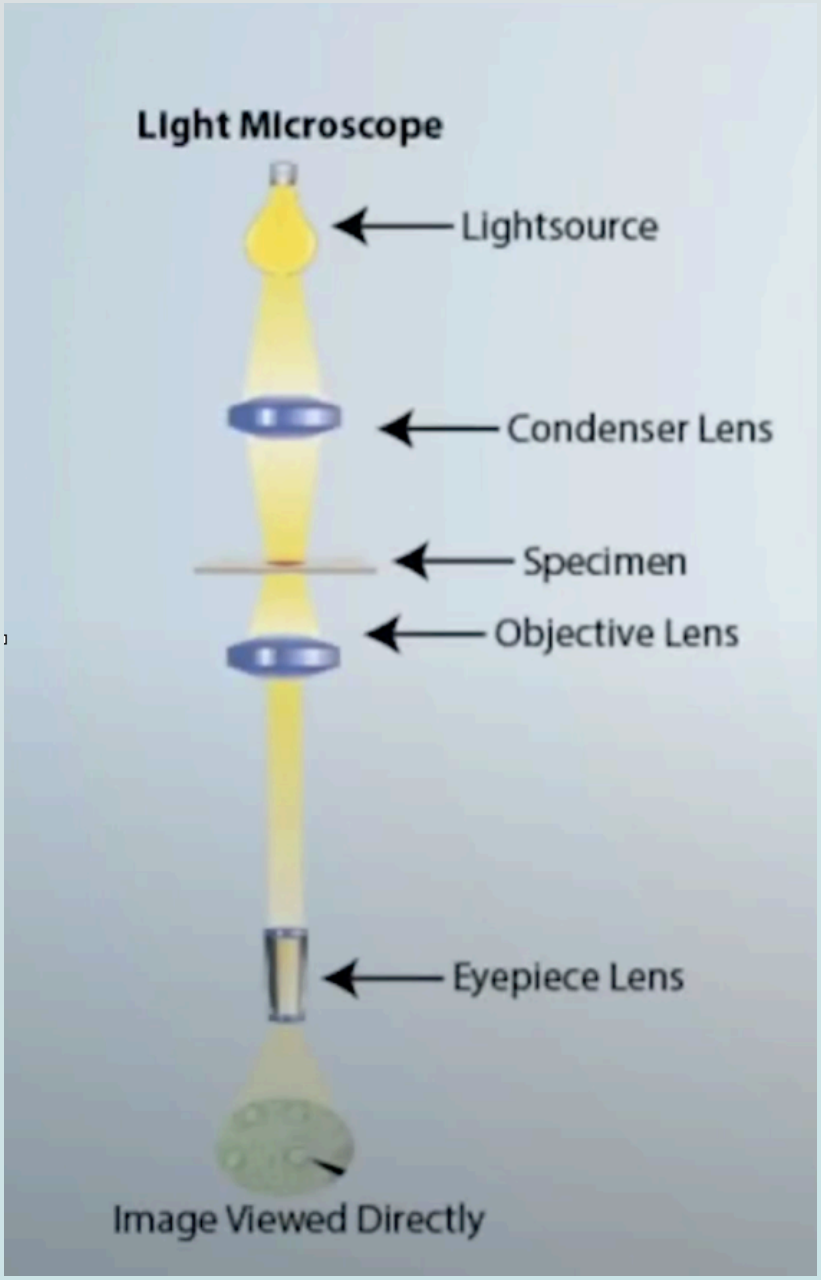
SUMMER INSTITUTE - ONLINE MODALITY CALENDAR 2022

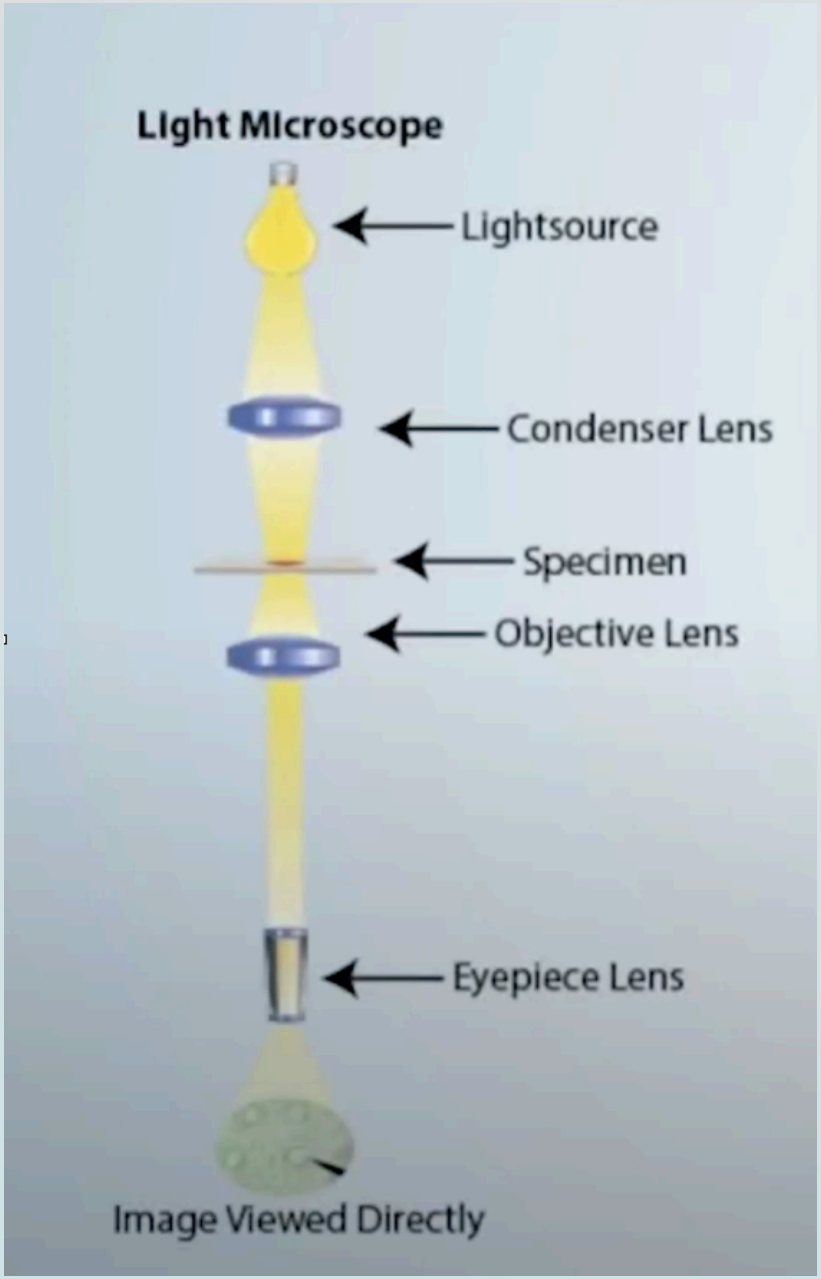
SUN	MON	TUE	WED	THU	FRI	SAT
June 26	27	28	29	30	31	July 02
	9:00-10:00am Virtual Program Orientation for Summer Institute Online Modality					
July 03	04	05	06	07	08	09
	Holiday (Independence Day)	8:30-10:00am -Welcome Reception and Buddy Meet & Greet Event	Free Day	Classes begin! 8:30-11am: BIOL4905 INTRODUCTION 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 DNA PREPARATION 8-10:20pm: Afternoon course	
10	11	12	13	14	15	16
	8:30-11am:BIOL4905 PROTEOMICS I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS II 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 PROTEOMICS III 8-10:20pm: Afternoon course	8:30-11am: BIOL4905 RNA PREPARATION 8-10:20pm: Afternoon course	Virtual Independence Day Activity	
17	18	19	20	21	22	23
	8:30-11am:BIOL4905 qPCR / ROBOTS 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 DNA Sequence Analysis 8-10:20pm: Afternoon course	Midterm Break		8:30-11am:BIOL4905 Next Gen. Sequencing 8-10:20pm: Afternoon course	
24	25	26	27	28	29	30
	8:30-11am:BIOL4905 Microarray I 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Nanostring 8-10:20pm: Afternoon course	8:30-11am:BIOL4905 Automated Microscopy /AFM	8:30-11am:BIOL4905 Flow Cytometry 8-10:20pm: Afternoon course	FINALS	
31	August 01	02	03			
	9:00-10:00am: Closing Reception		Grades available in PAWS			

Legend:
Orange: Courses Blue: Activities

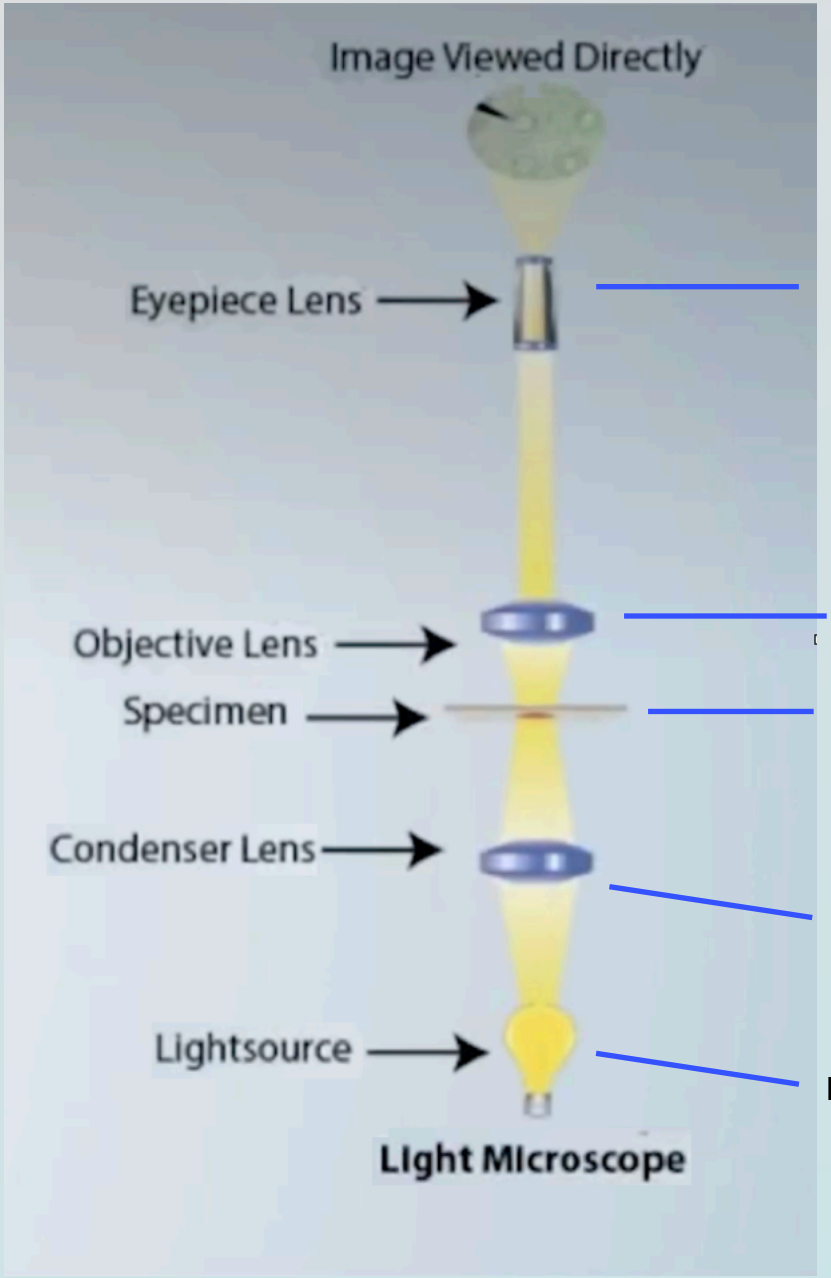
Light Microscopy

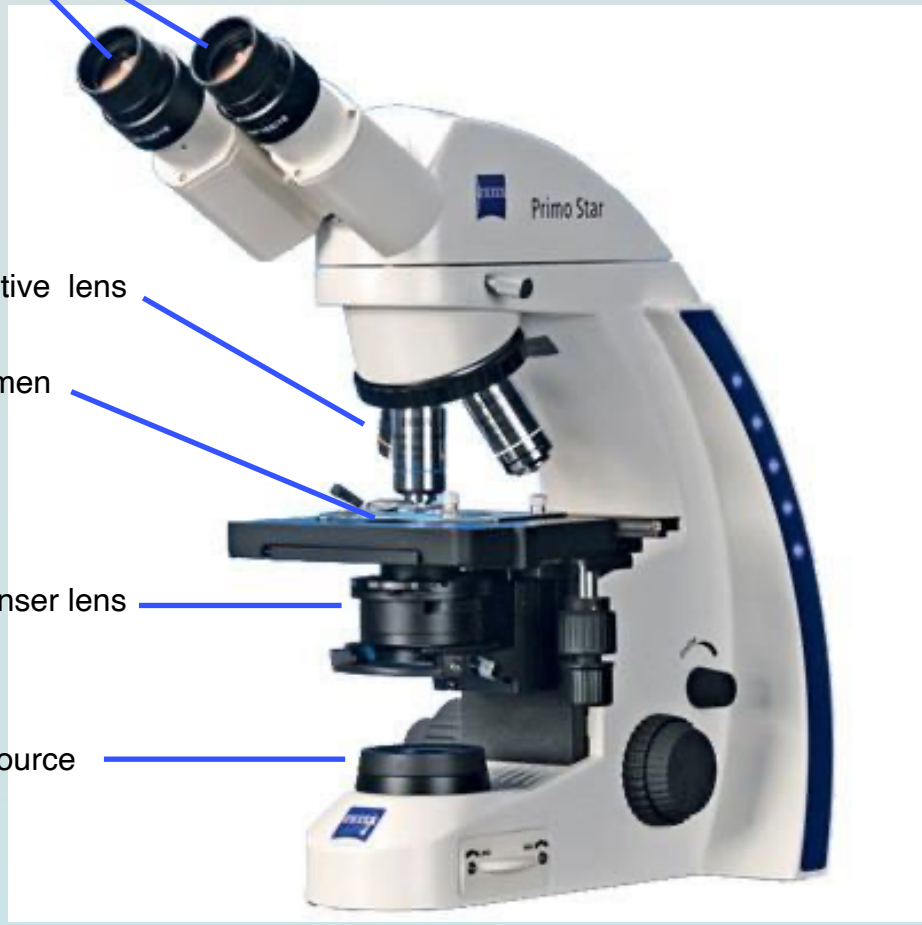


Zeiss Primo Star
Binocular Microscope



Zeiss Primo Star Binocular Microscope





Zeiss Primo Star Binocular Microscope

Fluorescence Microscopy

Epifluorescence Microscopy

The Majority of Fluorescence microscopes, especially those used in Biology, are of the epifluorescence design

Light of the excitation wavelength is focused on the specimen through the **objective** lens.

The fluorescence emitted by the specimen is also focused on the detector by the objective lens

Since most of the excitation light is transmitted through the specimen, ONLY reflected excitatory light reaches the objective -together with the emitted light.

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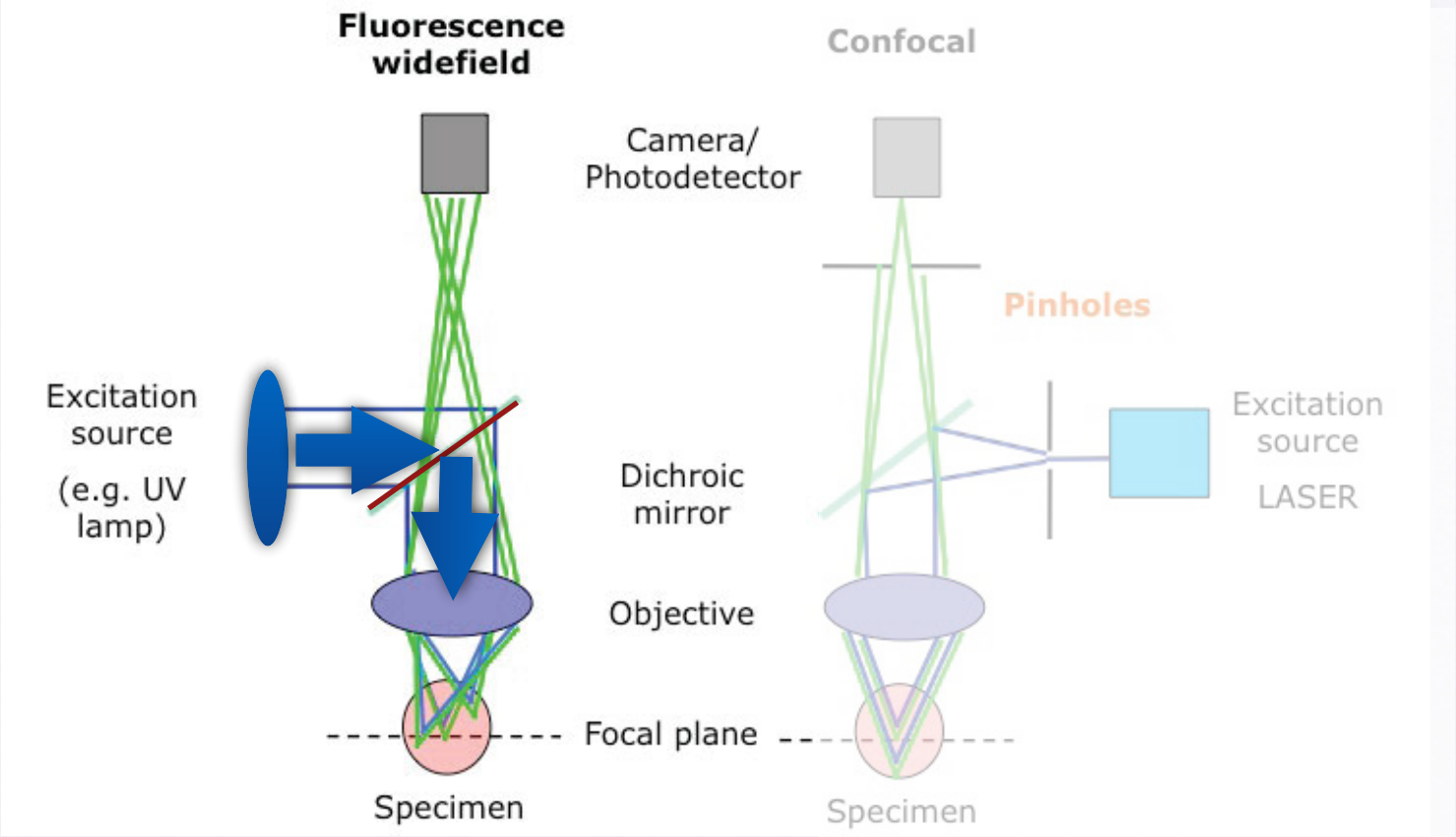
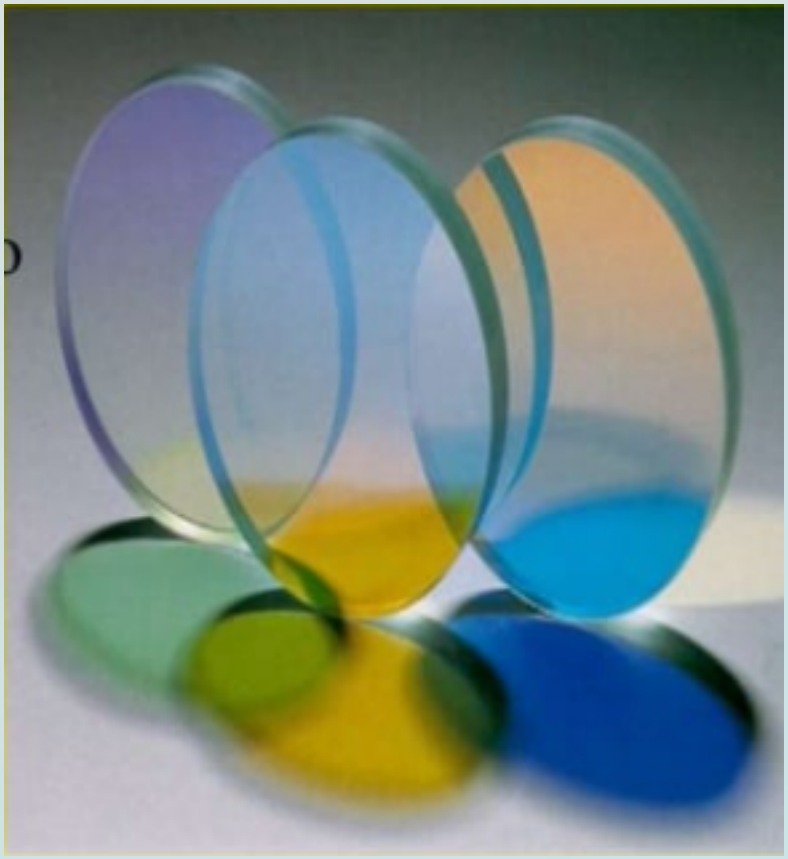


Two Common Forms of Fluorescence Microscopes

Epifluorescence Microscopy

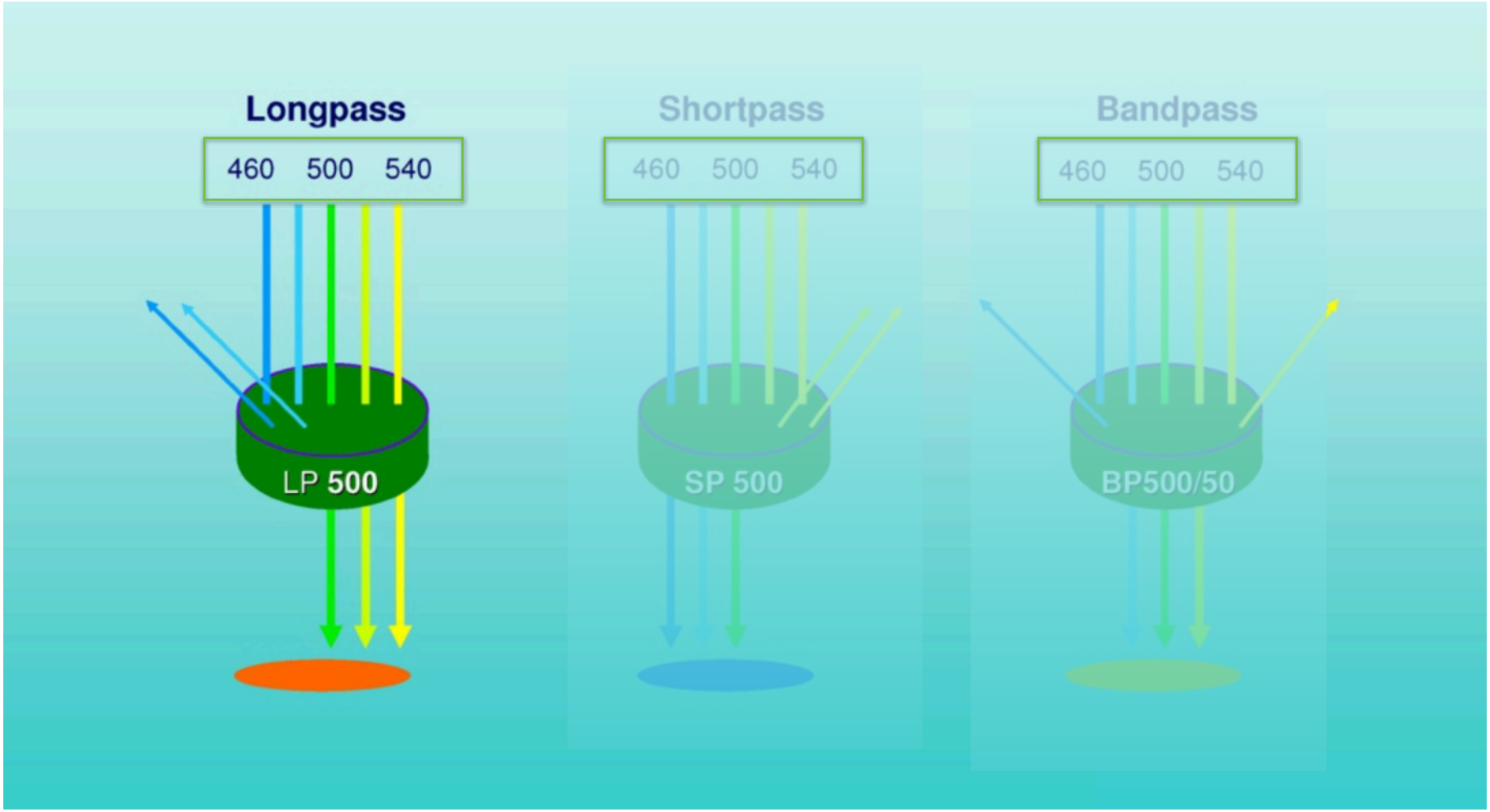
Zeiss Axioimager 2 Fluorescence Microscope

Optical Filters



A **dichroic filter**, or interference filter is a color filter used to selectively pass light of a small range of colors while reflecting other colors.

