Organ on chip approaches for Neuroscience: Neurofluidics reconstruction of neural networks and functional markers in drug discovery

TISSUE ENGINEERING FOR INDUSTRY, Adebiotech,
December 2021

Thibault Honegger, PhD
CEO & co-founder

NETRI

ETHICAL PRESSURE

POST COVID PRESSURE

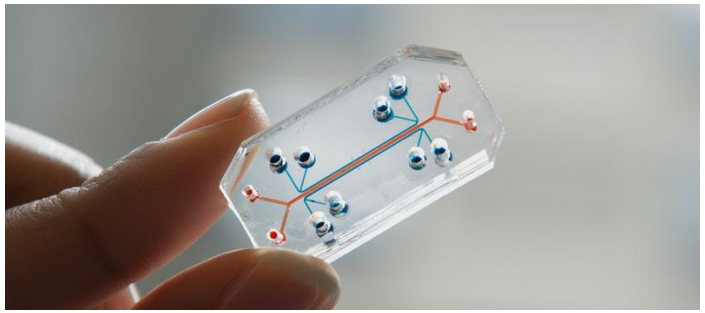
AGE PRESSURE

TECHNOLOGIES IN MATURATION

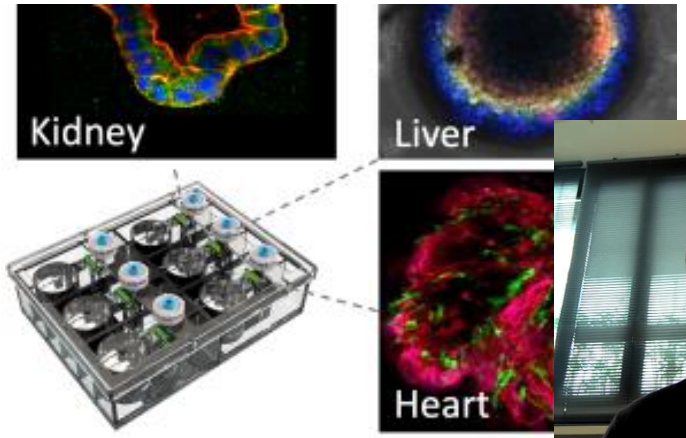
POSITIONED STAKEHOLDERS

REGULATORY INCENTIVES

PHARMA WILLINGNESS



THE REVOLUTION OF ORGAN-ON-CHIP IS COMING



Organs-on-chip

NETRI
PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY

Why ? Everything we do, we believe in challenging the way we discover new treatments

How ? We build our products with a mutlidisciplinary approach, simple to use and user friendly

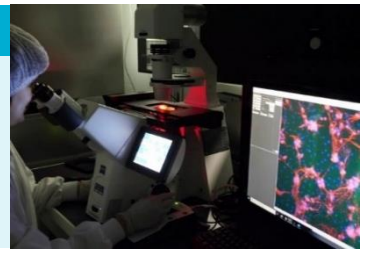
What ? We design Organs-on-a-chip to industrialize and standardize in-vitro human predictive models

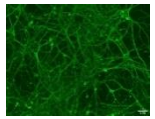
ENGINEERING

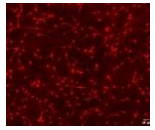
Patented technologies:
Control of the production line

BIOLOGY

Multi-organ biological validation:
Human Stem Cells

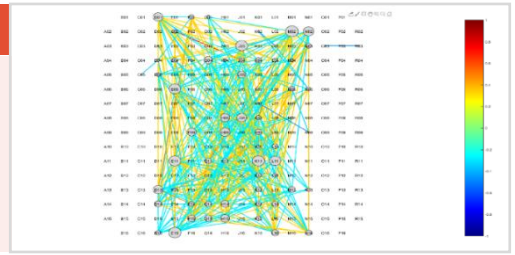






DIGITAL

Proprietary algorithms:
Analysis of neuronal activity



Organs-on-chip

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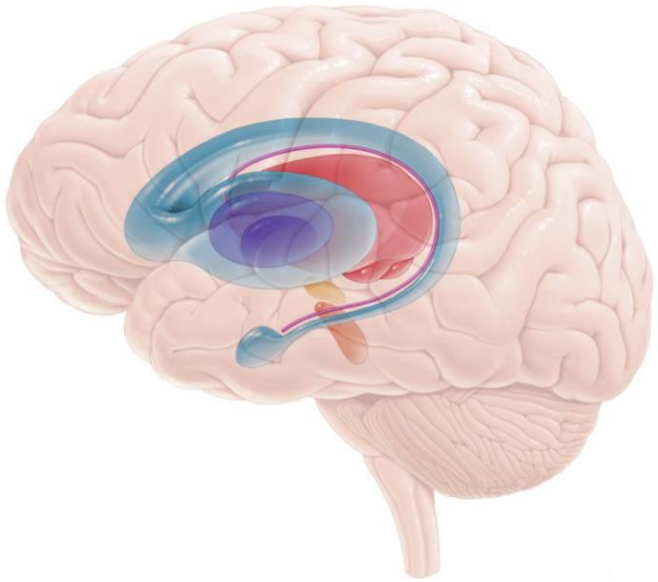
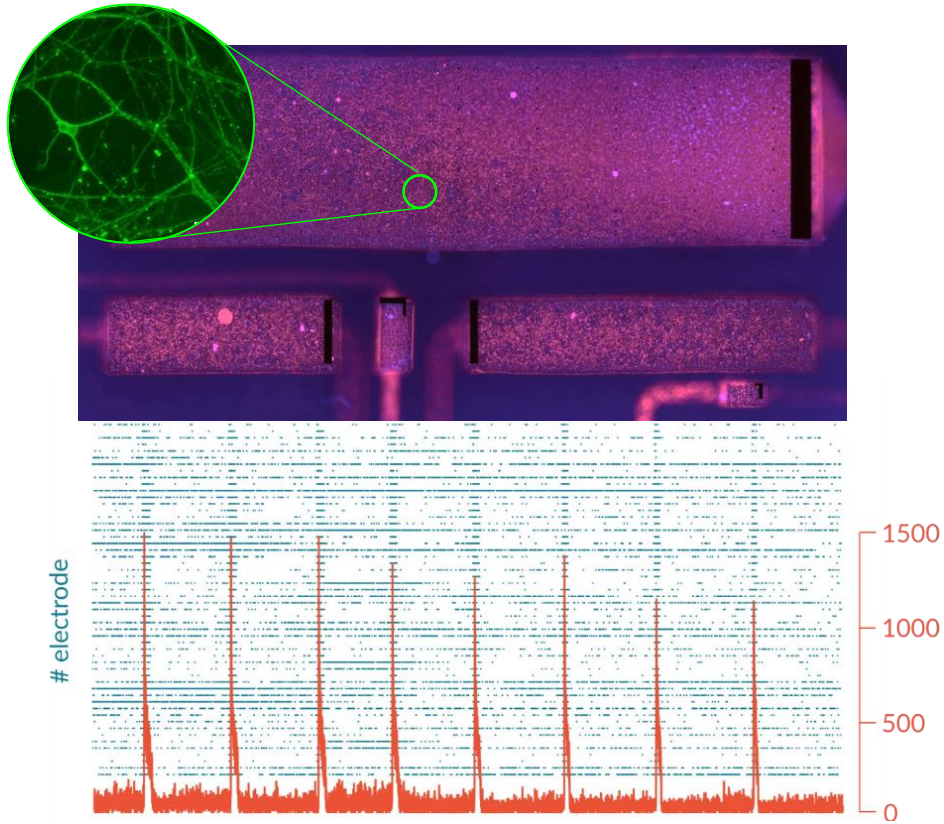


PHYSIOLOGY

A PHYSIOLOGY is a system of cell interaction observed in the real world, *in-vivo*.

