LECTURE 5: AFM IMAGING

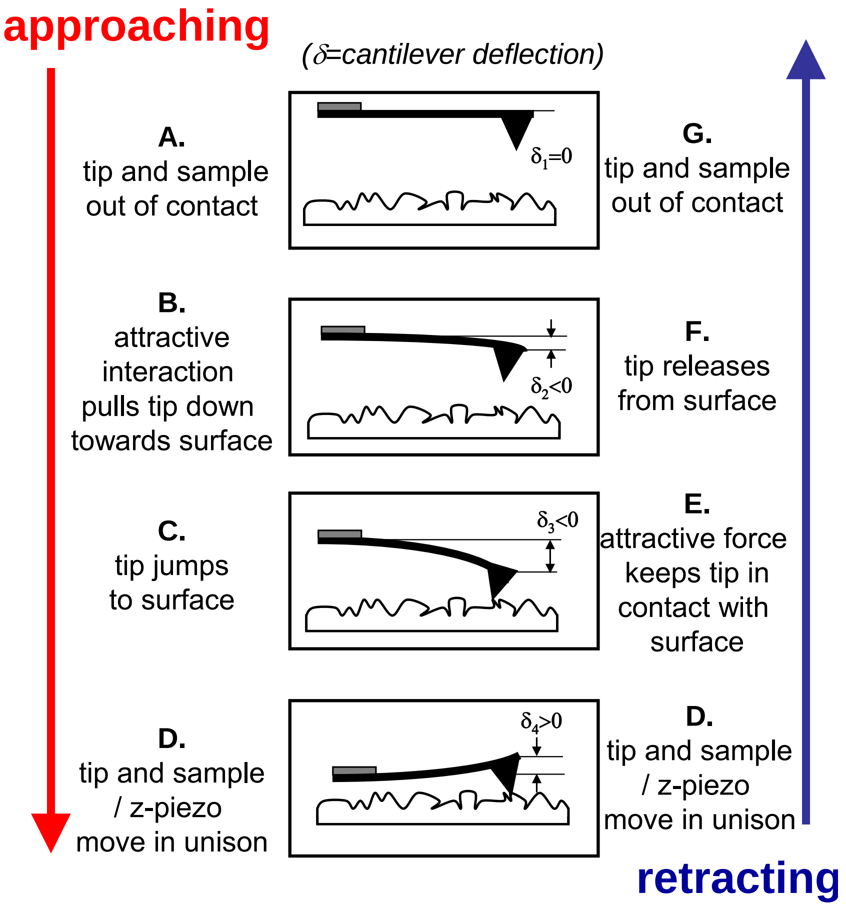
Outline :

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 posted on Stellar.

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



approaching

(δ=cantilever deflection)