Optical Filters



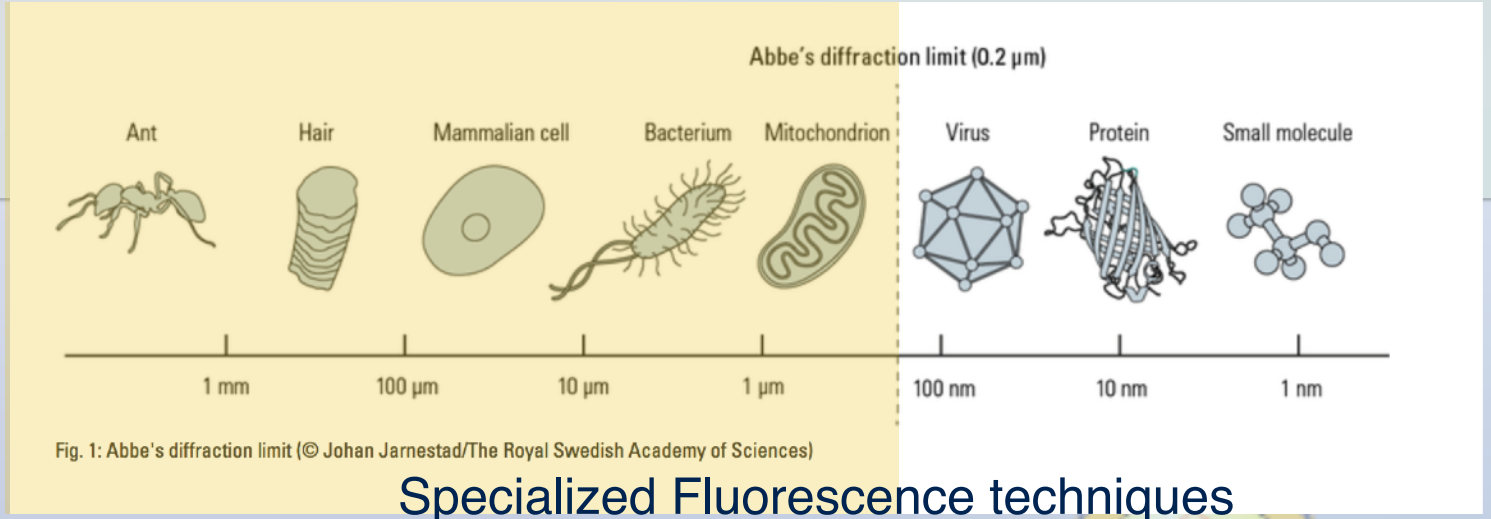
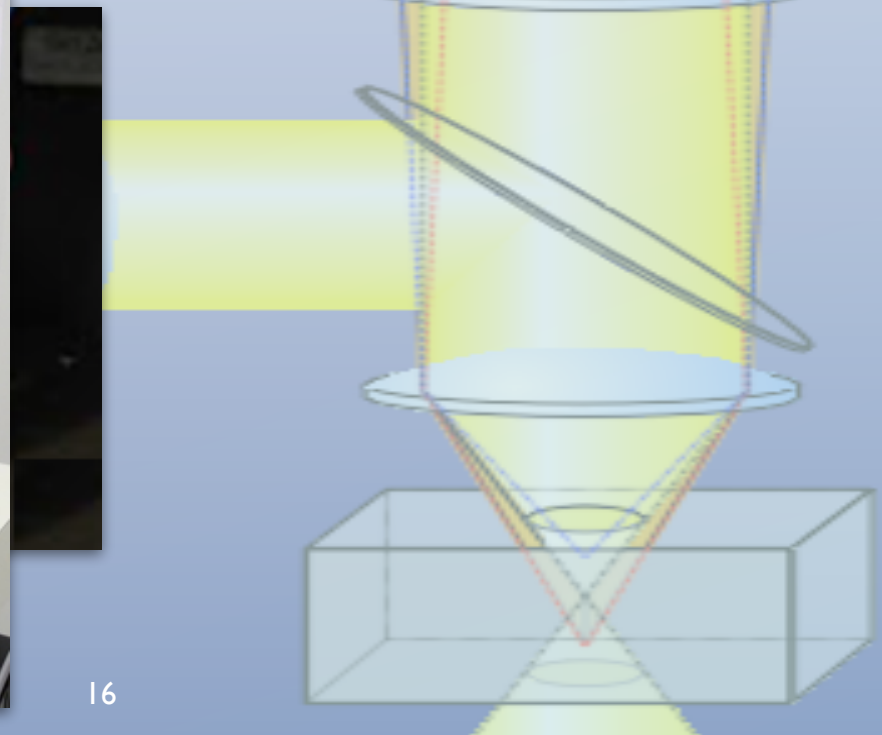
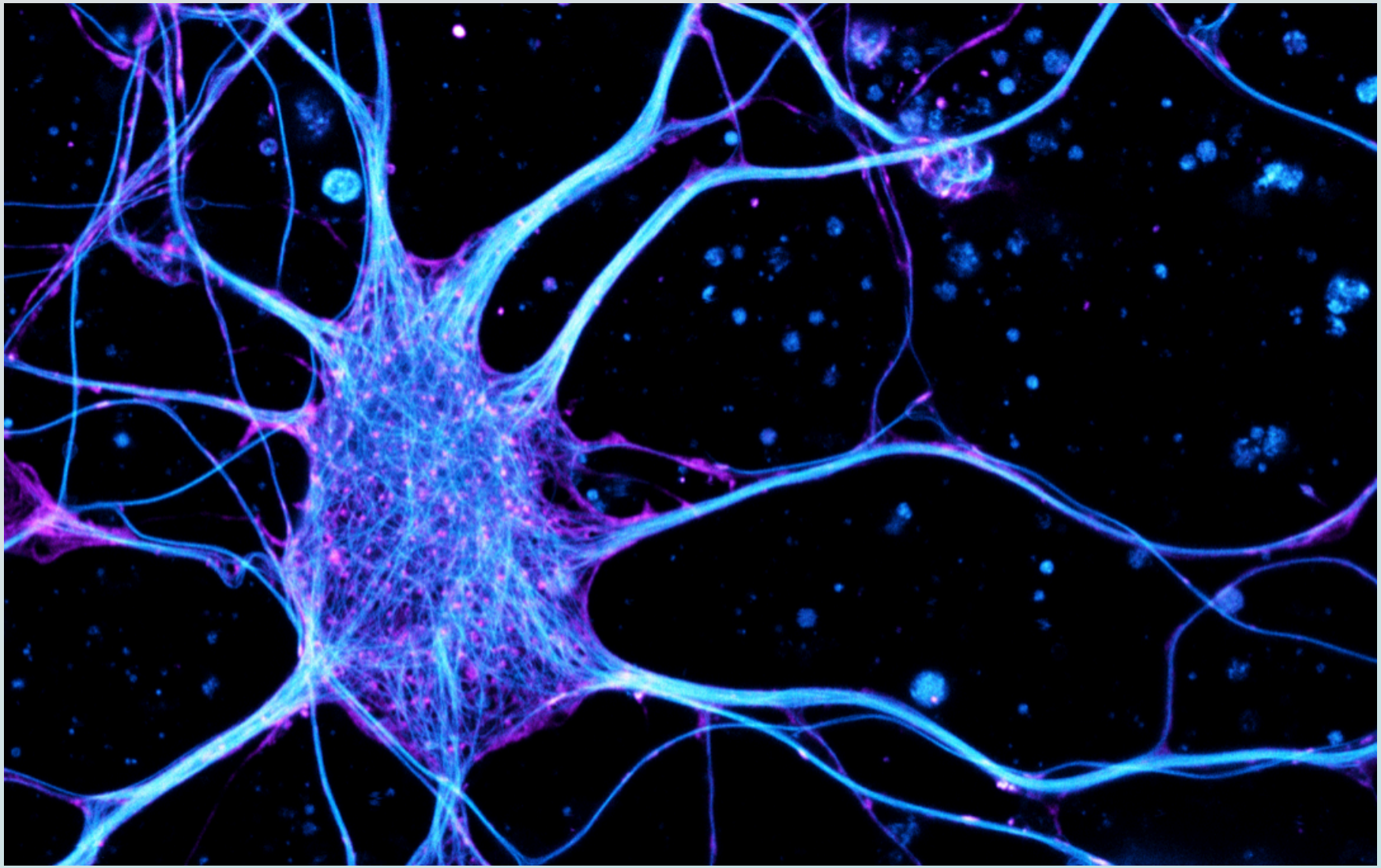


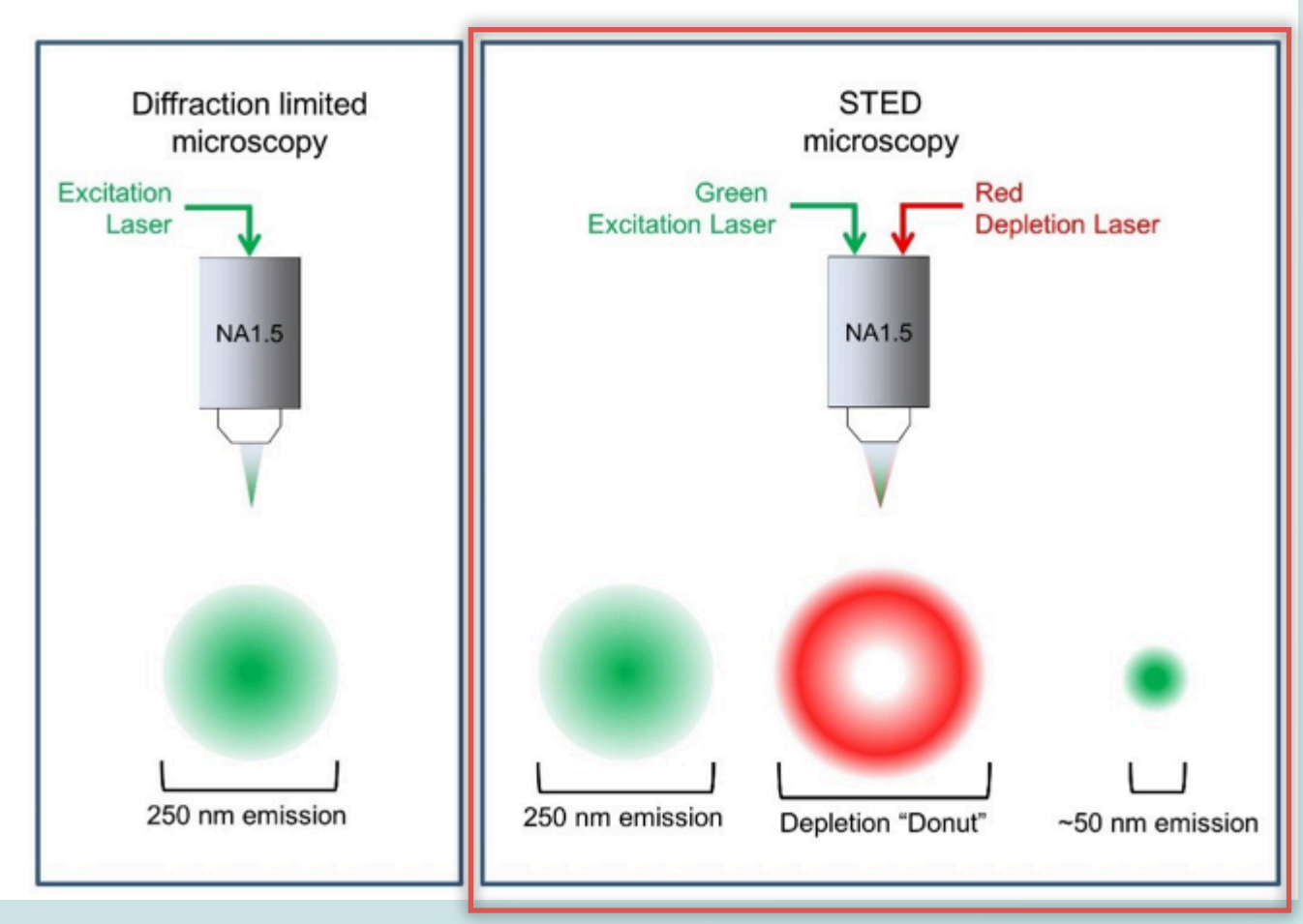
Fig. 1: Abbe's diffraction limit (© Johan Jarnestad/The Royal Swedish Academy of Sciences)

Specialized Fluorescence techniques (STimulated Emission Depletion Microscopy (STED)





A STED (stimulated emission/depletion) micrograph image revealing actin (magenta) and microtubules (cyan) of a young dissociated hippocampal neuron. Image by K. Jansen and E. Katrukha, Kapitein Lab, Molecular and Cellular Biophysics, Utrecht University, The Netherland



Specialized Fluorescence techniques
(STimulated Emission Depletion Microscopy (STED)

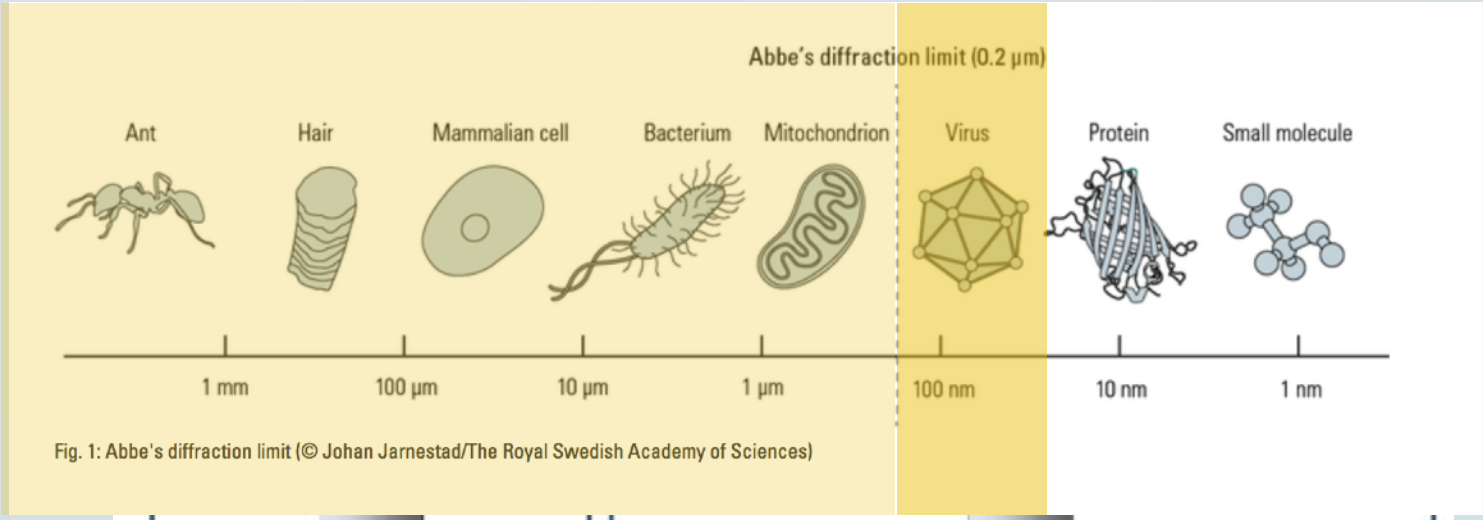
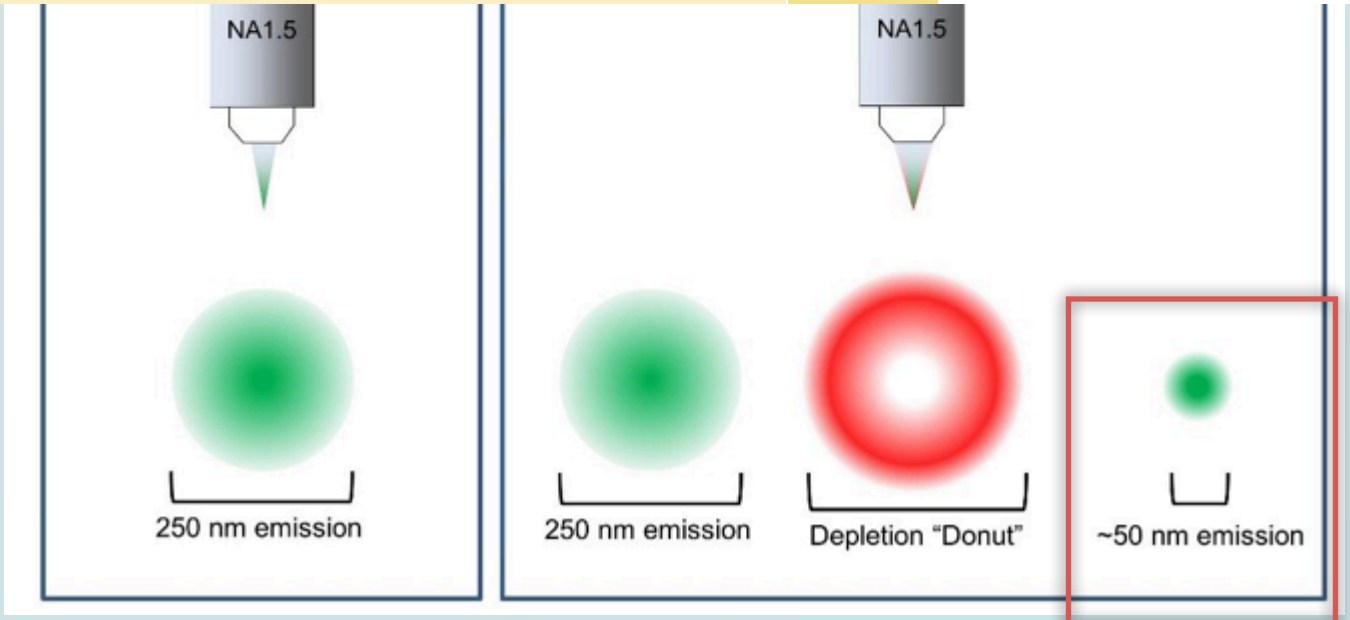


Fig. 1: Abbe's diffraction limit (© Johan Jarnestad/The Royal Swedish Academy of Sciences)



Specialized Fluorescence techniques
(STimulated Emission Depletion Microscopy (STED)

Keyence BZX-Series Automated Fluorescence Microscope

Keyence BZ X700
Fluorescence
High Resolution Optics
Automated Microscope

All-in-One Fluorescence Microscope

The BZ-X700 can be configured to accommodate specific research objectives. Capabilities include:

- Imaging in brightfield and phase contrast
- Automated XYZ stitching of large histology sections
- Screening and quantification of well plates
- Time-lapse incubation for cell culture

Keyence BZ X700
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High Resolution Optics
Automated Microscope

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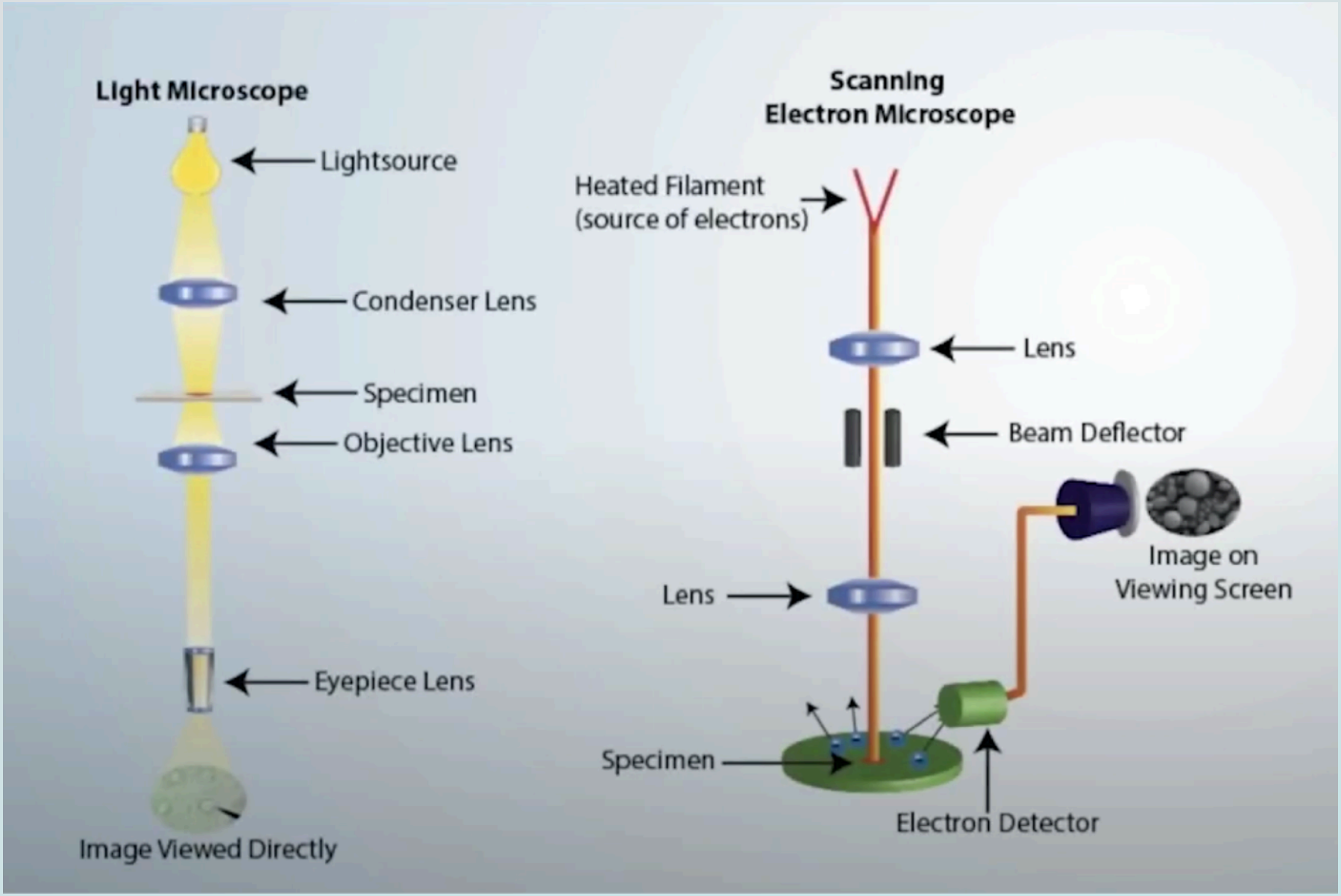
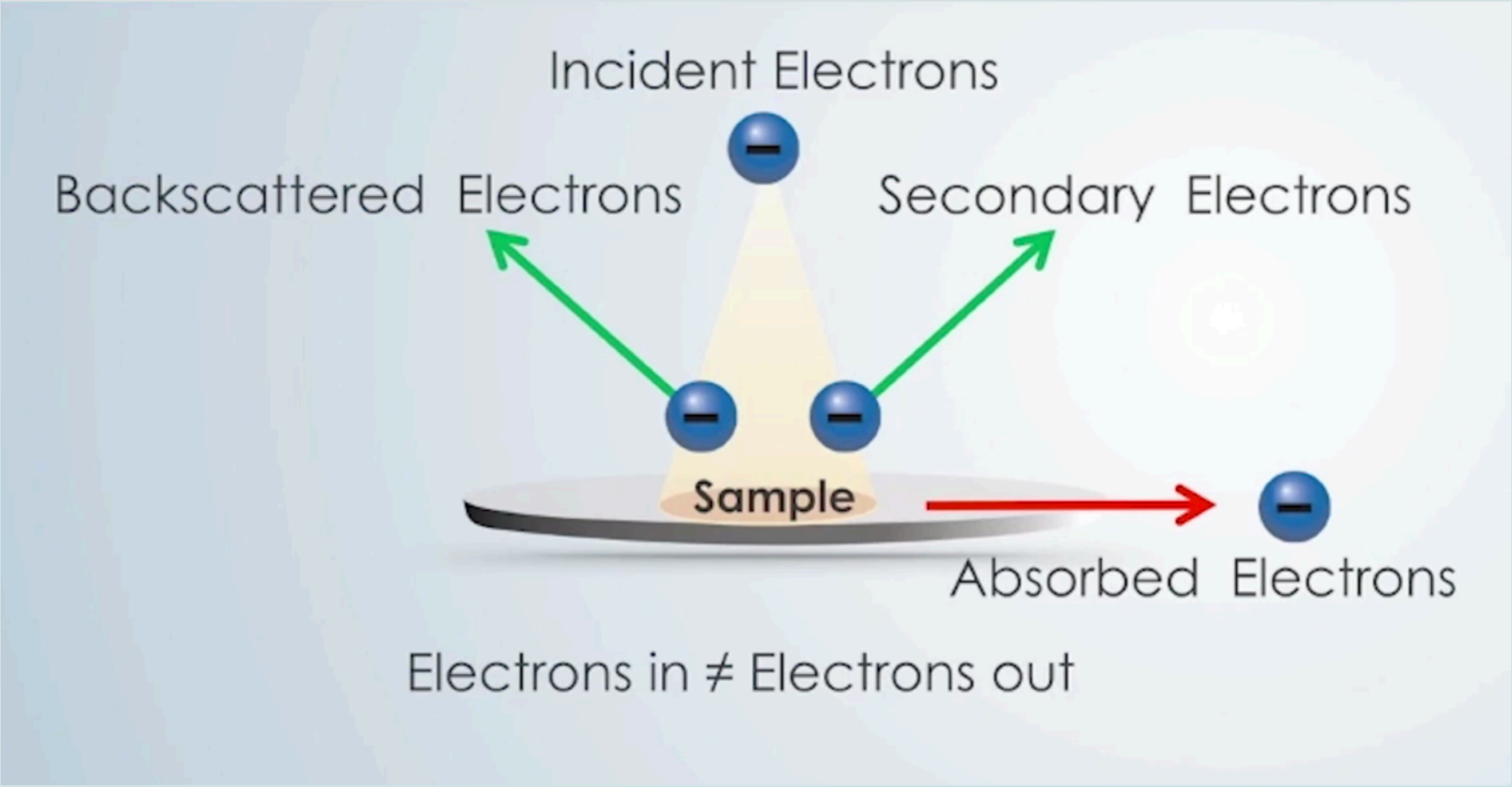
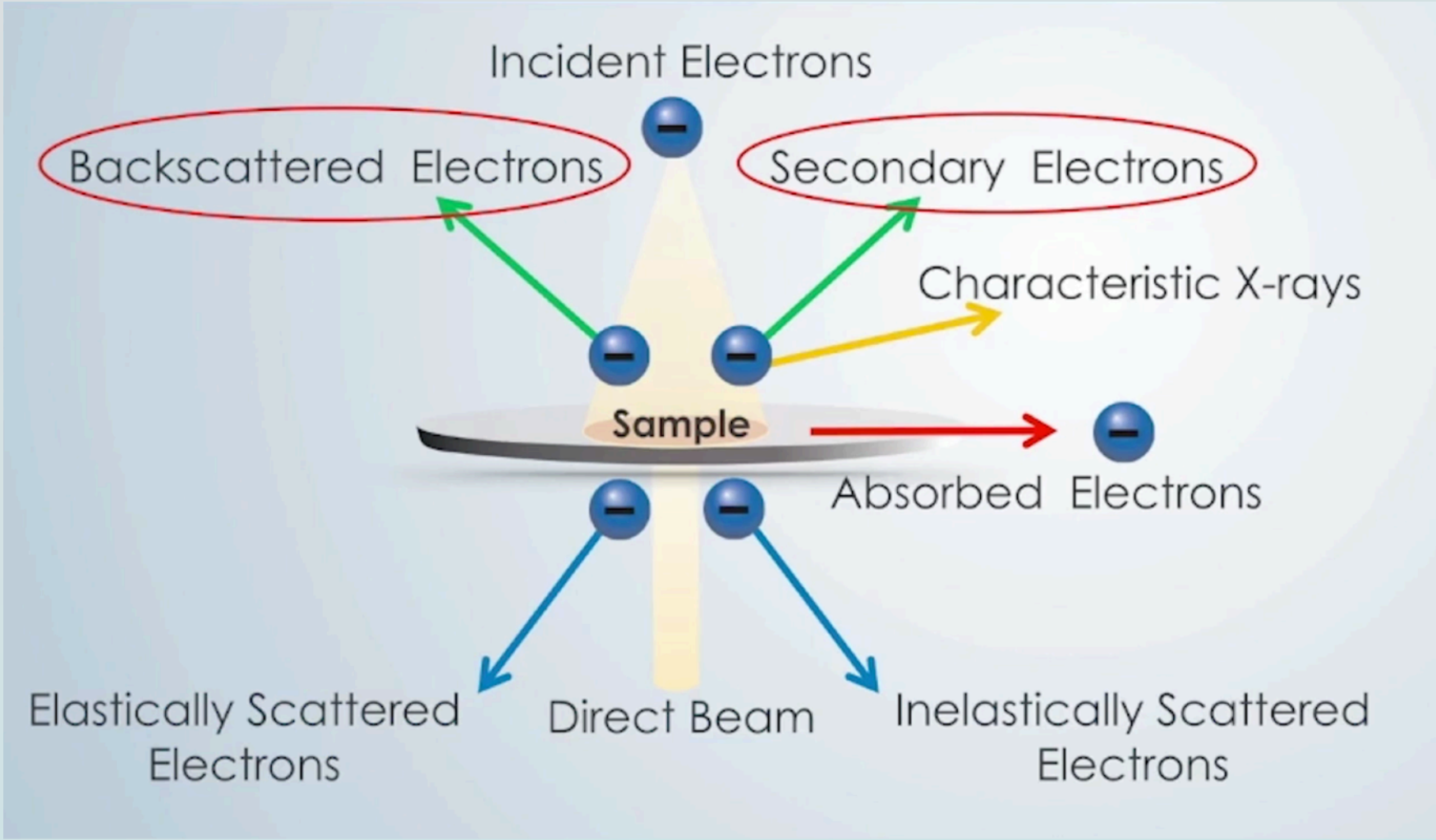
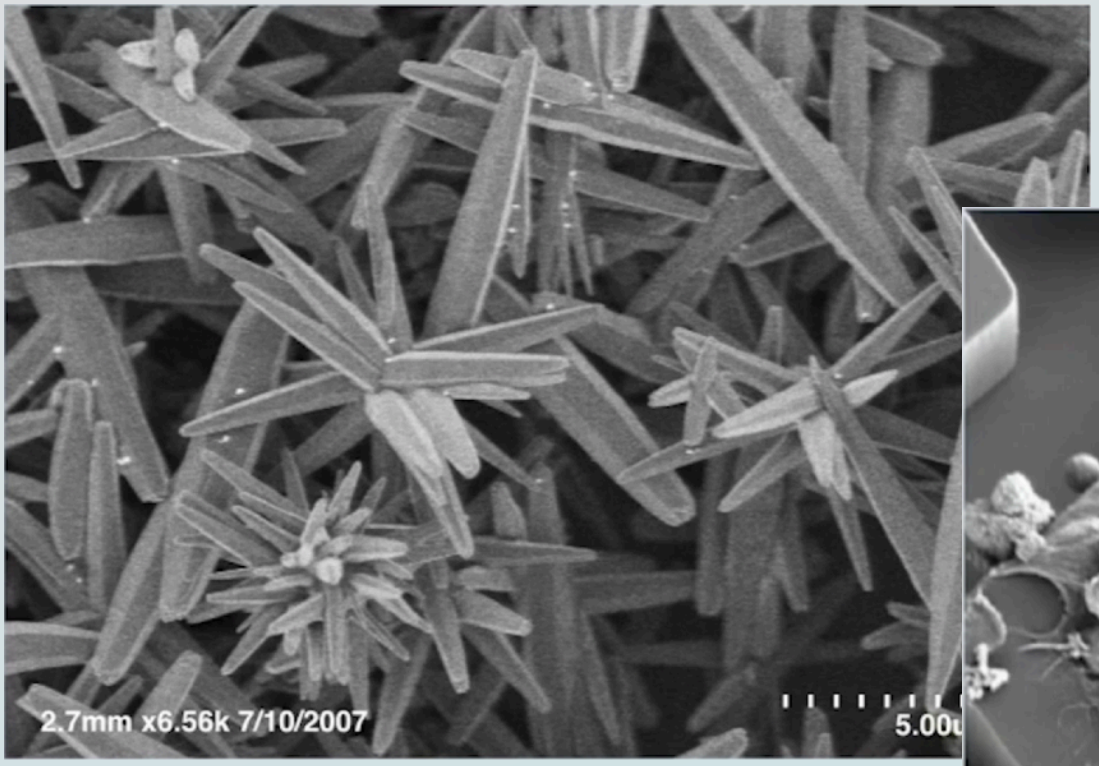
Keyence BZX-Series Microscopy

