Measuring Neural Network Activity on MEA:

iCell DopaNeurons and optional co-culture with iCell Astrocytes

Introduction

iCell® DopaNeurons, 01279 are a population of dopaminergic neurons derived from human induced pluripotent stem cells (iPSC). These cells have been fully differentiated, shown to be highly pure for various neuronal markers, and come cryopreserved so they are ready to use. iCell DopaNeurons are well suited to study neurodegeneration in the context of Parkinson's disease (PD). When in culture, these cells fire rapidly to form connected networks, displaying typical electrophysiological characteristics of cultured neurons.

iCell Astrocytes, 01434 are a subtype of glial cells that play essential but complex roles in the maintenance of a healthy central nervous system, including axon guidance, synapse formation, neural network maturation, and balancing glutamate homeostasis. Astrocytes play an essential but complex role in the maintenance of a healthy central nervous system. When combined, co-cultures of iCell DopaNeurons and iCell Astrocytes provide a readily accessible and consistent in vitro model of neural network function and communication.

Microelectrode array (MEA) technology is an important tool for plate-based, multi-well measurements of neuronal electrophysiology. iCell DopaNeurons in monoculture or in co-culture with iCell Astrocytes, 01434 in MEA plates can be easily maintained for extended periods of time. Since MEA recordings are non-invasive and label-free, the development of synchronously bursting neural networks can be monitored over the course of the experiment. The flexibility of this assay allows users to decide what day in culture is optimal for their intended application.

This Application Protocol describes how to maintain iCell DopaNeurons on either 48-well or 96-well MEA plates (with or without iCell Astrocytes in co-culture) and provides basic instructions on data acquisition and analysis on the Maestro Pro MEA system from Axion Biosystems. Importantly, FCDI provides iCell DopaNeurons from various iPSC lines, including patient-derived resources from the Parkinson's Progression Markers Initiative (PPMI), which enables the unique opportunity for disease modeling and the study of PD-related pathology *in vitro*.

Required Equipment, Consumables, and Software

The following equipment, consumables, and software are required in addition to the materials specified in the iCell DopaNeurons User's Guide and iCell Astrocytes, 01434 Quick Guide.

Item	Vendor	Catalog Number
Equipment		
12-channel Pipettor, 200 µl	Multiple Vendors	
Maestro Pro MEA System	Axion BioSystems	
Consumables		
iCell DopaNeurons Kit, 01279	FUJIFILM Cellular Dynamics, Inc.	R1032
• iCell Neural Supplement B, 2 ml (50X) †	FUJIFILM Cellular Dynamics, Inc.	(incl. in R1032)
• iCell Nervous System Supplement, 1 ml (10X) †	FUJIFILM Cellular Dynamics, Inc.	(incl. in R1032)
• iCell Neural Base Medium 1, 100 ml	FUJIFILM Cellular Dynamics, Inc.	(incl. in R1032)
iCell Astrocytes Kit, 01434	FUJIFILM Cellular Dynamics, Inc.	R1092
Borate Buffer (20X)	Thermo Fisher Scientific	28341
BrainPhys™ Neuronal Medium	STEMCELL Technologies	05790
Centrifuge Tubes, 1.5, 15, and 50 ml	Multiple Vendors	
DPBS, no calcium, no magnesium	Multiple Vendors	
Laminin Solution, from Mouse EHS Tumor ‡	FUJIFILM Wako Pure Chemical Corp.	120-05751
N-2 Supplement (100X)	Thermo Fisher Scientific	17502-048
Penicillin-Streptomycin (100X)	Thermo Fisher Scientific	15140-022
50% Polyethyleneimine (PEI) Solution §	Sigma-Aldrich	181978-100G
Sterile Disposable Reagent Reservoirs	Multiple Vendors	
0.22 um Sterile Vacuum Filter Unit	Multiple Vendors	
Sterile Water	Multiple Vendors	
BioCircuit MEA 48-well plate or 96-well plate	Axion BioSystems	M768-BIO-48 (or -96)
CytoView MEA 48-well plate or 96-well plate	Axion BioSystems	M768-tMEA-48 (or -96)
Software		
AxIS Navigator Software	Axion BioSystems	Latest Version
Axion Metric Plotting Tool	Axion BioSystems	Latest Version
Neural Metric Tool	Axion BioSystems	Latest Version

† Additional media supplements will be required to make enough complete BrainPhys medium to carry out the assay beyond two weeks. iCell GlutaNeurons Media Kit (Cat. No. R1149), which contains 2 ml of iCell Neural Supplement B and 1 ml of iCell Nervous System Supplement, can be purchased separately.

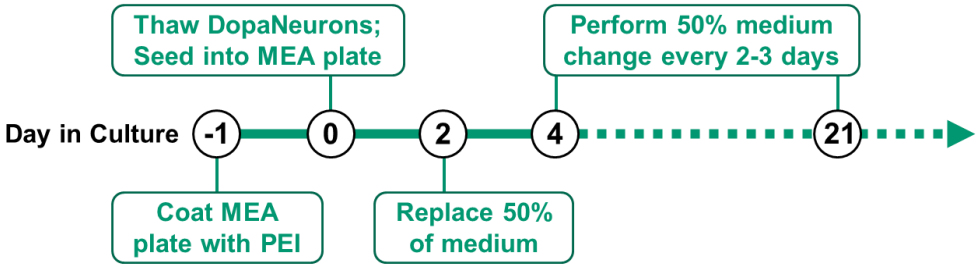
‡ Various sources of laminin have been tested with equivalent results, including Sigma-Aldrich Cat. No. L2020.

