Recenti sviluppi nella tecnica di microscopia a scansione di sonda: al di là della topografia

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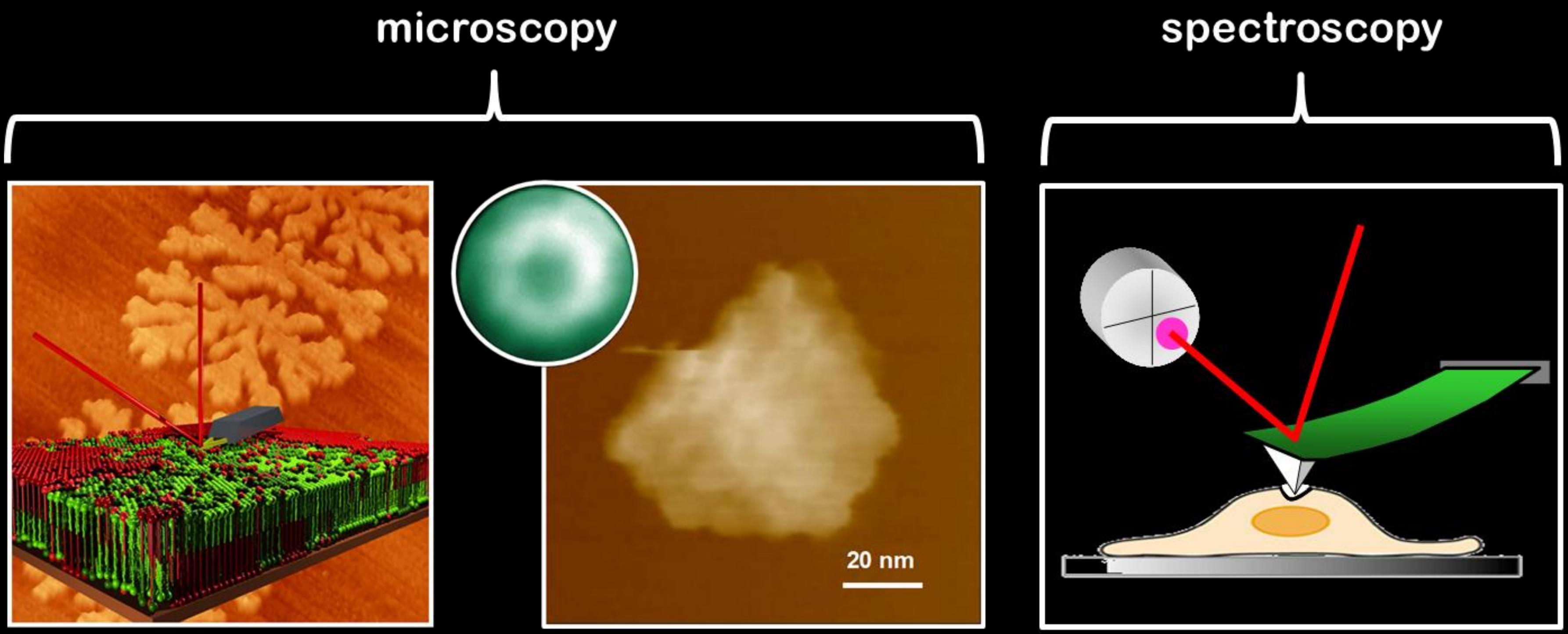
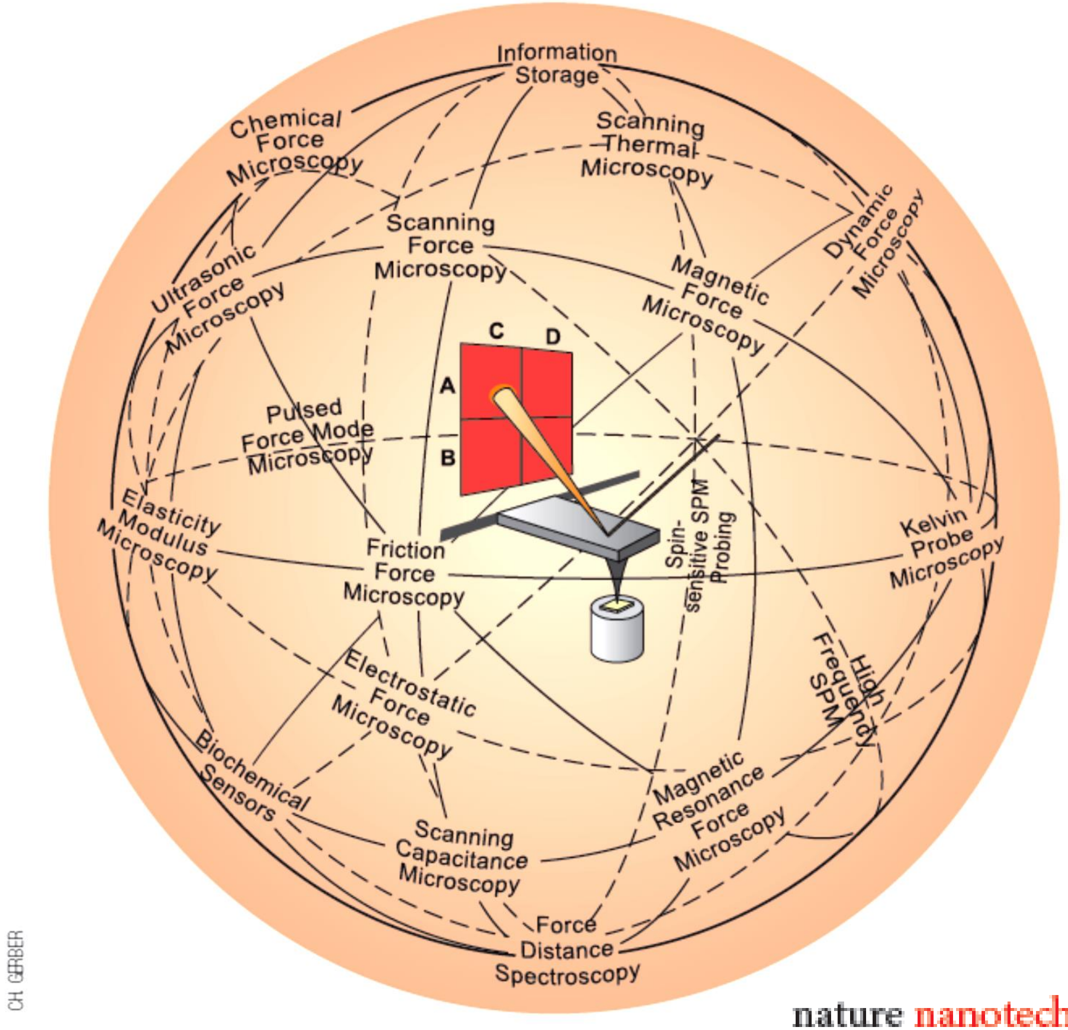


Scanning Probe Microscopy (SPM) ‘Family tree’

Starting from Scanning Tunneling Microscopy (STM)



The AFM has inspired a variety of other scanning probe techniques

nature nanotechnology | VOL 1 | OCTOBER 2006 |

THE NOBEL PRIZE IN PHYSICS 1986

The crucial role of scanning tunneling microscopy for our understanding of the material world, and its immanent significance for the future of scientific research and technical progress, has been duely appreciated by the awarding of the 1986 Nobel Prize in Physics to its developers Gerd Binnig and Heinrich Rohrer. It represented the latest stage in the series of developments of microscopies not based on light, which started with the development of the electron microscope by Ernst Ruska who shared that year Nobel Prize with them, opening the way to ***resolutions way beyond the limits set by the wavelength of light.***

