ESC7: Single molecule approaches

Atomic Force Microscopy (AFM)

Sourav Maity

rijksuniversiteit groningen

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ESC7: Single Molecule Approaches

Atomic Force Microscopy (AFM)

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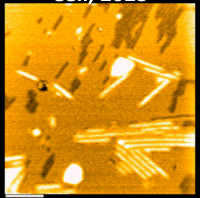
06/11/2023

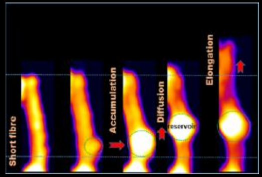
Department of Molecular Biophysics

Nature 2022
Nat. Comm, 2023,
Cell, 2023



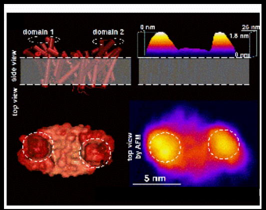
Surface active antibiotics

JACS, 2020



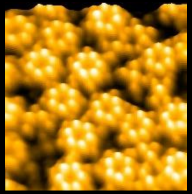
Assembly of supramolecular polymer

PNAS 2022



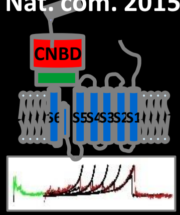
Membrane transporter

Science Advances 2021



Self-assembly of HBV

Nat. com. 2015



Membrane protein unfolding

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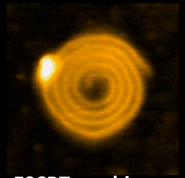
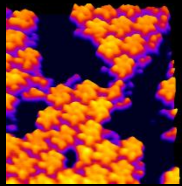
faculty of science and engineering
zernike institute for advanced materials

Nat. com. 2020



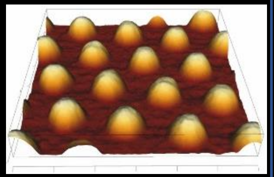
ESCRT machinery membrane remodelling

ACS Nano, 2020



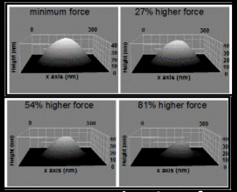
Self-assembly of HIV

Angewandte Chemie 2021



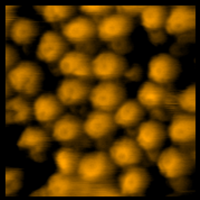
Organogel

BMC Biology, 2021



Nano-mechanics of nano-particles

Nano Letters



Protein Nanocage

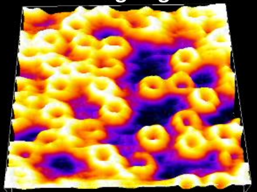
Ongoing

Virus–receptor interactions

Small. 2019

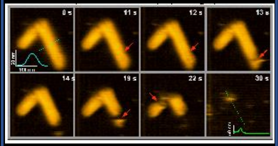
Polymer Chemistry: Structure and mechanics

Ongoing



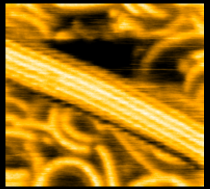
Self-assembly of nano pores

Science Advances 2019
Nat. Struct. Mol. Bio.



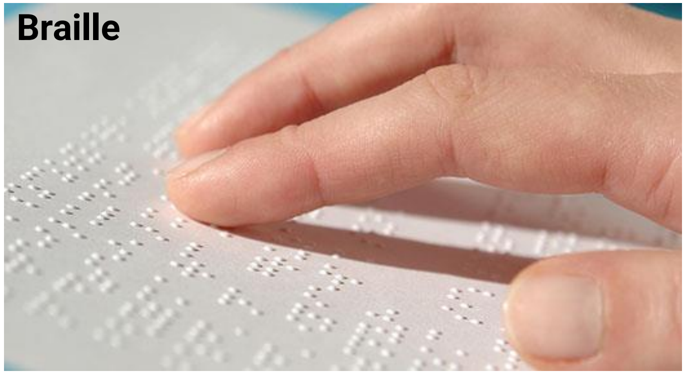
Membrane remodelling

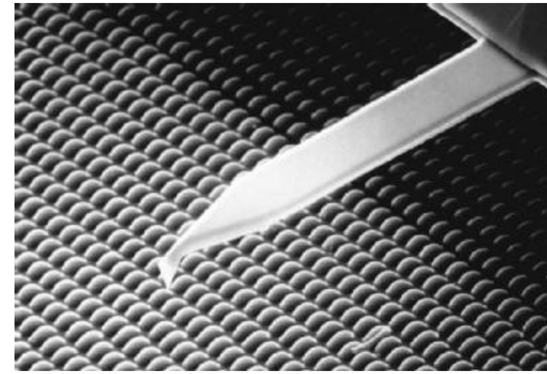
Ongoing

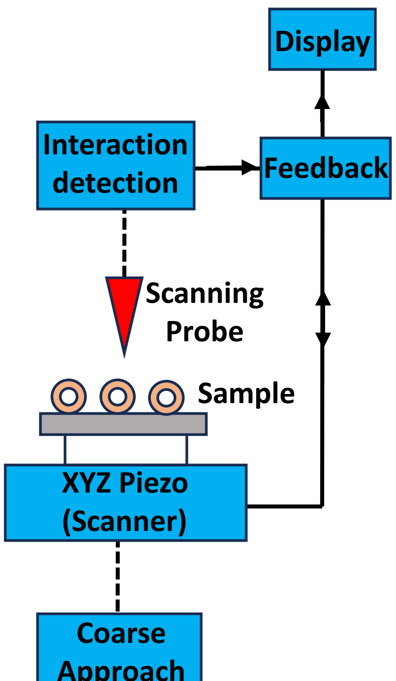


Microtubules

What is scanning probe microscope?







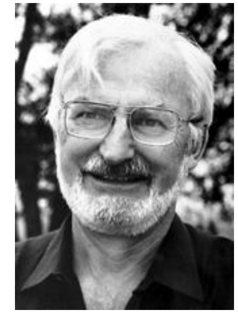
- **Some important types of scanning probe microscopy**
- AFM, atomic force microscopy
- EFM, electrostatic force microscope
- FMM, force modulation microscopy
- MFM, magnetic force microscopy
- STM, scanning tunneling microscopy
- SVM, scanning voltage microscopy
- SHPM, scanning Hall probe microscopy

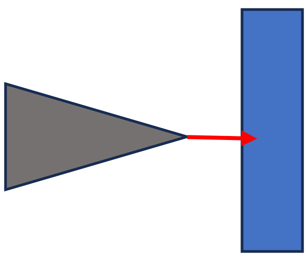
………………

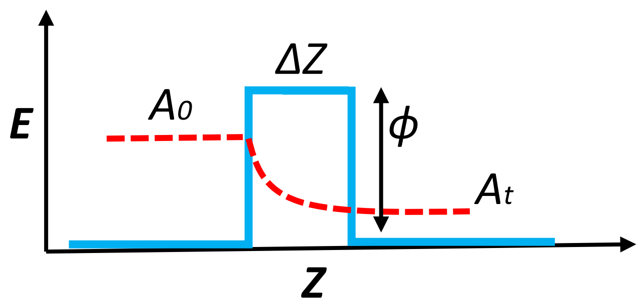
https://www.seewritehear.com/braille-reading/
https://playback.fm/record-player-turntable-buying-guide
Yongho Seo, Wonho Je; "Atomic force microscopy and spectroscopy", Rep. Prog. Phys. 71 (2008) 016101 (23pp).

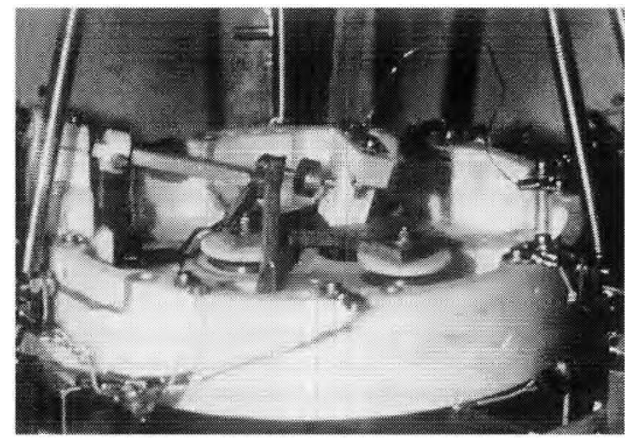
Scanning tunneling microscopy

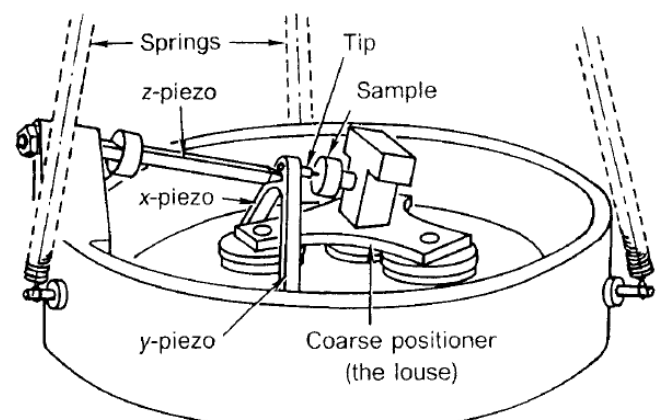
- 1981 – Swiss scientists Gerd Binnig and Heinrich Rohrer
- Atomic resolution
- 1986 – Nobel prize











https://www.slideshare.net/Atsew/atomic-force-microscopy-afm

Atomic force microscopy

university of groningen

Atomic force microscopy (AFM) was developed when people tried to extend STM technique to investigate the electrically non-conductive materials, like proteins.

In 1986, Binnig and Quate demonstrated for the first time the ideas of AFM, which used an ultra-small probe tip at the end of a cantilever (*Phys. Rev. Letters,* 1986, Vol. 56, p 930).

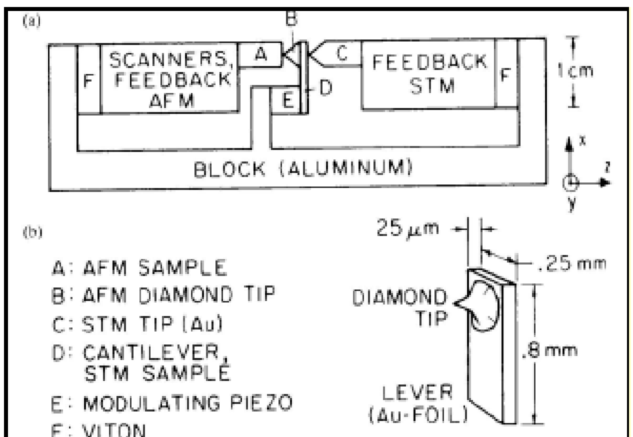


Fig. 2.3. Experimental set-up for the first AFM. The cantilever is not to scale in (*a*). Its dimensions are given in (*b*). The STM and AFM piezoelectric drives face each other, sandwiching the diamond tip that is glued to the cantilever (Binnig *et al.*, 1986c).

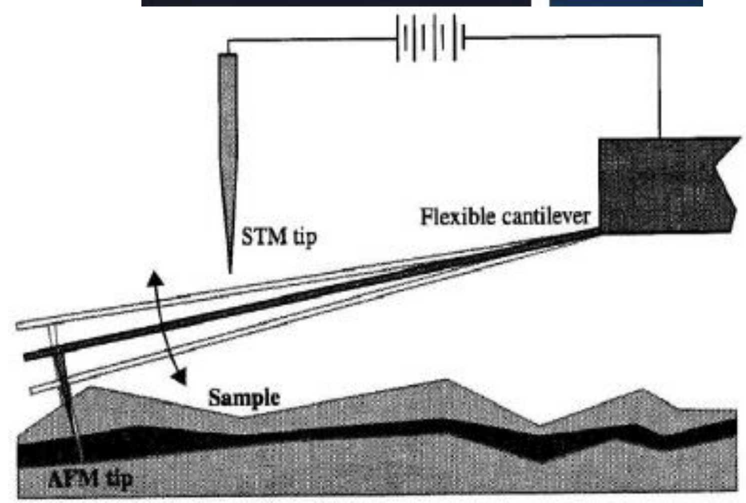
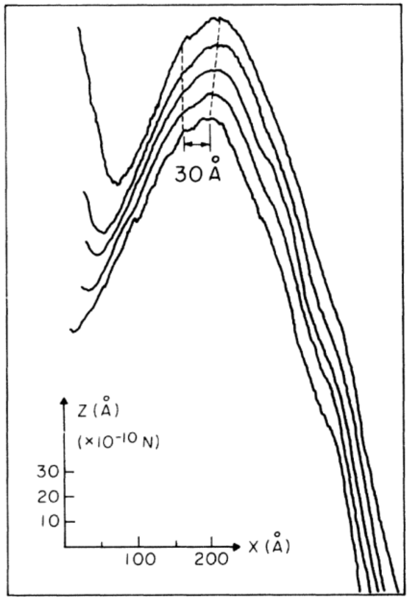
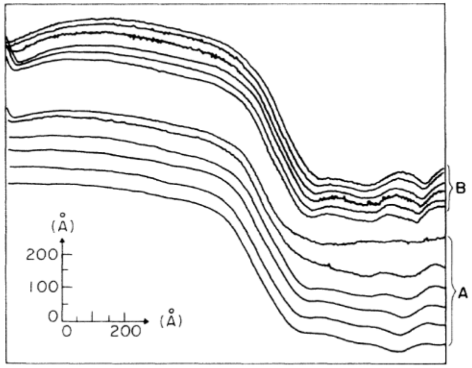


Figure 2.12. Early contact AFM which allowed imaging non-conductive samples. In this scheme, a contact AFM tip was monitored using the STM tip directly above it.

Phys. Rev. Letters, 1986, Vol. 56, p 930

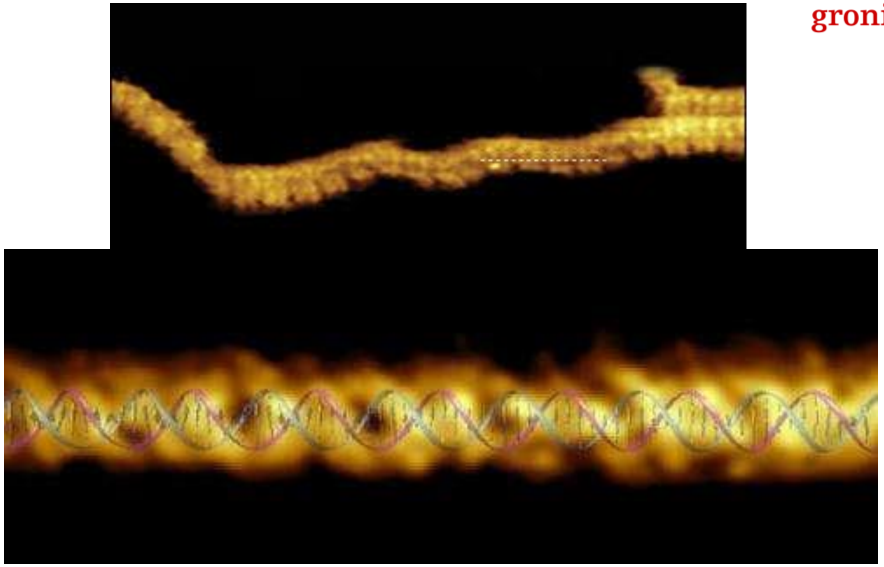
The past & the present





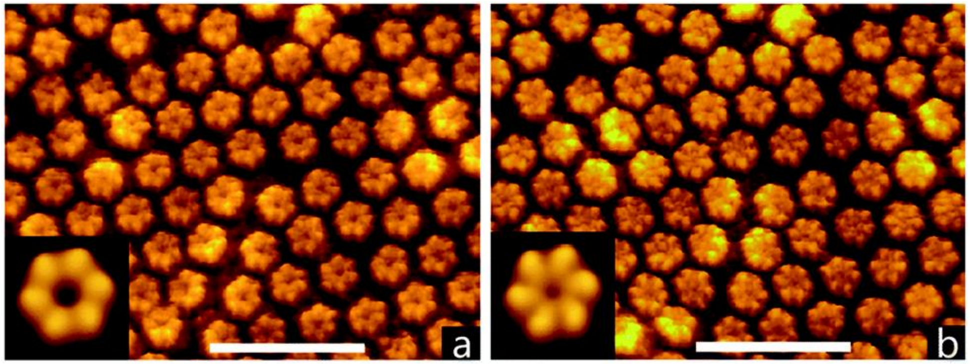
The first topographic image of a ceramic (Al_2O_3) surface Binnig and Quate (1986).

Phys. Rev. Letters, 1986, Vol. 56, p 930



DNA Helical structure

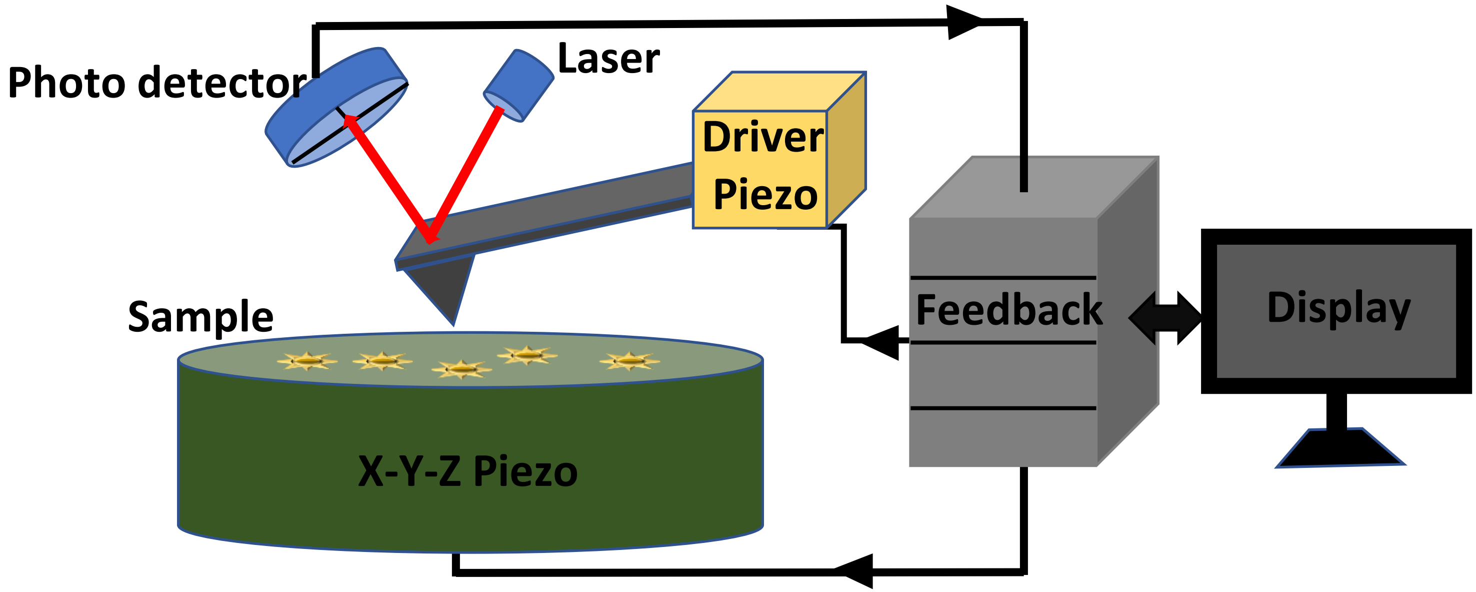
(Pyne, A. et al. Small 2014)



Membrane protein structure

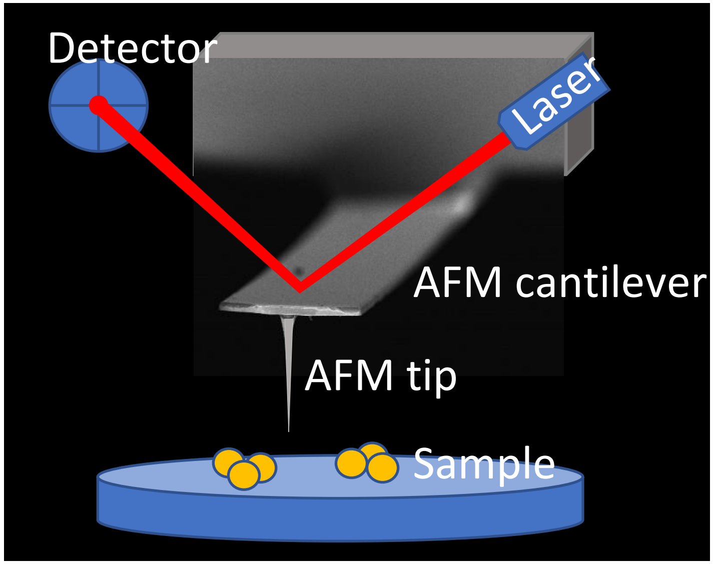
(D. J. Muller et al. *EMBO J* 2002)

How the AFM works?

