The Abdus Salam International Centre for Theoretical Physics

2268-3

Conference on Nanotechnology for Biological and Biomedical Applications (Nano-Bio-Med)

10 - 14 October 2011

Local control of cell positioning and migration by multiscale substrate patterning

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National Research Council of Italy

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Joint ICTP-KFAS
Conference on Nanotechnology
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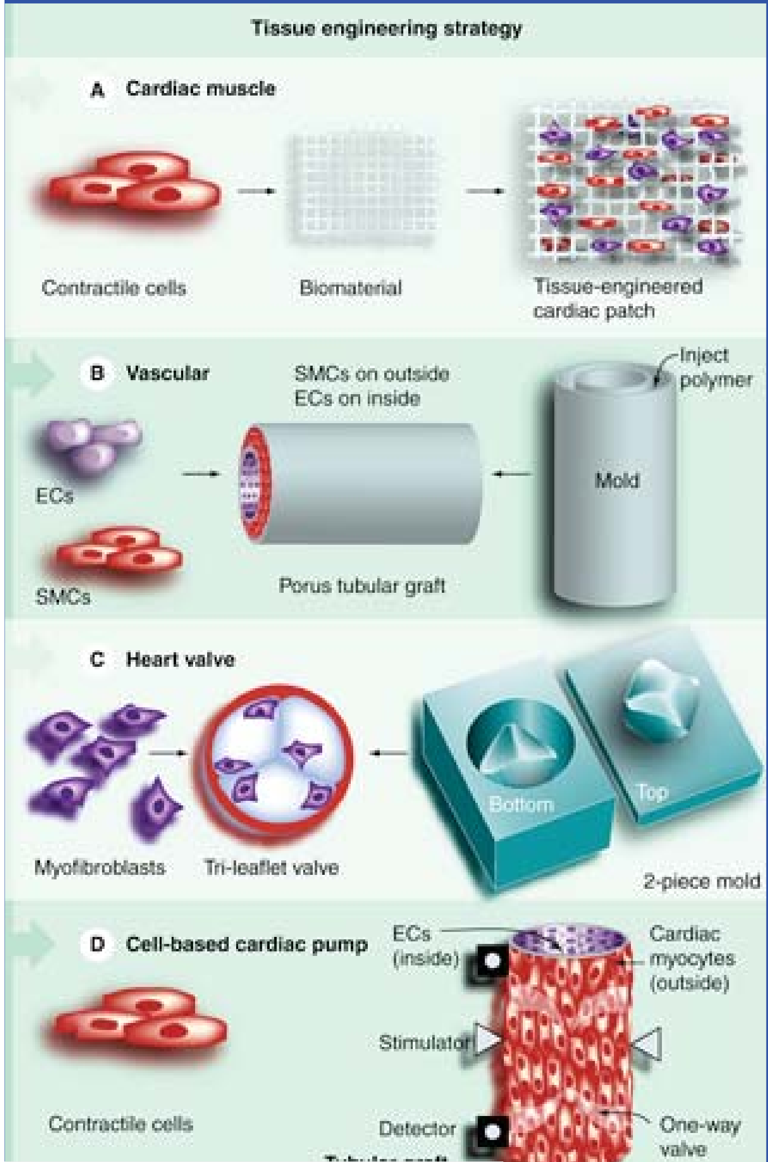
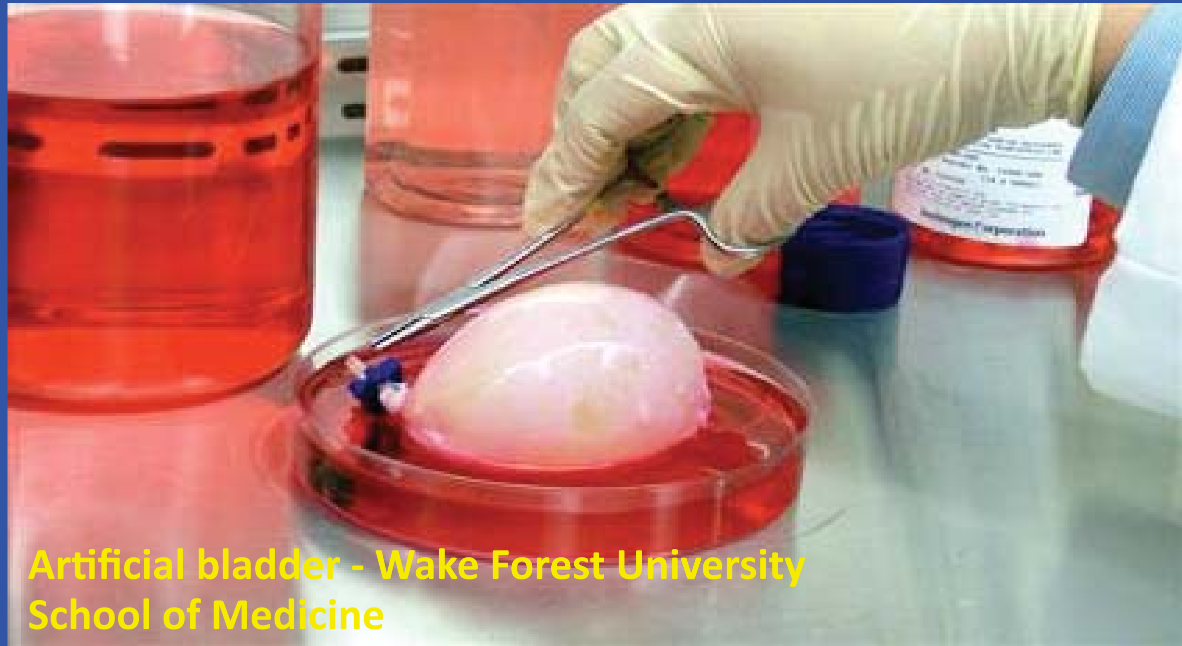
Miramare - Trieste, Italy

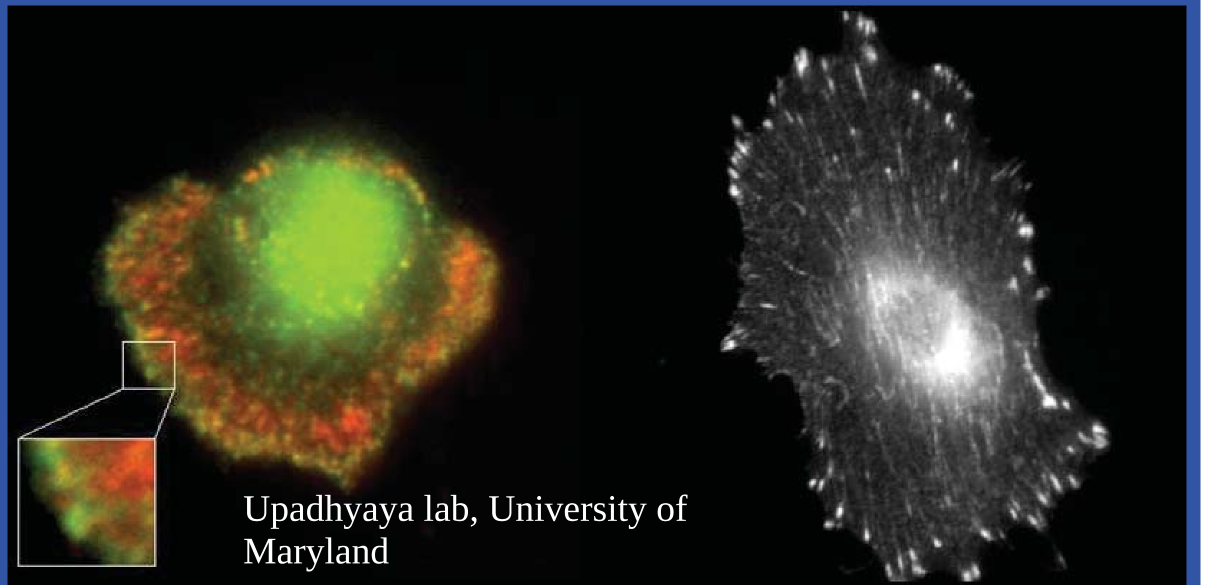
The Abdus Salam
International Centre for Theoretical Physics

IAEA

Controlling cell adhesion is crucial in regenerative medicine

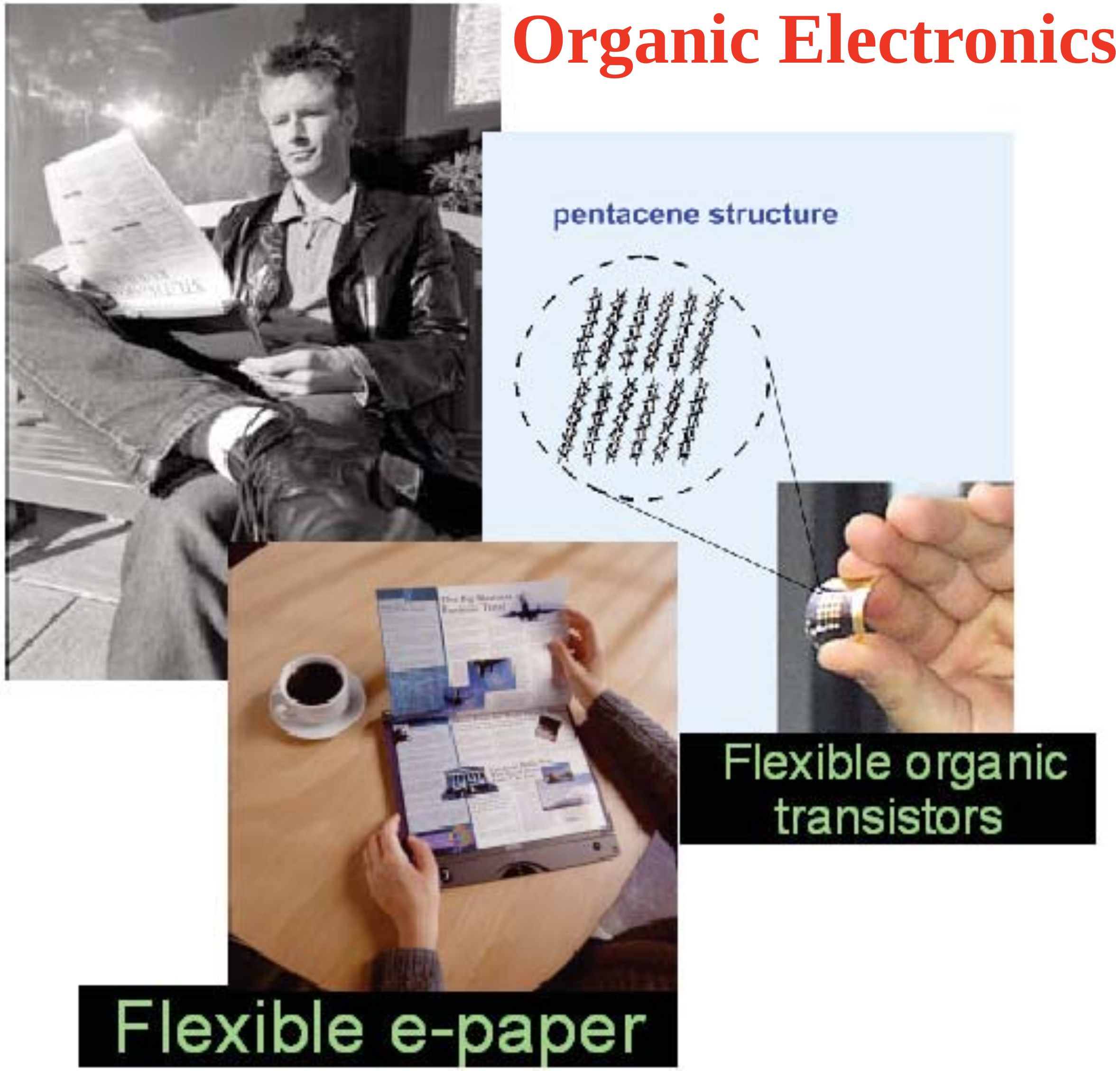


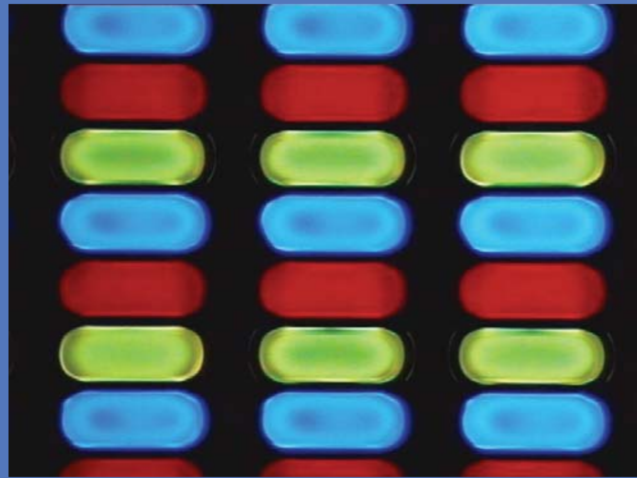




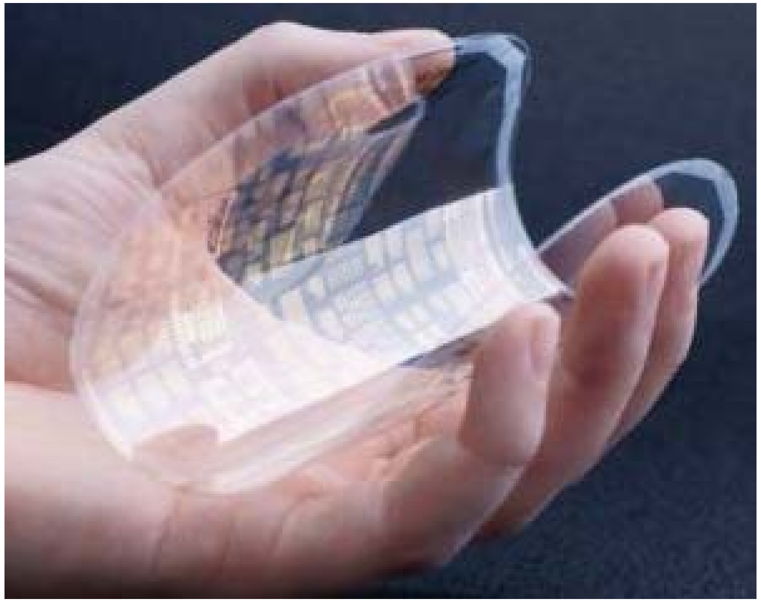
Sensing cell adhesion is also crucial in regenerative medicine

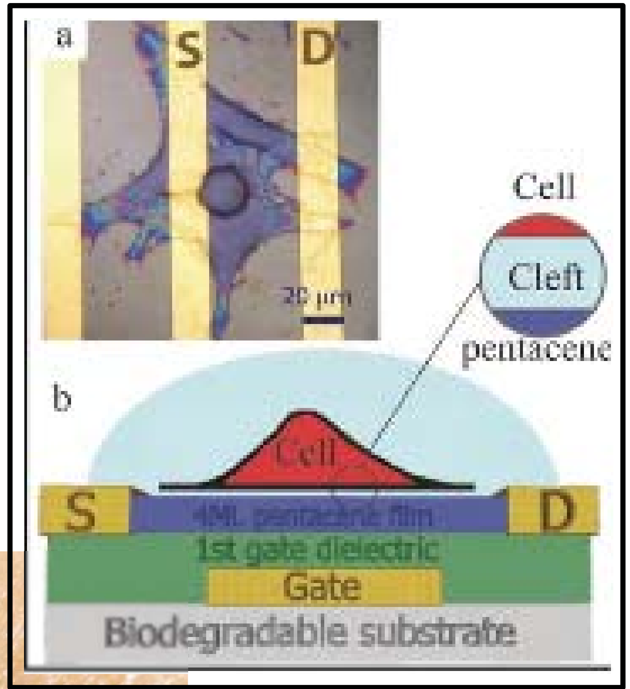
Organic Electronics

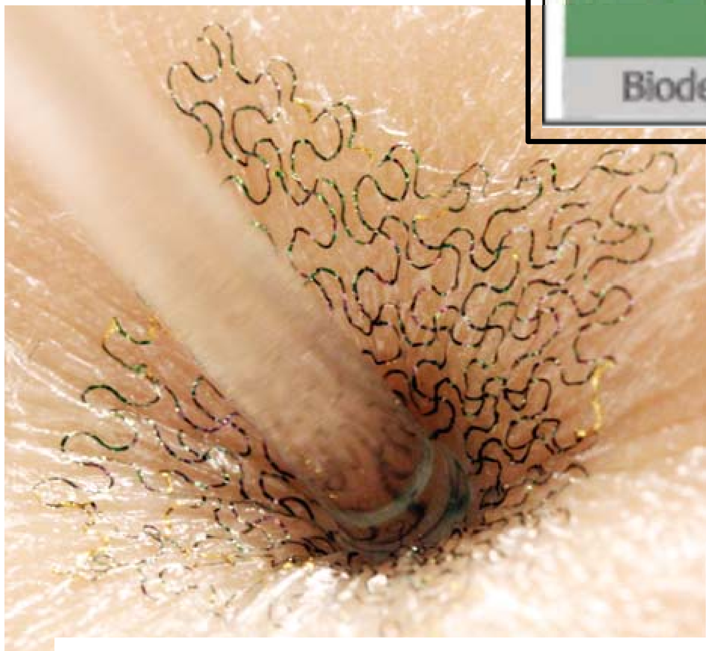


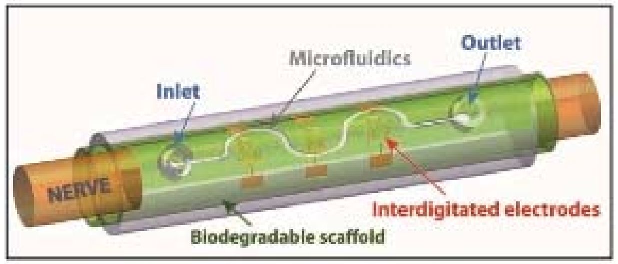


Flexible devices able to sense and to guide cell fate. Aiming to be implantable





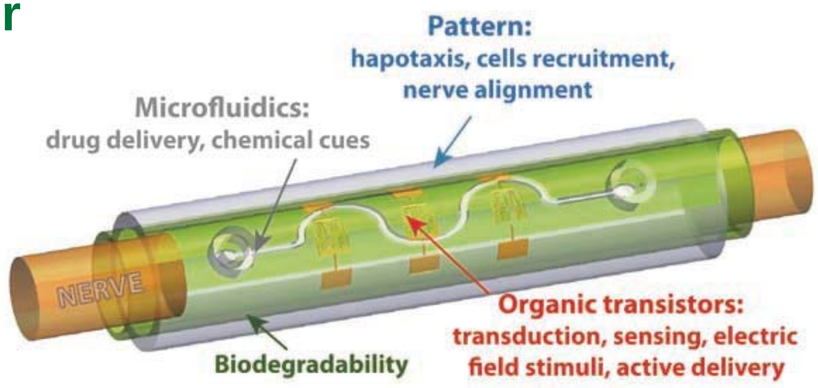


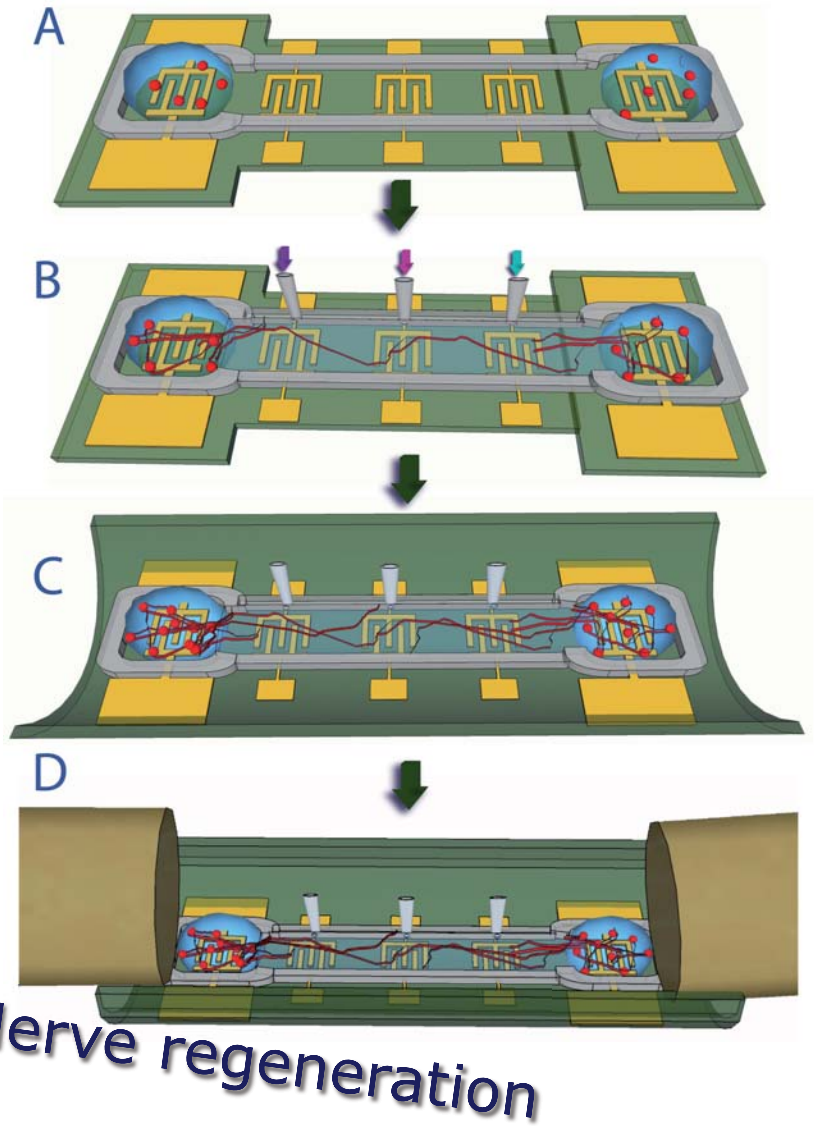


Prof. John Rogers, University of Illinois

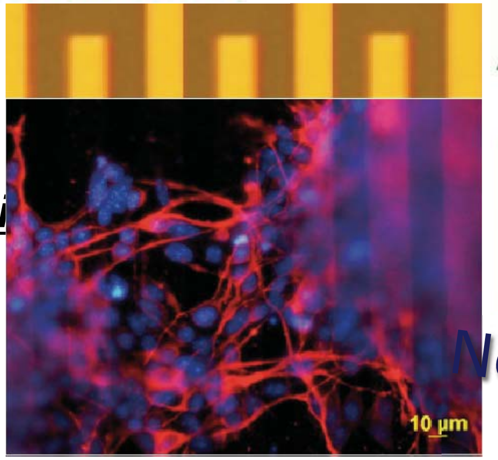
Implantable Organic Nano-Electronics
I-ONE

Fabricate an active multifunctional implantable organic devices as transducers and sensors on flexible substrates made of biocompatible polymers targeted to Spinal Cord Injury repair



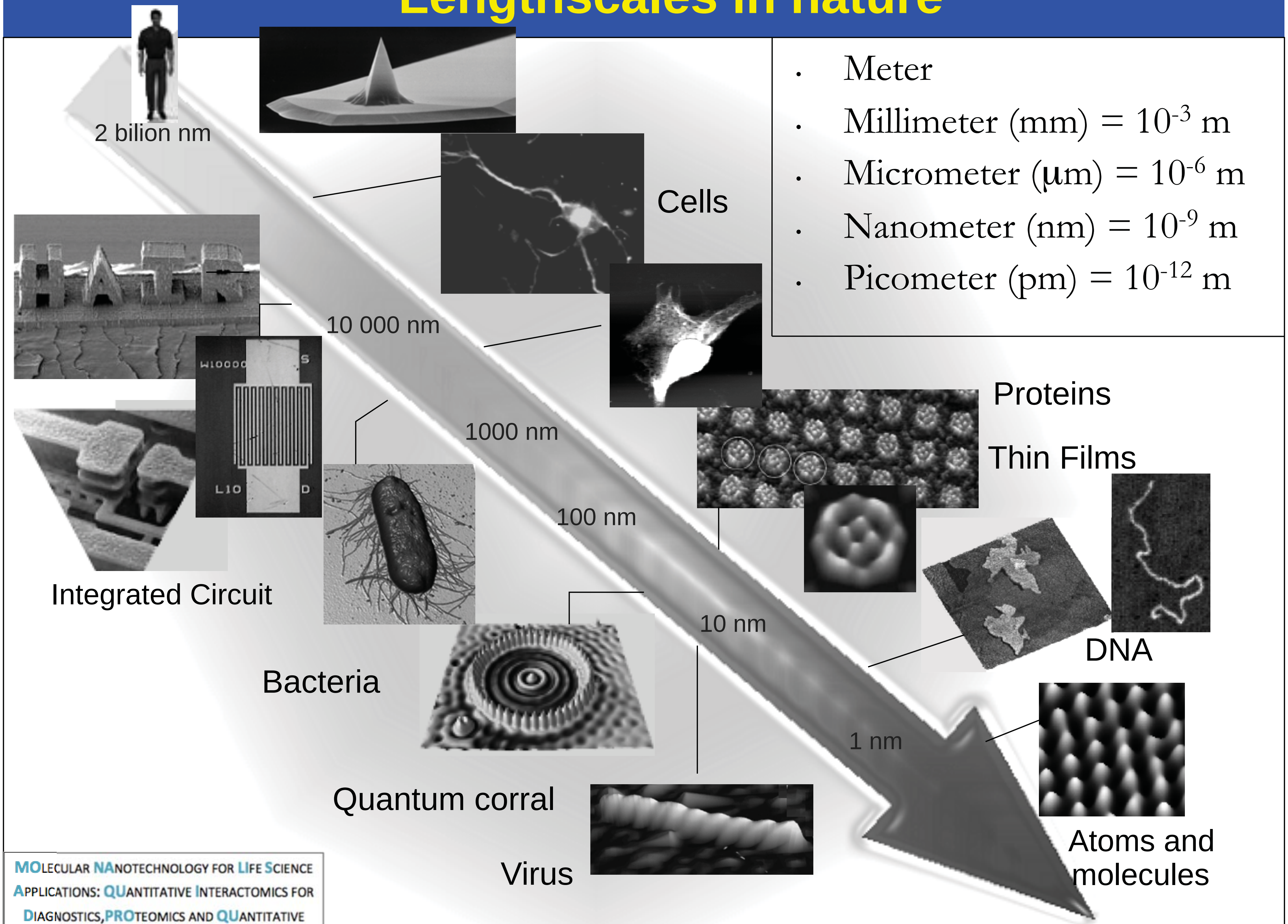


Supply a combination of topo-electro-chemical stimuli to the injured nerve region
resembling the complex microenvironment required for stem cell commitment.

