Utilizing HD-MEA to Interrogate the Diverse Functionality of Human iPSC-derived Neurons

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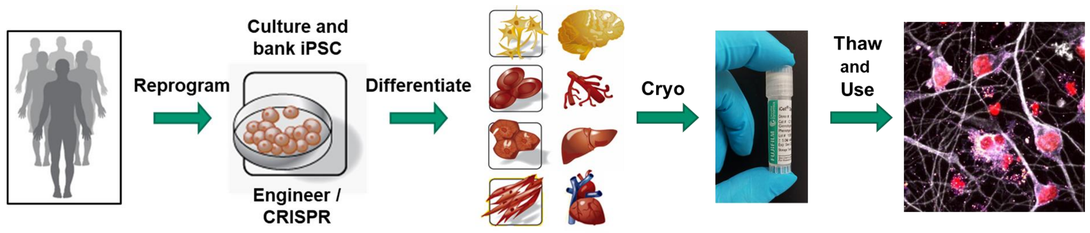
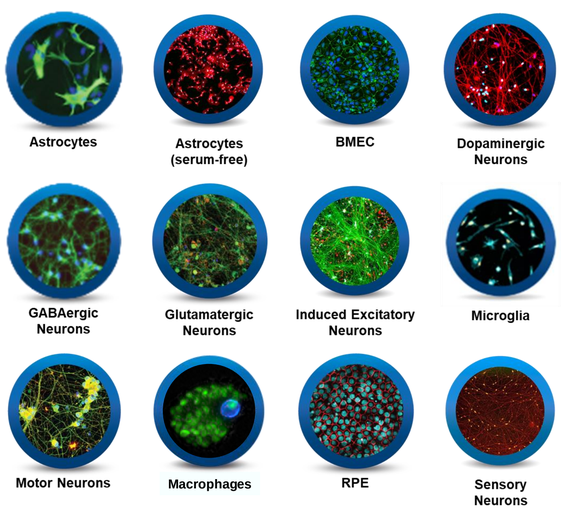
Overview

FUJIFILM Cellular Dynamics, Inc. (FCDI) is the global leader in large-scale manufacturing of specialized cell types derived from human induced pluripotent stem cells (iPSC) for use in basic research, safety/toxicity testing, and drug discovery applications. FCDI offers >20 terminally differentiated cell types as catalog products with a heavy emphasis on "iCell®" neurons, astrocytes, and microglia.



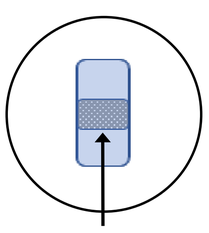
To study the function of human iPSC-derived neurons, electrophysiology techniques are essential. While patch clamp recordings are the gold standard for investigating ion channel behavior of electrically active cells, the experimental procedures are time consuming and can require great skill to perform. Instead, the high-density micro-electrode array (HD-MEA) MaxTwo system and 24-well plates from Maxwell Biosystems are uniquely suited to investigate the range of different cell types available in the FCDI catalog. In this poster, we will present example data from 2D mono- and co-culture of neurons and astrocytes, disease modeling for Parkinson's Disease and ALS cells, and even recordings from 3D spheroid cultures. The HD-MEA data generated here highlights how neuronal spike activity and network bursting can be impacted by the type of neurons tested, composition of the culture (numbers and ratios of cells), media and supplements used, disease genotype, and 2D vs 3D co-cultures.

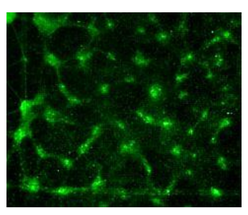


Materials and Methods


MaxTwo HD-MEA System


24w HD-MEA plate


Whole well area


Live-cell Imaging

HD-MEA Preparation
- Coat "whole-well" area of sterile 24w HD-MEA with 50 µL of 0.1% PEI or 50 ug/mL PDL for 1 h at 37 °C.
- Rinse wells 3 times with sterile water and dry in BSC for 1 hour.
- Coat whole-well area with 50 µL of Laminin solution for 1 h at 37 °C.

Cell Plating
- Thaw iCell "Neurons" and iCell Astrocytes 1.0 or 2.0 (C1037 or C1249) according to the User's Guides.
- Mix iCell "Neurons" with astrocytes at a density of 8-10 million cells/mL.
- Dispense 50 µL of cell suspension (400-500,000 total cells) per well.
- Culture cells in BrainPhys™ medium supplemented with M1029 or M1032, M1031, & N2 with 1% Laminin.