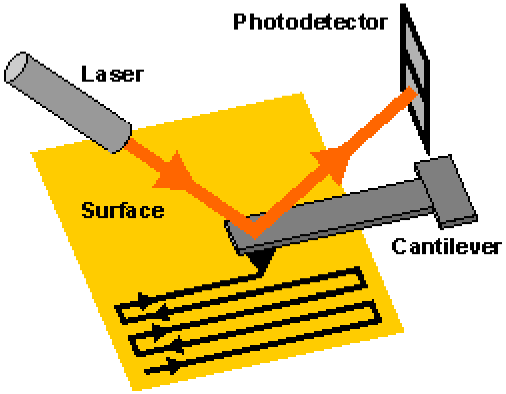


Generating an Image

Scanning Tip



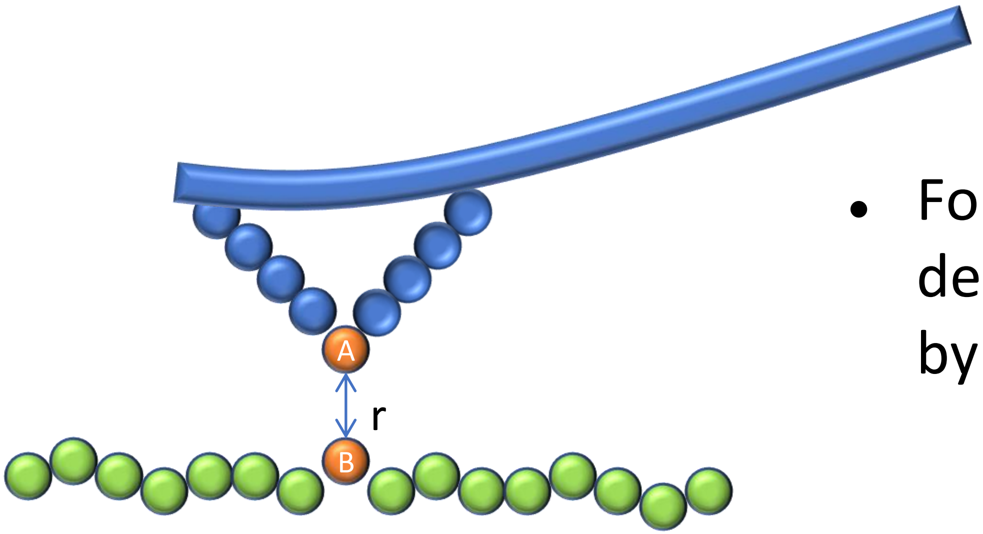
Raster Motion



- The tip passes back and forth in a straight line across the sample (think old typewriter or CRT)
- In the typical imaging mode, the tip-sample force is held constant by adjusting the vertical position of the tip (feedback).
- A topographic image is built up by the computer by recording the vertical position as the tip is rastered across the sample.

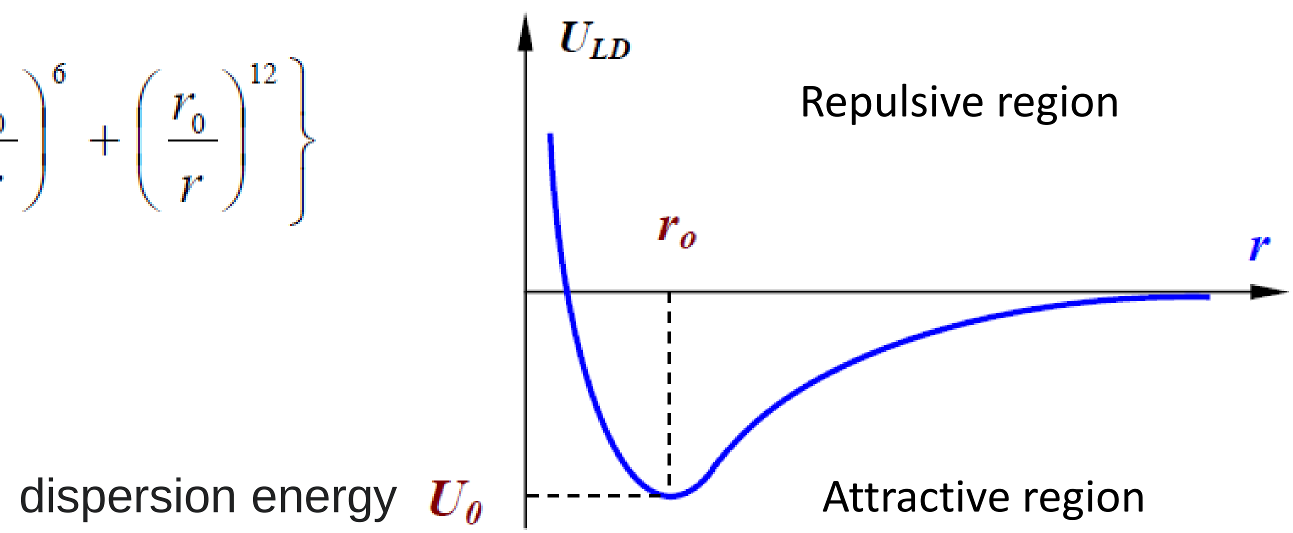
Bottom Image Courtesy of Stefanie Roes
(www.fz-borstel.de/biophysik/ de/methods/afm.html)

How the AFM works?



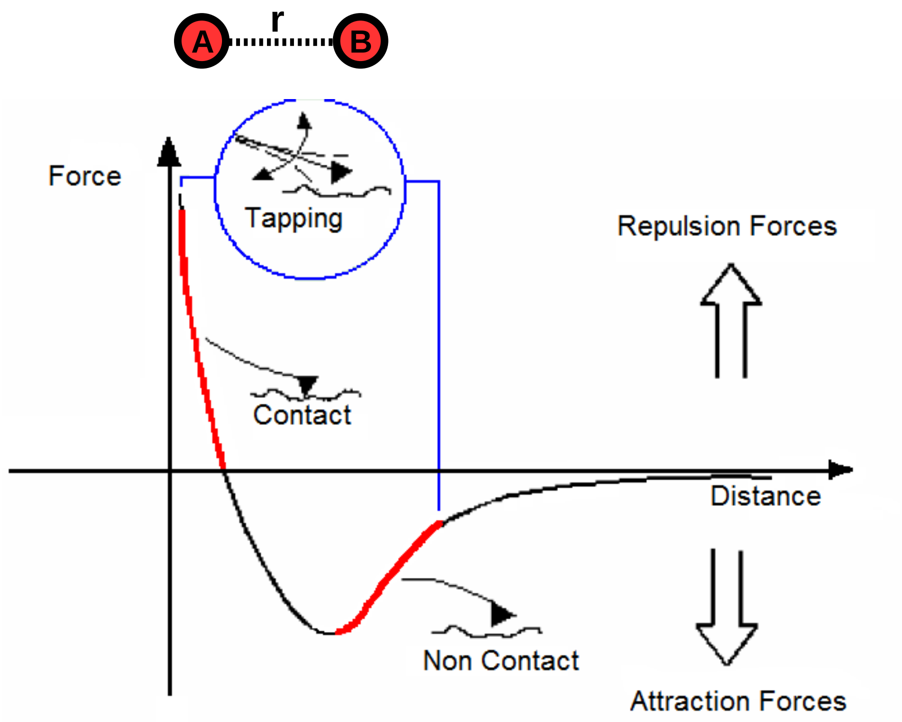
- Forces can be explained by e.g. van der Waals forces – approximated by Lennard-Jones potential

$$U_{LD}(r) = U_0 \left\{ -2\left(\frac{r_0}{r}\right)^6 + \left(\frac{r_0}{r}\right)^{12} \right\}$$



Imaging

Lennard-Jones potential



- Contact Mode
- Non-contact mode
- Intermittent contact mode (tapping mode)
- Force spectroscopy mode (jumping mode, QI mode, peak force etc.)

Cidade et al., 2003; https://www.intechopen.com/chapters/40431

Imaging: contact mode

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At very small tip-sample distances (a few angstroms) a very strong repulsive force appears between the tip and sample atoms. Its origin is the so-called **exchange interactions** due to the overlap of the electronic orbitals at atomic distances. When this repulsive force is predominant, the tip and sample are considered to be in “**contact**”.

Two ways - 'constant force' ……. feedback system moves tip in z direction to keep force Constant

'constant height'……. no feedback system - usually used when surface roughness small - higher scan speeds possible

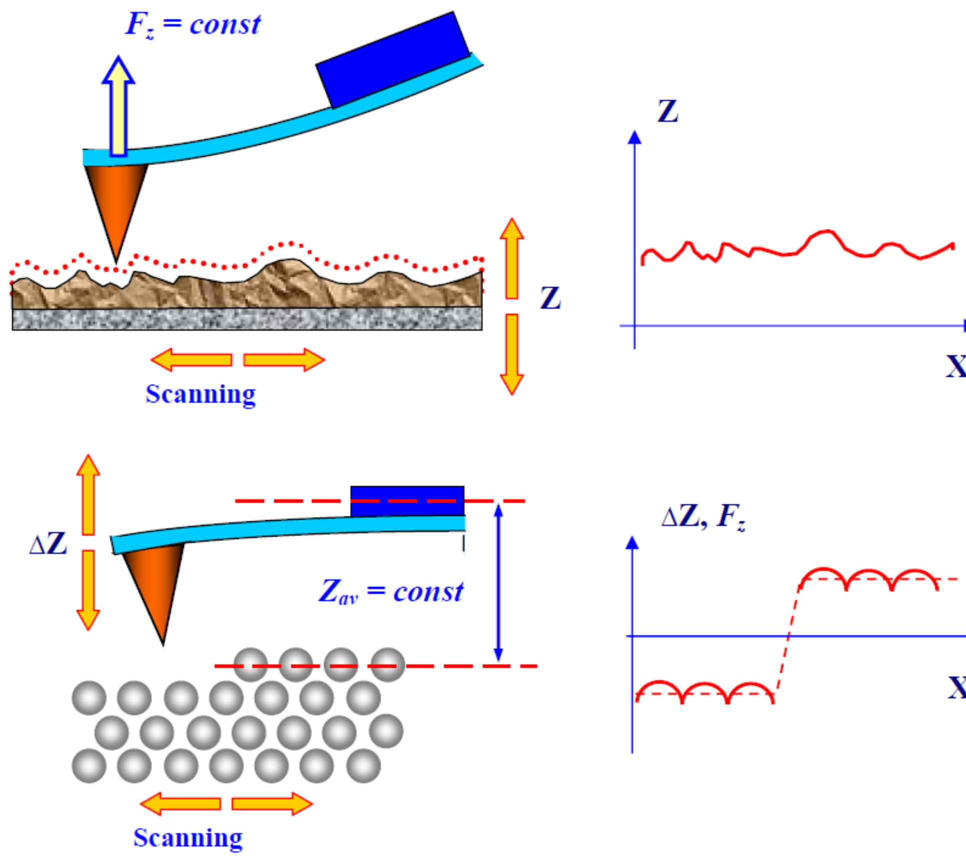
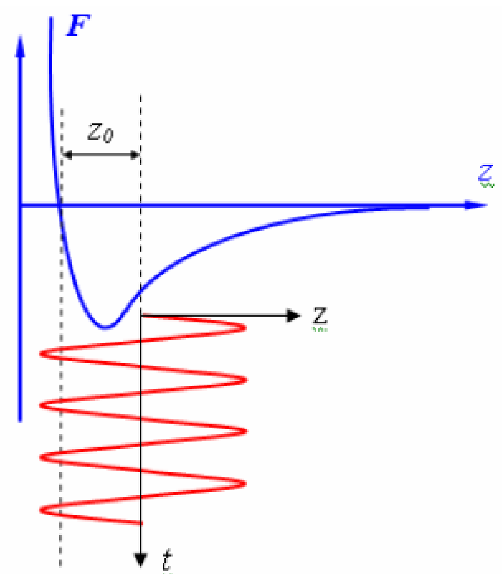


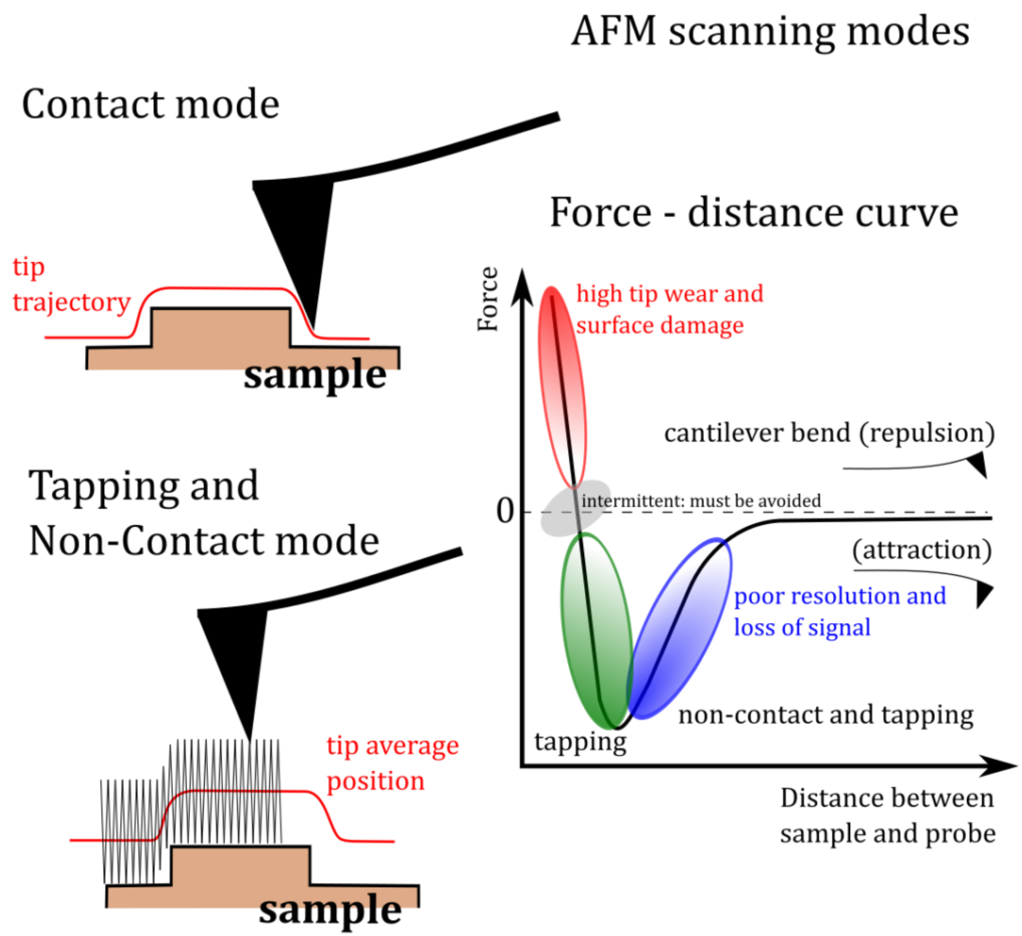
Image by Victor Mironov (2014)

Tapping mode

Attraction (Van der Waals, electrostatic, dipole-diploe):
A polarization interaction between atoms: An instantaneous polarization of an atom induces a polarization in nearby atoms – and therefore an attractive interaction.

