BT 601: Analytical Biotechnology

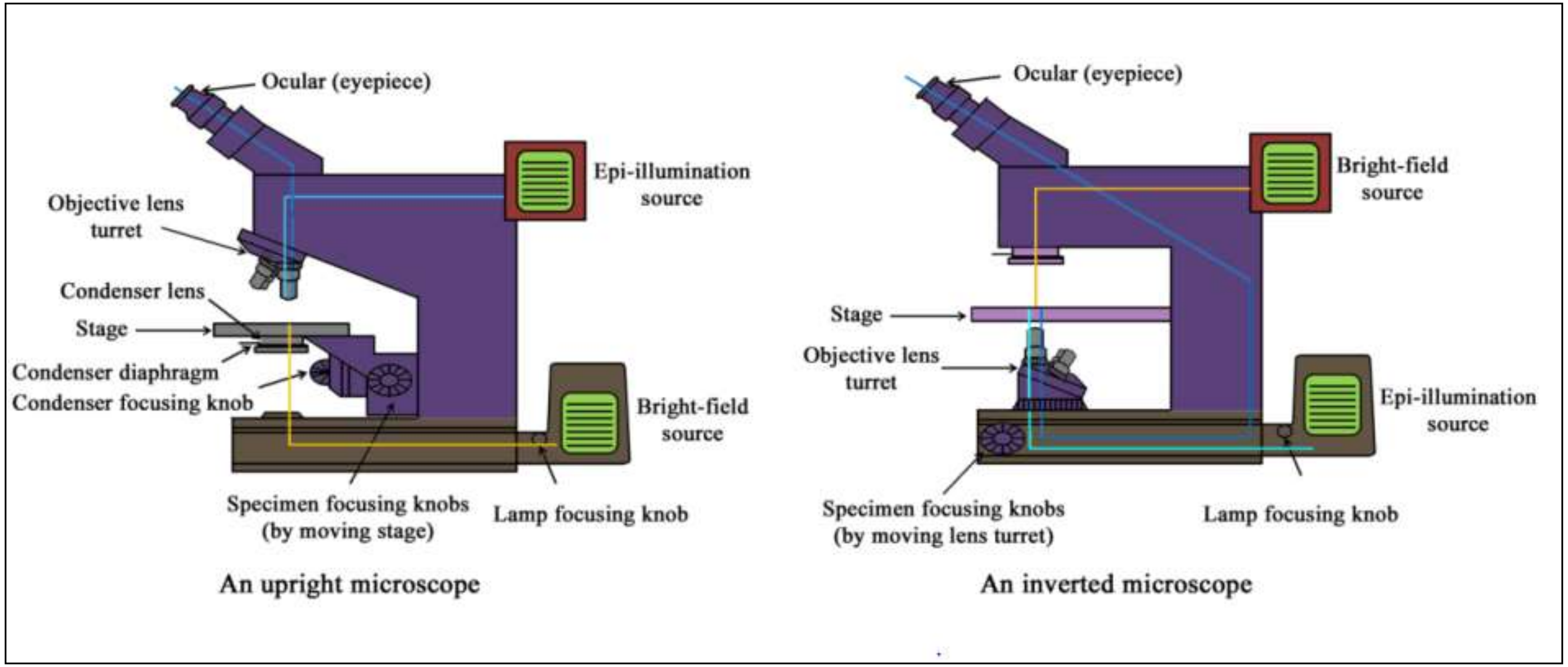
-Prof. Siddhartha Sankar Ghosh

Lec-15

Inverted and upright microscope

Light microscopes come in two designs: upright and inverted

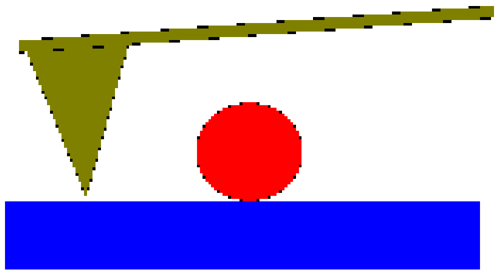
- In upright microscope, the objective turret is fixed and the image is focused by moving the sample stage up and down.
- In inverted microscope, the sample stage is fixed and objective turret is moved up and down to focus the final image.

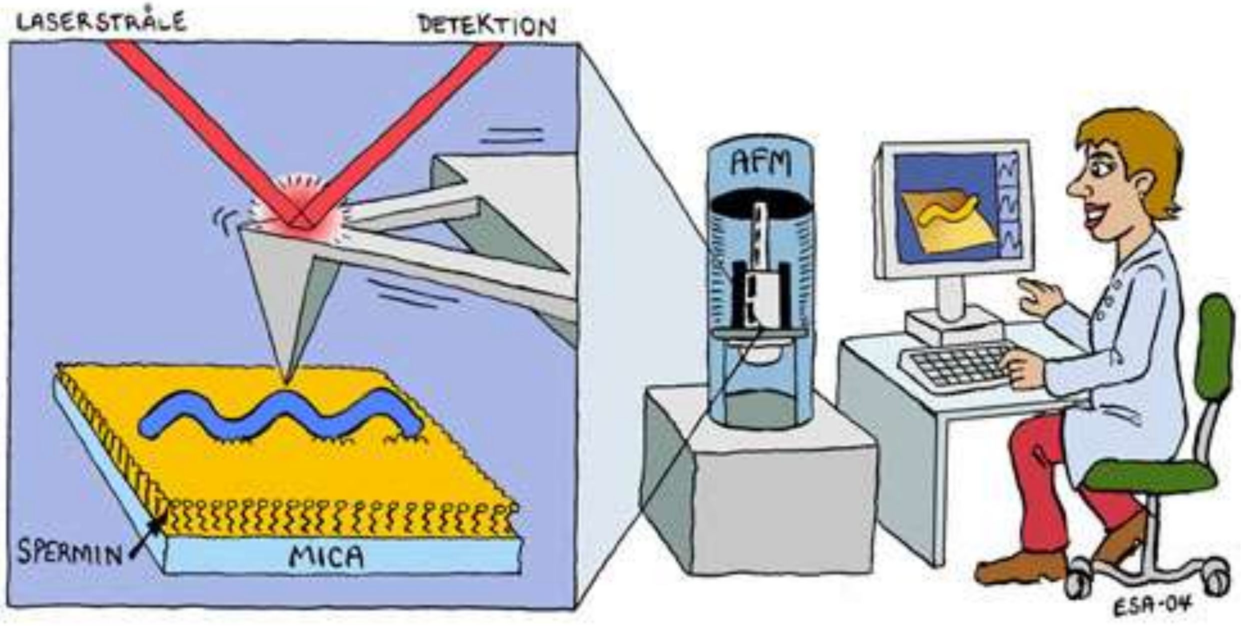


Design of upright (A) and inverted (B) microscopes

Cantilever System

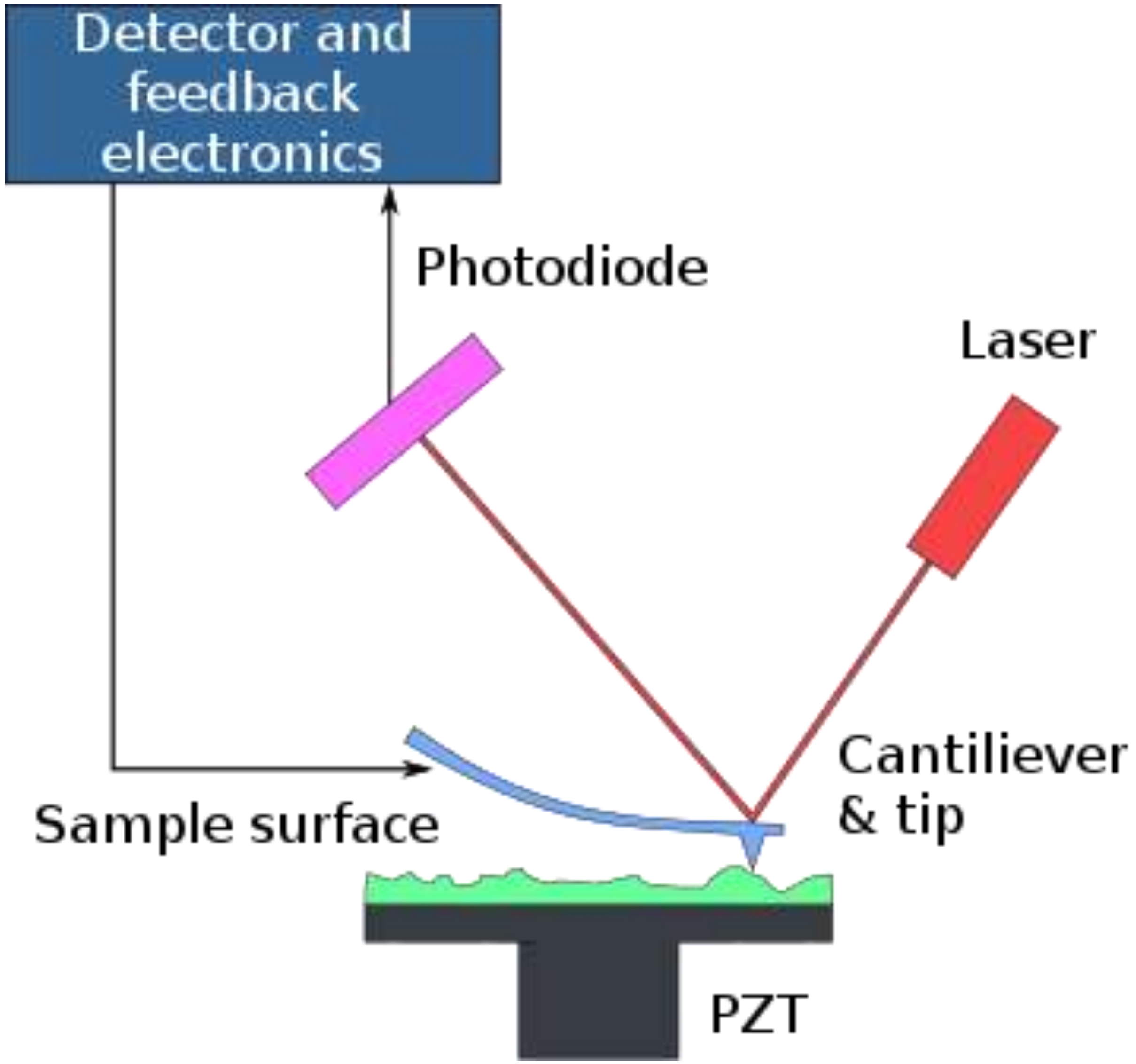
Atomic Force Microscopy (AFM)



