POLLEN
SEM AND TEM

Why Electron Microscope?

- Light Microscopes are limited by the physics of light to 500x or 1000x magnification and a resolution of 0.2 micrometers.

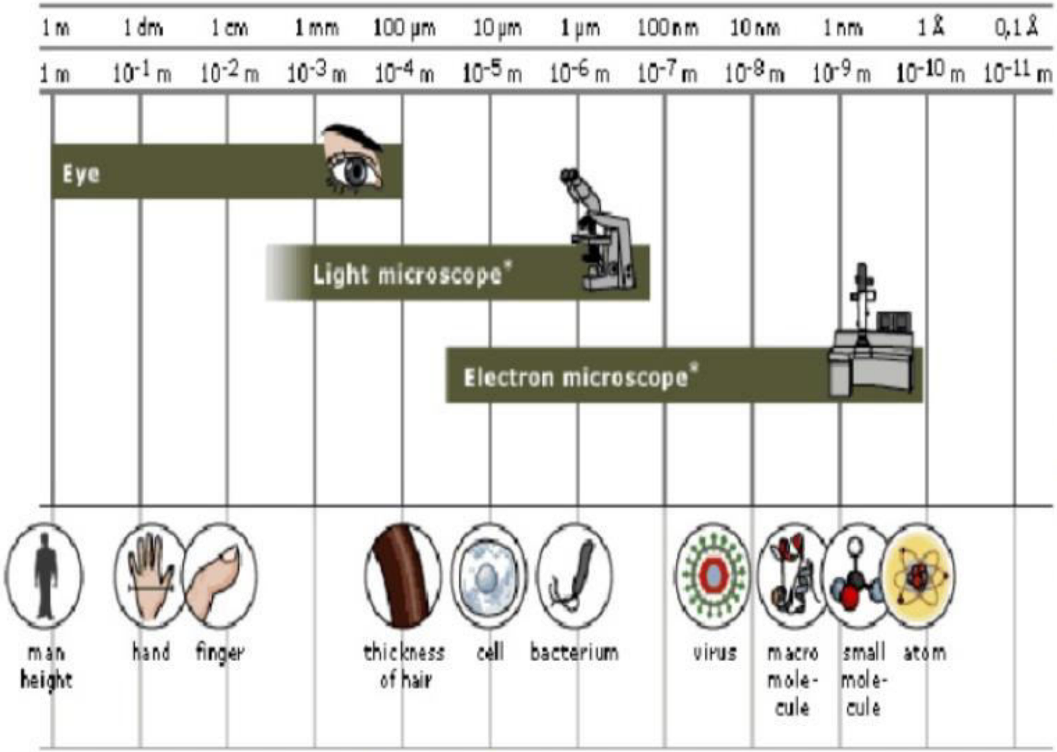
- In the early 1930's there was a scientific desire to see the fine details of the interior structures of organic cells (nucleus, mitochondria...etc.).

- This required 10,000x plus magnification which was just not possible using Light Microscopes.

DIFFERENCES BETWEEN OM AND EM

OPTICAL MICROSCOPE	ELECTRON MICROSCOPE
1. The source of light. 2. The specimen. 3. The lenses that makes the specimen seem bigger. 4. The magnified image of the specimen that you see.	1. The light source is replaced by a beam of very fast moving **electrons**. 2. The specimen usually has to be specially prepared and held inside a **vacuum chamber** from which the air has been pumped out (because electrons do not travel very far in air). 3. The lenses are replaced by a series of coil-shaped **electromagnets** through which the electron beam travels. 4. The image is formed as a photograph (called an electron micrograph) or as an image on a **TV screen**.

MICROSCOPE	RESOLUTION	MAGNIFICATION
OPTICAL	200 nm	1000x
TEM	0.2 nm	500000x



LM, resolving power ~0.25µm, maximum (useful) magnification is about 250µm/0.25µm = 1000X.

Any magnification above this value represents

empty magnification

TEM at 60,000 volts has a resolving power of about 0.0025 nm. Maximum useful magnification of about 100 million times!!!