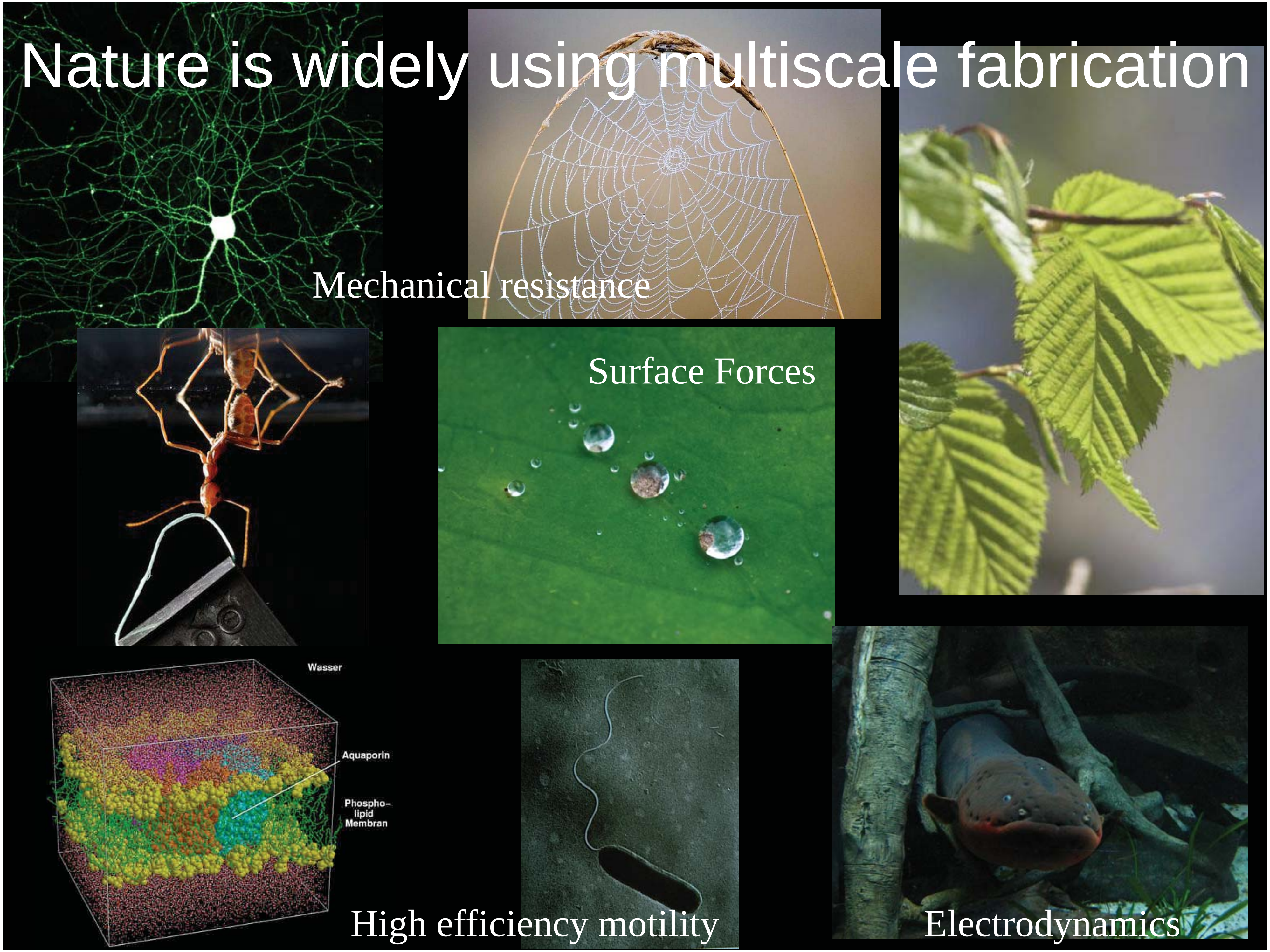


Lengthscales in nature

- Meter
- Millimeter (mm) = 10^{-3} m
- Micrometer (µm) = 10^{-6} m
- Nanometer (nm) = 10^{-9} m
- Picometer (pm) = 10^{-12} m

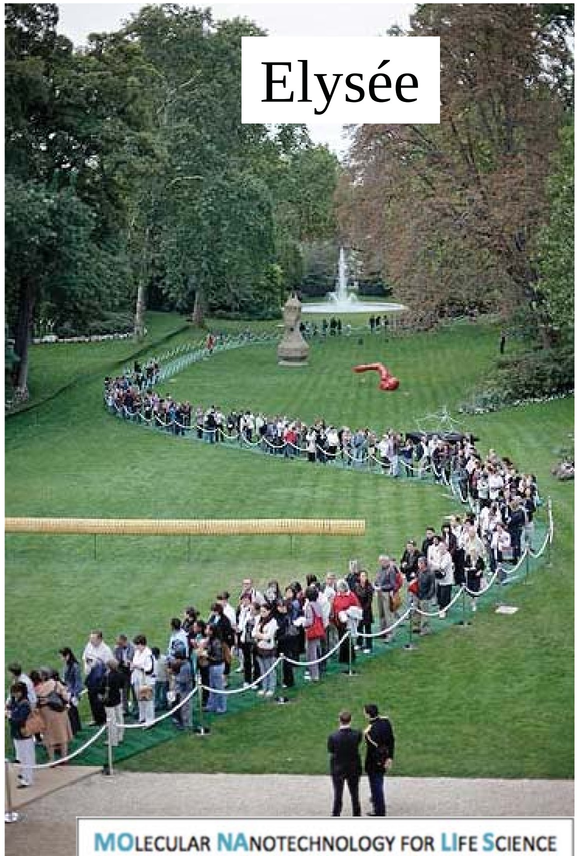


Nature is widely using multiscale fabrication



Controlling the fate…let's start by controlling the positioning

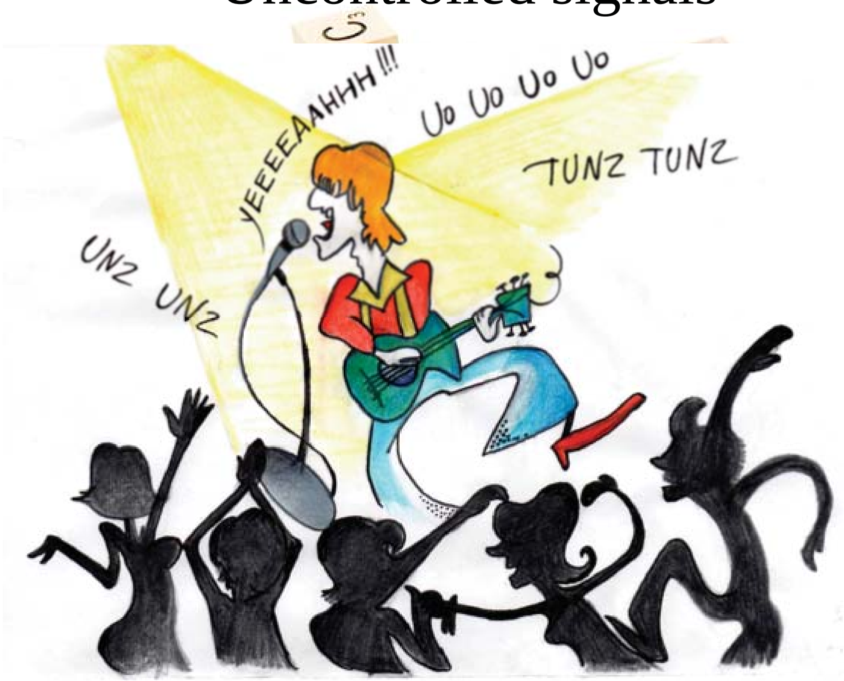

Palio, Siena


Elysée

MOLECULAR NANOTECHNOLOGY FOR LIFE SCIENCE APPLICATIONS: QUANTITATIVE INTERACTOMICS FOR DIAGNOSTICS,PROTEOMICS AND QUANTITATIVE

…we have to provide instructions to the cells:

Uncontrolled signals

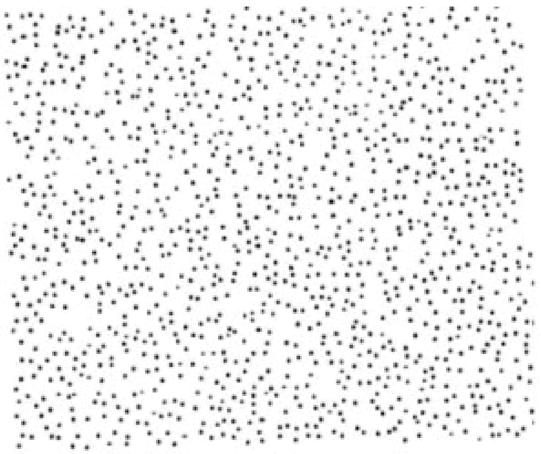


Fabricated patterns of signals

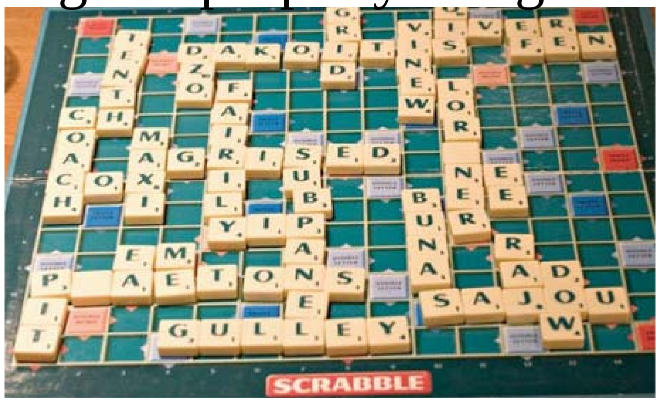
…we have to provide instructions to the cells:

Uncontrolled signals

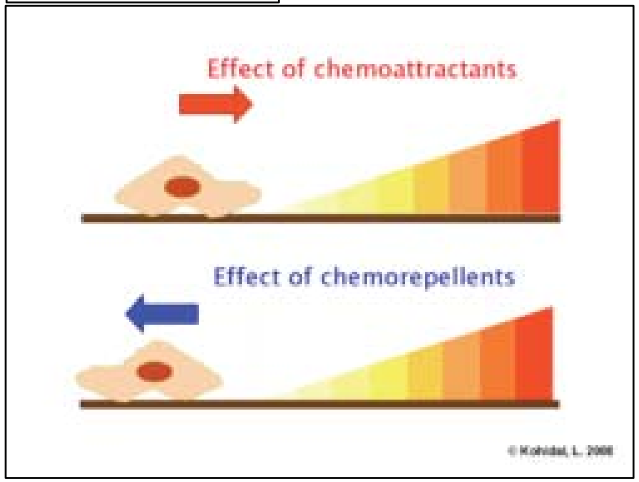
Random layer of cues

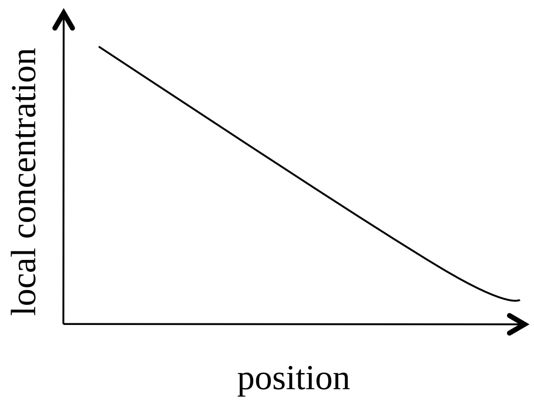


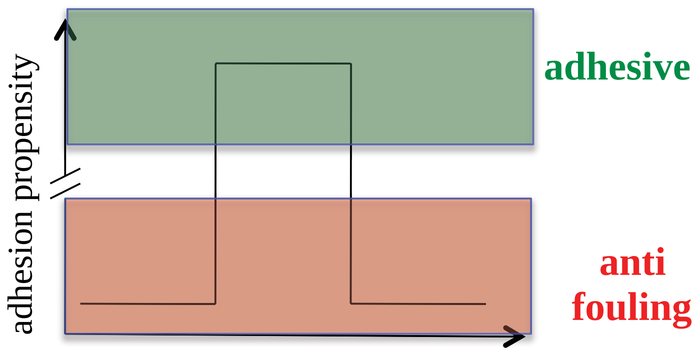
Signals properly designed



Gradients

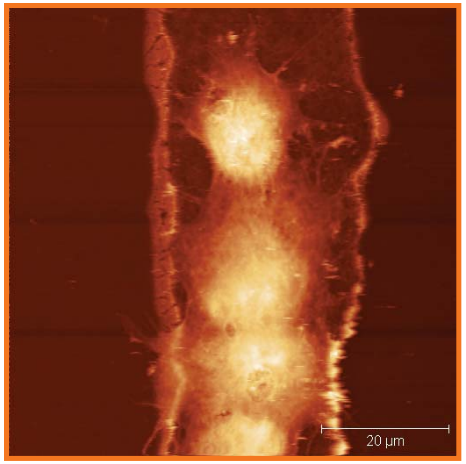




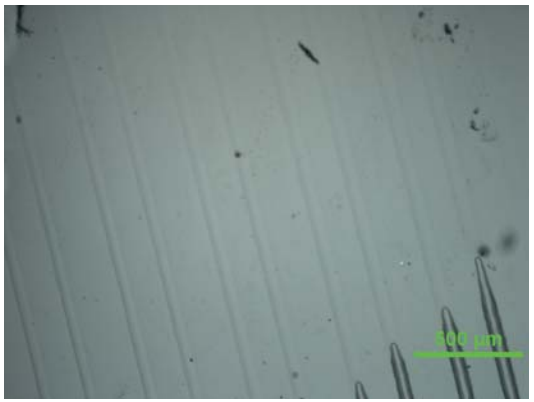


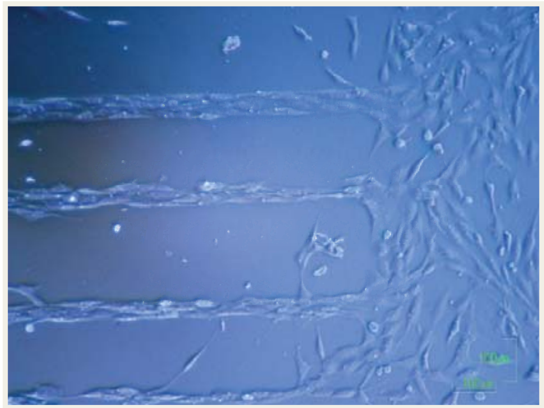
Which are the words:
Surface interactions influence cell fate

Surface property	Topography	Mechanics	Chemistry
Signals provided	Surface morphology, roughness	Stiffness, elasticity	Adhesion proteins and Growth factors
Fabrication strategy	Micro- and Nano-textured surfaces	Locally controlled Young's modulus	Soft lithography



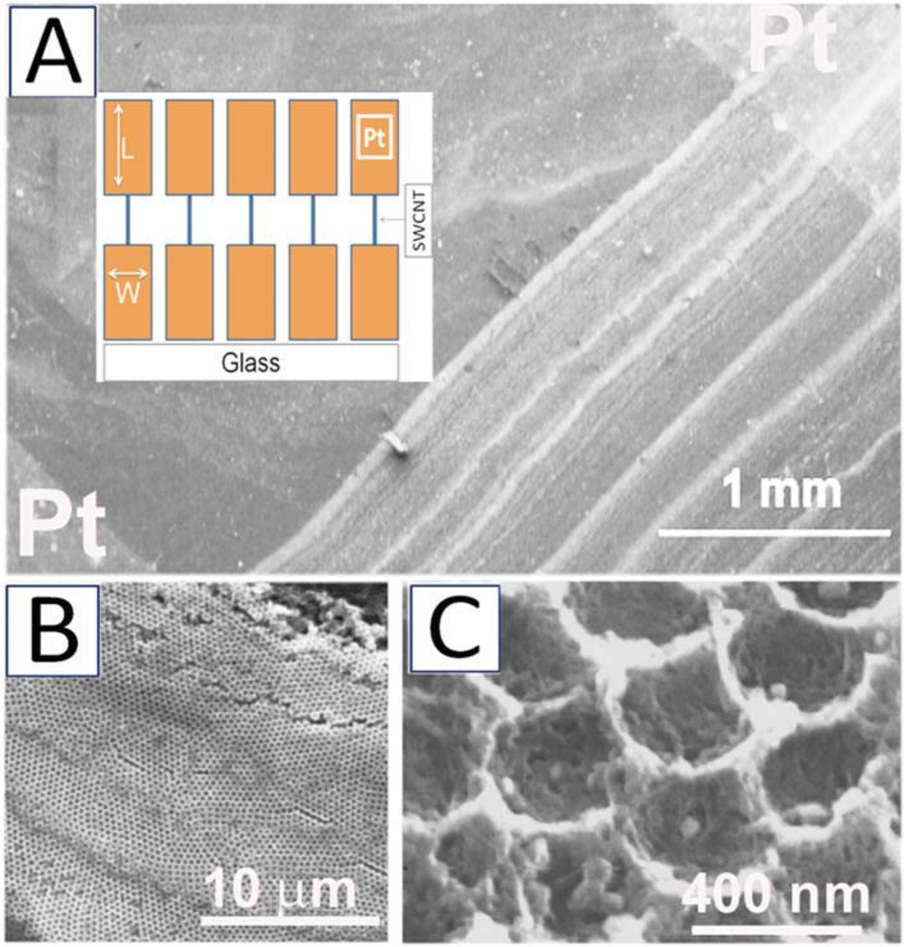
M. Bianchi et al 2010

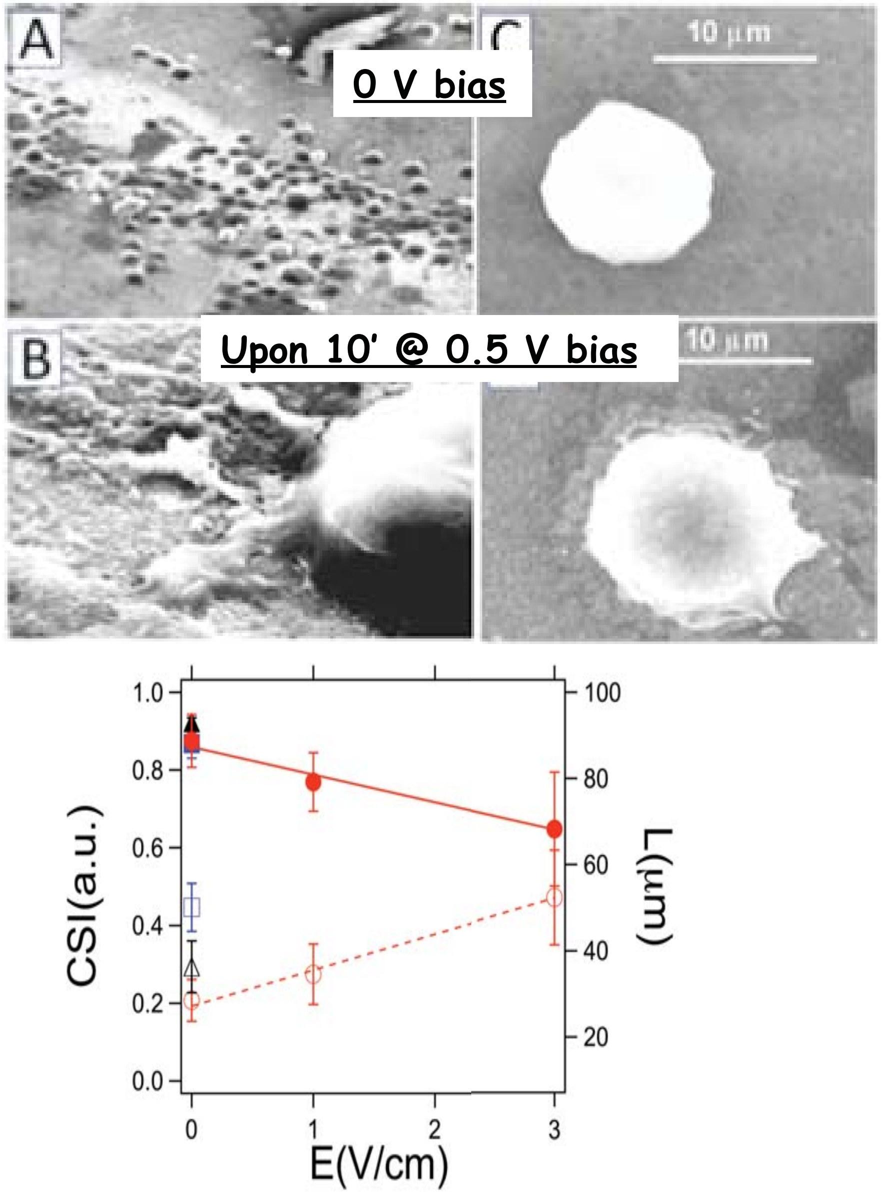




F. Valle et al Adv. Biomater. 2010

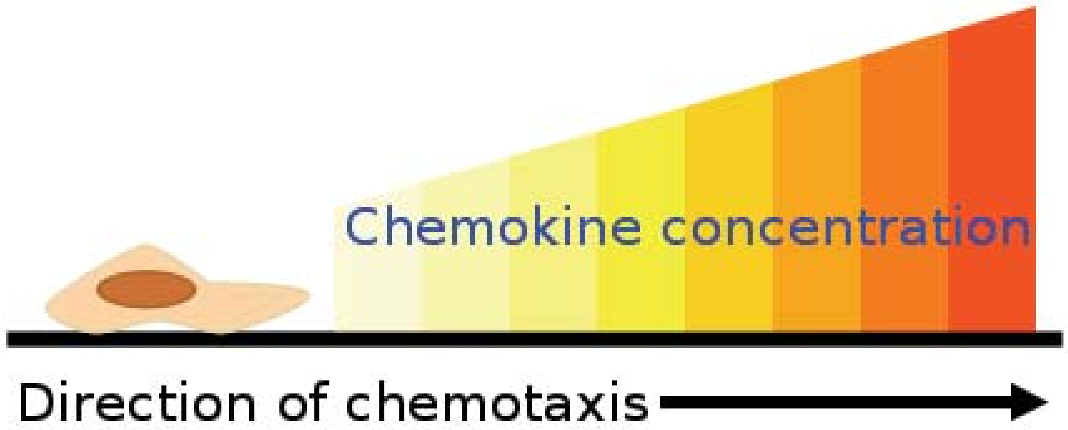
…and so does electric field: Neuronal cell adhesion on SWCNT patterns





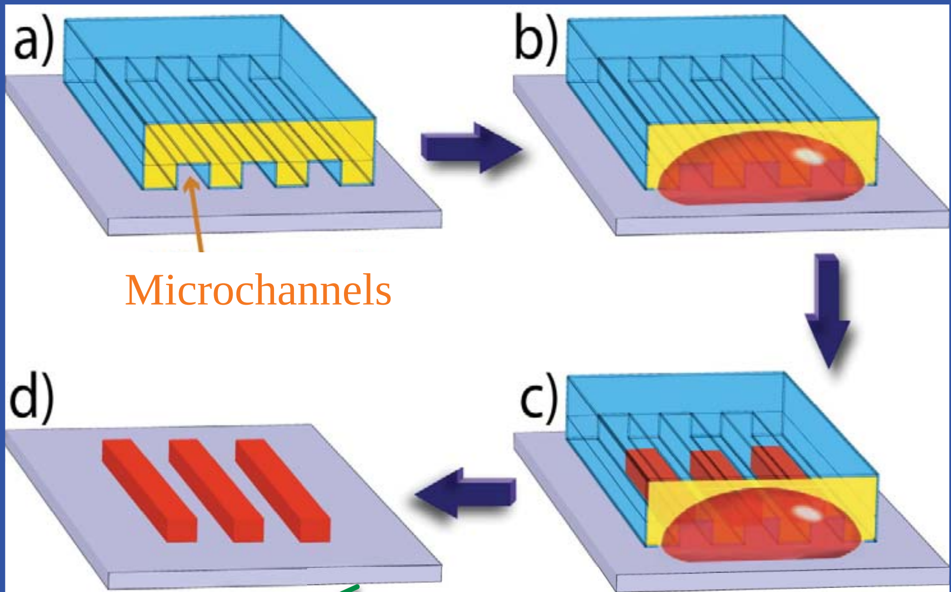
C. Dionigi et al. J. Mater. Chem. (2009)

Smooth gradients

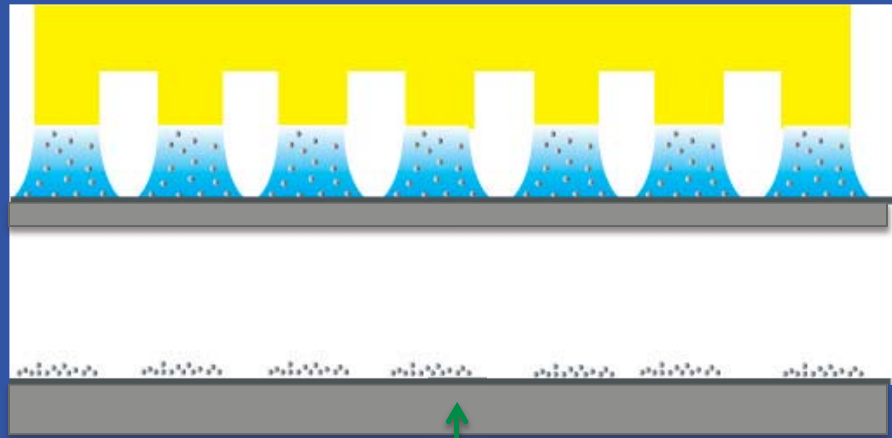


Soft-lithography to pattern proteins and chemical and topographic stimuli

MIMIC: Micro Molding in Capillaries



LCW: Lithographically Controlled Wetting



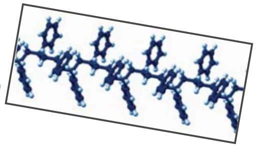
Patterned solute

M. Cavallini & F. Biscarini, Nano Letters 2003

Y. N. Xia & G. Whitesides, Angew. Chemie 1998

Patterning of Laminin on Tissue Culture Dish
by MIMIC

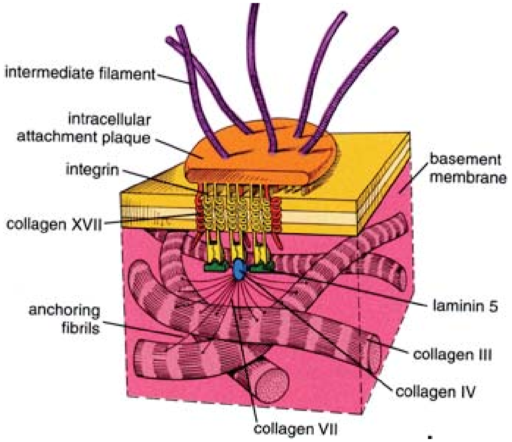
Polystyrene

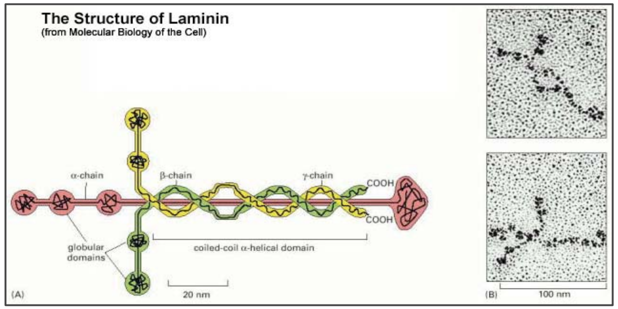


Roughness=6.9 nm

Contact Angle:

