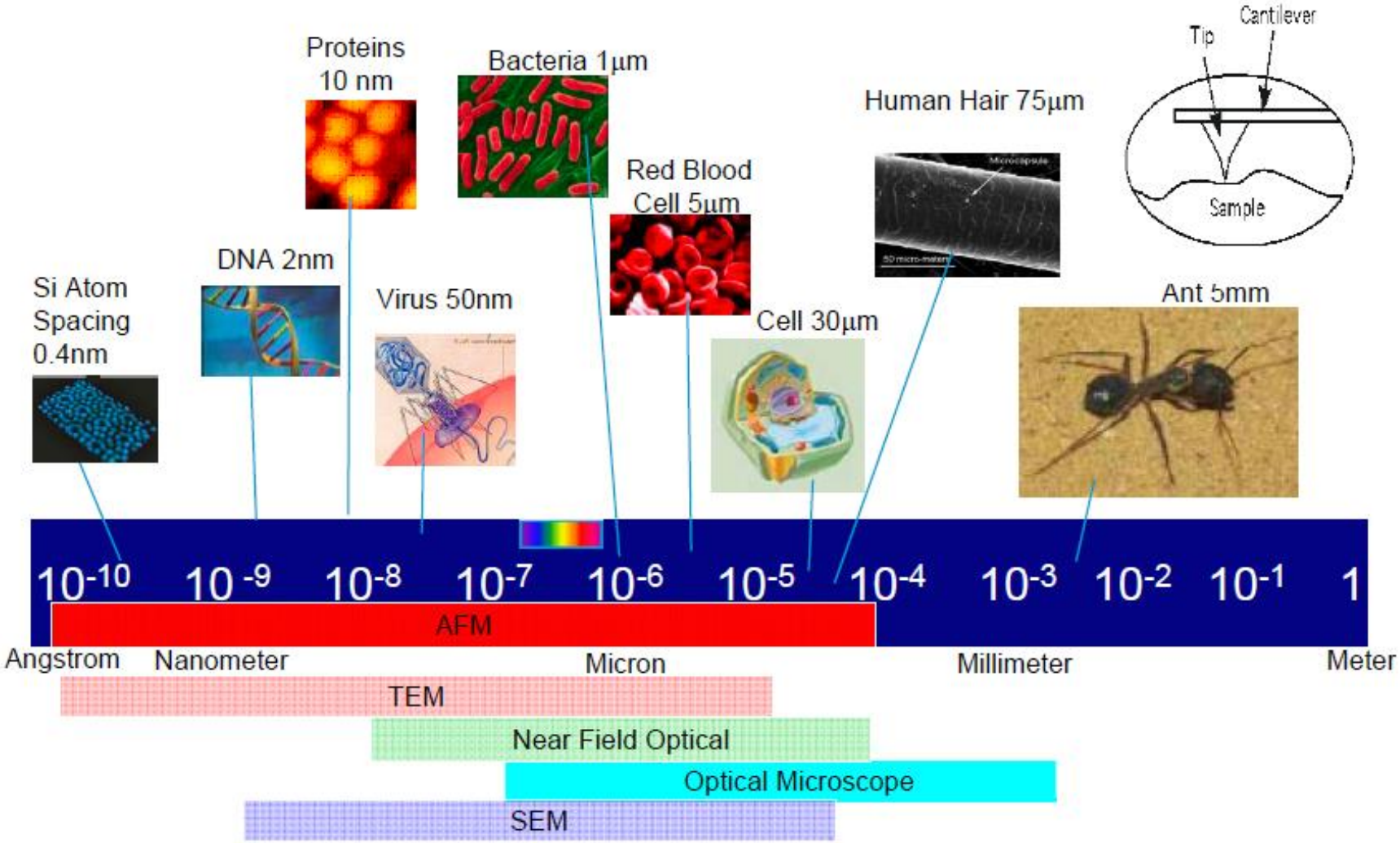
Imaging Techniques: Scales



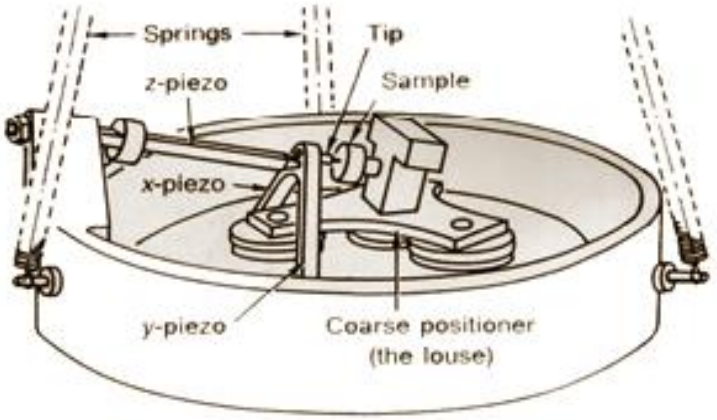
Atomic Force Microscopy, AFM
Scanning Force Microscopy (SFM)

History of SPM

1986 Nobel Physics Price

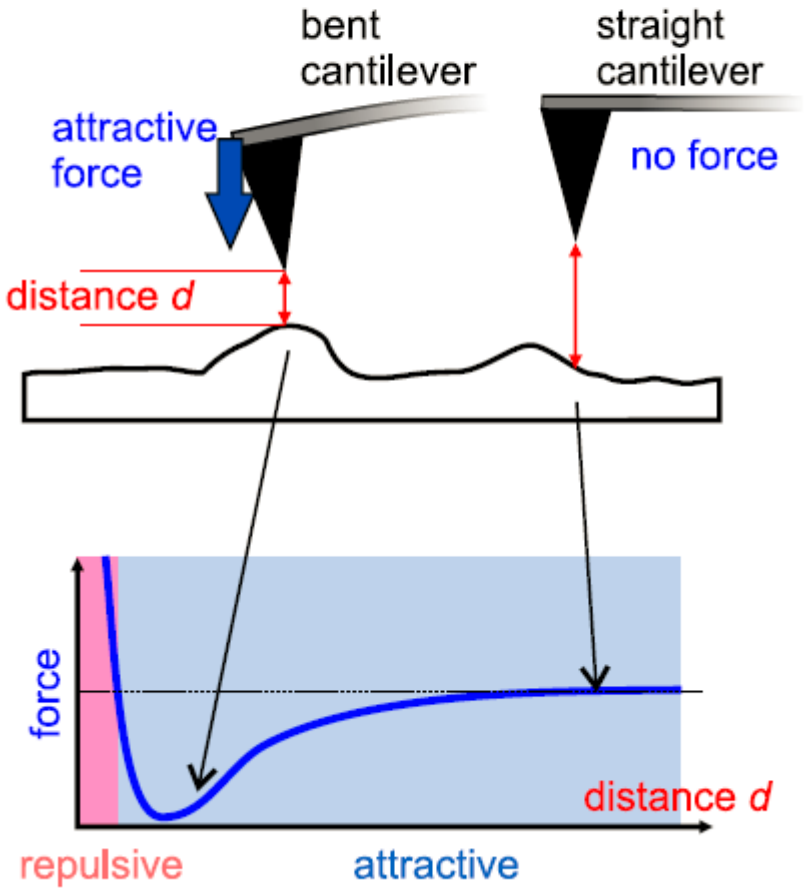
The IBM Zurich laboratory soccer team. Binnig (right) and Rohrer (left) are the two members holding flowers. The IBM team lost to Dow Chemical 2:4!

The first STM Instrumentation

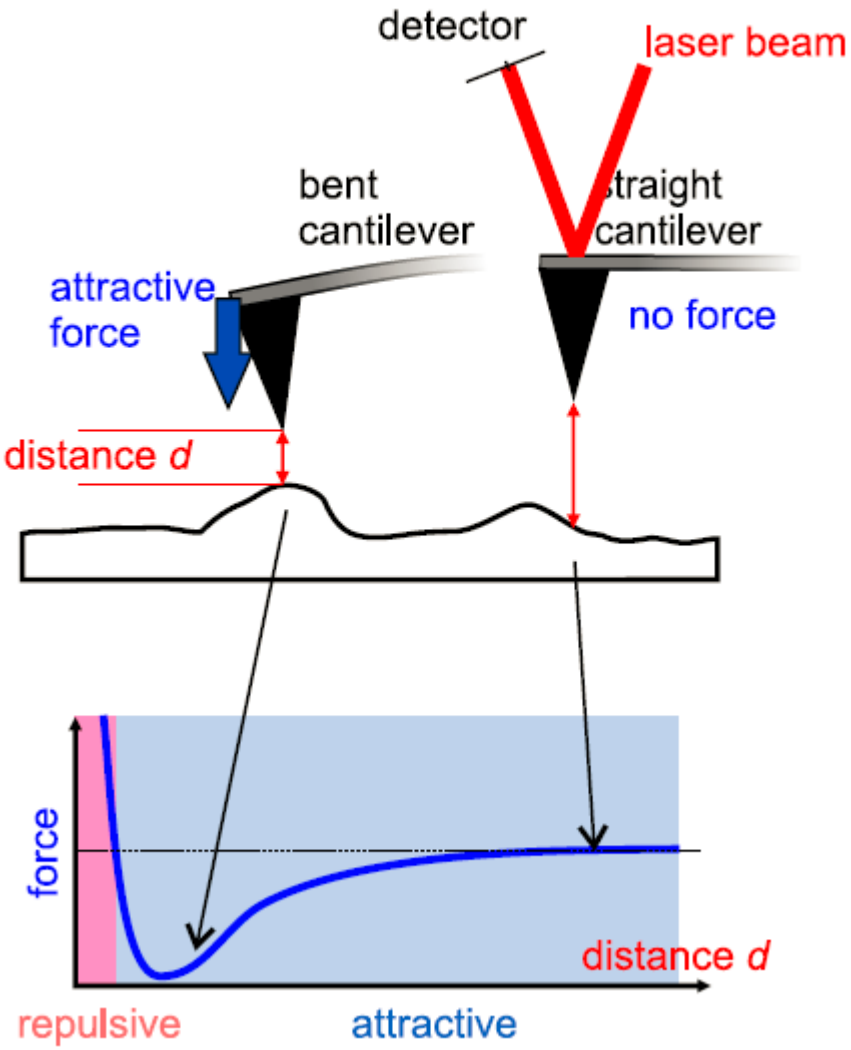


What is AFM? An analogue

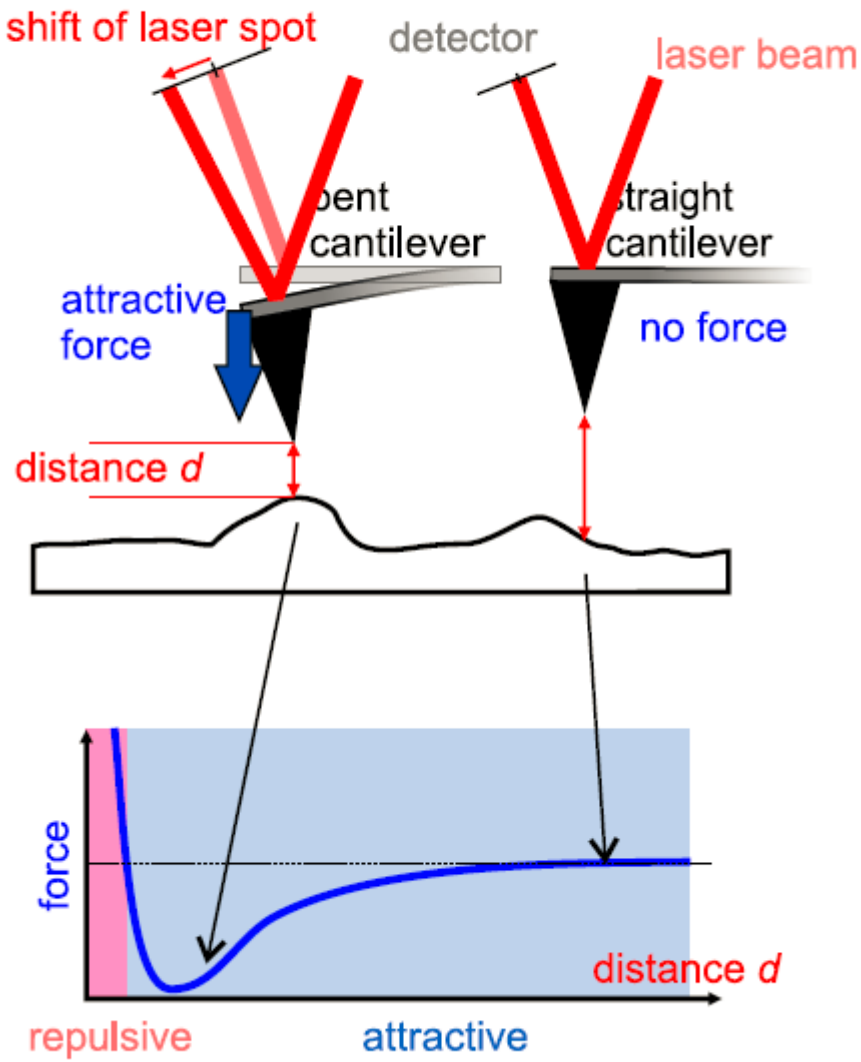
Atomic Force Microscopy (AFM): Tip & Surface



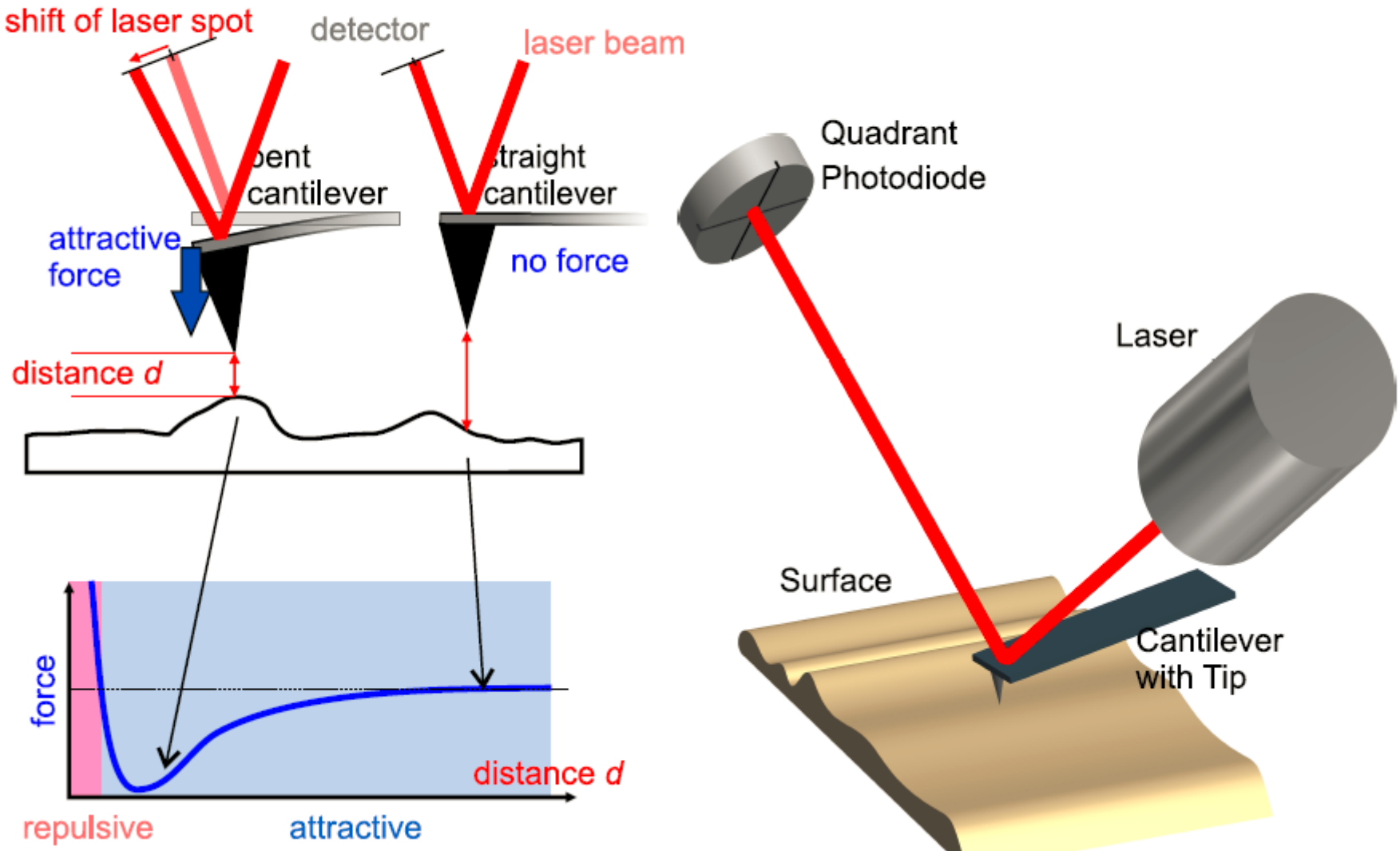
Atomic Force Microscopy (AFM): Tip & Surface



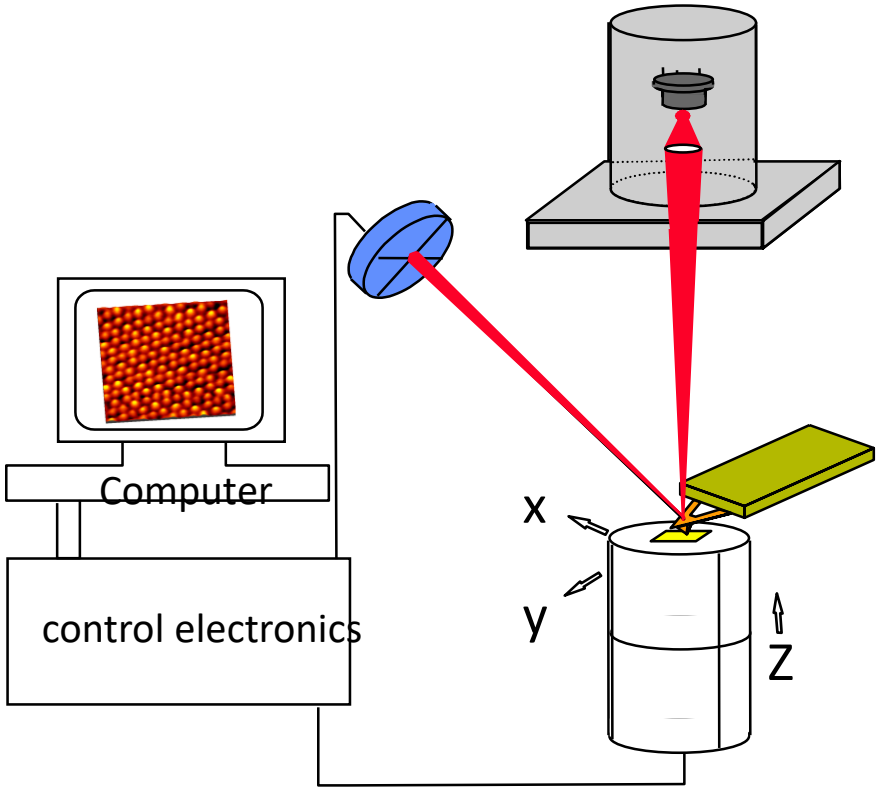
Atomic Force Microscopy (AFM): Tip & Surface

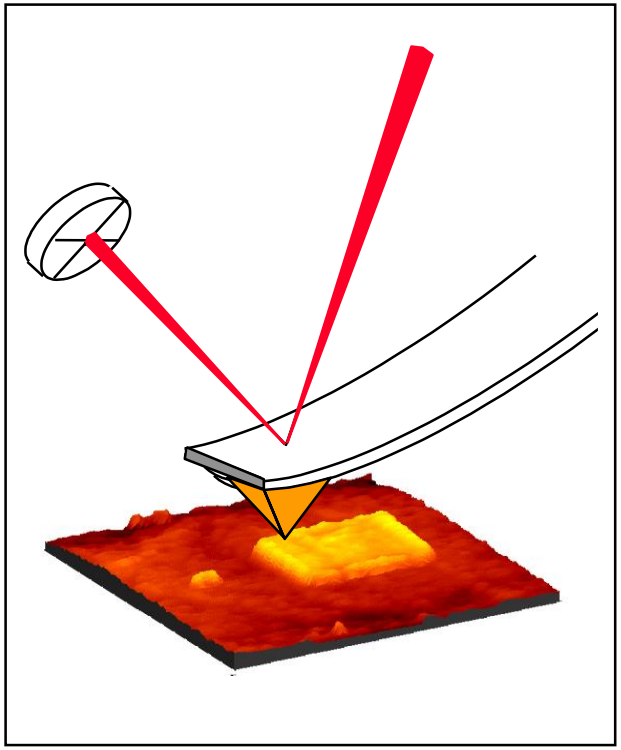


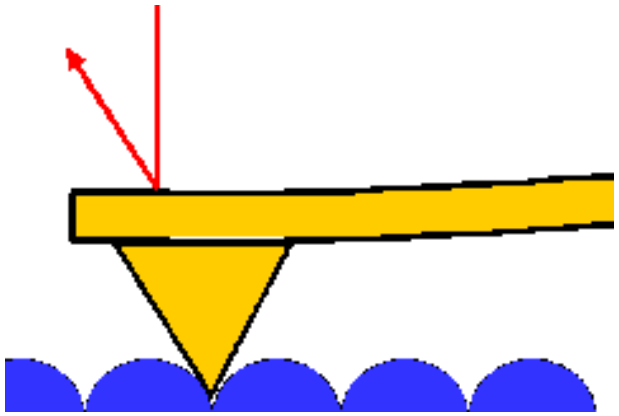
Atomic Force Microscopy (AFM): Tip & Surface



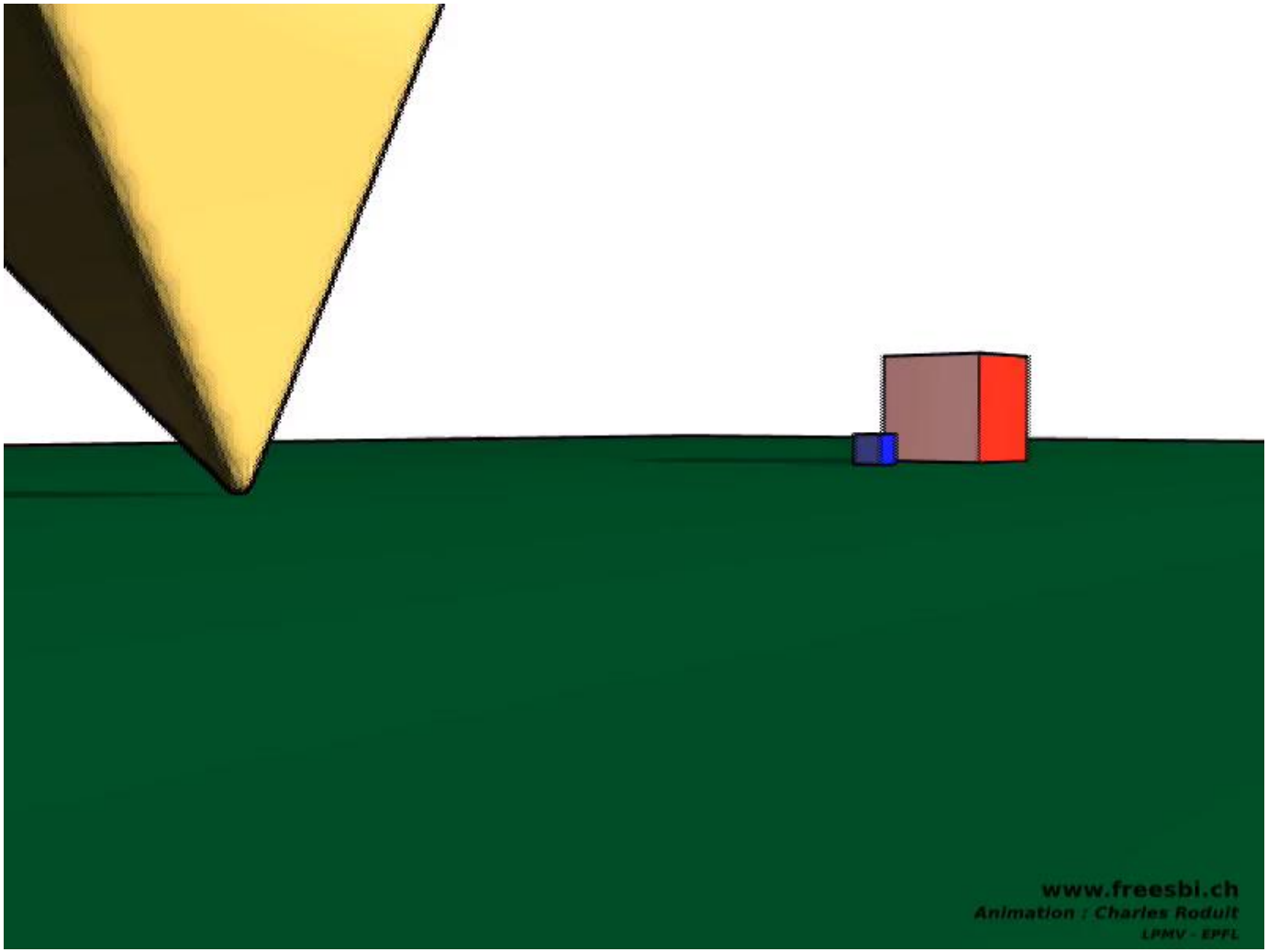
Laser beam deflection type AFM(most commercial AFM)







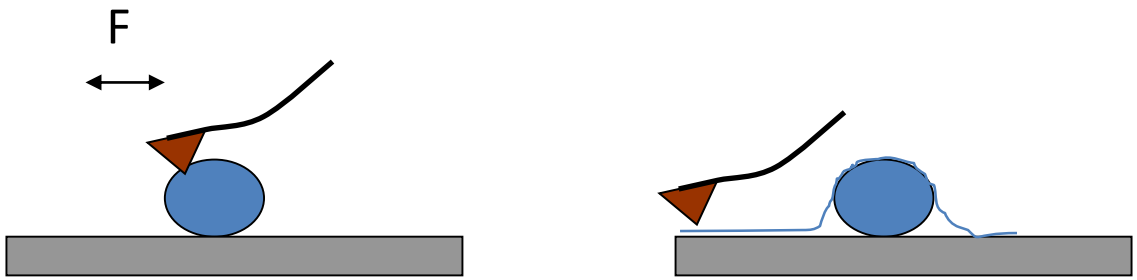
Contact



Basic AFM Imaging Modes

Contact mode AFM (1986)

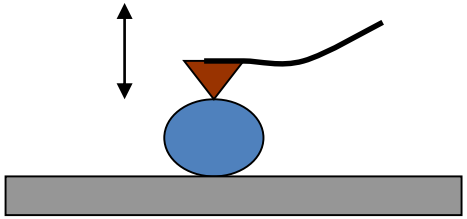
- Small vertical force, but probe dragging over the surface exerts lateral force
- Weakly bound soft samples move easily
- Images not clear

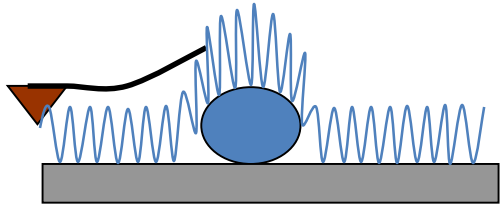


Intermittent-contact

Basic AFM Imaging Modes

AC Mode AFM (1993)

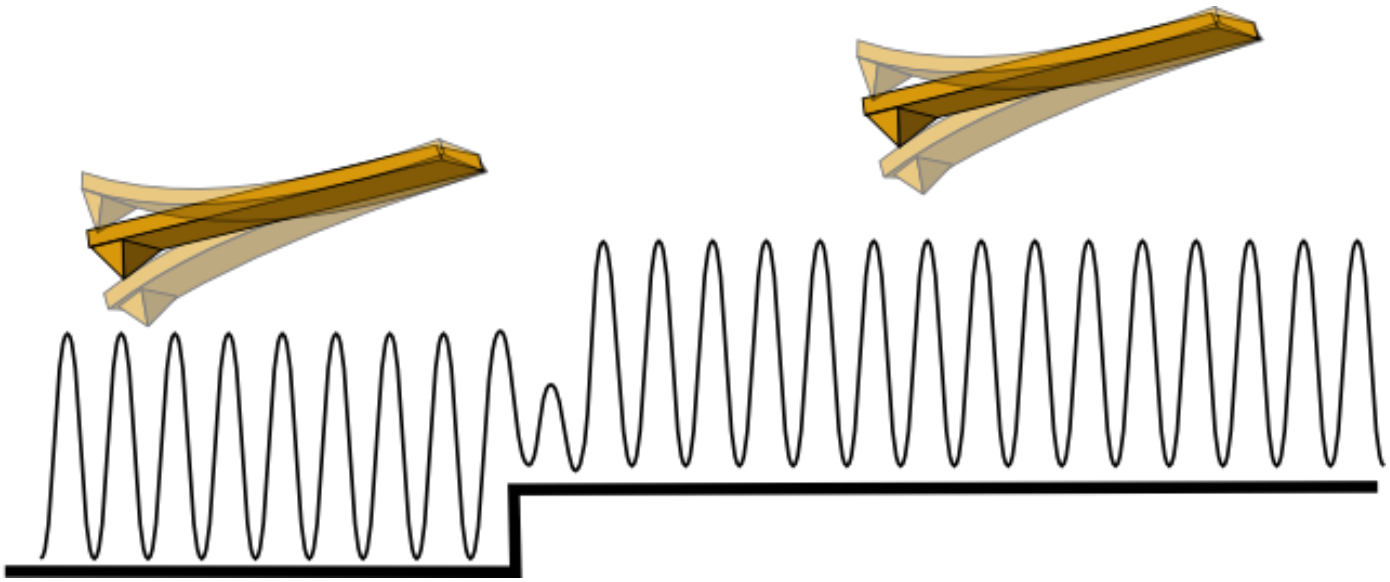




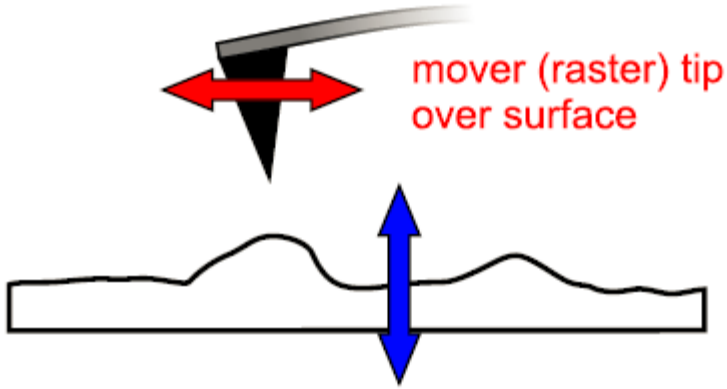
- Intermittent contact
- Soft surfaces stiffened by viscoelastic response.
- Impact predominately vertical, therefore large vertical force, but no lateral force.
- Images very clear

F

Tapping Mode



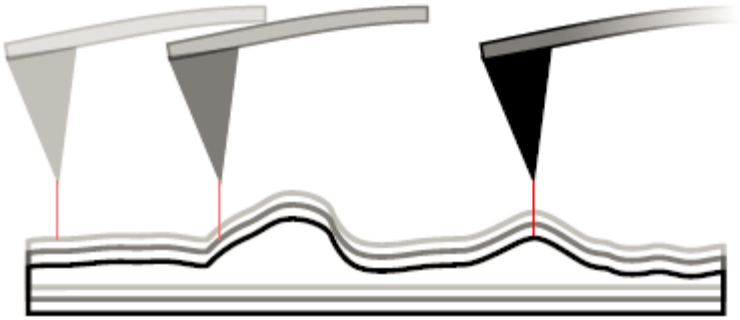
Atomic Force Microscopy (AFM): Imaging Mode



mover (raster) tip
over surface

move surface up/down,
so that distance is constant

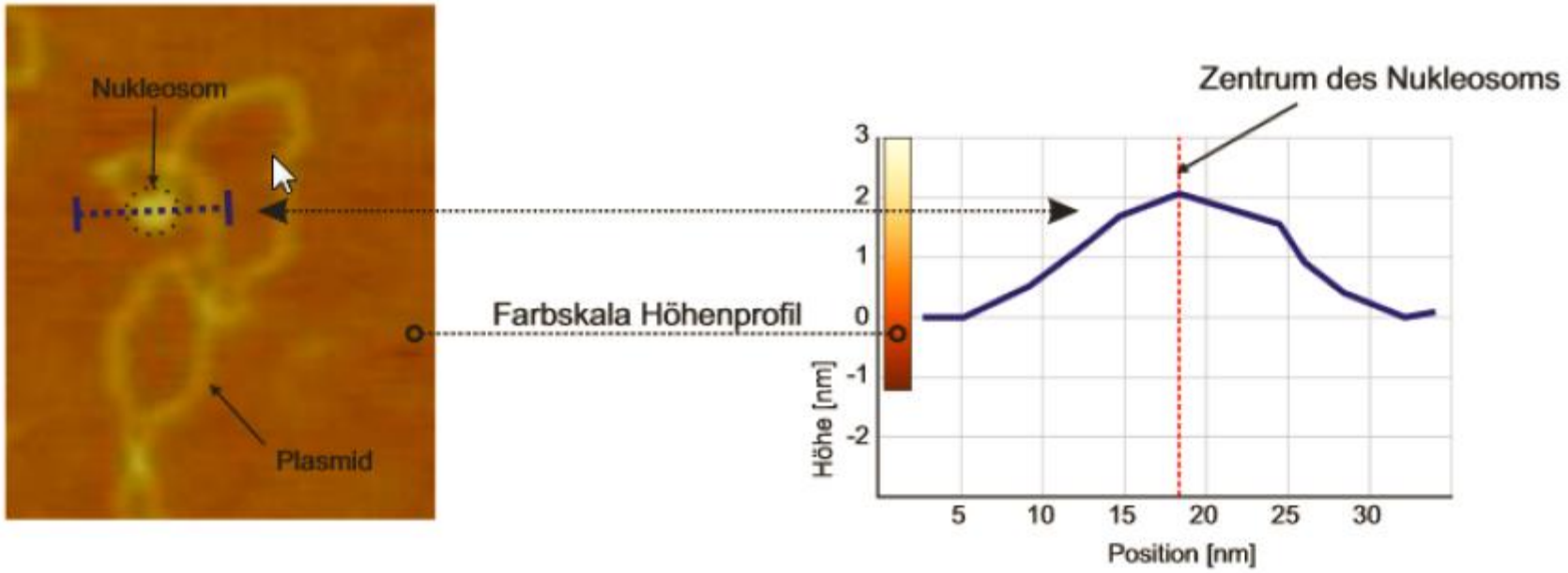
store for every position:
how much did you have
to move surface
=> height map

