# Micro and nanotechnologies for life sciences : single molecule combing, microfluidics for neurosciences and infrared stimulation of action potential in neurons.


**Benoît CHARLOT, Fabrice BARDIN**

*IES : Lambert PARIS, Patrice QUINTANA, Didier LAUX*
*INM : Jean VALMIER, Patrick CAROLL, Pascale BOMONT*
*MMDN : Christelle LASBLEIZ, Jean Michel VERDIER*
*IGMM : Etienne SCHWOB*
*GIN: Maxime CAZORLA, Frédéric SAUDOU*

Labex NUMEV

# Outline

1. Lab overview
2. Single molecule : DNA combing
3. **Microsystems for neurosciences**
   - Neuron growth containment
   - Microfluidics
   - Optical stimulation
   - Mechanical stimulation

# 1 Lab overview

**TIMA** (Grenoble) 1998-2005
Ph.D (MEMS testing)
Fingerprint sensor
Micro power sources

**LIMMS** (Tokyo) 2005-2007
Nanomechanical memories
Field emission microresonator

**IES** (Montpellier) 2007-2015
....
DNA combing
Microfluidics for Neurosciences

So I am not a neuroscientist....sorry

# 1 Lab overview

CNRS / Univ. Montpellier
159 persons
Faculty 64, CNRS 8, Post doc& Ph.D 58, technicians 29

3 research depts.

- Photonics and Waves
  IR lasers & detectors, Terahertz
- Energy, Reliability and Radiations
  Power electronics, Rad. Hard electronics
- Sensors, Devices and Systems
  MEMS, microfluidics, Photovoltaïcs, acoustics ...

ies l'institut d'électronique

Microfabrication facilities

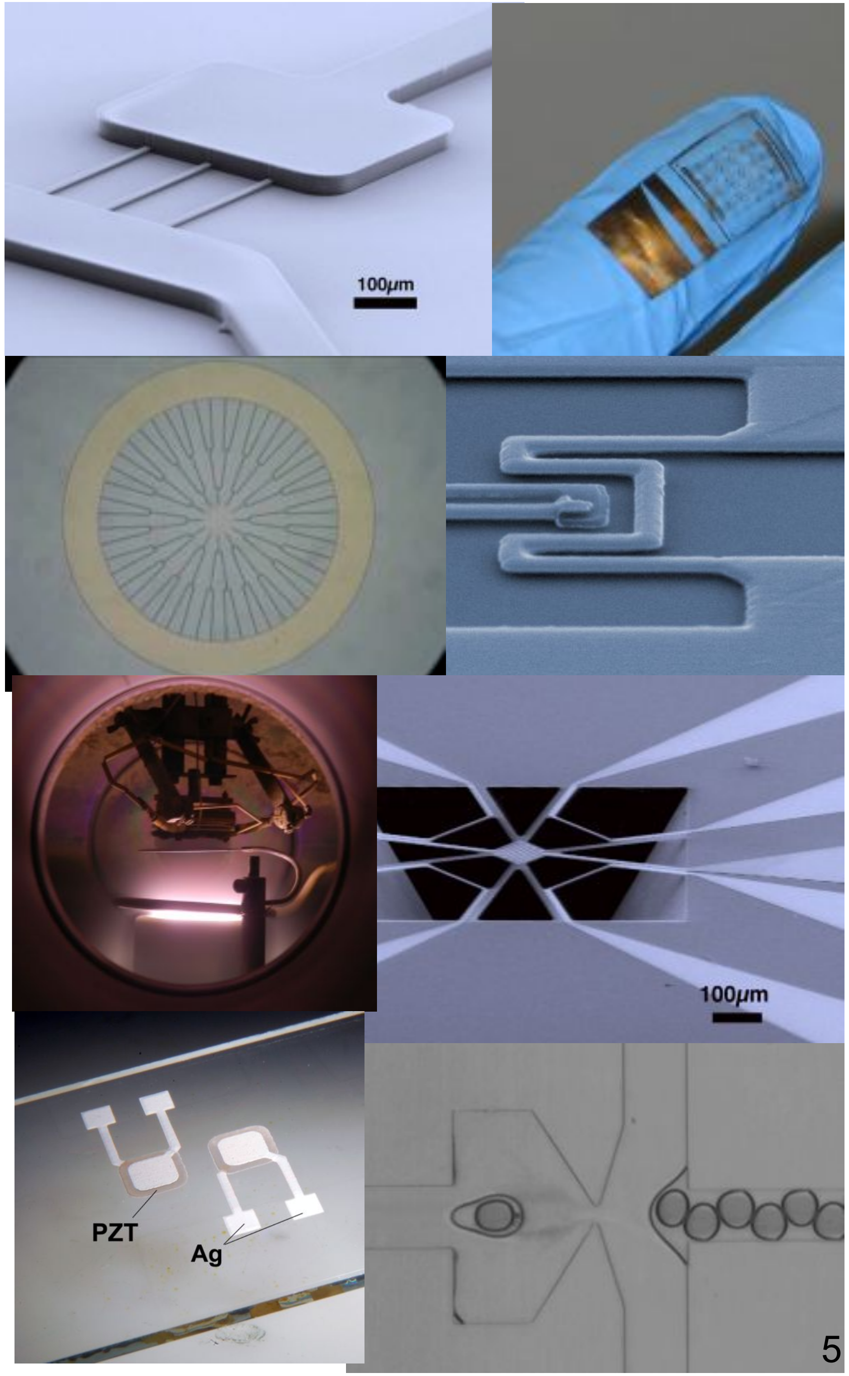
NEW !! 400 m$^2$ Clean room (Litho, Sputter, Evap, E-beam, RIE, PECVD, ....)
Microscopy (SEM, TEM, AFM, EDX ...)

# 1 Lab overview

## Dept. Sensors Devices and Systems

Research activities

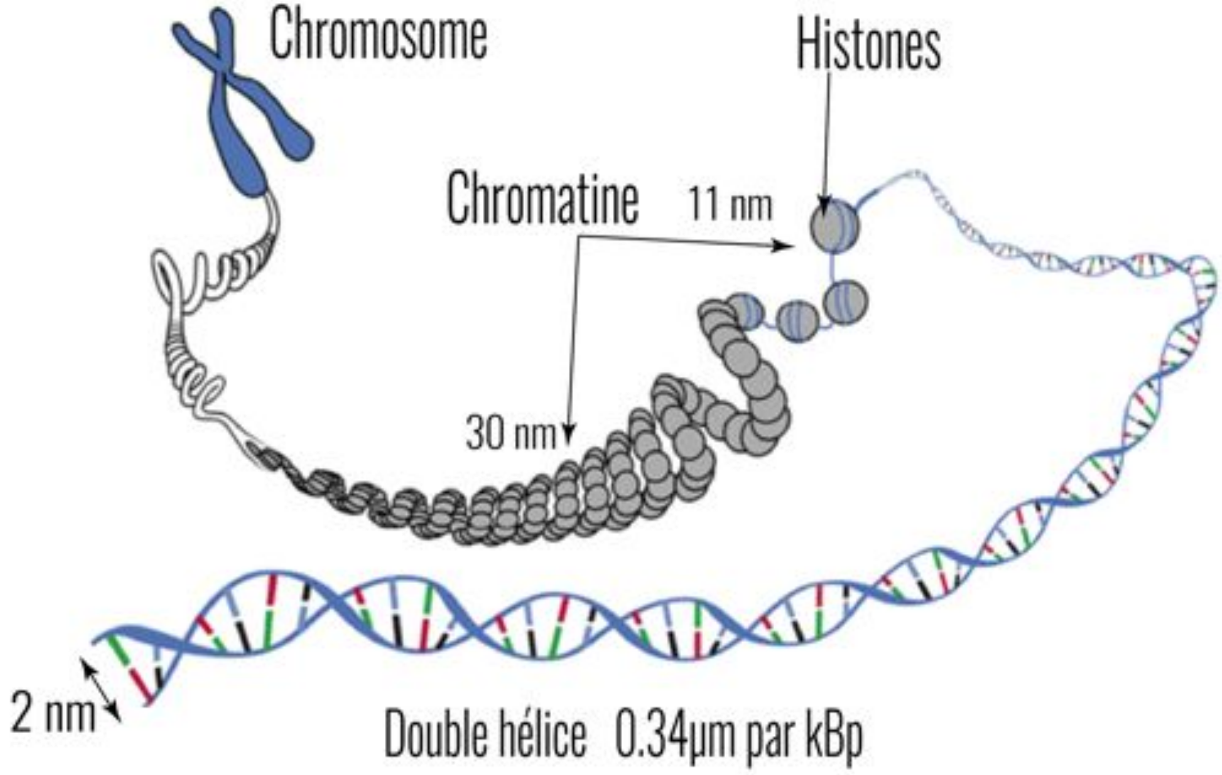
- Thin film Materials Piezo, Pyro, Thermoresistances
- Thermal MEMS accelerometers,thermoelectrics
- Photovoltaïcs concentrated PV
- Flex substrates, paper, polymer, RFid
- Acoustics, ultrasound, piezo
- **BioMEMS, Cell chips & microfluidics**



# Part 2. Single molecule handling : DNA combing

# 2 DNA Combing

➔ DNA organisation



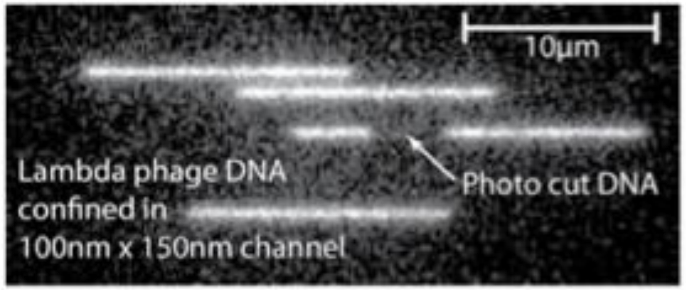

Persistance length: **100 nm** for dsDNA and **2 nm** for ssDNA

In solution : Pellets, Coiling need of uncoiling techniques for observation

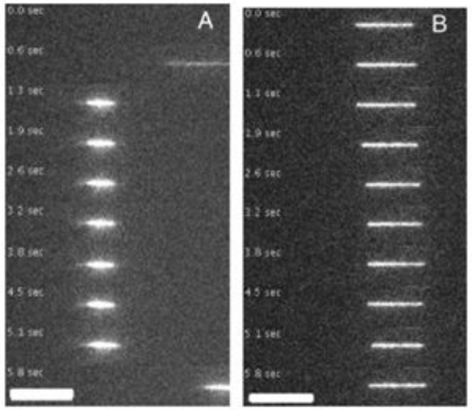
# 2 DNA Combing

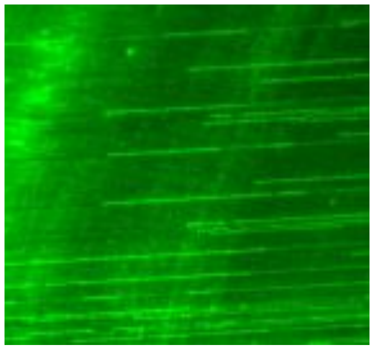
## How to uncoil DNA ?

### ➔ Nanofluidic containment



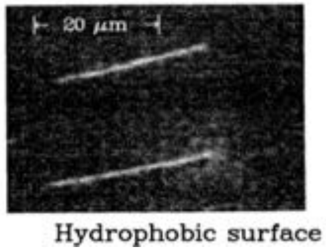

F. Westerlund,
Chalmers



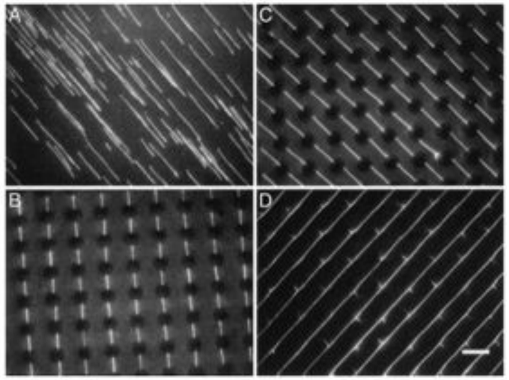



D.E.Streng; North
Carolina state Univ.

