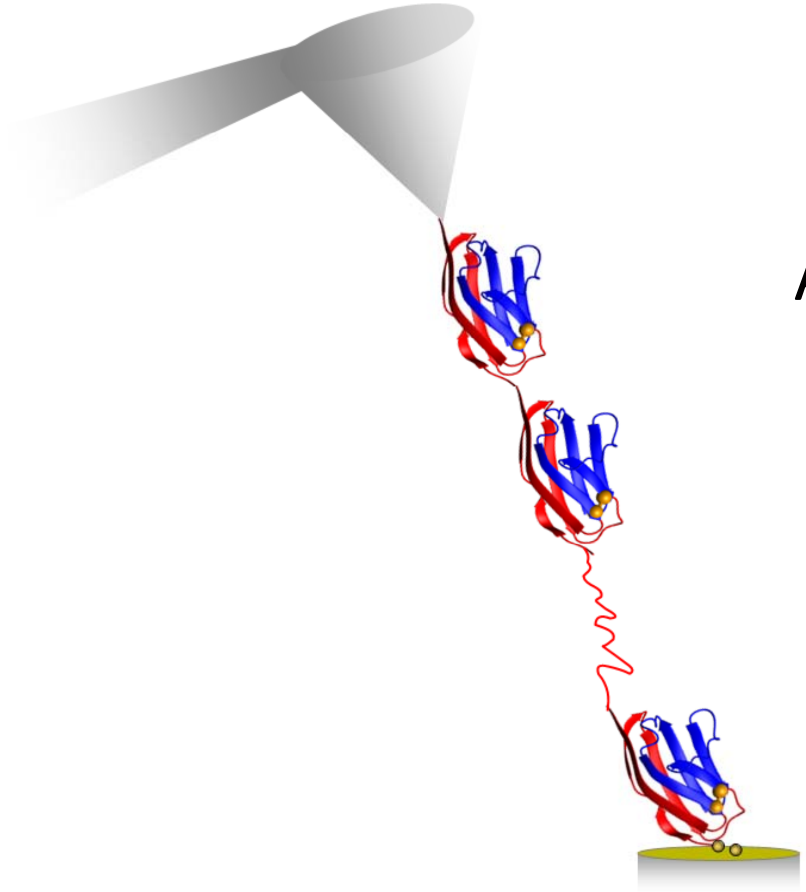
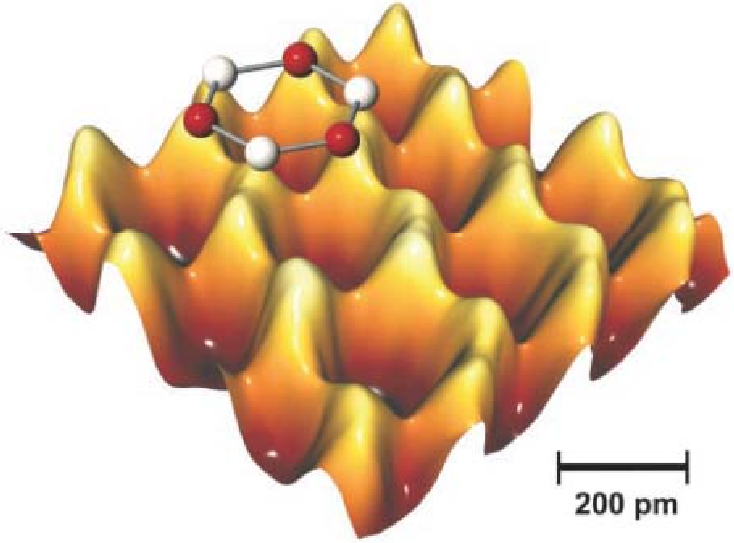
Atomic Force Microscopy:
A tool for high resolution imaging and force sensing

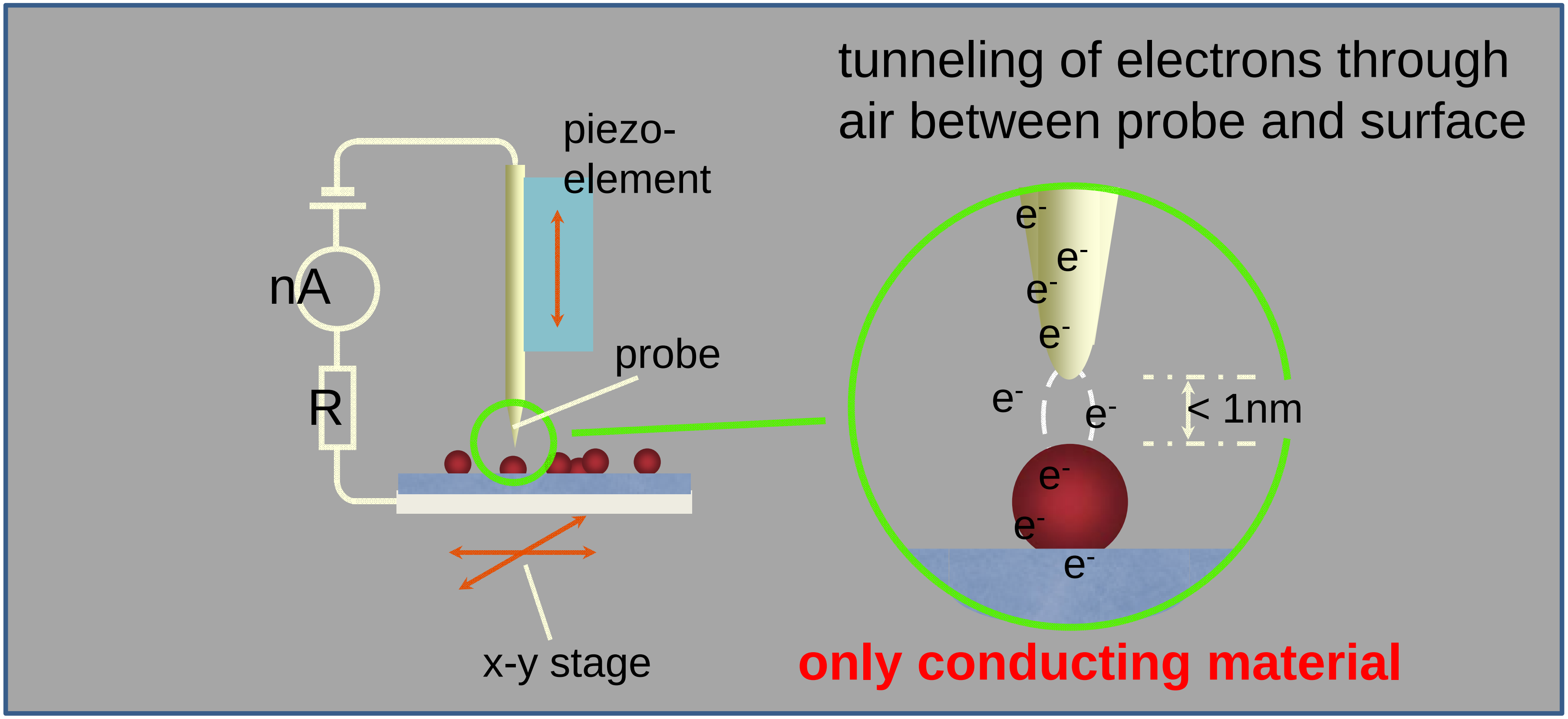


A.S.R. Koti
DCS, TIFR



ASET Colloquium
02 Sept 2011

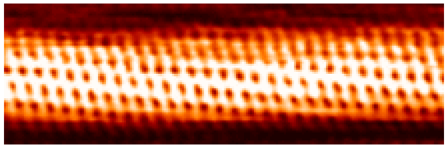
Scanning Tunneling Microscope (STM)
(Precursor to Atomic Force microscope)



tunneling of electrons through air between probe and surface

only conducting material

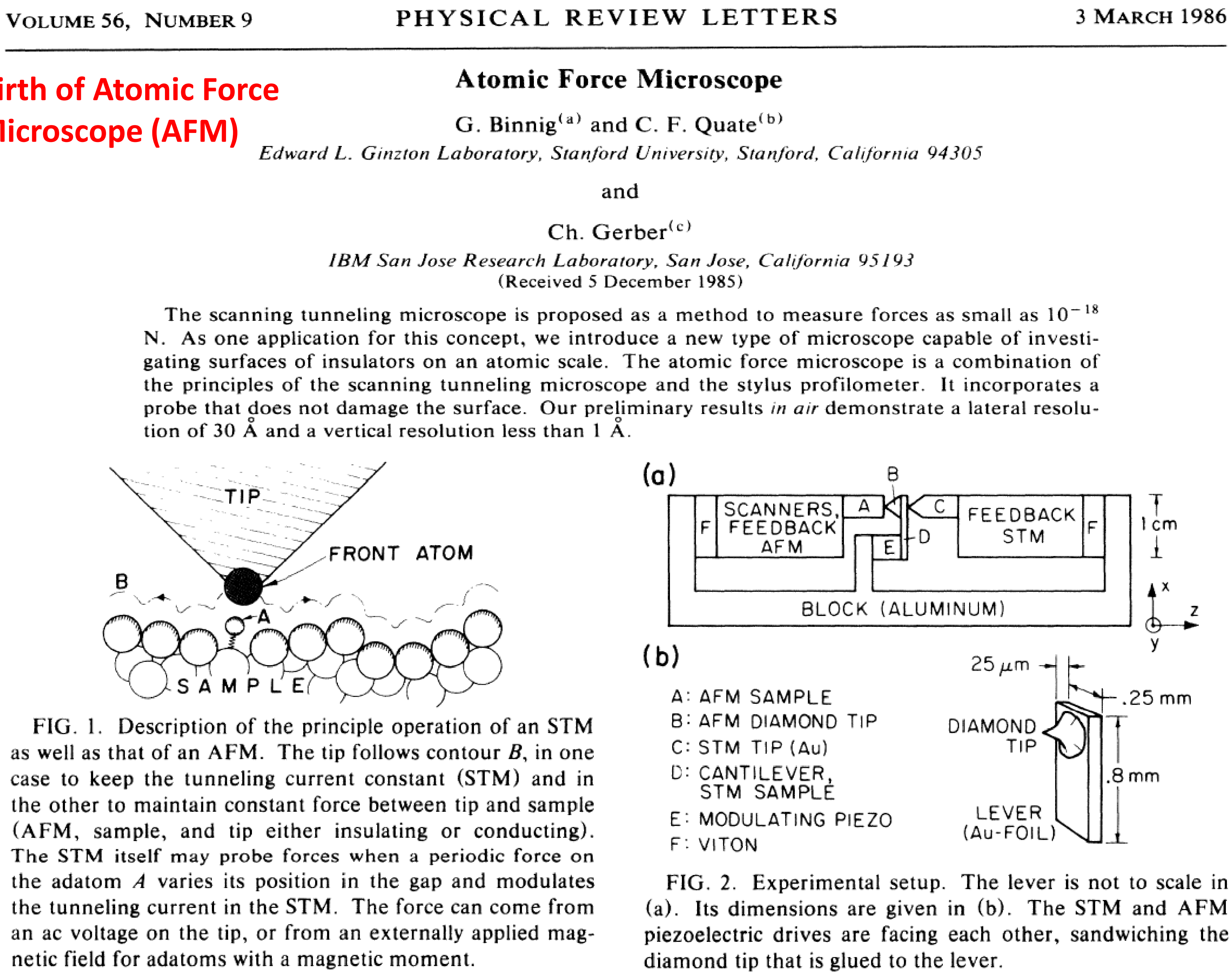
Developed by **Gerd Binnig** and **Heinrich Rohrer** in the early 1980s at IBM Research – Zurich
Awarded with the Nobel Prize for Physics in 1986.



