臺北醫學大學

Biomedical Imaging

生物醫學影像學

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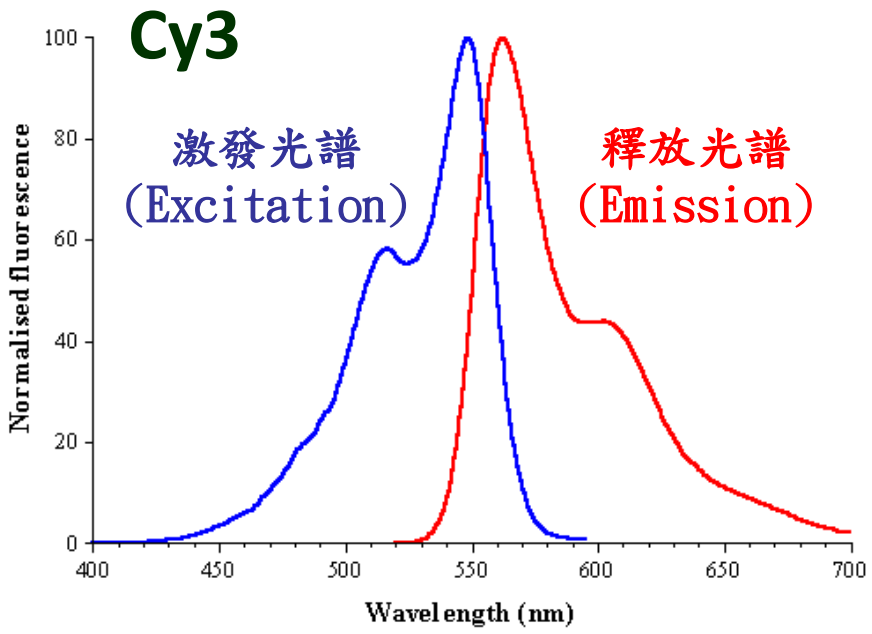
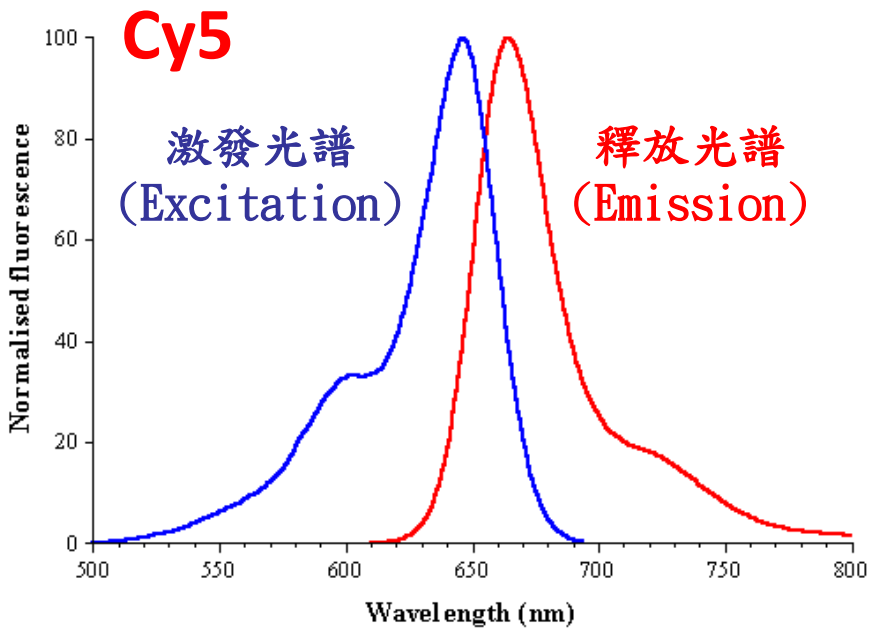
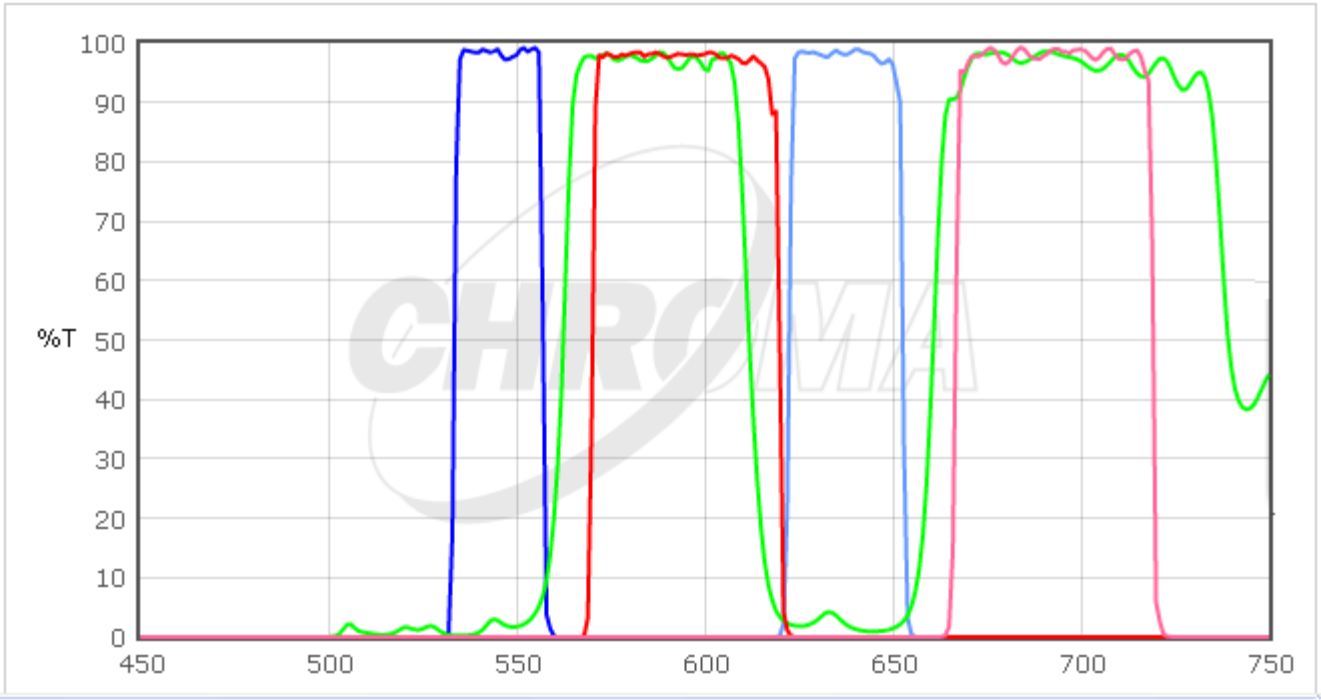
Course Outline

1. Course Introduction
2. Basic Optics and Light Microscopes
3. Fluorescence/Confocal/TIRF Microscopes
4. FRET Techniques and Photo-Spectroscopic Imaging
5. Single Molecule Detection
6. Cell Imaging
7. **Atomic Force Microscopy (AFM)**
8. Scanning Electron Microscope (SEM)
9. Transmission Electron Microscopy (TEM)
10. Digital Image Processing Using MATLAB







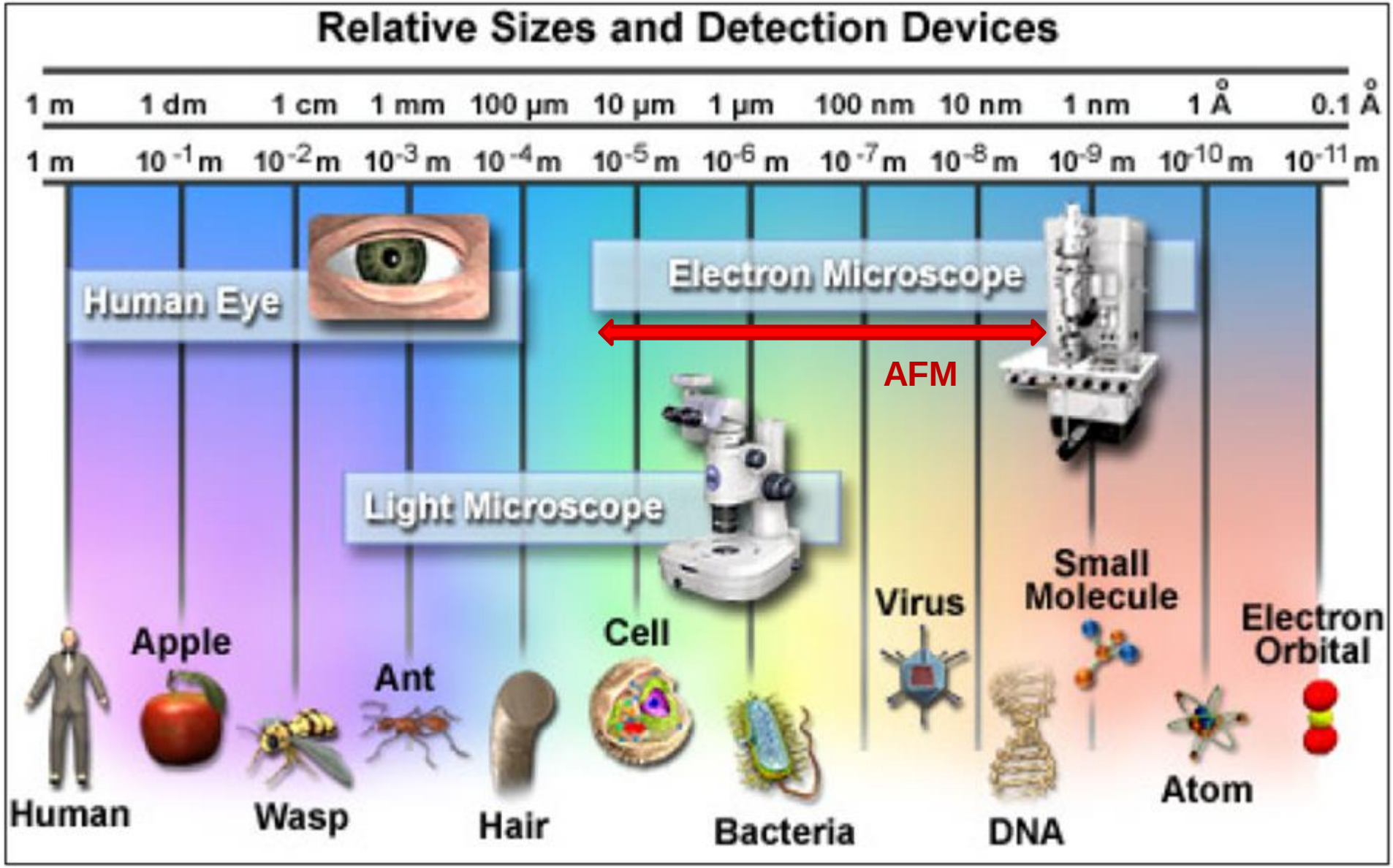
http://www.chroma.com/product/complete-filter-sets/widefield-microscopy/dual--89022--ET-Cy3-Cy5

Part I

Atomic Force Microscopy (AFM)

Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Diversity of Optical Bio-Imaging:
principles, technologies, information, applications

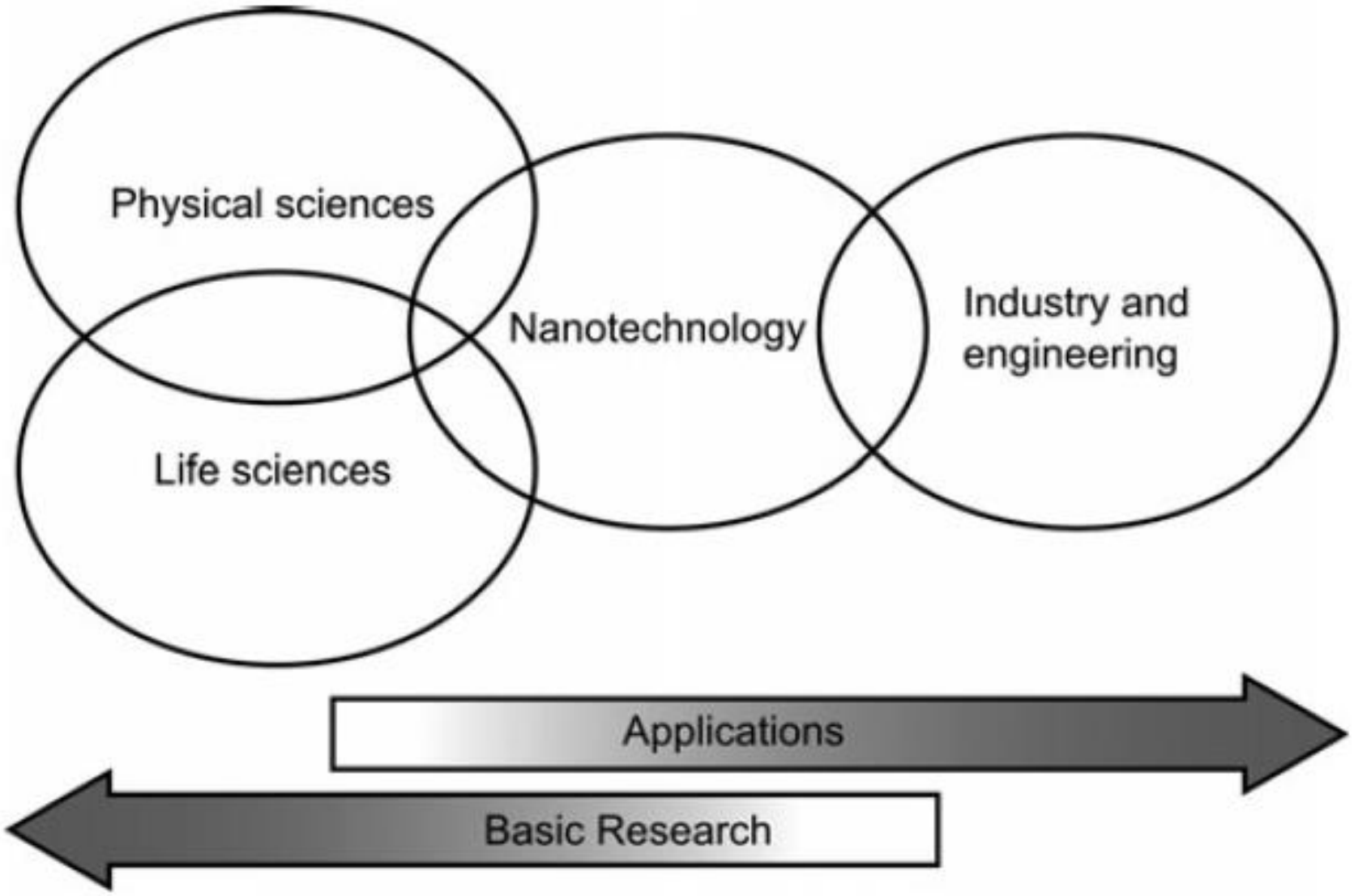


Characteristics of Measuring Techniques for Surface Morphology

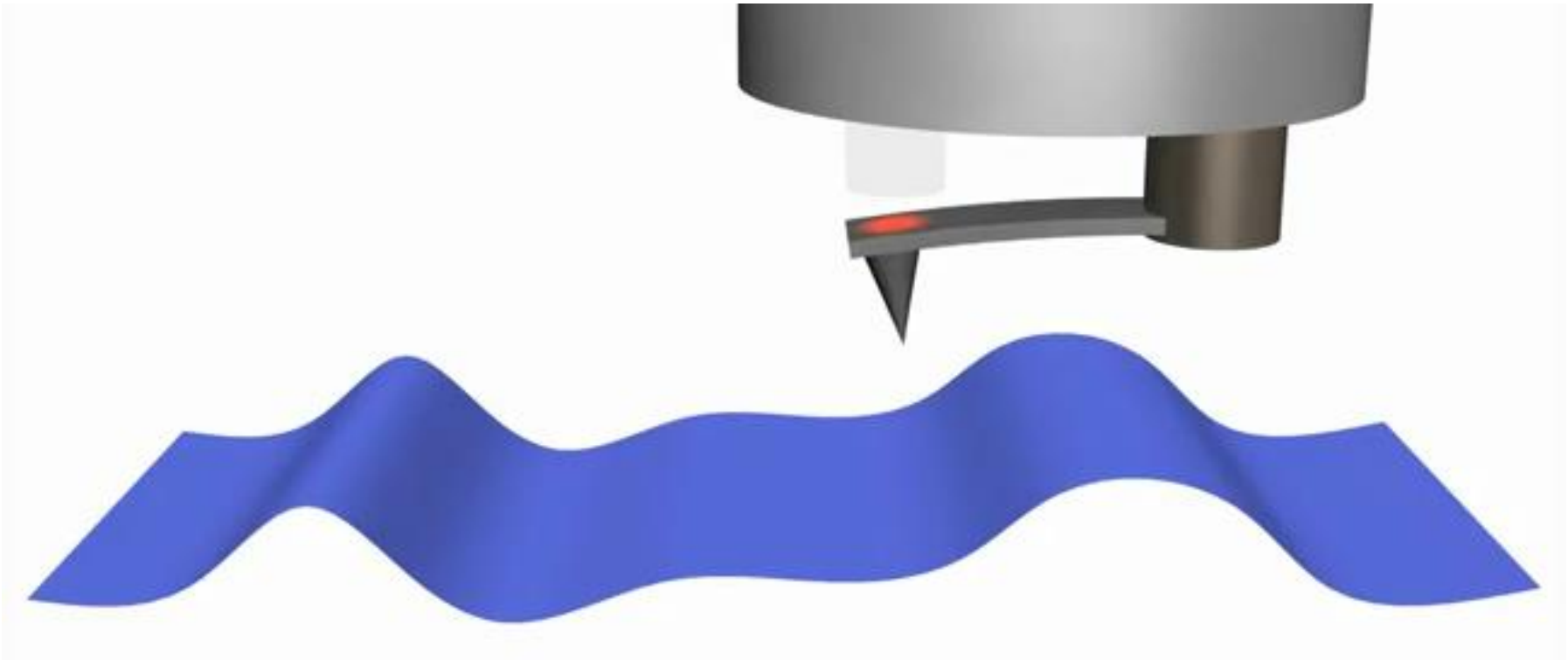
	Resolution X,Y	Resolution Z	Circumstance	Pretreatment required	Magnific-ation
Optical Microscope	0.2 μm	1 μm	air, liquid	none	10^3
Confocal Microscope	170 nm	500 nm	air, liquid	none	10^4
AFM	**0.1 nm**	**0.1 nm**	air, liquid, vacuum	**none**	**10^9**

- **AFM have high resolution and simply sample preparation.**

Application Areas Where Atomic Force Microscopy (AFM) is Most Commonly Used.

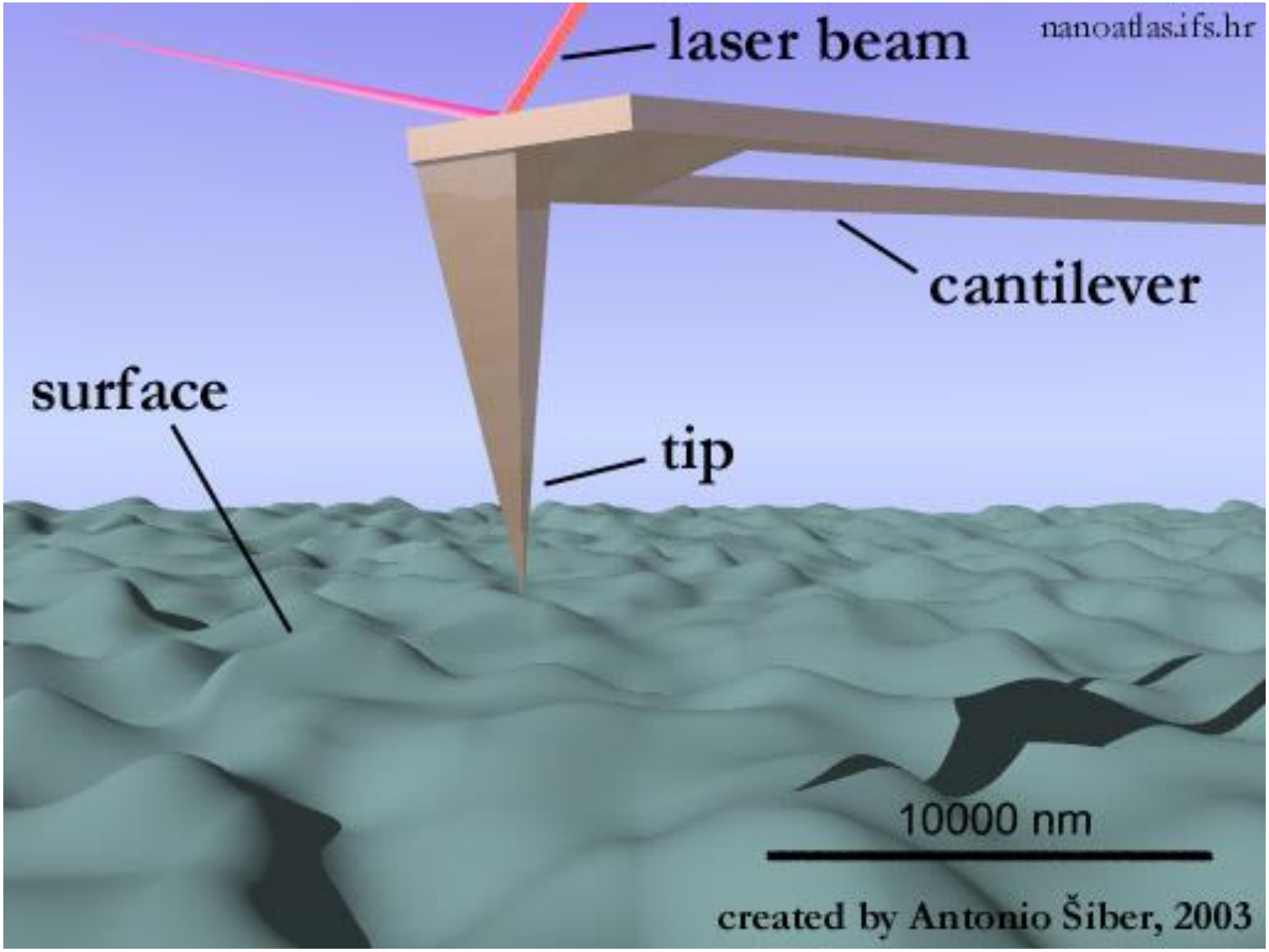


Atomic Force Microscopy (AFM)

