High-content imaging of brain-on-chip microfluidic devices using PreciScan intelligent acquisition.

We address:

- How to automate image acquisition of microfluidic devices
- How to set up a pre-scan re-scan routine for selected relevant channels within brain-on-chip devices
- How to increase throughput for quantitative microscopy-based screening of brain-on-chip models

Introduction

Brain-on-chips (BoCs) are pioneering research tools that simulate complex brain environments, significantly enhancing our understanding of neurodegenerative diseases (NDDs). These microfluidic devices not only enhance our insights into brain biology but also hold the potential to accelerate the development of novel therapeutic strategies.[1, 2] Compared to traditional 2D *in vitro* models, BoCs improve the cell micro-physiological environment, enhance the relevance of cell phenotypes and better replicate neuronal connectivity and neuronal electrical activity. This makes them invaluable for studying the intricate mechanisms of NDDs and testing new treatments.

Traditionally, organ-on-chip platforms have been designed in a format that allows for testing only one condition per chip, which limits their effectiveness in drug discovery. To enhance usability, increasing the throughput of these systems is crucial for advancing both drug discovery and disease modeling. Companies, such as NETRI, are now offering brain-on-chip platforms that can be assembled to meet SBS plate standards and that enable testing of up to 16 conditions per plate **(Figure 1)**.

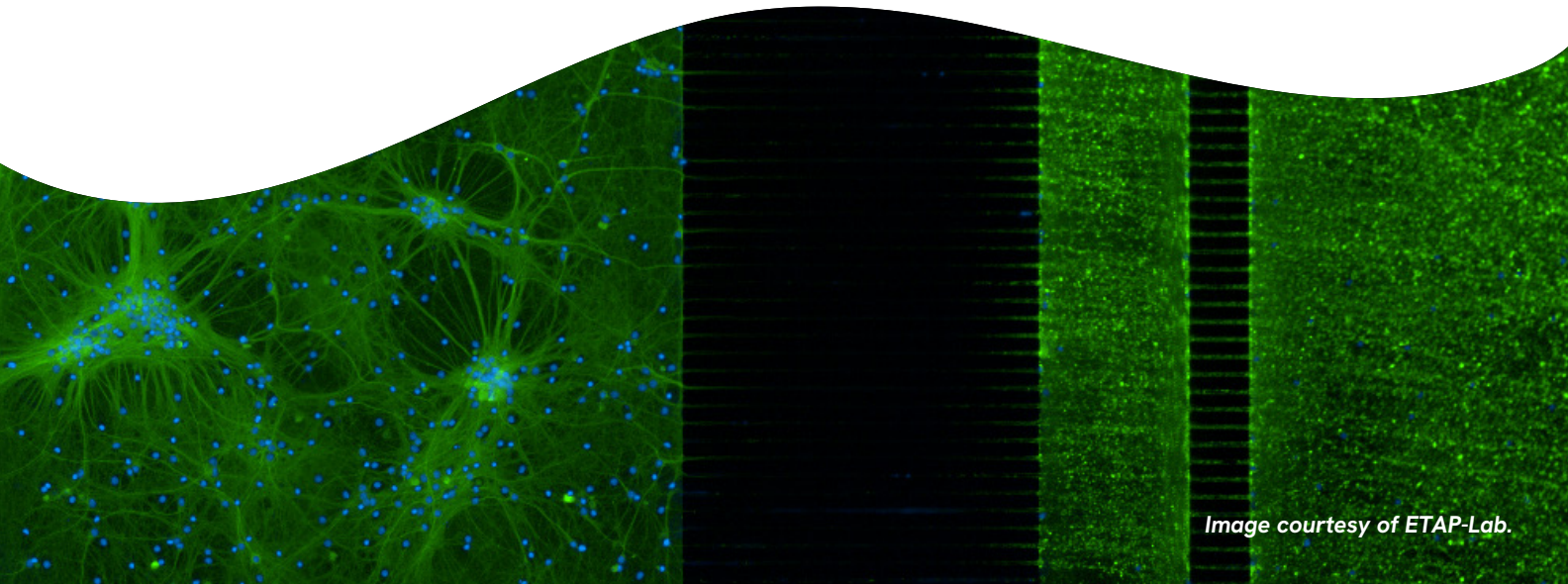


Image courtesy of ETAP-Lab.

