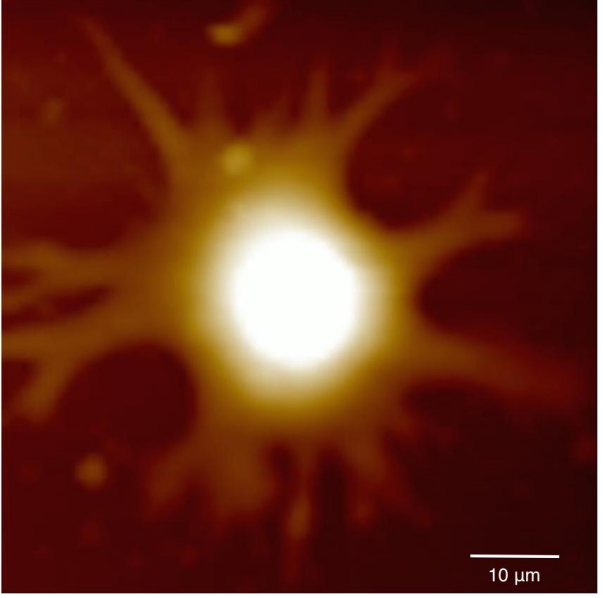


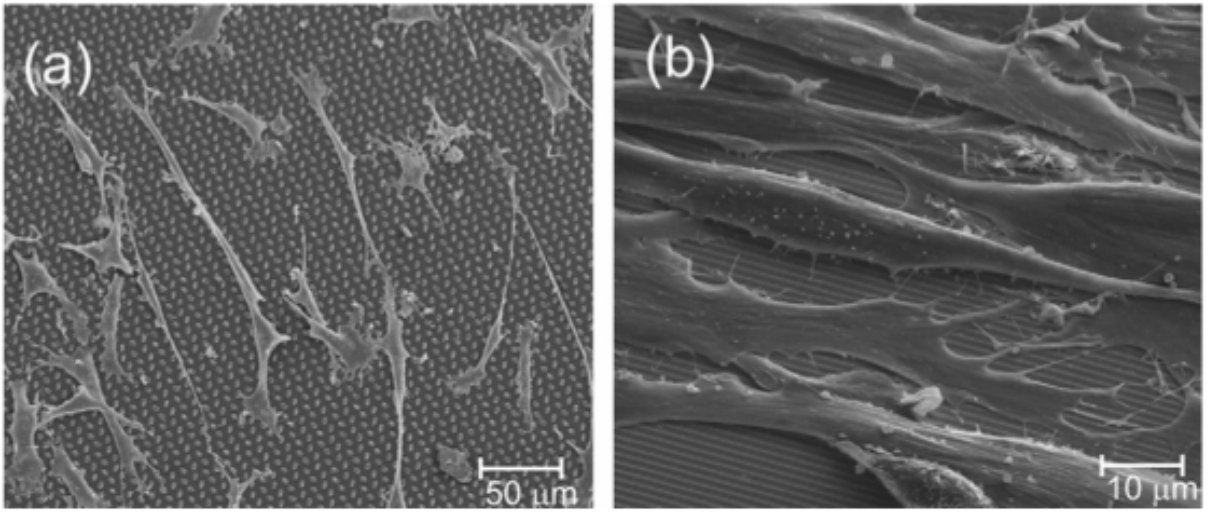
Mechanotransduction: A biophysical investigation of the *cell-microenvironment* interaction

Candidate: **Matteo Chighizola**

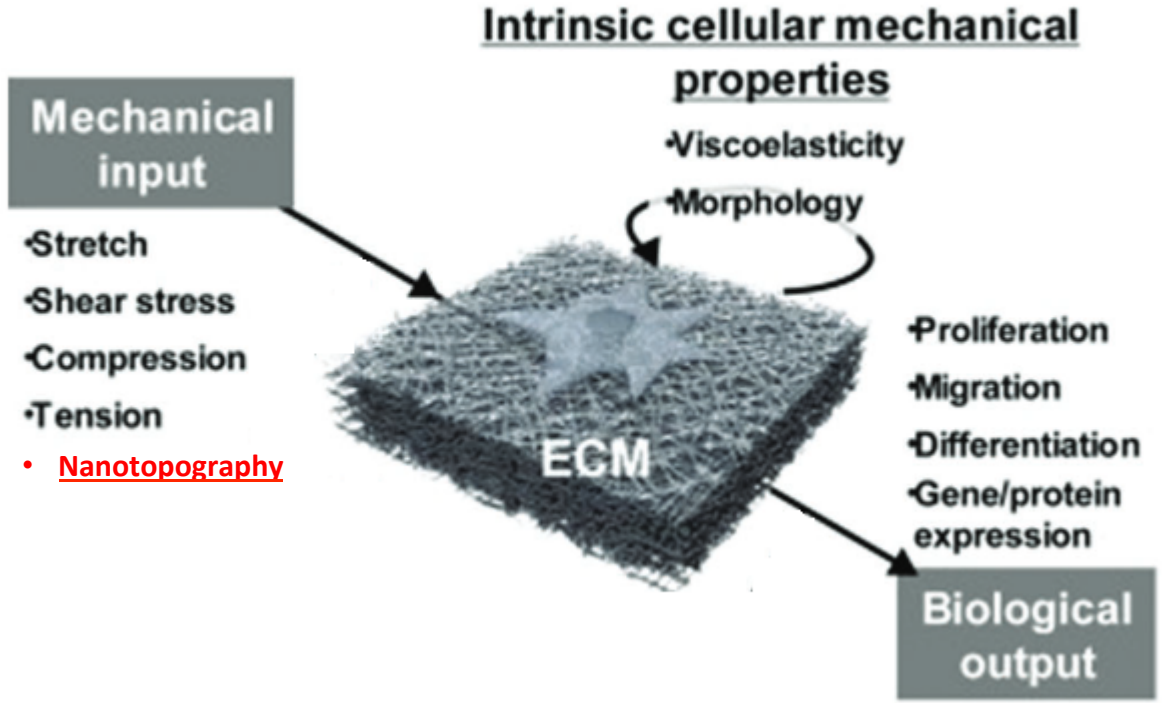
Tutor: **Prof. Alessandro Podestà**

Workshop 1° year Phd Course

Mechanotransduction refers to the processes through which **cells sense** and respond to **Physical** stimuli by converting them to biochemical signals that stimulate specific cellular **responses**.



Flemming R. et al *Biomaterials* **20,** 573–588 (1999)



Joseph Long et al. Journal of Materials Chemistry ***B5.13*** *(2017), pp. 2375–2389.c*

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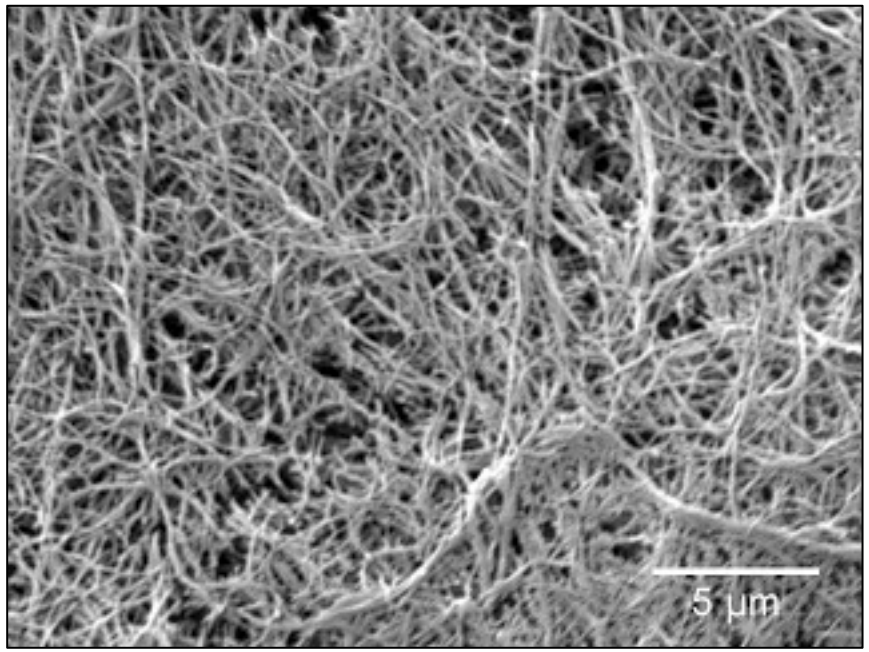
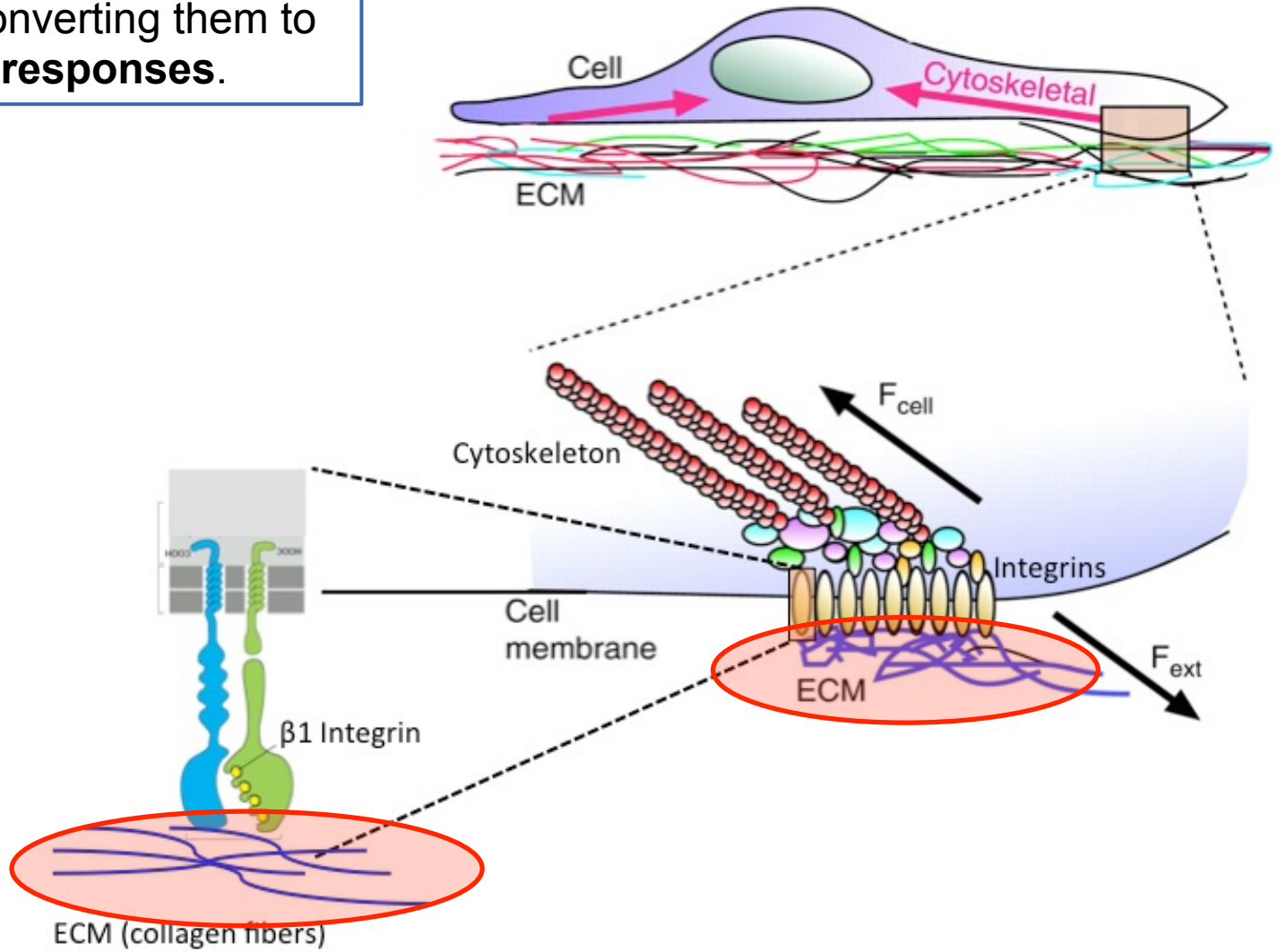
ECM: **Extracellular Matrix**



Schultz. *Proc. Natl. Acad. Sci.* **112,** E3757–E3764 (2015).



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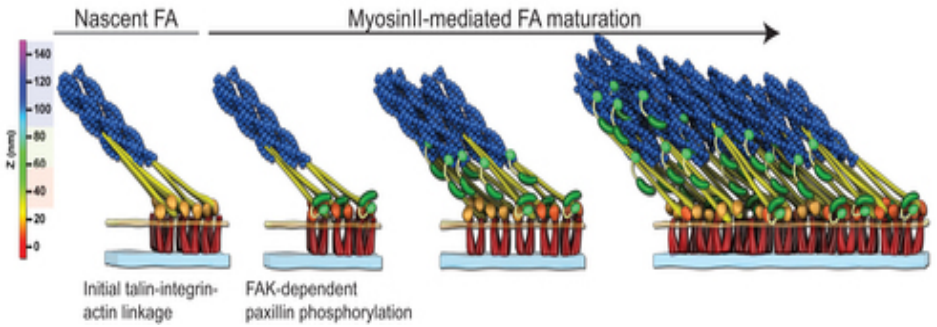

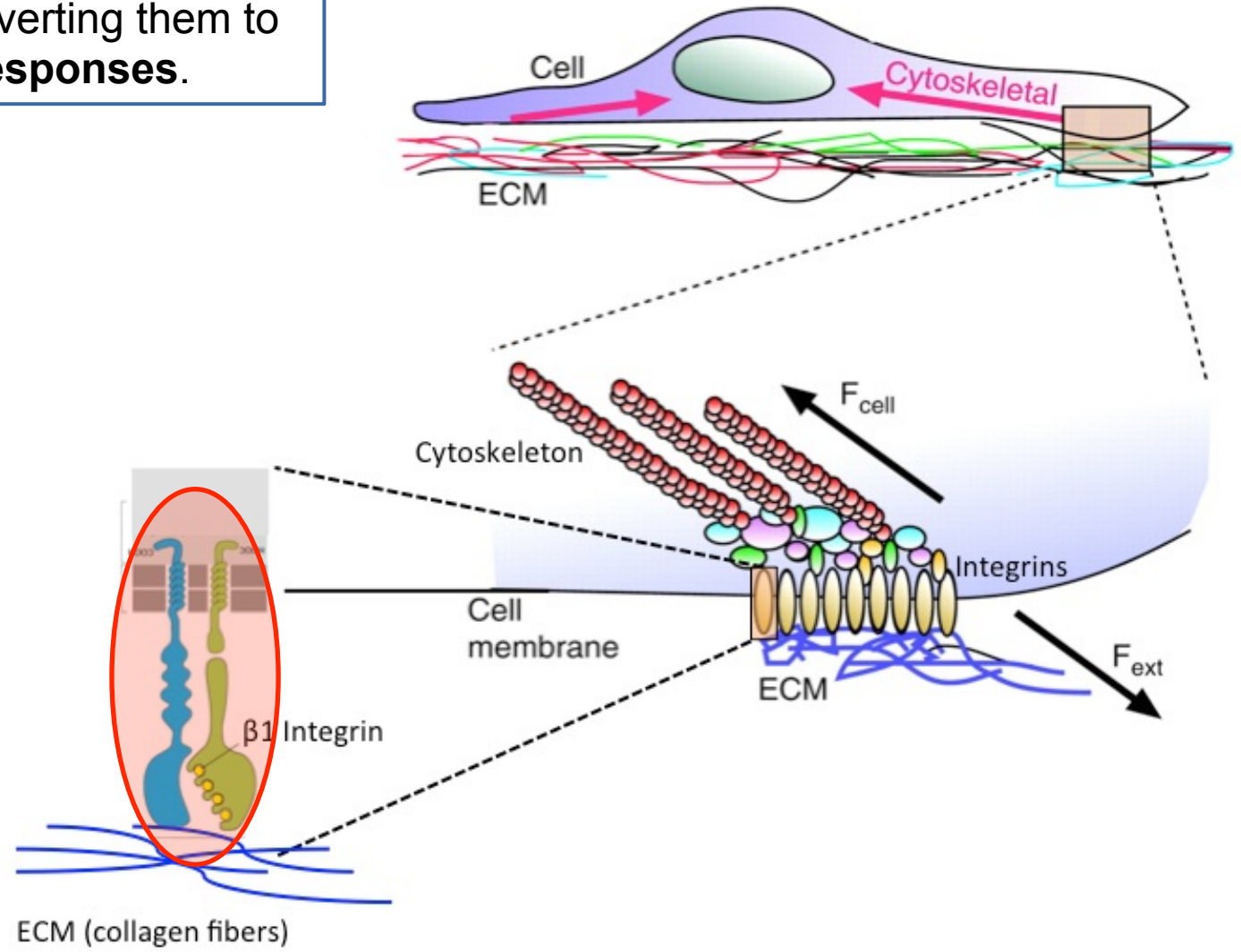

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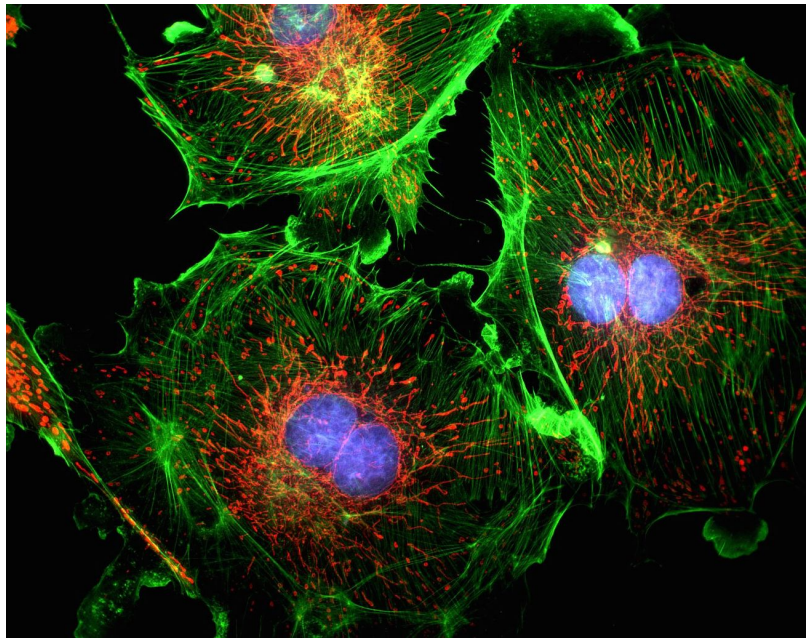
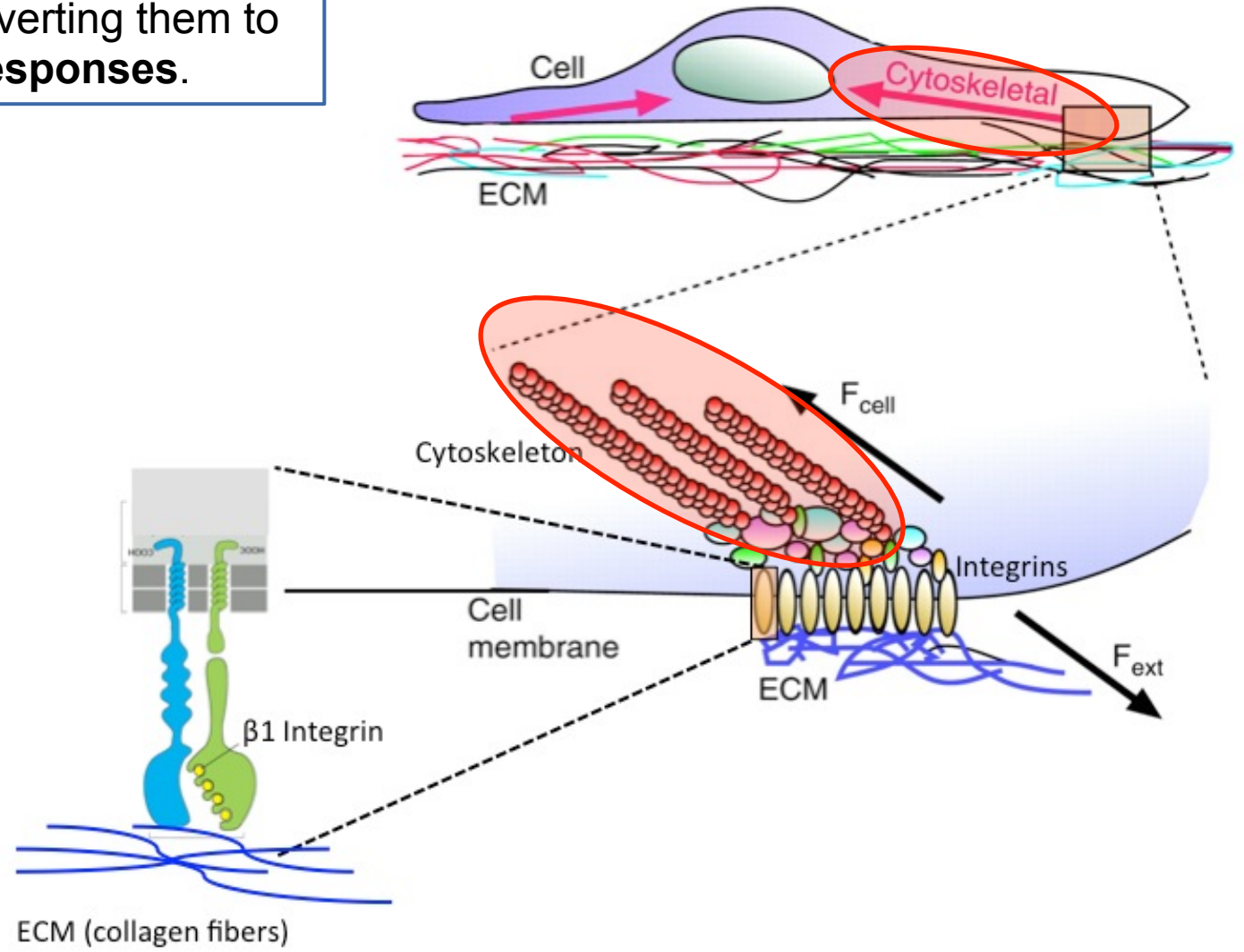
Cytoskeleton



Cell Fluorescence Image.
Microscopyu.com (Nikon)



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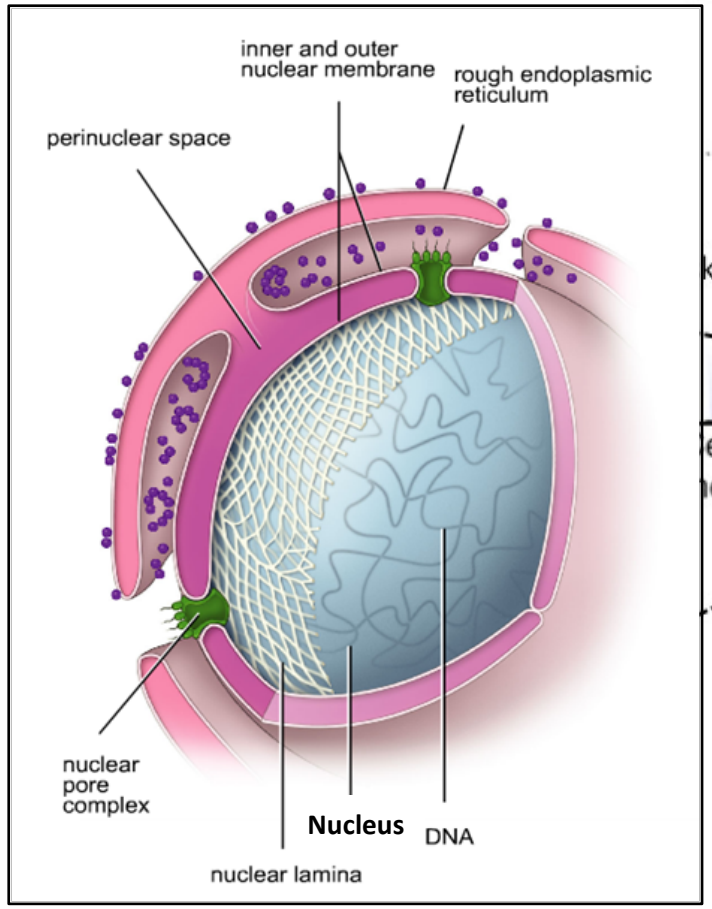