A.
tip and sample out of contact

B.
attractive interaction pulls tip down towards surface

C.
tip jumps to surface

D.
tip and sample / z-piezo move in unison

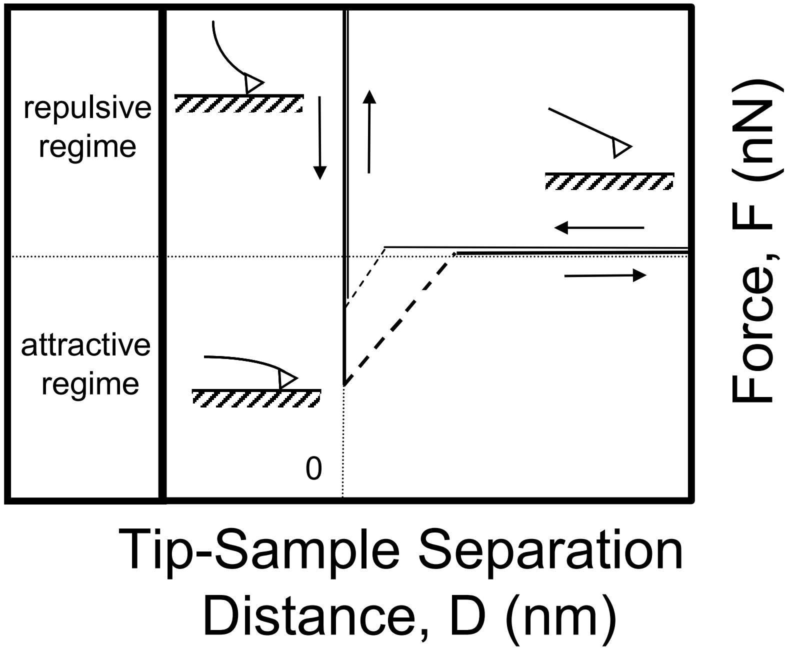
G.
tip and sample out of contact

F.
tip releases from surface

E.
attractive force keeps tip in contact with surface

D.
tip and sample / z-piezo move in unison

retracting



-Normal vs. Lateral Force Microscopy

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

$\delta=s/m$

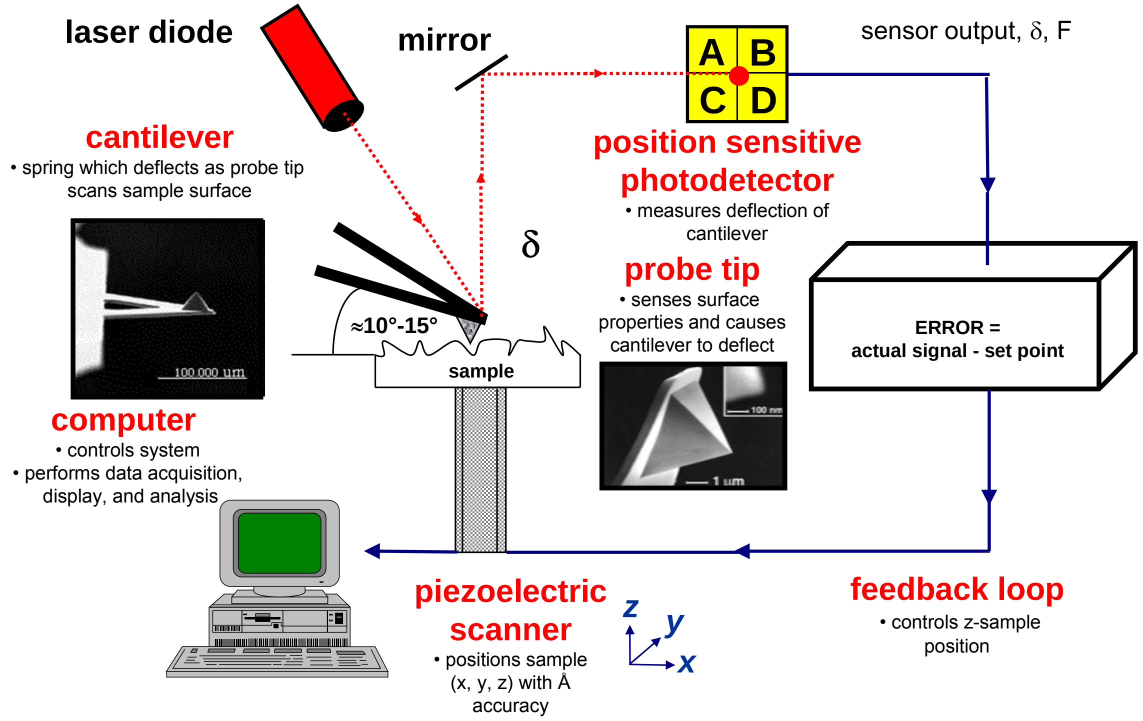
m= slope in constant compliance regime $=\Delta s/\Delta\delta$ (V/nm)

$F=k\delta$

$D=z\pm\delta$

-zeroing the baseline

ATOMIC FORCE MICROSCOPY : GENERAL COMPONENTS AND FUNCTIONS



laser diode

cantilever
- spring which deflects as probe tip scans sample surface

position sensitive photodetector
- measures deflection of cantilever

probe tip
- senses surface properties and causes cantilever to deflect

computer
- controls system
- performs data acquisition, display, and analysis

piezoelectric scanner
- positions sample (x, y, z) with Å accuracy

feedback loop
- controls z-sample position

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.)

-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**

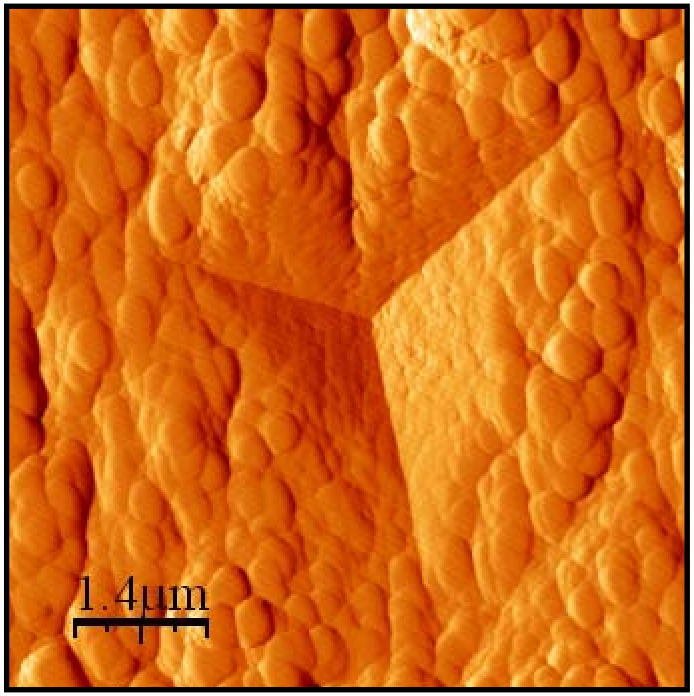
-System continuously changes in response to an experimental output (δ= cantilever deflection which is the feedback parameter)

