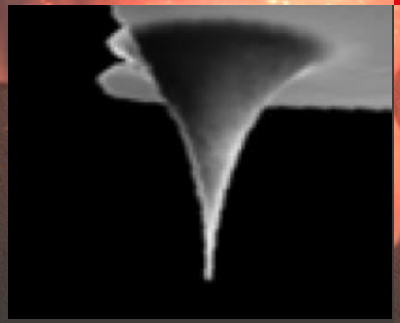
AFM
Atomic Force Microscopy

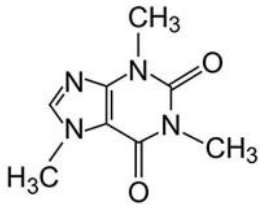


Georg E. Fantner

Laboratory for Bio- and Nano- Instrumentation

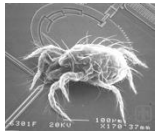
Why we need nanoscale microscopy

10^{-9}m 10^{-6}m 10^{-3}m 10^{0}m 10^{3}m 10^{6}m 10^{9}m



The Scale of Things – Nanometers and More

Things Natural

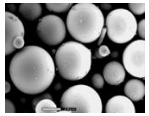


Dust mite

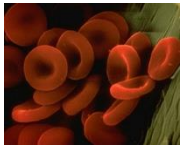
200 μm

Human hair
~ 60-120 μm wide

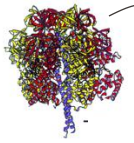
Ant
~ 5 mm



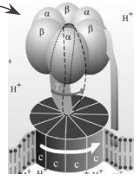
Fly ash
~ 10-20 μm



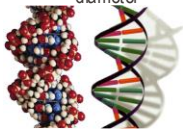
Red blood cells
(~7-8 μm)



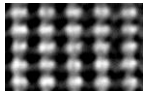
~10 nm diameter



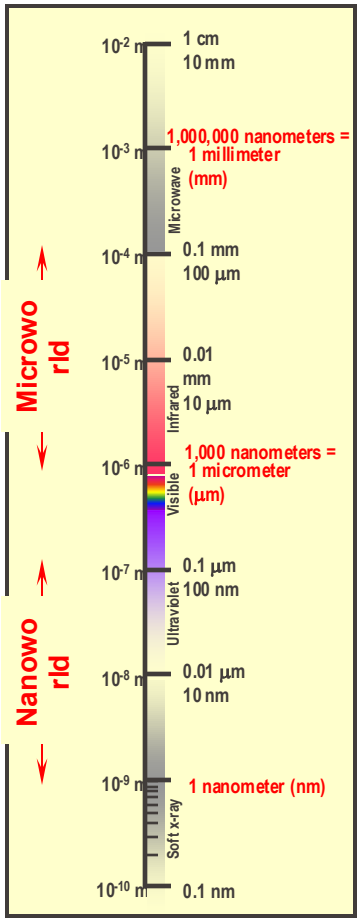
ATP synthase



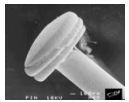
DNA
~2-1/2 nm diameter



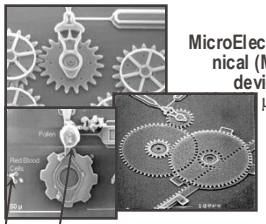
Atoms of silicon
spacing
0.078 nm



Things Manmade



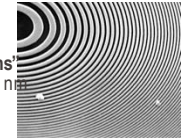
Head of a pin
1-2 mm



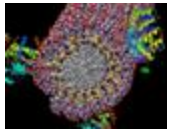
MicroElectroMechanical (MEMS) devices
μm wide

Pollen grain

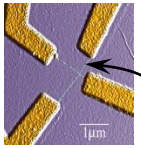
Red blood cells



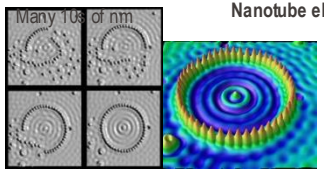
Zone plate x-ray "lens"
Outer ring spacing ~35 nm



Self-assembled, Nature-inspired structure



Nanotube electrode

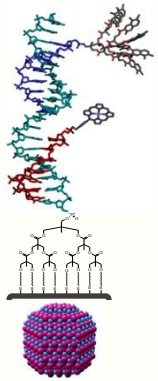


Quantum corral of 48 iron atoms on copper surface
positioned one at a time with an STM tip
Corral diameter 14 nm

Carbon nanotube
~1.3 nm diameter

Carbon buckyball
~1 nm diameter

The Challenge



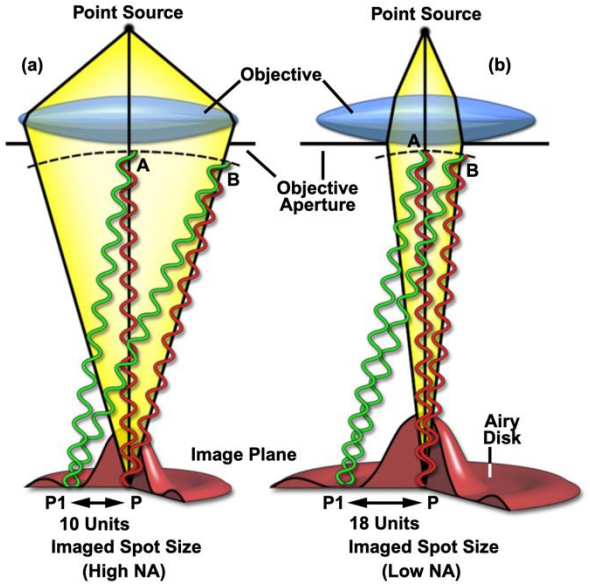
Fabricate and combine nanoscale building blocks to make useful devices, e.g., a photosynthetic reaction center with integral semiconductor storage.

Office of Basic Energy Sciences
Office of Science, U.S. DOE
Version 05-26-06, pmd

Why is it difficult to measure small things?
The diffraction limit

In any (far field) microscopy system where we create a magnified image of an object via an image projection using diffractive elements (such as lenses) we run into the *diffraction limit:*

Point sources (with zero size) are projected to an Airy disk with a certain size. Two point sources that are close together will result in two Airy disks close together. If the disks are too close together they can no longer be separated based on their intensity. That is then the resolution limit of the microscope.



What determines the achievable resolution

Abbe Resolution$_{x,y}$ = λ/2NA

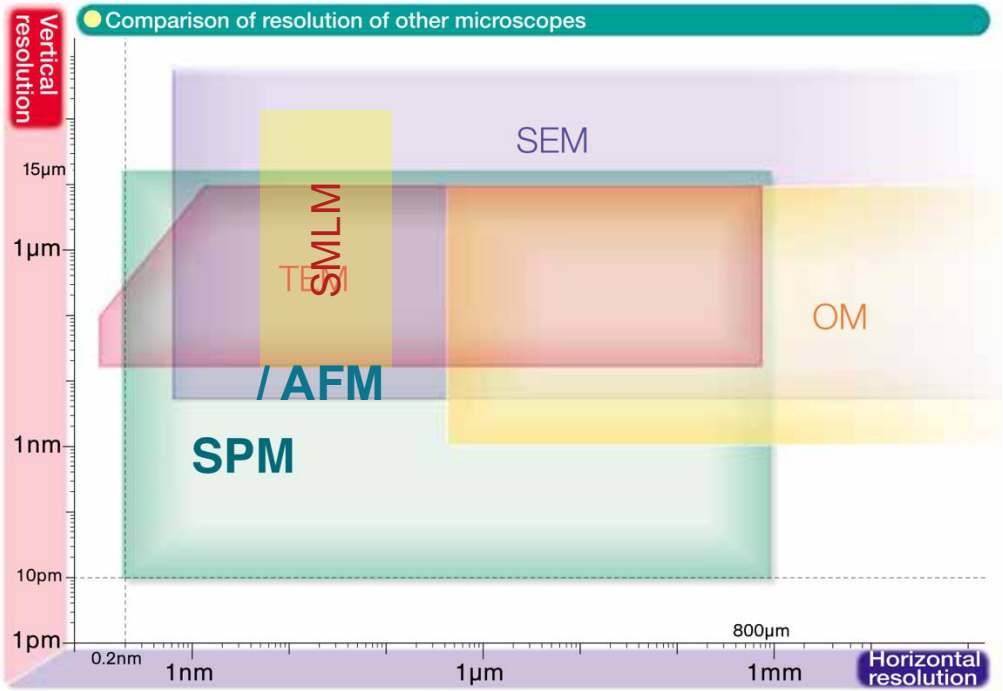
- λ... Wavelength
- NA … Numerical aperture

What can we do to get around this?

- Work with smaller wavelengths: instead of photons use particles with much smaller wavelength (such as electrons: de Broglie wavelength of an electron with acceleration voltage of 10kV = $1{,}22 \cdot 10^{-11}$m, which is 40’000 times smaller than that of a photon). That is what we use in electron microscopy
- Try to use non far field microscopy techniques (near field techniques or scanning probe techniques). This is wat we do in atomic force microscopy (AFM) or scanning near field optical microscopy (SNOM)

Resolution is NOT everything…

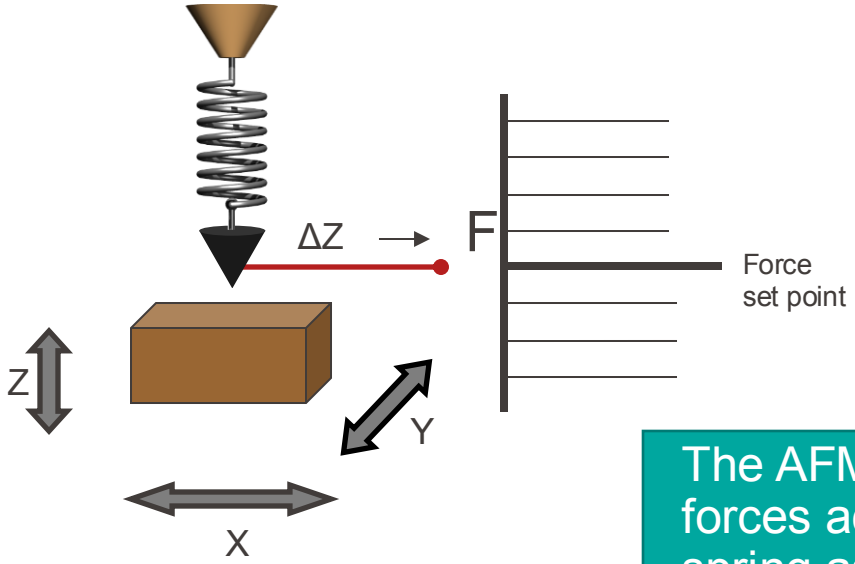
…but it's sure nice to have a good one



What is an AFM?

(don't be fooled by the word *atomic)*

“Scanning Force Microscopy “SFM

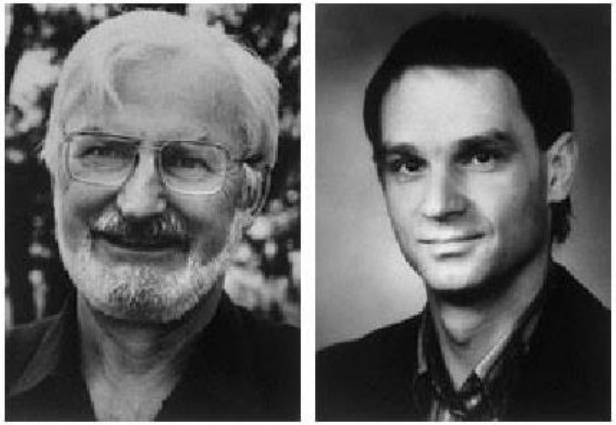


The AFM measures the effect of forces acting on the sharp tip on a spring as a function of the position on the surface. – ***sometimes*** these forces are due to topography

It all started with *Tunneling*…

- Binnig, Gerber, Rohrer, Wiebel *Tunneling through a controller vacuum gap.*(Applied Physics Letters **40**, 178 (1982)

- *"This investigation is the first step towards the development of scanning tunneling microscopy, where the surface is scanned by a tunnel current and should open the door to a new area of surface studies."*



Scanning tunnelling microscopy was invented by Gerd Binnig (right) and Heinrich Rohrer (left) in 1981. They were awarded the Nobel Prize in 1986.

…but only for conducting samples!

The AFM...

- *G. Binnig, C. F. Quate and Ch. Gerber, PRL 56, 930 (1986)*

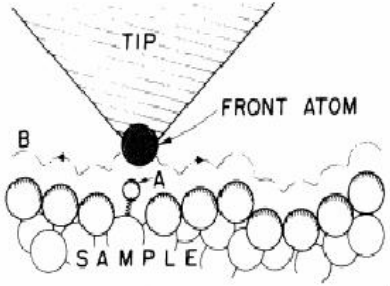


FIG. 1. Description of the principle operation of an STM as well as that of an AFM. The tip follows contour *B*, in one case to keep the tunneling current constant (STM) and in the other to maintain constant force between tip and sample (AFM, sample, and tip either insulating or conducting). The STM itself may probe forces when a periodic force on the adatom *A* varies its position in the gap and modulates the tunneling current in the STM. The force can come from an ac voltage on the tip, or from an externally applied magnetic field for adatoms with a magnetic moment.

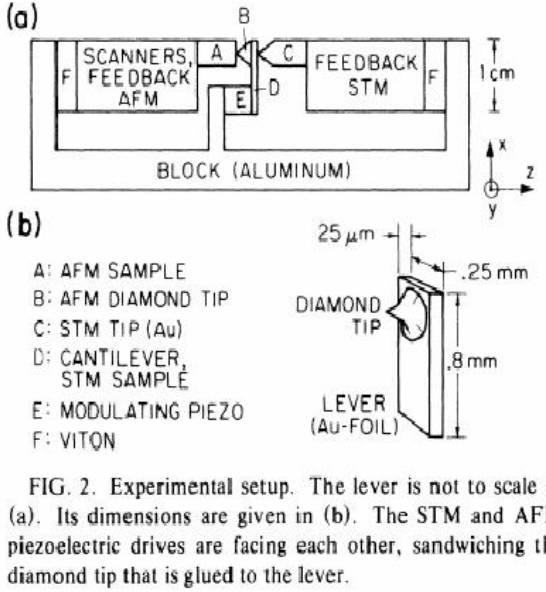
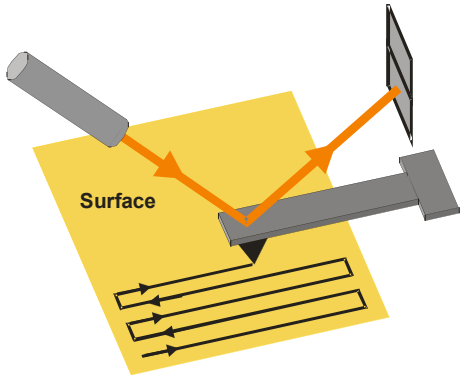
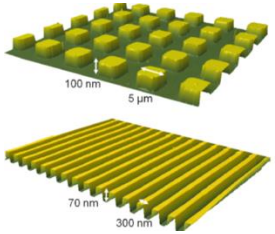


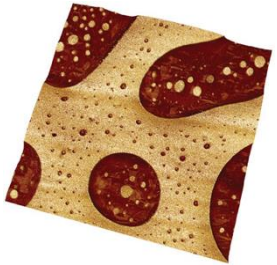
FIG. 2. Experimental setup. The lever is not to scale in (a). Its dimensions are given in (b). The STM and AFM piezoelectric drives are facing each other, sandwiching the diamond tip that is glued to the lever.