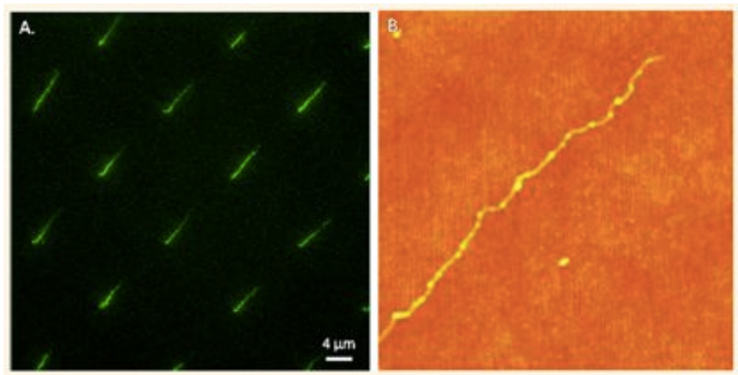
### ➔ Deposition by dewetting




D.Bensimon, ENS



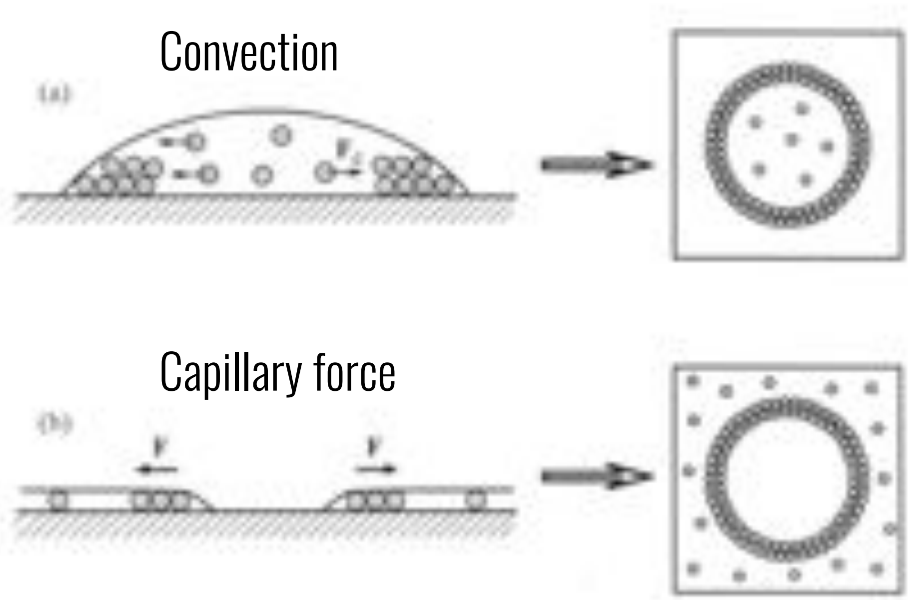

J. Guan and L. J.Lee
Ohio State University



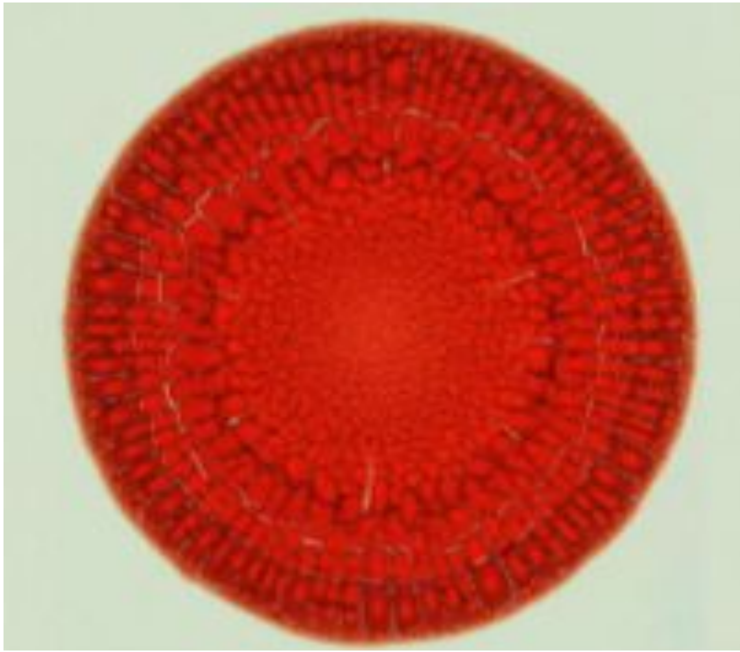

A.Cerf, LAAS CNRS

# 2 DNA Combing

Capillary force assembly ：
interactions between particles mediated by fluid interfaces.

