MICROSCOPES

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LIGHT MICROSCOPE

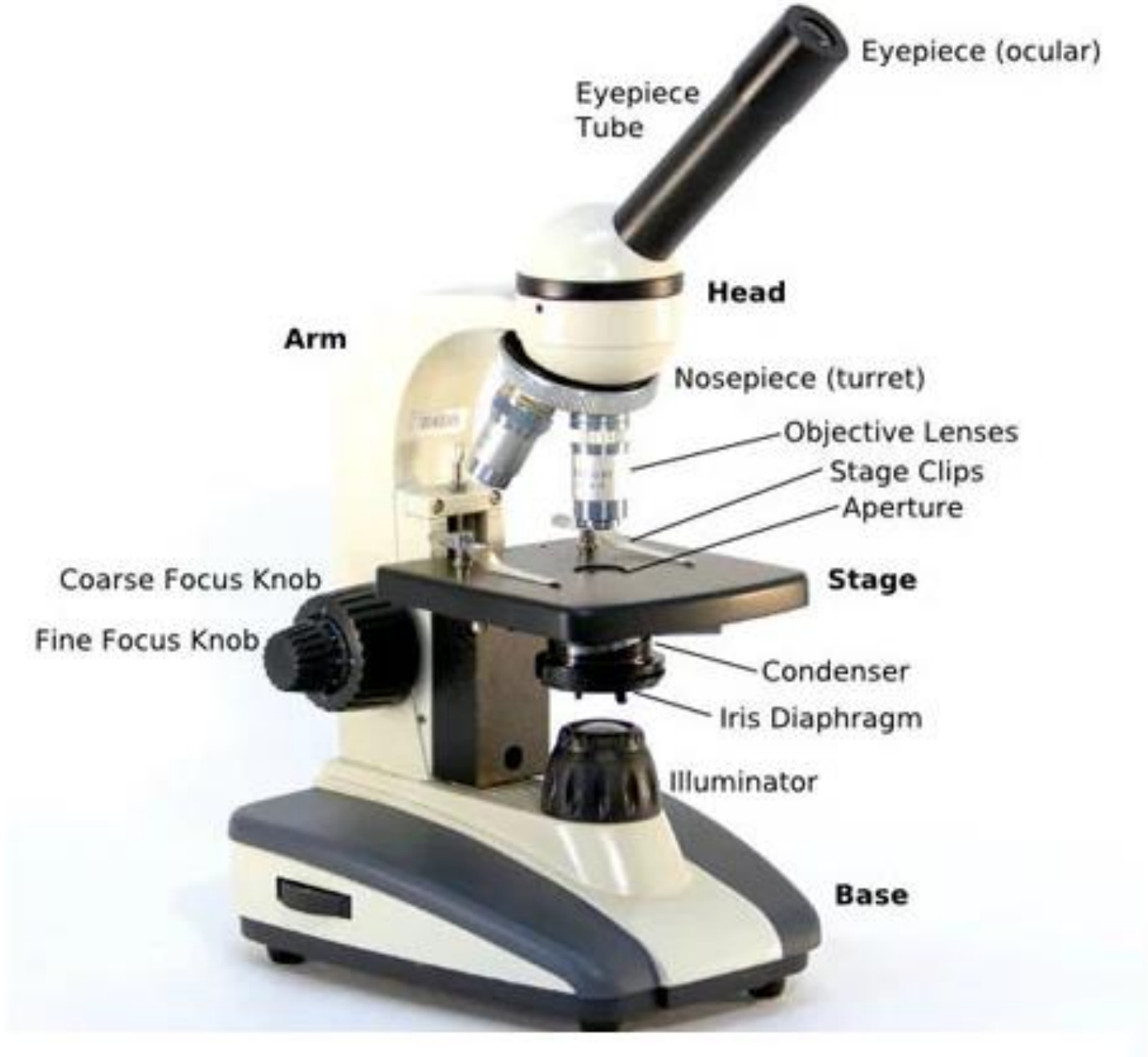
ELECTRON MICROSCOPE

Microscopes are available in different sizes and in particular usage. The most common types of microscopes are the light microscope and electron microscope. Each of these microscopes possesses distinct features and is appropriate for different purposes. Both light microscopes and electron microscopes use radiation to form detailed images of objects that a human eye cannot produce unaided. The main difference between light microscope and electron microscope is that beam of electrons is used for magnifying the image of an object while visible light is used in the light microscope to magnify images of tiny areas of materials or biological specimens. More differences between the two are listed below in a tabular column.