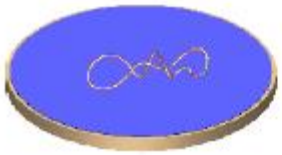


Atomic Force Microscopy (AFM): Superhelical DNA & Nucleosomes

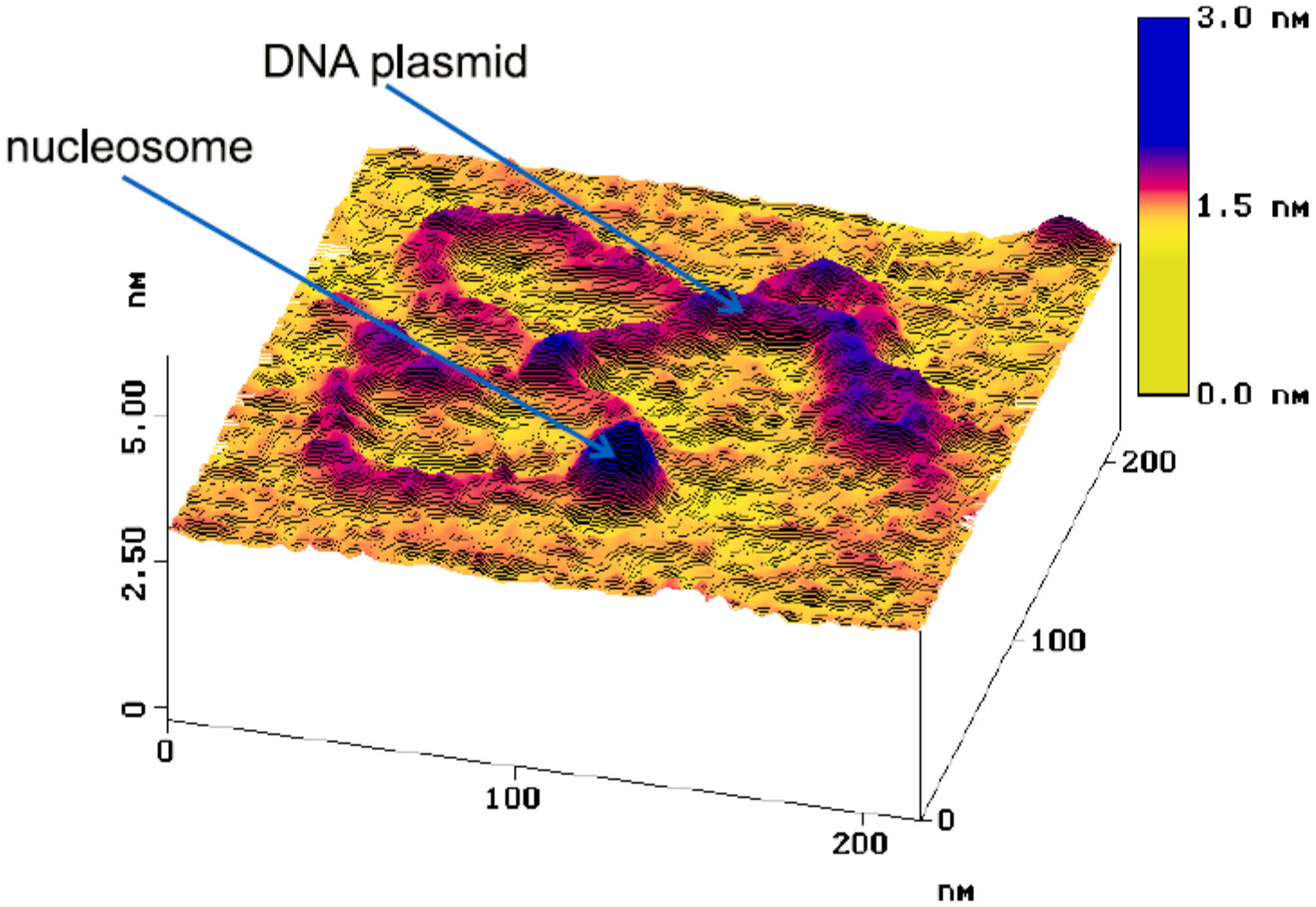
a) AFM-Topographie

b) Höhenprofil: Schnitt durch ein Nukleosom





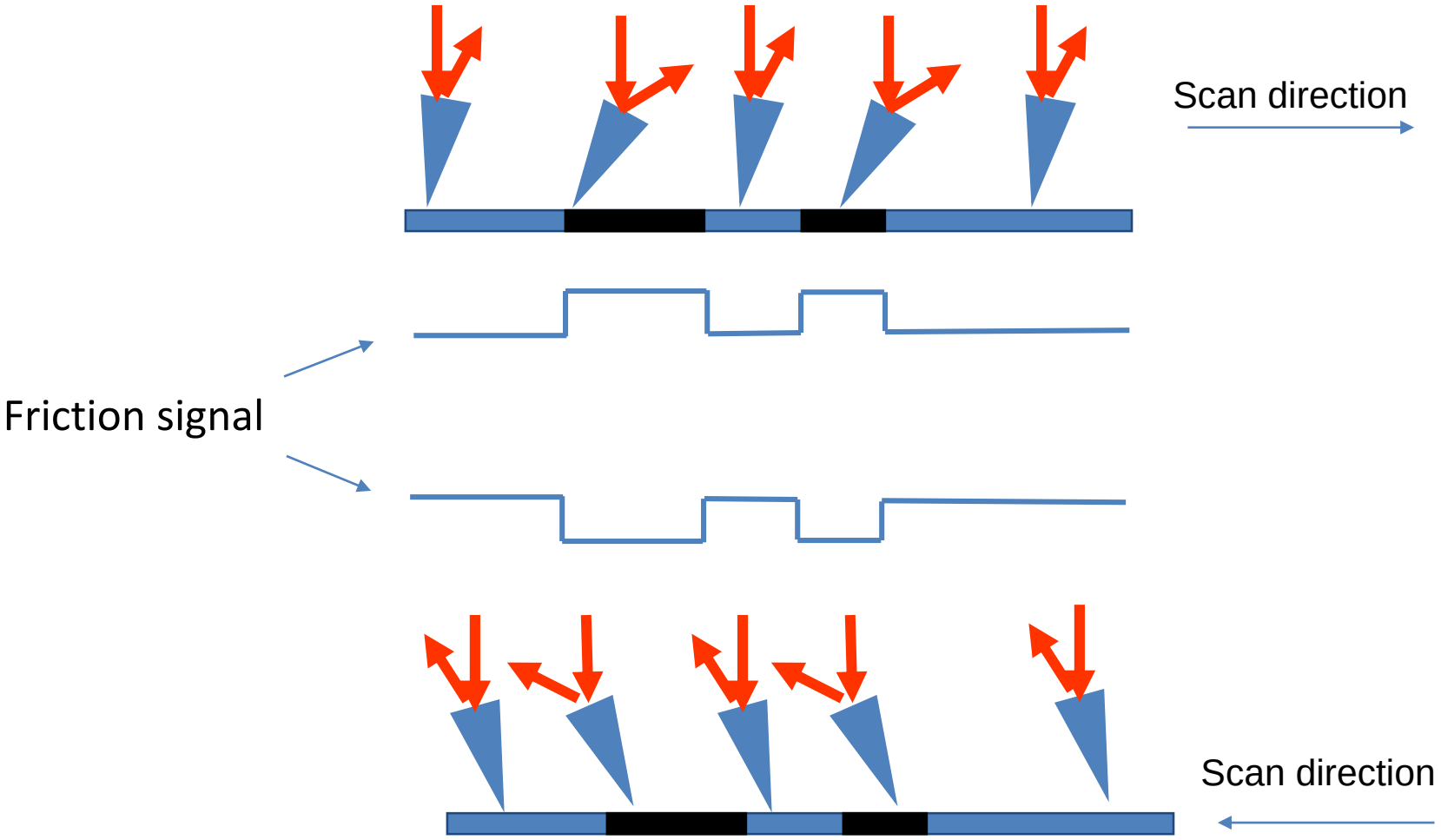
Atomic Force Microscopy (AFM): Example



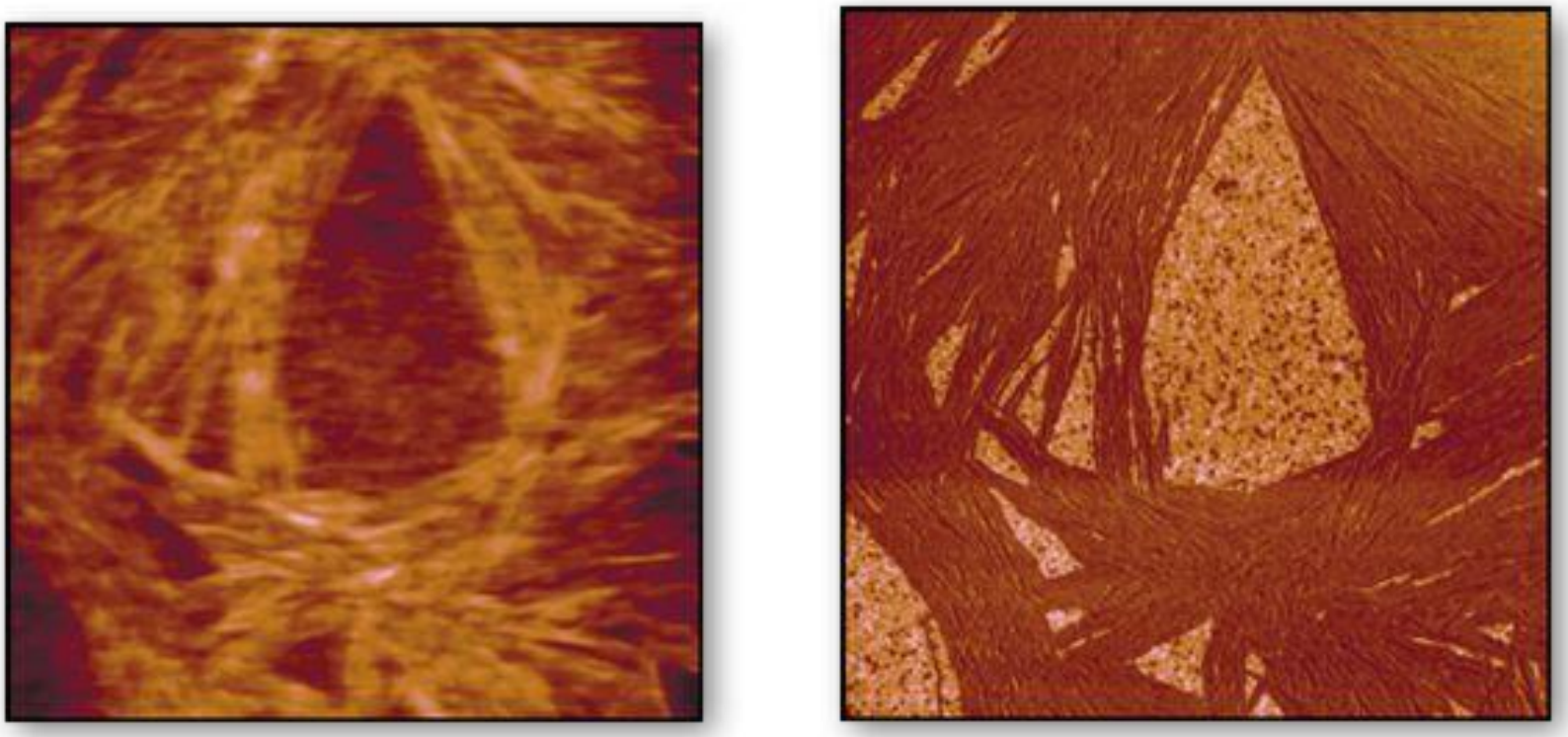
AFM image (height map) of a DNA molecule with a bound protein

Basic AFM Imaging Modes

Friction Imaging (operate at contact mode)



Polyvinyl Alcohol at 65C

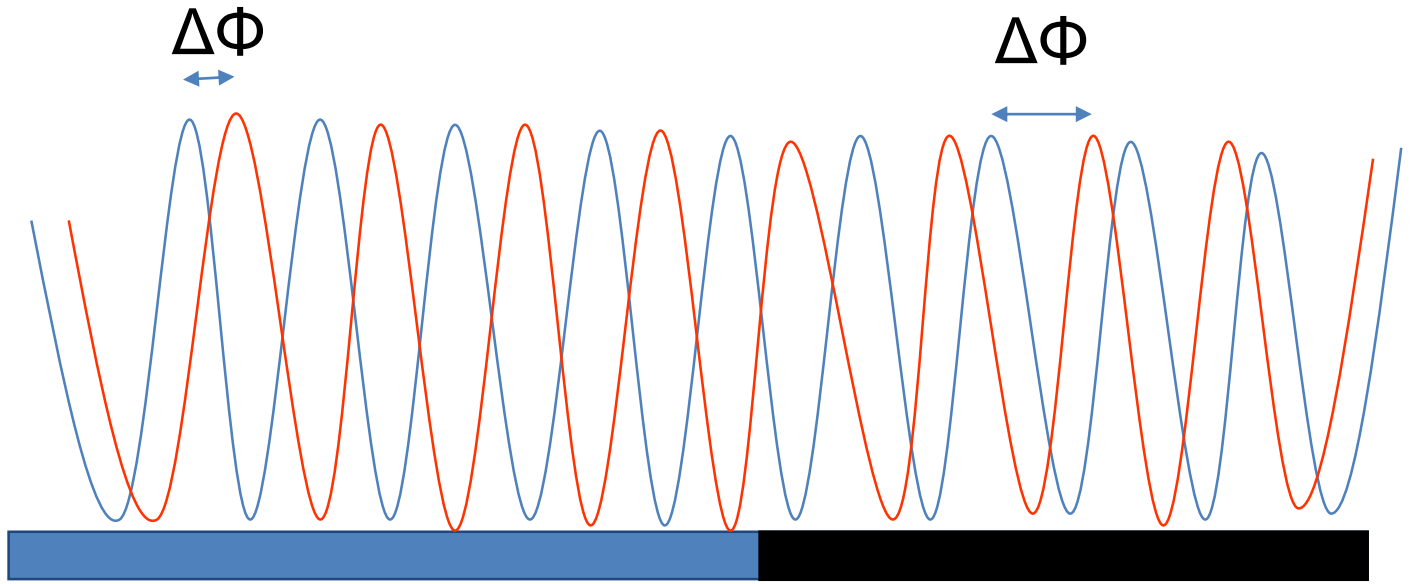


Topography (left) and lateral force (right) images of polyvinyl alcohol at 10% relative humidity.

More information abou these images can be found on MI's application note:
"Studies of Polyvinyl Alcohol under Temperature and Humidity control."

Basic AFM Imaging Modes

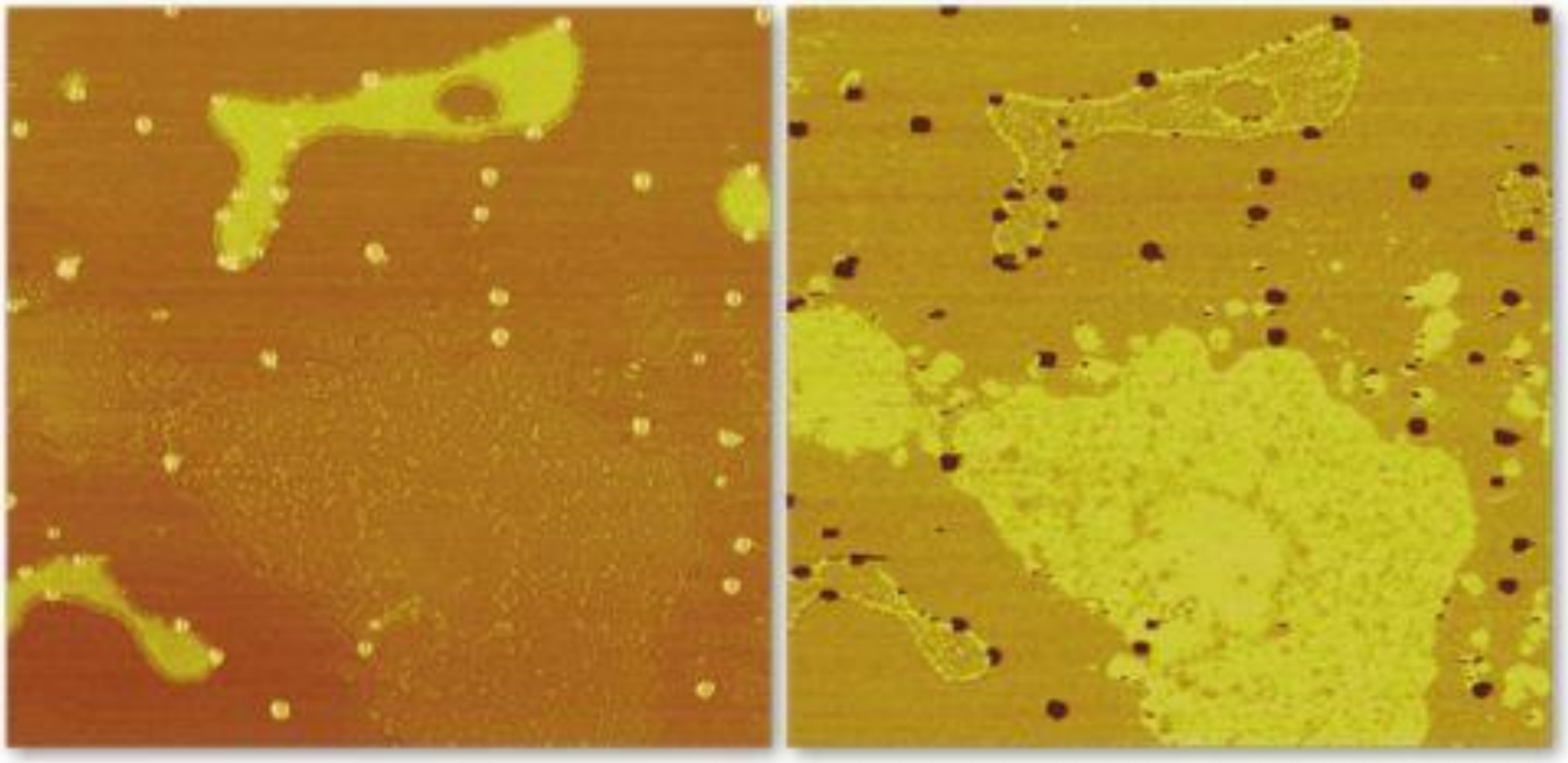
- **Phase image (operate at AC mode)**



- Measure the phase of tip oscillation relative to the signal drives the tip.

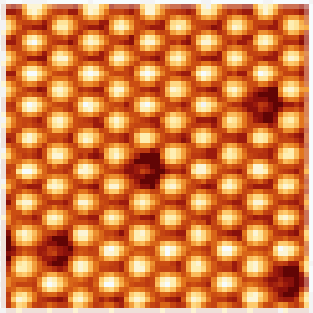
- Phase change corresponse to surface properties change such as composition, adhesive, stiffness, hydrophobicity, surface charge, etc

Height (left) and Phase (right) images of Polyvinyl Alcohol in air



Atoms

Imaging and manipulation of single atoms (Zn on Si)

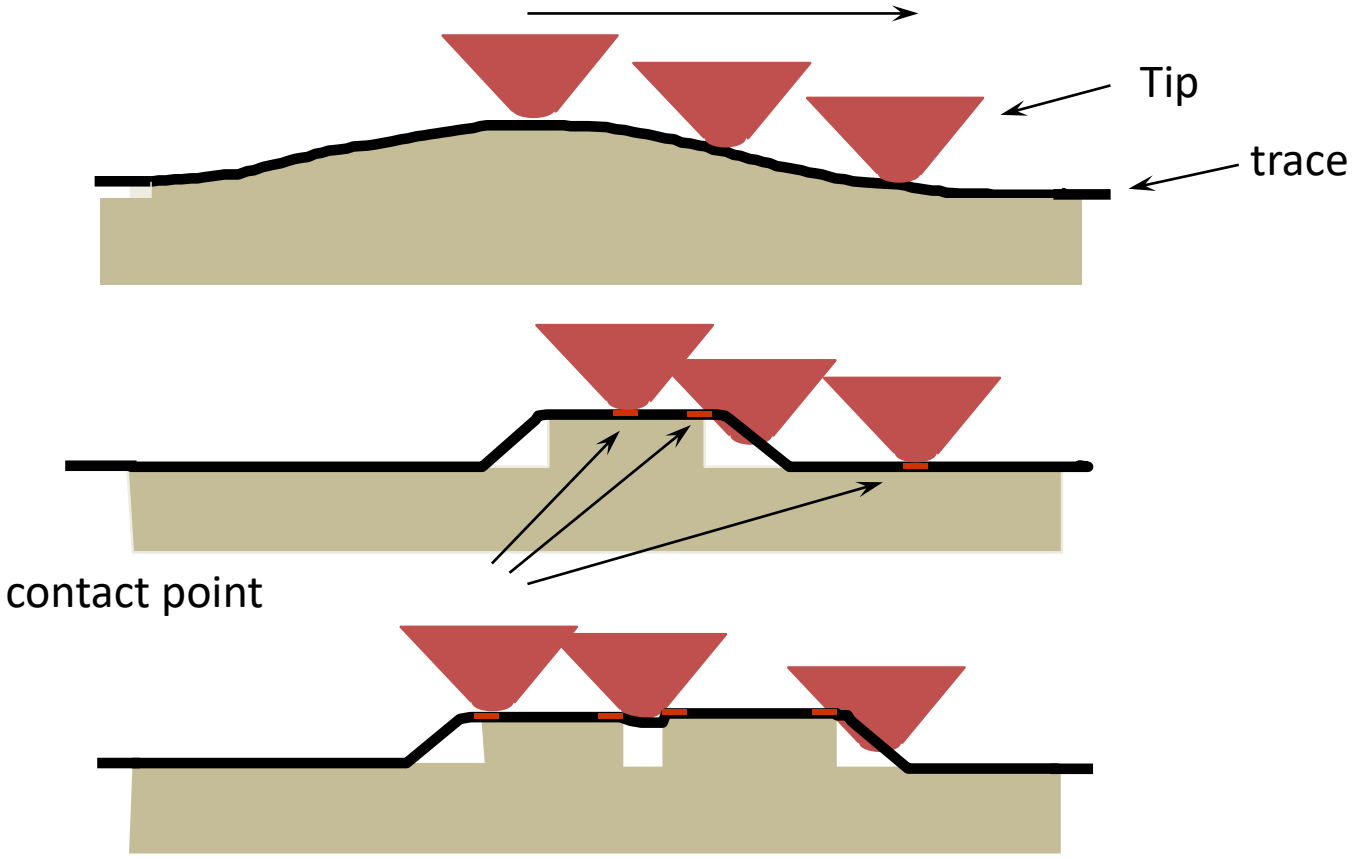


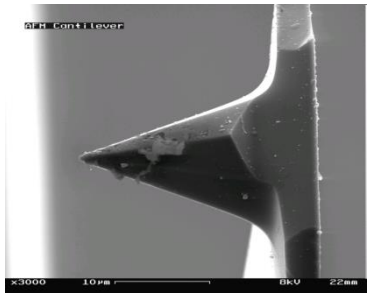
Y Sugimoto, *et al., Science,* ***322: 413–417 (2008).***

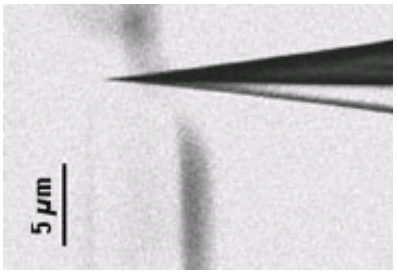
Cantilevers

Cantilever dimensions determine how easy to bend it is.

Image quality depends on tip size and shape

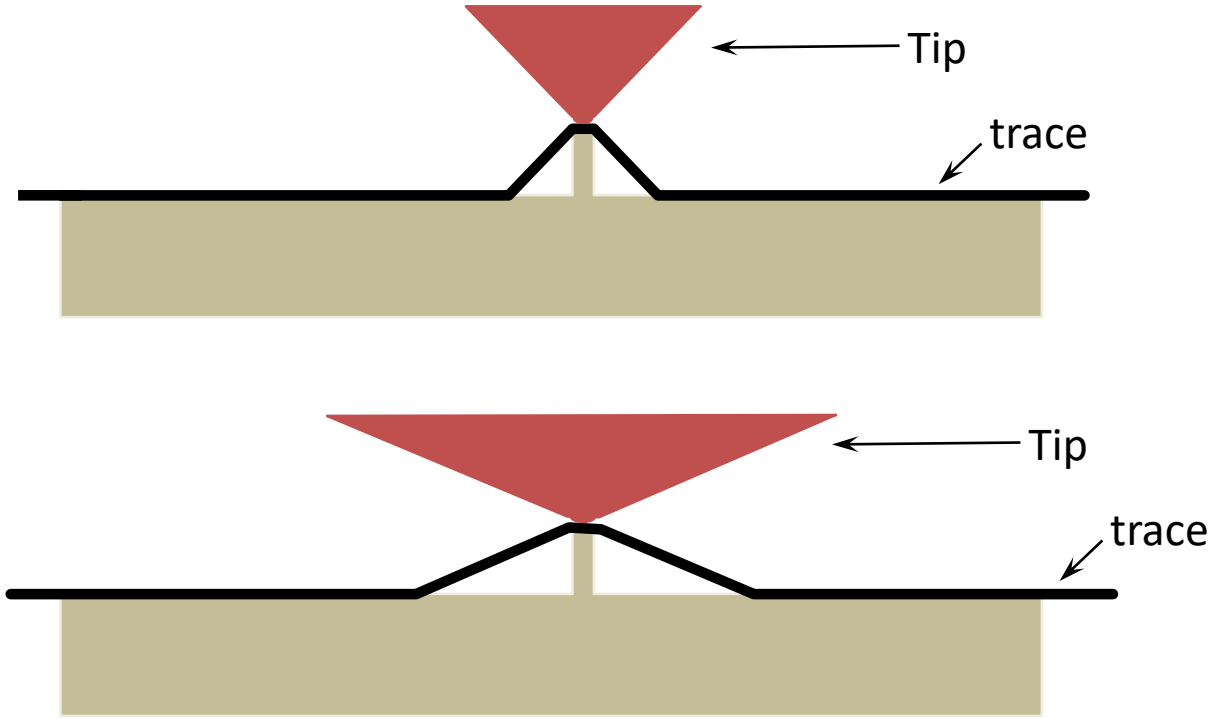






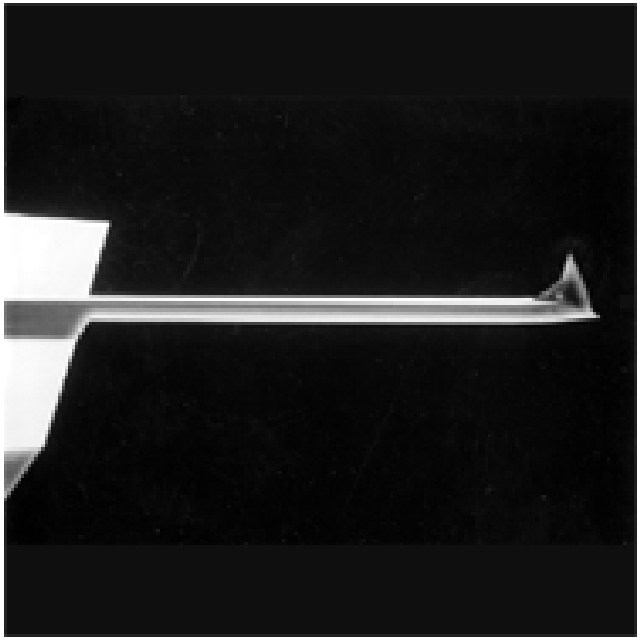
Slide courtesy of Duncan Sutherland

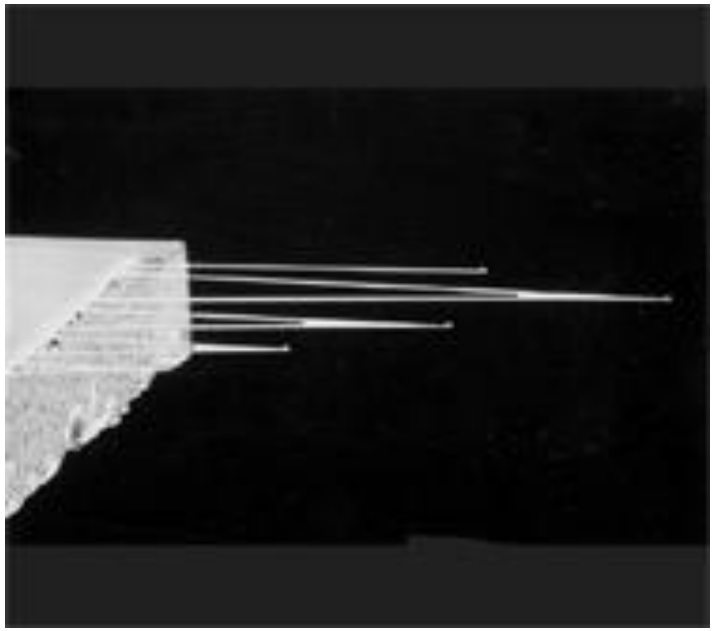
Influence of tip sharpness

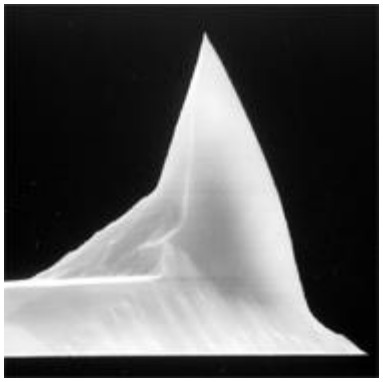


Slide courtesy of Duncan Sutherland

AFM cantilever and AFM tips



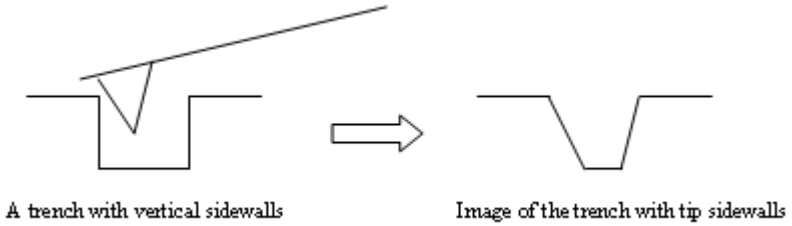


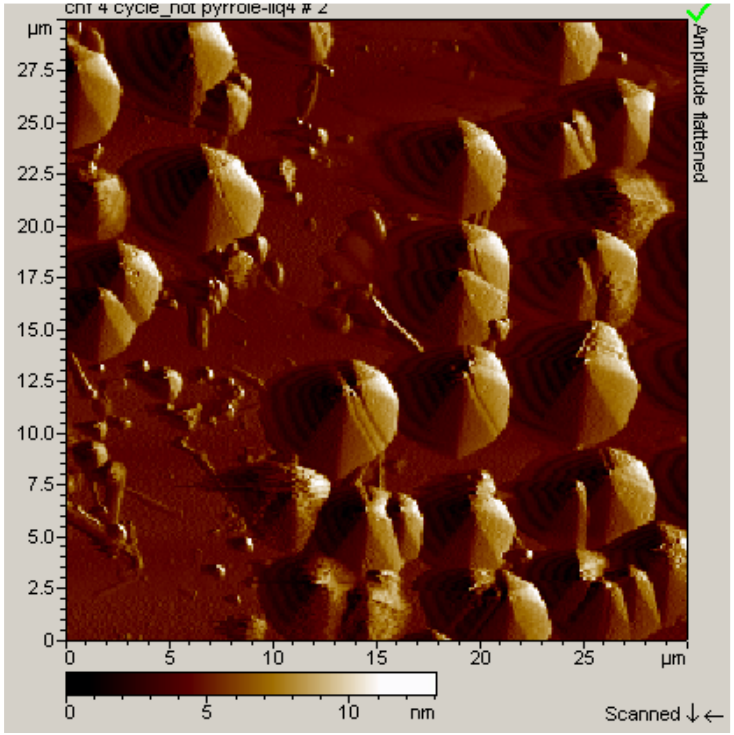


AFM artifacts

Probe Shape related artifacts:

The ability to image a deep trench, a step, or a small particle in AFM is limited by the sidewall angles of the tip. A good rule of thumb is that the tip is unable to image features that have greater sidewall angles than those of the tip. If the angles on the surface are greater than those on the tip the image will be of the tip sidewalls and not of the actual surface. Below is a schematic drawing of this artifact along with an image where the tip shape is easily observed.

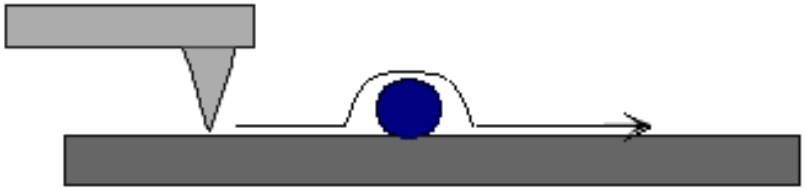


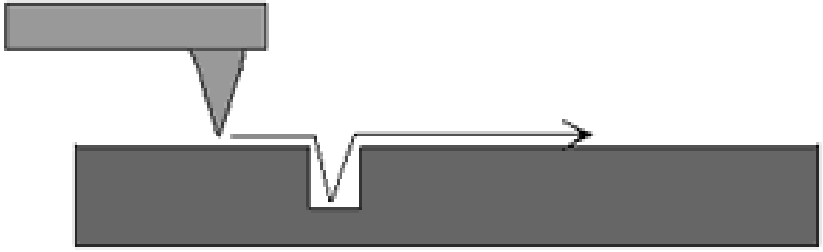


AFM artifacts

Probe Size related artifacts

Often the lateral size of small particles or thin structures appears to be larger than expected while the height of the feature appears to be correct. This is because the width of the AFM probe is being added to the width of the structure being measured. When measuring narrow trenches the trenches may appear to be narrower than expected for the same reason. The images below show the contributions of the tip to both of these measurements.

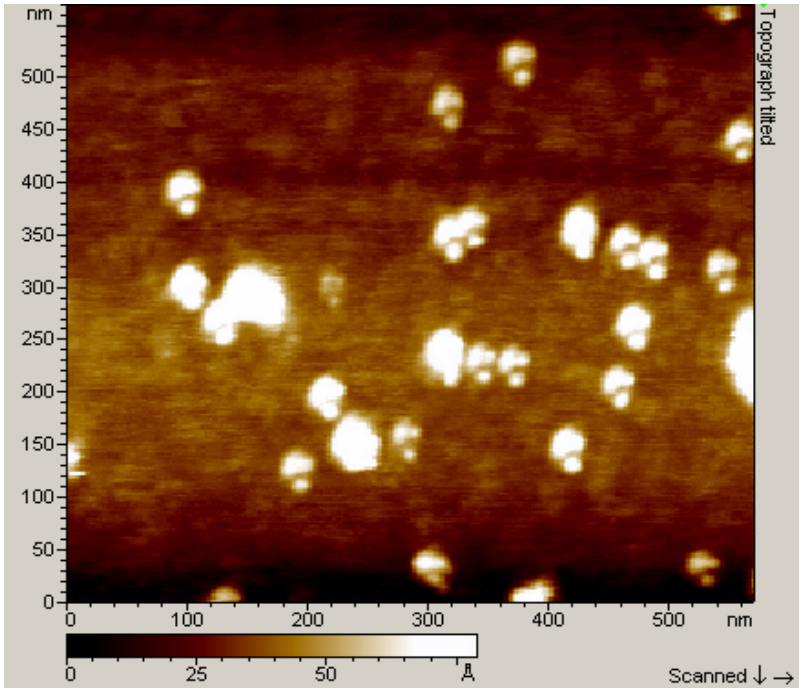




AFM artifacts

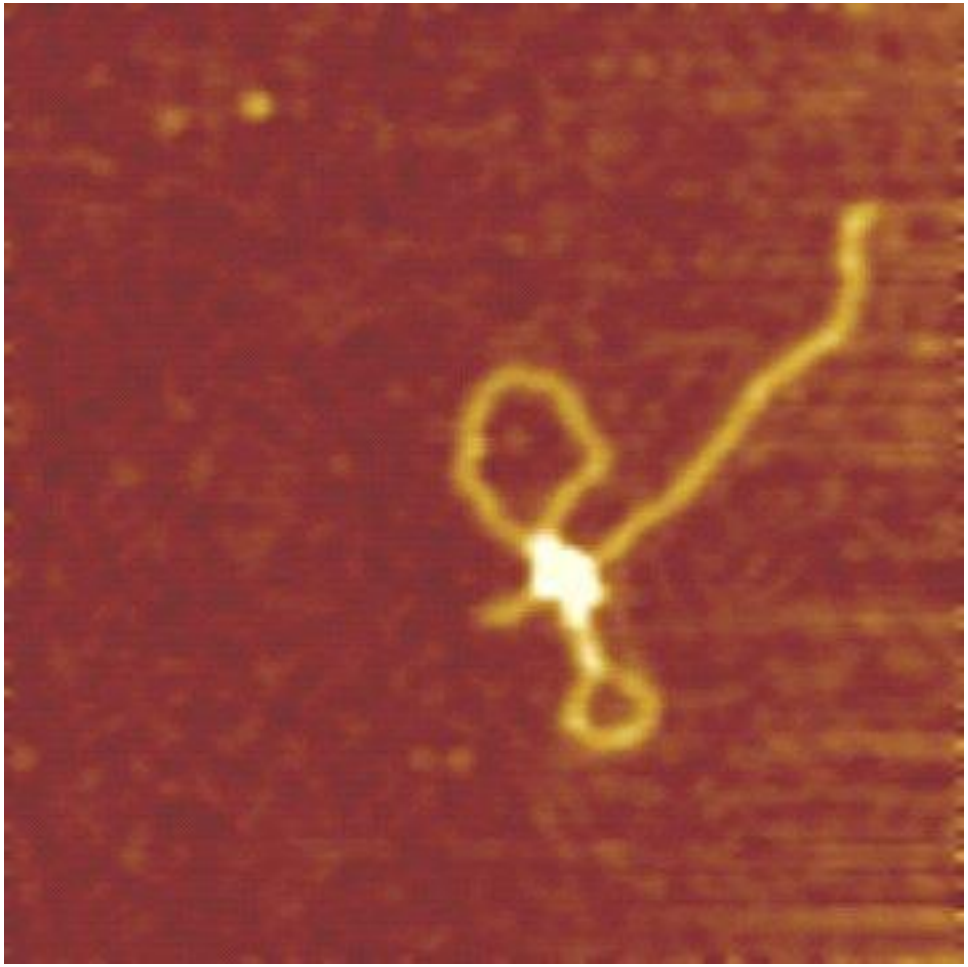
Broken tip or tip contamination -----Multiple tips:

If the tip has part broken off or is dirty it can produce strange results. Often when the tip is dirty the image will appear fuzzy or not in focus and changing the gains will only marginally help to resolve this. Dirty tips can also make the image appear to have strangely shaped or repeating structures. The image below clearly shows the results of a multiple tip or more specifically in this case “double tip”.