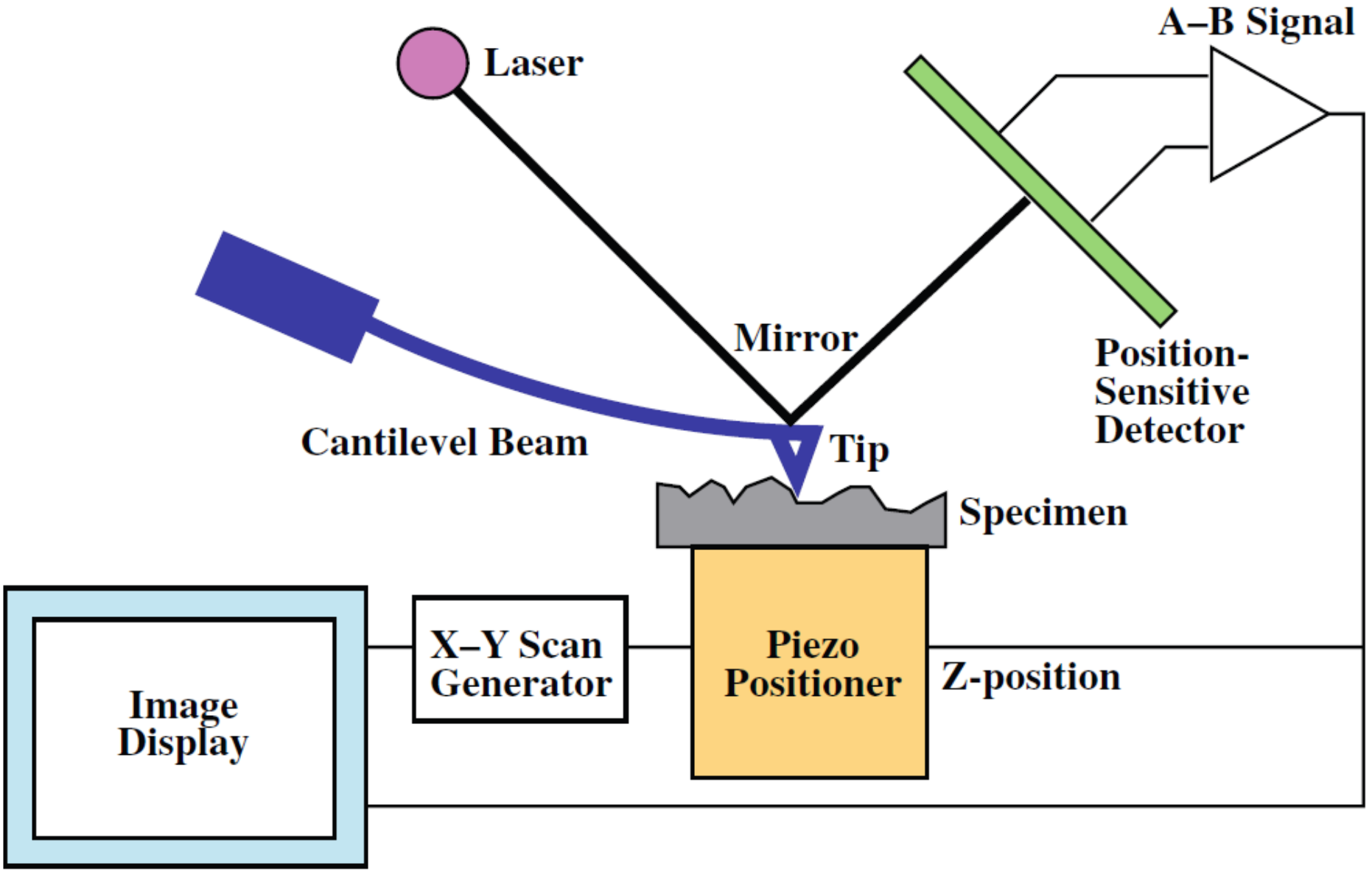


https://www.youtube.com/watch?v=Nfu4BPmbFoI

Atomic Force Microscopy (AFM)

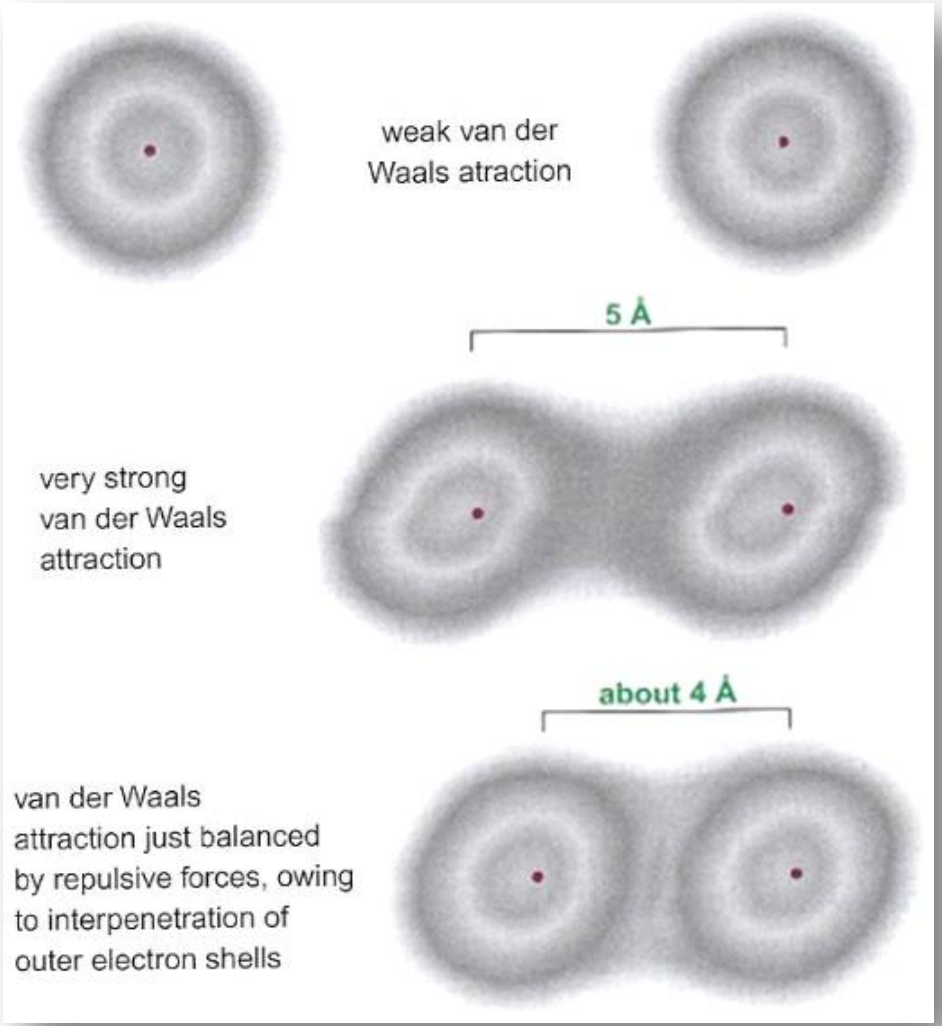


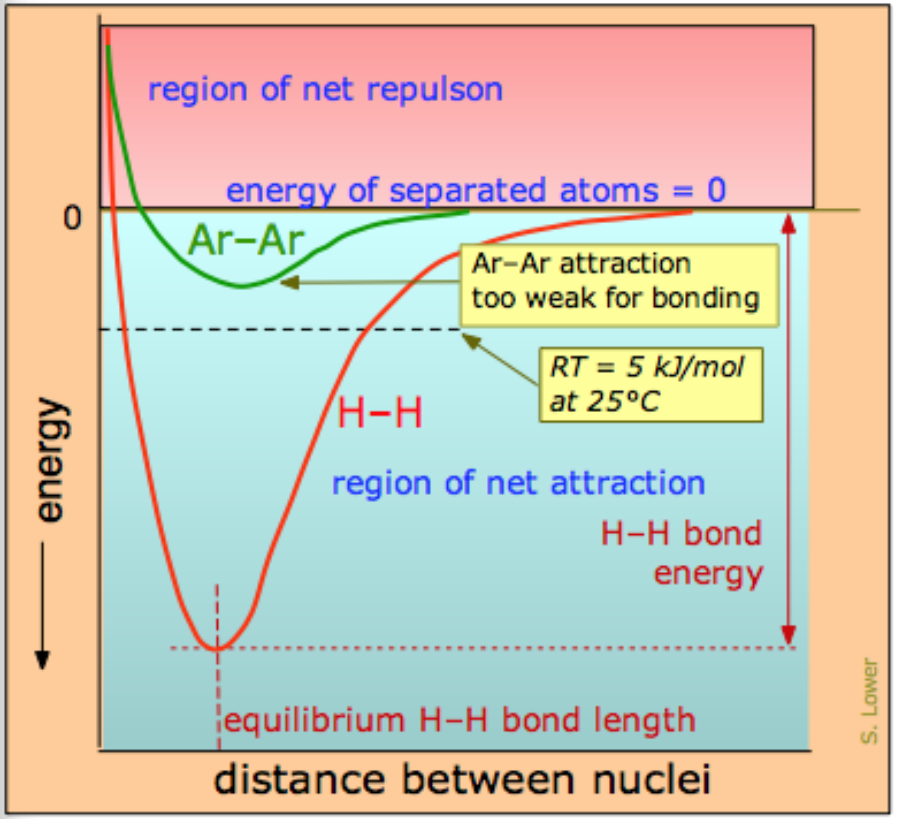
Schematic Diagram of an Atomic Force Microscope



Variation of van der Waals Forces with Distance

Gas argon





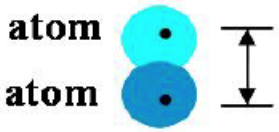
http://nipunaswchemistrypage.blogspot.tw/2013/02/liquids-and-solids-differ-from-gases-in.html

Atomic Force Microscopy to Visualize Nanosurfaces

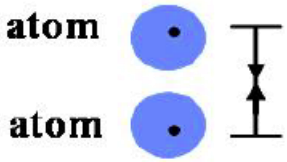
van der Waals' forces

$$E^{pair}(r) = 4\varepsilon[(\frac{\sigma}{r})^{12} - (\frac{\sigma}{r})^{6}]$$

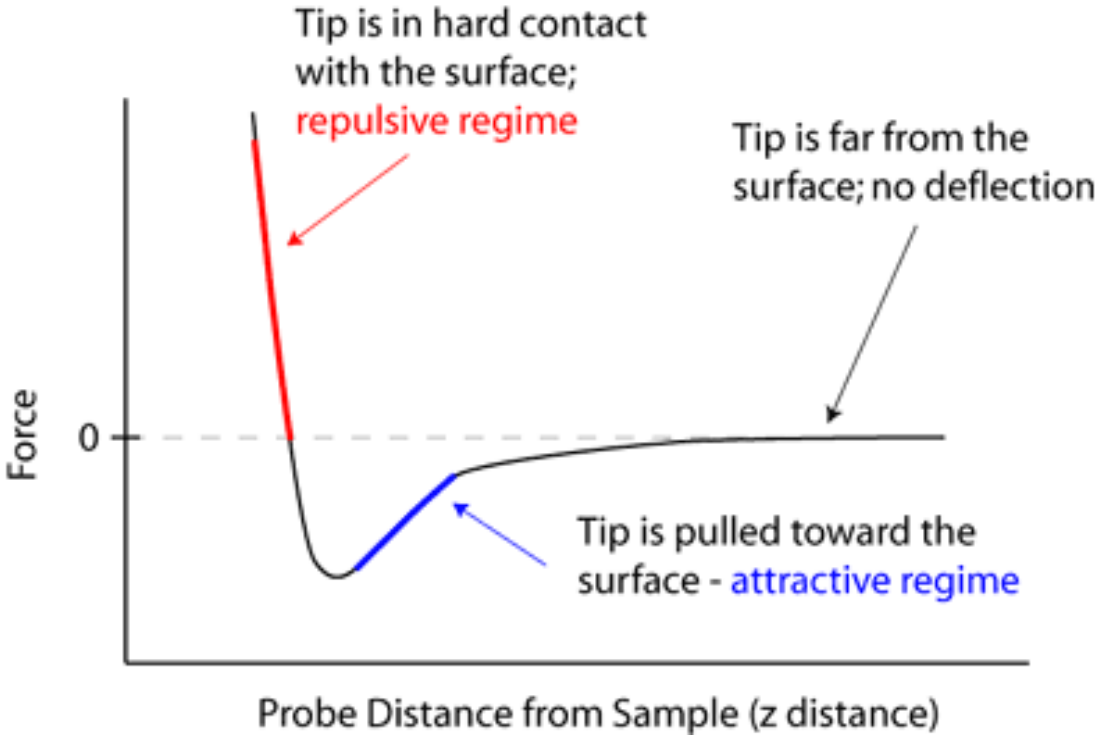
σ:原子半徑, r:兩原子間距, ε:介電常數

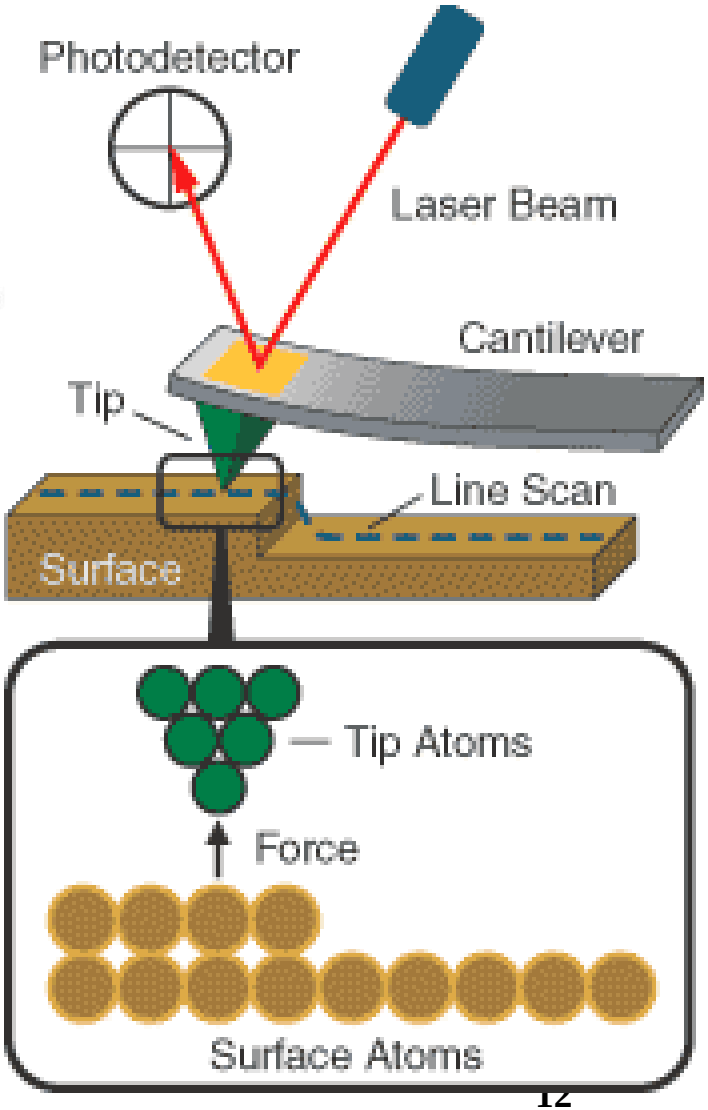


Expulsive force

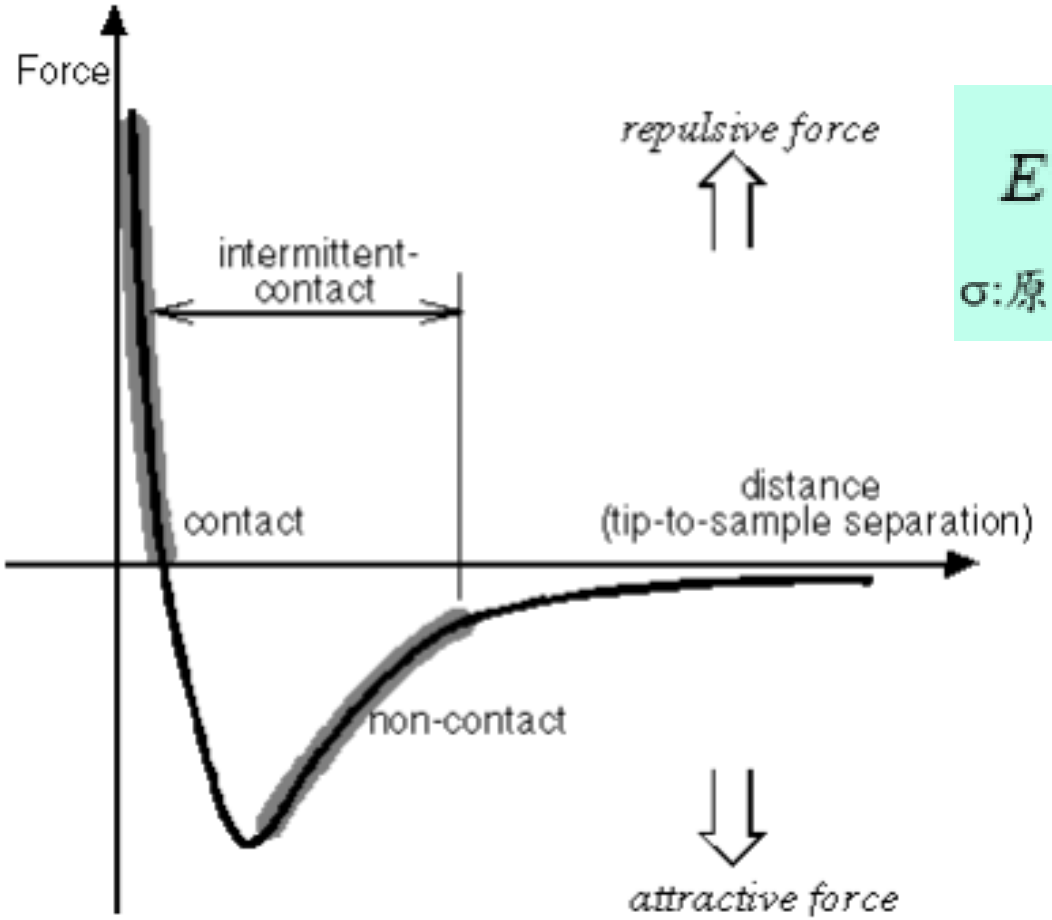


Attractive force





Comparison of AFM Contact, Semi-contact and Non-contact Modes



Interatomic force vs. distance curve.

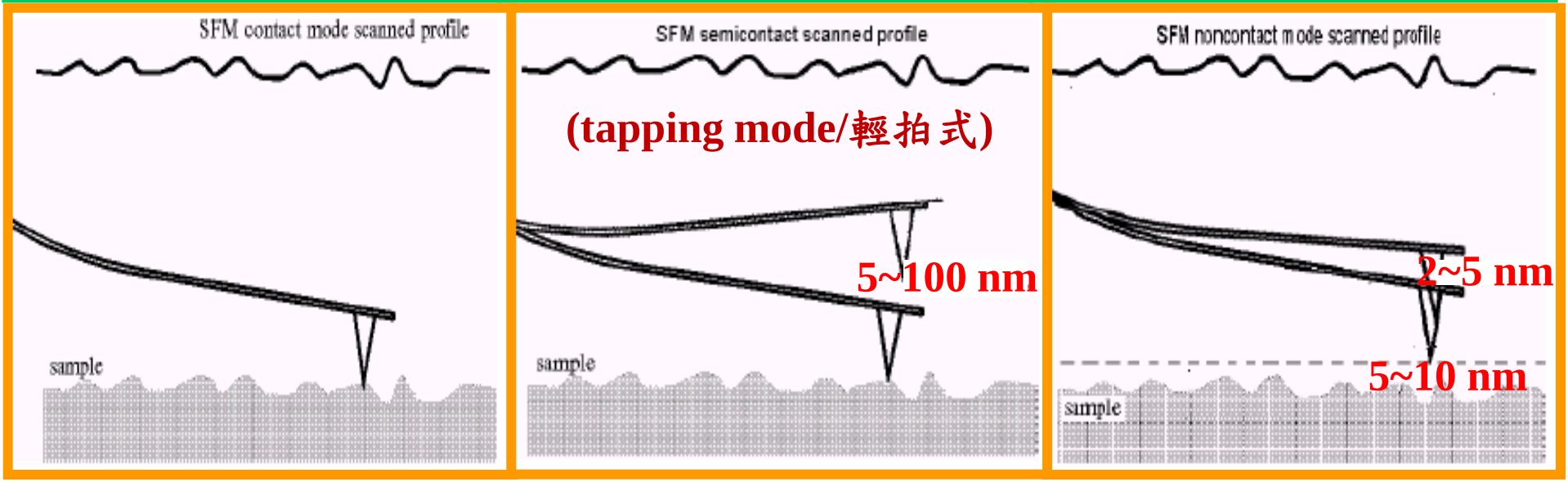
$$E^{pair}(r) = 4\varepsilon[(\frac{\sigma}{r})^{12} - (\frac{\sigma}{r})^{6}]$$

σ:原子半徑, r:兩原子間距, ε:介電常數

AFM image Resolution

Comparison of AFM Contact, Semi-contact and Non-contact Modes

Contact Mode	Semi-contact Mode	Non-contact Mode



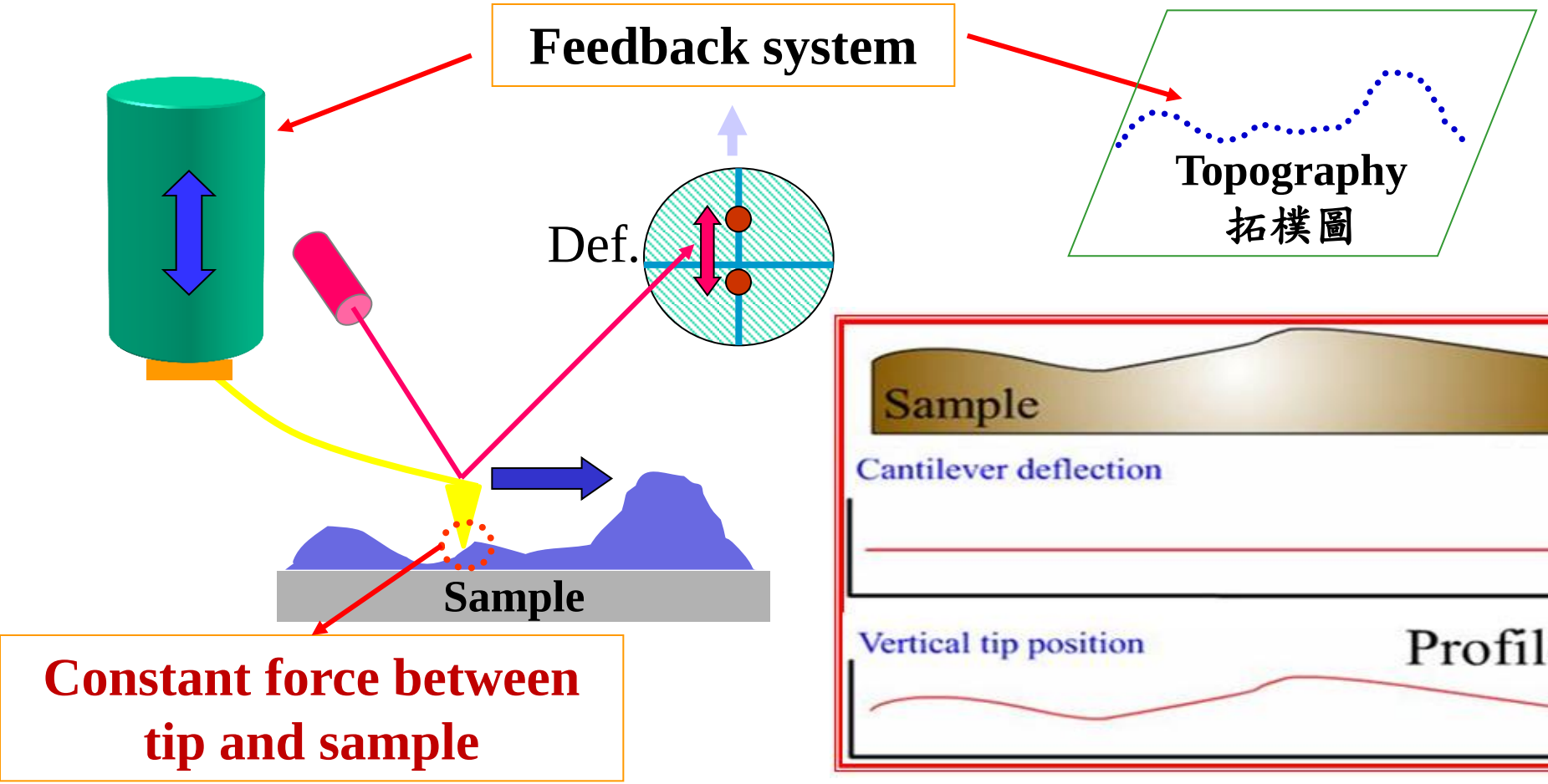
- Most of times, non-contact mode is operated as tapping mode.