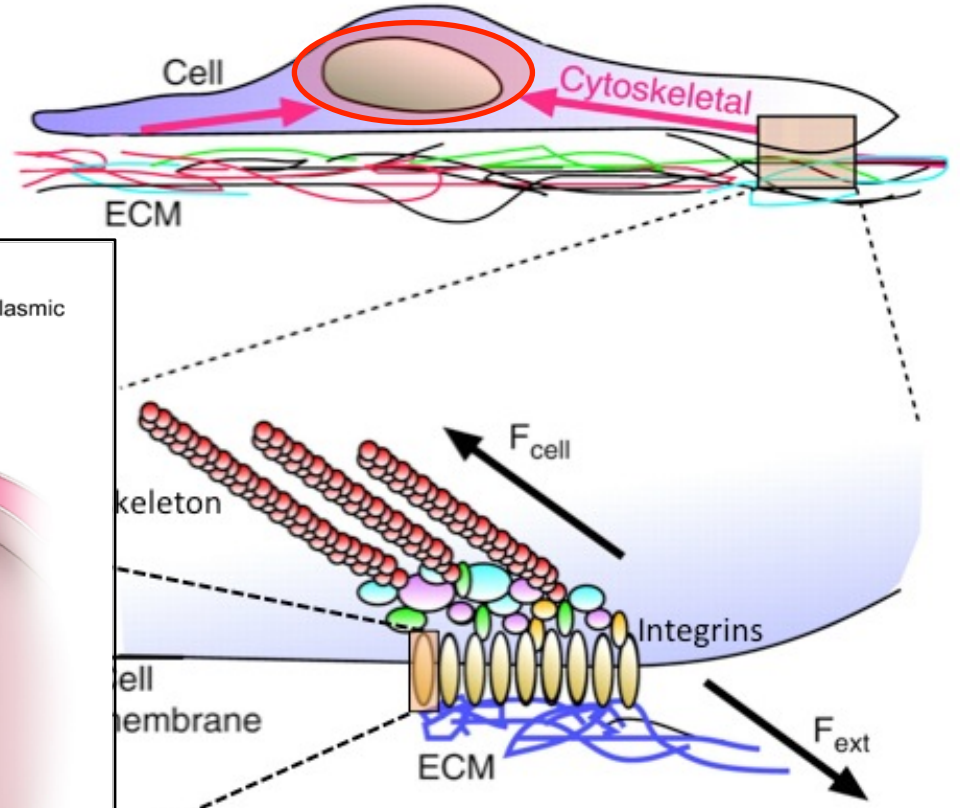

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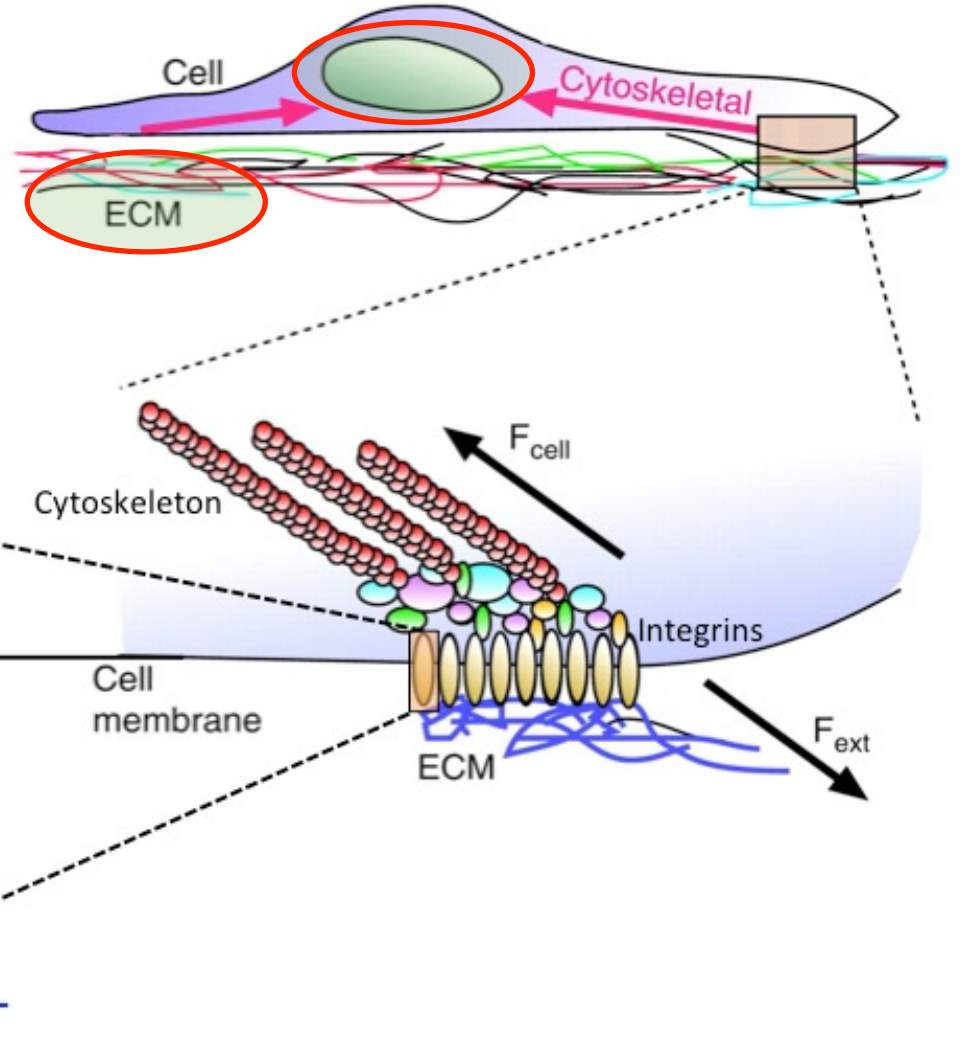
Nuclear Envelope

Micro-Environment
Mechanical Stimulus

Cell Nucleus
Biological Reaction



Connection between external **Environment** and **Nucleus.**

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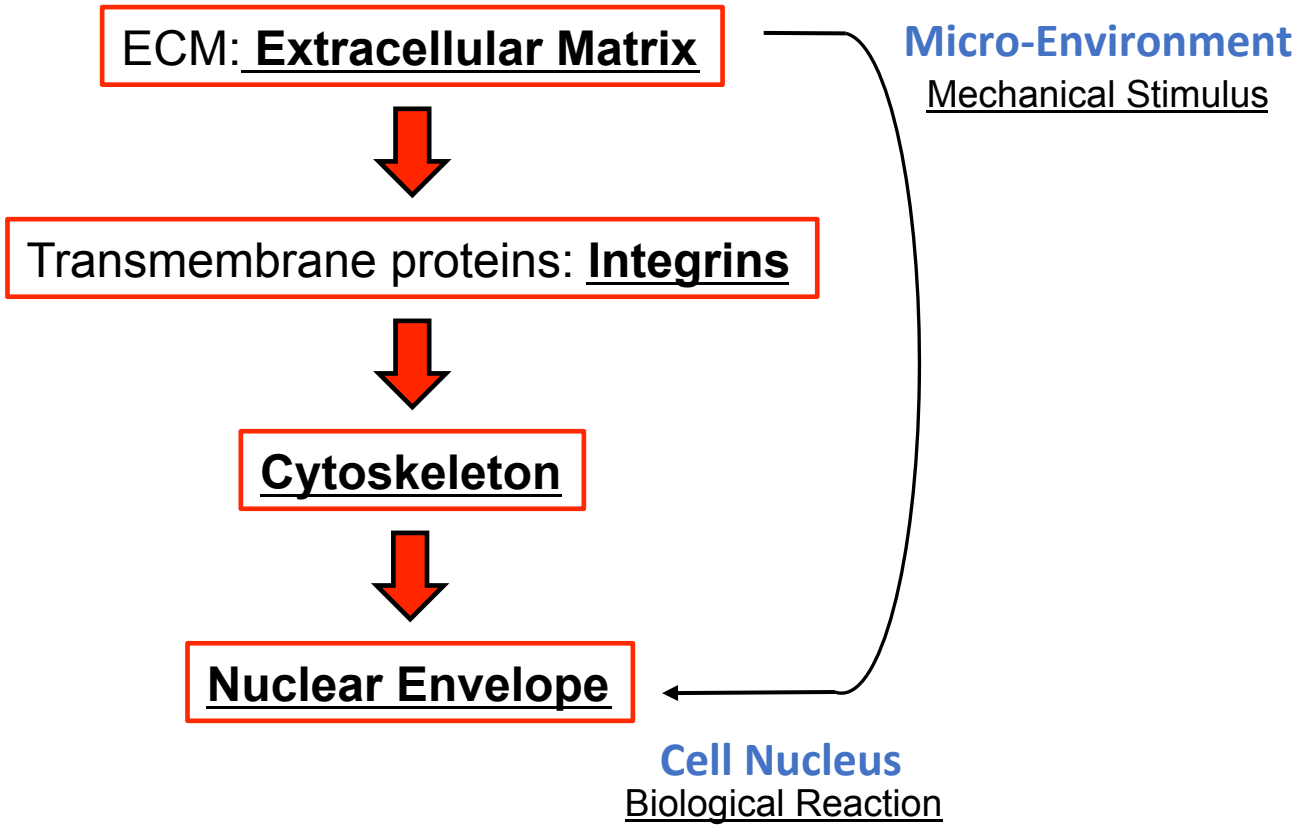

*Cells are capable of **sensing** the nanoscale topographical features and mechanical **properties** of the microenvironment.*

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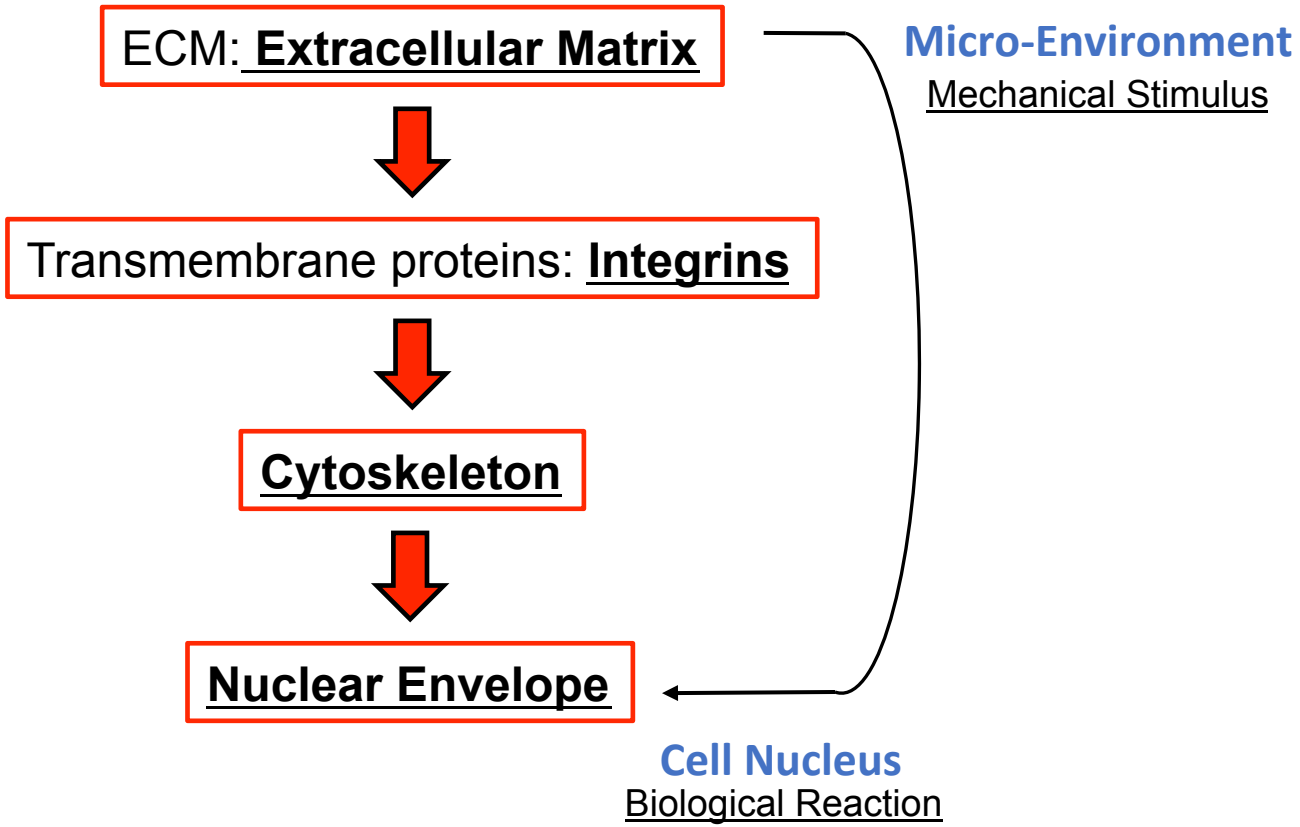

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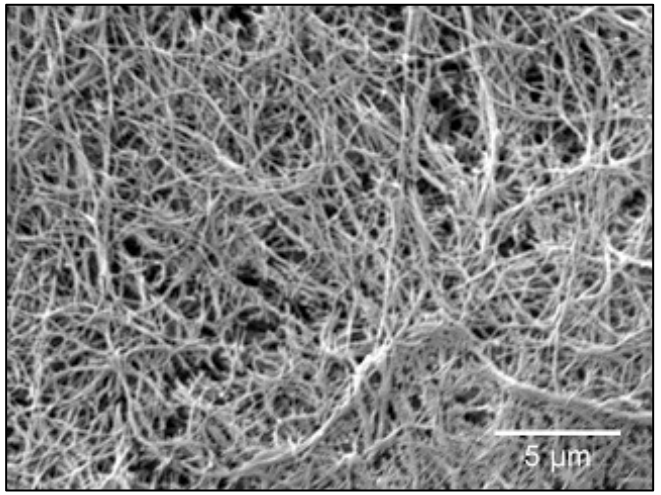

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Extracellular matrix is a complex and **Disordered** system.

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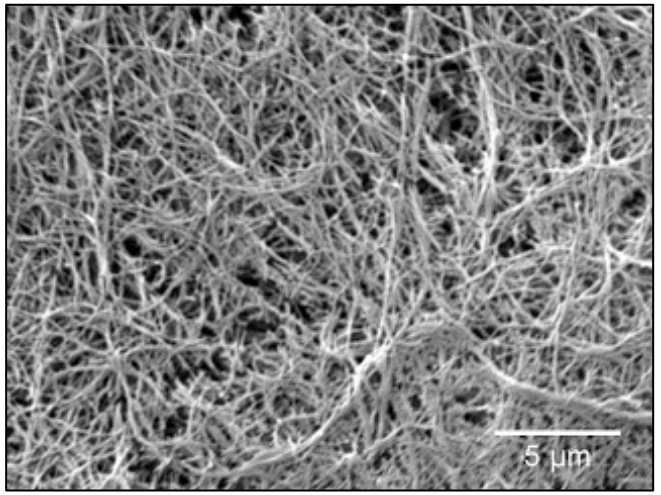

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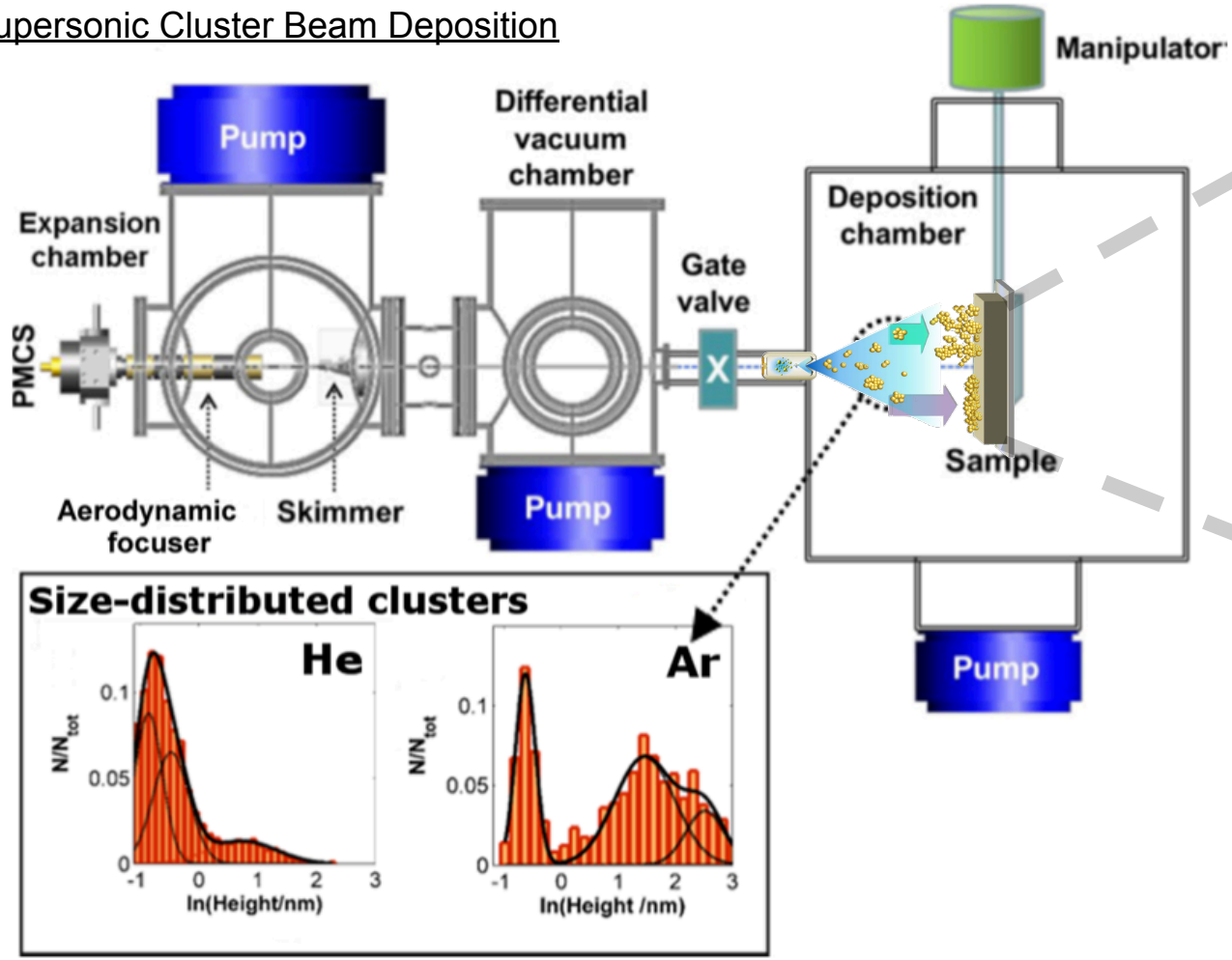
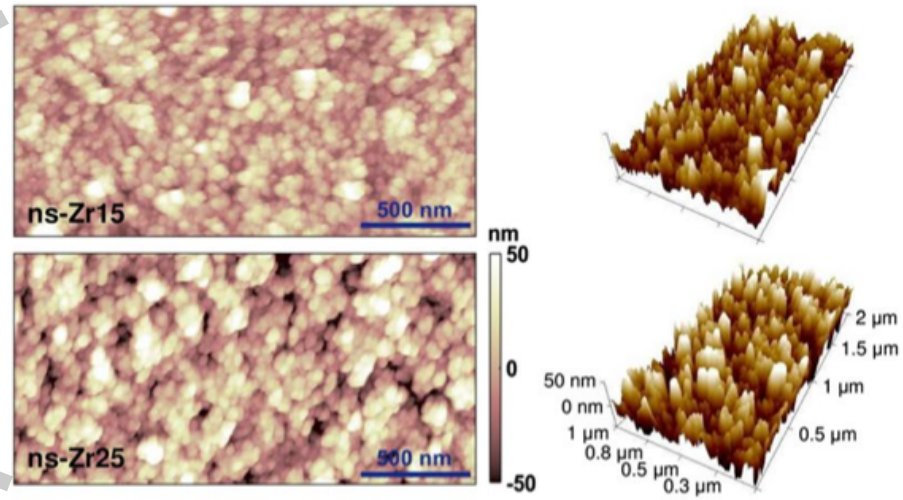
Reproduce this complexity on the **micro** and **nano**-scale

Nanostructured materials as tool to mimic ***ECM Complexity*** *and* ***Structure***

Supersonic Cluster Beam Deposition

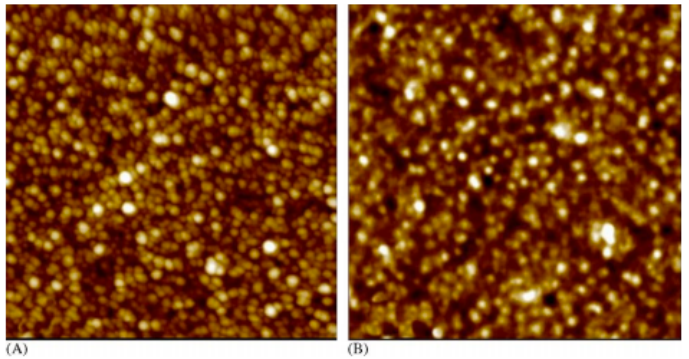


Cluster-Assembled Substrate (ZrO_2)



AFM Image:

Ns ZrOx **Gelatine Coated coverslip**



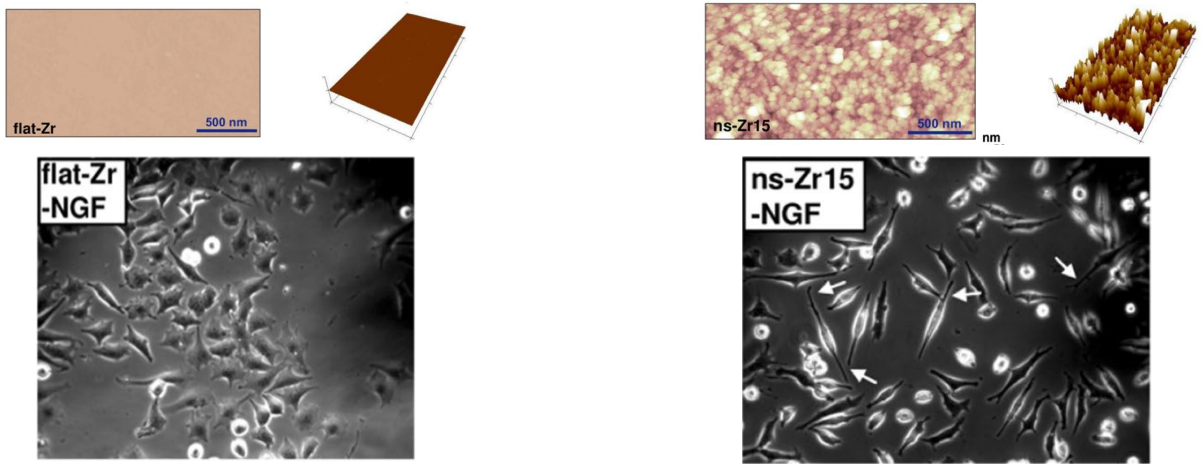
Schulte et al. A Chem. Res. **50**, 231-239 (2017)

Wegner et al. *J. Phys. Appl. Phys.* **39,** R439 (2006)

Carbone et al. *Biomaterials* **3221**, 9 (2006)

Differentiation through morphological Interaction

- **Experiment Details**



Schulte, C. *et al.J. Nanobiotechnology* **14,** 18 (2016).

Differentiation through morphological Interaction

Nanostructured surfaces induce differentiation without NGF!!