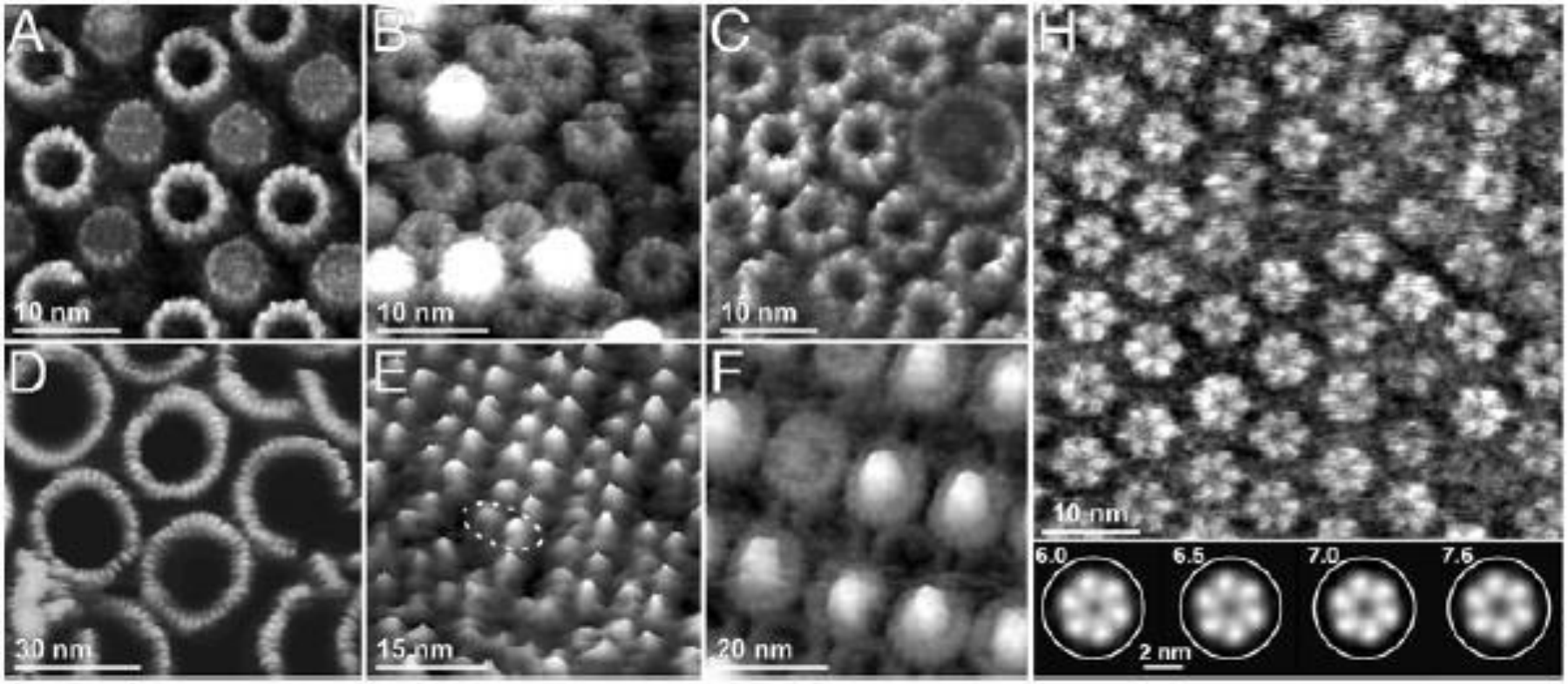


Biological Applications

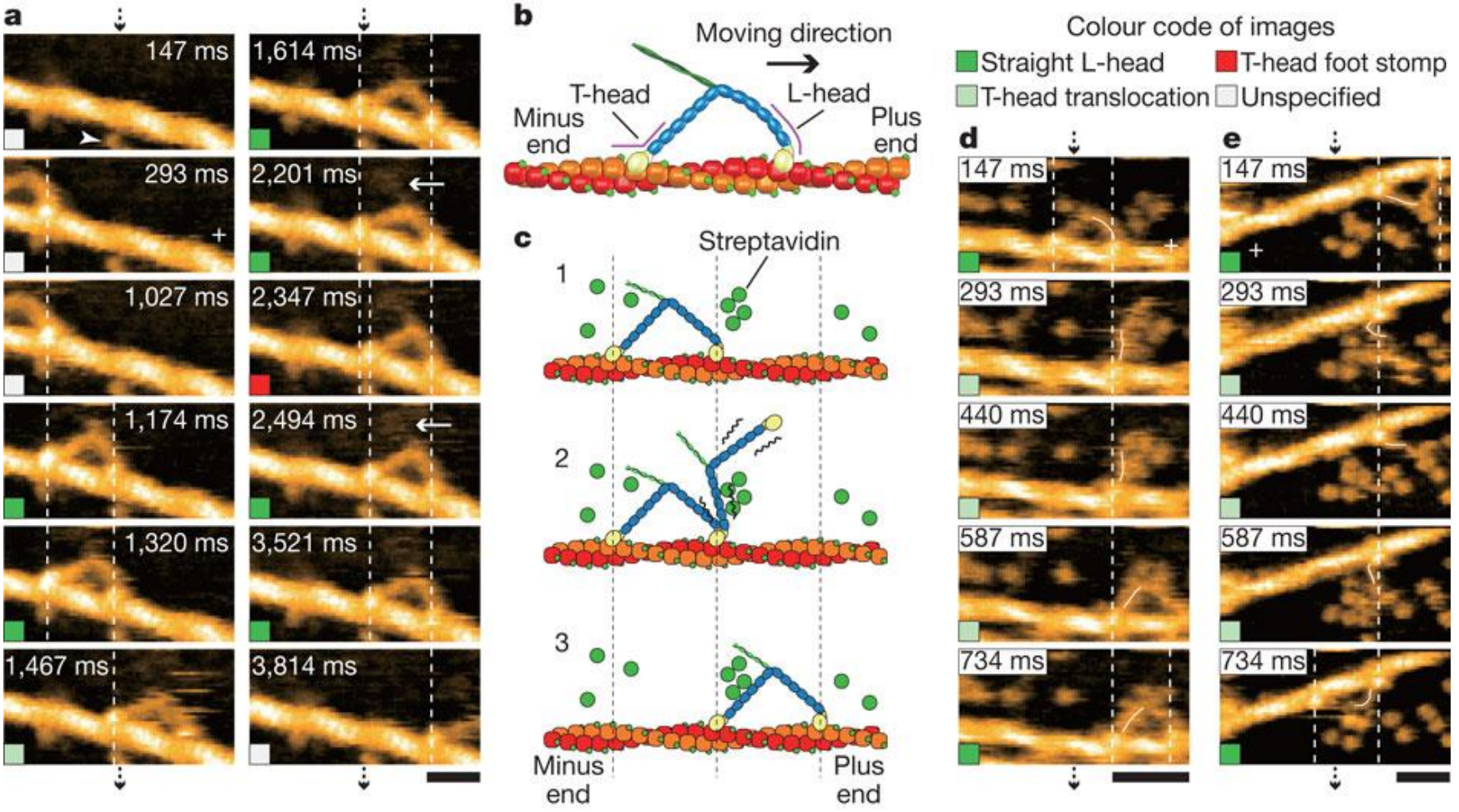
DNA + Proteim



Membran & Protein

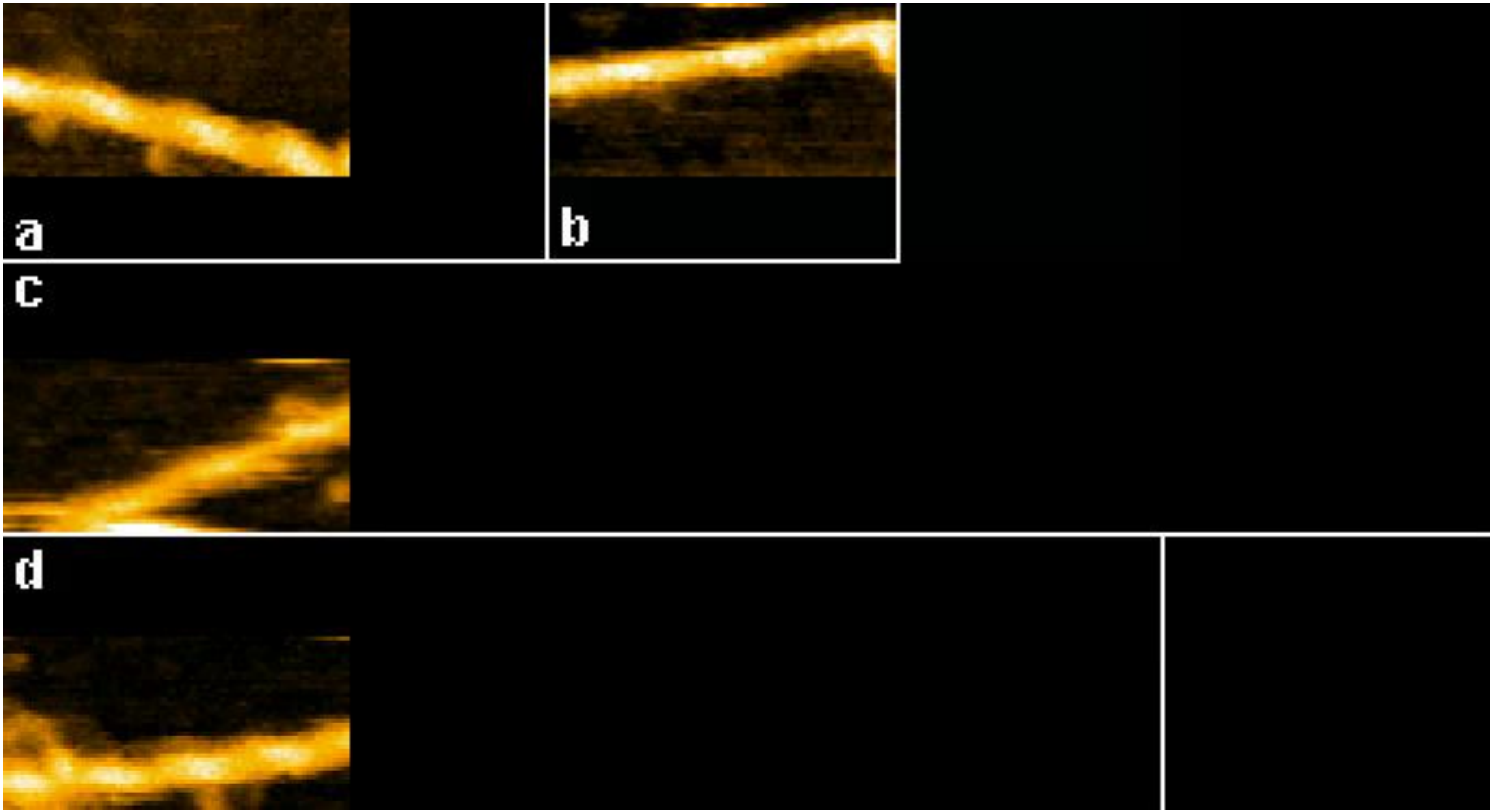


Directly visualized walking M5-HMM.

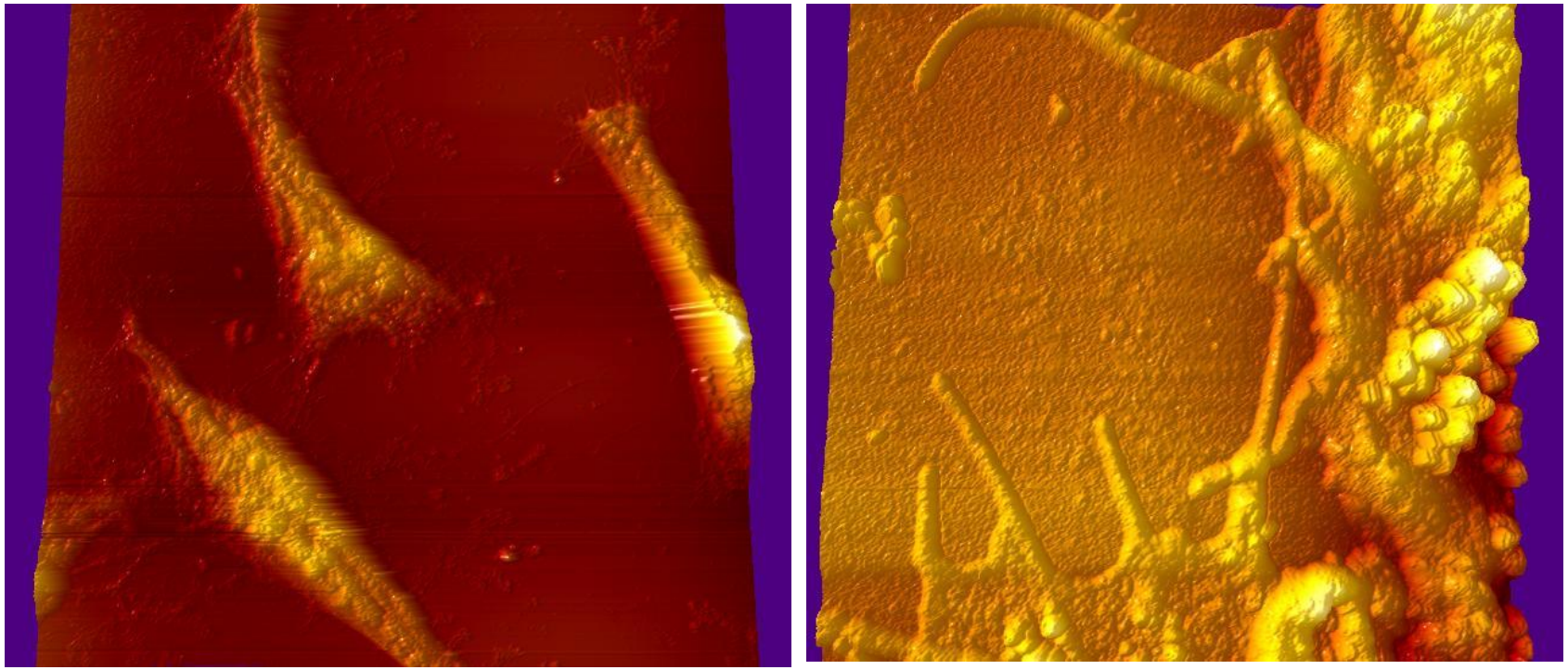


N Kodera *et al*. *Nature* **000**, 1-5 (2010) doi:10.1038/nature09450

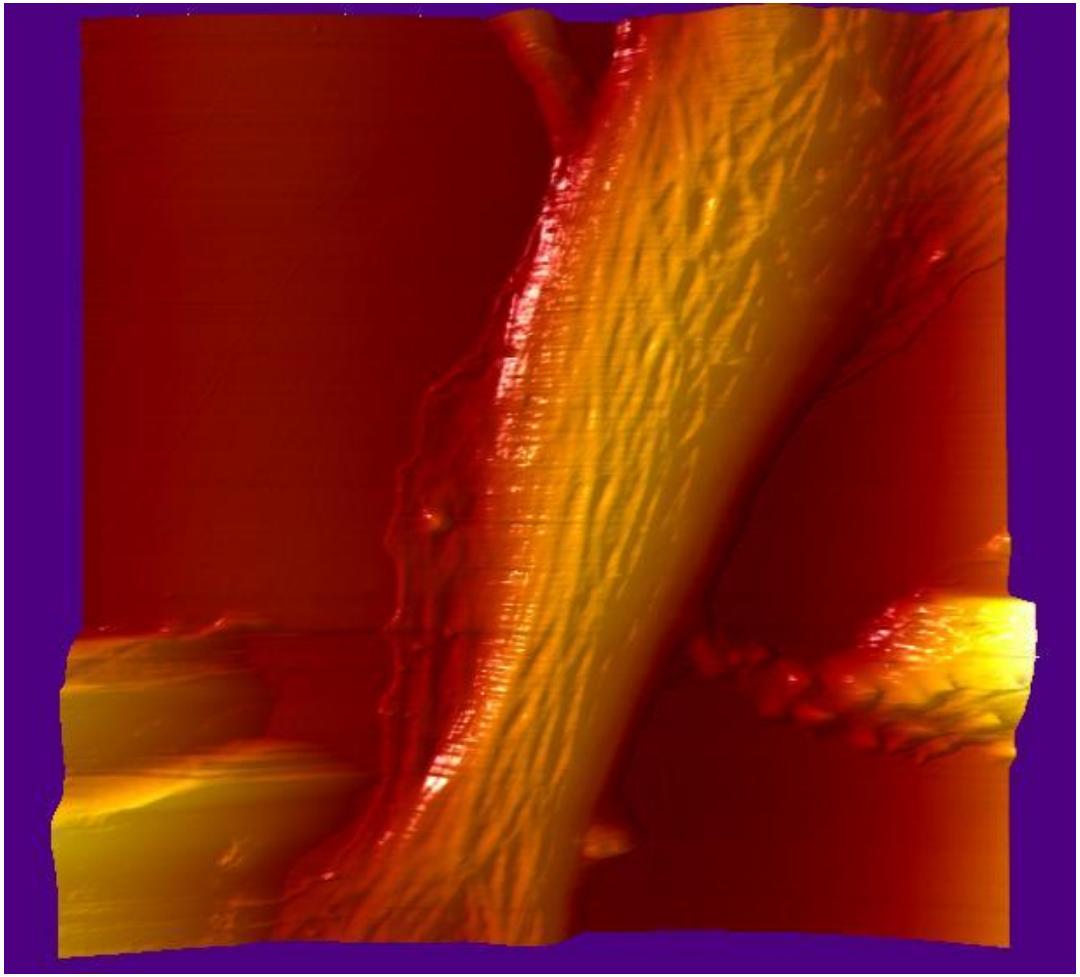
Directly visualized walking M5-HMM.



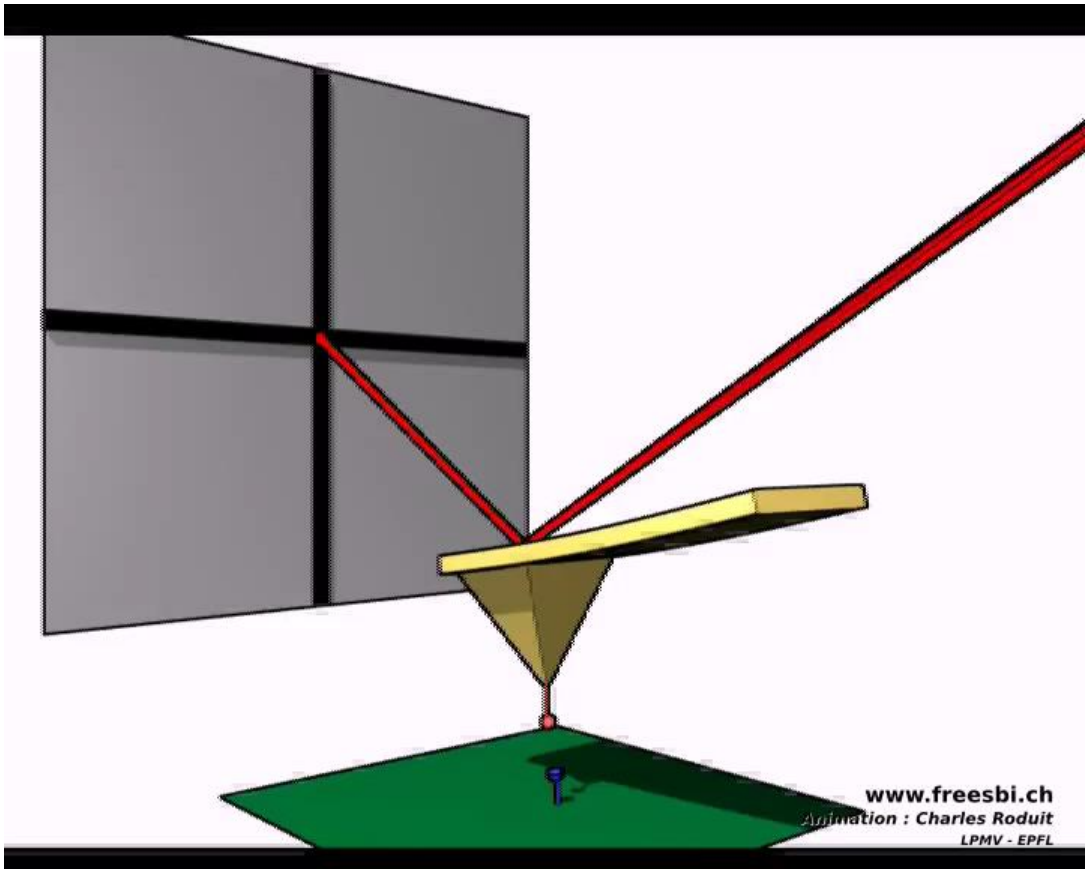
HELA cancer cell



Living Endothelial Cell



Force Spectroscopy



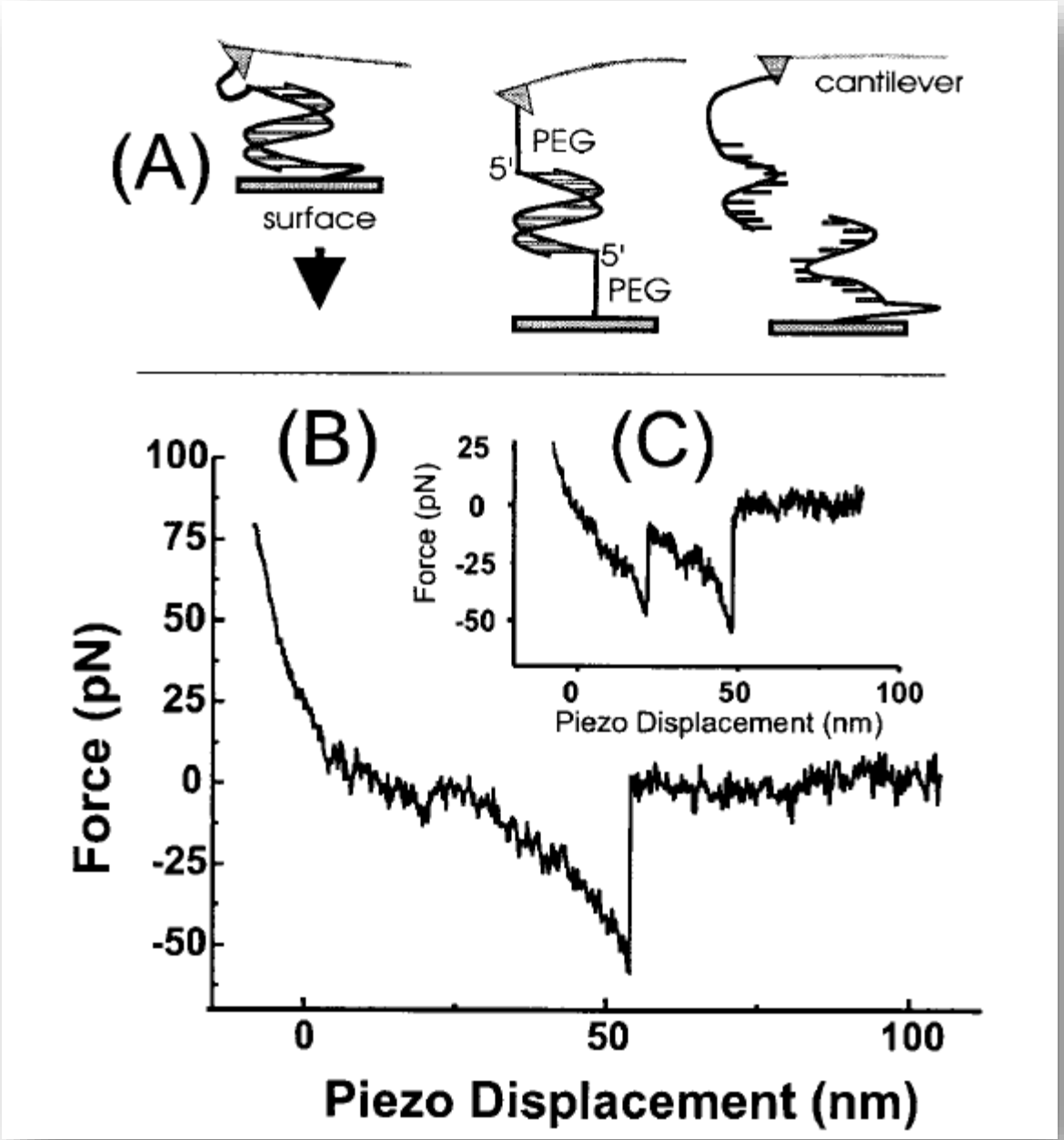
Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 11277–11282, September 1999
Biophysics

Dynamic force spectroscopy of single DNA molecules

TORSTEN STRUNZ*, KRISZTINA OROSZLAN, ROLF SCHÄFER, AND HANS-JOACHIM GÜNTHERODT

Department of Physics and Astronomy, University Basel, Klingelbergstrasse 82, 4056 Basel, Switzerland

Interaction force between individual DNA strands is measured

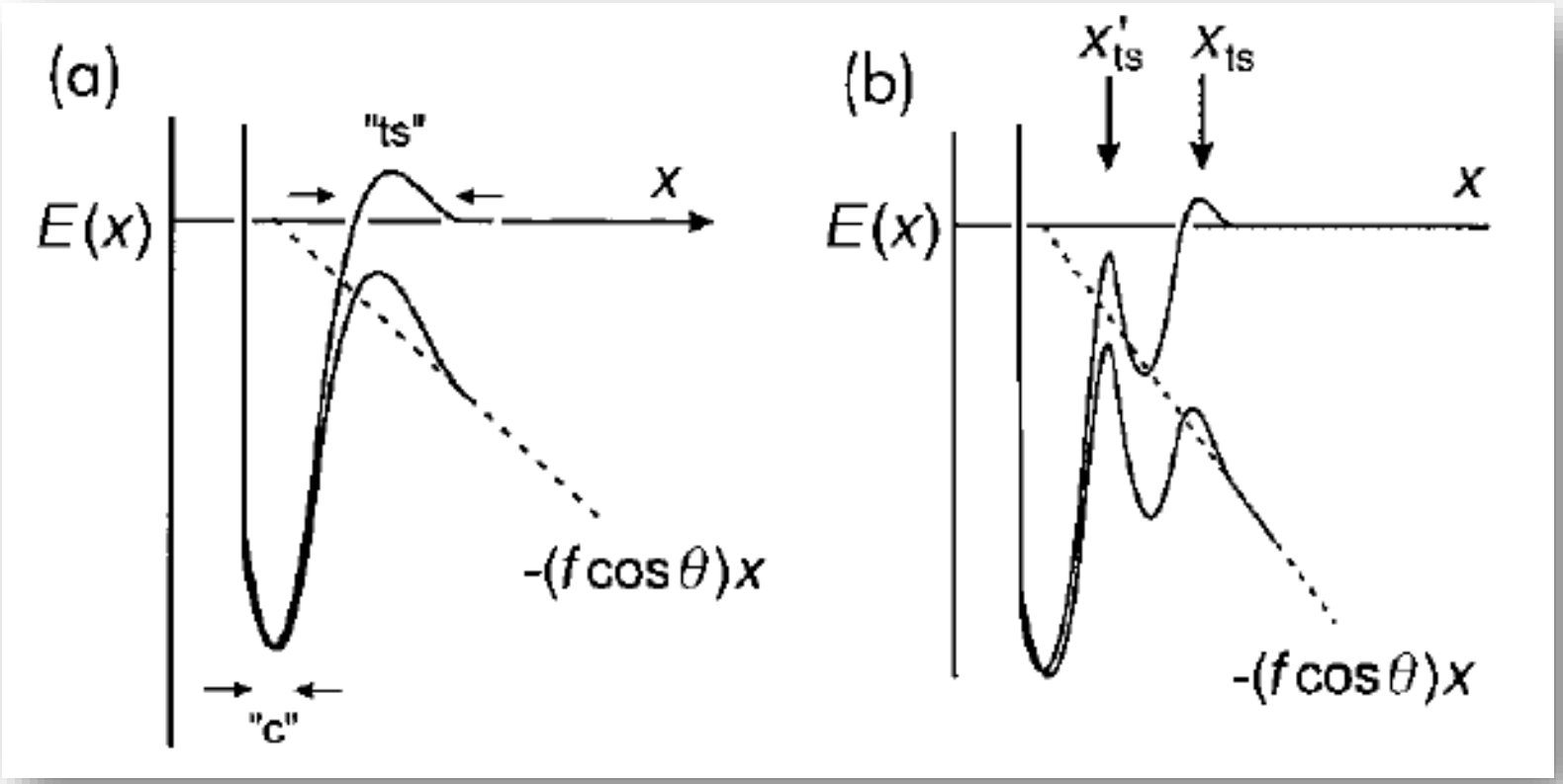


Biophysical Journal Volume 72 April 1997 1541–1555

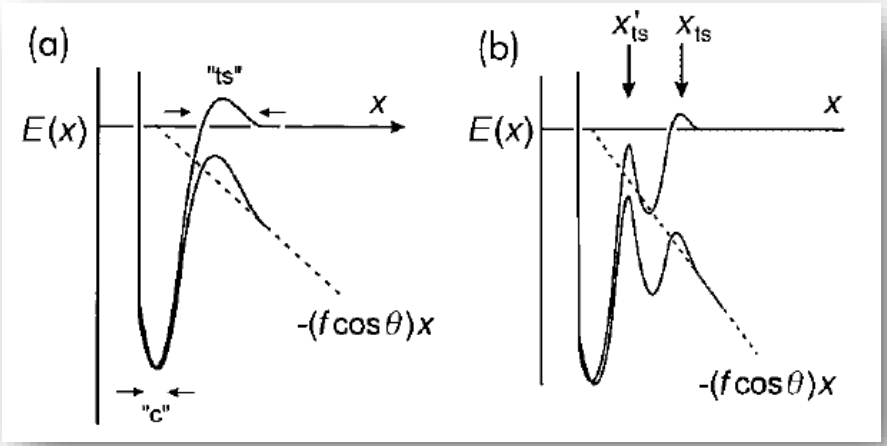
Dynamic Strength of Molecular Adhesion Bonds

Evan Evans and Ken Ritchie
Departments of Physics and Pathology, University of British Columbia, Vancouver, British Columbia V6T 1Z1 Canada

Force tilts the energy landscape



Force tilts the energy landscape, increasing the off-rate (Bell model)



λ: off-rate

k_{off}: off-rate in absence of force

$$\lambda = k_{off} \exp(F \Delta x / k_B T).$$

Δx: distance to transition state

$$F_{prob} = \frac{k_B T}{\Delta x} \ln \dot{F} + \frac{k_B T}{\Delta x} \ln \left(\frac{\Delta x}{k_{off}^{*} k_B T} \right).$$

$\dot{F}$: Rate of force application

Dynamic Force Spectroscopy

The rupture strengths for weak
biochemical bonds are not constants but instead depend on
the rate of force application and duration of loading.