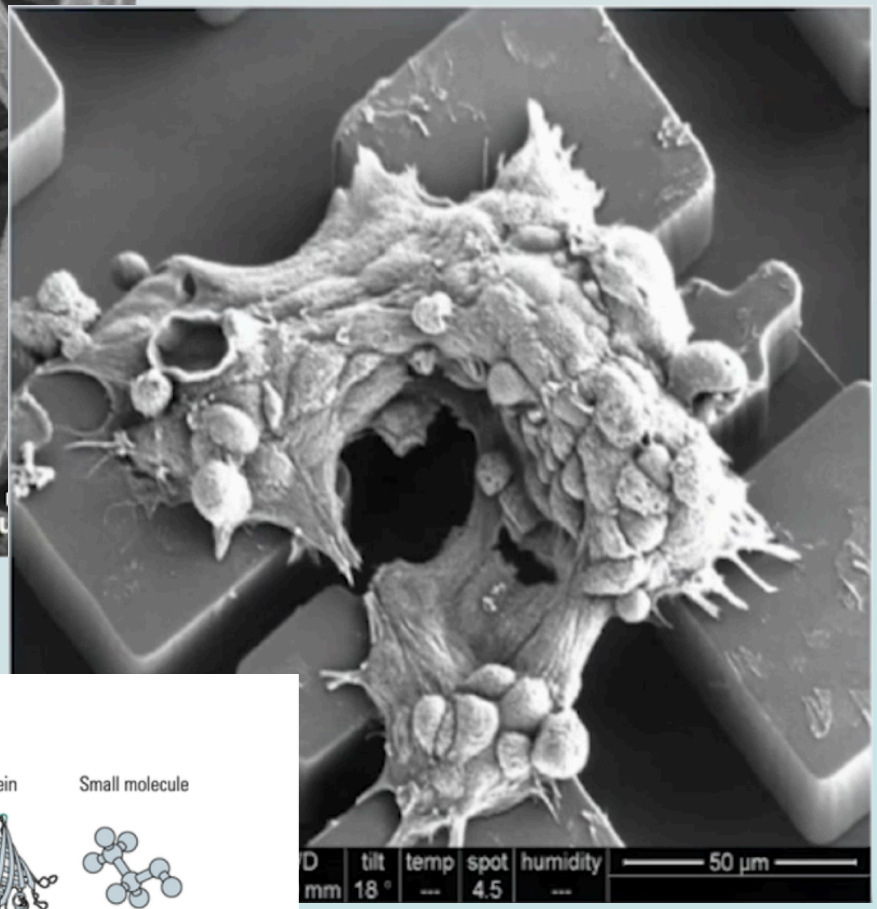
Scanning Electron Microscopy


Light Microscope
Lightsource
Condenser Lens
Specimen
Objective Lens
Eyepiece Lens
Image Viewed Directly
Scanning
Electron Microscope
Heated Filament
(source of electrons)
Lens
Beam Deflector
Image on
Viewing Screen
Lens
Specimen
Electron Detector


Incident Electrons
Backscattered Electrons
Secondary Electrons
Sample
Absorbed Electrons
Electrons in ≠ Electrons out


Incident Electrons
Backscattered Electrons
Secondary Electrons
Characteristic X-rays
Sample
Absorbed Electrons
Elastically Scattered Electrons
Direct Beam
Inelastically Scattered Electrons





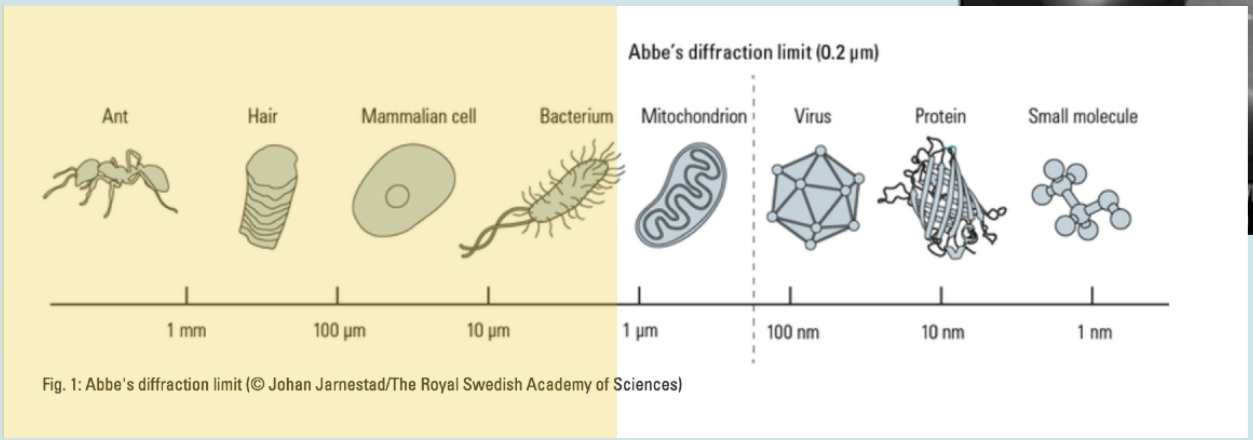
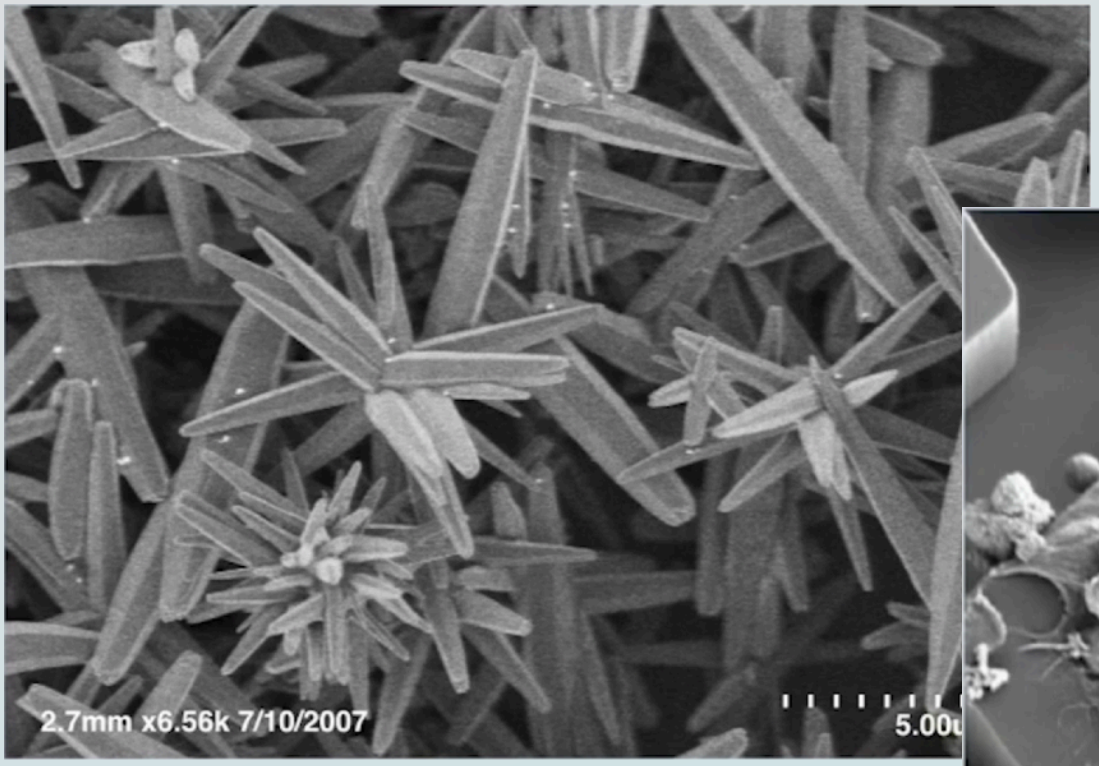
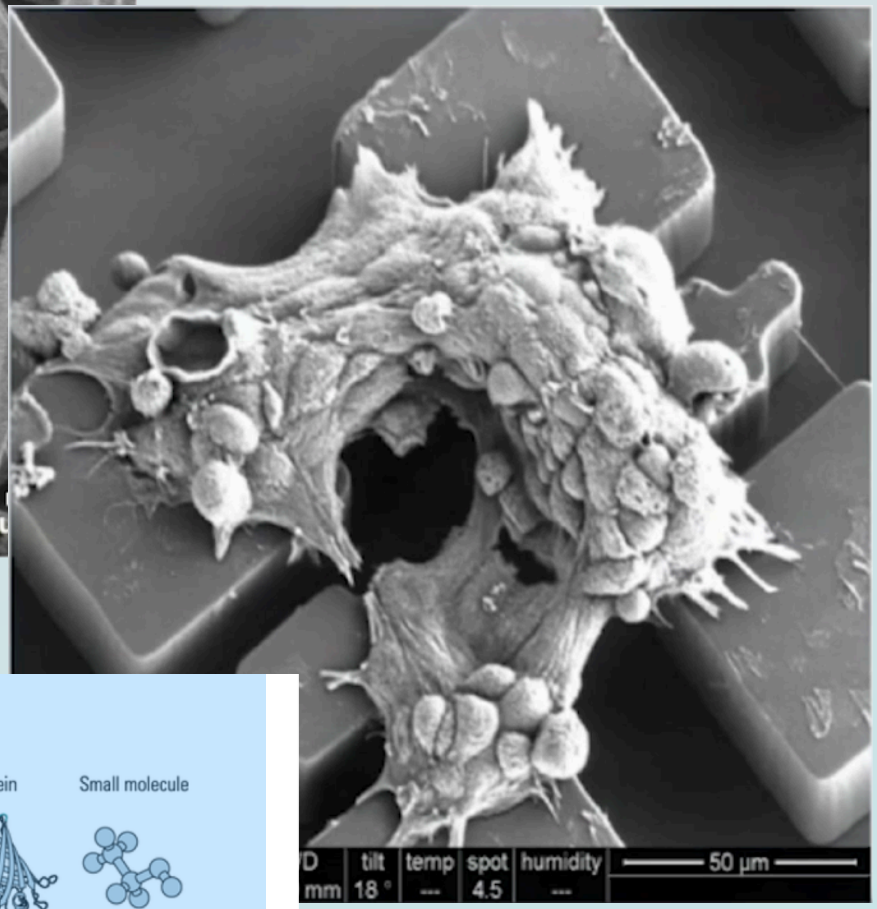


Fig. 1: Abbe's diffraction limit (© Johan Jarnestad/The Royal Swedish Academy of Sciences)





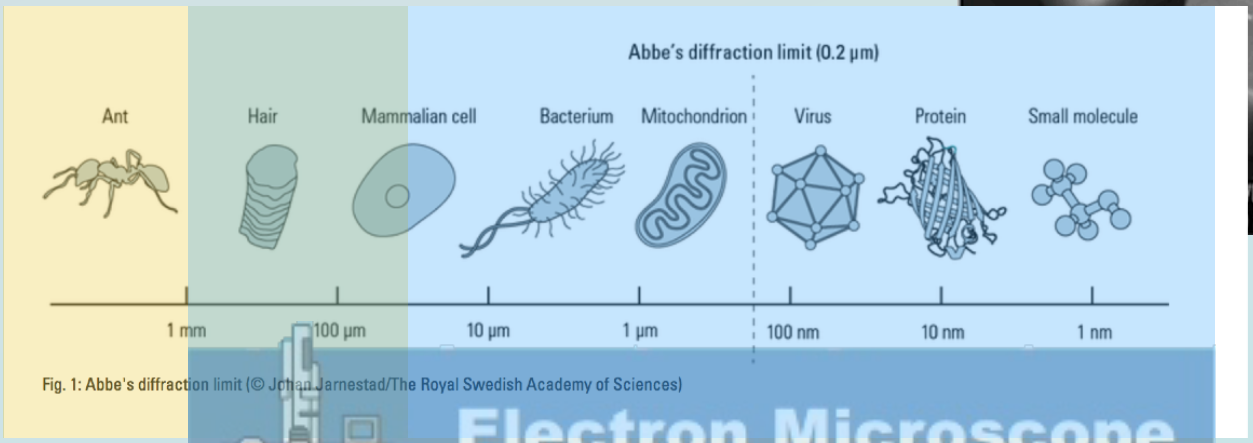


Fig. 1: Abbe's diffraction limit (© Johan Jarnestad/The Royal Swedish Academy of Sciences)

Electron Microscope

Atomic Force Microscopy

Introduction/History

Atomic Force Microscope (AFM) is part of a larger family of **Scanning Probe Microscopes** (SPM).

The **First SPM** was invented in 1981 by Gerd Binnig and Heinrich Rohrer (Nobel Prize, 1986) and acquired images by detecting the difference in electrical potential between two objects on a slide.

AFM technology, however, really came of age in 1986 when Gred Binnig, Calvin F. Quate, and Christoph Gerber, generated images based on the attraction and repulsion forces between the scanning tip and the objects on the slide, and then again…

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Review

Nature Nanotechnology **3**, 261 - 269 (2008)
doi:10.1038/nnano.2008.100

Subject Categories: Molecular machines and motors | Nanobiotechnology | Nanometrology and instrumentation | Structural properties

Atomic force microscopy as a multifunctional molecular toolbox in nanobiotechnology

Daniel J. Müller[1] & Yves F. Dufrêne[2]

With its ability to observe, manipulate and explore the functional components of the biological cell at subnanometre resolution, atomic force microscopy (AFM) has produced a wealth of new opportunities in nanobiotechnology. Evolving from an imaging technique to a multifunctional 'lab-on-a-tip', AFM-based force spectroscopy is increasingly used to study the mechanisms of molecular recognition and protein folding, and to probe the local elasticity, chemical groups and dynamics of receptor–ligand interactions in live cells. AFM cantilever arrays allow the detection of bioanalytes with picomolar sensitivity, opening new avenues for medical diagnostics and environmental monitoring. Here we review the fascinating opportunities offered by the rapid advances in AFM.

top

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Correspondence to: Daniel J. Müller[1] e-mail: daniel.mueller@biotec.tu-dresden.de

Correspondence to: Yves F. Dufrêne[2] e-mail: yves.dufrene@uclouvain.be

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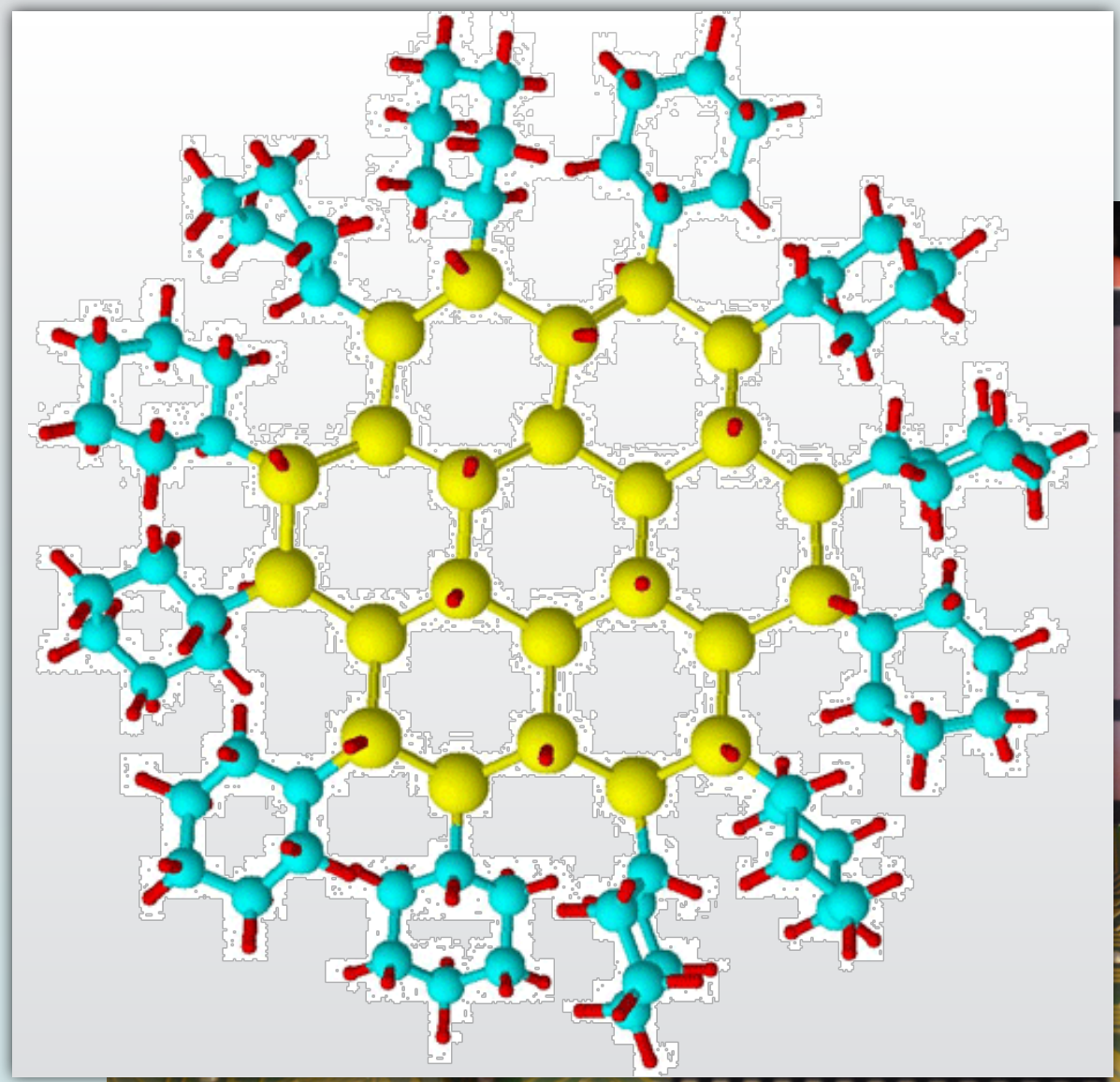
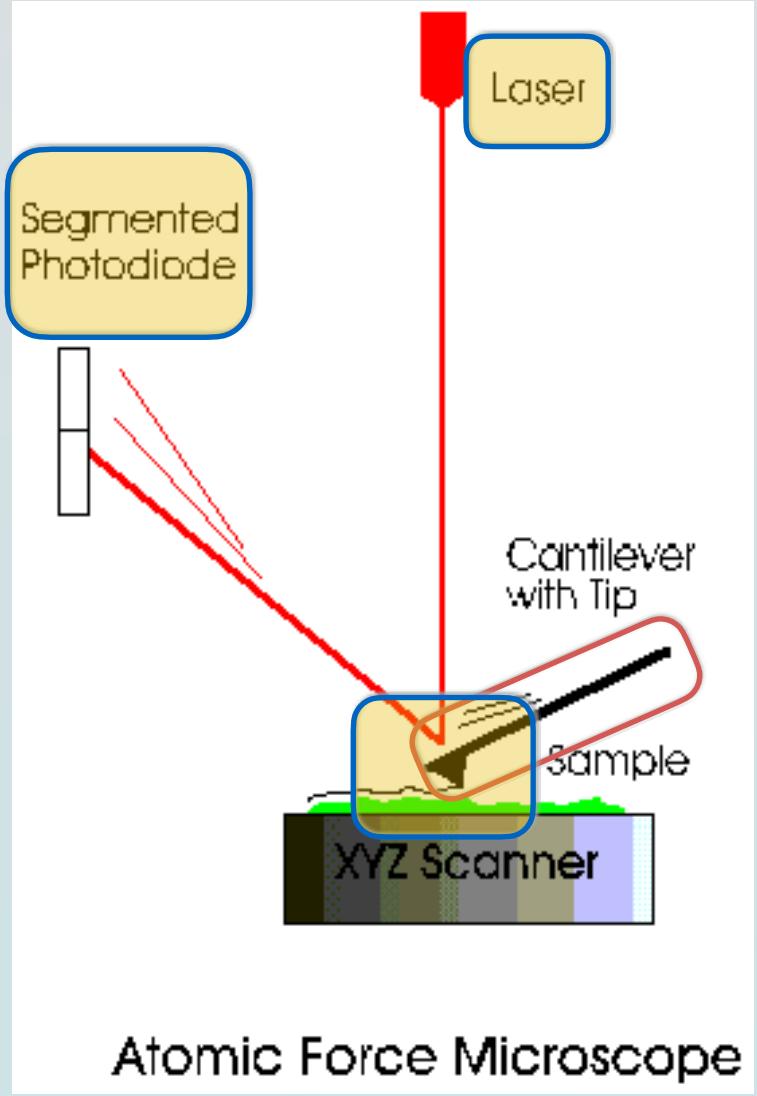


nano particles

AFM Principle



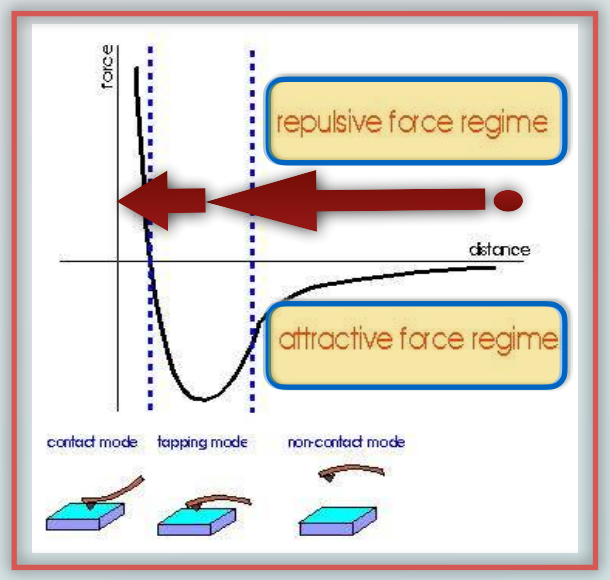
Images in AFM are acquired by **scanning the surface** of the sample with a sharp tip.

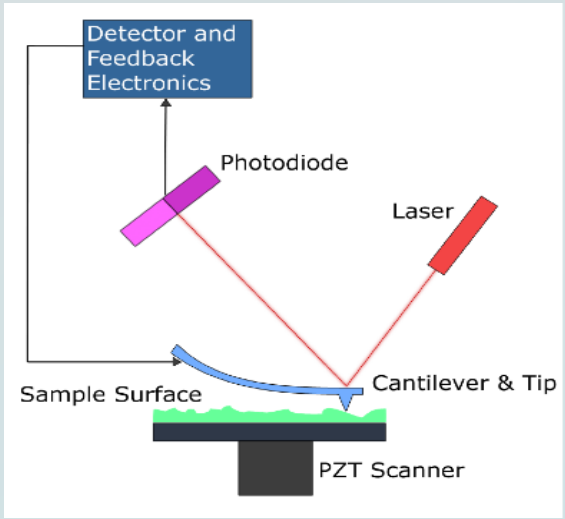
The tip is located at the free end of a flexible cantilever.

The cantilever's movements are detected by a laser beam that is reflected of the back of the cantilever to a photodiode.

The photodiode then relays the information to the computer which in turn generates a topographical image of the sample.

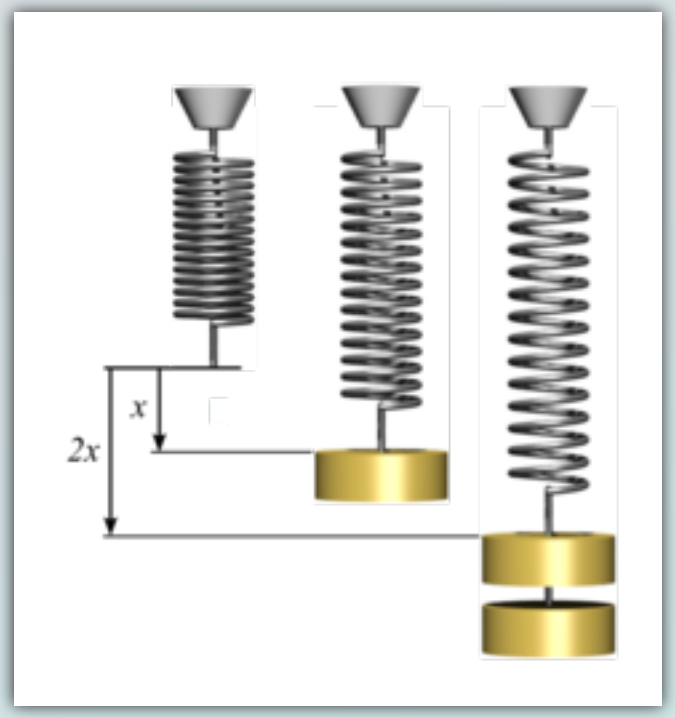
Forces between the tip and the sample (normally $< 10^{-9}$ N) cause the cantilever to deflect.





Atomic Force Microscope (AFM) operates by measuring attractive or repulsive forces between a probe or "tip" and the sample. The tip is located at the end of a leaf spring or "cantilever". A laser beam is reflected off the cantilever. Any angular deflection of the cantilever caused by the change of the force between tip and sample is represented by the angular deflection of the laser beam. Images are taken by scanning the sample relative to the tip and measuring the deflection of the cantilever as a function of lateral position. Different from traditional microscope, image from AFM is three dimensional.

Premise of AFM… Hooke's Law



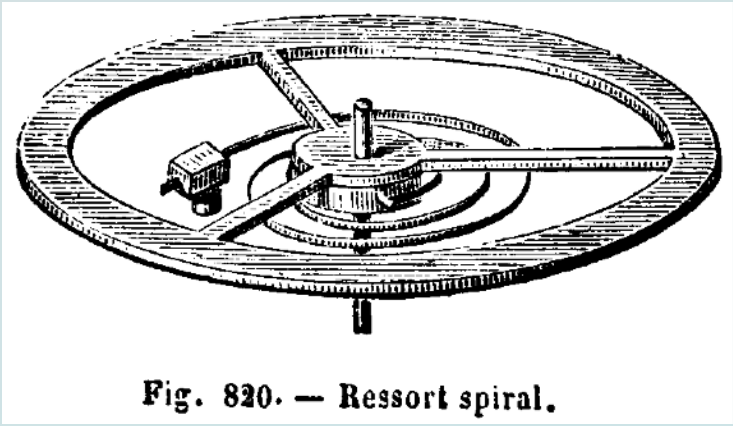
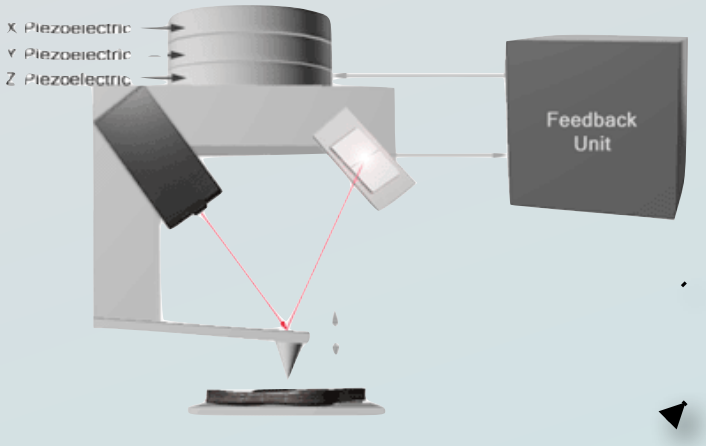
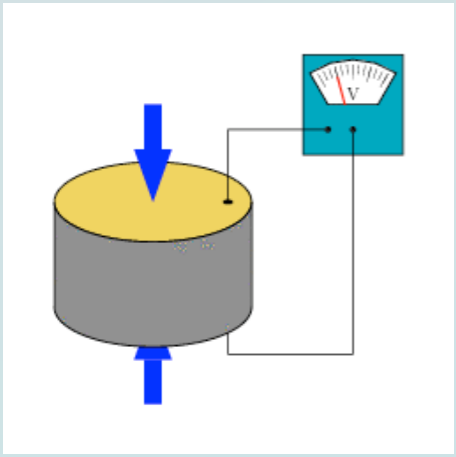


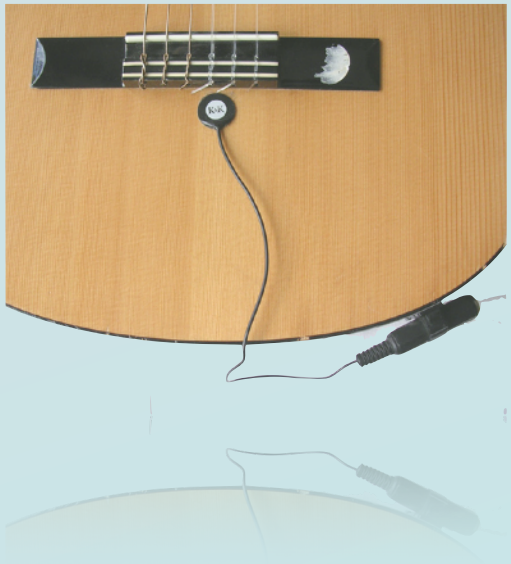
Fig. 820. — Ressort spiral.

