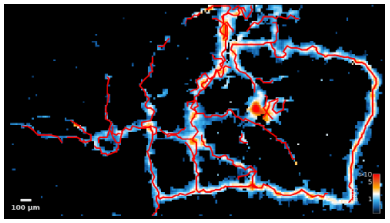
5 Footprint Extraction

Reconstruct the average extracellular profile across the electrode array for every identified neuron using the sorted spikes.



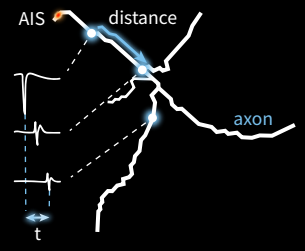
6 Axon Analysis

Identify individual axonal branches and reconstruct the morphology of the neurite outgrowth using an unsupervised object-tracking algorithm.

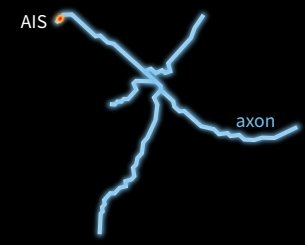


Metrics

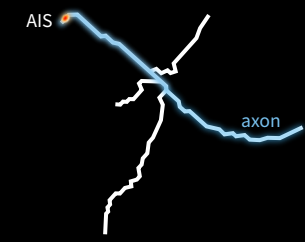
Neuron Conduction Velocity



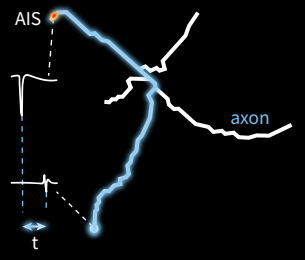
Total Axon Length



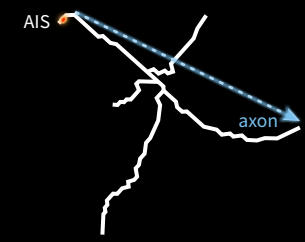
Longest Branch Length



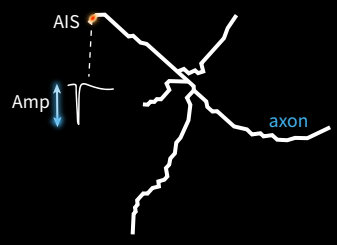
Longest Latency



Longest Distance from AIS



Amplitude at AIS

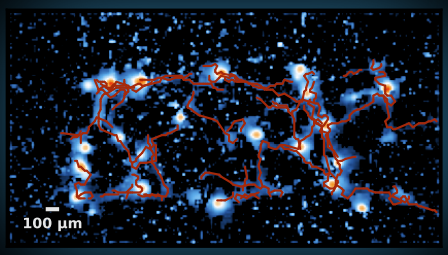


Results

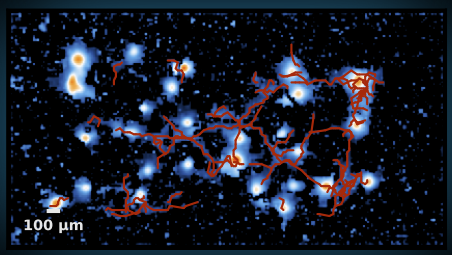
AxonTracking Assay in Human Neurons

Propagating APs along axonal processes recorded from different human induced pluripotent stem cells-derived (iPSC-derived) neuronal cell lines.