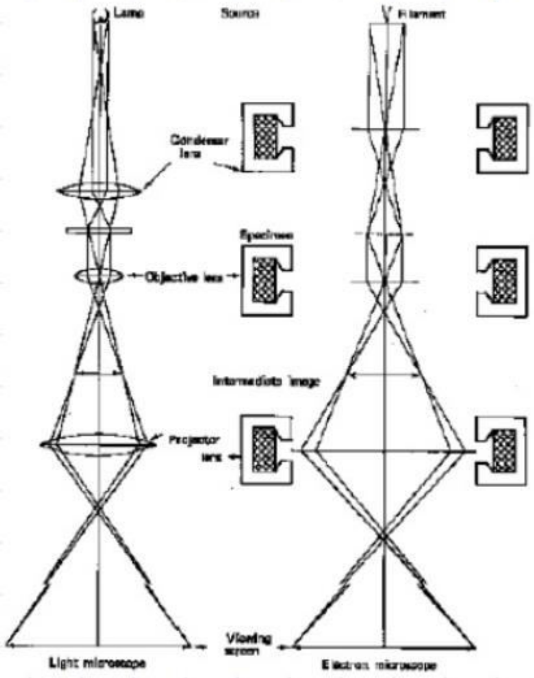
COMPARISION OF LIGHT AND ELECTRON MICROSCOPE

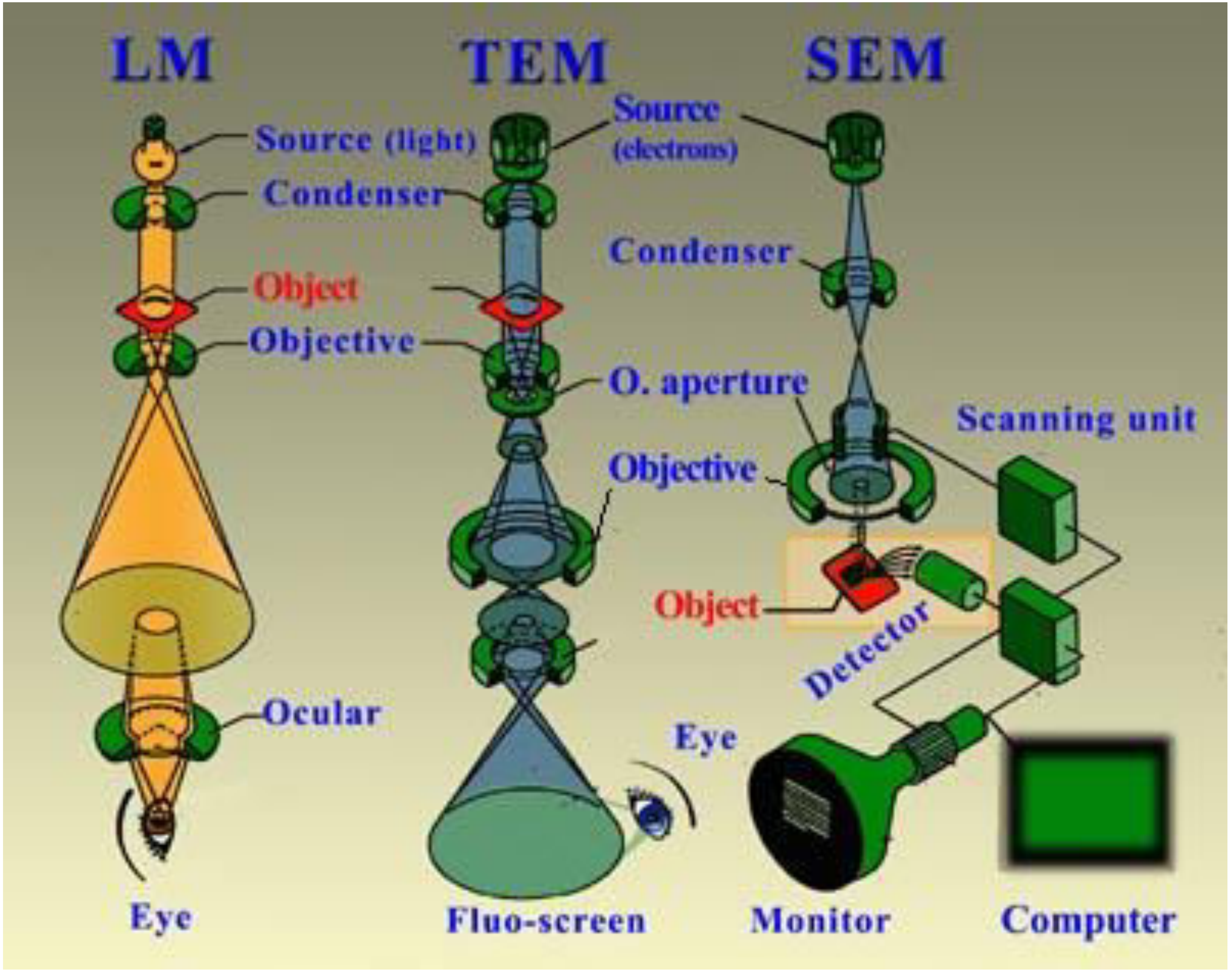
LIGHT MICROSCOPE

Optical glass lens, Small depth of Field, lower magnification, do not Require vacuum, Low price.

ELECTRON MICROSCOPE

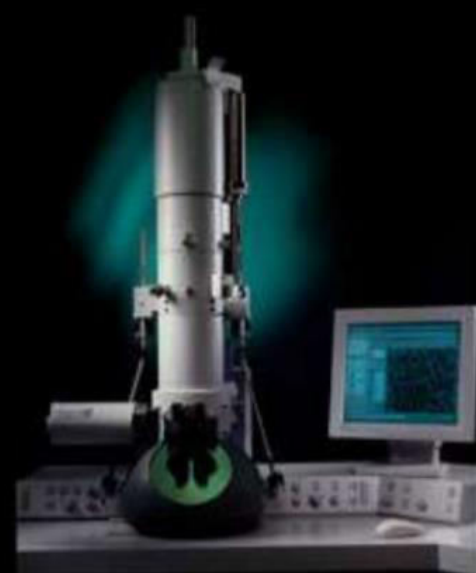
Magnetic lens, Large depth of field, Higher magnification and better Resolution, Operates in HIGH vacuum, Price tag.





LM
TEM
SEM
Source (light)
Source
(electrons)
Condenser
Condenser
Object
Objective
O. aperture
Scanning unit
Objective
Object
Detector
Ocular
Eye
Eye
Fluo-screen
Monitor
Computer

TRANSMISSION
&
SCANNING
ELECTRON MICROSCOPES



INTRODUCTION

- Electron Microscopes are scientific instruments that use a beam of highly energetic electrons to examine objects on a very fine scale.

- This examination can yield information about the topography , morphology, composition and crystallographic information.

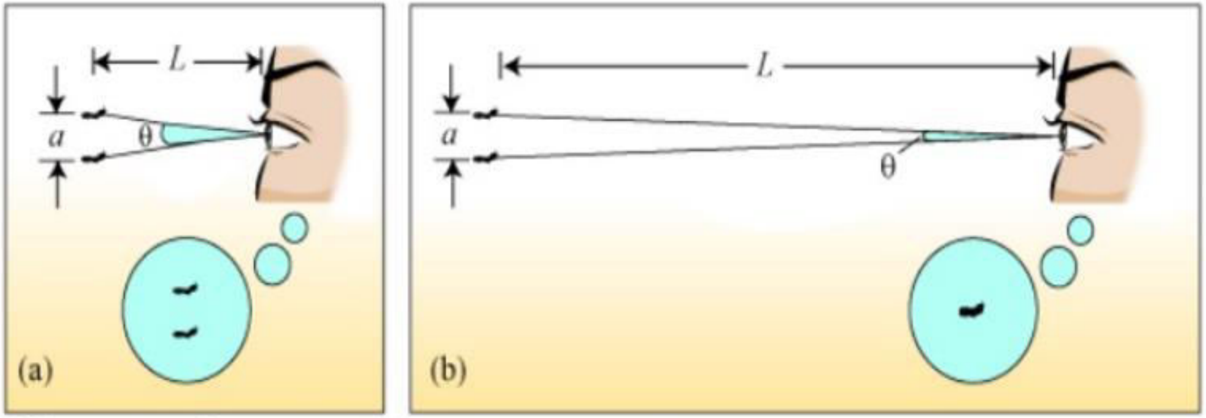
  Mainly 2 types:

- **Transmission Electron Microscope** (TEM) - allows one the study of the **inner structures.**

- **Scanning Electron Microscope** (SEM) **-** used to visualize the **surface** of objects.

RESOLVING POWER

Resolving power is the ability of an imaging device to see objects distinctly, that are located at a small angular distances.



Close to the eye – resolvable

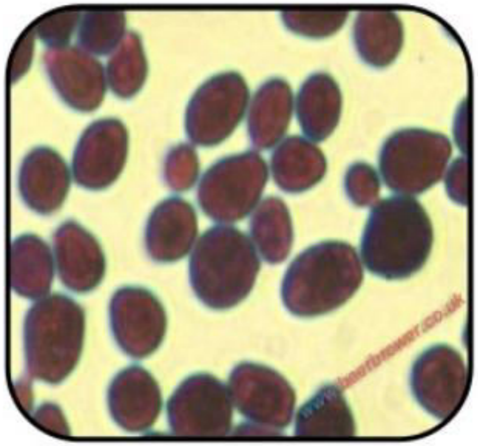
At larger distance -not resolvable .

Rayleigh's criterion:

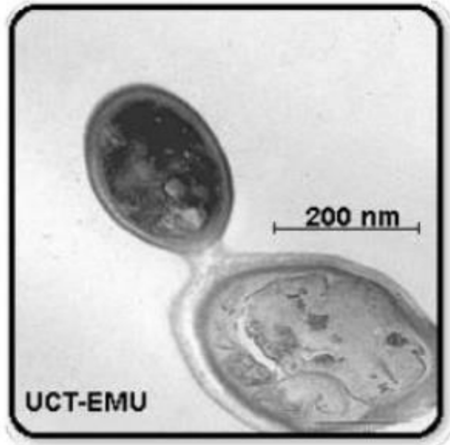
$$\text{Angular resolution} = 1.22\lambda / D$$

- Smaller value of angular resolution - instrument can resolve finer details & has a higher resolving power.
- Electrons have very small wavelength.
- Hence according to Rayleigh's criterion, electron wave can be used to resolve very small angular separations.