G. Binning and H. Rohrer, Helvetica Physica Acta **55**, 726 (1982).

A very brief history of AFM

1986 – Atomic force microscopy is invented

1988 – Development of the Optical Beam Deflection (OBD) method

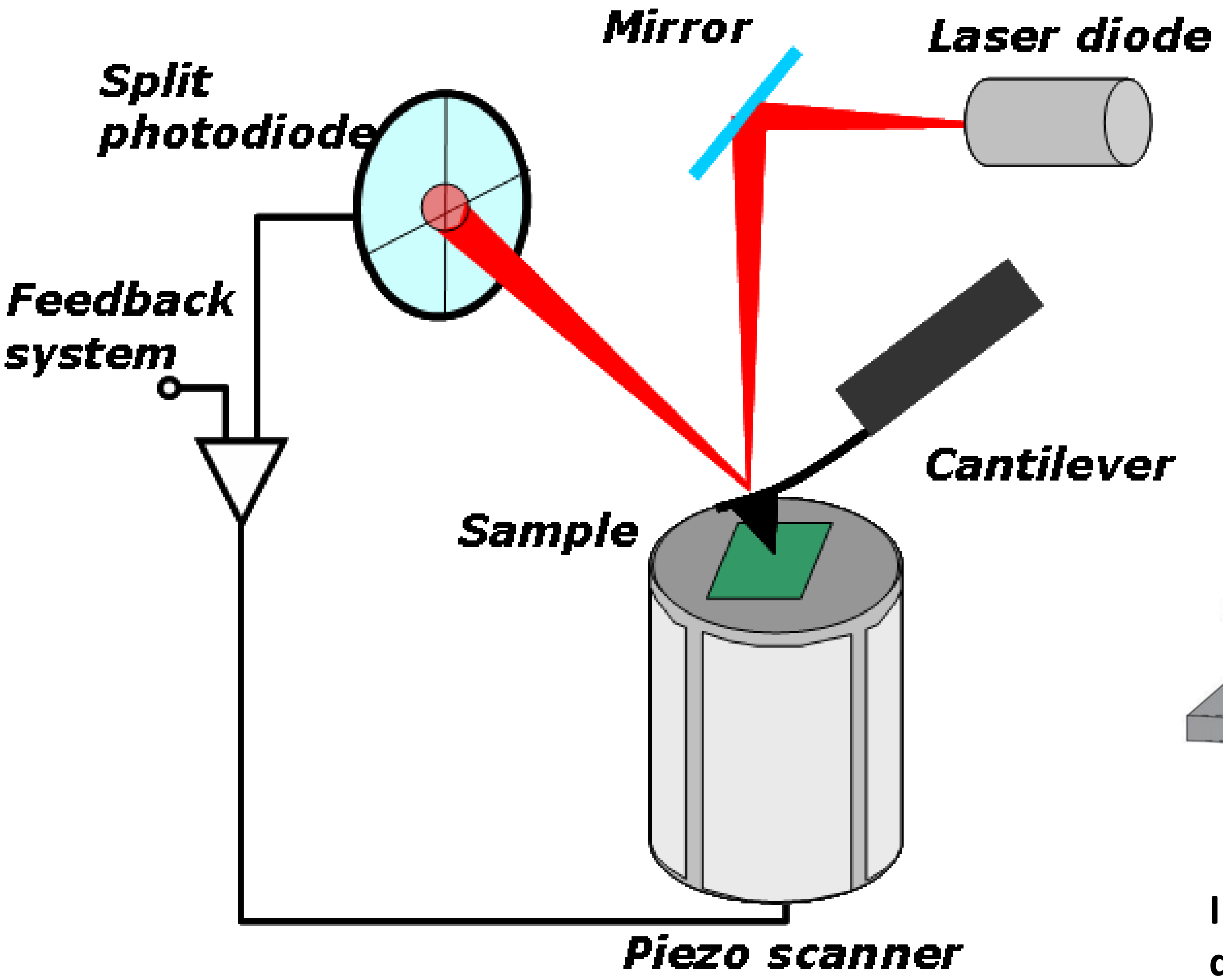
1991 – Microfabricated tips were developed

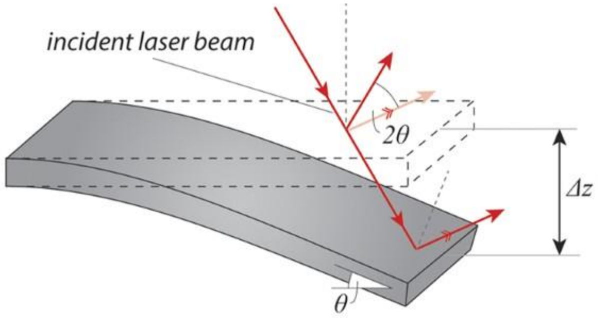
1993 – The Amplitude Modulated imaging mode is introduced

1994 – First Amplitude Modulated Imaging mode in liquid

1999-2001 – First attempts to develop small cantilevers for High Speed Atomic Force Microscopy

Very basic concepts of Atomic Force Microscopy





In the AFM technique the deflection of the cantilever is typically detected (OBD) and not the height of the tip

A. Alessandrini, P. Facci
AFM: a versatile tool in biophysics,
Measurement Science and Technology 16, R65-R92 (2005)

The Chemical Structure of a Molecule Resolved by Atomic Force Microscopy

Leo Gross,[1]* Fabian Mohn,[1] Nikolaj Moll,[1] Peter Liljeroth,[1,2] Gerhard Meyer[1]

Resolving individual atoms has always been the ultimate goal of surface microscopy. The scanning tunneling microscope images atomic-scale features on surfaces, but resolving single atoms within an adsorbed molecule remains a great challenge because the tunneling current is primarily sensitive to the local electron density of states close to the Fermi level. We demonstrate imaging of molecules with unprecedented atomic resolution by probing the short-range chemical forces with use of noncontact atomic force microscopy. The key step is functionalizing the microscope's tip apex with suitable, atomically well-defined terminations, such as CO molecules. Our experimental findings are corroborated by ab initio density functional theory calculations. Comparison with theory shows that Pauli repulsion is the source of the atomic resolution, whereas van der Waals and electrostatic forces only add a diffuse attractive background.

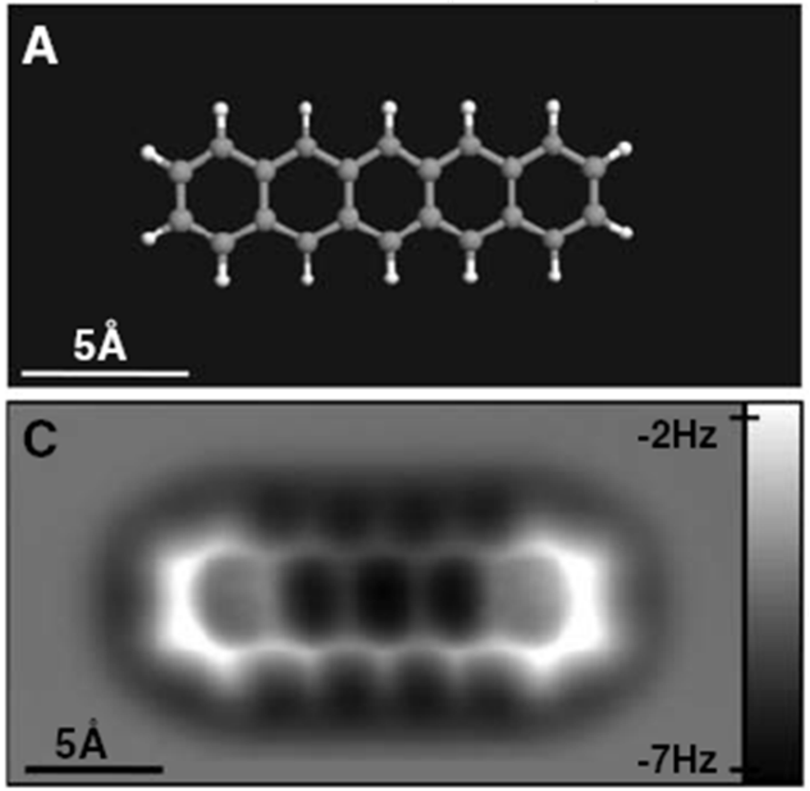
28 AUGUST 2009 VOL 325 **SCIENCE** www.sciencemag.org

STM is sensitive to the density of states near E_F, which extends over the entire molecule. This prevents the direct imaging of the atomic positions (or core electrons) in such planar aromatic molecules by STM.

Noncontact atomic force microscopy (NC-AFM), usually operated in frequency modulation mode, in which the frequency shift is measured

It works with cantilevers of high stiffness with oscillation amplitudes on the order of 1 Å

AFM imaging of pentacene on Cu(111)

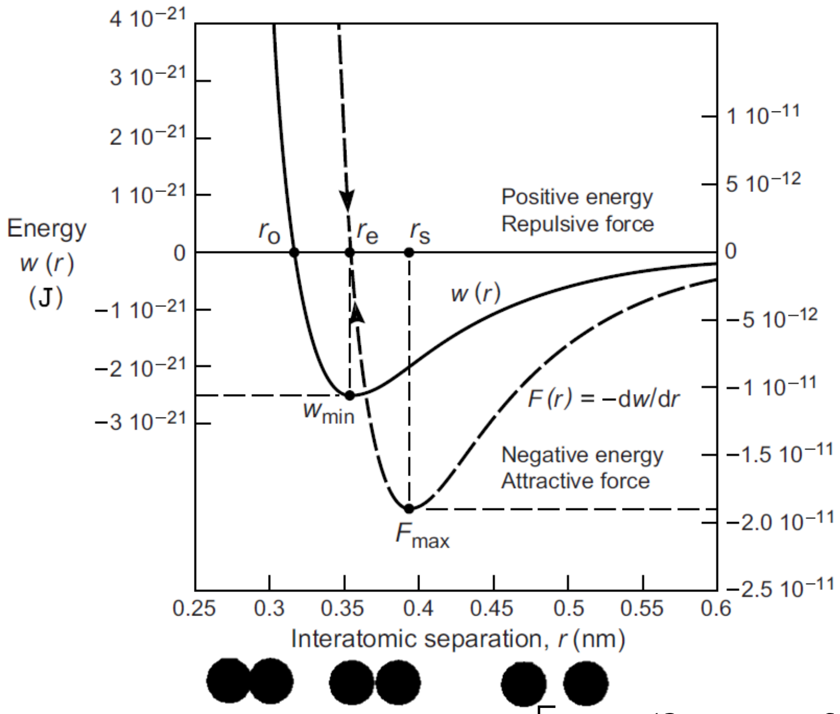


Pauli repulsion is the source of the atomic resolution

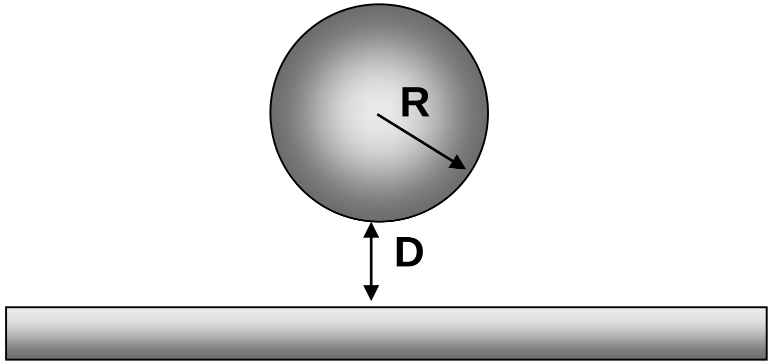
Why is it called Atomic Force Microscope?
Origin of contrast

Lennard-Jones View

- Repulsive part - contact
- Attractive part - non-contact



$$w(r)=\frac{A}{r^{12}}-\frac{B}{r^{6}}=4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12}-\left(\frac{\sigma}{r}\right)^{6}\right]$$



The van der Waals interaction between macroscopic bodies or surfaces is usually described in terms of the Hamaker constant H.

