LECTURE 5: AFM IMAGING

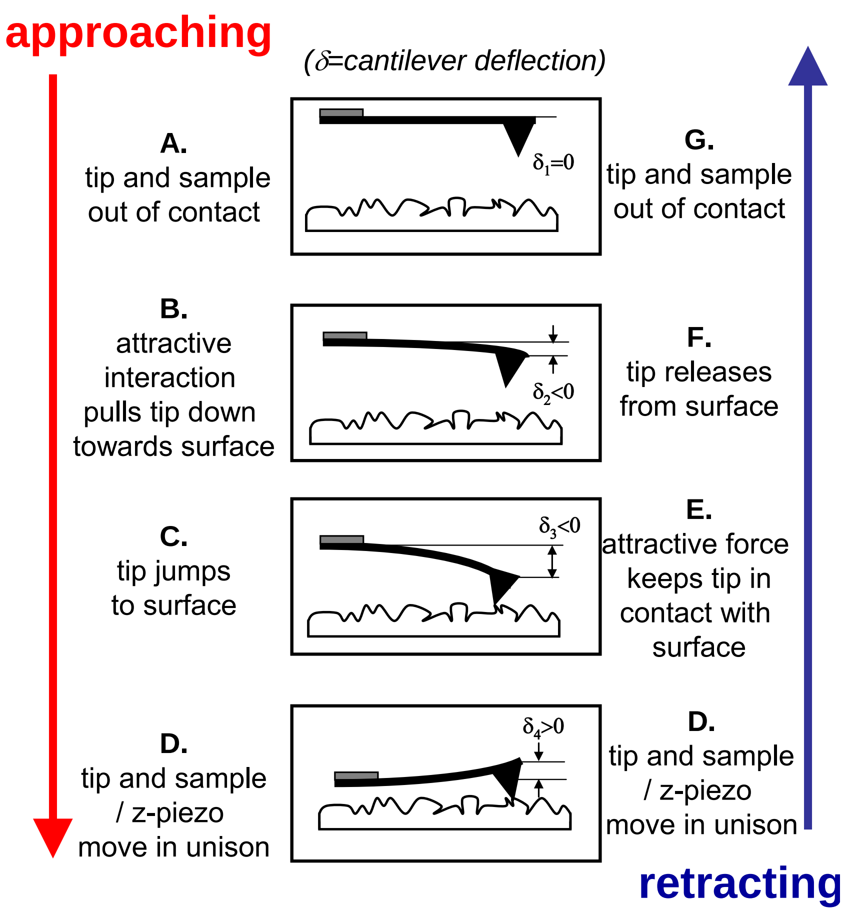
Outline :

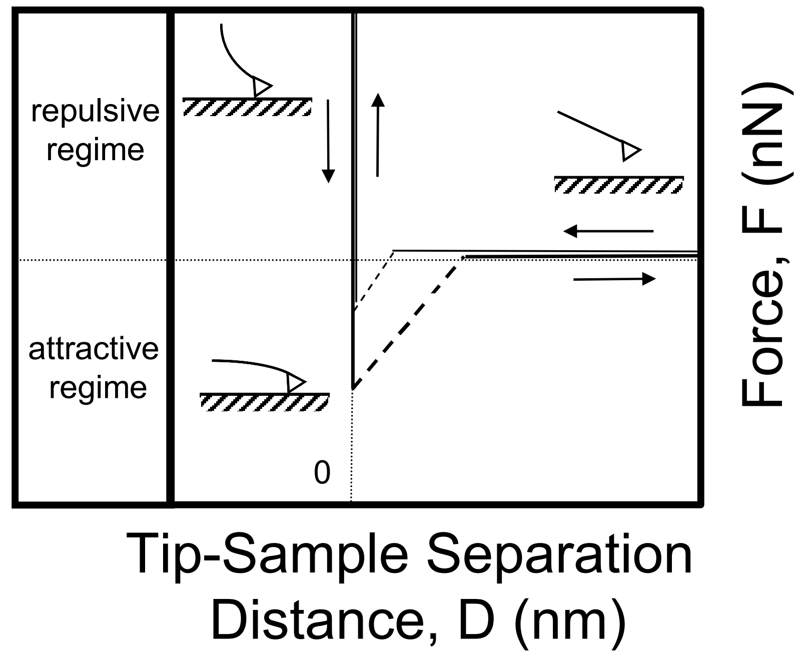
Objectives: To review the basic principles, capabilities, and current state of the art uses of the atomic force microscopy

Readings: Course Reader Document 12-15.

Multimedia : Watch *Introduction to AFM by Asylum Research, Inc. (Quicktime Movie)* for Lectures 4-5.