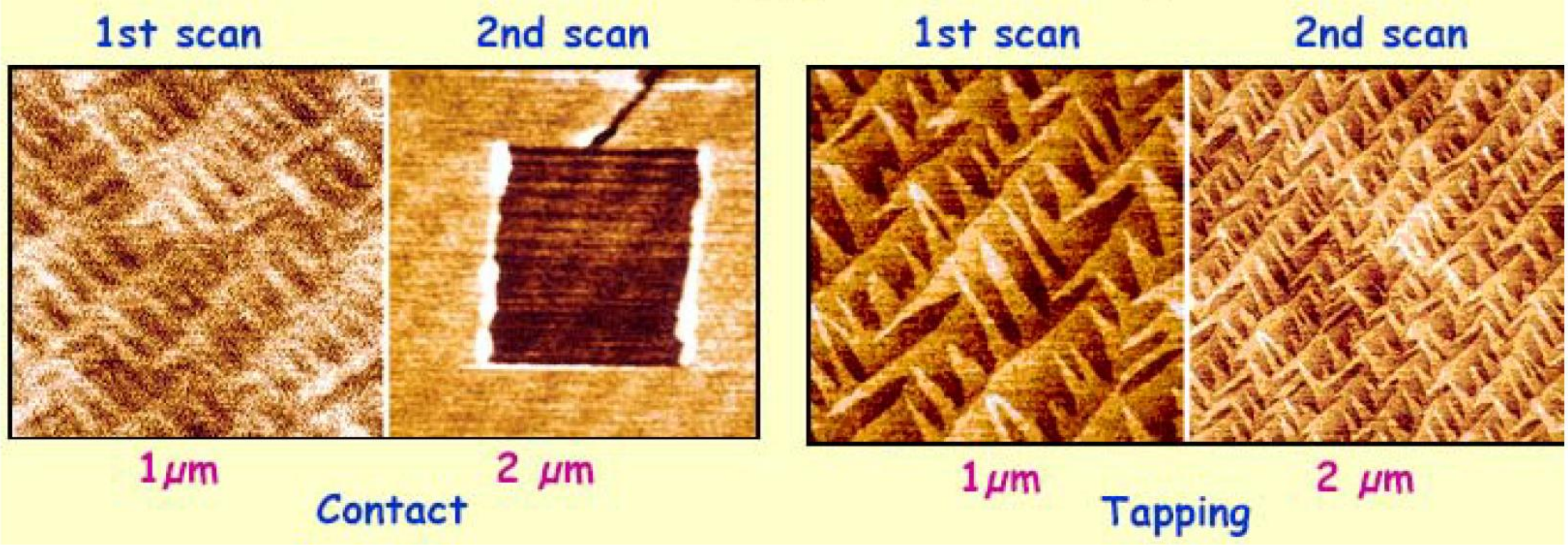


Cantilever oscillation



https://www.maxiv.lu.se/beamlines-accelerators/support-labs/microscopy-labs/atomic-force-microscope/afm-scanning-modes/

Contact mode Vs Tapping mode -Si (100)

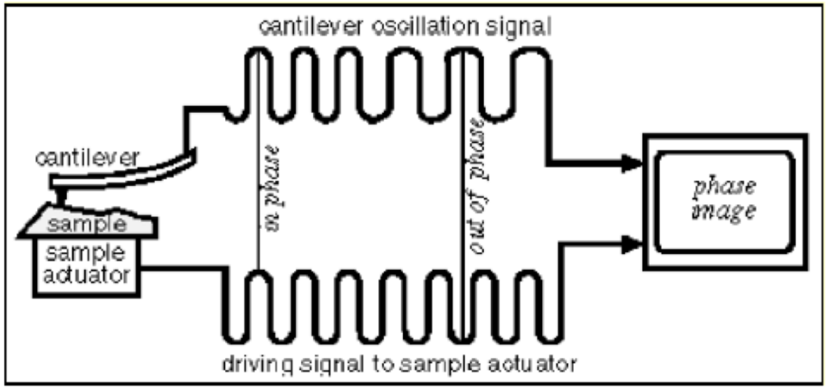


- **Contact mode** imaging works best for relatively hard and flat surface
- **Tapping mode** imaging is best suitable for all kind of sample and in air or in liquid. The resolution comes often the best. Tip contamination should be taken into consideration.

https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

Alternative imaging modes

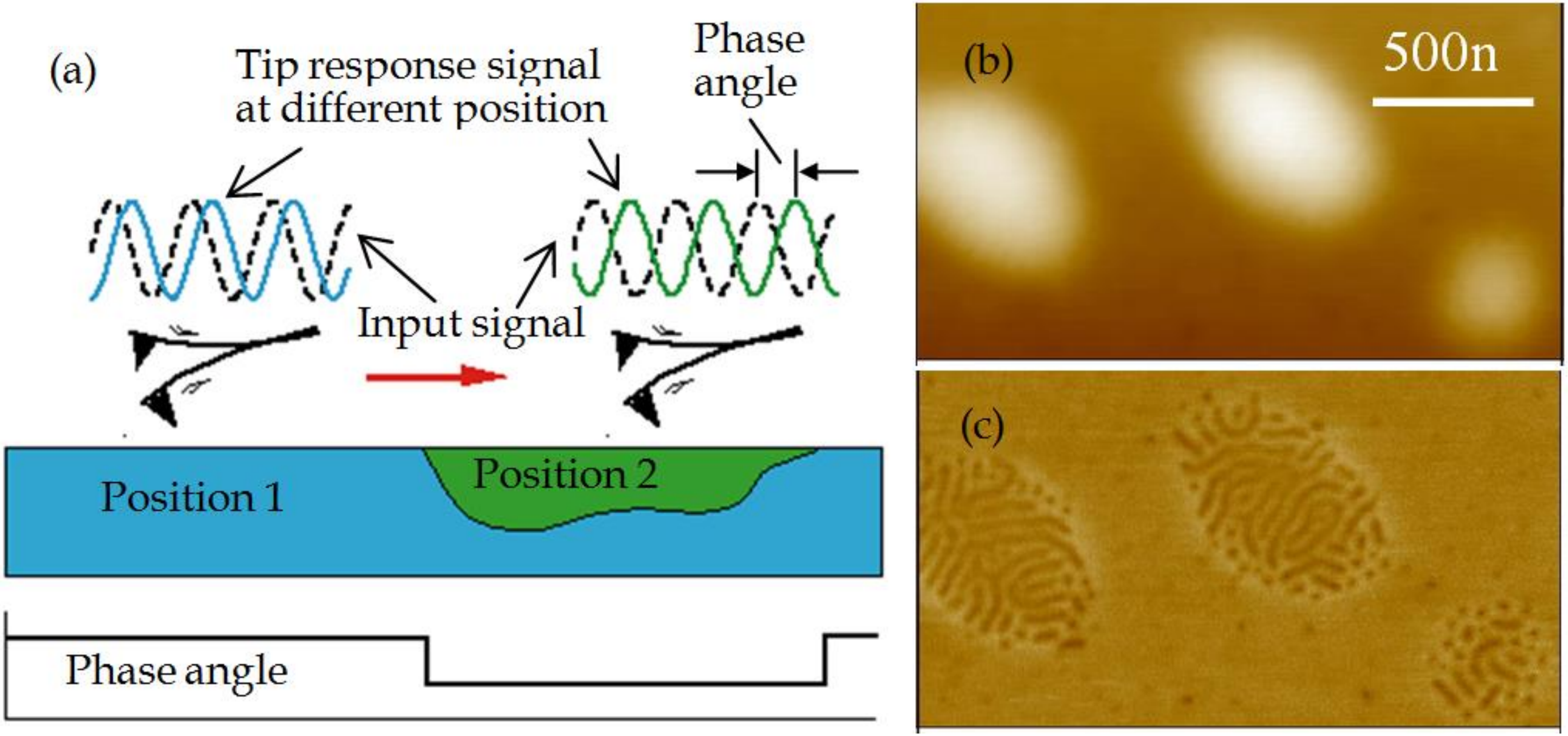
Phase imaging- Tapping mode



- Phase imaging monitors the phase lag between the signal that drives the cantilever to oscillate and the cantilever oscillation output signal. In Tapping-Mode AFM, the cantilever is excited into resonance oscillation with a piezoelectric driver.
- Phase imaging is used to map variations in surface properties such as elasticity, adhesion and friction, which all may cause the phase lag.
- Phase detection images can be produced while an instrument is operating in any vibrating cantilever mode, such as tapping mode AFM, MFM, EFM.
- The phase lag is monitored while the topographic image is being taken so that images of topography and material properties can be collected simultaneously ---- direct correlation between surface properties and topographies.

https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

Example- Phase imaging



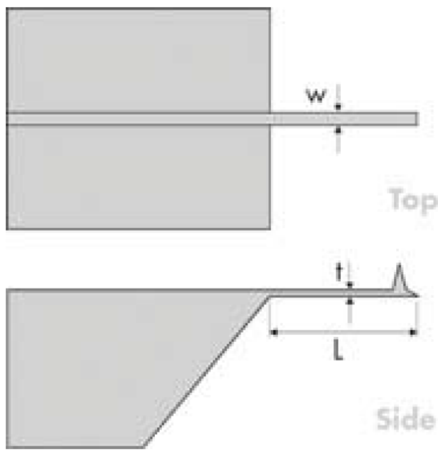
a) Phase angle in TM-AFM (b) topography and (c) phase images of copolymer. The height scale is 10nm and the phase angle scale is 20º .

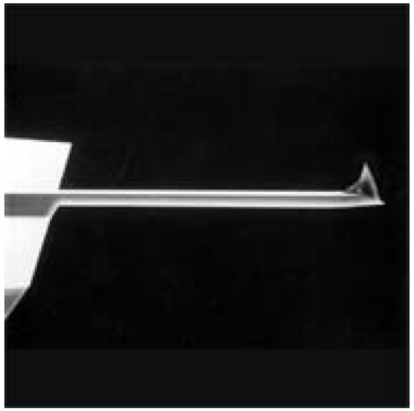
Fang et al., Measurement Science and Technology, 19 5, 0957-0233 (2008).

Effect of cantilever and tip on AFM scanning (limitation)

AFM cantilever

university of groningen





Common commercial cantilever: Si_3N_4 and SiO_2

Resonance frequency of the cantilever,

$$f_0 = \frac{1}{2\pi}\left(\frac{k}{m_0}\right)^{0.5} \qquad k = \frac{Ewt^3}{4l^3}$$

k: the spring constant, *E: Young module; t: thickness; l: length; w: width, m_0* the effective mass of the lever.

The softer the lever (smaller *k*), the better for sensing the deflection, but requires smaller mass to keep the high frequency. Why high f needed?

https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

Effect of cantilever

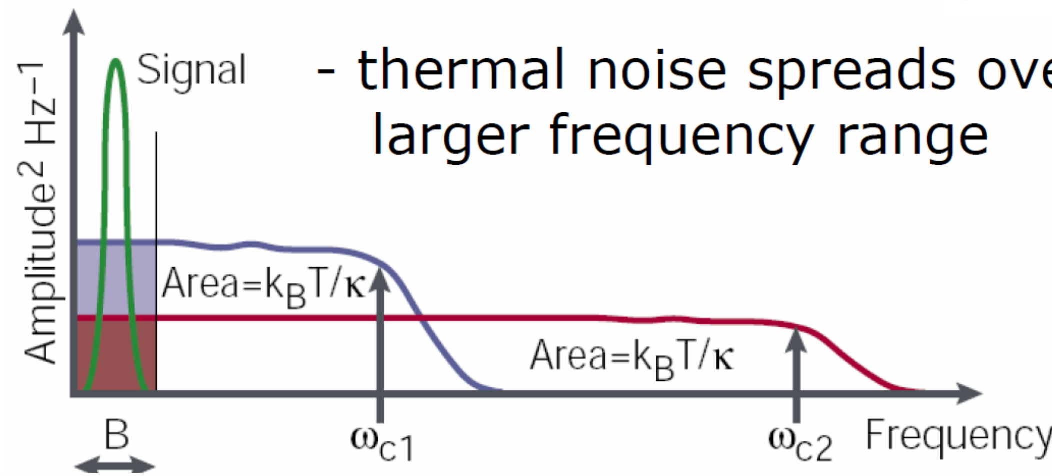
- Thermal noise:

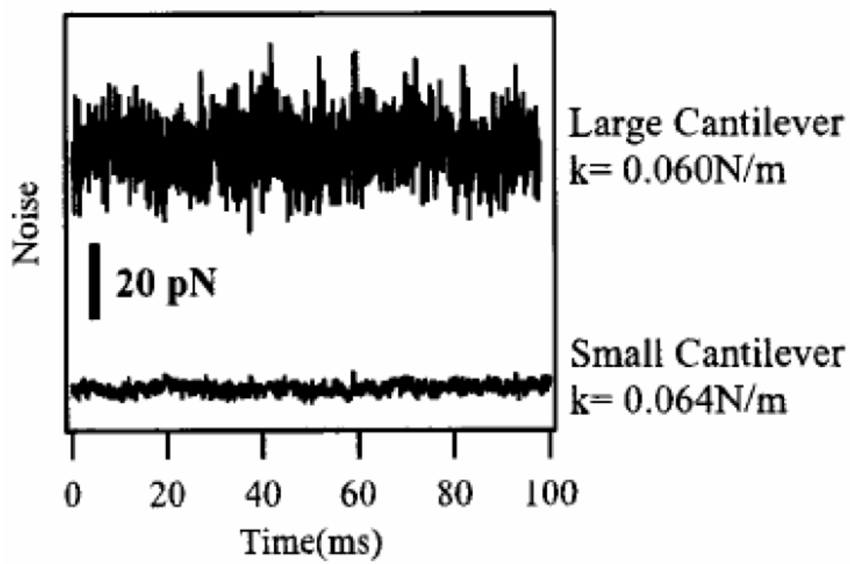
Equipartition theorem

$$k_{spring} \cdot \langle \Delta x^2 \rangle = k_B T$$

$$k_{small} = k_{large}$$

- thermal noise spreads over larger frequency range





small cantilever:
- better signal-to-noise
- faster measurements

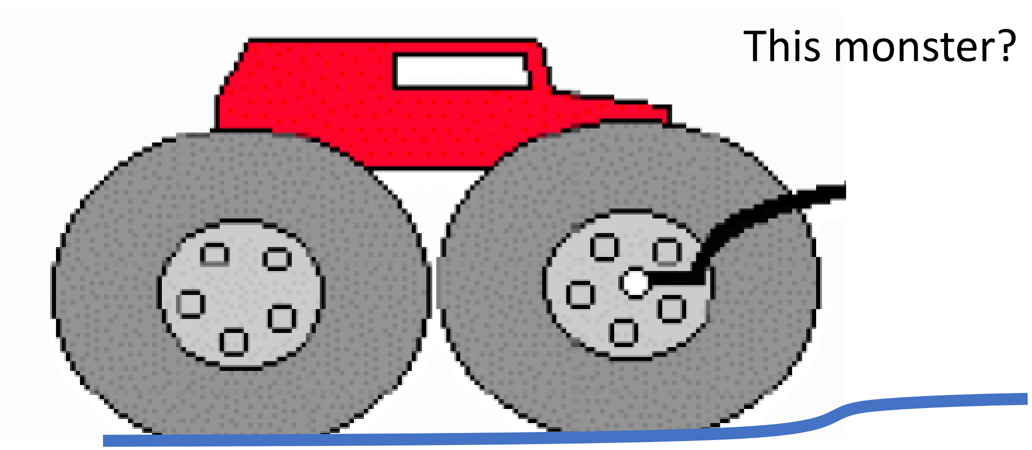
Viani et al, *APL* 1999

Bustamante et al., *Nature Rev. Mol. Cell Biol.* 2000

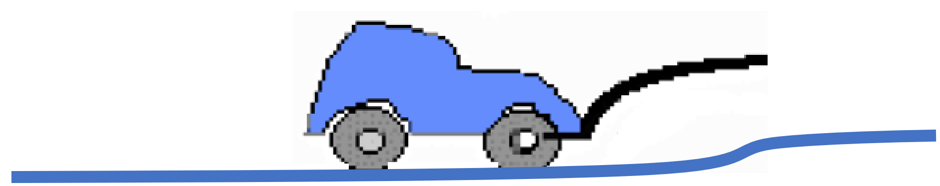
Effect of Tip on AFM scanning (limitation)

Who will feel the bump in the road?

This monster?

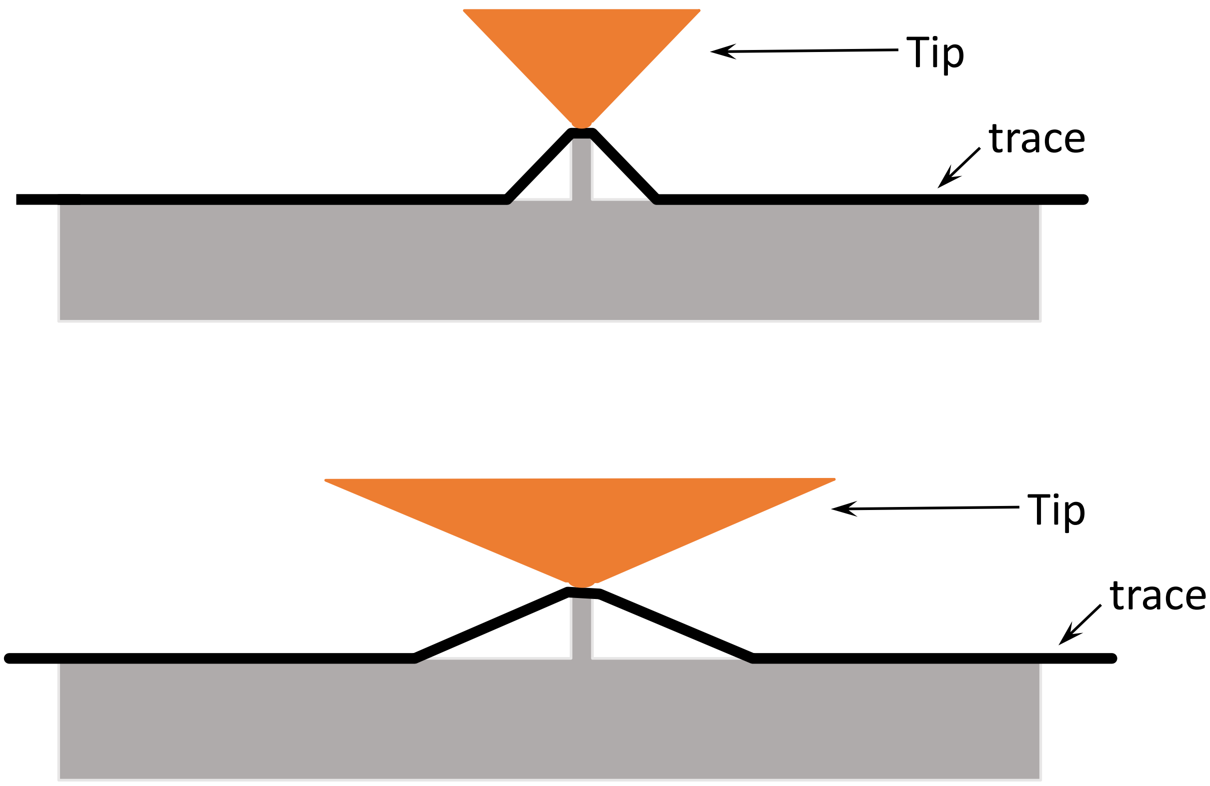


Or this bug?



