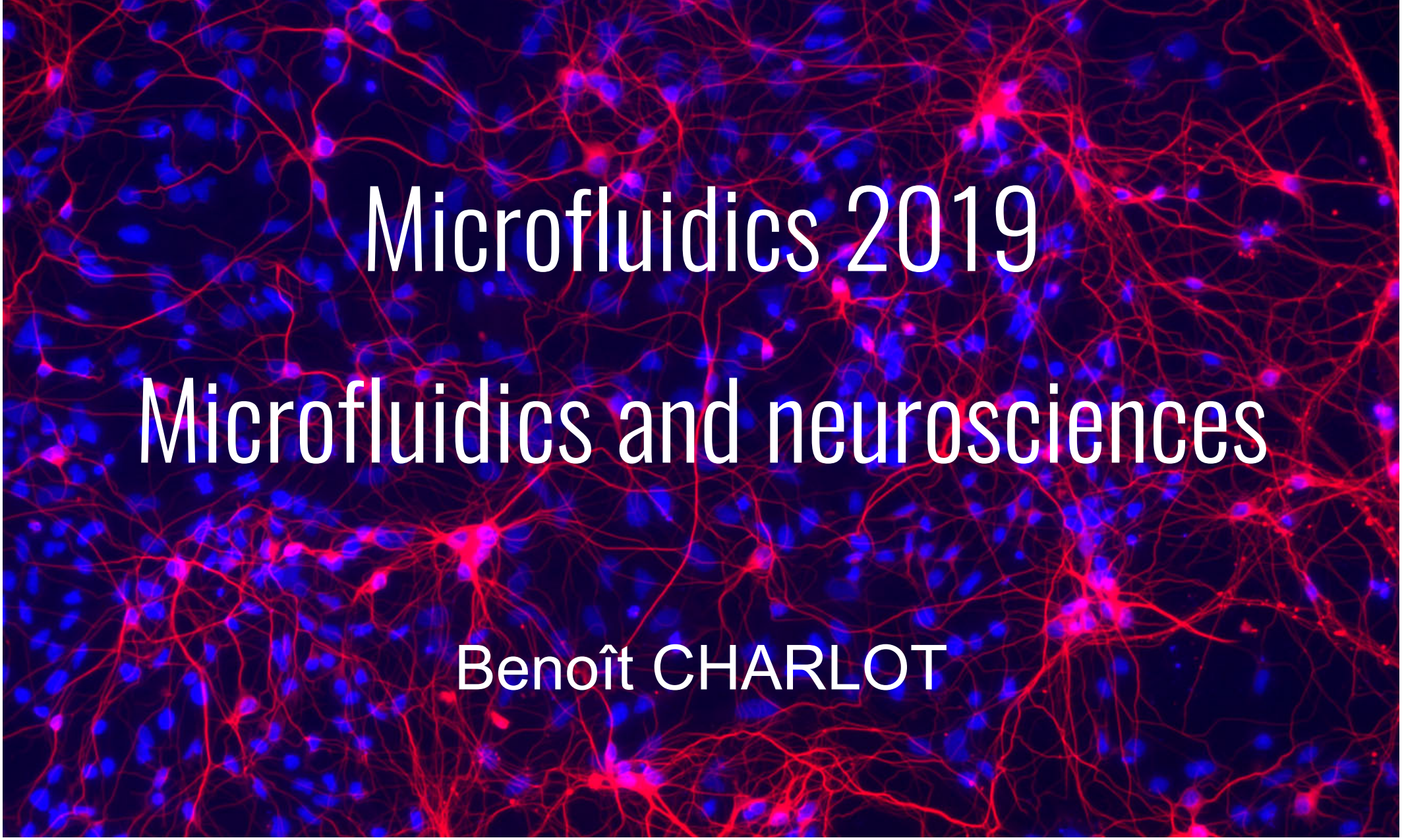


Microfluidics 2019

Microfluidics and neurosciences

Benoît CHARLOT

Outcome

1. Neurosciences for physicists
   - Neurons, synapses, Nervous influx
   - Action potentials and Hodgkin Huxley model

2. Iono electronic interfaces : electrodes

3. Neurofluidics

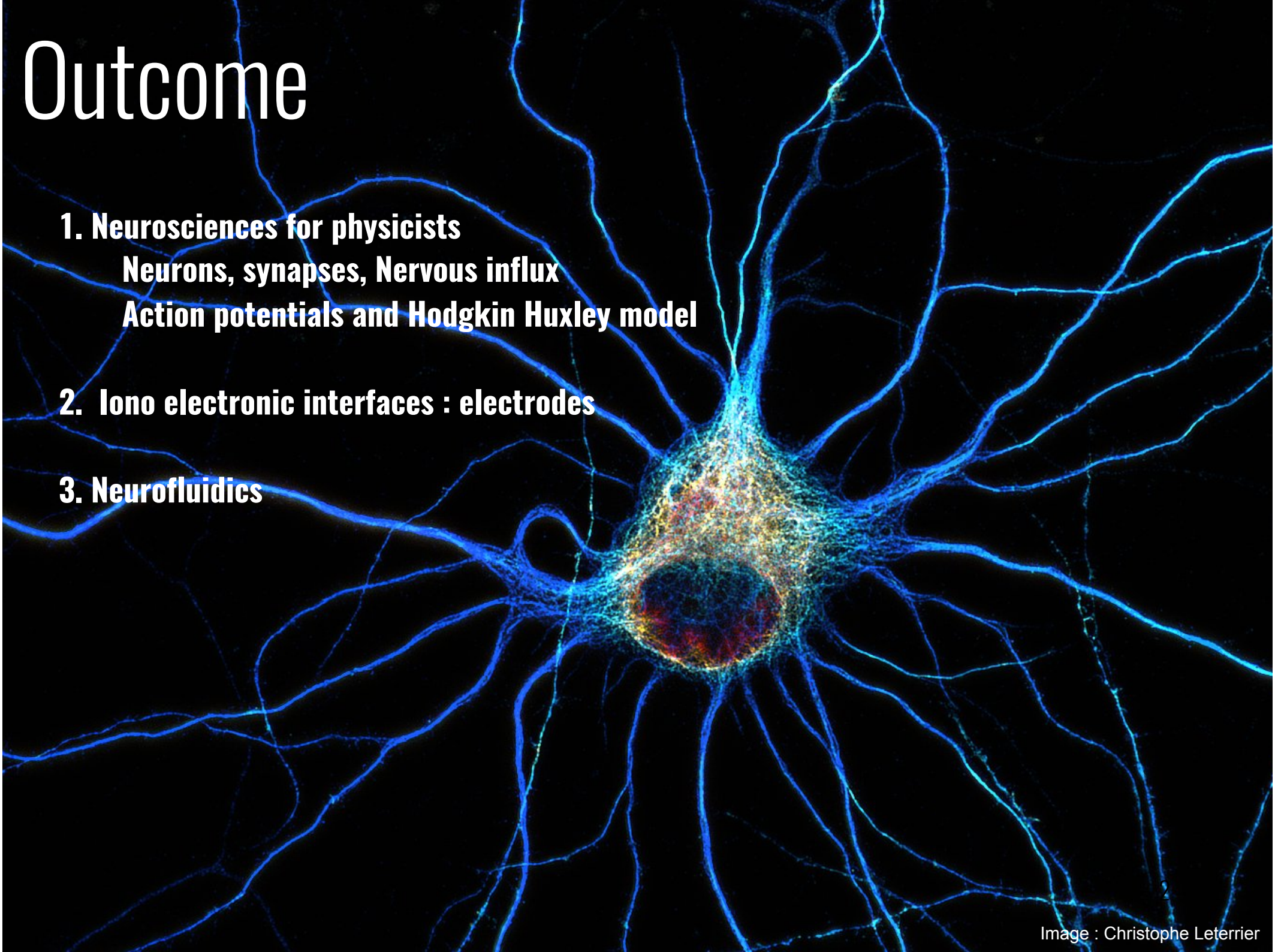


Image : Christophe Leterrier

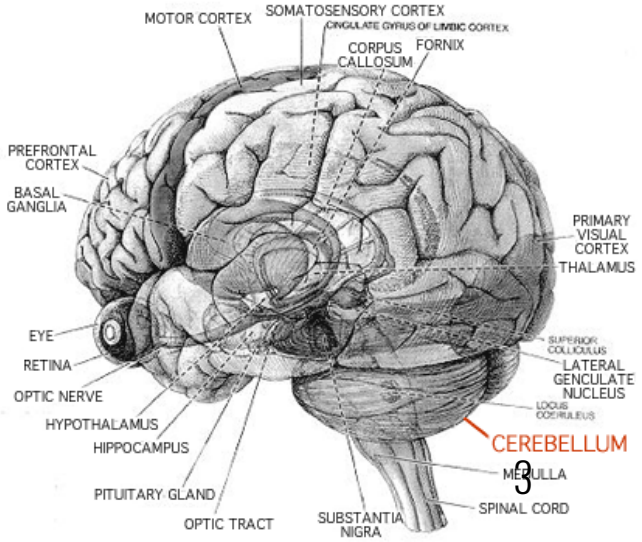
Human brain

Brain weight = **0.2 %** total weight

Brain blood flow = **15 to 20 %** total flow

20% of oxygen consumption of the body

20-30W over 100W entire body



« The brain consumes a great amount of energy doing nothing. It's a great mystery of neuroscience »

James Kozloski, Researcher, IBM

Neurons

One multipolar Neuron:

- **1** axon
- **Several** dendrites
- **1 000** synapses

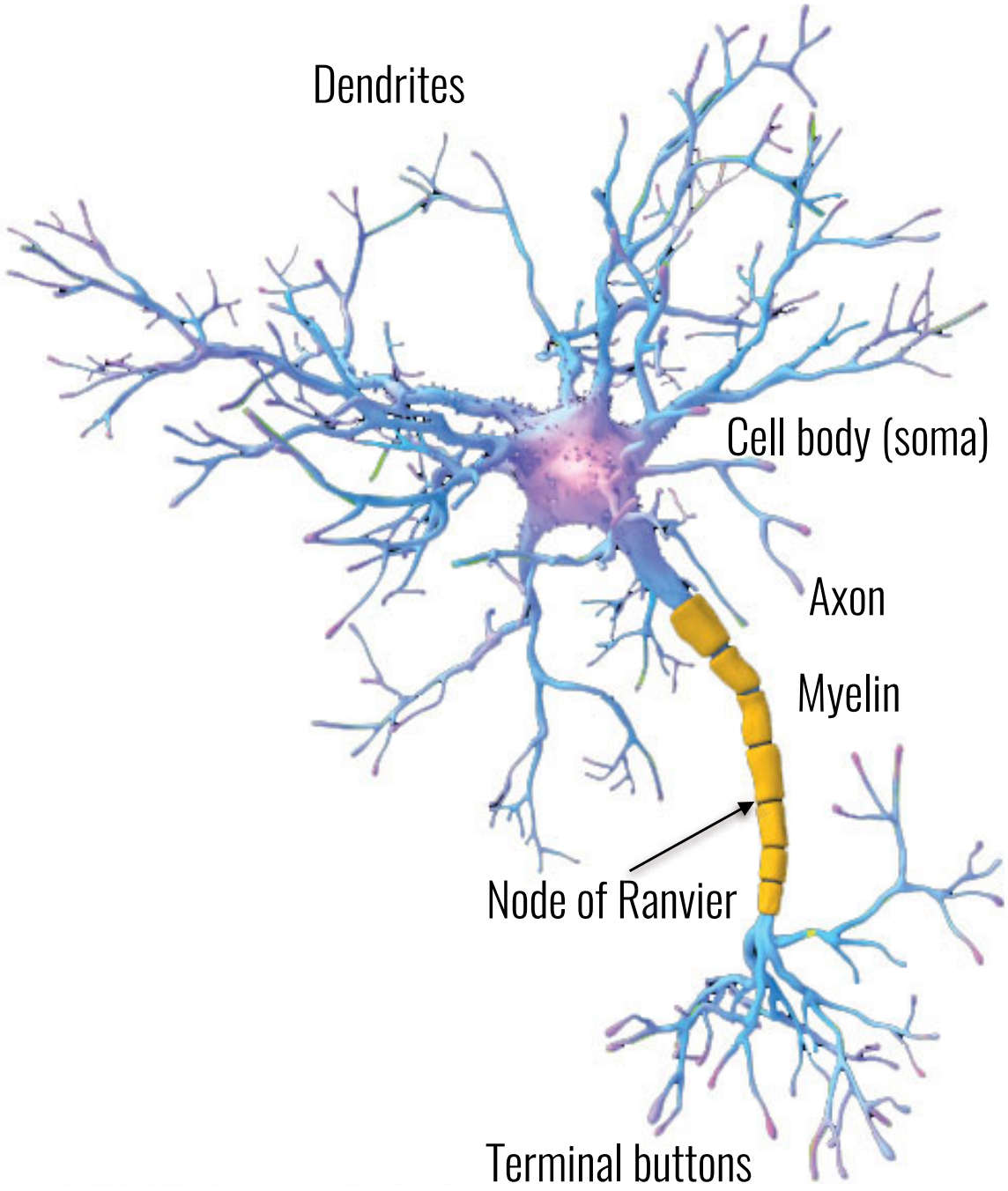
One human brain

- **100 Billions** neurons
- **10 000 billions** synapses

Types :

- Afferent (Sensitive periphery)
- Efferents (muscles and glands)
- Interneurons (Short and long)

+glial cells, astrocytes, oligodendricytes



Complete myelinisation of the brain : not before 25 years old !

Neurons

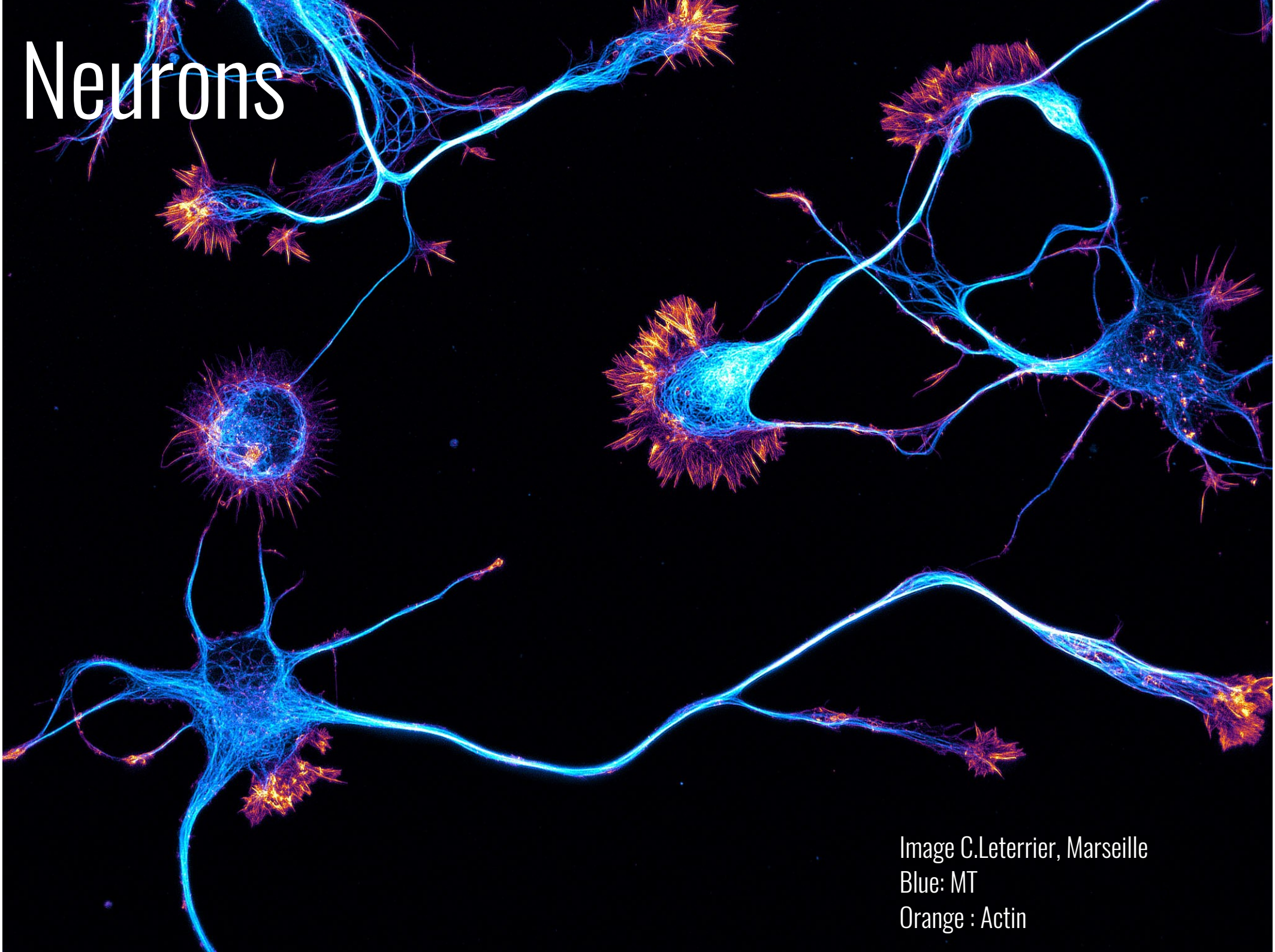
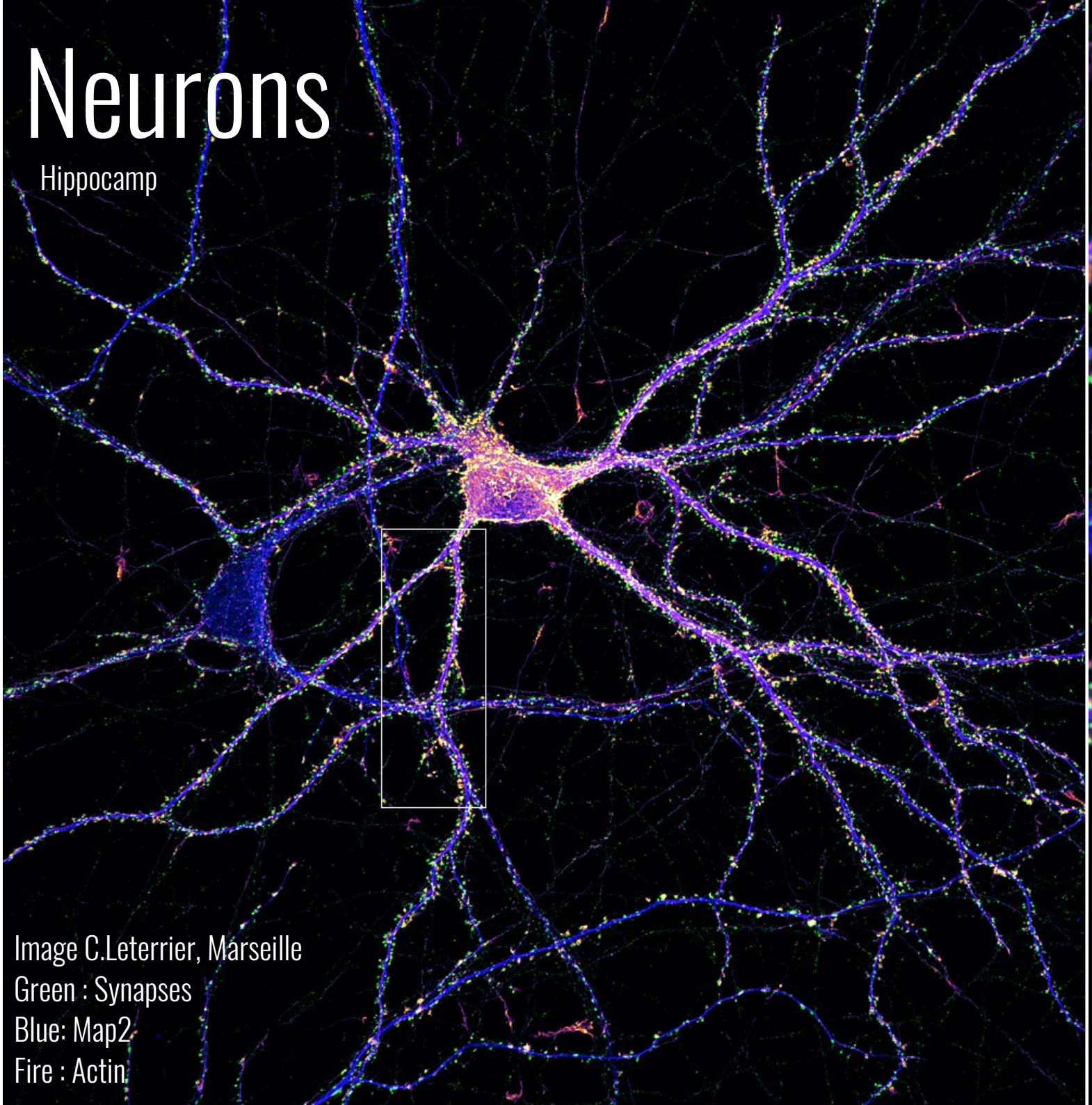


Image C.Leterrier, Marseille
Blue: MT
Orange : Actin

Neurons

Hippocamp



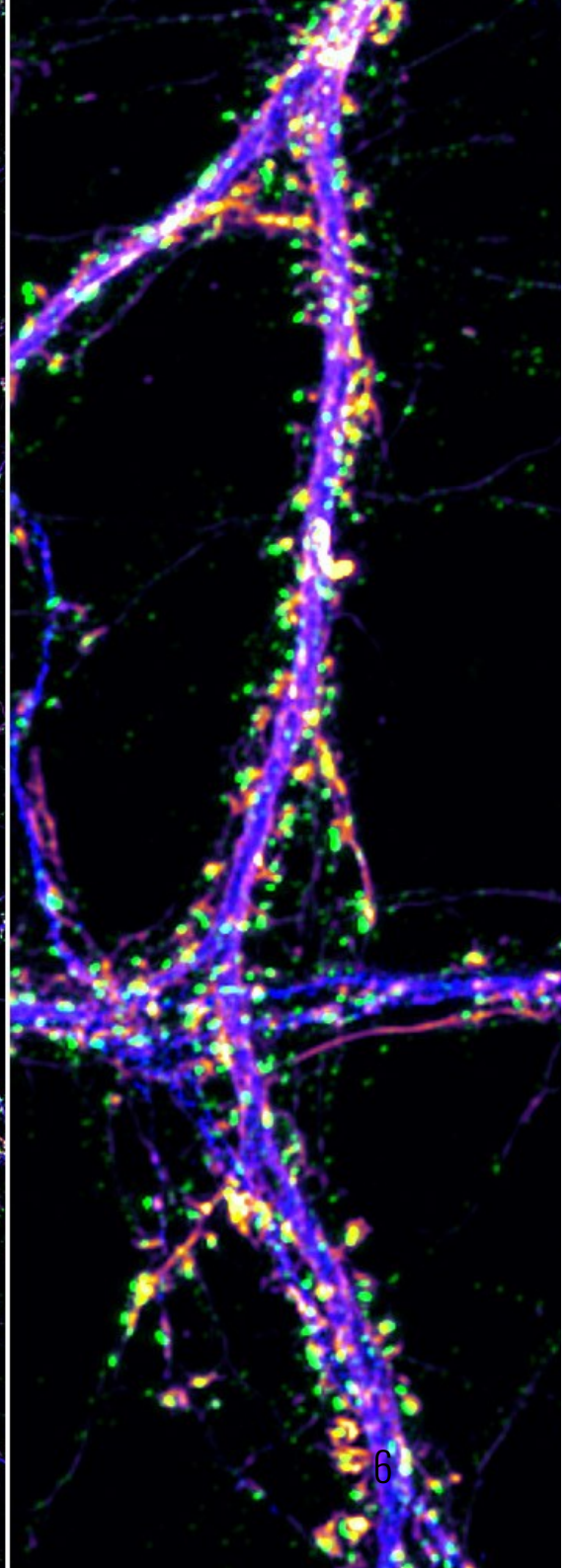
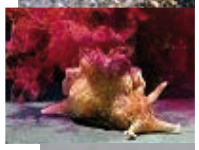


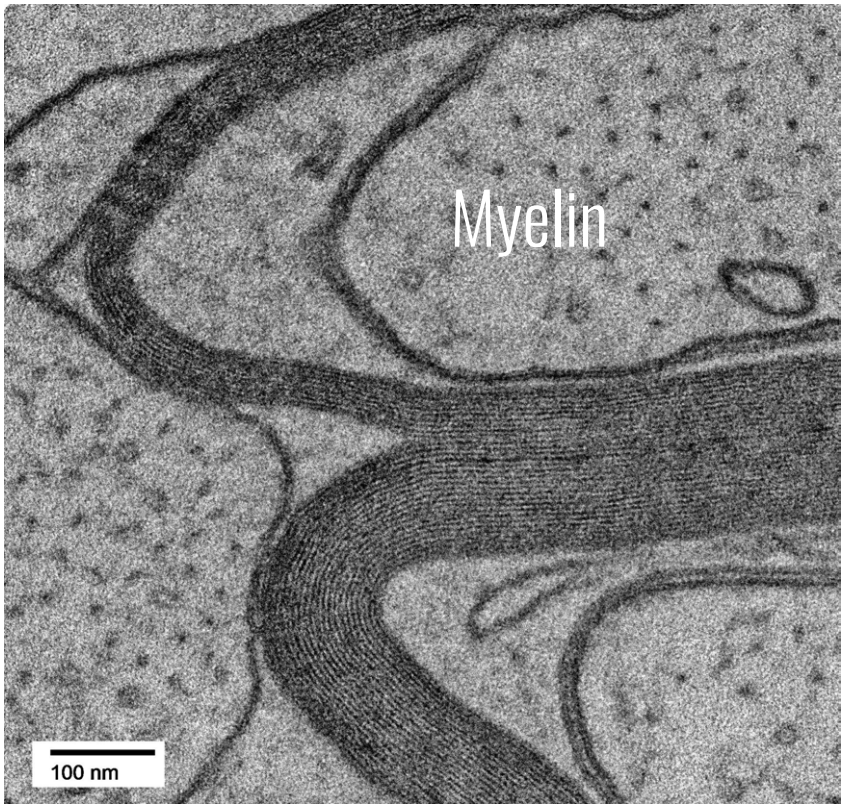
Image C.Leterrier, Marseille
Green : Synapses
Blue: Map2
Fire : Actin

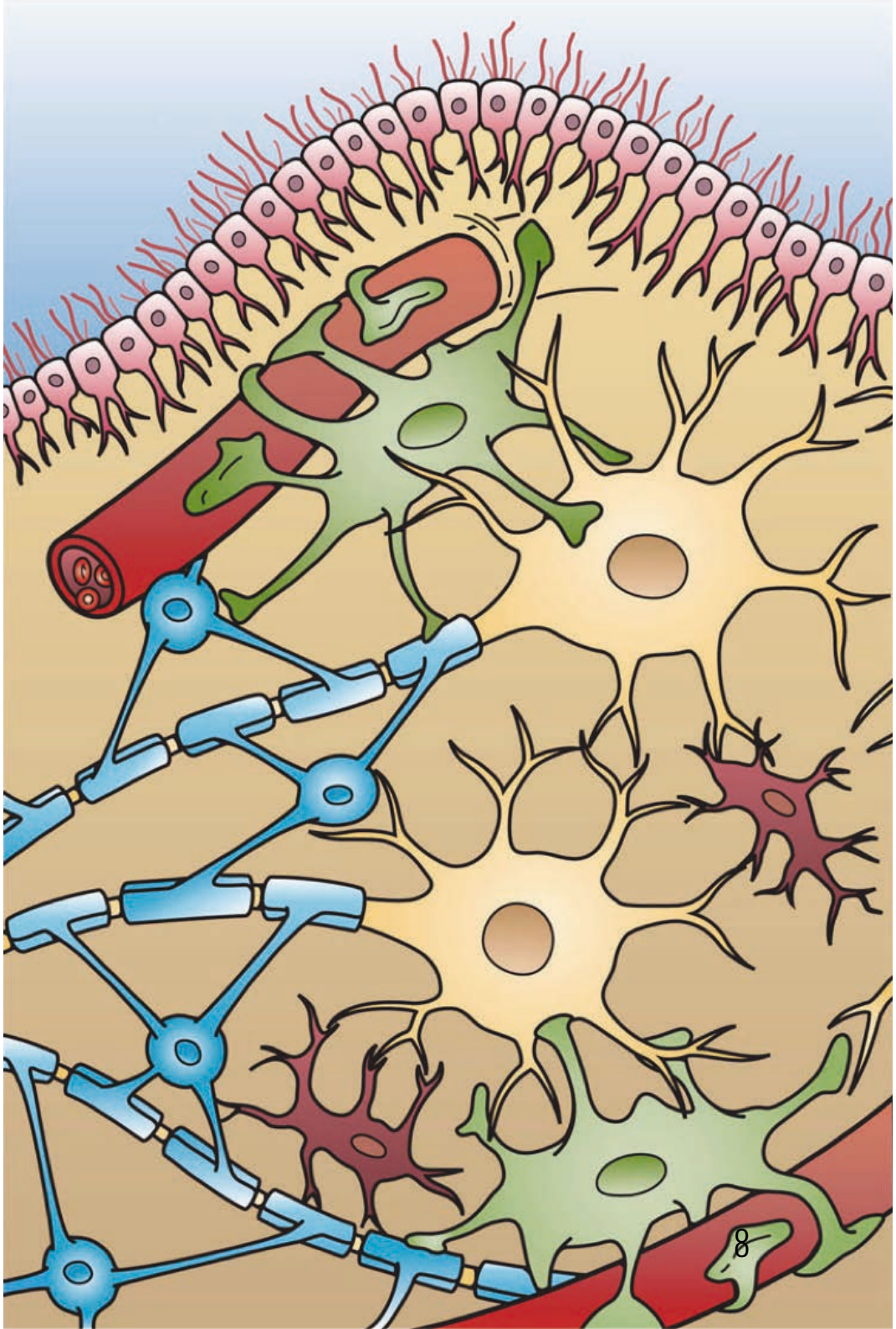
Number of neurons by species

	Caenorhabditis elegans	302
	Pond snail	11,000
	Aplysia	18,000
	Drosophila	100,000
	Lobster	100,000
	Master Student	2
	Ant	250,000
	Honey bee	960,000
	Cockroach	1,000,000
	Frog	16,000,000
	Octopus	300,000,000
	Human	100,000,000,000
	Elephant	200,000,000,000

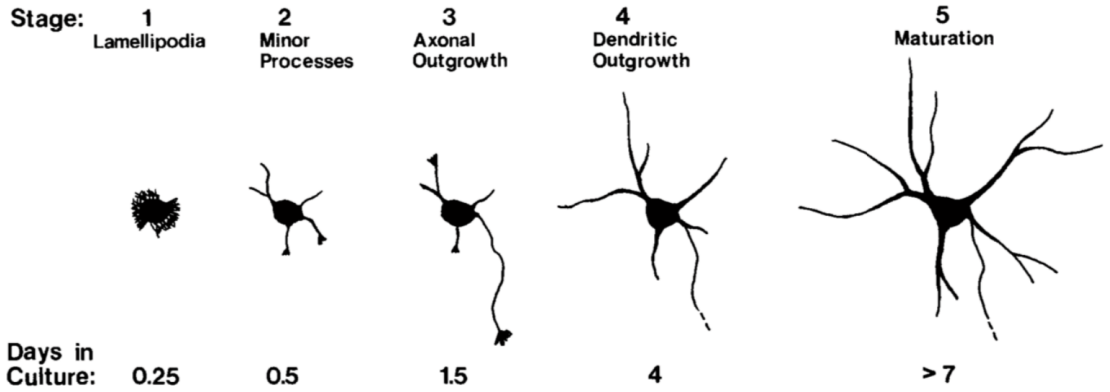
Glial cells

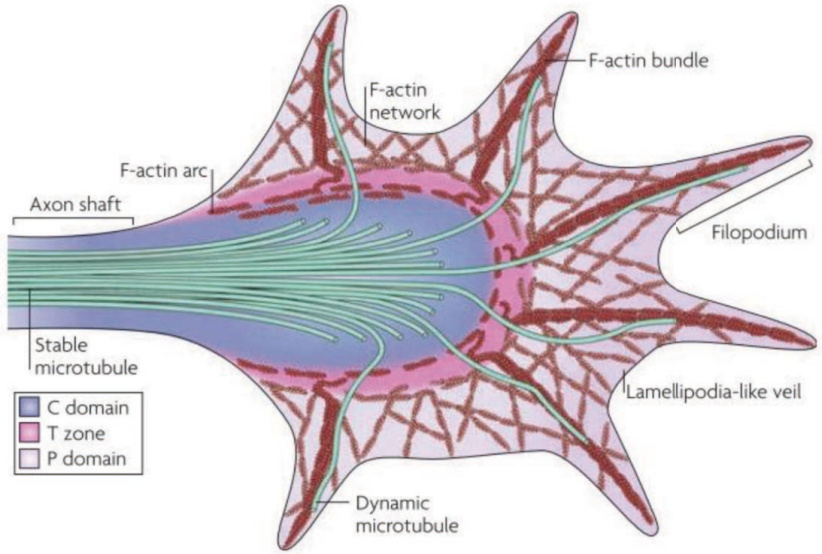
Astrocytes
Oligodendrocytes
Schwann cells
Microglia

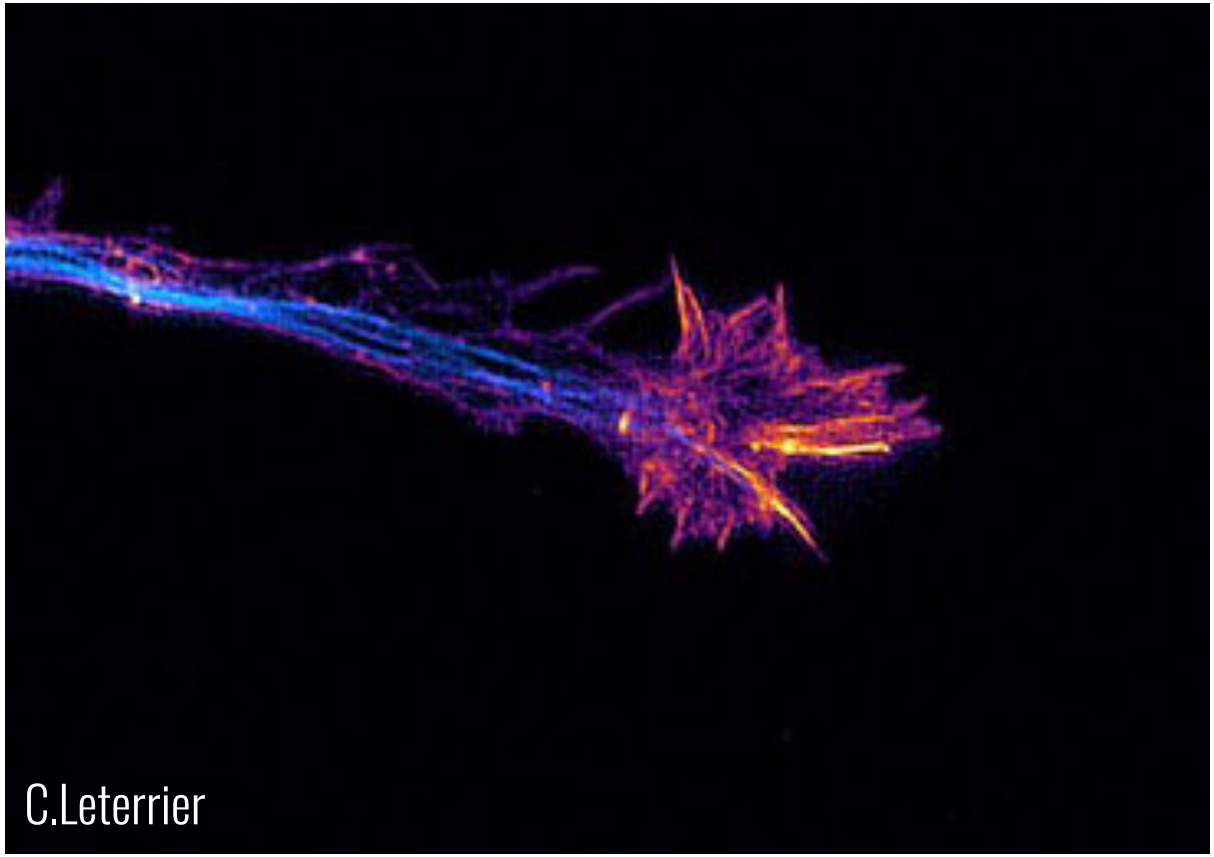


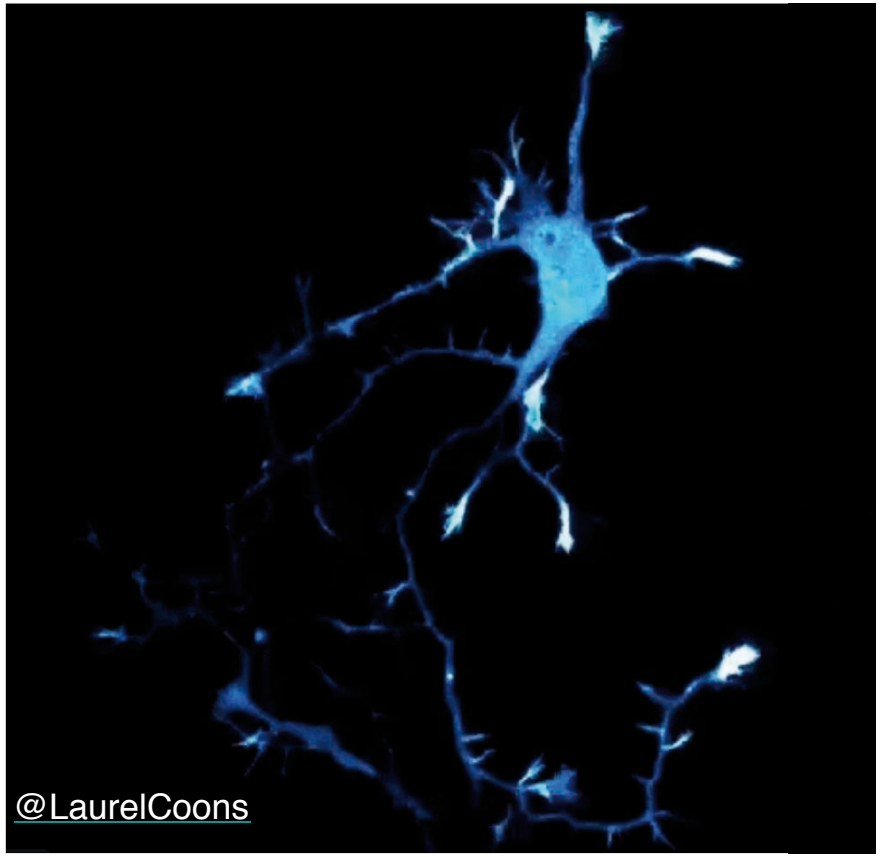


Growth / Growth Cone





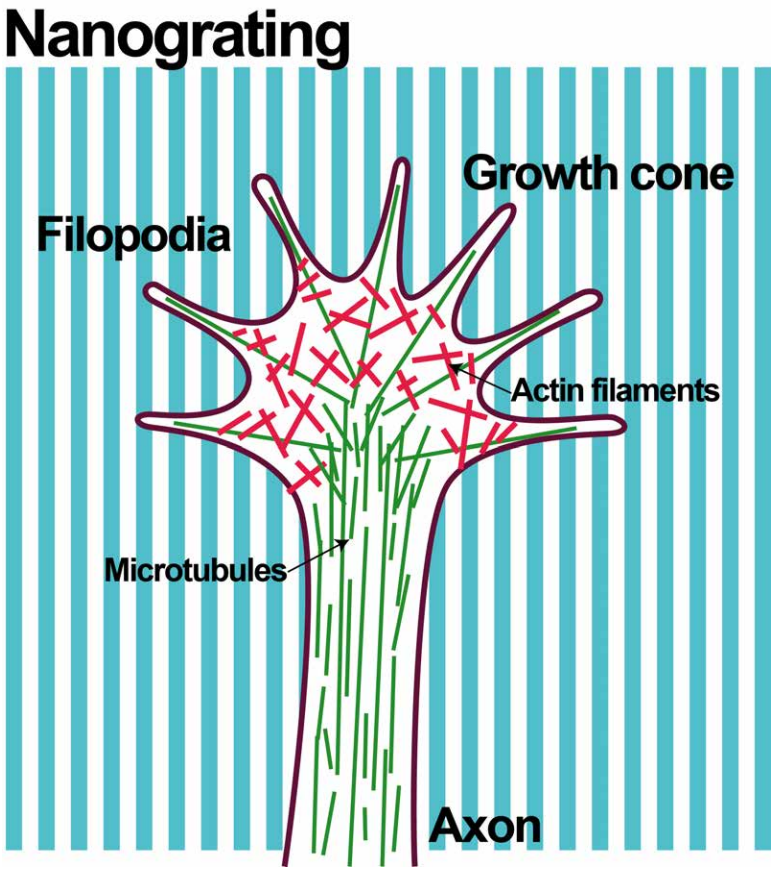


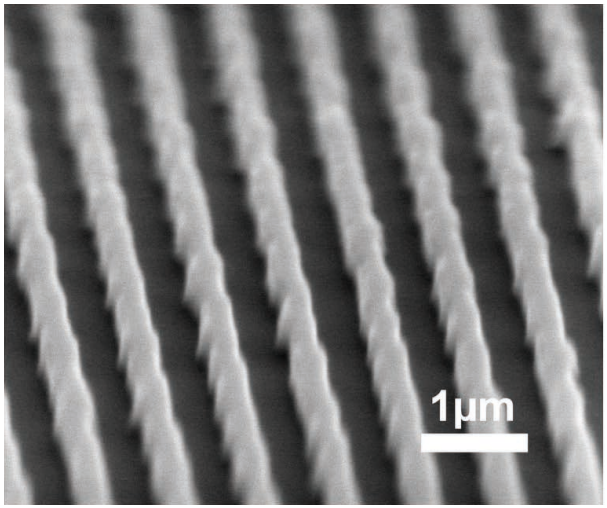


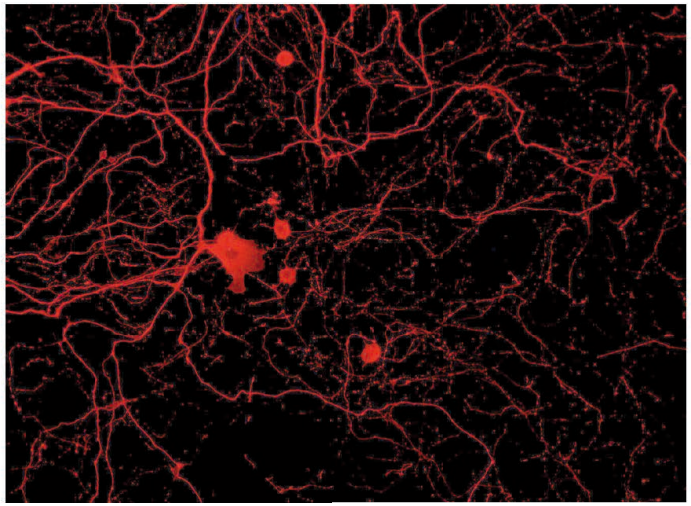
Growth cone

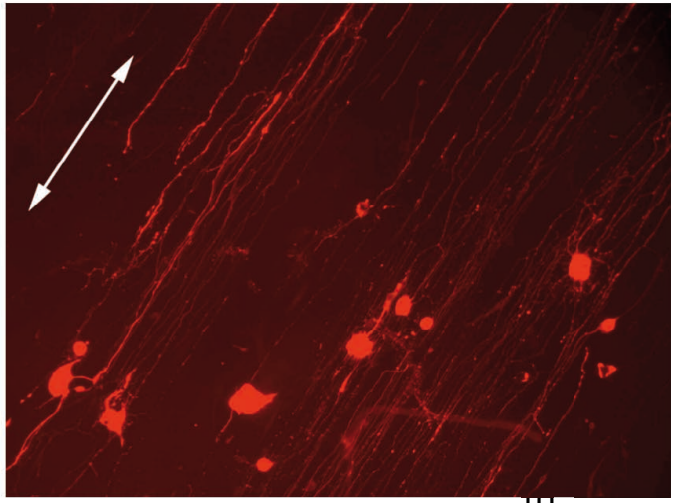
Topology guidance :

A nano grating allows to polarise cell growth



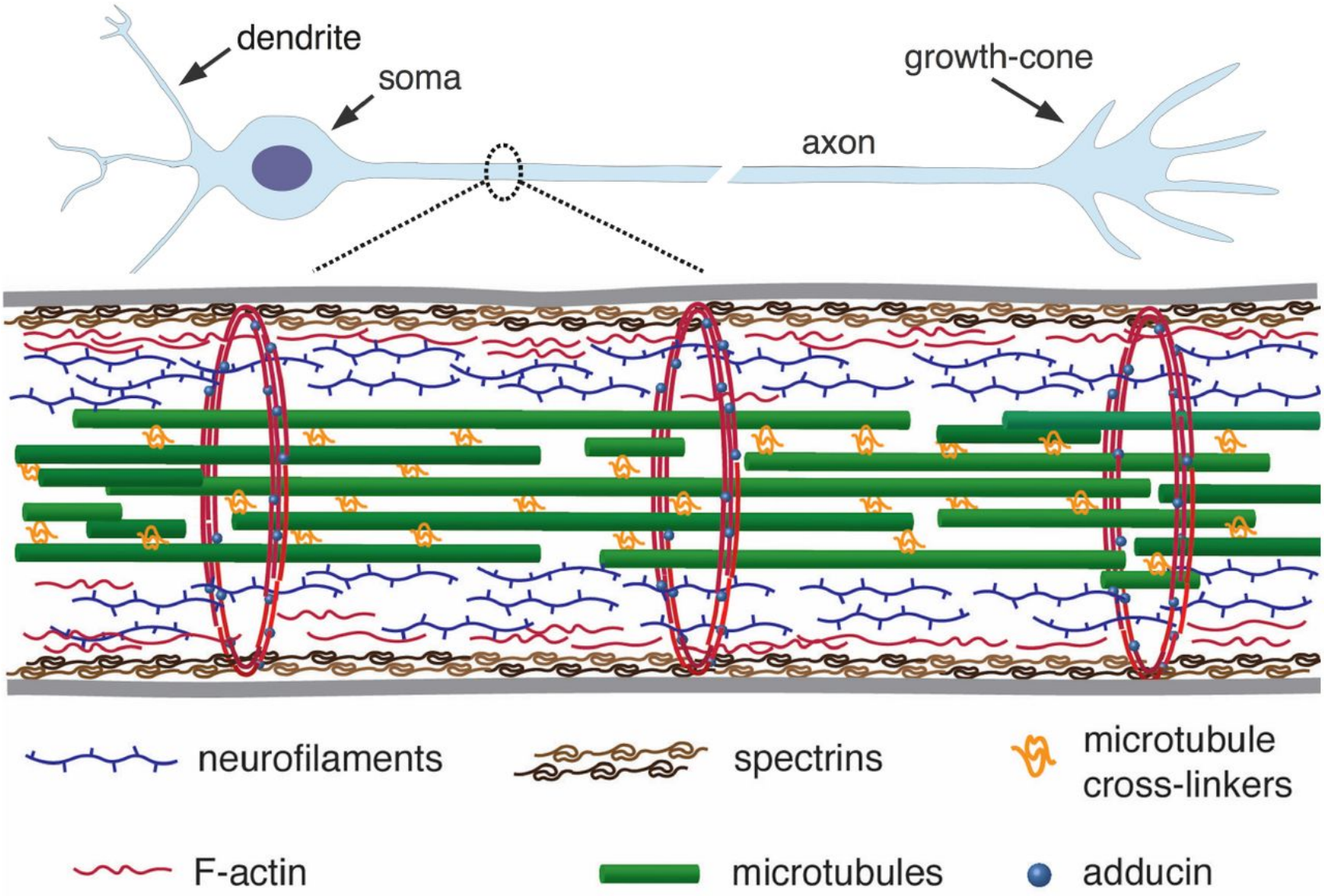






Cytoskeleton

Microtubule
Actin

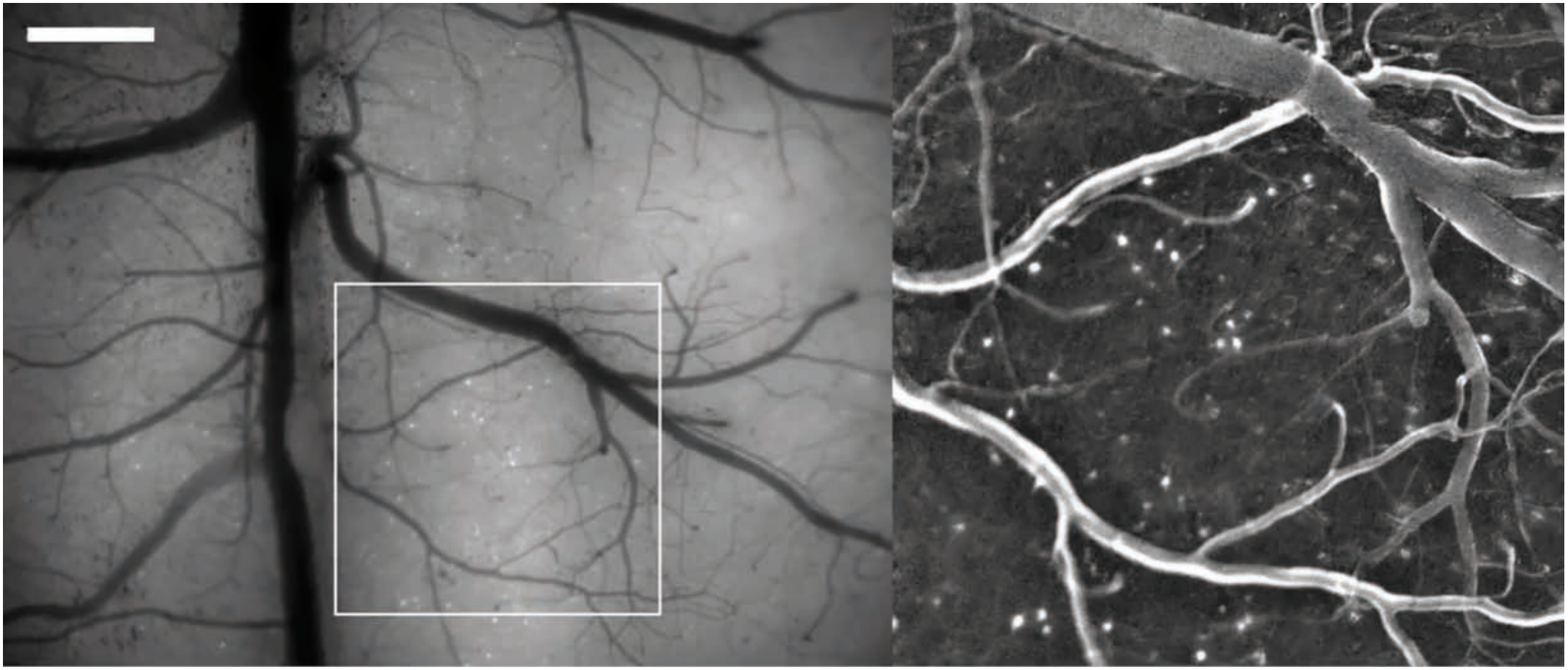


Sushil Dubey, The axonal actin-spectrin lattice acts as a tension buffering shock absorber, BioArXiv

A brain in action

Crystal skull

50,000 neurons in the outer layers of the brain.



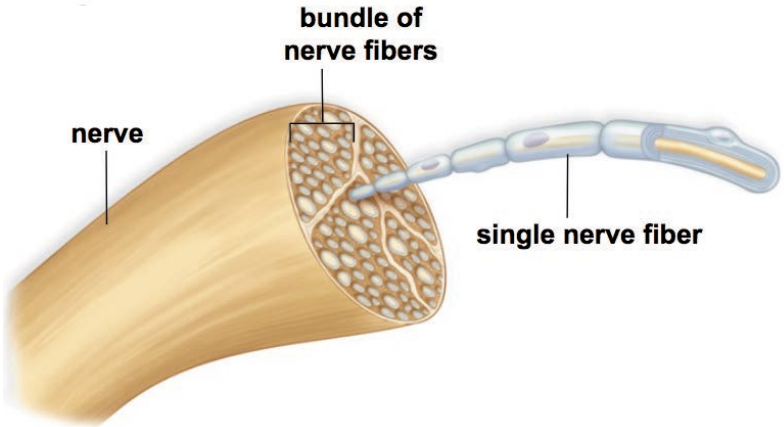
Kim T.H. et al. *Cell Rep.* 17, 3385-3394 (2016)

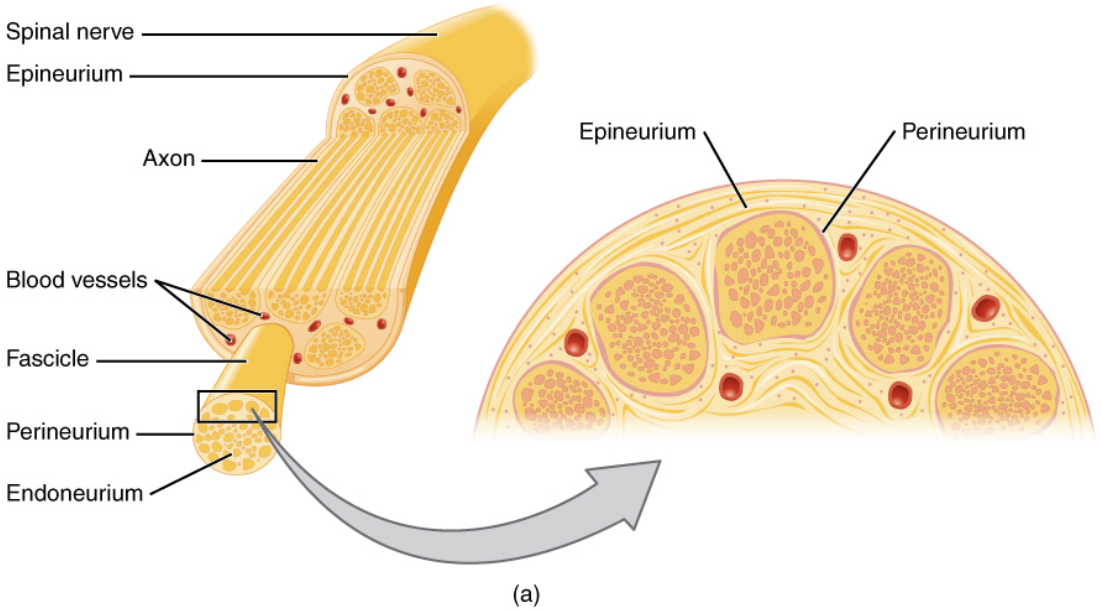
Nerves

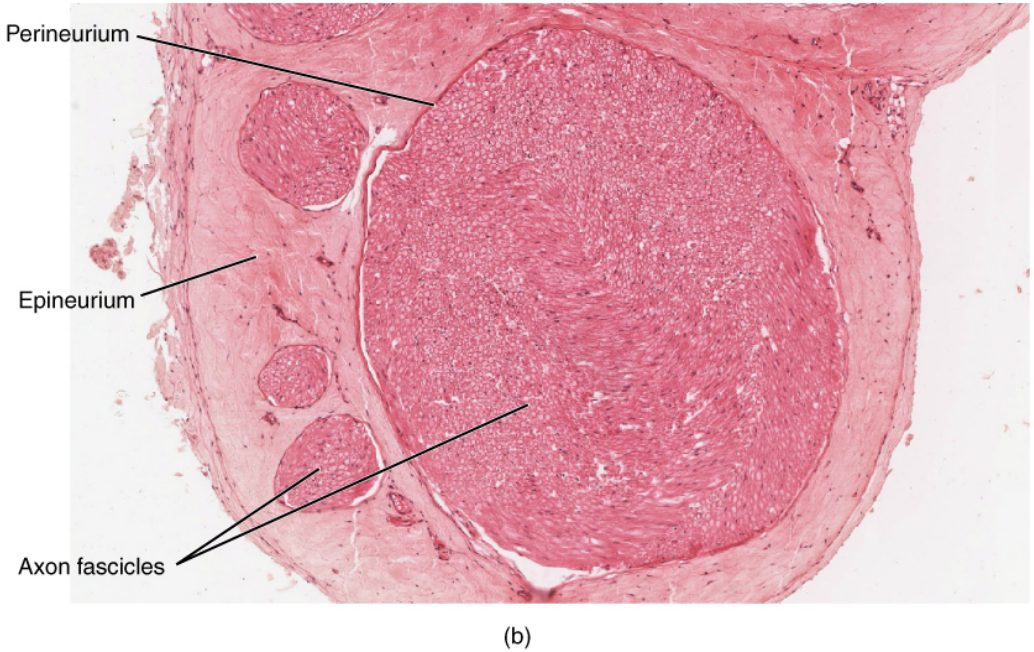
cable-like bundle of axons

Afferent nerves conduct signals from sensory neurons to the central nervous system, for example from the mechanoreceptors in skin.

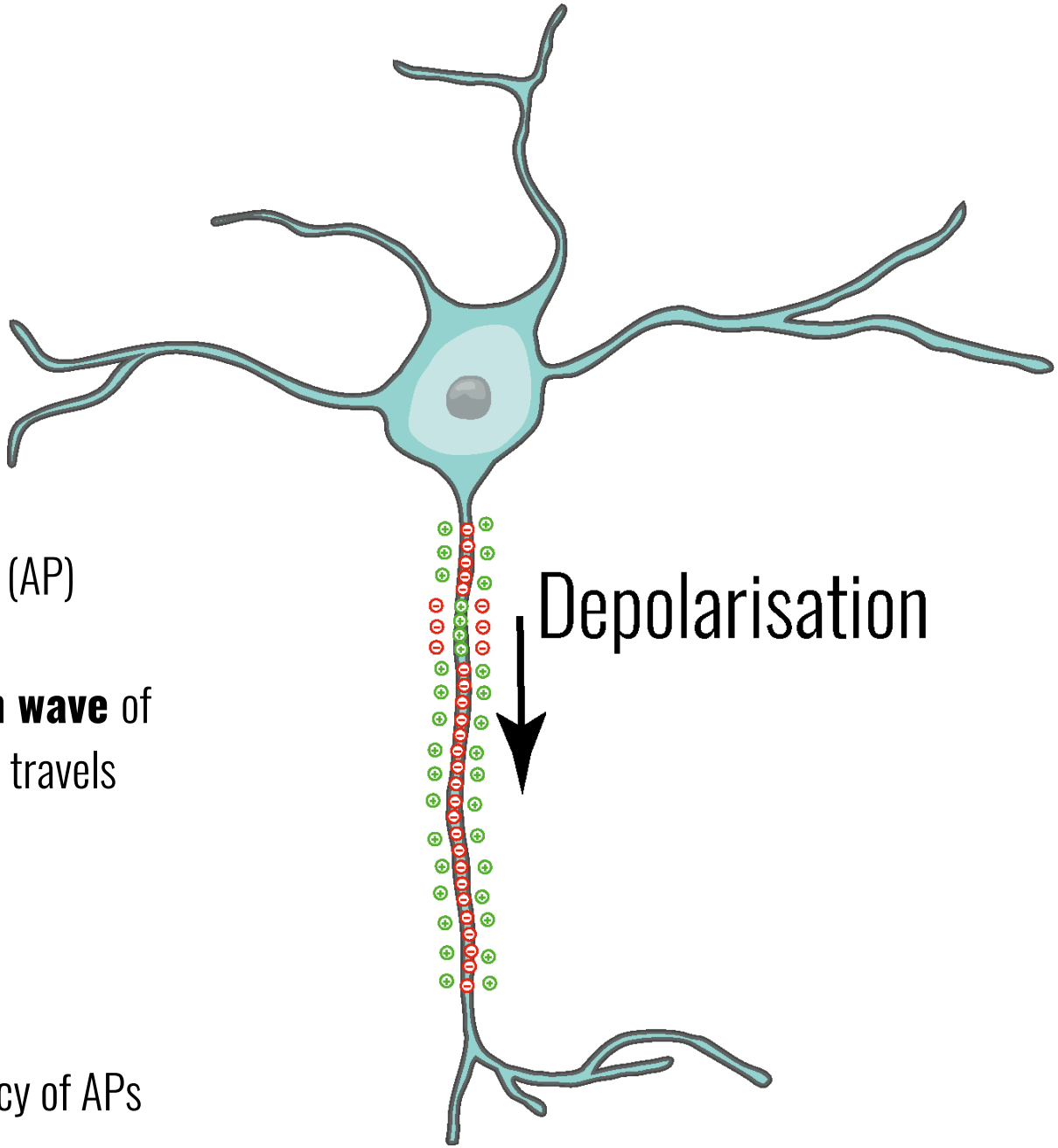
Efferent nerves conduct signals from the central nervous system along motor neurons to their target muscles and glands.