MICROFLUIDIC CHIP

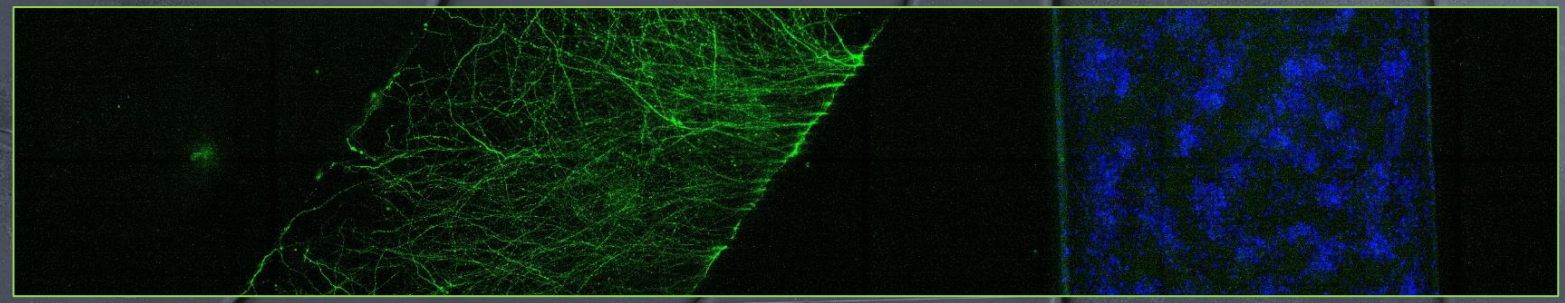
A CHIP is a collection of microfluidic functions capable of supporting an in-vitro MODEL.
A CHIP is an engineering product.



PREDICTIVE MODEL

A MODEL is a simplified but reliable *in-vitro* representa
PHYSIOLOGY in a C
A MODEL is a matter of
supported by Digit

Microchannels



Microchannels

- Connection of chambers by microchannels
- Unidirectional or bidirectional growth of neurites
- Fluidic isolation for axonal transport only
- Control of the number of connections
- Allow the maximise projection rate of neurites
- Recreate in a single chip the physiological behaviors of different area of the same organ, the interaction of a tissue with another one or complex neural networks
- Example :
  Neuron + Muscles = LAS (Laterale Amyotrophic Sclerosis)

3D-Deposition Chamber

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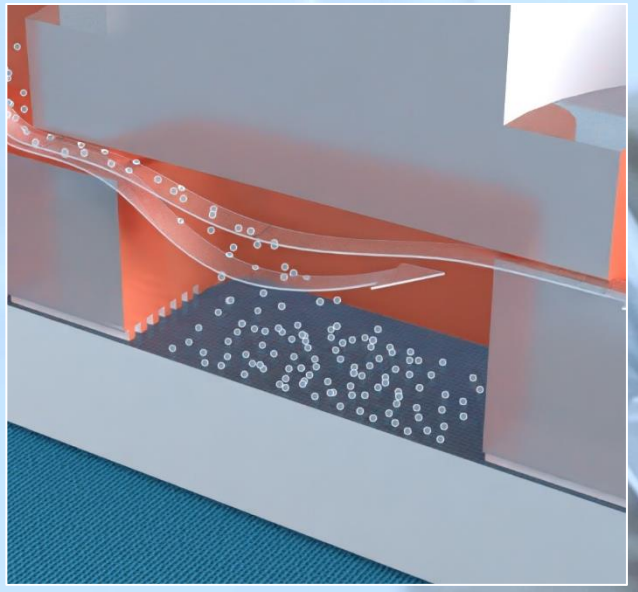

3D-Deposition Chamber

- Control of cells density (300-3000 cells/mm^2) and homogeneity
- Control of media change (50, 75 or 100%)
- Multiple seeding in the same cham

Cereb
Fœta

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

B. G. C. Maisonneuve[1], L. Libralesso[2], L. Miny[3], A. Batut[3], J. Rontard[3], M. Gleyzes[3], B. Boudra[3], J. Viera [3], D. Debis[3], F. Larramendy[1,3], V. Jost[2] and T. Honegger[1,3,*]

1 Univ. Grenoble Alpes, CNRS, LTM, 38000 Grenoble, France
2 Univ. Grenoble Alpes, CNRS, GSCOP, 38000 Grenoble, France
3 NETRI, 69007 Lyon, France

Membrane

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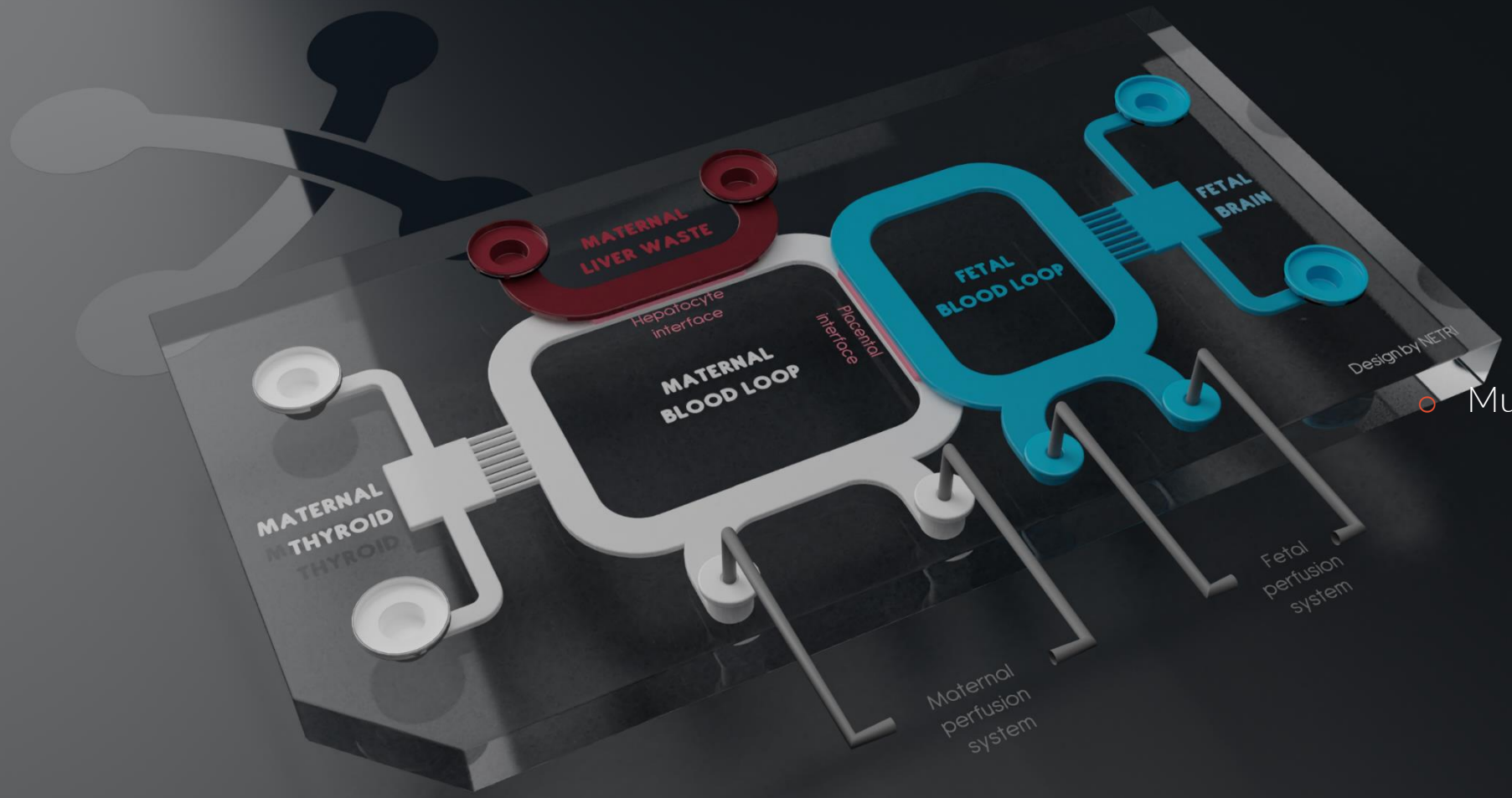
Membrane

