NETRI's APP NOTE

# SYNAPTIC TRANSMISSION INVESTIGATION USING ASYMMETRIC SHAPE MICROFLUIDIC DEVICE, DUALINK™ SHIFT

- Asymmetric-shape microfluidic device allowing to create a dendritic-axonal synapse compartment
- Neurites and synaptic isolation of two distinct cell types in co-culture
- Microfluidic device compatible with microscopy (classical inverted and high-resolution microscopes, and confocal)
- Compatibility with electrophysiological recordings – NeoBento™ Axion™

## OVERVIEW

Neurotransmission is the communication between neurons via chemicals or electrical signals across a synapse, called synaptic transmission. Several molecules as neurotransmitters, proteins or chemical molecules are involved in synapse formation and plasticity[1]. Progressive loss of synaptic connections could lead to neuronal dysfunction involved in many neurodegenerative disorders as Alzheimer's, Parkinson's, or Huntington's diseases[2].

NETRI has developed the DuaLink™ Shift. It is a 3-channel chip with asymmetrical shape connected by microchannels tunnels technology that allows discontinuous connectivity and synaptic isolation. 2 channels are for cell culture and 1 for synaptic isolation creation. Due to their micron scale, only cell extensions can grow within the microchannels, leaving the cell bodies withing the channels themselves (Figure 1).

This application note presents the DuaLink™ Shift chip with human neurons and their different applications and readouts such as immunostainings, Live/dead (LDA) assays, pharmaco-toxicology testing, synaptic propagation, and electrophysiological recordings.

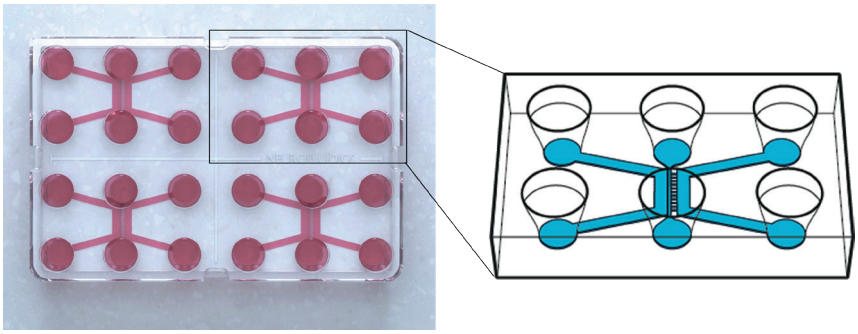

*Figure 1. Picture of a QuarterBento™ and a 3D diagram of the DuaLink Shift.*

# RESULTS

## Microfluidic architecture characteristics

DuaLink™ Shift microfluidic chip is composed of three channels connected by microchannels tunnels technology allowing fluidic isolation. Knowing that dendrites are approximately 200 µm in length and axons are more than 500 µm3, this particular architecture allows for a synaptic compartment in channel 2 with axonal projections from channel 3 and neurite projections from channel 1. DuaLink™ Shift microfluidic devices have been well characterized with NETRI manufacturing quality control (Figure 2) (DuaLink Shift & DuaLink Shift MEA – Technical specifications).

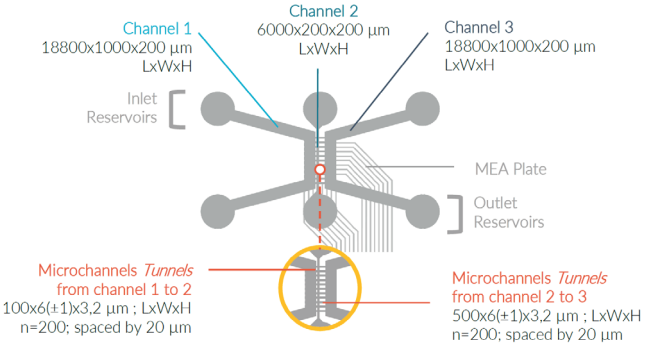


*Figure 2. Characteristic of the asymmetric microfluidic device. Schematic representation and dimensions of the DuaLink™ Shift.*