§ Various sources of PEI have been tested with equivalent results, including Sigma-Aldrich Cat. No. P3143 and Fujifilm Wako Pure Chemical Corp. Cat. No. 167-11951.

Workflow

Numerous factors contribute to the development of an electrically active and synchronously bursting neural network. As a result, there can be some variation in this assay for the optimal time point to start dosing with compounds. Since MEA technology is non-invasive and label-free, however, it is possible to measure the baseline activity of iCell DopaNeurons in monoculture (and with iCell Astrocytes, 01434 in co-culture) repeatedly over time. iCell DopaNeurons typically display robust synchronous activity after 2-3 weeks in culture. An example assay workflow is as follows:

- Day (-1): Coat 48-well or 96-well MEA plate with PEI. Allow to dry overnight.
- Day 0: Thaw iCell DopaNeurons in complete BrainPhys medium and seed/dot into the PEI-coated MEA plate. For co-culture with iCell Astrocytes, 01434, thaw cells separately and then combine them together to seed/dot into the PEI-coated MEA plate.
- Day 2: Replace 50% of the spent culture medium with complete BrainPhys medium.
- Day 4 and beyond: 50% medium changes every 2-3 days; perform baseline recordings to monitor neuronal activity.
- Day 21 or later ("day of assay"): perform a medium change, wait 2-4 hours, record baseline activity, dose with compounds, and record drug response.



Note: *MEA assays with iCell DopaNeurons and iCell Astrocytes, 01434 are compatible with a "weekend-free" assay workflow. Changing medium Friday late afternoon and then Monday morning is recommended.*

Tips Before Starting

1. Refer to the User's Guide for iCell DopaNeurons and Quick Guide for iCell Astrocytes, 01434 for information on storage and handling of the cells and media supplements.
2. Prepare the intermediate 10% PEI stock solution in advance. It is recommended to prepare the 0.1% PEI solution fresh on the day of coating the MEA plate.
3. Thaw the laminin solution at 4°C overnight prior to use. **Do not thaw at 37°C**.
4. Addition of Penicillin-Streptomycin into complete BrainPhys medium is optional but recommended.
5. Additional media supplements (Catalog No. R1149 for iCell Neural Supplement B and iCell Nervous System Supplement) are required for cell culture and MEA assay >14 days.
6. For consistency from assay-to-assay, it is recommended to determine cell counts from the Certificate of Analysis (CoA) for each specific lot of iCell DopaNeurons or iCell Astrocytes, 01434.
7. Recording of baseline MEA activity before the desired day of assay is recommended to determine that synchronous network bursting is present and that the cells are ready for assay. Neuronal activity typically improves over time and waiting 1-2 days later (for example) does not result in a significant difference in assay performance or compound response.

Methods

Preparing the PEI solution

1. Prepare an intermediate solution (10% w/w) of PEI by pouring ~2 ml of the 50% PEI stock solution into a tared 15 ml centrifuge tube. Centrifuge at 400 × g for 5 minutes.
2. Determine the weight (in grams) of PEI solution. By weighing out a known amount of PEI, the stock solution can be diluted with water to a known volume (density of water is 1.0 g/ml, thus 1 ml = 1 g).

   Note: *An example calculation is 2 g of the 50% PEI stock solution is diluted with 8 ml of sterile water to a final volume of 10 ml to obtain an intermediate 10% PEI solution (w/w).*
3. Mix the intermediate 10% PEI solution in the 15 ml centrifuge tube to dissolve.

   Note: *PEI is a viscous material and may require extended vortexing or mixing overnight on a benchtop rocker at room temperature to dissolve completely.*

   Note: *This intermediate 10% PEI solution may be aliquoted and stored at -20°C for future use.*
4. Prepare 100 ml of 1X borate buffer by diluting 5 ml of 20X borate buffer with 95 ml of sterile water.
5. Prepare a working 0.1% PEI solution by diluting 1 ml of the intermediate 10% PEI solution with 99 ml of 1X borate buffer. Sterile filter the working solution through a vacuum filter unit.

   Note: *It is recommended to make the 0.1% PEI working solution fresh before each use. If needed, the 0.1% PEI working solution can be made a day in advance and stored at 4°C for up to one week.*

Coating the MEA plate with PEI

1. Dispense 80 µl/well of the 0.1% PEI working solution to the 48-well or 96-well MEA plate directly over the electrodes at the center of each well with a multi-channel pipette. Incubate at 37°C for 1 hour.
2. Aspirate the PEI solution from the MEA plate. Do not allow the wells to dry out.
3. Immediately rinse the plate with **sterile water** two times with ≥500 µl/well for a 48-well MEA plate and two times with ≥300 µl/well for a 96-well MEA plate.
4. Rinse the plate with **DPBS** two times with ≥500 µl/well for a 48-well MEA plate and ≥300 µl/well for a 96-well MEA plate.
5. Allow the MEA plate to air-dry overnight in a sterile biological safety cabinet face down with the lid removed.

