Lab on Chip and Microfluidics

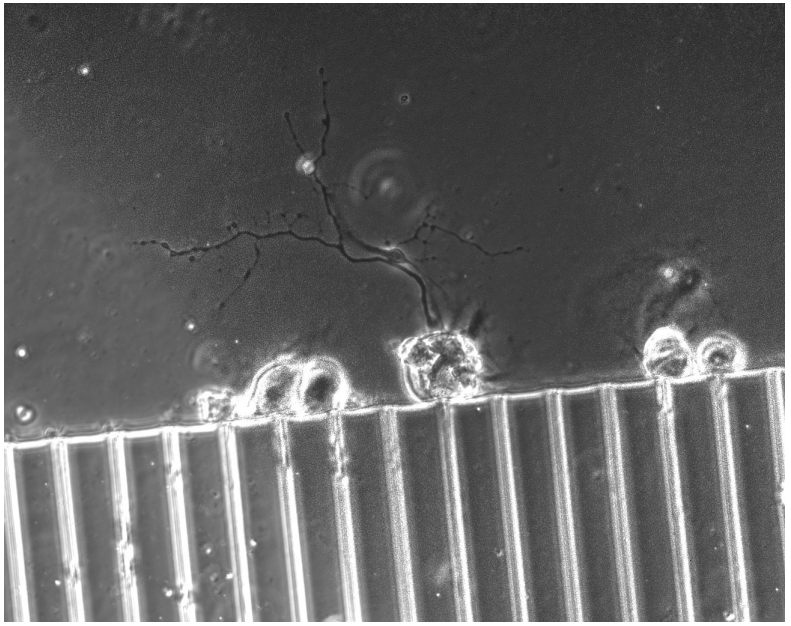
Benoît CHARLOT

Part IX. Cells on Chips

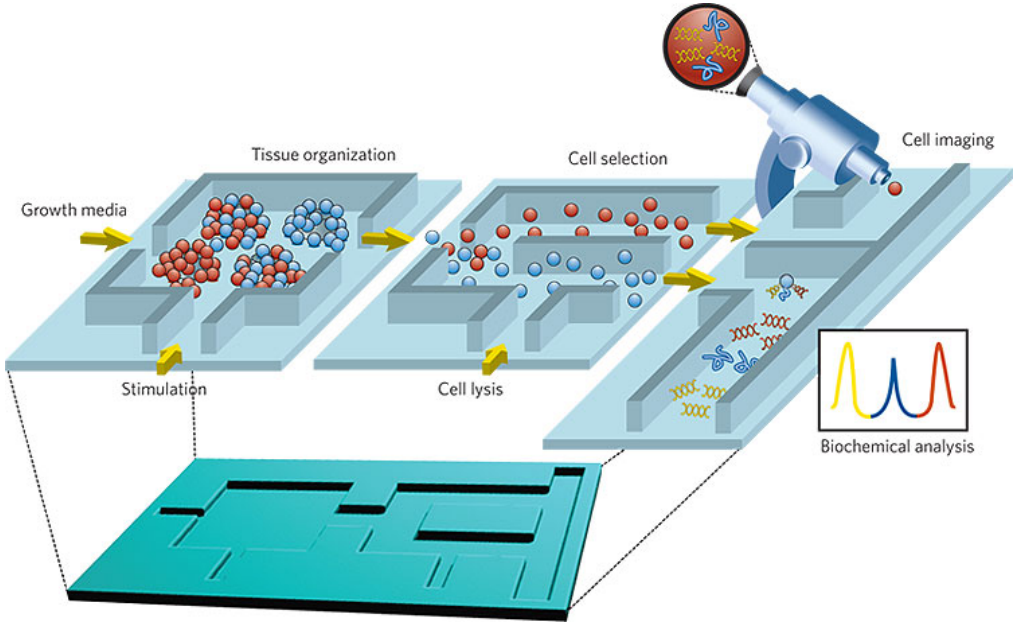
Cells On Chips

Systems for the spatial and temporal control of cell growth and stimuli

- Surfaces that mimic complex biochemistries and geometries of the extracellular matrix
-Microfluidic channels that regulate transport of fluids
-Diffusion of gaz in PDMS

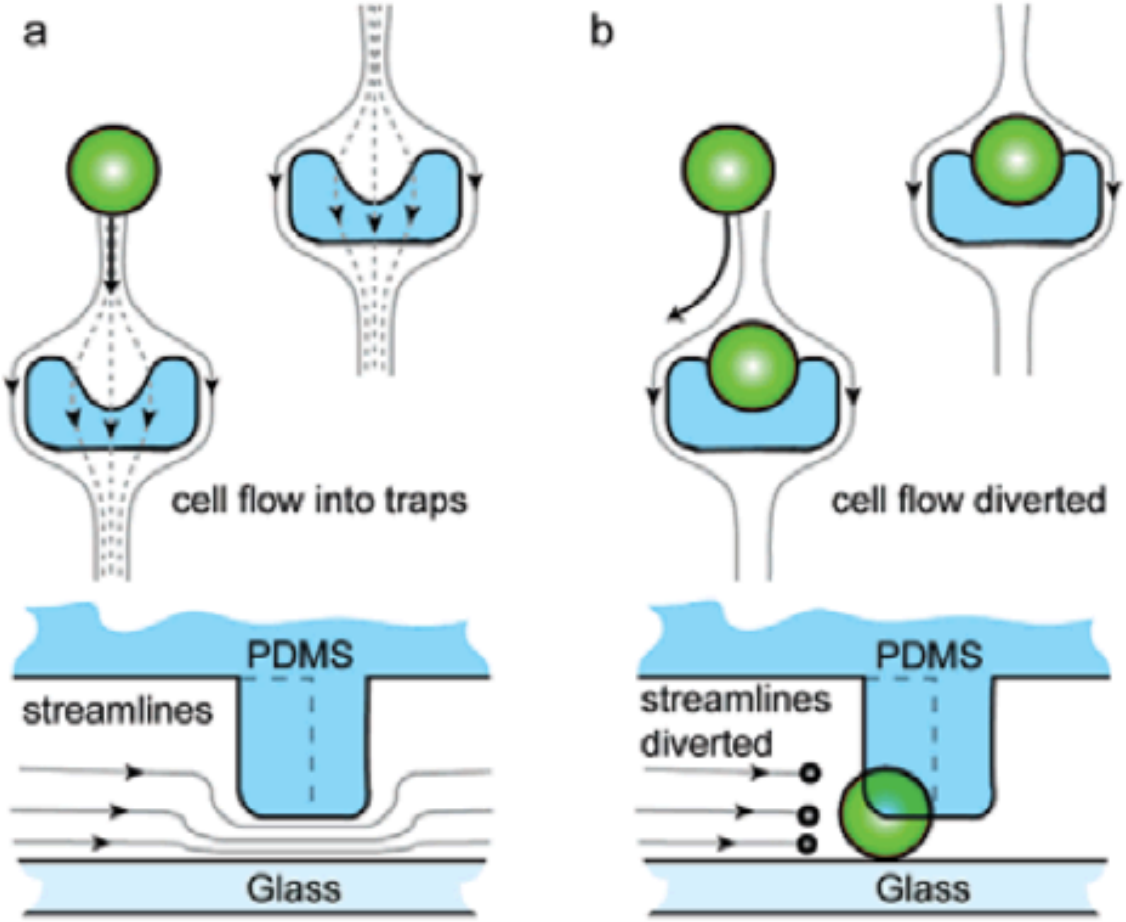


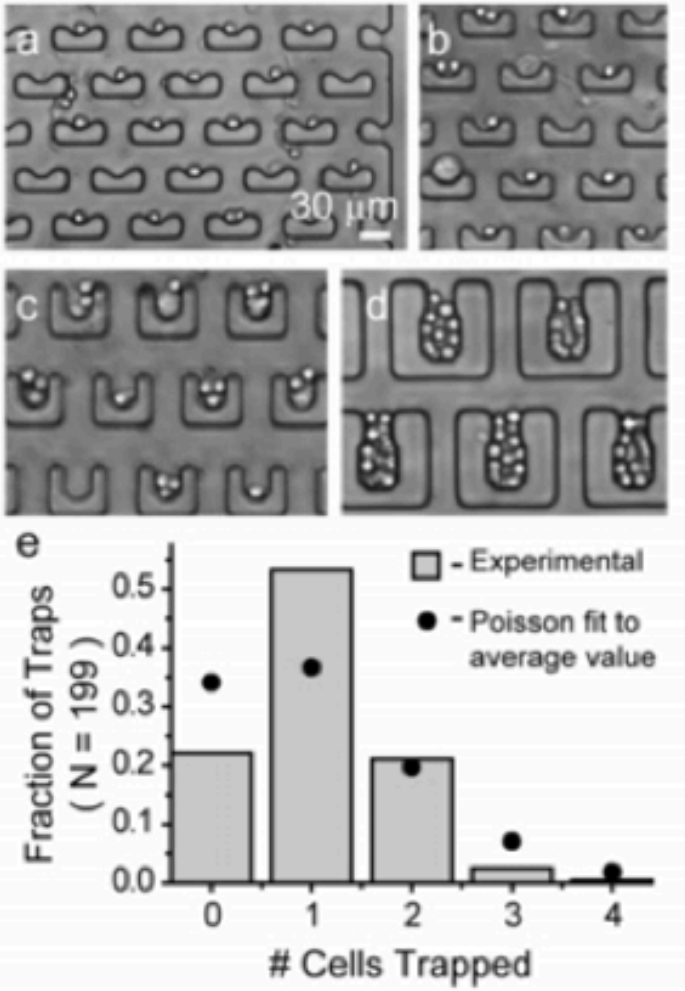
Cell trapping and sorting
Culture
Oriented growth (neurons)
Compartimented culture
Cytometry
Lysis
Electroporation
Mechanical /Electrical stimulation
Identification

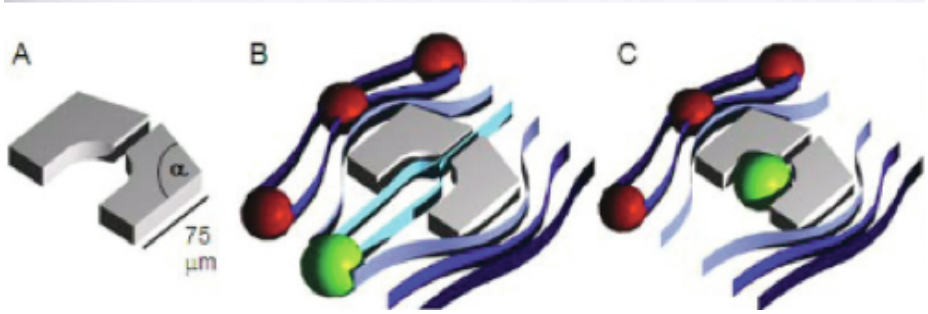


Jamil El-Ali et al. , Nature, 442, 2006

Cell Chips Capture and Sorting

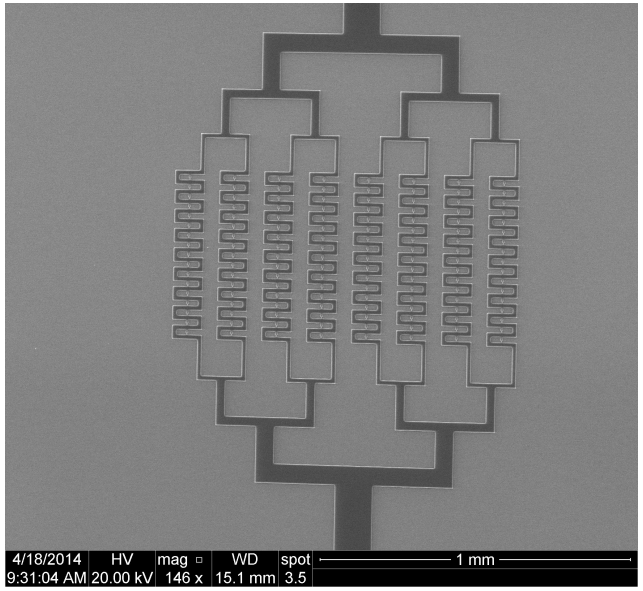


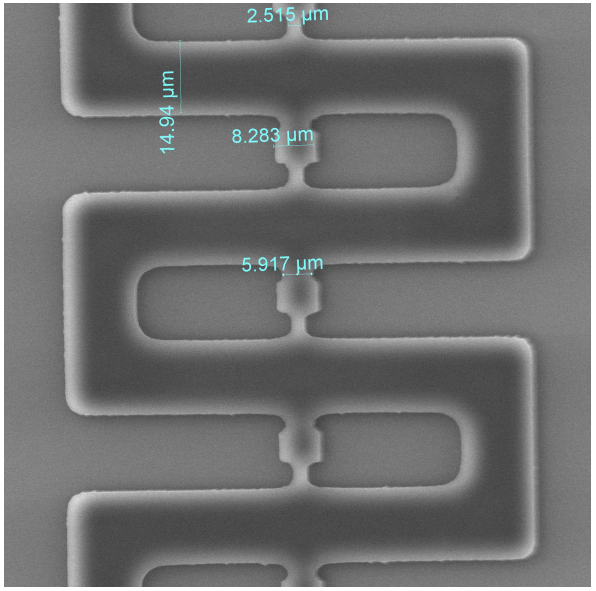


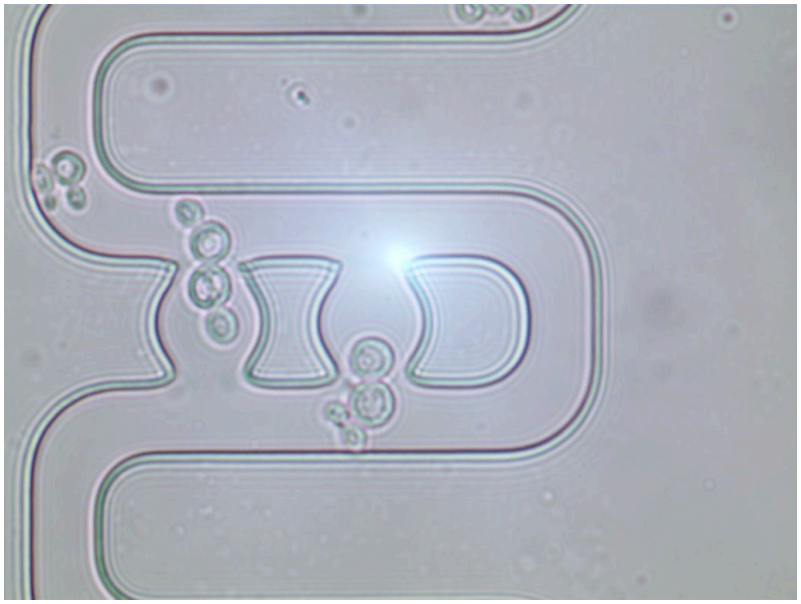


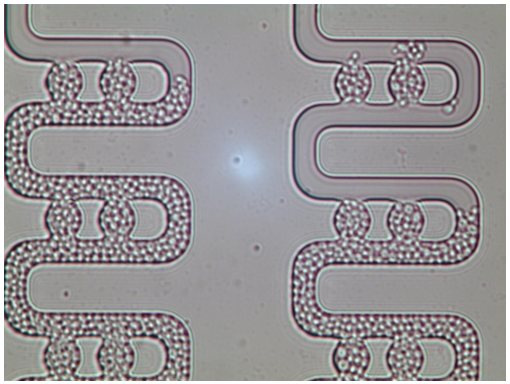
Cell Chips Capture and Sorting

Capture of yeast cells for duplication analysis (E.Schwob, B.Charlot)

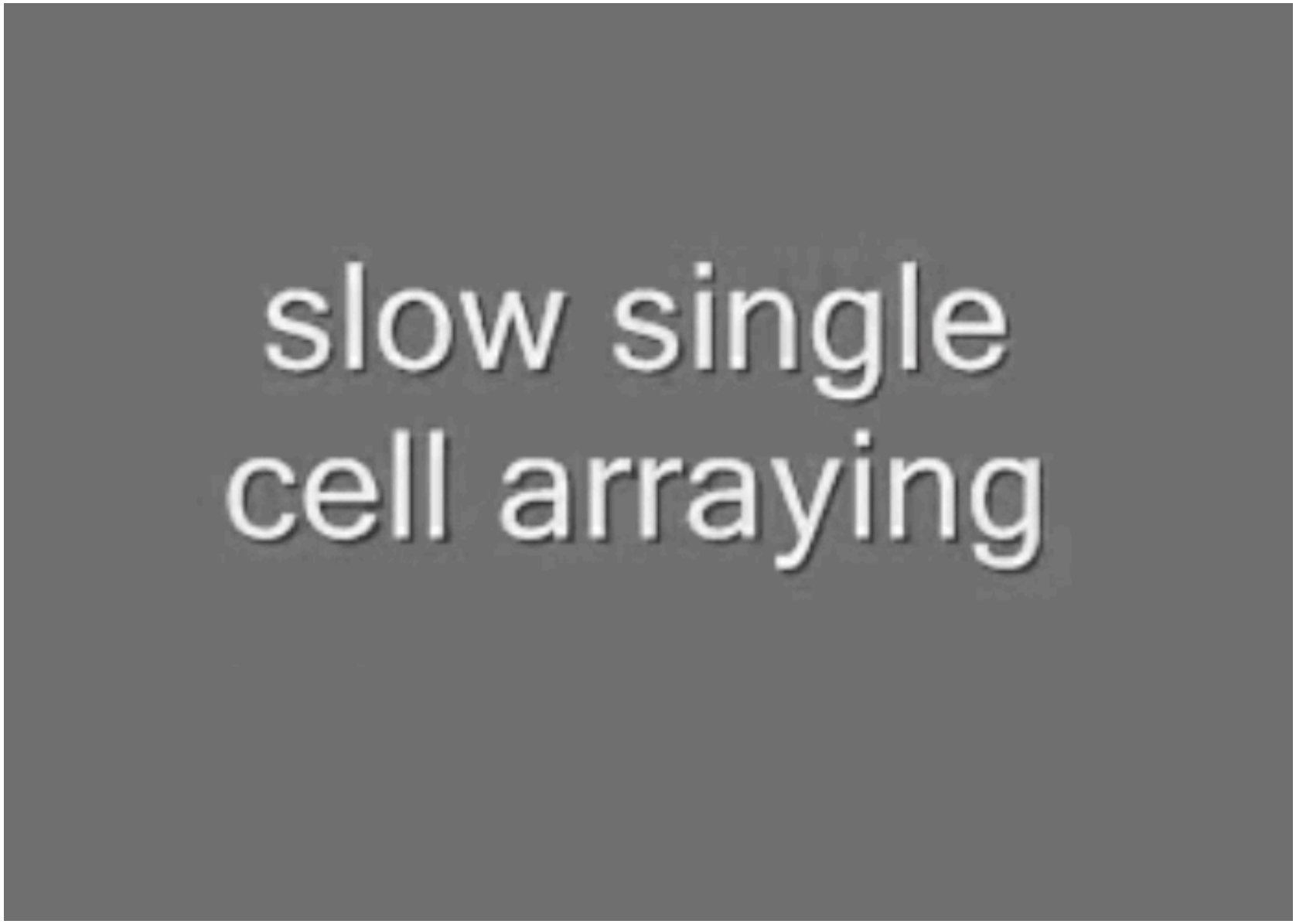








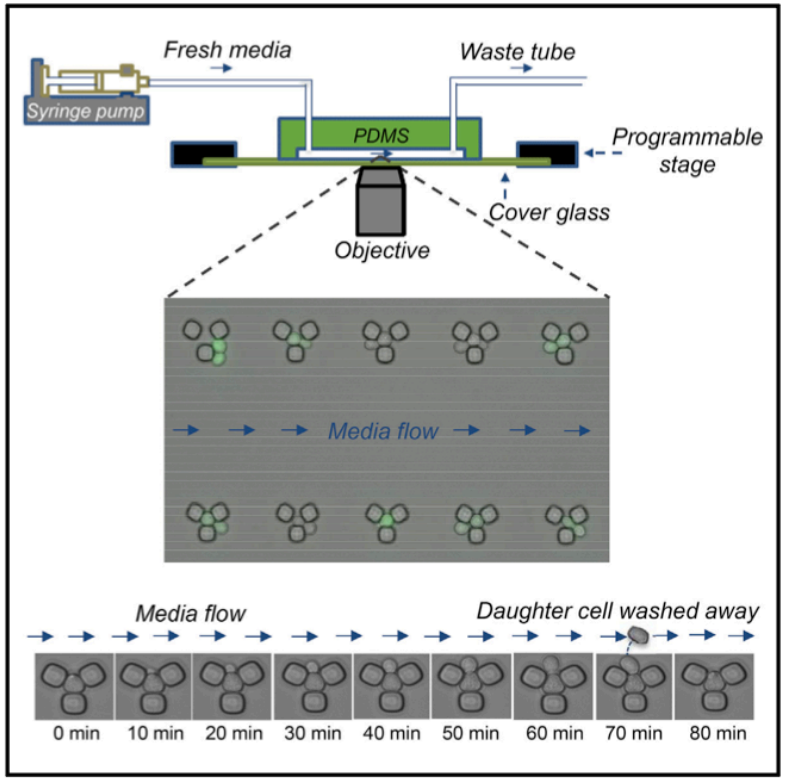
Cell Chips Capture and Sorting



Cell Chips Capture and Sorting

Ping Liu, Thomas Z. Young, Murat Acar

Cell Reports 13, 634–644



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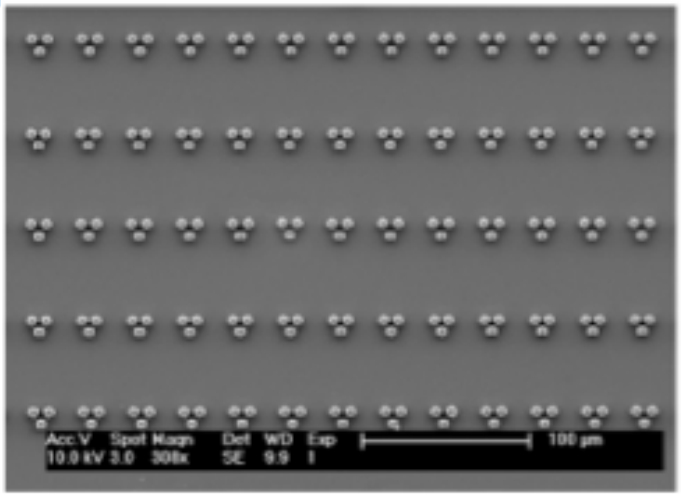

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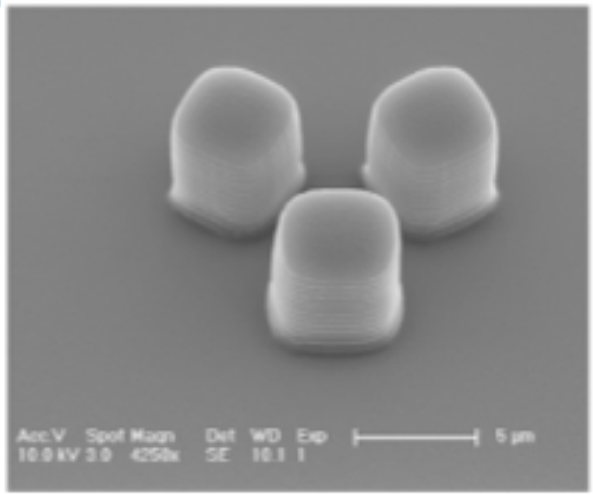

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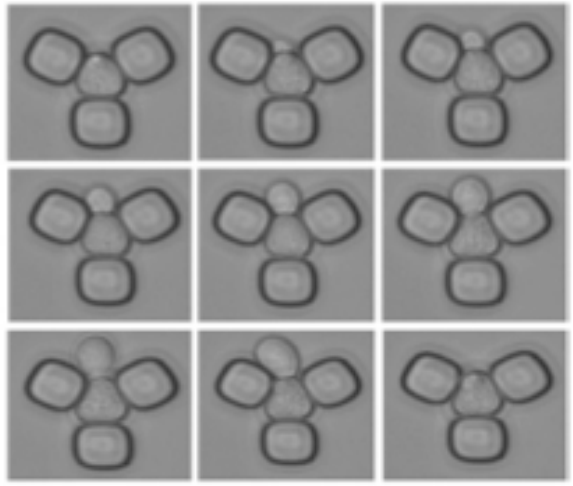
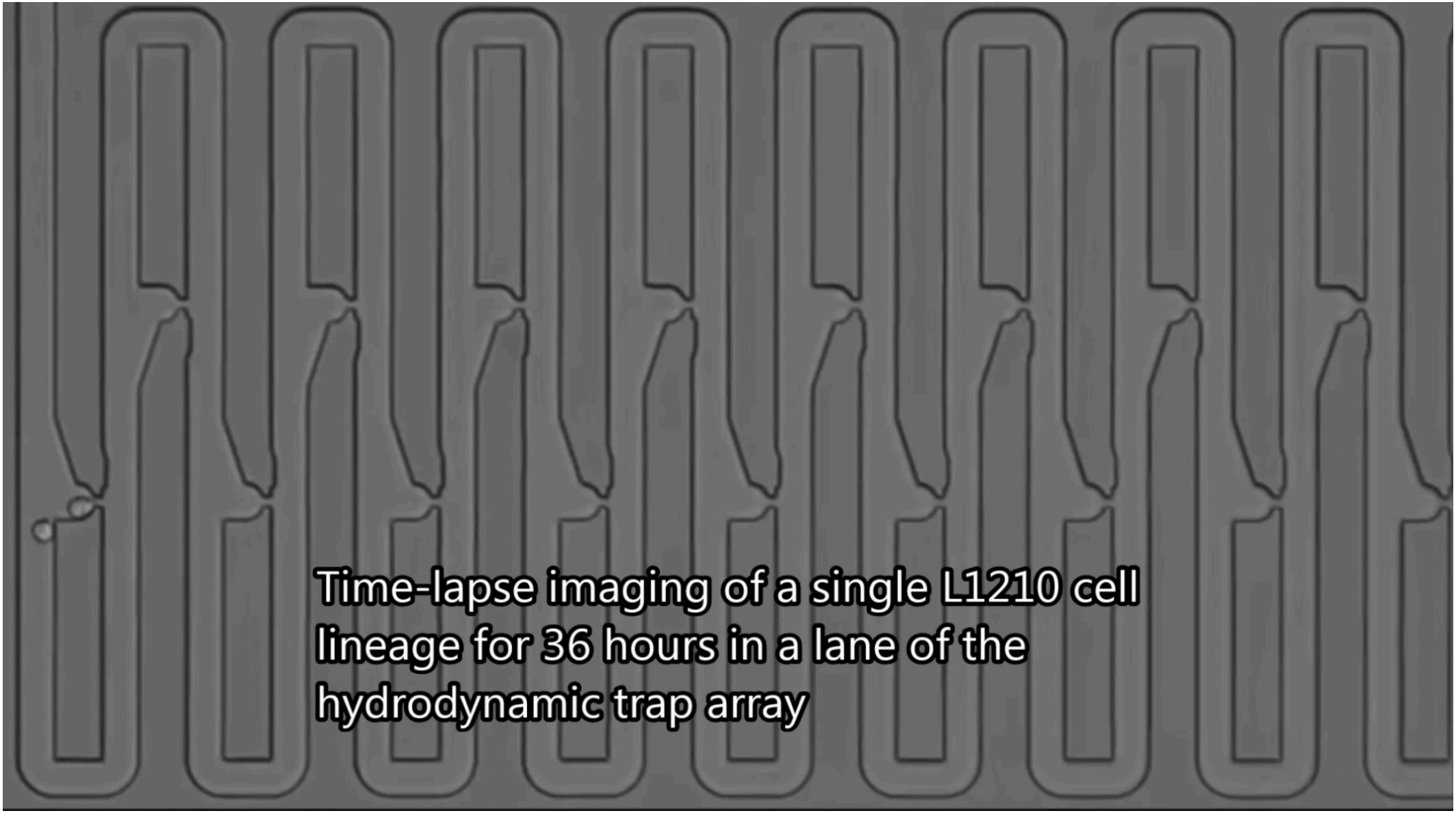
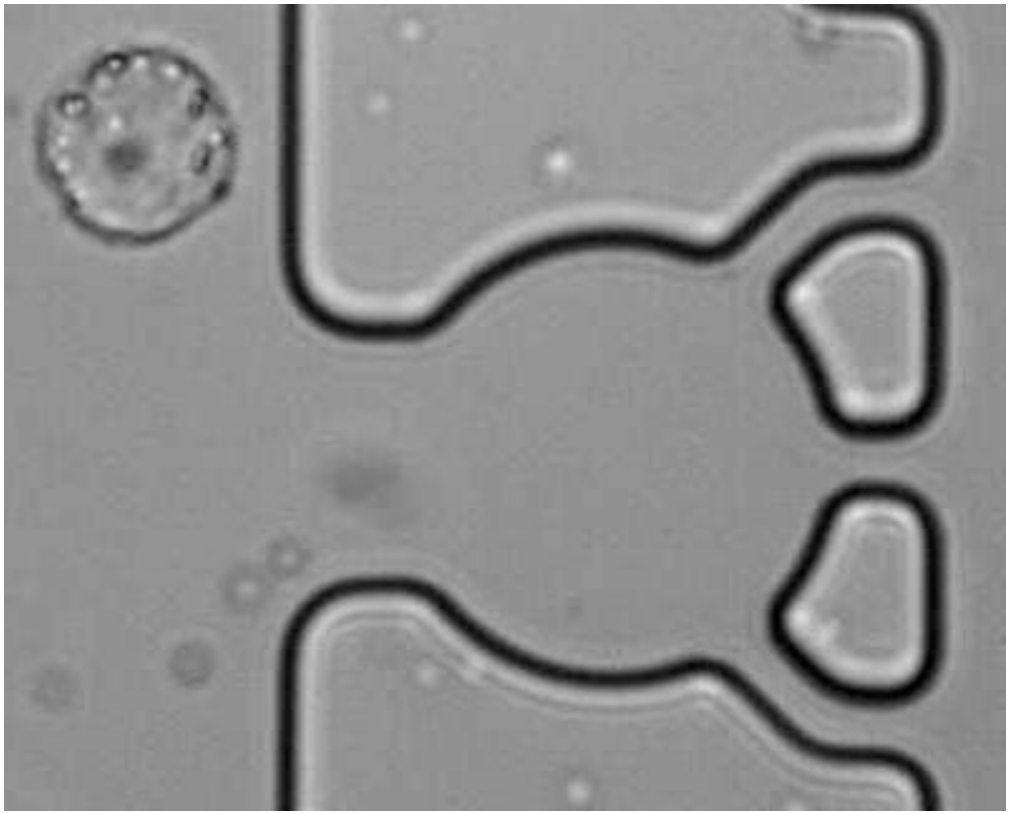


Cell Chips Capture and Sorting

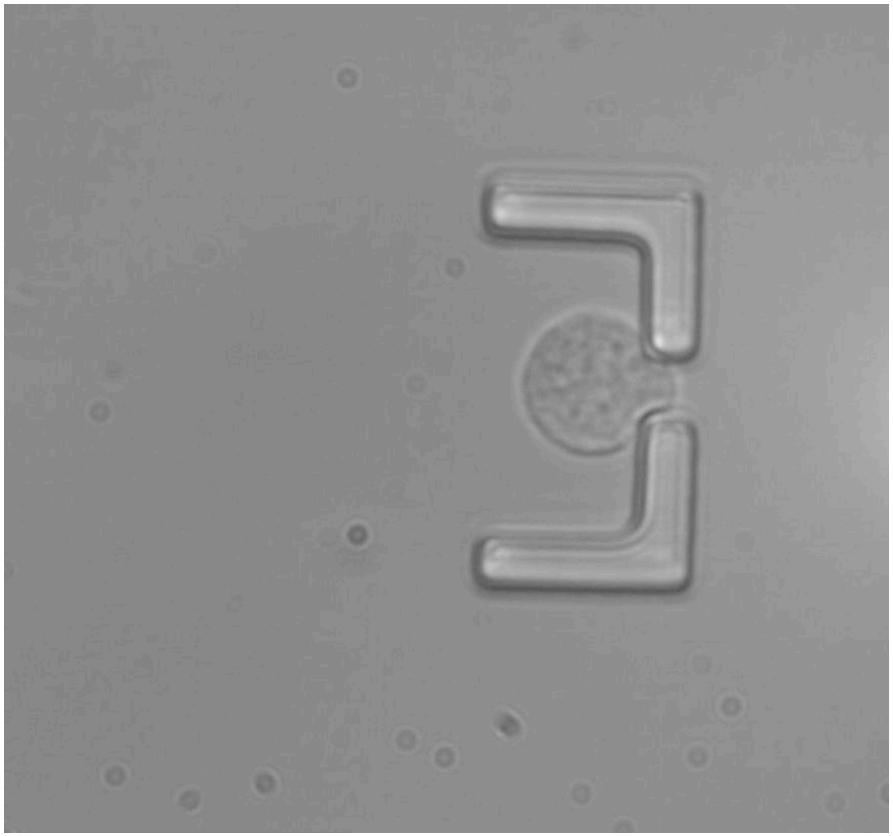


Robert J. Kimmerling et al (2016), Nature Communications

Cell Chips Capture and Sorting



HELA

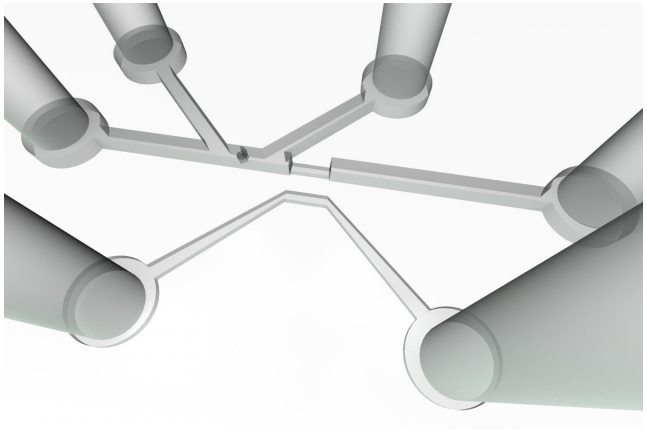


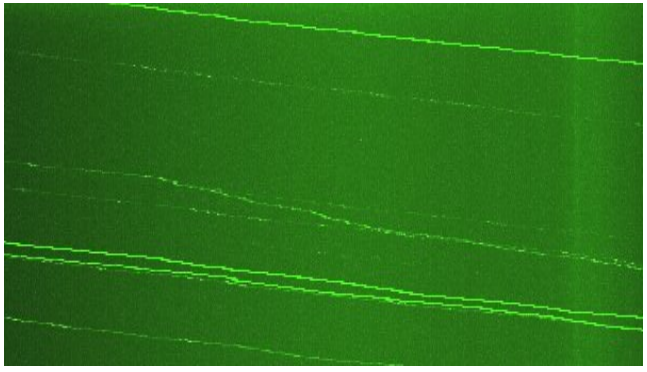
JURKAT

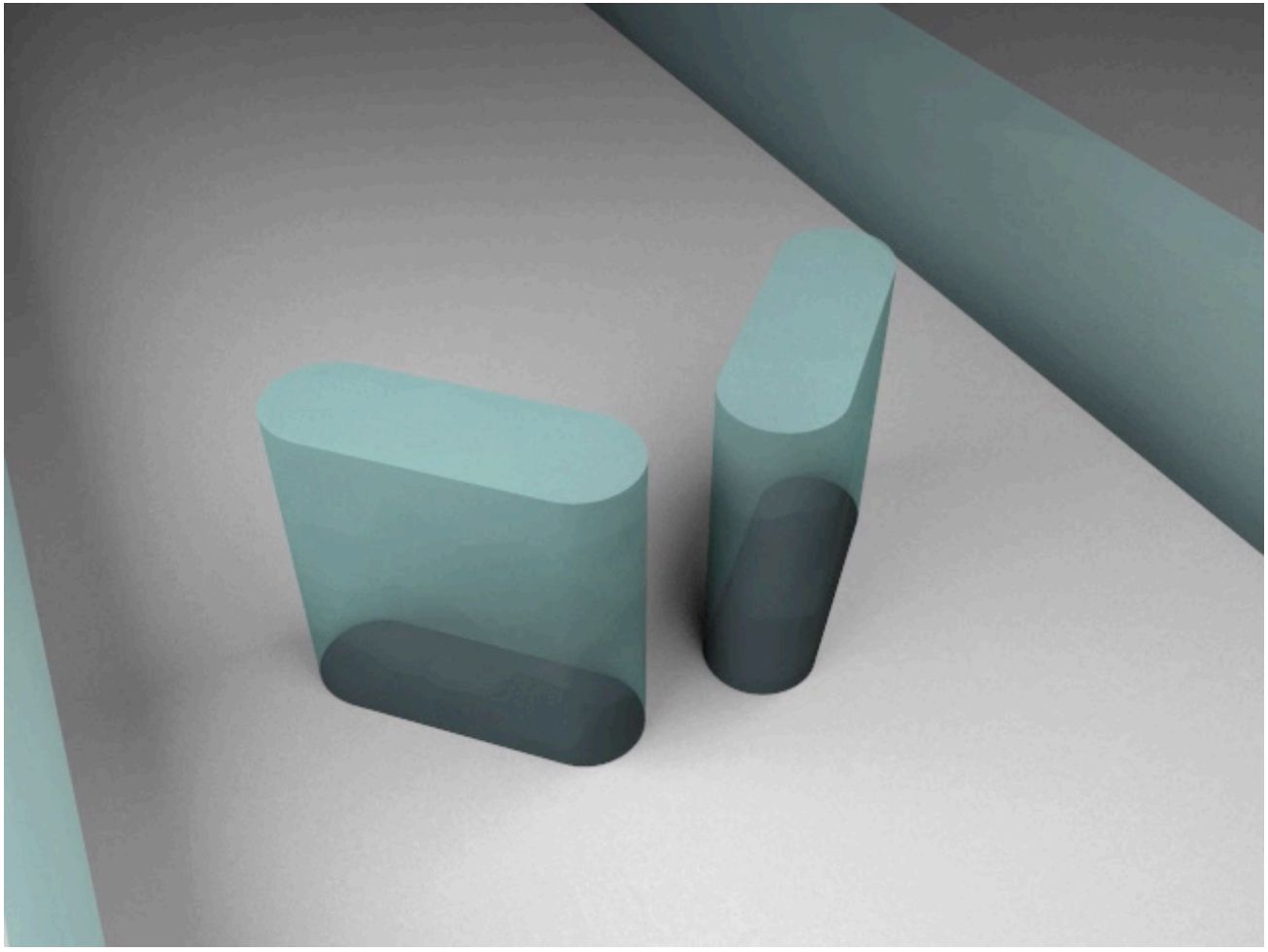
M.Socol, J.Eid, M.Mougel, IRIM
B.Charlot, IES