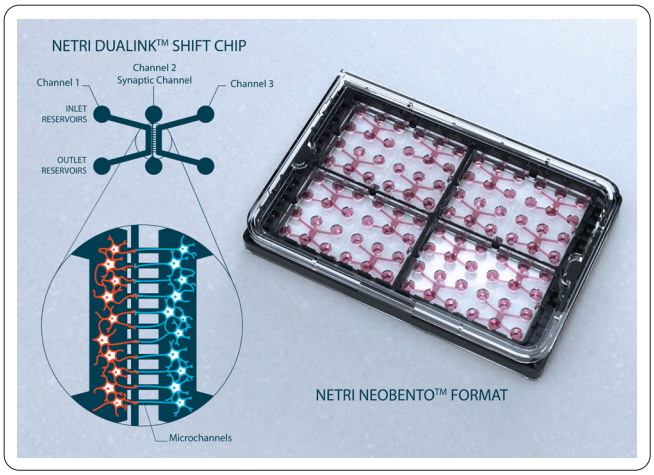


Figure 1: Schematic of a DuaLink™ Shift: Each chip is made of three channels (channels 1, 2 and 3), with 200 microchannels connecting adjacent channels; enabling compartmentalized fluidically-isolated neuronal culture. Sixteen chips made of polydimethylsiloxane (PDMS) with a thin bottom layer optimized for imaging are organized in the NeoBento™ format, which adheres to the SBS standards, ensuring compatibility with automated dispensing and high-content imaging.

To model diverse NDD pathophysiologies and meet the needs of different applications, a range of BoCs have been developed with various channel architectures. However, these varied architectures present challenges, particularly in automating image acquisition and analysis. Typically, the positioning of areas of interest, such as certain channels, vary between different chip models and sometimes between plate batches. High-content imaging acquisition protocols must be adaptable to effectively accommodate the diverse architectures and tolerances of various organ-on-chip systems.

Here, in collaboration with ETAP-Lab, we show an automated image acquisition protocol for NETRI DuaLink™ Shift microfluidic devices on the Operetta CLS™ high-content analysis system. Using PreciScan intelligent acquisition, channel 2 of the device could be robustly identified in a low-resolution prescan, followed by high-resolution rescan of only this region of interest with the 40x objective.

ABOUT ETAP-Lab

With 30 years of expertise in neuropharmacology and cell culture, associated with unique capabilities in producing human neurotoxins involved in NDDs, ETAP-Lab leads several research projects aiming at developing translational *in vitro* models of CNS diseases to improve drug development.[3]

Microfluidic device for neuronal research

The NETRI DuaLink™ Shift microfluidic device is designed to facilitate compartmentalized cell culture experiments. These chips allow the independent culture of different cell populations from various origins, including neuronal cells, cell lines, iPSCs or primary cells, in each of its three separate channels. Channels are connected by microchannels in which neurites can grow and connect to other channels' cell populations, while ensuring fluidic isolation between them. This mimics the specific architecture of the brain characterized by distant neuronal populations or tissues (i.e. with different local environments) connected *via* nervous tracts (**Figure 1**). Cells can be cultured for extended periods of time (up to 4 weeks for neuronal cells) until maturation. After biological experiments, cells can be fixed and stained for phenotypic readouts.

PreciScan intelligent acquisition setup

Chips were imaged using the Operetta CLS high-content analysis system, equipped with a 10x objective in widefield mode, with 5% image overlap (pre-scan). The SBS format allows for quick registration and accurate imaging positioning with Harmony™ 5.2. The 16 devices were imaged in 3 colors in less than 5 min. Analysis was conducted using the Global Image generated from the pre-scan acquisition. Channels and grooves can be identified using *Find Texture* Region, allowing for segmentation of the various compartments. The edges of the selection were smoothed by resizing the region (Select Region / Resize). Finally, each channel, based on its position in the chip or based on biological information (fluorescent signal for example) can be selected for subsequent re-scan acquisition (Determine Well Layout), here with the 40xW objective. The thin PDMS bottom devices are compatible with high magnification water immersion objectives (20xW and 40xW validated). (**Figures 2 & 3**).

ABOUT NETRI

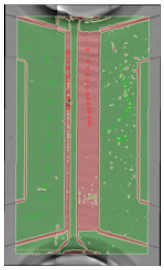
NETRI is an industrial start-up that supports the creation of human organs-on-chip for healthcare applications. Through advanced microfluidics and electrophysiology, their NeuroFluidics™ platform focuses on predicting drug efficacy or toxicity with AI, and supports pain, cosmetics, neurological disorders, and agricultural applications.