Budding yeast cell



Compound microscope image

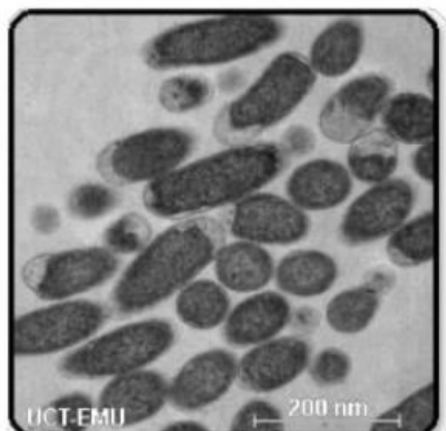


TEM image

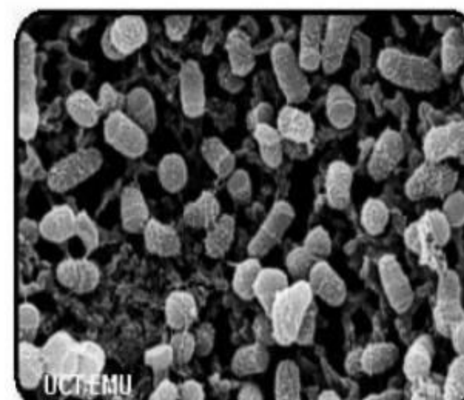
SEM image

***E. coli* bacteria**

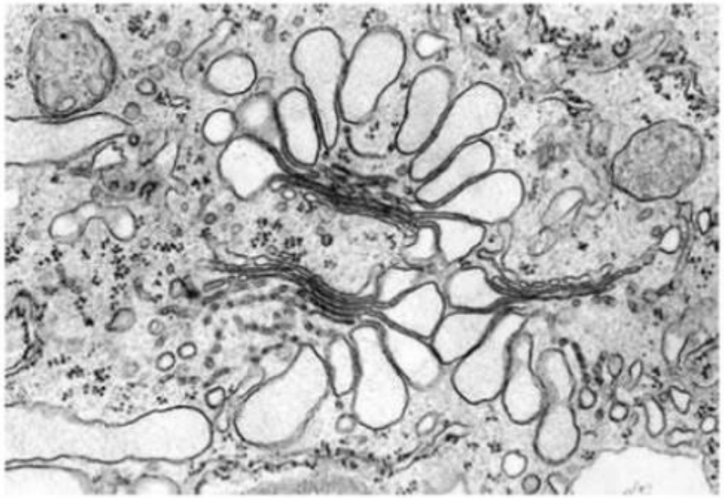
Compound microscope image



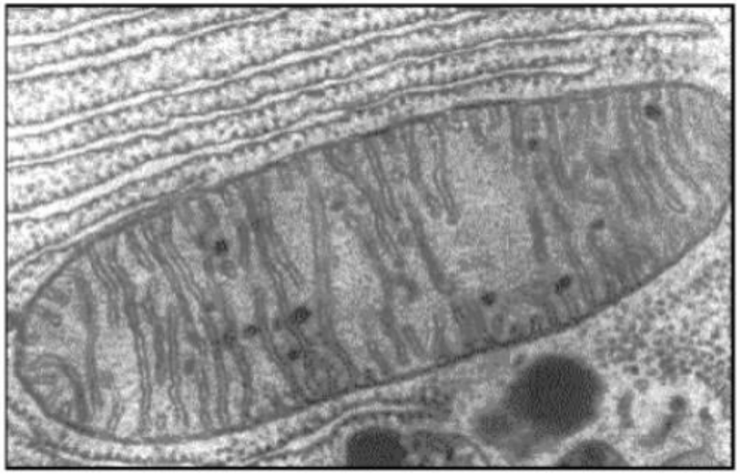
TEM image



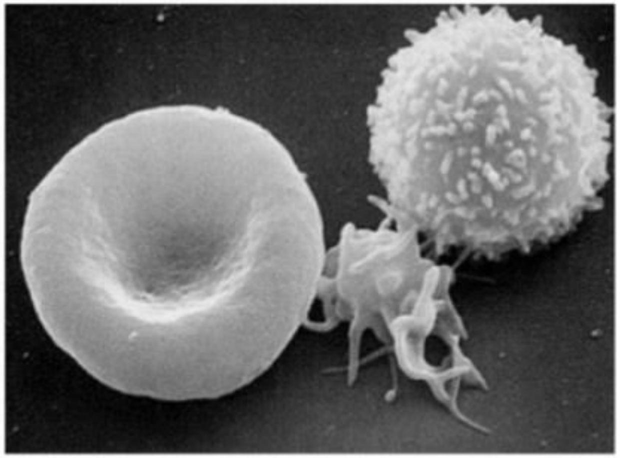
SEM image



TEM image of golgi complex

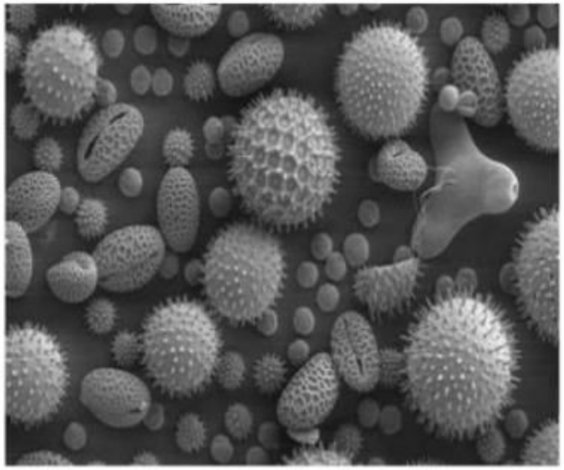


TEM image of mitochondria



SEM image of blood cells

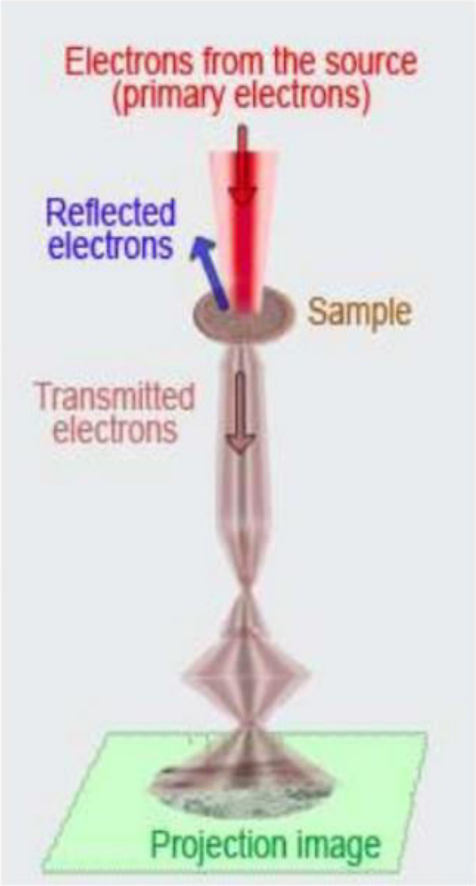
Drosophila's eye.