67.3 ± 1.5°

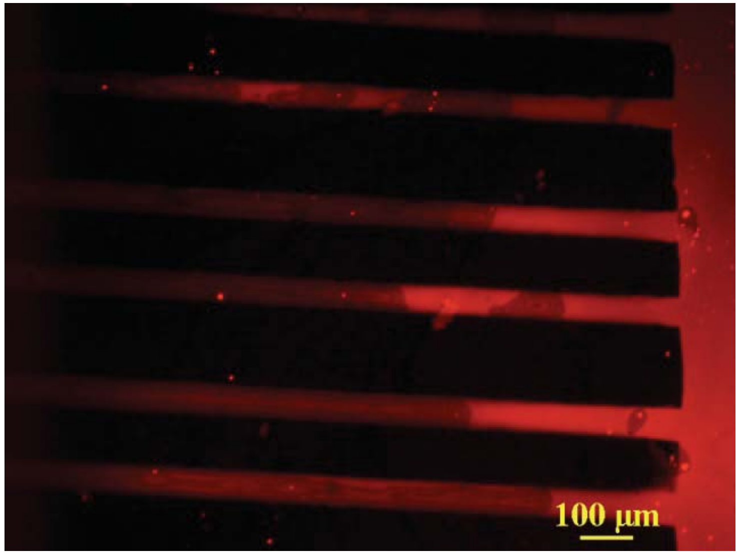




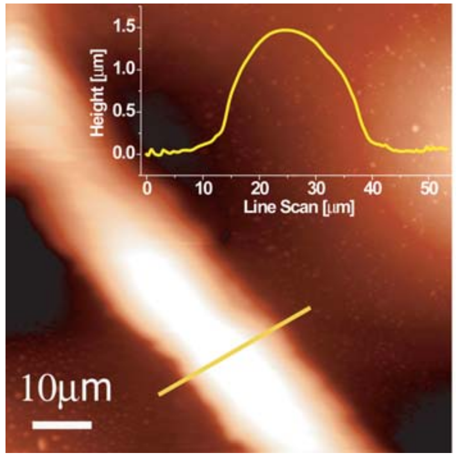
Univ. of Prince Edward Island

22 variabile channels from 17-70 μm

Optical micrograph of Laminin pattern on TC dish

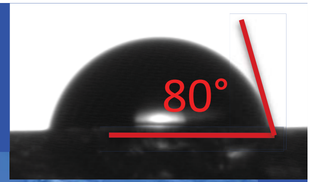


Laminin pattern visualized by anti-laminin immuno-fluorescence

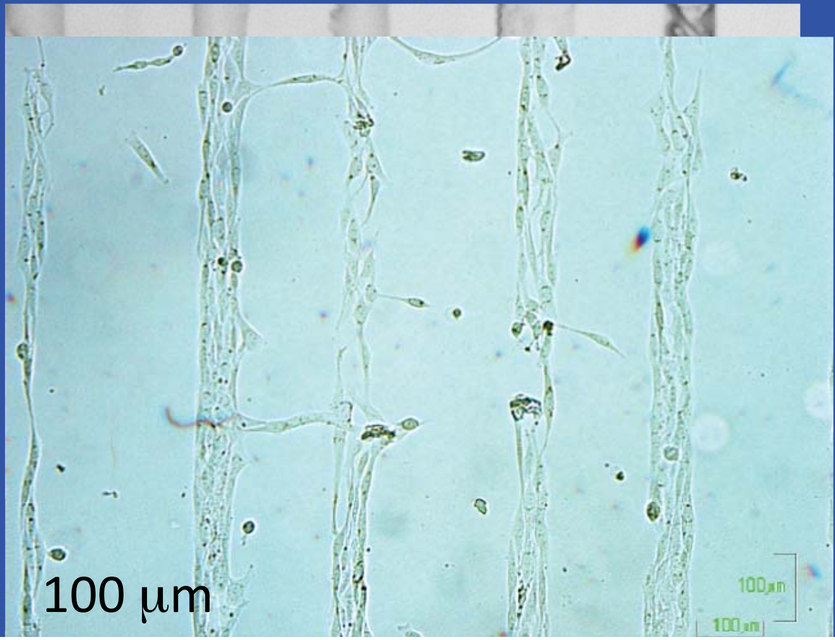


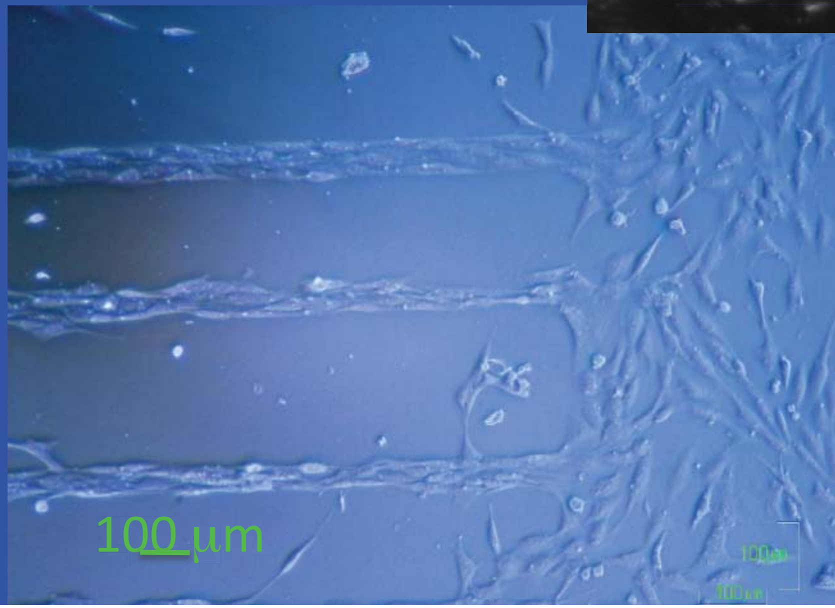
Detail of Laminin Microstructure by Atomic Force Microscopy (AFM)

Glial cells grown on patterned polystirene well

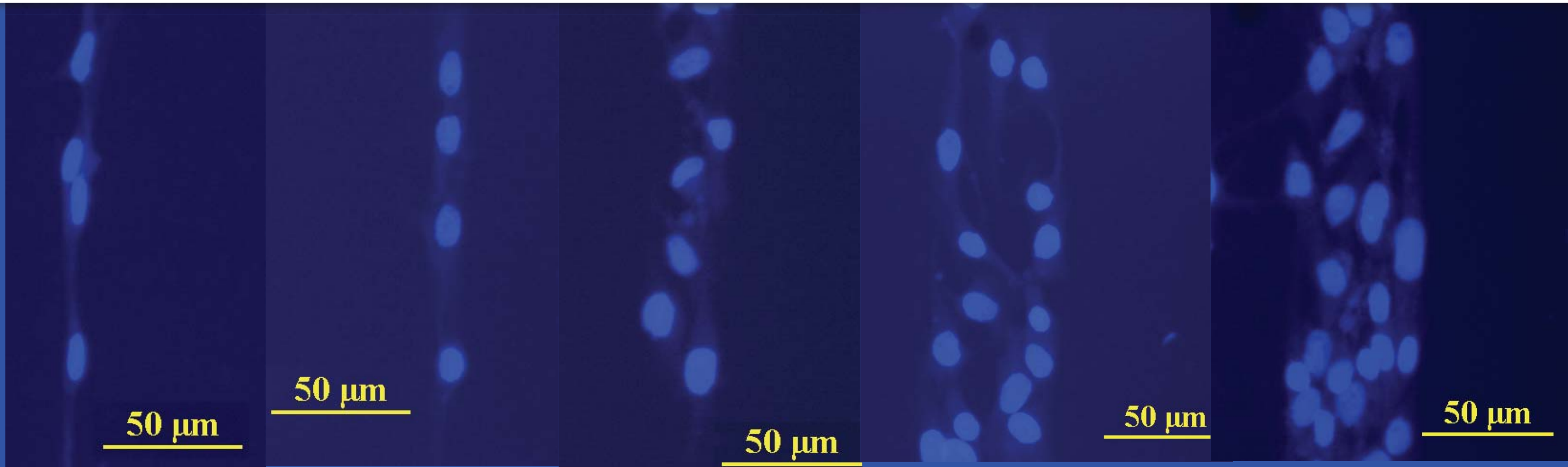


Cell growth after 6 days



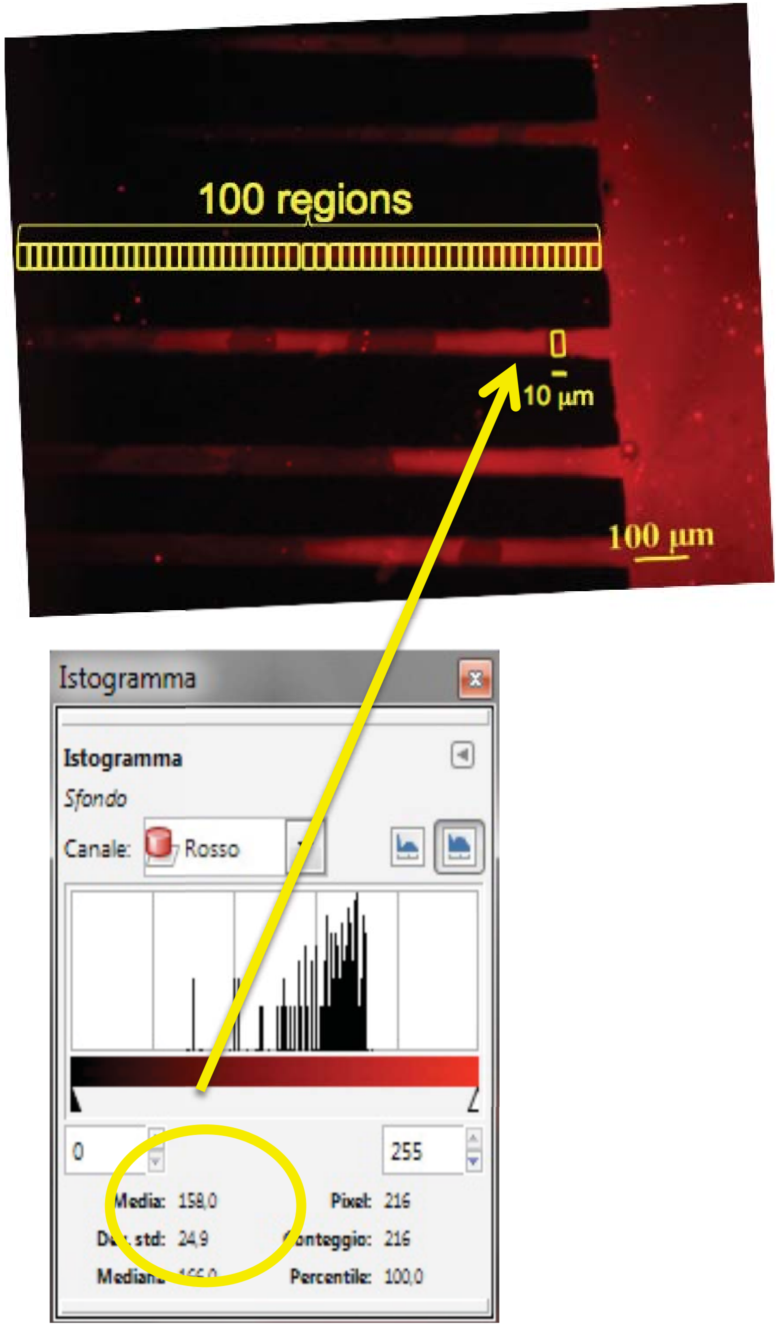


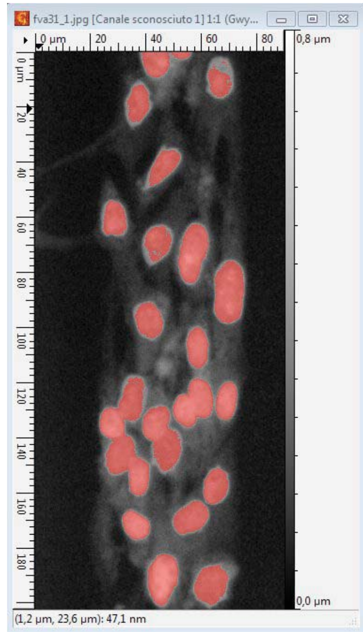
Cell number controlled by stripe width

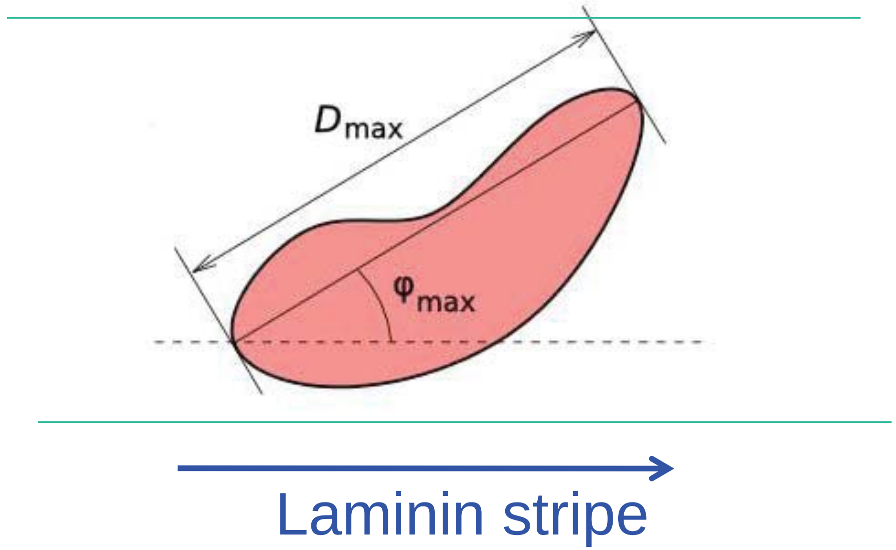


Immuno staining images in liquid: nuclei marker Hoechst 33342

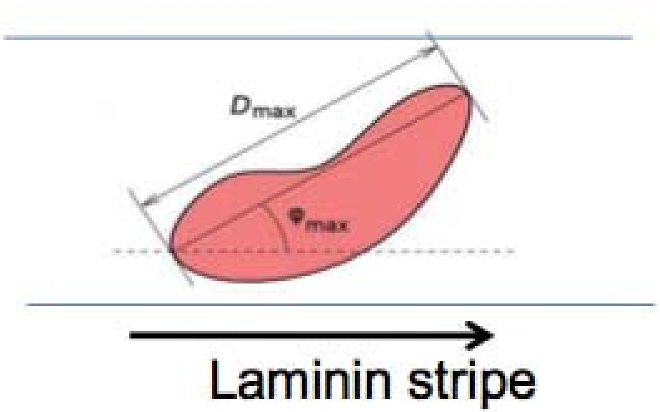
Signal gradients to guide cell positioning



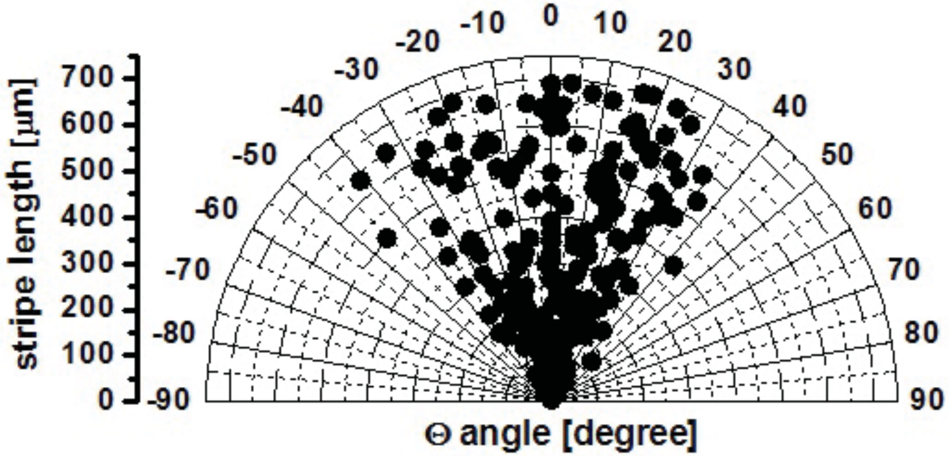




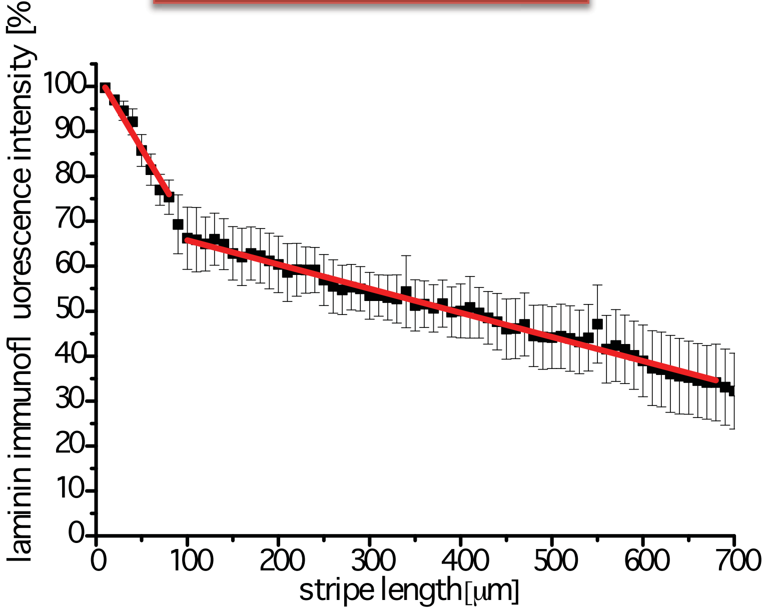
Signal gradients to guide cell positioning



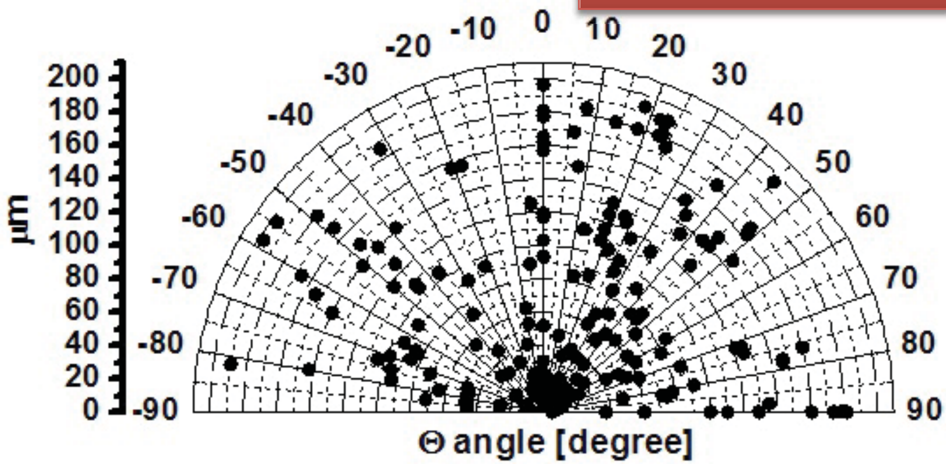
stripe width 25.8 μm



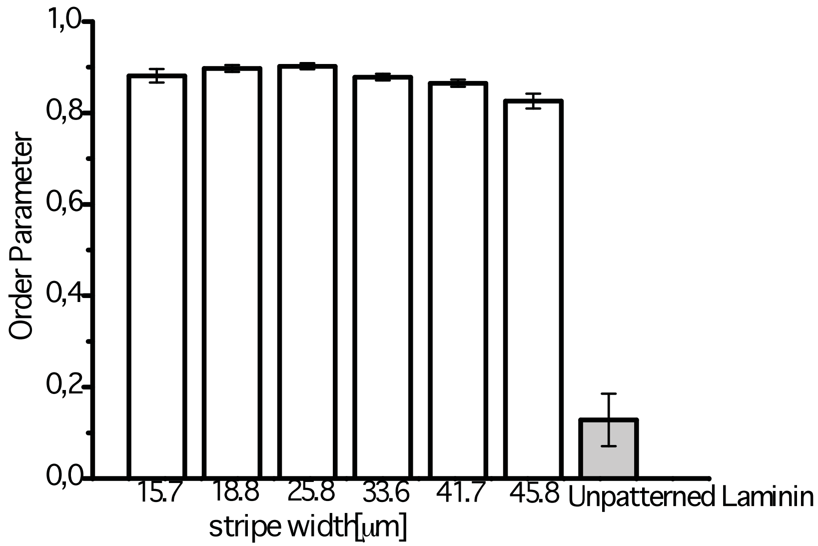
Stripe width 25.8 μm

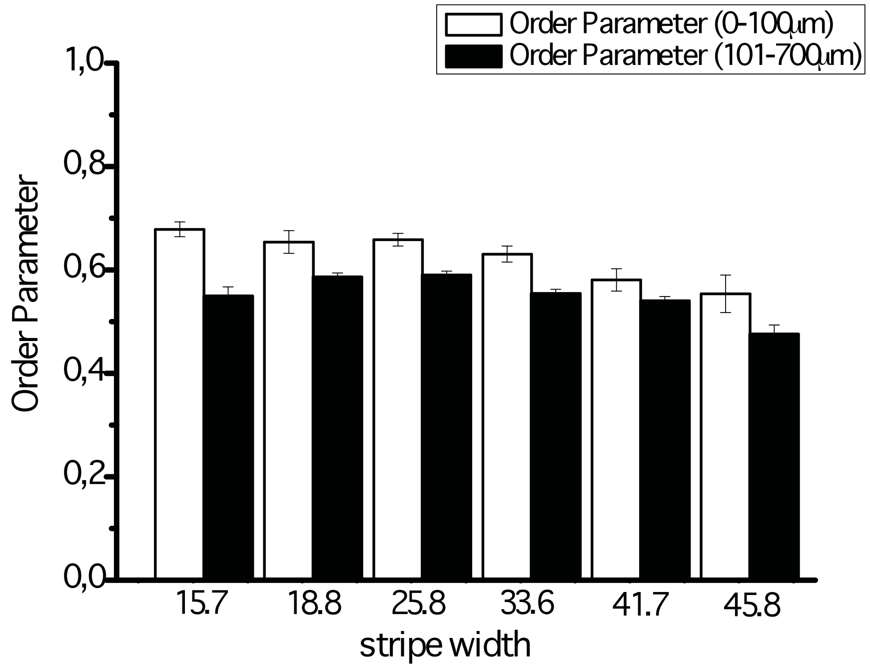


Unpatterned laminin

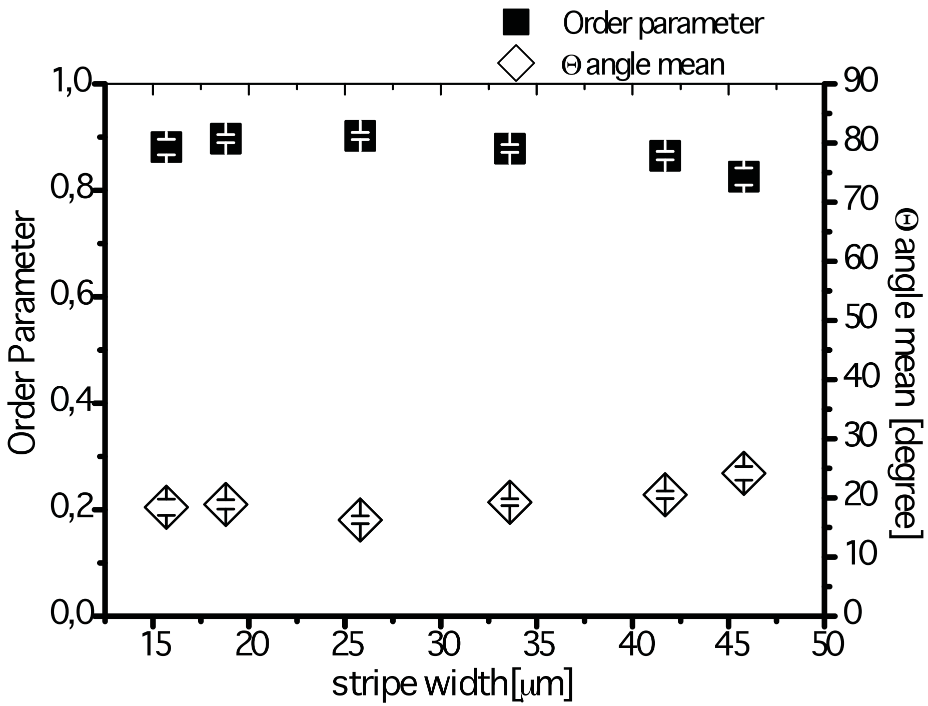


Order Parameter





$$S = \frac{3}{2} < \cos^2 \Theta > - \frac{1}{2} \qquad [0,1]$$



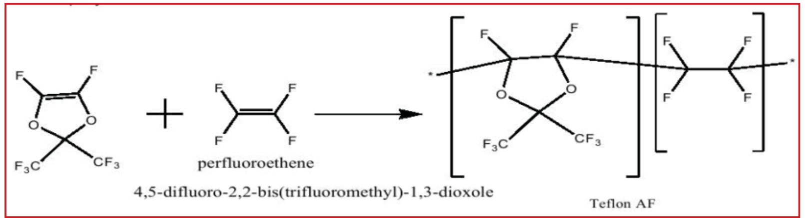
S=1 when the nucleus is perfectly aligned along the director.

S=0 when the nucleus is isotropic.