1

Input Image
Global Brightfield Image to visualise the chip

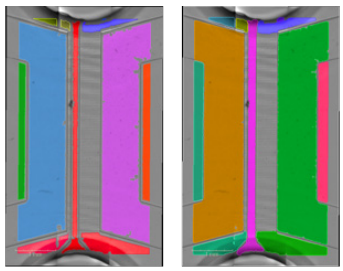
2

Resize
If relevant remove the inlets of injection of the image to segment *(Select Region)*



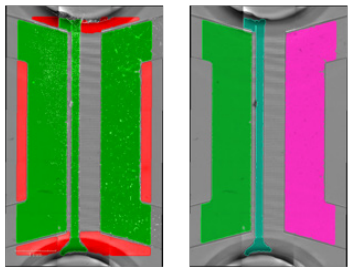
3

Supervised Machine Learning
On Brightfield Image, train the software to identify the channels and the grooves to enable individual detection of each channel *(Find Texture Region)*.



4

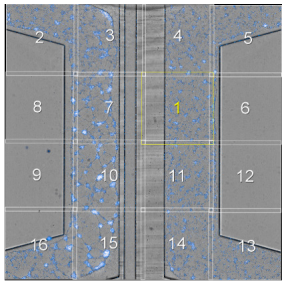
Smoothing
Holes can be filled in channels (*Modify Population*). Size can be adjusted to improve compartmentalization *(Select Region)*.



5

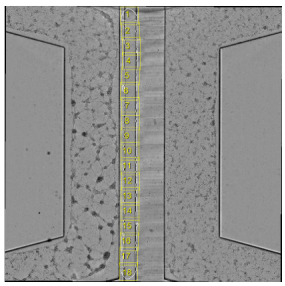
Selection
Channel of interest can be selected based on size and morphologies properties *(Calculate Morphology Properties, Select Population)*

Figure 2: Setup of the PreciScan Analysis Sequence. Using a low resolution pre-scan image, an analysis sequence is set up to identify the 3 channels of the DuaLink Shift™ microfluidic device. Once the 3 channels are properly detected, an automated re-scan can be set up to image only the channel of interest. This way, PreciScan reduces the image acquisition and analysis time and keeps the data volume manageable.



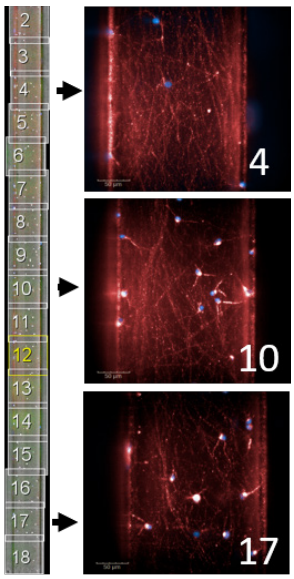
1

Pre-scan
On Brightfield image with 5% overlap, the whole chip is acquired



2

PreciScan Analysis
The image analysis sequence (Fig 2) is used to identify the channels. Here channel 2 is selected for re-scan with the 40x objective and FOVs are illustrated.



3

Re-scan
Highlight of the re-scan routine using the 40x objective and few example FOVs along the channel.

Figure 3: PreciScan intelligent acquisition of channel of interest in brain-on-chip microfluidic device. Primary cortical neurons were obtained from rat embryos' brain as previously described.[4] Dissociated neurons were seeded at 40,000 viable cells in Channel 3. After 2 weeks of maturation, neurites have grown through microchannels and invading Channel 2. After fixation, cells were stained (cytoskeleton and nuclei). Cell culture, immunostaining and imaging performed by ETAP-Lab.

Conclusion

Unlocking the great added value of microfluidic devices in replicating brain architectures requires overcoming imaging challenges compared to conventional 2D assays.

Here, we demonstrate that the Operetta CLS high-content analysis system allows automated image acquisition of neuronal cells in microfluidic devices conforming to SBS format. By utilizing PreciScan intelligent acquisition, the region of interest on the chips could be robustly identified, providing high-quality images of the relevant channels while avoiding capture of unnecessary data. Additionally, Harmony 5.2 software facilitates rapid and accurate analysis of a wide variety of cellular features within the spatially separated cellular compartments.

These results pave the way for wider adoption of BoCs in drug discovery programs. For further information on the work carried out by ETAP-Lab, read our interview article with CEO, Nicolas Violle.

References

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2. Miny L, Maisonneuve BGC, Quadrio I, Honegger T. Modeling Neurodegenerative Diseases Using *In Vitro* Compartmentalized Microfluidic Devices. Front Bioeng Biotechnol. 2022 Jun 24;10:919646.
3. Colin, J., Allouche, A., Hidalgo, S., Lager, E., Lemoine, S., Muller, C., ... & Violle, N. New Alzheimer models for drug screening based on improved human amyloid beta (1-42) oligomer preparations. *Alzheimer's & Dementia. 2021.* 17, e054152.
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