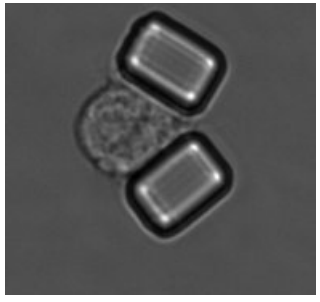
Cell Chips Capture and Sorting

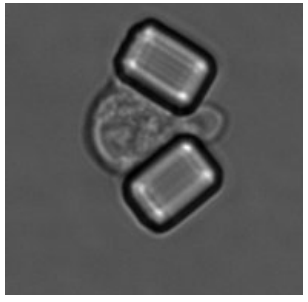
Virofluidics
Capturing single Cell
Studying virions release along time at single cell level











M.Socol, J.Eid, M.Mougel, IRIM
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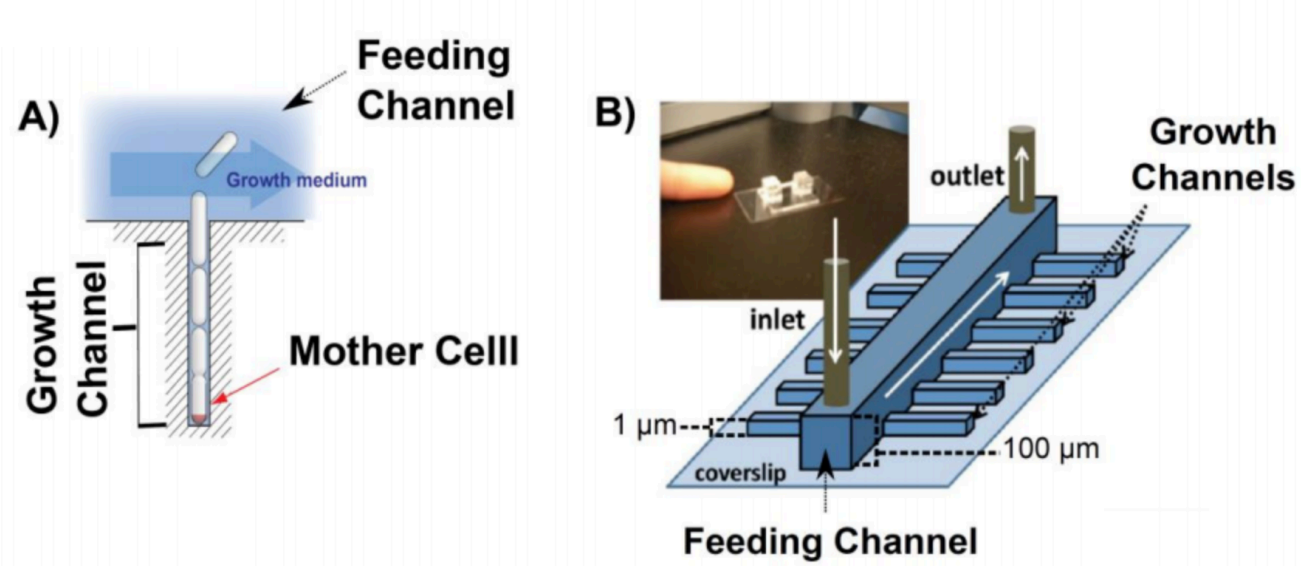
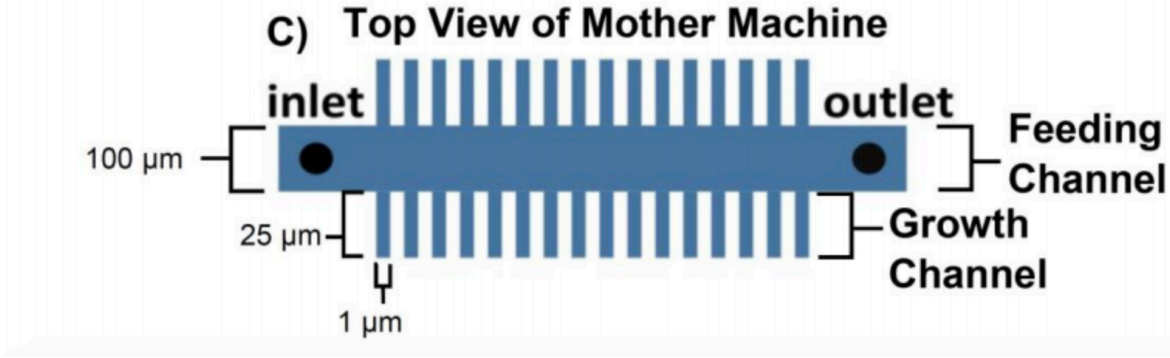
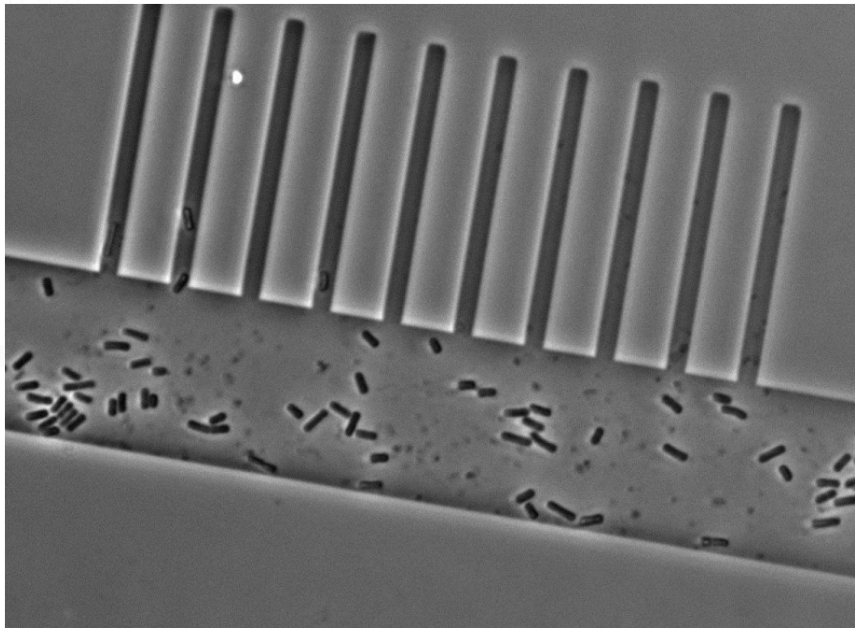
Cell Chips Capture and Sorting

The Green Mother Machine: a microfluidics device for cyanobacteria

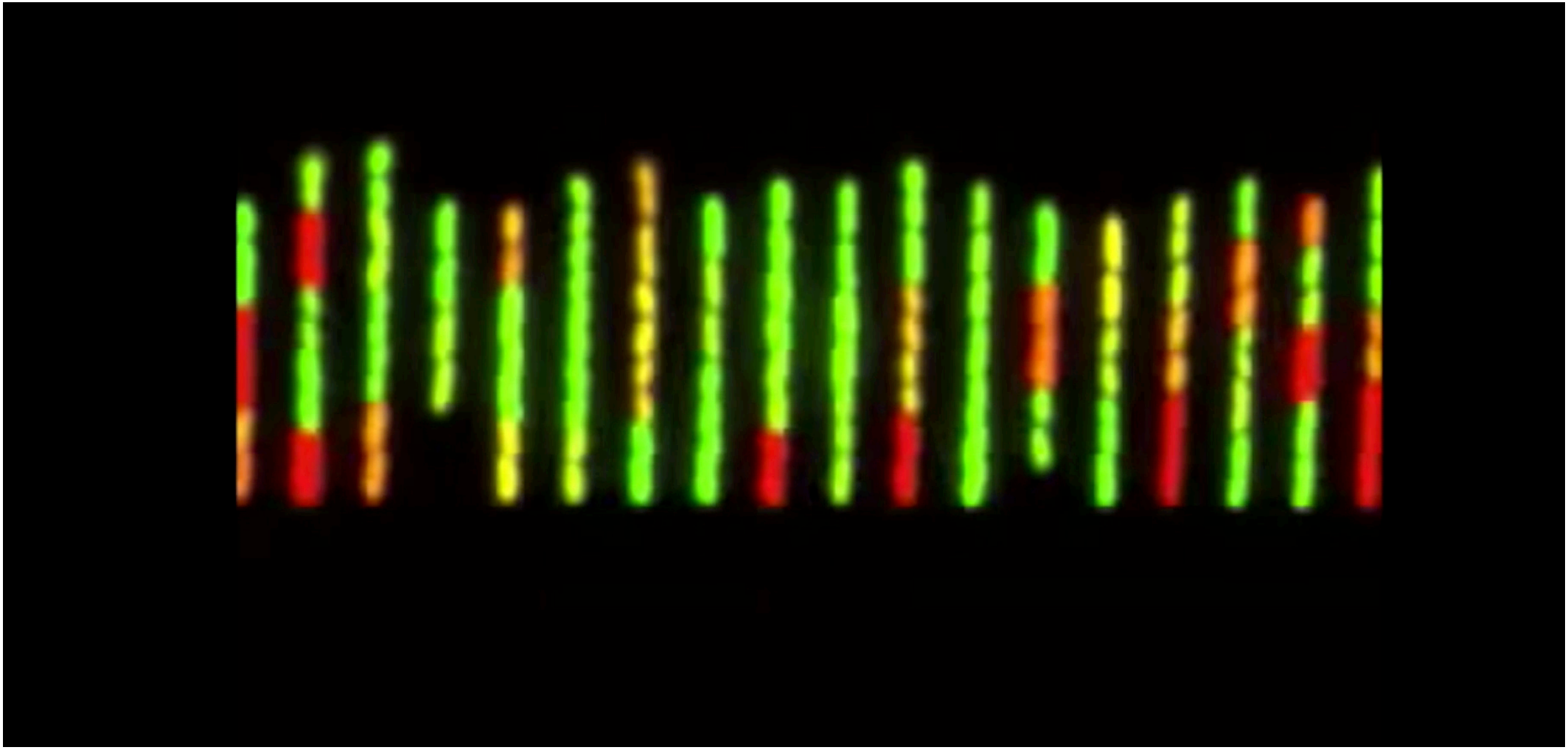




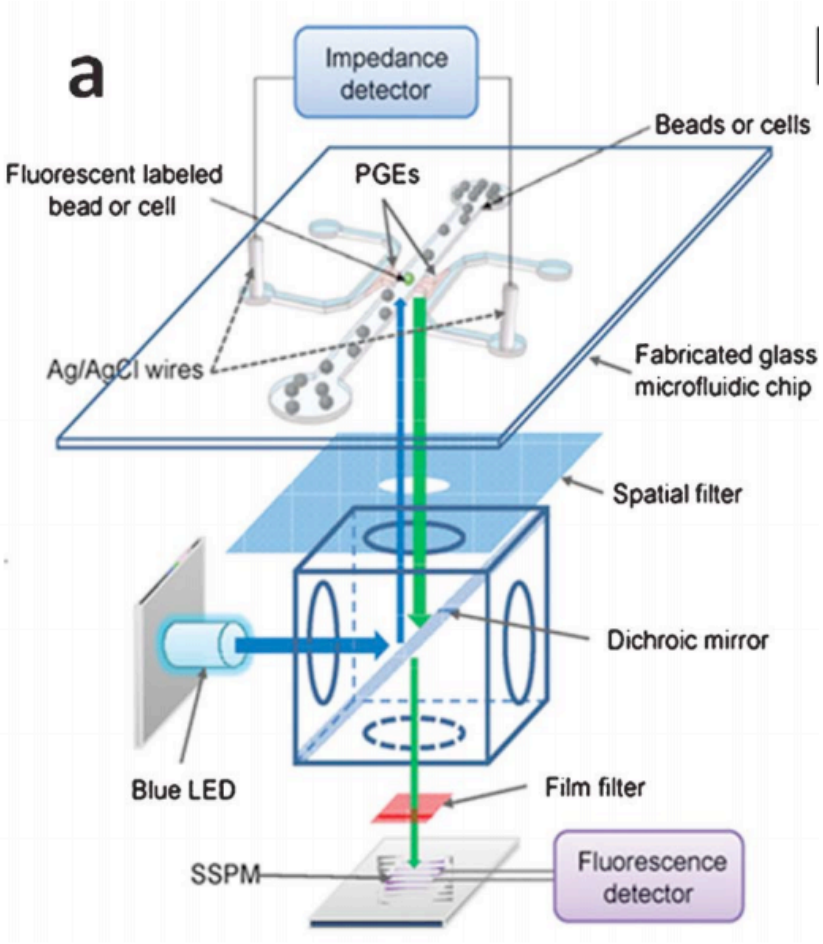


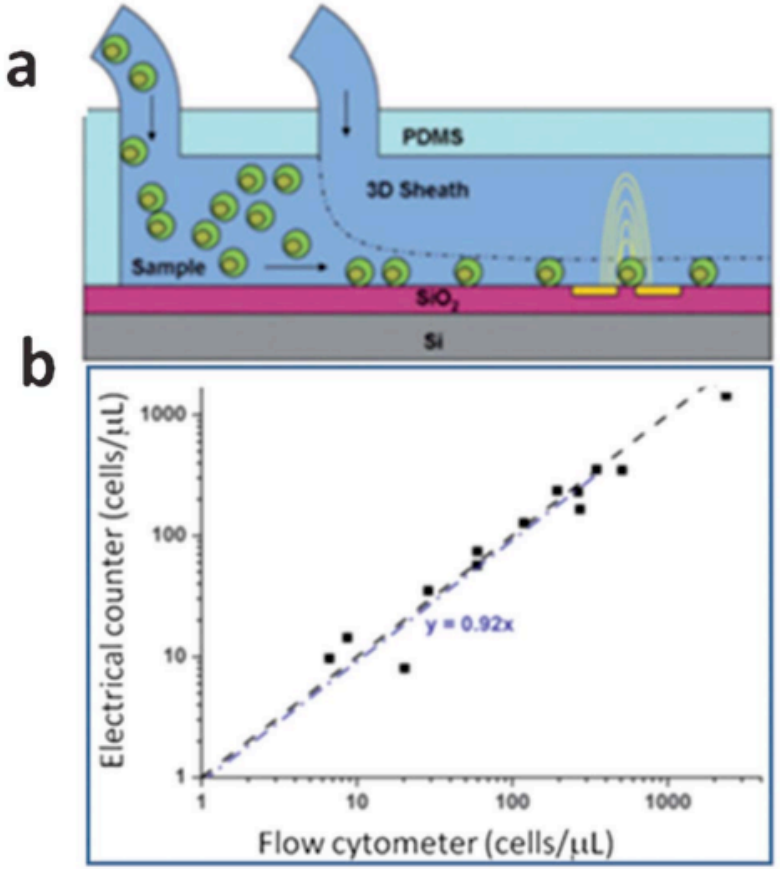
Cell Chips Capture and Sorting

The Green Mother Machine: a microfluidics device for cyanobacteria



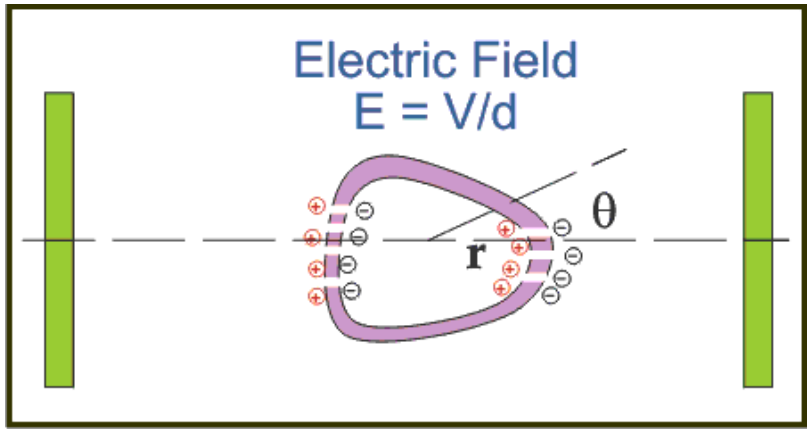
Cell Chips Cytometry

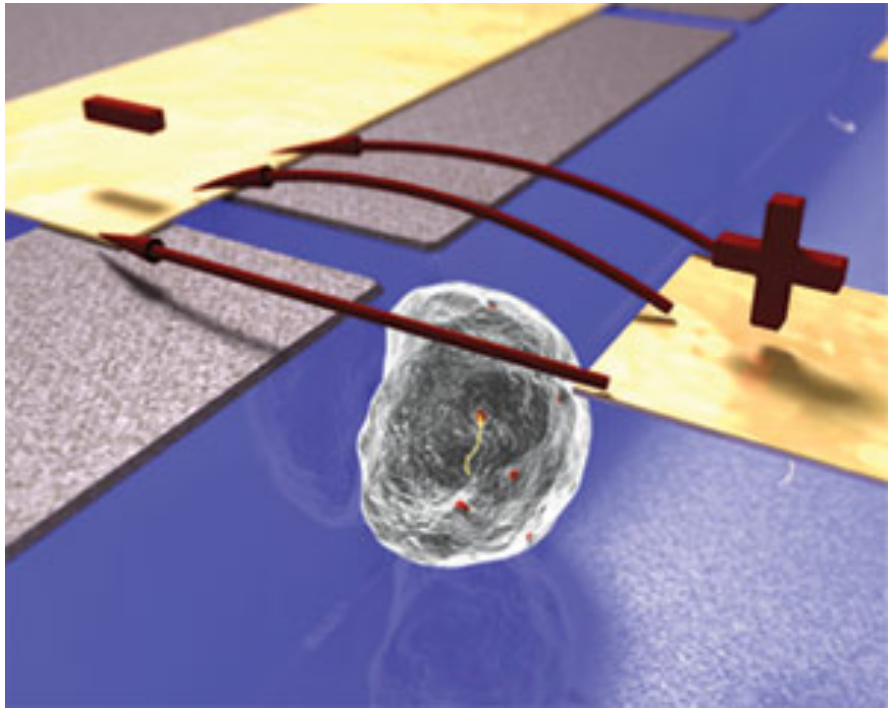




Cell Chips Electroporation

Electropermeabilization

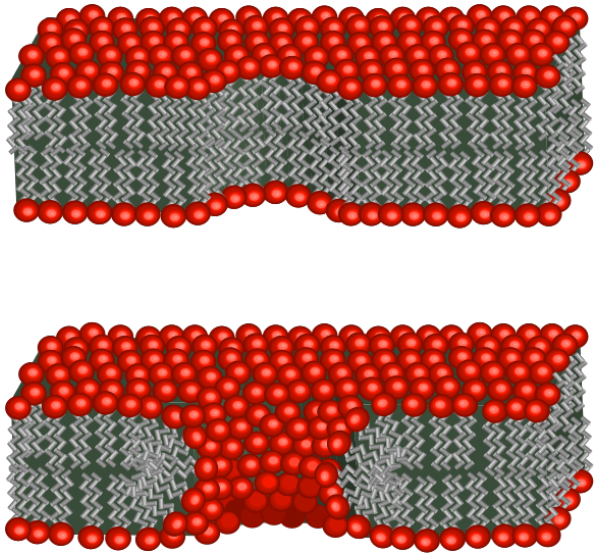




Cell Chips Electroporation

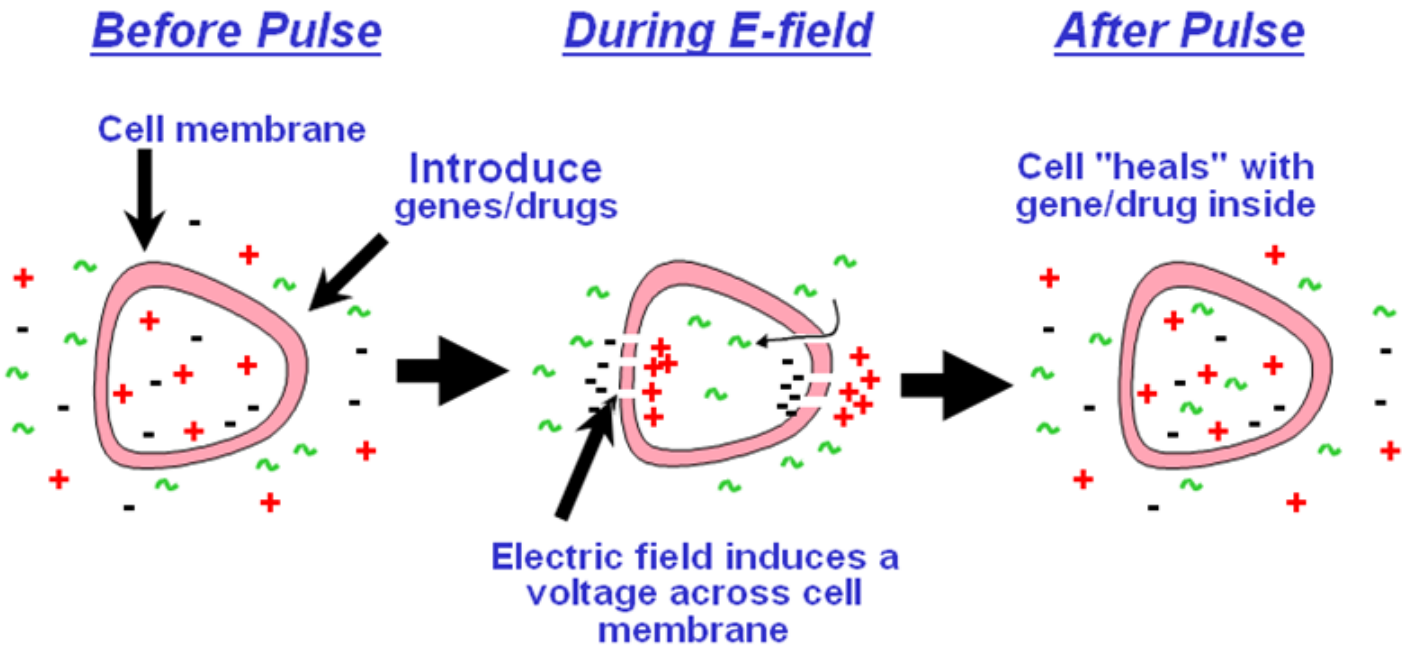
a.k.a Electropermeabilization

an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, or DNA to be introduced into the cell

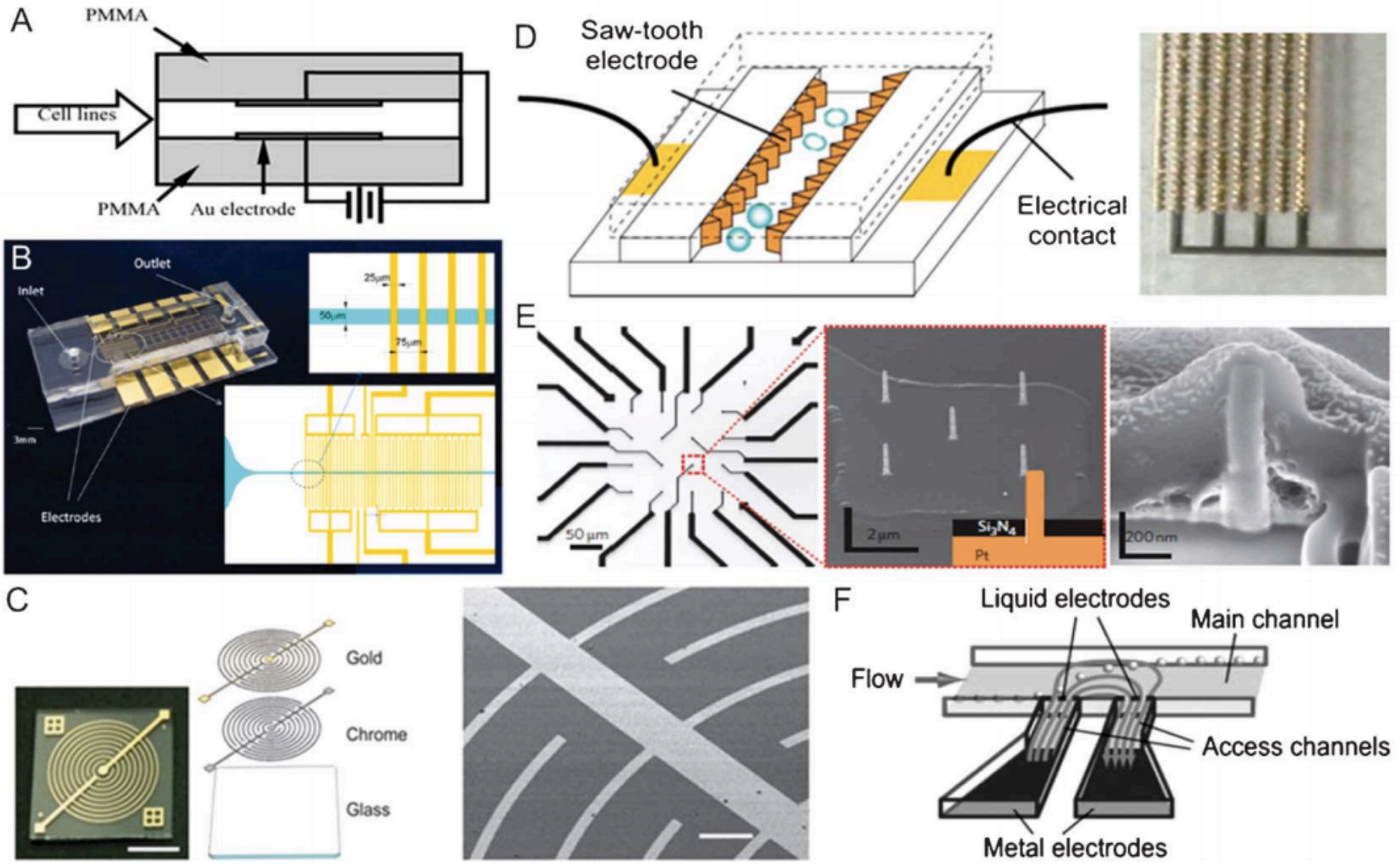


Transfection

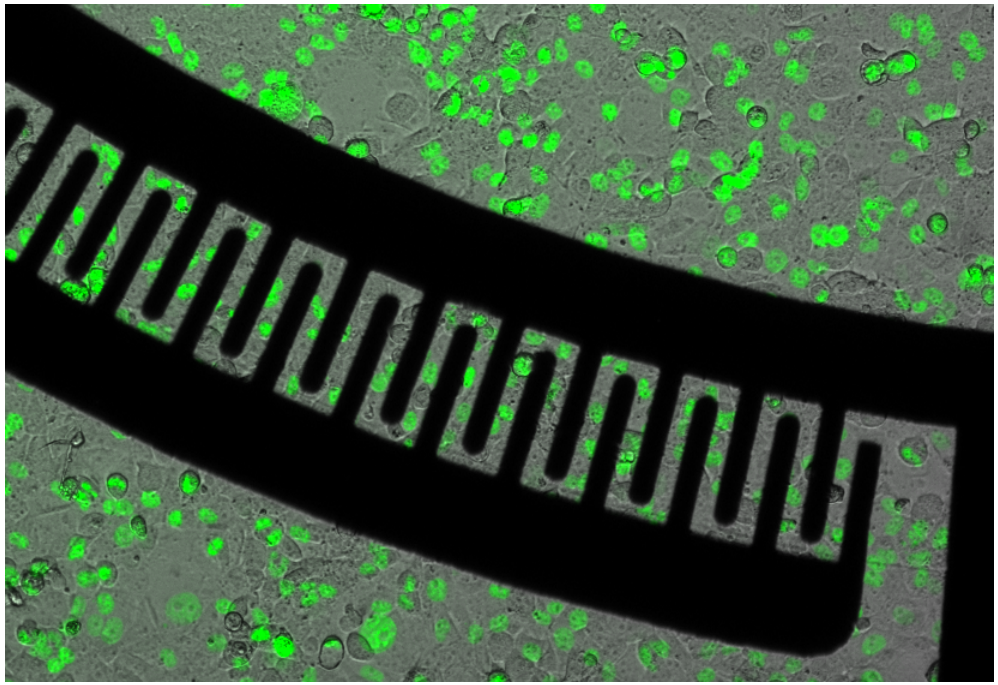
300-400 mV for < 1 ms (across the membrane)

