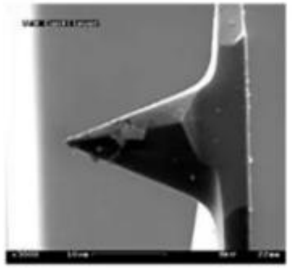
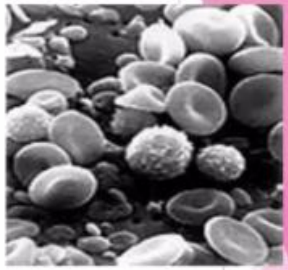
Scanning Electron Microscope (SEM)

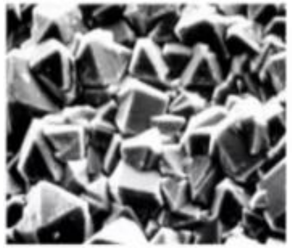


AFM Cantilever Tip

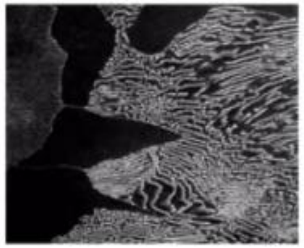
Ant Head



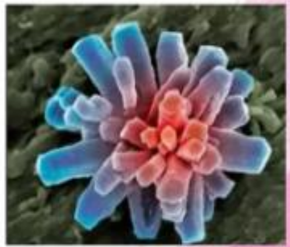
Blood Cells



Diamond Thin Film



Microstructure of a plain carbon steel



Calcium Phosphate Crystal

Scanning Electron Microscope (SEM)

- A scanning electron microscope (SEM) allows the observation of heterogeneous organic and inorganic materials on a nanometer to micrometer scale.

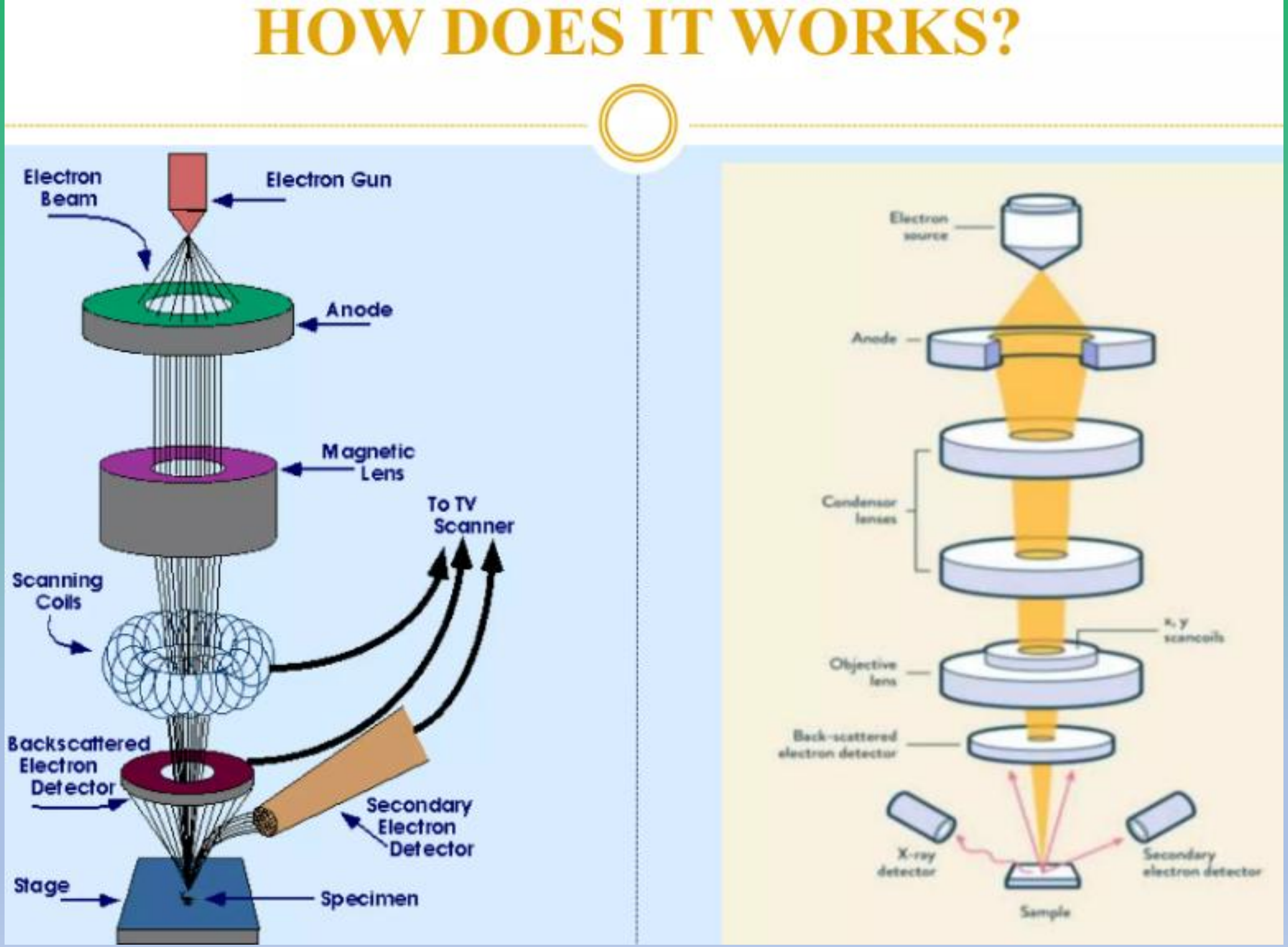
- SEM uses electron beam just like an optical microscope uses light rays to visualize the object.

- The interaction of electrons with the object can result in different effects which are utilized in imaging and chemical analysis performed by SEM.

- The SEM uses electrons instead of light to form an image.

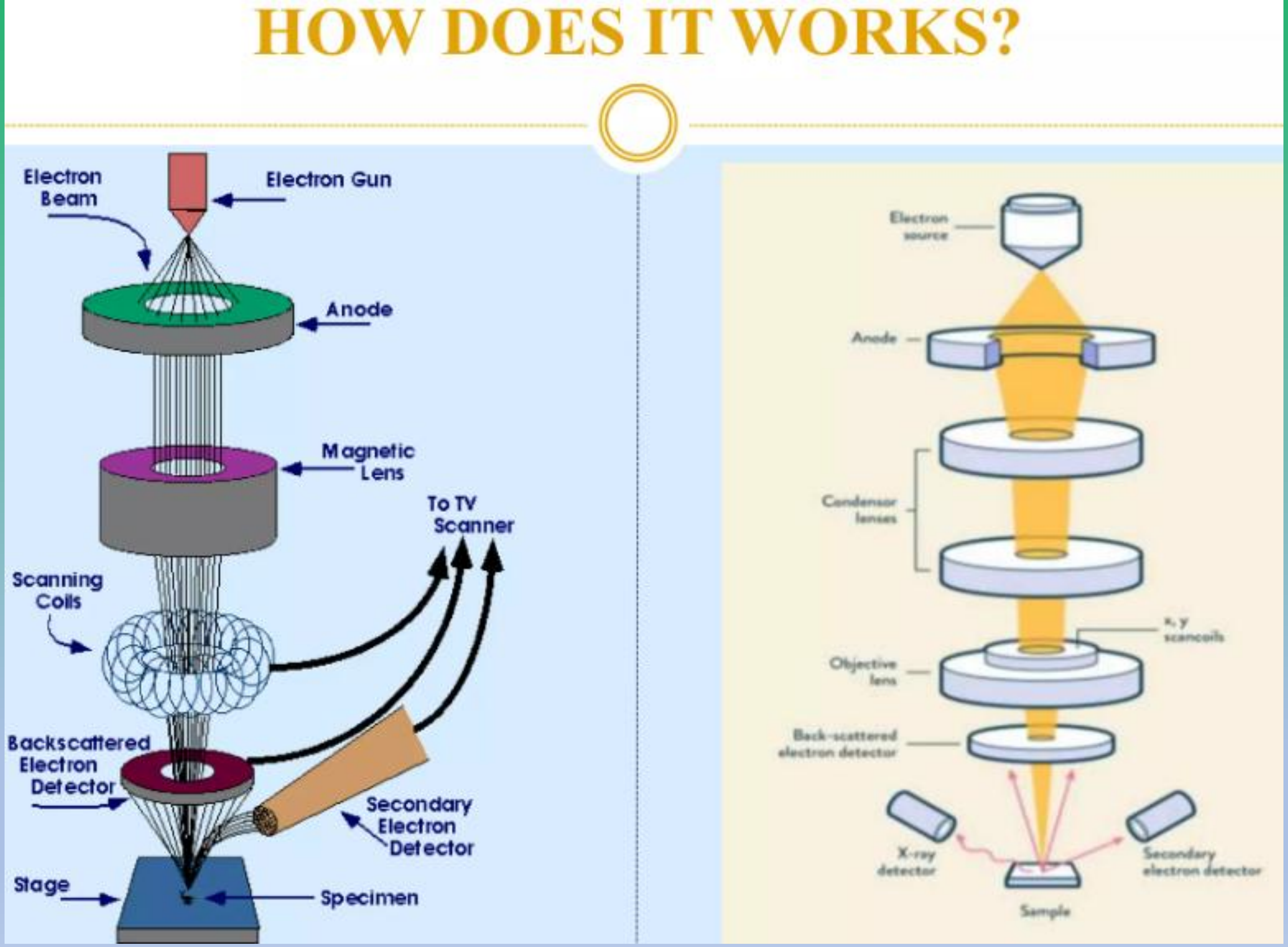
- A beam of electrons is produced at the top of the microscope by heating of a metalic filament.

- The electron beam follows a vertical path through the column of microscope. It makes its way through electromagnetic lenses which focus and directs the beam down towards the sample.