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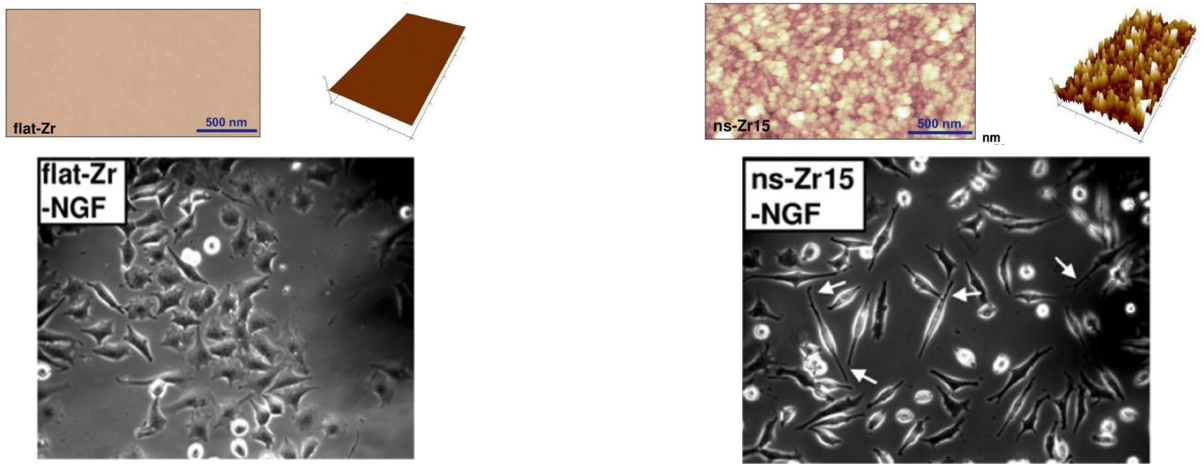
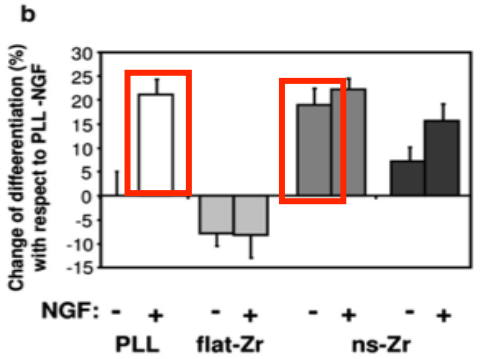


- **Results**



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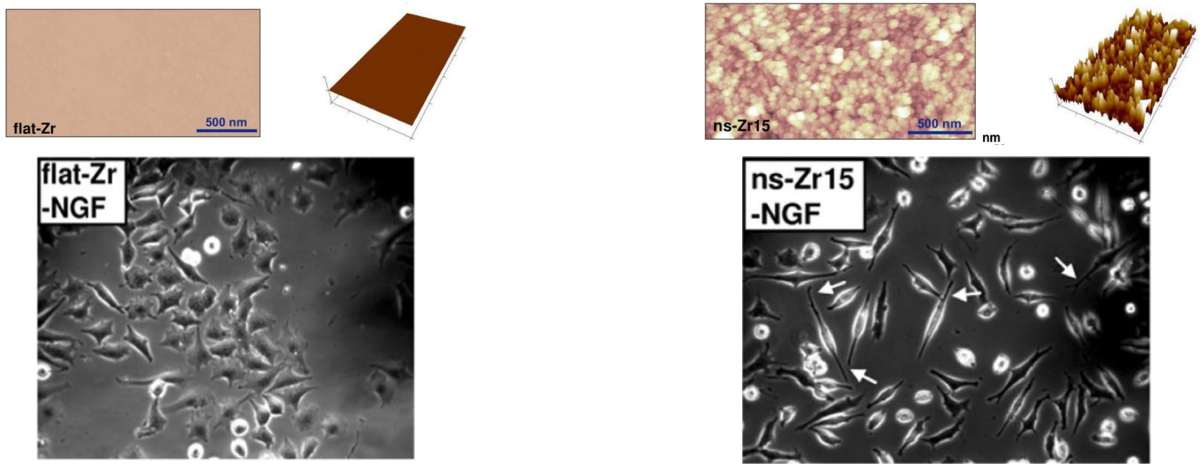

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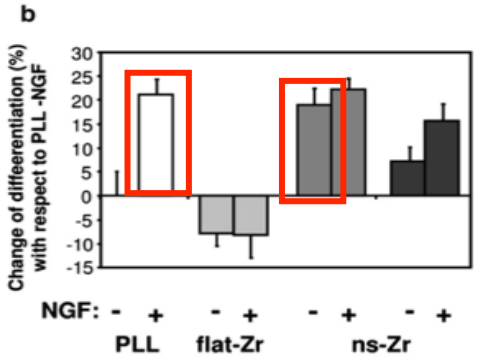

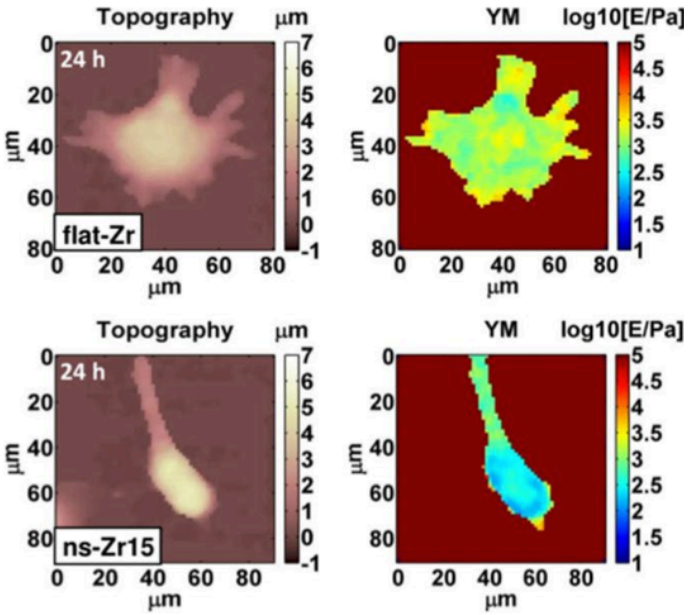

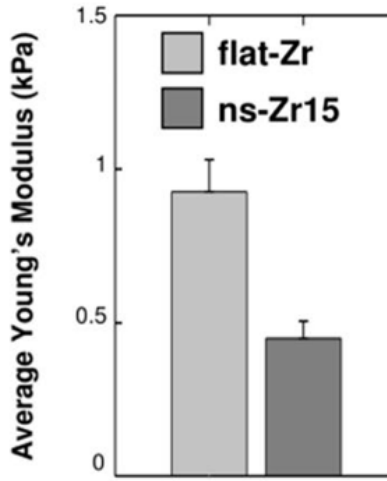

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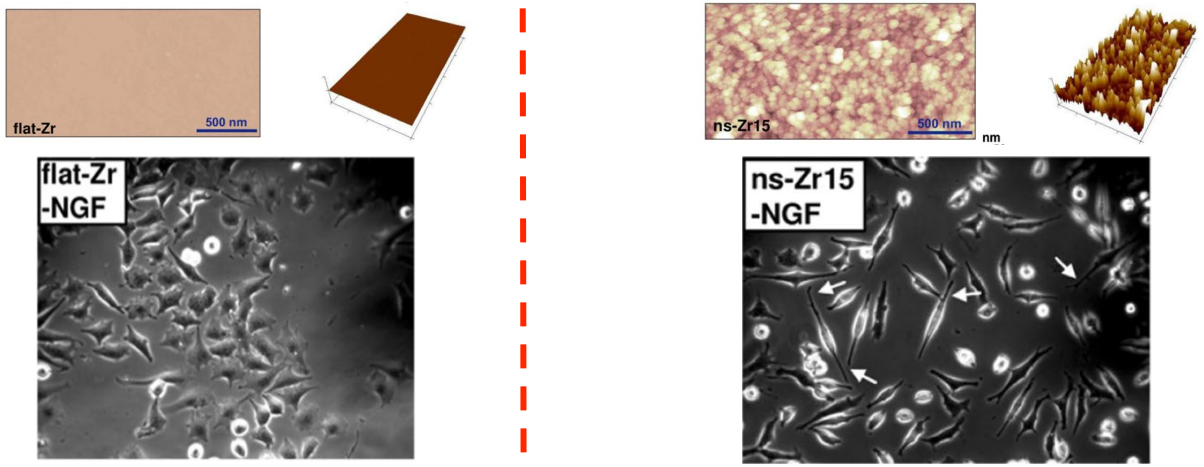

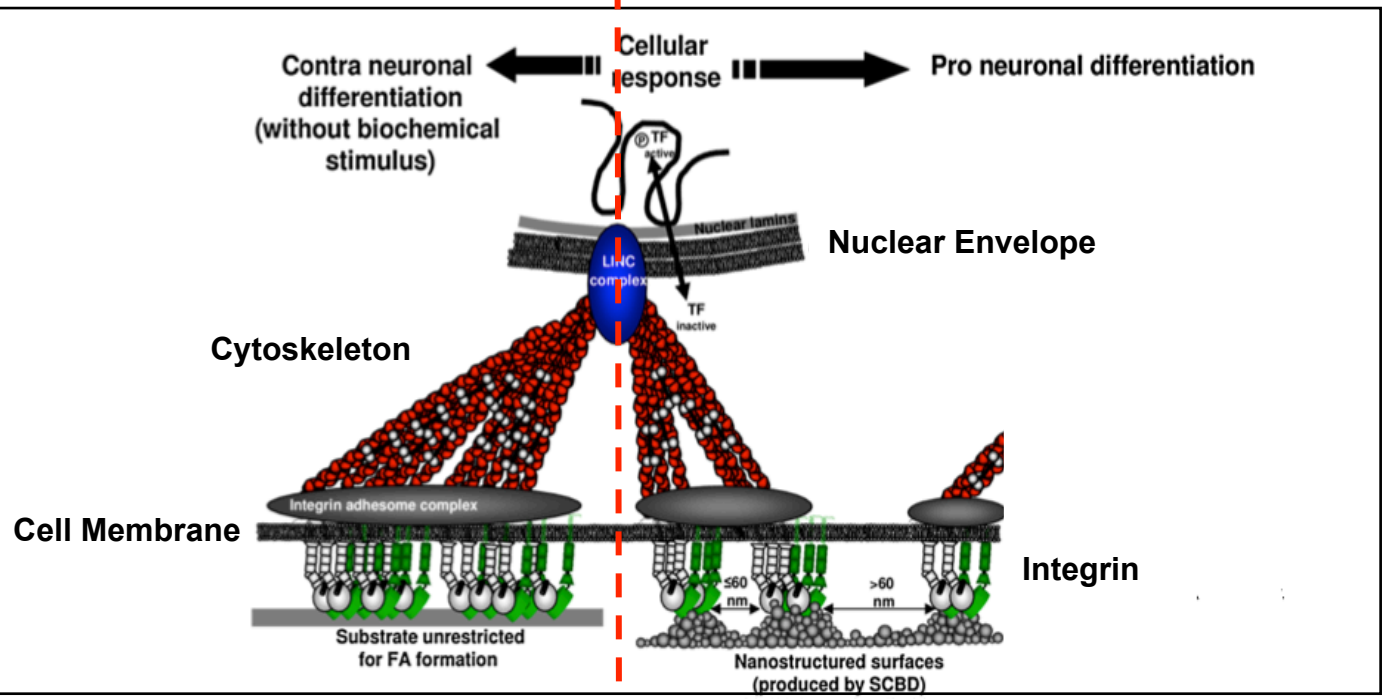

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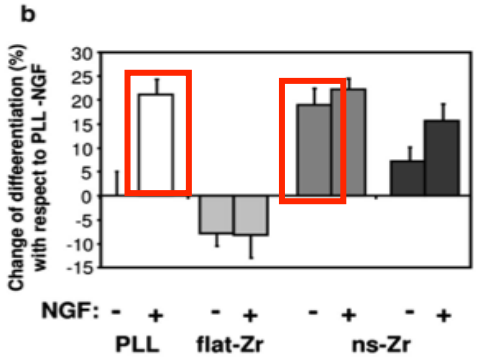

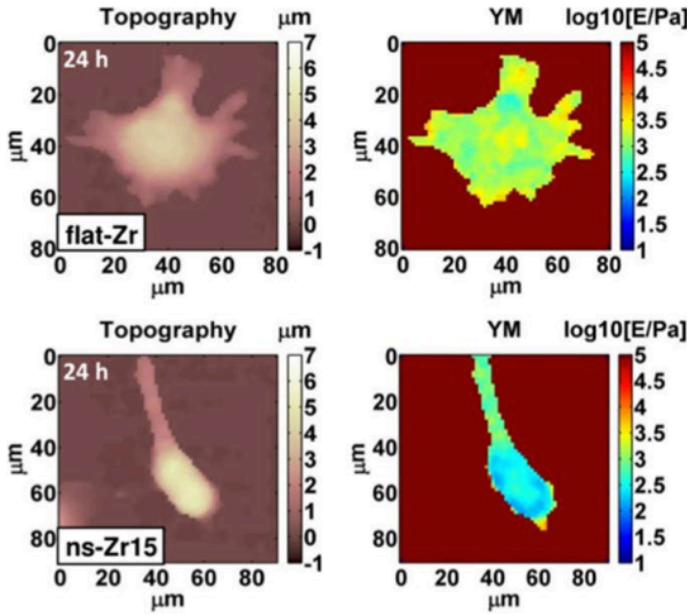

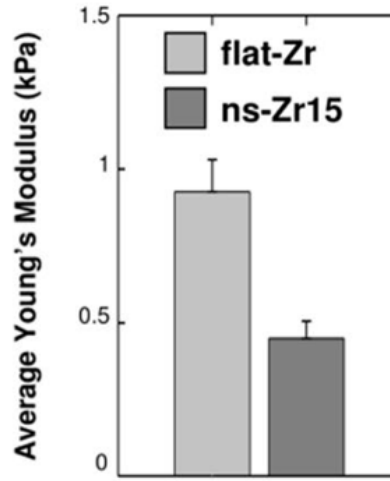

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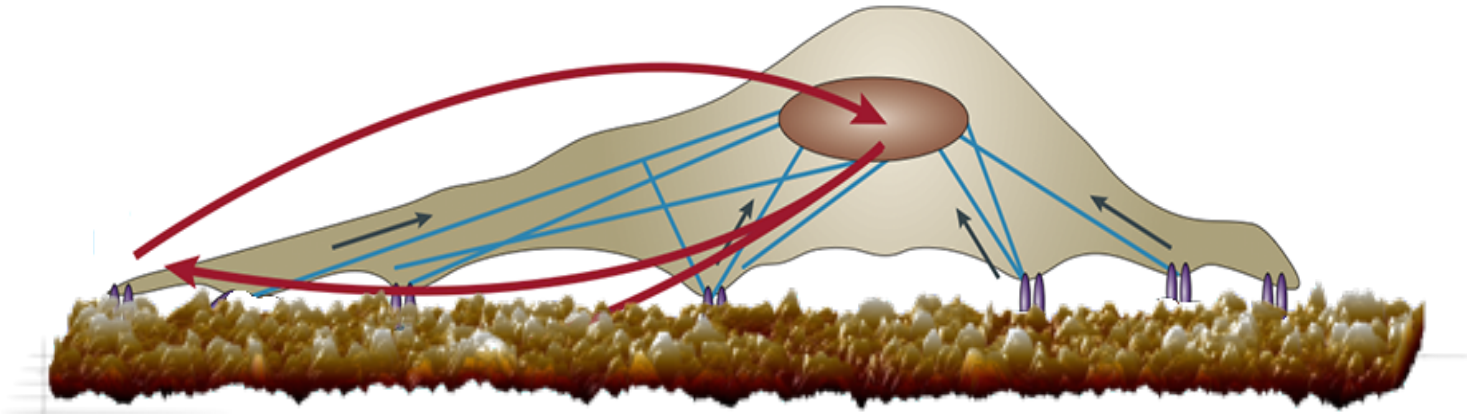
Study the step by step mechanical transmission of the external morphological stimulus through the whole path:

From **the interface** to **cell nucleus.**



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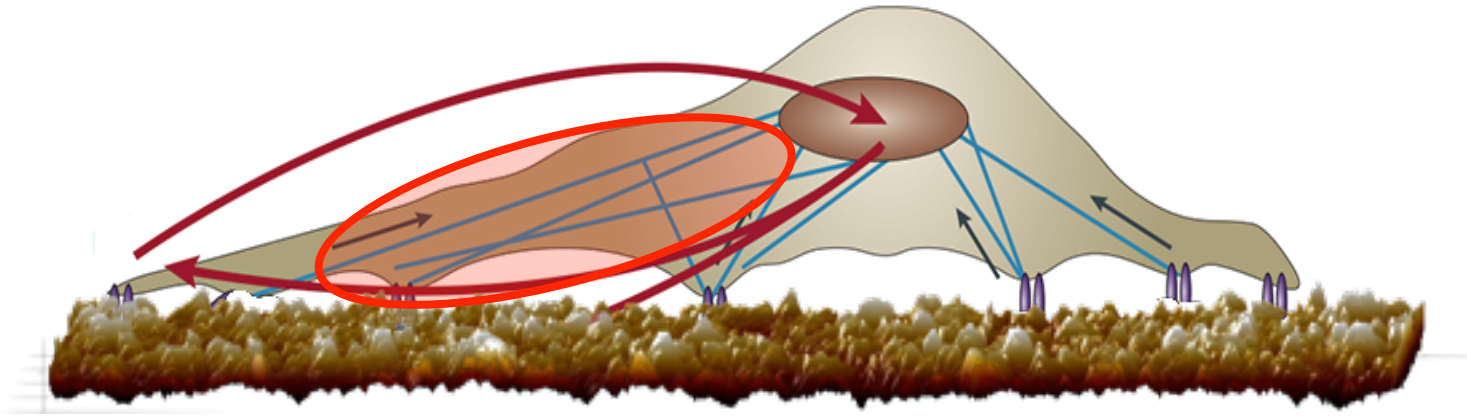
2 Cell **Nucleus**

3 Cell-Substrate **Interface**



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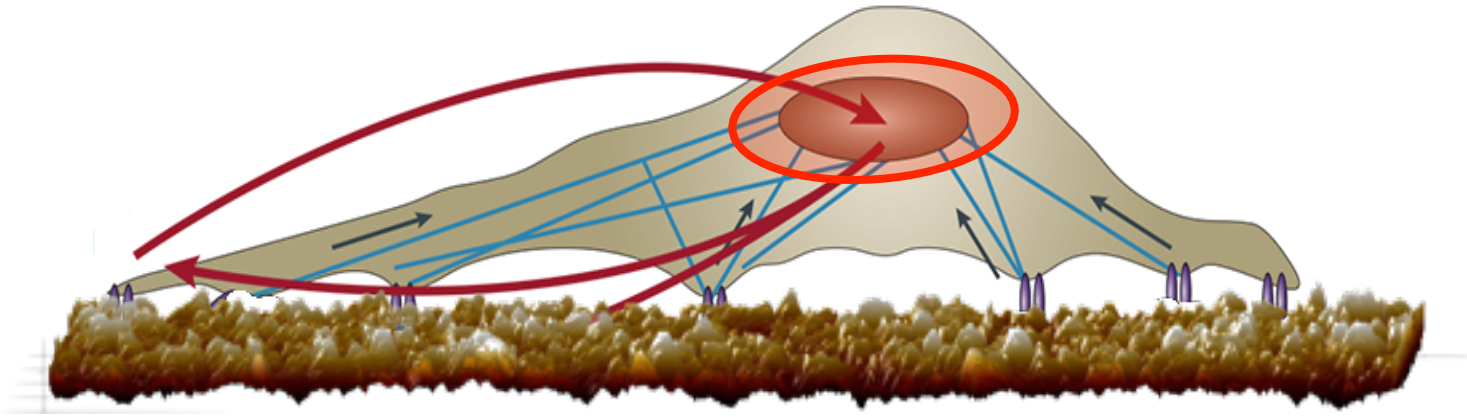

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- Witch are the adhesion condition (**size**, **distribution** and **strength** of the adhesion spots) of the cell that triggers the cellular differentiation.

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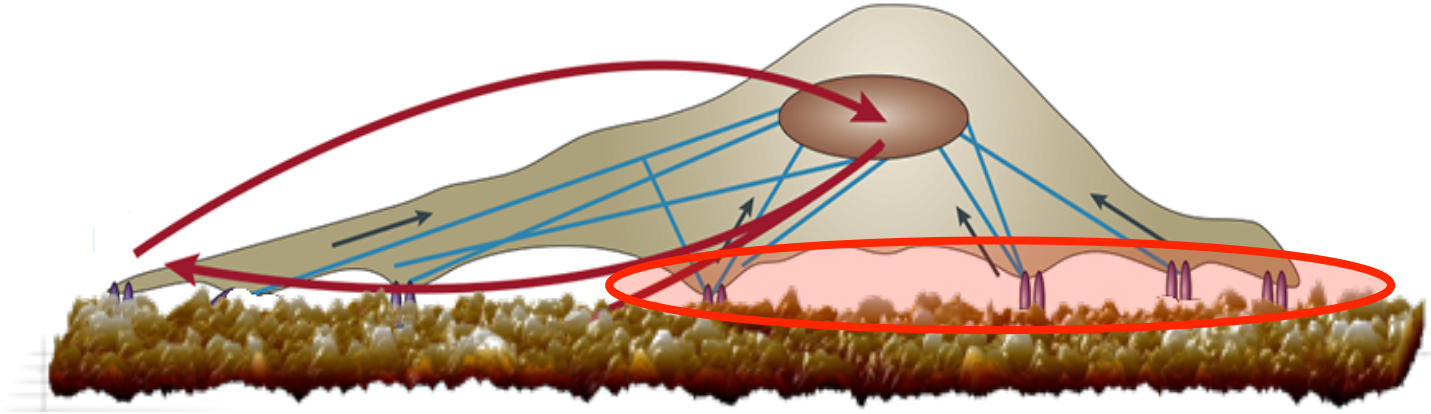
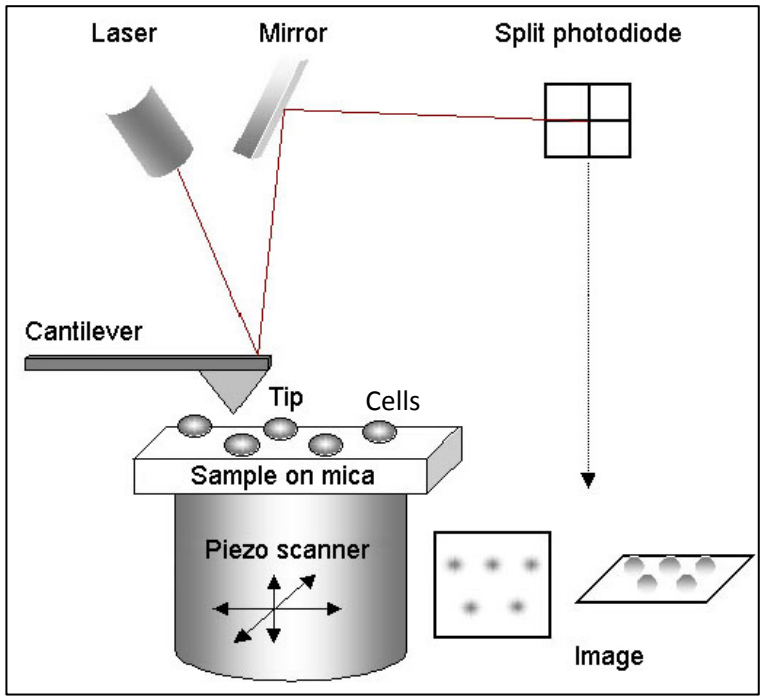
2 Cell **Nucleus**

3 Cell-Substrate **Interface**



Atomic Force Microscopy

- **AFM Imaging**



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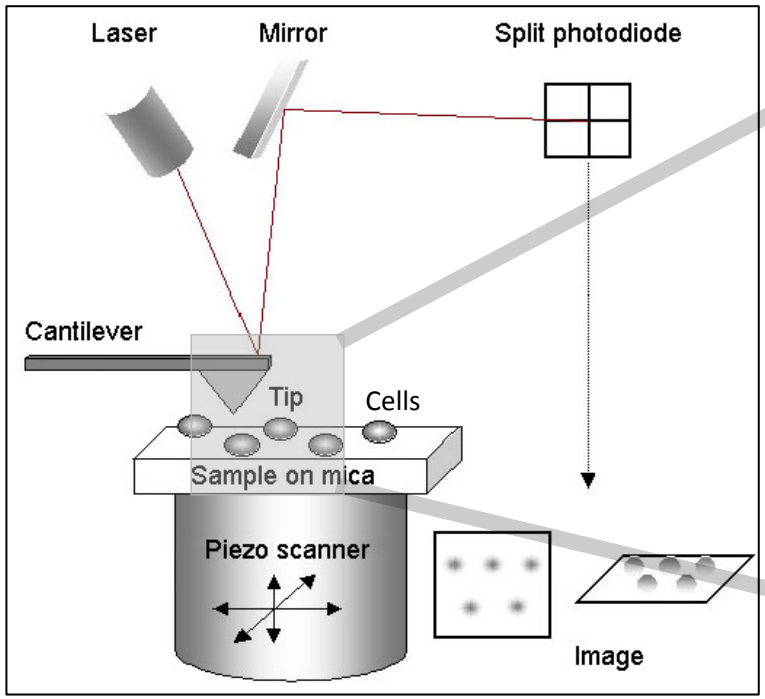

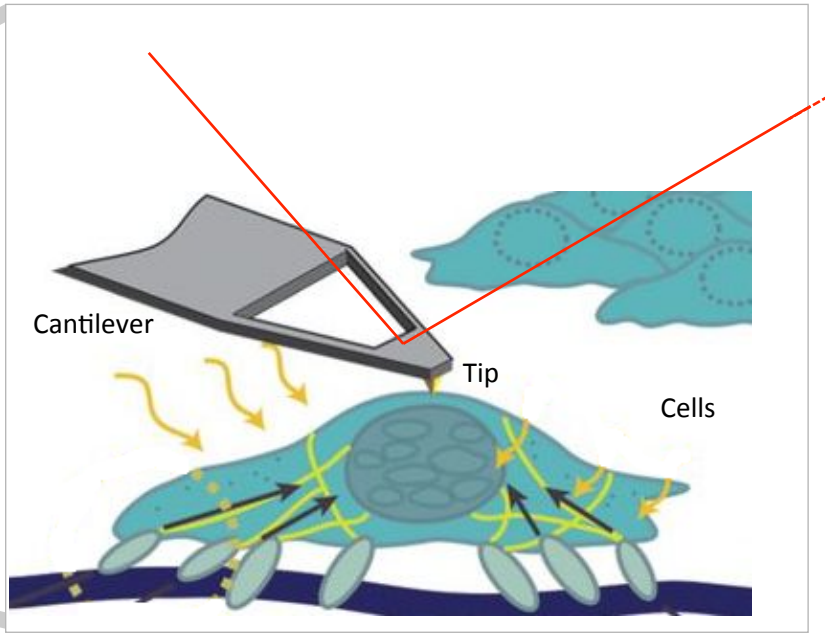

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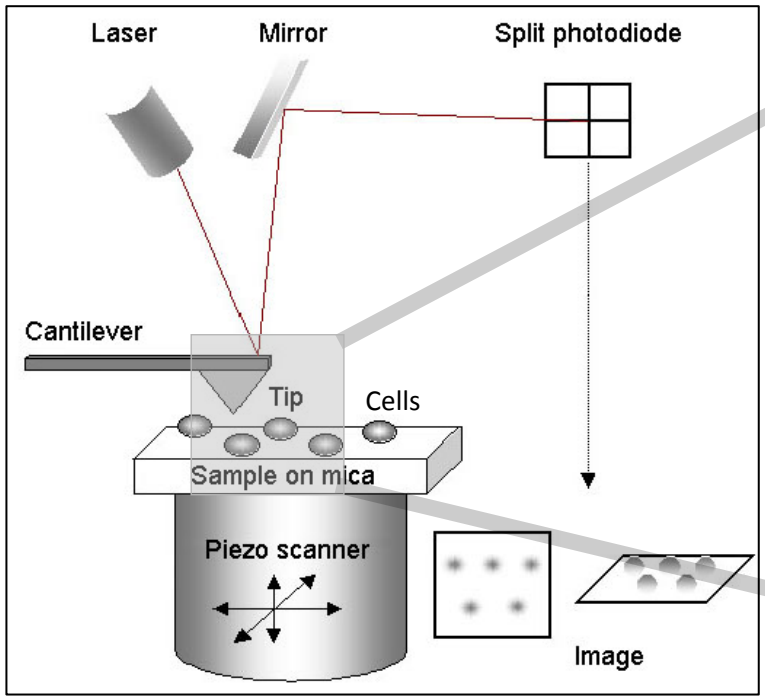
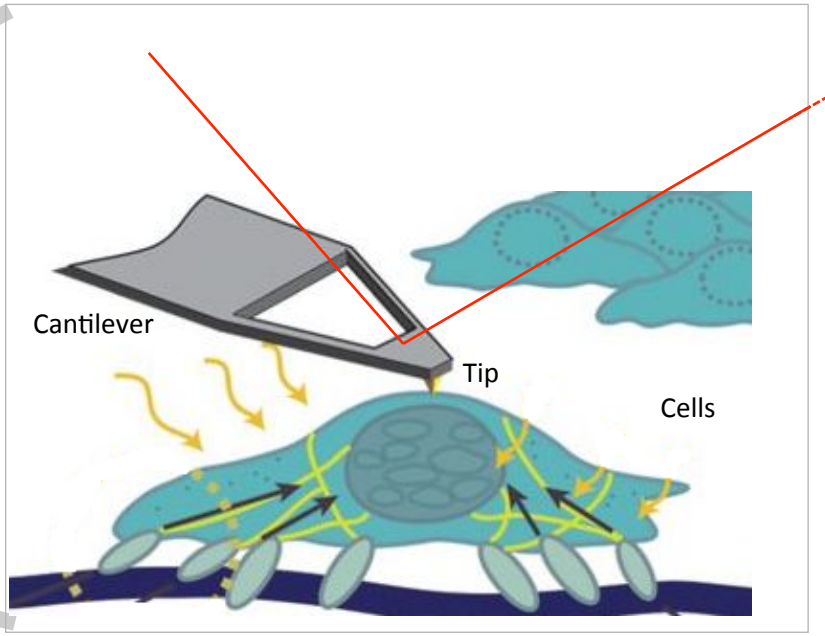
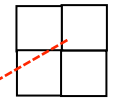
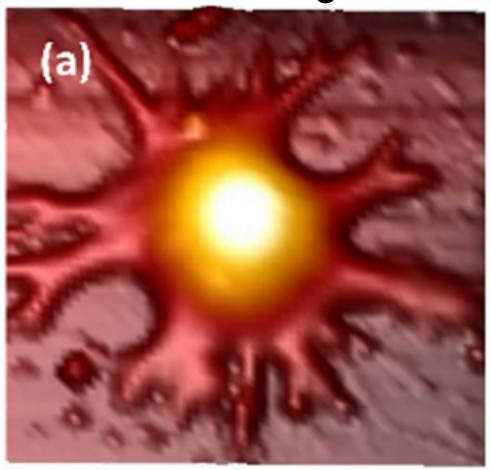