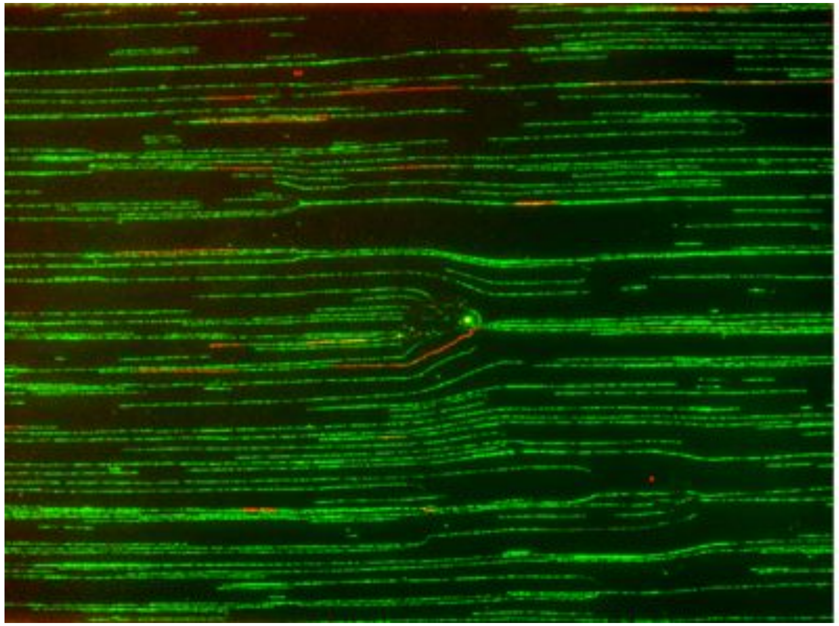

Peter A. Kralchevsky

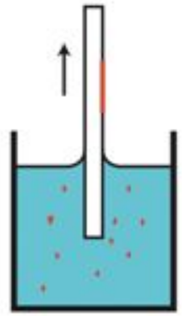


Blood droplet

# 2 DNA Combing

The standard DNA combing technique used everyday at IGMM





Human genomic DNA, YOYO tagged, 237 x 177 µm image

Substrate Silanization

DNA ends anchoring at pH 5,4

Slow dewetting

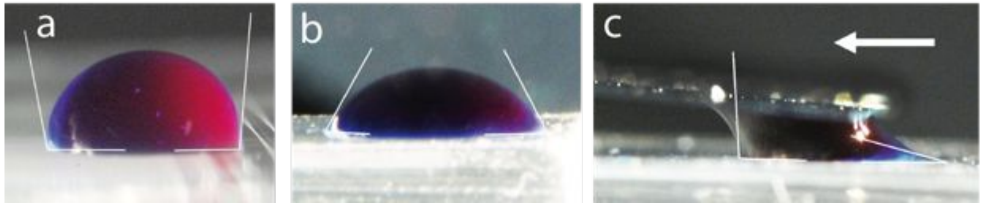
# 2 DNA Combing

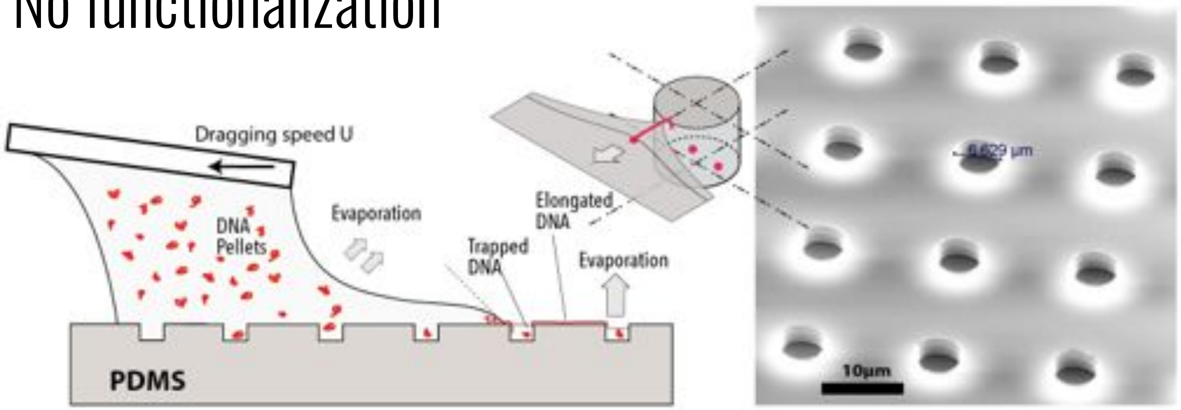
Ordered deposition

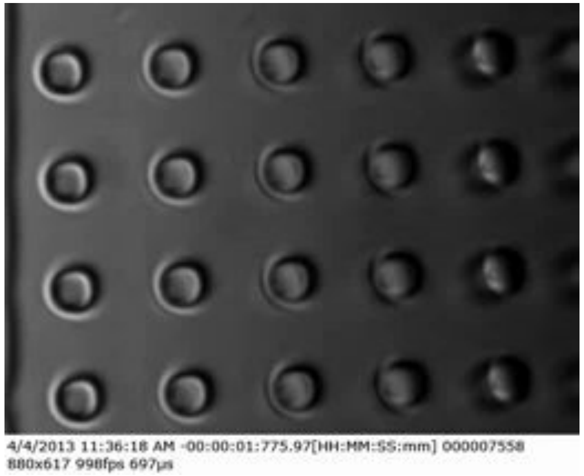
Forced dewetting on perturbations

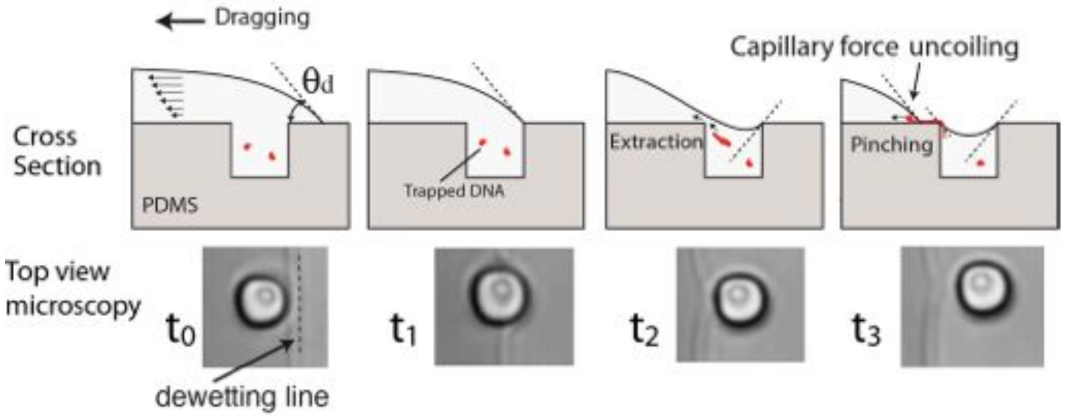
PDMS substrate

No functionalization

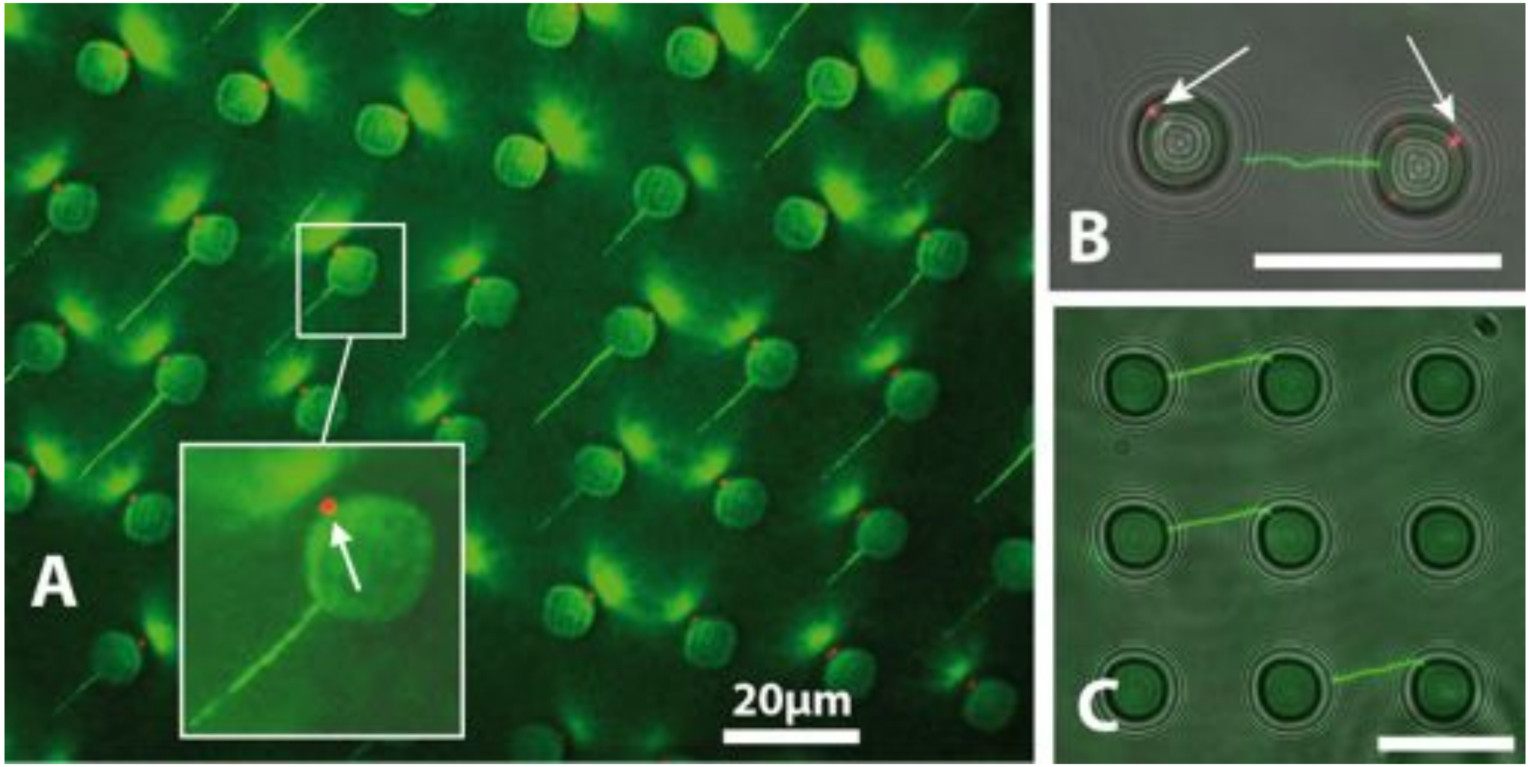


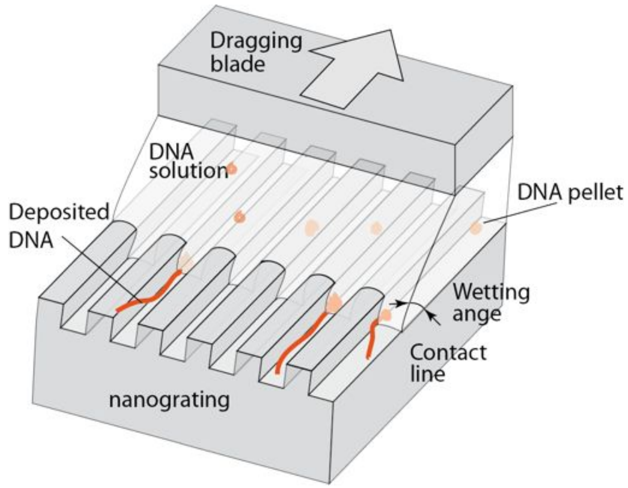
# 2 DNA Combing




composite Image (GFP Fluorescence + transmited light) of λ phage DNA (48kbp) tagged with YOYO intercalating dye. Dewetting 400µm/s. Red: uncoiled DNA pellets, 5µm below surface

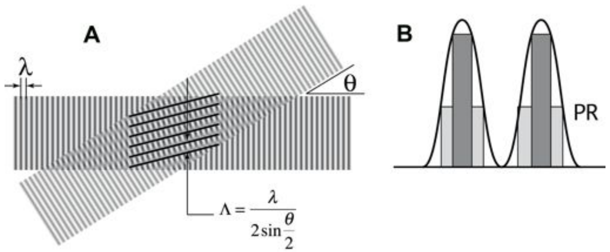
B. Charlot, et al. “Elongated unique DNA strand deposition on microstructured substrate by receding meniscus assembly and capillary force”, **Biomicrofluidics** 8, 014103 (2014).

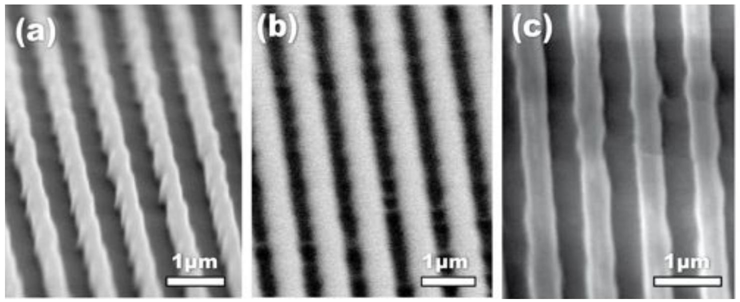
# 2 DNA Combing

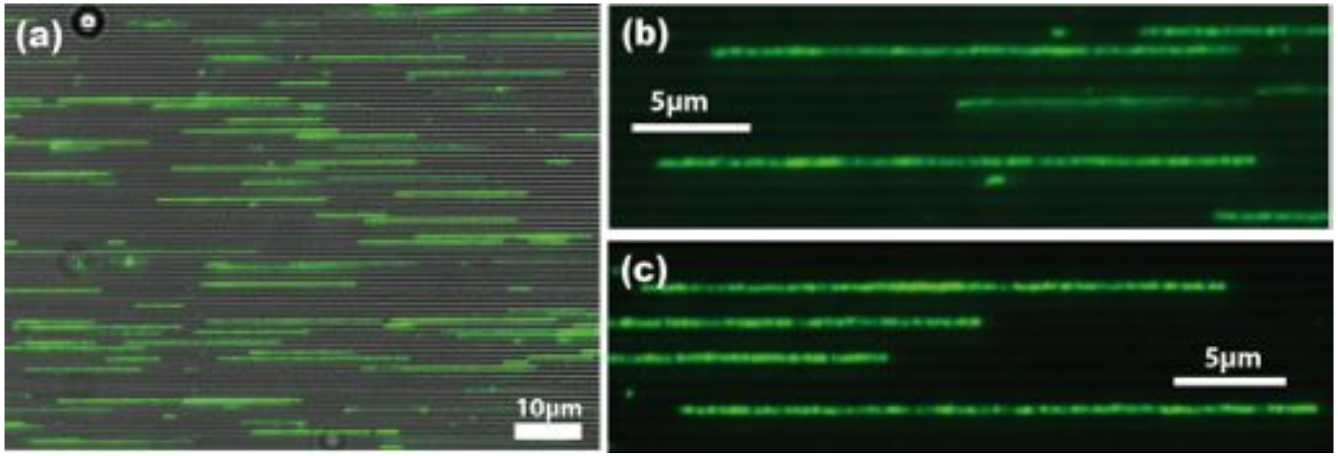


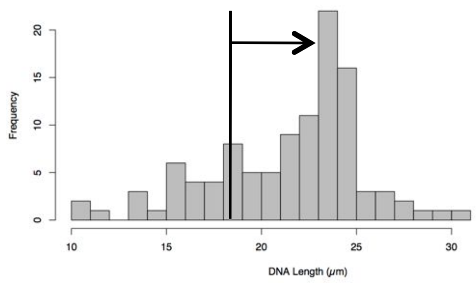

Combing on nanogratings : rectilinear conformation

800nm pitch nanogratings : Interference lithography




Stretching ratio 150%

B.Charlot, R.Teissier, M.Drac, E.Schwob, "DNA on rails: Combing DNA fibers on nanogratings", **Applied Physics Letters,** 105, 243701 (2014).

# 2 DNA Combing

Next steps :

Combination of perturbation dewetting (PDMS) + nanogratings

Long dsDNA fragments

Chromatin combing

# Part 3. Microsystems for Neuroscience

# 3 Microsystems for neuroscience

**Objectives** : analysis of the effect of physical stimulations on neurons

- Optical stimulations (Lasers, LED, IR, visible) ! ~~optogenetics~~
- Mechanical / acoustic stimulations
- Thermal / thermodynamics of the nervous influx