- 接觸式(contact mode): 距離約0.5 nm，作用力為排斥力
- 半接觸模式(Semi-contact mode): 介於接觸式和非接觸式之間
- 非接觸式(non-contact mode): 距離約數十奈米，作用力為吸引力

http://www.eng.utah.edu/~lzang/images/Lecture_10_AFM.pdf

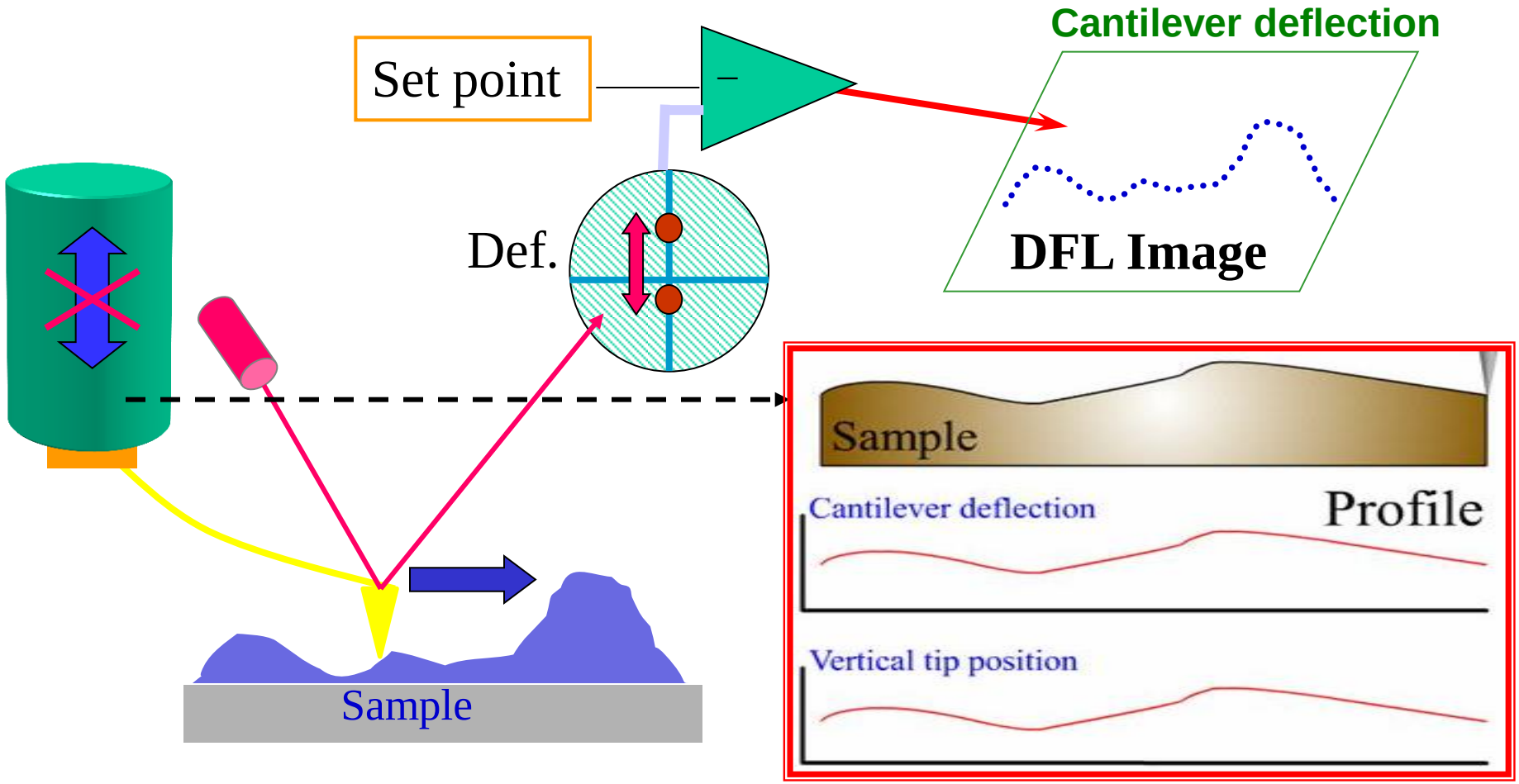
Contact Mode AFM (1.固定力模式)

- **Topography (constant force mode):**
  - To detect the change in the **sample height**
  - Suitable for measuring marked samples

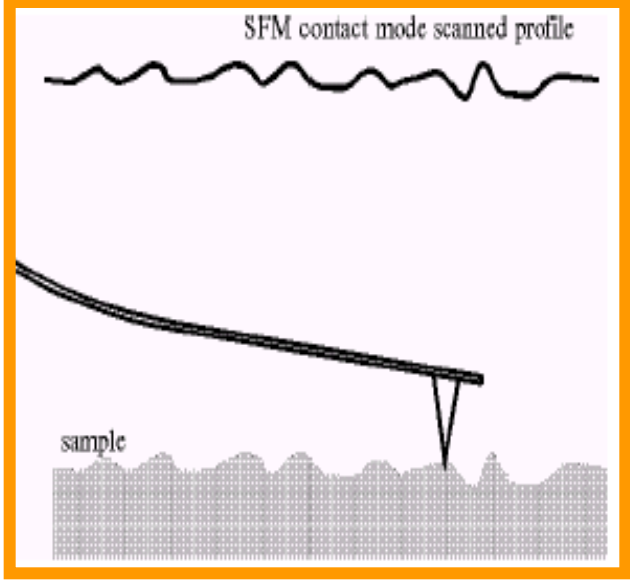
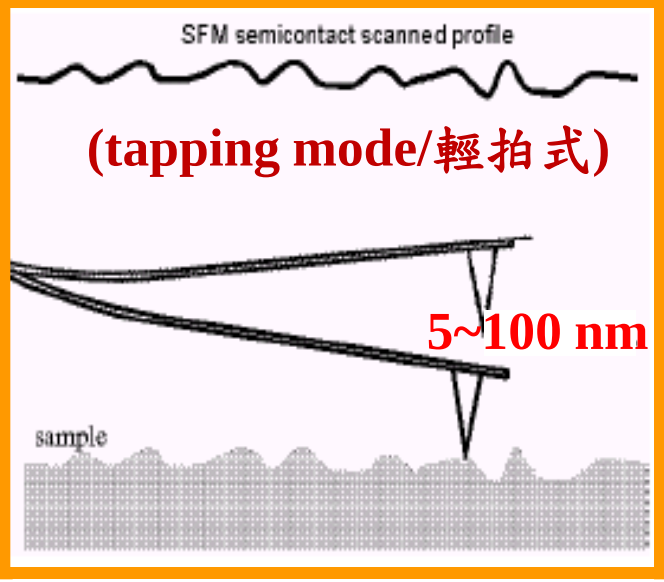
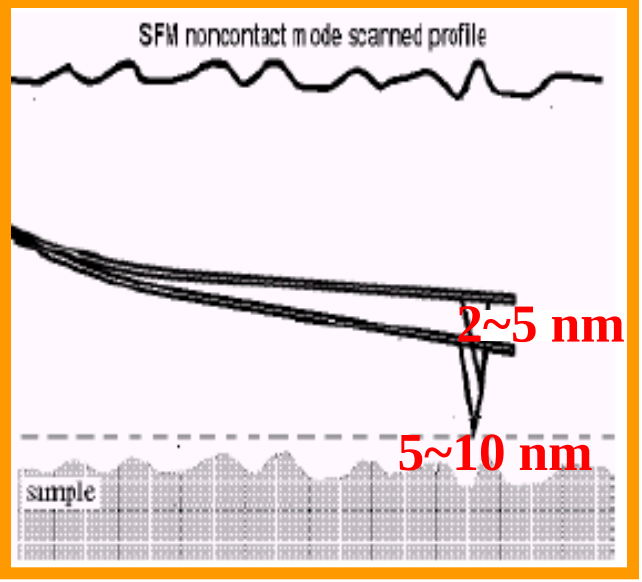


Contact Mode AFM (2.固定高模式)

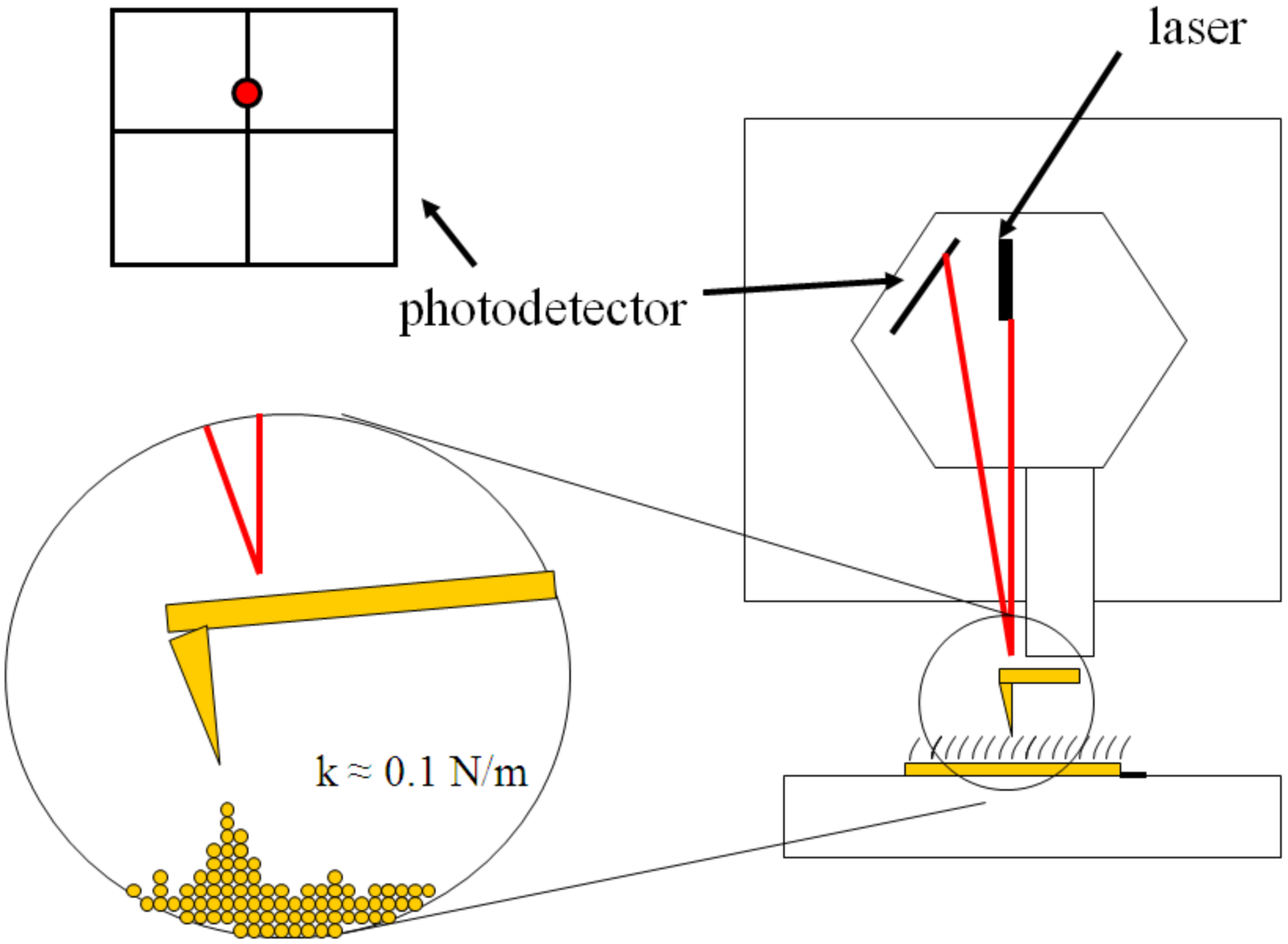
- **Constant height mode:**
  - To detect the **force change**
  - Suitable for the observation of tiny structure



Comparison of AFM Contact, Semi-contact and Non-contact Modes

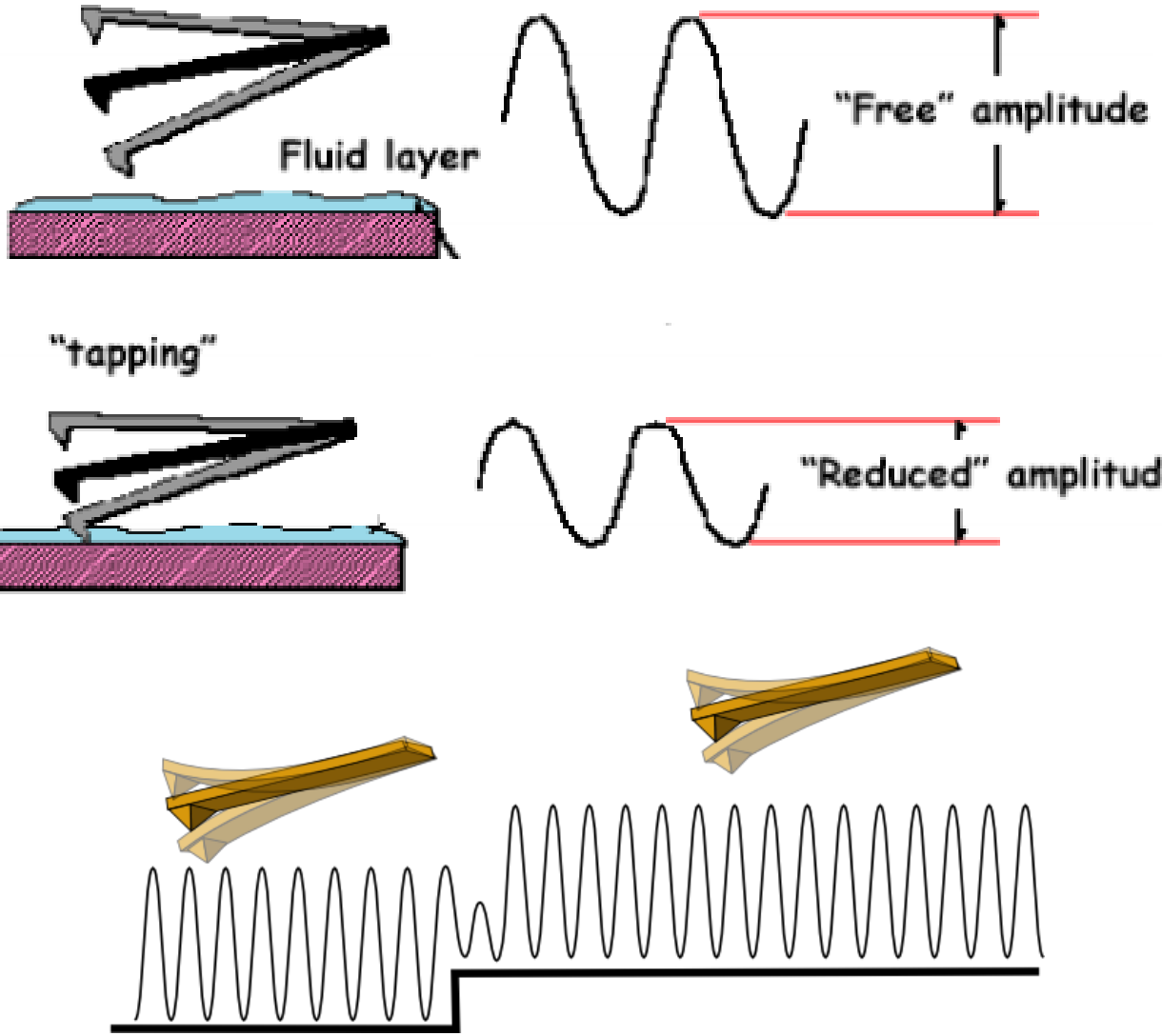
Contact Mode	Semi-contact Mode	Non-contact Mode
		
1）間解析度較佳。 2）此過大的作用力可能會造成樣品表面損壞。	1）解析度介於接觸式與非接觸式間。 2）其操作模式適用於表面較軟的材料或是液樣中掃描的樣本可以降低水層的干擾或是毛細現象的產生。	1）由於探針和樣品沒有接觸，所以樣品不易被損壞。 2）但由於探針和樣品並未接觸，因此非接觸模式的呈像之空間解析度較差，為其缺點。

Contact Mode AFM (2.固定高模式)



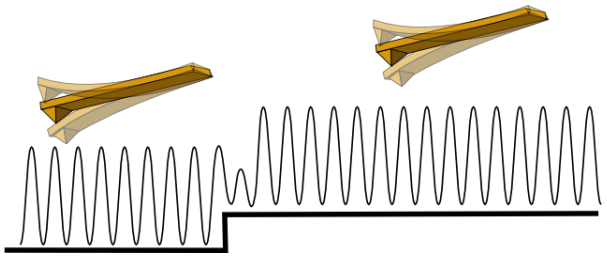
Atomic Force Microscopy (AFM)

Non-contact Mode

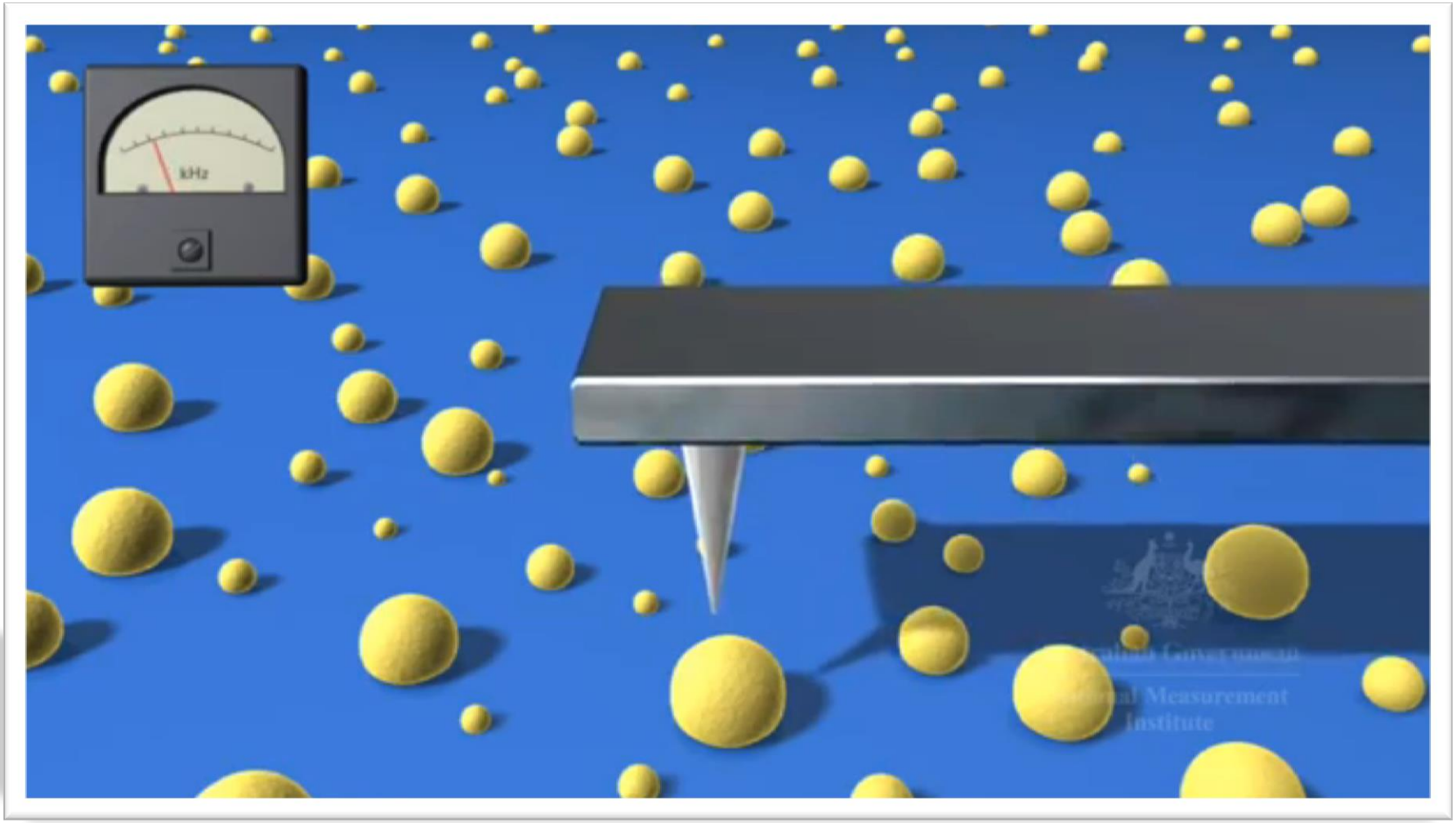


Atomic Force Microscopy (AFM)