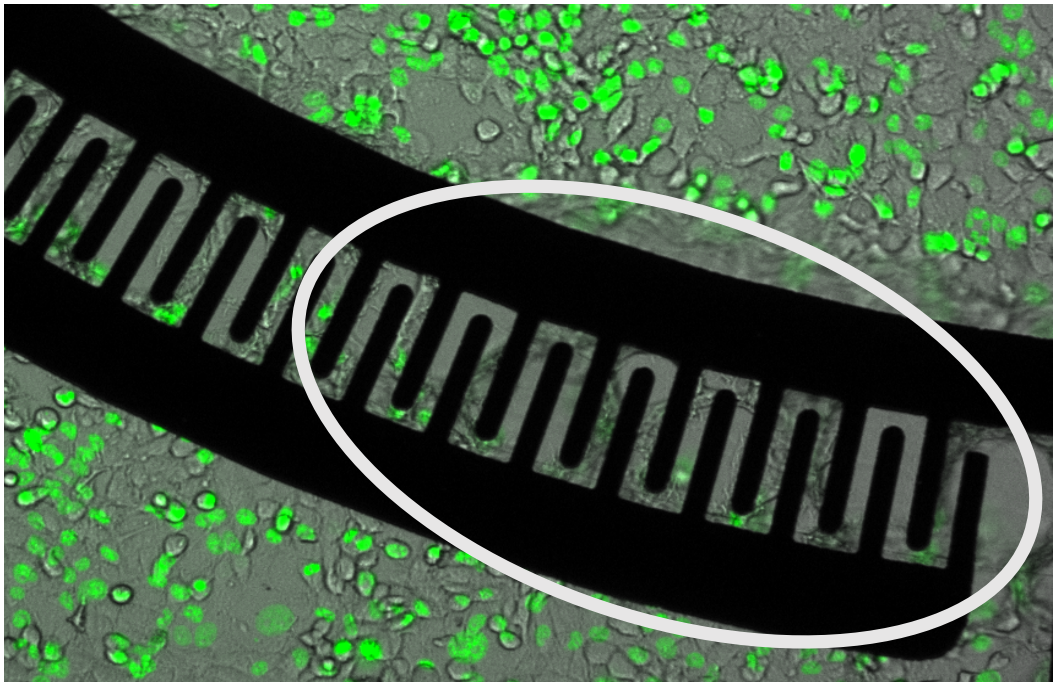


Cell Chips Electroporation



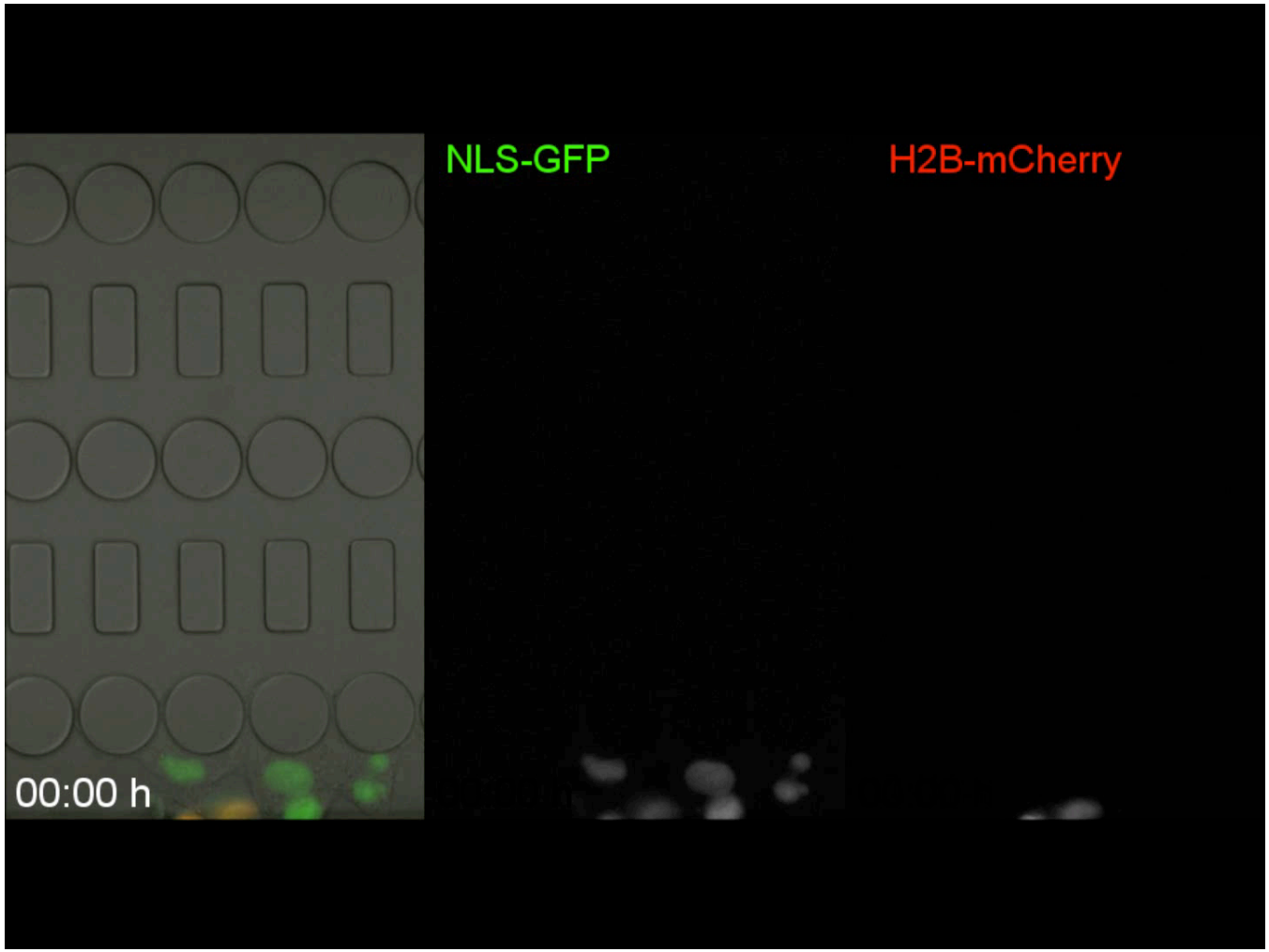
Cell Chips Electroporation



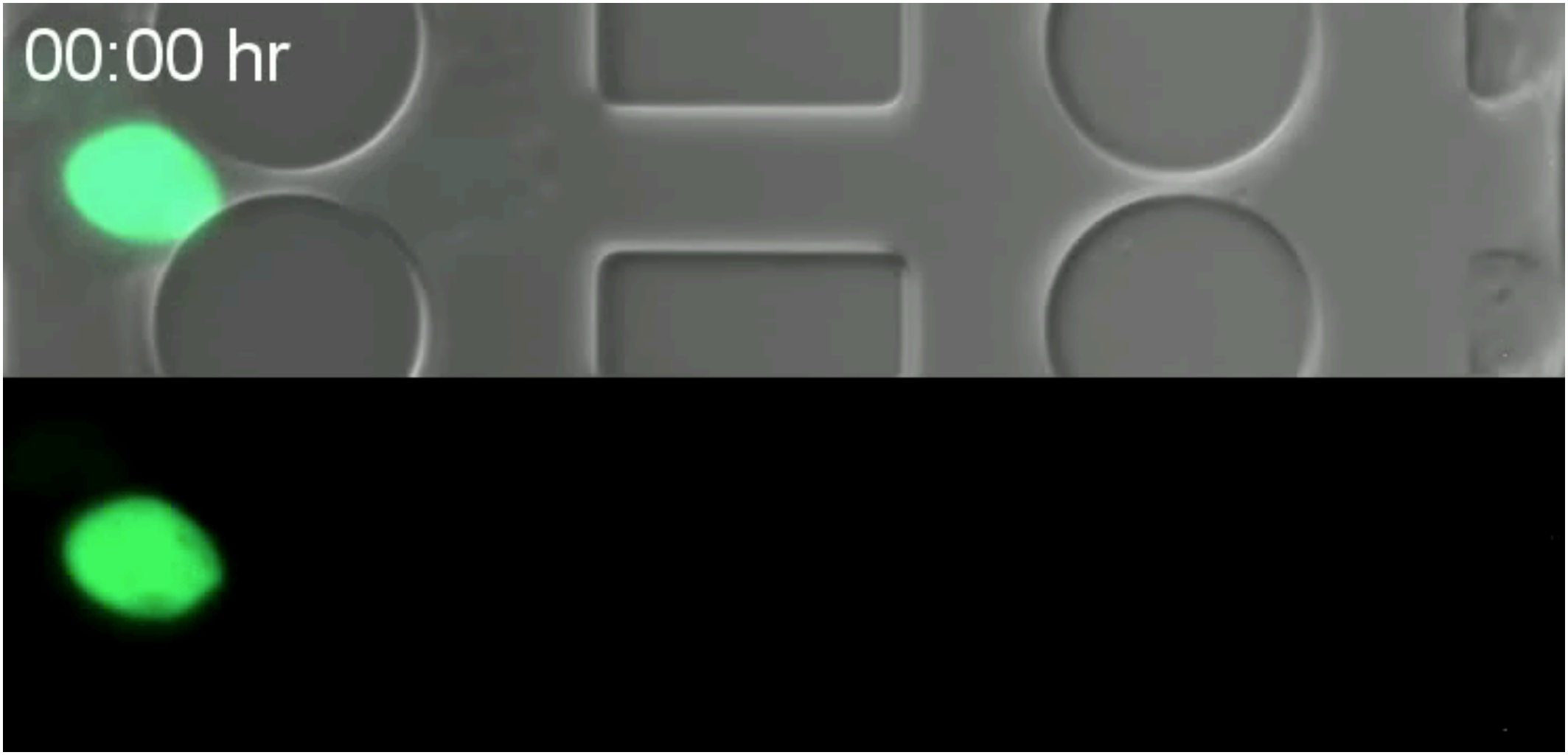


Cell migration

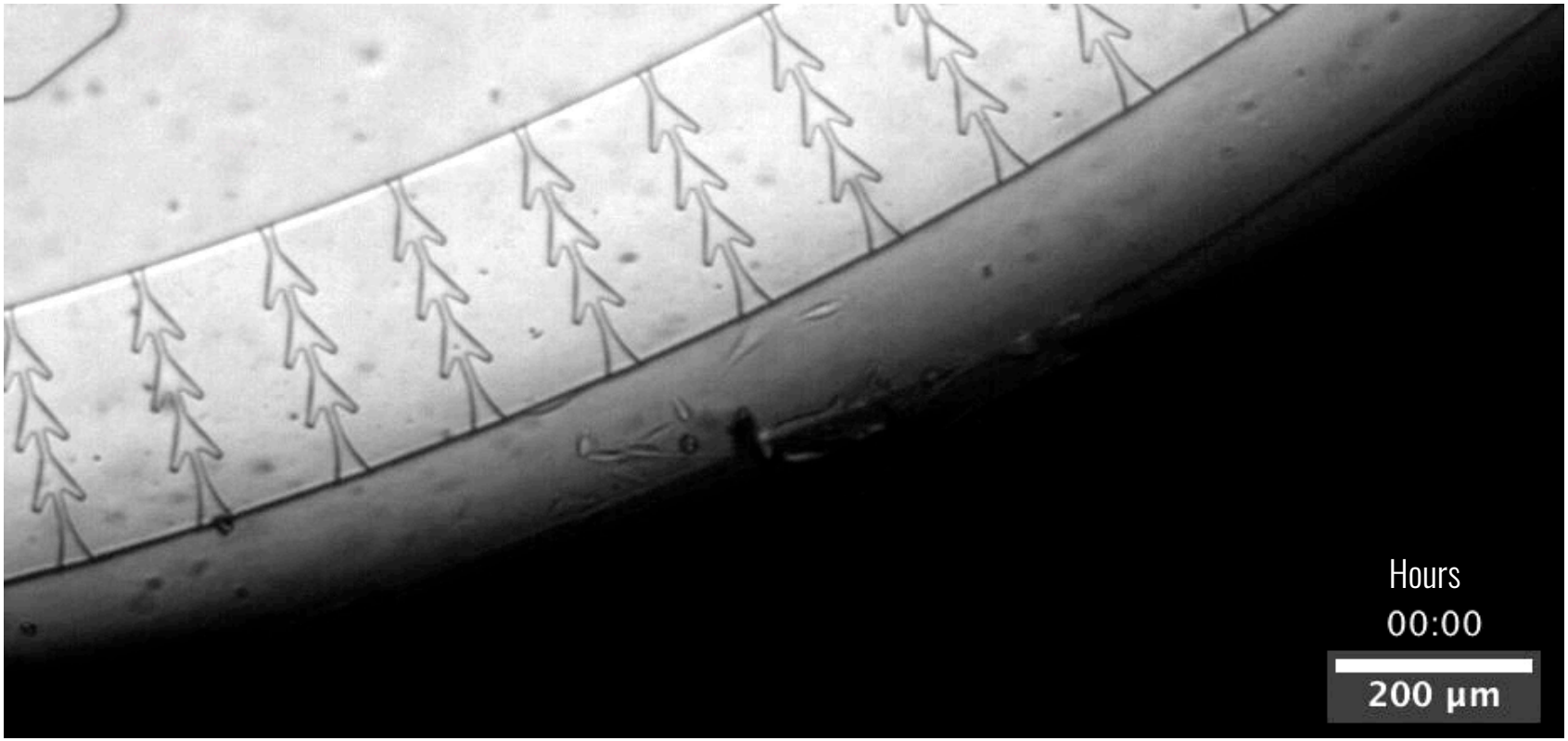
Cell migration in microengineered tumor Environments, Eujin Um et al. Lab Chip DOI: 10.1039/c7lc00555e



Cell migration

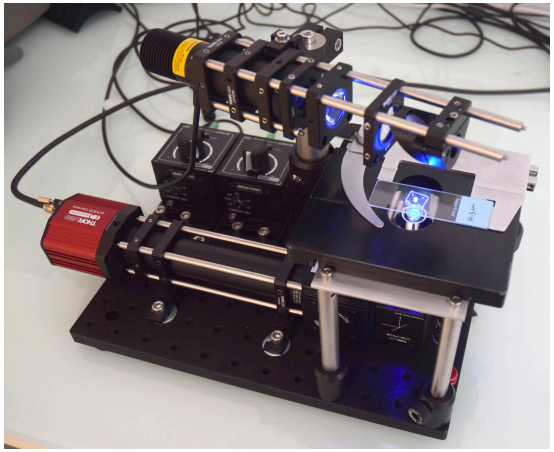


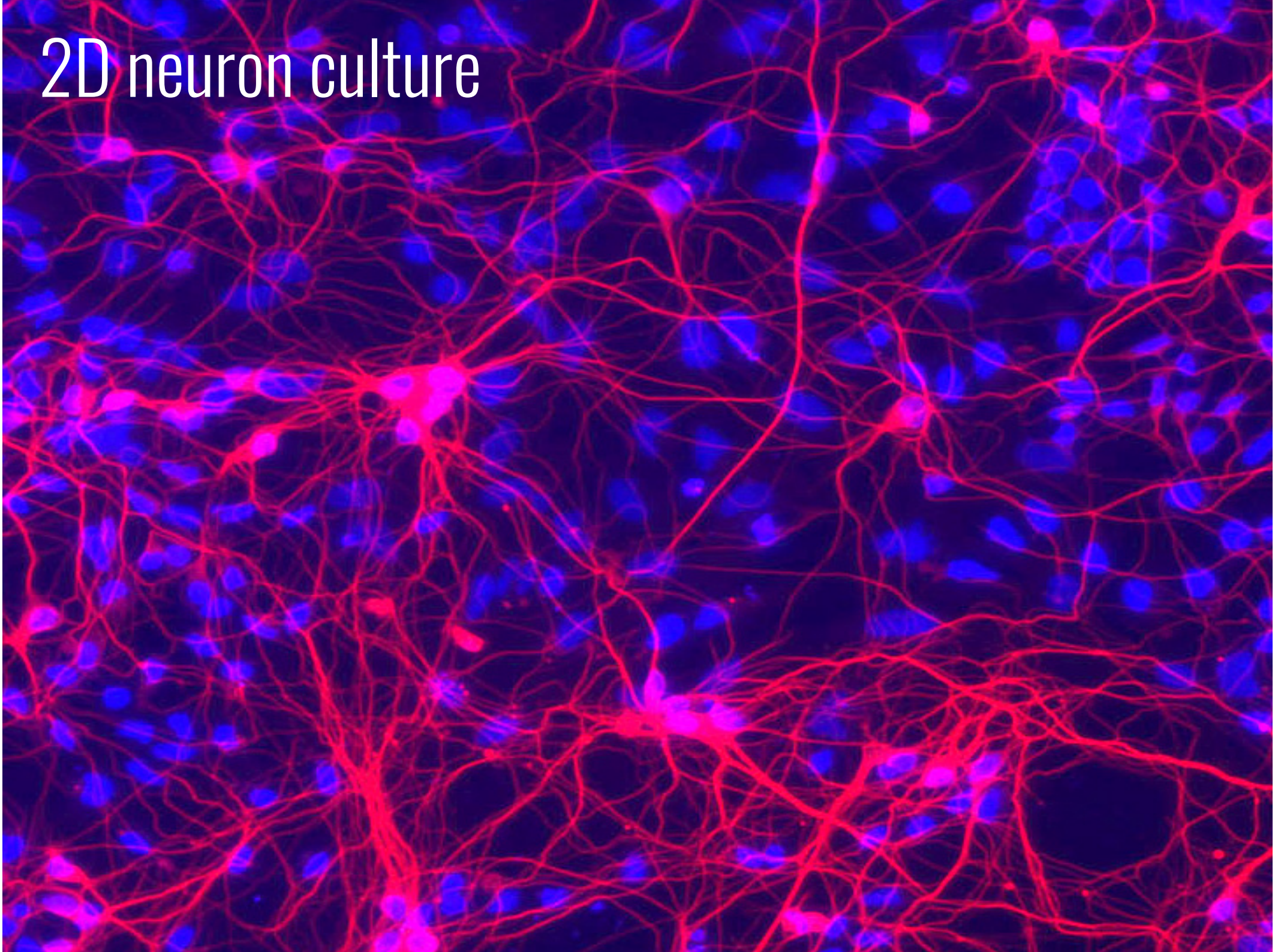
Cell migration



Cancer cells migrating through a microfluidic circuit

DIY microscope



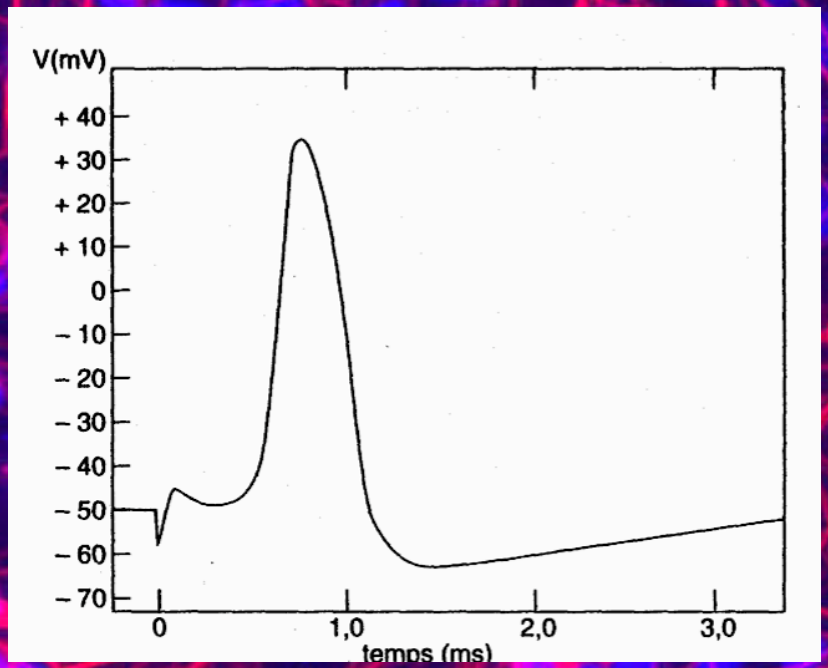

2D neuron culture

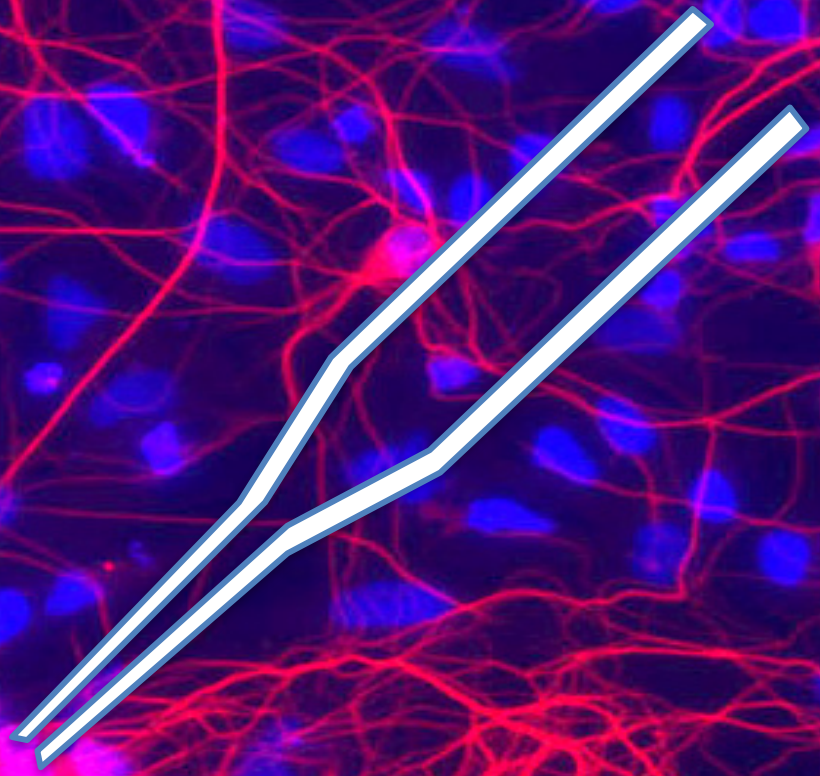
How to measure electrical activity of the network?

Patch clamp

One cell at a time

Intracellular recording



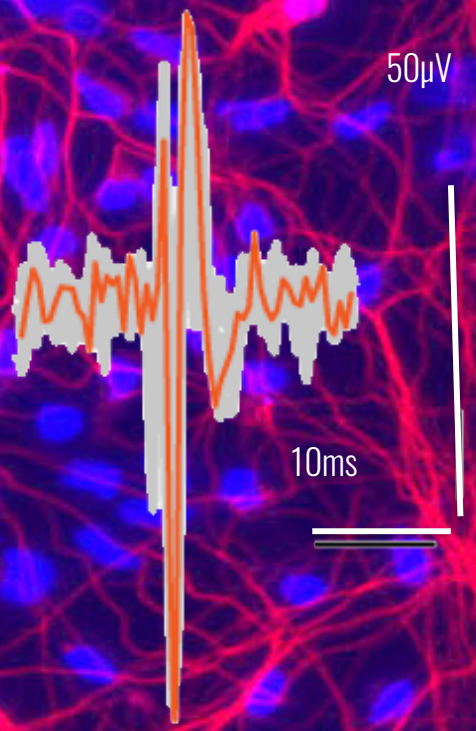


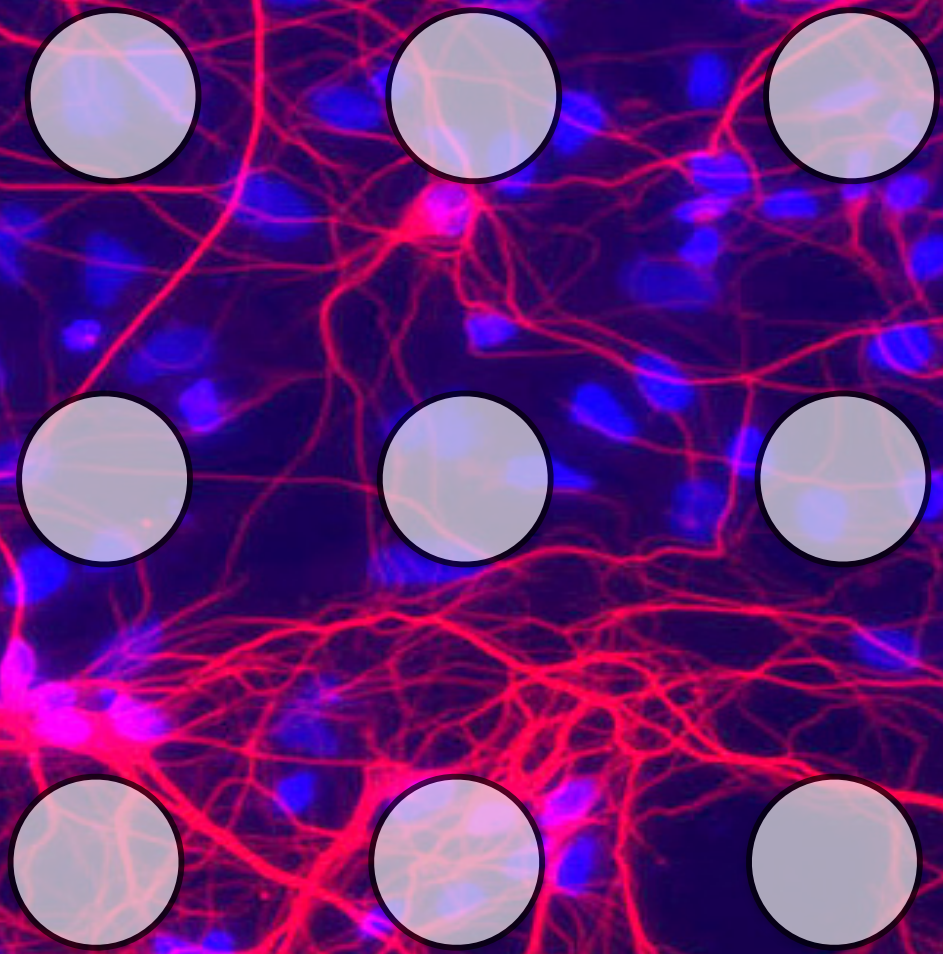
How to measure electrical activity of the network?

Micro Electrode array

Array of fixed electrodes

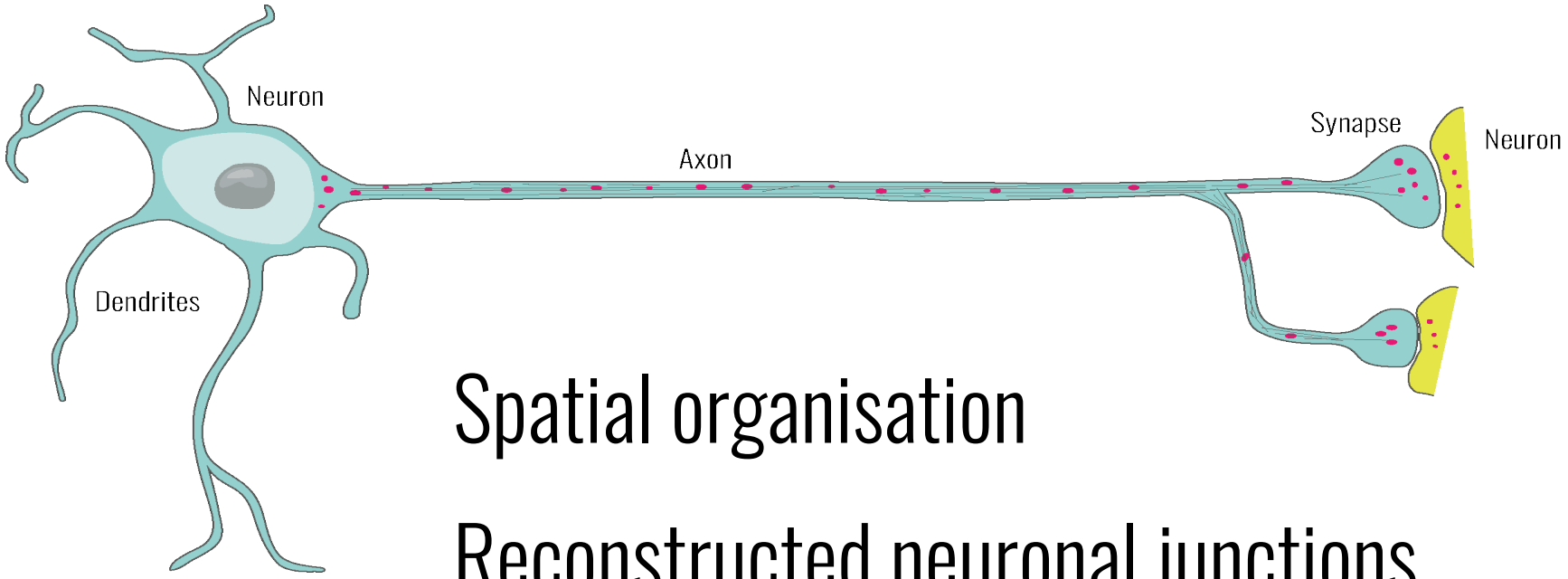
Extracellular potential





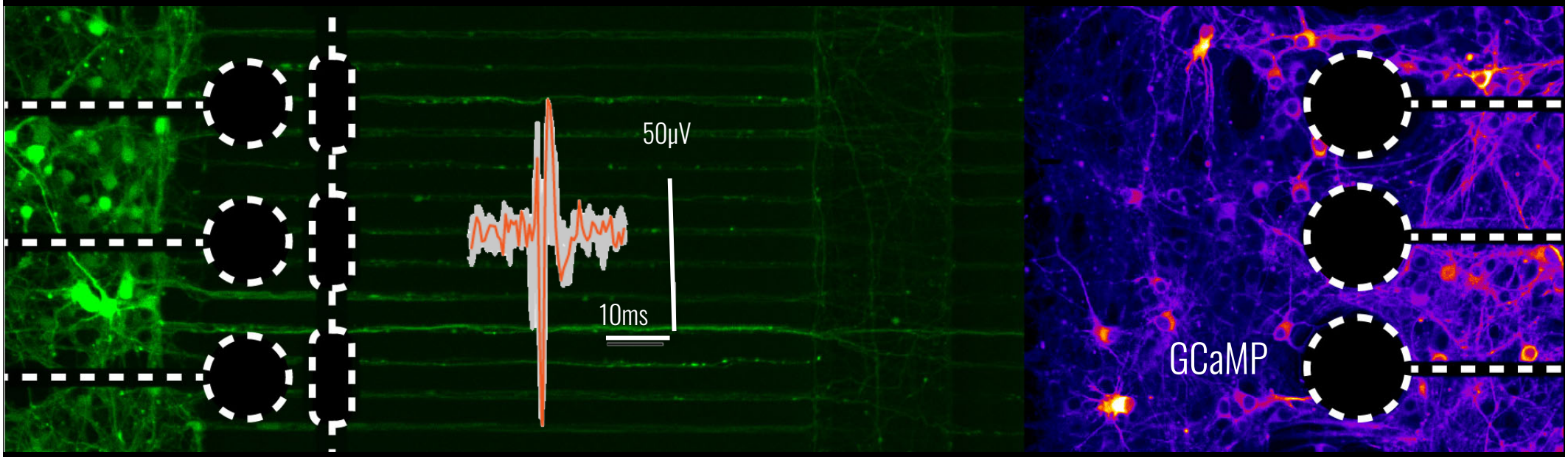
How to organise the network?

Neurofluidics



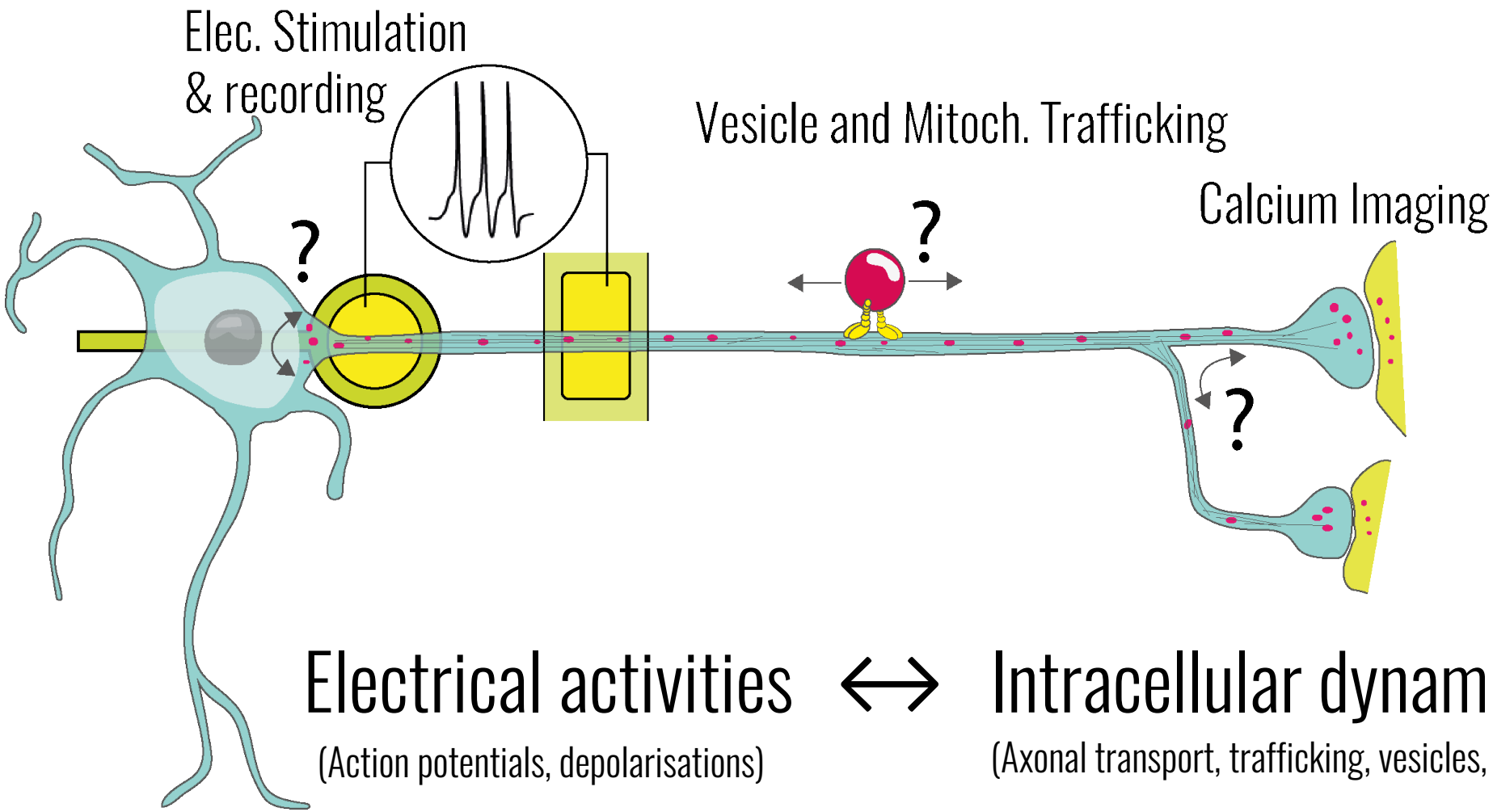
Spatial organisation

Reconstructed neuronal junctions



- Reconstruction of neuronal junctions ➔ **Microfluidics**
- Stimulation and monitoring neuronal junctions ➔ **Micro Electrodes**
- Observation of axonal transport ➔ **Spinning Disc Fluorescence Microscopy**

Neurofluidics + Extracel. electrodes

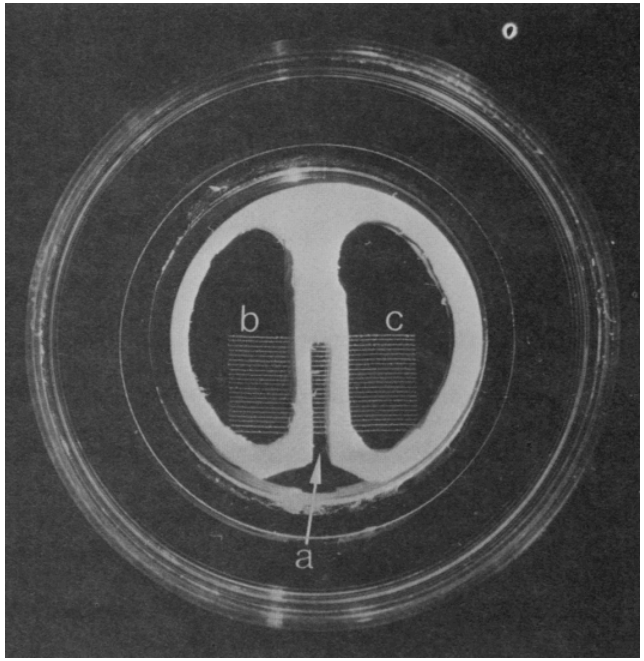


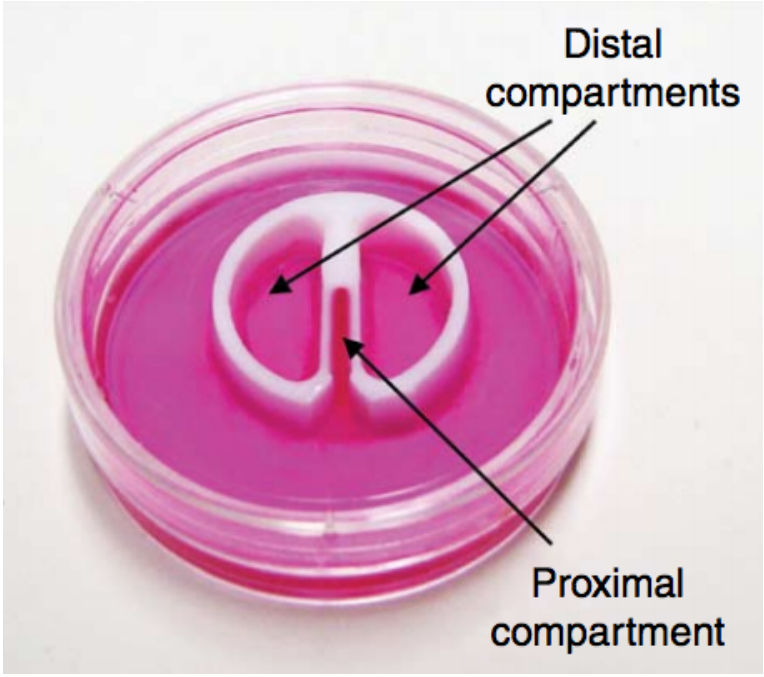
Electrical activities ↔ Intracellular dynamics

(Action potentials, depolarisations) (Axonal transport, trafficking, vesicles, RNA)

Neurodegenerative disease : HD Huntington disease, ALS, SMA

Campenot Chambers, 1977



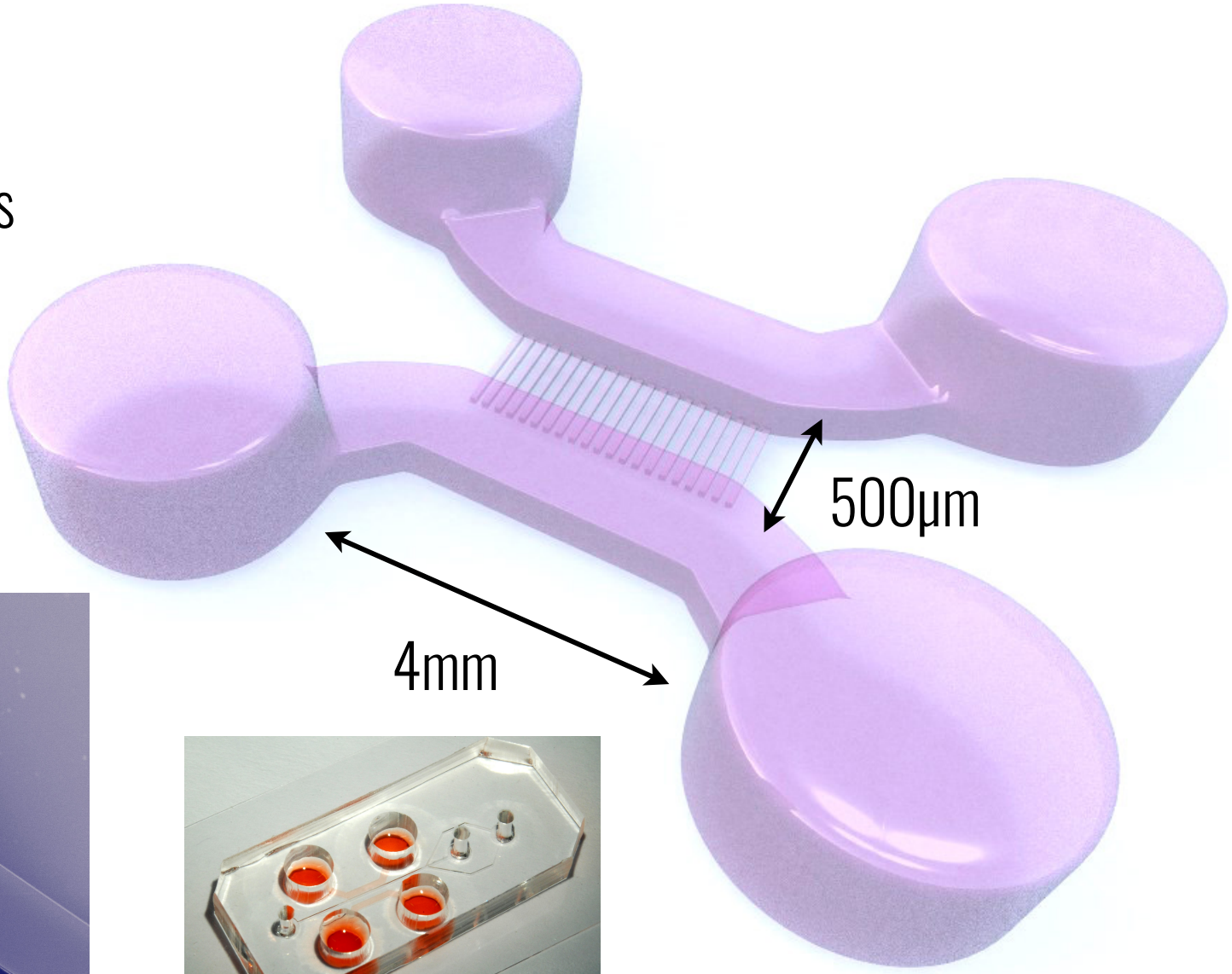


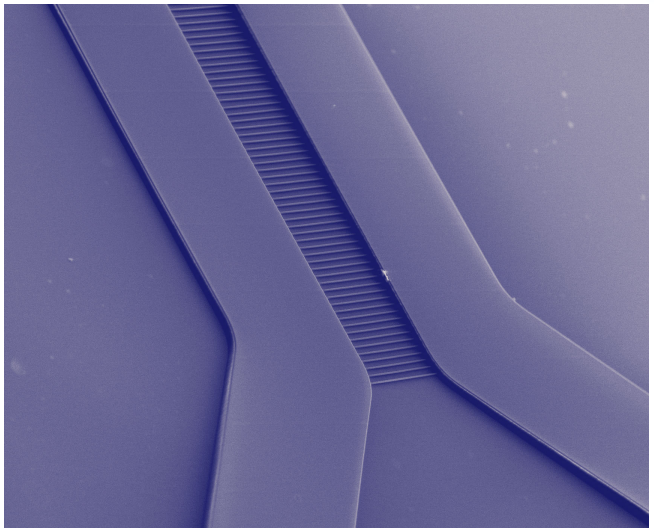
Robert. B. Campenot,
PNAS 1977 Oct; 74(10): 4516–4519.

A rake made by cementing together twenty insect pins was used to make 20 parallel scratches about 200 μm apart on the collagen-coated coverslip.