Through the use of MEMS, BioMEMS, microfluidics, nanostuff

Micro sensors and actuators, piezos, electrodes

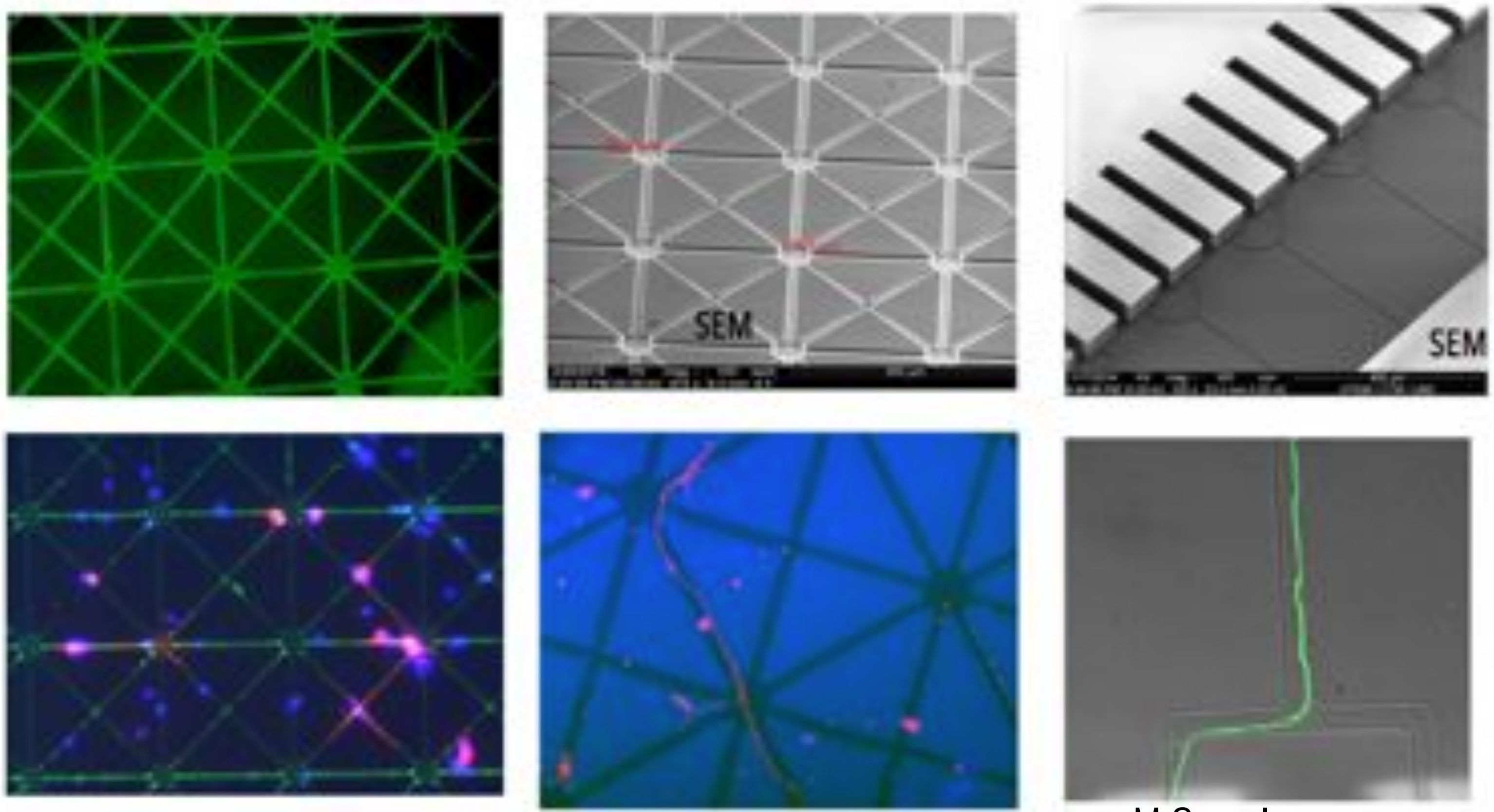
And several collaborations…

# 3.1 Neuron Growth Engineering

Protein stamping
**2D** containment

PEG-DMA microstructures
**2,5D** containment

PDMS microfluidics
**3D** containment

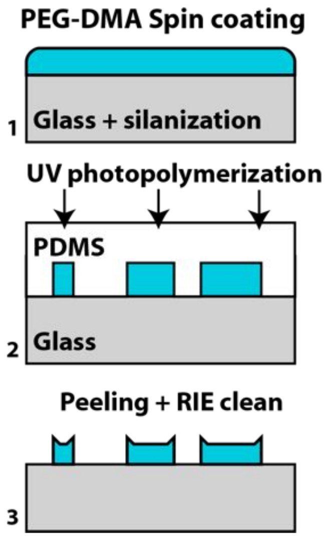


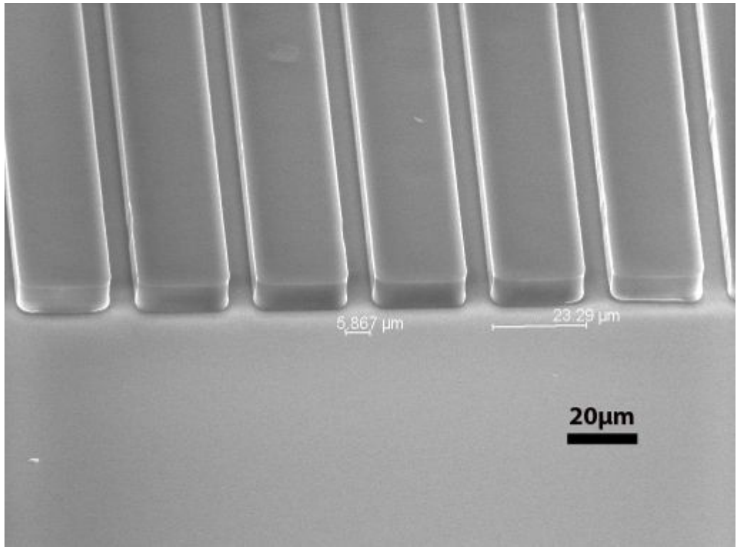

M.Cazorla

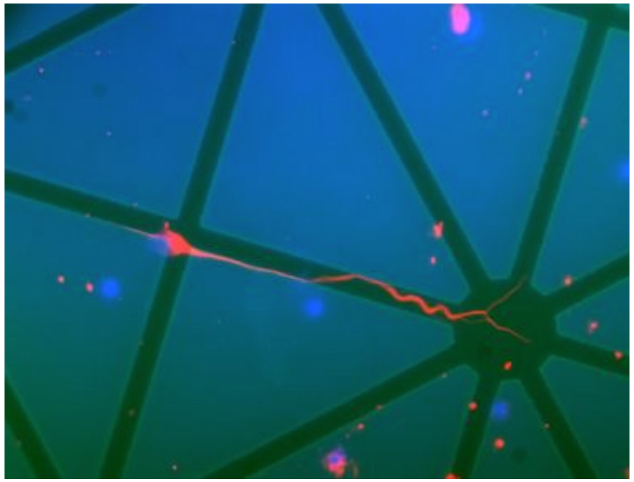
# 3.1 PEG-DMA scaffolds

Non-immunogenicity
Non-antigenicity
Protein rejection

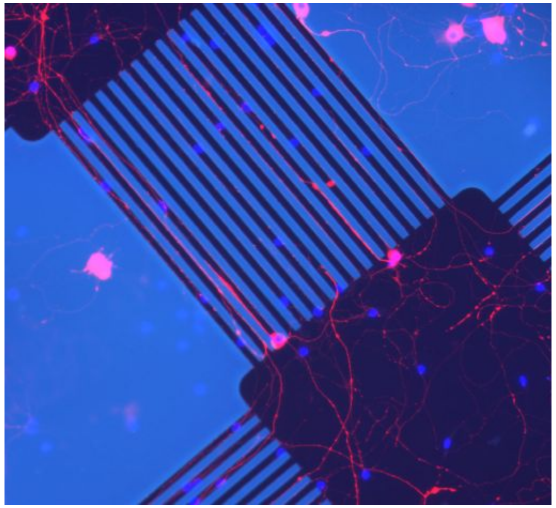
2,5 D cell culture pattern
Confinement
Cell adhesion selectivity



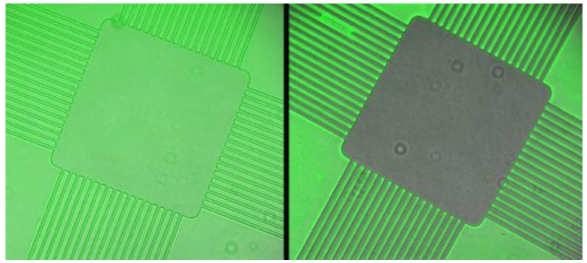



Cortical



DRG

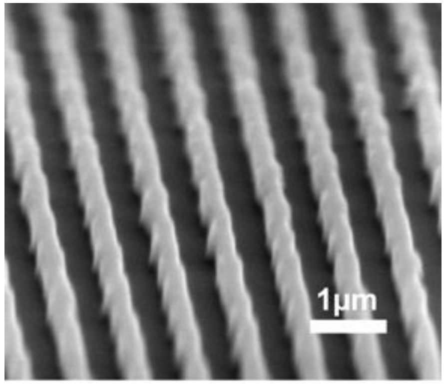


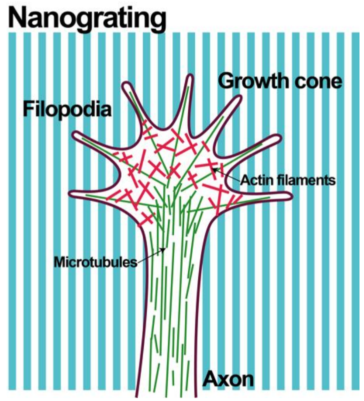
ß tubulin
DAPI
PEG-DMA autofluorescence

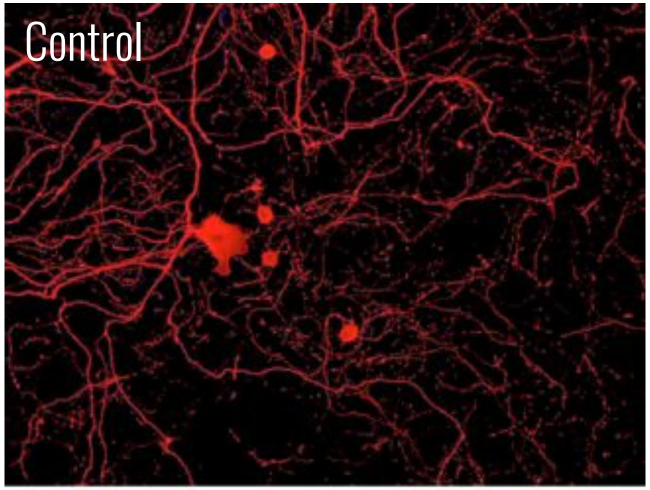
# 3.1 Neuron growth on nanogratings

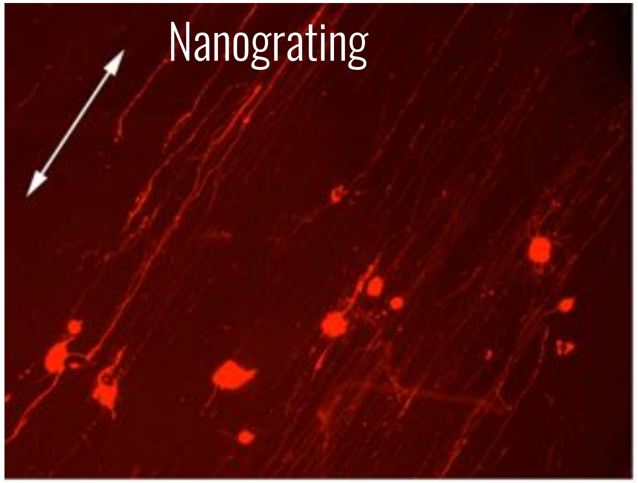
## Regenerative medecine

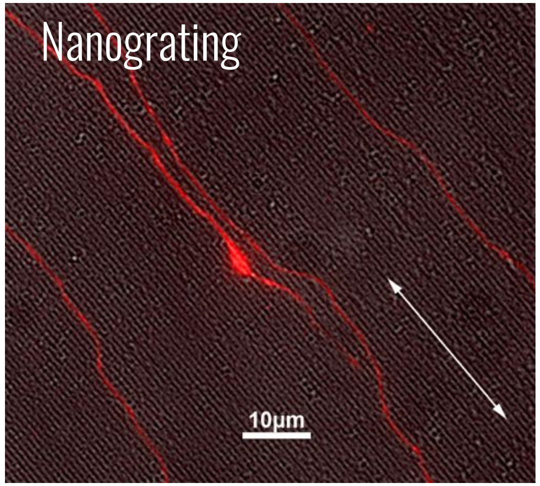
- Dorsal Root Ganglions neurons (axotomized)
- « straight line » axonal growth
- Interference lithography



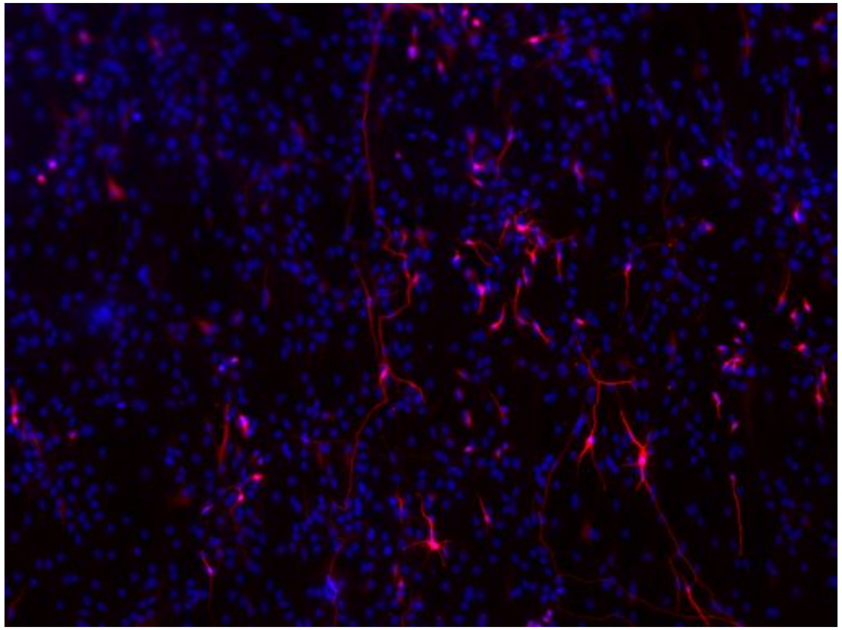



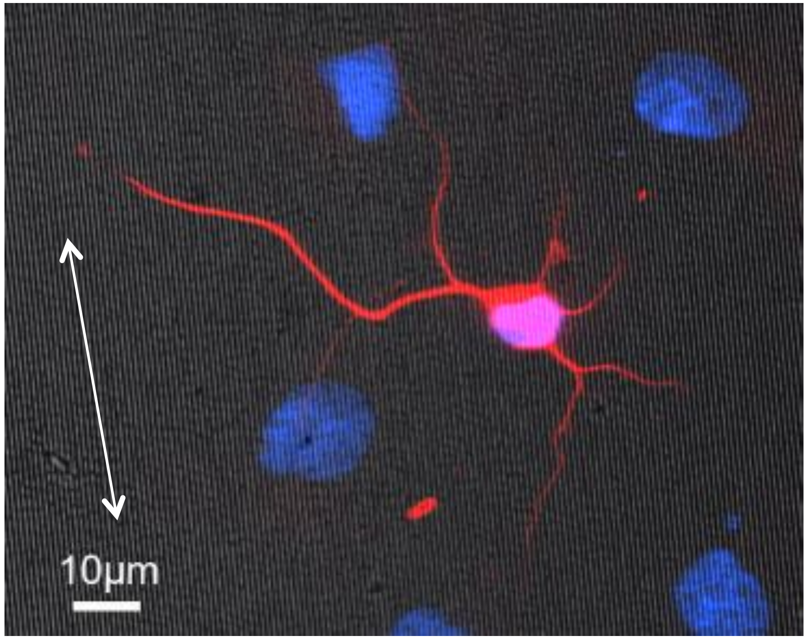




B.Charlot, et al., "Axonal growth guidance by surface nano-topology for the regeneration of sensori motor neurons", **IEEE EMBS Neural engineering conference**, Montpellier, France, (2015)