AFM Image



Living Cells

Atomic Force Microscopy

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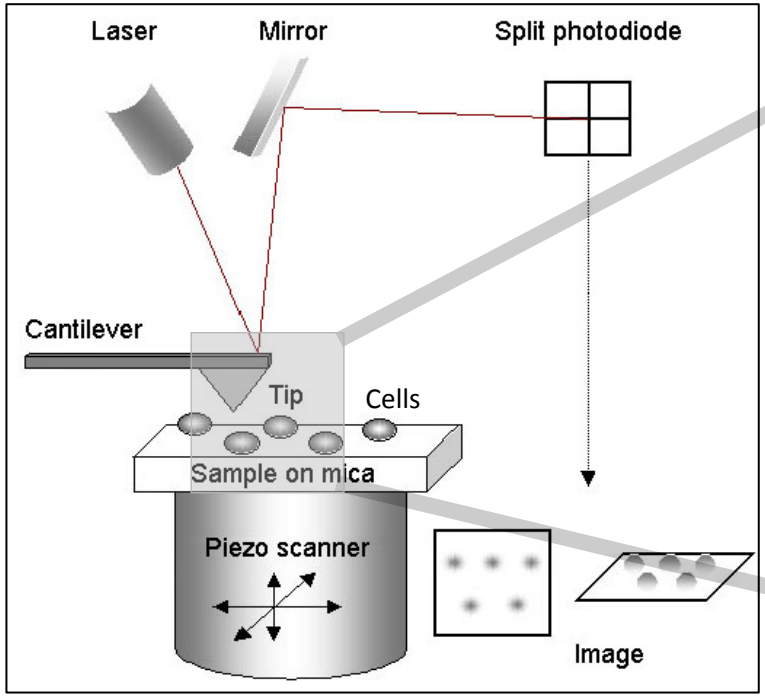

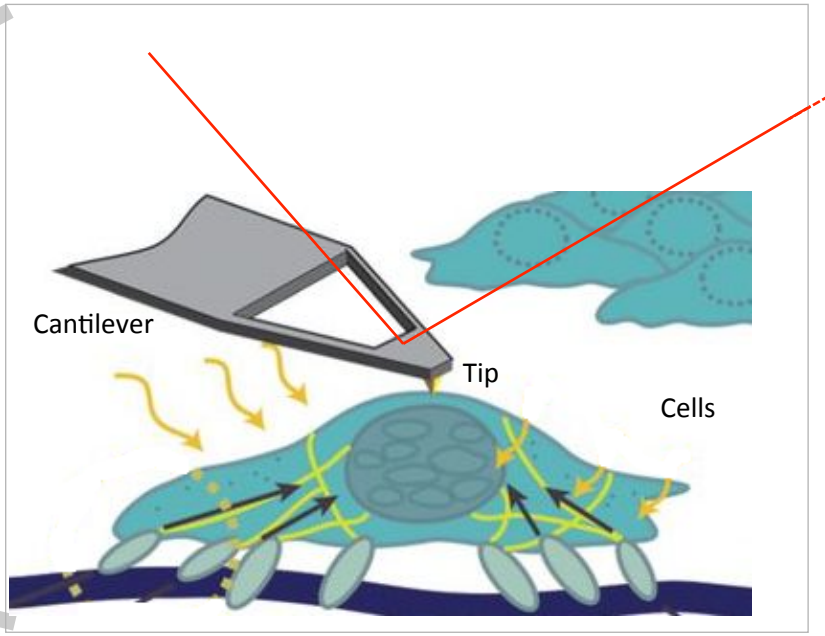

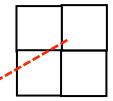

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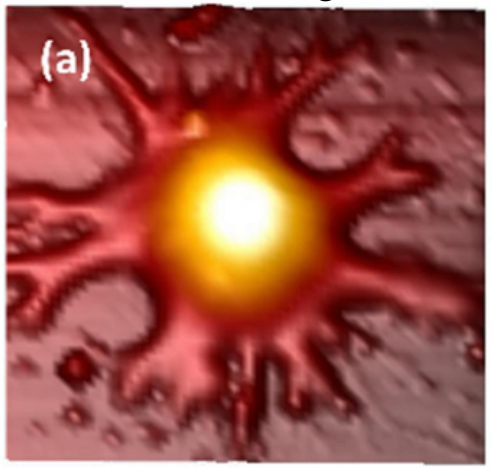
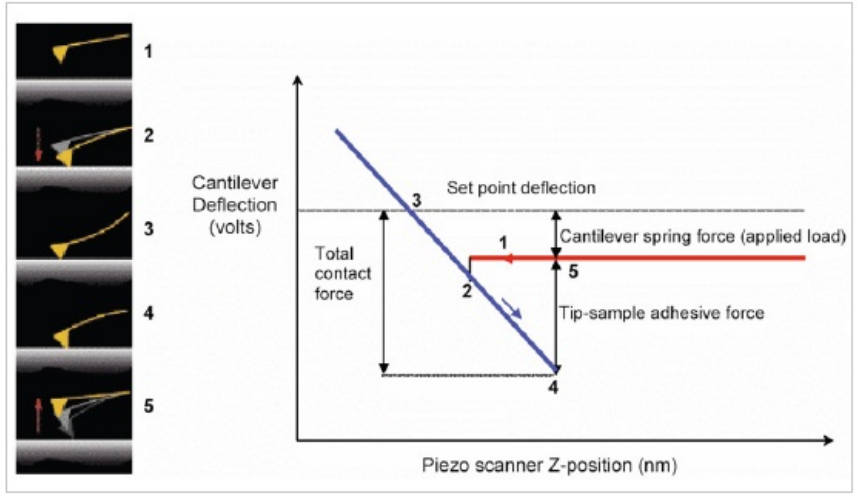


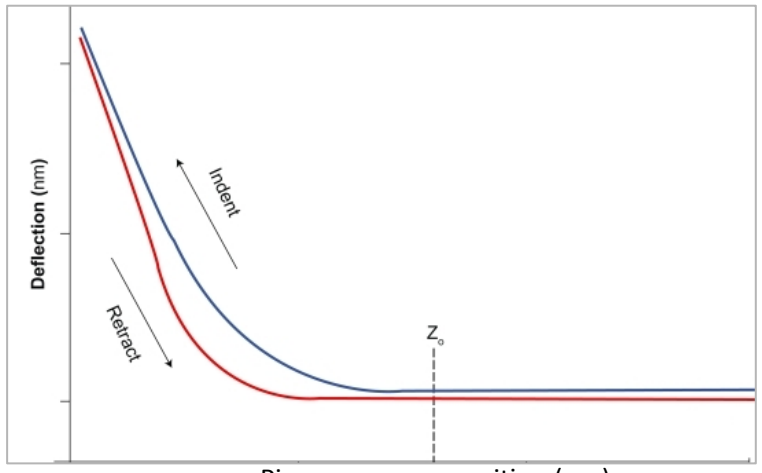
Living Cells

- **AFM Force Spectroscopy**

Hard Substrate

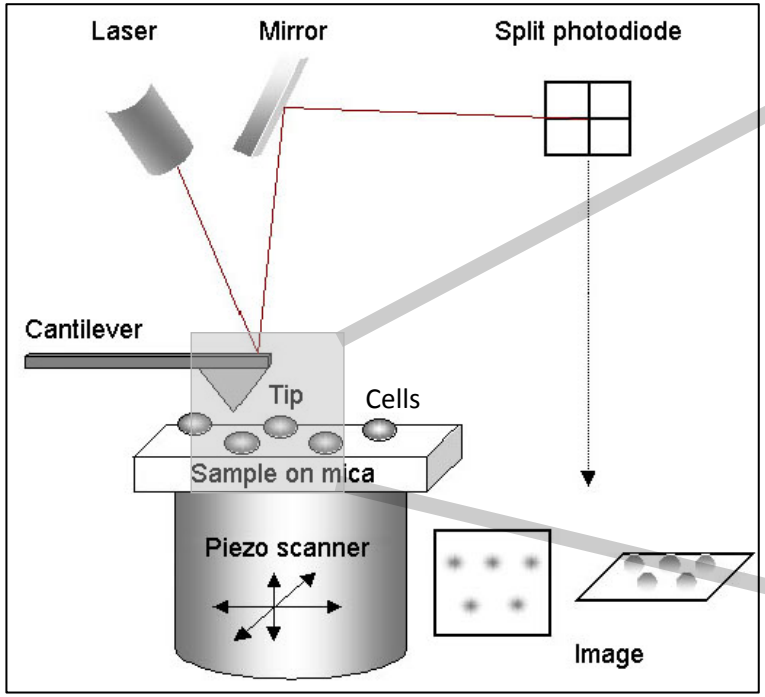


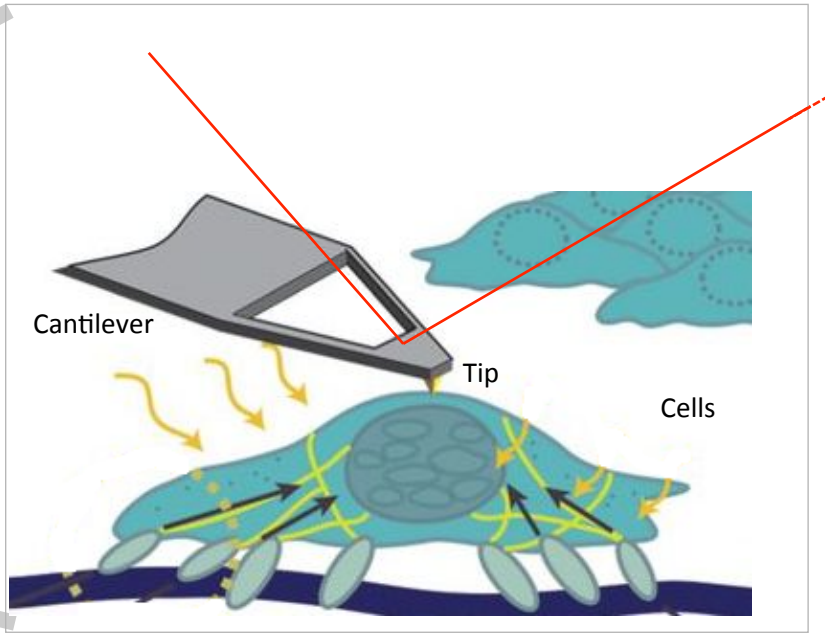
Soft Substrate

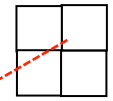


Atomic Force Microscopy

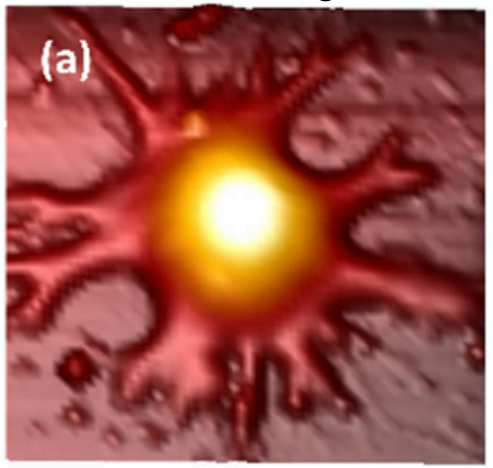
- **AFM Imaging**







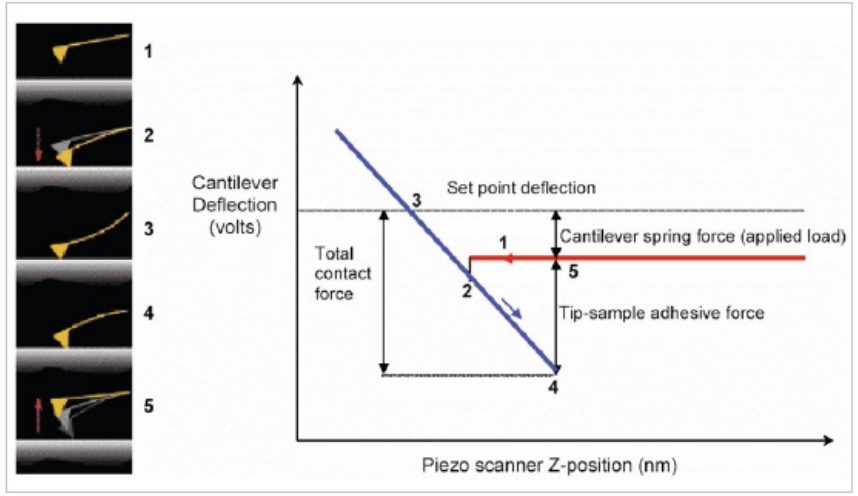
AFM Image



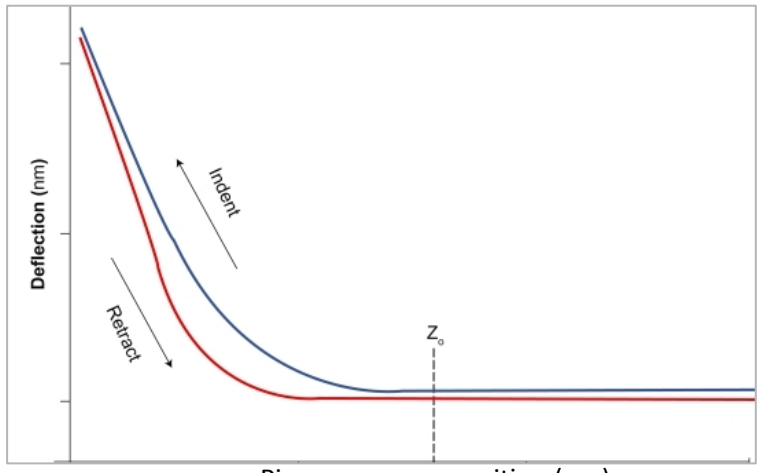
Living Cells

- **AFM Force Spectroscopy**

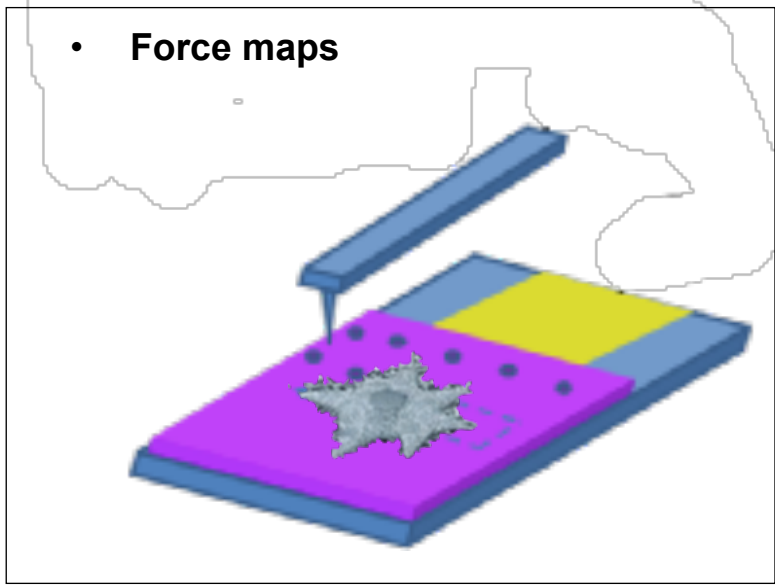
Hard Substrate



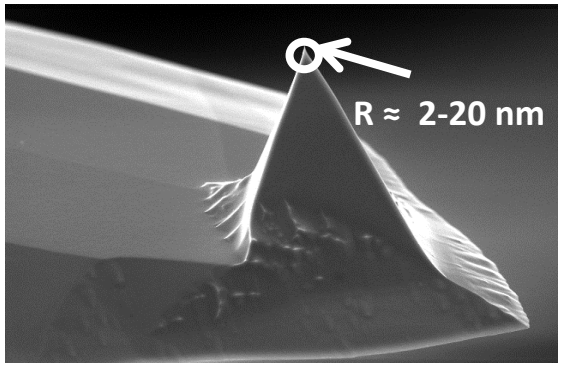
Soft Substrate



- **Force maps**

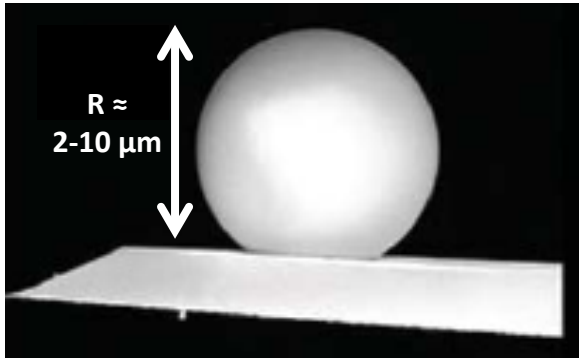


AFM Indentation Measurement

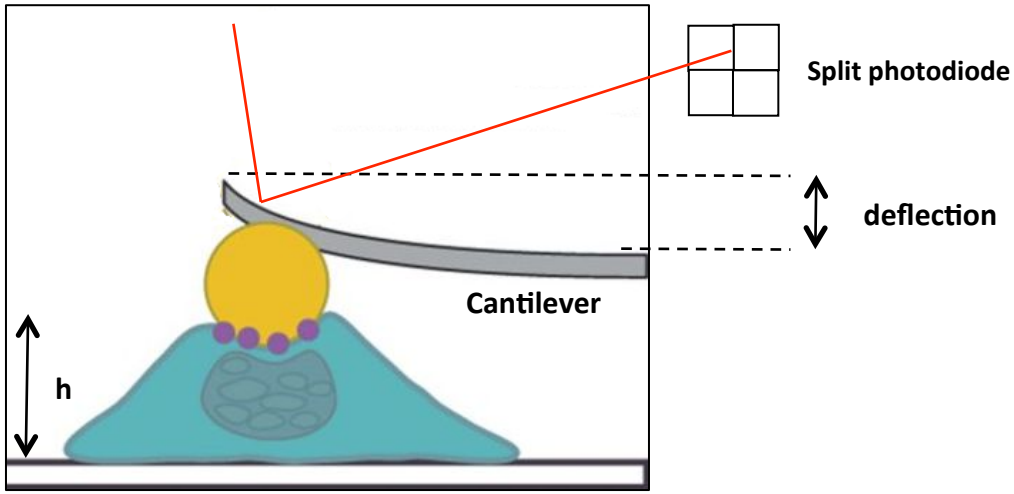


Sharp Probe

VS

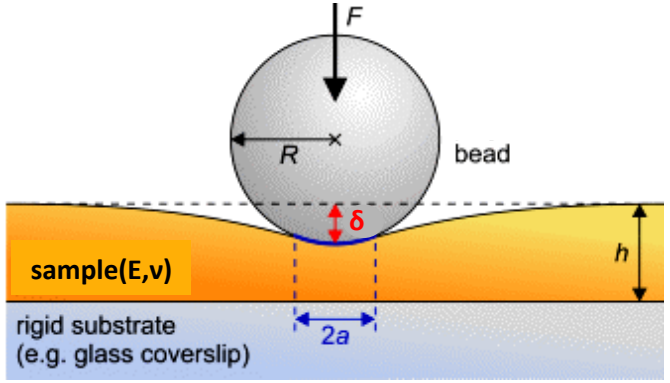


Colloidal Probe



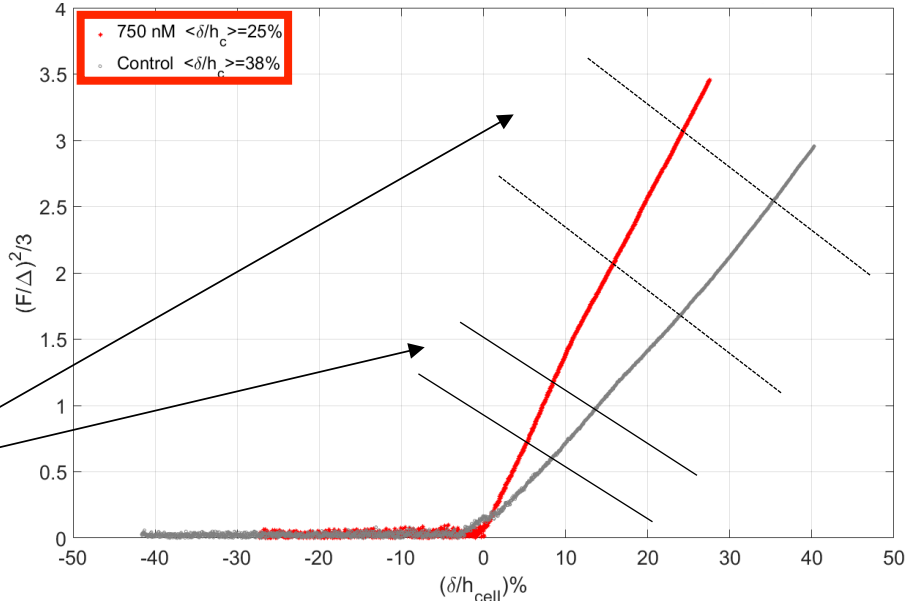
HERTZ model $$F = \frac{4}{3}\frac{E}{1-\nu^2}R^{\frac{1}{2}}\delta^{\frac{3}{2}}$$

F ... applied force
R ... radius of the probe
δ ... indentation of the sample
E ... elastic modulus
ν ... POISSON's ratio



$$E = \frac{stress}{strain} = \frac{F/A}{\Delta l/l_o}$$

Elastic Range measured respect to the cells height

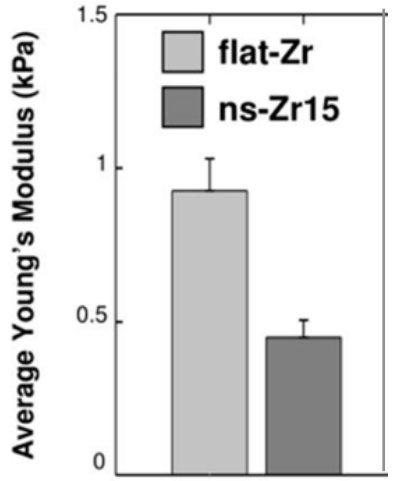


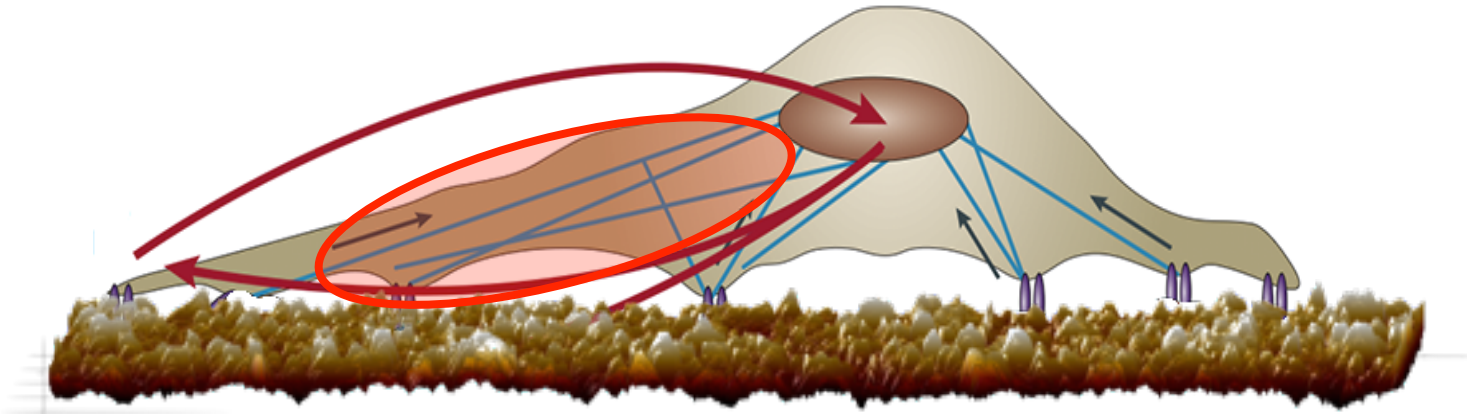
Two different elastic regimes are clearly visible.