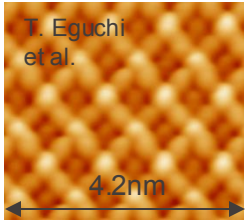
- Binnig invented the AFM in 1986, and while Binnig and Gerber were on a sabbatical in IBM Almaden they collaborated with Calvin Quate (Stanford) to produce the first working prototype

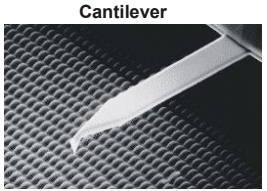
… Became a Versatile Tool for Nanoscale Measurements







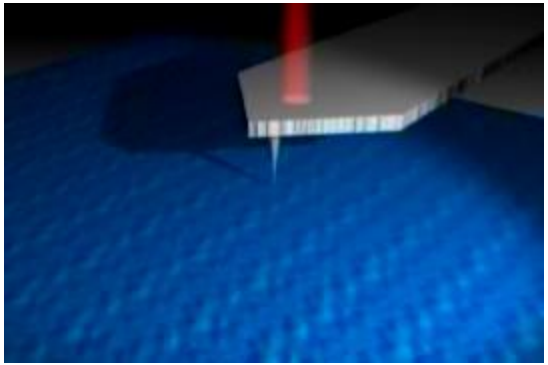




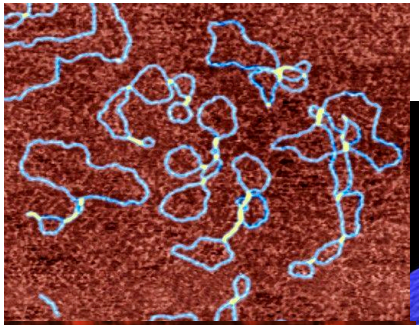
conductivity, surface potential, electrochemical potential, ion currents, magnetism, NMR….and many more

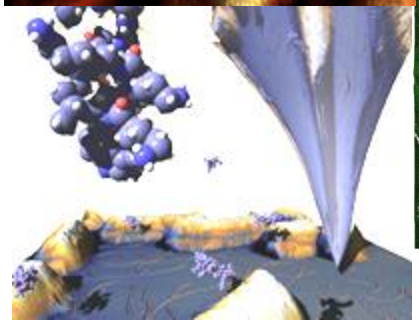
Atomic Force Microscopy

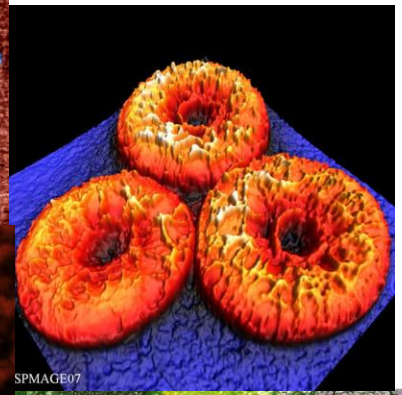
- a Versatile Tool for Nanoscale Biology

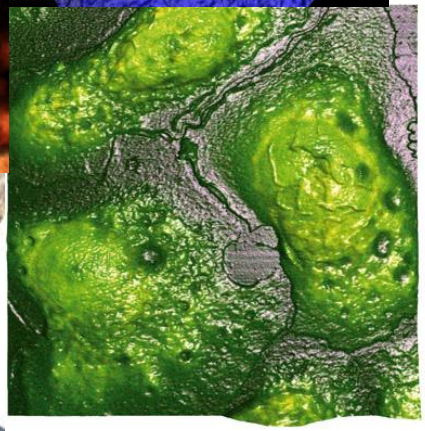


- Single molecule resolution
- High resolution imaging in aqueous solution
- Nanomanipulation
- Single molecule mechanics
- Imaging of living cells



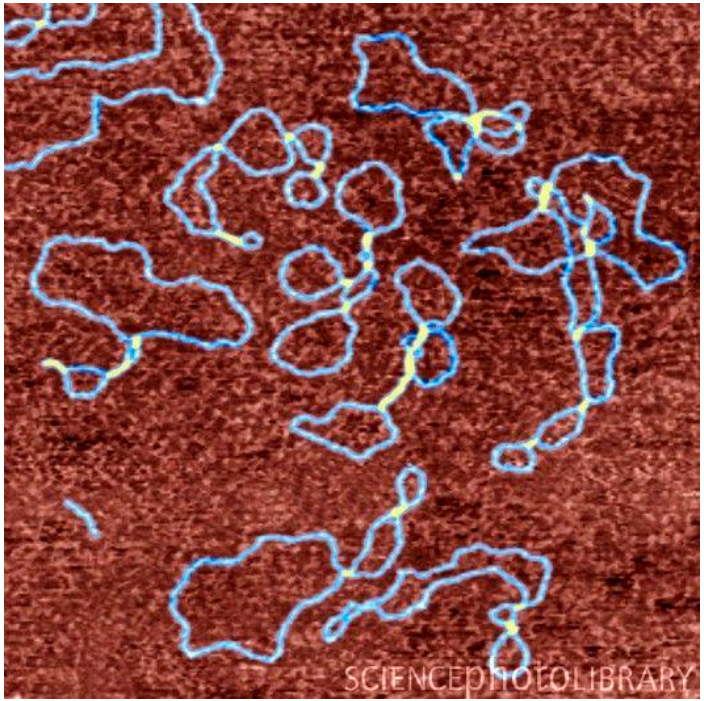






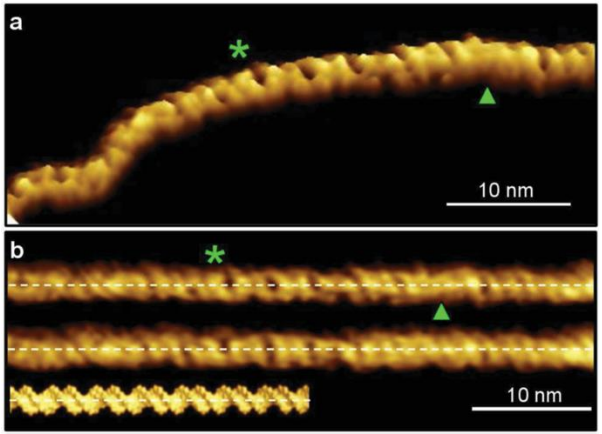
Single molecule resolution

Plasmid DNA on mica



Source: SciencePhotoLibrary

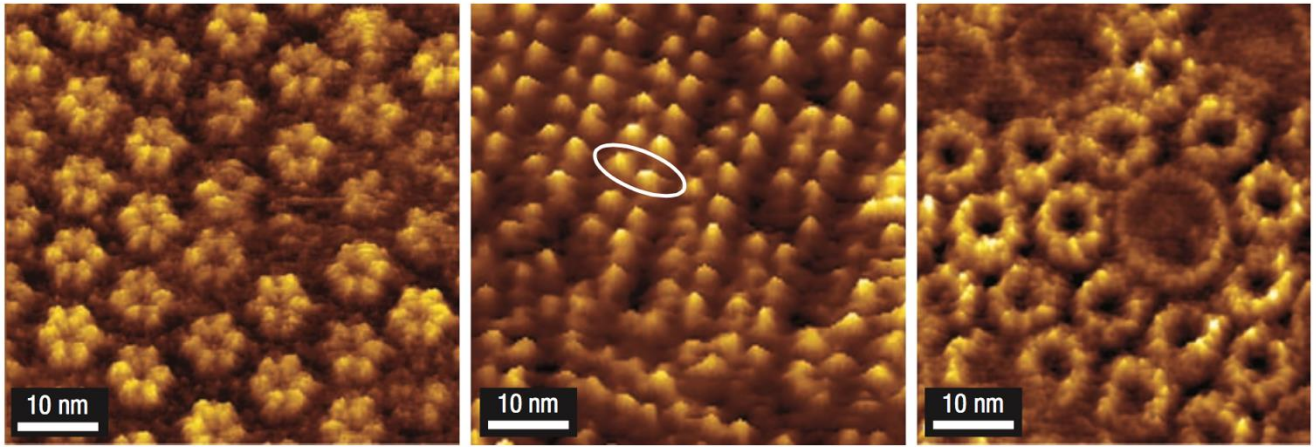
- Single molecules can be easily resolved
- Even the double helix!



Pyne et al. *Small, 10, Nr16, 2014*

High resolution images in fluid of proteins

- Imaging of membranes and membrane bound proteins
- Imaging of live cells



From Review Nature Nanotechnology 2008,D. Müller and I. Dufren,

AFM can be used for Nanomanipulation

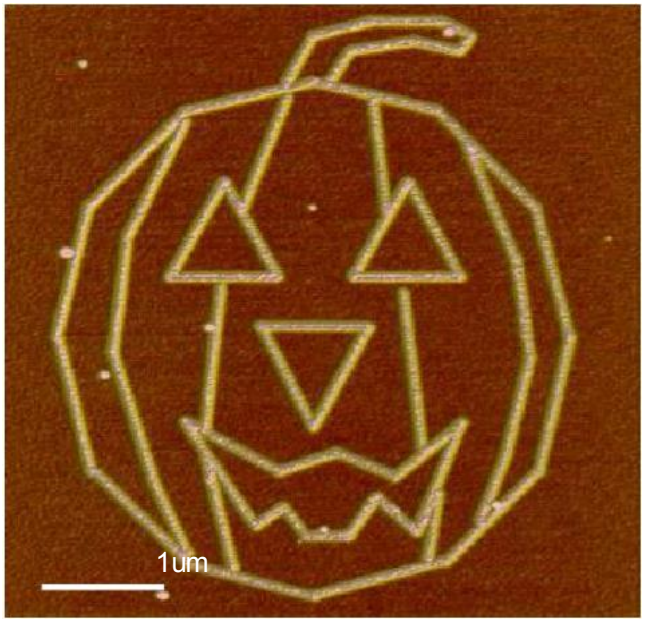


Image from:
http://www.veeco.com/library/nanotheater

- AFM patterning of a silicon surface using anodic oxidation
- Other approaches have been developed such as
  - dip-pen nanolithography and
  - Thermal scanning probe lithography (tSPL)

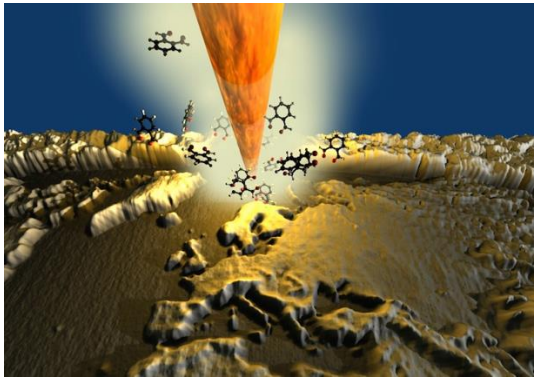


Image from: https://www.swisslitho.com

Different types of scanning probe microscopes

- SPM = scanning probe microscopy
- AFM= Atomic force microscopy (AFM), also known as
- SFM =scanning force microscopy
- STM scanning tunneling microscopy
- …
- SSETM = scanning single-Electron transistor microscopy

Wikipedia lists 41 different SPM modes!

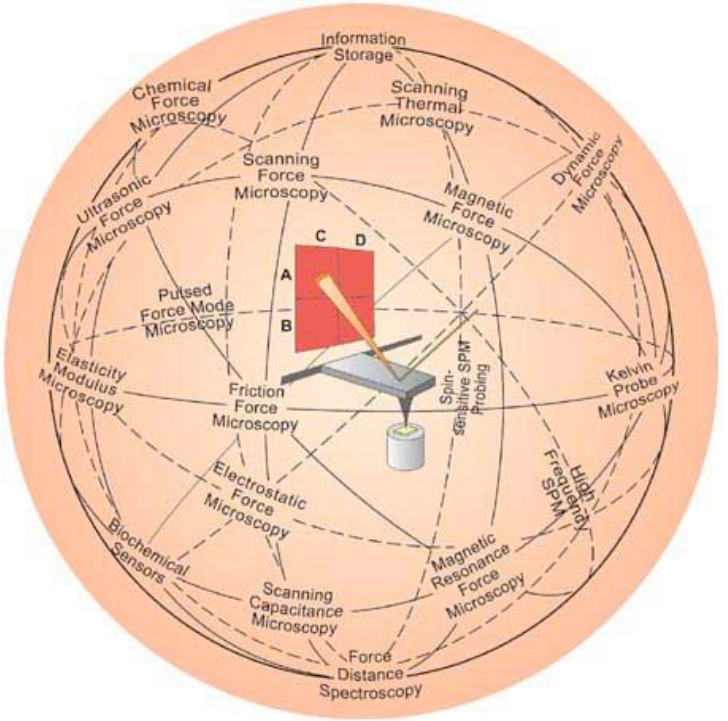
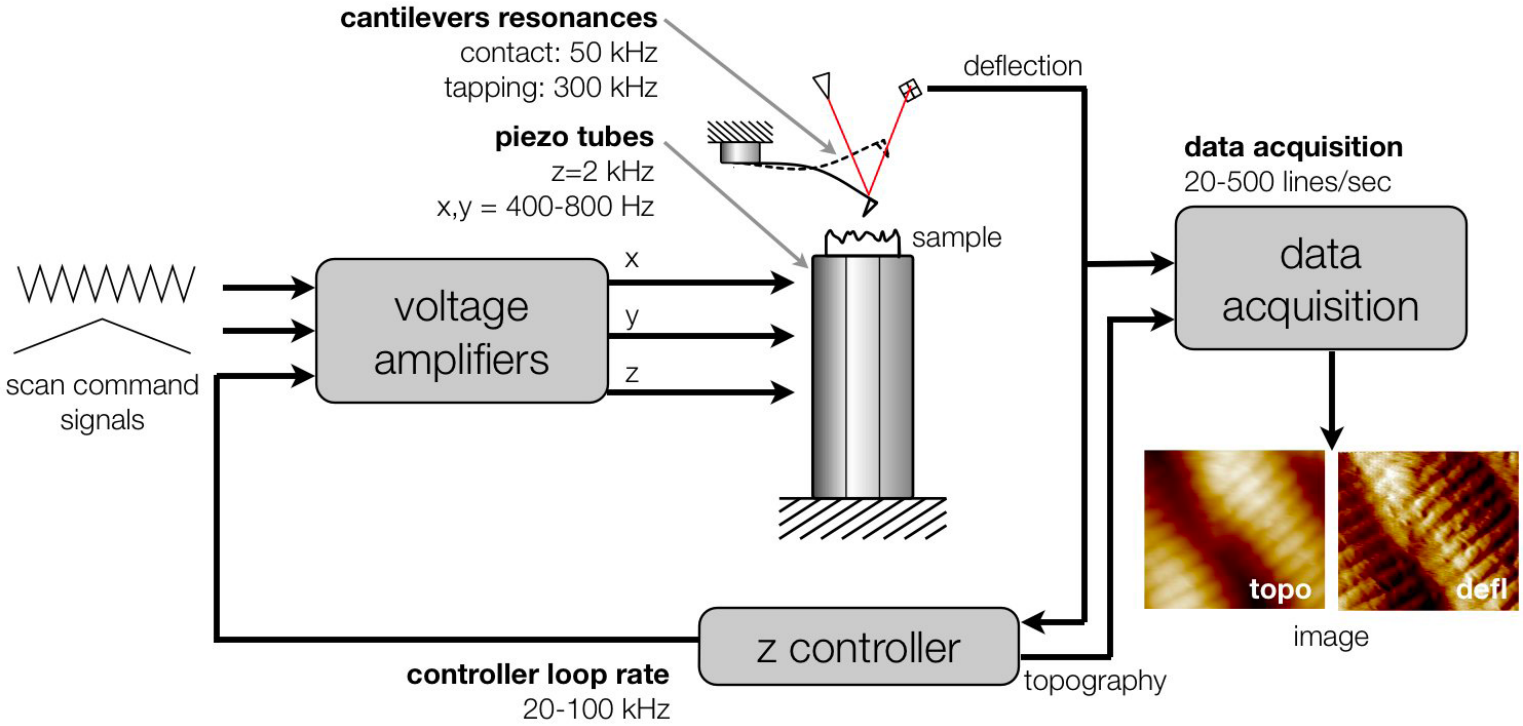