- Porous polycarbonate membranes integrated into 3D Deposition Chamber
- Creation of compartmentalized and connected barriers
- Physiological separation
- Perfusion/diffusion
- Example :
  Intestinal Barrier =
  Trials on microbiota

Modular integration



Modular integration

- Multi-organs/organoids architecture
- Multi-technology integration
- Membrane integration
- Co-culture in the same chip

Impact of a mo
treatment on her

MEA Compatibility

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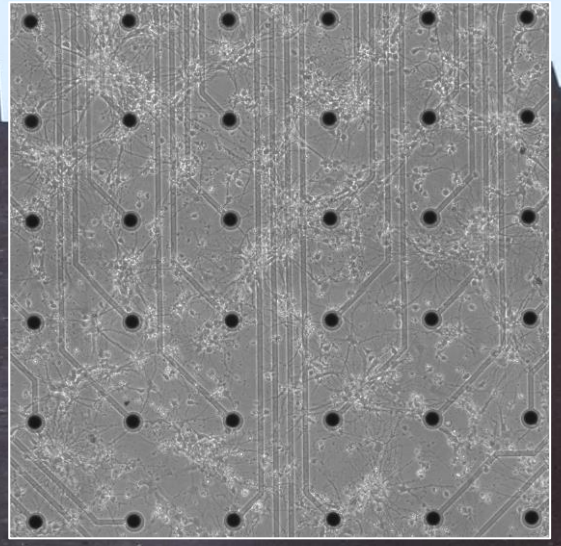

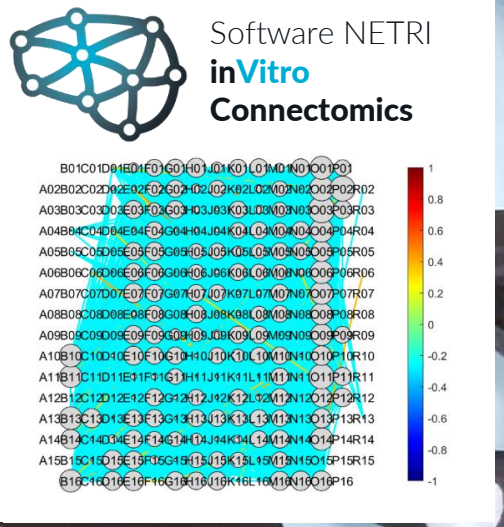

MEA Compatibility

- Detecting electrophysiological signals
- Continuous recordings of functional activity and network dynamics
- Functional activity of the entire network
- Proprietary InVitro Connectomics (IVC) algorithms to extract functional network markers
- Example :
  Network impact of a drug on Parkinson

NeuroBento™

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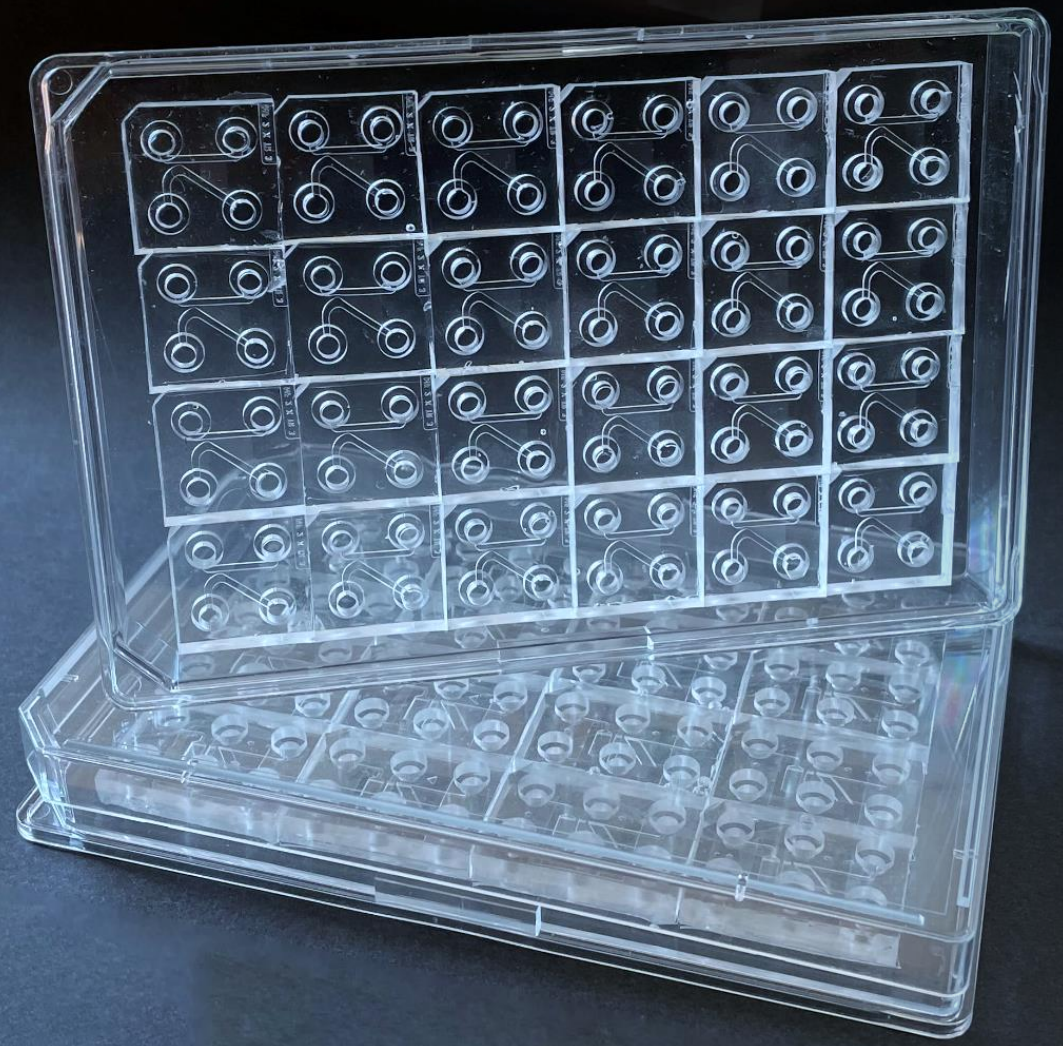

NeuroBento™

- Standard SBS alignment on 96-well microplates format
- Compatible with HTS & all 96-well microscope jigs
- Optical transparency
- Compatible with all transmissive imaging methods or any microscope, direct or inverted
- No pump or mechanical stirrer needed
- Optimized design to ensure the maturation of cell