High local contrast of adhesion propensity

Patterning of Laminin on Teflon-AF
by combined LCW-MIMIC

TEFLON-AF *Poly[4,5-difluoro-2.2-bis (trifluoromethyl)-1,3-dioxole-cotetrafluorethylene]*



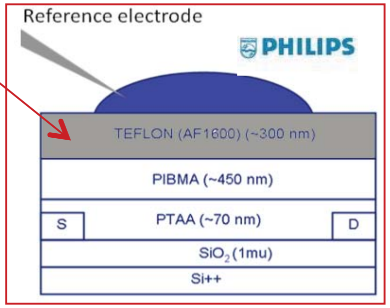
Roughness= 2.5 nm

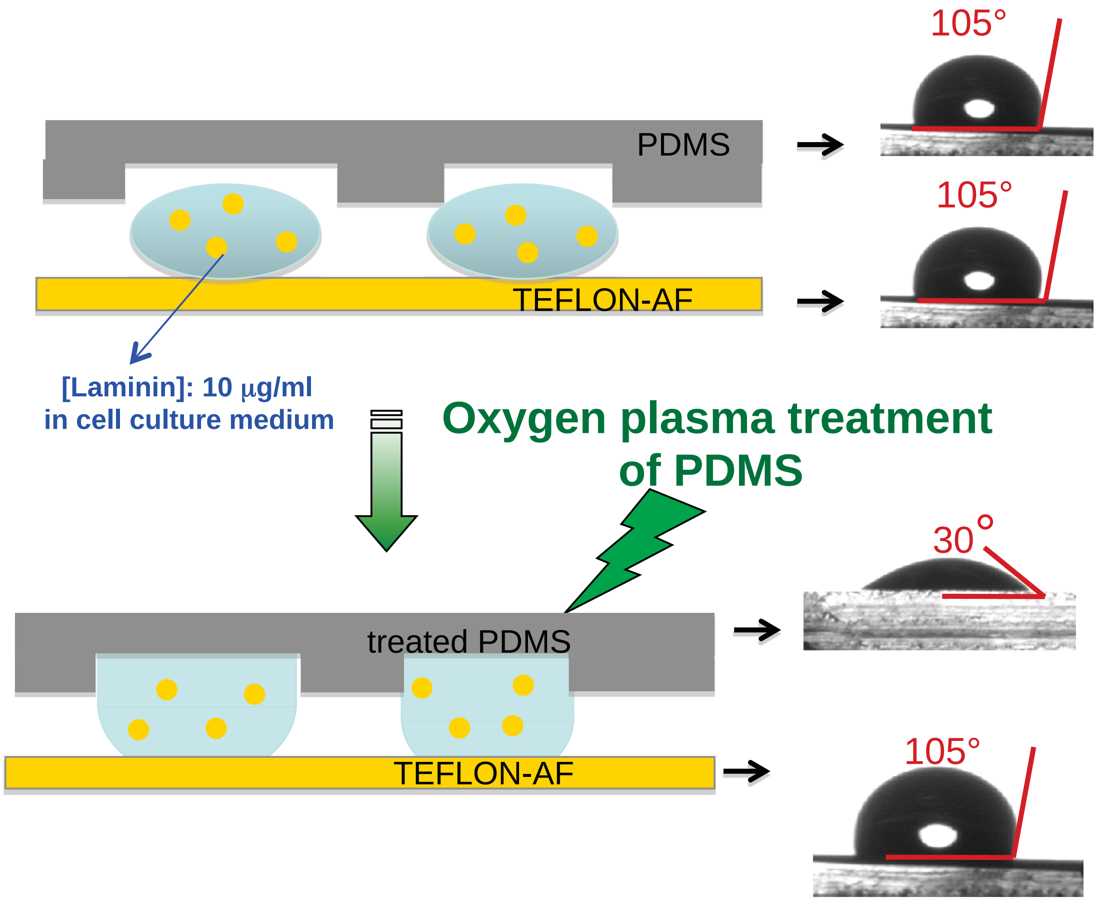
In Regenerative Medicine:

- biocompatible
- chemically inert
- antifouling
- employed as safety material for medical tools and instruments

In Biosensing:

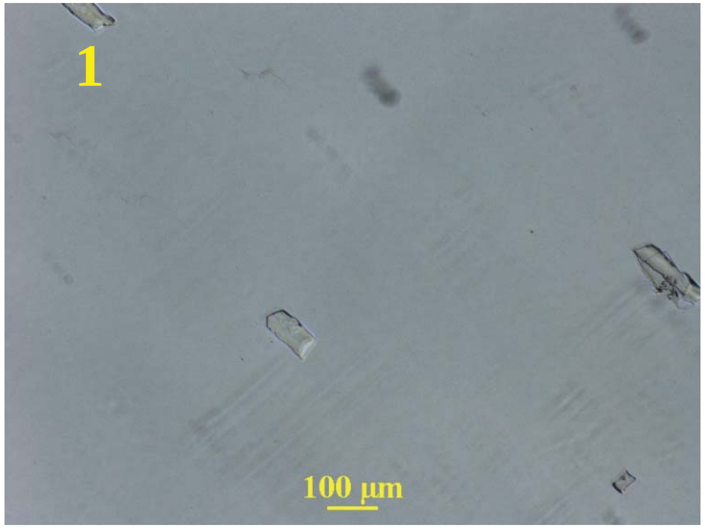
- good second dielectric

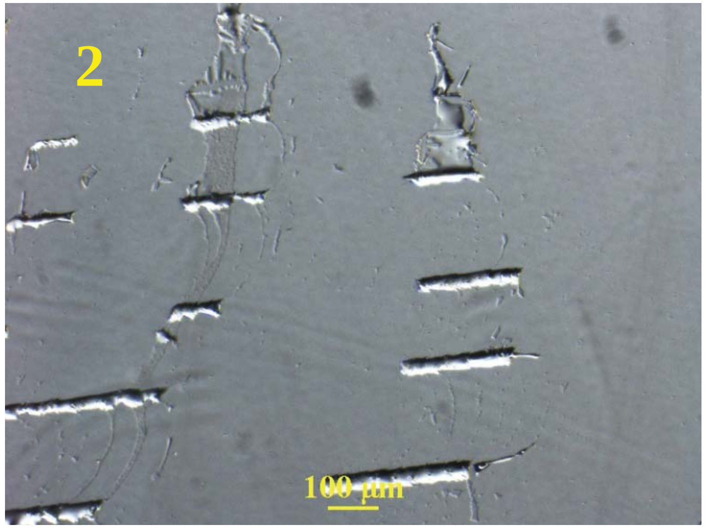




F. Valle, B. Chelli et al .Adv Biomaterials 2010

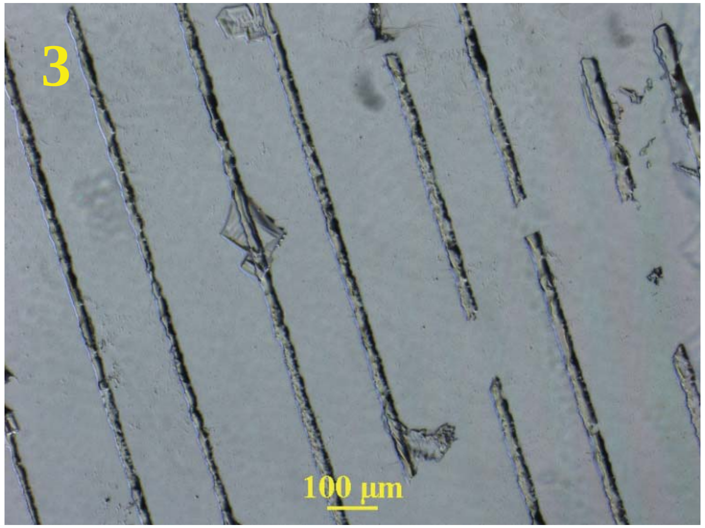
Control of the stripe length and density in addition to the lateral size

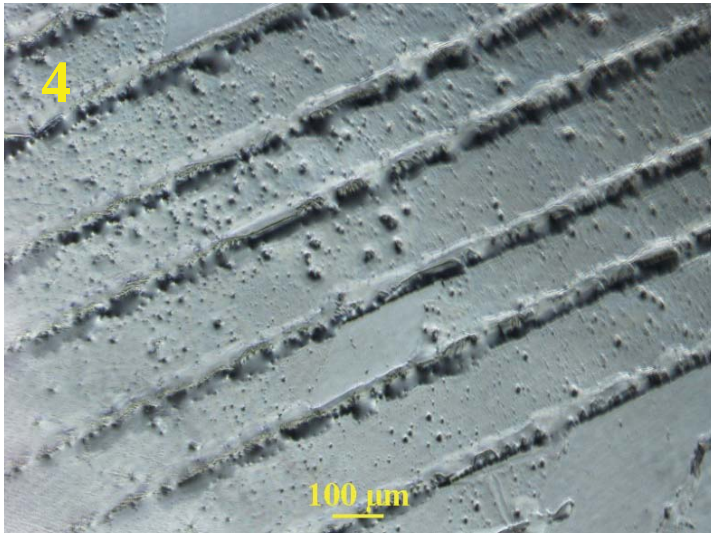




Controlling the hydrophobicity ratio Hr_i between the stamp and the surface one obtain longer and more dense pattern

$Hr_1 < Hr_2 < Hr_3 < Hr_4 = 1$

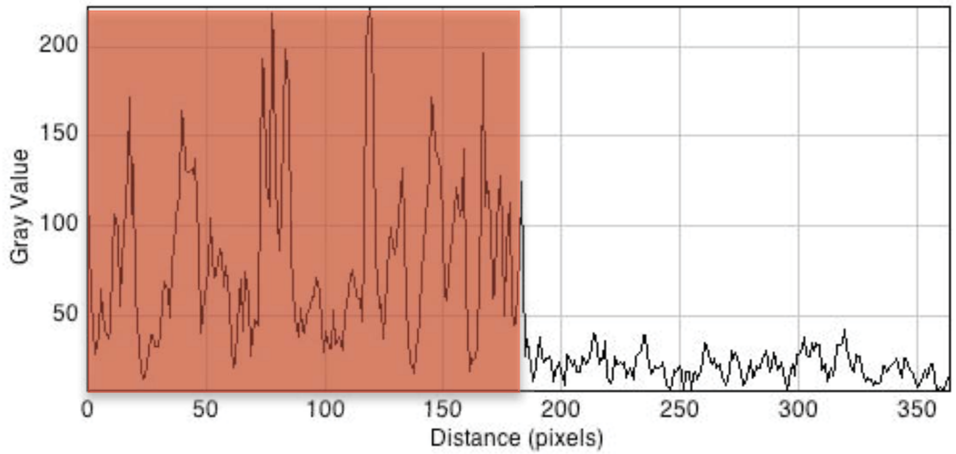


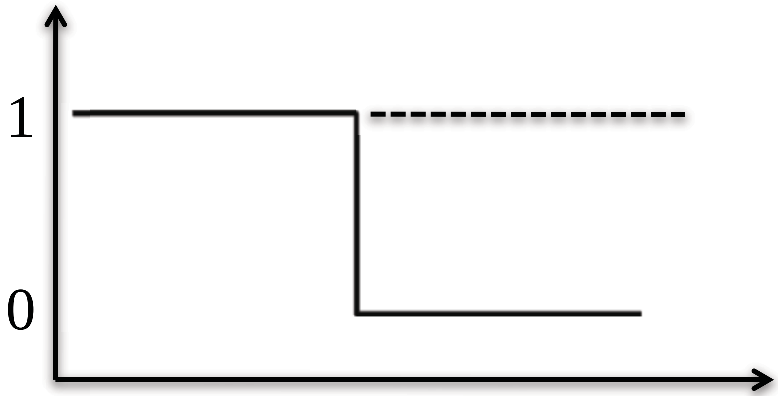


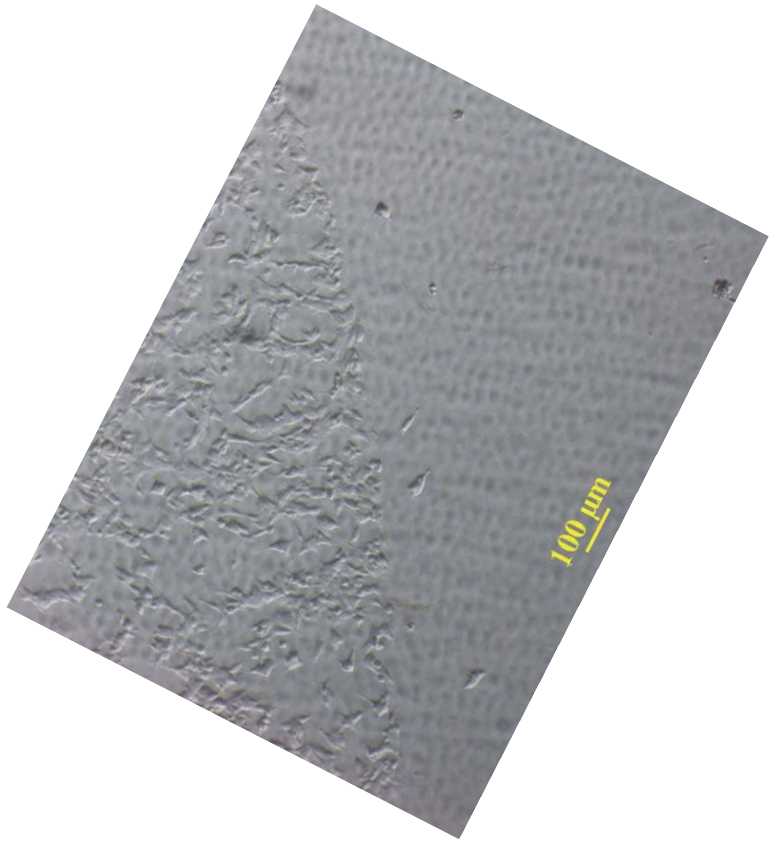
The presence of neighboring anti-fouling and adhesion promoting regions is crucial for increasing the stringency

Laminin TEFLON

neuroblastoma

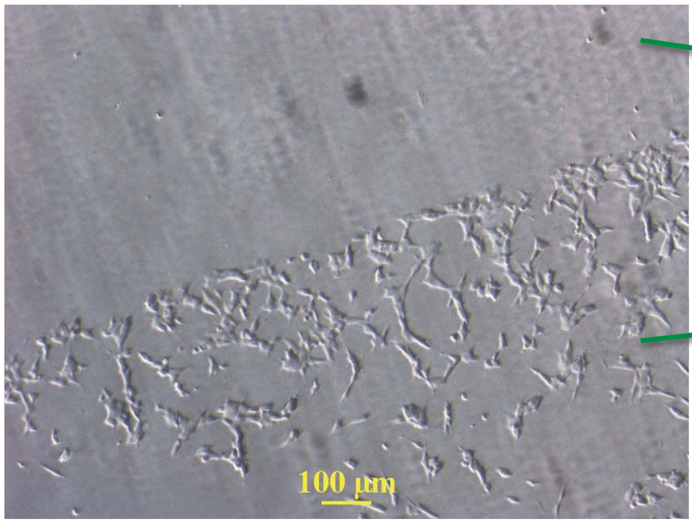






Patterning adhesive molecules as a strategy for reaching a controlled adhesion on teflon substrates

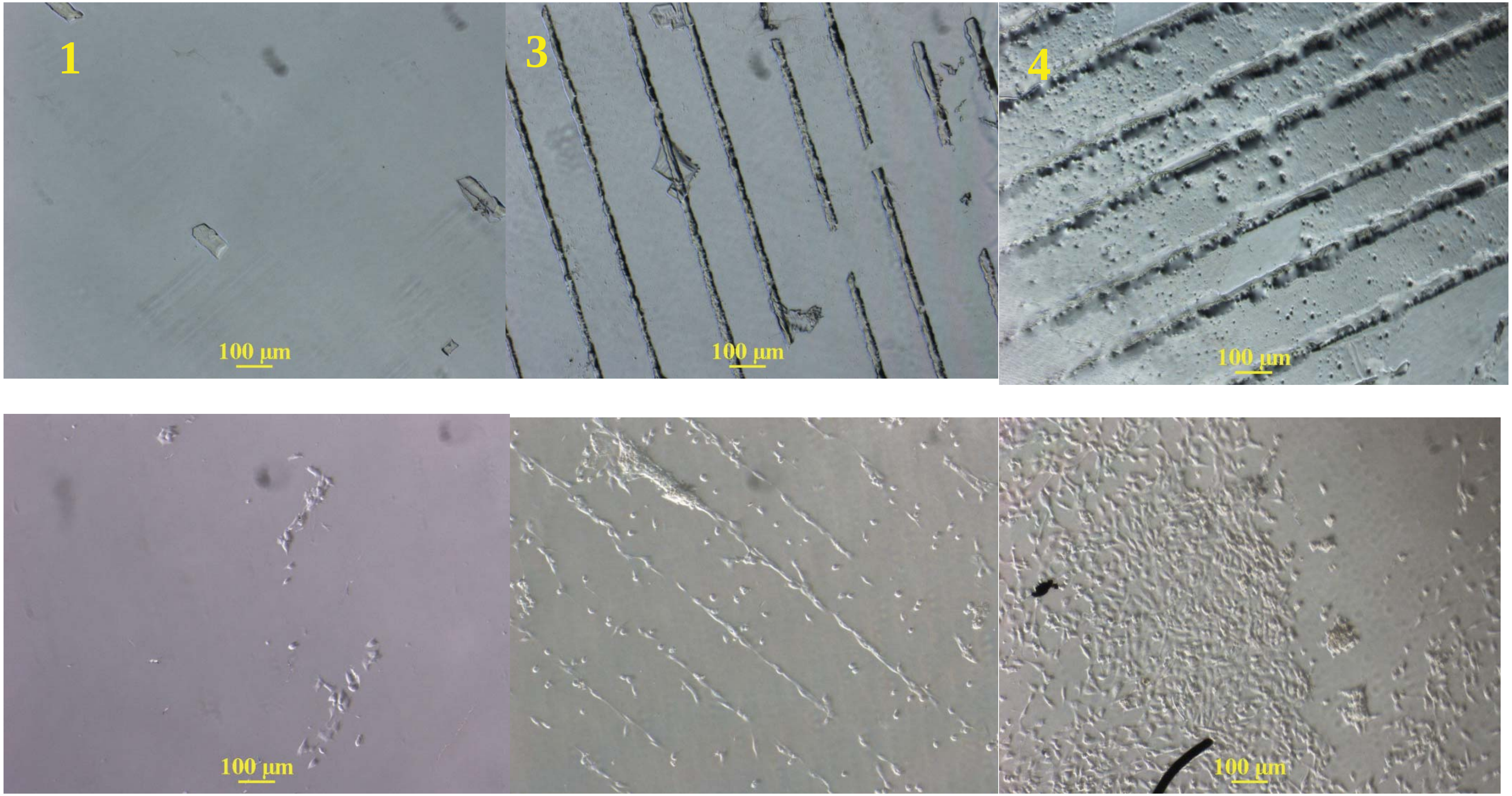
24h – 48 h after seeding



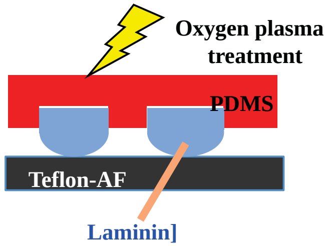
Teflon

Laminin layer

Tuning the cell adhesion by hydrophobicity controlled patterning



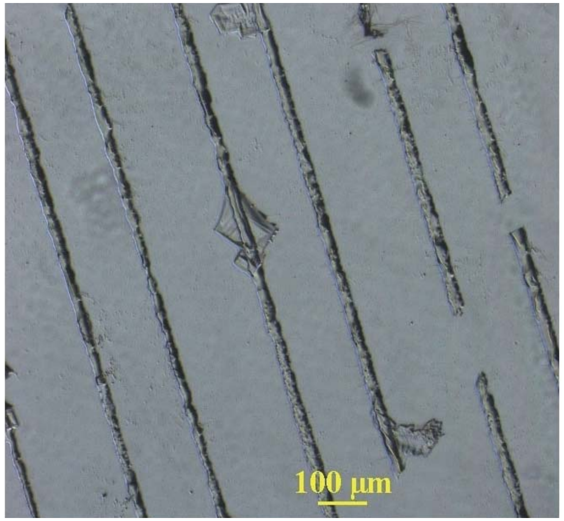
$Hr_1 < Hr_3 < Hr_4 = 1$



Neural Cells Grown
on Laminin pre-patterned TEFLON-AF

After 24h under standard cell incubation conditions

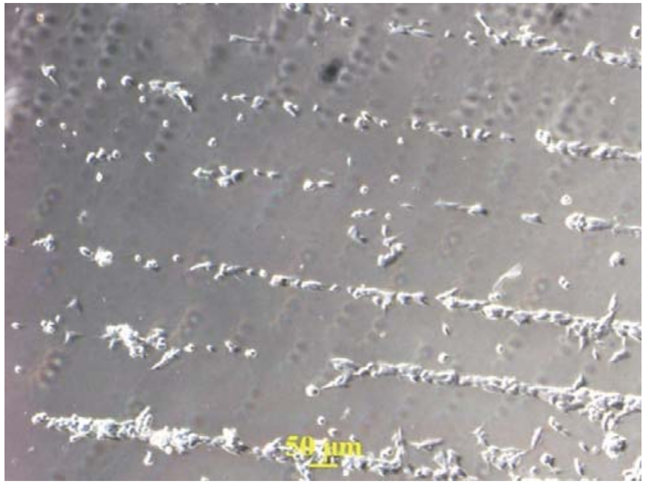
Laminin pattern

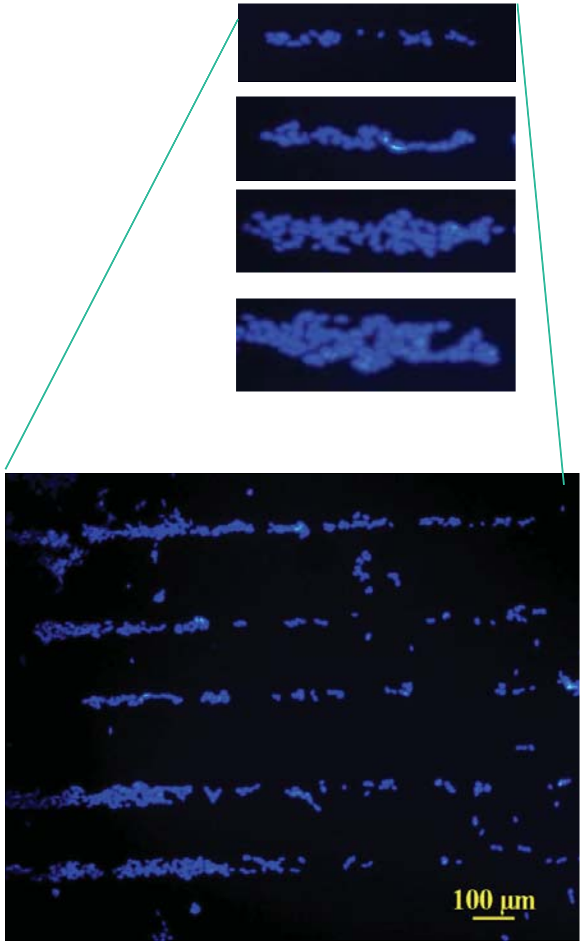


Optical micrograph

Fluorescence micrograph

1321N1 Astrocytoma cells

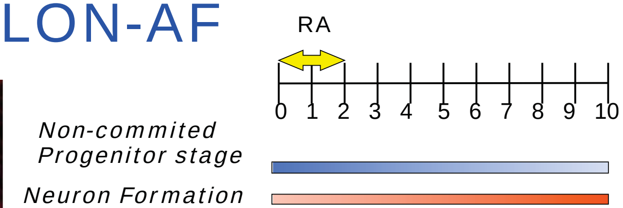




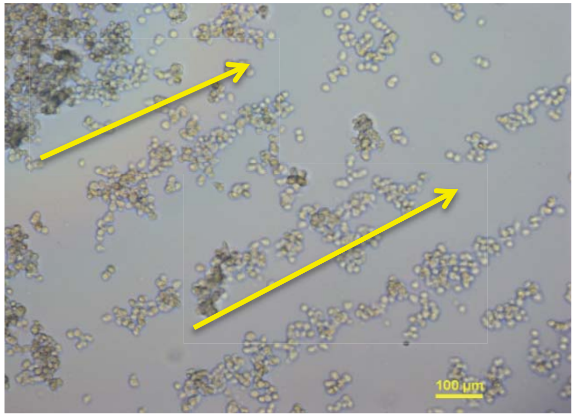
SHSY5Y Neuroblastoma cells

F. Valle, B. Chelli et al .Adv Biomaterials 2010

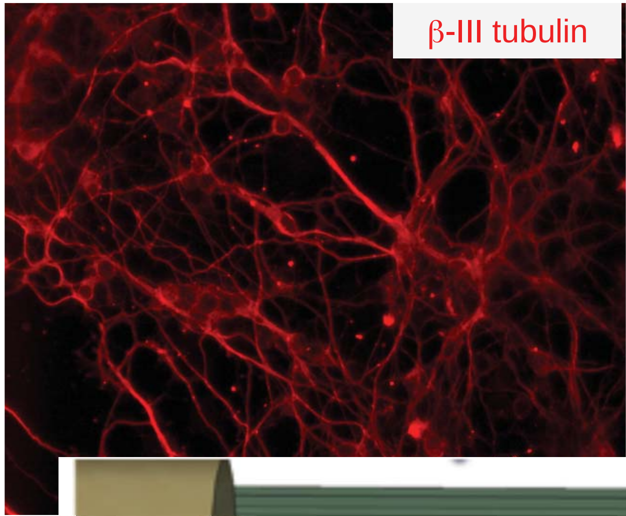
Differentiated Neurons
on Laminin pre-patterned TEFLON-AF

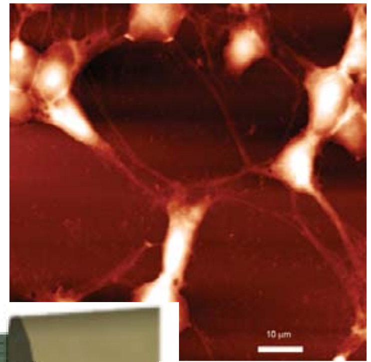


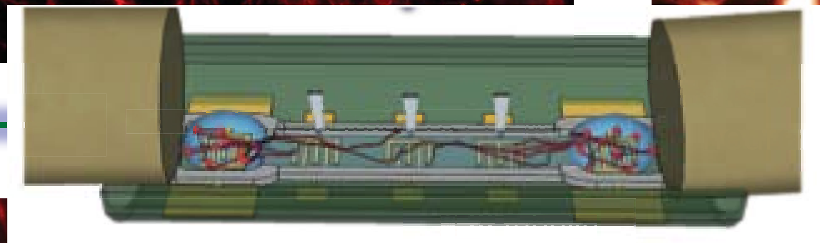
From Stem cells…



Neural NE4C stem cells after 3h incubation.