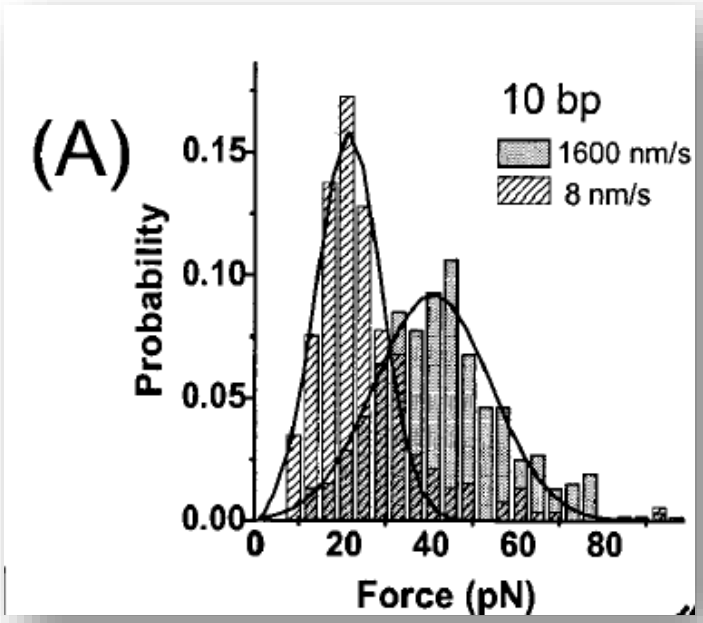
Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 11277–11282, September 1999
Biophysics

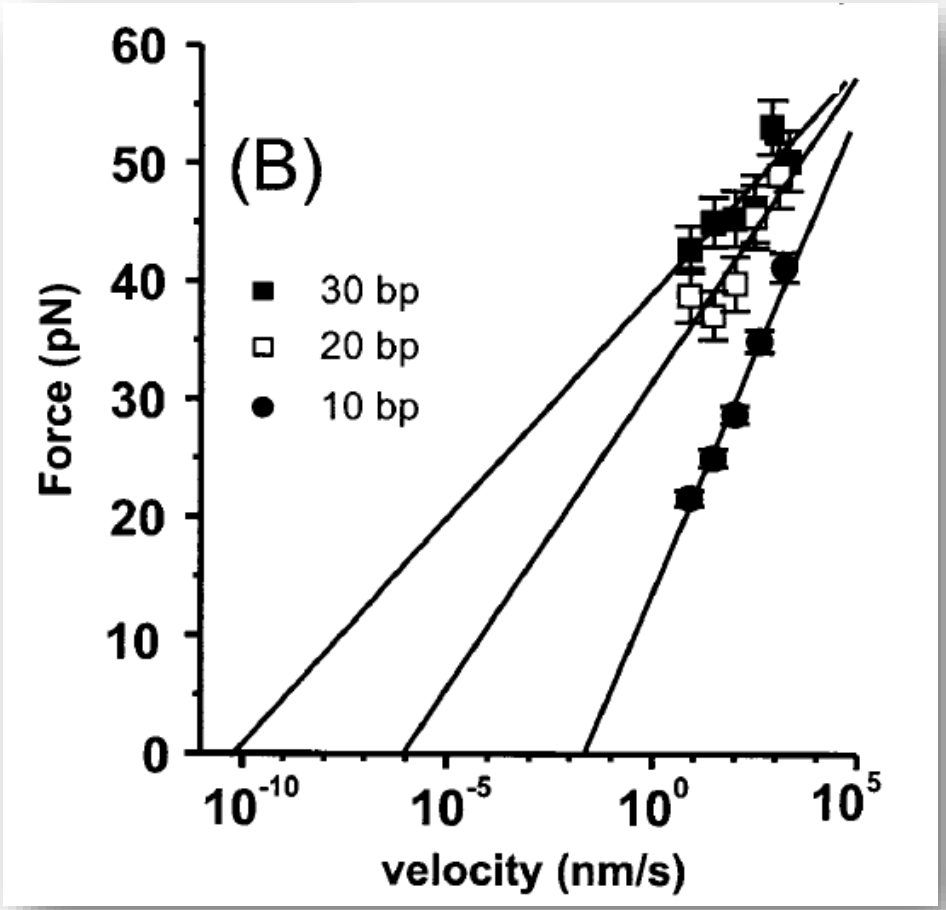
Dynamic force spectroscopy of single DNA molecules

TORSTEN STRUNZ*, KRISZTINA OROSZLAN, ROLF SCHÄFER, AND HANS-JOACHIM GÜNTHERODT

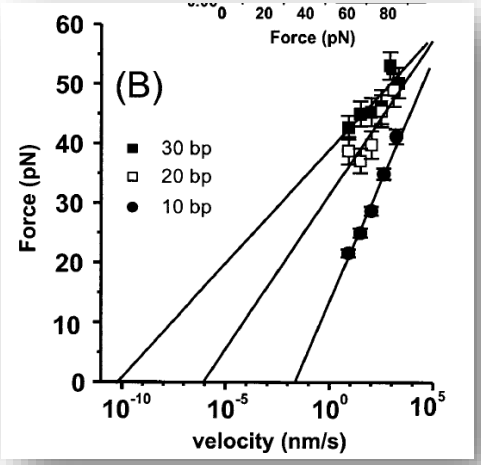
Department of Physics and Astronomy, University Basel, Klingelbergstrasse 82, 4056 Basel, Switzerland

Different rupture force distributions at different loading rates

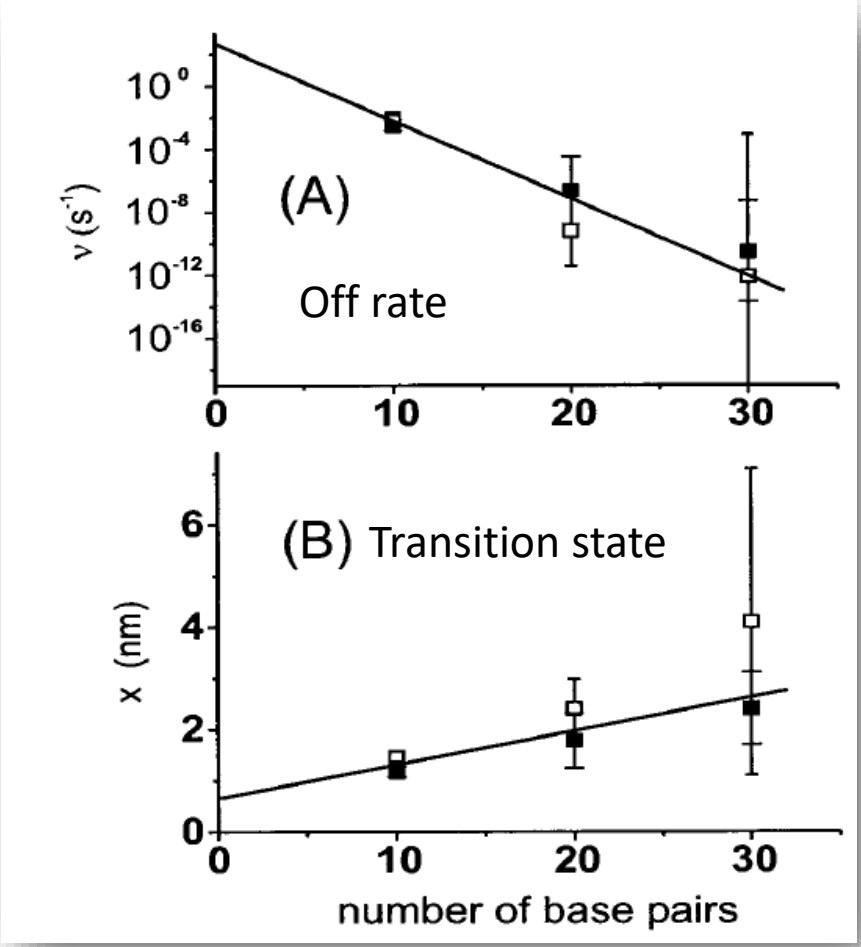




Dynamic force spectroscopy reveals energy landscape of bonds



$$F_{prob} = \frac{k_B T}{\Delta x} \ln \dot{F} + \frac{k_B T}{\Delta x} \ln \left(\frac{\Delta x}{k_{off}^{*} k_B T} \right)$$

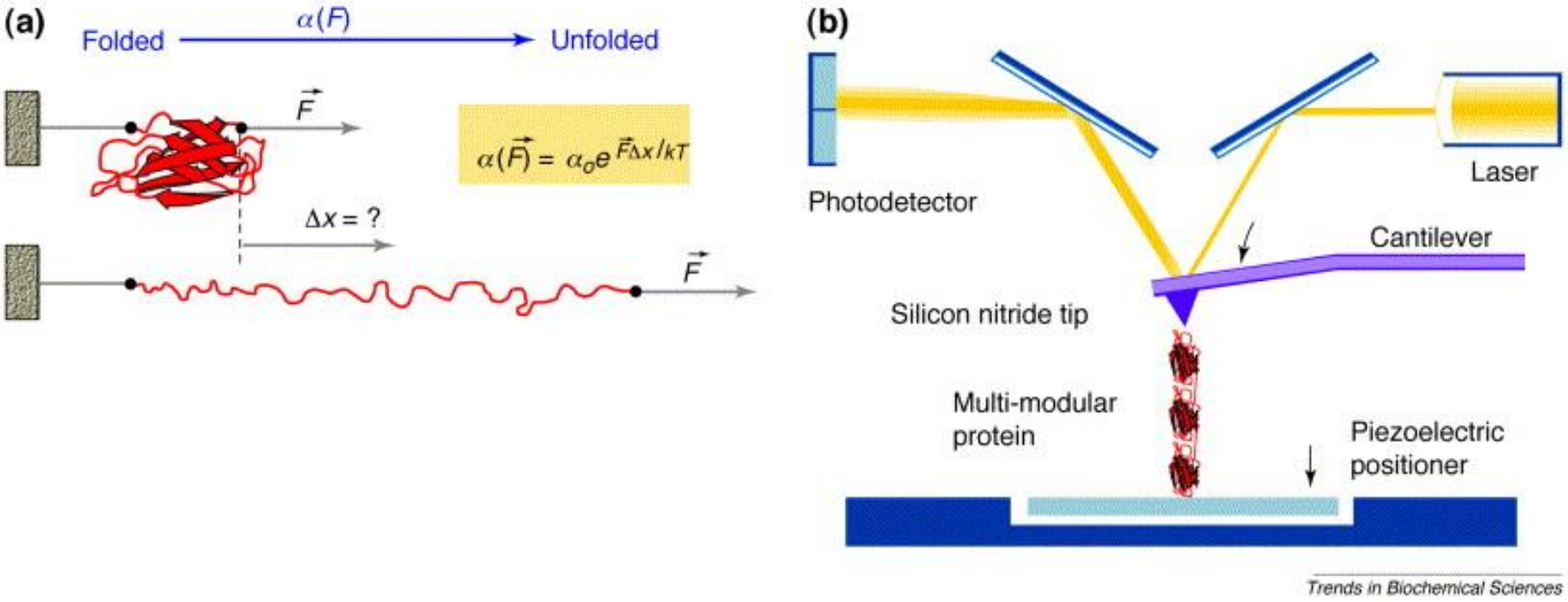


The study of protein mechanics with the atomic force microscope

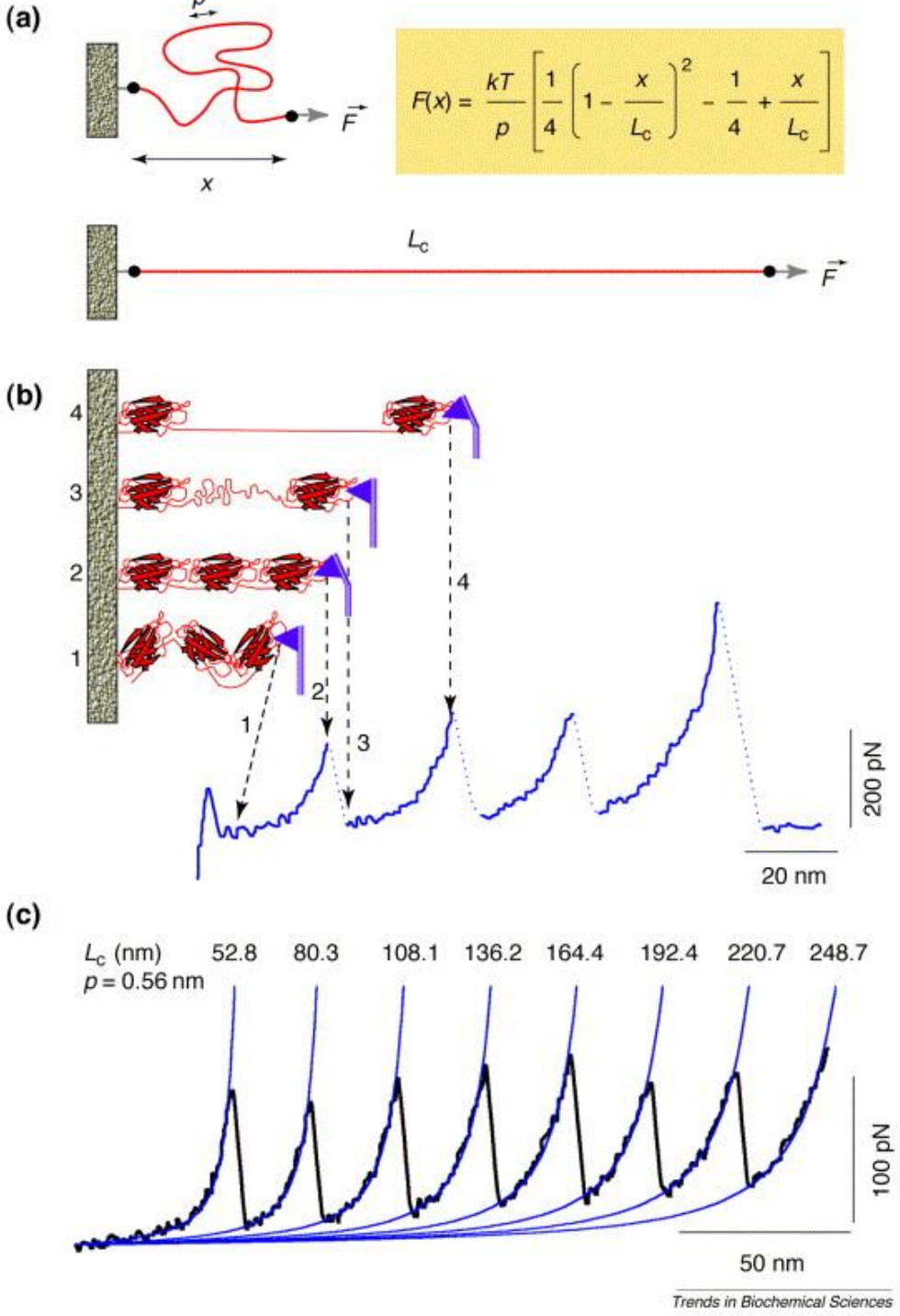
Thomas E Fisher, Andres F Oberhauser, Mariano Carrion-Vazquez, Piotr E Marszalek, Julio M Fernandez

Trends in Biochemical Sciences
Volume 24, Issue 10, Pages 379-384 (October 1999)
DOI: 10.1016/S0968-0004(99)01453-X

The unfolding of protein domains by an external force

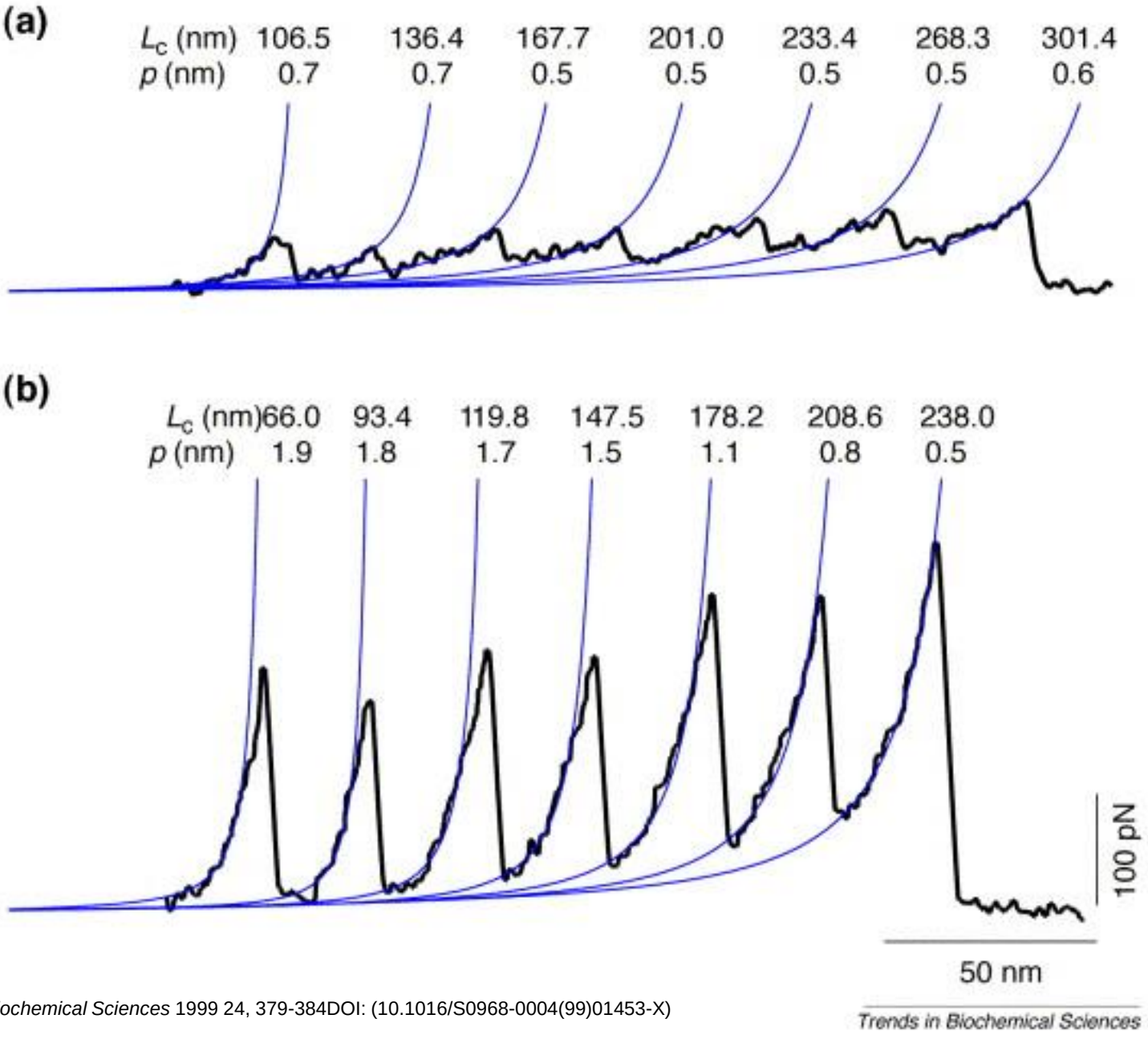


Trends in Biochemical Sciences 1999 24, 379-384DOI: (10.1016/S0968-0004(99)01453-X)



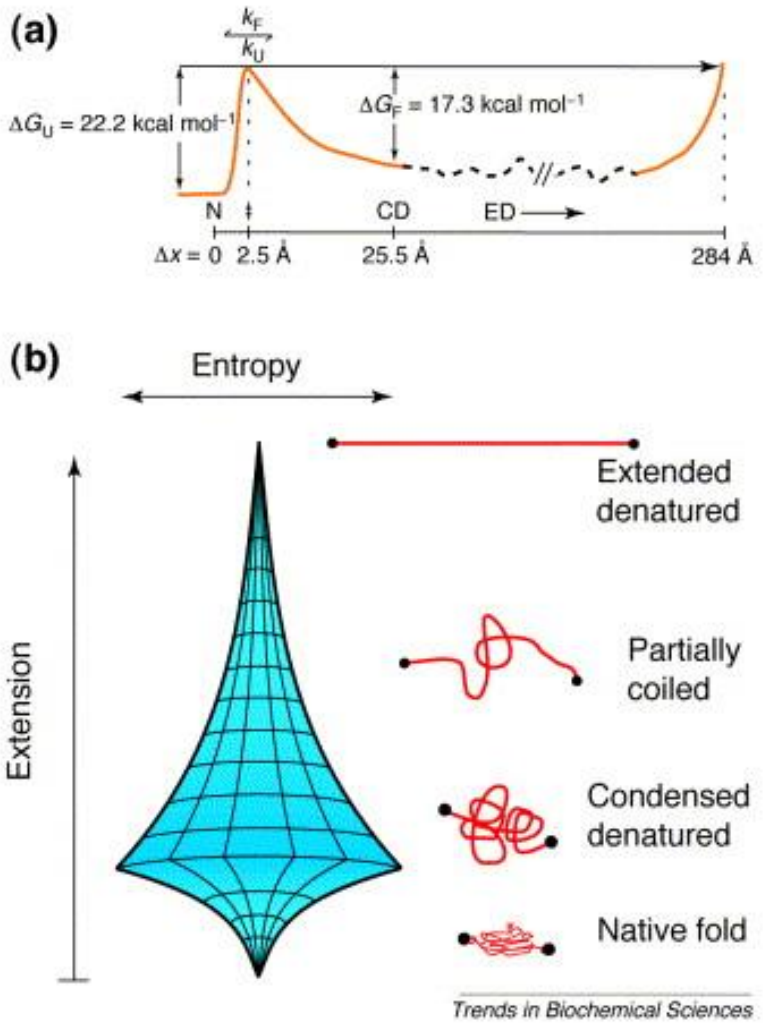
Trends in Biochemical Sciences

Figure 3



Trends in Biochemical Sciences 1999 24, 379-384DOI: (10.1016/S0968-0004(99)01453-X)

Figure 6

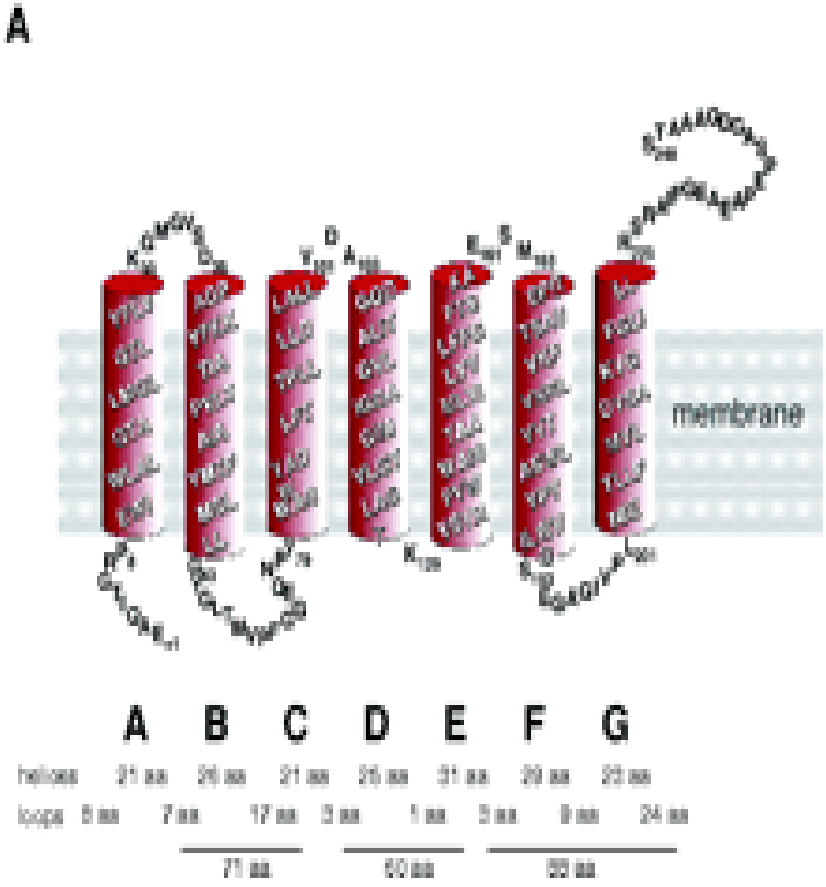


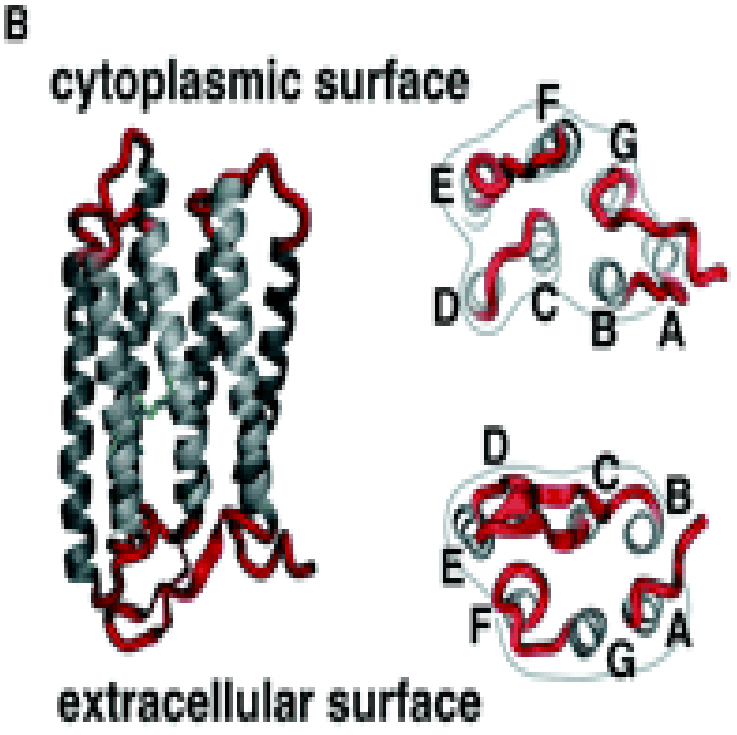
Trends in Biochemical Sciences 1999 24, 379-384DOI: (10.1016/S0968-0004(99)01453-X)

Unfolding Pathways of Individual Bacteriorhodopsins

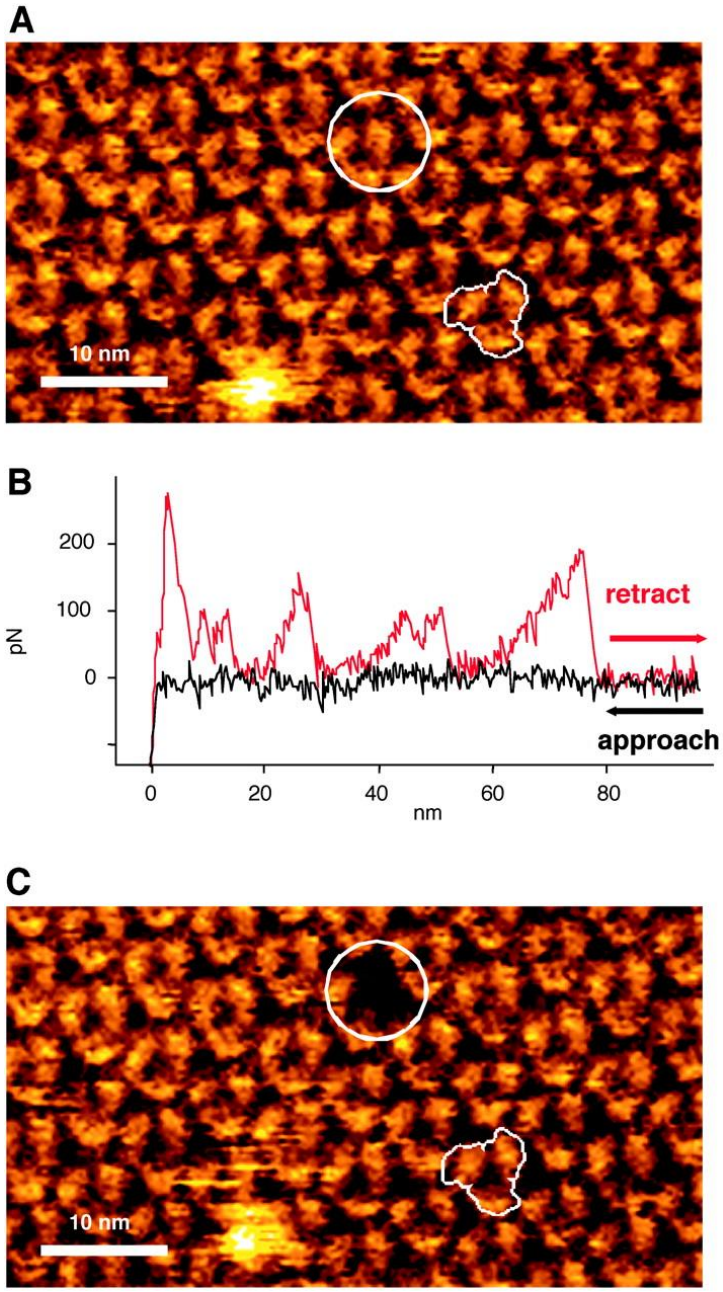
F. Oesterhelt,[1] D. Oesterhelt,[2] M. Pfeiffer,[2] A. Engel,[3]
H. E. Gaub,[1]* D. J. Müller[3,4]

www.sciencemag.org SCIENCE VOL 288 7 APRIL 2000

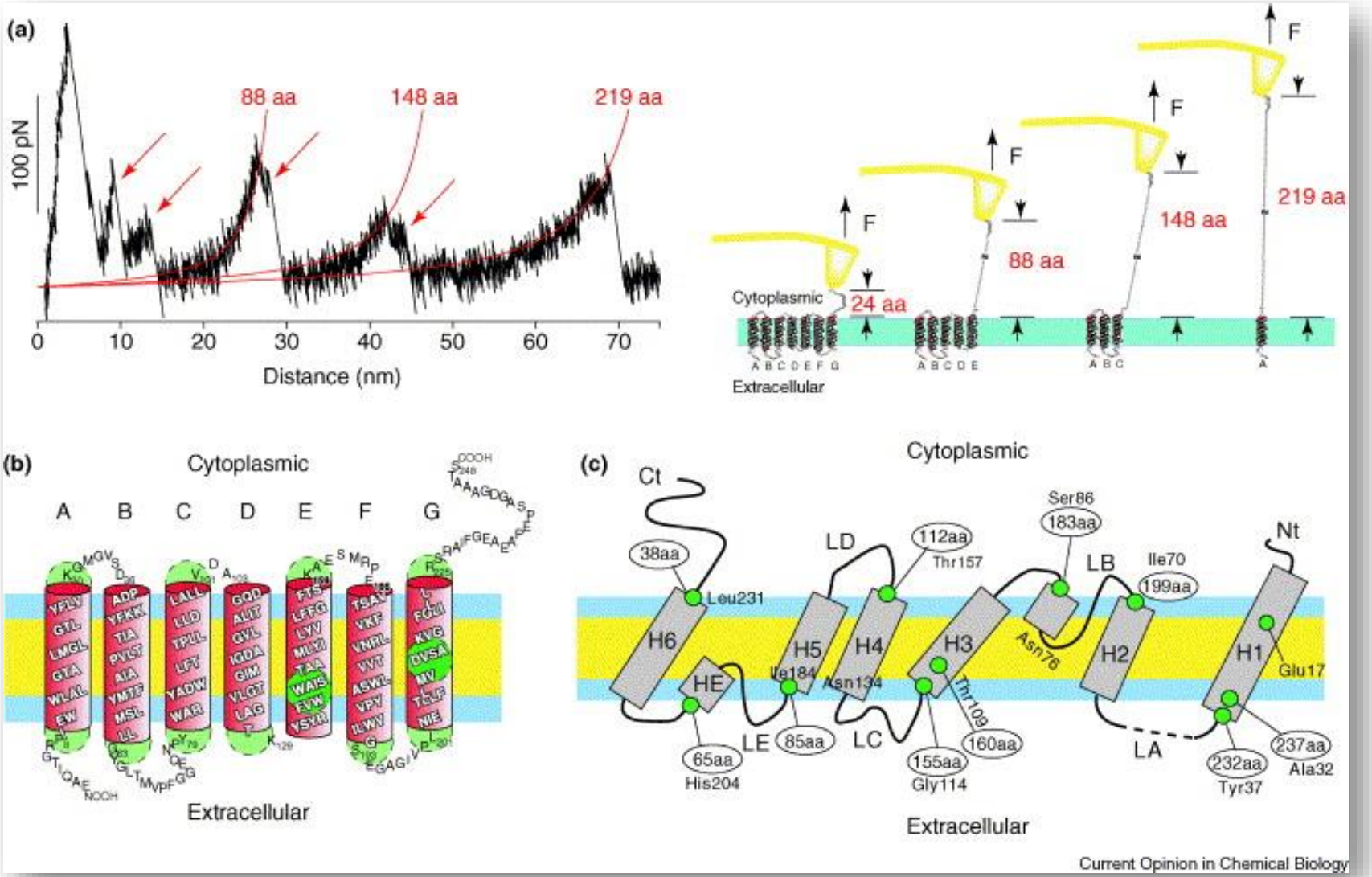




Science 2000, 288, 7, 143



Science 2000, 288, 7, 143



Science 2000, 288, 7, 143

Summary I

1. Single Molecule Force Microscopy is a powerful toll to study mechanical properties in nanoscopic piconewton scales.

2. The technique allow the controlled manipulation of individual molecules

3. With the revealed detail of molecular conformations, it could be a powerful tool to study biological molecules.

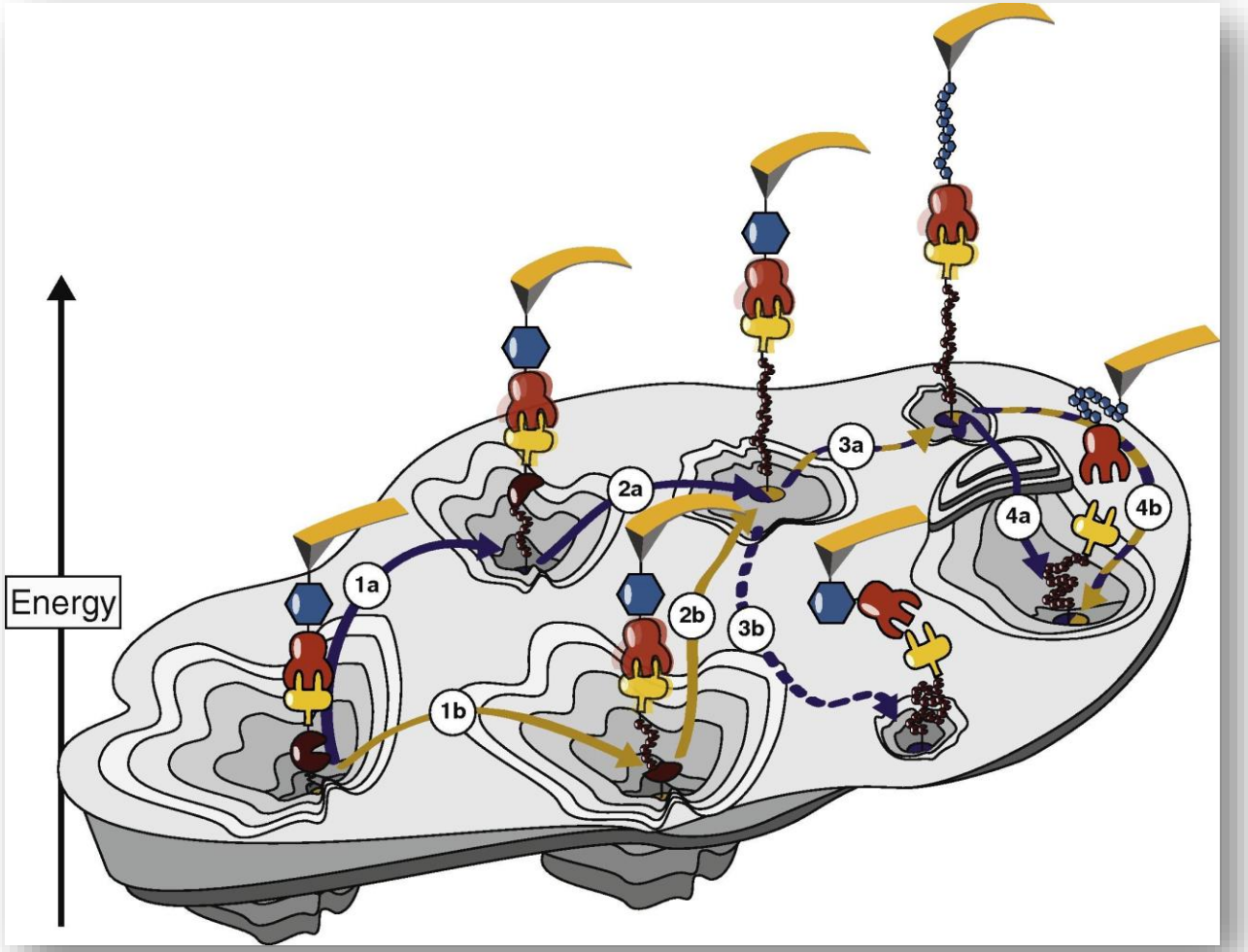
Summary II

1. Single molecular force microscopy provide unprecedented resolutions to study the unfolding of single proteins.

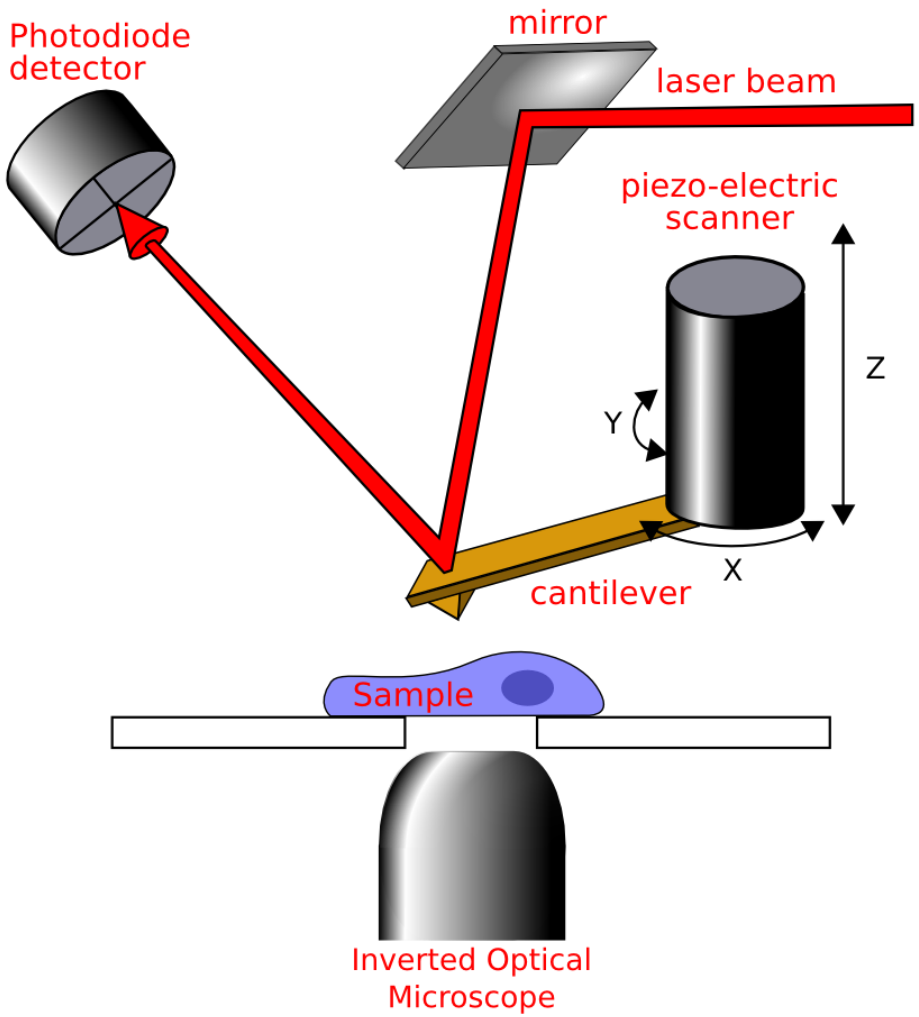
2. Single molecular force microscopy can reveal a very detailed and map of the unfolding pathways and local interactions in the membranes..

3. The structure information form single molecular force microscopy make it a promising tool in studying protein secondary structures.