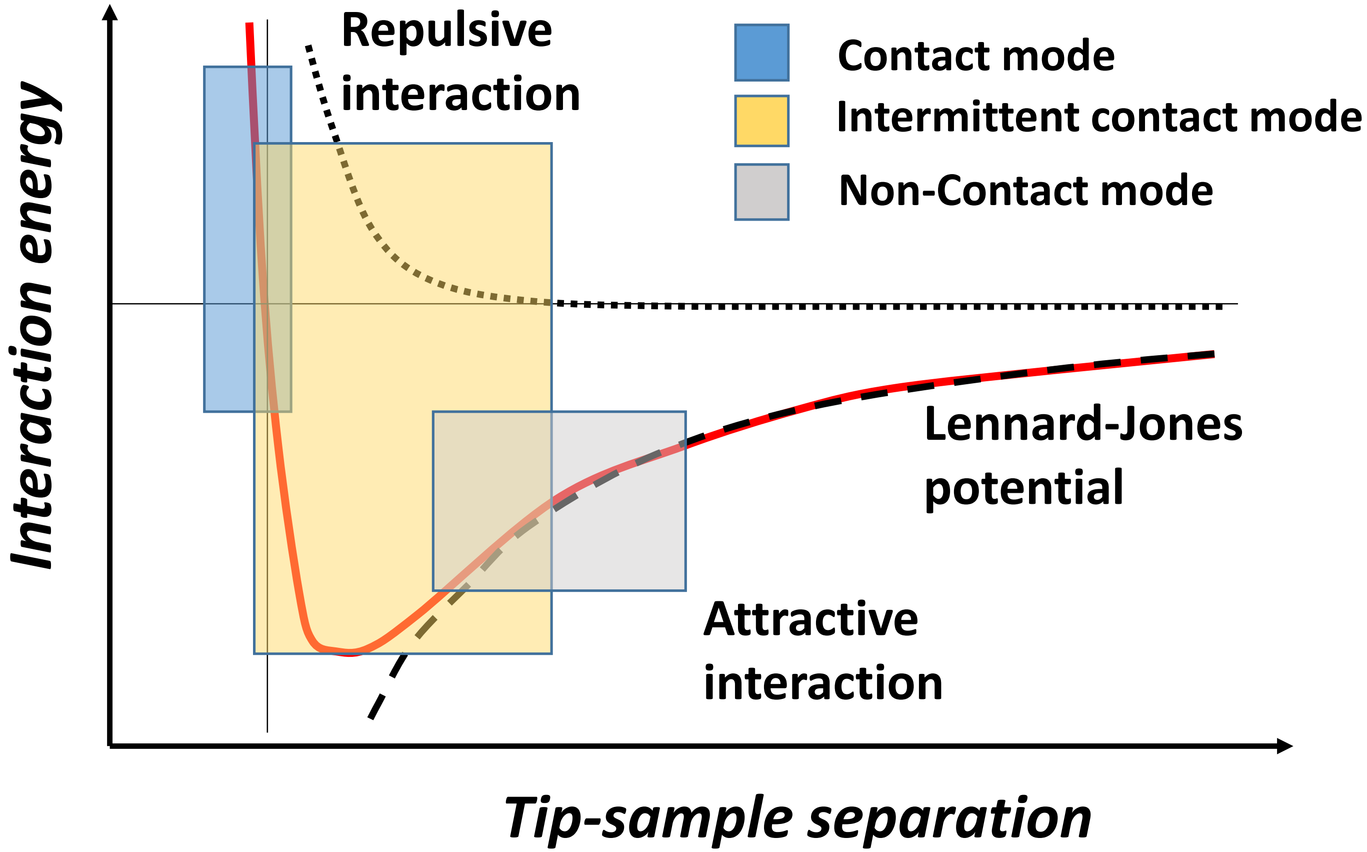
For a sphere on a flat surface, for R>> D:

$$W(D)=-\frac{HR}{6D}$$

The Lennard-Jones potential is additive. In the case of a sphere and a flat plane the result is:

$$W(D)=\left(\frac{HR_{tip}}{6\sigma}\right)\left[\frac{1}{210}\left(\frac{\sigma}{D}\right)^{7}-\frac{\sigma}{D}\right]$$

The Lennard-Jones potential



1) *Non-contact AFM vdW attraction 10-100Å tip-surface separation*
2) *Contact AFM e^--e^- repulsion <5Å tip-surface separation*
3) *Intermittent contact AFM 5-30Å tip-surface separation*

Latest advances in Atomic Force Microscopy and Spectroscopy

The new generation of scanning probe microscopes enables a wide range of new imaging modalities, increased imaging speed and coupling with optical techniques.

I will concentrate on:

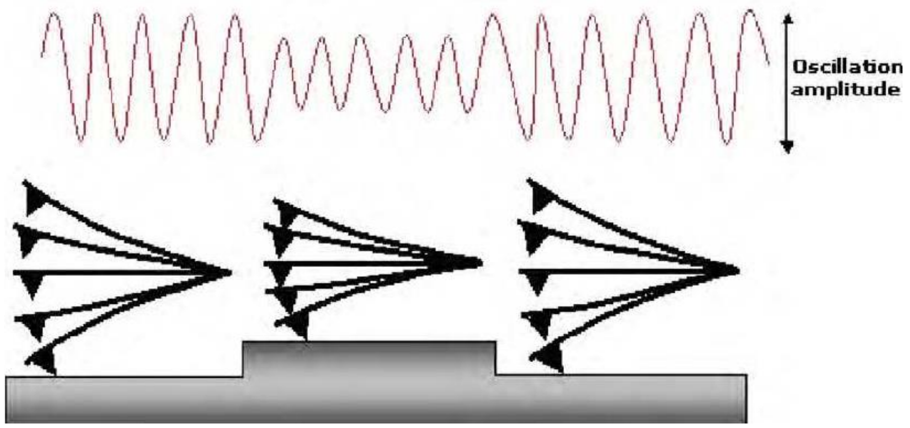
- **Better control of tip/sample interaction force**
- **High-speed imaging possibilities**
- **Combined AFM-optical microscope**

- **Better control of tip/sample interaction force**

Dynamic methods - Intermittent contact imaging method (pulsed-force mode operation)

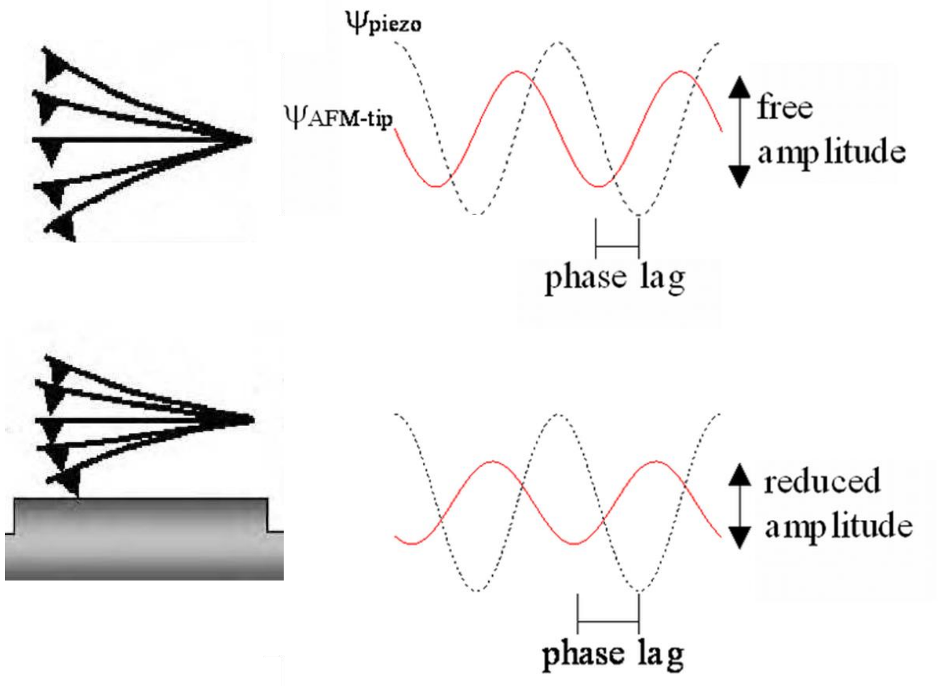
In dynamic methods the cantilever is driven to oscillate at a specific frequency or frequencies. Probably the most familiar dynamic mode is tapping mode for imaging topography.