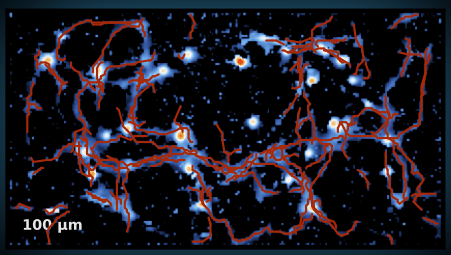
Human Glutamatergic Neurons



Human Dopaminergic Neurons



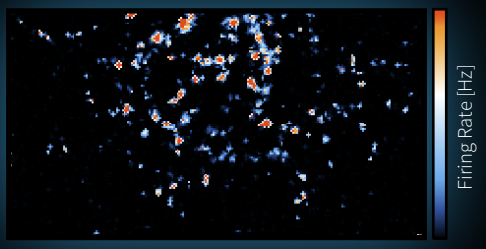
Human Motor Neurons



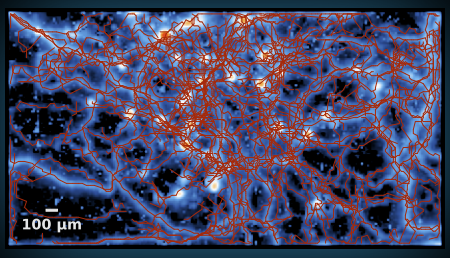
Axon Tracking Assay in Long-Term Neuronal Cultures

Neurons and propagating APs can be resolved even in cultures with long and dense axonal processes (iPSC-derived glutamatergic neurons, DIV 63).

