

Jose F. Saenz, Ph.D.

AFM

MICROSCOPIA A FORZA ATOMICA E APPLICAZIONI NELLA BIOINGEGNERIA



UNIVERSITÀ DEGLI STUDI DI CAGLIARI

DIPARTIMENTO DI INGEGNERIA ELETTRICA ED
ELETTRONICA

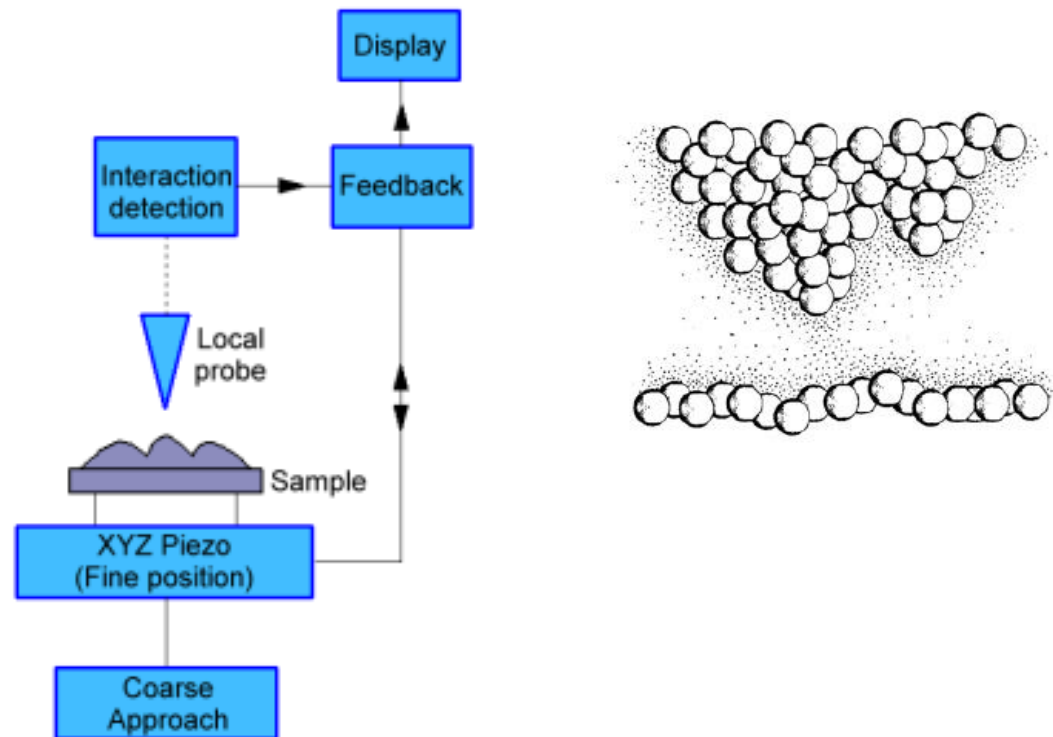


24-11-2015

Microscopia a scansione di sonda (SPM)

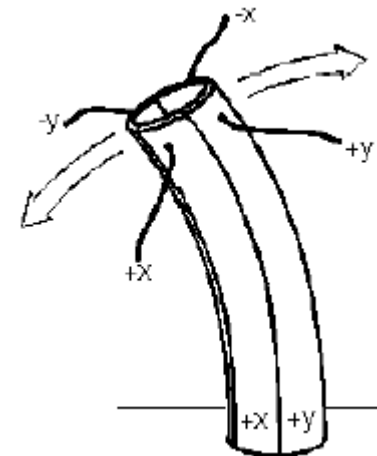
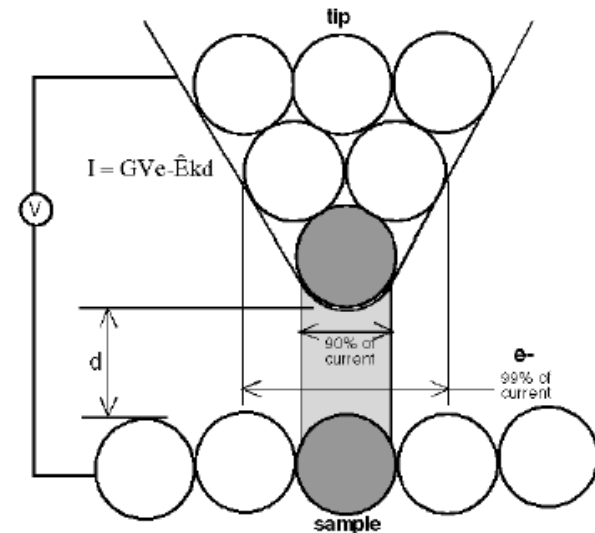
- SPM: Famiglia di tecniche di microscopia che ricavano le immagini di superfici usando una sonda fisica che esegue la scansione del campione.
- Si misurano le interazioni sonda-superficie.

Generalised schematic of a scanning probe microscope



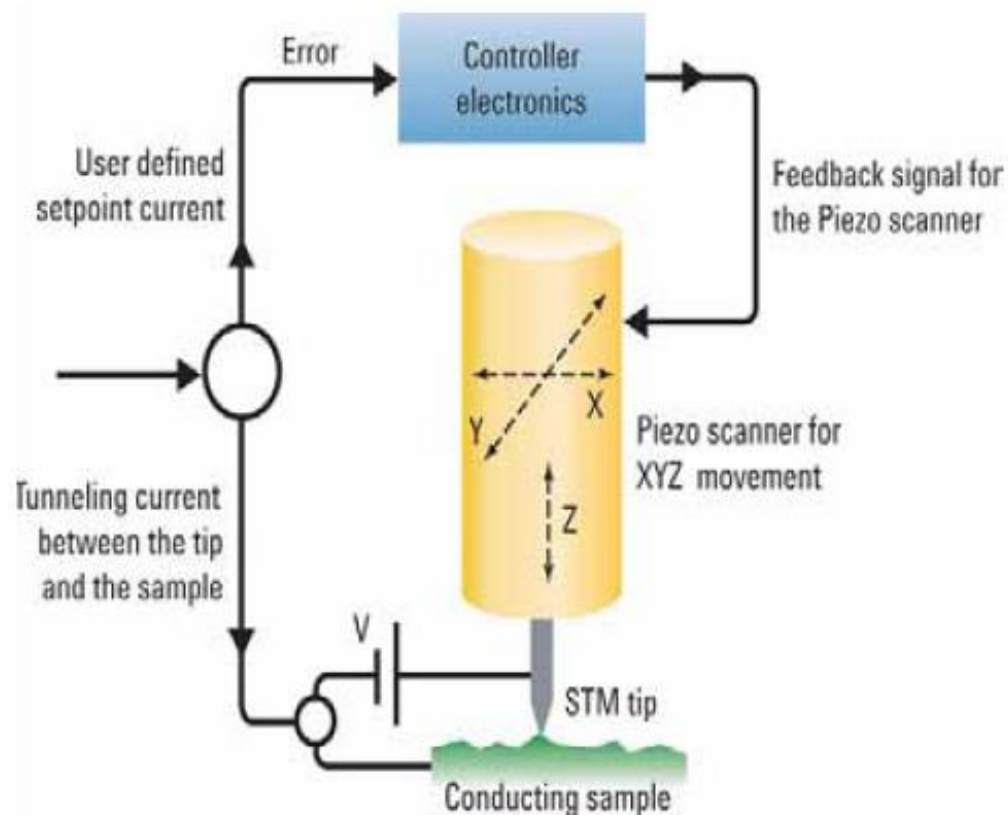
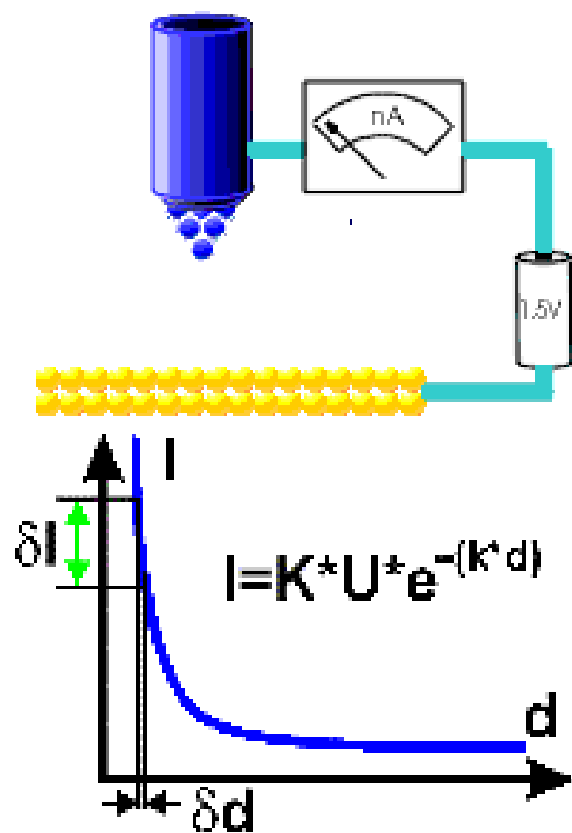
Principio generale di funzionamento delle tecniche SPM

- Sonda che permette rilevare le interazioni locali
- Posizionamento a alta risoluzione
 - Ceramica piezoelettrica (scanner XYZ)
 - $\Delta V \Rightarrow \Delta x$; $\Delta x \Rightarrow \Delta V$



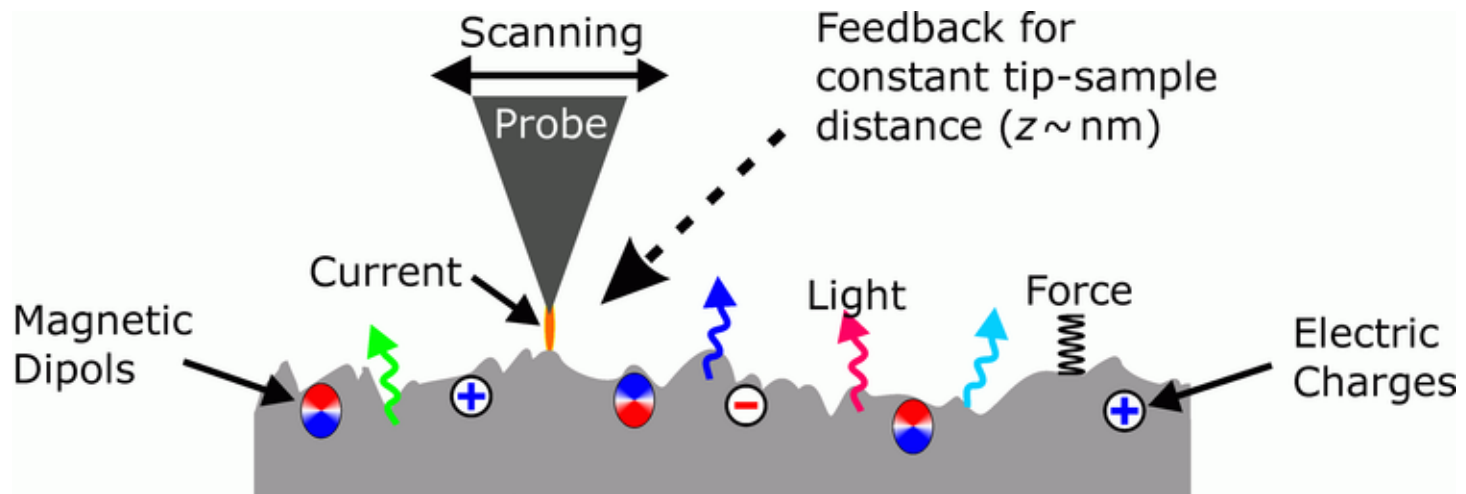
Microscopio a effetto tunnel (STM)

- Si basa sulla misurazione della corrente a effetto tunnel tra la punta e un campione conduttivo



Tecniche di SPM

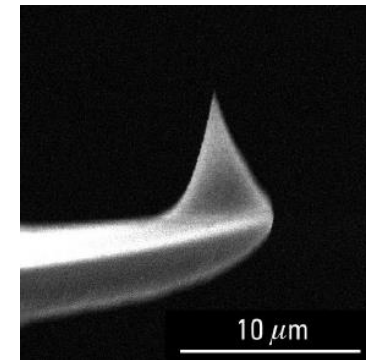
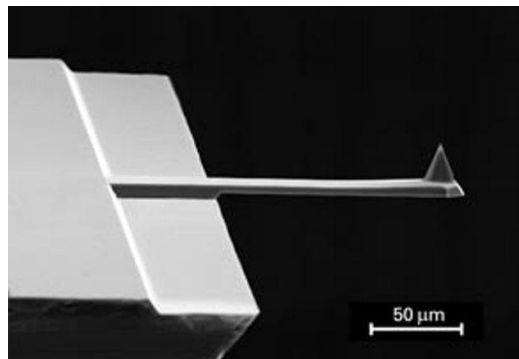
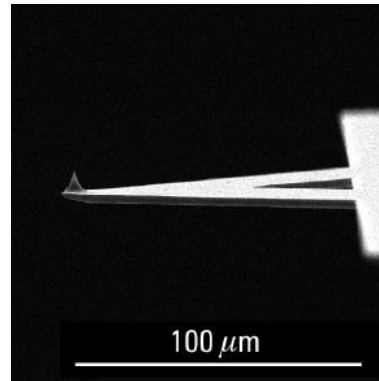
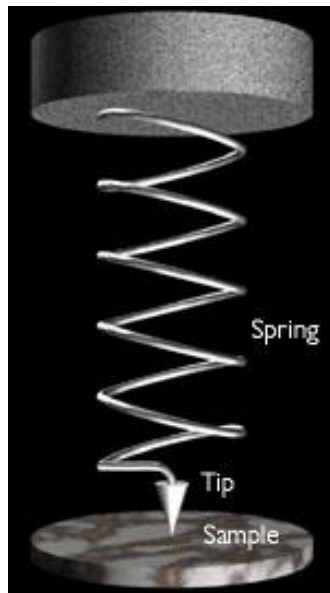
- **AFM, atomic force microscopy**
- BEEM, ballistic electron emission microscopy
- CFM, chemical force microscopy
- C-AFM, conductive atomic force microscopy
- EFM, electrostatic force microscopy
- ESTM electrochemical scanning tunneling microscope
- FMM, force modulation microscopy
- KPFM, kelvin probe force microscopy
- MFM, magnetic force microscopy
- MRFM, magnetic resonance force microscopy
- NSOM, near-field scanning optical microscopy (or SNOM, scanning near-field optical microscopy)
- PFM, Piezoresponse Force Microscopy
- PSTM, photon scanning tunneling microscopy
- PTMS, photothermal microspectroscopy/microscopy
- SECM, scanning electrochemical microscopy
- SCM, scanning capacitance microscopy
- SGM, scanning gate microscopy
- SICM, scanning ion-conductance microscopy
- SPSM spin polarized scanning tunneling microscopy
- SSRM, scanning spreading resistance microscopy
- SThM, scanning thermal microscopy
- **STM, scanning tunneling microscopy**
- SVM, scanning voltage microscopy
- SHPM, scanning Hall probe microscopy
- SXSTM synchrotron x-ray scanning tunneling microscopy



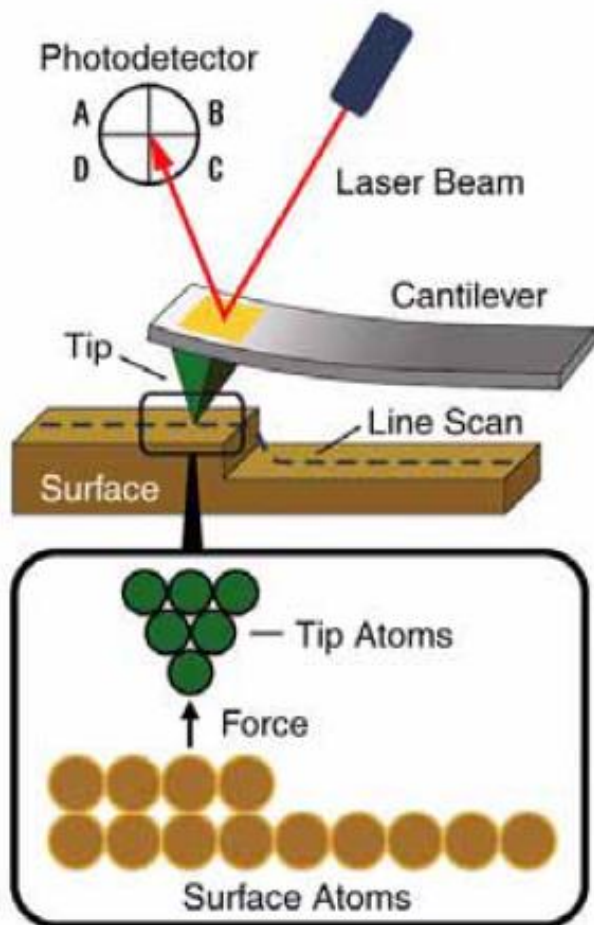
Microscopio a Forza Atomica (AFM)

- Misura le forze tra gli atomi della punta e quelli del substrato
- Le sonde o cantilevers funzionano d'accordo alla legge di Hooke.
- Materiali tipici: Si, SiN. $K = 0.01 - 100 \text{ N/m}$
- La deflessione del cantilever viene ricavata solitamente tramite leva ottica.

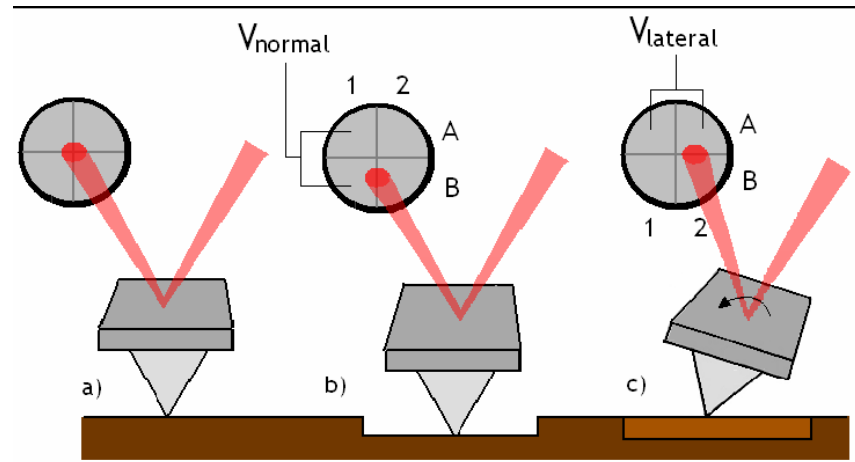
$$F_{tot} = -Kd$$



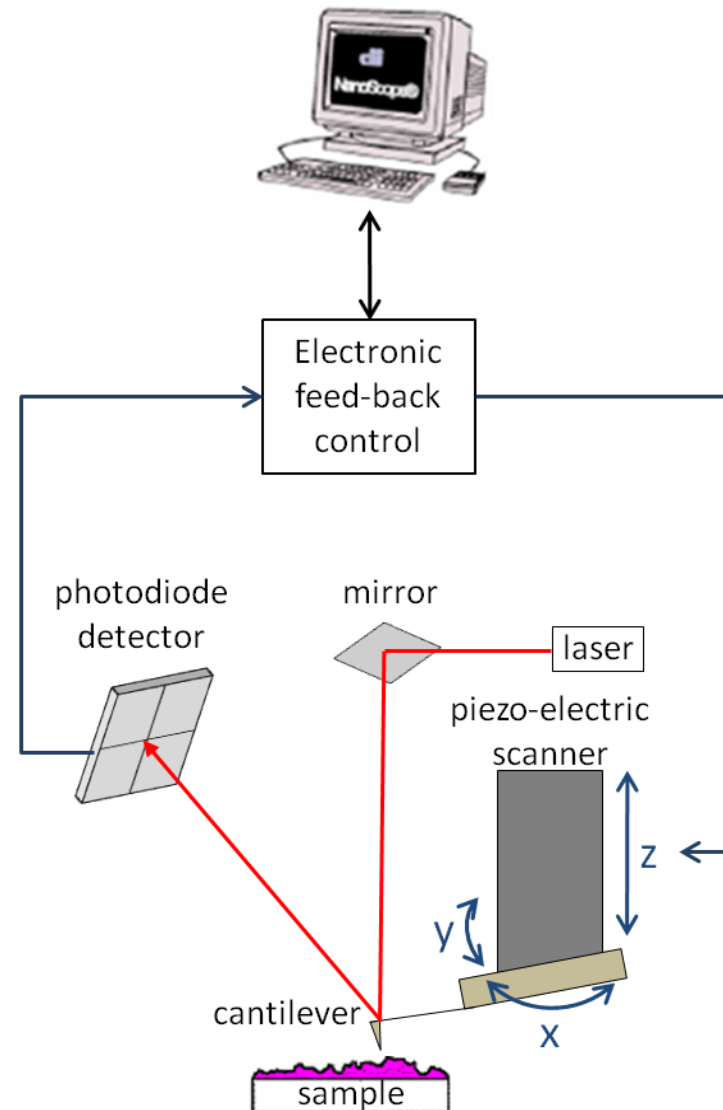
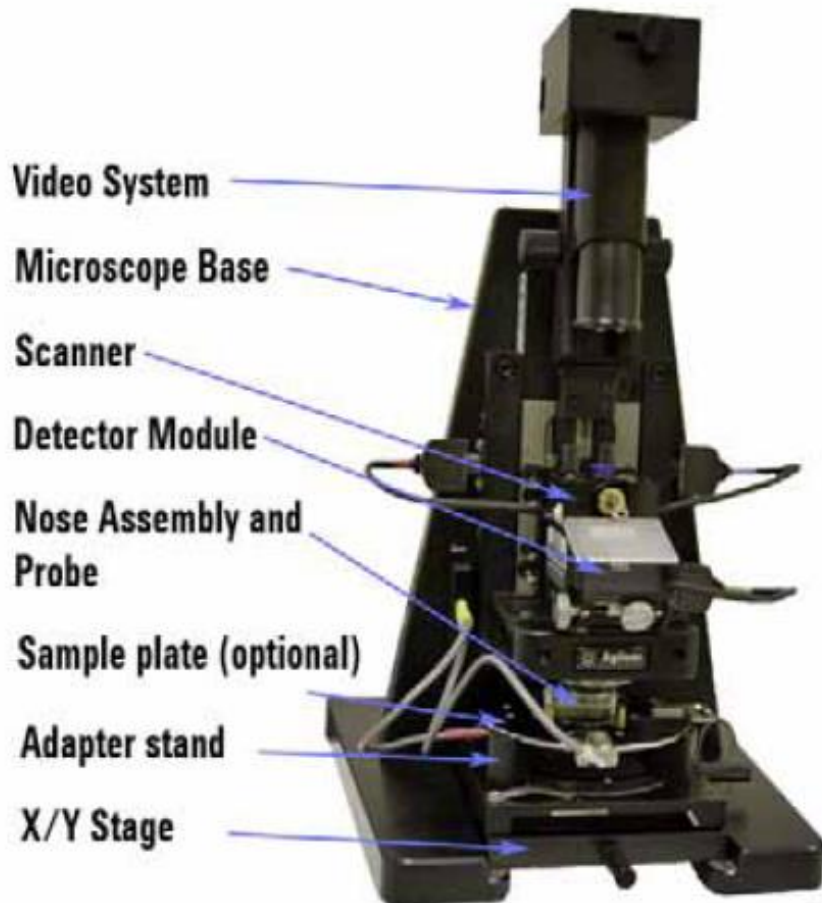
Principio de funcionamiento del AFM



$$F_{\text{normal}}(z) = \frac{k_z [V^{\text{normal}}(z) - V_{\text{FS}}^{\text{normal}}]}{S_z}$$