Light Microscope vs Electron Microscope

Difference Between Electron Microscope And Light Microscope	
Light Microscope	Electron Microscope
Uses light (approx 400-700 nm) as an illuminating source	Uses electron beams (approx 1 nm) as an illuminating source.
Lower magnification than an electron microscope	Higher magnification
No risk of radiation leakage	Risk of radiation leakage
Specimen preparation takes about a few minutes or an hour	Specimen preparation takes several days
Both live and dead specimen can be seen	Only dead and the dried specimen can be seen
The image formation depends upon the light absorption from the different zones of the specimen	The image formation depends upon the electron scattering
The image is seen through the ocular lens. No screen needed	The image is received on a zinc sulfate fluorescent screen
Useful magnification of 500x to 1500x	Direct magnification as high as 16000x and photographic magnification as high as 1000000 x
Low resolution	High resolution
Inexpensive and requires a low maintenance cost	Expensive and high maintenance

COMPOUND LIGHT MICROSCOPE



- The compound microscope uses lenses and light to enlarge the image and is also called an optical or light microscope (versus an electron microscope).
- The simplest optical microscope is the magnifying glass and is good to about ten times (10x) magnification.

- The compound microscope has two systems of lenses for greater magnification:
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- 1. Ocular eyepiece lens to look through.
  2. Objective lens, closest to the object. Before purchasing or using a microscope, it is important to know the functions of each part. This information is presented below. Links will take you to additional information and images.
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- **The Functions of a Microscope**

- **Eyepiece Lens**: the lens at the top that you look through, usually 10x or 15x power.
- **Tube**: Connects the eyepiece to the objective lenses.
- **Arm**: Supports the tube and connects it to the base.
- **Base**: The bottom of the microscope, used for support.
- **Illuminator**: A steady light source (110 volts) used in place of a mirror. If your microscope has a mirror, it is used to reflect light from an external light source up through the bottom of the stage.
- **Stage with Stage Clips**: The flat platform where you place your slides. Stage clips hold the slides in place. If your microscope has a mechanical stage, you will be able to move the slide around by turning two knobs. One moves it left and right, the other moves it up and down.
- **Revolving Nosepiece or Turret**: This is the part that holds two or more objective lenses and can be rotated to easily change power.

- **Objective Lenses**: Usually you will find 3 or 4 objective lenses on a microscope. They almost always consist of 4x, 10x, 40x and 100x powers.
- When coupled with a 10x (most common) eyepiece lens, total magnification is 40x (4x times 10x), 100x , 400x and 1000x. To have good resolution at 1000x, you will need a relatively sophisticated microscope with an Abbe condenser.
- An Abbe condenser is composed of two lenses that control the light that passes through the specimen before entering the objective lens on the microscope.
- The shortest lens is the lowest power, the longest one is the lens with the greatest power. Lenses are color coded and if built to DIN standards are interchangeable between microscopes.
- "DIN" is an abbreviation of "Deutsche Industrial Normen". This is a German standard that has been adopted internationally as an optical standard used in most quality microscopes. A typical DIN standard microscope objective lens has a 0.7965" (20.1mm) diameter threads, 36 TPI (threads per inch), and a 55º Whitworth.
- Many high power objective lenses are retractable (i.e. 40XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide. All good quality microscopes have achromatic, parcentered, parfocal lenses.

- **Rack Stop**: This is an adjustment that determines how close the objective lens can get to the slide. It is set at the factory and keeps students from cranking the high power objective lens down into the slide and breaking things.