Hooke's law is a principle of physics which states that the force **F** needed to extend or compress a spring by some distance **X** is proportional to that distance.

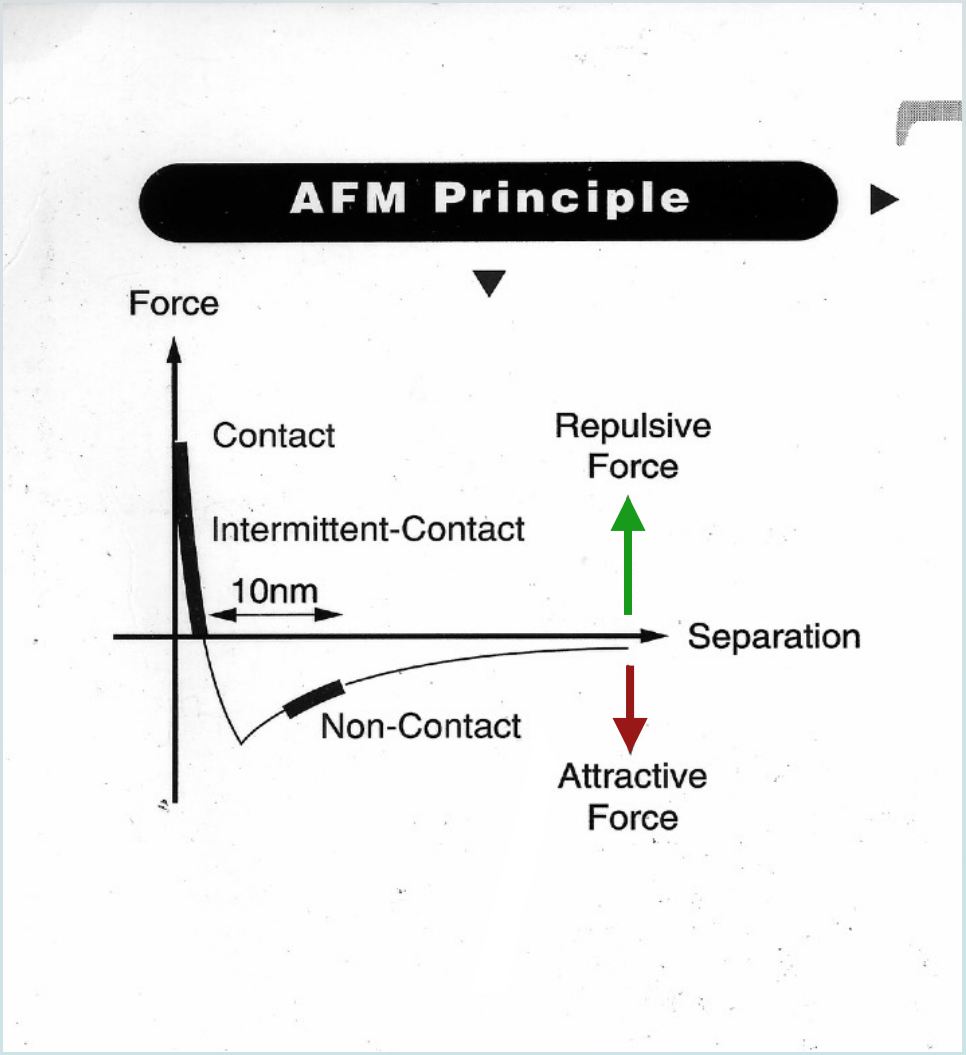
That is: **F = *k*X** where ***k*** is a constant factor characteristic of the spring, its "stiffness".


X Piezoelectric
Y Piezoelectric
Z Piezoelectric
Feedback
Unit


V



Modes of AFM Operation

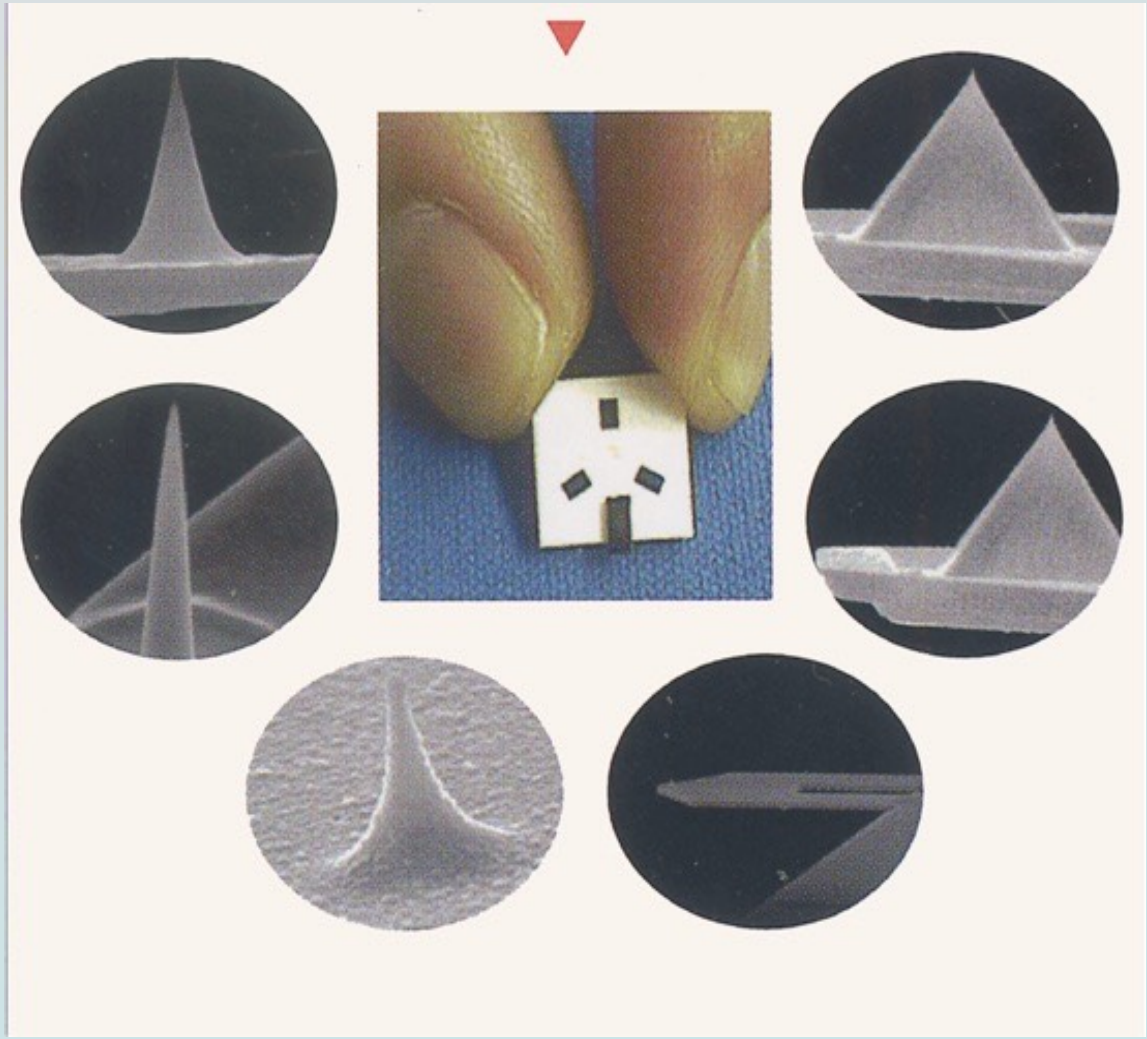


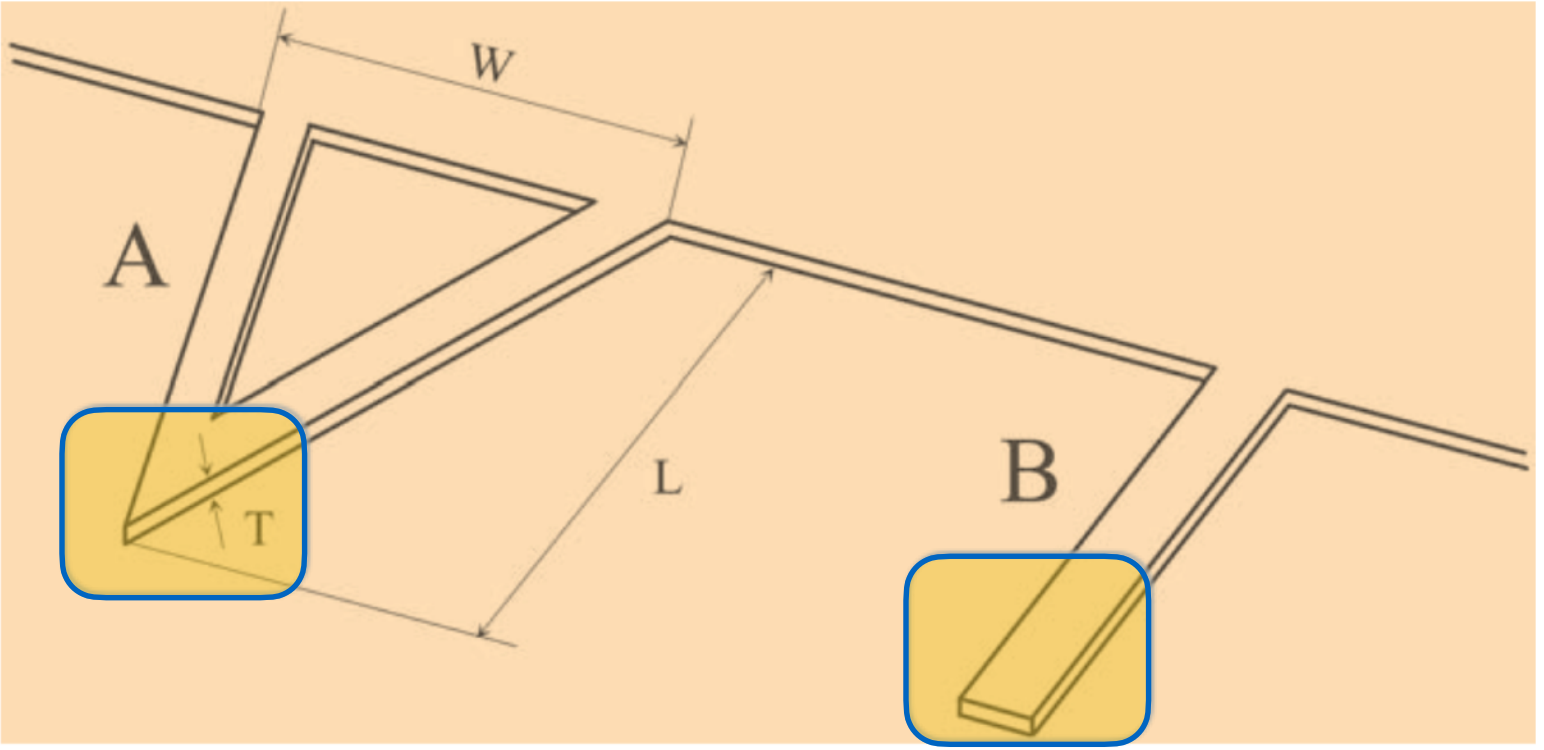
Contact Mode

Non Contact Mode

Tapping Mode

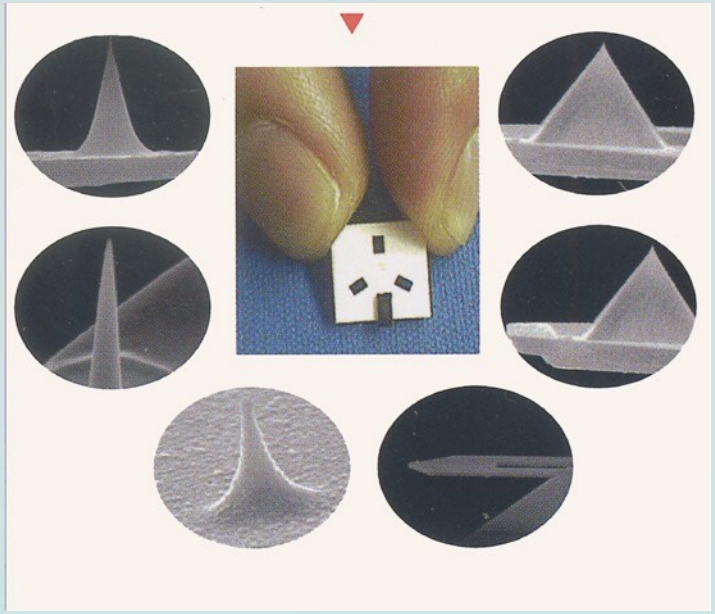
CP II





The cantilever carrying the tip is attached to a small glass “chip” that allows easy handling.

There are essentially two designs for cantilevers, the “V” shaped and the single-arm kind, which have different torsional properties. The length, width, and thickness of the beam(s) determines the mechanical properties of the cantilever and provides for a variety of types that are essentially classified by their force (or spring) constant and resonance frequency: soft and low-resonance frequency cantilevers (A) are more suitable for imaging in contact (and resonance mode in liquid), whereas stiff and high-resonance frequency cantilevers (B) are more appropriate for resonance mode in air.

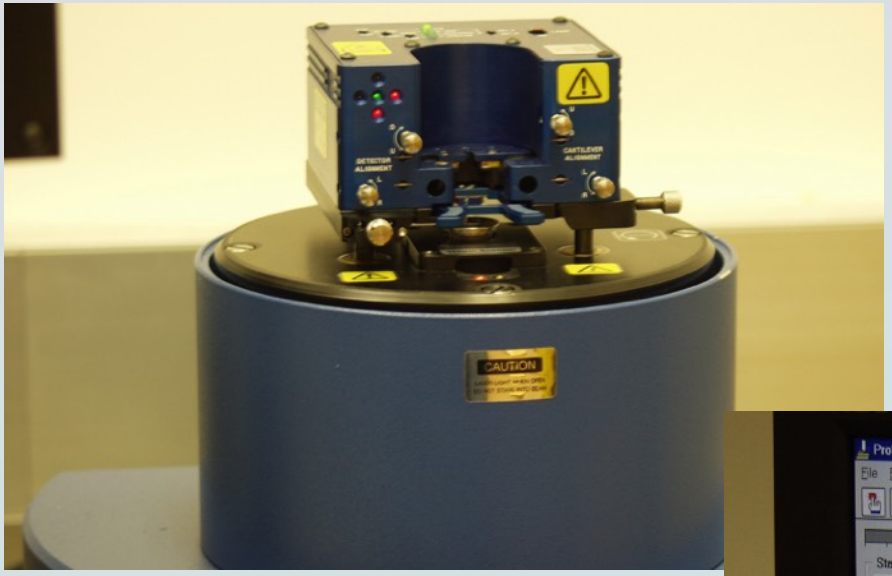


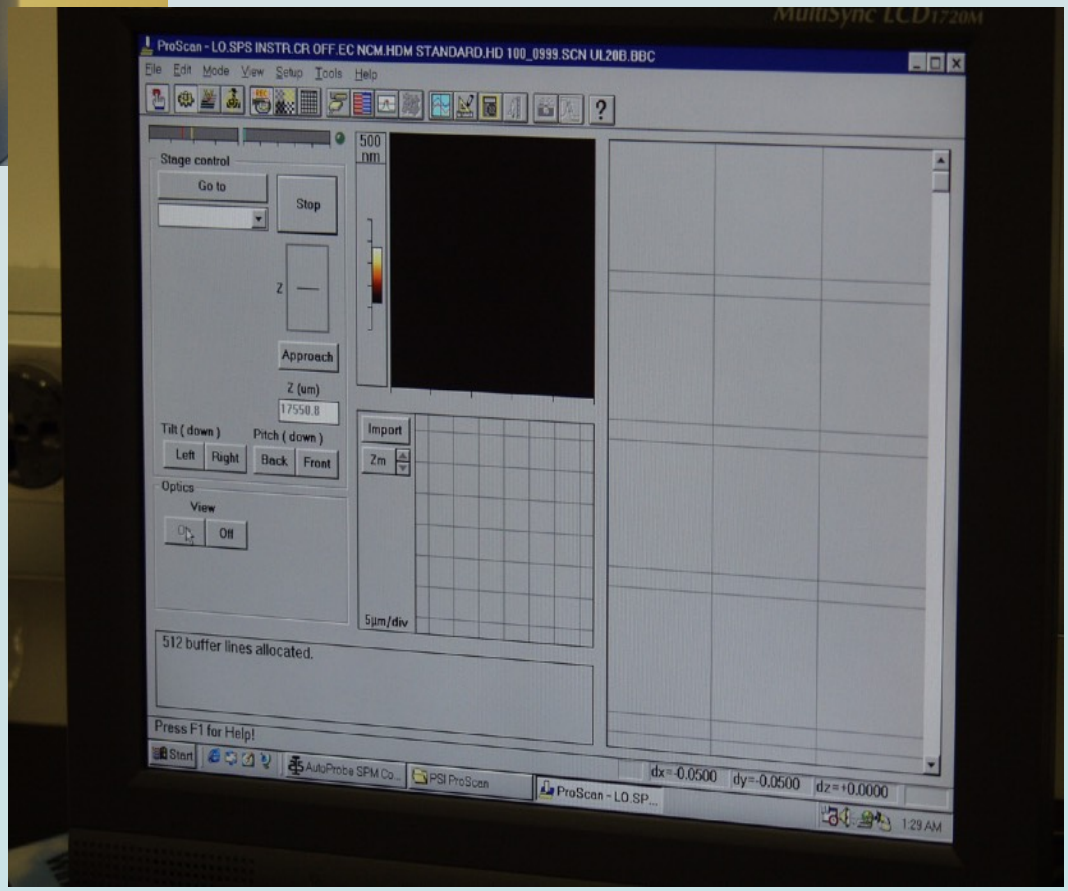
CP II

Viewing the scanning tip using attached optics

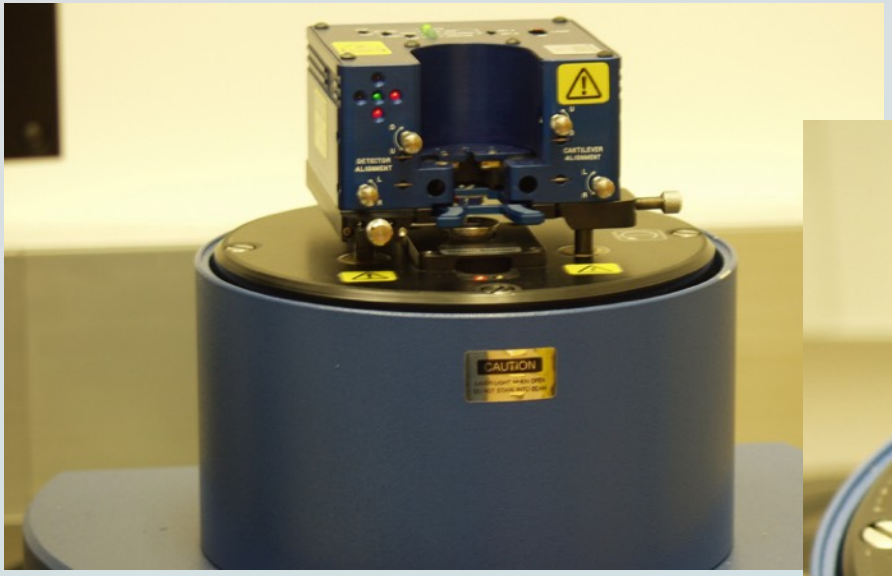
CP II

General Manipulation of the Scanning Platform





CP II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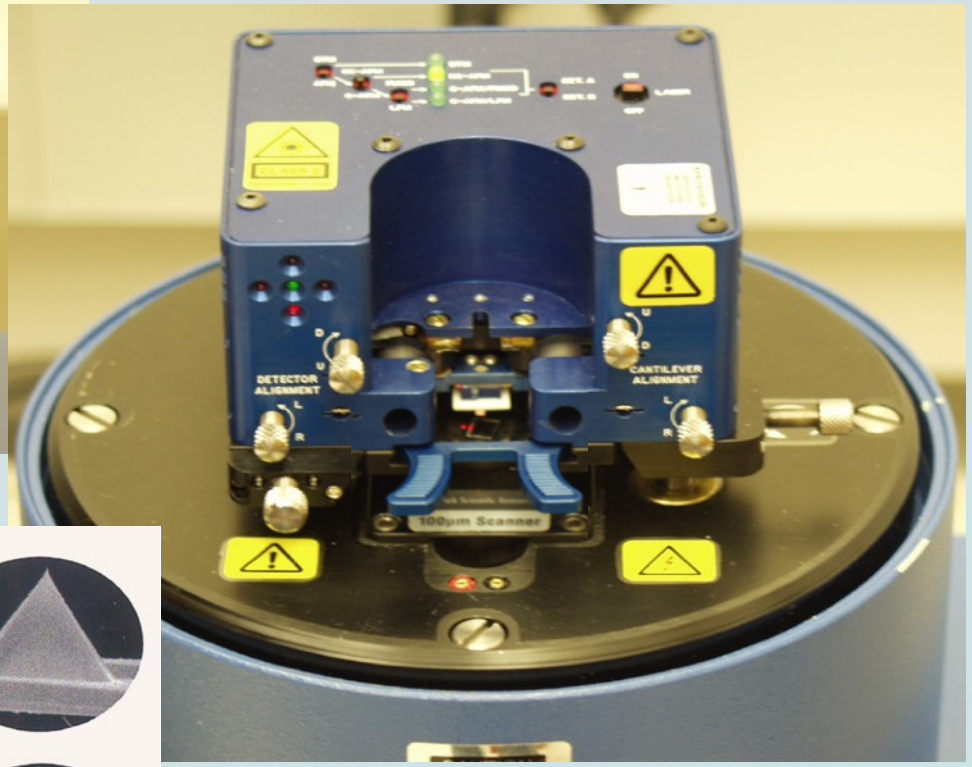
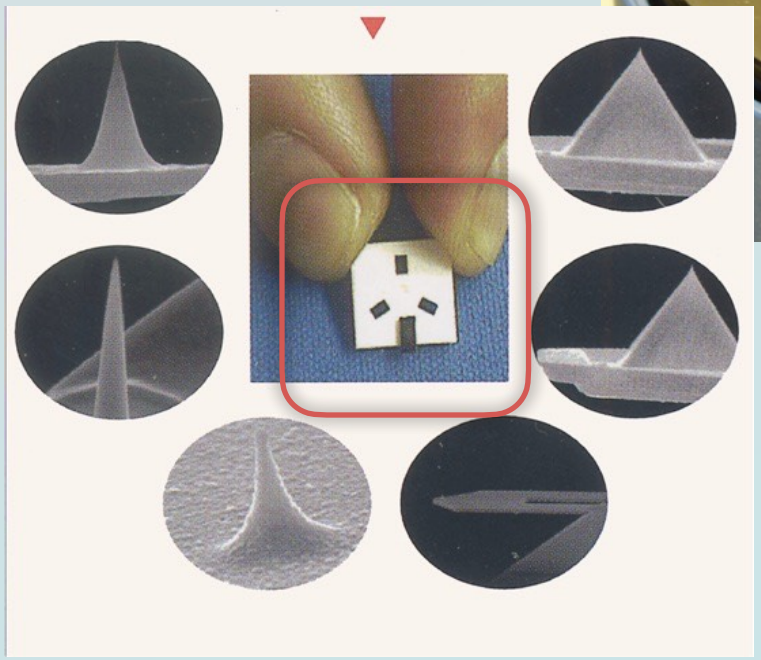
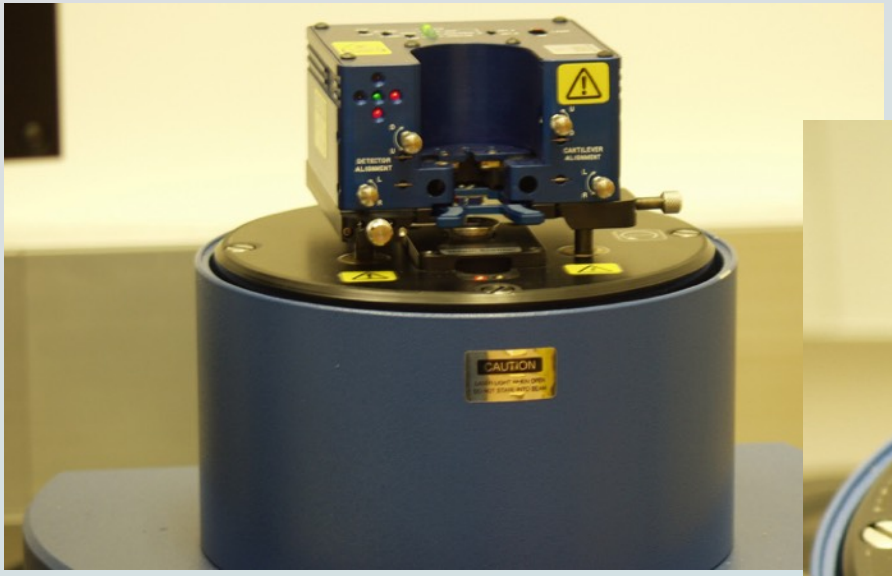