Nervous influx



The Nervous influx is a set of Action Potentials (AP)

An AP is the propagation of a **depolarisation wave** of the membrane that initiate in the cell body and travels down the terminations

~digital signal

Information coding by the number and frequency of APs

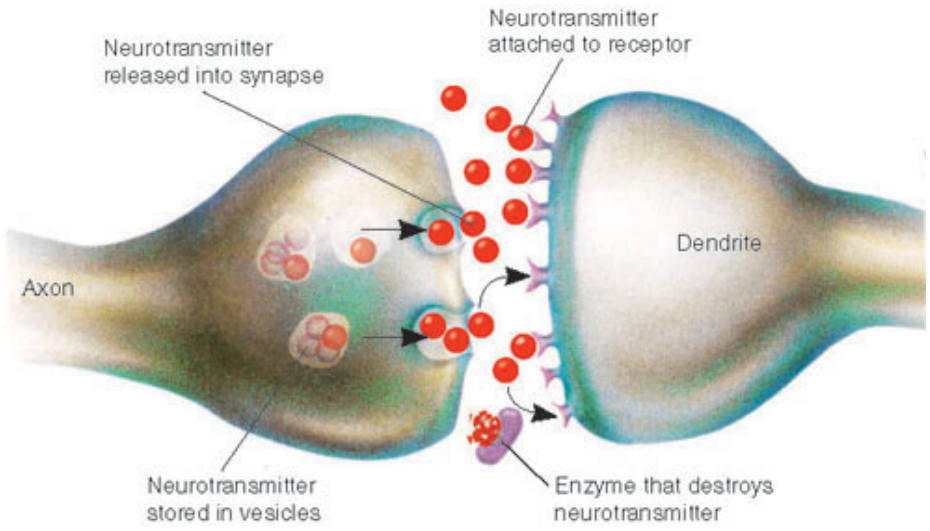
Synapses

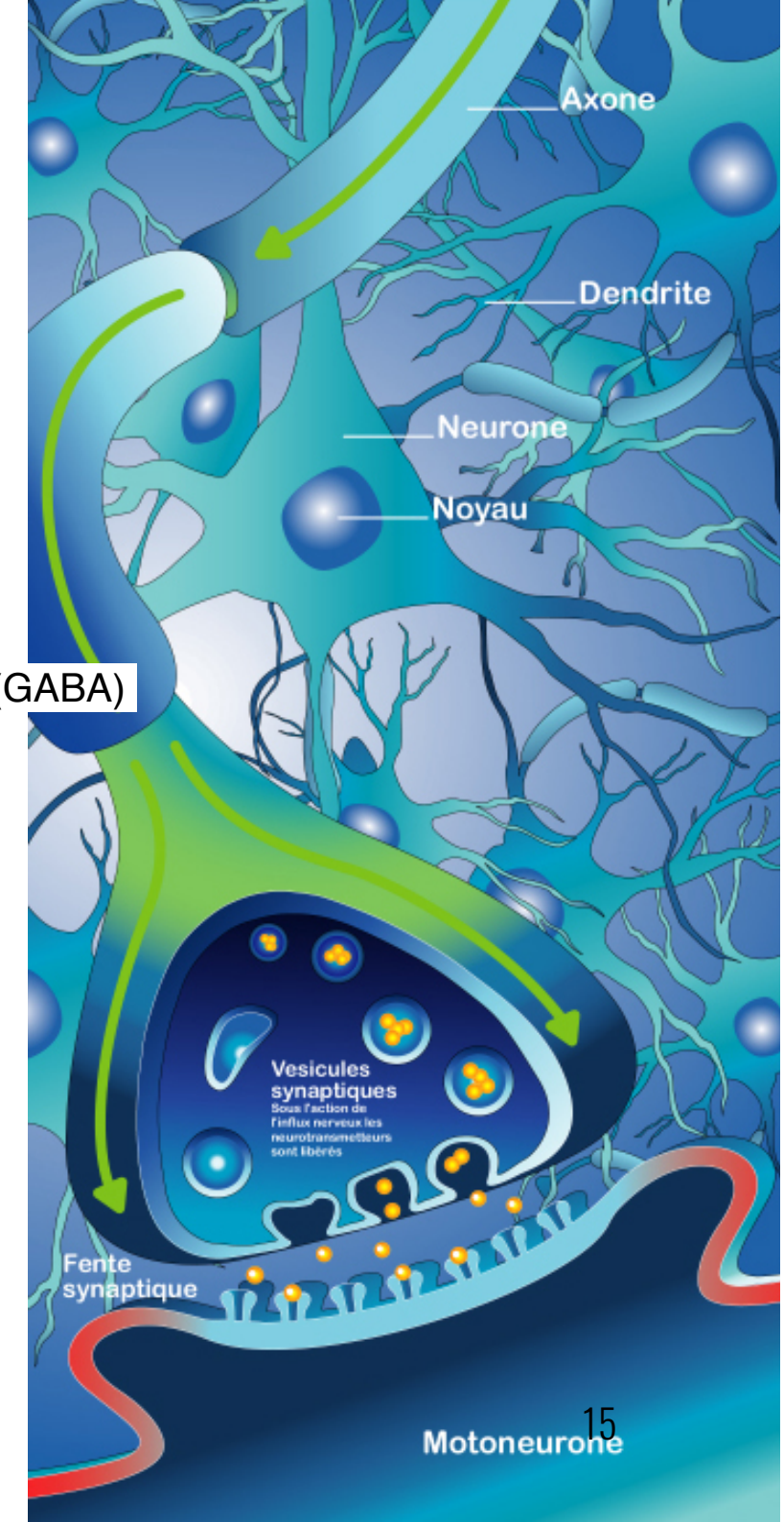
It is the zone of contact that spread between two neurons
or between one neuron and another cell

Chemical synapse uses neurotransmitters
Electrical synapse

Synaptic cleft : between **10 and 40 nm**

glutamate
Serotonin
Dopamine
Adrenaline
γ-aminobutyric acid (GABA)
Acetylcholine
Endorphin
Oxytocin
Glycine
….





Synapses

Synapses transmit AP from one cell to another

Briefly :

The arrival of one AP in the synaptic cleft induces the progressive delivery of neurotransmitters

Diffusion of neurotransmitters in the cleft

Neurotransmitters are captured by receptors

Excitation or inhibition

Synapses

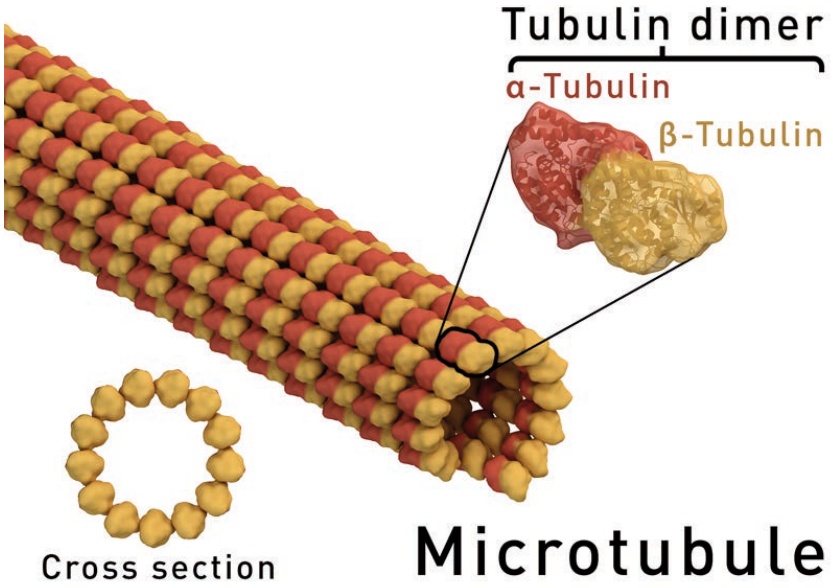
The **synaptic potential** is
- Weak (0.1-10 mV)
- progressive (~analogic)
- Passive propagation (diffusion driven)
- hyperpolarisation, or depolarisation

The **Action potential** is
- High (70-110 mV)
- « all or nothing» (~digital)
- Active propagation
- depolarisation

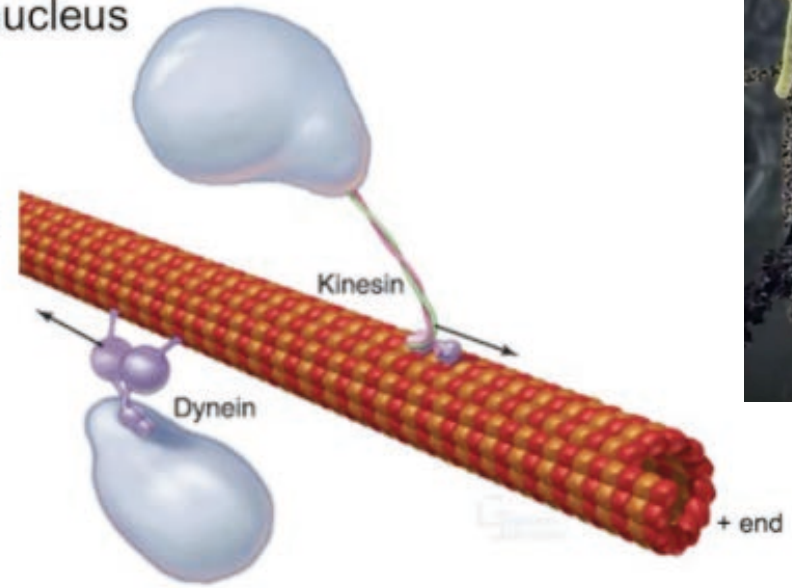
Axonal transport

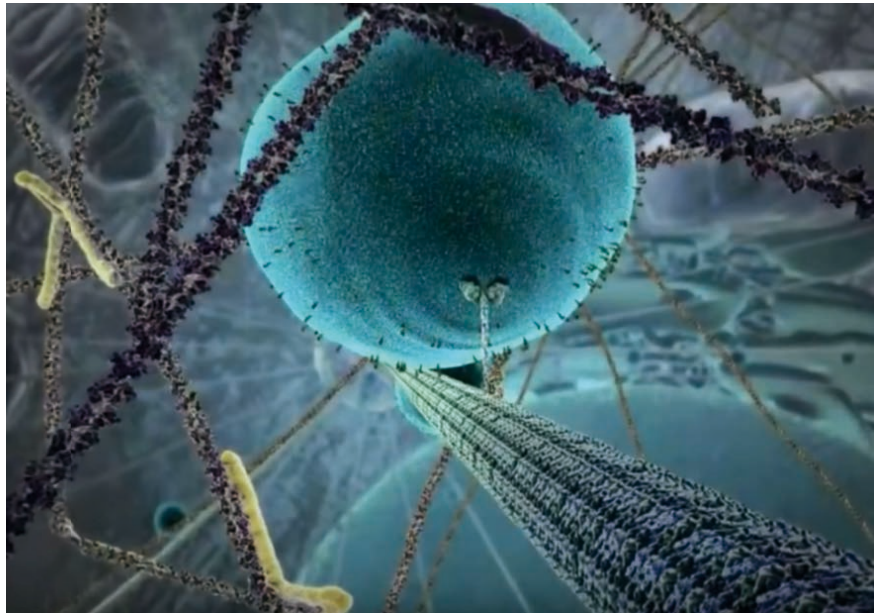
Neurotransmitters are synthesised in the cell body
Diffusion is too long
Some axons can extend up to 1 meter

How NT are sent from the cell body down to the synapses?

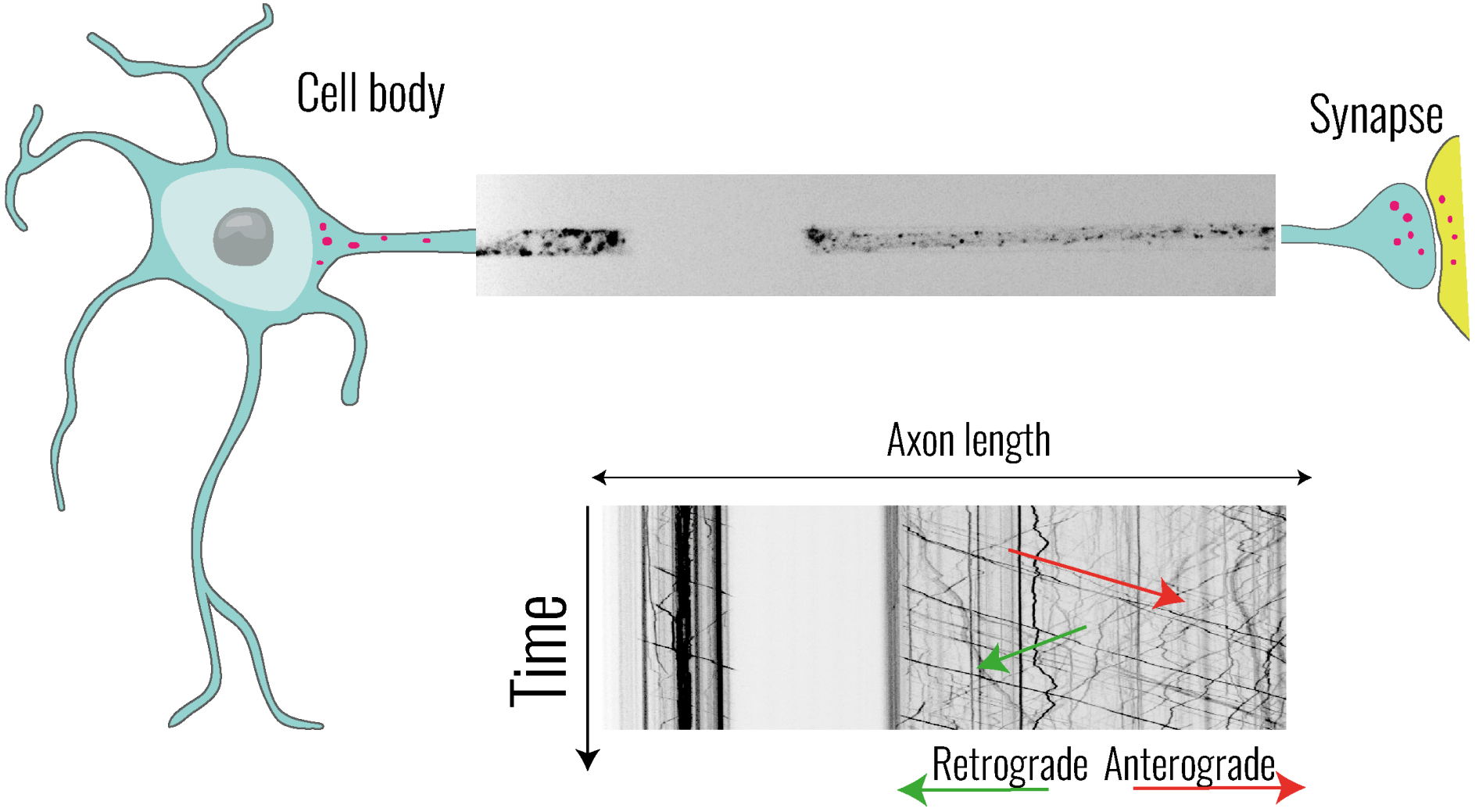


- Vesicular transport
  - Kinesin
    - Toward +
    - Away from nucleus
  - Dynein
    - Toward –
    - Toward Nuc
  - 0.1-1 um/s





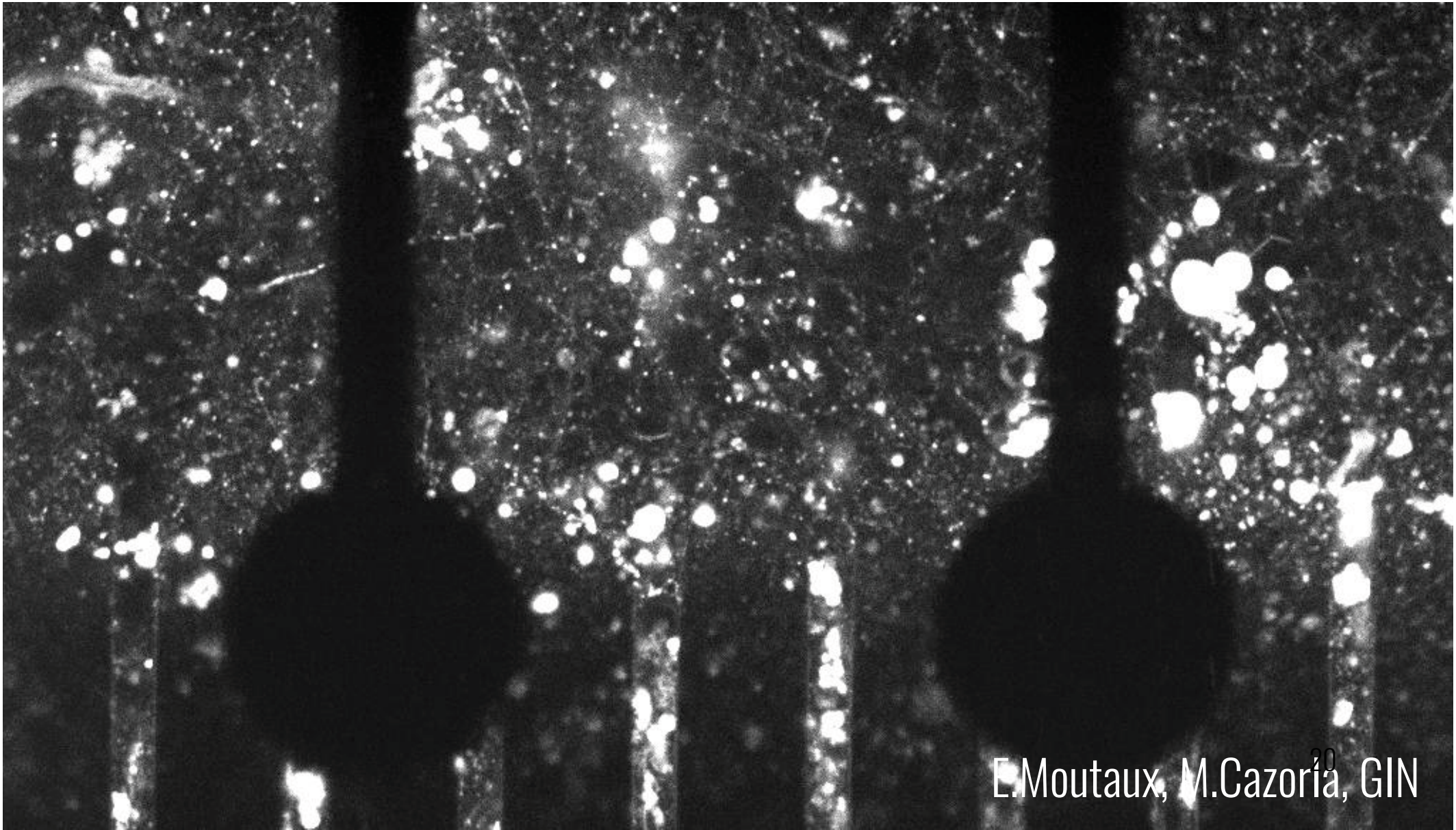
Axonal transport



E.Moutaux, M.Cazorla, GIN

Kymographe

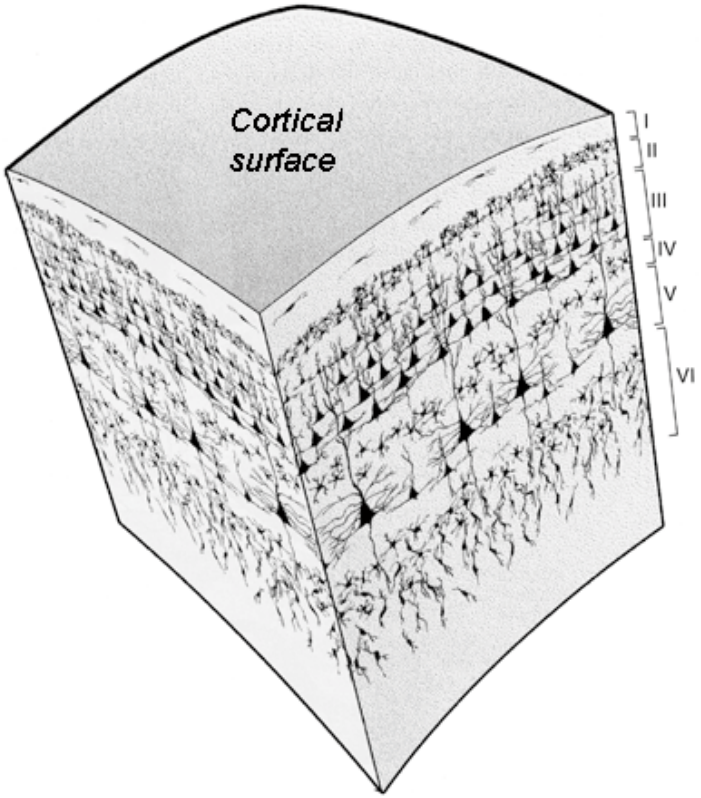
Axonal transport

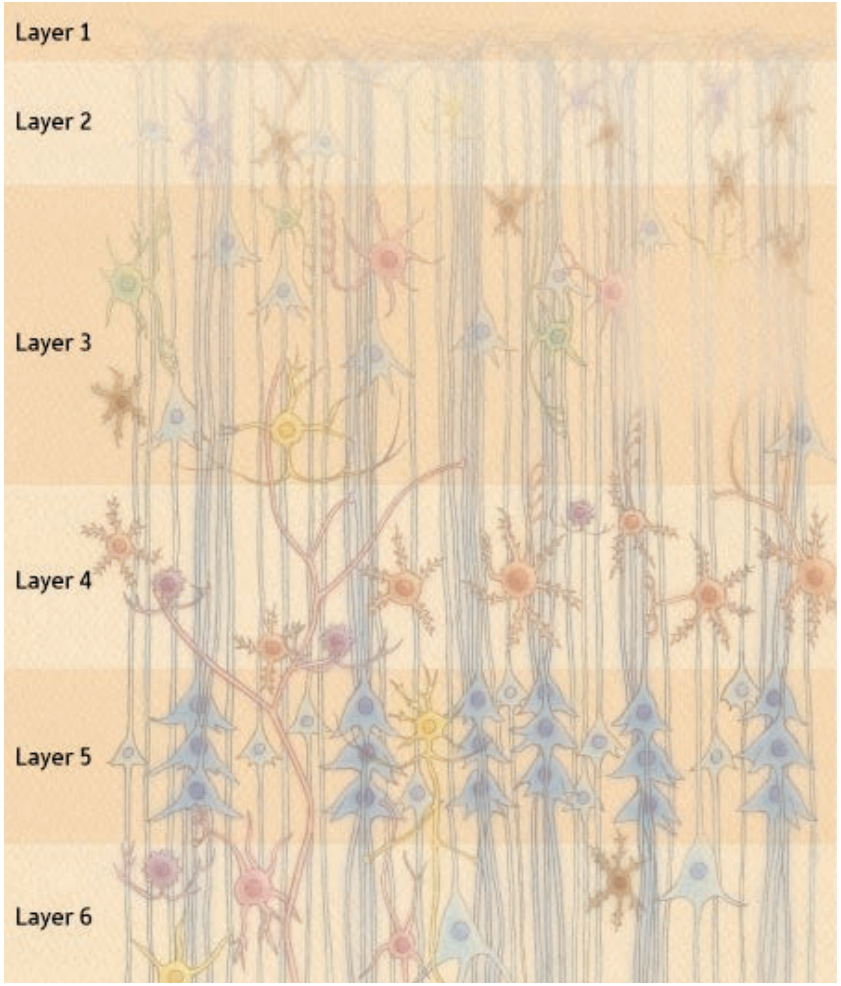


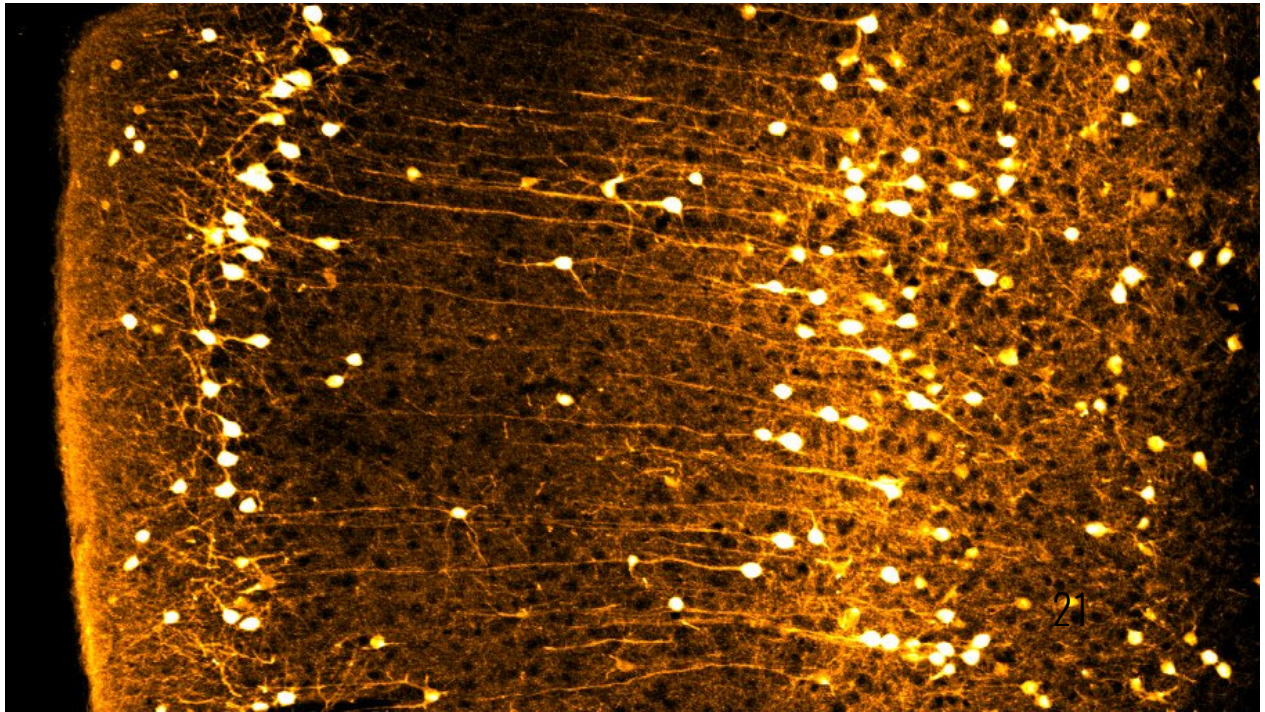
E.Moutaux, M.Cazoria, GIN

Neuronal Network

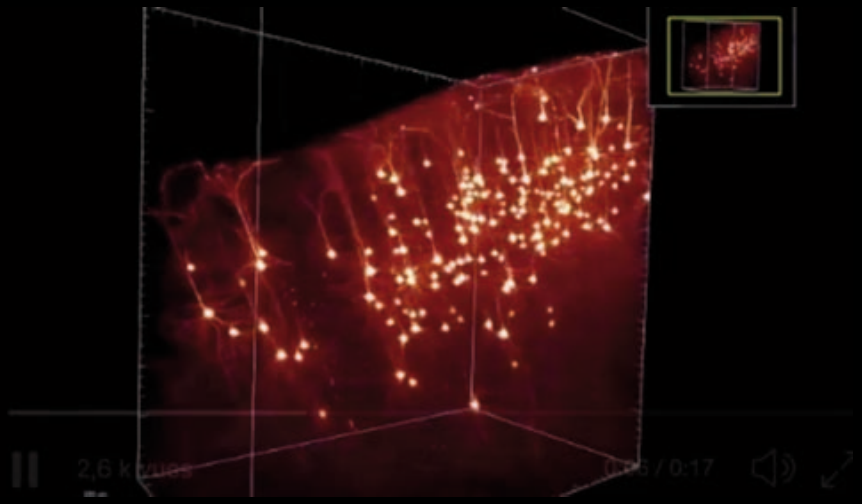
Cortical layers

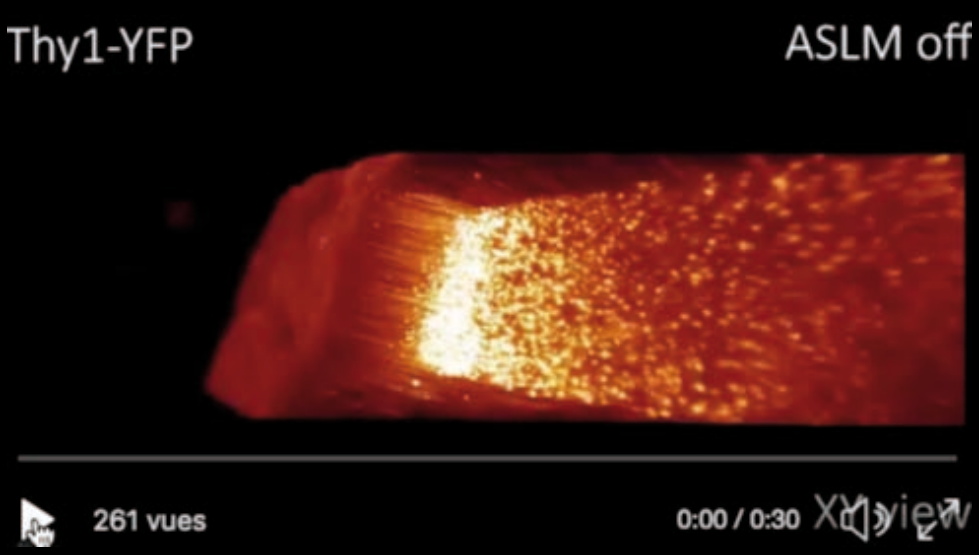






Neuronal Network

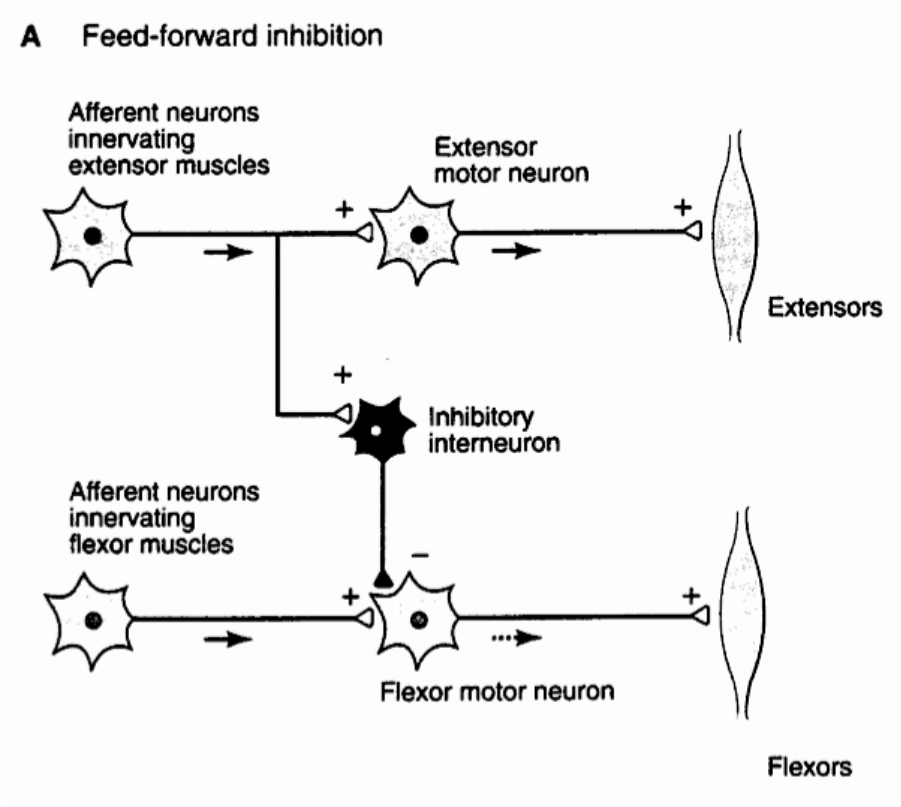


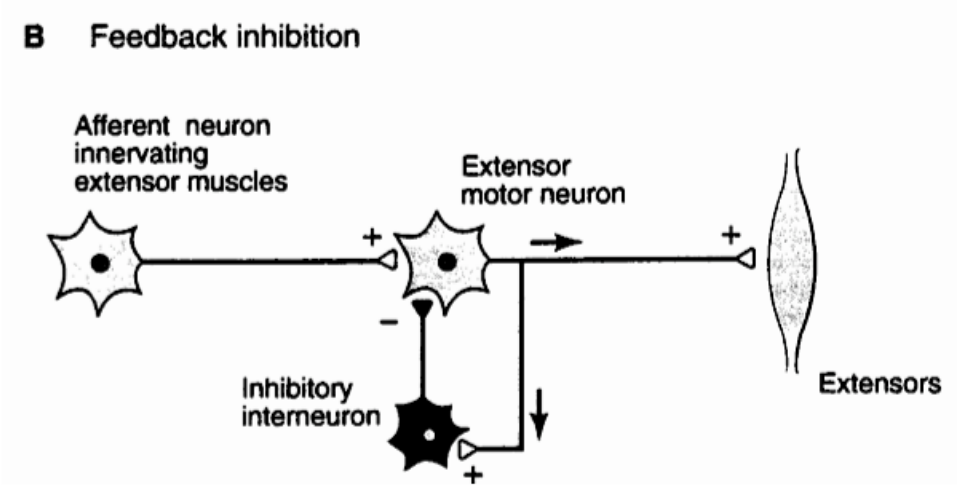


mesospim.org

Neuronal Network

Loops, positive Feedback , excitation and inhibition





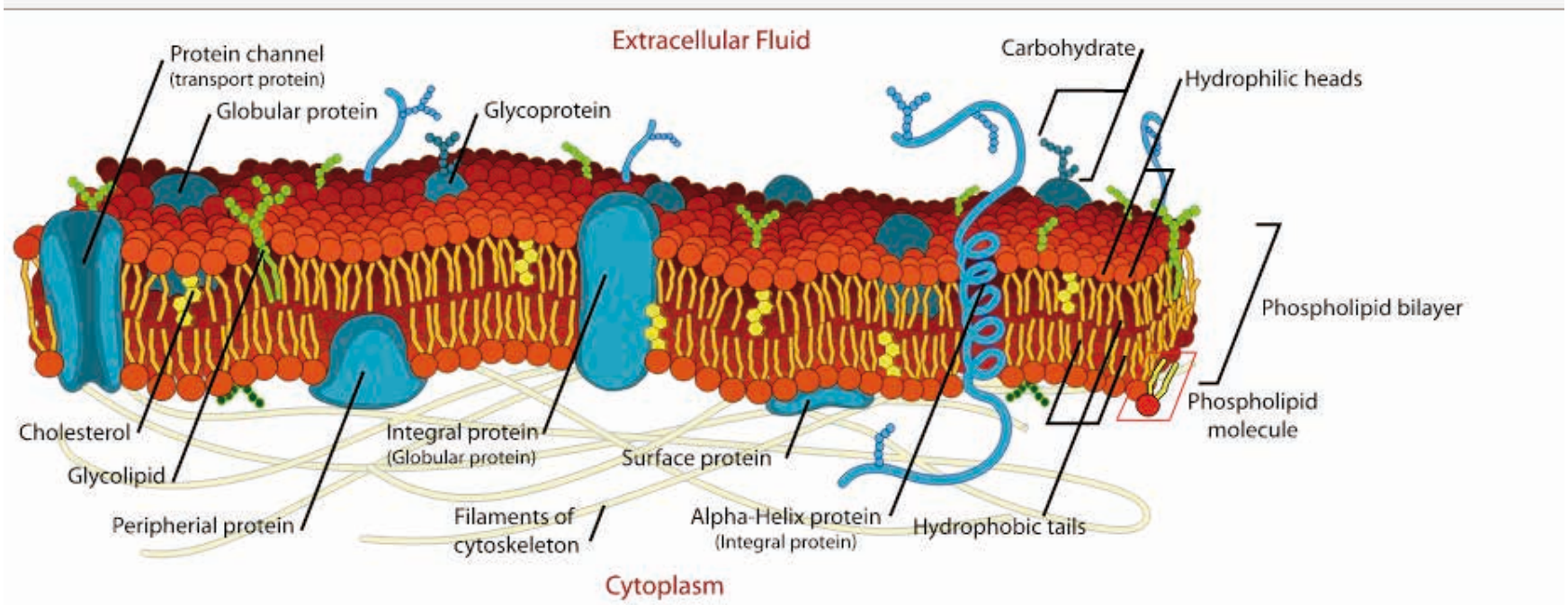
P.Fromherz

Neuroelectronic interfacing: Semiconductor chips with ion schannels, nerve cells, and brain
Nanoelectronics and Information technology. Wiley-VCH 781-810

Cell Membrane

Thickness **7nm**

Phospholipids
Lipid bilayer



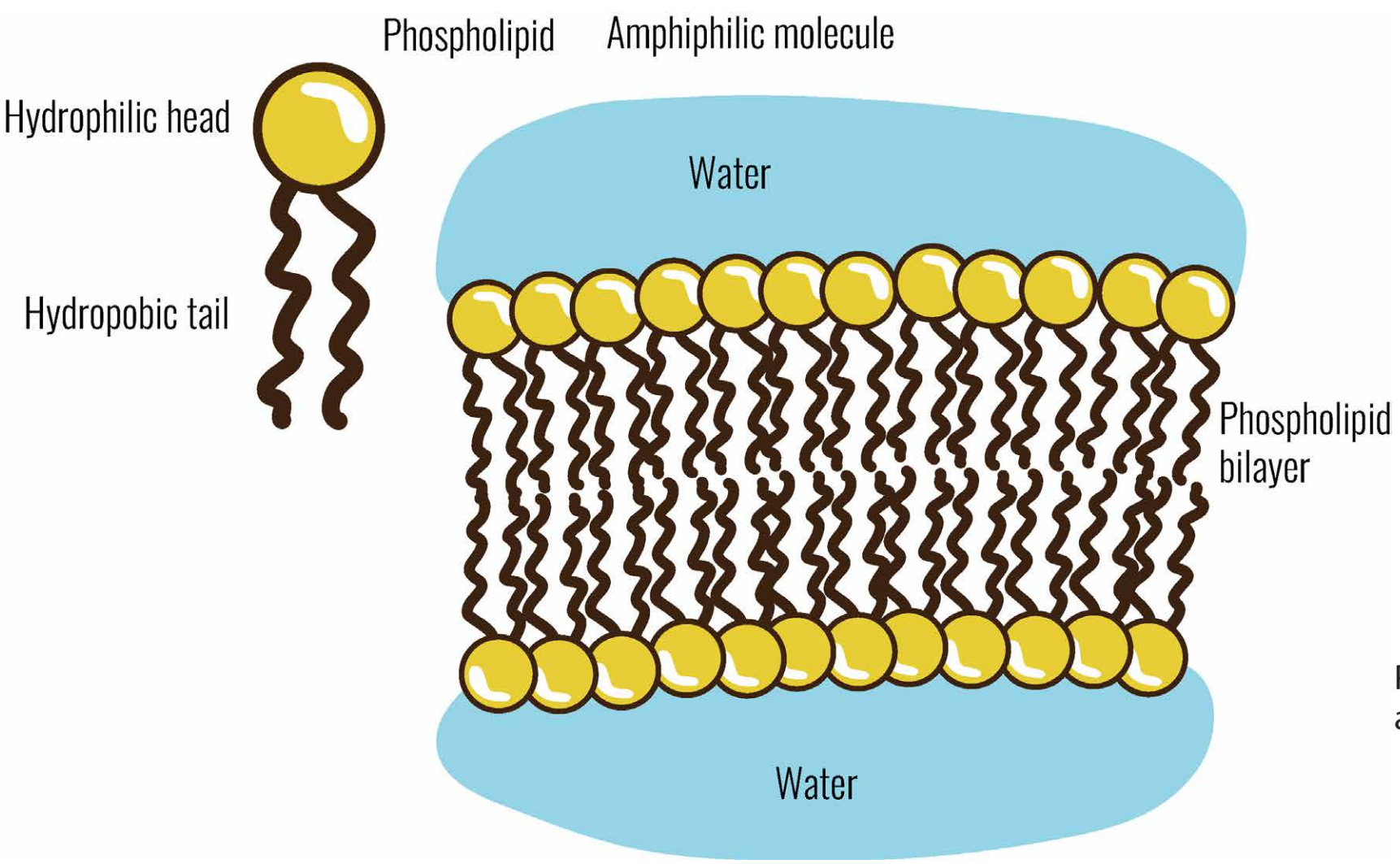
By LadyofHats Mariana Ruiz - Own work. Image renamed from File:Cell membrane detailed diagram.svg, Public Domain, https://commons.wikimedia.org/w/index.php?curid=6027169

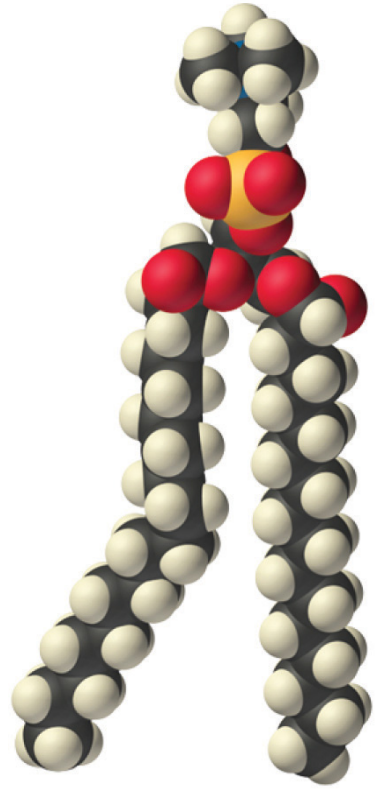
Cellular membrane divide intra and extracellular compartments

Ion concentrations in these compartments are different

This difference induce a voltage across the membrane

Phospholipids





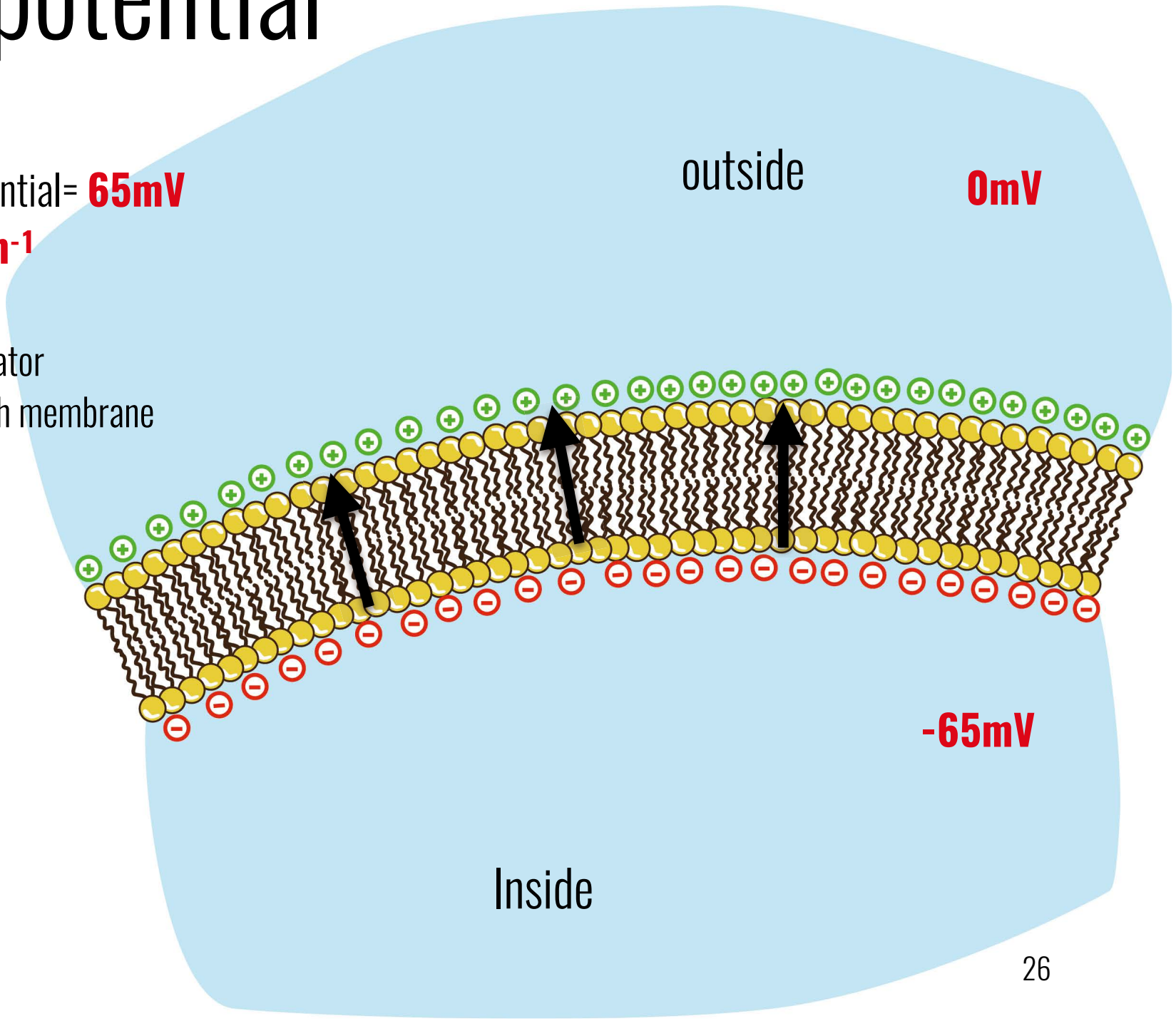
Phosphatidylcholine:
a phospholipid

Resting potential

Membrane resting potential= **65mV**
Electric field : **10MV.m^{-1}**

The membrane is an insulator
Ions can pass through with membrane proteins (with leaks)

Capacitance
of membrane : 1 µF/cm^2



Ion charges

The electrochemical potential can be computed with **Nernst equation**

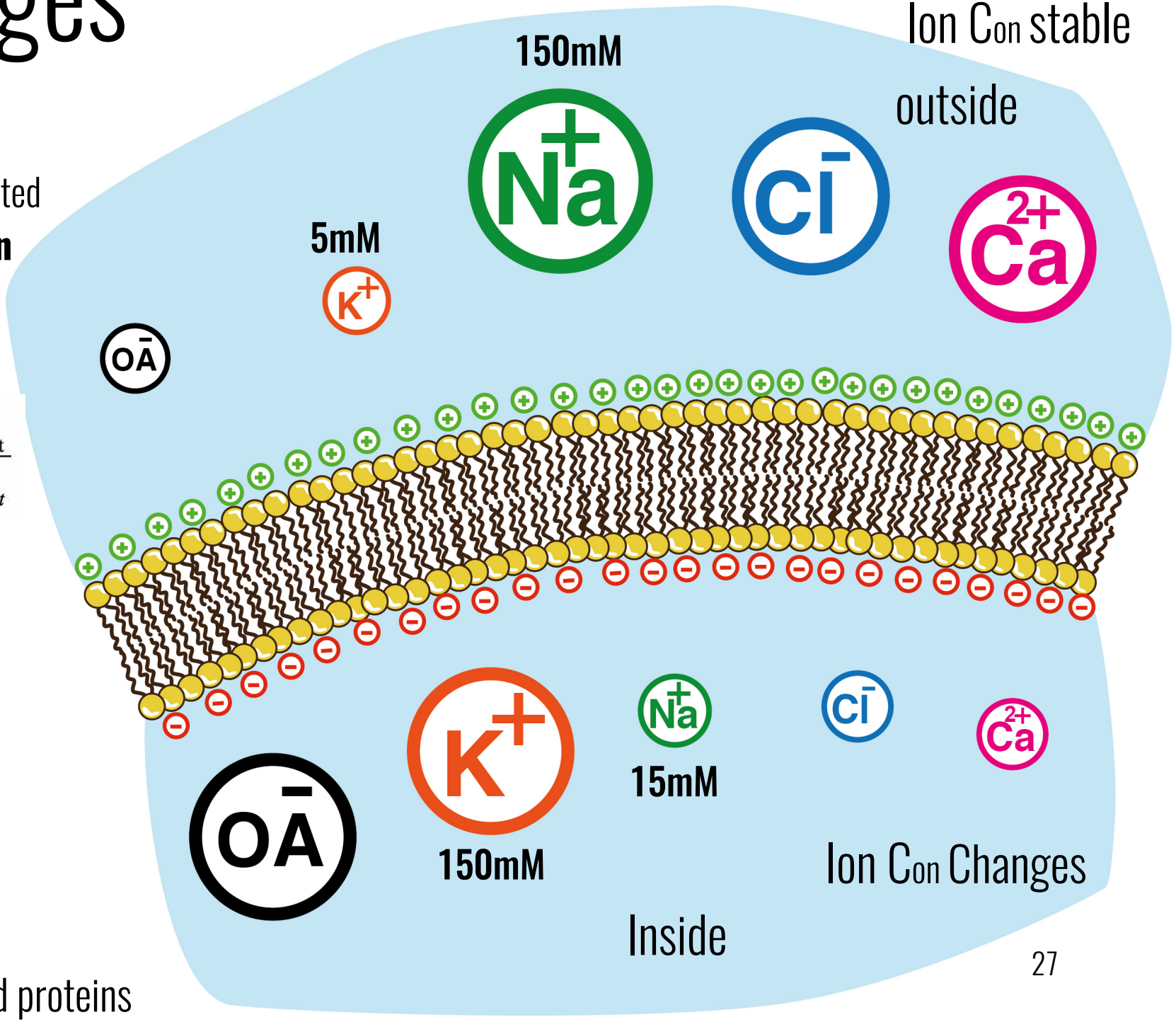
$$E_K = -\frac{RT}{ZF}\log\frac{[K]_{int}}{[K]_{ext}}$$

R : Perfect gazes constant
T : Absolute temperature
Zx: valence of ion ;
F : Faraday number

E_{Na}= + 64 mV

E_K= -90 mV

Organic Ions : charged proteins



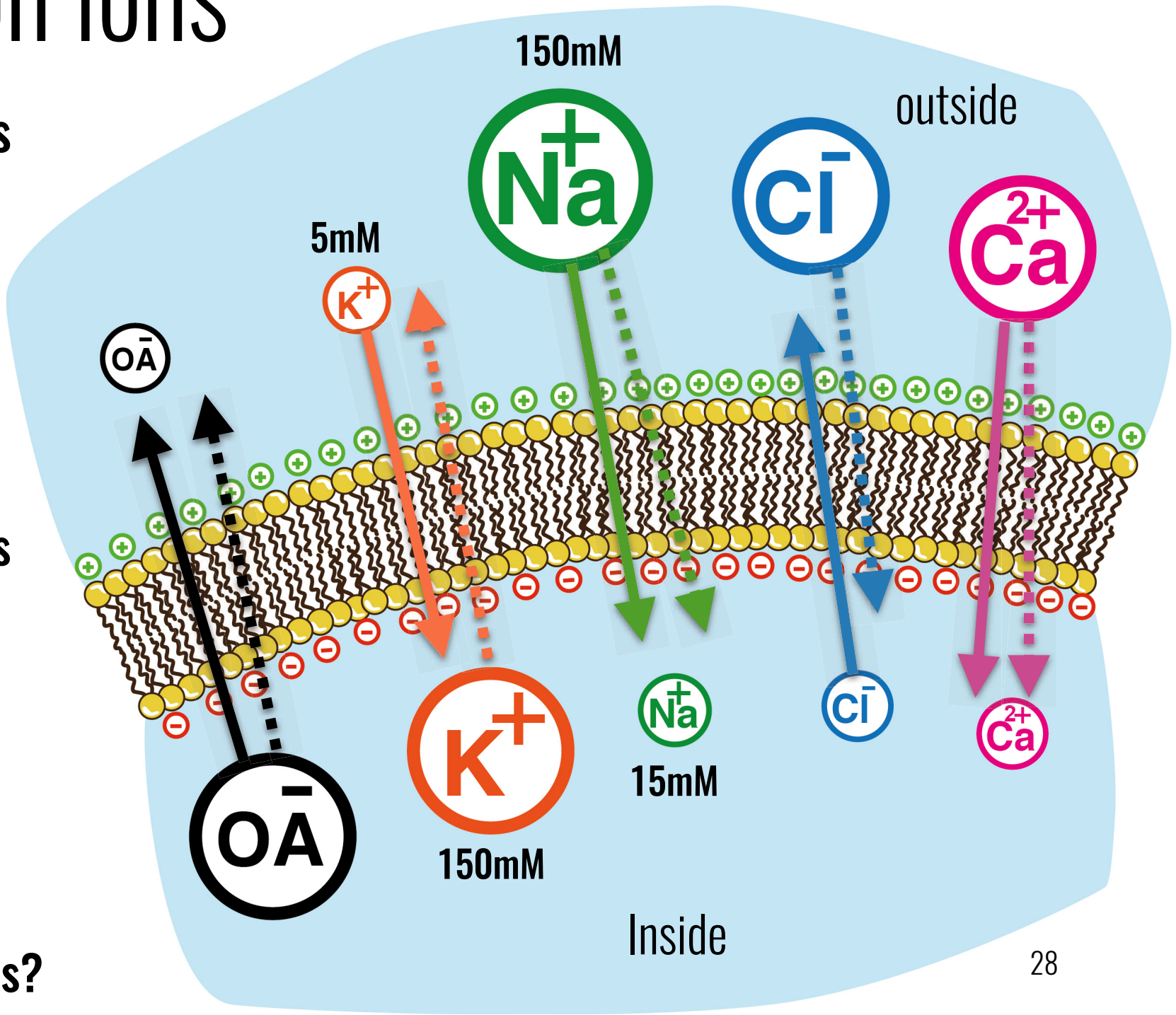
Forces on ions

Electrical forces

From - to +

Diffusion forces

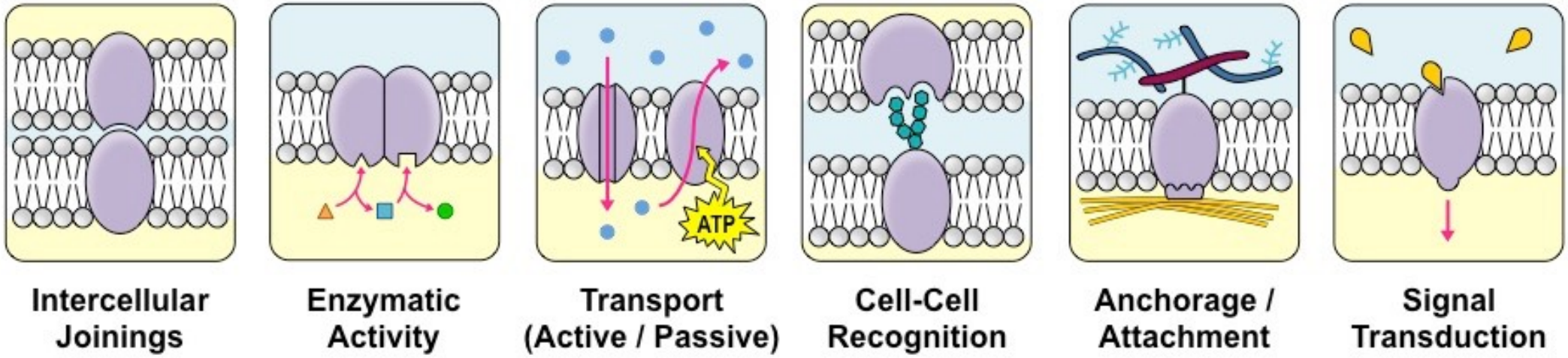
From high to low C



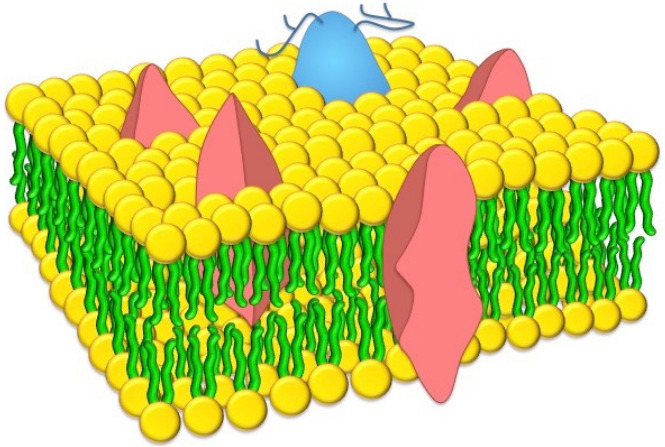
How ions passes?

Membrane Proteins

Molecules encaged in the membrane, several functions



These ones are interesting for Action Potential



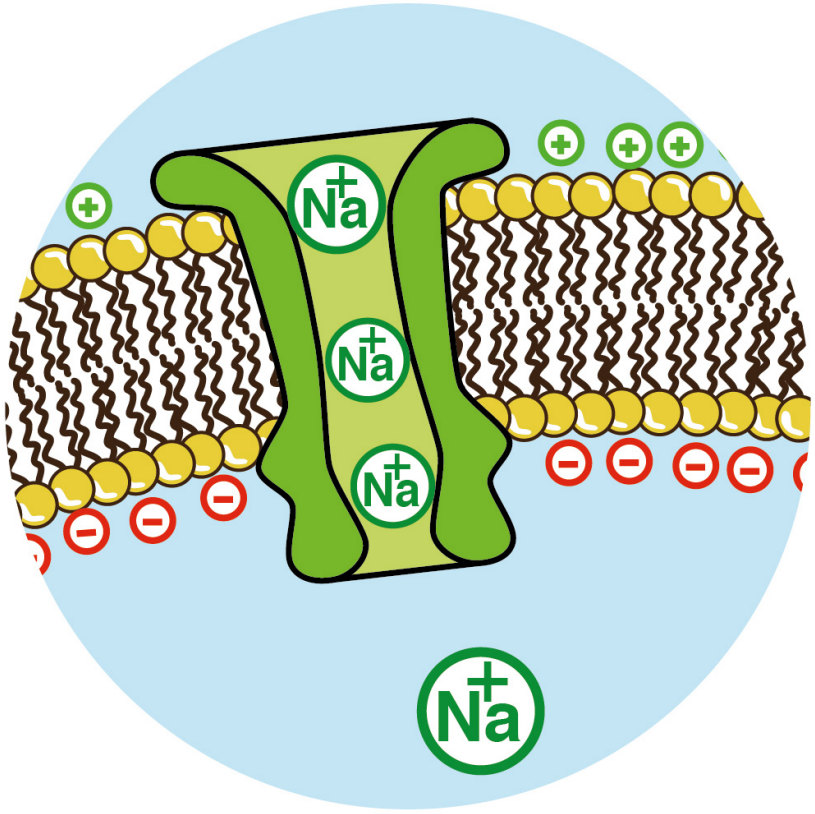
Ion Channels

Ion Channels are membrane proteins gating the flow of ions across the cell membrane

Selective valves permeable to unique ion species

The rate of ion transport : **10^6** ions/s

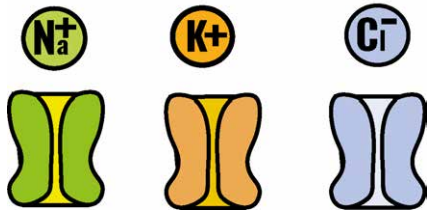
There are over **300** types of ion channels in a living cell



Passive Ion Channels
Found on dendrites, cell body, and axon.