Typically piezoelectric excitation

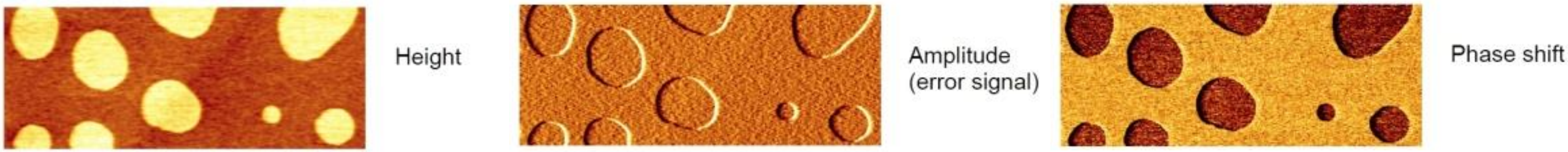


amplitude-modulated method

Phase signal: material contrast



The phase lag of the tip relative to the excitation signal is monitored and recorded while the feedback keeps the amplitude at a fixed value



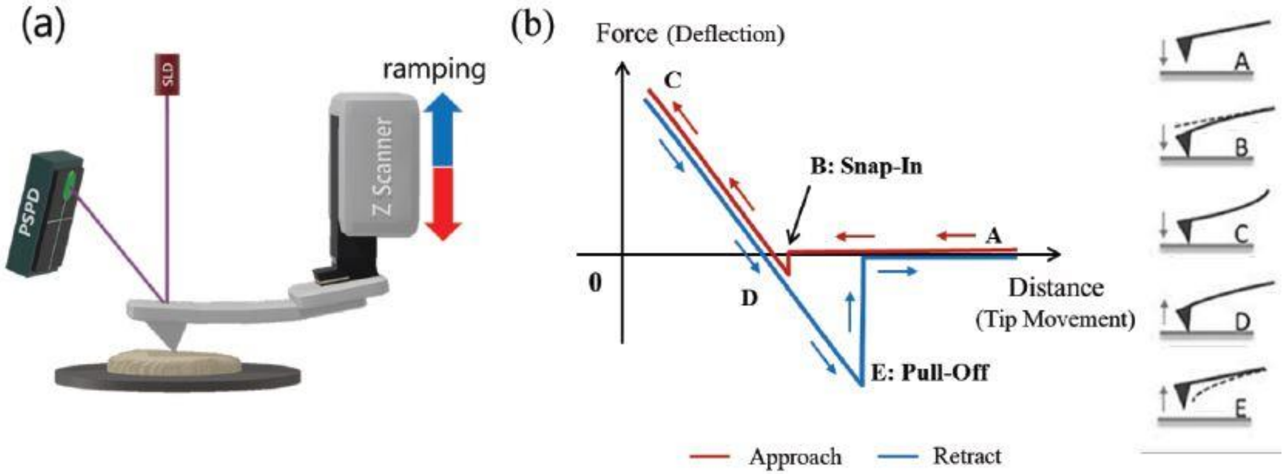
The height image shows the arrangement of the domains in a SLB, with a consistent height difference between the lipid phases. The amplitude signal shows contrast only at the sides of the domains. The phase image shows a stable phase contrast between the two lipid phases. From: Wiley Analytical Science Magazine 12 August 2013

Intermittent contact mode enabled AFM imaging for a much wider range of samples, its one drawback compared to contact mode is that it cannot directly measure forces.

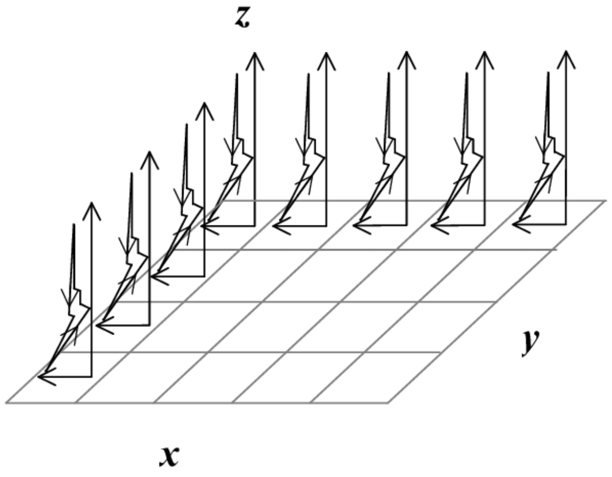
The need for quantitative data from AFM and maps of mechanical properties

Atomic force microscopy (AFM) is a powerful investigation and diagnostic tool, especially in force mode. Nevertheless, force spectroscopy suffers from several limitations, including the speed of acquisition, a relative lack of resolution, and the fact that it doesn't directly provide quantitative information.

Force-distance curve

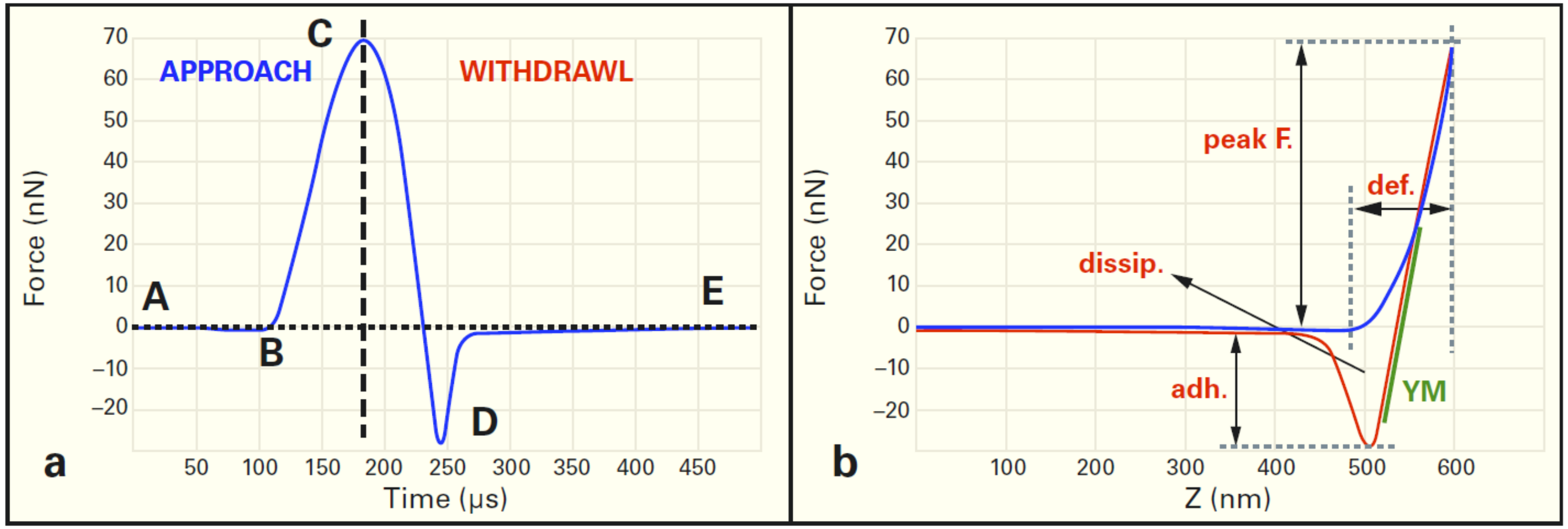


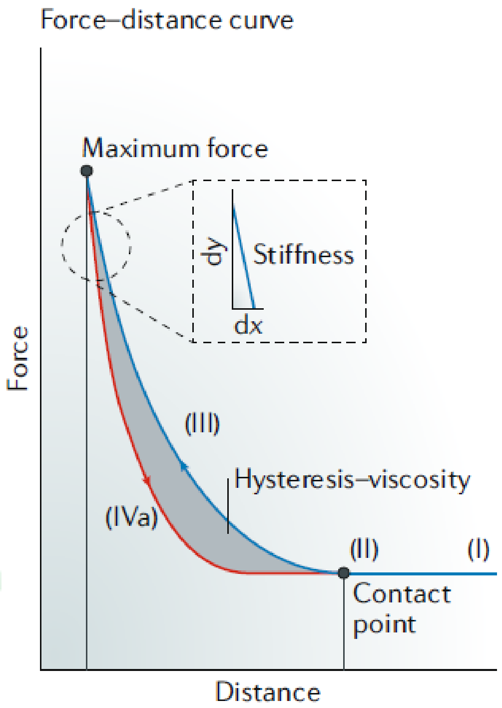
Traditional force volume



Off-resonance Tapping

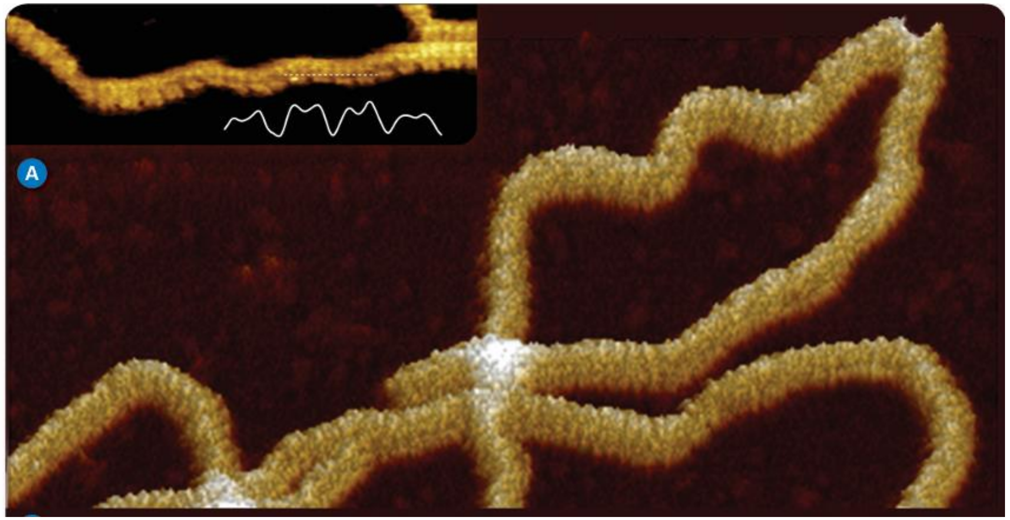
Oscillation far below the resonance frequency