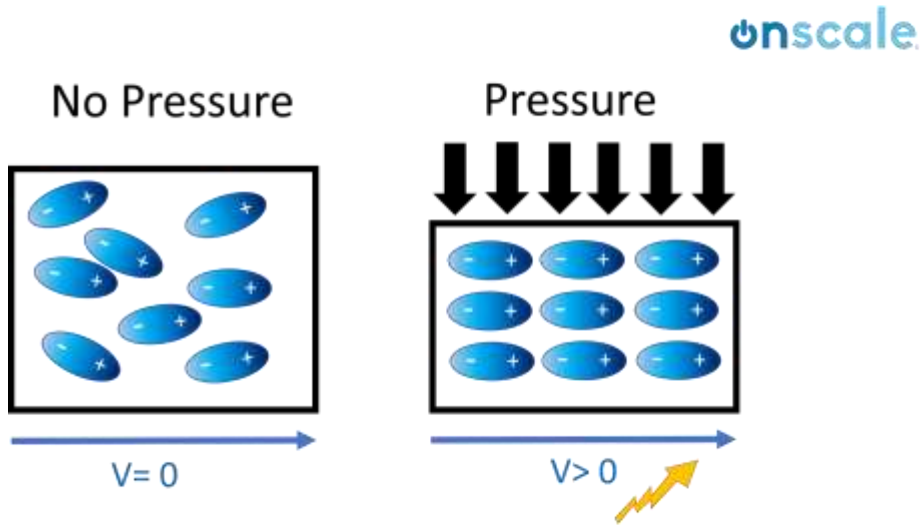


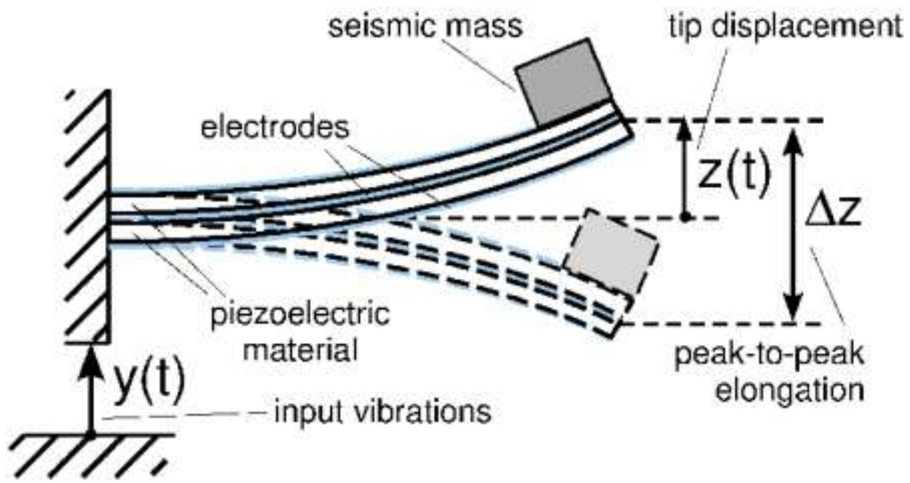
AFM tips made of silicon nitride

An AFM generates images by scanning a small cantilever over the surface of a sample. The sharp tip on the end of the cantilever contacts the surface, bending the cantilever and changing the amount of laser light reflected into the photodiode. The height of the cantilever is then adjusted to restore the response signal resulting in the measured cantilever height tracing the surface.



Piezoelectric Effect is the ability of certain materials to generate an electric charge in response to applied mechanical stress

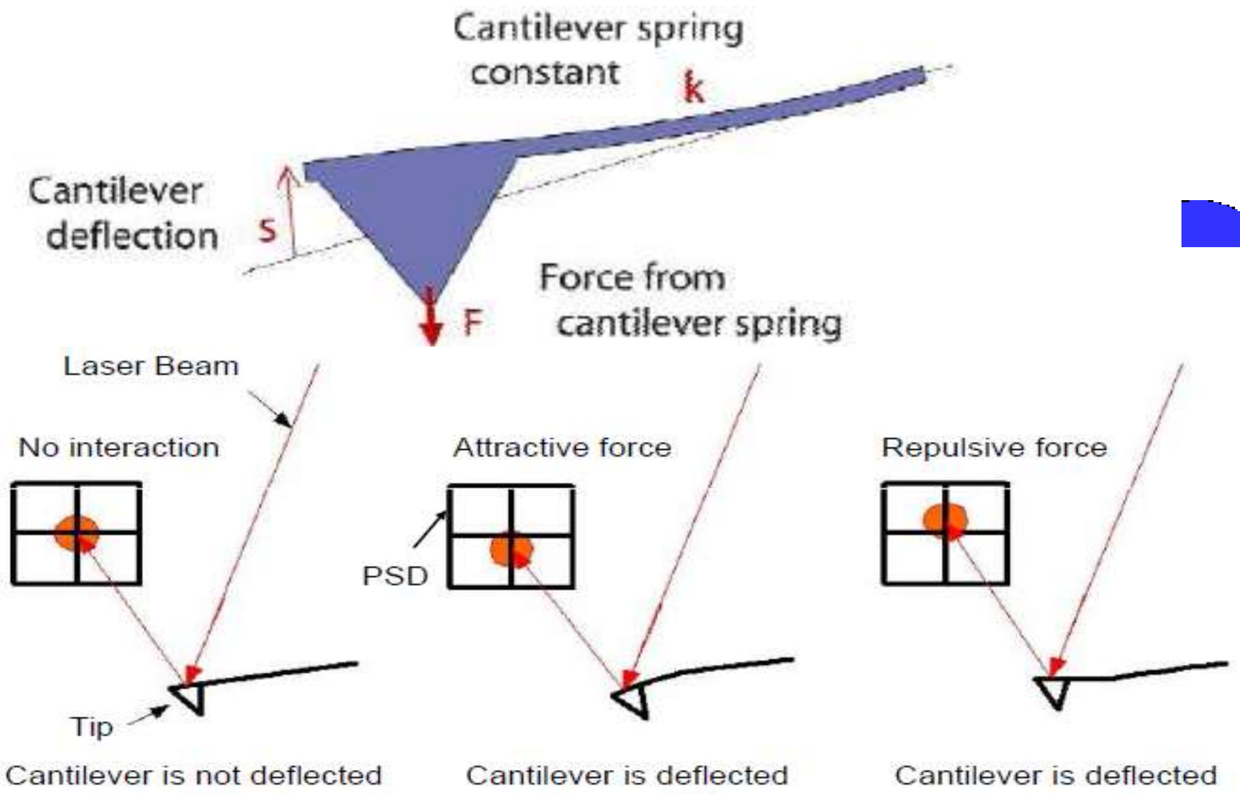


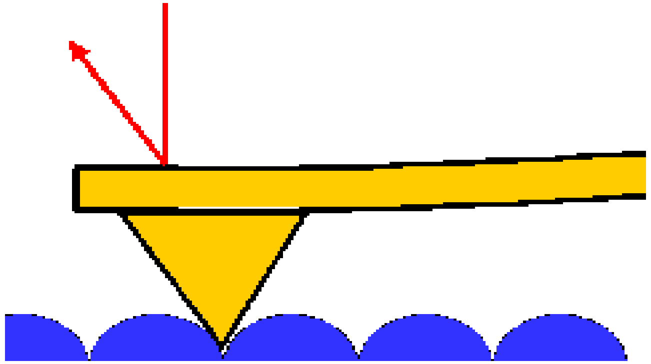


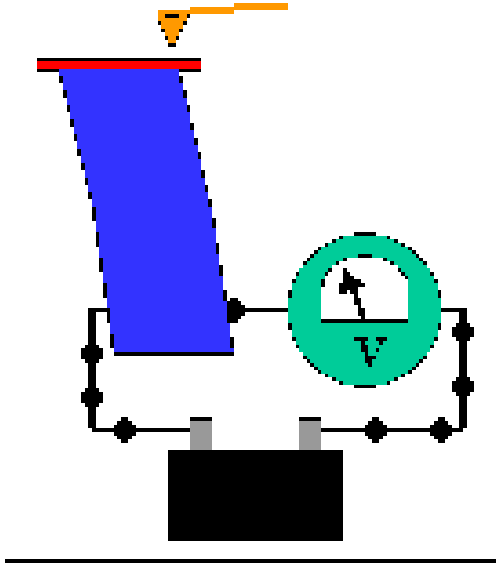
Crystalline materials produce small amounts of electricity when a force is applied that changes their shape in some way. When small amounts of pressure are applied to a quartz crystal, a small voltage is produced from the changing charge created by the moving electrons.

How are forces measured ?

