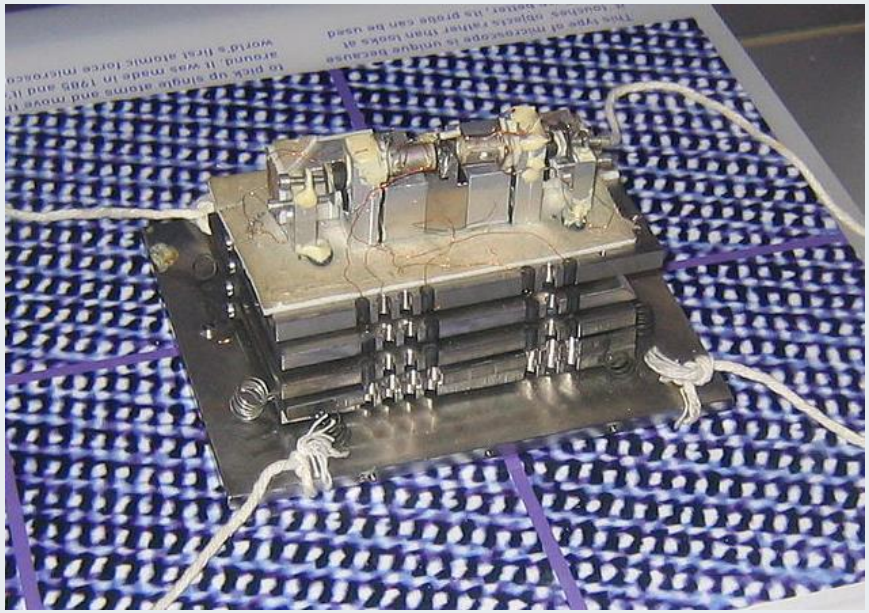

qbio
quantitative
biology

Atomic Force Microscopy and Scanning Probe Microscopy

1981 - Invention of the STM

1986 - Invention of the AFM



The Nobel Prize in Physics 1986
Ernst Ruska, Gerd Binnig, Heinrich Rohrer

Share this: 14

The Nobel Prize in Physics 1986

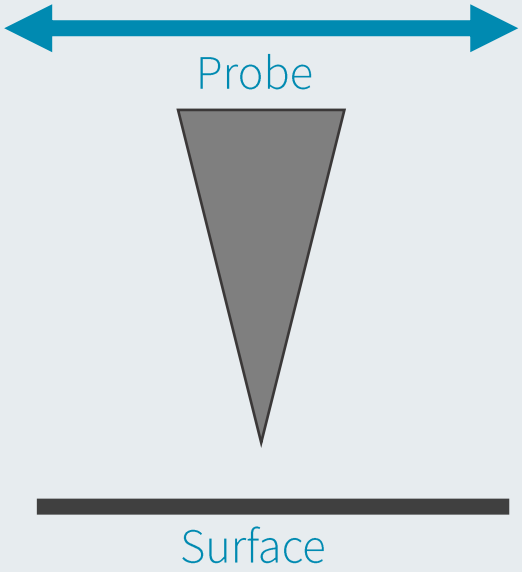
Ernst Ruska
Prize share: 1/2

Gerd Binnig
Prize share: 1/4

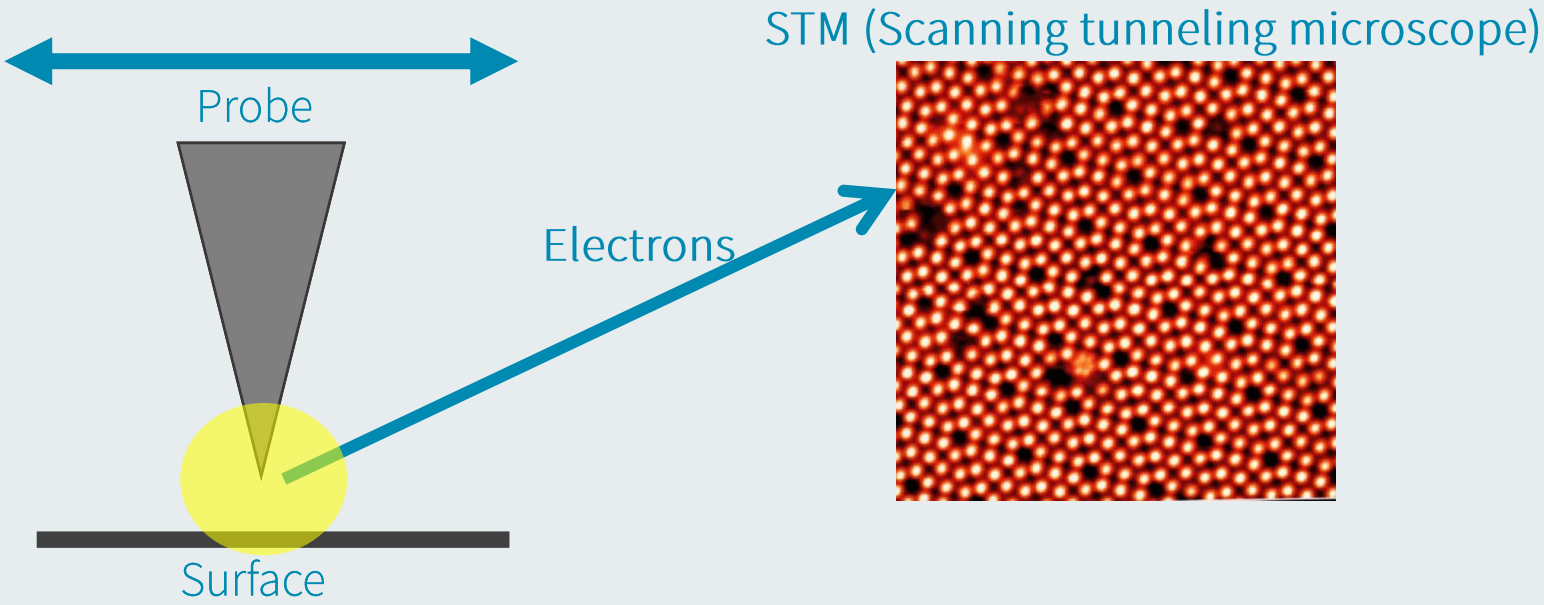
Heinrich Rohrer
Prize share: 1/4

Binnig G, Quate CF, Gerber C. Atomic force microscope. *Phys. Rev. Lett.* 1986 Mar 3;56(9):930-933

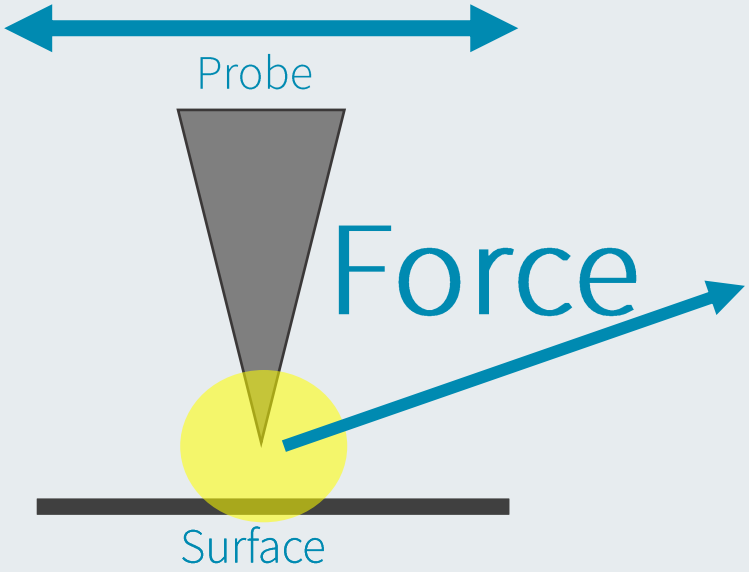
Atomic Force Microscopy and Scanning Probe Microscopy



Atomic Force Microscopy and Scanning Probe Microscopy

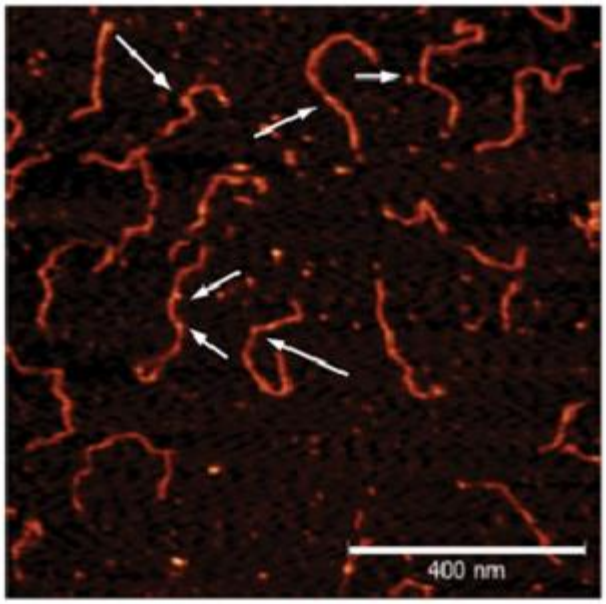


Atomic Force Microscopy and Scanning Probe Microscopy



STM (Scanning tunneling microscope)

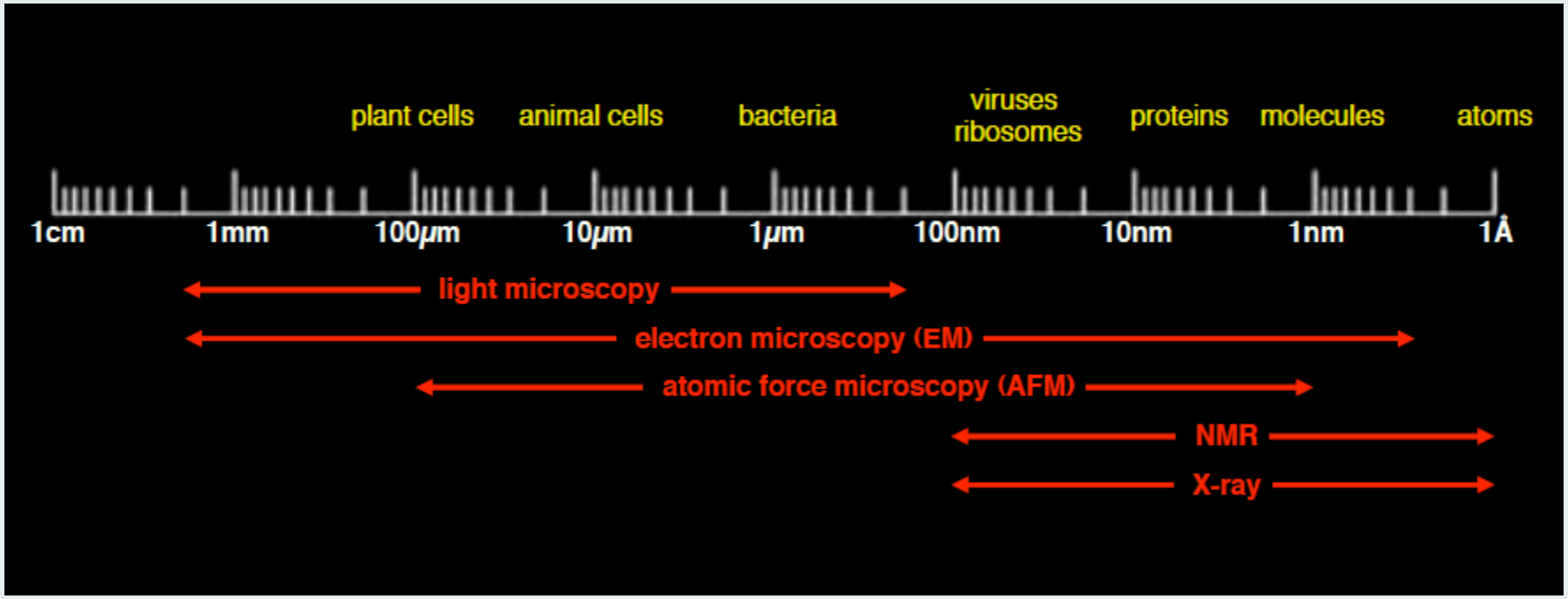
AFM (Atomic Force Microscope)



Puranik et al. *Plant Cell*, 2014

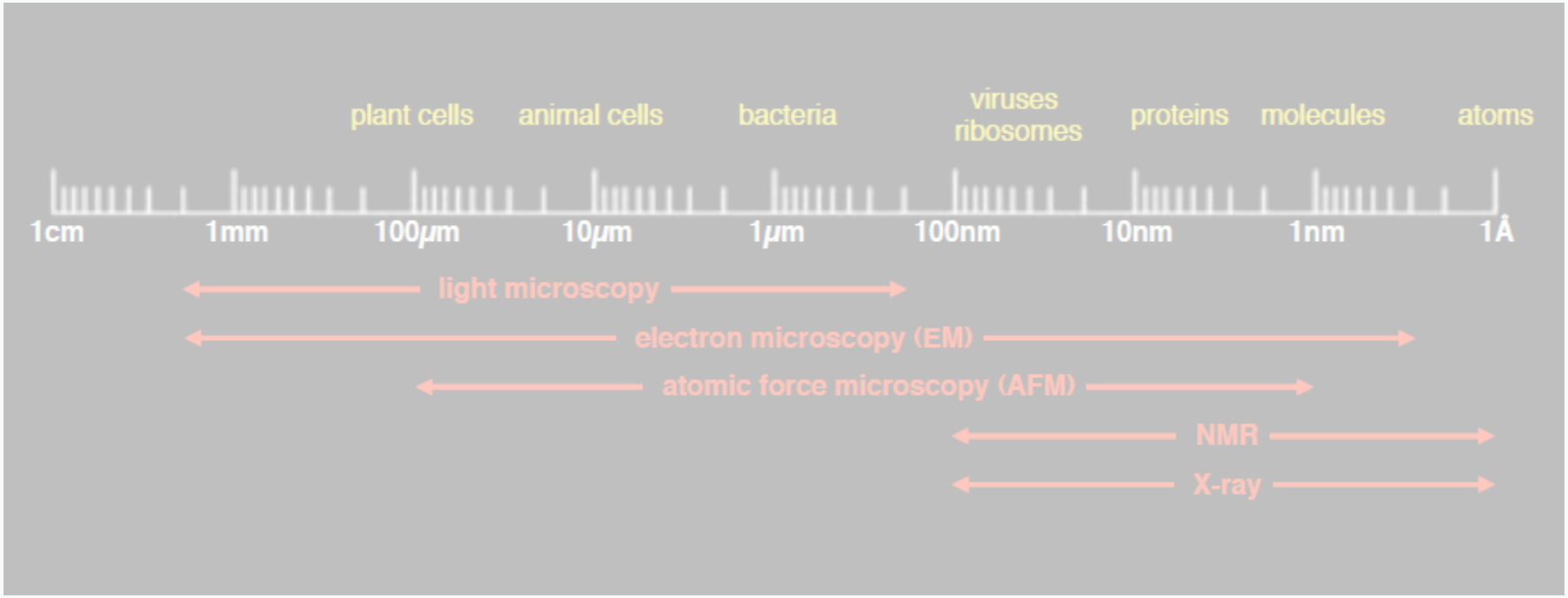
Techniques in Structural Biology

Dimensions in life science – what technique is appropriate



S. Scheuring, AFMBioMed Summer School, Marcoule 2011

Dimensions in life science – what technique is appropriate



S. Scheuring, AFMBioMed Summer School, Marcoule 2011

LENGHTSCALE
TIMESCALE

Outline

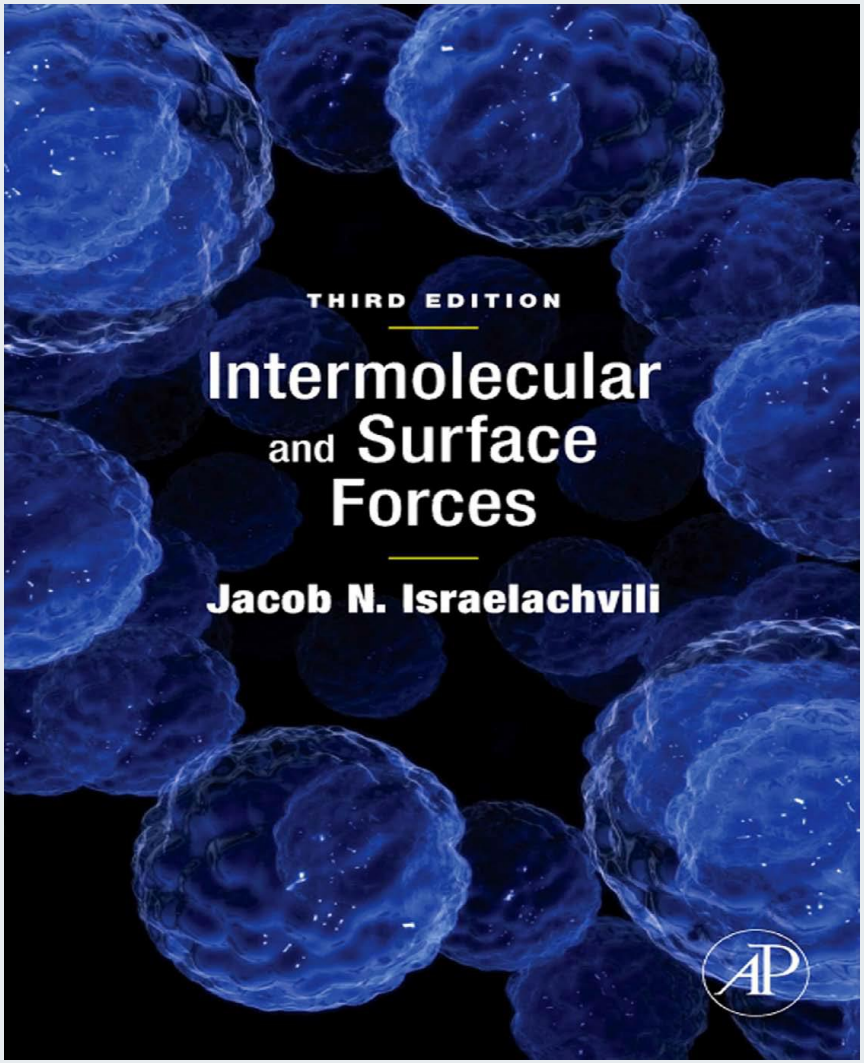
1) Introduction to Scanning Probe Microscopy

2) The Force 

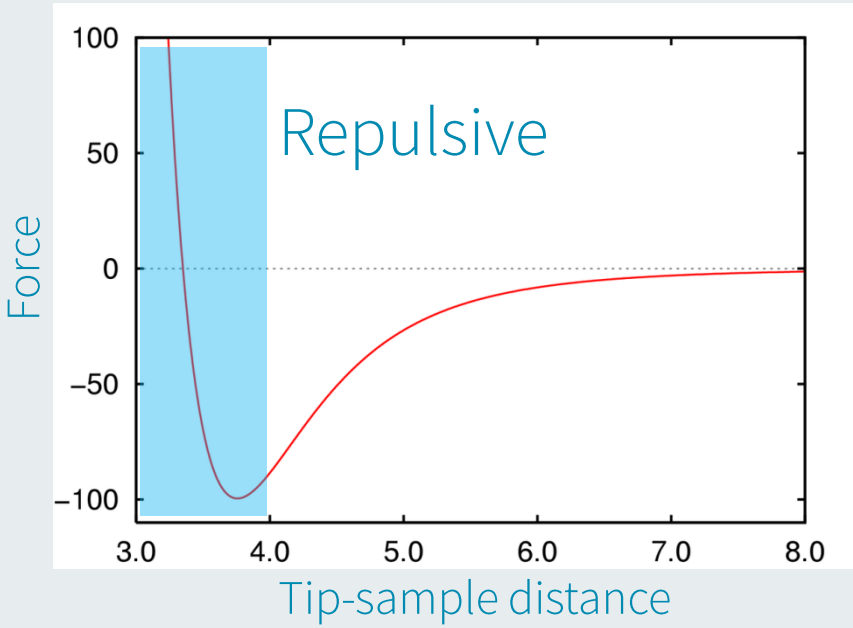
3) Methods
Cantilevers
Static and Dynamic AFM