Compatibility with industria

Standard products compatibility with HTS equipements

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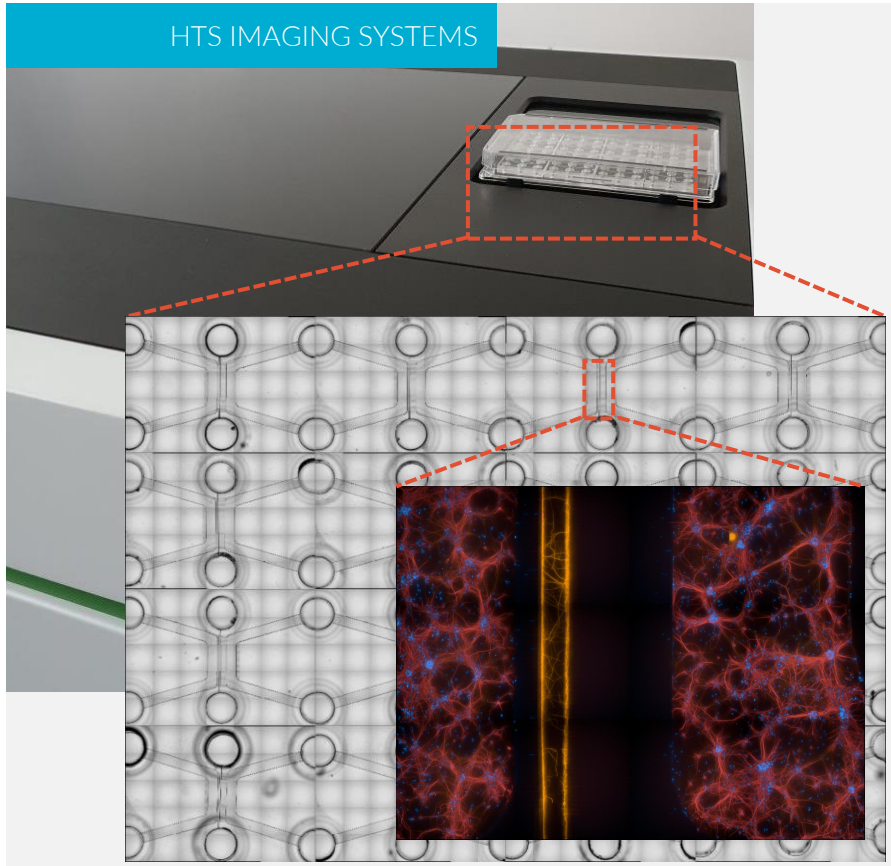
HTS IMAGING SYSTEMS