- **Condenser Lens**: The purpose of the condenser lens is to focus the light onto the specimen.
- Condenser lenses are most useful at the highest powers (400x and above). Microscopes with in-stage condenser lenses render a sharper image than those with no lens (at 400x).
- If your microscope has a maximum power of 400x, you will get the maximum benefit by using a condenser lenses rated at 0.65 NA or greater. 0.65 NA condenser lenses may be mounted in the stage and work quite well.
- A big advantage to a stage mounted lens is that there is one less focusing item to deal with. If you go to 1000x then you should have a condenser lens with an N.A. of 1.25 or greater. All of our 1000x microscopes use 1.25 Abbe condenser lens systems.
- The Abbe condenser lens can be moved up and down. It is set very close to the slide at 1000x and moved further away at the lower powers.

- **Diaphragm or Iris**:
- Many microscopes have a rotating disk under the stage.
- This diaphragm has different sized holes and is used to vary the intensity and size of the cone of light that is projected upward into the slide.
- There is no set rule regarding which setting to use for a particular power.
- Rather, the setting is a function of the transparency of the specimen, the degree of contrast you desire and the particular objective lens in use.

- **Magnification**
- The objective lenses are the main lenses used for focusing the image, on the condenser. This produces an enlarged clear image that is then magnified again by the eyepiece to form the primary image that is seen by the eyes.
- During imaging, the objective lenses remain parfocal in that, even when the objective lens has changed the image still remains focused. The image seen at the eyepiece is the enlarged clear image of the specimen, known as the virtual image.
- The magnification of the image is determined by the magnification of the objective against the magnification of the eyepiece lens. The objectives have a magnification power of 40x-1000x depending on the type of brightfield microscope while the eyepiece lens has a standard magnification power of 10x.
- Therefore to calculate:
- **Total Magnification power = Magnification of the objective lens x Magnification of the eyepiece**

- **Its applications include:**

1. Used to visualize and study the animal cells
2. Used to visualize and study plant cells.
3. Used to visualize and study the morphologies of bacterial cells
4. Used to identify parasitic protozoans such as *Paramecium.*