Compartimentalized Microfluidics

Two large chambers
A set of Microchannels





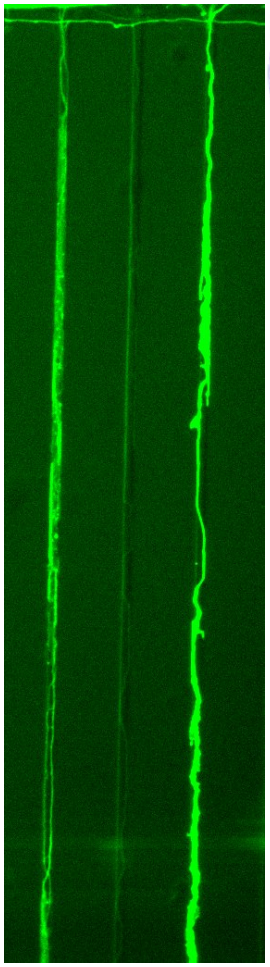
Dual thickness SU8 / PDMS

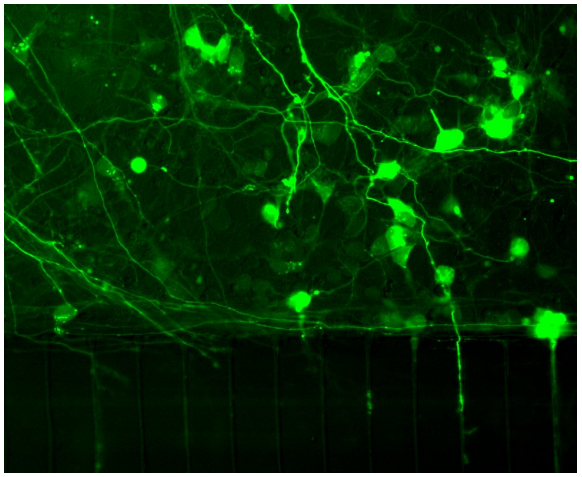
A.M.Taylor et al. Langmuir 19, 2003

A.M.Taylor et al. Nat. Methods 2, 2005

Compartimentalized Microfluidics

PDL/Laminin coating
Cell seeding
Incubator
Neurites and Axons
(if L>500µm)

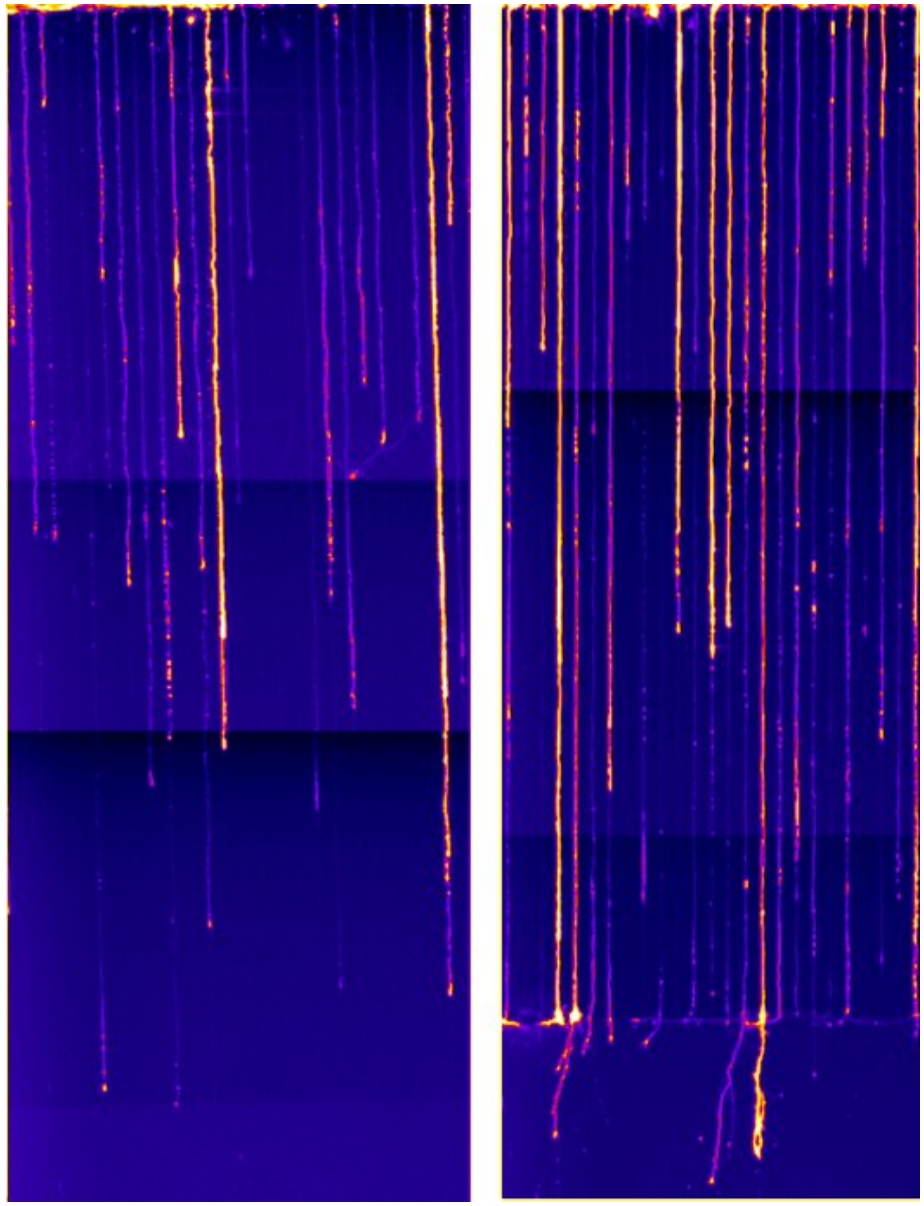




Compartimentalized Microfluidics

Long micro channels : 1,5 mm
Analysis of growth rate analysis under different stimulations

- Chimioatractant
- Mechanotransduction
- Electric fields
- **Light**



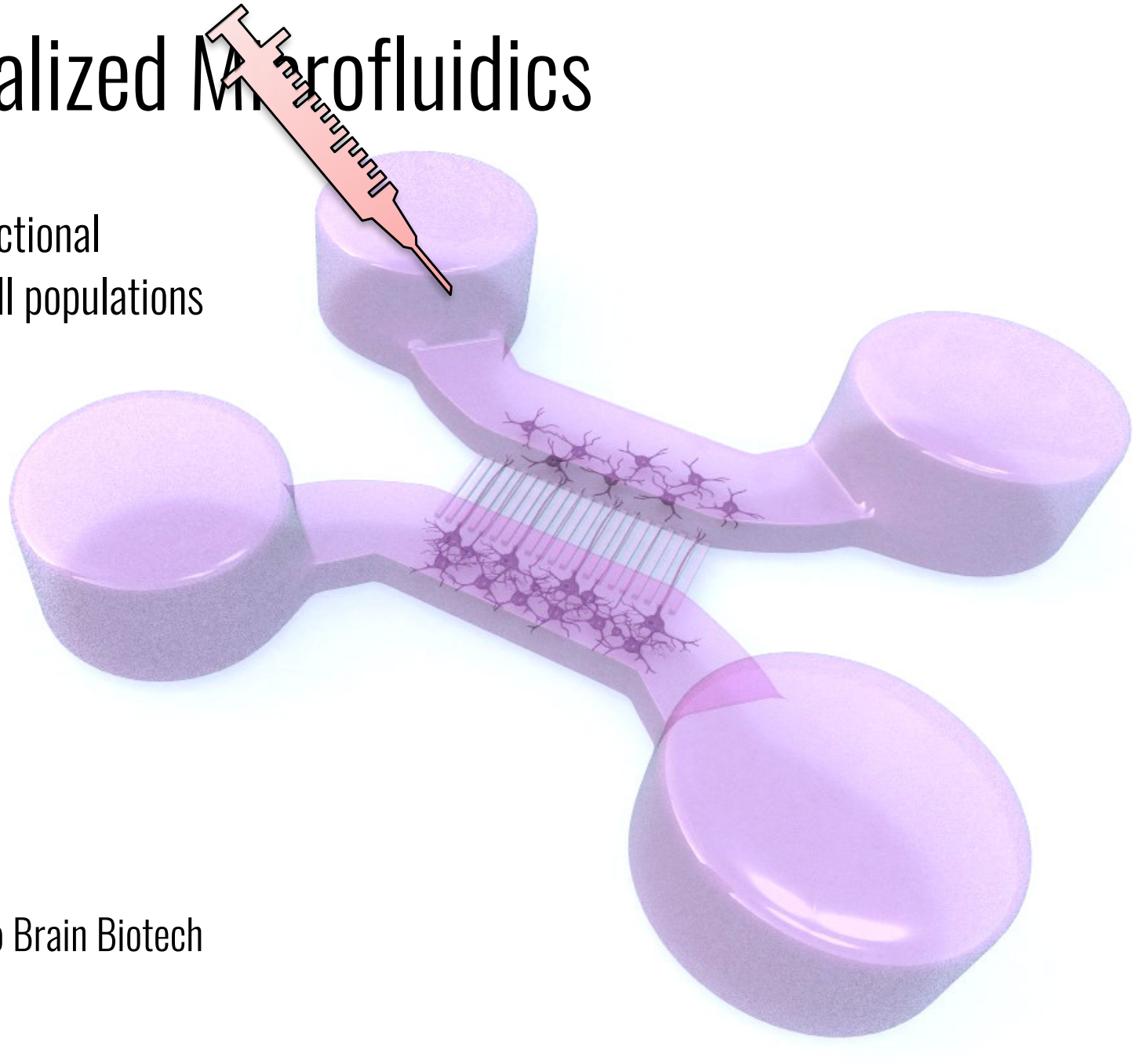
Control

Optical stimulation

Compartimentalized Microfluidics

in-vitro reconstitution of functional connections between two cell populations

cortico-cortical
cortico-Striatal
cortico-hippocampal,
hippocampo-hippocampal,
...

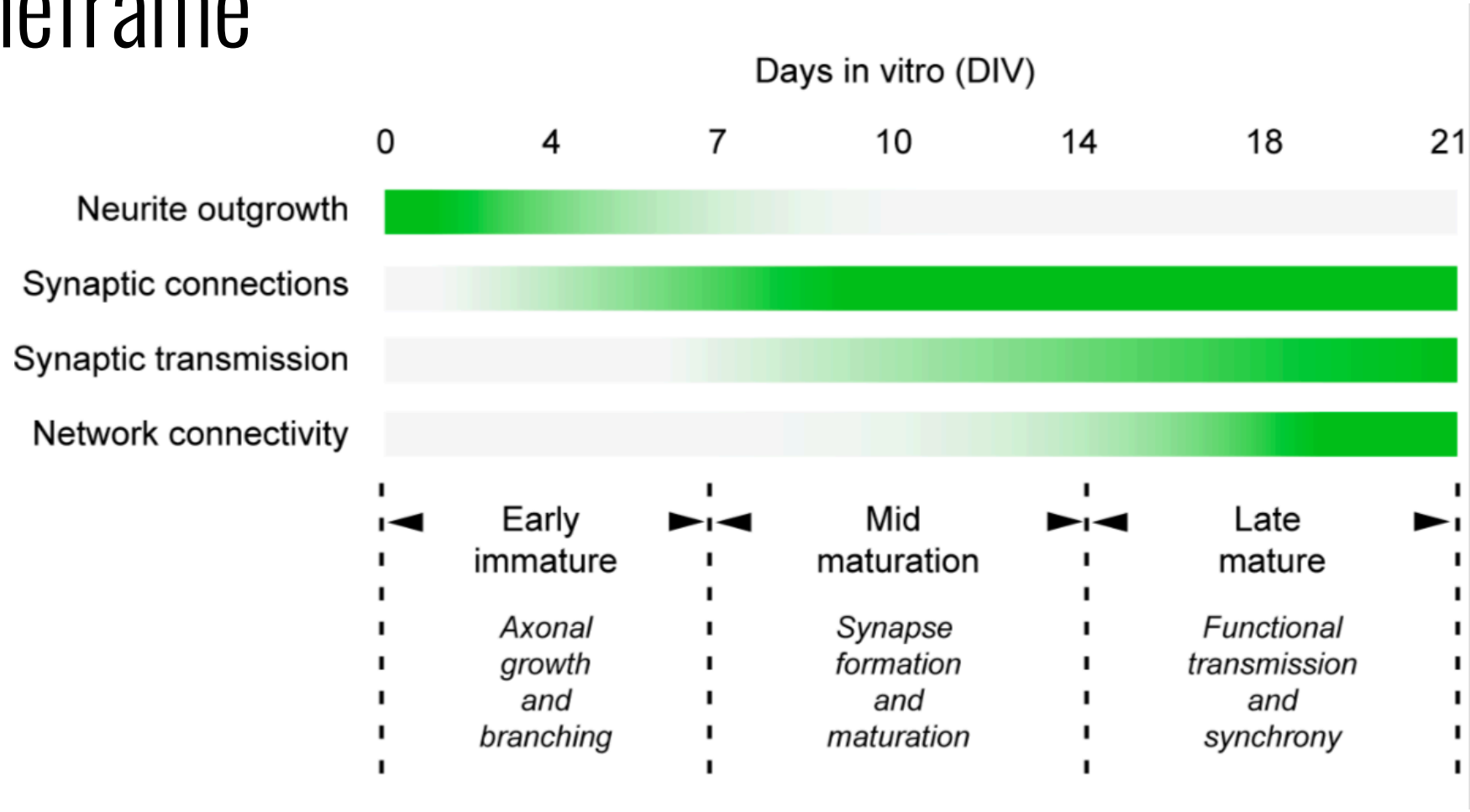


Xona, Millipore, Ananda, Micro Brain Biotech

+gradient of laminin/poly-d-lysin coating

Compartimentalized Microfluidics

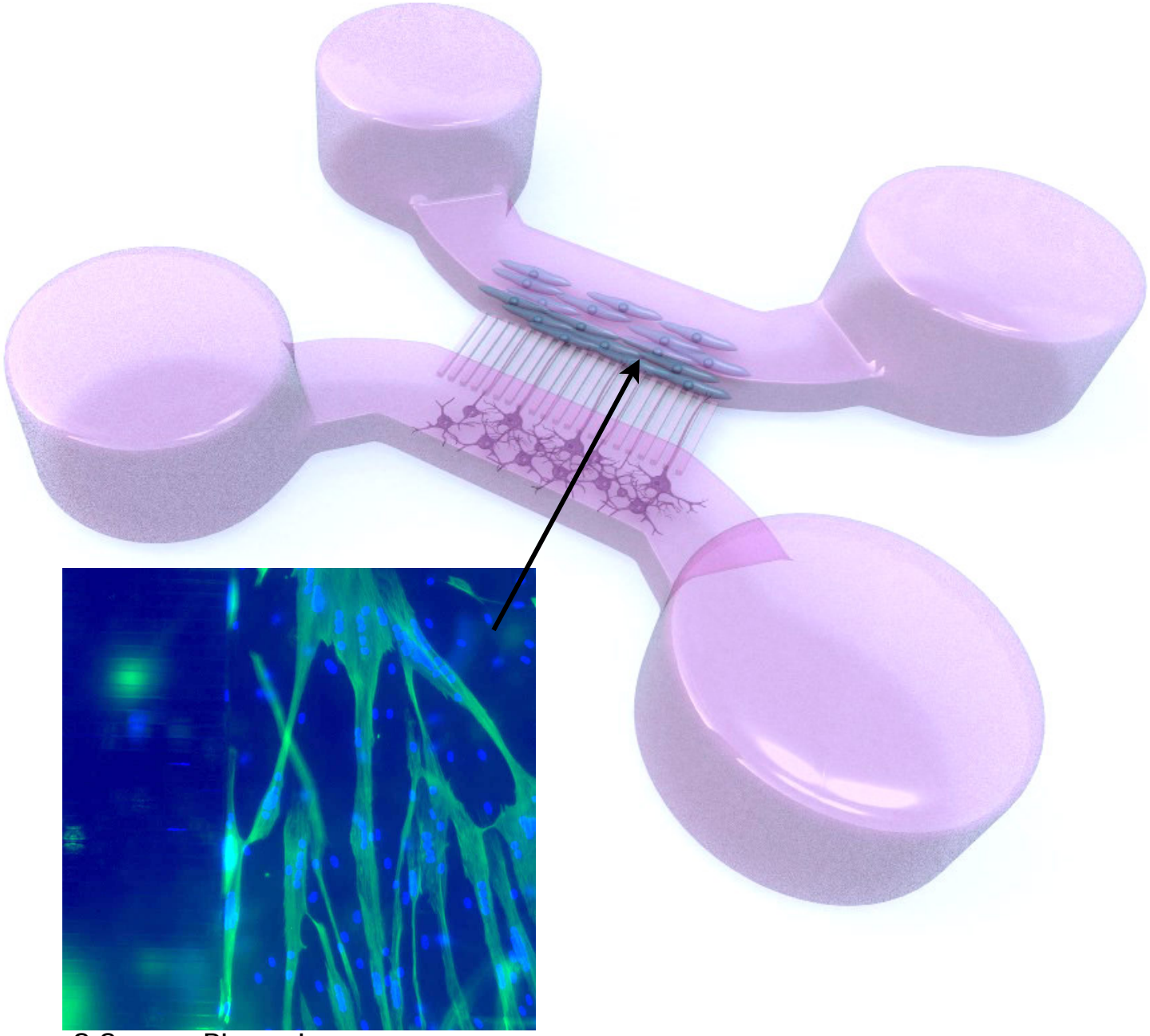
Timeframe



Compartimentalized Microfluidics

Co-Cultures

Neuron-skin
Neuron-bone
Motoneuron-muscle
...

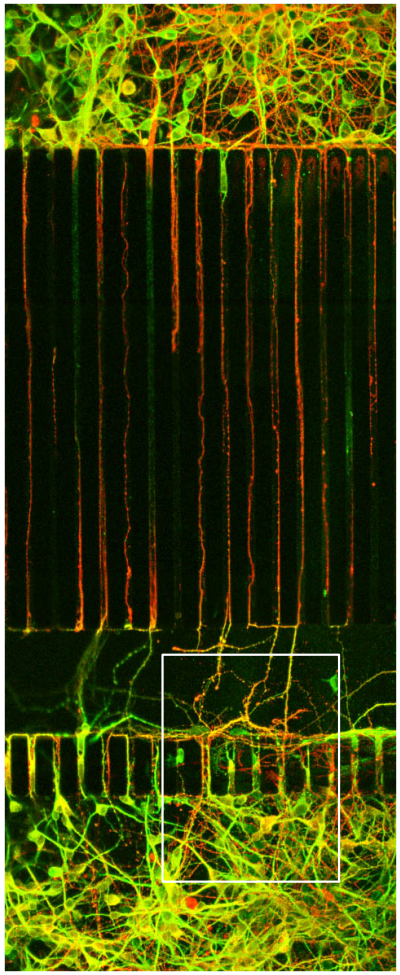


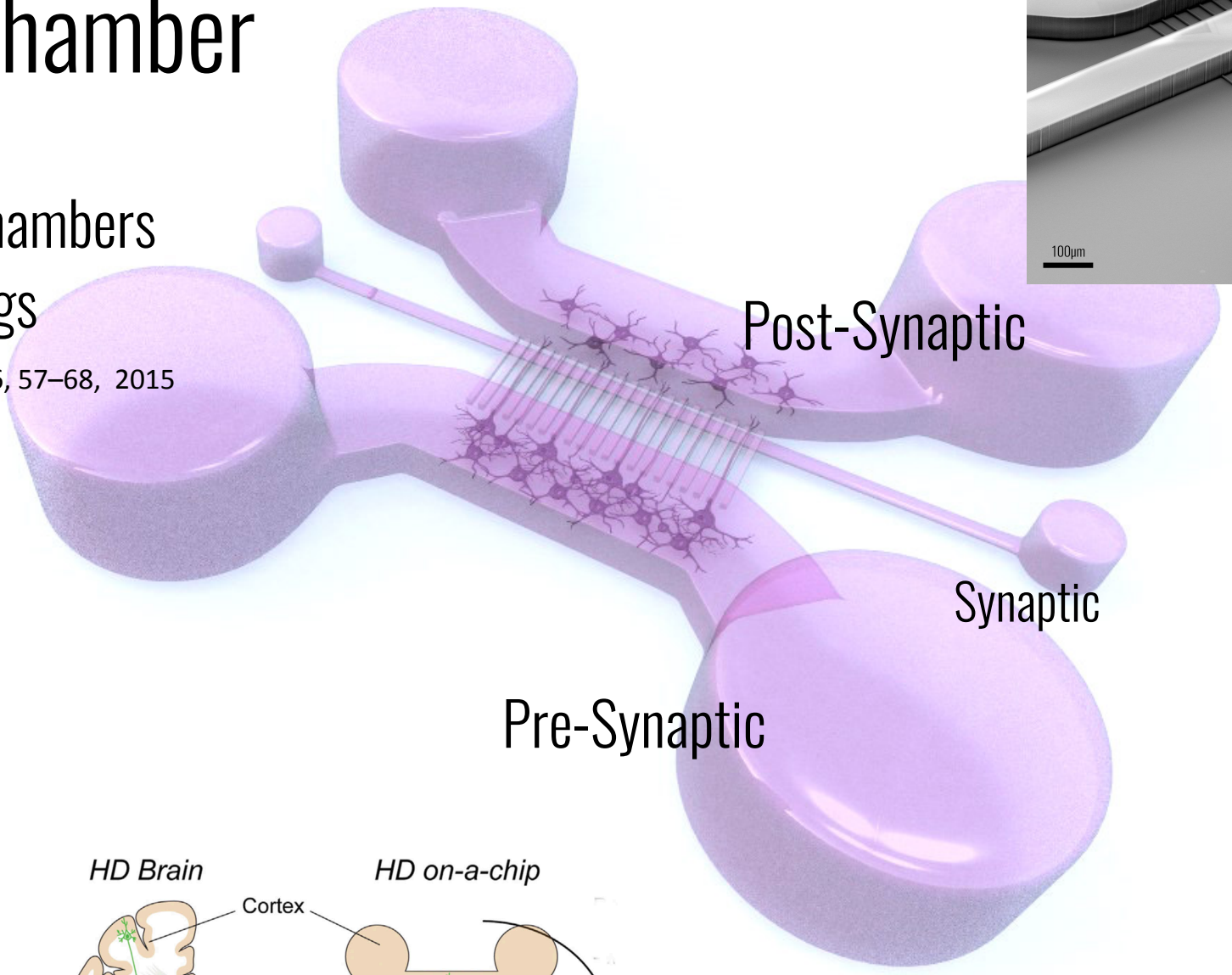
G.Carnac, Phymedexp

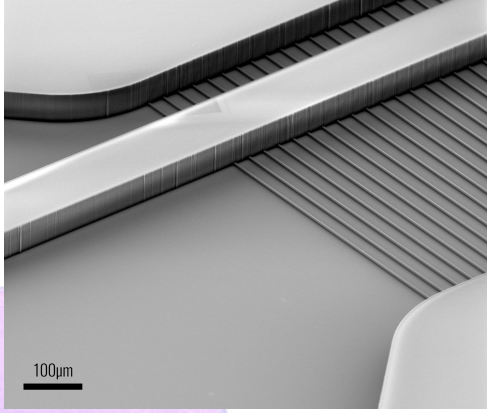
Synaptic chamber

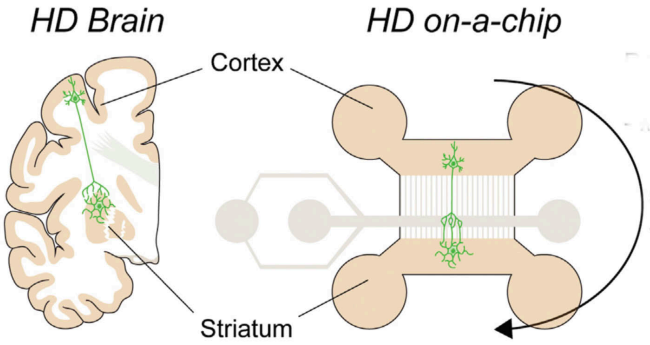
Design with 3 chambers
Perfusion of drugs

A.M. Taylor et al. Neuron 66, 57–68, 2015









A.Virlogeux et al. Cell Reports 22-1 (2018)

Microfluidics + Micro Electrode Array (MEA)

Organisation

Axons along microchannels

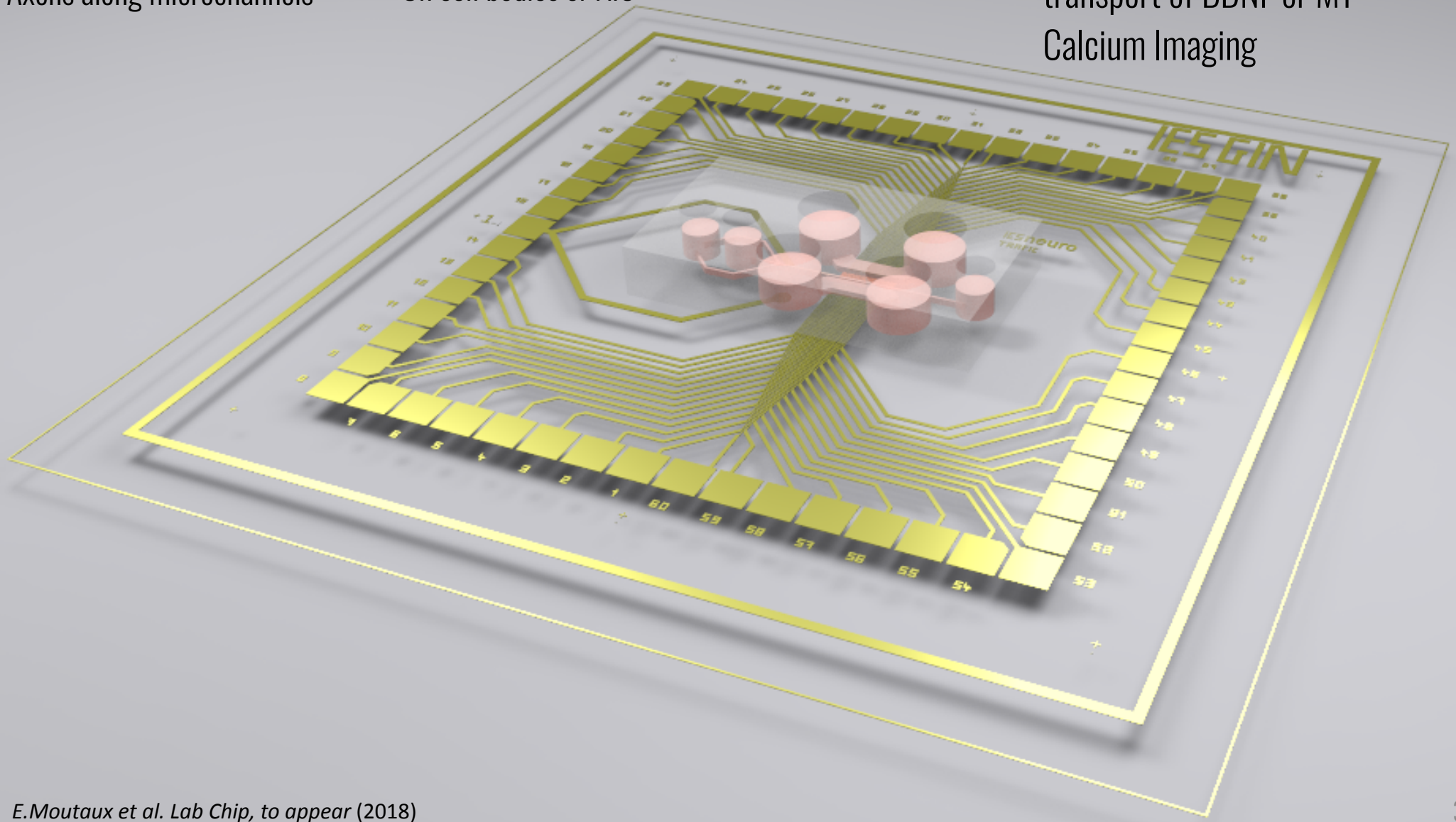
+ Stimulation & Recording

On cell bodies or AIS

+ Observation

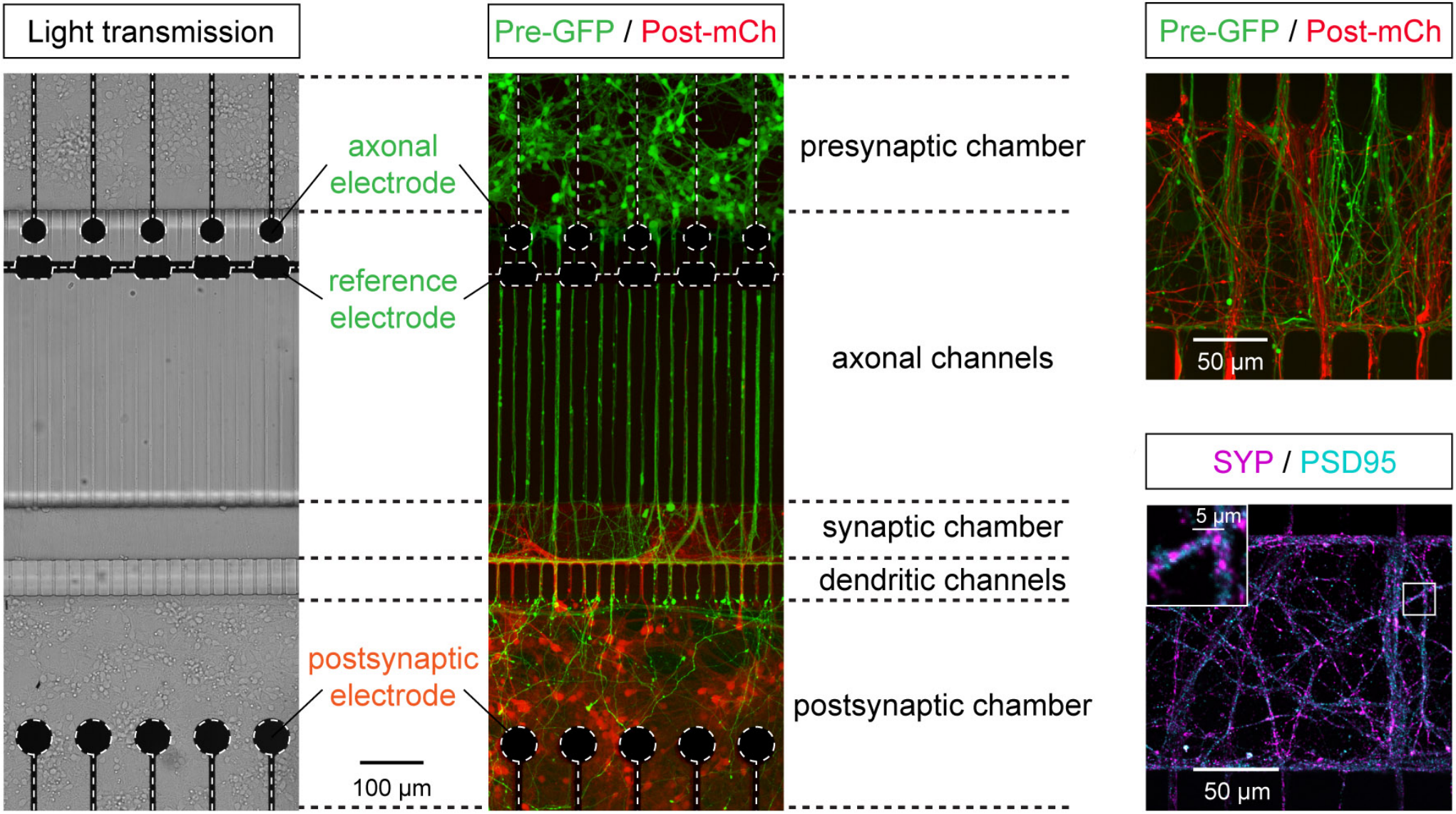
transport of BDNF or MT

Calcium Imaging



E.Moutaux et al. Lab Chip, to appear (2018)

Electrode arrangement



MEA microfabrication

Thin glass substrate : 5x5cm 170µm

Mask1 Ti/Pt electrodes (Electron gun evap. + Lift Off)