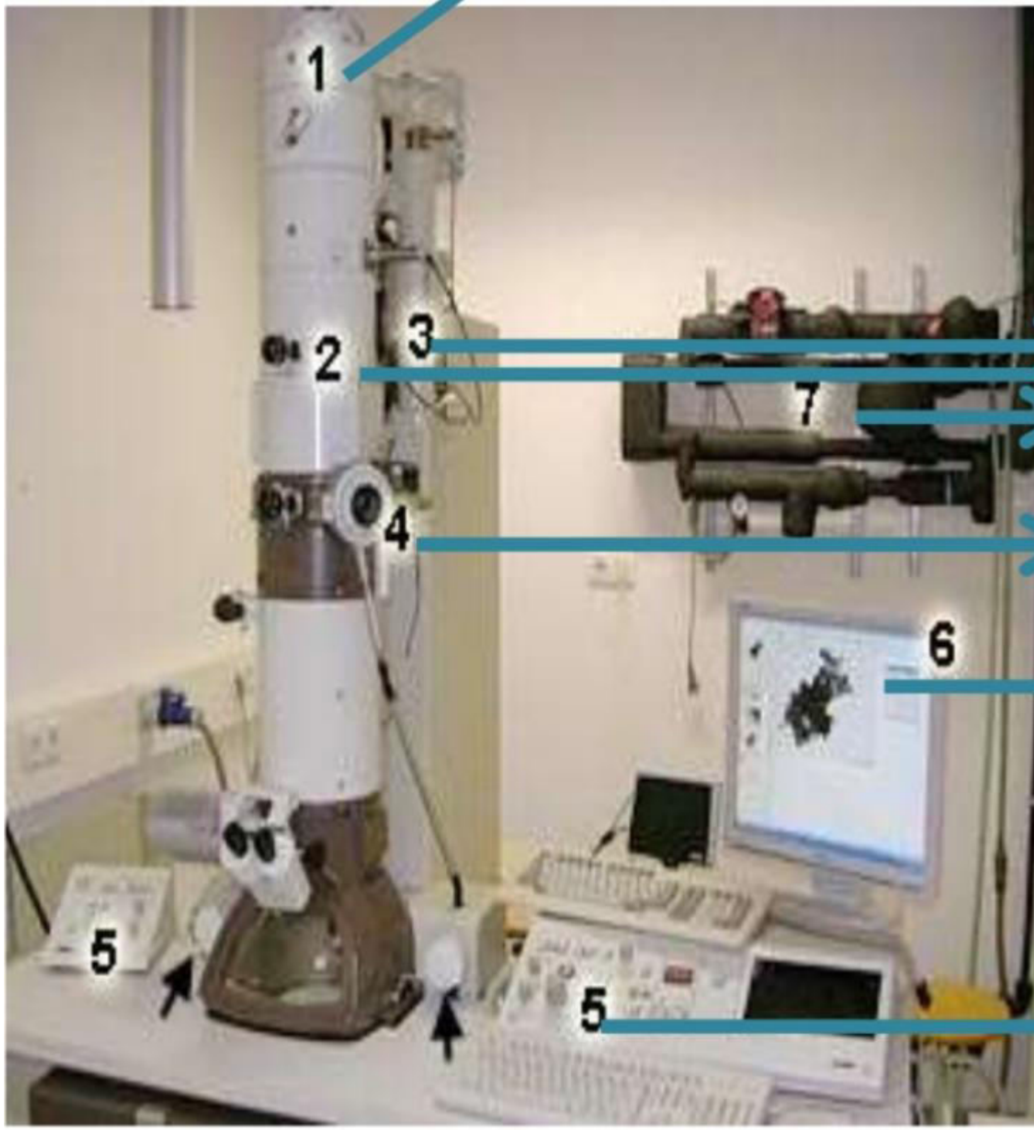
SEM image of pollen grains

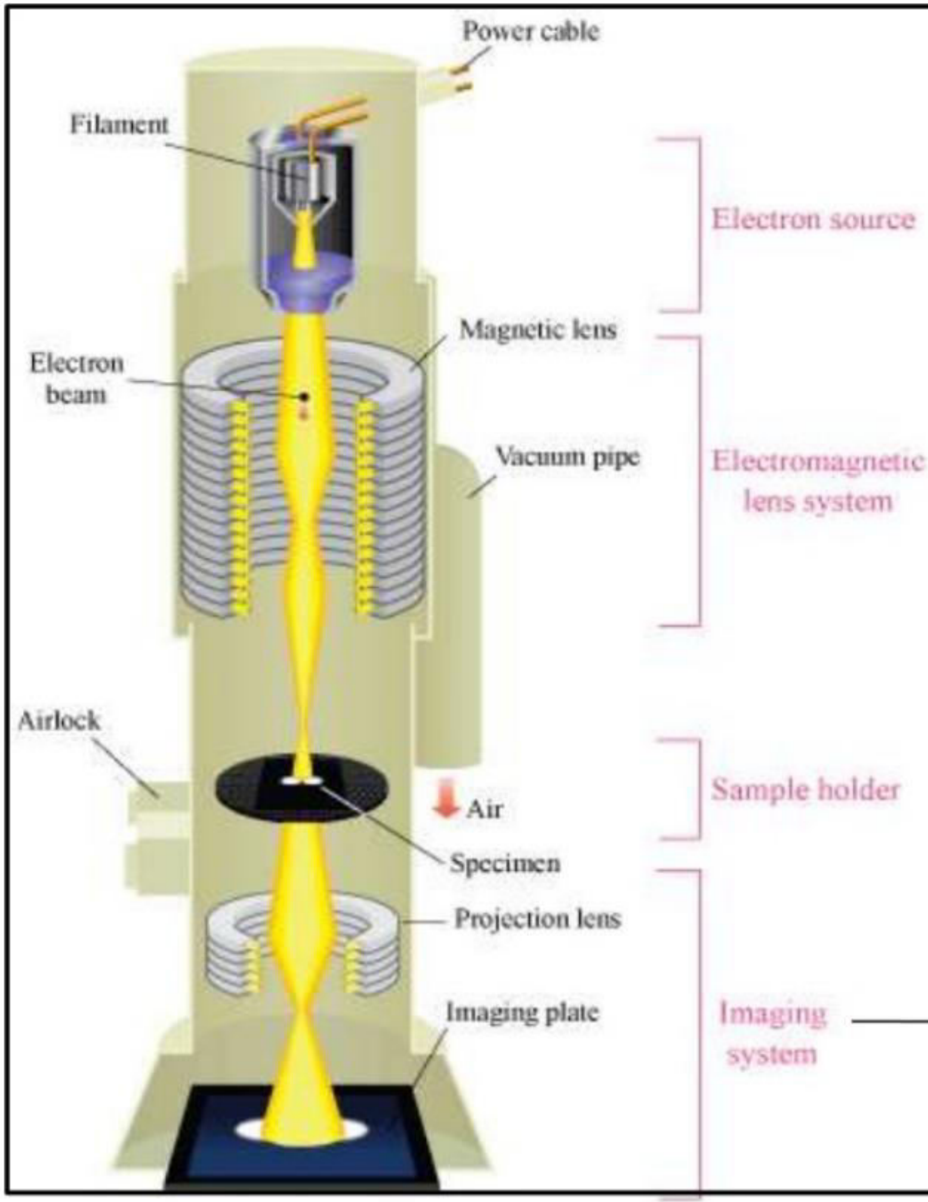
PRINCIPLE OF WORKING OF TEM

- Electrons possess a wave like character.
- Electrons emitted into vacuum from a heated filament with increased accelerating potential will have small wavelength.
- Such higher-energy electrons can penetrate distances of several microns into a solid.
- If these transmitted electrons could be focused - images with much better resolution.
- Focusing relies on the fact that, electrons also behave as negatively charged particles and are therefore deflected by electric or magnetic fields.



PARTS OF TEM


1: Electron cannon.
3: Vacuum pumps system
7: Water supply to cool the instrument
rid
igh-
vacuum chamber for
observation..
6: Screen for menu and image display
5: Operation panels



- The electron source consists of a cathode and an anode.
- Cathode - tungsten filament which emits electrons when being heated.
- A negative cap confines the electrons into a loosely focused beam
- The beam is then accelerated towards the specimen by the positive anode

