Forum des Microscopies à Sondes Locales – 18 mars 2019

AFM et mécanobiologie

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Mechanobiology

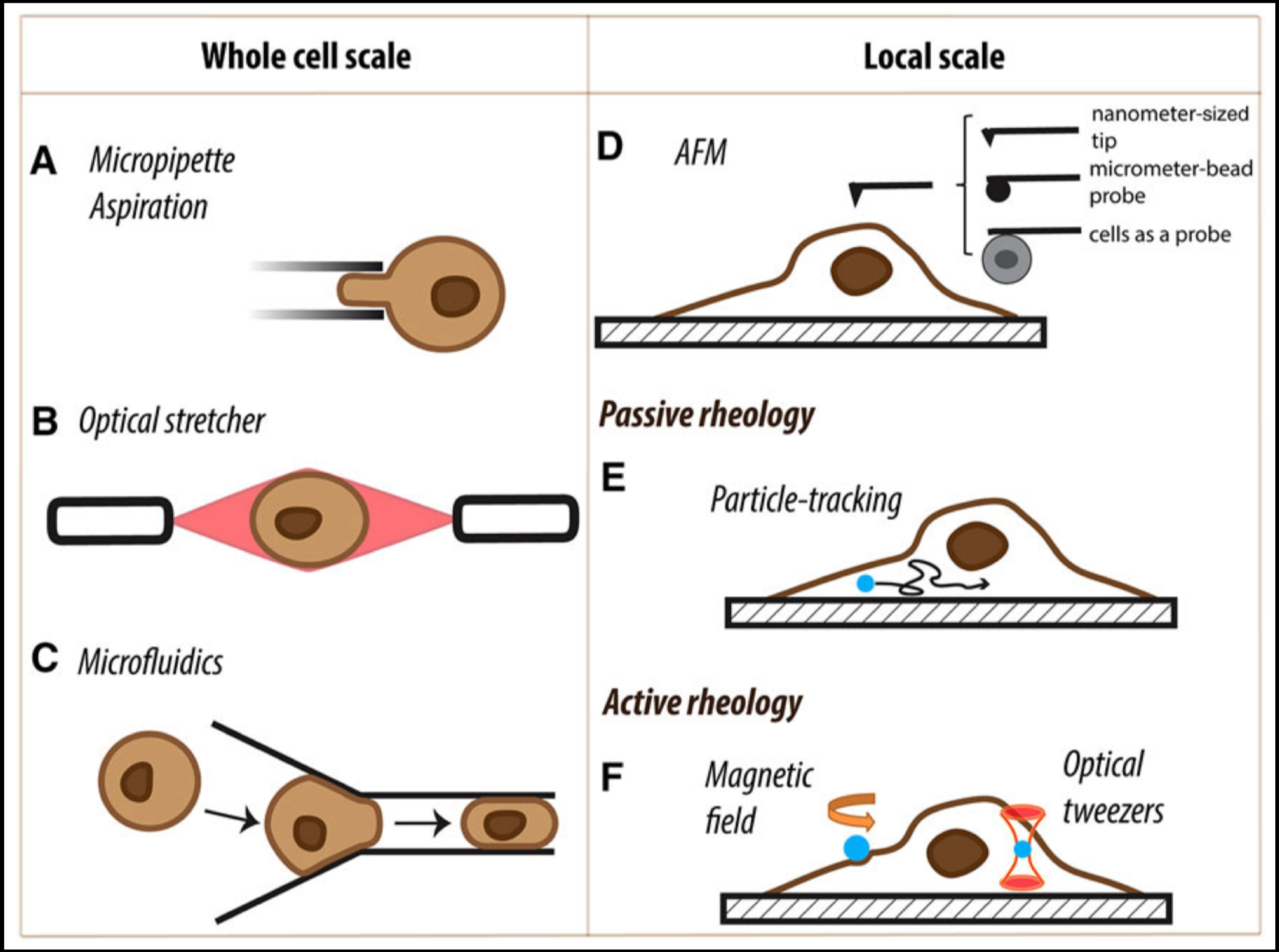
How cells could sense and respond to the physical properties of their environment ?

→ dependent on dynamic subcellular systems that can generate and transduce mechanical force.

Cellular function modification ⟷ Mechanical properties
(biochemical processes or diseases)

indicator of their biological status

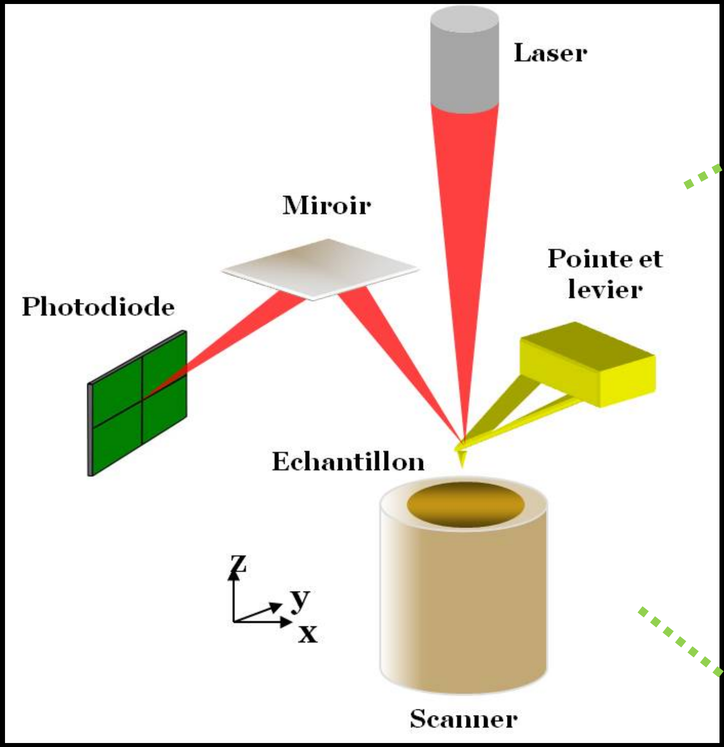
Mechanobiology



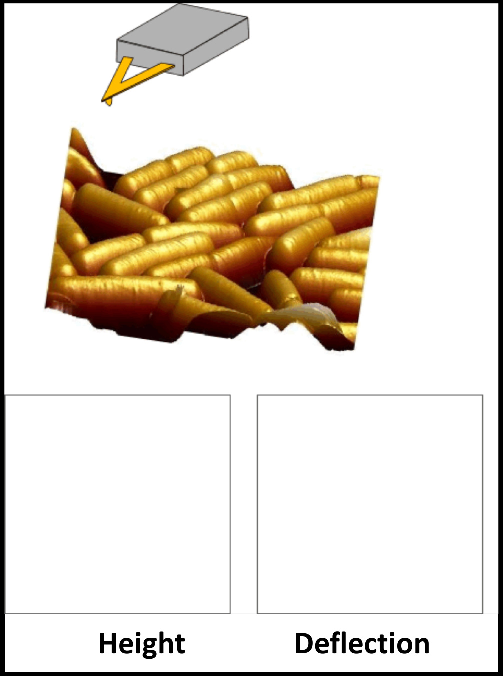
Alibert *et al*., Biol. Cell (2017) 109, 167-189

1) How can we measure cell mechanics by AFM ?
2) Why (cell mechanics in diseases) ?
3) Perspectives

Atomic Force Microscopy : principle

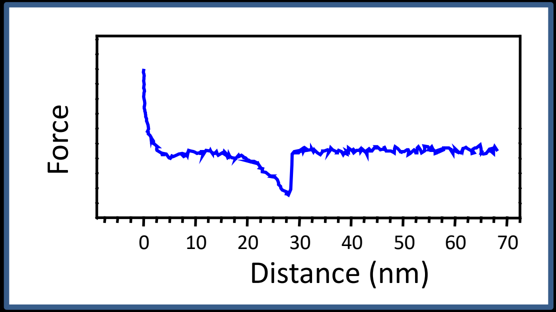


- **Imaging**



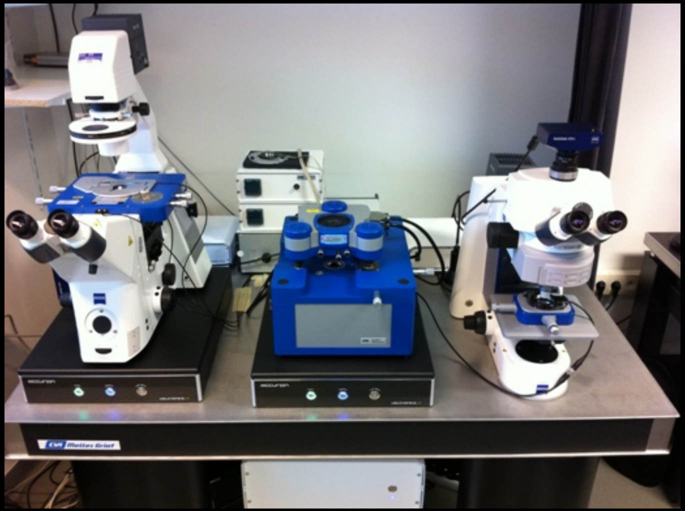
- resolution : nm
- minimal sample prep
- in buffer
- ~ real time

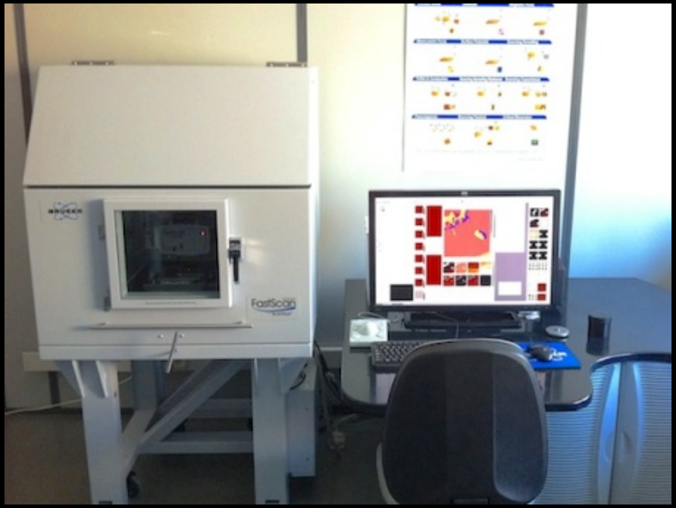
- **Force spectroscopy**

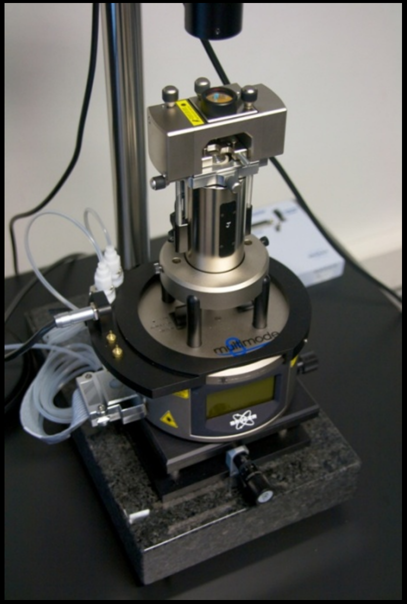


- pN sensitivity
- single molecule
- Physical properties
- cartography

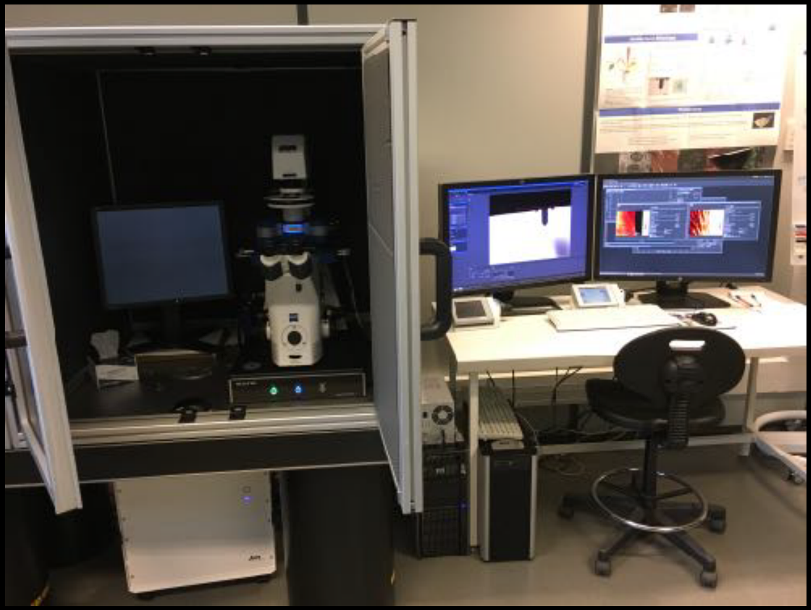
Different models for different applications

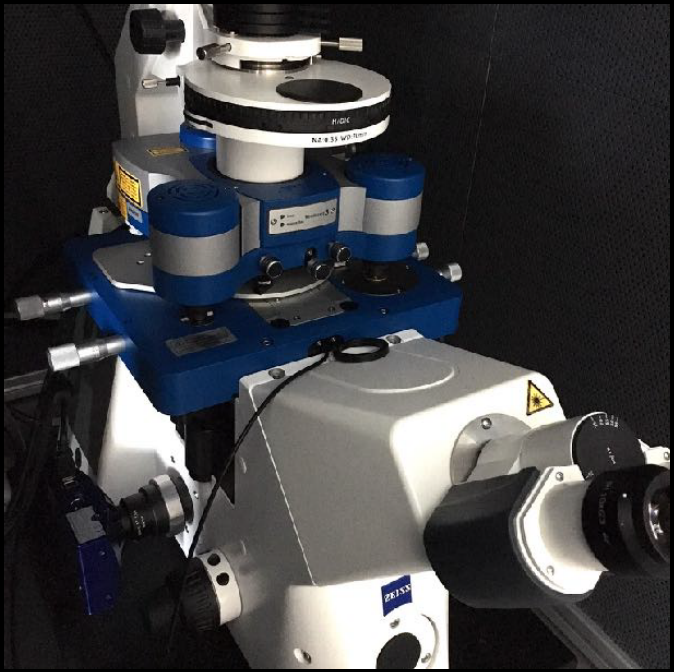






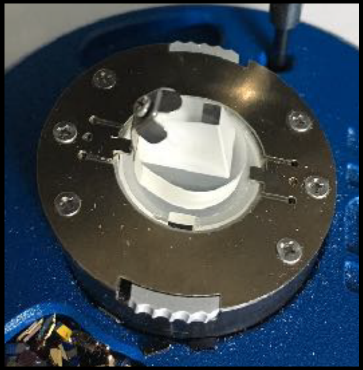
JPK NanoWizard III AFM





AFM scanning head (xyz) and stage (z)

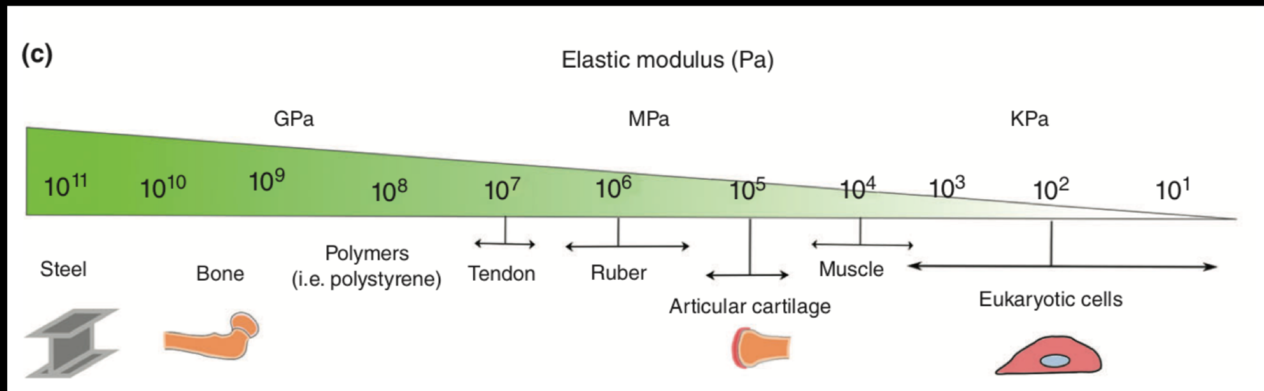
Petri dish heater



Cantilever holder

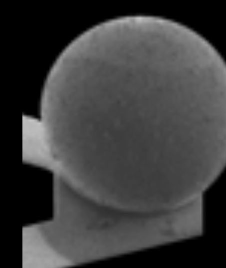
Choice of the probe

→ **Stiffness of the cantilever (spring constant k)**



→ **Tip shape**

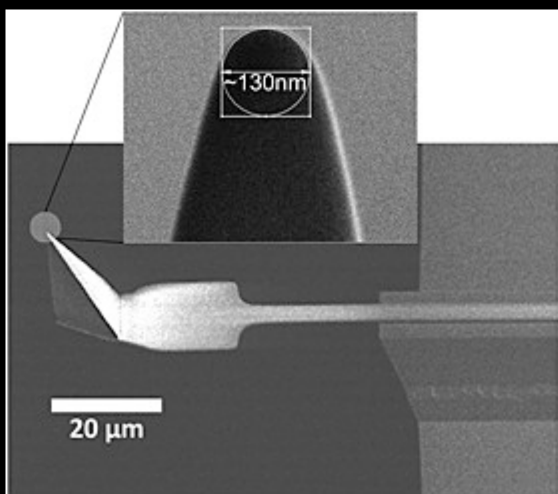
tip radius : 2-500 nm
tip height : 3-20 μm



Choice of the probe

Long tips enabling imaging of cell surfaces even with large height differences

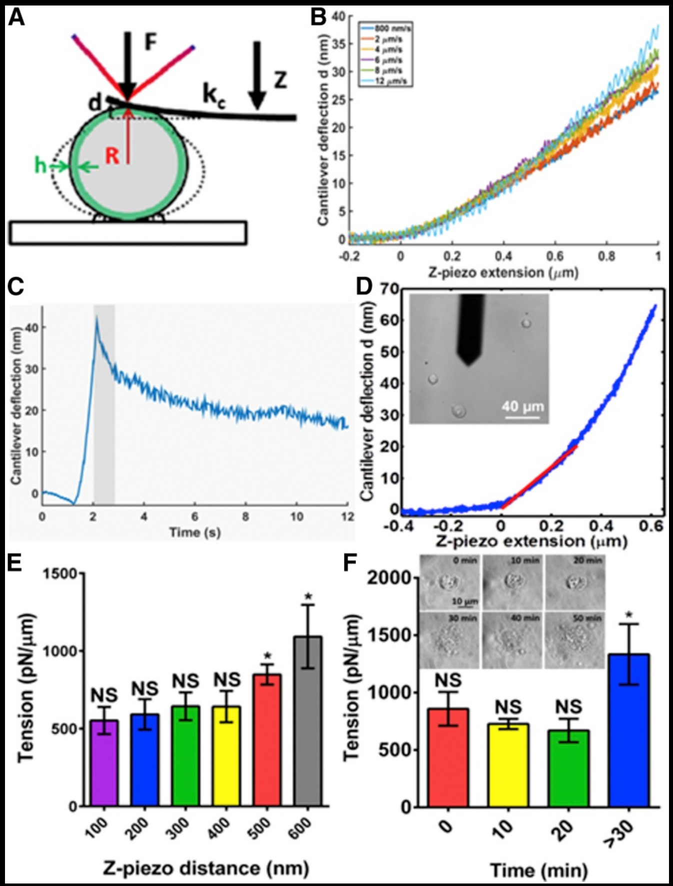
→ **PeakForce QNM-Live Cell probe**



Schillers *et al.*, J. Mol. Rec., 2016

→ short cantilever with a 17-µm-long tip (minimizes hydrodynamic effects between the cantilever and the sample surface)