Chemically-gated Ion Channels
Found on dendrites & cell body

Voltage-gated Ion Channels
Found on axon hillock, unmyelinated axons and at nodes of Ranvier on myelinated axons.



Ion Channels

Ion channels are **passive** valves (driven by electrochemical gradient)

≠ membrane pumps

No use of metabolic energy

-Voltage dependants

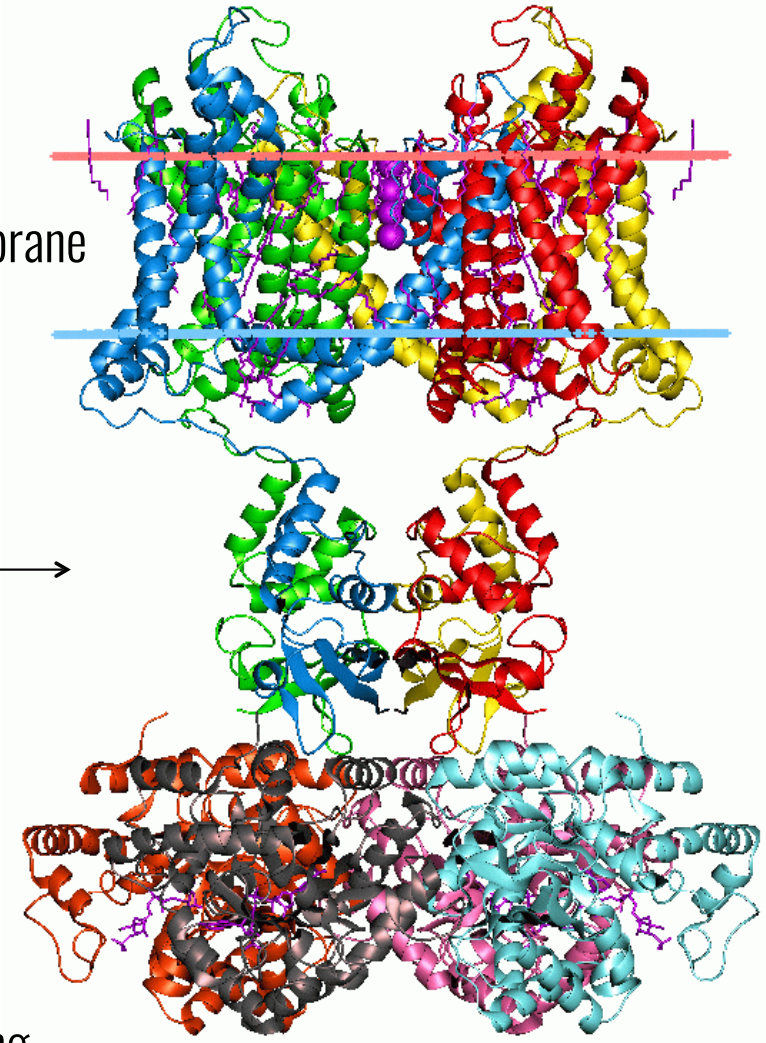
Na^+, Sodium

Ca^{2+}, Calcium

K^+, Potassium

Cl^-, Chloride

H^+, protons



The opening and closing of the channels are triggered by changing ion concentration

Ion Channels

Other type of Ion channels

-Ligand-gated ion channel : opens with the binding of neurotransmitters : GABA, Glutamate, serotonin, ATP, nicotin...

-Inwardly rectifying potassium channels (Cl, K, Na, Ca, H)

-Calcium-activated potassium channel

-Light-gated ion channel : **Channelrhodopsin** → Optogenetics !!

-Mechanosensitive channels (Piezo, TREK)

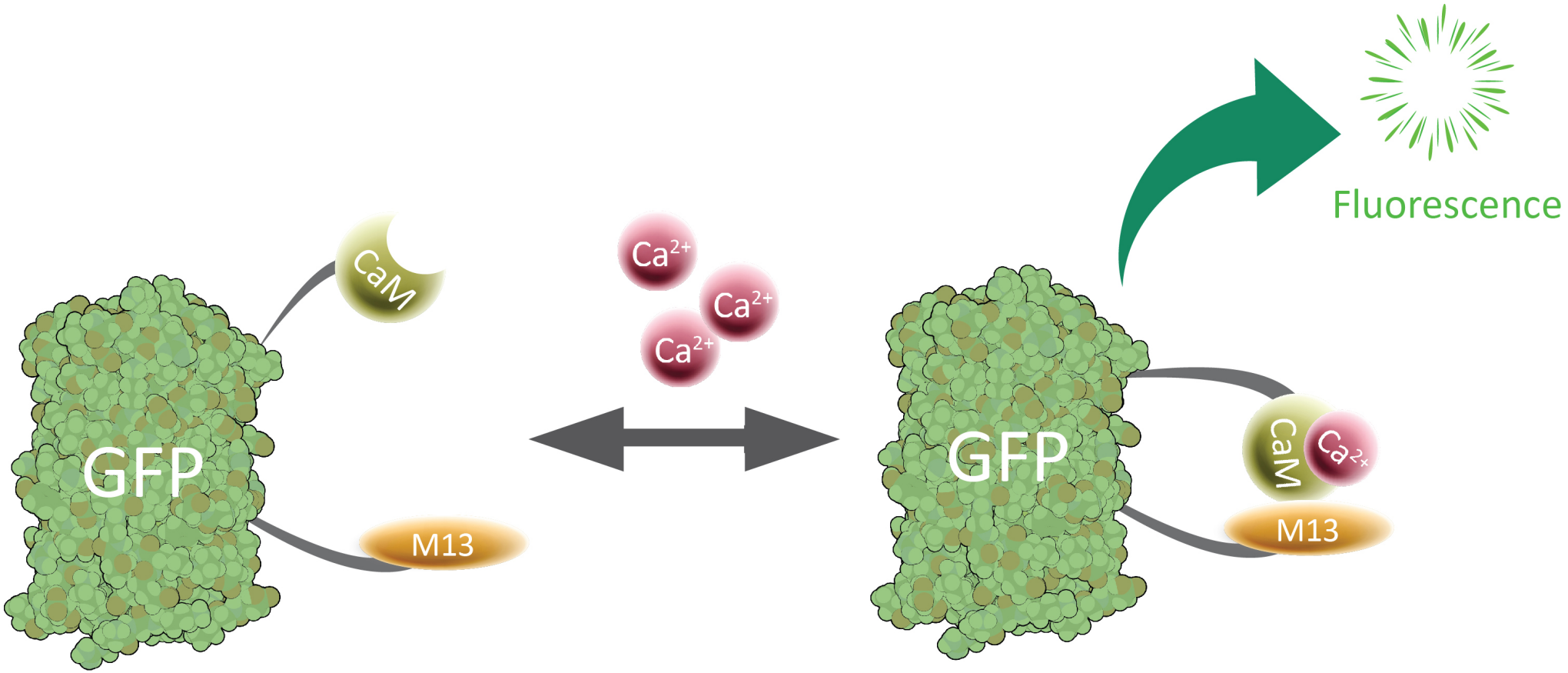
-Temperature-gated channels (TRPV) → Thermal stimulation

We can observe them at work !!

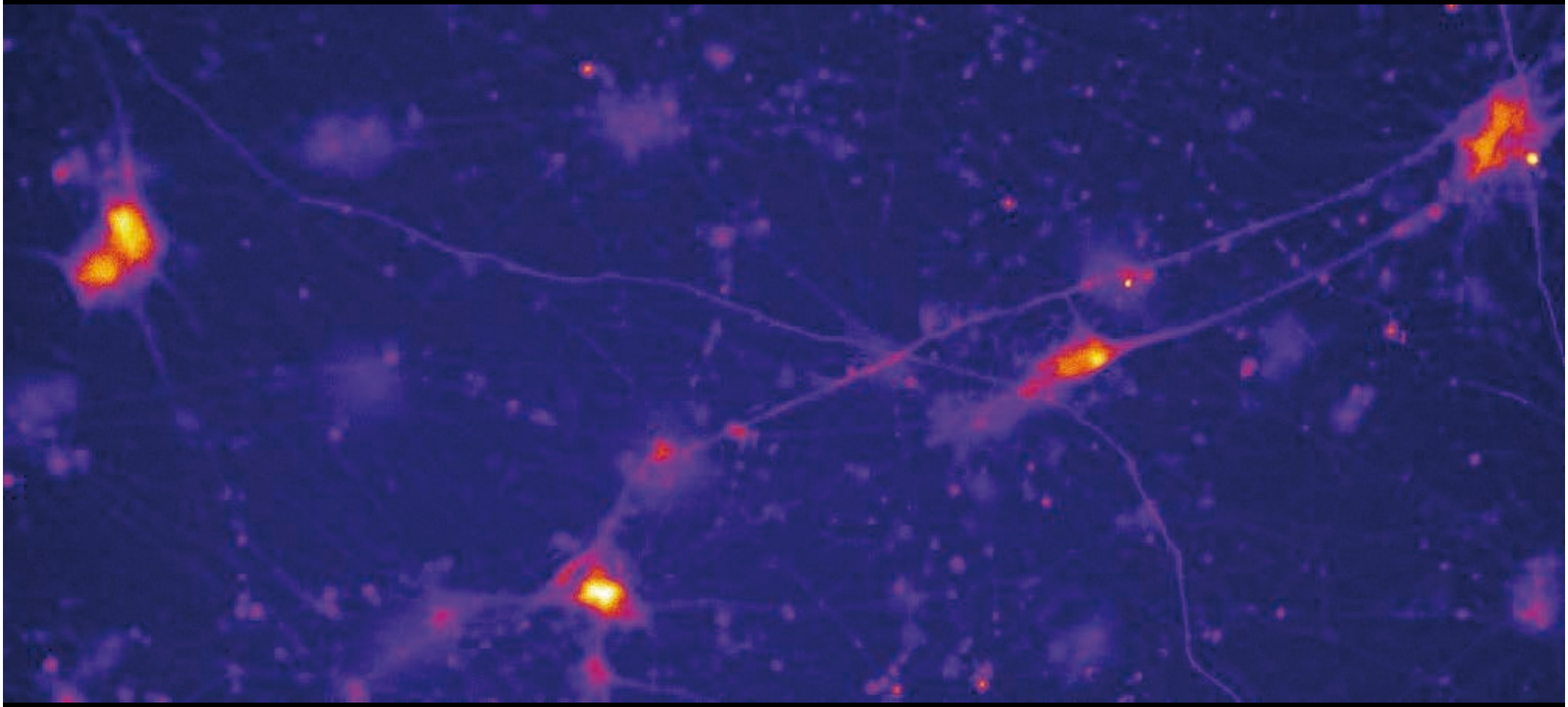
Calcium imaging

fluorescent molecules that can respond to the binding of Ca^{2+} ions by changing their fluorescence properties.

Chemical indicators (FURA, FLUO 3,…)
Genetically encoded calcium indicator (GCaMP)



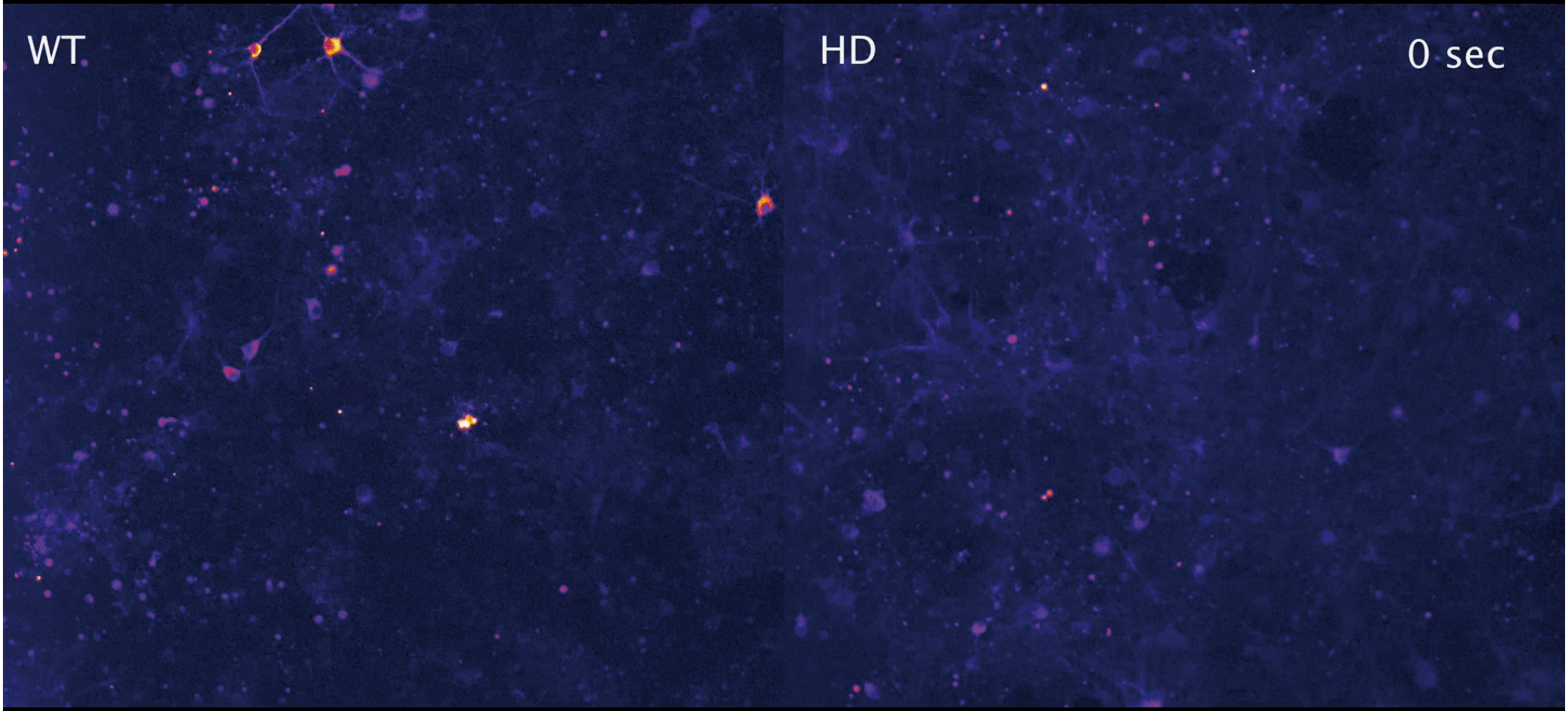
Calcium imaging



GCaMP 6F calcium indicator

P.Duc, F.Rage IGMM
B.Charlot IES

Calcium imaging

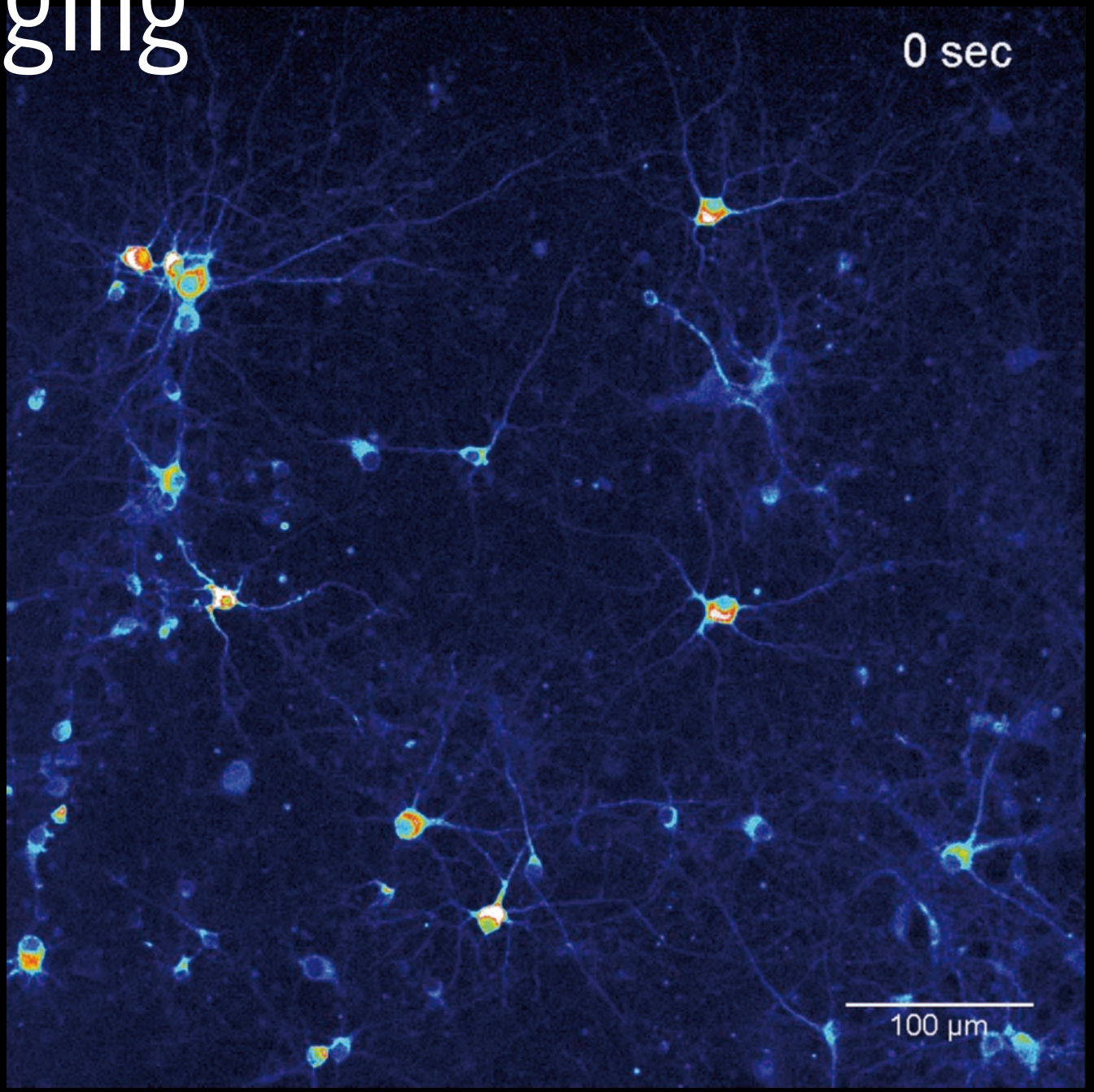


GCaMP 6F calcium indicator

F.Saudou GIN

Calcium imaging

Synchrony



GCaMP 6F calcium indicator

M.Cazorla, E.Moutaux GIN

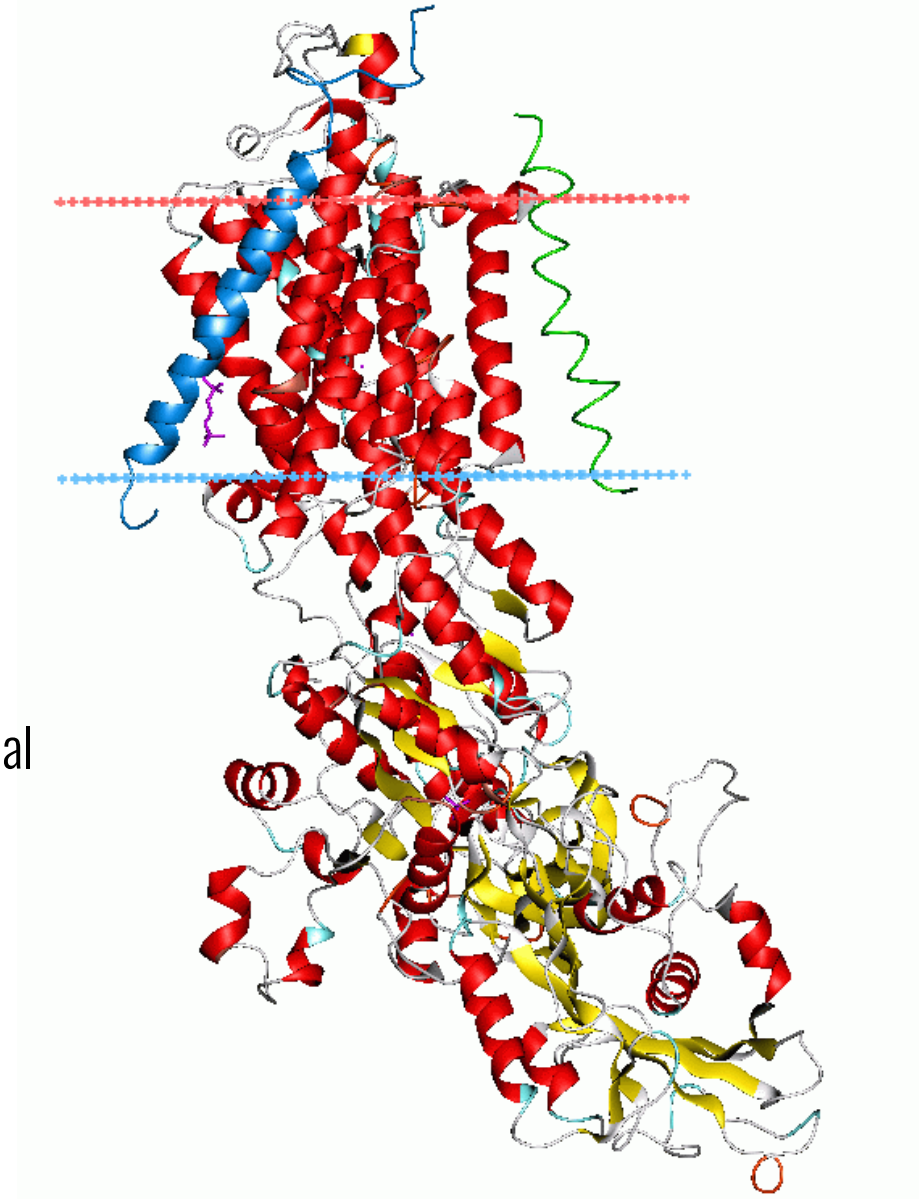
Ion pumps

Na+/K+ -ATPase

Ion pumps are enzymes that pumps sodium out of cells while pumping potassium into cells, both against their concentration gradients.

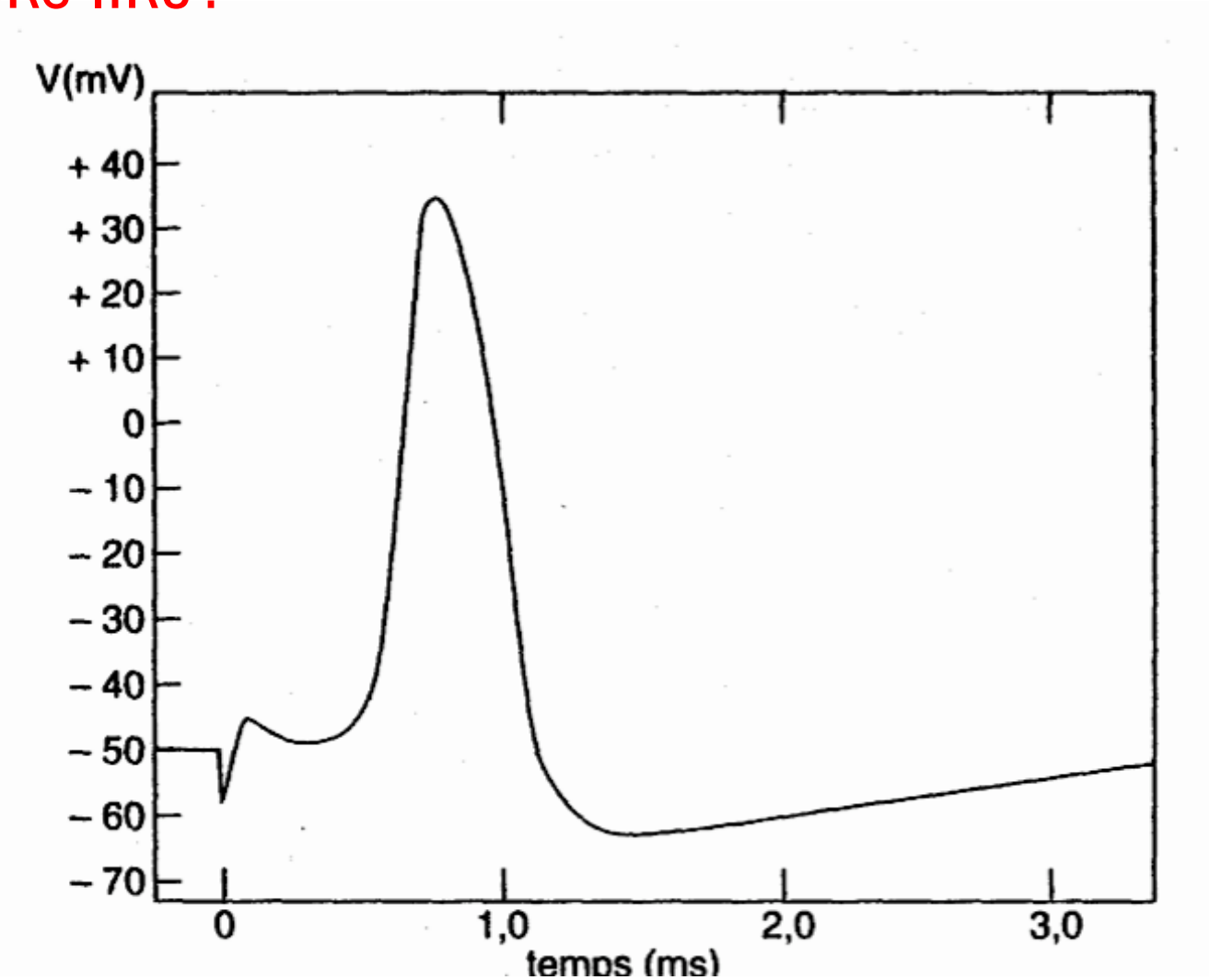
Active process (consumes ATP)

Responsible for the generation of the resting membrane potential



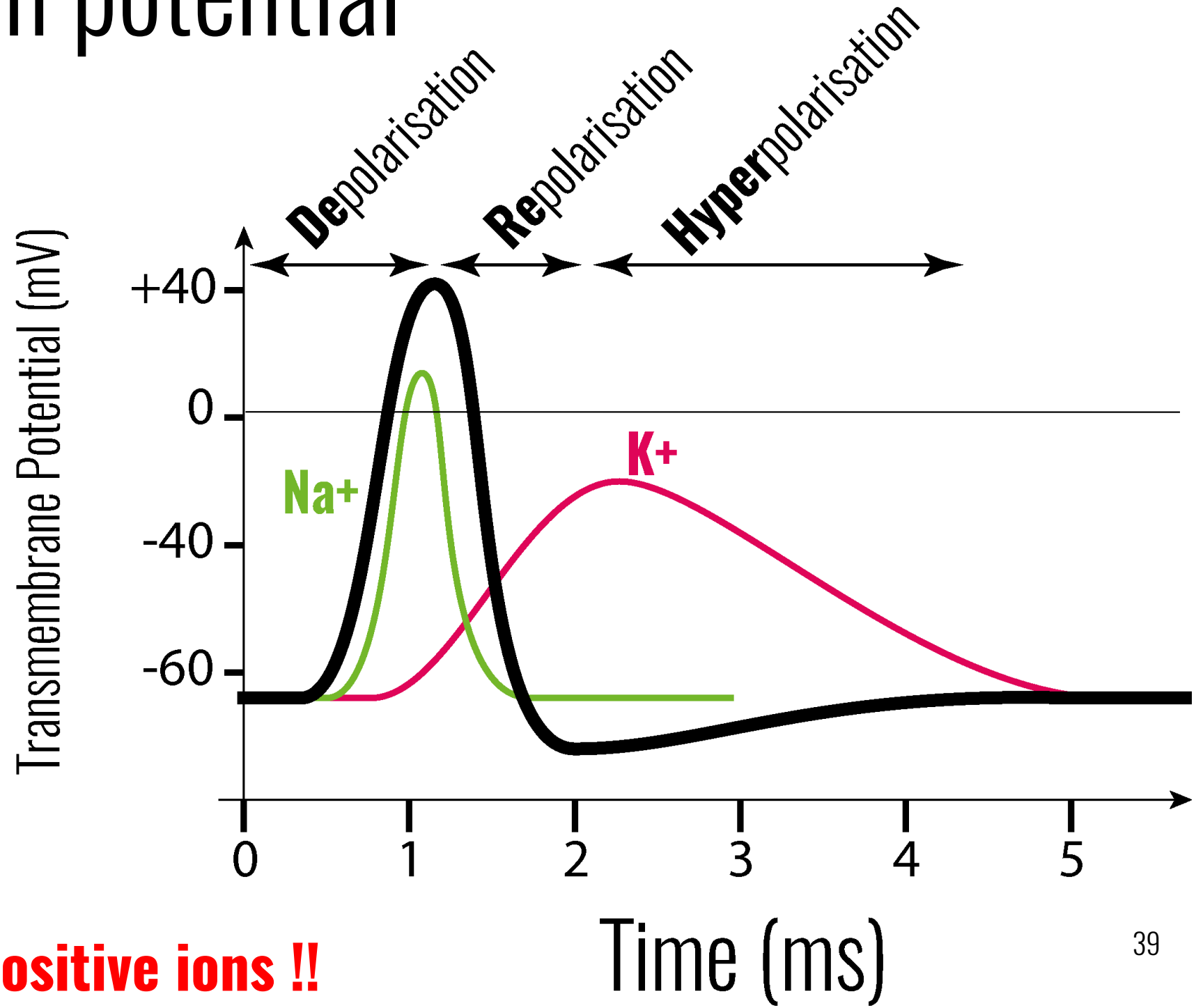
Action potential

What it looks like?



Intracellular recording of a giant squid axon under current Stimulation

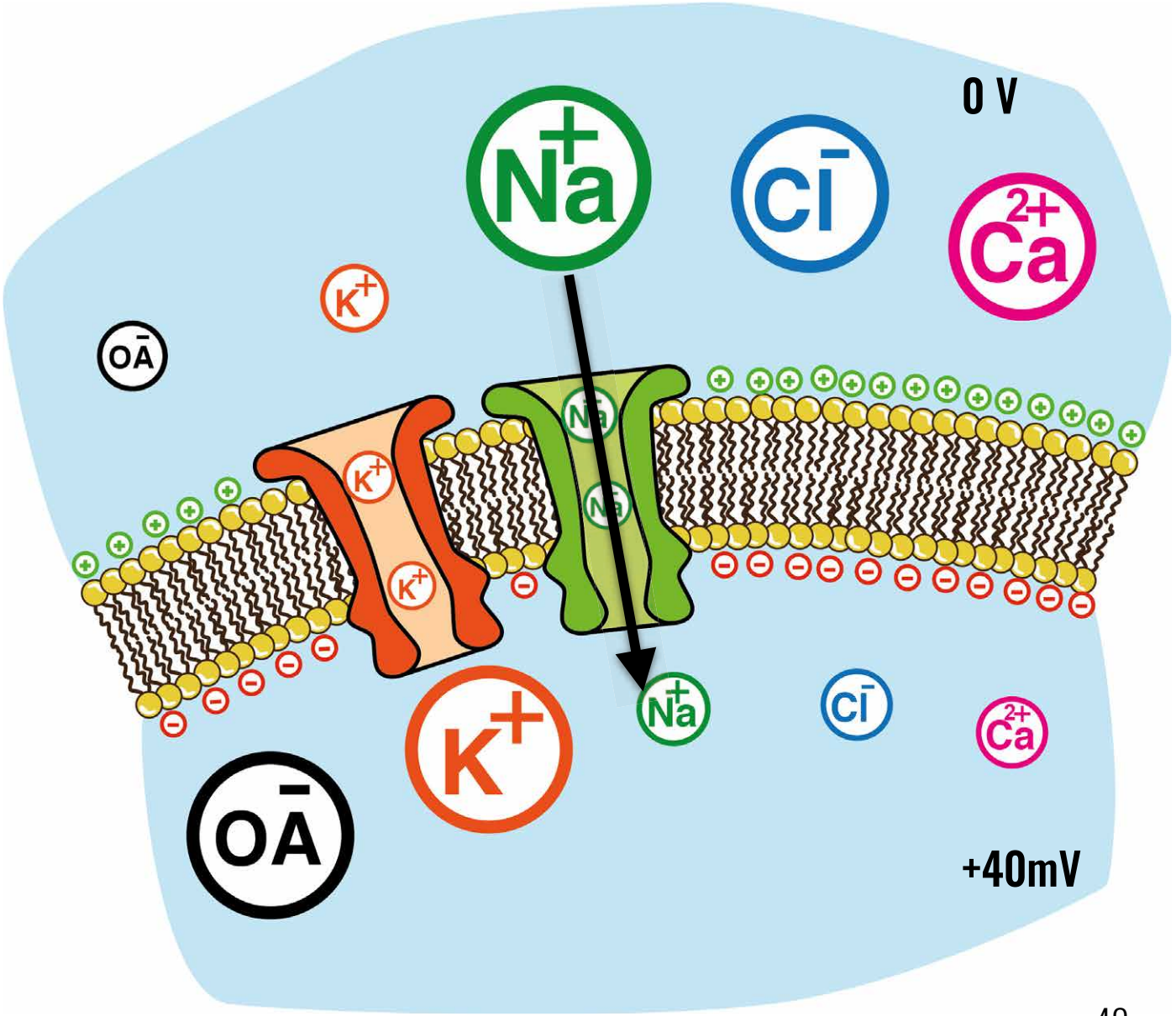
Action potential



Only positive ions !!

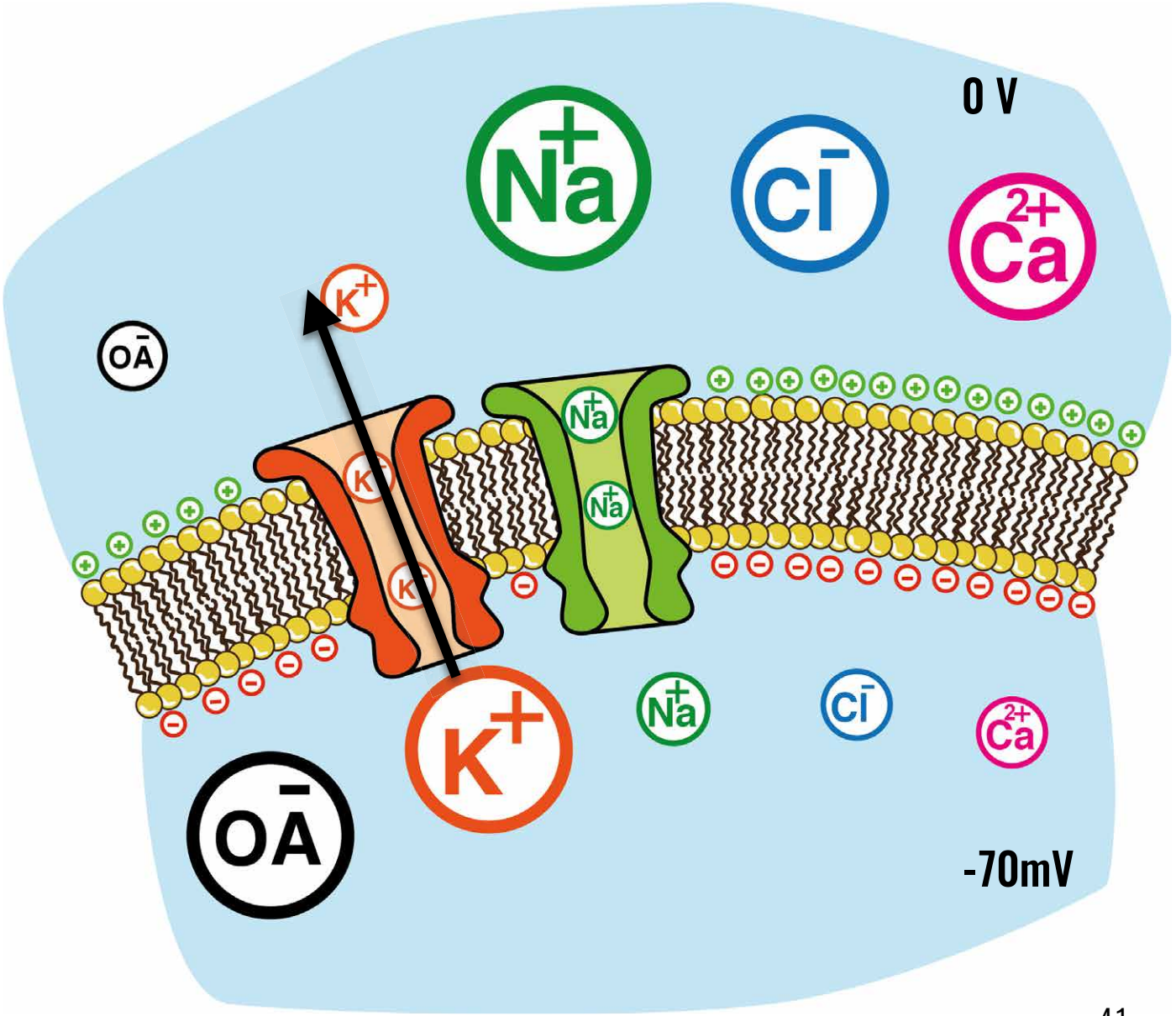
Action potential

Depolarisation



Action potential

Repolarisation

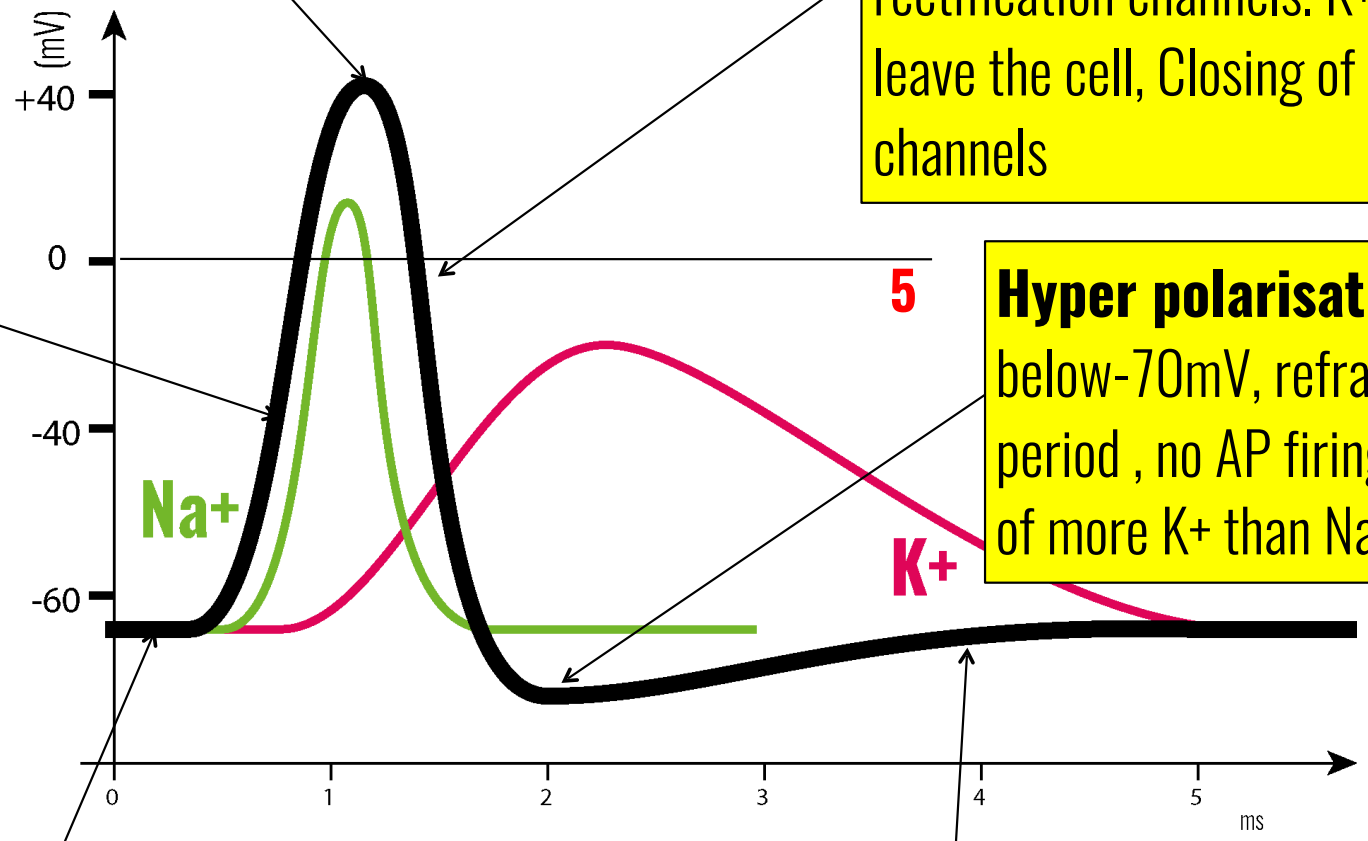


Action potential

3 **Maximum** : All Na+ channels are open, maximum concentration, minimum gradient

4 **Repolarisation** : return to -70mV opening of voltage gated rectification channels. K+ ions leave the cell, Closing of Sodium channels

2 **Depolarisation** : rise up to +30mV opening of voltage gated sodium channels, Na+ ions enter the cell



5 **Hyper polarisation** : below-70mV, refractive period , no AP firing PA, exit of more K+ than Na+

1 **Rest** : transmemb. potential = -65mV

6 **Restauration** of initial concentrations with Ionic pumps ATP dependant : K+ enters, Na+ exits

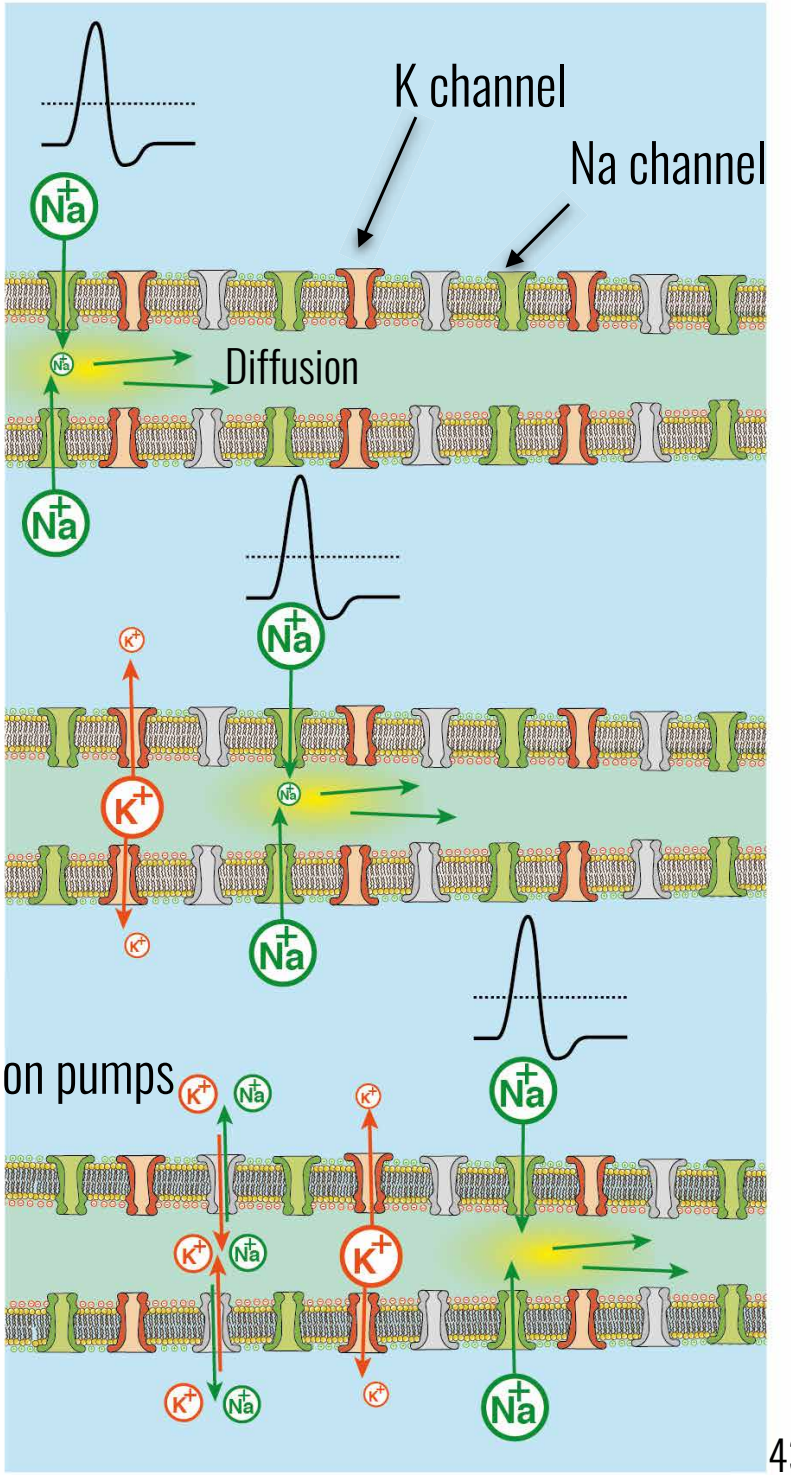
Action potential propagation

100 m/s for myelinated axons

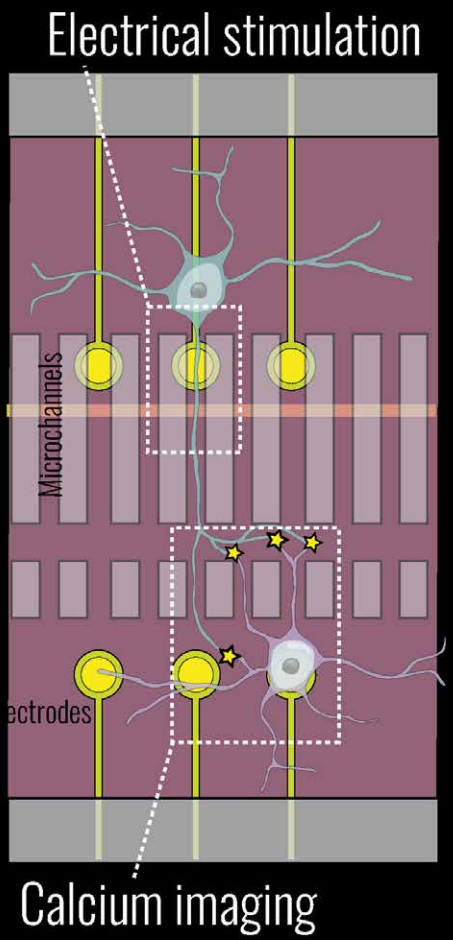
25 m/s for non myelinated axons

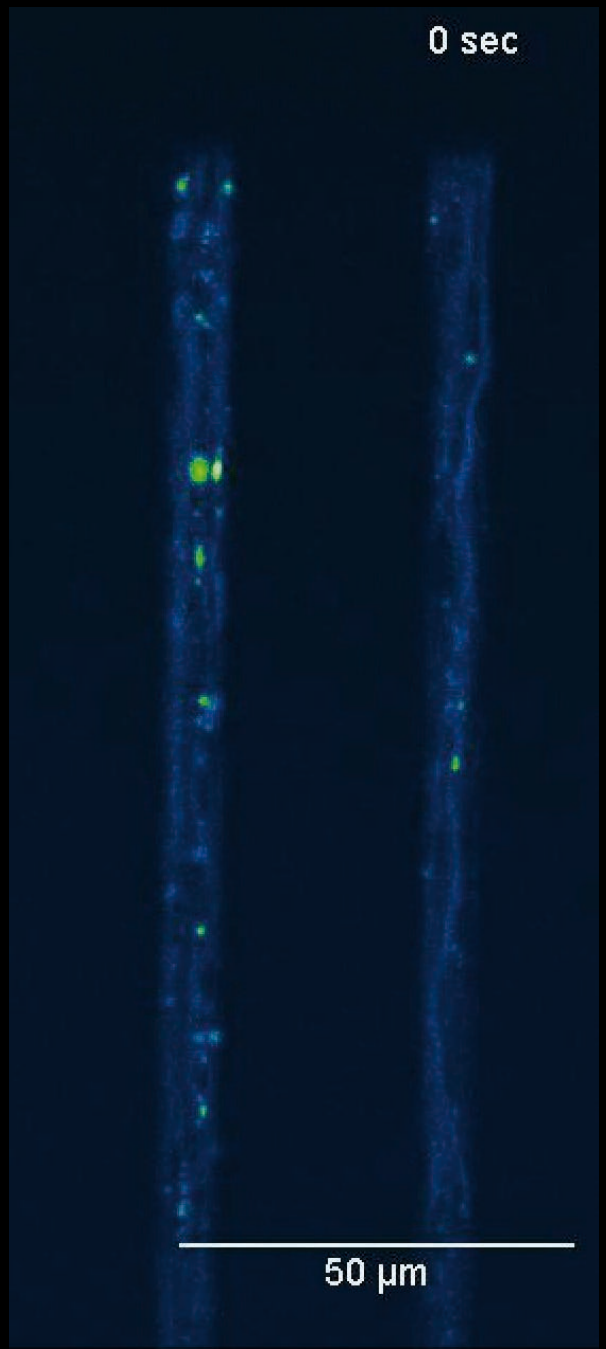
With myelin :
Saltatory conduction

Typical pulse length of about **10 cm.**



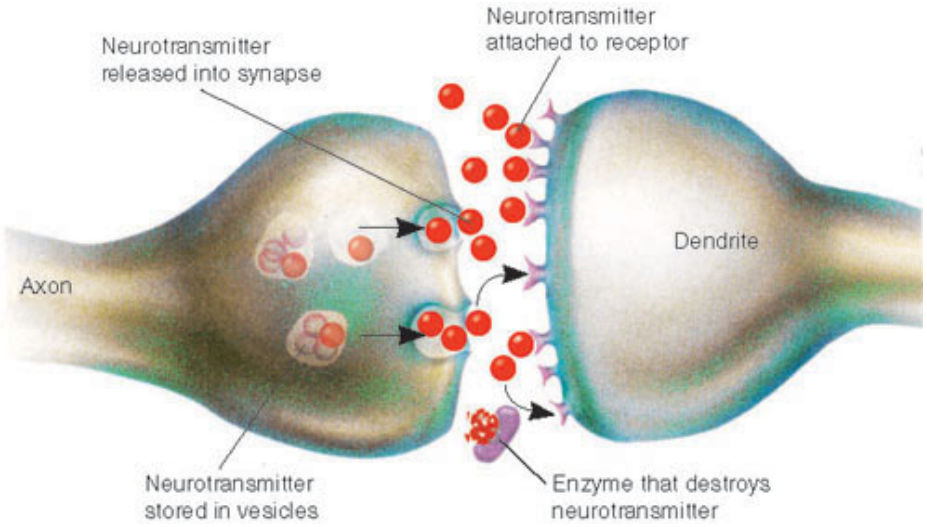
Action potential propagation





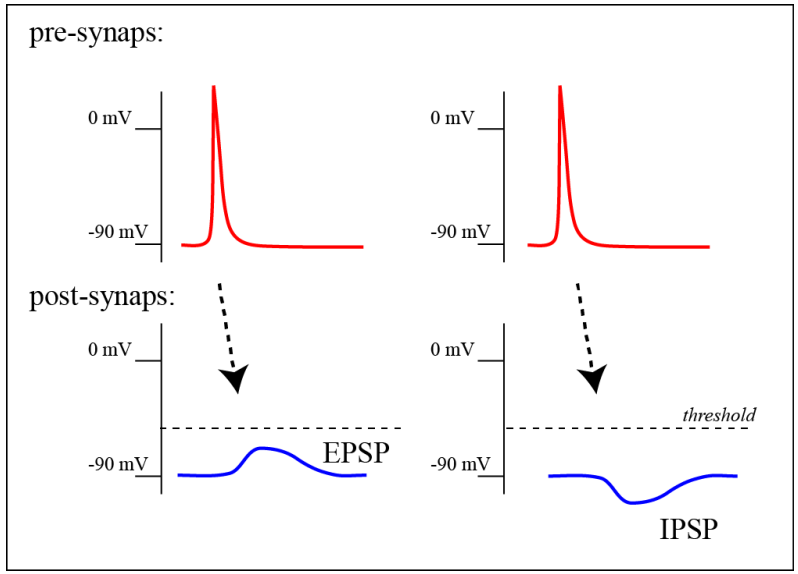
E.Moutaux, M.Cazorla, GIN

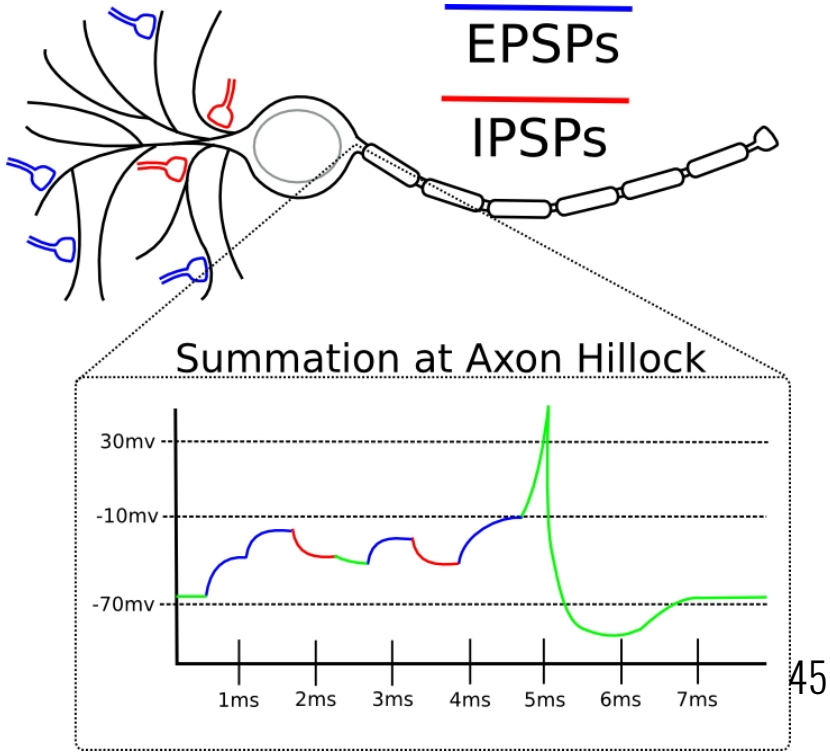
After the synapse



EPSP Excitatory Post Synaptic Potential

IPSP Inhibitory Post Synaptic Potential





Summary

Action potential is movements of ions trough channels

Mainly positive ions

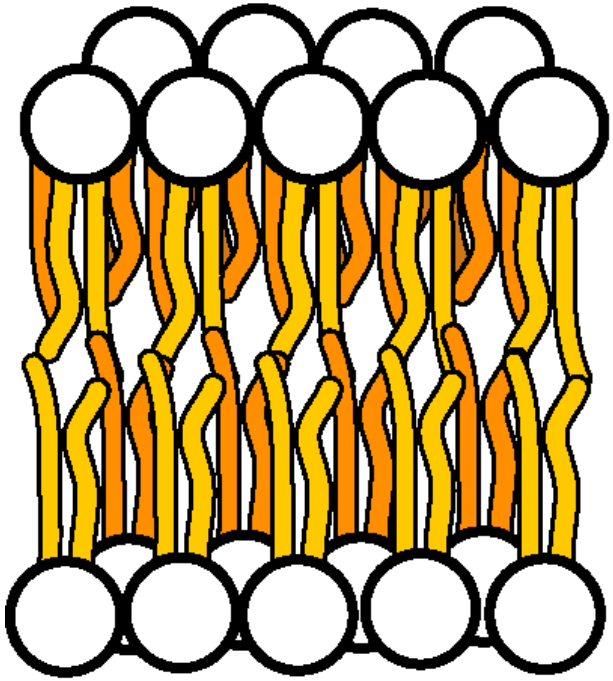
Ions are going down concentration gradients