   Note: *It is critical to thoroughly rinse the MEA plate (finishing with DPBS) and then allow it to dry overnight to achieve optimal cell attachment and maximal assay performance.*

Preparing the Media

1. Thaw the iCell Neural Supplement B and iCell Nervous System Supplement at room temperature and the laminin stock solution at 4°C overnight.

   Note: *Do not vortex or thaw the stock laminin solution in a 37°C water bath.*
2. Prepare **complete BrainPhys medium** according to the table below. Refer to the User's Guide for iCell DopaNeurons for additional information.

Note: *Complete medium can be stored at 4°C for up to two weeks. Given the workflow in this application protocol, additional medium will need to be prepared over the course of the assay.*

Complete BrainPhys Medium – 100 ml

Component	Volume	Final Concentration
BrainPhys Neuronal Medium	95 ml	N/A
iCell Neural Supplement B (50X)	2 ml	1X
iCell Nervous System Supplement (100X)	1 ml	1X
N-2 Supplement (100X)	1 ml	1X
Laminin Solution (1 mg/ml)*	~0.1 ml	1 µg/ml
Penicillin-streptomycin (100X) **<optional>**	1 ml	1X

*It is recommended to calculate volumes of laminin that is needed from the Protein Content (mg/ml) found on the manufacturer's CoA.

3. Filter sterilize the complete BrainPhys medium using a 0.22 µm filter before use and store at 4°C, protected from light, for up to two weeks.
4. Prepare an aliquot of **dotting medium** for the subsequent cell seeding steps using the materials and volumes indicated in the table below.

Dotting Medium – 1 ml

Component	Volume	Final Concentration
Complete BrainPhys Medium	0.9 ml	N/A
Laminin Solution (1 mg/ml)*	~0.1 ml	0.1 mg/ml

*It is recommended to calculate volumes of laminin that is needed from the Protein Content (mg/ml) found on the manufacturer's CoA.

Thawing iCell DopaNeurons

The following procedure details the thawing of one vial of iCell DopaNeurons, 01279 into a 48-well MEA plate at a density of 120,000 cells/well. Scale volumes accordingly when plating into a 96-well MEA plate at the same density (two vials of iCell DopaNeurons is needed). Thaw no more than 2 vials of iCell DopaNeurons at one time.

1. Obtain the number of viable cells per vial from the Certificate of Analysis (CoA) for the specific lot of iCell DopaNeurons.

   Note: *Each CoA can be found online: fujifilmcdi.com/resources/coa-lookup/*
2. Allow complete BrainPhys medium to equilibrate to room temperature for 10-15 minutes prior to use.
3. Thaw iCell DopaNeurons, 01279 into a sterile 50 ml centrifuge tube according to the iCell DopaNeurons User's Guide and dilute cell suspension with complete BrainPhys medium to a final volume of 10 ml.
4. Remove a sample of the cell suspension to manually count the cells using a hemocytometer to confirm viability and total number of viable cells before plating.

   Note: *With proper handling, expect to recover about 20% of the values listed in the CoA. Utilizing an automated cell counter without prior optimization for iCell DopaNeurons is not recommended.*
5. Transfer the iCell DopaNeurons cell suspension from the 50 ml centrifuge tube to a sterile 15 ml centrifuge tube.
6. Concentrate the cells by spinning the centrifuge tube at 400 x g for 5 minutes.
7. Determine the volume of dotting medium required to obtain a cell density of **$15x10^6$ iCell DopaNeurons/ml** based on the manual cell counts measured in step 4. Subtract 50 µl from this number.
8. Aspirate the supernatant being careful not to disturb the cell pellet, leaving approximately 50 µl above the cell pellet.

9. Gently resuspend the cell pellet of iCell DopaNeurons with the final volume of **dotting medium** determined above in step 7.
10. Transfer the iCell DopaNeurons cell suspension to a sterile 1.5 ml centrifuge tube and label accordingly.

    Note: *The cell suspension volume of iCell DopaNeurons from the ≥5M vial size should be ≥400 µl.*

Optional Step: Thawing iCell Astrocytes for Co-culture in the MEA Assay

The following optional procedure details the thawing of one vial of iCell Astrocytes, 01434 into a 48-well MEA plate at a density of 20,000 cells/well for co-culture with iCell DopaNeurons, 01279. Scale volumes accordingly when plating into a 96-well MEA plate at the same density (two vials of iCell Astrocytes, 01434 is needed). Thaw no more than 2 vials of iCell Astrocytes, 01434 at one time. Incorporating astrocytes into dopaminergic neuron cultures benefit network maturity and improve well-to-well consistency (Figure 6). Contact Technical Support for co-culture with iCell Astrocytes 2.0.

1. Obtain the number of viable cells per vial from the Certificate of Analysis (CoA) for the specific lot of iCell Astrocytes, 01434.