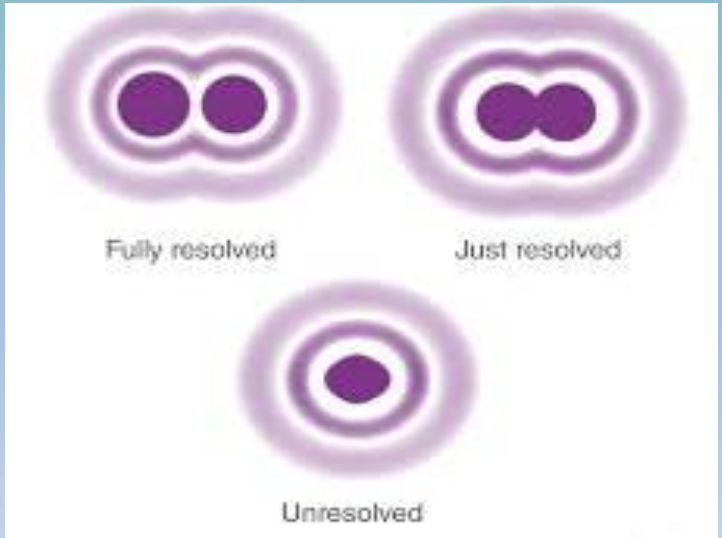
- Once it hits the sample, other electrons (secondary, backscatterd and so on) are emitted from the sample.

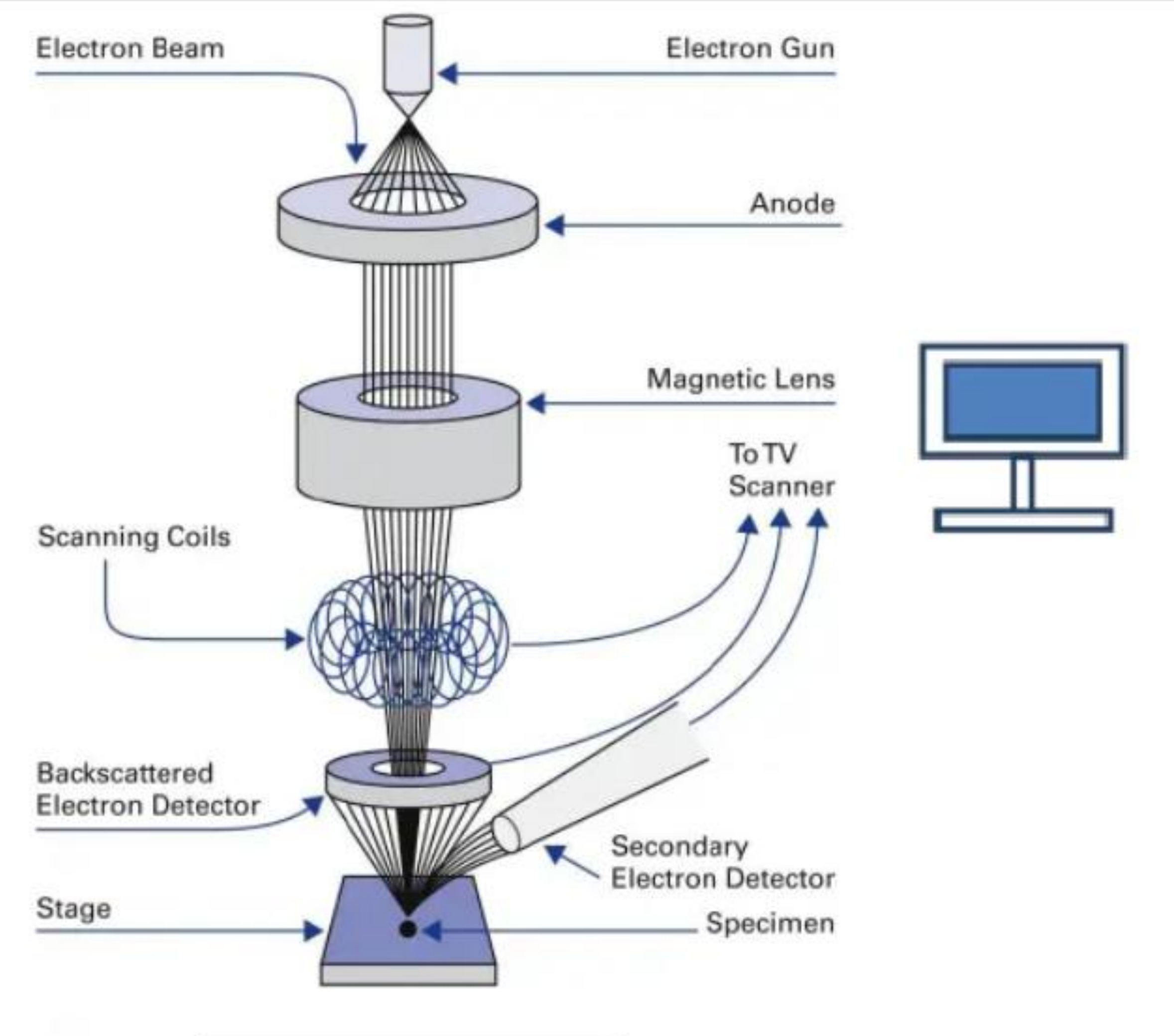
- Detectors collect the secondary or backscattered electrons, and convert them into a signal that is sent to a viewing screen to produce an image.



HOW DOES IT WORKS?

- Electron microscope was developed to overcome detection limits of optical microscope.
- Since microscopy is the study of objects illuminated by radiation or particles the wave length of illuminating electron or light beam limits the resolving power of the microscope.
- Electrons have the wavelength much shorter than the wavelength of light rays resulting in that the resolving power of electron microscope is orders of magnitude better than for the optical microscope.
- Resolving power is the smallest distance between two points that microscope can observe separately.

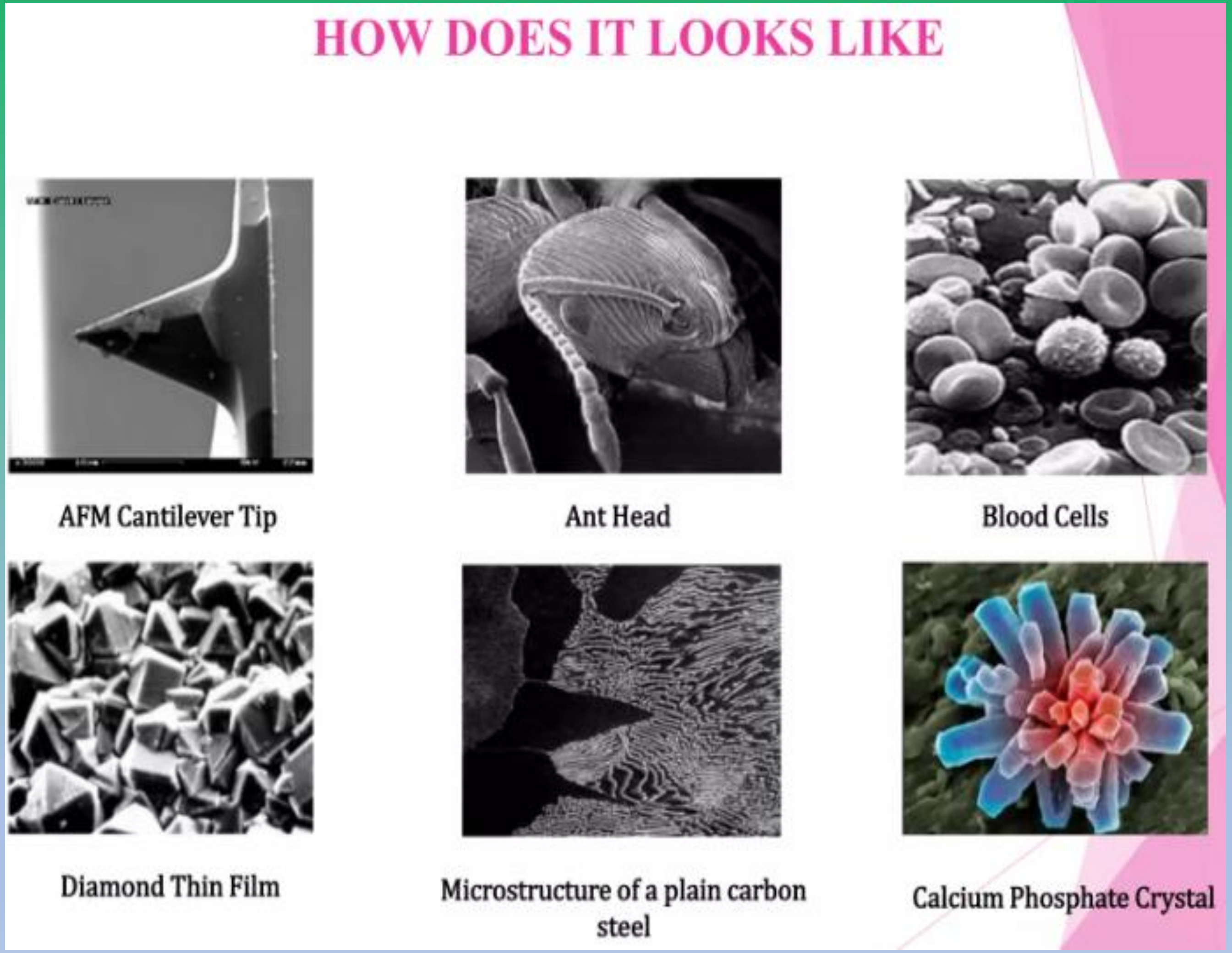




Blog diagram of SEM

- The smallest features that you can resolve by SEM are 1 to 20 nm apart, while theoretical limit of resolution (not visibility) of the light microscope in white light of about 200 – 250 nm.