Following the Mechano-Transductive Path

- The connection between mechanical properties of the cell and cytoskeletal organization.

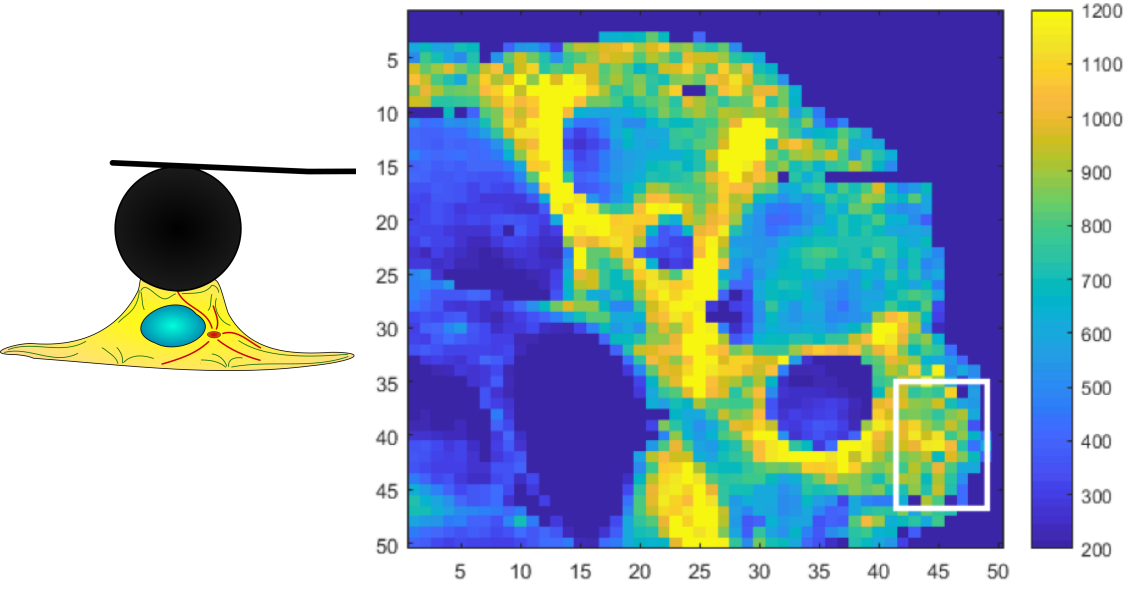
1. **Cytoskeleton**
2. Cell **Nucleus**
3. Cell-Substrate **Interface**





Cytoskeletal Organization

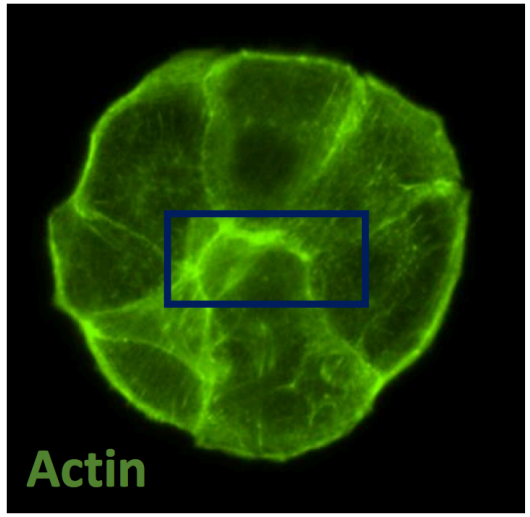
Elasticity map shallow indentation



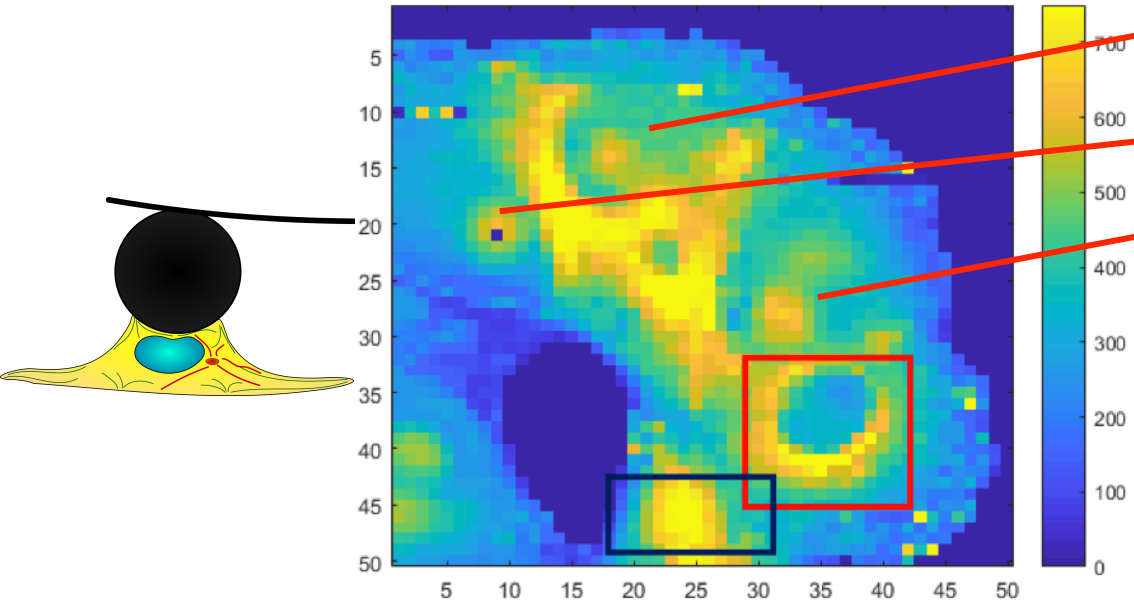
YM(Pa)

- **Du145 Cell Line**
- **Brain Metastases of Prostate Cancer**
- ***Prominent Cytoskeleton***

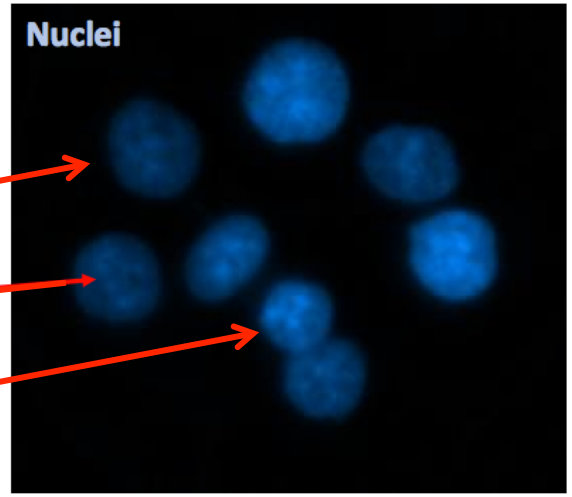
Flurescence Image

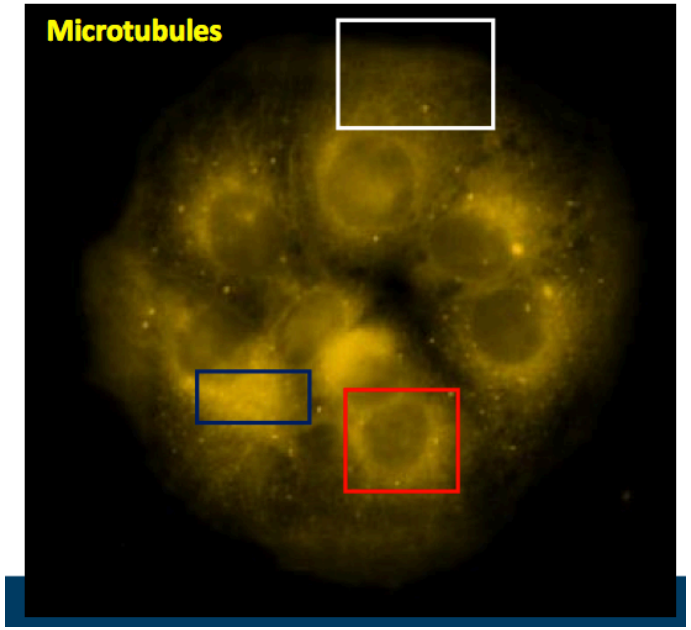


Elasticity map deep indentation



YM(Pa)

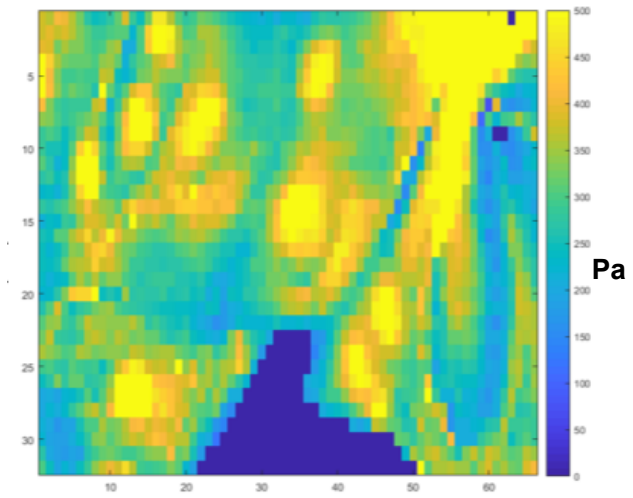


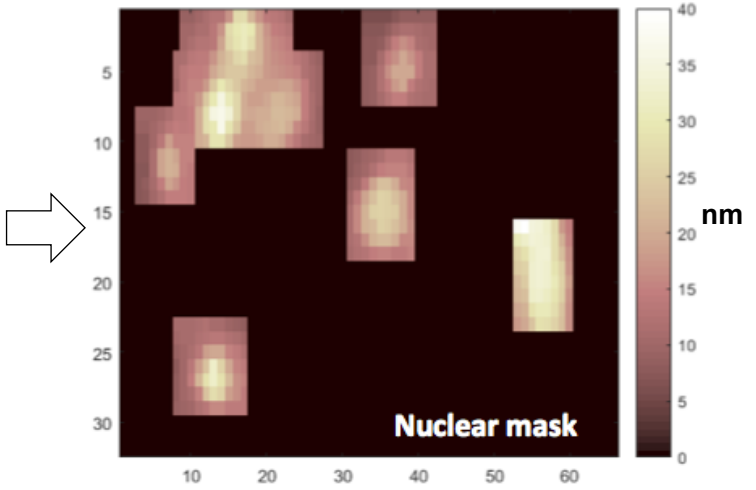


Cytoskeletal Organization

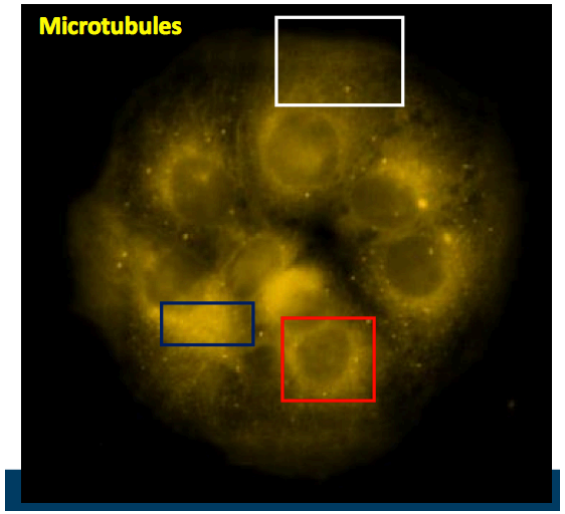
Du145 Cell Line
Treated with Vinflunine (VFL)
Anti-Cancer Drugs

Elasticity map

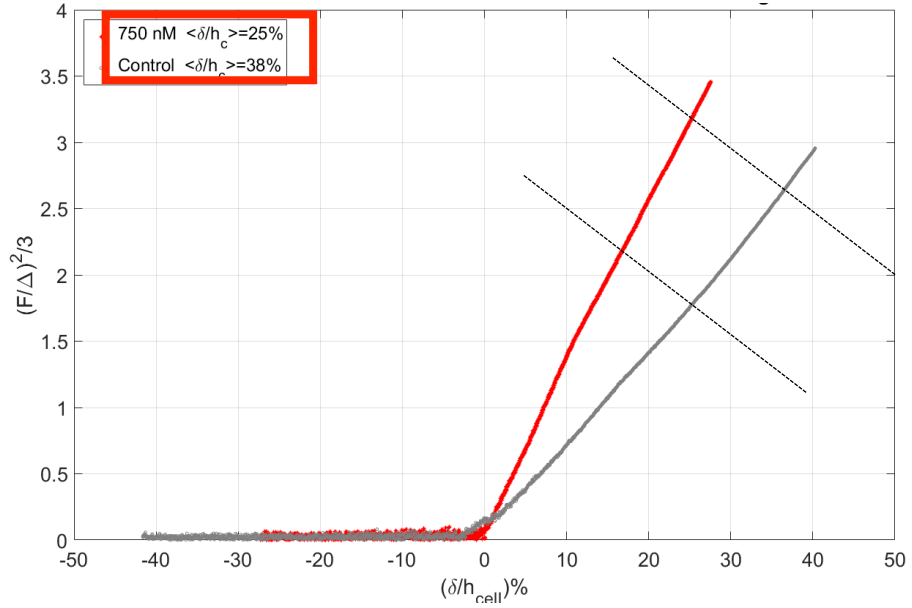


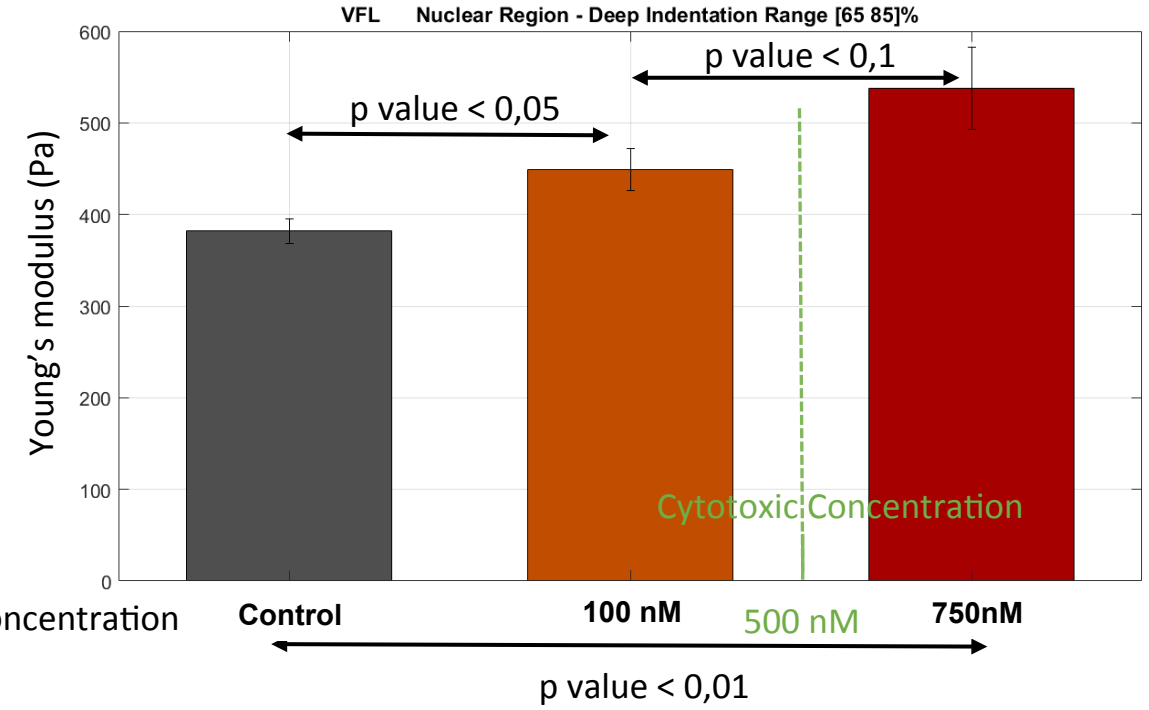


Flurescence Image



Elastic Range measured respect to the cells height





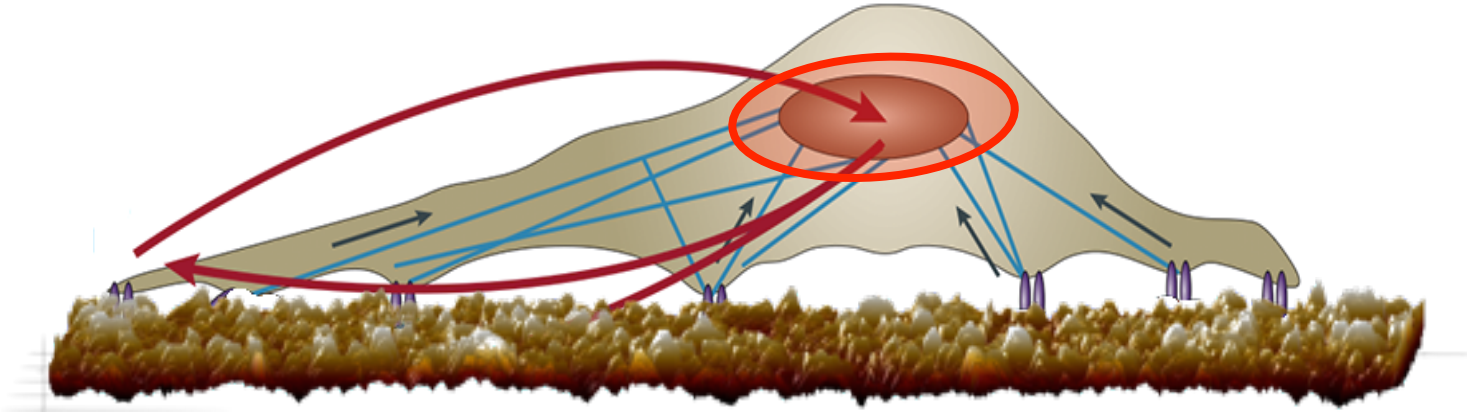
Following the Mechano-Transductive Path

Phd Project Objective:

Study the step by step mechanism through witch cells are capable to convert an external and **mechanical** stimulus into a **biological** reaction, tuning the **cell's fate**.

- How the cytoskeleton modulates then the nuclear Architecture.

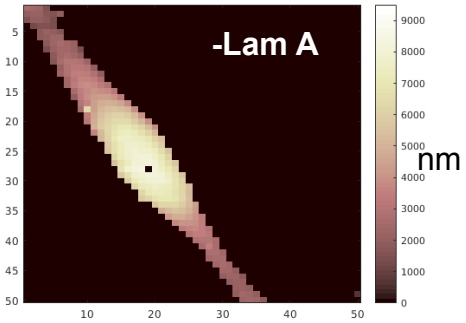
1. **Cytoskeleton**
2. Cell **Nucleus**
3. Cell-Substrate **Interface**

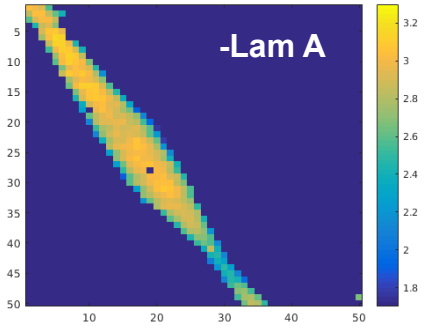


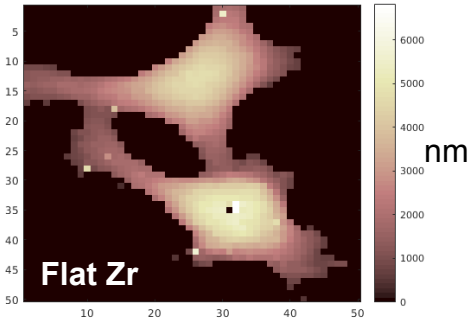
Cytoskeletal organization influence the Nuclear Architecture

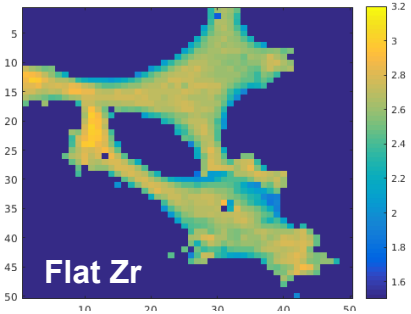
Reconstructed Cell Topography

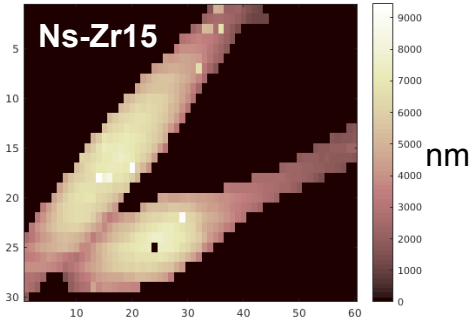
Elasticity map

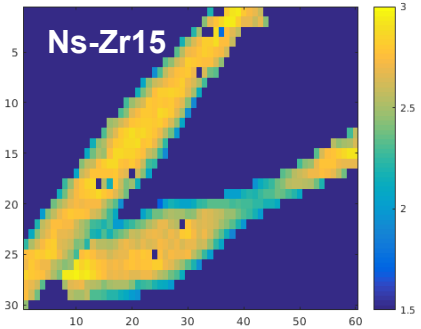




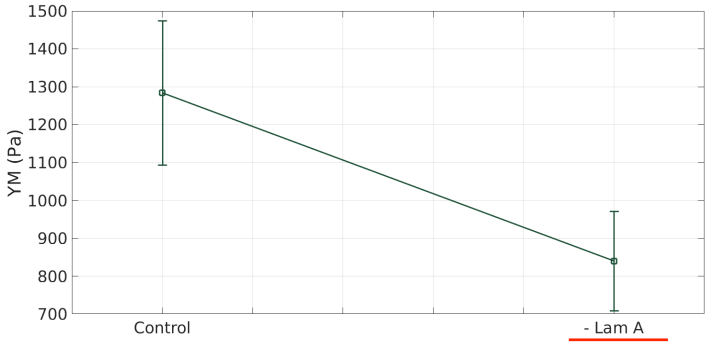


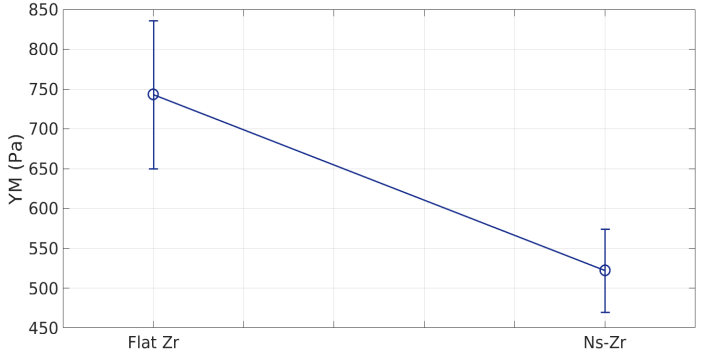


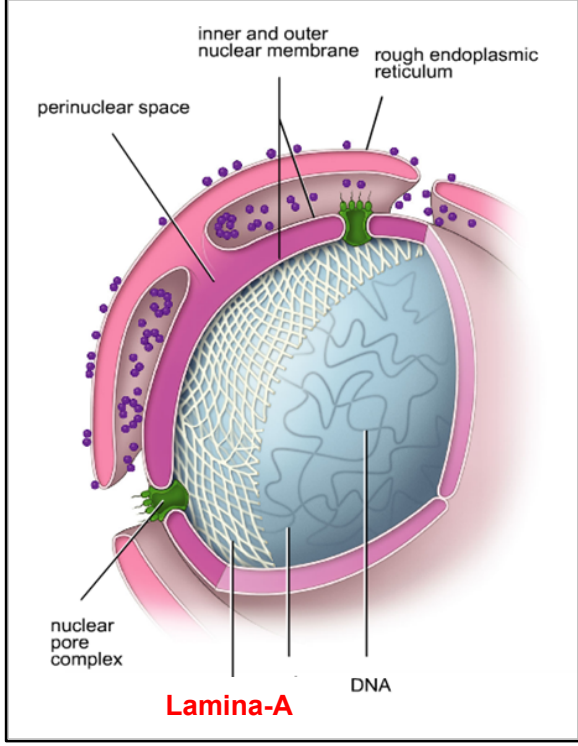




Young's Modulus of Cell Nucleus







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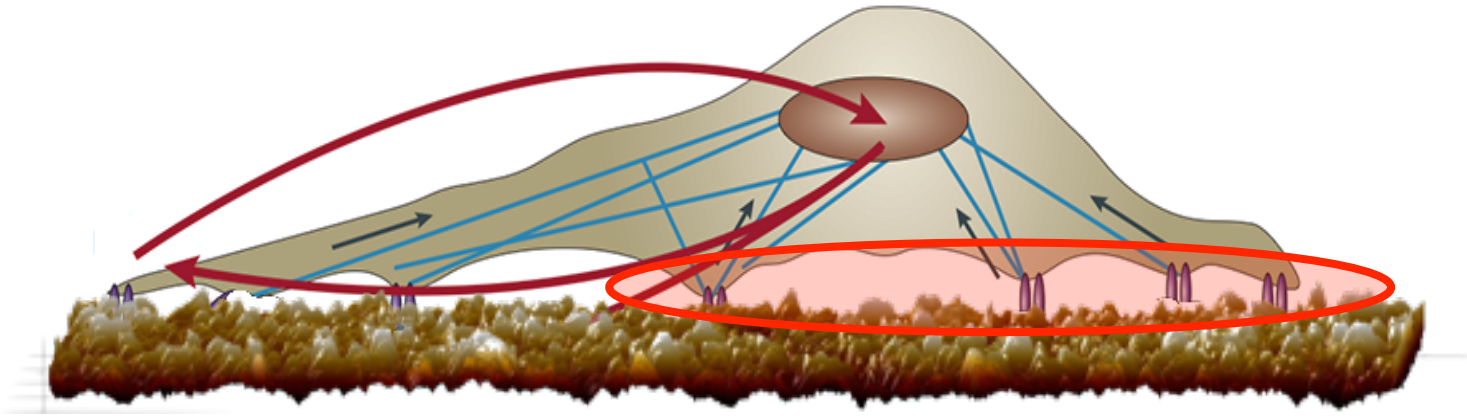
Study the step by step mechanism through witch cells are capable to convert an external and **mechanical** stimulus into a **biological** reaction, tuning the **cell's fate**.