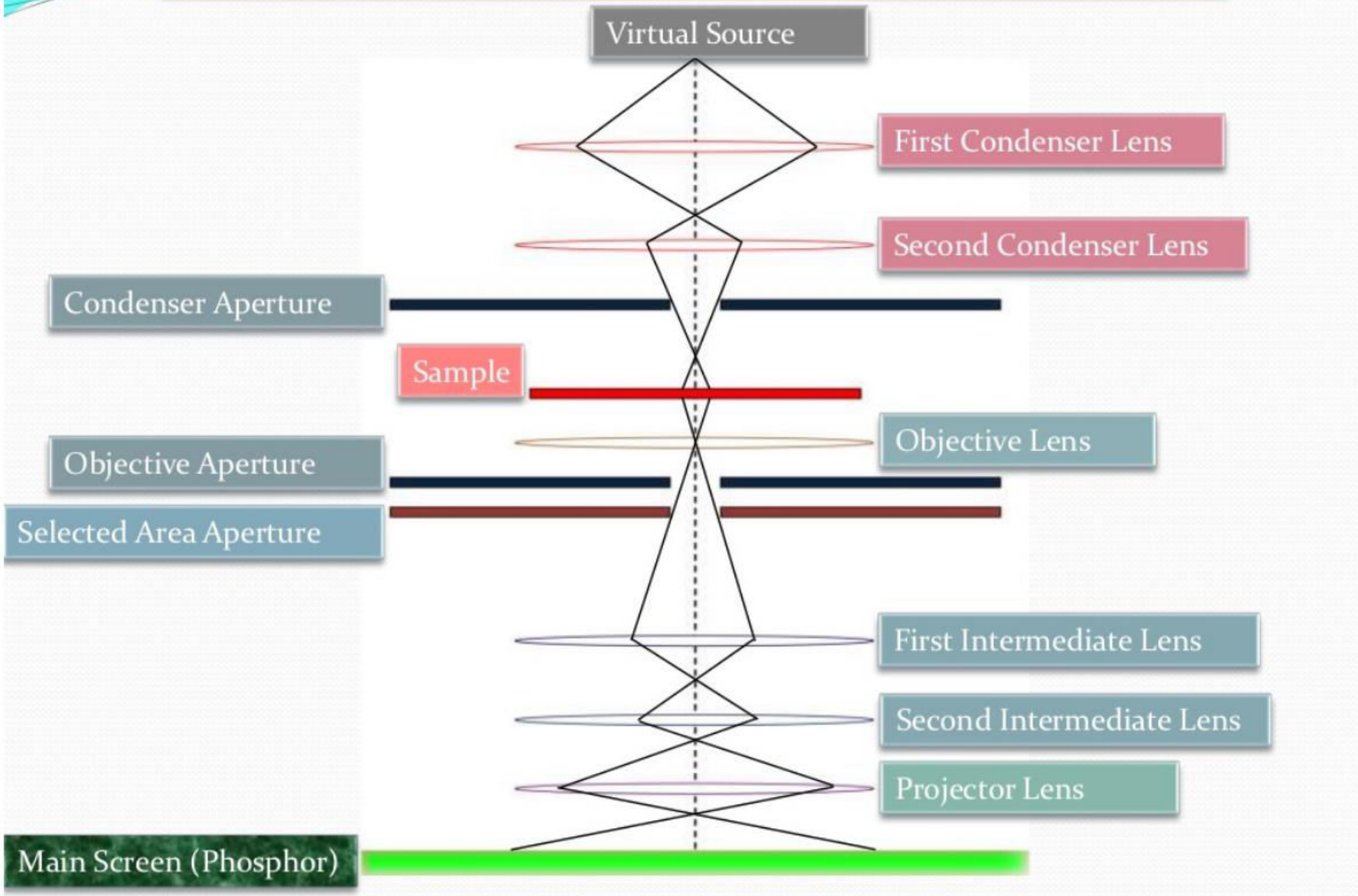
Electron beam is tightly focused using electromagnetic lens and metal apertures.

A platform equipped with a mechanical arm for holding the specimen and controlling its position.

→ Electromagnetic lens system
  - Objective lens
  - Projector lens

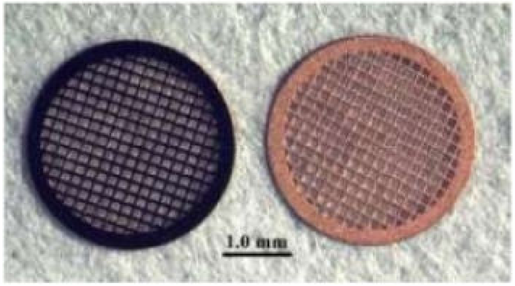
→ Phosphorescent Screen

DIAGRAM TO REPRESENT TEM'S WORKING



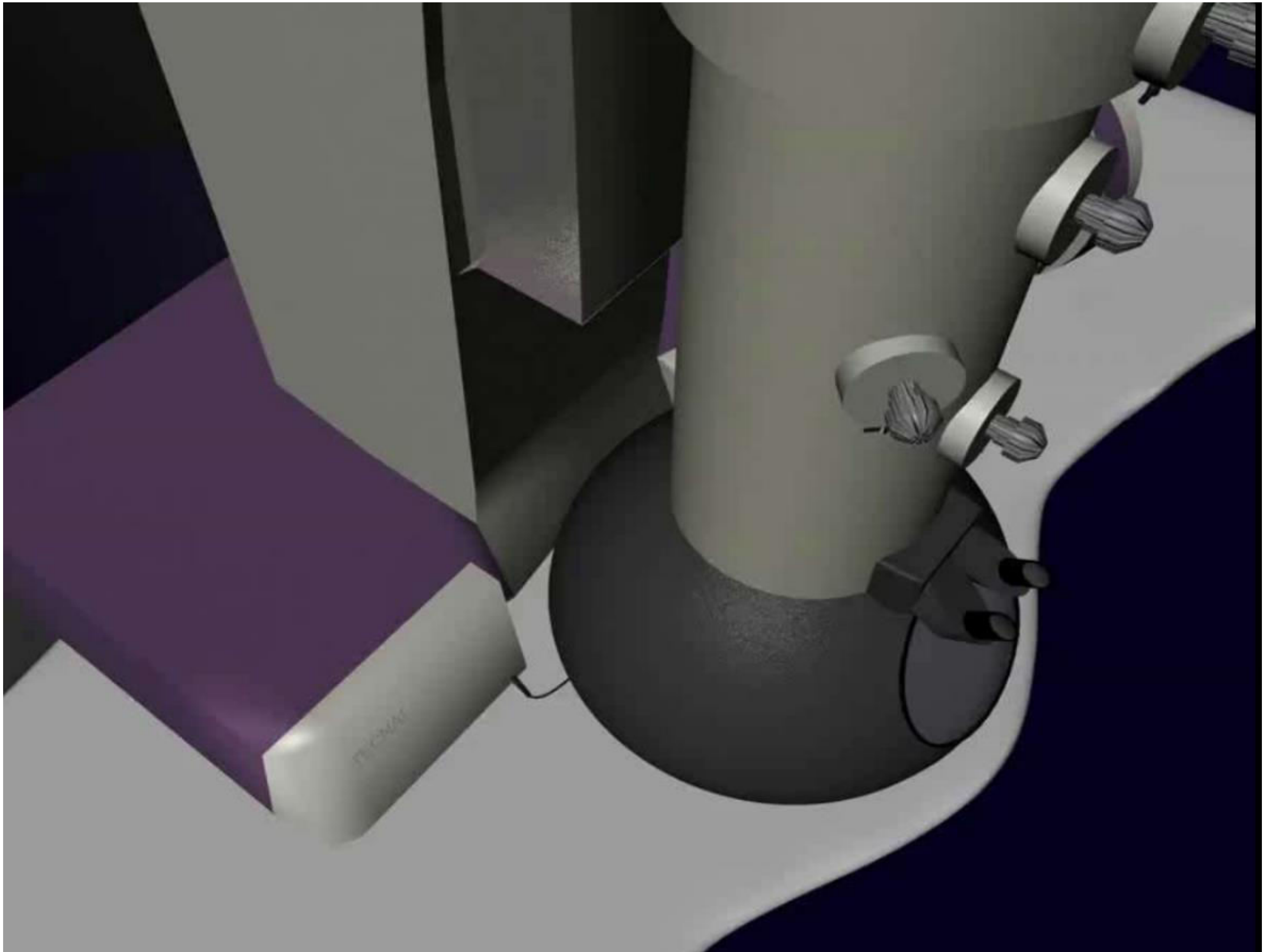
SAMPLE PREPARATION FOR TEM

- **Fixation -** fixed with chemical products (e.g. glutaraldehyde)
- **Rinsing and 'staining' -** treated with heavy metal compounds.
- **Dehydration -** washing with increasing ethanol concentration, followed by final wash in another a polar substance like propylene oxide.
- **Embedding in resin -** material is gradually infiltrated with the still unpolymerized resin .Little pieces of resin-infiltrated material are placed in small holders.
- **Trimming of resin block and ultrathin sectioning -** sections with a thickness of about 70 nm are cut with special knifes of cleaved glass . The cutting is done with a ultra-microtome.
- **Collection of sections on grid**



WORKING OF TEM

- Specimen is bombarded by a beam of electrons, the **primary electrons** . The bombarding electrons are focussed to a bundle onto the object.
- In areas in the object where these electrons encounter atoms with a heavy atomic nucleus, they rebound.
- In regions where the material consists of lighter atoms , the electron are able to pass through.
- The fine pattern of electrons leaving the object , reaches the objective lens forms the image
- It is then greatly enlarged by projector lens.
- Eventually, the traversing electrons (transmission) reach the scintillator plate at the base of the column of the microscope.
- The scintillator contains phosphor compounds that can absorb the energy of the stricking electrons and convert it to light flashes.
- Thus a contrasted image is formed on this plate.