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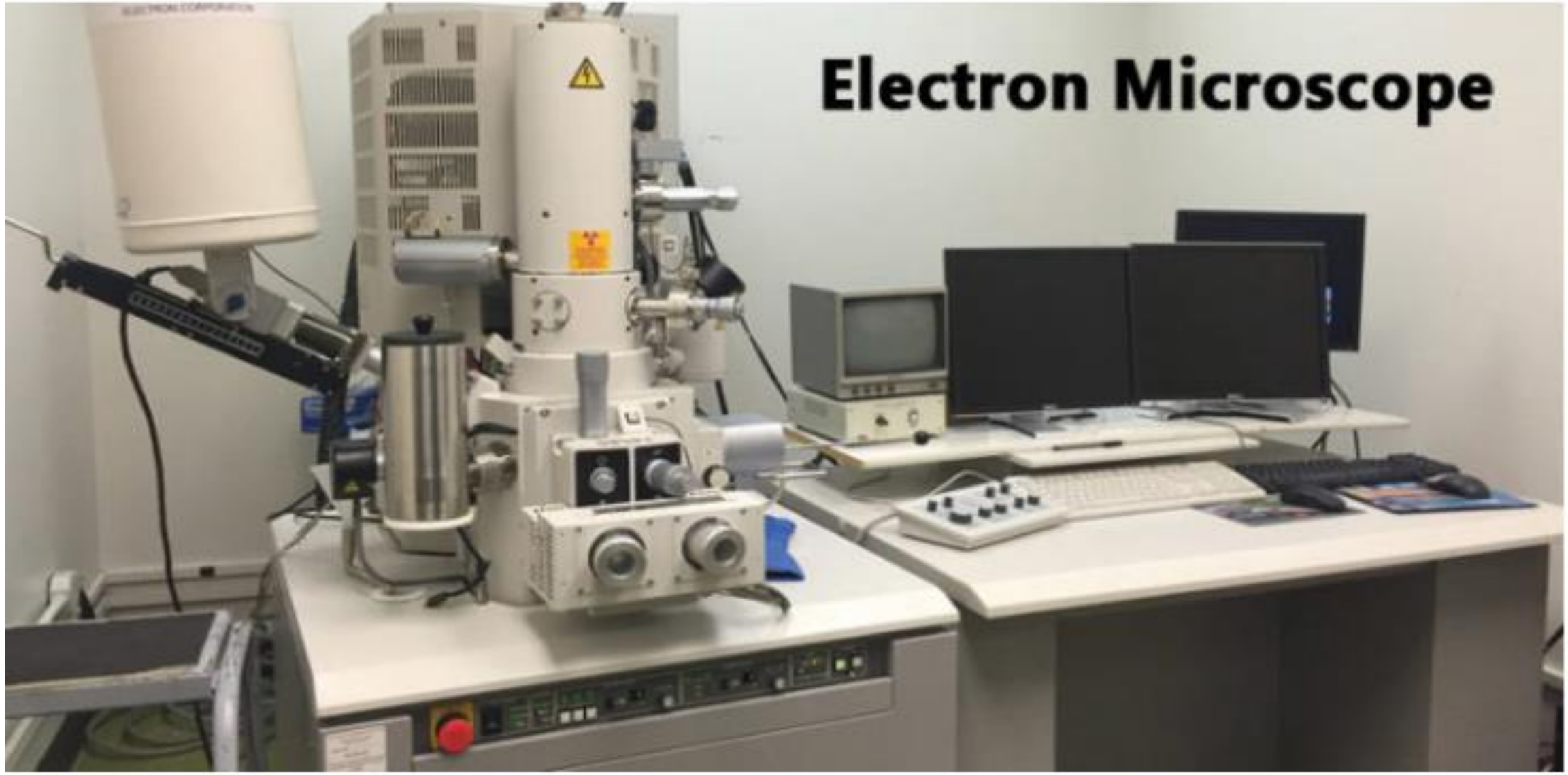
- **Electron microscope definition**
- An electron microscope is a microscope that uses a beam of accelerated electrons as a source of illumination.
- It is a special type of microscope having a high resolution of images, able to magnify objects in nanometres, which are formed by controlled use of electrons in vacuum captured on a phosphorescent screen.