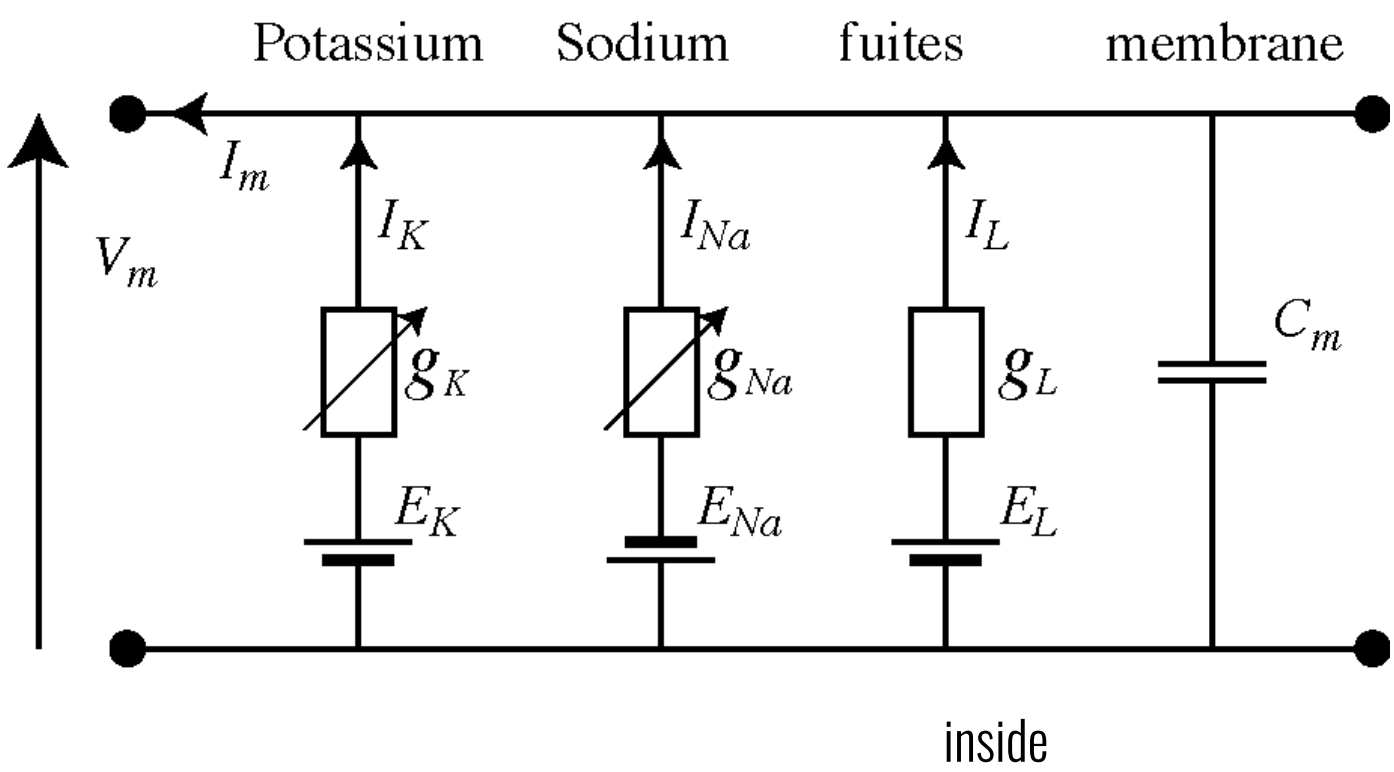
Only ion pumps consume ATP

Summation of excitation and inhibition after the synapse

Part.II Iono-electronic interface

Hodgkin-Huxley Model





$$I_m = I_K + I_{Na} + I_L + C_m \frac{dV_m}{dt}$$

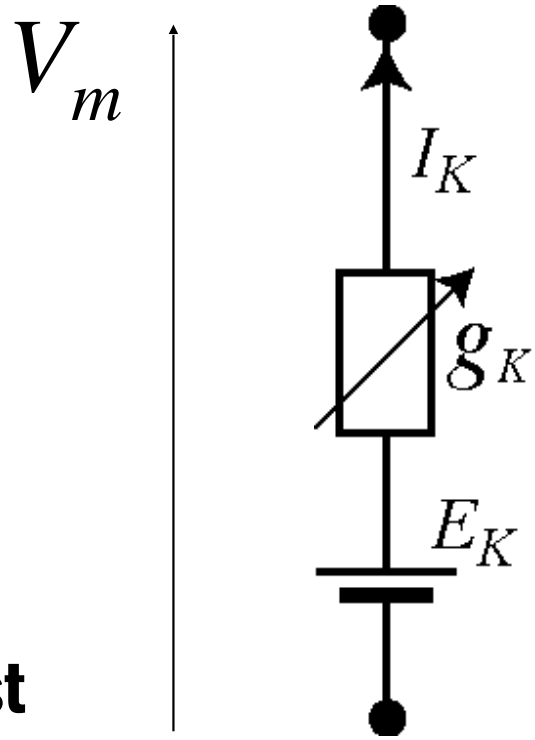
Hodgkin-Huxley Model

Ionic Currents

$$V = RI \quad \text{Loi d'Ohm}$$

$$I = gV \quad \text{conductance}$$

$$I_K = g_K(V_m - E_K)$$



Battery / resistance model

Electrochemical potential can be computed with **Nernst** equation

$$E_K = -\frac{RT}{ZF}\log\frac{[K]_{\text{int}}}{[K]_{ext}}$$

$$g_K = \bar{g}_K n^4$$

$$g_{Na} = \bar{g}_{Na} m^3 h$$

E_{Na}= + 64 mV E_K= -90 mV

N, m, h : activation gates

Hodgkin-Huxley Model

$$g_K = \bar{g}_K n^4$$

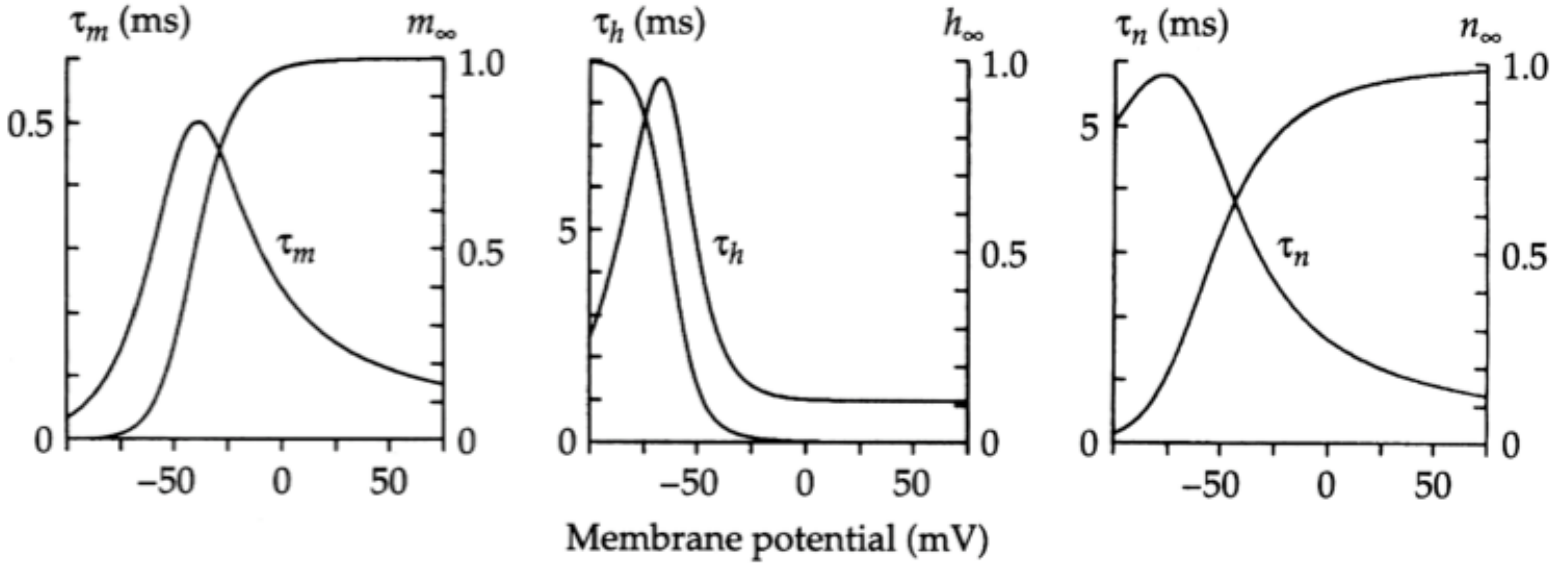
$$g_{Na} = \bar{g}_{Na} m^3 h$$

$$n, m, h = f(V_M, t)$$

$$\frac{dm}{dt} = \frac{m_\infty(V_m) - m}{\tau_m(V_m)}$$

$$\frac{dh}{dt} = \frac{h_\infty(V_m) - h}{\tau_h(V_m)}$$

$$\frac{dn}{dt} = \frac{n_\infty(V_m) - n}{\tau_n(V_m)}$$



Hodgkin-Huxley Model

$m_\infty = A_m/(A_m+B_m)$ and $\tau_m = 1/(A_m+B_m)$

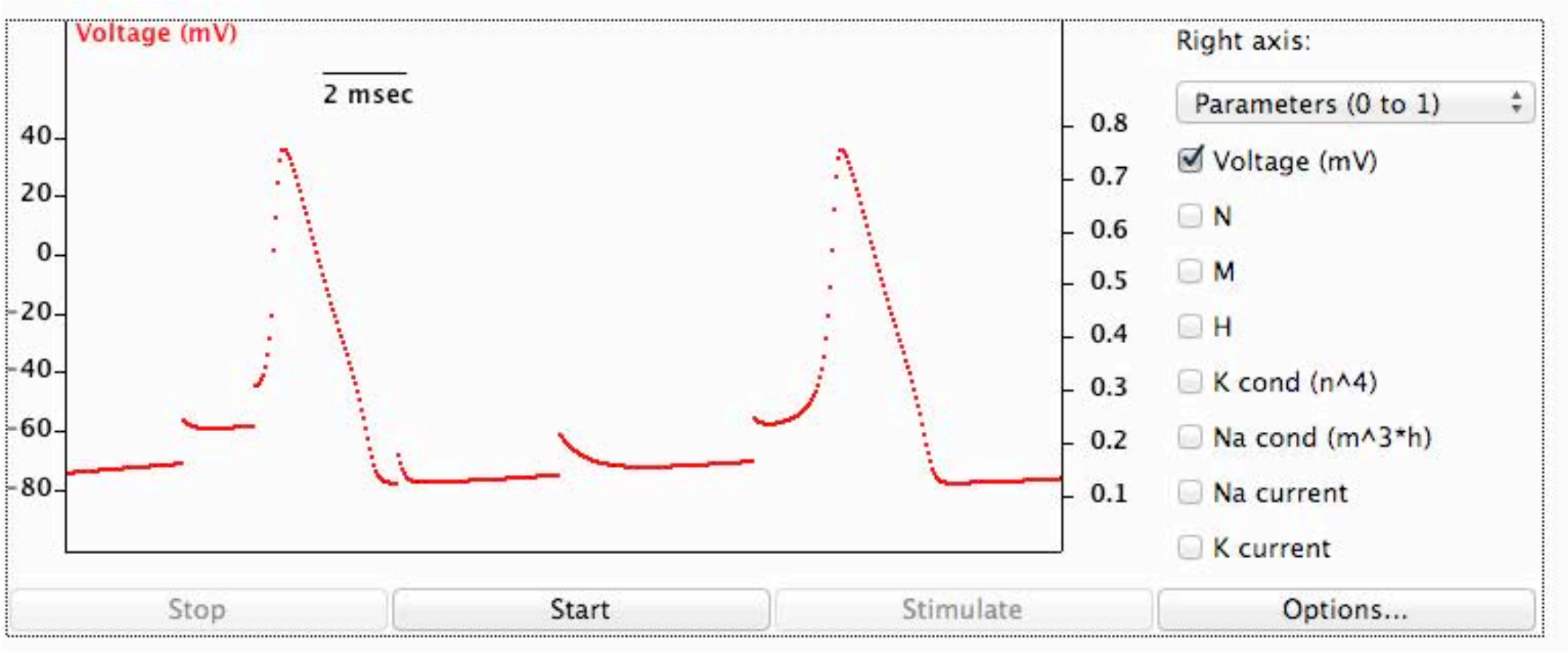
$$\frac{dm}{dt} = A_m(V)[1-m] - B_m(V)m \qquad A_m(V) = \frac{\alpha_m(V-V_{\alpha m})}{1-e^{-(V-V_{\alpha m})/K_{\alpha m}}} \qquad B_m(V) = \beta_m e^{-(V-V_{\beta m})/K_{\beta m}}$$

$$\frac{dh}{dt} = A_h(V)[1-h] - B_h(V)h \qquad A_h(V) == \alpha_h e^{-(V-V_{\alpha h})/K_{\alpha h}} \qquad B_h(V) = \frac{\beta_h}{1-e^{-(V-V_{\beta h})/K_{\beta h}}}$$

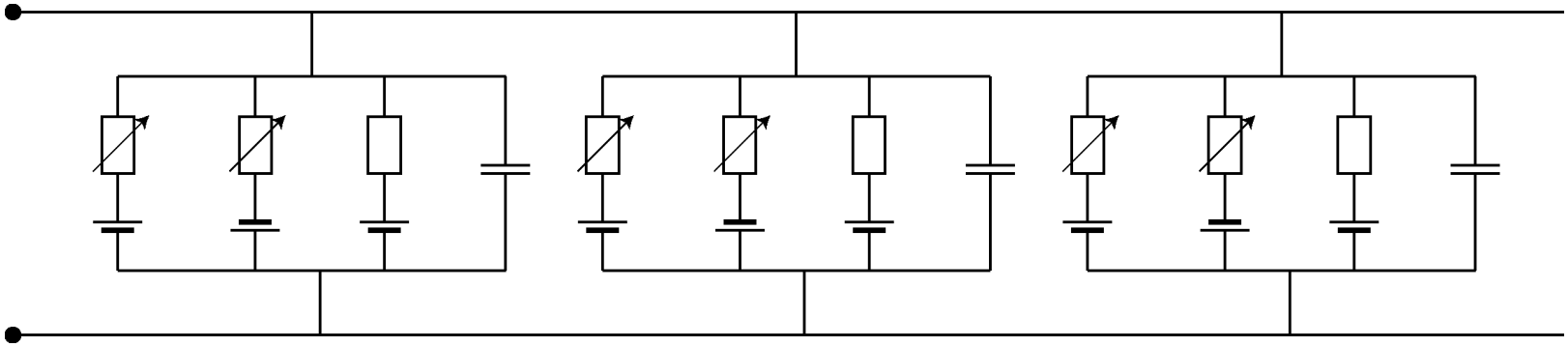
$$\frac{dn}{dt} = A_n(V)[1-n] - B_n(V)n \qquad A_n(V) = \frac{\alpha_n(V-V_{\alpha n})}{1-e^{-(V-V_{\alpha n})/K_{\alpha n}}} \qquad B_n(V) = \beta_n e^{-(V-V_{\beta n})/K_{\beta n}}$$

$\bar{G}_L$	0.3	0.75	mS/cm^2	α_h	0.07	0.0081	ms^{-1}
$\bar{G}_K$	36	21.6	mS/cm^2	β_h	1	4.38	ms^{-1}
$\bar{G}_{Na}$	120	150	mS/cm^2	$V_{\alpha h}$	-60	-45	mV
C	1	4	µFd/cm^2	$V_{\beta h}$	-30	-45	mV
E_L	-87	*	mV	$K_{\alpha h}$	20	14.7	mV
E_K	-95.3	-72	mV	$K_{\beta h}$	10	9	mV
E_{Na}	36.7	55	mV				
α_m	0.1	0.288	ms^{-1}	α_n	0.01	0.0131	ms^{-1}
β_m	4	1.38	ms^{-1}	β_n	0.125	0.067	ms^{-1}
$V_{\alpha m}$	-36	-46	mV	$V_{\alpha n}$	-50	-40	mV
$V_{\beta m}$	-60	-46	mV	$V_{\beta n}$	-60	-40	mV
$K_{\alpha m}$	10	10	mV	$K_{\alpha n}$	10	7	mV
$K_{\beta m}$	18	18	mV	$K_{\beta n}$	80	40	mV

Hodgkin-Huxley Model



Hodgkin-Huxley Model

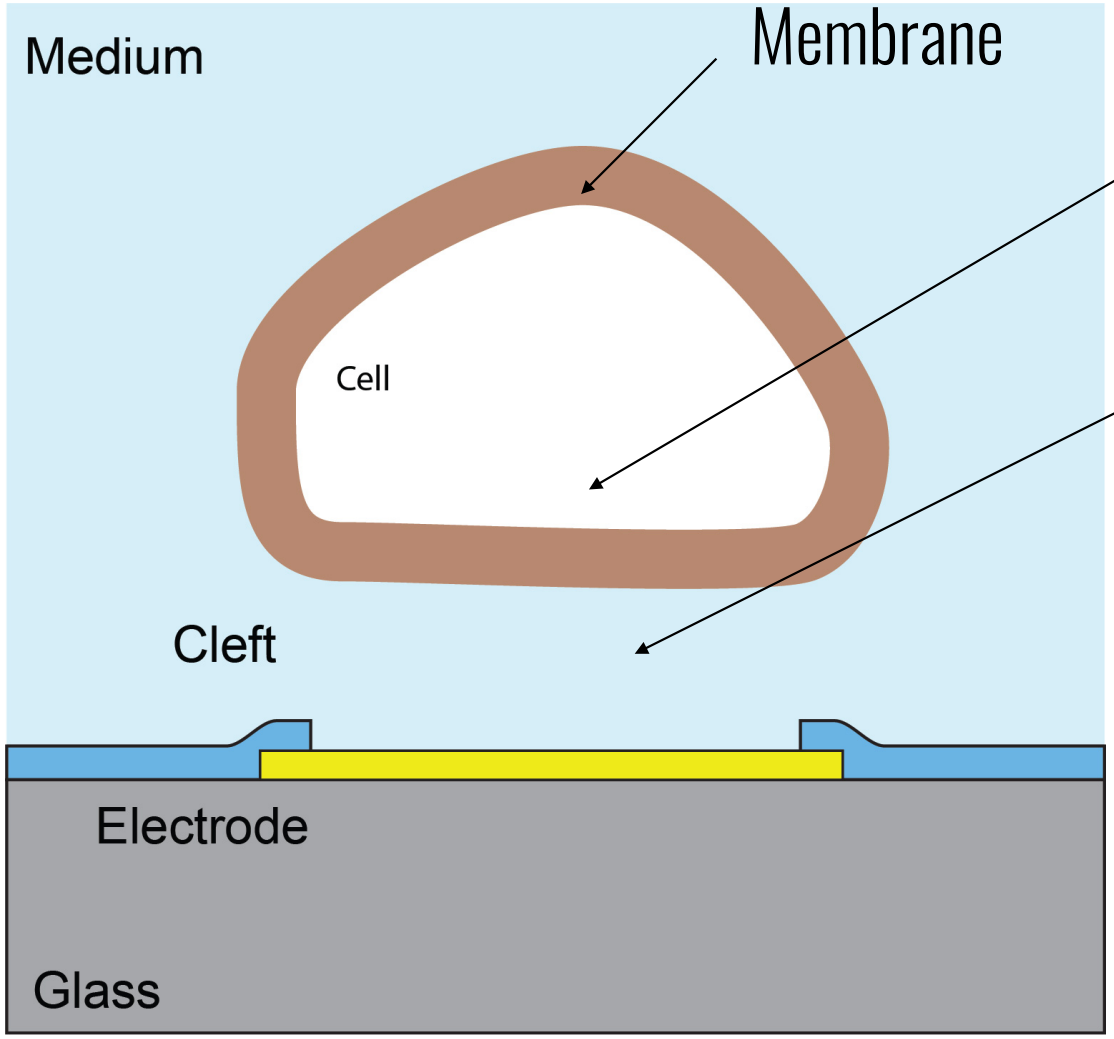


$$\frac{a}{2R_\mathrm{i}}\frac{\partial^2 U}{dt^2} = C_\mathrm{m}\frac{\partial U}{dt} + g_\mathrm{K}(U - E_\mathrm{K}) + g_\mathrm{Na}(U - E_\mathrm{Na})$$

Cable equation

Iono-Electronic interface

Let's put an electrically active cell on top of an electrode

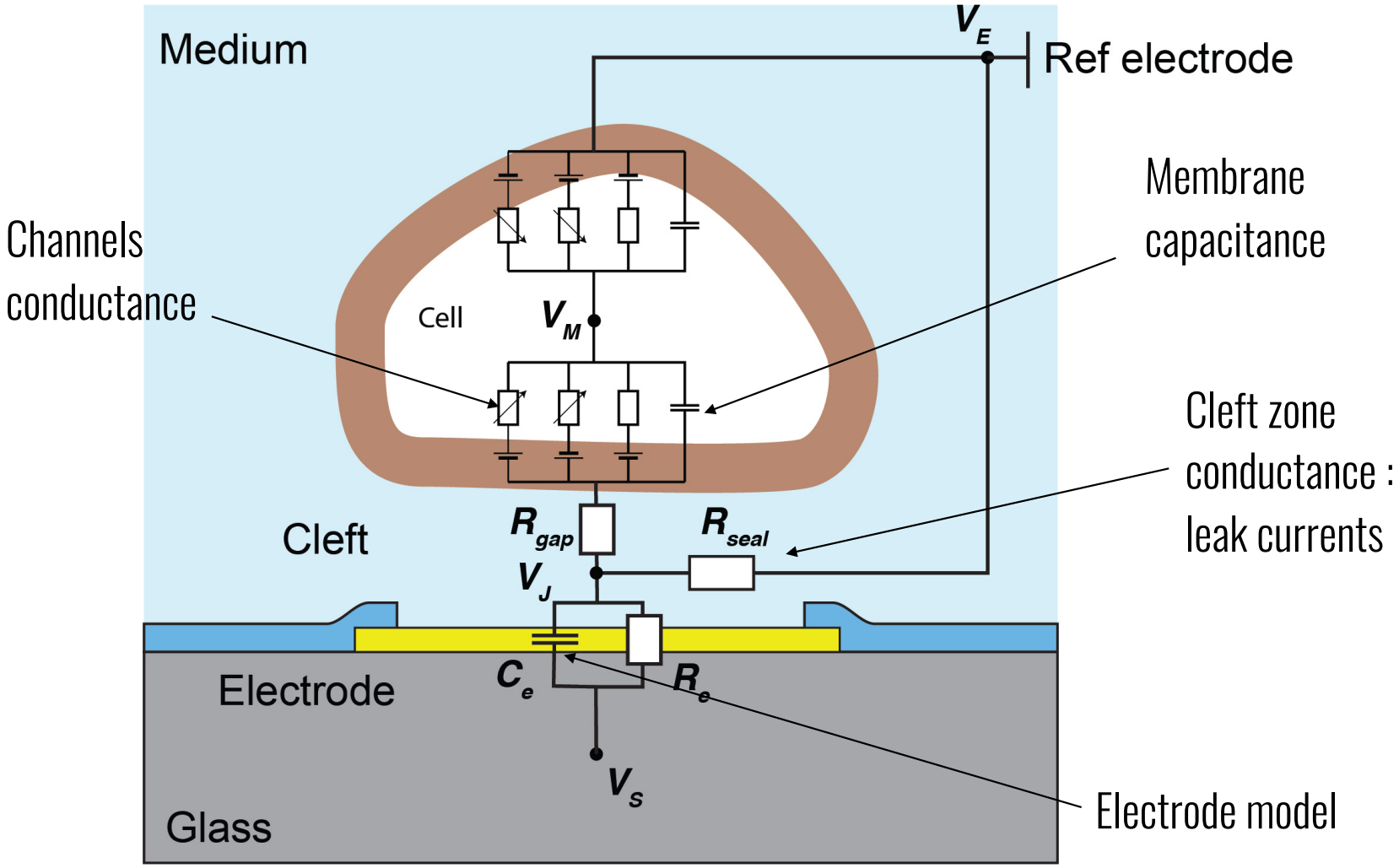


intracellular potential

Cleft : contact zone (proteins,..)

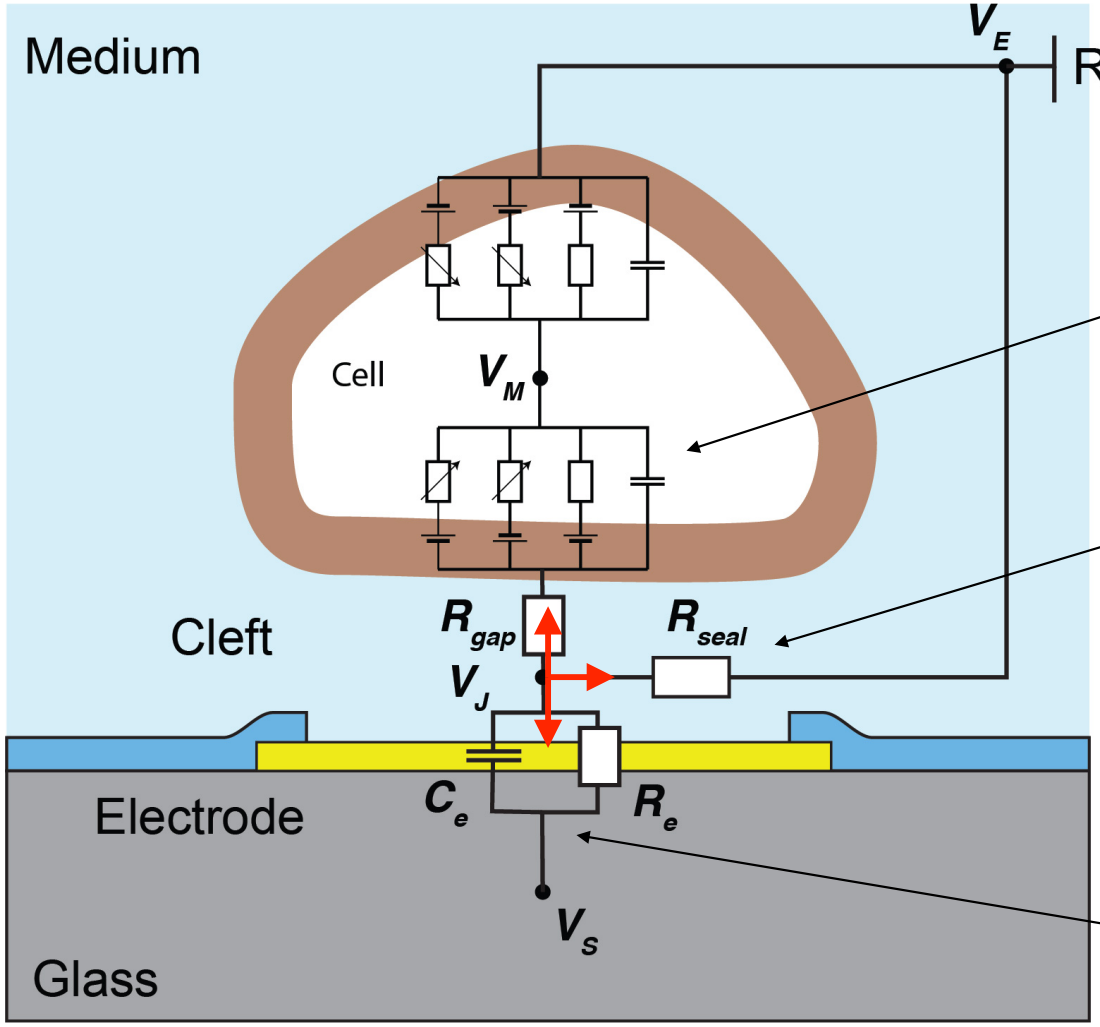
Iono-Electronic interface

Let's put an electrically active cell on top of an electrode



Iono-Electronic interface

Let's put an electrically active cell on top of an electrode



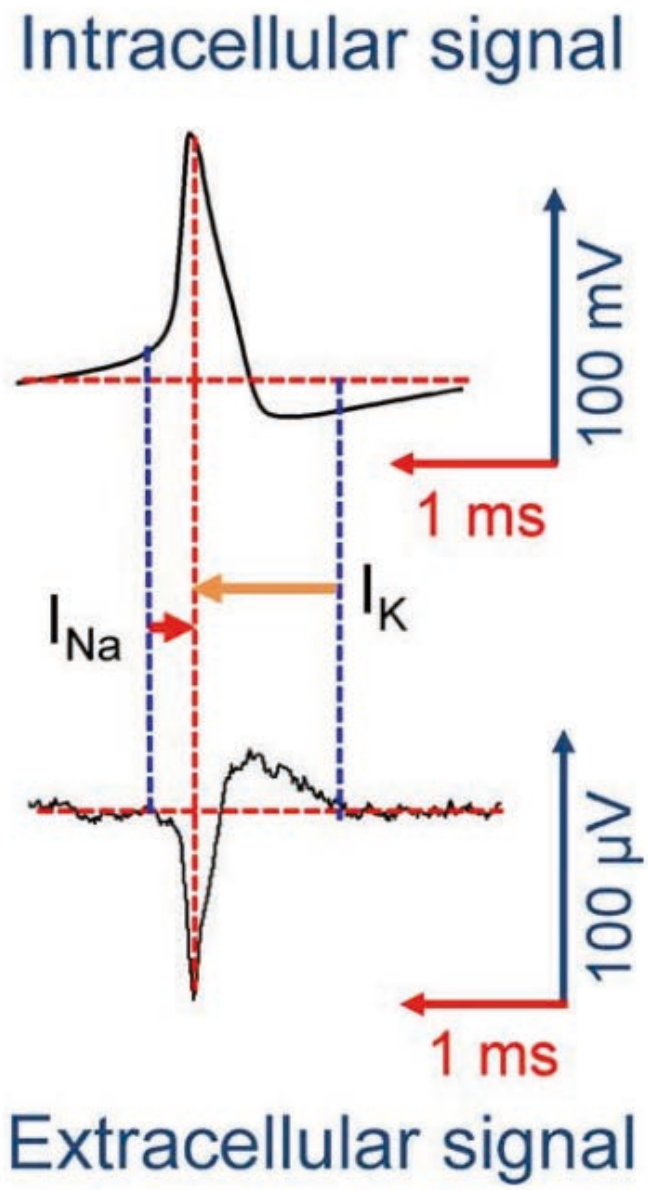
$$I_M = \sum_i g_{JM}^i \left(V_M - V_J - V_0^i\right)$$

$$+C_M \left(\frac{dV_M}{dt} - \frac{dV_J}{dt}\right)$$

$$I_{cleft} = g_J \left(V_J - V_E\right)$$

$$I_S = C_S \left(\frac{dV_S}{dt} - \frac{dV_J}{dt}\right)$$

Iono-Electronic interface

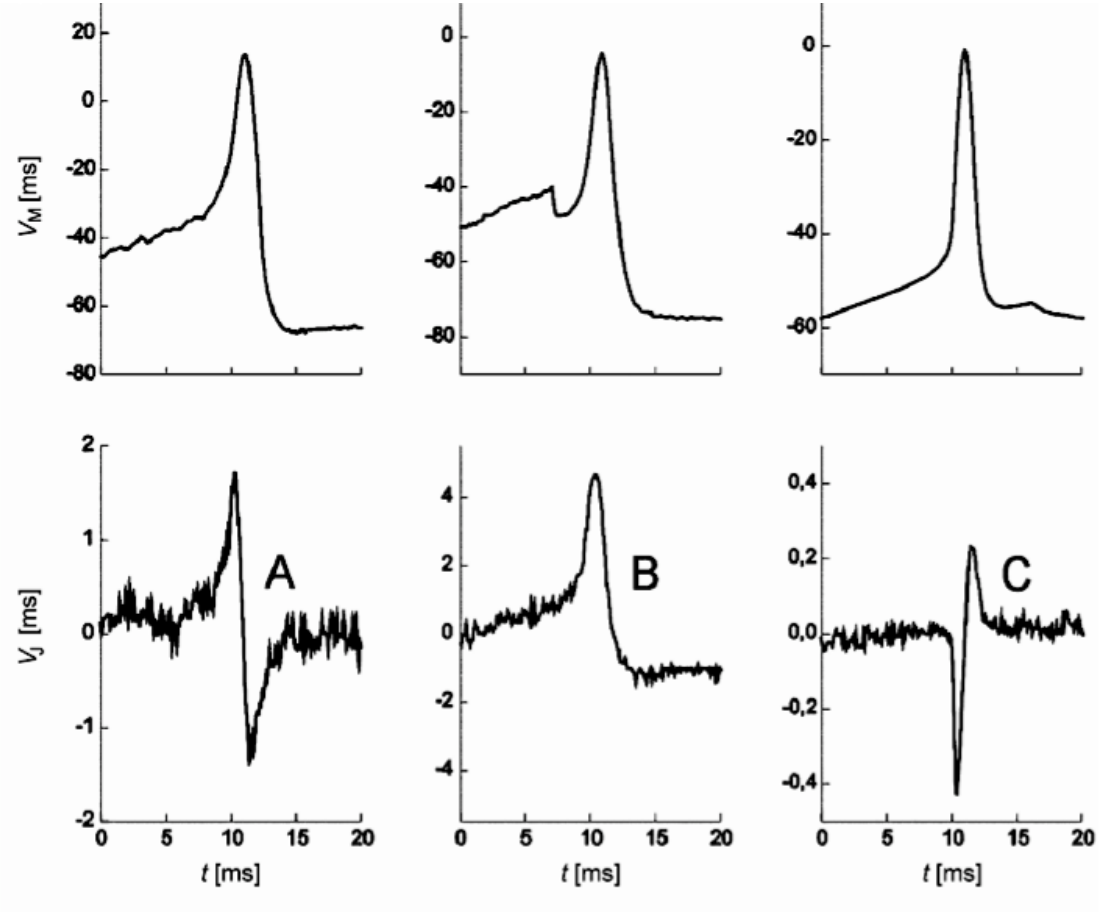


More importantly, the intracell recording signal is always positive whereas the extracell recording shows different polarities according to the electrode position. If the electrode 'looks' at where current enters, the signal is negative, whereas if it looks at where the current leaves, the signal is positive.

Iono-Electronic interface

Intracellular Potential V_M

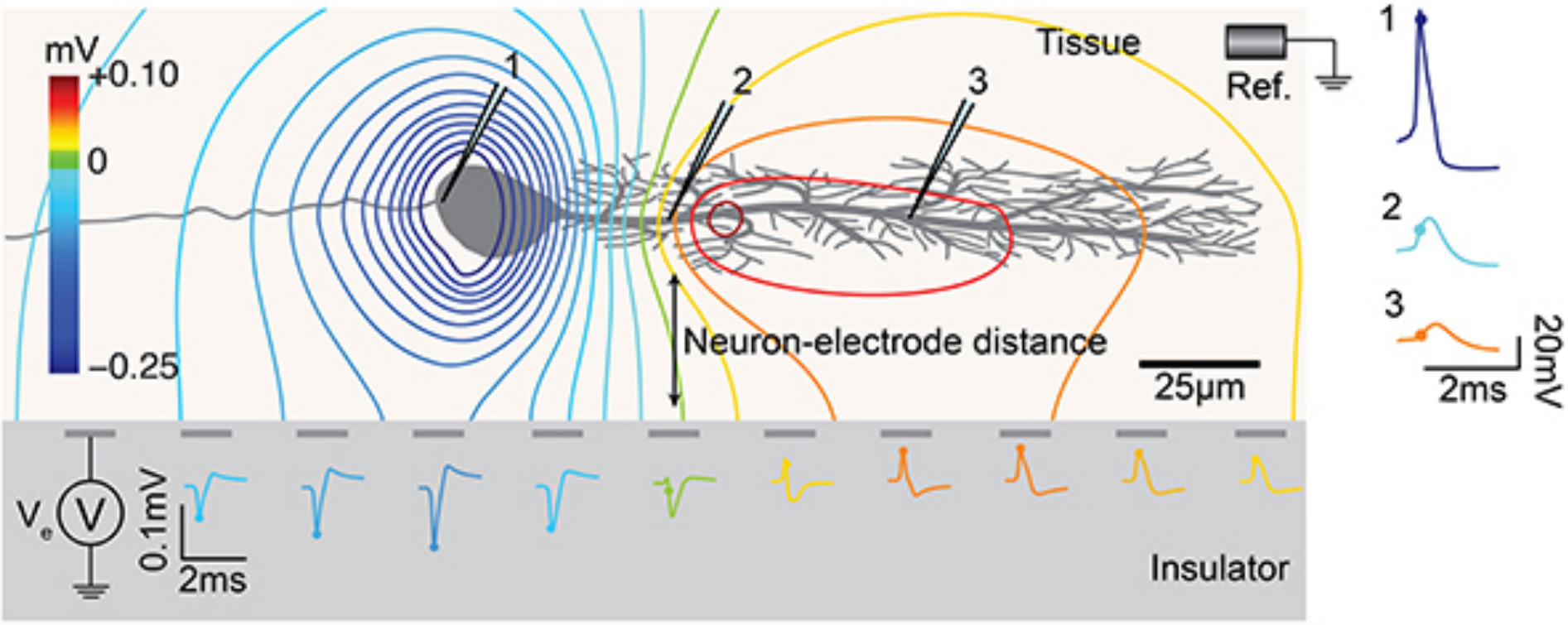
Extracellular Potential V_J



~ Dérivative of V_M ~ V_M

Capacitive junction Ohmique junction

Local field potential



Intracell recording signal is always positive whereas

Extracell recording shows different polarities according to the electrode position.

Summary

Extracell electrodes record changes in Ion concertration around a cell

HH models using K and Na channels allows to model the electrical activity of neurons

It is possible to record APs with extracellular electrode

- weak signal (50µV)
- depends on position to neuron

We can evoke APs with voltage steps

2D neuron culture

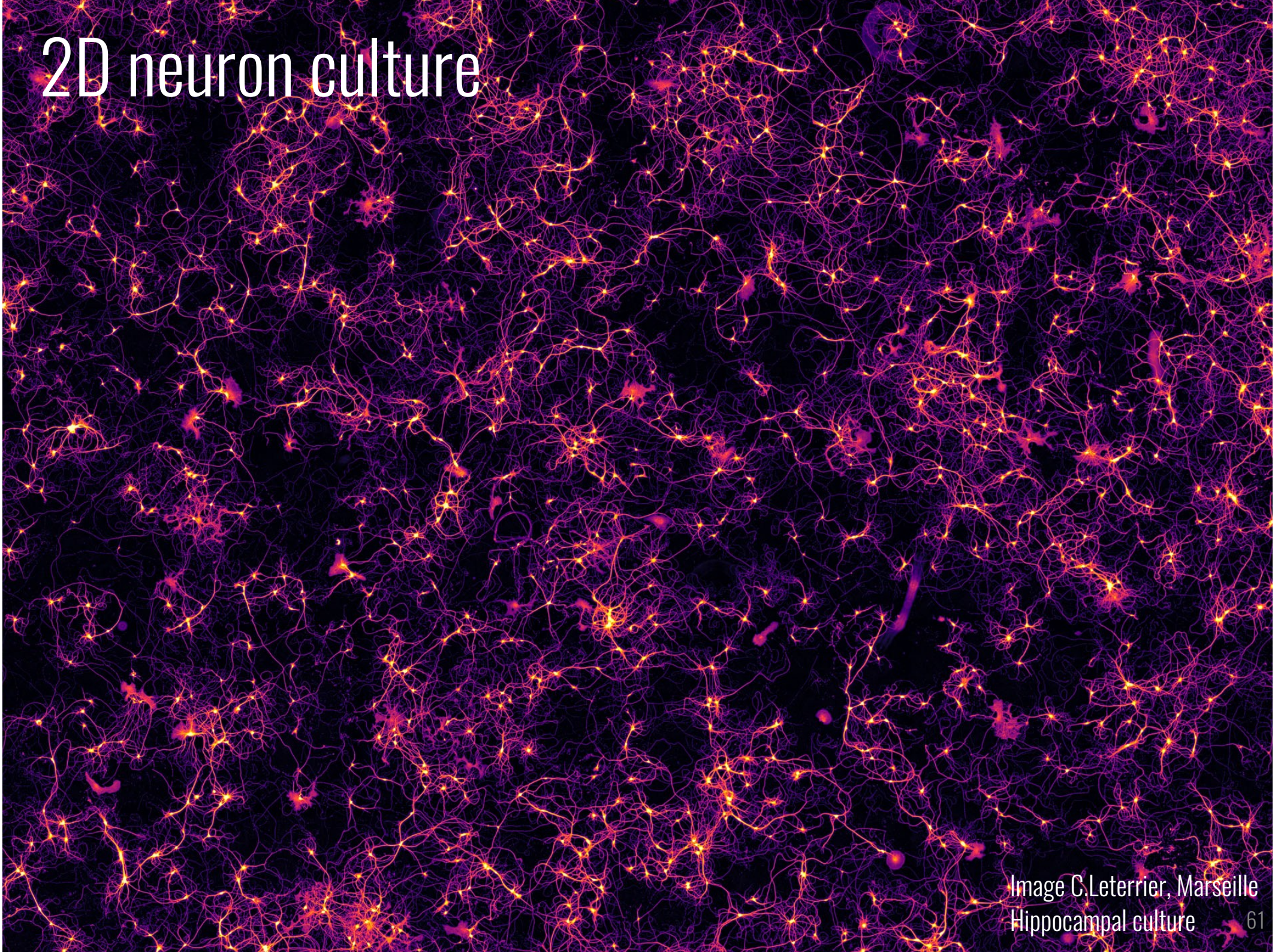


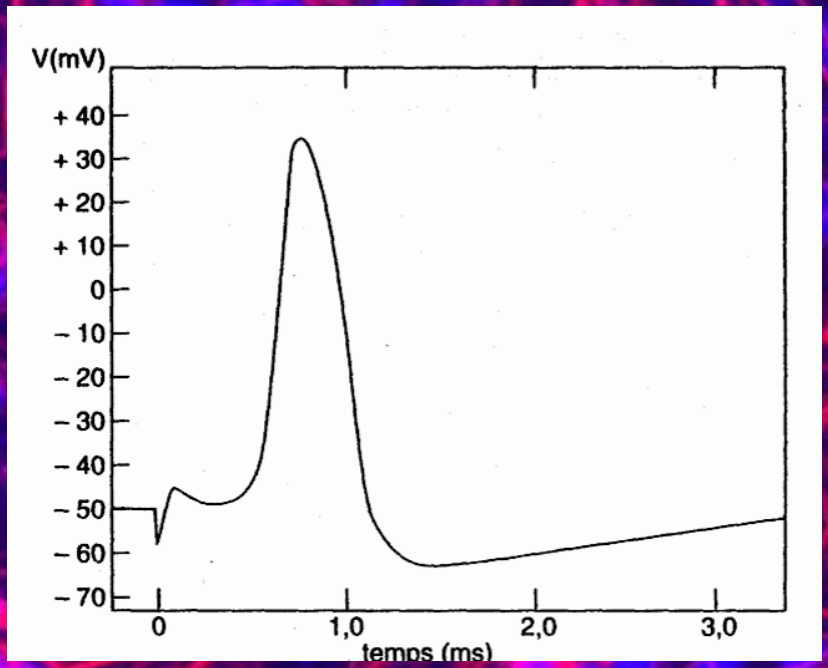
Image C.Leterrier, Marseille
Hippocampal culture

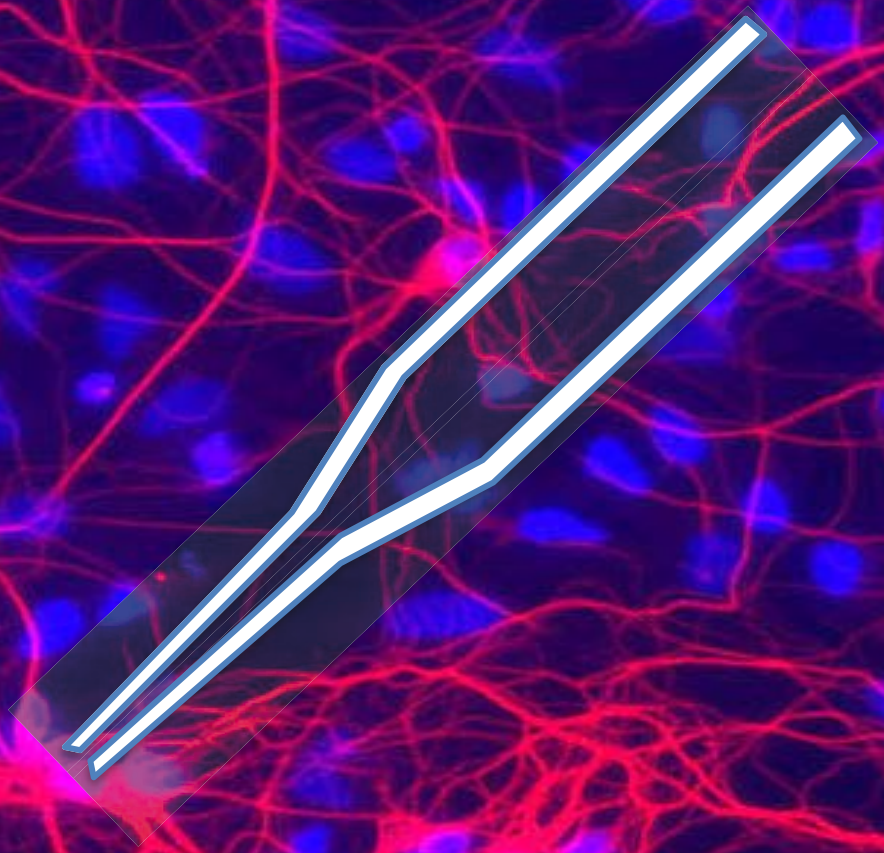
How to measure electrical activity of the network?

Patch clamp

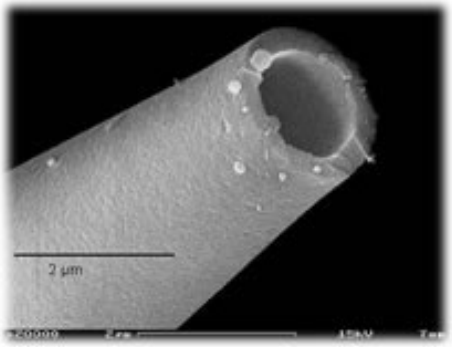
One cell at a time

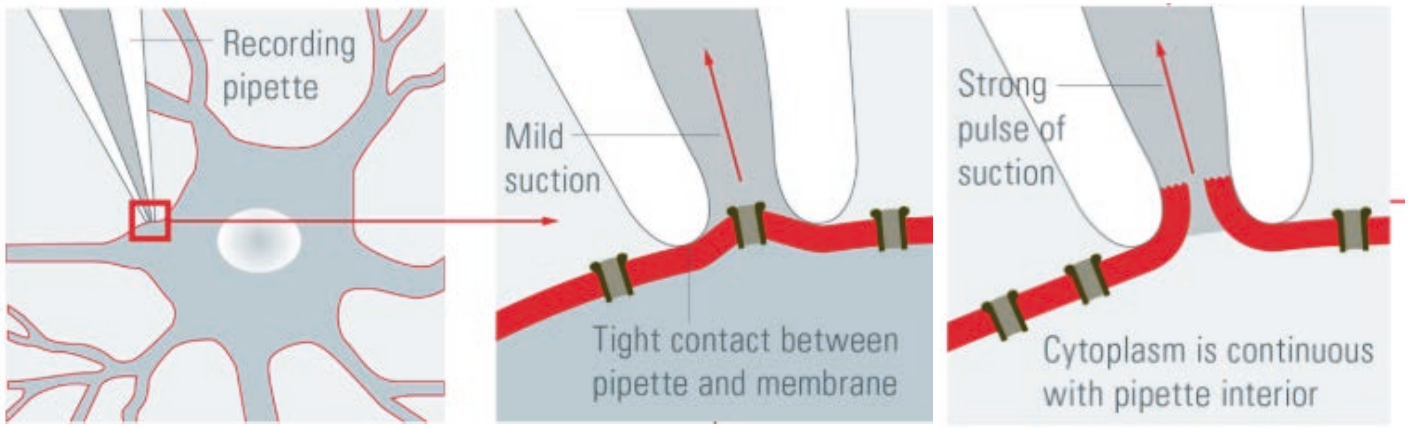
Intracellular recording



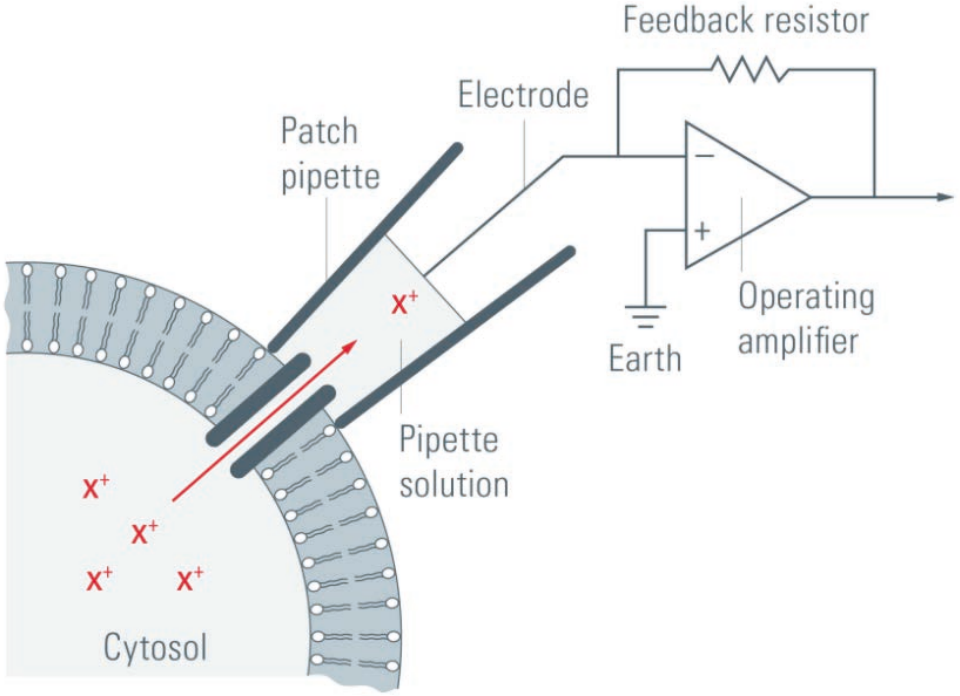


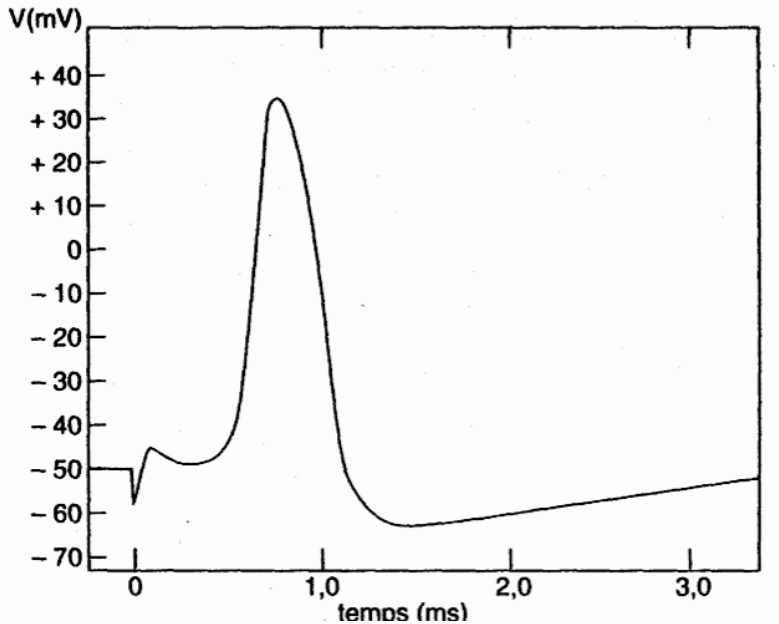
Patch Clamp



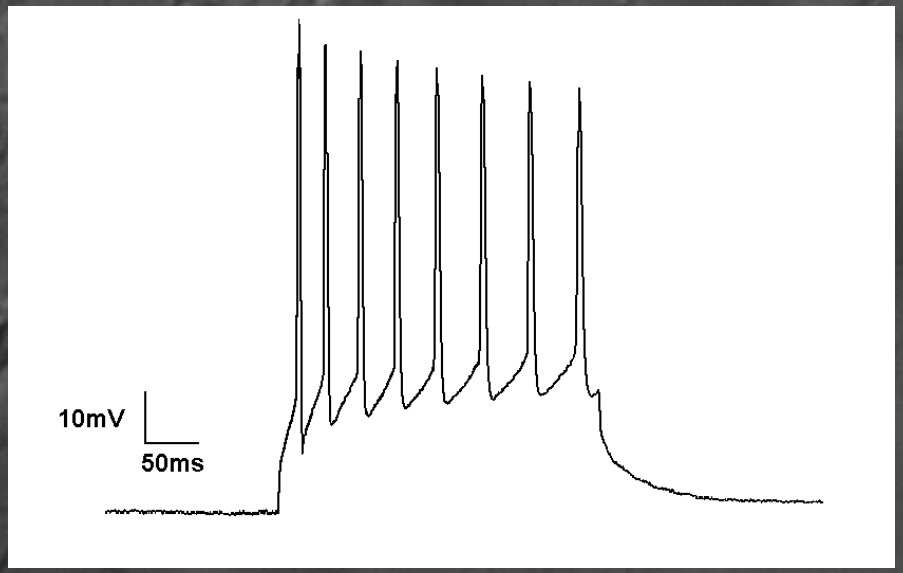


@Leica microsystems





Patch Clamp



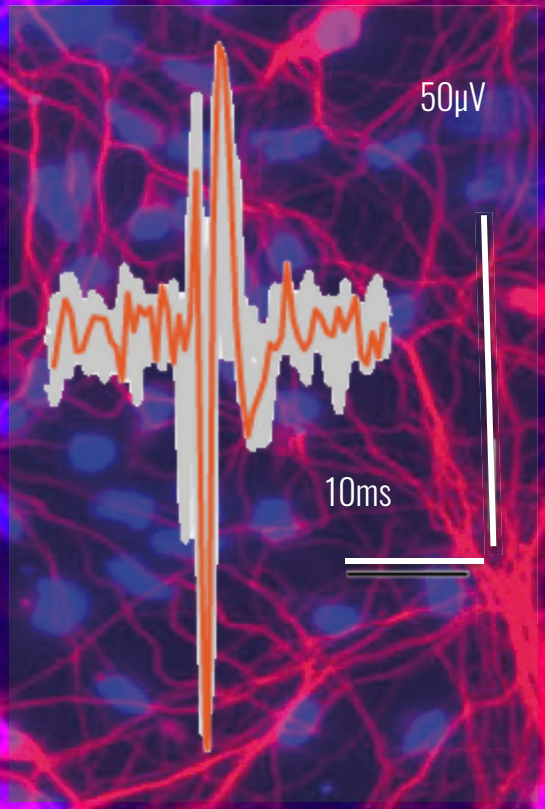
Dr. Ainhara Aguado, Ruhr University Bochum, Germany

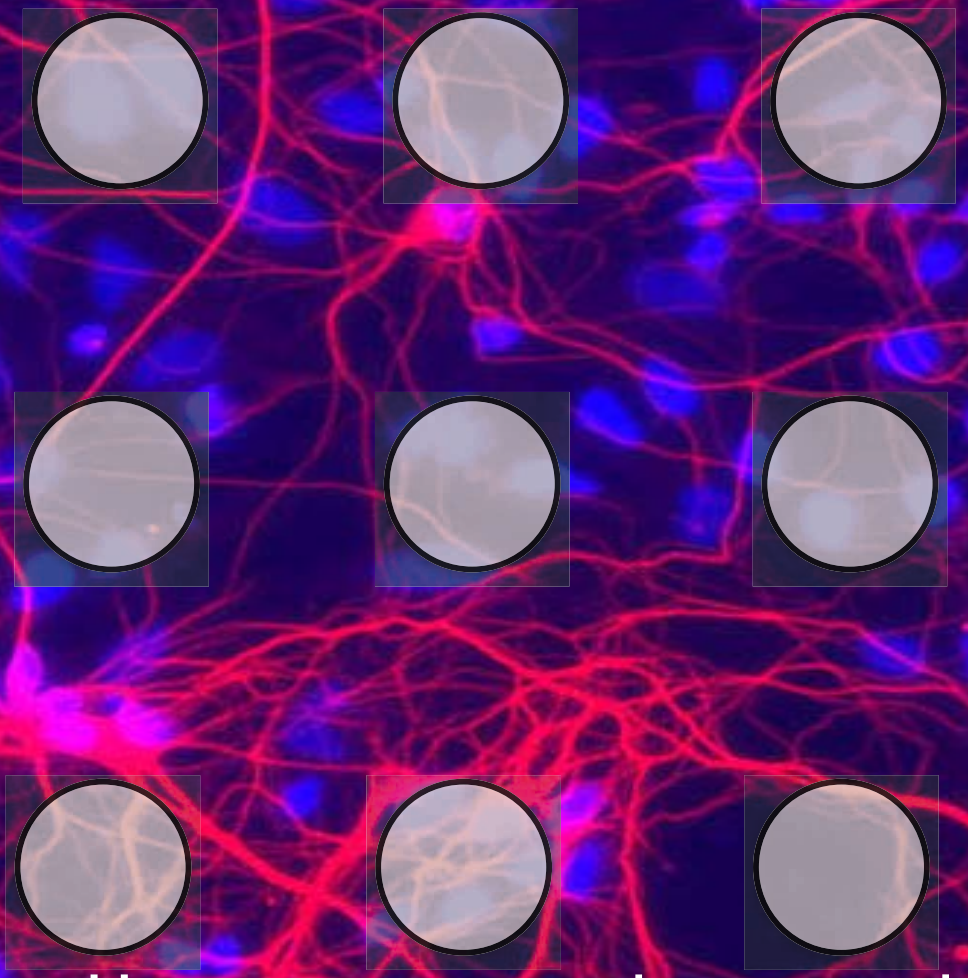
How to measure electrical activity of the network?

Micro Electrode array

Array of fixed electrodes

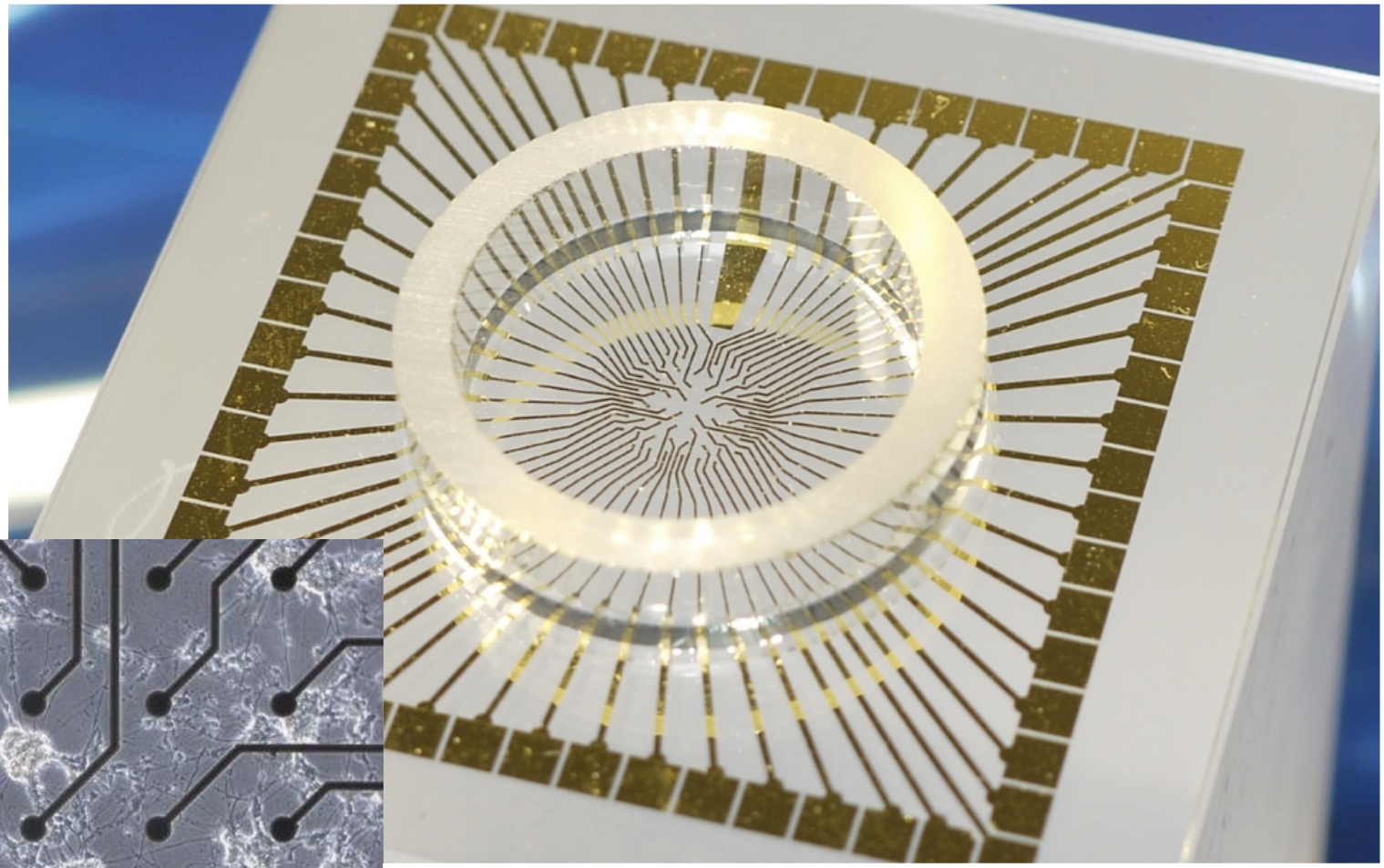
Extracellular potential

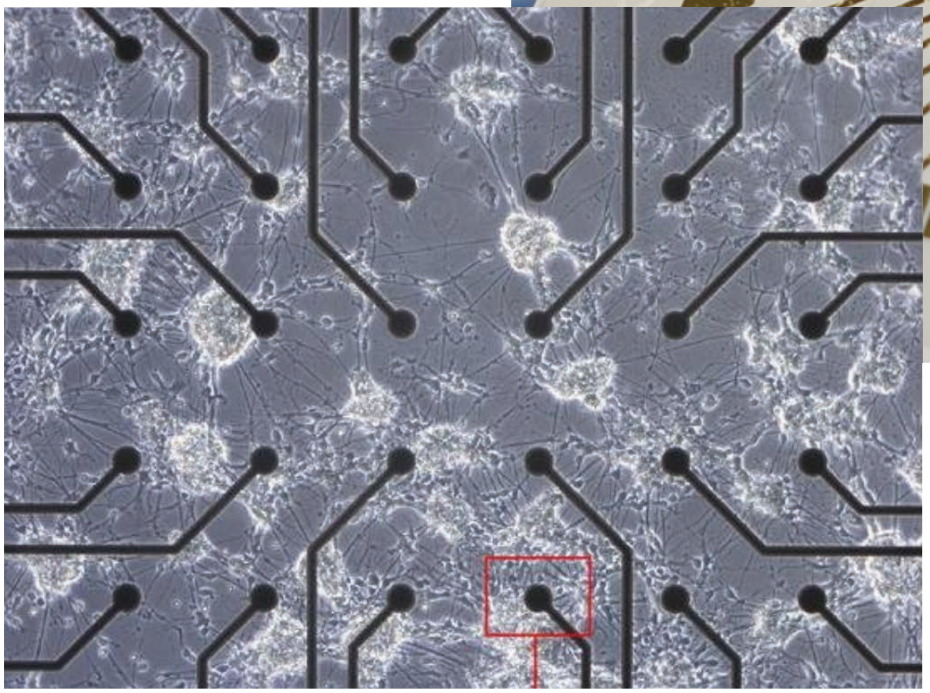




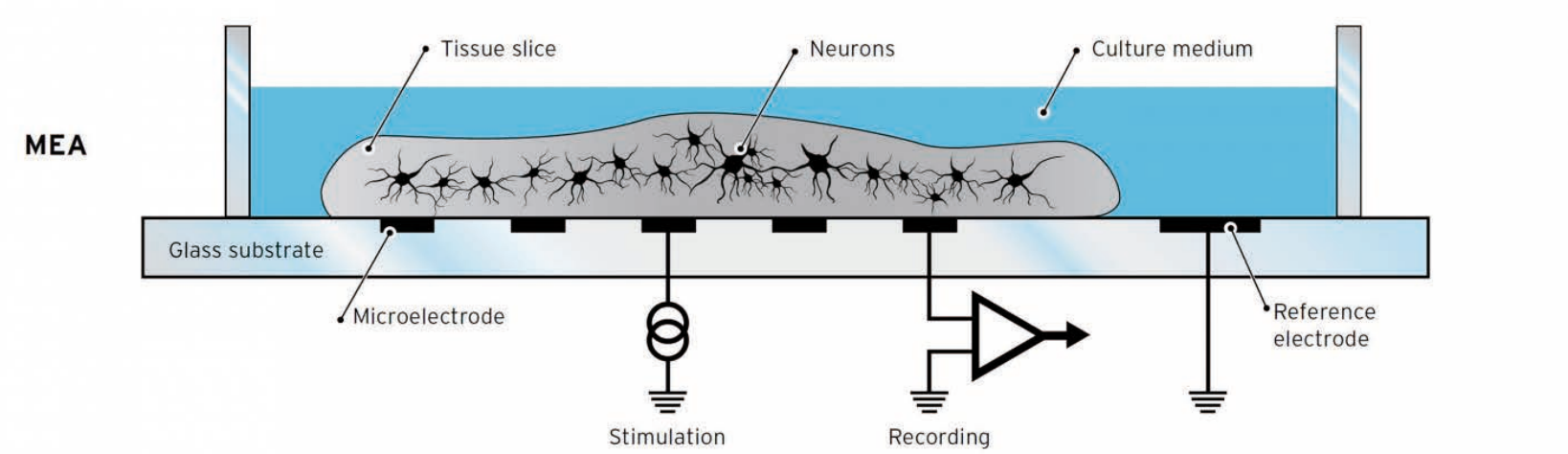
How to organise the network?