ADVANTAGES & DISADVANTAGES OF TEM

Advantages

- TEMs offer very powerful magnification and resolution.
- TEMs have a wide-range of applications and can be utilized in a variety of different scientific, educational and industrial fields
- TEMs provide information on element and compound structure .
- Images are high-quality and detailed.

Disadvantages

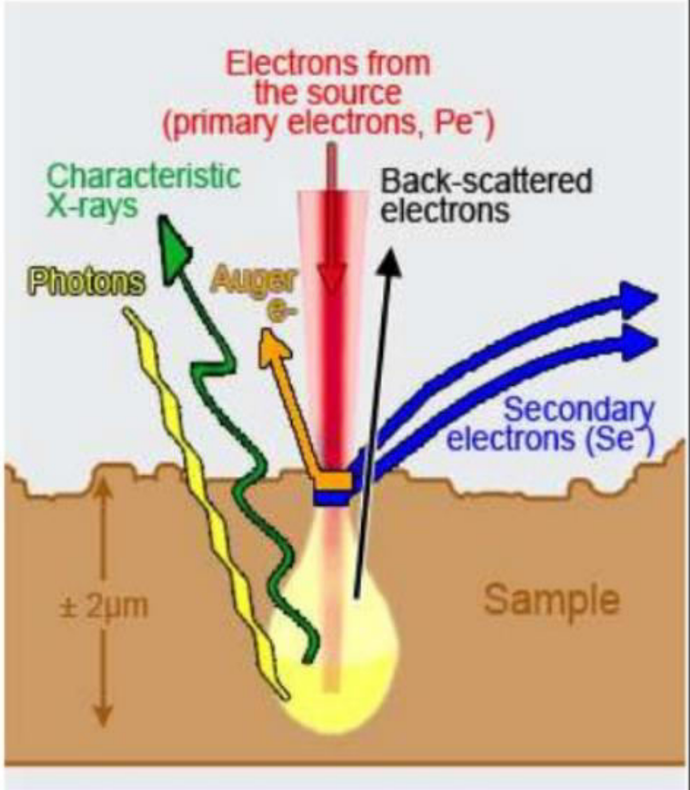
- TEMs are large and very expensive.
- Laborious sample preparation.
- Operation and analysis requires special training.
- Samples are limited to those that are electron transparent.
- TEMs require special housing and maintenance.
- Images are black and white .

BIOLOGICAL APPLICATIONS OF TEM

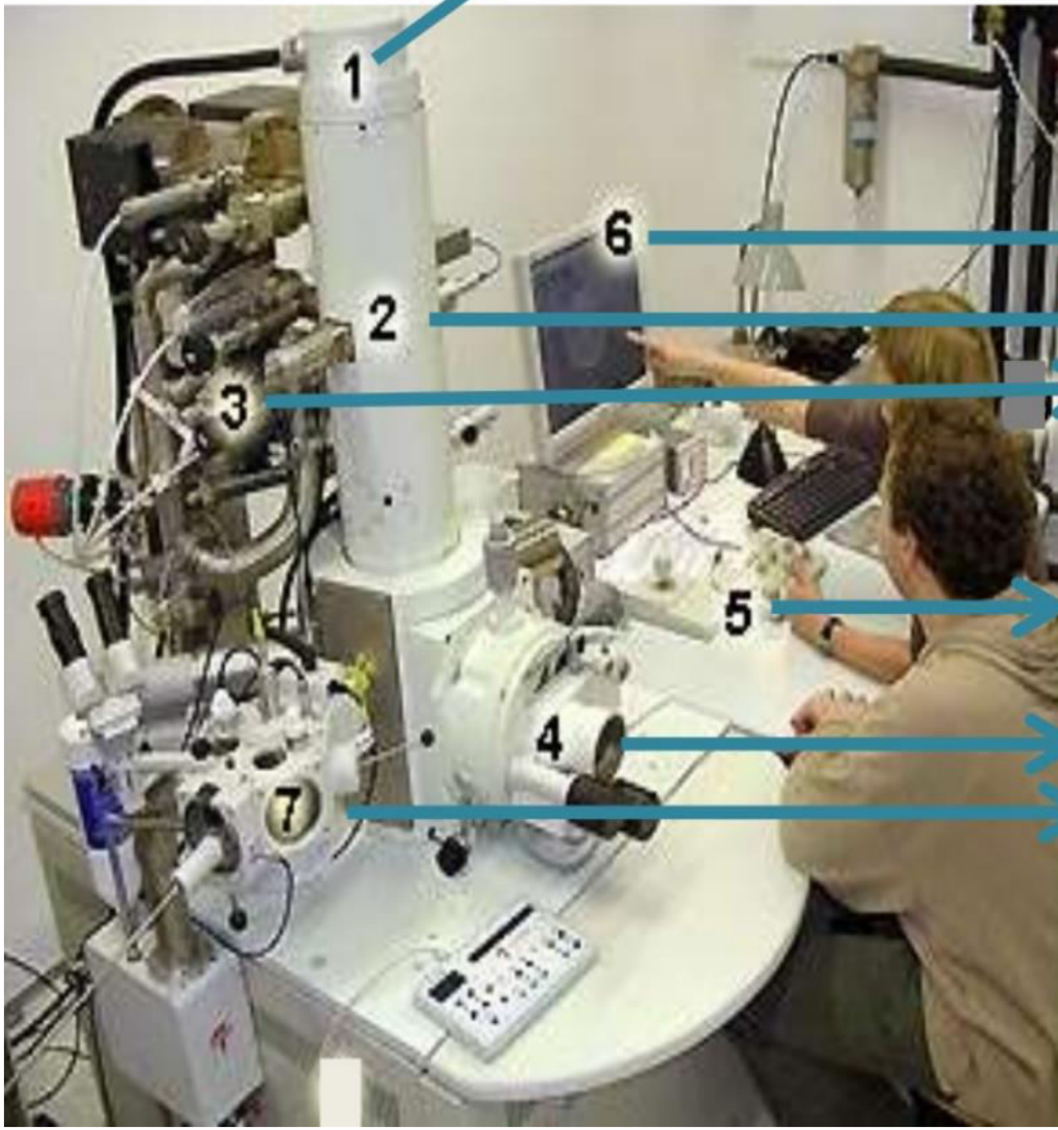
- In medicine as a **diagnostic tool** – important in renal biopsies.
- **Cellular tomography**
  - Tomography refers to imaging by sectioning, through the use of any kind of penetrating wave.
  - Information is collected and used to assemble a three dimensional image of the target.
  - Used for obtaining detailed 3D structures of subcellular macromolecular objects.
- **Cancer research** - studies of tumor cell ultrastructure .
- **Toxicology** – to study the impacts of environmental pollution on the different levels of biological organization.

PRINCIPLE OF WORKING OF SEM

- Incoming (primary) electrons
  - can be "reflected" (backscattered) from a bulk specimen.
  - can release secondary electrons.
- Primary electrons are focused into a small-diameter electron probe that is scanned across the specimen.
- Electrostatic or magnetic fields, applied at right angles to the beam, can be used to change its direction of travel.
- By scanning simultaneously in two perpendicular directions, a square or rectangular area of specimen (known as a **raster**) can be covered.
- Image of this area can be formed by collecting secondary electrons from each point on the specimen.