## Human neurons in an asymmetric shaped microfluidic device

NETRI has been established relevant and robust cell culture protocols allowing long- term culture of several cell type such as human induced pluripotent stem cell (iPSC)-derived neurons. Human neuronal culture can be performed in DuaLink™ Shift thanks to Poly-dimethyl-siloxane (PDMS), a transparent and biocompatible materials used to manufacture devices. Well characterized human glutamatergic neurons were seeded in DuakLink™ Shift, in channels 1 and 3 where homogeneity has been verified. To promote human glutamatergic neurons attachment and long-term viability, up to 28 days, microfluidic devices were previously coated with Poly-D-Lysine (PDL) and Geltrex and changes cell culture media adaptation to microfluidic technology. Long-term viability and cell homogeneity and could be maintained with 50% of media change up to 28 days twice a week (Cell types protocol – Glutamatergic neurons BrainXell & Glutamatergic neurons MEA BrainXell). Several experiments, such as evaluation of stem cells pluripotency and biological markers and functional recordings were performed to validate the cell culture conditions in microfluidic devices[4].

Pictures of neuronal culture seeded in the DuaLink™ Shift can be done in brightfield contrast and fluorescence (Operating protocol – Immuno Staining). We have been performed several readouts with human glutamatergic neurons previously seeded in the DuaLink™ Shift (Figure 3):

- Brightfield pictures to validate neuronal morphology and homogeneity cell seeding (Figure 3B),
- LDA pictures in fluorescence to evaluate the neuronal viability. Staining in green represent live cells and staining in red the dead ones (Figure 3C),
- Immunofluorescence pictures with specific markers of glutamatergic neurons such as vglut1 which are a vesicular marker of glutamine present in the pre-synaptic part (Figure 3D).

## Evaluation of synaptic markers expression

The asymmetric shape of the microfluidic device allows to isolate synapses between two cell type. Length of microchannels between the channel 1 and channel 2 is about 100 µm allowing throughout of dendrites and axons projections while microchannels between channel 3 and channel 2 are 500 µm enabling only axons projections (Figure 2). Thanks to a dendritic staining as MAP2, it has been shown that dendrites from channel 1 pass through the channel 2 but not those from channel 3 (Figure 4).

To mark synapses with fluorescence in the channel 2, we have been used antibody anti- Homer to visualize post-synaptic part (Figure 4A) and anti-Synapsin for stain pre-synaptic part (Figure 4B). We have shown that pre and post synaptic part can be visualized in DuaLink™ Shift. This microfluidic isolation could permit to perform several experiments on synaptic part of the neuronal connection. For example, neurotransmitters assays, injury with addition of misfolding protein and disease modelling with use of patient derived hiPSCs, drug transport[2] and electrophysiological recordings.

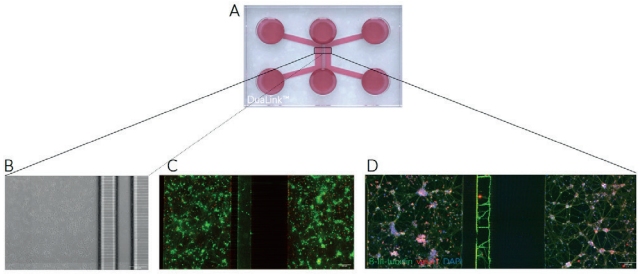


*Figure 3. Human glutamatergic neurons seeded in DuaLink™ Shift.*
*(A) Schematic representation of DuaLink™ Shift.*
*(B) Brightfield picture of glutamatergic neurons at day 12 in a DuaLink™ Link.*
*(C) LDA picture of glutamatergic neurons at day 12 in DuaLink™ Shift.*
*(D) Immunofluorescence of glutamatergic neurons with β-III-tubulin (1/200 in green) for neuronal staining, vglut1 (1/100 in red) and DAPI (in blue) markers at day 22 in DuaLink™ Shift. Scale bars represent 200 µm. Pictures were analyzed with ImageJ software[5]. LDA: Live/ Dead Assay*