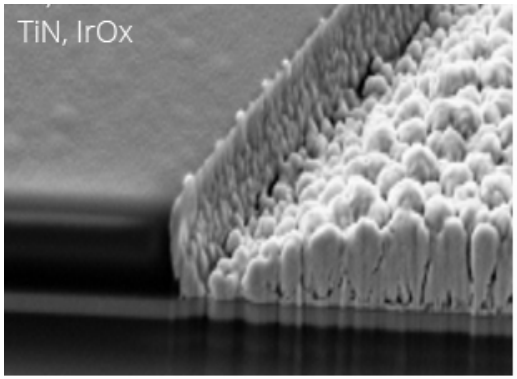
Micro Electrode array

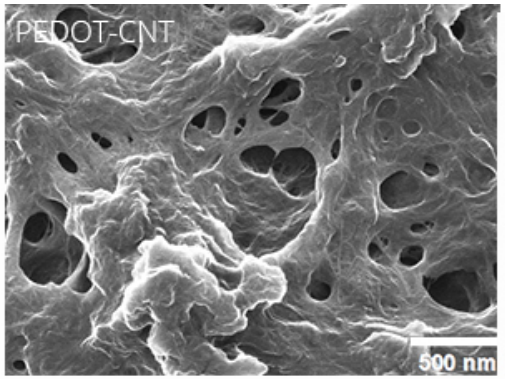


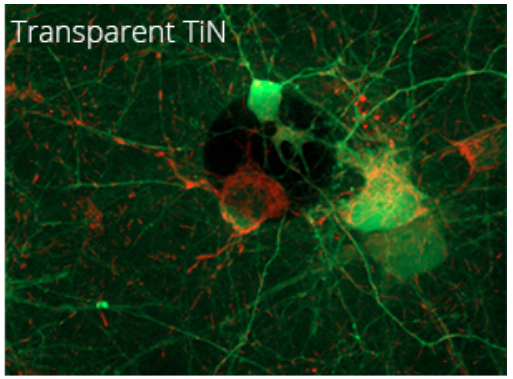


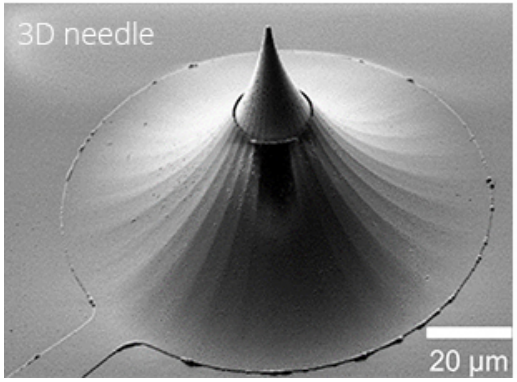
Micro Electrode array

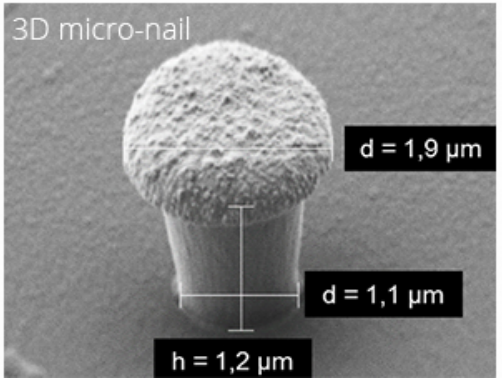


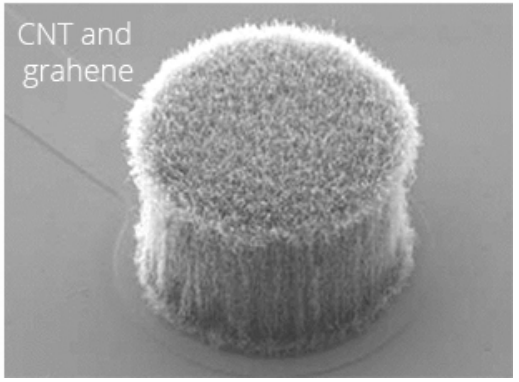




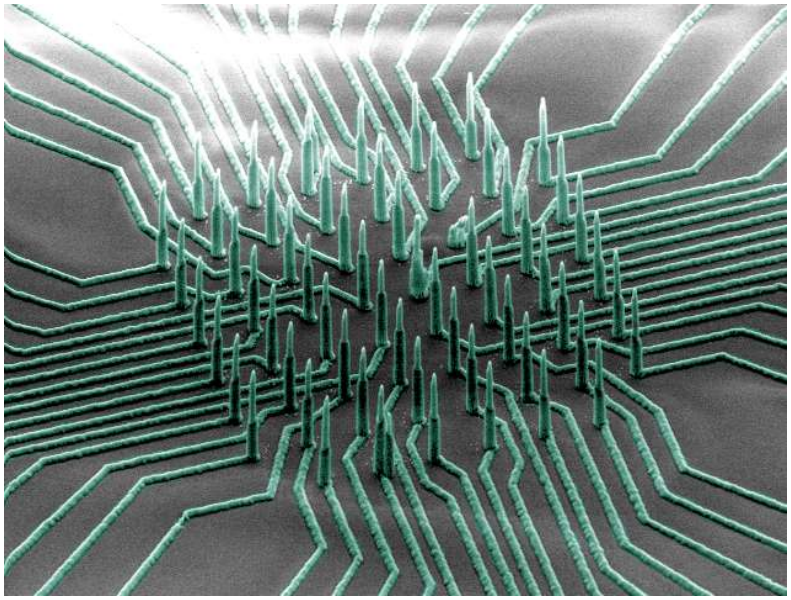


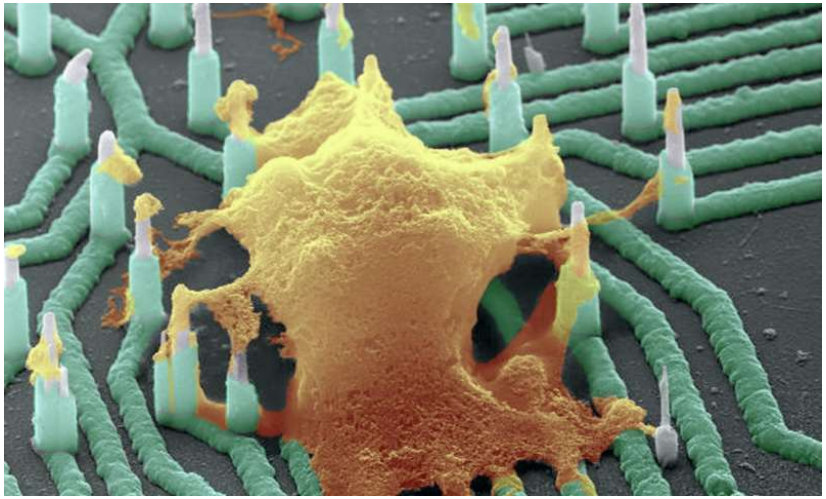




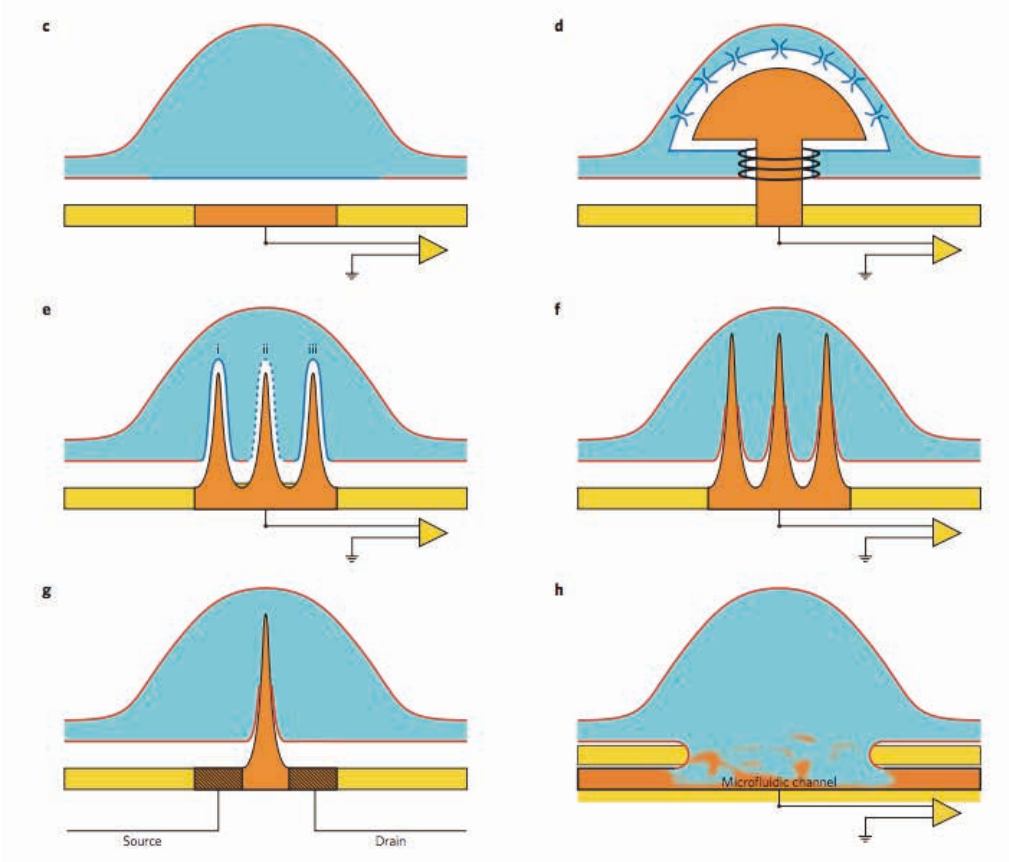


Nanowires





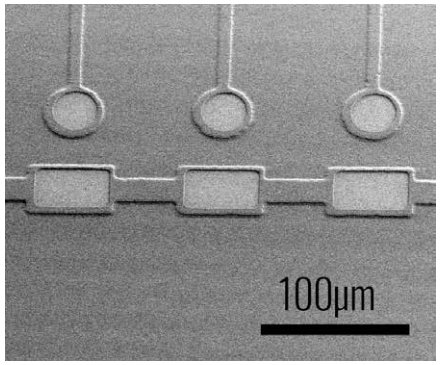
Ren Liu et al, High Density Individually Addressable Nanowire Arrays Record Intracellular Activity from Primary Rodent and Human Stem Cell Derived Neurons, **Nano Letters (2017)**

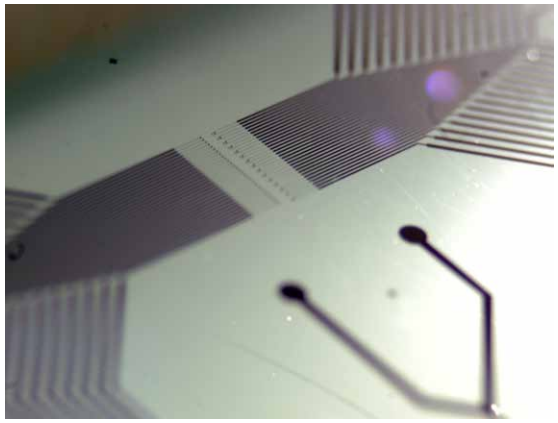


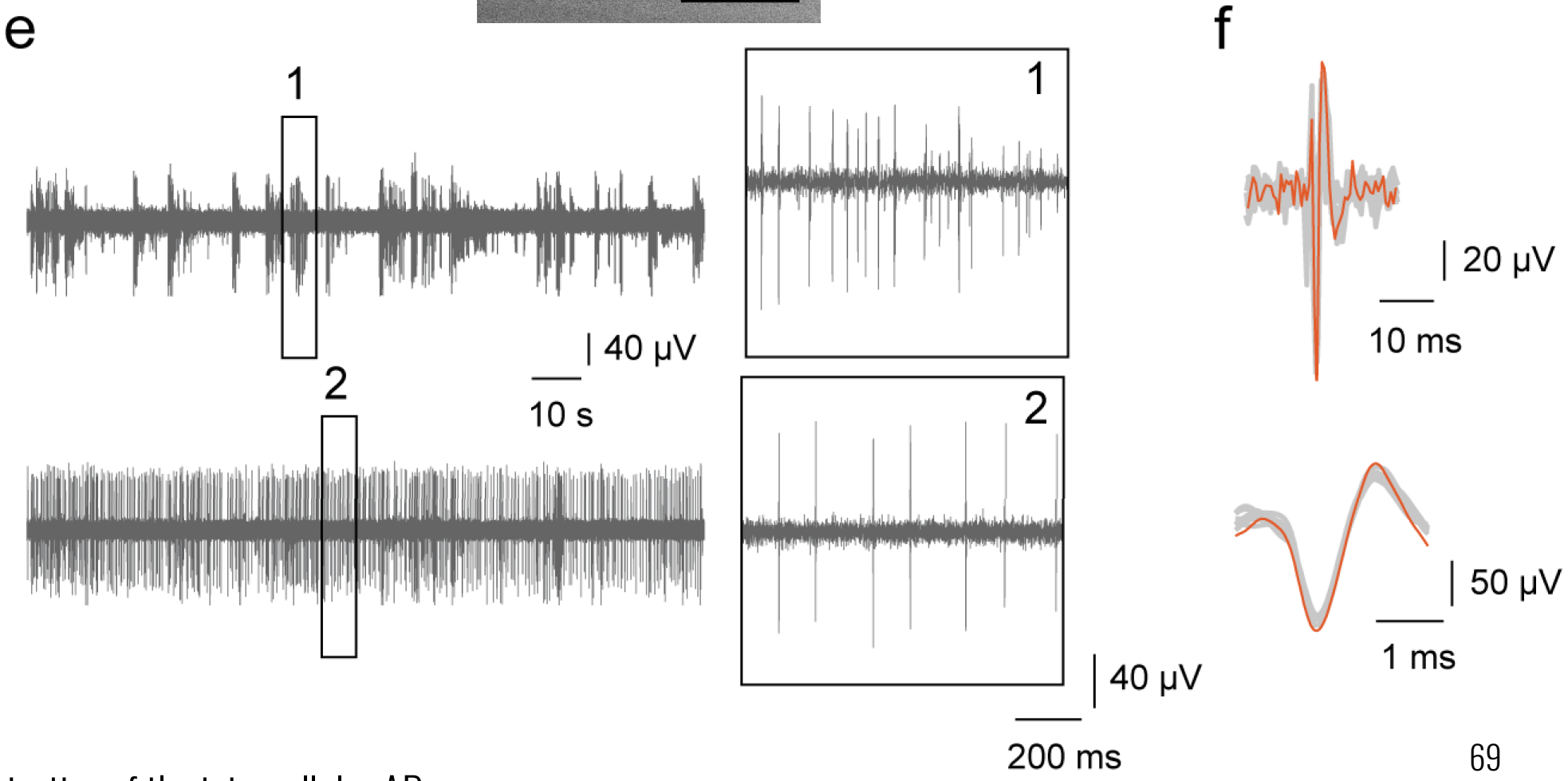
Micha E. Spira, NATURE NANOTECHNOLOGY | VOL 8 | FEBRUARY 2013

Stimulation and recording

Microelectrode: **extracellular**



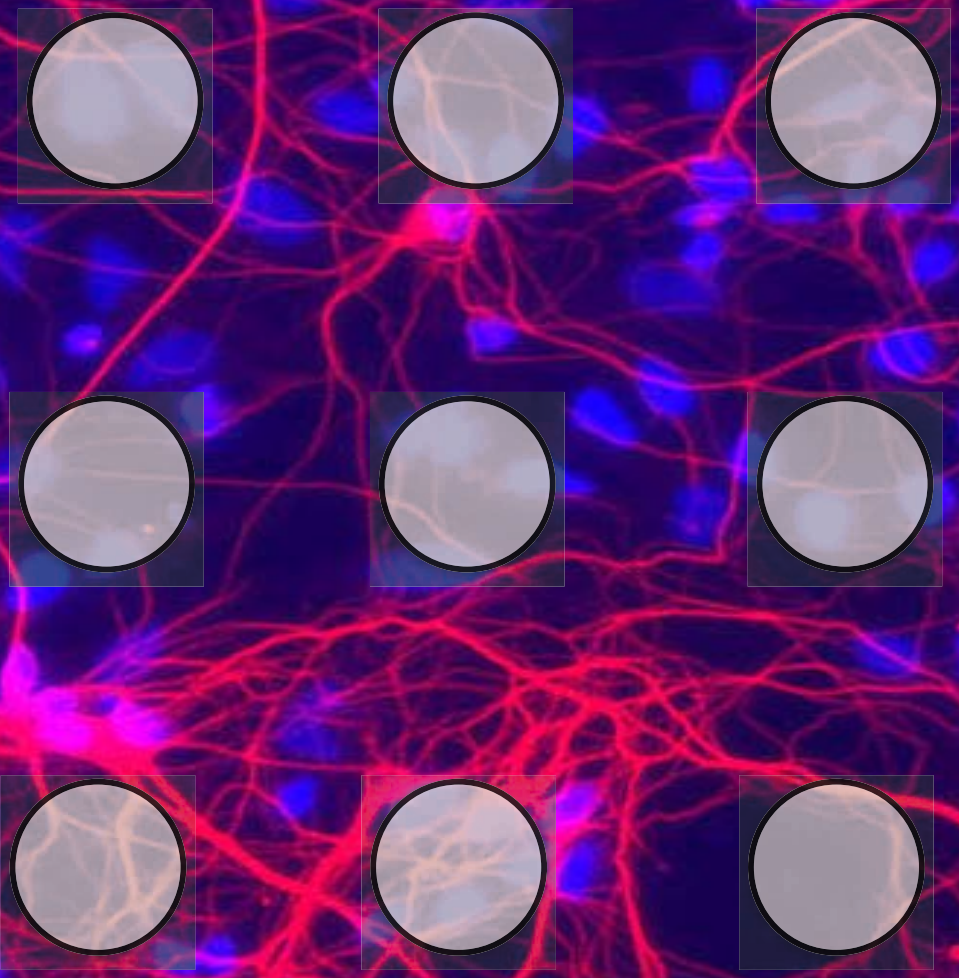




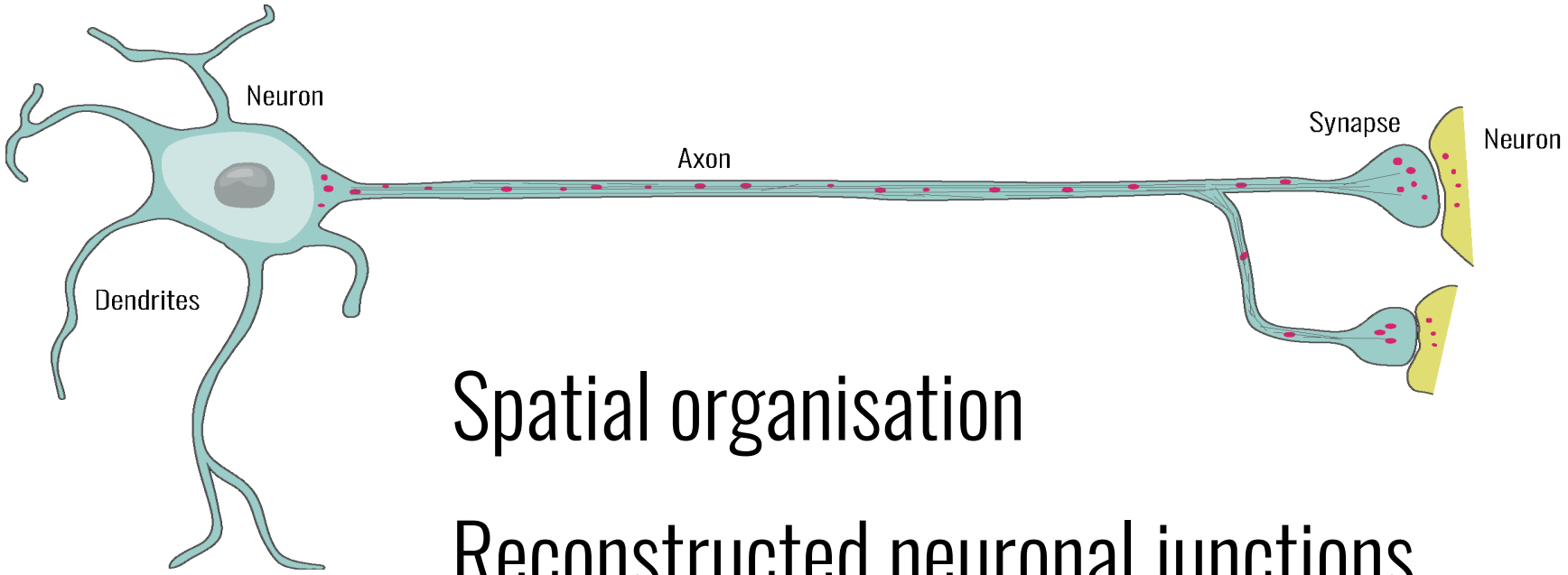
Derivative of the intracellular AP

Part.III Neurofluidics

How to organise the network?



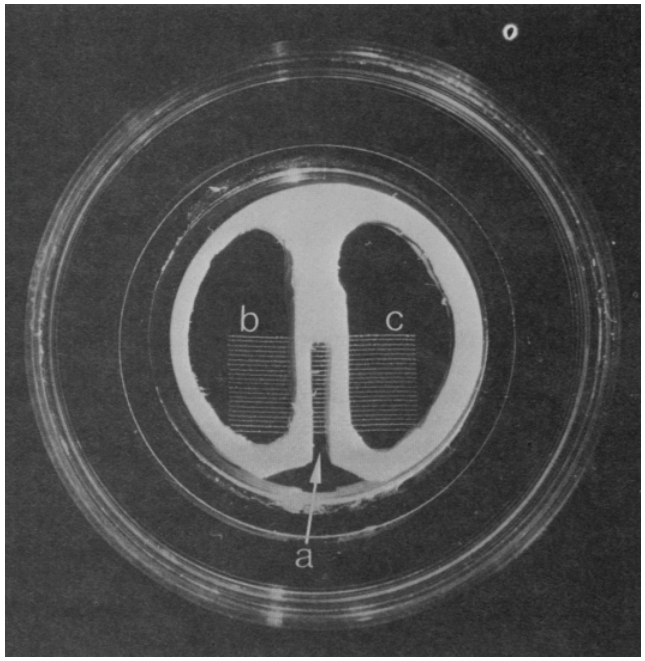
Neurofluidics

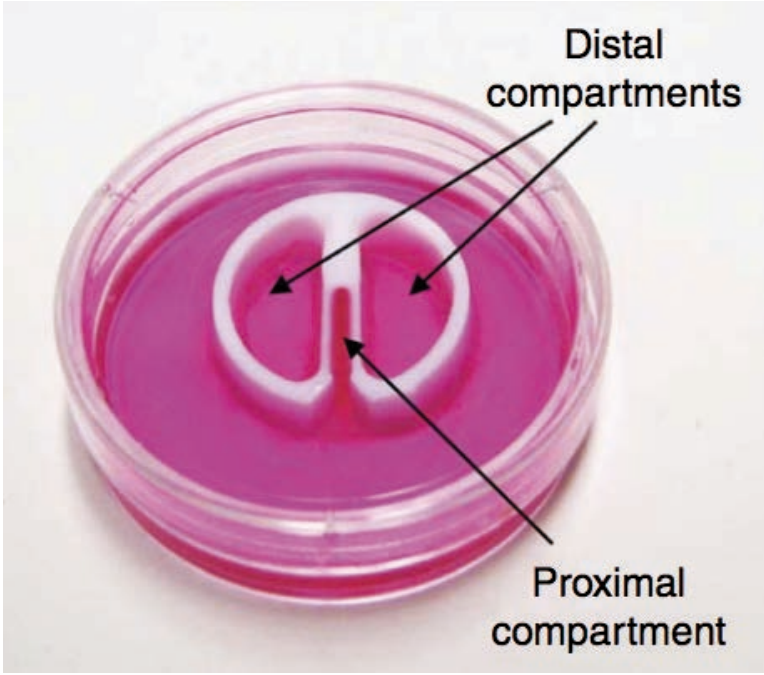


Spatial organisation

Reconstructed neuronal junctions

Campenot Chambers, 1977



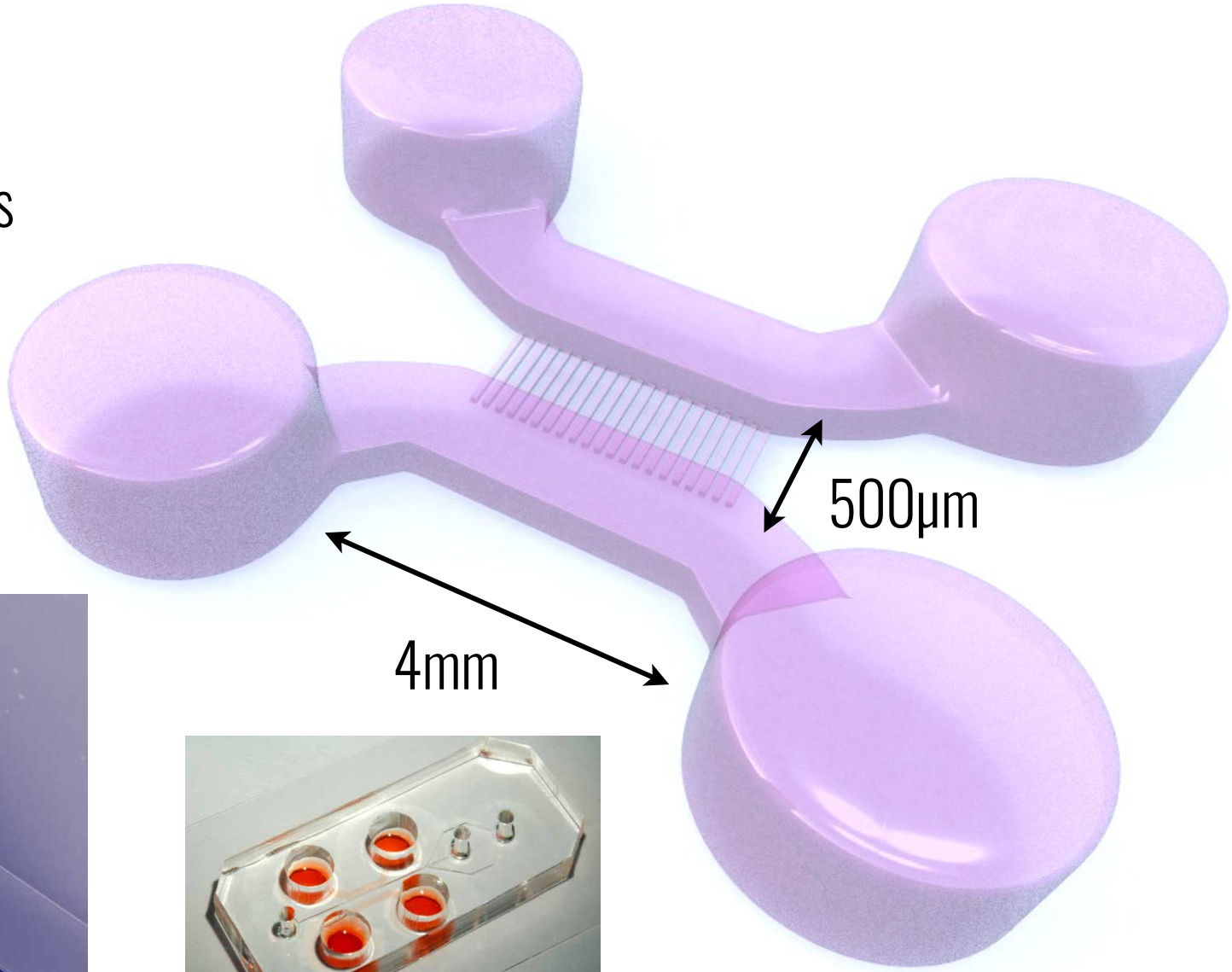


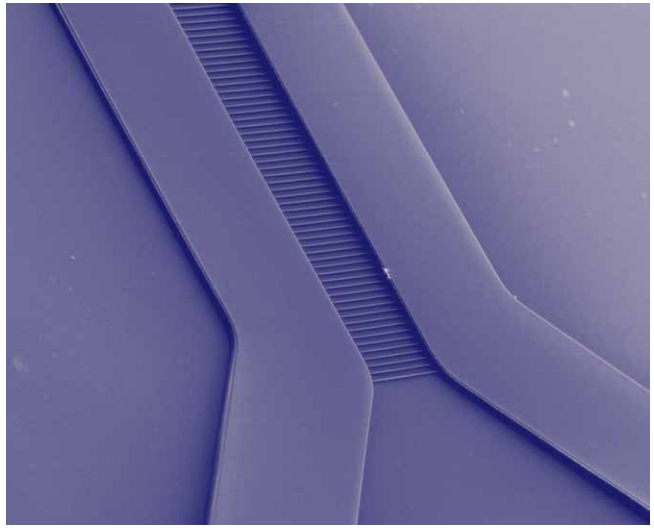
Robert. B. Campenot,
PNAS 1977 Oct; 74(10): 4516–4519.

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





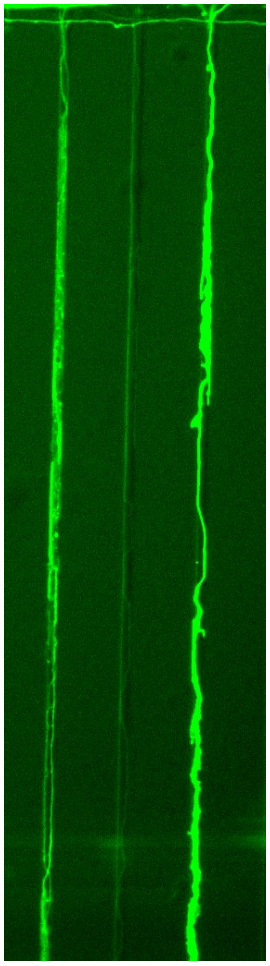
Dual thickness SU8 / PDMS

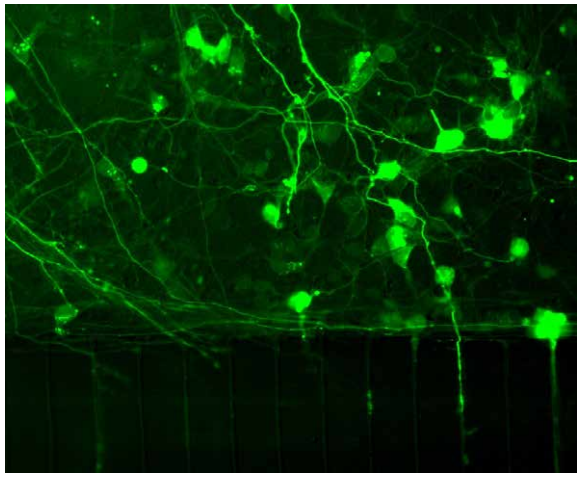
A.M.Taylor et al. Langmuir 19, 2003

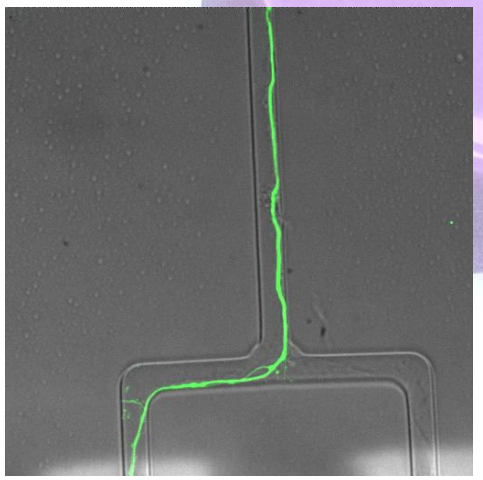
A.M.Taylor et al. Nat. Methods 2, 2005

Compartimentalized Microfluidics

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



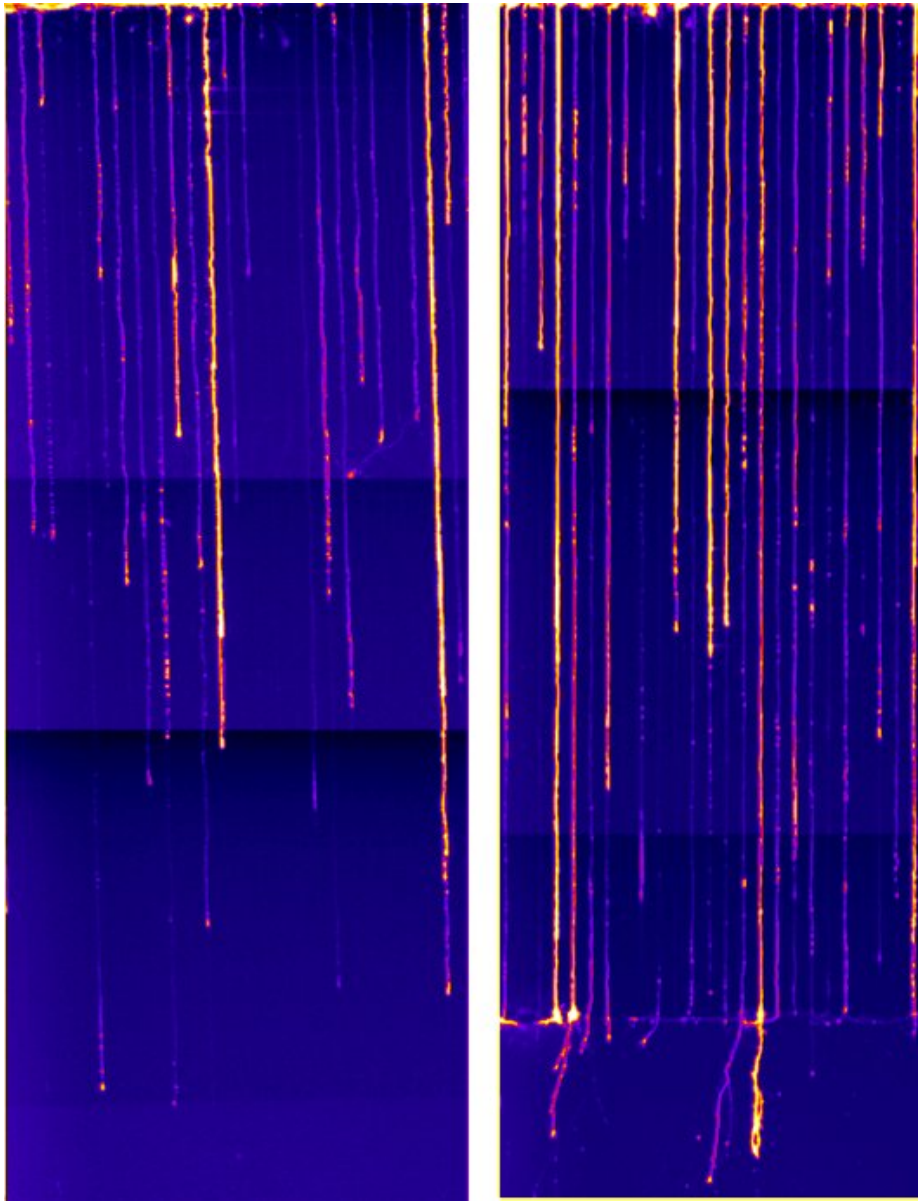




Compartimentalized Microfluidics

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

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



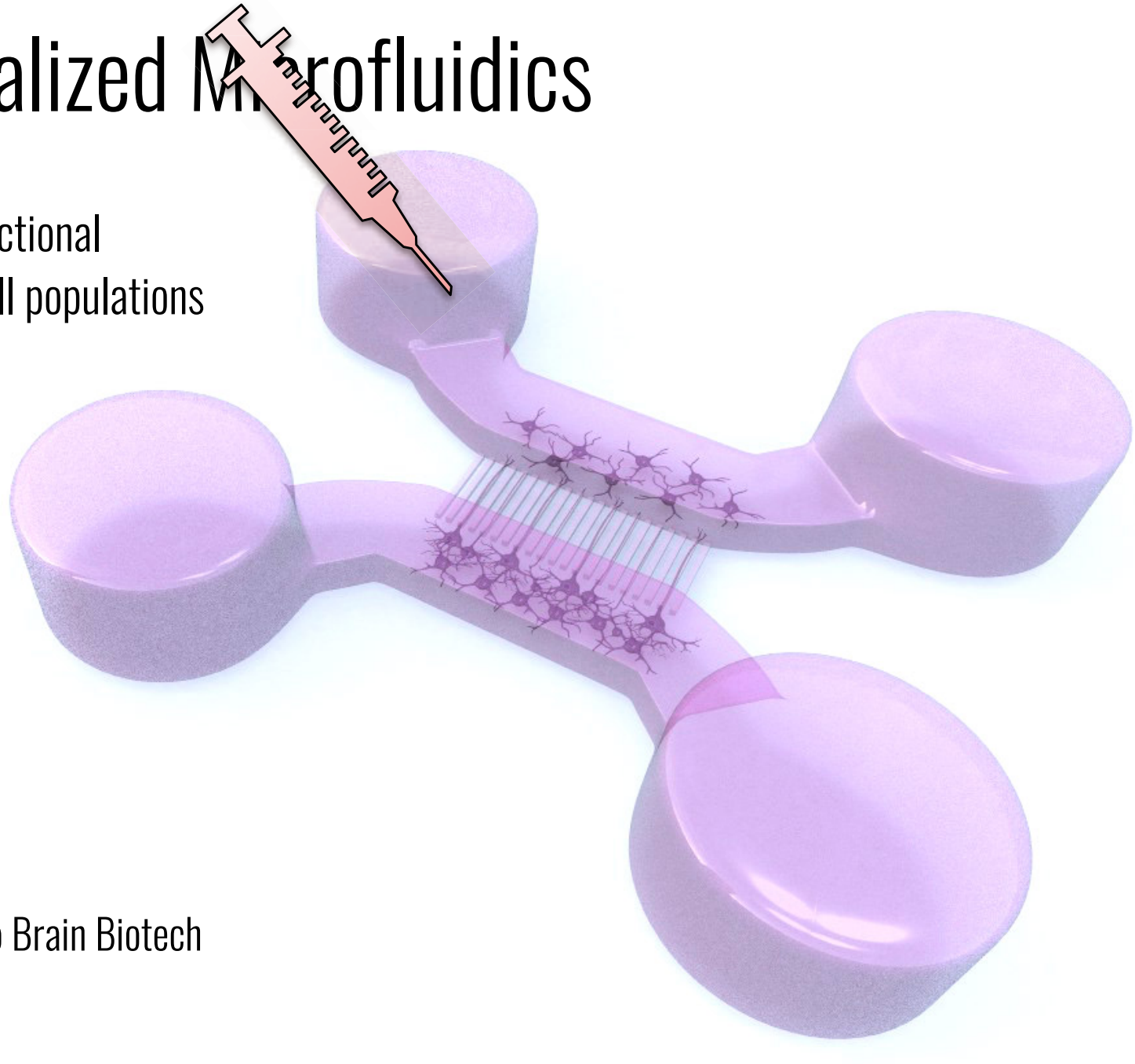
Control

Optical stimulation

Compartimentalized Microfluidics

in-vitro reconstitution of functional connections between two cell populations

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

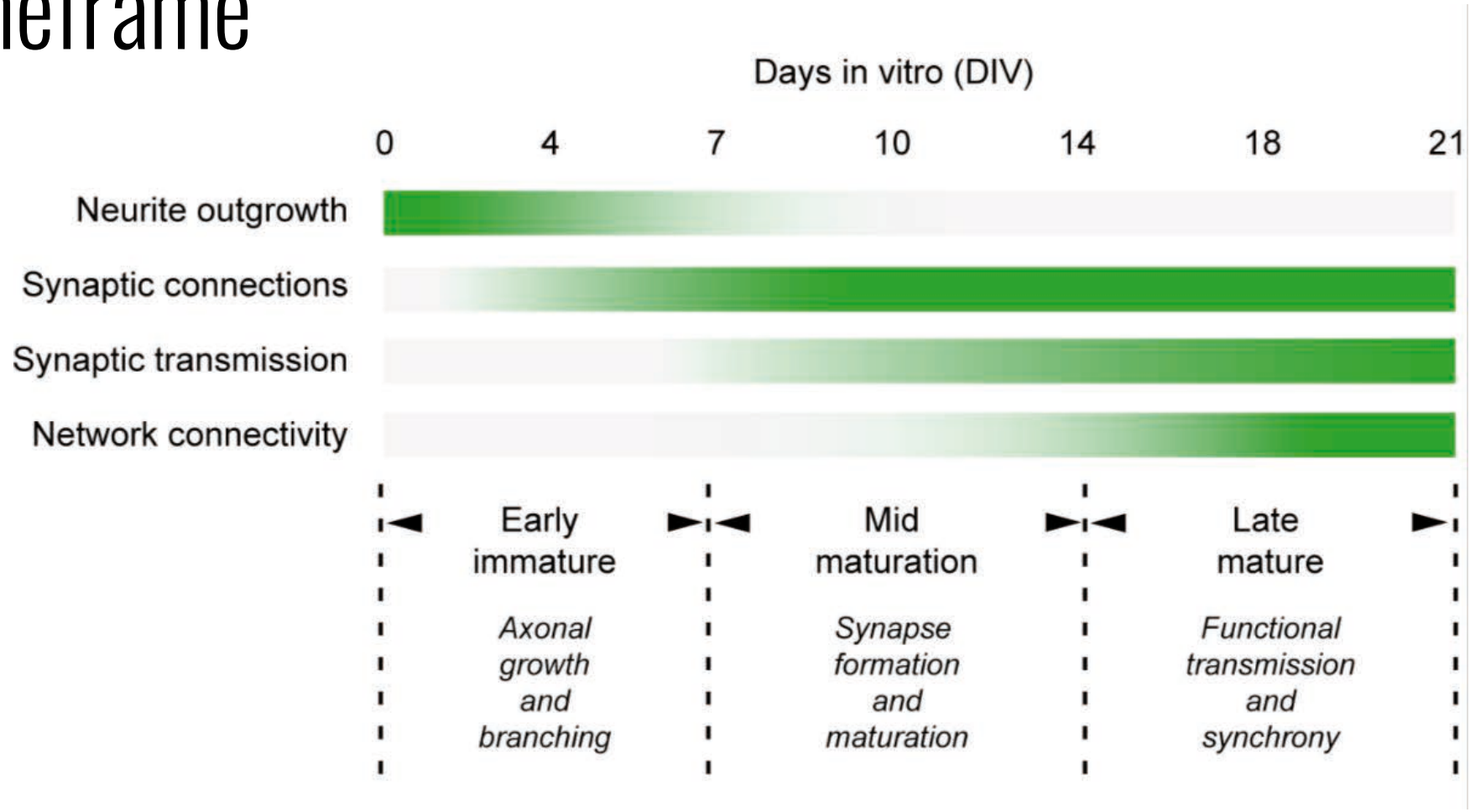


Xona, Millipore, Ananda, Micro Brain Biotech

+gradient of laminin/poly-d-lysin coating

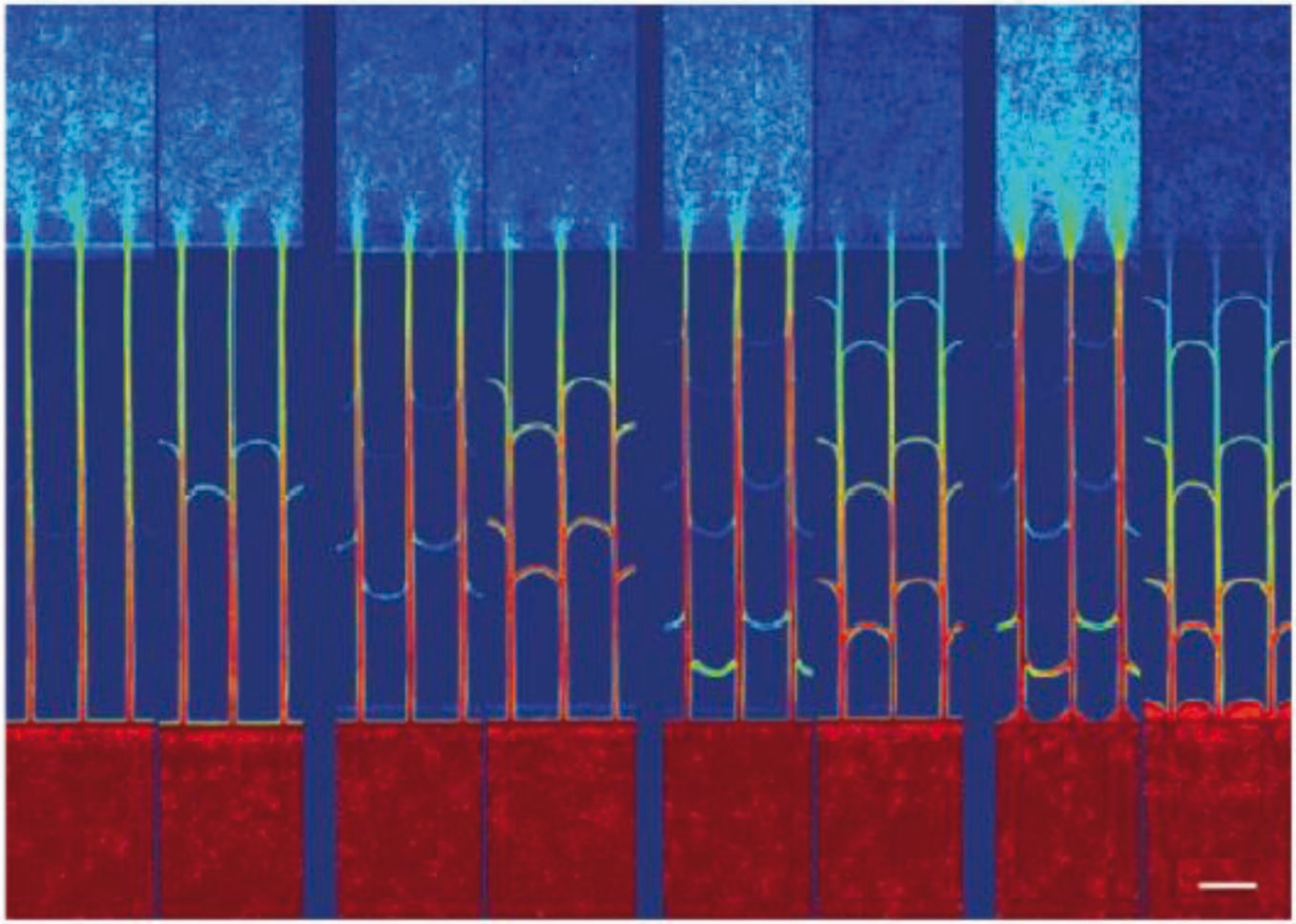
Compartimentalized Microfluidics

Timeframe



Compartimentalized Microfluidics

Arches

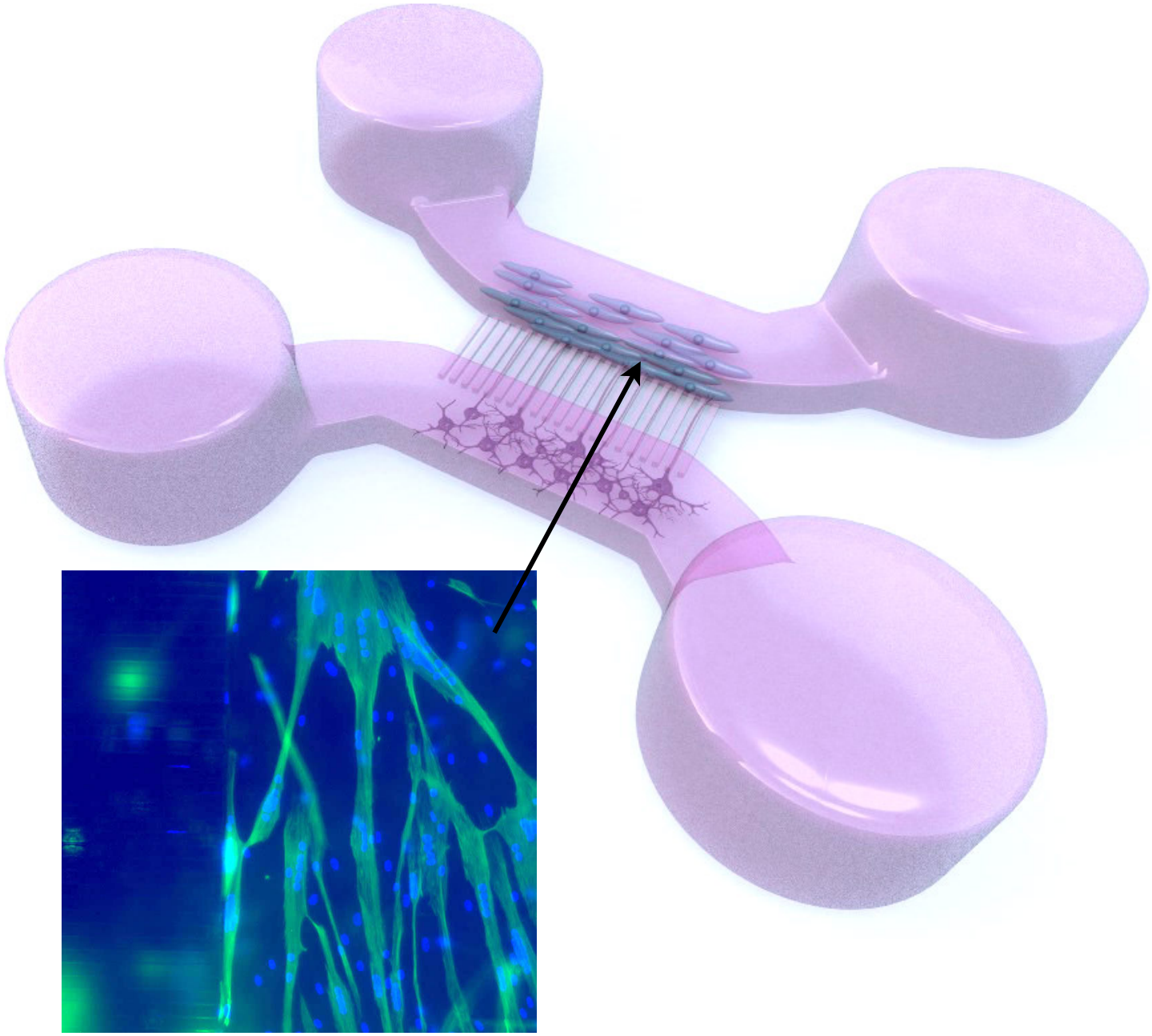


C.Villard, IPGG

Compartimentalized Microfluidics

Co-Cultures

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

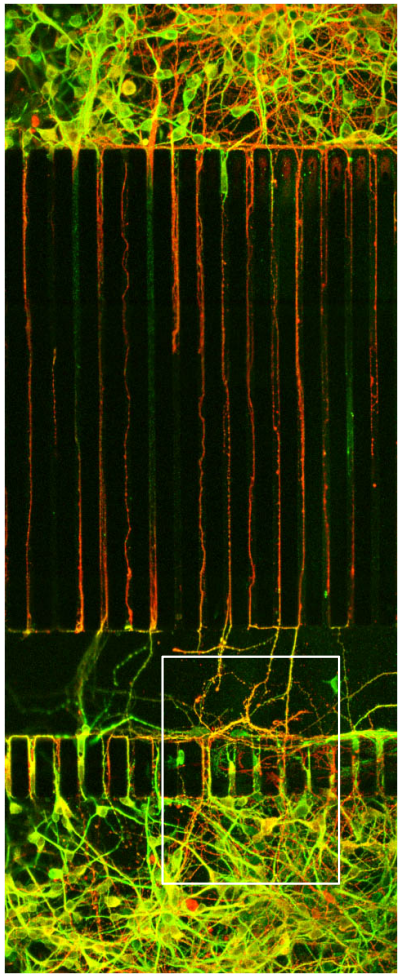


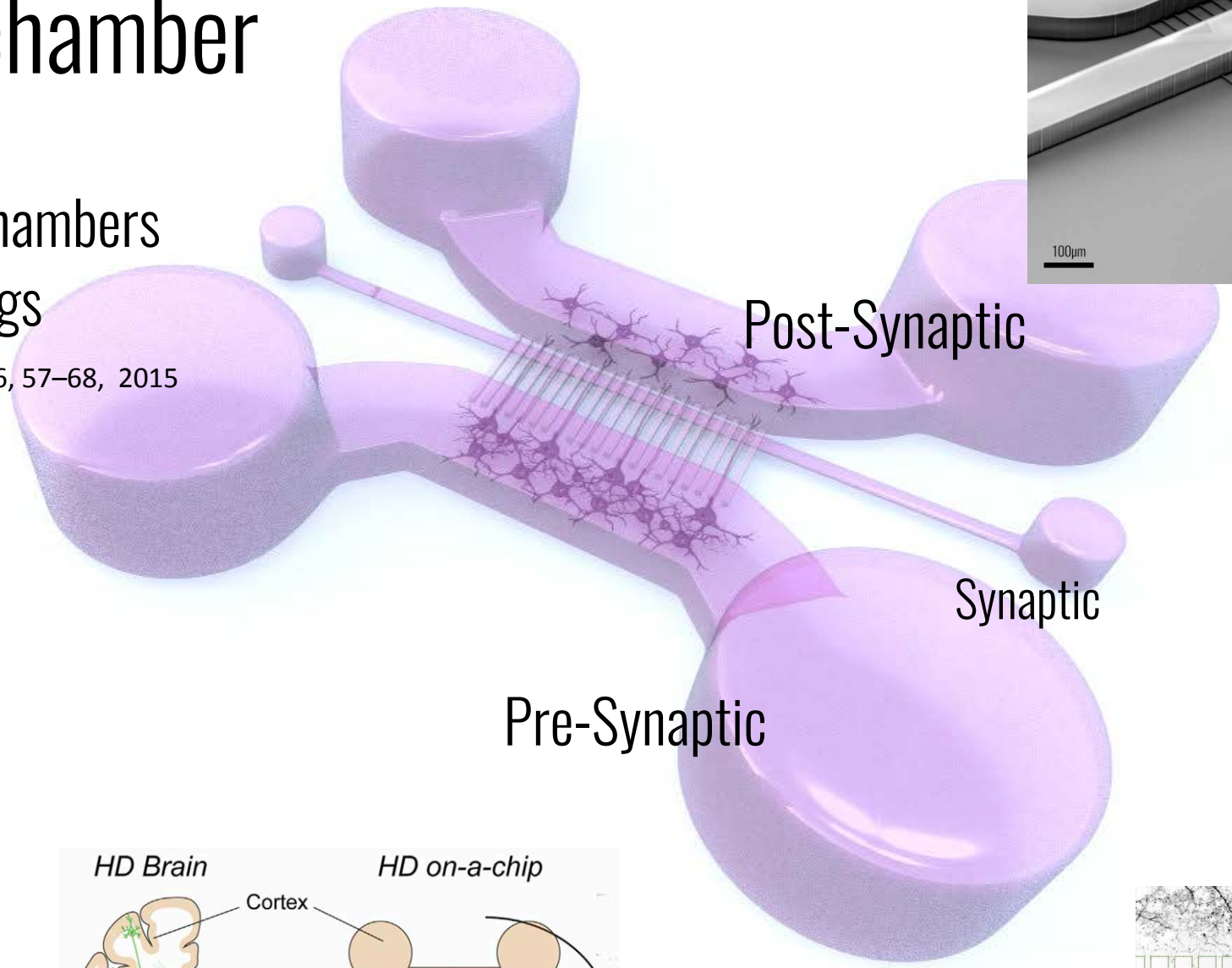
G.Carnac, Phymedexp

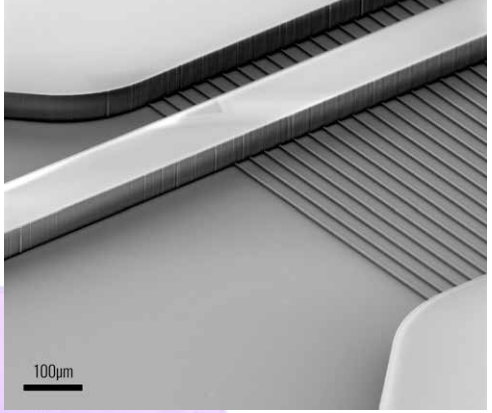
Synaptic chamber

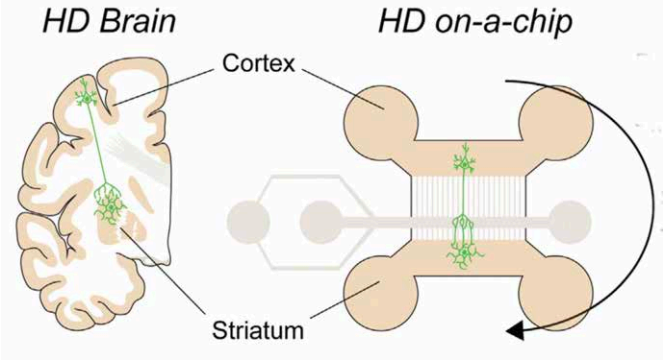
Design with 3 chambers
Perfusion of drugs