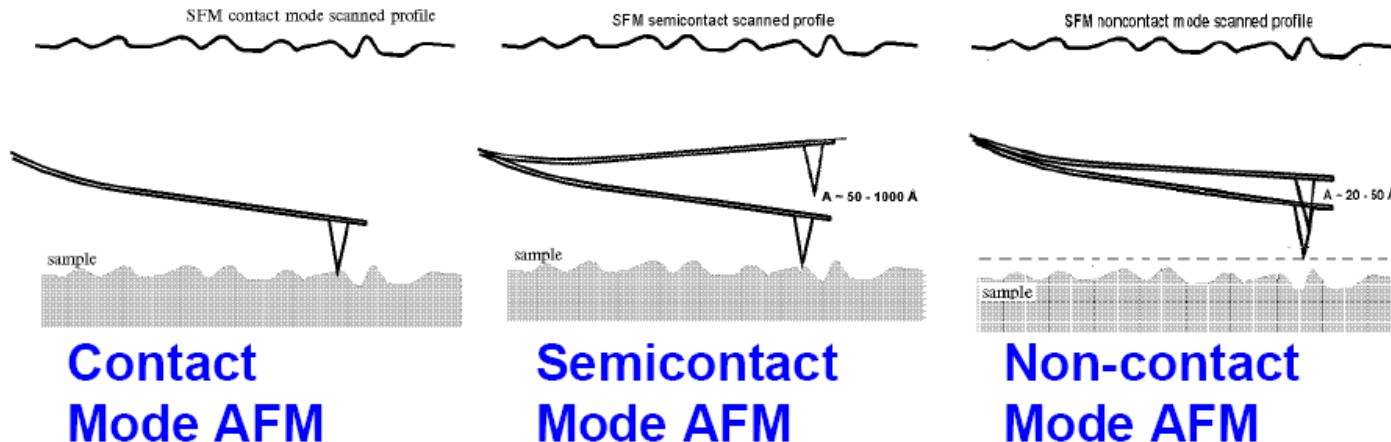
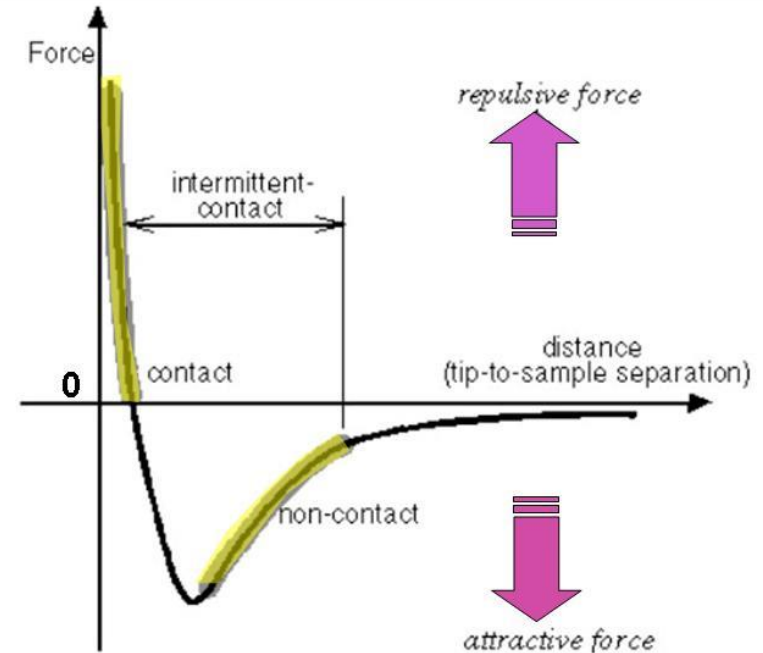


Principio de funcionamiento del AFM

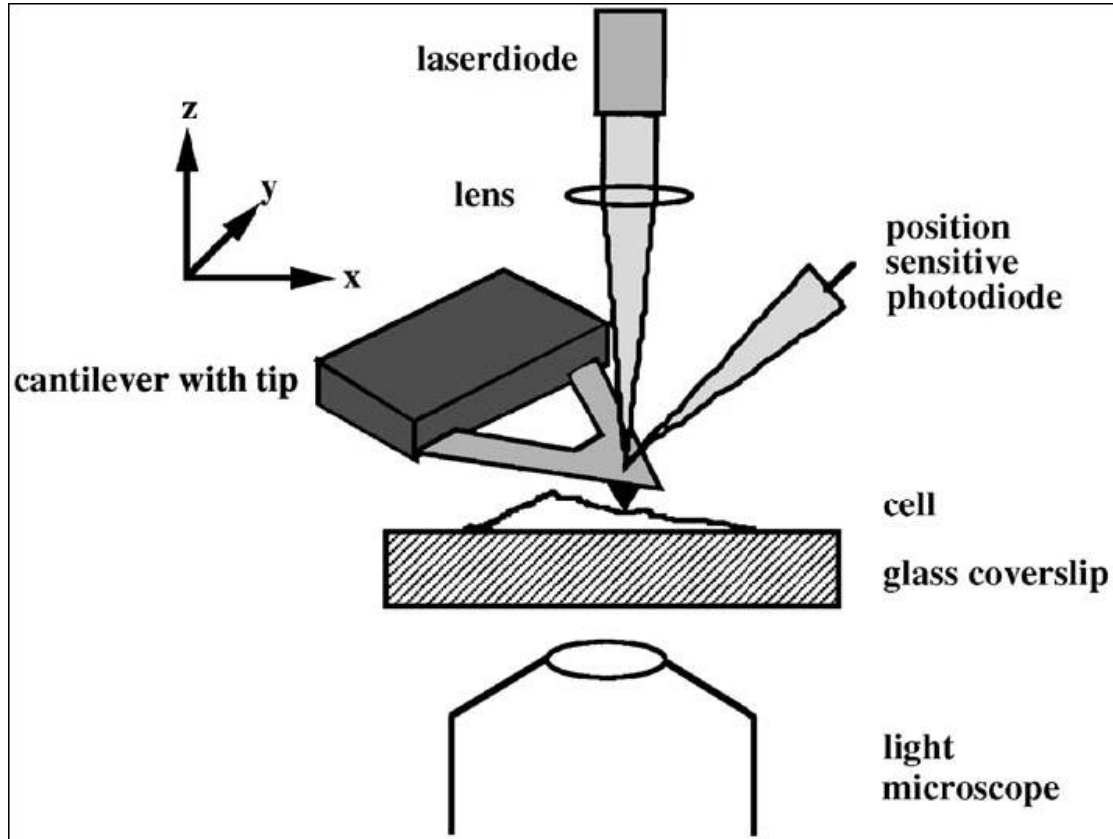


Modalità di funzionamento del AFM

- Dipendendo del materiale del campione e della punta la interazione tra di loro può dipendere di diversi tipi di forze:
 - Forza di van der Waal
 - Forza di capillarità
 - Forza elettrostatica
 - Forza Magnetica
 - Legami chimici
 - ...

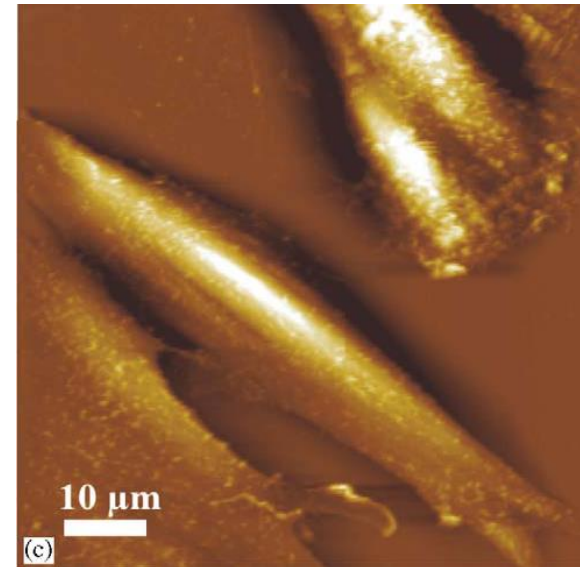
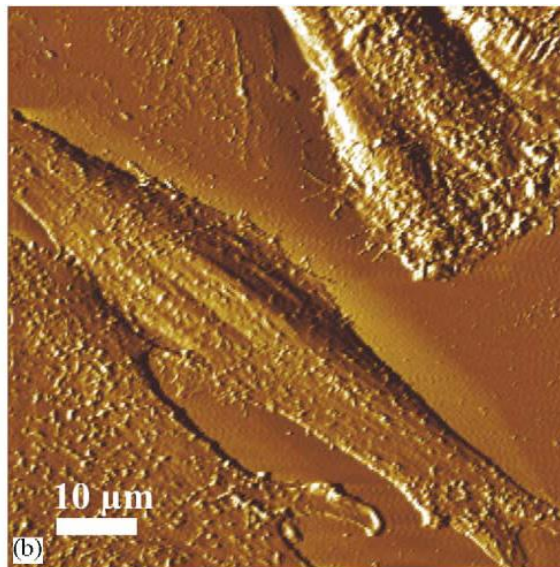
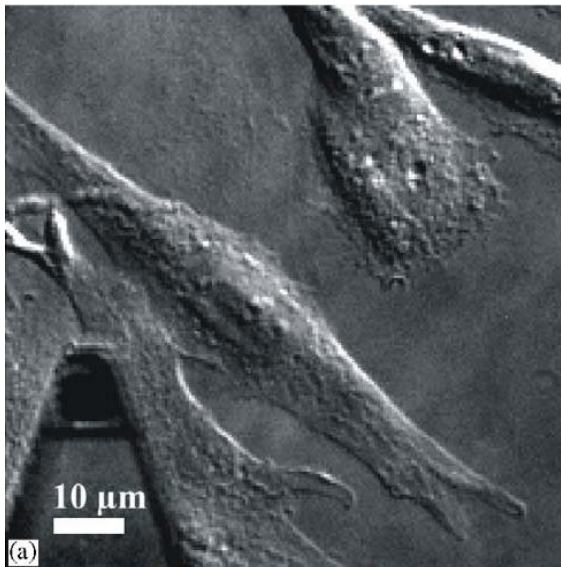


L'AFM nello studio cellulare



Modalità Contatto

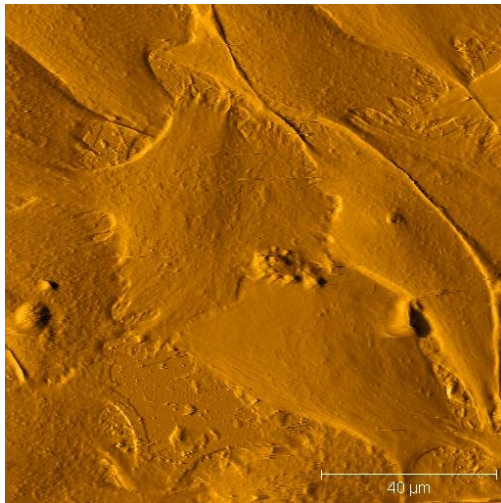
- Altezza costante. Distanza scanner-campione fissa.
 - Superficie piate.
 - Immagini con risoluzione atomica.
 - Immagini in tempo reale di superfici che cambiano nel tempo
- Forza costante. Deflessione del cantilever fissa usando feedback elettronico.
 - Superfici con alta variabilità di altezza (centinaia di micrometri).
 - Velocità e risoluzione inferiore al modo di altezza costante.



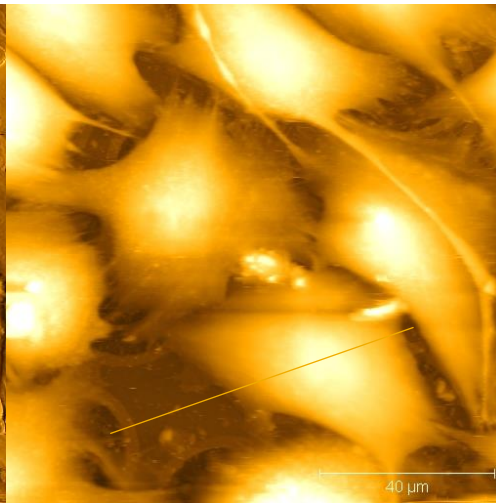
Madl J. et al. A combined optical and atomic force microscope for live cell investigations. Ultramicroscopy 106 (2006), 645-651

Imaging

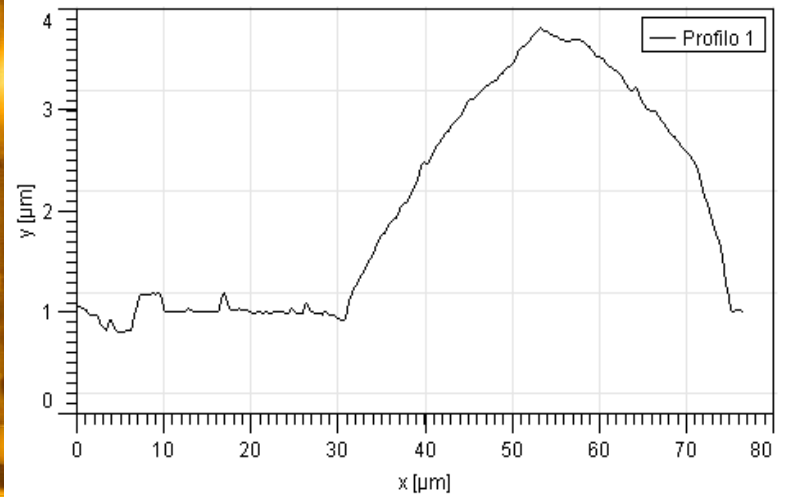
Error image



Topography image



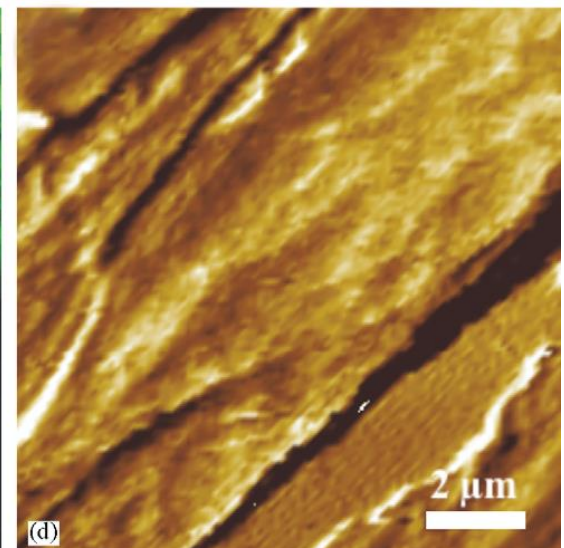
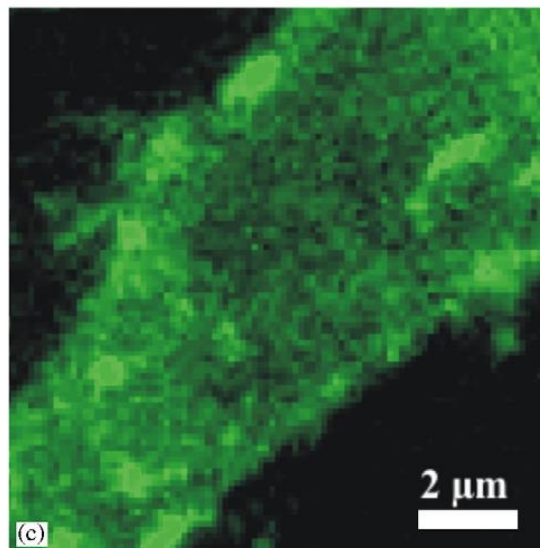
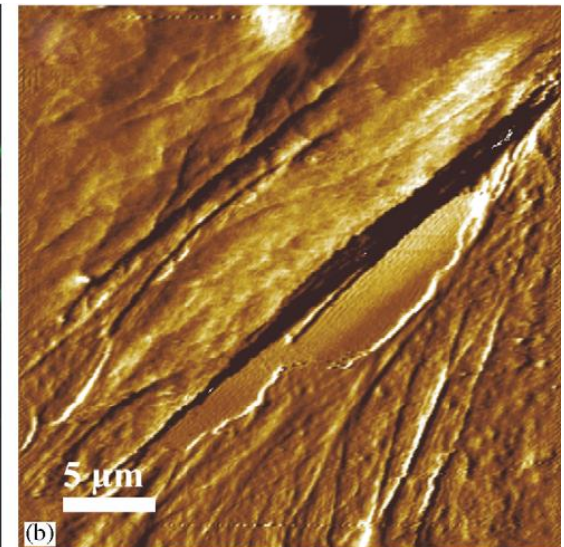
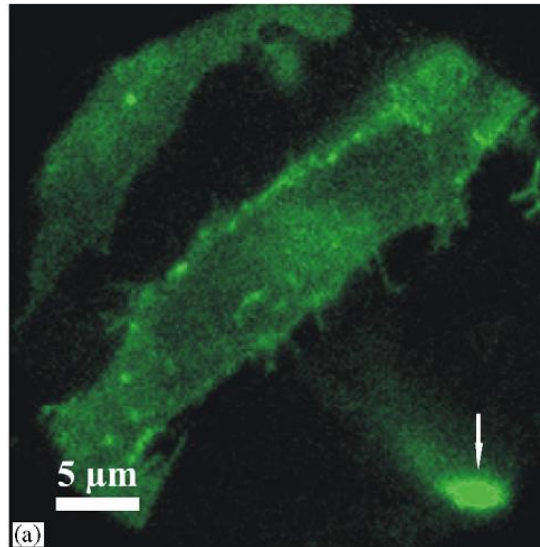
Heigh profile



Culture of fibroblasts

Vedere e toccare

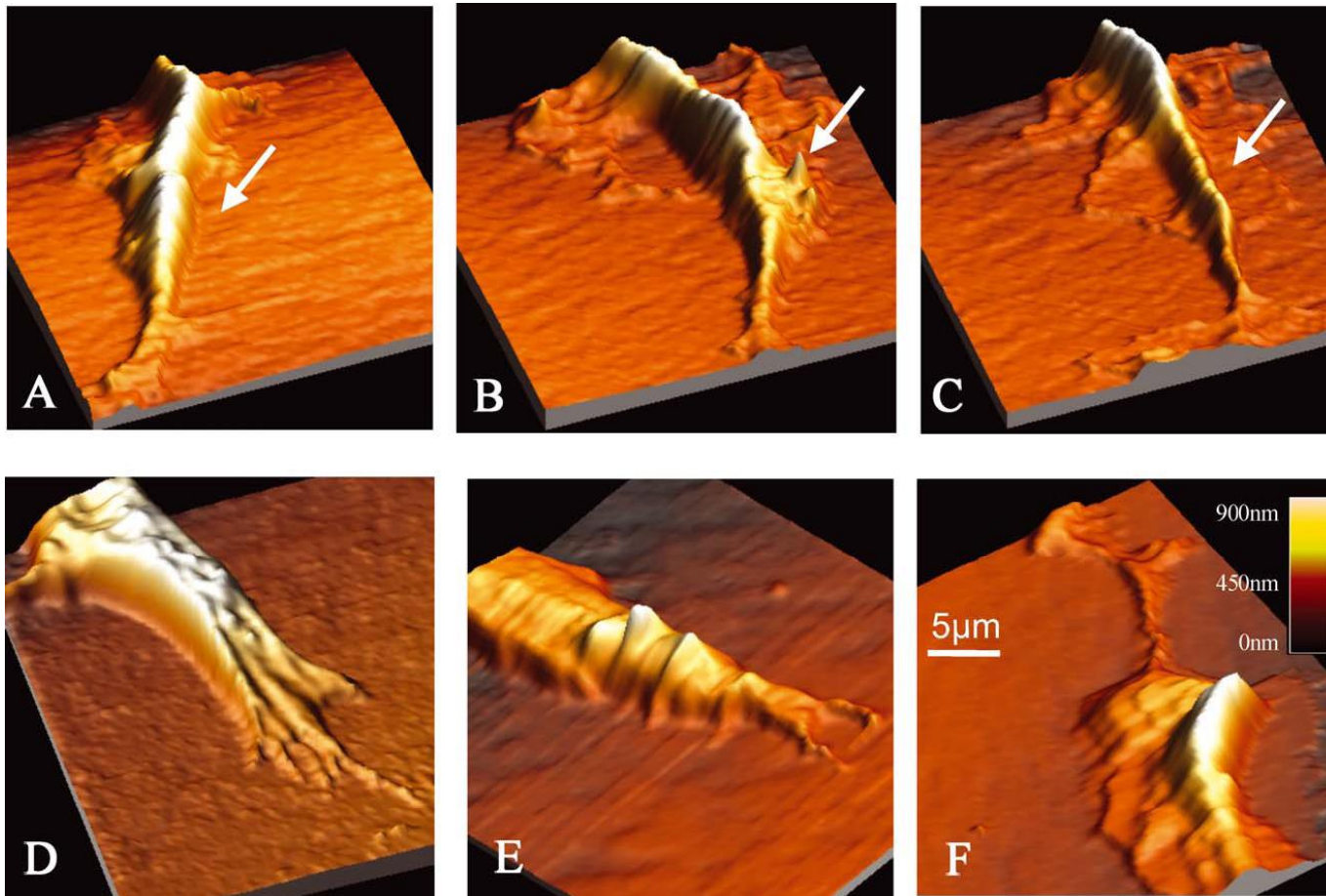
- Immagini in fluorescenza e AFM in modo contatto
- La topografia delle cellule può essere correlata all'immagini in fluorescenza



Madl J. et al. A combined optical and atomic force microscope for live cell investigations. Ultramicroscopy 106 (2006), 645-651

Ricostruzione topografica 3D

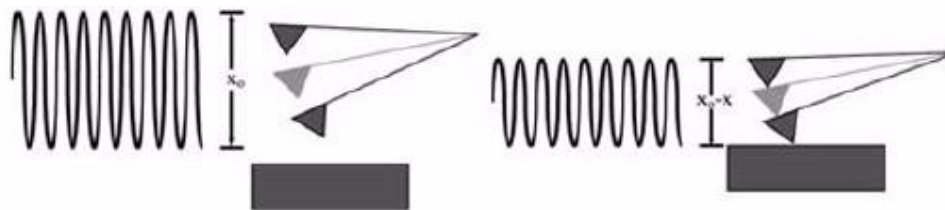
- Le immagini A-C sono state fate 5min tra di loro



McNally, H. A. and R. B. Borgens (2004). "Three-dimensional imaging of living and dying neurons with atomic force microscopy." *Journal of neurocytology* 33(2): 251-258.