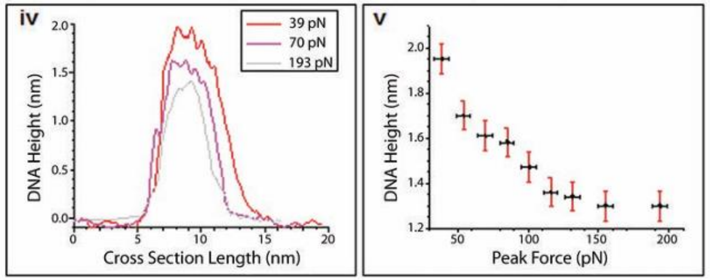




The probe periodically taps the sample and the pN-level interaction force is measured directly by the deflection of the cantilever. A feedback loop keeps the peak force down to 10 pN at actuation rates up to 8 kHz, in air and fluid.

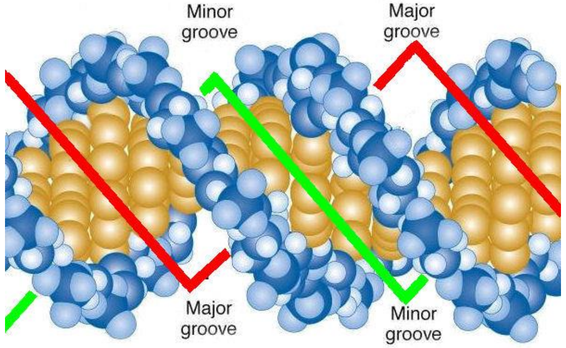
PeakForce Tapping also uses sinusoidal ramping rather than linear ramping so that as the tip moves closer to the surface, its velocity approaches zero.

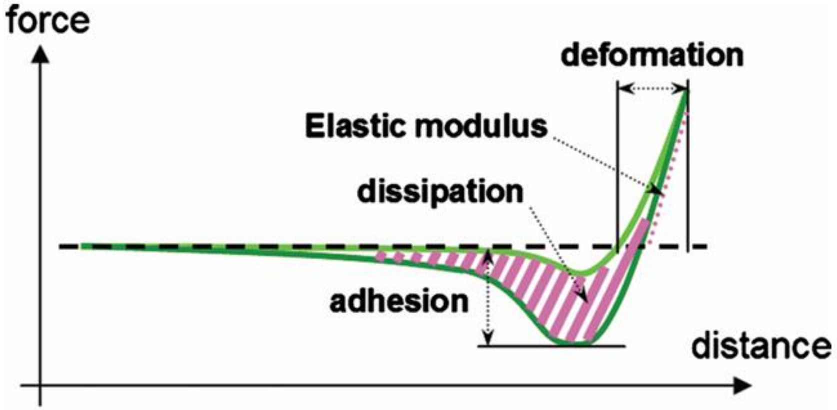


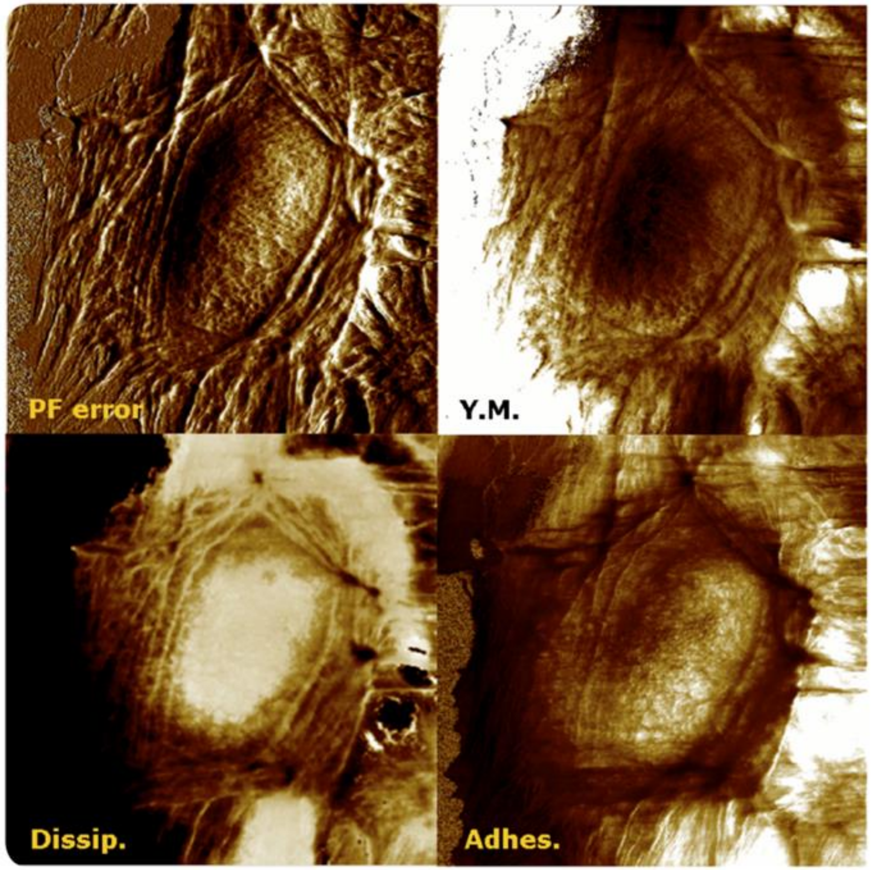


Height profiles measured across the DNA, as indicated by the dashed line in the inset of B, for different peak forces.