image: Christoph Gerber; copyright Nature Publishing Group

What's in an AFM?



A few principles we should understand

- Optical lever detection
- Piezos
- Feedback
- Force curves

Optical lever detection

Transduces cantilever deflection into a voltage

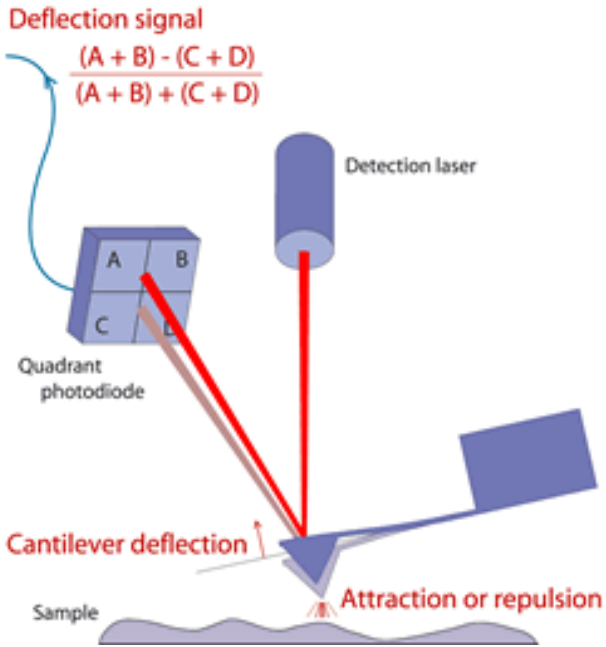
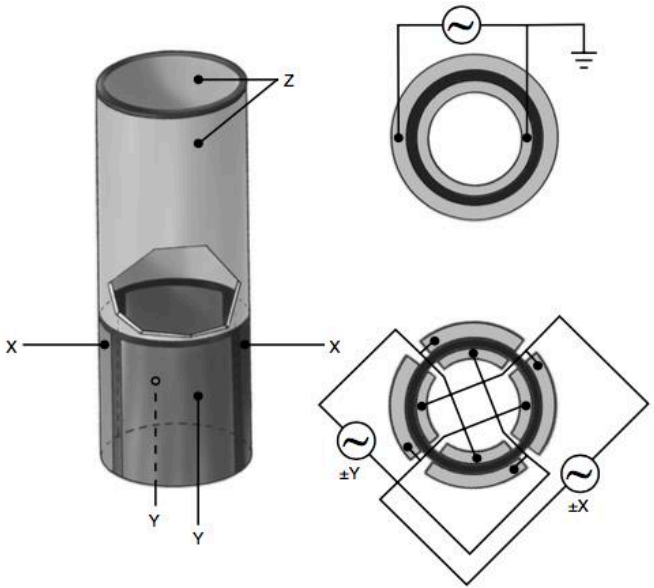


Image source: http://usa.jpk.com

- A very sensitive way to measure cantilever ***angle change***
- The change of angle is amplified by the distance from the cantilever tip to the 4-quadrant photodiode
- Each quadrant creates a current which is turned into a voltage using a transimpedance amplifier (I/V converter)
- The cantilever deflection is the ***normalized*** difference of the top quadrants minus the bottom quadrants

Piezo scanners

Piezo materials expand when a voltage is applied



Piezo scanners can be:

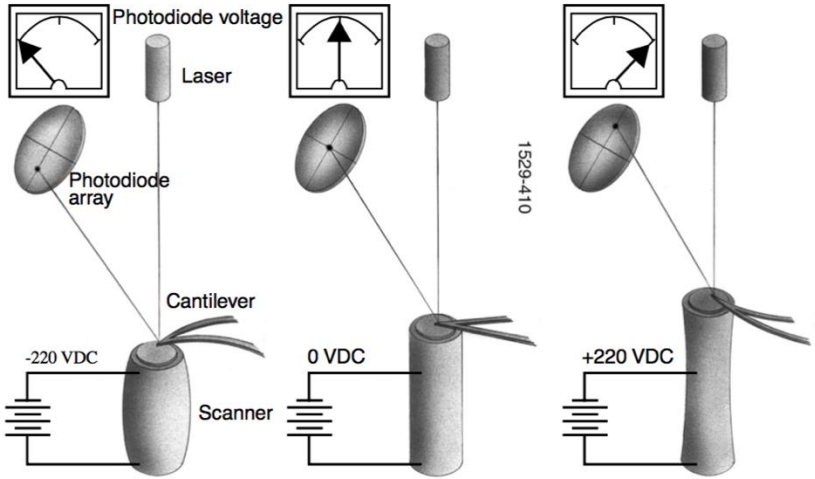
- Tubes
- Stacks
- Plates
- Monolythic piezo blocks

Or other types of actuation can be used:

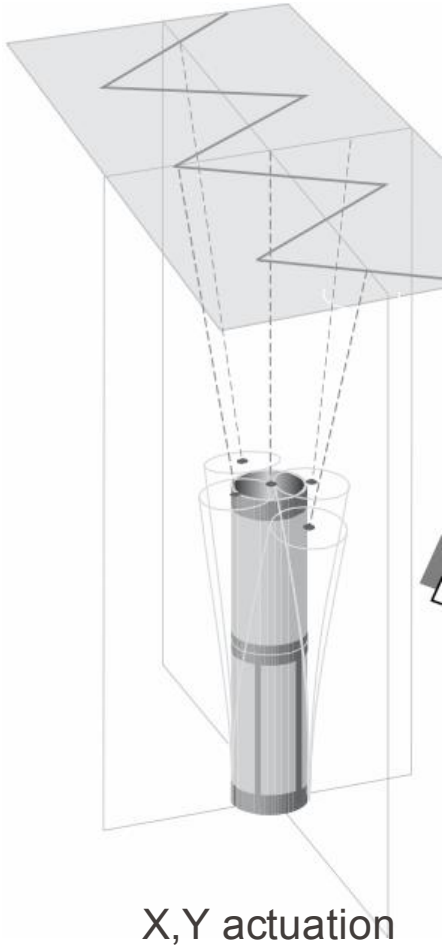
- Voice coil actuation
- Electrostatic combs
- Linear magnetic motors

Piezo scanners

Piezo materials expand when a voltage is applied



Z actuation



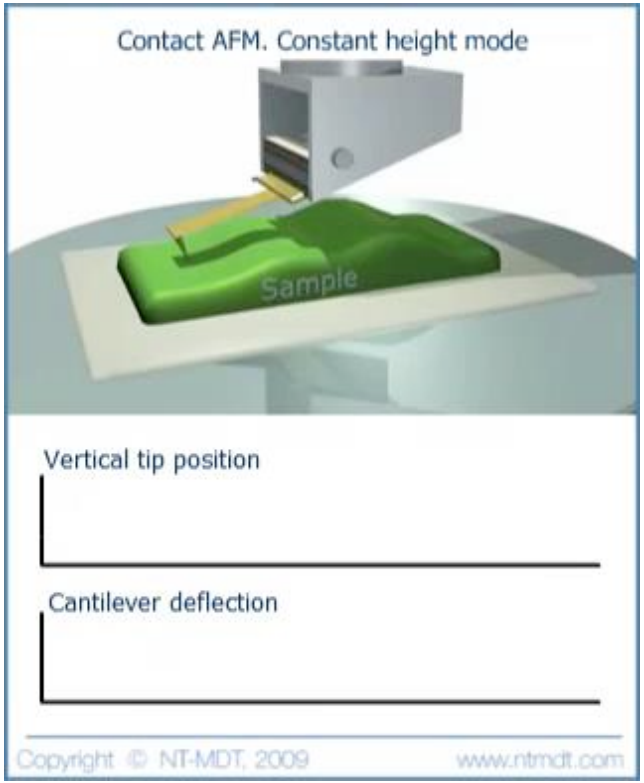
X,Y actuation

Images: Bruker Multimode Manual

Feedback

Why do we need feedback?

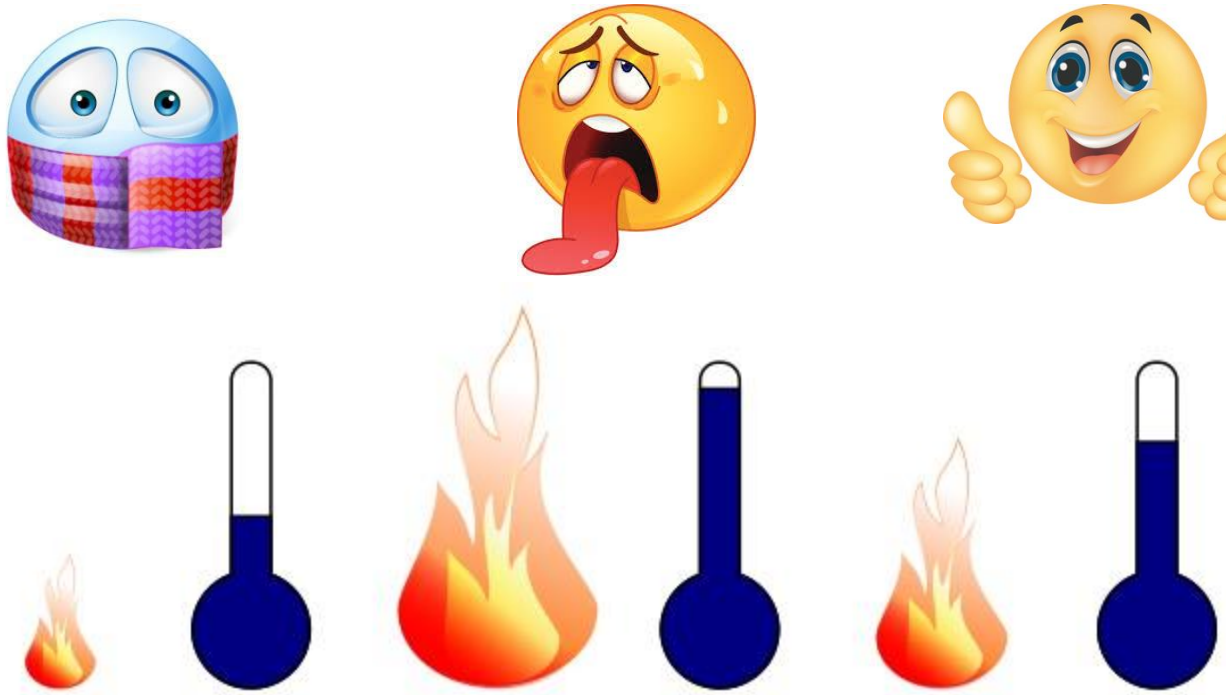
Constant height mode



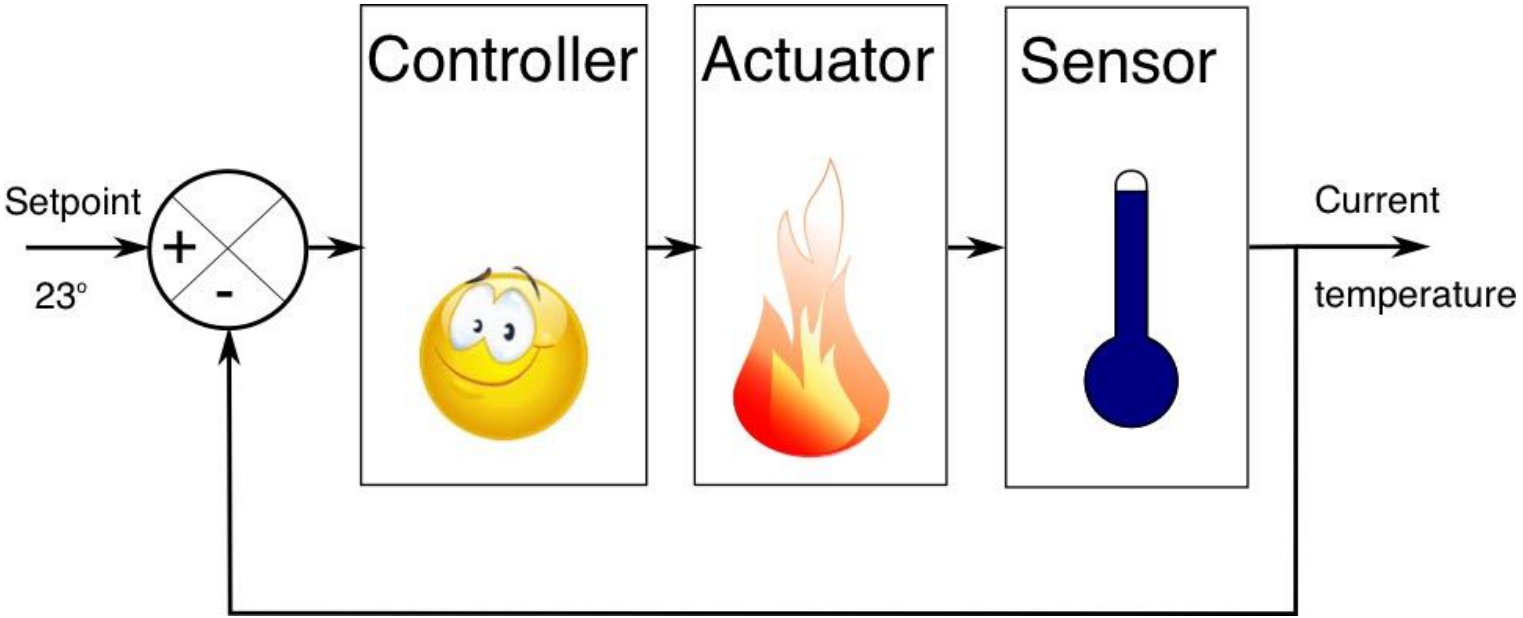
Why don't we just drag the cantilever over the surface?