- In theory an electron has an equivalent wavelength less than 1 nanometer, which can enable the resolving power of ordinary electron microscope to be 0.1 nm, however the construction details of the microscope and specimen characteristics diminish the resolving power.



HOW DOES IT LOOKS LIKE

AFM Cantilever Tip

Ant Head

Blood Cells

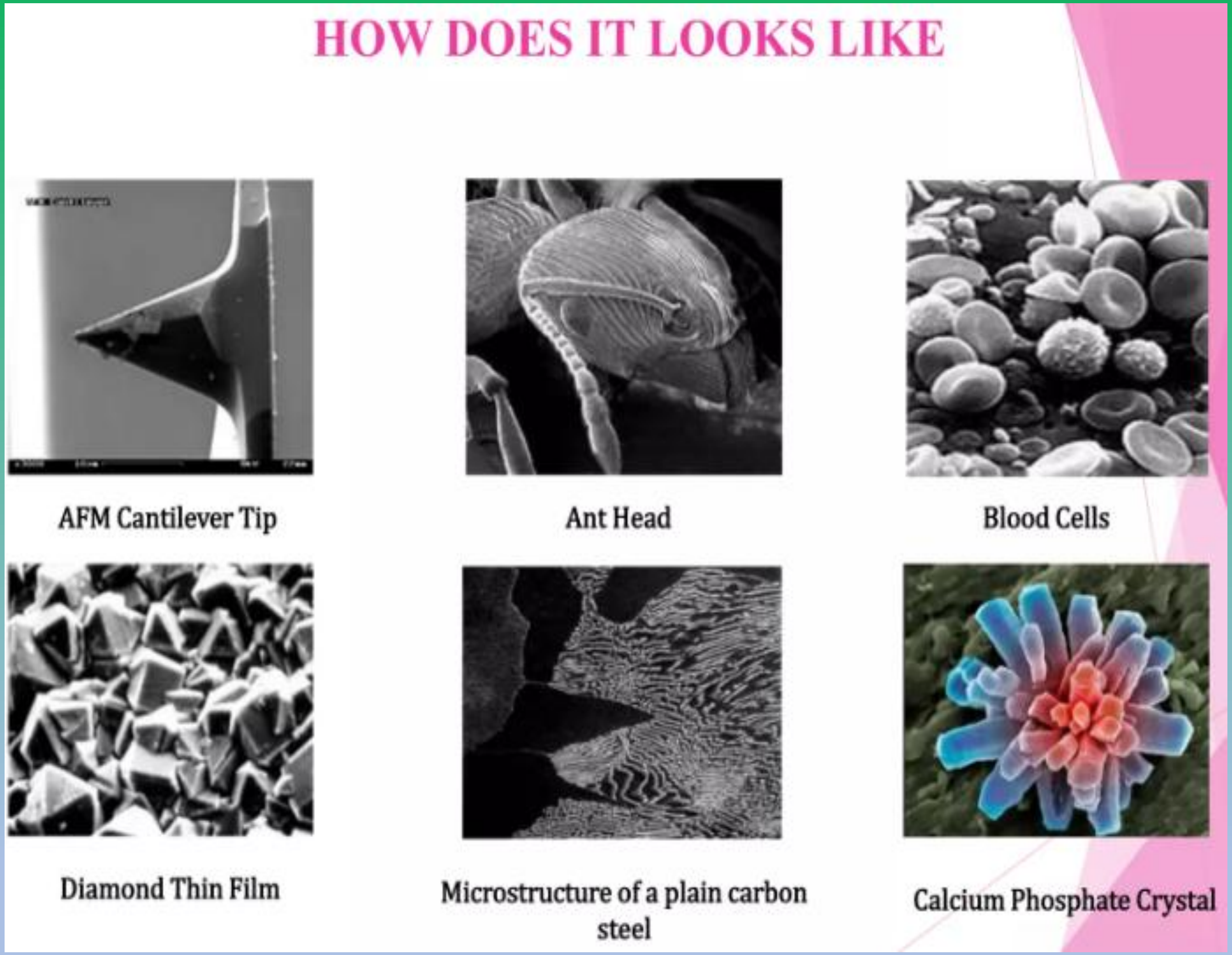
Diamond Thin Film

Microstructure of a plain carbon steel

Calcium Phosphate Crystal

- In scanning electron microscopy signal detection begins when electron beam enters a specimen.

- The interaction of an electron beam and the atoms composing the specimen produce various kinds of information.



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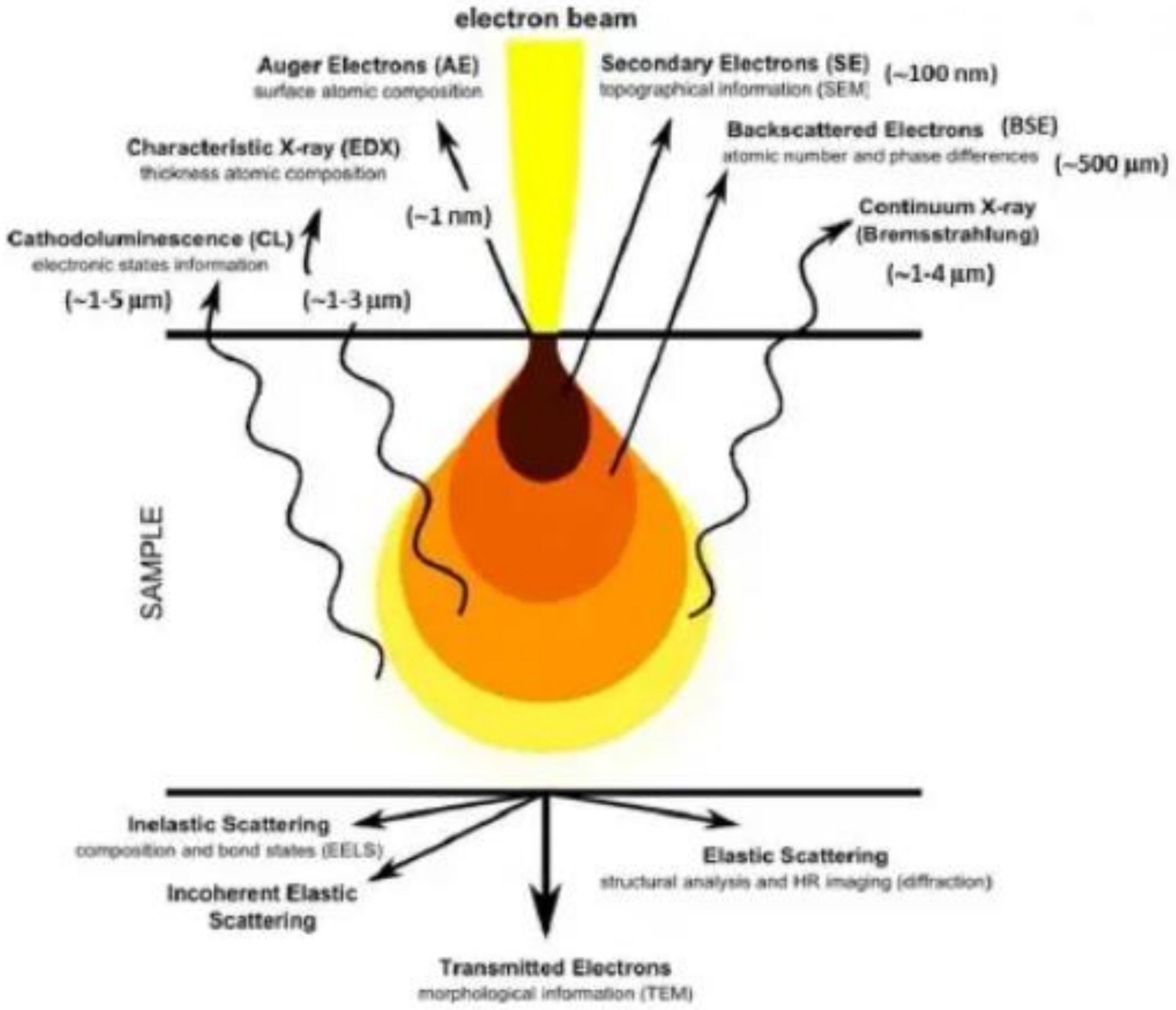
- When the primary electron enters a specimen it will penetrate into it for some distance before colliding with another particle.

- After colliding with an electron, nucleus or other particle, the primary electron will follow a new trajectory, known as scattering.

- It is the components of scattering events that are detected by SEM.