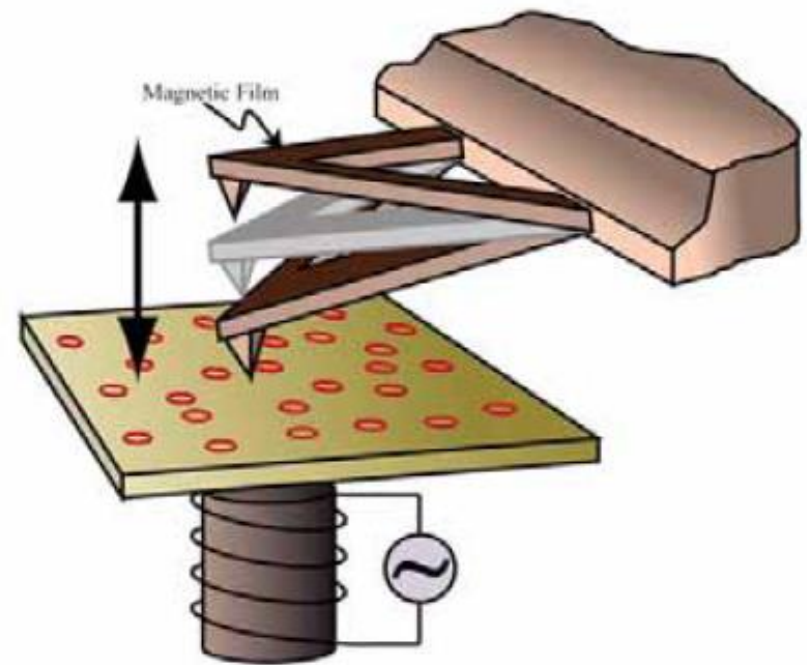
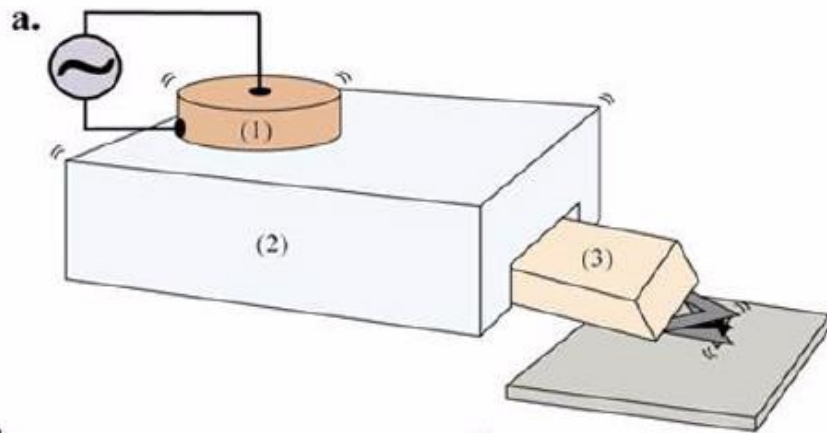
Modalità contatto intermittente (tapping)

- Si fa oscillare il cantilever circa alla sua frequenza di risonanza
- L'interazione con la superficie produce cambiamenti nella ampiezza, fase e frequenza di risonanza
- L'immagini di ampiezza e fase possono dare dell'informazione aggiuntiva non presente nelle immagini di topografia
- La forza laterale durante la scansione si riduce sostanzialmente
- È più lento e complicato di aggiustare della modalità di contatto



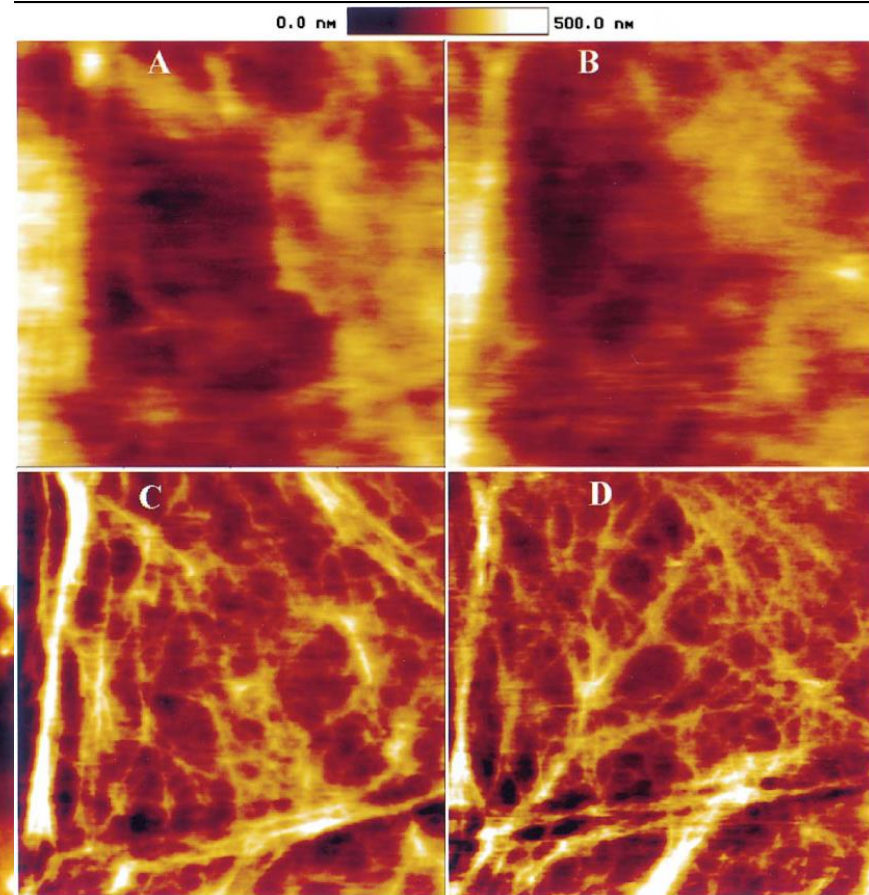
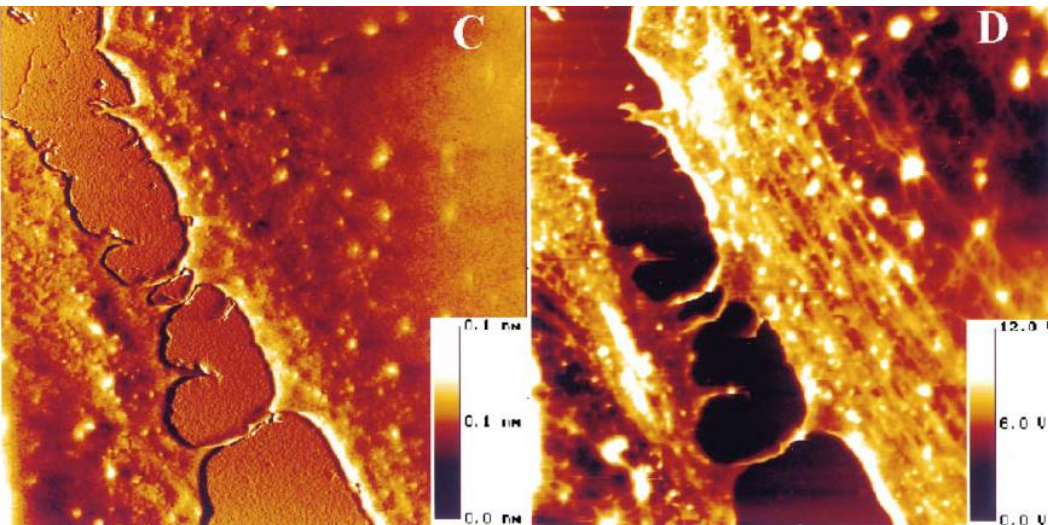
Oscillazione del cantilever

- Oscillazione acustica vs. magnetica



Immagini in tapping mode

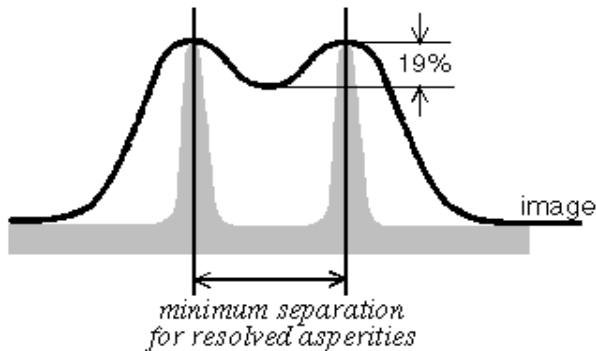
- Cell surface and membrane skeleton reorganizations between successive scans.
- In contrast with contact mode, the visualization of structures like the membrane skeleton does not require the use of high (nN range) scanning forces.



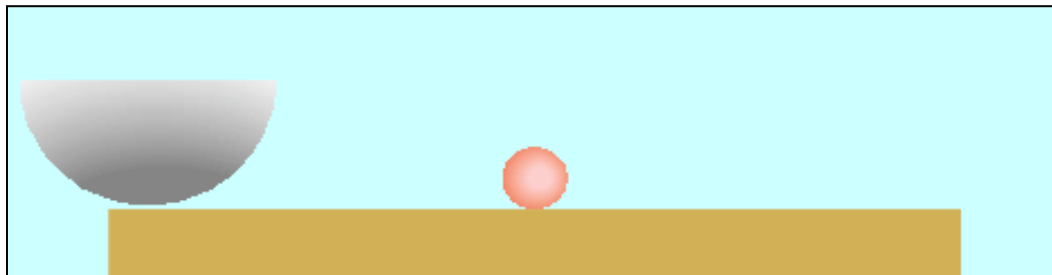
Vié V. et al. Tapping-mode atomic force microscopy on intact cells. *Ultramicroscopy* 82 (2000) 279-288

Risoluzione laterale del AFM

Criterio di Rayleigh



Effetto de convoluzione campione

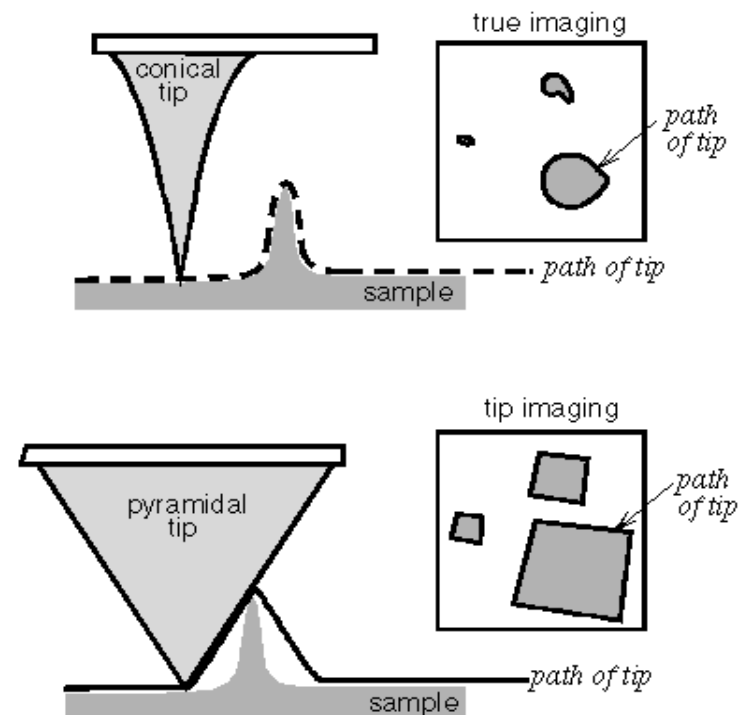


Punte migliori: radio de curvatura 5 nm

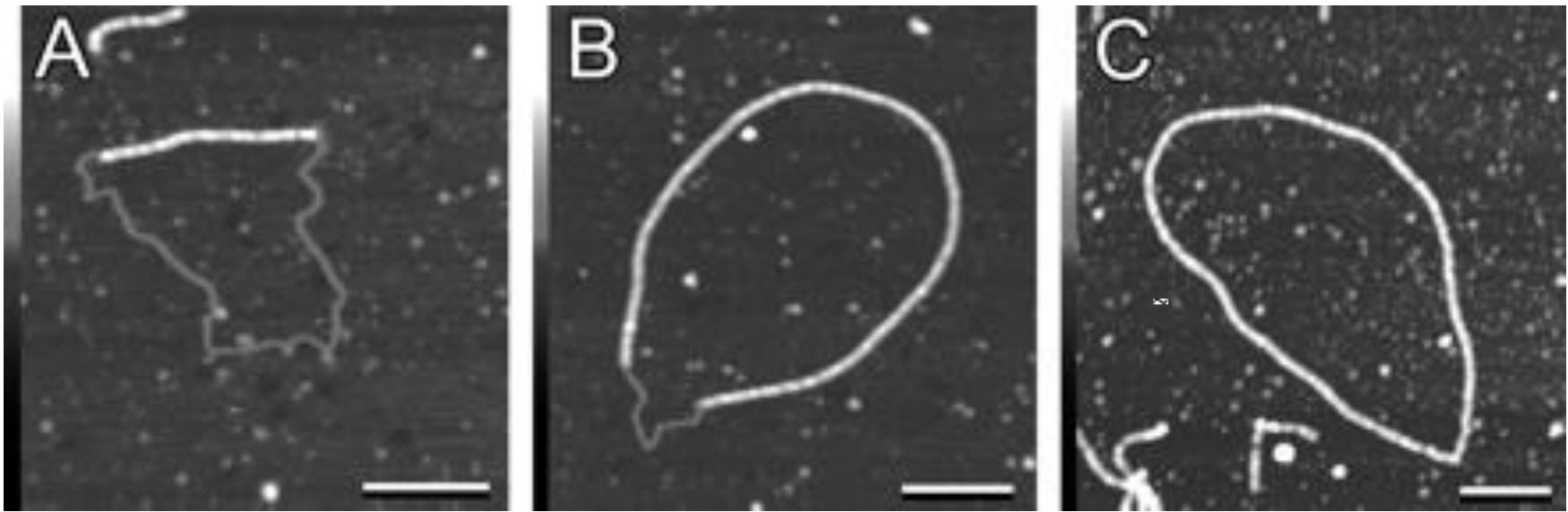


risoluzioni laterale: 1-2 nm

Convoluzione e topografia



Immagini della formazione di complessi RecA/DNA con el AFM



Typical RecA/dsDNA complexes observed at different incubation times, at 37°C: (A) after 30 min— 38% coverage of DNA by RecA; (B) 45 min— 88% coverage; and (C) 60 min—DNA fully coated by RecA. Topographic images acquired in air: scale bar = 250 nm; z scale = 10 nm (*dark to light*).

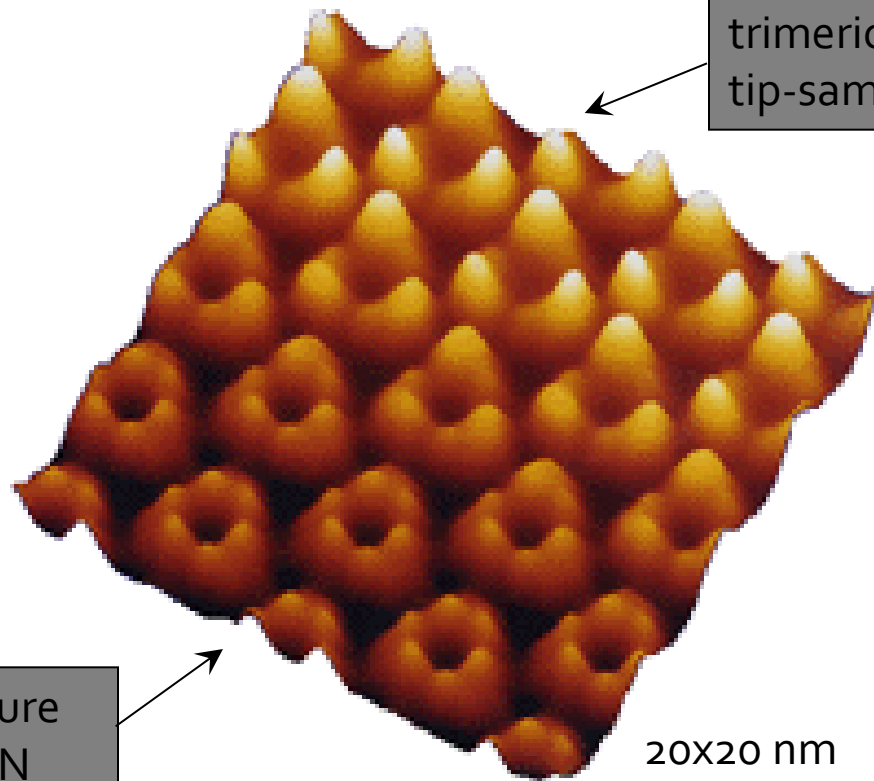
Ref: Bernie D. Sattin and M. Cynthia Goh, *Biophys J.*, 87:3430 (2004)

RecA is a 38-kDa globular protein *Escherichia coli* protein essential for the repair and maintenance of DNA and other functions.