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

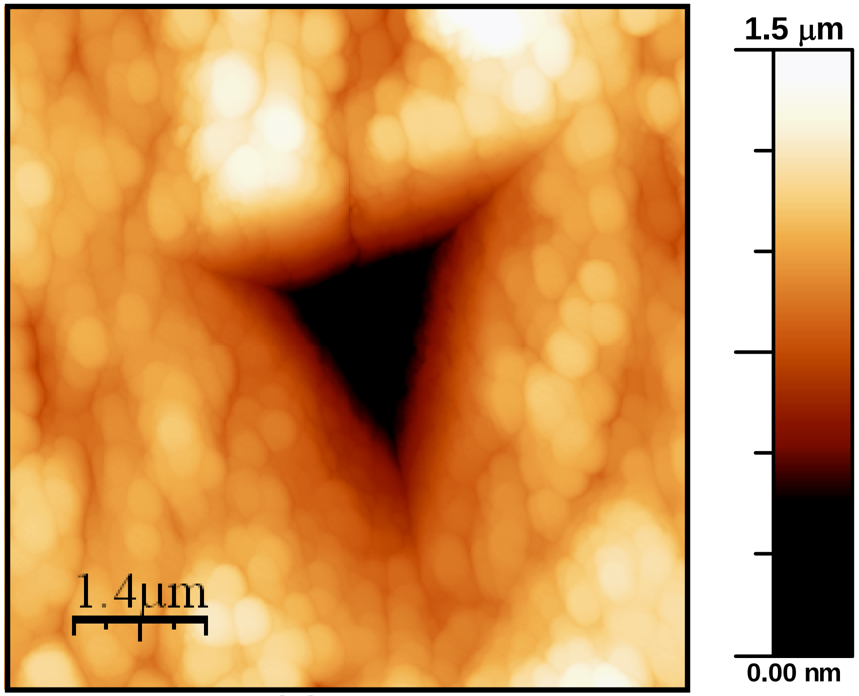
↓

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

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

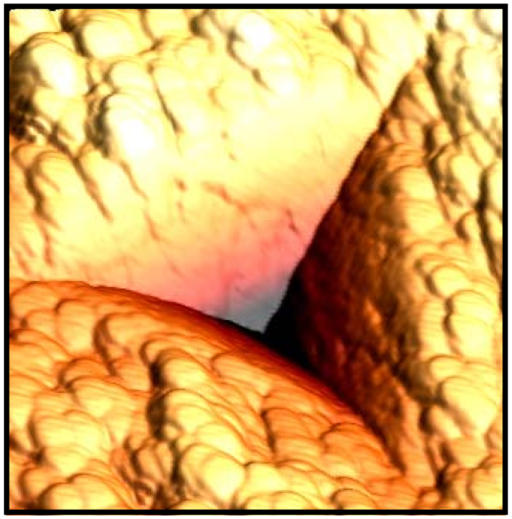


Height Image:
-Processed data from z-piezo, less clear compared to deflection image, maximize your feedback system, can quantify the height of structural features, in 2D image corresponds to brightness