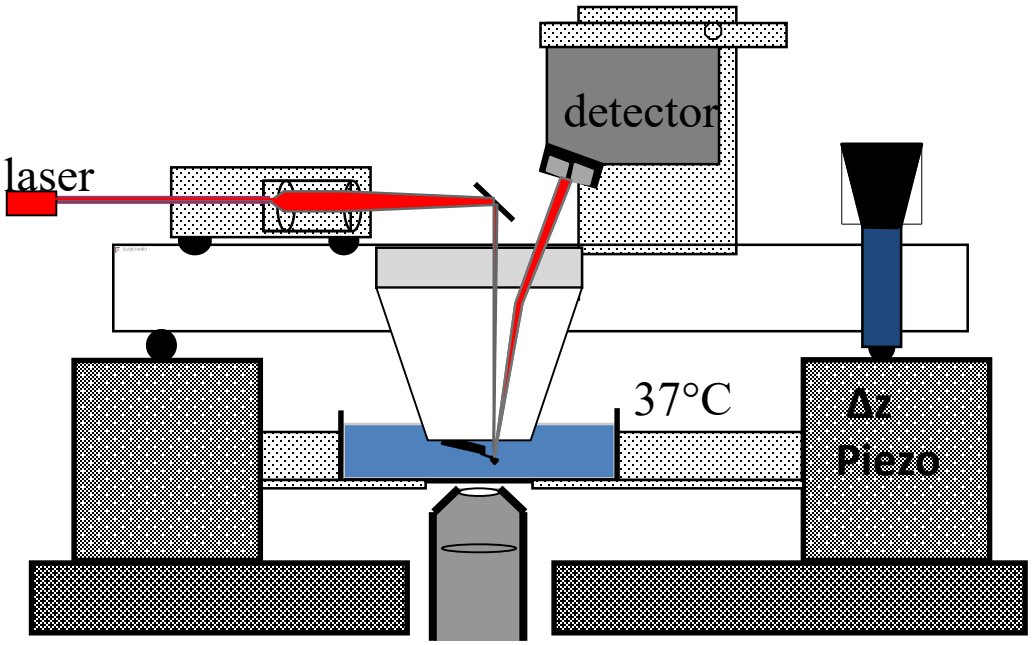
Thermal unfolding/unbinding and forced unfolding/unbinding may probe different transitions states.



AFM & Biological cells



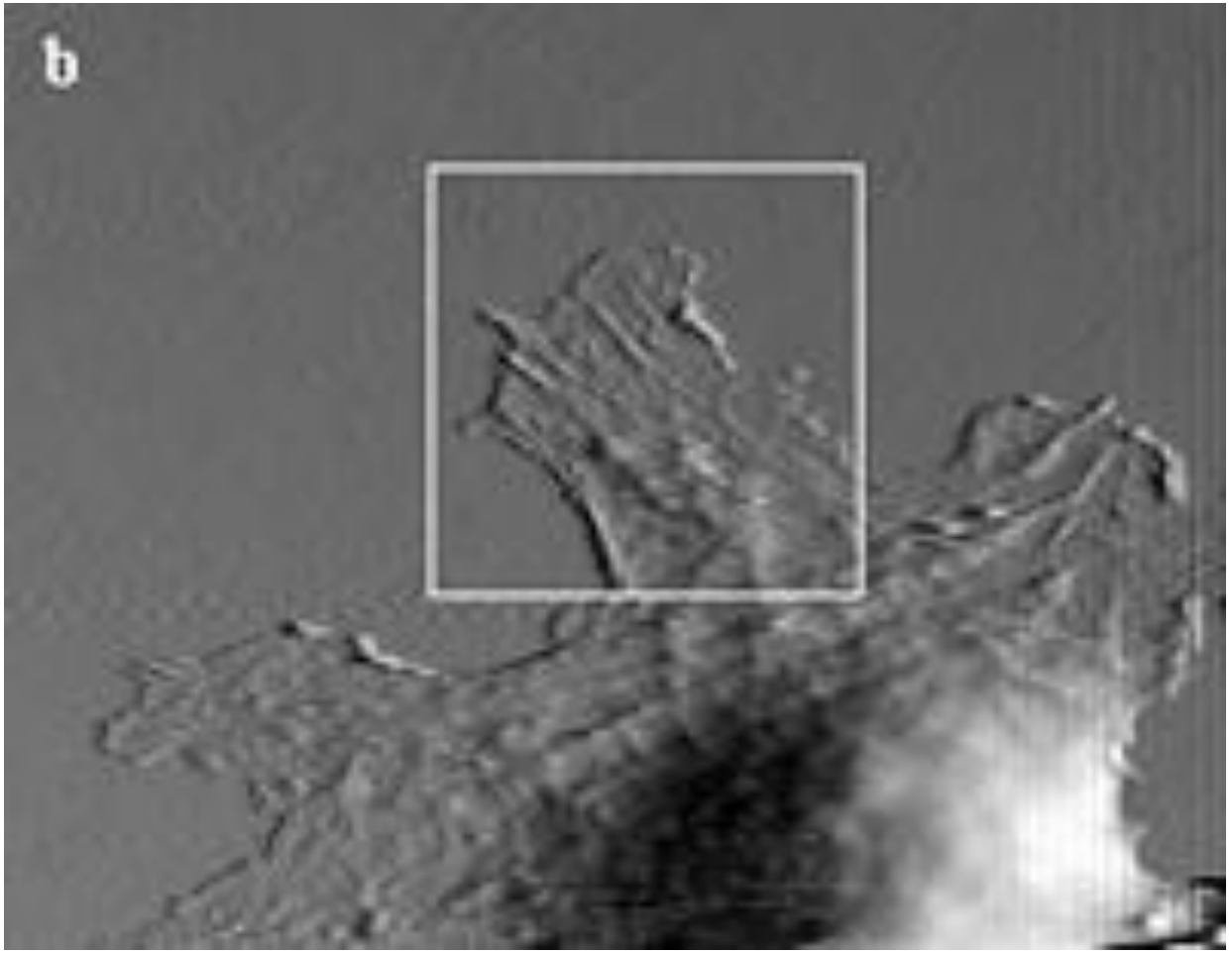
AFM



Force Spectroscopy
on living cells:

- temperature control
- controlled contact time and force

Imaging of cells

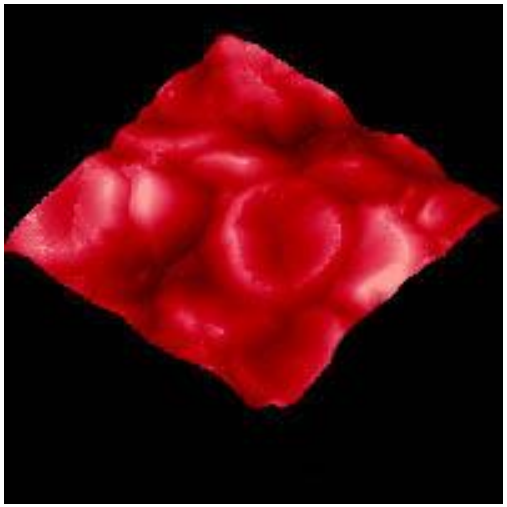


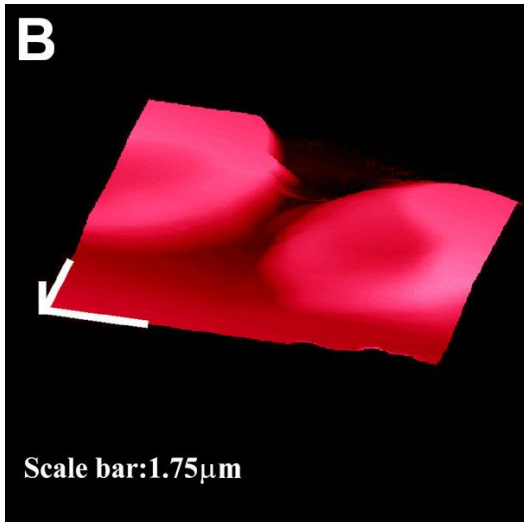
Biological Applications: AFM Images of Cells

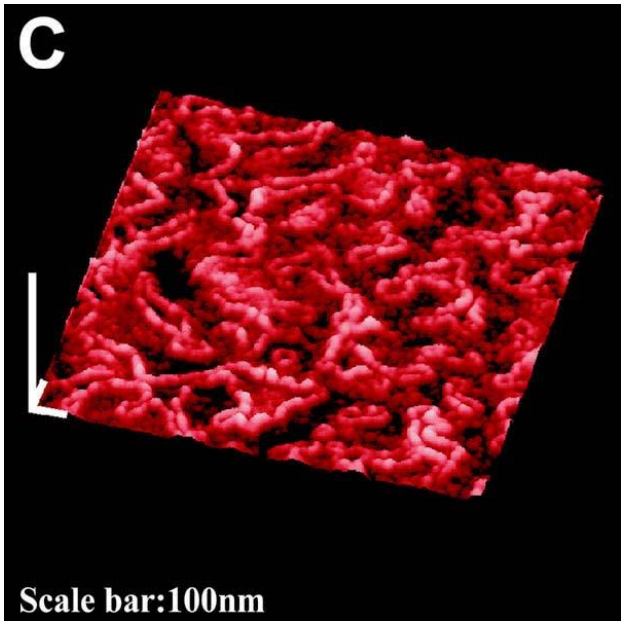
Contact mode image of **human red blood cells** - note cytoskeleton is visible. blood obtained from Johathan Ashmore, Professor of Physiology University College, London. A false color table has been used here, as professorial blood is in fact blue. 15µm scan courtesy M. Miles and J. Ashmore, University of Bristol, U.K.

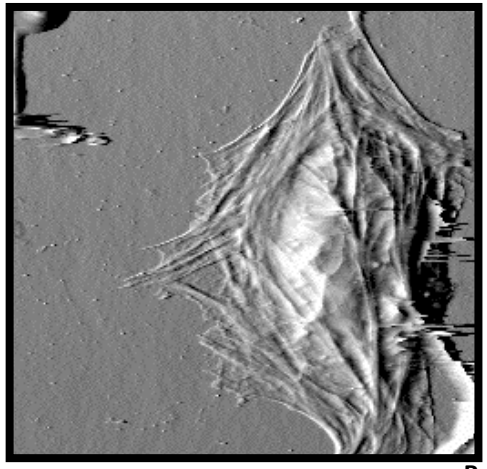
Red Blood Cells

Shao, et al., : http://www.people.virginia.edu/~js6s/zsfig/random.html

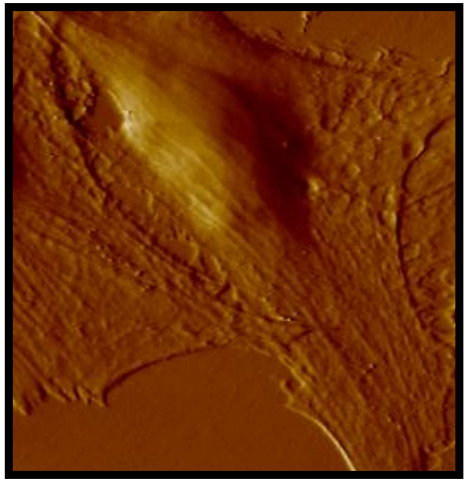




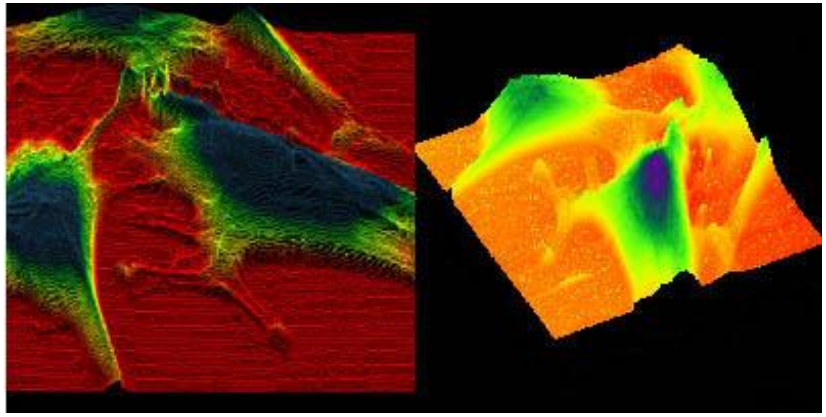




Radmacher, et al., **Cardiac Cells**
http://www.physik3.gwdg.de/~radmacher/

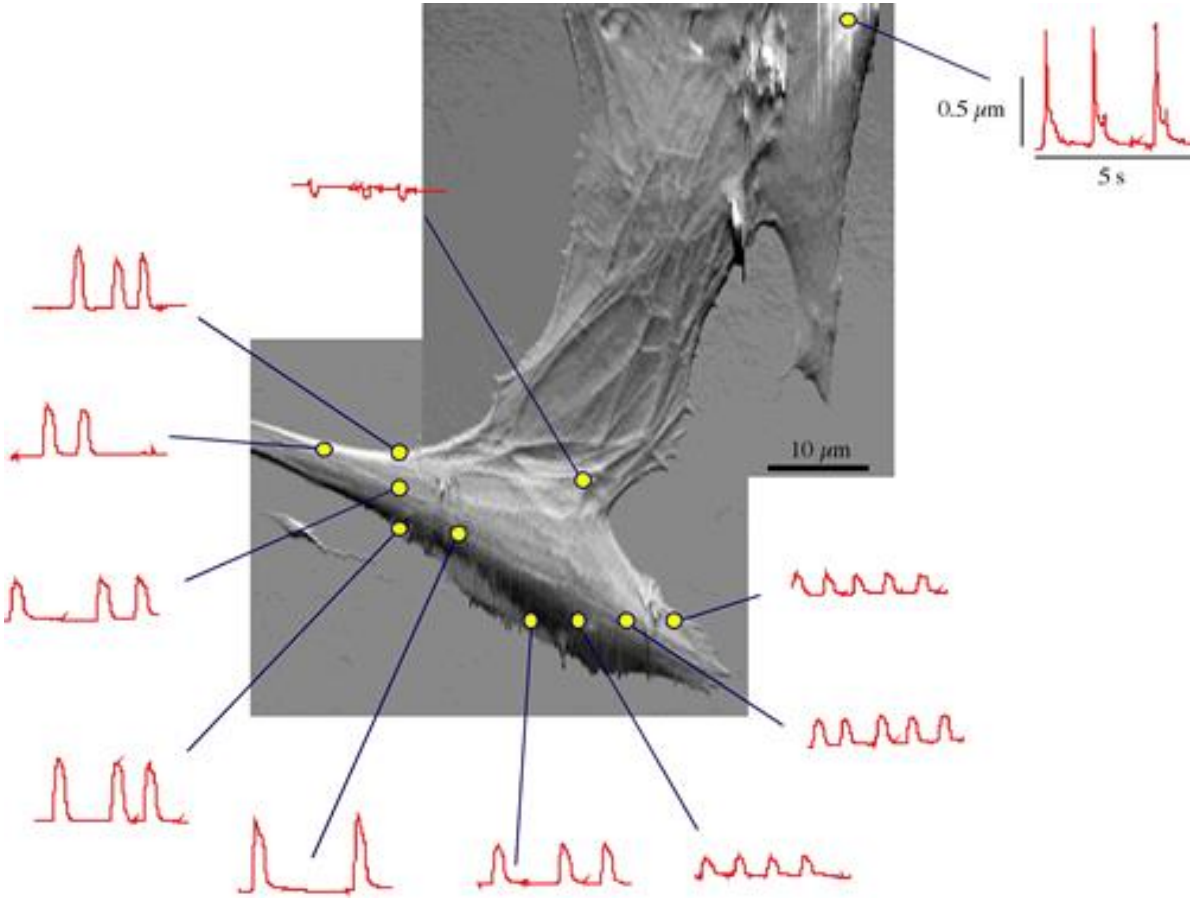


Rat Embryo Fibroblast*(*M. Stolz,C. Schoenenberger, M.E. Müller Institute, Biozentrum, Basel Switzerland)*



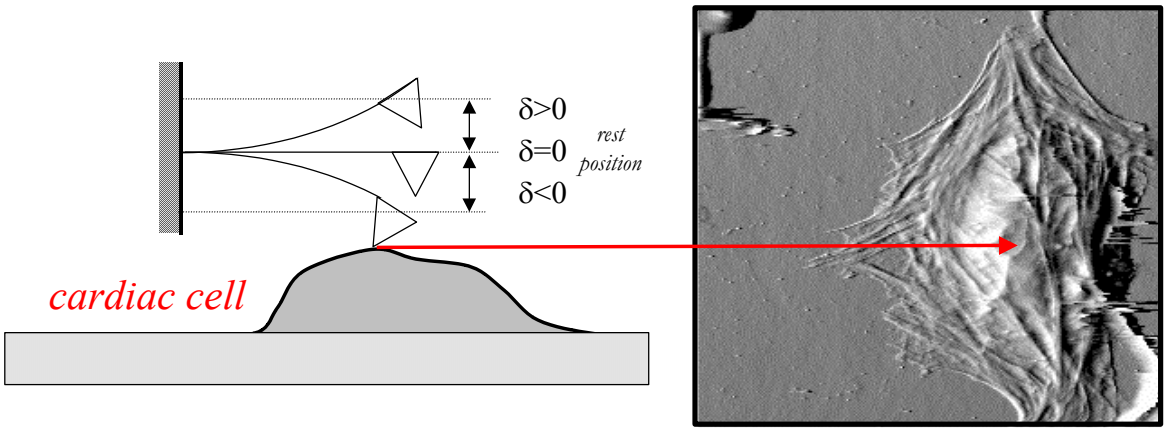
Height image of **endothelial cells** taking in fluid using Contact Mode AFM. 65 µm scan courtesy J. Struckmeier, S. Hohlbauch, P. Fowler, Digital Intruments/Veeco Metrology, Santa Barbara, USA.

Image & Force

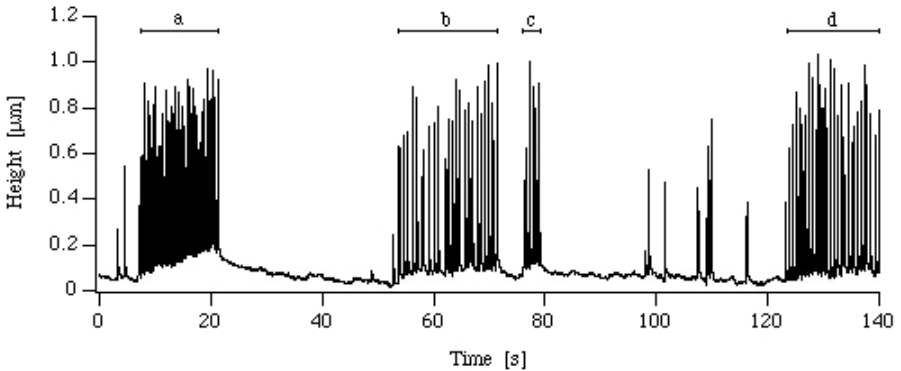


Biological Applications: Manipulation of Living Cells

- rest cantilever on top of cell and monitor cantilever deflection up and down = beating of cell



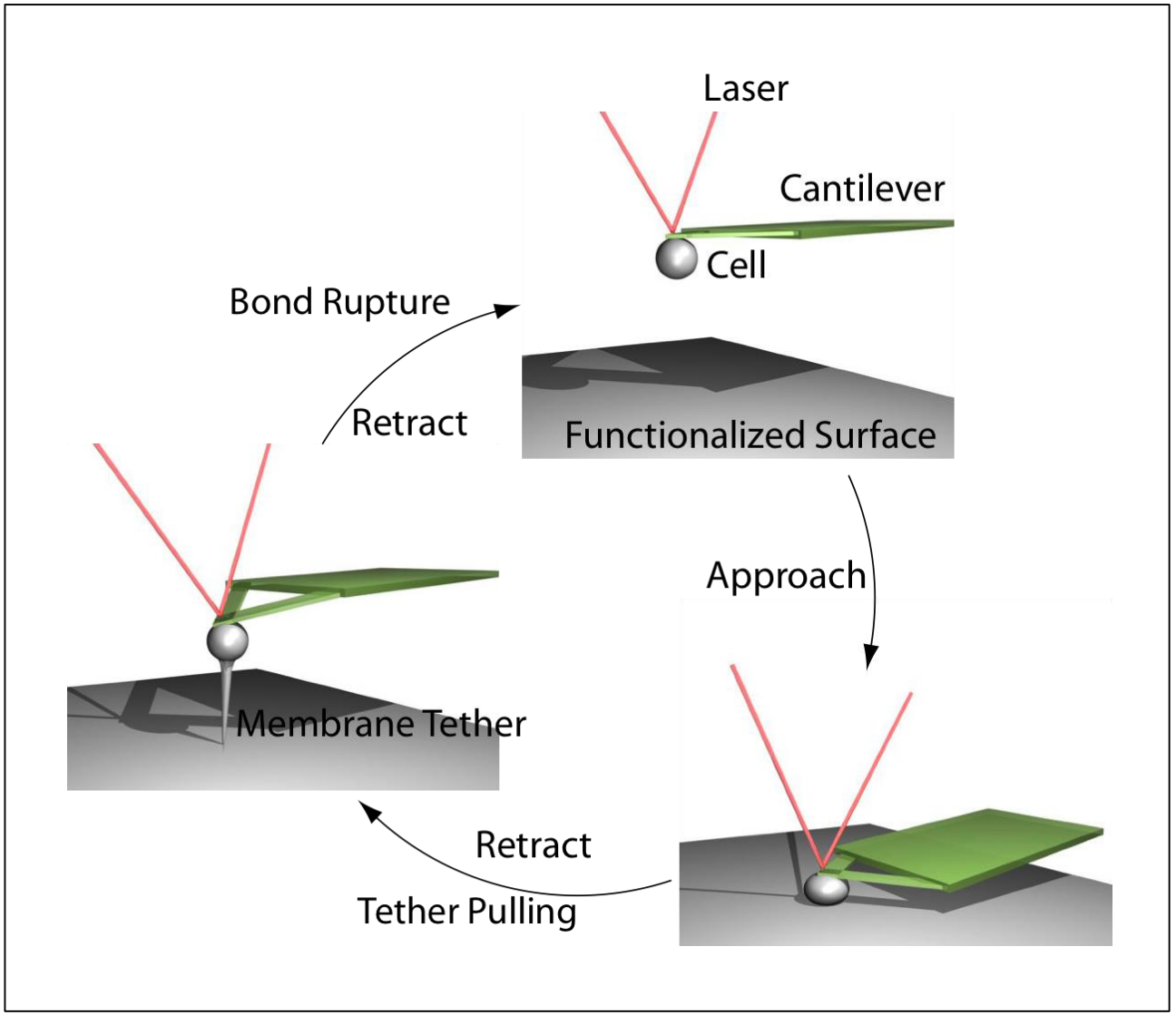
- **I. confluent layer of cells :** beat regularly in terms of frequency and amplitude, enormous stability of pulsing, cell are synchronized and coupled together : diverse pulse shapes due to macroscopic moving centers of contraction and relaxation
- **II. individual cell :** sequences of high mechanical activity alternate with times of quietness, irregular beating which often last for minutes, active sequences were irregular in frequency and amplitude
- **III. group of cells:** "pulse mapping"



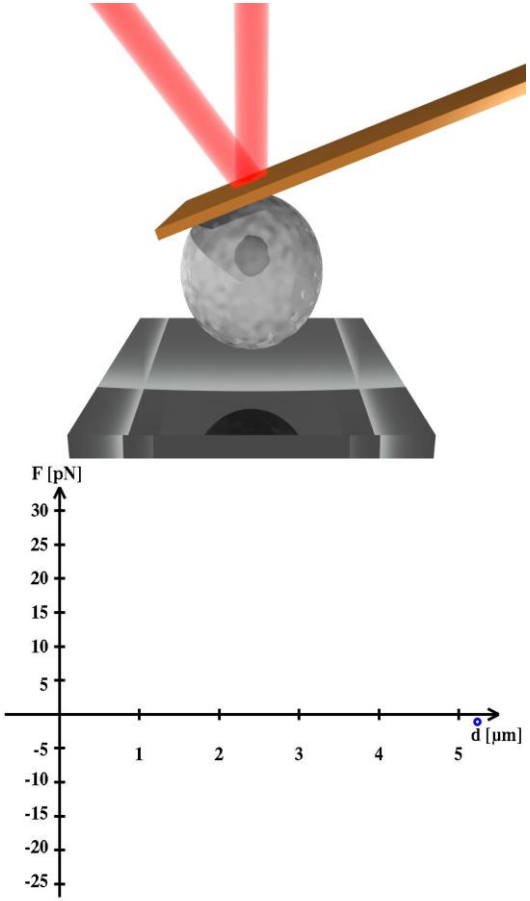
Ortega-Esteban, A. *et al*. Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue. *Sci. Rep.* 3, 1434; DOI:10.1038/srep01434 (2013).

Studying early stages of fibronectin fibrillogenesis in living cells by atomic force microscopy

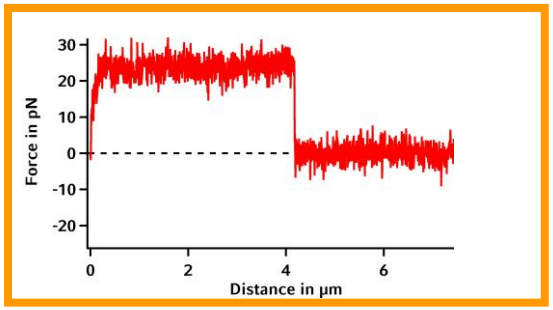
AFM & Cell Adhesion



Single molecules on cells

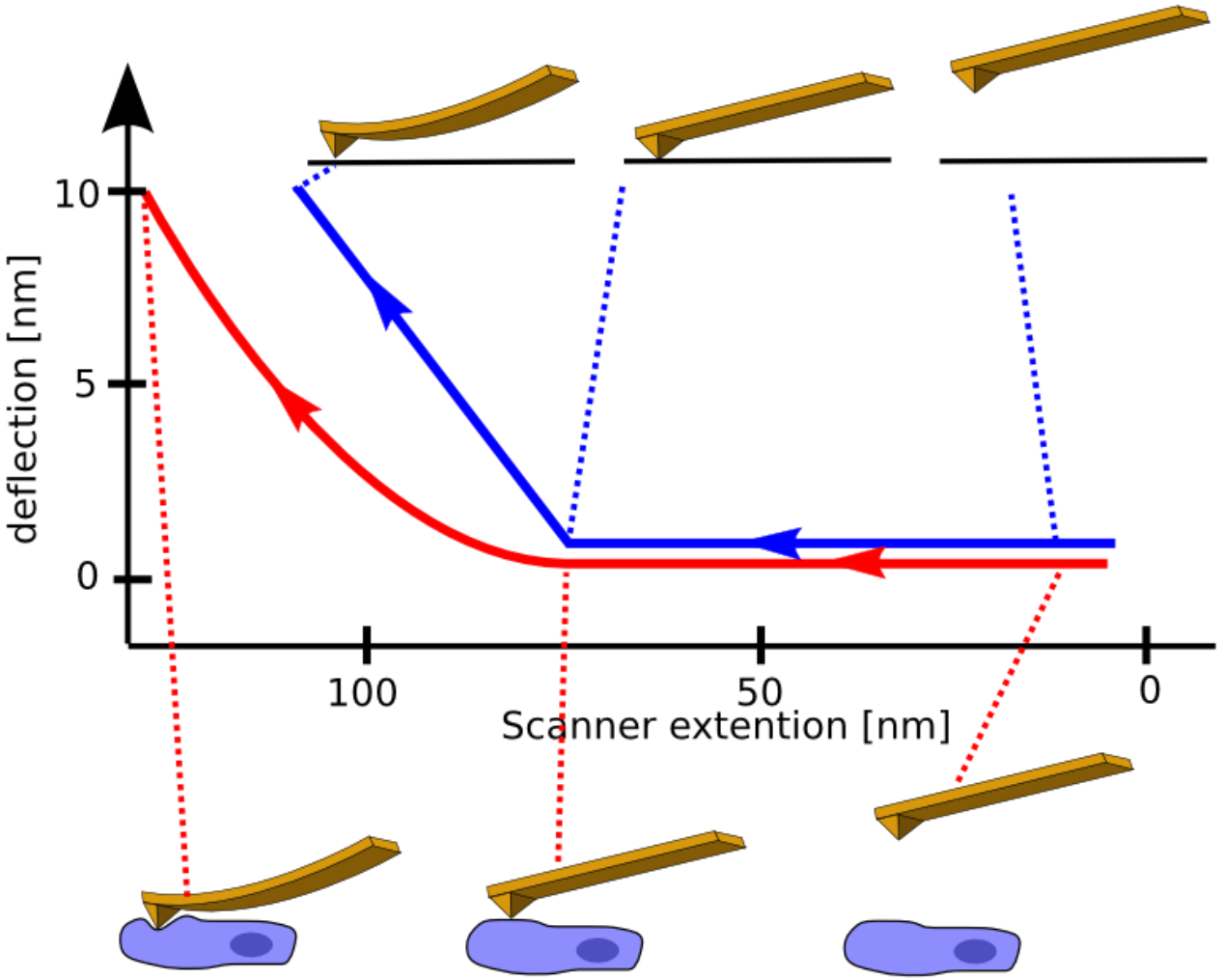


Curve on cells

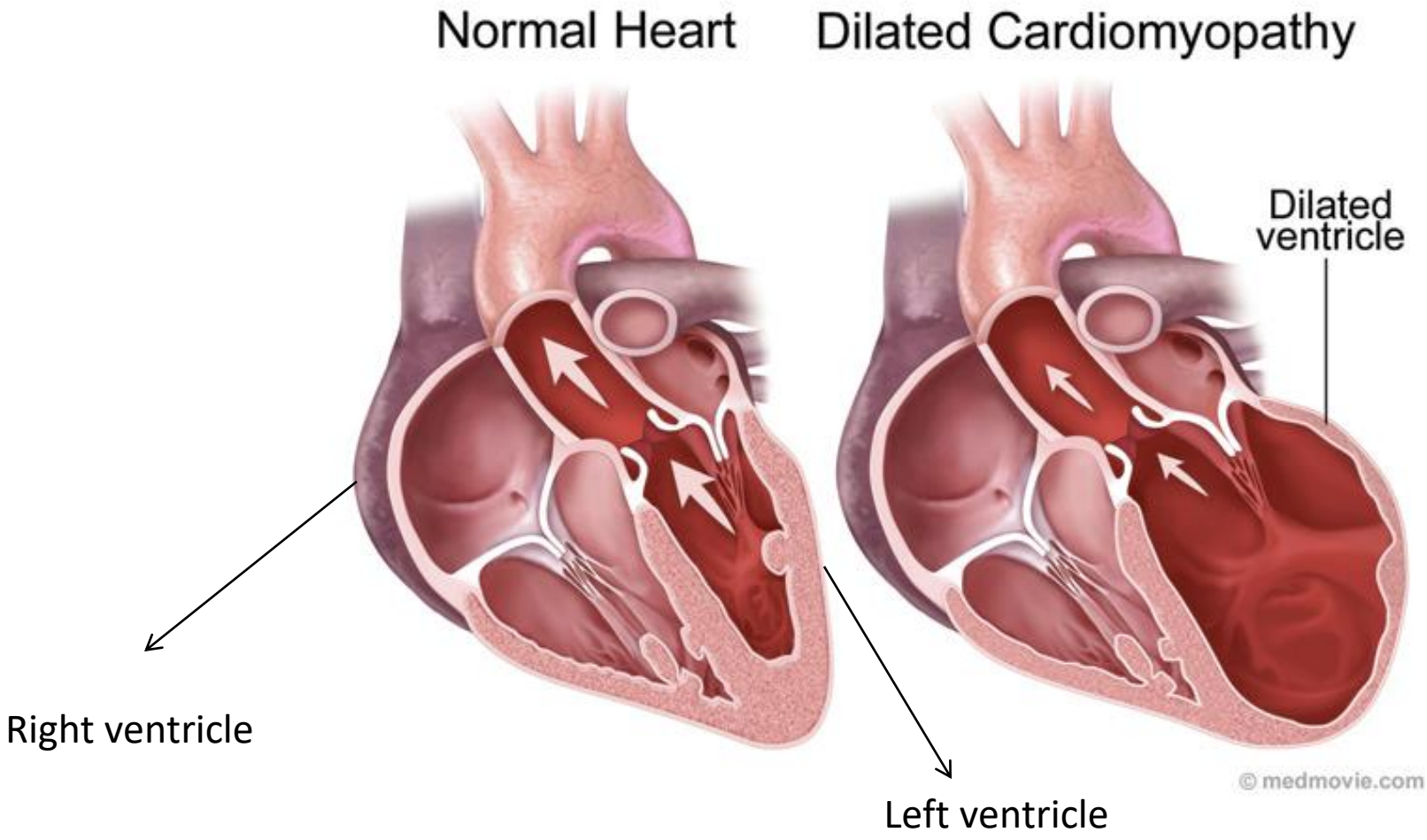


- Initial elastic response
- Followed by viscuous flow

Mechanik



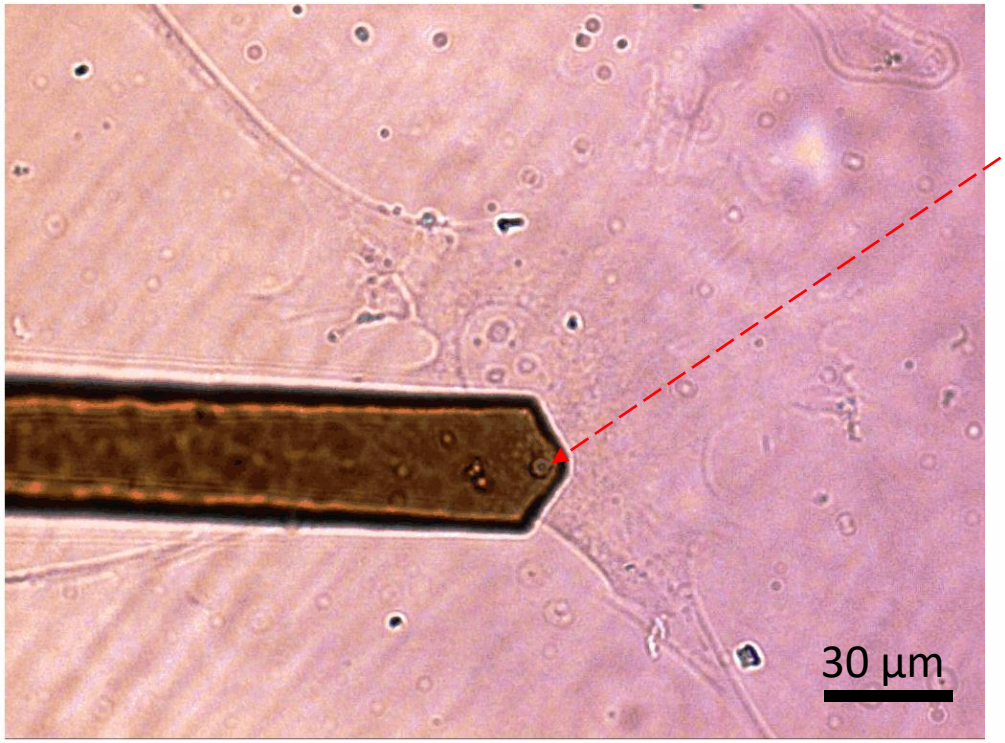
Dilatetd Cardiomyopathy (DCM) May Affect Cell Elasticity



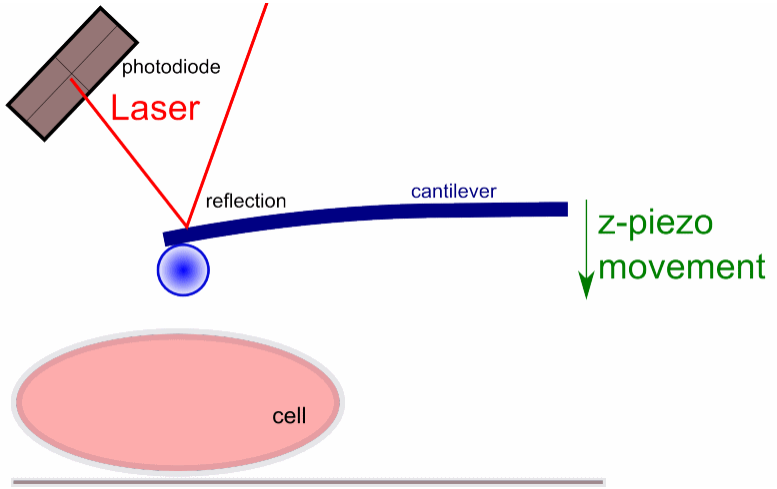
http://www.genedx.com/site/dilated-cardiomyopathy-genetic-testing