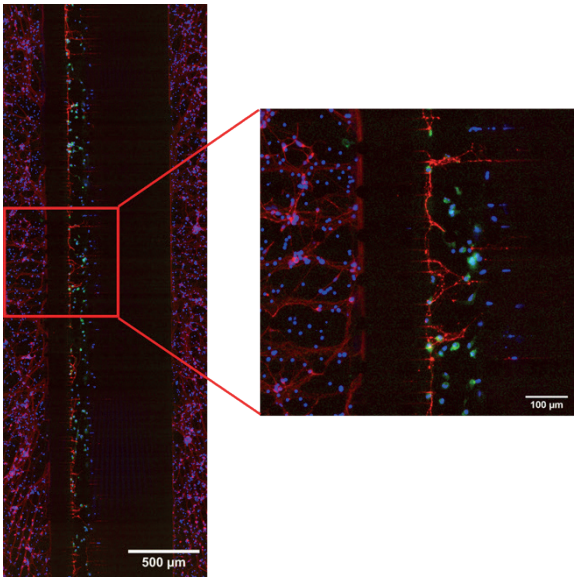


*Figure 4. Pictures of DuaLink™ Shift with synapses staining. Immunofluorescent pictures of glutamatergic at day 22 neurons with CD209 (1/50 in green) for immune cells, MAP2 (1/100 in red) and DAPI (in blue) markers with a zoom picture on the channel 2.*

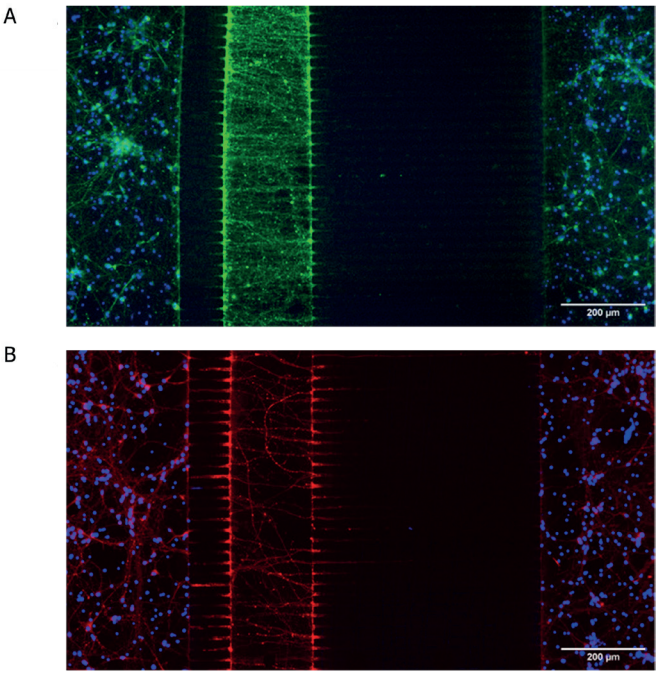


*Figure 5. Immunofluorescent pictures of glutamatergic neurons at day 21 with (A) Homer (1/100, in green) and DAPI (in blue) marker and (B) Synapsin (1/200, in green) and DAPI (in blue) marker.*

## Analysis of electrophysiological recordings using Multi-Electrode Array (MEA)

The functional activity of human glutamatergic neurons was recorded in a microfluidic device on microelectrode array (MEA). This technology allows the extracellular recording, in contrast of patch clamp that records intracellular activity. All data were recorded with a MEA2100-256-Systems commercially available from MultiChannel Systems (Reutlingen, Germany) composed of a 256-channels amplifier head-stage. Recording was performed using the commercially available software (Multi Channel Experimenter, MultiChannel Systems). All the experiments were carried out with 256MEA100/30iR- ITO-w/o (MCS) that consist of 30-µm- diameter electrodes spaced by 100 µm. Raw data were off-line filtered by a Bandpass Butterworth filter 2nd order (with cut-off frequencies of 100 Hz and 2500 Hz) and plotted with a homemade program based on Matlab (The Mathworks co.).

DuaLink Shift™ microfluidic devices are compatible with MEA. Neurons and more particularly, synapses (stained in red), are in direct contact with electrodes (black spots) (Figure 6A). The extracellular activity of human glutamatergic neurons has been recorded and analyzed with a proprietary software showing action potential (red point) during record time (Figure 6B).

Moreover, electrophysiological analyses allow us to have a visualization of functional activity of the recorded neurons during time. We can have a representation of occurrences of action potentials during 10 min (60 seconds). In this recording, 9 electrodes were activated (9 blue lines) and each blue point represent an action potential detected at one time point.