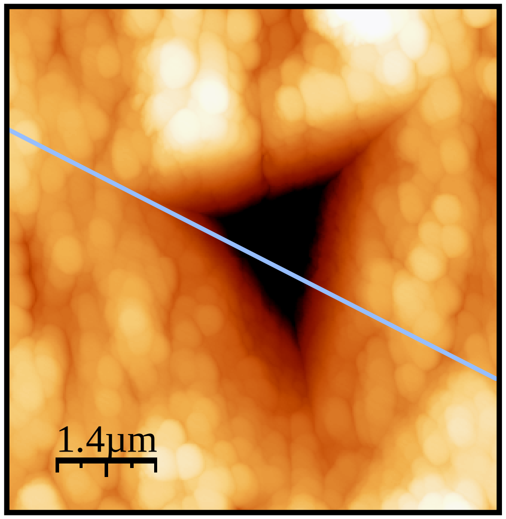
z-scale bar

*(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**)*

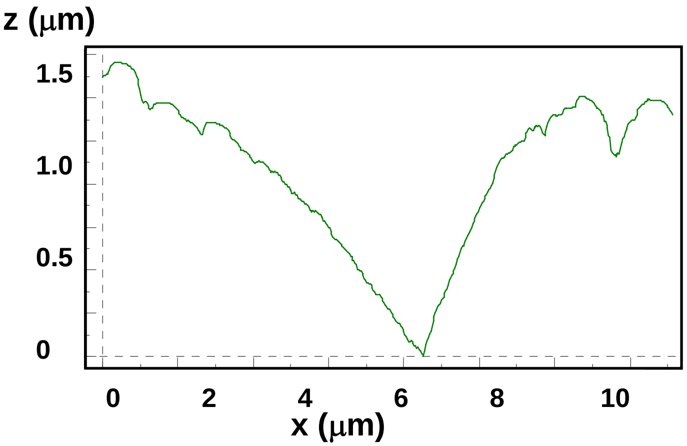
ATOMIC FORCE MICROSCOPY : 3D PLOTS AND 2D SECTION PROFILES



3D Height image



2D Height image



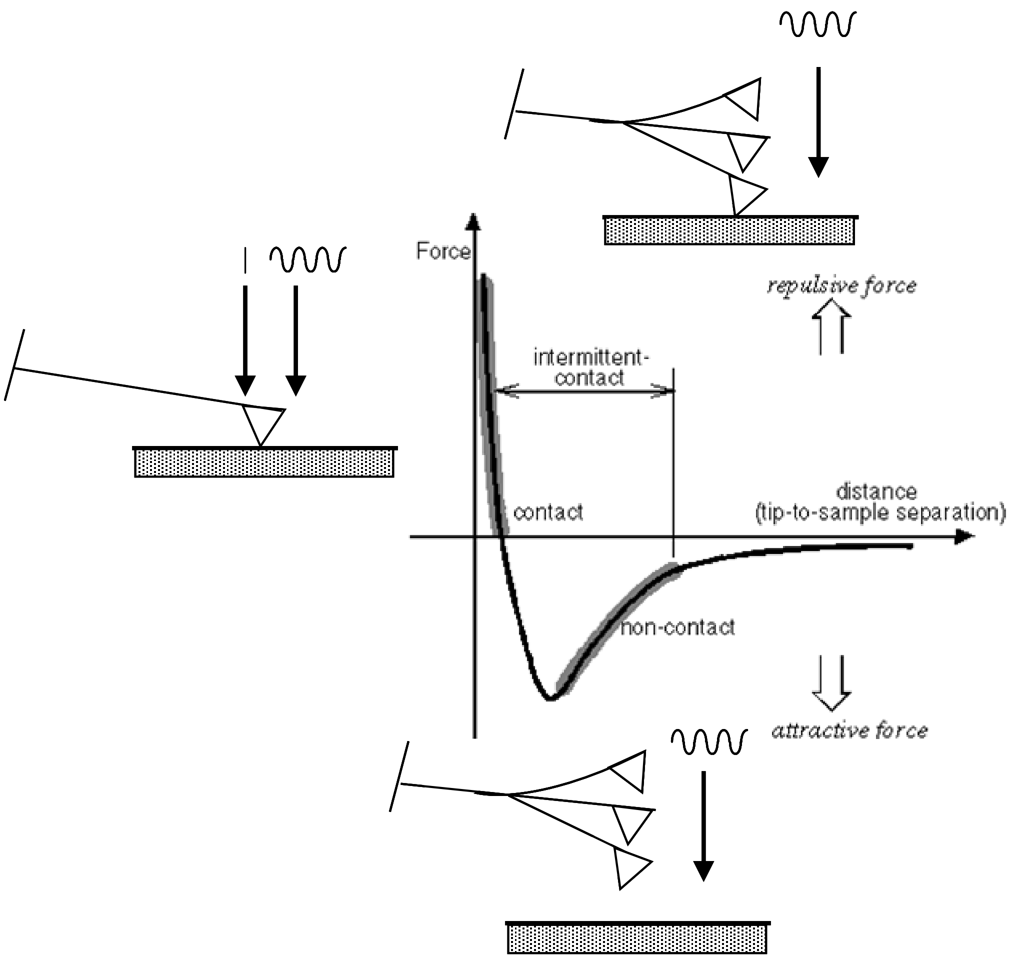
2D Section Profile

-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



(Schematic taken from 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 :

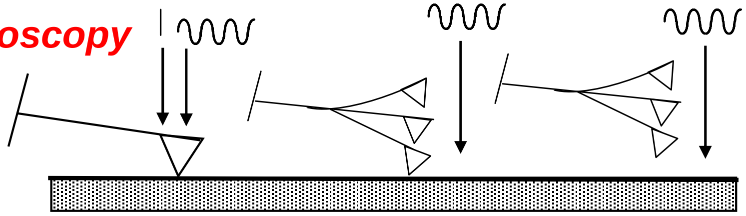
- tip is oscillated near its resonant frequency and "taps" on and off the surface
- feedback signal, oscillation amplitude or phase
- less destructive due to reduction of lateral forces
- loss of spatial resolution

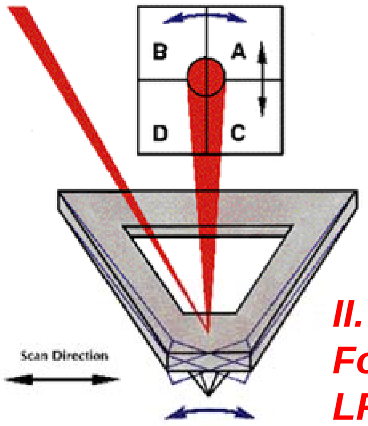
Noncontact (AC) Mode :

- 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

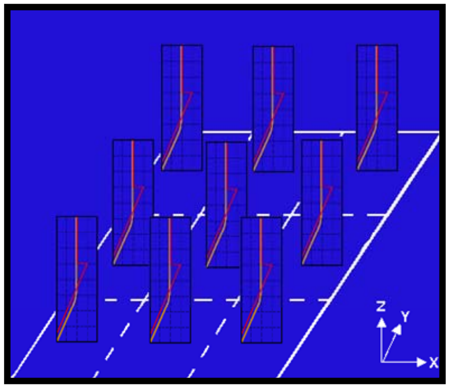
II. Friction or Lateral Force Microscopy (FFM/ LFM)