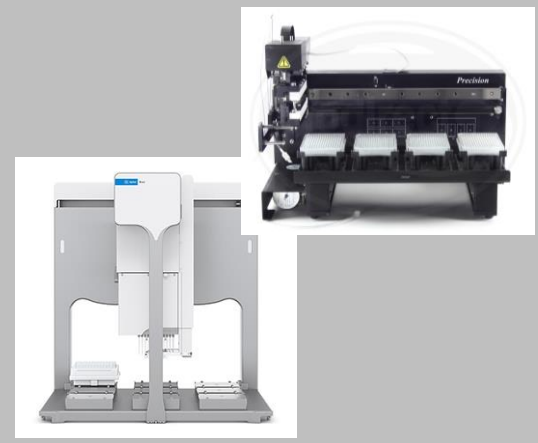


PerkinElmer
ZEISS
ThermoFisher SCIENTIFIC
MOLECULAR DEVICES

HTS MEA RECORDING

AXION BIOSYSTEMS

LIQUID HANDLING ROBOTS

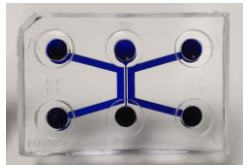


Agilent Technologies
opentrons
ThermoF SCIENT

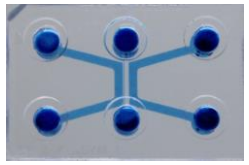
Current portfolio

NETRI
PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY

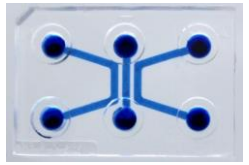
8 MICROFLUIDIC CHIPS

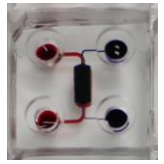

NF_ 3_X_NFI

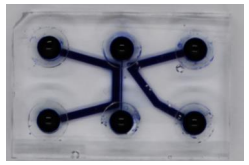

NF_1_CD_100


NF_3_X_ASYM


NF_4_M2


NF_ 3_X_SBS

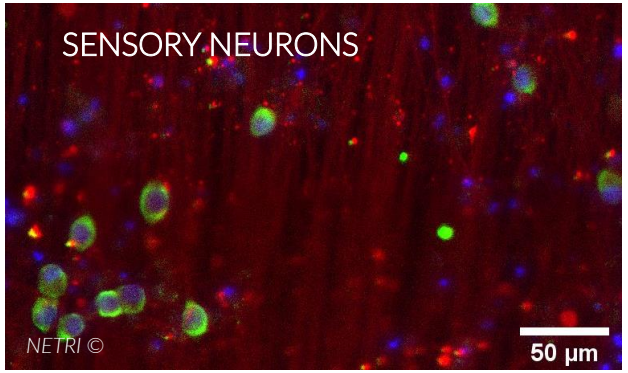

NFM_2_XCD_BBB

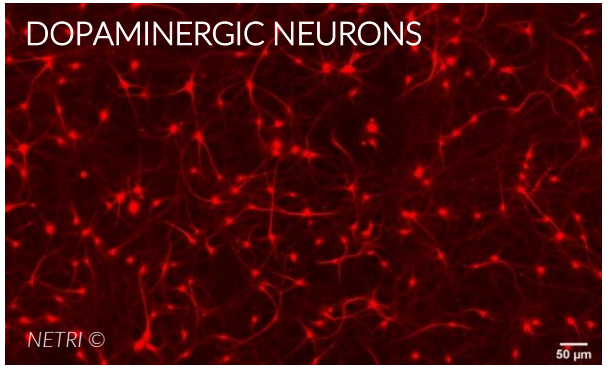

NF_ 3_X_TR

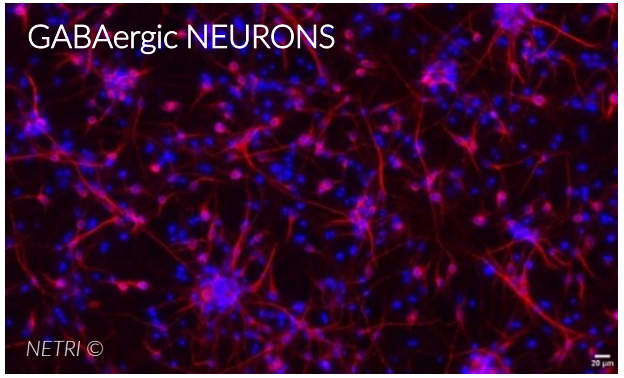

NFM_4_XCD_Skin

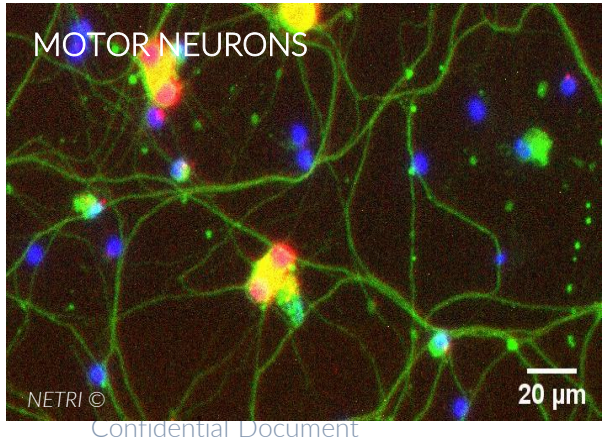
5 HUMAN CELLS TYPES VALIDATED

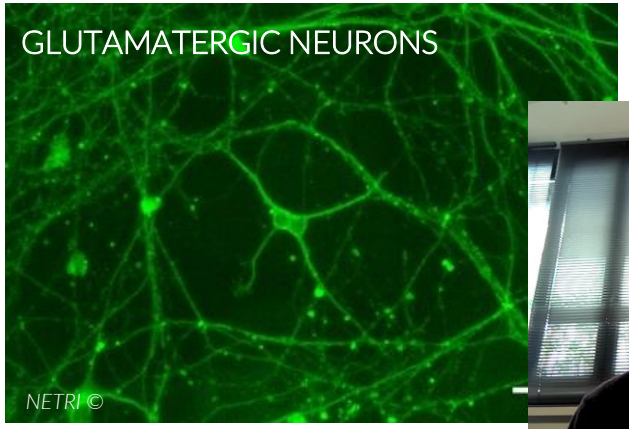
A solid foundation for more complex models



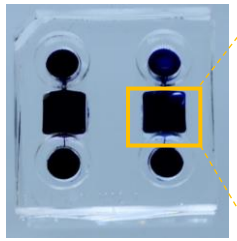


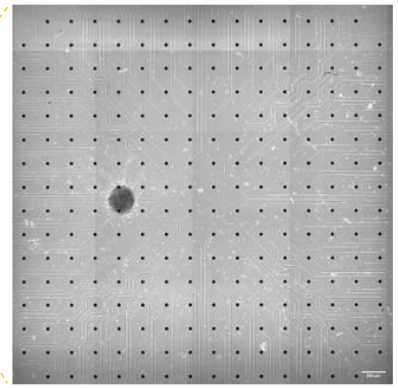




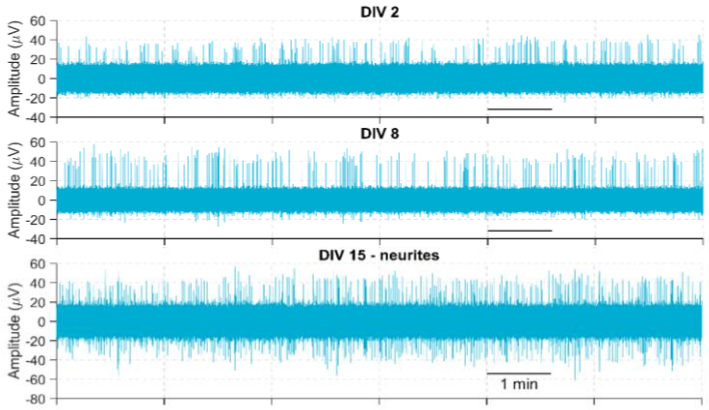


Organoids/explants-on-chip

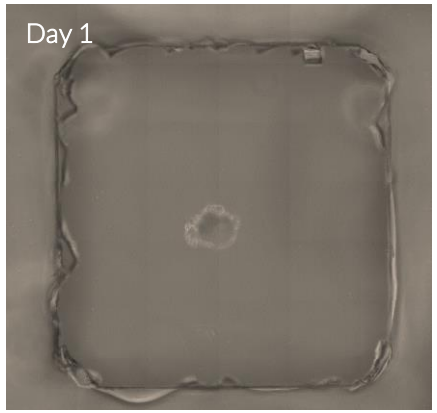




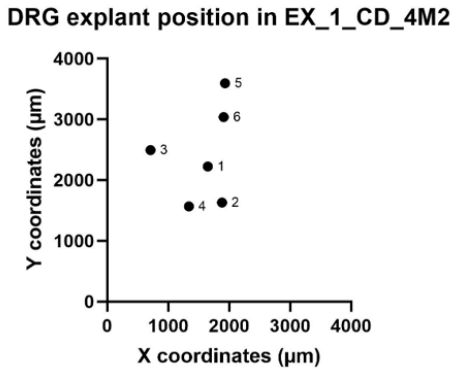
Picture of DRG seeding in EX_1_CD_4M2 device coupled with MEA electrodes.



Recording of spontaneous activity of DRG explant in EX_1_CD_4M2 device coupled with Multi Electrod Array (MEA).



Brightfield pictures of DRG explant in EX_1_CD_4M2 device.



Graph DRG coordinates according to the tested conditions

Conditions	% of positionning DRG in the microfluidic chamber (total assays)
1	25 (8)
2	38 (8)
3	13 (15)
4	88 (8)
5	21 (14)
6	25 (12)
7-12	0

Table of condition tested with successful DRG positioning in deposition chamber. Dorsal Root Ganglia: DRG.

3D-Deposition Chamber Chip
4000 µm width

ORGANOIDS

Challenge: How to grow and maintain an organoid in a microfluidic device?

Solution: Use a microfluidic device large enough to allow any 3D cell culture, with an initial diameter of less than 500 µm, to be positioned in a microfluidic chamber and grown to 4000 µm under physiological conditions and to record the functional activity of the entire 3D cell culture.

Possible applications

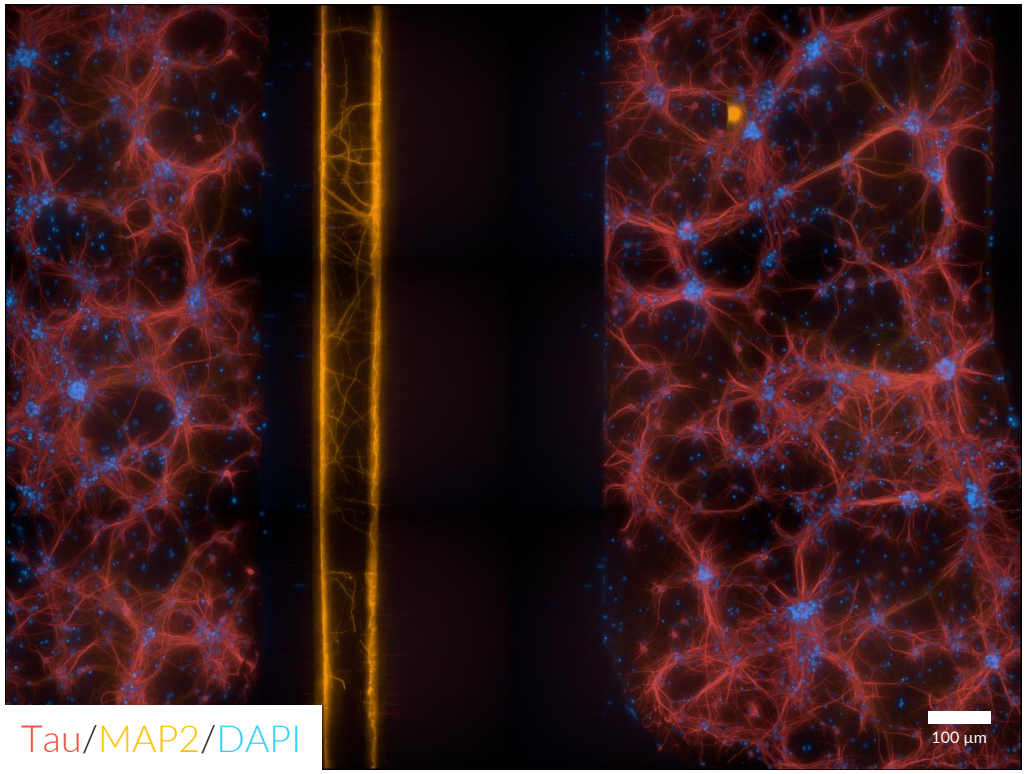
- Seed of an one organoid/explant (with an initial diameter <500 µm) per chamber with realiable positioning protocol
- Long term culture in microfluidic device up to a diameter of 4000 µm
- Recording func
entire surface
- Compatibility w
Assays – up to
multiplexed