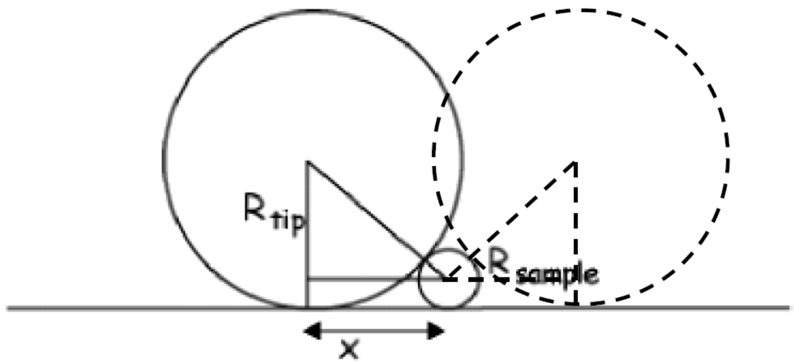
https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

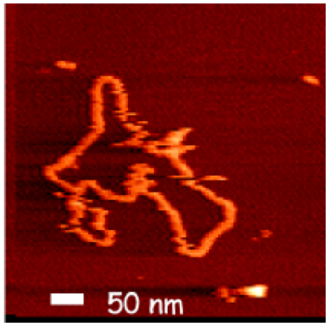
Fat tip effect



Slide courtesy of Duncan Sutherland

Fat tip effect





$$x^2 = (R_{tip} + R_{sample})^2 - (R_{tip} - R_{sample})^2$$

$$x^2 = \cancel{R_{tip}^2} + 2R_{tip}R_{sample} + \cancel{R_{sample}^2} - \cancel{R_{tip}^2} + 2R_{tip}R_{sample} - \cancel{R_{sample}^2}$$

$$x = 2\sqrt{R_{tip}R_{sample}}$$

$$w = 2x = 4\sqrt{R_{tip}R_{sample}}$$

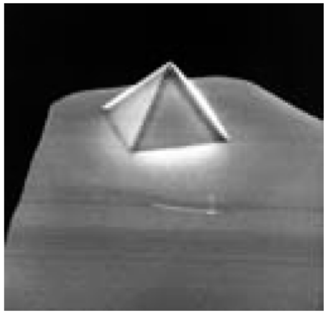
DNA: 2 nm.

tip ~ 20 nm => w = 25 nm
tip ~ 10 nm => w = 18 nm

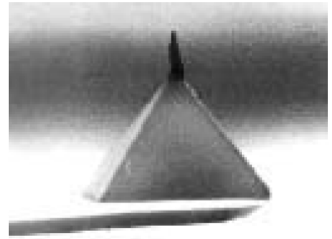
- Measured width: distance between the 1st and last tip/sample contact;
- The smaller the tip (R_{tip}), the smaller the measured width;
- When R_{tip} ~ ¼ R_{sample}, measured width = $2R_{sample}$;
- For a 5 nm feature (say a particle), the tip apex size must be ~ 1 nm to get a reliable lateral measurement --- quite challenging!
- Normal tip size, ~ 20 nm or larger.
- Another challenge for lateral imaging: to differentiate two adjacent features.

https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

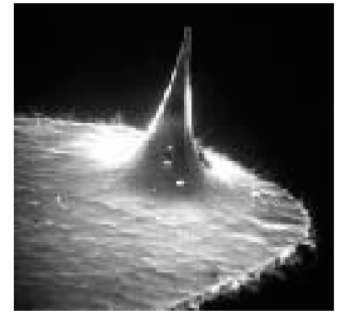
AFM tips



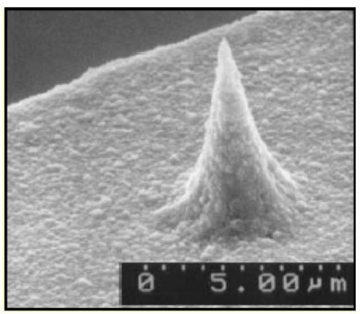
Normal Tip



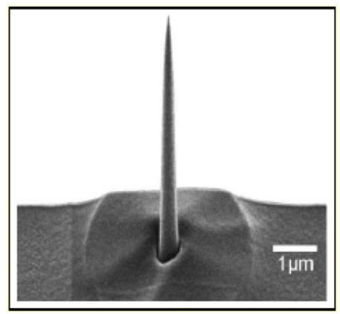
Supertip



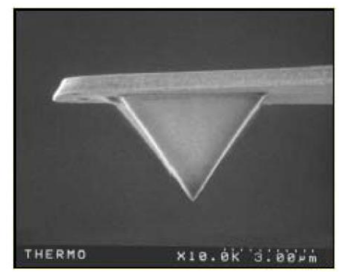
Ultralever



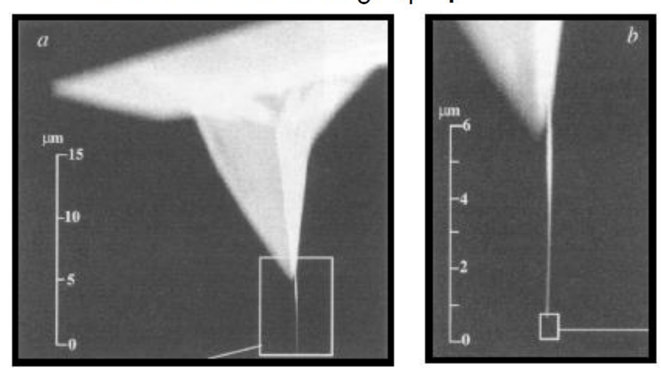
Diamond-coated tip



FIB-sharpened tip



Gold-coated Si_3N_4 tip

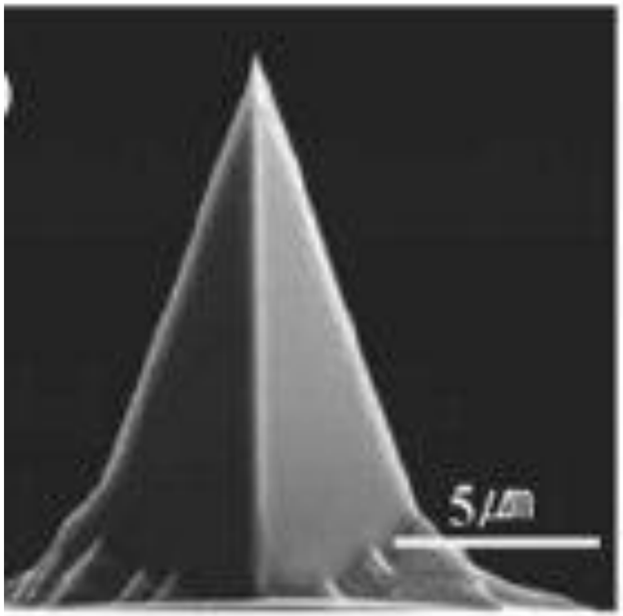


Carbon nanotube

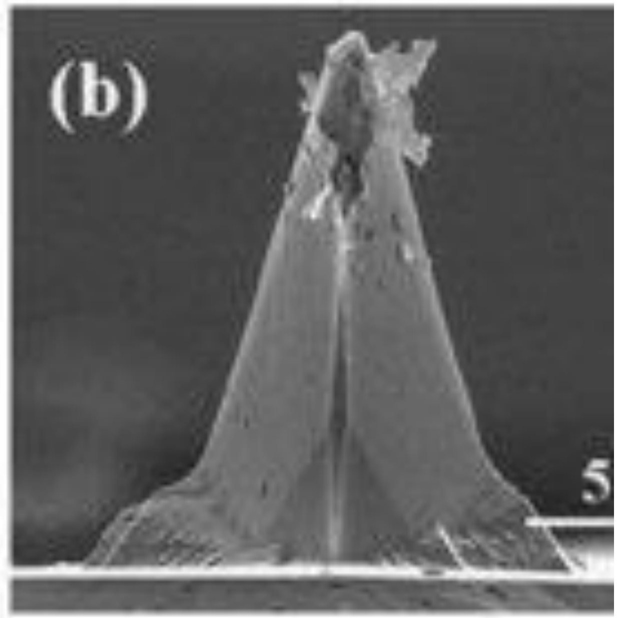
https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

Tip Contamination and image Artefacts

New tip



Contaminated tip

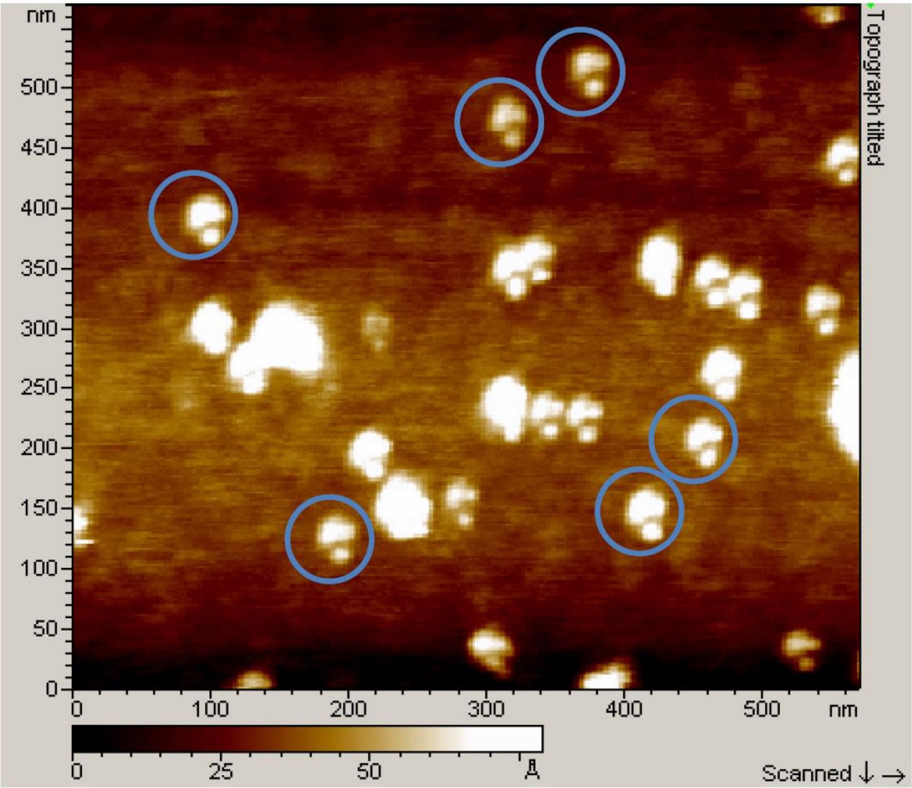


What kind of image would you expect from these tips?

Tip Contamination and image Artefacts

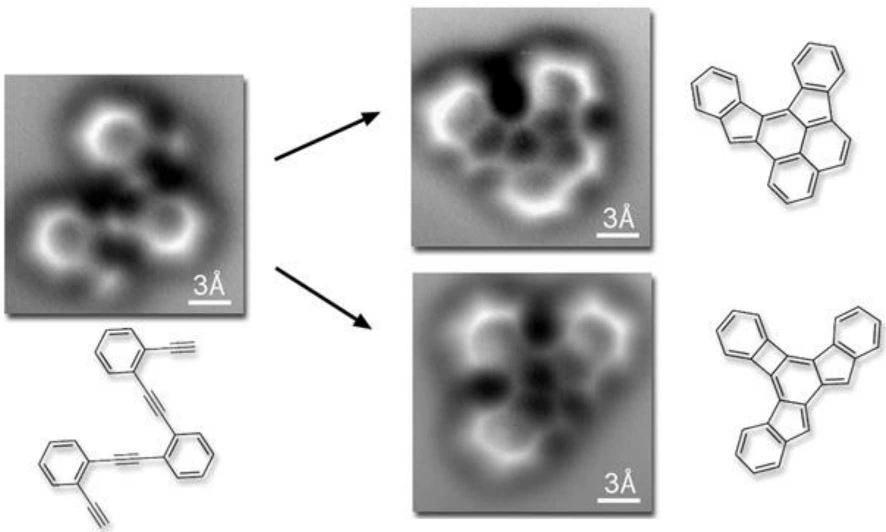
university of groningen

Multiple tips: Usually appears as all small features in image appearing identical. Sample is imaging tip rather than the other way around.



https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

If everything goes fine



Felix Fischer, Berkeley 2013

Chemical reaction observed with AFM

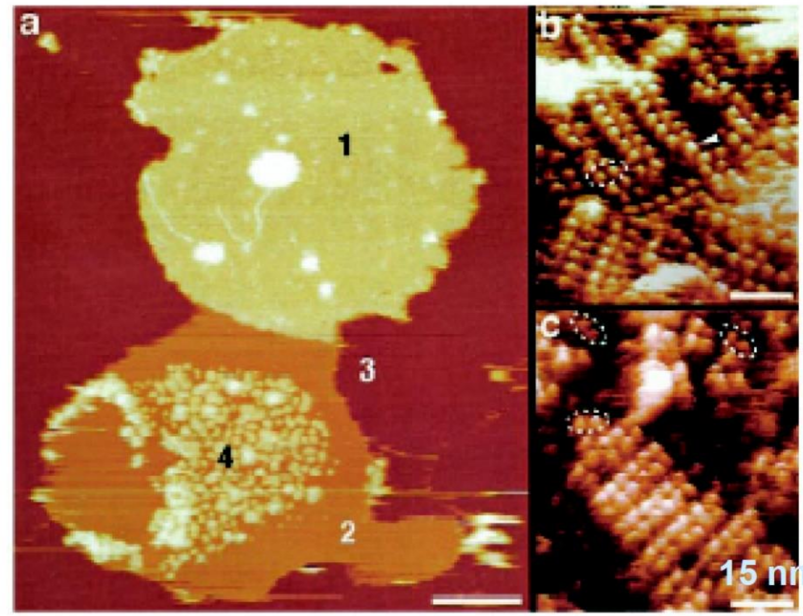
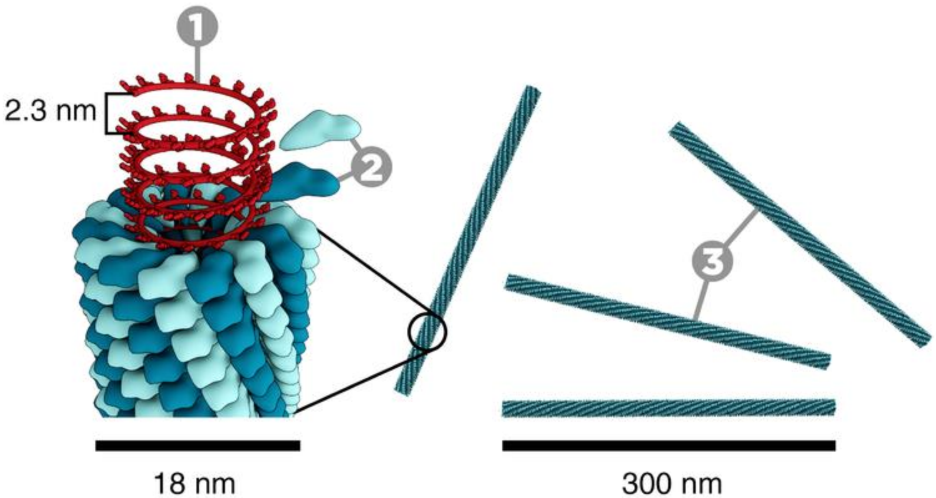


FIG. 3. **Topography of an open, spread-flattened disk adsorbed to mica and imaged in buffer solution.** *a*, height image of the open, spread-flattened disk. Four different surface types are evident: the cytoplasmic surface of the disk (types *1* and *4*), lipid (type *2*), and mica (type *3*). The topographies of regions 1 (*b*) and 4 (*c*) at higher magnification reveal densely packed rows of rhodopsin dimers. Besides paracrystals, single rhodopsin dimers (*broken ellipses*) and occasional rhodopsin monomers (*arrowhead*) are discerned floating in the lipid bilayer. *Scale bars*: 250 nm (*a*) and 15 nm (*b* and *c*). Vertical brightness ranges: 22 nm (*a*) and 2.0 nm (*b* and *c*).

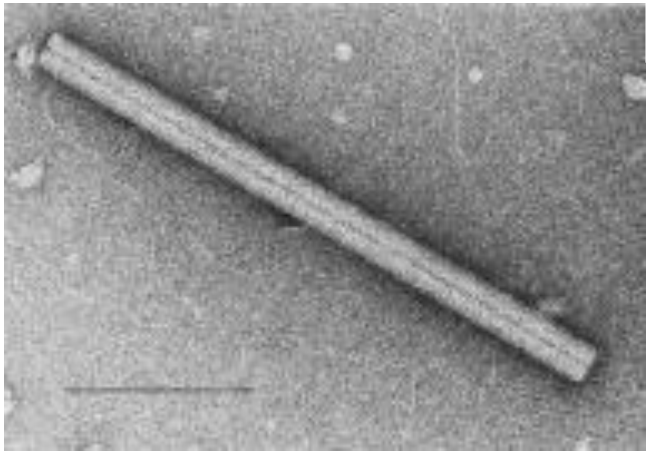
Imaging small features and scanning small area at high resolution require ultra-sharp tips.