Imaging of DNA Submolecular Structure

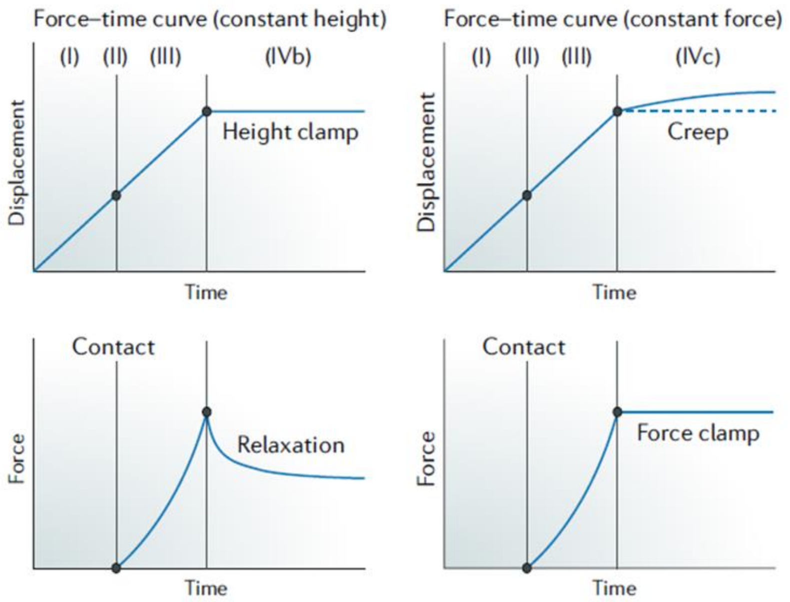


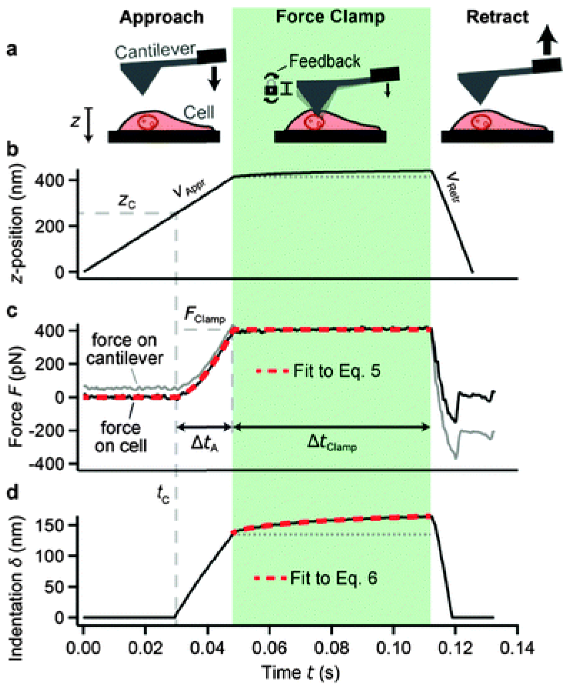


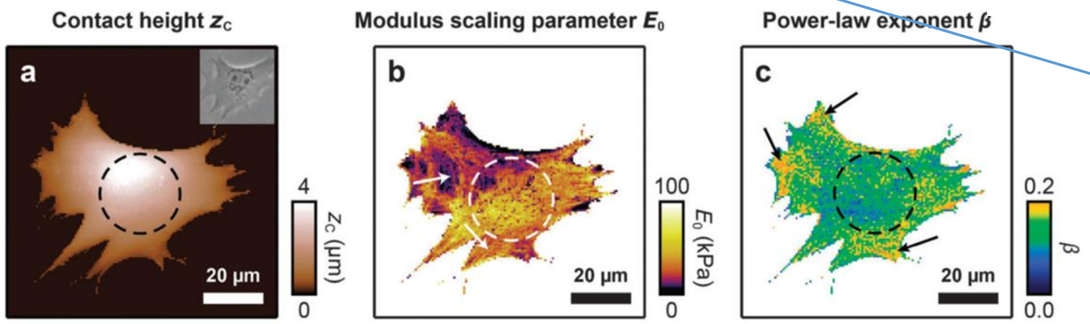


Rheological analyses of materials with nanometric lateral resolution

An analysis similar to DMA can be performed. The new generation microscopes allow you to use closed loop control of the vertical movements of the tip, to modulate the position using scripts that can be developed by the user. Quantitative information is extracted on the modulus of elasticity and the viscous component of the analyzed materials (frequency analysis or stress relaxation analysis...)
Force spectroscopy experiments (force ramps, force-clamp) can also be carried out both on single molecules and on single cells.







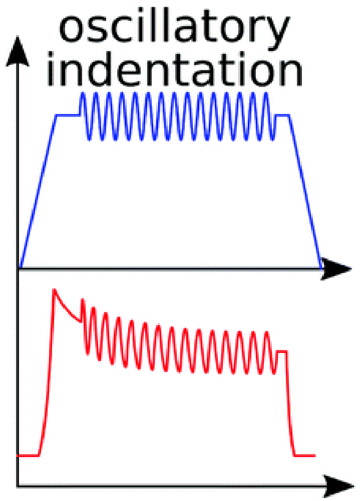

Soft Matter

PAPER

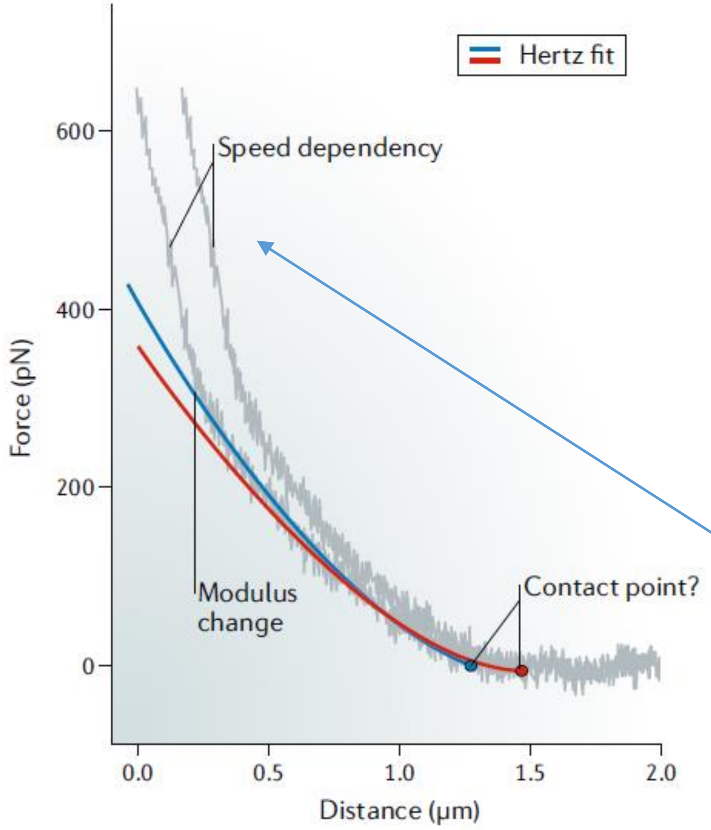
Cite this: Soft Matter, 2015, 11, 4584

Imaging viscoelastic properties of live cells by AFM: power-law rheology on the nanoscale†

Fabian M. Hecht,‡[a] Johannes Rheinlaender,‡[a] Nicolas Schierbaum,[a] Wolfgang H. Goldmann,[b] Ben Fabry[b] and Tilman E. Schäffer*[a]



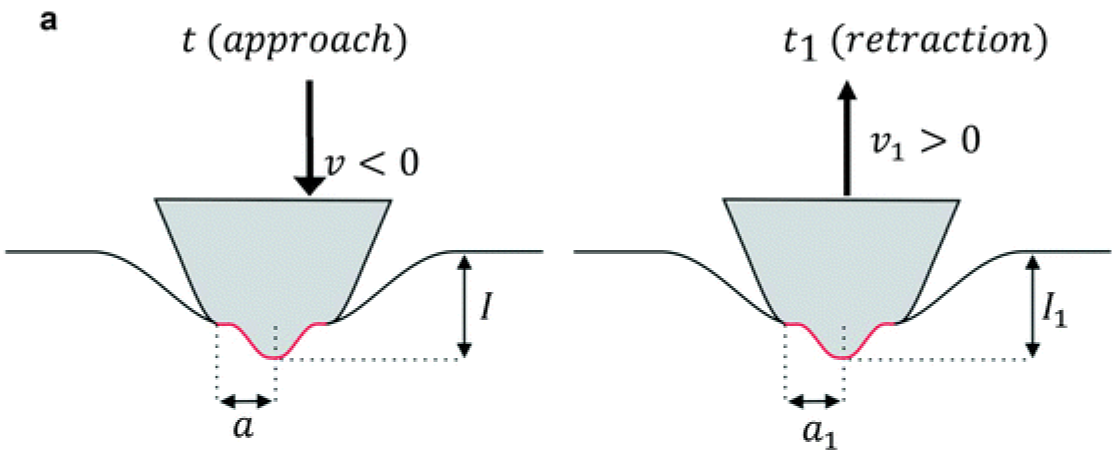
Rheological maps at high spatial and time resolution can be obtained on live cells



Typical challenges encountered when analysing FD (or force–time (FT)) curves are defining the contact point, fitting the slope of the approach curve (different fits lead to different elastic moduli). Blue and red curves are fits based on the Hertz model, which assume different contact points for the left- most grey curve

A speed- dependent behaviour indicates that the sample is viscoelastic. In this example, the grey curves are data acquired at different speeds.

Ting's method assumes that for a given time t of the retraction curve there is an equivalent time $t_1(t)$ in the approach curve that has the same contact area. The key feature of the method is that the force at time t can be obtained from the equation of the force history by replacing the upper limit of the integral from t to $t_1(t)$. This requires to solve the integral equation which links the current time t_1 with a previous time t.



b

$t \dashrightarrow t_1(t)$

$$\int_{t_1(t)}^{t} \varphi_E(t-t')\frac{d}{dt'}(I(t'))dt' = 0.$$

$a = a_1$

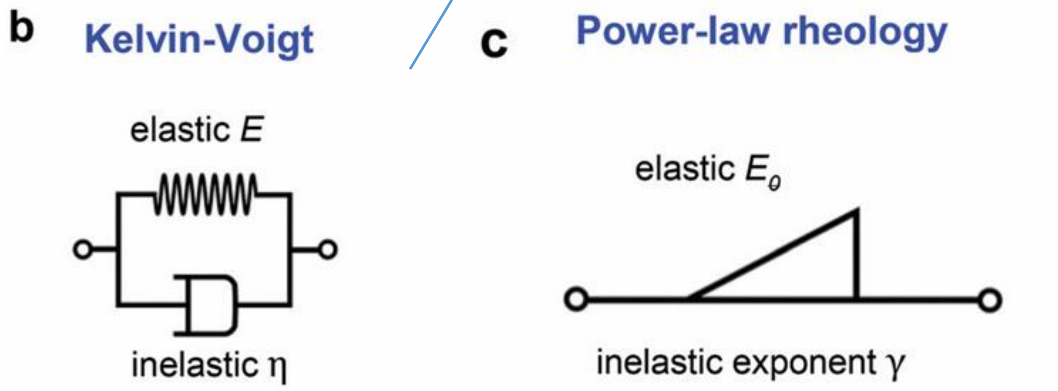
$F = F_1$

$v \neq v_1$

$I \neq I_1$

Garcia, Nanoscale, 2020, 12, 9133-9143

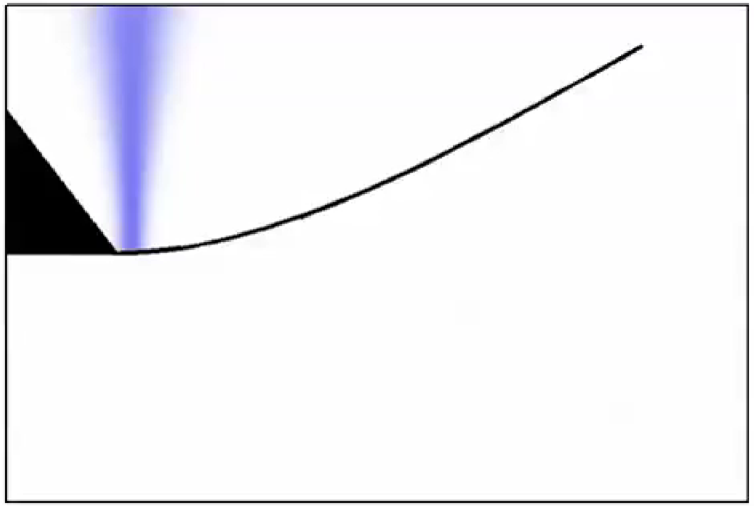
$$F(t) = \sum_{j=0}^{N} \alpha_j \int_0^t \varphi_E(t-t')\frac{d}{dt'}\left(I(t')^{\beta_j}\right)dt'$$