References

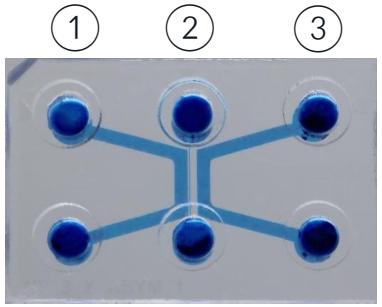
- Application Note: DR
- Operating Protocol: D
- Technical Sheets: DR

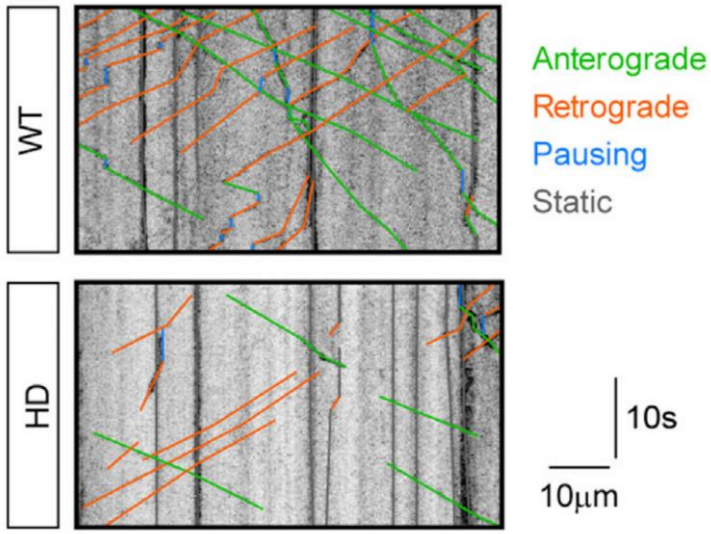
Huntington on chip

① Cortical chamber ② Synaptic chamber ③ Striatal chamber



Tau/MAP2/DAPI





Adapted from Virlogeux *et al.*, 2018, Cell Reports 22, 110–122

Asymetric chip

SYNAPTIC ISOLATION

Challenge: How to assess the involvement of cortico-striatal synapses in Huntington's disease?

Solution: Co-culture of 3 types of cells using NF_3_X_ASYM chip which is composed of 3 compartments connected by microchannels.

Possible applications

- Evaluation of synaptic markers (Pre- & Post-)
- Quantification of vesicular neurotransmitters released in surnatant
- Quantification of dendrites vs axons
- Possible dopa
  hiPSC derive

References

- Product references:
- Operating Protocol:
- Technical Sheet: DR

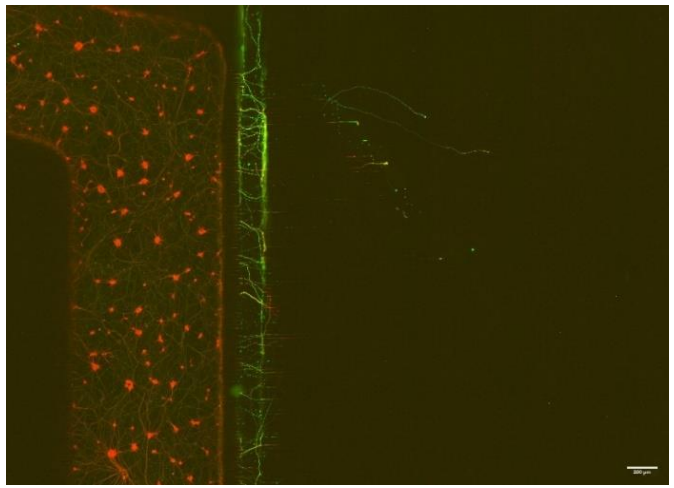
Peripheral Pain on chip

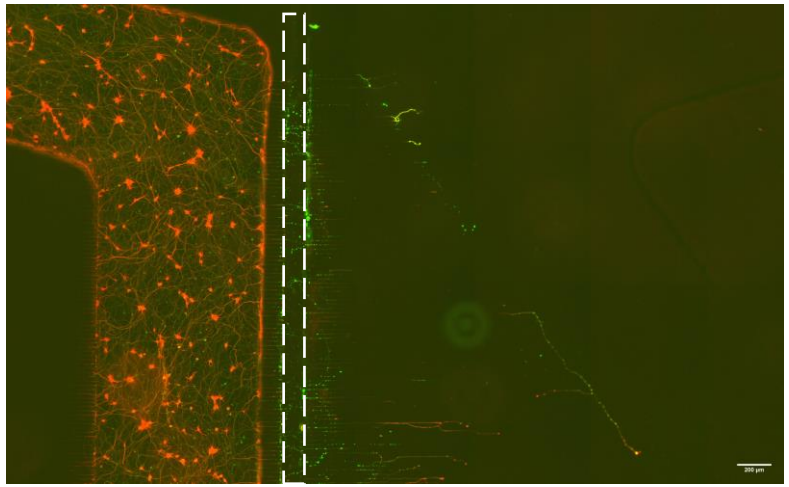
Before Axonal injury (Day 30)

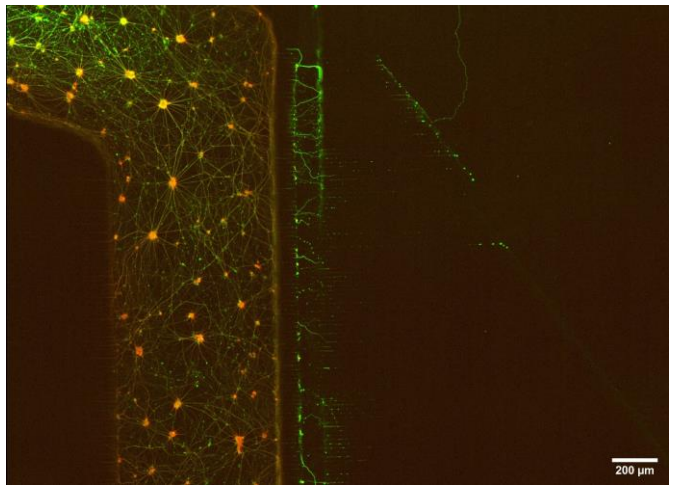
Axonal injury (Day 30)

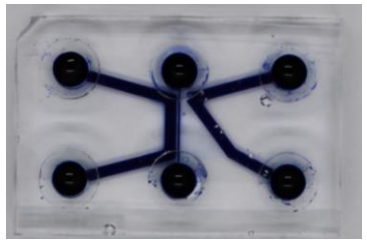
17 Days Post Exposure (Day 47)