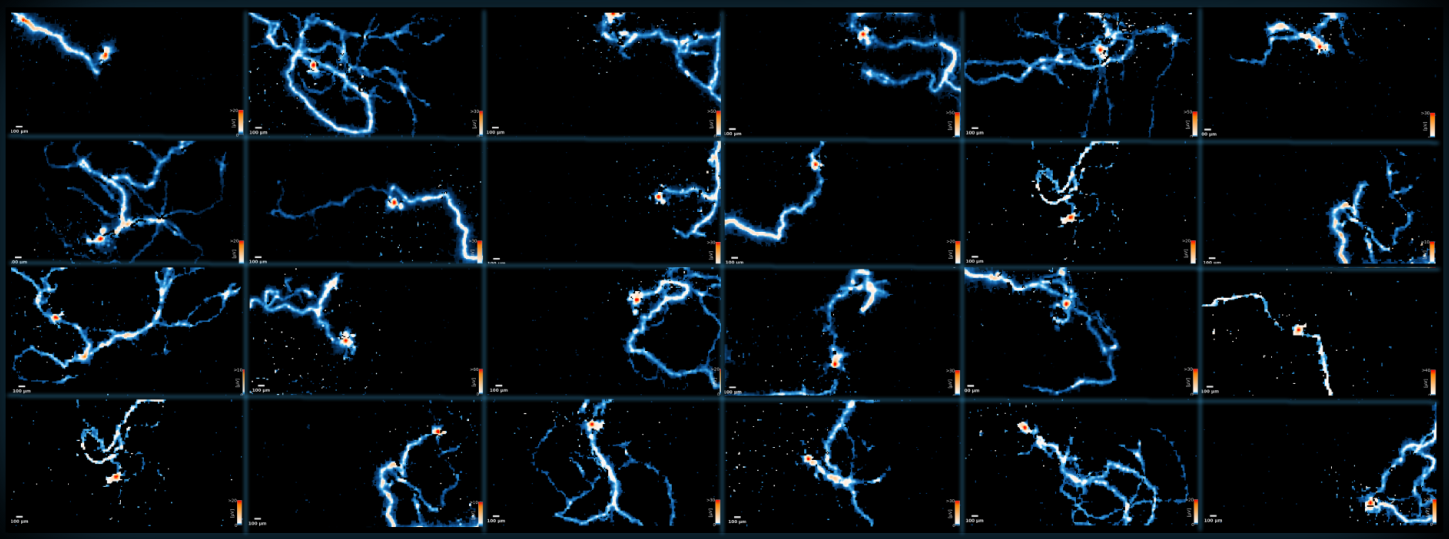
Activity map



AxonTracking Assay

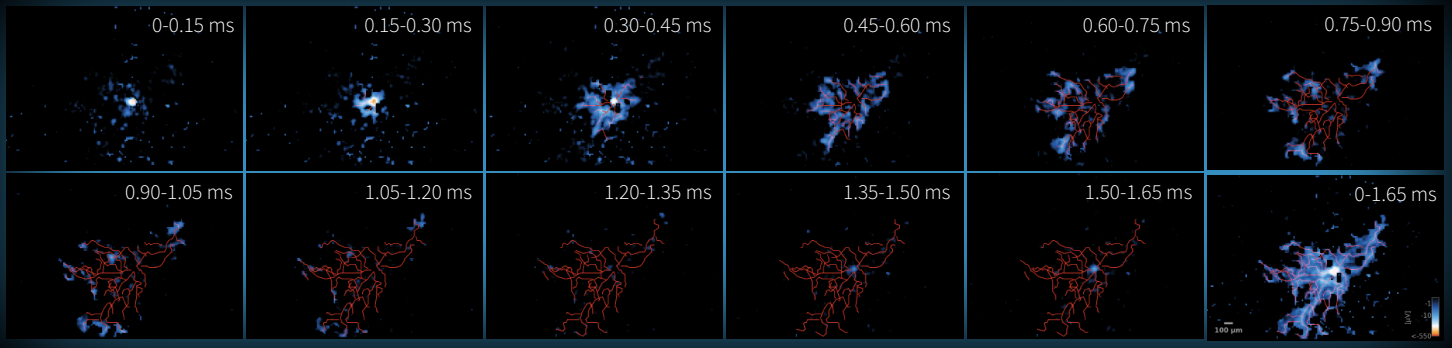


Propagating AP of individual neurons resolved:



AP Propagation Along Axons

Propagating APs along the axonal processes of a rat primary cortical neuron shown in a time-series.



Case Study

Electrophysiological Characterization of Neurons Modeling Neurological Diseases using High-Density Microelectrode Arrays

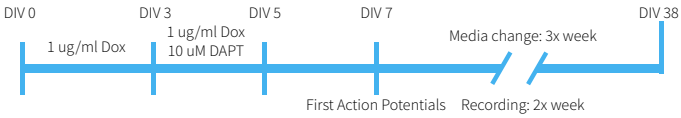
ioGlutamatergic neurons

ioGlutamatergic neurons (hGN) are human induced-pluripotent-stem-cell(iPSC)-derived glutamatergic neurons (bit-bio,UK), obtained by direct reprogramming using the opti-ox™1 procedure [1]. The opti-ox™1 procedure makes use of doxycycline to induce NGN2 gene overexpression. Disease lines are engineered using CRISPR/Cas9 genome editing.

ioGlutamatergic modeling Huntington's Disease

- hGN-HD carry an insertion of 50CAG repeats in the HTT gene
- One heterozygous clone was tested

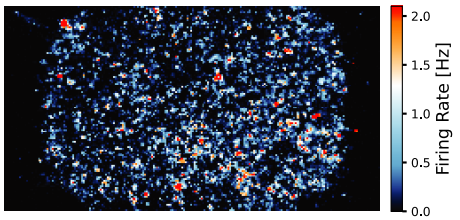
Protocol



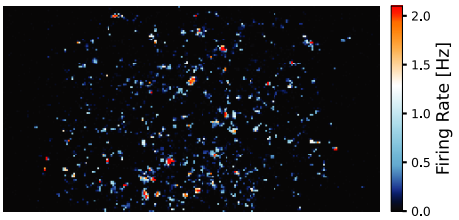
200k ioGlutamatergic neurons (bit.bio, UK); 40k iCell Astrocytes (Fujifilm Cellular Dynamics, Inc., USA)