- Cantilever deflection isn't linear → height measurement is distorted
- Force on cantilever is not constant → tip and sample can get damaged

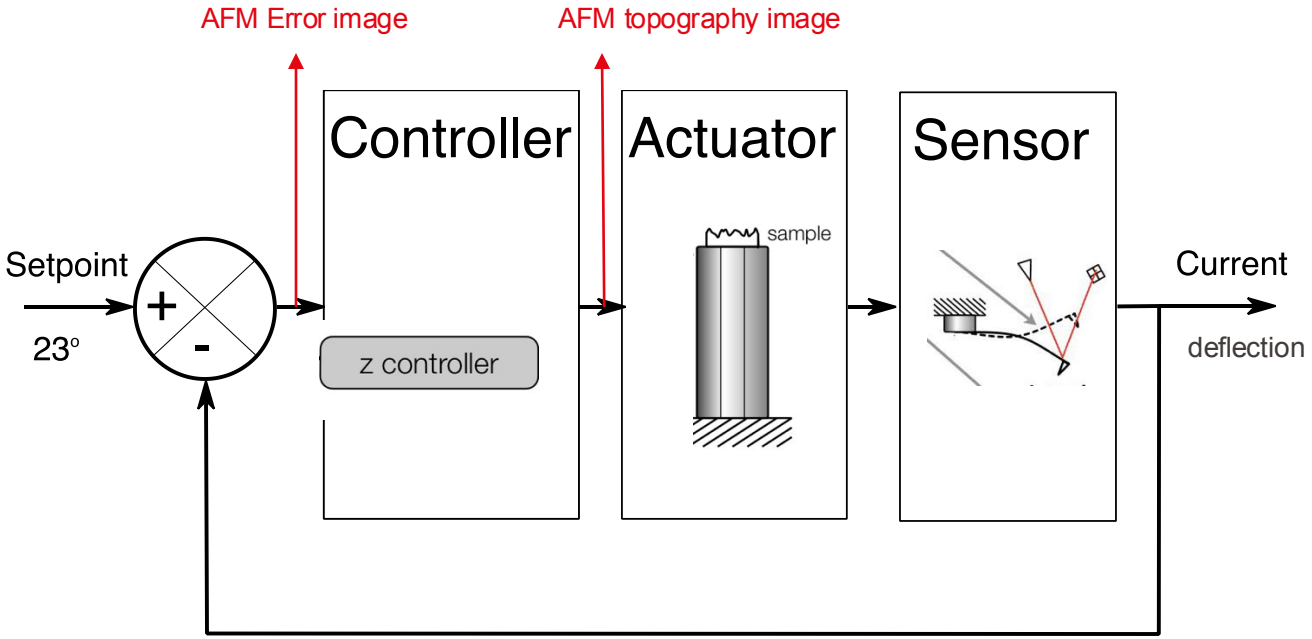
What do you do if you are cold?



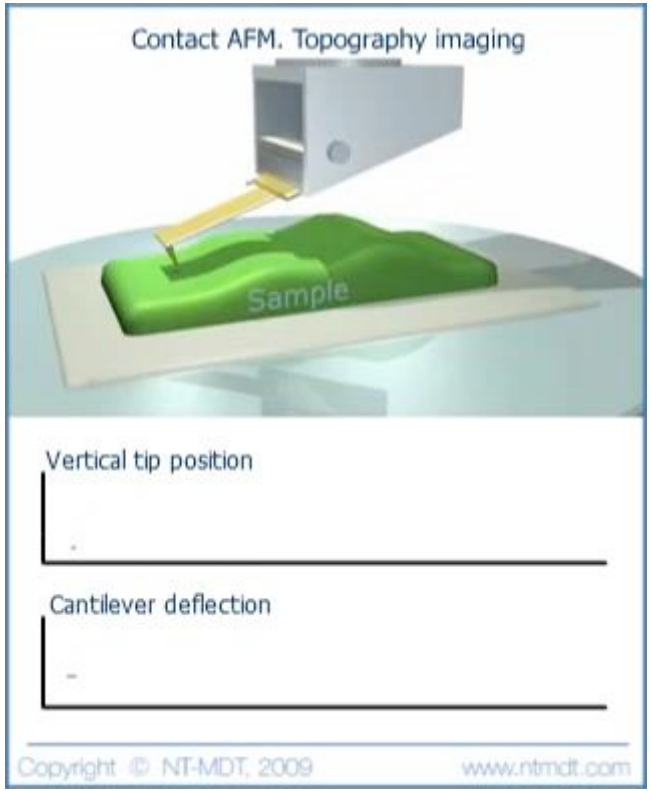
Rearranging into a feedback loop



Rearranging into a feedback loop



Feedback keeps the tip/sample interaction constant

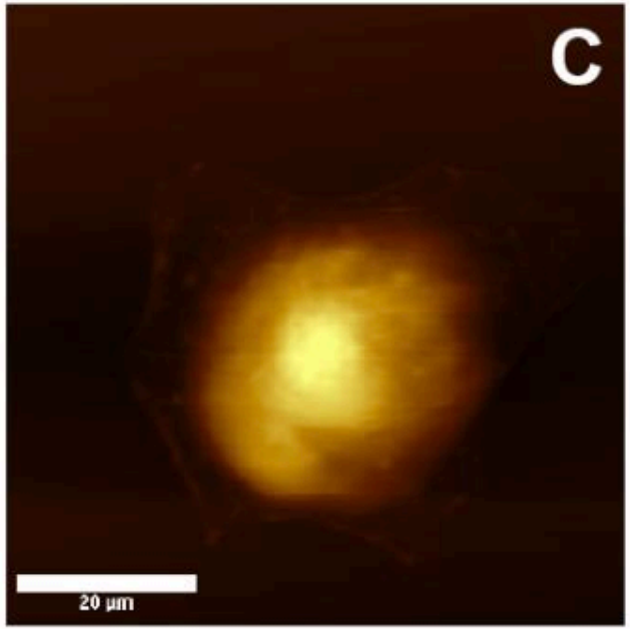


Benefits of operating in feedback:

- Cantilever deflection varies only slightly around setpoint
- The amount that the controller has to move the piezo up or down *approximates* the topography of the sample

Height image vs error image

Height



Error

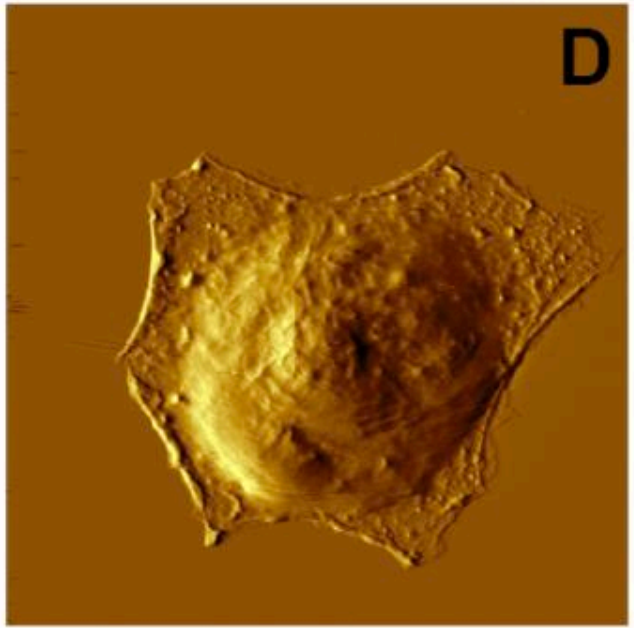


Image source: http://www.ntmdt.com

What is the meaning of the error signal?

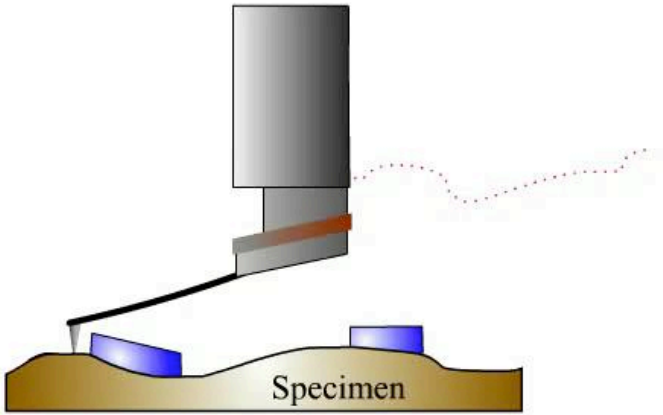


Fig. A. Sample and scanning probe.

Fig. B. Profile of scanner moving.

Fig. C. Profile of cantilever deflection changing.

- The deflection/error signal is as much part of the AFM image as the topography image (also called height image)!
- It accentuates edges and features with small spatial frequencies
- The height image combined with the error image represent the “true topography”

Imaging modes (dynamic modes)

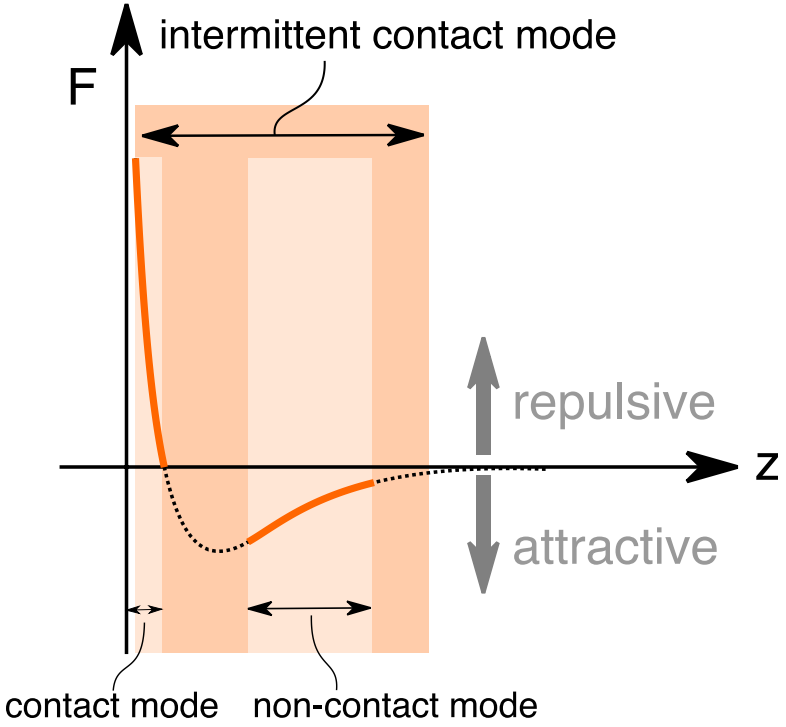
Dynamic modes

Reduces tip sample interactions

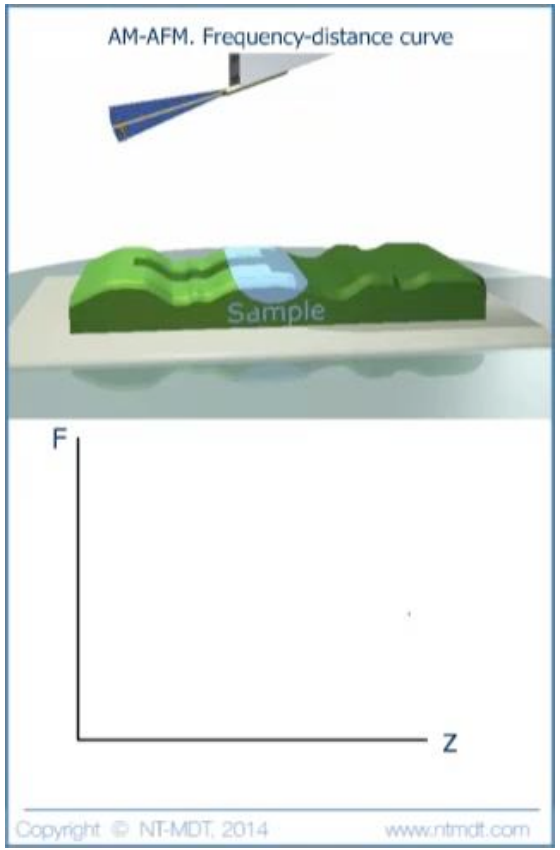
- Tapping mode™ (intermittent contact mode, amplitude modulation mode, dynamic mode,…)
- Non-contact mode
- Off resonance modes (Peak Force Tapping™, QI mode™, hopping mode™, HybriD mode™,…)

Lennard-Jones potential

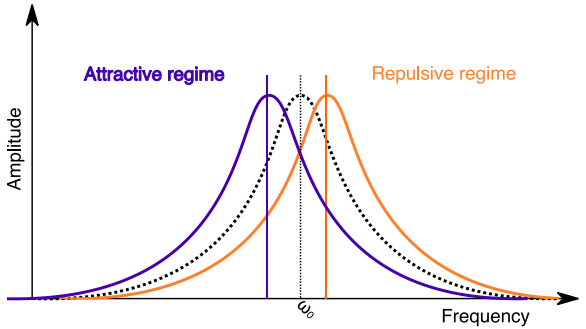
The cantilever feels different force regimes