Cell Culture Maintenance
- Culture cells in 0.6 mL of Complete BrainPhys medium (1.2 mL for 6w HD-MEA plate).
- Perform 50% media change every 2-3 days.
- Cover 24w HD-MEA plate with Breathe-Easy® sealing membrane while in incubator.
- Image live cells on HD-MEA plate with NeuroFluor™ NeuO probe (STEMCELL Technologies #01801).

HD-MEA Recordings
- Record neuronal electrophysiology on a MaxTwo HD-MEA system.
- Acquire, process, analyze co-culture data with MaxLab Live Software.

HD-MEA Analysis of iCell Induced Excitatory Neurons

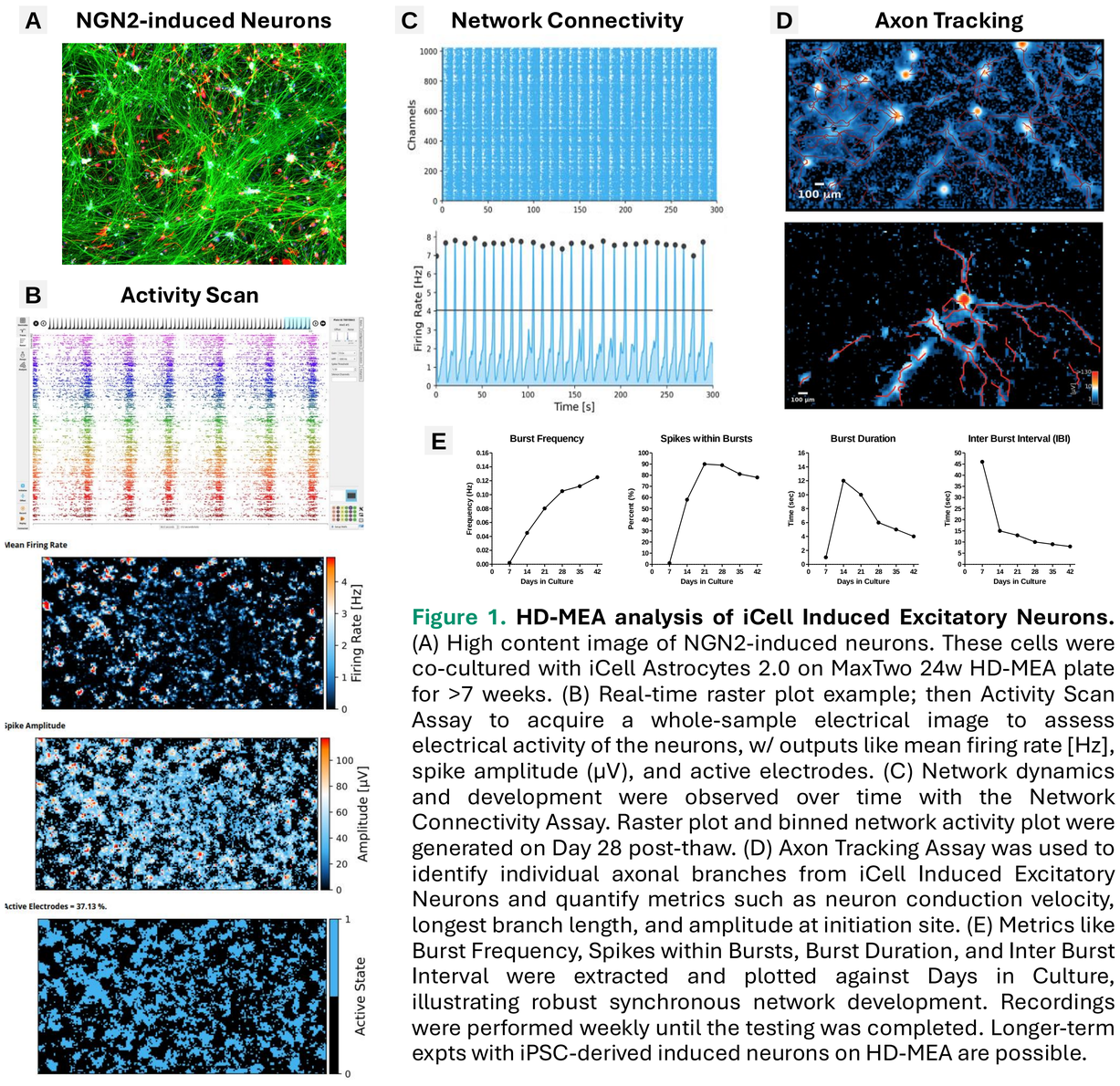


Figure 1. HD-MEA analysis of iCell Induced Excitatory Neurons. (A) High content image of NGN2-induced neurons. These cells were co-cultured with iCell Astrocytes 2.0 on MaxTwo 24w HD-MEA plate for >7 weeks. (B) Real-time raster plot example; then Activity Scan Assay to acquire a whole-sample electrical image to assess electrical activity of the neurons, w/ outputs like mean firing rate [Hz], spike amplitude (µV), and active electrodes. (C) Network dynamics and development were observed over time with the Network Connectivity Assay. Raster plot and binned network activity plot were generated on Day 28 post-thaw. (D) Axon Tracking Assay was used to identify individual axonal branches from iCell Induced Excitatory Neurons and quantify metrics such as neuron conduction velocity, longest