   Note: *Each CoA can be found online: fujifilmcdi.com/resources/coa-lookup/*
2. Thaw iCell Astrocytes, 01434 into a sterile 50 ml centrifuge tube according to the iCell Astrocytes, 01434 Quick Guide and dilute cell suspension with complete BrainPhys medium to a final volume of 5 ml.
3. Remove a sample of the cell suspension to manually count the cells using a hemocytometer to confirm viability and total number of viable cells before plating.

   Note: *With proper handling, expect to recover >1x10^6 viable cells.*
4. Transfer the iCell Astrocytes, 01434 cell suspension to a sterile 15 ml centrifuge tube.
5. Concentrate the cells by spinning the centrifuge tube at 400 x g for 5 minutes.
6. Determine the volume of dotting medium required to obtain a cell density of **6.7x10^6 iCell Astrocytes/ml** based on the manual cell counts measured in step 3. Subtract 50 µl from this number.
7. Aspirate the supernatant being careful not to disturb the cell pellet, leaving approximately 50 µl above the cell pellet.
8. Gently resuspend the cell pellet of iCell Astrocytes (either donor) with the final volume of **dotting medium** determined above in step 6.
9. Transfer the iCell Astrocytes cell suspension to a sterile 1.5 ml centrifuge tube and label accordingly.

   Note: *The cell suspension volume of iCell Astrocytes, 01434 from the ≥1M vial size should be ≥150 µl.*

Plating iCell DopaNeurons (and iCell Astrocytes) into the MEA plate

The following procedure describes the plating (or dotting) of cells into the MEA plate. If co-culturing with iCell Astrocytes, cell suspensions are mixed to yield a 6:1 ratio of dopaminergic neurons to astrocytes (EX: 400 µl + 150 µl).

1. Mix the cell suspension by gently inverting the centrifuge tube 2-3 times.
2. Dispense a droplet of the cells over the recording electrode area of each well with the MEA plate tilted at a 30° angle so that the bottom of the well is visible (see **Figure 1** for droplet placement).

For iCell DopaNeurons monoculture on MEA, droplet size is 8 µl. For co-culture with iCell Astrocytes, 01434 on MEA, droplet size is 11 µl.

Note: *FCDI recommends using 20 µl pipette tips with a long thin ending for this step to facilitate droplet formation. Additionally, dispensing one row (or one column) at a time and mixing the cell suspension between each row helps to ensure even distribution of cells in each well across the plate.*

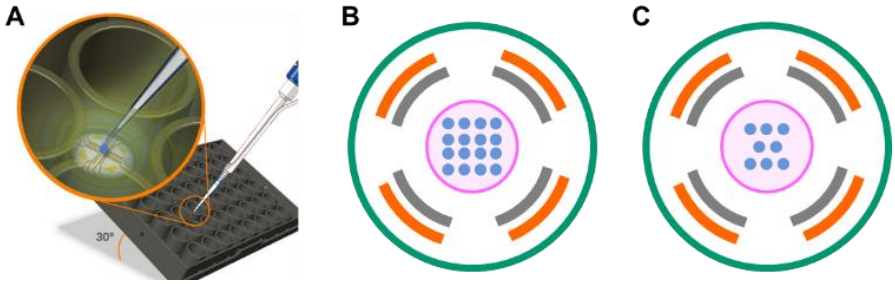


Figure 1. Dotting of cells in an MEA plate. A) Tilt the plate at a 30° angle to aid in visualization of the plate bottom; B) schematic of the droplet location (highlighted in pink) over the 16 electrodes in one well of a 48-well MEA plate; C) schematic of the droplet location (highlighted in pink) over the 8 electrodes in one well of a 96-well MEA plate.

3. Repeat dotting step until all wells of the MEA plate have been filled with the cells.
4. Add 2-3 ml of sterile water to the area surrounding the wells of the MEA plate to limit evaporation. Do not allow water into the wells.

   Note: *FCDI recommends adding the water after dotting the cell suspension to avoid water leaking into wells when the MEA plate is tilted.*
5. Cover the MEA plate with the lid and incubate it in a cell culture incubator at 37°C, 5% CO_2 for approximately 1 hour.

   Note: *Do not allow the MEA plate to incubate for more than 1 hour to prevent evaporation.*
6. Remove the MEA plate from the cell culture incubator and work in a biological safety cabinet.
7. Load a 12-channel pipettor with sterile tips, removing tips from the positions identified in **Figure 2** to deliver medium to the plate.