Oscillating approach curves

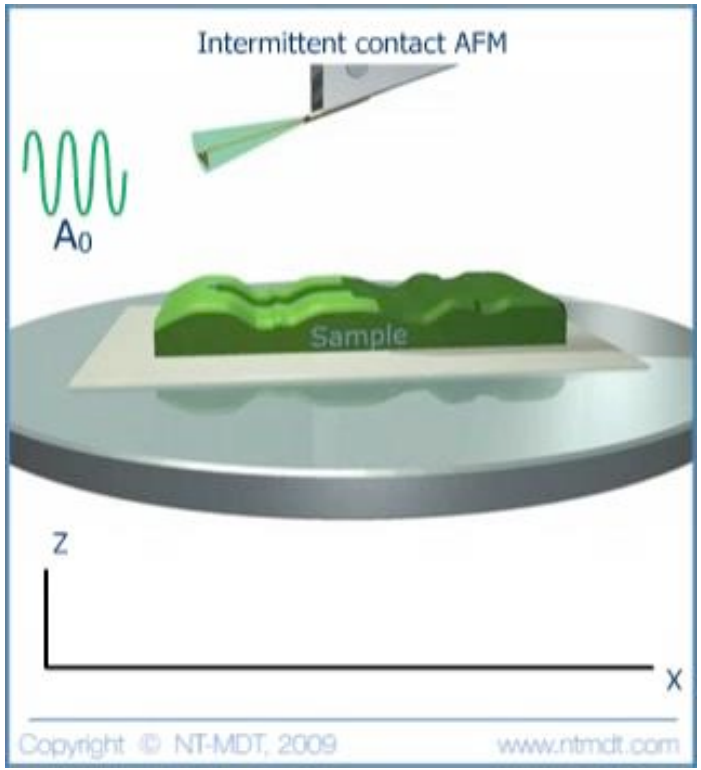


- As the cantilever approaches the surface it feels different forces (due to the Leonard Jones potential)
- When the cantilever is in the attractive regime the resonance frequency decreases
- When the cantilever is in the repulsive regime the resonance frequency increases

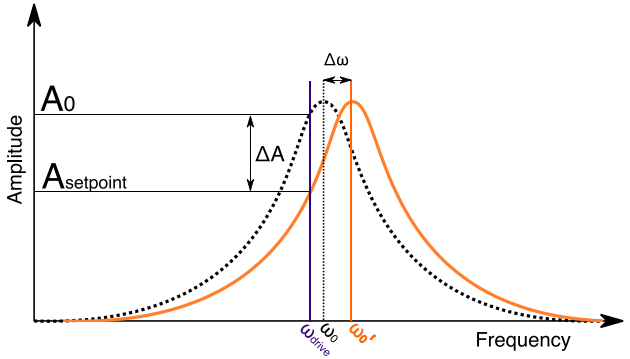


Amplitude modulation

(a.k.a. Tapping mode™)

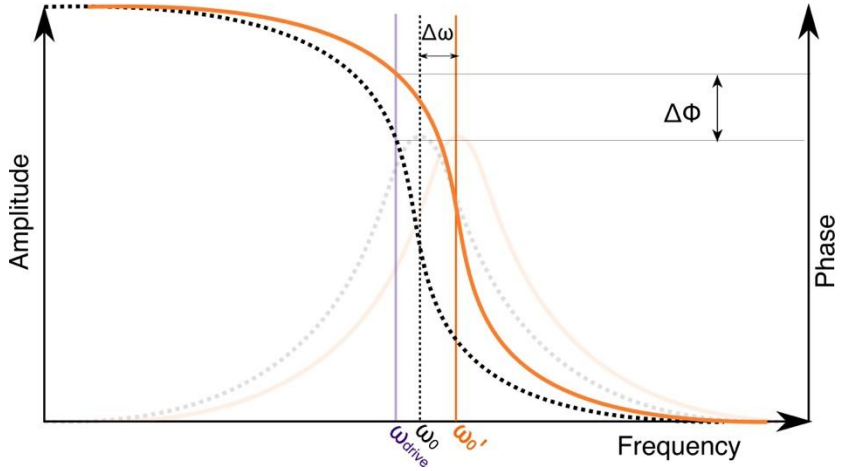


- In tapping mode we excite the cantilever at a fixed frequency ω_{drive} slightly below its resonance frequency
- As the cantilever approaches into the repulsive regime, the resonance frequency (of cantilever + sample force) increases.
- At the fixed frequency ω, the resulting amplitude will therefore drop as we enter the repulsive regime
- The amplitude error is used for the feedback parameter



Phase imaging

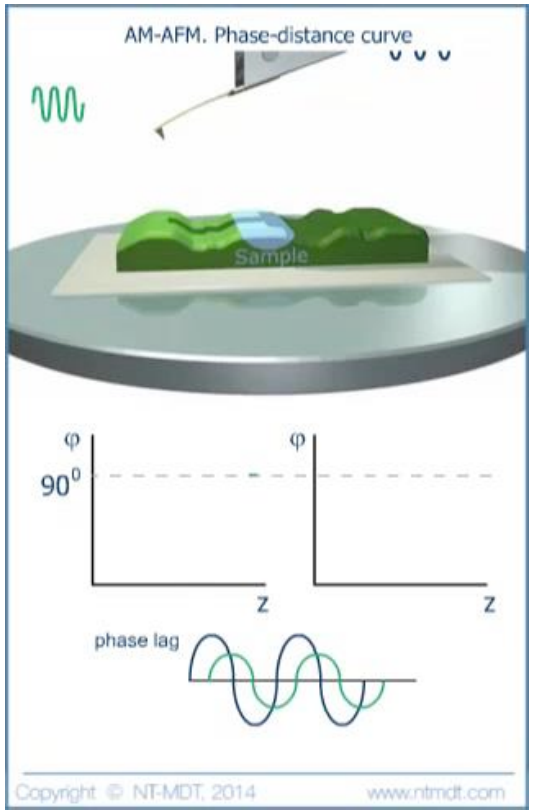
In tapping mode



- The phase difference between the cantilever drive signal and the cantilever oscillation is called the “phase signal”
- The resonance shift Δω also introduces a phase shift Δφ at the driving frequency ω_{drive}
- This shift could also be used for feedback, but…
- … other factors such as materials properties affect phase as well

Phase imaging

In tapping mode



- The phase signal “represents” the damping that the cantilever feels due to the tip sample interaction
- This damping can be due to topography (especially side walls)
- Or it could be due to damping by the sample material

Force Curves

Tip sample interactions

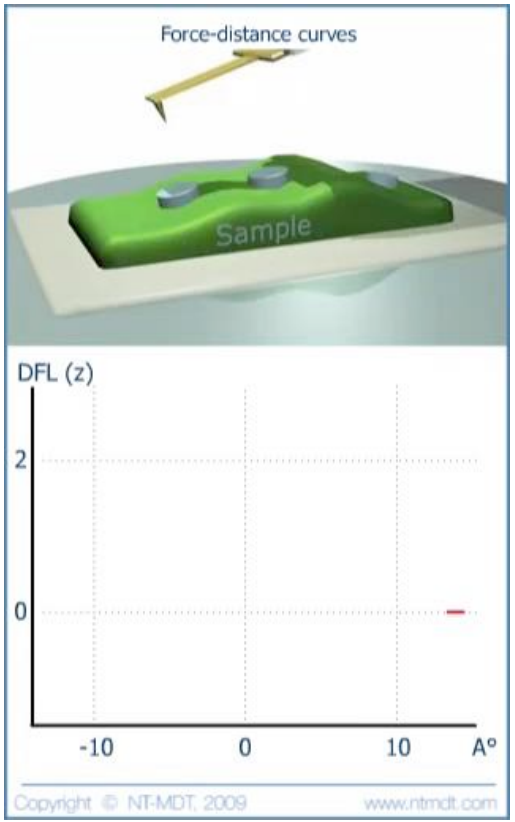
Force curves

There are many forces that can act between the tip and the sample

- Van Der Waals forces (attractive)
- Pauli repulsion (repulsive)
- Electrostatic forces (attractive or repulsive)
- Capillary forces (attractive)
- Magnetic forces (attractive or repulsive)
- …

We can measure what forces act on a cantilever as a function of distance from the surface by measuring a ***Force Curve***

Force curves



Force curves can tell us a lot about the tip sample interaction:

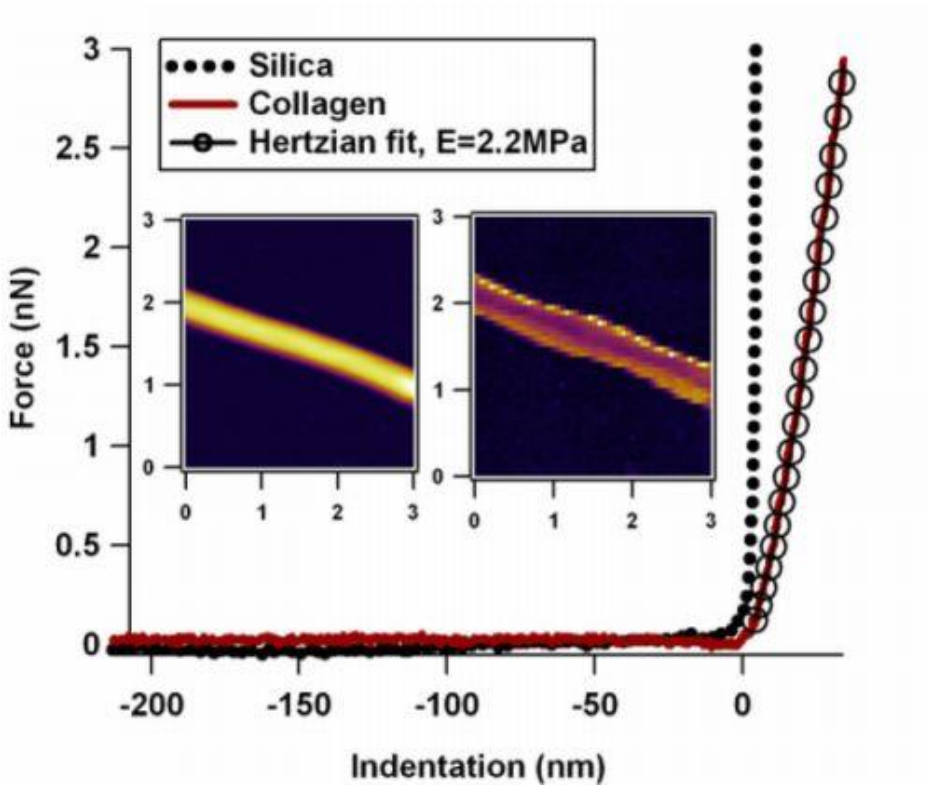
- What is the adhesion of tip to sample
- What is the hardness of the sample
- What is the energy dissipation per cycle

Or about our measurement setup

- What is the deflection sensitivity (how many nm do we have to deflect the cantilever to measure 1V shift in the 4-quadrant photodiode)