1. **Cytoskeleton**
2. Cell **Nucleus**
3. Cell-Substrate **Interface**

- Witch are the adhesion condition of the cell that triggers the cellular differentiation.

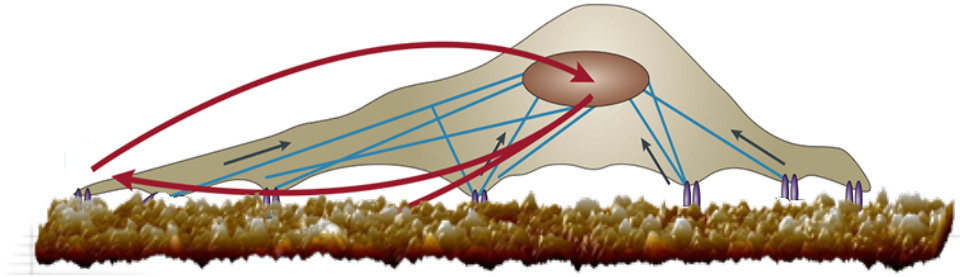


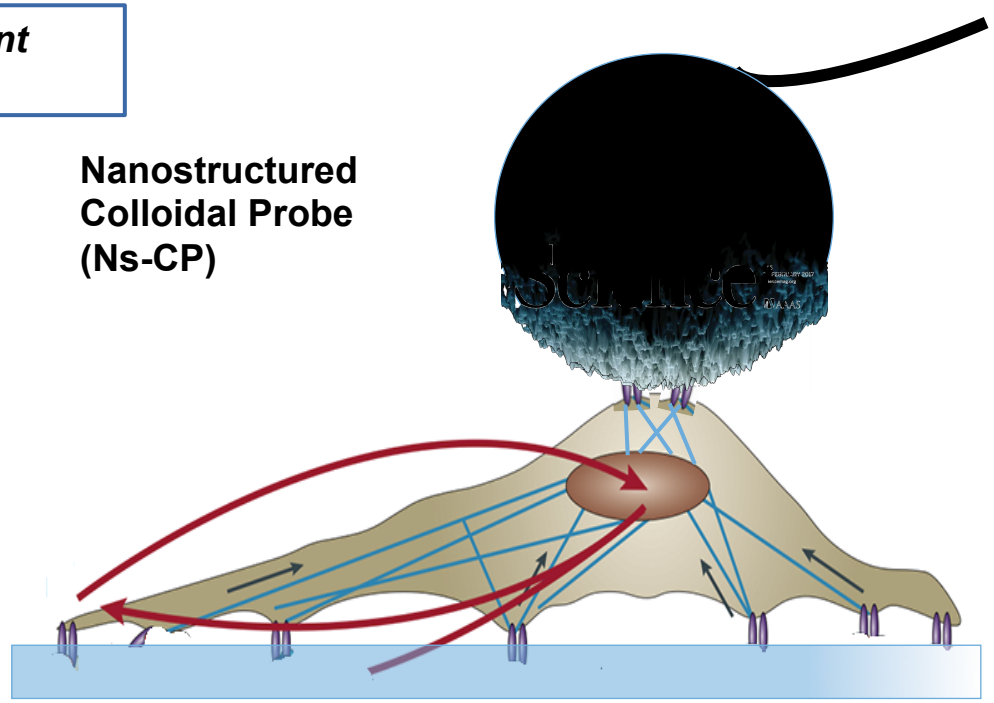
Membrane-Microenvironment Interface

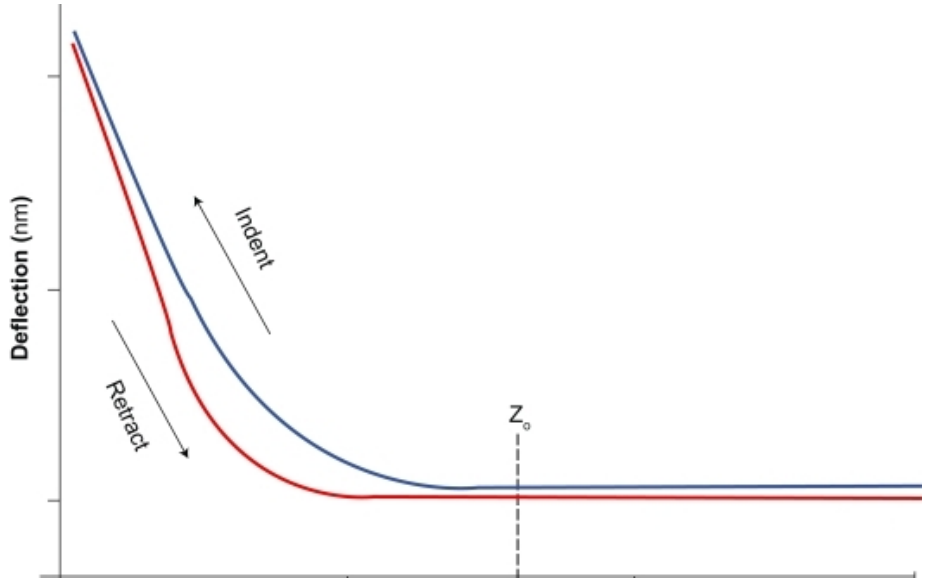
- **Microscopic investigation of cell adhesion:**

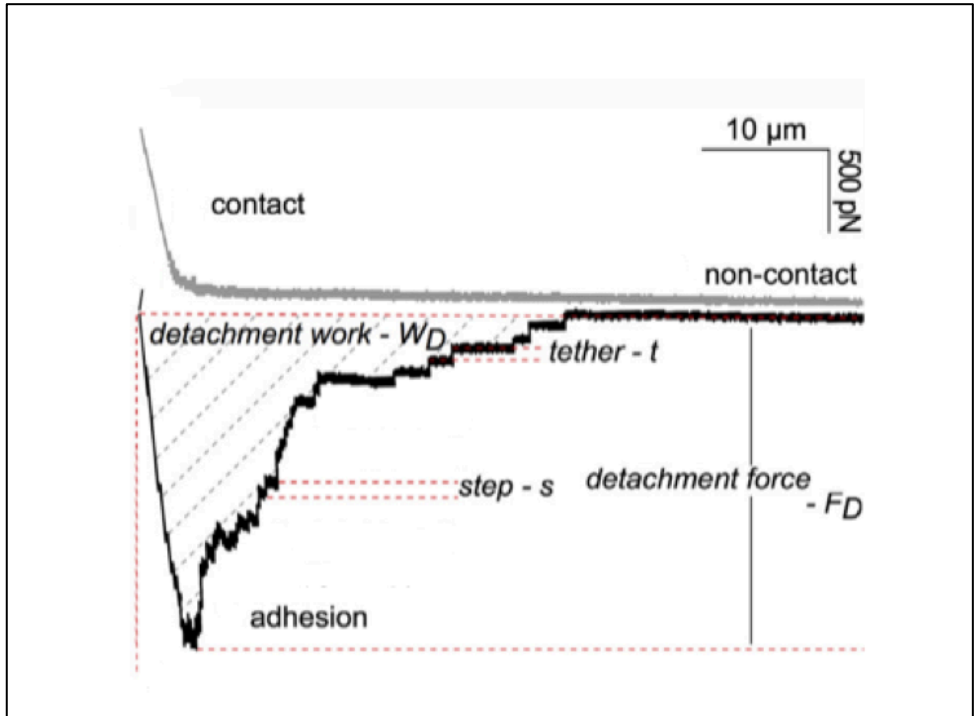
AFM FORCE SPECTROSCOPY

Nanostructured Colloidal Probe (Ns-CP)





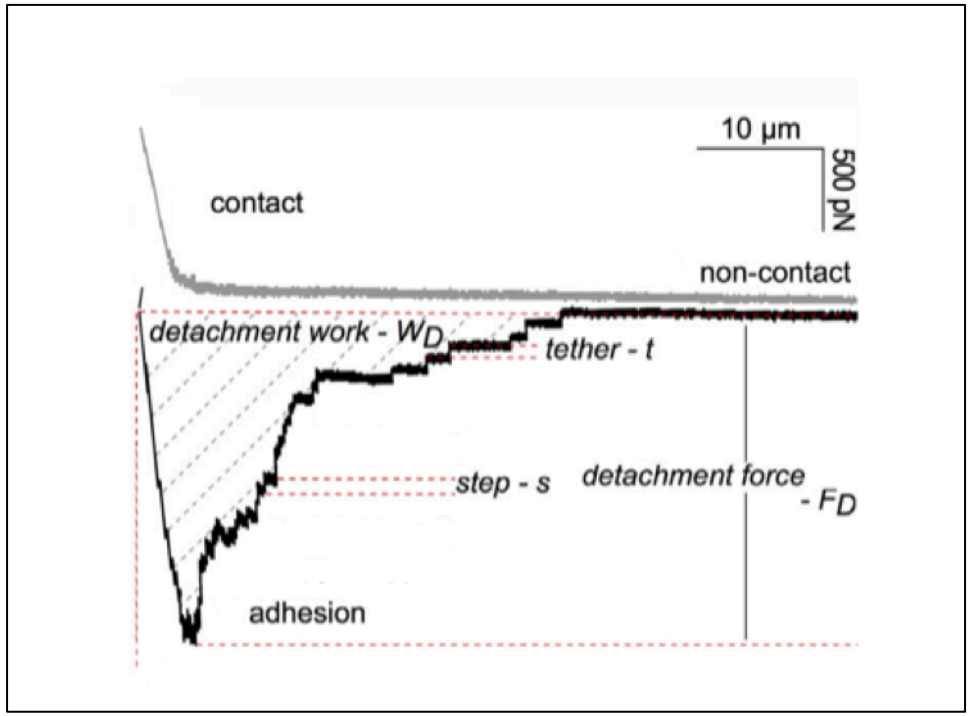




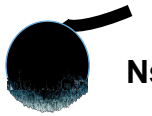
Membrane-Microenvironment Interface

- **Microscopic investigation of cell adhesion:**

RESUSLTS



Flat Probe

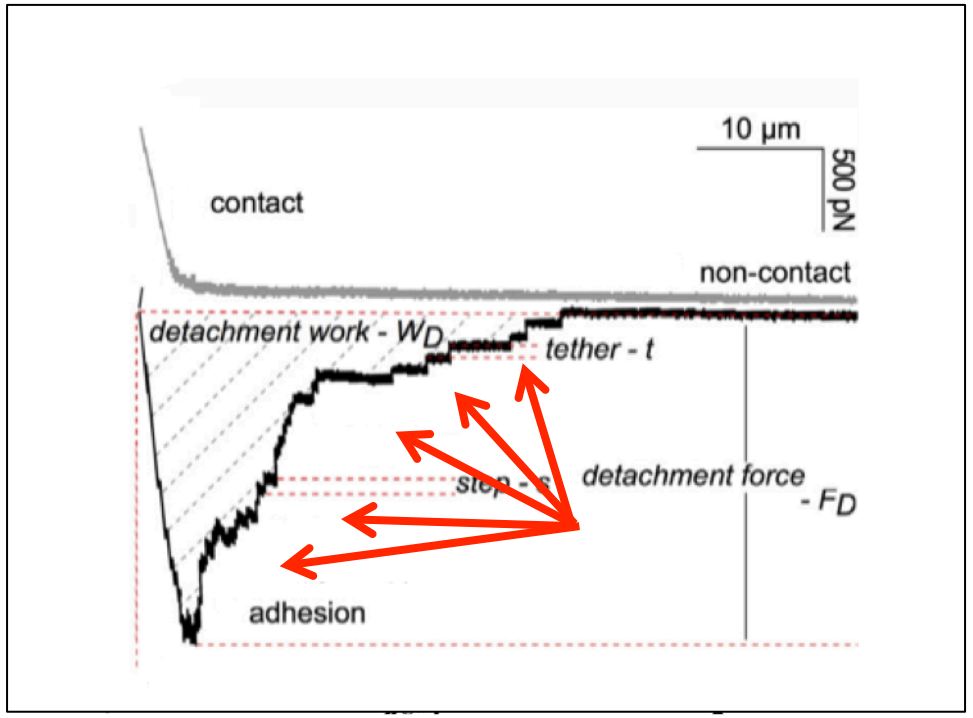


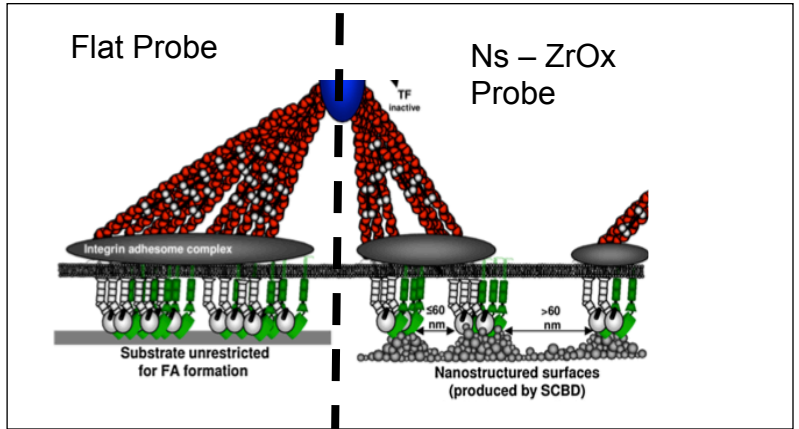
Ns-ZrOxProbe

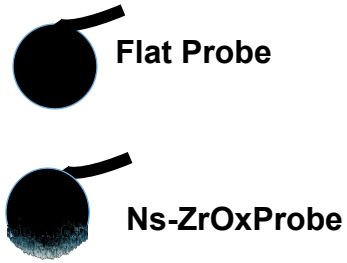
Membrane-Microenvironment Interface

- **Microscopic investigation of cell adhesion:**

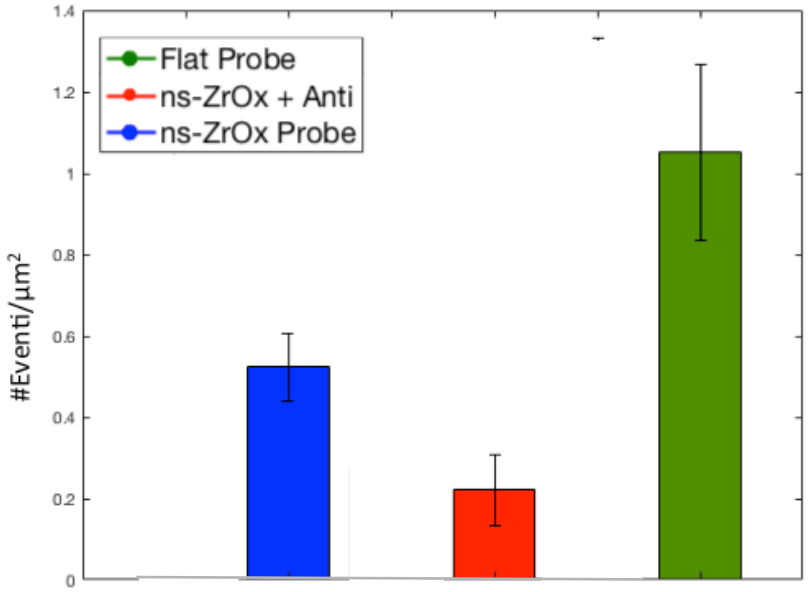
RESUSLTS







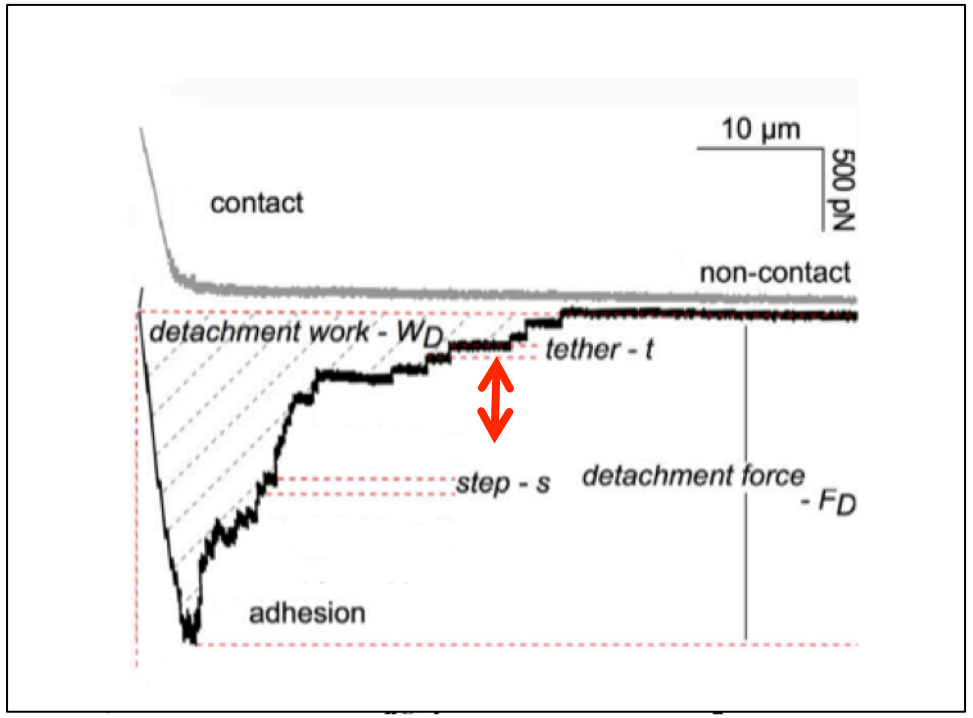
Density of unbinding events

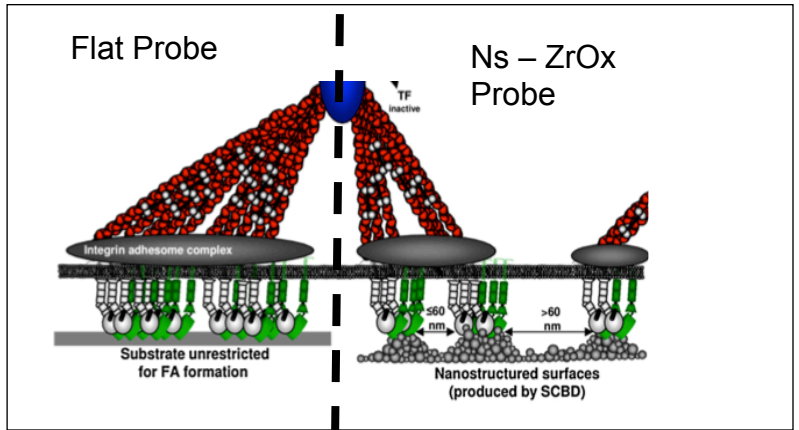


Membrane-Microenvironment Interface

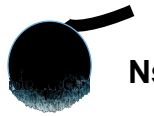
- **Microscopic investigation of cell adhesion:**

RESUSLTS



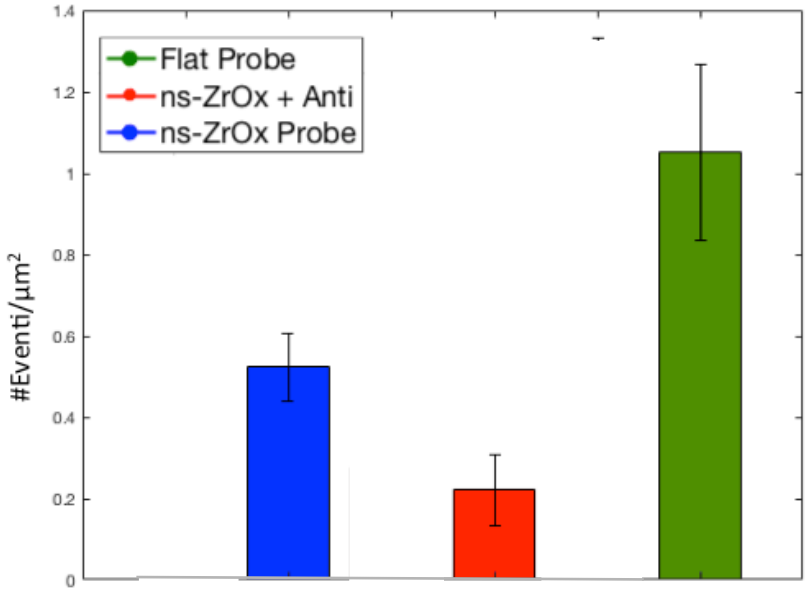


Flat Probe

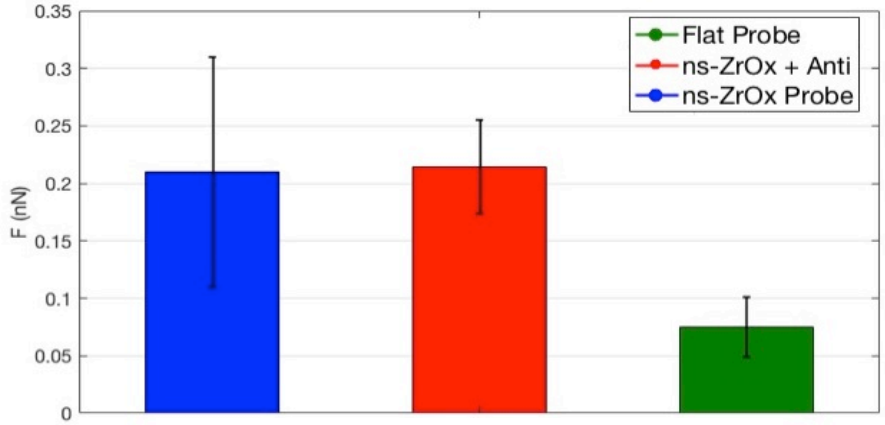


Ns-ZrOxProbe

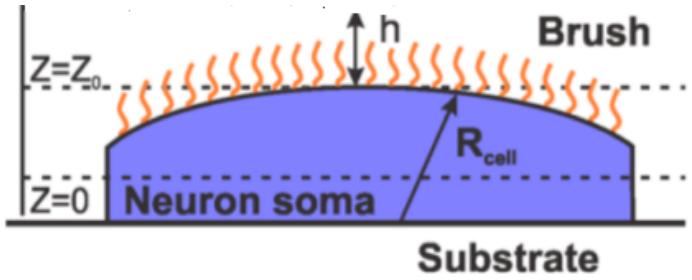
Density of unbinding events

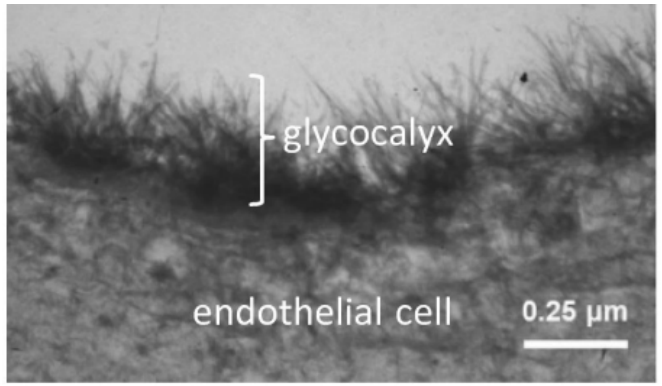


Force of unbinding events

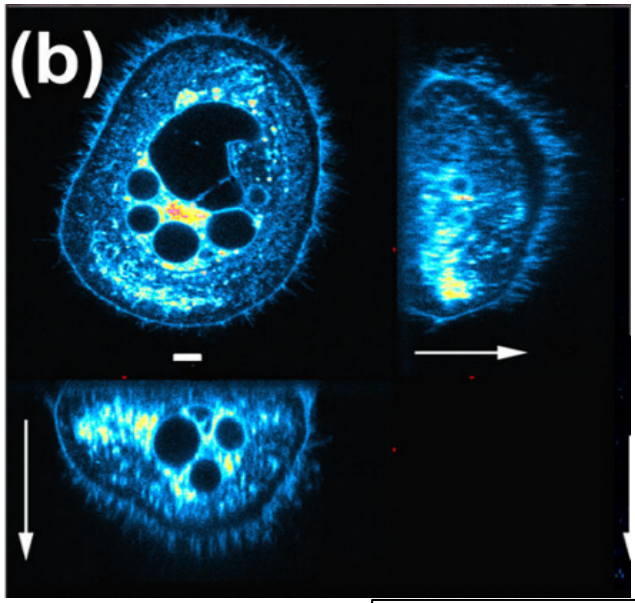


The Surface cellular Brush
(Glycocalyx)