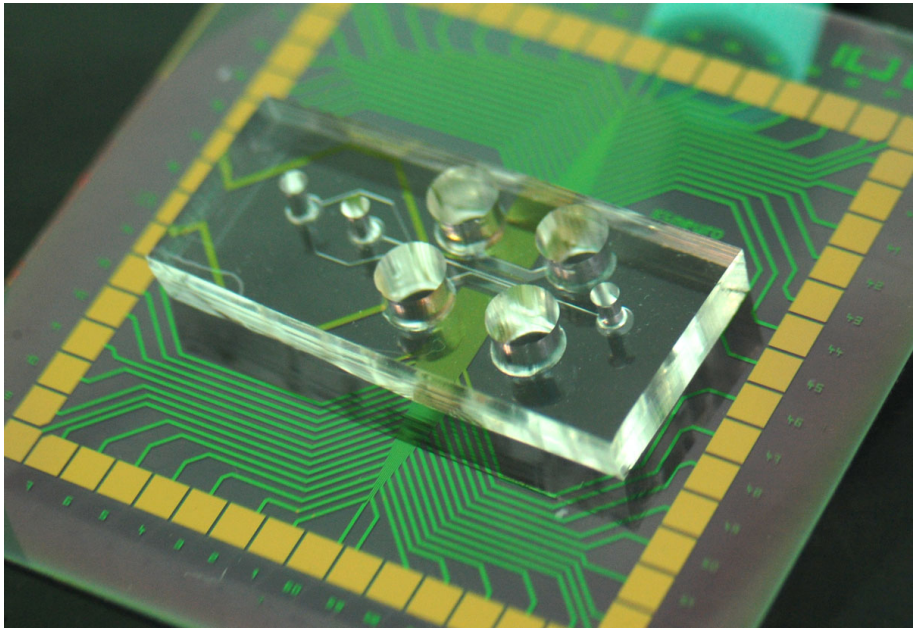
SiNx PECVD

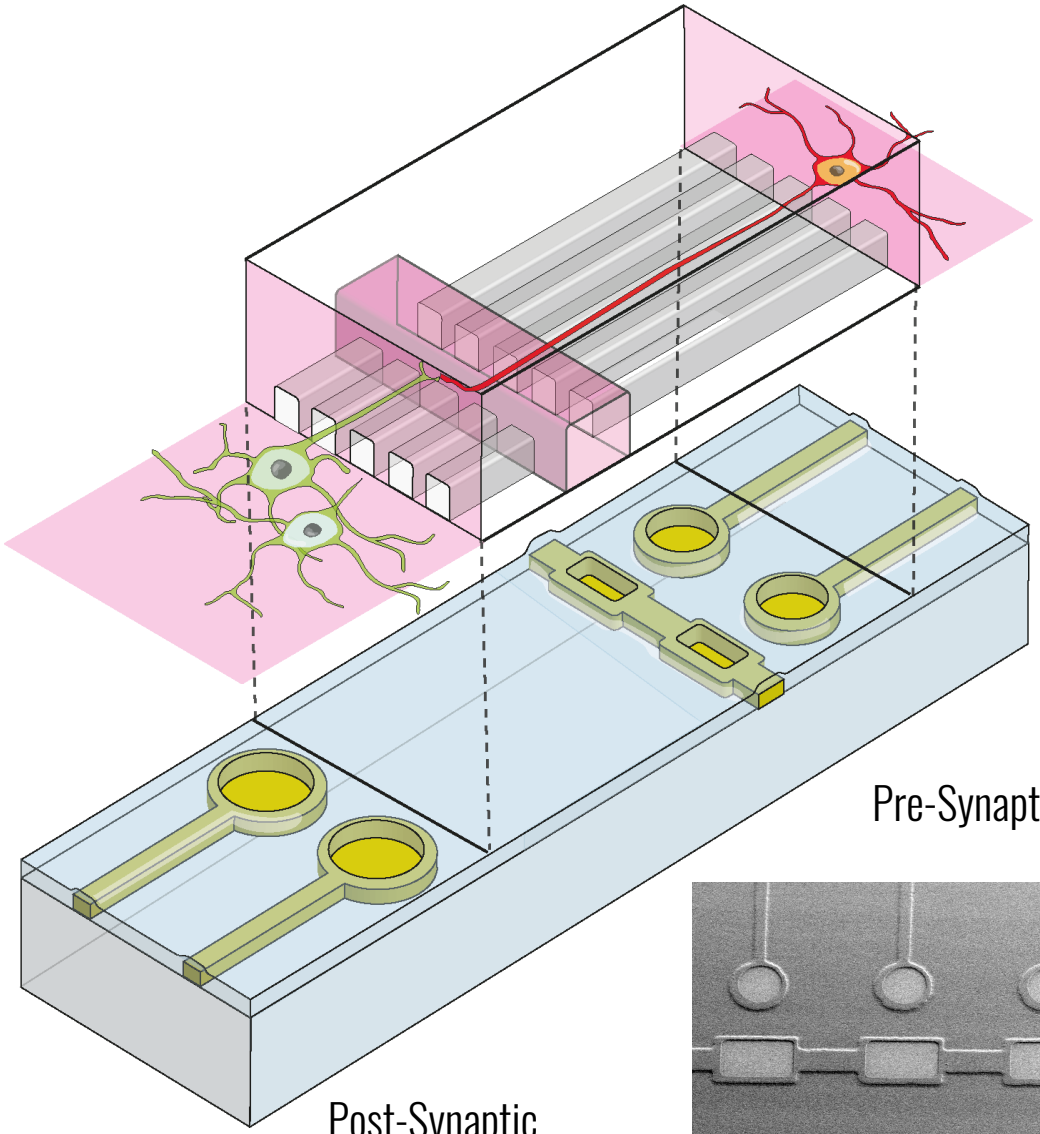
Mask2+ Alignment + RIE etching

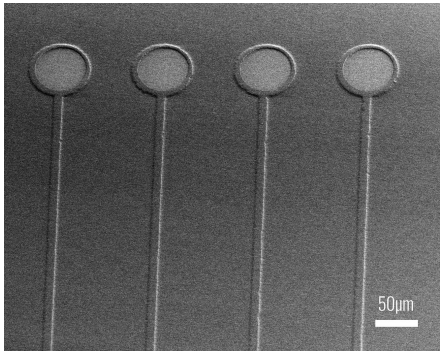
Cleaning, PDMS alignment and Bonding

Simple and Stable process

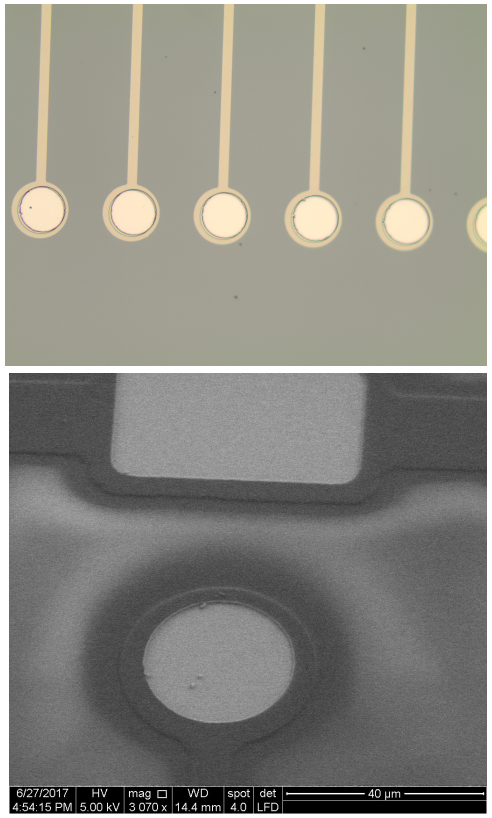
For series > 100 samples

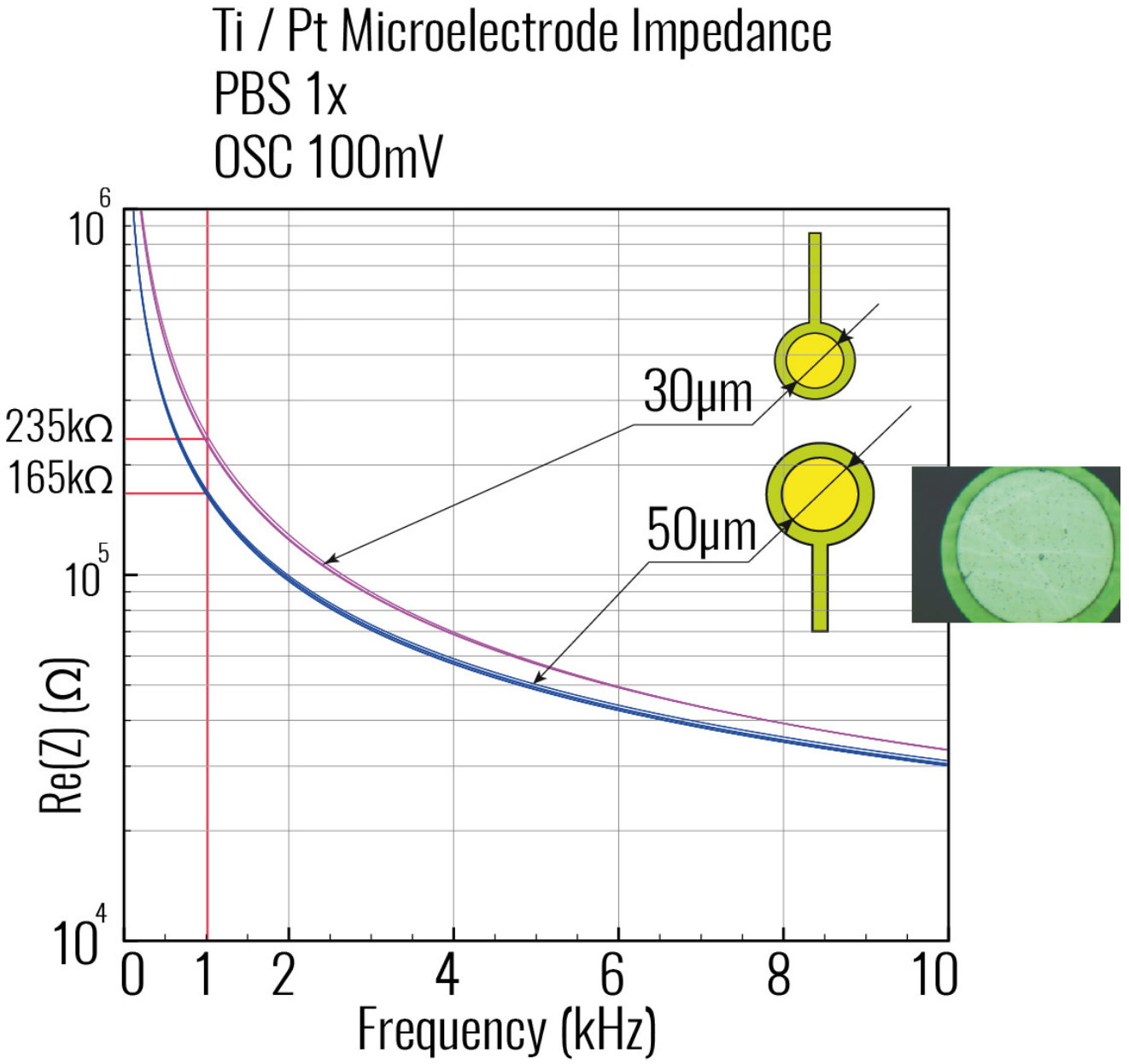






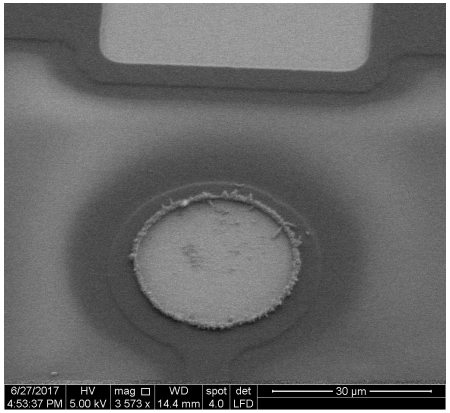
Impedance

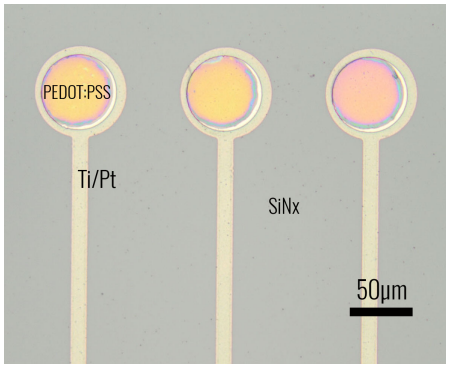

6/27/2017 4:54:15 PM
HV 5.00 kV
mag 3 070 x
WD 14.4 mm
spot 4.0
det LFD
40 µm

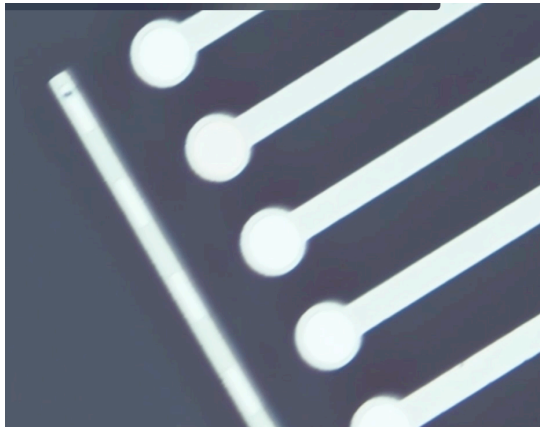

Ti / Pt Microelectrode Impedance
PBS 1x
OSC 100mV
10^6
235kΩ
165kΩ
10^5
10^4
Re(Z) (Ω)
0
1
2
4
6
8
10
Frequency (kHz)
30µm
50µm

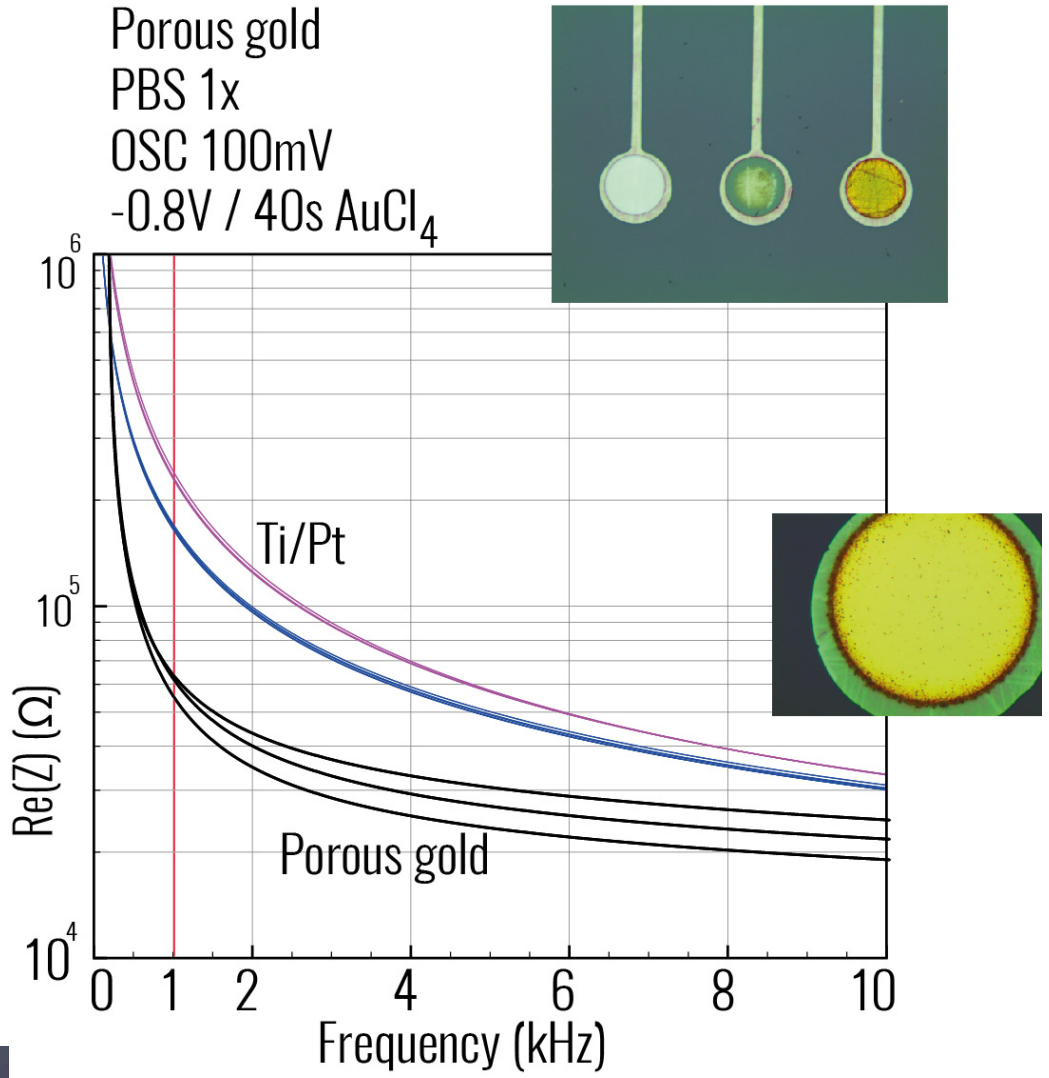
Impedance lowering

Ti/Pt
TiN
PEDOT:PSS
Porous gold Electrodeposition







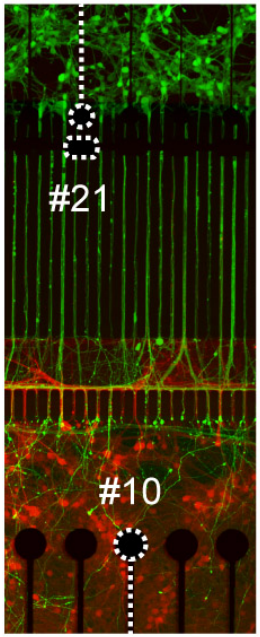


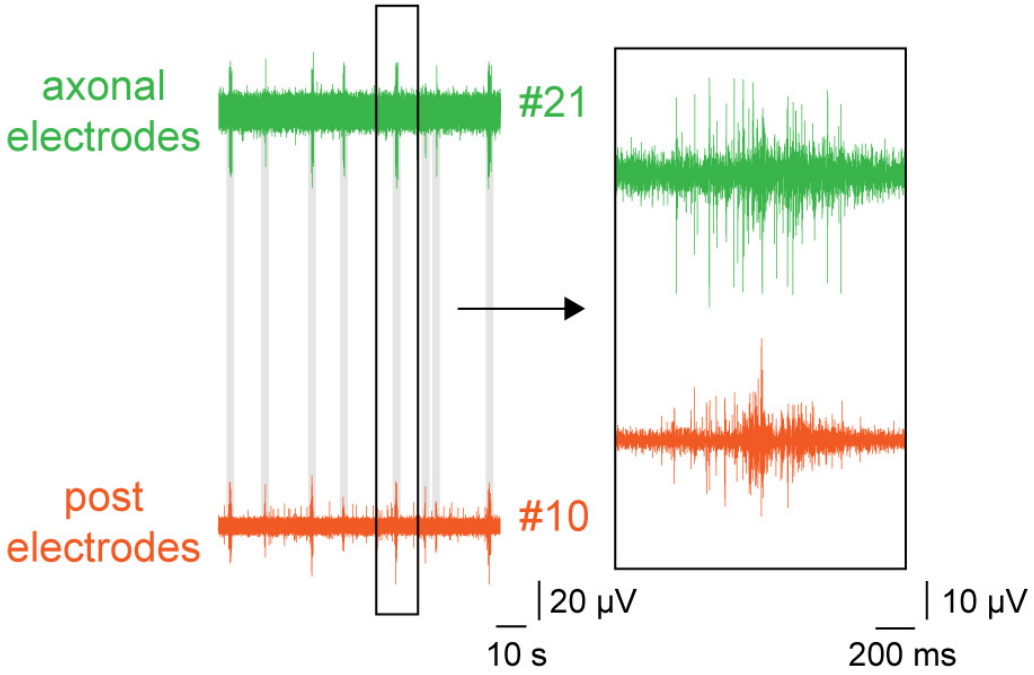
But..... Platinum for stability and reproducibility

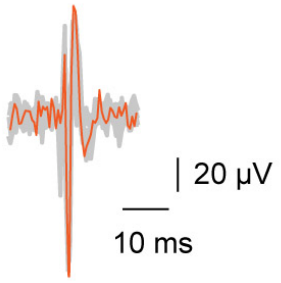
Extracellular Recording

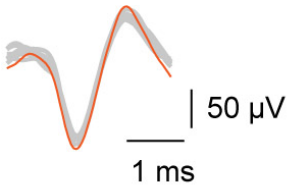
Spikes detection (SNR>5)

Spontaneous activity DIV10



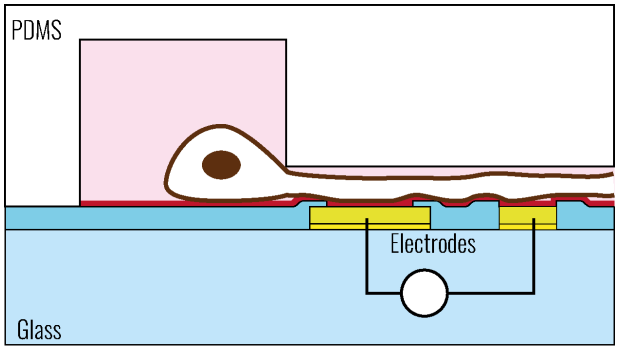




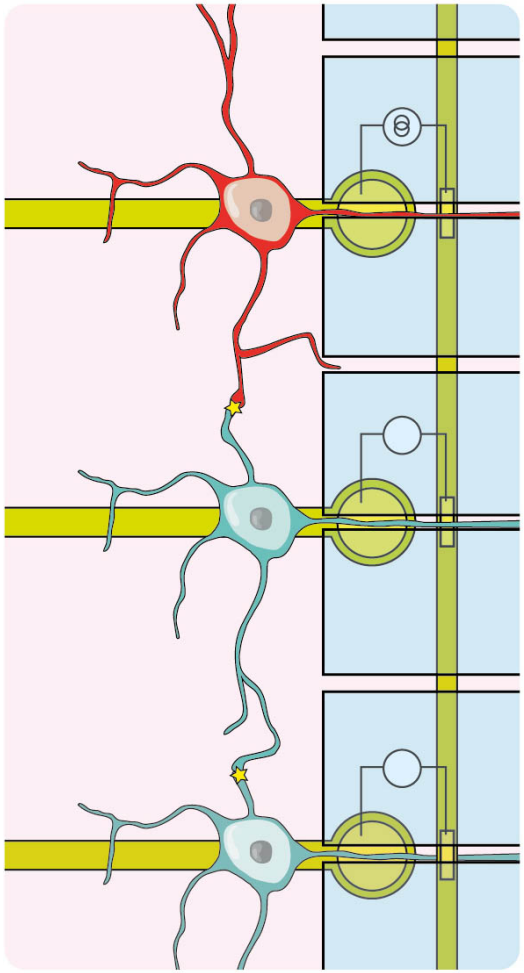


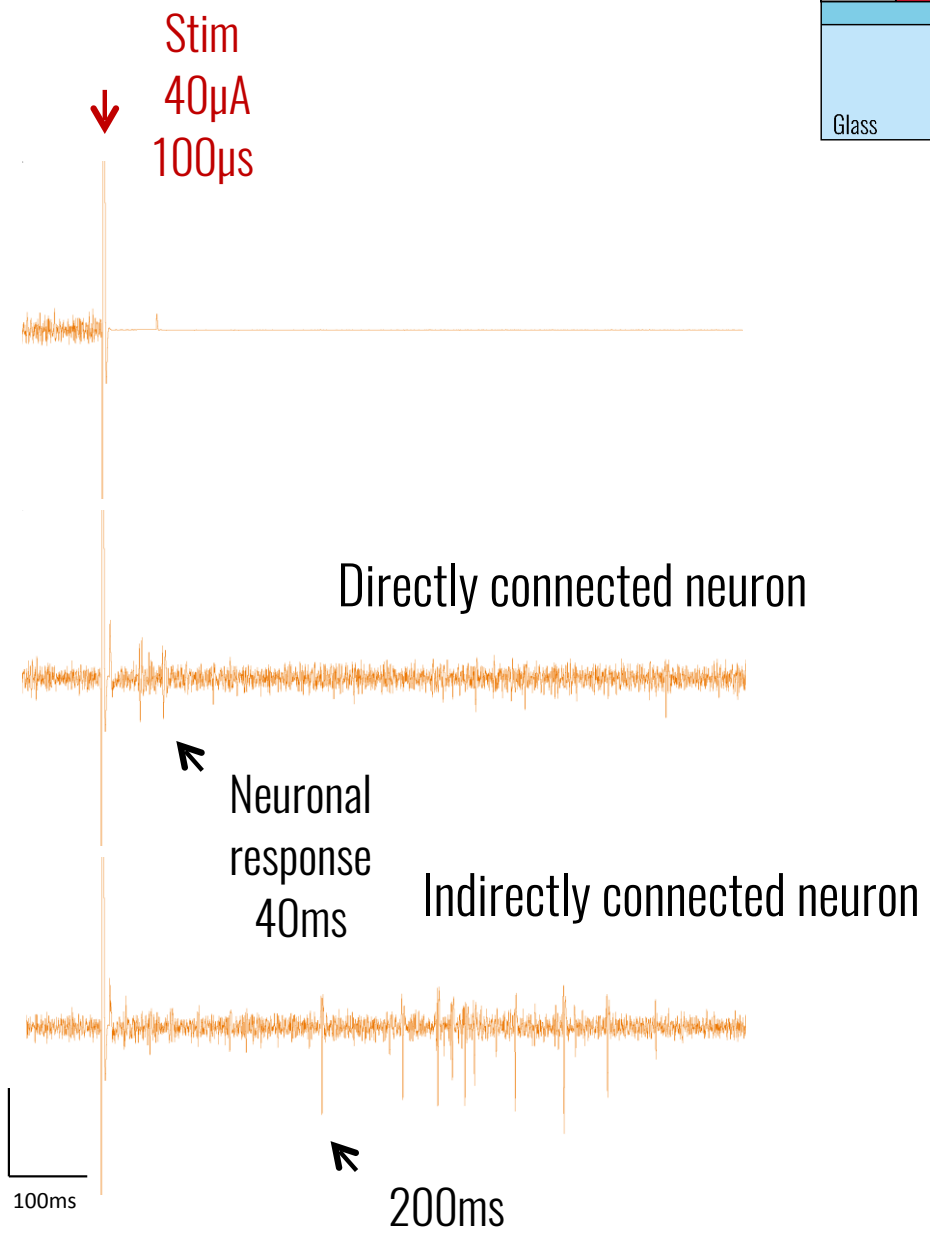
Self-organization and synchrony of the network

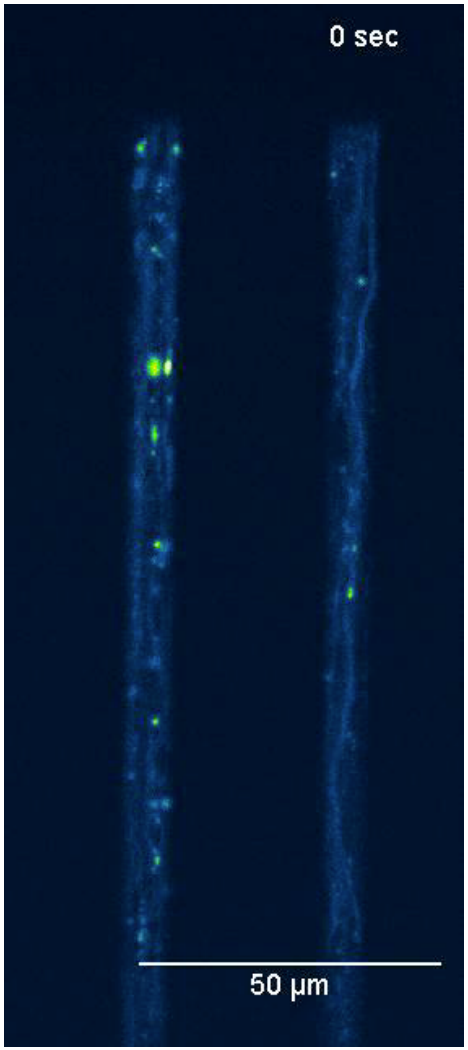
Extracellular Stimulation



Current stimulation
Axonal (AIS) stimulation



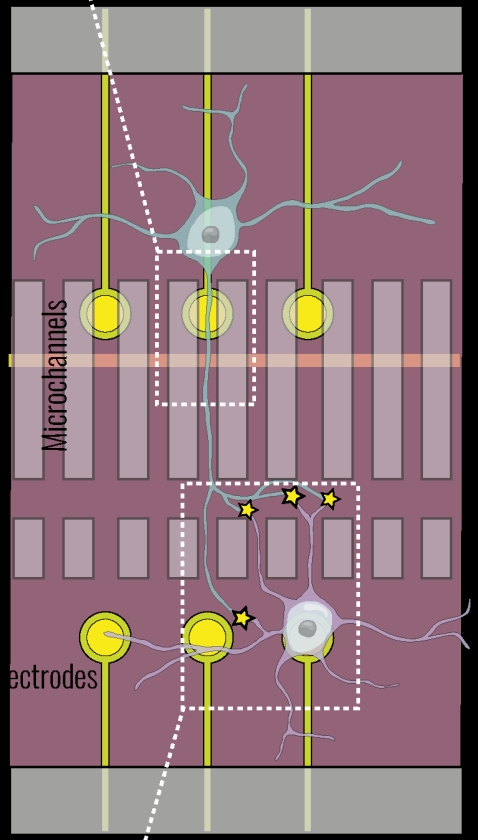




Stimulation+ GCaMP6f visualisation

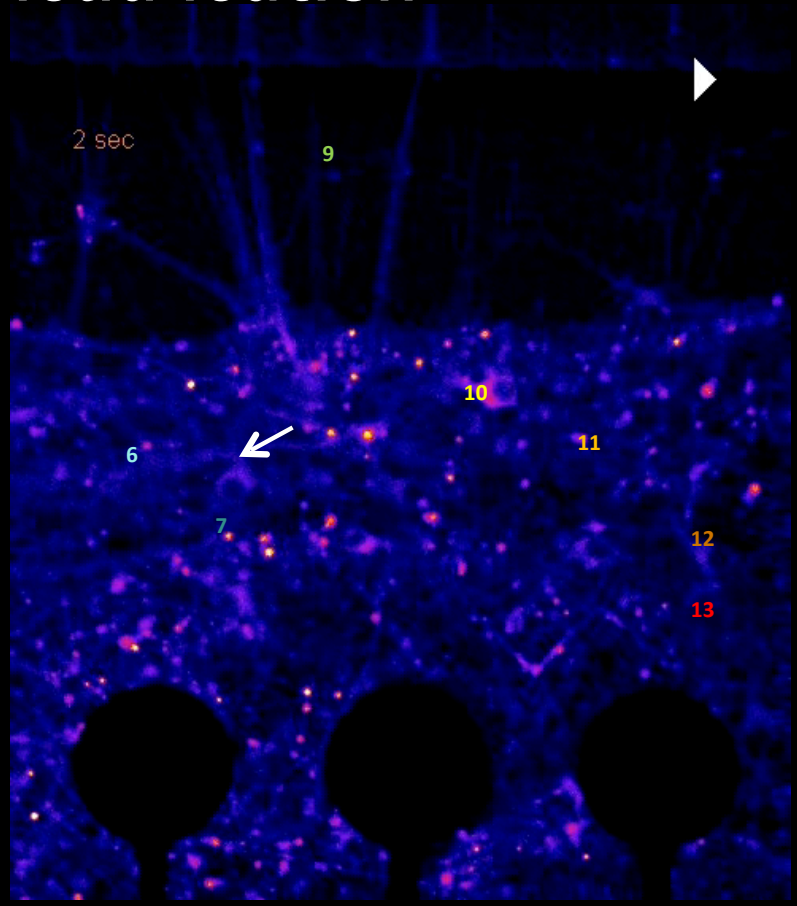
Genetically Encoded Calcium Indicators

Electrical stimulation

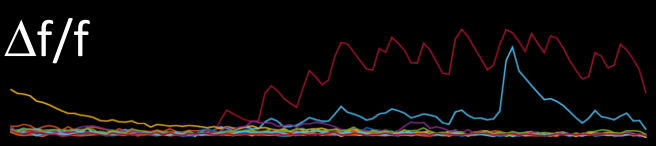


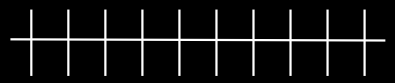
Calcium imaging

1Hz



Δf/f





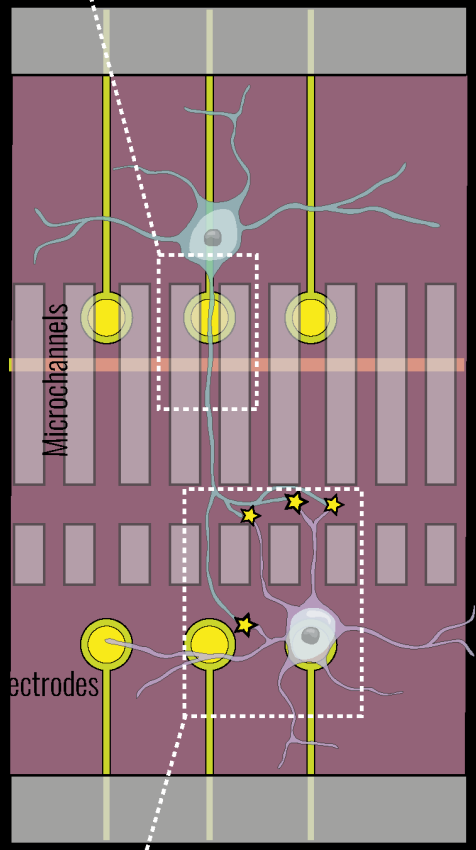
Small amplitude, repeated

LTD long term depression : Decrease in synaptic strength induced by LF stimulation of presynaptic afferents

Stimulation+ GCaMP6f visualisation

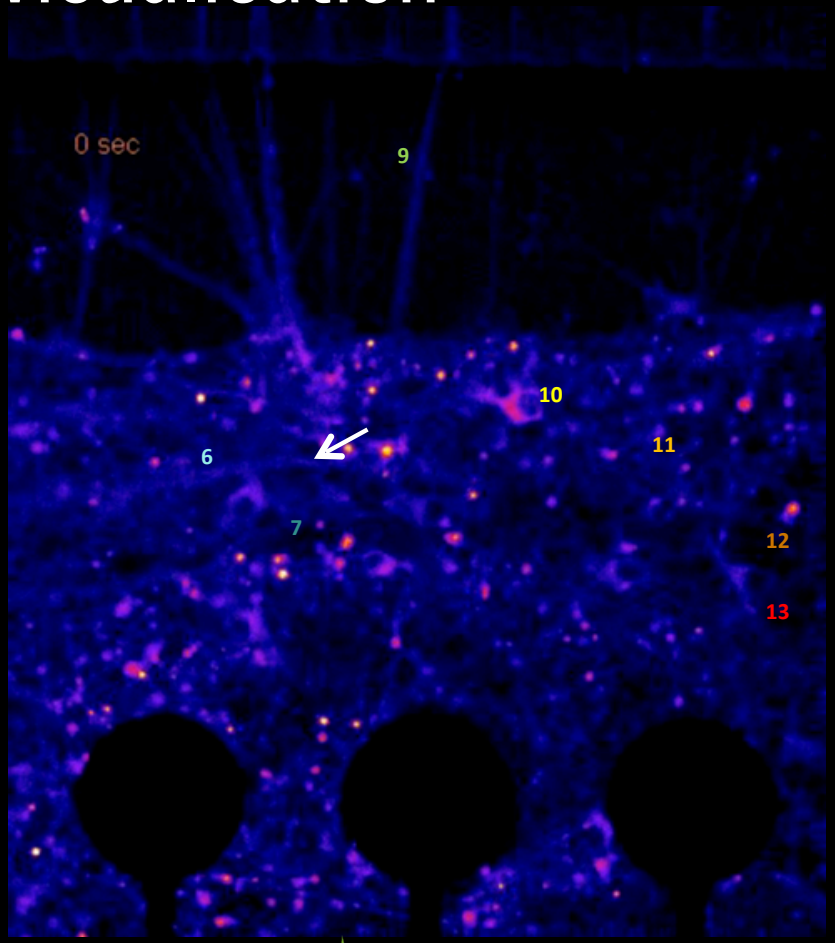
Genetically Encoded Calcium Indicators

Electrical stimulation

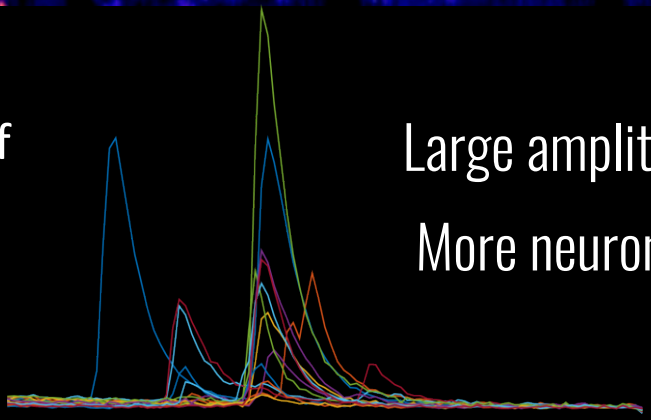


Calcium imaging

50Hz



Δf/f

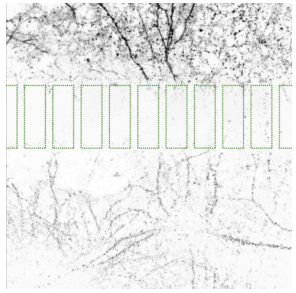


Large amplitude, long signal

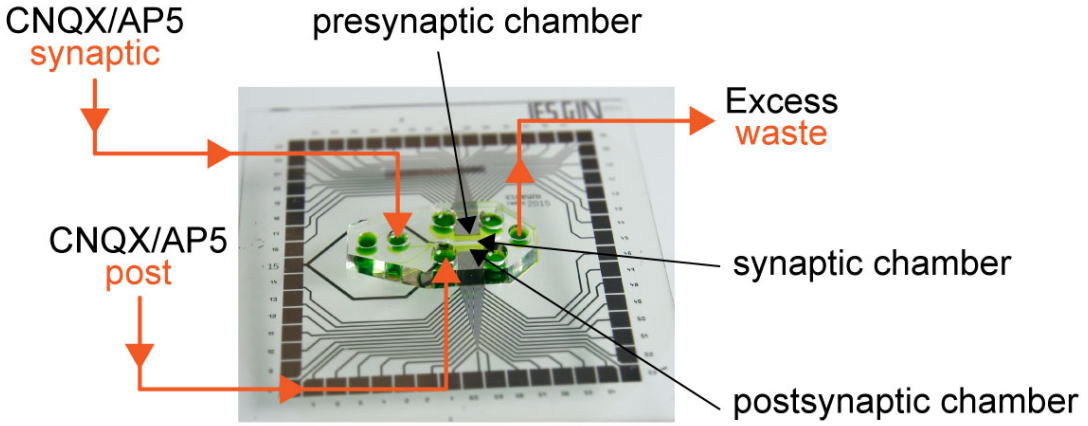
More neurons are recruted

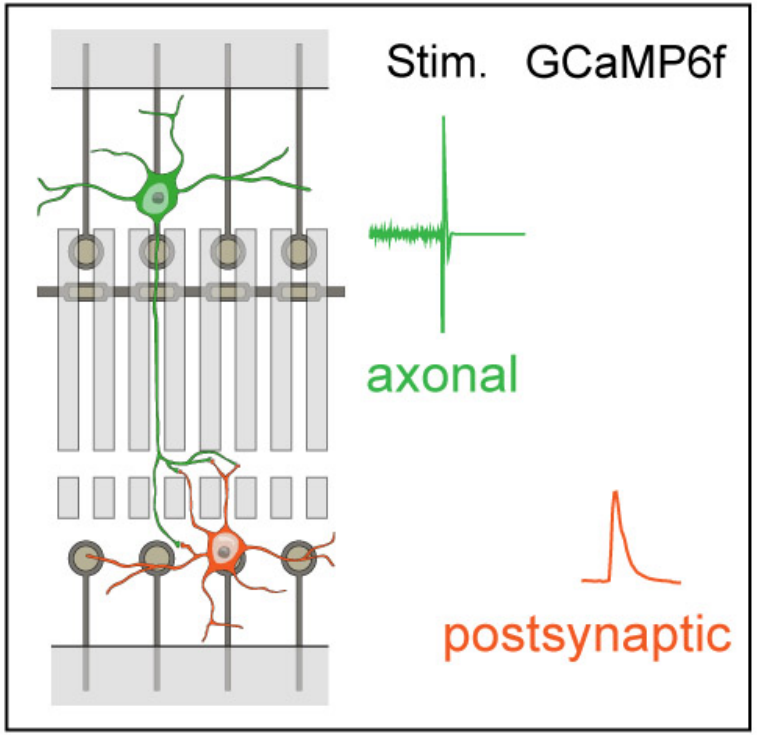
LTP long term potentiation: Persistent increase in synaptic efficacy produced by high-frequency stimulation

Manipulating activity-dependent transmission using local application of drugs at the synapse