- Hooke's law: F= -ks





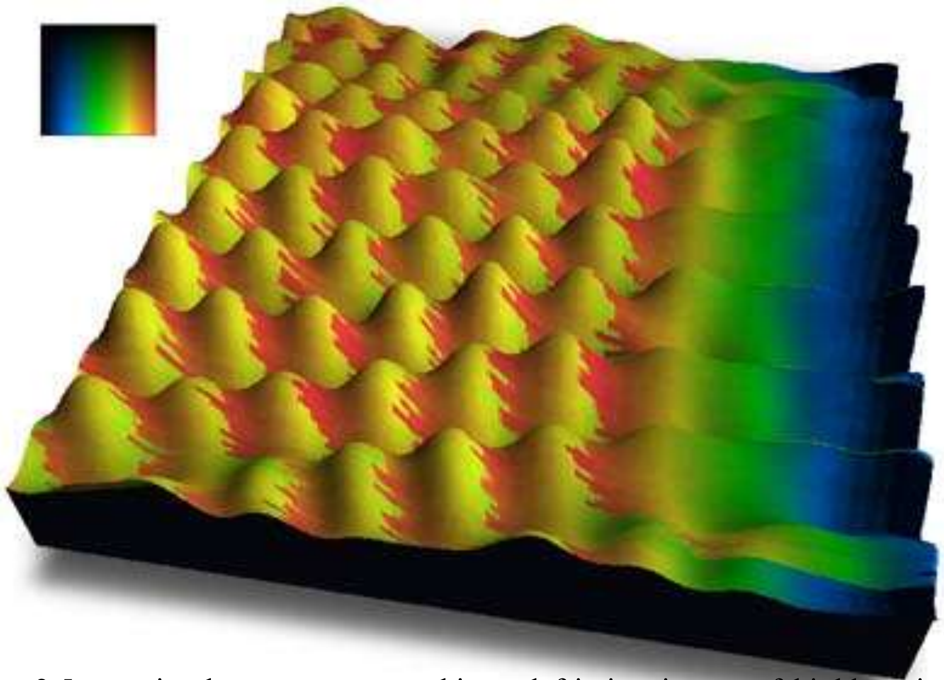


- Laser Beam Deflection Method

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

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

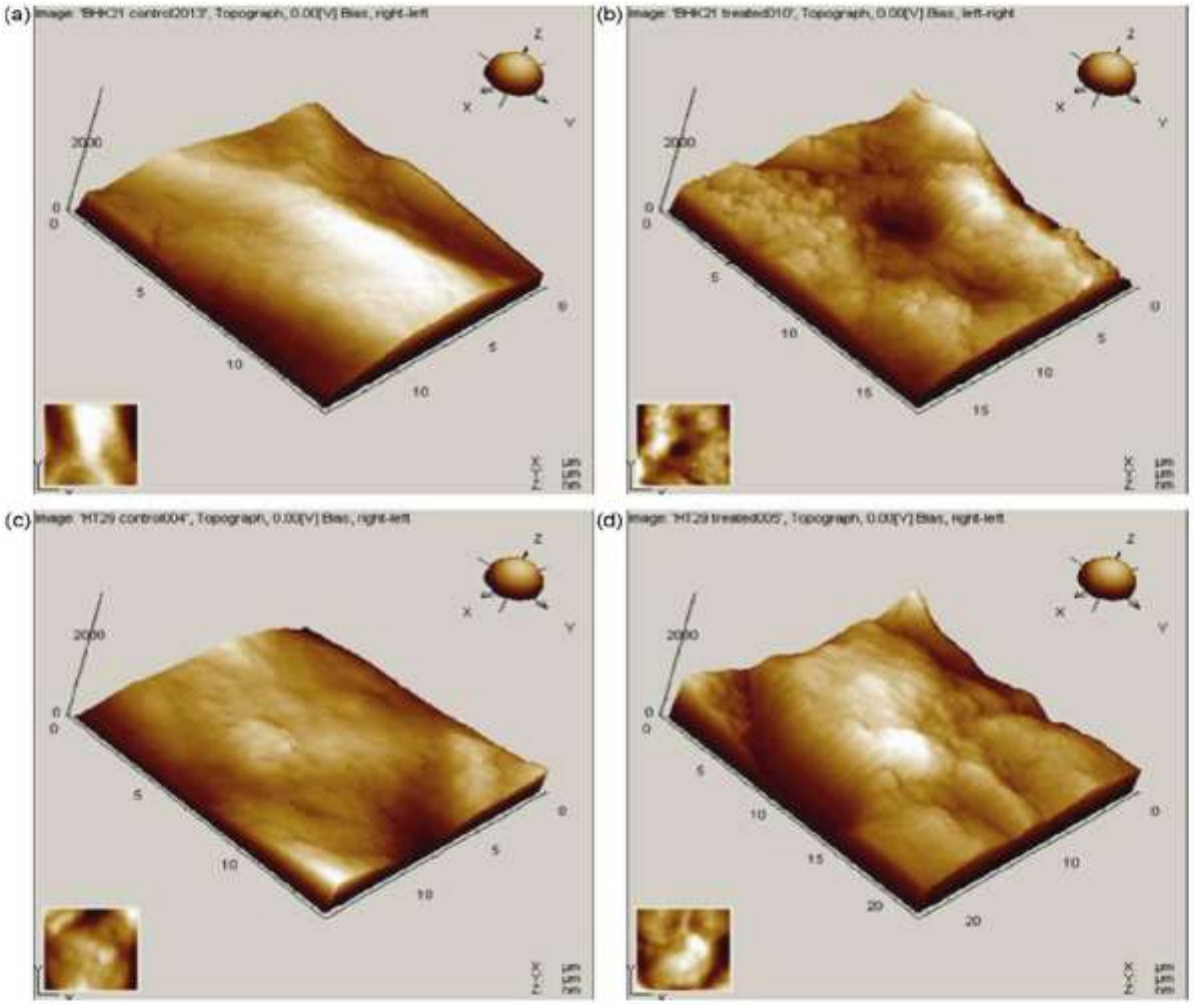


2.5 x 2.5 nm simultaneous topographic and friction image of highly oriented pyrolytic graphic (HOPG). The bumps represent the topographic atomic corrugation, while the coloring reflects the lateral forces on the tip. The scan direction was right to left

Applications of AFM

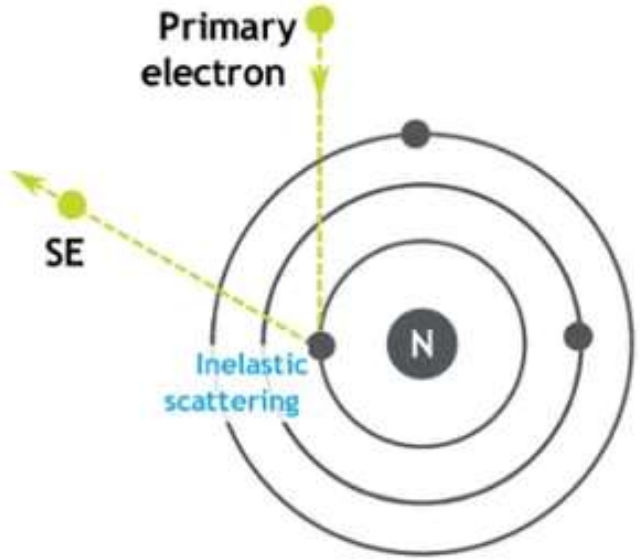
- Digitally image a topographical surface
- Determine the roughness of a surface sample or to measure the thickness of a crystal growth layer
- Image non-conducting surfaces such as proteins and DNA
- Study the dynamic behavior of living and fixed cells
- Can be done in liquid /gas mediums; Vacuum not required.
- No special treatment of sample.
- High resolution: 0.1nm vertically &~ 1nm X-Y direction.

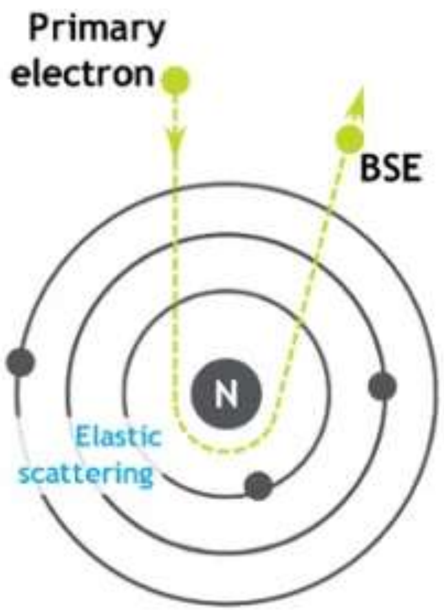
AFM analysis of Ag NPs treated BHK21 and HT29 cells. Atomic force microscope images showed three-dimensional surface topography of BHK21 and HT29 cell membrane under 10m×10m fields of view, (**a and c**) untreated BHK21 and HT29 cells respectively; (b and d) Ag NPs treated BHK21 and HT29 cells respectively.

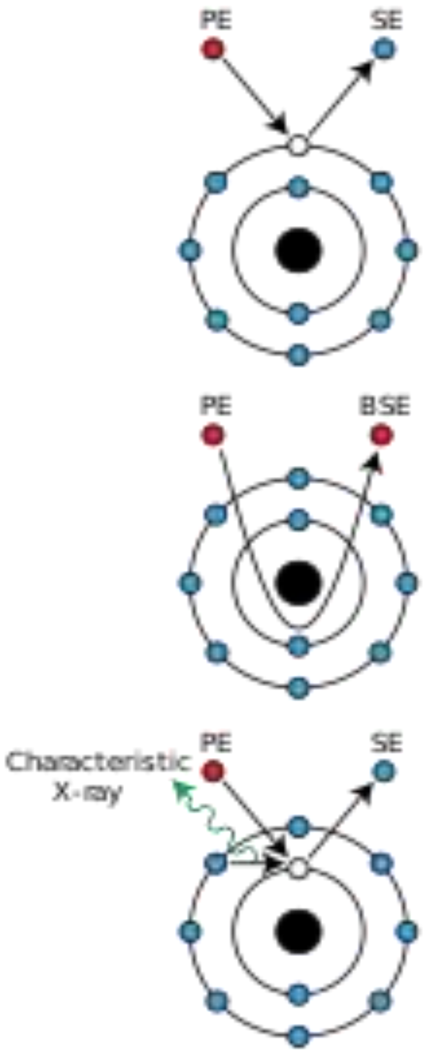


Scanning Electron Microscope (SEM)