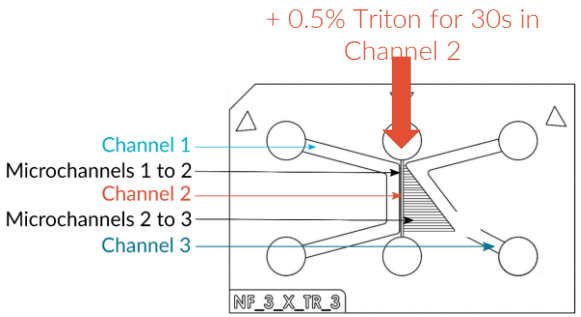
MAP2/ BetaIII-Tubulin



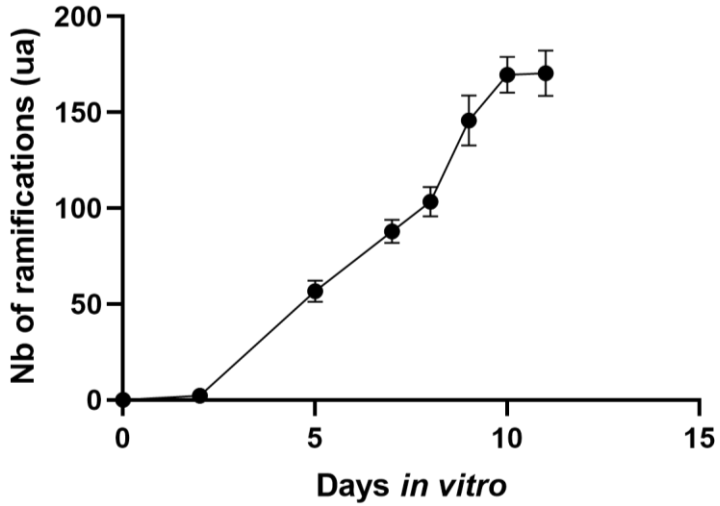




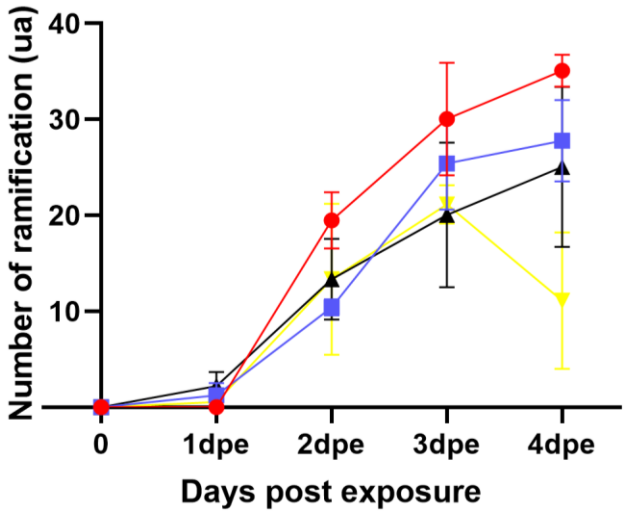


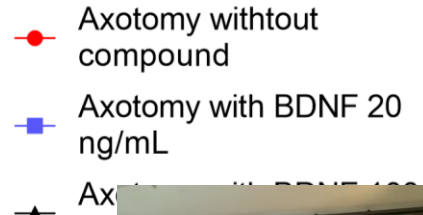


Without axotomy

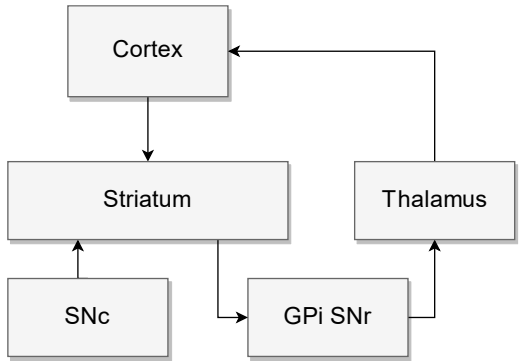


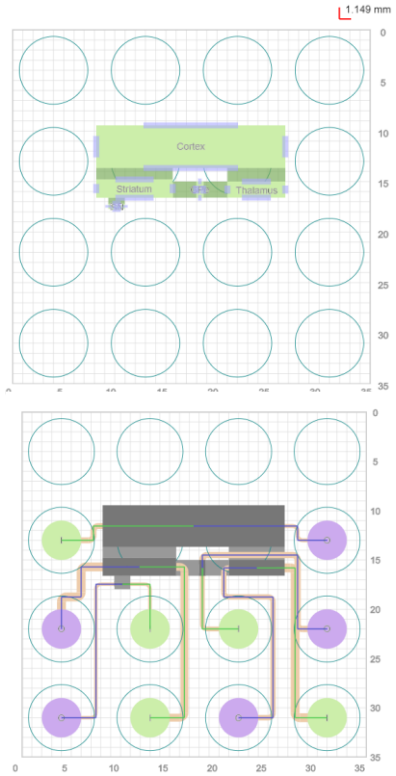
Post-axotomy

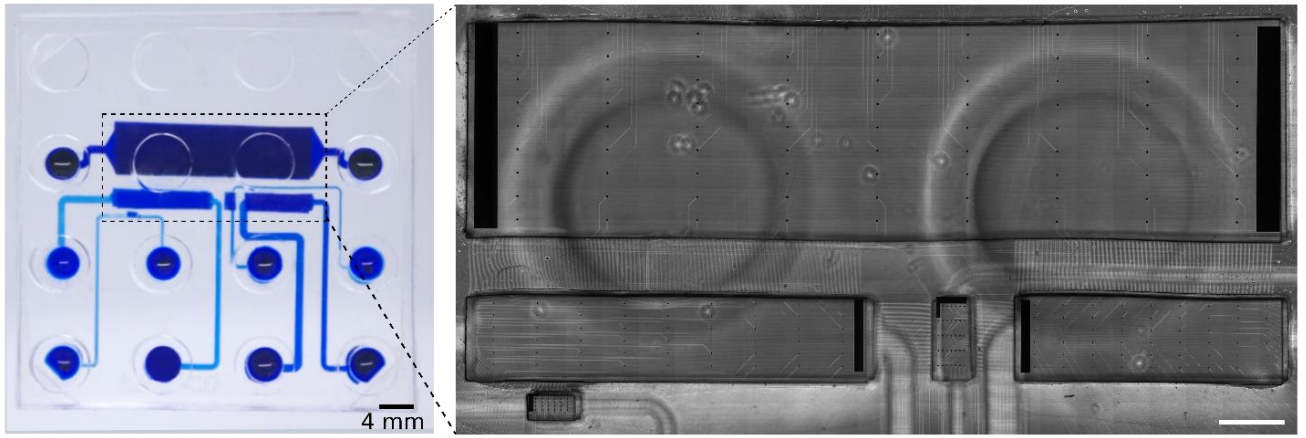


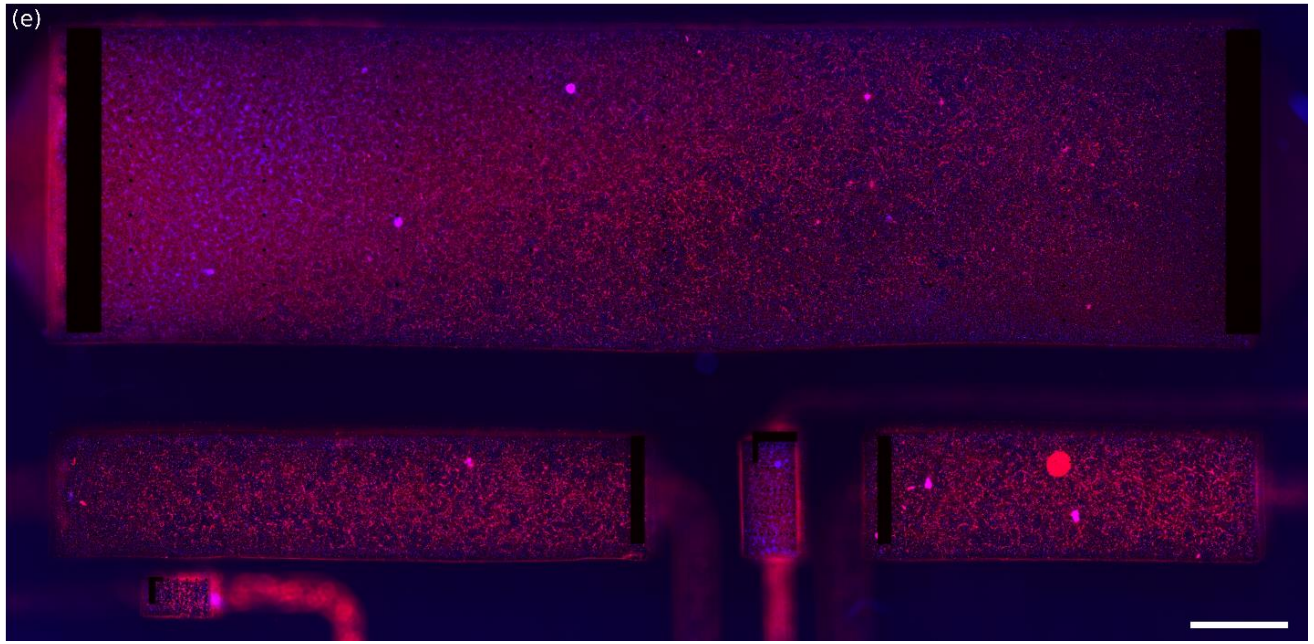


Basal Ganglia direct way on chip









BG5 chip

BASAL GANGLIA LOOP

Challenge: How to assess the involvement the basal ganglia direct way in Parkinson Disease

Solution: Co-culture of 5 types of cells using NF_5_CD_BG5 chip which is composed of 5 compartments connected by microchannels.

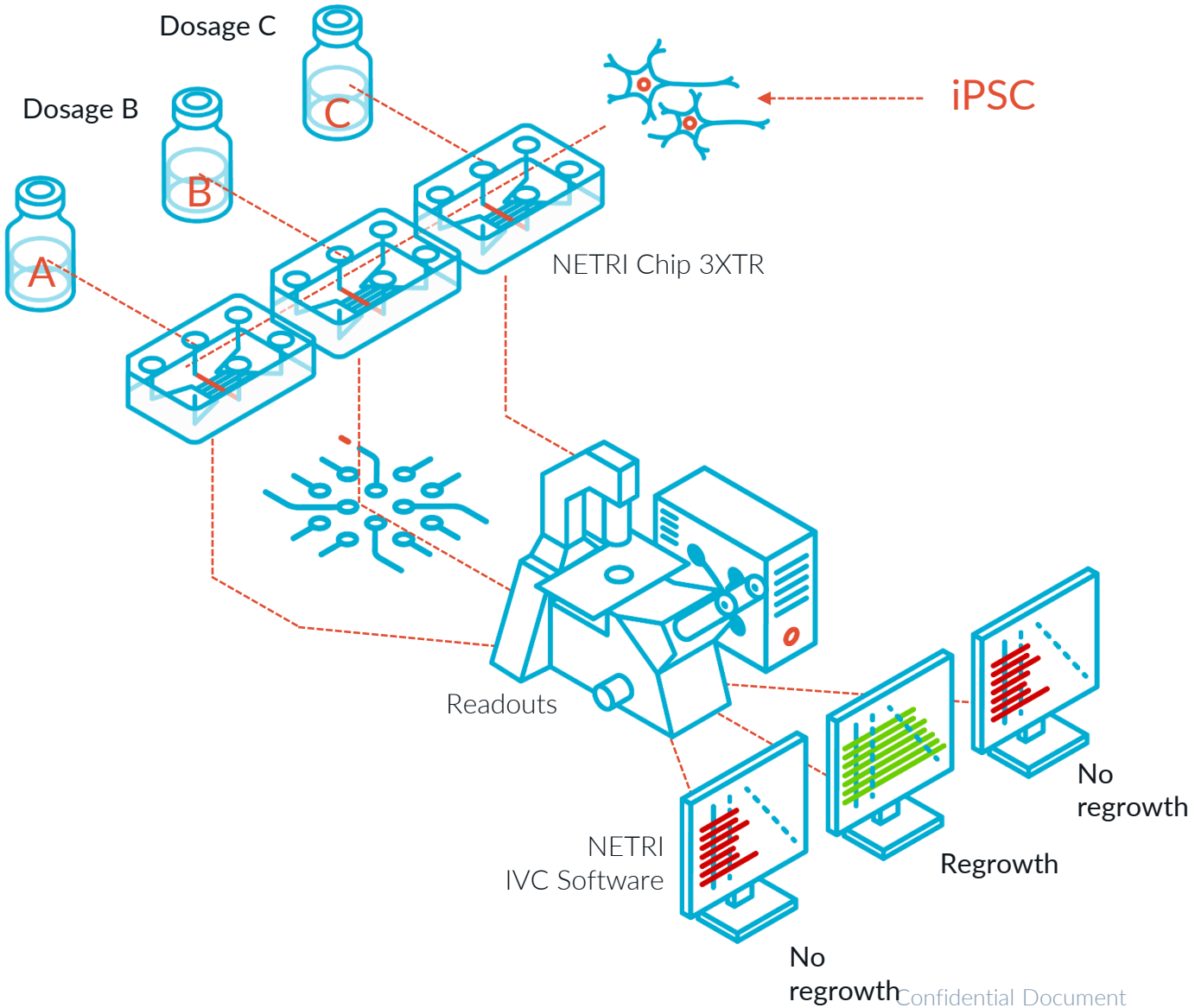