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

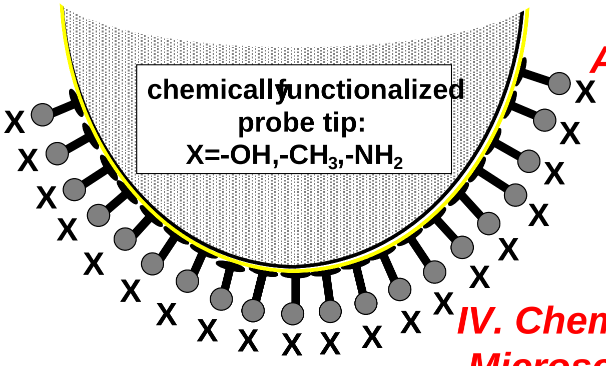
http://www.di.com/AppNotes/LatChem/LatChemMain.html

III. Force / Volume Adhesion Microscopy

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



http://www.di.com/AppNotes/ForceVol/FV.array.html



chemically functionalized probe tip:
X=-OH,-CH$_3$,-NH$_2$

IV. Chemical Force Microscopy (CFM)

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

Surface Maps:

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

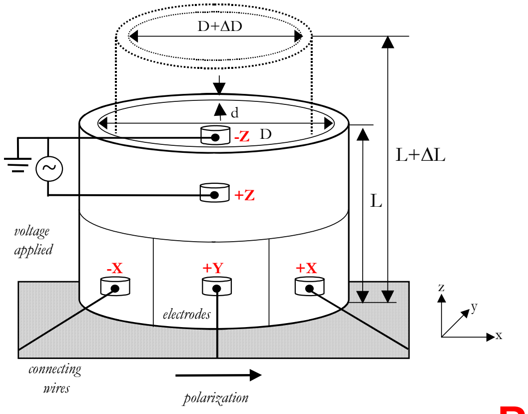
Dynamic Processes :

Erosion, Degradation, Protein-DNA Interactions

ATOMIC FORCE MICROSCOPY IMAGING : FACTORS AFFECTING RESOLUTION

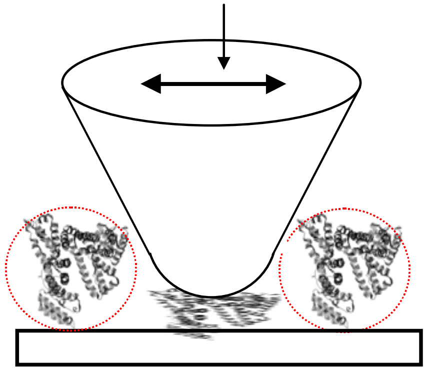
PIEZO AMPLIFIER, SENSOR AND CONTROL ELECTRONICS, MECHANICAL PARAMETERS

Physik Instruments, *Nanopositioning* **1998**



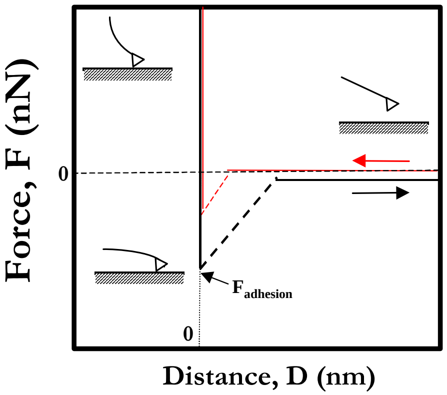
SPECIMEN DEFORMATION & THERMAL FLUCTUATIONS

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



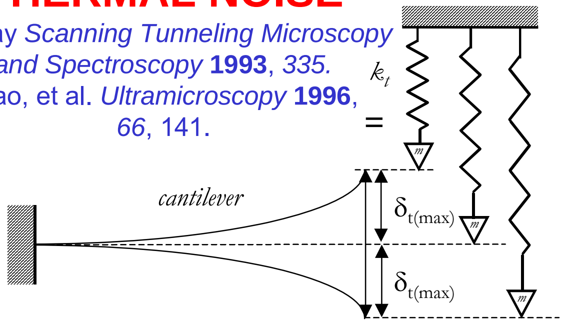
ADHESION FORCE

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



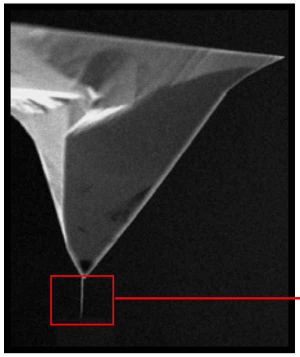
CANTILEVER THERMAL NOISE

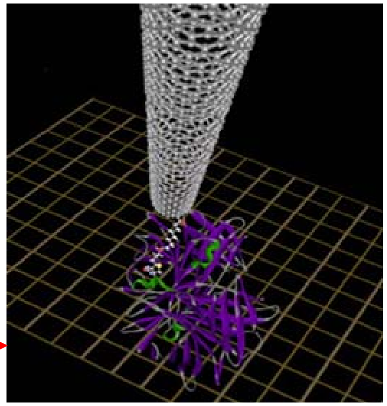
Lindsay *Scanning Tunneling Microscopy and Spectroscopy* **1993**, *335.*
Shao, et al. *Ultramicroscopy* **1996**, *66*, 141.