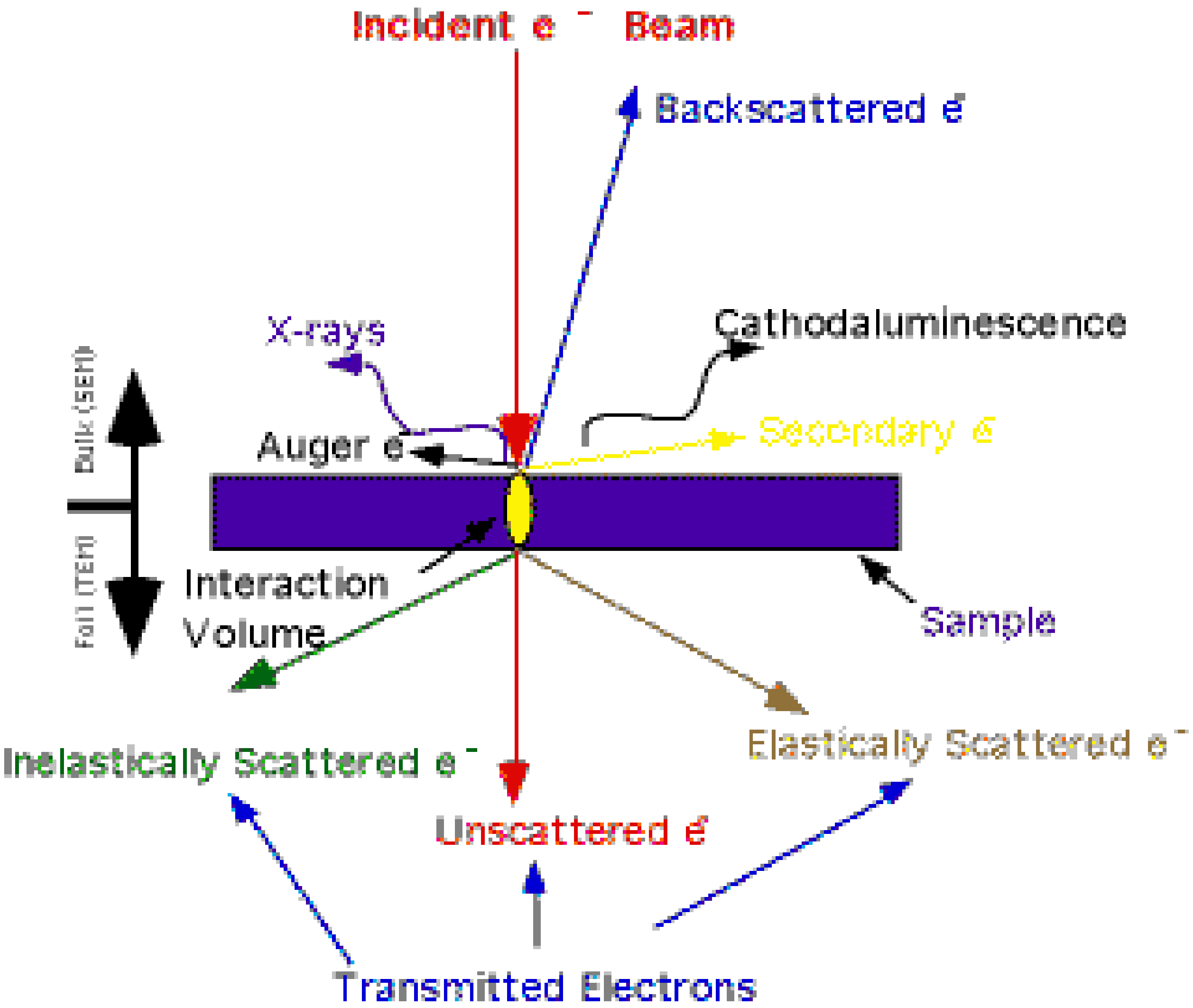
Secondary and Back Scattered Electron



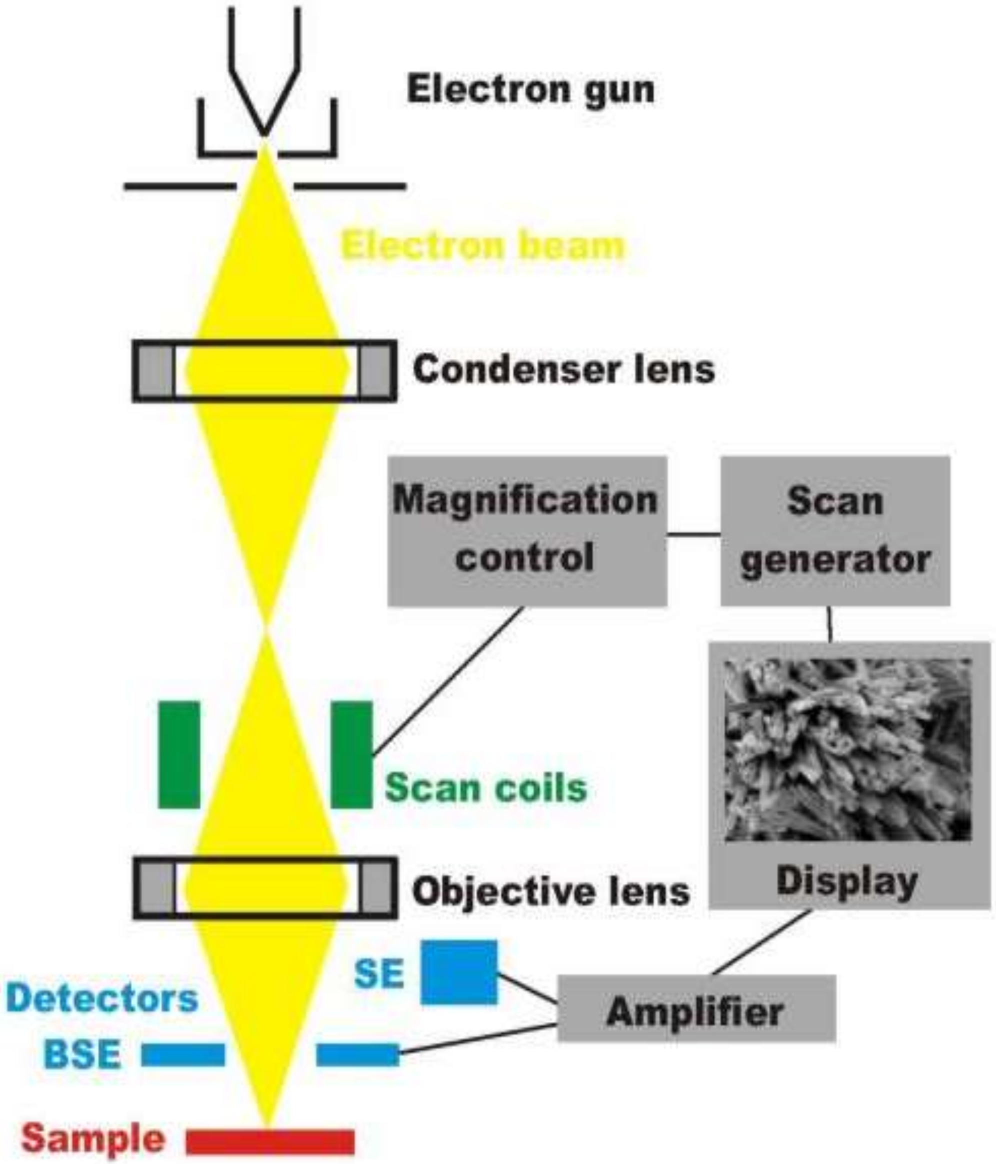




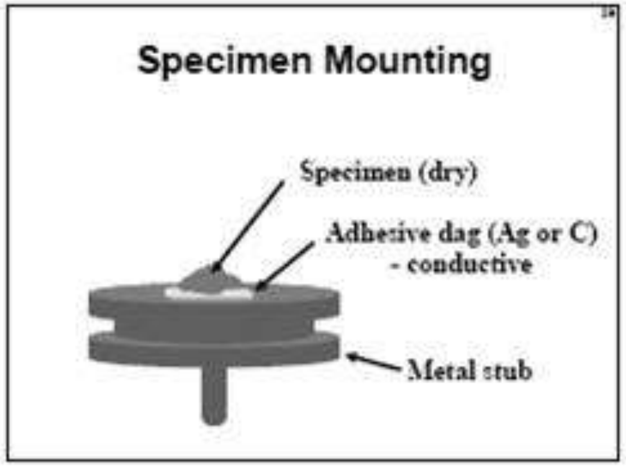
Specimen Information



How the SEM works?



SEM stub

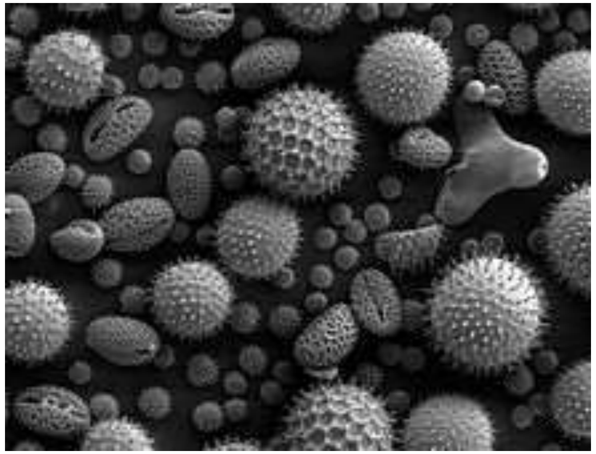


What is SEM ?

- It is a microscope that produces an image by using an electron beam that scans the surface of a specimen inside a vacuum chamber.
- The SEM functions much like an optical microscope but uses electrons instead of visible light waves.
- The SEM uses a series a series of EM coils as lenses to focus and manipulate the electron beam.
- Samples must be dehydrated and made conductive.
- Images are black and white.

What can we study in a SEM?

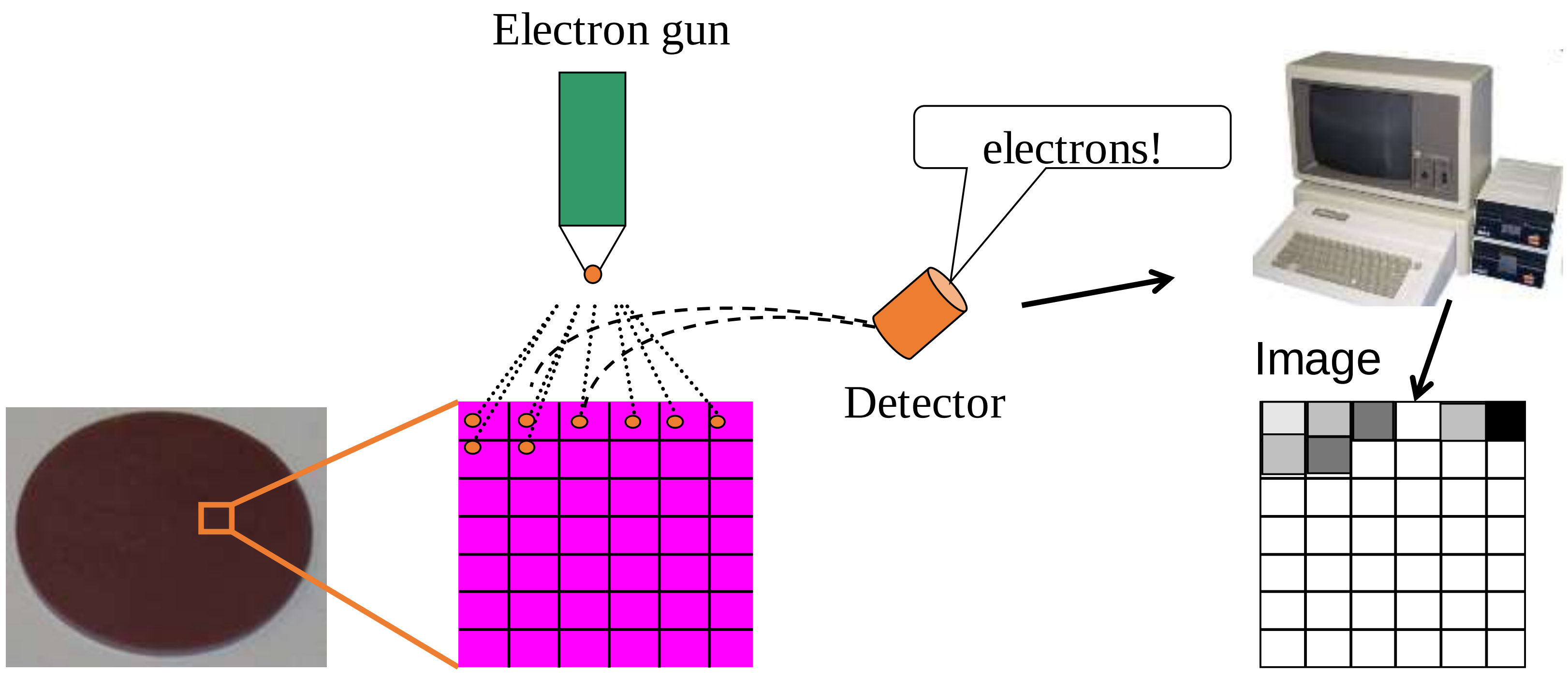
- Topography and morphology
- Elemental composition
- Crystallography & Orientation of grains