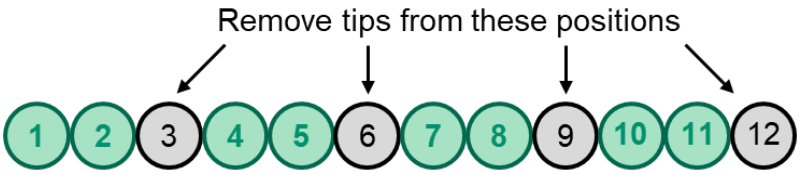


Figure 2. Tip loading strategy for using a 12-channel pipettor with a 48-well MEA plate. Load a 12-channel pipettor with eight (8) sterile 200 µl pipette tips are arranged in the highlighted positions (green circles) for easy addition and removal of medium to a 48-well plate; the other four (4) positions (grey circles) are left empty. ***Note:*** *Use all 12 positions with a 96-well MEA plate.*

8. Tilt the MEA plate at a steep (>45°) angle and gently add complete BrainPhys medium down the side of the wells one row at a time. For a 48-well MEA plate, add 150 µl/well. For a 96-well MEA plate, add 100 µl/well. Adding the medium too quickly can dislodge the cells.
9. Slowly return the MEA plate to a flat position to allow the medium to gently cover the droplet after it has been added to all rows.
10. Repeat steps 8 and 9 above. The final volume of medium needed for culturing cells in a 48-well MEA plate is 300 µl/well, while 200 µl/well is recommended for a 96-well MEA plate.
11. Add an additional 2-3 ml of sterile water to the area surrounding the wells of the MEA plate to limit evaporation.
12. Cover the MEA plate with the lid and place it in a cell culture incubator at 37°C, 5% CO_2.

Maintenance of iCell DopaNeurons (and iCell Astrocytes) on the MEA plate

1. Equilibrate complete BrainPhys medium to room temperature prior to use.
2. On Day 2, perform a 50% complete medium change. Slowly aspirate the spent medium from the MEA plate using a 12-channel pipettor and replace with the same volume of complete BrainPhys medium.
3. Maintain the monoculture of iCell DopaNeurons (or co-culture with iCell Astrocytes, 01434) in the MEA plate by replacing 50% of the spent medium with complete BrainPhys medium every 2-3 days.
4. Incubate the MEA plate in a cell culture incubator at 37°C, 5% CO_2 after changing the medium.

Data Acquisition and Analysis

Data Acquisition

Baseline activity of iCell DopaNeurons in monoculture (and with iCell Astrocytes, 01434 in co-culture) can be viewed at any point in time during the MEA assay. Please refer to the AxIS Navigator software User Guide (located under the “Help” menu) and the Maestro Pro MEA system manual for detailed instructions on how to acquire data. For consistent measurements of neural network activity, however, the following steps are recommended:

1. On the day of recording, replace 50% of the spent medium with complete BrainPhys medium approximately 2-4 hours before data acquisition.
2. Allow the MEA plate to equilibrate at 37°C and 5% CO_2 directly on the Maestro Pro instrument for at least 10 minutes prior to recording.
3. Record for 5-10 minutes (300-600 seconds) to adequately capture network bursting behavior.

Data Analysis

Neuronal activity on an MEA is analyzed by identifying the individual action potentials (or “spikes”) and by quantifying spike timing and coordination of the spikes across the co-culture. Each MEA recording produces an output of continuous voltage in a data file called “AxIS Raw” (or .raw) that must be further processed for downstream analysis.

AxIS Navigator software converts the .raw file to an “AxIS Spike” (or .spk) file, which contains spike times and voltage waveforms organized by electrode. The .spk file is the required output file for the Neural Metric Tool (NMT) software. To begin a batch process in AxIS Navigator, click File → New Batch Process in the Menu bar and add the desired .raw files(s) to be converted. Then, right click on the batch process that appears in the Streams window to apply the desired configuration as outlined in **Figure 3** below:

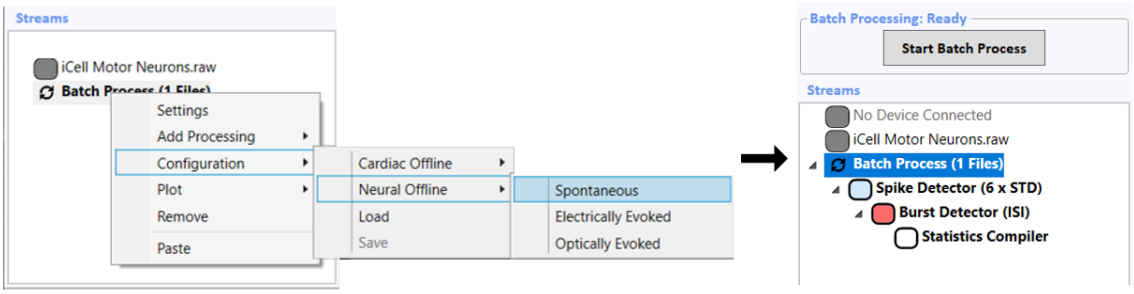


Figure 3: Batch processing of AxIS RAW data. The recorded MEA data (.raw file) must be converted to a “spike” (.spk) file for downstream analysis though a batch processing step performed in AxIS Navigator software. The screenshot above illustrates the process.

For network burst detection of iCell DopaNeurons in monoculture (and with iCell Astrocytes, 01434 in co-culture), the .spk file must be loaded into the NMT software. It is recommended to use the “Envelope” settings with a “Threshold Factor” of 3 in the Bursting window. Typically, the “Min Electrodes (%)” and the “Burst Inclusion (%)” can be set at 50% or greater. It is also important to select “All” from the dropdown menu for “Additional Synchrony Metrics” in the Synchrony window as this is not the default setting. See **Figure 4** below for a screenshot from the NMT analysis window.