JOURNAL OF BIOLOGICAL CHEMISTRY,
Vol. 278, No. 24, Issue of June 13, pp. 21655–21662, 2003

Example – Tobacco mosaic virus

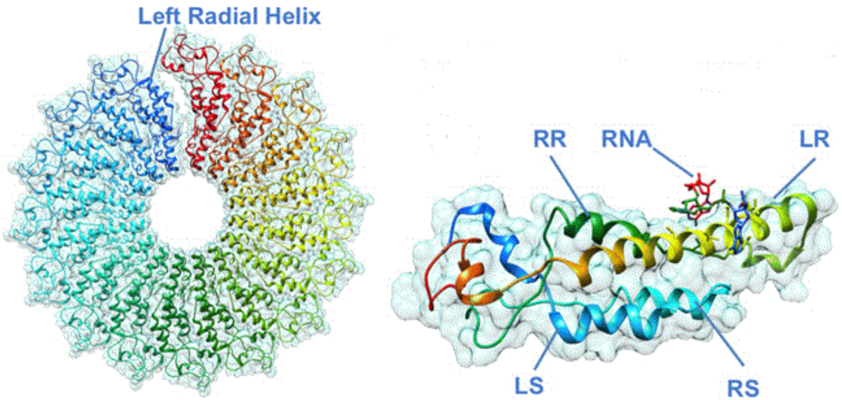


Molecular arrangement of TMV capsid



SEM image of TMV

http://www.virology.net/Big_Virology/BVunassignplant.html

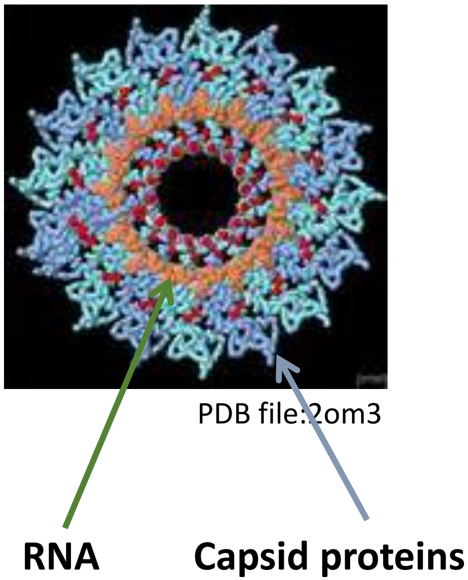


Single ring of 17 Capsid proteins **Capsid proteins (CP)**

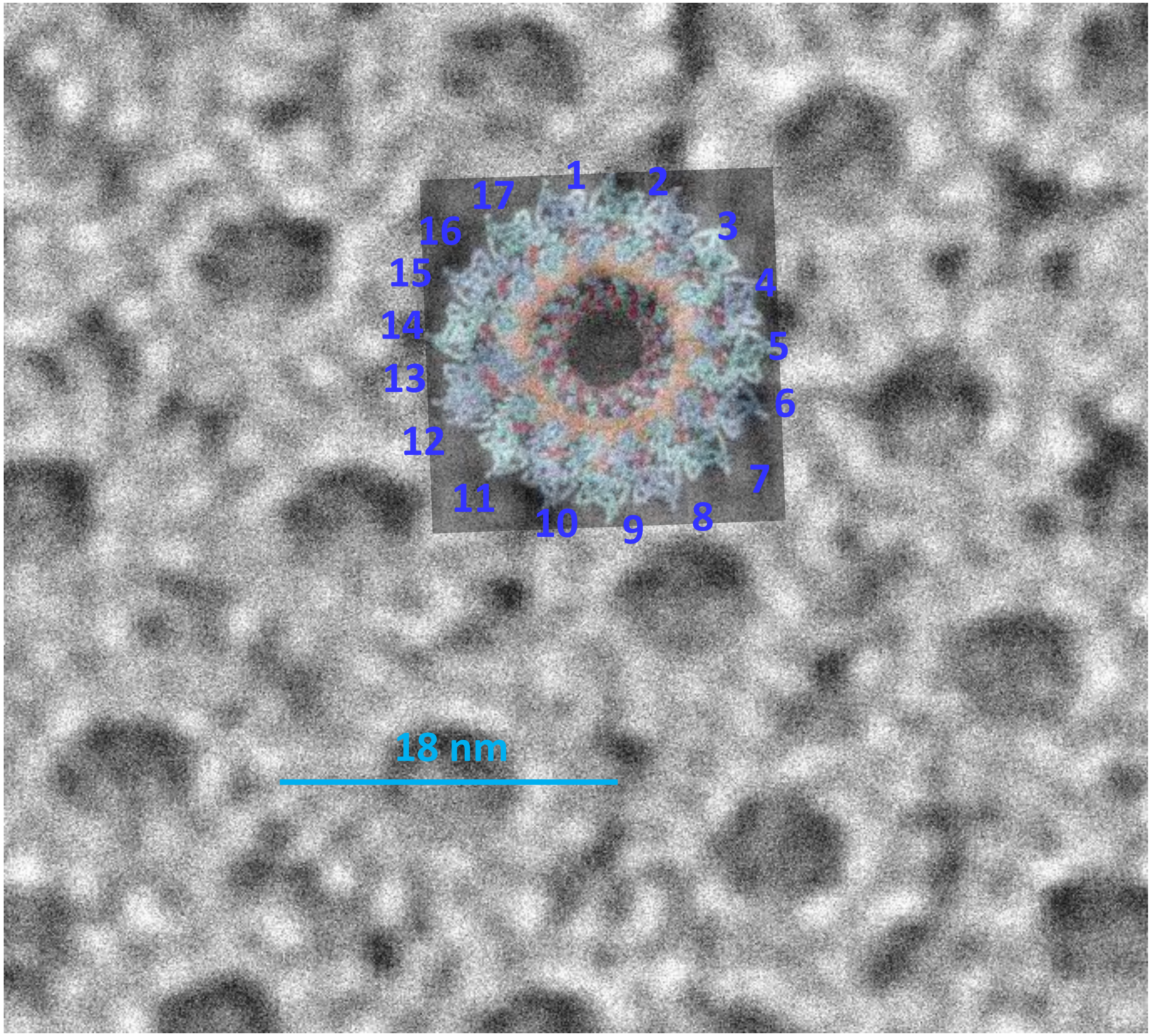
Modified from Annalisa Calò et. al. 2016 (PDB file 3J06)

Example – Tobacco mosaic virus

university of groningen



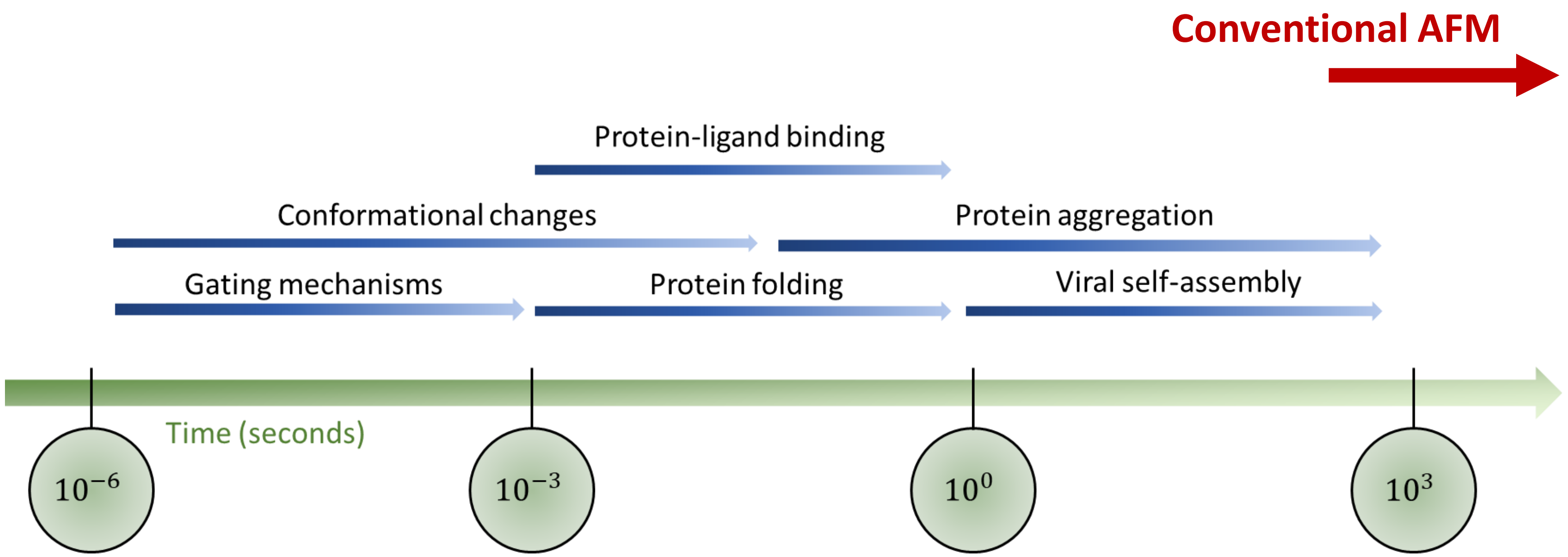
TMV single ring structure solved by X-ray fibre diffraction



TMV single (or double) ring structure solved by HS-AFM

Limitation in conventional AFM

So… We can see single molecules in ambient (in liquid) conditions…



Recent advancement : High speed -AFM

Can image 1000 times faster than conventional AFM

A vision from the inventors…

Ultramicroscopy 42–44 (1992) 7–15
North-Holland

ultramicroscopy

Force microscopy

G. Binnig
Physics Group München, IBM Research Division, Schellingstrasse 4, 8000 München 40, Germany

Received 31 January 1992

Gerd Binnig

"AFM will probably be used more frequently in biology…. where <u>biological processes and events can be filmed</u> on a scale not accessible with other techniques."

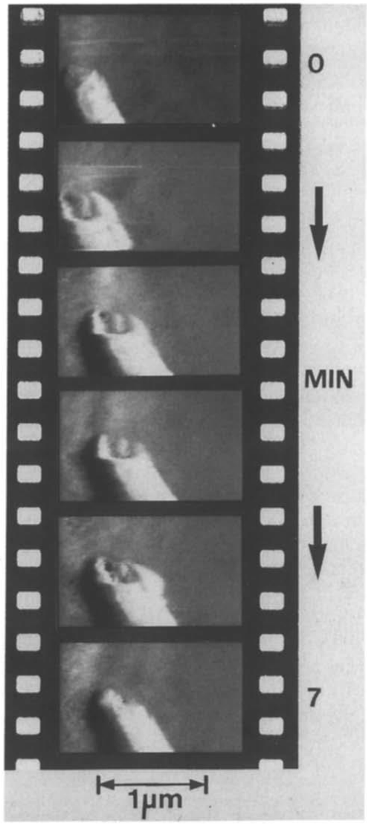
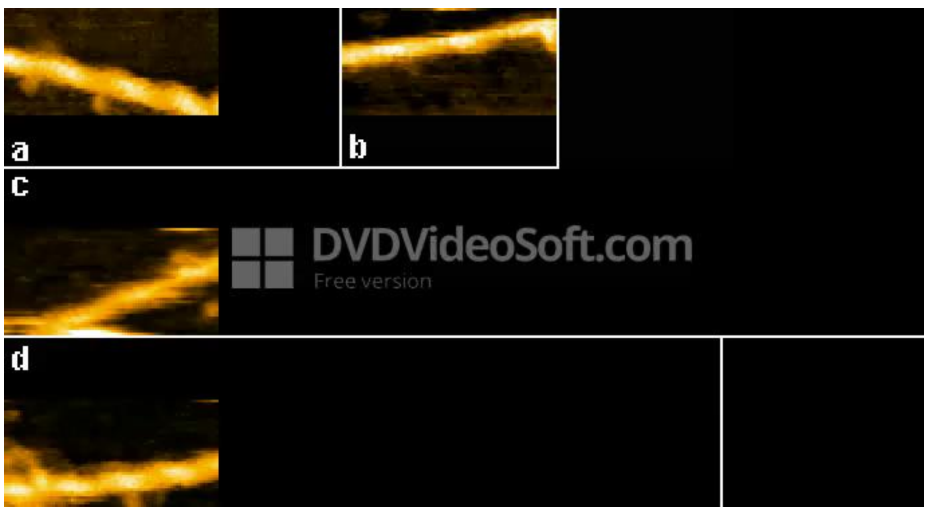


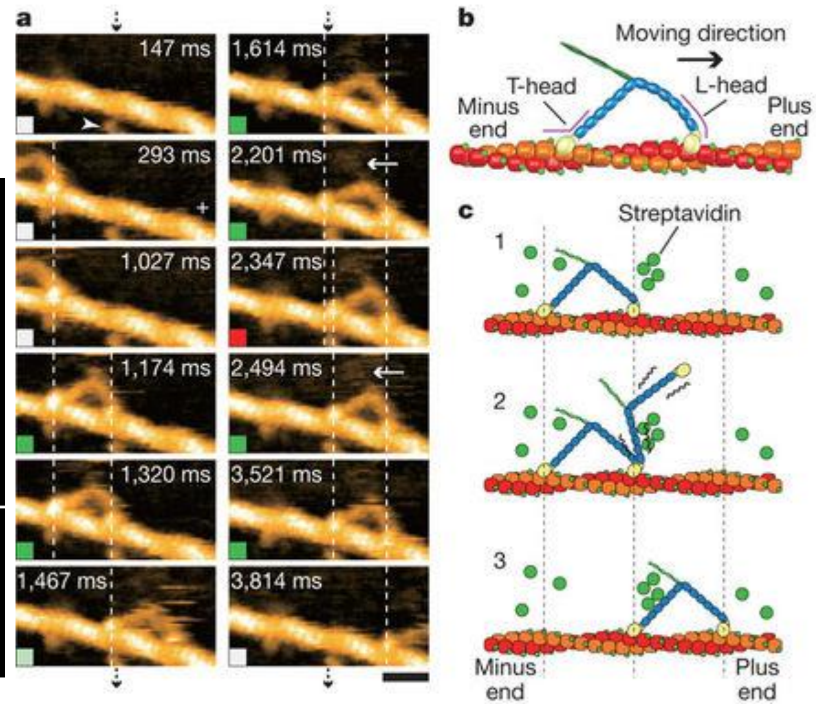
Image of a single living cell twenty hours after its infection by a pox virus.

A movie that surprised the world…

walking myosin V

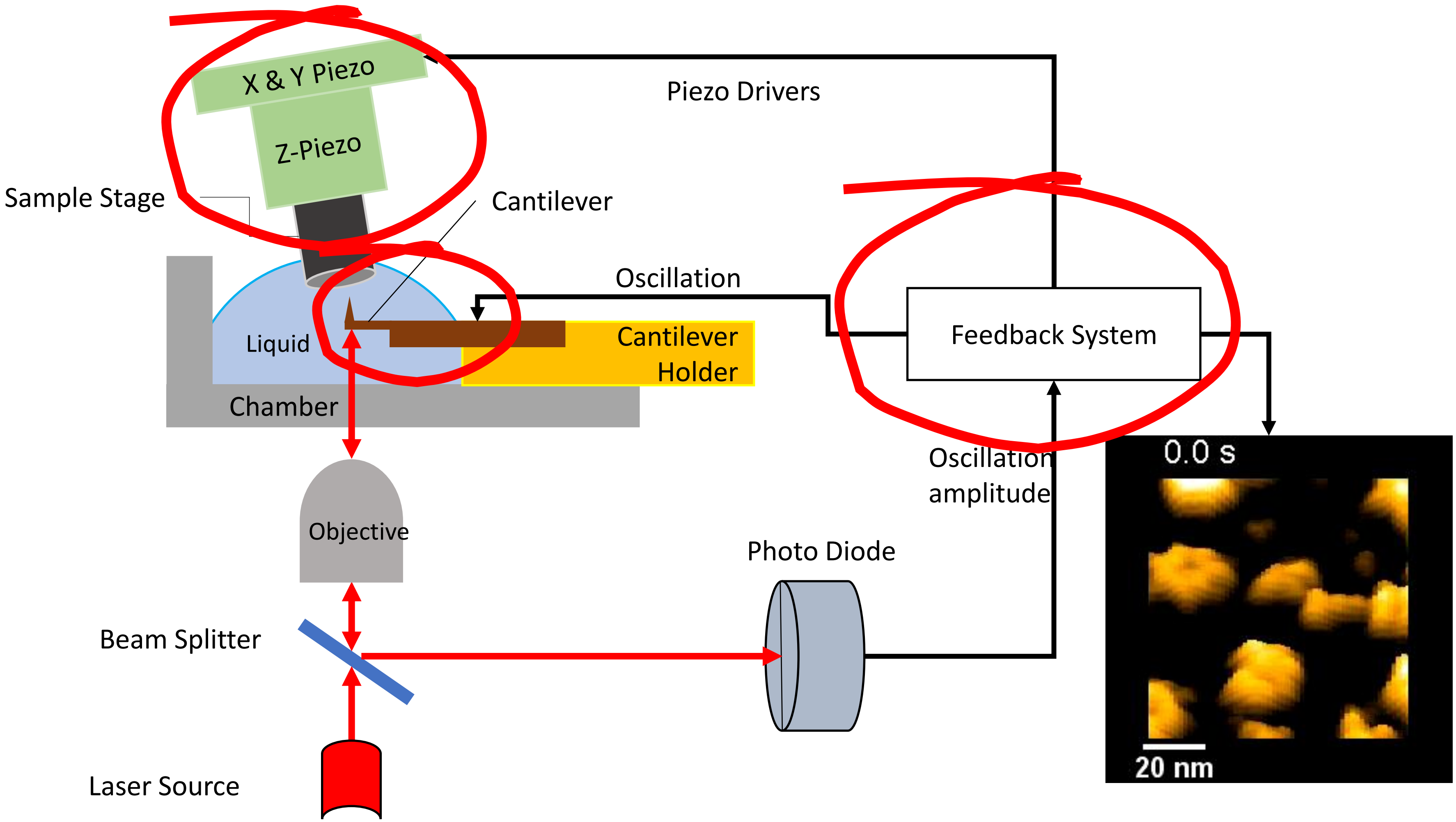
Prof. Toshio Ando





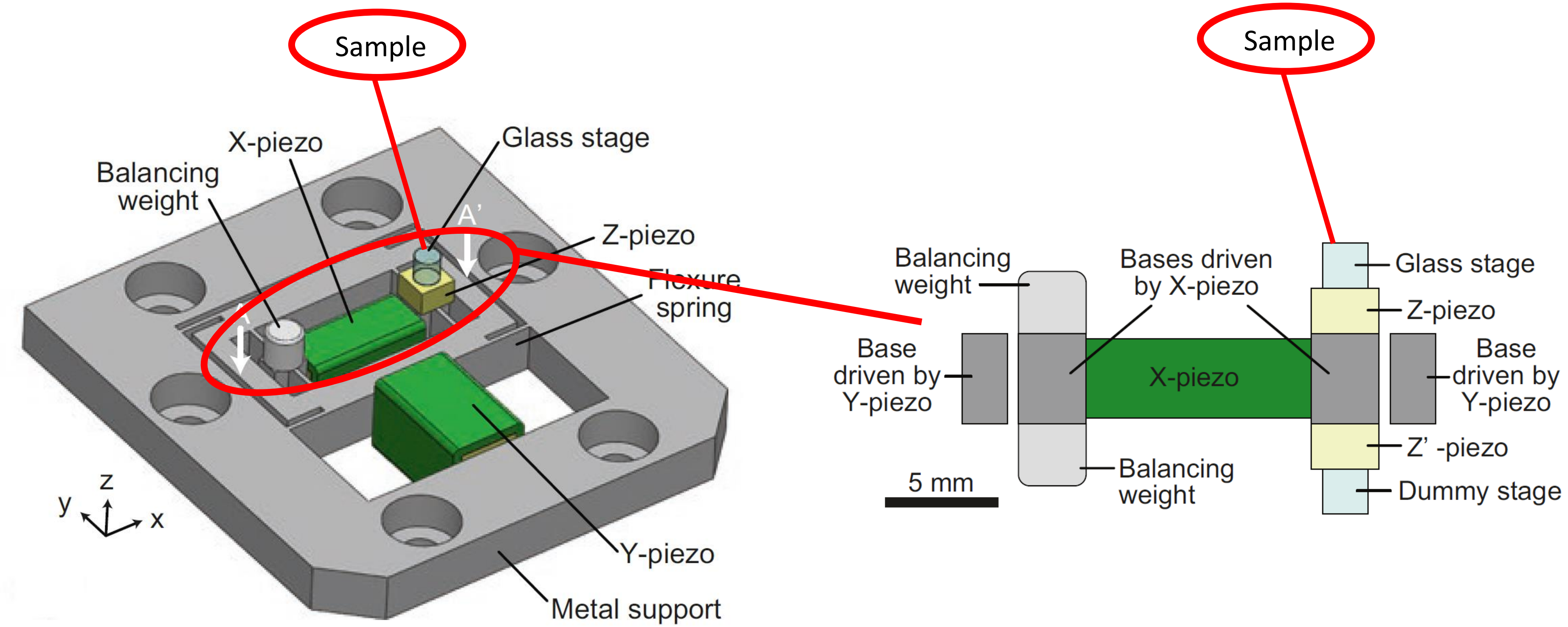
Kodera et al., Nature, 2010

Simplified design of HS-AFM (Ando type)