PROBE TIP SHARPNESS

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

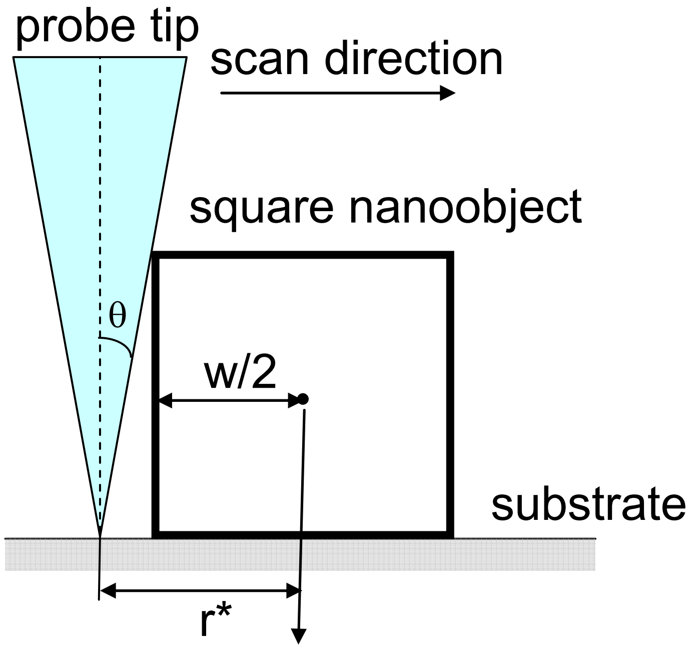


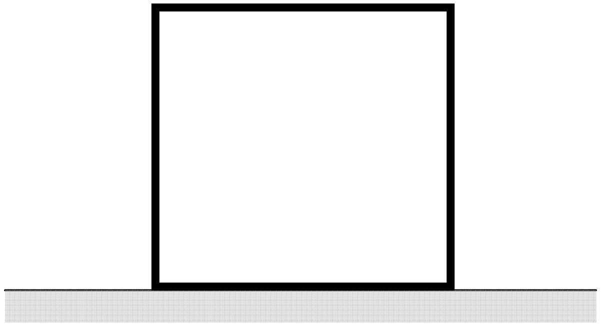


*(*http://cnst.rice.edu/pics.html Lieber, et al., 2000)*

ATOMIC FORCE MICROSCOPY IMAGING : TIP DECONVOLUTION

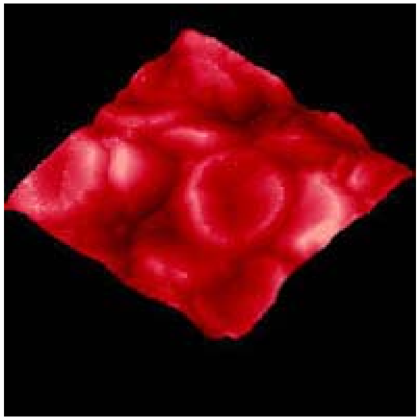
-Imaging very sharp vertical surfaces is influenced by the sharpness of the tip. Only a tip with sufficient sharpness can properly image a given z-gradient. Some gradients will be steeper or sharper than any tip can be expected to image without artifact. False images are generated that reflect the self-image 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.





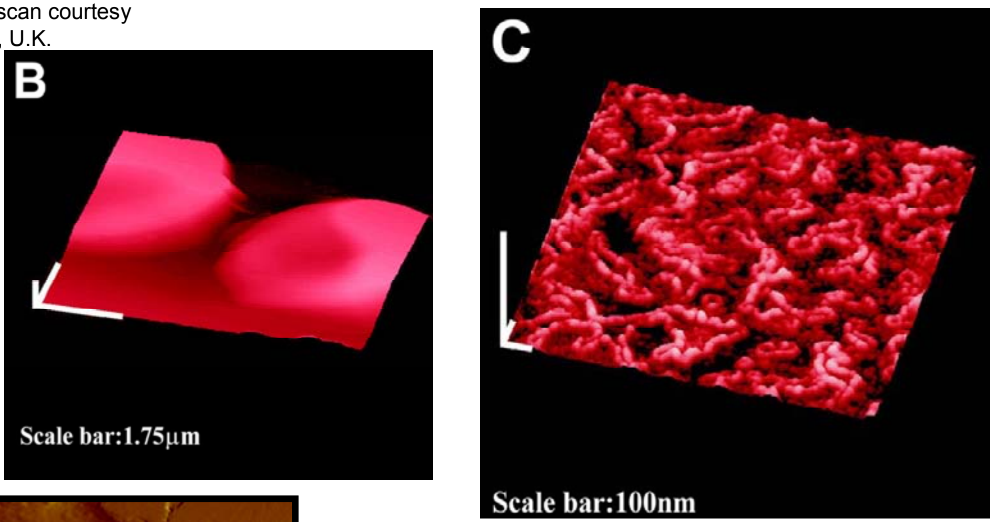
ATOMIC FORCE MICROSCOPY IMAGING OF CELLS

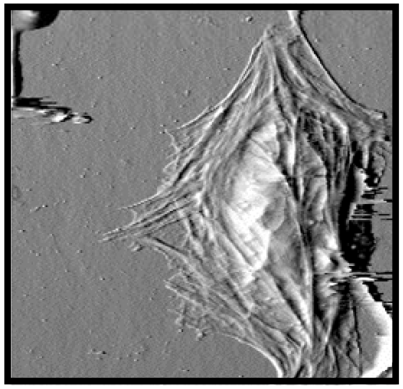
Contact mode image of **human red blood cells** - note cytoskeleton is visible. blood obtained from Johathan Ashmore, Professor of Physiology University College, London. A false color table has been used here, as professorial blood is in fact blue. 15μm scan courtesy M. Miles and J. Ashmore, University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