ActivityScan Assay

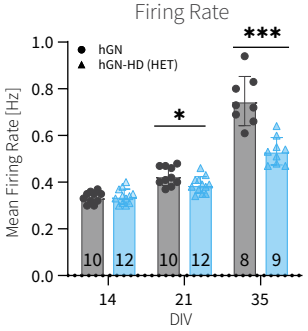
ioGlutamatergic Neurons
hGN - DIV 35



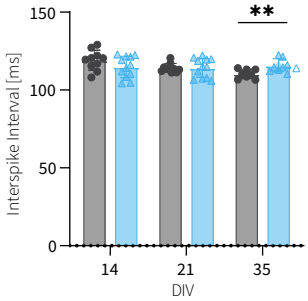
ioGlutamatergic Neurons Huntington's Disease
hGN-HD (HET) - DIV 35



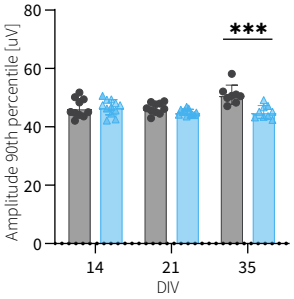
Firing Rate



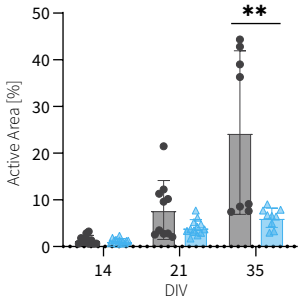
Inter-Spike Interval



90th Percentile Amplitude

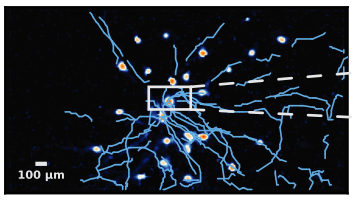


Active Area

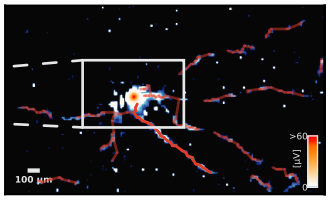


AxonTracking Assay

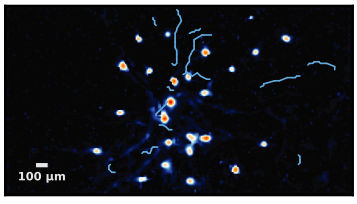
hGN - Multiple Neuronal Units



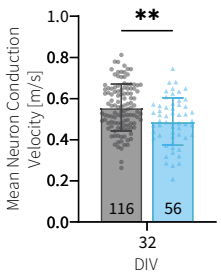
hGN - Single Neuronal Unit



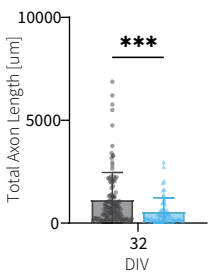
hGN-HD (HET) - Multiple Neuronal Units



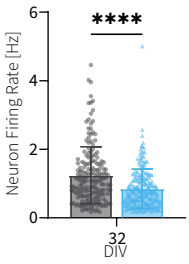
Conduction Velocity



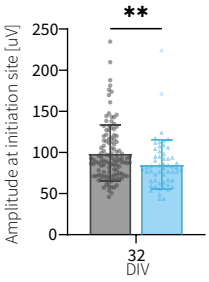
Total Axon Length



Neuron Firing Rate



Amplitude at Initiation Site



Statistics used: Kurskal-Wallis test with Dunn's correction

Data was recorded at Early Discovery at Charles River Laboratories, United Kingdom.

Conclusions

The disease line hGN-HD (HET) showed slower maturation compared to the control hGN. Highly reproducible differences in actitvity and axonal maturation were obtained comparing the disease line to the control.

References

[1] Pawlowski, M., Ortmann, D., Bertero, A., Tavares, J. M., Pedersen, R. A., Vallier, L., & Kotter, M. R. "Inducible and deterministic forward programming of human pluripotent stem cells into neurons, skeletal myocytes, and oligodendrocytes." Stem cell reports, 8(4), 803-812 (2017).

MaxWell Biosystems AG
@mxwbio
Switzerland

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