Buzon et al. Sci. Adv. 2022

Step I: The high-speed scanner…



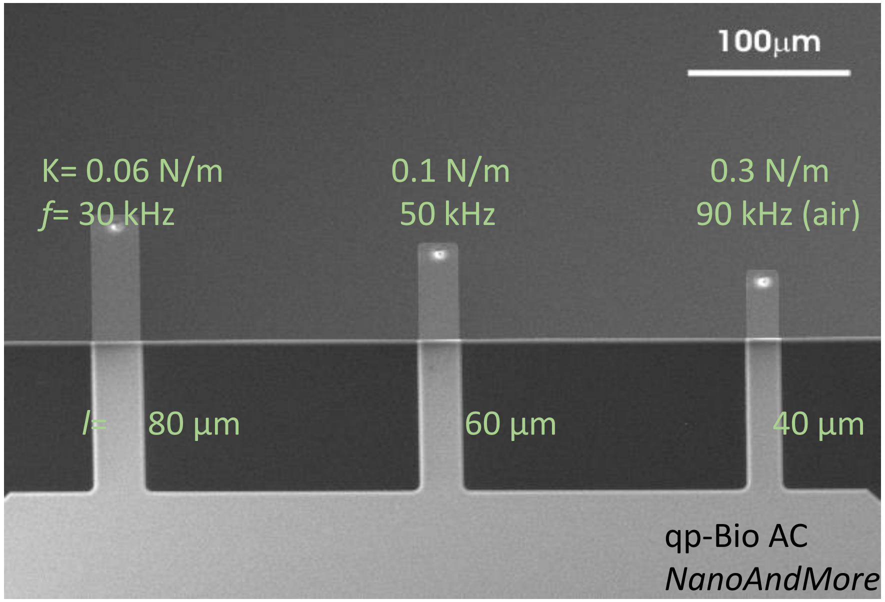
Kodera N., Ando T. (2020): Coluccio L. (eds) Myosins. Advances in Experimental Medicine and Biology, vol 1239. Springer, Cham

Step II: The cantilever effect…

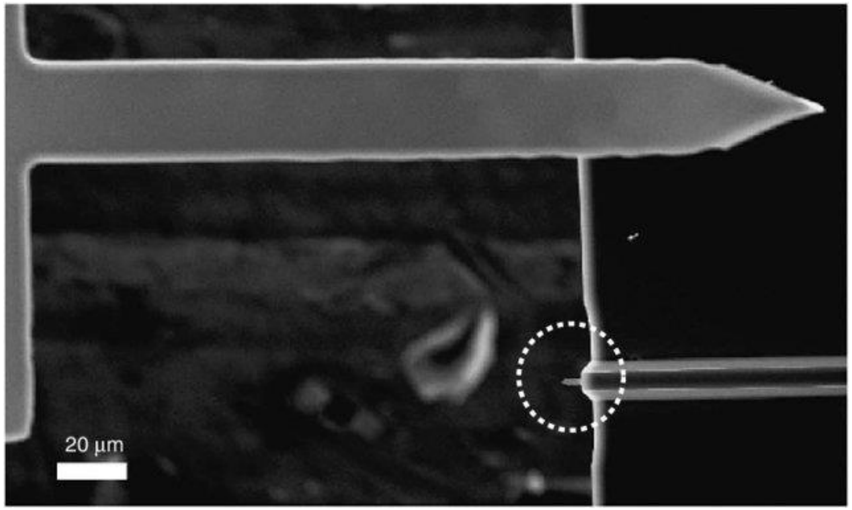
Resonance frequency of the cantilever,

$$f_0 = \frac{1}{2\pi}\left(\frac{k}{m_0}\right)^{0.5} \qquad k = \frac{Ewt^3}{4l^3}$$

K: the spring constant, *E*: Young modulus, *t*: thickness, *l*: length, *w*: width, m_0: the effective mass of the cantilever.



The high speed cantilever



Uchihashi et al., Nat. Protocol, 2012

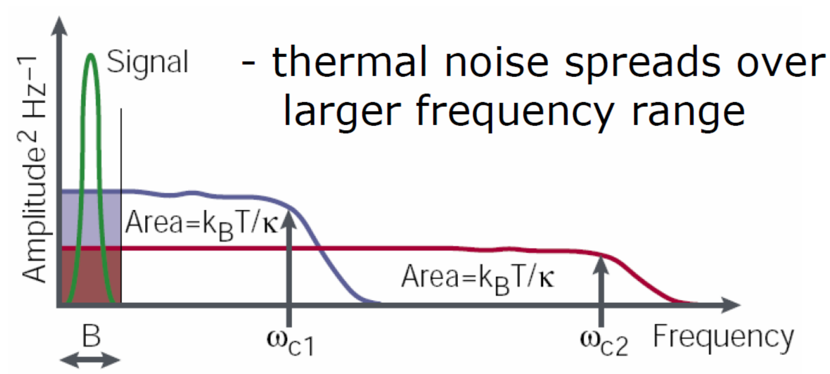
Step II: The cantilever effect…

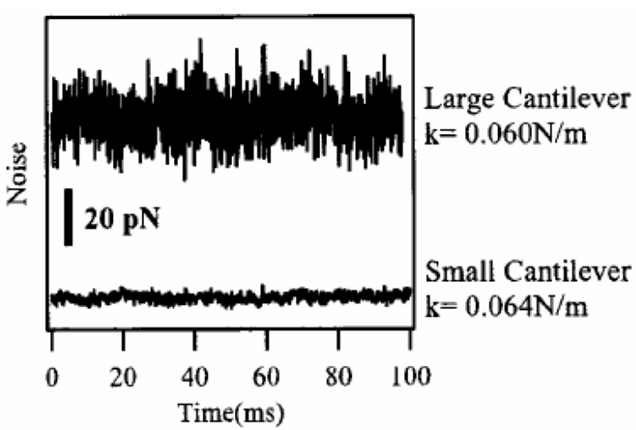
- Thermal noise:

Equipartition theorem

$$k_{spring} \cdot \langle \Delta x^2 \rangle = k_B T$$

$$k_{small} = k_{large}$$





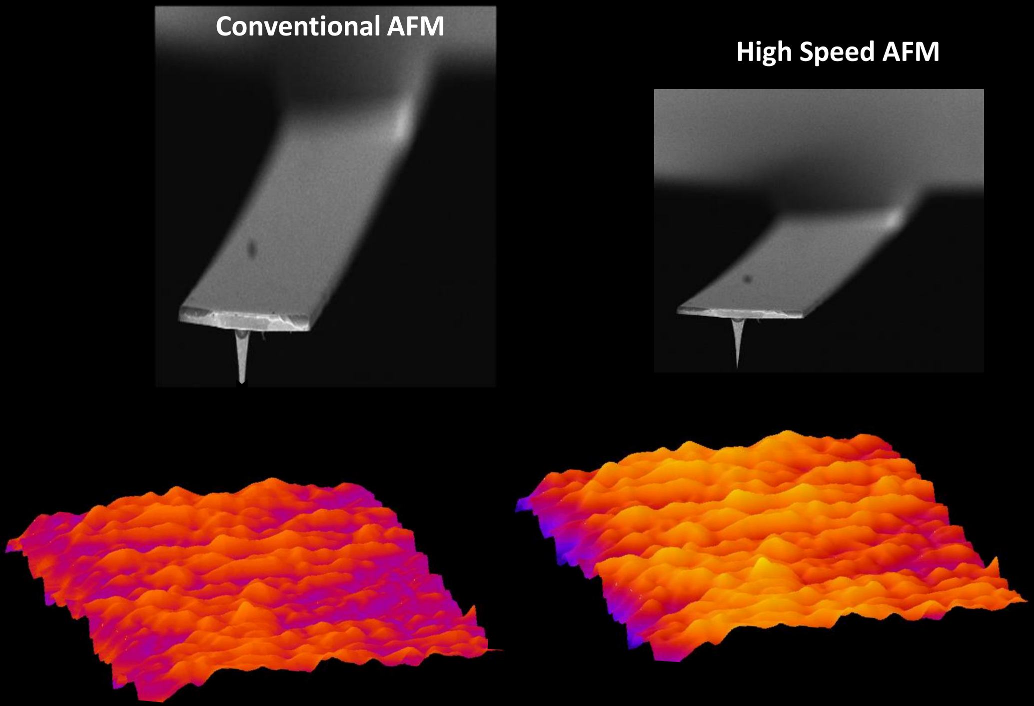
Small cantilever:

- **Better signal-to-noise**
- **Faster measurements**

Viani et al, *APL* 1999

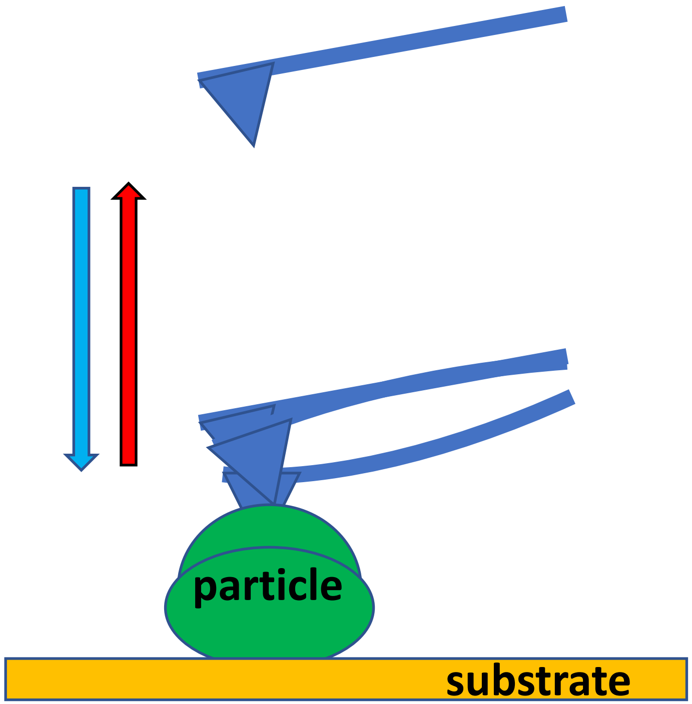
Bustamante et al., *Nature Rev. Mol. Cell Biol.* 2000

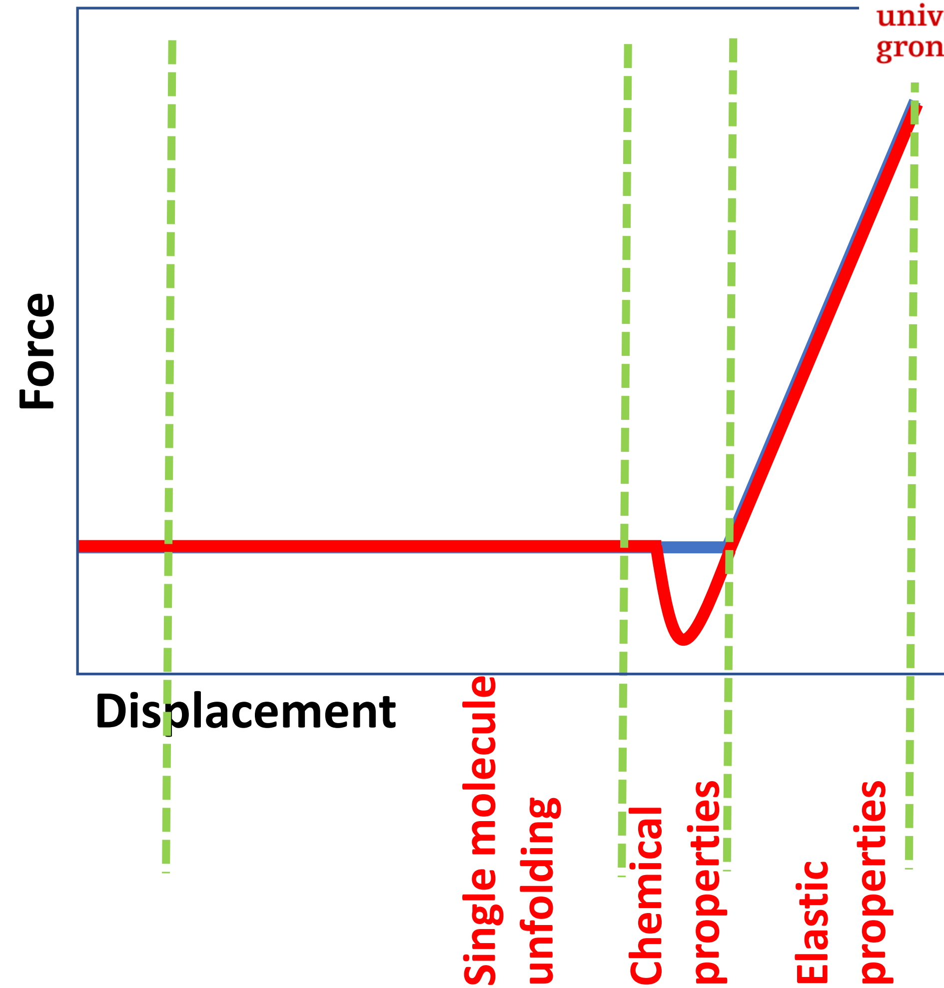
No speculations…. No deduction… direct visualisation!