4) Instruments

5) Advanced and novel AFM methods

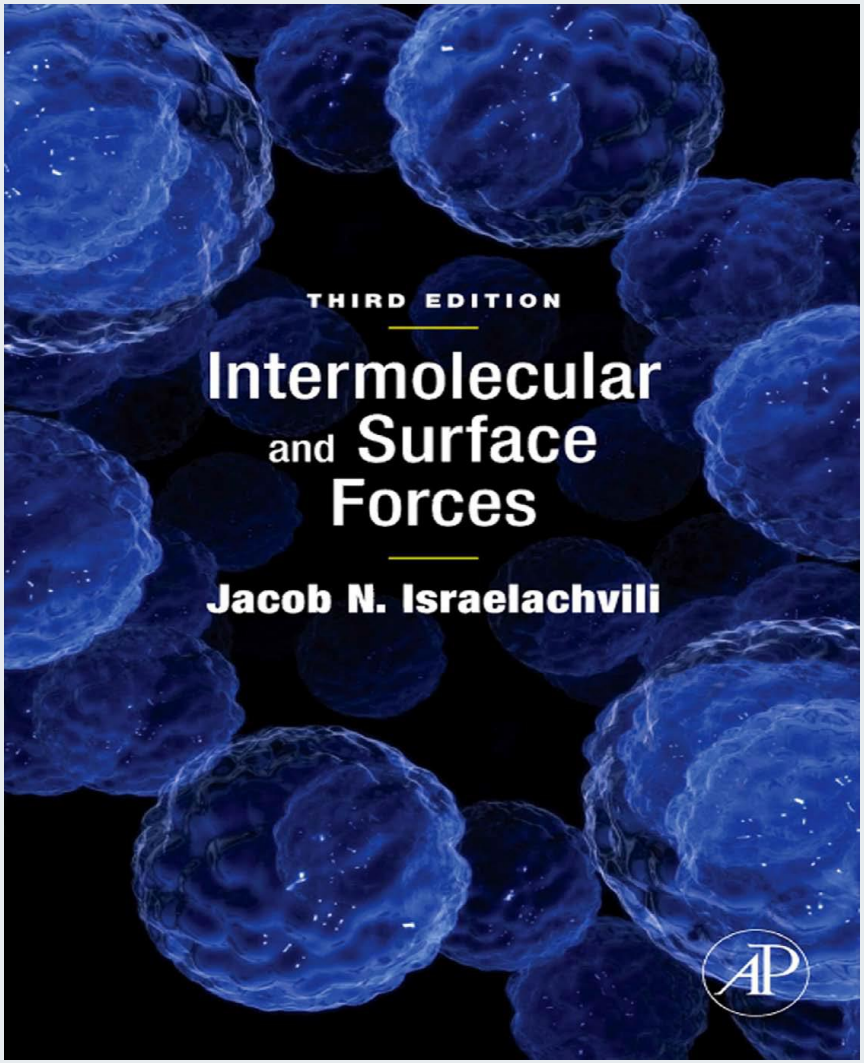


Force

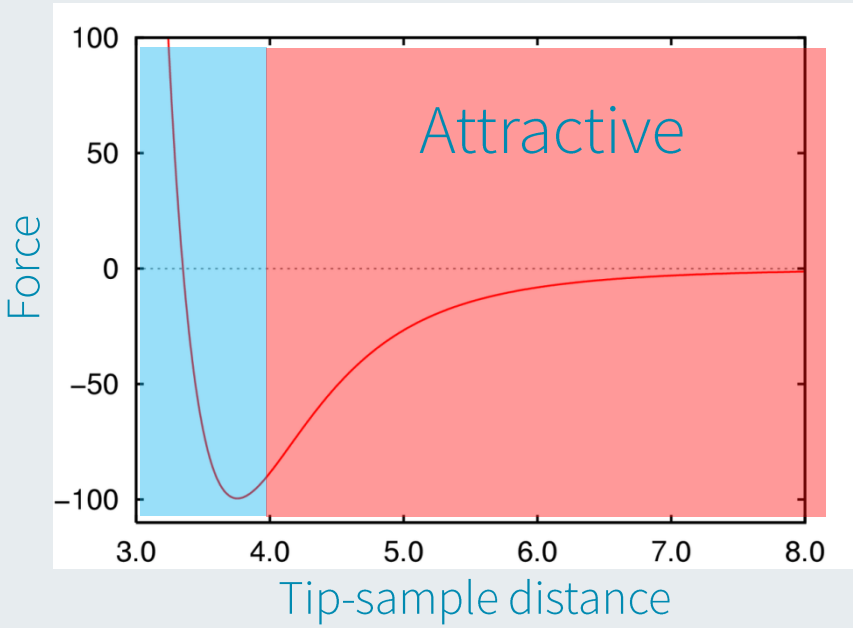


Repulsion due to overlap of tip-sample electronic shells

Interaction Forces at nanoscale



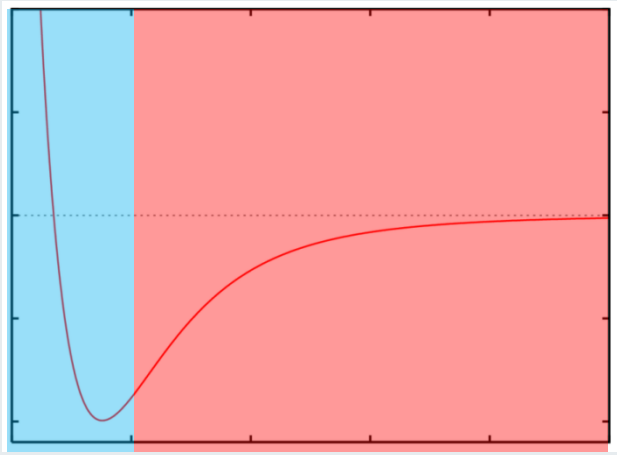
Force



Attraction due to
Van der Waals, capillarity….

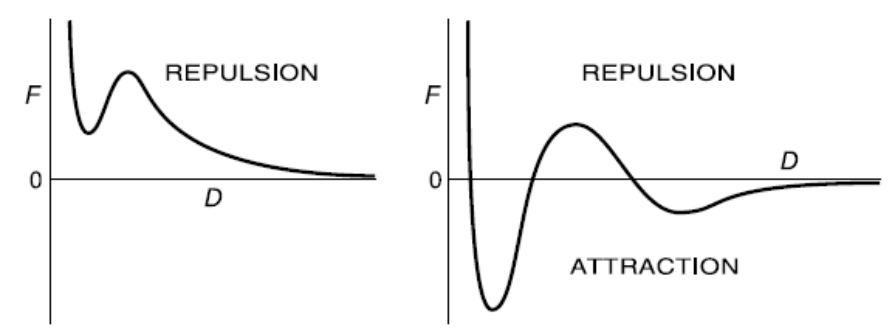
Interaction Forces at nanoscale

Richard P. Feynman (1918 – 1988)



"…If, in some cataclysm, all of scientific knowledge were to be destroyed, and only one sentence passed on to the next generations of creatures, what statement would contain the most information in the fewest words? I believe it is the atomic hypothesis (or the atomic fact, or whatever you wish to call it) that all things are made of atoms—little particles that move around in perpetual motion, attracting each other when they are a little distance apart, but repelling upon being squeezed into one another..."

Interaction Forces at nanoscale



Tip sample Interaction Force depends on

1) Tip and sample geometry

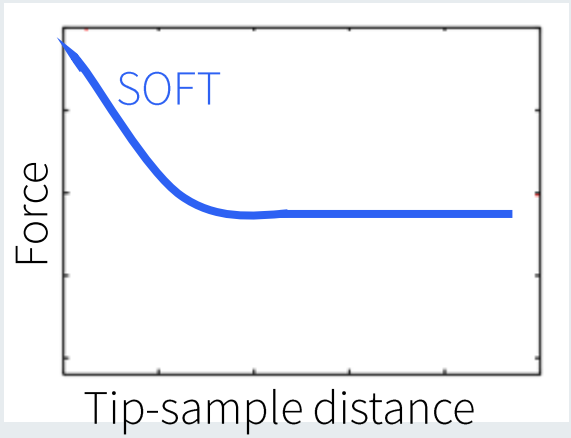
2) Tip and sample nature (chemical affinity, charges)

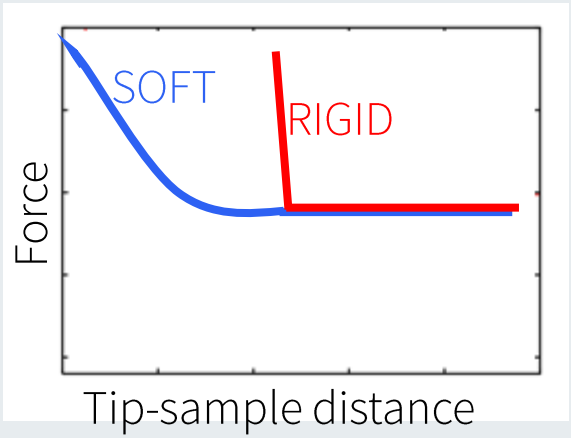
3) Tip and/or Sample stiffness

4) Environment (liquid, air, gas, vacuum)

Interaction Forces at nanoscale

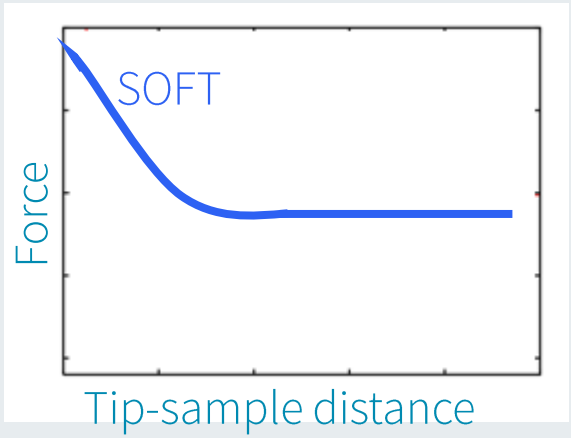
Tip and/or Sample stiffness

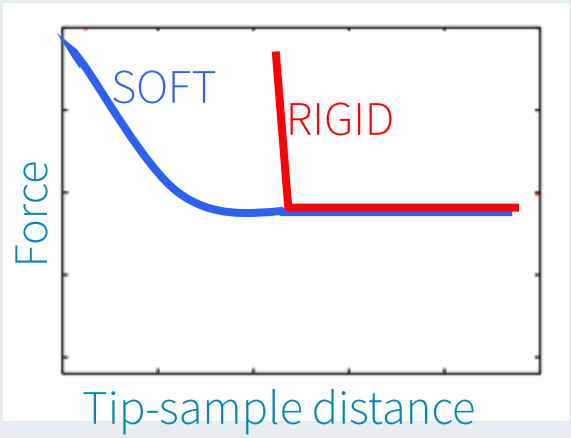




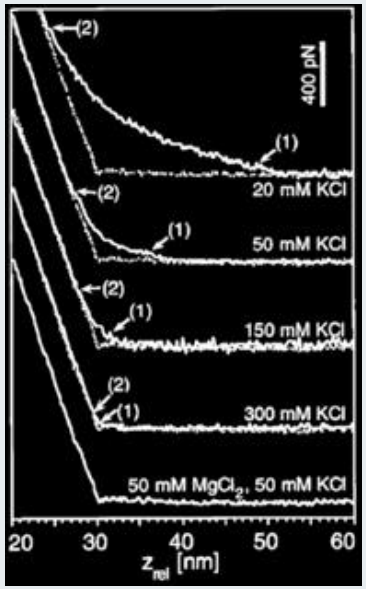
Interaction Forces at nanoscale

Tip and/or Sample stiffness

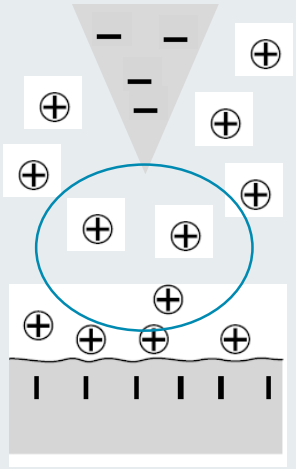




Environment: liquid + salt



IONS ARE IMPORTANT!!!



Daniel J Müller, Dimitrios Fotiadis, Simon Scheuring, Shirley A Müller & Andreas Engel *Electrostatically balanced subnanometer imaging of biological specimens by atomic force microscopy Biophys J*, 1999, 76 (2): 1101-1111

Outline

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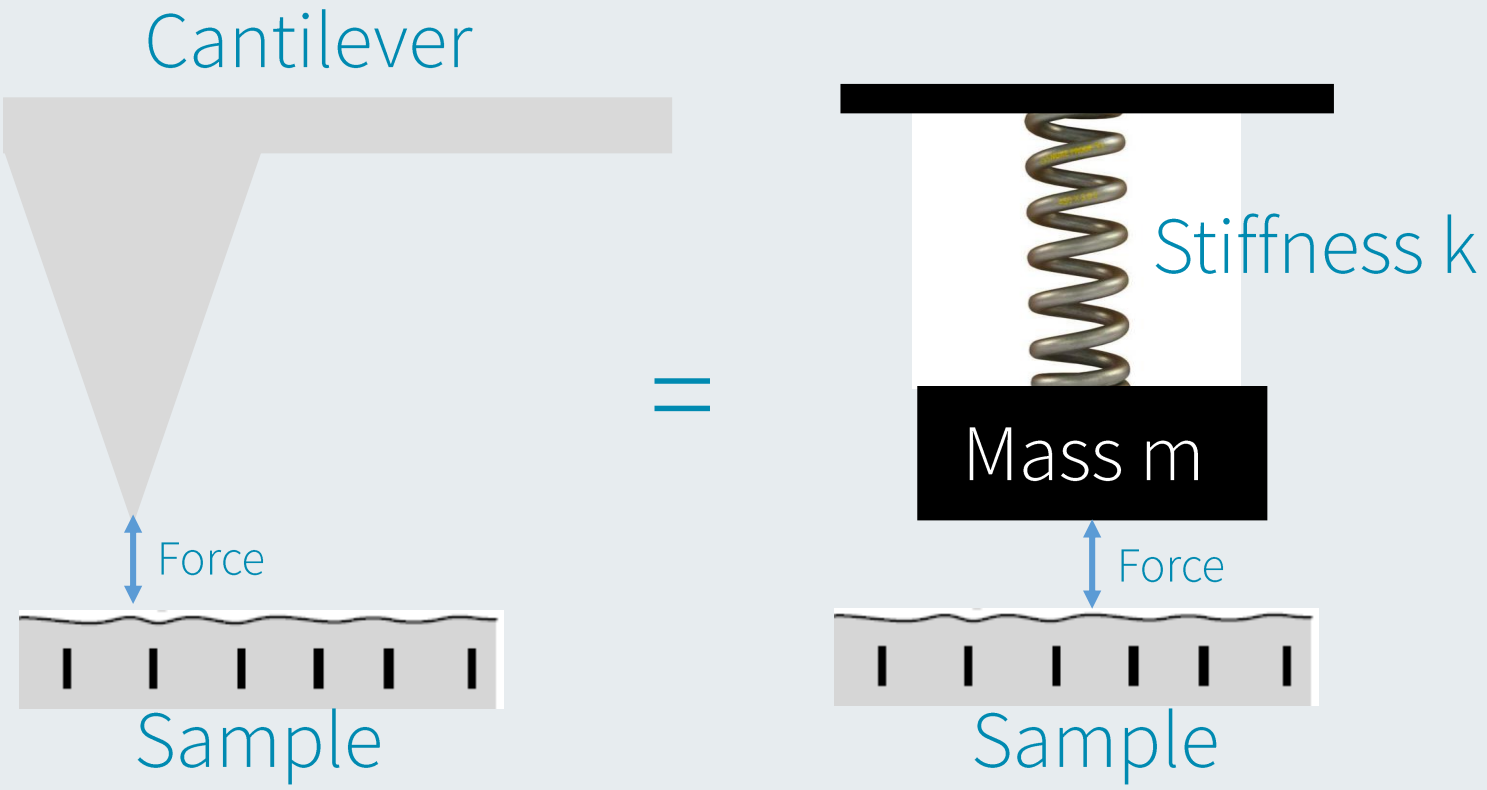
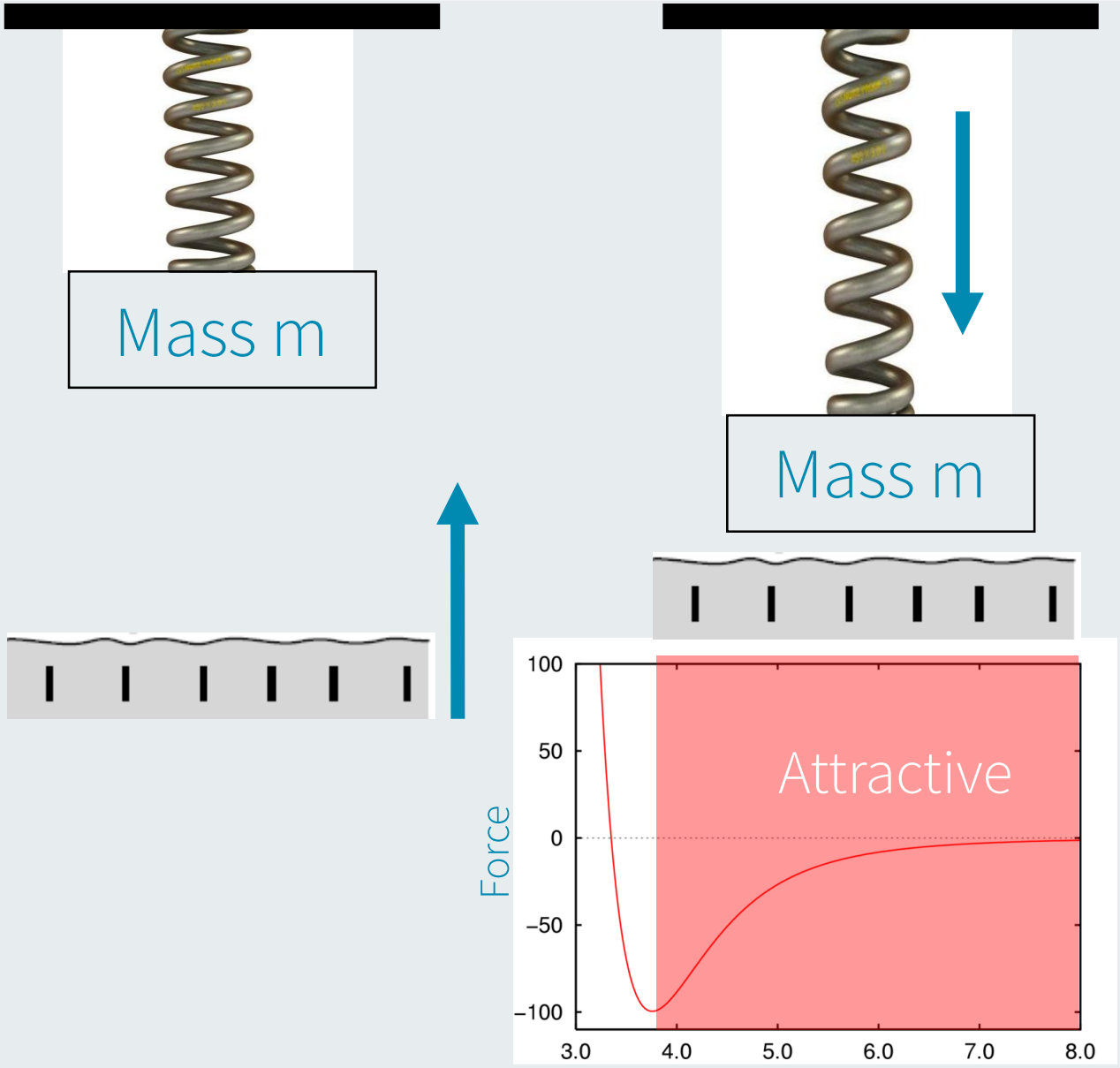
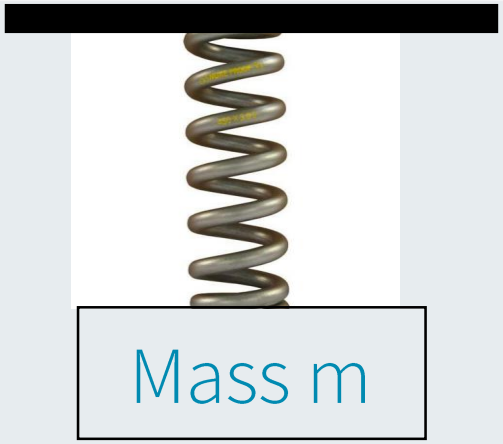
Cantilevers