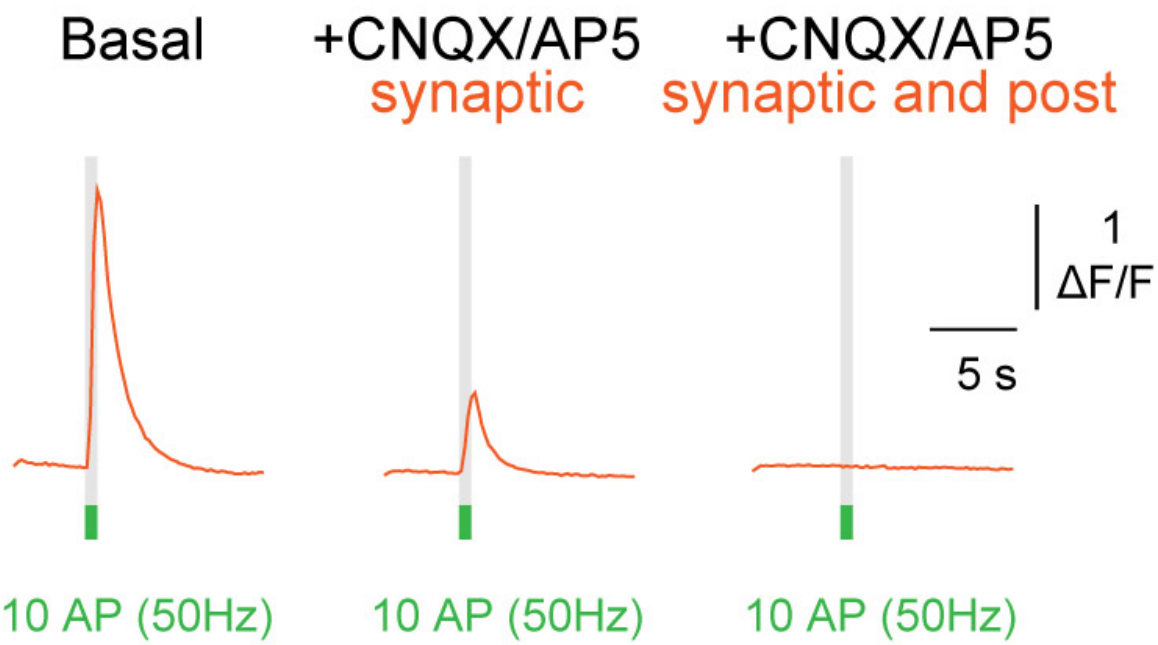


Synaptophysin

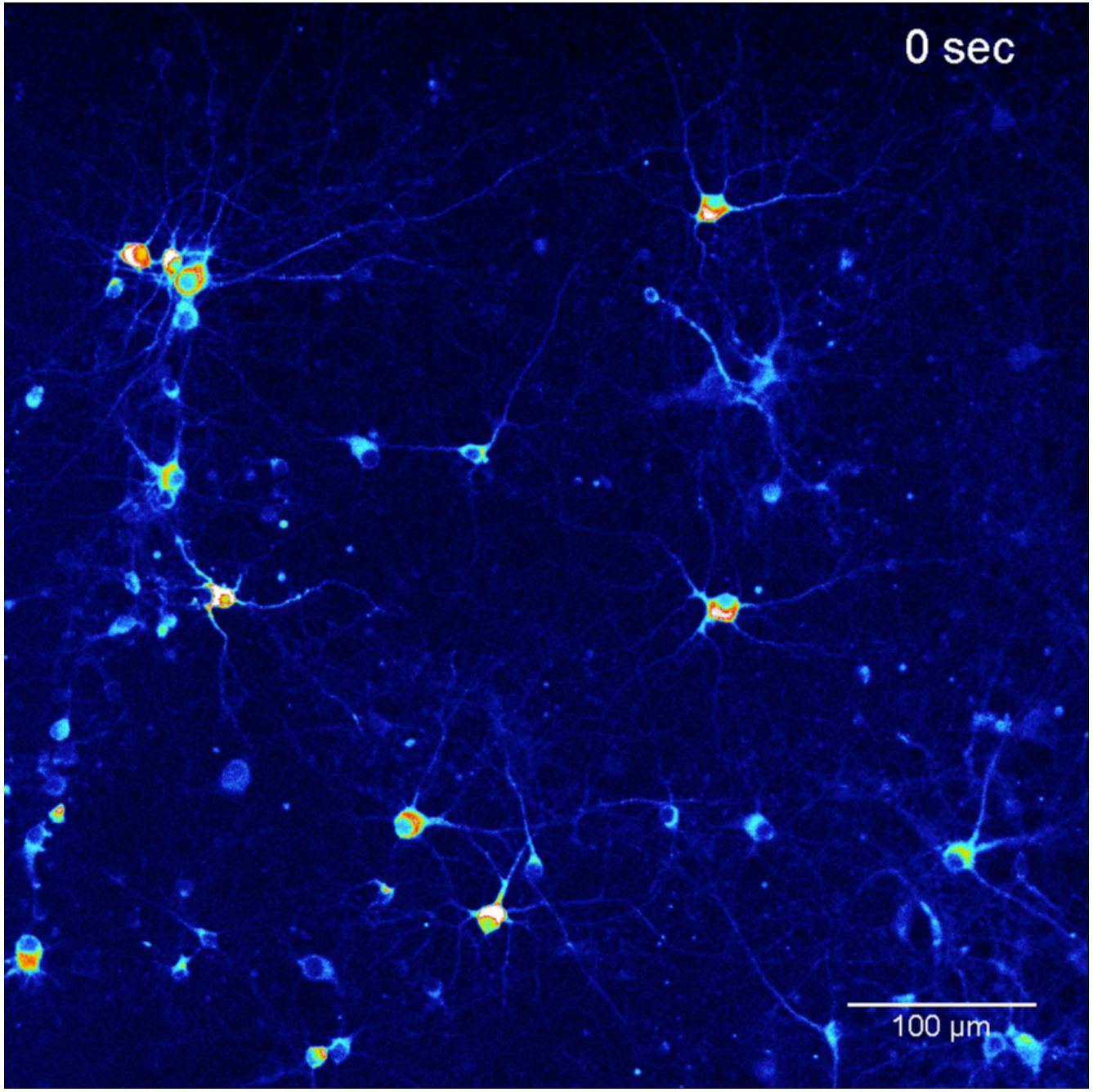




E.Moutaux et al. Lab on Chip 2018



AMPA/Kainate and NMDA receptor antagonist


0 sec
100 µm

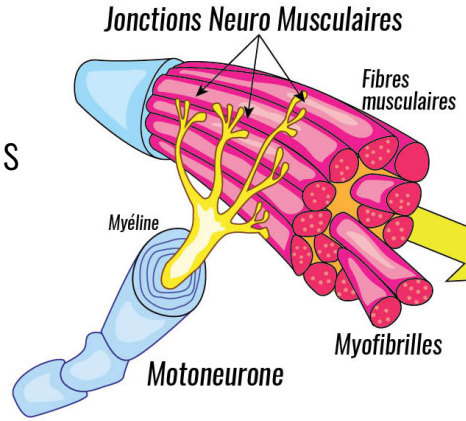
On going : Neuro Muscular Junctions

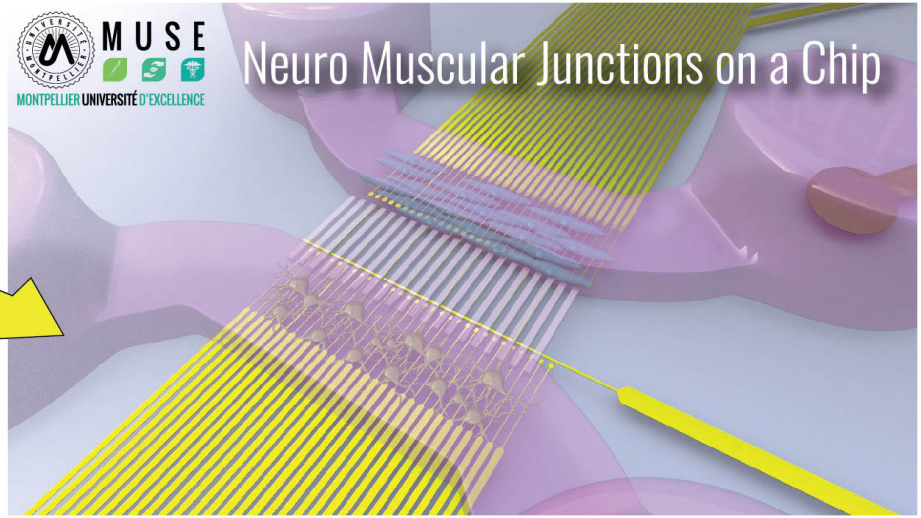
iPS cells -> motoneurons / myocytes -> myofibrils

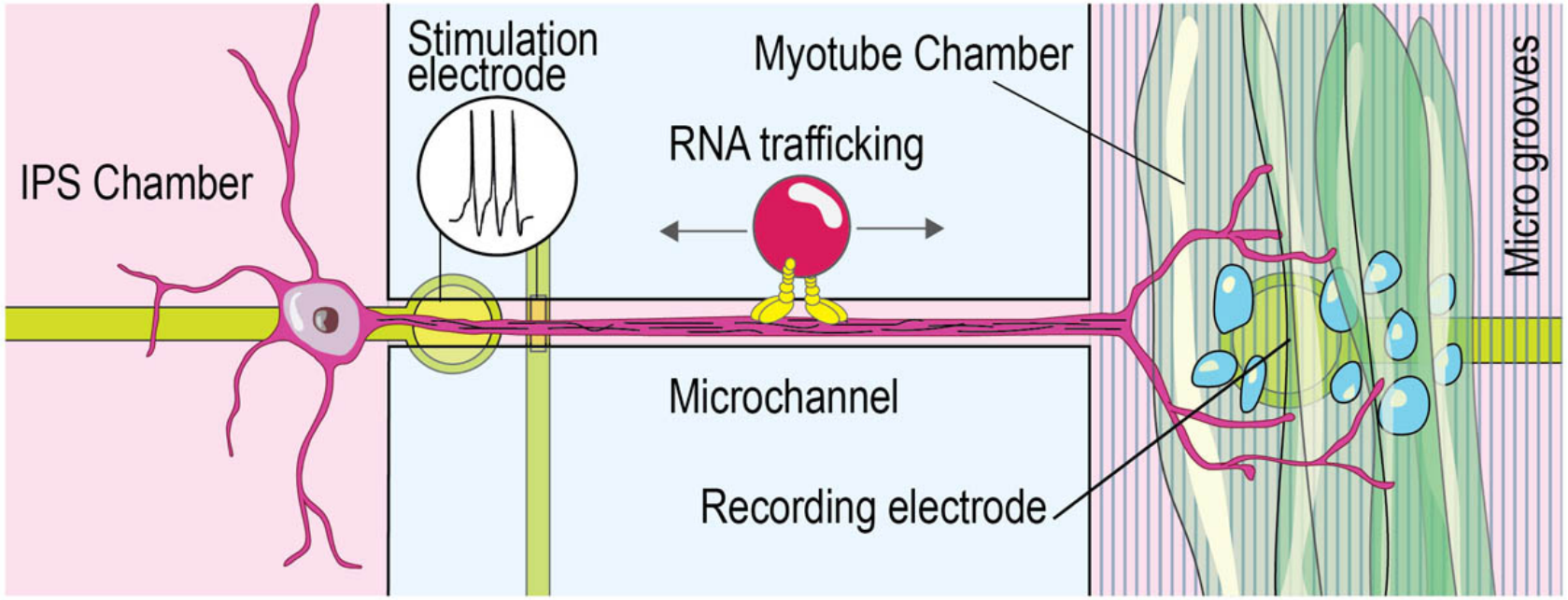
All human model

ALS model : Amyotrophic lateral sclerosis

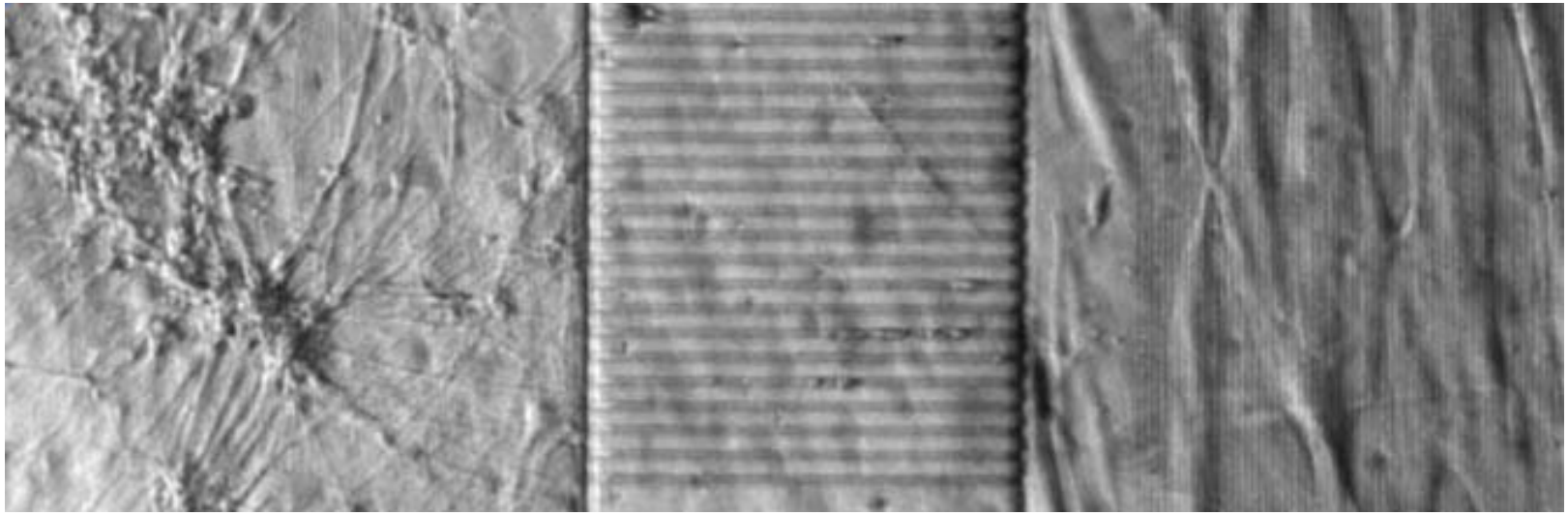
SMA model : Spinal Muscular Atrophy

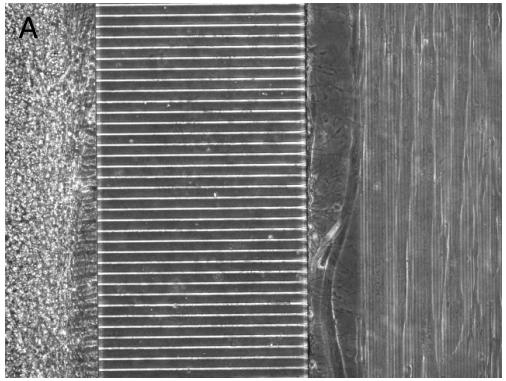


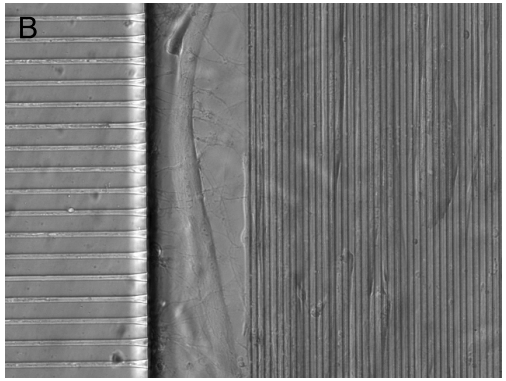


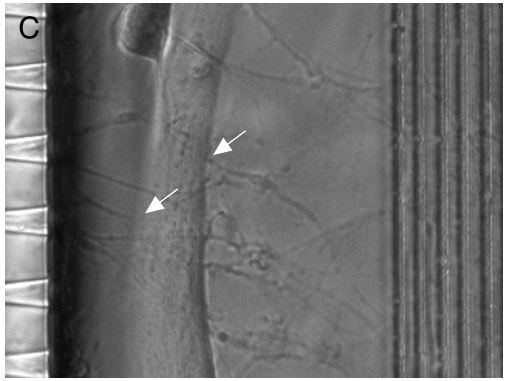


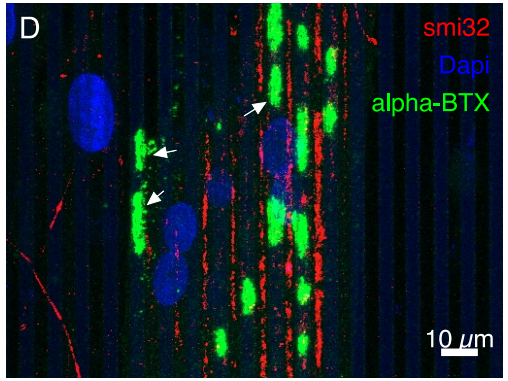
On going : Neuro Muscular Junctions





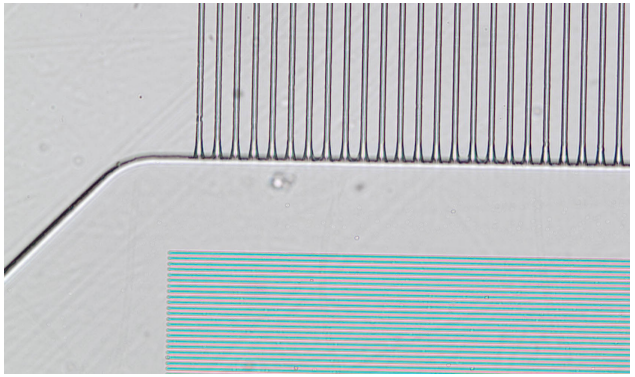






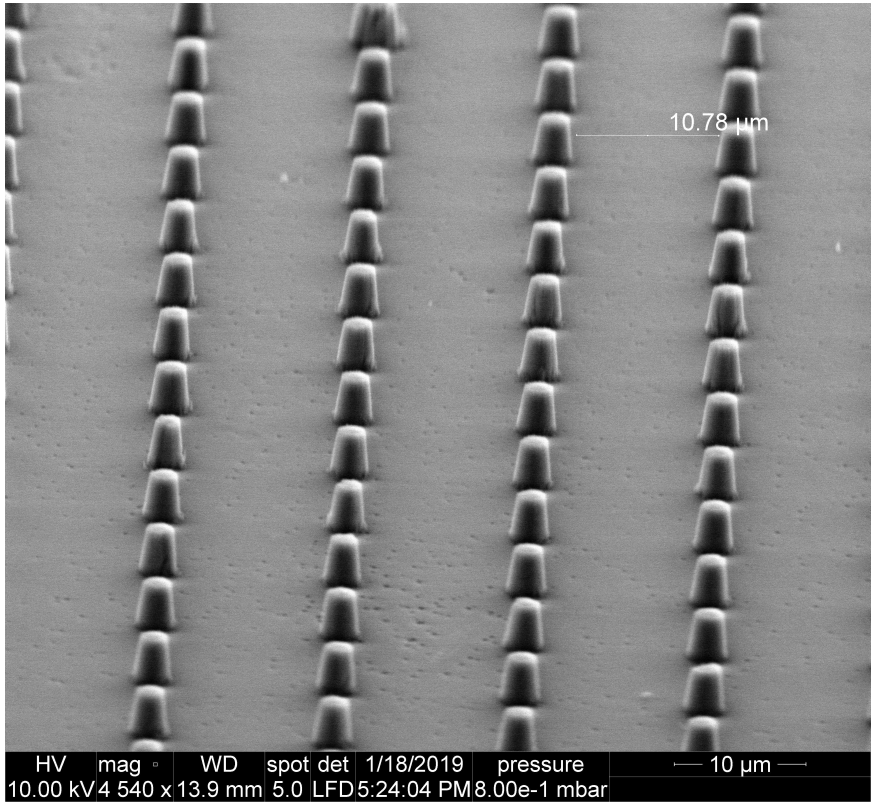
Micro grooves in the Myo chamber

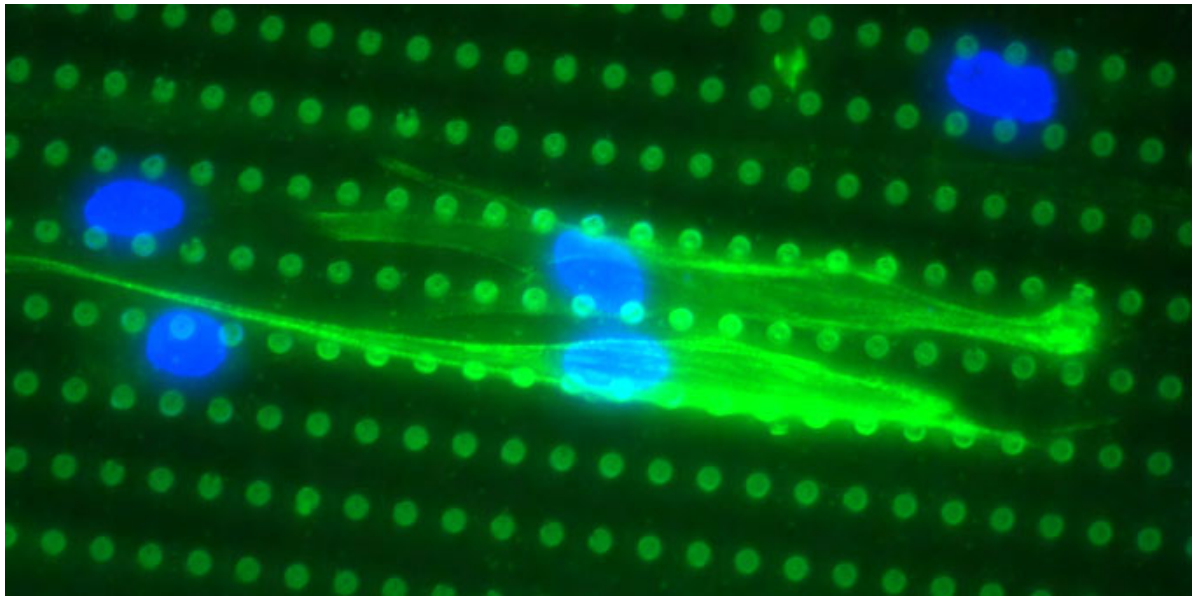
Alignment of Myocytes -> fusion toward Myotubes



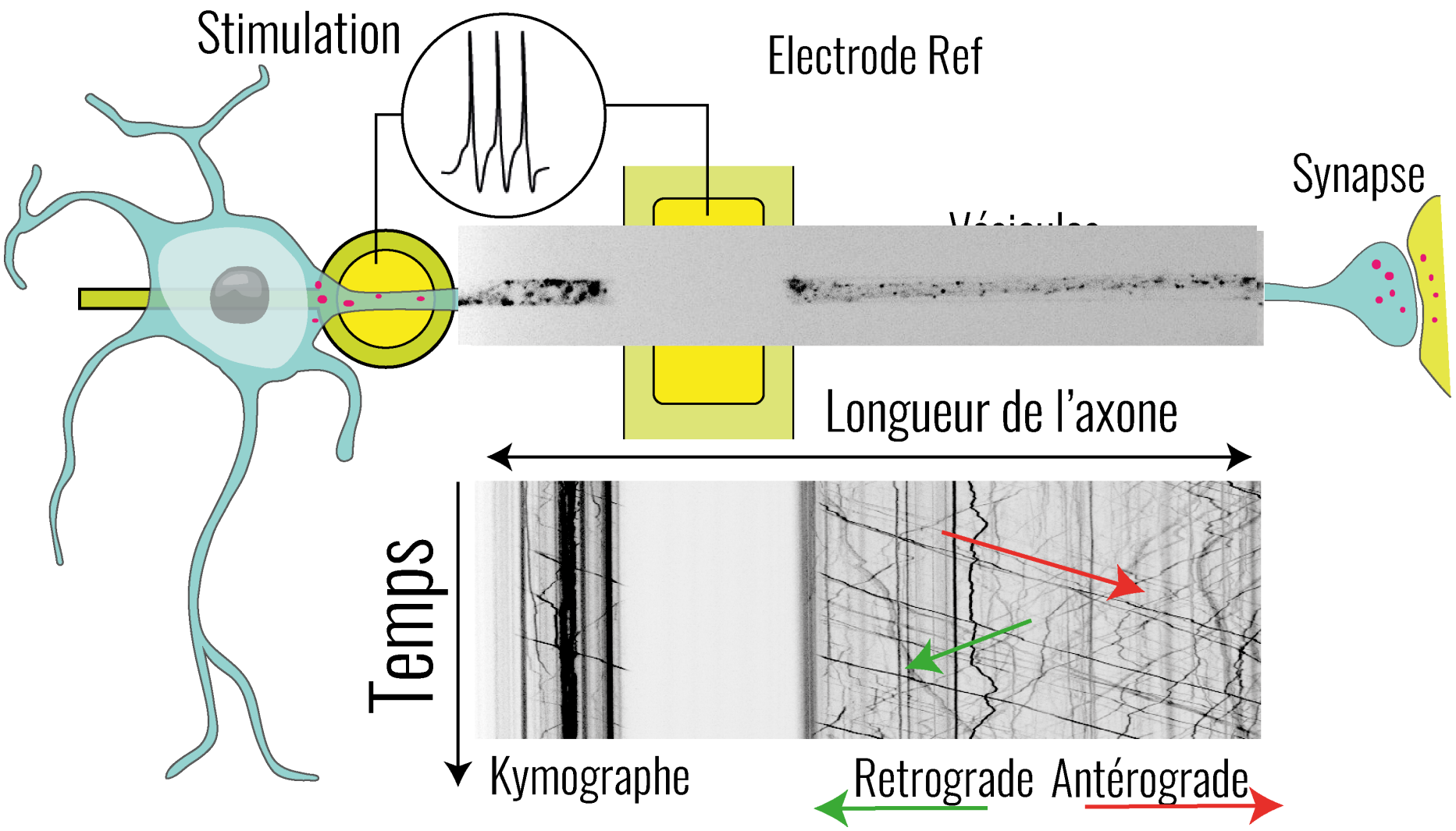
On going : Neuro Muscular Junctions

Micro pillars for cell alignment/fusion

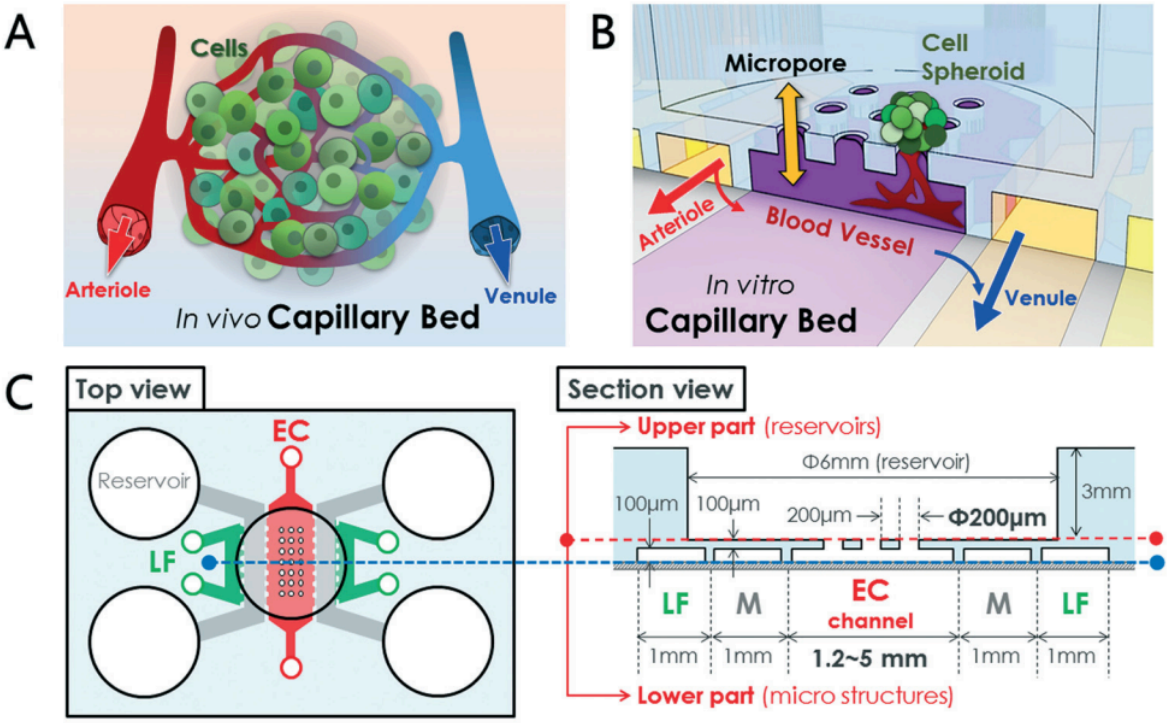


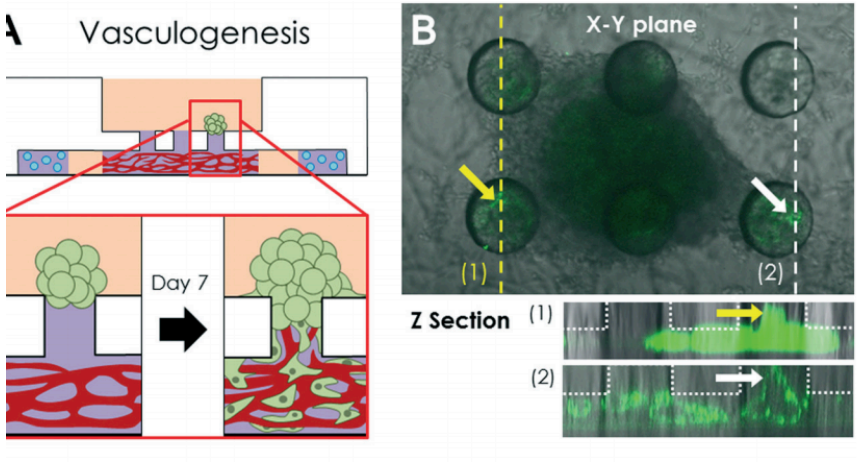


Axonal Transport under Stimulation

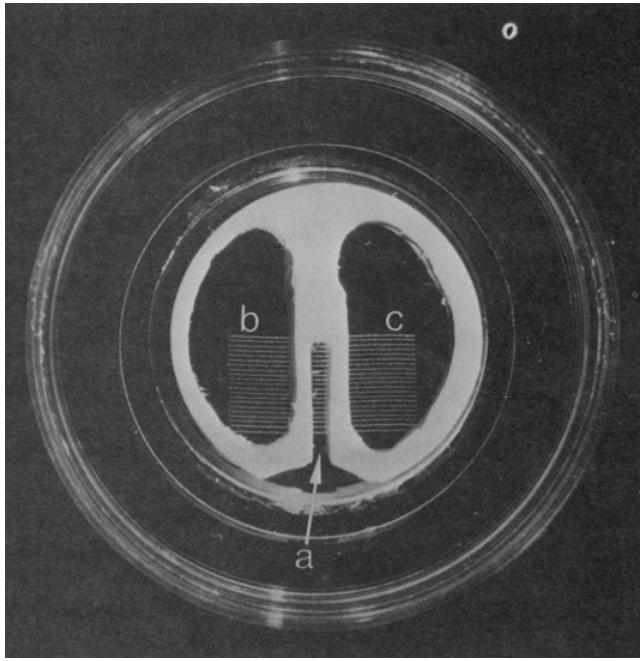


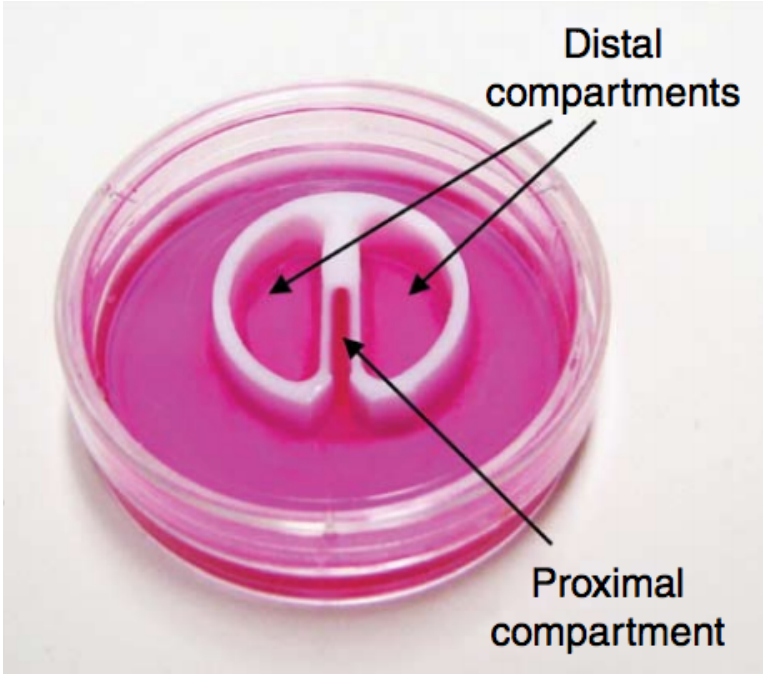
Angiogenesis on chip





Campenot Chambers, 1977



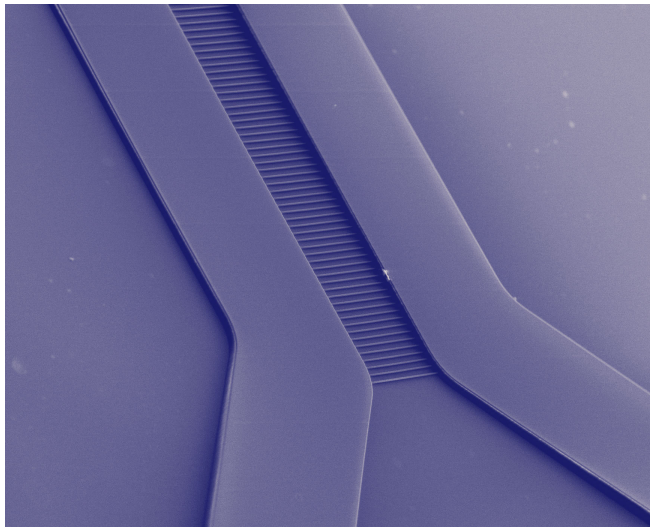


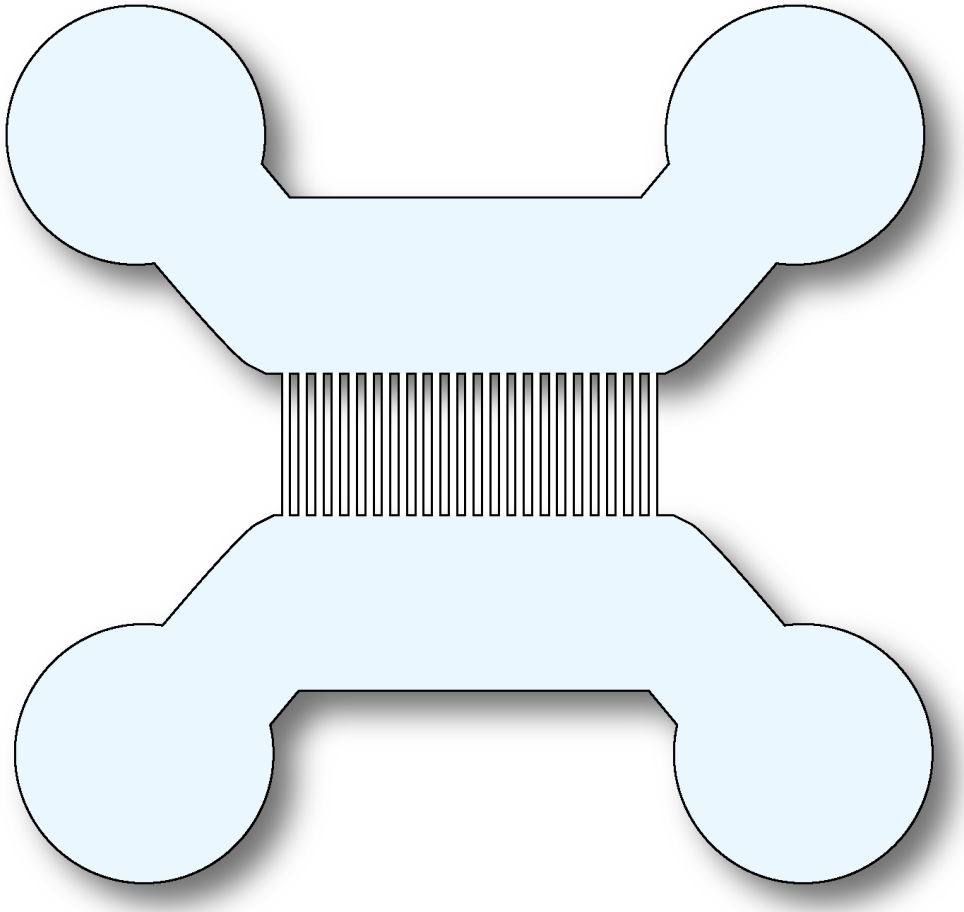
Robert. B. Campenot,
PNAS 1977 Oct; 74(10): 4516–4519.

A rake made by cementing together twenty insect pins was used to make 20 parallel scratches about 200 μm apart on the collagen-coated coverslip.