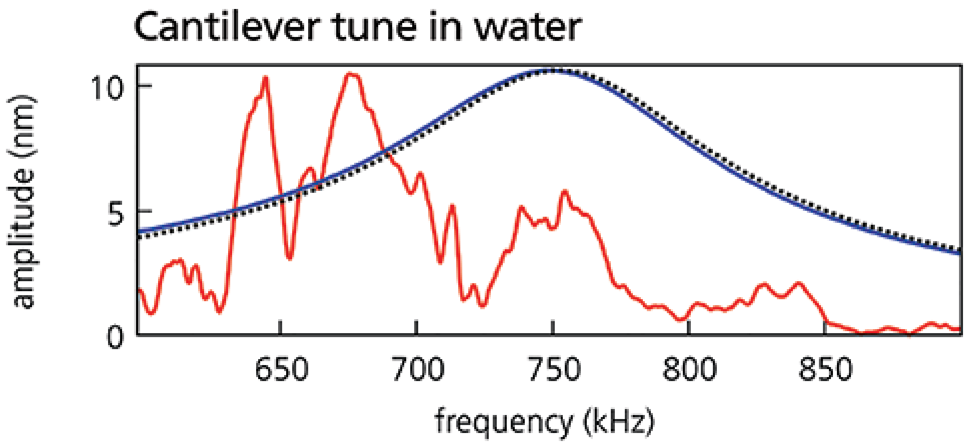
https://doi.org/10.5281/zenodo.10529399.
An alternative python code is also available at https://github.com/yu-efremov/ViscoIndent.

New methods for cantilever excitation

Phototherma l excitation technique replaces the tapping piezo with a laser that is focused on the base of the cantilever. Its power is modulated at the desired drive frequency, exciting the cantilever oscillation photothermally.



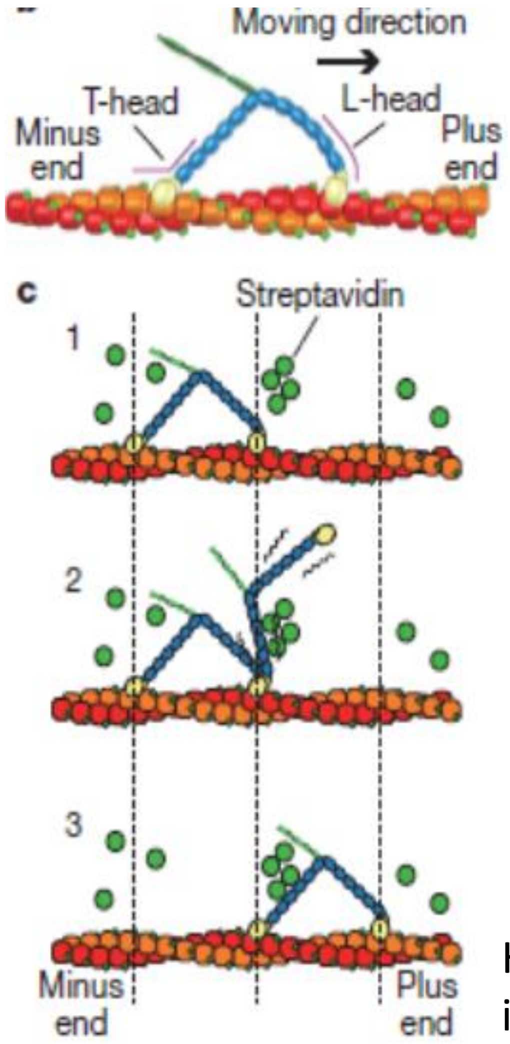
no other resonances are excited

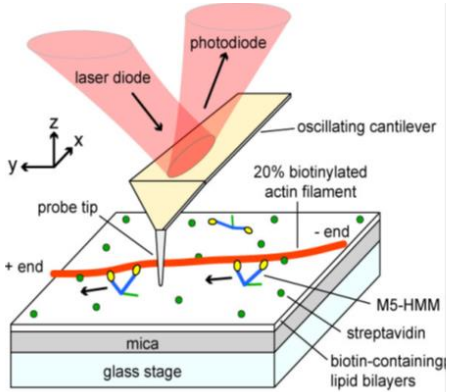


From Oxford Instuments, Asylum Research

- **High-speed imaging possibilities**

Video imaging of walking myosin V by high-speed atomic force microscopy





Video imaging of walking myosin V by high-speed atomic force microscopy

Nature 2010 Nov 4;468(7320):72-6. doi: 10.1038/nature09450.

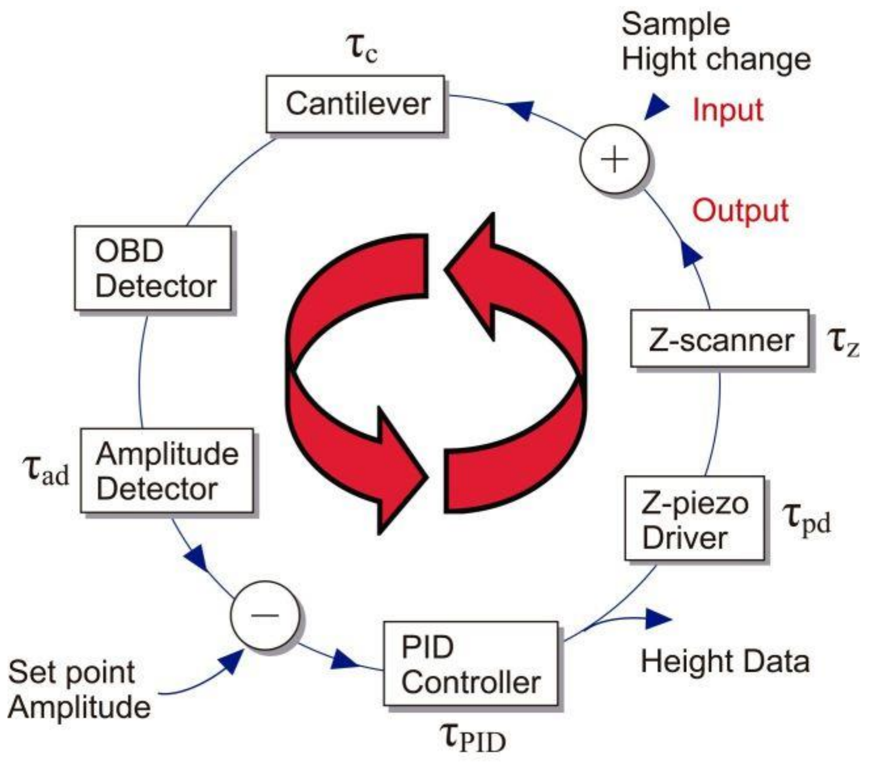
High-speed AFM made it possible in the tapping mode of operation to capture a 100x100 pixel image within 80ms

HS-AFM instrument at a speed about 1000 times faster than conventional AFM systems

Hardware developments connected to HS-AFM

The imaging speed at optimal quality strongly depends on the sample, probe, imaging mode, scan size, and interaction force

Typically speed comes at the expense of increased interaction-force

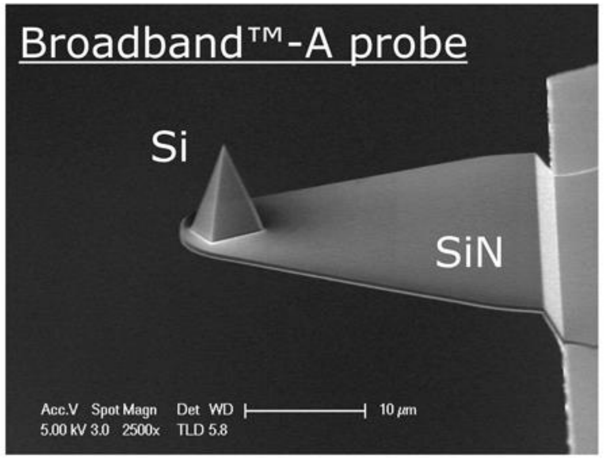


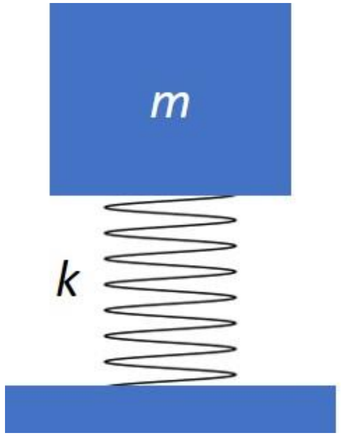
The AFM tracks the sample surface using a feedback loop that observes and maintains the interaction of the AFM probe with the sample surface during scanning by adjusting the tip-sample separation. The components involved are the AFM probe, photodiode and electronics, controller, amplifier, and Z actuator. Each component in the feedback loop introduces its own dynamics (e.g., a delay)