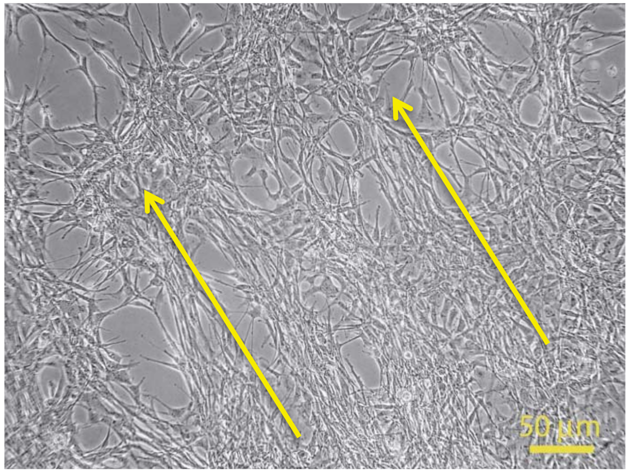




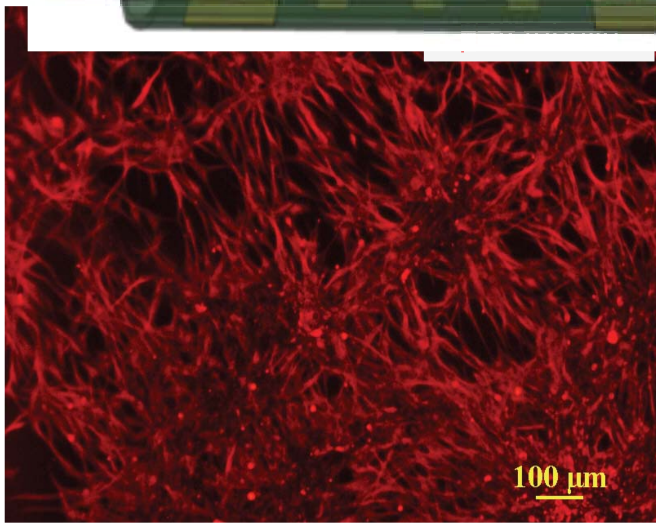


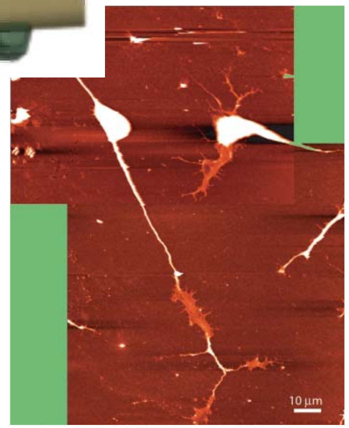
ifferentiated
s by AFM

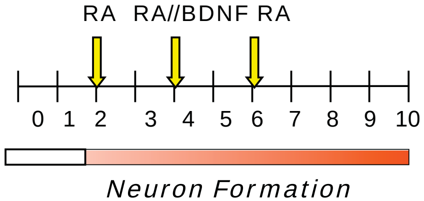
From Neuroblastoma Cells…



SHSY5Y neuroblastoma cells after 5 days Retinoic Acid (RA).treatment





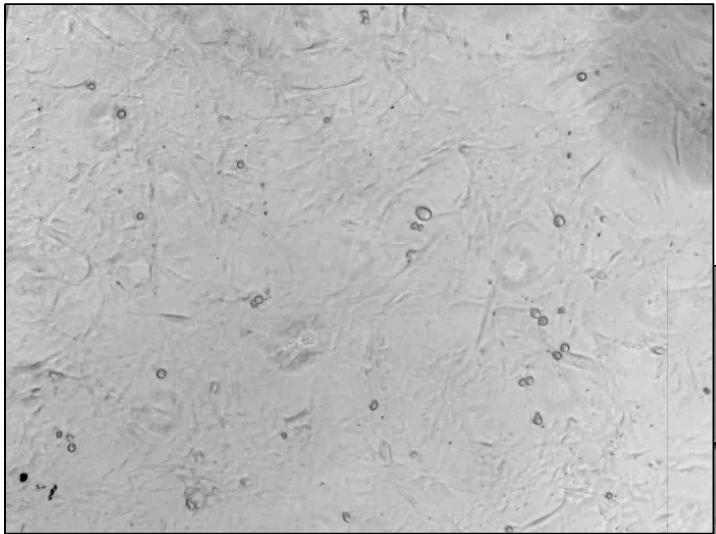
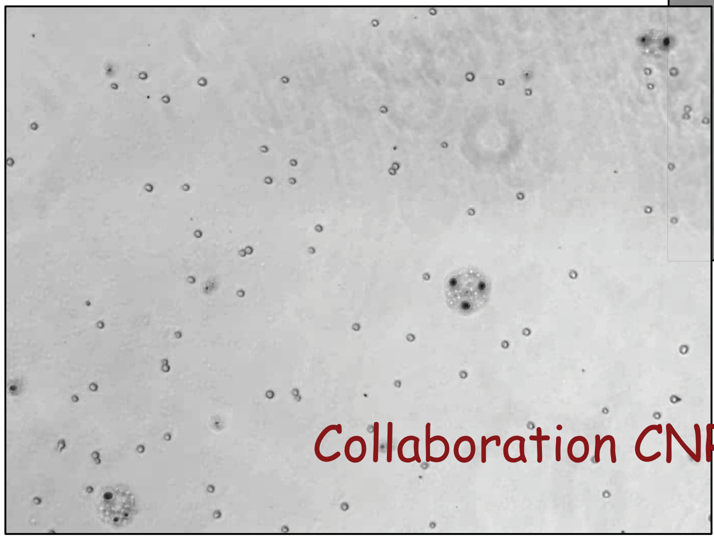
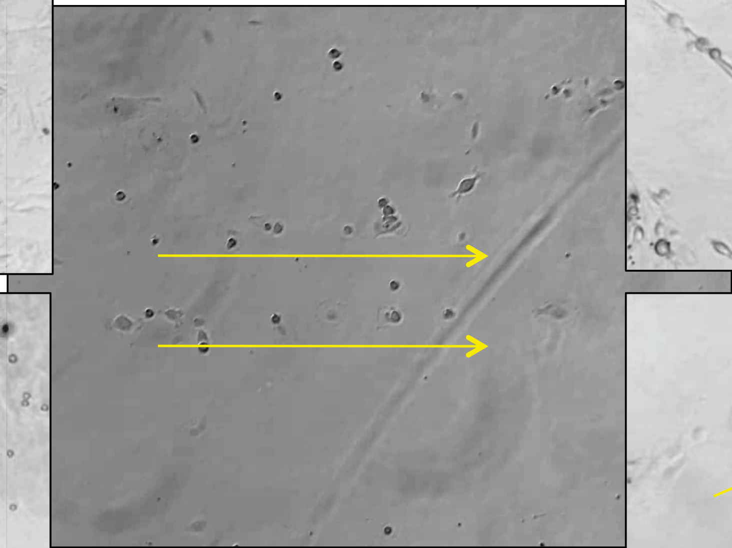
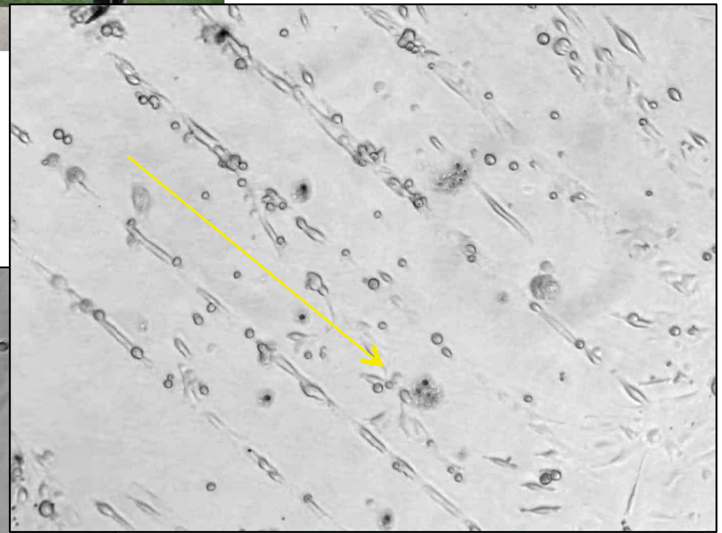
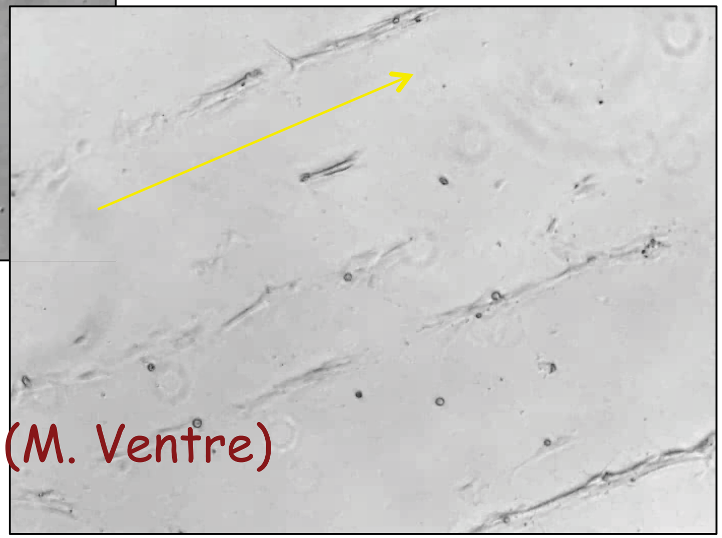


B. Chelli et al .2010, in preparation

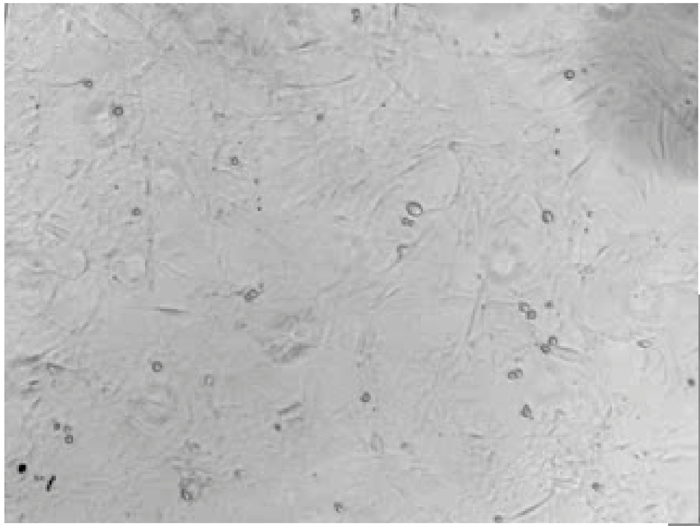
Positioning → migration

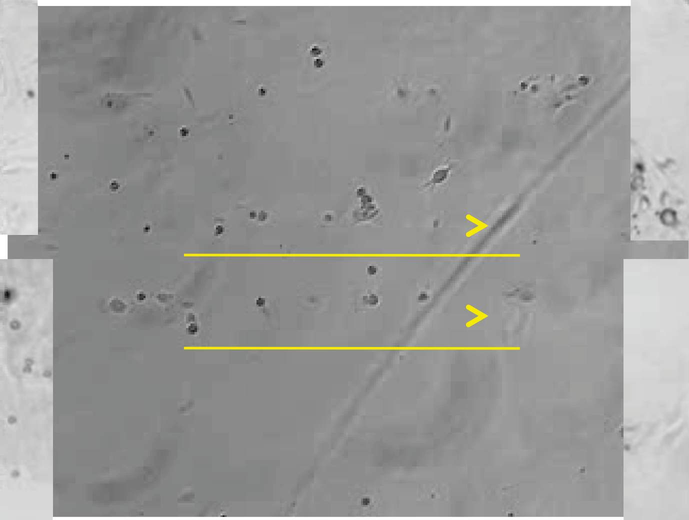
Following the trajectories of the cells migrating in the presence of patterned signals

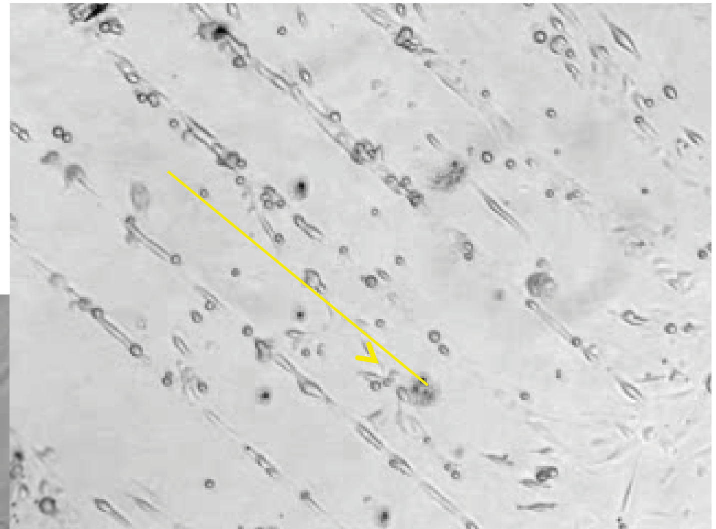


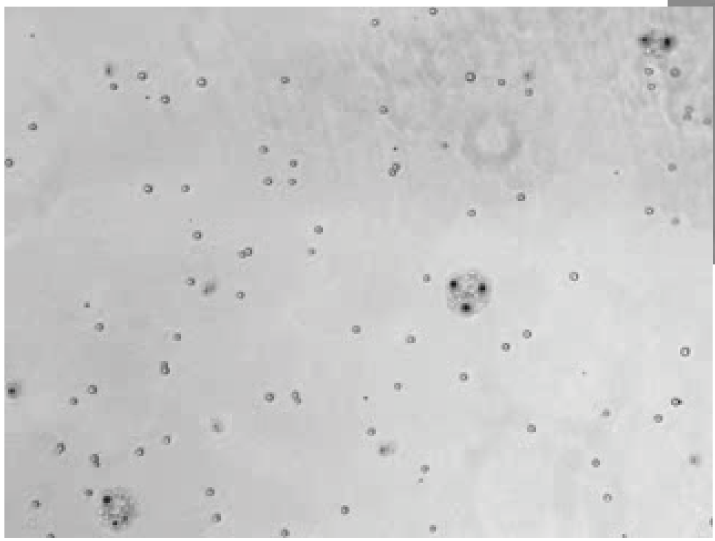







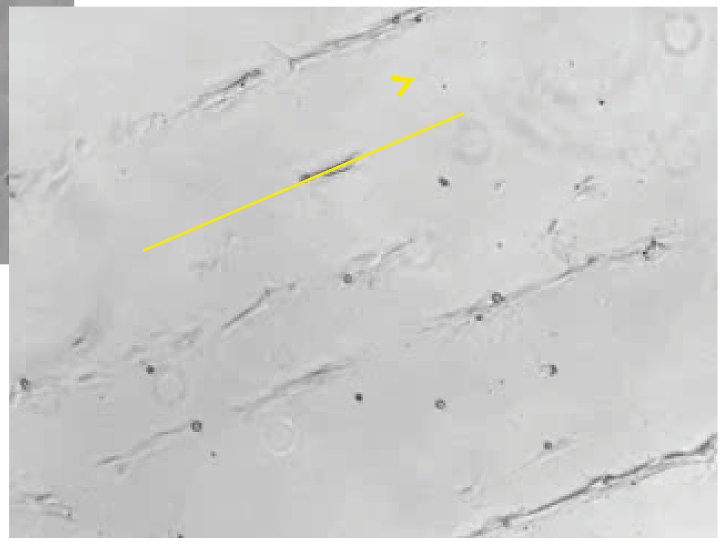
Collaboration CNR (F. Valle)-Univ. Naples (M. Ventre)





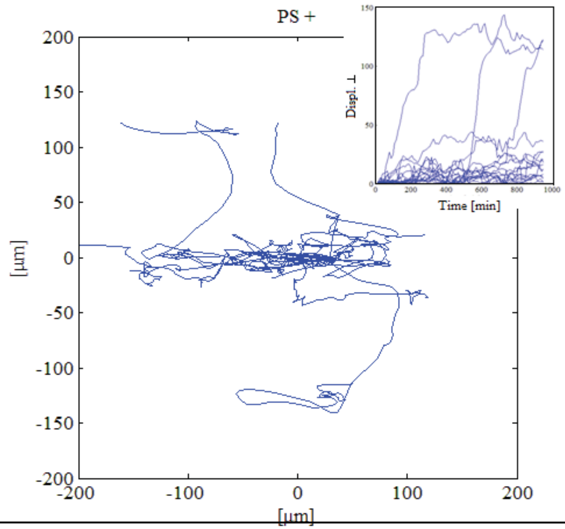
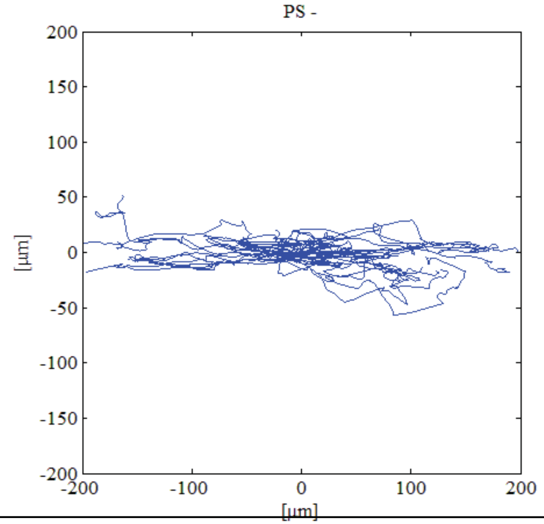
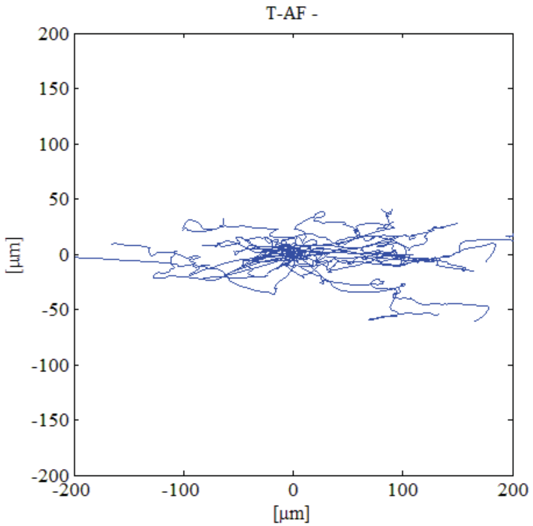
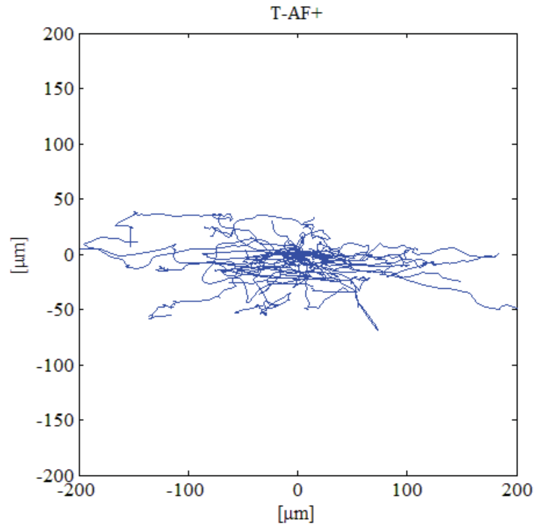






Collaboration CNR (F. Valle)-Univ. Naples (M. Ventre)

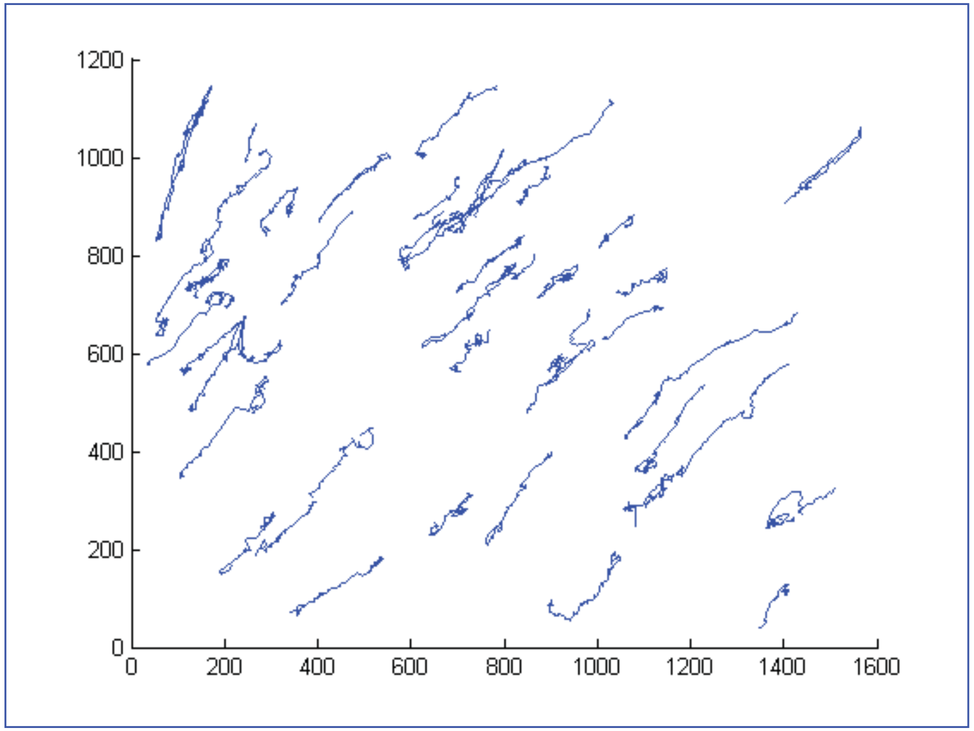
avi

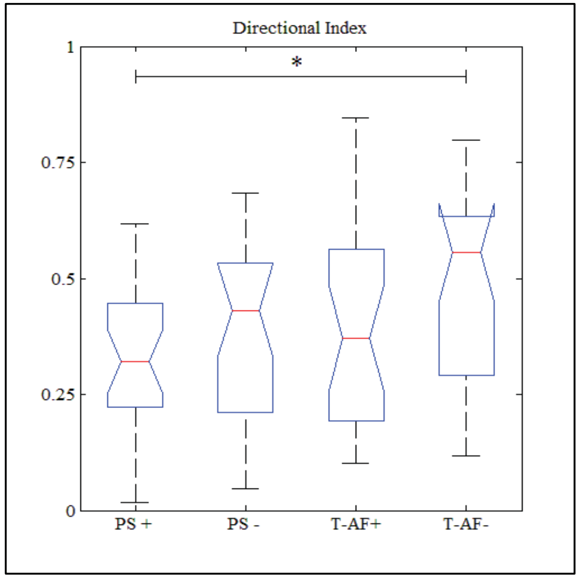
	DMEM+ Serum	DMEM
Petri		
Teflon		

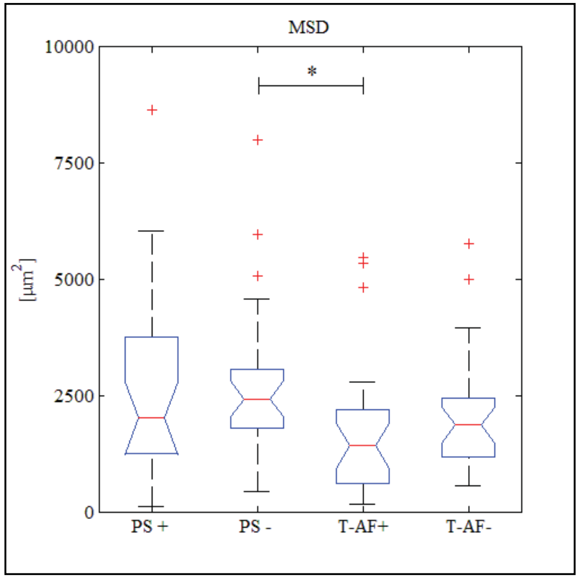
Serum proteins may adsorb on the PS surface thus leading to jump through neighboring stripes.

The antifouling behavior of Teflon prevents this phenomenon.

Accurate cell guidance achieved by local control of the adhesion contrast





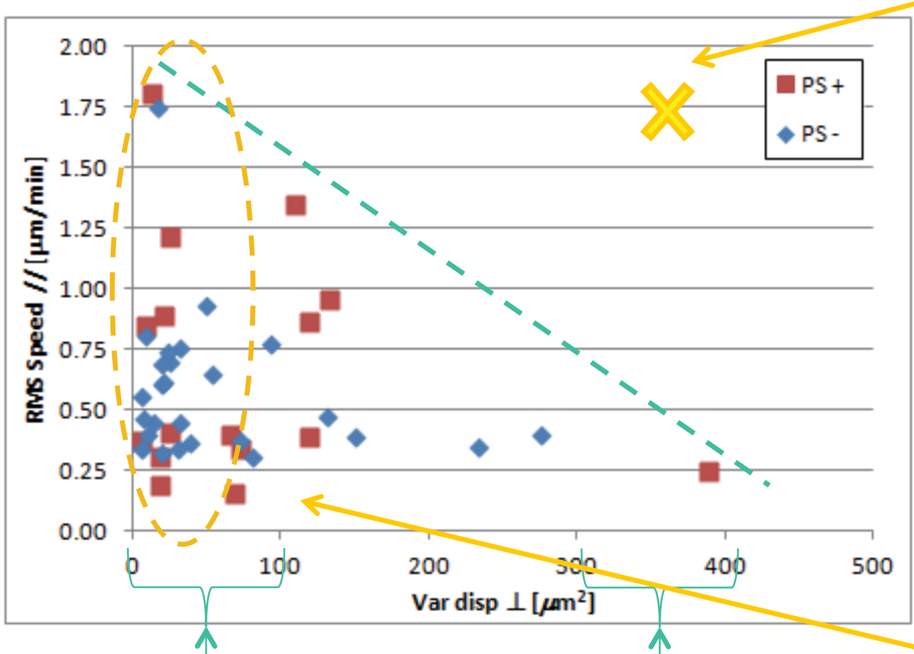


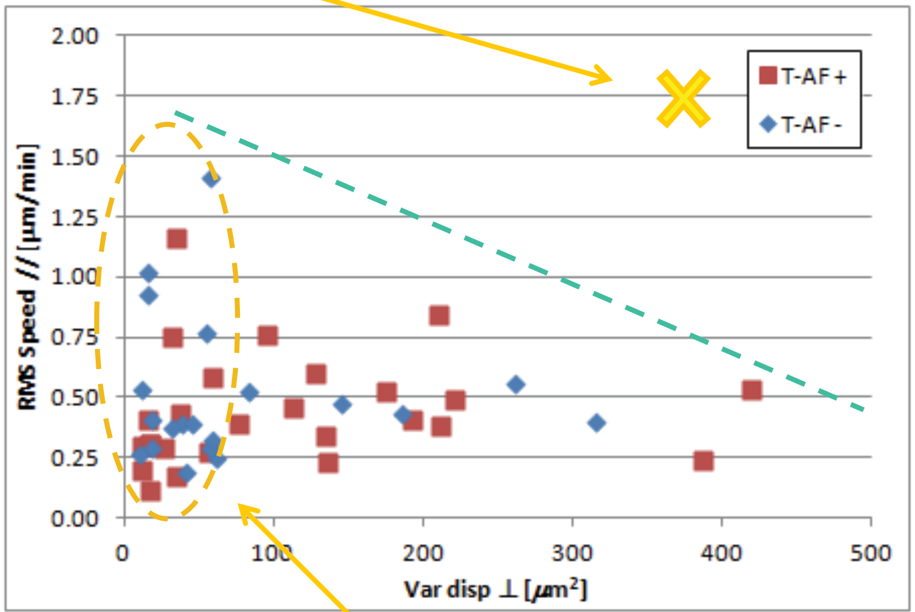
It is not only important what we design but also how the local environment is altering the scaffold

Confinement affects the cell speed

X-axis: variance of the displacements distribution othogonal to the pattern
Y-axis: RMS speed along the lanes

Fast moving cells are not observed on wider lanes





small oscillations:
small lanes

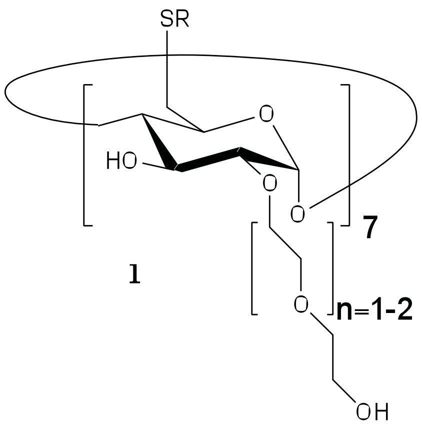
large oscillations:
large lanes

High migration speed (along the pattern) are invariably associated with small orthogonal oscillation which are observed on small lanes. Cells on wider lanes may display small oscillations, which has been especially observed when they reach the edge of the lane. In this case, however we still record small speeds. It might be that cells moving on narrow lanes are still slow (maybe they are dying or they got stuck on other cells) but we were not able to exactly measure lane width.