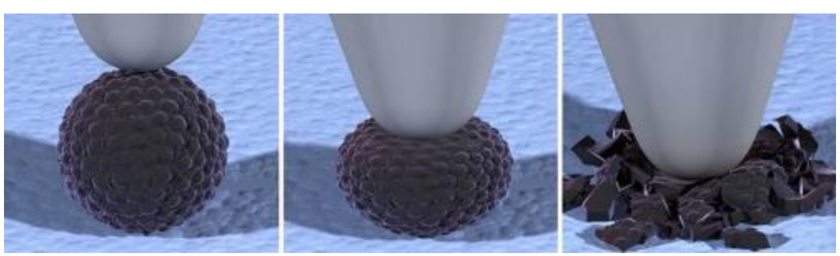


Force spectroscopy

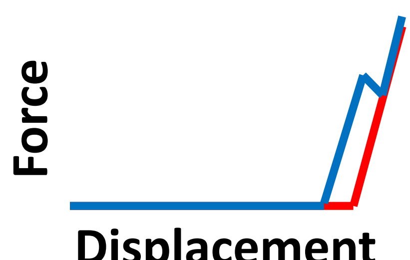
Force spectroscopy



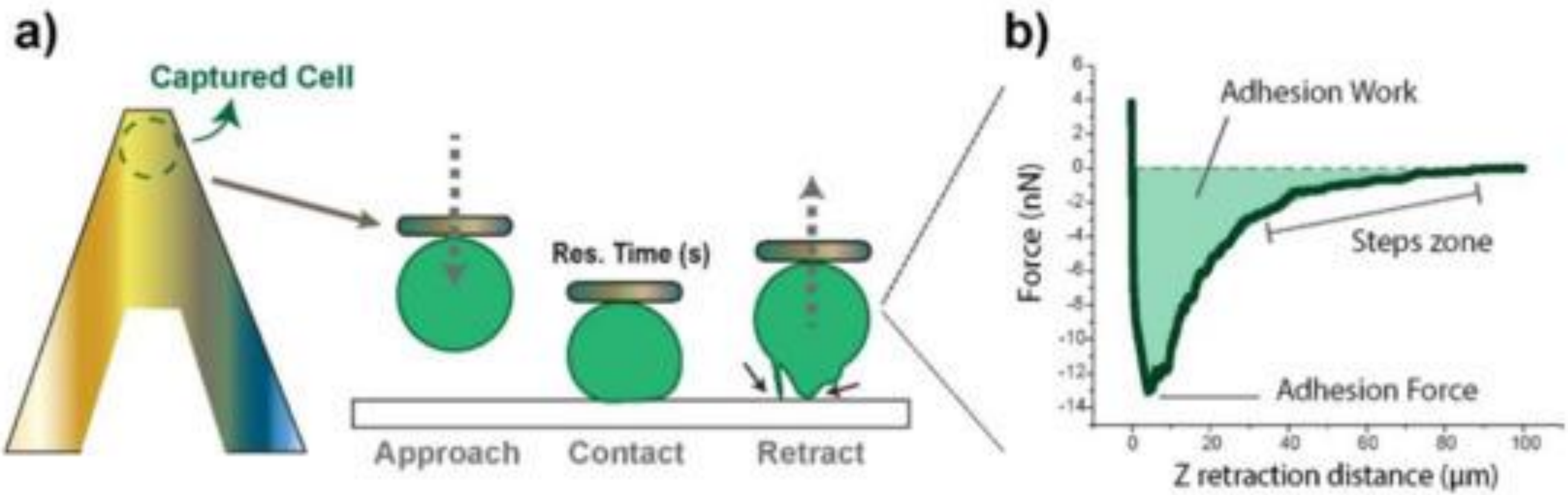




M Marchetti et al. Current Opinion in Virology2016



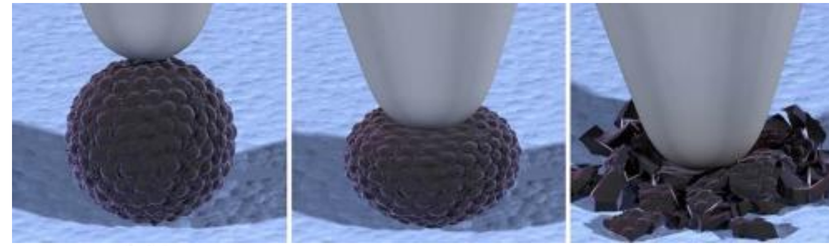
Adhesive force measurements



Iturri et al., *Cells,* 2020

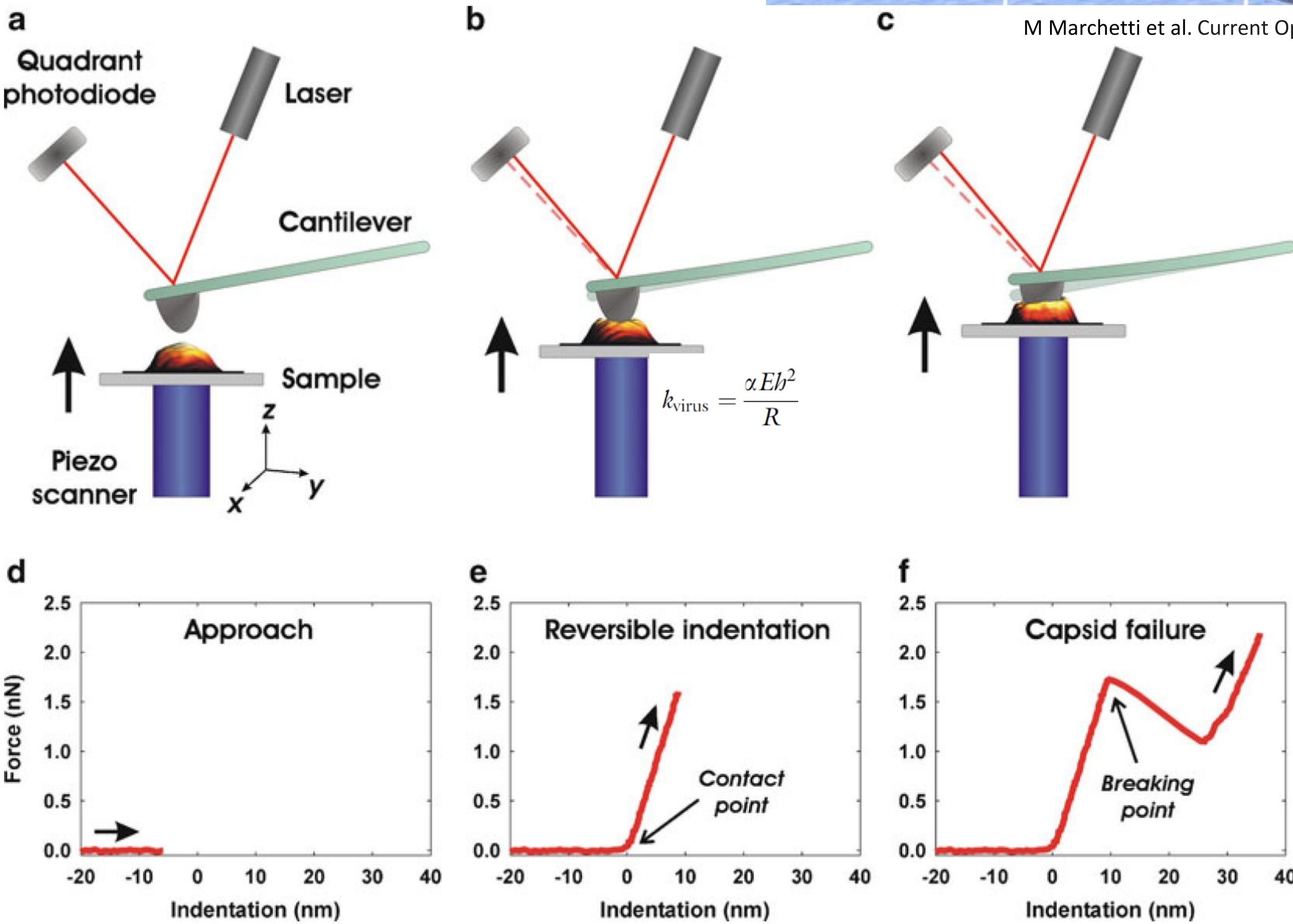
Nanoindentation

Nanoindentation



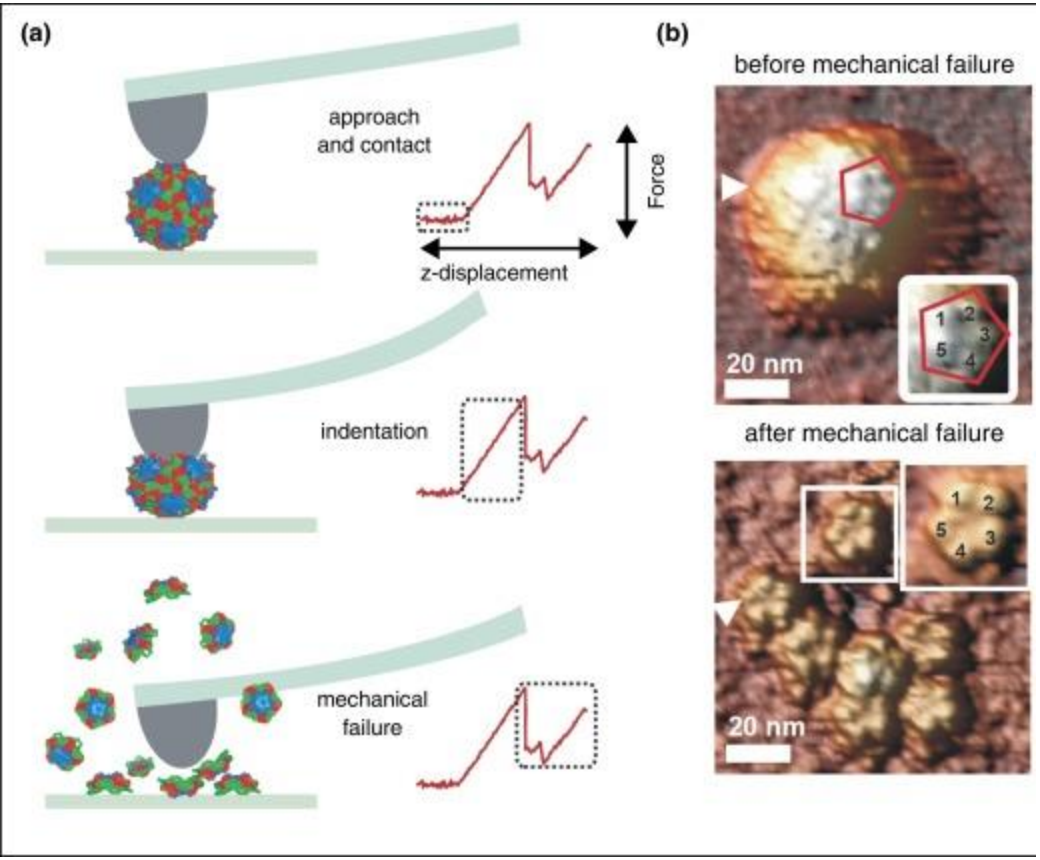
Force

M Marchetti et al. Current Opinion in Virology2016



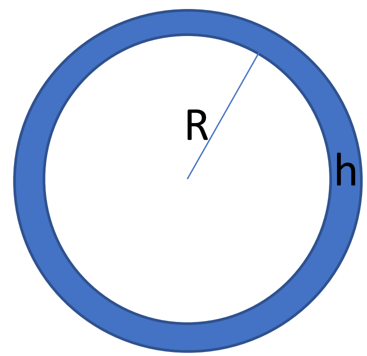
W. Roos, Methods Mol Biol, 2011

Nanoindentation



Hooke's law:
Two springs in series
$1/k_{effective}=1/k_{cantilever}+1/k_{particle}$

$$\text{Height}_{\text{real}} = \text{Height}_{\text{measured}} + \frac{F_{\text{imaging}}}{k_{\text{virus}}}$$



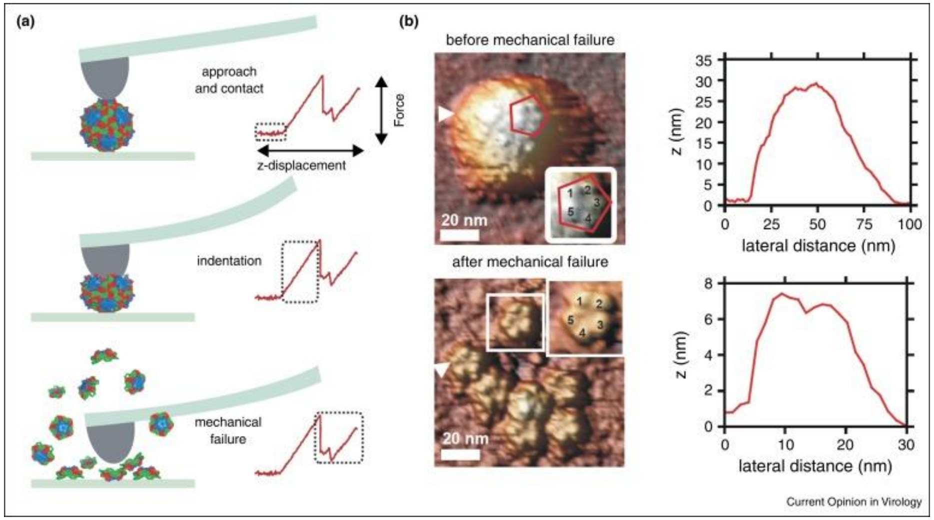
$$k_{\text{virus}} = \frac{\alpha E h^2}{R}$$

h= thickness
R= radius
α= proportionality factor (=1 good approximation)
E=Youngs modulus

M Marchetti et al. Current Opinion in Virology2016

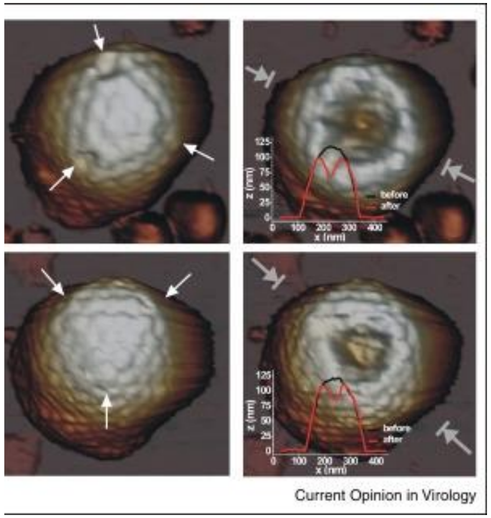
Nanoindentation

Triatoma virus



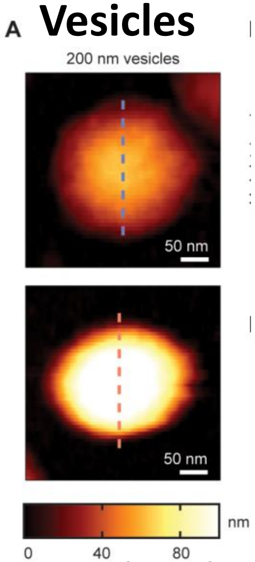
Snijder et al. Nat Chem 2013

Herpes simplex virus 1



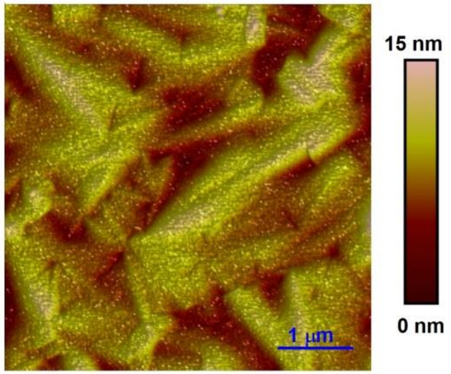
Roos et al. PNAS 2009

Vesicles

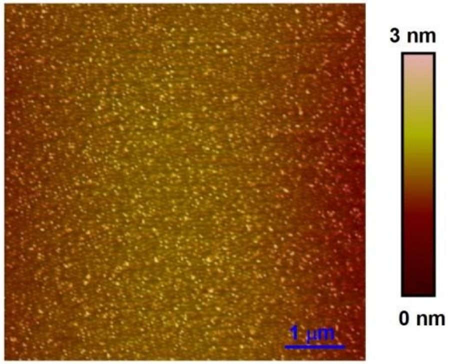


Daan Vorselen et al. Acs Nano 2017

ds DNA Azobenzene liquid crystal



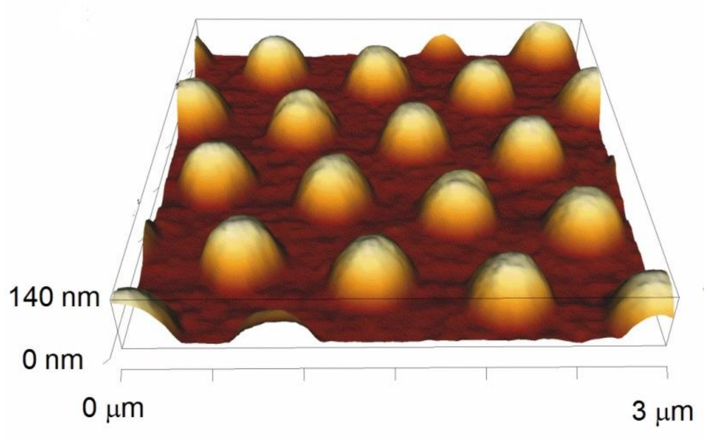
AZO-beforeUV



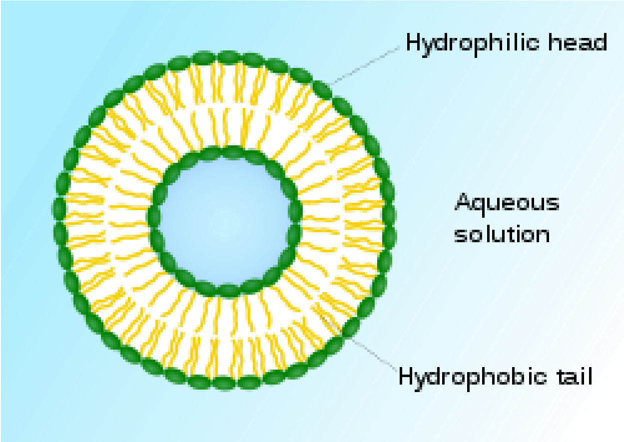
AZO-AfterUV

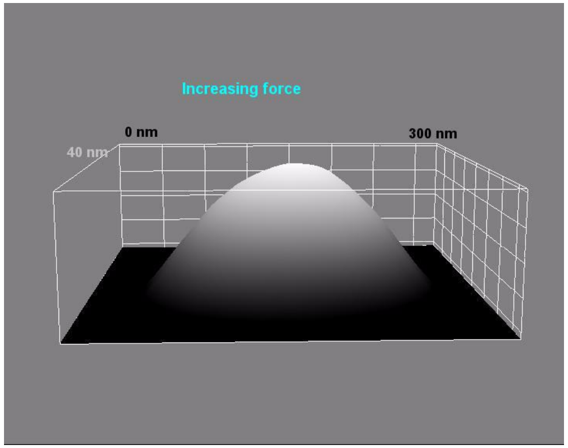
Lei Zhang et al. small 2017

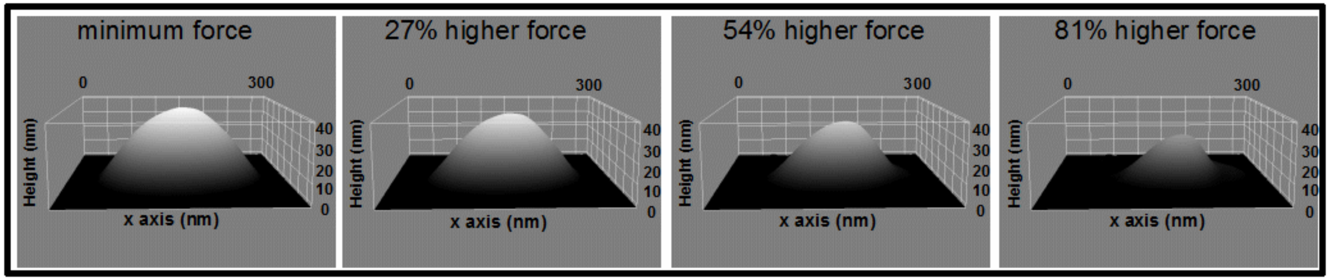
DNA organogel nanopillars

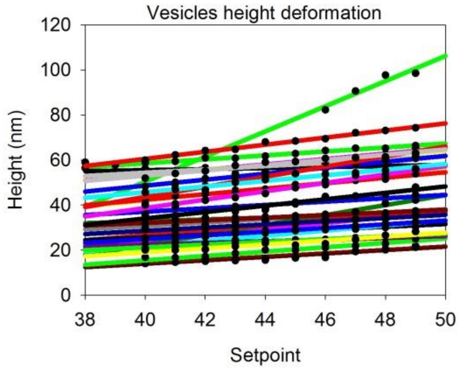


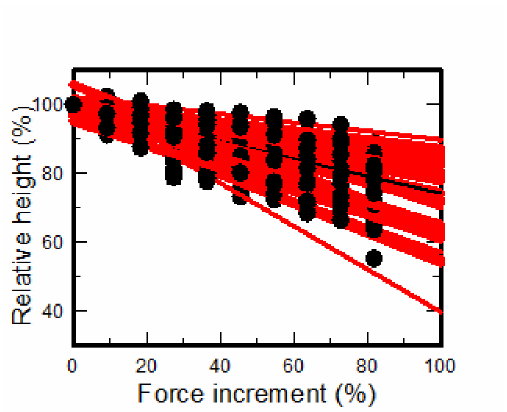
High throughput mechanical study











BMC biology, (2021). 19(1), pp.1-18

Advantages and disadvantages

- Easy sample preparation
- Accurate height information
- Works in vacuum, air, and liquids
- Living systems can be studied

- Limited vertical range
- Limited magnification range
- Data not independent of tip
- Tip or sample can be damaged

The limit?!