Force volume mode

Creating mechanical properties maps



http://www.imaging-git.com

Single molecule force spectroscopy

Force curves as a tool for single molecule mechanics

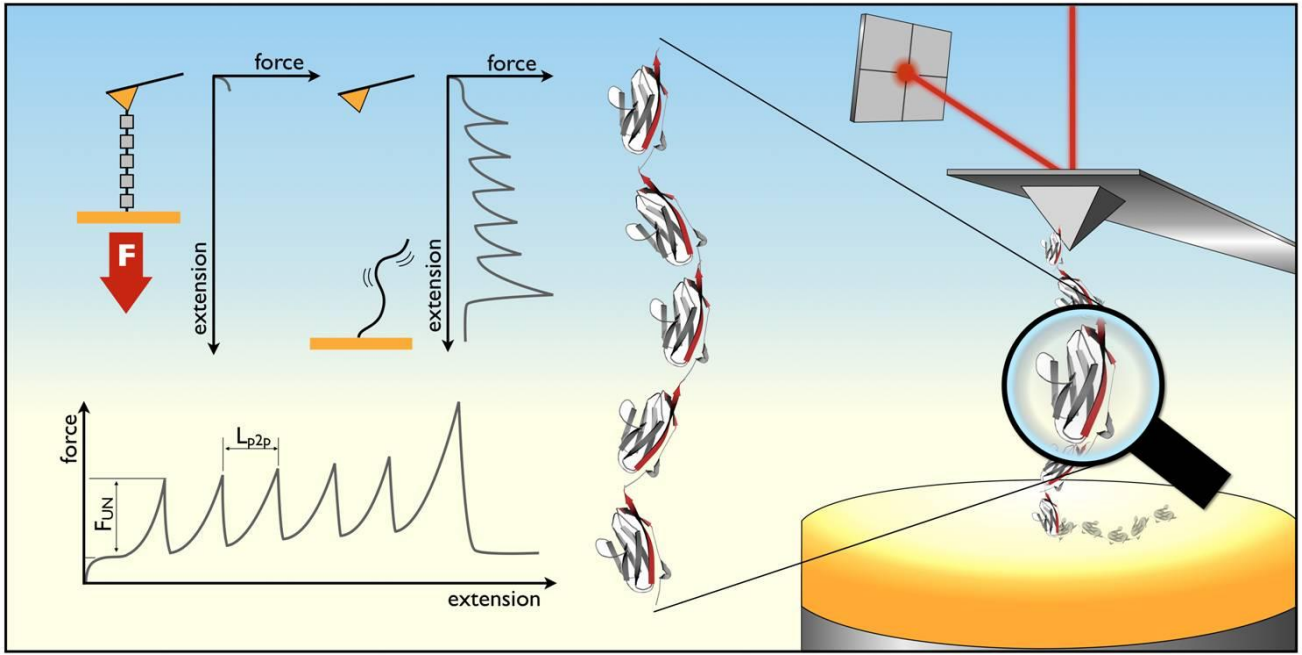
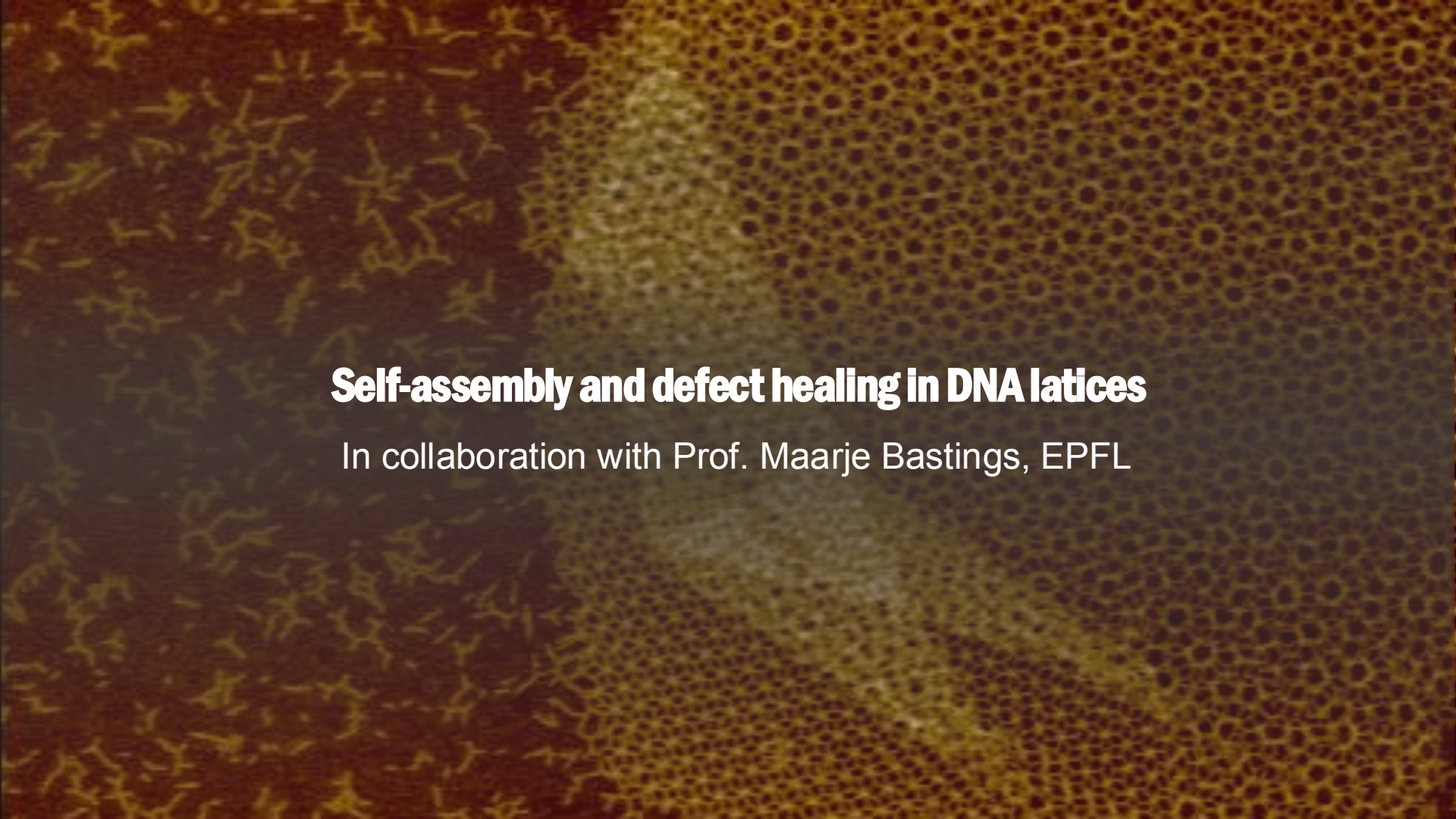
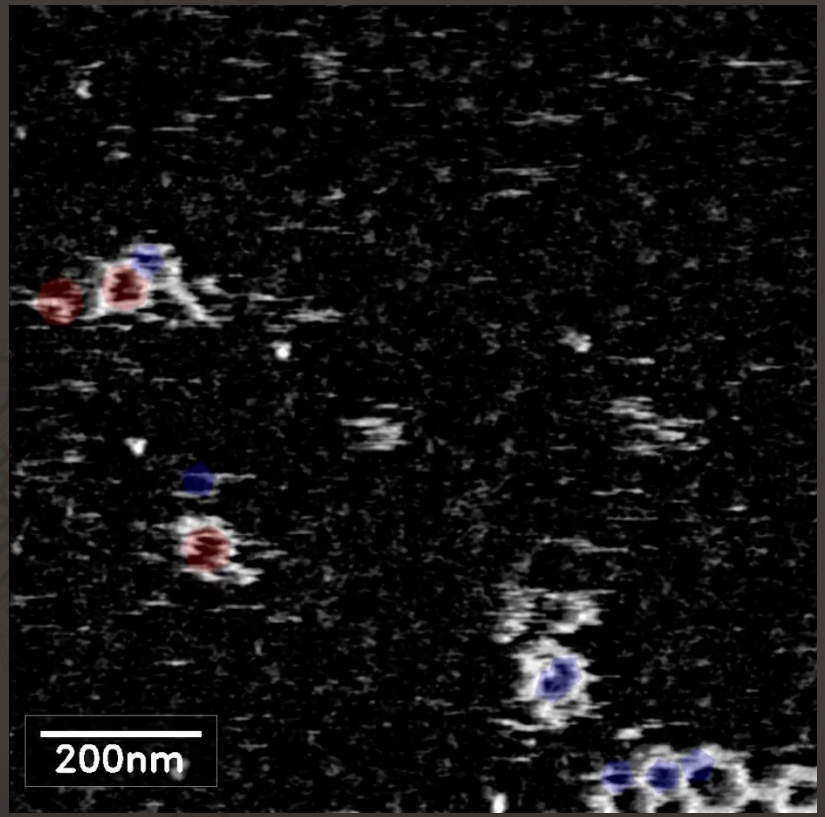


Image source: Dr Lorna Dougan - http://www1.mnp.leeds.ac.uk/ldougan/


Self-assembly and defect healing in DNA latices
In collaboration with Prof. Maarje Bastings, EPFL

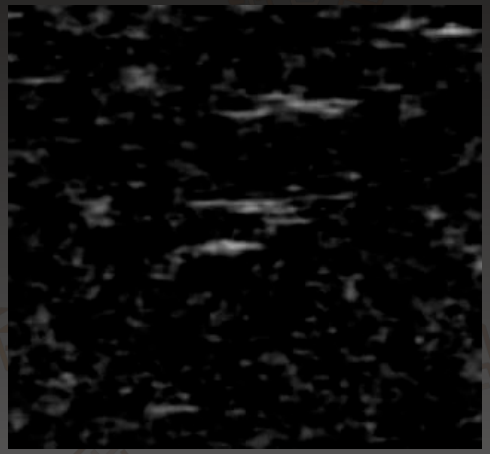
Defect healing in DNA-latices

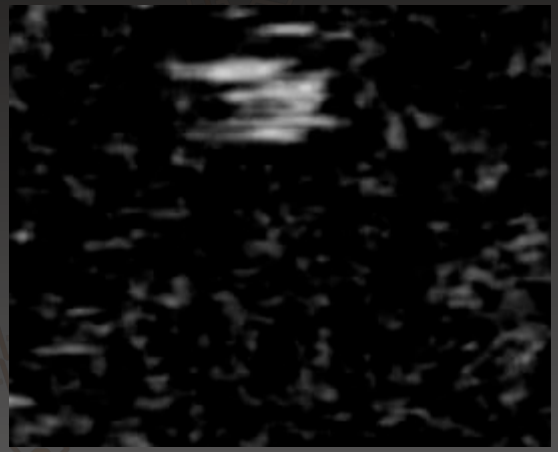


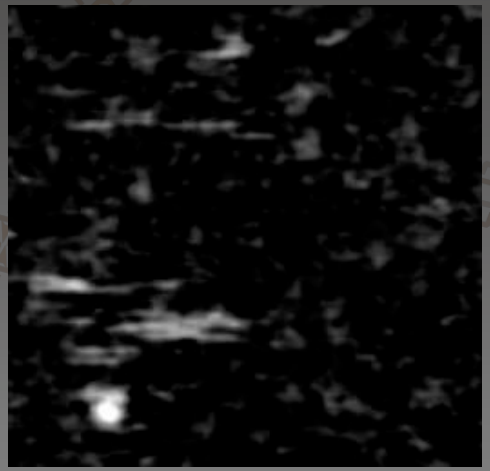
100kHz OBT rate, 80kHz line rate (512x512 pixel) (tube, XY=140nm)

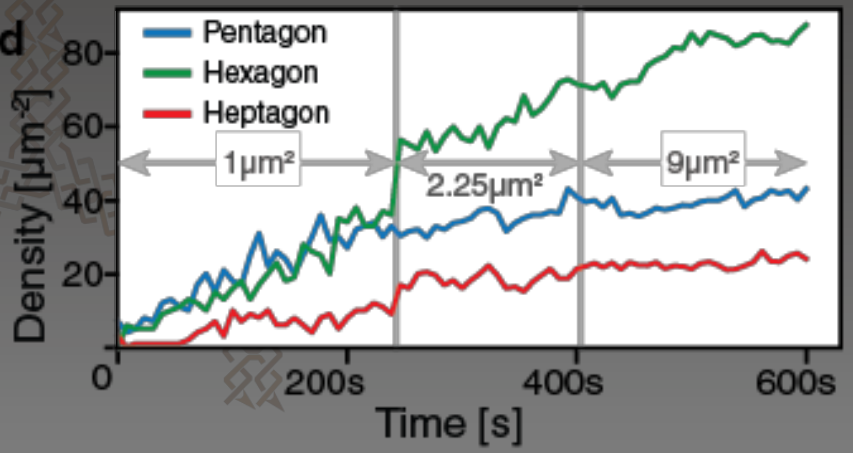
Nievergelt et al, *Small Methods* 2019

Characterization of defect formation and healing









Centrioles are at the heart of the mitotic spindle

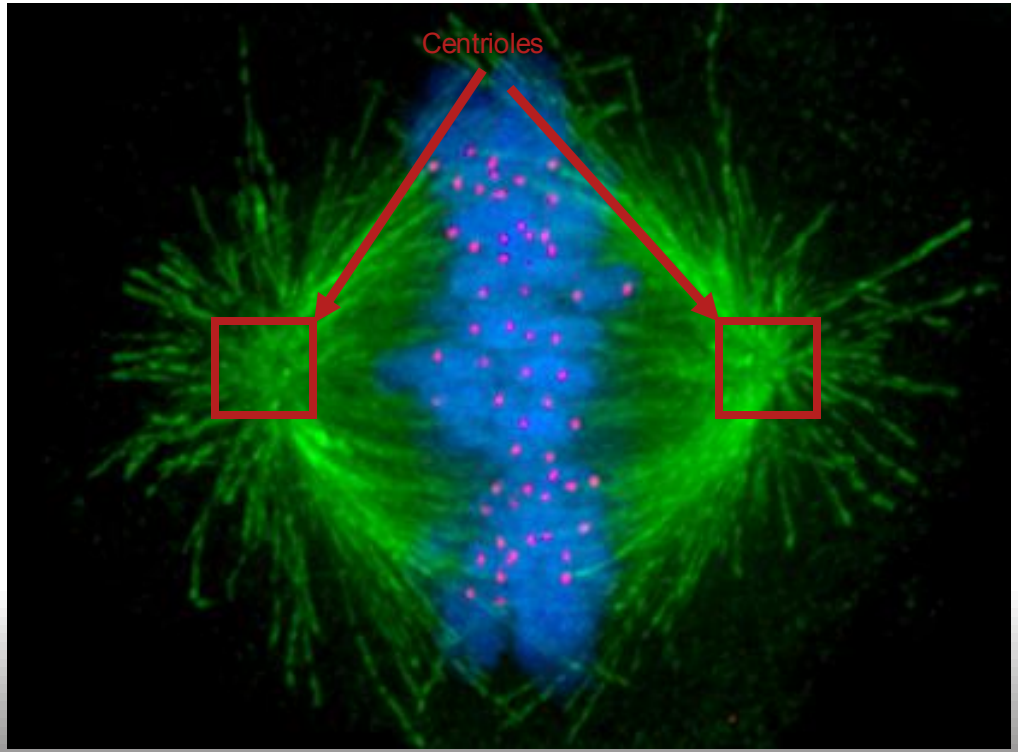
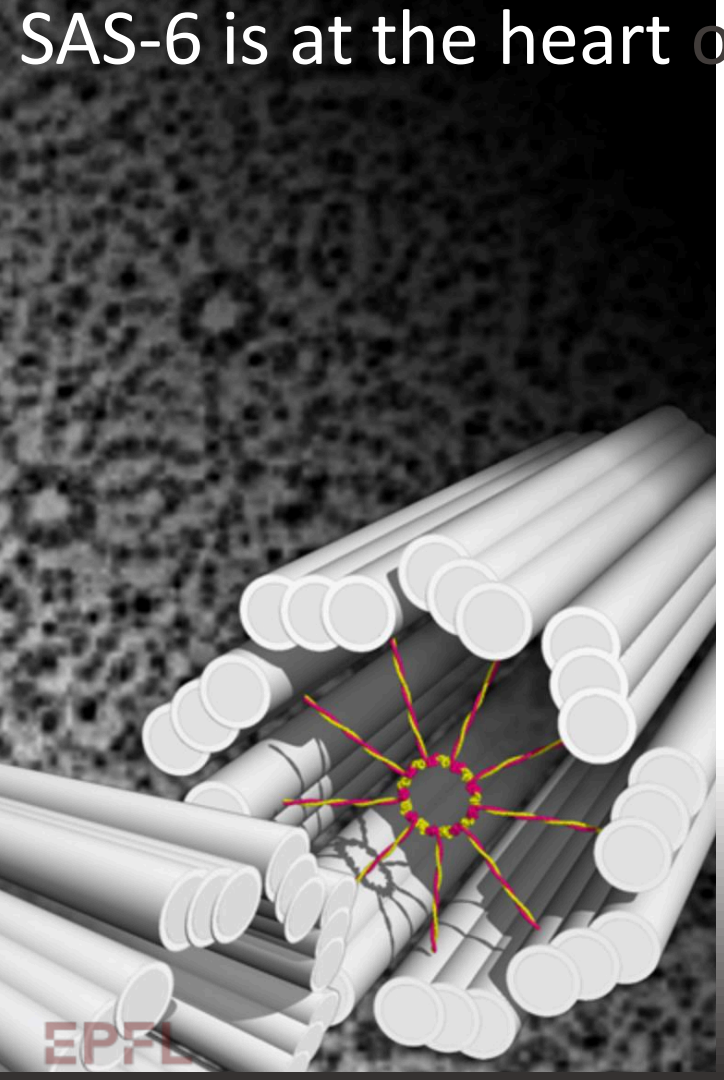
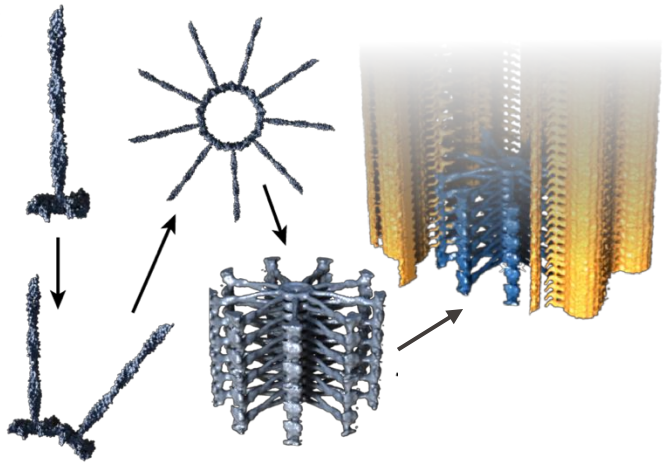


Image source: wikipedia.com

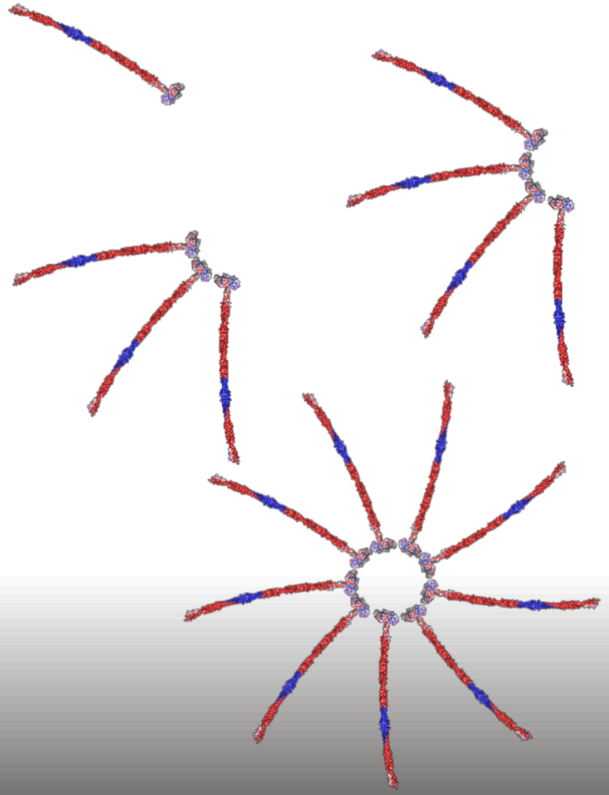
SAS-6 is at the heart of the centrioles





Guichard et al. ***Science*** Vol. 337, Issue 6094, pp. 553, 2012
Hilbert et al, ***Nature Cell Biology***, vol. 18, num. 4, p. 393-+, 2016.
Guichard et al, ***Nature Communications***, vol. 8, 2017.