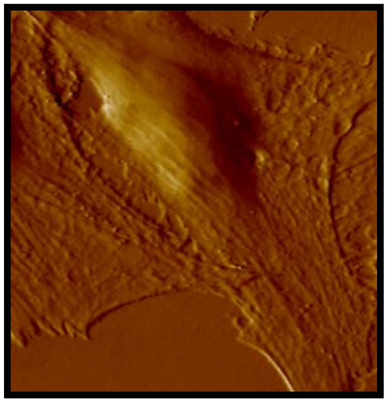
Red Blood Cells

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

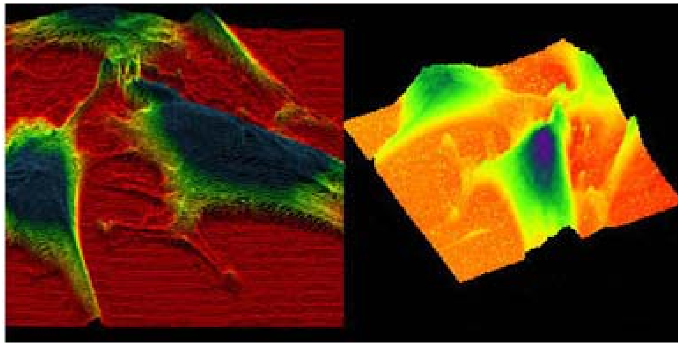




Radmacher, et al., **Cardiac Cells**
http://www.physik3.gwdg.de/~radmacher/



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

TappingMode image of nucleosomal DNA. Image courtesy of Y. Lyubchenko.

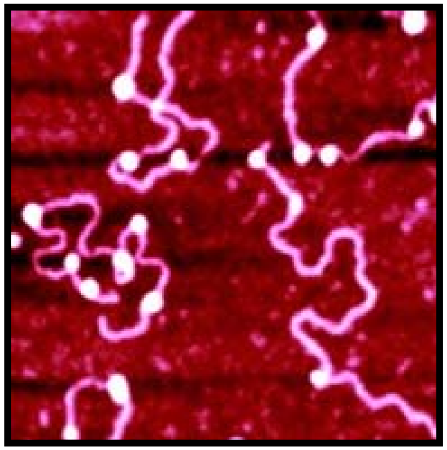
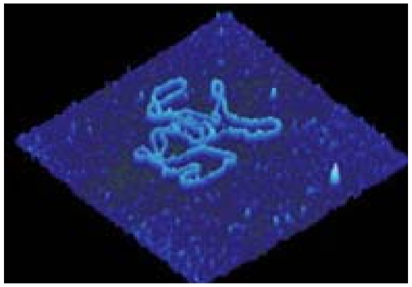
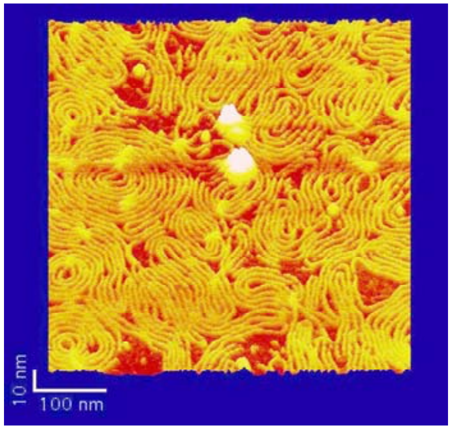
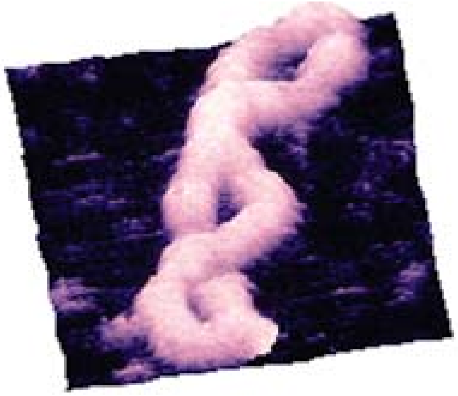


Image of PtyrTlac supercoiled DNA. 750 nm scan courtesy C. Tolksdorf, Digital Instruments/Veeco, Santa Barbara, USA, and R. Schneider and G. Muskhelishvili, Istitut für Genetik und Mikrobiologie, Germany.