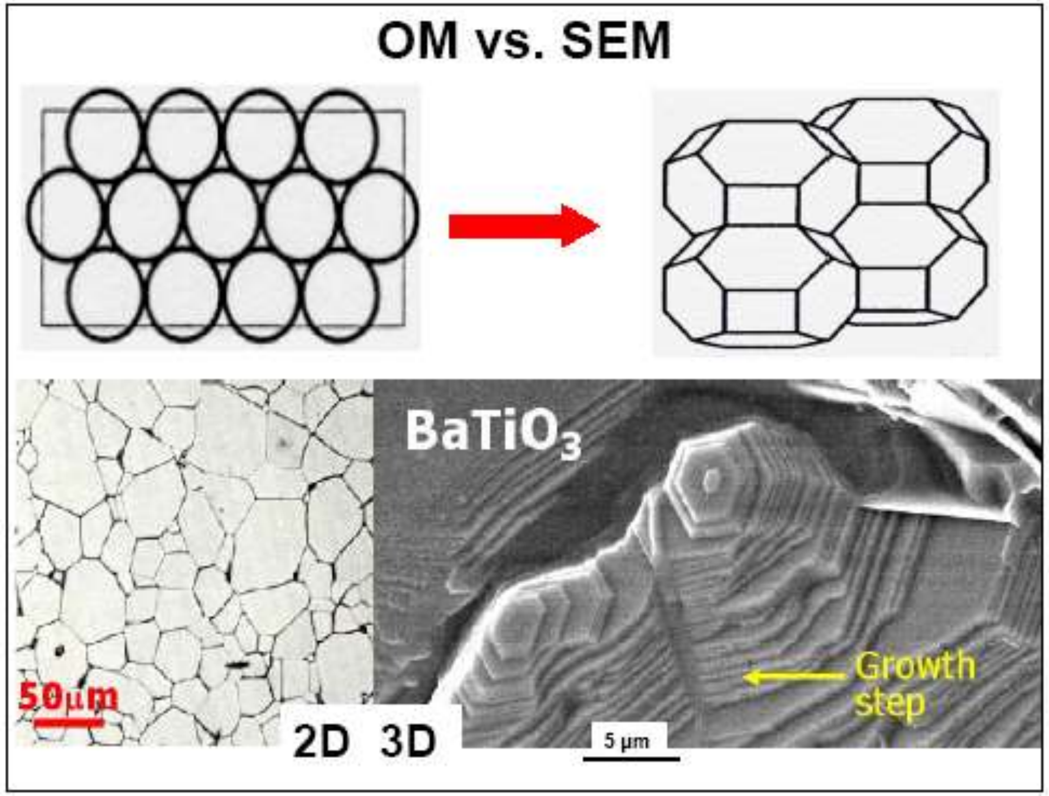
How the SEM works?

- The SEM uses electrons instead of light to form an image.
- A beam of electrons is produced at the top of the microscope by heating of a metallic filament.
- The electron beam follows a vertical path through the column of the microscope. It makes its way through electromagnetic lenses which focus and direct the beam down towards the sample.
- Once it hits the sample, other electrons (backscattered or secondary) are ejected from the sample. Detectors collect the secondary or backscattered electrons, and convert them to a signal that is sent to a viewing screen similar to the one in an ordinary television, producing an image.
- Voltage in range 0.1 KV to 40KV (10KV for biological samples) and 20KV for non-biological samples.

How do we get an image?



Optical microscope vs SEM



ADVANTAGES:

1. Improved resolution
2. Good depth of field

LIMITATIONS:

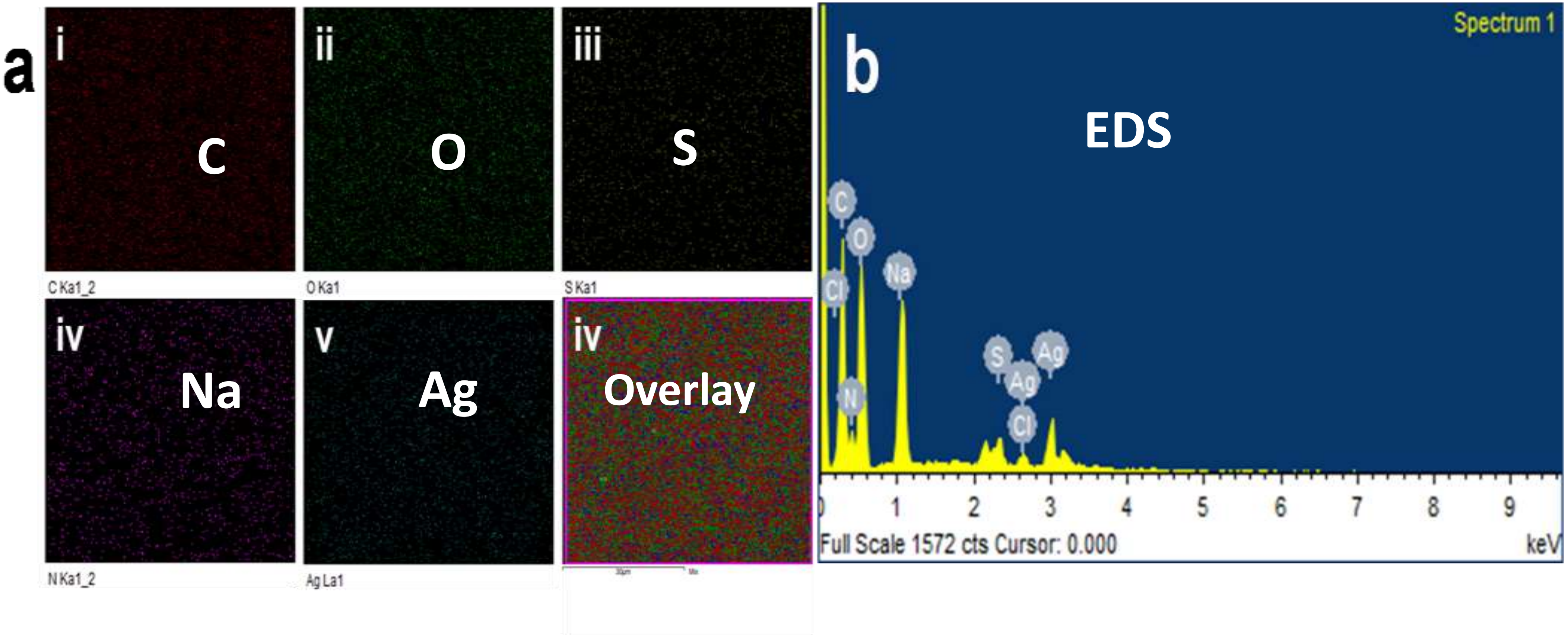
1. Vacuum required.
2. Expensive

Energy dispersive analysis of x-rays(EDAX)

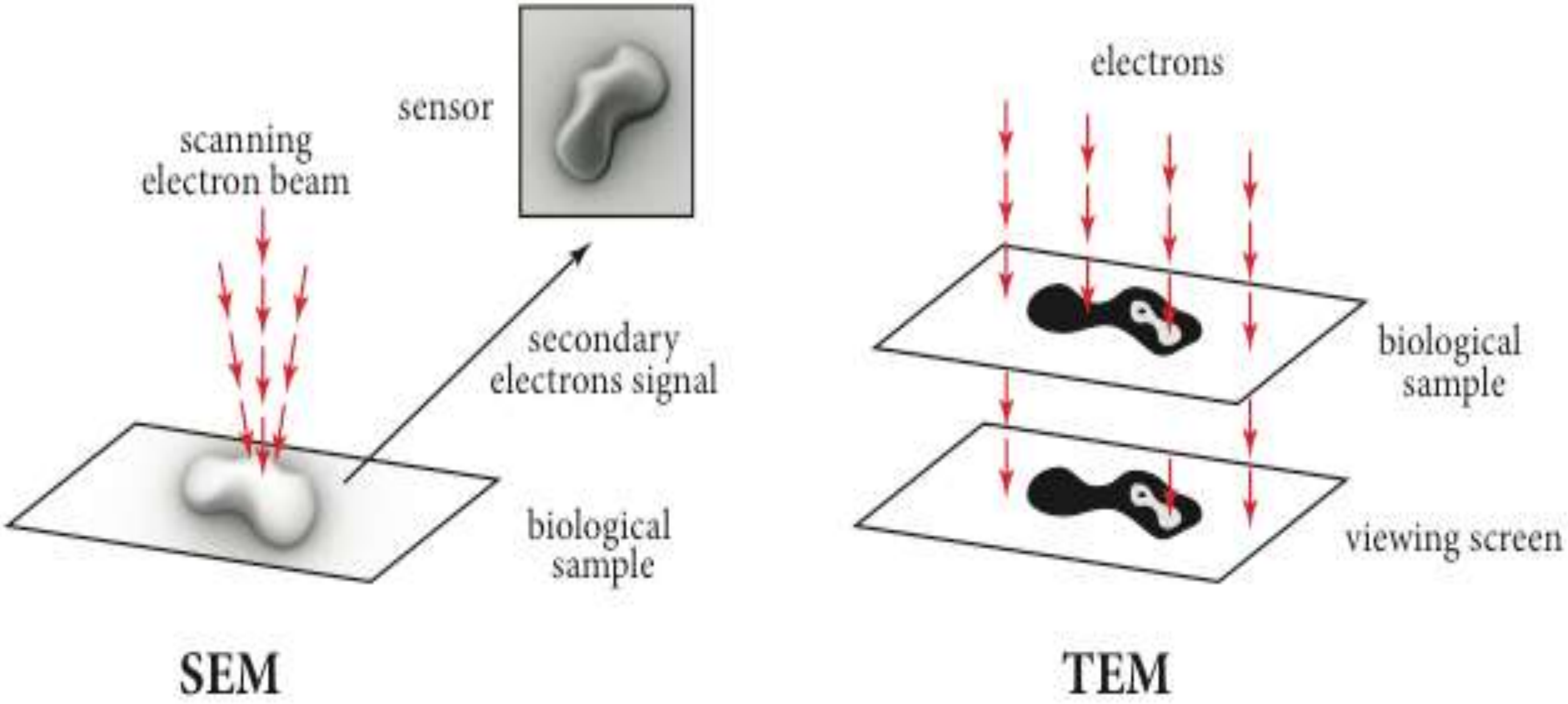
PRINCIPLE: Each element has a unique electronic structure and thus interacts uniquely with electromagnetic radiation.

USED FOR: Chemical characterization of a substance.