DETECTOR ALIGNMENT
CANTILEVER ALIGNMENT
100µm Scanner

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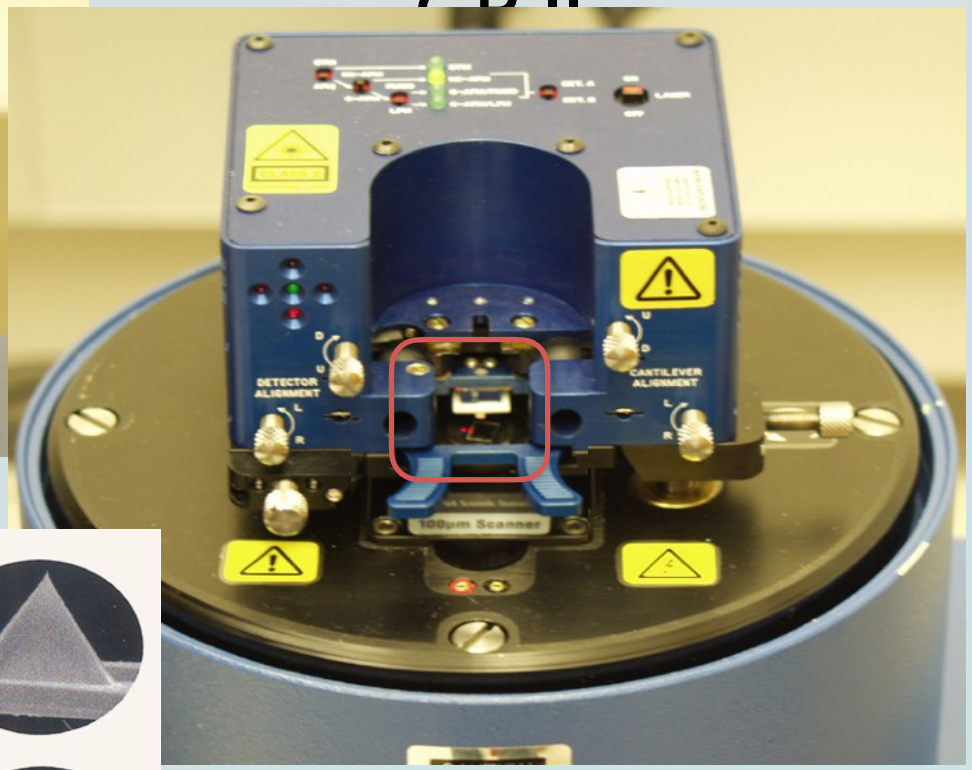
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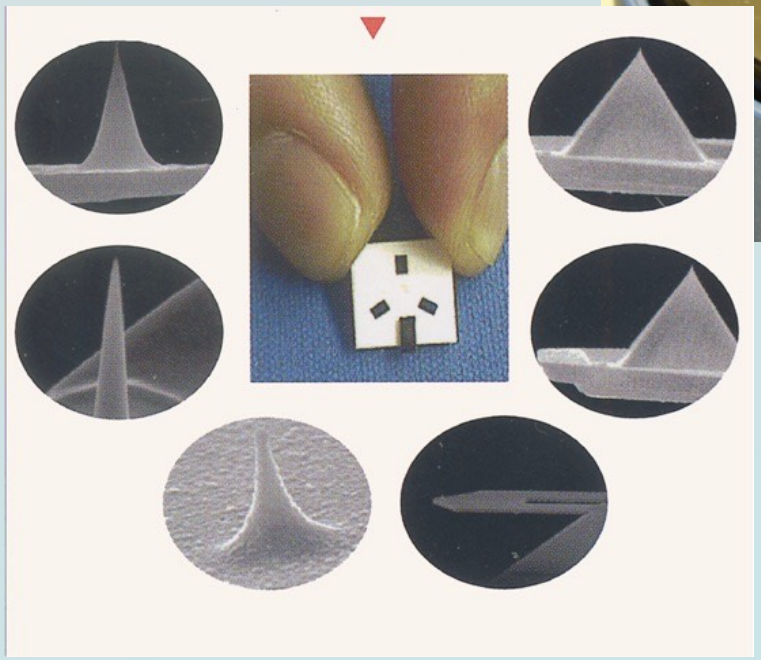
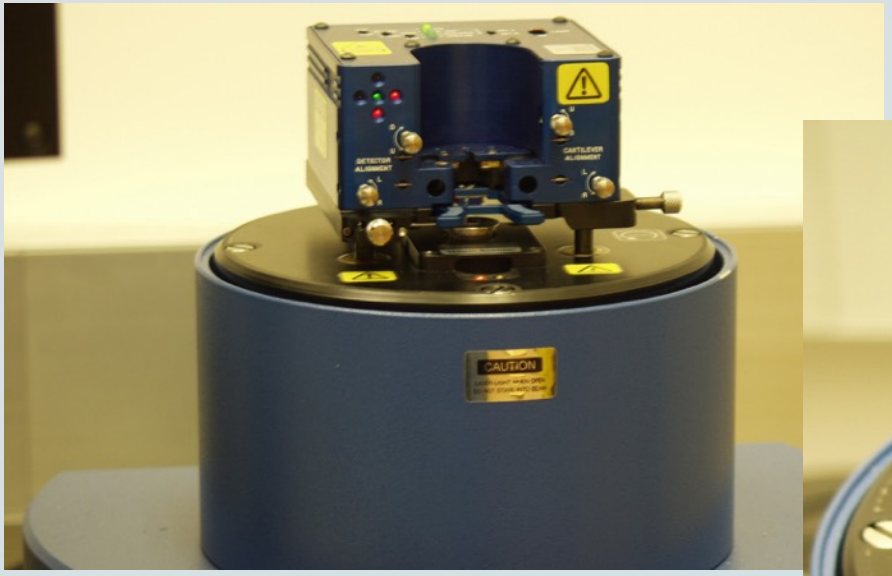
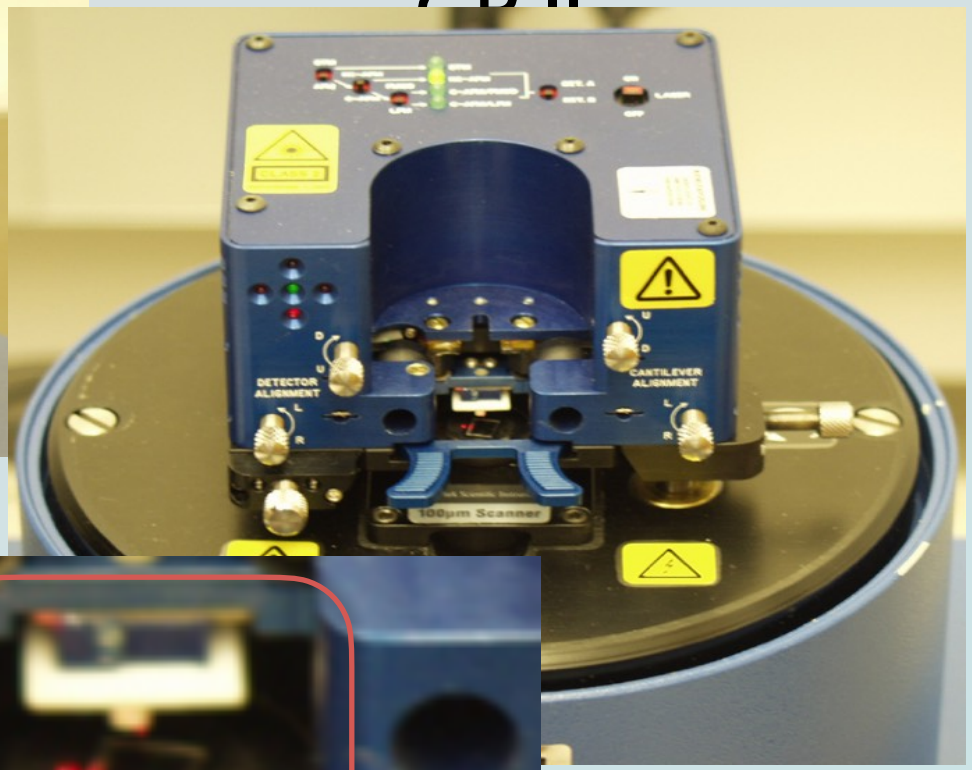
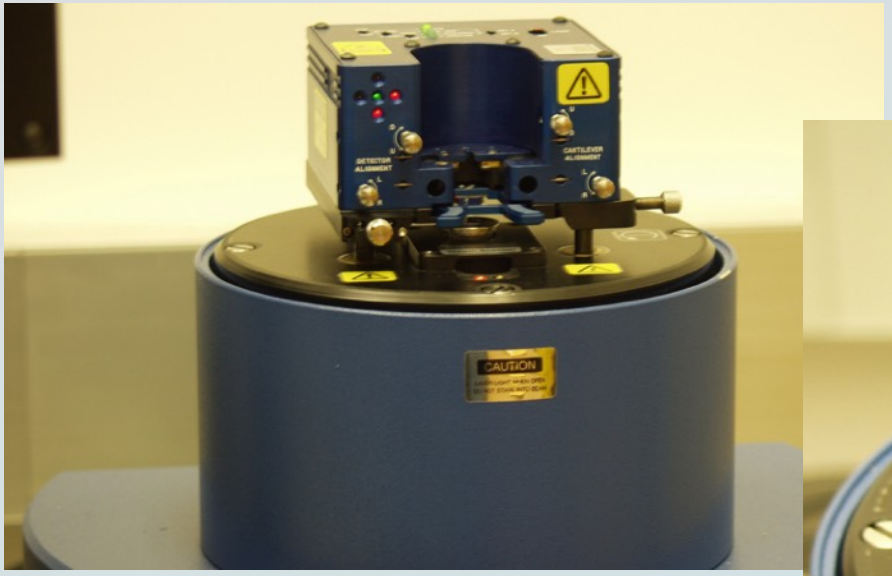
CANTILEVER ALIGNMENT
100µm Scanner

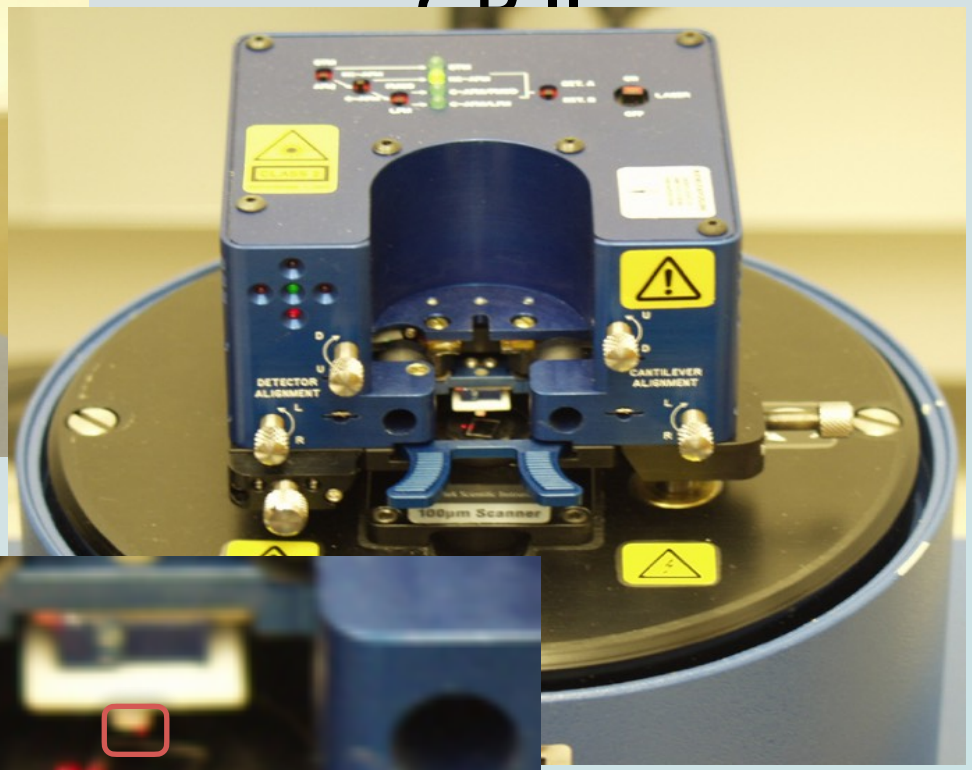


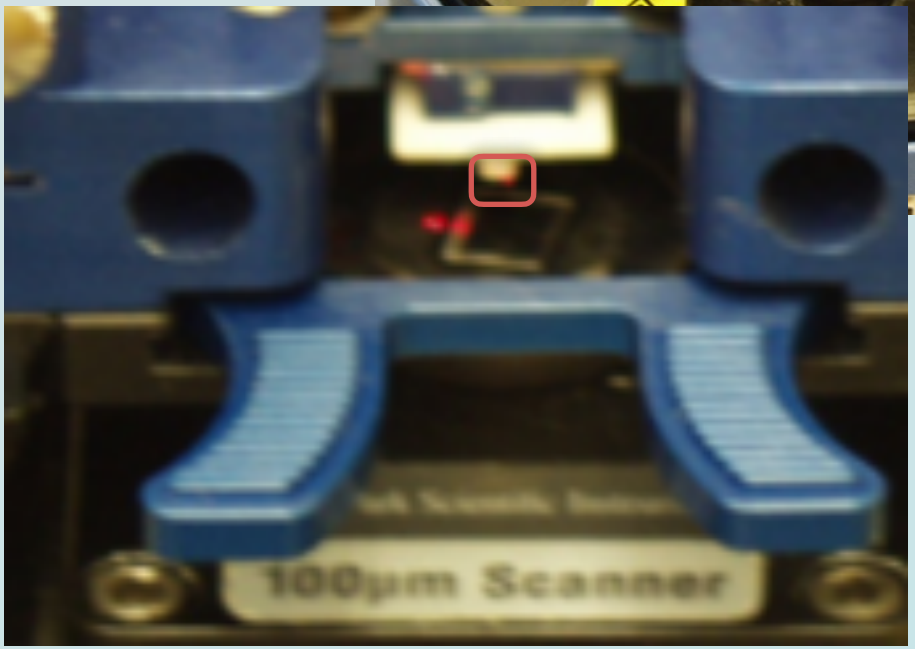

CAUTION

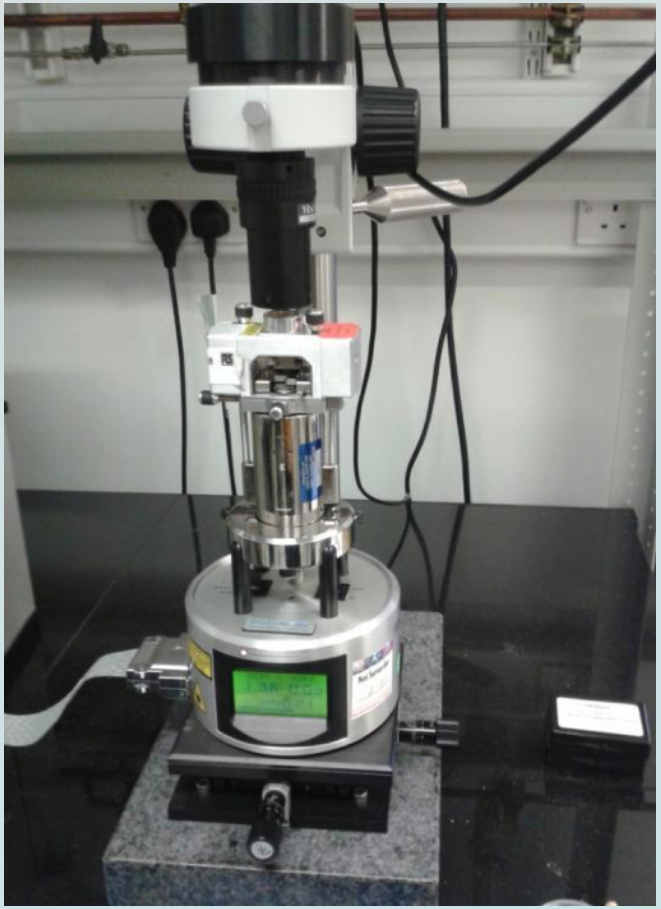

DETECTOR ALIGNMENT
CANTILEVER ALIGNMENT
100µm Scanner


100µm Scanner


CAUTION

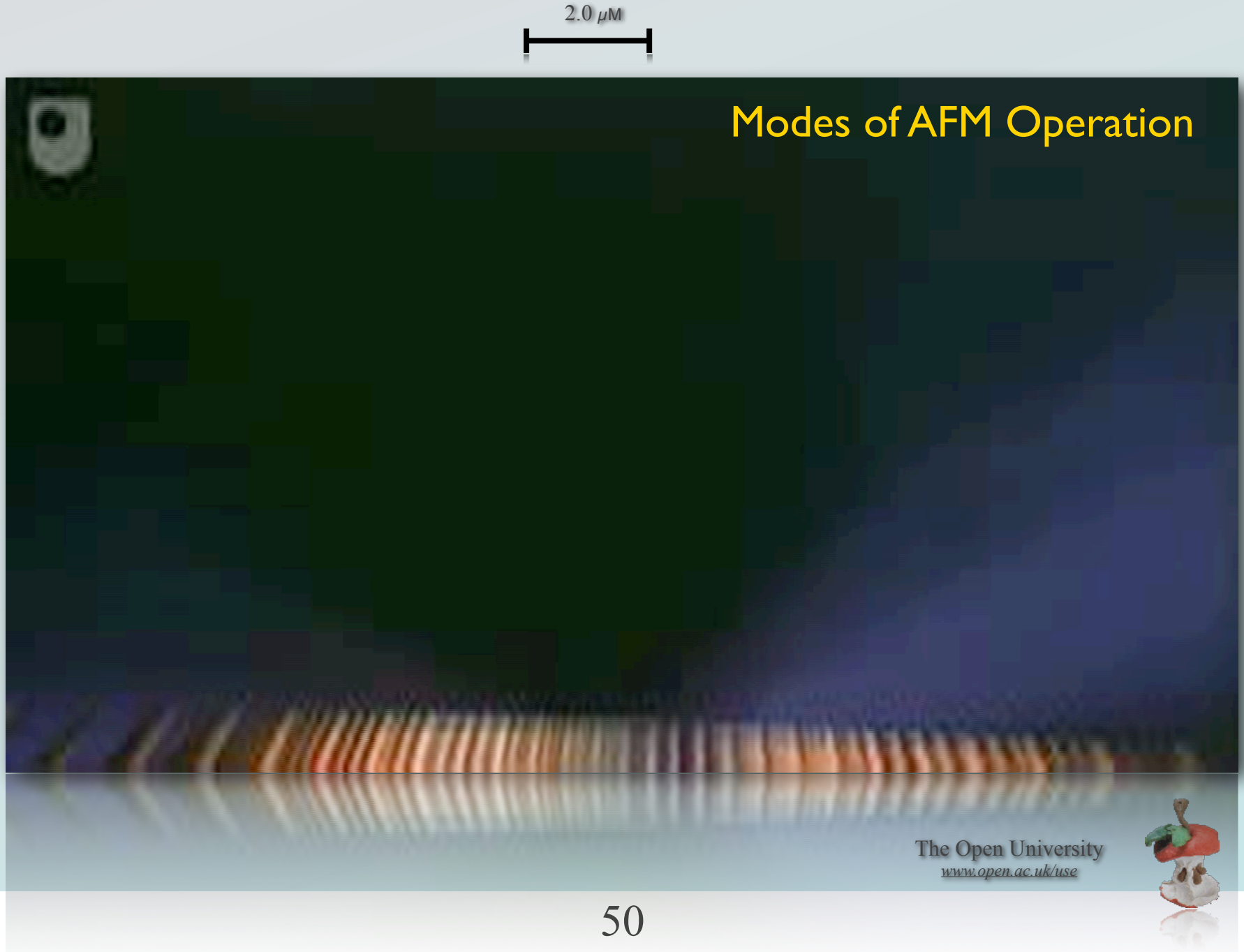

DETECTOR ALIGNMENT
CANTILEVER ALIGNMENT
100µm Scanner


100µm Scanner



MultiMode VIII from Bruker

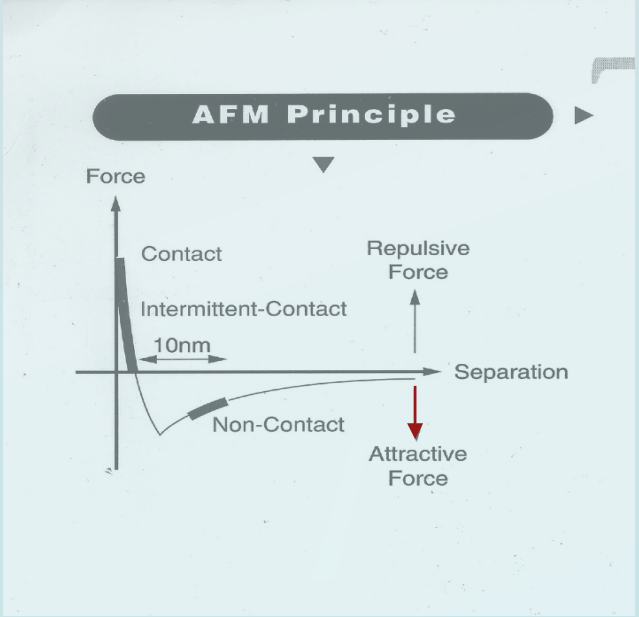
The MultiMode VIII represents the “next generation” of the most field-proven SPM. It performs the full range of atomic force microscopy (AFM) and scanning tunneling microscopy (STM) techniques to measure surface characteristics like topography, elasticity, friction, adhesion, and magnetic/electrical fields.


2.0 μM
Modes of AFM Operation

Contact Mode

The contact mode where the tip scans the sample in close contact with the surface is the common mode used in the atomic force microscope. At this proximity to the object being examined the force on the tip is “repulsive”, with a mean value approximating
1×10^{-9} N.

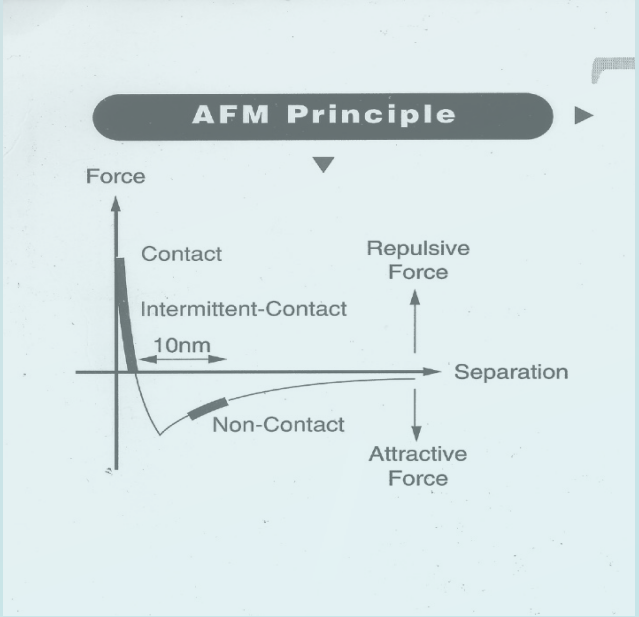
This **force** is set by **pushing the cantilever** against the sample surface with a piezoelectric positioning element. The flexible cantilever scans across the surface and the tip is repulsed from the surface by strong “repulsive” forces... which are countered by the piezoelectric capability of the AFM and thus measuring the contours of the molecules at the surface.



Non Contact Mode

Non Contact mode is used in situations where the tip contact might alter the sample in subtle ways. In this mode the tip is held in a "hovering" pattern -vibrating slightly above its resonance frequency- just above the sample surface. Attractive Van der Waals forces, acting between the tip and the sample, are detected and topographic images of the undulations of these forces are constructed as the tip scans over the surface.

Unfortunately these "**attractive**" forces from the sample are substantially weaker than the forces used in **contact mode**. Thus, the tip is given a small oscillation so that any significant change in oscillations can be used to amplify changes of small forces between the tip and the sample by measuring the change in amplitude, phase, or frequency of the oscillating cantilever in response to force gradients that are set up between the cantilever and the sample.



“Tapping” Mode

Tapping mode is achieved by oscillating the cantilever assembly at or near the cantilever's resonant frequency. This “piezo” motion causes the cantilever to oscillate with a high amplitude (typically greater than 20nm, but less than 200nm).

As the oscillating tip is then moved toward the surface it begins to almost (but not quite) lightly touch, or “tap” the surface, the vertically oscillating tip alternately contacts the surface and lifts off (generally at a frequency of 50,000 to 500,000 cycles per second). This transient “contact” with the surface reduces the oscillation amplitude, which can be used to identify and measure surface features.

As it moves toward the sample the attractive/repulsive forces alter the amplitude for the piezoelectric oscillations, which are detected and compensated for by the instrument.

Unlike “contact” and “non-contact” modes, when the tip “contacts” the surface, it has sufficient oscillation amplitude to overcome the tip to sample adhesion forces. Consequently, the use of tapping mode (as opposed to full contact mode) prevents the tip from sticking to the surface and thus reduce damage to the tip during scanning.

Measurement of Various Forces To Define “Texture” of Samples

1. The cantilever starts out not touching the surface. If the cantilever - in this region - feels a long-range attractive (or repulsive) force it will deflect downwards (or upwards) before making contact with the surface.

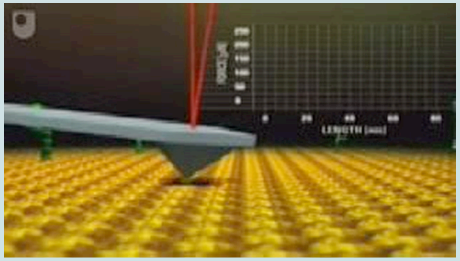
2. As the probe tip is brought very close to the surface, it may jump into contact if it feels sufficient “attractive force” from the sample.

3. Once the tip is in contact with the surface, cantilever deflection will increase as the fixed end of the cantilever is brought closer to the sample.

   If the cantilever is sufficiently “stiff”, the probe tip may indent into the surface at this point. In this case, the slope or shape of the contact part of the force curve can provide information as to the elasticity of the sample surface.

4. After loading the cantilever to a desired force value, the process is reversed. As the cantilever is withdrawn, adhesion or bonds formed during contact with the surface may cause the cantilever to adhere to the sample at some distance past the initial contact point on the approach curve.

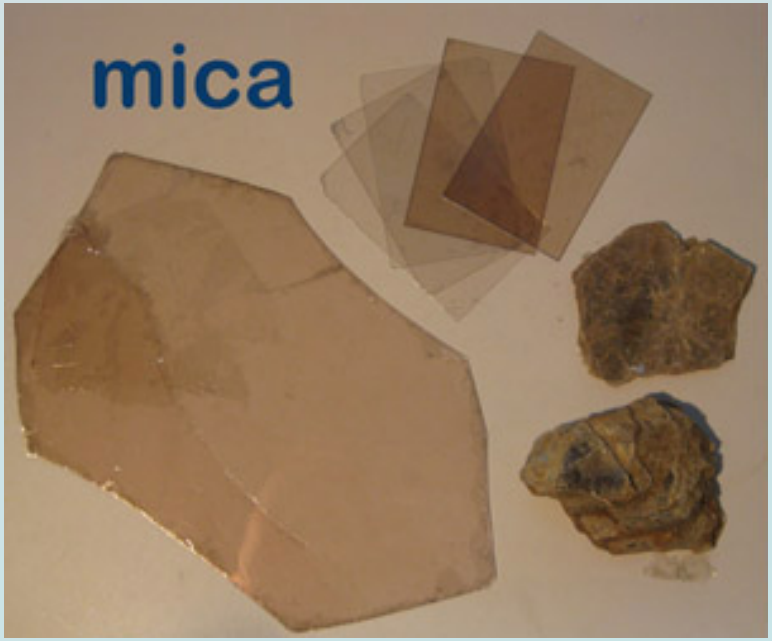
5. A key measurement of the AFM force curve is the point at which the tip to surface adhesion is broken and the cantilever comes free from the surface. This can be used to measure the “rupture force” required to break the bond or adhesion, which again can be used to define some form of texture to the material or sample under analysis.



One of the first uses of force measurements was to improve the quality of AFM images by monitoring and minimizing the attractive forces between the tip and sample.

Sample Preparation -Basic analysis

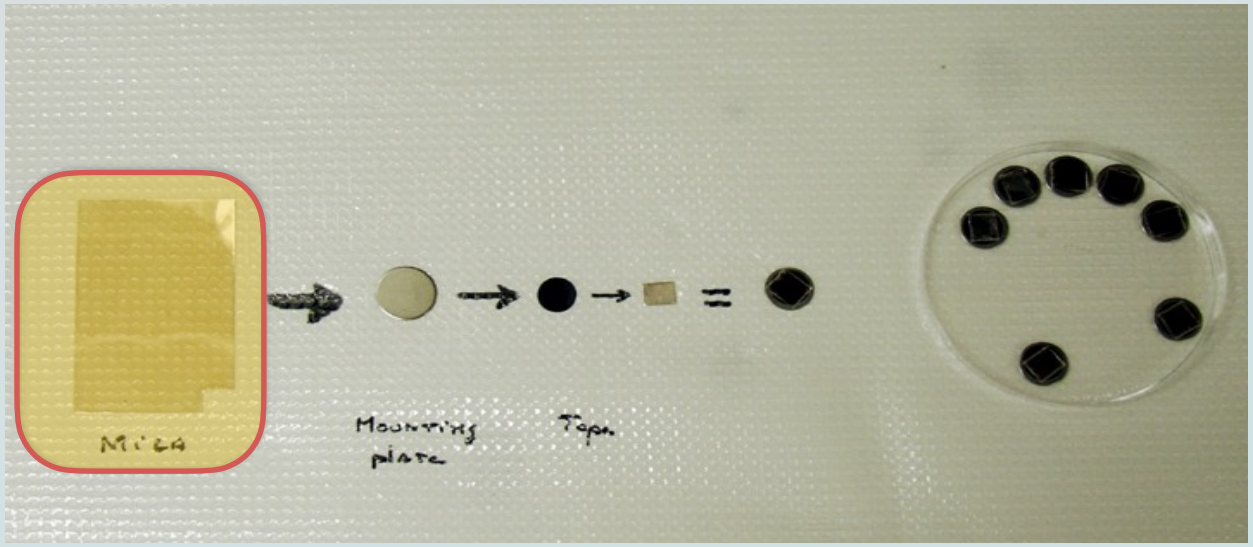
Deposition of buffer containing a divalent cation (eg. $Ca^{2+)}$ to allow charged particles to adhere to the flat surface upon which the sample is being analyzed..



Flat substrate:

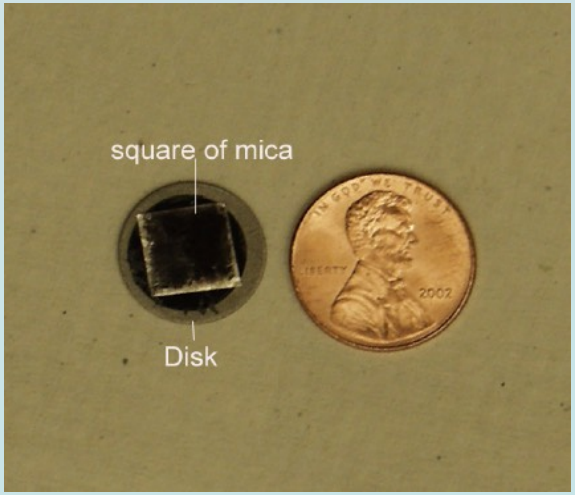
1. Plain mica
2. Aminopropyltrimethoxy saline (APTES)-treated mica
3. Glow discharged mica.
4. highly oriented pyrolytic graphic (HOPG).