branch length, and amplitude at initiation site. (E) Metrics like Burst Frequency, Spikes within Bursts, Burst Duration, and Inter Burst Interval were extracted and plotted against Days in Culture, illustrating robust synchronous network development. Recordings were performed weekly until the testing was completed. Longer-term expts with iPSC-derived induced neurons on HD-MEA are possible.

ALS Disease Modeling with iCell Motor Neurons

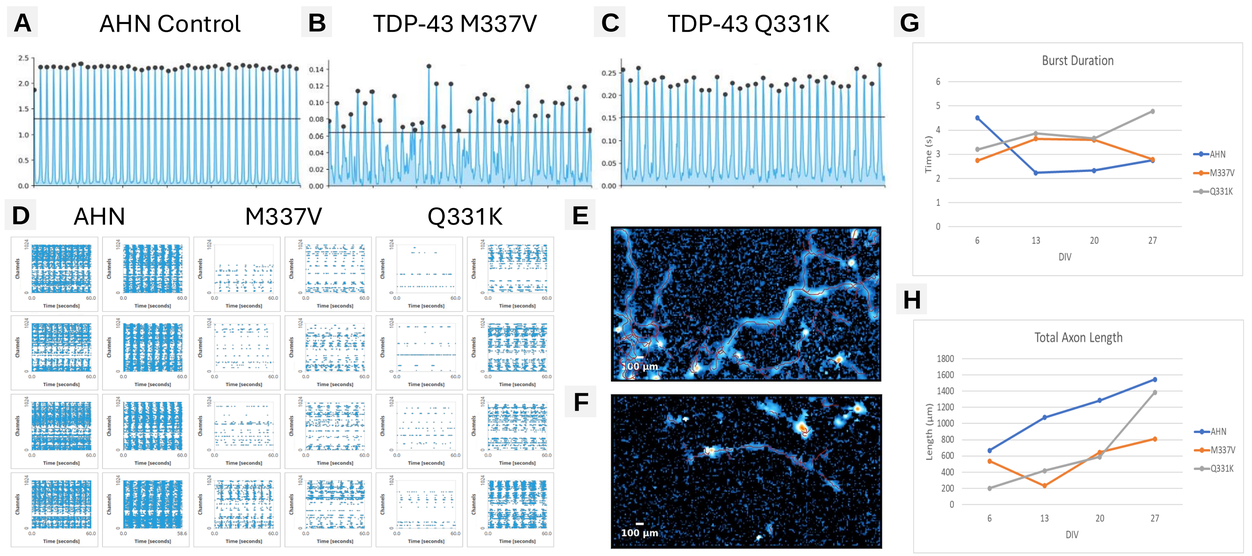


Figure 2. ALS Disease Modeling with iCell Motor Neurons. Representative Network Activity plots recorded on DIV 21 from (A) Apparently Healthy Normal (AHN) iCell Motor Neurons, 01279 (C1048) and iPSC-derived motor neurons with mutations in TAR DNA-binding protein 43 (TDP-43) at (B) M337V (C1162) or (C) Q331K (C1161). All neurons were co-cultured with iCell Astrocytes 2.0. (D) Scatter Plots of all wells from the 24w plate highlights well-to-well consistency (conditions grouped column-wise). Axon Tracing examples from (E) AHN control vs. (F) TDP-43 M337V ALS disease model. HD-MEA data was analyzed over time (4 weeks in culture) to show changes in (G) Burst Duration or (H) Total Axon Length. Healthy motor neurons maintained a network bursting phenotype with a regular but shorter burst duration and an overall longer total axon length as compared to both TDP-43 mutant lines.