- Ernst Ruska (1906-1988), a German engineer and academic professor, built the first Electron Microscope in 1931, and the same principles behind his prototype still govern modern EMs.


Electron Microscope

- **Working Principle of Electron microscope**

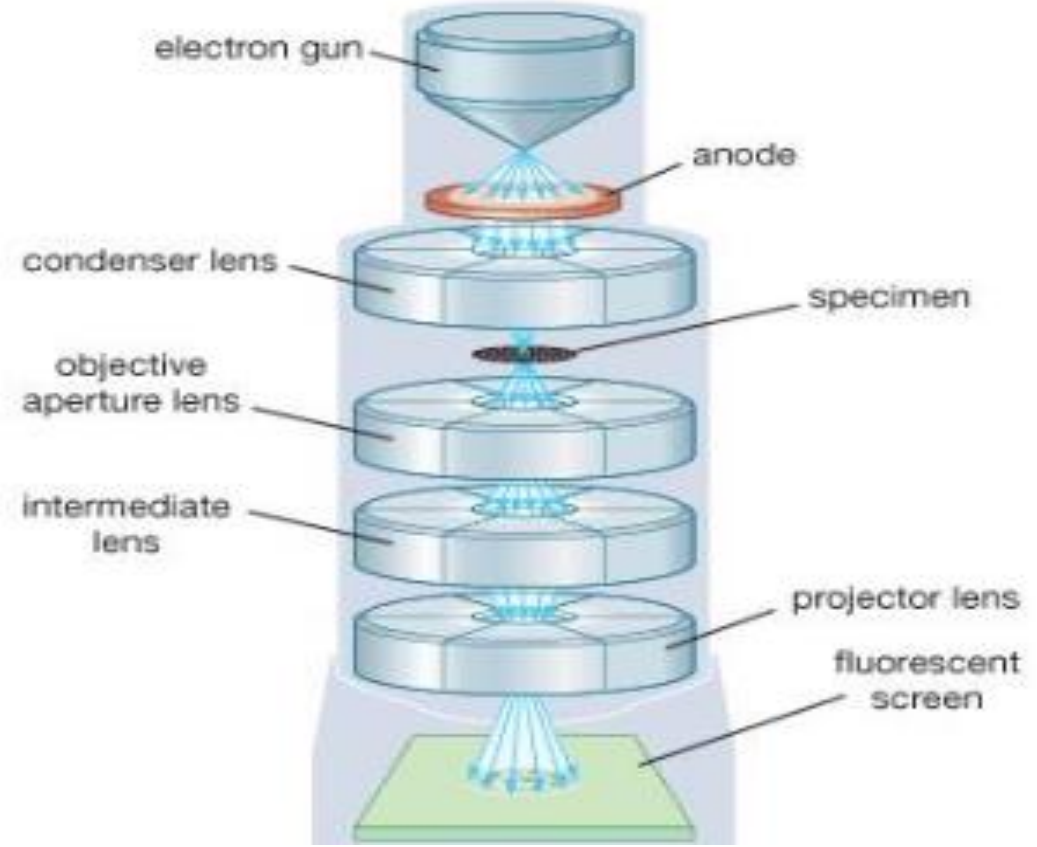
Electron microscopes use signals arising from the interaction of an electron beam with the sample to obtain information about structure, morphology, and composition.