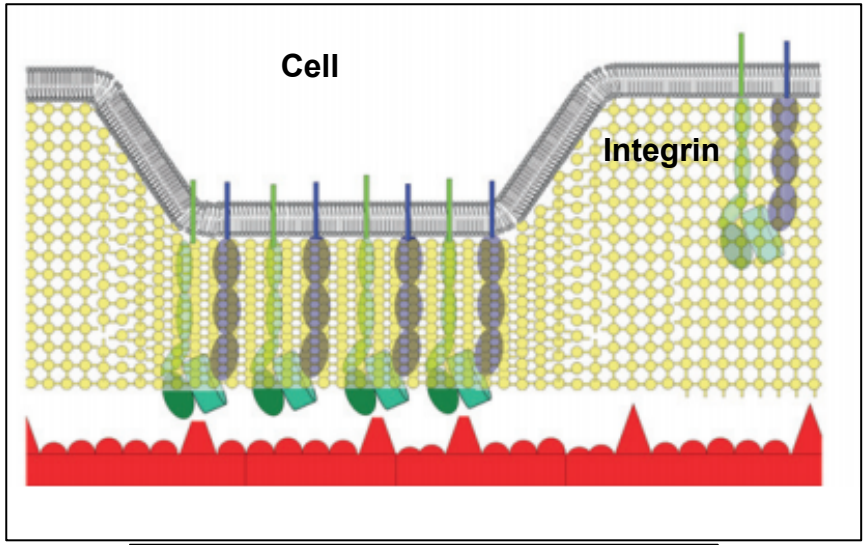


Wiesinger et al. *PLOS ONE.* **8,** (2011)

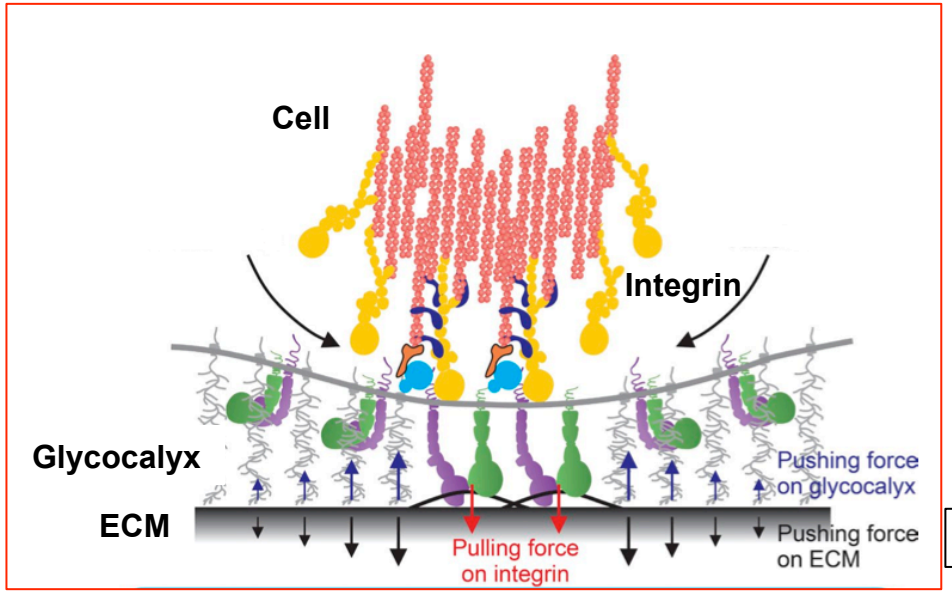


Sokolov et al. *Appl. Phys. Lett.* **91,** (2007)

- **Glycocalyx mechanically prime <u>integrin Clustering</u>**



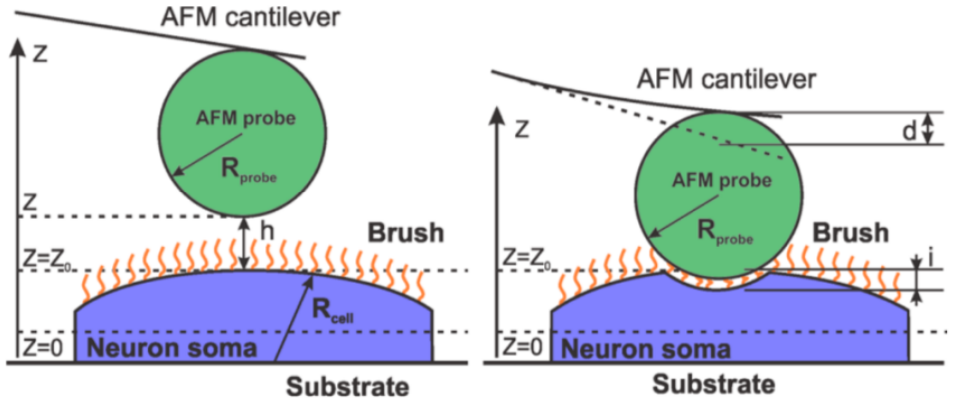
Boettinger. *Cell Biol.* **24,** 592-599, (2012)



Paszek, *Nature.* **511,** (2014)

The Surface cellular Brush
(Glycocalyx)

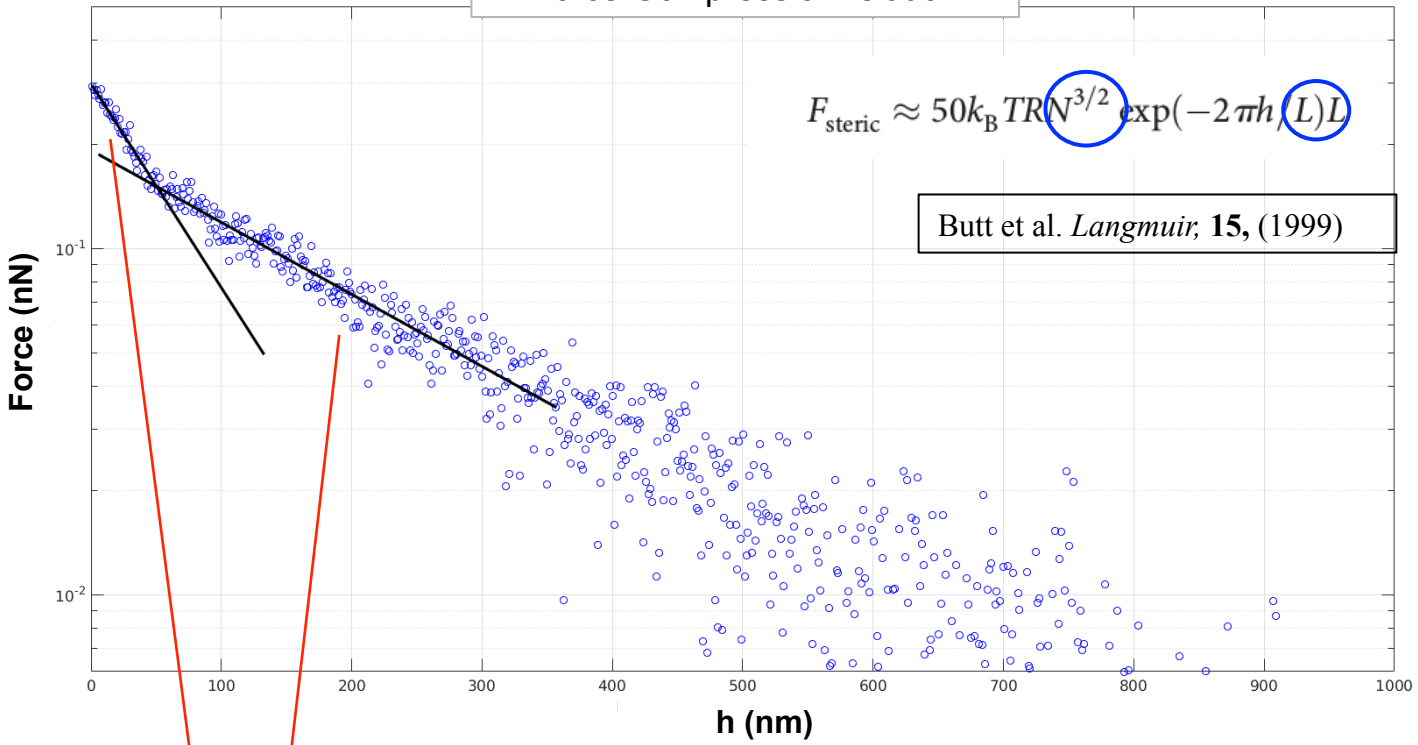
- **Lenght and Density of Glycocalyx in PC12 Cell line**



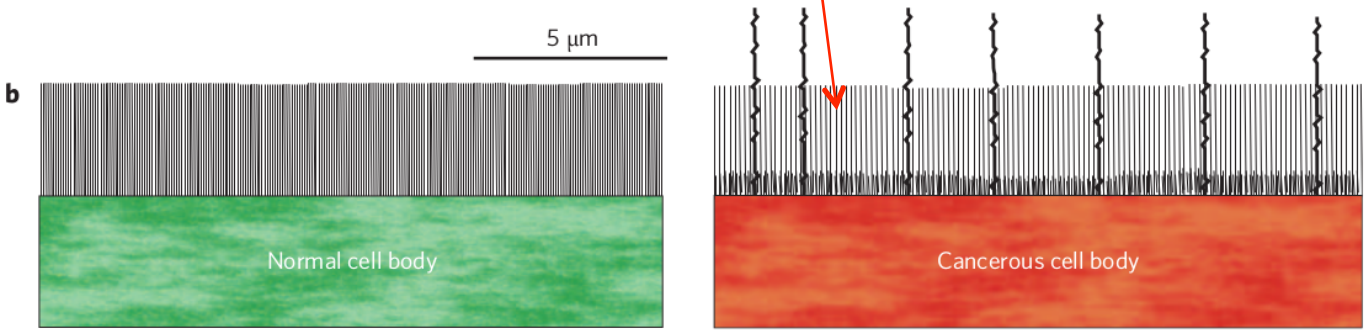
$$h = Z - Z_0 + \left[\frac{3k(1-\nu^2)}{4E}\left(\frac{R_{probe}+R_{cell}}{R_{probe}R_{cell}}\right)^{1/2}\right]^{2/3} d^{2/3} + d$$

Sokolov et al. *App. Phys. Lett.* **91,** (2007)

Force-Compression relation



Butt et al. *Langmuir,* **15,** (1999)



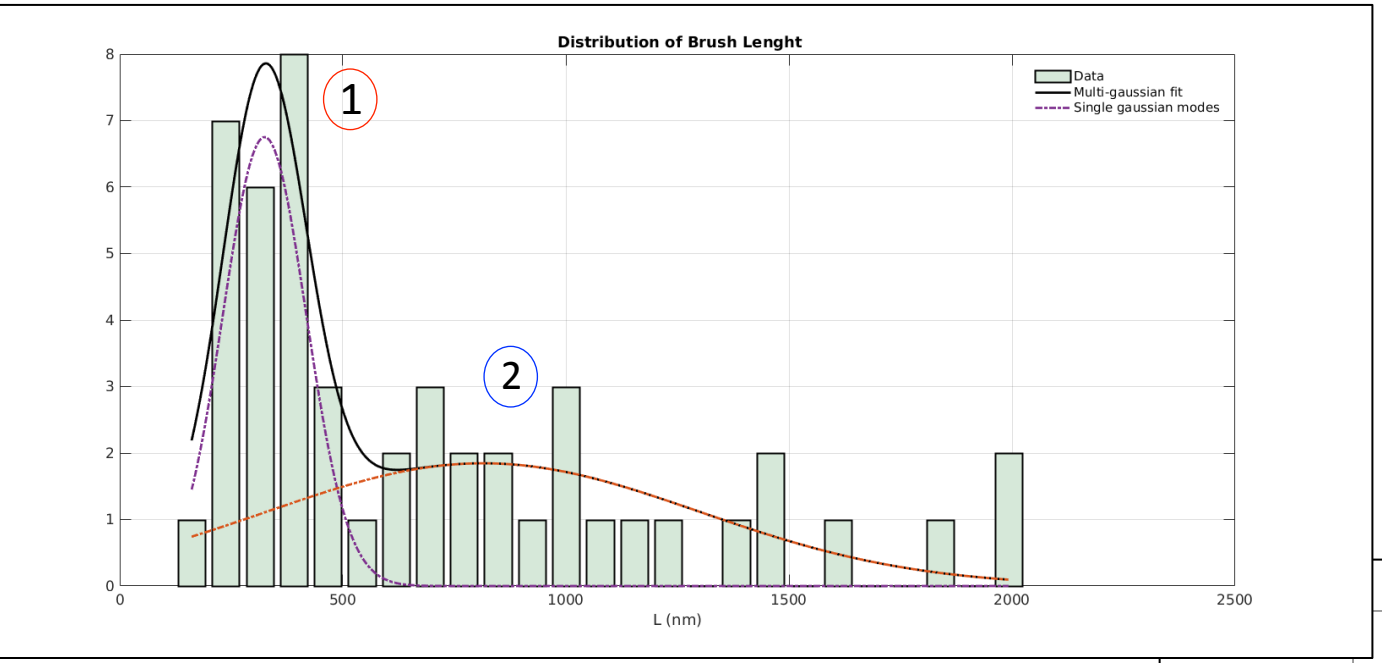
Iyer et al. *Nature Tech.* **4,** (2009)

The Surface cellular Brush
(Glycocalyx)

$$F_{steric} \approx 50 k_B T R N^{3/2} \exp(-2\pi h/L) L$$

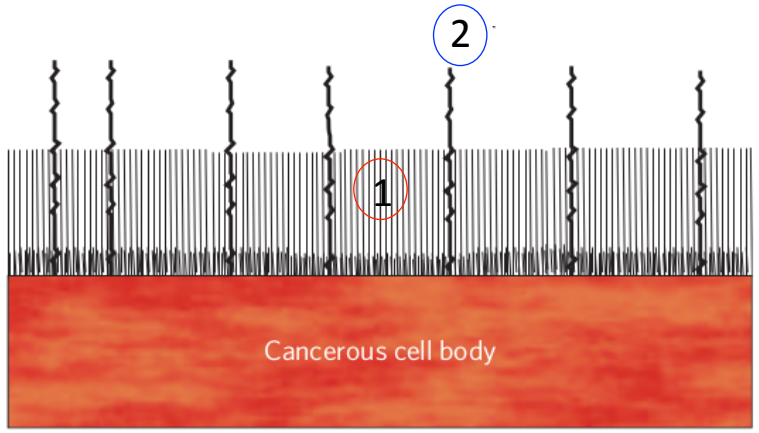
Butt et al. *Langmuir,* **15,** (1999)

Brush ***Lengh*** Distribution (L)



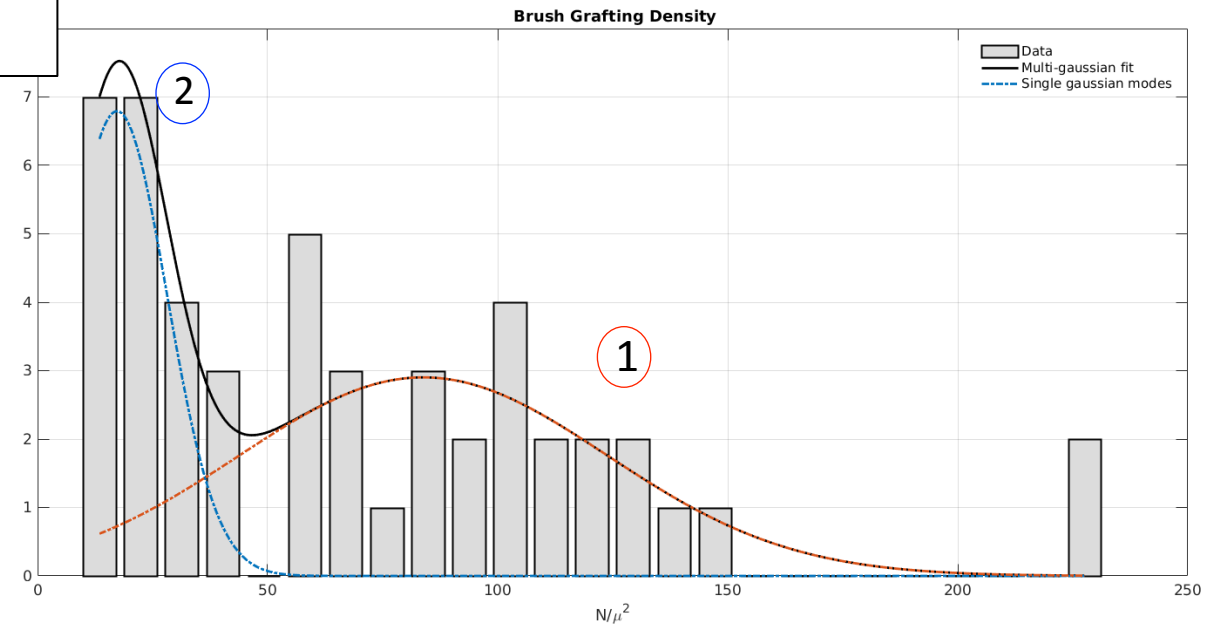
Bimodal Distribution:

- L_1= **336** ± 54 nm , N_1= **83** ± 20 N/μ²
- L_2= **965** ± 202nm , N_2= **17**± 7 N/μ²



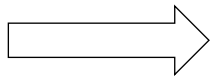
Iyer et al. *Nature Tech.* **4,** (2009)

Brush ***Density*** Distribution (N)



Conclusions and future perspectives

- The stiffness and **topography** of the **micro-environment** influence the distribution and the composition of the **adhesion sites**.
- The adhesion spots feedbacks on the force transmission, **cytoskeletal organization** and **mechanical properties** of the cell.
- The variation of the cellular **biophysical state** impacts on the **nuclear architecture** and mechano-sensitive transcription factors witch eventually **modulate the cell fate.**



- Further investigation on the role of the different **glycocalyx components** in the growth of adhesion spots and relates it with the **confinement** action of the **nanostructured surfaces.**
- Select different morphological properties of the **ns-CP** for a complete characterization of the **Integrine clustering** and the cellular response.

C.I.Ma.I.Na

UNIVERSITAS STUDIORUM MEDIOLANENSIS

Matteo Chighizola

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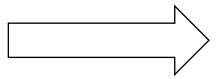

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Thank for your Attention!

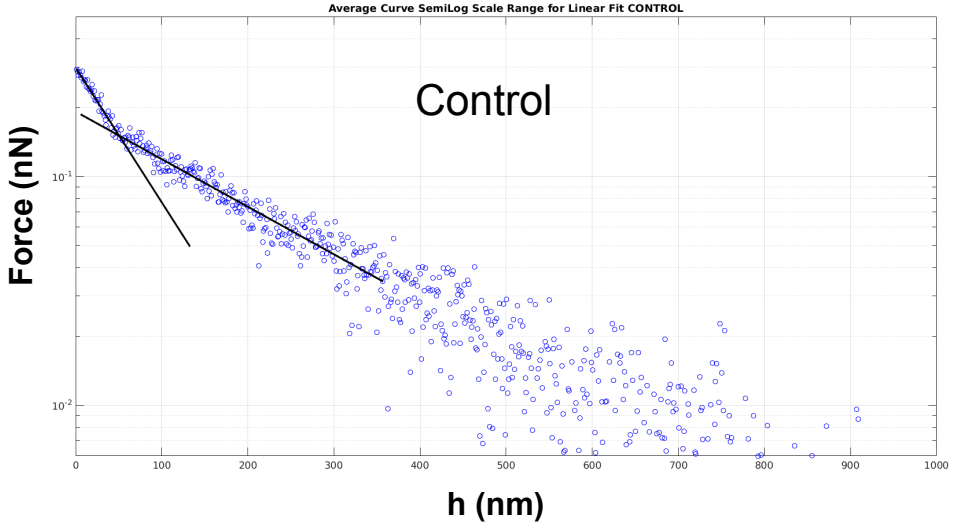
C.I.Ma.I.Na

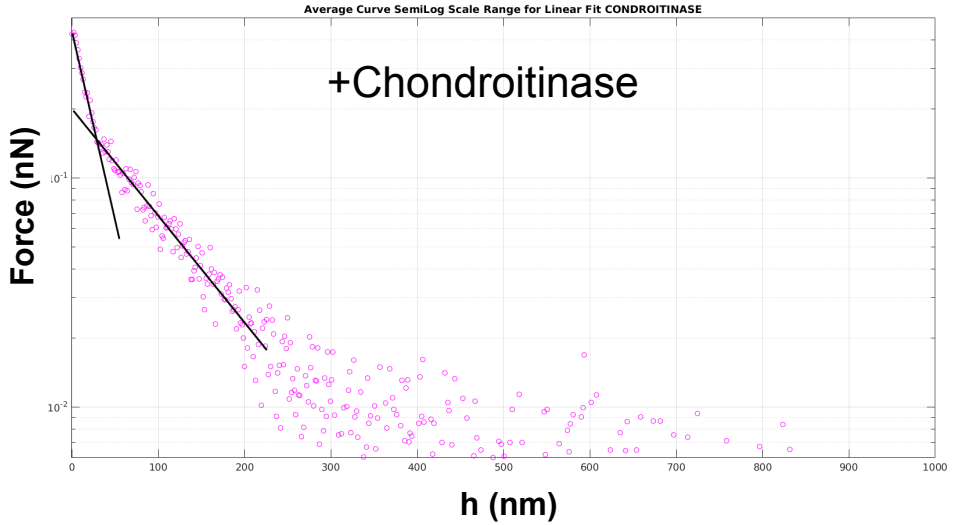
UNIVERSITAS STUDIORUM MEDIOLANENSIS

Matteo Chighizola

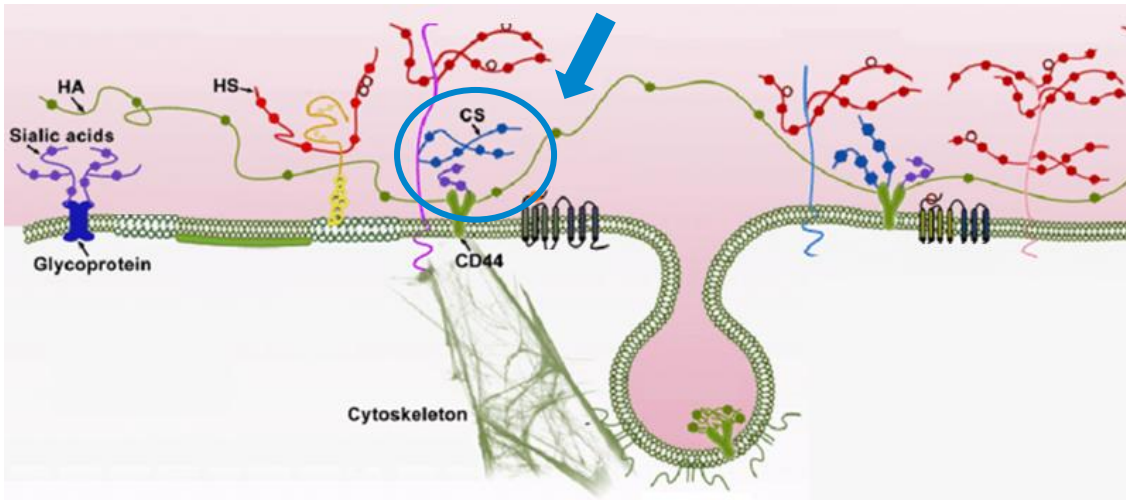
The Surface cellular Brush
(Glycocalyx)

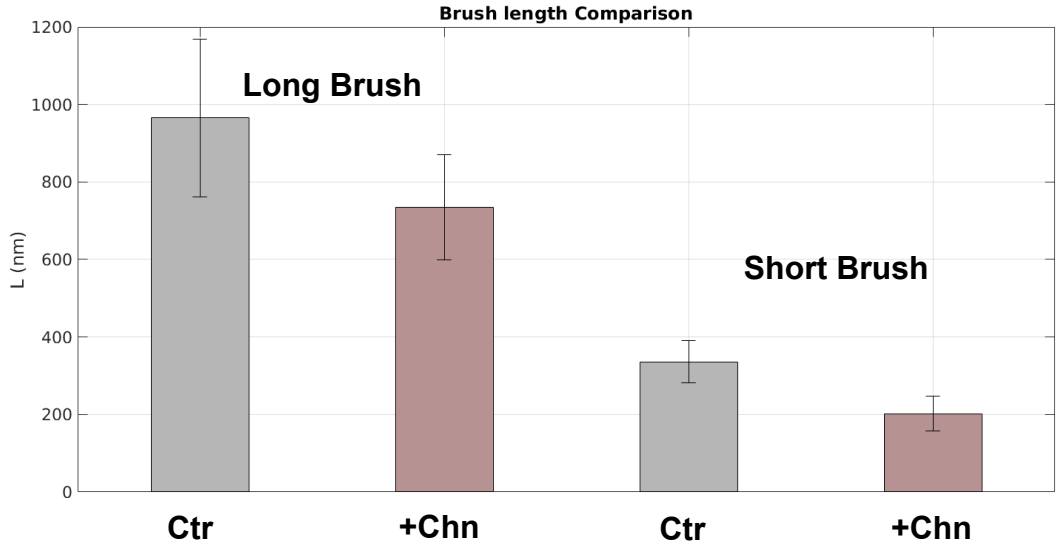
- **Control Measurement:** *Cutting the Glycocalyx*