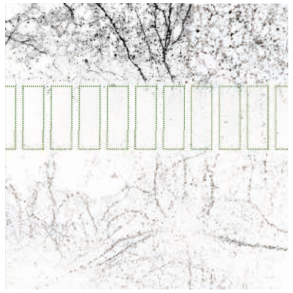
A.M. Taylor et al. Neuron 66, 57–68, 2015







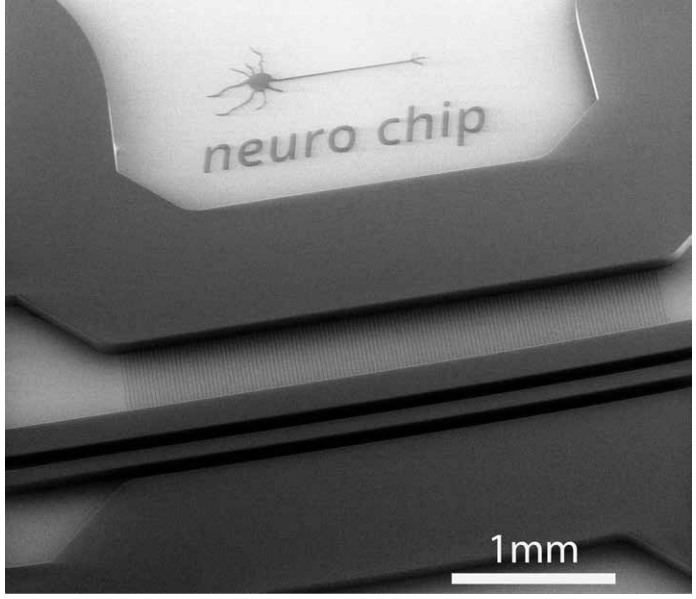


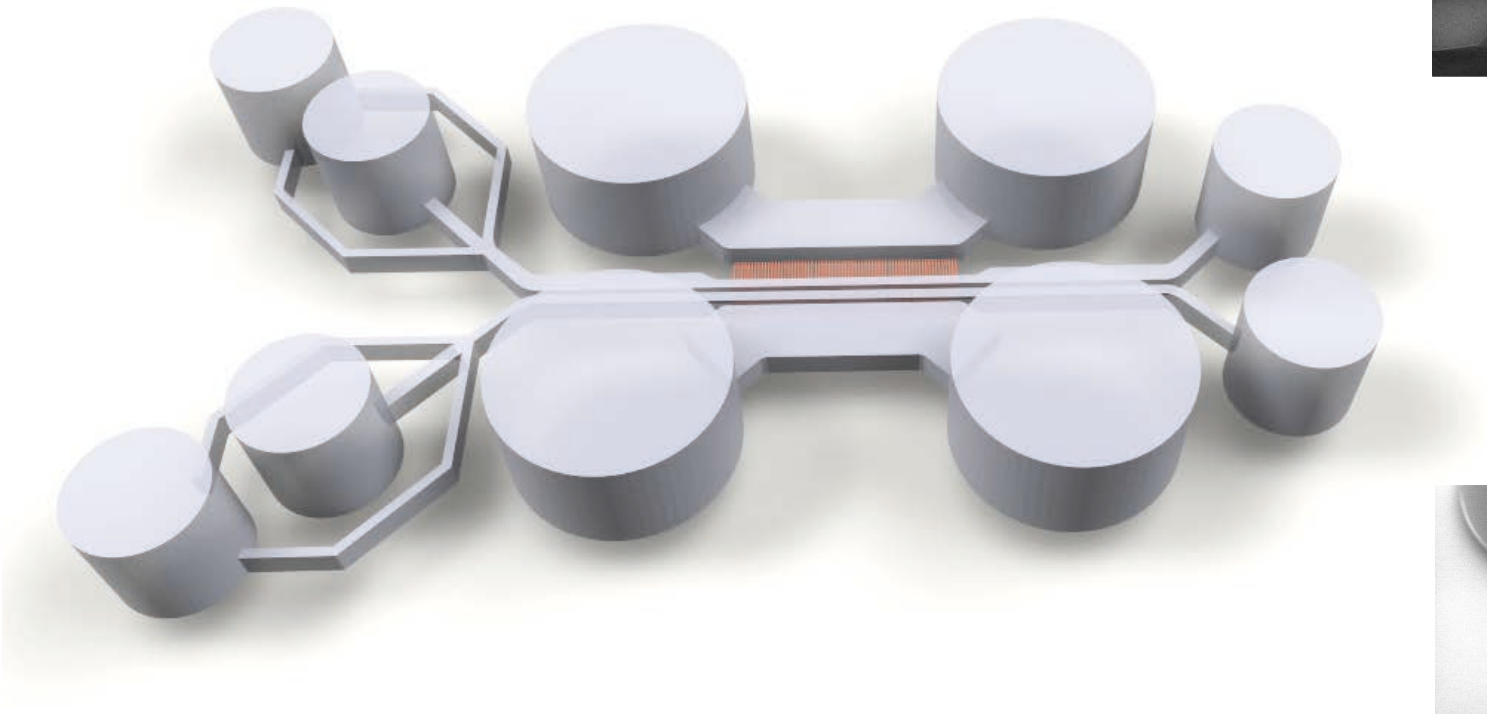


Synaptophysin

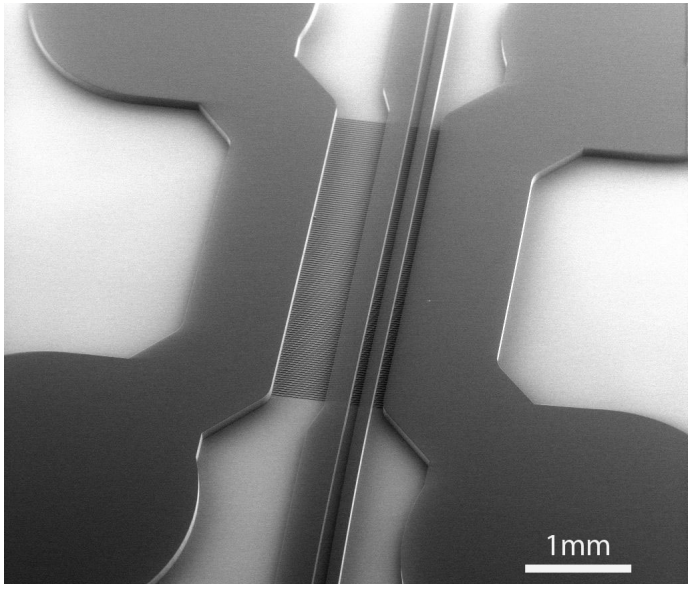
A.Virlogeux et al. Cell Reports 22-1 (2018)

Axotomy chamber

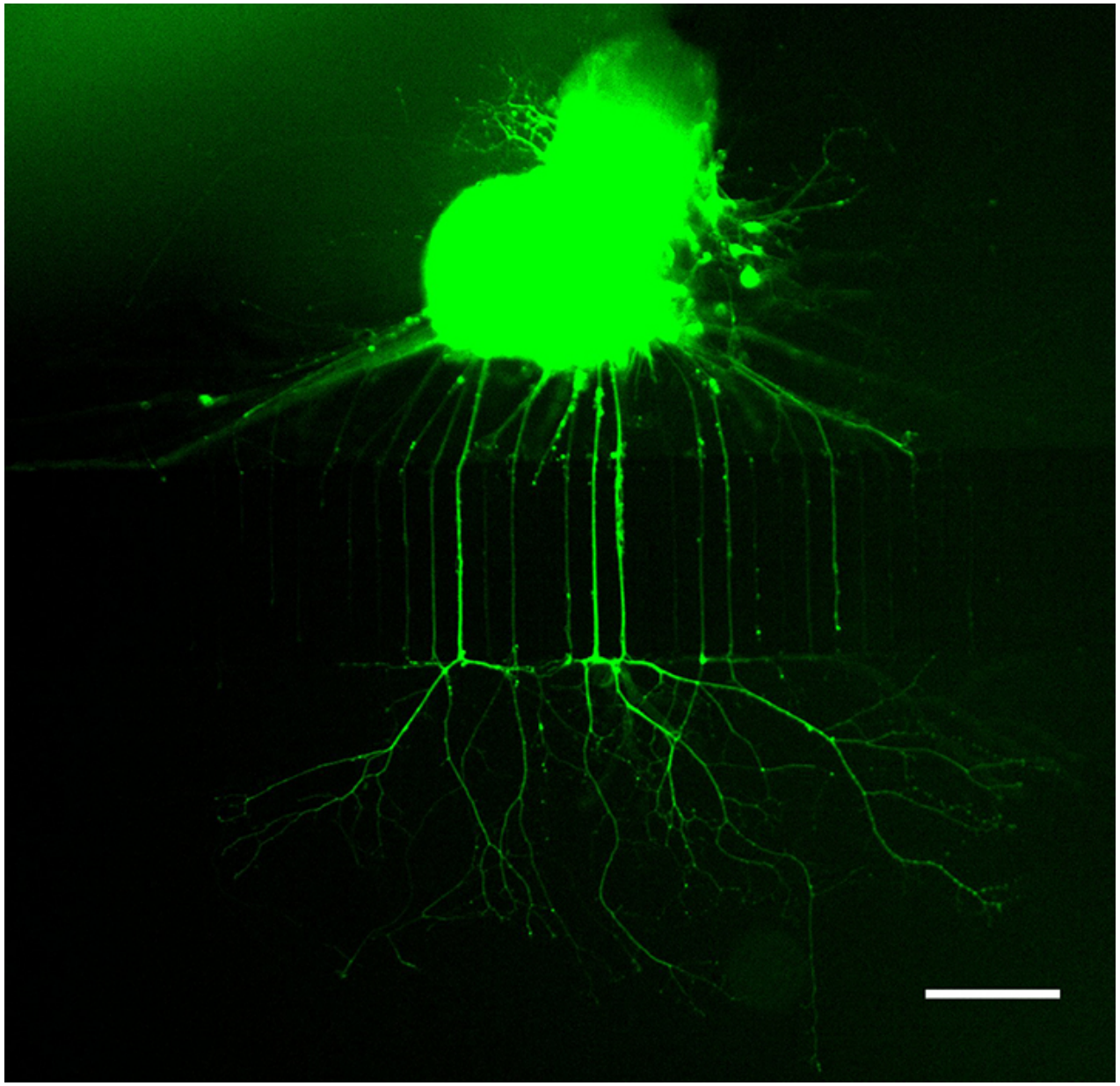




Culture chambers
Synaptic chamber
Axotomy chamber



Explant in chamber



Eran Perlson, U.Tel Aviv

Microfluidics + Micro Electrode Array (MEA)

Organisation

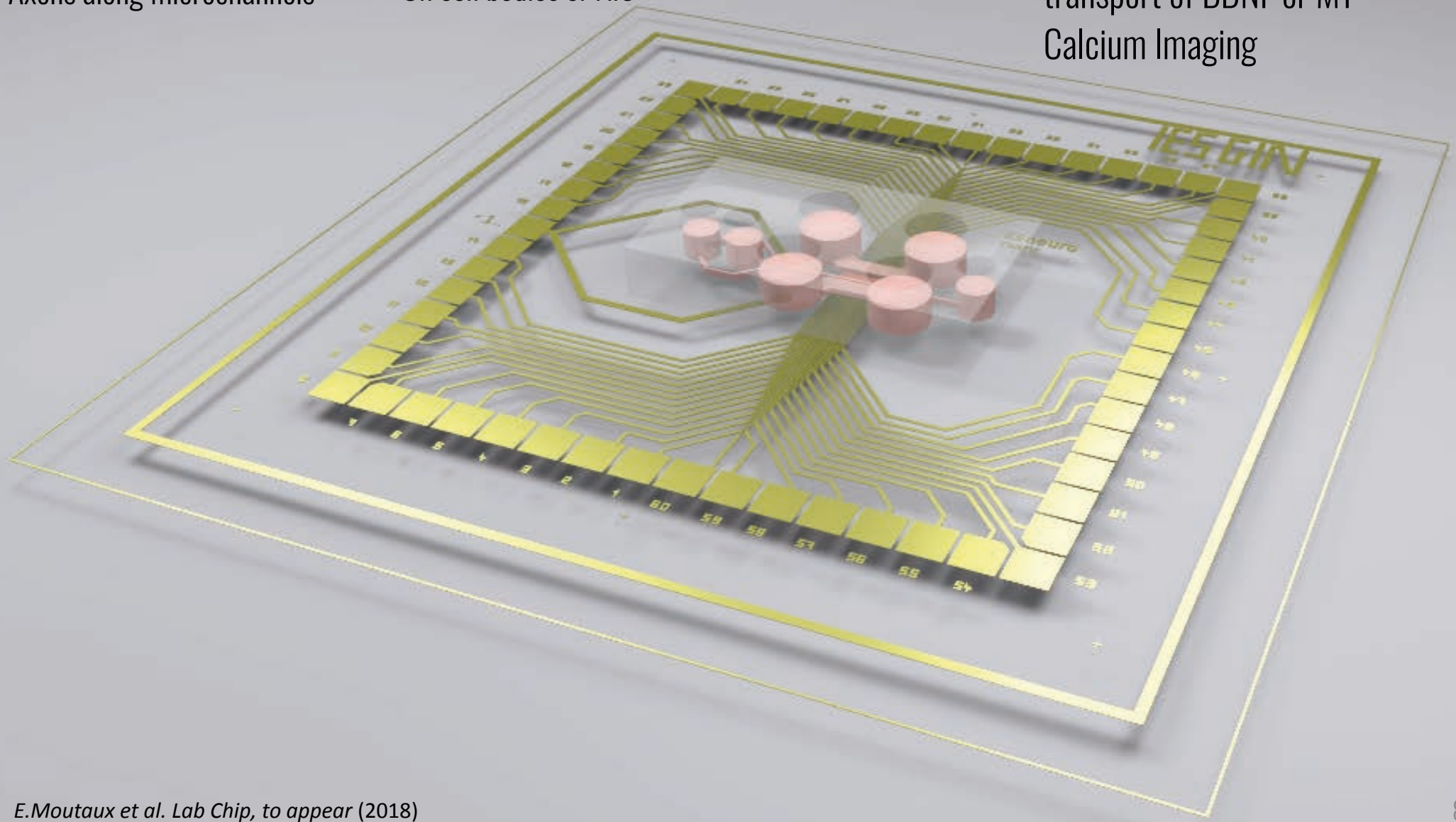
Axons along microchannels

+ Stimulation & Recording

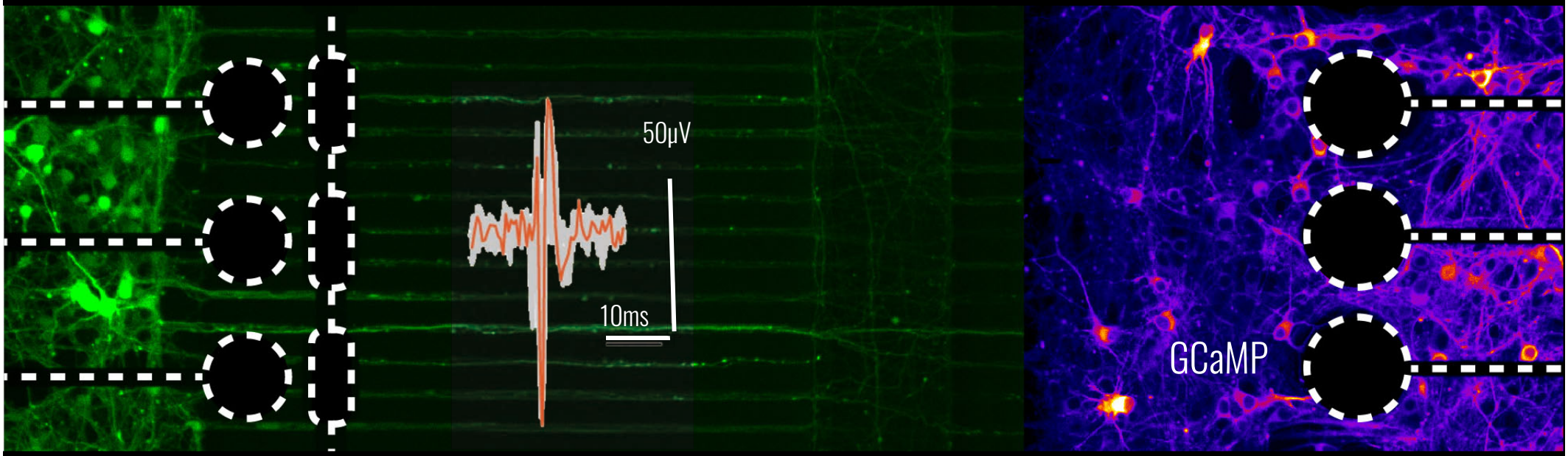
On cell bodies or AIS

+ Observation

transport of BDNF or MT
Calcium Imaging

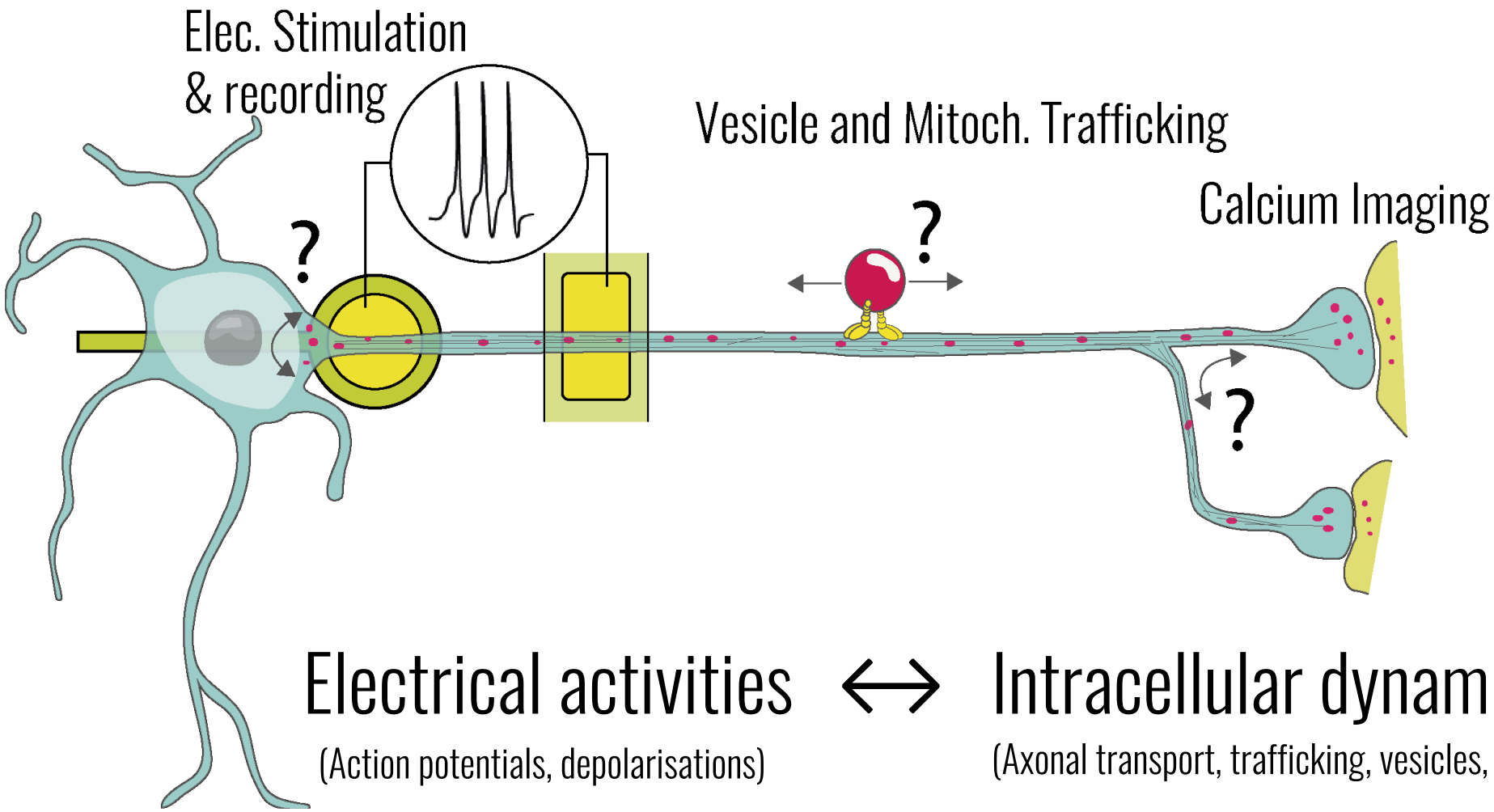


E.Moutaux et al. Lab Chip, to appear (2018)



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

Neurofluidics + Extracel. electrodes

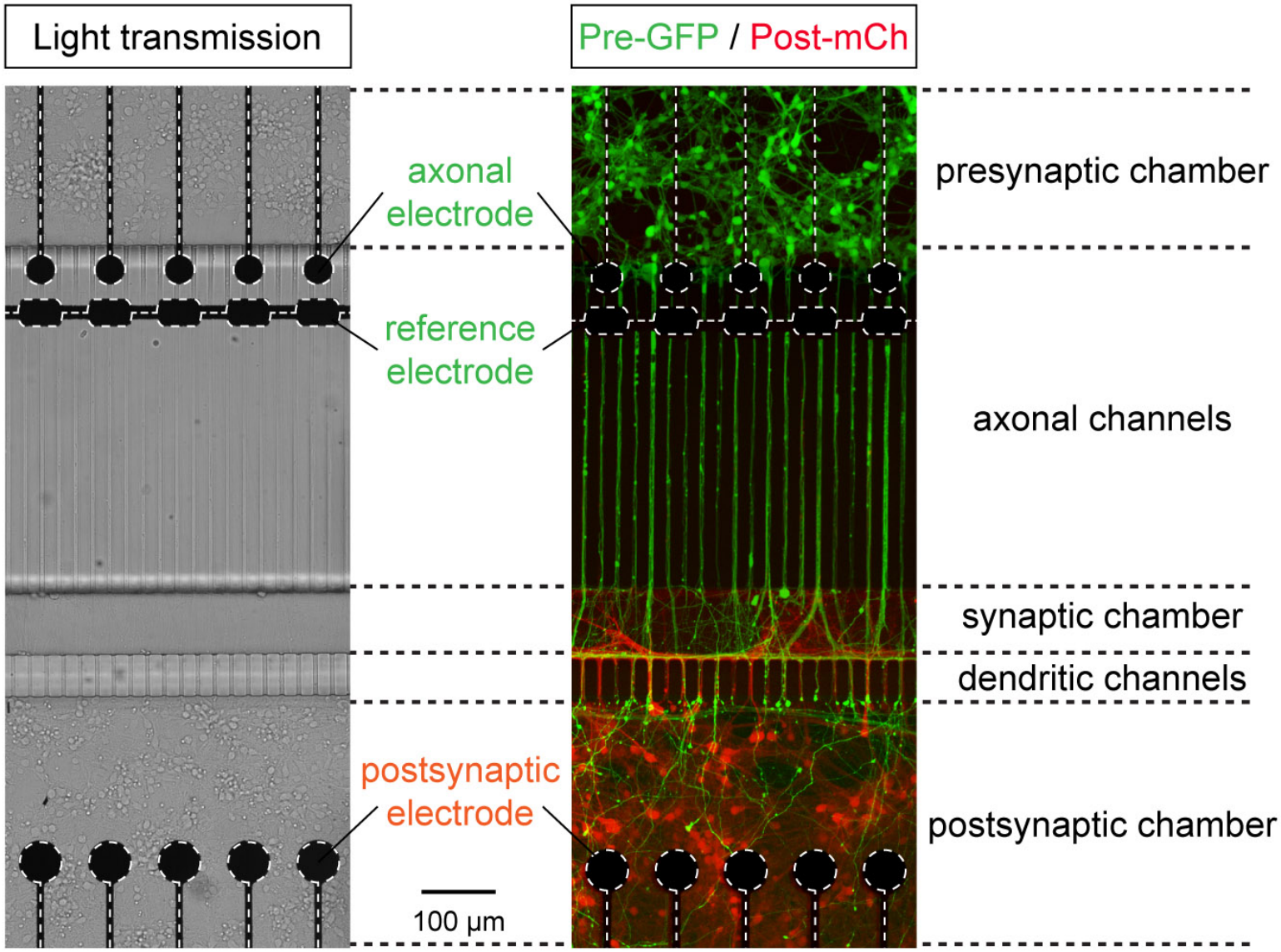


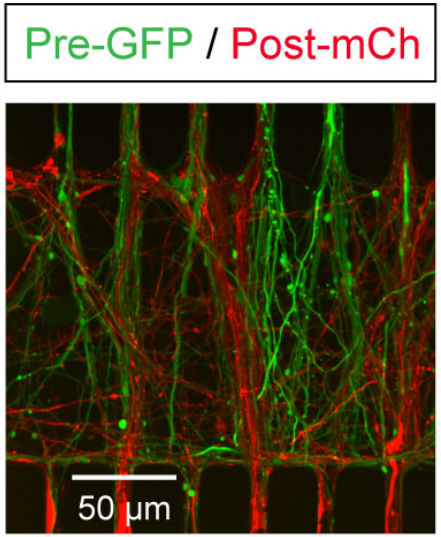
Electrical activities ↔ Intracellular dynamics

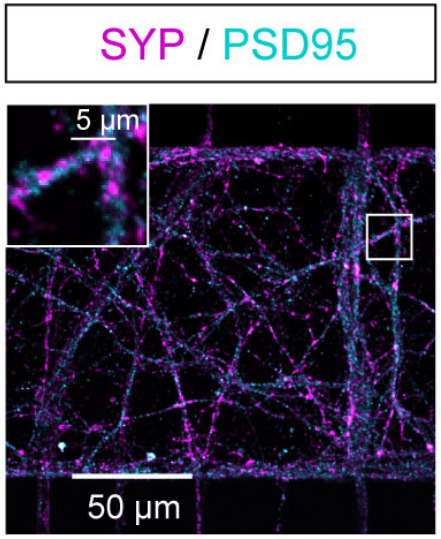
(Action potentials, depolarisations) (Axonal transport, trafficking, vesicles, RNA)

Neurodegenerative disease : HD Huntington disease, ALS, SMA

Electrode arrangement







MEA microfabrication

Thin glass substrate : 5x5cm 170µm

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

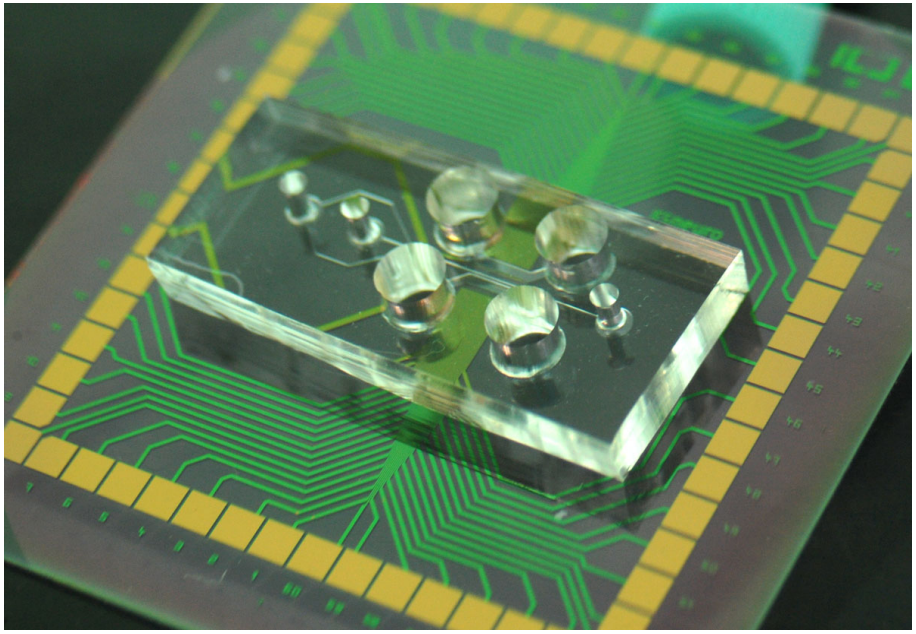
SiNx PECVD

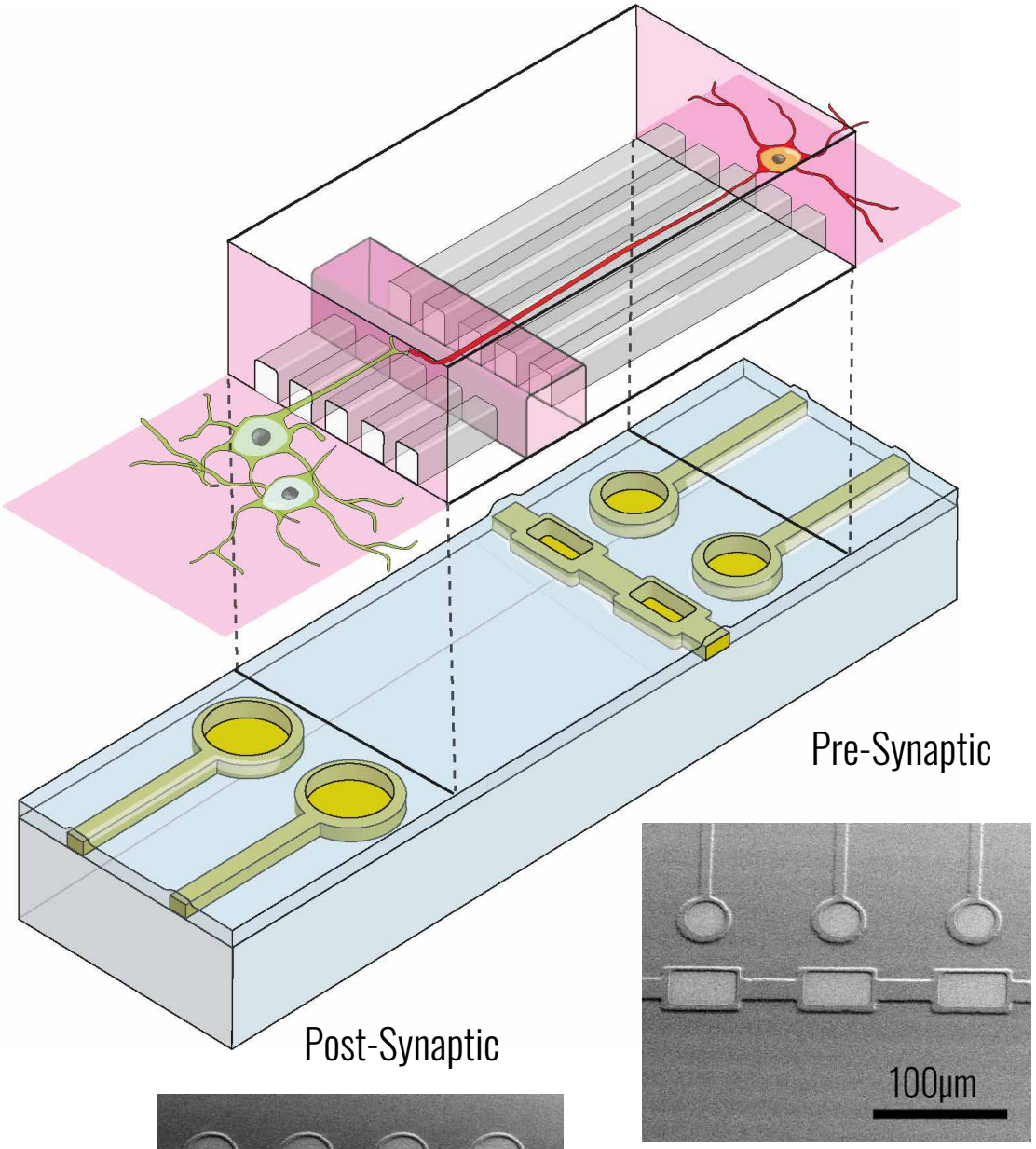
Mask2+ Alignment + RIE etching

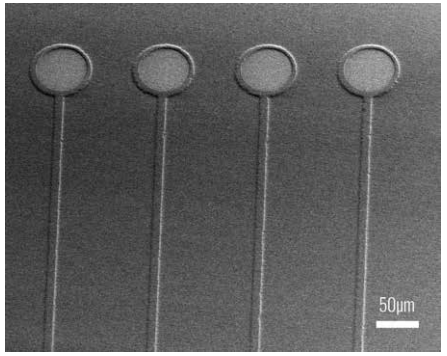
Cleaning, PDMS alignment and Bonding

Simple and Stable process

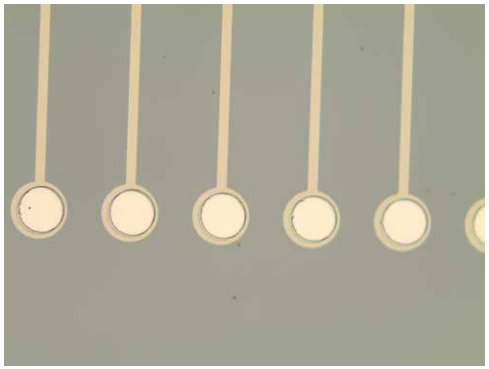
For series > 100 samples

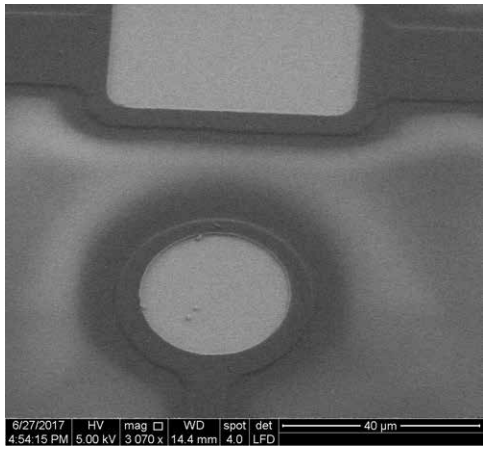


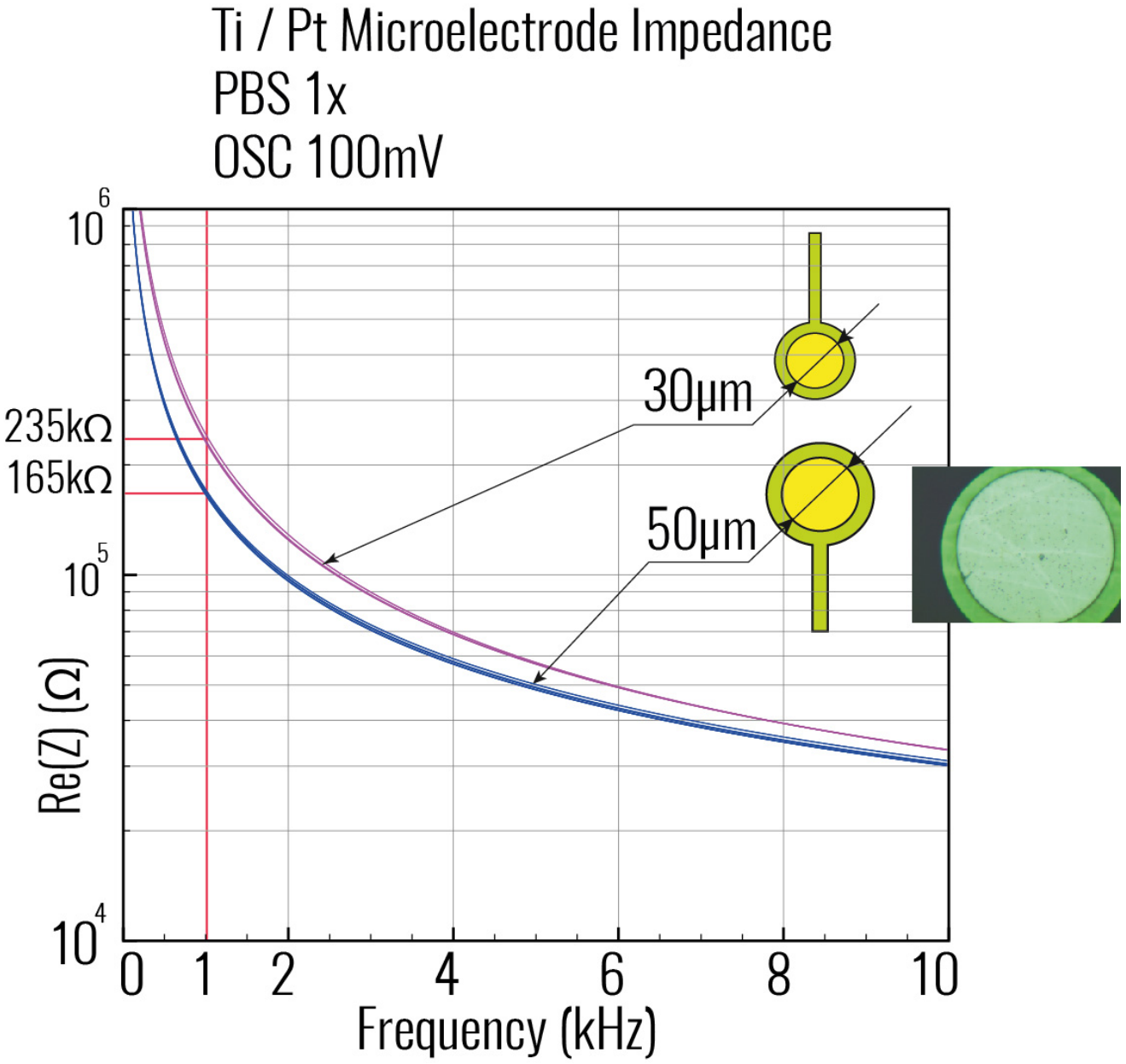




Impedance

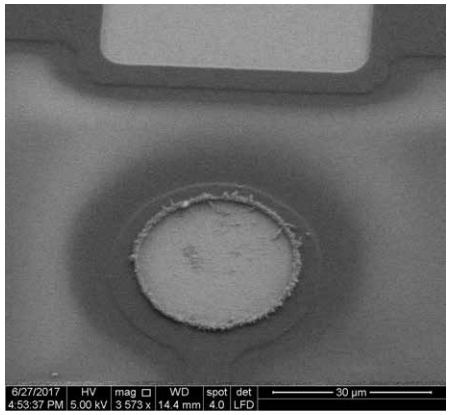


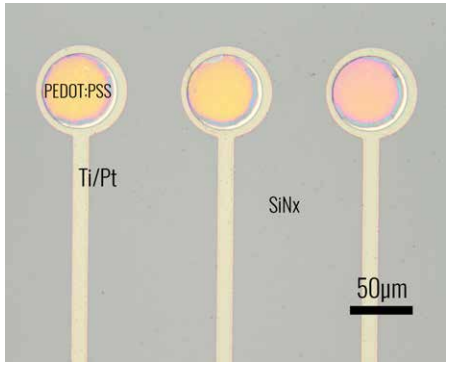

6/27/2017 4:54:15 PM
HV 5.00 kV
mag 3 070 x
WD 14.4 mm
spot 4.0
det LFD
40 µm

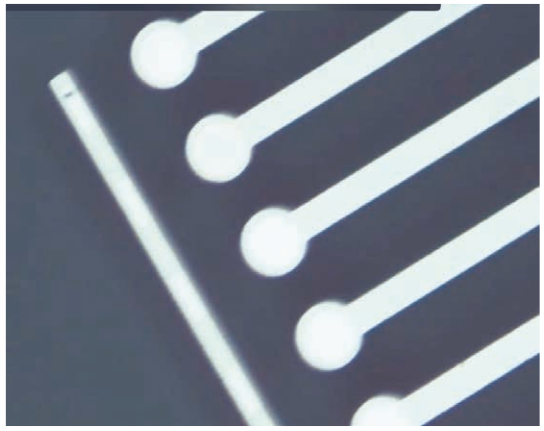

Ti / Pt Microelectrode Impedance
PBS 1x
OSC 100mV
10^6
235kΩ
165kΩ
10^5
10^4
Re(Z) (Ω)
0
1
2
4
6
8
10
Frequency (kHz)
30µm
50µm

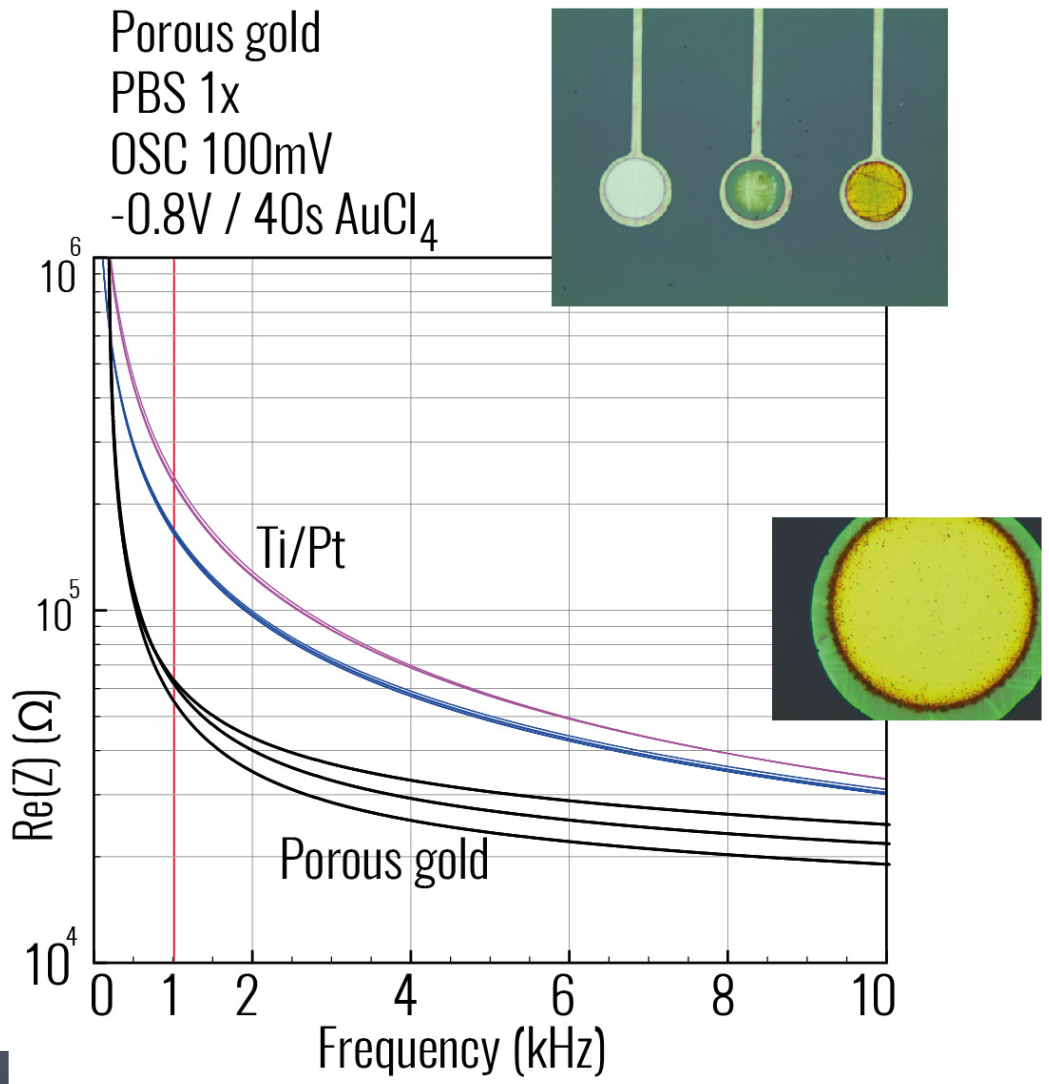
Impedance lowering

Ti/Pt
TiN
PEDOT:PSS
Porous gold Electrodeposition







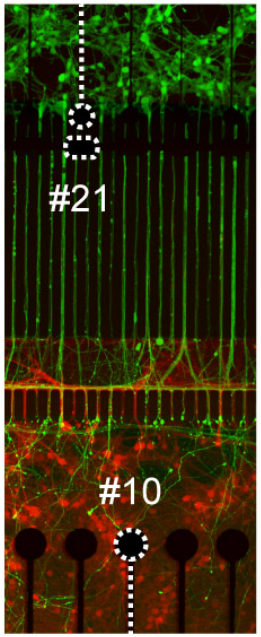


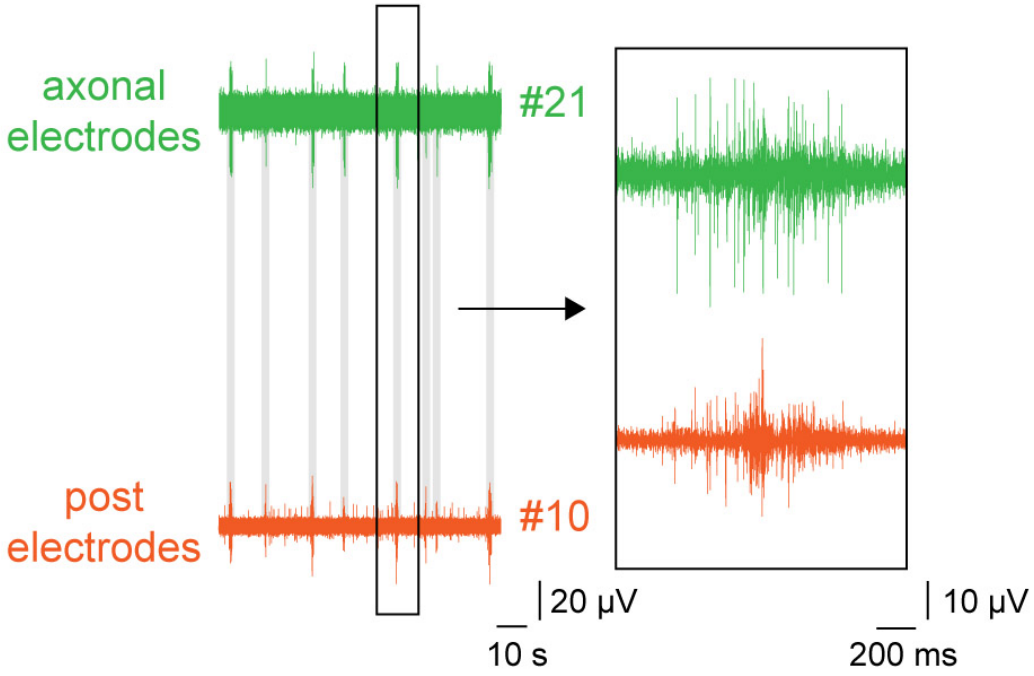
But..... Platinum for stability and reproducibility

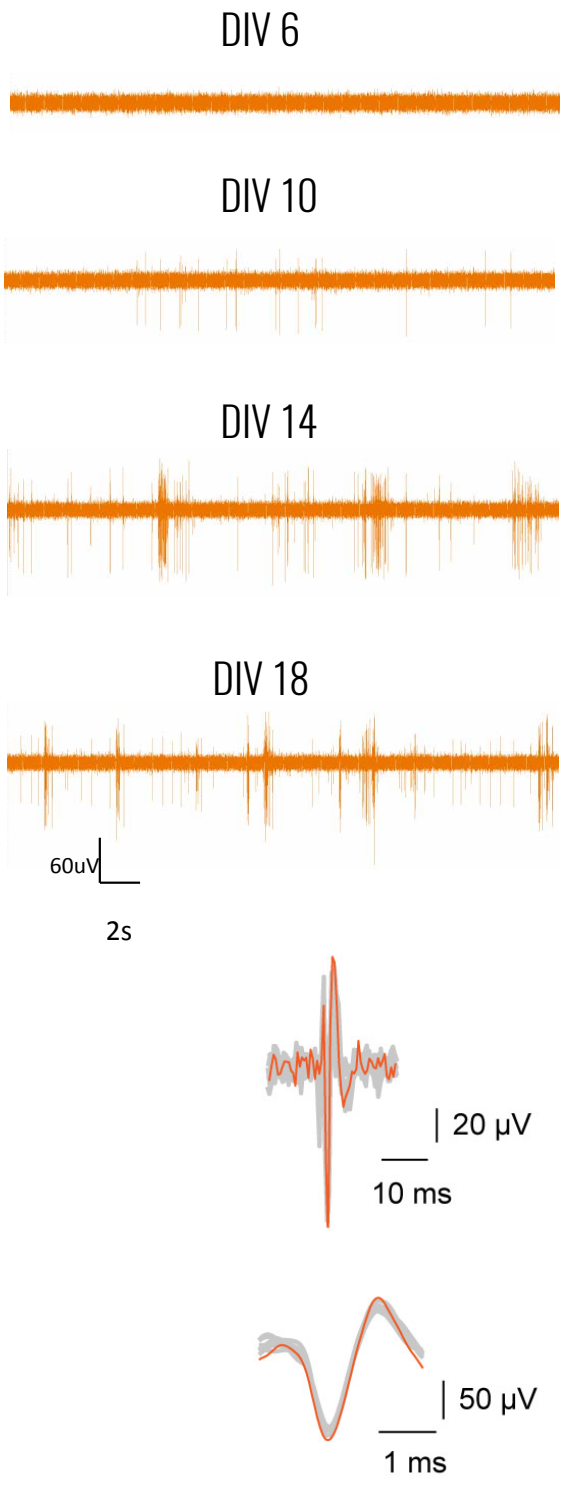
Extracellular Recording

Spikes detection (SNR>5)

Spontaneous activity DIV10

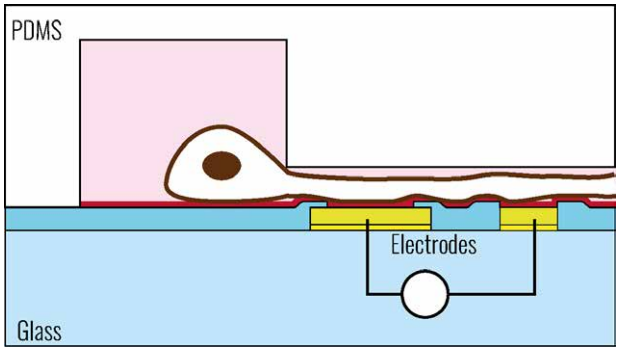




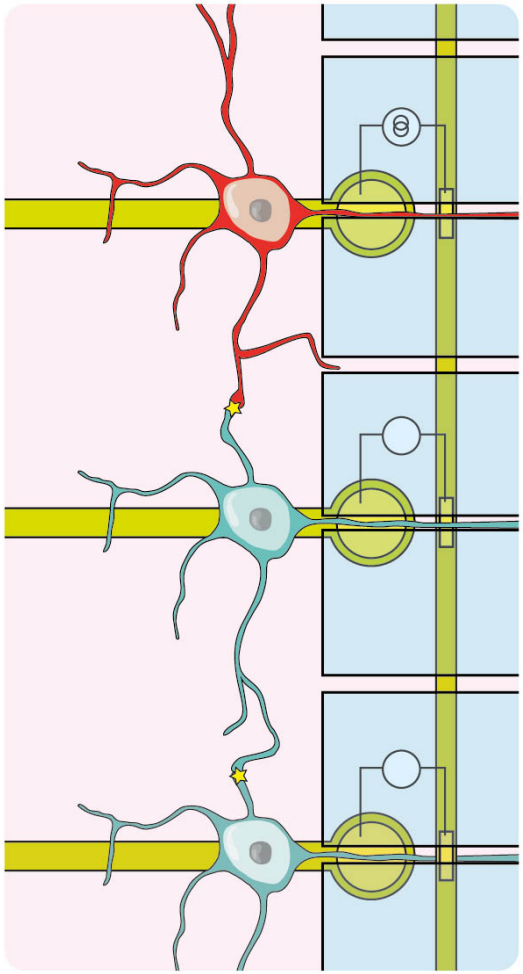


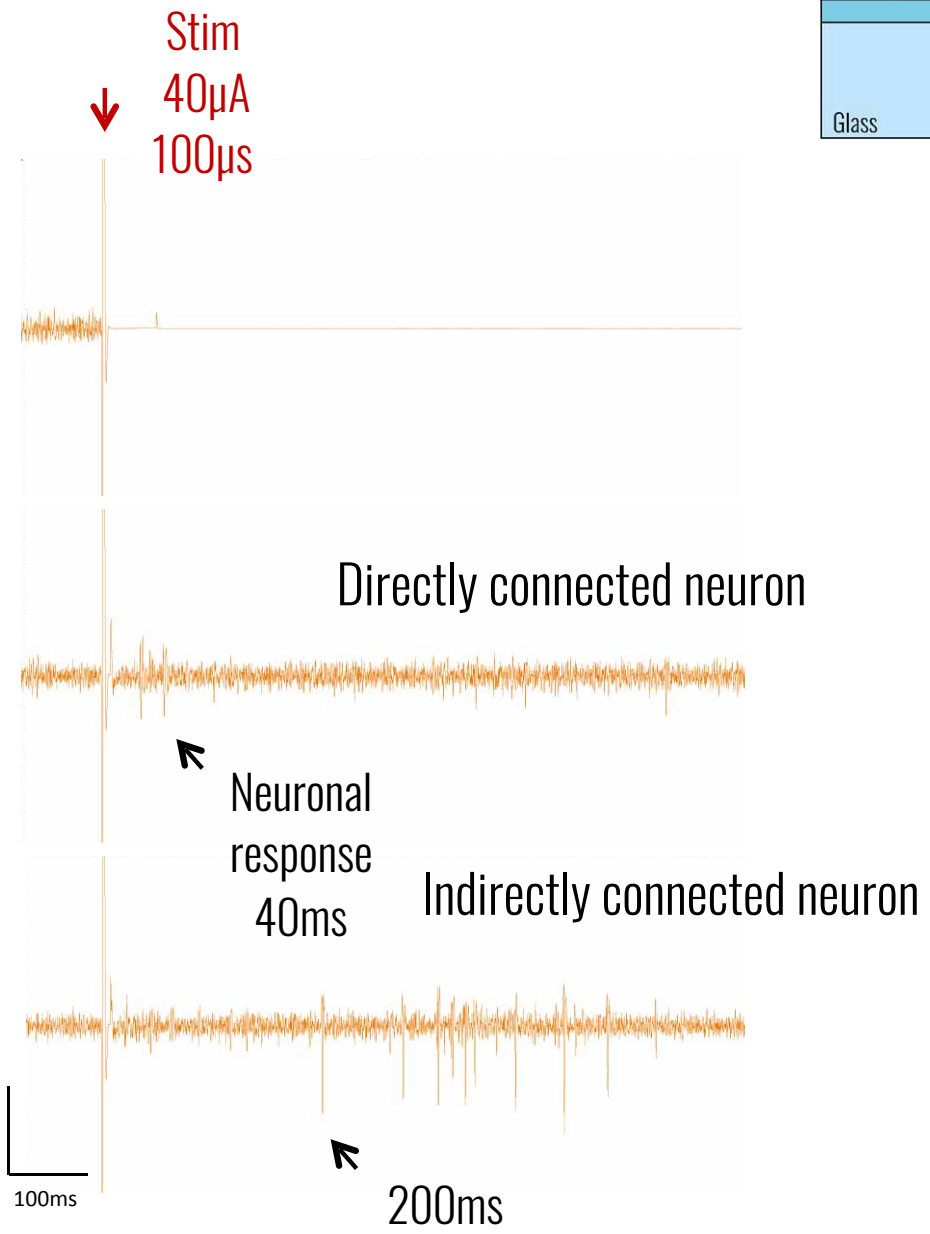
Self-organization and synchrony of the network