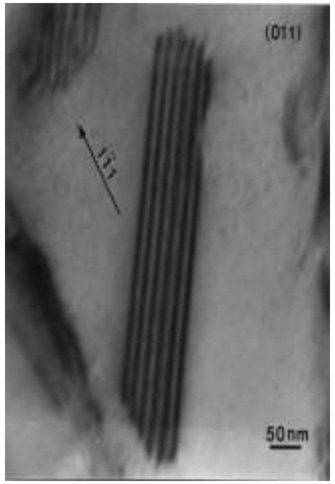
Energy dispersive X-ray spectroscopy (EDS) and elemental analysis

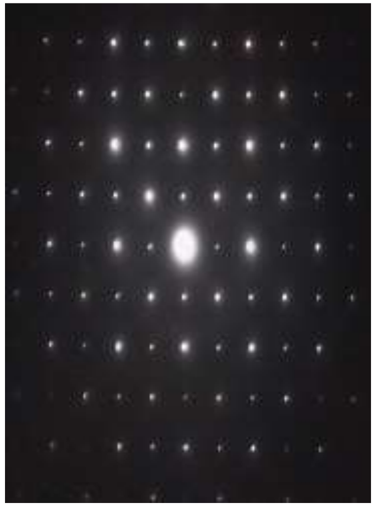


Basic principles of electron microscope



Transmission Electron Microscopy(TEM)

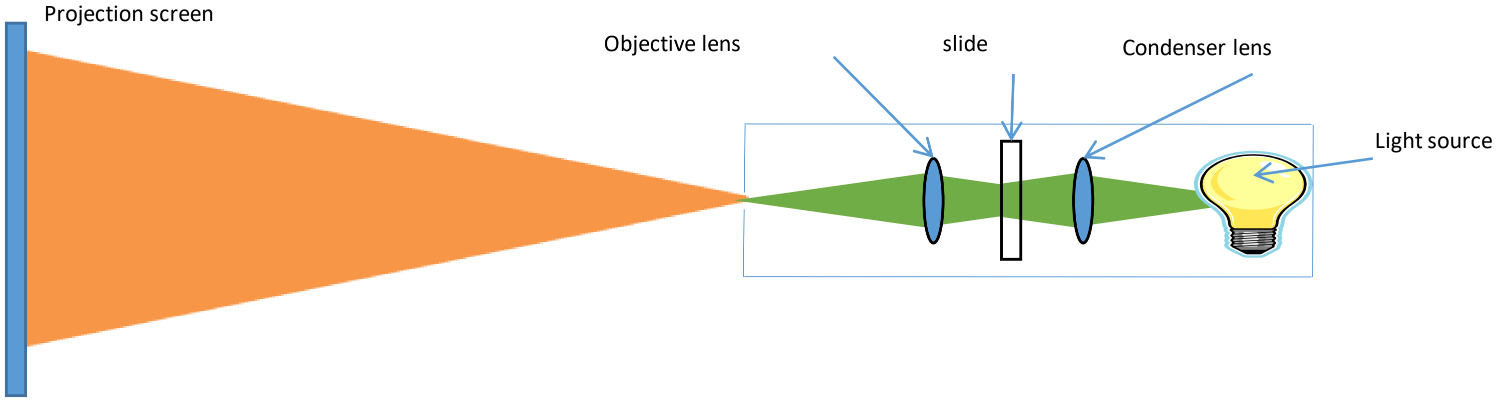




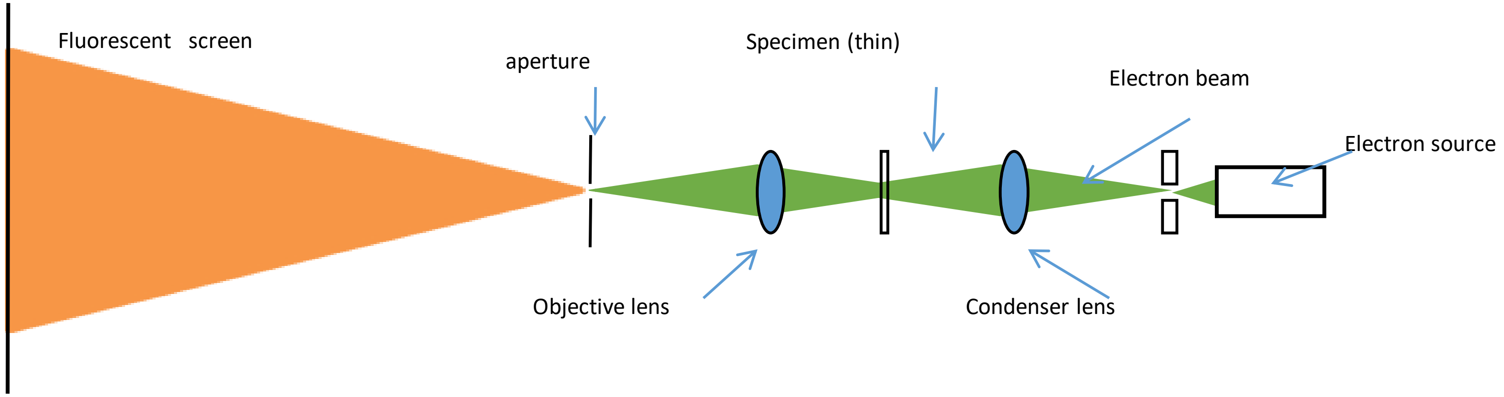
Working principle of TEM

- Electrons pass through a (very thin) sample (i.e. are transmitted) to form an image.
- Simplistically, In its operation a TEM can be thought of as analogous to a slide projector:-

Slide projector



TEM



Transmission Electron Microscopy (TEM)

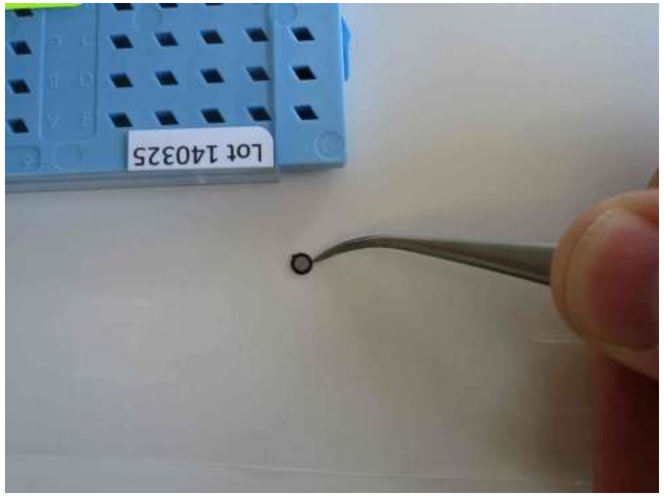
- The TEM is a complex viewing system equipped with a set of electromagnetic lenses used to control the imaging electrons in order to generate the extremely fine structural details that are usually recorded on photographic film.

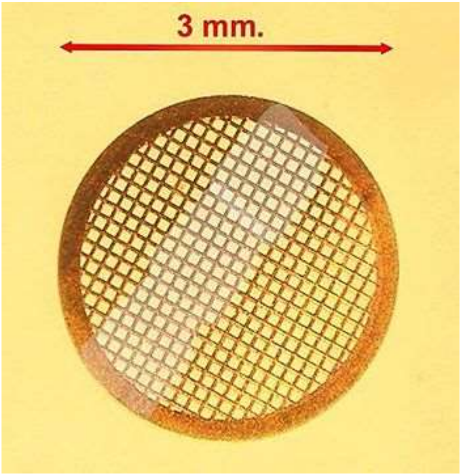
- Since the illuminating electrons pass *through* the specimens, the information is said to be a *transmitted* image.

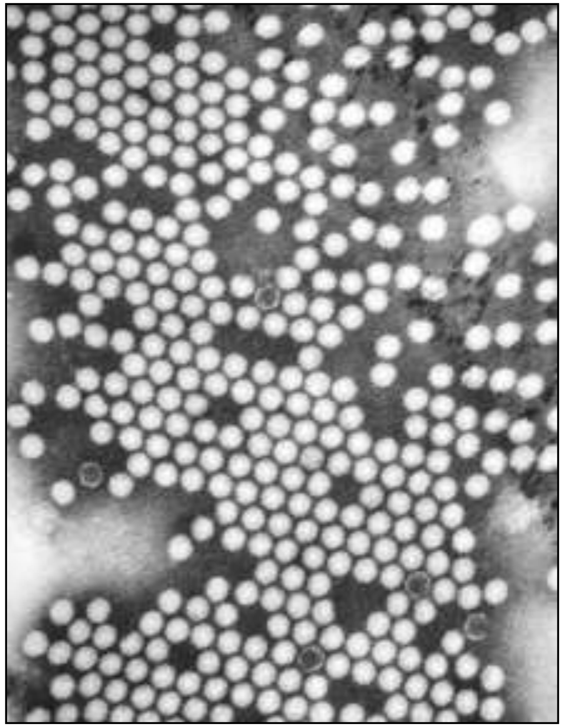
- The modern TEM can achieve magnifications of one million times with resolutions of 0.1 nm.

Transmission Electron Microscopy (TEM)

- Specimen is very thin
- Beam passes through it.
- **Gives information about a) the size of NPs, b) morphology, c) crystallographic information & d) compositional information.**





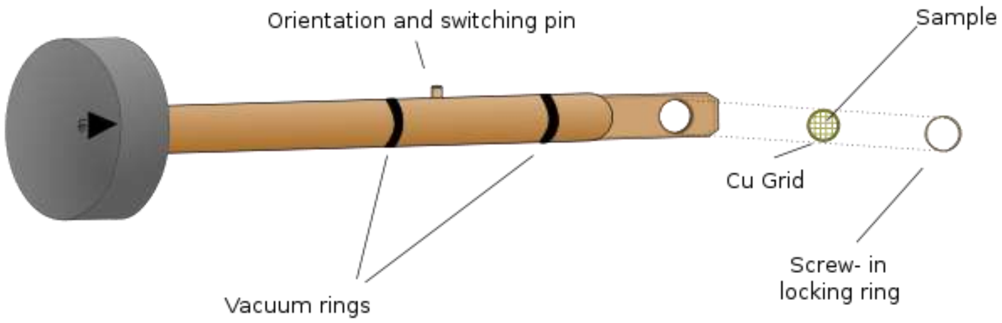


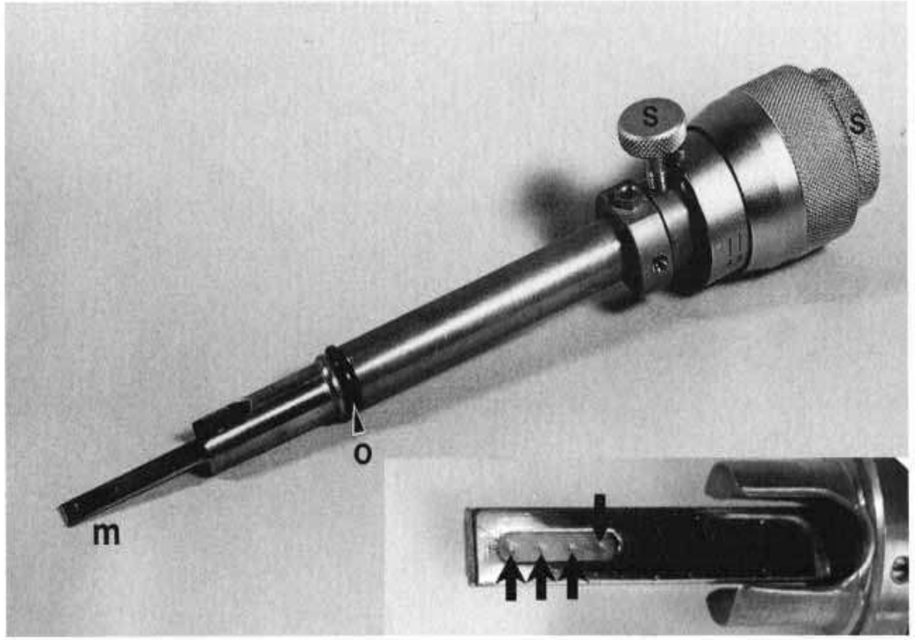
TEM image of the polio virus.
The polio virus is 30 nm in size.