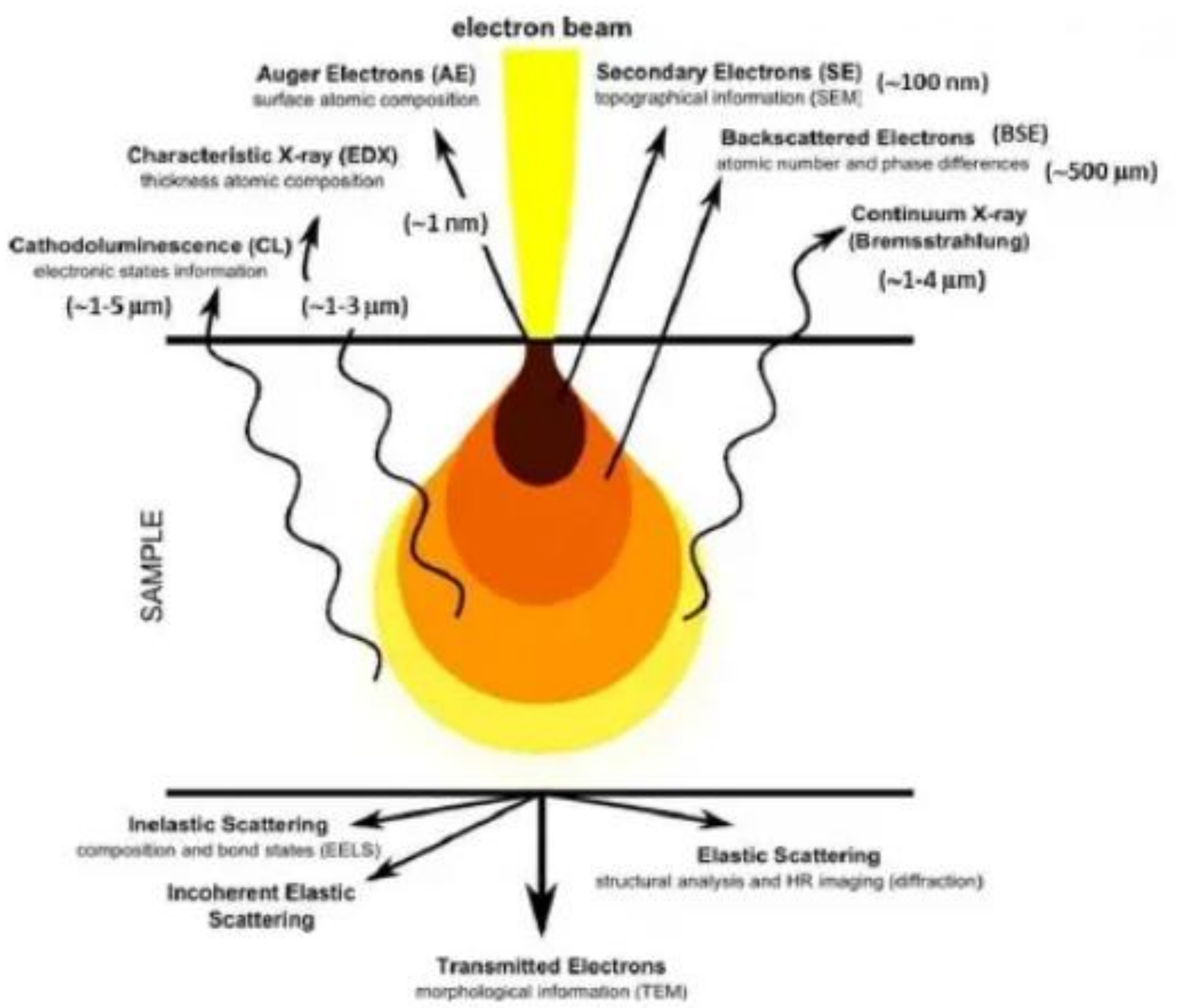


Schematic of electron beam interaction.

- Scattering components include secondary electrons (SE), backscattered electrons (BSE), Auger electrons (AE), XRays, cathode luminescence and other phenomena.

- They arise from different depth of the specimen and provide microscopic information about the specimen.

- This forms a basis for another advantage of the SEM, described as depth of focus or an ability of a microscope to bring the features of an object at different depths into focus.



Schematic of electron beam interaction.

- **Backscattered electron** emission occurs due to the scattering of primary electrons in such a way that they escape back from the sample without going through the sample.

- Since backscattered electrons are original primary electrons, they possess high energy level near that of a gun voltage.

- These type of electrons conserve their energy, but their trajectory has been modified by size of the atoms as well as by density of the atoms.

- The regular arrangement of atoms in crystals can influence the backscattering of electrons compared to those when same atoms randomly distributed in an amorphous material.

- **X-rays** are generated due to the de-energizing of the atom in the sample after secondary electron emission occurred.

- Soon after the ejection of secondary electron the newly formed vacant orbit is filled by the electron dropping down from a higher energy orbital level.

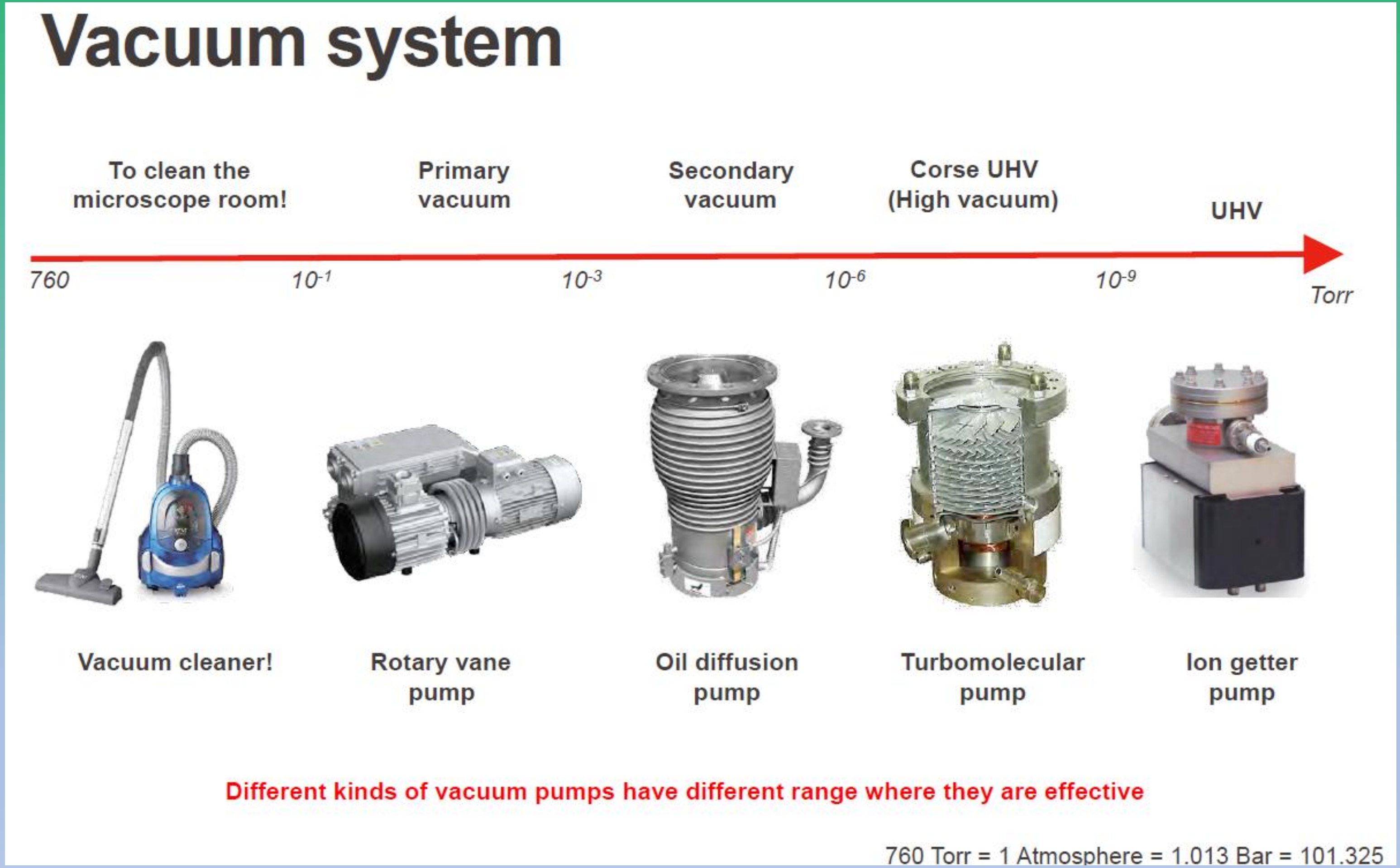
- **Specimen current** is important characteristic of electron- sample interaction.

- Most of the time the primary electrons undergo multiple scattering events and lessen their energy level to the point where the electrons are absorbed by the sample, phenomena known as specimen current.

- Changes in specimen current can influence imaging substantially and can be regulated to improve the imaging quality.

VACUUM SYSTEM

- The SEM chamber operates in high vacuum to ensure that electrons don’t scatter or disperse due to collisions with other molecules from the air.
- Atmospheric pressure at sea level is equal to 760 millimeters of mercury.
- A pressure of 1 millimeter of mercury is call a Torr.



Vacuum system

To clean the microscope room!	Primary vacuum	Secondary vacuum	Corse UHV (High vacuum)	UHV
760 – 10^{-1}	10^{-1} – 10^{-3}	10^{-3} – 10^{-6}	10^{-6} – 10^{-9}	below 10^{-9}
Vacuum cleaner!	Rotary vane pump	Oil diffusion pump	Turbomolecular pump	Ion getter pump