# 3.1 Neuron growth on nanogratings

On Cortical neurons…. Less effect…



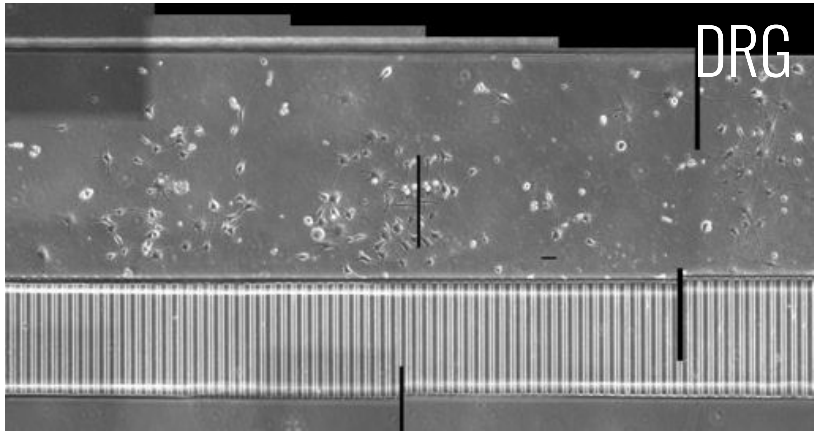


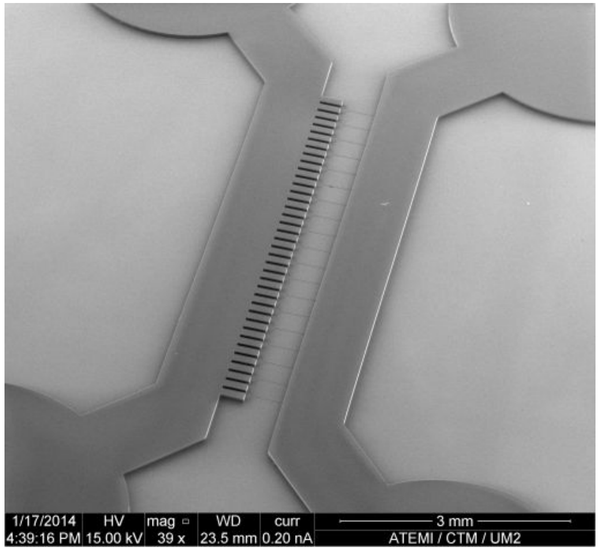
# 3.2 Microfluidics for Neurosciences

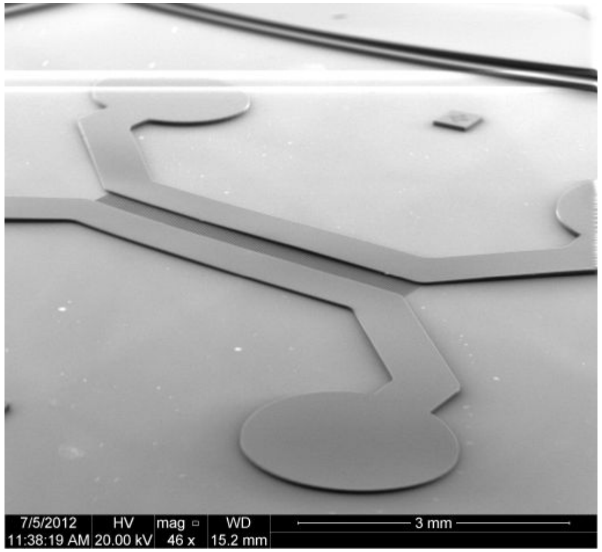
Microfluidic Compartiment cell culture chambers

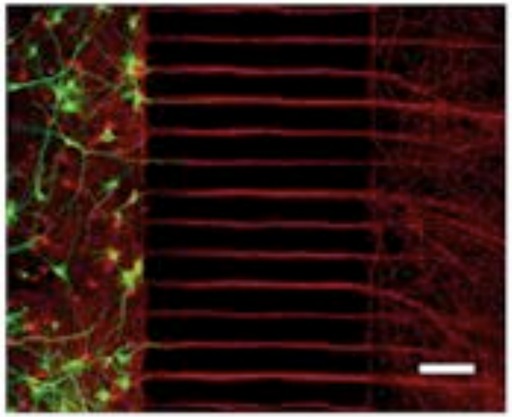
- Campenot cell design
- Oriented neuronal networks / guided growth
- Microchannels for Soma / Axon separation

-SU8 /PDMS replica molding



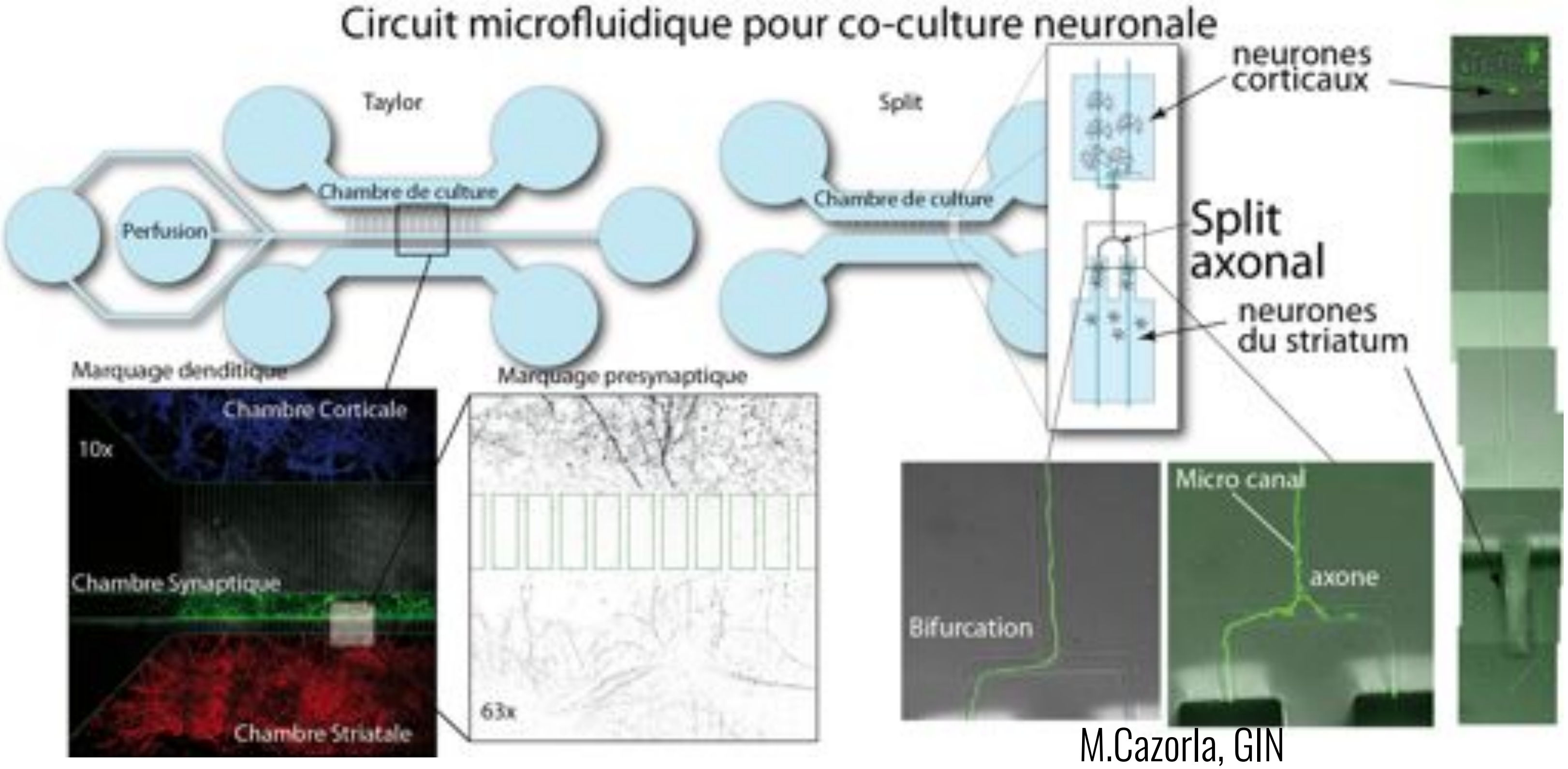



Taylor et al.

# 3.2 Microfluidics for Neurosciences

## In-vitro Neuronal junctions reconstruction

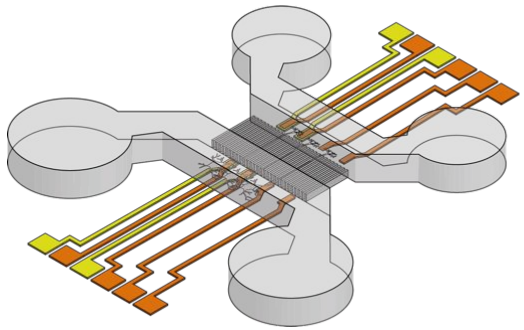
- Pathologic brain model (Alzheimer, Huntington..)
- Cortico-striatal junctions
- Electrophysiologie+ HR microscopy
- Drug perfusion in synaptic chambers
- Axonal Transport (pre-synaptic vesicles)
-+ localised Physical stimulation



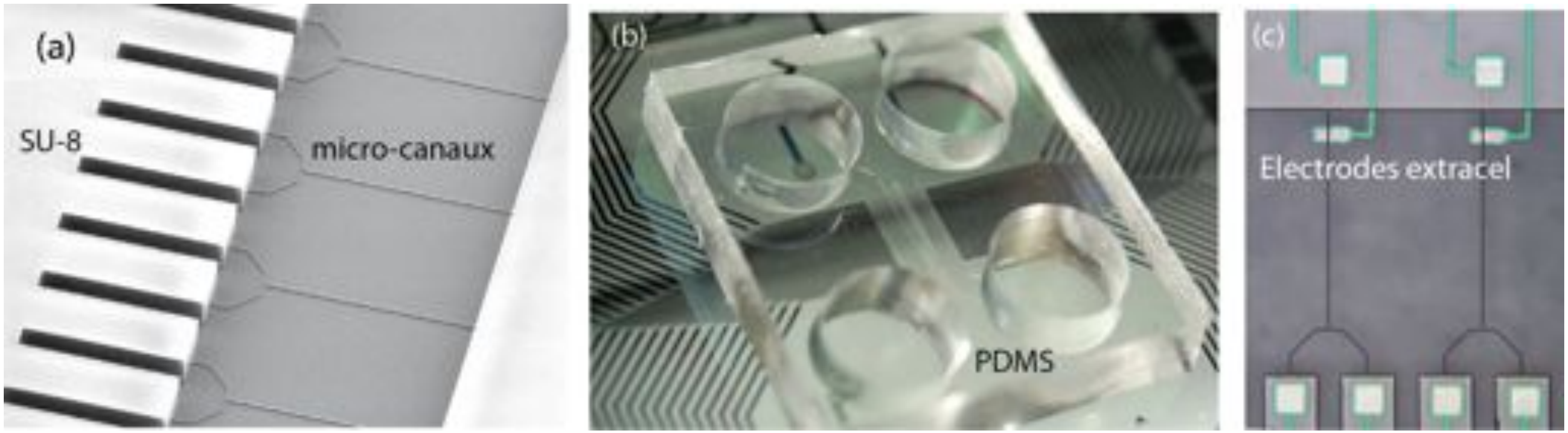

M.Cazorla, GIN

# 3.2 Microfluidics for Neurosciences

## Microfluidic chamber + Micro Electrode Array



- 64 channels (plugged on Multi Channel System)
- Electrode network : Chrome/ Platinum
- 5x5 cm glass chip, 170µm thick for microscopy
- Branch design for axonal splitting