Non-contact Mode



➢ **Constant oscillation amplitude**

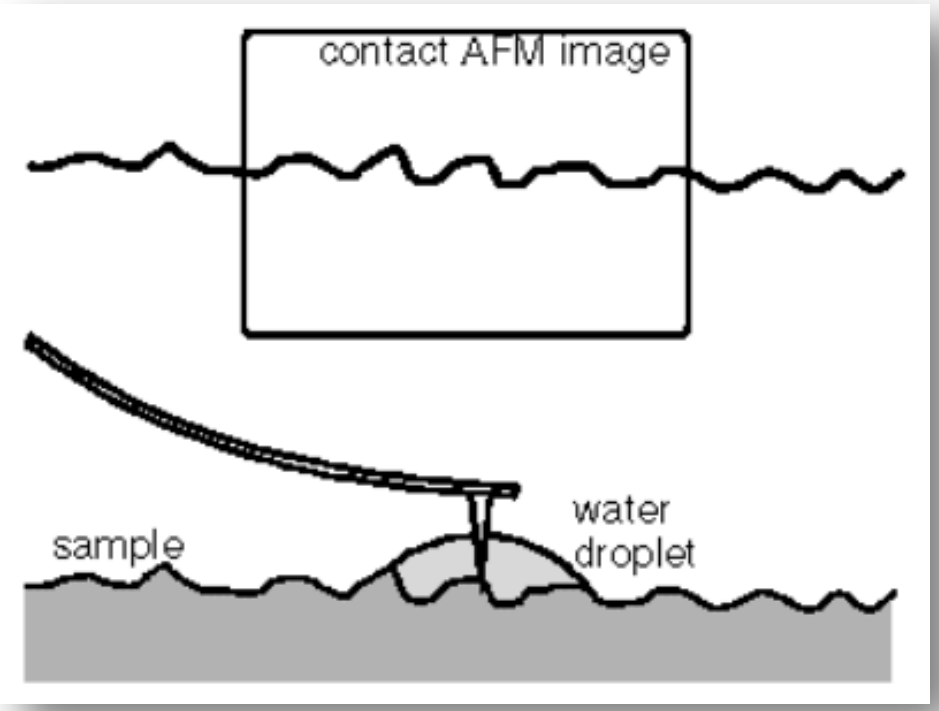


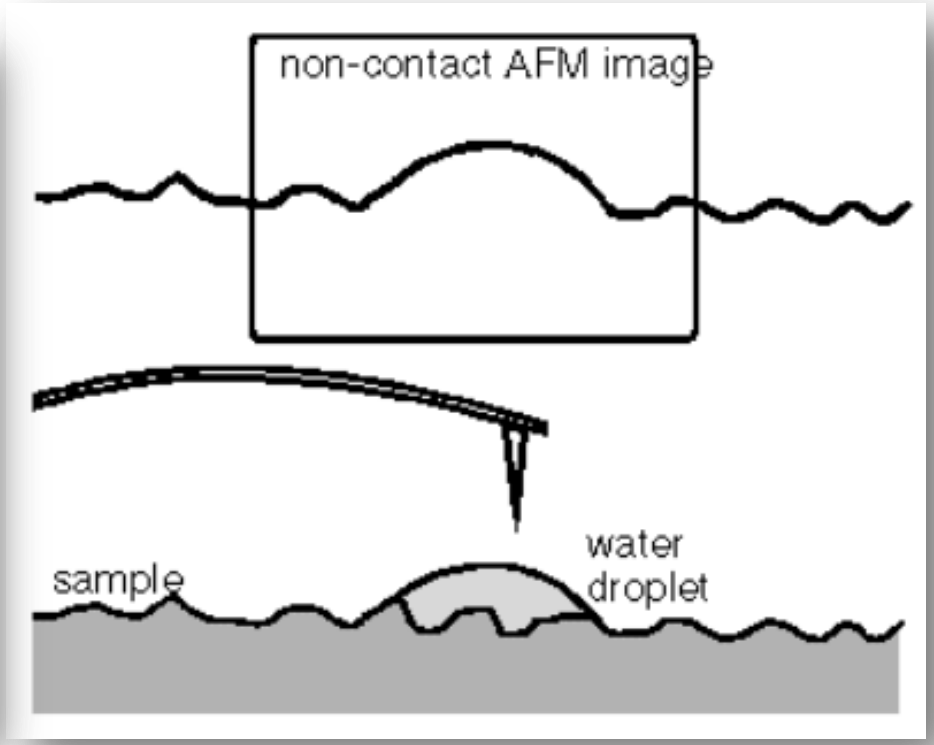
http://www.freesbi.ch/en/illustration/figures

https://www.youtube.com/watch?v=Ha53tFTsmW8

Comparison of AFM Contact, Semi-contact and Non-contact Modes

Contact and non-contact AFM images of a surface with a droplet of water.

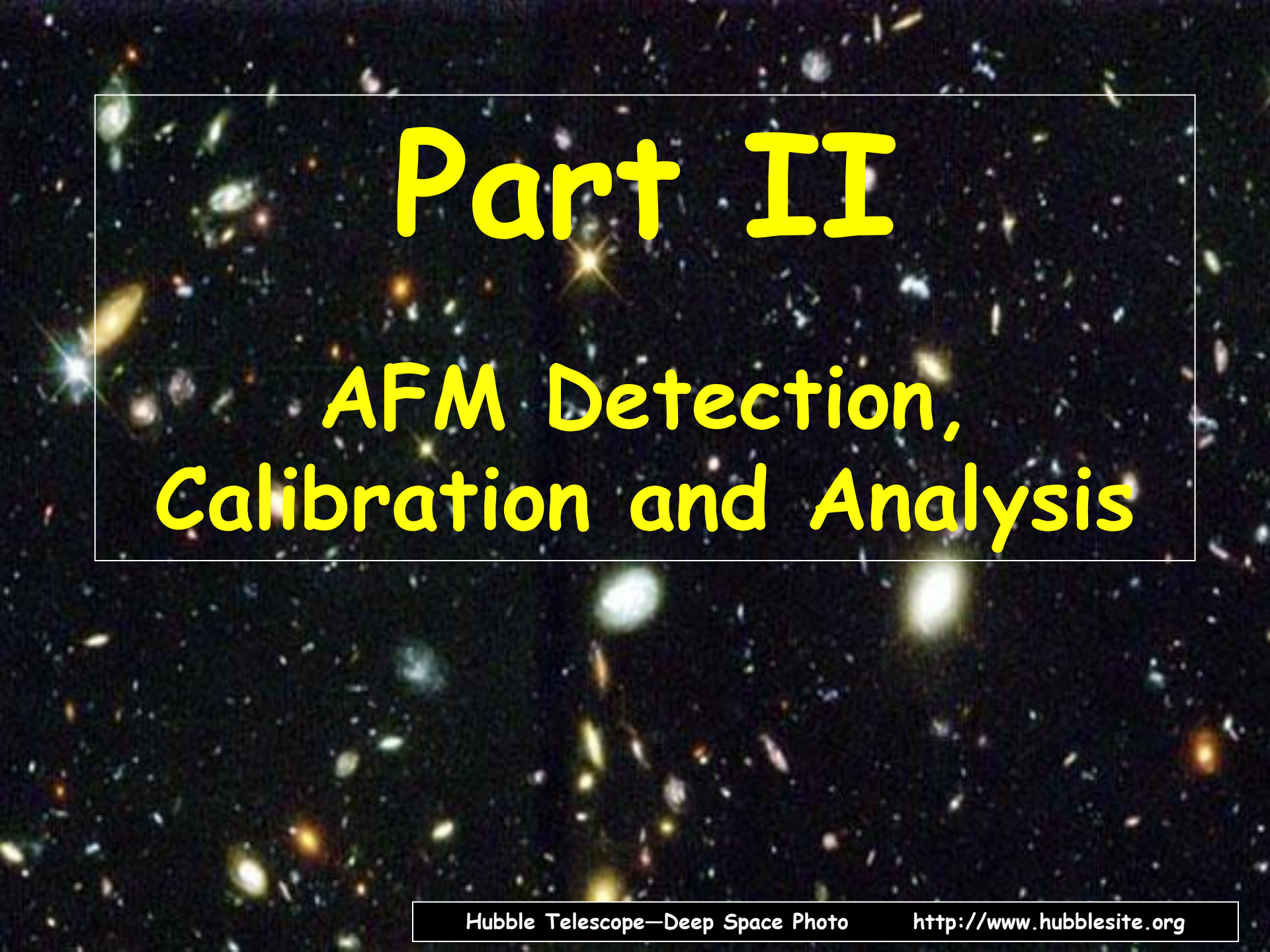




http://os.tnw.utwente.nl/otonly/Practical%20guide%20to%20scanning%20probe%20microscopy.pdf

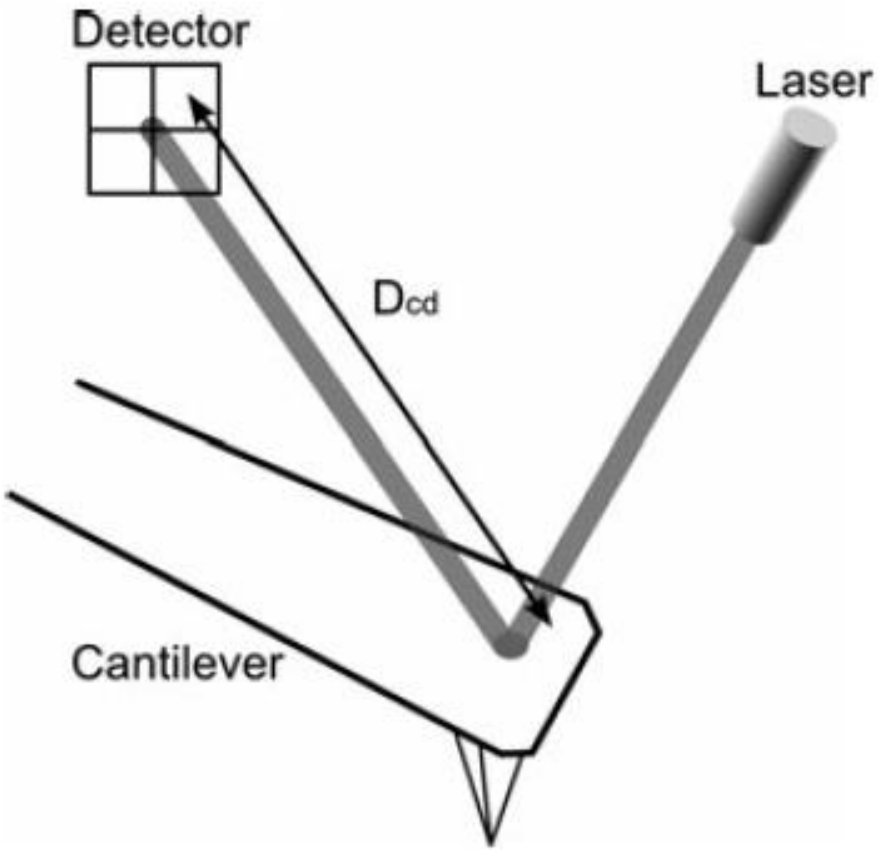
Part II

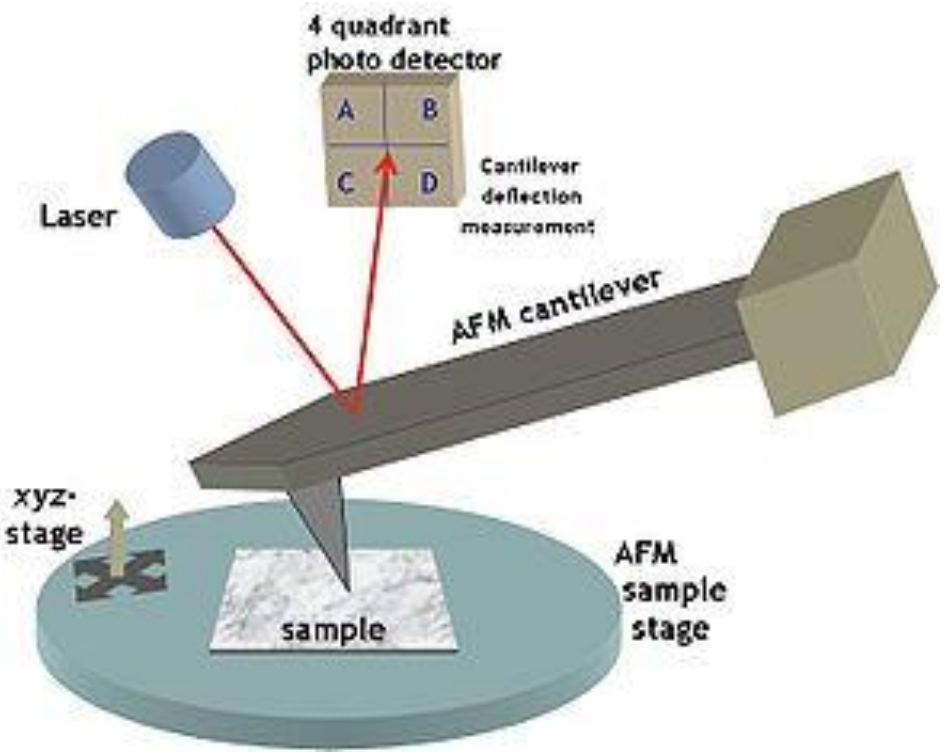
AFM Detection, Calibration and Analysis



Hubble Telescope—Deep Space Photo http://www.hubblesite.org

Schematic Diagram of the Optical Lever Sensor

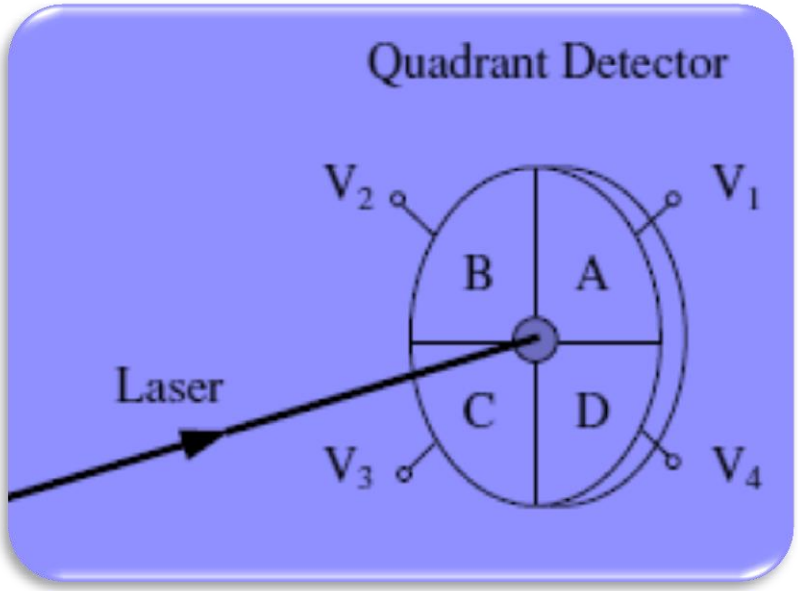


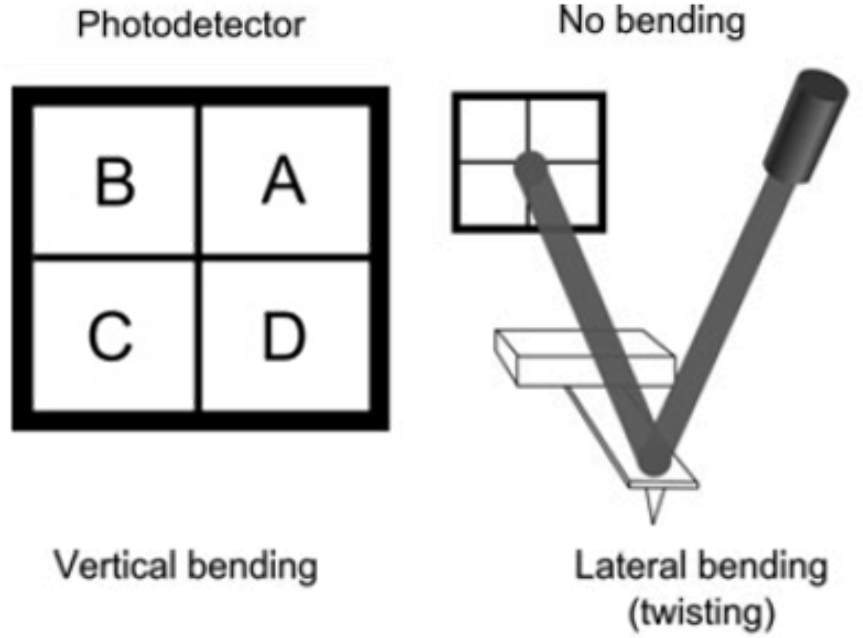


In an optical lever, as the end of the cantilever bends the position of the laser spot on the detector changes. As the cantilever – detector distance Dcd is large, a small movement of the cantilever causes a large change in the laser spot position at the detector.

Schematic Diagram of the Optical Lever Sensor

Illustration of how the photodetector detects vertical and horizontal bending of the cantilever.





$$X = K\frac{(V_1 + V_4) - (V_2 + V_3)}{V_s}$$

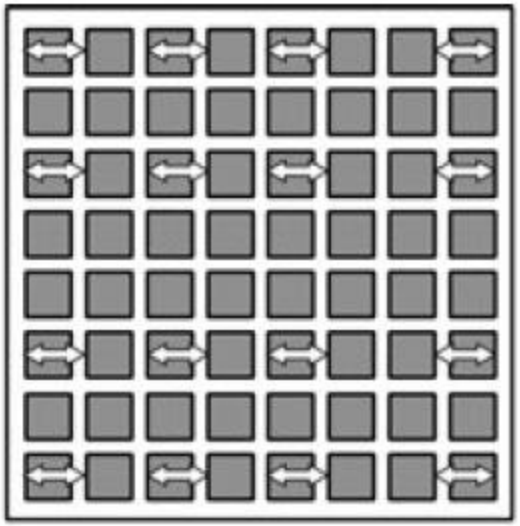
$$Y = K\frac{(V_1 + V_2) - (V_3 + V_4)}{V_s}$$

K is a constant

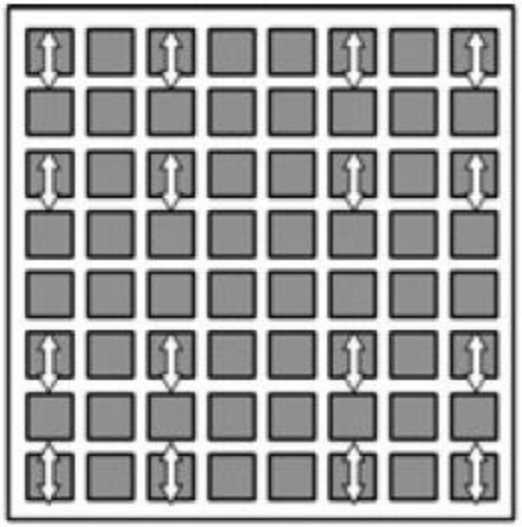
$$V_s = V_1 + V_2 + V_3 + V_4$$

Scanner calibration and certification procedures

Measurements to determine x and y scan linearity.

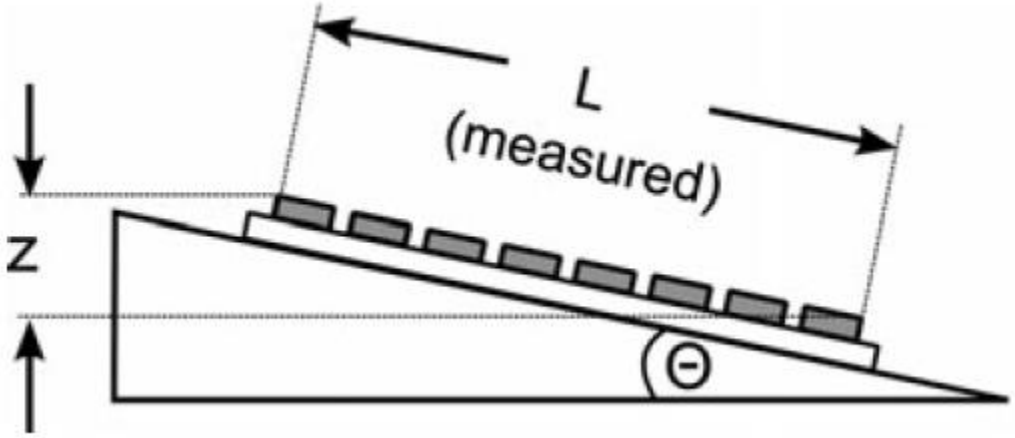


Pitch measurements for x linearity



Pitch measurements for y linearity

Method to measure z range.