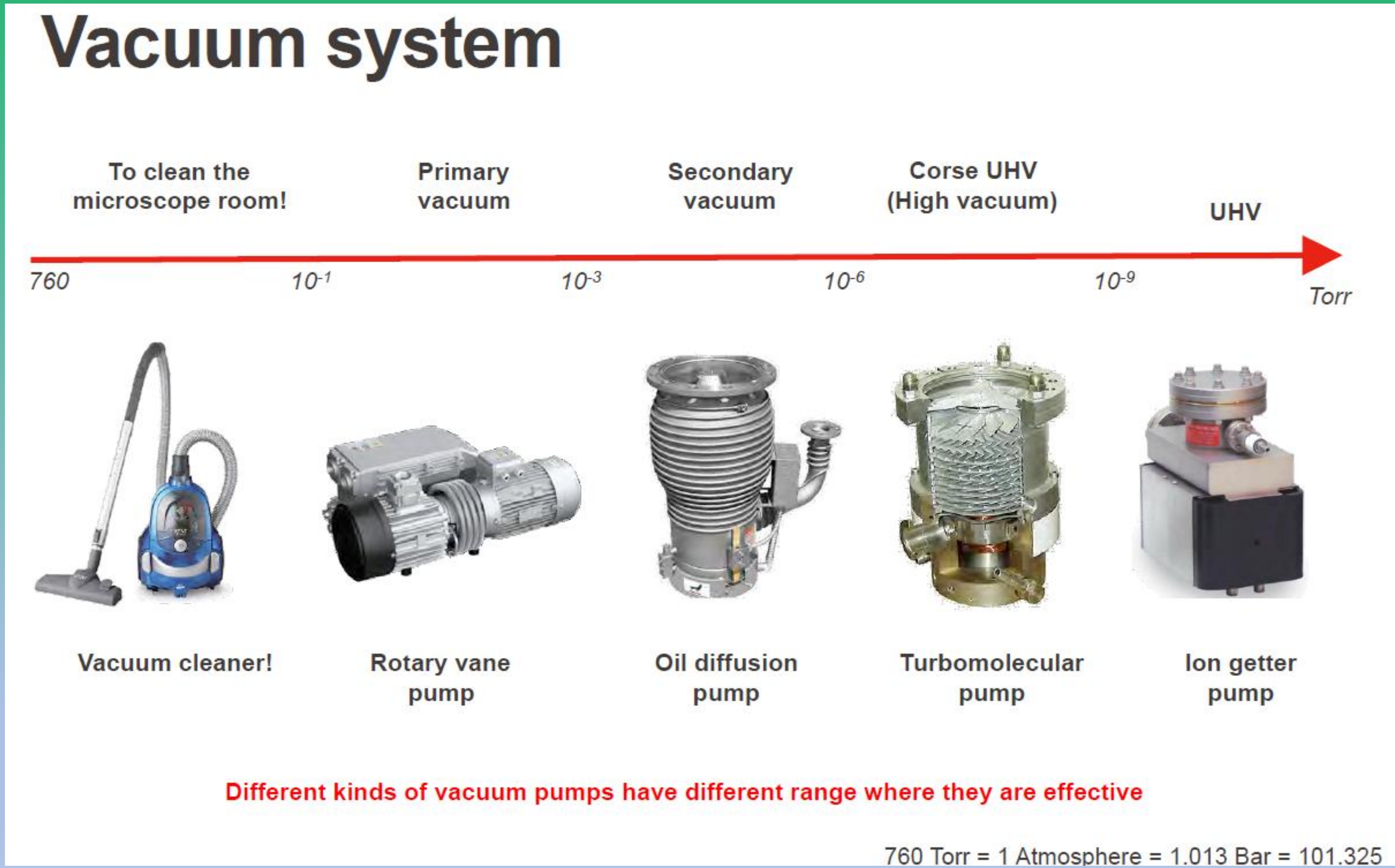
Different kinds of vacuum pumps have different range where they are effective

760 Torr = 1 Atmosphere = 1.013 Bar = 101.325

VACUUM SYSTEM

- The minimum vacuum requirement for SEM operation is 10-4 Torr, although most microscopes operate at 10-6 Torr or greater vacuum.

- The higher the vacuum (the lower the pressure), the better the microscope will function.



Vacuum system

Different kinds of vacuum pumps have different range where they are effective

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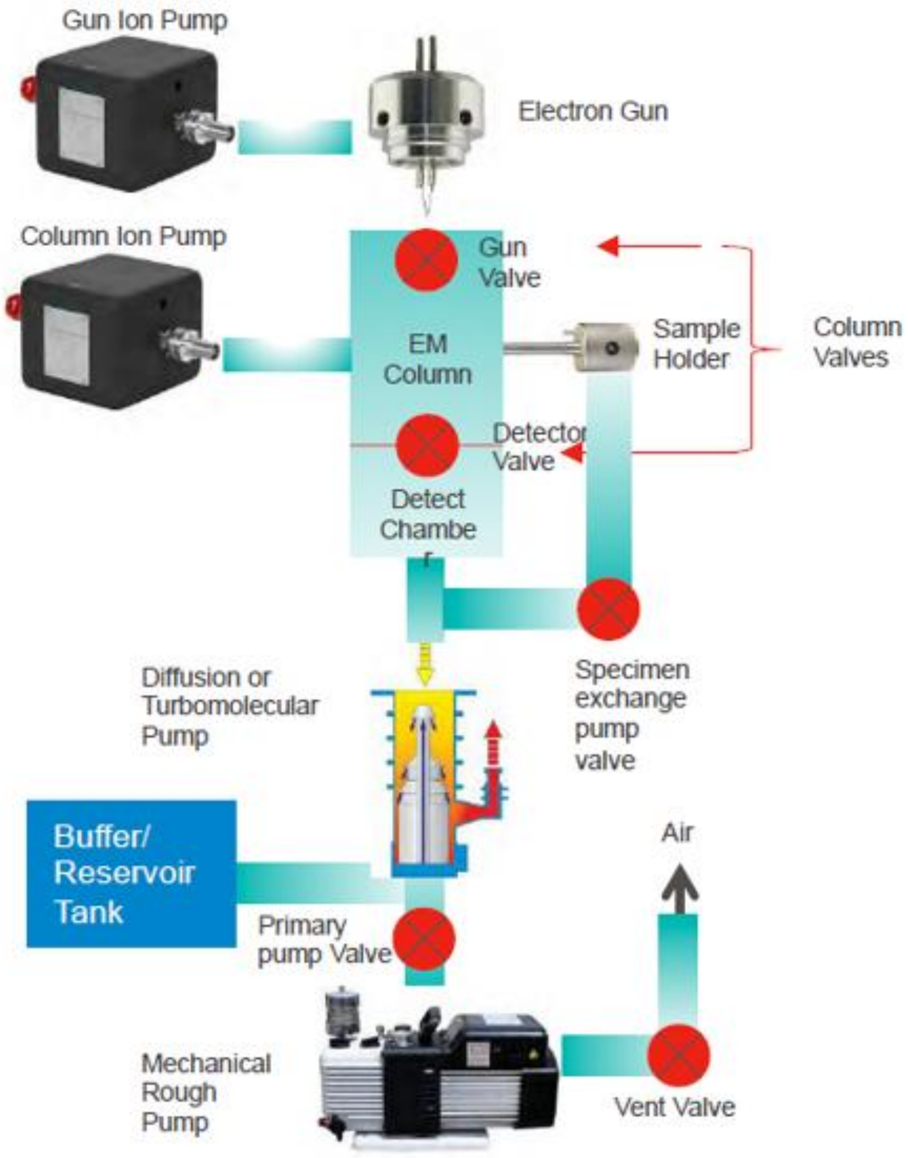
Vacuum system



UHV is not needed in all parts of the microscope

- **Primary vacuum (>0.1 Pa)**
  - Mechanical pump
- **Secondary to high vacuum**

  Detector or viewing chamber (<10^{-4} Pa)
  - Oil diffusion pump
  - Turbomolecular pump
- **High and ultra-high vacuum**

  Gun & specimen area (<10^{-6} Pa)
  - Ion getter pump
  - Cold trap

Vaccum level in space: 1 Pa at 100km above earth
760 Torr = 1 Atmosphere = 1.013 Bar = 101.325 KPa

Electron beam generation:

- The electron beam is generated by the electron gun.
- Purpose: To create a narrow intense beam of electrons

3 types of electron guns:

- Thermionic (Thermal)

Heat only

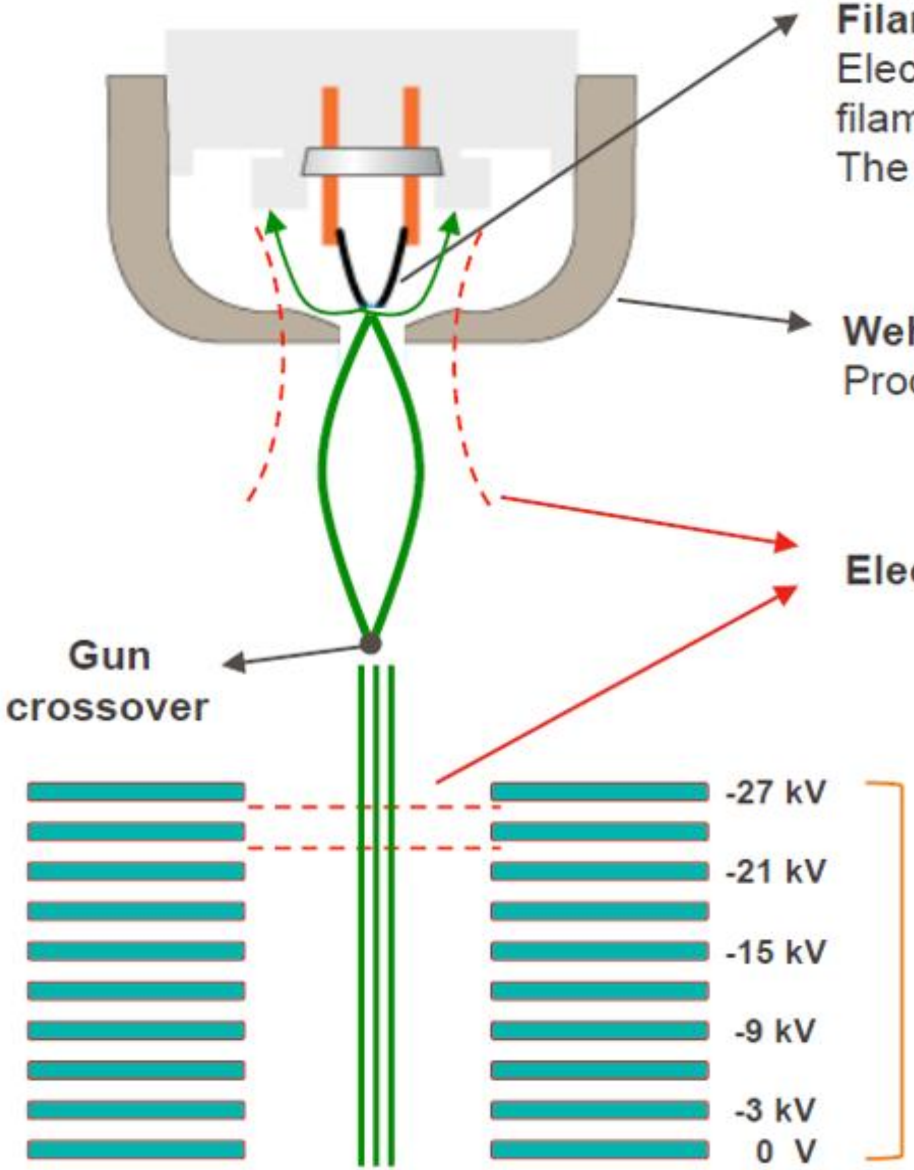
- Cold field emission

Electrical field: Potential (voltage) difference

- Heat assisted field emission: Schottky emitter

Heat+Electric field

Thermionic gun



Filament (cathode) @ V_0

Electrons receive enough energy to escape the filament (work function of the filament)

