# Deposition chamber technology as building blocks for a standardized brain-on-chip framework

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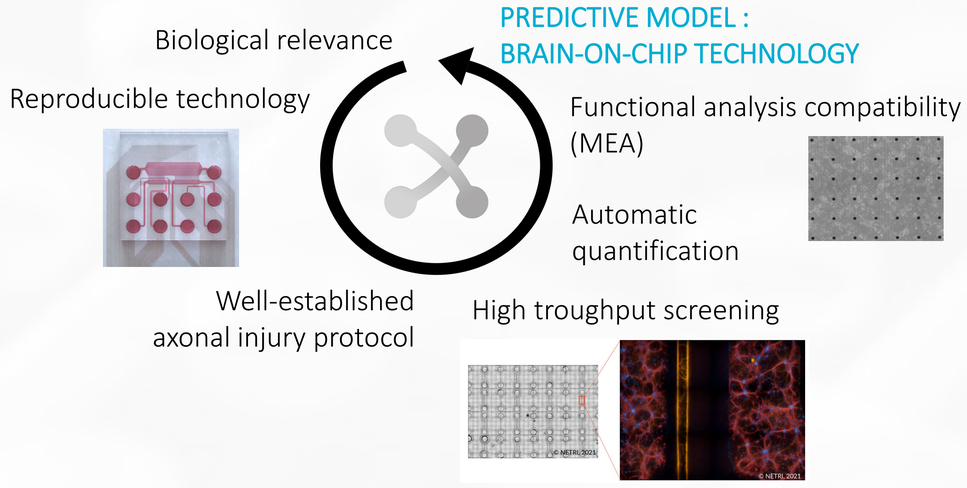
## Background

Animal modelling is currently the gold standard when studying neuropatholgies, for lack of a better alterntives. In the last decade, microfluidic based models have started to appear, and have the potential to be able to model human brain connectomes, while offering scalable platforms potentially comptible with:

- high throughput automated technologies,
- high resolution microscopy,
- functional analysis.

Such models have to recapitulate several key features, such as the types of human cells used in relevant proportions, directional connectivity between these populations etc. One of these key factors, namely the relevant proportions between the various cell types, has remained unexplored. Here, we present an innovative microfluidic design, called a 3D-Deposition Chamber, that allows to control the seeding of several neuronal populations in microfluidic devices, in terms of number, density and, homogeniety, while supporting their culture and maturation.




## Results

### 1 What is a 3D-Deposition Chamber?

The device is constituted of 3D Depositon Chambers (one or more) each connected to an inlet and outlet with precisely tailored hydraulic resistance to ensure control over the seeding of neurons. Each 3D-Deposition Chamber can then be connected via microchannels, ensuring control over the interconnectivity between the seeded populations and fluidic isolation.

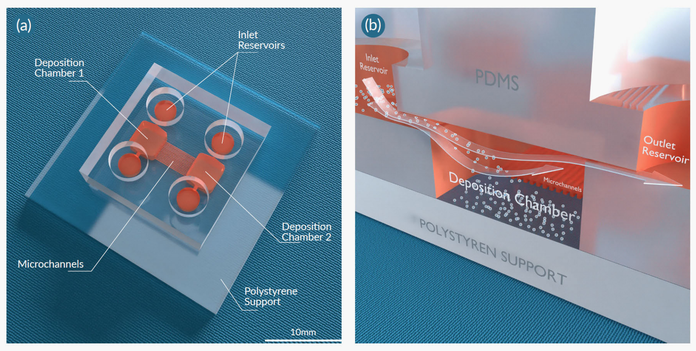



**Fig. 1: NETRI's 3D-Deposition Chamber microfluidic device. (a)** Artist view of the microfluidic design of brain OoC and **(b)** of a 3D-Deposition Chamber.