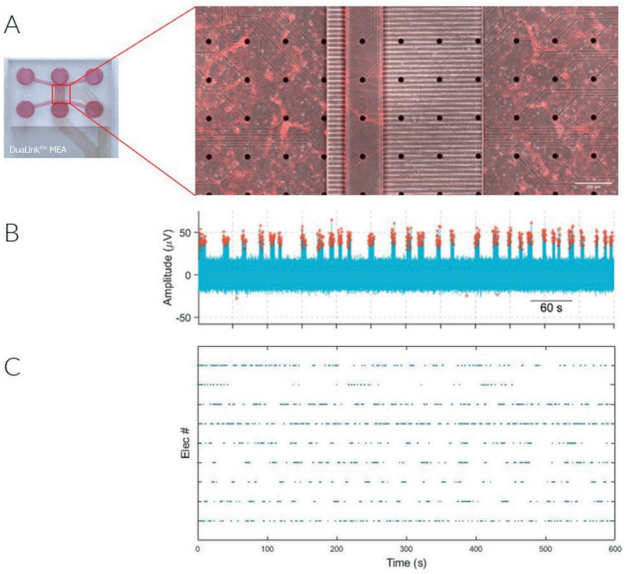


*Figure 6. Electrophysiological recordings in DuaLink Shift™ MEA.*
*(A) Schematic visualization of a DuaLink Shift™ MEA with a composite picture of glutamatergic neurons at day 21 seeded on MEA in brightfield contrast and immunofluorescence. Synapses were marked with Synapsin (1/200, in red).*
*(B) Plot of functional activity recorded by one electrode during 10 min. The red points are action potential detected.*
*(C) Raster plot of glutamatergic neurons functional activity recorded. The blue points represent action potential detected on active electrodes during time.*

## Drug testing

DuaLink™ microfluidic device can be used to isolate synaptic part of specific neurons co- culture. It can be useful to target specific components of synapses, such as receptor, pre- or post-synaptic part, or ionic channel. Thanks to his asymmetrical shaped, drug testing can be performed on each channel of the microfluidic device (several conditions) and electrophysiological activity of neurons seeded has been recorded. For example, it is possible to use reference compound, specific drug targeting synaptic receptor (NMDA, AMPA...) or synaptic inhibitor as TTX, calcium ion channel blocker, in parallel to pharmacological drug to test. Thanks to our proprietary software for neuronal network analysis, it is possible to analyze each channel separately or have a mean of the activity recorded by all the electrodes (corresponding to "DuaLink Shift" on Figure 7).

Relative mean firing rate is a metric of functional activity and corresponding to the number of spikes (action potential) per second. The graph present in Figure 6 shows that functional activity of the human glutamatergic culture is focused on channel 2, i.e., on the synaptic compartment.

Several experiments have been performed with different conditions of recording that allow to compare results between function activity from the whole neuronal culture and from neuronal parts in each channel. The relative mean firing rate (corresponding at the number of spikes per seconds) is concentrated in channel 2 which means that synaptic parts have an important functional activity (Figure 7). It is also possible to analyze the supernatant to quantify the release of neurotransmitters and inflammatory factors in each channel (data not shown).

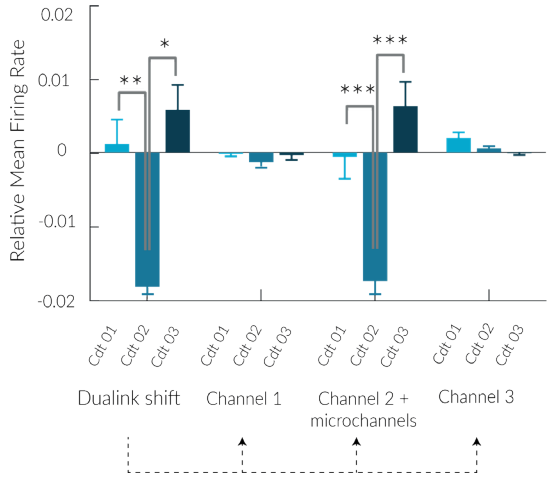


*Figure 7. Data plot of relative mean fire rate (spikes/s) quantification with three different conditions of the whole device and of each channels recorded.*