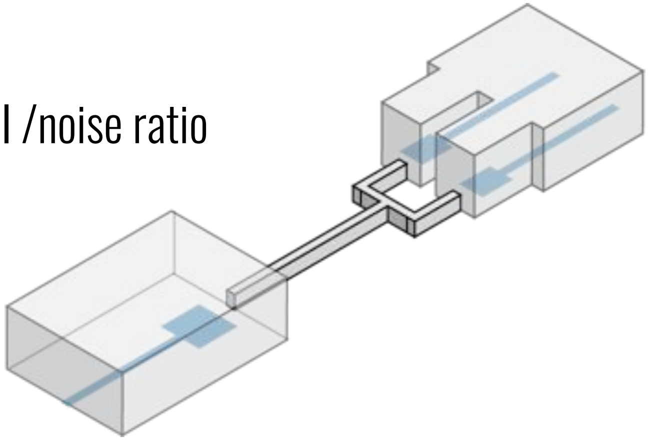
# 3.2 Microfluidics for Neurosciences

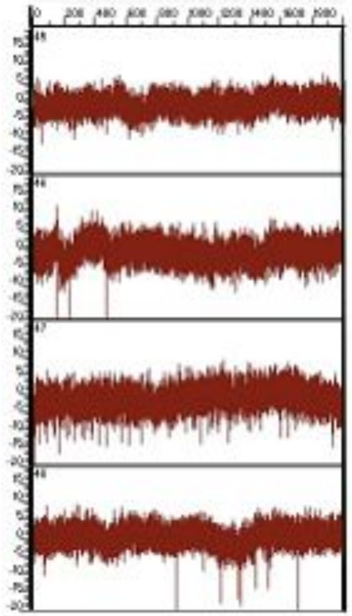
Extracel recording and stimulations

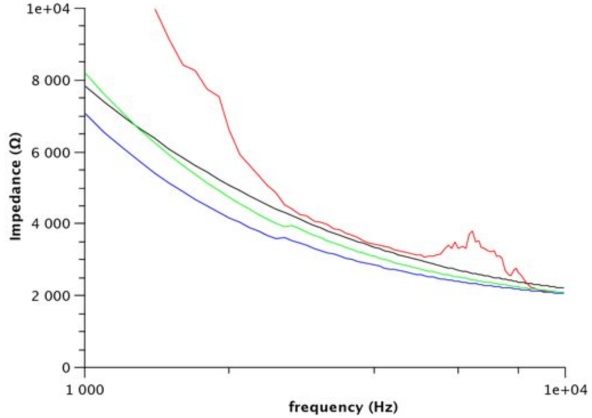
-Impedance

-Noise

-Signal /noise ratio



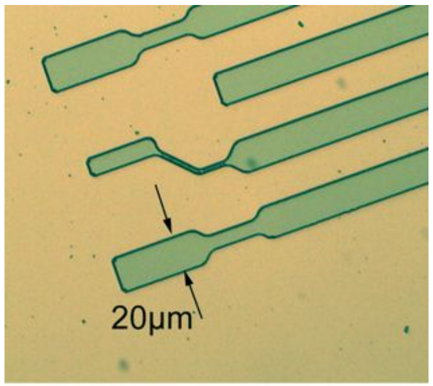





Pt and Au electrode
Impedance measurement

To improve S/N : lowering of the impedance

- Black Platinum, black gold
- Nanostructured electrodes
- PEDOT:PSS conductive polymer




PEDOT:PSS electrodes

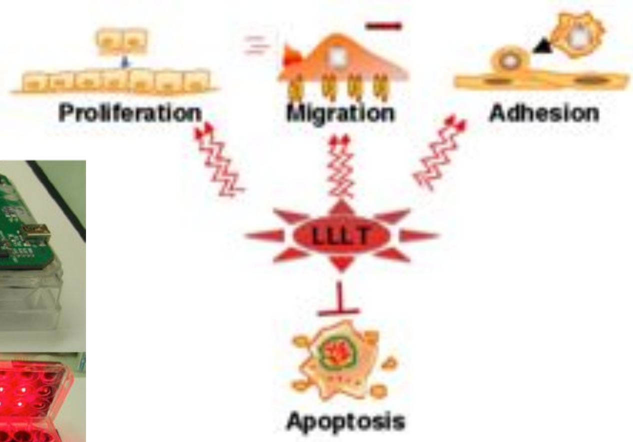
# 3.3 Optical stimulation

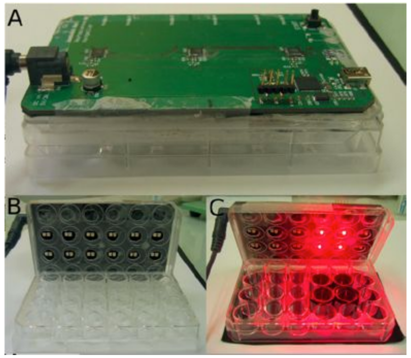
645nm **low level** Light

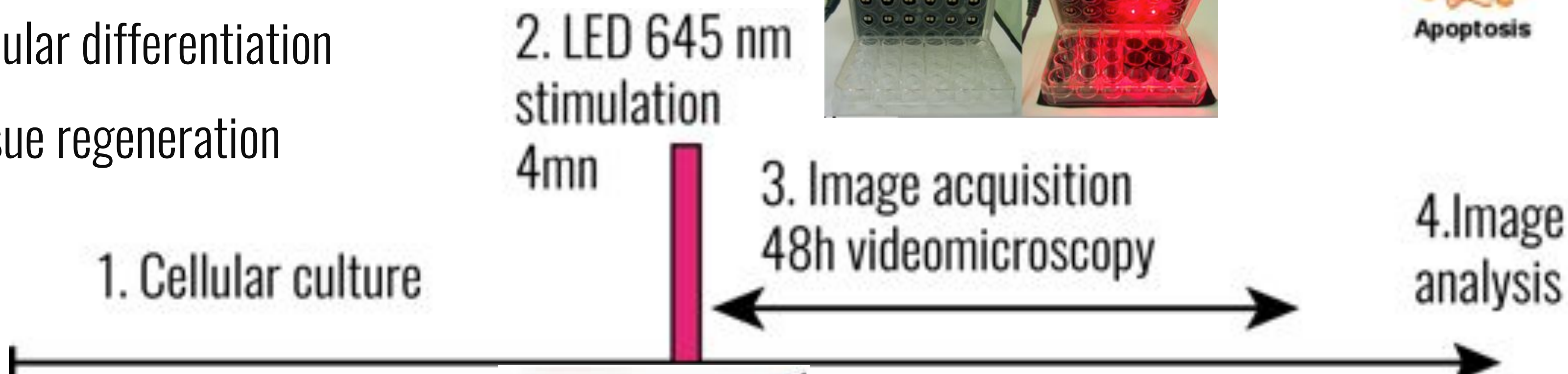
Proliferation stimulation

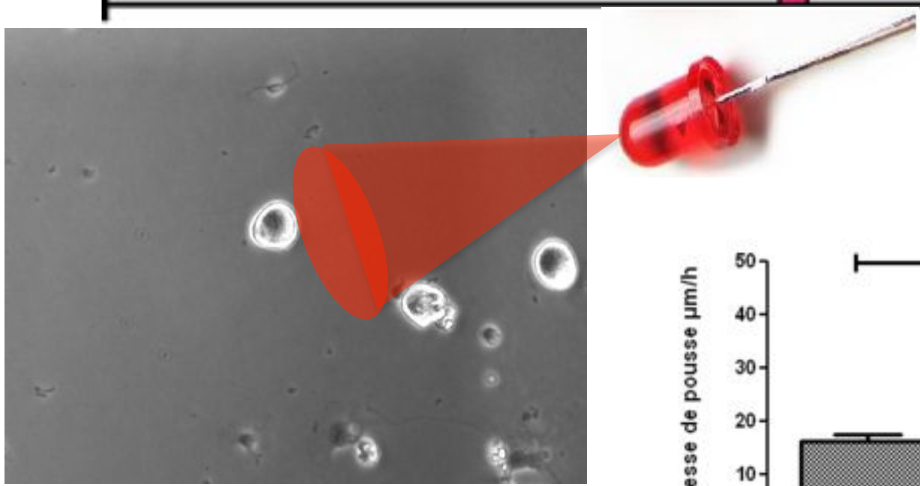
Cellular differentiation

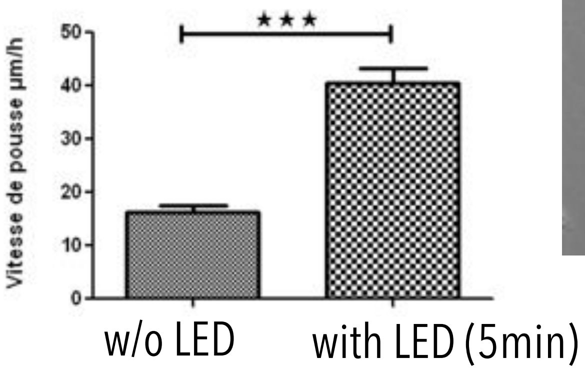
Tissue regeneration

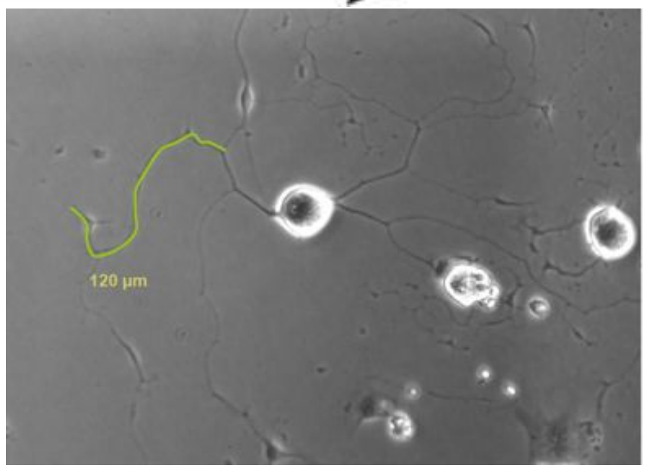




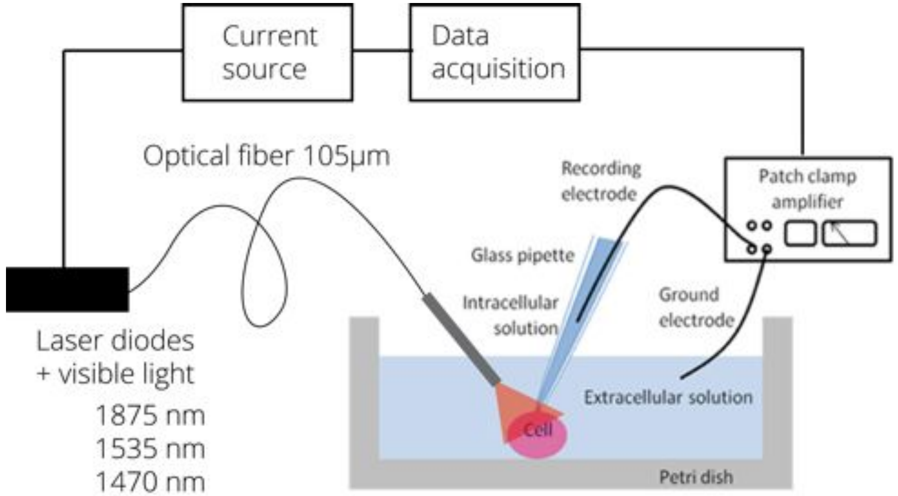




L.Paris, et al. "Neurite growth acceleration of adult Dorsal Root Ganglion neurons illuminated by low-level Light Emitting Diode light at 645 nm", **Journal of Biophotonics** 1–9 (2014).

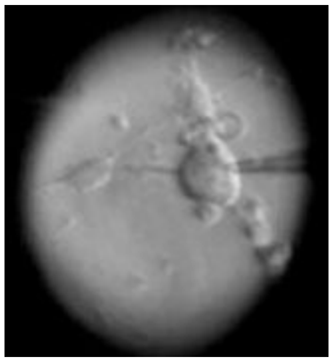
→ Neuron growth rate increasing

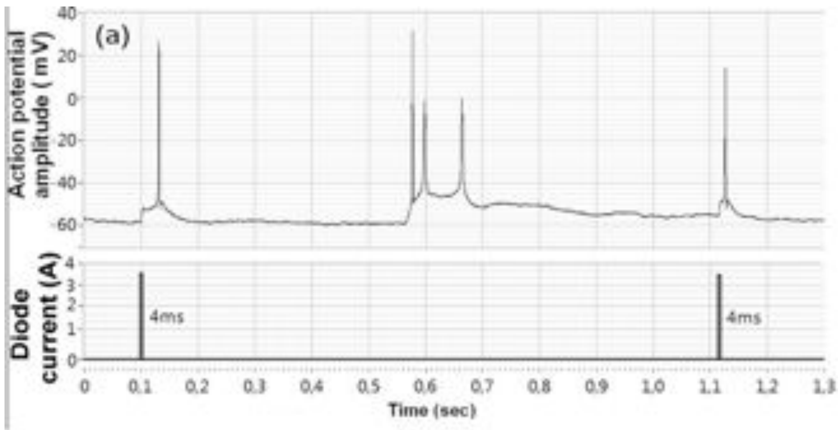
# 3.3 Optical stimulation

**Firing of action potentials** and depolarizations by infrared (1875nm) millisecond laser pulses

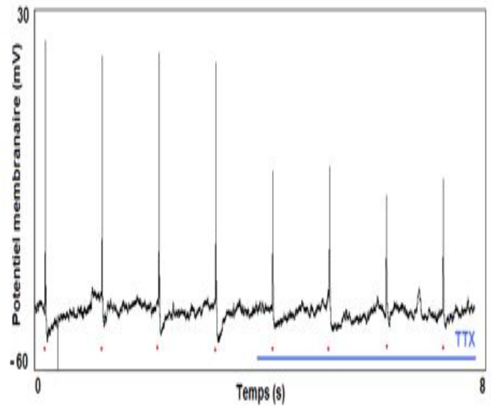



On dorsal root ganglion, vestibular and retinal ganglion cells






Patch clamp recording with whole cell configuration of laser stimulation pulses on cultured retinal ganglion cells

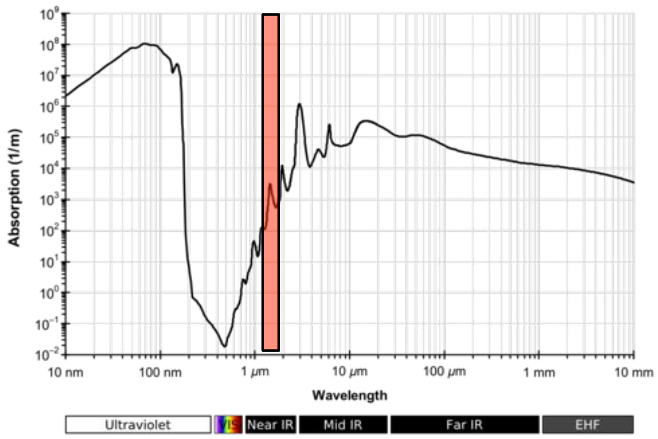



Energy threshold for AP generation It works….. Ok, But why?