Possible applications

- Disease model of Parkinson disease (including motor & mood disfunctions)
- Quantification of propagation of alpha-synuclein
- Quantification of global electrical activity disfu
  network

References

- Maisonneuve

Organs on chip in pre clinical trials



Standardization

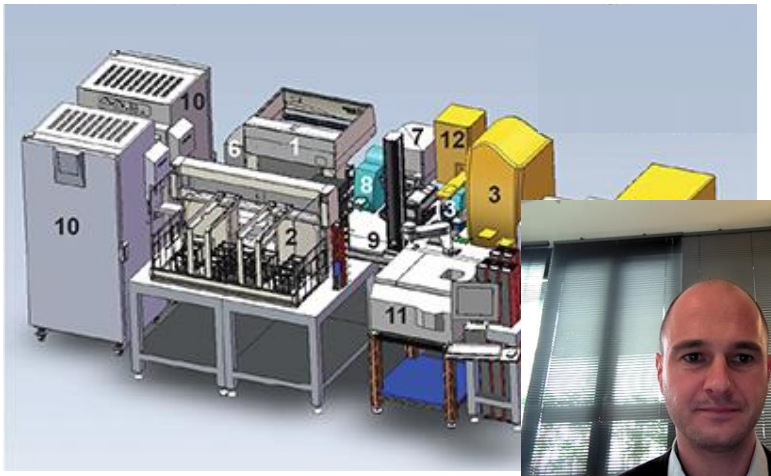
1. Architectures (ANSI SBS)
2. Cells (SOP for markers)
3. Models (reproducibility)

Industrialisation

1. Cell culture automation (ML + electrophysioly)
2. HTS compatibility

Open new field of therapeutic application

1. Neurological troubles
2. Comsetics
3. Nutrition



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Merci.

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