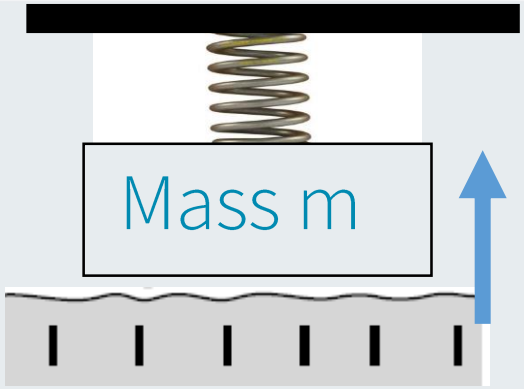


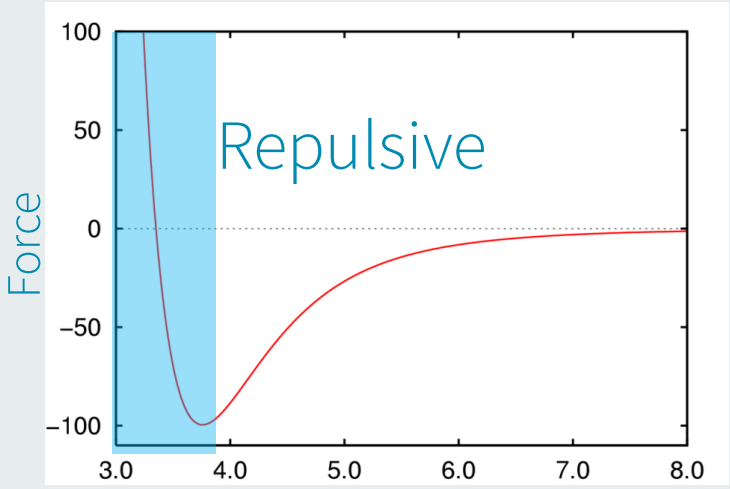
Cantilevers



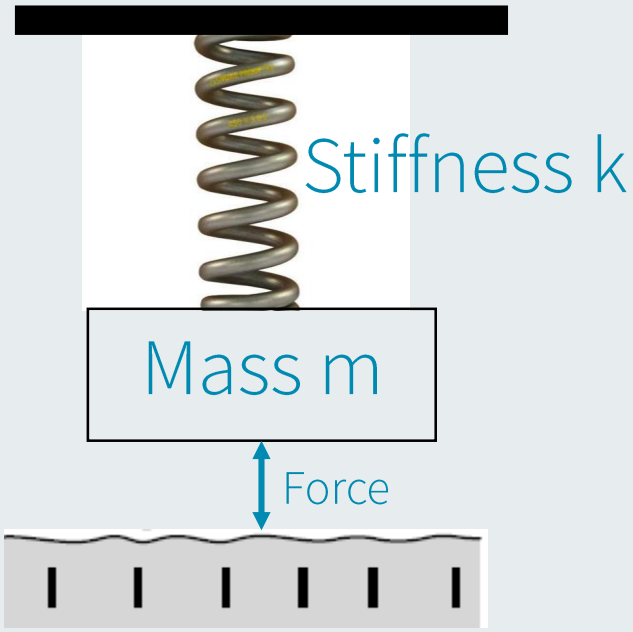
Cantilevers

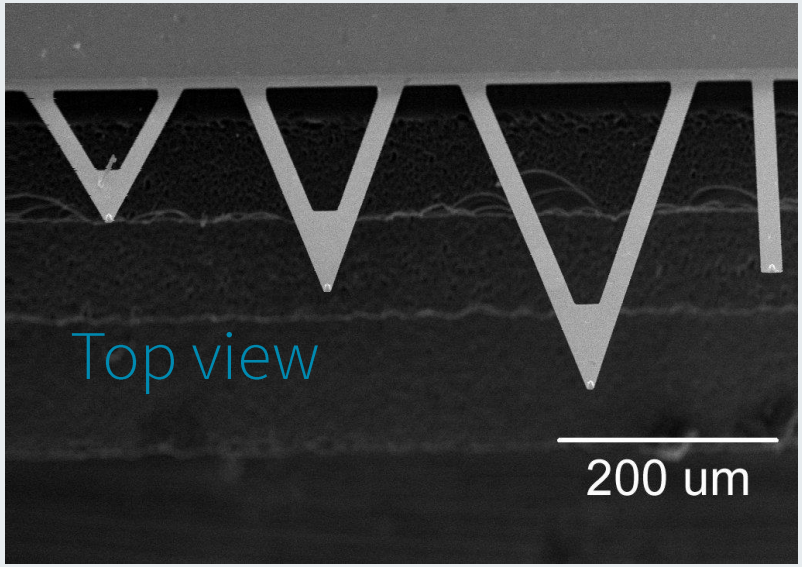


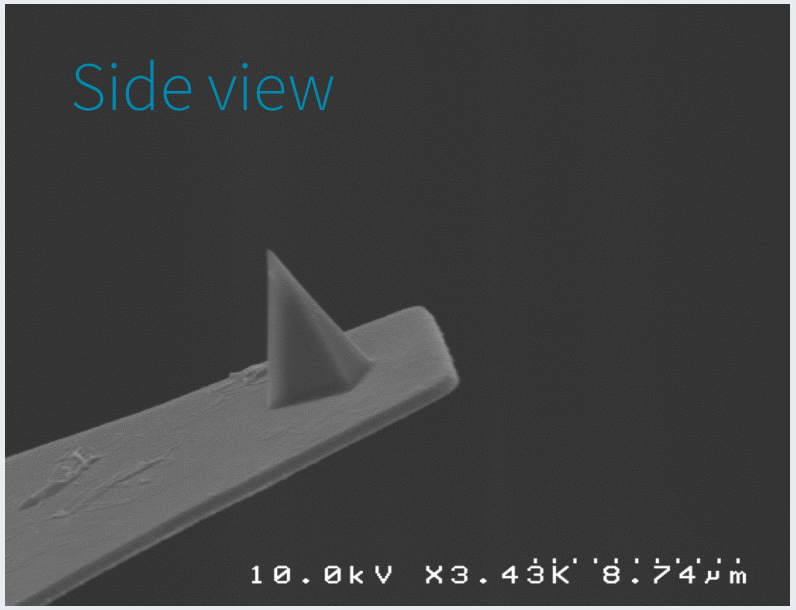


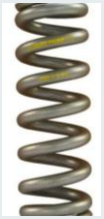


Cantilevers



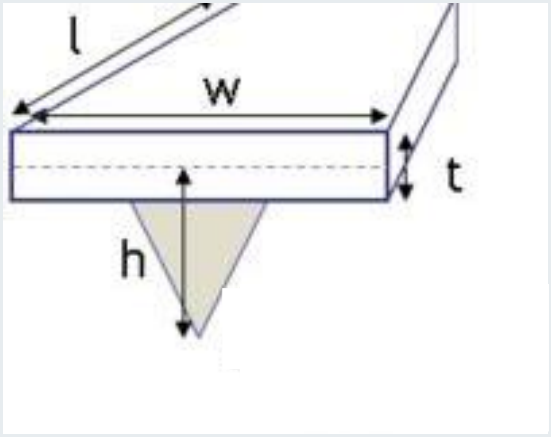






Stiffness k: from 0.006 N/m to 100 N/m

Extrinsic property



$$k = \frac{Ewt^3}{4L^3}$$

k must be calibrated !!

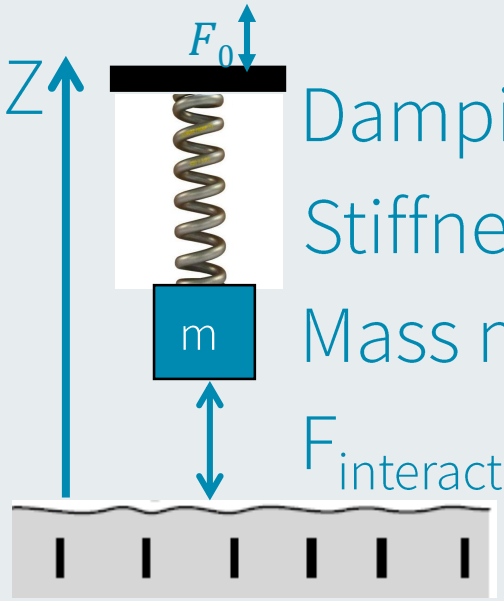
E = Young's modulus [Pa] intrinsic property

Length L: from 10 µm to 500 µm

Materials: Silicon and Silicon Nitride

Cantilevers and harmonic oscillators

$$m\ddot{z} + \delta\dot{z} + kz = F_{interaction}(z) + F_0(\omega t)$$



Damping δ [N s /m]: environment, viscosity

Stiffness k [N/m]

Mass m [Kg]

$F_{interaction}$ [N]

Cantilevers and harmonic oscillators

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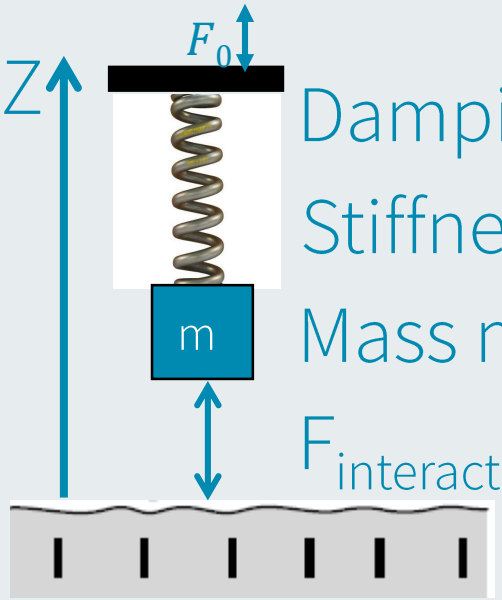

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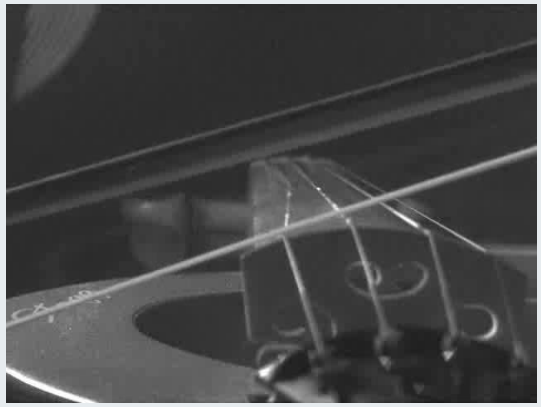

STATIC AFM: tip in permanent contact with the sample

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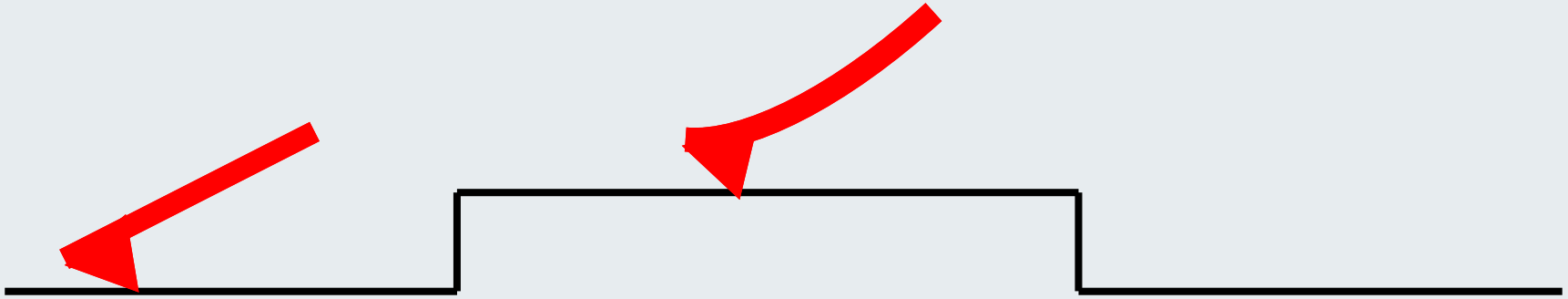

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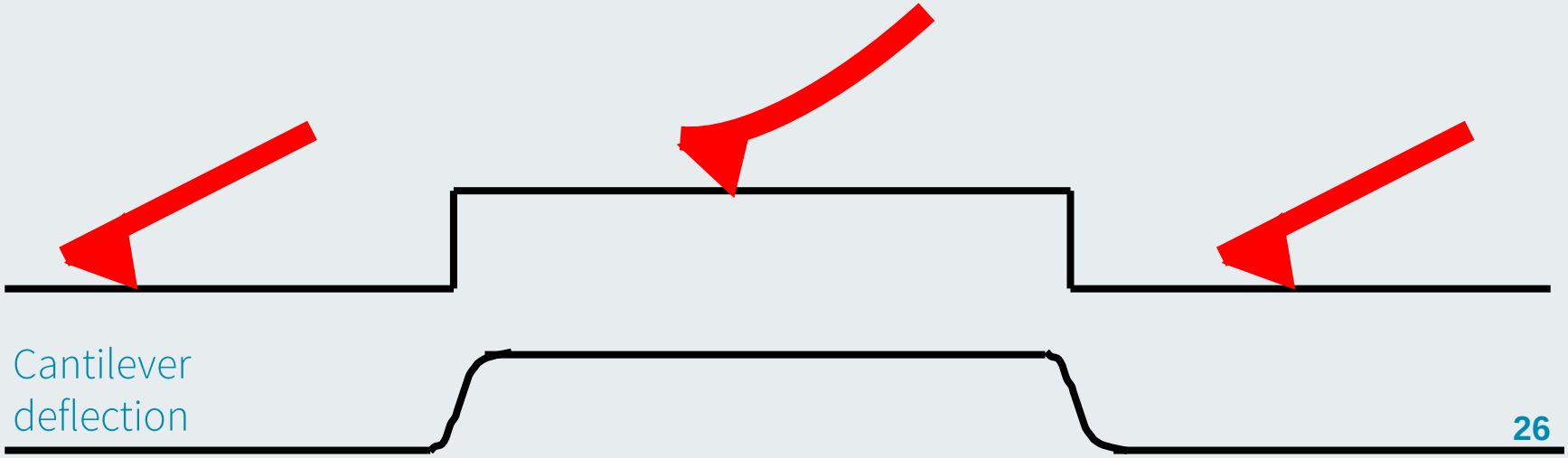
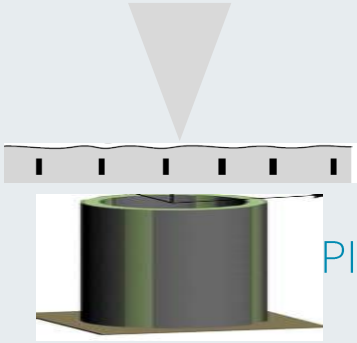


Static AFM mode (contact mode) – The closed loop



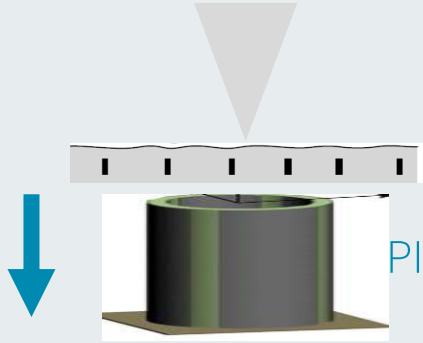
PIEZO: X/Y/Z scanner

Scan direction

Cantilever deflection

Z scanner position

Static AFM mode (contact mode) – The closed loop



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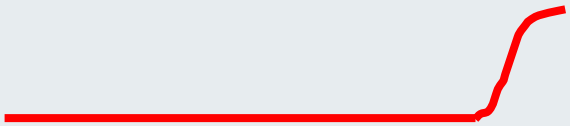

Cantilever deflection

Z scanner position

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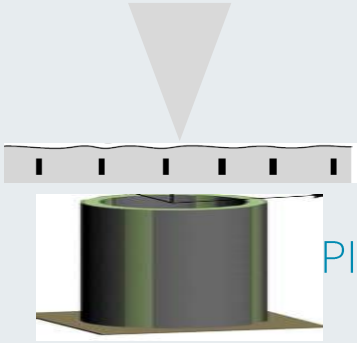

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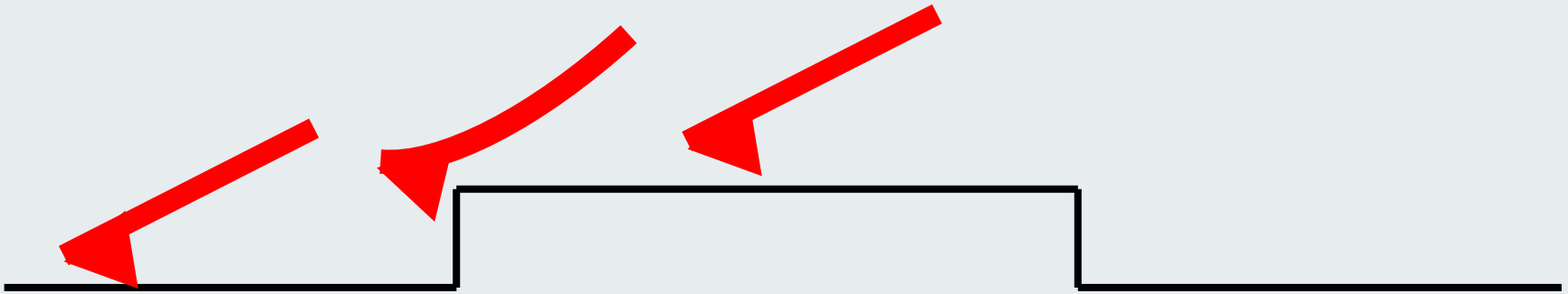

Cantilever deflection

Z scanner position

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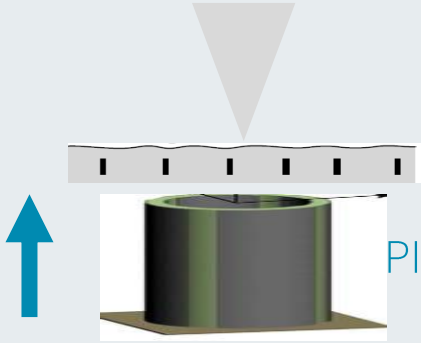
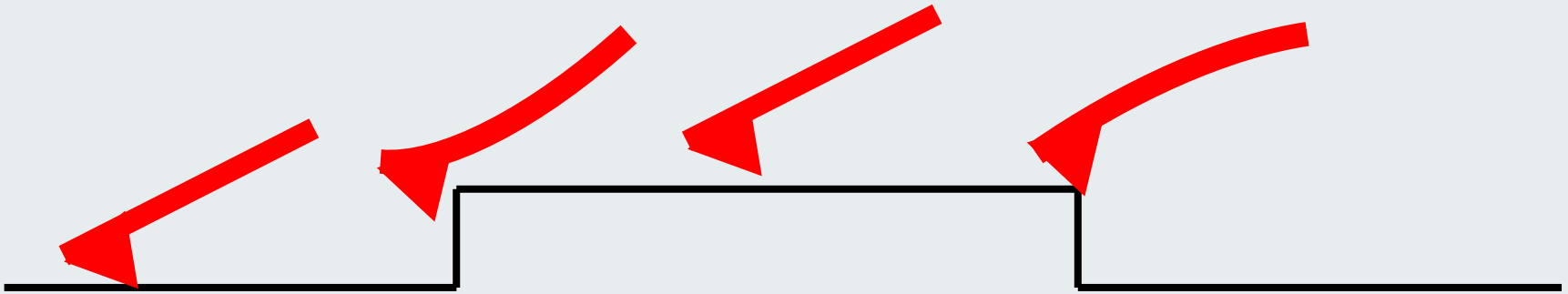
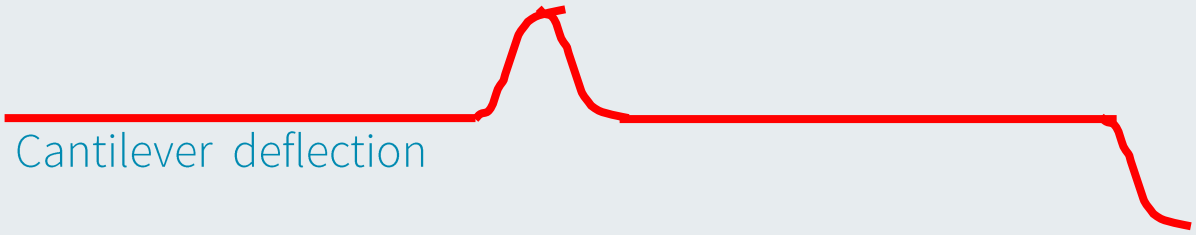


PIEZO: X/Y/Z scanner

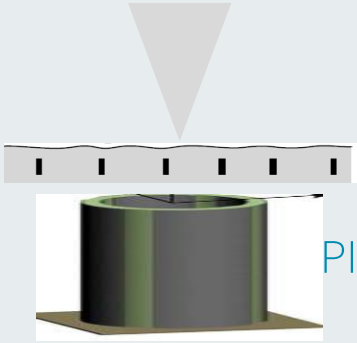




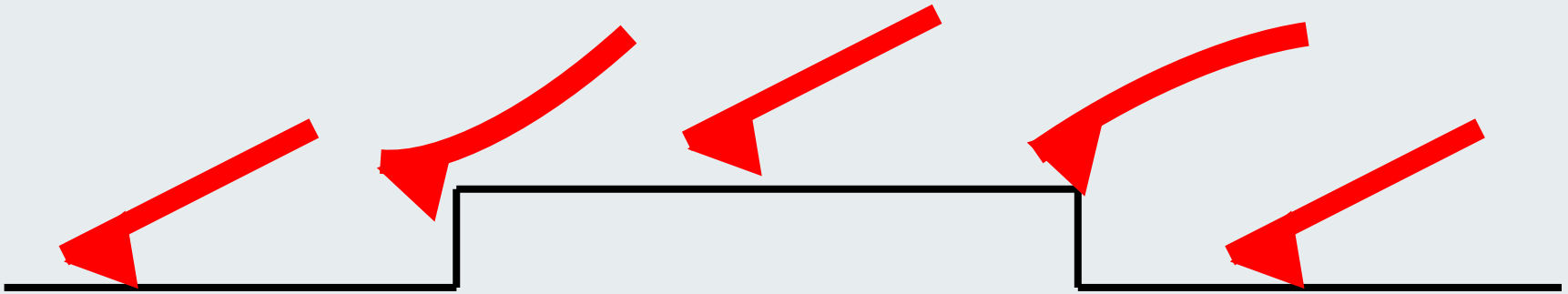
Cantilever deflection

Z scanner position

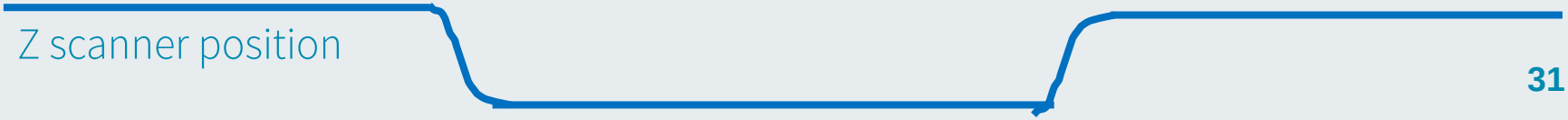
Static AFM mode (contact mode) – The closed loop