Tipless cantilever



Cartagena-Rivera, Biophys.J, 2016

Calibration

Force measurements in practice : calibration of force distance curves

Cantilever : Hookean spring

$$F = k \times d$$

→ Sensitivity (of the optical detection system)

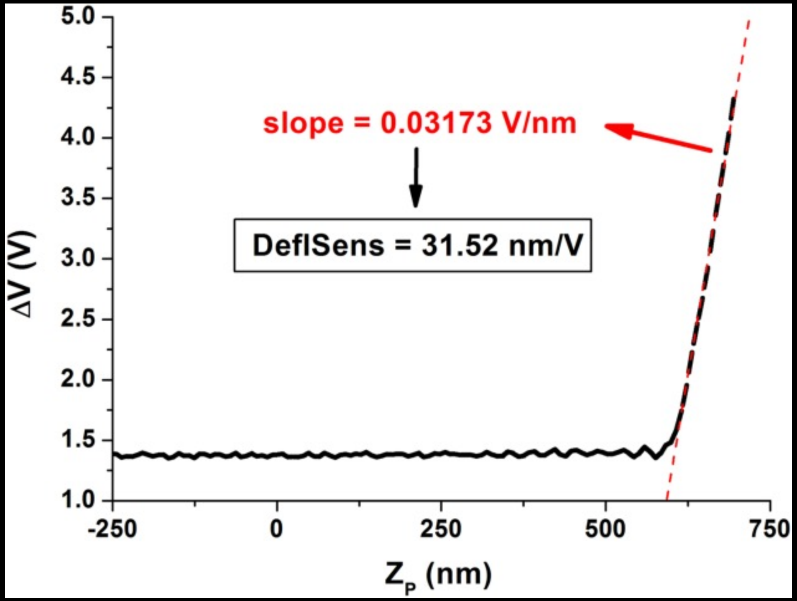
→ Cantilever spring constant k

Calibration

Deflection sensitivity (nm/V)

- voltage signal detected by the photodiode (V)
- conversion factor : V → nm

OLS (optical lever sensitivity)



Gavara, Micros. Res. Tech., 2016

→ calculated from the slope in a force-distance curve obtained on a hard substrate (such as glass)

⇒ InvOLS : nm/V

Calibration

Cantilever stiffness : spring constant (k)

→ Nominal stiffness

→ Sader method

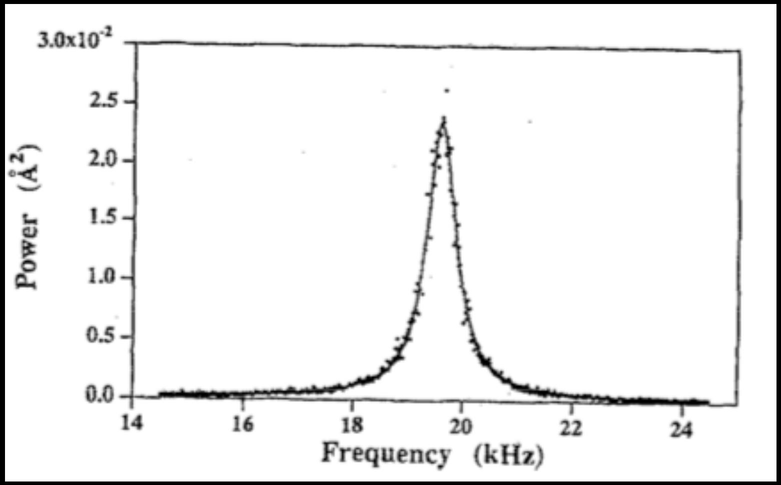
→ Thermal fluctuations method

Equipartition theorem

$$\frac{1}{2}k_B T = \frac{1}{2}k\left\langle \Delta x^2 \right\rangle$$

k : spring constant
k_B : Boltzmann constant
T : temperature (K)
$\langle \Delta x^2 \rangle$: thermal noise

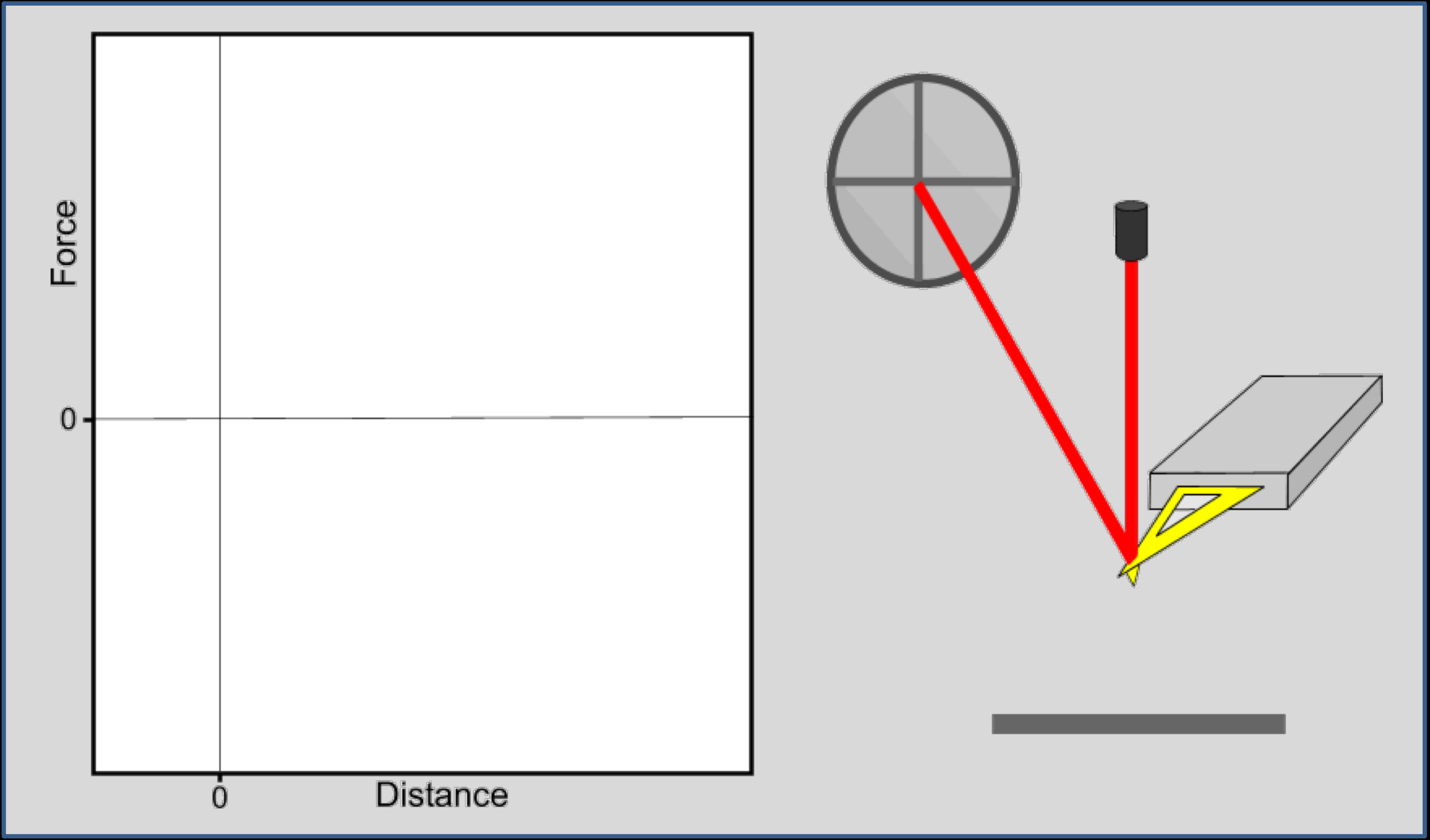
→ power spectrum



To be carried out at each cantilever change and after each laser adjustment

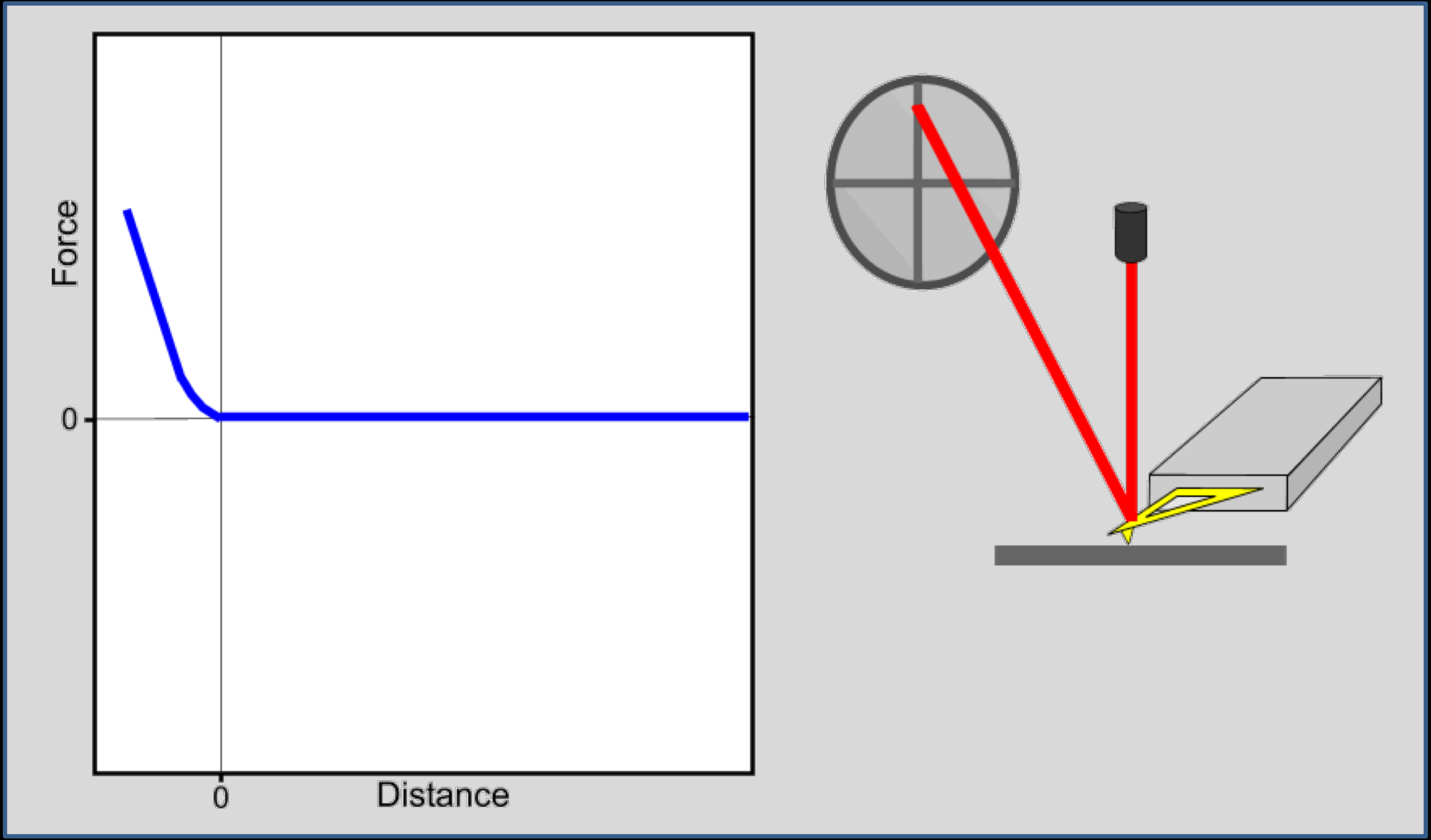
Force spectroscopy

→ Force-distance curve (approach)



Force spectroscopy

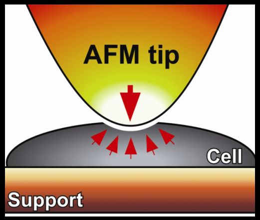
→ Force-distance curve (retract)



Force spectroscopy

One force-curve : two different parts

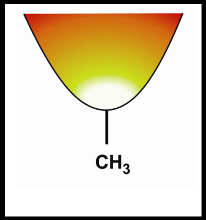
Approach curve
⇒ mechanical properties

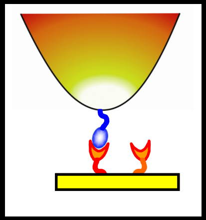


Elasticity (Young's modulus)

Viscosity (visco-elasticity)

Retract curve
⇒ physico-chemical properties





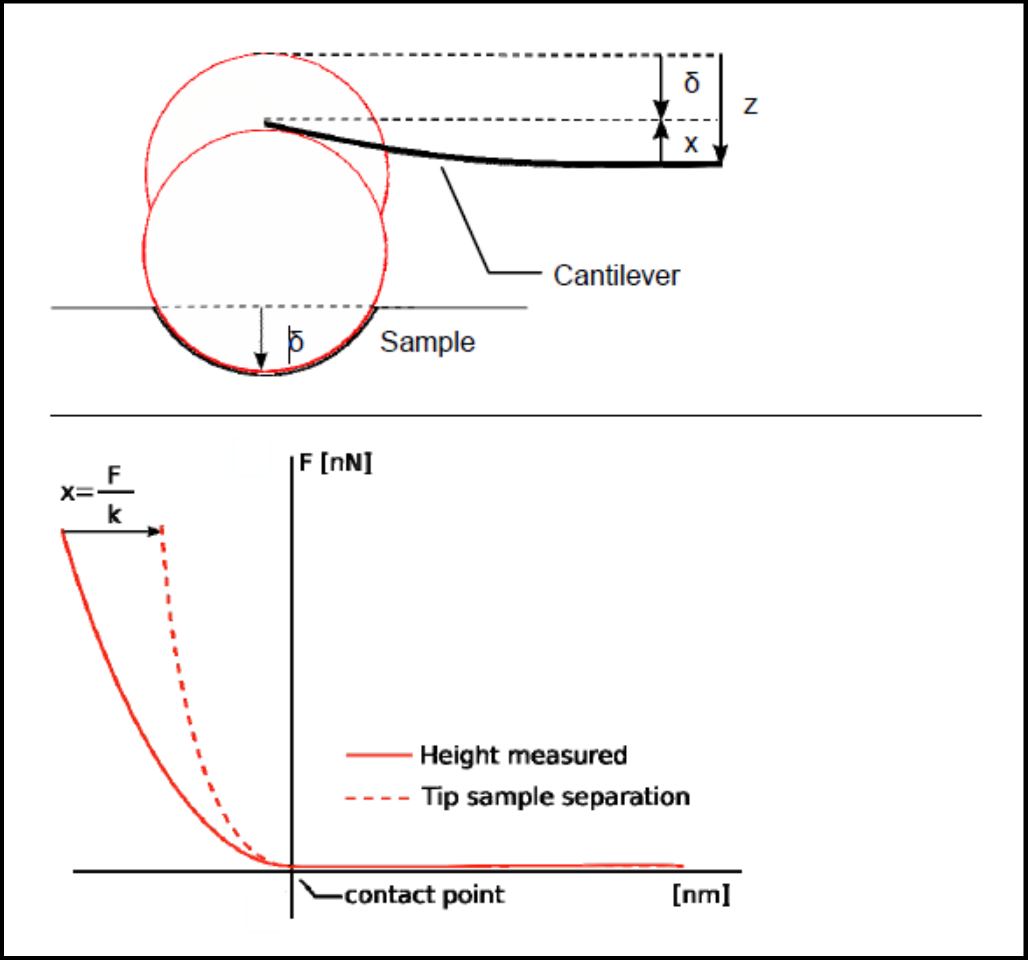
Single mol. manipulation (unfolding)

Hydrophobicity (CFM)

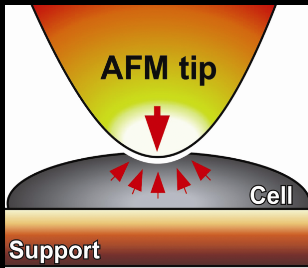
Molecular recognition imaging
(adhesion maps)

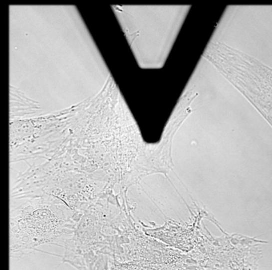
Polymer conformational analysis
(elasticity, length)

Mechanical properties : Young modulus

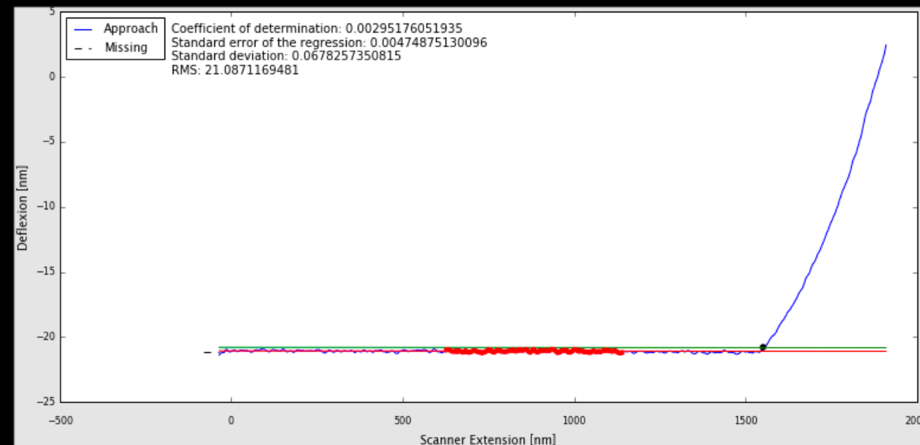


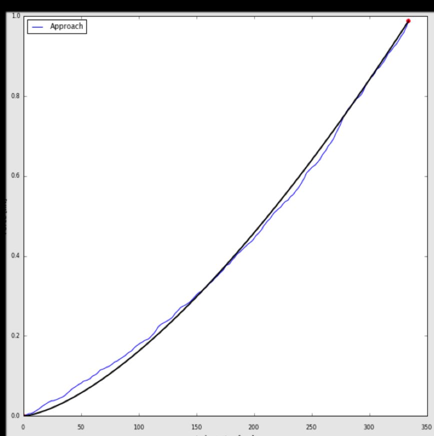
Mechanical properties : Young modulus





Force *vs* indentation curves





Hertz model

$$F = \frac{4}{3}\frac{E}{1-\upsilon^2}\sqrt{R\delta^3}$$

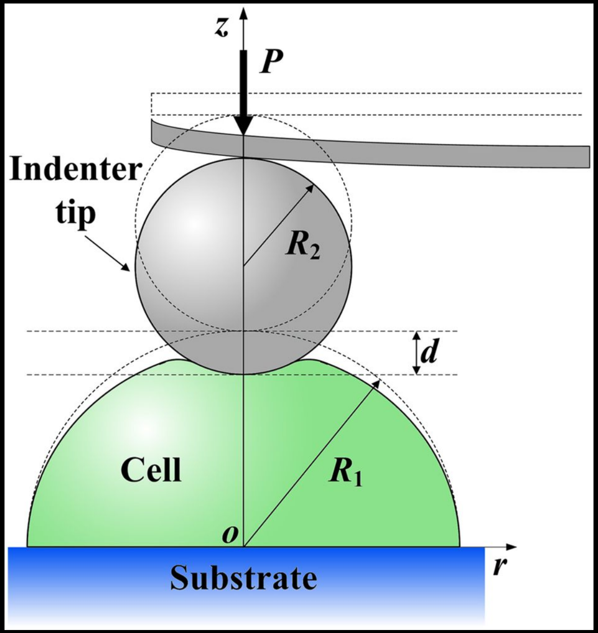
F : applied force
R : radius of the probe
δ : indentation
E : elastic modulus
ν : Poisson’s ratio