Parkinson's Disease (PD) Modeling with iCell DopaNeurons

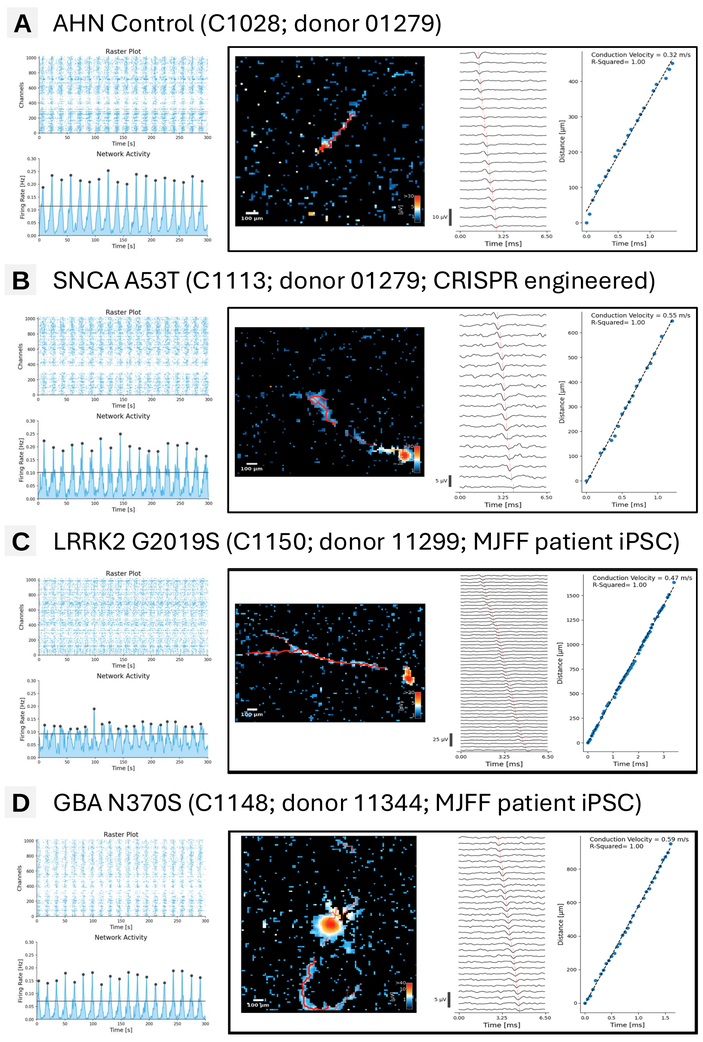


Figure 3. Human iPSC-based PD Modeling. In partnership with the Parkinson's Progression Markers Initiative (PPMI) and The Michael J. Fox Foundation (MJFF), FUJIFILM CDI generated iPSC lines from clinically symptomatic PD patients carrying known risk-associated gene mutations. CRISPR engineering is a strategy to create disease models, as well. The resulting iPSC-derived dopaminergic midbrain neurons are highly pure cell populations that were co-cultured with iCell Astrocytes 2.0 on 24w HD-MEA plates. Raster plots and network activity plots (DIV 14) and branch level plots (DIV 28) from four different PD model iPSC lines are shown: (A) AHN control vs. (B) alpha-synuclein (SNCA) A53T, (C) leucine-rich repeat kinase 2 (LRRK2) G2019S, and (D) glucocerebrosidase (GBA) N370S mutations. Not only are there differences observed in spike rate and network activity, but HD-MEA enables in-depth analysis of action potential propagation and axonal conduction velocity across the panel of cells. MaxLab Live software tracked the branching of neurons and calculated peak time differences & cumulative distances across electrodes to estimate velocities with high linear regressions $r^2 \approx 1$. Rank order of Vel. (m/s) for the samples: GBA (0.59), SNCA (0.55), LRRK2 (0.47), and AHN (0.32). Previously published data <Ronchi *et al.* 2021> using iCell DopaNeurons (AHN vs. SNCA) on HD-MEA obtained similar results. This platform provides accurate readouts at the single-cell and sub-cellular level, so when combined with disease-relevant iPSC-derived cell types it is an extremely helpful tool for the assessment of neurological disorders.