PIEZO: X/Y/Z scanner

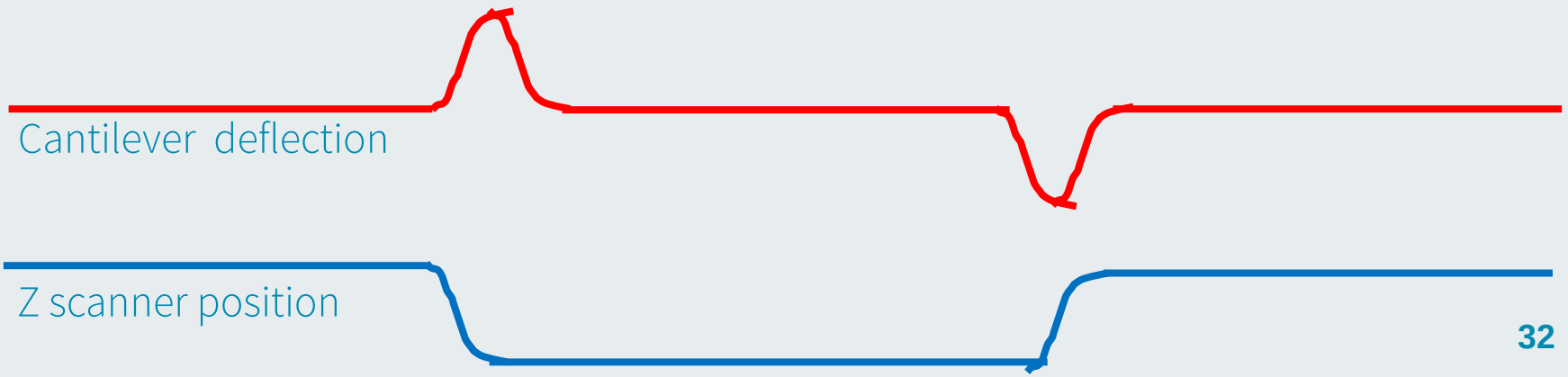


Cantilever deflection

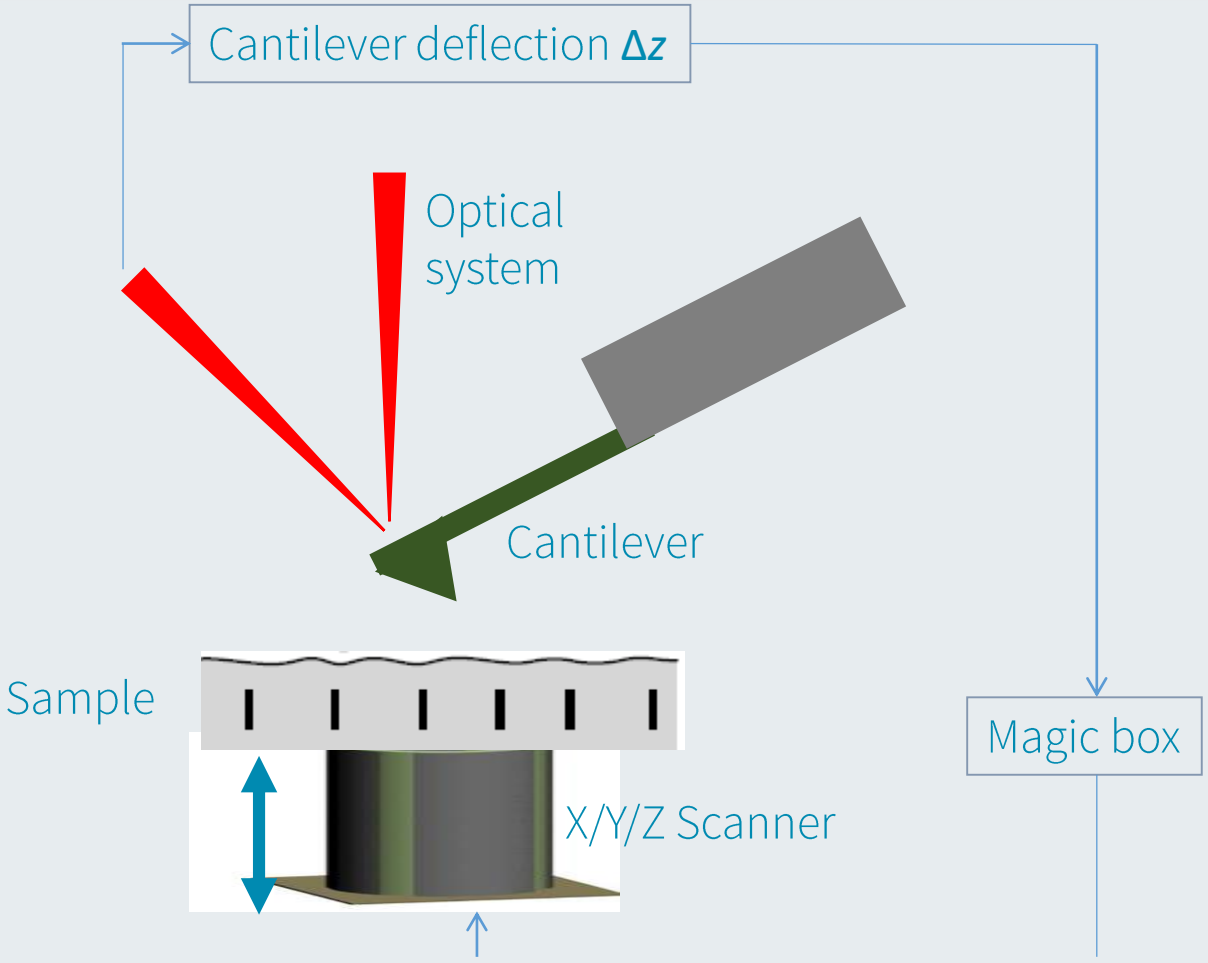


Z scanner position

Constant deflection



Static AFM mode (contact mode) – Operational scheme

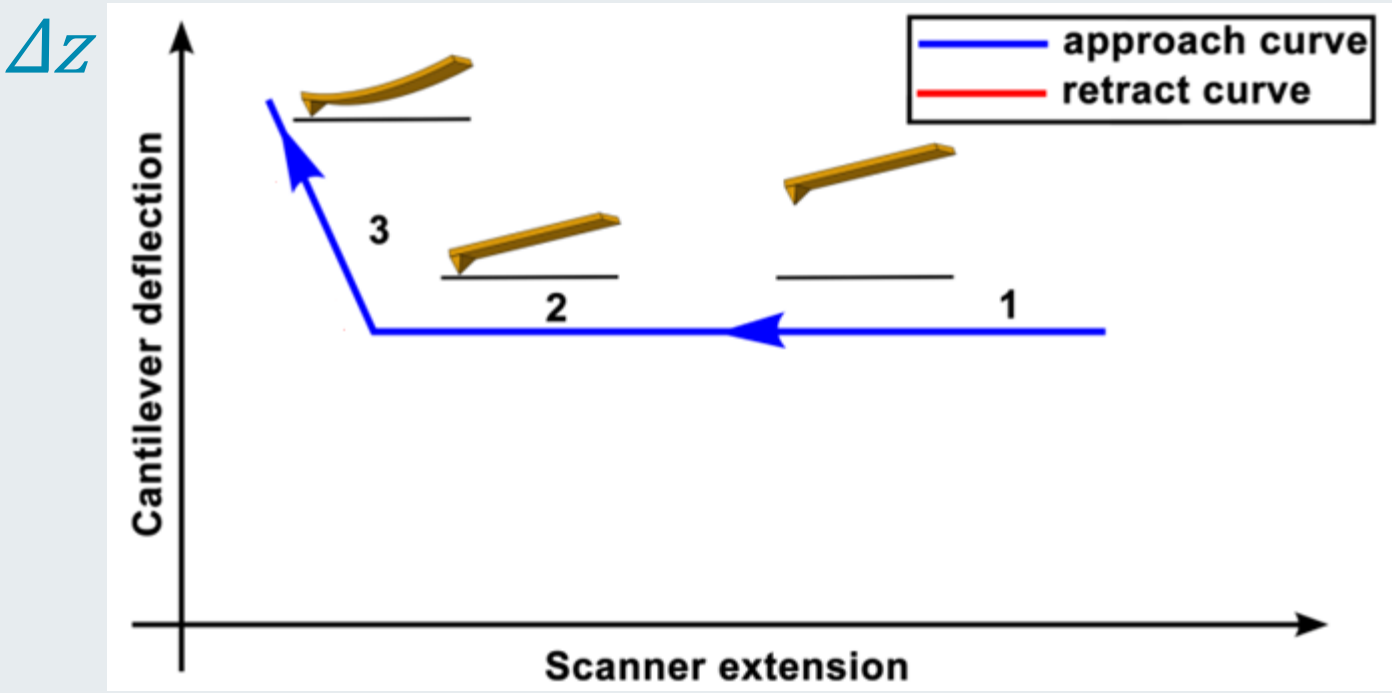


$$m\ddot{z} + \delta\dot{z} + kz = F_{interaction}(z) + F_0(\omega t)$$

$$\ddot{z} = 0, \dot{z} = 0, F_0(\omega t) = 0 \rightarrow \boxed{F_{interaction}(z) = k\Delta z}$$

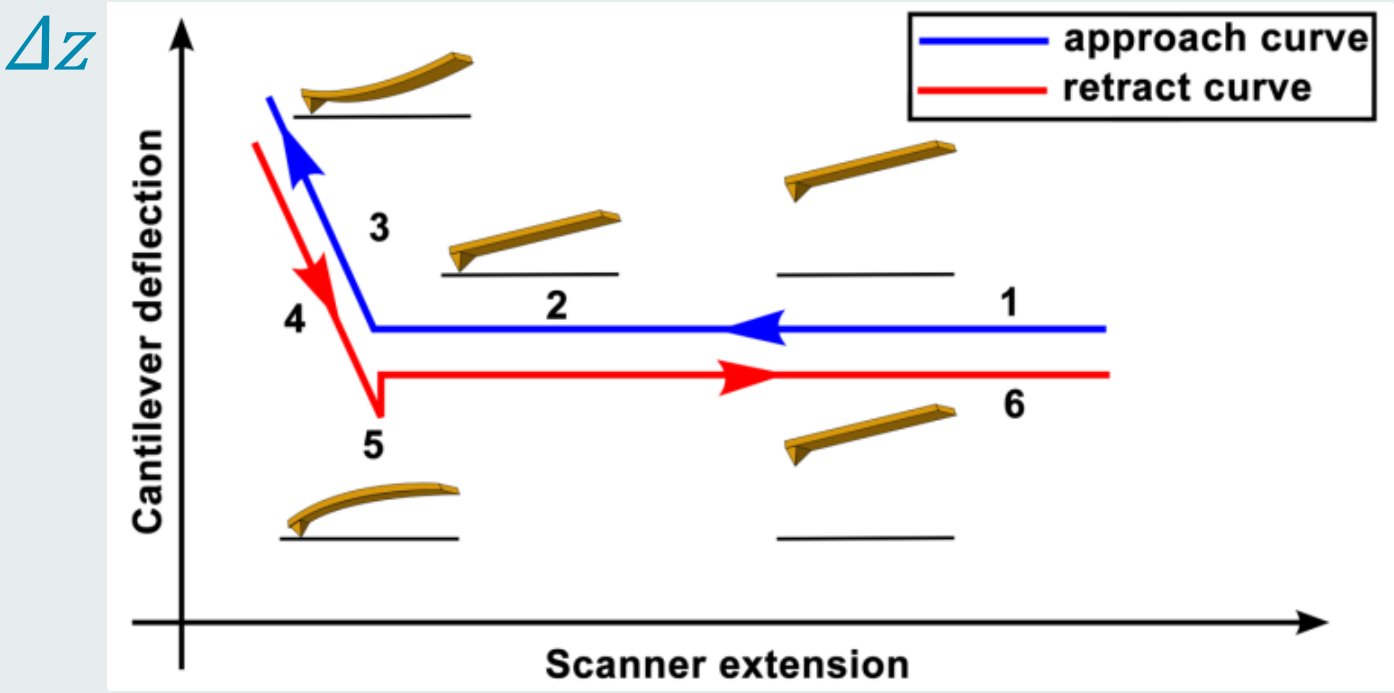
Hook Law

Static AFM mode (contact mode) – Force curves



Adapted from Polymer Science, Chapter 4, U. Maver et al., Intech, 2013

Static AFM mode (contact mode) – Force curves



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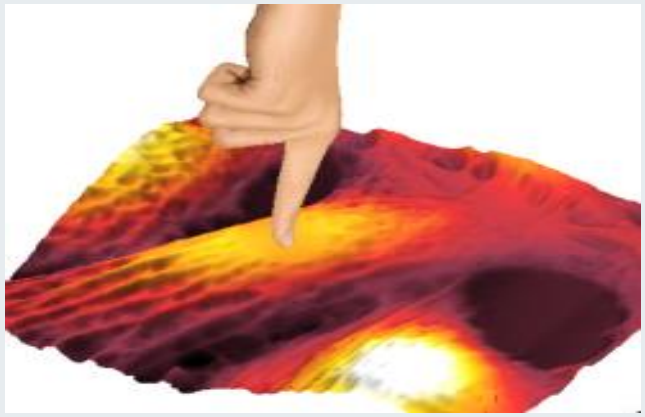
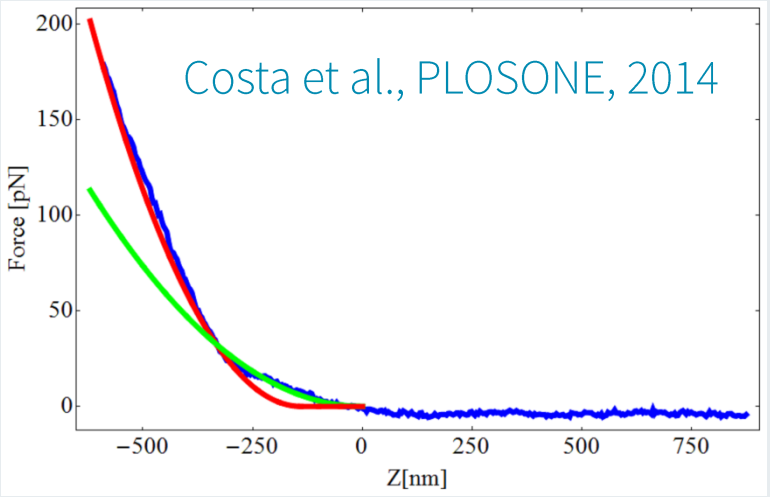
H.-J. Butt, B. Cappella and M. Kappl,
Surface Science Reports, 59, 1-152, 2005

Static AFM mode (contact mode) – Force curves

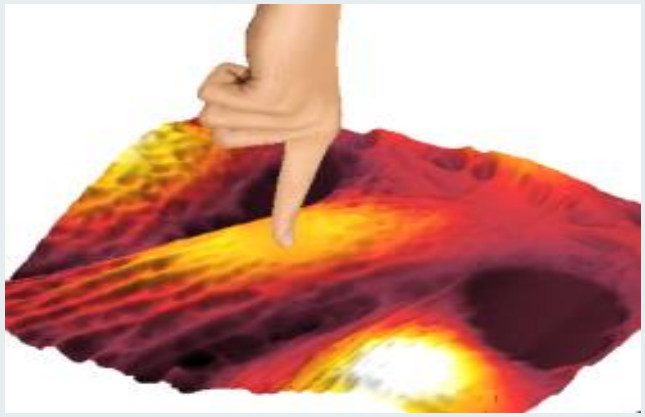
Mechanics

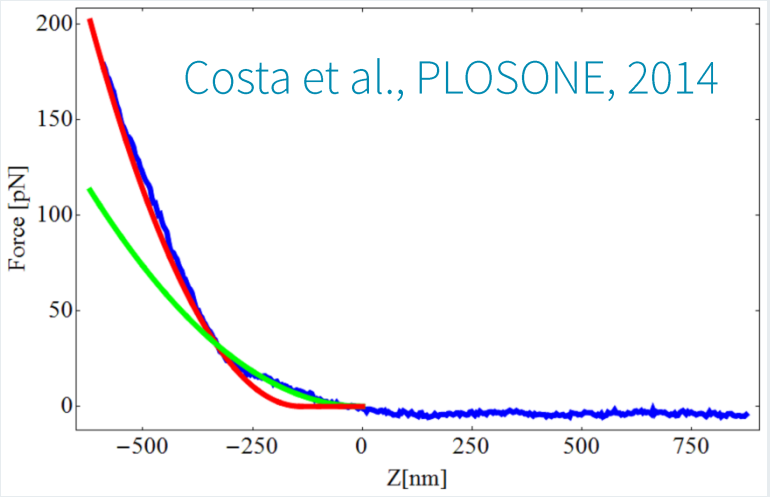




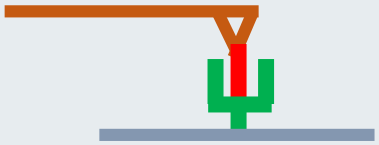
Static AFM mode (contact mode) – Force curves

Mechanics





Molecular recognition

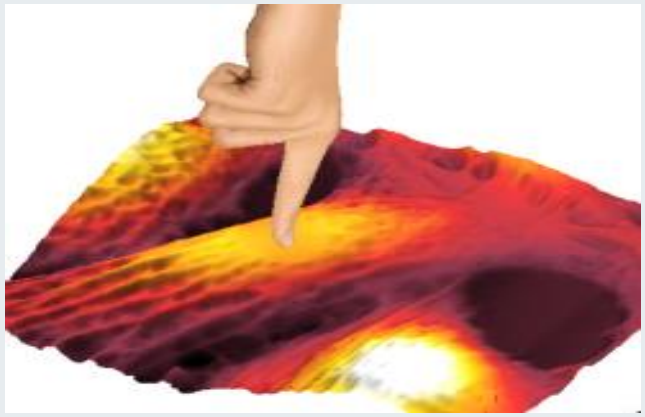


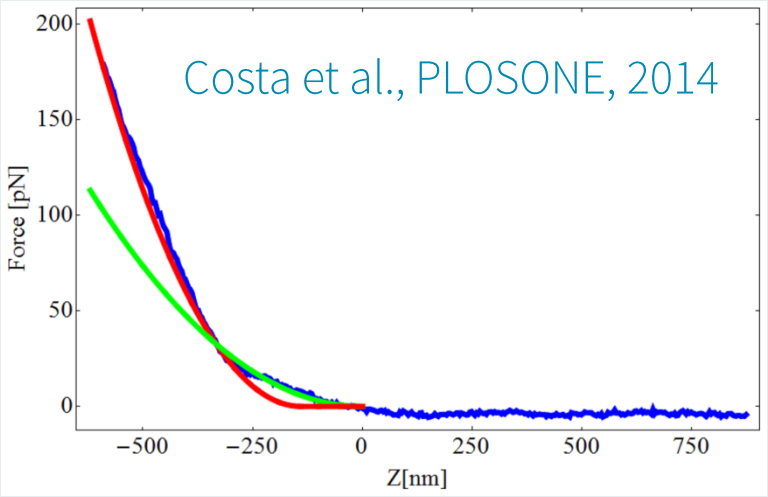
Ligand

Receptor

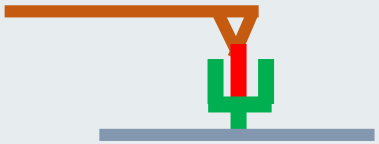
Static AFM mode (contact mode) – Force curves

Mechanics





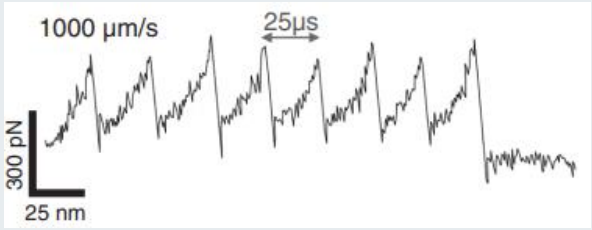
Molecular recognition



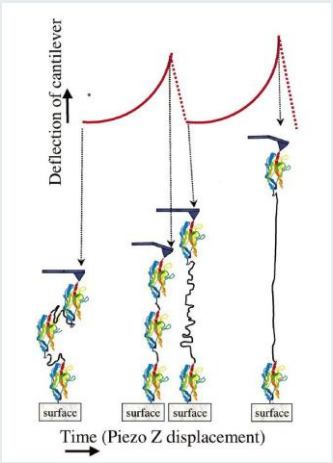
Ligand

Receptor

Protein unfolding



F. Rico et al. Science, 342(6159), 741-743, 2013



Luigi Bubacco website

Outline

1) Introduction to Scanning Probe Microscopy

2) The Force

3) Methods

Cantilevers

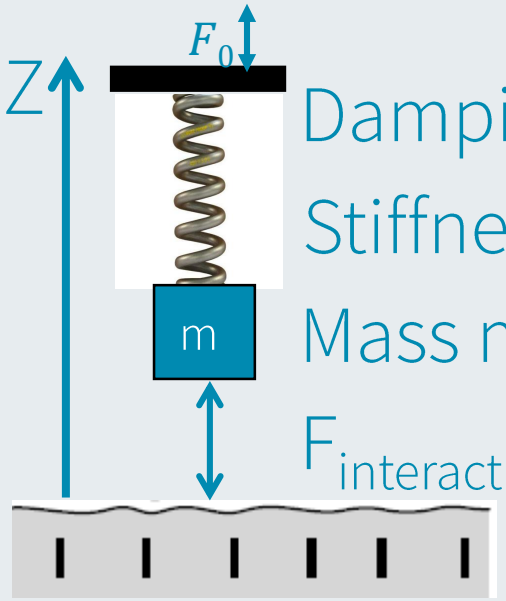
Static and Dynamic AFM

4) Instruments

5) Advanced and novel AFM methods

Dynamic AFM mode (Amplitude Modulation mode) – AM-AFM

$$m\ddot{z} + \delta\dot{z} + kz = F_{interaction}(z) + F_0(\omega t)$$



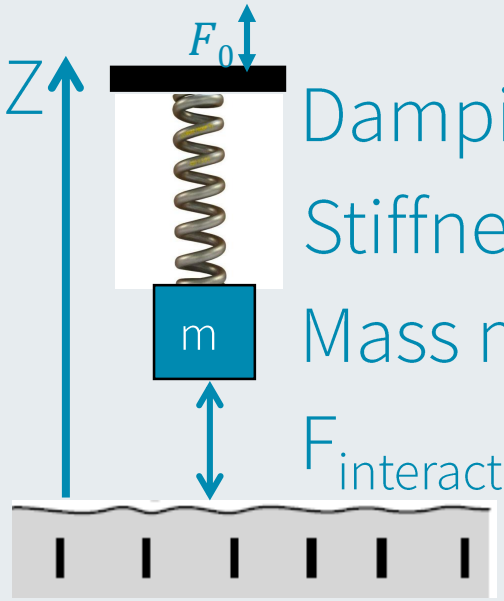
Damping δ [N s /m]: environment, viscosity