Therefore, it takes time until the completion of the feedback Z-scanning at each height change event. In HS-AFM, all devices are optimized for their fast response

The cantilever

The cantilever response time τ_c is given by $\tau_c = Q_c/(\pi f_c)$





$$f = \frac{1}{2\pi}\sqrt{\frac{k}{m}}$$

The inverse of the settling time for cantilevers depends on the cantilever’s first resonance frequency (*f*), divided by its quality factor (*Q*). Therefore, to make a cantilever for faster imaging, *f* must be increased or *Q* reduced

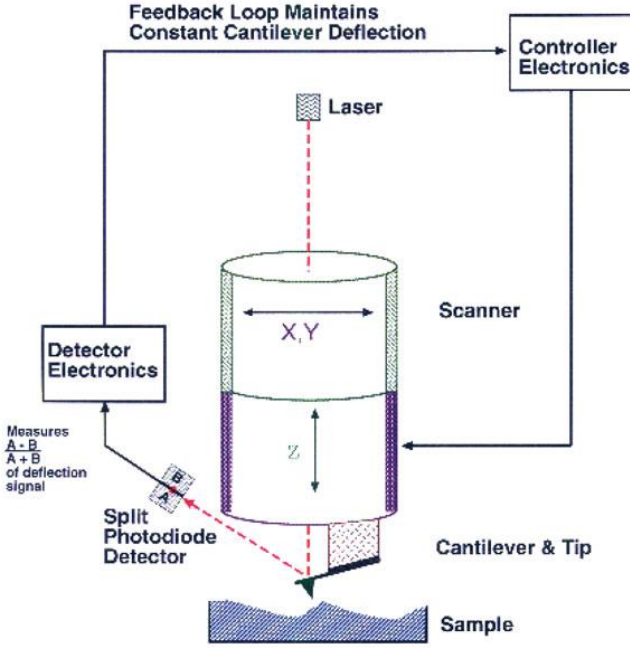
The spring constant has to be maintained at a small value (~0.1 N/m for biological samples). This requirement is fulfilled only by the miniaturization of the cantilever. Small cantilevers (10 µm long, 2 µm wide and ~100 nm thick) are now commercially available

Reducing Q can be obtained by increasing the dampening of the cantilever. One way to do this is to give the cantilever a wide, closed shape and to reduce tip length

In the pursuit of high-speed AFMs, smaller cantilevers were recognized as the key to higher imaging speeds from the beginning. However, designing a cantilever that balances all these factors well, while still being able to be manufactured in large quantities and at reasonable cost, within the tighter tolerance requirements imposed by smaller cantilevers, has been one of the biggest obstacles to commercial high-speed AFM technology.

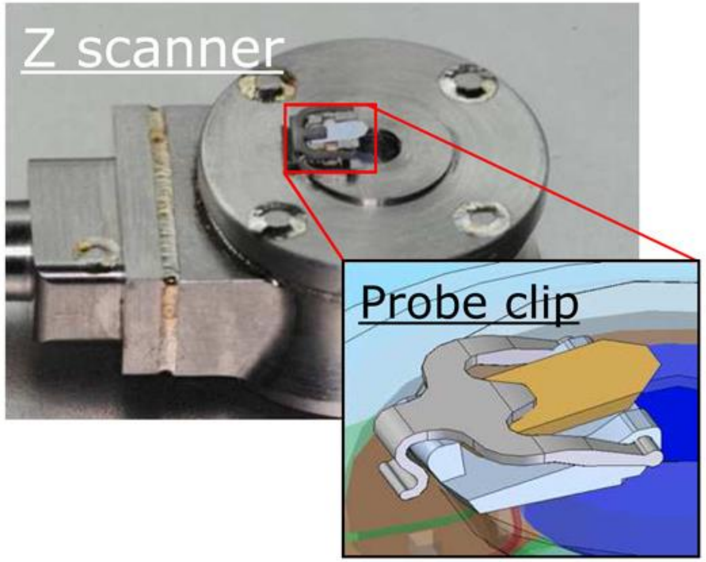
Z Scanner

The response time of the Z-scanner τ_z is approximately determined by the piezo actuator, and expressed as $\tau_z = Q_z/(\pi f_p)$. For a travel range of 0.5–1 µm, usually it is possible to achieve $f_p \approx 200$ kHz when one end of the piezo actuator is held. The quality factor of piezo actuators is large, and therefore, Q_z is reduced to ~1 by a Q-control method



In traditional AFM tube scanners, the Z scanner was one with the XY scanner. Therefore, it did not have separate dynamics that could be maximized independently.

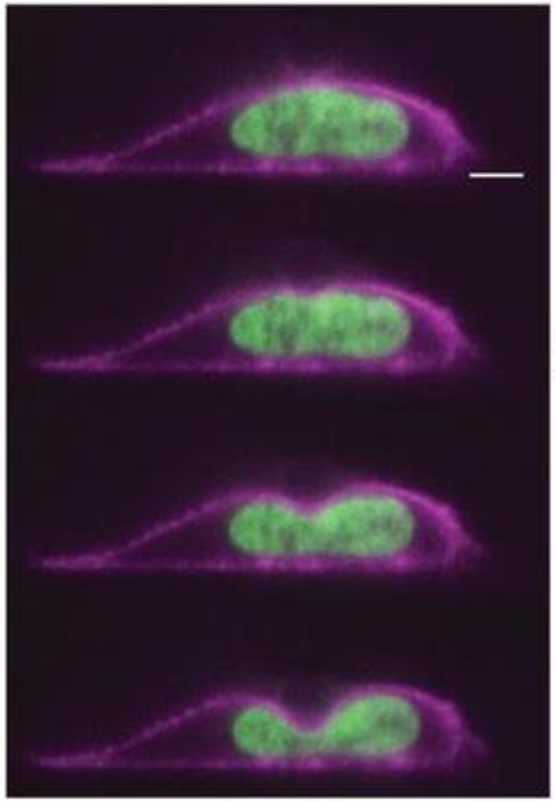
The relatively slower X and Y axis can move a fast, low-mass, low-inertia Z scanner around. The mass difference of the XY scanner and the mass moved by the Z scanner can be sufficient to allow the Z scanner to have its own isolated, fast dynamics

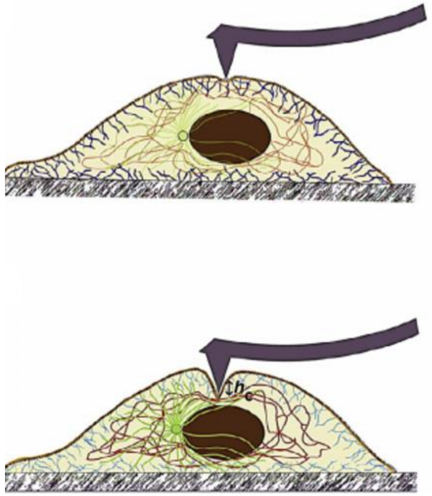


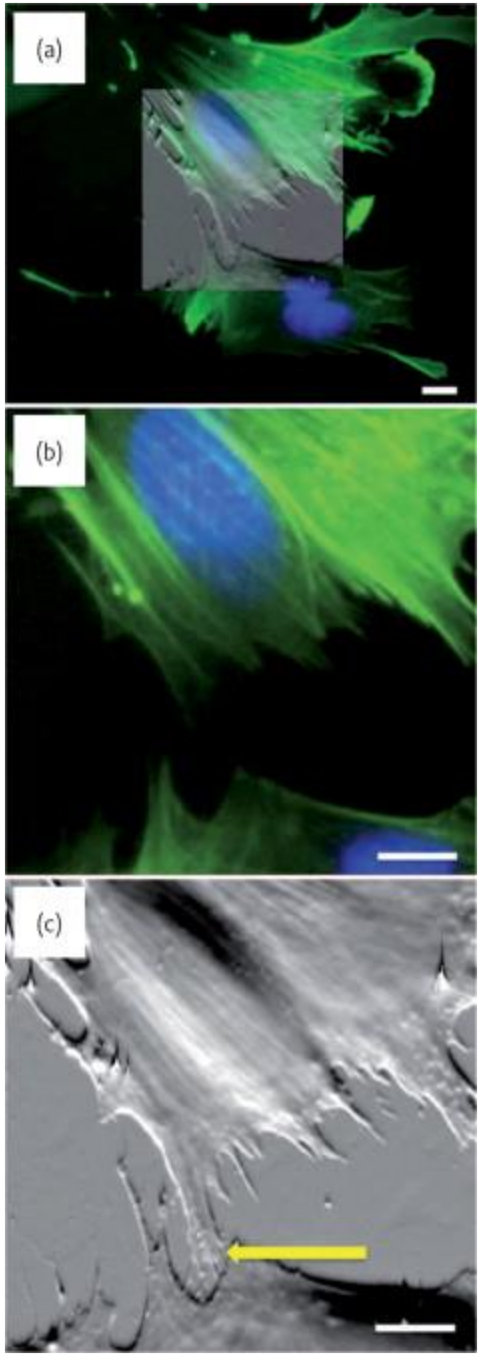
- **Combined AFM-optical microscope**

A combined AFM-optical microscope

(c)







A combined AFM-optical microscope is an excellent instrument for characterizing various samples. Optical microscopy's chemical specificity and ability to image live processes within the depth of a sample is well complemented by the higher resolution capability of the AFM

Thank you for your attention