Immagini a risoluzione atomica di proteine

- Cambiamenti strutturali indotti per la forza nella bacteriorodopsina

10Å lateral and 1Å vertical resolution

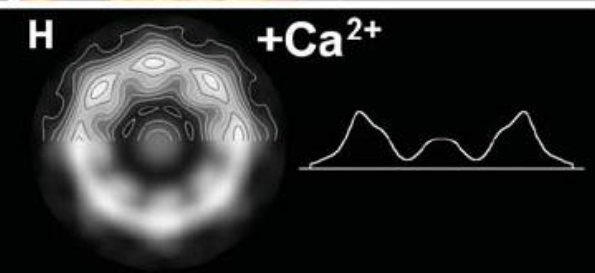
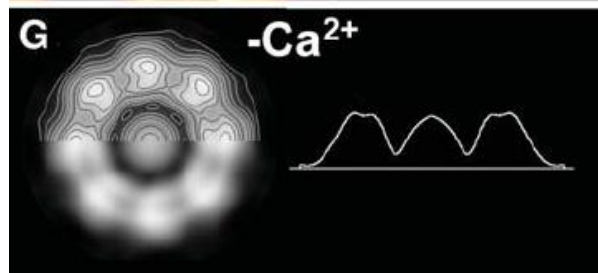
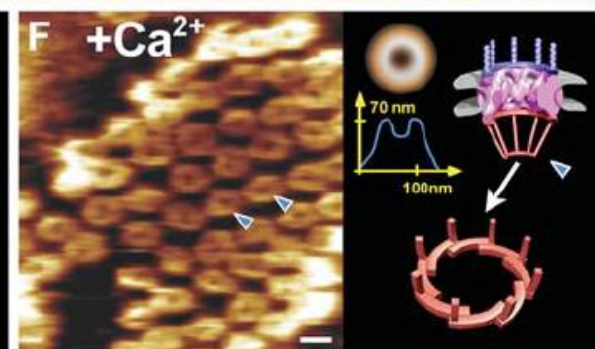
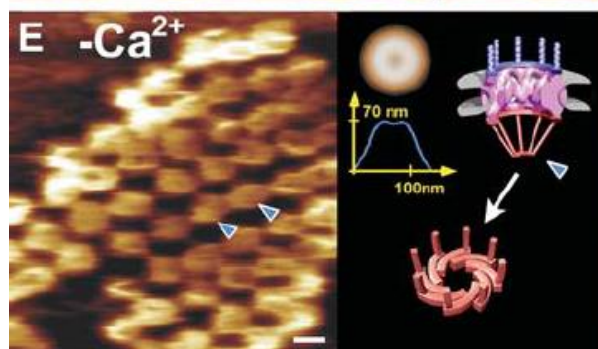
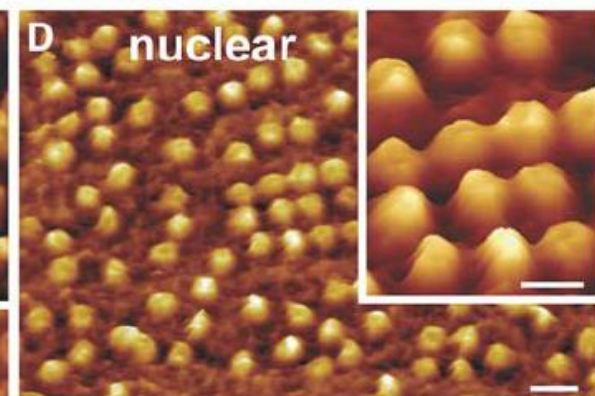
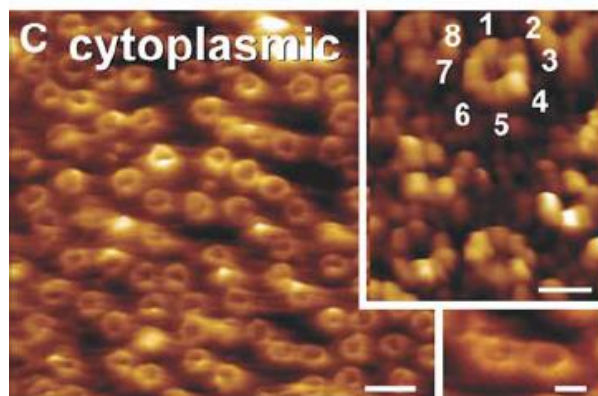
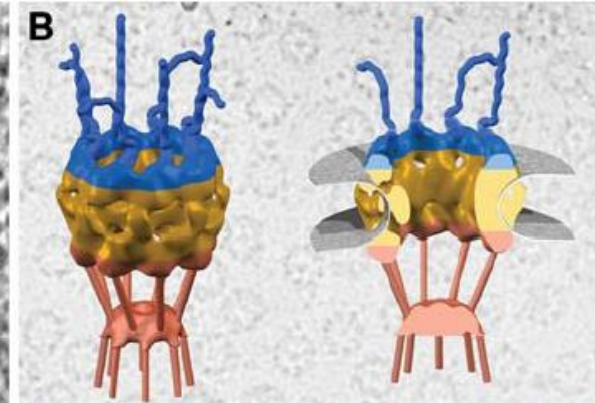
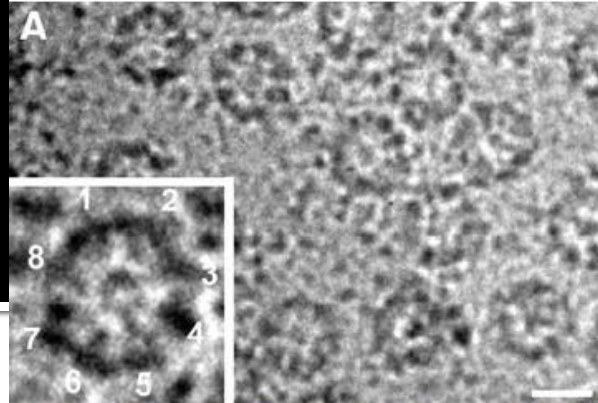


trimeric structure
tip-sample force = 100 pN

hexameric, donut structure
tip-sample force = 300 pN

20x20 nm

AFM in Alta risoluzione

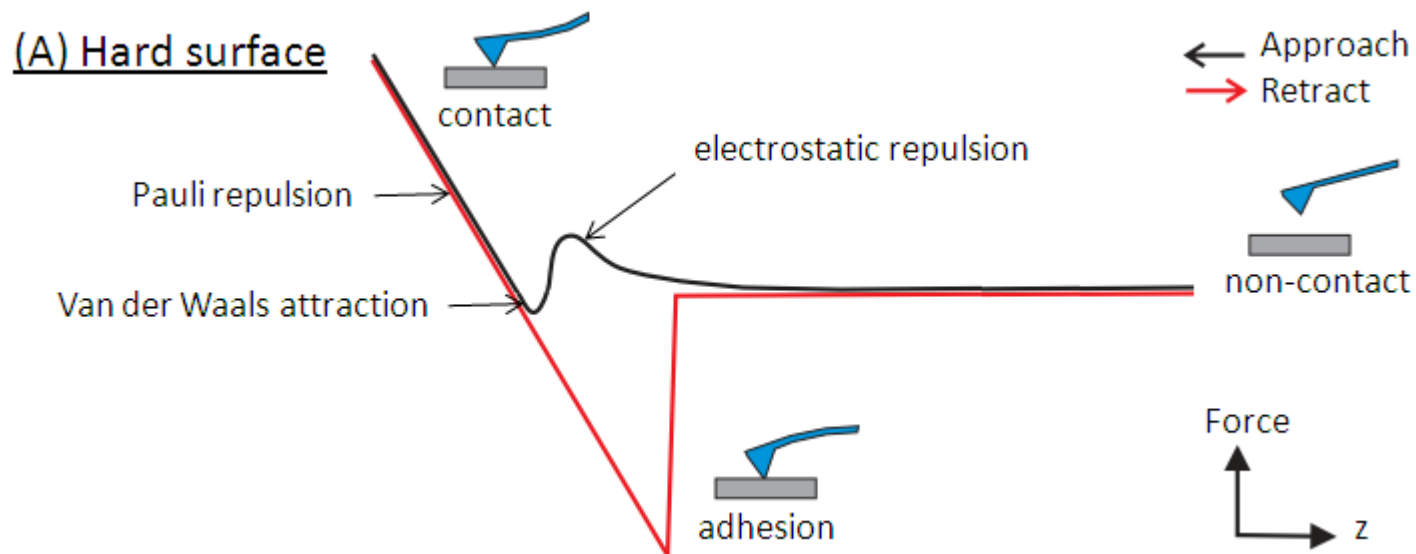


Nuclear pore complexes



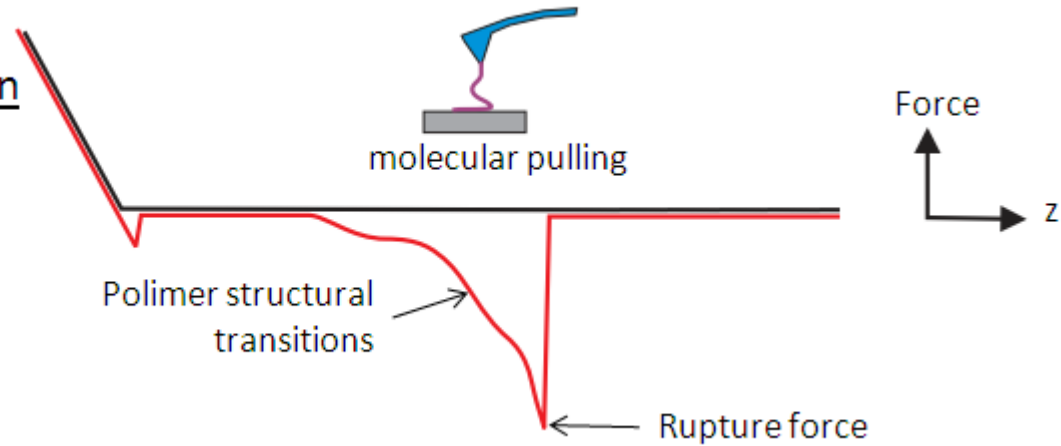
Modalità di Forza

- Utile per misurare le proprietà elastiche o di adesione di una superficie generando curve forza-distanza (F-D)
- Le curve F-D sono generate controllando il movimento verticale senza spostamento laterale

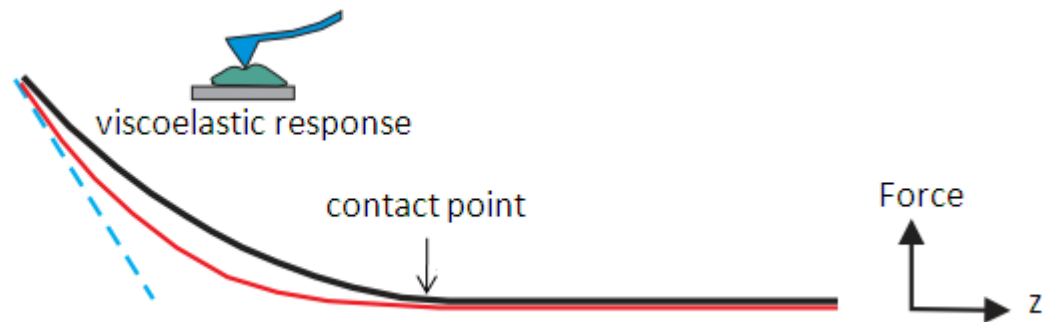


Curve forza-distanza

(B) Molecular binding/extension

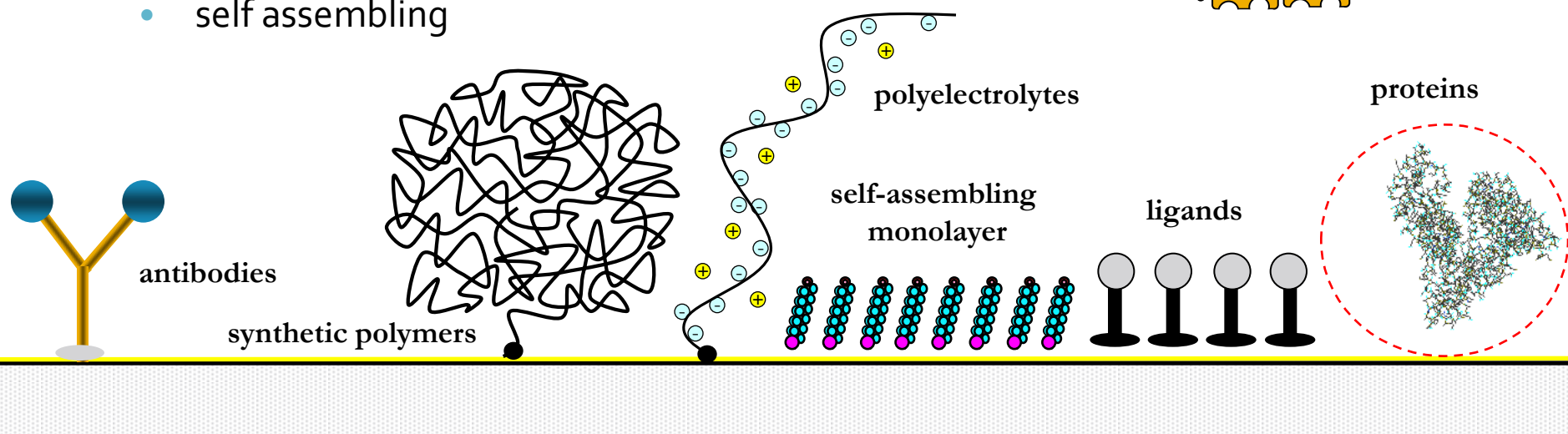


(C) Indentation

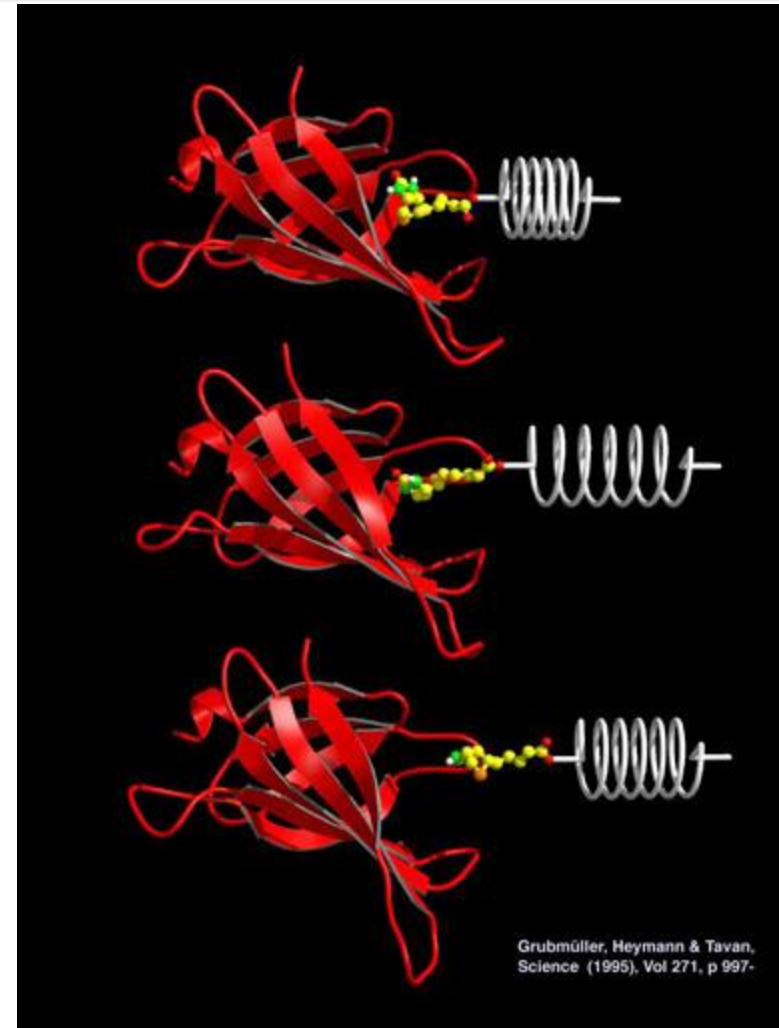
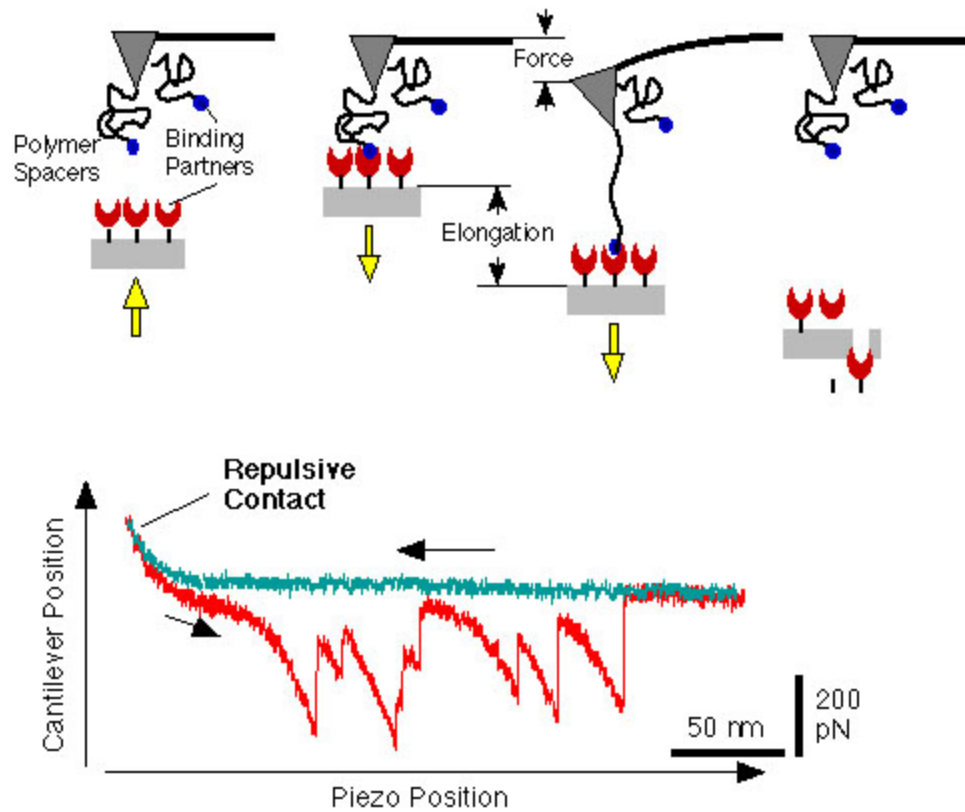


Single molecule force spectroscopy

- Funzionalizzazione di superfici
- Studio delle proprietà delle singole molecole o copie molecolari (e.g. antigene/anticorpo)
 - Allungamento, torsione, folding/unfolding
 - Struttura molecolare / Energia di legame
- surface (bio)chemistry techniques:
 - covalent bonds
 - self assembling

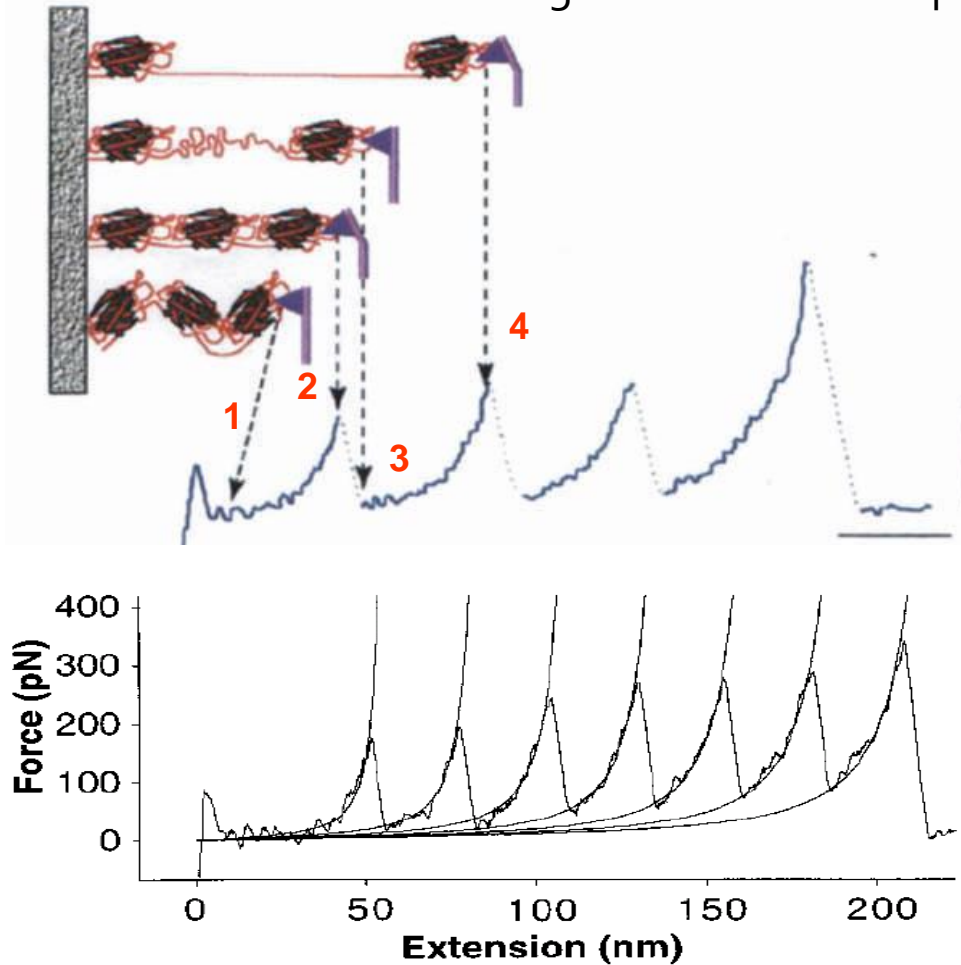


Misura della forza tra singole molecole



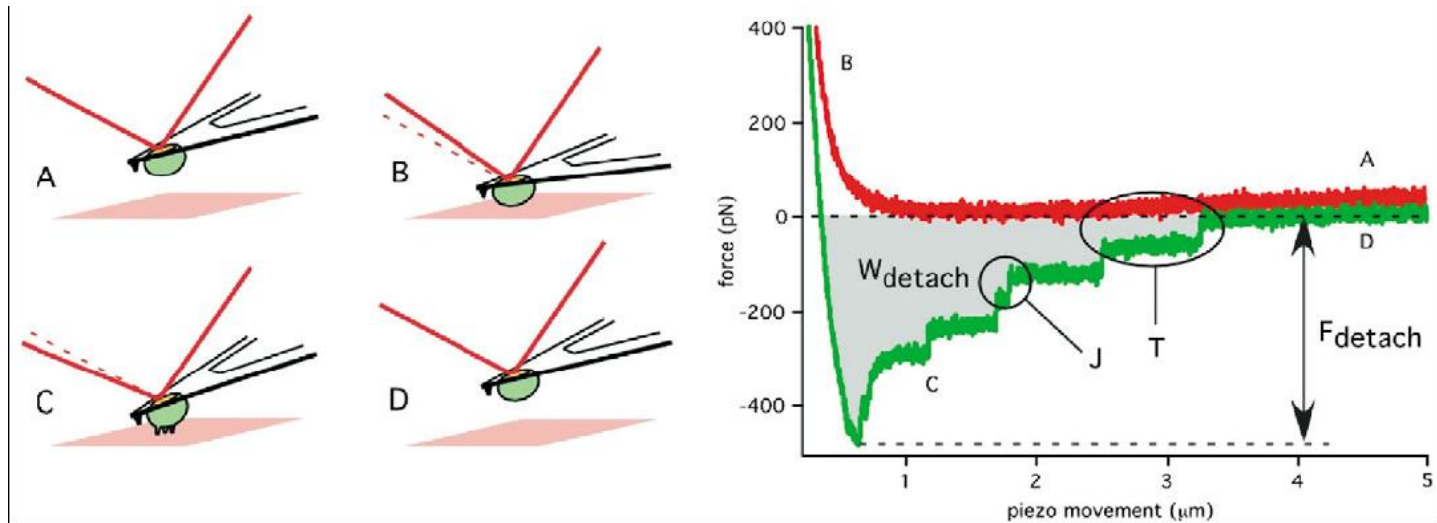
Unfolding di proteine con l'AFM

unfolding of a multidomain protein under stretching



The sawtooth pattern of peaks that is observed when force is applied to extend the protein corresponds to a sequential deformation and unfolding of individual domains. When the distance between substrate and cantilever increases (from state 1 to state 2), the protein elongates, generating a restoring force that bends the cantilever. When a domain unfolds (state 3), the free length of the protein increases, reducing the force on the cantilever to almost zero. Further extension again result in force on the cantilever (state 4). The consecutive domain deformation of the molecule (titin) obeys the worm-like chain model, with the persistence length p fixed at 0.56 nm and the contour length L_c for each peak adjusted as shown.