Background and History of AFM

- 1st AFM made by Gerd Binnig, C.F. Quate and Cristoph Gerber in 1985
- Constructed by gluing tiny shard of diamond onto one end of tiny strip of gold foil
- Small hook at end of the tip pressed against sample surface
- Sample scanned by tracking deflection of cantilever by monitoring tunneling current to 2nd tip position above cantilever
- Developed in order to examine insulating surfaces

Birth of Atomic Force Microscope (AFM)



VOLUME 56, NUMBER 9 PHYSICAL REVIEW LETTERS 3 MARCH 1986

Atomic Force Microscope

G. Binnig[a] and C. F. Quate[b]
Edward L. Ginzton Laboratory, Stanford University, Stanford, California 94305

and

Ch. Gerber[c]
IBM San Jose Research Laboratory, San Jose, California 95193

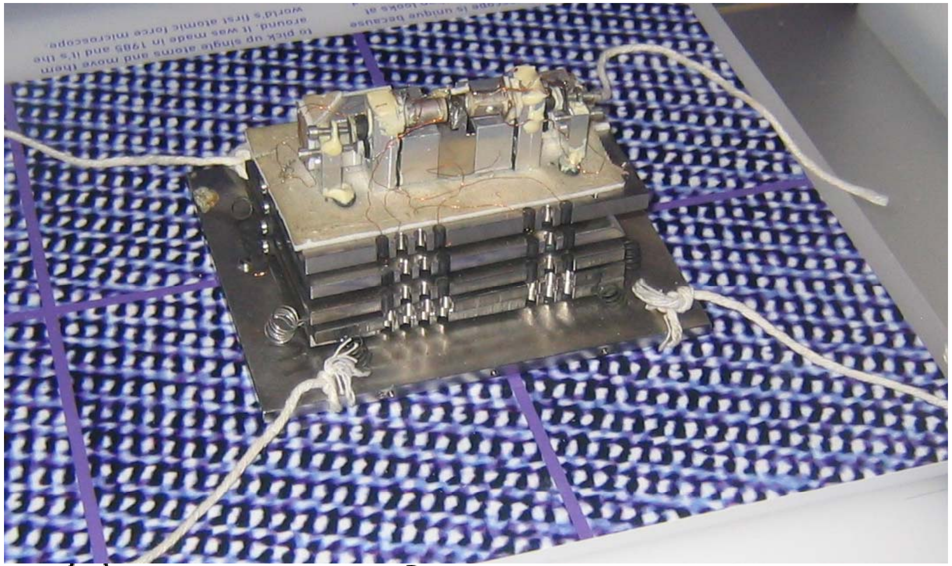
(Received 5 December 1985)

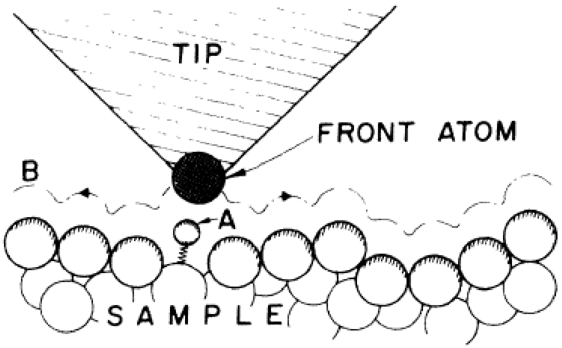
The scanning tunneling microscope is proposed as a method to measure forces as small as 10^{-18} N. As one application for this concept, we introduce a new type of microscope capable of investigating surfaces of insulators on an atomic scale. The atomic force microscope is a combination of the principles of the scanning tunneling microscope and the stylus profilometer. It incorporates a probe that does not damage the surface. Our preliminary results *in air* demonstrate a lateral resolution of 30 Å and a vertical resolution less than 1 Å.

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.

The first AFM; ‘Touching’ microscope





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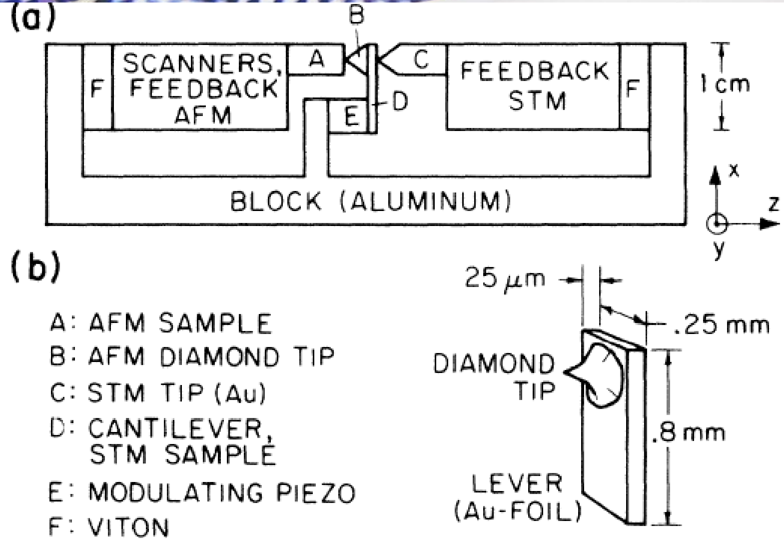

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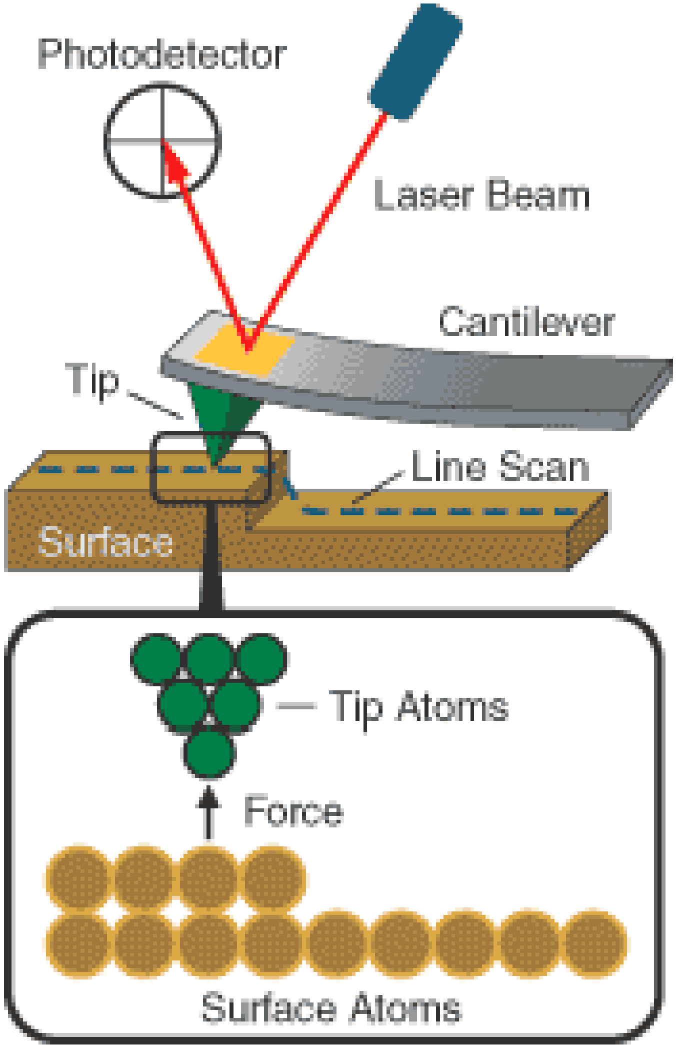
Current generation AFM



http://www.molec.com/what_is_afm.html

- Cantilever
- Tip
- Surface
- Laser
- Multi-segment photodetector

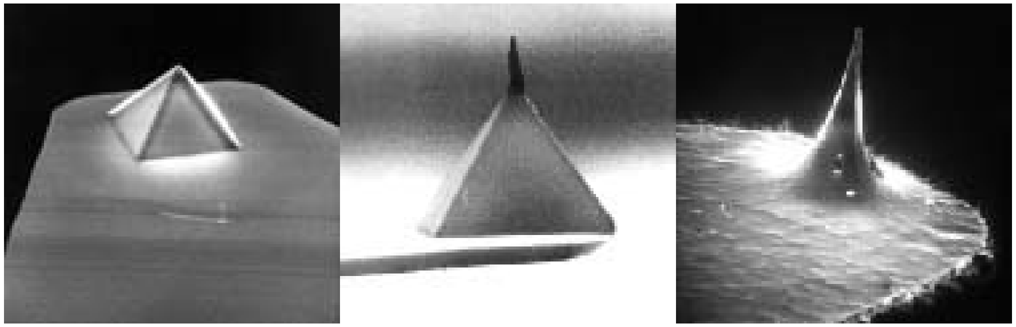
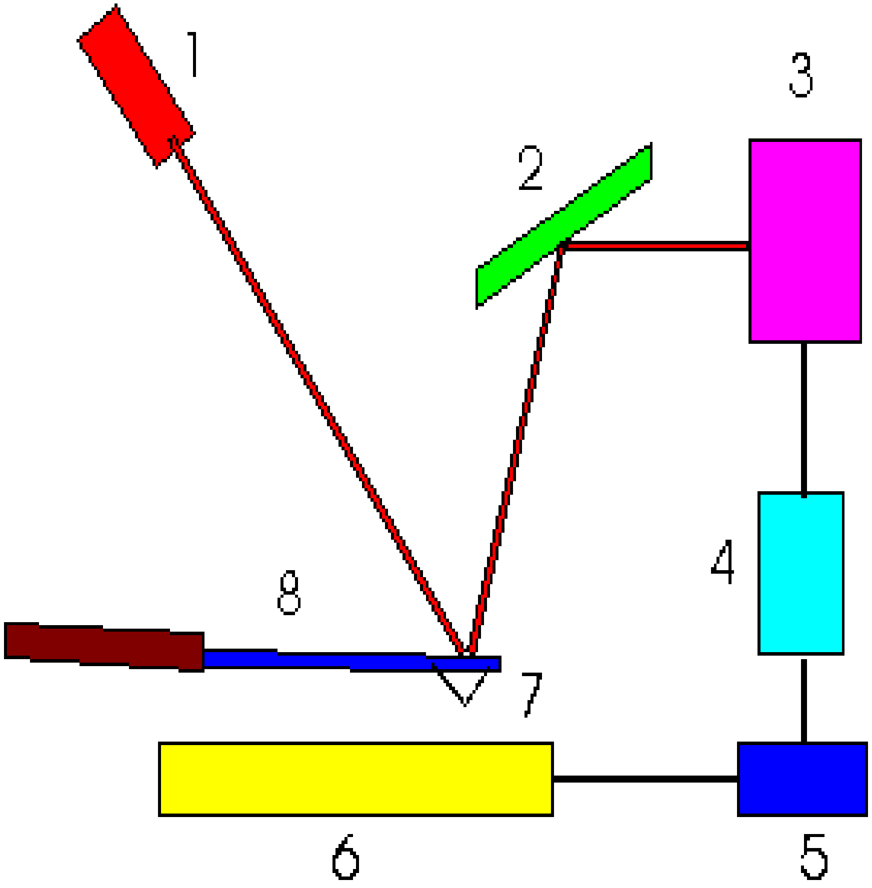