Nanoindentation probes Mechanics of Primary Cardiac Fibroblasts

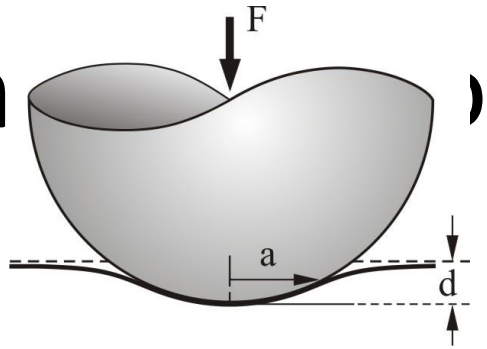
Messvorgang



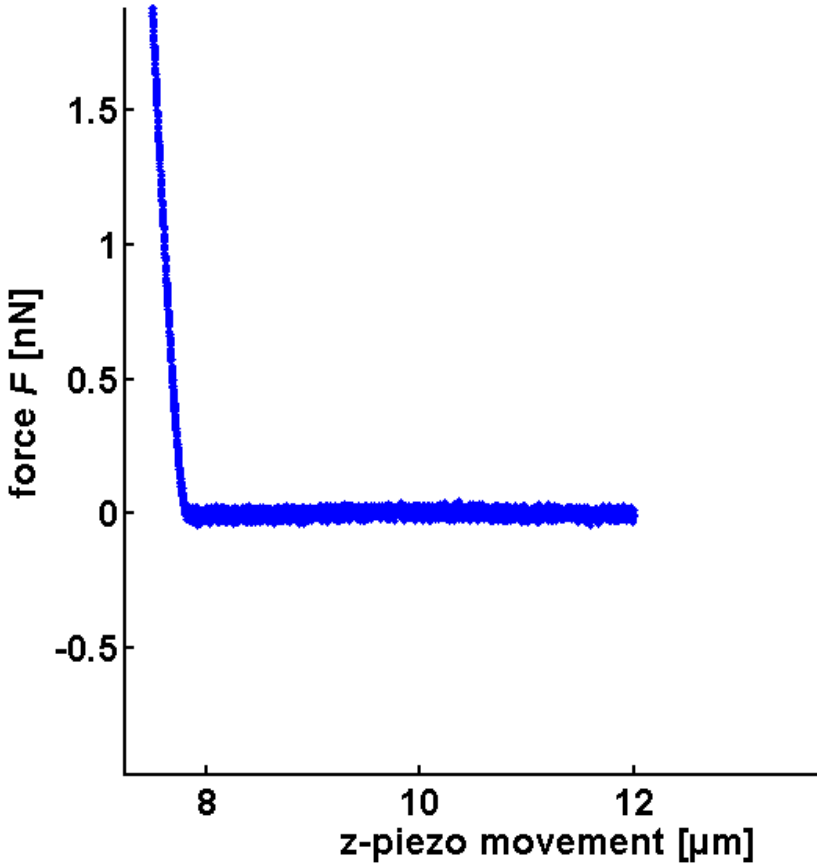
colloidal probes (*R=2.5 µm*)



Data Evaluated with odel



http://de.academic.ru/pictures/dewiki/75/Kontakt_Kugel_Ebene.jpg



$$F = \frac{16}{9} E \sqrt{R} d^{3/2}$$

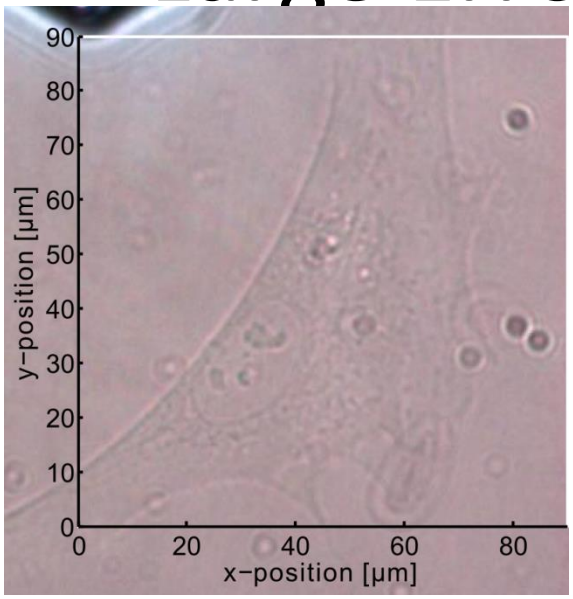
F :	Indentation Force
d :	Indentation Depth
E :	Young Modulus
R:	Sphere Radius

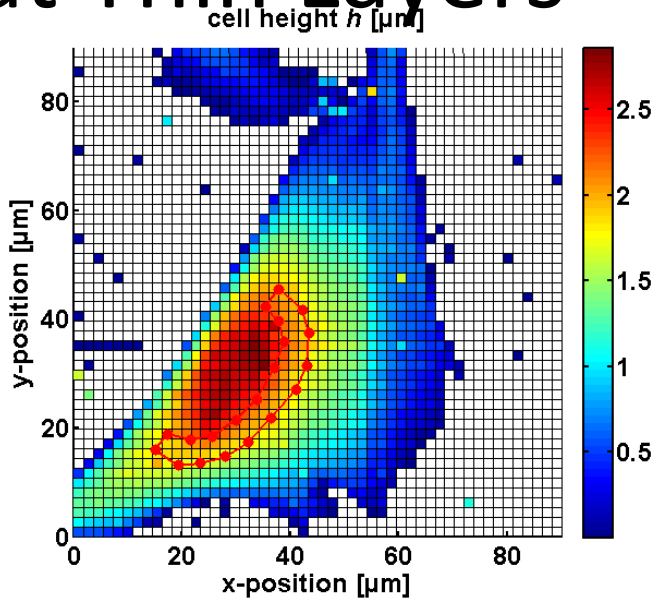
Homogeneous elastic half-space

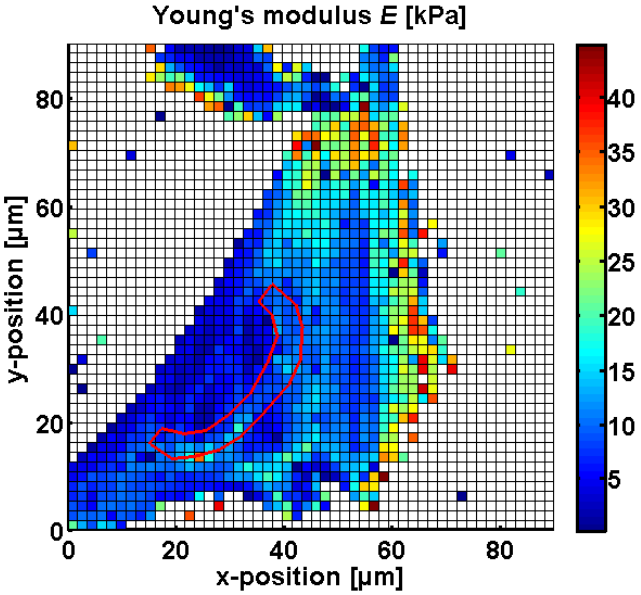
$R >> d$

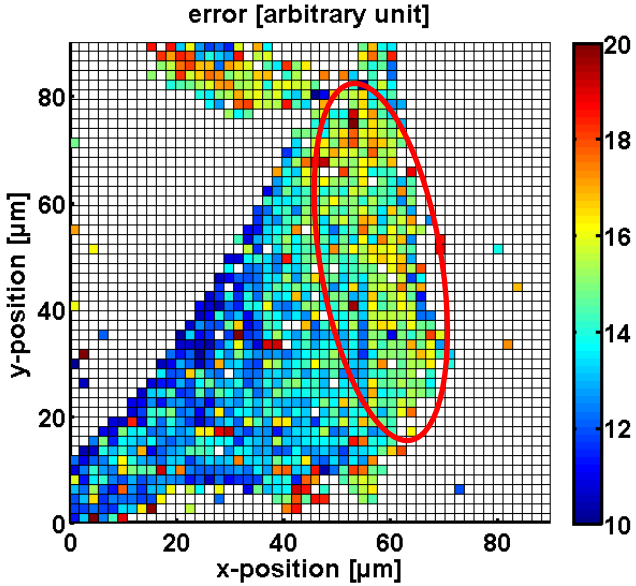
No Adhesion

Large Errors at Thin Layers

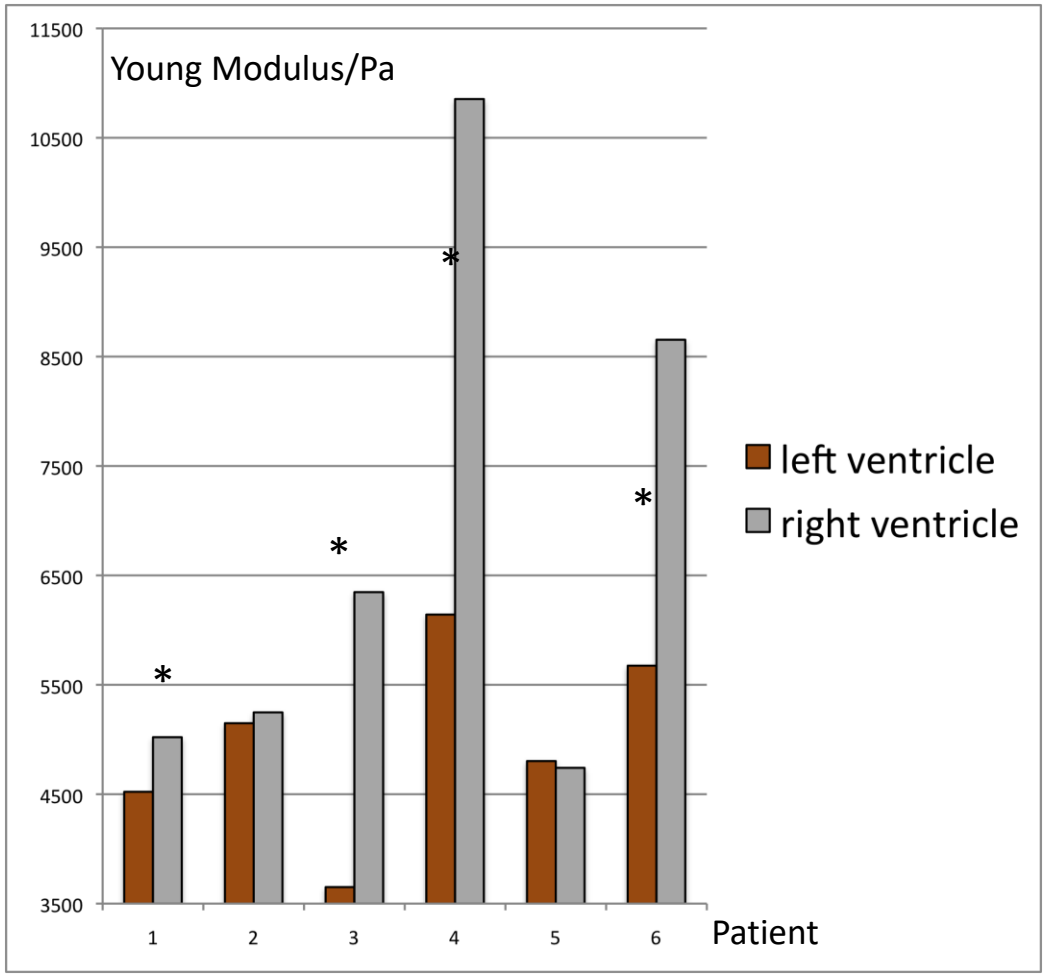


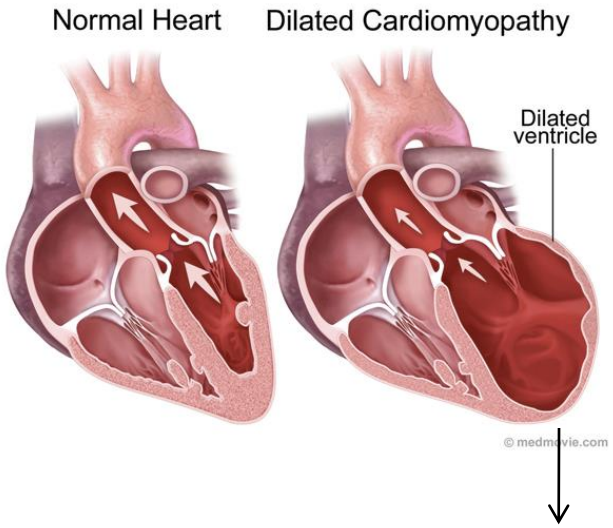






In DCM Patients, Left Ventricular Fibroblasts are Less Elastic

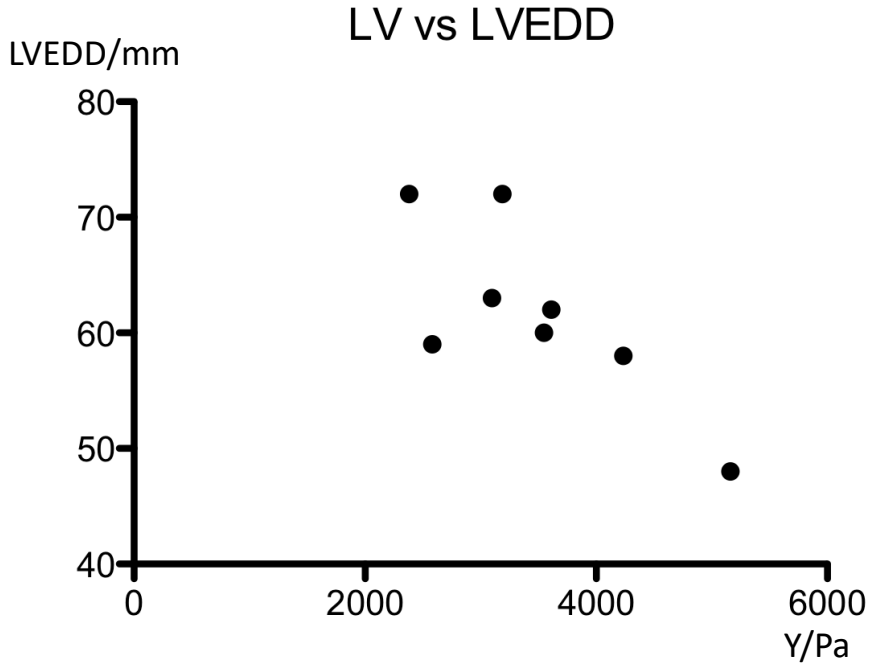


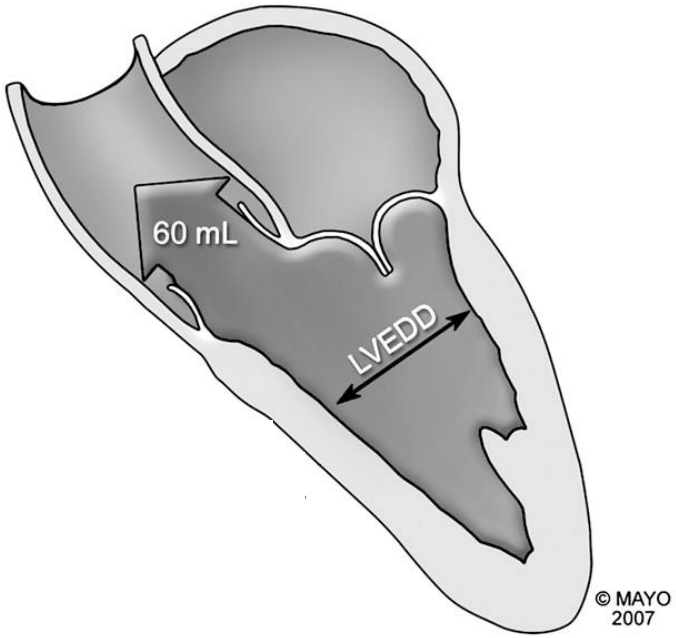


Left ventricle

Fibroblast Elasticity Correlates with Pathology

LVEDD: Left Ventricle End Diastolic Dimension





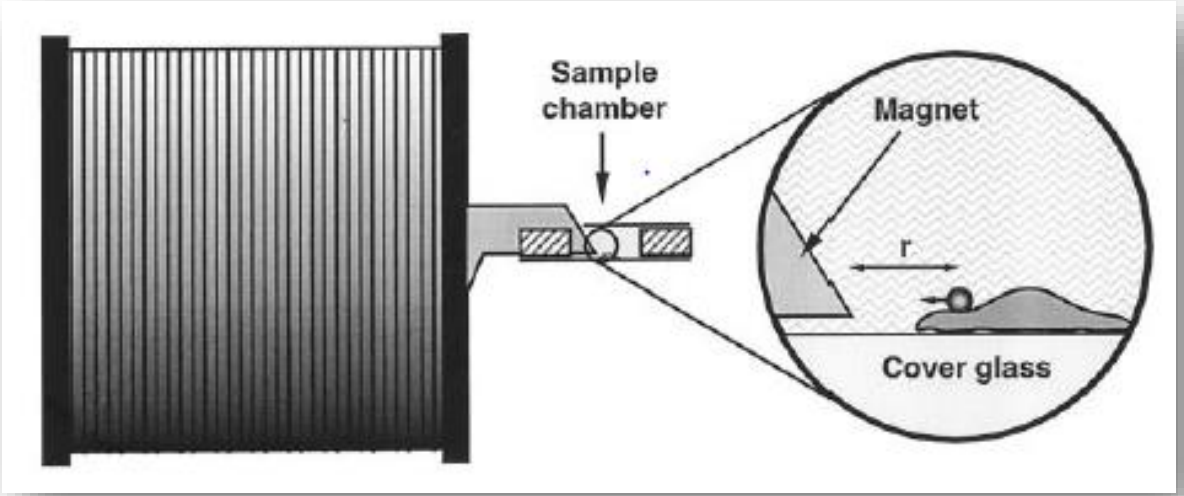
Conclusion

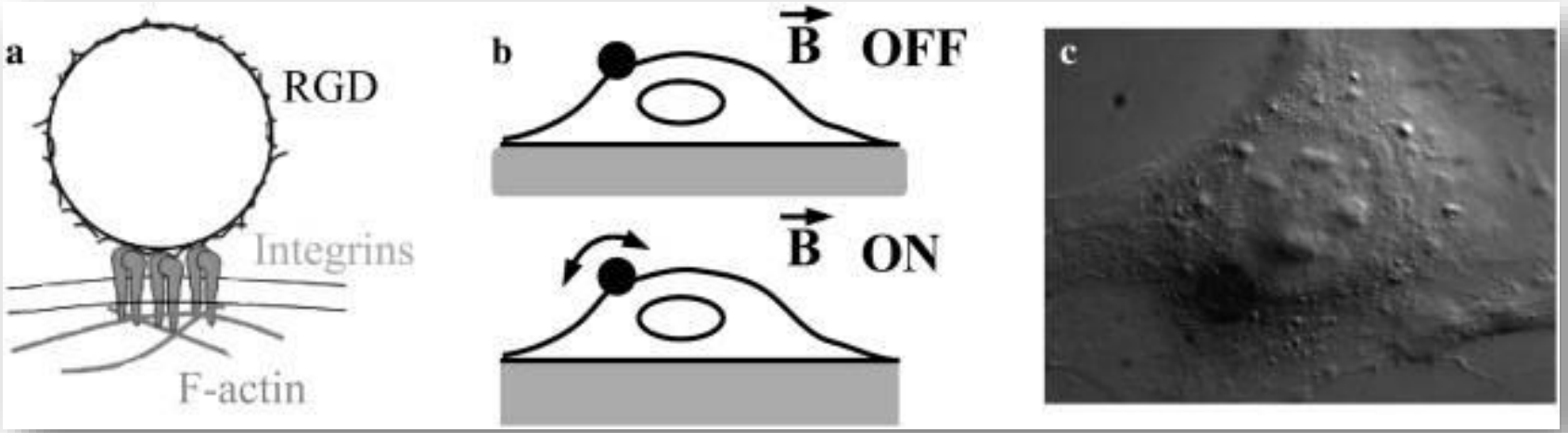
Scanning Probe Microscopy is a powerful tool for high-resolution biological studies.

Data interpretation is not always straightforward.

Mechanical Properties are important for physiological behavior.