PARTS OF SEM



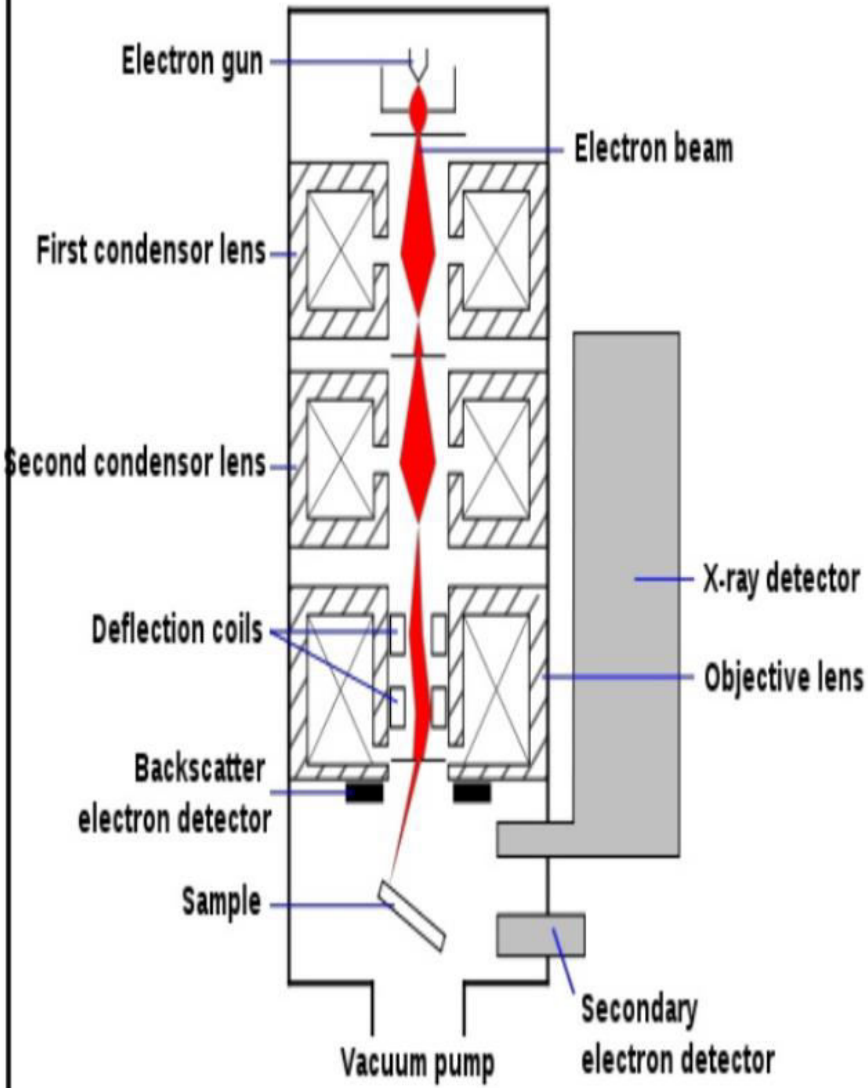
1.Electron cannon.

6. Screen for menu and image display

3. Vacuum pumps system

5. Operation panel with focus, alignment and magnification tools and a joystick for positioning

7.Cryo-unit to prepare frozen material before insertion in the observation chamber in Cryo-SEM mode



- Electron gun consisting of cathode and anode.
- The condenser lens controls the amount of electrons travelling down the column
- The objective lens focuses the beam into a spot on the sample.
- Deflection coil helps to deflect the electron beam.
- SED attracts the secondary electrons.
- Additional sensors detect backscattered electrons and X-rays.

SEM SAMPLE PREPARATION

•Sample coated with a thin layer of conductive material.

•Done using a device called a "**sputter coater.”**

• Sample placed in a small chamber that is at a vacuum .

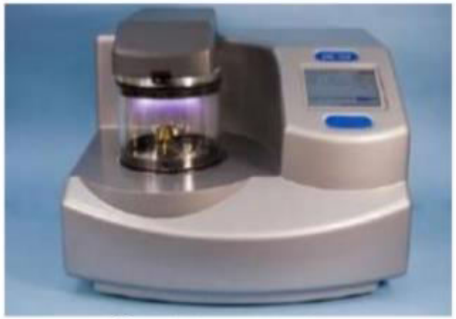
•Gold foil is placed in the instrument.

• Argon gas and an electric field cause an electron to be removed from the argon, making the atoms positively charged.

• The argon ions then become attracted to a negatively charged gold foil.

•The argon ions knock gold atoms from the surface of the gold foil.

• These gold atoms fall and settle onto the surface of the sample producing a thin gold coating.



Sputter coater

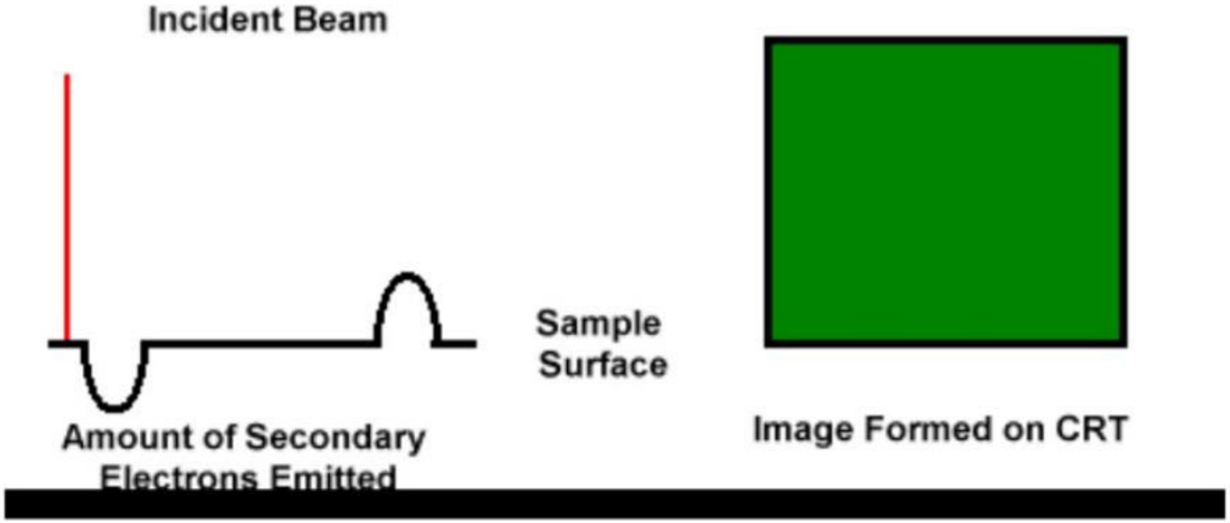
A spider coated in gold

13mm radius aluminium stubs

SEM WORKING

- The electron gun produces an electron beam when tungsten wire is heated by current.
- This beam is accelerated by the anode.
- The beam travels through electromagnetic fields and lenses, which focus the beam down toward the sample.
- A mechanism of deflection coils enables to guide the beam so that it scans the surface of the sample in a rectangular frame.
- When the beam touches the surface of the sample, it produces:
  - Secondary electrons (SE)
  - Back scattered electrons (BSE)
  - X - Rays...
- The emitted SE is collected by SED and convert it into signal that is sent to a screen which produces final image.
- Additional detectors collect these X-rays, BSE and produce corresponding images.

- A secondary electron detector attracts the scattered electrons and, depending on the number of electrons that reach the detector, registers different levels of brightness on a monitor.



- By reducing the size of the area scanned by the scan coils, the SEM changes the magnification of the image.

ADVANTAGES & DISADVANTAGES OF SEM

Advantages

- It gives detailed 3D and topographical imaging and the versatile information garnered from different detectors.
- This instrument works very fast.
- Modern SEMs allow for the generation of data in digital form.
- Most SEM samples require minimal preparation actions.