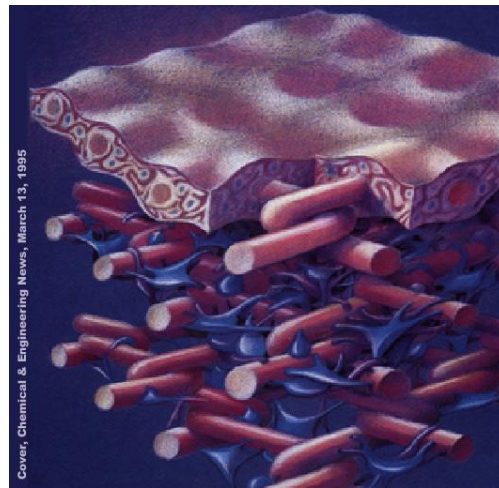
Spettroscopia di forza nello studio della adesione cellulare



Puech, P.-H., A. Taubenberger, et al. (2005). "Measuring cell adhesion forces of primary gastrulating cells from zebrafish using atomic force microscopy." *J Cell Sci* 118(18): 4199-4206.

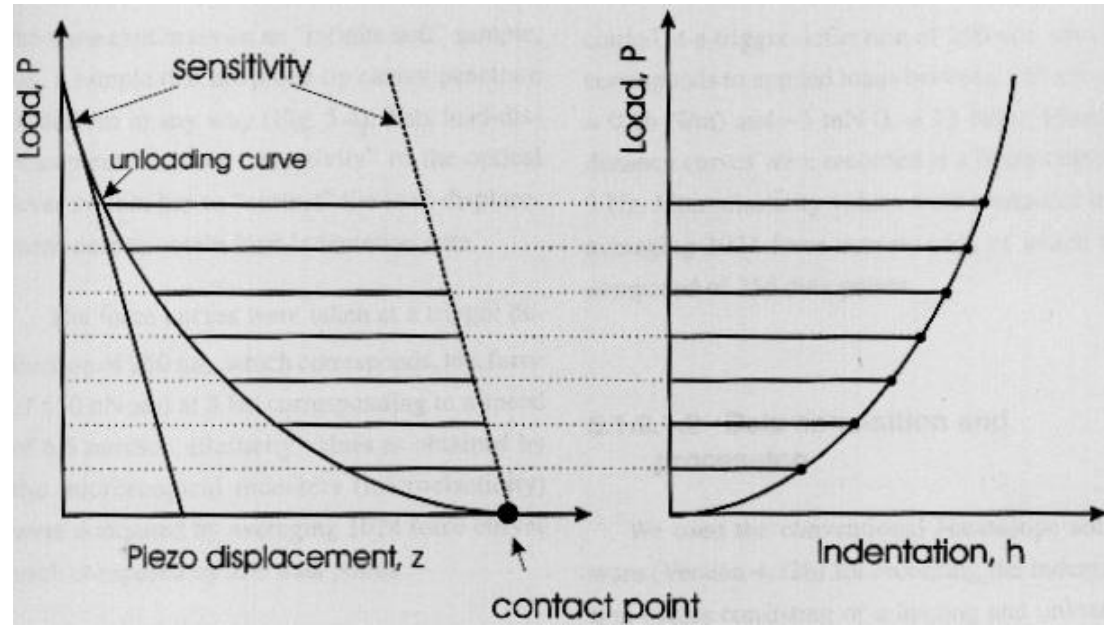
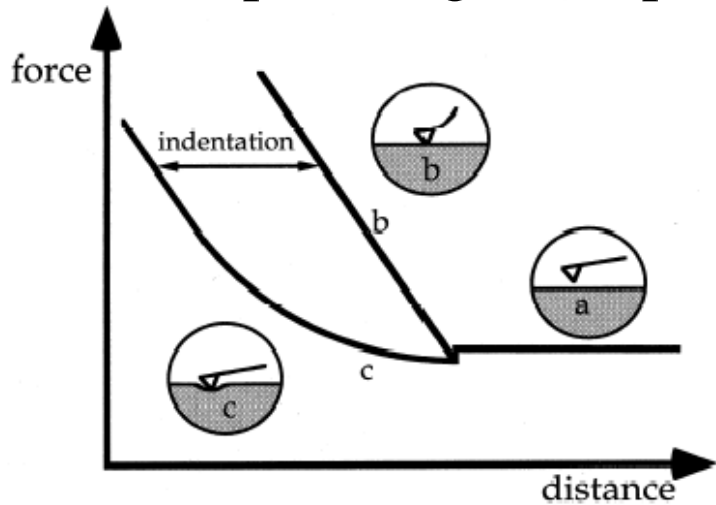
L'AFM nello studio nano-meccanico di tessuti biologici

- Motivazione
 - Studio delle proprietà biofisiche delle cellule
 - Diagnostico
 - Cambiamenti nelle proprietà meccaniche correlati a patologie
 - Ingegneria di tessuti
 - Ottimizzazione delle proprietà funzionali / controllo della qualità dei tessuti ingegnerizzati

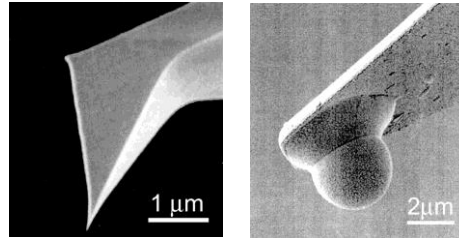
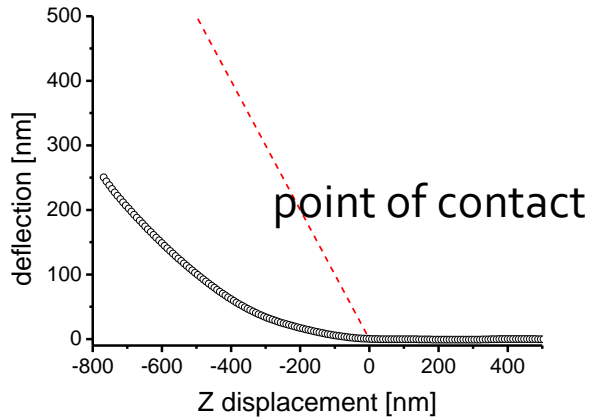


Indentazione usando l'AFM

Soft sample Rigid sample



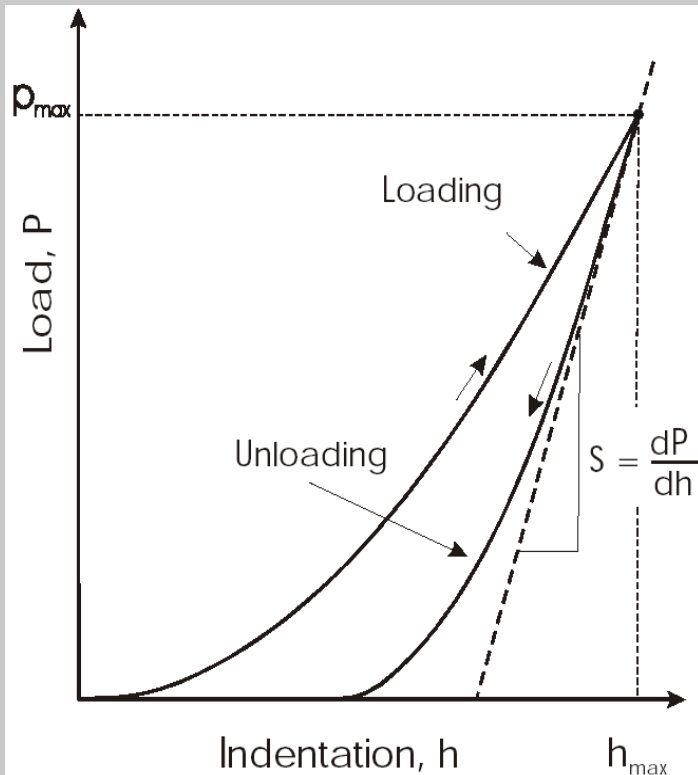
Practical concerns:



- tip geometry
- cantilever spring constant

Indentazione usando l'AFM

Procedure used: W.C. Oliver, G.M. Pharr, *J. of Mat. Res.*, 7, 1564-1583 (1992).



$$P = A(h - h_f)^m$$

for a spherical indenting tip $m = 1.5$

$$E = \frac{\sqrt{\pi}}{2} (1 - \nu^2) \frac{S}{\sqrt{A}}$$

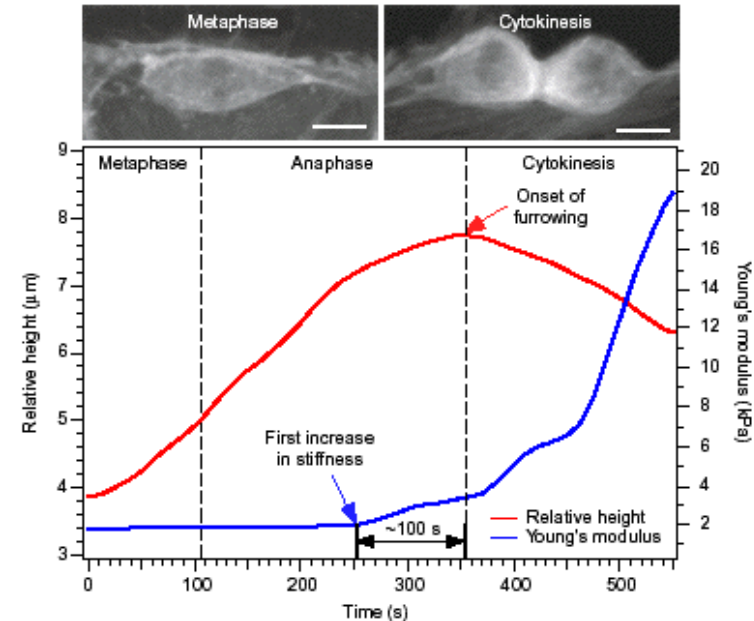
ν = Poisson ratio

S = contact stiffness

A = projected tip contact area

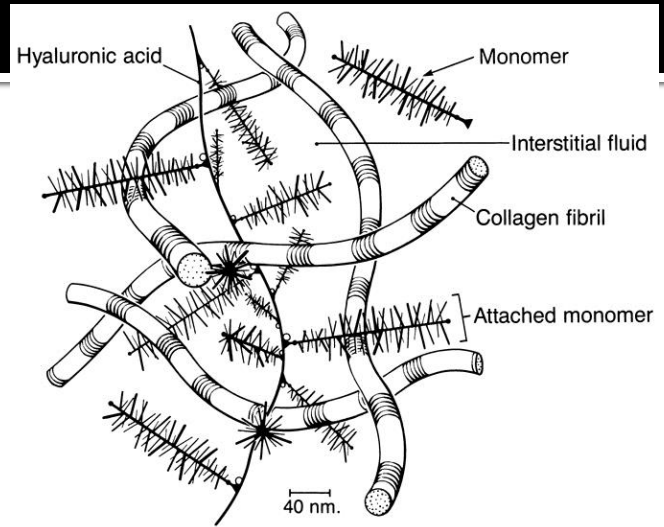
Vantaggi dell'indentazione usando l'AFM

- nanometer range indentations (thin films)
- low applied loads (soft materials)
- high force resolution (high sensitivity)
- small contact area (high spatial resolution)
- non destructive measurement
- miniaturized probe (*in vivo* measurements)

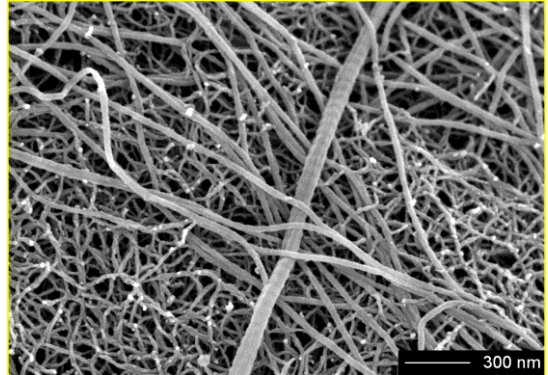
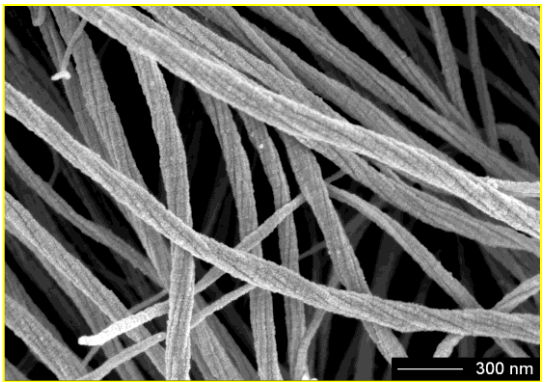
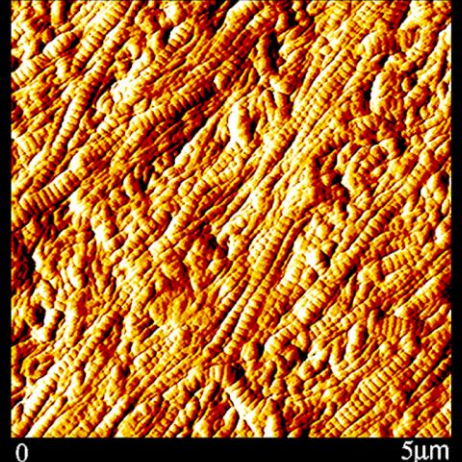
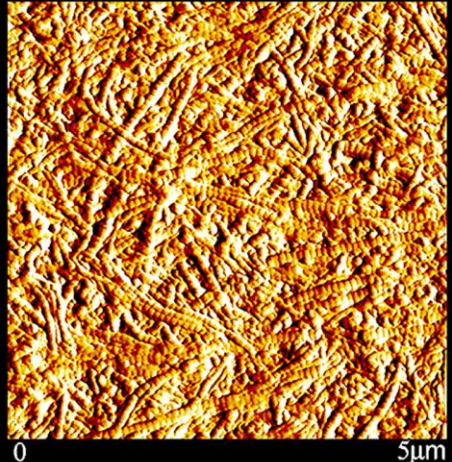


Matzke, K. Jacobson and M. Radmacher, "Direct, high resolution measurement of furrow stiffening during the division of adherent cells", [*Nature Cell Biol.*, \(2001\), 3\(6\) 607-610](#)

AFM mechanical testing of articular cartilage



pathological cartilage
healthy state osteoarthritic



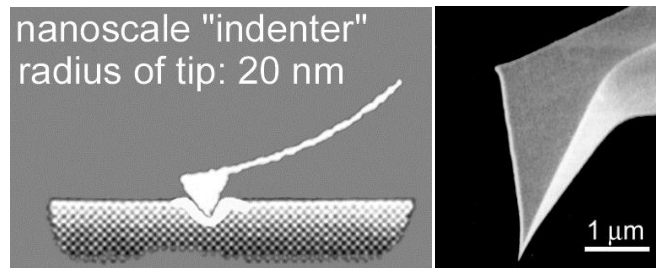
Biomechanics



Biochemistry

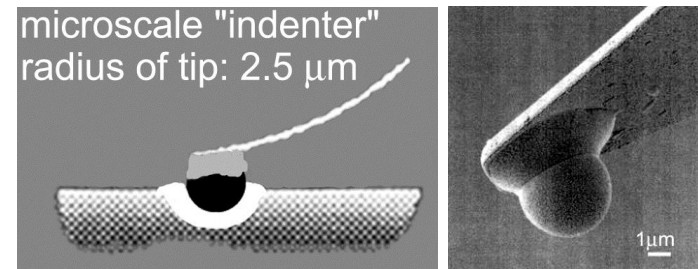
Comparison of cartilage elasticity monitored at the **nm** versus the **μm** scale by AFM

nm



E-module **nm** = 0.021 MPa

μm



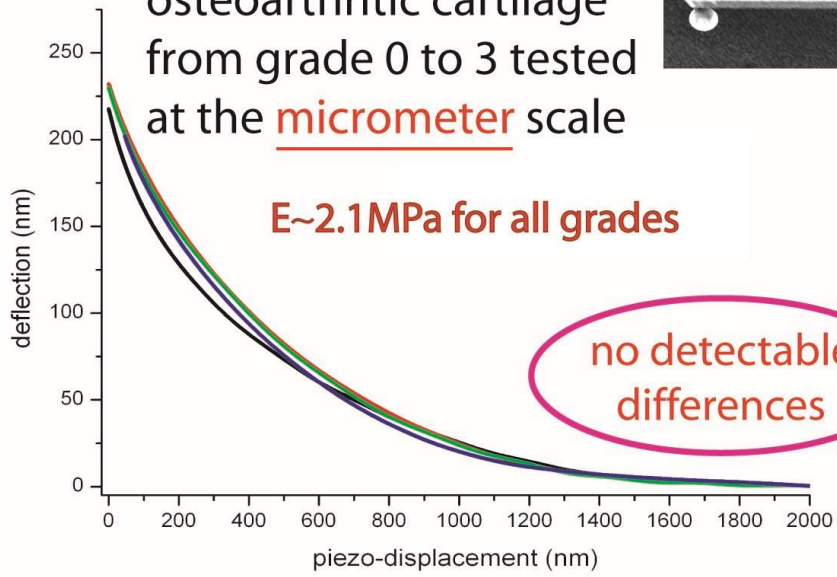
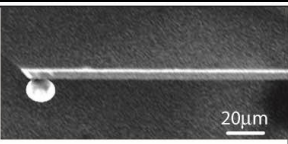
E-module **μm** = 2.6 MPa

Reported values: 1.5 to 9.7 MPa

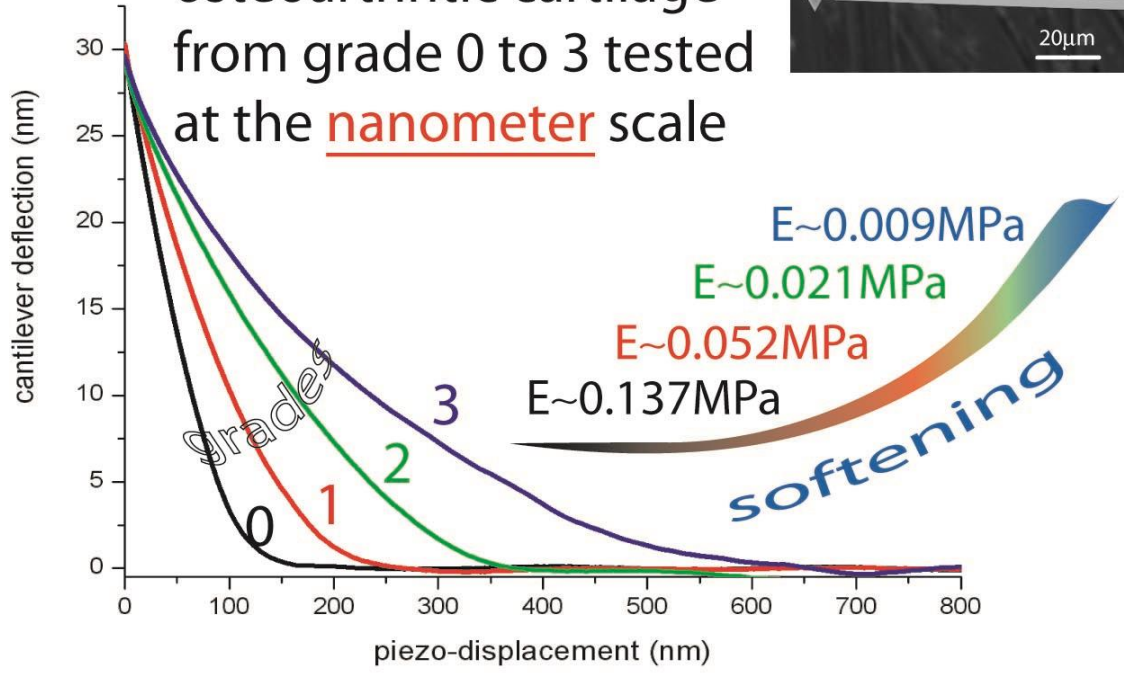
Cartilage is a hierarchically organized material

M. Stolz *et al.*, Biophys. J. 2004; 86 3269-3283

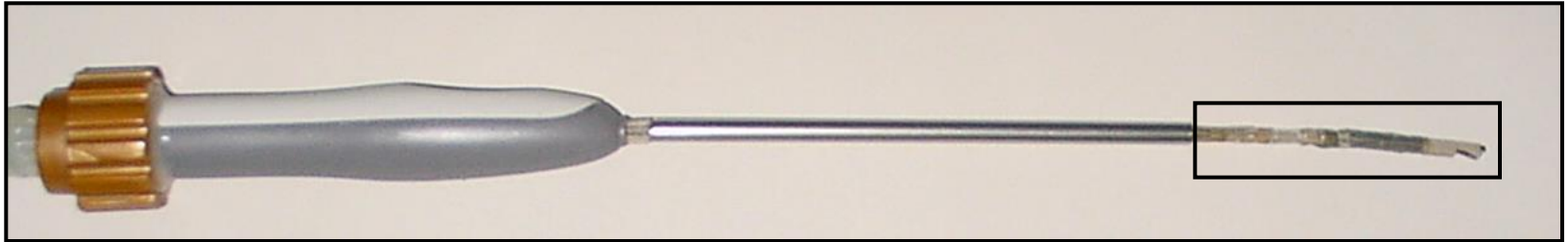
osteoarthritic cartilage
from grade 0 to 3 tested
at the micrometer scale