HIGH RESOLUTION FORCE SPECTROSCOPY (HRFS) EXPERIMENT : FORCE-DISTANCE CURVES





- **Conversion of raw data**; sensor output, s (Volts) vs. z-piezo displacement/deflection, δ (nm) to Force, F, versus tip-sample separation distance, D :

$\delta = s/m$

m= slope in constant compliance regime $=\Delta s/\Delta\delta$ (V/nm)

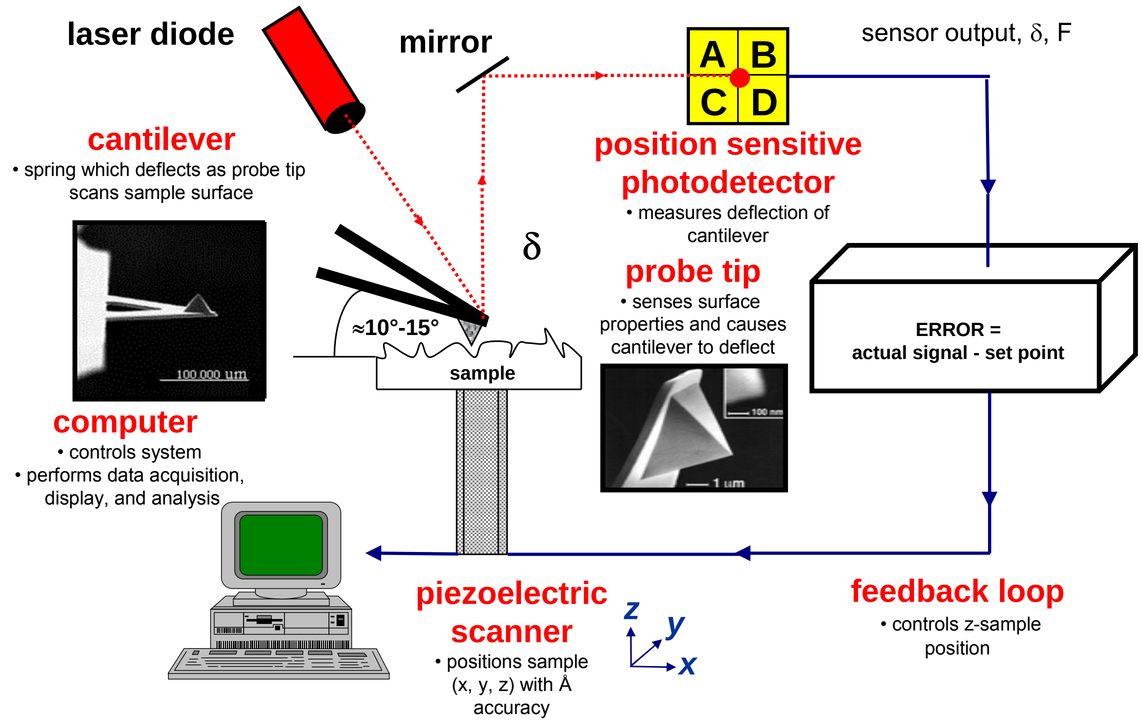
$F = k\delta$

$D = z \pm \delta$

-zeroing the baseline

-Normal vs. Lateral Force Microscopy

ATOMIC FORCE MICROSCOPY : GENERAL COMPONENTS AND FUNCTIONS



-Piezo rasters or scans in the x-y direction across the sample surface

↓

-Cantilever deflects (δ) in response to an a topographical feature (hill or valley)

↓ **Feedback loop**

-System continuously changes in response to an experimental output (δ= cantilever deflection which is the feedback parameter)

-Computer adjusts the piezo z-displacement to keep δ constant = "setpoint"

↓

Error signal (actual signal-set point) used to produce a topographical (height) map in the z-direction of the surface

Advantages : 1) Unlike electron microscopes, samples do not need to be coated or stained, minimal damage, 2) Unlike electron microscopes, samples can be imaged in fluid environments (near-physiological conditions), 3) Unlike STM samples do not need to be conductive, 4) Sub-nm resolutions have been achieved on biological samples (detailed information on the molecular conformation, spatial arrangement, structural dimensions, rate dependent processes, etc.)

ATOMIC FORCE MICROSCOPY : DEFLECTION VS HEIGHT IMAGES

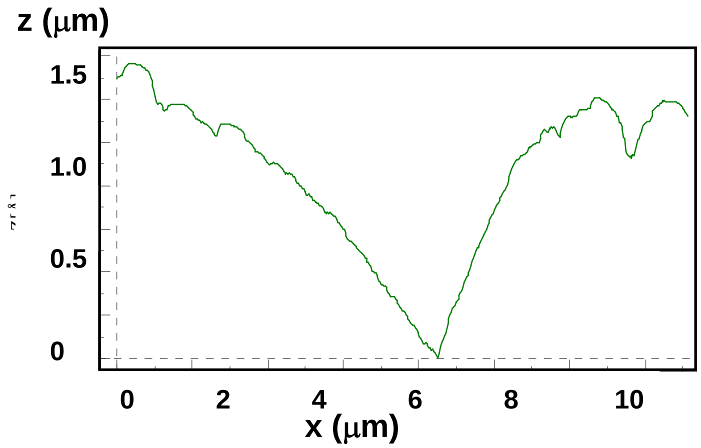
Deflection Image:
-Raw data output of cantilever deflection from photodiode, very clear, the less feedback the clearer, One can identify and measure high resolution (x/y) spatial dimensions of structural features