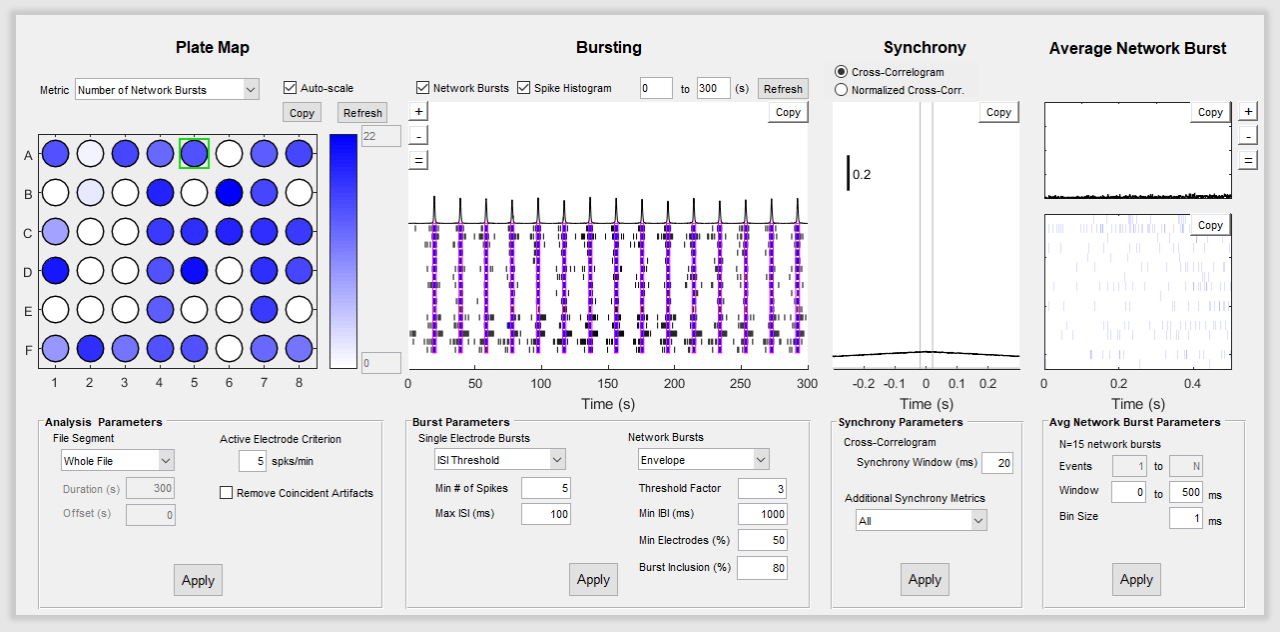


Figure 4. Neural Metric Tool analysis window. Screenshot of software window with representative data from a 48-well CytoView MEA plate including typical settings for analysis. Values for Network Burst Parameters are editable and may be changed as needed.

After using the NMT software for advanced burst detection and calculation of synchrony metrics, the data can be exported as a Comma Separated Value (or .csv) file. The AxIS Metric Plotting Tool (AMPT) can then be used to visualize the experimental results. Please refer to the User Guides for in-depth information on how to use each of these different software tools from Axion Biosystems.

Compound Dosing (optional)

iCell DopaNeurons plated on MEA in monoculture can be used for compound testing and drug screening (Figure 8). Follow this section for guidance on the day of the assay.

Media Change on the Day of Assay

1. Equilibrate complete BrainPhys medium to room temperature prior to use.
2. Remove the MEA plate from the cell culture incubator. Obtain a sterile cell culture plate to match the dimensions of the MEA plate (48- or 96-well).
3. Transfer 150 µl of the spent medium from the MEA plate to the sterile cell culture plate, matching the plate layout.
4. Add an equal volume (150 µl) of complete BrainPhys medium to the sterile cell culture plate to prepare a 50:50 mixture of spent/fresh culture medium. Total volume should be 300 µl.

5. Aspirate the remaining spent medium from the MEA plate and discard.
6. Transfer 250 µl of the 50:50 mixture of spent/fresh culture medium from the cell culture plate back to the MEA plate, again preserving the plate layout.

   Note: *It is recommended to perform steps 3-6 for 2 rows at a time until all wells have been completed.*

7. Return the MEA plate to the cell culture incubator for at least 2-4 hours prior to compound addition.

Compound Preparation

There are various approaches to compound dosing. This section offers the end user a *recommended workflow*. If the medium was changed as described above, 6X working dilutions of compound(s) are prepared as described below on the day of the assay.

1. Prepare an initial stock solution of compound in DMSO at ≥1000X the max concentration intended for use (for example 10 mM). Store the stock solution at -20°C until use.
2. Dilute the compound stock with complete BrainPhys medium to 6X the target concentration.