The filament is hold at a negative high voltage e.g. – 30 kV

Wehnelt cup and anode @ $V_0 + V_{bias}$

Produces a small negative bias (e.g. -31 kV) to focus electrons to crossover

Electrostatic field

Accelerator

To give the electrons some speed

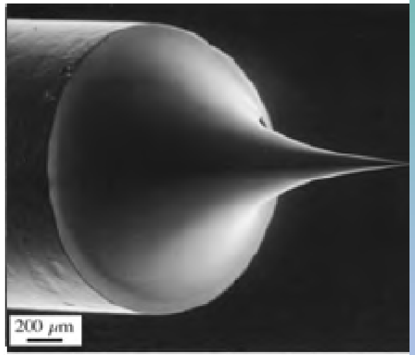
Stacks are isolated by SF_4 gas → Heavier than air

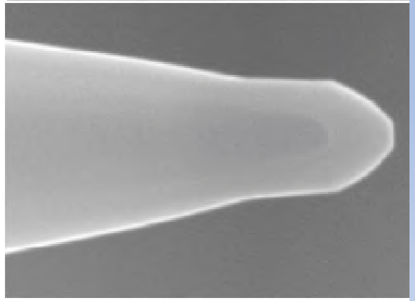
Field Emission Gun (FEG)

By applying an electric field of very high field strength at the surface of a metal, electrons are emitted even without heating the metal: *Cold field emission*

- Sharp tip is needed (less than 100 nm)
  - The strength of an electric field E is considerably increased at sharp points.
- Electrons can tunnel straight from the Fermi level out of the filament (usually tungsten).
- Surface has to be pristine (no contamination or oxide)

- 2 types of FEGs:
  - Cold FEG (Ultra-high vacuum condition needed)
    - $E \approx 10^9$ V/m
    - W mono-crystal with sharp tip (radius ~100 nm)
  - Heat assisted FEG: Schottky effect* (high vacuum is usually enough)
    - W crystal with ZrO surface treatments to lower the work-function
    - Can work with slightly poorer vacuum





*Schottky effect is the effective decrease of the work function when an external field is applied at the metal surface.

Electron gun

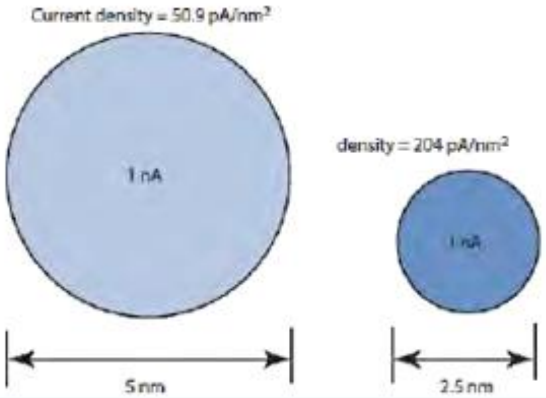
Spatial coherency:
Do all the electrons com form the same direction?
An electron beam emanating from a small source size is said to have high **spatial coherency.**

Temporal coherency:
Do all the electrons have exactly the same speed/energy?
A beam with high **temporal coherency** will have electrons of the same wavelength.

Important parameters

- Source and crossover size: determines the probe size (→ resolution)
- Energy spread: temporal coherency
- Emitted current and current density
- Brightness: current per surface unit and per solid angle
- Current stability
- Vacuum needed

$$\beta \mid \text{brightness} = \frac{\text{Beam current}}{(\text{Area})\ (\text{Solid angle})}$$



Filament current and its saturation point:

- Electron beam generation by thermal emission gun uses heat to excite the electrons off the filament.

- Electrical current in the filament causes filament heating and with increasing its temperature the number of electrons emitted by the filament will increase up to a point.

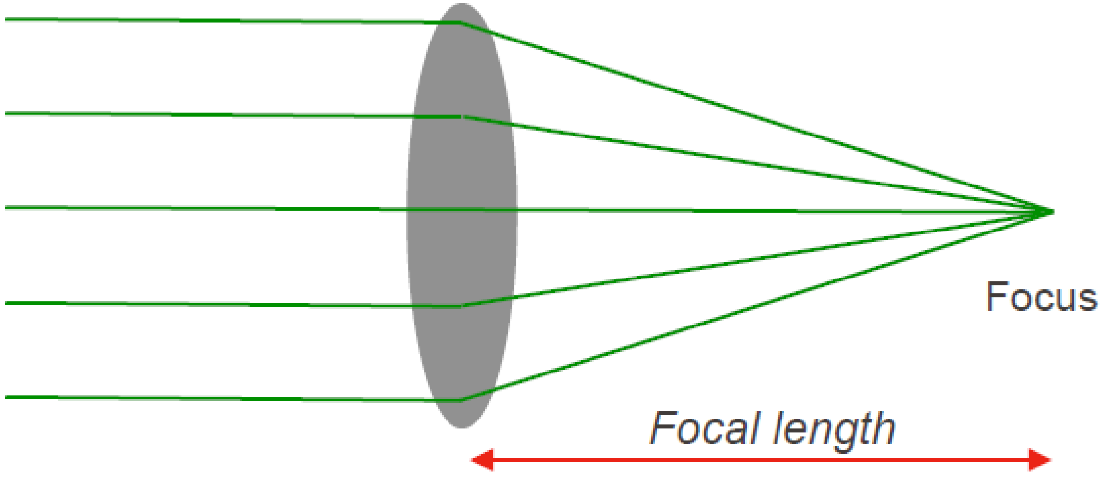
- After this point, called the saturation point, the filament current will only increase the filament temperature while will be no increase in emitted electrons.

Electron beam manipulation by lenses:

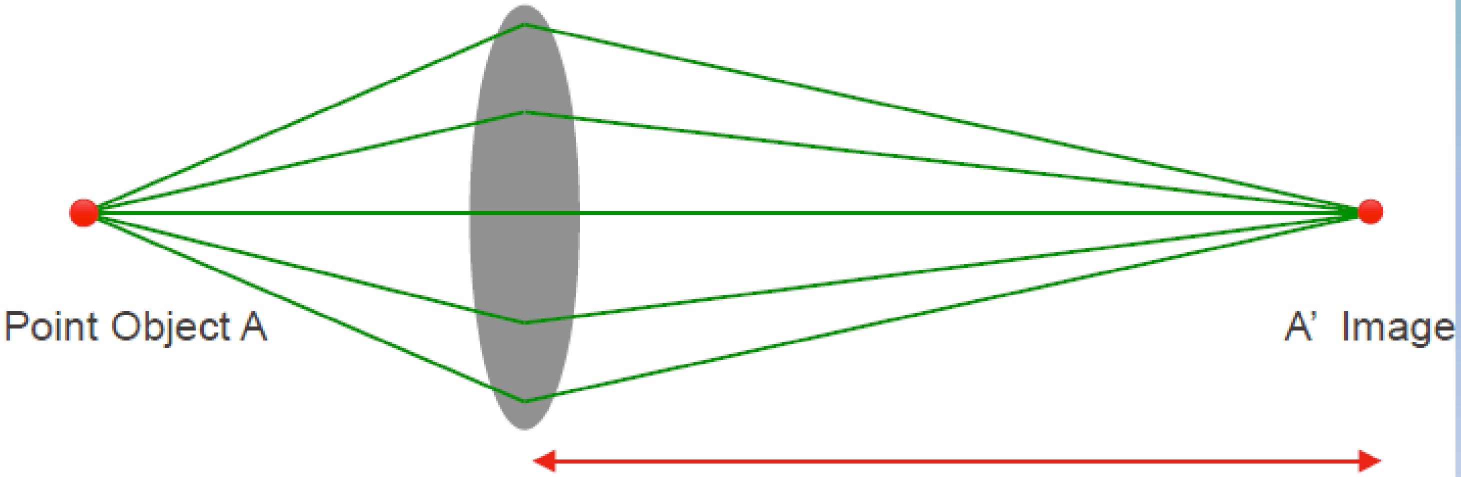
- Most SEMs use one to three magnetic lenses to reduce size of electron beam.
- The lenses operate by passing electric current through a coper wire.
- These are known as condenser lenses and since they possess spherical aberration they can limit microscope resolution similar to optical microscope lenses.
- There are other set of lenses that correct astigmatism and alignment.

Lenses

Lens bends beams to focus it to a point.



The rays emanating from a point in the object plane come to one common well defined point in image plane.