Basic systems making up a TEM

Variable aperture holder from a TEM.

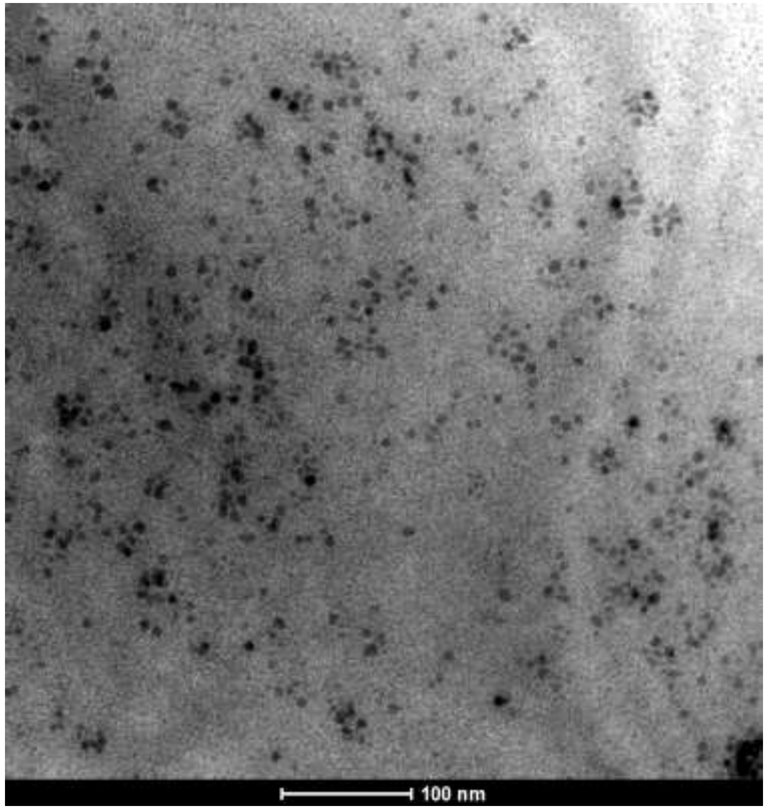
- The rod contains a molybdenum strip (m) with apertures of various sizes.
- Positioning screws (s) permit the precise alignment of the apertures in the electron beam.
- An O-ring seal (o) permits the aperture to be sealed off inside the vacuum of the microscope column.
- Insert shows enlargement of the molybdenum aperture strip held in place by a brass retainer clip. Arrows point to apertures in the strip.

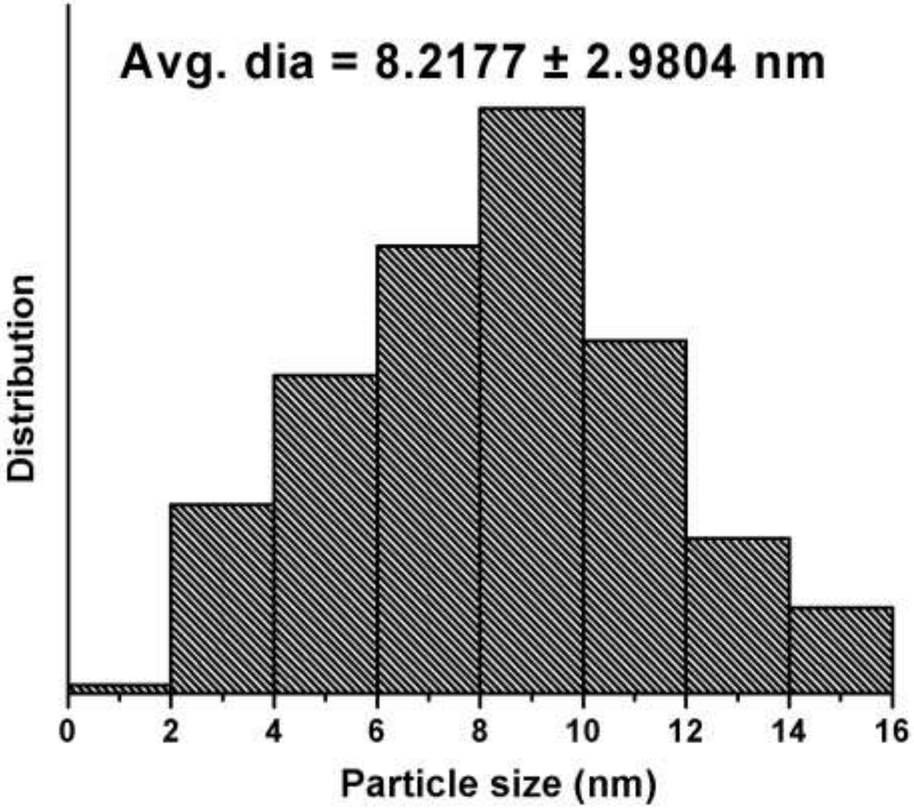




TEM image and particle size

a) morphology b) the size of NPs

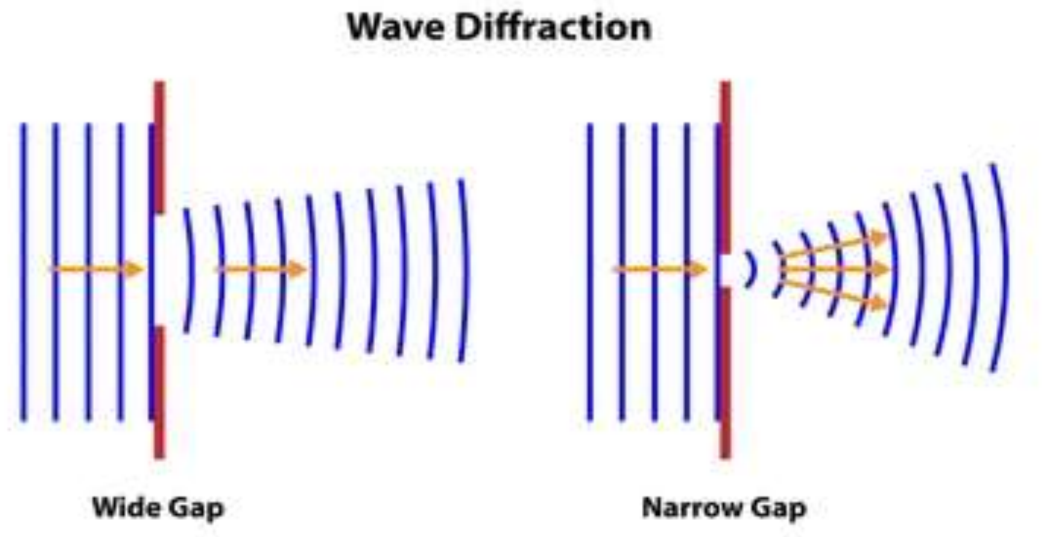




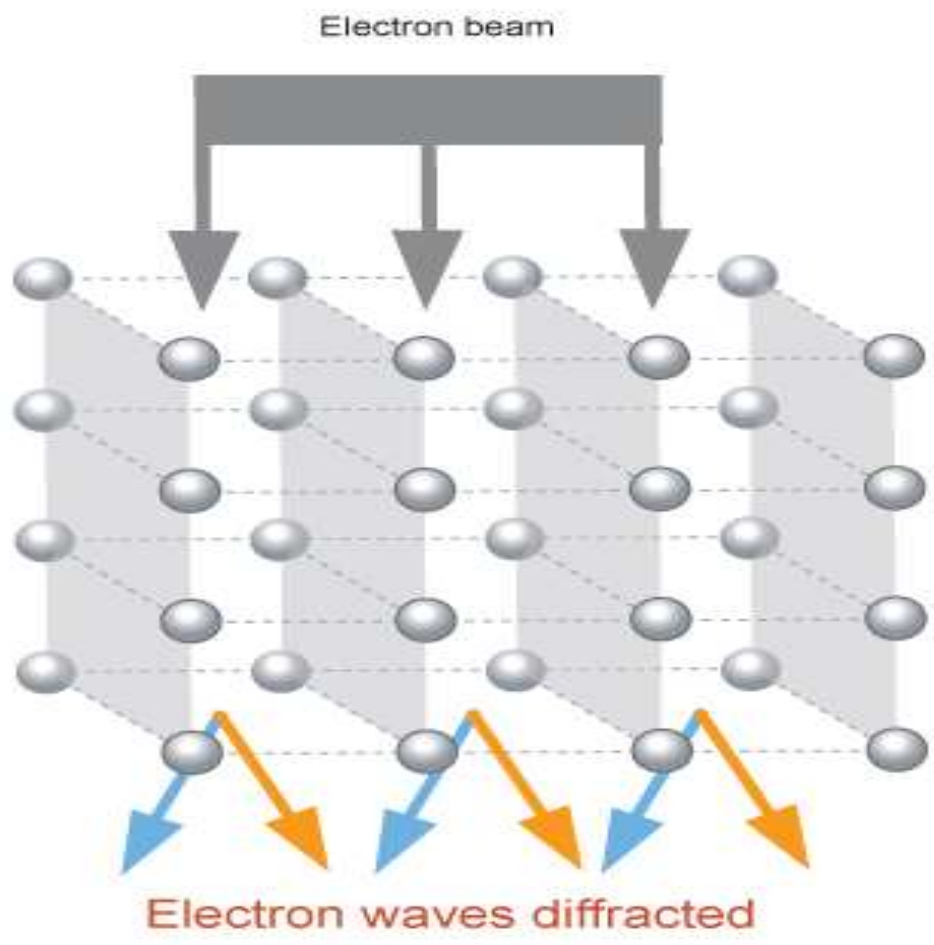
c) Crystallographic Information

Diffraction:

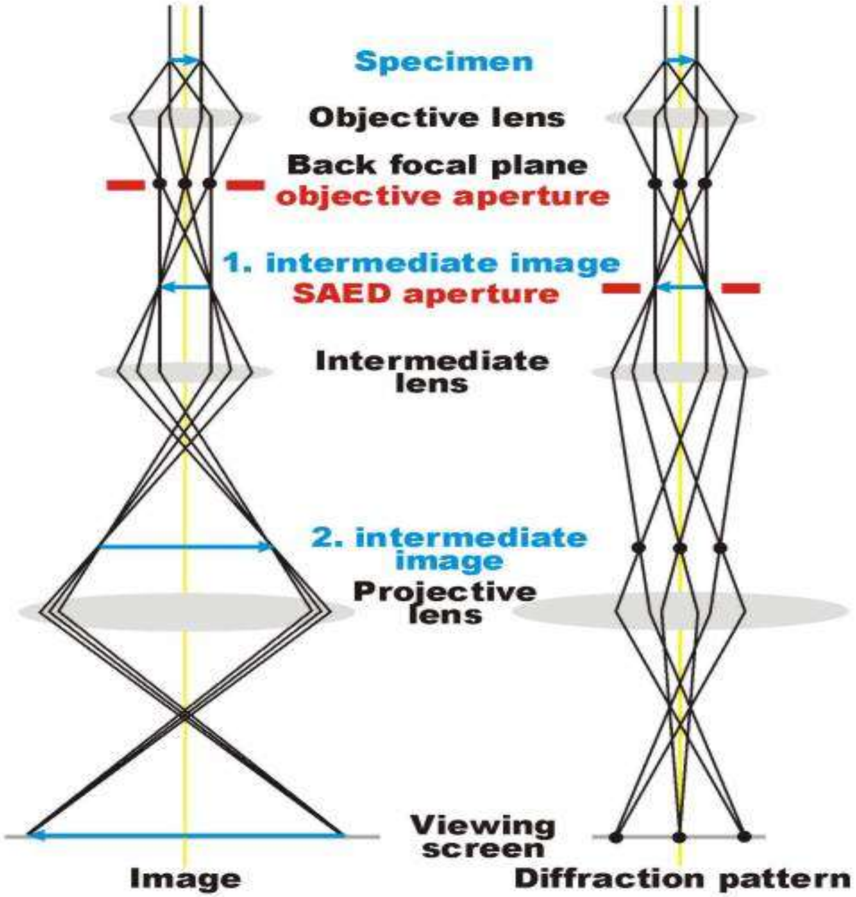
It is the slight bending of light as it passes around the edge of an object.