   Note: *For example, to dose cells with picrotoxin at a final 1X concentration of 10 µM (and a final percentage of DMSO at 0.1%), dilute 6 µl of the 10 mM DMSO stock of picrotoxin into 1000 µl of complete BrainPhys medium to obtain 60 µM compound in 0.6% DMSO.*

3. Repeat steps 1-2 for all compounds and all concentrations to be tested. Allow the 6X compound solutions to equilibrate to 37°C in an incubator for ≥15 minutes.
4. Perform a baseline recording after at least 2-4 hours post-medium change on the day of assay.
5. Transfer 50 ul of the 6X working dilution of compound to the appropriate wells of the MEA plate. Gently mix the total volume of 300 µl by pipetting 2-3 times using the same tips.
6. Perform a post-dose recording 30 minutes after compound treatment.

Representative Data

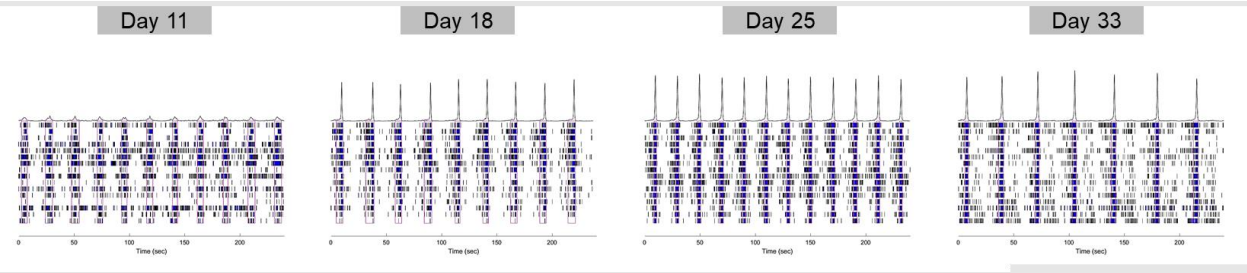


Figure 5. Development of synchronous bursting networks over time. Representative raster plots taken from the same well of iCell DopaNeurons in monoculture on a 48-well CytoView MEA plate at different days post-thaw. Every spike in the raster plots indicates an action potential and it is evident that the neuronal cultures are highly active and starting to show organization early on (Day 11; numerous black and blue tick marks). Soon, MEA activity transitions into more organized, synchronously bursting networks (Day 18-25; indicated by pink boxes) aligned across every electrode. Bursting becomes much stronger and more regular over time, with fewer spikes in between the bursts (Day 33). The ability to observe network development over time on the MEA plate enables flexibility for assay timing and design.

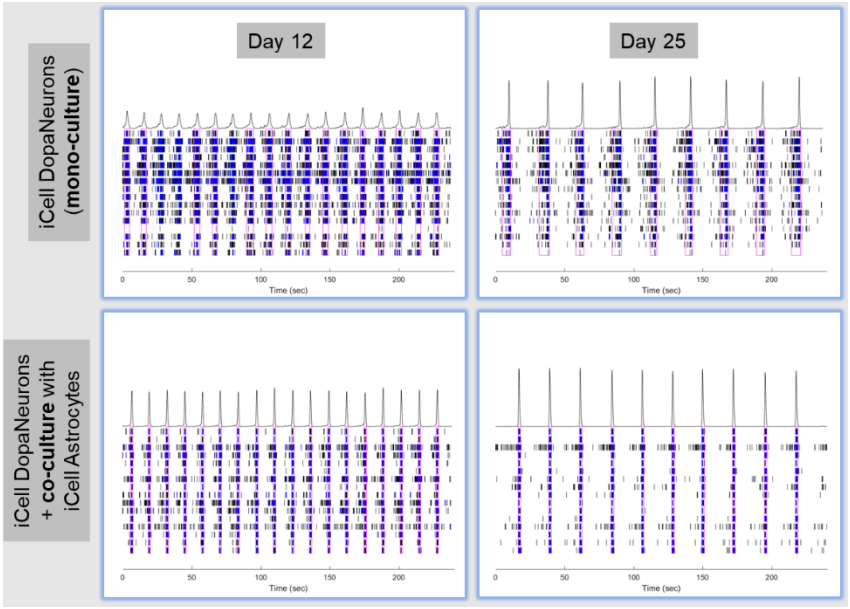


Figure 6. Comparison of iCell DopaNeurons in mono- vs. co-culture. iCell DopaNeurons were dotted on a 48-well CytoView MEA plate in the absence (top row: mono-culture) or presence (bottom row: co-culture) of iCell Astrocytes, 01434. Representative raster plots acquired on Day 12 and Day 25 are presented to illustrate the impact of glial cells in culture with dopaminergic neurons. Both examples demonstrate high electrical activity (EX: all electrodes are active) and robust synchronous network bursting both time points. iCell Astrocytes, 01434 support neuronal network development by quieting the number of spikes between bursts and increasing the number of spikes within a network burst. The addition of iCell Astrocytes, 01434 to cultures of iCell DopaNeurons on MEA is application dependent.