Height Image:
-Processed data from z-piezo, less clear compared to deflection image, maximize your feedback system, can quantify the height of structural features, in 2D image corresponds to brightness

*(e.g. images are a large residual nanoindent of bone using an instrumented indenter and Berkovich diamond probe showing plastic deformation of mineralite nanogranular structure, K. Tai and C. Ortiz Nano Letters, **2006**)*

ATOMIC FORCE MICROSCOPY : 3D PLOTS AND 2D SECTION PROFILES

Two images removed due to copyright restrictions. See Tai and Ortiz, Nano Letters 2006



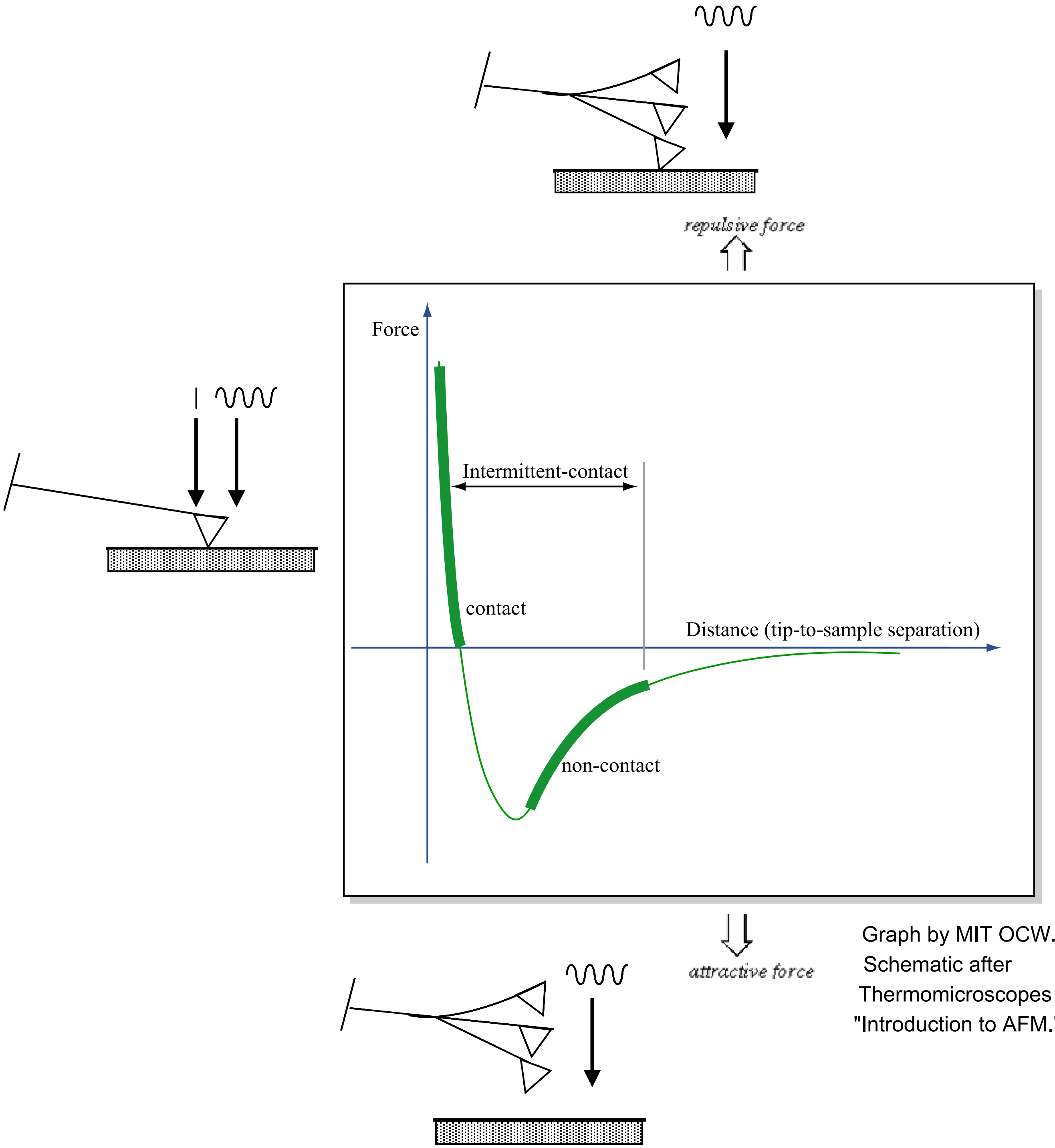
3D Height image **2D Height image** **2D Section Profile**

-Select linear region of plot and plot 2D section profile (height along line) z vs. x to get quantitative mathematical functional form of topography. For example, we can see the profile of the deformation of indent plus form of plastically deformed "pileup" regions"→ one can use these profiles in conjunction with modeling to extract out material properties such as modulus, yield stress, anisotropy, and strain hardening behavior.