Pierre Cörazy



Pierre Cörtzy

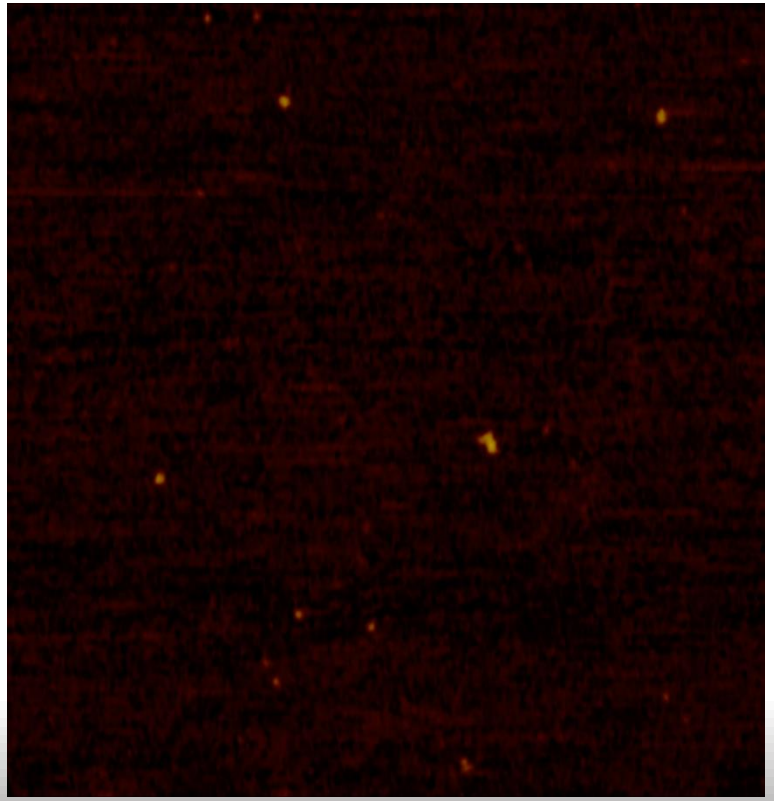
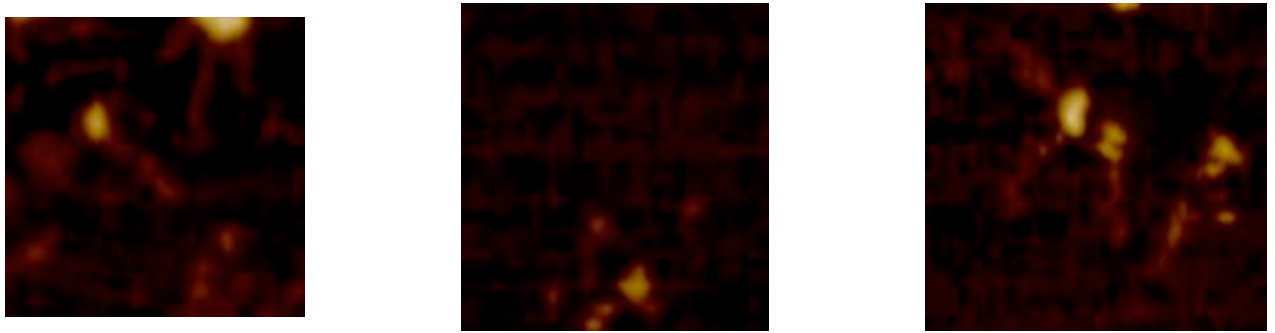
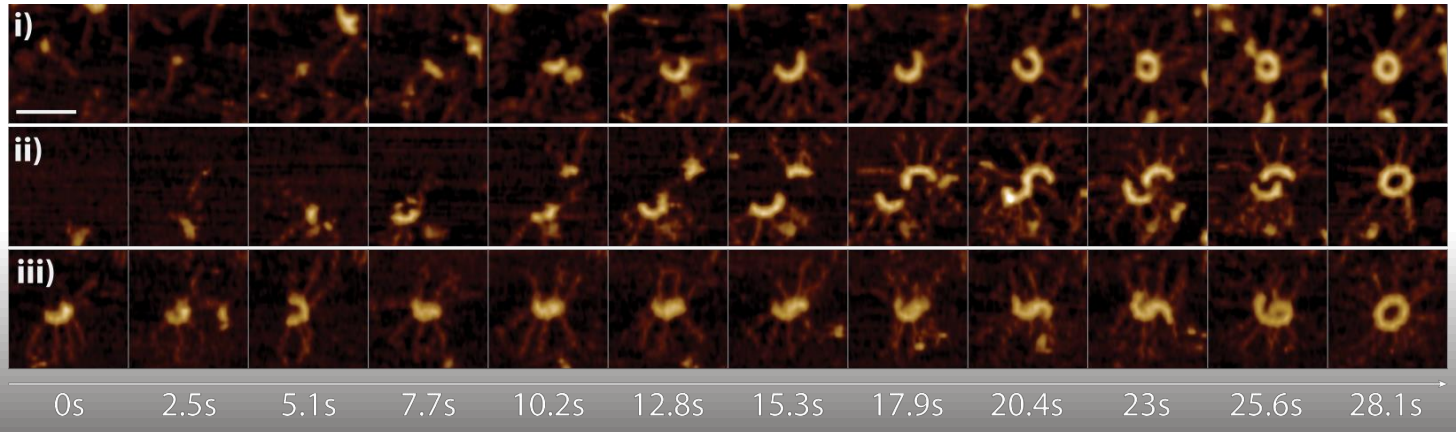


Image: 512x256, 150 um/s, 100 lines/s, PORT mode AC-10

Nievergelt et al. ***Nat. Nanotechnol. 2018***

Multiple pathways for SAS-6 cartwheel assembly

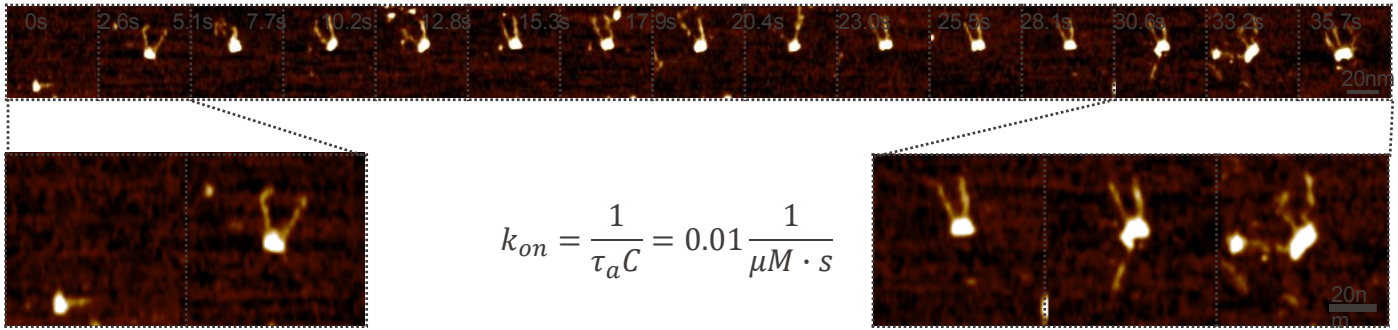




Nievergelt et al. ***Nat. Nanotechnol. 2018***

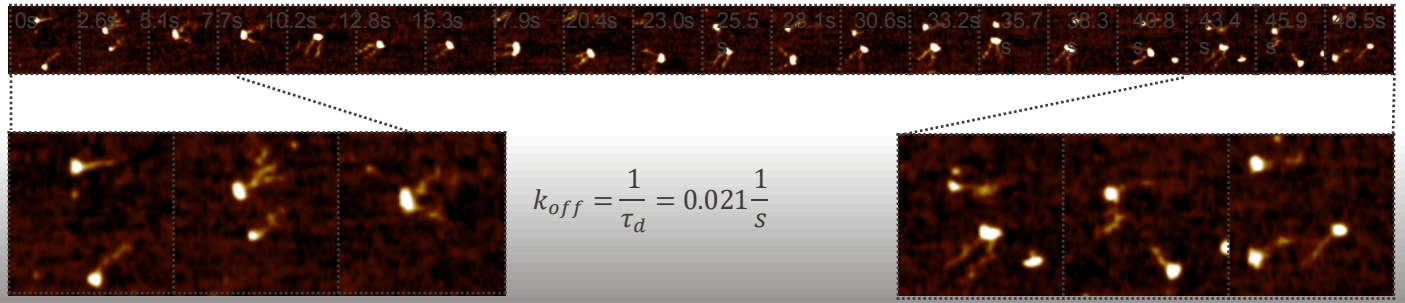
Measurement of k_{on} and k_{off}

Measured the time τ_a between two successive association events



$$k_{on} = \frac{1}{\tau_a C} = 0.01 \frac{1}{\mu M \cdot s}$$

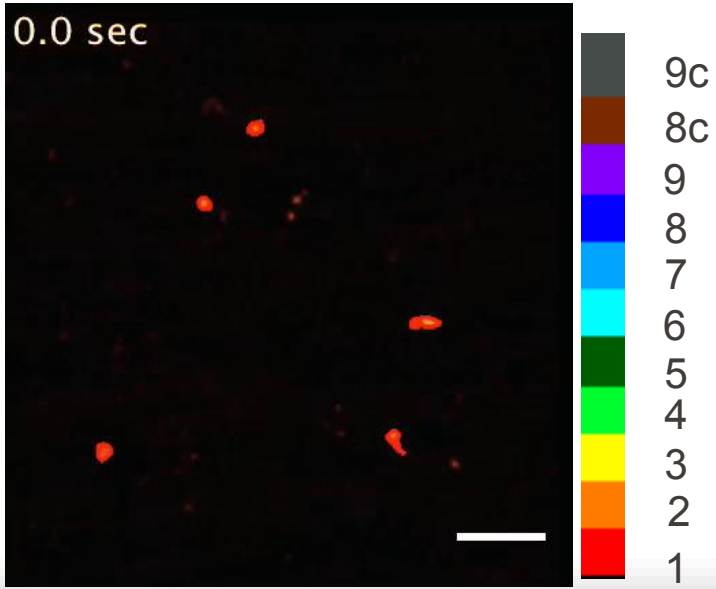
Measured the time τ_d of dimer dissociation in low concentration movies

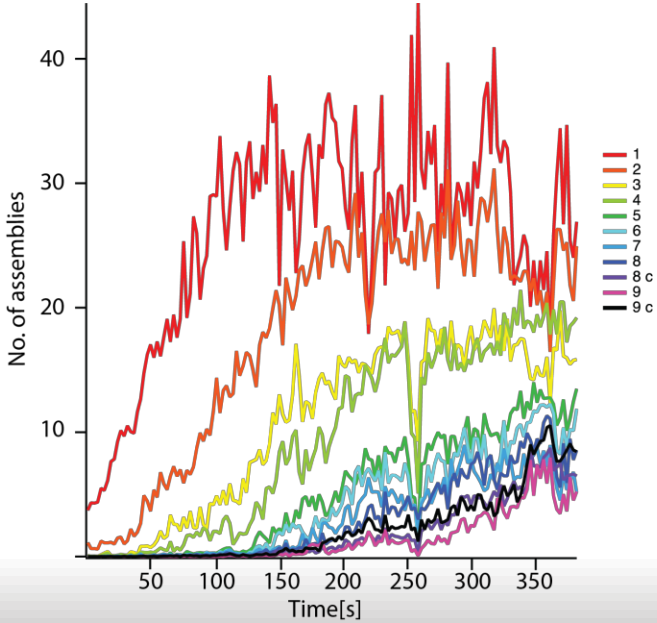


$$k_{off} = \frac{1}{\tau_d} = 0.021 \frac{1}{s}$$

Banterle et al. In preparation

Automatic classification





Banterle et al. In preparation

Bacterial Nanoscopy:

A closer look at the growth and division of *M.Smegmatis*

Very basic things we don't know about *Mycobacteria*...

How do *Mycobacteria* grow?

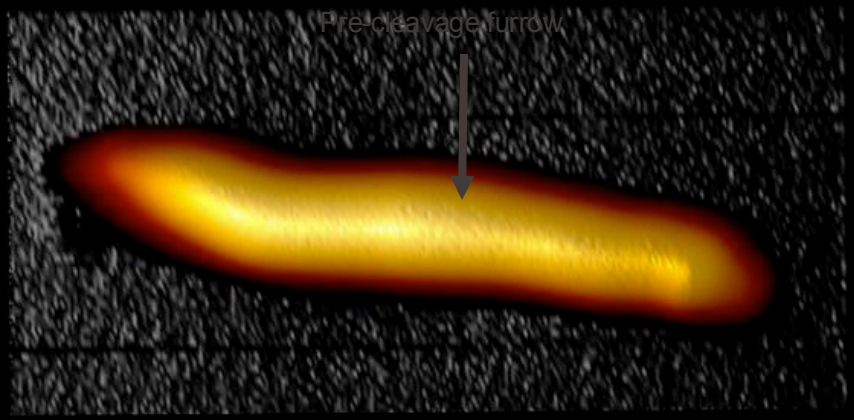
How do *Mycobacteria* divide?

