FUJIFILM
Value from Innovation

Cellular Dynamics

Enhancing the Function of iCell® Sensory Neurons with OoC Devices

iCell Lab Note

Introduction.

The availability of human iPSC-derived sensory neurons presents a valuable source of cells for applications in pain drug discovery and research. Compartmentalization of cells into microfluidic "organ-on-a-chip" (OoC) systems enables precise control of cellular microenvironments and compound application to specific cellular features (soma vs. neurite). Because of microelectrode array (MEA) technology for measuring neuronal function, the introduction of NeuroFluidics™ DuaLink MEA devices (NETRI) for use on the Maestro Pro™ MEA system (Axion BioSystems) merges electrophysiology and microfluidics for next-level testing. Pairing iCell® Sensory Neurons with OoC MEA technology accelerates the utility of these cells and elevates their biological impact.

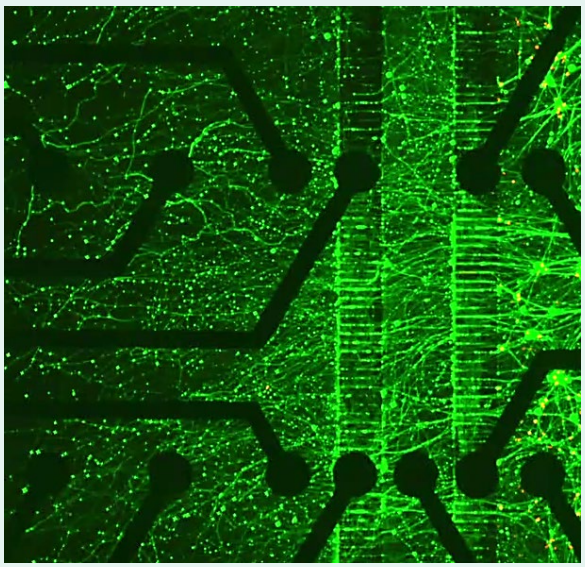


Figure 1. Organ-on-a-Chip and iPSC technologies together. iCell Sensory Neurons were plated into channel 3 of the DuaLink MEA microfluidic device where neurites readily grow and project through the microchannels (µTunnels) over time. Pictured here are sensory neurons stained with the live cell dye Calcein AM on DIV 29. *Image courtesy of NETRI.*

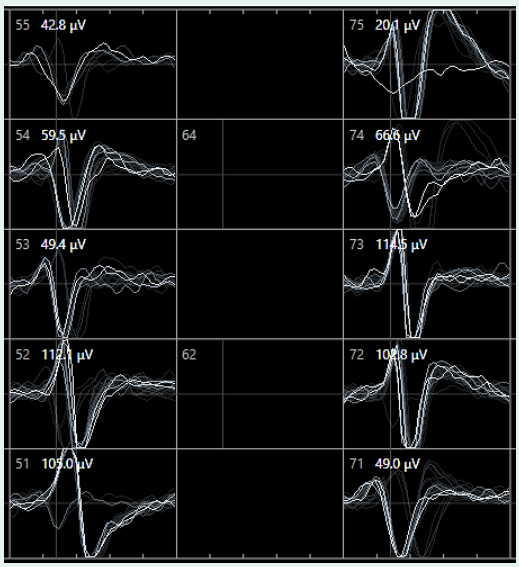


Figure 2. Neuronal electrophysiology is enhanced in microchannels. iCell Sensory Neurons exhibit low levels of spontaneous spike activity when maintained on standard MEA plates, but on the DuaLink MEA chip, action potentials with spike amplitudes averaging >70 µV can be recorded as early as DIV 14 and then carried out beyond DIV 50.

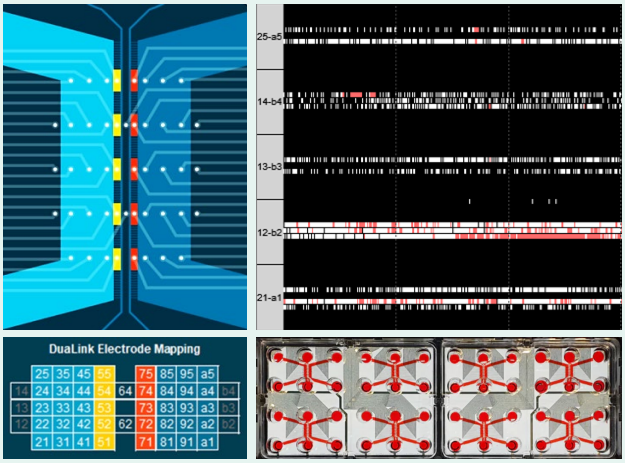
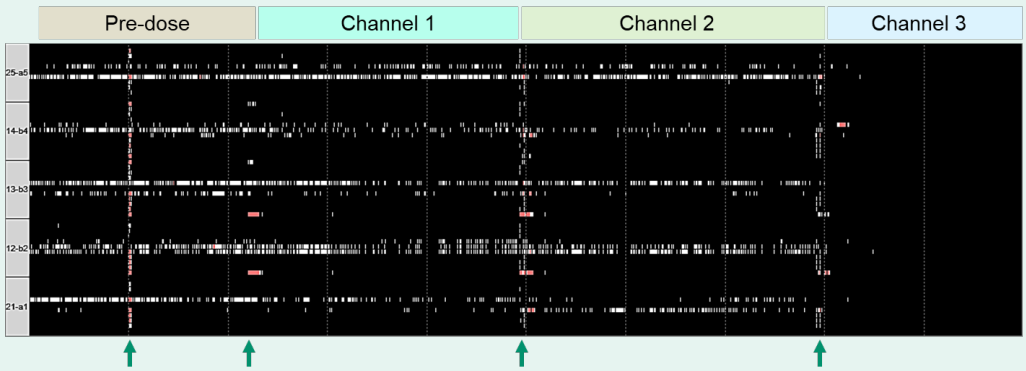