-In the next assignment, you will have to use a section profile on nanoparticles to estimate the probe tip radius.

*(e.g. images are a large residual nanoindent of bone using an instrumented indenter and Berkovich diamond probe showing plastic deformation of mineralite nanogranular structure, K. Tai and C. Ortiz Nano Letters, **2006**)*

ATOMIC FORCE MICROSCOPY IMAGING : NORMAL MODES OF OPERATION



Graph by MIT OCW. Schematic after Thermomicroscopes "Introduction to AFM."

Contact (DC and AC or Force Modulation) :

- tip remains in contact with sample
- feedback signal, δ_c
- potentially destructive due to high lateral (x/y) forces
- high resolution

Intermittent Contact (AC, Tapping) Mode :

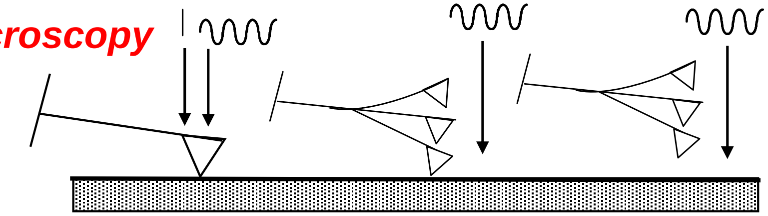
- tip is oscillated near its resonant frequency and "taps" on and off the surface
- feedback signal, oscillation amplitude or phase
- less destructive due to reduction of lateral forces
- loss of spatial resolution

Noncontact (AC) Mode :

- tip is oscillated near its resonant frequency without touching the surface
- feedback signal, oscillation amplitude
- nondestructive
- loss of spatial resolution
- difficult in practice

ATOMIC FORCE MICROSCOPY IMAGING : OTHER MODES OF OPERATION

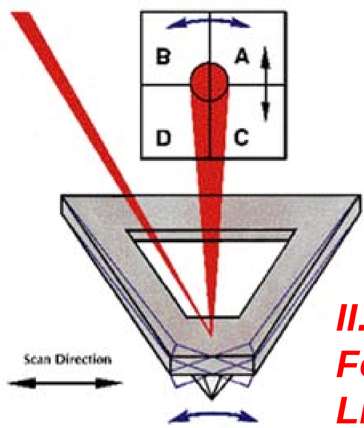
I. Normal Force Microscopy



Contact DC and AC (Force Modulation Microscopy (FMM), Phase Imaging) Hansma, *et al.*, **1991**

Intermittant Contact/ Tapping / Lift (AC) Hansma, *et al.*, **1994**

Noncontact (NC) **1995**

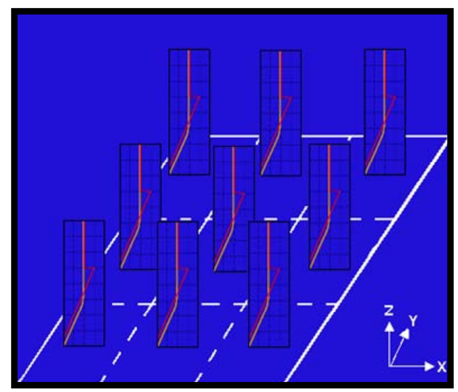


II. Friction or Lateral Force Microscopy (FFM/ LFM) Frisbie, *et al.*, **1994**

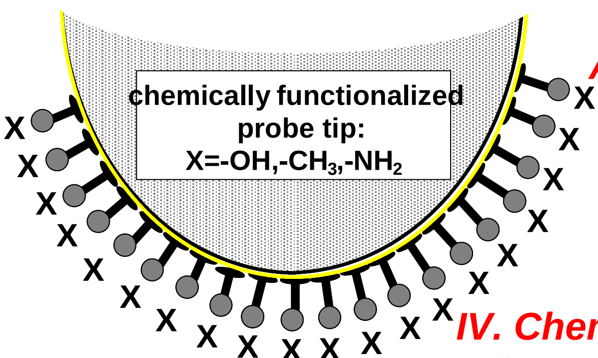
Courtesy of Veeco Instruments. Used with permission.
http://www.di.com/AppNotes/LatChem/LatChemMain.html

III. Force / Volume Adhesion Microscopy

Radmacher, *et al.*, **1994**



Courtesy of Veeco Instruments. Used with permission.
http://www.di.com/AppNotes/ForceVol/FV.array.html



IV. Chemical Force Microscopy (CFM)

Frisbie, *et al.*, **1994**

Surface Maps:

Topography & Roughness, Electrostatic Interactions, Friction
Chemical, Adhesion , Hardness, Elasticity /Viscoelasticity