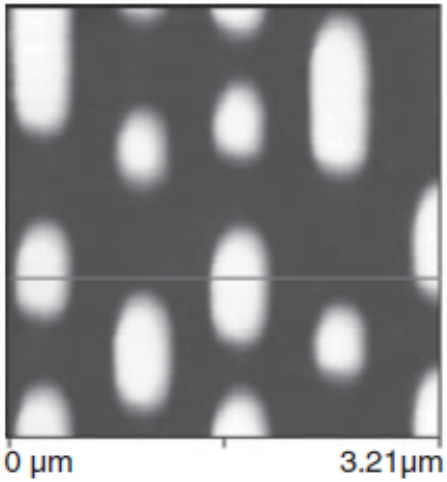


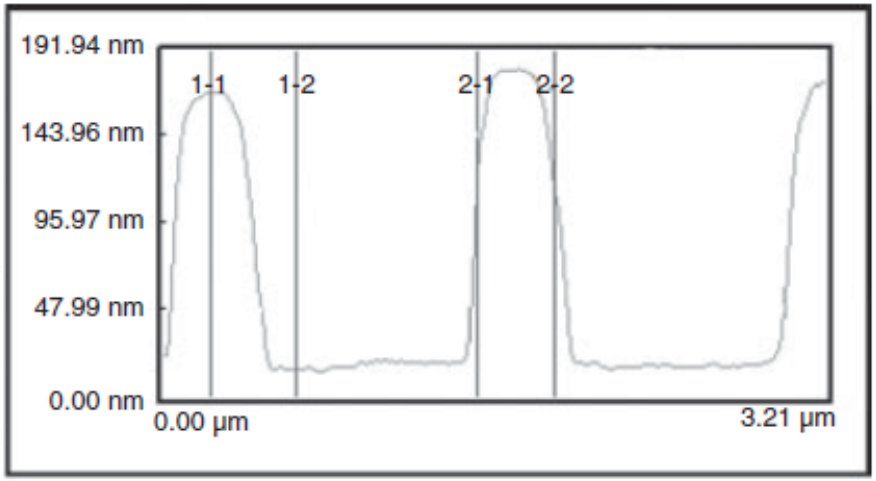
z is calculated as

$z = L \sin \Theta$

Analysing AFM Images

Line profiles



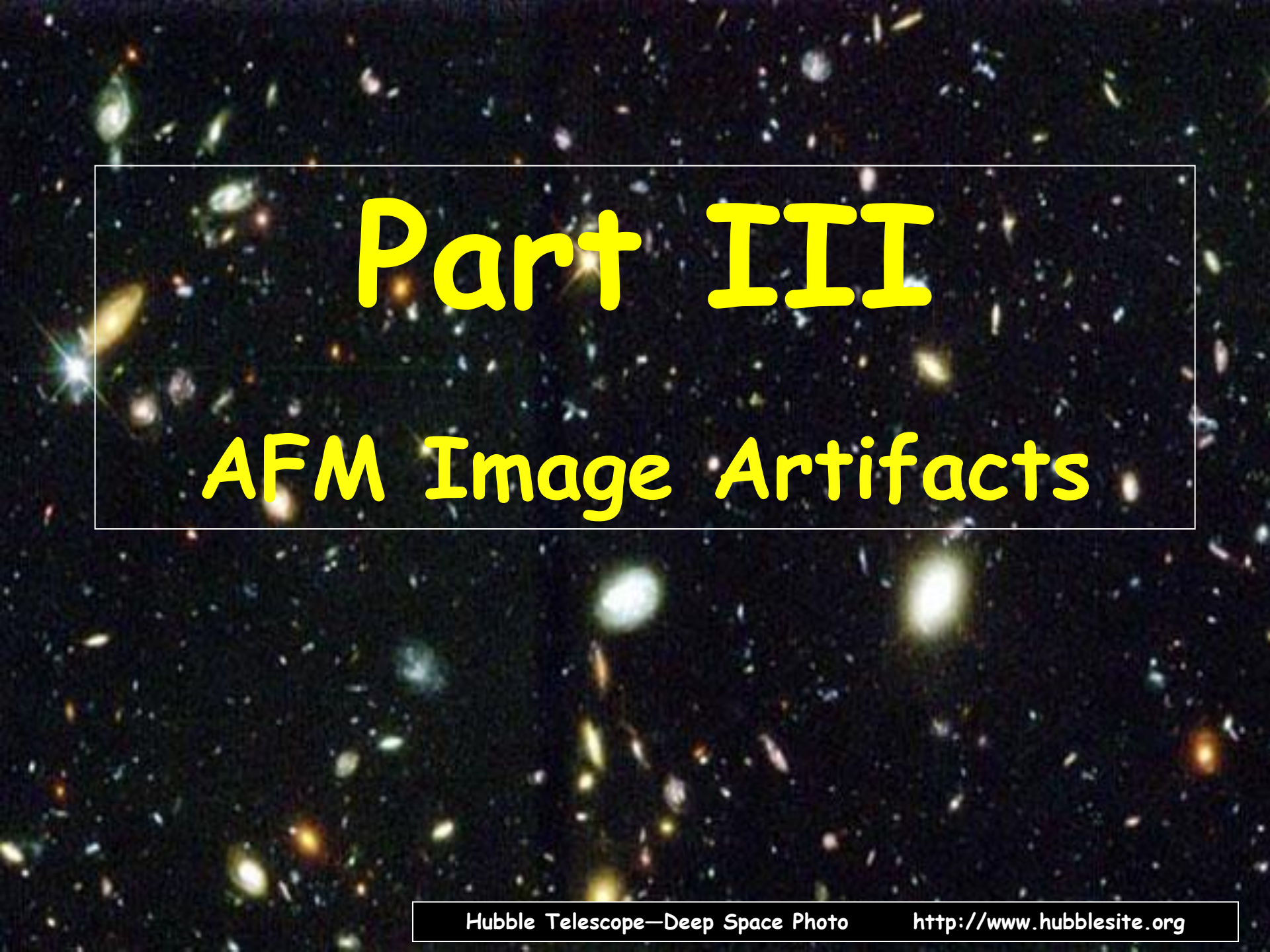


		pair 1		pair 2	
		x (µm)	Z (nm)	x (µm)	Z (nm)
Line 1	1	0.228	169.469	1.514	98.600
	2	0.638	15.000	1.890	125.404
	distance	0.410	–154.470	0.376	26.804
	angle	–20.65°		4.08°	

Line profile analysis of an AFM image of DVD bits. The height (indicated by Z in the image) and width (indicated by X) of the surface features can be measured from the line profiles.

Part III

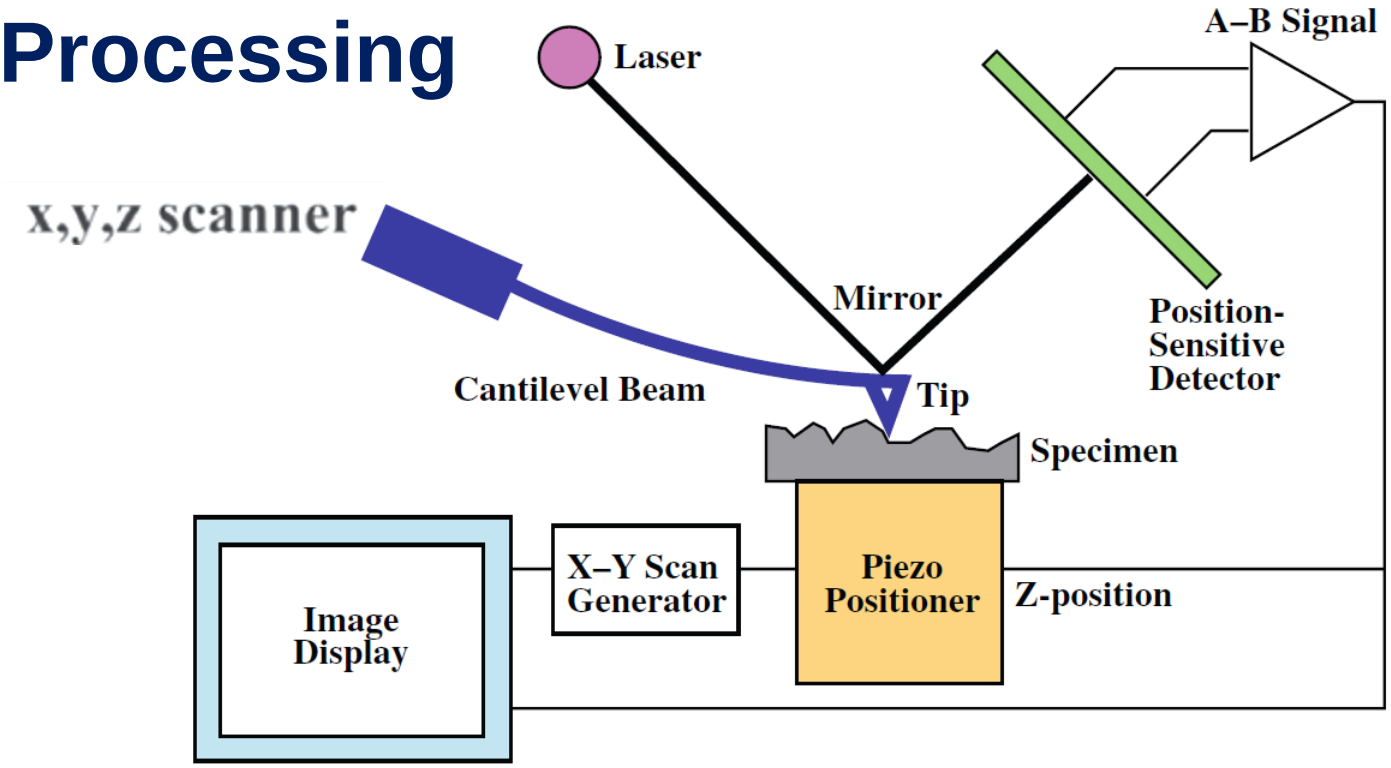
AFM Image Artifacts



Hubble Telescope—Deep Space Photo http://www.hubblesite.org

AFM Image Artifacts

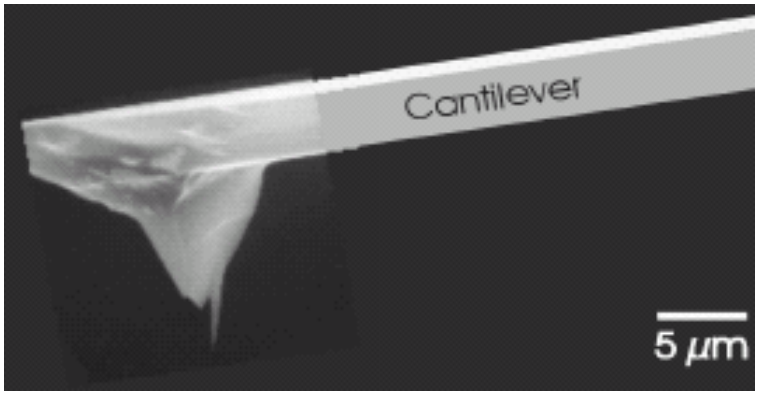
1) Probe Artifacts
2) Scanner Artifacts
3) Image Processing Artifacts
4) Vibrations/Contamination/Electronic
5) Laser Interference
6) Image Processing

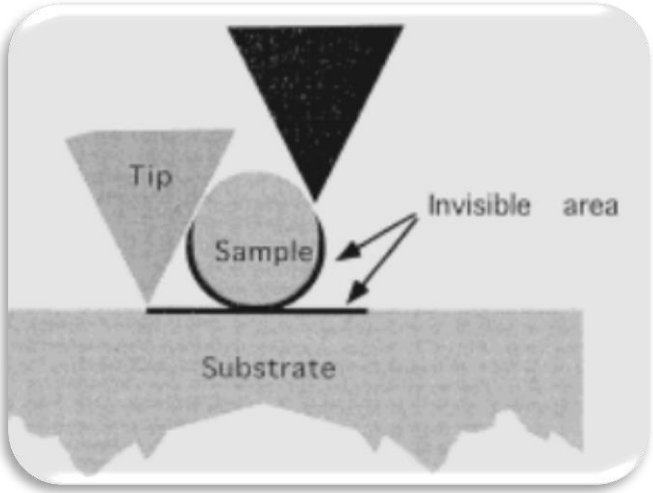


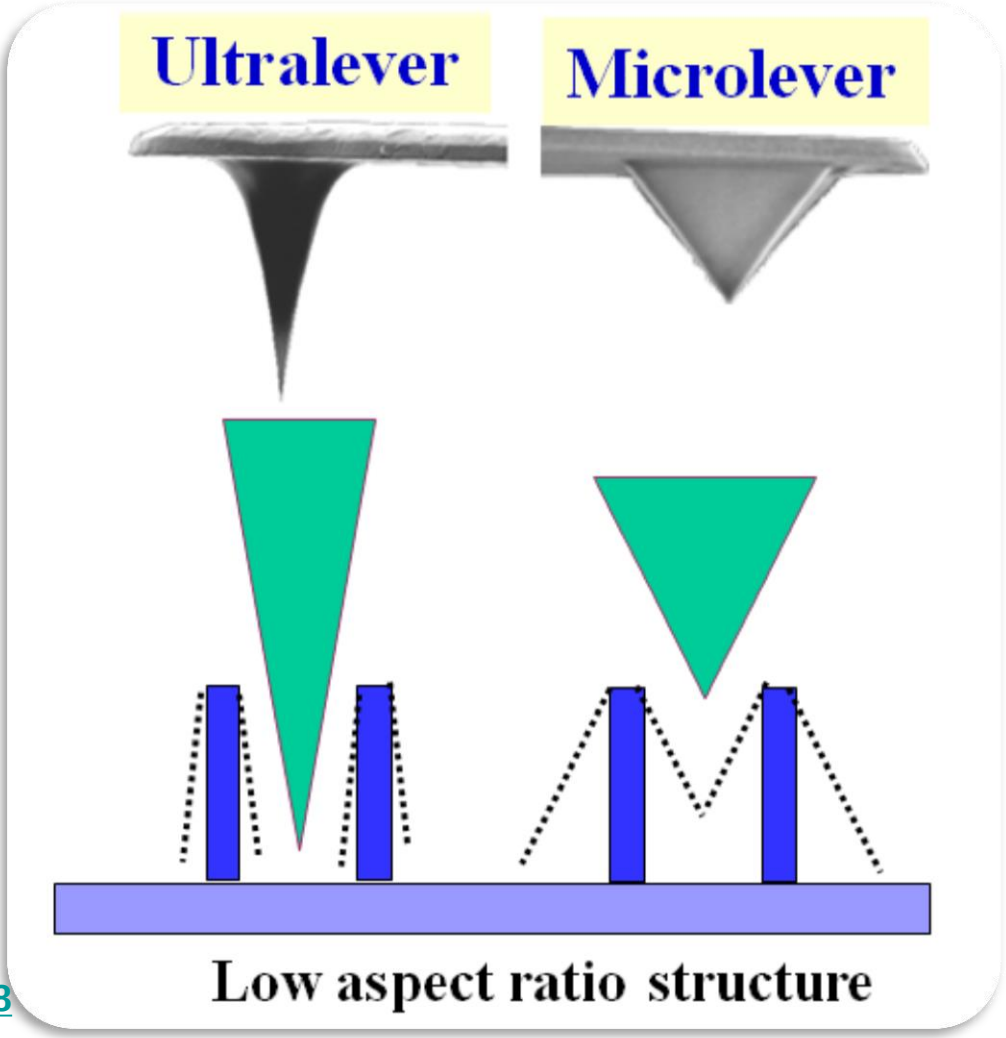
(1) The Effects of Tip Shape in Image

- **AFM image is the convolution of tip shape and actual size of tested sample.**

high aspect ratio tip







https://www.youtube.com/watch?v=veTskO7EWM8

https://www.youtube.com/watch?v=yvZIeHfF364

(1-1) Blunt Probes

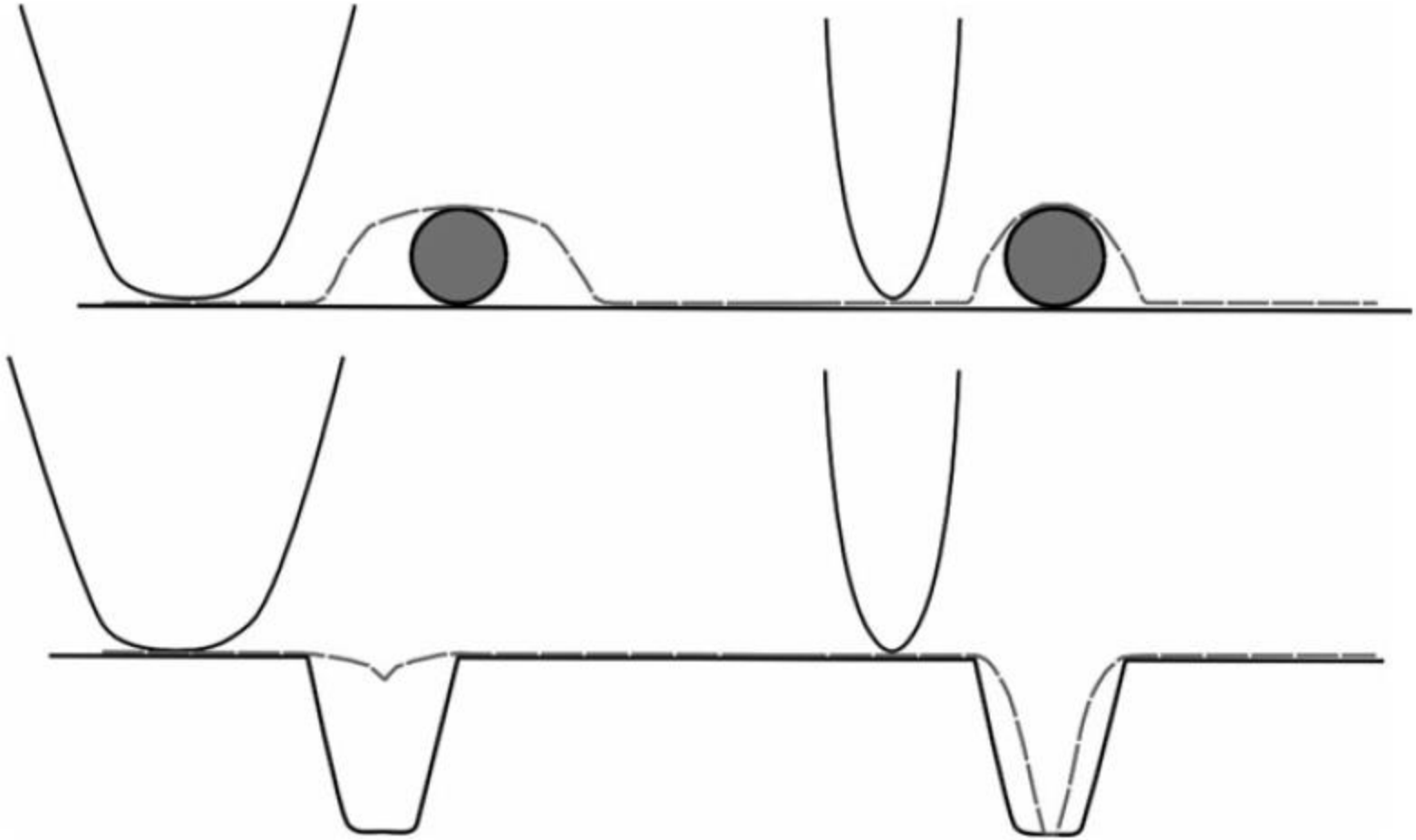


Illustration of probe-based dilation. Convex features such as particles tend to appear wider with blunter probes, although feature height may be accurate. Concave features such as pits tend to appear smaller (both less wide and less deep) with blunter probes.

(1-1) Blunt Probes

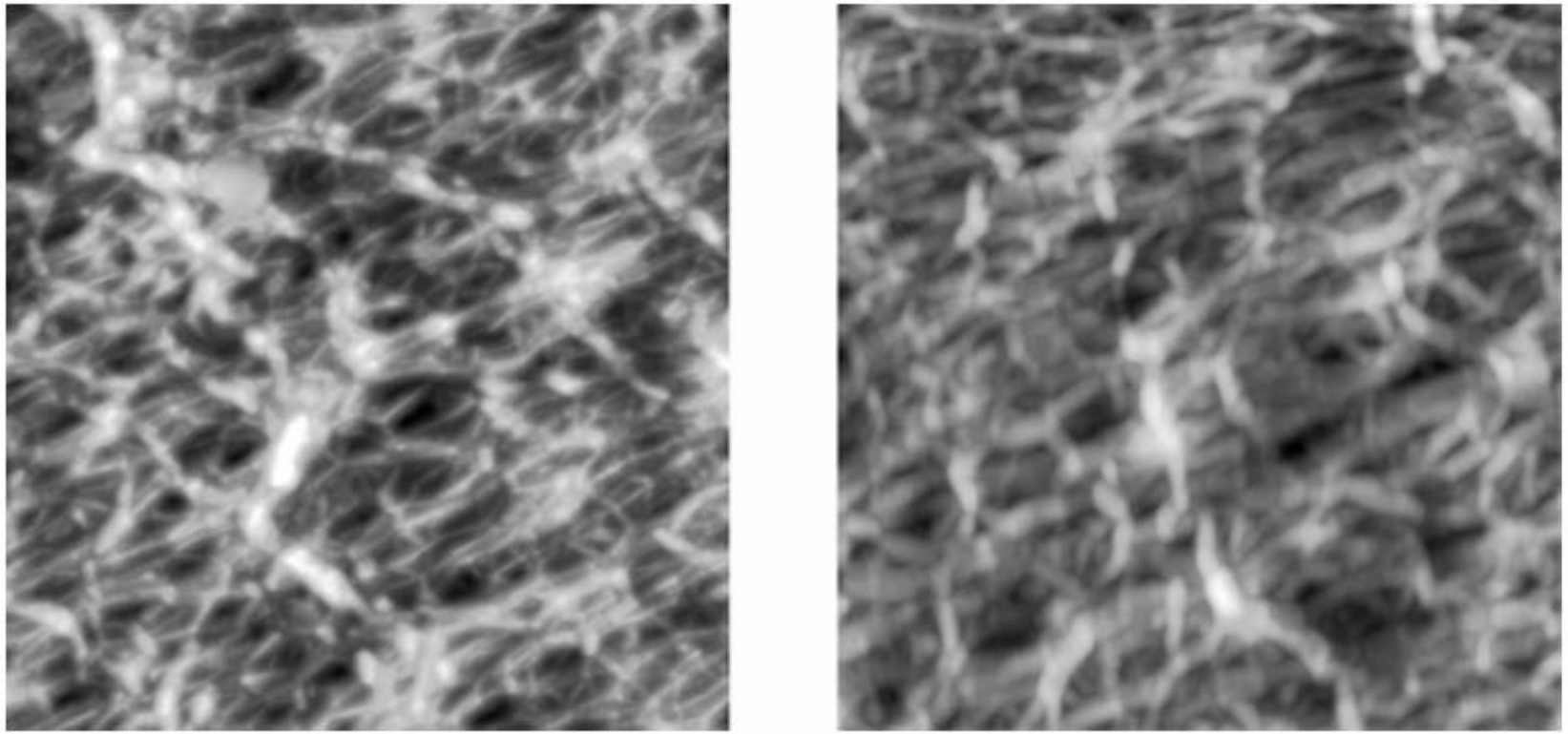
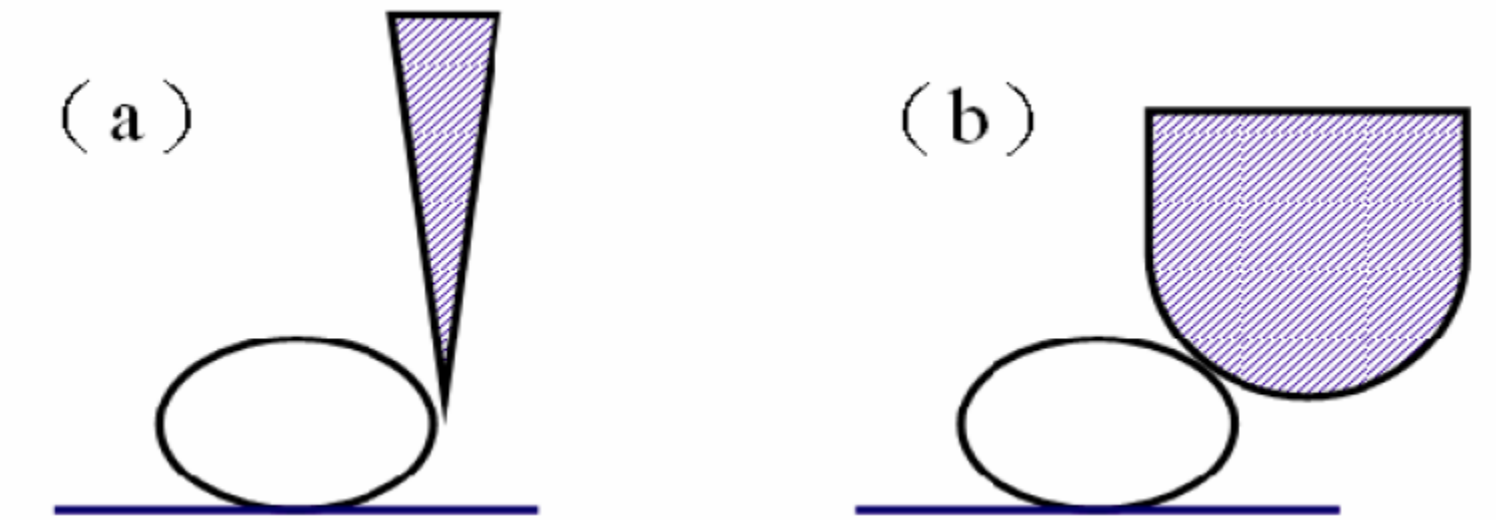


Illustration of the effect of using a blunt probe. These two images are of the same sample, and both are 1.5 μm×1.5 μm × 40 nm. The image on the left was taken with a sharp probe, the image on the right with a blunt probe.