Sample Preparation cont'd

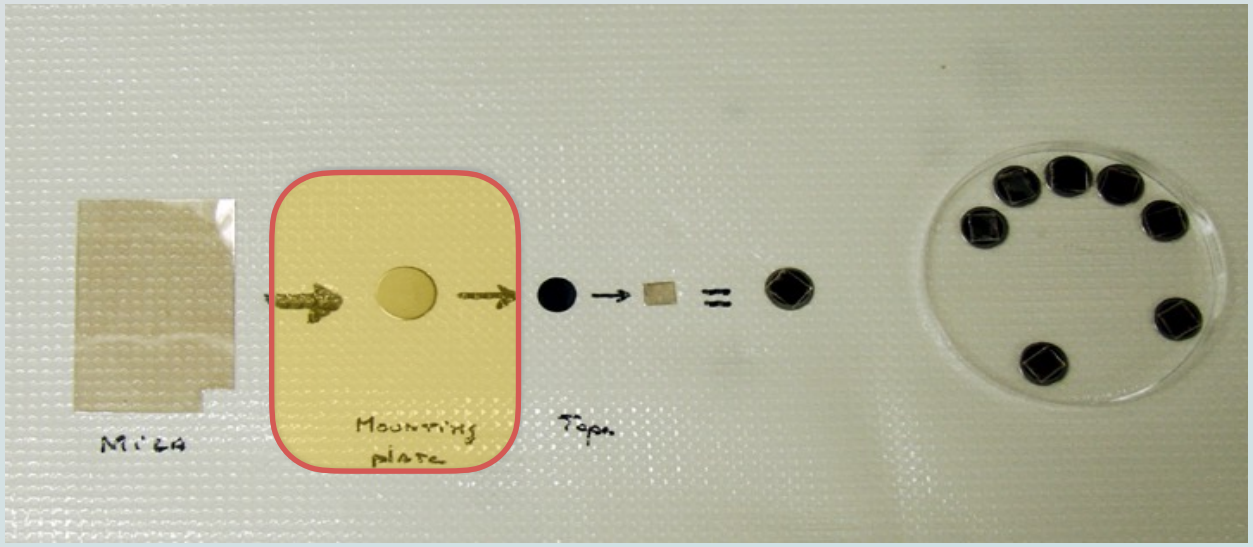


- mica
- sticky tape
- metal disk

Add sample and dry with condensed air or in dessicator for ~ 2 hours to overnight



Sample Preparation cont'd



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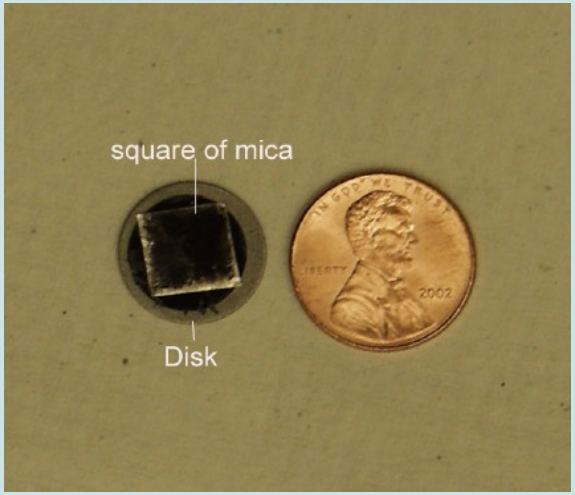
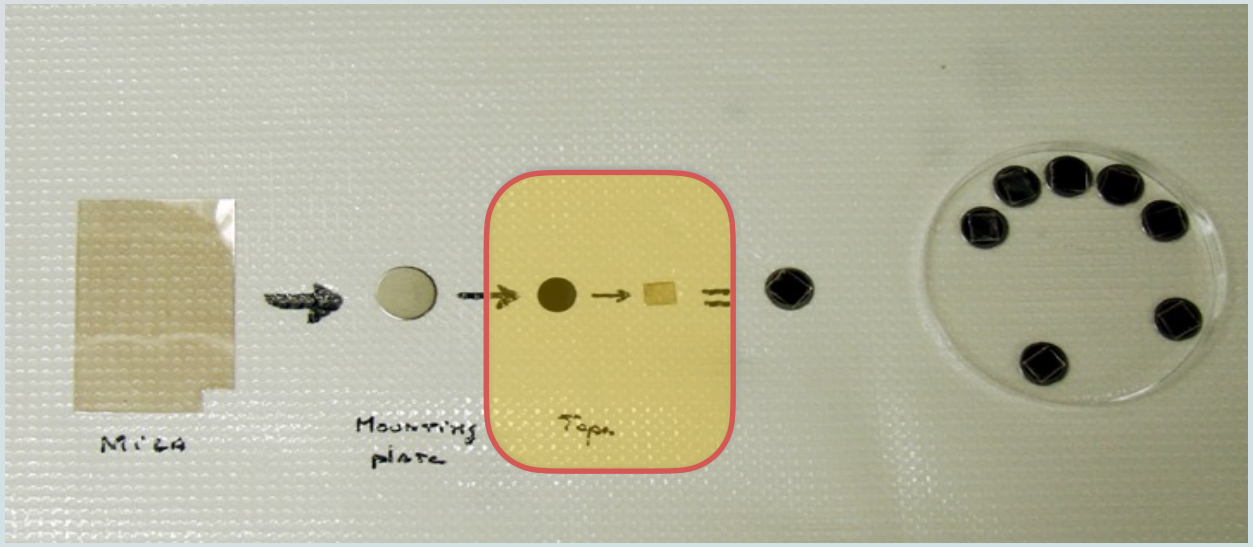


Sample Preparation cont'd



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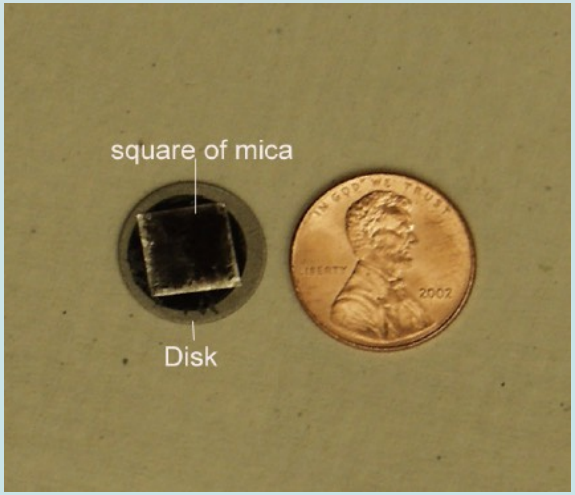
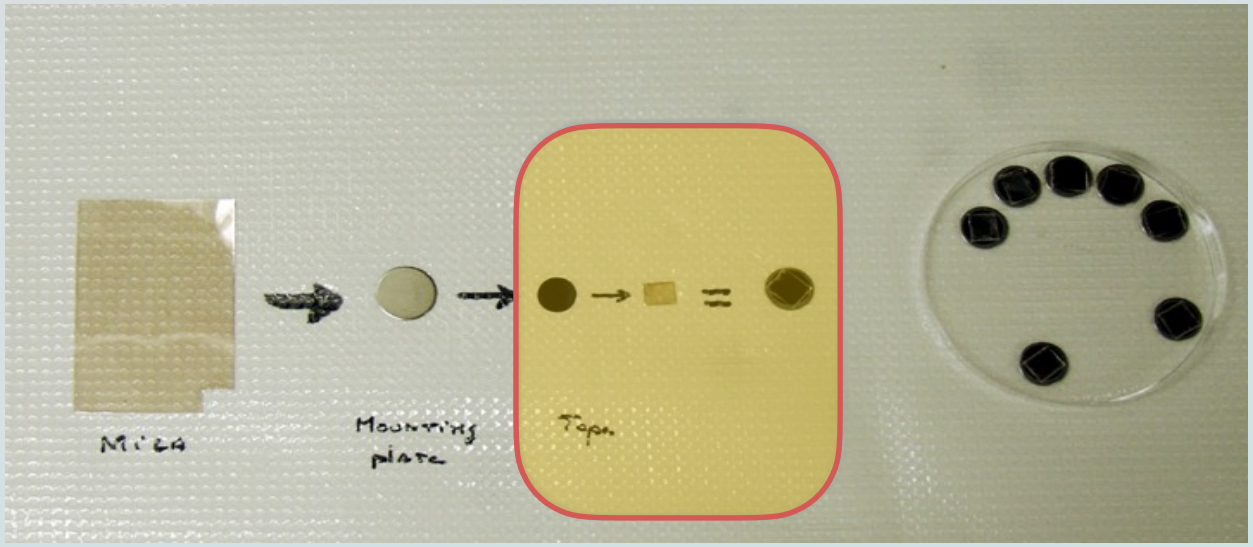


Sample Preparation cont'd



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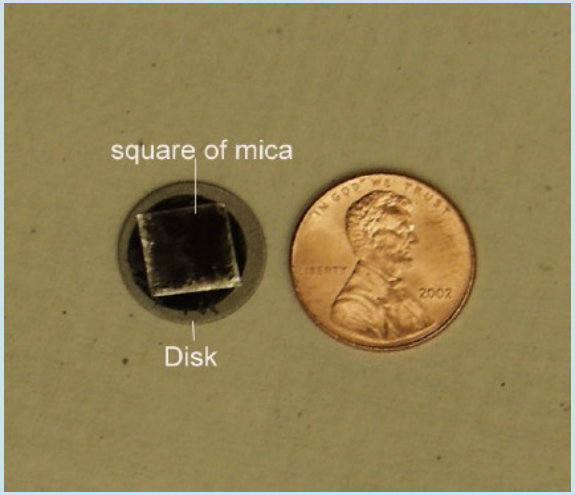
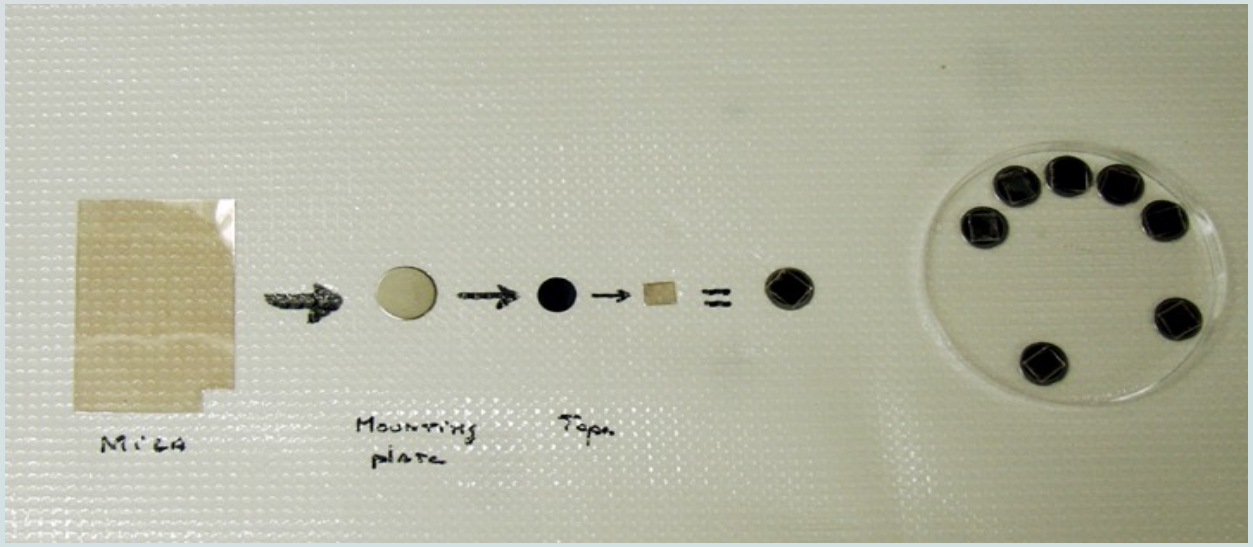


Sample Preparation cont'd



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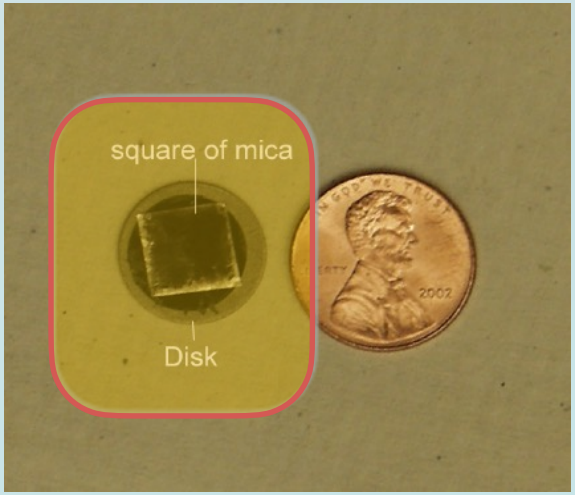


Resolution of AFM

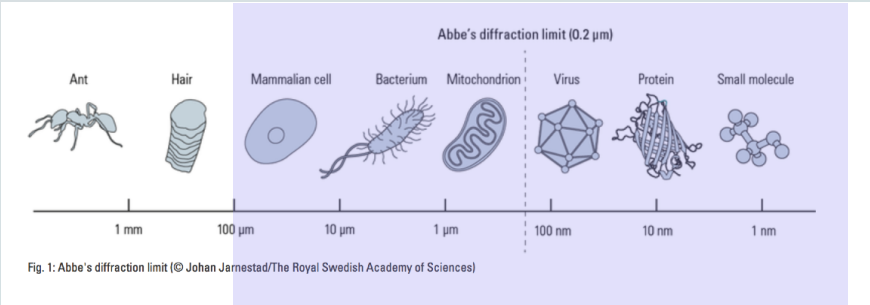


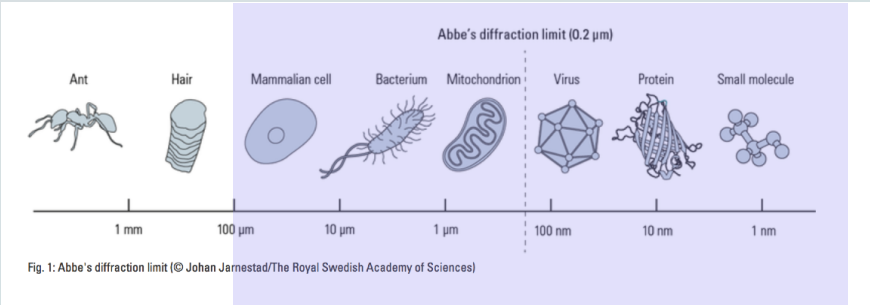
Fig. 1: Abbe's diffraction limit (© Johan Jarnestad/The Royal Swedish Academy of Sciences)

Resolution between 0.1 and 1 Angstrom can be achieved through AFM technology -although even greater resolutions can be captured in vacuo or in liquid

Such resolution is dependent upon a variety of factors, however:

- The mode of analysis: contact vs. non-contact etc.
- The sharpness of the tip.
- The distances between the objects to be resolved.
- The height of the two objects that are being resolved.

Resolution of AFM



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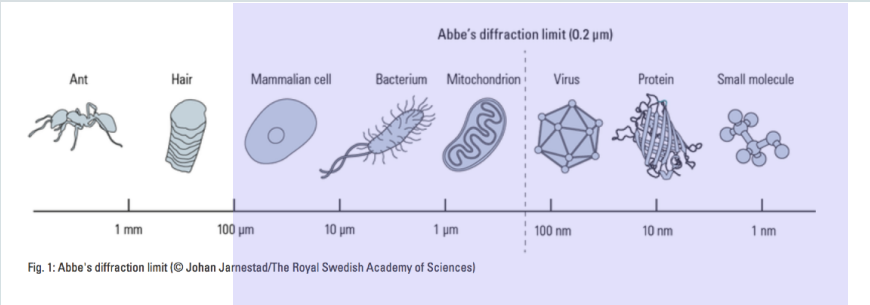

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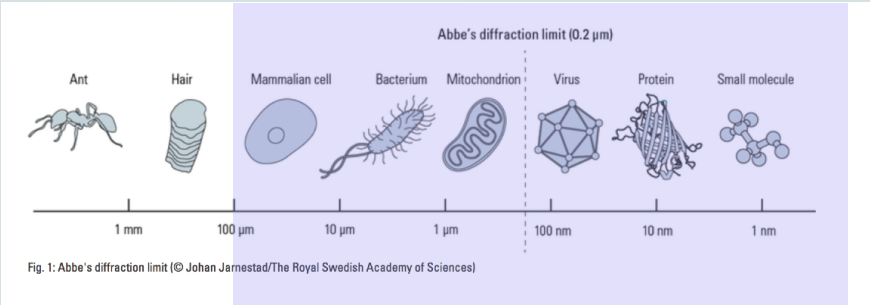

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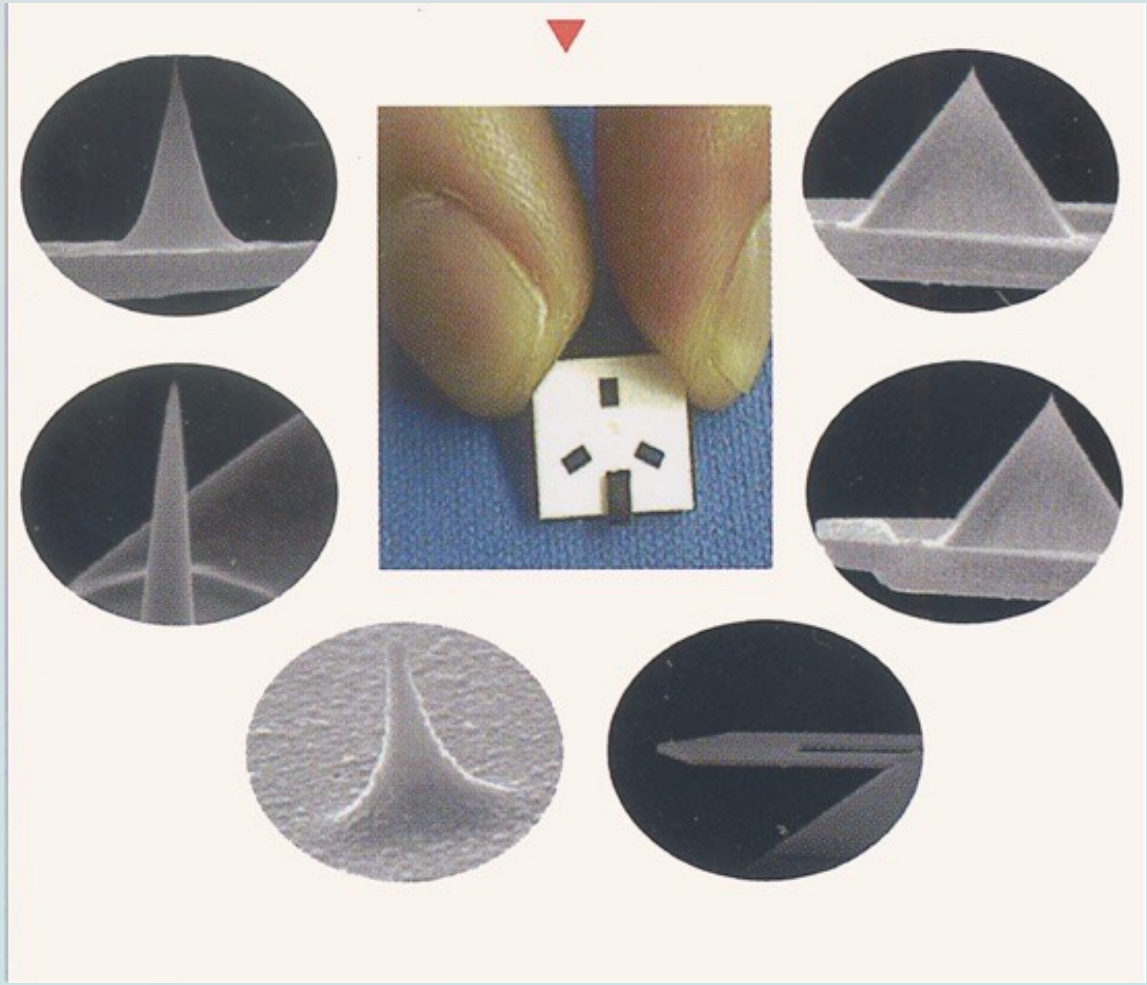
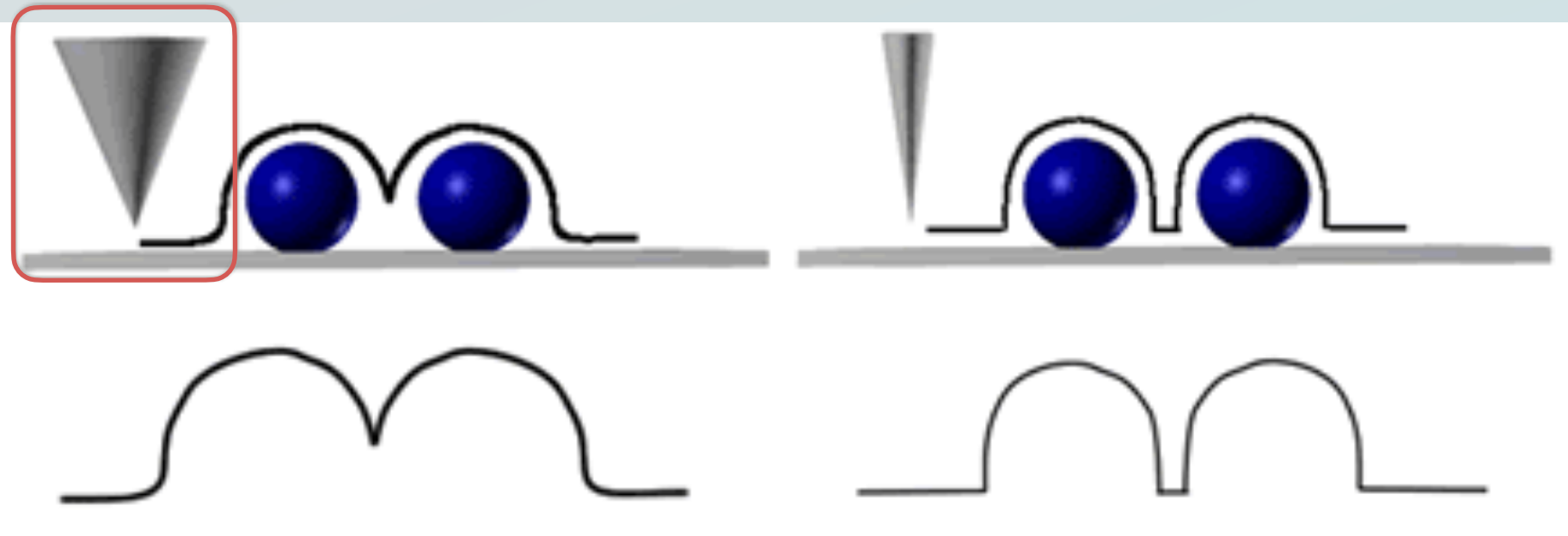
Different Tips for Different Jobs



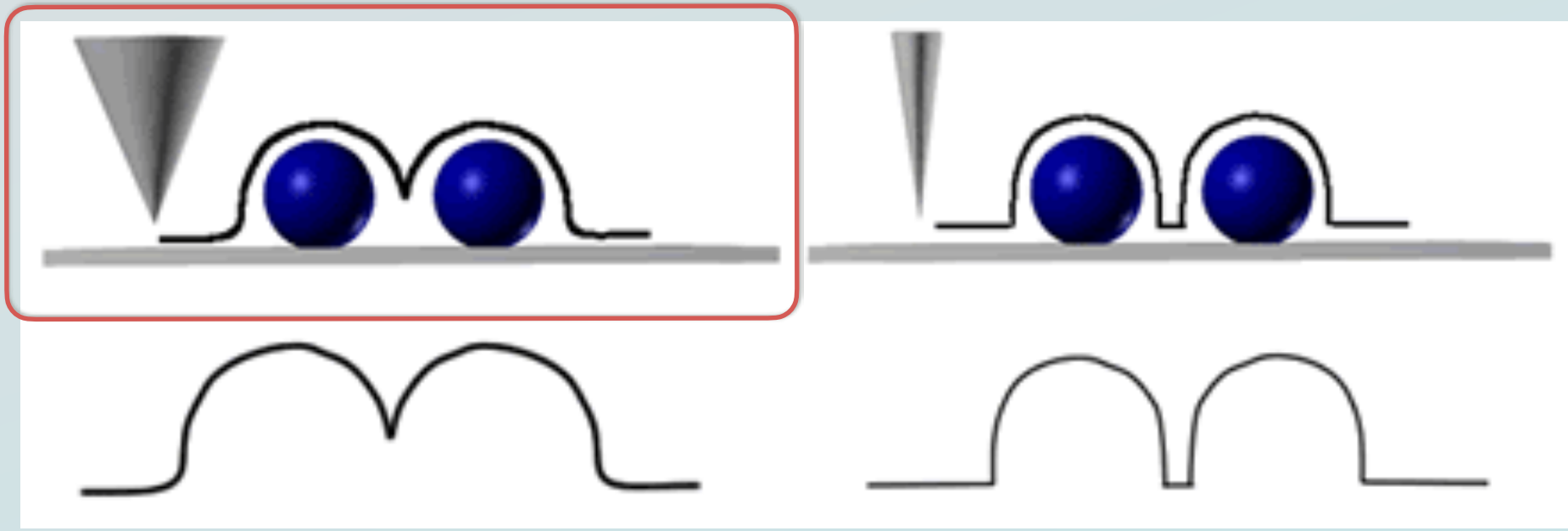
Resolution of the AFM

The sharpness of the tip



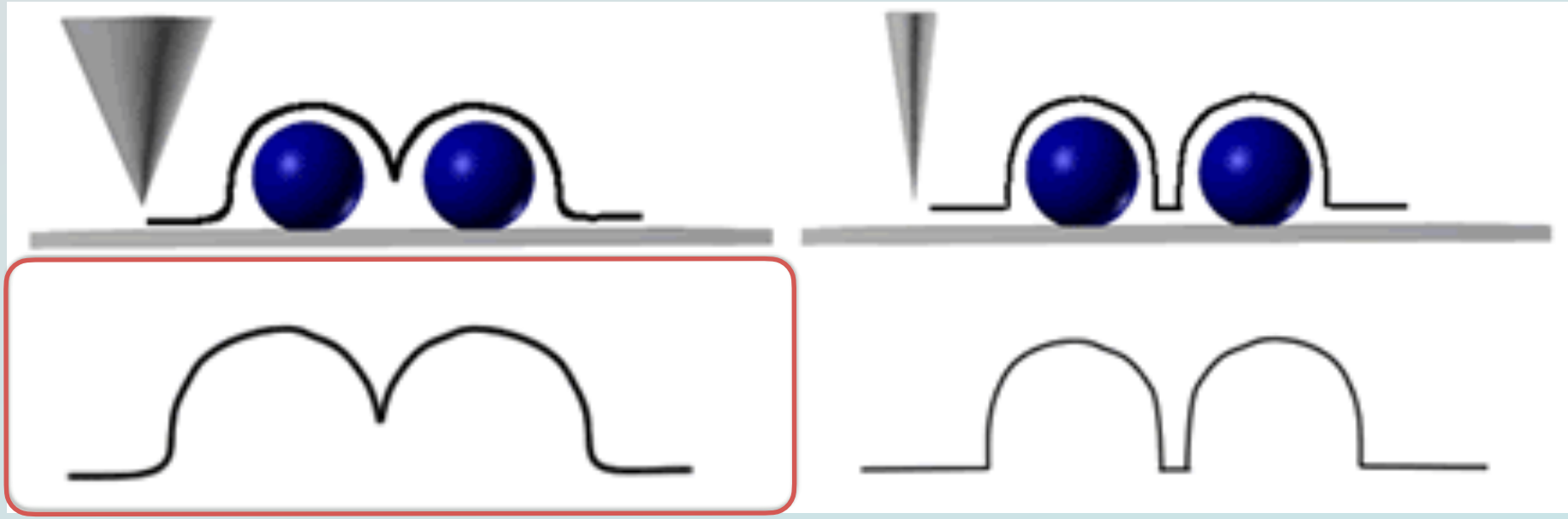
A schematic diagram showing the factors that affect resolution in AFM