Figure 4. Three common types of AFM tip. (a) normal tip (3 µm tall); (b) supertip; (c) Ultralever (also 3 µm tall). Electron micrographs by Jean-Paul Revel, Caltech. Tips from Park Scientific Instruments; supertip made by Jean-Paul Revel.

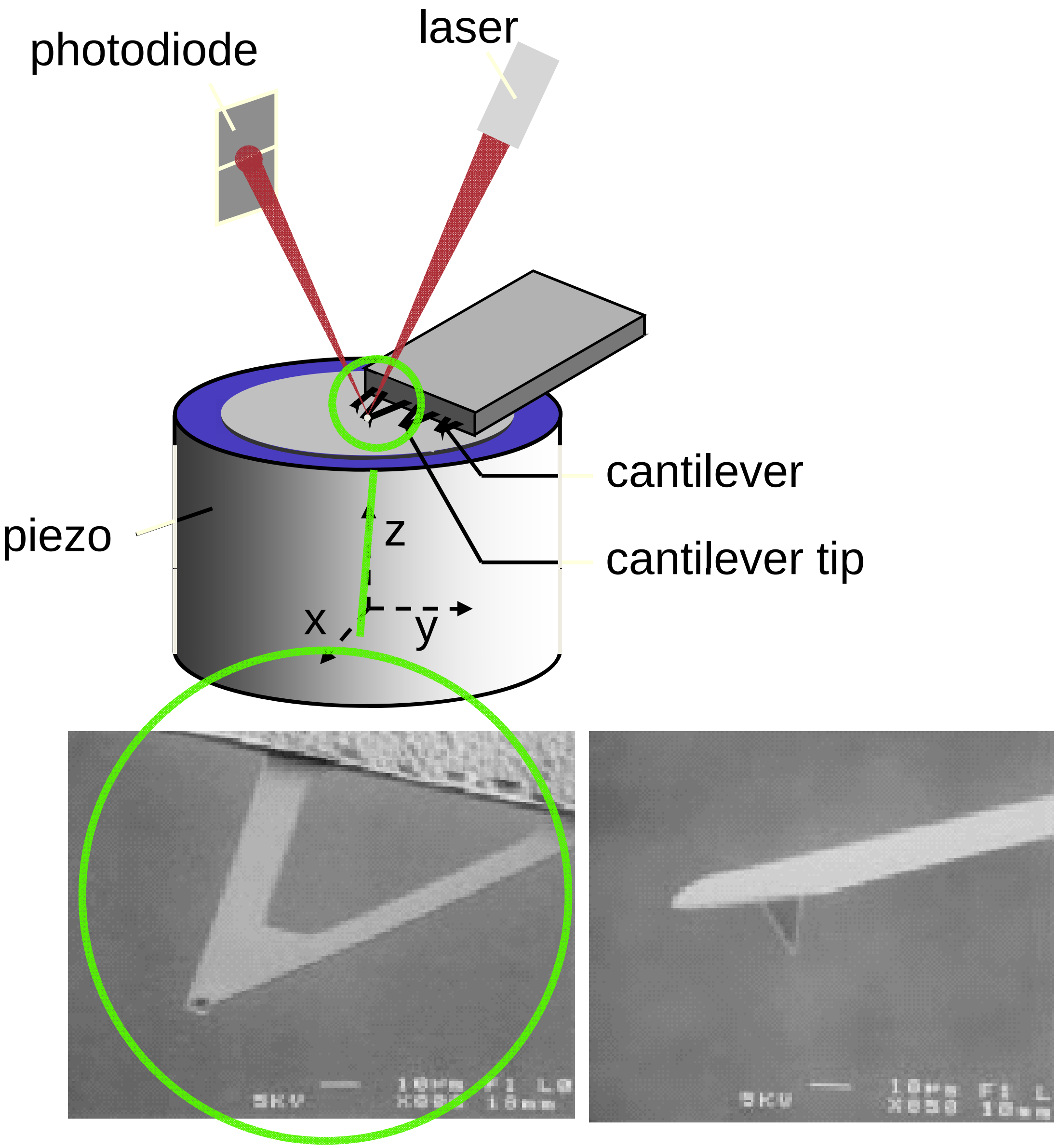
http://stm2.nrl.navy.mil/how-afm/how-afm.html#imaging%20modes

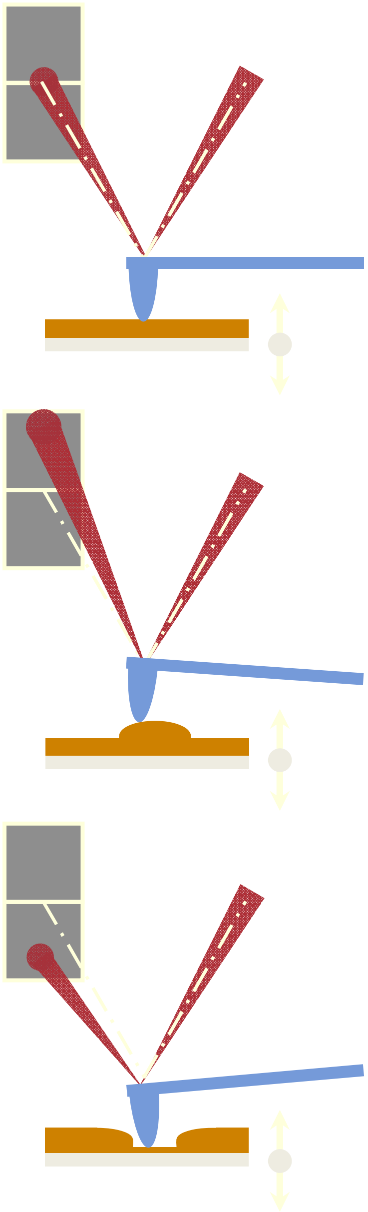
Parts of AFM

- 1. **Laser** – deflected off cantilever
- 2. **Mirror** –reflects laser beam to photodetector
- 3. **Photodetector** –dual element photodiode that measures differences in light intensity and converts to voltage
- 4. **Amplifier**
- 5. **Register**
- 6. **Sample**
- 7. **Probe** –tip that scans sample made of Si
- 8. **Cantilever** –moves as scanned over sample and deflects laser beam

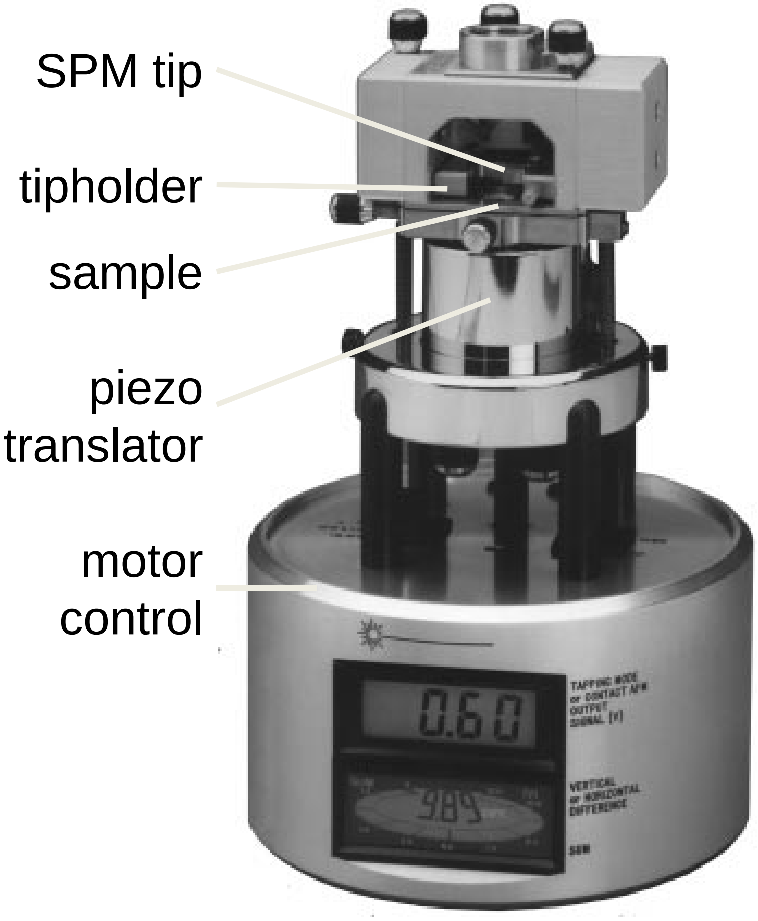


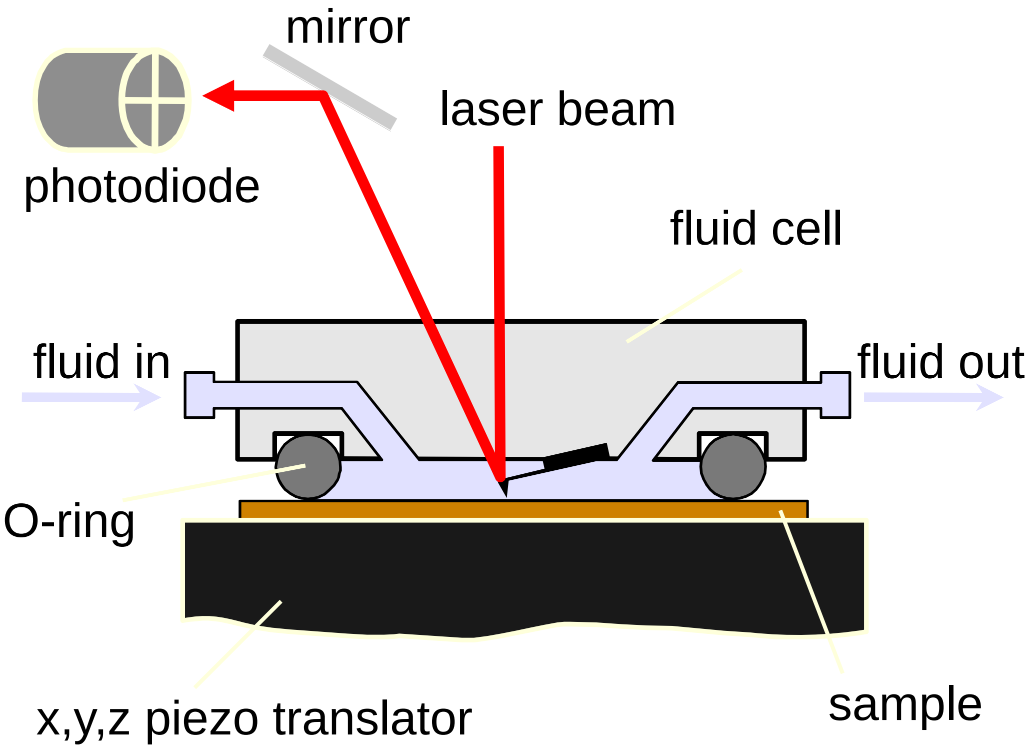
Atomic Force Microscope





Atomic Force Microscope





in air and in buffer solutions

General Applications

- Materials Investigated: Thin and thick film coatings, ceramics, composites, glasses, synthetic and biological membranes, metals, polymers, and semiconductors.

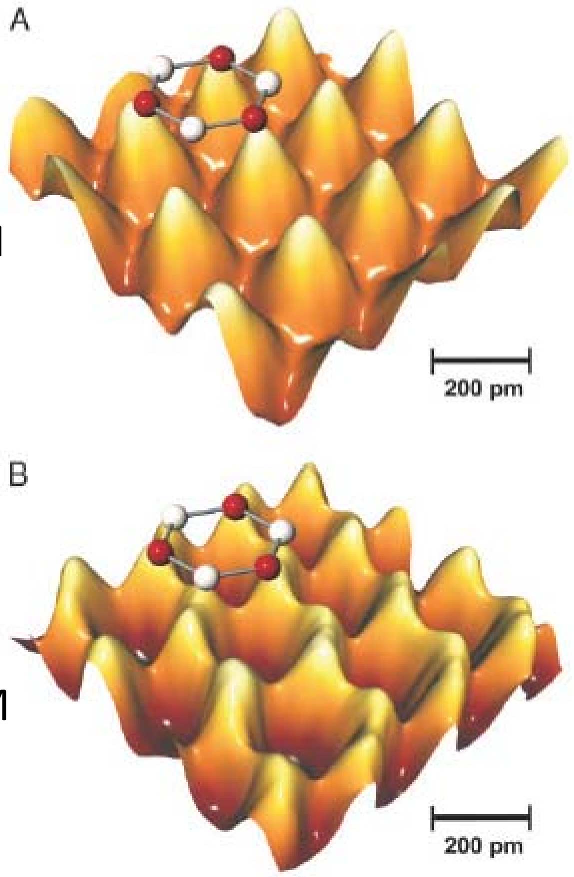
- Used to study phenomena of: Abrasion, adhesion, cleaning, corrosion, etching, friction, lubricating, plating, and polishing.

- AFM can image surface of material in atomic resolution and also measure force at the nano-Newton scale.

Hexagonal lattice of Graphite - 'Missing atoms in STM' are seen by AFM!

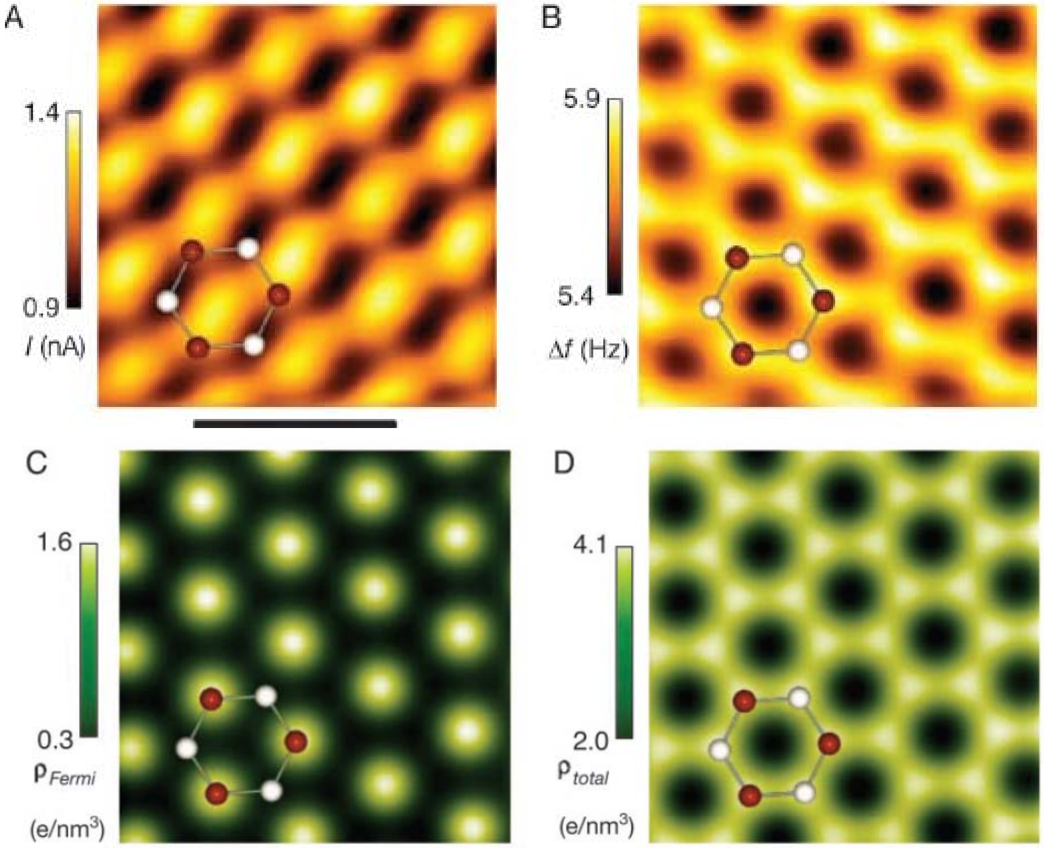
STM

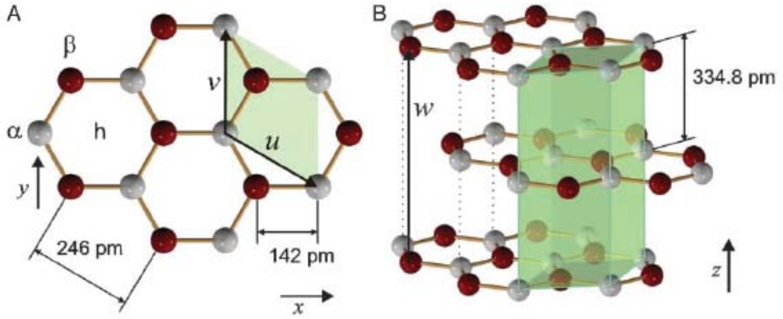
AFM



STM

AFM

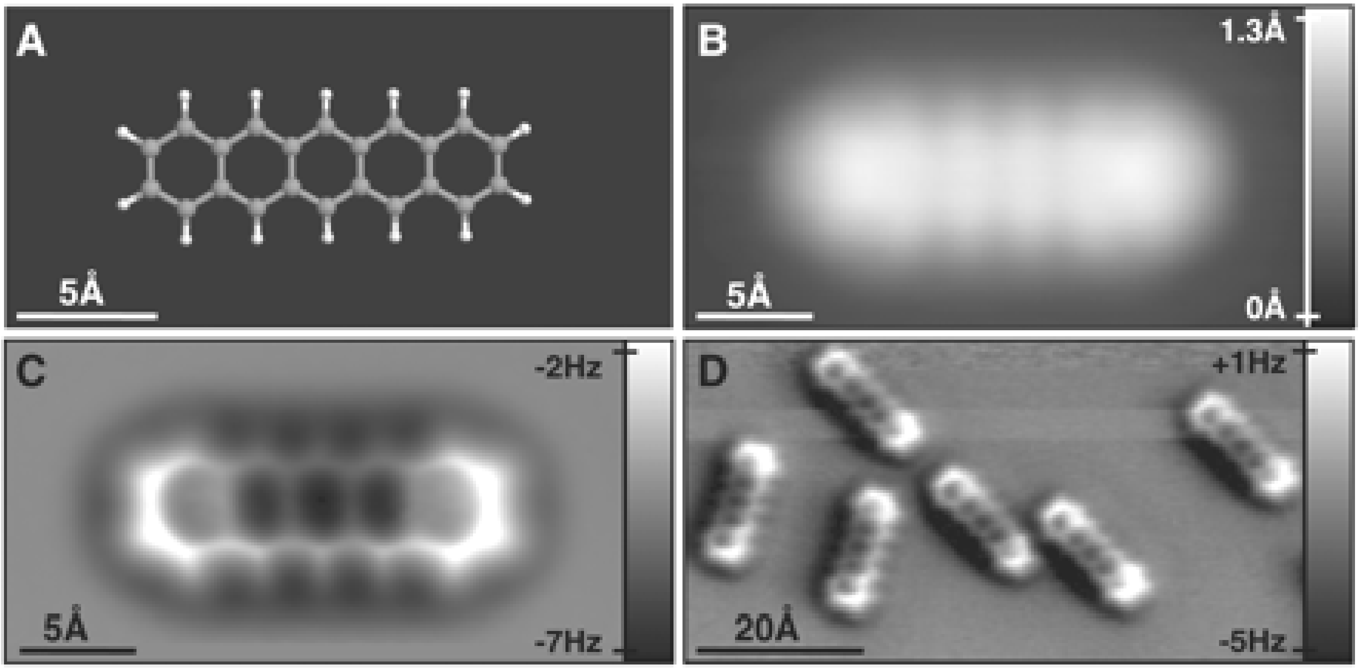




S Hembacher, F.J. Giessibl, J. Mannhart, C.F. Quate
PNAS(2003)

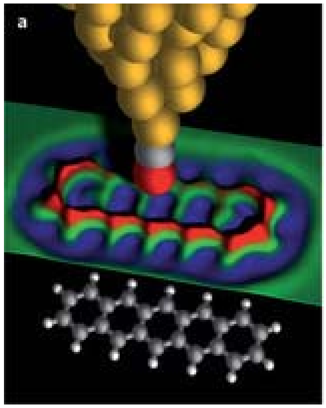
Pentacene ($C_{22}H_{14}$)

STM



AFM

AFM



Probe with CO molecules at the apex

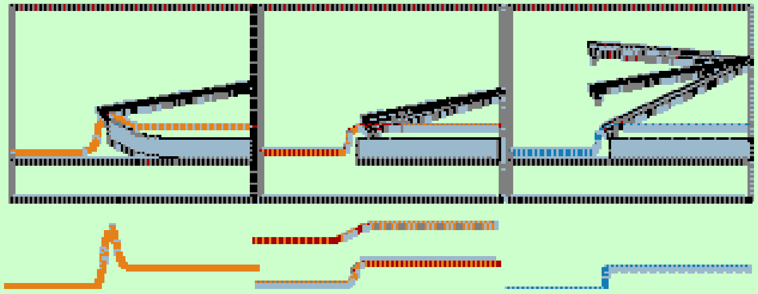
L. Gross, F Mohn, N. Moll, P. Liljeroth, G. Meyer. Science (2009)

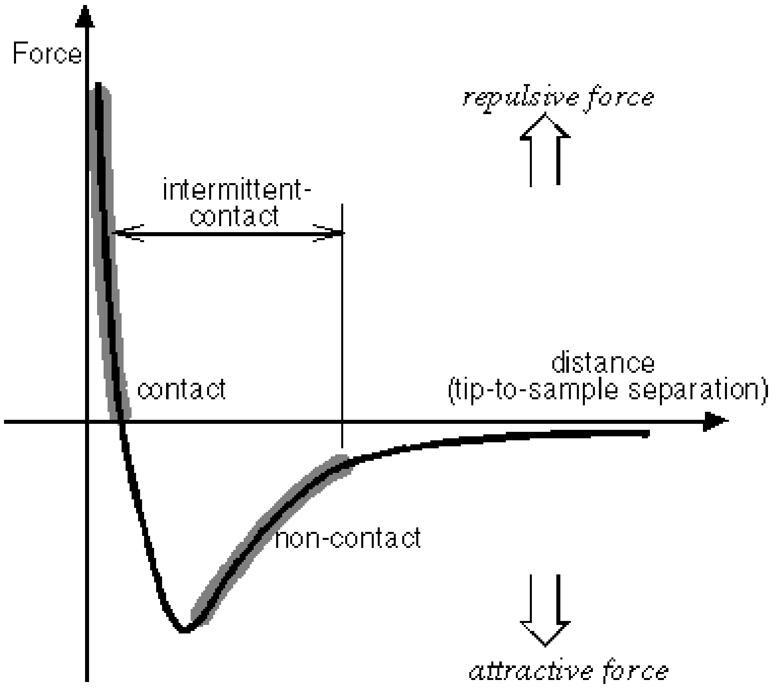
Modes of AFM

Contact Mode

Non-Contact Mode

Tapping (Intermittent contact) Mode





Contact Mode

- Measures repulsion between tip and sample
- Force of tip against sample remains constant
- Feedback regulation keeps cantilever deflection constant
- Voltage required indicates height of sample
- Problems: excessive tracking forces applied by probe to sample

Non-Contact Mode

- Measures attractive forces between tip and sample
- Tip doesn’t touch sample
- Van der Waals forces between tip and sample detected
- Problems: Can’t use with samples in fluid
- Used to analyze semiconductors
- Doesn’t degrade or interfere with sample- better for soft samples

Tapping (Intermittent-Contact) Mode

- Tip vertically oscillates between contacting sample surface and lifting of at frequency of 50,000 to 500,000 cycles/sec.
- Oscillation amplitude reduced as probe contacts surface due to loss of energy caused by tip contacting surface
- Advantages: overcomes problems associated with friction, adhesion, electrostatic forces
- More effective for larger scan sizes

Figures of Merit

- Can measure surface features with dimensions ranging from inter-atomic spacing to 0.1mm

- Resolution limited by size of tip (2-3nm)

- Resolution of imaging 5nm lateral and .01nm vertical

Advantages of AFM

- AFM versus STM (scanning tunneling microscope): both conductors and insulators

- AFM versus SEM (scanning electron microscope): greater topographic contrast

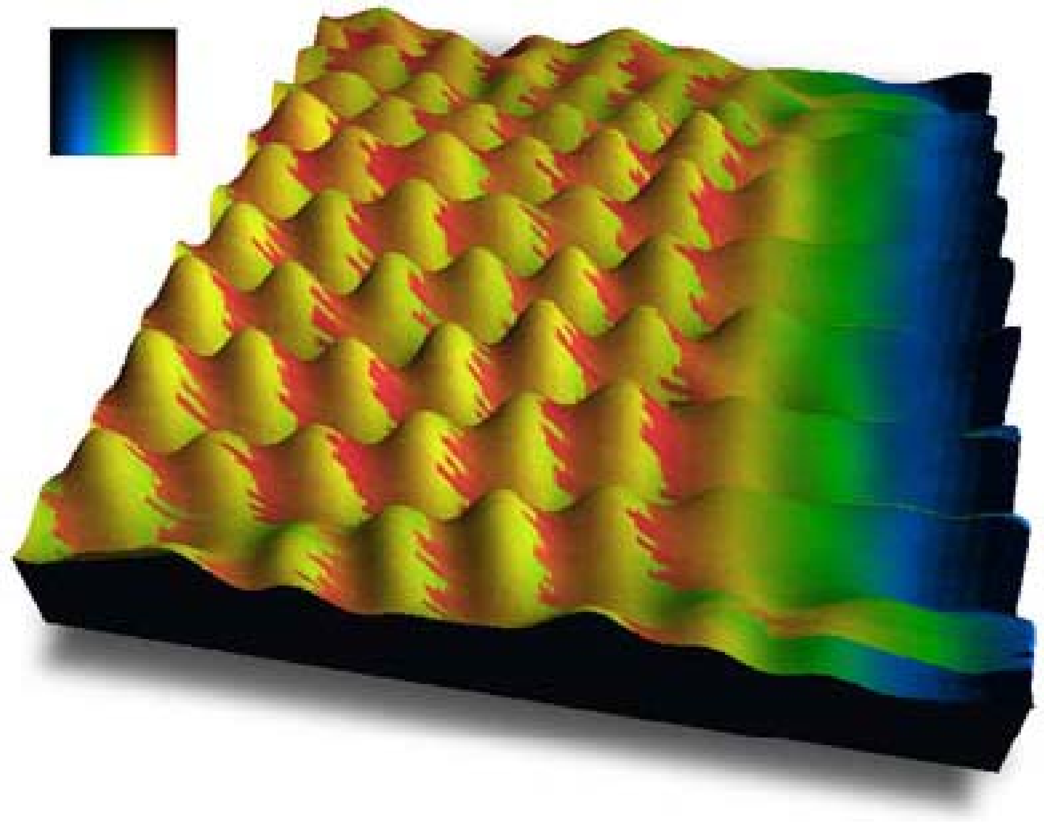
- AFM versus TEM (transmission electron microscope): no expensive sample prep

Biological Applications

- Used to analyze DNA, RNA, protein-nucleic acid complexes, chromosomes, cell membranes, proteins and peptides, molecular crystals, polymers, biomaterials, ligand-receptor binding
- Little sample prep required
- Nanometer resolved images of nucleic acids
- Imaging of cells
- Quantification of molecular interactions in biological systems
- Quantification of electrical surface charge

Topography

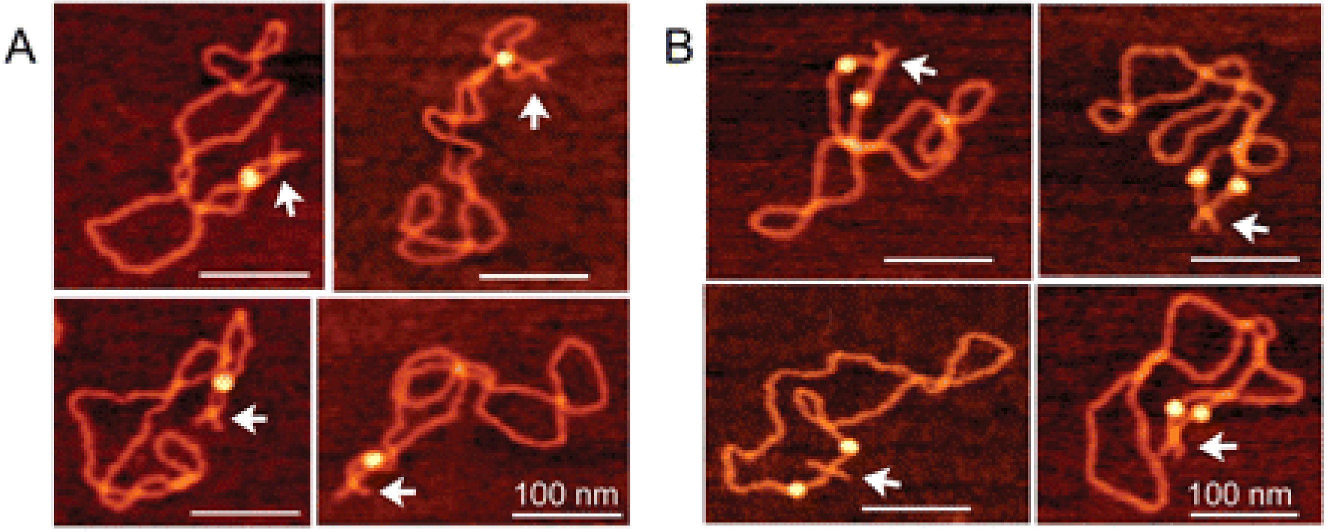
- Contact Mode
  - High resolution
  - Damage to sample
  - Can measure frictional forces
- Non-Contact Mode
  - Lower resolution
  - No damage to sample
- Tapping Mode
  - Better resolution
  - Minimal damage to sample



2.5 x 2.5 nm simultaneous topographic and friction image of highly oriented pyrolytic graphic (HOPG). The bumps represent the topographic atomic corrugation, while the coloring reflects the lateral forces on the tip. The scan direction was right to left

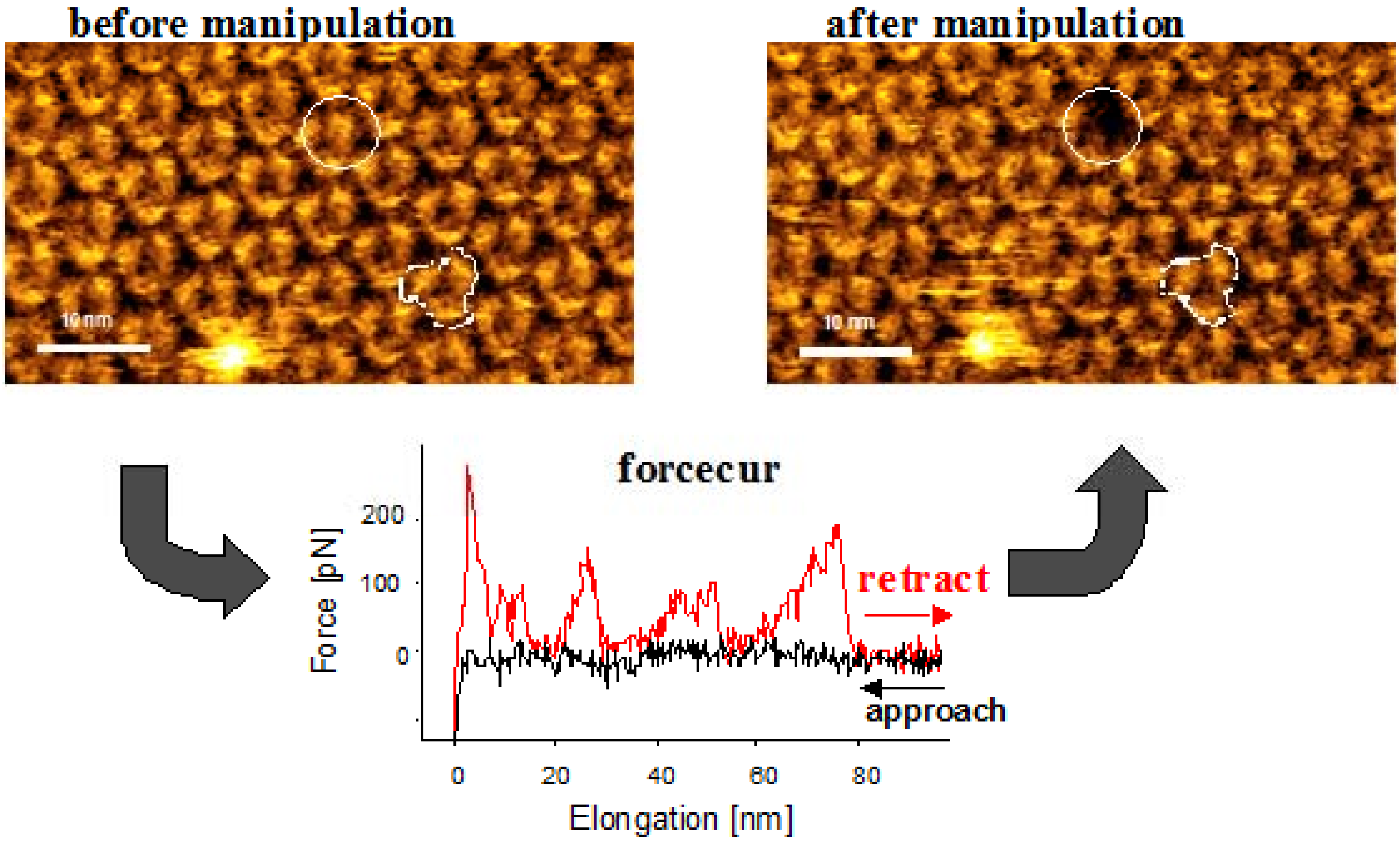
http://stm2.nrl.navy.mil/how-afm/how-afm.html#imaging%20modes

Imaging of Macromolecules in Dry and solution



AY. Lushnikov, VN. Potaman and YL.
Lyubchenko. Nucl. Acid Res. (2006)

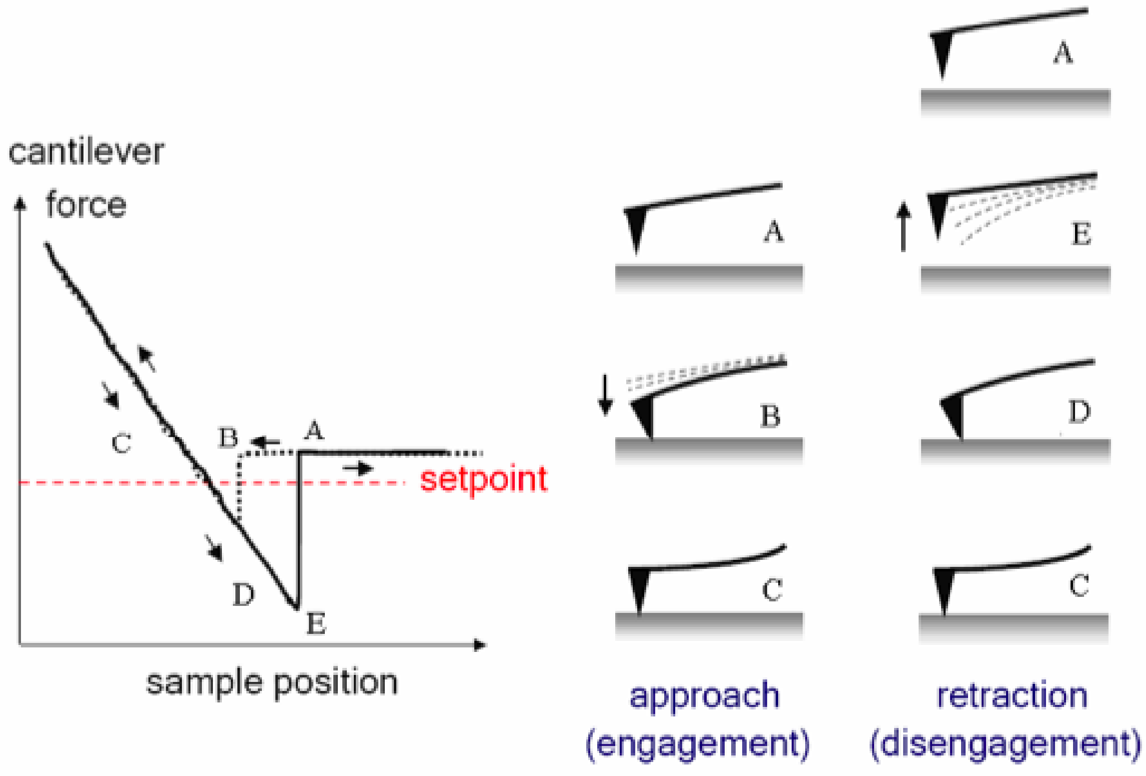
Images ofBacteriorhodopsin – purple membrane



from AG Oesterhelt’s webpage

Force Spectroscopy

Force – displacement curves

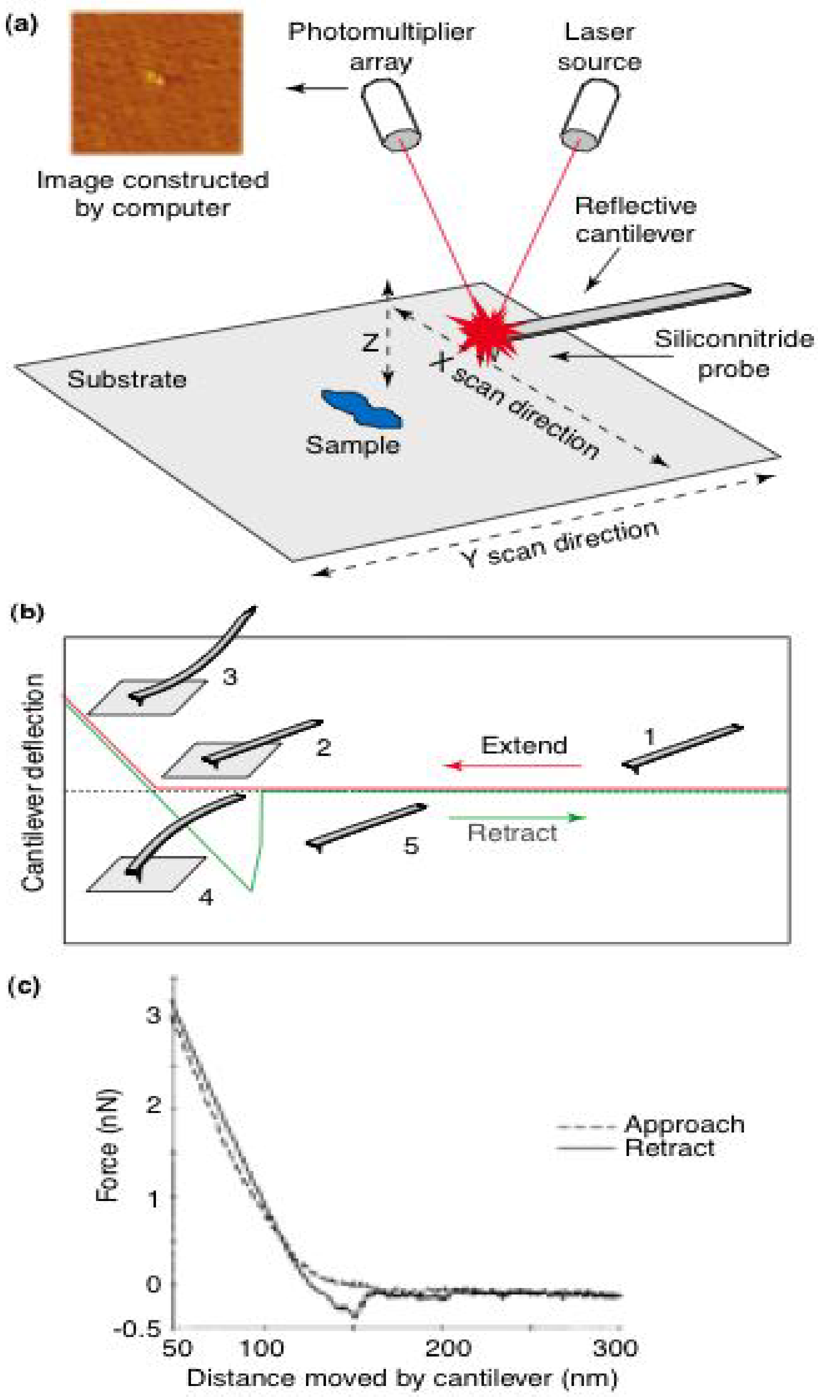


Approach

- In the approach the tip is not yet in contact with the surface
- Attractive forces maybe
- Repulsive forces definitely
  - Due to contact
  - Gives information about the elasticity or stiffness of sample

Retraction

- Attractive forces again during the retraction phase
  - Chemical and/or electrostatic
- Break of attractive forces due to retraction of the tip > characteristic “jump” in force curve



Drug Discovery Today

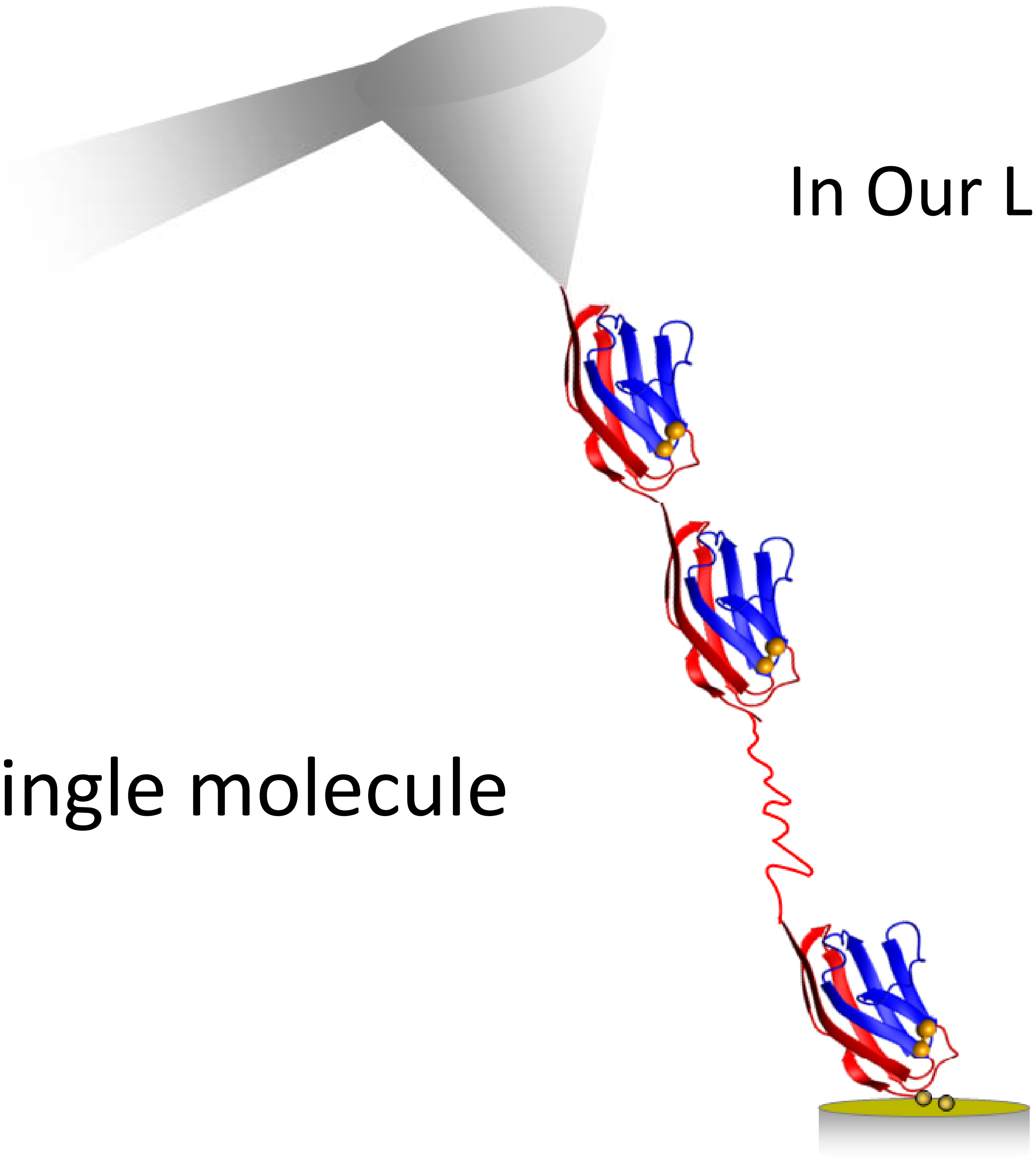
Figure 1. Operation of the atomic force microscope. **(a)** The principle of AFM. A fine pyramidal silicon nitride tip at the end of a reflective cantilever is scanned back and forth over the substrate in a raster pattern. As the tip is deflected by the sample, the cantilever also deflects, and the magnitude of the deflection is registered by the change in direction of a laser beam that is reflected off the end of the cantilever and detected by a photomultiplier array. In this way, a topological map of the surface is constructed. **(b)** The principle of the force curve. The tip is held stationary over the substrate and then oscillated up and down ('extended' and 'retracted'). At point '1' the tip is not in contact with any substrate and so no deflection is registered. At '2' the probe meets the substrate, and at '3' it is advanced further downwards onto the substrate and so the cantilever bearing the probe is deflected. This is shown in the 'y' axis of the curve. The piezo drivers then begin to withdraw the probe upwards ('retract'). Because the probe and substrate are physically attracted, they maintain contact '4', even when the probe has been withdrawn before the point where it originally made contact with the substrate. At point '5', the probe loses contact with the substrate and jumps back to its original position, as does the 'retract' curve. The measure of the force of attraction between tip and substrate is given broadly by the size and shape of the triangle lying below the dotted line in the diagram. **(c)** The measurement of the force between a biotinylated AFM tip and a streptavidin-coated substrate. The 'retract' trace shows the breakage of the attachment between streptavidin and biotin and the force measured is approximately 300 pN. Part **(c)** of this figure is adapted from Reference [5].

J. Michael Edwardson and Robert M. Henderson

DDT Vol. 9, No. 2 January 2004

Applications

- Study Unfolding Of Proteins
- Imagining Of Biomolecules &Force Measurements In Real Solvent Environments
- Antibody-Antigen Binding Studies
- Ligand-Receptor Binding Studies
- Binding Forces Of Complimentary DNA Strands
- Study Surface Frictional Forces