Compartimentalized Microfluidics

Two large chambers
A set of Microchannels





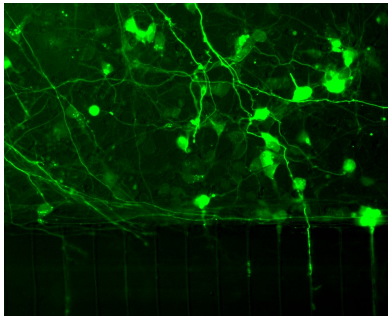
Dual thickness SU8 / PDMS

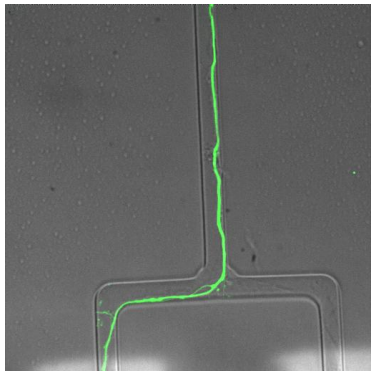
A.M.Taylor et al. Langmuir 19, 2003

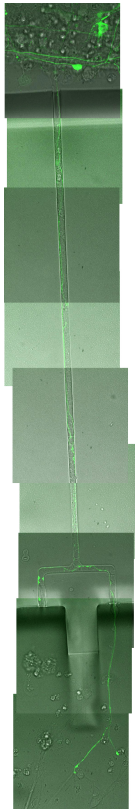
A.M.Taylor et al. Nat. Methods 2, 2005

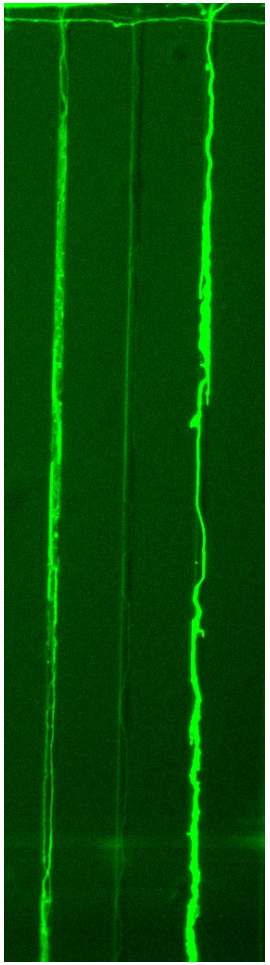
Compartimentalized Microfluidics

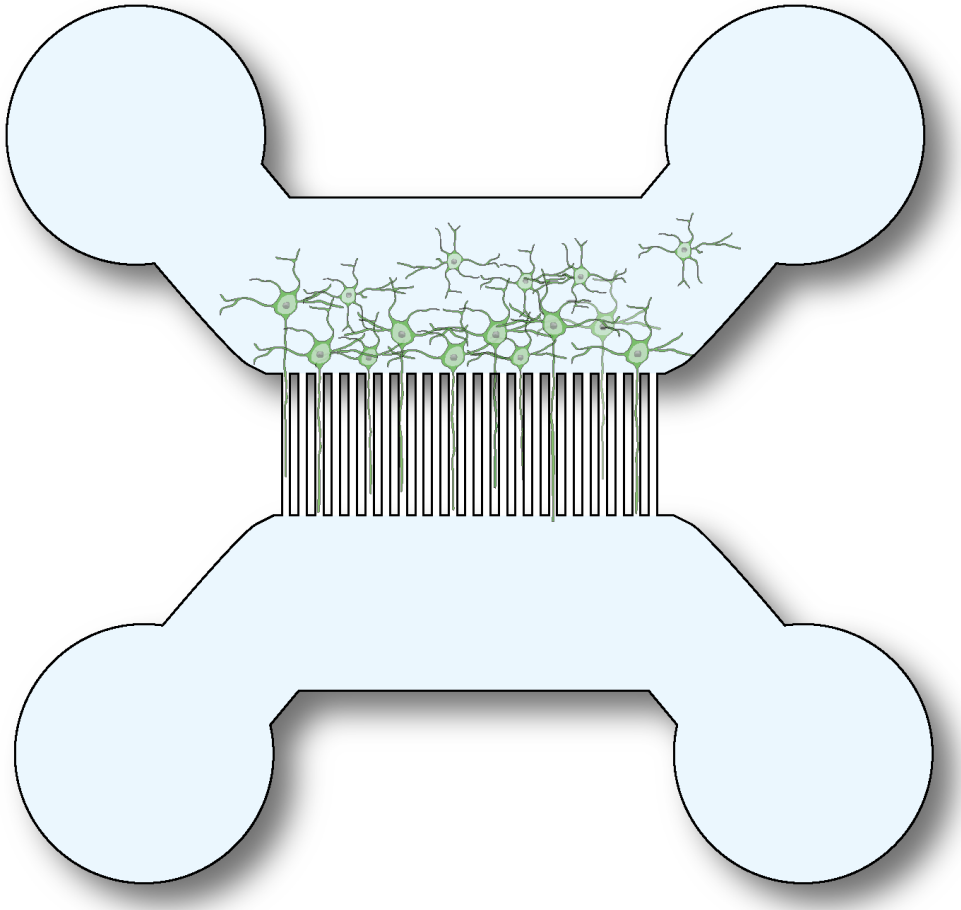
PDL/Laminin coating
Cell seeding
Incubator
Neurites and Axons
(if L>500µm)









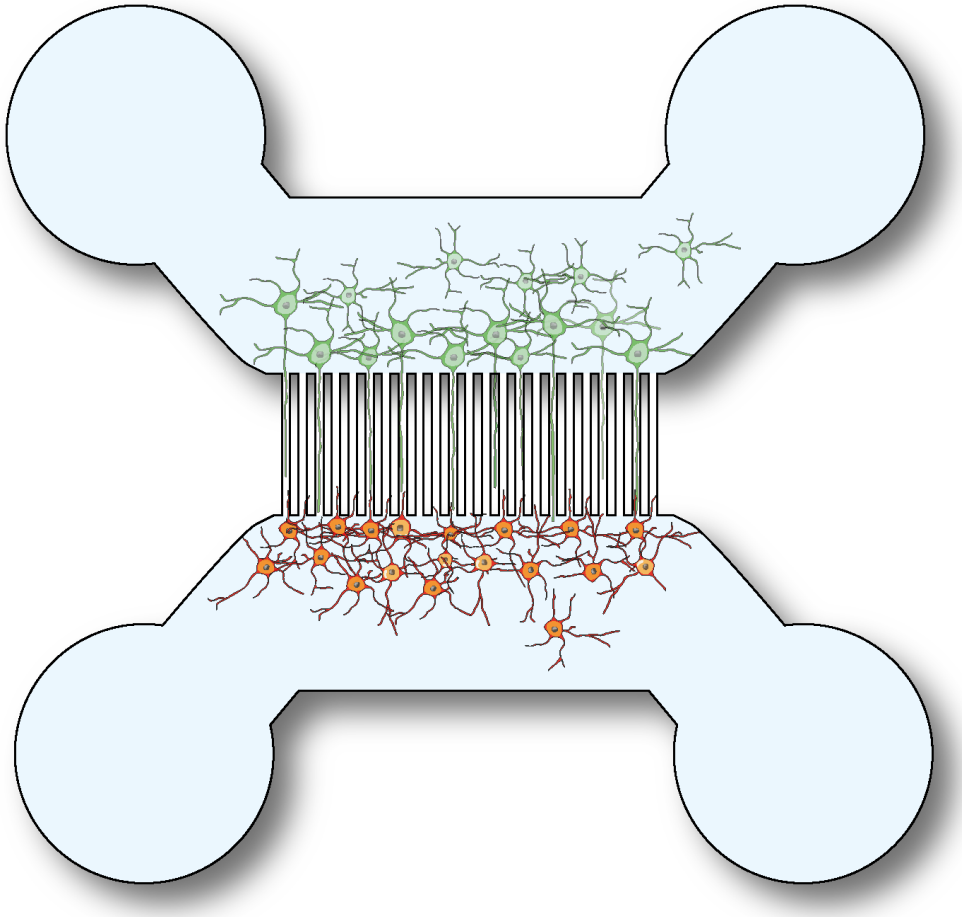


Compartimentalized Microfluidics

in-vitro reconstitution of functional connections between two cell populations

cortico-cortical
cortico-Striatal
cortico-hippocampal,
hippocampo-hippocampal,
neuron-muscle
…

Xona, Millipore, Ananda, Micro Brain Biotech



"the only widely used microfluidics device for neuro"

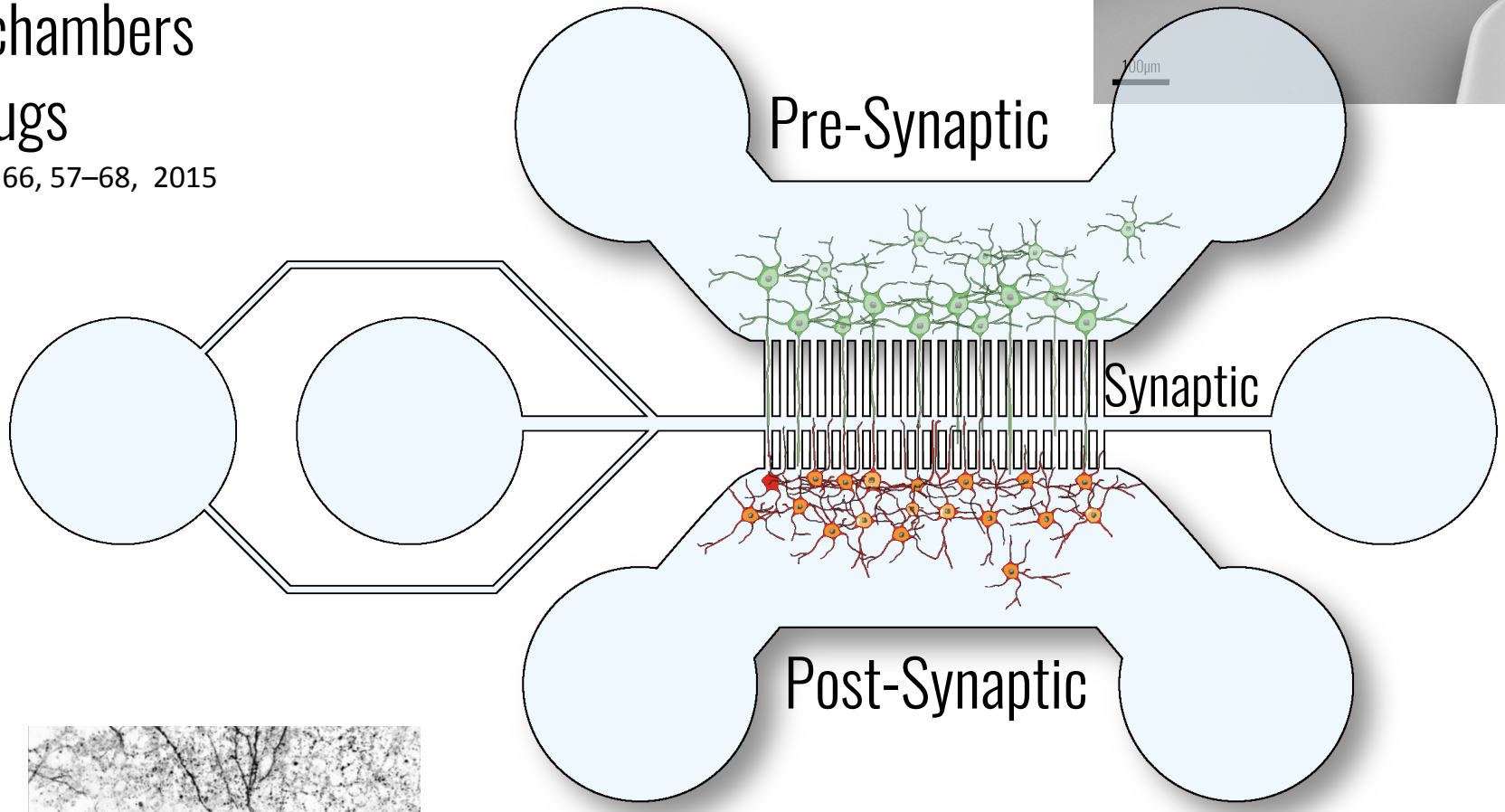
C.Leterrier

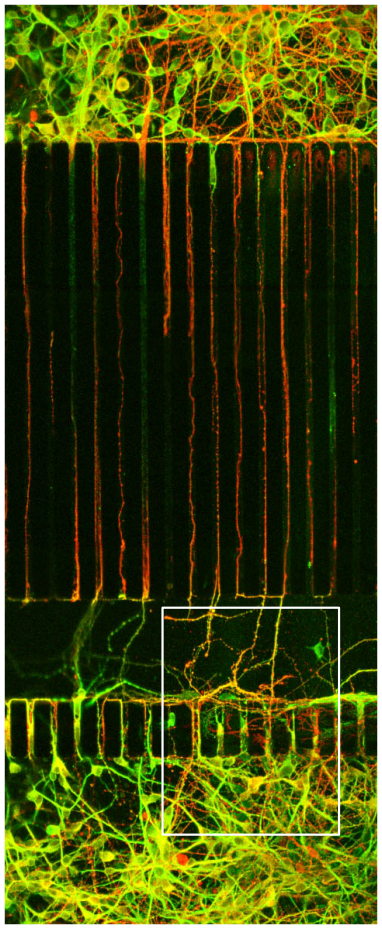
Synaptic chamber

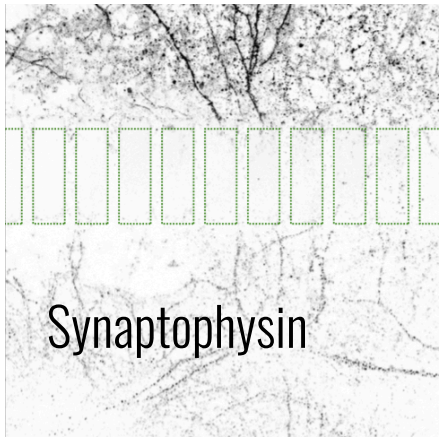
Design with 3 chambers

Perfusion of drugs

A.M. Taylor et al. Neuron 66, 57–68, 2015

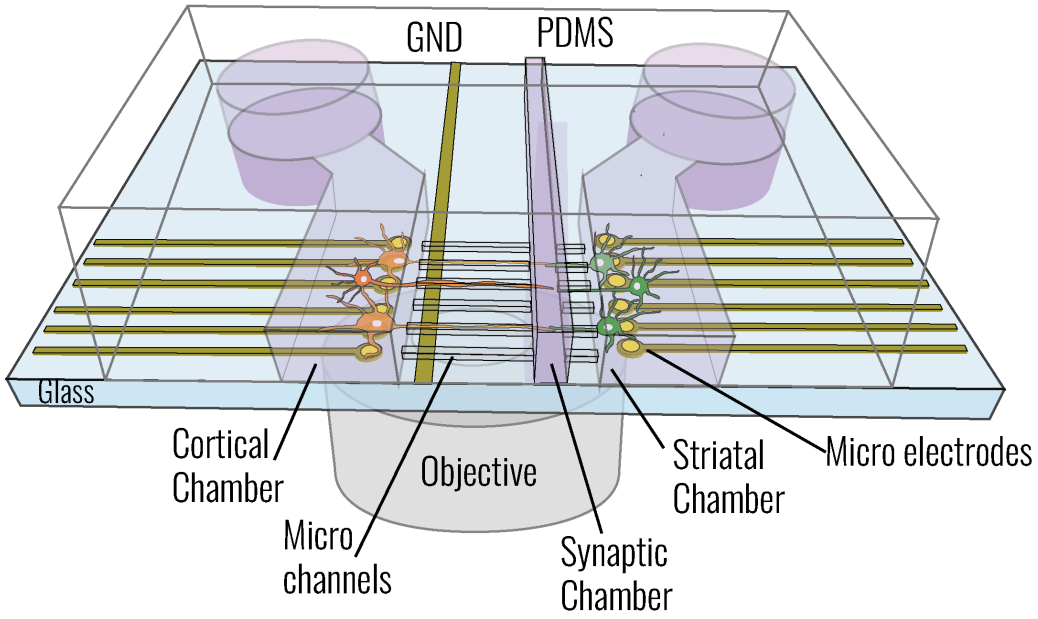


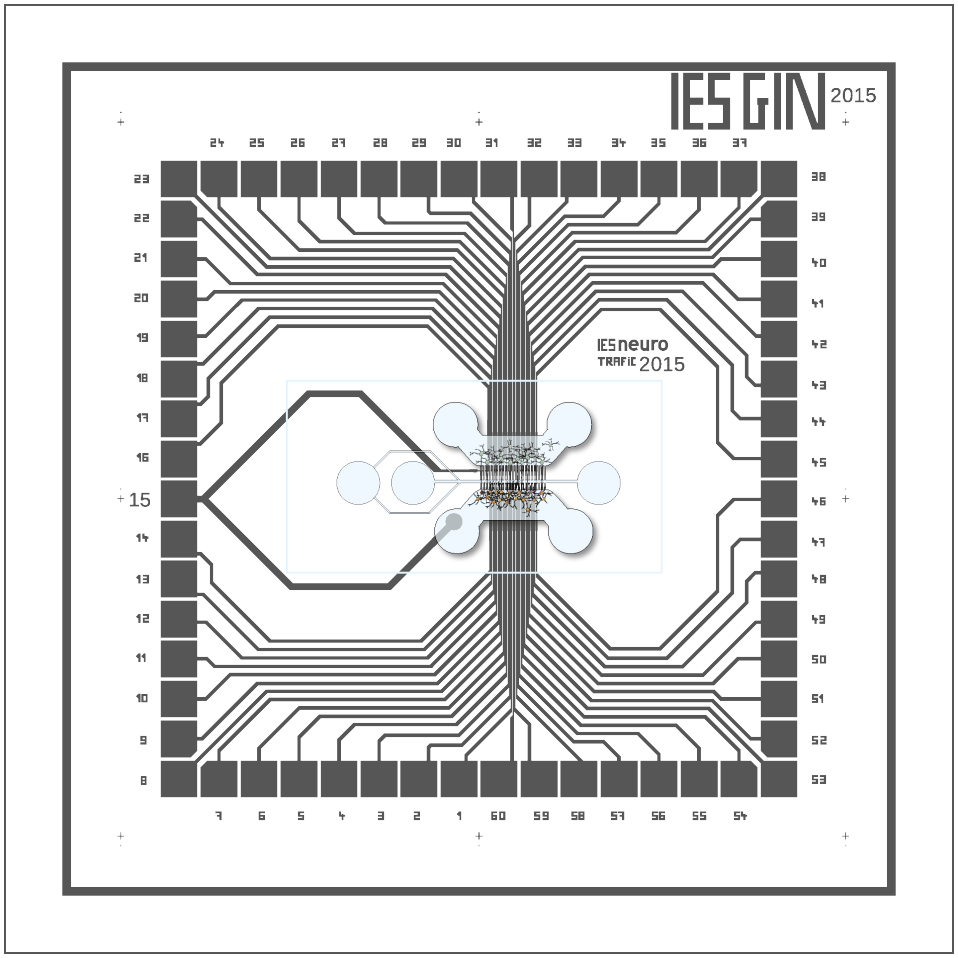




Passive device...

Microfluidics + dedicated MEA





Organisation

Axons along microchannels

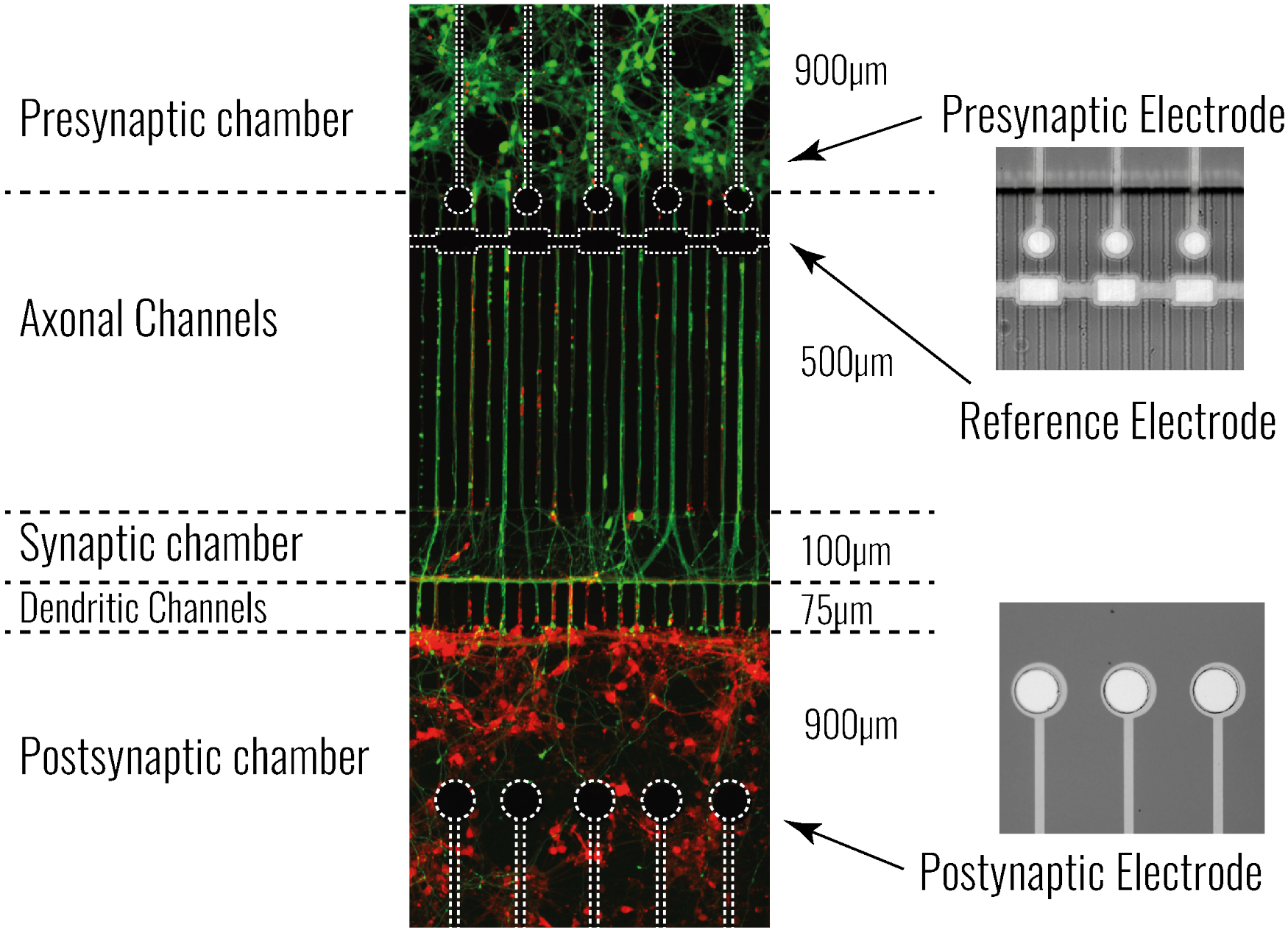
+ Stimulation & Recording

On cell bodies or AIS

+ Observation

transport of BDNF or MT

MEA alignment



MEA microfabrication

Thin glass substrate : 5x5cm 170µm

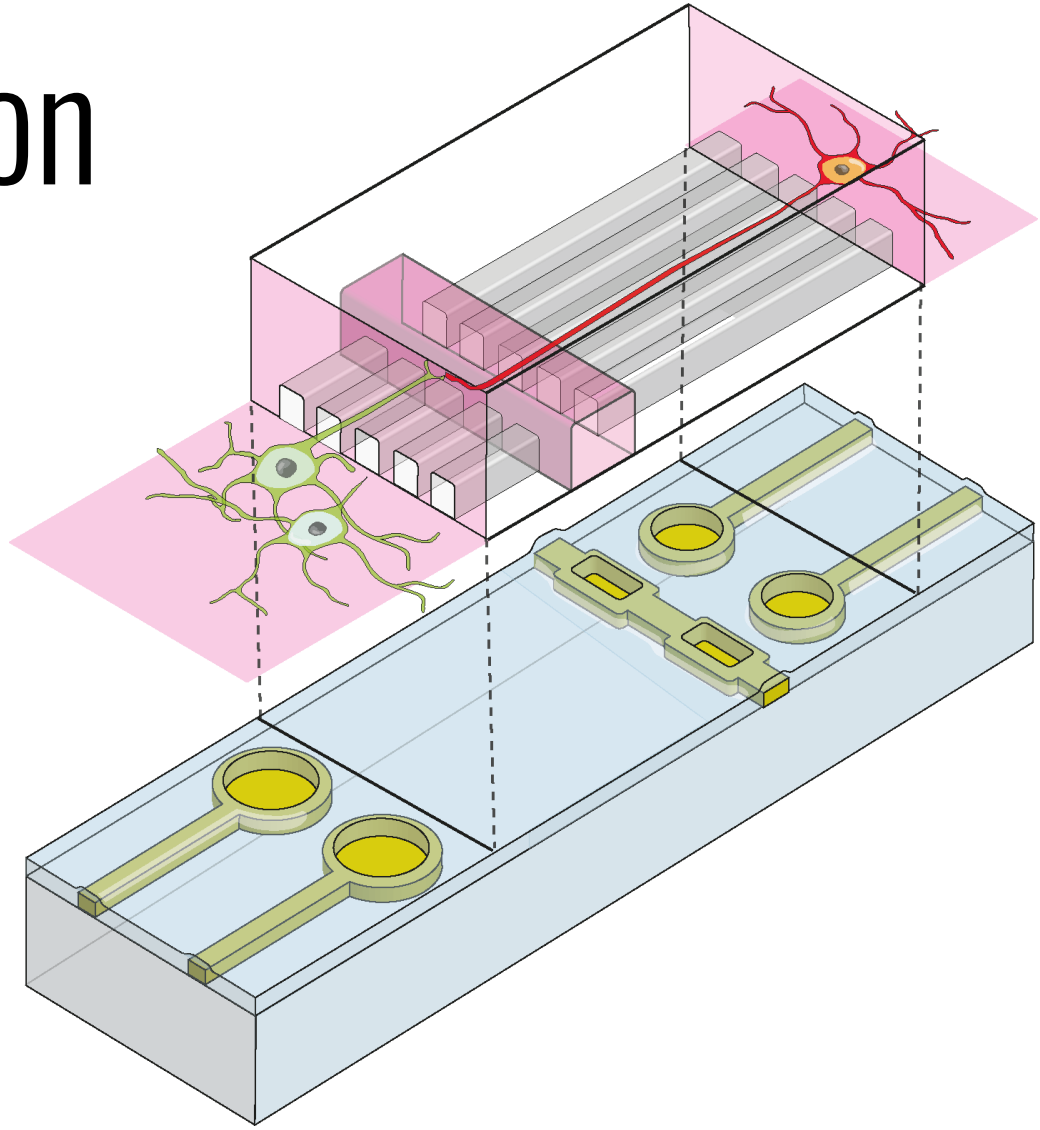
Mask1 Ti/Pt electrodes (Electron gun evap. + Lift Off)

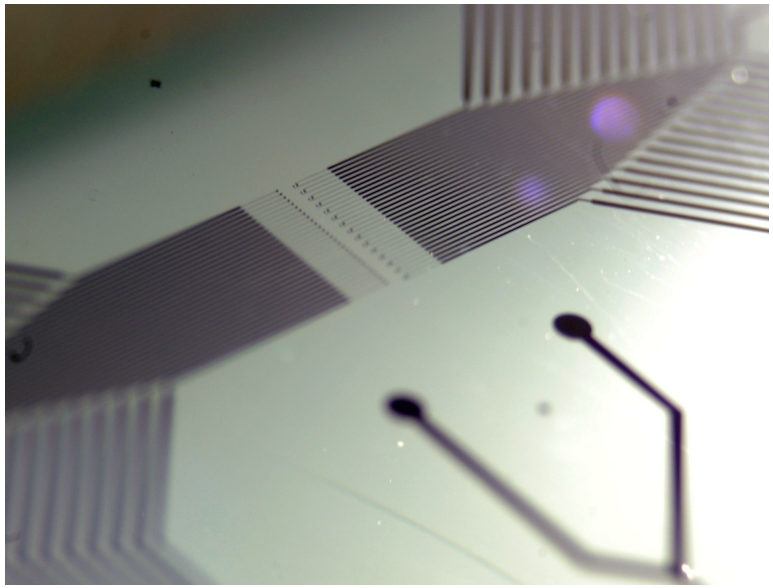
SiNx PECVD

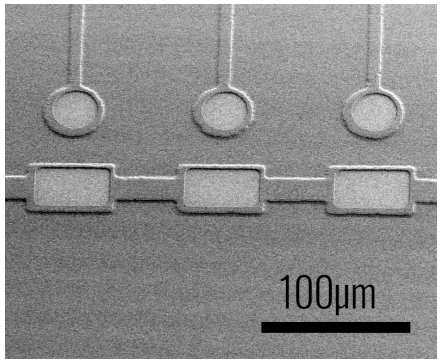
Mask2+ Alignment + RIE etching

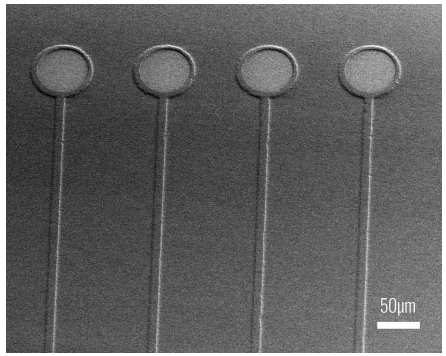
Simple and Stable process

For series > 100 samples

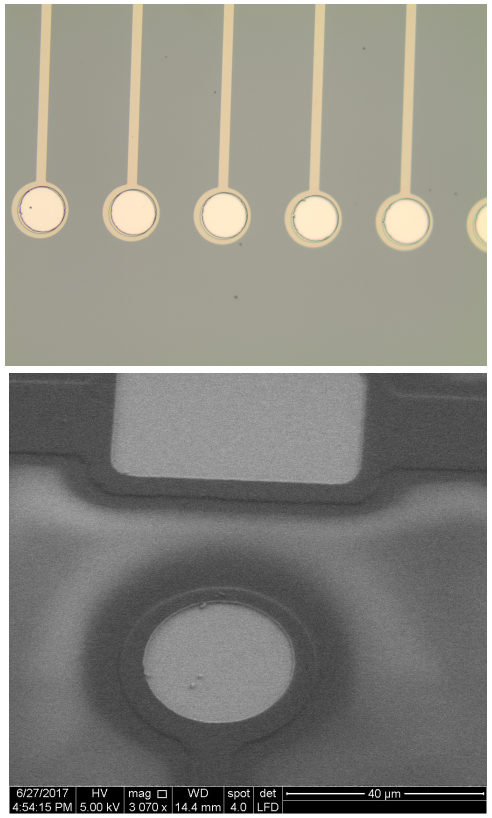


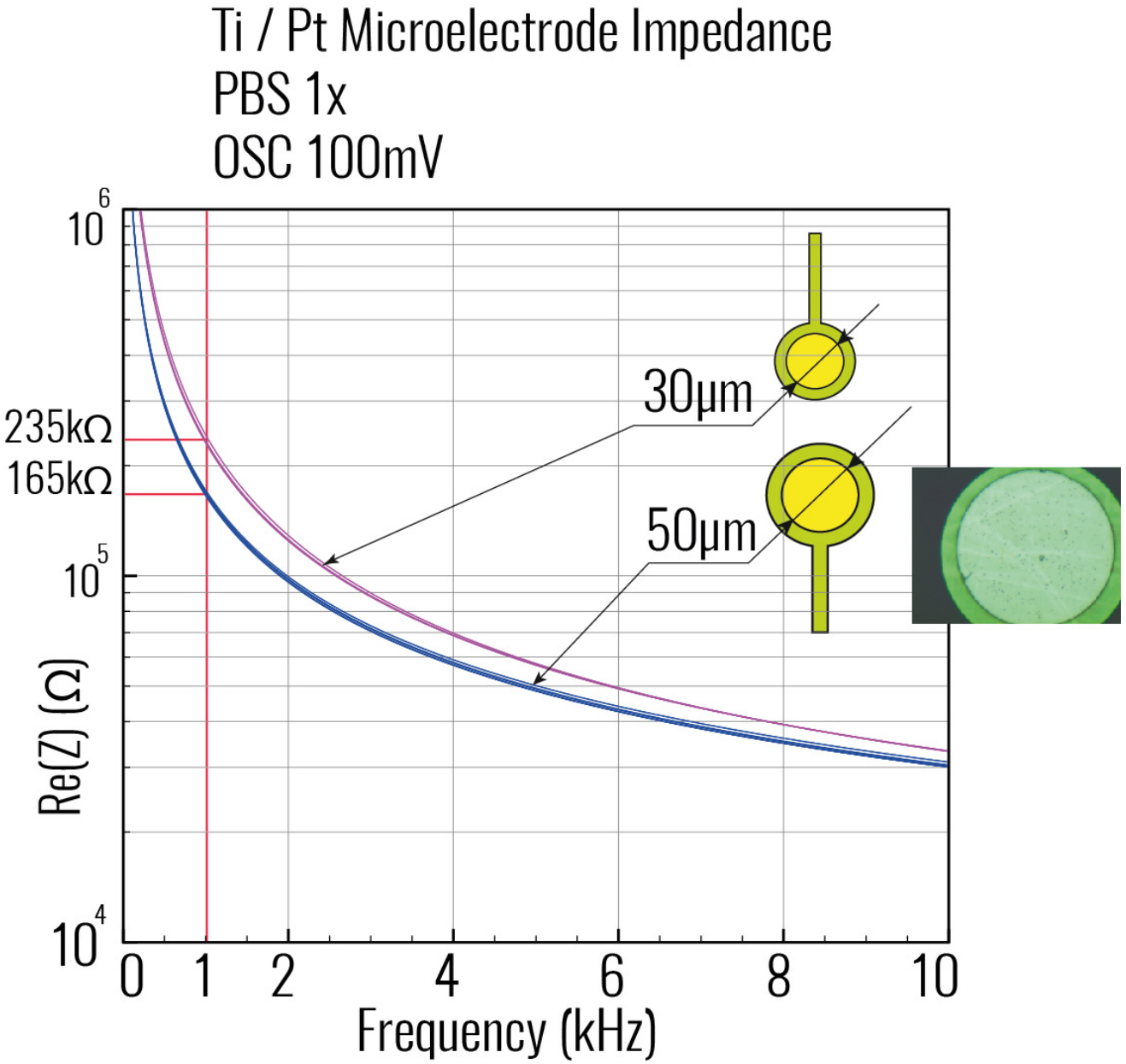






Impedance


6/27/2017
4:54:15 PM
HV
5.00 kV
mag
3 070 x
WD
14.4 mm
spot
4.0
det
LFD
40 µm


Ti / Pt Microelectrode Impedance
PBS 1x
OSC 100mV
10^6
235kΩ
165kΩ
10^5
10^4
Re(Z) (Ω)
30µm
50µm
0
1
2
4
6
8
10
Frequency (kHz)

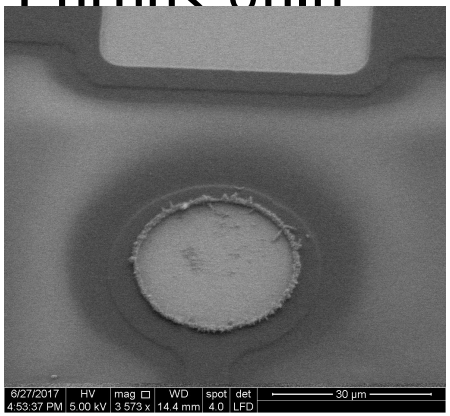
Impedance

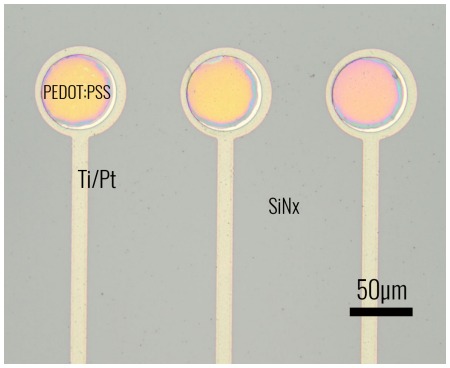
Lowering of the impedance : better Signal to noise ration