Resolution of the AFM
The sharpness of the tip



A schematic diagram showing the factors that affect resolution in AFM

Resolution of the AFM
The sharpness of the tip



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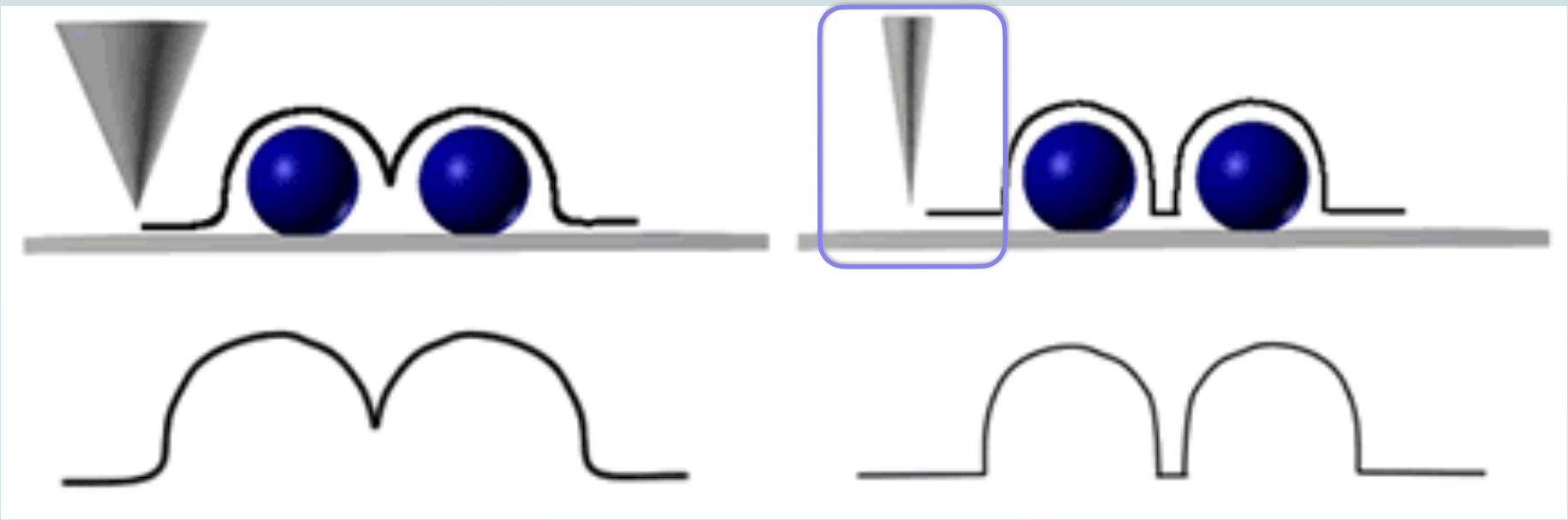
Resolution of the AFM

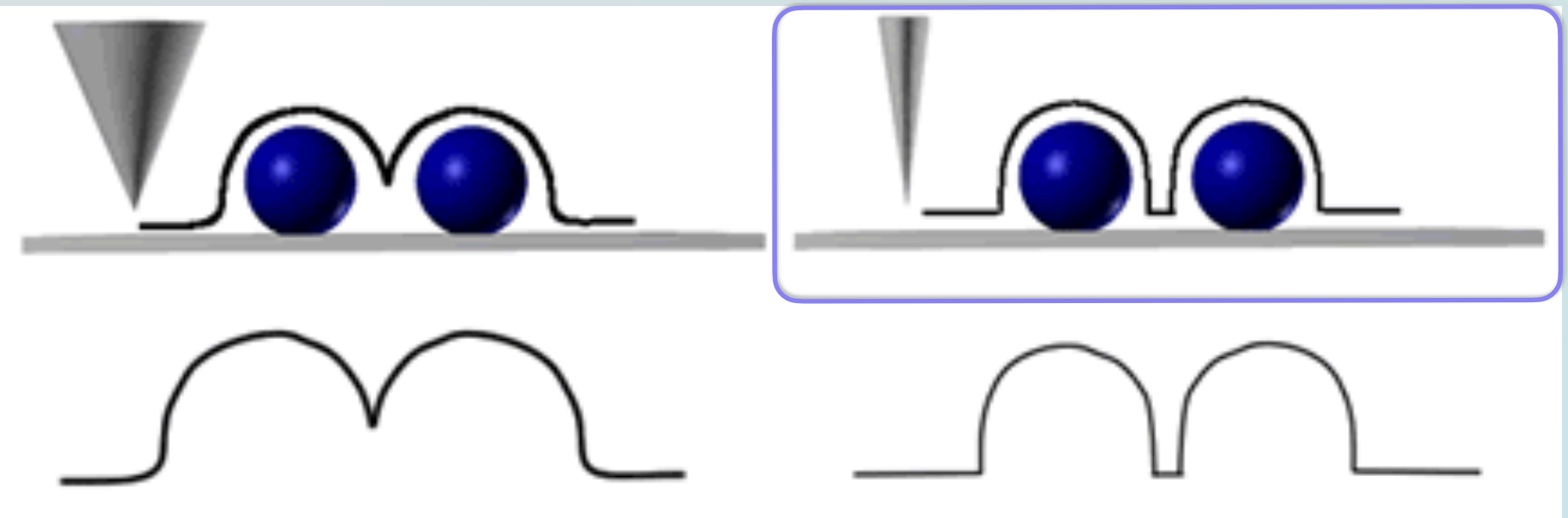
The sharpness of the tip



A schematic diagram showing the factors that affect resolution in AFM

Resolution of the AFM

The sharpness of the tip



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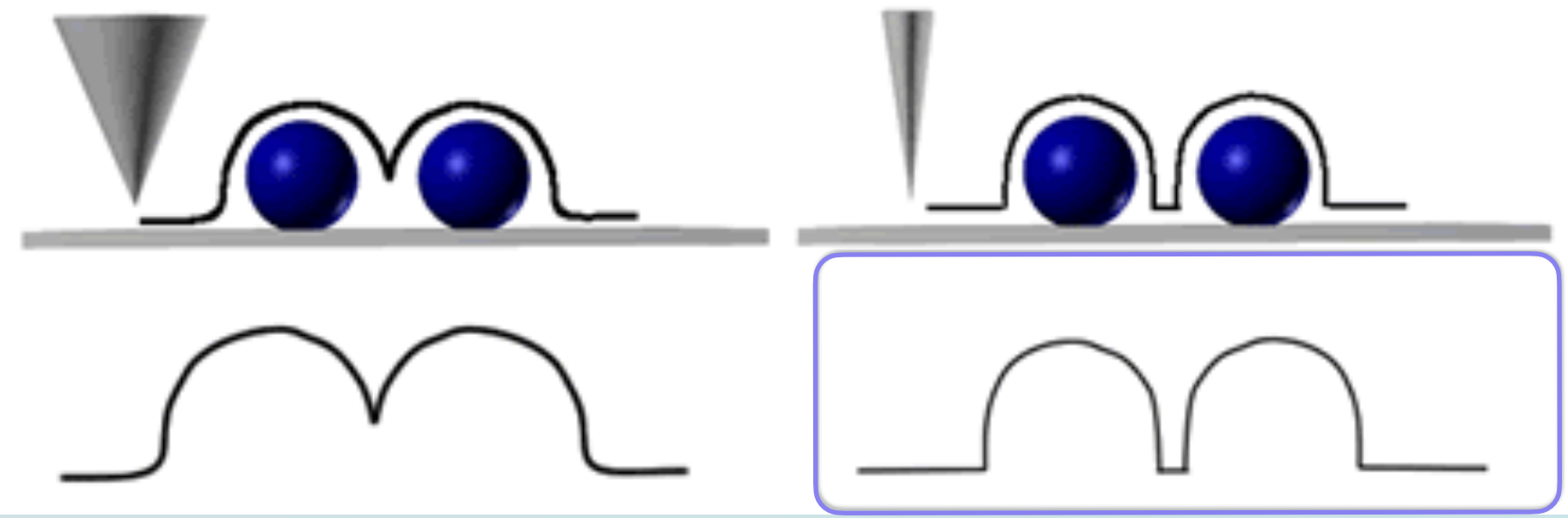
Resolution of the AFM

The sharpness of the tip



A schematic diagram showing the factors that affect resolution in AFM

Resolution of the AFM

The height of the objects to be resolved

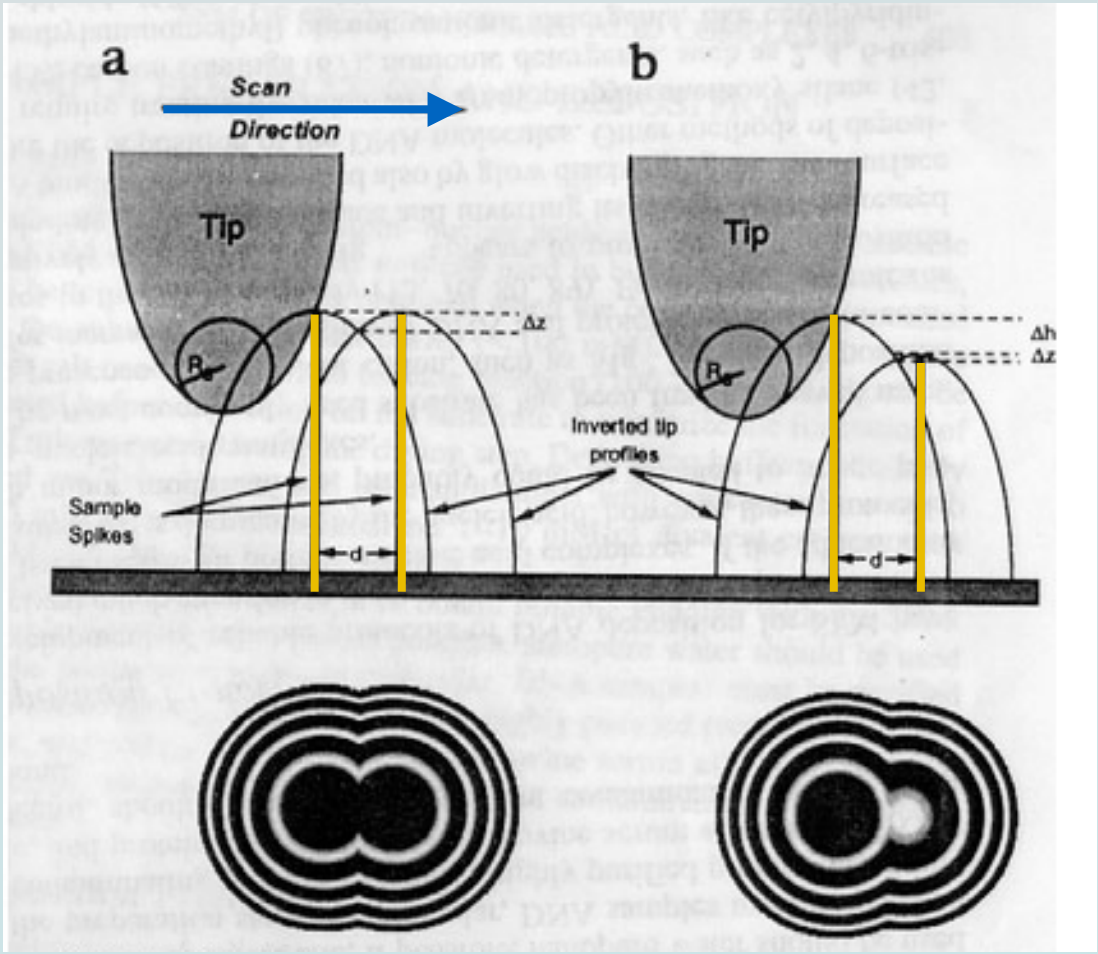


Figure adopted from Bustamante and Revetti 1996

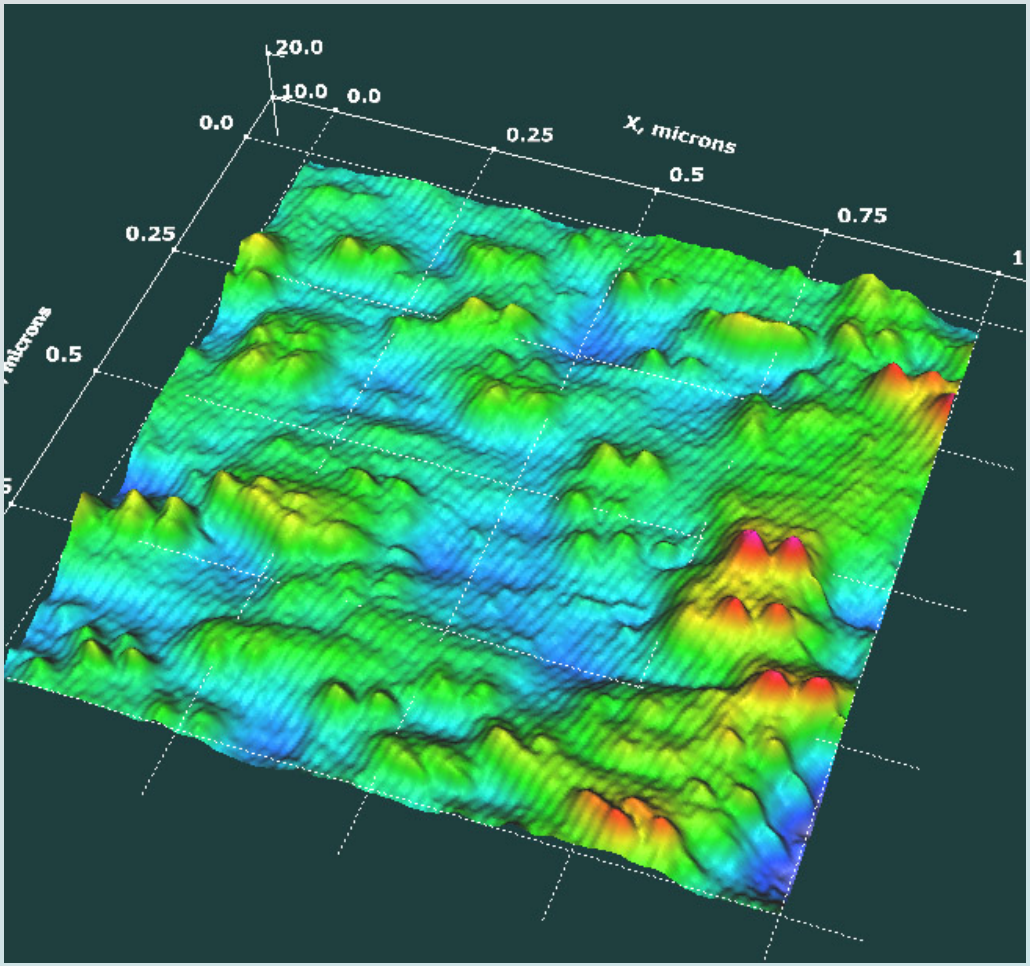
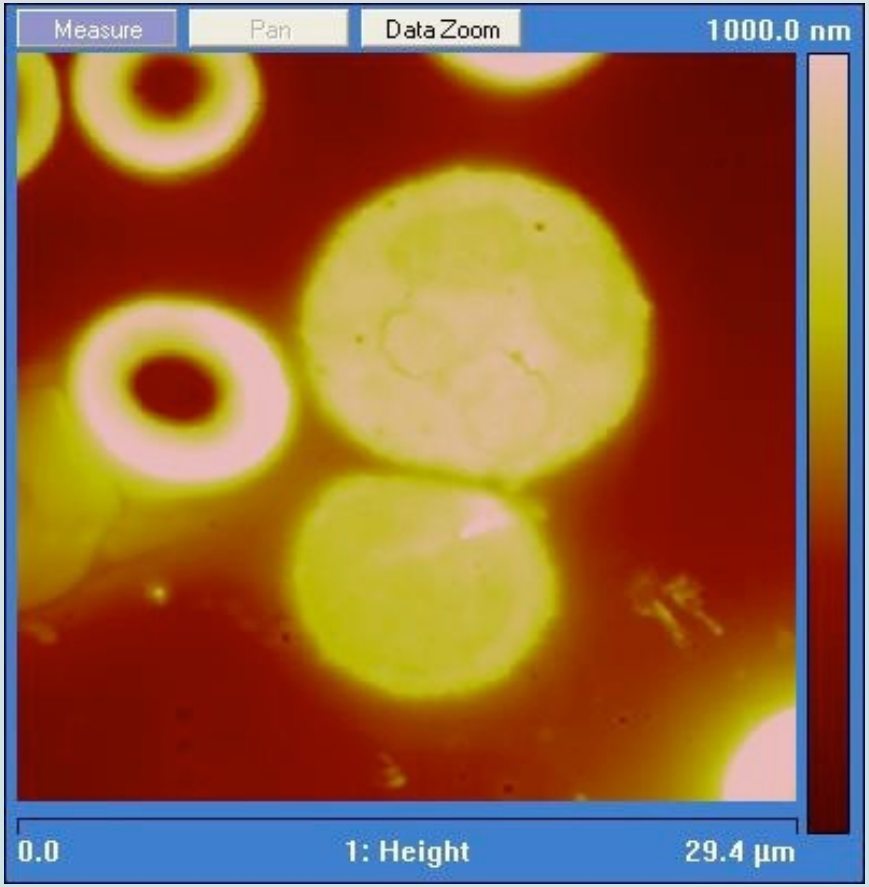


Fig.1. 3D AFM height image of nanoparticles sample. Raw atomic force microscope data is visualized as 3D surface.
AFM data courtesy **Dr. Kannan Raghuraman,** University of Missouri-Columbia.

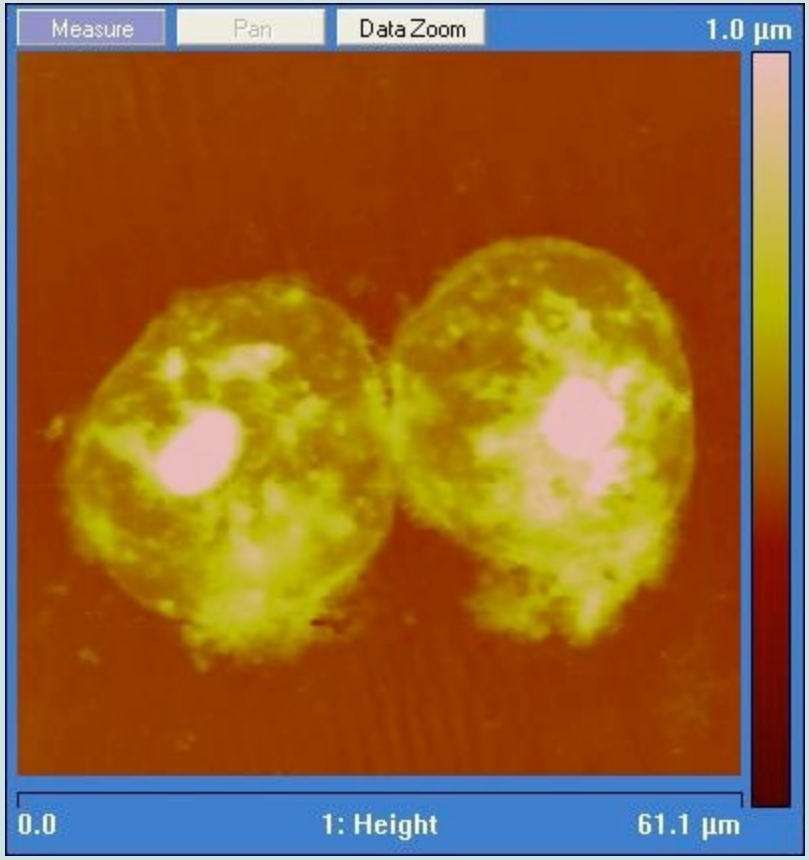
Biological Applications

Countless biological processes - DNA replication, protein synthesis, drug interaction (to name but a few)- are governed by intermolecular forces in the nano newton range that can be detected by the AFM and used to:

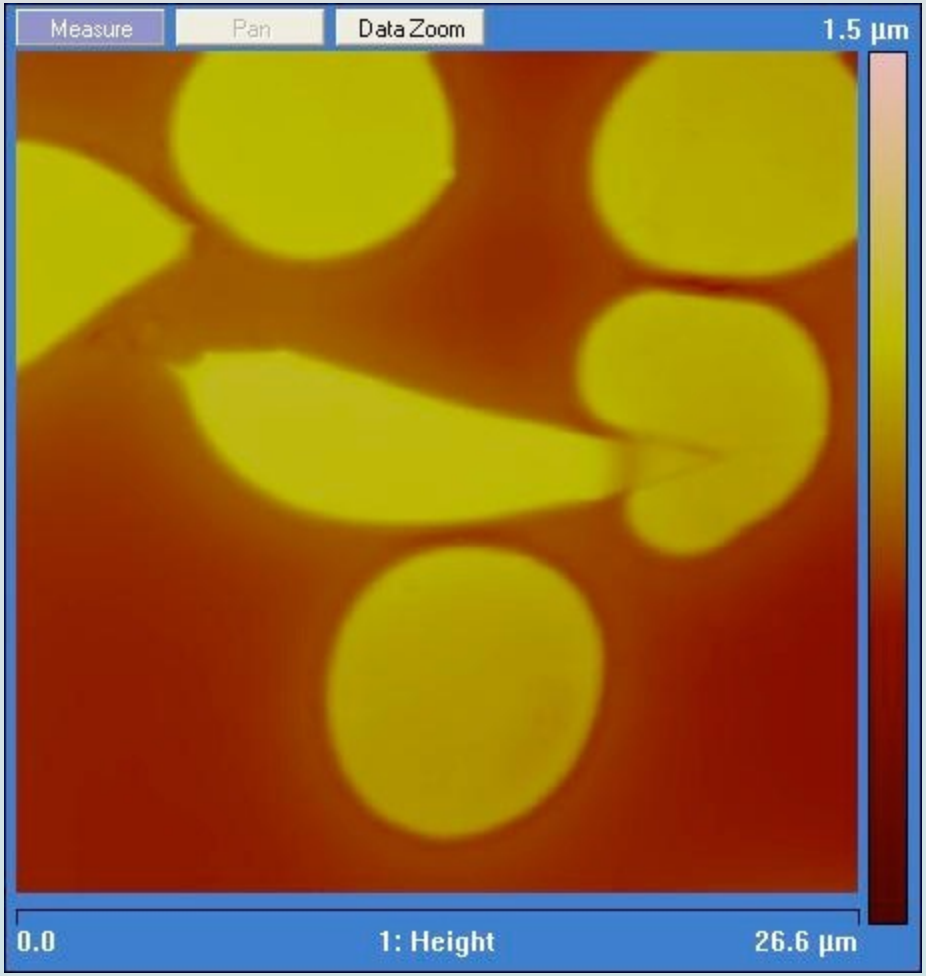
- Study of the structure and function of membrane proteins.
- Study of DNA-Protein interactions.
- Image and analysis of Protein “overt” functions reactions as they proceed in “real time”.
- Cell structures
- Other applications….