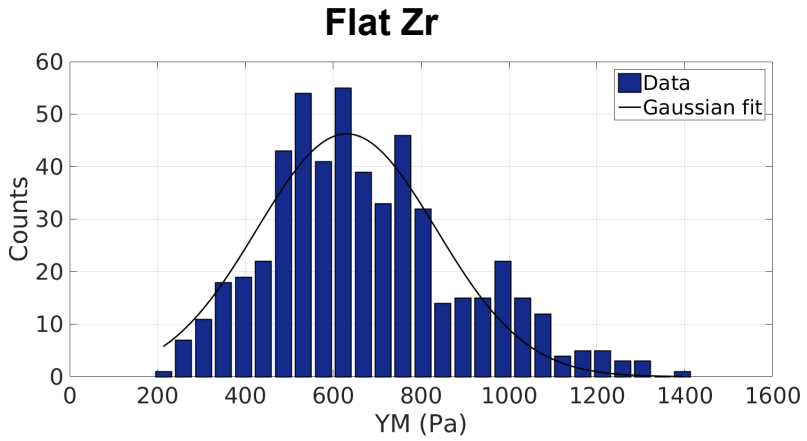


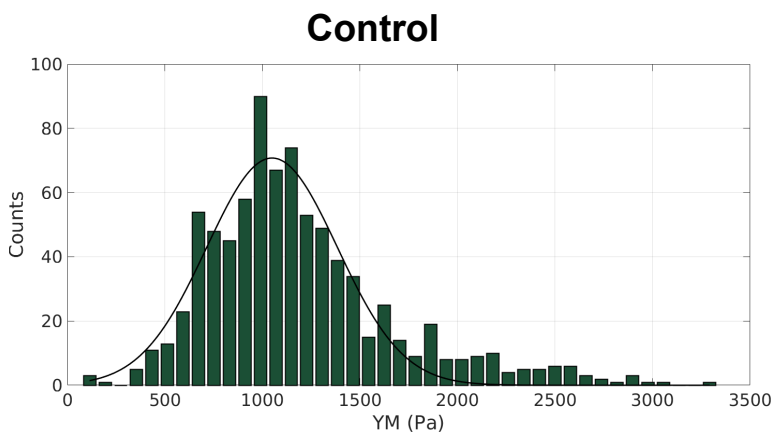
Chondroitinase Action

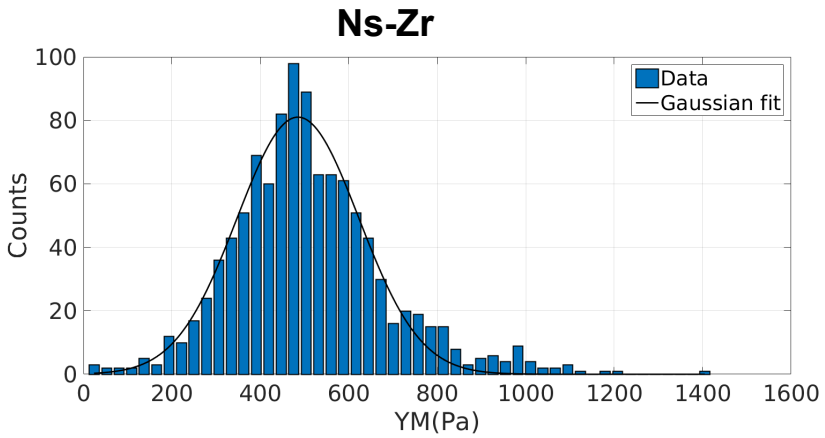


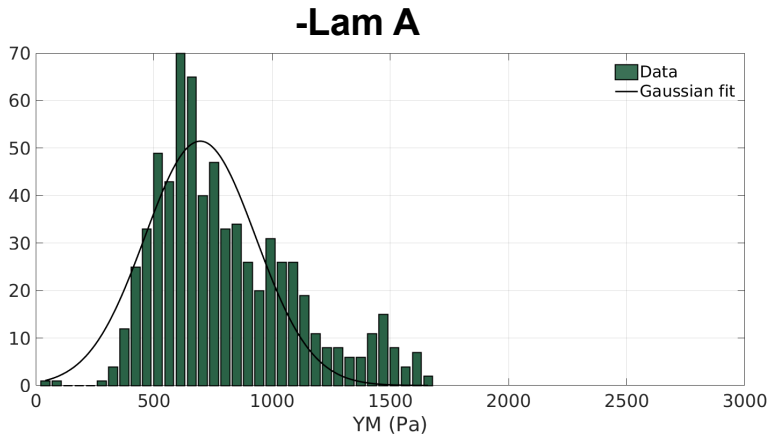


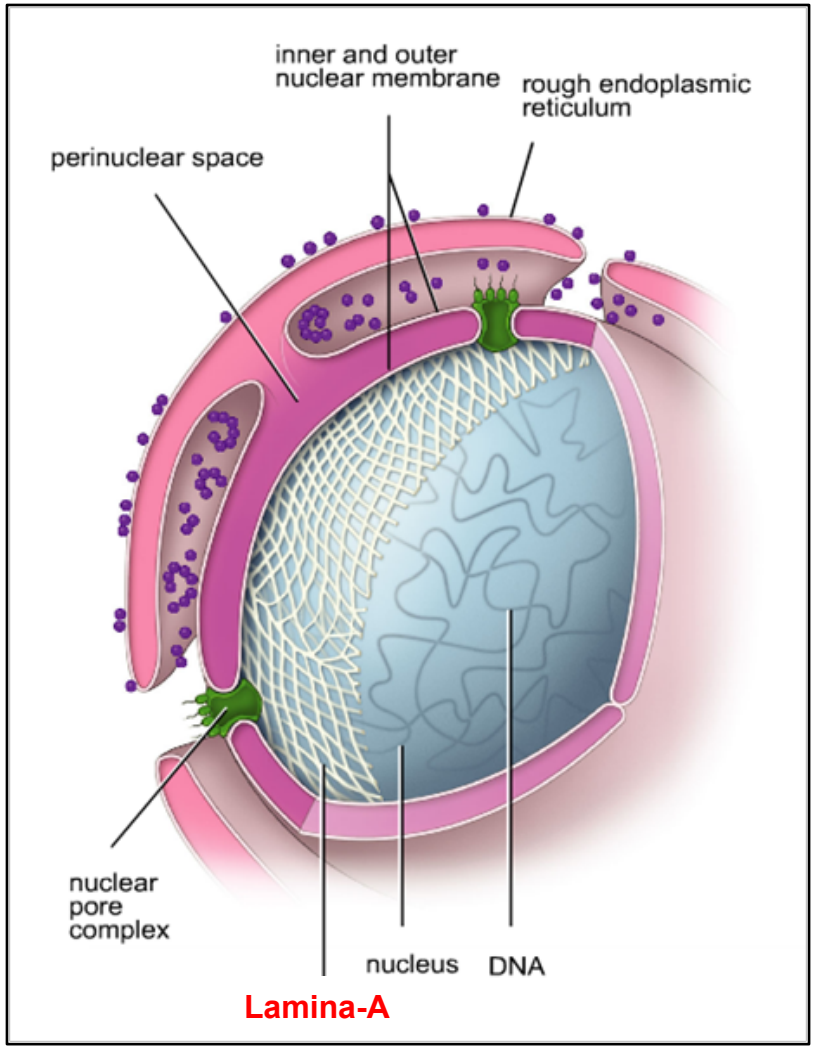
Nanotopography influence the Nuclear Architecture





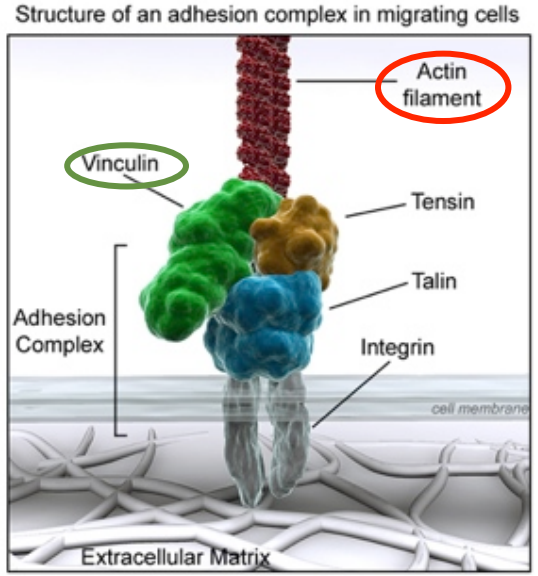






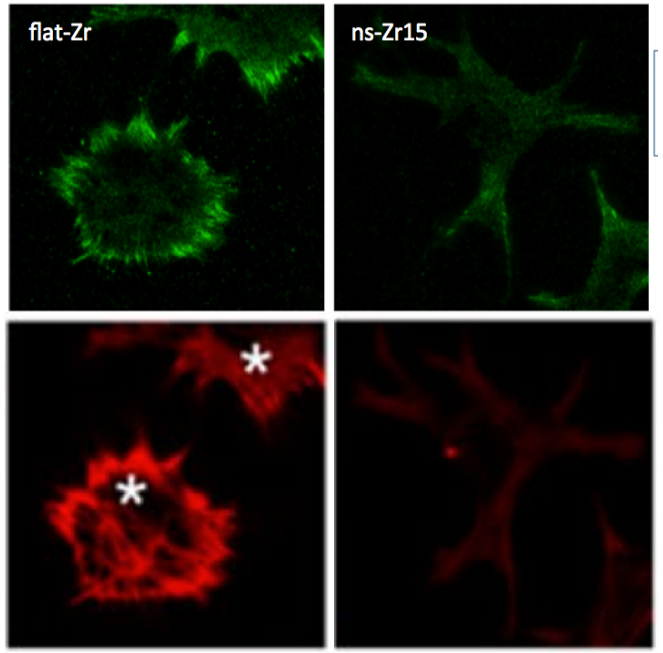
Differentiation through morphological Interaction

- **Fluorescence Analysis**

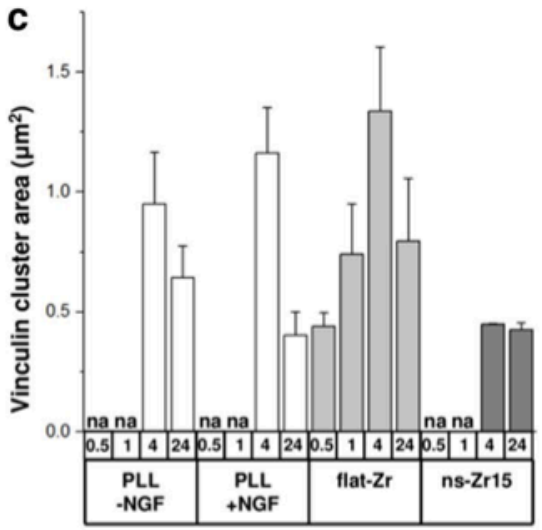


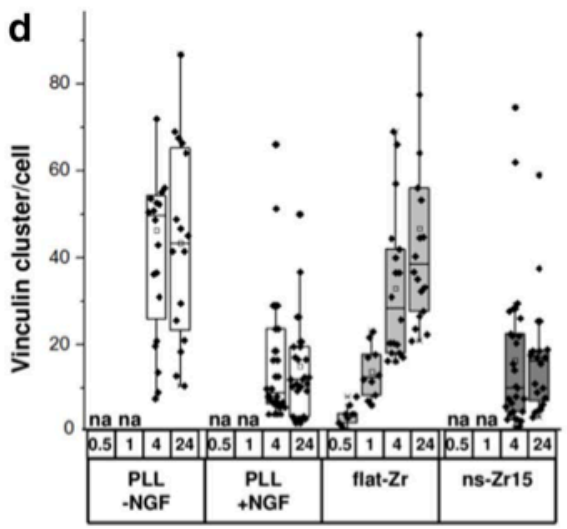
Vinculine Staining

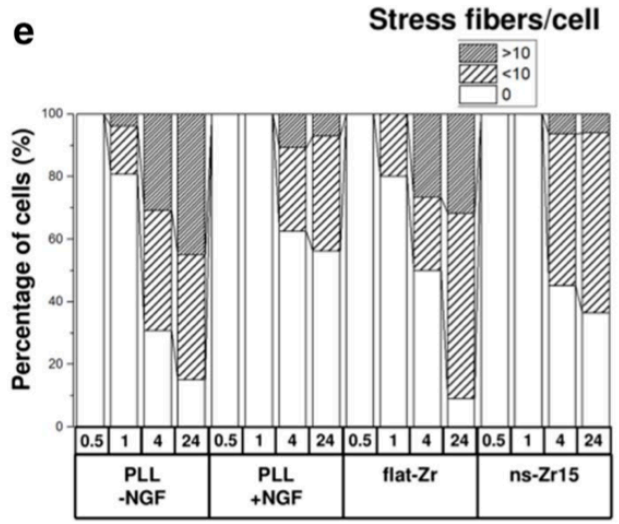
Actin Staining



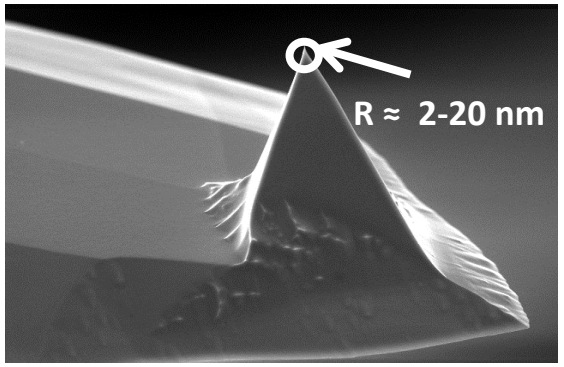
Img alta res Carsten





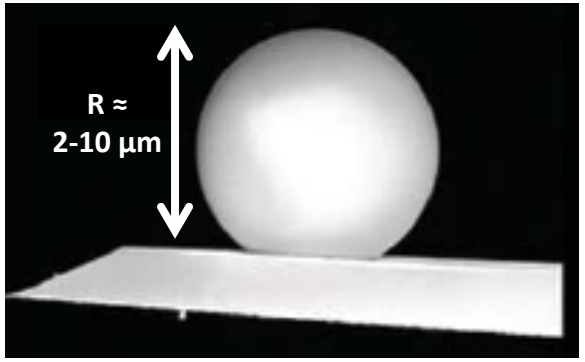


AFM Indentation Measurement

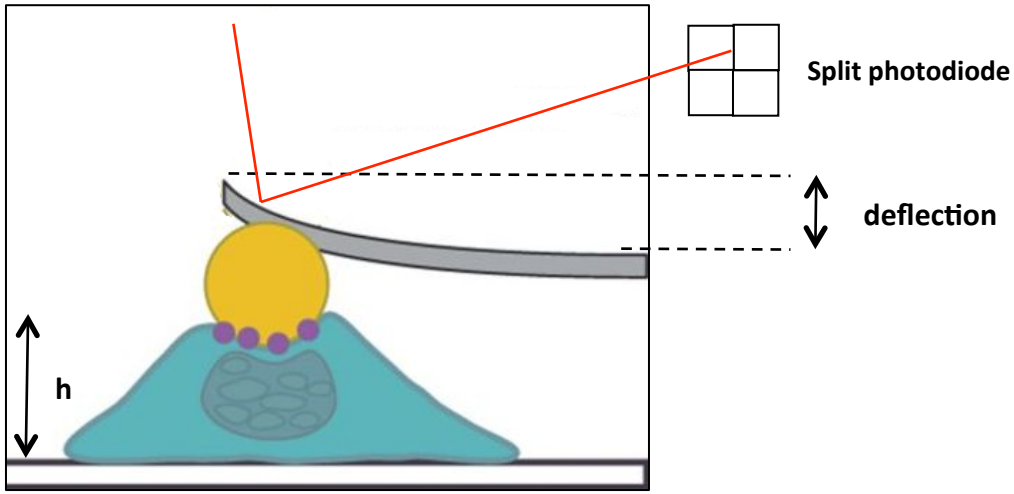


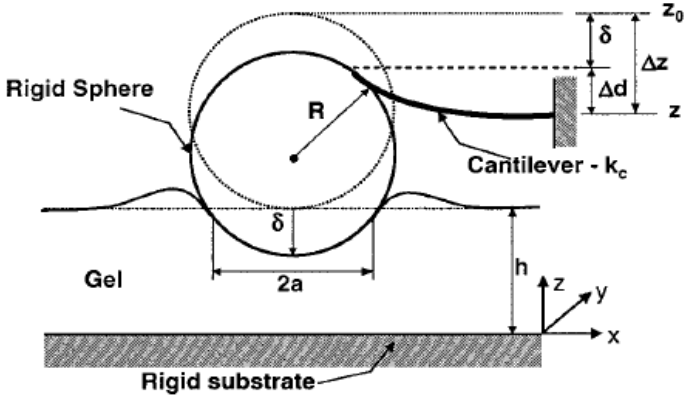
Sharp Probe

VS



Colloidal Probe

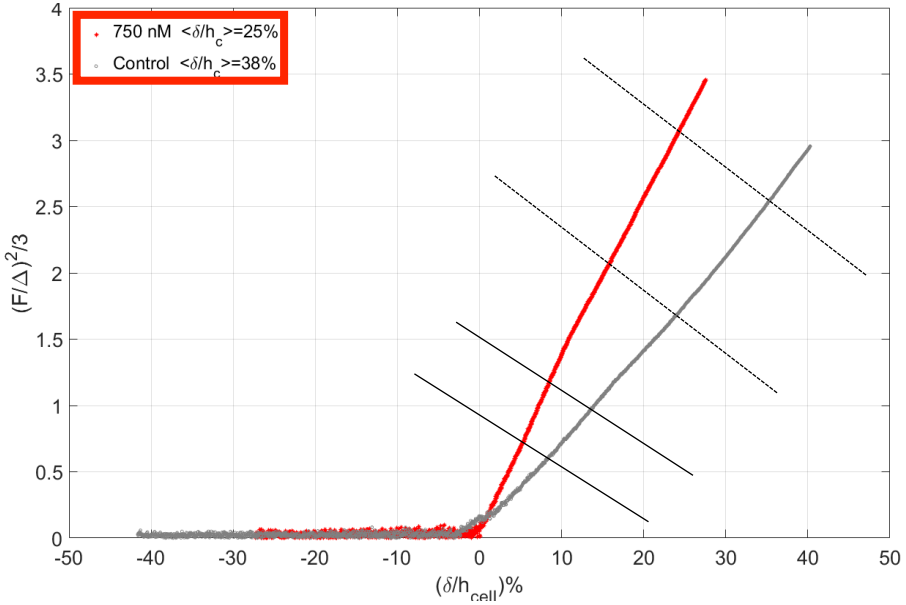


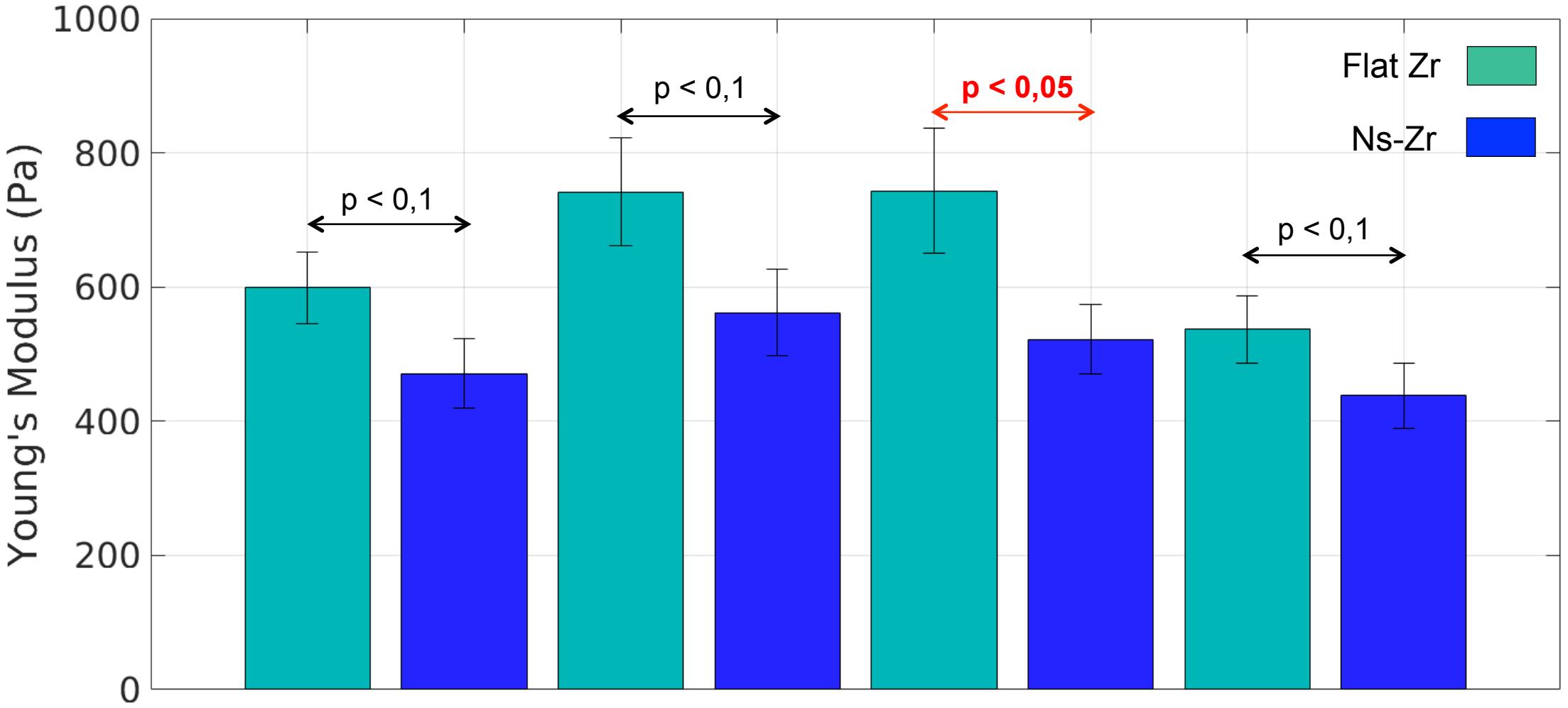


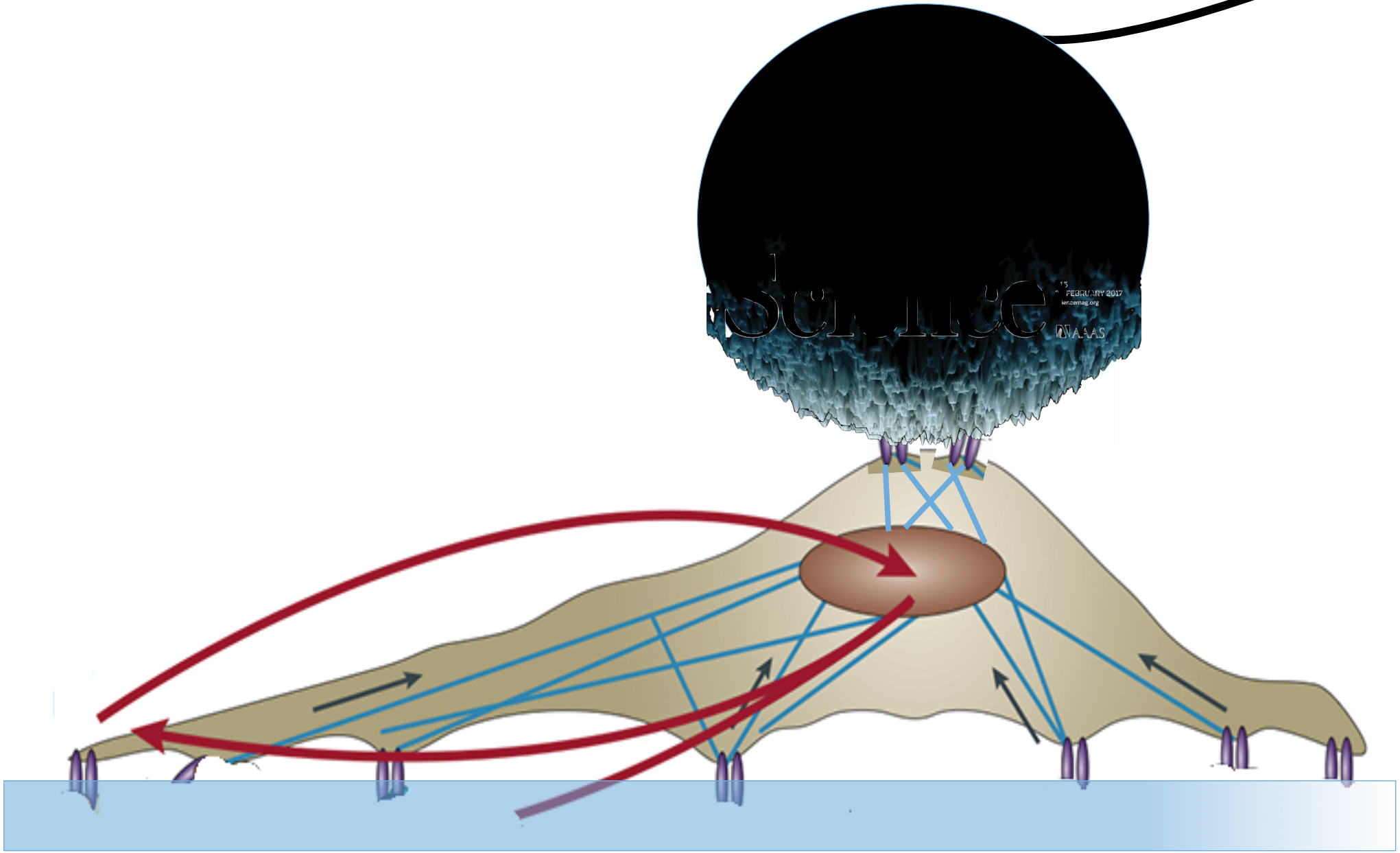
$$E = \frac{stress}{strain} = \frac{F/A}{\Delta l/l_o}$$

- δ = sample indentation
- δ_0 = contact point
- F_I = applied force
- R = sphere radius
- a = contact radius
- ν = Poisson ratio
- E = Young's modulus

Elastic Range measured respect to the cells height




1000
800
600
400
200
0
Young's Modulus (Pa)
Flat Zr
Ns-Zr
p < 0,1
p < 0,1
p < 0,05
p < 0,1


SCIENCE
15
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The general meaning of this concept is that the stiffness and the topography of the environment influence the architecture and composition of adhesions sites (e.g. integrin clustering) which feedbacks on the force transmission, cytoskeletal organization and mechanical properties of the cell. The variation of the cellular biophysical state impacts on the nuclear architecture and mechano-sensitive transcription factors which eventually modulate the cell fate.