Stiffness k [N/m]

Mass m [Kg]

$F_{interaction}$ [N]

$$m\ddot{z} + \delta\dot{z} + kz = F_{interaction}(z) + F_0(\omega t)$$



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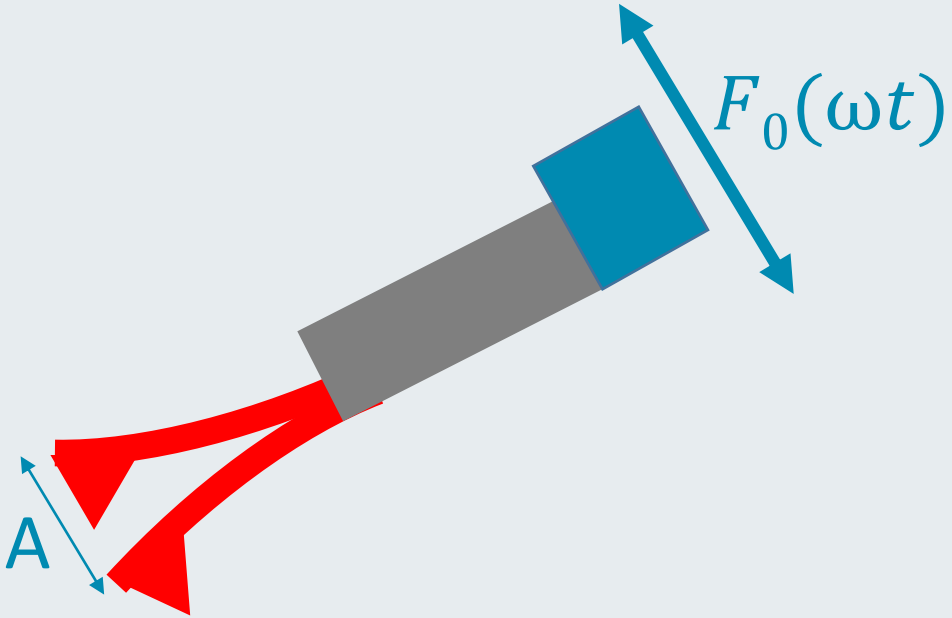

Resonance frequency

$$\omega_R = \sqrt{\frac{k}{m}}$$

$$m\ddot{z} + \delta\dot{z} + kz = F_{interaction}(z) + F_0(\omega t)$$

$$F_{interaction}(z) = 0 \quad meaning\ no\ sample$$

$$solution:\ z = z_0 e^{i\omega t} = z_0\left(\cos(\omega t) + \mathrm{i}\sin(\omega t)\right)$$

$$z_0 = F_0 / (-m\omega^2 + i\delta\omega + k)$$

Dynamic AFM mode – Harmonic Oscillator

$$z_0 = F_0 / (-m\omega^2 + i\delta\omega + k)$$

$$z = a + ib$$

Modulus: $|z| = \sqrt{a^2 + b^2}$

Phase: $\varphi = 2\arctan\left(\frac{b}{a+\sqrt{a^2+b^2}}\right)$

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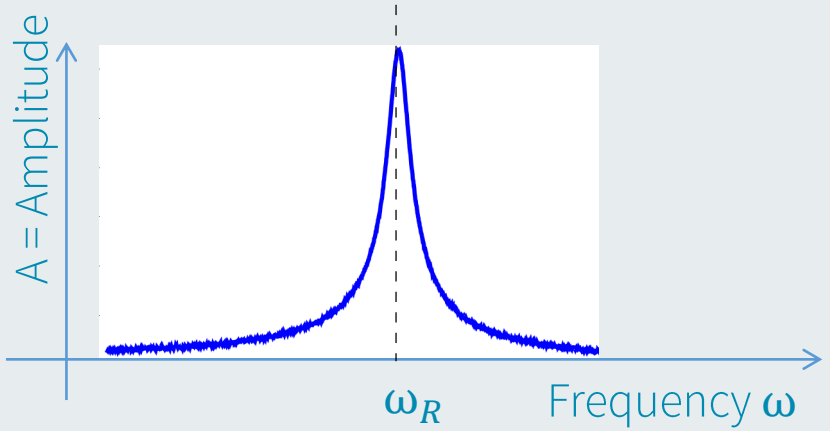
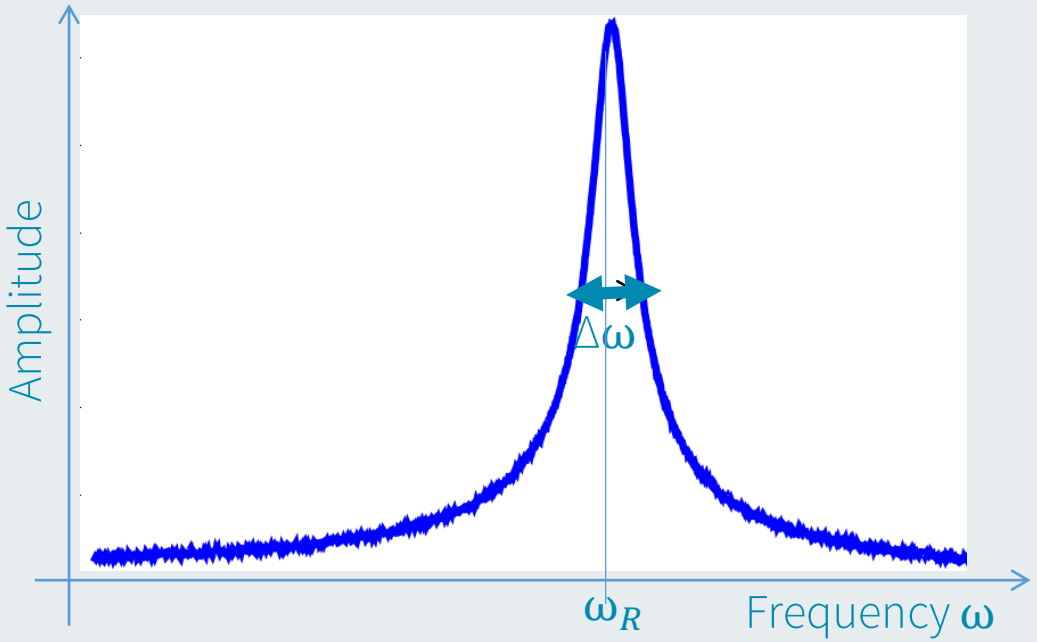
$$A = |z| = \frac{\frac{F_0}{m}}{\sqrt{(\omega_R{}^2 - \omega^2)^2 + \omega^2\delta^2}}$$

$$\text{Phase} = \arctan(\varphi) = \frac{\delta\omega}{\omega_R{}^2 - \omega^2}$$



Dynamic AFM mode – Harmonic Oscillator (Q factor)

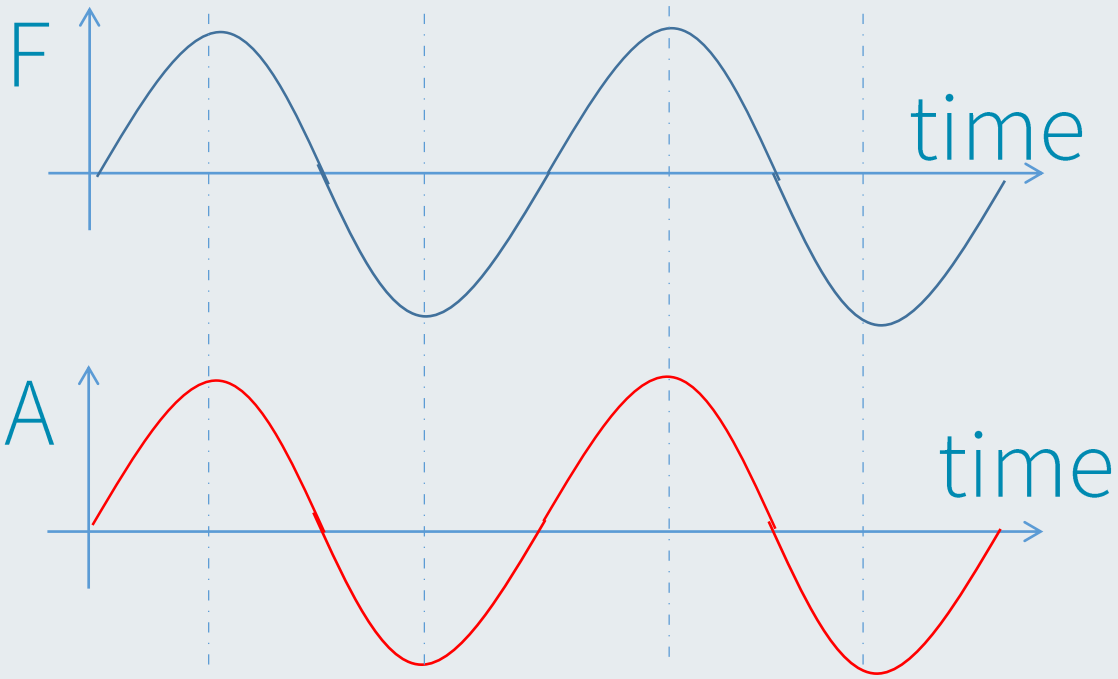


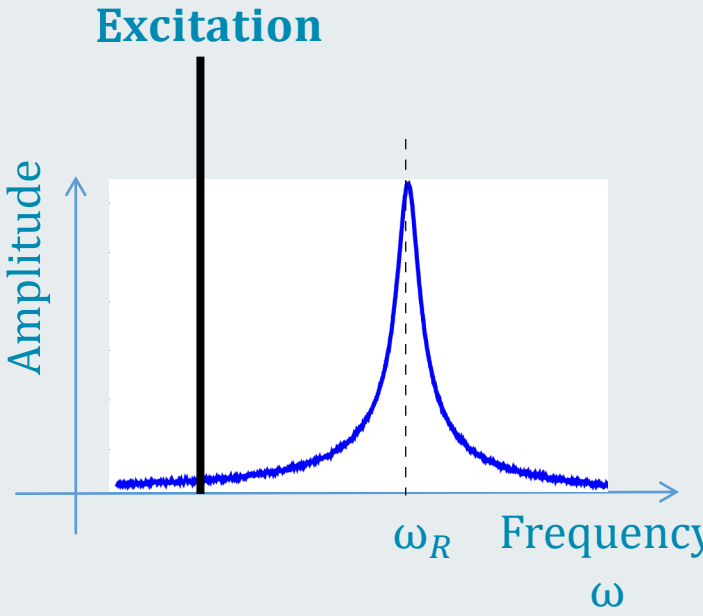
Q factor

$$Q = \frac{\omega_R}{\Delta\omega}$$

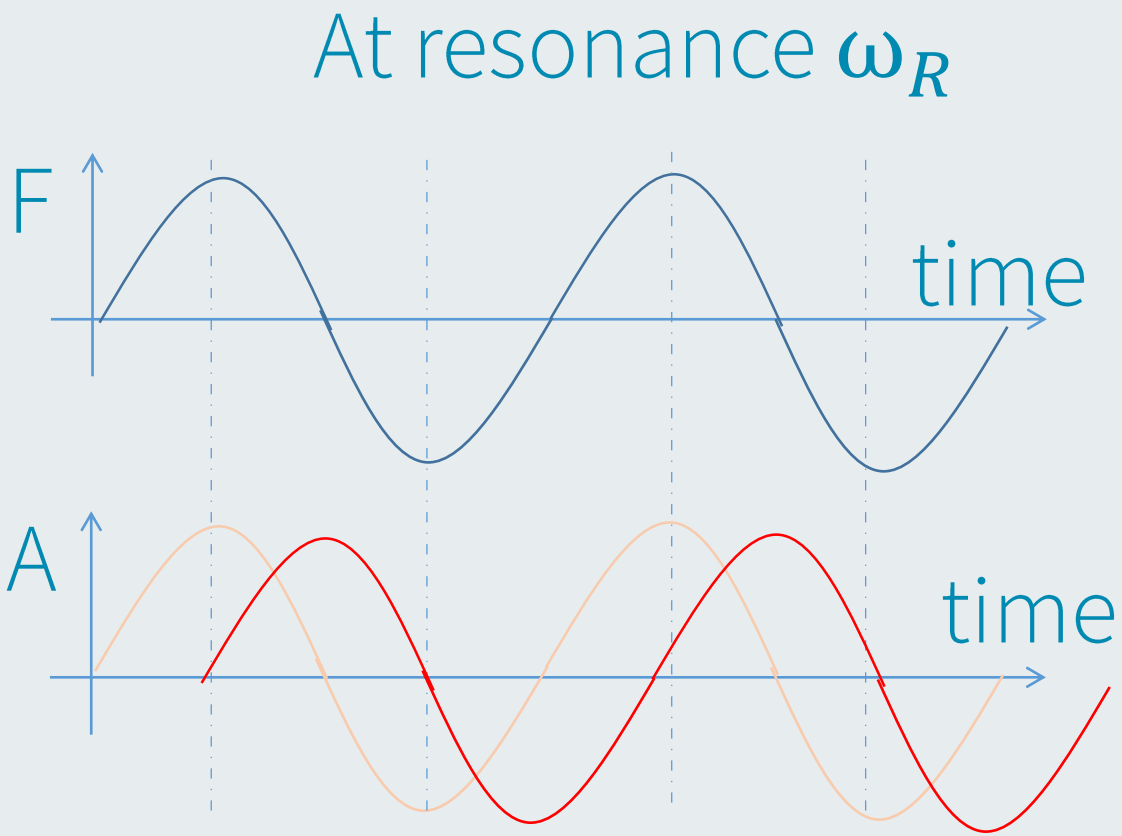
Dynamic AFM mode – Harmonic Oscillator (phase)

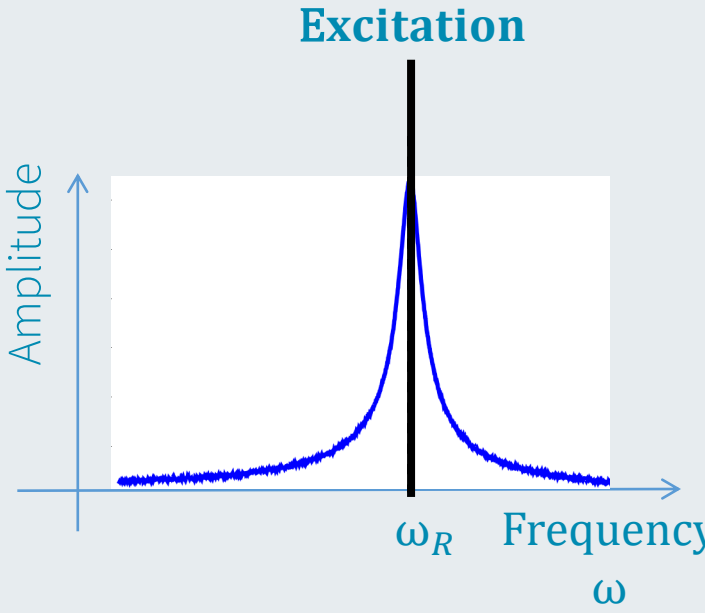
At low frequency





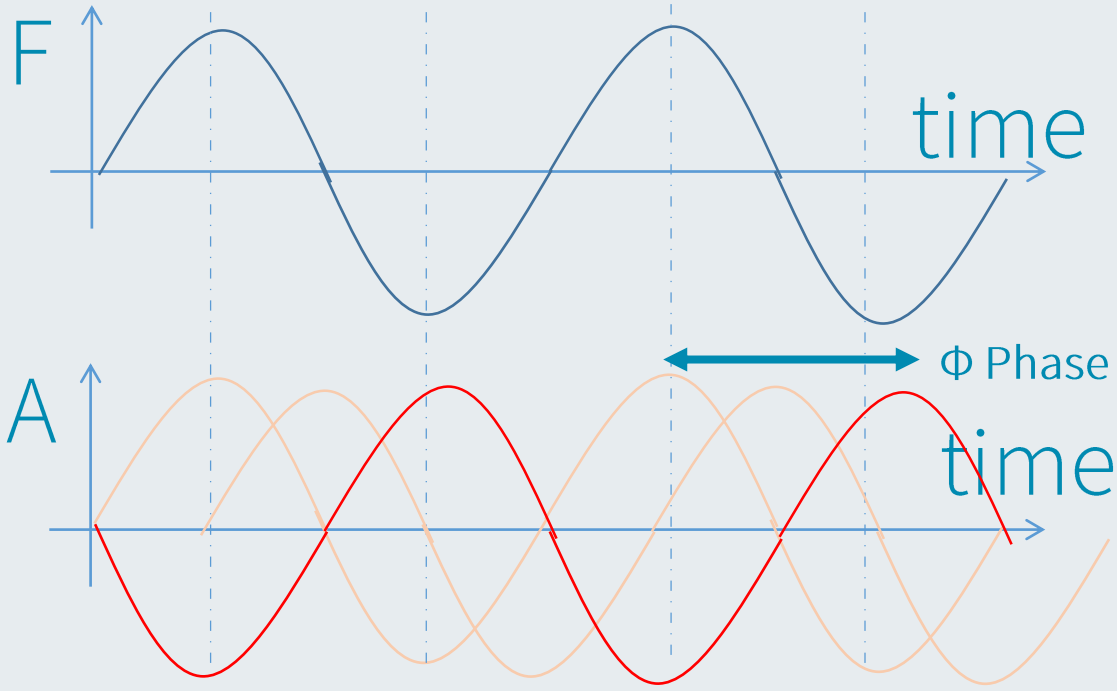
Dynamic AFM mode – Harmonic Oscillator (phase)

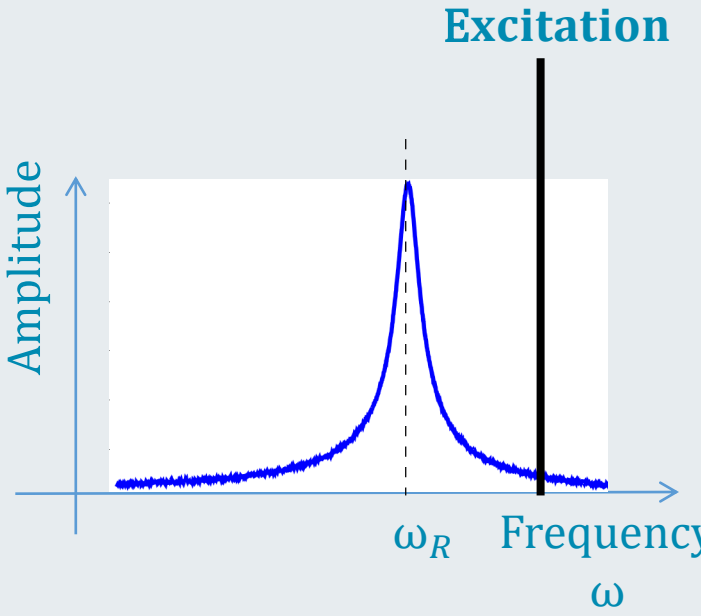




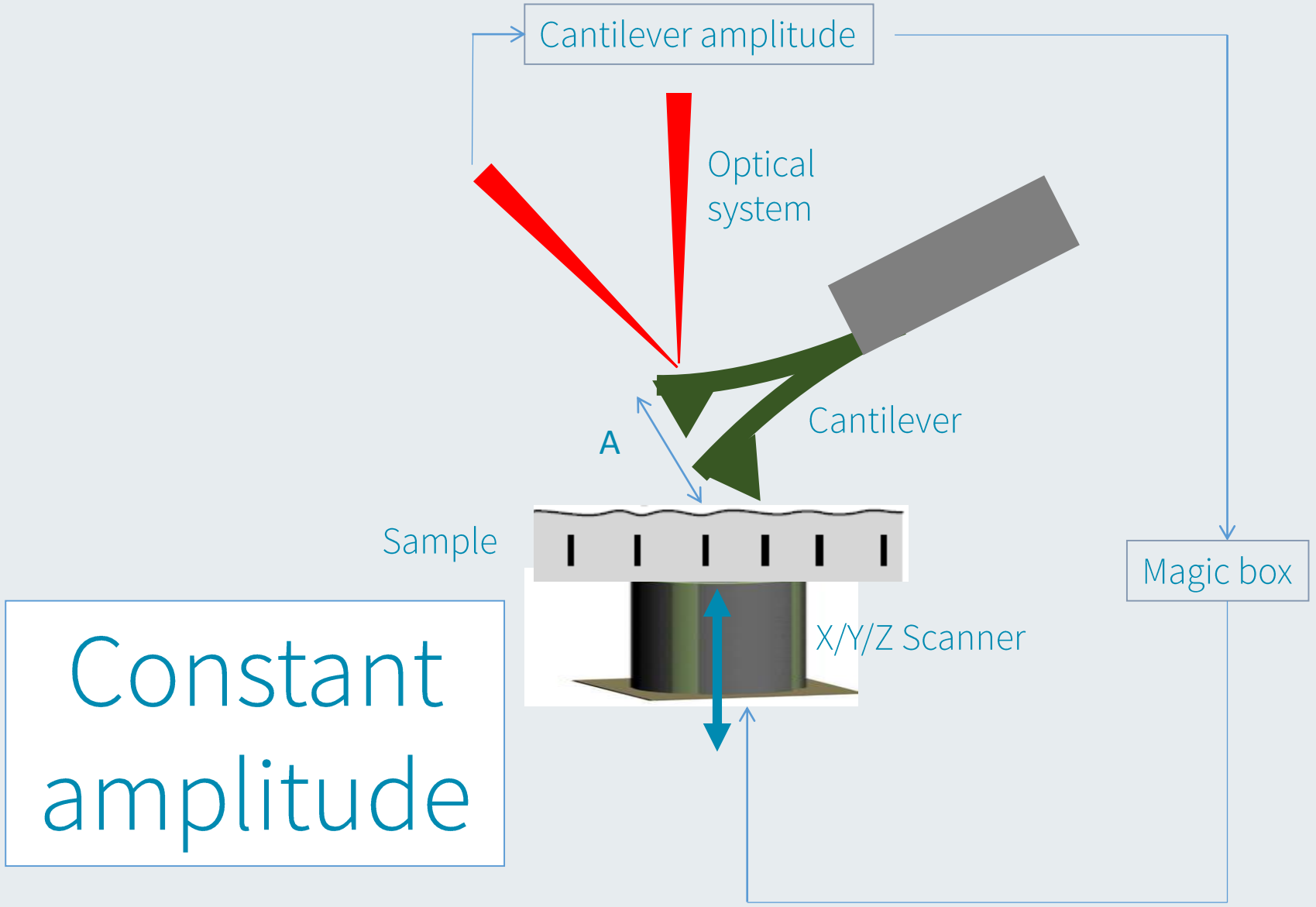
Dynamic AFM mode – Harmonic Oscillator (phase)