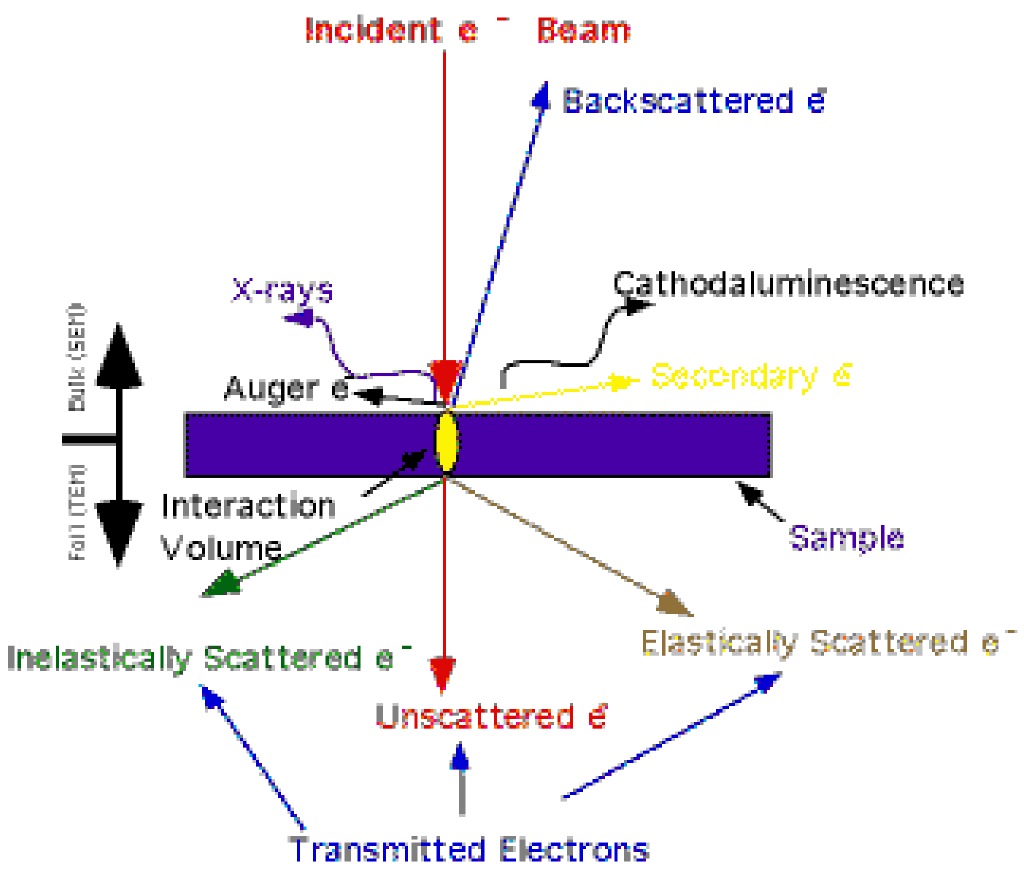


Diffraction in the TEM



Diffraction in the TEM





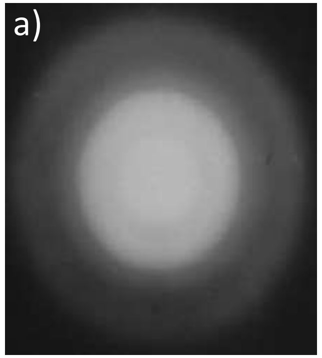
Specimen Interaction

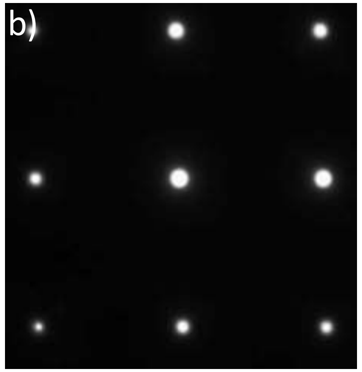
Switching from image mode to diffraction mode is easily achieved by changing the strength of the intermediate lens.

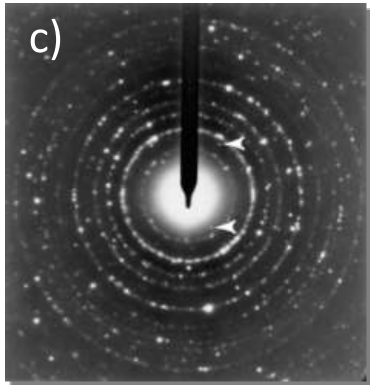
Electron diffraction

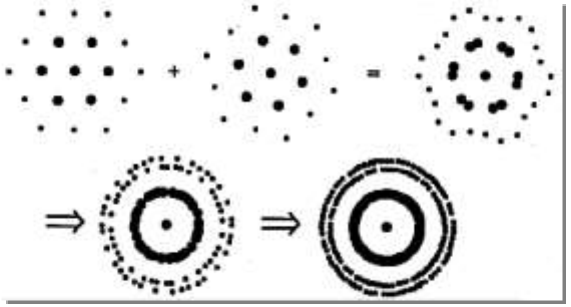
•Diffraction patterns give crystallographic information about a material from site specific small volumes

•Can determine if a material is a) amorphous, b) crystalline, c) poly crystalline quickly and effectively.





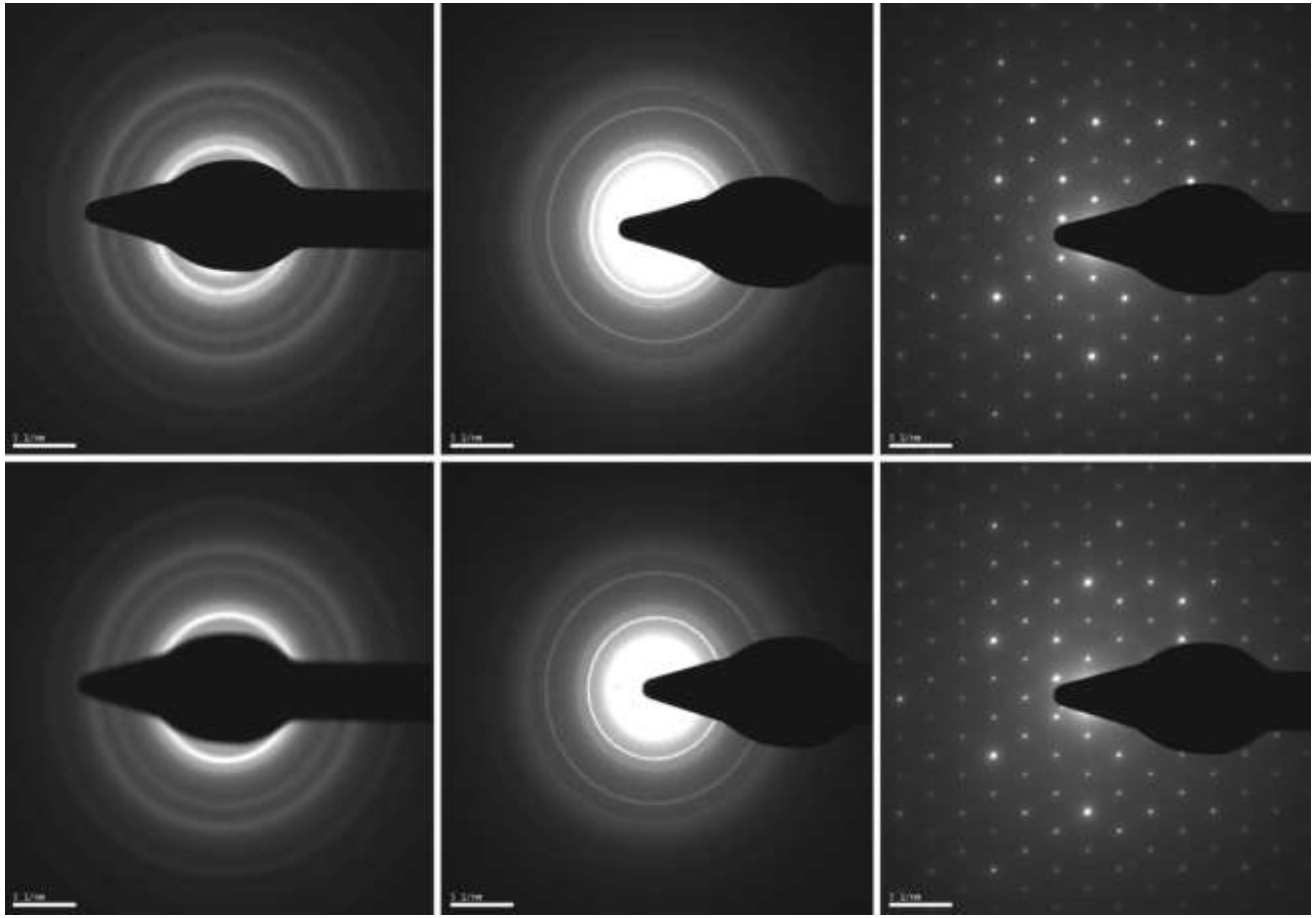




a)Amorphous material b) Crystalline material c) polycrystalline material

TEM diffraction patterns

Amorphous carbon | Polycrystalline aluminium | Single-crystal gold



PNAS 2005;102:7069-7073

Applications of TEM

- The TEM is used heavily in both material science/metallurgy and the biological sciences.
- micro structural analysis
- crystal structure
- magnifications up to 1,000,000 X => atomic resolution
- small region elemental analysis

The observation of materials by transmission electron microscopy requires the use of very thin samples, transparent to electrons accelerated to 100 keV-300 keV.

The specimen thickness needs to be less than 200 nm

THANK YOU