Assessment of 3D spheroids/organoids on HD-MEA

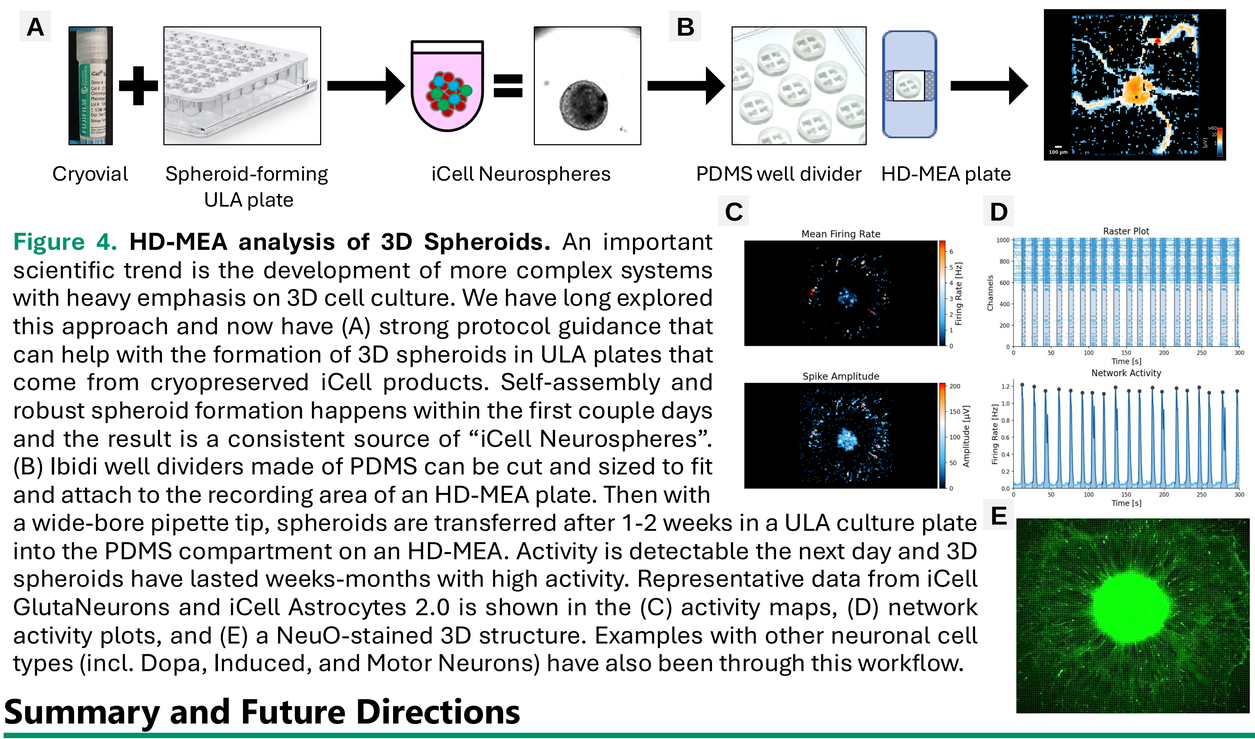
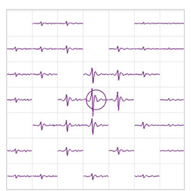


Figure 4. HD-MEA analysis of 3D Spheroids. An important scientific trend is the development of more complex systems with heavy emphasis on 3D cell culture. We have long explored this approach and now have (A) strong protocol guidance that can help with the formation of 3D spheroids in ULA plates that come from cryopreserved iCell products. Self-assembly and robust spheroid formation happens within the first couple days and the result is a consistent source of "iCell Neurospheres". (B) Ibidi well dividers made of PDMS can be cut and sized to fit and attach to the recording area of an HD-MEA plate. Then with a wide-bore pipette tip, spheroids are transferred after 1-2 weeks in a ULA culture plate into the PDMS compartment on an HD-MEA. Activity is detectable the next day and 3D spheroids have lasted weeks-months with high activity. Representative data from iCell GlutaNeurons and iCell Astrocytes 2.0 is shown in the (C) activity maps, (D) network activity plots, and (E) a NeuO-stained 3D structure. Examples with other neuronal cell types (incl. Dopa, Induced, and Motor Neurons) have also been through this workflow.

Summary and Future Directions



FUJIFILM CDI and Maxwell Biosystems have had a positive and rewarding collaboration for many years now. HD-MEA is a powerful technology that when combined with iPSC-derived cell types has the potential to unlock information about the function of human neurons like nothing else can. With the increased throughput provided by MaxTwo 24-well HD-MEA plates and their continuously improving software modules and features, we have been able to highlight the features of numerous iCell products and spotlight some important cell types for modeling neurodegenerative diseases. Additionally, we have iPSC-derived microglia (with AD-relevant APOE or TREM2 mutations) that we are excited to integrate into this system – in both 2D and 3D spheroid formats.