## CONCLUSION

NETRI's microfluidic devices DuaLink Shift™ allow to create an isolate synaptic compartment between two cell populations. This microfluidic device offers many advantages and uses compared to classical cell culture plate:

- Asymmetric shaped microfluidic device with 100 µm and 500 µm length of microchannel allowing selection of neurites throughout from one channel to another.
- Isolation of synaptic part for assays experiments, neurotransmitters or factors released assay, injuries, and disease modelling.
- Microfluidic device allowing all types of microscope compatibility and techniques such as brightfield and fluorescent pictures in high resolutive microscopy or confocal (Figure 8), calcium imaging and optogenetic.
- Seeding well high-throughput compatible allowing the use with cell culture automates.
- Device allowing electrophysiological recordings and analyses of each channel.

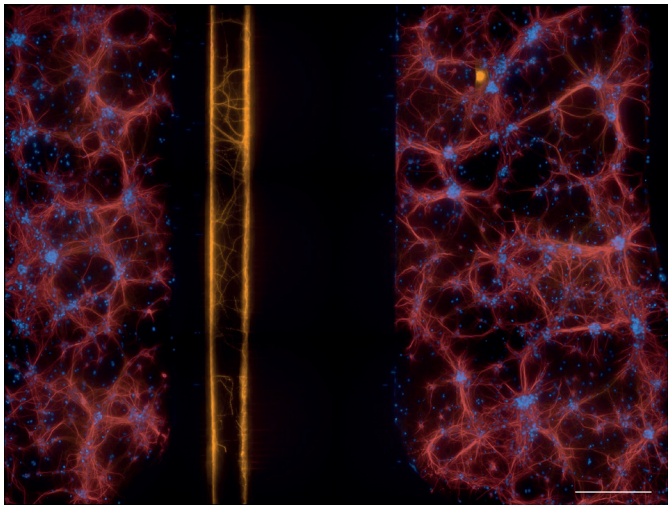

*Figure 8. Immunofluorescent picture of rodent hippocampal neurons at day 21 with MAP2 (1/100, in red) and Tau (1/100, in yellow) staining. Scale bar represent 200 µm.*

## RESOURCES

*Available upon request*

- Cell types Protocol - Glutamatergic neurons - Brainxell
- Cell types Protocol – Glutamatergic neurons MEA – BrainXell
- Cell types Protocol - Glutamatergic neurons - FujiFilm CDI
- Operating Protocol - Immuno-Staining
- Glutamatergic neurons (BrainXell) - Data Sheets
- Technical specifications - DuaLink Shift MEA
- Poster - Modeling the human Brain-on-Chip with human iPSC-derived Glutamatergic neurons
- Poster - Standardization criteria of hiPSC-derived neurons for Brain-on-Chip applications

*1. Shi, P. et al. Synapse microarray identification of small molecules that enhance synaptogenesis. Nat. Commun. 2, 510 (2011).*
*2. Virlogeux, A. et al. Reconstituting Corticostriatal Network on-a-Chip Reveals the Contribution of the Presynaptic Compartment to Huntington's Disease. Cell Rep. 22, 110–122 (2018).*
*3. Taylor, A. M. et al. Microfluidic multicompartment device for neuroscience research. Langmuir 19, 1551–1556 (2003).*
*4. Maisonneuve, B. G. C. et al. Deposition chamber technology as building blocks for a standardized brain-on-chip framework. bioRxiv 2021.06.21.449231 (2021) doi:10.1101/2021.06.21.449231.*
*5. Schindelin, J. et al. Fiji: An open- source platform for biological-image analysis. Nature Methods vol. 9 676–682 (2012).*

Based on 10 years of scientific research, NETRI has developed a unique know-how in designing organs/organoids-on-Chip by integrating disruptive building blocks into the same microfluidic devices, while maintaining industrial production standards compatible with pharma industry equipments & requirements.

Thanks to our patented technologies, we are capable of manufacturing prototypes and validating their biological function using primary animal or human induced pluripotent stem cells differentiated in our chip. Our unique infrastructure allows us also to scale up chip production for mass production.

Need more information about NETRI's products and services or how Organs-on-Chip revolutionize pre-clinical trials and human *in-vitro* models? Contact us!

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