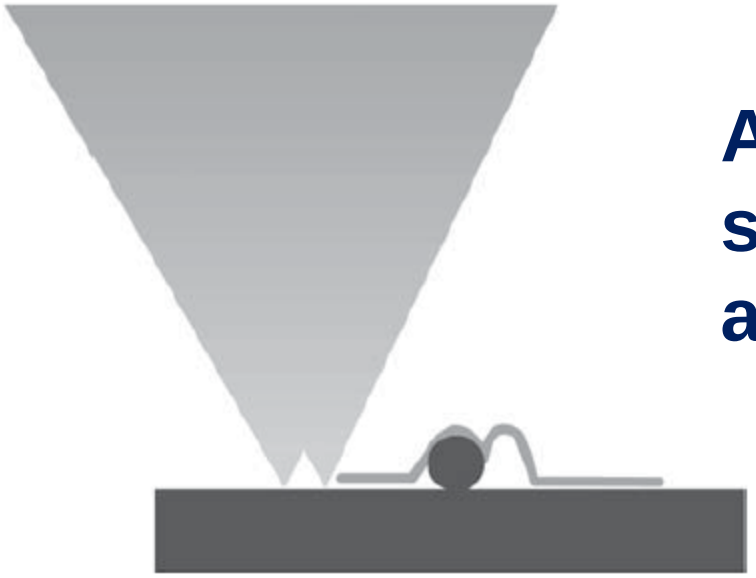
The Effects of Tip Shape in Image

- **AFM image is the convolution of tip shape and actual size of tested sample.**



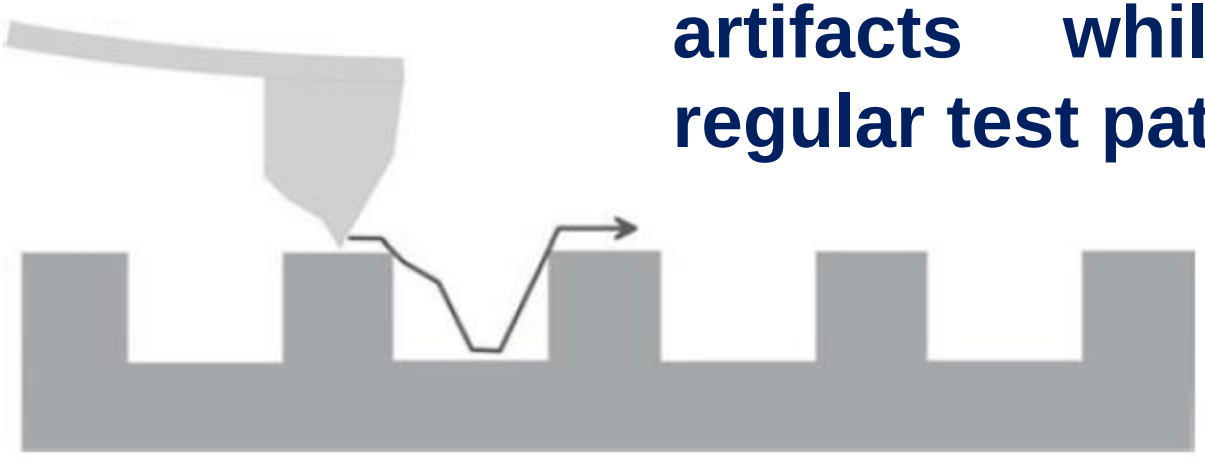
AFM image

(1-2) The Effects of Tip Shape in Image

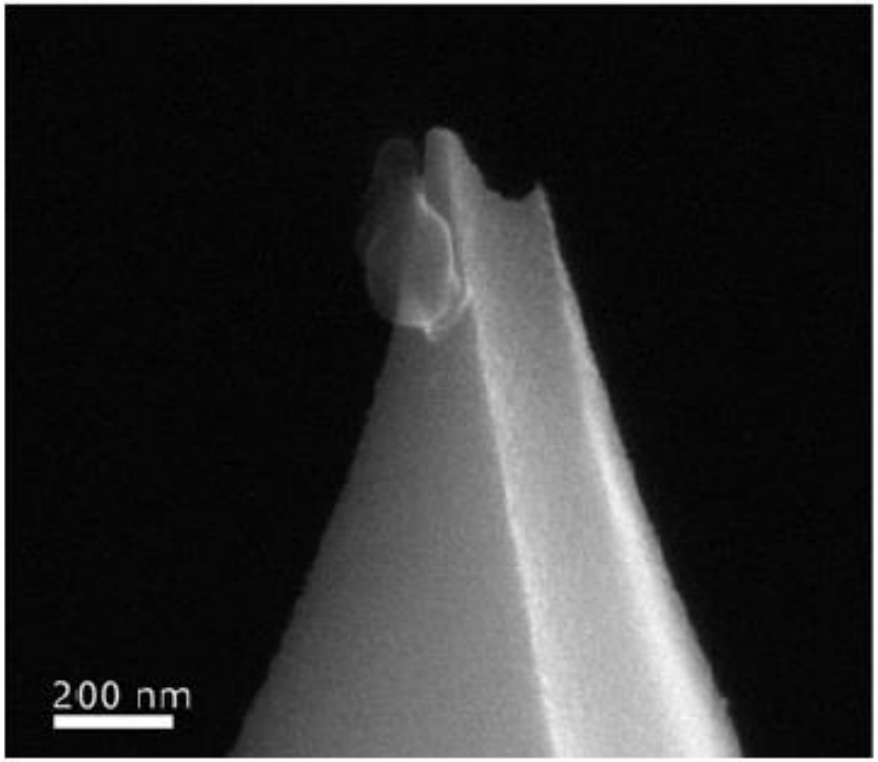


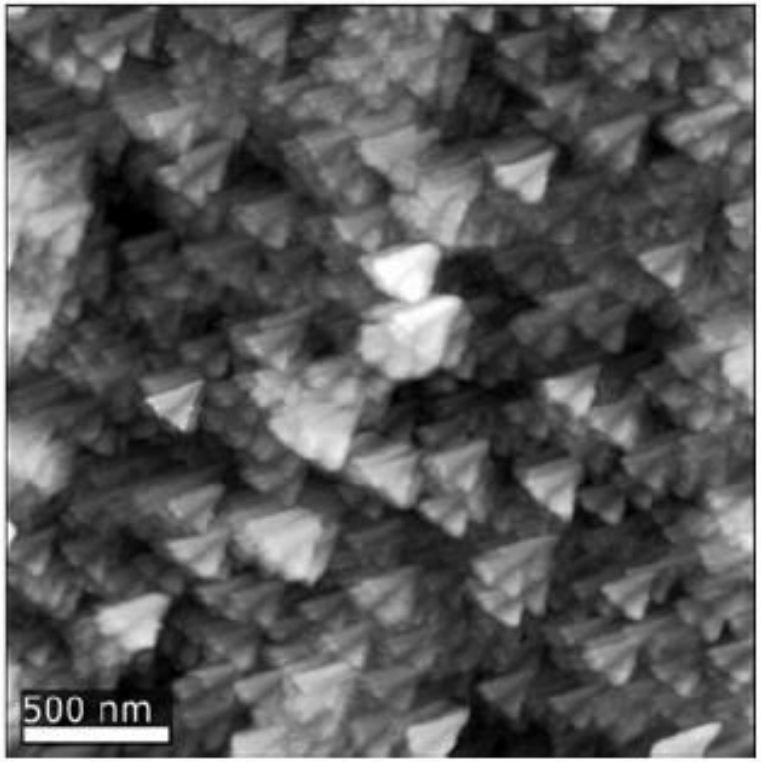
A double tip will cause a shadow or double image along the scanning direction.

A badly damaged tip creates artifacts while scanning a regular test pattern.



(1-3) Contaminated or Broken Probes

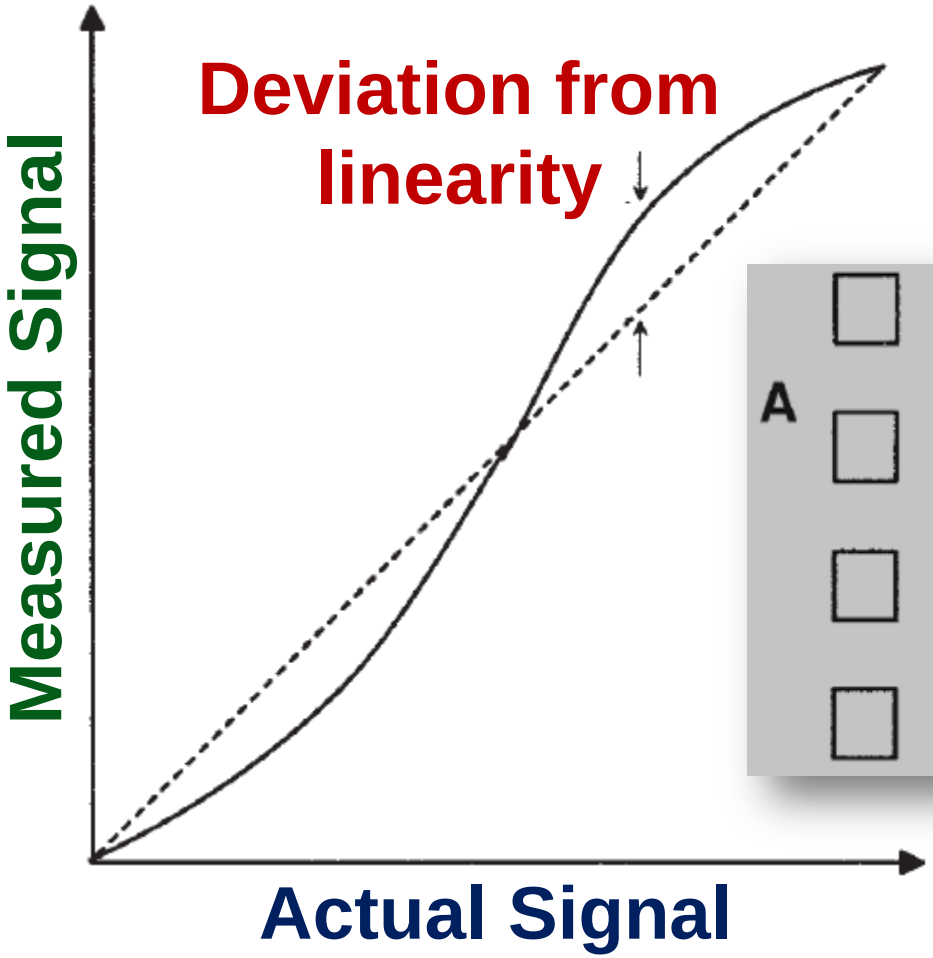




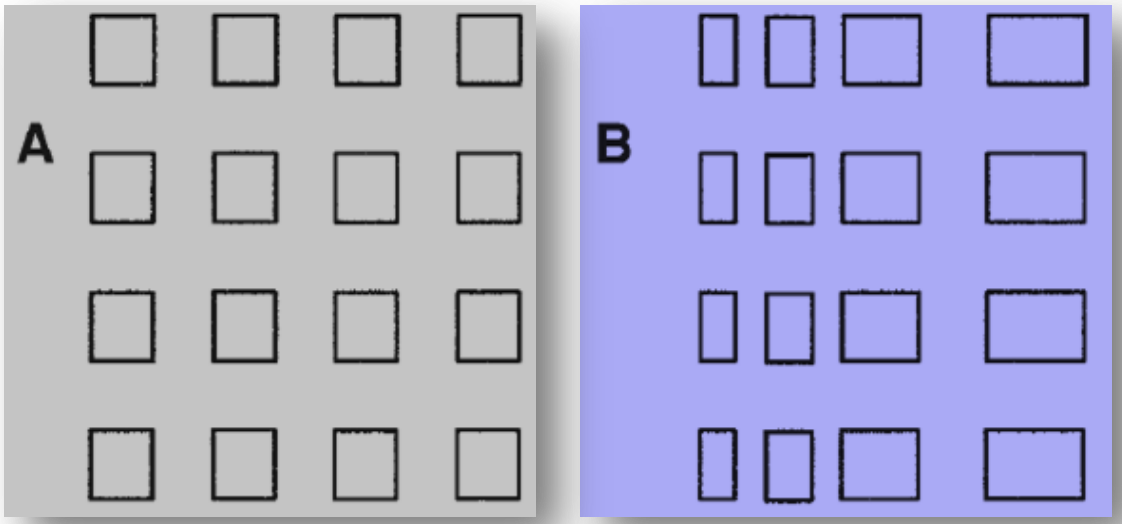
Examples of how images produced with broken or dirty probes show repeating patterns in the images. Left: SEM images of damaged and dirty probes. Right: AFM images produced using the probes shown on the left. Images with repeating patterns like these are usually due to broken or dirty probes.

(2) Scanner Probes

Plot of the scanner extension vs. driving signal. Notice the large deviation from linearity.

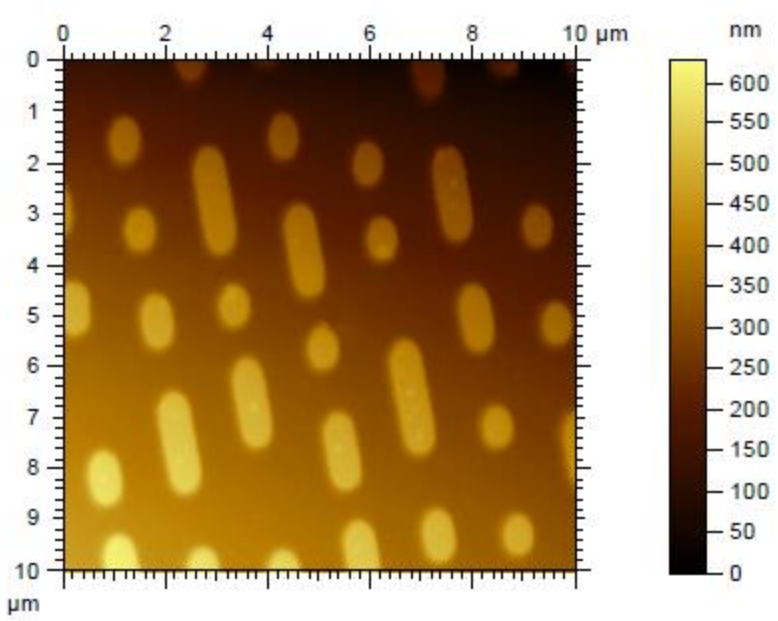


Distortion of a test pattern caused by scanner nonlinearity.

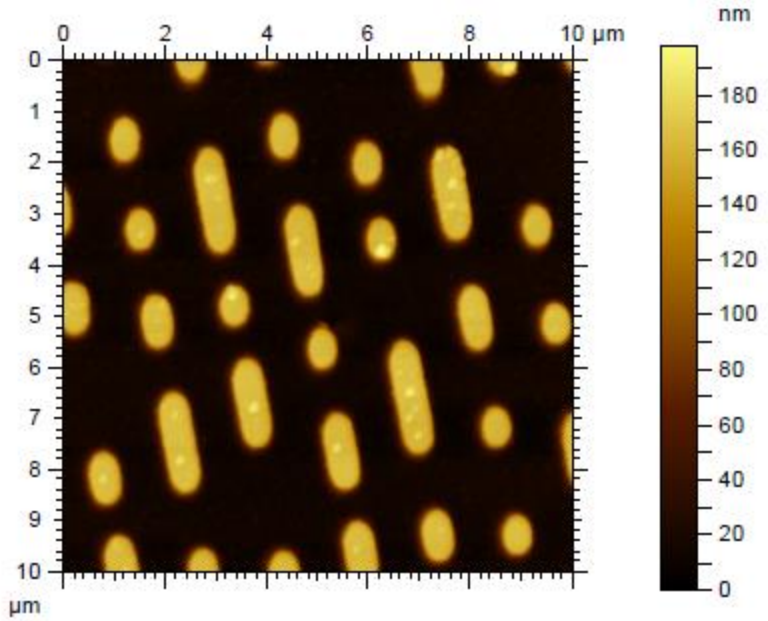


(3) Image Processing Artifacts

Most all AFM images require a basic leveling to remove inevitable artifacts from image acquisition (sample tilt, scanner bow / nonlinearities, z-drift, line skips, etc.).



Original raw image



After leveling process

(3-1) Line by Line Flattening

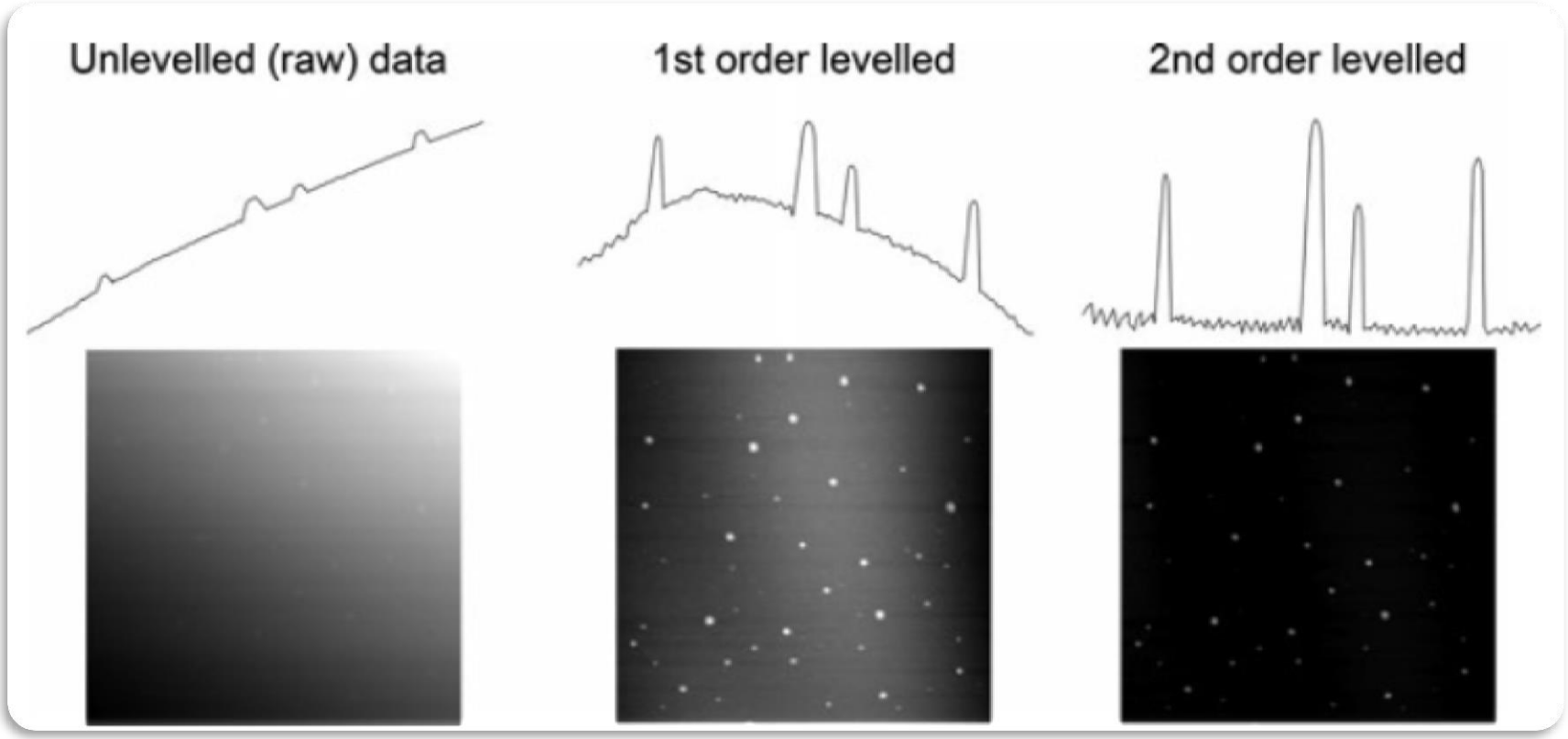
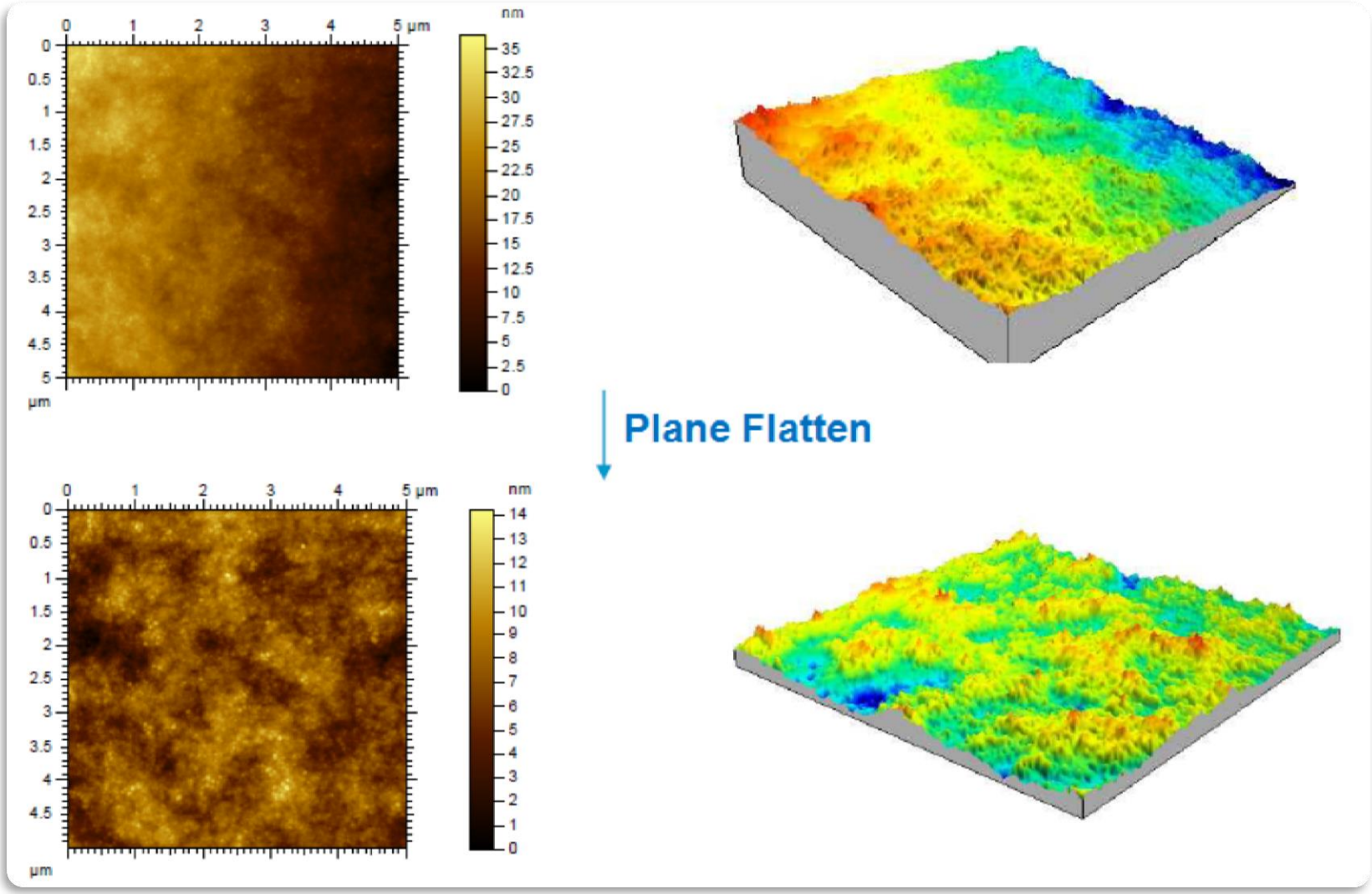


Illustration (top: line profiles; below: images) of the effect of first and second order polynomial line levelling. Left: unlevelled (raw) AFM image of nanoparticles showing tilt and scanner bow. Middle: effect of first order horizontal levelling – the nanoparticles are much clearer, but the curvature of the background due to scanner bow is still evident. Right: the effect of second order levelling with exclusions, the background is now flat.