1. The electron gun generates electrons.
2. Two sets of condenser lenses focus the electron beam on the specimen and then into a thin tight beam.
3. To move electrons down the column, an accelerating voltage (mostly between 100 kV-1000 kV) is applied between tungsten filament and anode.
4. The specimen to be examined is made extremely thin, at least 200 times thinner than those used in the optical microscope. Ultra-thin sections of 20-100 nm are cut which is already placed on the specimen holder.
5. The electronic beam passes through the specimen and electrons are scattered depending upon the thickness or refractive index of different parts of the specimen.
6. The denser regions in the specimen scatter more electrons and therefore appear darker in the image since fewer electrons strike that area of the screen. In contrast, transparent regions are brighter.
7. The electron beam coming out of the specimen passes to the objective lens, which has high power and forms the intermediate magnified image.
8. The ocular lenses then produce the final further magnified image.

- **Types of Electron microscope**

There are two types of electron microscopes, with different operating styles:

1. **The transmission electron microscope (TEM)**

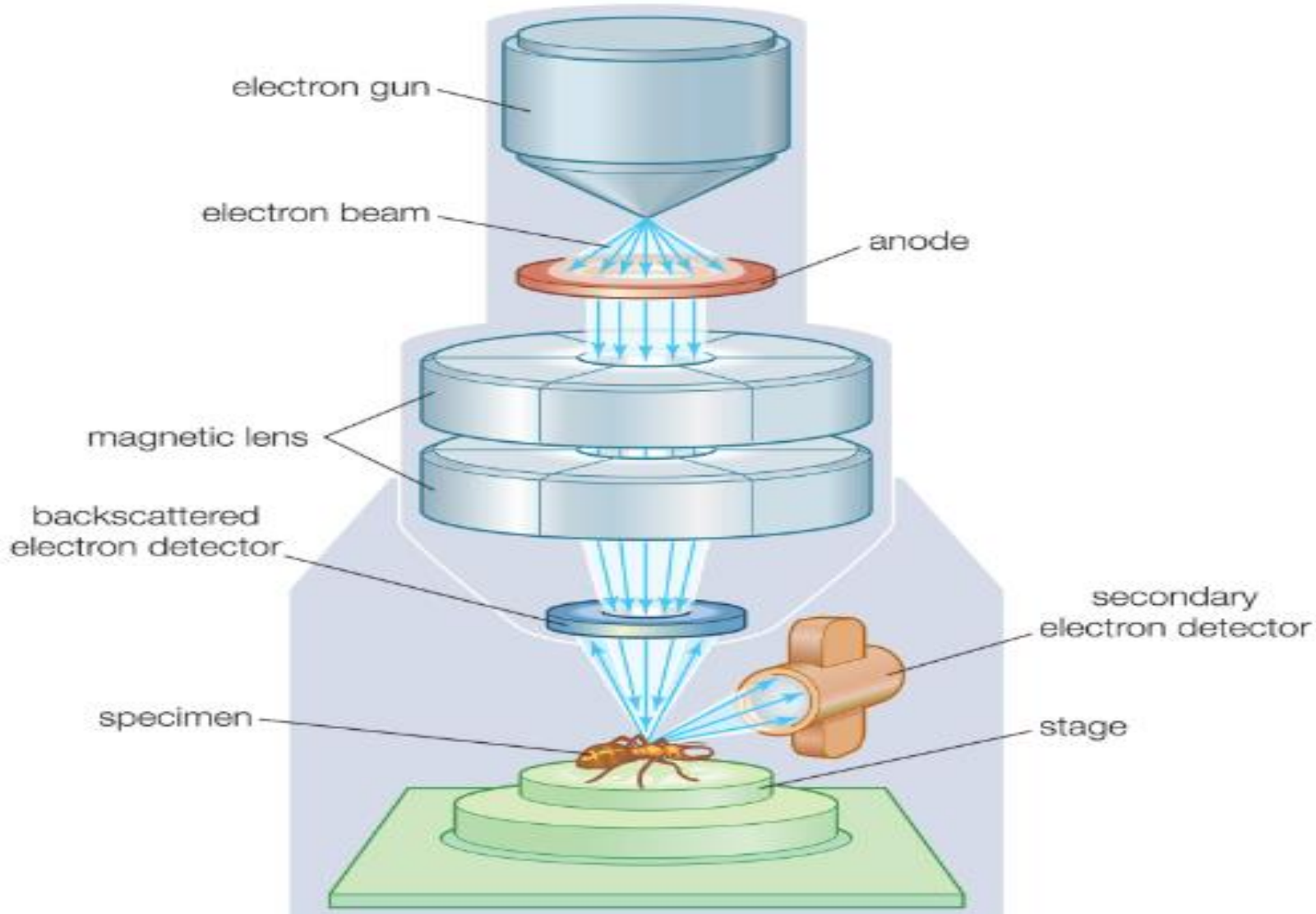


- The transmission electron microscope is used to view thin specimens through which electrons can pass generating a projection image.

- The TEM is analogous in many ways to the conventional (compound) light microscope.

- TEM is used, among other things, to image the interior of cells (in thin sections), the structure of protein molecules (contrasted by metal shadowing), the organization of molecules in viruses and cytoskeletal filaments (prepared by the negative staining technique), and the arrangement of protein molecules in cell membranes (by freeze-fracture).

2. The scanning electron microscope (SEM)



2.The scanning electron microscope (SEM)

- Conventional scanning electron microscopy depends on the emission of secondary electrons from the surface of a specimen.
- Because of its great depth of focus, a scanning electron microscope is the EM analog of a stereo light microscope.
- It provides detailed images of the surfaces of cells and whole organisms that are not possible by TEM. It can also be used for particle counting and size determination, and for process control.
- It is termed a scanning electron microscope because the image is formed by scanning a focused electron beam onto the surface of the specimen in a raster pattern.

- **Parts of Electron microscope**
- EM is in the form of a tall vacuum column which is vertically mounted. It has the following components:

1.Electron gun

- The electron gun is a heated tungsten filament, which generates electrons.

2.Electromagnetic lenses

- **Condenser lens** focuses the electron beam on the specimen. A second condenser lens forms the electrons into a thin tight beam.
- The electron beam coming out of the specimen passes down the second of magnetic coils called the **objective lens**, which has high power and forms the intermediate magnified image.
- The third set of magnetic lenses called **projector (ocular) lenses** produce the final further magnified image.
- Each of these lenses acts as an image magnifier all the while maintaining an incredible level of detail and resolution.

3.Specimen Holder

- The specimen holder is an extremely thin film of carbon or collodion held by a metal grid.

4.Image viewing and Recording System.