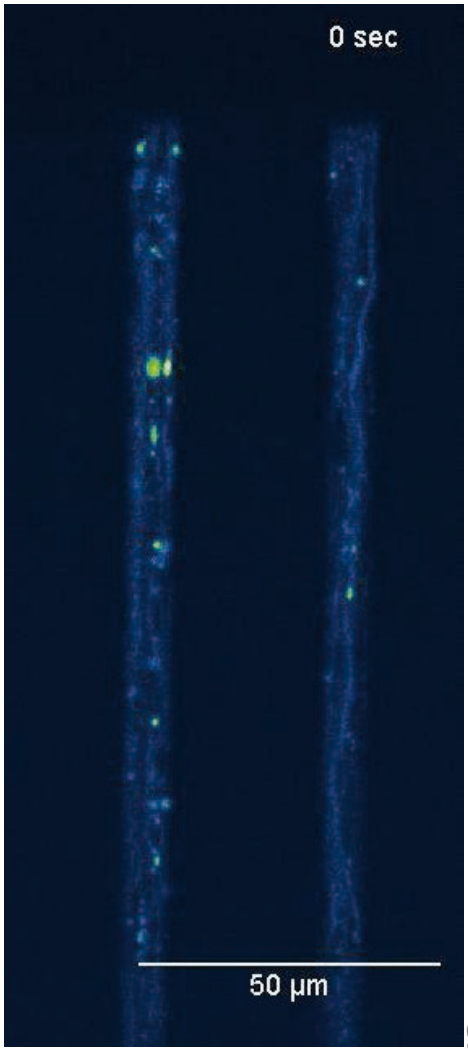
Extracellular Stimulation



Current stimulation
Axonal (AIS) stimulation

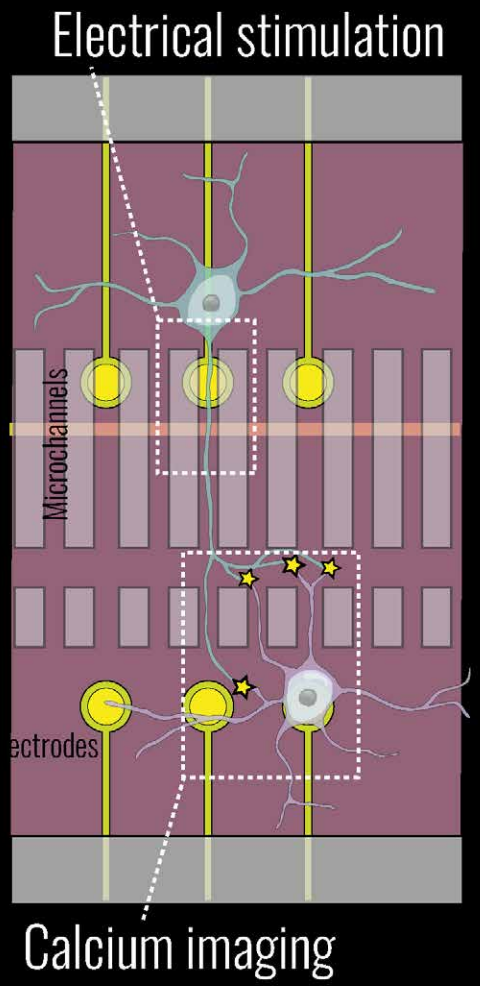




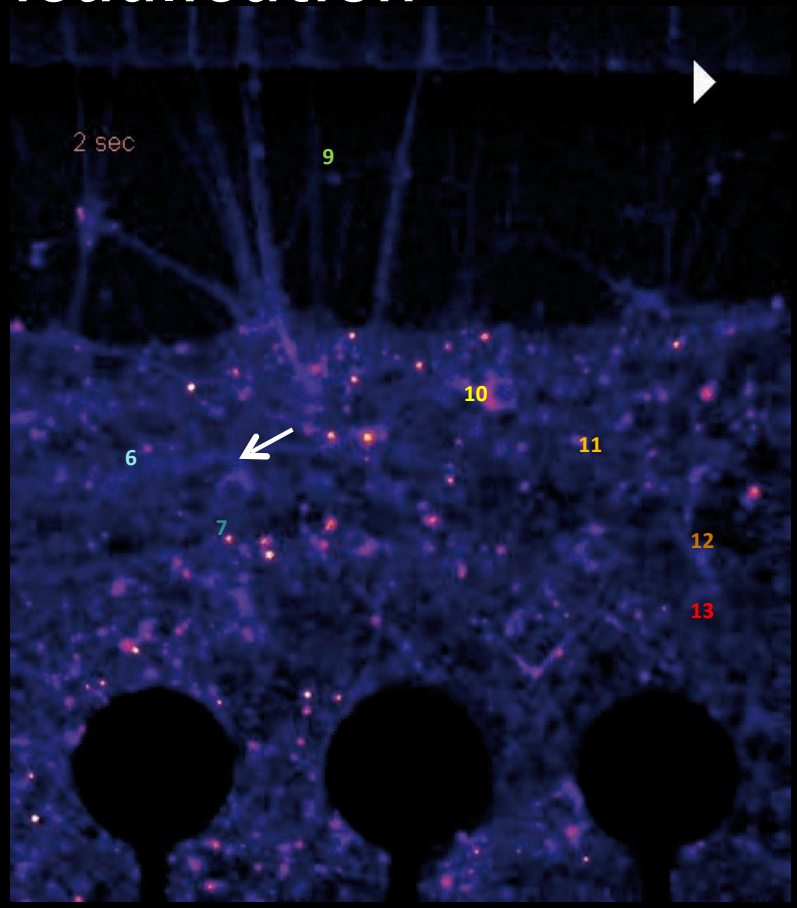


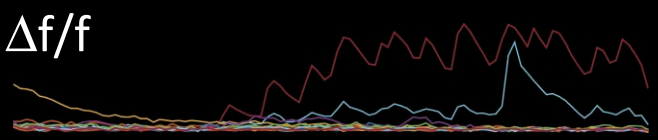
Stimulation+ GCaMP6f visualisation

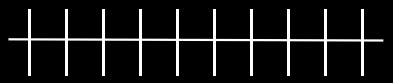
Genetically Encoded Calcium Indicators



1Hz





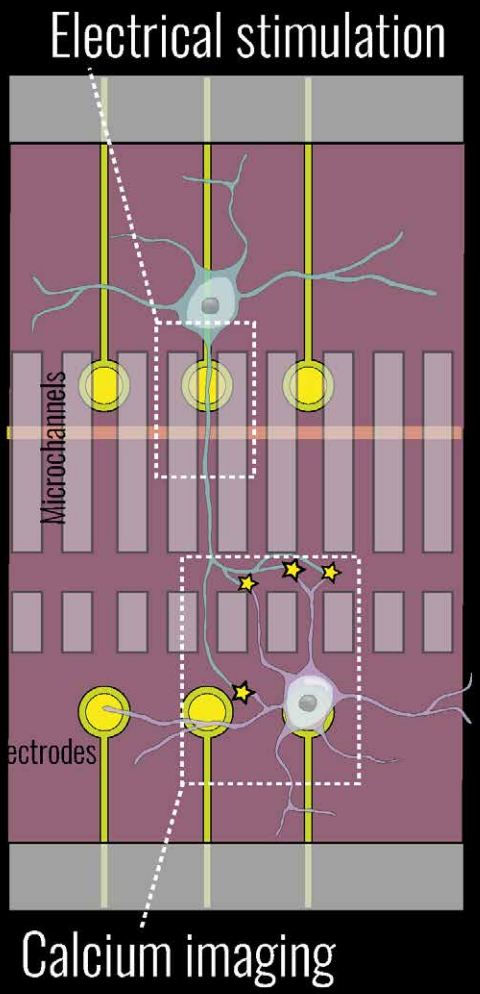


Small amplitude, repeated

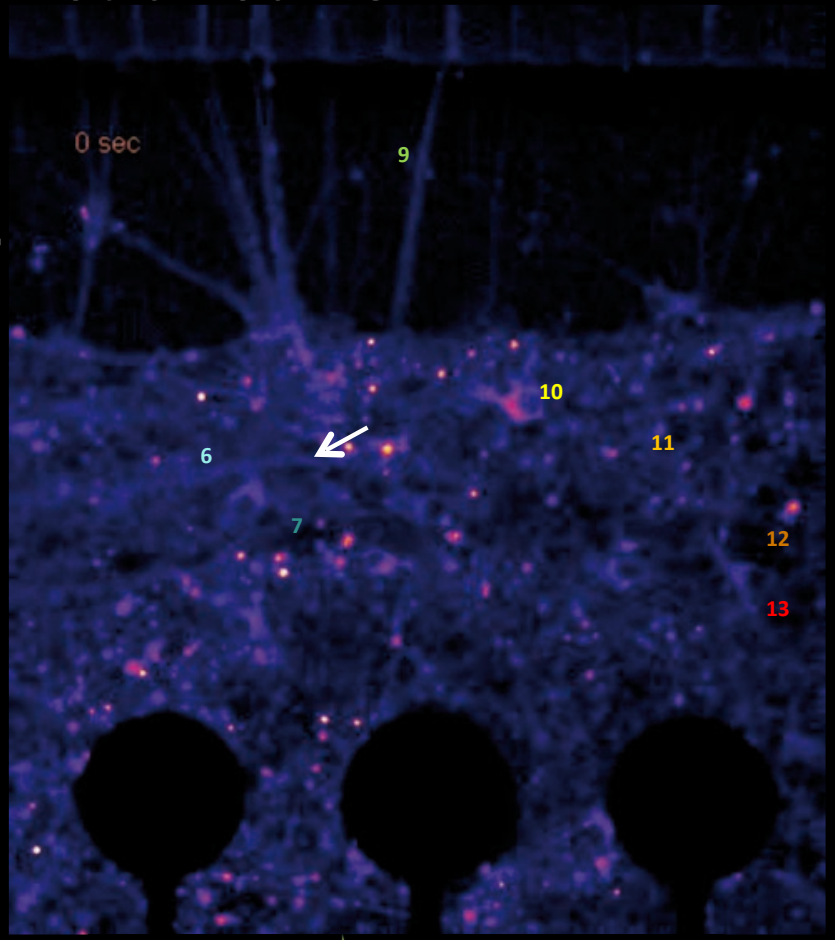
LTD long term depression : Decrease in synaptic strength induced by LF stimulation of presynaptic afferents

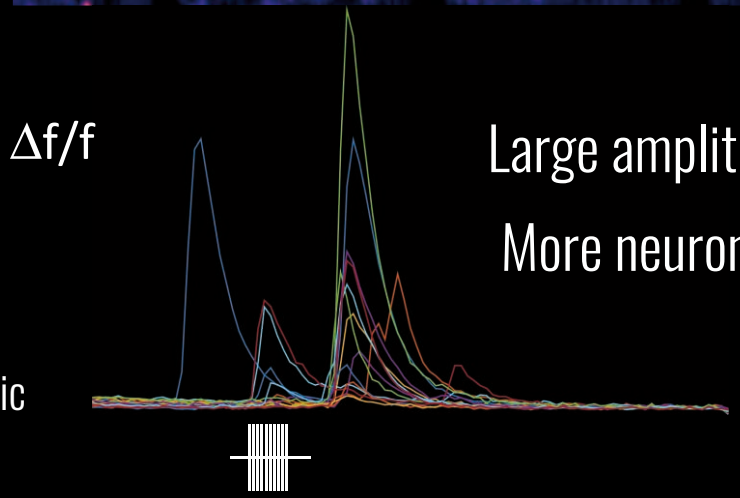
Stimulation+ GCaMP6f visualisation

Genetically Encoded Calcium Indicators



50Hz



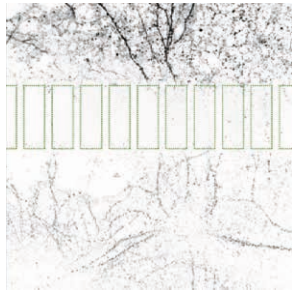


Large amplitude, long signal

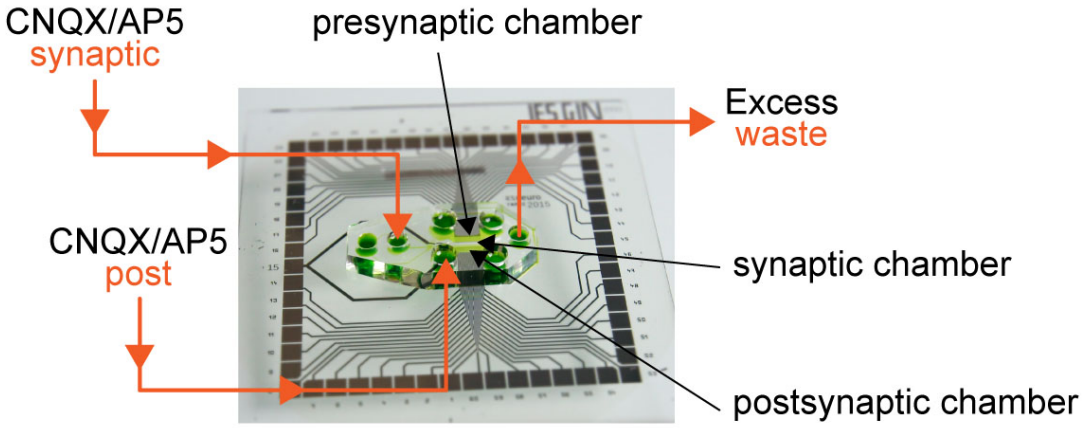
More neurons are recruted

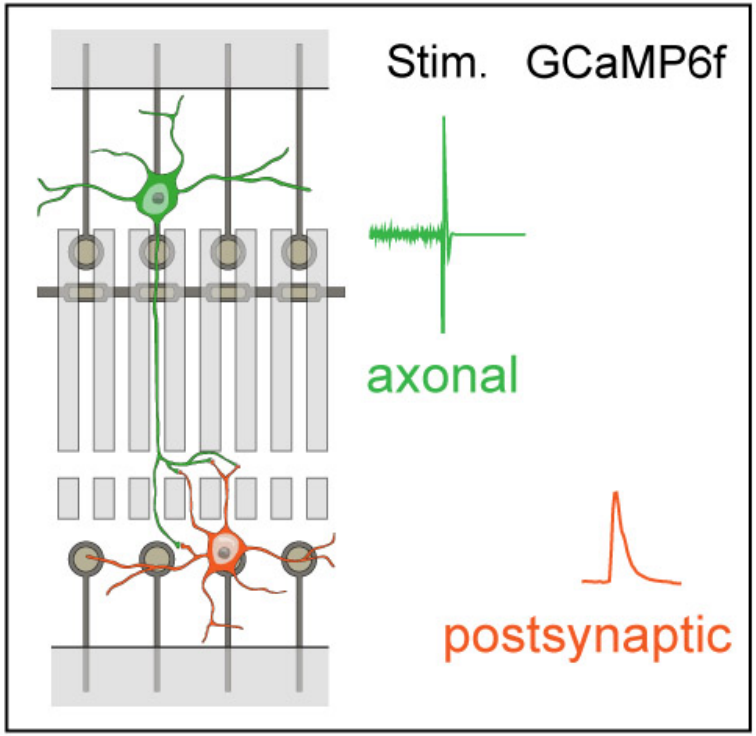
LTP long term potentiation: Persistent increase in synaptic efficacy produced by high-frequency stimulation

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

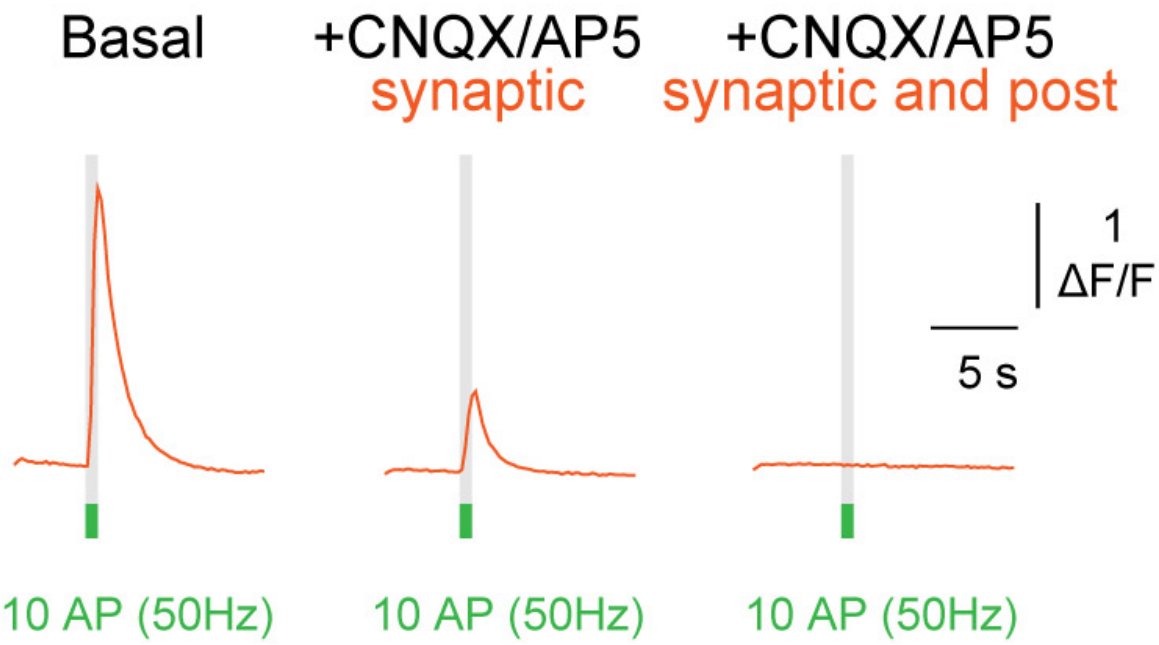


Synaptophysin



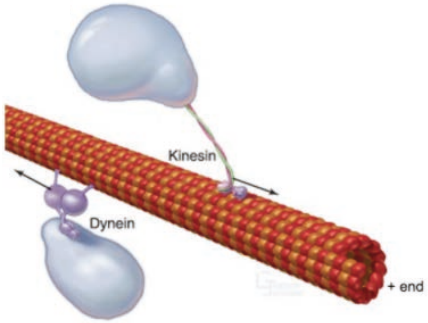


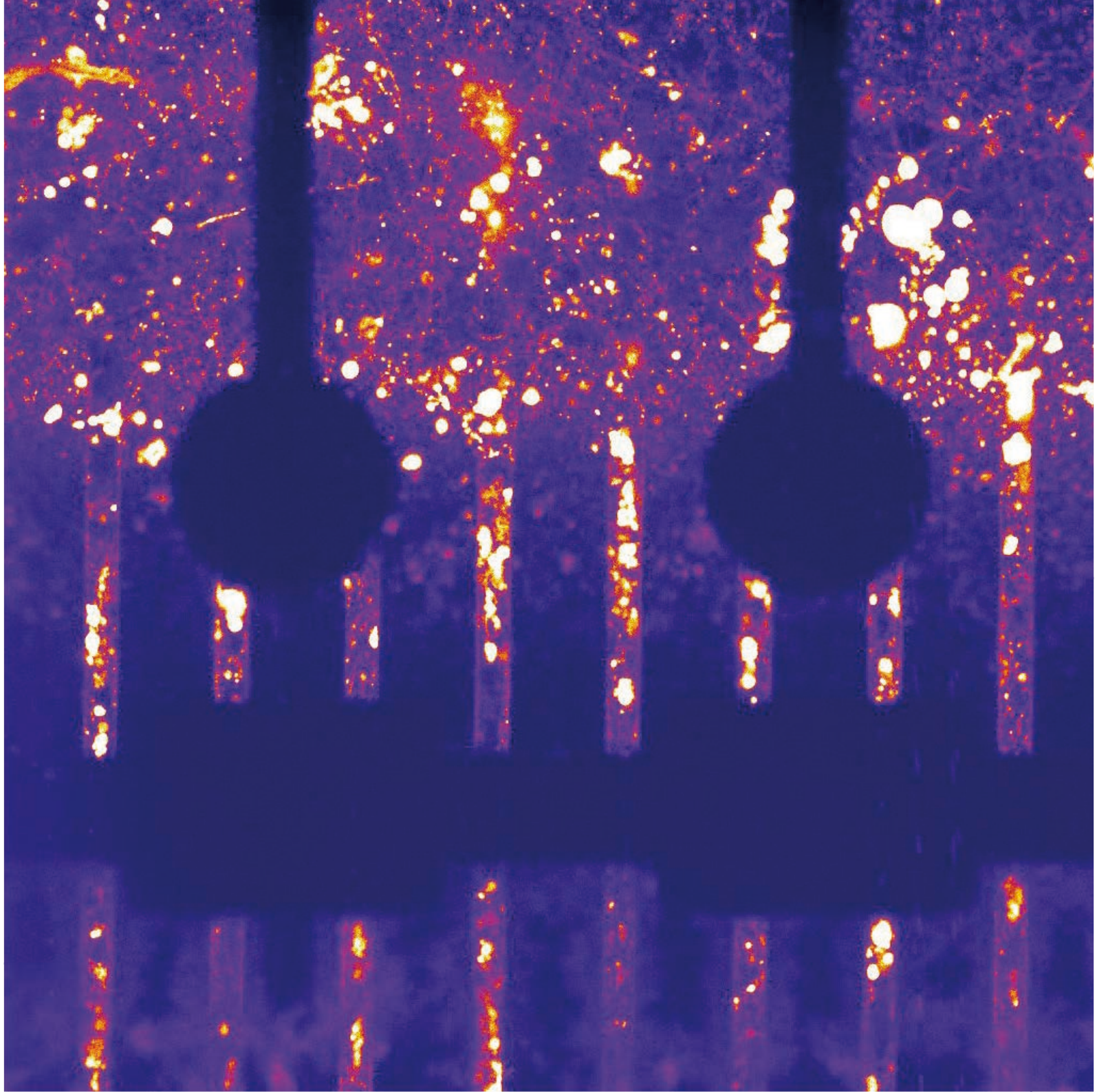
E.Moutaux et al. Lab on Chip 2018



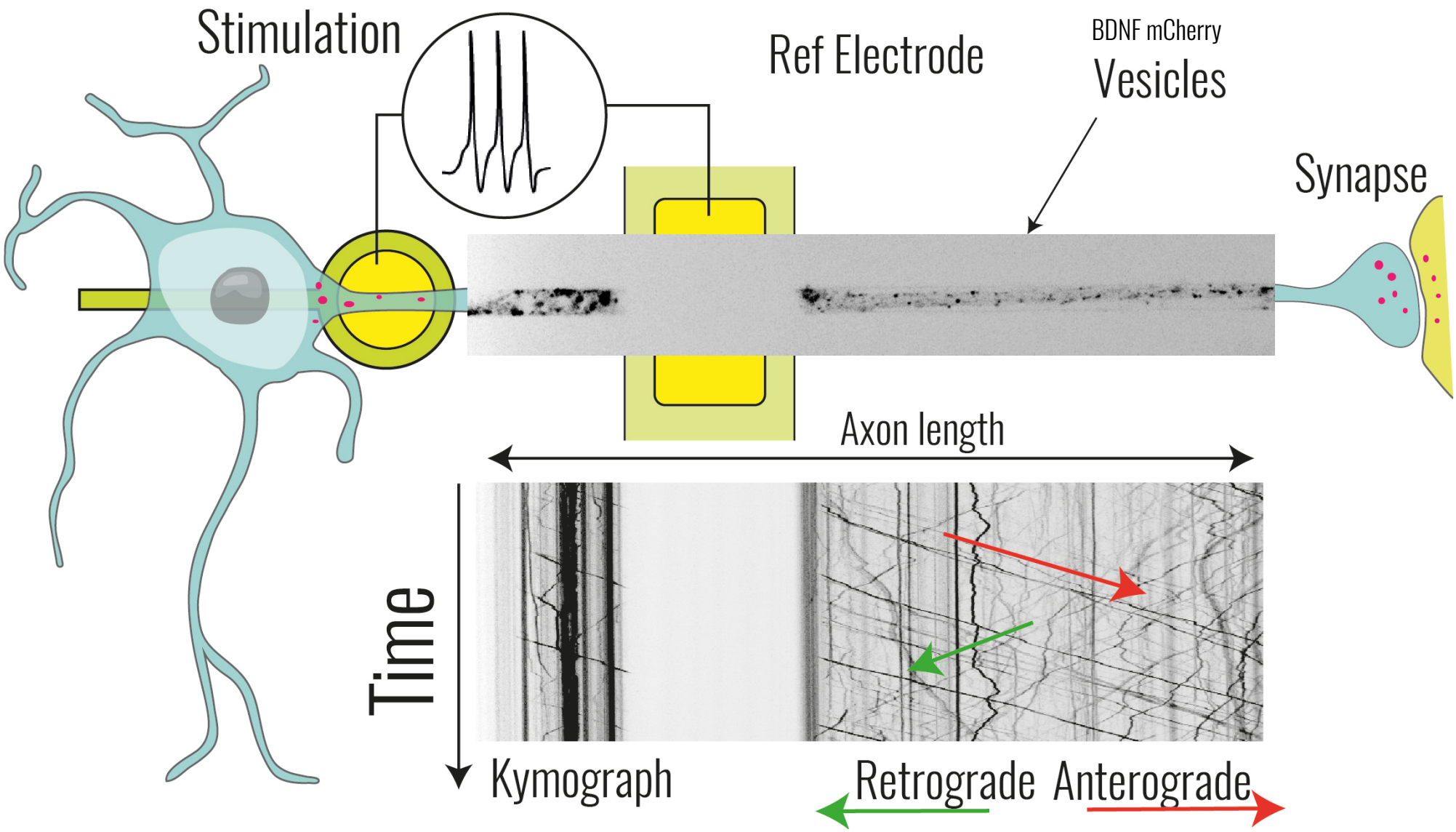
AMPA/Kainate and NMDA receptor antagonist

Axonal Transport



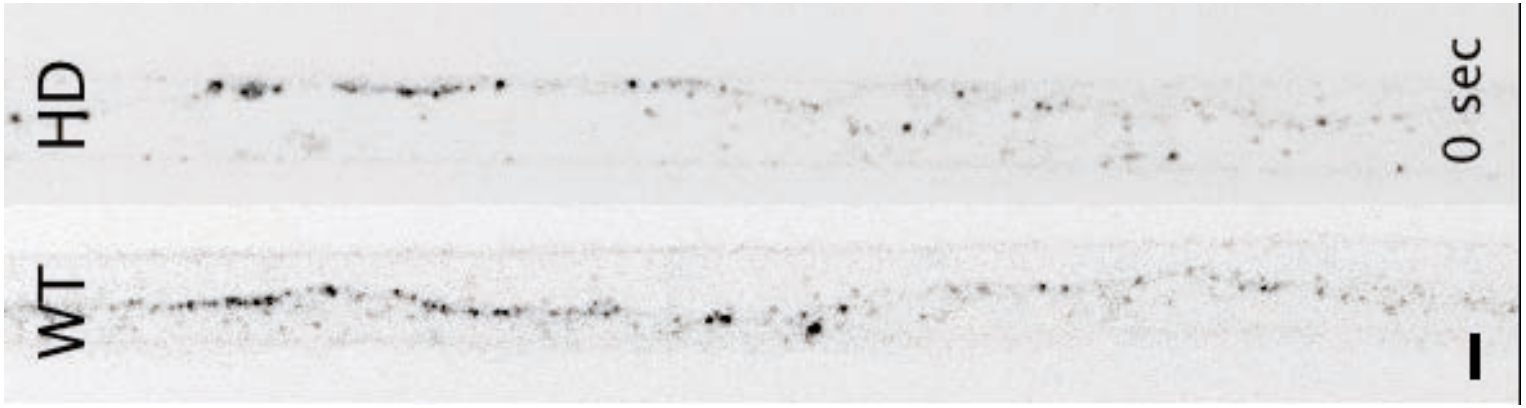


Axonal Transport under Stimulation

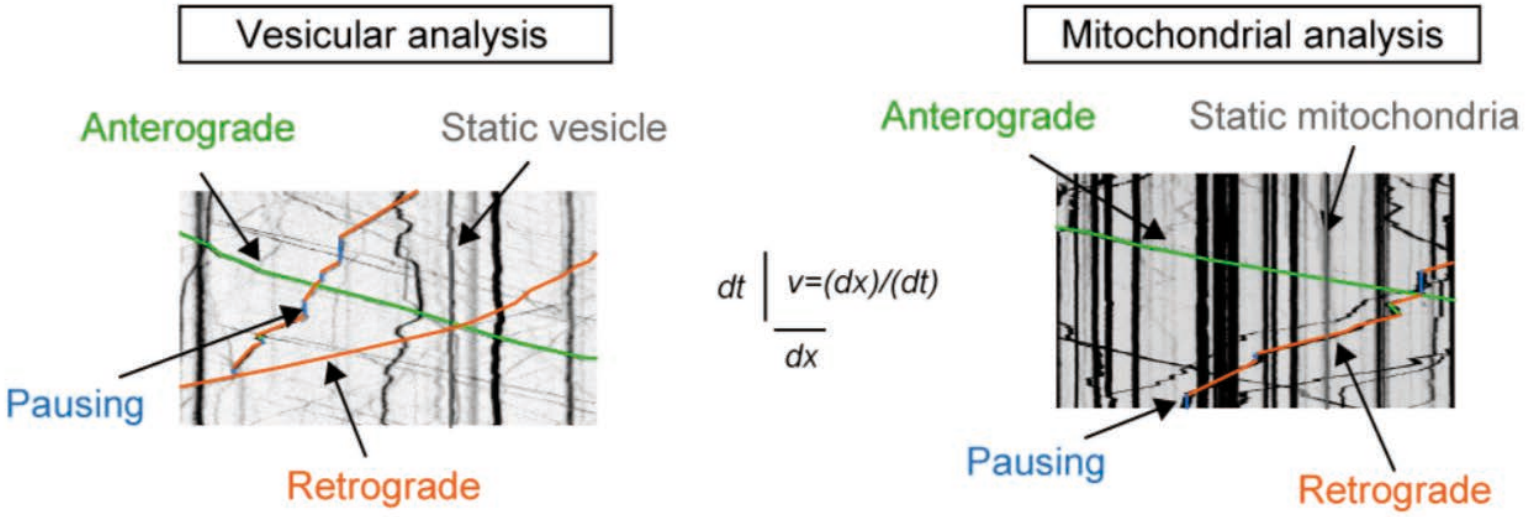


How the neuronal activity will be decoded and translated into a **regulation of axonal transport**

Axonal Transport



Defects in fast axonal transport (BDNF) in Huntington disease



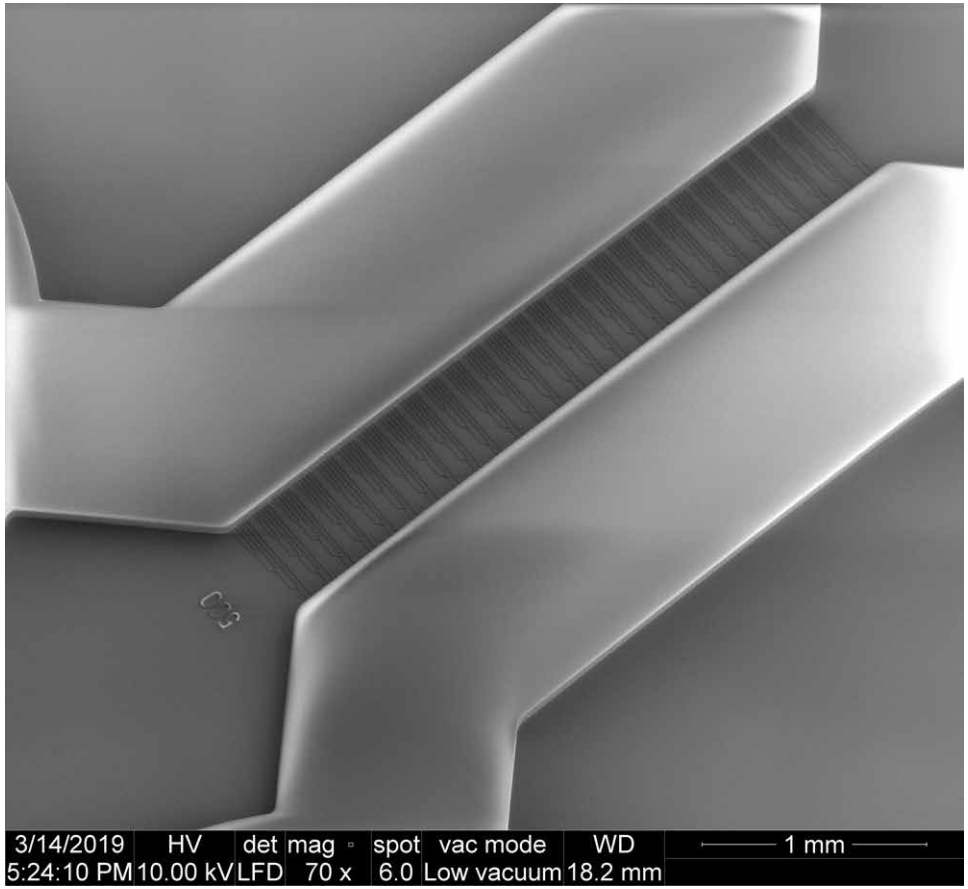
Vesicular motility increases with neuronal network maturation

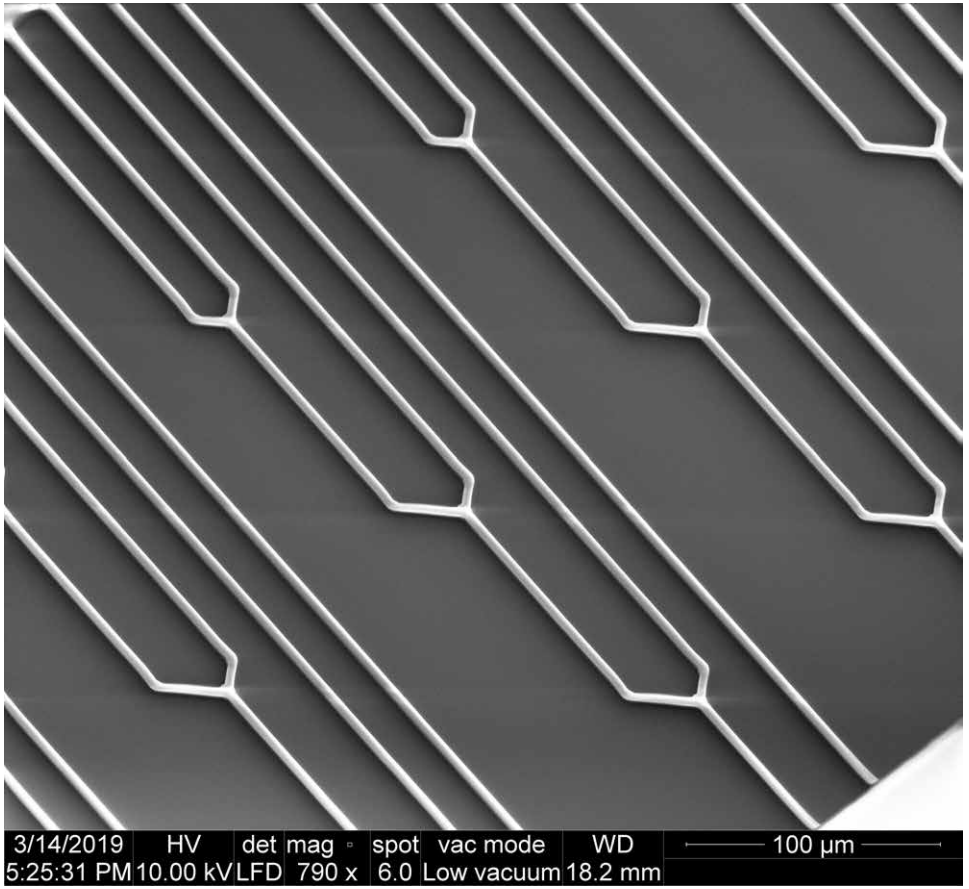
Mitochondrial dynamics decreases with network maturation

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

E.Moutaux et al. Sci. Rep., to appear (2018)

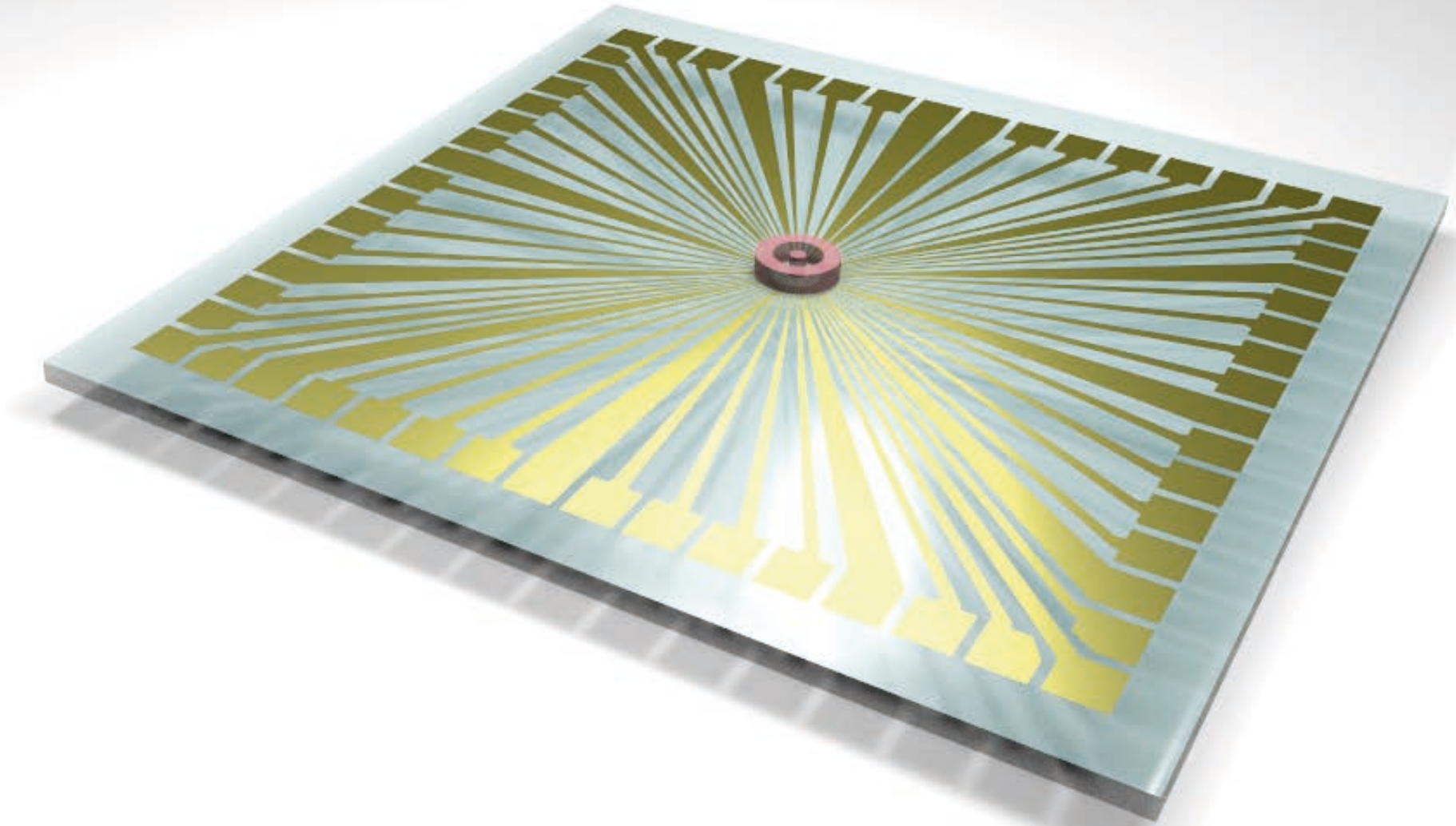
Axonal Transport





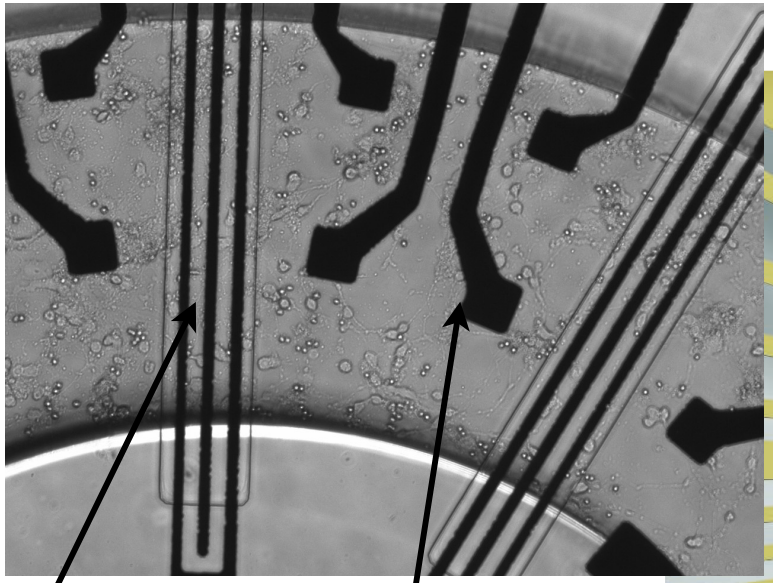
On going : Chronic electrical stimulation

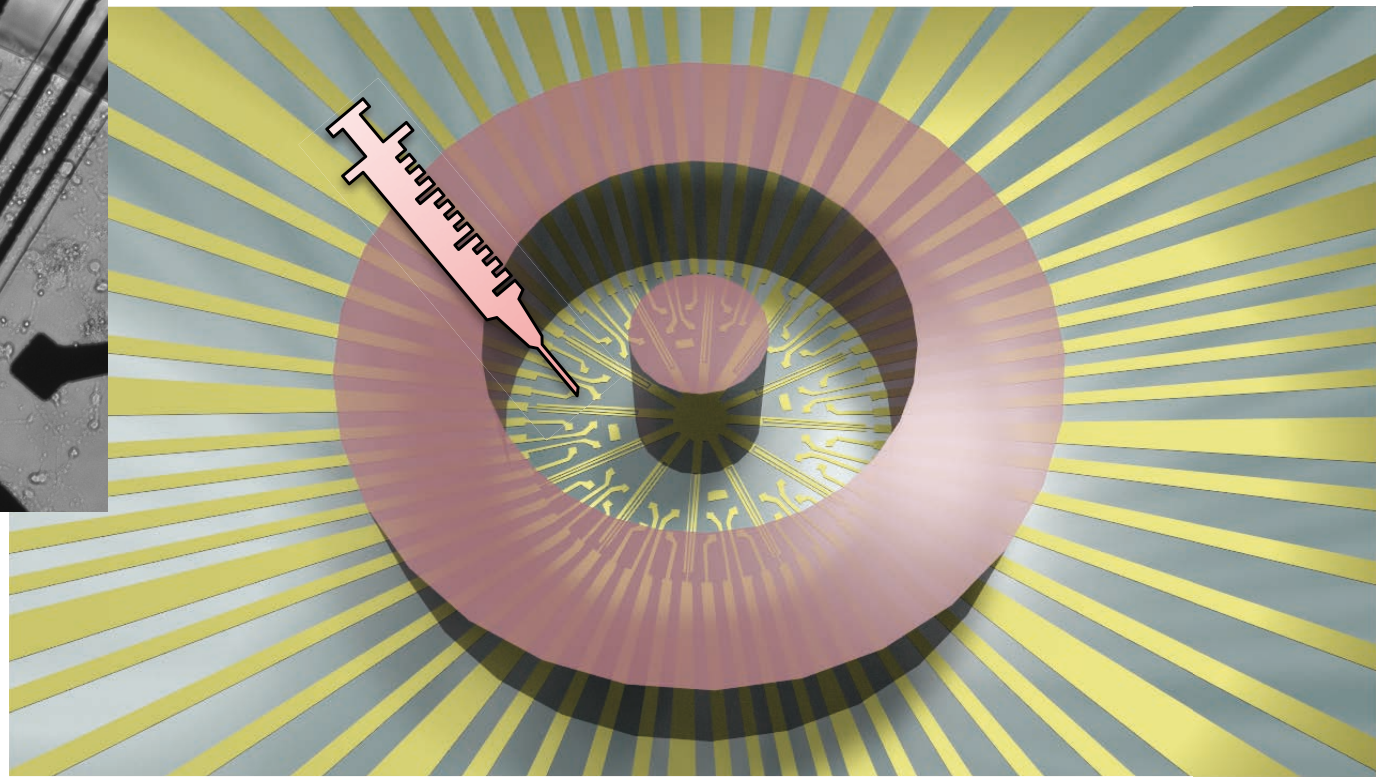
Understand the role of chronic neural oscillatory and synchronisation on neural networks



Malishev, E. *et al.*. *J. Phys. Conf. Ser.* **643**, 012025,

On going : Chronic electrical stimulation



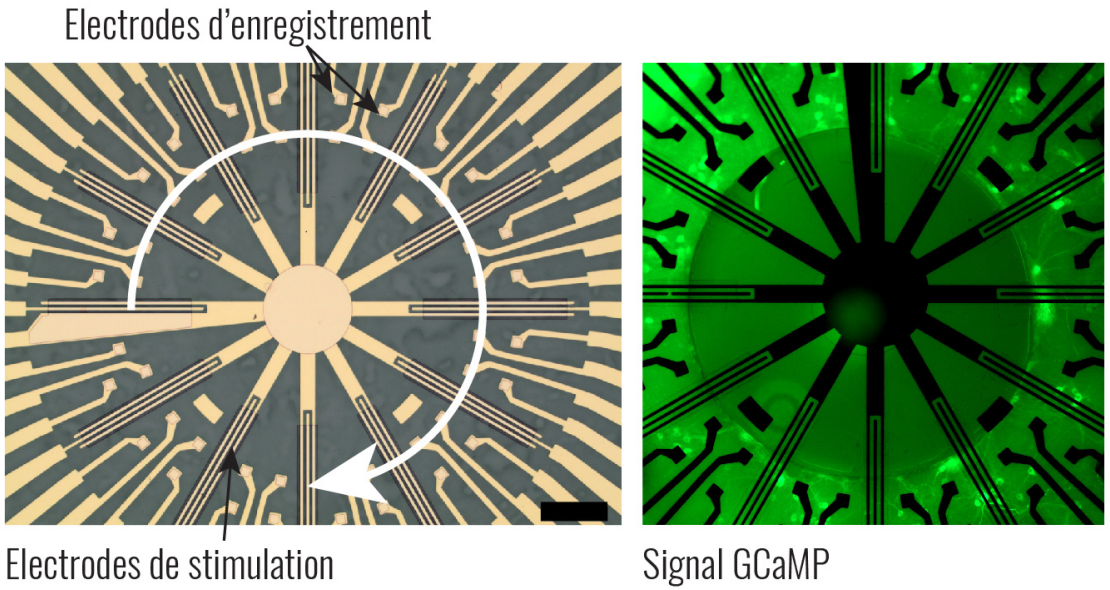


apply 1000 - 10 000 stimuli per day during the culture development

On going : Chronic electrical stimulation

Collab. Florence Rage

Neuronal Oscillator



Chambre de culture torique

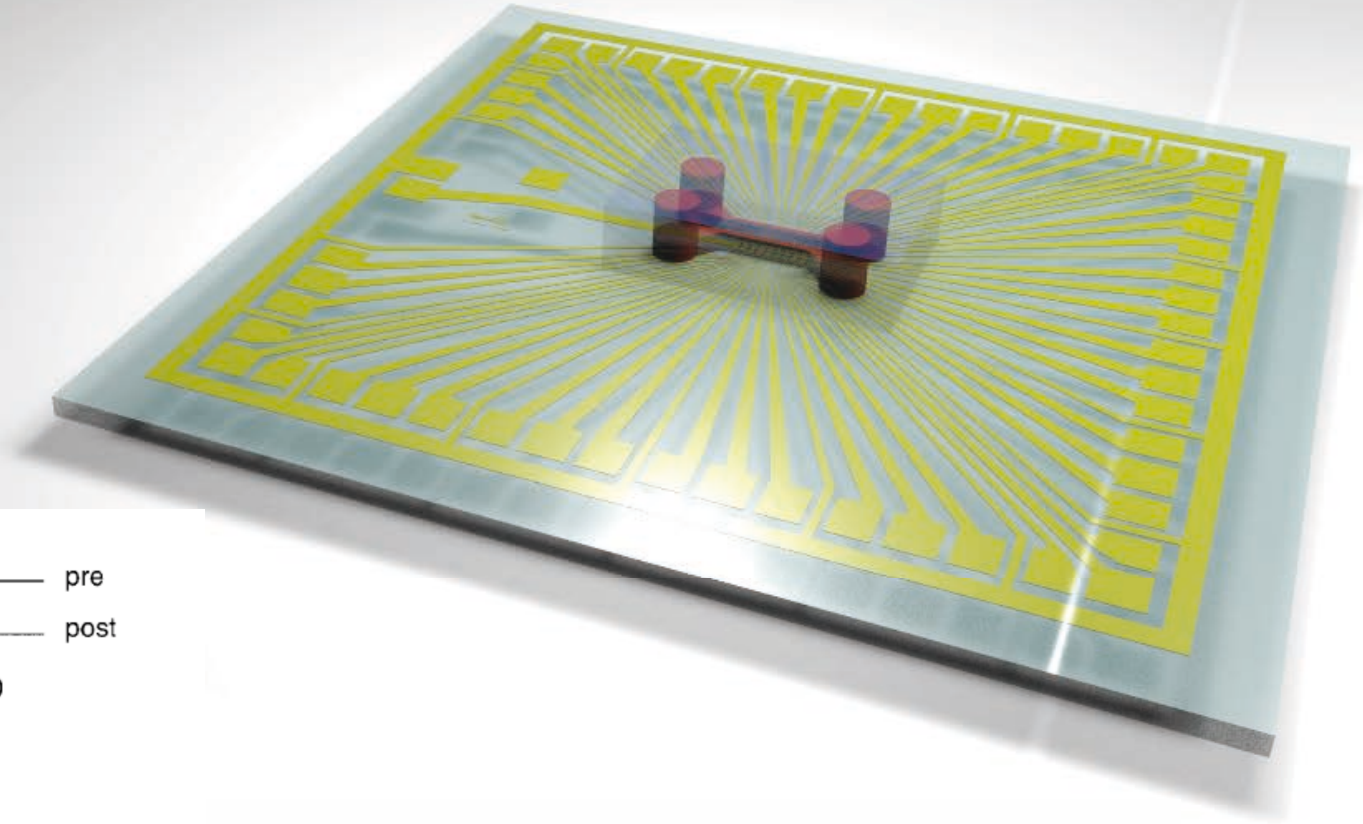
Evocation de vagues d'activité « tournantes »

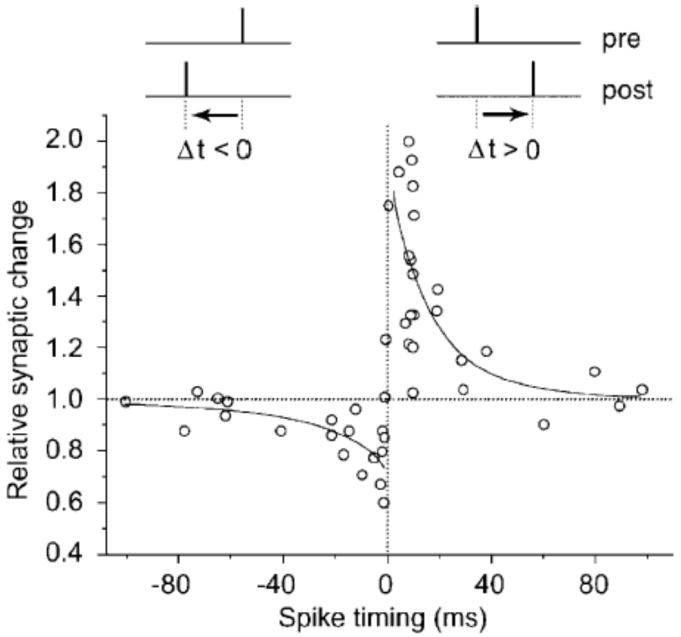
Etude des évènements synchrones

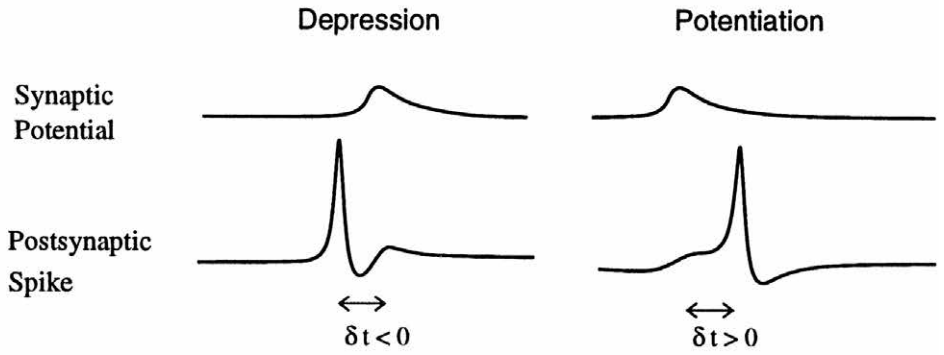
Etude de la stimulation sur le développement du réseau

On going : STDP Spike-timing-dependent plasticity

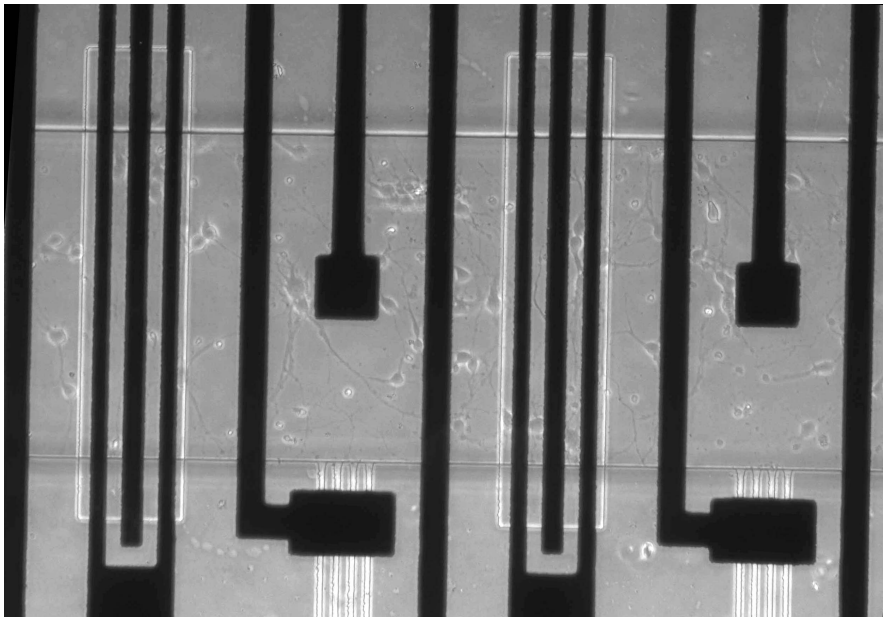
Long-term strengthening of synapses occurs if presynaptic action potentials precede postsynaptic firing by no more than about 50 ms

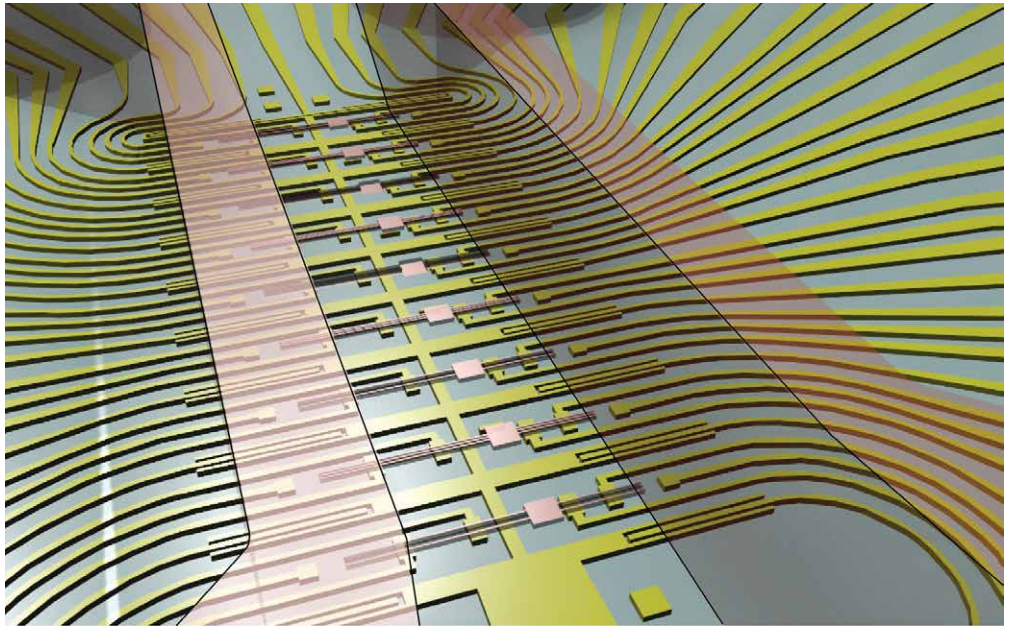


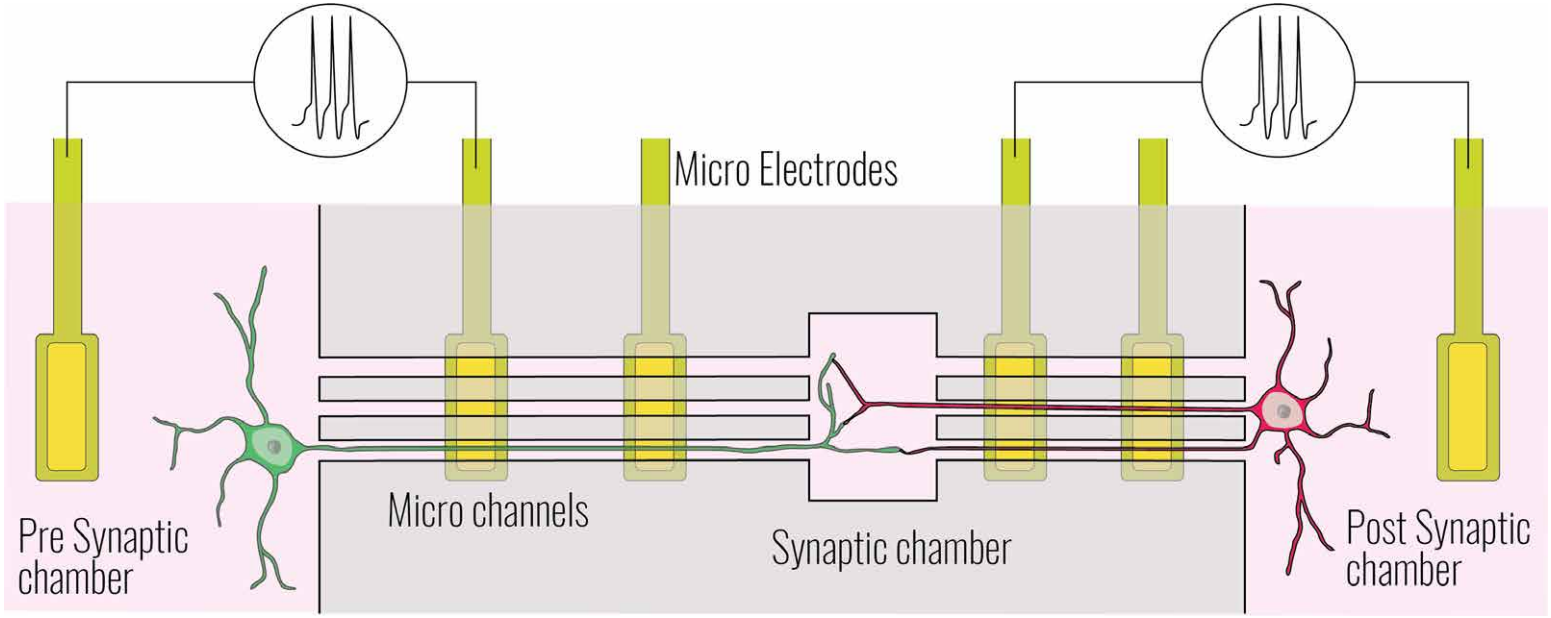




On going : Spike-timing-dependent plasticity



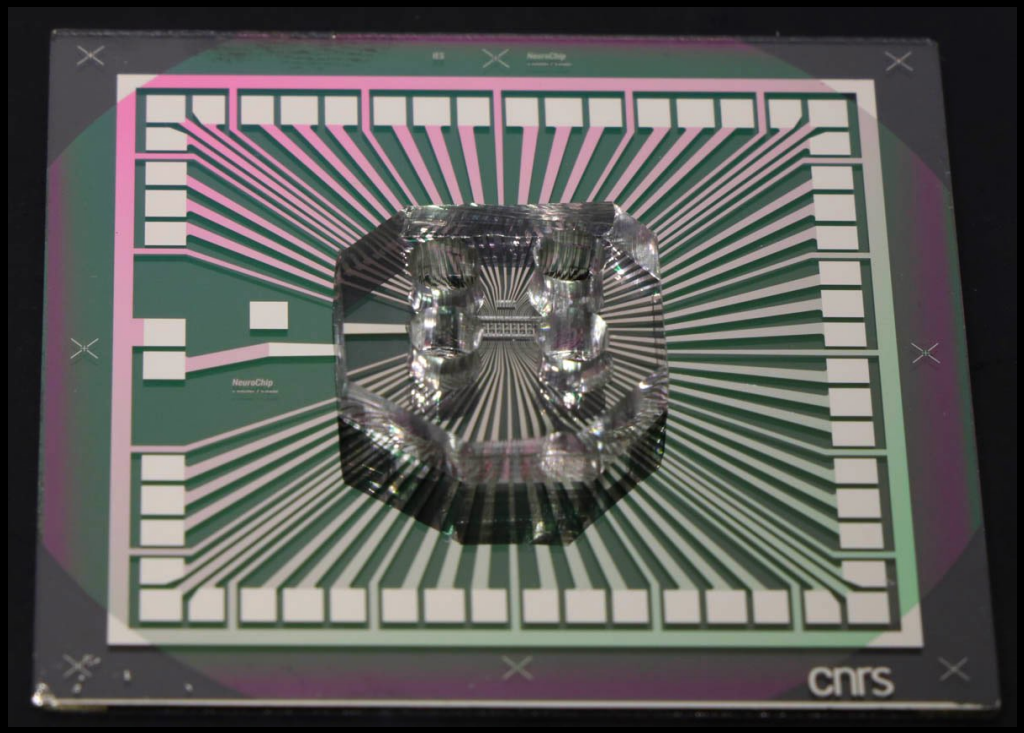


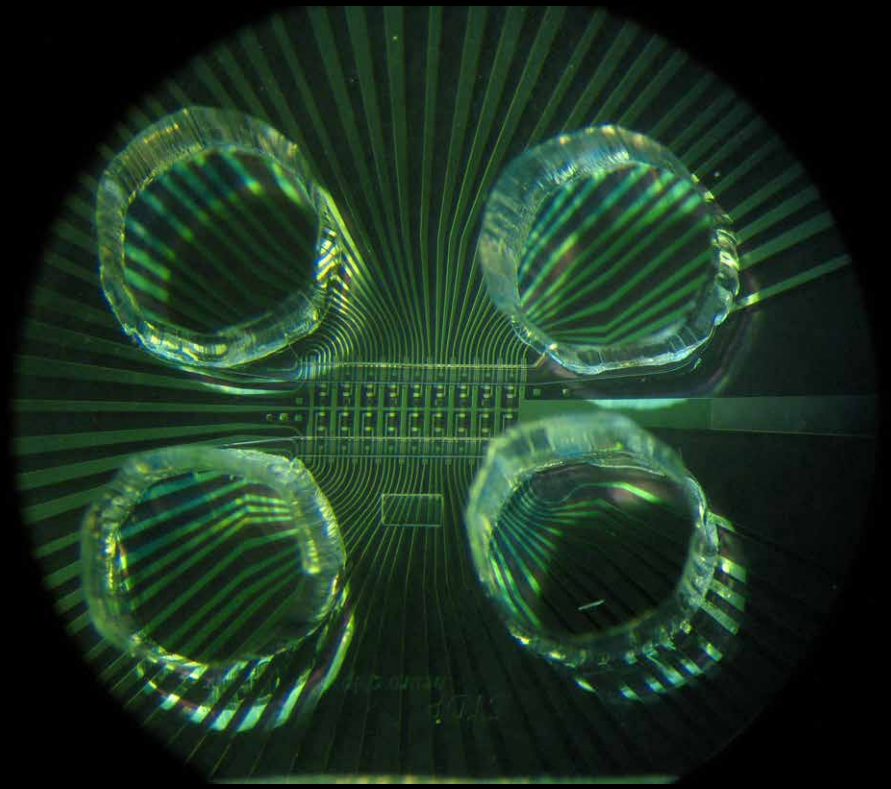


Observation of synaptic strengthening

On going : Spike-timing-dependent plasticity

Device





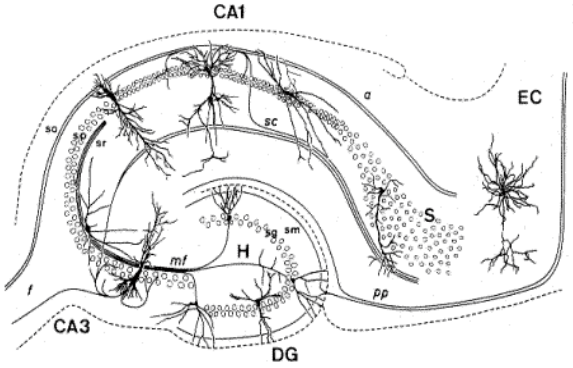
Synaptic plasticity on chip

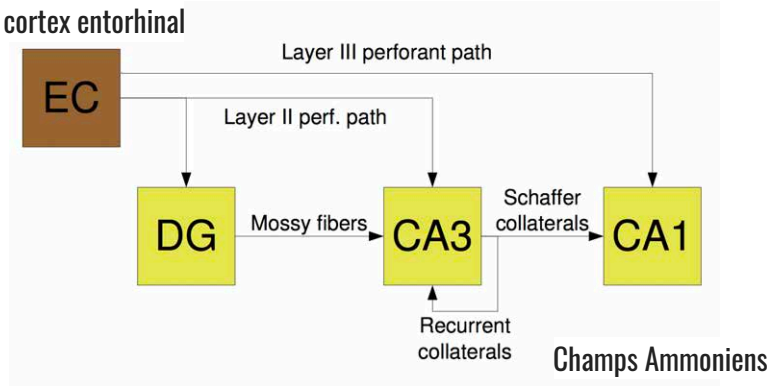
Collab. Florence Rage

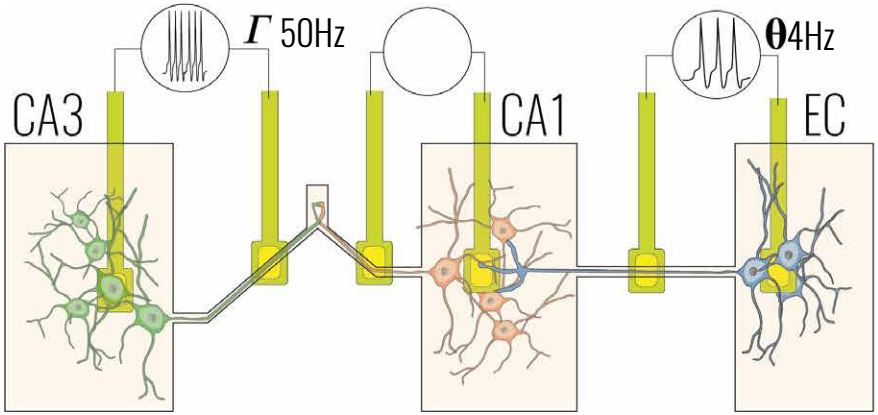
Reconstruction of neuronal junctions within hippocamp zones
(spatial navigation, episodic memory)