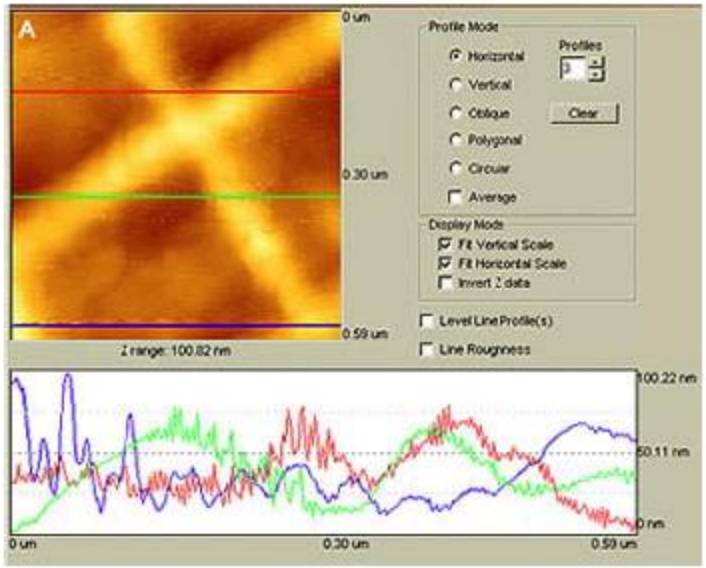
(3-2) Plane Flatten

Simplest approach – a linear plane is subtracted from surface. Useful when there is very minimal curvature relative to the surface topography.

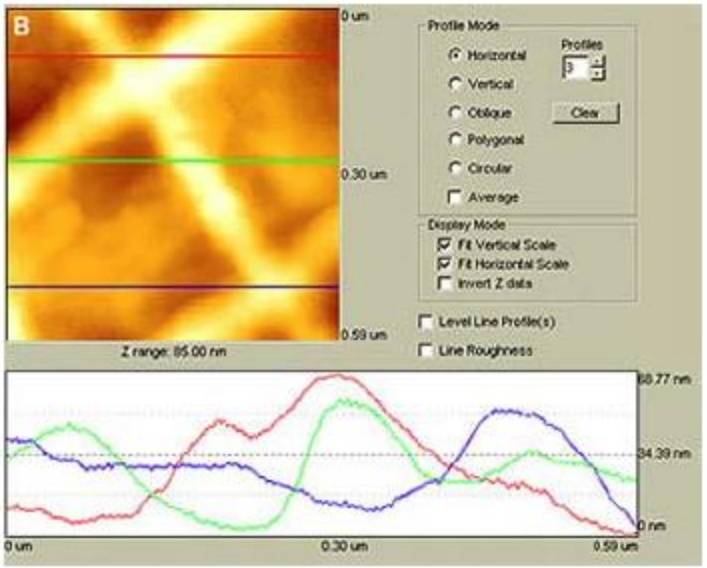


(4) Vibrations/Contamination/Electronic

Vibrations

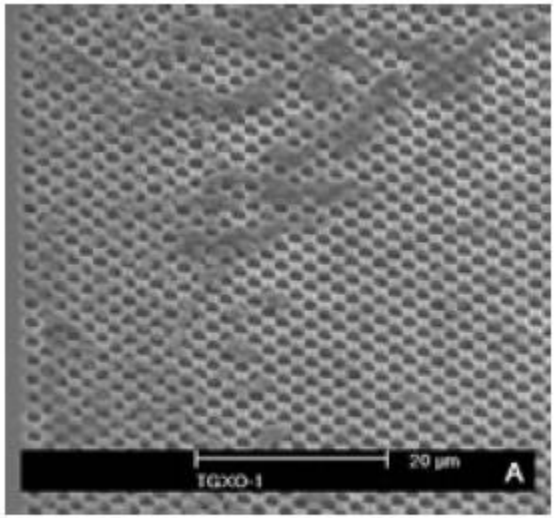


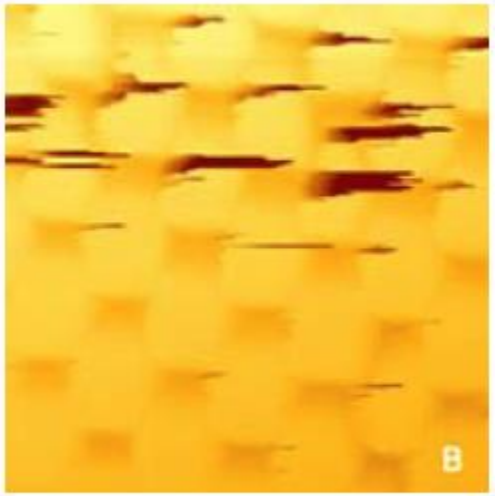
With vibrations



Without Vibrations

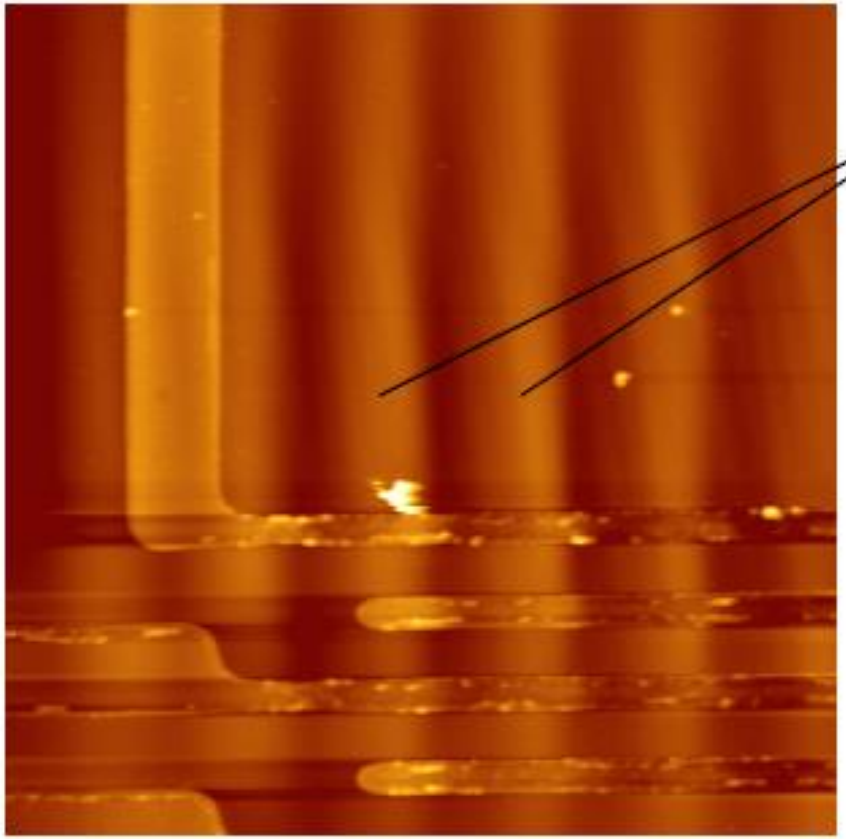
Surface Contamination



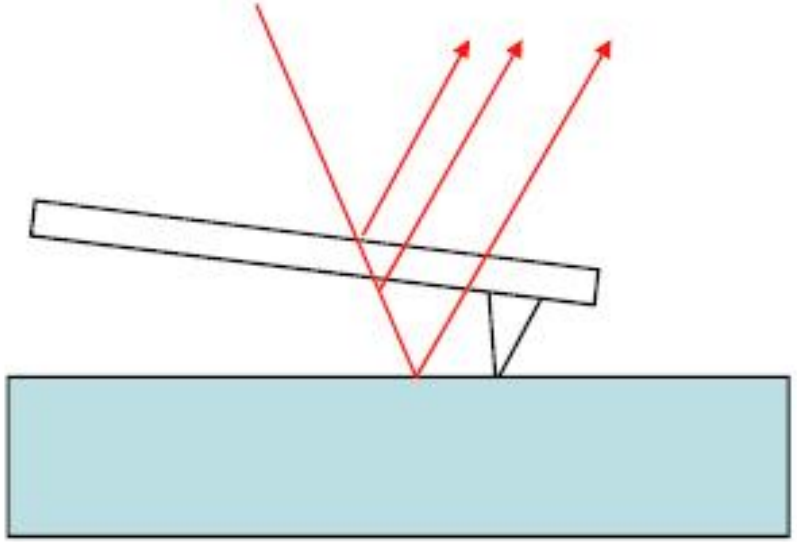


Surface Contamination

(5) Laser Interference

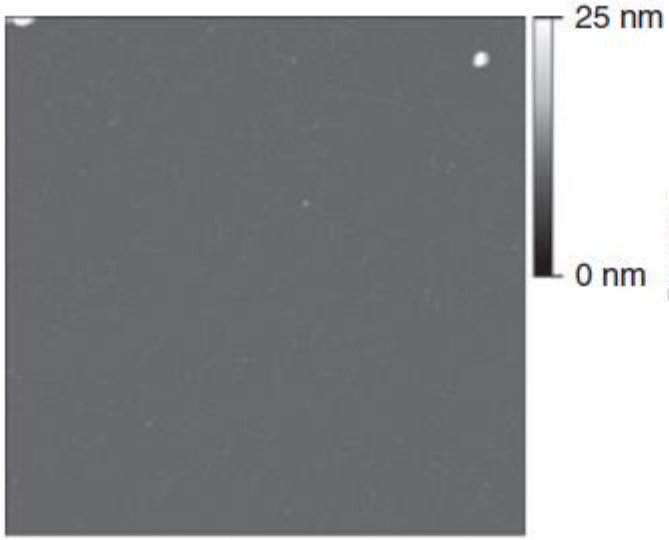


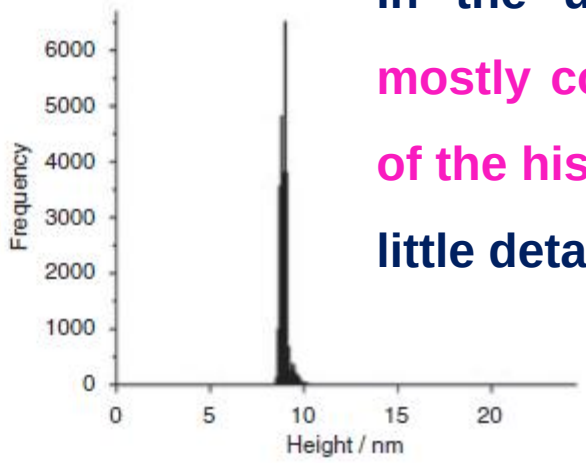
Oscillations caused by laser diffraction in the LL force sensor



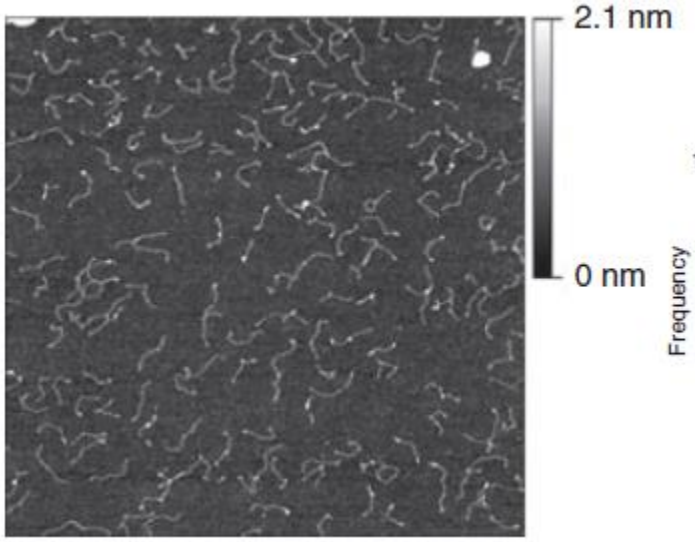
(6) Image Processing

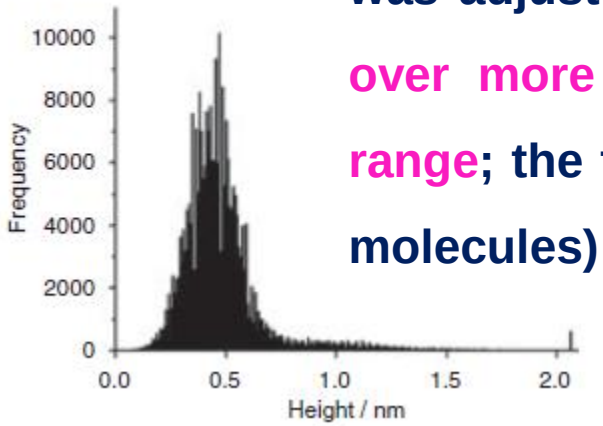
Example of histogram adjustment





In the upper case, the data is mostly concentrated in the centre of the histogram; the image shows little detail.





In the lower case, the colour range was adjusted so the data stretches over more of the available colour range; the features of interest (DNA molecules) are much clearer.

Part II

The Application of AFM in Research Field

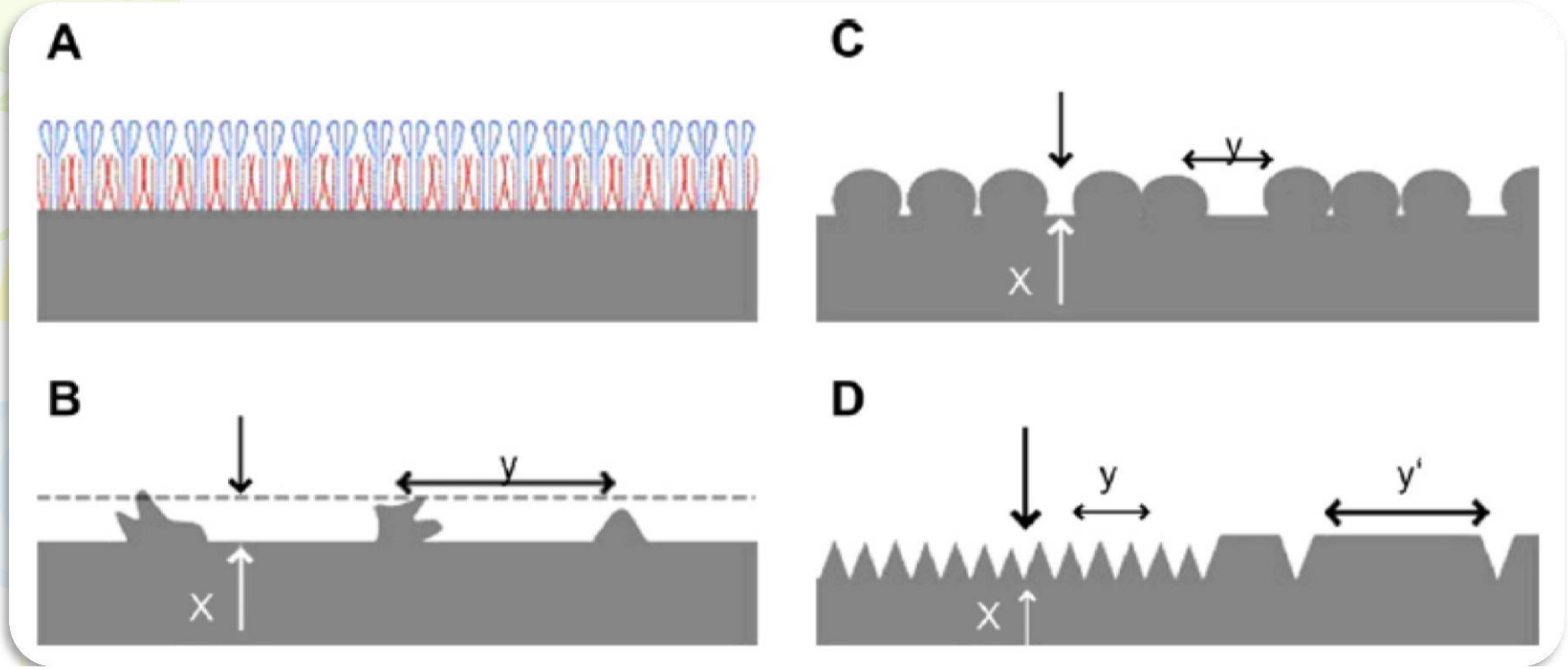
Hubble Telescope—Deep Space Photo http://www.hubblesite.org

The Application of AFM in Research Field

1. **Surface science**
2. **Materials engineering**
3. **Semiconductor analysis**
4. **Biochemistry study**
5. **Biology filed**

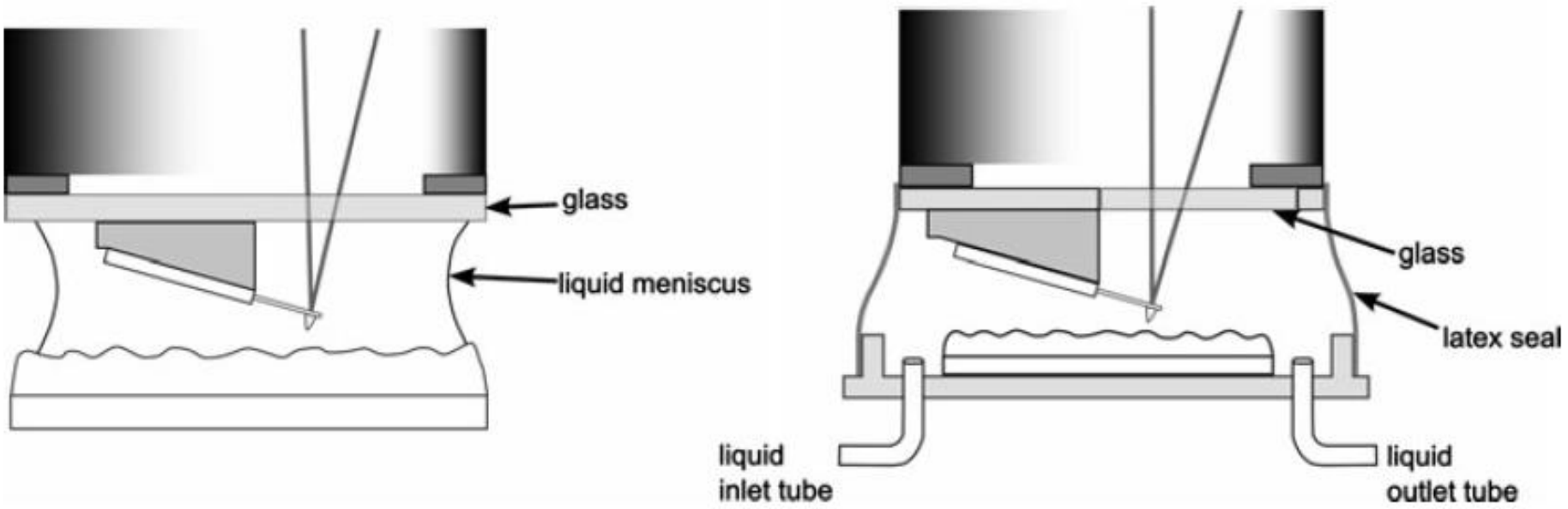
From Cappella, B., Dietler, G., “Force-distance curves by atomic force microscopy”, Surface Science Reports, 1999, 34, 1-104

Some Nanosurfaces Mimic Natural Cell Environments



Nanoscale surface modification. (A) Self-assembled monolayers (SAMs) can change the topography and chemistry of a surface to impart novel physical and/or biochemical properties. (B) Deposition (沉積) or chemical modification techniques can apply nanoscale features (x≤100 nm) in a manner that are distributed in micron-scale (y > 100 nm). (C) Other deposition or compaction methods can place nanoscale features in nanoscale distribution. The cell response to surfaces represented by (B) or (C) may be different. (D) Isotropic surfaces can be created in the nanoscale (x ≤ 100 nm) by subtractive or additive methods. The distribution can be in either the nano- (y) or micron-scale (y').