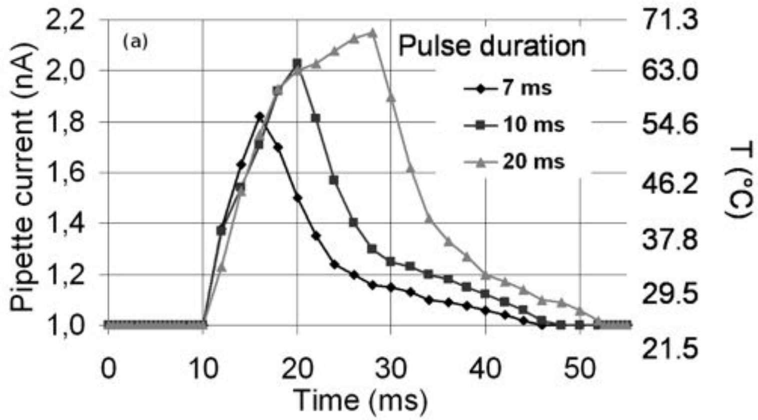
# 3.3 Optical stimulation

- Infrared light absorption in water
- High power density (200mW to 1W)

➔Temperature induced phenomenon?

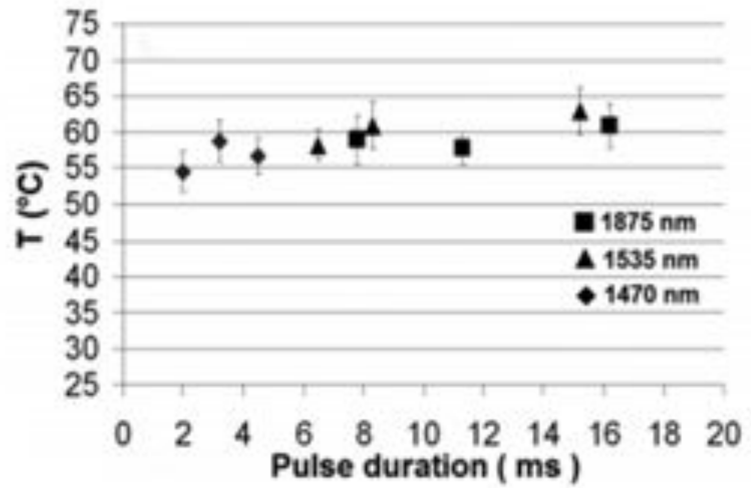



Temperature rise in the vicinity of neurons during Laser pulses




- Open Patch method : Laser burst on the pipette→ modification of its impedance and measured current as function of voltage steps

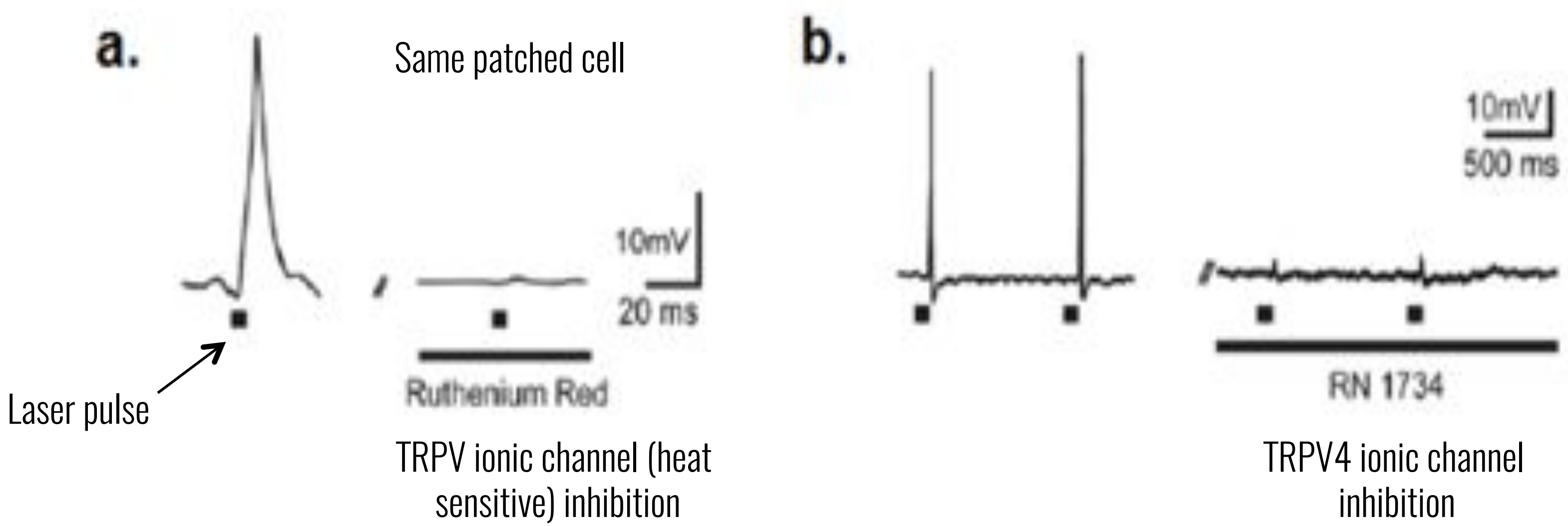
Temperature rise for AP triggering is equivalent for different laser conditions : 55°C- 60°C




➔Temperature seems to be involved, but what is the link with neurons ? Ion channels?

# 3.3 Optical stimulation

Temperature sensitive ion channel activation by IR laser
TRPV (Transient Potential Receptor Vanilloid channels)
(Albert et al., 2012; Bec et al. , 2012)



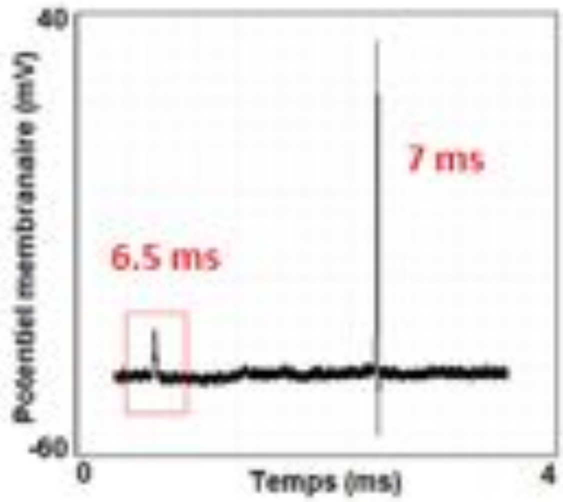

Inhibition of TRPV channels stops the laser induced AP generation

# 3.3 Optical stimulation

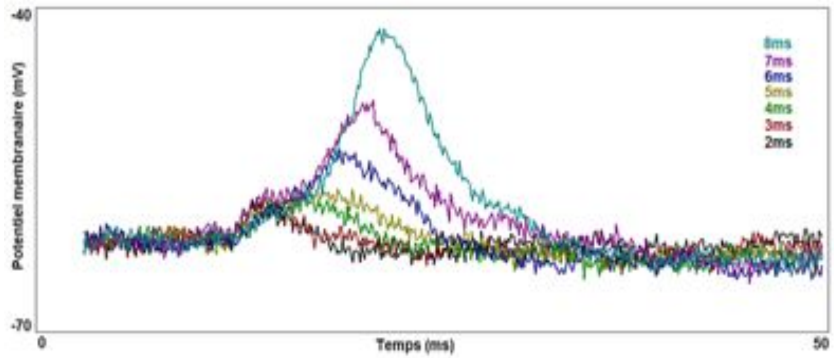
What happens below the threshold?

Action potential triggering

-Threshold (n=15)
10,5±1,23 mV




Modification of membrane potential induced by laser (IR 1875nm)
- The level of depolarization is function of the optical energy received

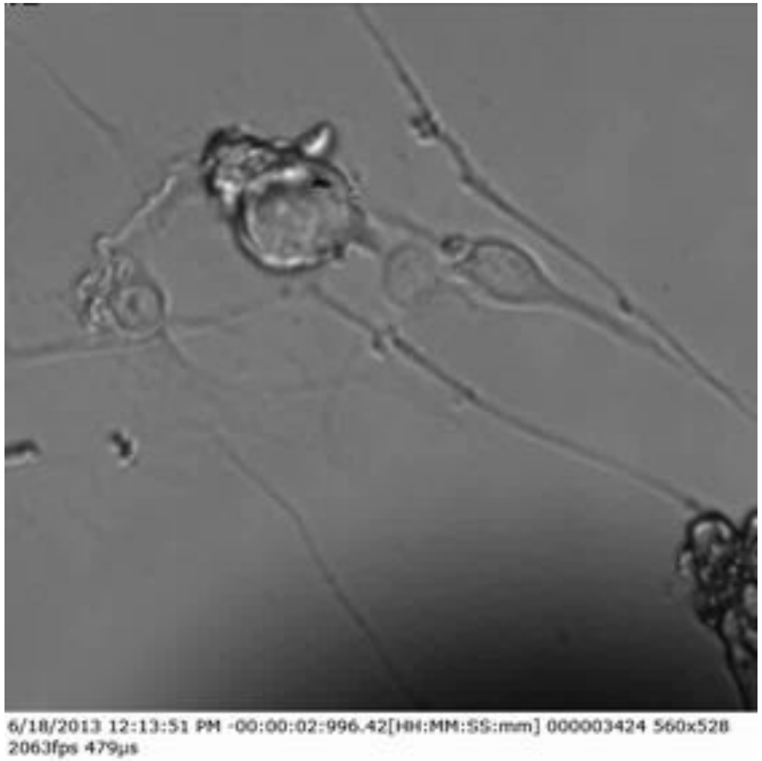



Two distinct mechanisms :

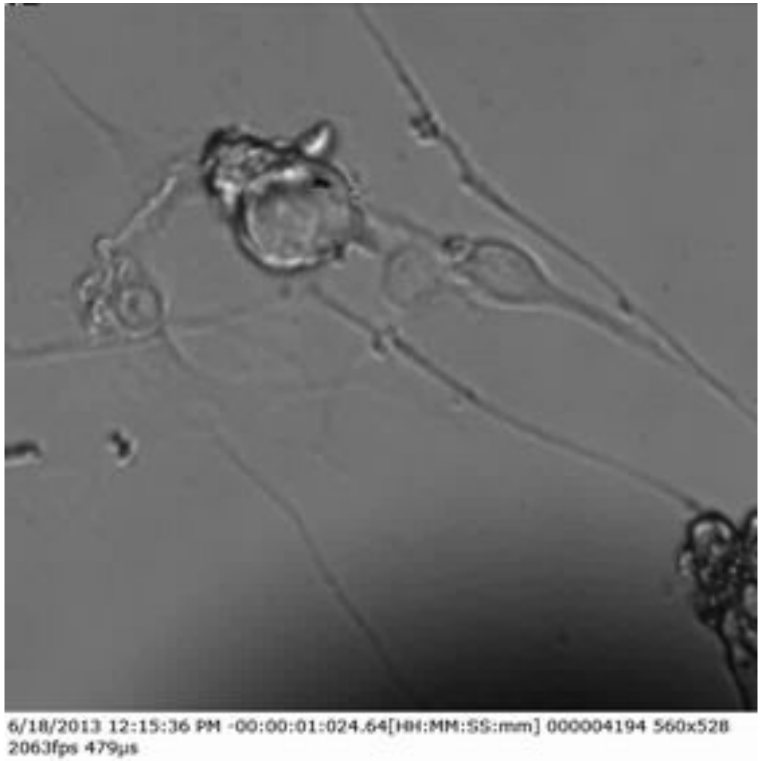
- Change in membrane potential with the temperature increase (capacitive effect)
- Action potential generation with TRPV Channels