nature

16th June 2021

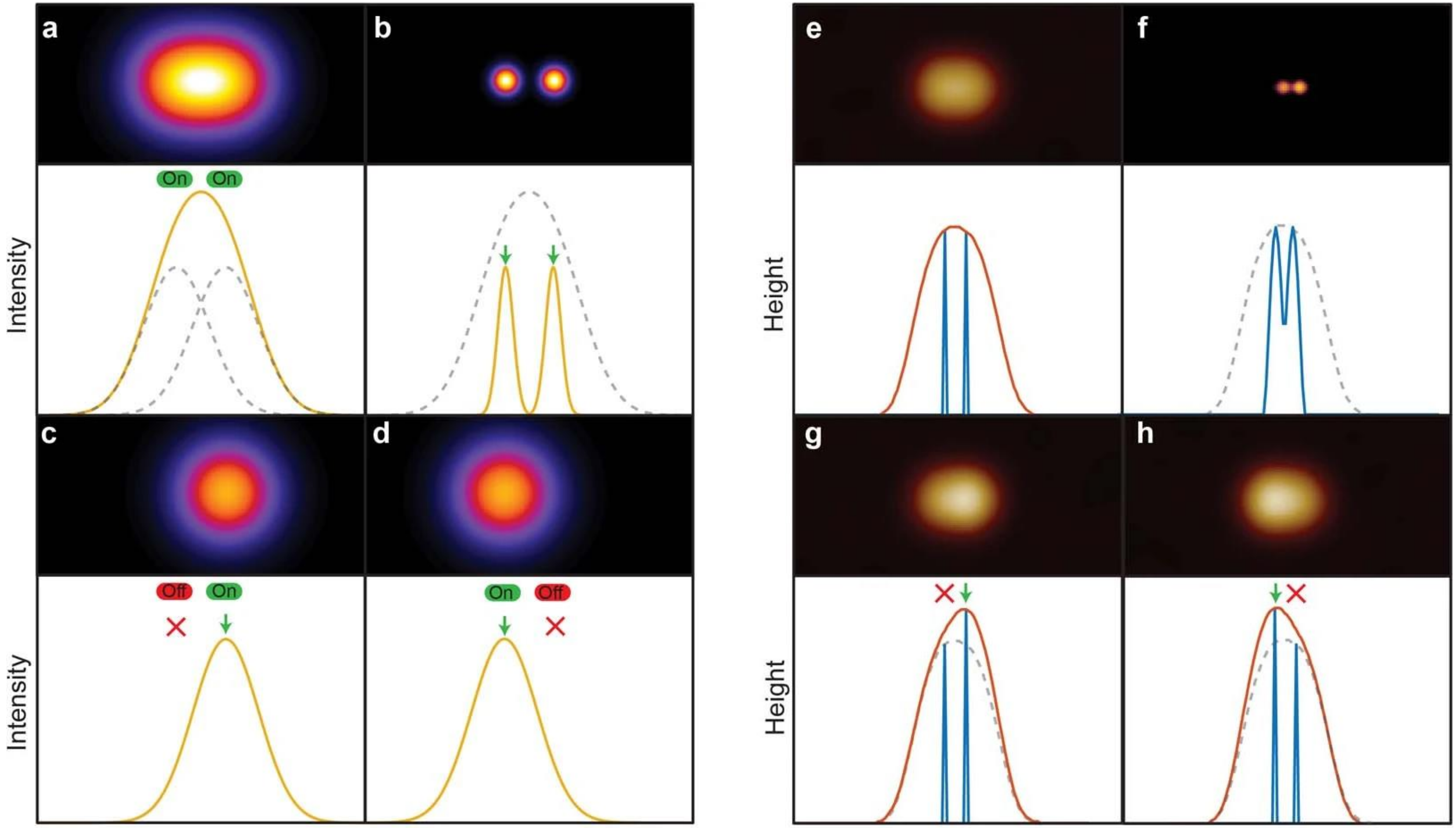
Article

Localization atomic force microscopy

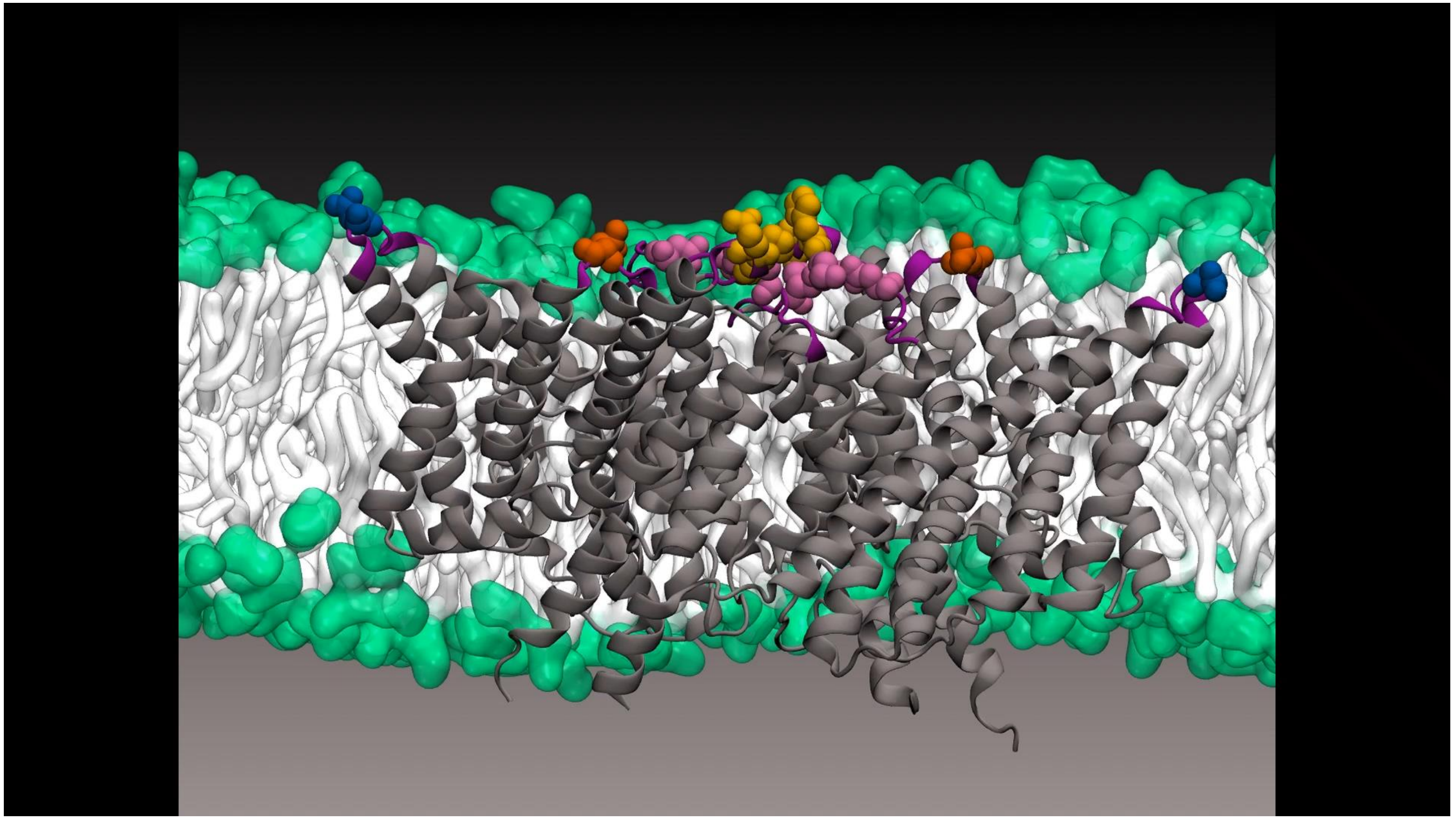
https://doi.org/10.1038/s41586-021-03551-x

George R. Heath[1,4], Ekaterina Kots[2], Janice L. Robertson[3], Shifra Lansky[1], George Khelashvili[2], Harel Weinstein[2] & Simon Scheuring[1,2]✉

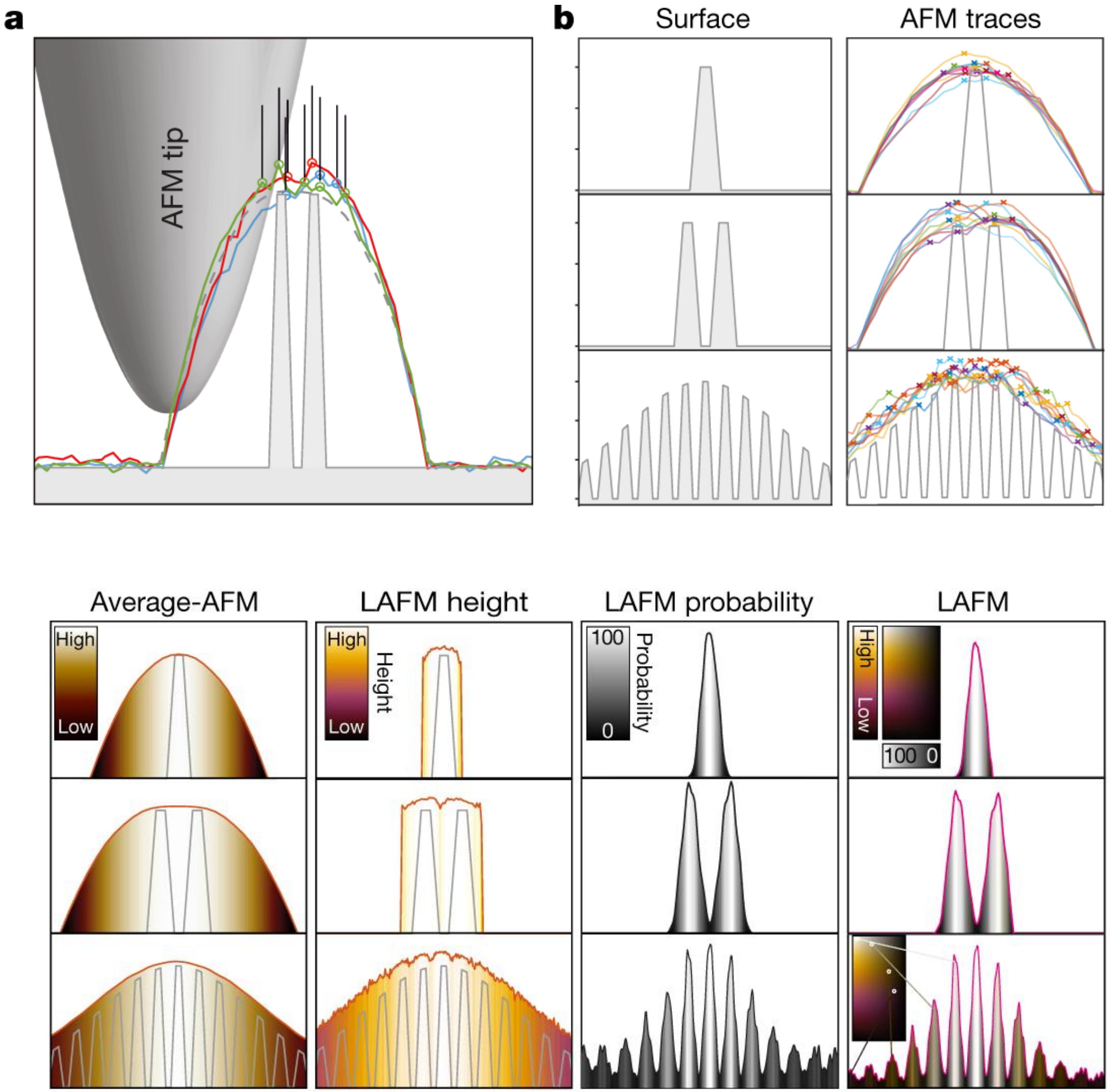
Localization principles in PALM and LAFM



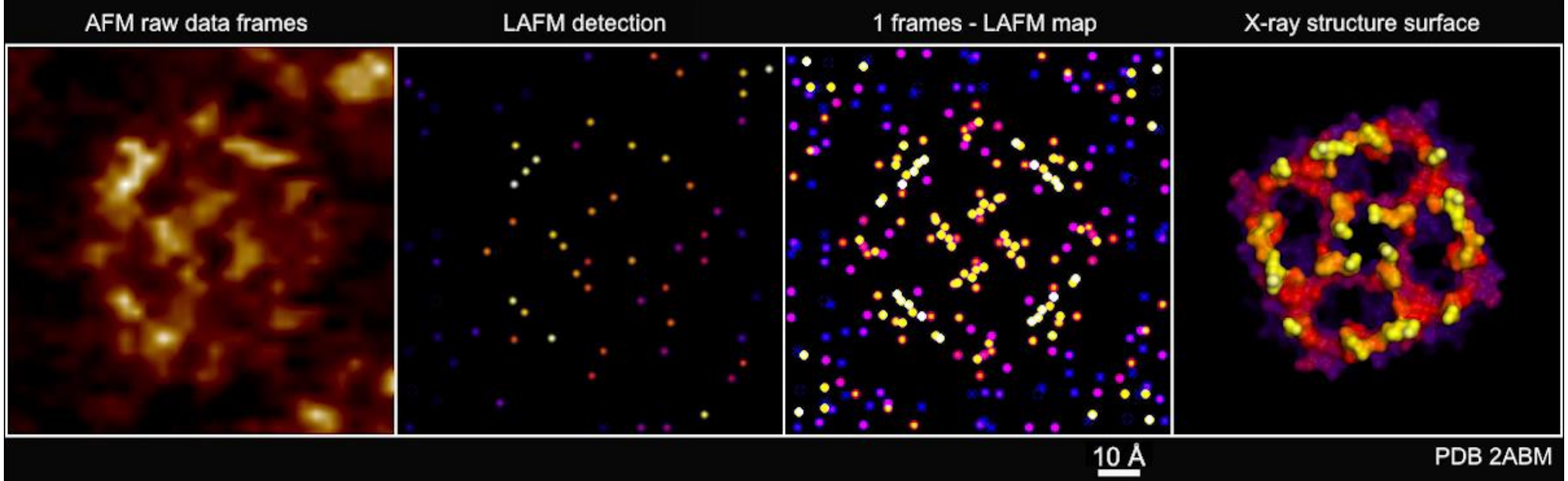
MD simulation of CLC-ec1 fluctuation trajectories



Principle of LAFM

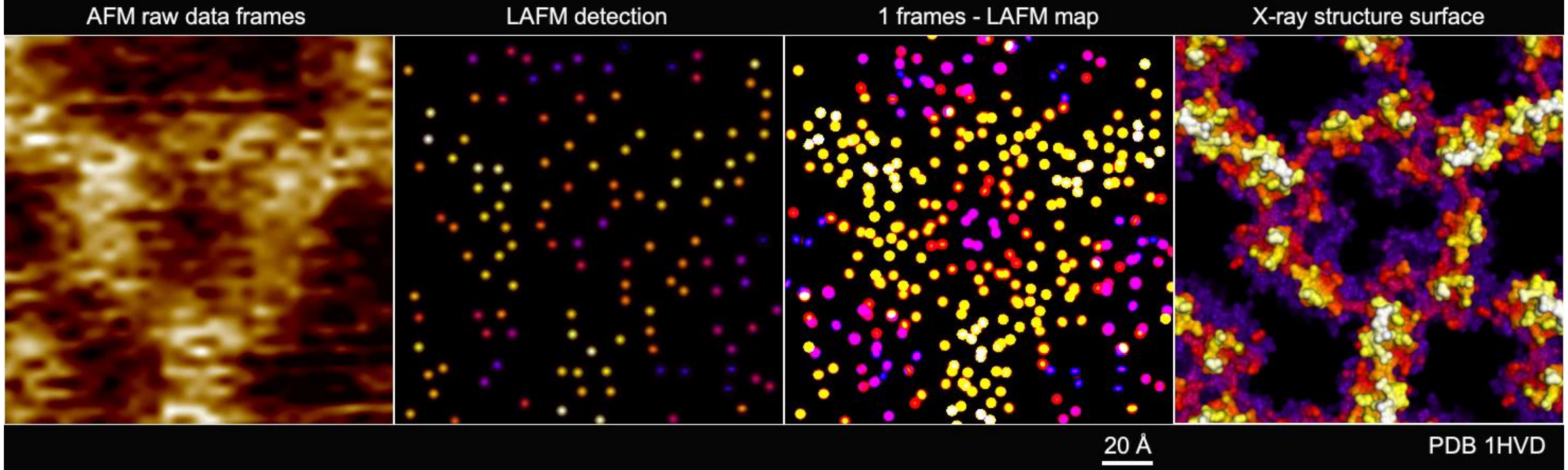


Example 1: aquaporin-Z by contact-mode AFM using a Nanoscope-III AFM

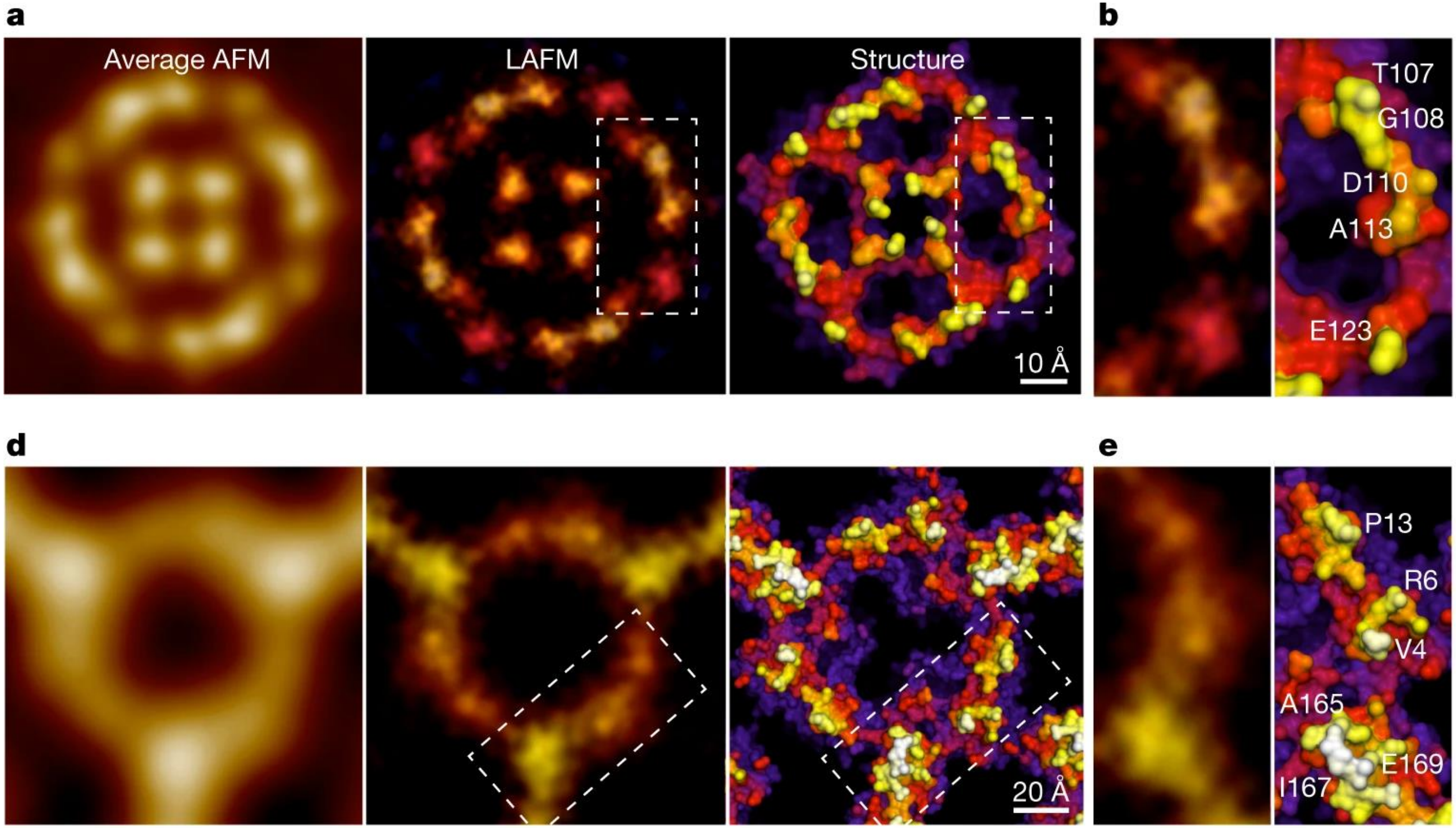


frame size: 10 nm, image capture: 3 pixels/nm, scan speed: 6.8 lines/s

Example 2: annexin-V by amplitude modulation HS-AFM



frame size: 19 nm, image capture: 4 pixels/nm, scan speed: 1 frame/s.


a
Average AFM
LAFM
Structure
10 Å
b
T107
G108
D110
A113
E123
d
20 Å
e
P13
R6
V4
A165
E169
I167

References

- Li, Hong-Qiang. "Atomic Force Microscopy". http://www.chembio.uoguelph.ca/educmat/chm729.afm.htm
- Baselt, David. "Atomic force microscopy". http://stm2.nrl.navy.mil/how-afm/how-afm.html
- Atomic Force Microscopy. http://www.topometrix.com/spmguide/1-2-0.htm
- An Introduction to Atomic Force Microscopy
- http://www.wpi.edu/academics/Depts/Physics/AFM/Pdfs/PosterIntro.pdf
- Basic Theory Atomic Force Microscopy (AFM) http://asdlib.org/onlineArticles/ecourseware/Bullen/SPMModule_BasicTheoryAFM.pdf
- Atomic Force Microscopy: An Introduction: M. Piontek & W.H. Roos 2018

- Some slides were adapted from

  https://my.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf