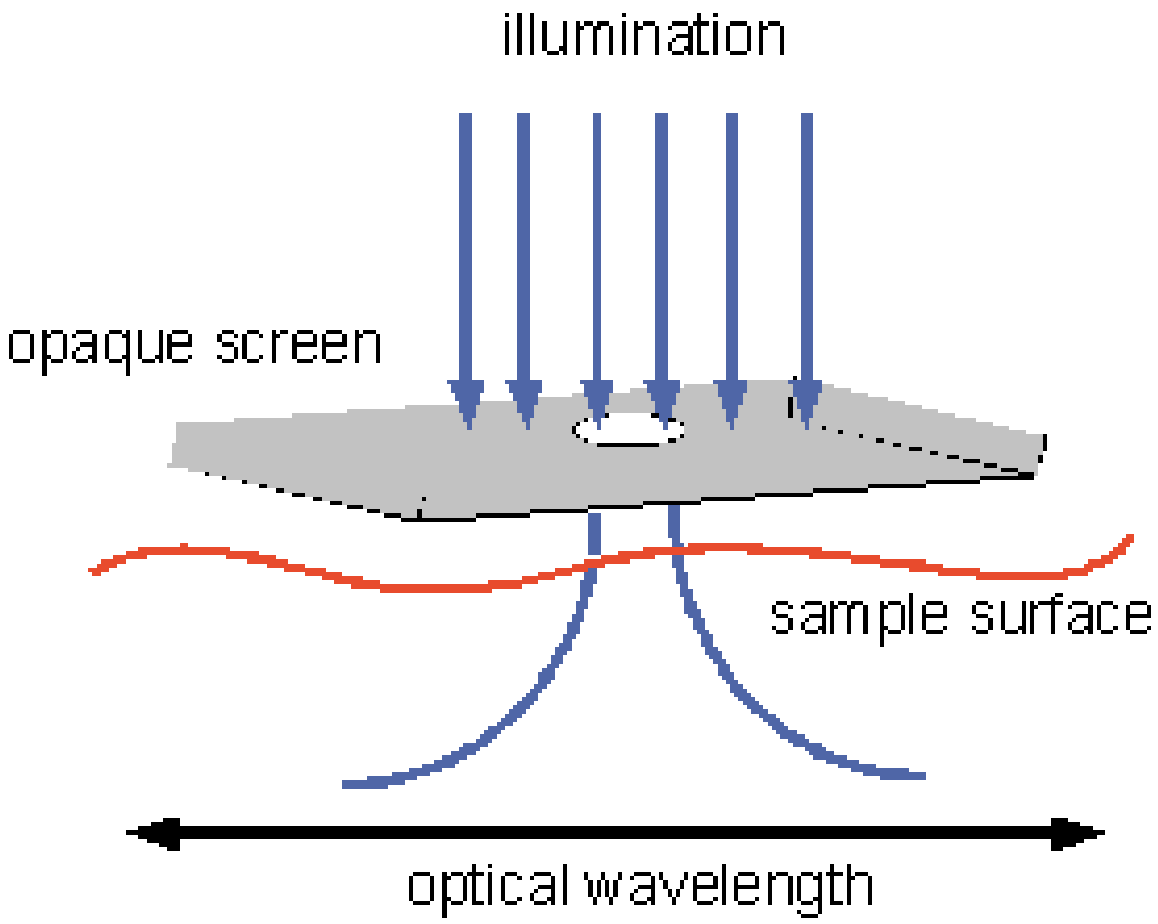
Atomic Force Microscopy

Andrew Rouff and Kyle Chau

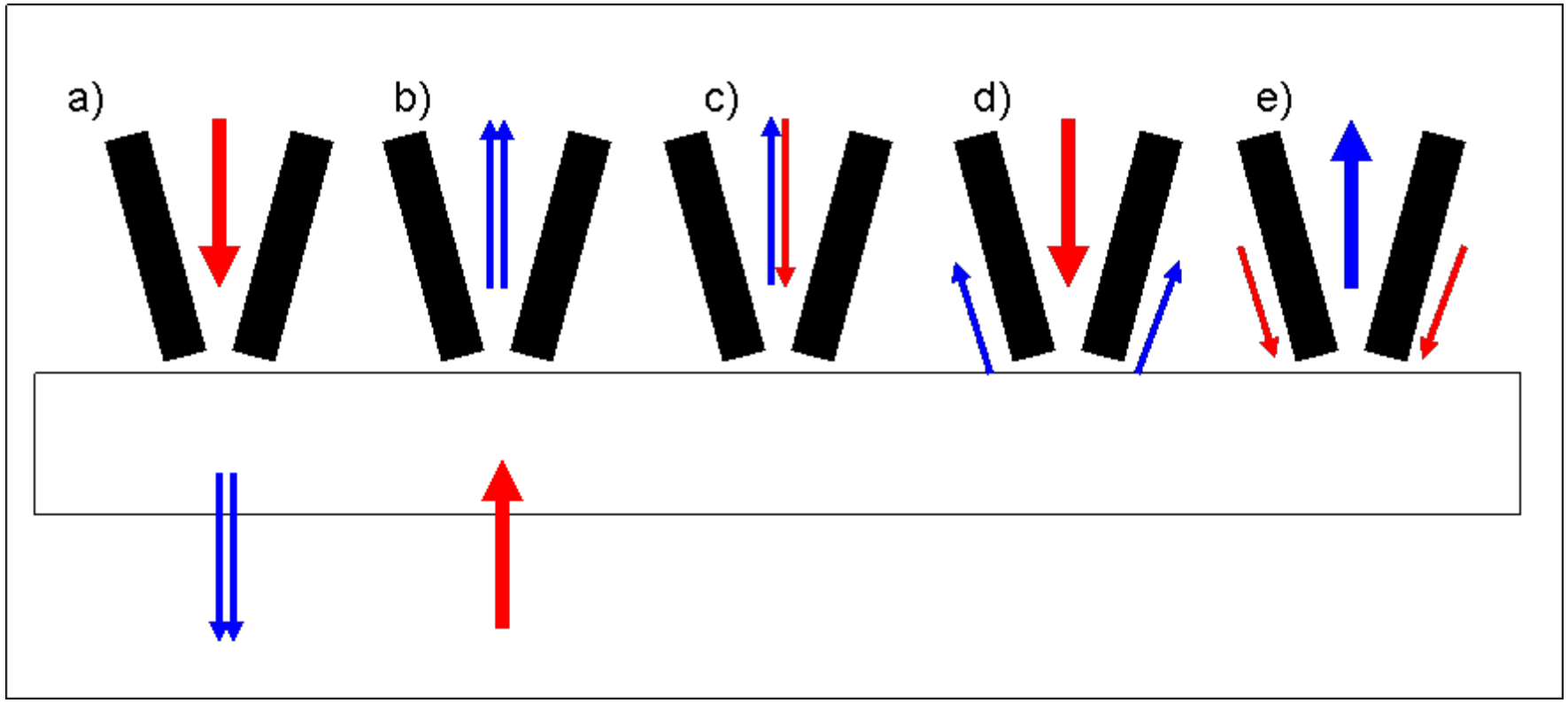
Light Microscopy, Near-Field Scanning Microscopy



NSOM

- Incident radiation is illuminated through aperture
- Sample is closer to opaque screen than one wavelength of light
- Opaque screen must be thick enough to stop all light from being detected

Ways of Detecting Light



a) = illumination
b) = collection
c) = illumination collection
d) = reflection
e) = reflection collection

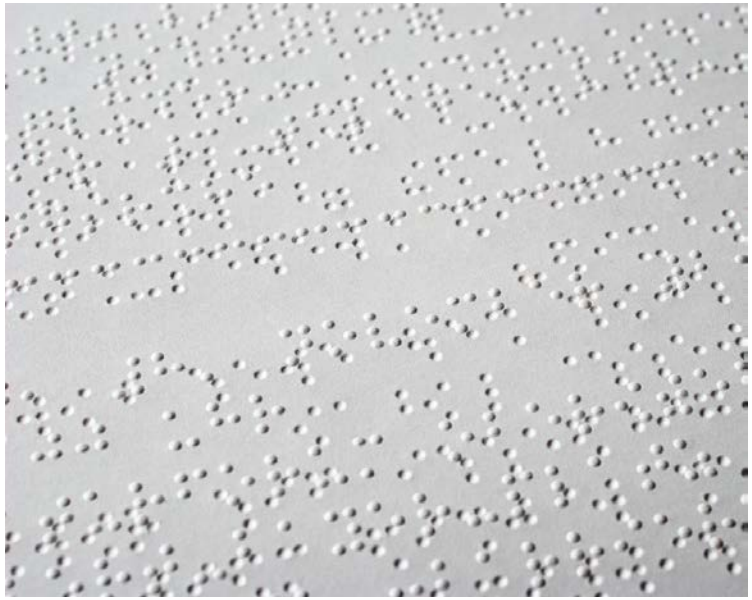
Atomic Mass Microscopy, History

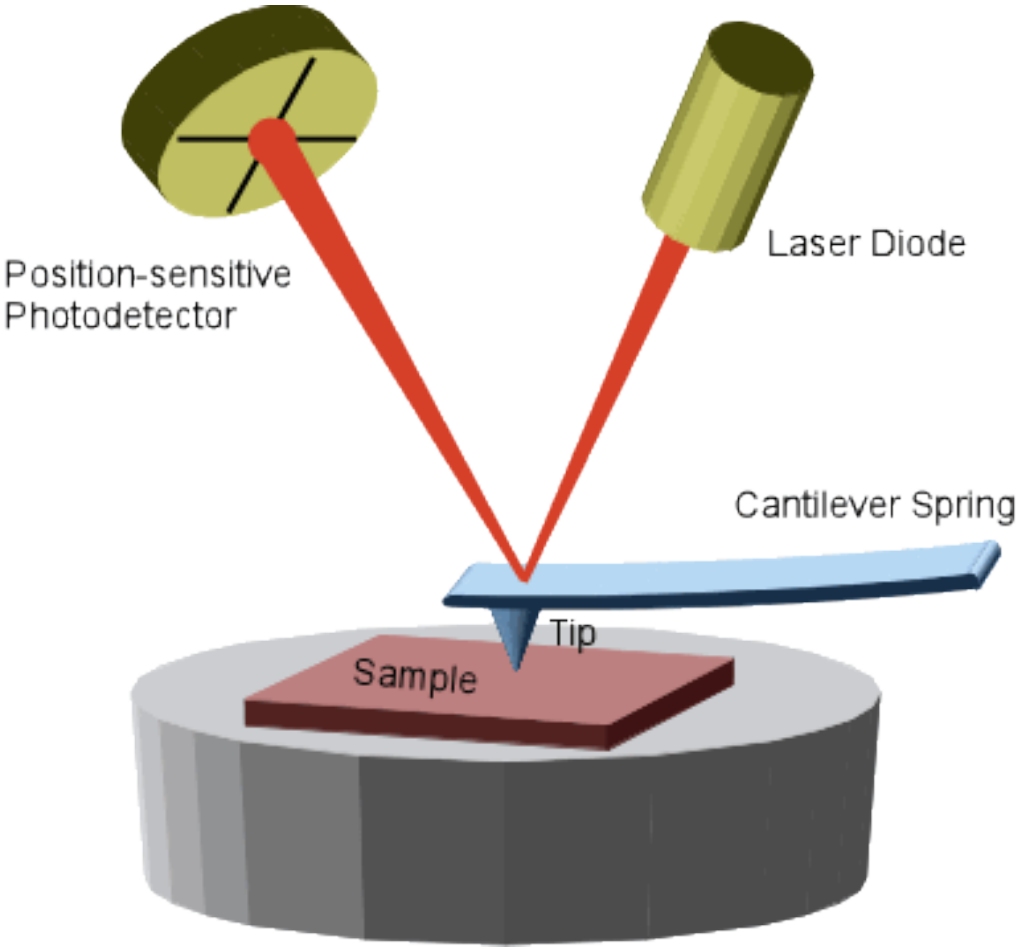
- Early 1980's- Binning and Rohrer propose scanning tunneling microscope (STM), win Nobel Prize. First attempt at imaging biological molecules with STM

- 1986- Binning, Quate, and Gerber invent scanning force microscope (SFM)

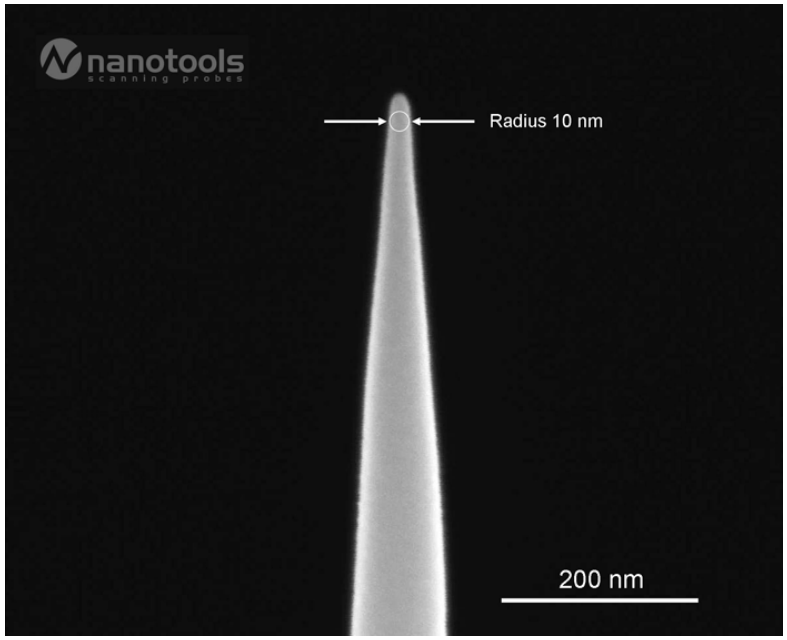
- Early 1990's- Keller, Shong, and Putman invent tapping mode AFM

General Principles of Atomic Force Microscopy





The Tip



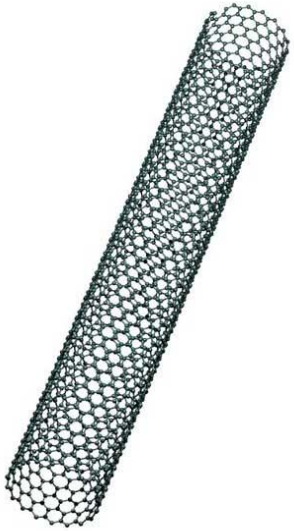
Φ= Angle at bottom of picture

R_c= radius shown

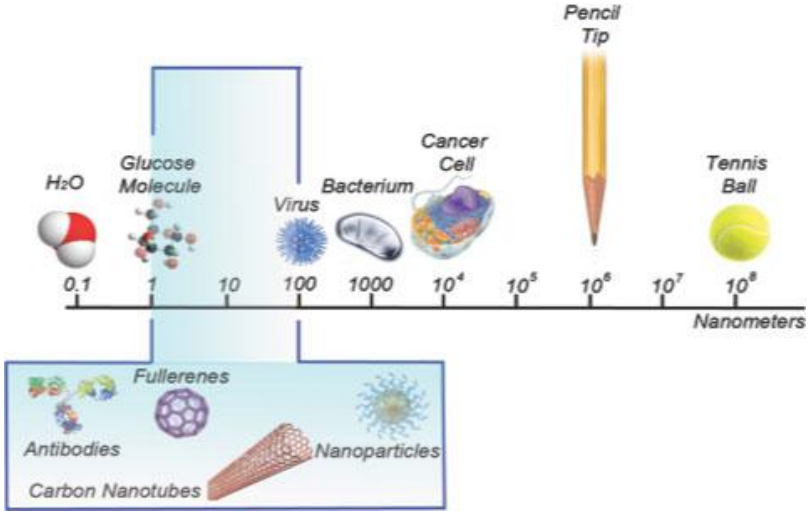
Ideal tip has Φ and R_c as small as possible

Material of Tip

Carbon Nanotubes are best material



Are made of SP^2 Carbon Molecules, which makes them flexible enough to avoid damage, but slender enough to get into narrow areas



Spring Constant of Atoms vs. Cantilever

If Cantilever has higher spring constant than atoms in a molecule, the force would break the molecule apart. eg. a chisel cracking ice

The smaller the interaction between the sample and the tip of the cantilever, the better

$K_{atom} = \omega^2 m$

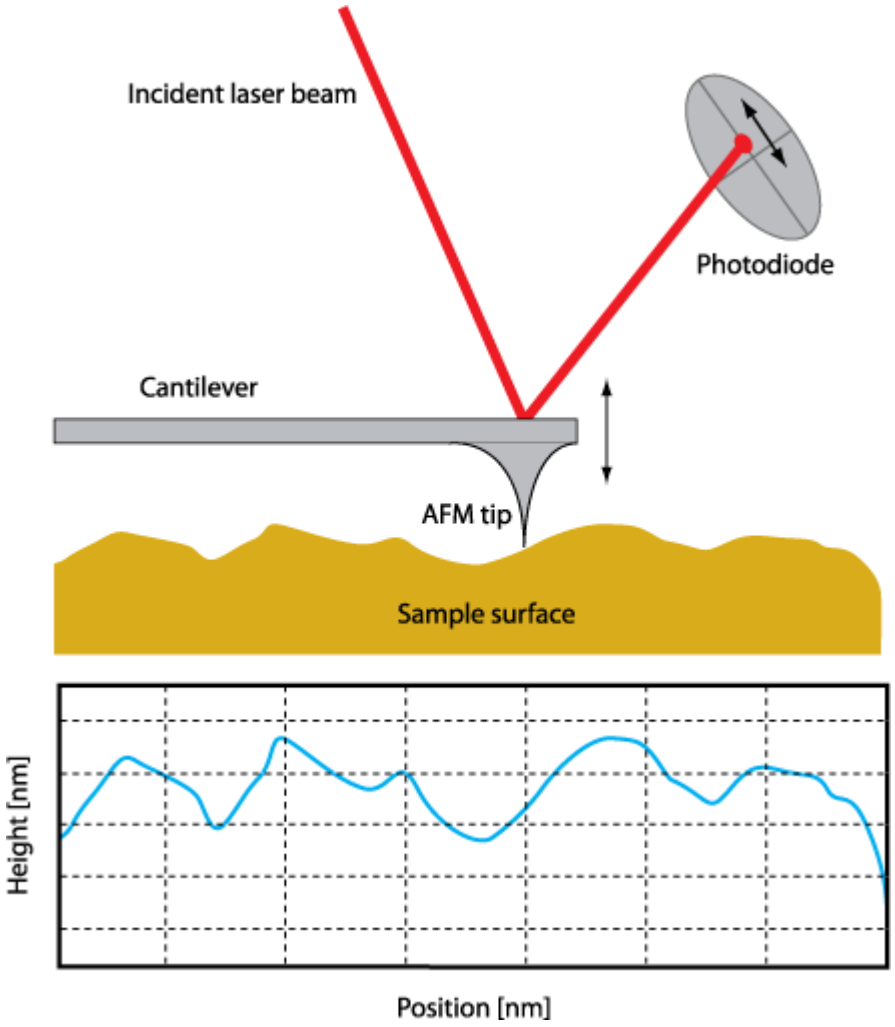
ω in atom generally 10^{13}Hz

$K_{atom} = (10^{13}\text{Hz})^2(10^{-25}\text{kg})$

$K_{atom} = 10\text{Nm}^{-1}$ roughly

Spring constant of aluminum foil 4mm long and 1mm wide is 1Nm^{-1}

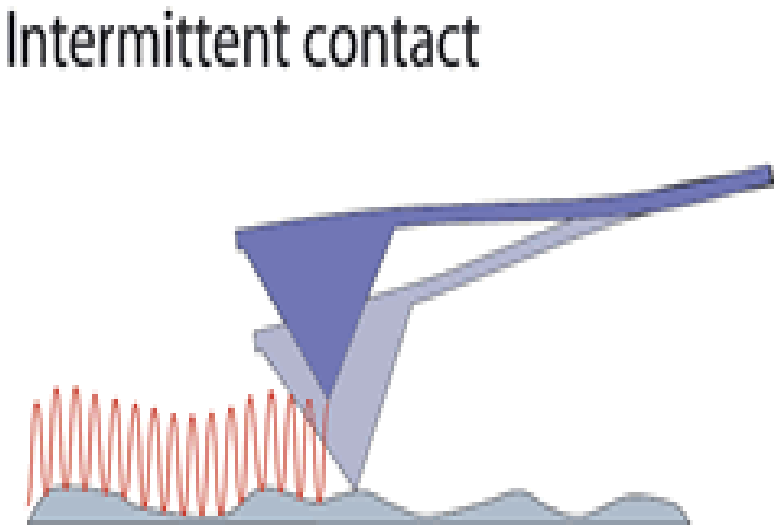
Contact (constant force) mode



Tip is always in contact with sample. When incident laser beam angle changes, cantilever moves to make it go back.

Cantilever movement is measured, sample can be imaged from known cantilever height changes

Oscillation (tapping) Mode

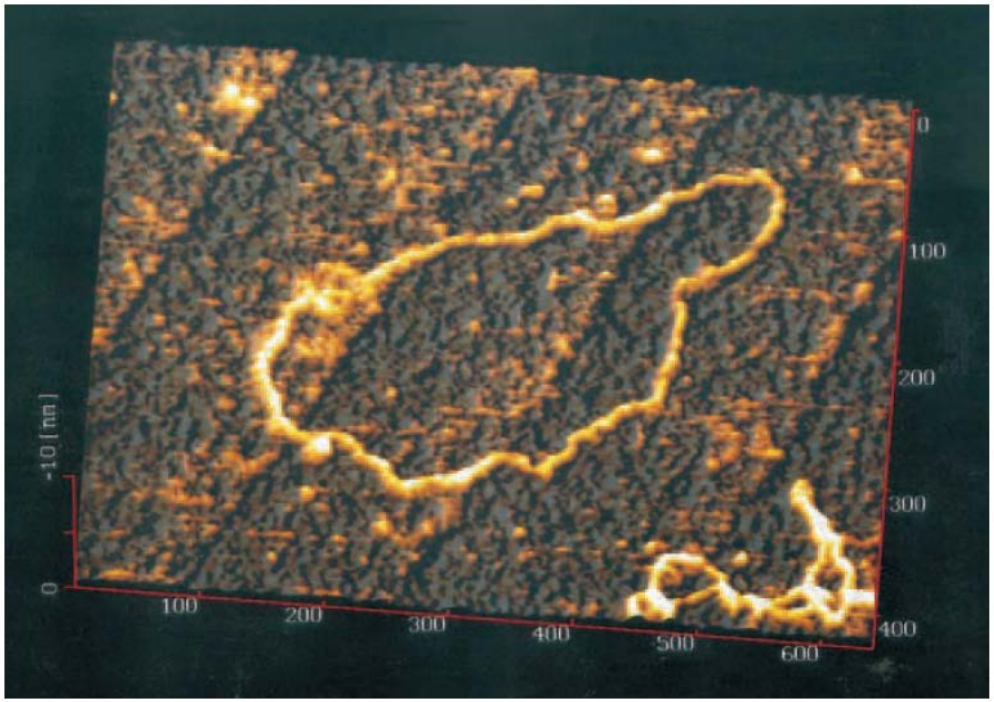


Tip “vibrates” up and down so it is only in contact with sample for short periods of time

Height of Cantilever is measured just like in contact force mode, but distance it moves down is measured instead of distance moved up

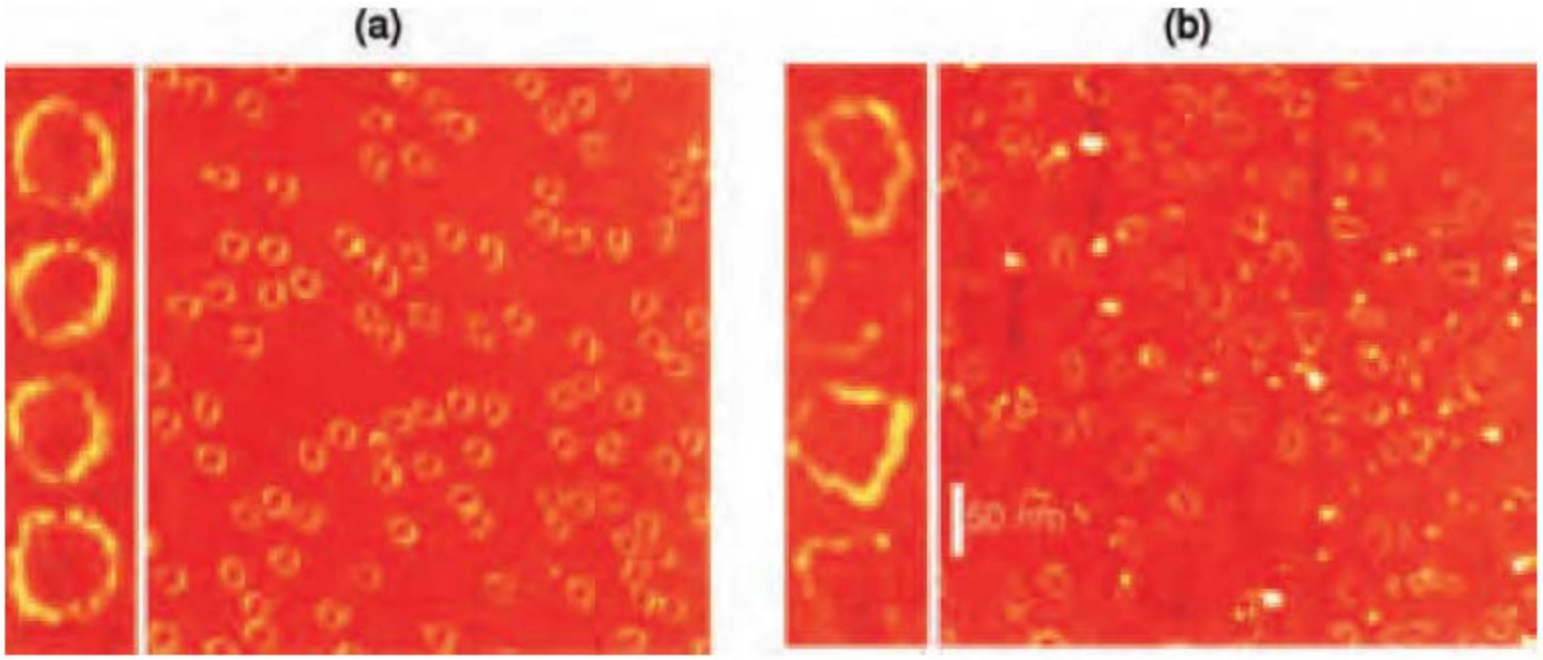
Imaging by AFM

DNA in aqueous media



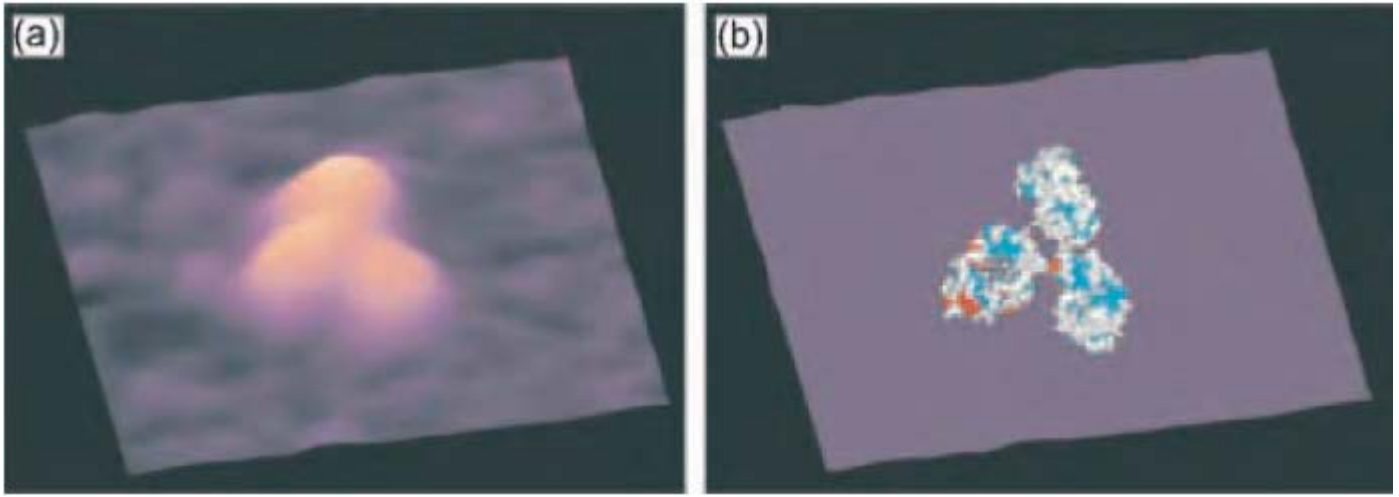
-
- Requires the binding of the sample to the surface be stronger than the contact interaction between the top and the sample.
    - Increasing the attachment of the sample to the substrate
    - Reduce the tip-sample force

Resolution of AFM



- AFM in situ environment
- Structural changes in different solutions
- Visualisation of kinked DNA in situ
  - DNA in 1mM MgCl2
  - DNA in 1mM ZnBr2

Protein

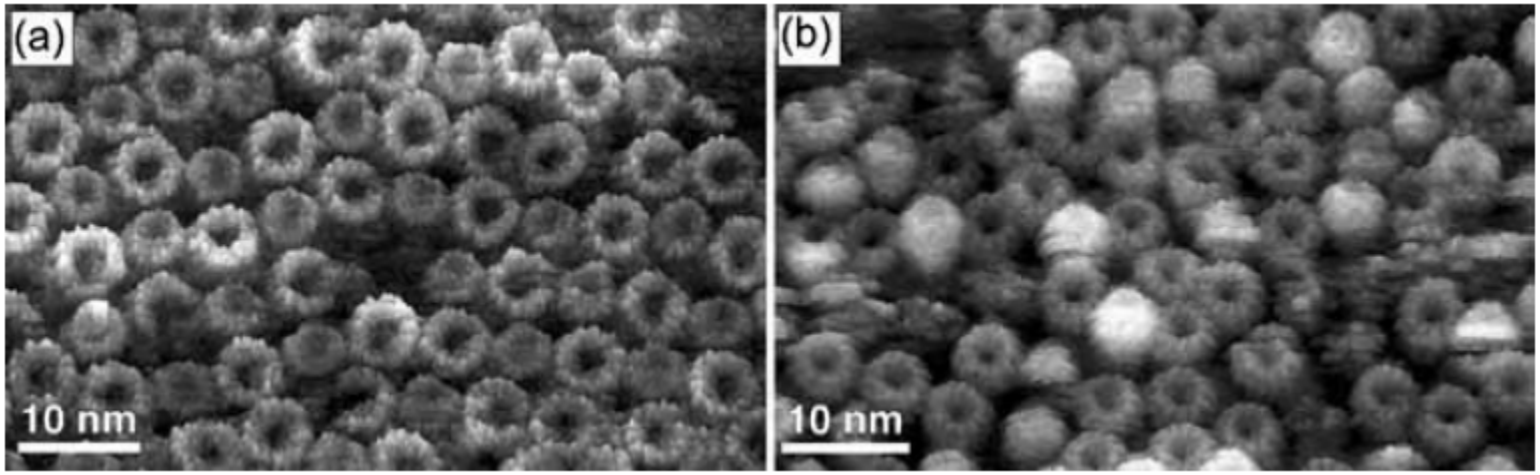


- Used a nanotube tip
- Immunoglobulin G
  - Y-shaped structure
- Compared with the crystal structure

Macromolecules in 2-Dimension

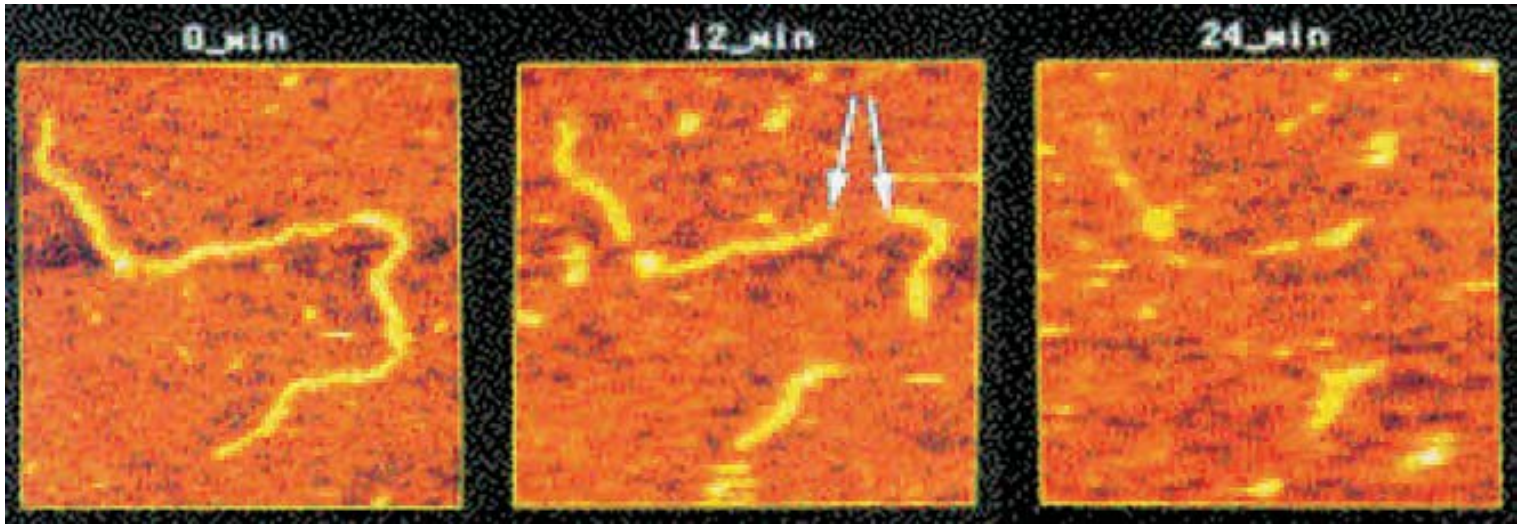
Ilyobacter Tartaricus

Spinach Chloroplast



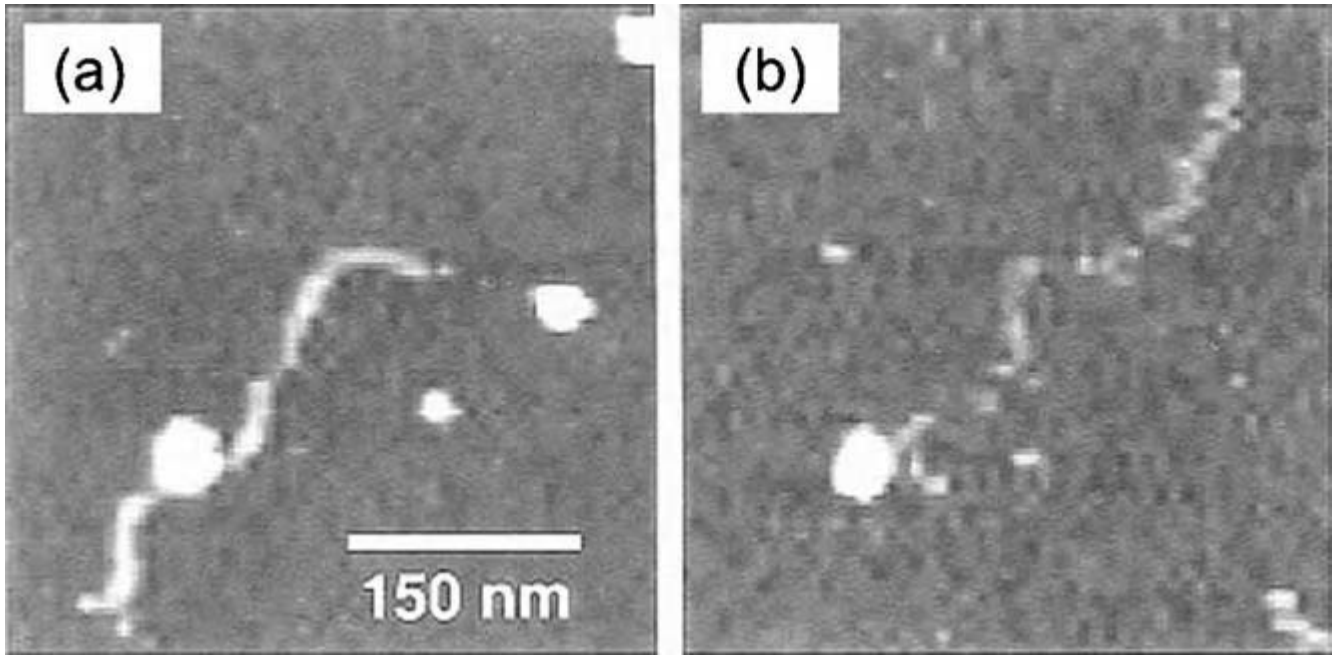
- F0F1-ATP rotor comprises of 12 subunits.
- Ilyobacter Tartaricus rotor consists of 11 subunits.
- Spinach Chloroplast rotor consists of 14 subunits.
- Subunit determine the rotor diameter and thereby constrain how many subunits would fit into the rotor.

Dynamics of Biological System



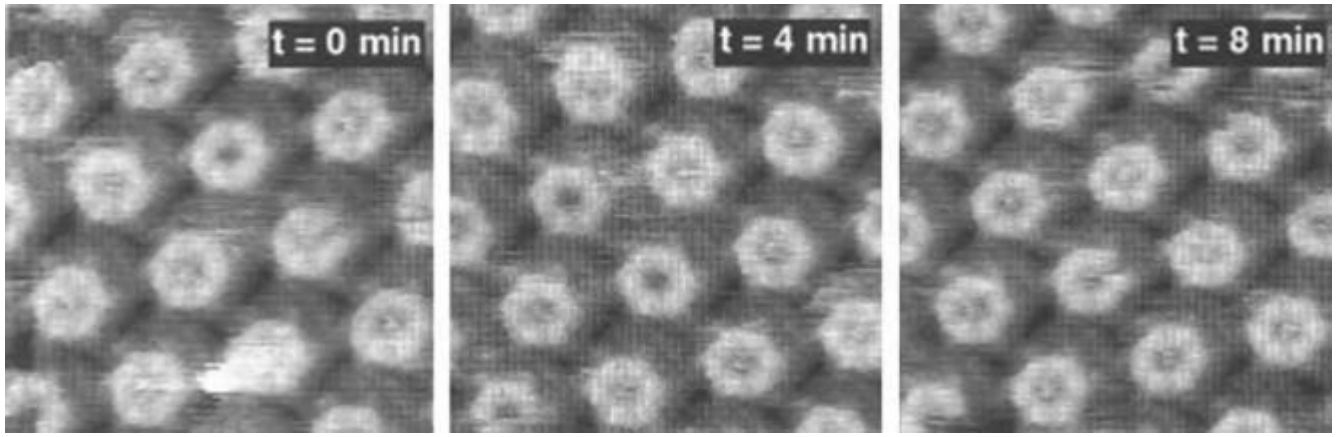
- Use the AFM to follow biological processes at the molecular level.
- Time-lapse sequence of the digestion of a DNA fragment by BAL 31 nuclease.
- As the DNA is digested, it disappears from the image,leaving an increasingly large gap in the molecule.

Biological Processes



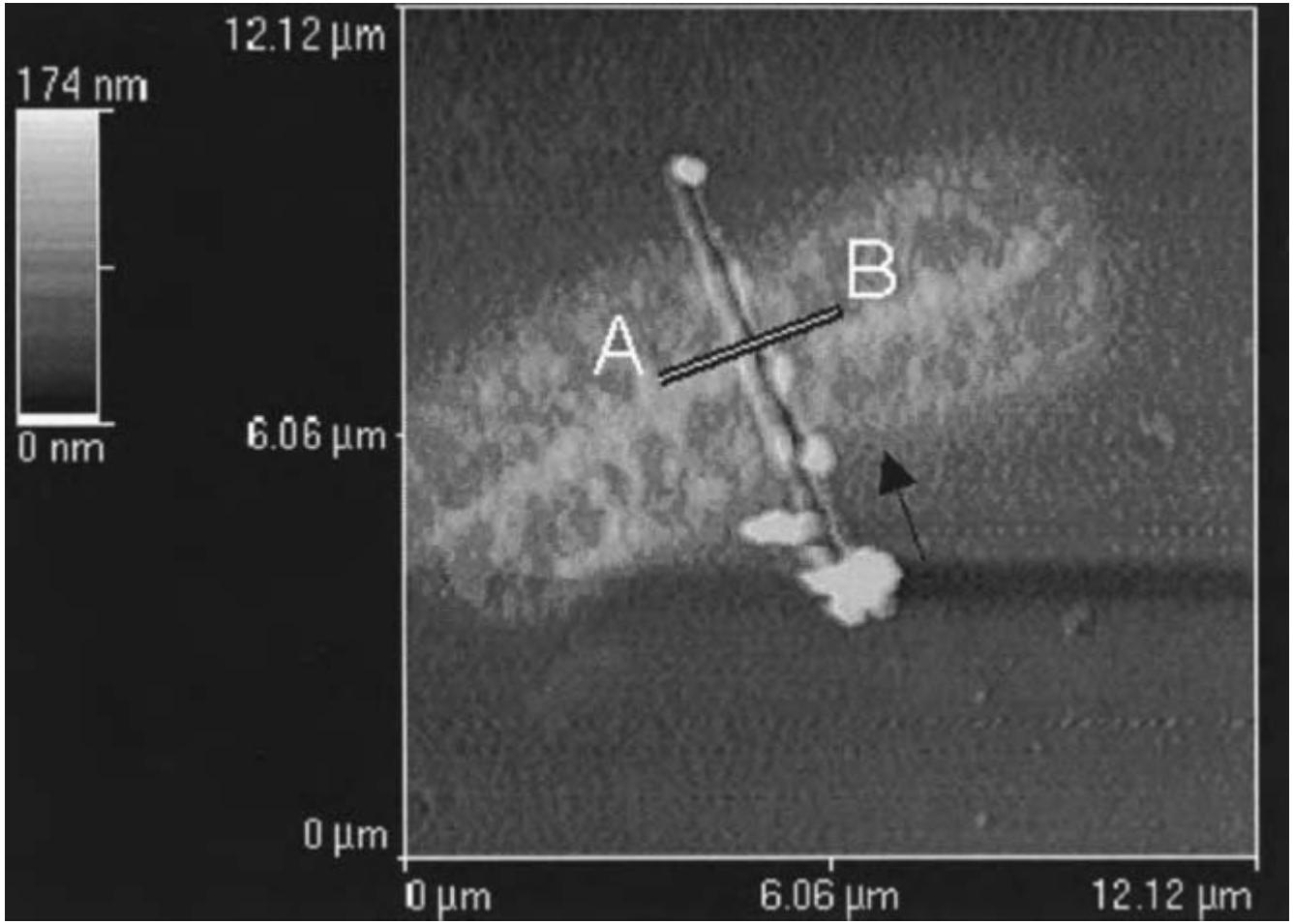
- DNA transcription can be observed over time using AFM.
- Adhesion of DNA to mica is adjusted through adjusting the Zn2+ concentration in the reaction chamber.
- DNA appears to have been pulled through RNAp.
  - Approximately 0.5 - 2 bases per second

Conformational Changes in Protein

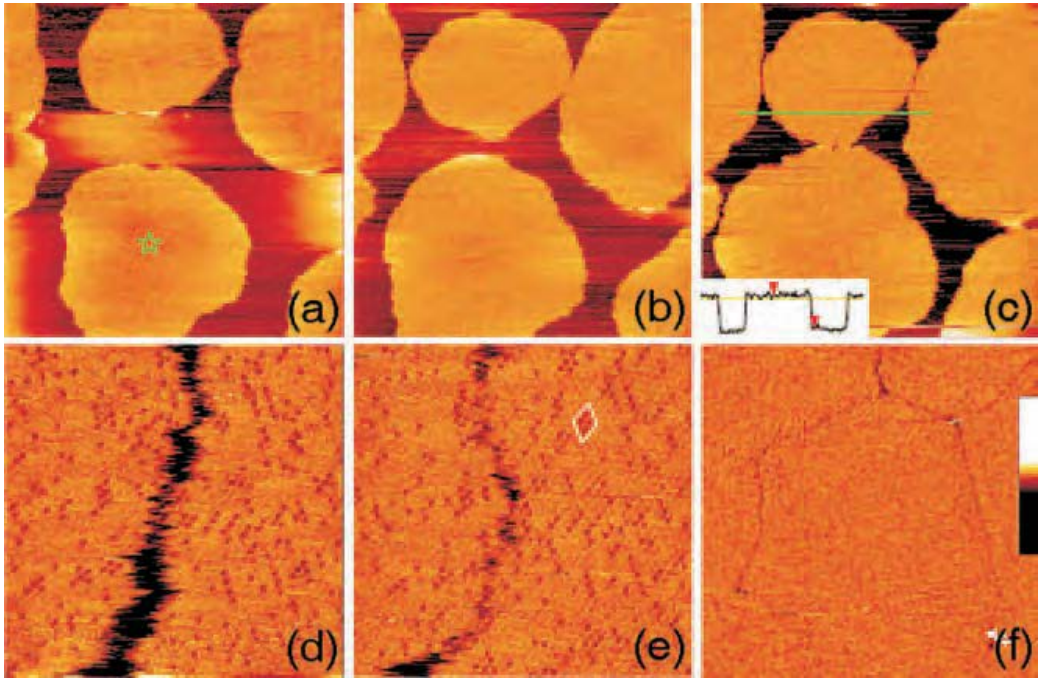


- A sequence of a scans over one area of the intermediate layer of Dienococus radiodurance.
- Same surface at 4-min intervals.
- Some pores changed their conformation from unplugged to plugged.

AFM Probe as Nanoscalpel



Crystal Growth



- Macromolecular crystals in situ.
- Contain three-dimensional information.
- AFM enables us to observe the process of two-dimensional crystal growths in real time.
- (a) 19 min; (b) 23 min; (c) 26 min; (d) 29 min (e) 31 min; (f) 34 min.

Combination of NSOM and AFM