After resonance





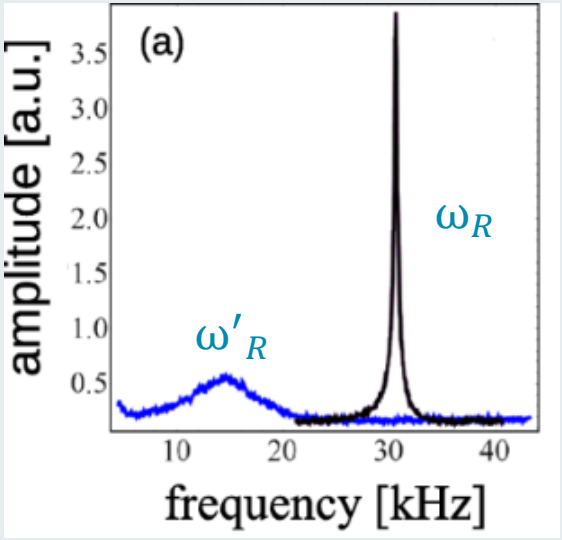
Dynamic AFM mode (AM-AFM) – Operational scheme



Constant amplitude

Dynamic AFM mode (AM-AFM) – in liquid, for biology

1) Part of the liquid participates (coupled) to cantilever motion



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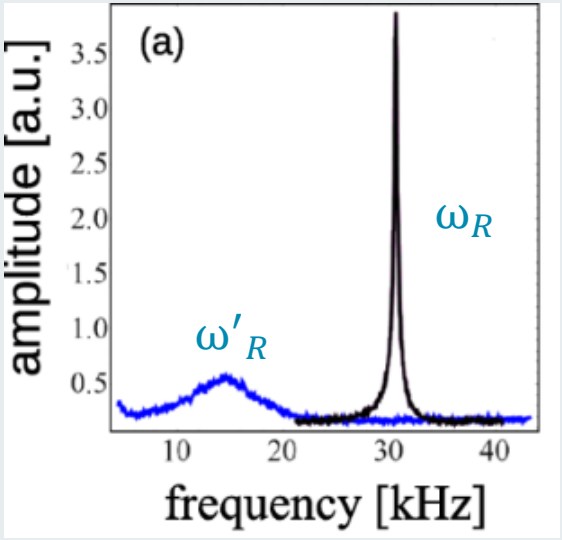

$$\omega_R = \sqrt{\frac{k}{m}} \longrightarrow \omega'_R = \sqrt{\frac{k}{m + m_L}}$$

2) The resonance gets broader → decrease of Q factor. That's because water has higher viscosity than air $\delta_{liquid} > \delta_{air}$

$$m\ddot{z} + \delta\dot{z} + kz = F_{interaction}(z) + F_0(\omega t)$$

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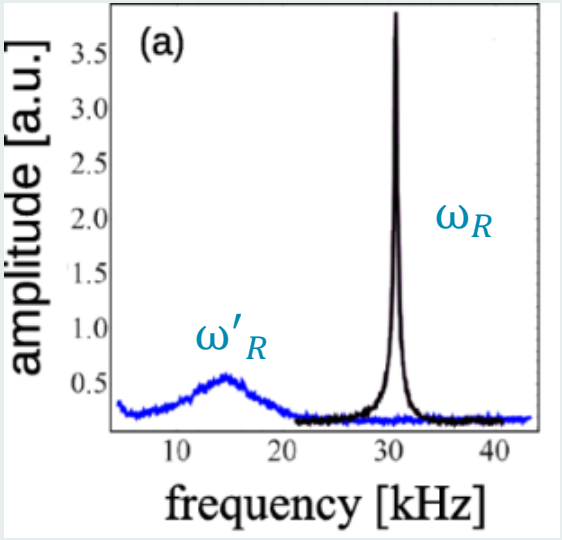

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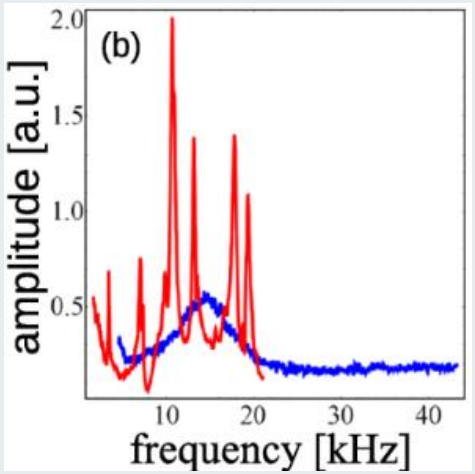

3) When excited mechanically,
the response of the tip is plenty of spurious peaks

Outline

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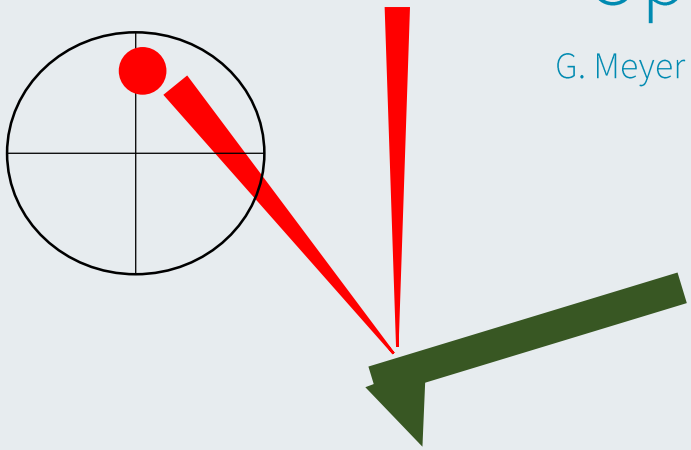
Optical beam deflection

G. Meyer and N. M. Amer, Appl. Phys. Lett. 53, 1045 (1988).



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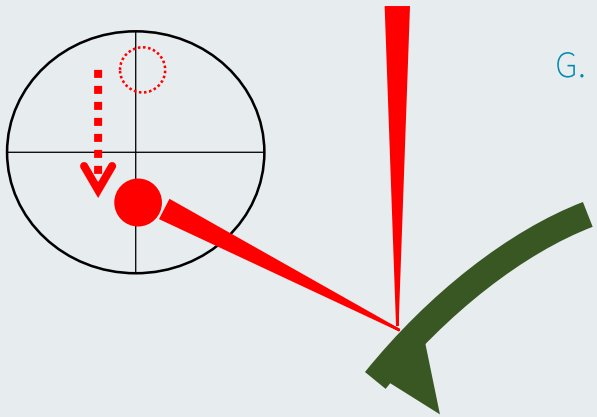

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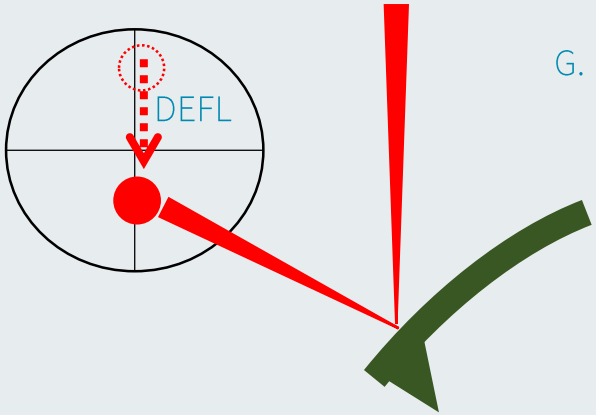

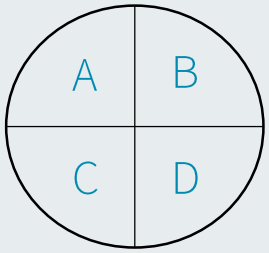

$$\mathrm{DEFL} = \frac{(A+B)-(C+D)}{A+B+C+D}$$

$$\mathrm{SUM} = A + B + C + D$$

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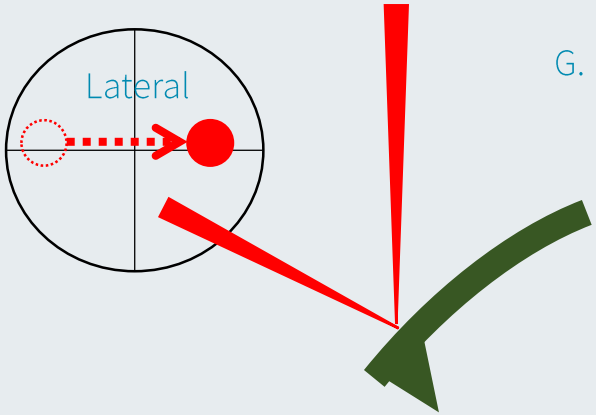
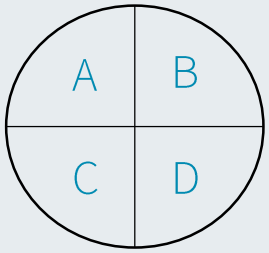




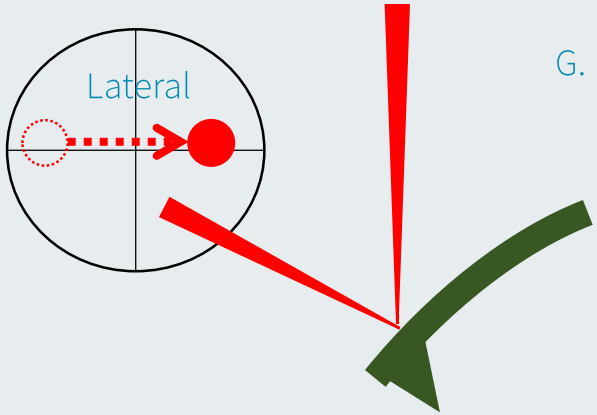
$$\mathrm{DEFL} = \frac{(A+B)-(C+D)}{A+B+C+D}$$

$$\mathrm{SUM} = A + B + C + D$$

$$\mathrm{Lateral} = \frac{(A+C)-(B+D)}{A+B+C+D}$$

Tip position measurement

Optical beam deflection



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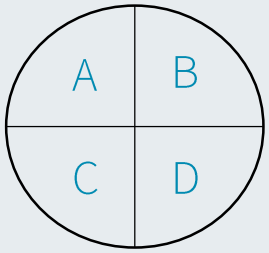

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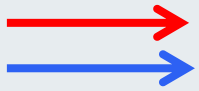
Interferometry

D. Rugar, H. J. Mamin, R. Erlandsson, J. E. Stern, and B. D. Terris, Rev. Sci. Instrum. 59, 2337 (1988).

Drawback:
DRIFT!!!

Interference of red and blue beams

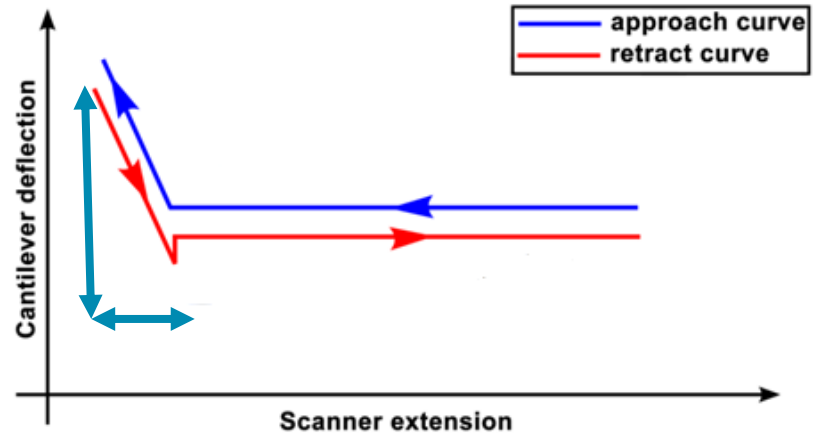


$I(d)$

$$I(d) = I_0 + \Delta I_0 sin\left(\frac{4\pi}{\lambda}d + \phi\right)$$

-1- Detector sensitivity

Acquire a force curve on a very rigid surface (i.e. mica or silicon)



Condition:
scanner extension (nm) = cantilever deflection (nm)
scanner extension (nm) = Φ x cantilever deflection (V)

$$\Phi = \frac{scanner\ extension}{cantilever\ deflection}\ [\mathrm{nm/V}]$$

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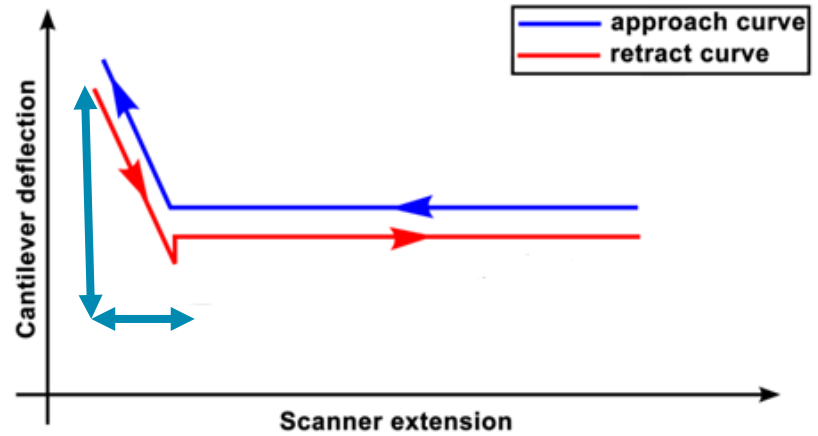

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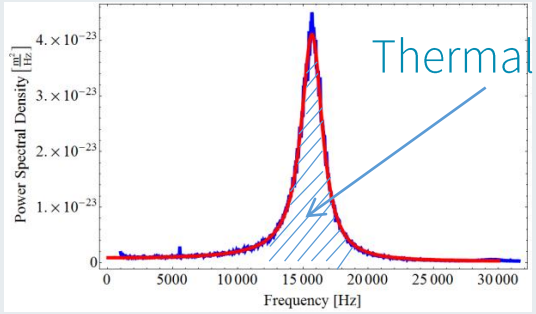
$$\Phi = \frac{scanner\ extension}{cantilever\ deflection} \quad [nm/V]$$

-2- Cantilever stiffness

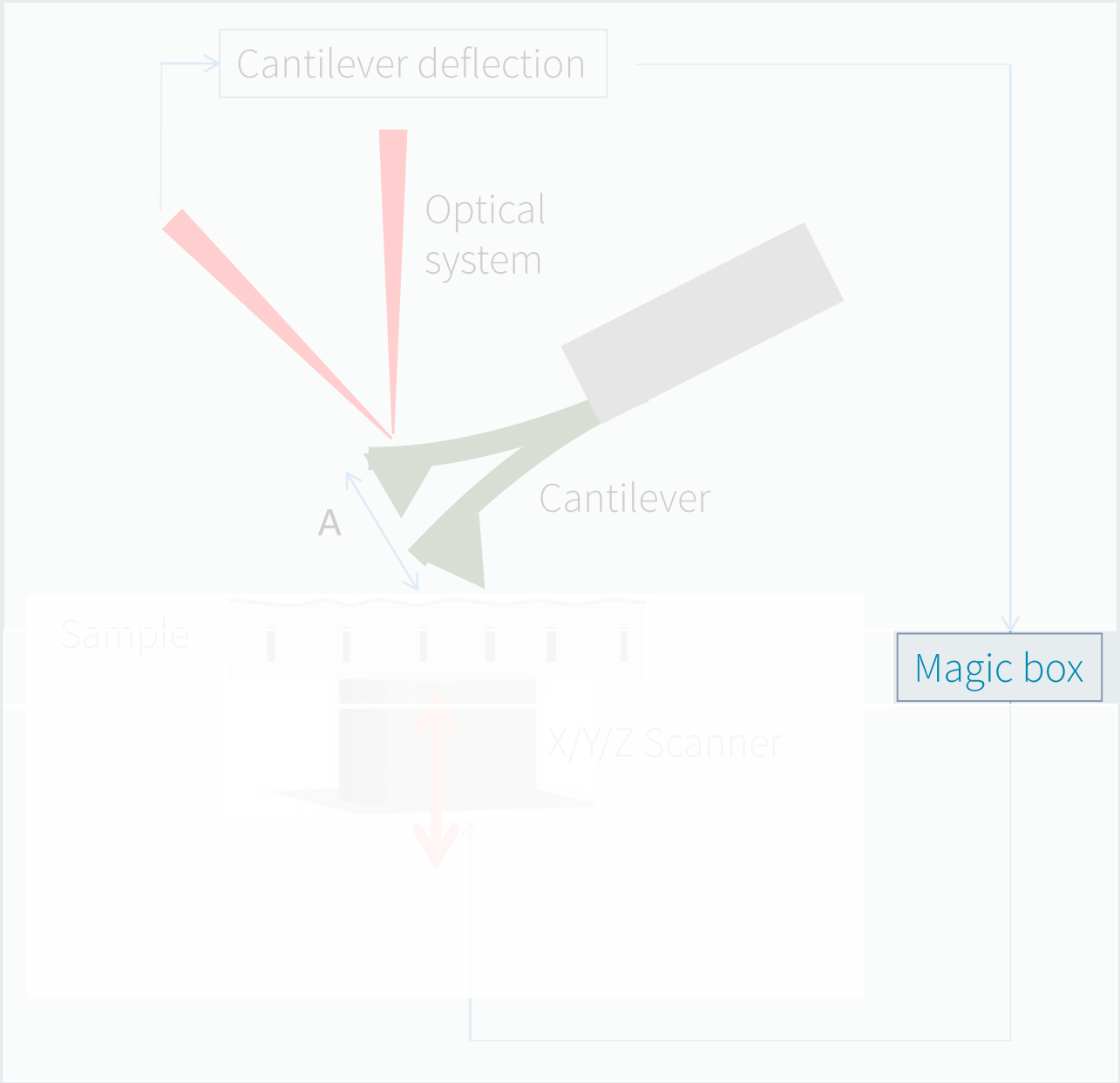
Thermal method

Thermal energy = ½ $K_B T$ = Cantilever energy = ½ k deflection^2

$$k = \frac{K_B T}{deflection^2(\omega)} \quad [N/m]$$



The « magic box »