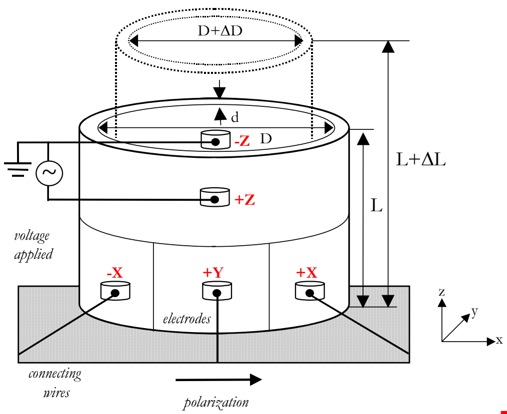
Dynamic Processes :

Erosion, Degradation, Protein-DNA Interactions

ATOMIC FORCE MICROSCOPY IMAGING : FACTORS AFFECTING RESOLUTION

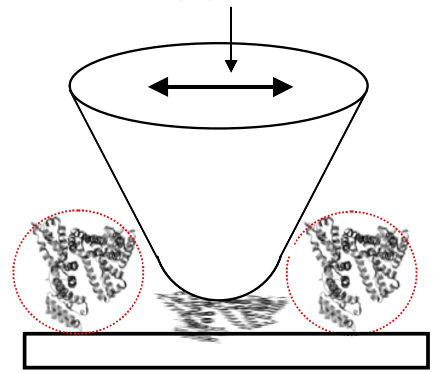
PIEZO AMPLIFIER, SENSOR AND CONTROL ELECTRONICS, MECHANICAL PARAMETERS

Physik Instruments, *Nanopositioning* **1998**



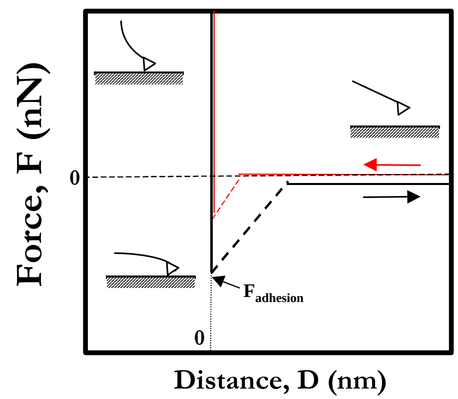
SPECIMEN DEFORMATION & THERMAL FLUCTUATIONS

Hoh, et al. *Biophys. J.* **1998**, *75*, 1076.



ADHESION FORCE

Yang, et al. *Ultramicroscopy* **1993**, *50*, 157



PROBE TIP SHARPNESS

Sheng, et al. *J. Microscopy* **1999**, *196*, 1.

CANTILEVER THERMAL NOISE

Lindsay *Scanning Tunneling Microscopy and Spectroscopy* **1993**, *335.*
Shao, et al. *Ultramicroscopy* **1996**, *66*, 141.

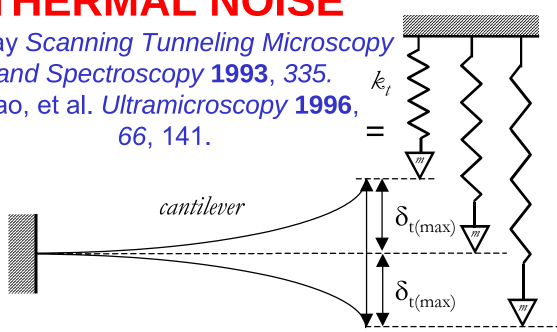
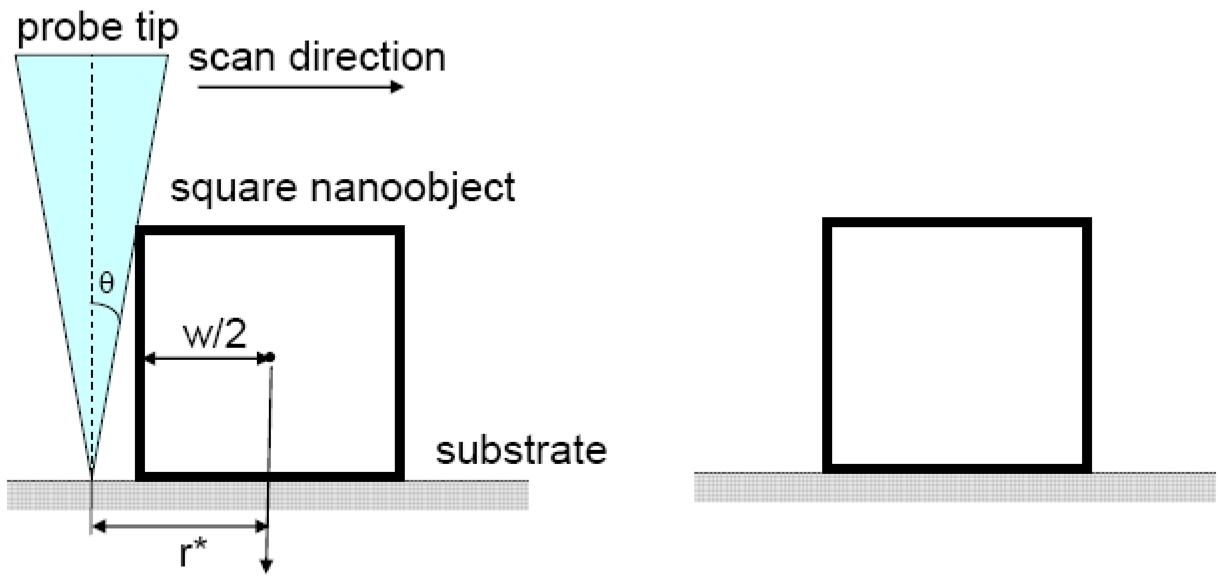


Image removed due to copyright restrictions.