Disadvantages

- SEMs are expensive and large.
- Special training is required to operate an SEM.
- The preparation of samples can result in artifacts.
- SEMs are limited to solid samples.
- SEMs carry a small risk of radiation exposure associated with the electrons that scatter from beneath the sample surface.

BIOLOGICAL APPLICATIONS OF SEM

- **Virology** - for investigations of virus structure
- **Cryo-electron microscopy** – Images can be made of the surface of frozen materials.
- **3D tissue imaging -**
  - Helps to know how cells are organized in a 3D network
  - Their organization determines how cells can interact.
- **Forensics -** SEM reveals the presence of materials on evidences that is otherwise undetectable
- SEM renders detailed 3-D images
  - extremely small microorganisms
  - anatomical pictures of insect, worm, spore, or other organic structures

Differences between SEM and TEM

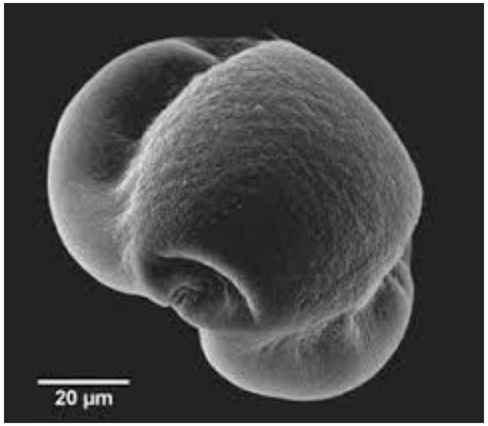
TEM	SEM
Electron beam passes through thin sample.	Electron beam scans over surface of sample.
Specially prepared thin samples are supported on TEM grids.	Sample can be any thickness and is mounted on an aluminum stub.
Specimen stage halfway down column.	Specimen stage in the chamber at the bottom of the column.
Image shown on fluorescent screen.	Image shown on TV monitor.
Image is a two dimensional projection of the sample.	Image is of the surface of the sample

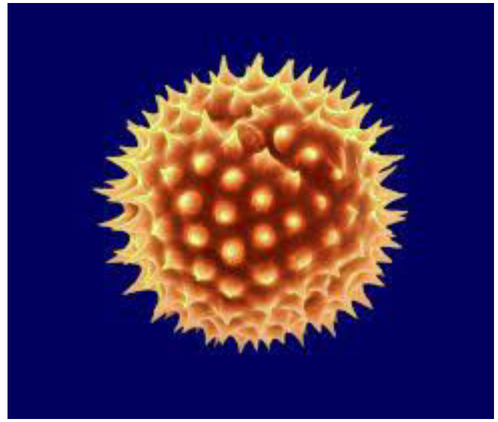
RECENT DEVELOPMENTS

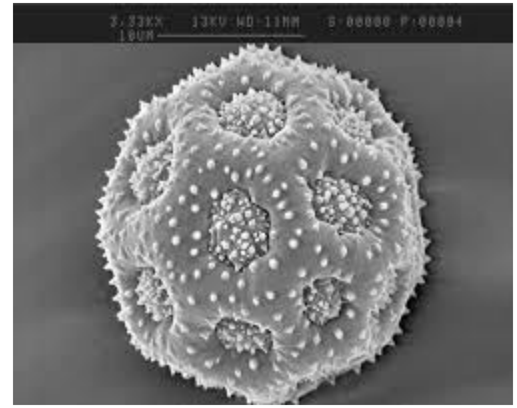
- Three famous physicists, **Harald H. Rose** , **Knut W. Urban** and **Maximillian Haider** have received the **Wolf-prize in physics 2011** for the realization of **aberration-corrected electron microscopy**.
- Aberrations are intrinsic imperfections of electron lenses.
- Those aberrations are reduced by installing in a microscope a set of specially designed auxiliary "lenses" which are called aberration correctors.
- They designed a novel aberration corrector thereby improving resolution of transmission electron microscope.

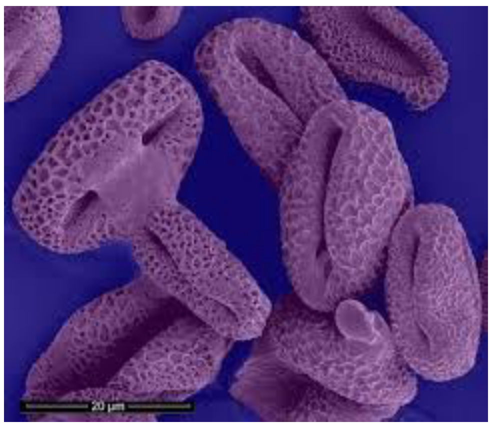
CONCLUSION

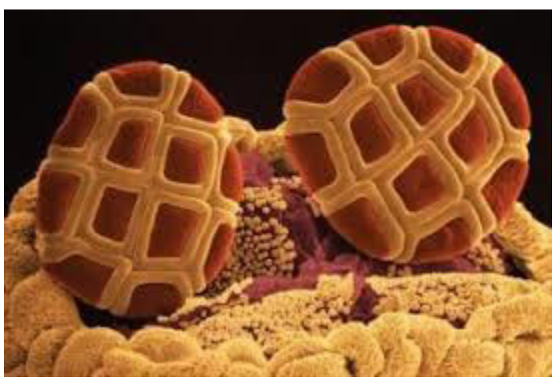
- Since its invention, electron microscope has been a valuable tool in the development of scientific theory
- It has contributed greatly to biology, medicine and material sciences.
- This wide spread use because they permit the observation of materials on a nanometer (nm) to micrometer (μm) scale.
- Although SEMs and TEMs are large, expensive pieces of equipments, they remain popular among researchers due to the high-resolution and detailed images they produce.

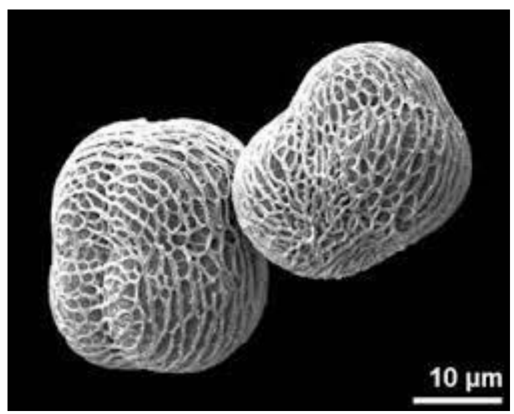












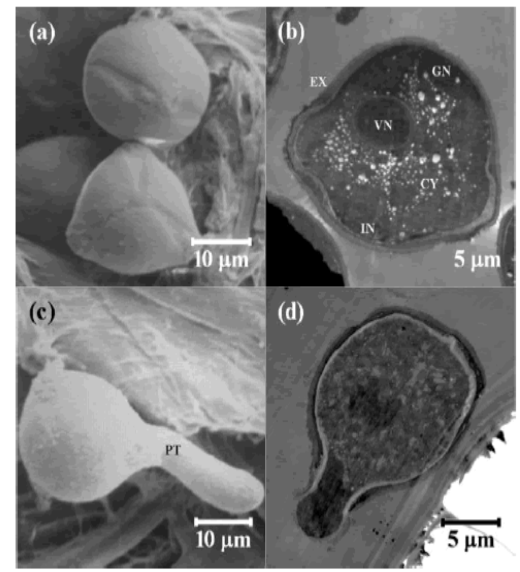


Figure 1: (a) Polar view of a non-germinated pollen, with no pesticide effects, (b) A 100 nm thick pollen section. EX: exine, IN: intine, VN: vegetative nucleus, GN: generative nucleus, CY: cytoplasm, (c) A germinated pollen grain showing the pollen tube (PT) structure growing in one of the apertures, (d) Section of germinated pollen showing its nuclei and other internal structures.

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