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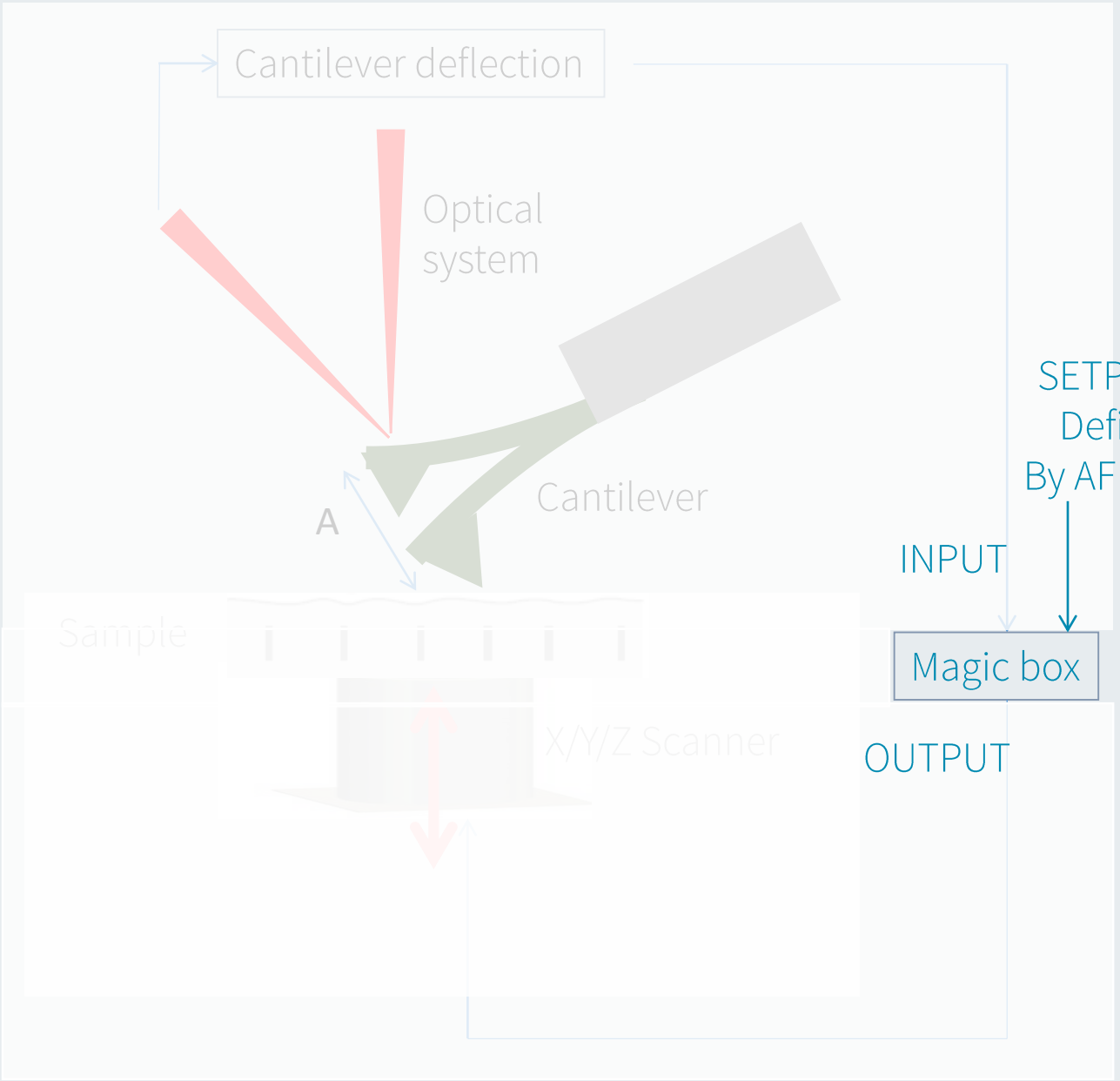


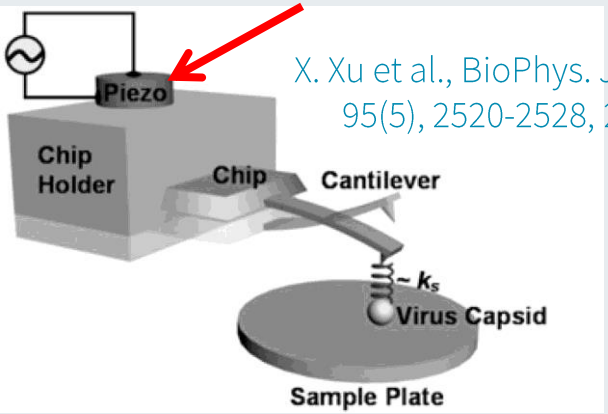
PID
=
Proportional – Integral – Derivative loop

output= P x (setpoint-input) +
I x ∫(setpoint-input)dt +
D x d(setpoint-input)/dt

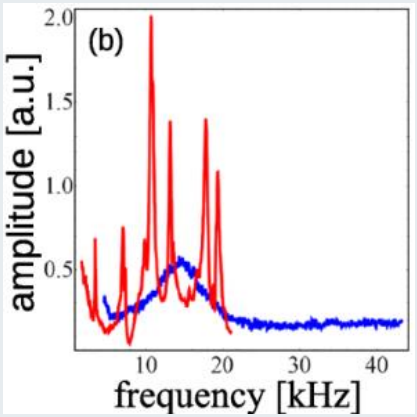
Tip excitation

-1- Piezo-dither mode

Present in all AFMs
Drawback:
Excitation in liquid



X. Xu et al., BioPhys. Journal, 95(5), 2520-2528, 2008



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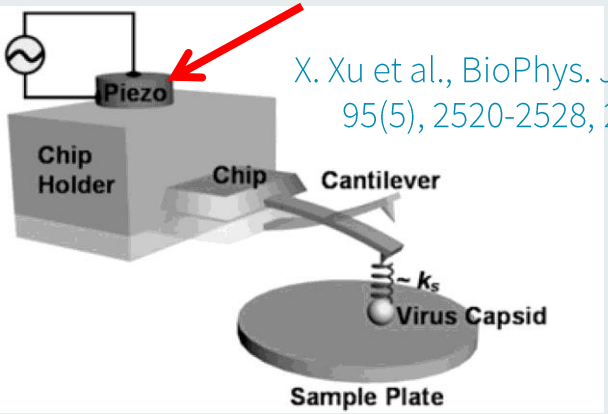

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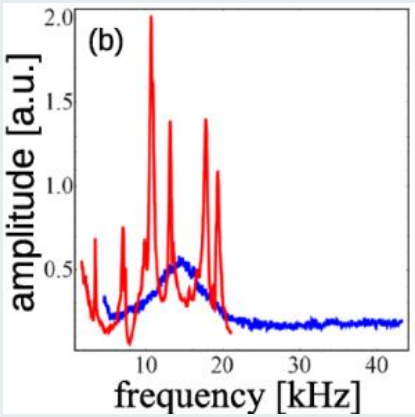
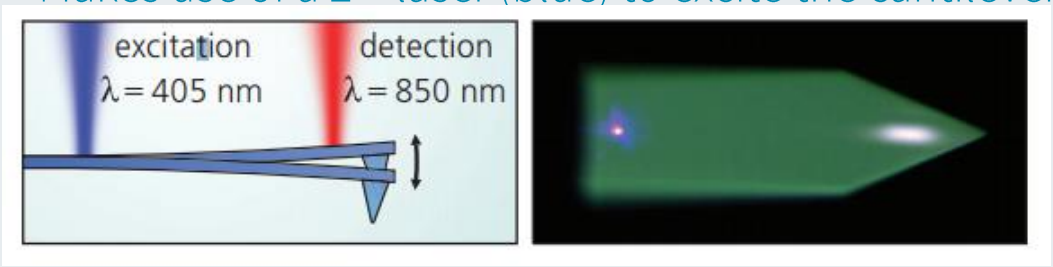
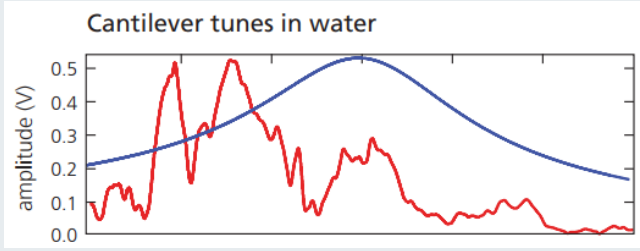


-2- Photothermal excitation

Provided by Asylum Research
Makes use of a 2nd laser (blue) to excite the cantilever base





From Asylum Research website

Tip excitation

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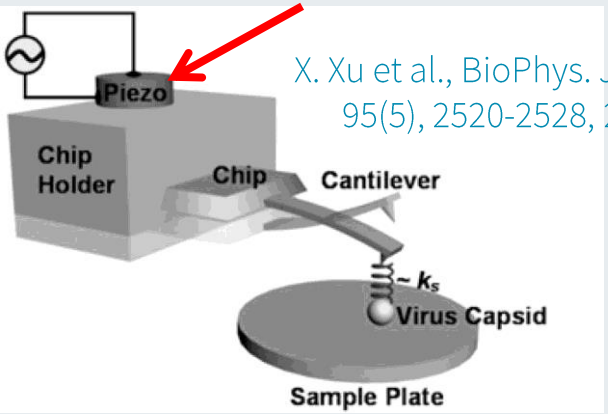
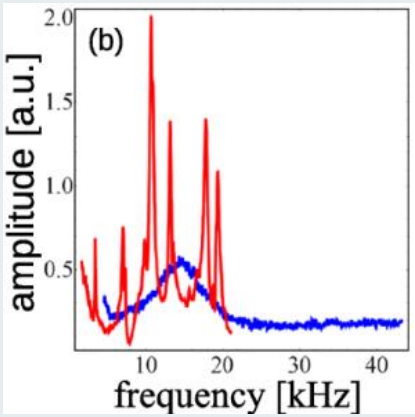
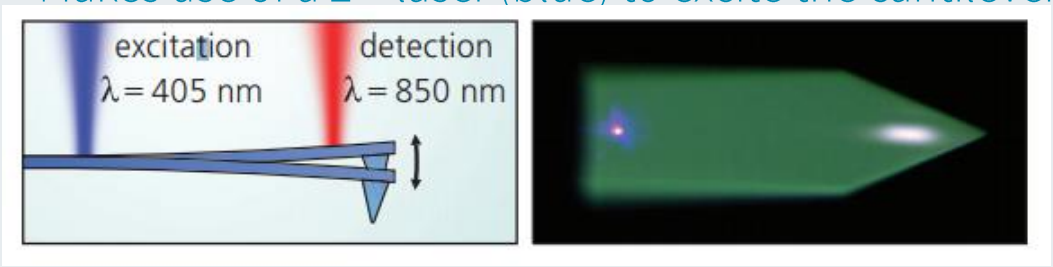


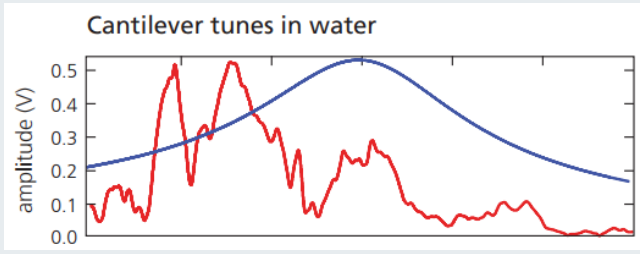
X. Xu et al., BioPhys. Journal, 95(5), 2520-2528, 2008



-2- Photothermal excitation

Provided by Asylum Research
Makes use of a 2nd laser (blue) to excite the cantilever base



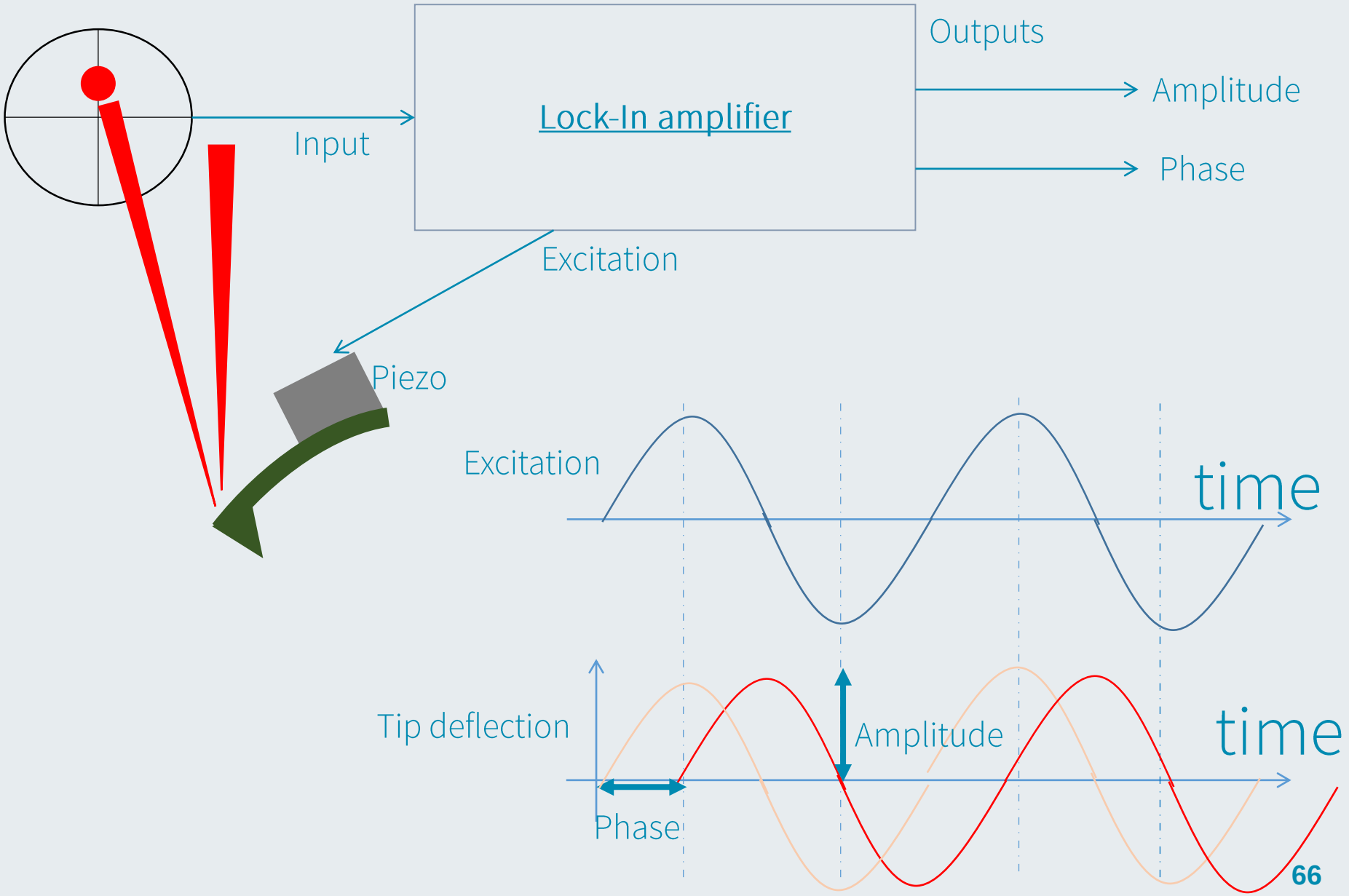


From Asylum Research website

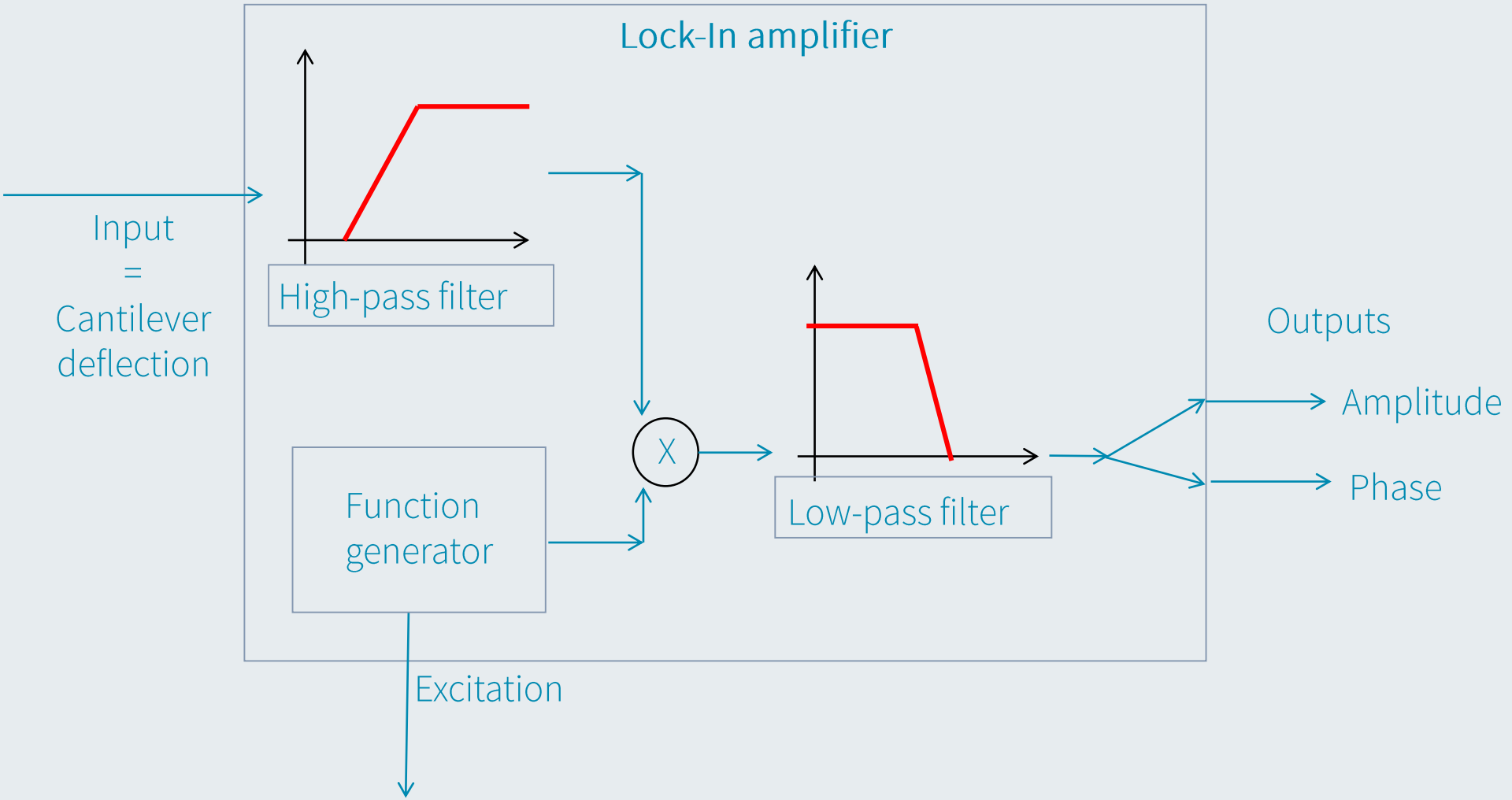
-3- Magnetic excitation

Provided by AGILENT
Makes use of a magnetic tip
Cantilever stiffness limitations

Lock-in Amplifier



Lock-in Amplifier



Outline

1) Introduction to Scanning Probe Microscopy

2) The Force

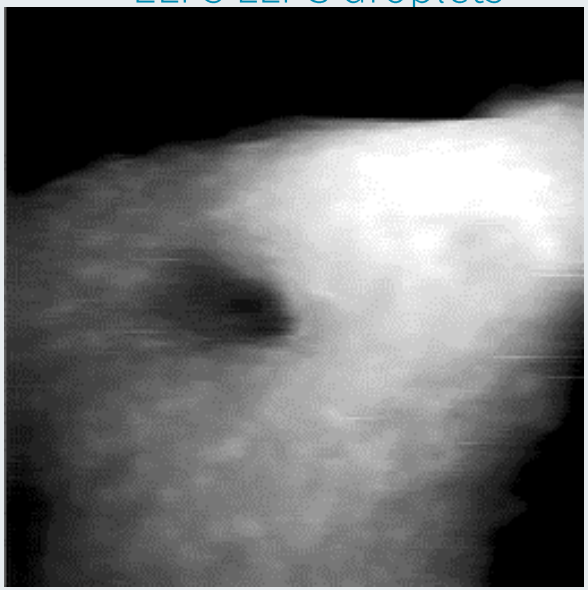
3) Methods
Cantilevers
Static and Dynamic AFM

4) Instruments

5) Advanced and novel AFM methods

High-Speed AFM

ELF3 LLPS droplets



Nessim Louafi

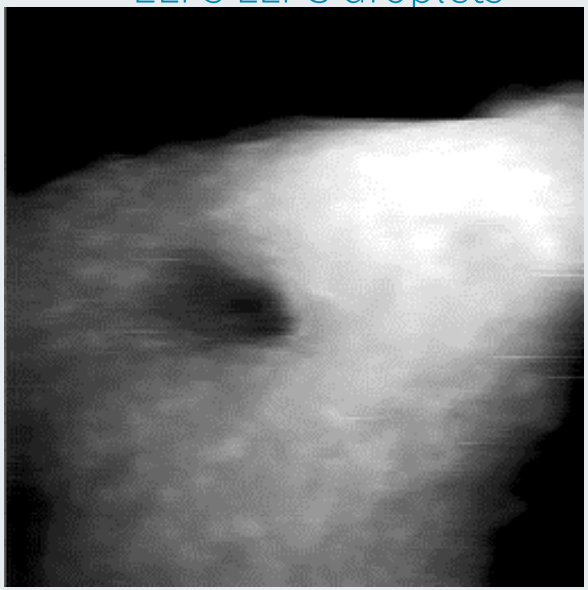
Walking myosin

Toshio Ando

Ando T. et al, PNAS 98(22), 12468-12472, 2001

High-Speed AFM

ELF3 LLPS droplets



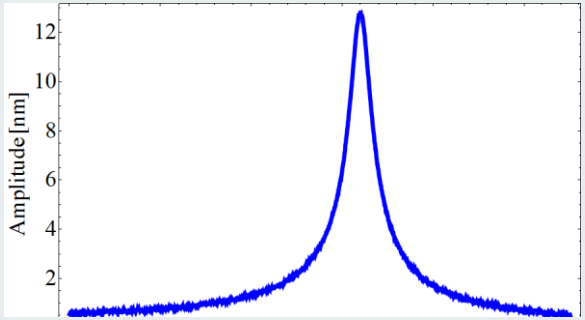
Nessim Louafi

Walking myosin

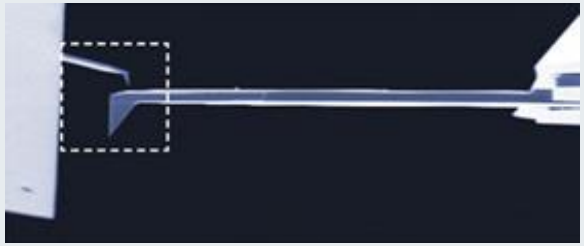
Toshio Ando

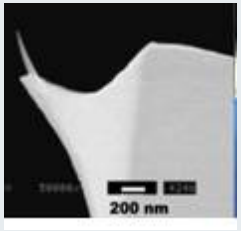
Ando T. et al, PNAS 98(22), 12468-12472, 2001