### 2 The 3D-Deposition Chambers allow reproducible control over the number of cells seeded and their homogeneity

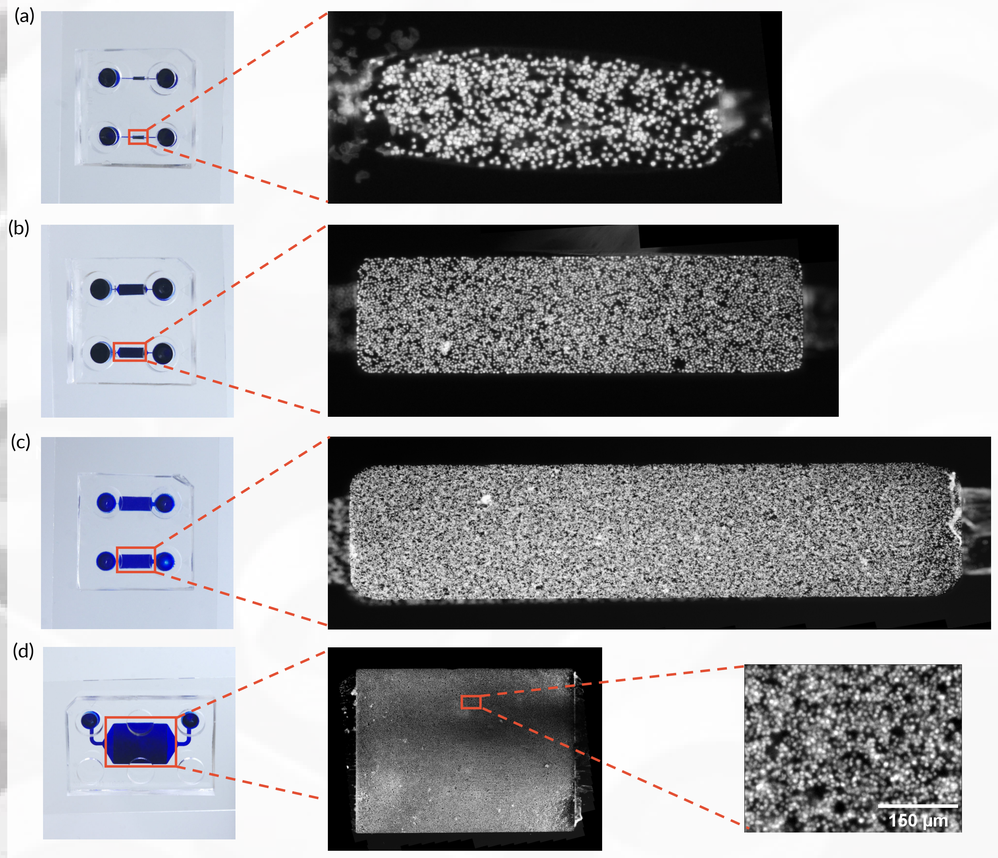



**Fig. 2: Example of 3D-Deposition Chambers with seeded with neurons.** The 3D-Deposition Chambers contain **(a)** $10^4$ neurons, **(b)** $5.10^4$ neurons, **(c)** $10^5$ neurons and **(d)** $10^6$ neurons.

### 3 3D-Deposition Chambers enable long term neuronal cultures with excellent viability

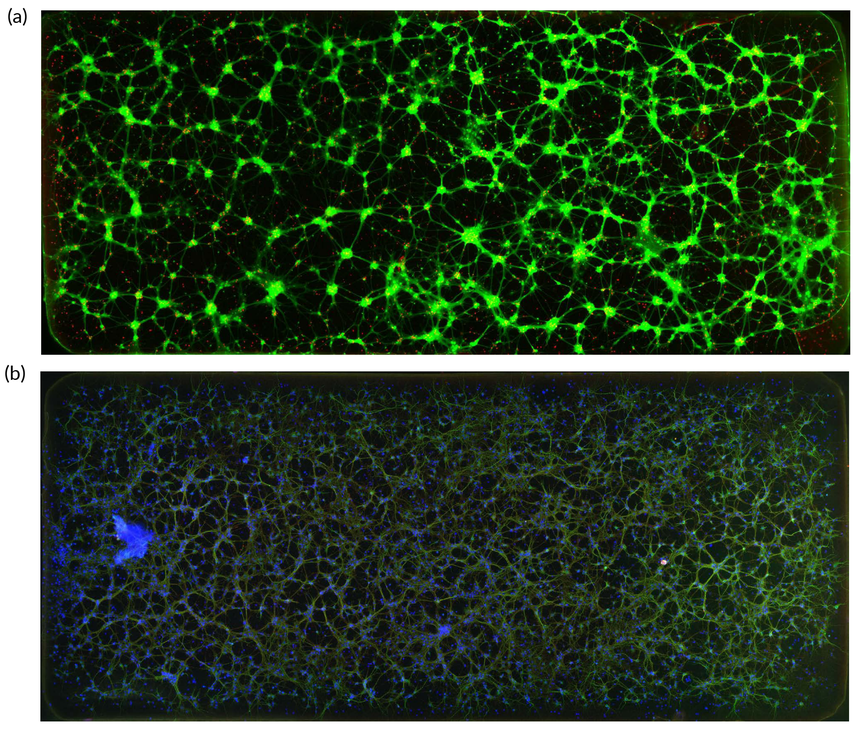



**Fig. 3: Illustrative pictures of embryonic rat hippocampal cell culture at 18 DIV. (a)** Staining with the LIVE/DEAD® Viability/Cytotoxicity Kit for the assessment of alive cells (green) and dead cells (red). **(b)** Visualization of axons Tau (red) and dendrites MAP2 (green) against and and counterstained with DAPI (blue). Scale bars indicate 200 µm.