…

4 µm

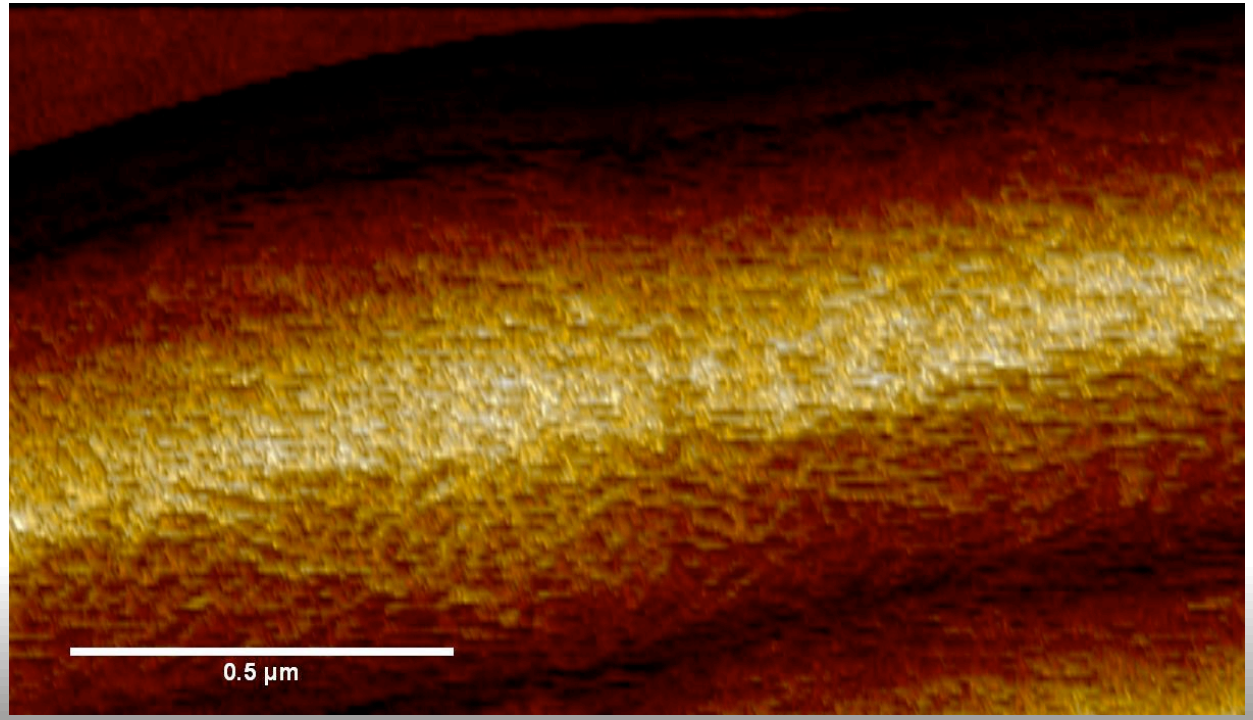
Adapted from:
Santi,…, McKinney, *Nat..Communications 2013* **4**, Article number: 2470

0h 00min



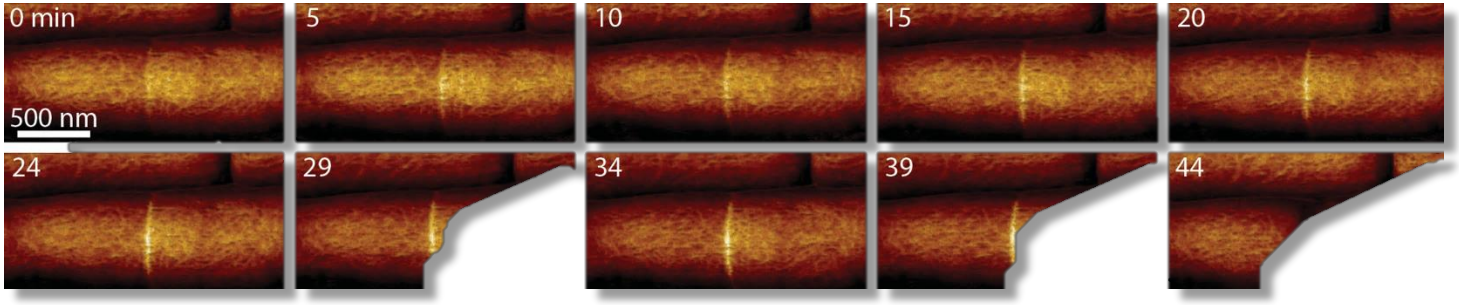
4 µm

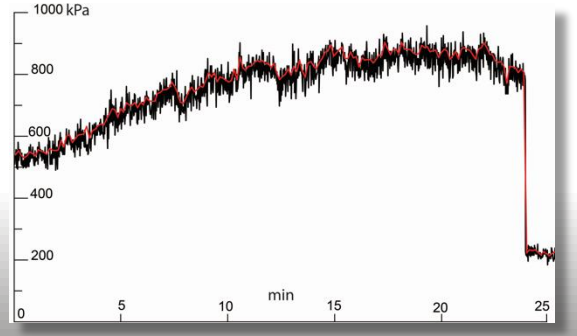
What Happens During Cell Seperation?

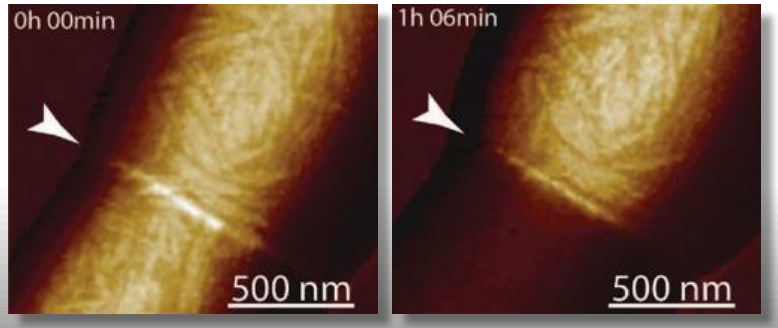


Odermatt et al. Nature Physics 2019

Tensile stress induced stiffening of the cell wall?





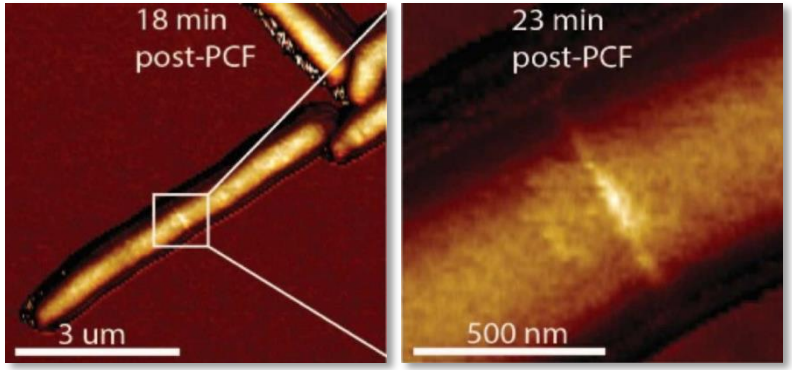


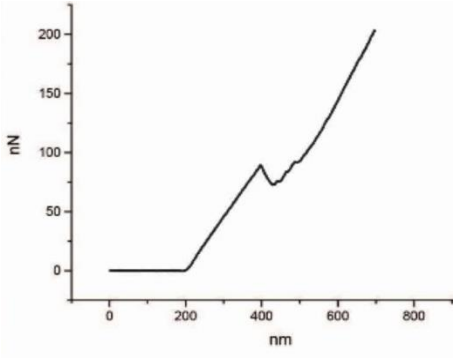
Odermatt et al. Nature Physics 2019

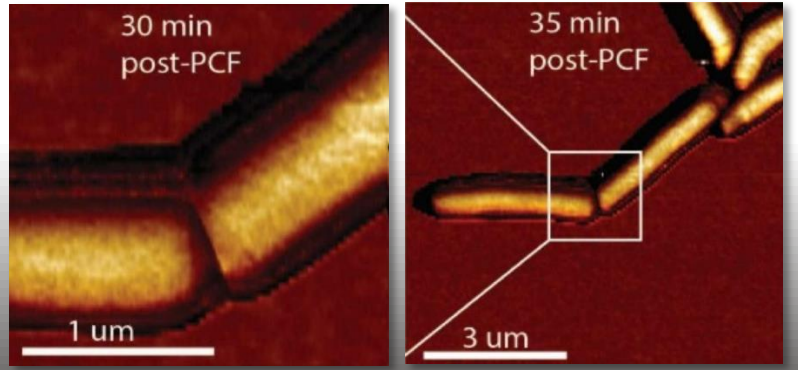
Hypothesis:

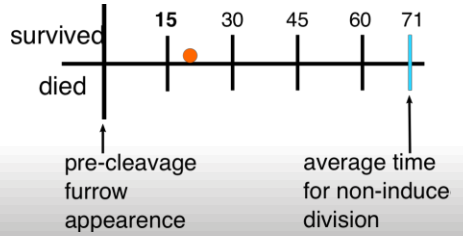
Cells separate once the tensile stress in the cell wall exceeds the ultimate tensile strength of the cell wall material

Mechanically induced cell









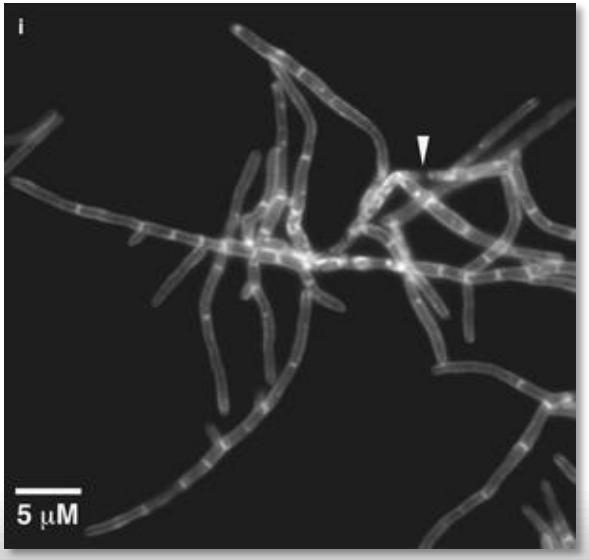
Odermatt et al. Nature Physics 2019

Hypothesis:

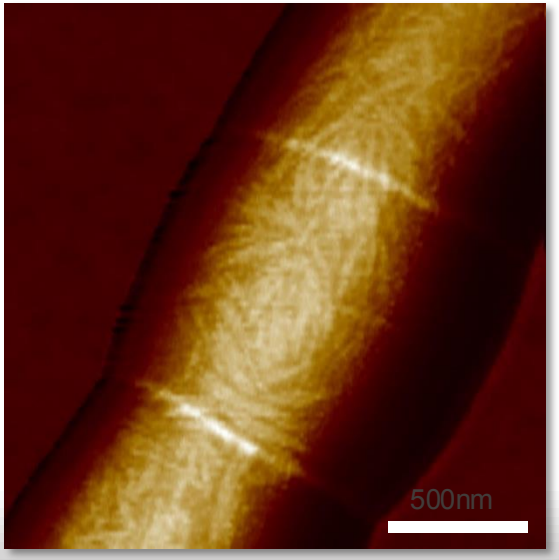
Cells separate once the tensile stress in the cell wall exceeds the ultimate tensile strength of the cell wall material

Why does the cell need hydrolases?

RipA Depletion Prevents Natural cell Separation

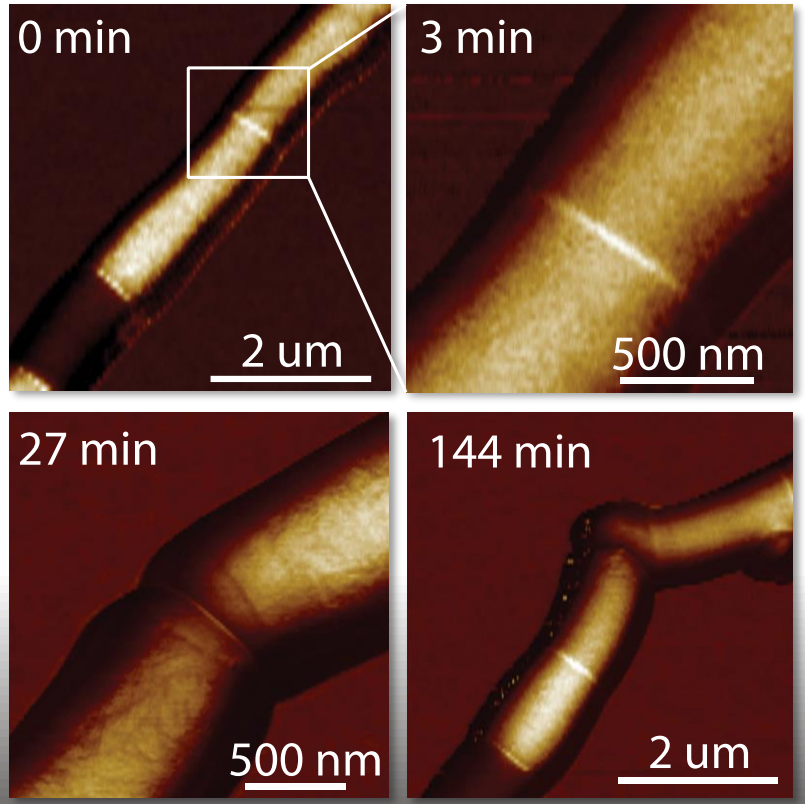


Hett EC et al, (2008) A Mycobacterial Enzyme Essential for Cell Division Synergizes with Resuscitation-Promoting Factor. PLoS Pathog 4(2): e1000001.



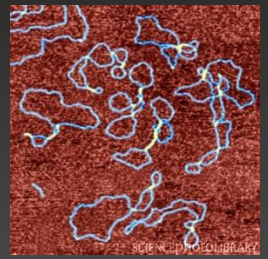
Odermat et al. in preparation

Mechanical stress can make up for missing enzymes

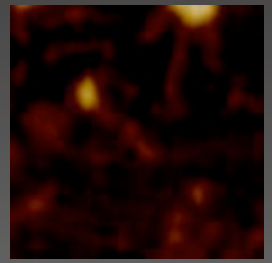


Odermatt et al. Nature Physics 2019

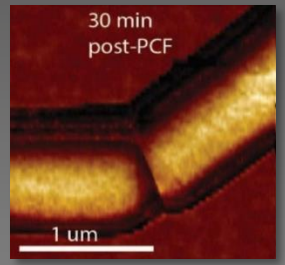
Conclusions



- AFM is a versatile tool for studying biological samples at high resolution in physiological conditions



- High-speed AFM can be used to image single molecule dynamics



- AFM is an essential tool for cellular mechanobiology