Lenses

- Lenses for light
  - Glass or polymer lenses
  - Deflection of light through changing refraction index

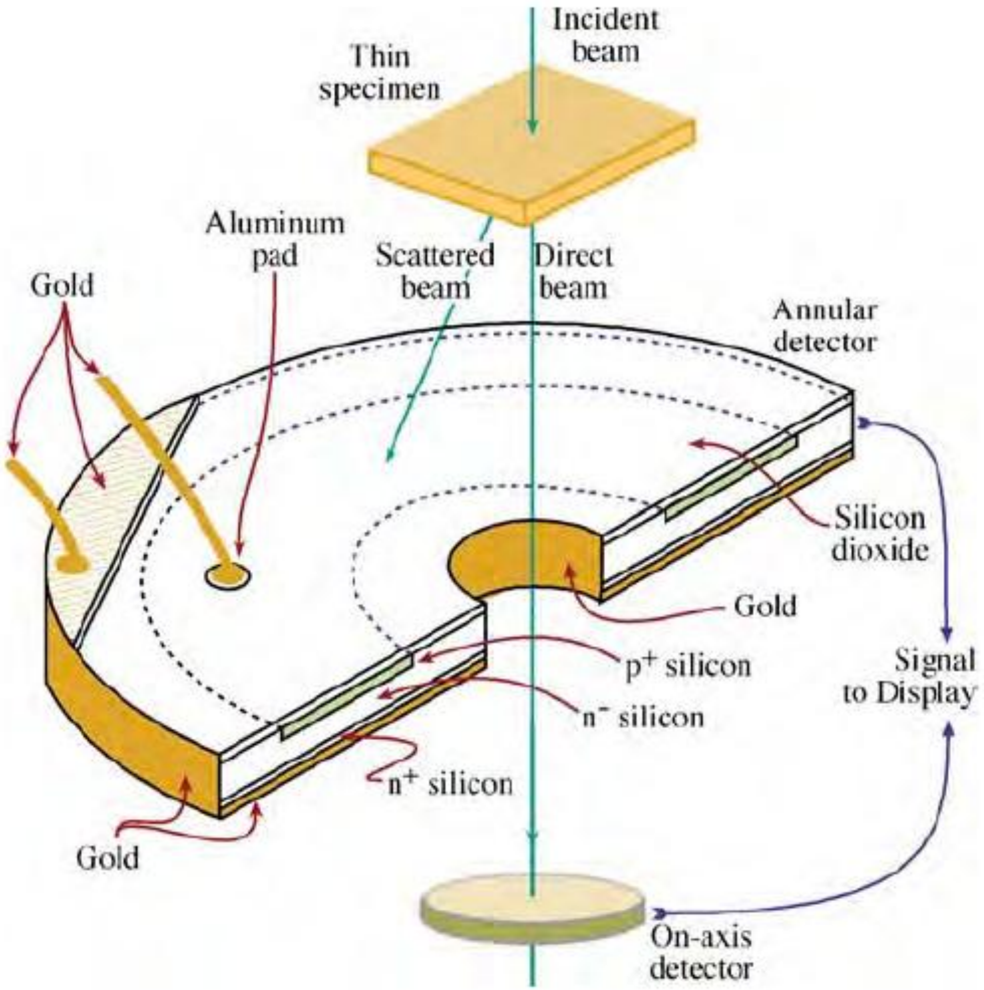
- Lenses for electrons
  - Variable focus
  - Electrostatic
  - Electromagnetic: Lorentz force

Detectors
How to “See” electrons

- **Semiconductor detectors**
- **Scintillator-Photomultiplier detectors**
- Charge-Coupled Device (CCD) Detectors
- Complementary metal-oxide semiconductors (CMOS)
- Direct electron cameras

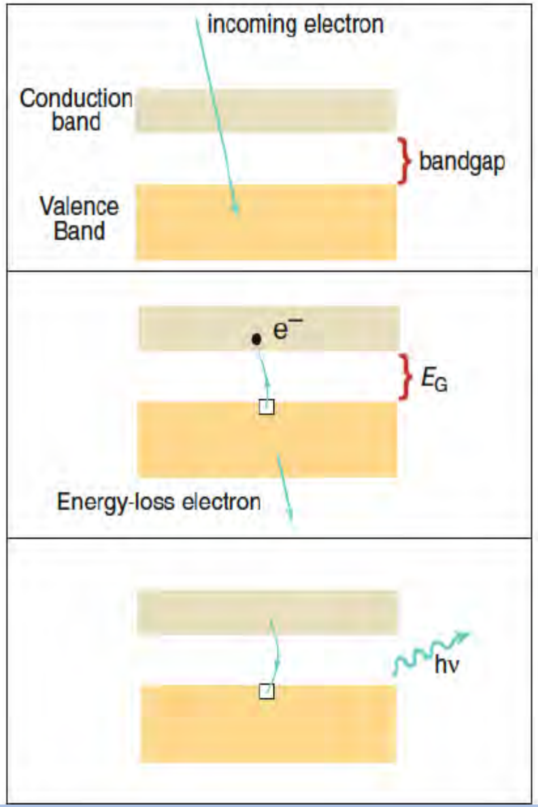
Electron detectors
Semiconductor detectors

- Si diode with a p-n junction close to its surface collects
  - By doping the Si (e.g., by ion implantation of n-type impurity atoms into p-type Si or vice versa).
  - n-type | Gives free electrons to semiconductor
  - By evaporating a thin layer of Au on the surface of high-resistivity n-type Si, or evaporating Al onto p-type Si (i.e. surface-barrier detector or a Schottky diode).

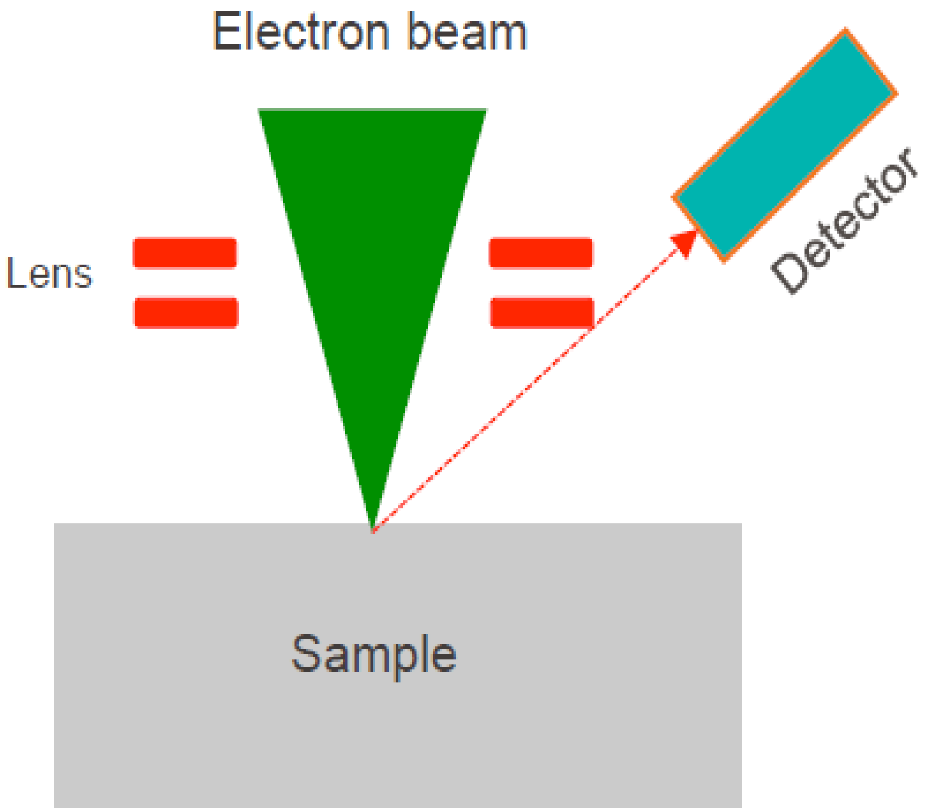


Electron detectors
Semiconductor detectors

- When struck by the high-energy electrons, most of the beam energy is transferred to valence-band electrons in the Si which are excited across the band gap into the conduction band thus creating electron-hole pairs (3.6 eV / electron-hole pair).
- 10 kev → ~2800 electrons
- Thus, the incoming electron signal is converted to a current in the external circuit between the surface contacts.



SEM



SPECIMEN PREPARATION AND OBSERVATION TECHNIQUES

Because of wide variety of SEM application field, there many kinds of specimen that are observed. Therefore, many different specimen preparation techniques have been developed depending on the object and the purpose of the research. In every case though, there are major points that must be taken into consideration during specimen preparation.

1. The specimen surface must be clean. A SEM observes the surface of a specimen. Therefore, it is essential that the specimen surface be clean. In order to observe the inner structure, fracturing, polishing and cutting are commonly performed. In some cases, ion etching or chemical etching are done to remove an unwanted film coating on the specimen surface.

2. The orginal morphologic construction must be maintained.

SPECIMEN PREPARATION AND OBSERVATION TECHNIQUES

3. The specimen must not acquire an electrostatic charge.

- When the specimen is irradiated with an electron beam, some electrons are emitted from the specimen as secondary electrons and backscattered electrons.
- The rest of the irradiated electrons may be absorbed in the specimen. However, if the specimen has no electric conductivity, the absorbed electrons can charge the specimen.
- This charge cause many errors in observations.
- Metal coating, observation with low accelerating voltage, or observation under low vacuum are done to prevent a specimen from acquiring an electric charge.

Sample mounting procedure:

- Specimens are mounted on the surface of the stub using double sided carbon adhesive tabs. Sample surface should be as flat as possible. Specimens must be mounted onto the standard sample holders and never should be mounted directly onto specimen stage.

- There are variety of sample holders available. Specimen can be adhered to 10 mm or 25 mm diameter stubs. Specimens of odd shape or greater than 25 mm can be mounted onto large sample holder.