### 4 Example of 3D-Deposition Chambers to create minimalistic Basal Ganglia Loop models on MEA substrates

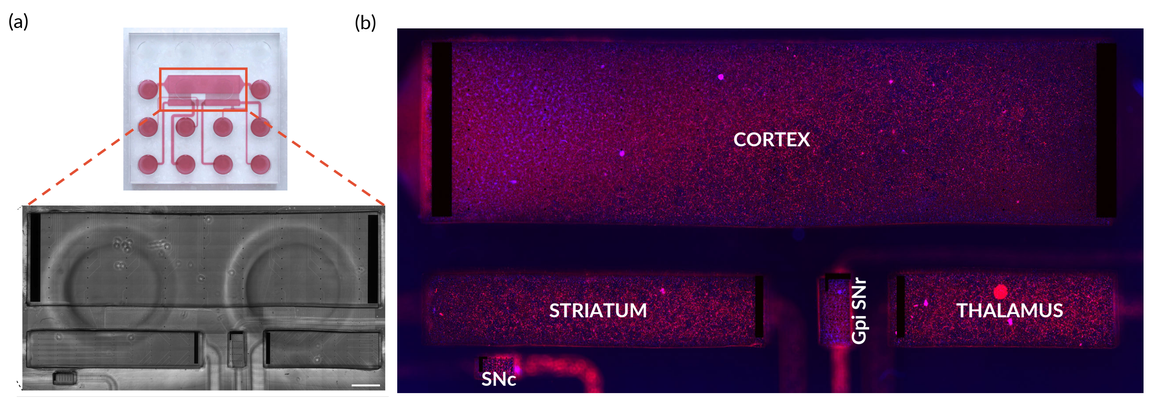



**Fig. 4: 3D-Deposition Chambers to create a model of basal ganglia circuit on a chip. (a)** Scheme representing the regions and connections within the in vivo circuit. GPi: Glubulus Pallidus internal, SNr: Substantia Nigra reticularis. SNc: Substantia Nigra compacta. **(b)** Image of the reconstructed basal ganglia circuit on a chip (filled with blue ink). Inset: Transmission light microscope image of the MEA aligned on the neurofluidic architecture. **(c)** Immunofluorescent pictures of 18 DIV embryonic rat hippocampal with anti-MAP2 (Red) and with DAPI (blue). All images were obtained using a 10x objective.

## Conclusions and Perspectives

**NETRI'S MICROFLUIDIC DEVICE FOR A STANDARDIZED BRAIN-ON-CHIP FRAMEWORK**

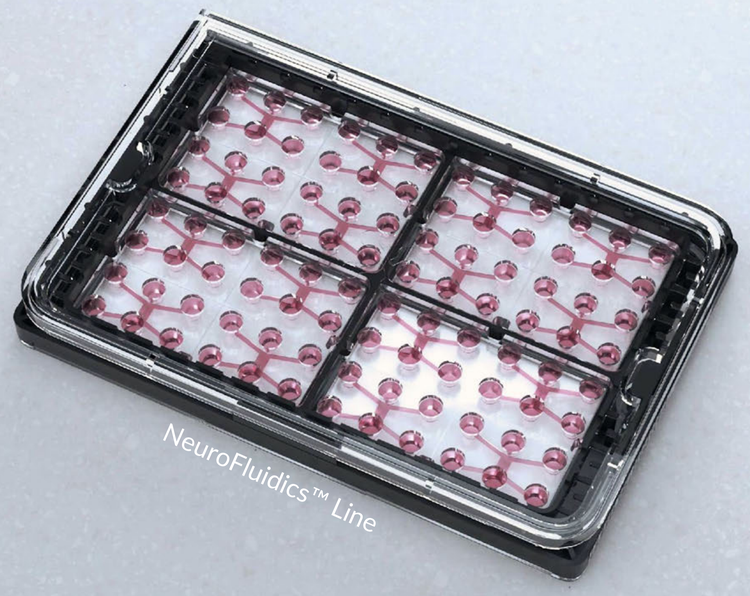
OoC technologies are state-of-the-art research tools that allow the construction of *in vitro* models with an accurate structural design at the organ level. This innovant microfluidic 3D-deposition chamber :

- Allows to model complex neural circuitry present in the intact brain
- Provides the scientific community with standards matching industrial applications,
- Allows a faster standardization and adoption of OoC by the pharmaceutical industry.

There are still challenges for the validation of the OoC Basal Ganglia loop model as :

- Including accurate neural subtype seeding in each node,
- Controlled directional connectivity between nodes,
- Network wide electrophysiological recordings and connectivity mapping.

We applied such innovative system to build an *in vitro* multi-nodal depiction of the basal ganglia loop of the brain, whose dysfunction leads to neurodegeneration such as Parkinson's disease.

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**Other NETRI publication:**

- Miny L, Maisonneuve BGC, Quadrio I and Honegger T (2022) Modeling Neurodegenerative Diseases Using In Vitro Compartmentalized Microfluidic Devices. Front. Bioeng. Biotechnol. 10:919646.doi: 10.3389/fbioe.2022.919646
- Guichard, A., Remoué, N., & Honegger, T. (2022). In Vitro Sensitive Skin Models: Review of the Standard Methods and Introduction to a New Disruptive Technology. Cosmetics, 9(4), 67. https://doi.org/10.3390/cosmetics9040067

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