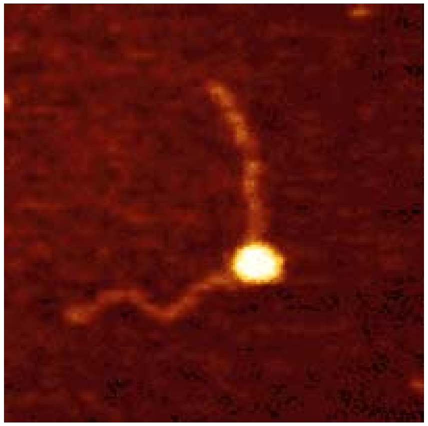




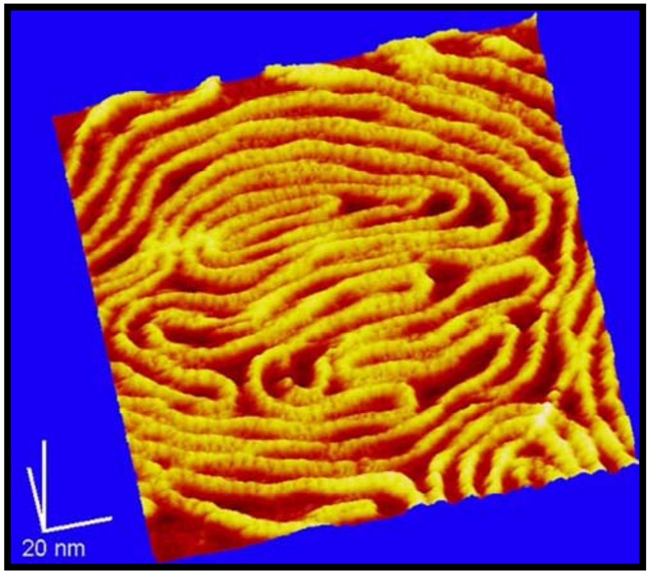
http://www.people.virginia.edu/~js6s/zsfig/DNA.html



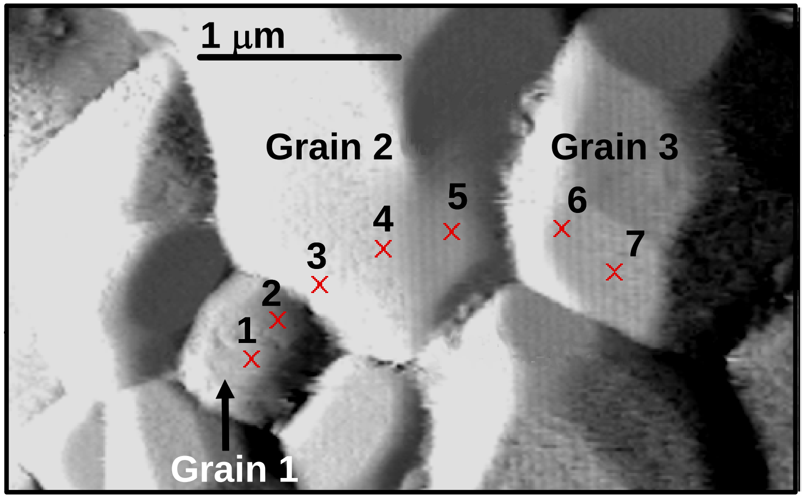
The high resolution of the SPM is able to discern very subtle features such as these two linear dsDNA molecules overlapping each other. 155nm scan. Image courtesy of W. Blaine Stine

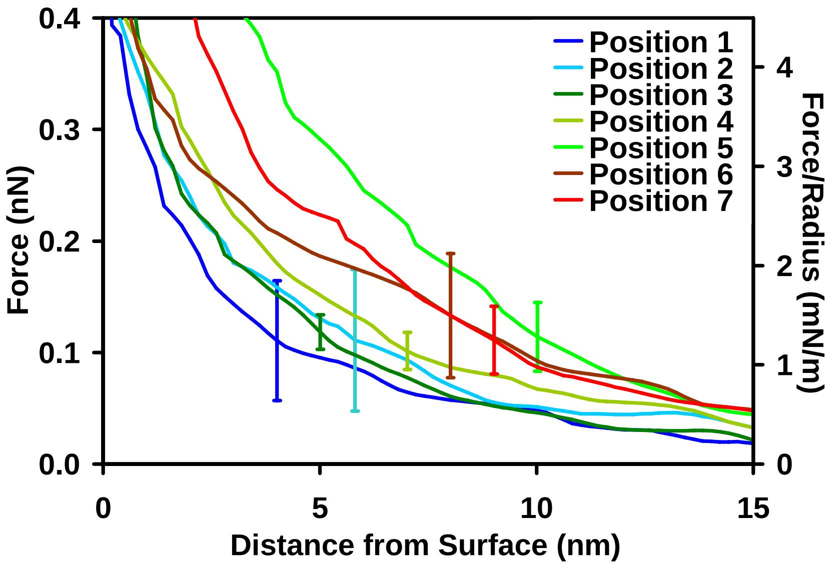


AFM image of short DNA fragment with RNA polymerase molecule bound to transcription recognition site. 238nm scan size. Courtesy of Bustamante Lab, Chemistry Department, University of Oregon, Eugene OR



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).