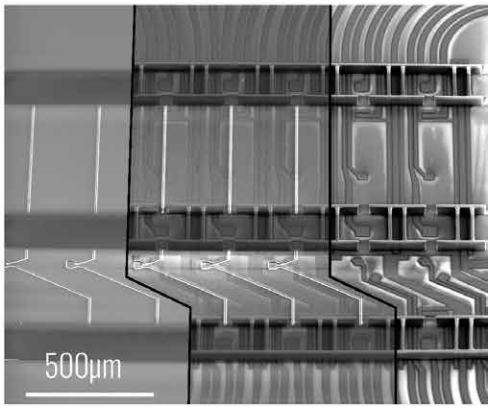
Conjonction of stimulations by cortical oscillations

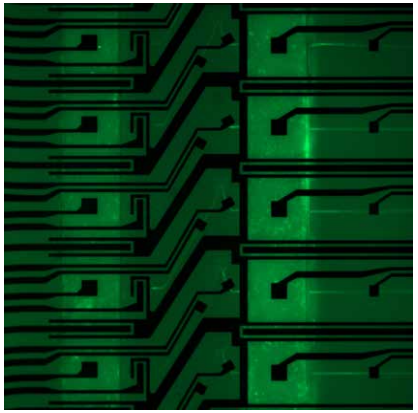
Gamma and Theta waves











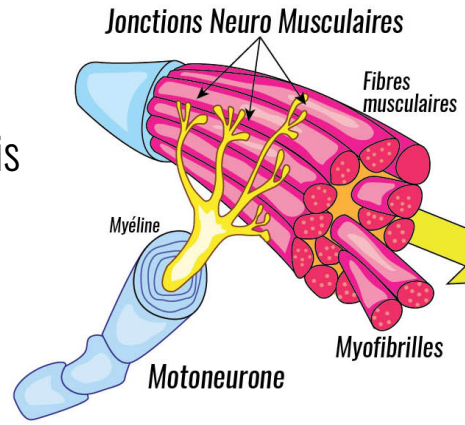
Neuro Muscular Junctions

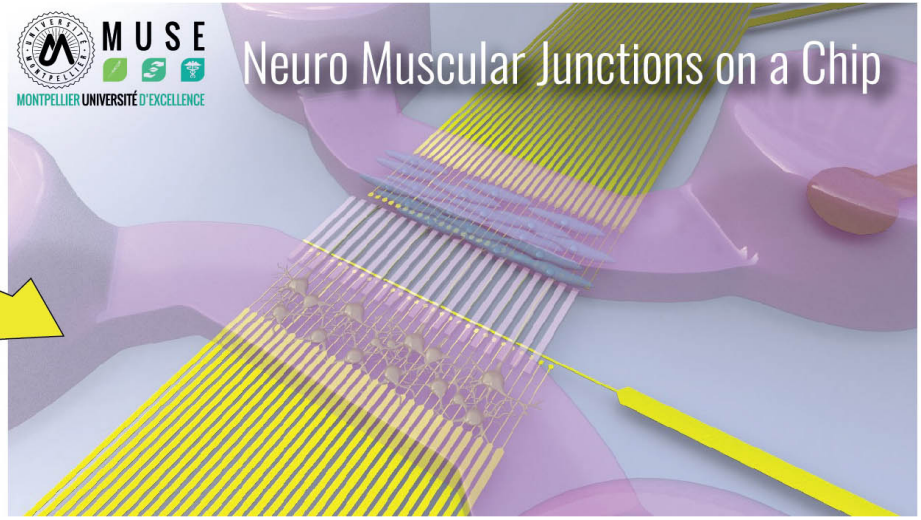
iPS cells -> motoneurons / myocytes -> myofibrils

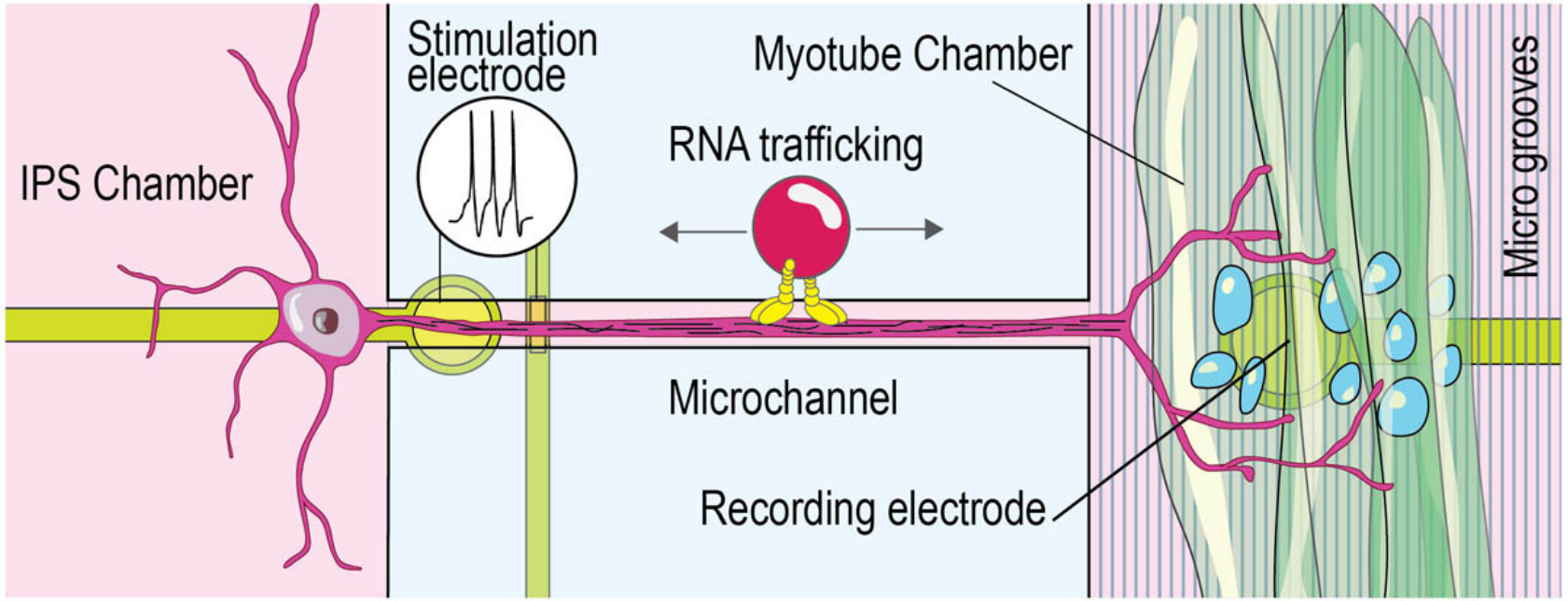
All human model

ALS model : Amyotrophic lateral sclerosis

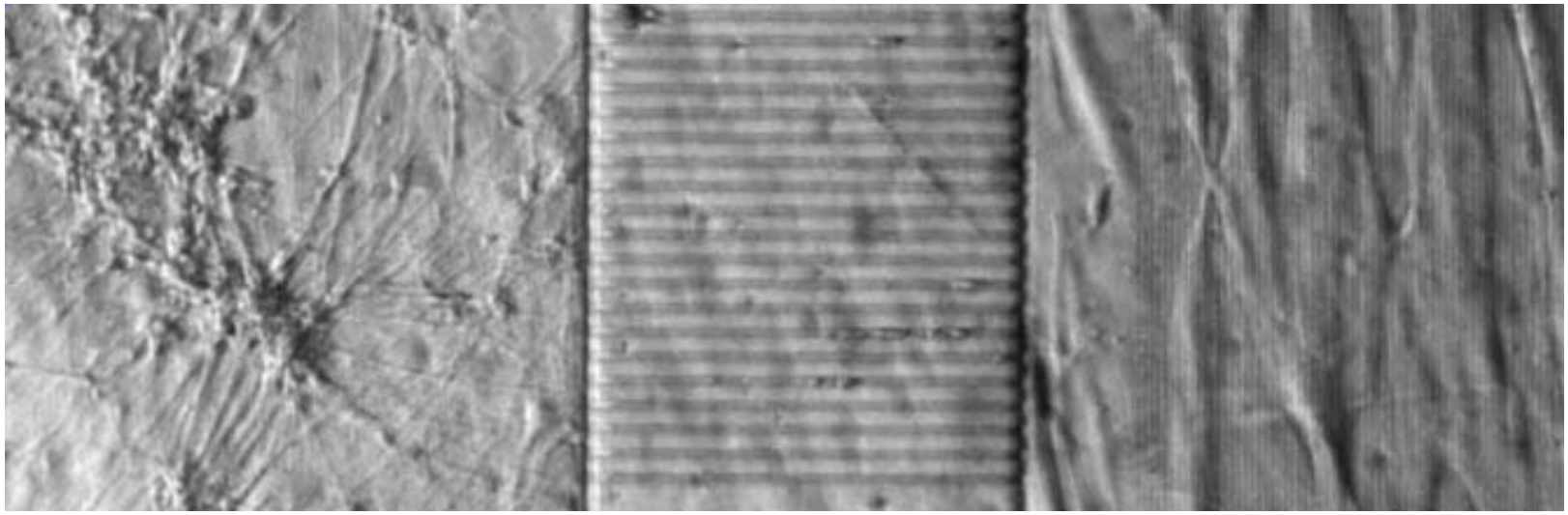
SMA model : Spinal Muscular Atrophy

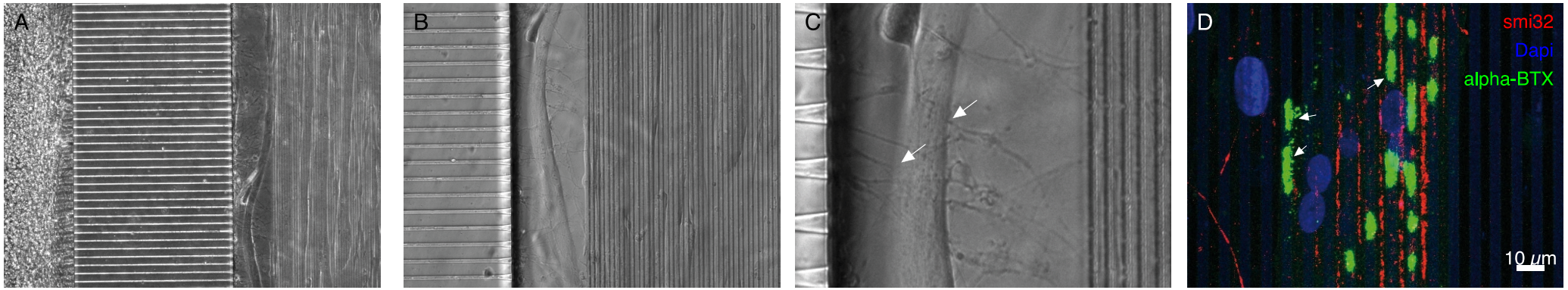






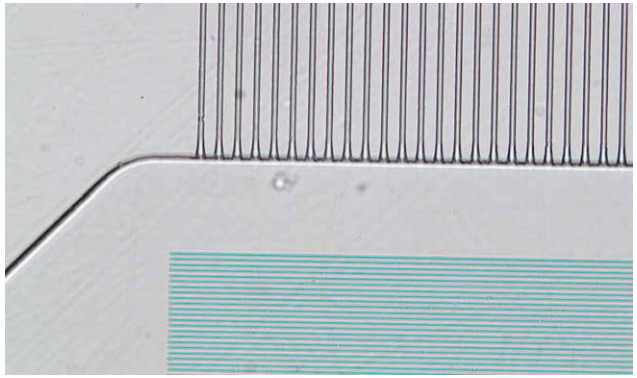
Neuro Muscular Junctions





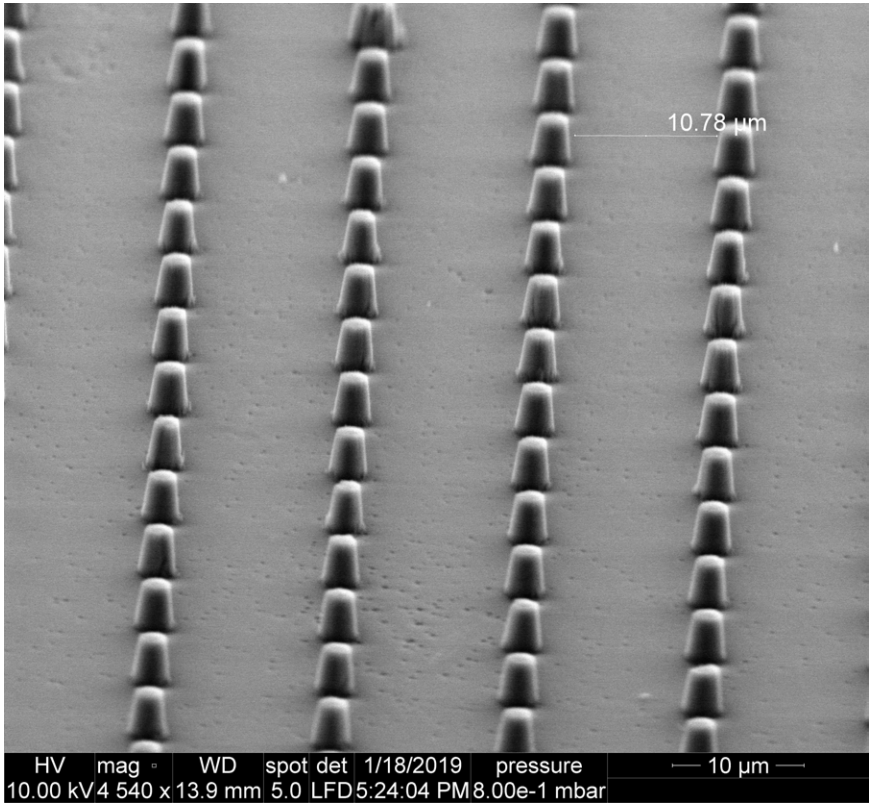
Micro grooves in the Myo chamber

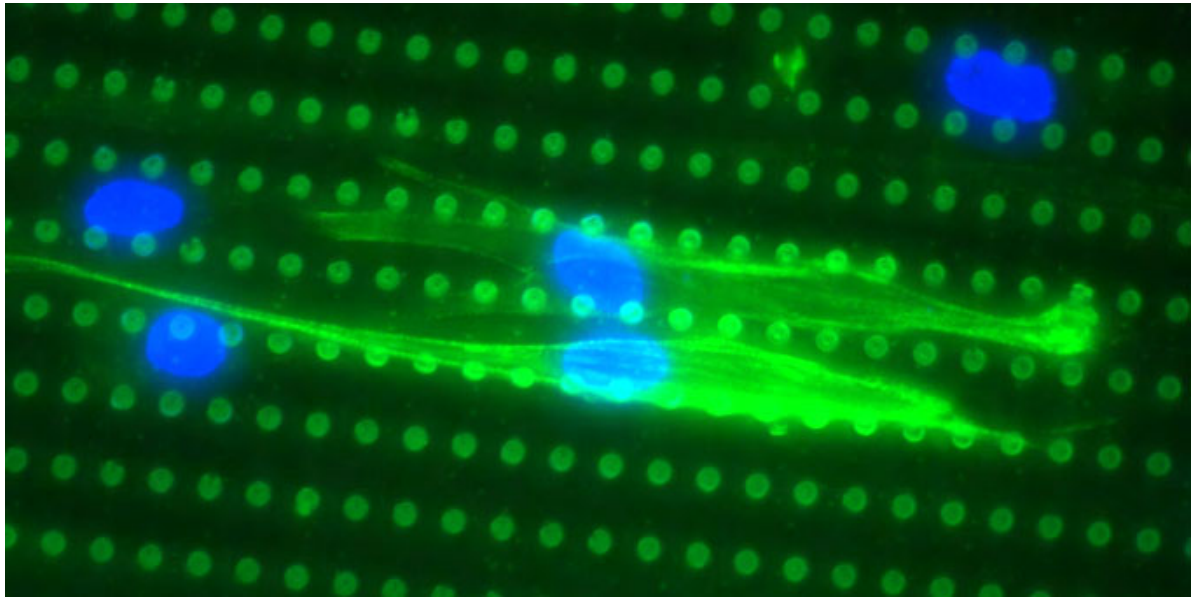
Alignment of Myocytes -> fusion toward Myotubes



Neuro Muscular Junctions

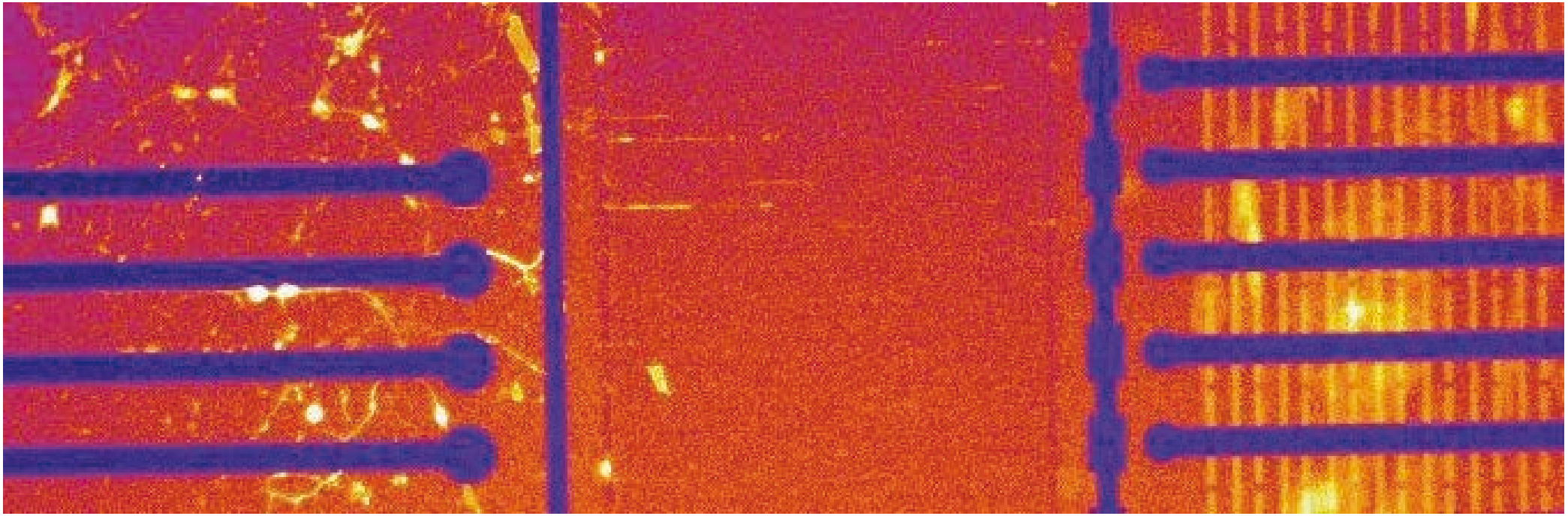
Micro pillars for cell alignment/fusion





Neuro Muscular Junctions

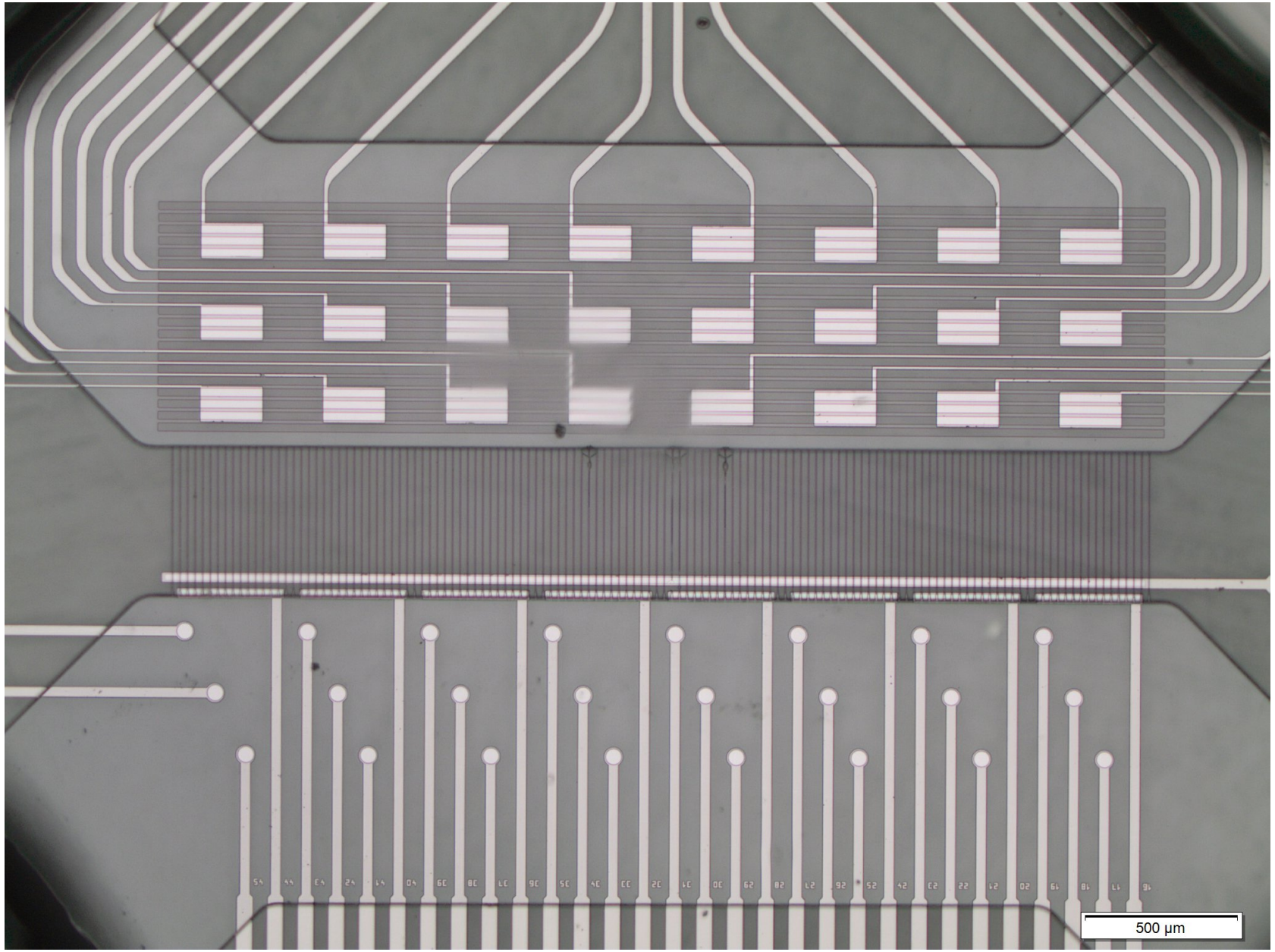
Neuron stimulation



Neuro Muscular Junctions

Muscle chamber

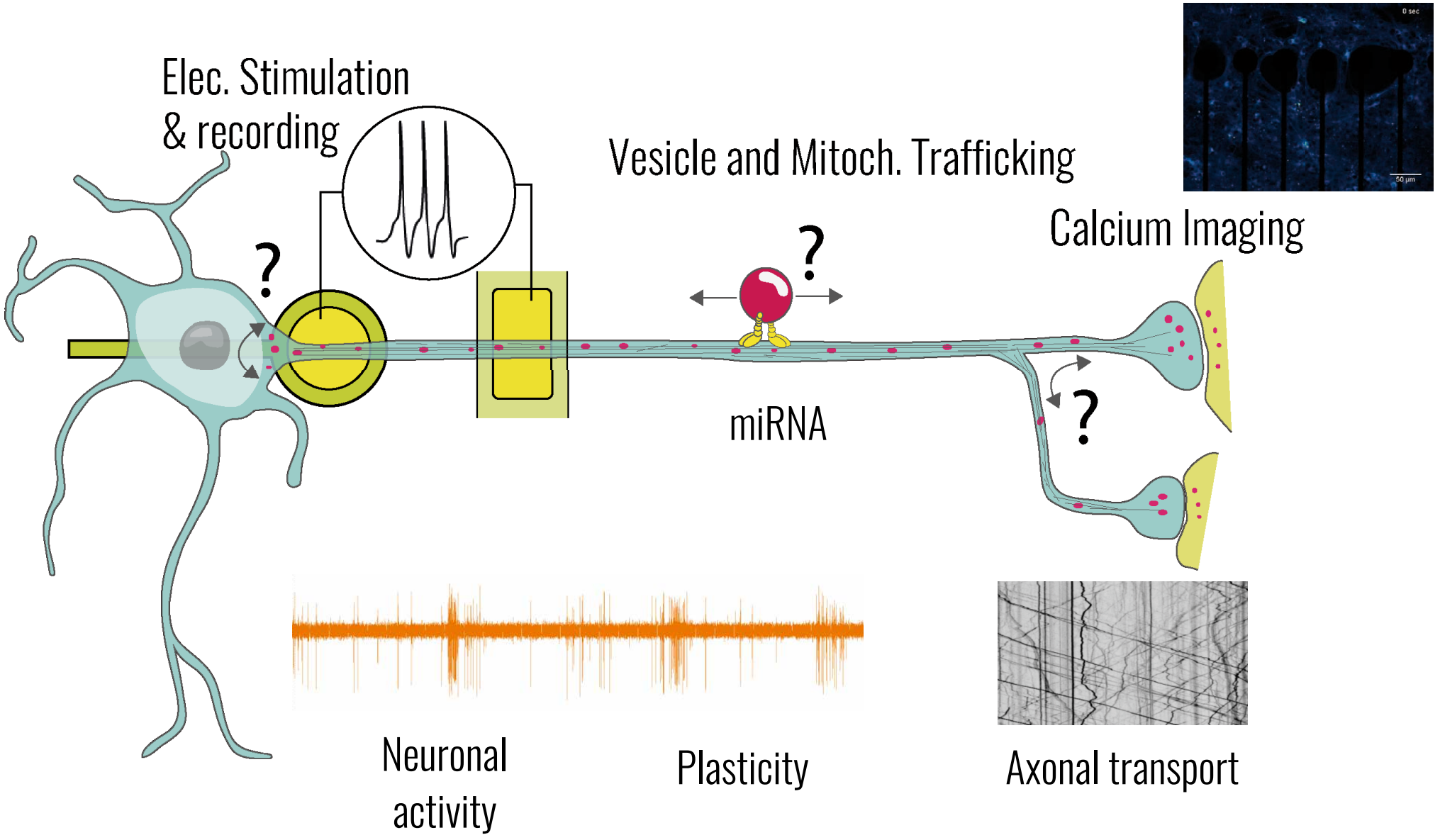
Motoneuron chamber



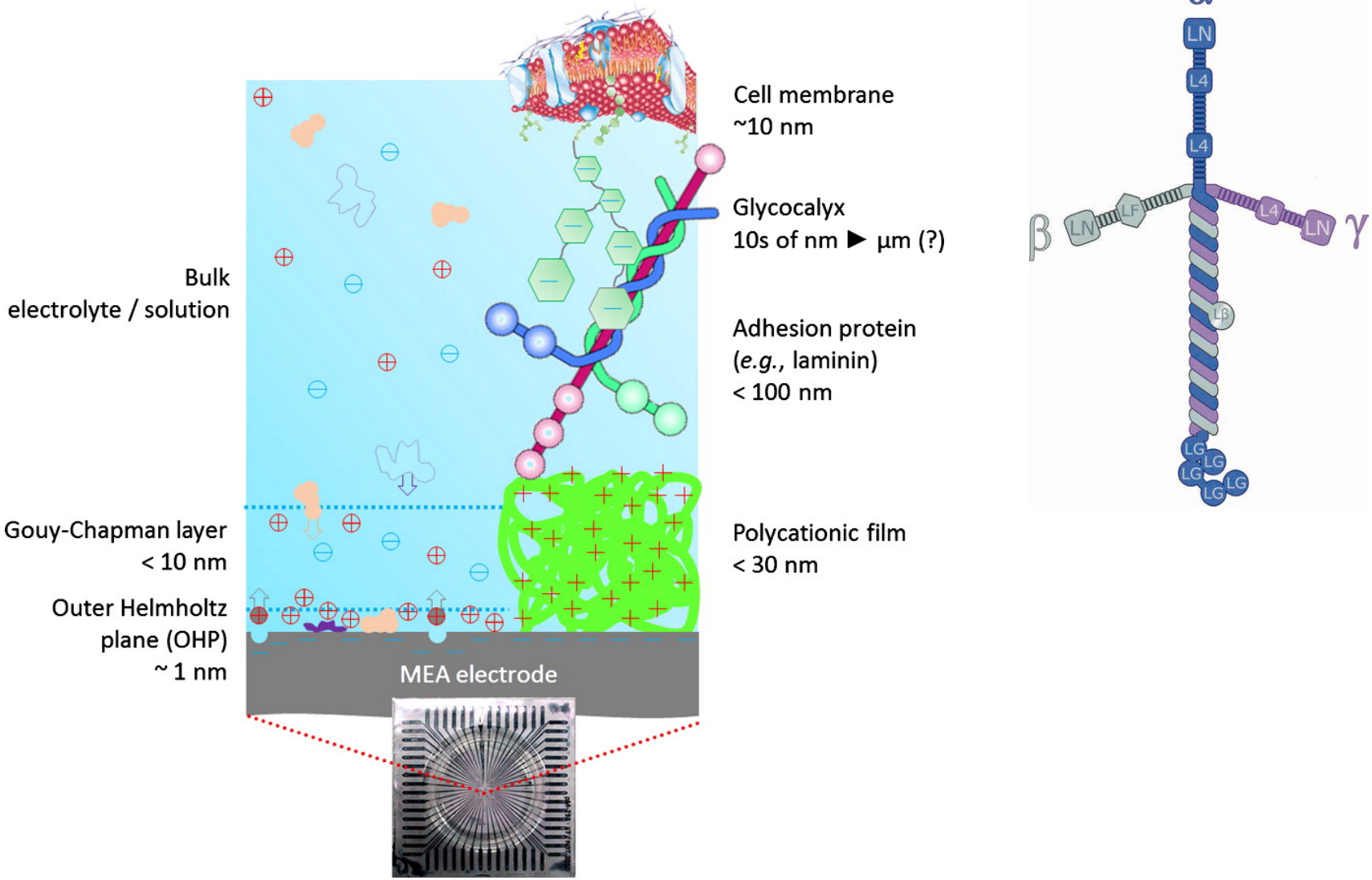
On going...

Summary

Integration of Microfluidic and Micro Electrode Arrays



Cell adhesion



Integrins are transmembrane receptors that facilitate cell-extracellular matrix (ECM) adhesion.

Micro contact printing

PDMS can be used for deposition by contact prining

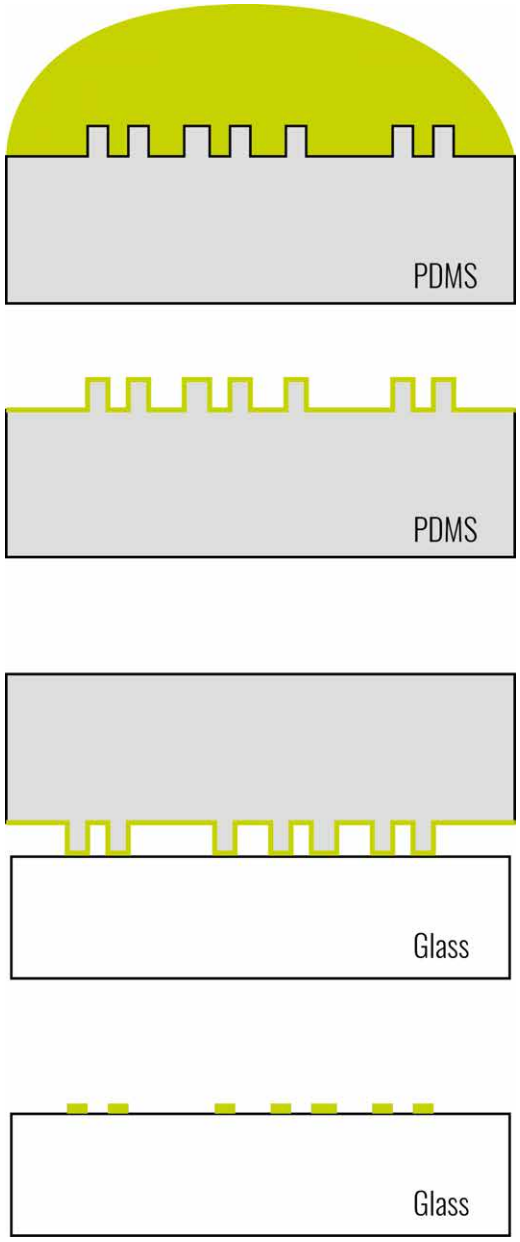
Surface activation O_2 Plasma

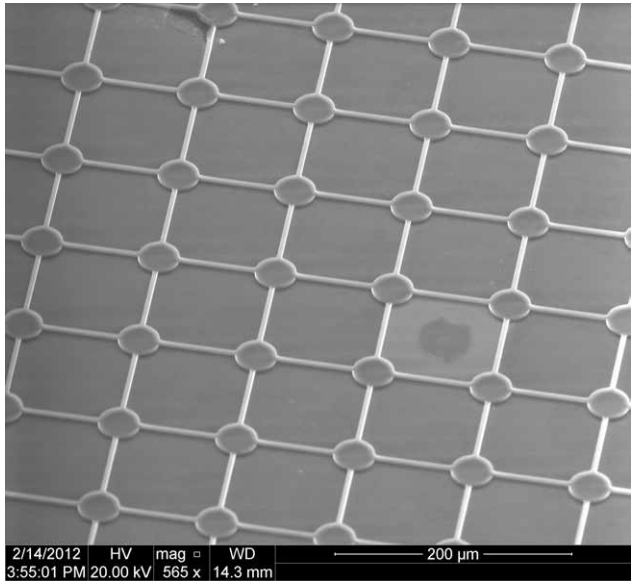
Wetting on PDMS 20mn

Rinse, blow drying N_2

Stamping

Passivation (PLL-PEG)

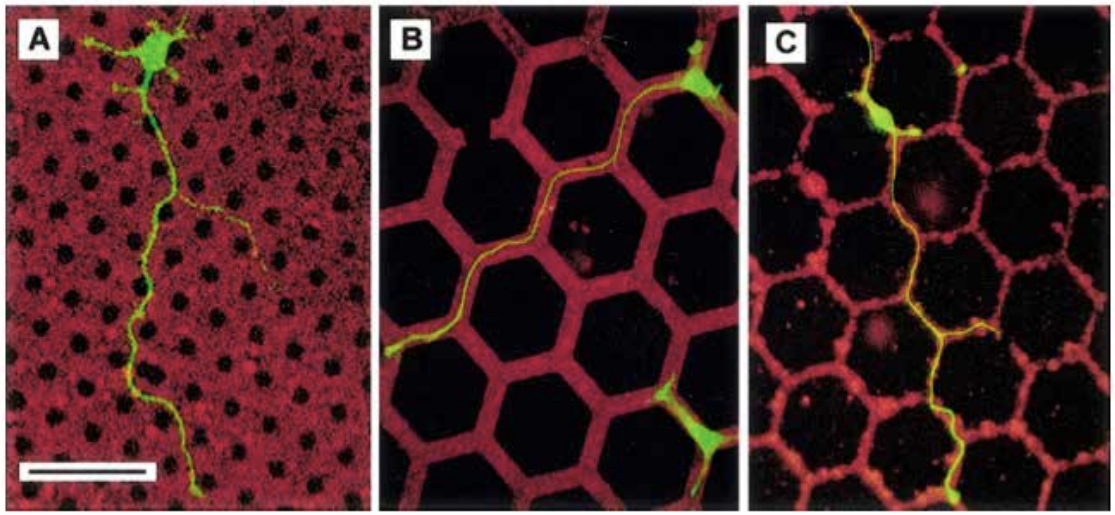


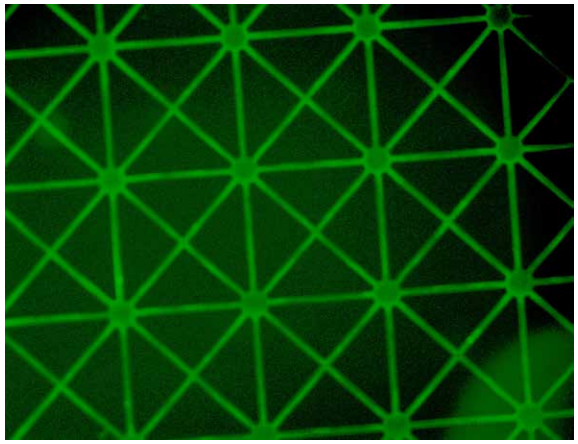


Micro contact printing

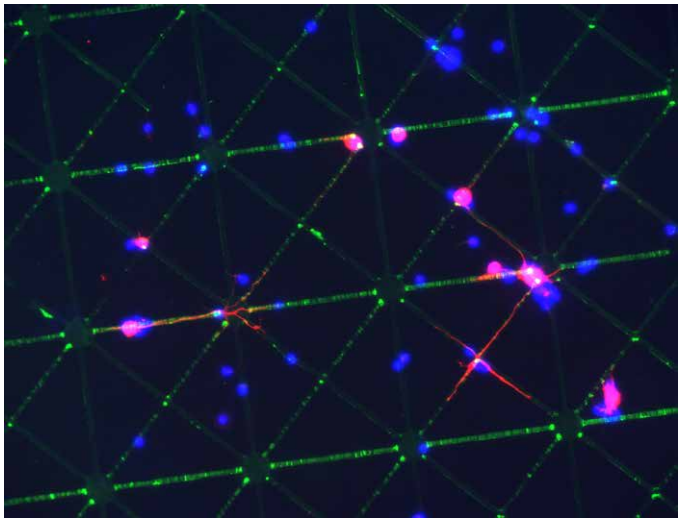
Proteins used for cellular culture

- Poly-L-Lysine
- Polyornithine
- Laminine
- Fibronectine





PLL-FITC



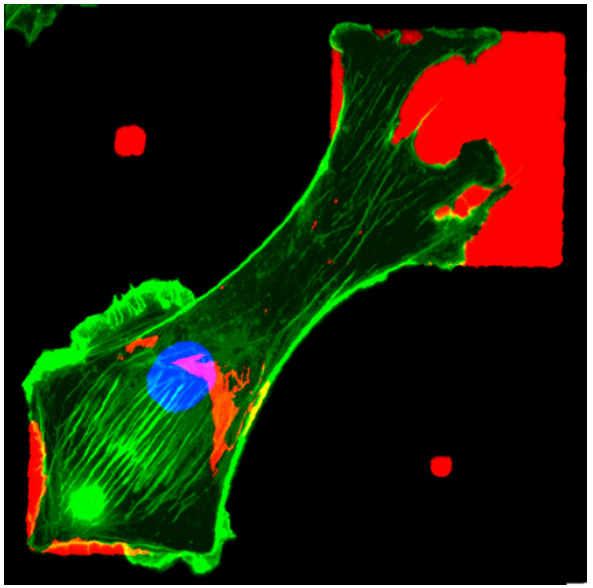


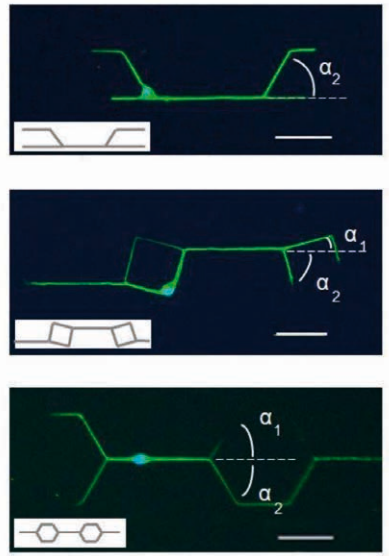
Image : (actin cytoskeleton shown in green; nucleus in blue) initially was plated on a single square (50 x 50 µm) extracellular matrix adhesive island (red) that was created with a microcontact printing technique.

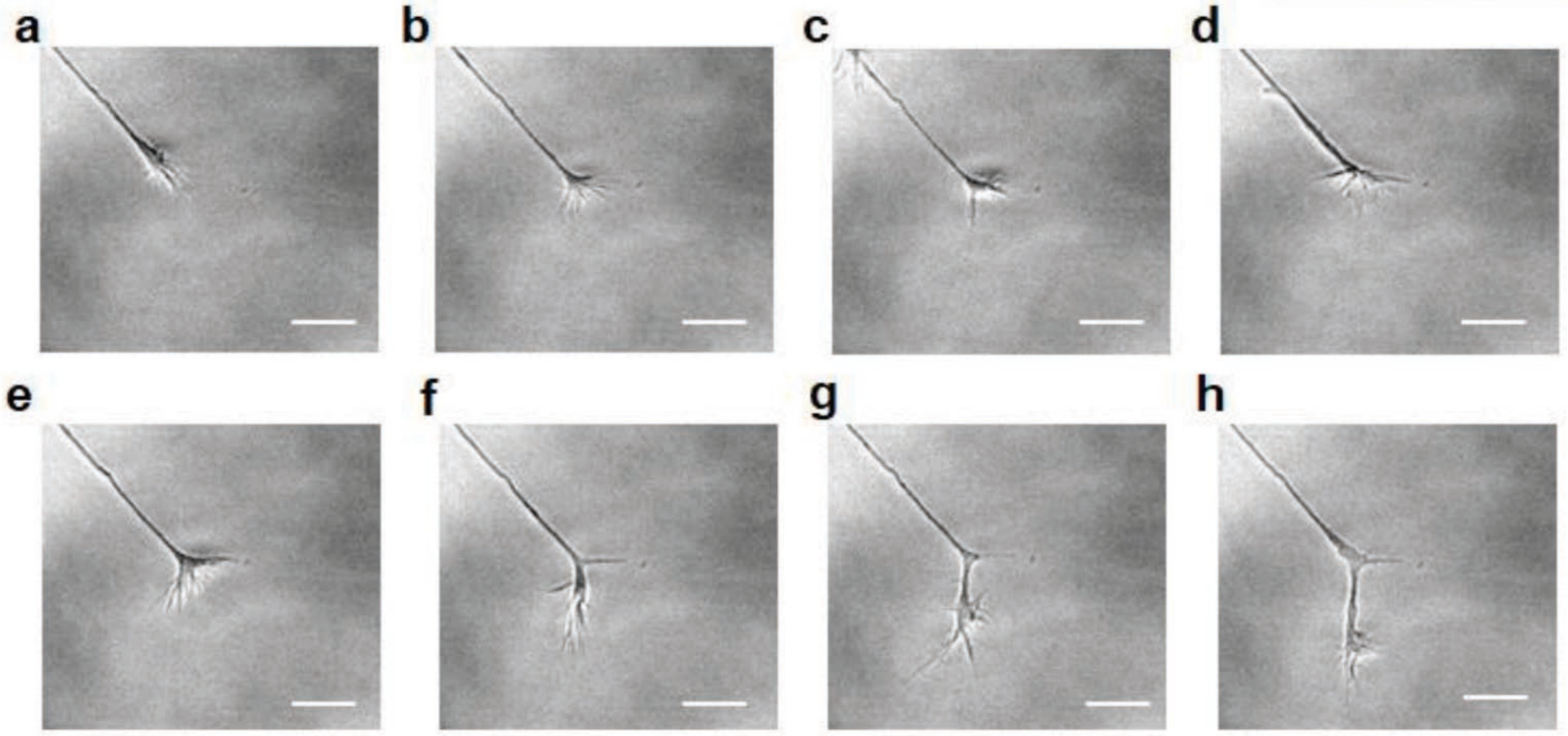
Cliff Brangwynne in the Ingber Lab

Micro contact printing

F.Cohen, C.Villard, IPGG

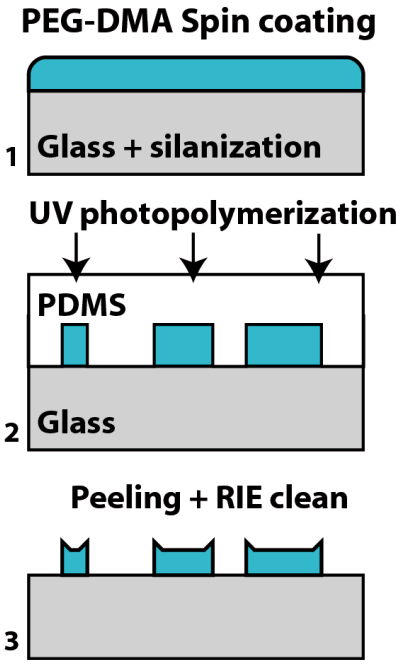
Neuronal branching on patterned structures

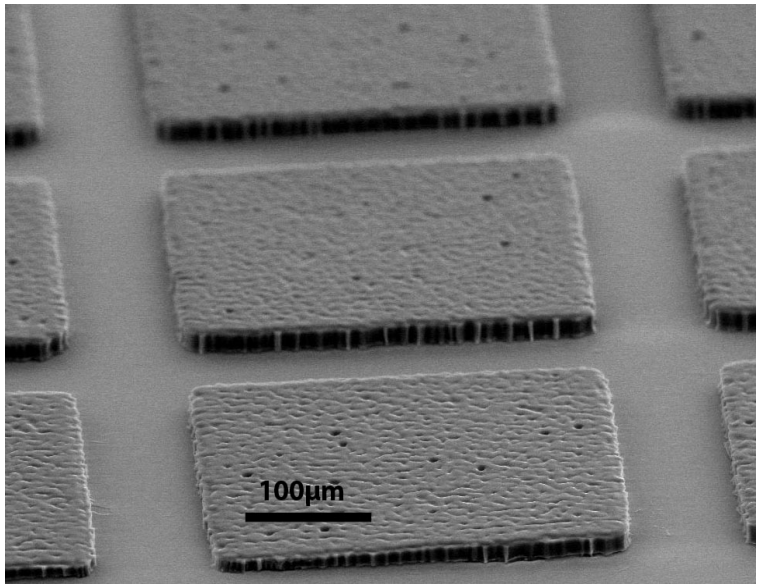


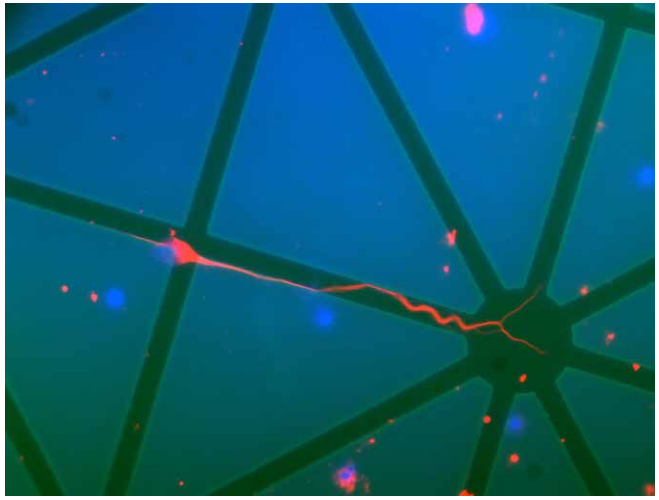


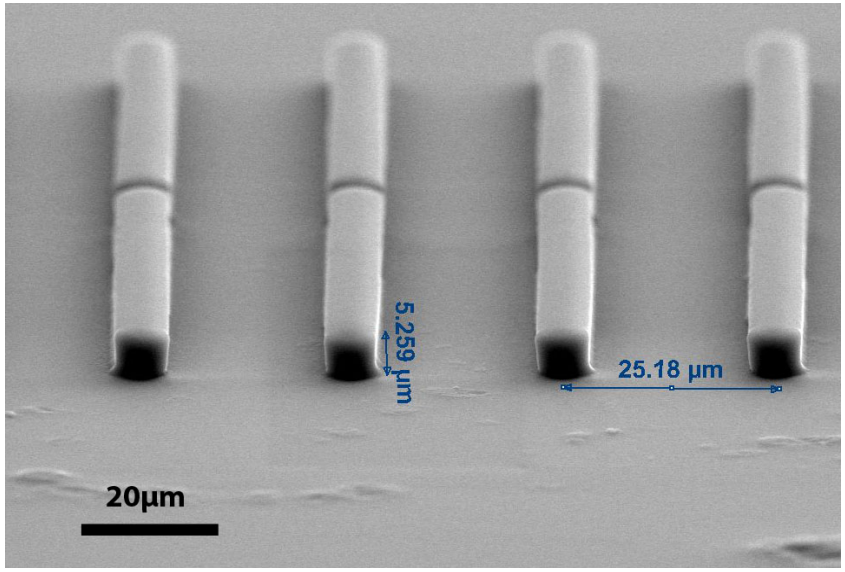
Micro contact printing PEG-DMA

Non-immunogenicity
Non-antigenicity
Protein rejection
2,5 D cell culture pattern
Confinement
Cell adhesion selectivity





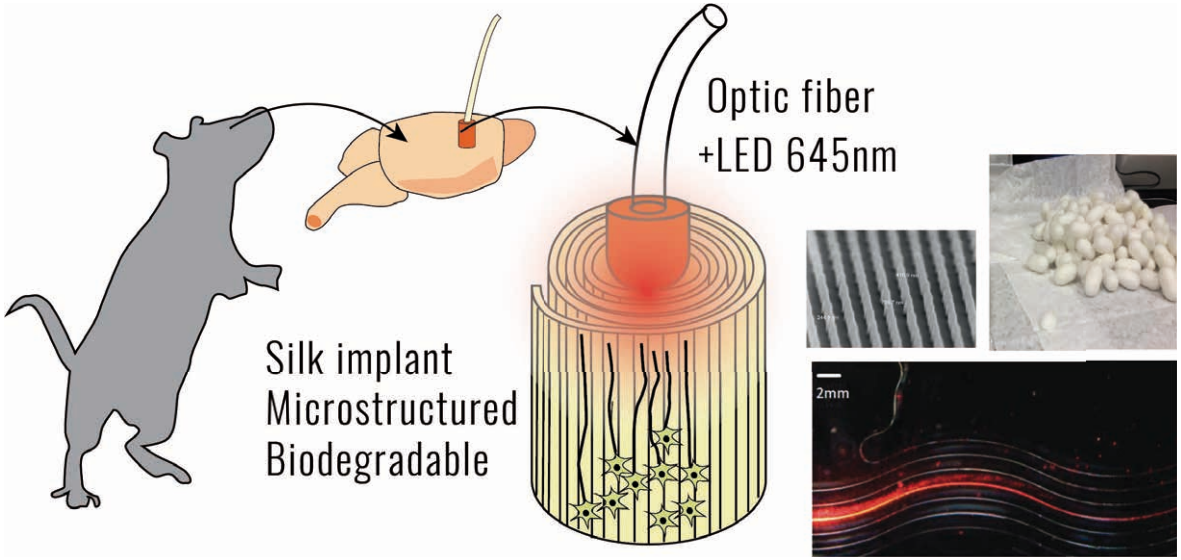


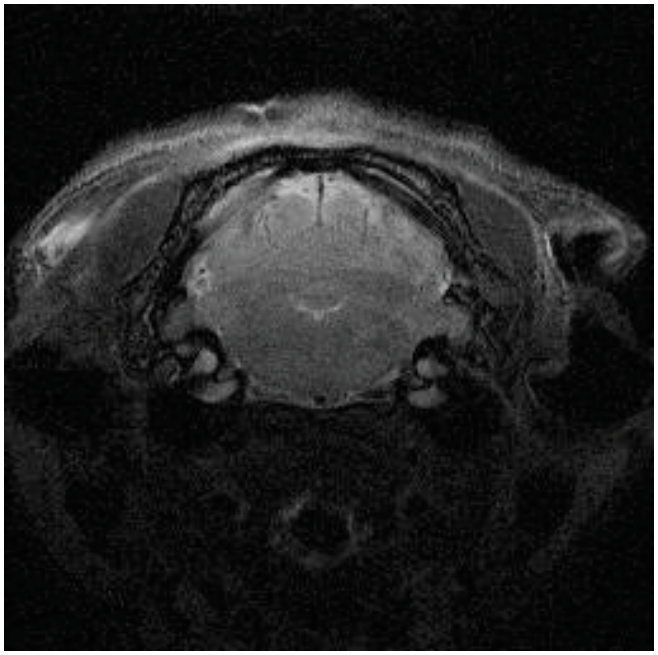


Toward In Vivo

Braindage project (FRM, GIN, IES)

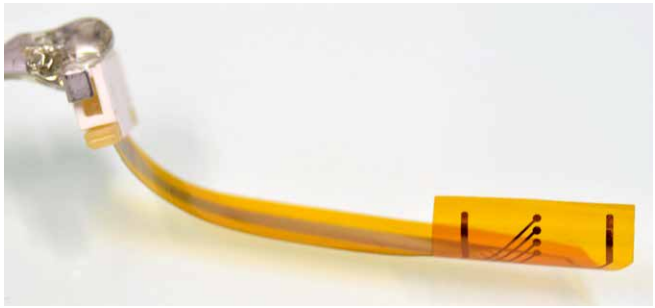
Optical stimulation + topography growth guidance

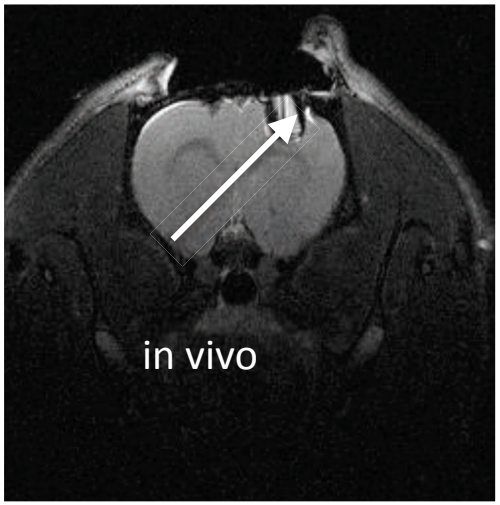




Spine stimulation with deformable electrodes

Collaboration D.Guiraud, C.Azevedo LIRMM INRIA





Aknowledgments

Florence RAGE
Pauline DUC
Johan Soret

Eve MOUTAUX
Maxime CAZORLA
Frederic SAUDOU

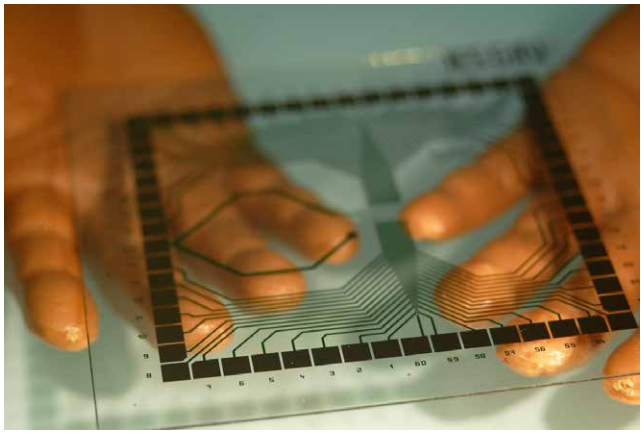
Jean Valmier
Hassan Boukhaddaoui

National center for scientific research

National Institute for medical research

Universities :
Grenoble
Montpellier

Fondation for medical research



Neurofluidics 2019
Montpellier, France, November 28-29, 2019