Young's modulus → cell elastic properties

Mechanical properties : Hertz Model

H. Hertz, Über die Berührung fester elastischer Körper, Journal für die reine und angewandte Mathematik 92, 156-171 (1881)

→ Two elastic, deformable spheres

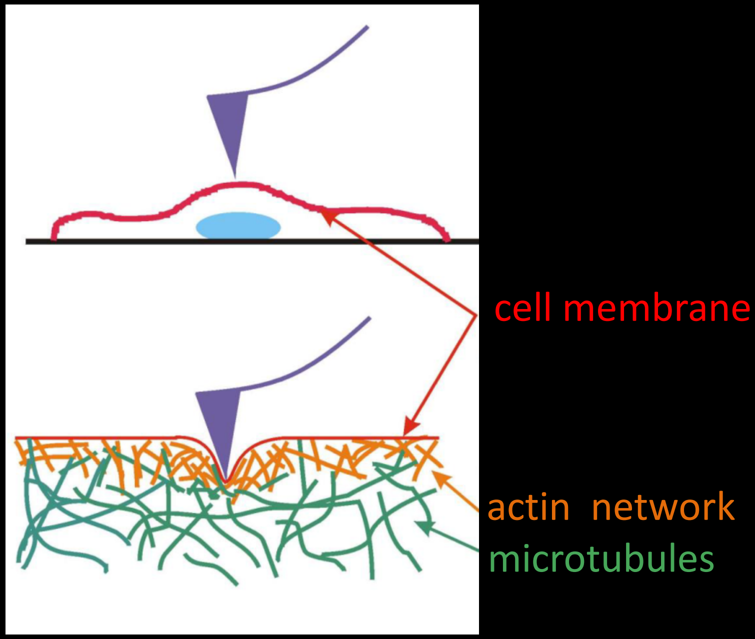


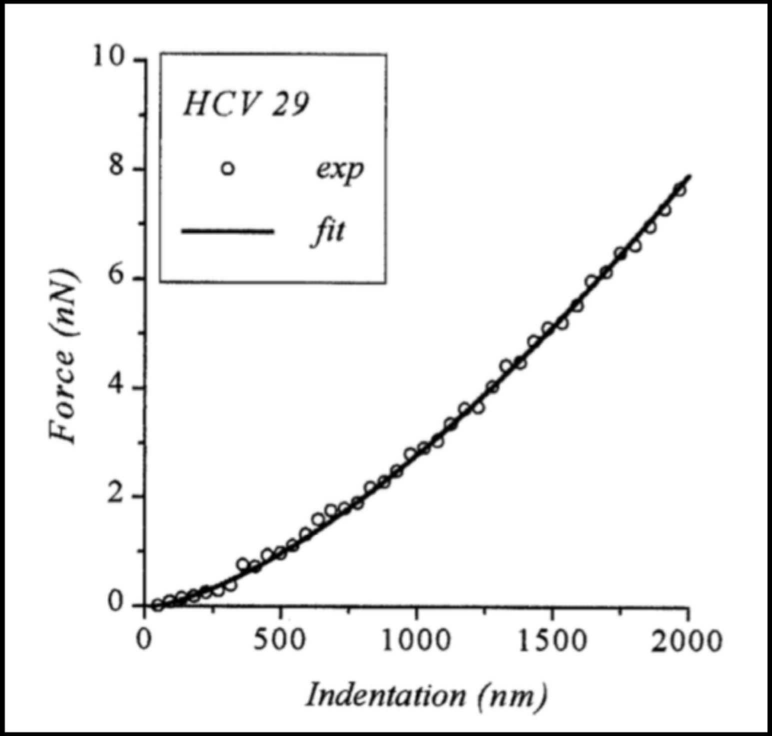
Assumptions of the Hertz theory :

- **Homogeneous and isotropic material**
- **Linear elastic material properties**
- **Non-adhesive tip-sample contact**
- **Infinite sample thickness**
- **Small stresses (infinitesimal deformations)**

Mechanical properties : Hertz Model

But cells are heterogeneous…



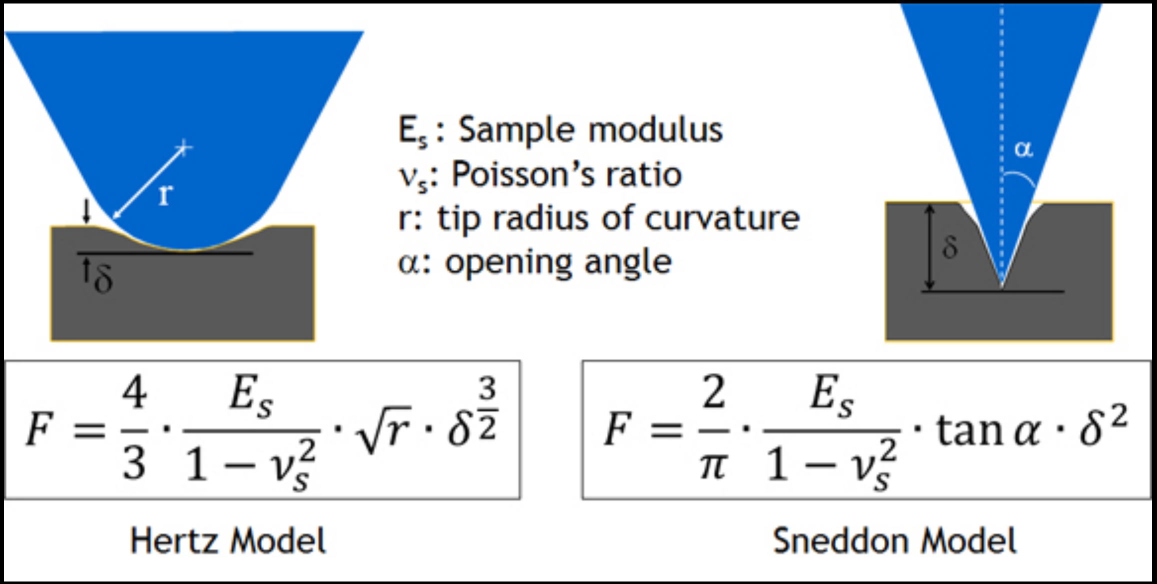


Lekka *et al.*, Eur Biophys J (1999)

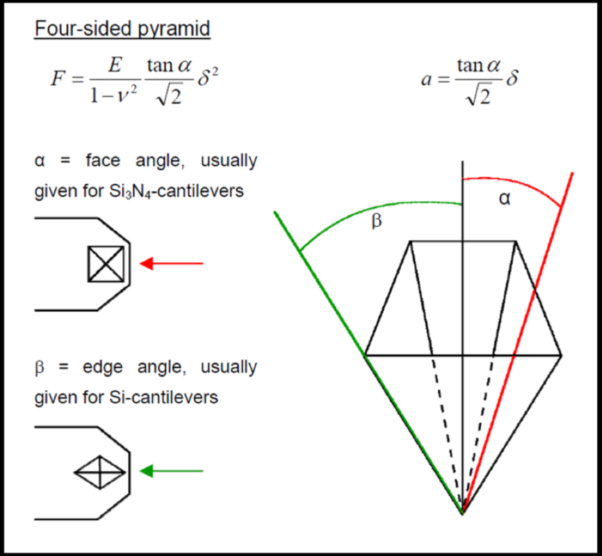
→ The Hertz fit works surprisingly well…

Mechanical properties : Hertz Model

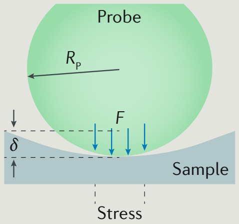
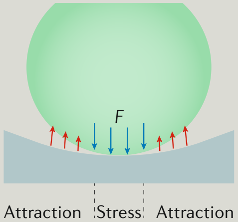
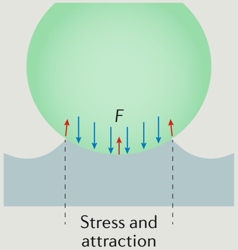
There are variants of the Hertz model for different probe geometries



http://www.iupui.edu/~bbml/



JPK, Application Note

Model	Probe geometry	Force	Additional assumptions	Schematic representation
Hertz	Spherical	$F = E_{eff} \cdot \left[(a^2+R_P^2) \cdot \ln\left(\frac{R_P+a}{R_P-a}\right) - 2aR_P\right]$ $\delta = \frac{a}{2}\ln\frac{R_P+a}{R_P-a}$	No surface forces	
	Cylindrical	$F(\delta) = 2E_{eff} \cdot R_Z\delta$	Smooth punch profile (no edges)	–
	Conical (Sneddon model)	$F(\delta) = E_{eff} \cdot 2\tan(\theta)/\pi \cdot \delta^2$	Infinitely sharp probe	–
	Parabolic	$F(\delta) = E_{eff} \cdot \frac{4\sqrt{R_P}}{3}\delta^{\frac{3}{2}}$	$R_C > \delta$	–
	Blunted pyramidal	$F(\delta) = 2E_{eff} \cdot \left[\delta a - \frac{\sqrt{2}}{\pi}\frac{a^2}{\tan\theta}\left(\frac{\pi}{2} - \arcsin\frac{b}{a}\right) - \frac{a^3}{3R_P} + \sqrt{(a^2-b^2)} \cdot \left(\frac{\sqrt{2}}{\pi}\frac{b}{\tan\theta} + \frac{a^2-b^2}{3R_P}\right)\right]$	Cross section of pyramid modelled as a circle	–
Derjaguin–Müller–Toporov	Spherical	$F = F_{Hertz} - F_{det}$ $\delta = \frac{a}{2}\ln\frac{R_P+a}{R_P-a}$	• Long-range surface forces outside the contact area • Valid for stiff materials, small spheres and weak adhesion	
Johnson–Kendall–Roberts	Spherical approximated with a paraboloid	$F = F_{Hertz} - 4\sqrt{\frac{E_{eff} \cdot F_{det}a^3}{3R_P}}$ $\delta = \frac{a^2}{R_P} - 2\sqrt{\frac{F_{det} \cdot a}{3E_{eff}R_P}}$	• Short-range surface forces inside the contact area • Valid for compliant materials, large spheres and strong adhesion	
Non-Hertzian contact models				
Cortical shell liquid core	Spherical	$F = \left[2T_c\left(\frac{1}{R_c} + \frac{1}{R_P}\right) \cdot 2\pi R_P\right] \cdot \delta$	• Linear force–displacement curves; large membrane reservoir • Variable: T_C	–
Standard linear solid	Conical, spherical	Analytical[175] and numerical[97] expressions	• Time-varying elastic modulus • Variable: viscosity	–
Poroelastic	Spherical	$F \approx e^{-D_p \cdot t\ R_P \cdot \delta}$	• Constant volume; timescale <0.5 s • Variable: $D_p \approx E\xi^2/\eta$	–
Thin shell	Conical	$F \approx \delta \cdot E \cdot h^2/R_S$	• Point probe; $h << R_S$ • Variables: R_S and h	–

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Krieg *et al*. Nat Rev Physics, 2019

Influence of the substrate

Hertz model : infinite sample thickness (neglectable indentation)

$\rightarrow$ BEC model (Bottom Effect Correction)

(Take into account the mechanical contribution of the stiff substrate)

- **Bead (spherical tips)**

$$E = \frac{9F}{16}\frac{1}{R^{1/2}\delta^{3/2}}\frac{1}{\left(1+1.133\chi+1.283\chi^2+0.769\chi^3+0.0975\chi^4\right)}$$

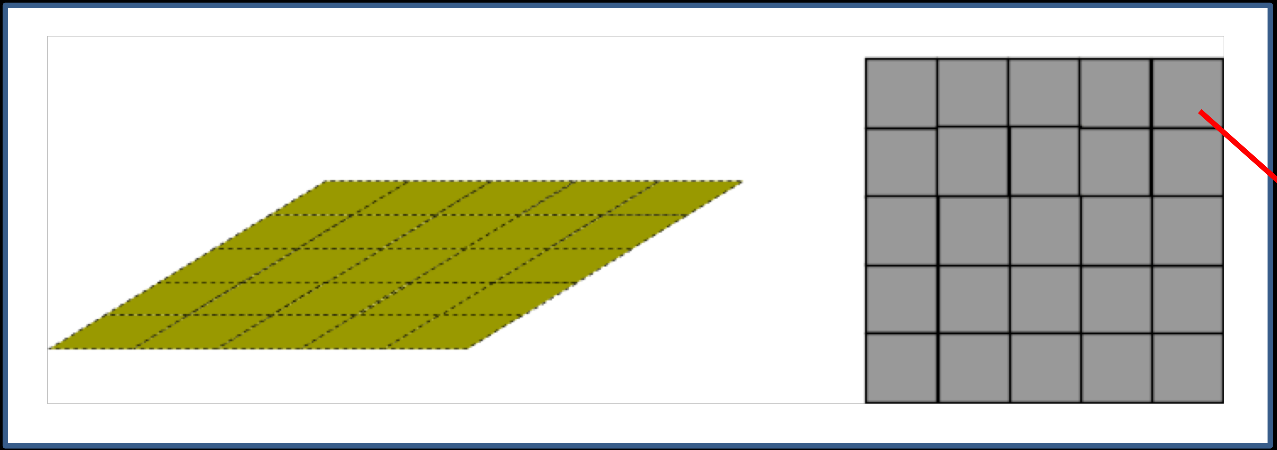
Dimitriadis *et al.*, *Biophys J*, 2002

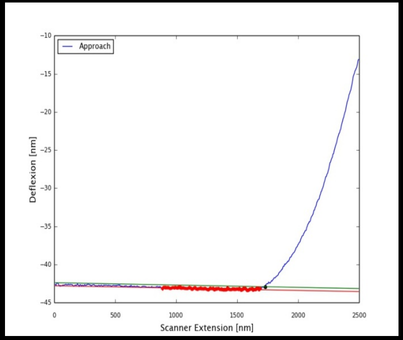
- **Cone shaped tips**

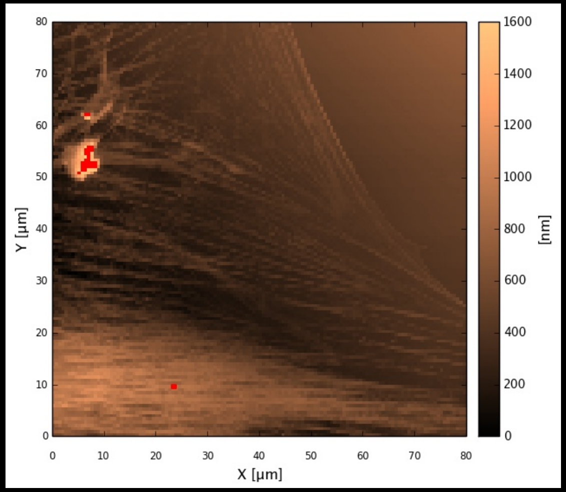
$$F = \frac{8E\tan\theta\delta^2}{3\pi}x\left\{1+1.7795\frac{2\tan\theta}{\pi^2}\frac{\delta}{h}+16(1.7795)^2\tan^2\theta\frac{\delta^2}{h^2}+O\left(\frac{\delta^3}{h^3}\right)\right\}$$

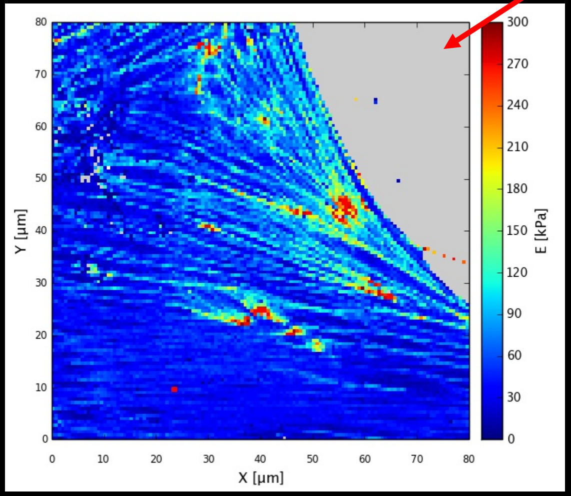
Gavara & Chadwick, *Nature Nanotech*, 2012

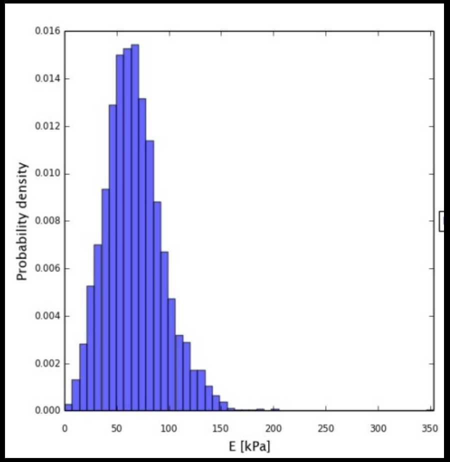
Mechanical properties : elasticity maps



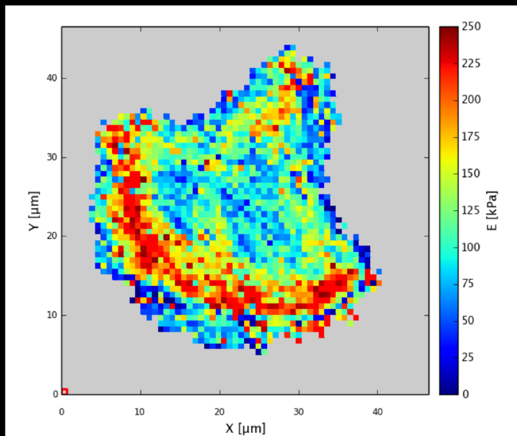






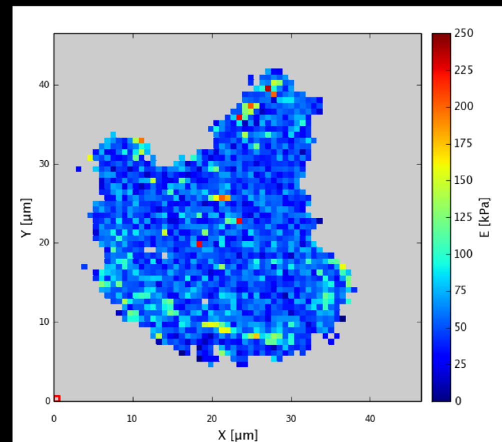


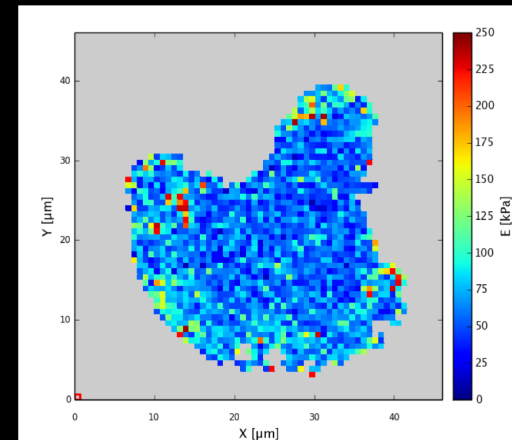
Mechanical properties : elasticity maps

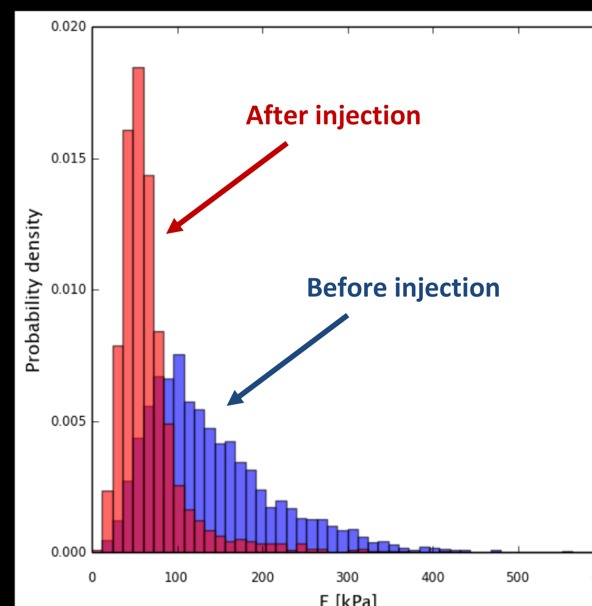


RPE1 cells

After injection of latrunculin A (0.2µM)





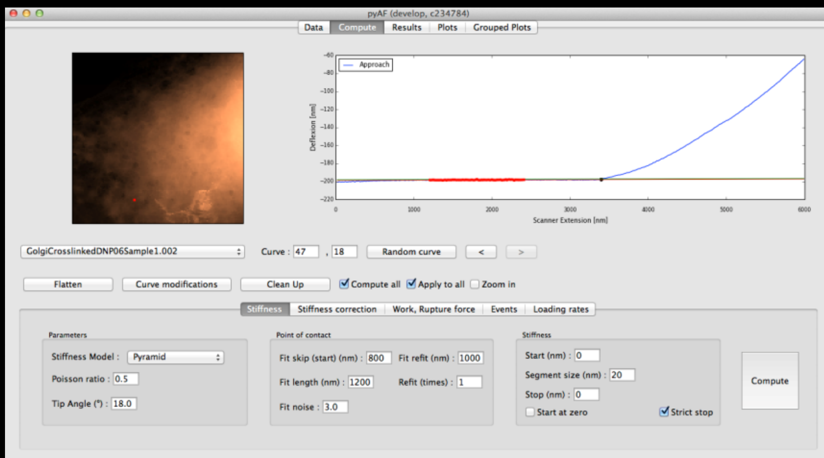


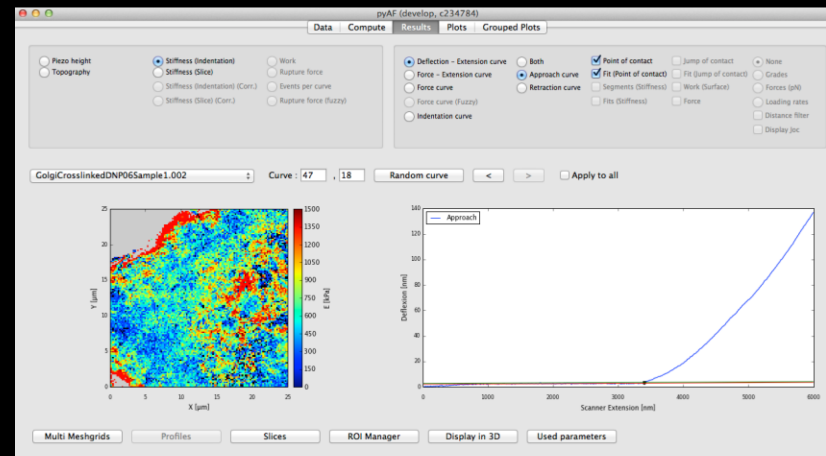
Analysis

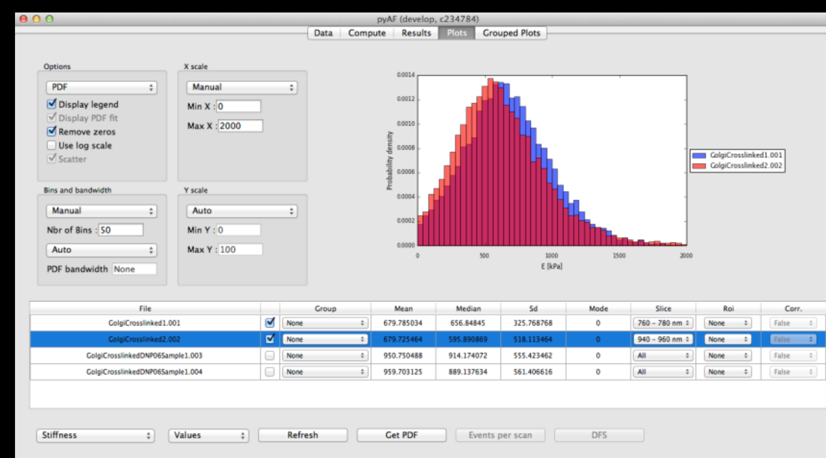
manual vs auto → home-built software

pyAF

Elasticity mapping

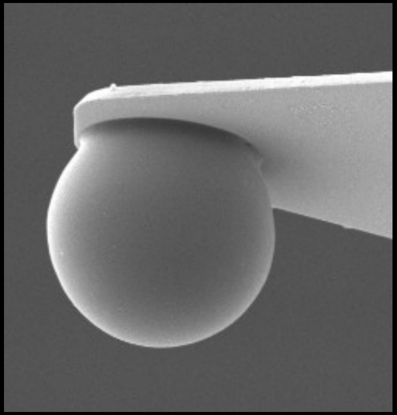


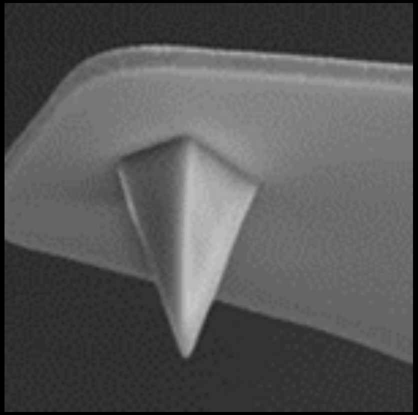




Histograms (stats)

Sharp tips or colloidal probes





Colloidal probes :

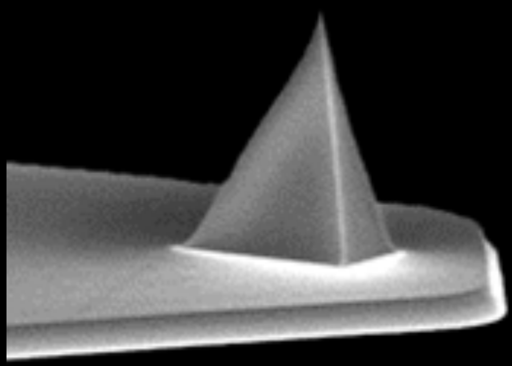
- Whole cell elasticity
- Well defined geometry
- Large contact area

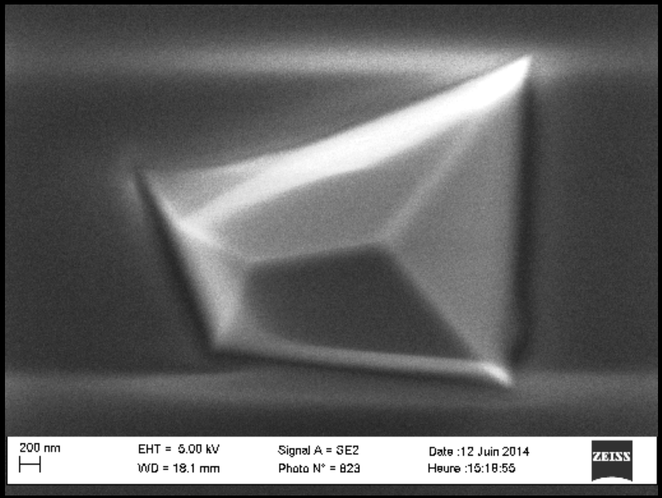
Sharp tip :

- Force mapping
- Precise tip geometry unclear
- Strain stiffening
- Damage a cell at high LR

Limitations : Elasticity measurements

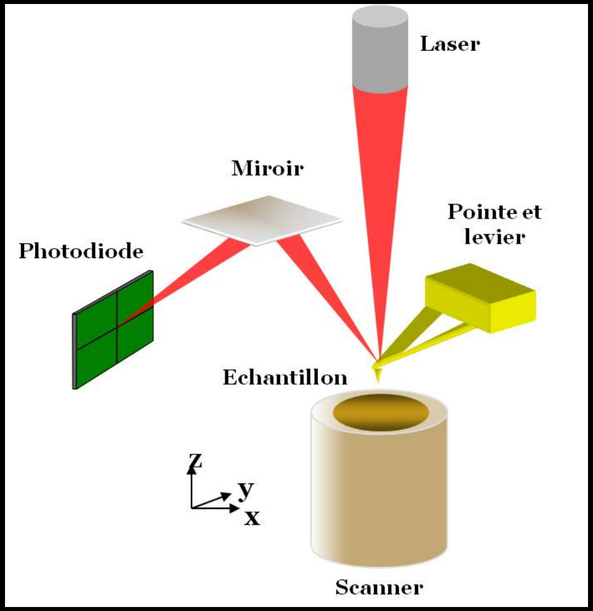
- Models in function of the tip geometry (Force, contact area …)
- Determination of the shear modulus
- Determination of the viscous drag
- Determination of the hydrodynamic drag on the cantilever
- Quality of the probe





Sources of errors

Determination of deflection sensitivity is a critical step in cantilever calibration



position of the laser spot on the detector (V)

↓

z-piezo movement (nm)

(how much cantilever bending corresponds to 1 V detector signal)

→ **Deflection sensitivity (nm/V)**

⇒ **a small variation of the deflection sensitivity value causes a significant change in spring constant k (and then the calculated force F)**

Calibration limits

→ SNAP protocol (2017)

SCIENTIFIC REPORTS

OPEN

Standardized Nanomechanical Atomic Force Microscopy Procedure (SNAP) for Measuring Soft and Biological Samples

Received: 10 January 2017
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Published online: 11 July 2017

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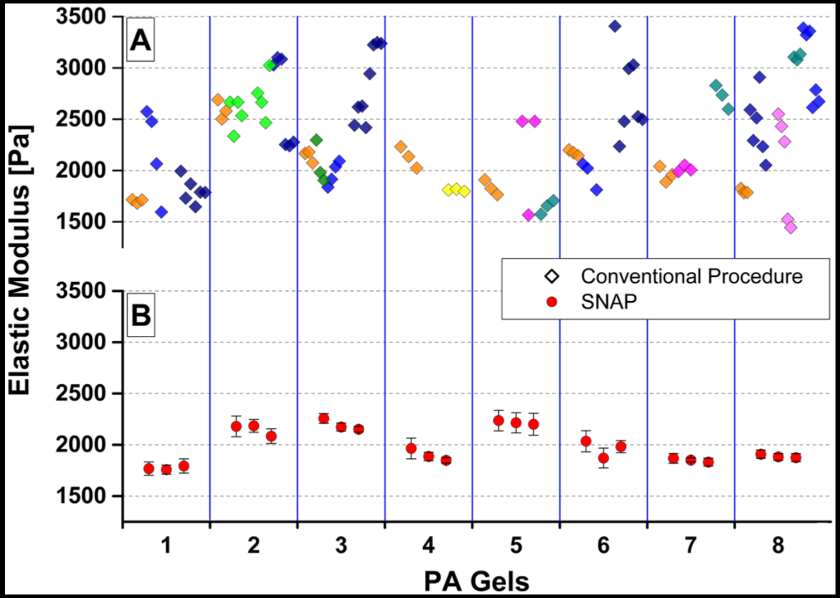
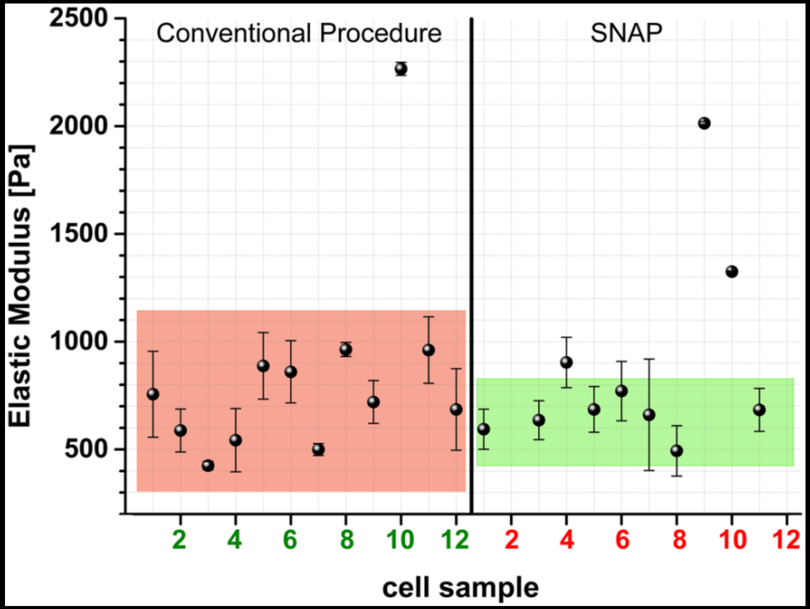
SNAP procedure

- Calibrated cantilever (known k ↔ interferometer)
- Thermal
- Deflection sensitivity





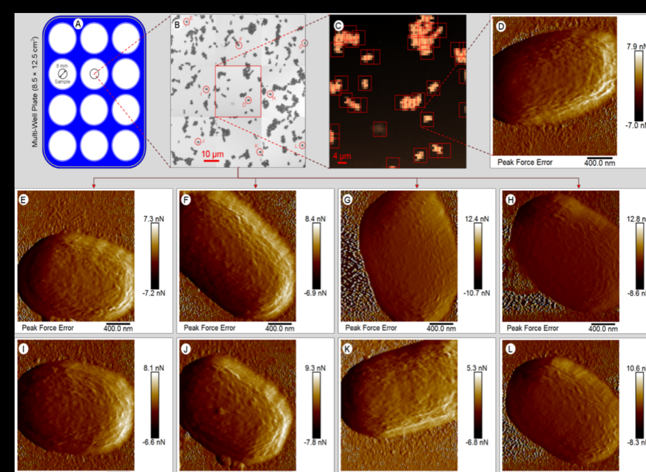
⇒ the errors present in gel measurements decreased from 30% down to 1% while in cell measurements the consistency increased by a factor of 2.

Statistics

	Colloidal probe	Sharp tip (mapping)
Cells per condition (sample)	~ 50-100	~ 10-20
Force curves recorded per single cell	~ 1-10	~ 100-10000

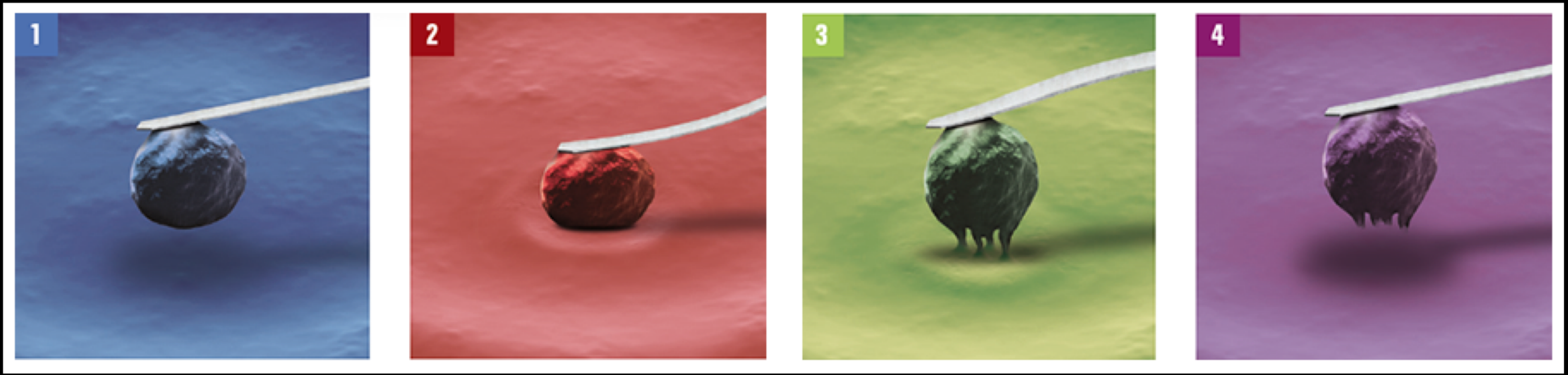
⇒ Several thousand of force curves to be analyzed

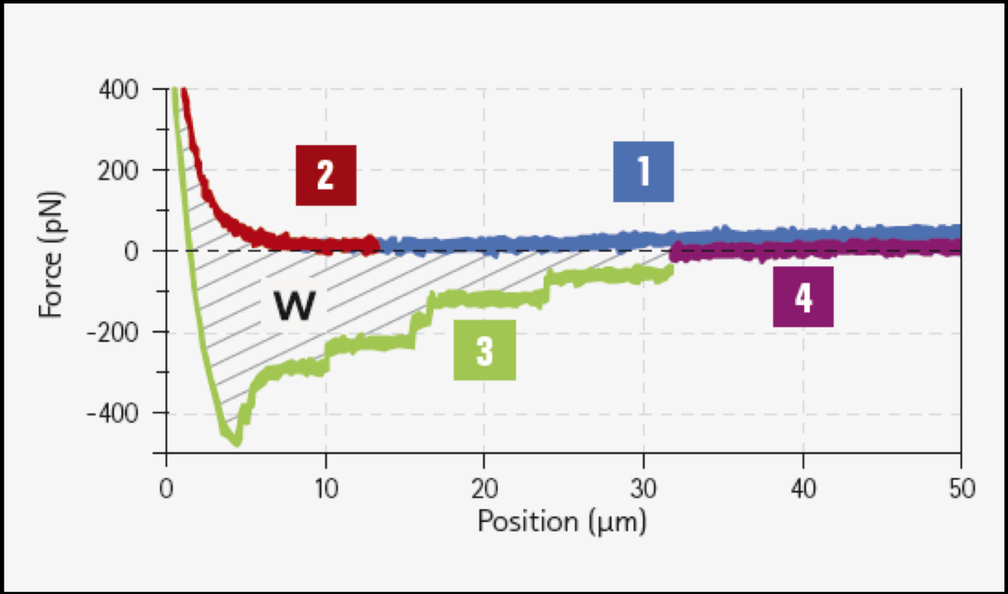
→ Fully automated multi-sample analysis using AFM



Dujardin *et al.*, PloS One (2019)

Cell Hesion (JPK)





Summary

Working conditions for a cell mechanics experiment :

→ stiffness of the cantilever (k)
→ shape of the indenting tip (⇒ model)
→ location on the cellular surface (whole cell, nucleus, …)

→ fix all the parameters :

- ✓ setpoint (indenting force / depth)
- ✓ tip velocity (ramp, frequency, …)

For best results in mechanical measurements :

→ use colloidal probes
→ spring constant calibrated by an interferometer
→ set the deflection sensitivity according to the SNAP procedure

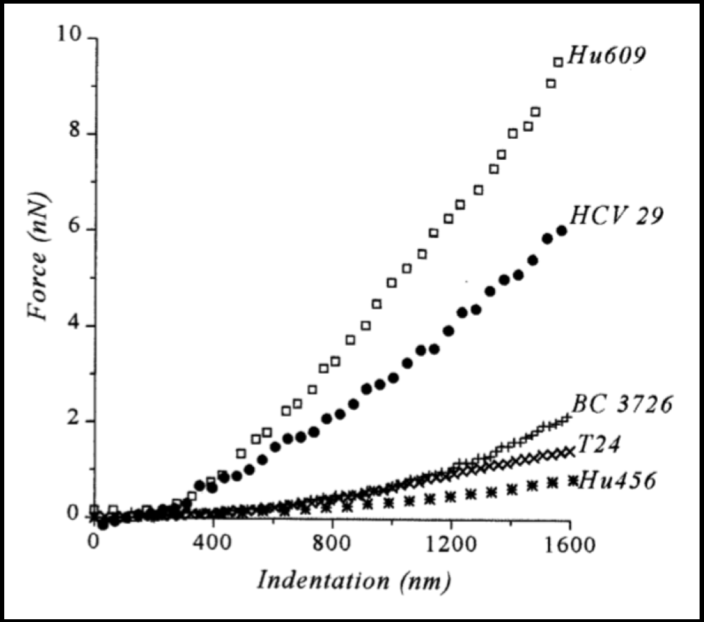
→ if possible, use the same cantilever…

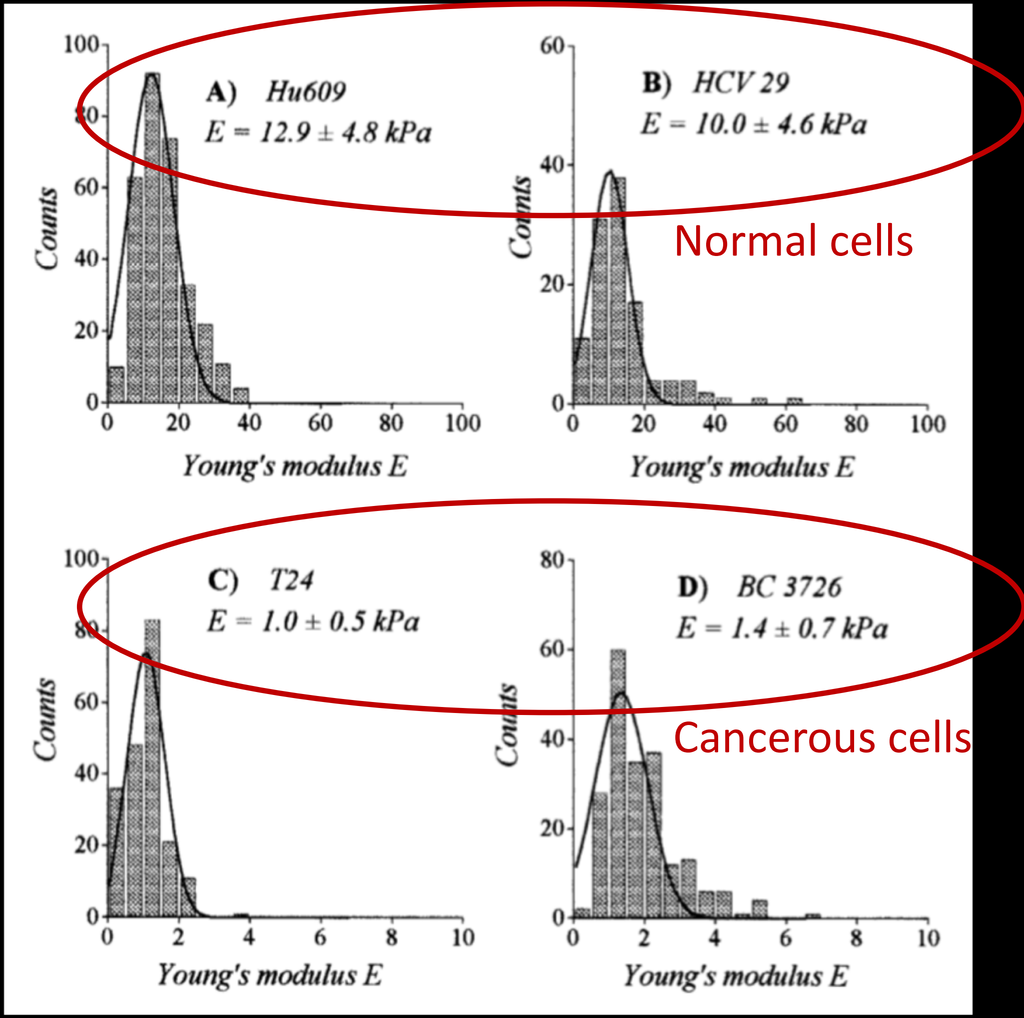
⇒ Absolute values of the Young's modulus are difficult to be obtained

1) How can we measure cell mechanics by AFM ?
2) Why (cell mechanics in diseases) ?
3) Perspectives

Relevance in Medicine and Physiology

→ **Cell stiffness changes during cancer**





Lekka *et al.*, Eur Biophys J (1999) 28, 312-316

Micron 43 (2012) 1259–1266

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Cancer cell recognition – Mechanical phenotype

Małgorzata Lekka[a,*], Katarzyna Pogoda[a], Justyna Gostek[a,b], Olesya Klymenko[a], Szymon Prauzner-Bechcicki[a], Joanna Wiltowska-Zuber[a], Justyna Jaczewska[a,c], Janusz Lekki[a], Zbigniew Stachura[a]

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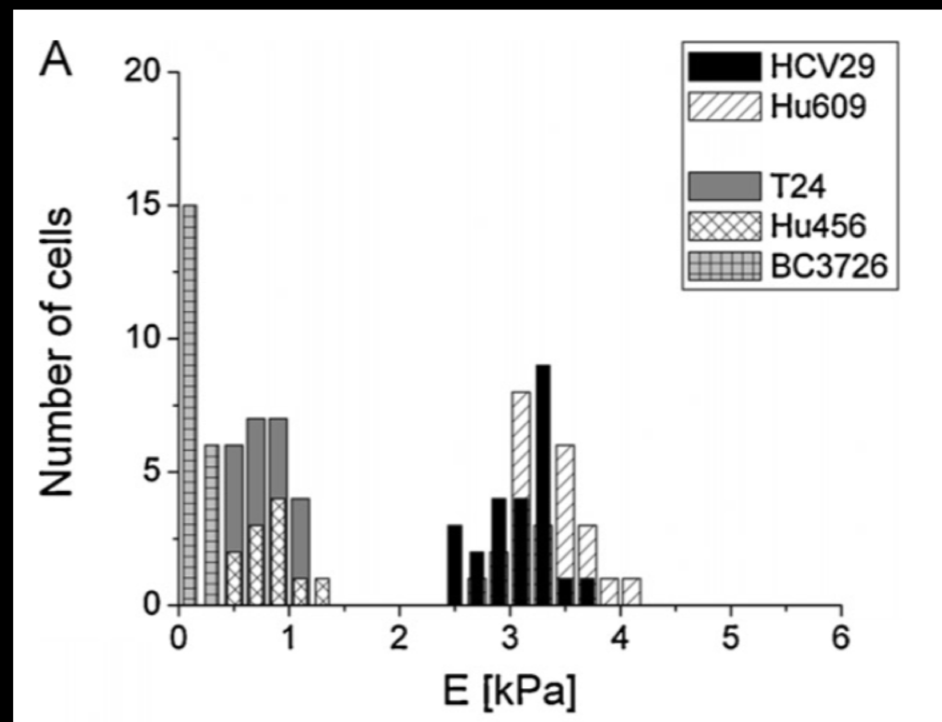
[c] Florida Atlantic University, Charles E. Schmidt College of Medicine, 777 Glades Rd., Boca Raton, FL 33431, United States



Relevance in Medicine and Physiology

→ **Cell stiffness changes during cancer (tissue)**

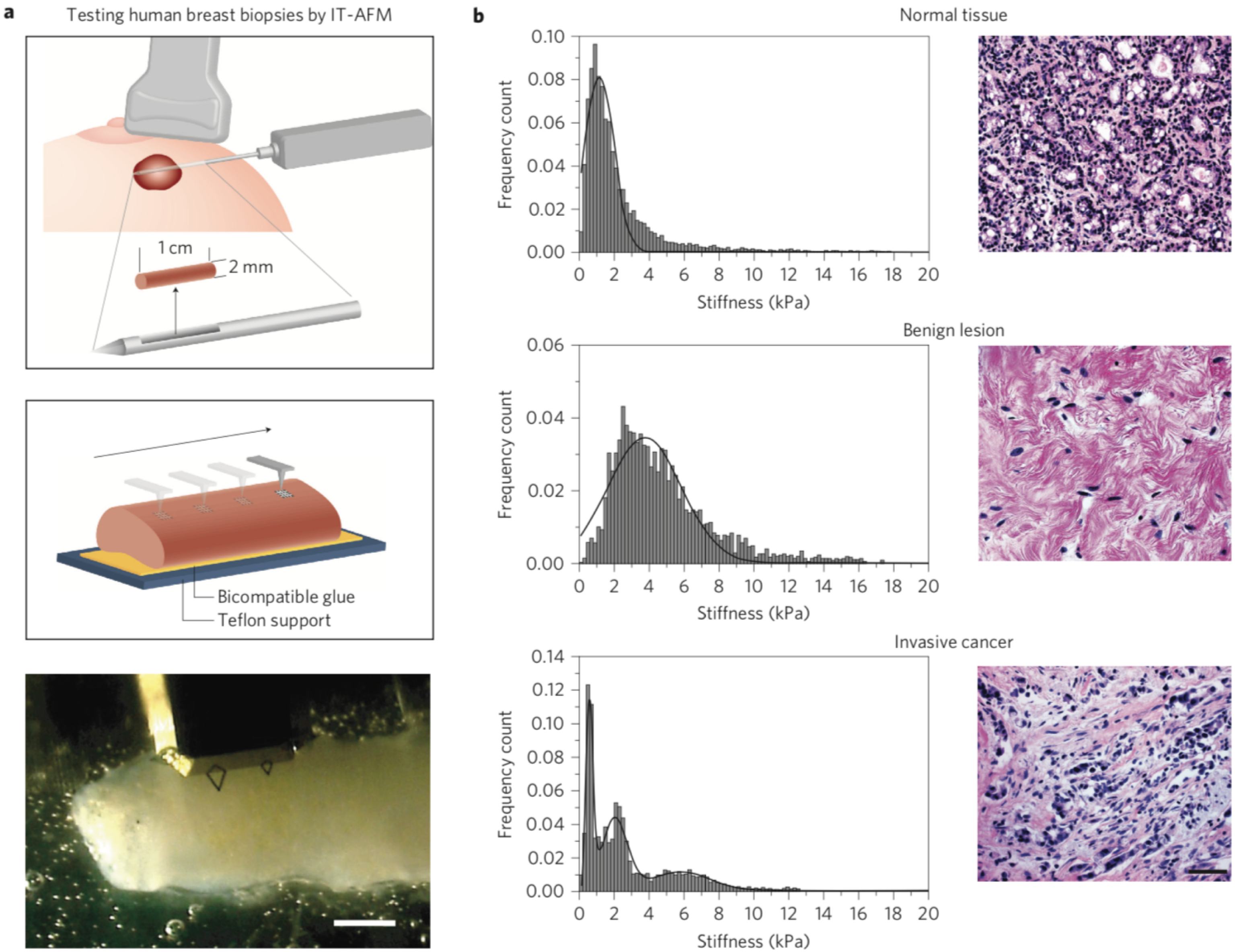
nature nanotechnology

ARTICLES

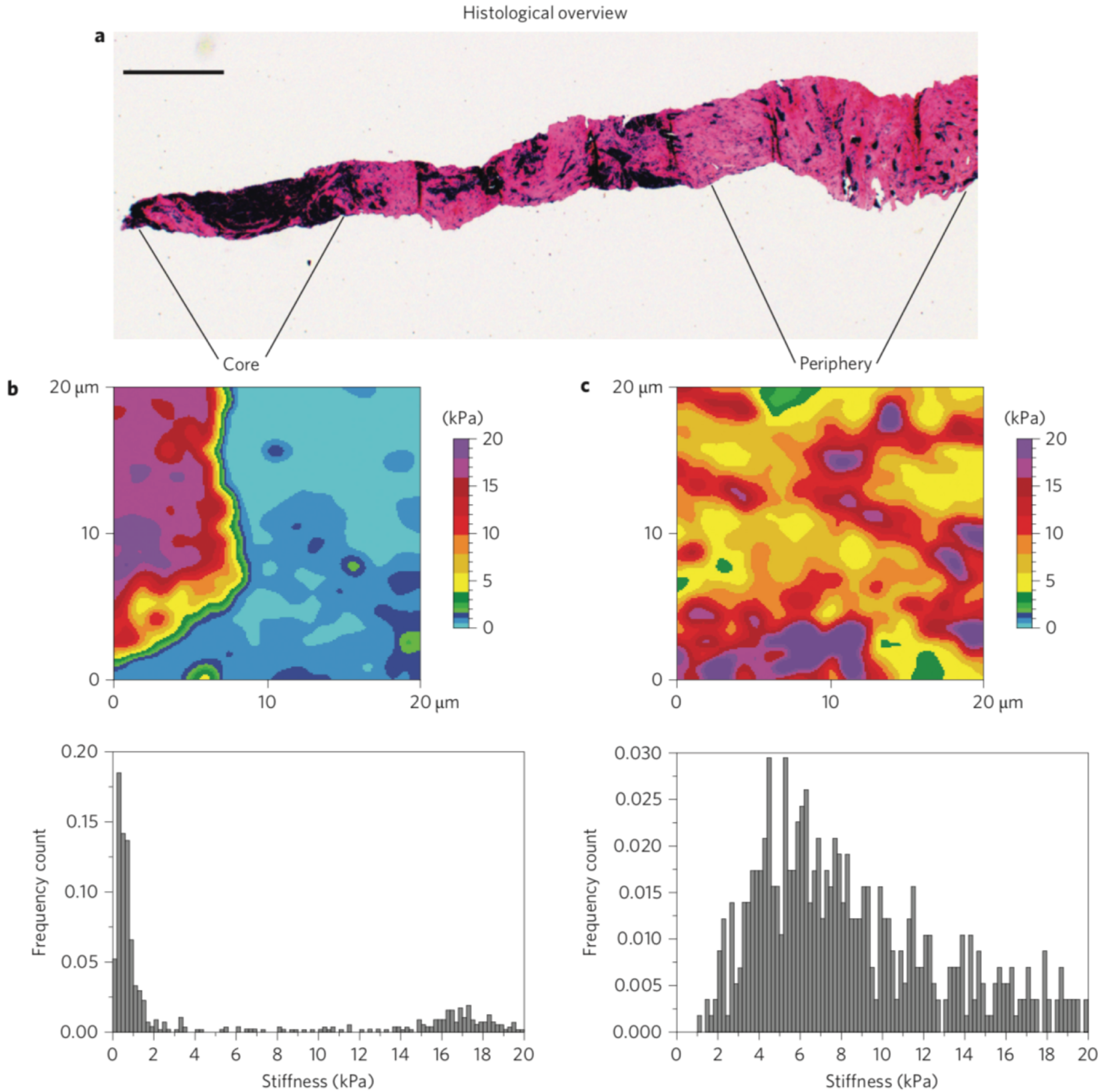
PUBLISHED ONLINE: 21 OCTOBER 2012 | DOI: 10.1038/NNANO.2012.167

The nanomechanical signature of breast cancer

Marija Plodinec[1,2], Marko Loparic[1,2†], Christophe A. Monnier[1†], Ellen C. Obermann[3†], Rosanna Zanetti-Dallenbach[4†], Philipp Oertle[1], Janne T. Hyotyla[1], Ueli Aebi[2], Mohamed Bentires-Alj[5], Roderick Y. H. Lim[1*] and Cora-Ann Schoenenberger[2]



a
Testing human breast biopsies by IT-AFM
1 cm
2 mm
Bicompatible glue
Teflon support
b
Normal tissue
Benign lesion
Invasive cancer
Frequency count
Stiffness (kPa)

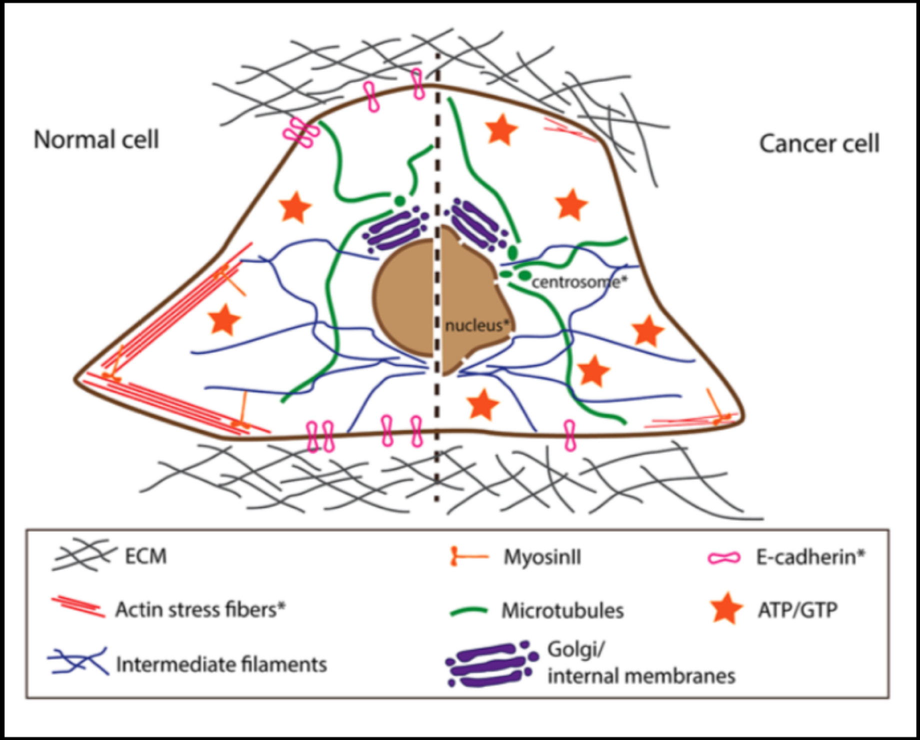


Histological overview
a
Core
Periphery
b
20 µm
10
0
0
10
20 µm
(kPa)
20
15
10
5
0
0.20
0.15
0.10
0.05
0.00
Frequency count
0 2 4 6 8 10 12 14 16 18 20
Stiffness (kPa)
c
20 µm
10
0
0
10
20 µm
(kPa)
20
15
10
5
0
0.030
0.025
0.020
0.015
0.010
0.005
0.000
Frequency count
0 2 4 6 8 10 12 14 16 18 20
Stiffness (kPa)

Are cancer cells really softer than normal cells?

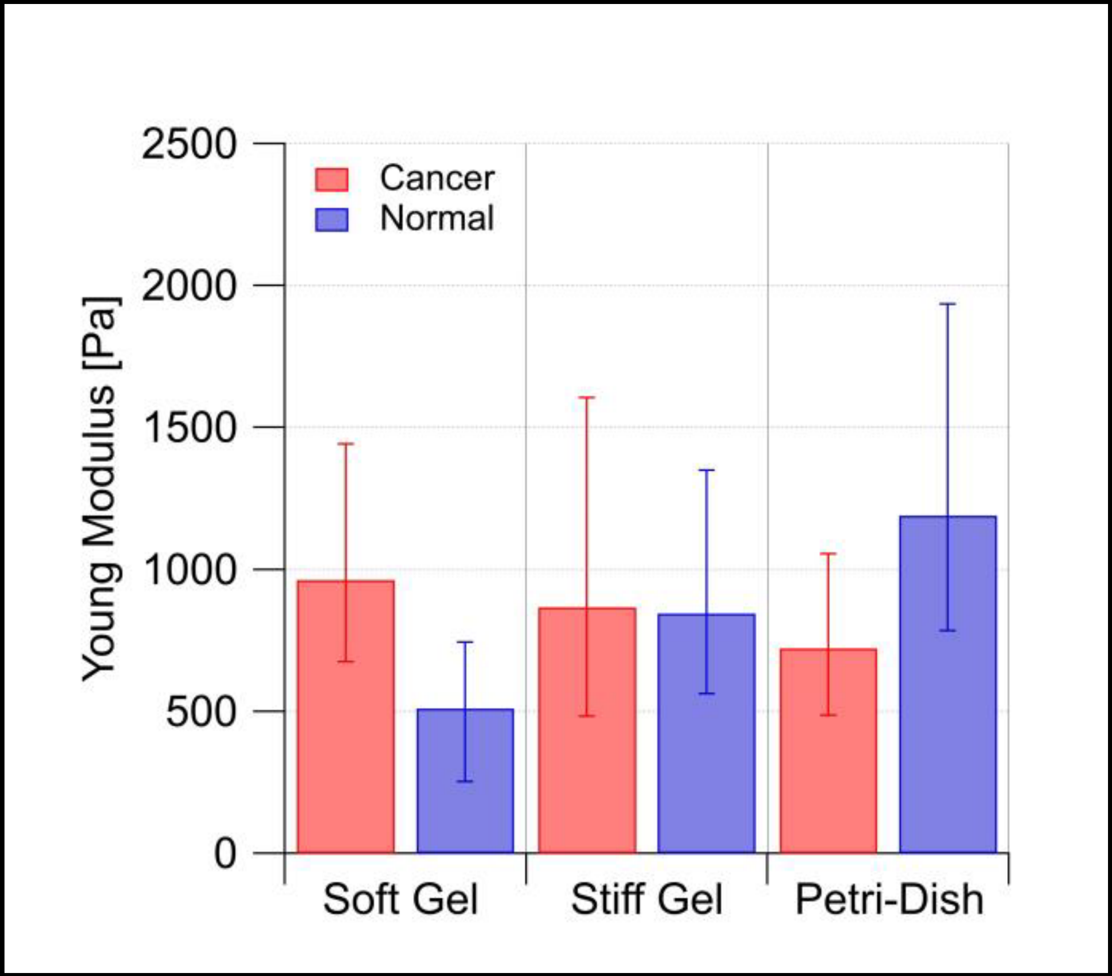
Charlotte Alibert*†, Bruno Goud*† and Jean-Baptiste Manneville*†[1]
*Institut Curie, PSL Research University, CNRS, UMR 144, Paris, France and †Sorbonne Universités, UPMC University Paris 06, CNRS, UMR 144, Paris, France

Biol. Cell (2017) 109, 167-189



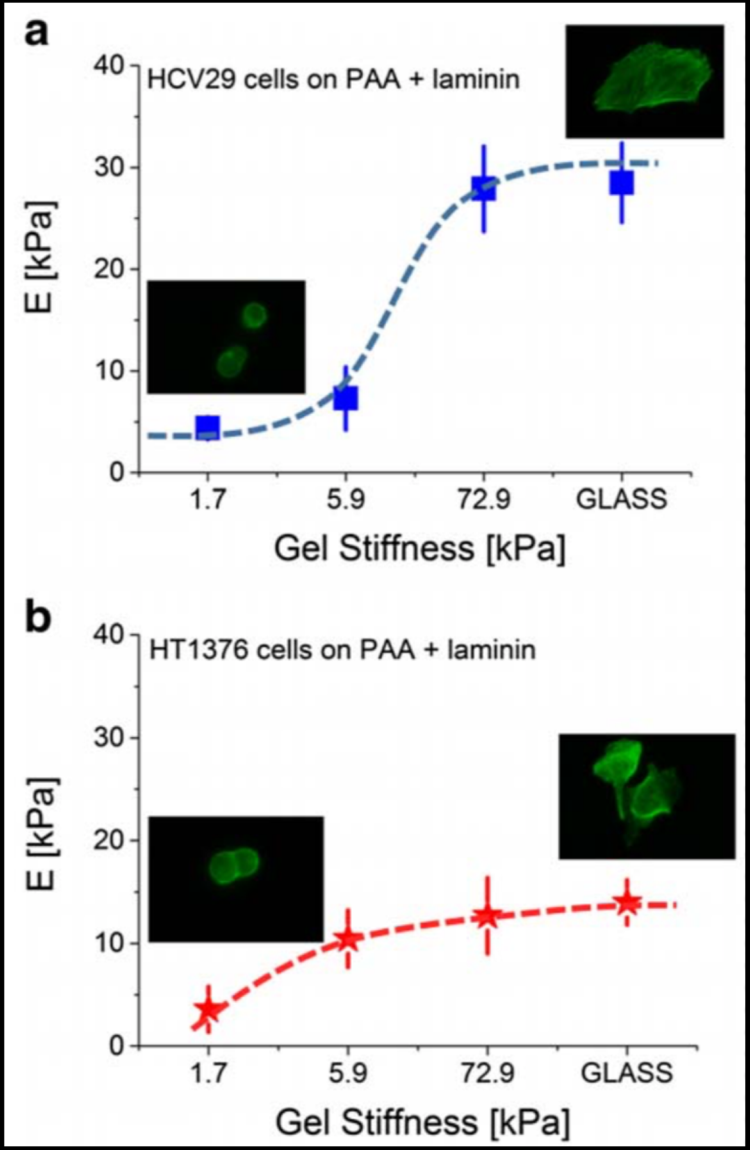
"The mechanical properties of cancer cells could thus be used as novel diagnostic and/or prognostic markers to complement histological examinations and genetic phenotyping of the tumour"

Cells on different stiffness substrates



C. Rianna, M. Radmacher, Eur. Biophys. J. (2016)

Cells on different stiffness substrates

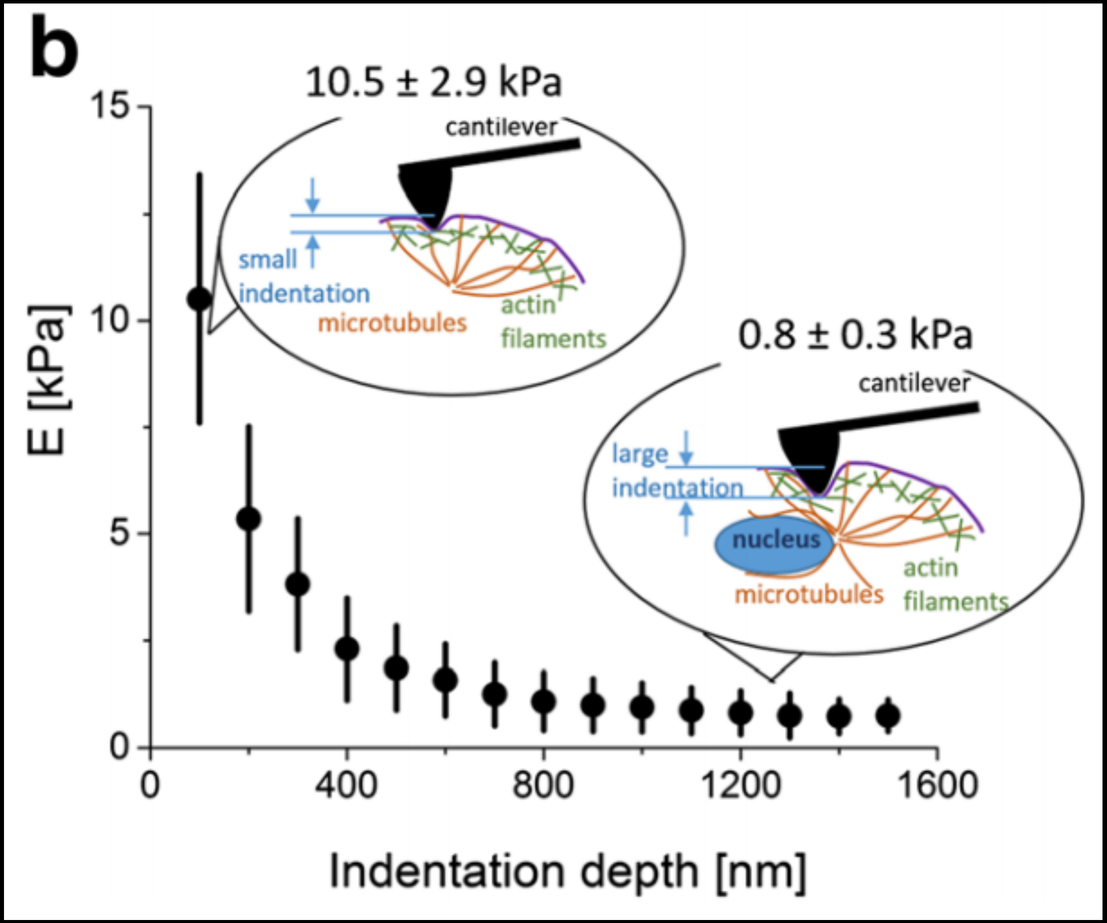


Lekka M. Bionanoscience (2016)

non-malignant cancer cells HCV29

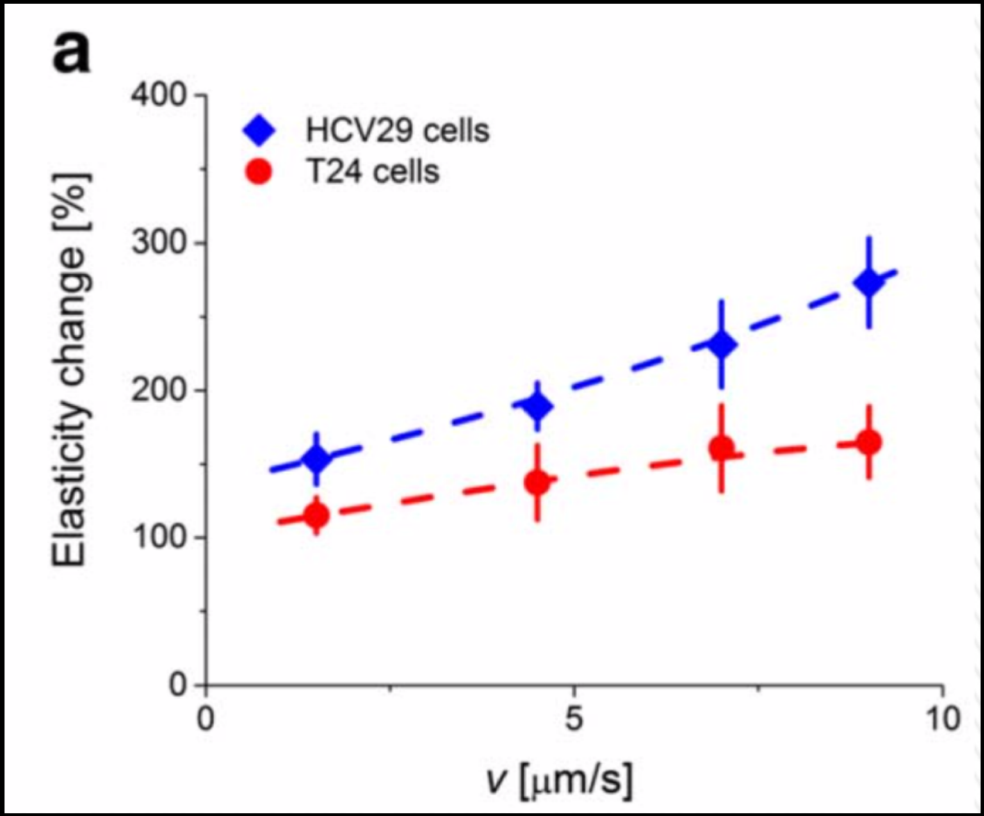
human bladder cancer cell HT1376

Indentation depth



- small indentation depths <200nm: mechanical response of the actin network
- larger indentation depths: the overall elasticity of the whole cell

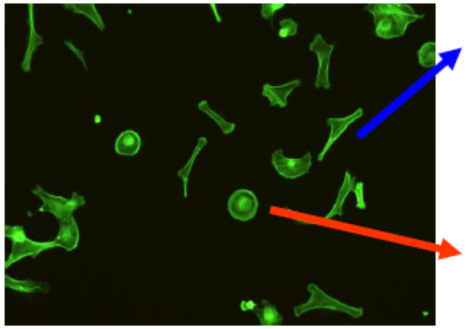
Tip velocity

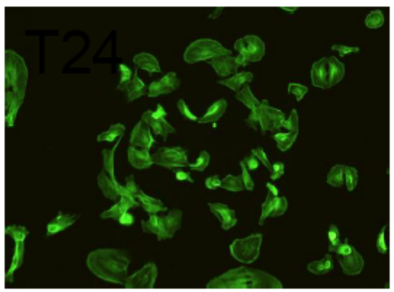


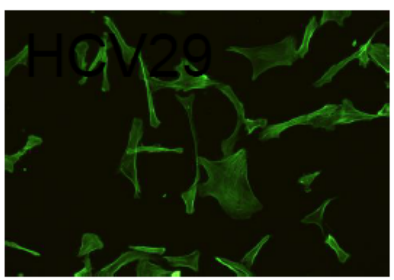
⇒ Measured Young's modulus depends on approach speed (tip velocity)

⇒ Cells appear stiffer at higher velocities

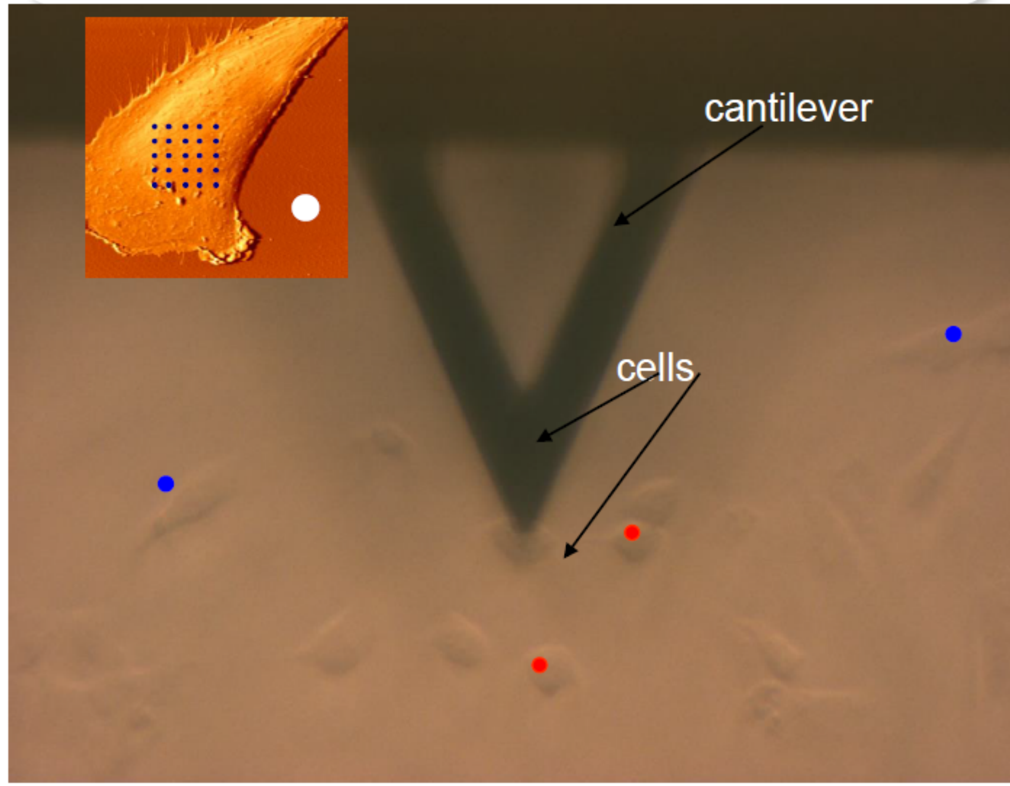
Cancer cell recognition







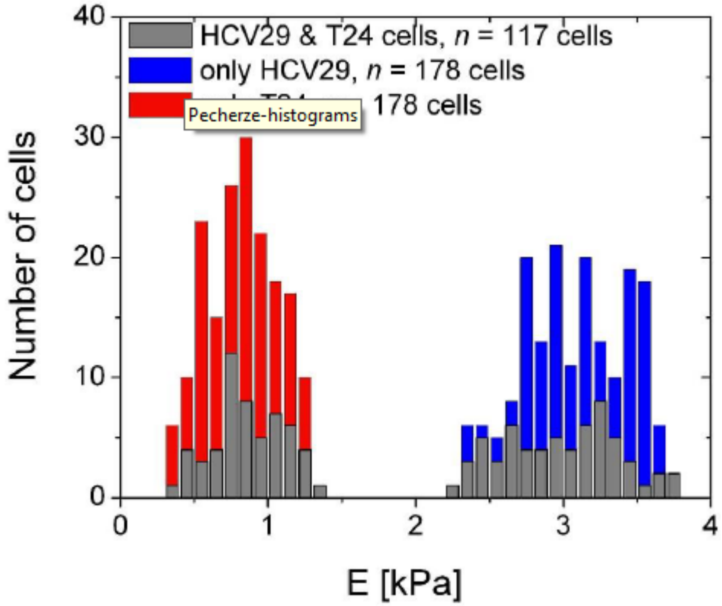
Can we recognized mechanically altered cell in the environment of other cells ?

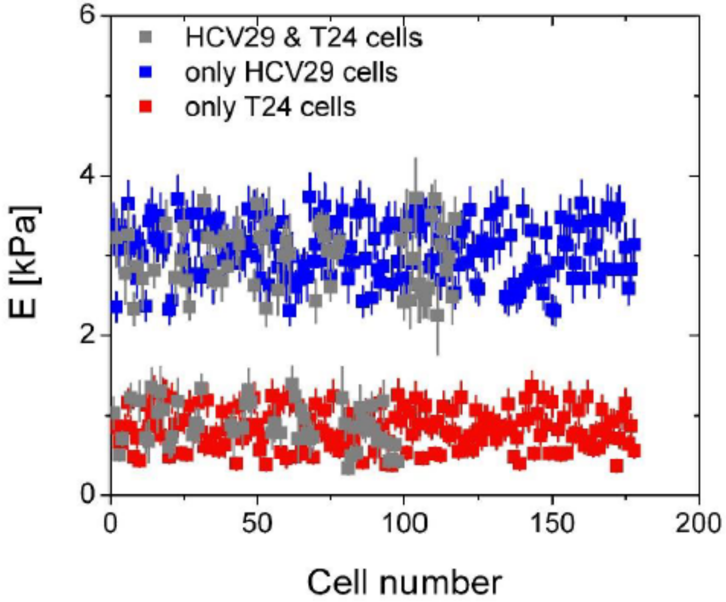


initial cell number
44000 (T24) and 67000 (HCv29) per ml; 24h of growth

Lekka et al., Micron (2012)

Cancer cell recognition





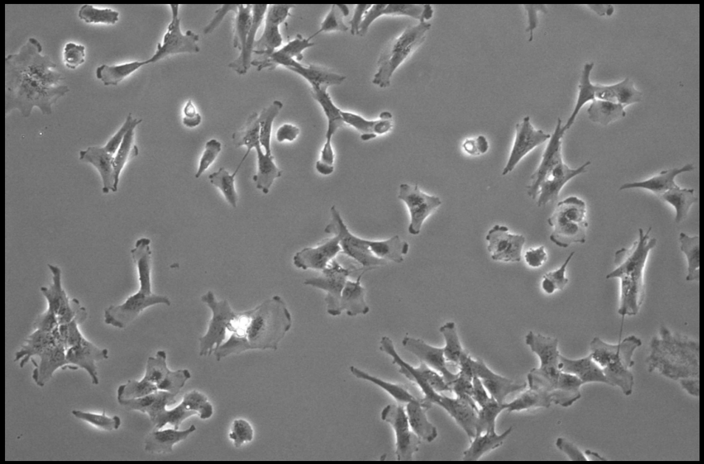
initial cell number 44000 (T24) and 67000 (HCV29) per ml; ratio 1:0.7; 96h

Same results as for separate cell lines

Lekka et al., Micron (2012)

Autophagy and cell mechanics

Cells seeded on glass surfaces adopt different shapes

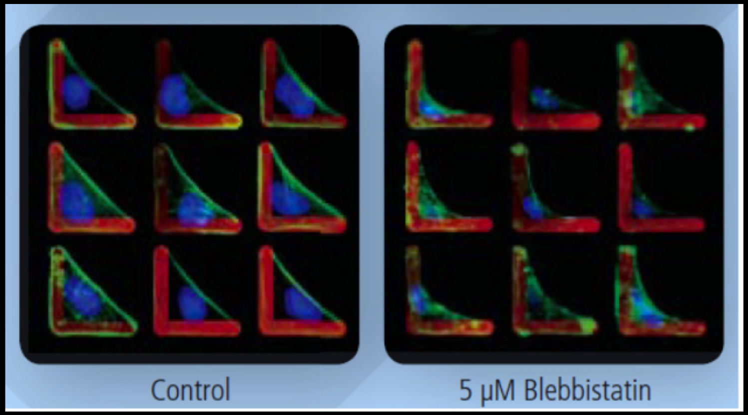


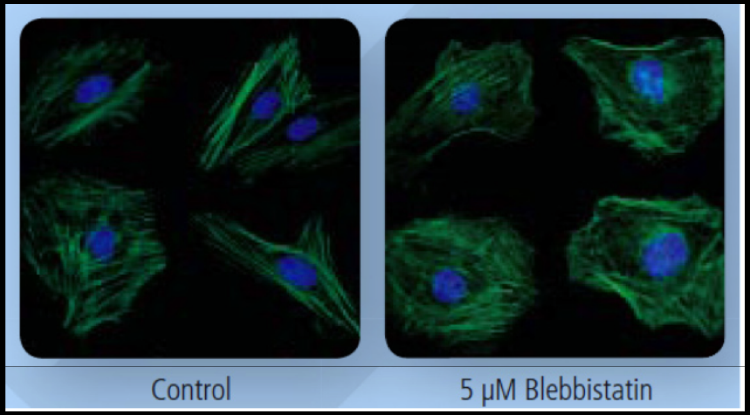
→ not convenient to easily quantify drug effect on cells

→ **Micropatterning**

Autophagy and cell mechanics

By growing cells on adhesive micropatterns, a well defined and confined environment is imposed that drives cells to adopt **regular shapes** and **cytoskeletal organization.**





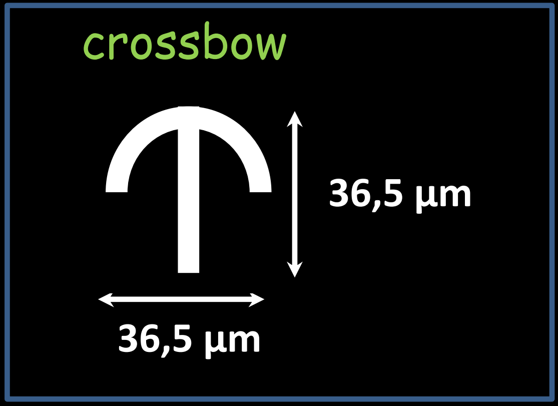
https://cytoo.com

→ Defined cell adhesion geometry (to control cellular processes or to mimic spatial constraints that a cell is exposed inside a tissue)

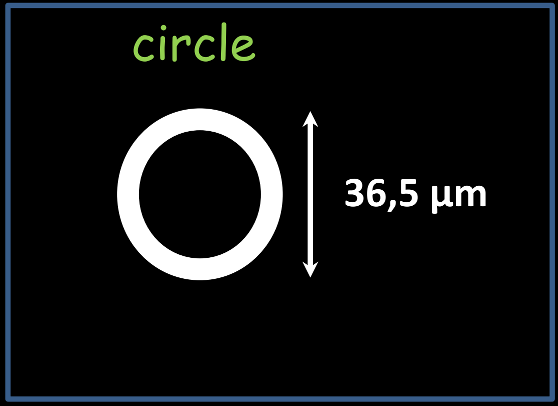
Autophagy and cell mechanics

Patterning

Micropatterns :



→ Strong polarization



→ Non-polarized

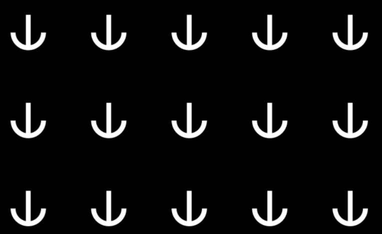
simulate the shape of a migrating cell with the rounded edge mimicking a lamellipodium at the front of a migrating cell.

→ cells **of controlled and regular shapes**

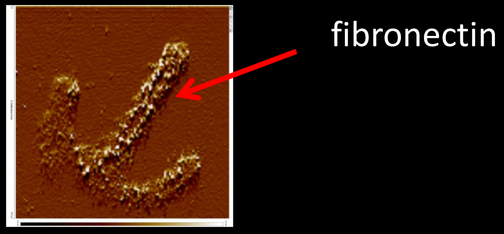
Autophagy and cell mechanics

→ Glass coverslips coated with poly-L-lysine functionalized with poly-ethylen glycol chains (PLL-g-PEG)

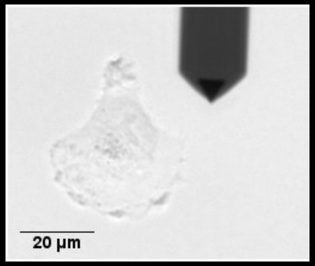
→ Coating removed at precise locations with a defined shape (crossbow) by illumination through a photo mask (or PRIMO).



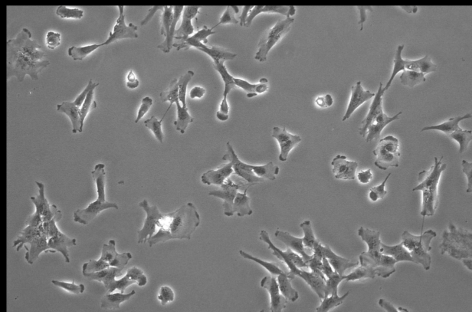
→ The resulting « holes » were then filled with fibronectin



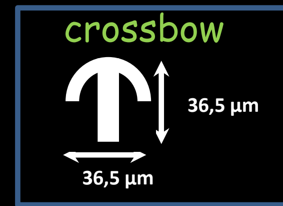
→ RPE1 cells were then seeded onto the micro-patterns

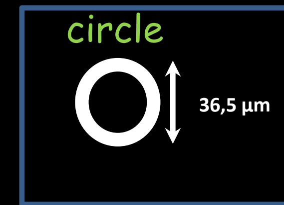


Influence of autophagy on cell mechanics



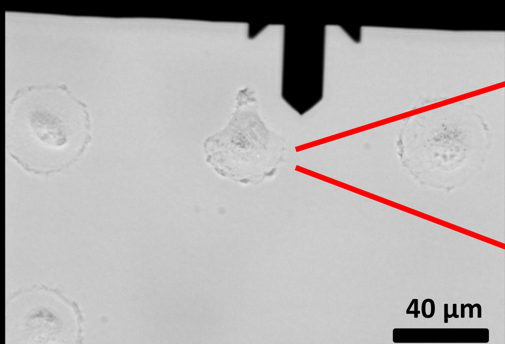
→ **Micropatterning**



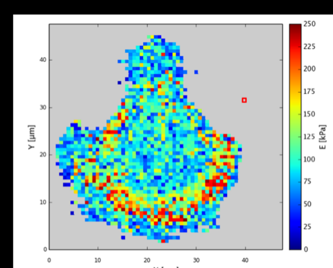


→ cells of **controlled and regular shapes**

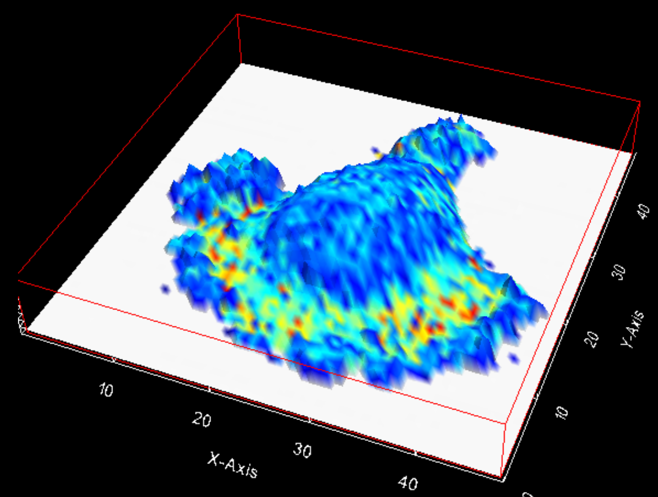
Cell mechanics



E (kPa)
(Sneddon model)

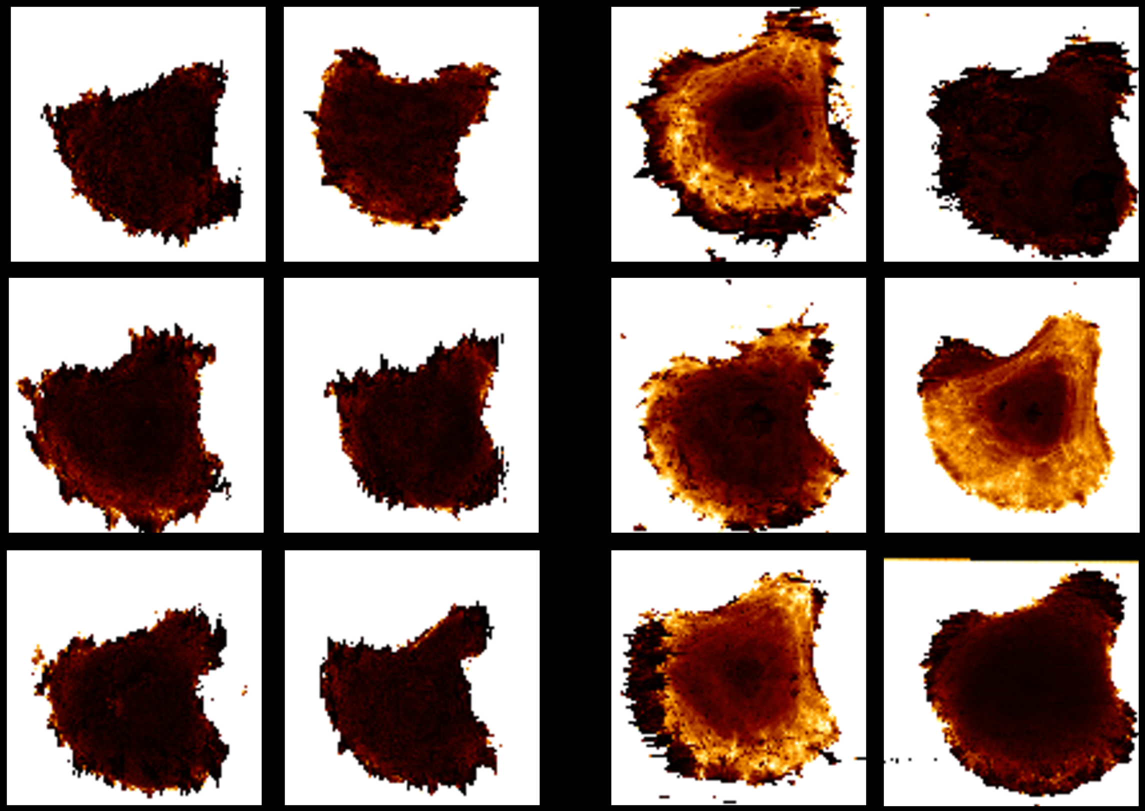


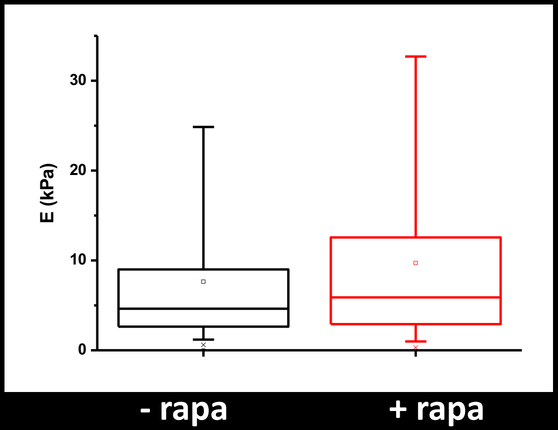
Elasticity map

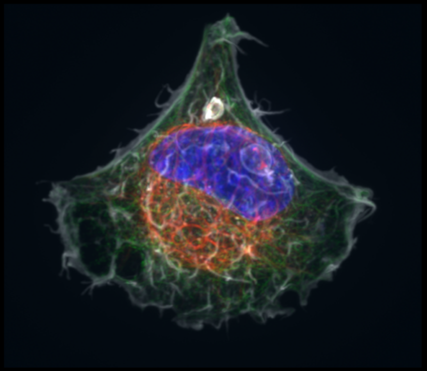


Influence of autophagy on cell mechanics

Without rapamycin *With rapamycin*



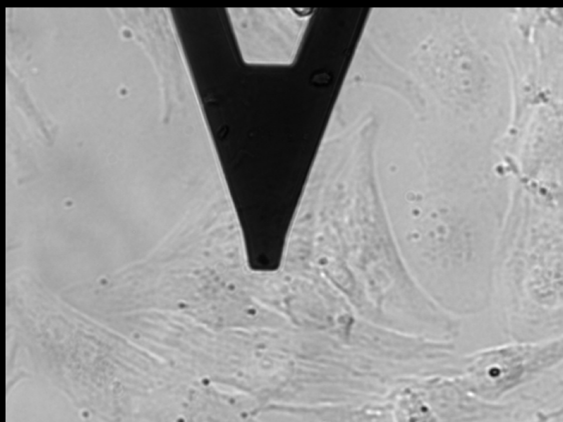


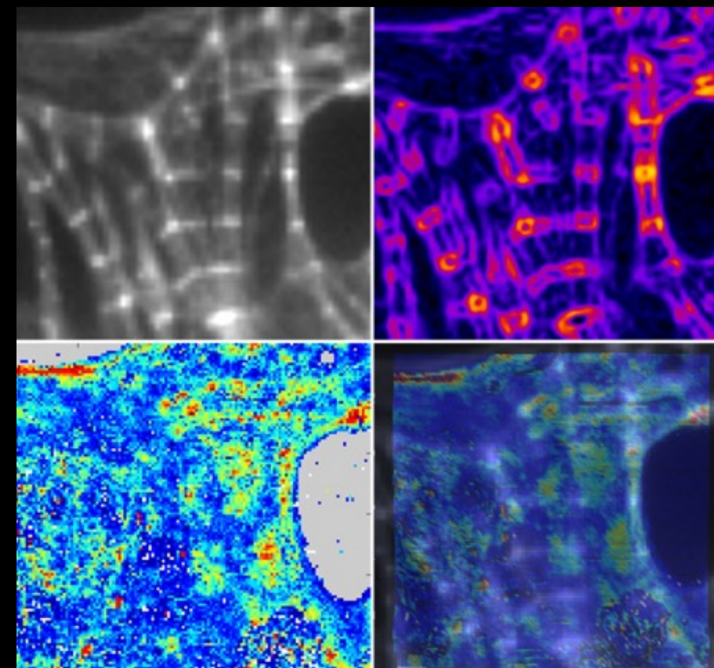


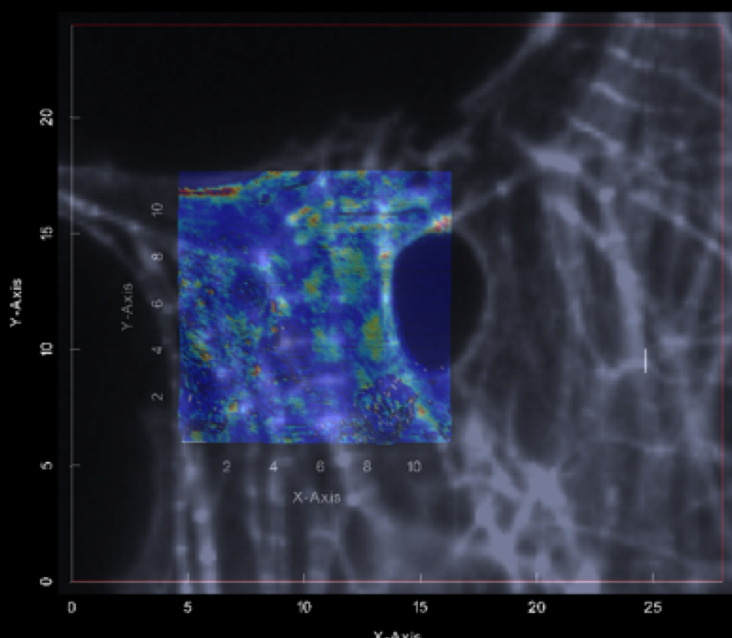
→ Cytoskeleton organization : High resolution imaging (STED)