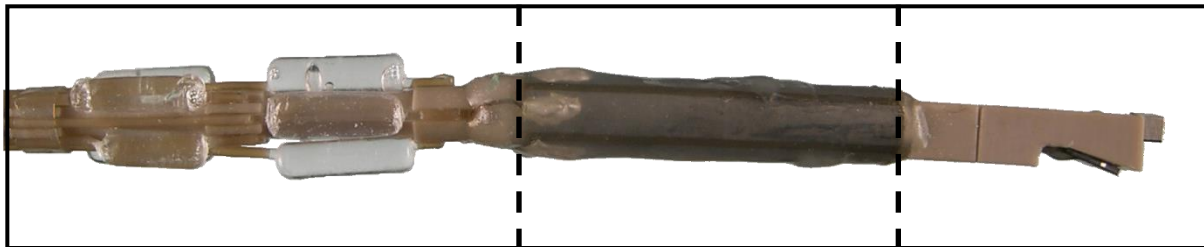
osteoarthritic cartilage
from grade 0 to 3 tested
at the nanometer scale



A scanning force arthroscope



R. Imer *et al.*, Jpn. J. Appl. Phys., **45**, 2319–2323 (2006)



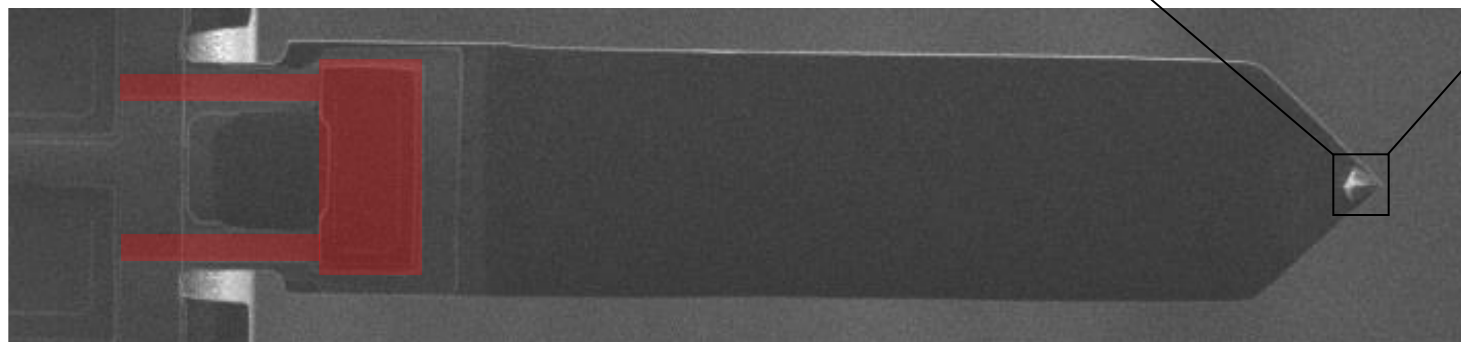
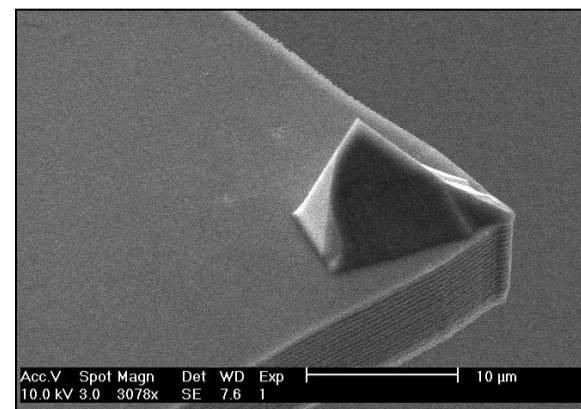
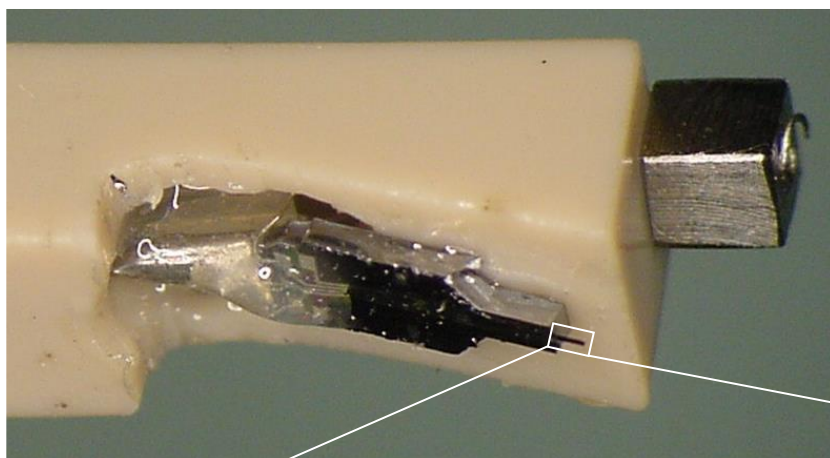
Stabilization
stage

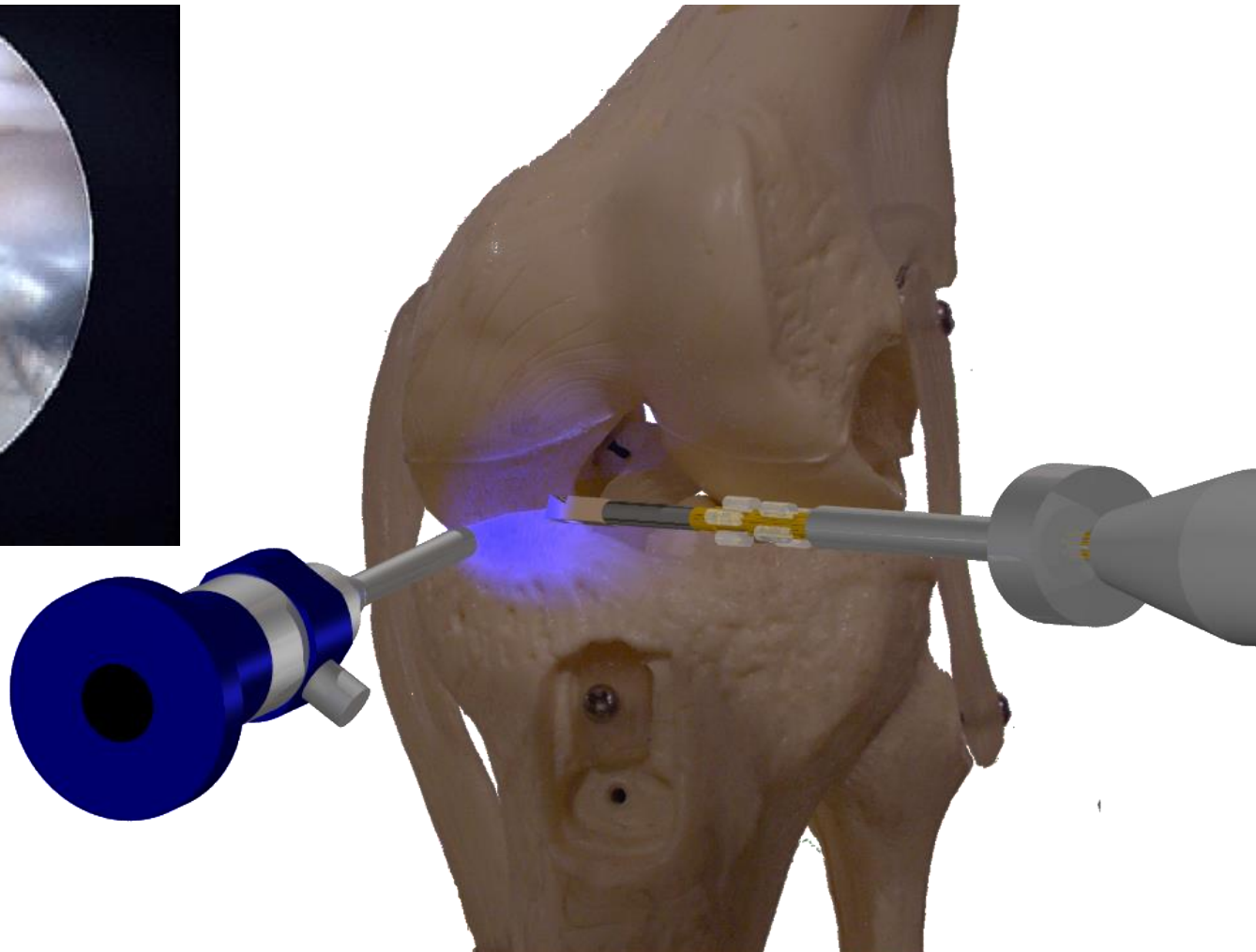
Scanning
stage

Indentation
stage

Instrument

Indentation stage





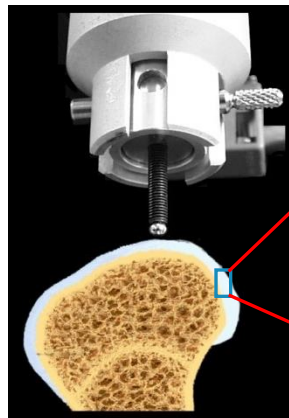
Comparison with conventional methods:

MRI



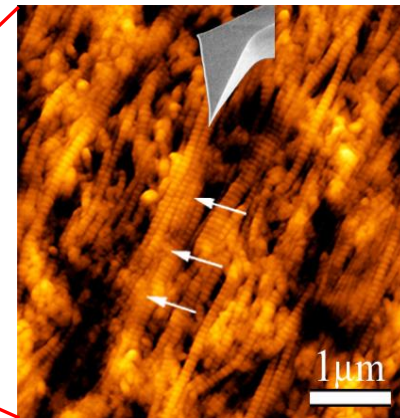
- + non-invasive, overview of the entire joint surface
- only recognition of large-scale defects
- no early detection of cartilage diseases

Classical Indentation Testing



- only limited information about the health status of cartilage
- no early detection of cartilage diseases

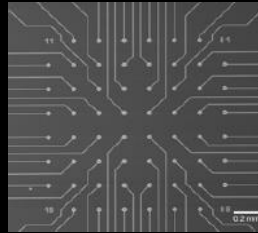
Indentation-type AFM



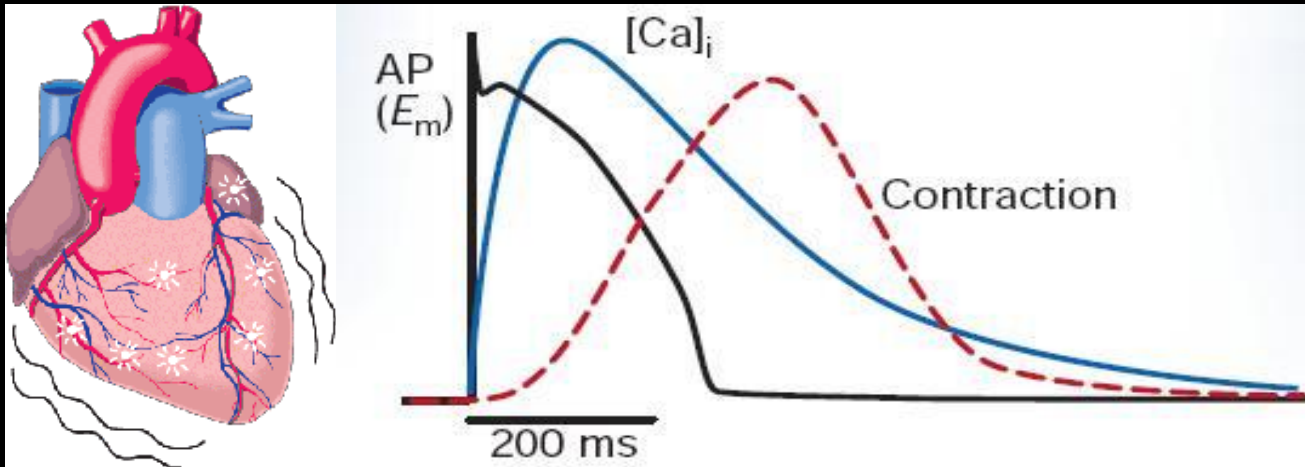
- + non-destructive
- + higher spatial resolved structural and functional analysis
- + IT SFM allows for an early detection of Osteoarthritis!

Understanding Cardiac Electro-Mechanics

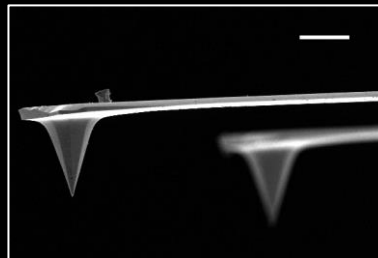
Electro-physiological domain



Micro-Electrode Arrays



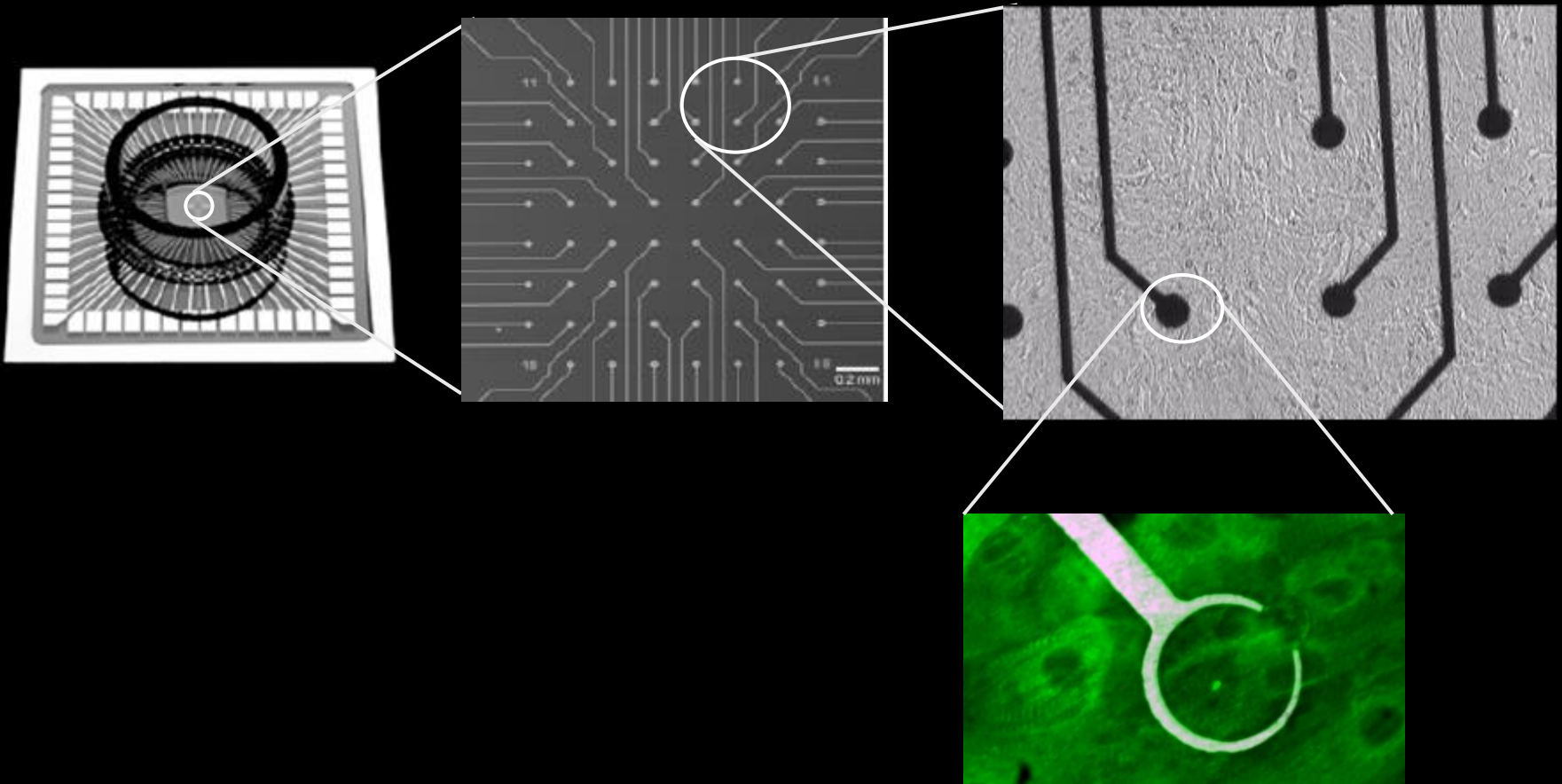
Mechano-physiological domain



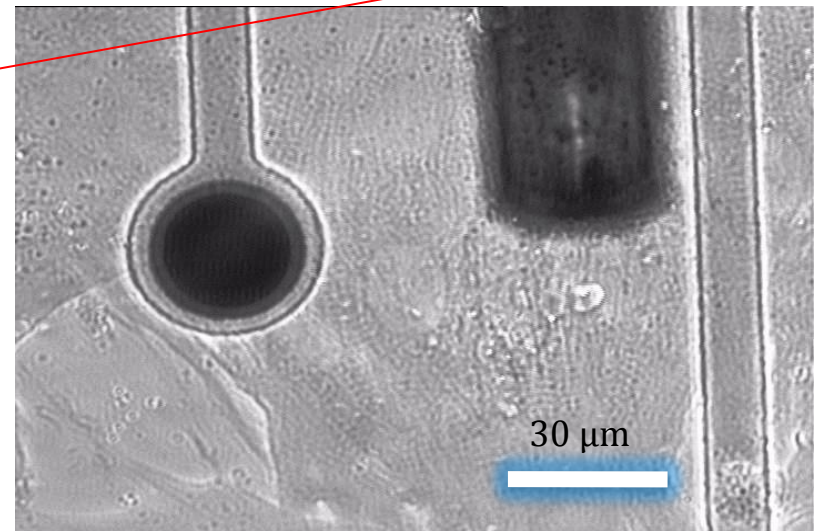
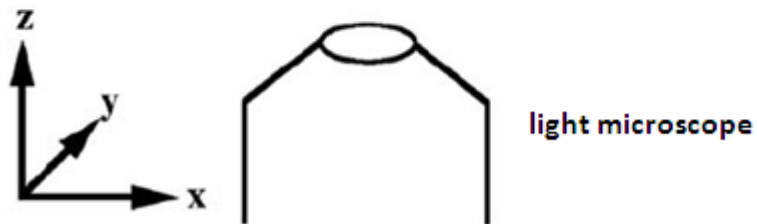
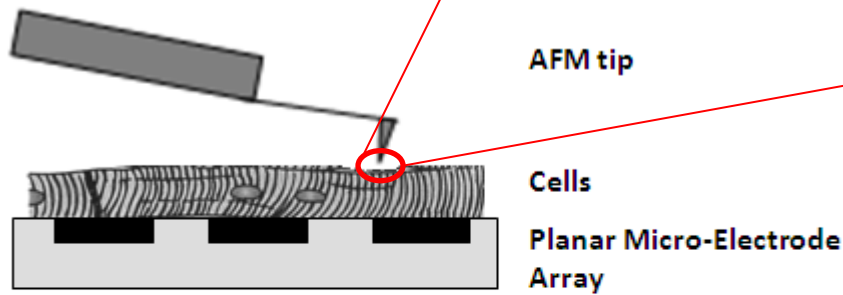
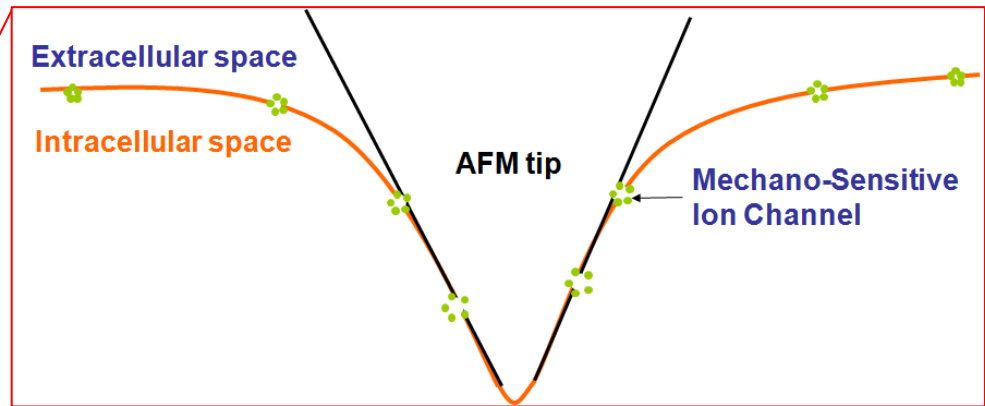
Atomic Force
Microscopy

Micro-Electrode Arrays (MEAs)

- Array of 60 planar titanium microelectrodes
- Multipoint extra-cellular recording of action potentials