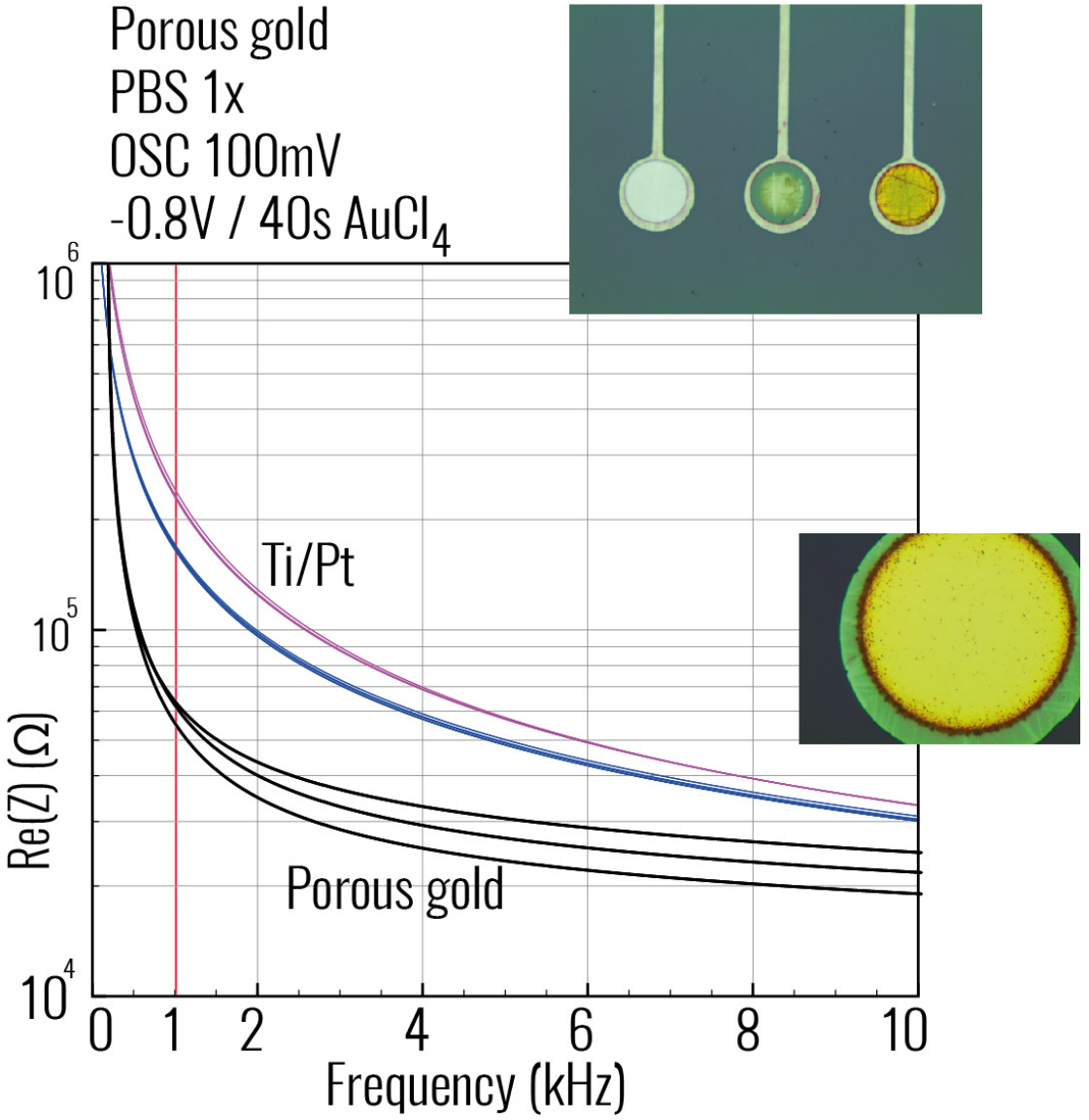
TiN

PEDOT:PSS

Porous gold







But..... Platinum for stability and reproducibility

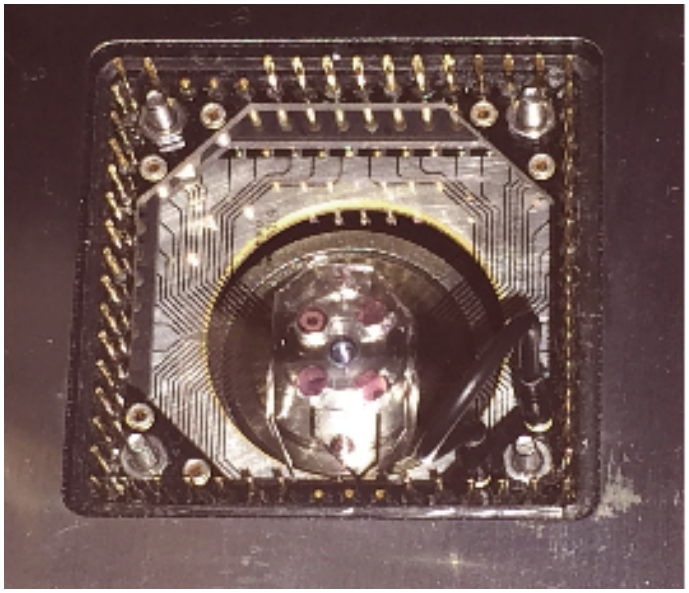
Experimental Setup

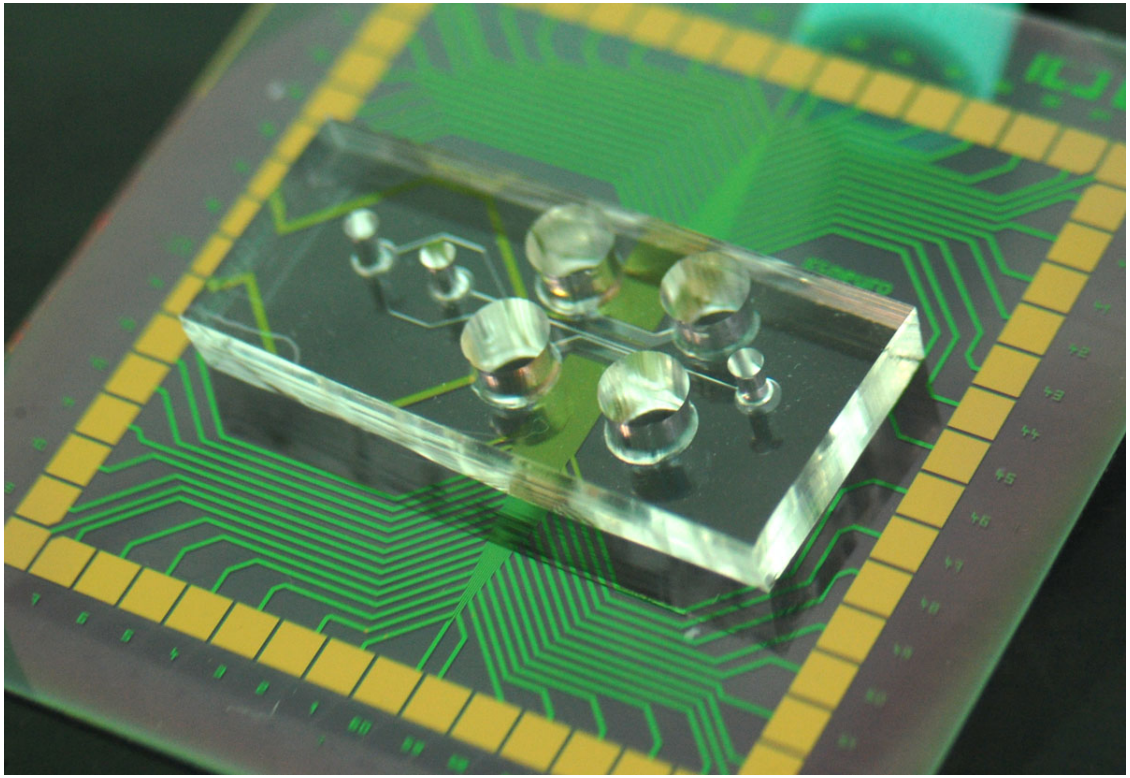
PDMS Microfluidic chamber

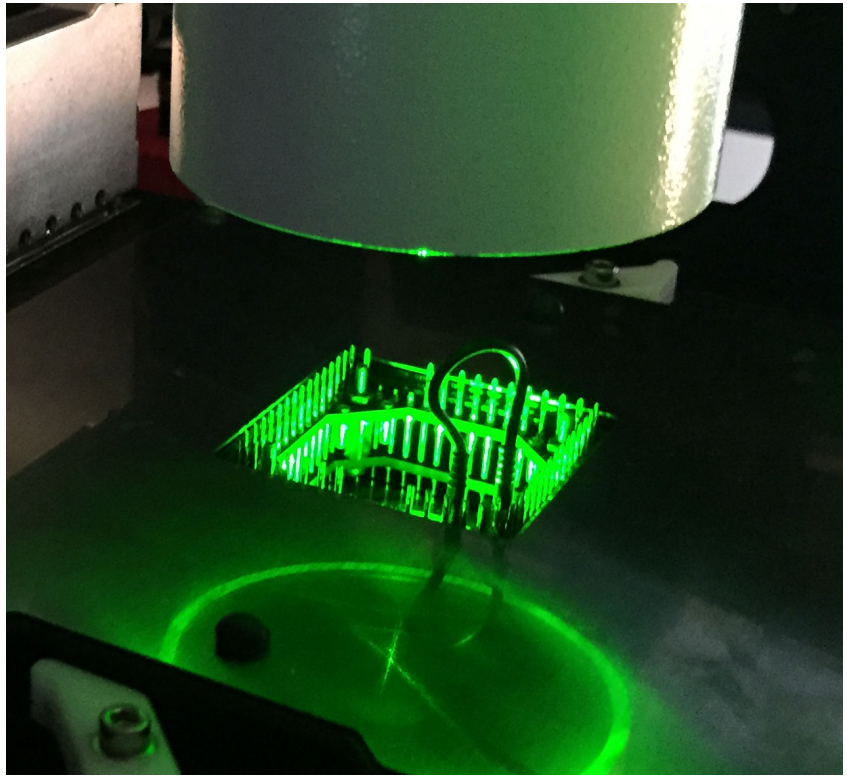
Aligned on specific MEA chip

Connected to a MCS 64 channel system

Under Spinning disc microscope





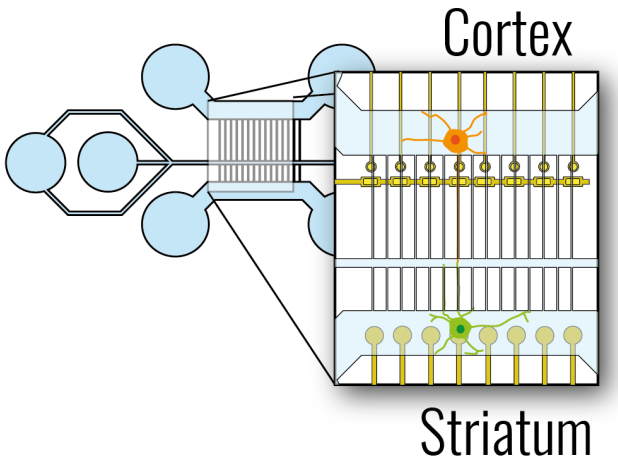


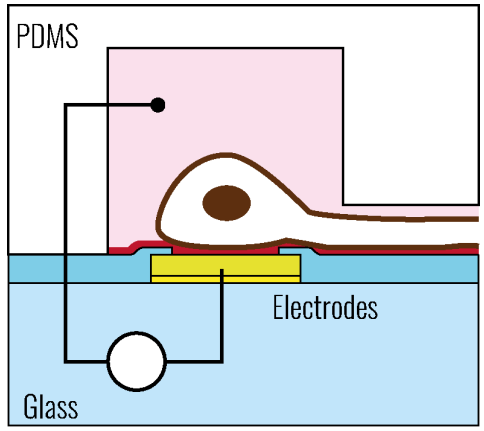
Extracellular Recording

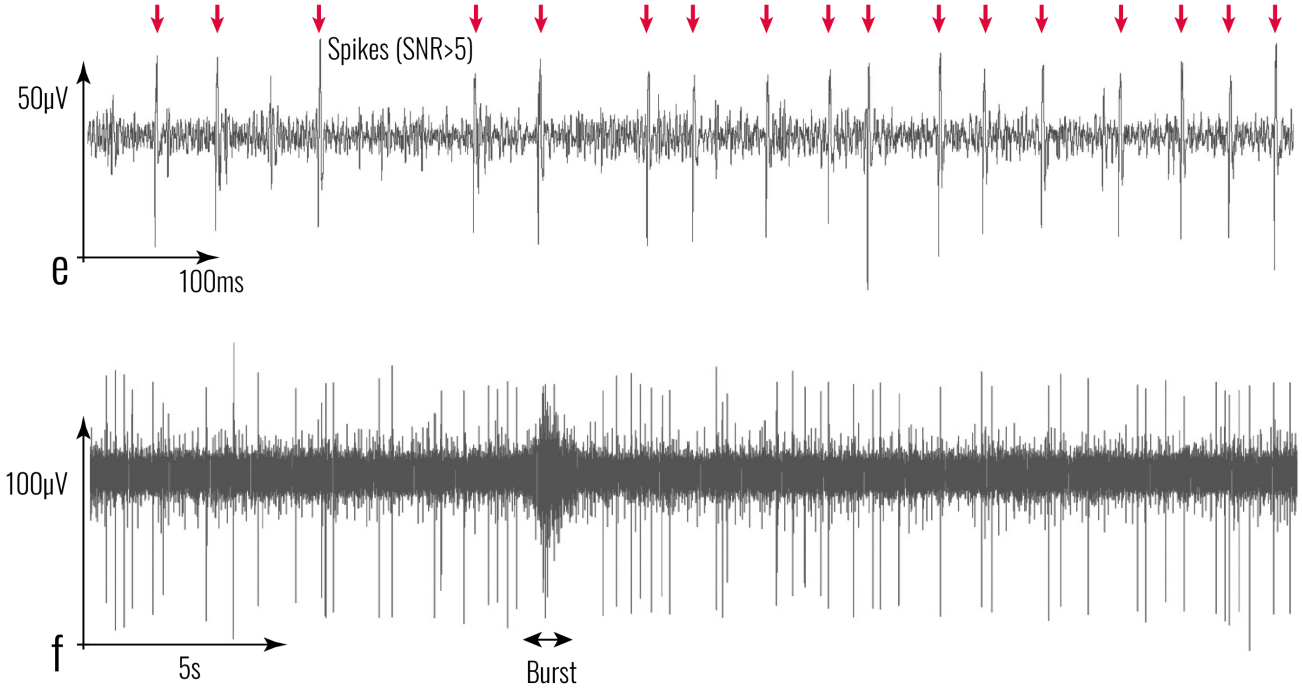
μV range

Spikes detection (SNR>5)

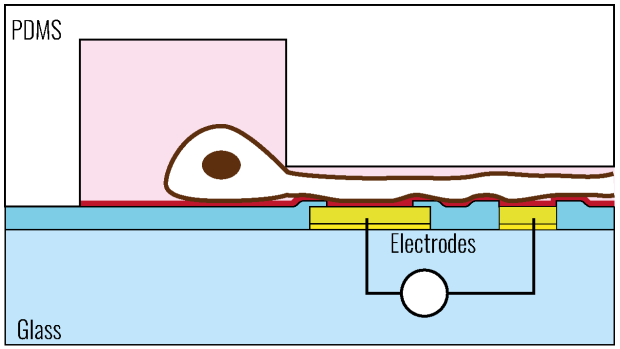
Spontaneous activity DIV10





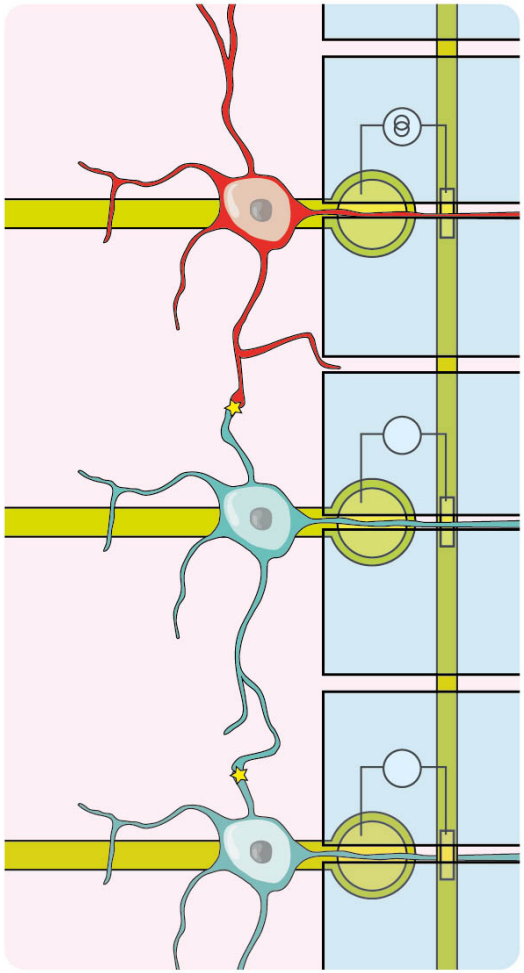


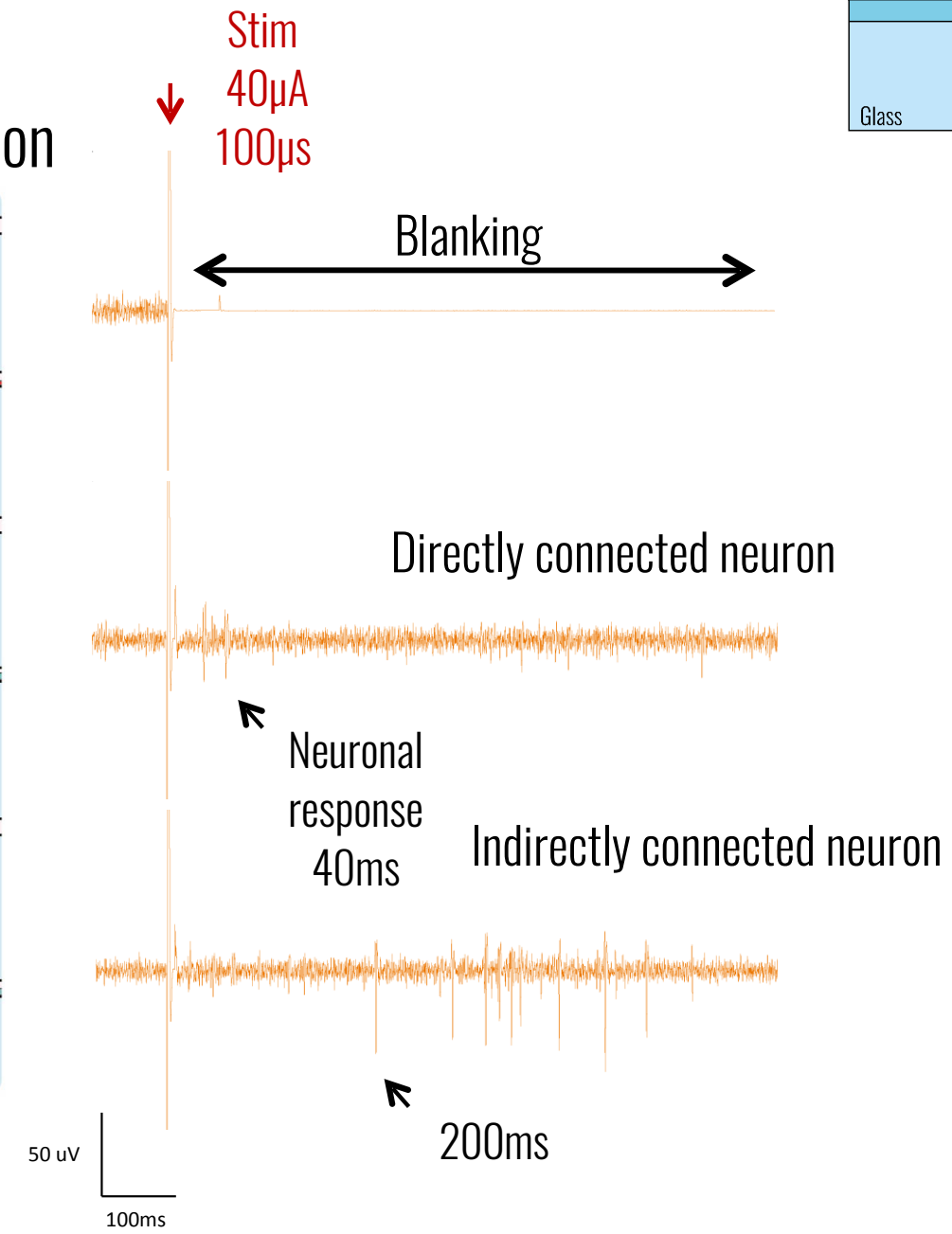
Extracellular Stimulation



Current stimulation

Axonal (AIS) stimulation



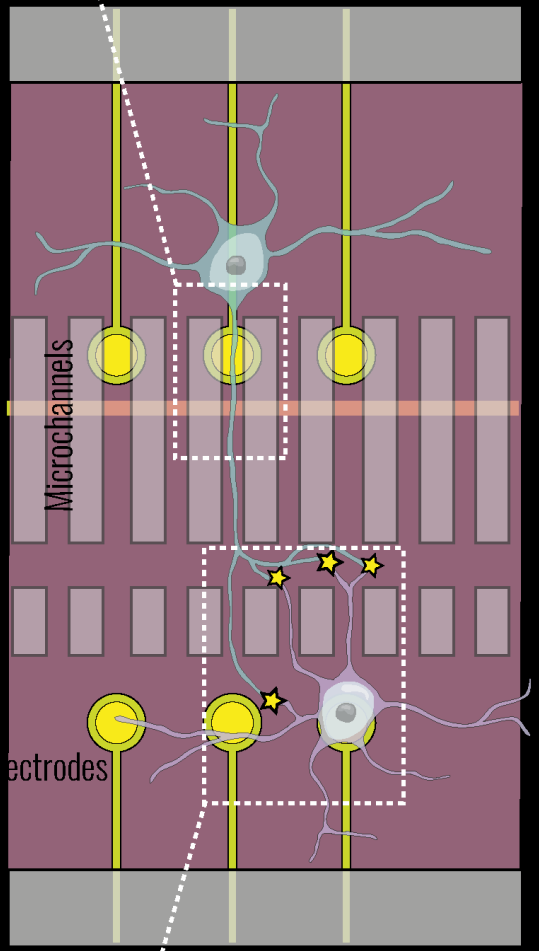


Stimulation+ GCaMP6f visualisation

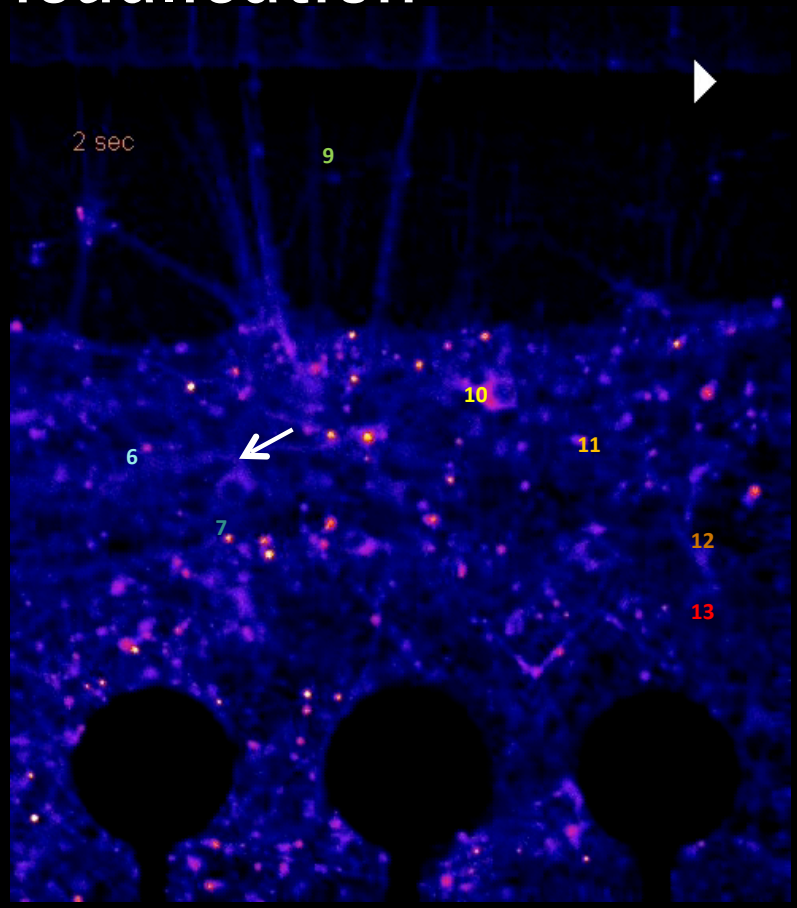
Genetically Encoded Calcium Indicators

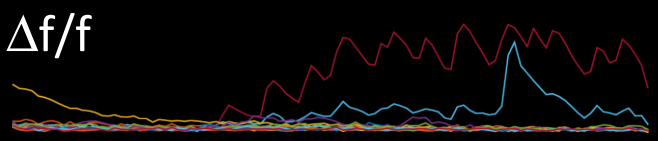
Electrical stimulation

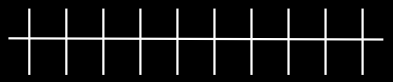
1Hz



Calcium imaging







Small amplitude, repeated

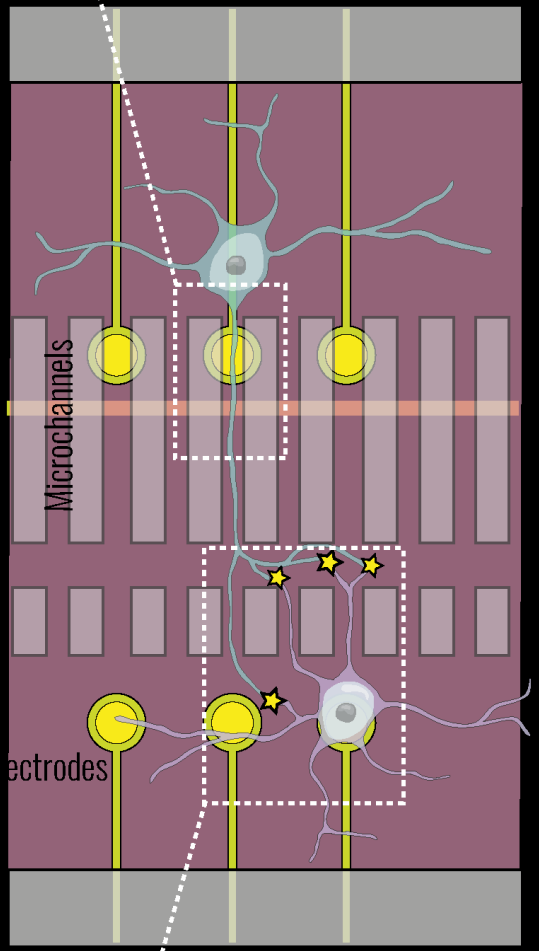
LTD long term depression

Stimulation+ GCaMP6f visualisation

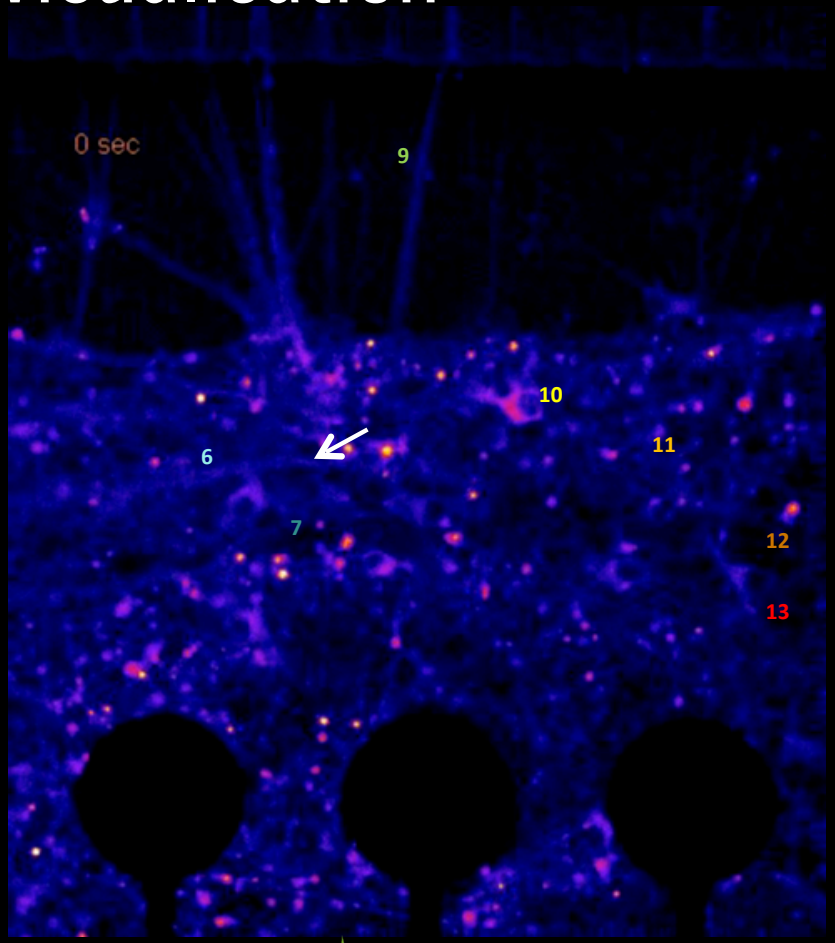
Genetically Encoded Calcium Indicators

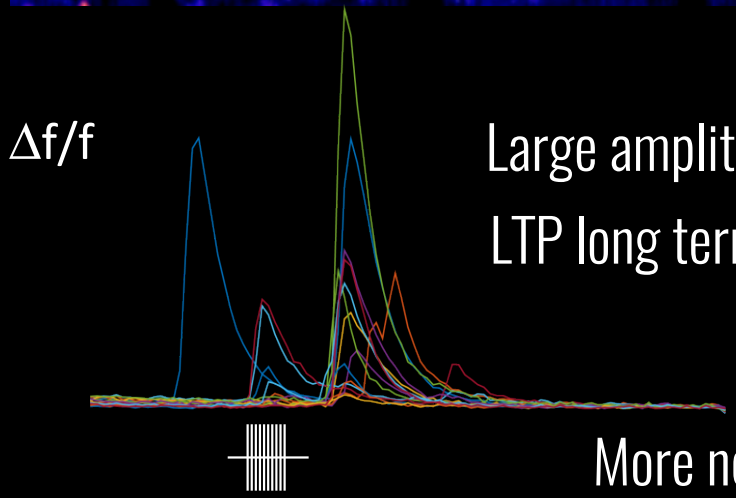
Electrical stimulation

50Hz



Calcium imaging





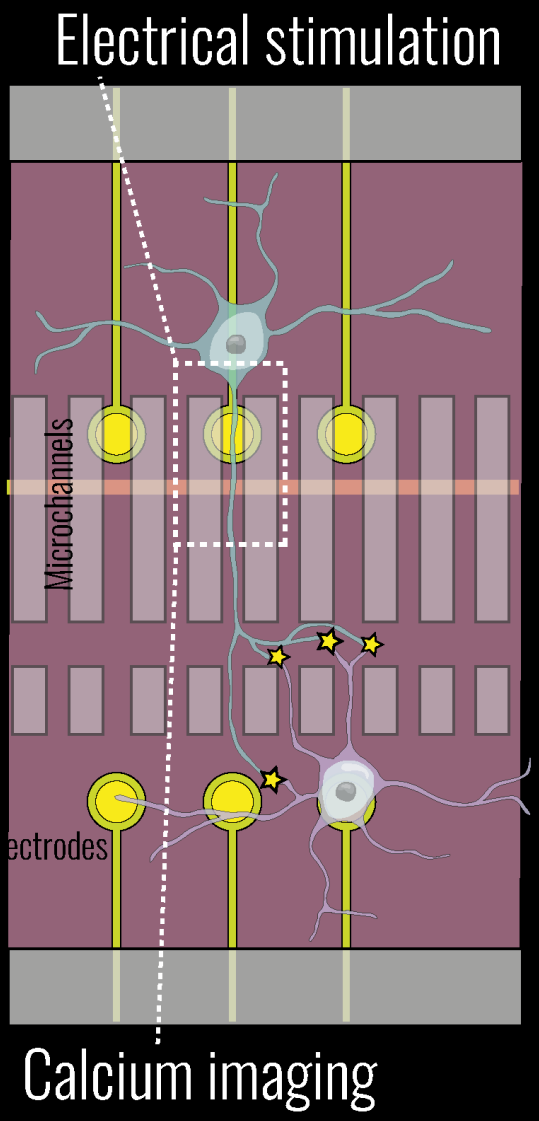
Large amplitude, long signal

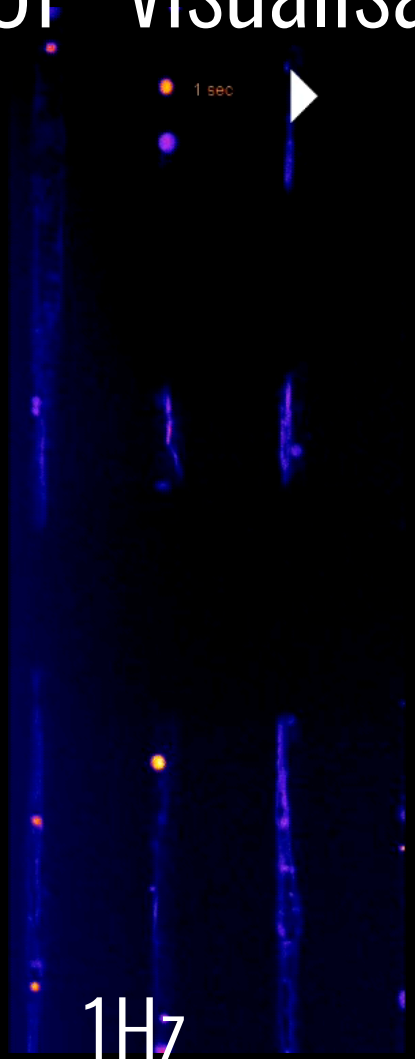
LTP long term potentiation

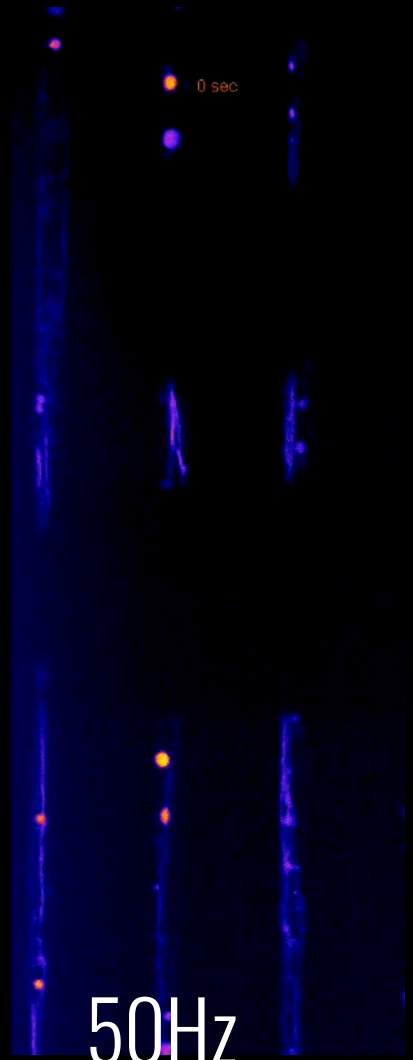
More neurons are recruted

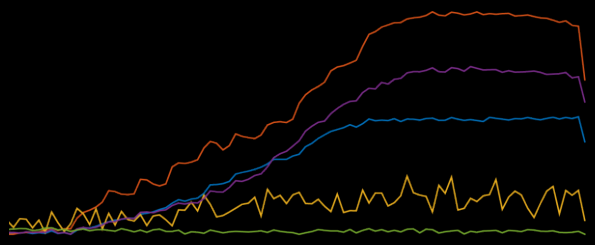
Stimulation+ GCaMP6f visualisation

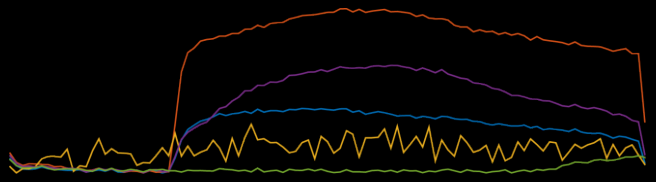
Axons Inside Microchannels



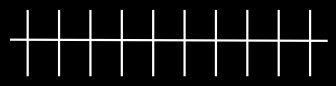




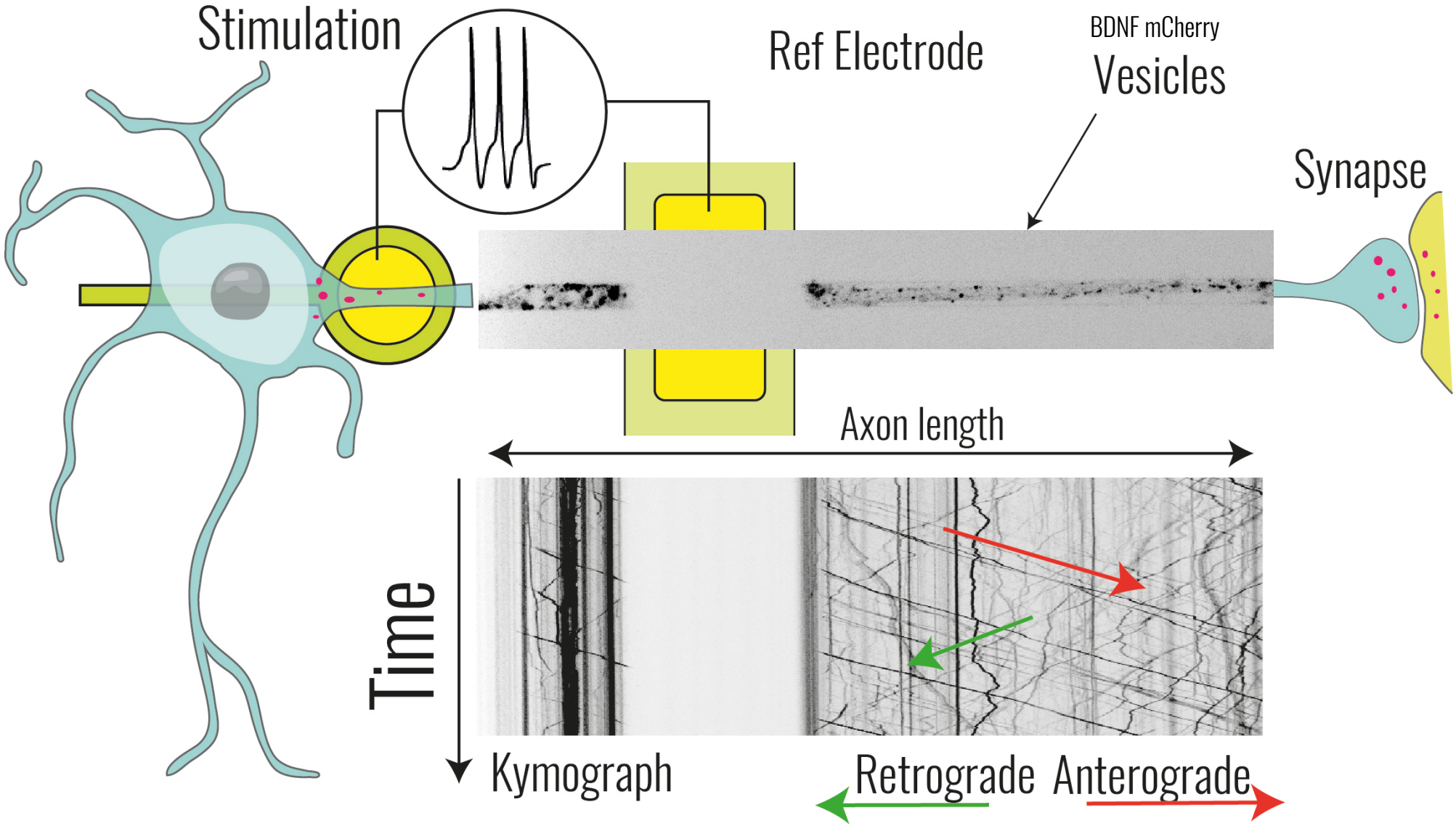




Δf/f



Axonal Transport under Stimulation



How the neuronal activity will be decoded and translated into a **regulation of axonal**

Summary

Integration of Microfluidic and Micro Electrode Arrays