FAST (SMALL) CANTILEVERS



MEGAHERTZ





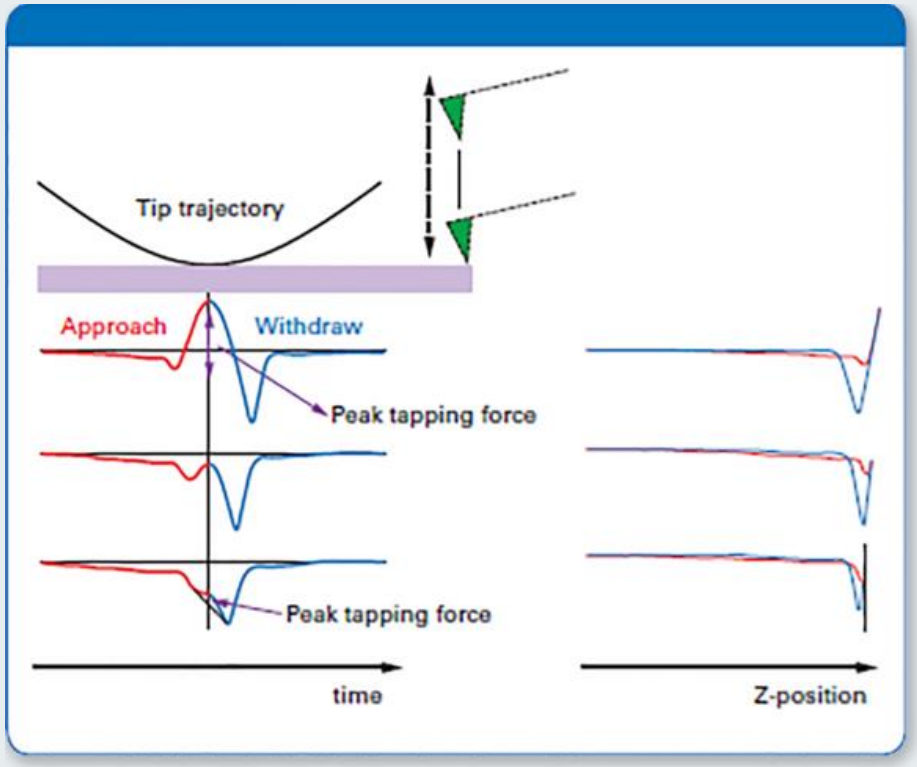
FAST XYZ SCANNER

Fast Force-Curves Imaging

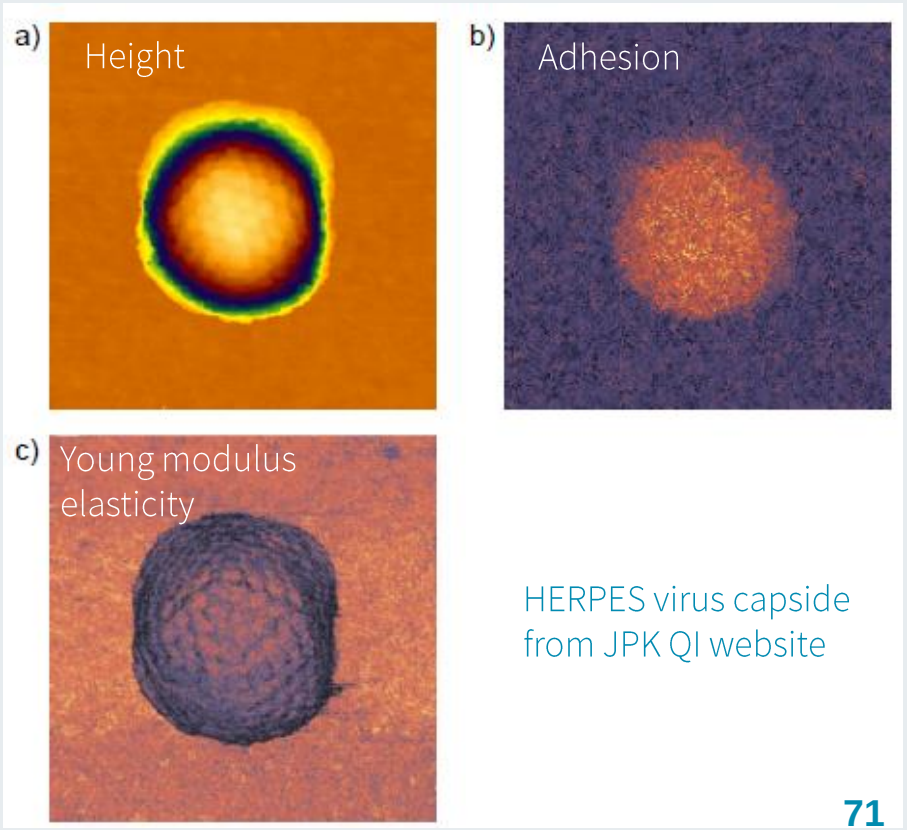
Bruker: PeakForce Tapping

JPK: QI quantitative imaging

Asylum Research: Fast Force Mapping



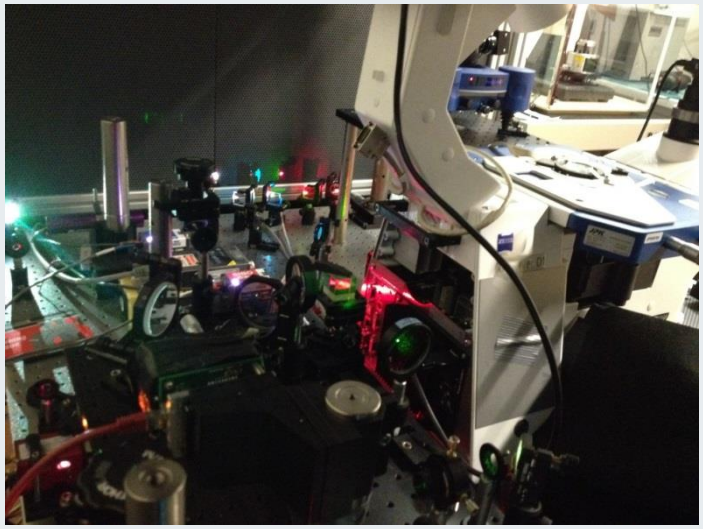
From Bruker website



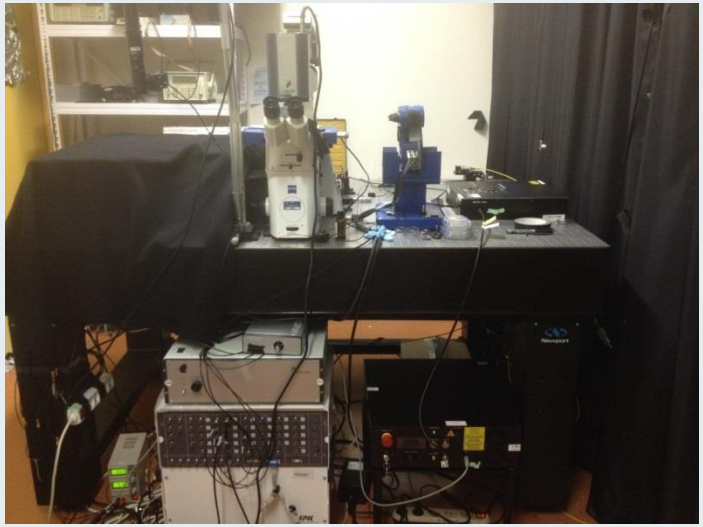
HERPES virus capside
from JPK QI website

Correlative AFM - Fluorescence

AFM + Fluorescence → Wide field illumination

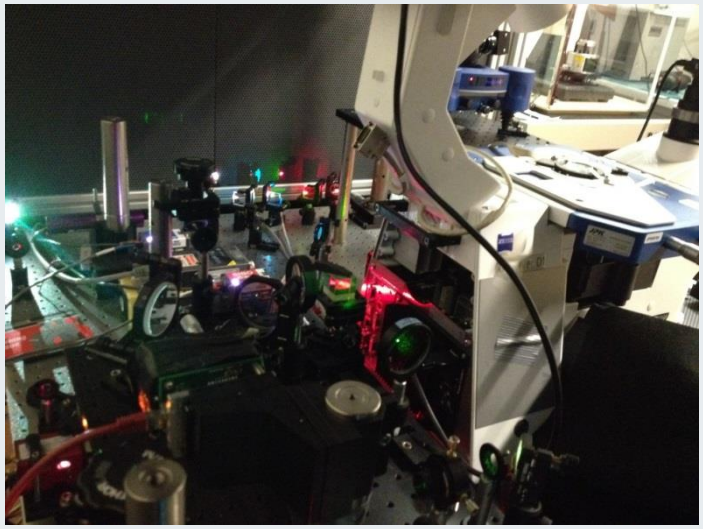


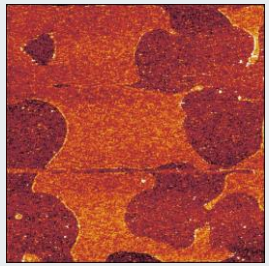
AFM + Fluorescence → Confocal illumination



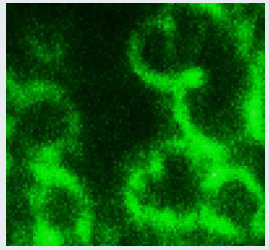
Correlative AFM - Fluorescence

AFM + Fluorescence → Wide field illumination



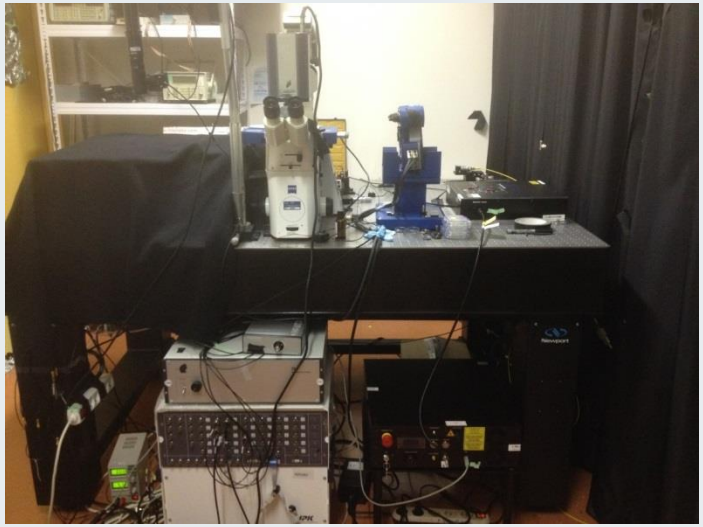


AFM



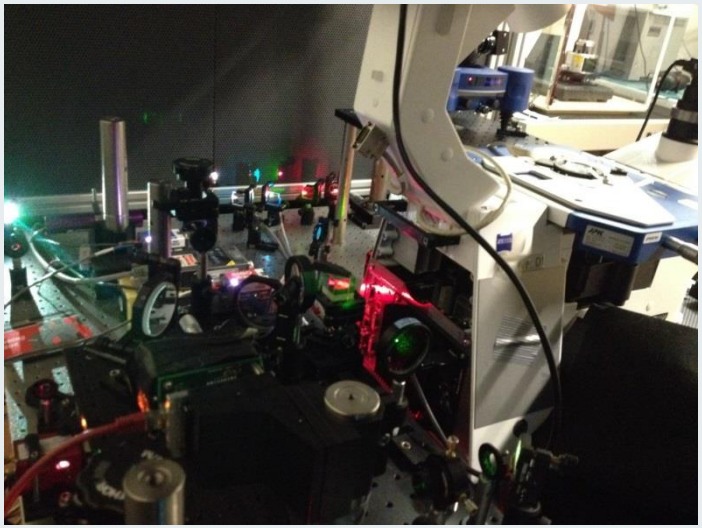
TIRF Fluorescence

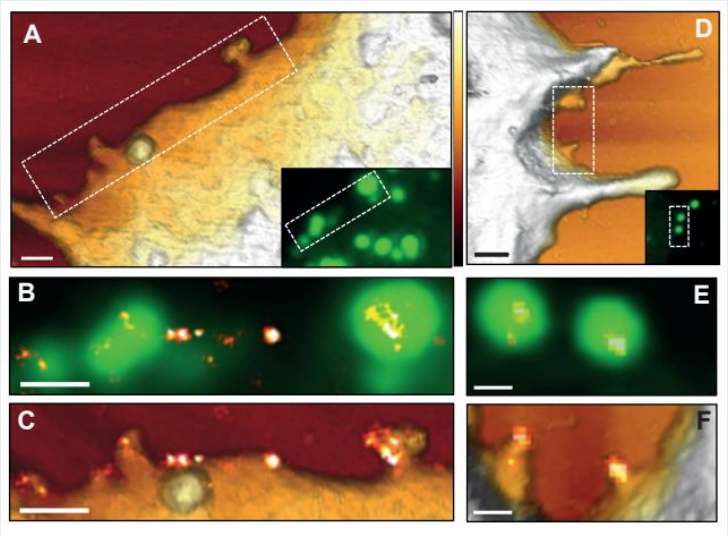
AFM + Fluorescence → Confocal illumination



Correlative AFM - Fluorescence

AFM + Fluorescence → Wide field illumination

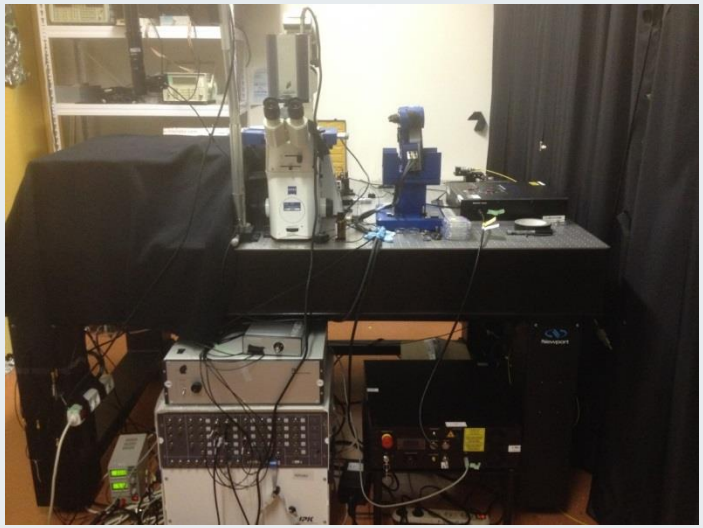




AFM and STORM

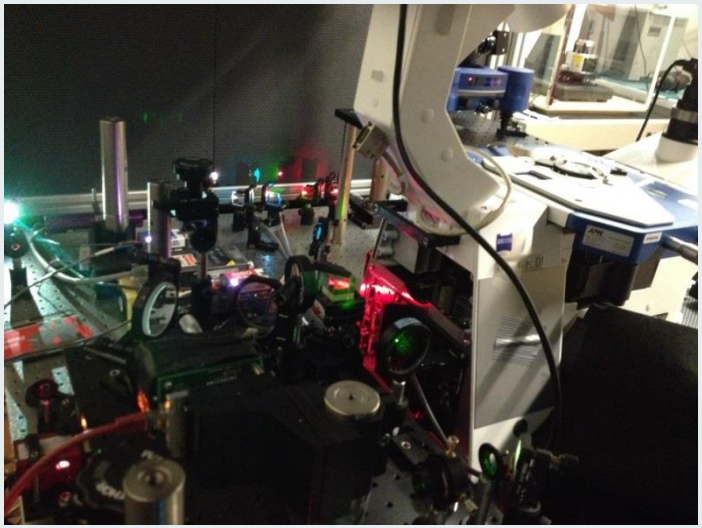
Dahmane, S. et al. , Nanoscale, 2019

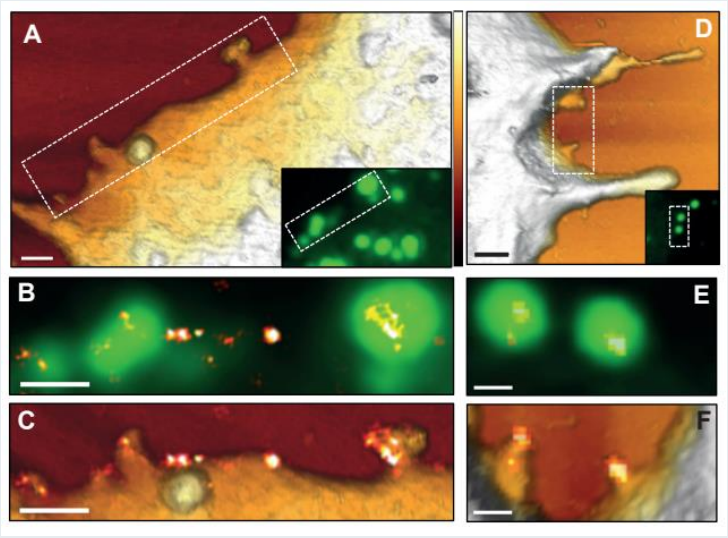
AFM + Fluorescence → Confocal illumination



Correlative AFM - Fluorescence

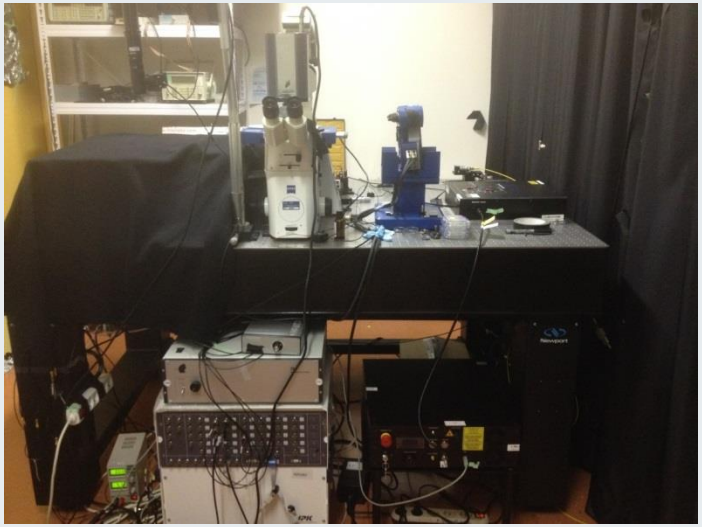
AFM + Fluorescence → Wide field illumination

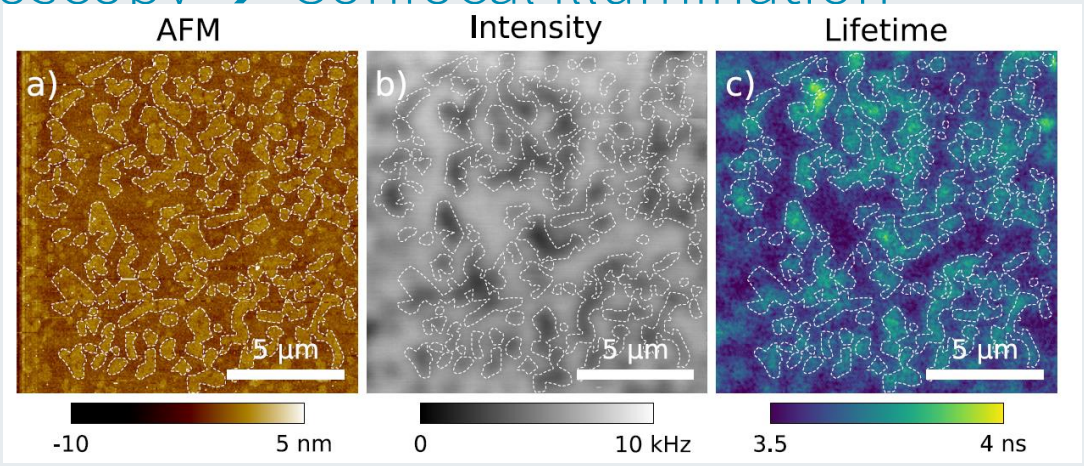




AFM and STORM

AFM + Fluorescence Spectroscopy → Confocal illumination





Fernandes T. et al., Scientific Reports, 2020

Tips for High-Resolution AFM imaging

1) The instrumental noise should be smaller than the aimed resolution.

2) The lateral resolution cannot be smaller than the size of the tip apex.

3) The decay length of the tip surface interaction should be smaller than the tip apex.

4) Imaging isolated three dimensional objects imposes limits on the lateral resolution.

5) The adhesion energy should be minimized. This is usually achieved by using **sharp tips.**

6) The applied force should be as small as possible.

7) Under identical conditions (tip radius and applied force), the stiffer the sample the better the lateral resolution.

Ricardo Garcia, Amplitude Modulation Atomic Force Microscopy, Wiley, 2010

Contacts

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https://integrativebiophysicsofmembranes.wordpress.com/