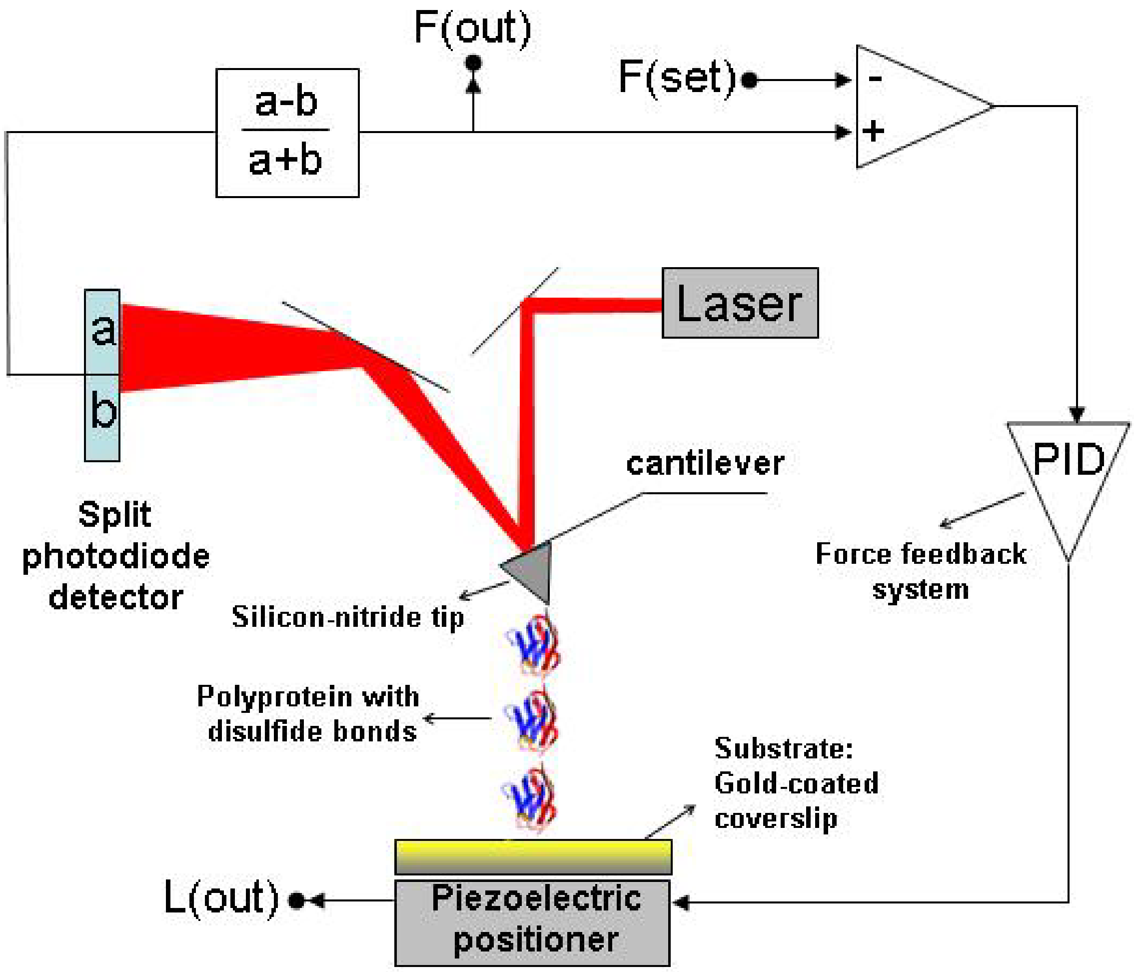


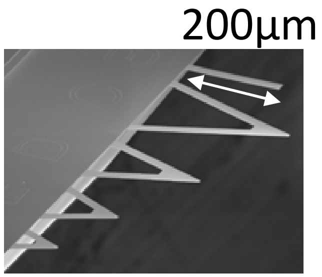
In Our Lab

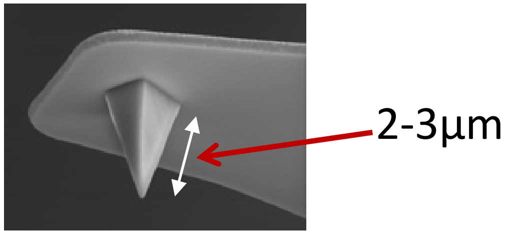
Pulling a single molecule

Technique to mechanical unfold proteins

Single-molecule stretching using AFM

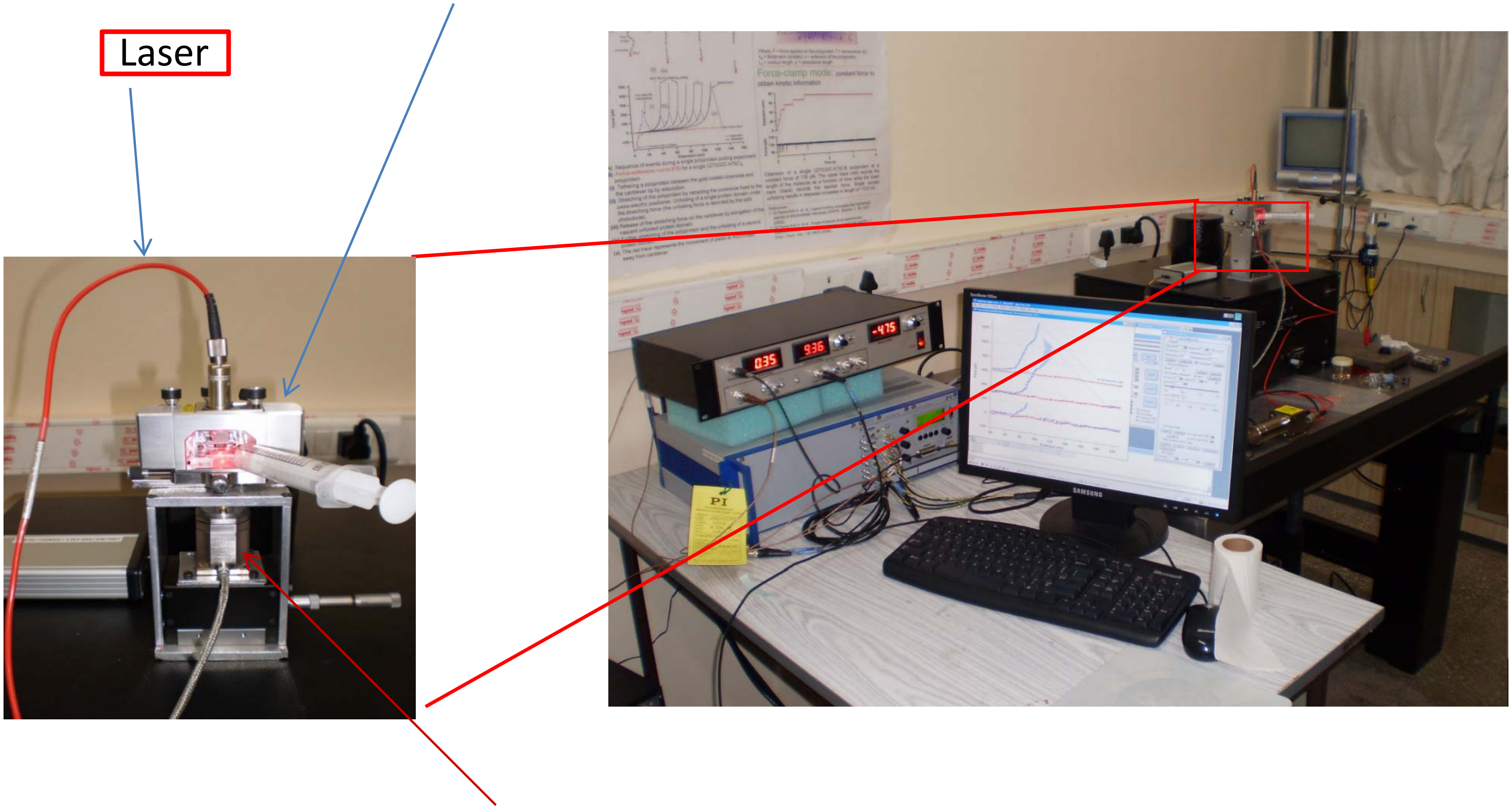






Custom-built AFM for single-molecule pulling

Room: D222



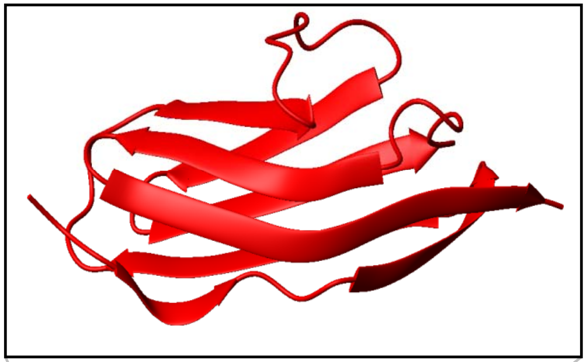
AFM head

Laser

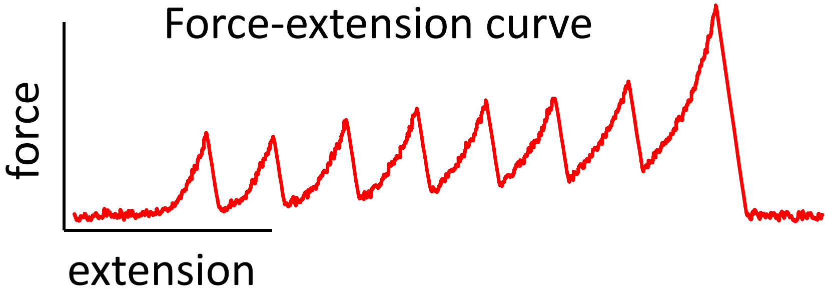
Piezo positioner

Engineering tailor-made Polyproteins: Proteins covalently linked in tandem

Protein = I27



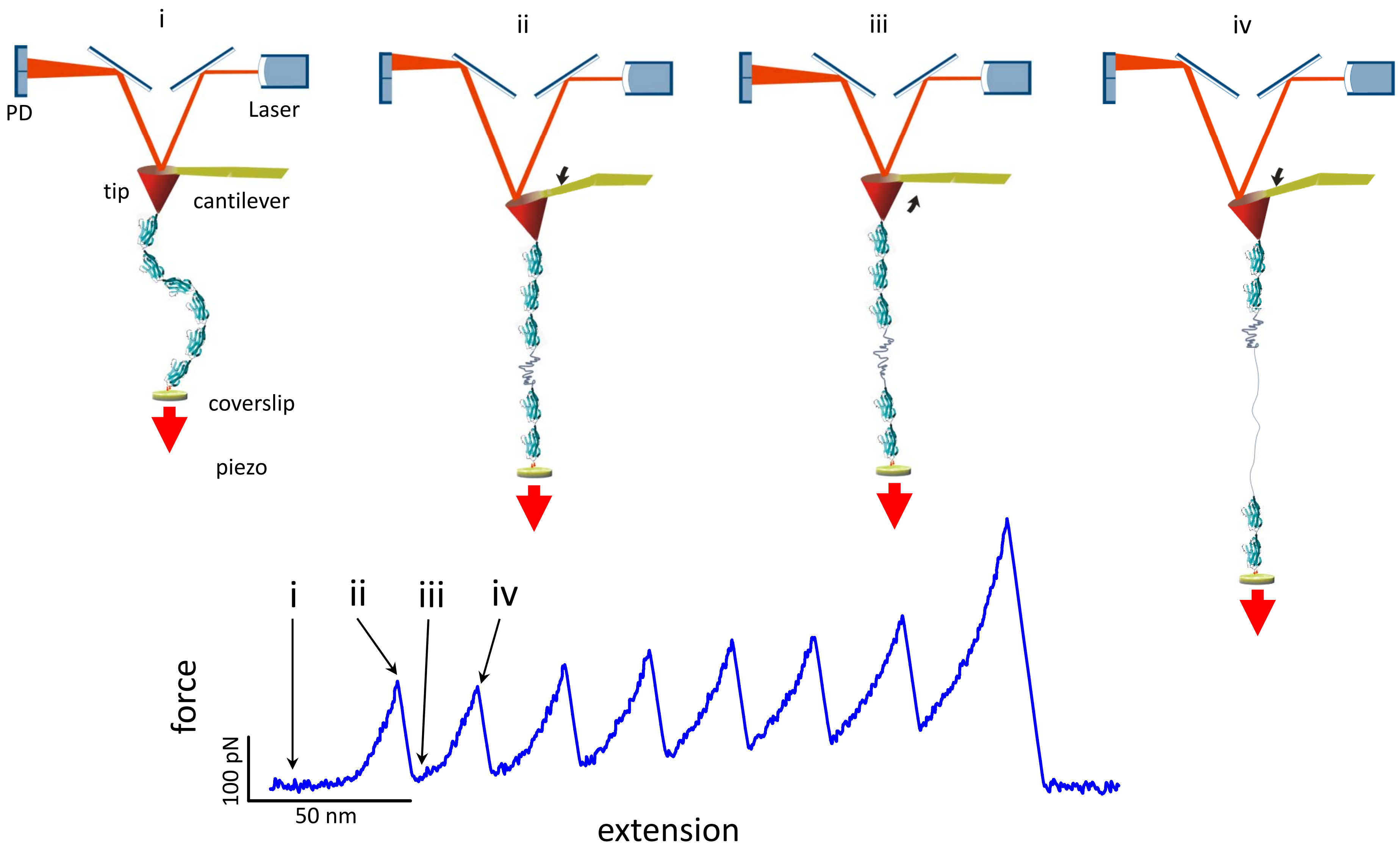
Saw-tooth pattern
Force-extension curve



Polyprotein = $(I27)_8$

Polyproteins provide unambiguous fingerprints

Sequence of events in a pulling experiment



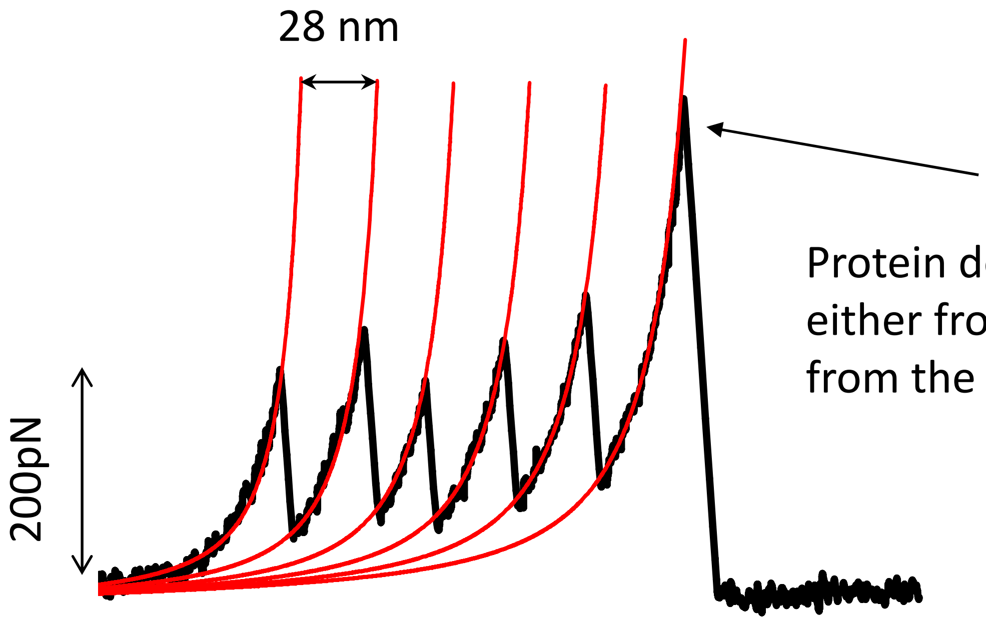
Data Analysis:

extraction of physical quantities
(unfolding force, contour length etc.)

Polymer elasticity:
Worm-like chain (WLC) model

$$F(x) = \frac{kT}{p}\left[\frac{1}{4}\left(1-\frac{x}{L_c}\right)^{-2} - \frac{1}{4} + \frac{x}{L_c}\right]$$

F = force
x = extension
p = persistence length
L_c= contour length



Protein detachment either from the tip or from the coverslip.

Thank You