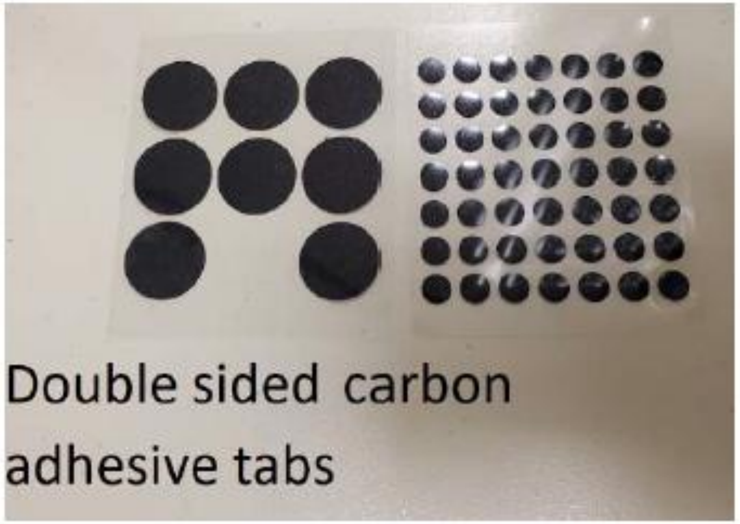
- If there are four different samples, then they can be mounted onto 4 small stubs and placed into 4 stub sample holder.

Sample stubs; specimens are mounted onto them using double sided carbon adhesive tabs

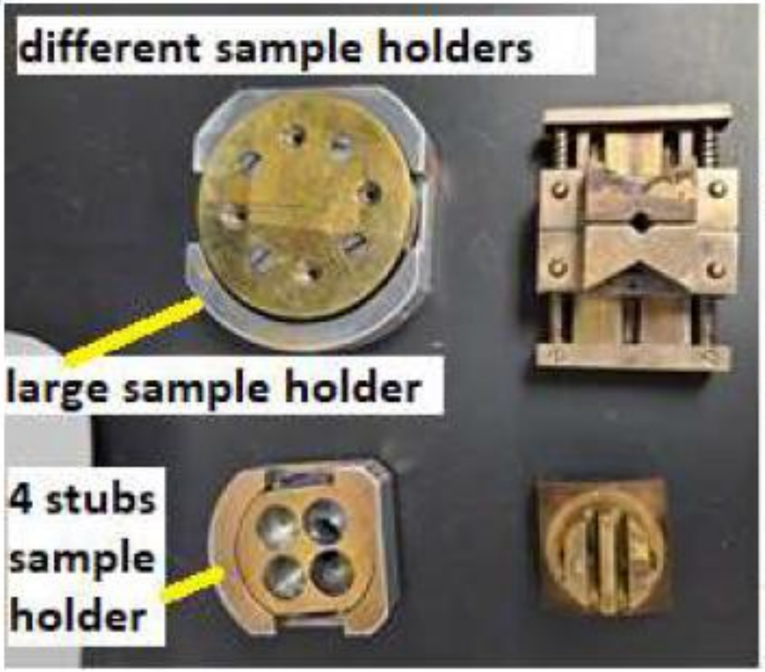
Wear gloves when handling anything that will go inside the SEM instrument. Keep surfaces clean and dust free.

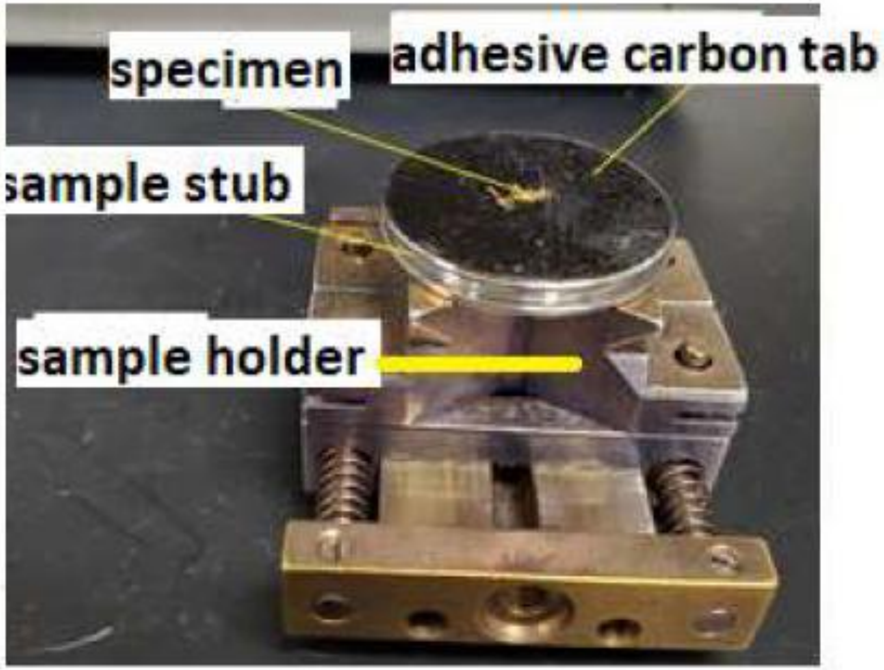
Following material must never be placed in the SEM instrument:

- ✓ Wet samples either from water or a solvent
- ✓ Porous materials that outgas water vapor or toxic gases



Double sided carbon adhesive tabs



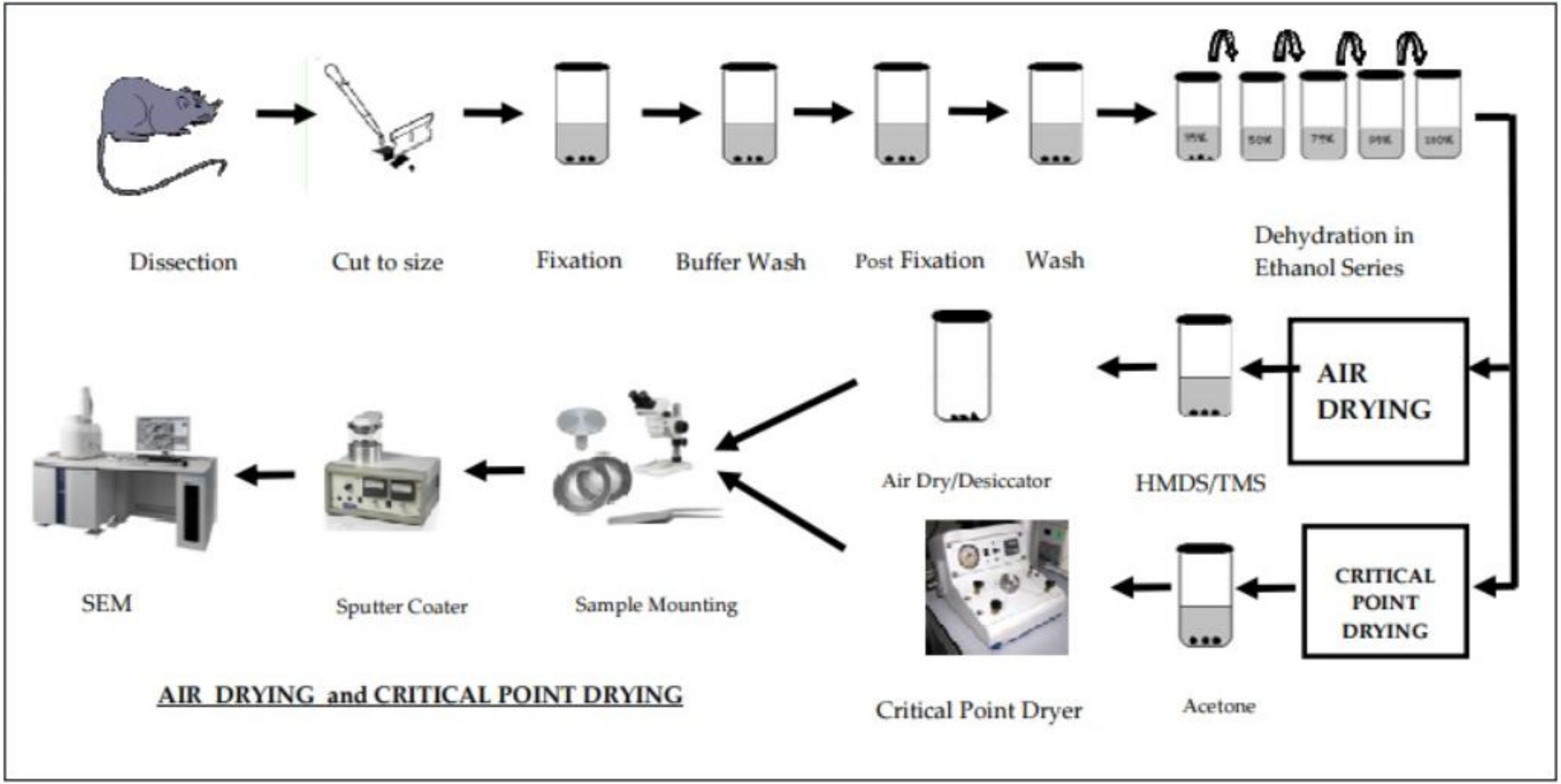


- Biological samples should undergo fixation and dehydration processes, such as Critical Point Drying (CPD) or freezdrying, and then dried to remove the water inside them.

- This will allow higher vacuum SEM imaging and clearer images. Moist samples that cannot be dehydrated are able to be imaged by cryo-SEM systems.

- Traditional methods of dealing with nonconductive materials was to sputter coat or evaporate a thin layer of a metal such as **gold, gold-palladium, platinum** or evaporate **a thin layer of conductive carbon** on the surface of the sample.

Note: The coating thickness must be enough to prevent charging, while preserving specimen surface details (around 10 nm).



Dissection

Cut to size

Fixation

Buffer Wash

Post Fixation

Wash

Dehydration in
Ethanol Series

AIR
DRYING

HMDS/TMS

Air Dry/Desiccator

CRITICAL
POINT
DRYING

Acetone

Critical Point Dryer

Sample Mounting

Sputter Coater

SEM

AIR DRYING and CRITICAL POINT DRYING

THANK YOU

FOR

YOUR ATTENTION