Image removed due to copyright restrictions.
3-D model of sharp probe tip on a protein, from Lieber et al, 2000 (http://cnst.rice.edu)

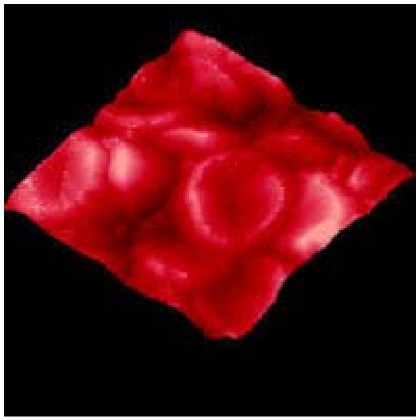
ATOMIC FORCE MICROSCOPY IMAGING : TIP DECONVOLUTION

-Imaging very sharp vertical surfaces is influenced by the sharpness of the tip. Only a tip with sufficient sharpness can properly image a given z-gradient. Some gradients will be steeper or sharper than any tip can be expected to image without artifact. False images are generated that reflect the self-image of the tip surface, rather than the object surface. Mathematical methods of tip deconvolution can be employed for image restoration. The effectiveness of these methods will depend on the specific characteristics of the sample and the probe tip.



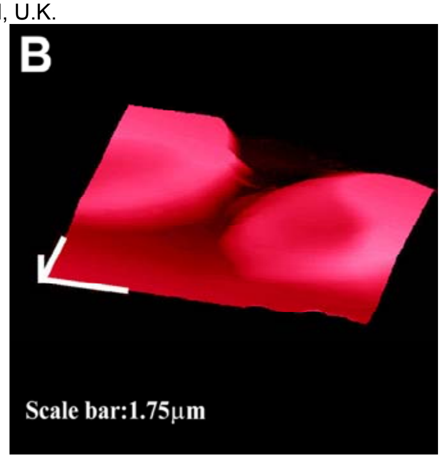
ATOMIC FORCE MICROSCOPY IMAGING OF CELLS

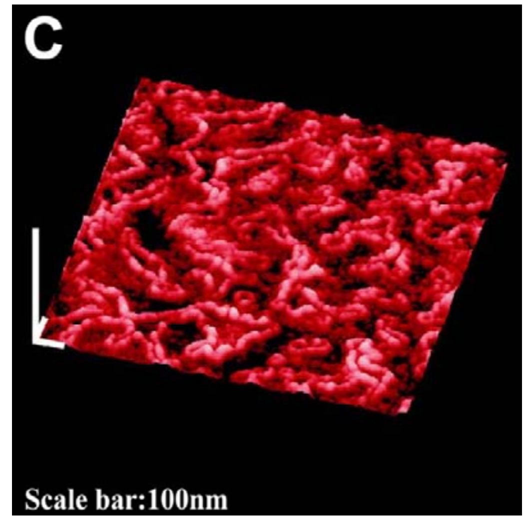
Contact mode image of **human red blood cells** - note cytoskeleton is visible. blood obtained from Johathan Ashmore, Professor of Physiology University College, London. A false color table has been used here, as professorial blood is in fact blue. 15µm scan courtesy M. Miles and J. Ashmore, University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

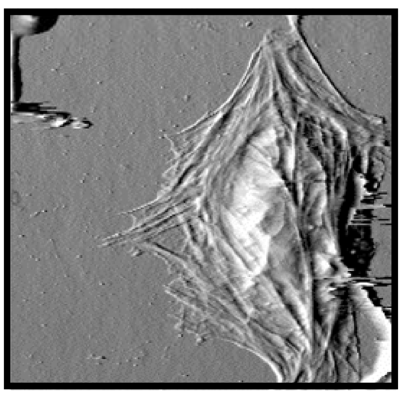


Red Blood Cells

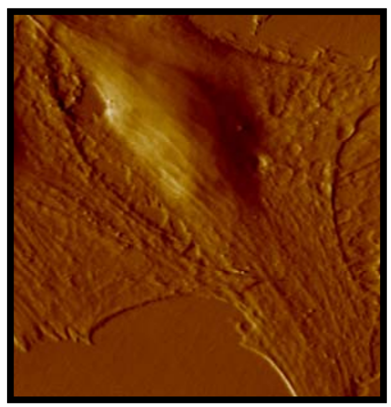
Shao, et al., : http://www.people.virginia.edu/~js6s/zsfig/random.html