- The final image is projected on a fluorescent screen.
- Below the fluorescent screen is a camera for recording the image.

- **Applications**

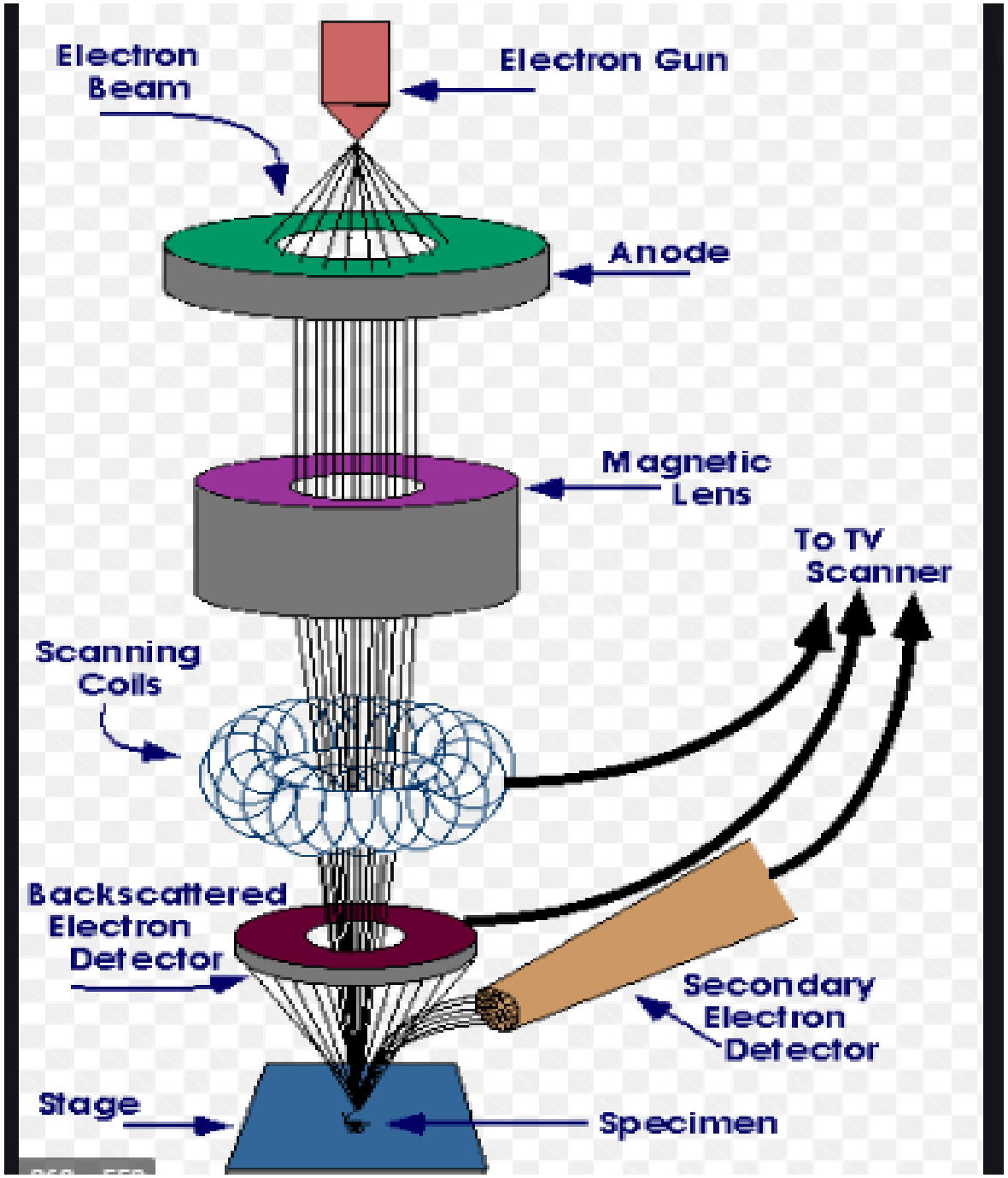
Electron microscopes are used to investigate the ultrastructure of a wide range of biological and inorganic specimens including microorganisms, cells, large molecules, biopsy samples, metals, and crystals.