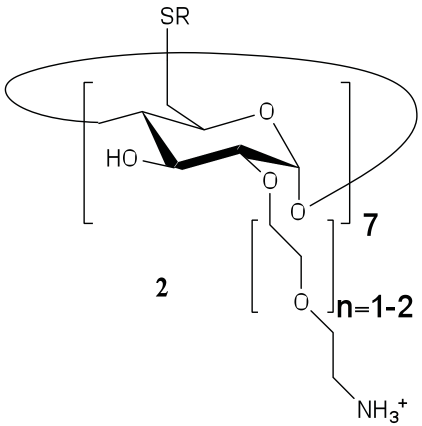
Novel strategies: designing and employing new carriers

Novel molecules for carrying the most suitable cues

Design of novel targeted ACyD for targeting cancer cells



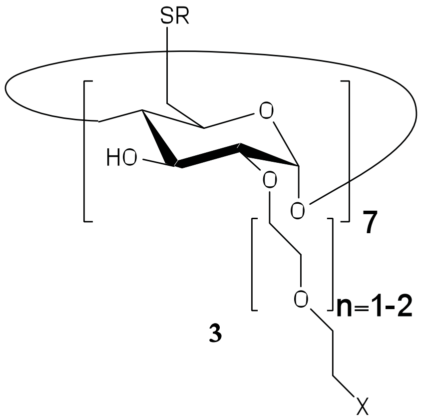
1



2

1 ACyD with antifouling cues
2 ACyD with fouling cues
3 ACyD with targeted cues

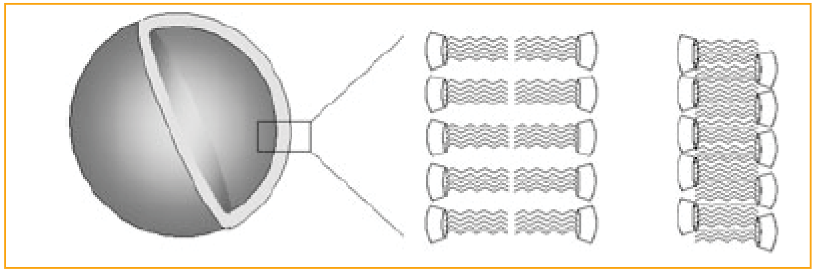
Charge modulation by mixing nanoassemblies of 1 and/or 2 and/or 3



3

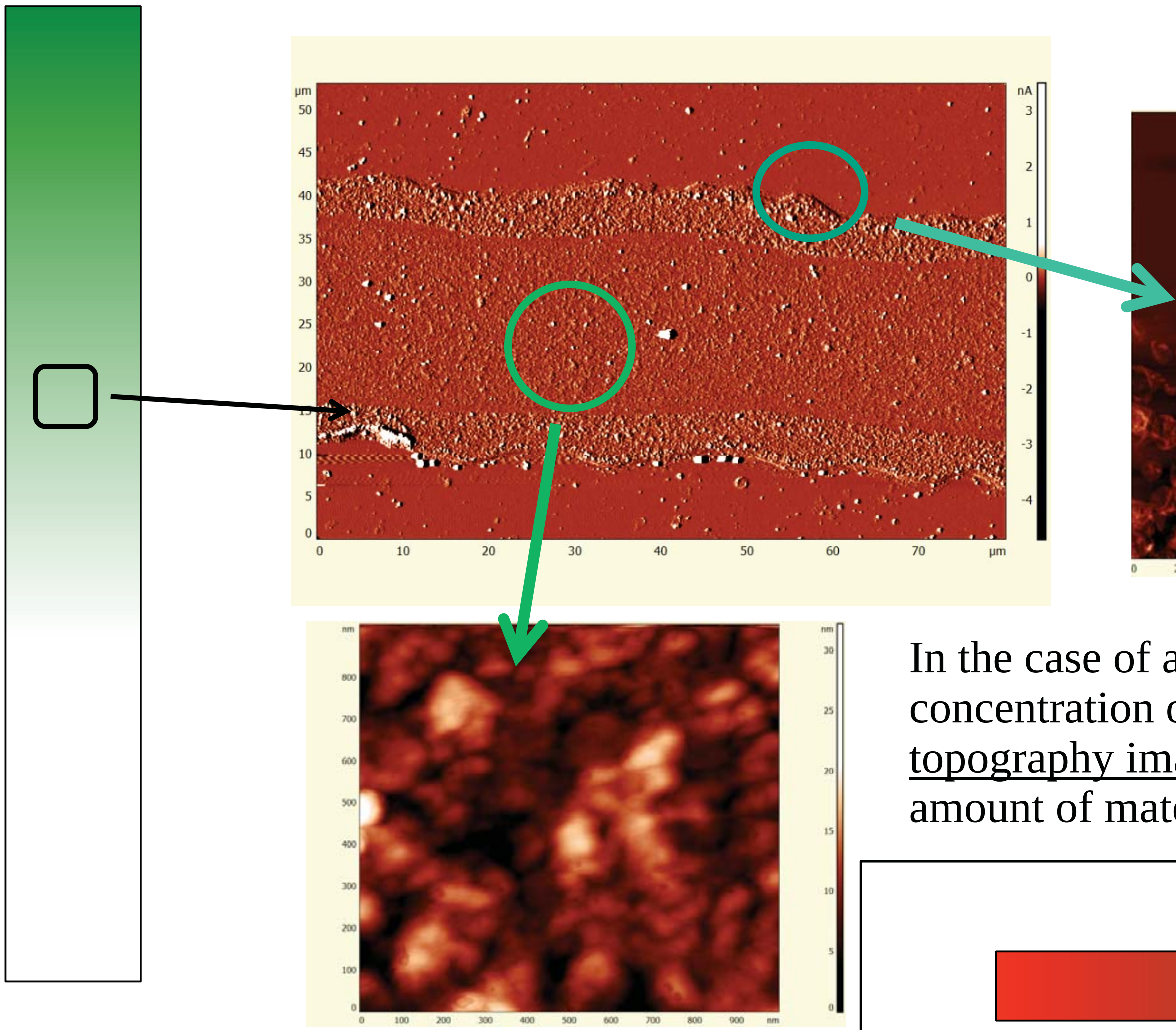
R= $-C_6H_{13}$
$-C_{12}H_{25}$
$-C_{16}H_{33}$

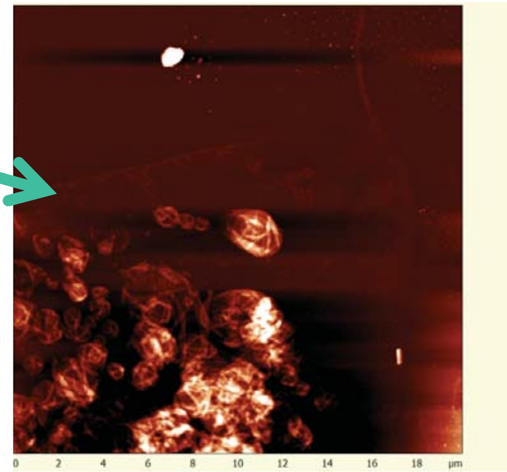
X= -Galactose
-Mannose
-folate
-Anisamide derivatives



Galactose: i.e. HepG2-cell lines
Mannose: i.e. sinusoidal endothelial cells, Kupferr cells
Folate: i.e. KB cells , MCF7 cells
Anisamide: Prostate cancer cells

Characterization of the deposited gradient by **<u>AFM imaging</u>**



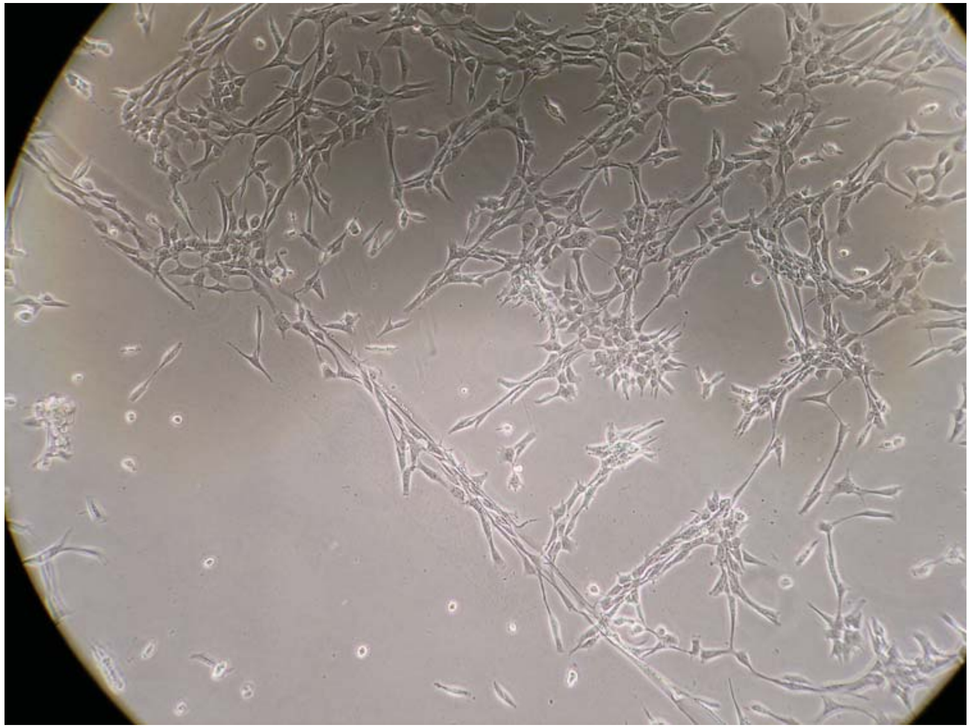


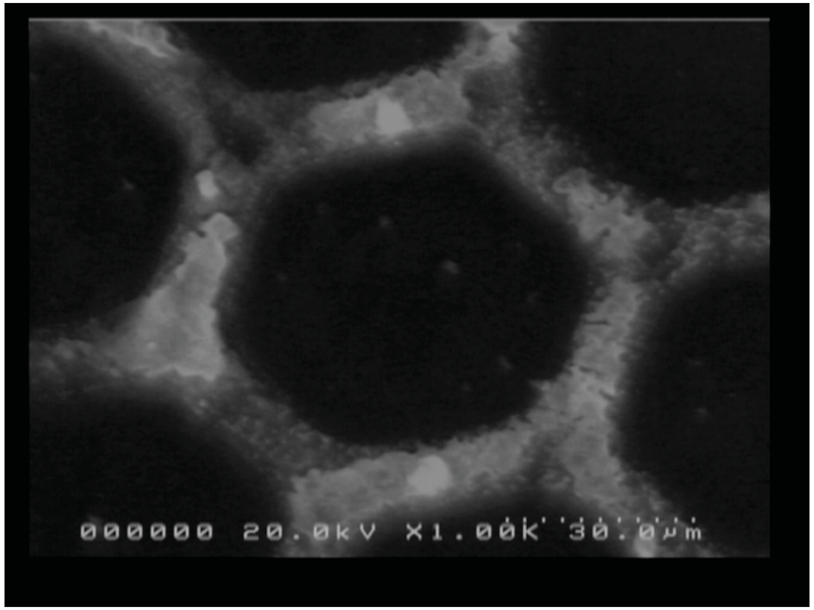
In the case of a simple gradient, i.e. a decreasing concentration of a single species, AFM <u>topography imaging</u> will monitor the local amount of material

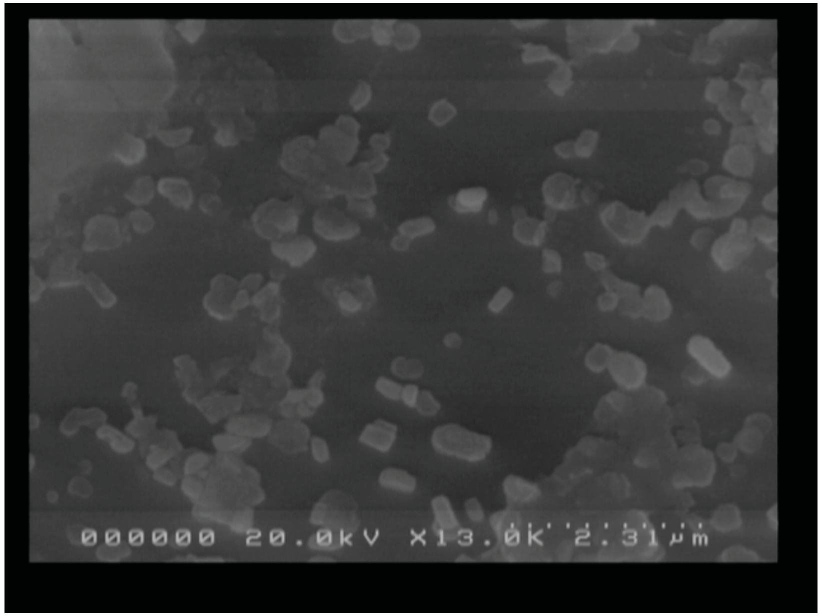
In the case of a complex gradient, i.e. a crossing decreasing concentration of two species, <u>functional AFM imaging</u> (phase, force spectroscopy, Trec) will monitor the local composition

Preliminary results illustrating:

1) how SEM can also be a suitable technique for characterizing the pattern
2) how cells (glia) arranges on our very preliminary patterns







Collab. Dr. Mazzaglia ISMN Messina

Topographic signals

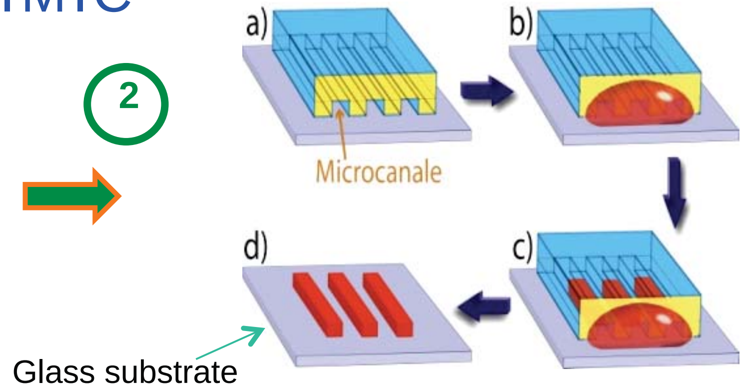
Important when dealing with hard tissue or with sensing devices

Patterning of macro-mesoporous TiO_2 onto glass by MIMIC

1

TALH/H_2O bis(ammonium lactate)titanium dihydroxide + PS/H_2O Polystyrene beads (Ø ~ 250 nm)

2

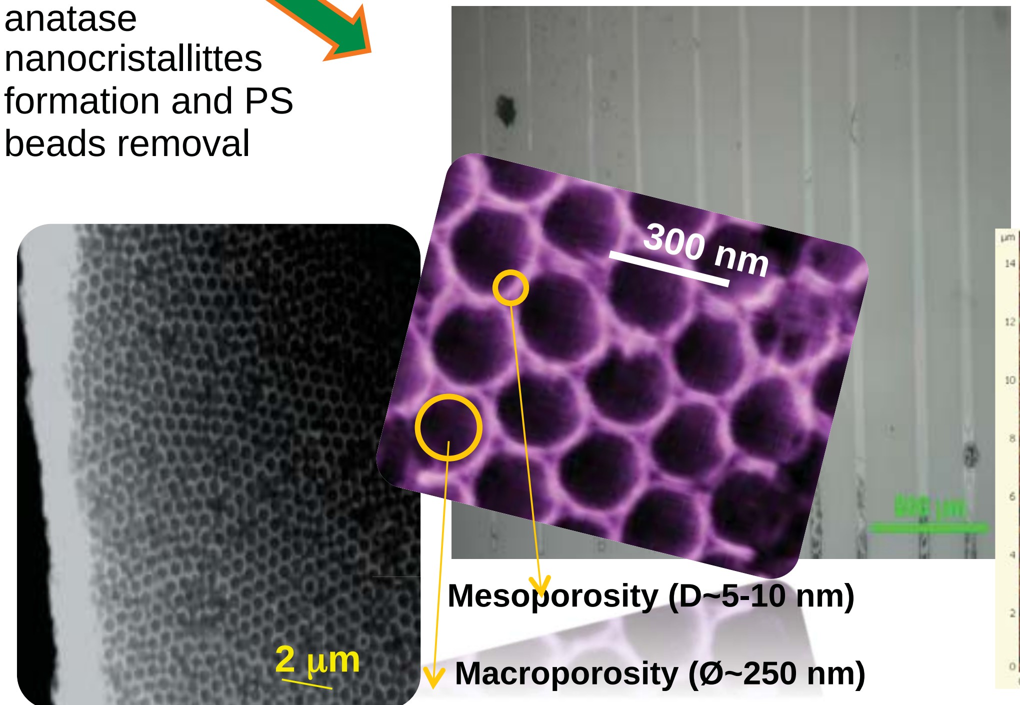


Dionigi, Biscarini et al. Chem. Mat. 2008

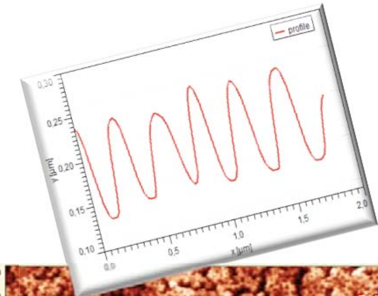
3

450°C in air

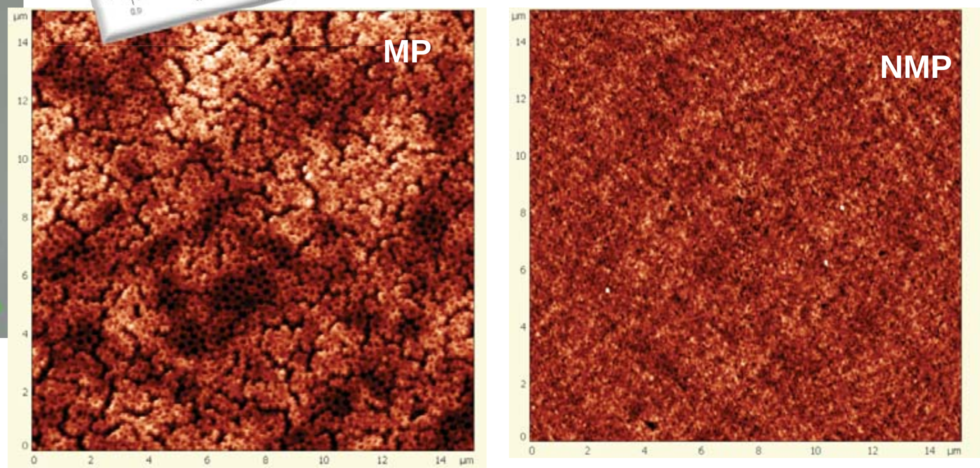
TiO_2 pattern anatase nanocristallittes formation and PS beads removal



SEM micrographs of TiO2 pattern

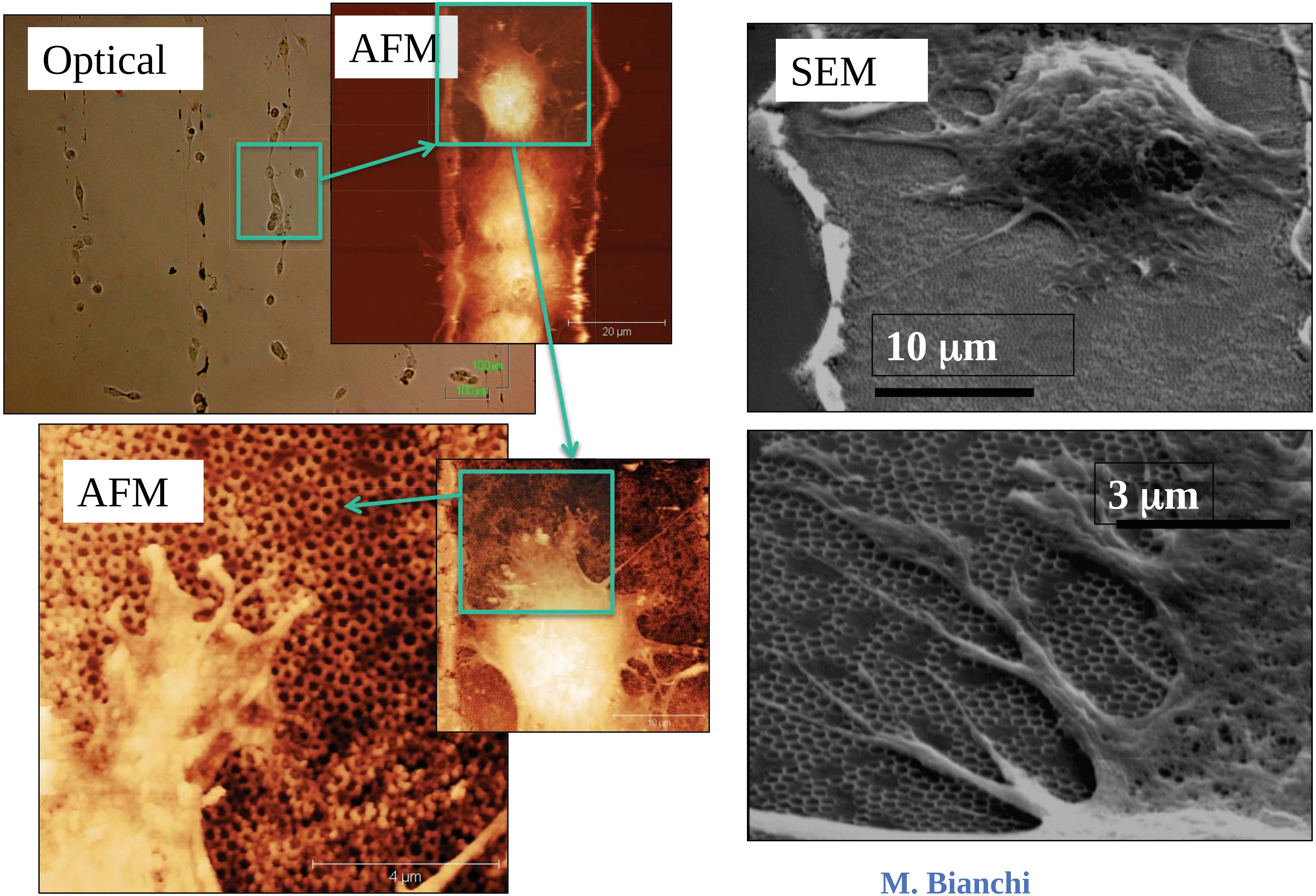


	MP	NMP
Roughness :	73 nm	0.5 nm
Contact Angle:	1±1 °	28±2 °



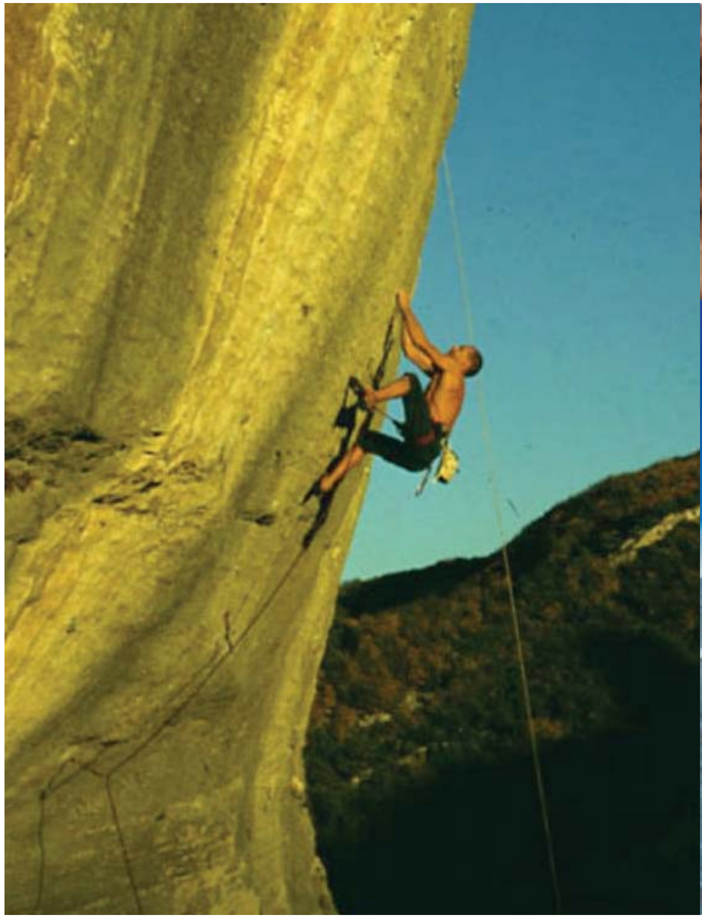
AFM images of macropourous (MP) and no macropouros (NMP) TiO_2 pattern