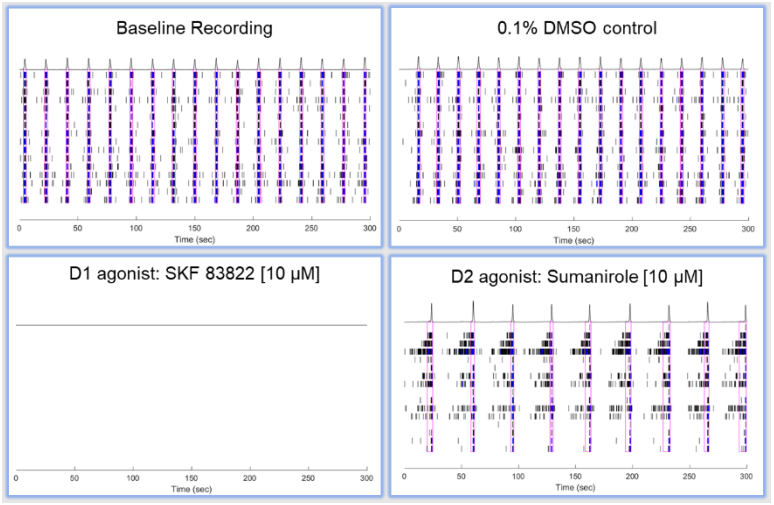


Figure 7. Dopamine receptor pharmacology. Representative raster plots of iCell DopaNeurons in mono-culture maintained on a 48-well CytoView MEA plate until Day 50 and then dosed with different agonists for the D1 and D2 dopamine receptors. The MEA signal is very stable following treatment with vehicle control (0.1% DMSO), as illustrated by the nearly identical spike / burst pattern to the baseline recording. However, exposure to 10 µM of the D1 agonist SKF 83822 hydrochloride (Tocris Cat. No. 2075) for 30 minutes shut down all electrical activity, whereas dosing with 10 µM of the D2 agonist Sumanirole (Tocris Cat. No. 2773) for 30 minutes noticeably altered the bursting phenotype on MEA. The ability to observe the electrophysiological and functional response of iPSC-derived dopaminergic neurons in culture is a feature benefit of this MEA assay with iCell DopaNeurons.

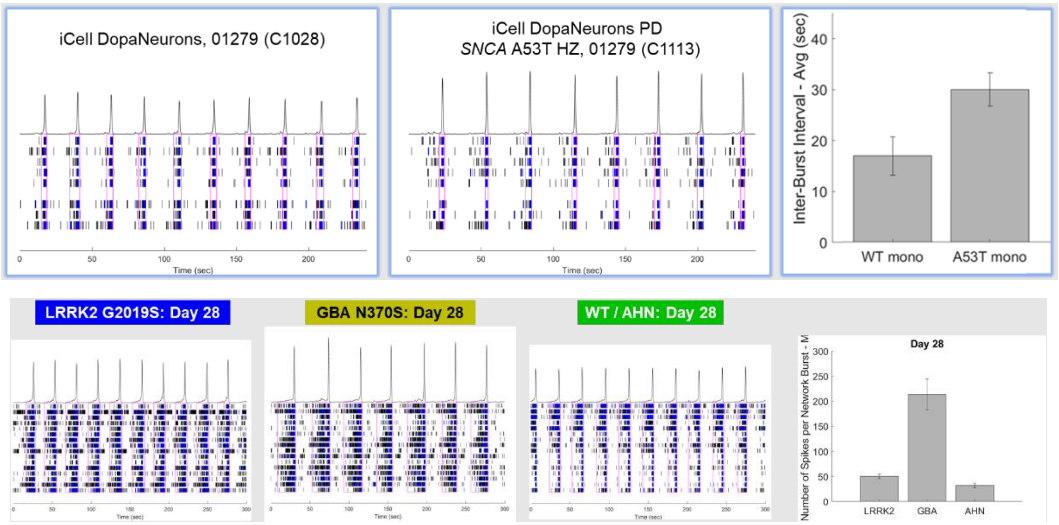


Figure 8. Disease modeling with iCell DopaNeurons. Representative raster plots comparing iCell DopaNeurons, 01279 (AHN or "WT") to the gene-edited, isogenic iCell DopaNeurons PD SNCA A53T HZ, 01279 cells (both in mono-culture) maintained on a 96-well CytoView MEA plate until Day 33. Both iPSC-derived dopaminergic neurons demonstrate robust activity across multiple time points, yet various MEA metrics, including inter-burst interval (IBI), illustrate some of the subtle differences in this disease model. Additional data from iCell DopaNeurons PD LRRK2 G2019S and GBA N370S were generated on 48-well CytoView MEA plates on Day 28 following this MEA Application Protocol. Data analysis here suggests that the number of spikes per network burst is 3-4-fold greater for GBA line.

Summary

iCell DopaNeurons, 01279 are a highly active population of human iPSC-derived dopaminergic neurons that can be used for numerous *in vitro* applications. Electrical spike activity from these cells can be detected on the MEA within a few days post-plating and synchronously bursting networks will develop after about two weeks in culture. iCell DopaNeurons in monoculture (and with iCell Astrocytes, 01434 in co-culture) can last for months with proper maintenance and the MEA signal can be recorded repeatedly due to the label-free and non-invasive nature of the technology. Importantly, iCell DopaNeurons, 01279 are responsive to D1/D2 receptor pharmacology, which can be tested in more detail following the guidelines in this application protocol.

Revision History
Version 1.0: December 2023
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