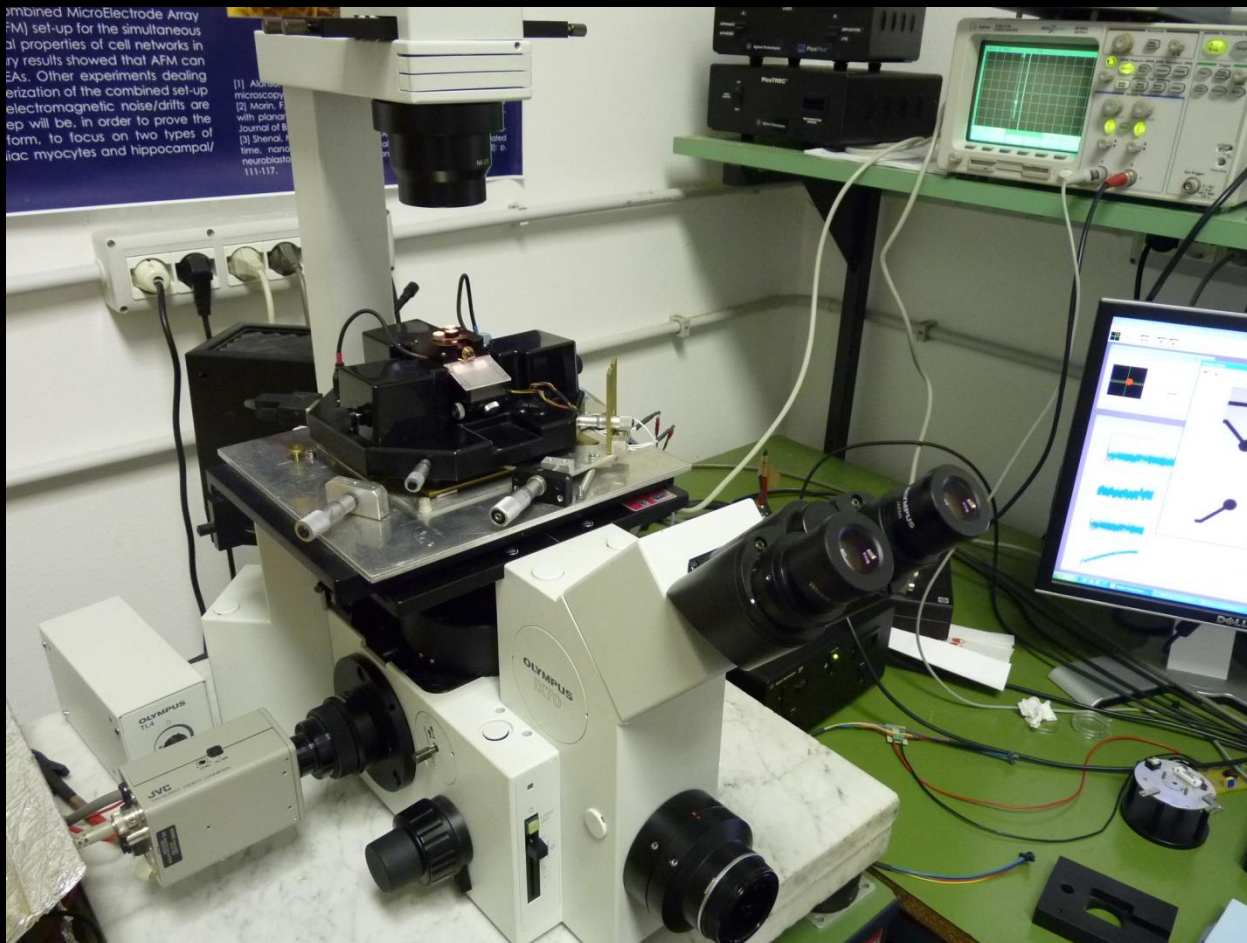


AFM/MEA combined set-up

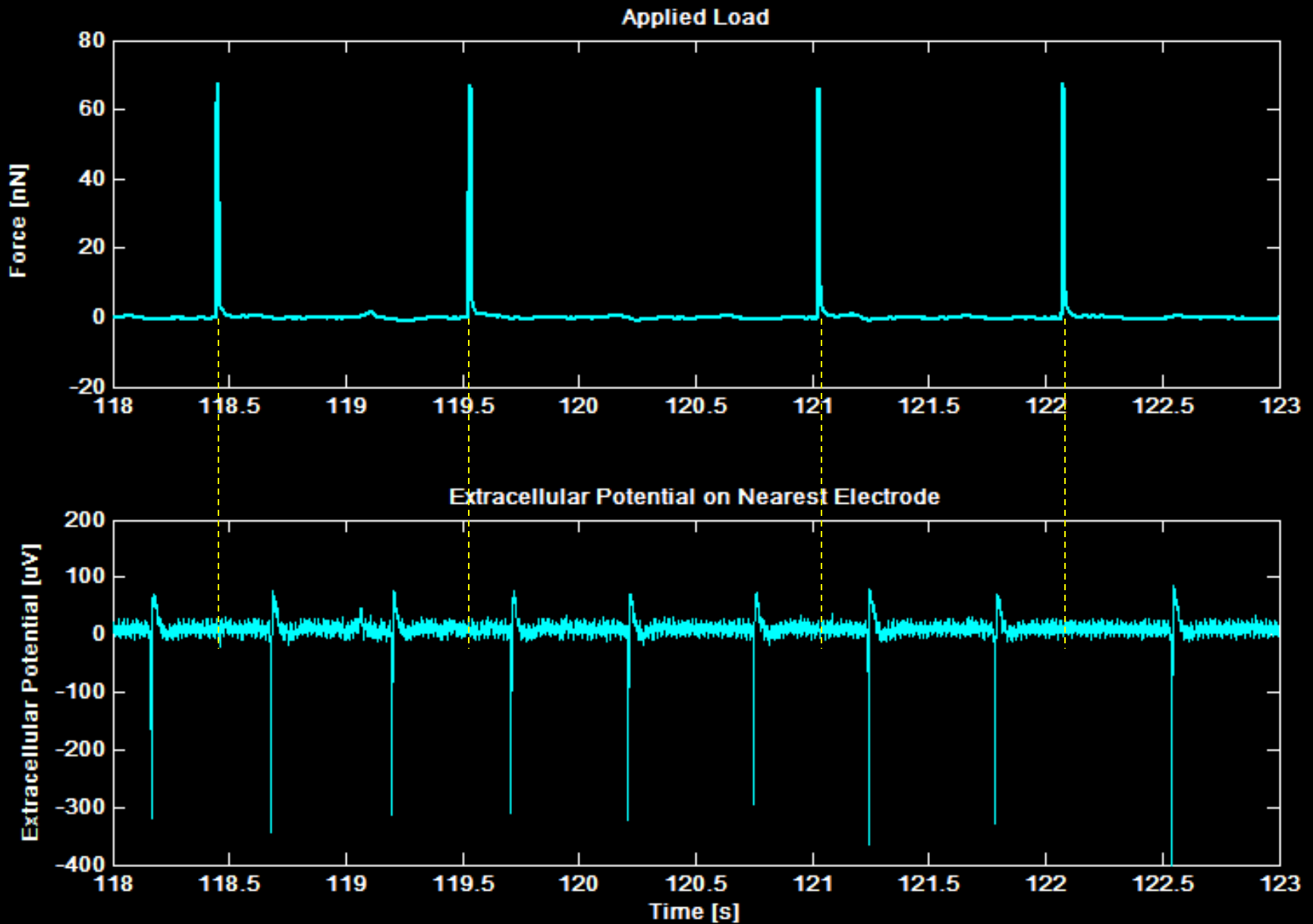


AFM/MEA combined set-up

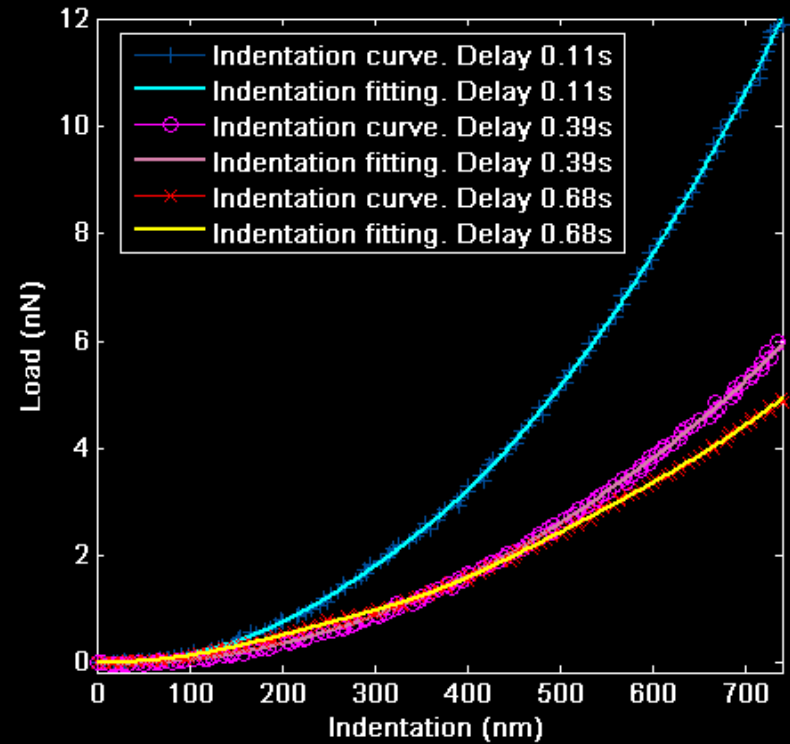
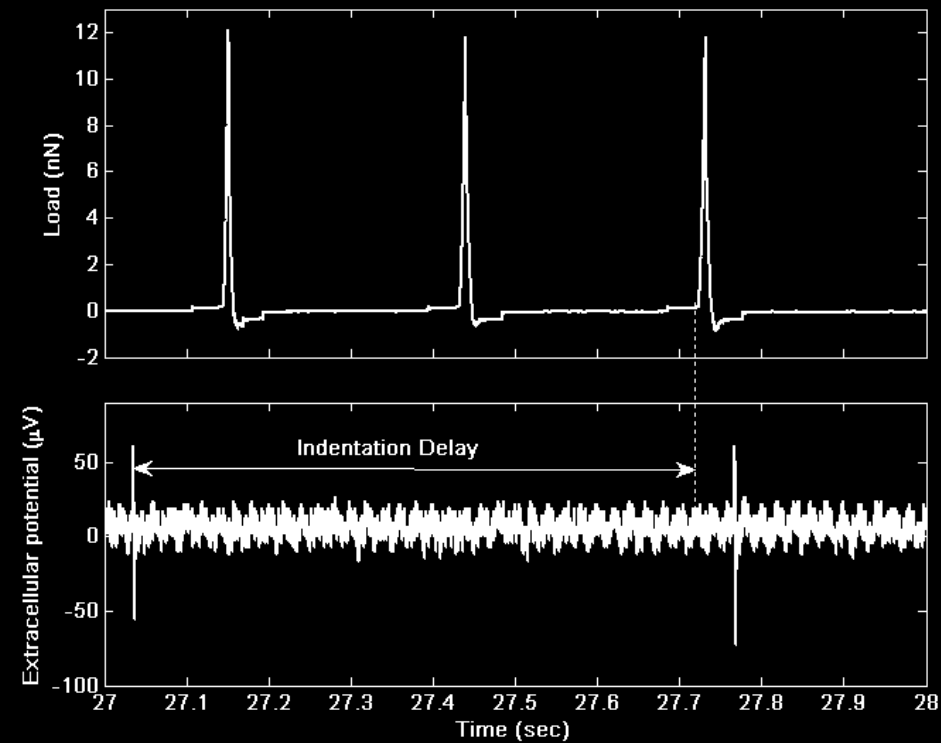
- Multi-Channel Systems MEA on the custom-made MEA stage
- Agilent 5500 AFM onto the MEA stage
- Inverted light microscope Olympus IX70



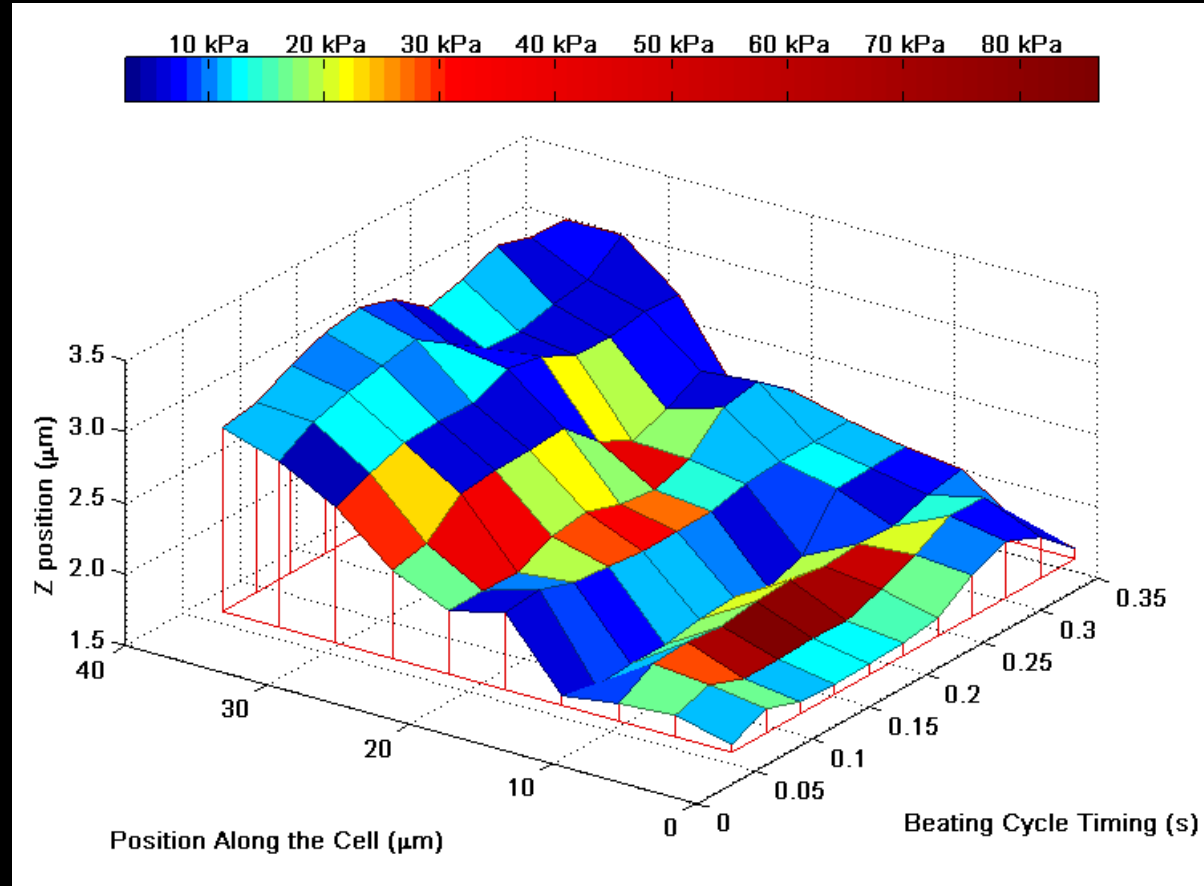
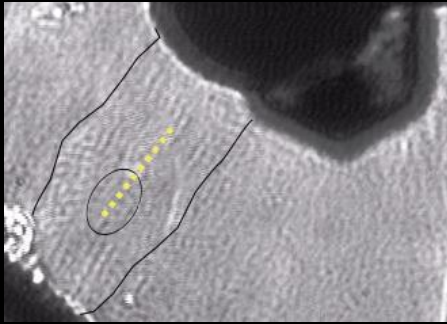
Synchronous Indentations



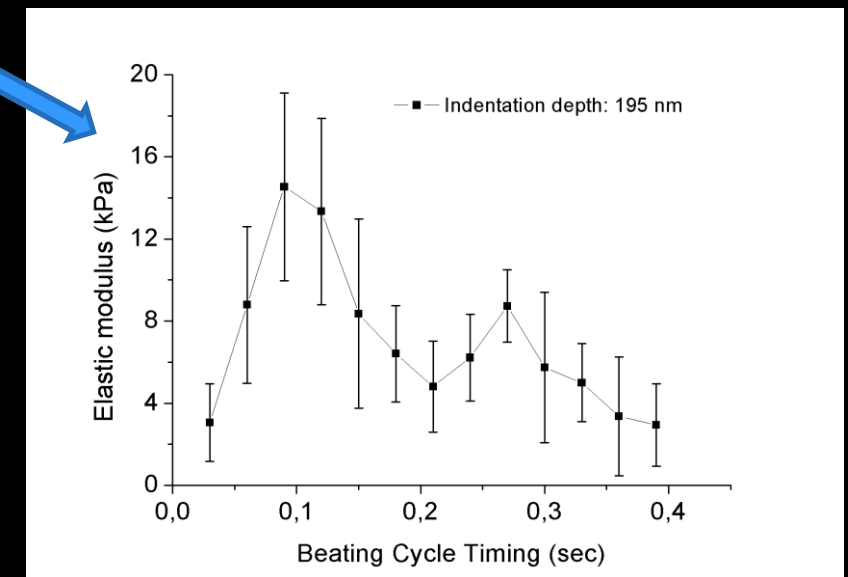
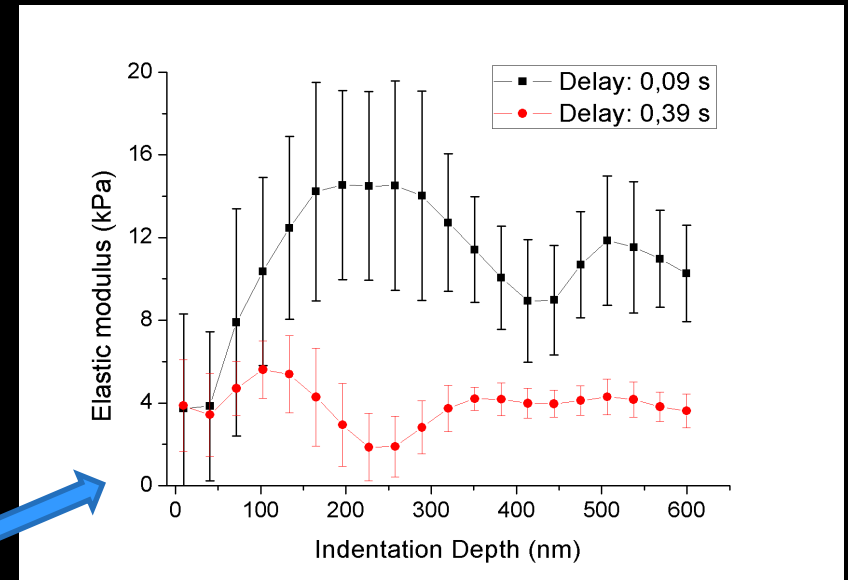
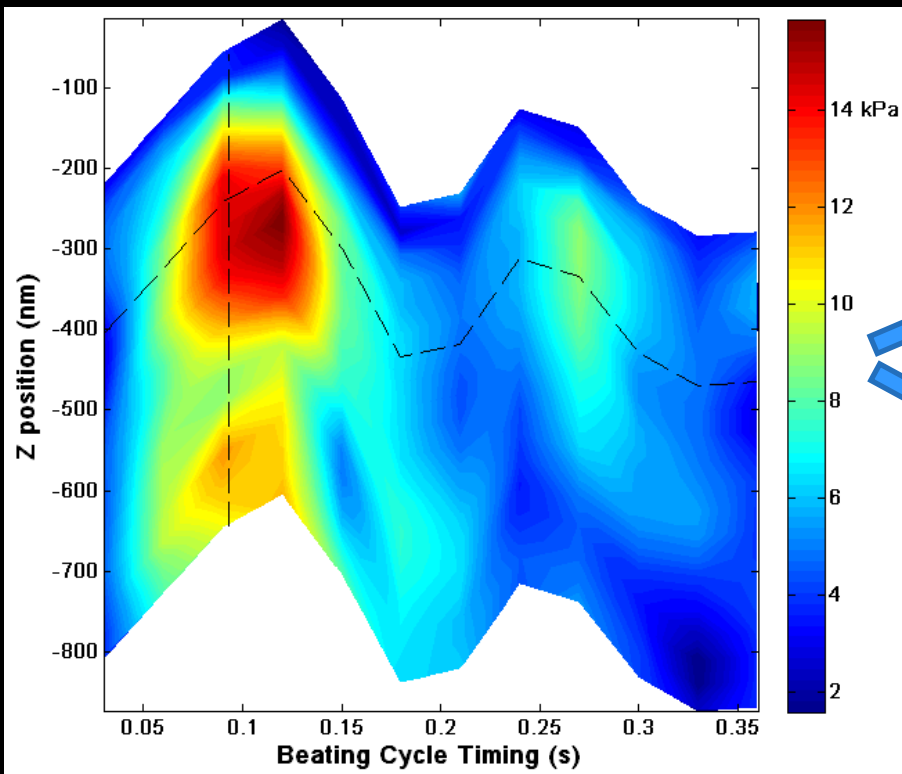
Data Analysis



Changes in morphology and elasticity during the beating cycle



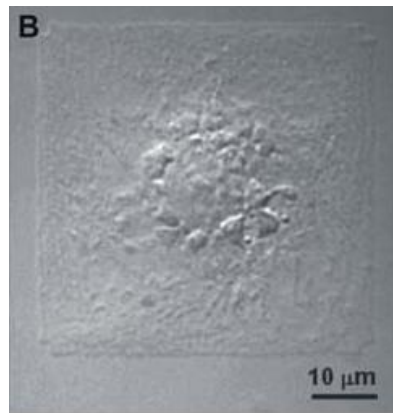
The colors of the facets represent the average elastic modulus calculated at a constant indentation depth of 195nm.



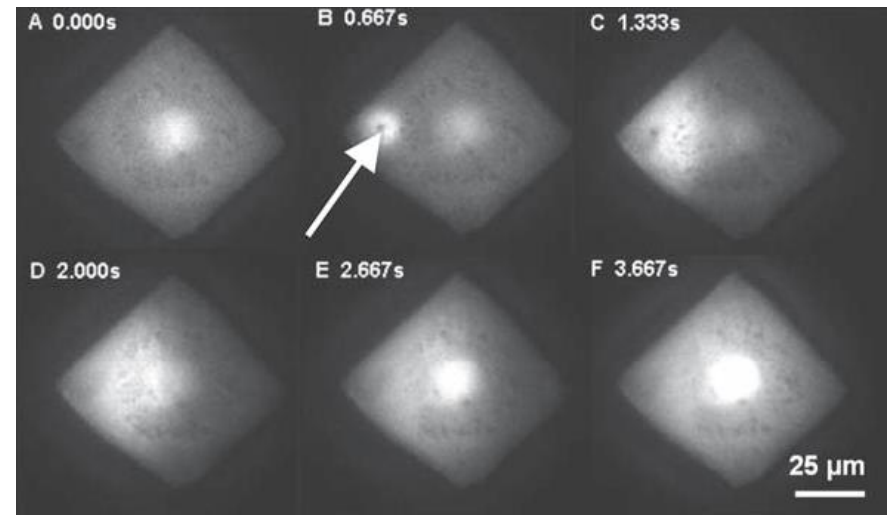
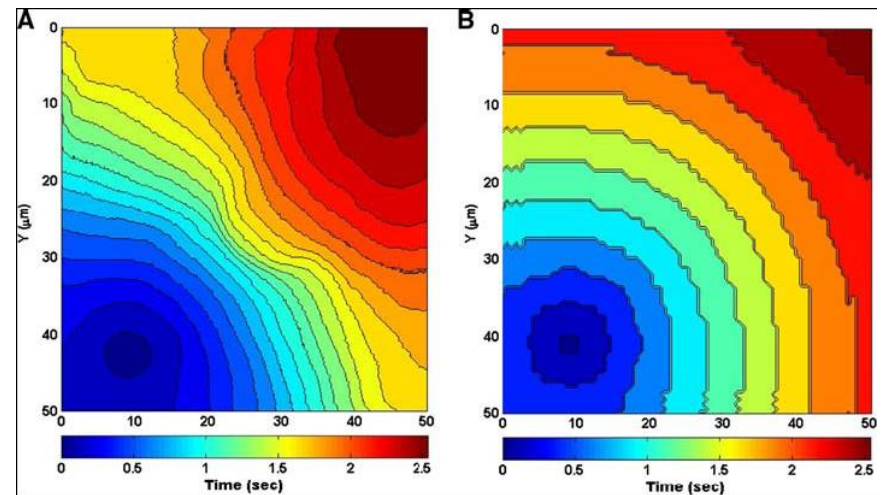
Saenz Cogollo J.F., et al. 2011. A new integrated AFM-MEA system for Measuring the Mechanical Properties of Living Cardiac Myocytes. *Biomedical Micro-Devices Journal*.