# 3.3 Optical stimulation

Any mechanical aspect?  (thermo mechanical wave)




11ms

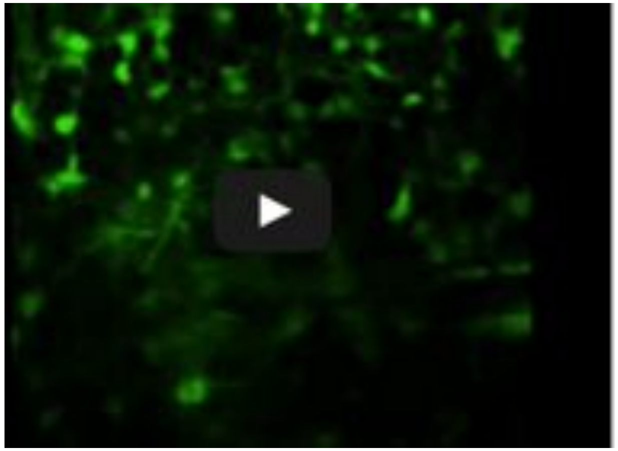



12ms

# 3.4 Mechanical stimulation

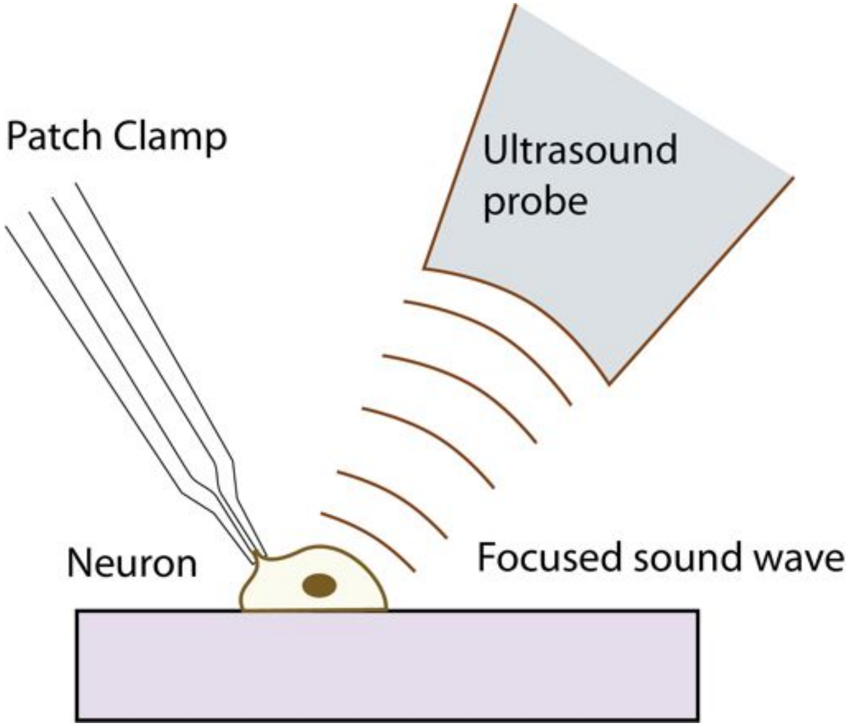
Goal : replace laser pulses by focused pulsed ultrasound

William J. Tyler
Arizona State University



low-intensity, low-frequency ultrasound 440kHz

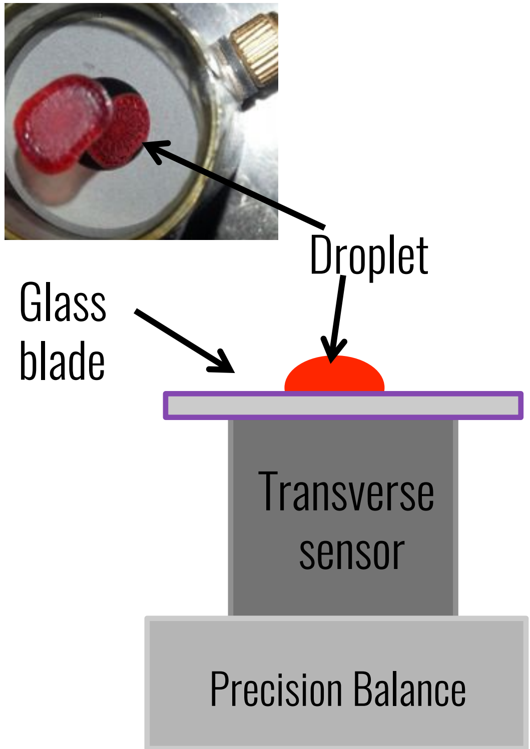
time-lapse confocal imaging of calcium transients in hippocampal slice cultures



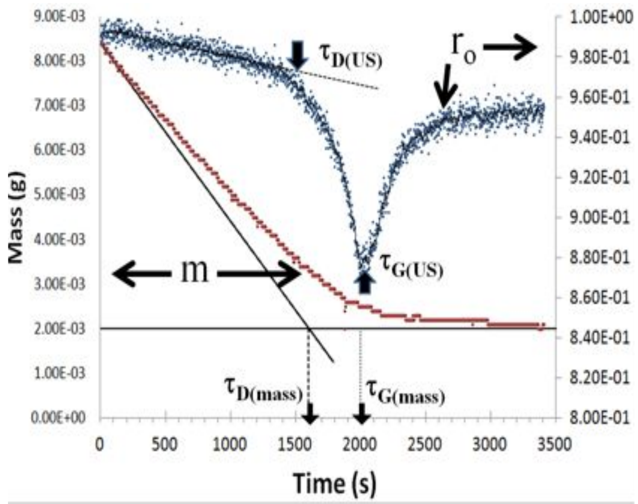

AP generation on single cell?

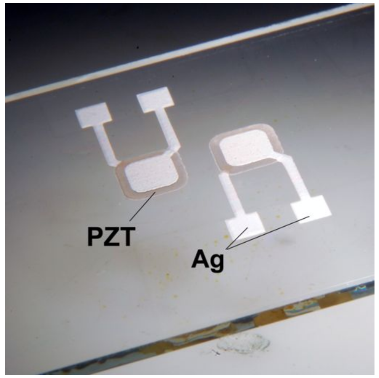
# 3.4 Mechanical stimulation
## Acoustics and rheology

- Viscoelasticity of drying blood
- Transverse ultrasound analysis at blood / glass interface

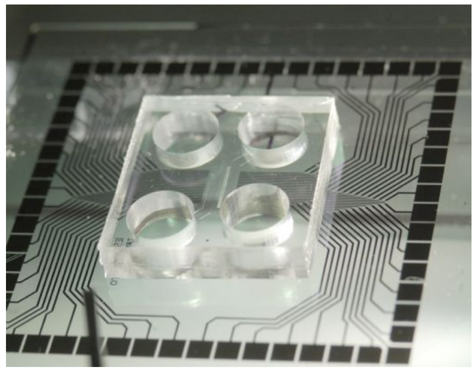



Mass and reflexion transverse coef. vs time




Screen printed PZT piezo on glass



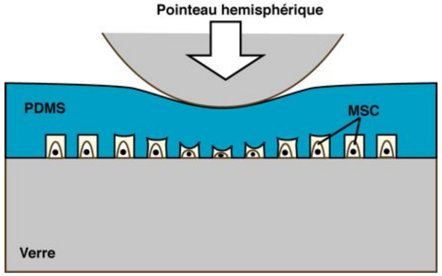
Ultrasound generator to be plugged below MEA?

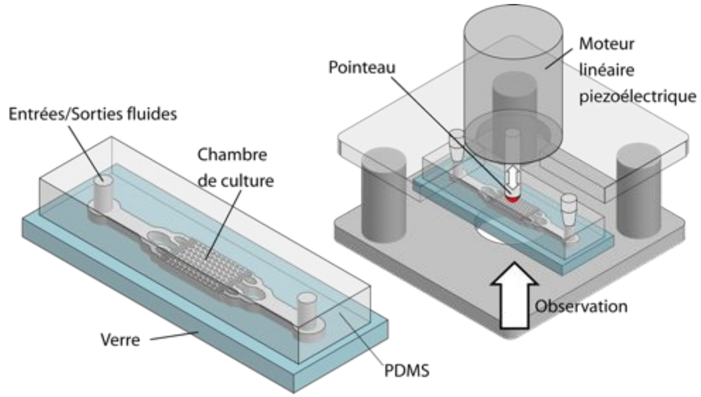
# 3.4 Mechanical stimulation

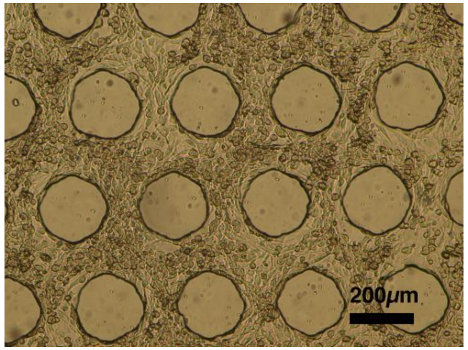
Mesenchymal Stem Cells (MSC)
Differentiation by mechanical stimulation.

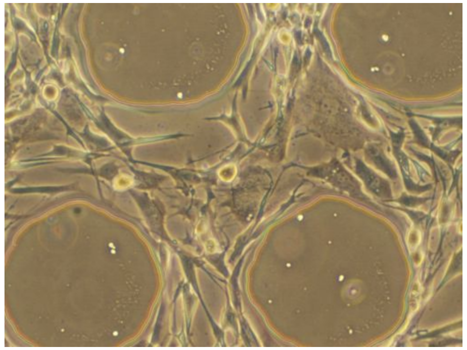
STIMDIF project

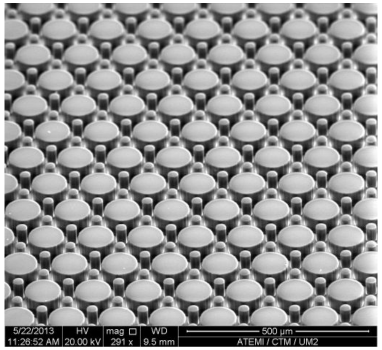
*(PEPS CNRS)*



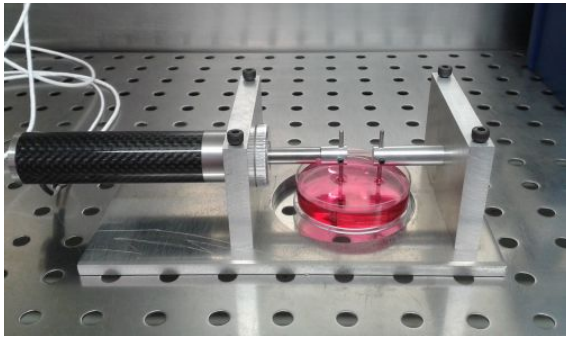





Biodegradable copolymer scaffold

Mechanical stimulation of MSC differentiation

A.Leroy, C.Bony, C.Pinese, B.Charlot, X.Garric, D.Noël, J.Coudane, "PLA-poloxamer/poloxamine copolymers for ligament tissue engineering: sound macromolecular design for degradable scaffolds and MSC differentiation", Biomaterials Science, DOI: 10.1039/C4BM00433G, (2015)



# 4 Summary

- DNA combing by capillary force: use of nanogratings
- Neurosciences :
    - Axonal guidance by micro engineered substrates: PEG-DMA / nanogratings
    - Microfluidic MEA platform for in-vitro reconstruction of neural junctions.
    - Physical stimulations :
        - Laser pulses : thermo mechanical effect to be analysed
        - Mechanical Stim and Ultrasound pulses : to be applied to neurons
        - Thermal : to be developped

# Thank you