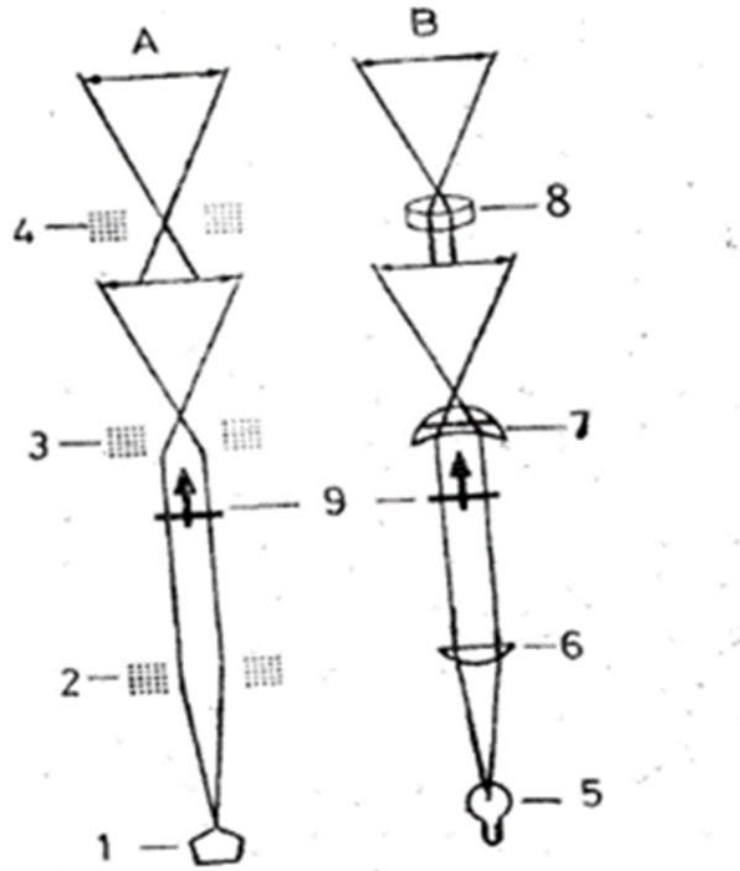
- Industrially, electron microscopes are often used for quality control and failure analysis.
- Modern electron microscopes produce electron micrographs using specialized digital cameras and frame grabbers to capture the images.
- Science of microbiology owes its development to the electron microscope. Study of microorganisms like bacteria, virus and other pathogens have made the treatment of diseases very effective.

- **Advantages**
- Very high magnification
- Incredibly high resolution
- Material rarely distorted by preparation
- It is possible to investigate a greater depth of field
- Diverse applications

LIMITATIONS

- The live specimen cannot be observed.
- As the penetration power of the electron beam is very low, the object should be ultra-thin. For this, the specimen is dried and cut into ultra-thin sections before observation.
- As the EM works in a vacuum, the specimen should be completely dry.
- Expensive to build and maintain
- Requiring researcher training
- Image artifacts resulting from specimen preparation.
- This type of microscope is a large, cumbersome extremely sensitive to vibration and external magnetic fields.


Electron Beam
Electron Gun
Anode
Magnetic Lens
To TV Scanner
Scanning Coils
Backscattered Electron Detector
Secondary Electron Detector
Stage
Specimen



படம் 11 – ஒளி மற்றும் லெக்ட்ரான் நுண்ணோக்கிகளின் ஒளிப் பாதைகள்

A-லெக்ட்ரான் நுண்ணோக்கி
1. லெக்ட்ரான் மூலம்
3. பொருளருகு லென்ஸ்
5. ஒளி மூலம்
7. பொருளருகு லென்ஸ்
9. பொருள்

B- ஒளி நுண்ணோக்கி
2. லெக்ட்ரான் குவியம்
4. நிறைப்பருதி
6. ஒளிக்குவியம்
8. கண்ணருகு பருதி