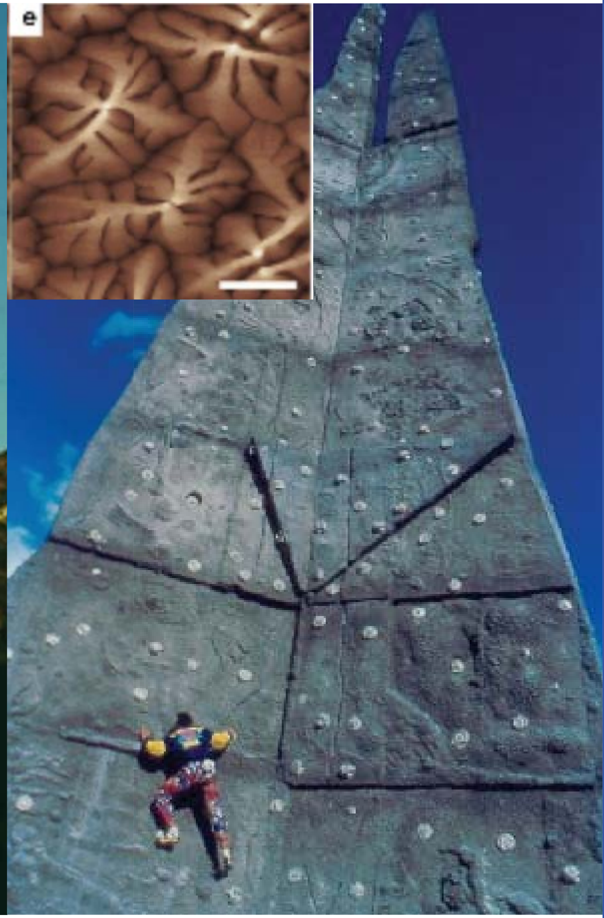
Cell guided adhesion: TiO_2 stripes with controlled porosity



M. Bianchi

Topography and morphology

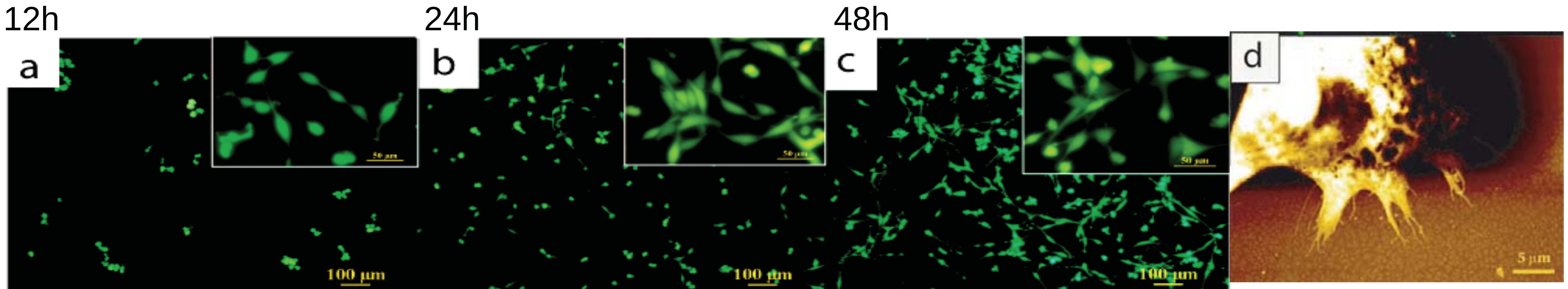


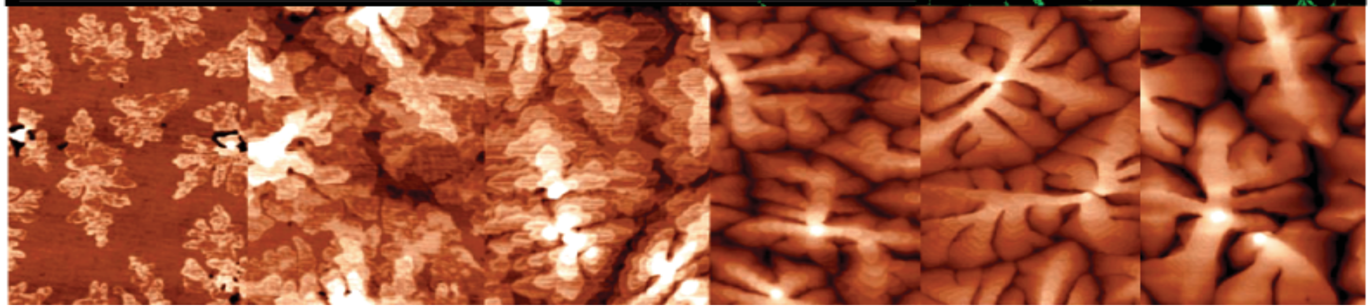


Coupling cells with organic semiconductor devices

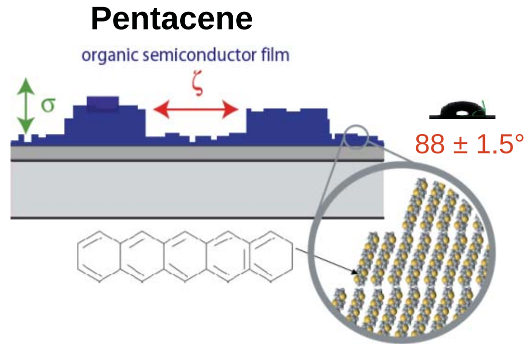
Scaffolds coupled with sensing devices

Neural Cells Adhesion & Proliferation on Pentacene Thin Films

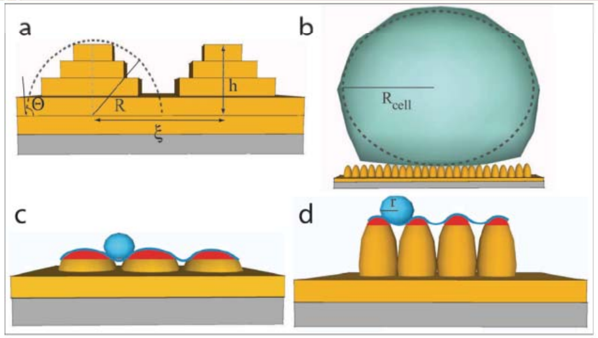


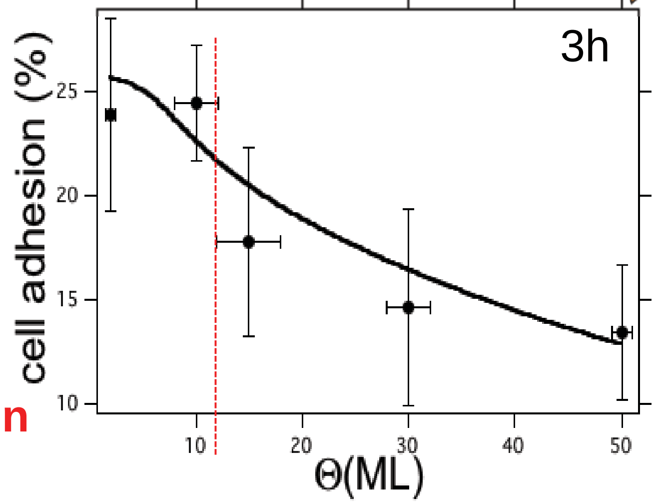


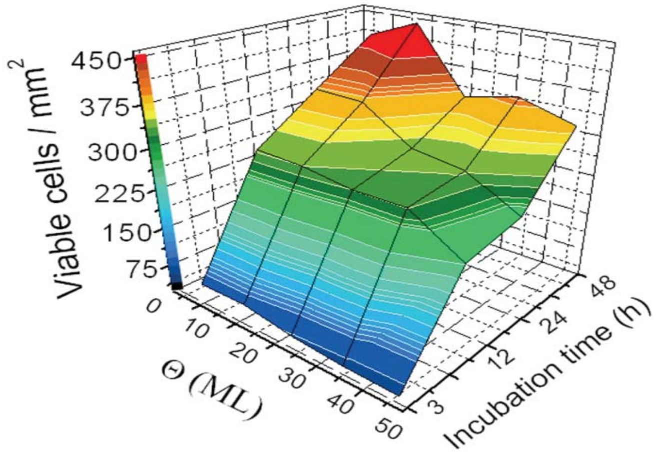
1.5 ML 4 ML 6 ML 13 ML 20 ML 60 ML



Film Thickness (θ)
(expressed in ML)





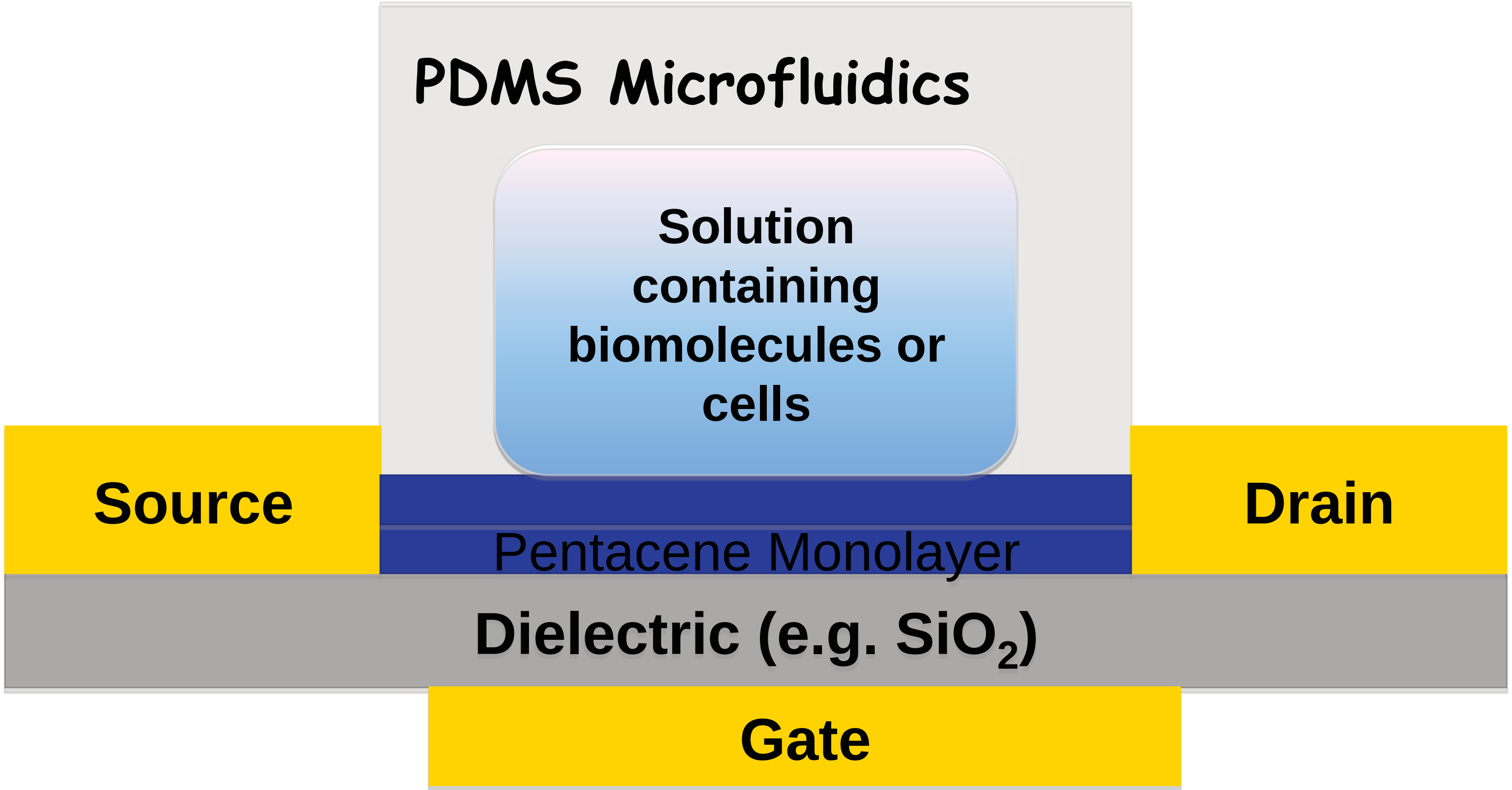


Cell adhesion and proliferation are enhanced for < 10 ML.

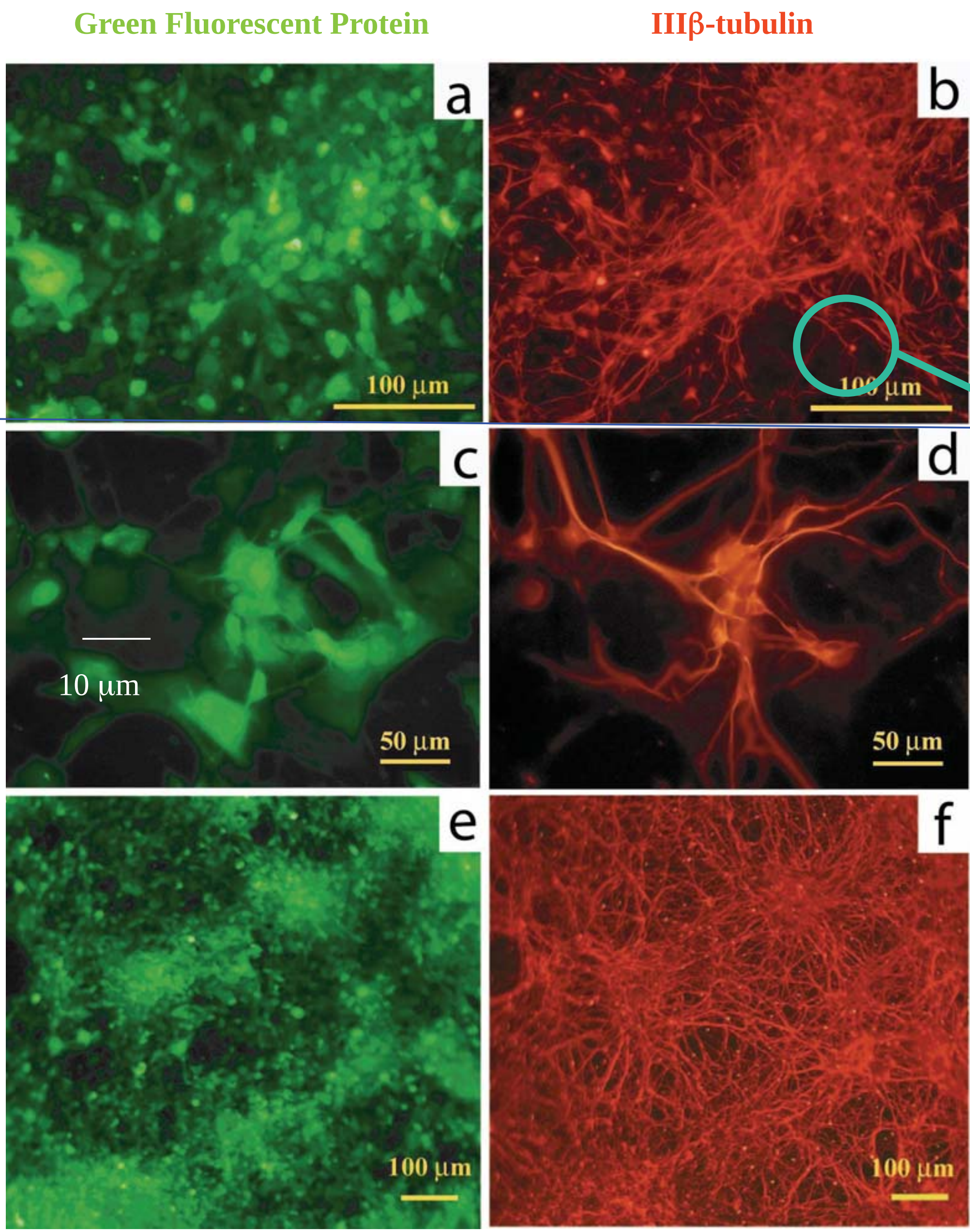
Optimum morphology for cell deployment is for σ ≤ 6 nm, ξ>500 nm, *df >2.45.*

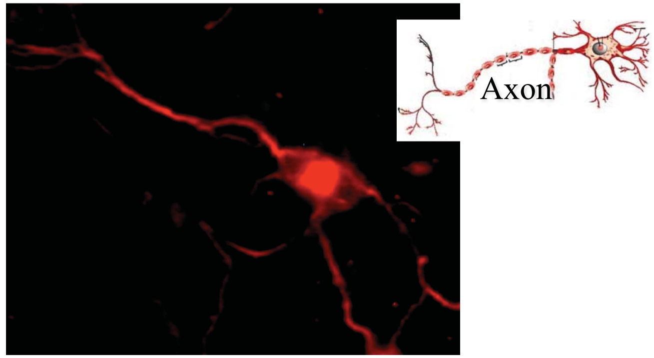
I. Tonazzini et al. 2010, Biophysical Journal

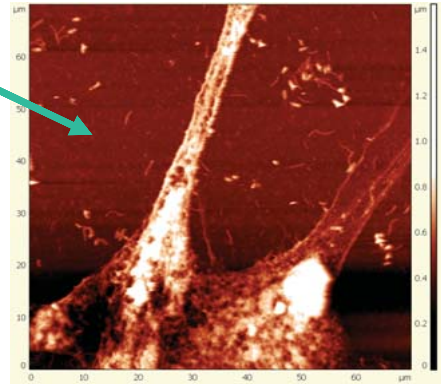
Ultra-thin film OFET transducer

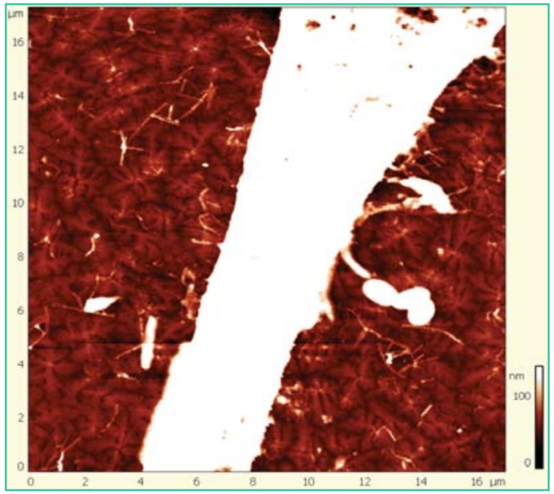


Stem cells to neural networks





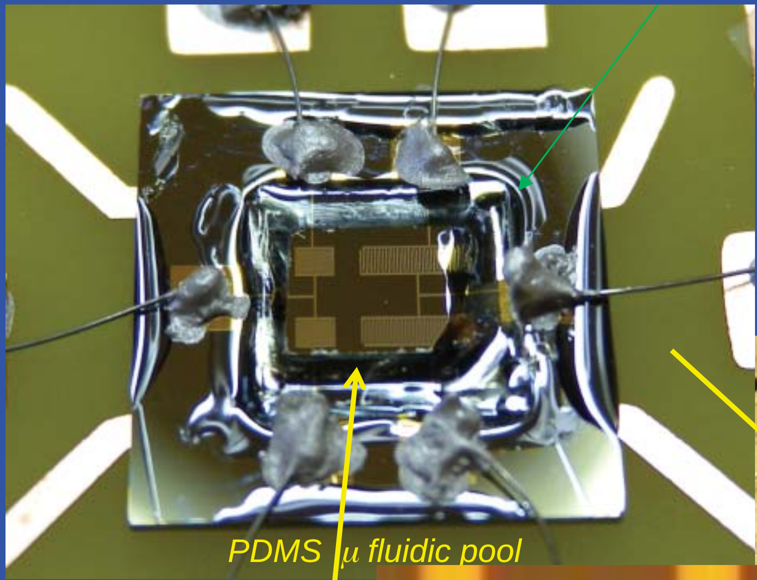


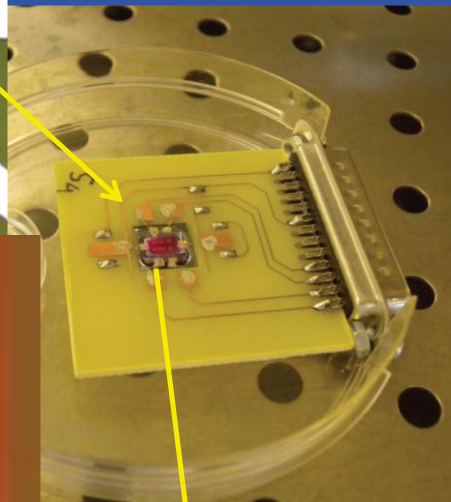


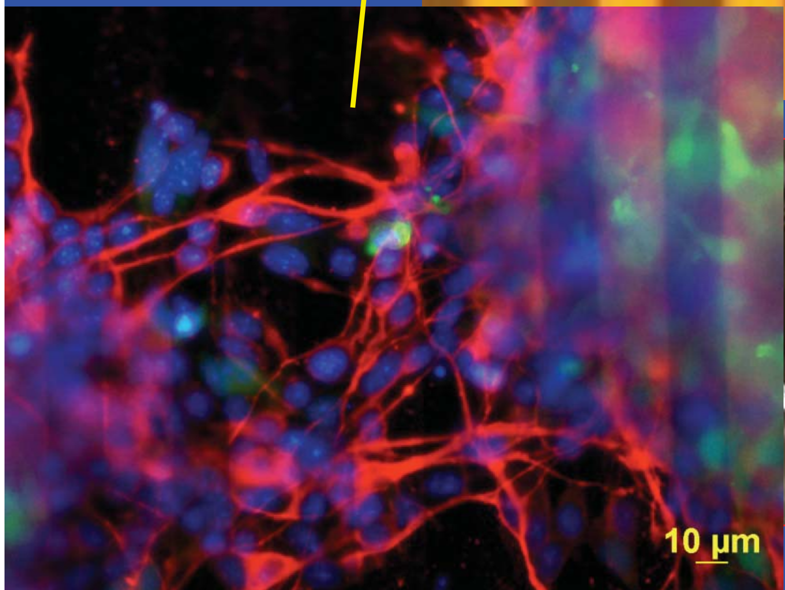
E. Bystrenova et al. ADVANCED FUNCTIONAL MATERIALS (2008)

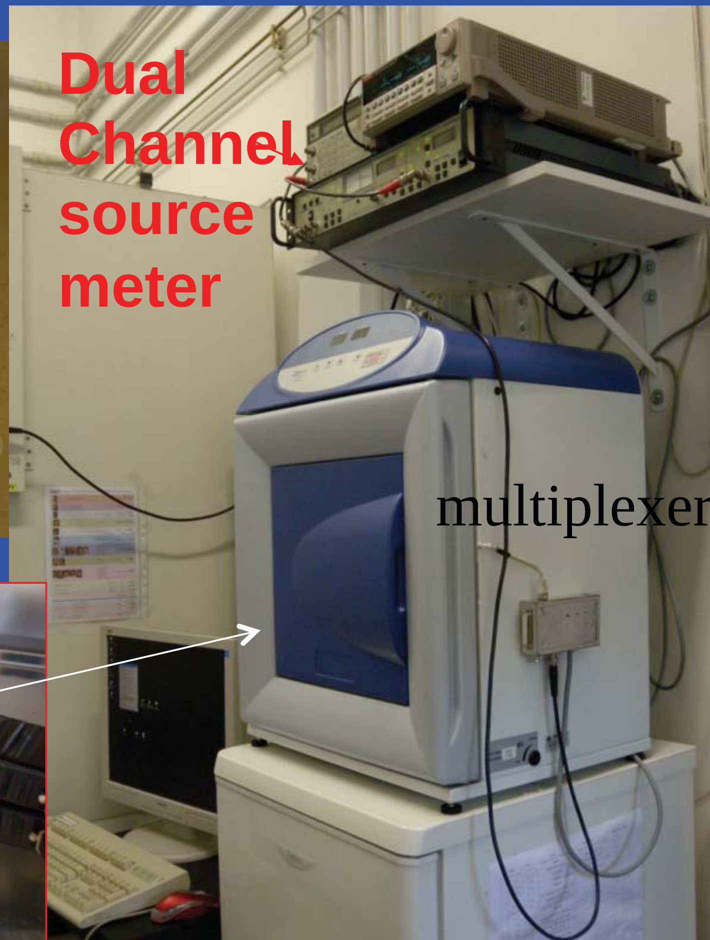
Signals from differentiated stem cells

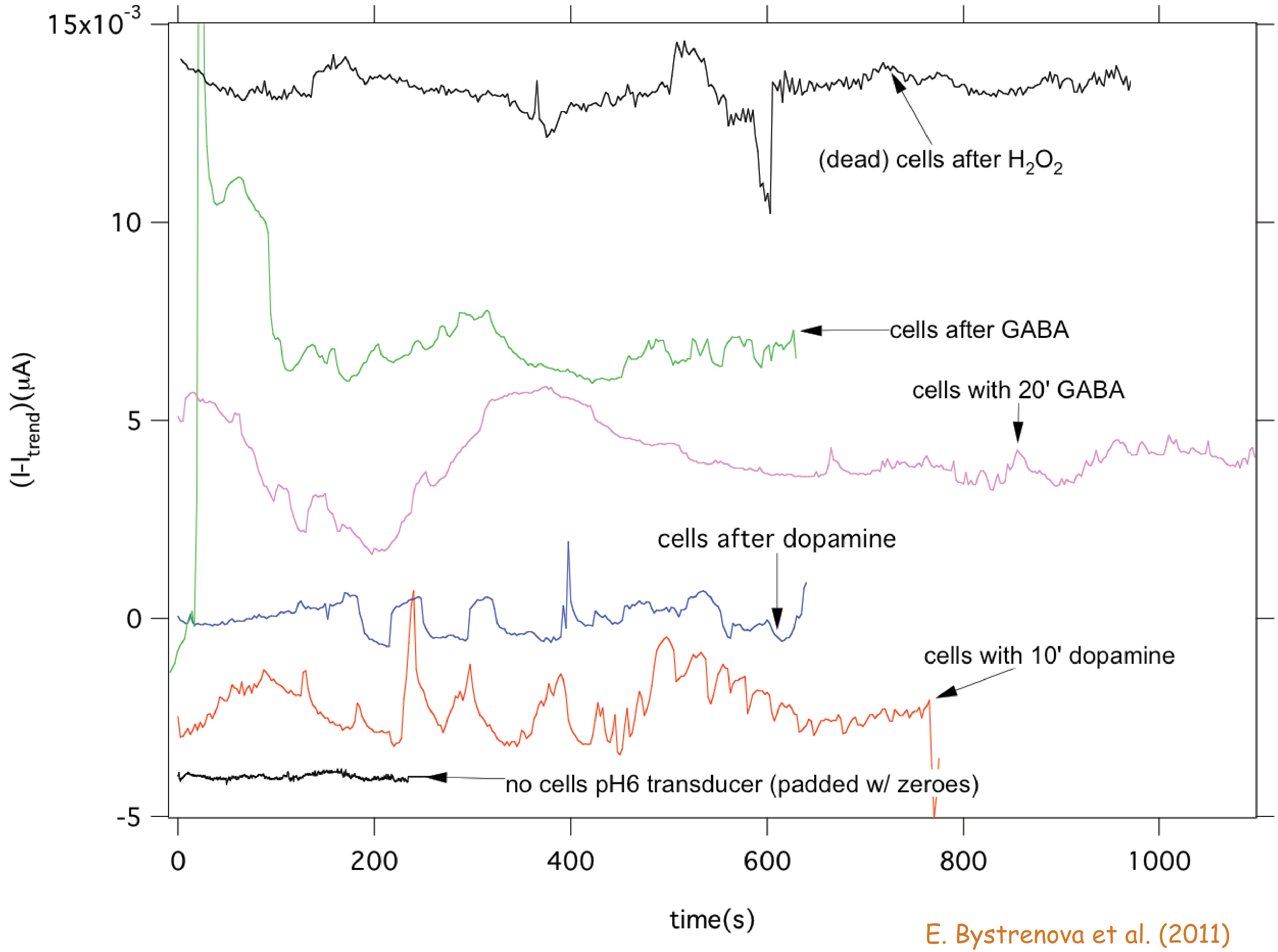
- controlled humidity
- controlled temperature
- controlled CO_2
- in situ el. measurement