Figure 3. Architecture of the DuaLink MEA chip. Each microfluidic device has 48 electrodes embedded within it. While iCell Sensory Neurons are cultured across all surfaces of the chip, spontaneous spike activity is detected primarily from electrodes that are near the microchannels (numbered as 51-55 and 71-75, colored in yellow & red on the map). Raster plots can be imaged via AxiS Navigator software (shown here) or generated with UpLink software after recordings.



		Number of Spikes			
		pre-dose	Ch1	Ch2	Ch3
2µ3	75	222	220	179	4
2µ3	74	5	24	3	1
2µ3	73	62	61	6	0
2µ3	72	92	146	179	3
2µ3	71	17	5	80	0
Ch2	64	136	108	32	0
Ch2	62	89	140	61	1
1µ2	55	45	52	45	3
1µ2	54	20	21	2	30
1µ2	53	175	206	100	2
1µ2	52	13	15	2	2
1µ2	51	191	125	12	2
TOTAL		1067	1123	701	48

Figure 4. Compound Dosing. iCell Sensory Neurons were treated with Lidocaine (1 mM) in each individual channel (1, 2, and 3) sequentially. Time between doses was ~90 sec and artifacts from touching the plate are indicated by the arrows. Spike activity across the entire chip was completely silenced when drug was added to Ch 3 (where cells were seeded initially). These data suggest that the effect of blocking sodium channel activity and inhibiting sensory neuron function with Lidocaine is stronger at the soma (Ch 3) vs. the axons (located in Ch 1 and 2).

Methods.

- Follow the NeuroFluidics Line DuaLink MEA Operating Protocol to prepare and interact with the device.
- Make Complete Medium and thaw iCell Sensory Neurons according to the User's Guide (see QR code).
- Coat all channels with PDL and Laminin-511.

  Note: *Dilute PDL to 50 µg/ml; Supplement complete media with Laminin-511 at 20 µg/ml for coating and cell seeding.*
- Concentrate cells to a density of $15x10^6$ cells per ml and seed 3 µl into either Ch 1 or 3 (45K cells per device).
- Change 50% of media (~80 µl) every 2-3 days according to recommended protocol from NETRI.

  Note: *Add Laminin-511 at 1 µg/ml to media during culture.*
- Record baseline spike activity on Maestro MEA.
- For dosing, remove 16 µl from outlet and add 16 µl of 10X compound to inlet.
- Analyze .spk files with UpLink software.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell Sensory Neurons Kit, 01279 [1]	FCDI	R1250
▪ iCell Sensory Neurons Base Medium	incl. in kit	M1052
▪ iCell Sensory Neurons Supplement	incl. in kit	M1053
Poly-D-Lysine (PDL) Solution	GIBCO	A3890401
Recombinant Laminin iMatrix-511 Silk	AMSBIO	AMS.892021
DuaLink MEA (Edge or Pro) [2, 3]	NETRI	N/A
Lidocaine [4]	Tocris	3057
Maestro (Edge or Pro) MEA System	Axion	N/A
AxiS Navigator and UpLink software [5]	Axion/Netri	Latest Version

[1] iCell Sensory Neurons from Donor 21527 (R1252) are also available.
[2] Other NeuroFluidics MEAs (DuaLink Shift or TriaLink) have not been tested.
[3] Visit www.netri.com/resources/ for protocol guidance to use the DuaLink MEA.
[4] Cells have been shown to respond to increased temperature or other sensory agonists (i.e., capsaicin, menthol, or PIEZO compounds) in calcium flux assays.
[5] Download UpLink software at www.netri.com/customers-support.

Summary.

This Lab Note provides technical recommendations for using iCell Sensory Neurons on the NeuroFluidics DuaLink MEA device. Neuronal activity can be recorded over time on the Maestro Pro MEA system and analyzed using Netri's UpLink software. By combining these technologies together, MEA activity can be recorded more robustly and the electrophysiological properties of sensory neurons can be interrogated with more confidence. This enables testing of pharmacological agents in a highly controlled and scalable manner. This application bridges critical gaps in translational neuroscience, providing invaluable insights into sensory neuron biology and therapeutic screening.

Highlights.

Human iPSC-derived iCell Sensory Neurons provide a consistent cell model that is ideal for advancing pain research.

Compartmentalization of cells with NETRI OoC devices offers a powerful new approach to observing neuronal function.

This MEA-compatible microfluidic chip enables sensitive measurements of sensory neuron activity that were previously not accessible.

Scan here to download the iCell Sensory Neurons User's Guide.

Contact **Technical Support** for more protocol details and supportive data.
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LN-X4013_0725