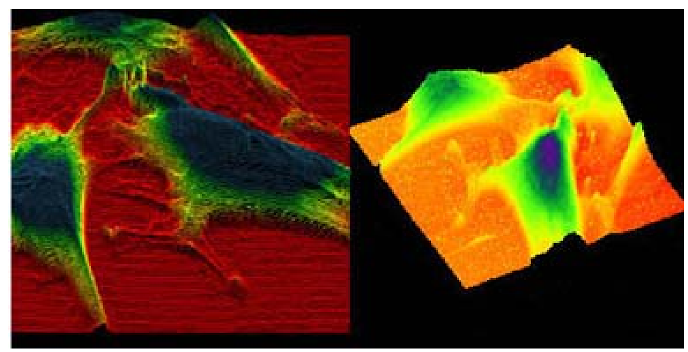




Radmacher, et al., **Cardiac Cells**
http://www.physik3.gwdg.de/~radmacher/



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



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

ATOMIC FORCE MICROSCOPY IMAGING OF DNA

Tapping Mode image of nucleosomal DNA.
Courtesy of Yuri Lyubchenko. Used with permission.

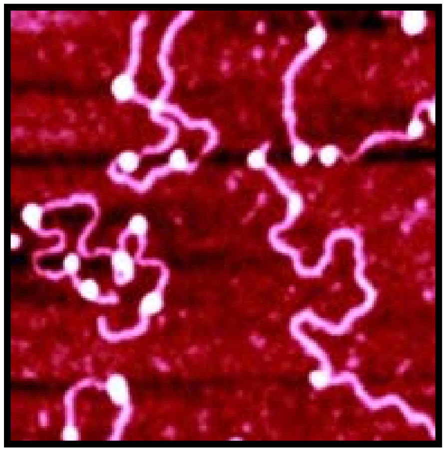
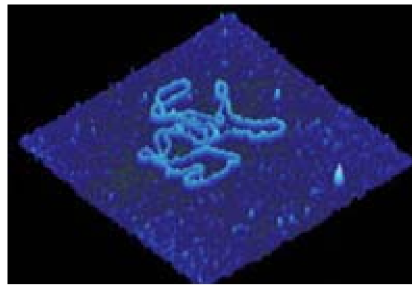
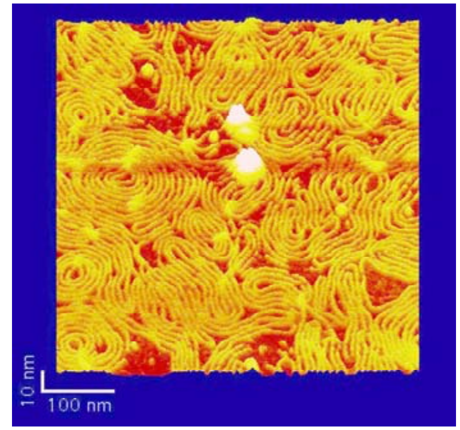


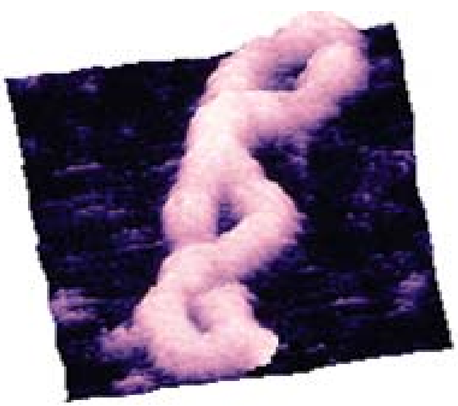
Image of PtyrTlac supercoiled DNA. 750 nm scan courtesy C. Tolksdorf, Digital Instruments/Veeco, Santa Barbara, USA, and R. Schneider and G. Muskhelishvili, Istitut für Genetik und Mikrobiologie, Germany.



Curtesy of Veeco Instruments and G. Muskhelishvili. Used with permission.

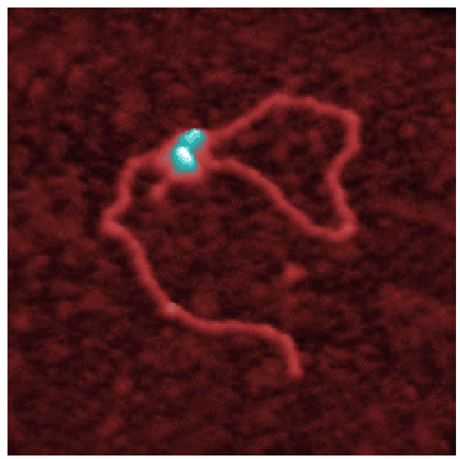


http://people.virginia.edu/~zs9q/zsfig/DNA.html
Courtesy of Zhifeng Shao. Used with permission.