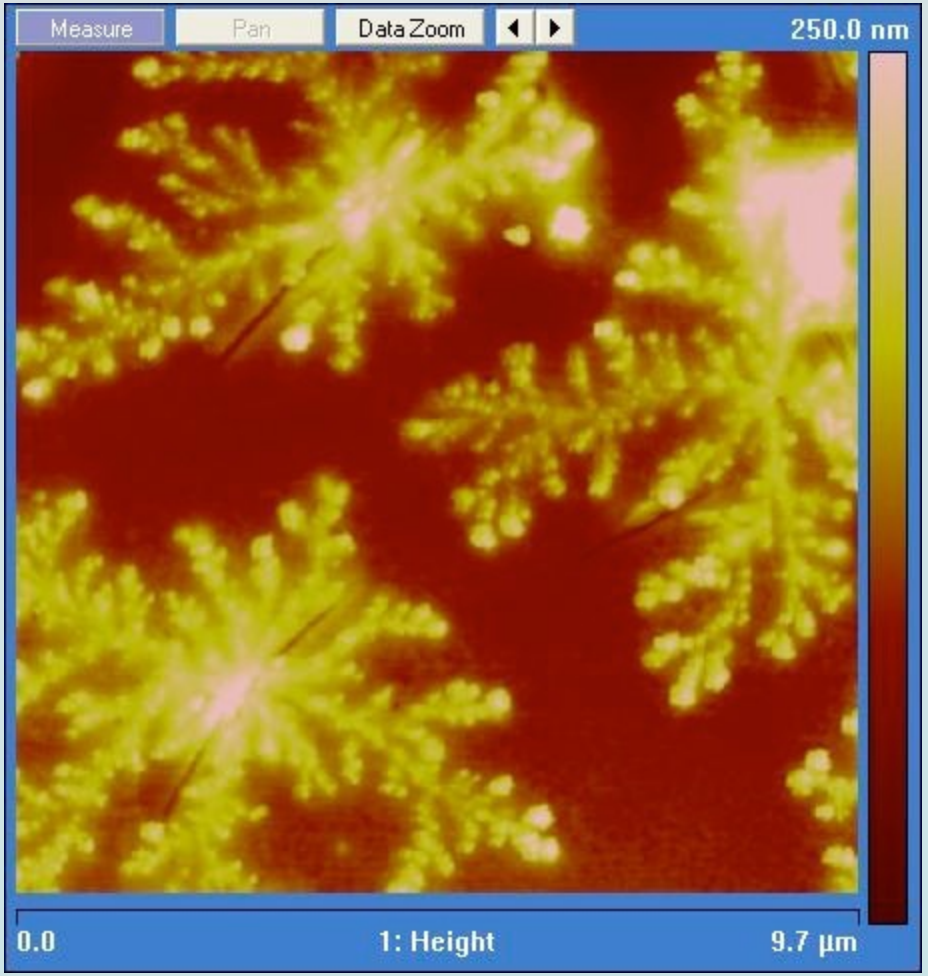
Blood cell



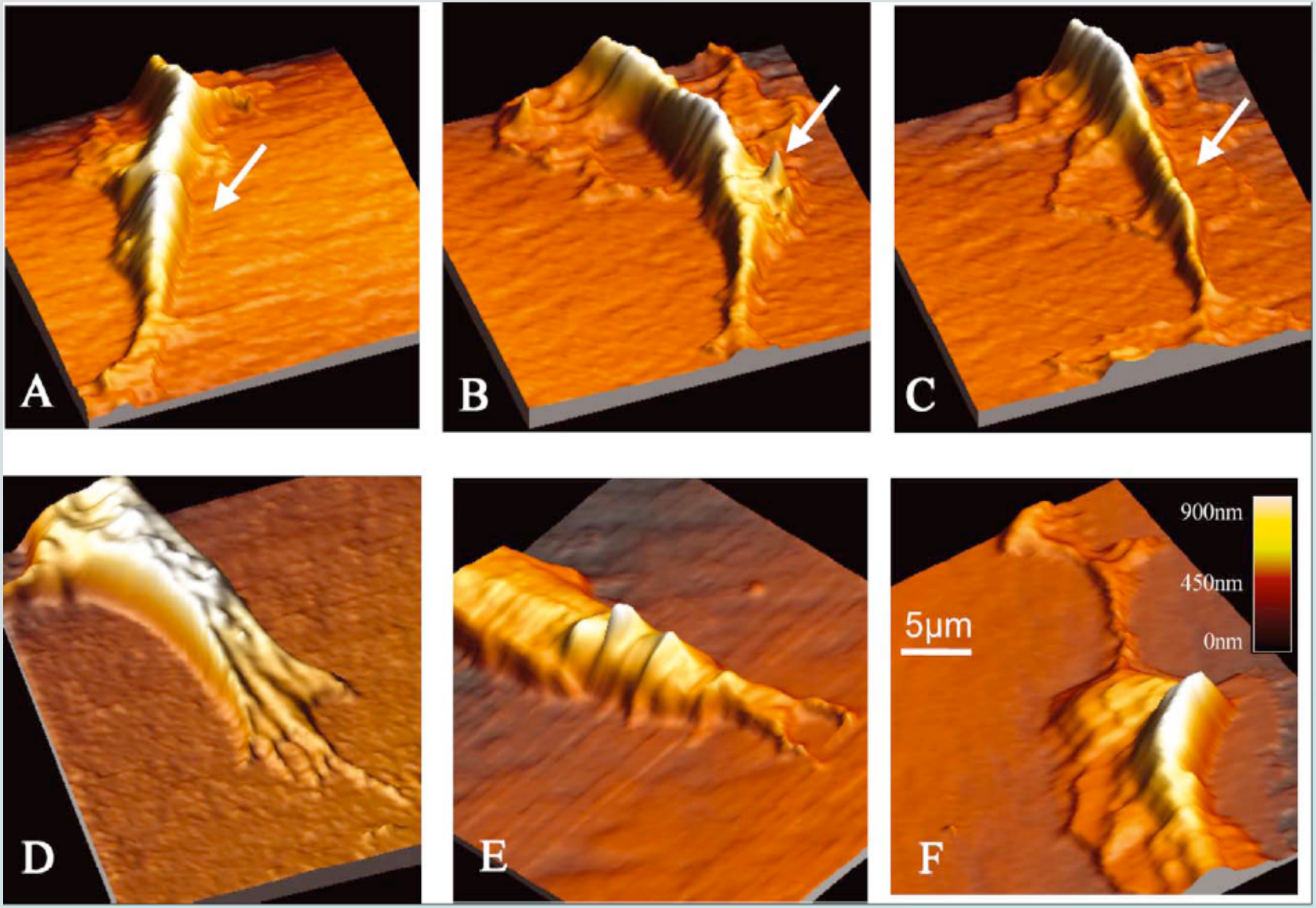
Cancer cell



Sickle cell

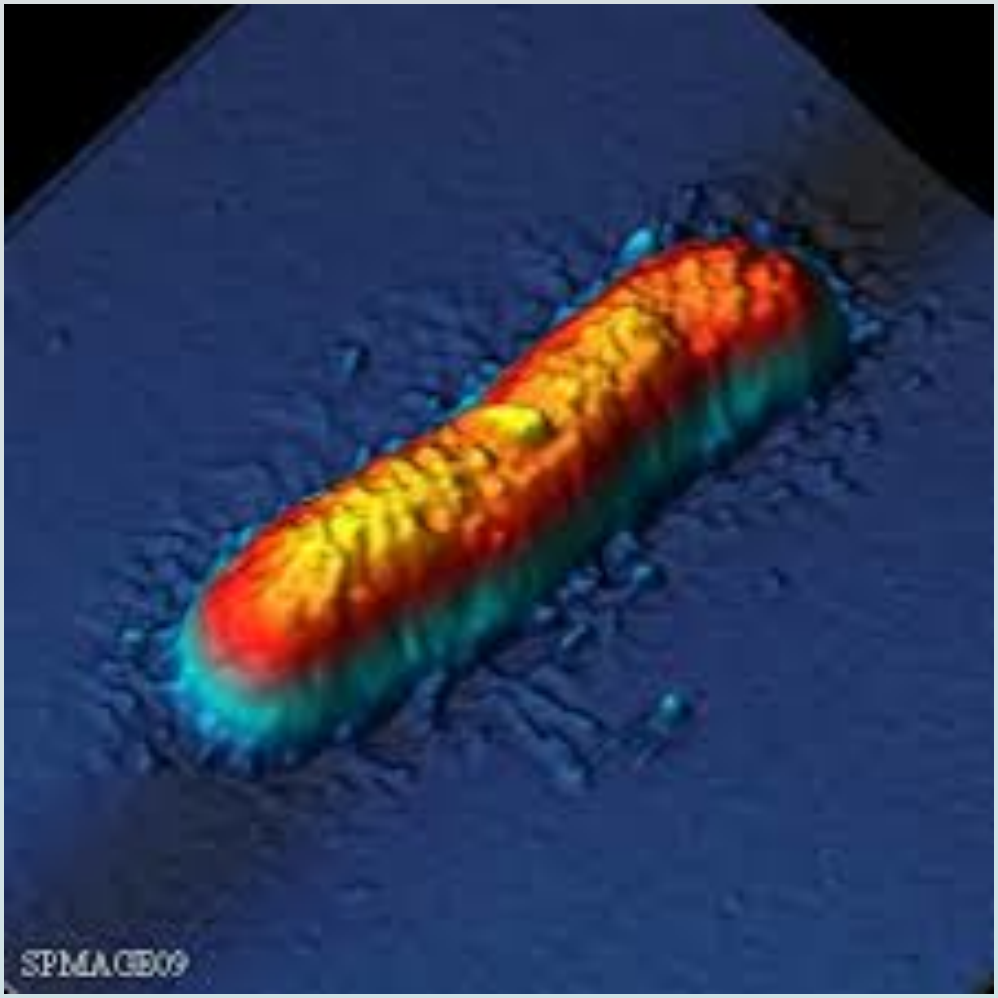


Salt Crystals



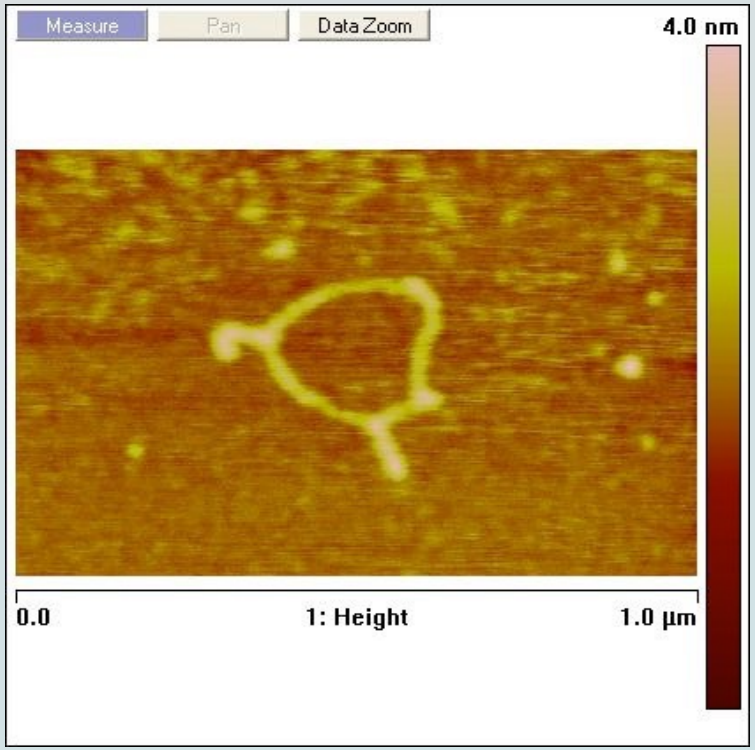
Growing tips of dorsal root ganglion neurites. The three-dimensional shape of the growing tip of a single neurite is shown in A–C, images acquired approximately 5 minutes apart. While the rapid extension and retraction of cytoplasm extending horizontal to the plane of the substrate has been revealed by other imaging techniques in living cells, the relatively high ridges and more singular spines (depicted by arrow in B, but not in A & C) which dynamically reshape themselves on the order of minutes have not. Panels D–F are three-dimensional reconstructions of additional sympathetic (D) and DRG (E, F) growth cones. Vertical projections as well as horizontal spines are present in each case and yet dramatically different. The scale bar applies to X and Y dimensions and the color bar applies to the Z dimension. These are shown only in the F panel, but apply to all panels as the dimensions are very similar.

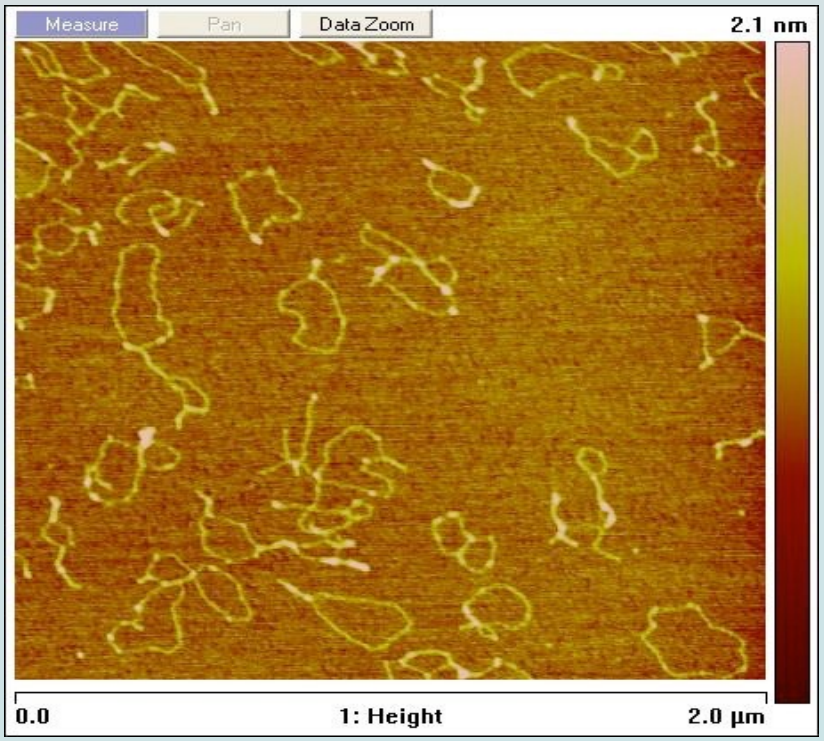
McNally and Borgens, 2004, Journal of Neurocytology 33, 251-258



Rachel Soon. Monash Institute of Pharmaceutical Sciences (**Australia**)

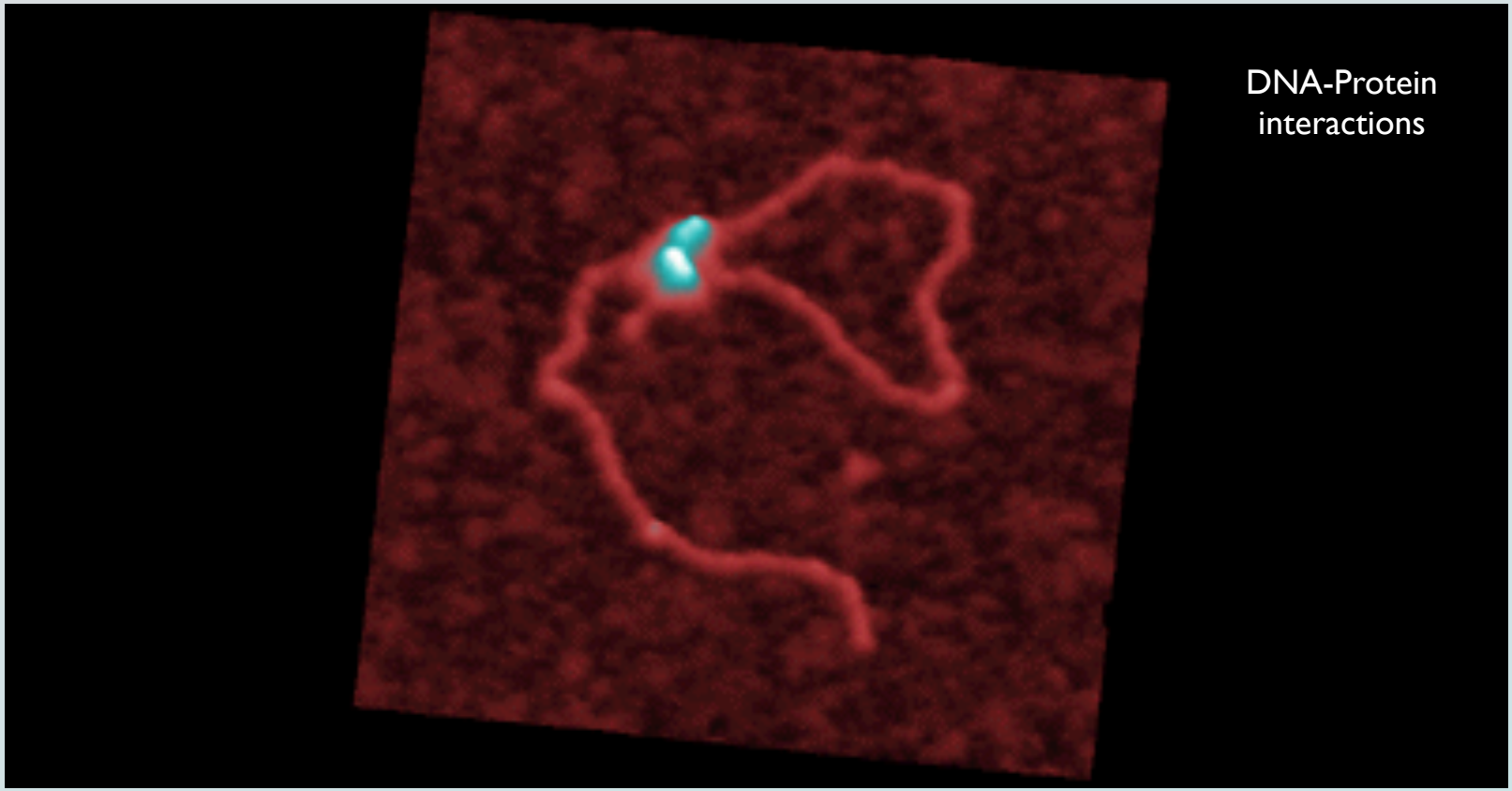
5 x 5um tapping mode AFM image of a gram negative bacterium (A. baumannii) immobilized on a glass surface following treatment with a peptide antibiotic. The image displays outer membrane disruption and leakage of intracellular contents, providing information into the mechanism of action of the antibiotic. A Veeco Dimension 3000 AFM was utilized to obtain this image in ambient conditions.





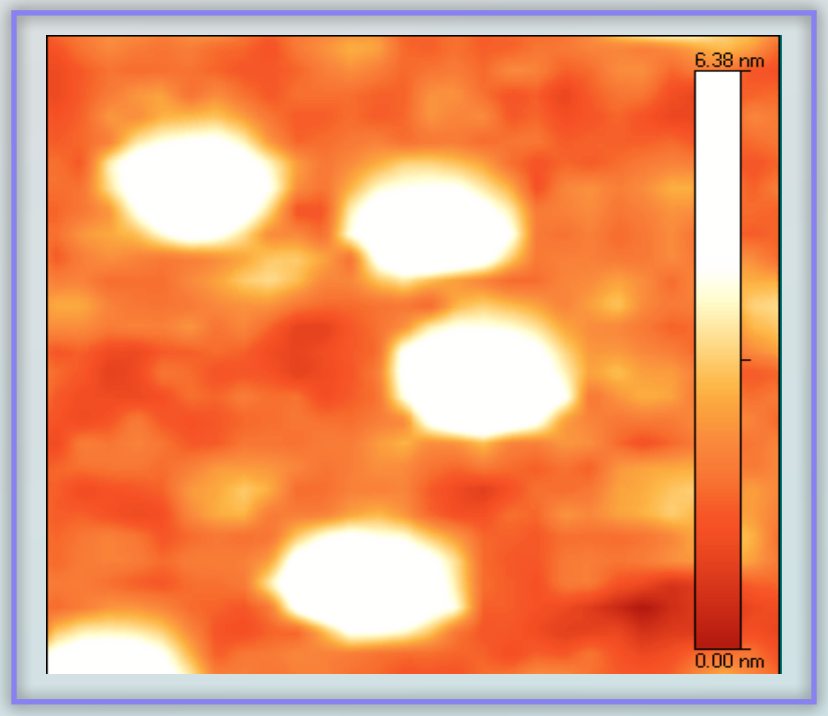
Two different kinds of plasmid scanned in air.

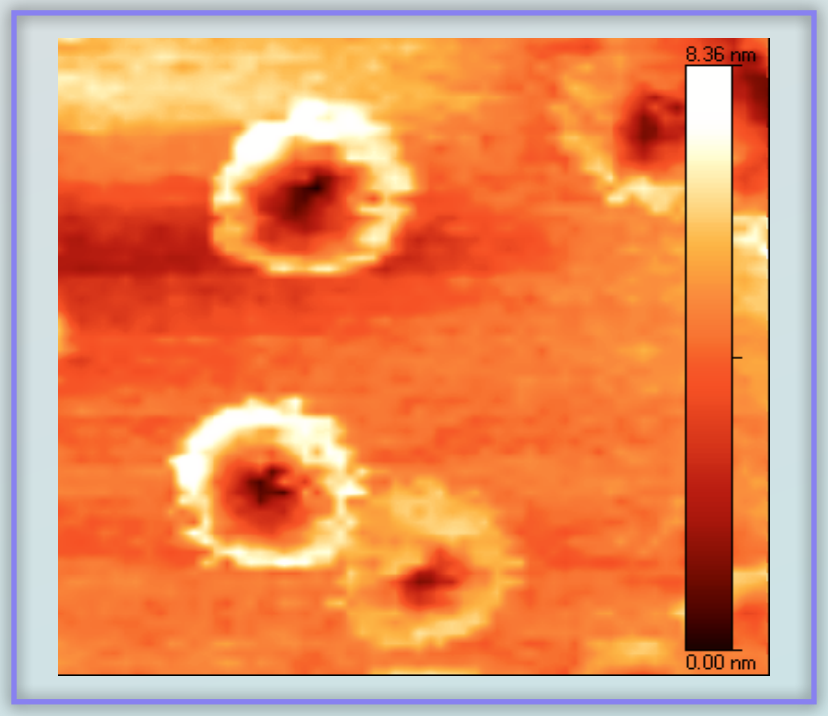
The first one is **954 nm** total in length.
The second is pGEM3Zf(+) vector, **3,197bp**; the average length being **674 nm.**



"Tapping Mode" AFM image of an individual human transcription factor 2 DNA complex. Protein:protein interactions of two regulatory proteins which facilitate the looping of the DNA, allowing two distal DNA sites to be combined.

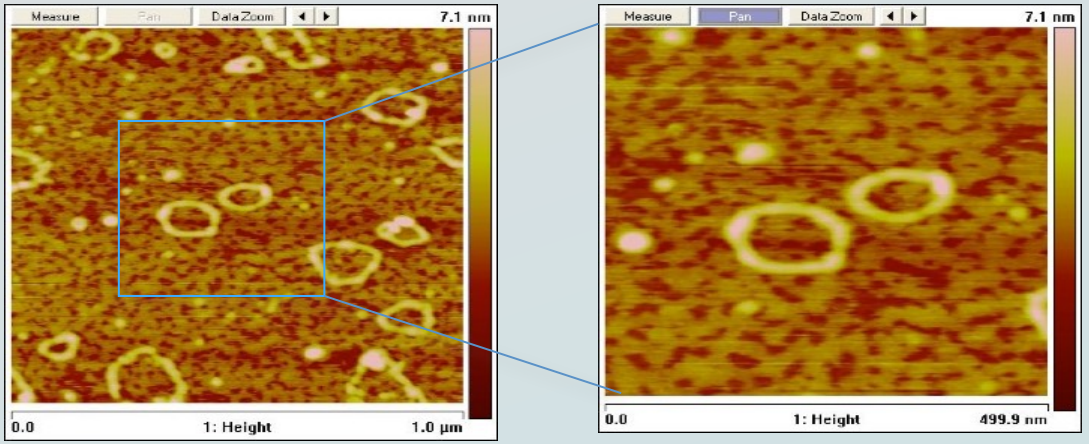
Image courtesy of Bustamante Lab, Institute of Molecular Biology, University of Oregon, Eugene http://thunder.temple.edu/~lkhrizma/transfactor.htm

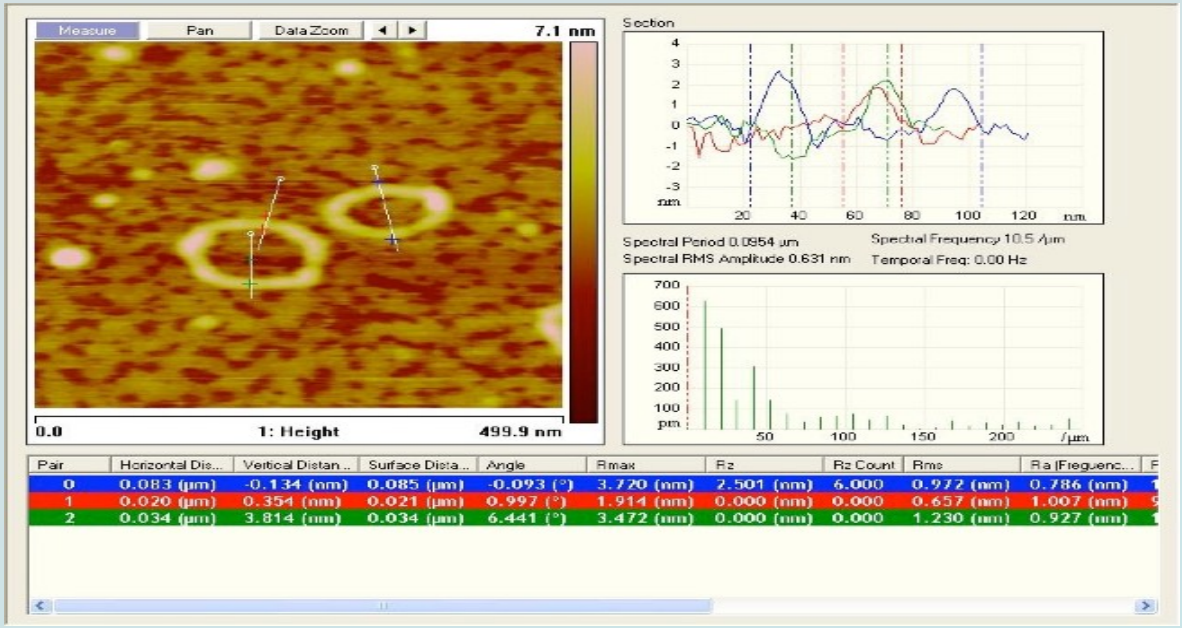




A high resolution (Raw data) AFM image taken by Dr. Hsiuchin Yang (GSU) of the SecA protein from *E. coli*.

The protein was analyzed in solution (left panel) and bound to lipid (right panel); These contrasting views demonstrate the two different, environmentally-dependent forms of the protein.

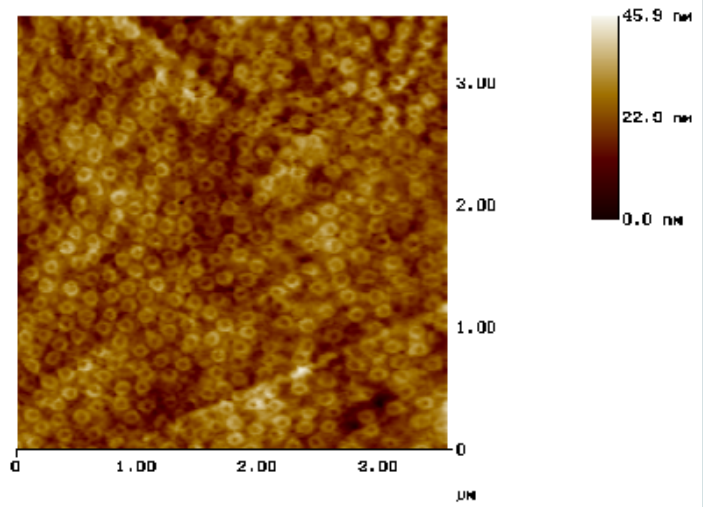




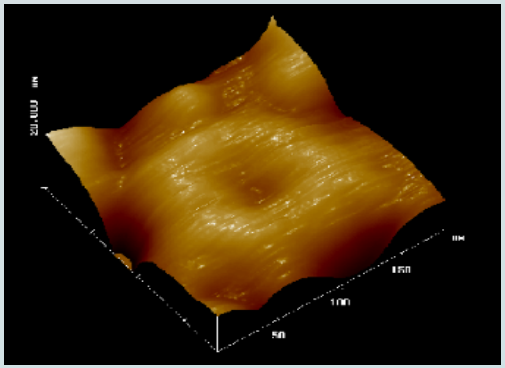
SecA with lipid

SecA ATPase is the major protein in the Sec-dependent protein translocation pathway on the cytoplasmic membrane of *Escherichia coli*. With the driving force provided by ATP, secretory proteins can cross the membrane through the channel formed by SecA.

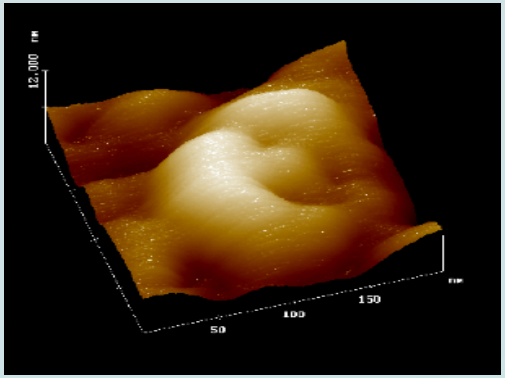
SecA can form channel structure with lipid, either with open ring or closed ring. Purified SecA was incubated with lipid and mounted on freshly cleaved mica. The width of the whole ring was 0.083µm, the width of single ridge was 0.020 µm, and the height of the ridge was 3.814nm.



A high resolution AFM image of the nuclear membrane of a *Xenopus* oocyte. A high density of nuclear pore complexes can be clearly seen as the round donut-like structures.

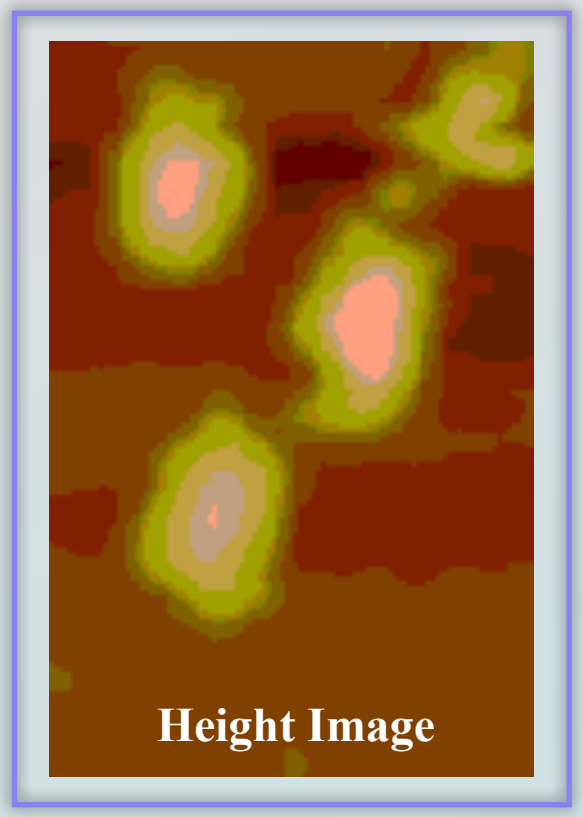


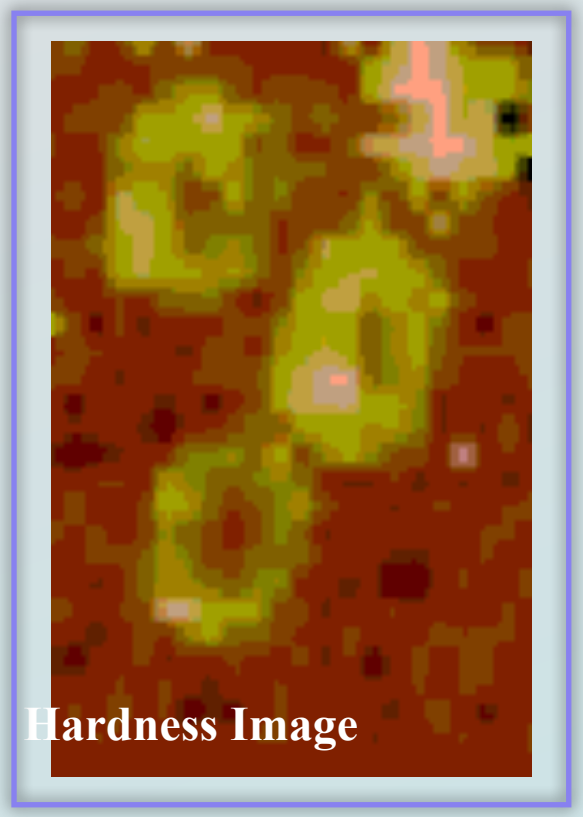
An AFM image, 250nm x 250nm scan, of a single nuclear pore complex in the nuclear membrane of a *Xenopus* oocyte in the cytoplasmic face. This NPC is in the open state.



After calcium depletion using EGTA, an effective calcium chelator, a conformational change takes place as evidenced by the emergence of the central plug in the pore region. This nuclear pore complex is in the closed state. Species between 20 and 40 kDal no longer enter the pore.

Lee, M. Annie; Dunn, Robert C.; Clapham, David E.; Stehno-Bittel, Lisa. **Calcium regulation of nuclear pore permeability.** Cell Calcium (1998), 23(2/3), 91-101.





The AFM can measure the elasticity of materials. These synaptic vesicles are high (white) in the center in the height image but dark in the center in the hardness image, because their centers are harder than their edges. The vesicles are on a hard surface and are from the electric organ of Torpedo californica, a marine ray. They are about 108nm in diameter.

Laney DE, Garcia RA, Parsons SM, Hansma HG. Biophys J. 1997 Feb;72(2 Pt 1):806-13. Changes in the elastic properties of cholinergic synaptic vesicles as measured by atomic force microscopy.

Advantages of AFM technology

AFM vs SEM

Advantages:

1. AFM provides a true three-dimensional surface profile.
2. Samples viewed by AFM do not require any special treatments (such as metal/carbon coatings) that would irreversibly change or damage the sample.
3. AFM does not require vacuum environment or a conductive sample: good for biological samples
4. AFM can provide higher resolution than SEM.
5. SEM can only scan dead samples.

Disadvantages:

1. Scanning size of AFM (150 x150 μm) is smaller than SEM
2. An incorrect choice of tip for the required resolution can lead to image artifacts.
3. Scan slower (several minutes) than SEM (near real-time).
4. AFM images can also be affected by **hysteresis** of the piezoelectric material (Lapshin, 1995) and cross-talk between the (x,y,z) axes that may require software enhancement and filtering.
5. AFM probes cannot normally measure steep walls or overhanging structures.

Questions?