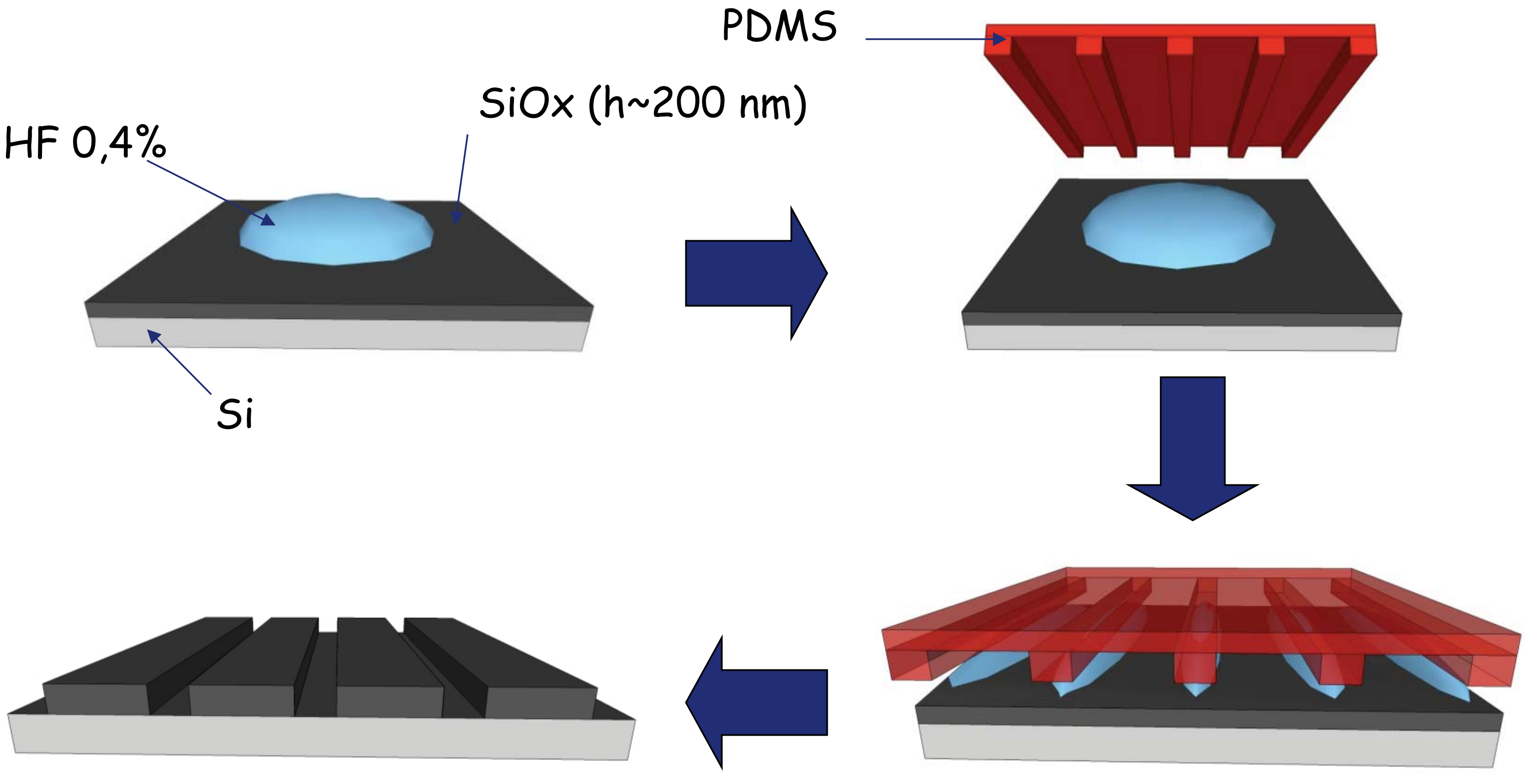


E. Bystrenova et al. (2011)

Topography and Chemistry together…

Lithographically Controlled Etching (LCE)

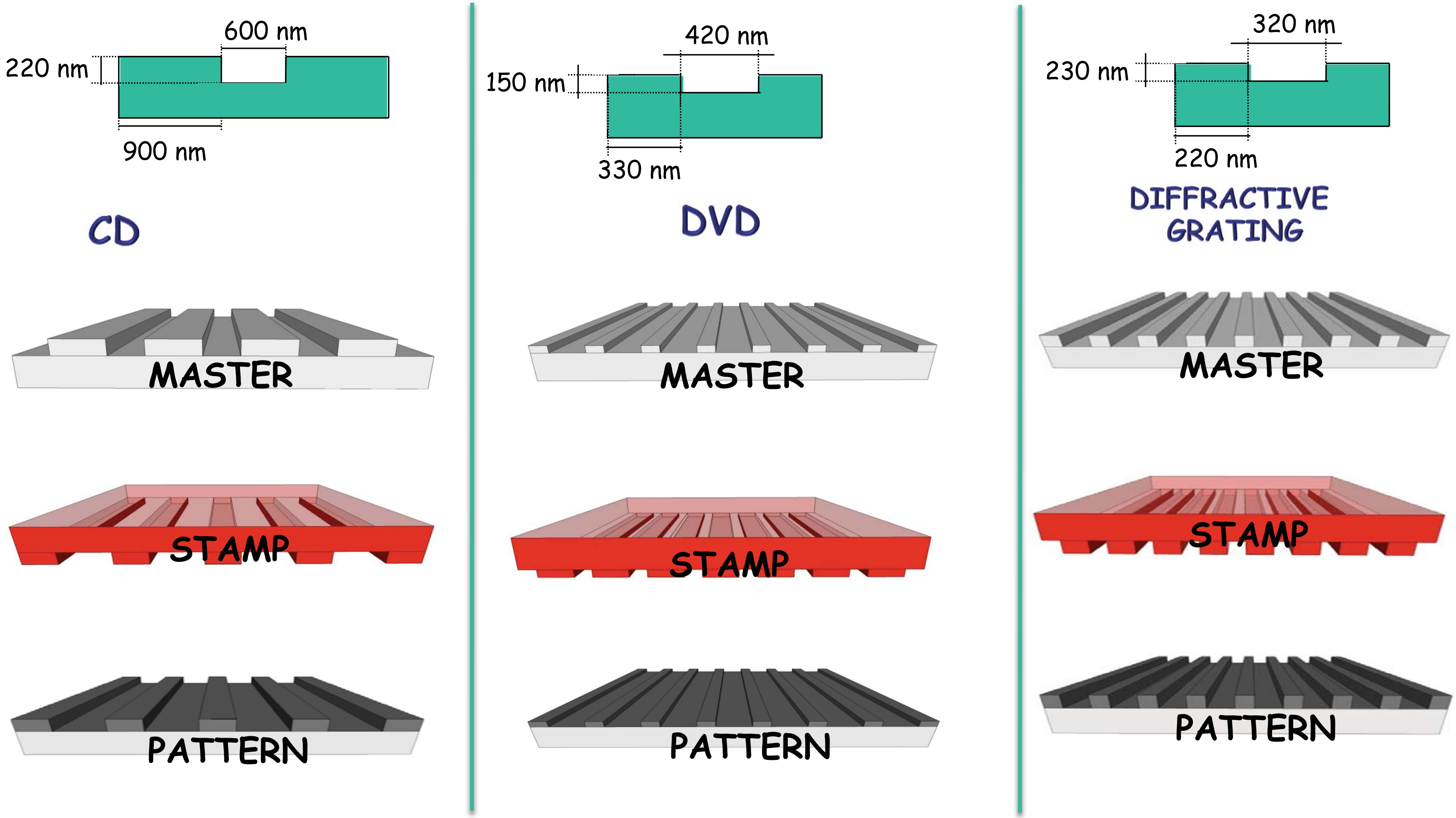
fluorhydric acid (HF) aqueous solution etches a silicon oxide surface



Daniel Limones Poster # 22

Fine tuning the fabrication parameters

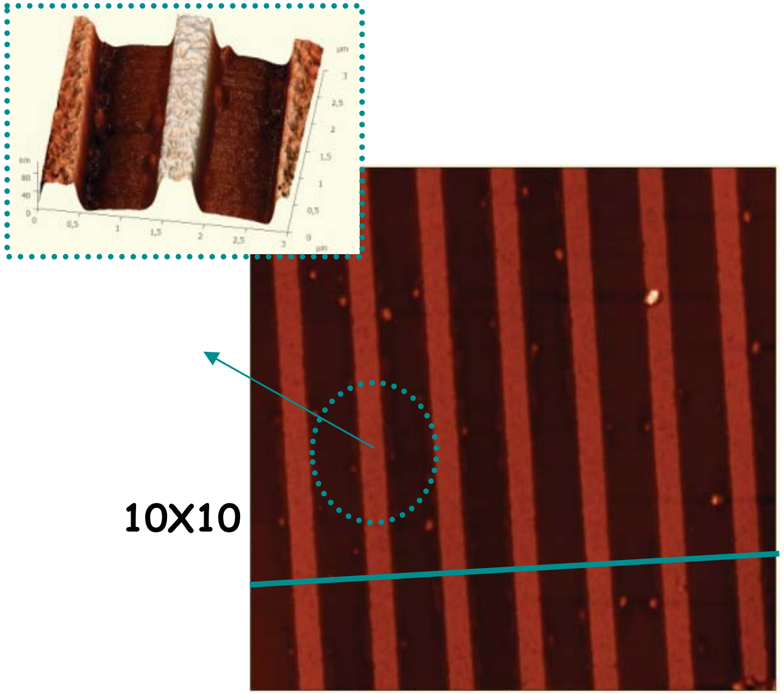
Different stamps → different areas of the recesses → different solution confinement

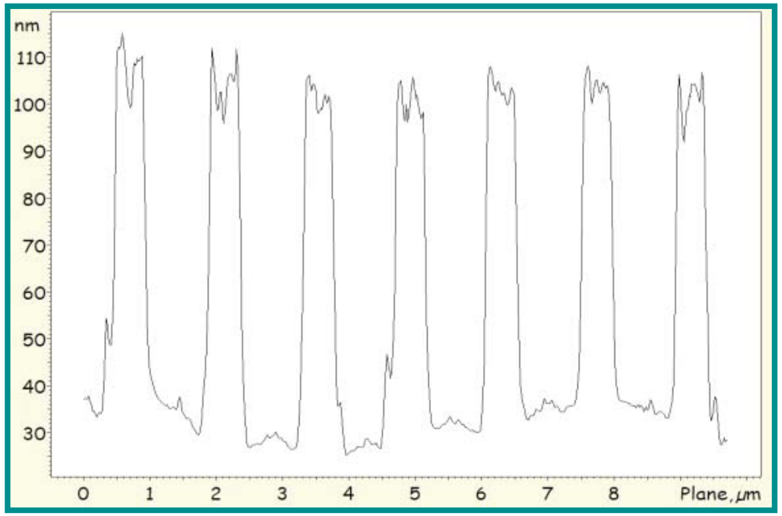


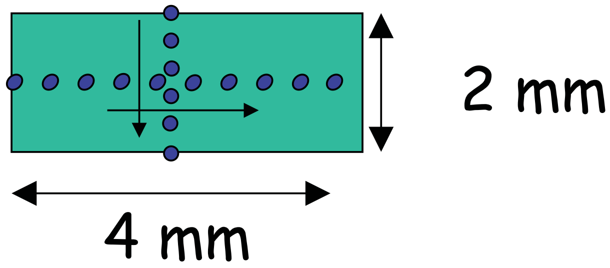
Controlling the engraved regions

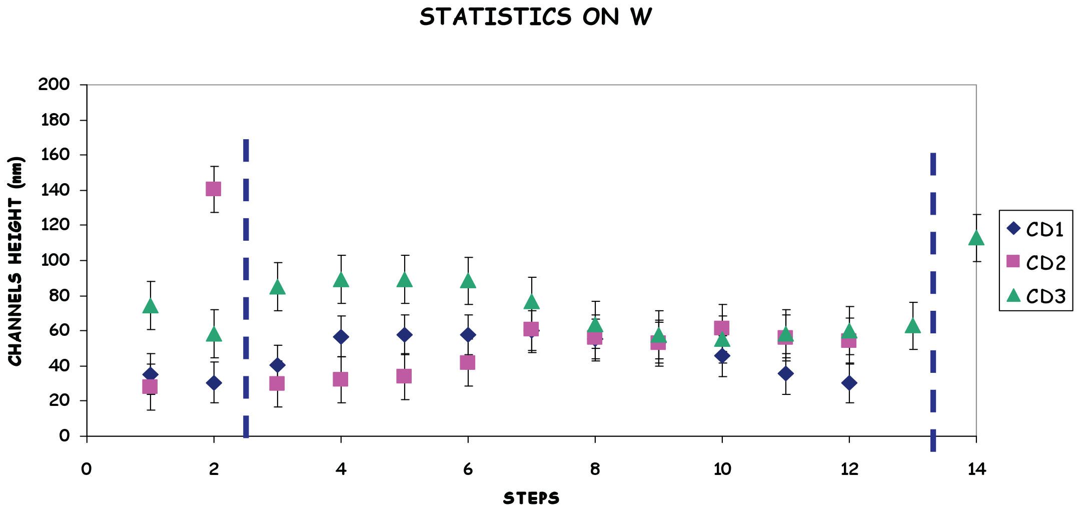
Highly controlled fabrication method

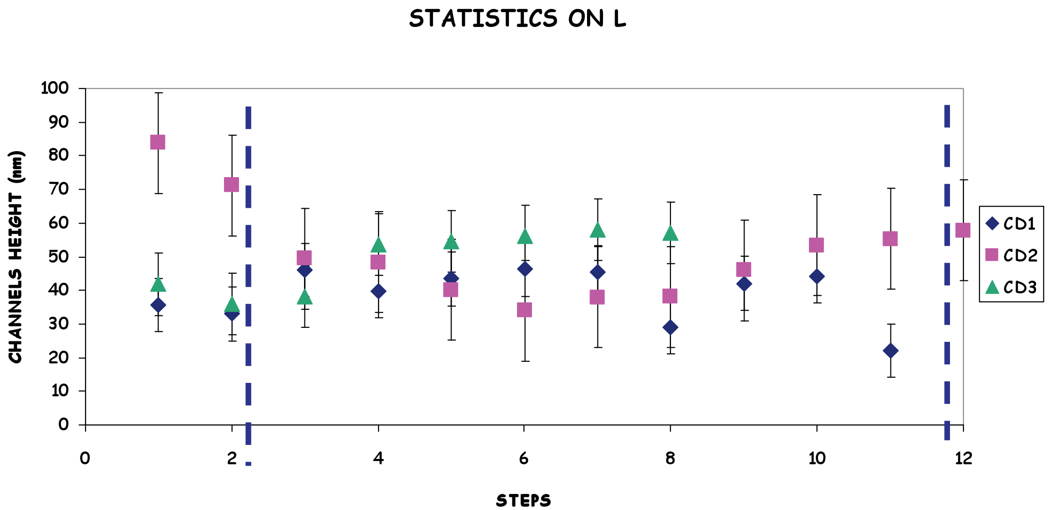
THERMAL SiOx SUBSTRATE PATTERNED BY PDMS-CD STAMP



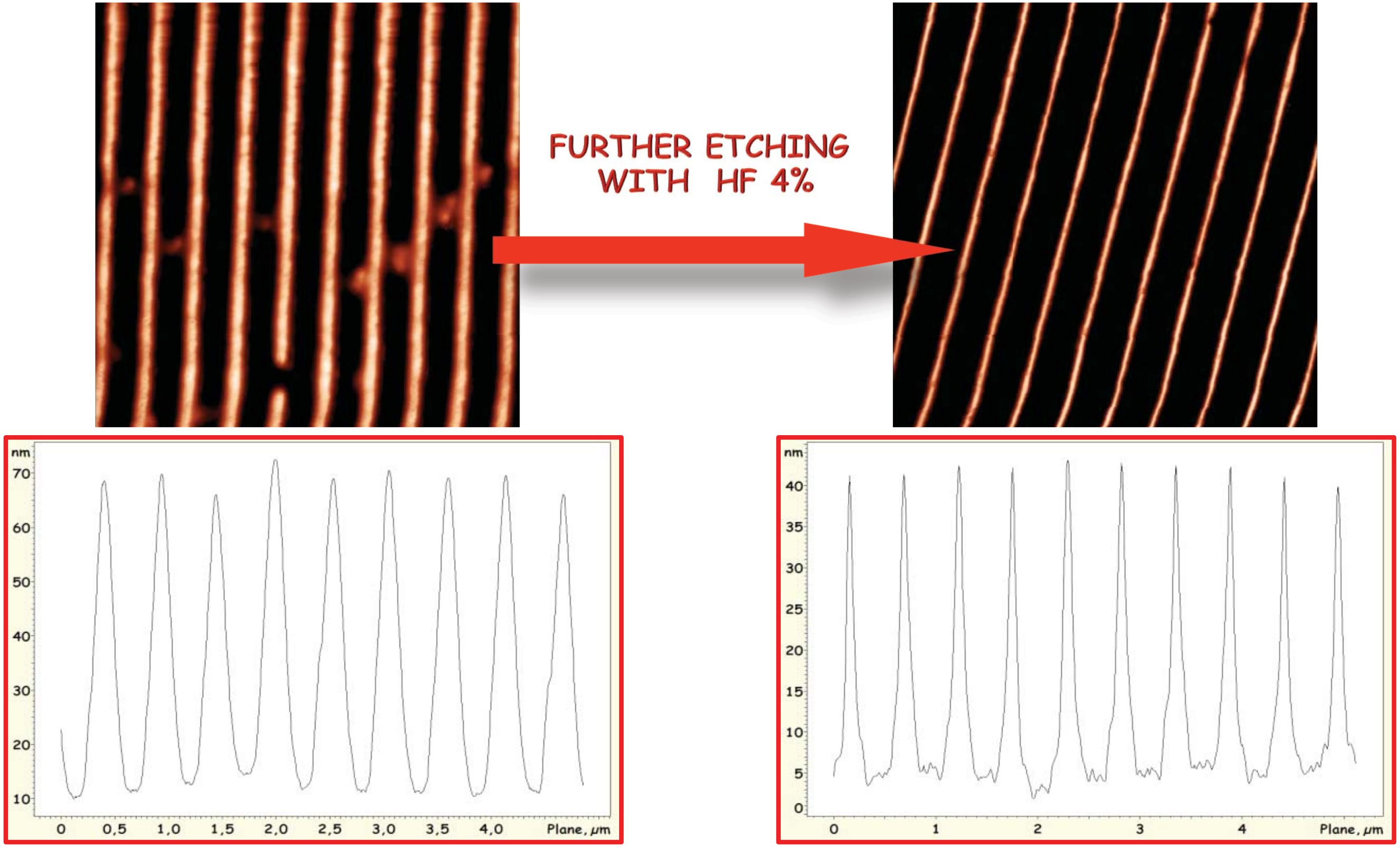






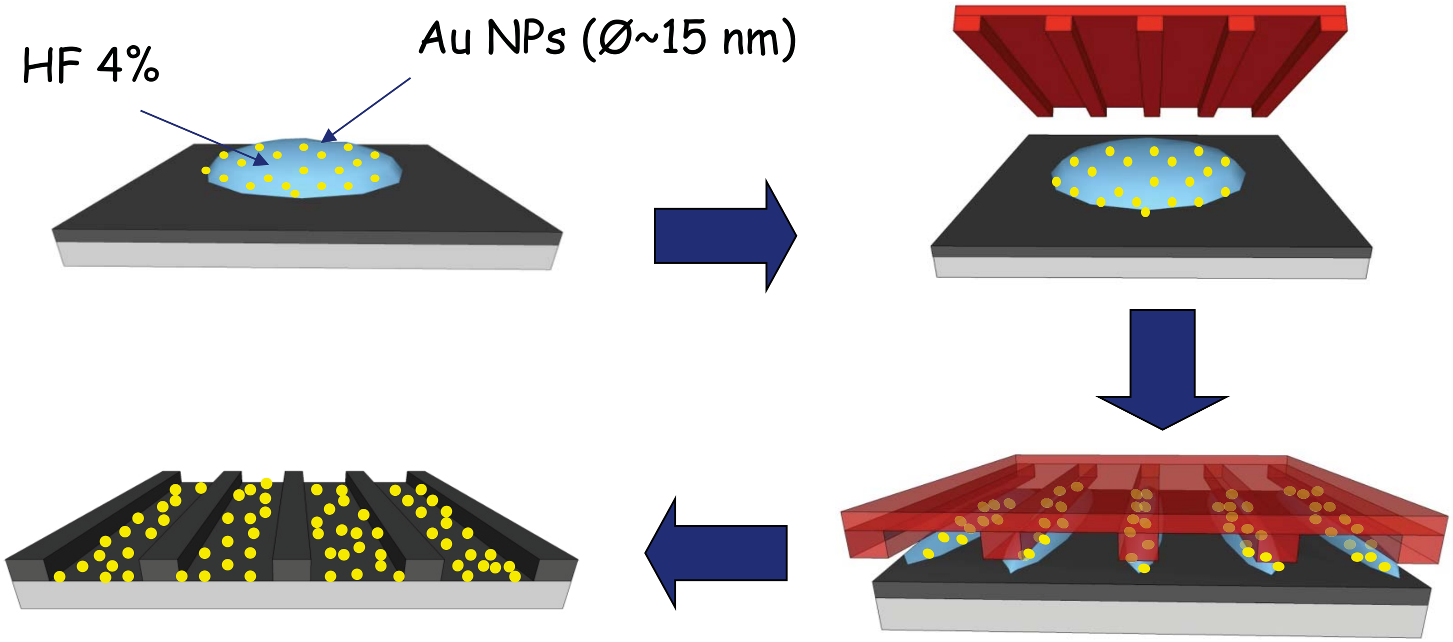


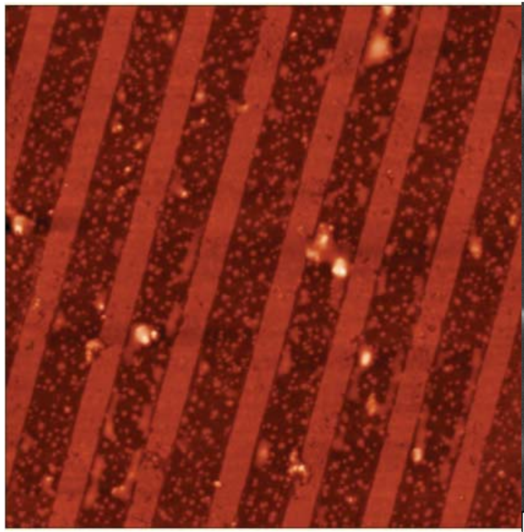
SiOx Nanowires fabrication:
pattern obtained by Diffractive grating replica

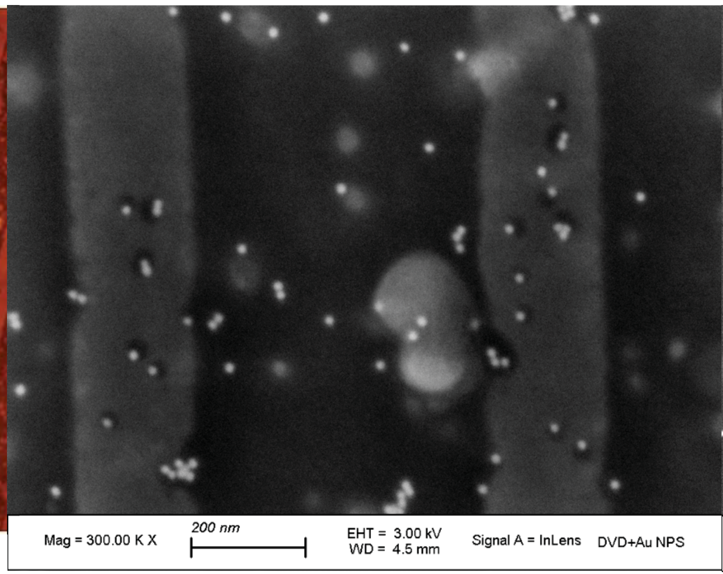


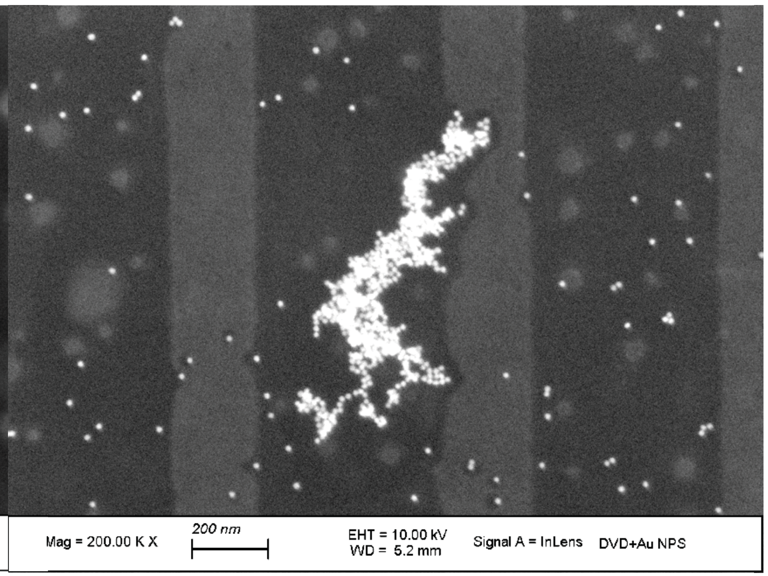
220 nm **protrusions** **75 nm**

One-step engraving and functionalizing a surface









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