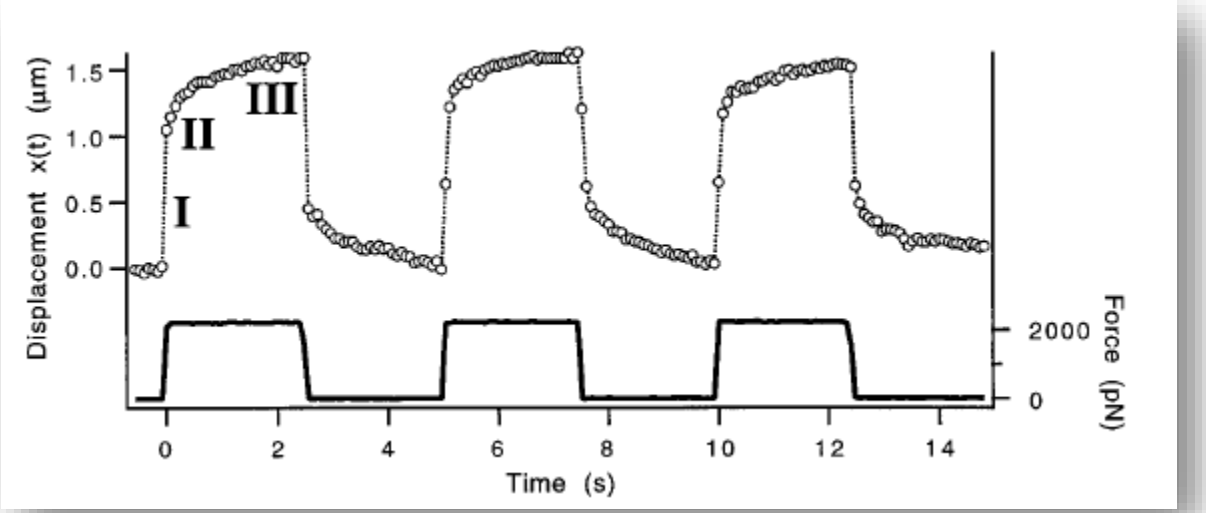
Magnetic Bead Twisting Rheology

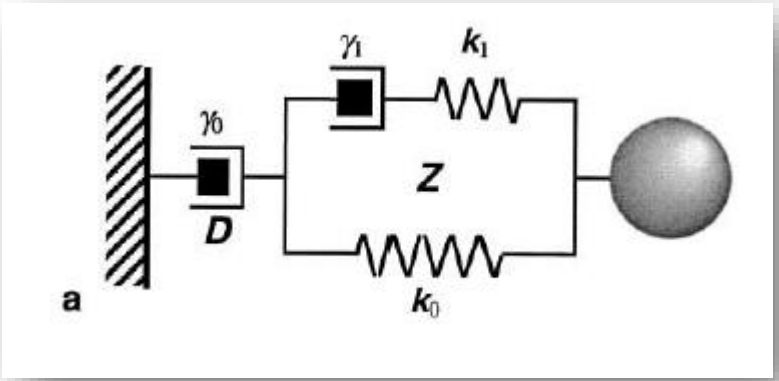




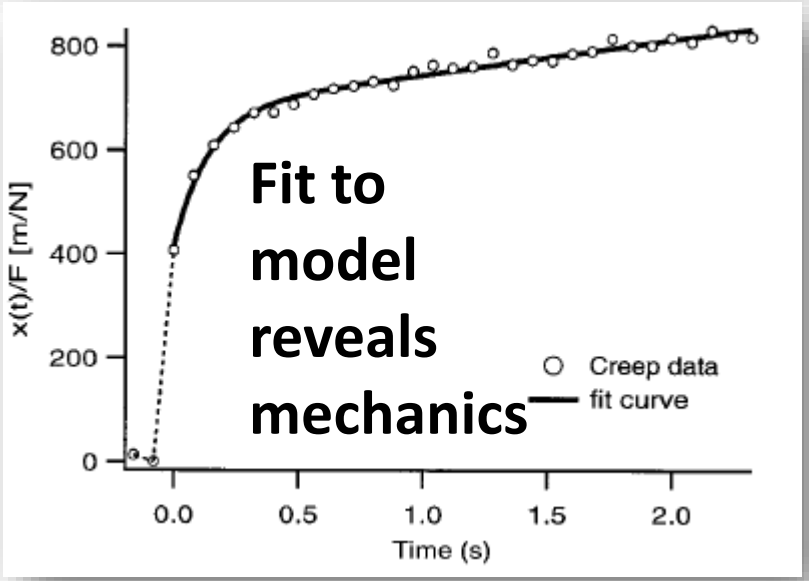
Typical Experimental Results

Creep Response

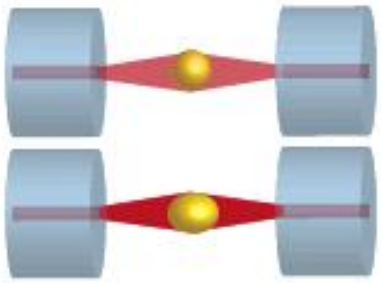




Mechanical Model



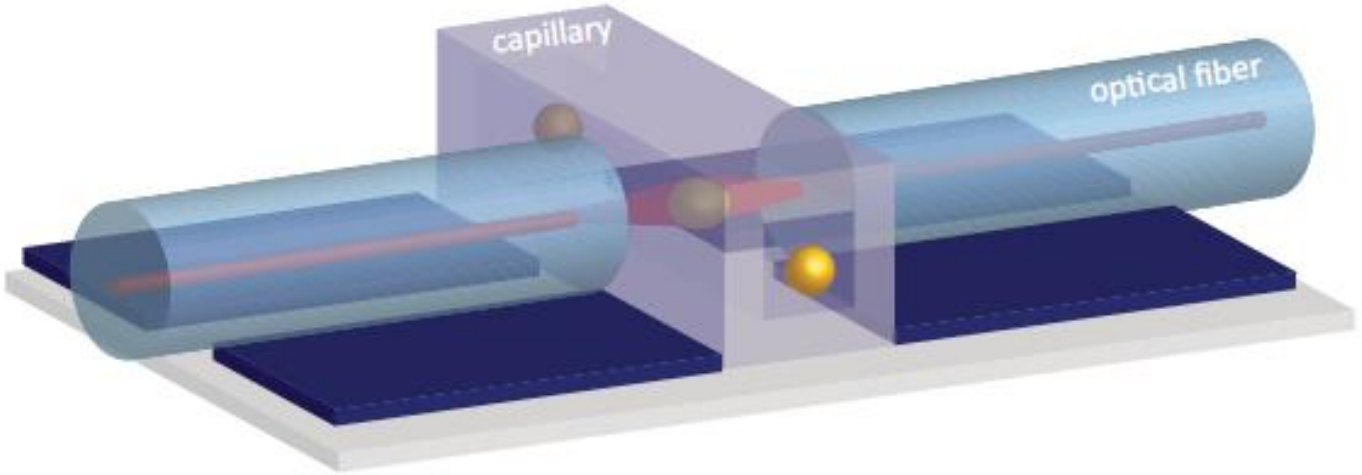
Optical Stretcher



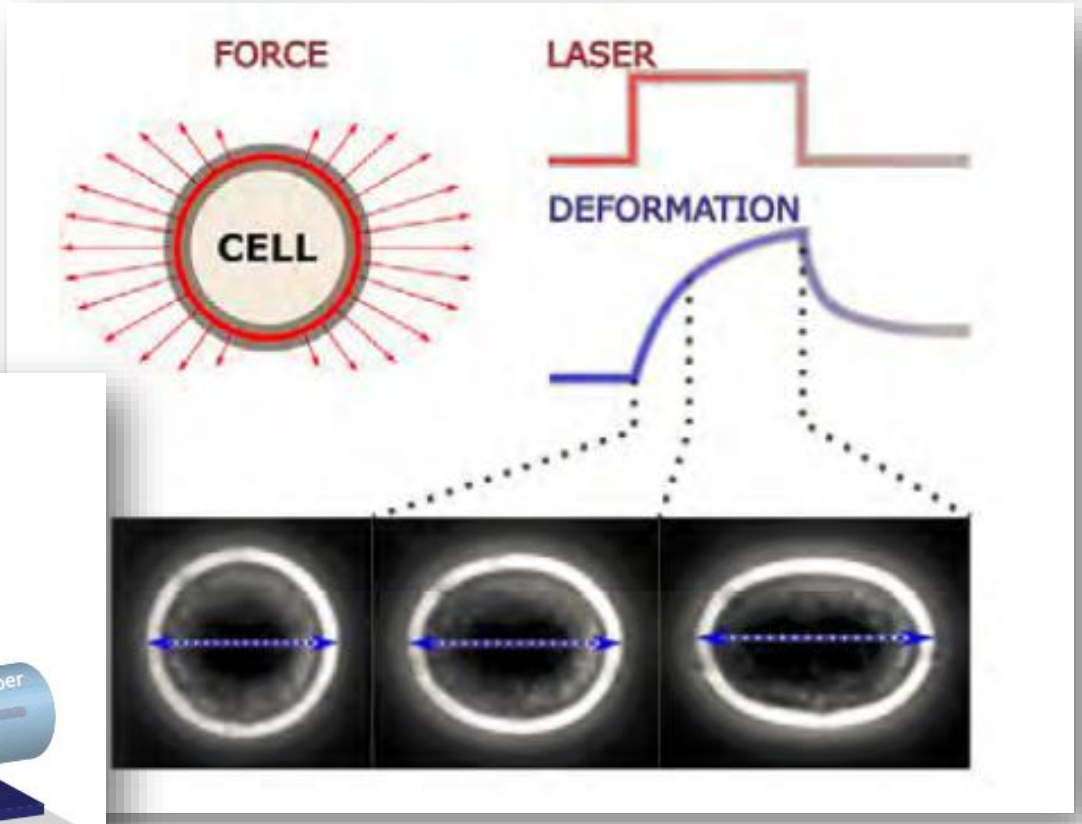
trapping at
low laser power

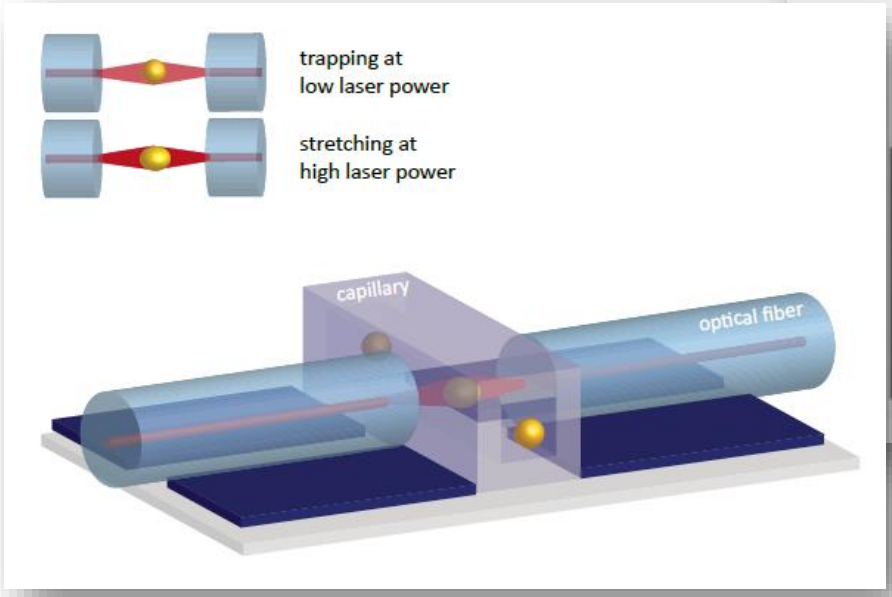
stretching at
high laser power

Individual cells are trapped in dual laser beam trap



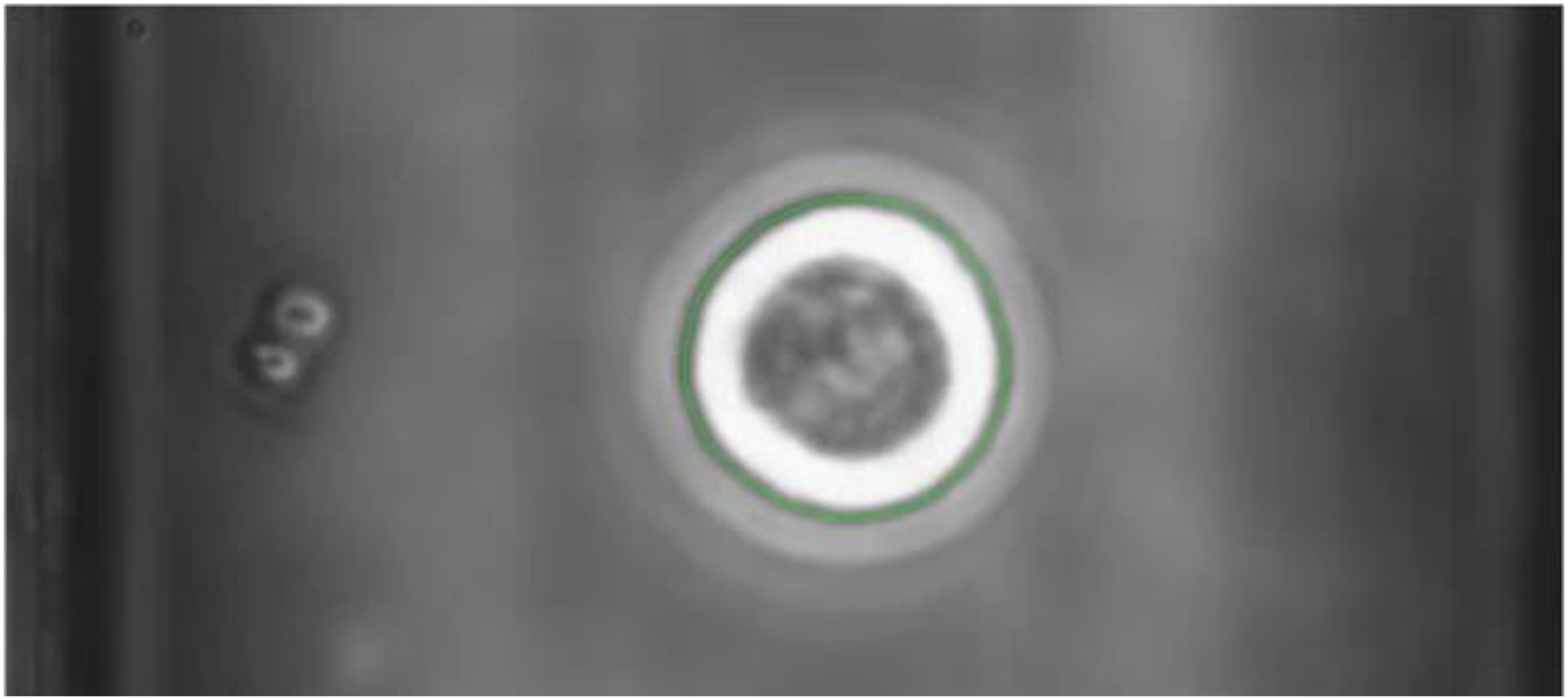
Optical Stretcher



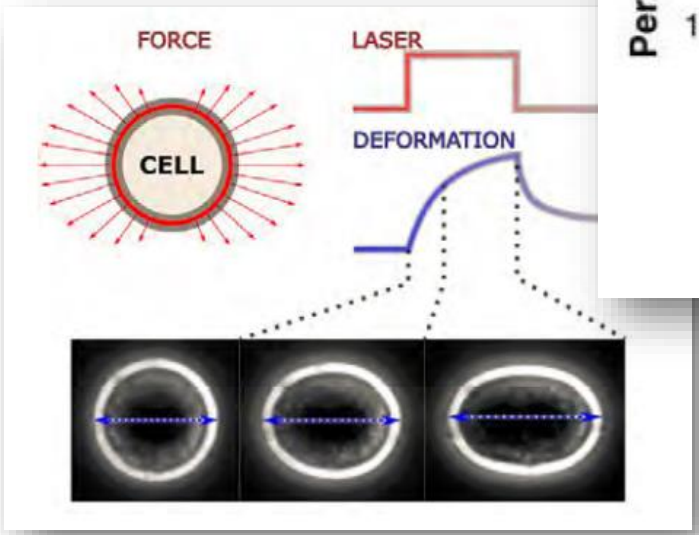


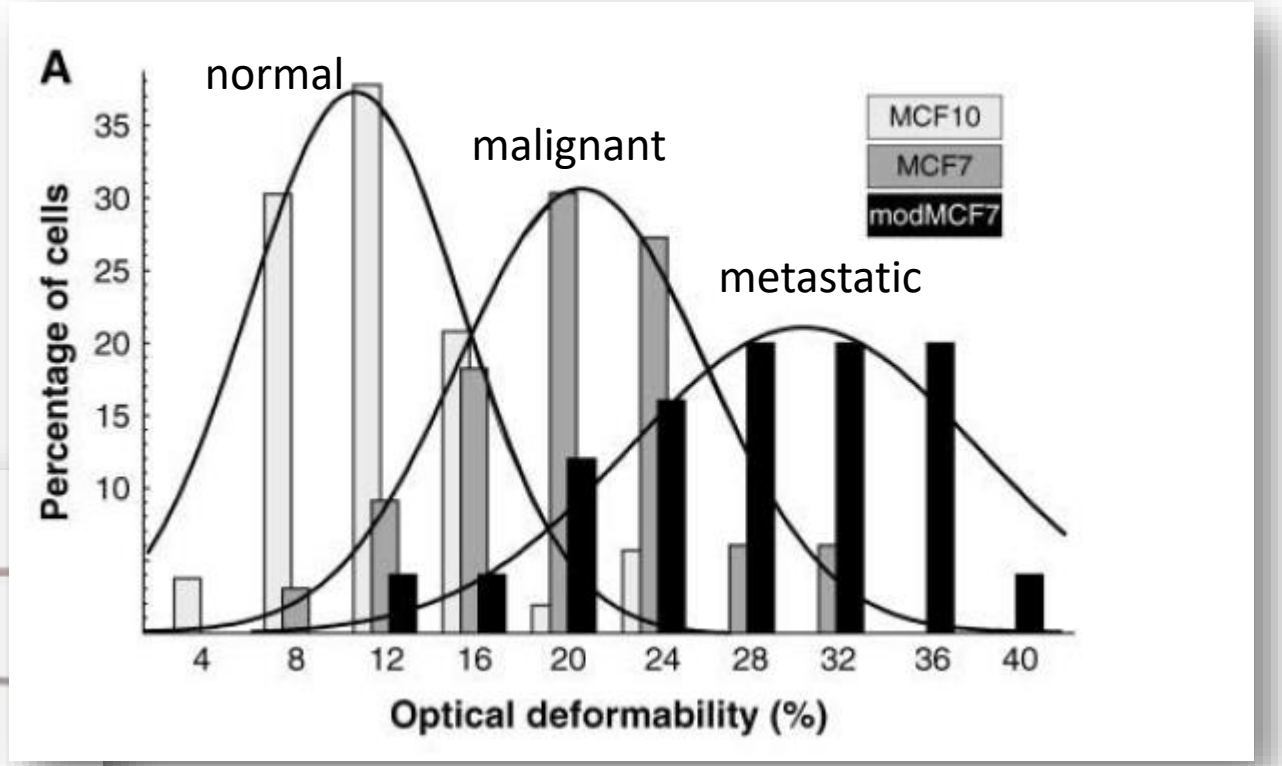
Optical Stretcher

Optical Stretcher



Optical Stretcher



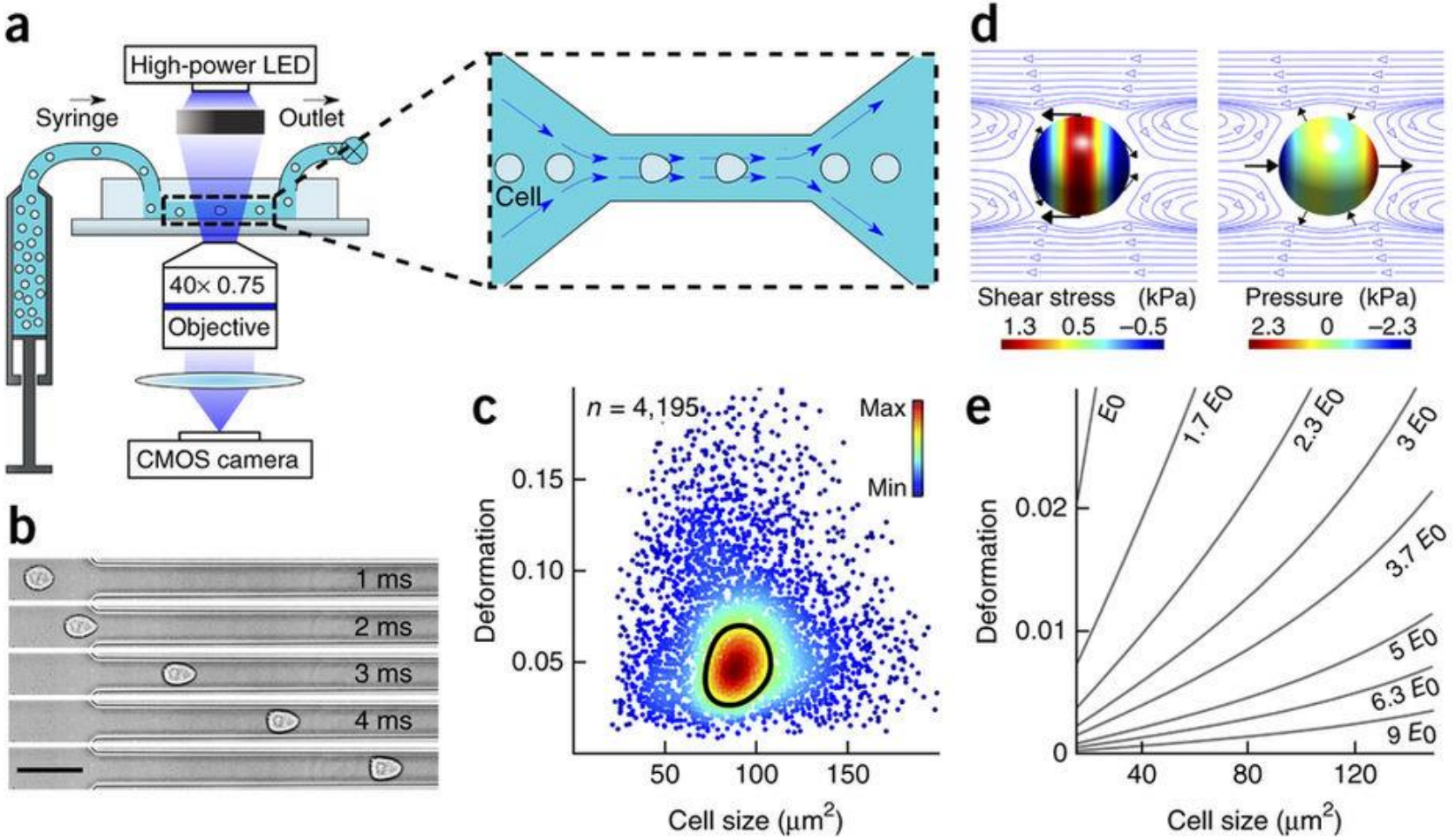


Real-time deformability cytometry: on-the-fly cell mechanical phenotyping

Oliver Otto, Philipp Rosendahl, Alexander Mietke, Stefan Golfier, Christoph Herold, Daniel Klaue, Salvatore Girardo, Stefano Pagliara, Andrew Ekpenyong, Angela Jacobi, Manja Wobus, Nicole Töpfner, Ulrich F Keyser, Jörg Mansfeld, Elisabeth Fischer-Friedrich & Jochen Guck

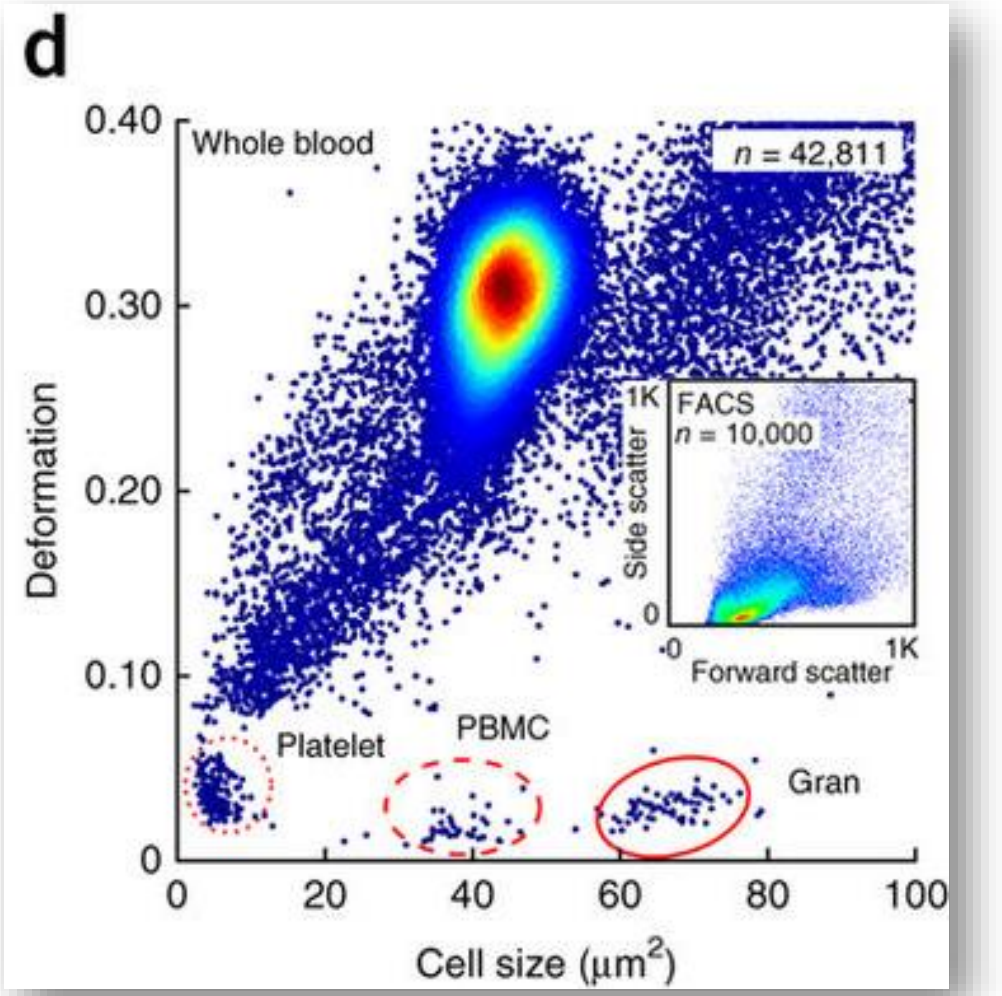
Nature Methods **12**, 199–202 (2015)
doi:10.1038/nmeth.3281
Download Citation

Received: 30 July 2014
Accepted: 23 December 2014
Published online: 02 February 2015


a
High-power LED
Syringe
Outlet
Cell
40× 0.75
Objective
CMOS camera
b
1 ms
2 ms
3 ms
4 ms
c
n = 4,195
Max
Min
Deformation
0.15
0.10
0.05
50
100
150
Cell size (μm²)
d
Shear stress (kPa)
1.3 0.5 −0.5
Pressure (kPa)
2.3 0 −2.3
e
E0
1.7 E0
2.3 E0
3 E0
3.7 E0
5 E0
6.3 E0
9 E0
Deformation
0.02
0.01
0
40
80
120
Cell size (μm²)

Video

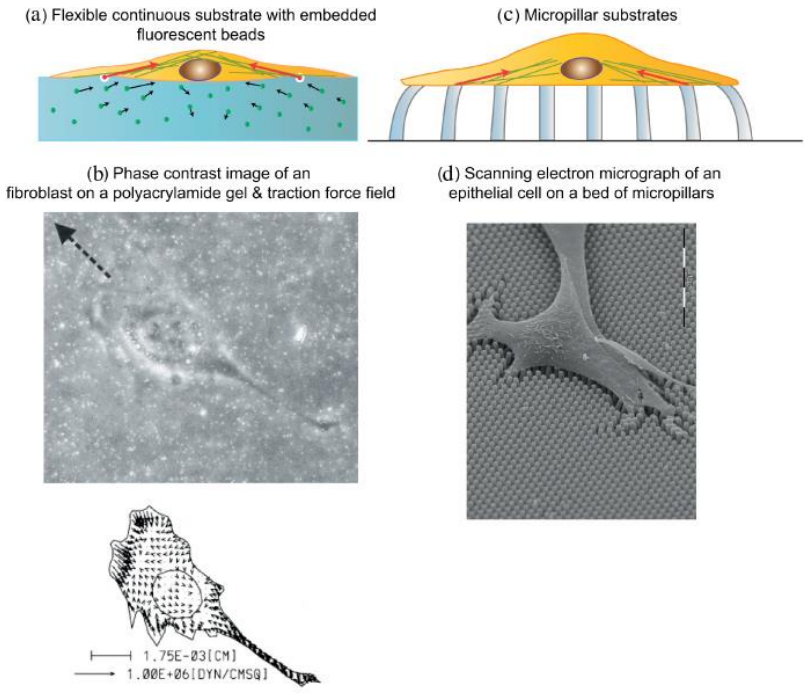
Analysis of whole blood possible



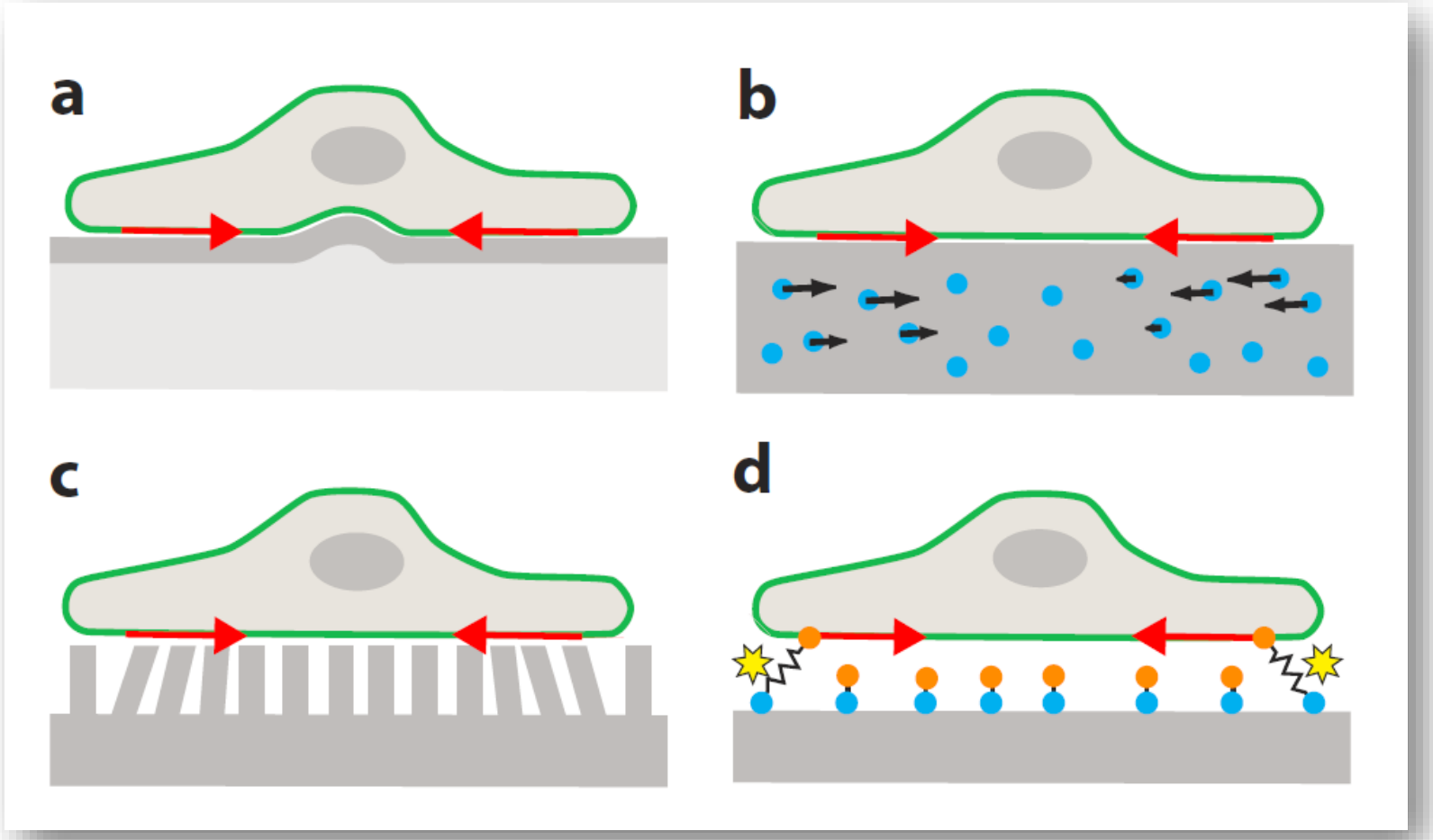
Mechanics & Migration

- Cells feel their mechanical environment and adapt accordingly
- Cells actively probe their environment mechanically
- Therefore, cells need to exert forces on the environment.

Which forces occur during migration? Traction Force Microscopy

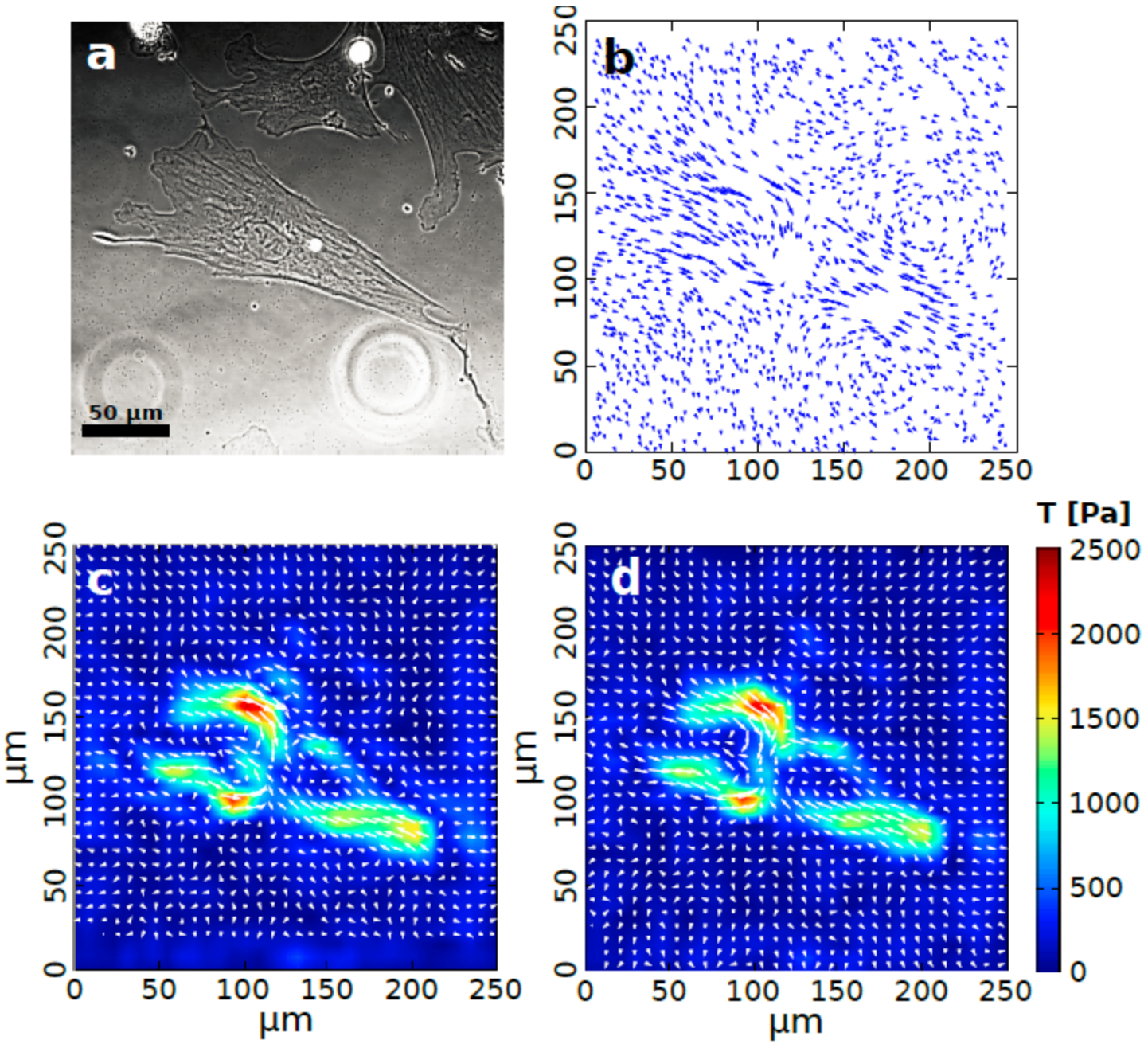


Traction force microscopy

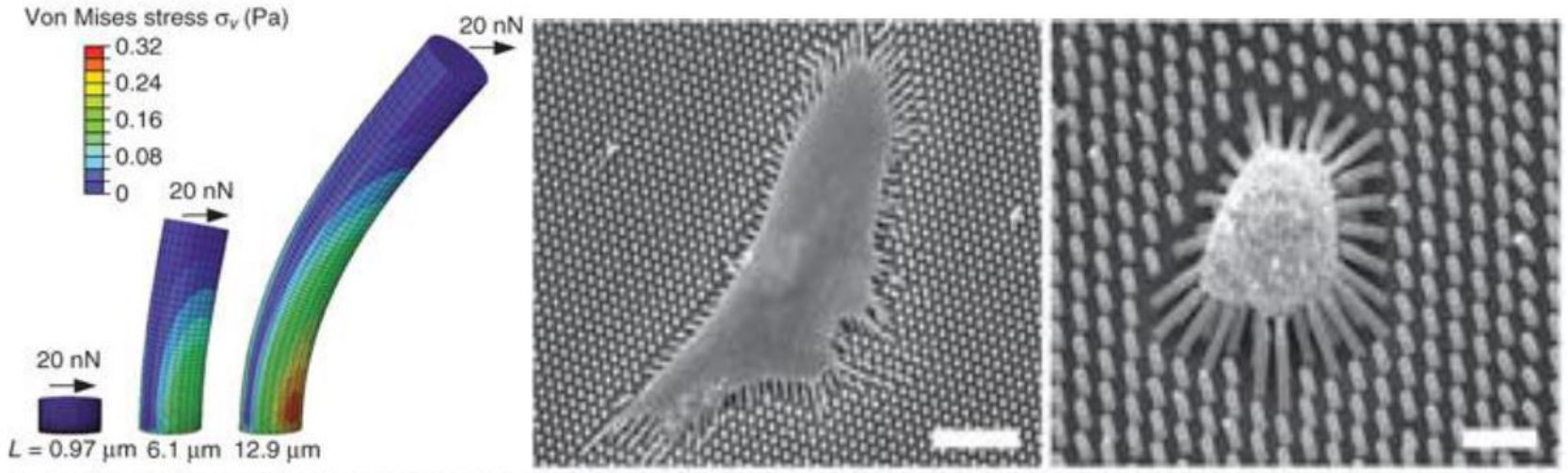


- Web page

http://www.cellmechanics.de/Methods/Tractions.html


a
50 µm
b
0
50
100
150
200
250
c
d
µm
T [Pa]
2500
2000
1500
1000
500
0

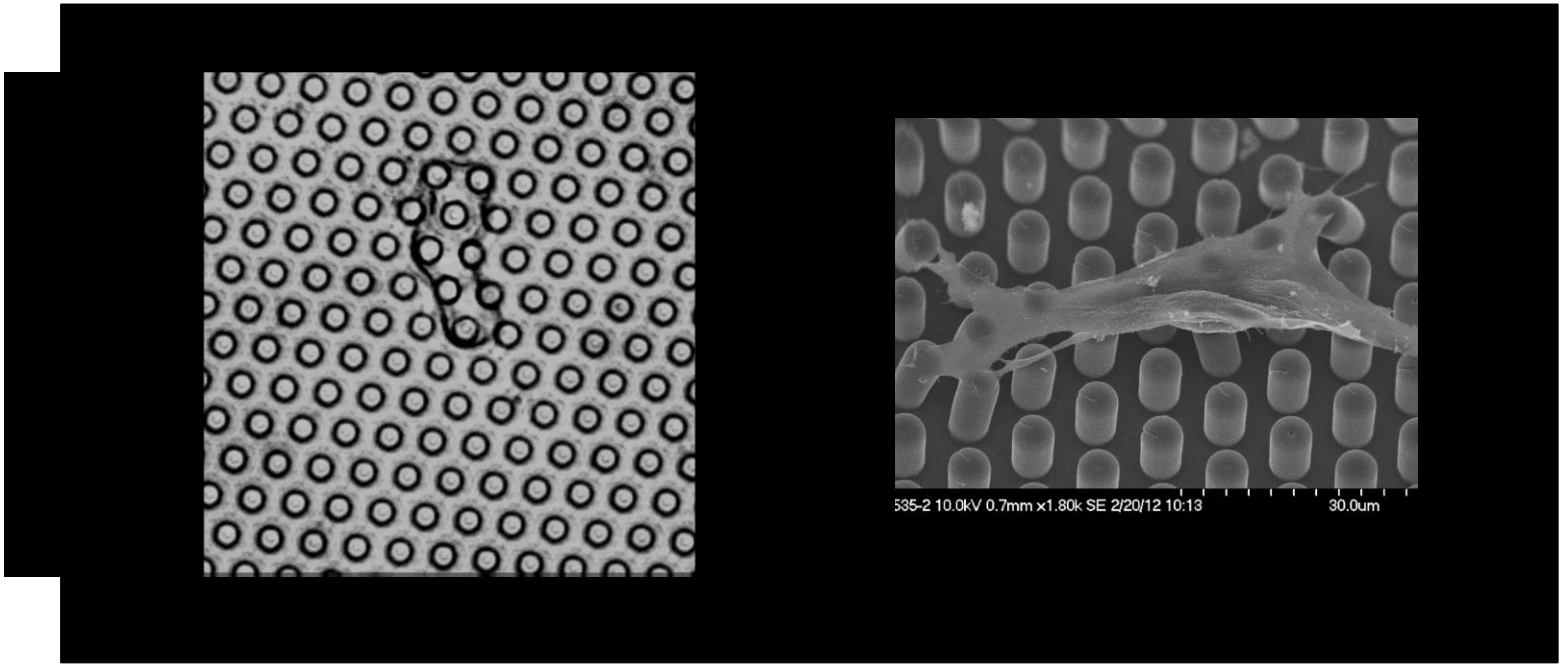
Micropost deflection



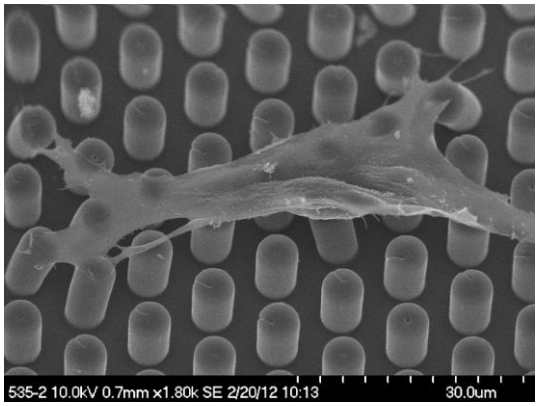
from Fu et al., **Nat Methods**, 2010

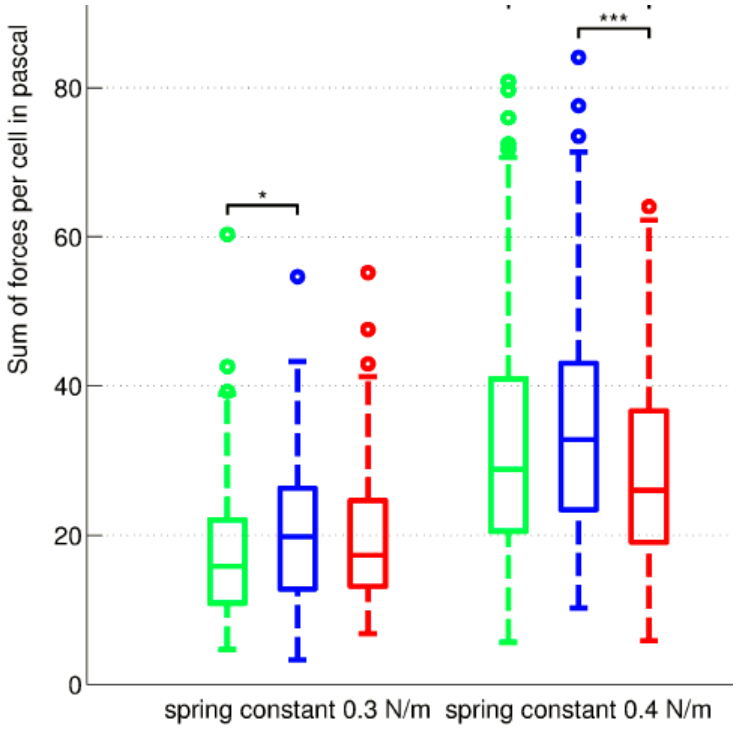
- **traction force microscopy is used to determine how much force is exerted by the cell onto an extracellular substrate**

Traction force microscopy



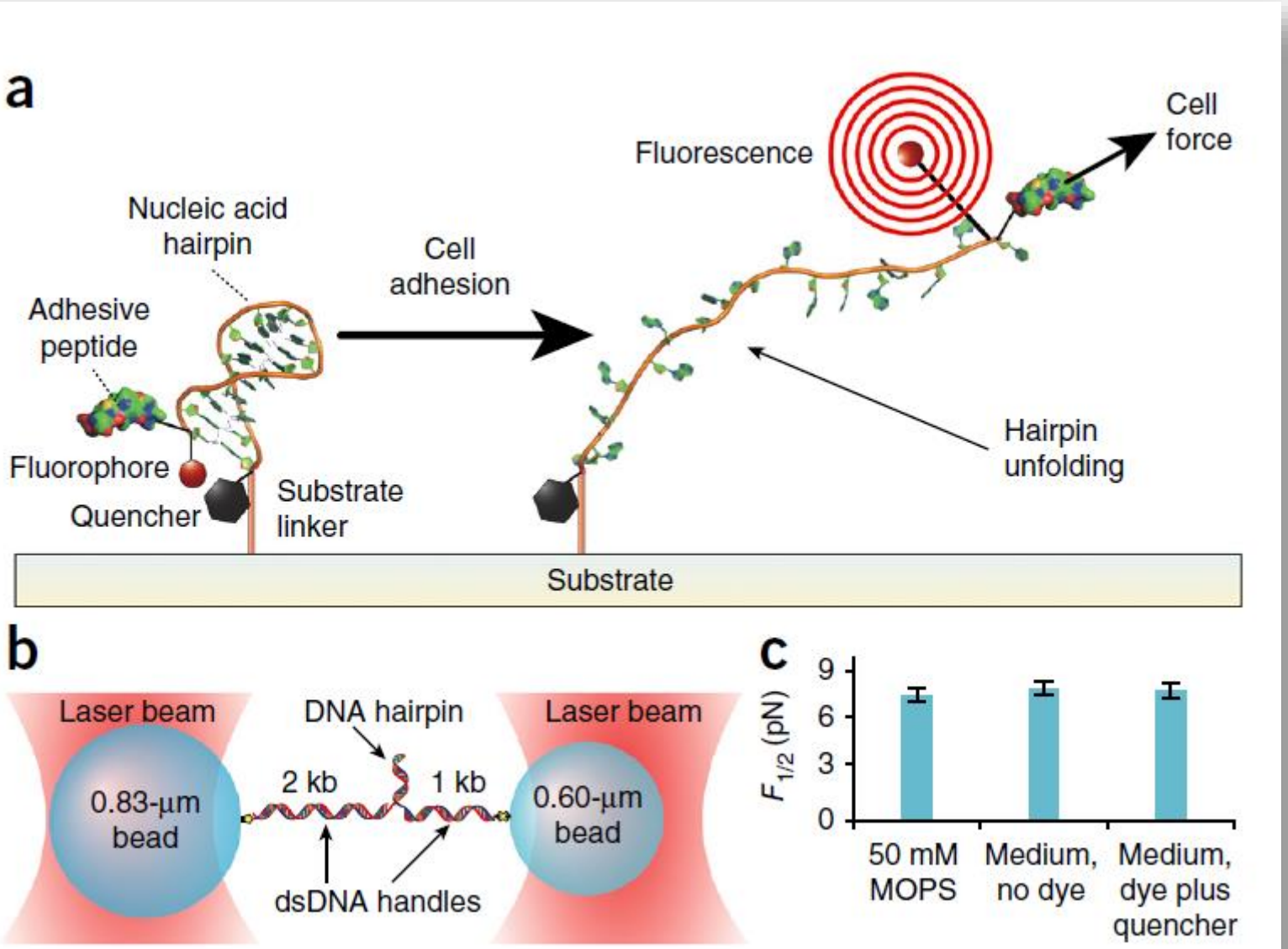
Higher rigidity leads to higer forces

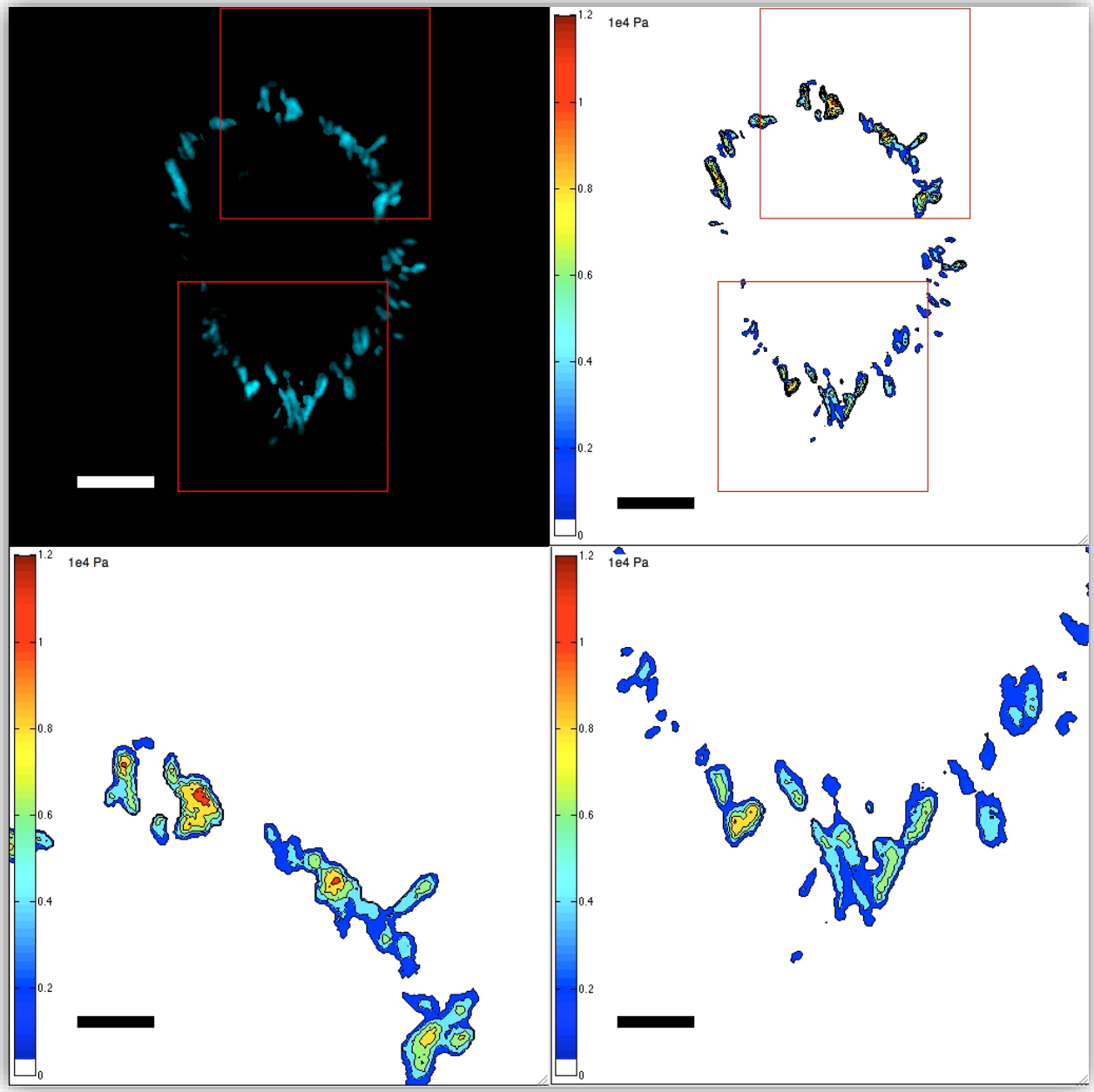




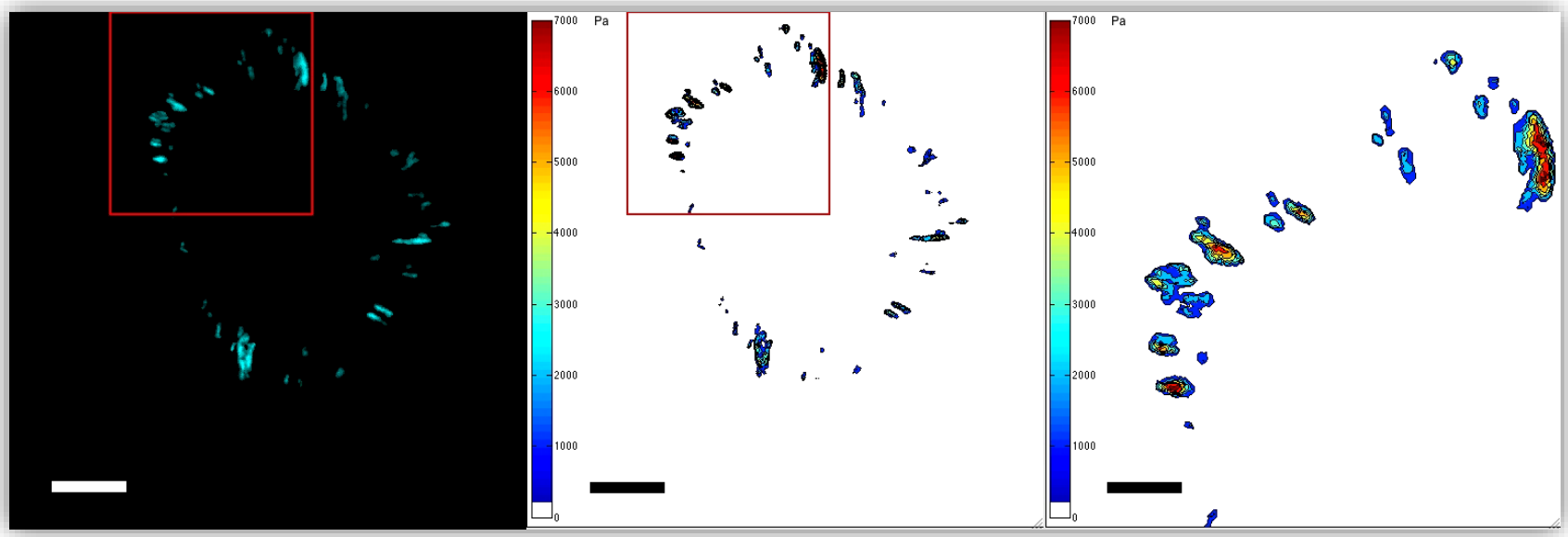
A DNA-based molecular probe for optically reporting cellular traction forces