Simulazione nano-meccanica

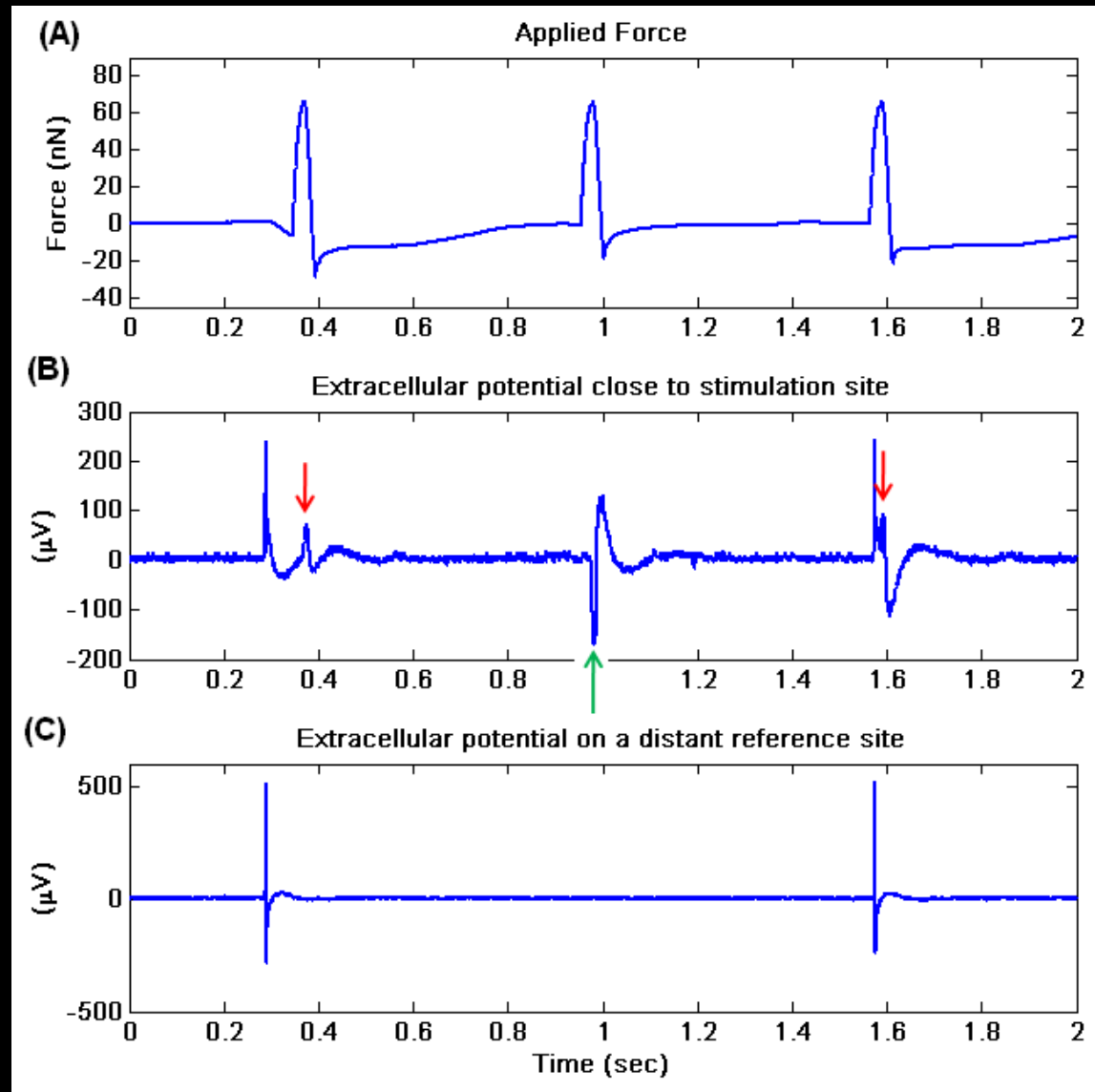
- To test physiological function of engineered myocytes, slow Ca^{2+} wave propagation was activated by mechanical perturbation of a myocyte with an AFM during high speed, fluorescence video microscopy.



Adams, W., T. Pong, et al. (2007). "Engineering design of a cardiac myocyte." Journal of Computer-Aided Materials Design **14**(1): 19-29.

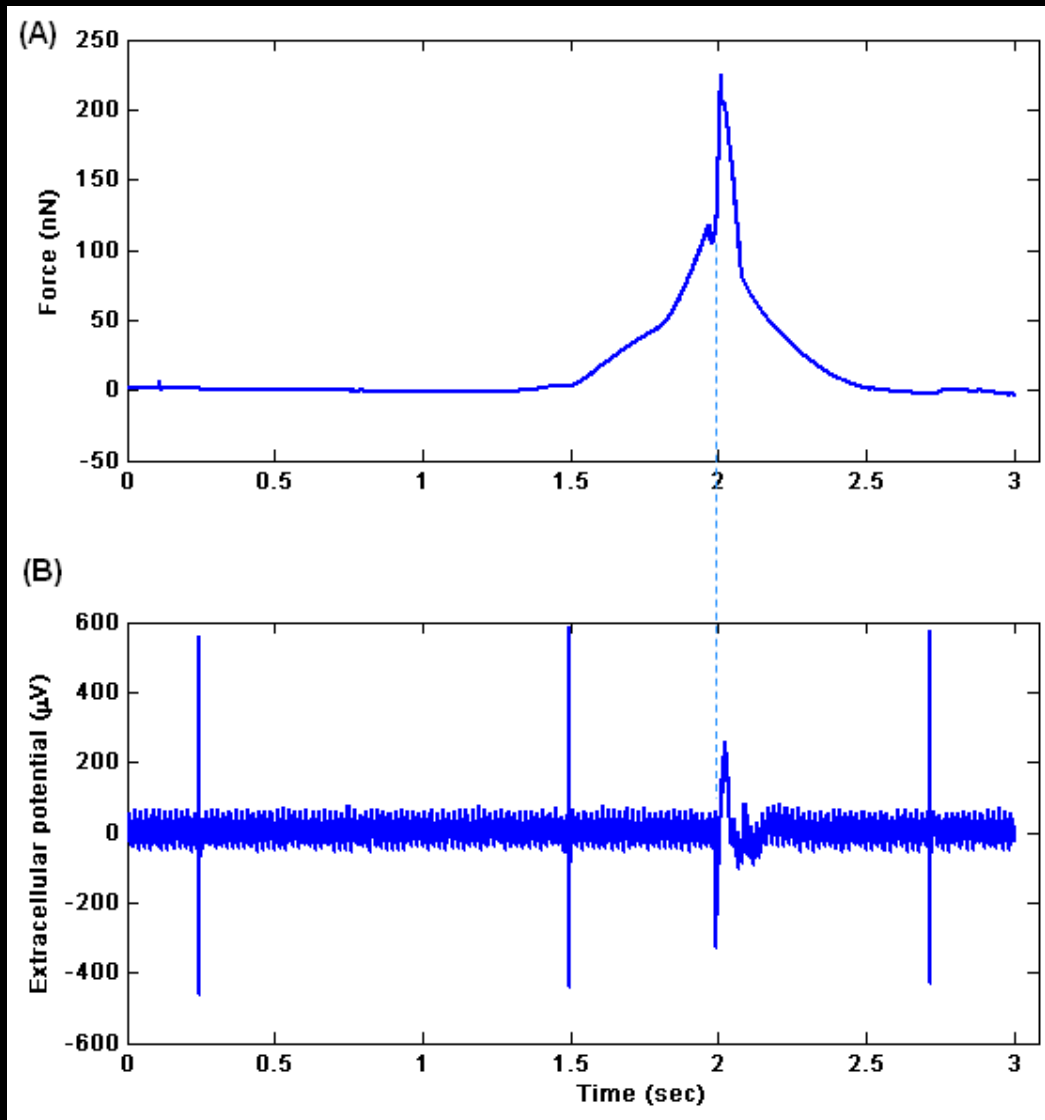


Effect of the mechanical stimulation

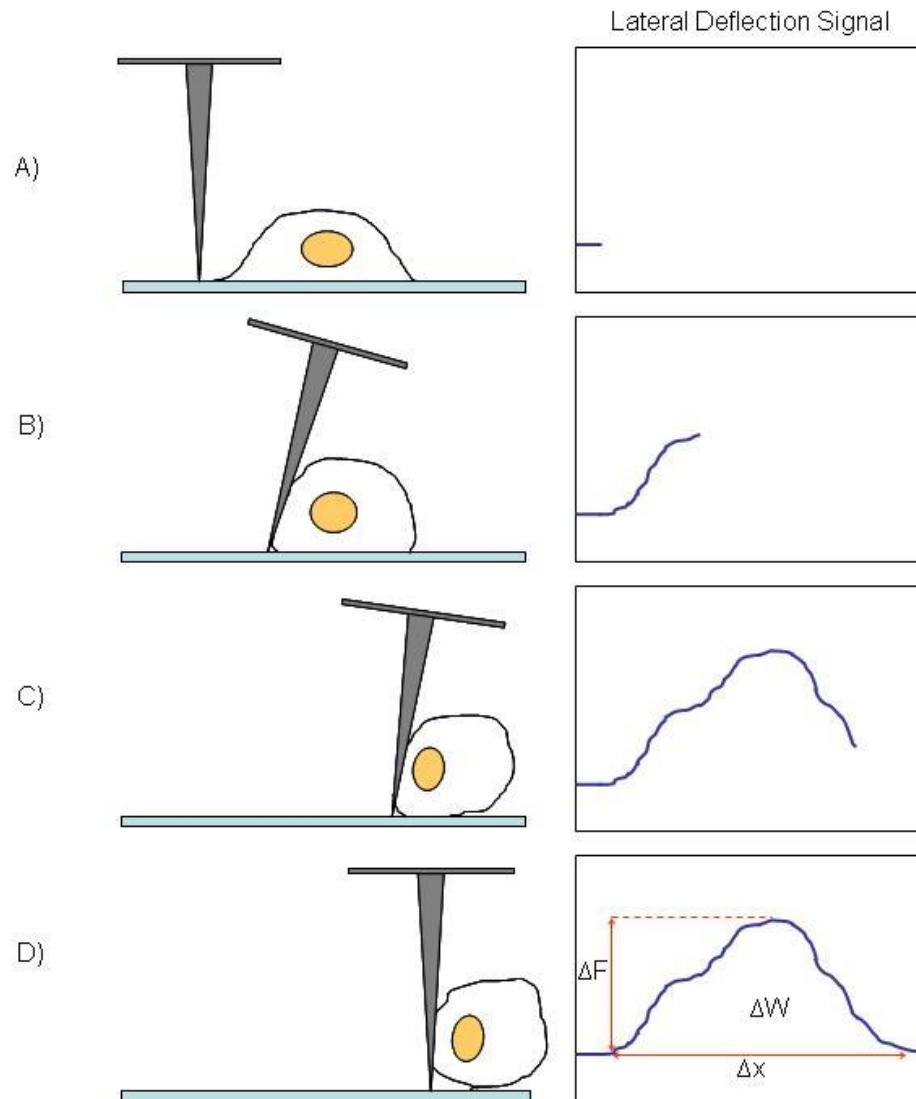
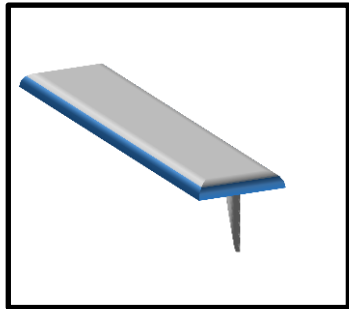


Observed induced responses seems to be produced by membrane perforations of the AFM tip

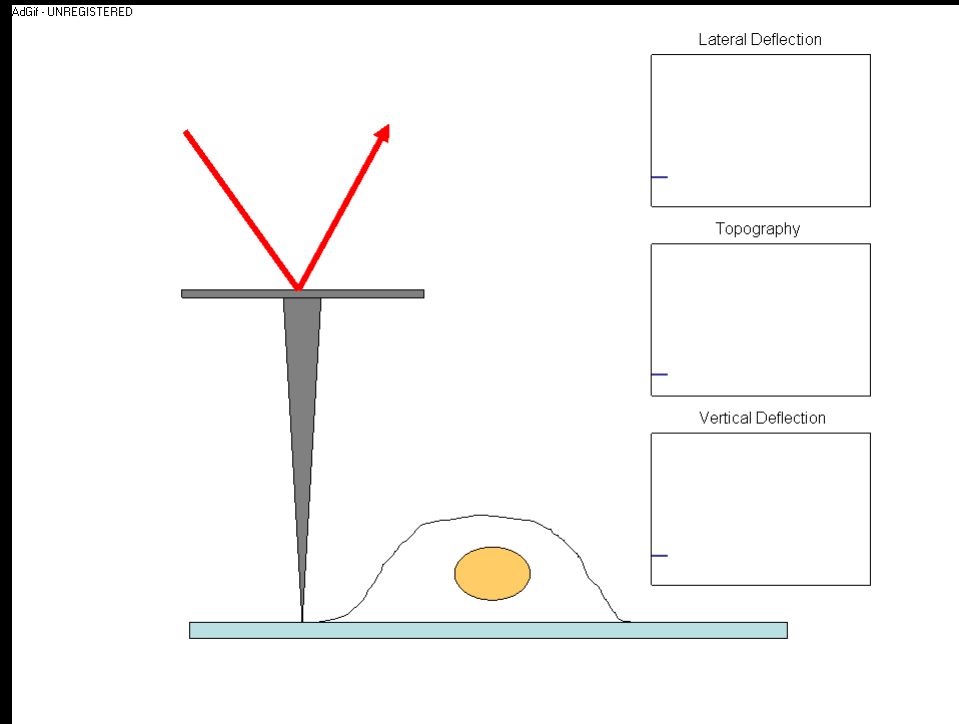
Using blebbistatin as an inhibitor of muscle contraction



The AFM in the quantification of the single cell-substrate adhesion strength



Investigation of the effects of integrin expression in the cell-substrate adhesion of osteoblast-like cells

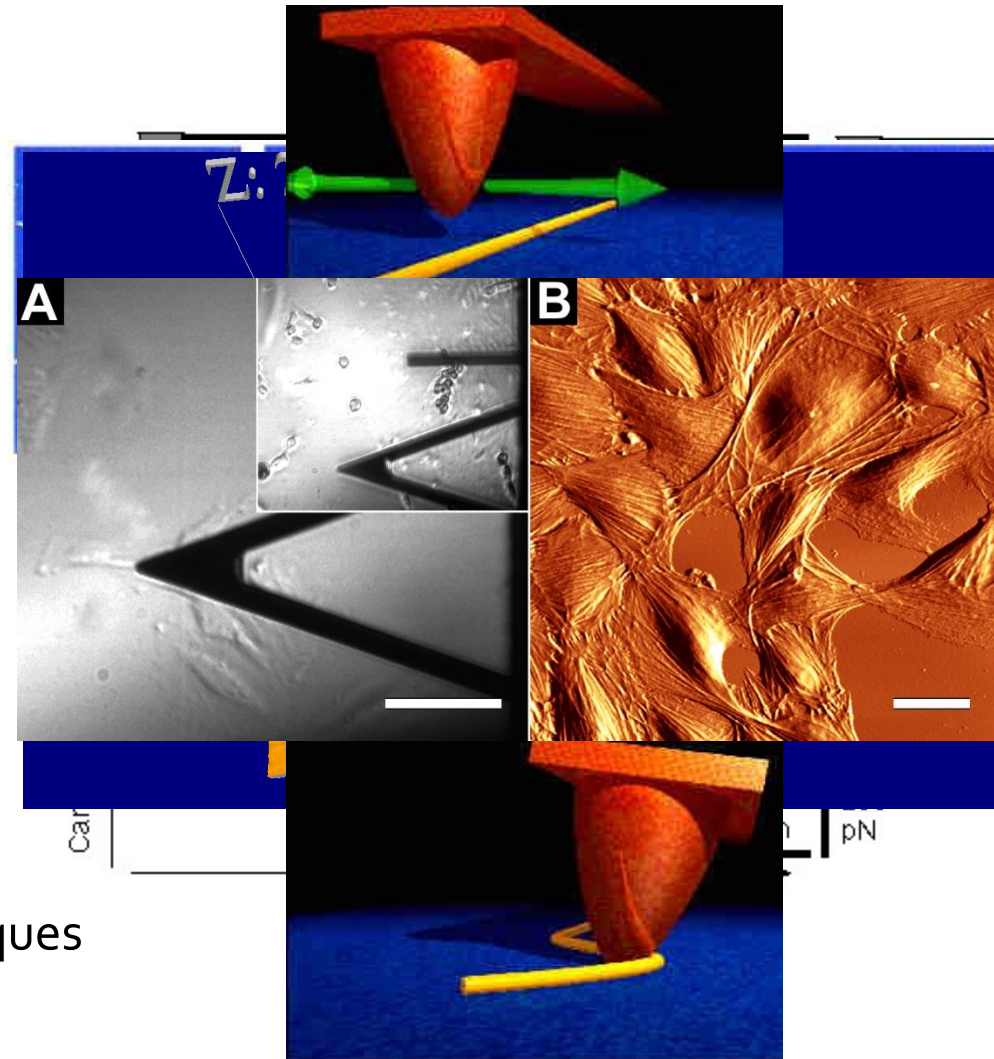


Cell type	Force ΔF [nN]	Work ΔW [nN/ μm]
Non-treated ($n = 24$)	138.09 ± 44.48	7305 ± 2242
Treated with TGF β 1 ($n = 23$)	78.44 ± 22.38	5234 ± 1706

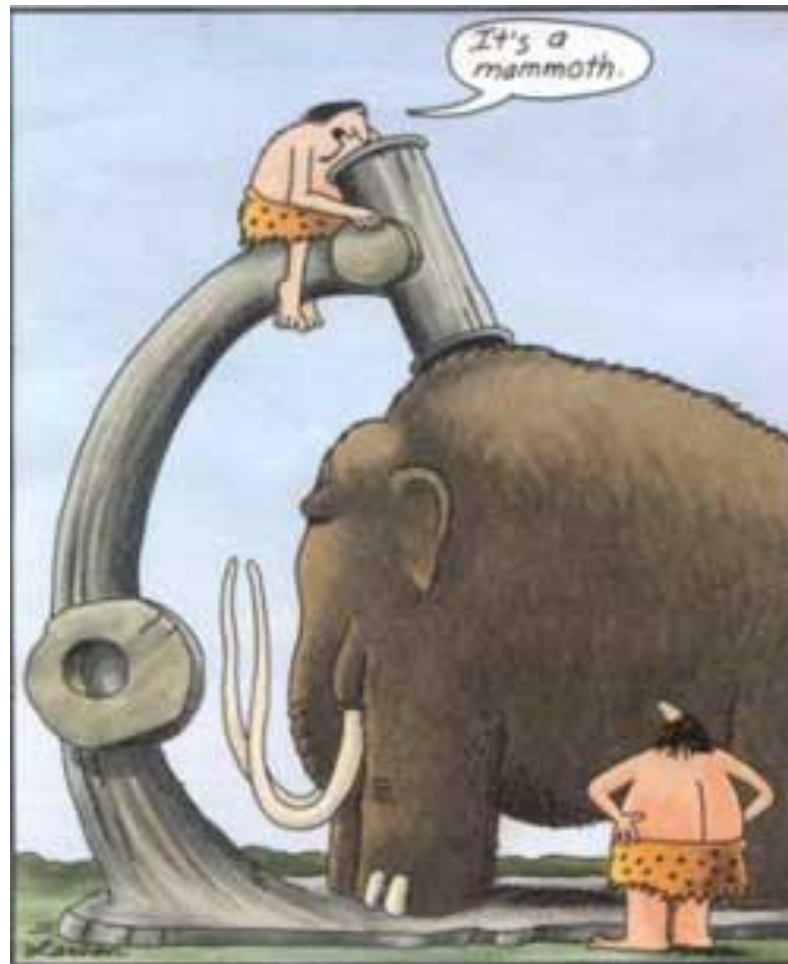
Caneva Soumetz, F., Saenz J. et al., 2010. Investigation of integrin expression on the surface of osteoblast-like cells by atomic force microscopy. *Ultramicroscopy*, 110(4), pp.330-8.

Riassunto delle possibilità dell'AFM

- high (molecular) resolution
- high flexibility
 - vacuum/ambient/liquid
 - no sample preparation
 - non destructive imaging
- true 3D topography
- functional imaging (electric, magnetic, mechanical, chemical properties)
- quantitative measurement of interaction forces
- nano- actuation/manipulation
- Coupling AFM with optical techniques



Domande?



Early microscopes