Coupling

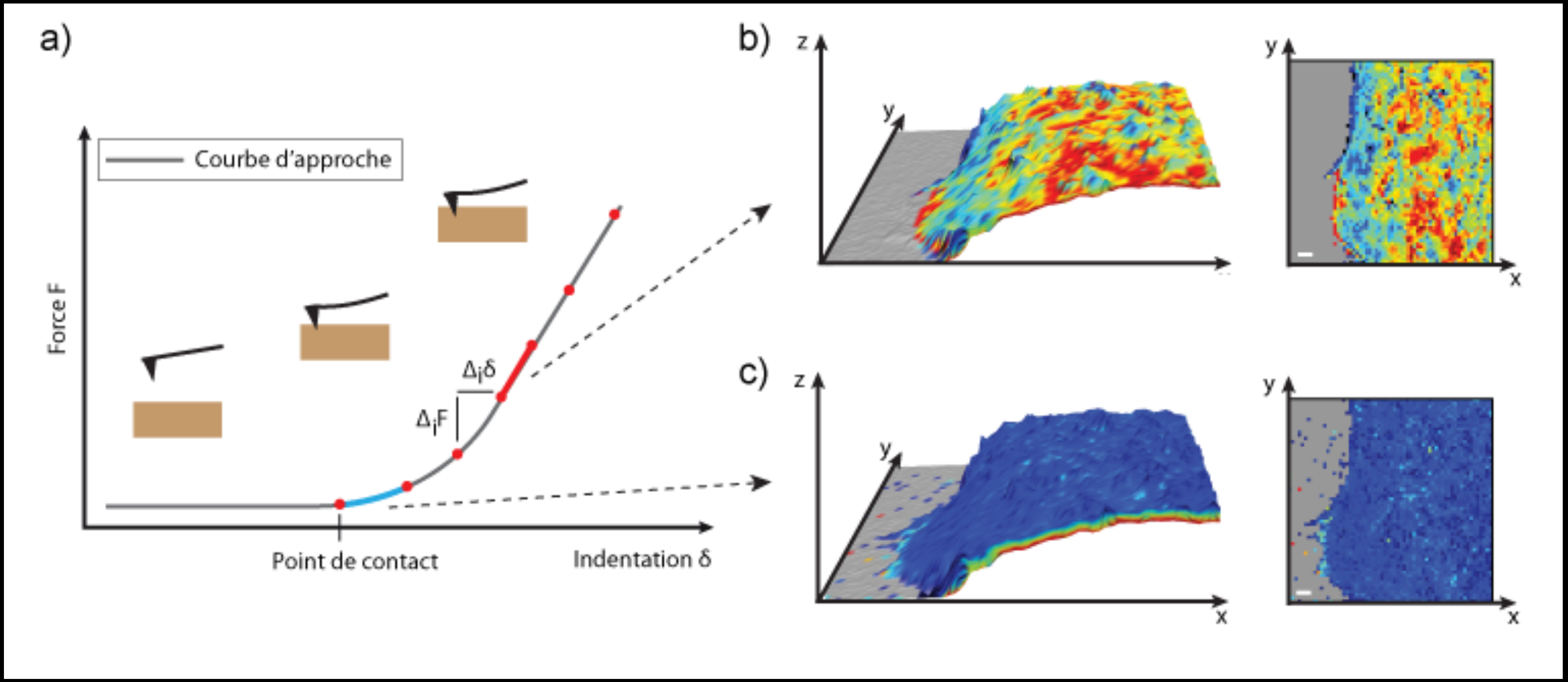
Cardiomyocytes







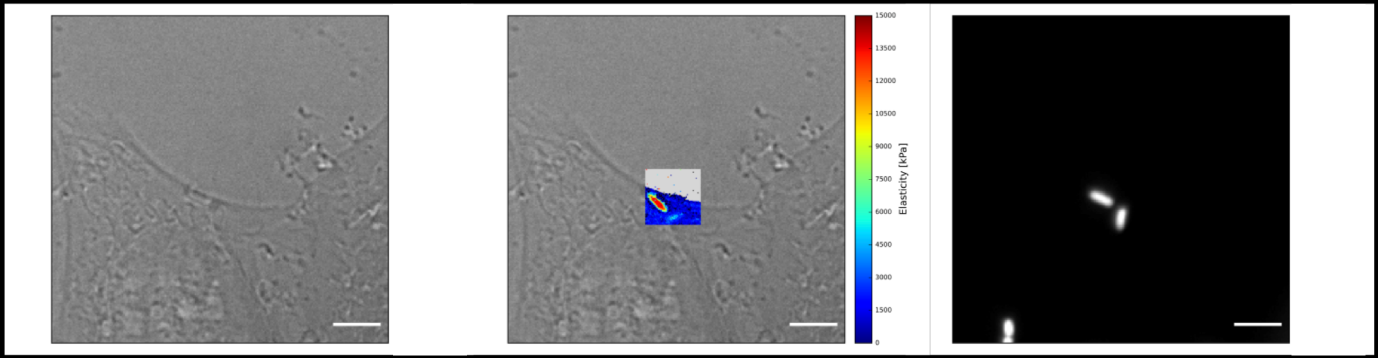
Stiffness tomography



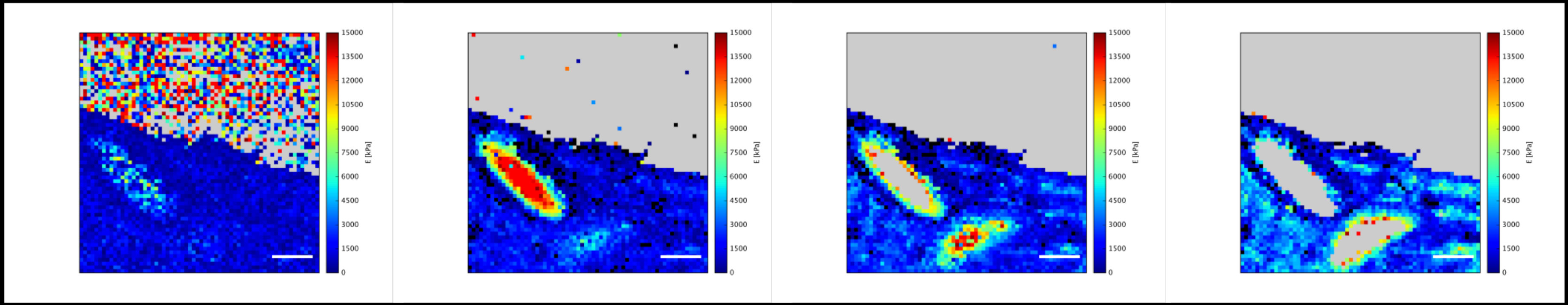
Stiffness tomography

SITE D'ENTREE DE YERSINIA

Infection de cellules HeLa par *Y. pseudotuberculosis*



Lumière transmise Corrélation Fluorescence



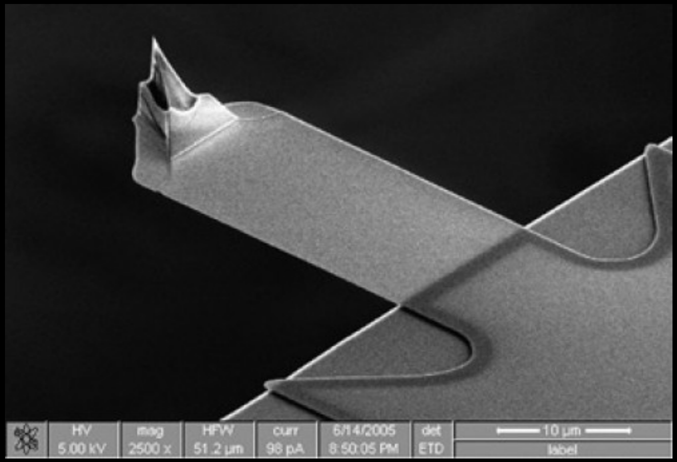
Élasticité 0-10 nm Élasticité 30-40 nm Élasticité 70-80 nm Élasticité 130-140 nm

1) How can we measure cell mechanics by AFM ?
2) Why (cell mechanics in diseases) ?
3) Perspectives

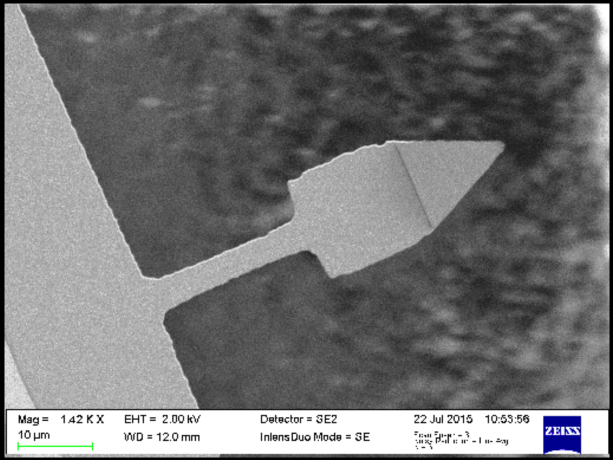
Perspectives : resolution

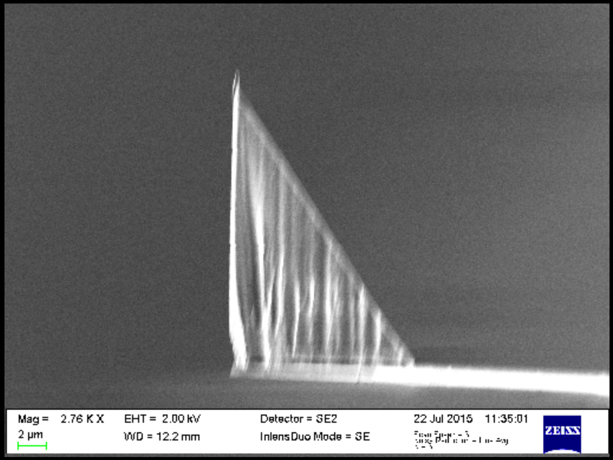
Recent AFM developments :

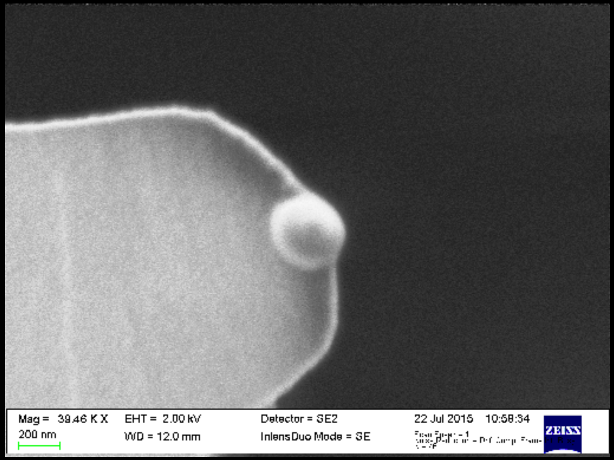
- new probes
- new modes (FV → QI, PFT)



BioLever Mini (AC40 – Bruker)
110 kHz Air, 25 kHz Fluid, 0.1 N/m







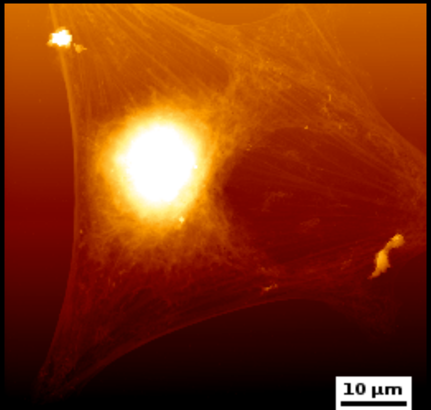
PeakForce QNM-LC (Bruker)

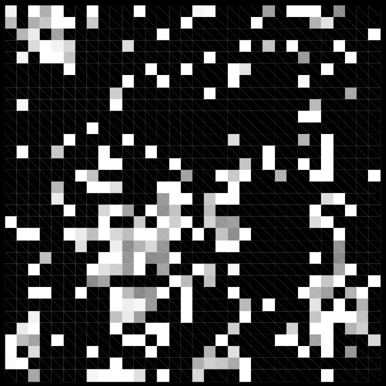
Perspectives : resolution

<u>Recent AFM developments</u> :

- new probes

- new modes (FV → QI, PFT)

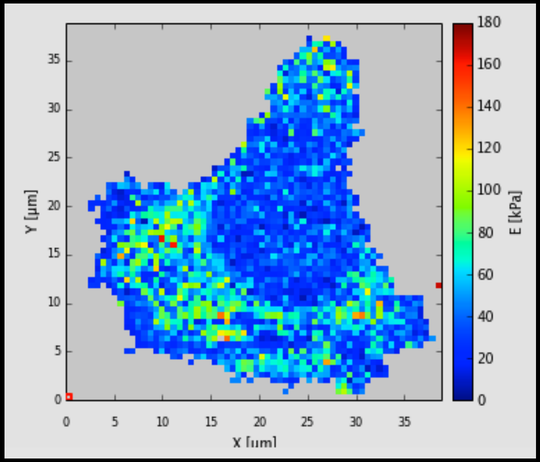




FV 32*32

Acquisition : 30 à 45min

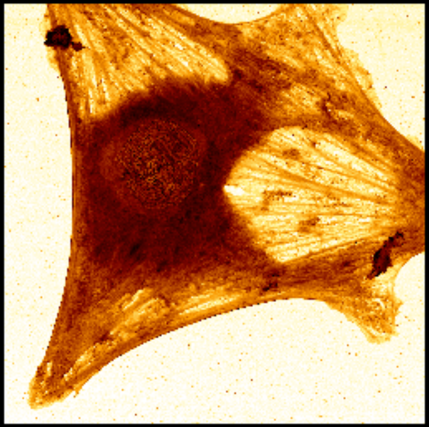
Treatment : ≈ 3-4 heures



QI 64*64

Acquisition : ≈ 5 min

Treatment : ≈ qq minutes



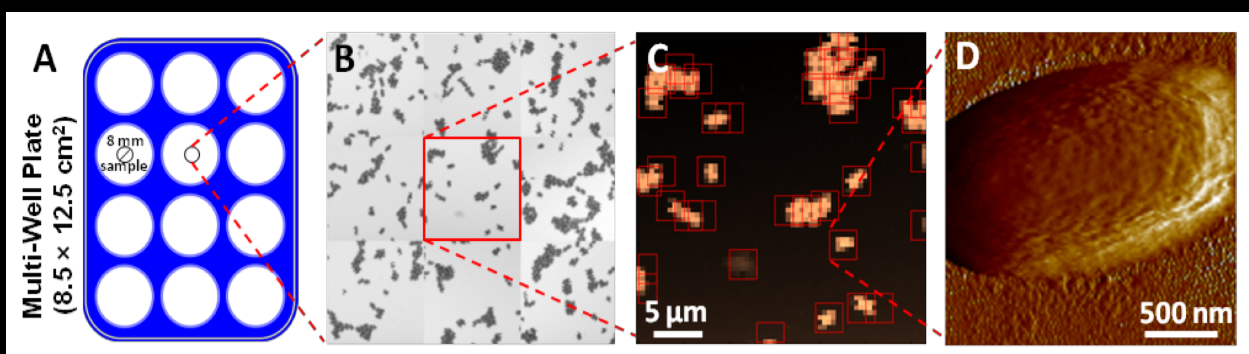
QI 256*256

Acquisition : ≈ 10 min

Treatment : ≈ qq minutes

Perspectives

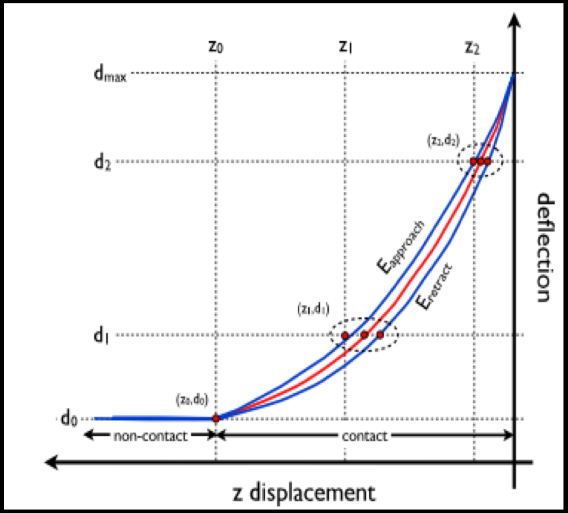
→ automation (middle – high-troughput)



Dujardin *et al.*, PloS One (2019)

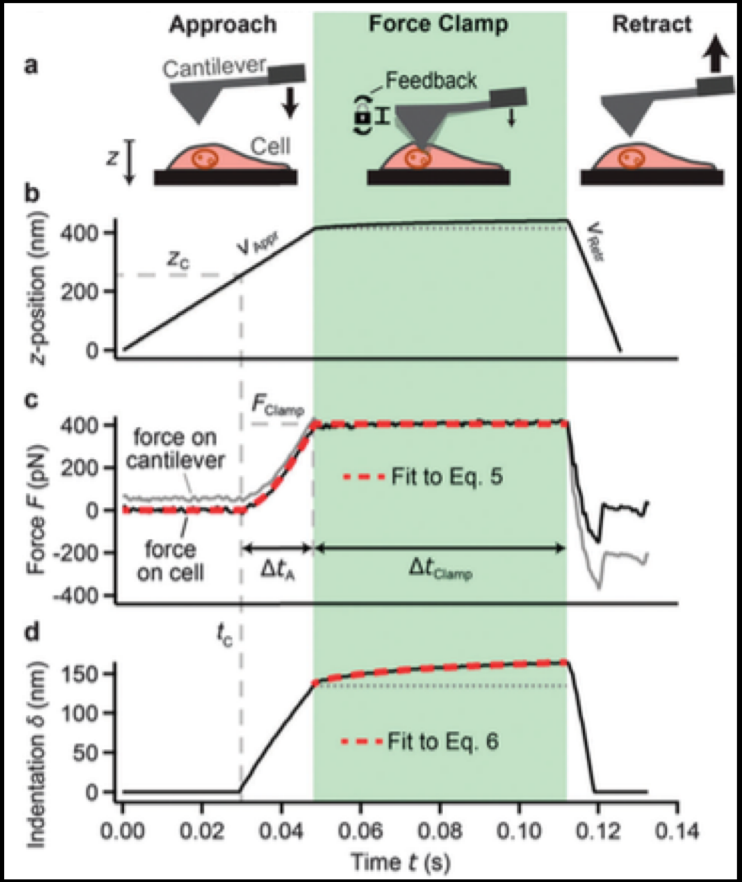
Perspectives

→ **viscosity (viscoelastic properties)**

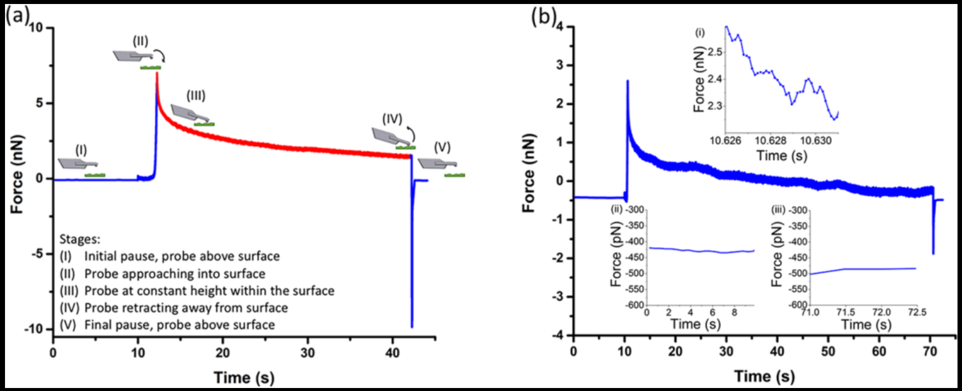


Rebelo *et al.*, Nanotechnology (2013)

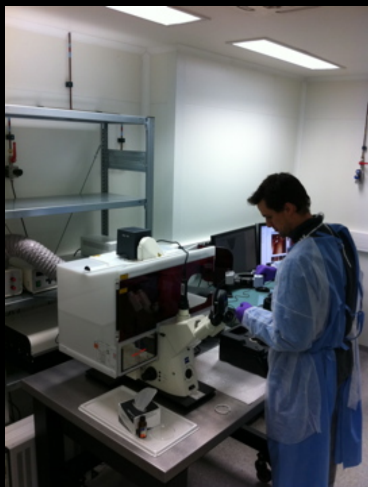
Force Clamp Force Mapping



Hecht *et al.*, Soft Matter (2015)



Chim *et al.*, Sci. Rep (2018)



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Mardi 19 mars 2019

Atelier 1 : Mécano biologie

(S. Janel)