Brandon L Blakely[1,10], Christoph E Dumelin[2,10], Britta Trappmann[3,4,10], Lynn M McGregor[5,6], Colin K Choi[3,4], Peter C Anthony[7], Van K Duesterberg[7], Brendon M Baker[3,4], Steven M Block[8,9], David R Liu[5,6] & Christopher S Chen[1,3,4]

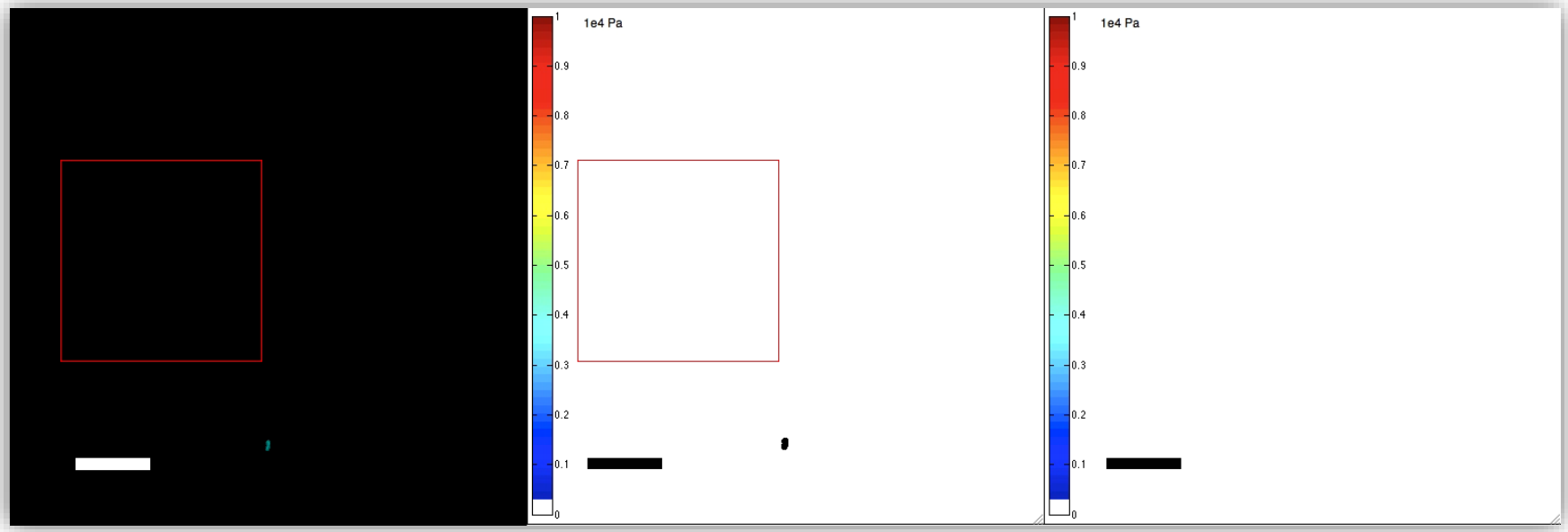

a
Fluorescence
Cell force
Nucleic acid hairpin
Cell adhesion
Adhesive peptide
Fluorophore
Quencher
Substrate linker
Hairpin unfolding
Substrate
b
Laser beam
DNA hairpin
Laser beam
0.83-µm bead
2 kb
1 kb
0.60-µm bead
dsDNA handles
c
$F_{1/2}$ (pN)
9
6
3
0
50 mM MOPS
Medium, no dye
Medium, dye plus quencher


1e4 Pa
1.2
1
0.8
0.6
0.4
0.2
0
1e4 Pa
1.2
1
0.8
0.6
0.4
0.2
0
1e4 Pa
1.2
1
0.8
0.6
0.4
0.2
0

Rock Inhibition (Loss of traction forces)



Force Increase by Lysophosphatic Acid



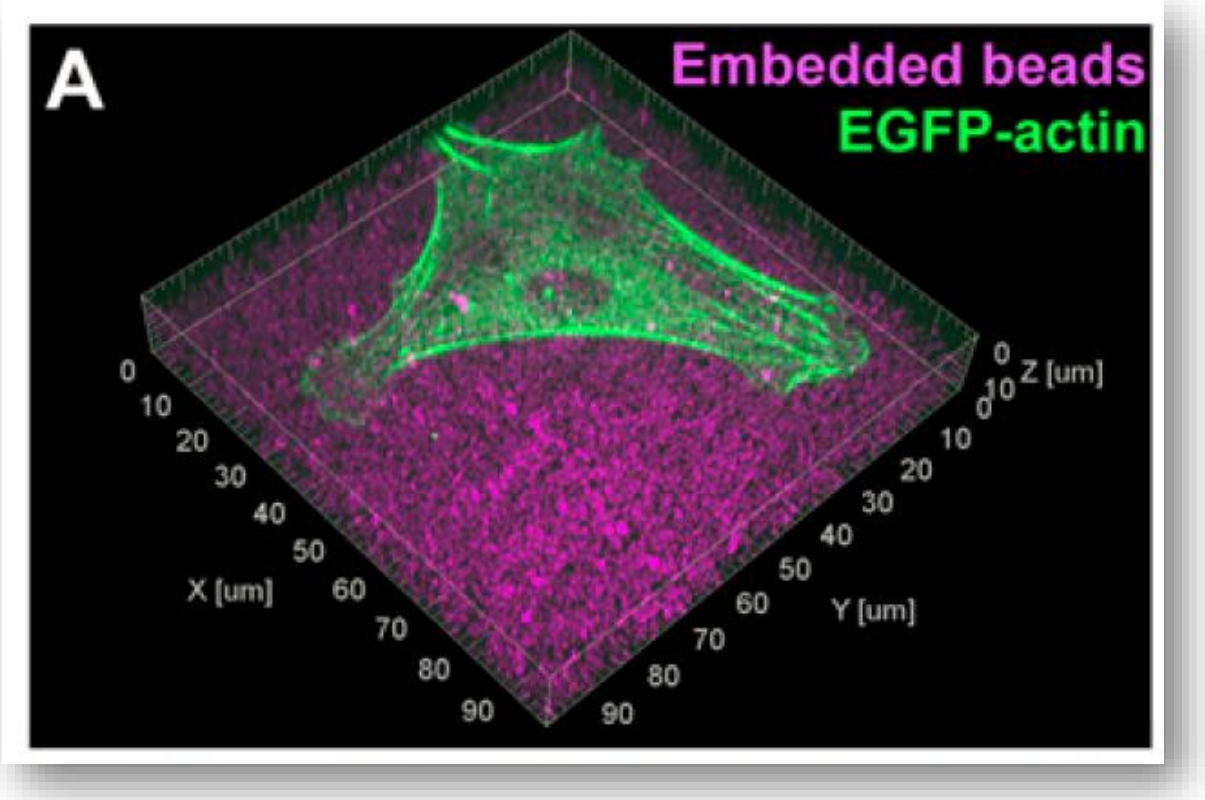
Traction Forces in 2.5 D

Multidimensional traction force microscopy reveals out-of-plane rotational moments about focal adhesions

Wesley R. Legant[a,1], Colin K. Choi[a,1], Jordan S. Miller[a], Lin Shao[b], Liang Gao[b], Eric Betzig[b], and Christopher S. Chen[a,2]

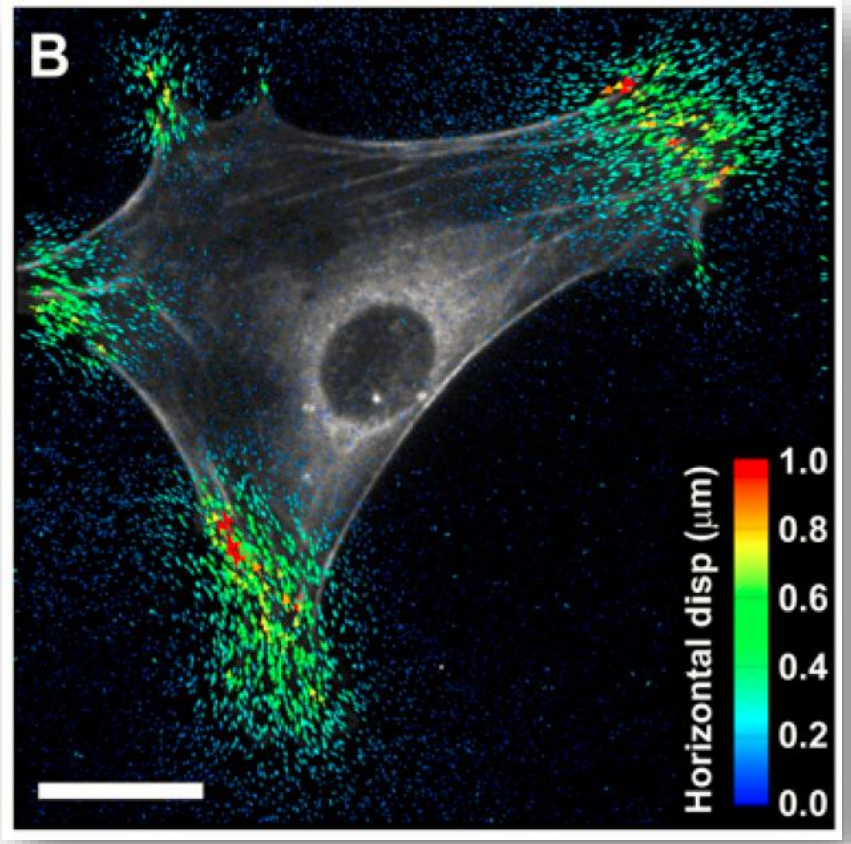
PNAS | January 15, 2013 | vol. 110 | no. 3 | 881–886

Beads embedded in slap and tracked in 3D with confocal

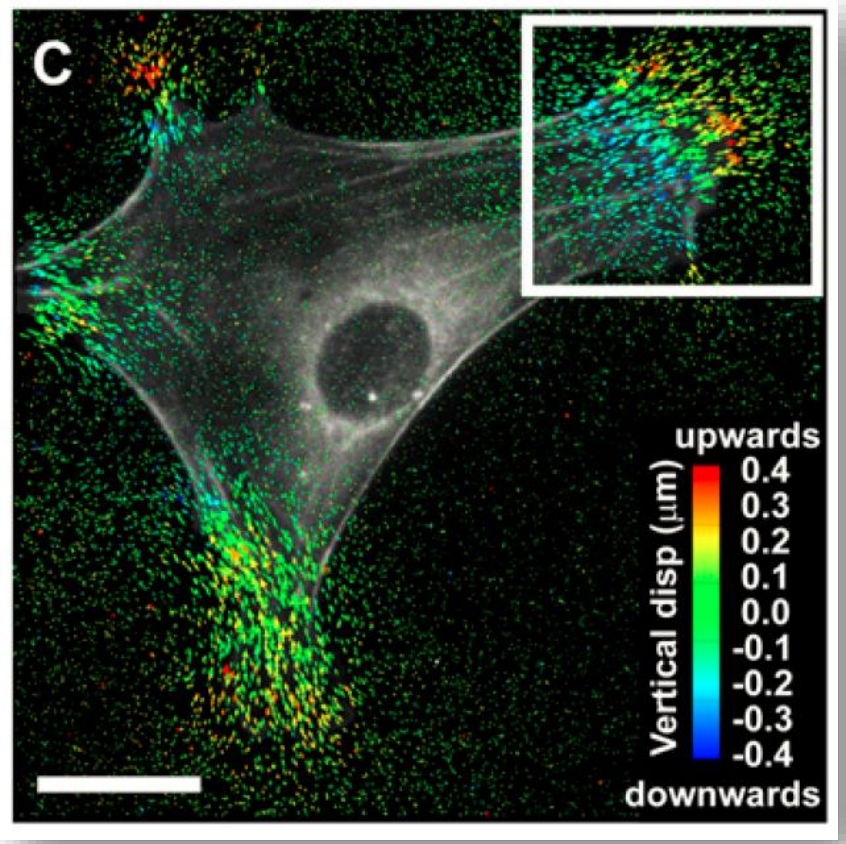


Video

Decomposing force vector in horizontal and vertical component

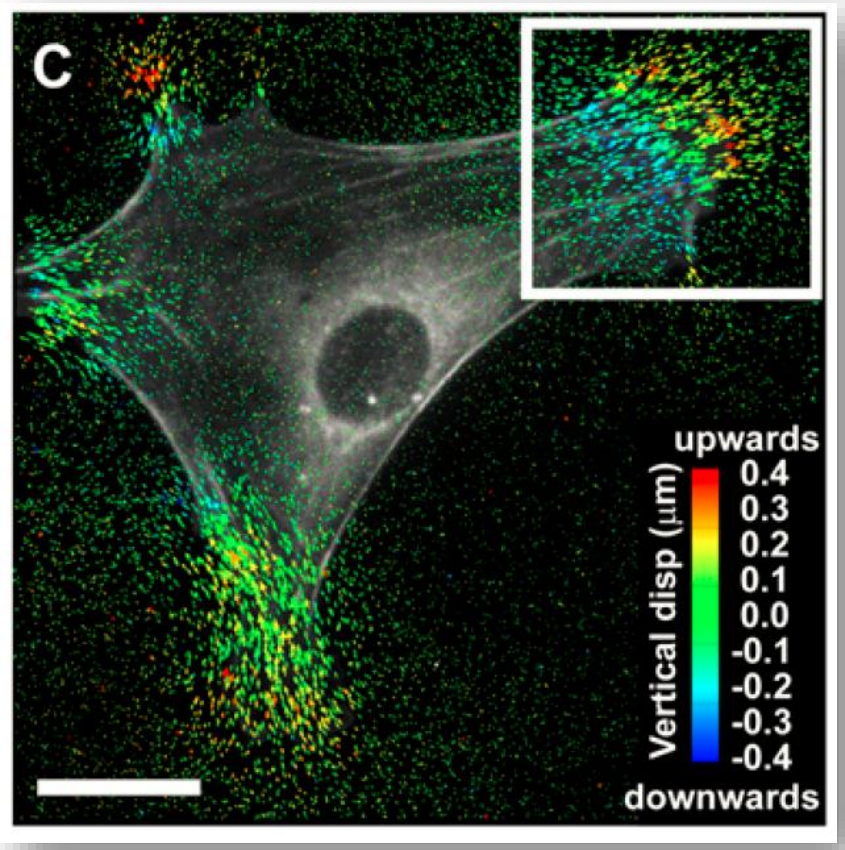


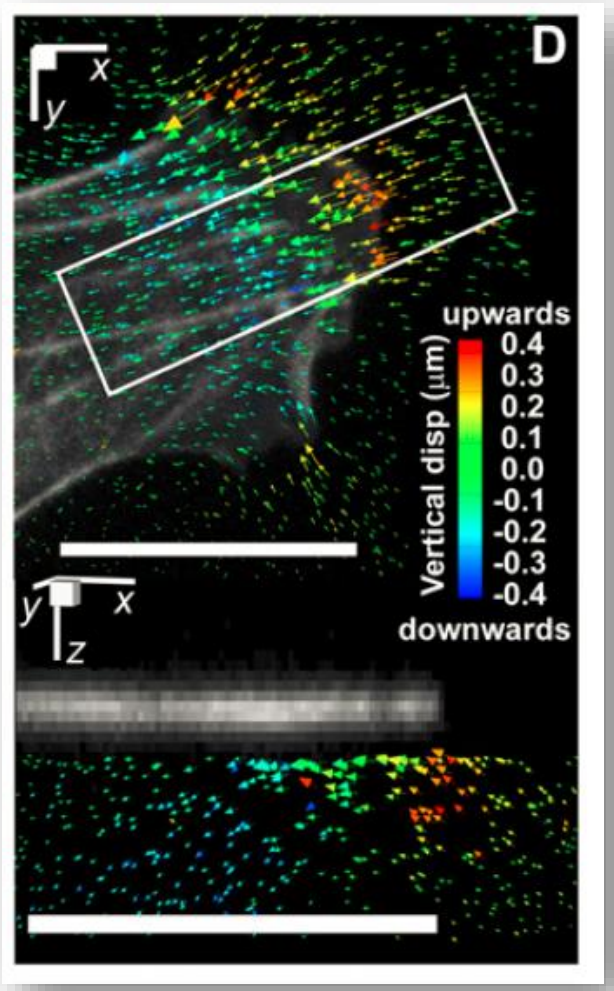
Horizontal



Vertical

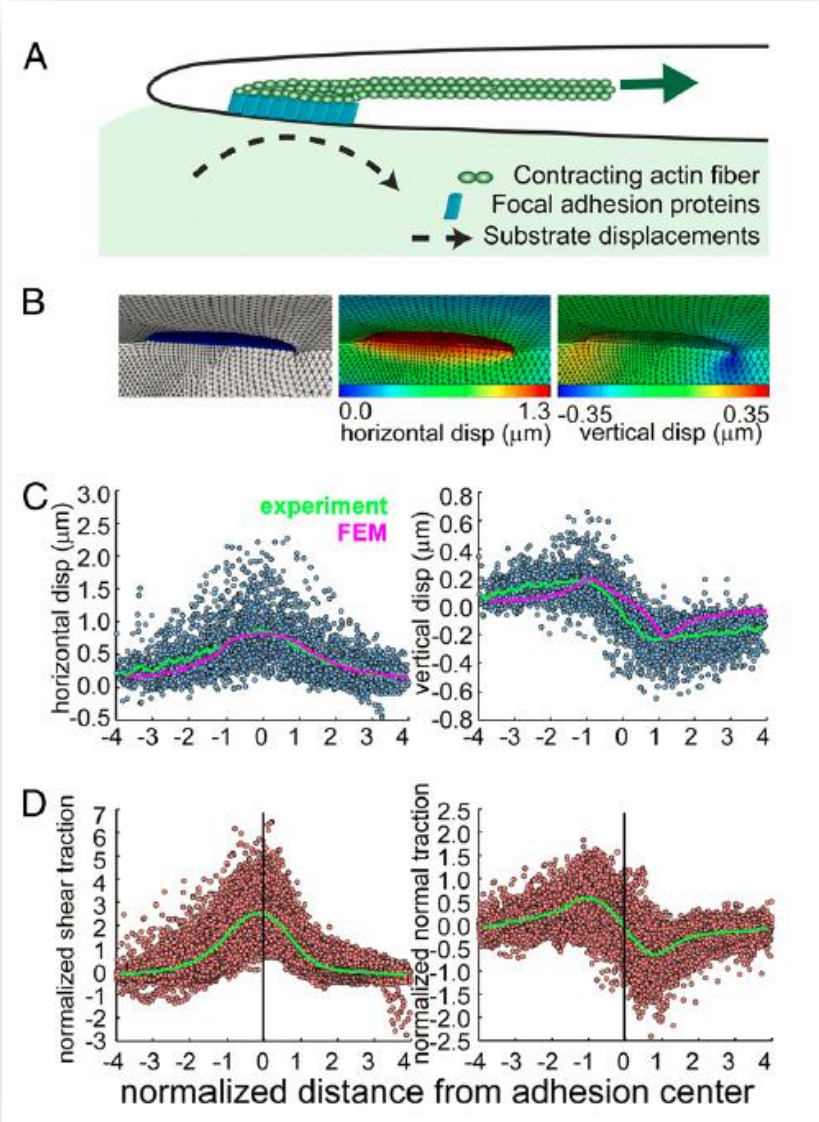
3-Dimensional bead displacement





Video

Focal adhesion lead to torque



PLOS ONE
TENTH ANNIVERSARY

OPEN ACCESS PEER-REVIEWED

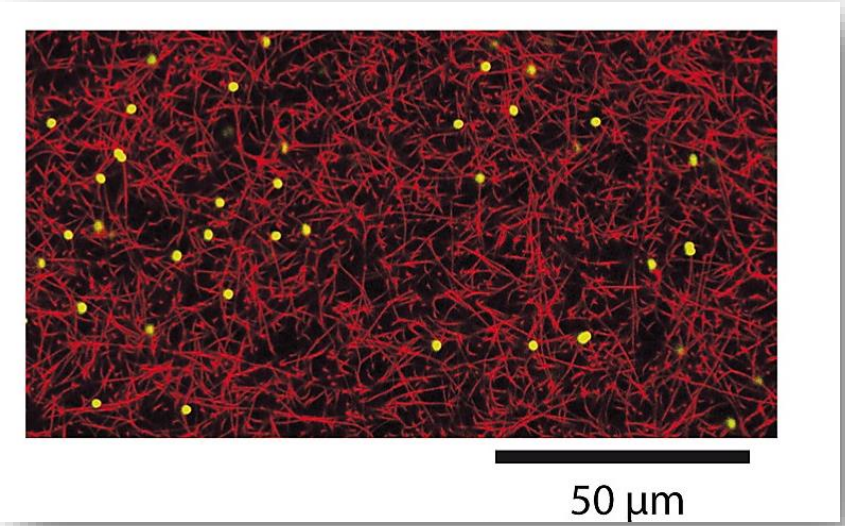
RESEARCH ARTICLE

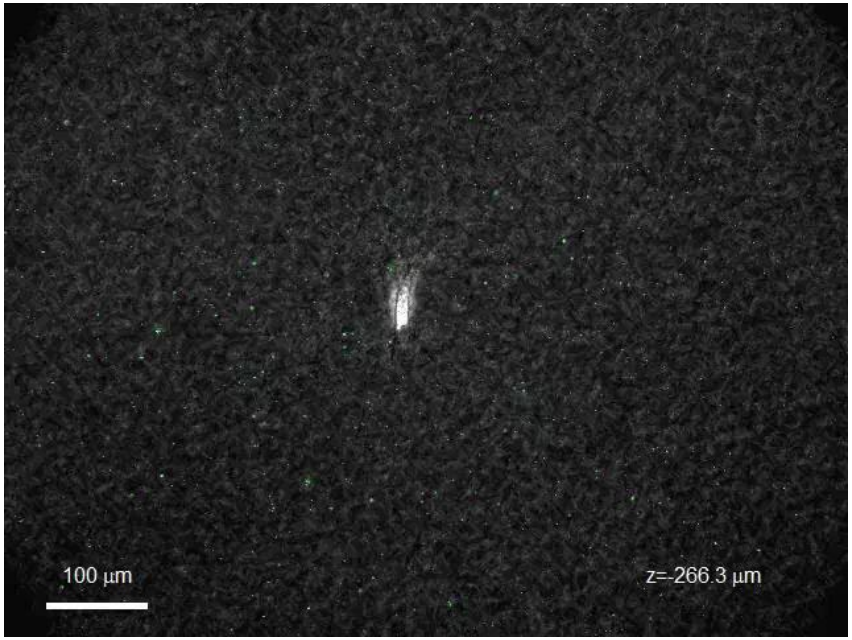
3D Traction Forces in Cancer Cell Invasion

Thorsten M. Koch, Stefan Münster, Navid Bonakdar, James P. Butler, Ben Fabry

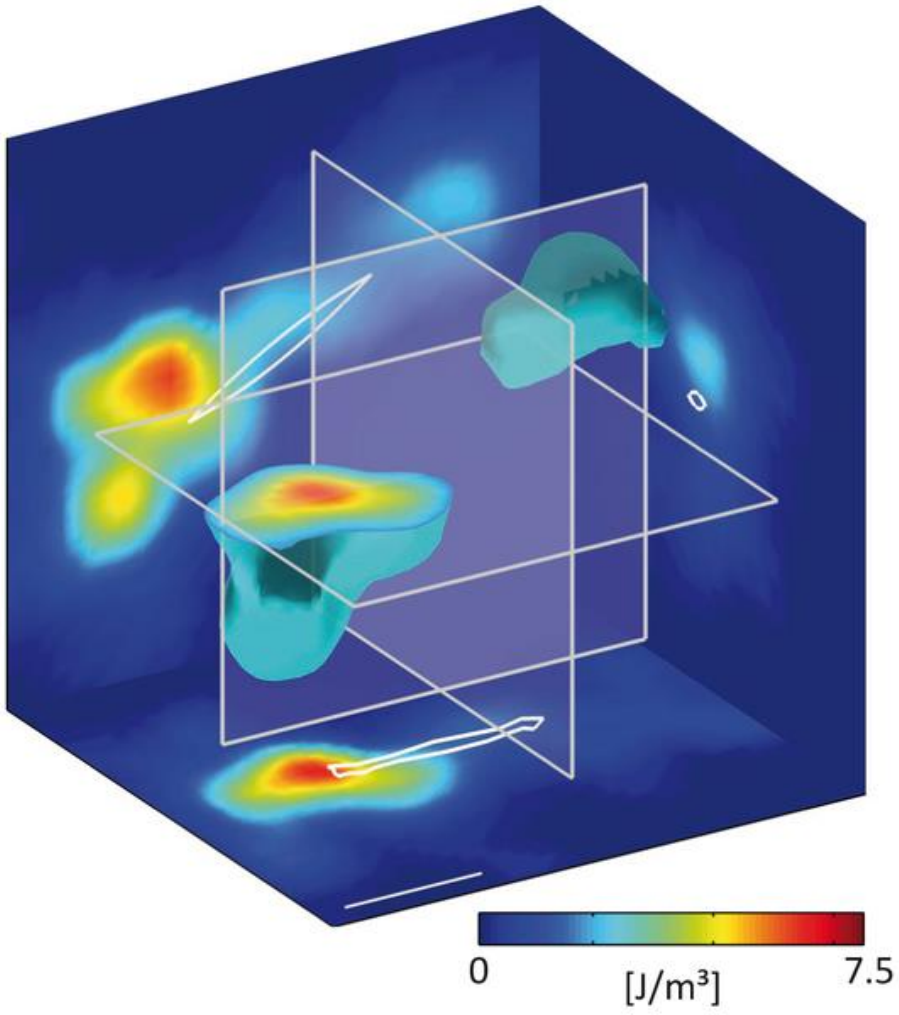
Published: March 30, 2012 • https://doi.org/10.1371/journal.pone.0033476

3D traction forces: cells embedded in fluorescently labeled collagen gel.



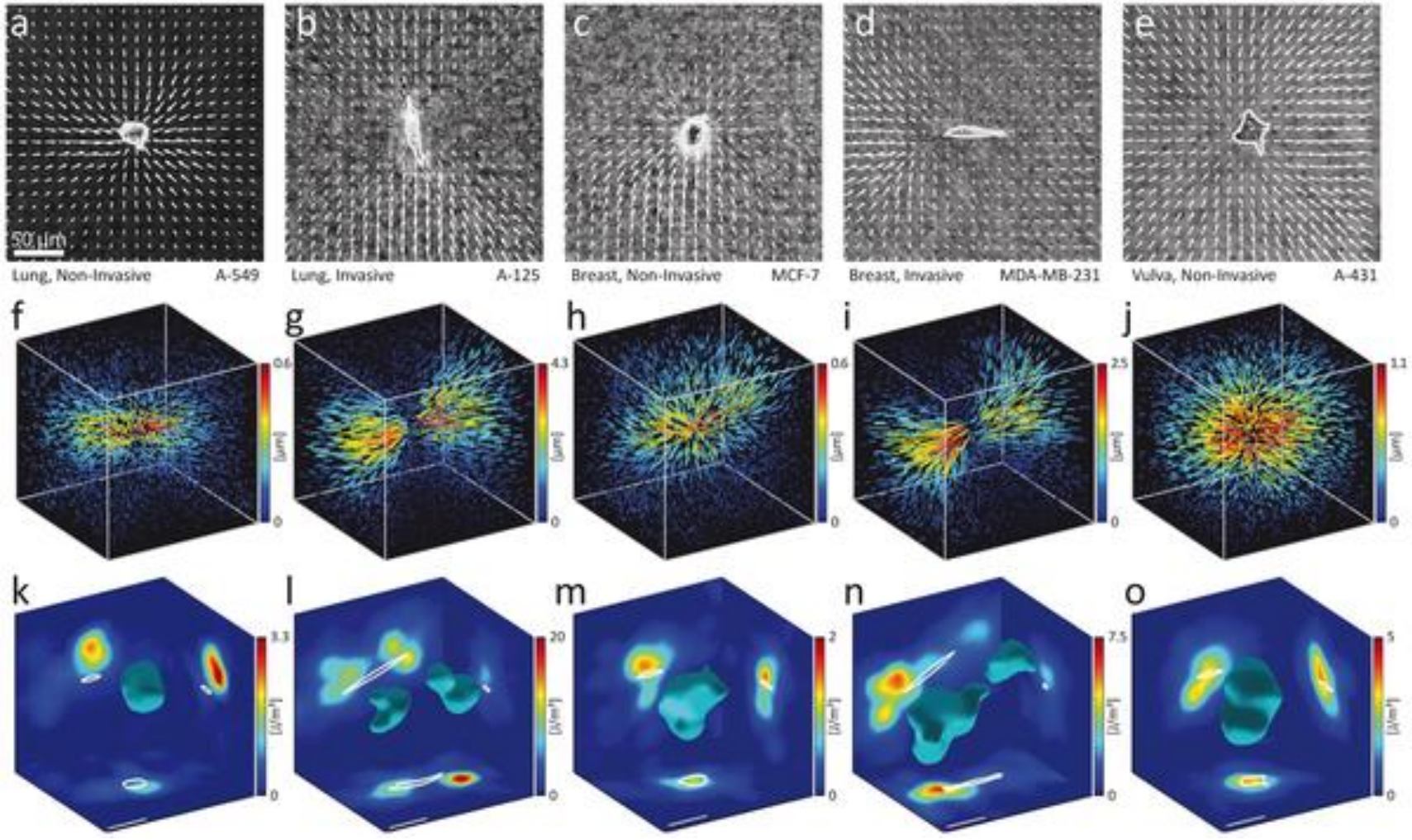


Bead displacements allow to calculate strain displacements.



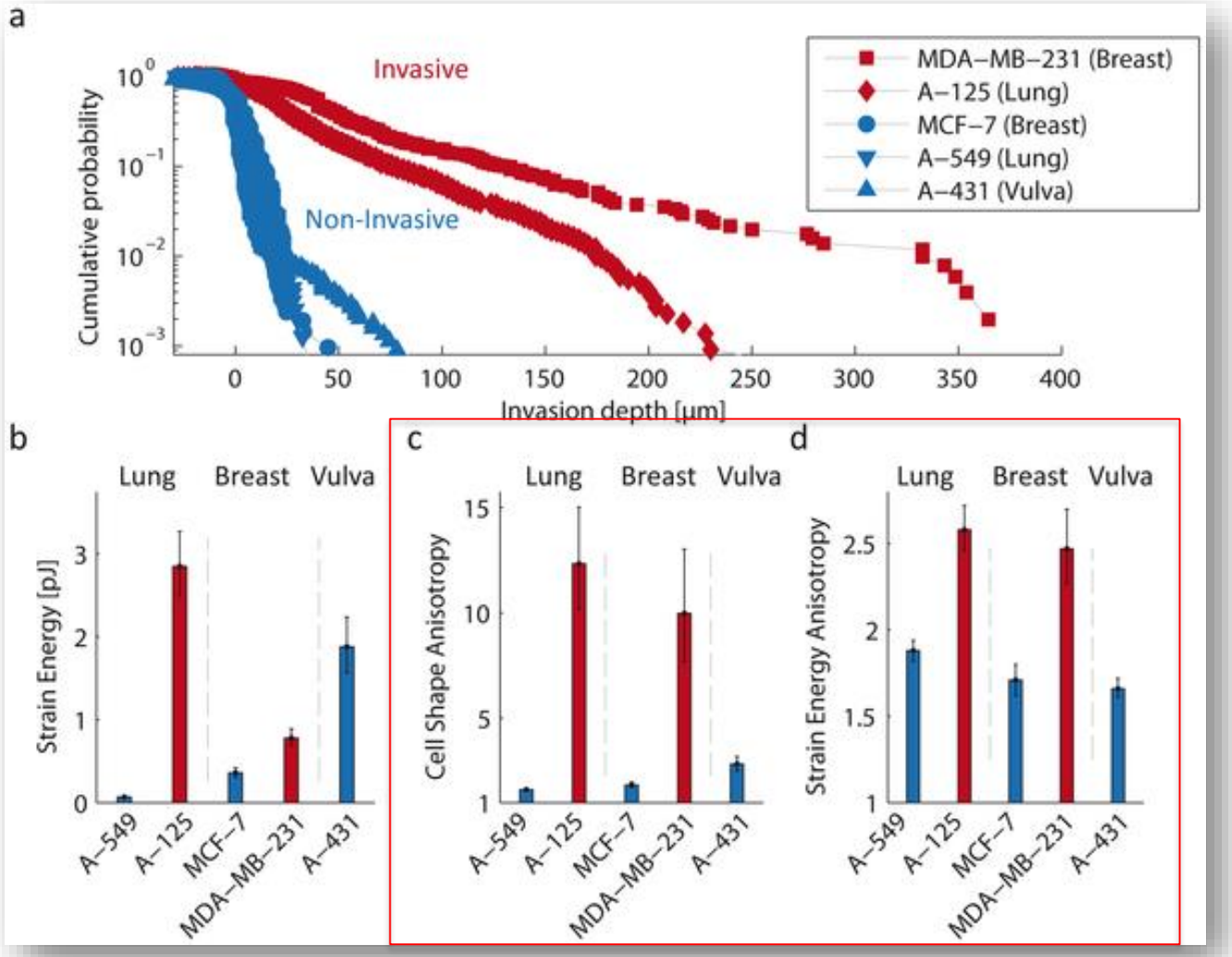
Koch TM, Münster S, Bonakdar N, Butler JP, Fabry B (2012) 3D Traction Forces in Cancer Cell Invasion. PLOS ONE 7(3): e33476. https://doi.org/10.1371/journal.pone.0033476
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0033476

Figure 3. Displacements and strain energy density around non-invasive and invasive cells.



Koch TM, Münster S, Bonakdar N, Butler JP, Fabry B (2012) 3D Traction Forces in Cancer Cell Invasion. PLOS ONE 7(3): e33476. https://doi.org/10.1371/journal.pone.0033476
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0033476

Strain energy and anisotropy of non-invasive and invasive cells.



Koch TM, Münster S, Bonakdar N, Butler JP, Fabry B (2012) 3D Traction Forces in Cancer Cell Invasion. PLOS ONE 7(3): e33476. https://doi.org/10.1371/journal.pone.0033476
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0033476