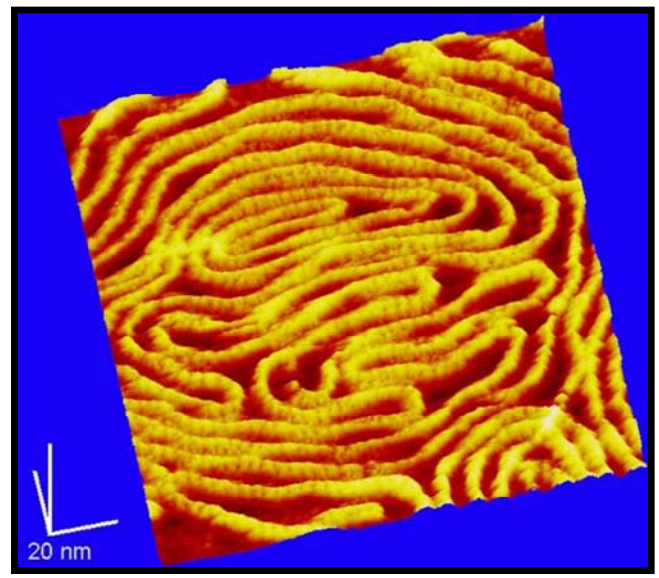
The high resolution of the SPM is able to discern very subtle features such as these two linear dsDNA molecules overlapping each other. 155nm scan.

Courtesy of W. Blaine Stine. Used with permission.



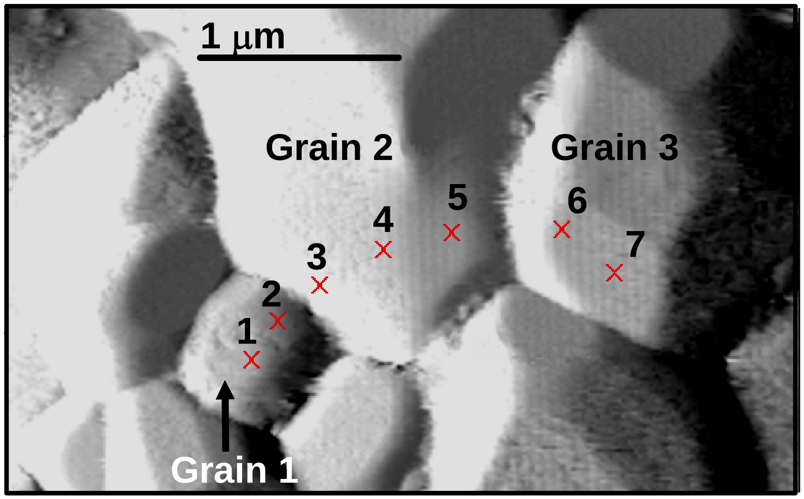
AFM image of short DNA fragment with RNA polymerase molecule bound to transcription recognition site. 238 nm size. Courtesy of Bustamante Lab. Chemistry Department. University of Oregon, Europe OR

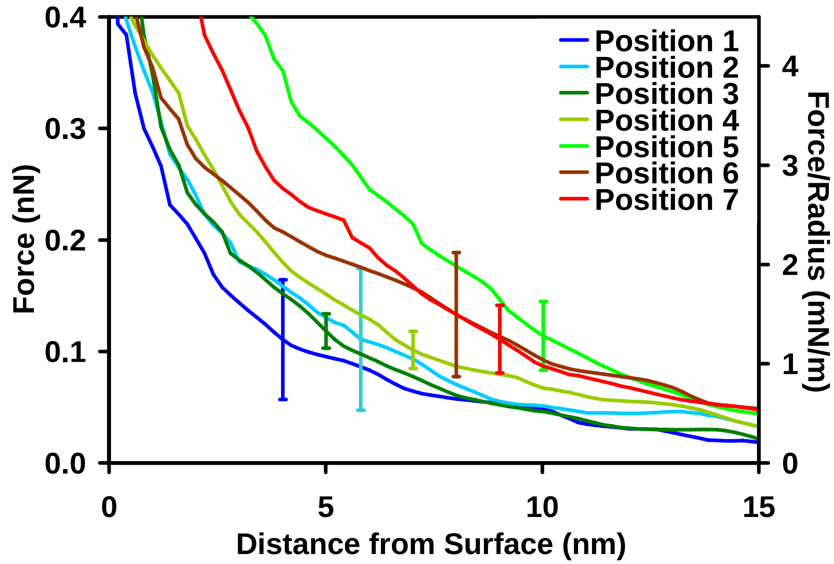
Courtesy of Prof. Carlos Bustamante. Used with permission.



Courtesy of Zhifeng Shao. Used with permission.

HRFS COMBINED WITH AFM : SPATIALLY SPECIFIC SURFACES INTERACTION INFORMATION





● AFM can be combined with high resolution force spectroscopy and nanoindentation since cantilever probe tip can be employed for both imaging and nanomechanical measurements→ nanomechanical measurements with positional accuracy down to the nanoscale (Vandiver, et al. *Biomaterials* 26 (**2005**) 271–283).