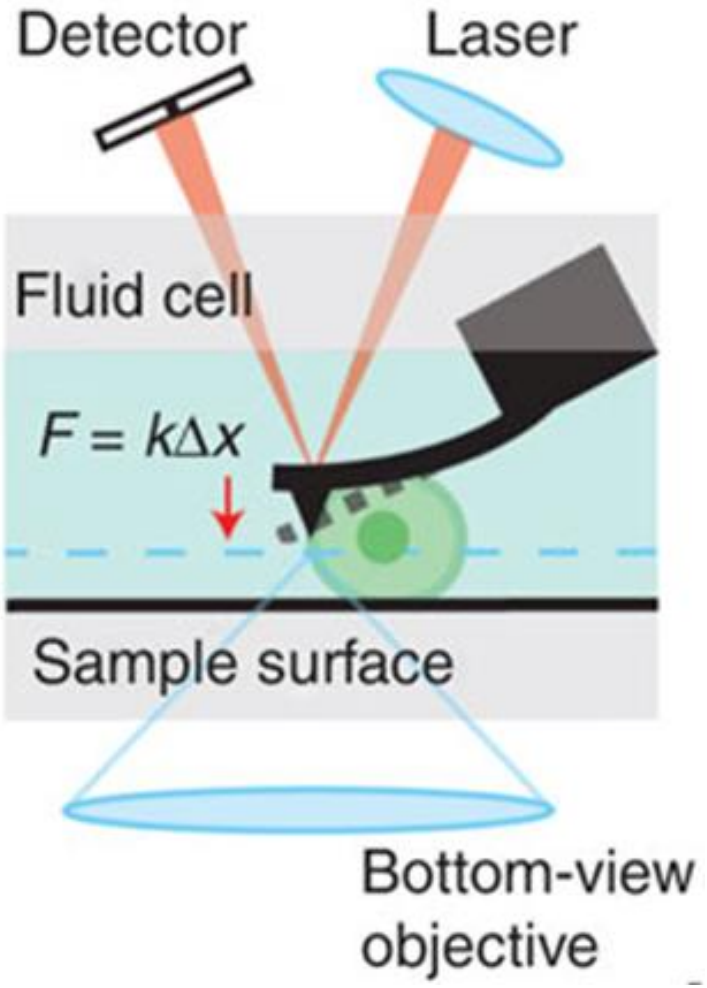
Combining AFM with Optical Microscopy (AFM/ILM)

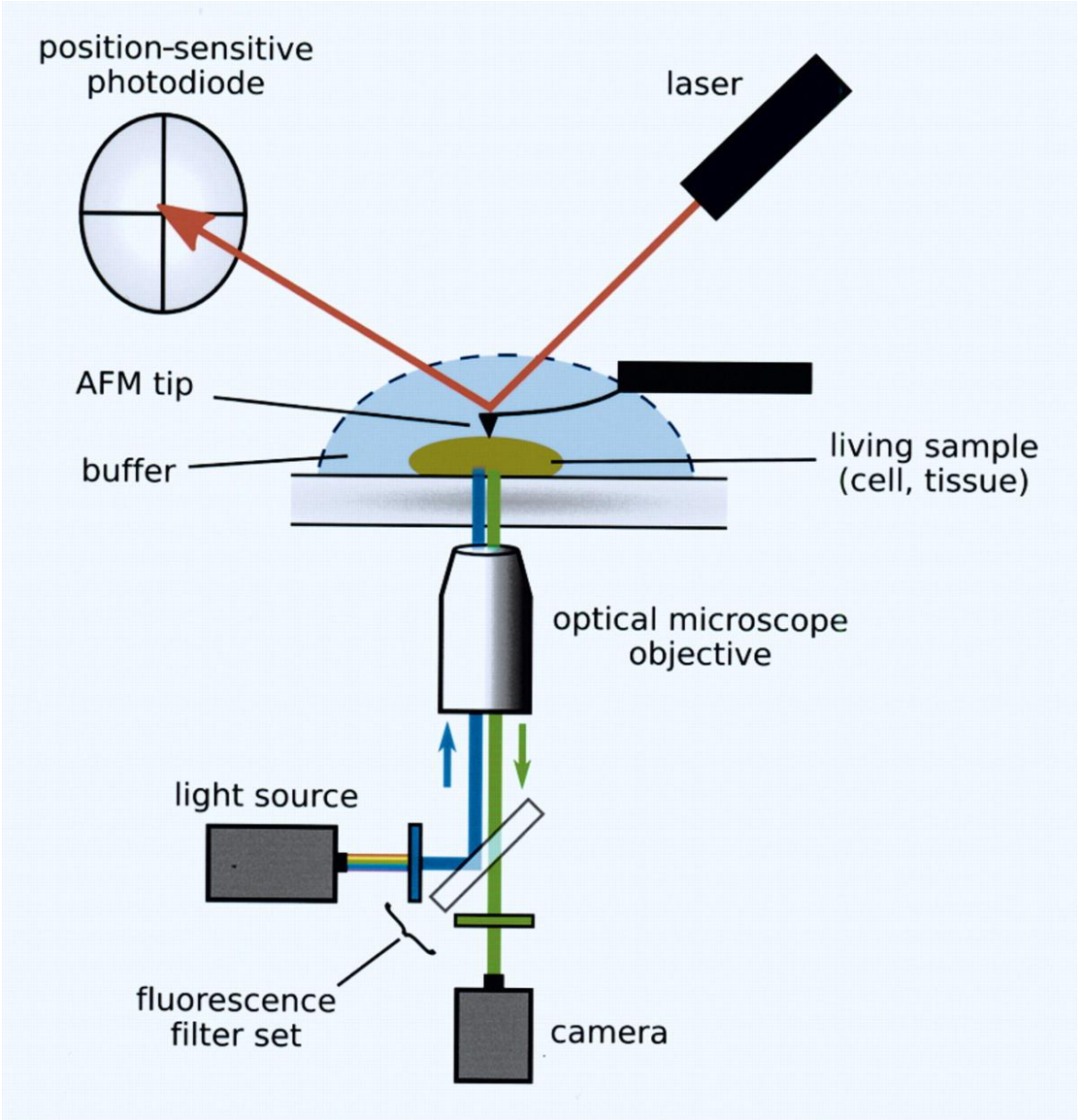


Two approaches to AFM scanning in liquid with a probe-scanning microscope.

1) **Scanning in a droplet of water (left) is simpler, but allows less control of the solution.**
2) **Scanning in an AFM fluid cell (right) allows exchange of fluids, and control of fluid temperature, ionic strength, etc.**

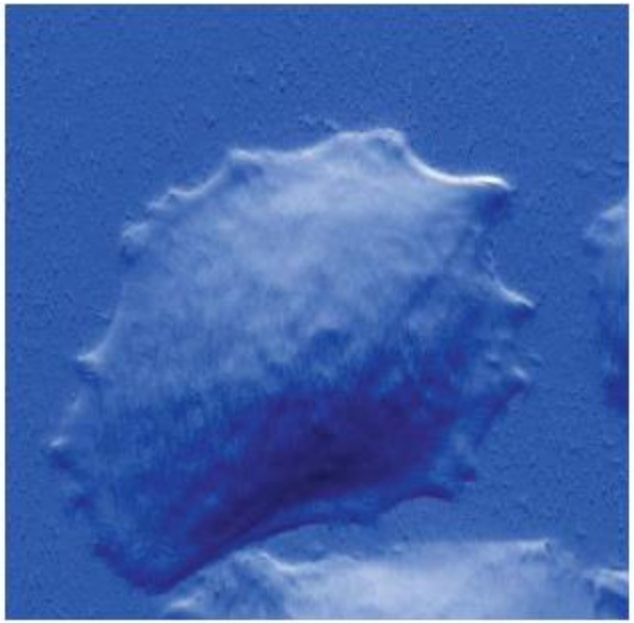
Combining AFM with Optical Microscopy (AFM/ILM)





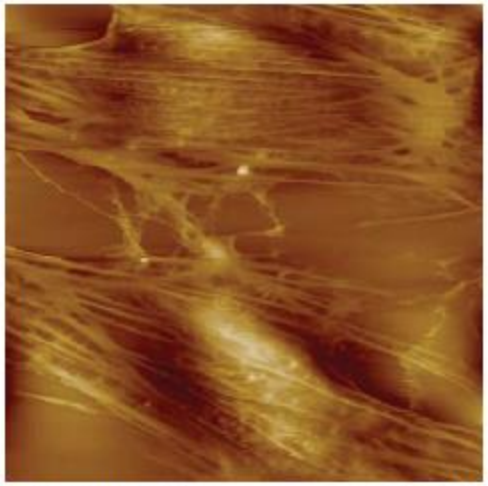
http://cshprotocols.cshlp.org/content/2010/10/pdb.prot5500/F3.large.jpg

Combining AFM with Optical Microscopy (AFM/ILM)

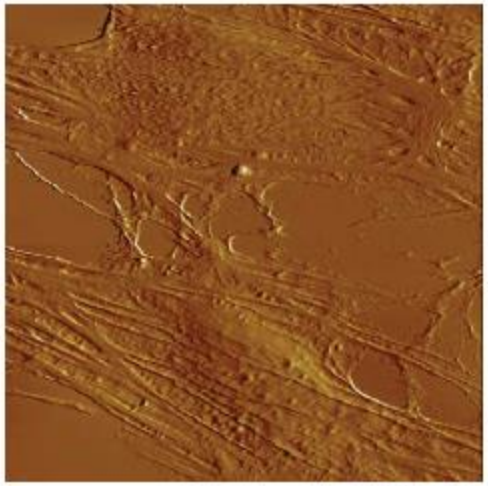


Human lung cancer cell.
Scan size: 45 µm.

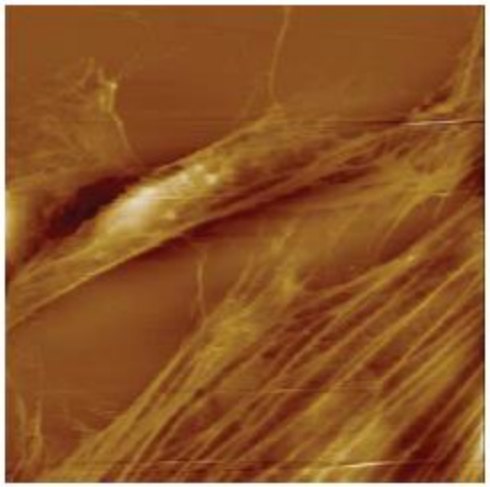
Bovine capillary endothelial cells.



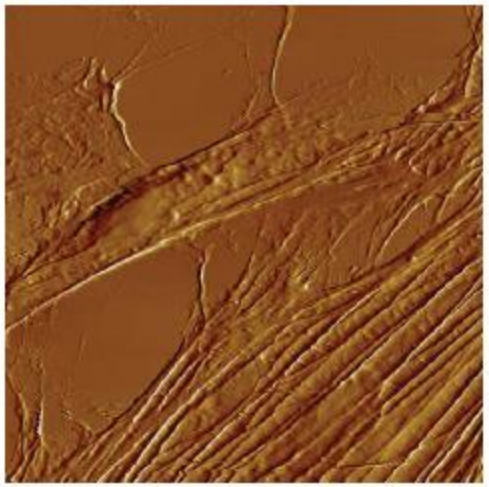
Scan size: 105 µm. Topograph



Deflection



Scan size: 56 µm. Topograph

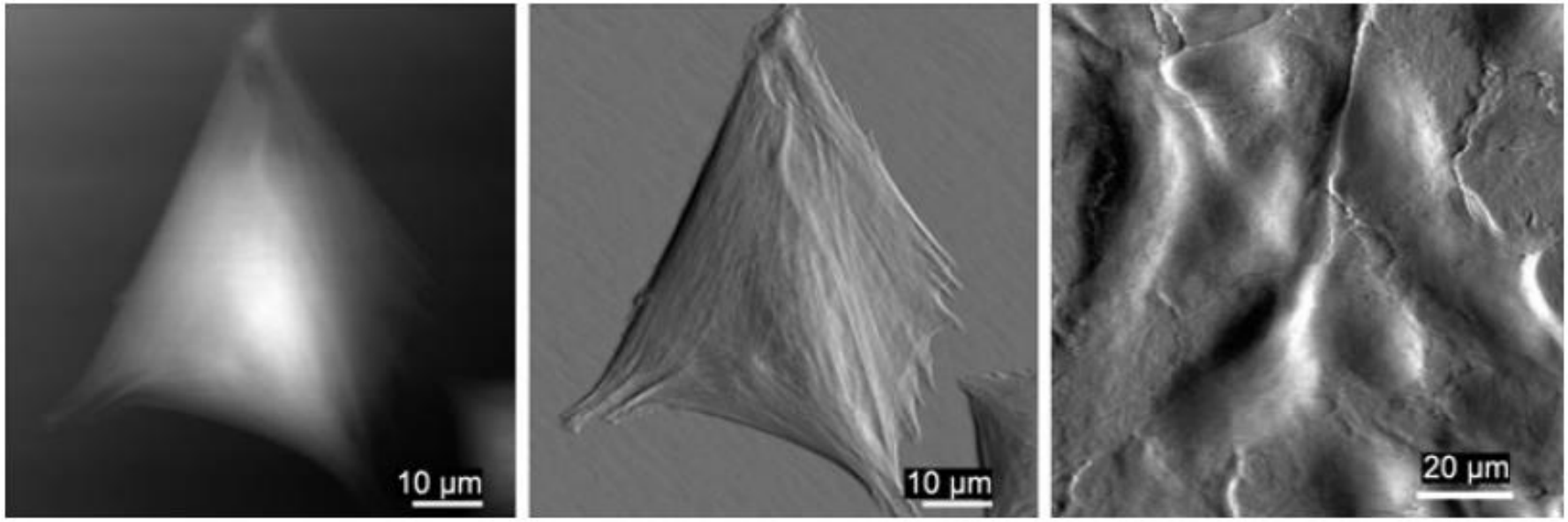


Deflection

http://www.htest.cz/download/AFM_Live%20cell%20imaging%20with%20ILM.pdf

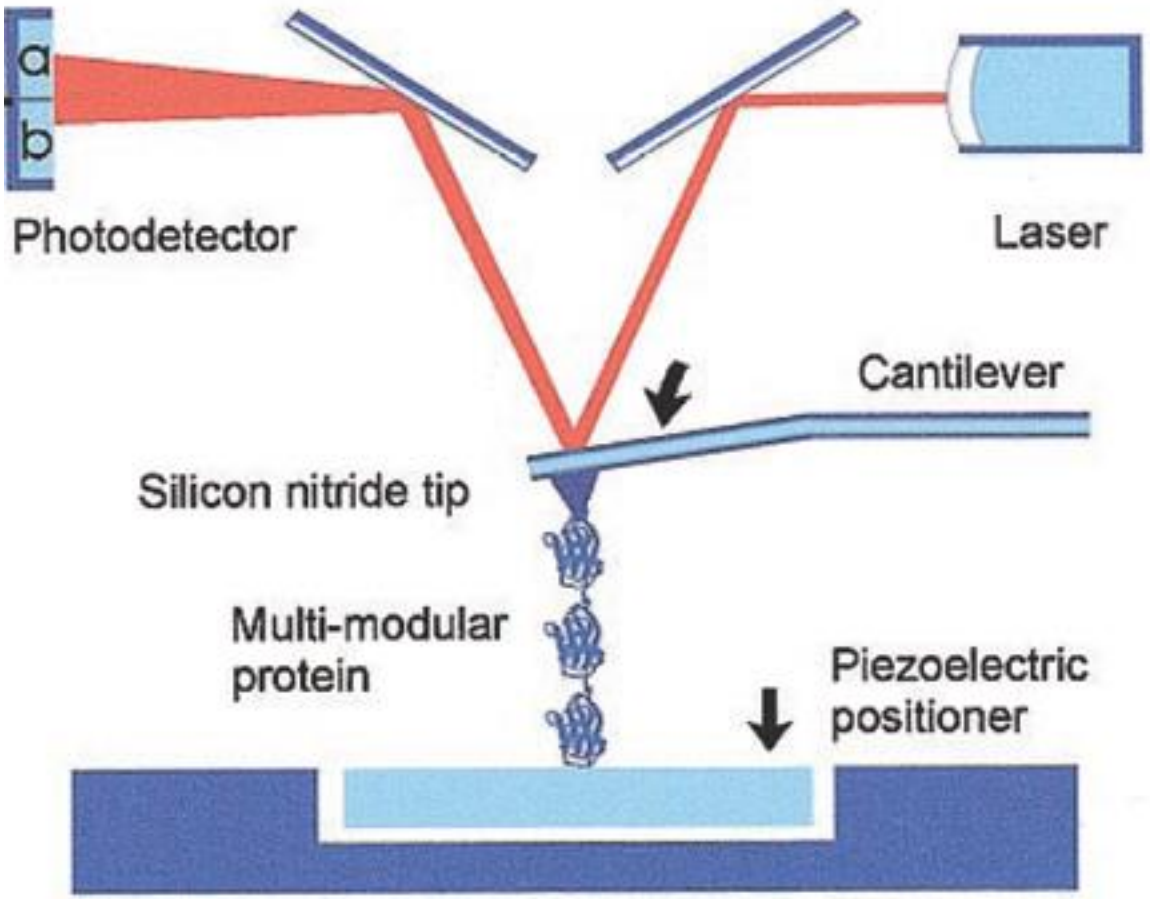
Combining AFM with Optical Microscopy (AFM/ILM)

Examples of AFM imaging of live animal cells

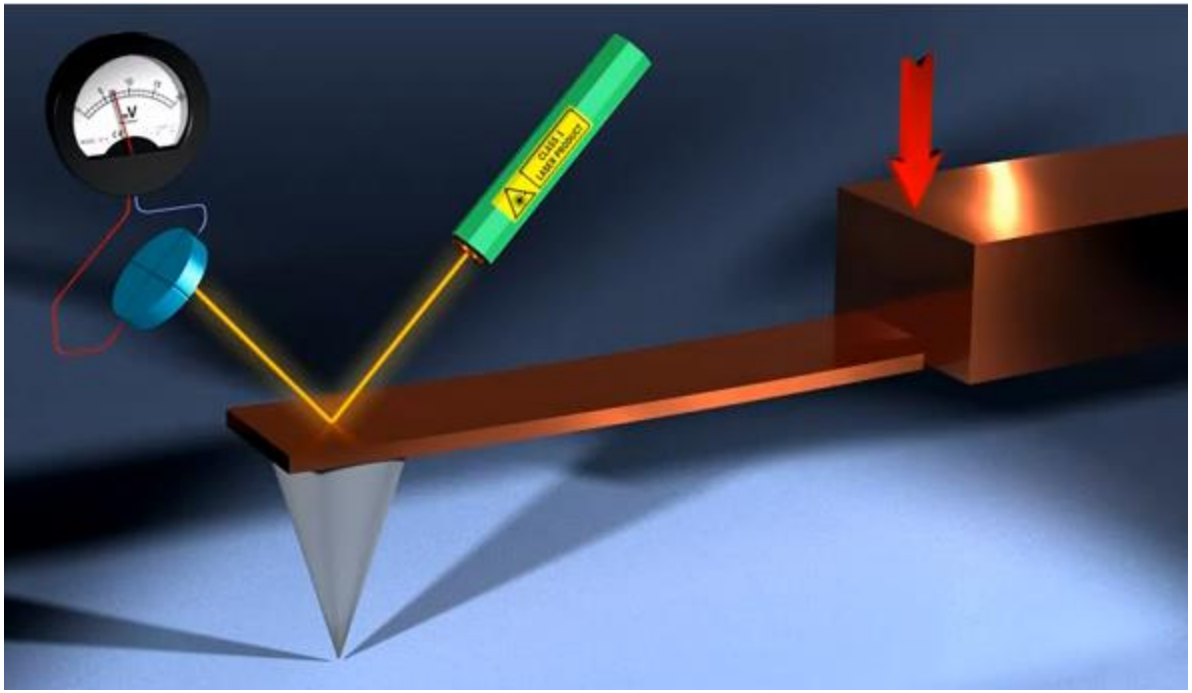


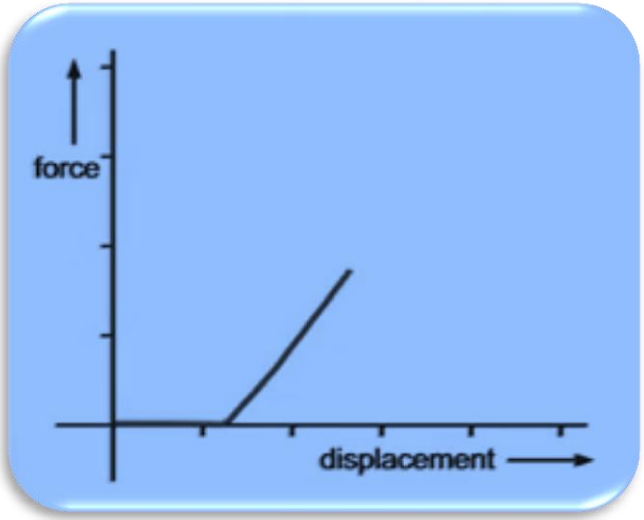
Left: contact-mode images; height and deflection images of osteoblast cells on a polystyrene culture dish (height image z-scale: 3.9 µm). Right: IC-AFM image (amplitude image) living fibroblast cell layer on PLL-coated glass slide. With cells, error signal images such as the deflection and amplitude images shown here are usually presented as they show more details.

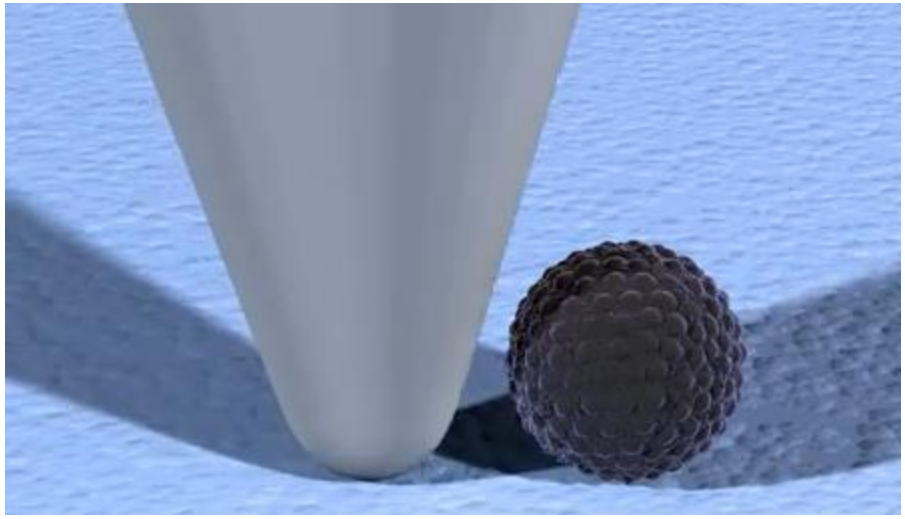
Biological Applications of the AFM: From Single Molecules to Organs

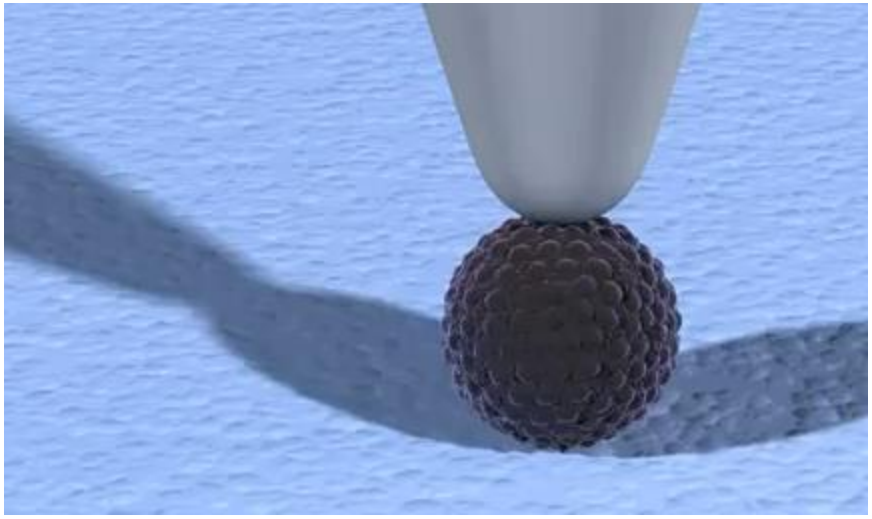


Virus Nanoindentation by AFM









https://www.youtube.com/watch?v=MZb8C0f7Kdg

Thanks For Your Attention