University of Al-Maarif

Medical Laboratory Techniques Department

Laboratory instruments

First stage

Lab(3)

Microscope

The Fluorescence Microscope

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4- The Fluorescence Microscope: -

Is an optical microscope that uses fluorescence to study properties of organic or inorganic substances.

Parts:

They are the same parts of the light microscope, but this microscope differs from the light microscope in that it contains several light filters, in addition to that the sample must be stained with fluorescent dyes.

The Fluorescence Microscope

Mercury arc lamp.

This lamp emits white light (with high energy and short wavelengths).

Excitation filter.

It is a type of bandpass filter, which passes the wavelengths absorbed by the fluorophore dyes.

Dichromic mirror.

It only passes small range of colors and reflects other colors.

Emission Filter.

It only passes those wavelengths are emitted from a fluorophore, it blocks all unwanted wavelengths, it creates a dark background.

Mercury arc lamp

Note: -

- The specimen must be stained with the fluorescent dyes.

Work Principle of the Fluorescence Microscope:

- Energy is absorbed by the atom which becomes excited.
- The electron jumps to a higher energy level.
- The electron returns back to its original position, and releases a photon (or beam of light).

How does the Fluorescence Microscope work?

1- Fluorescence microscope uses a high-intensity Mercury lamp as a Source of light. This lamp emits white light..

2- The exciter filter transmits only blue lights to the specimen and blocks out all other colors.

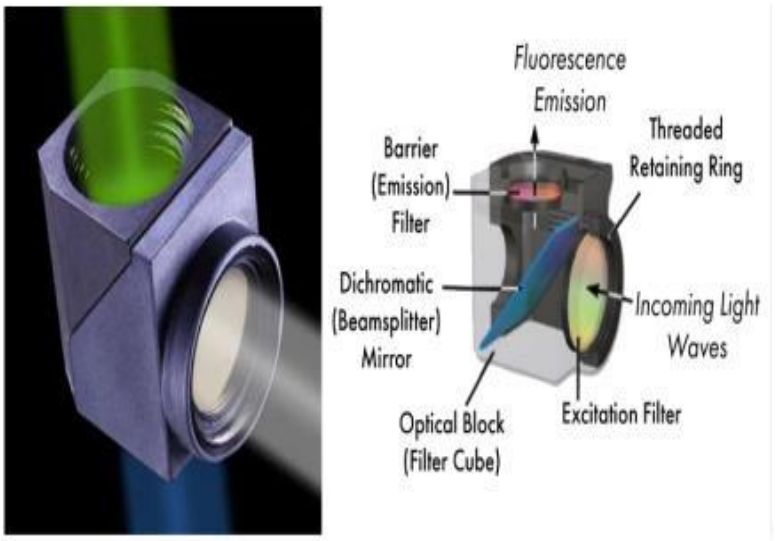
3- The blue light is reflected downward to the specimen by a dichroic mirror.

4- The specimen is stained with a fluorescent dye, certain portions of the specimen keep the dye but others do not.

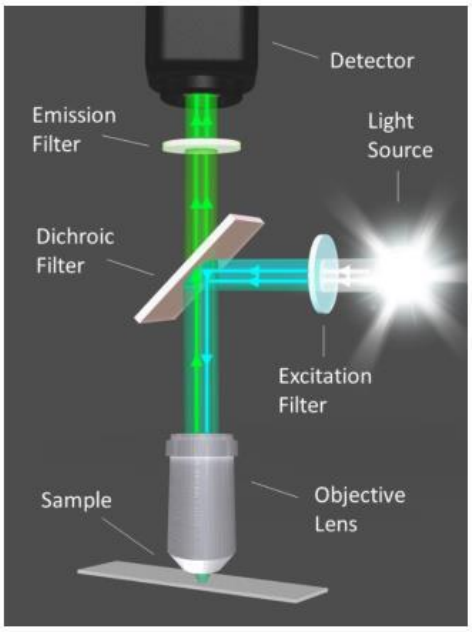
5- The stained portion absorbs blue light and emits green light, which passes upward penetrates the dichroic mirror and reaches the barrier filter.

6- This filter allows the green light to pass to the eye; however, it blocks out any residual blue lights from the specimen which may not have been completely deflected by the dichroic mirror.

7- Thus the eye perceives the stained portion of the specimen as glowing green against a black background whereas the unstained portion of the specimen is invisible.



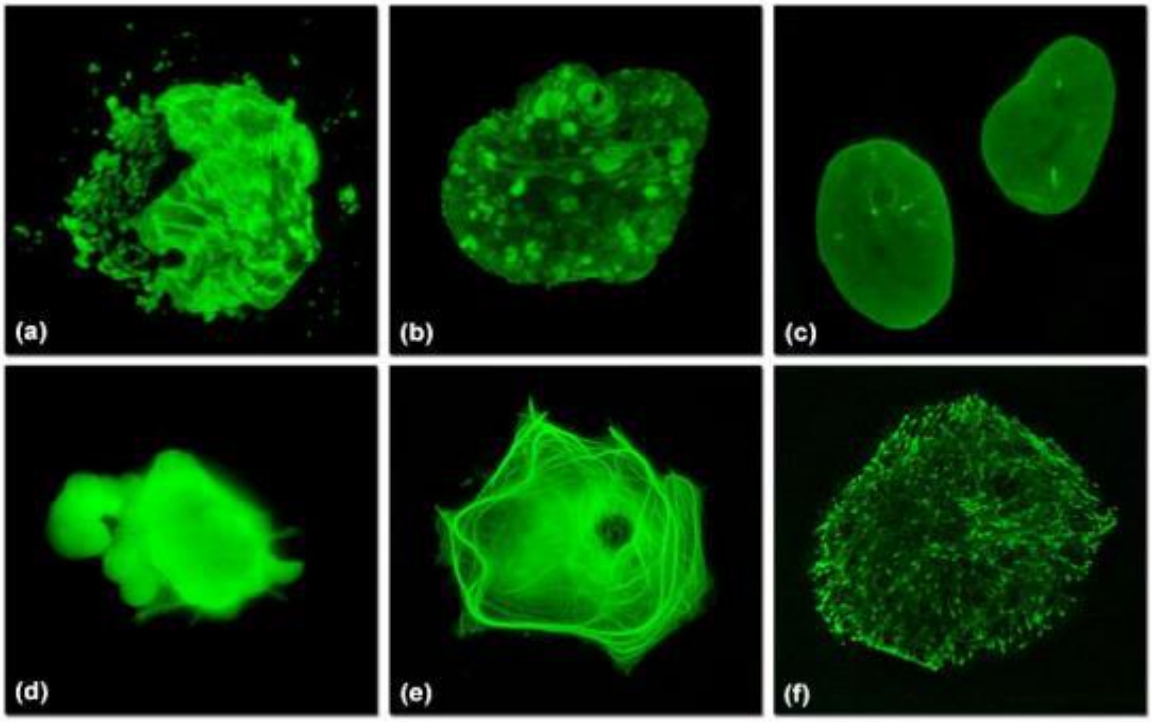
Anatomy of filtration cubic



Work Principle of the Fluorescence Microscope

Users:

- Detection of antigen- antibody reaction.
- Imaging structural components of small specimen, such as cells.
- Imaging the genetic material within a cell (DNA & RNA).
- It can trace the location of a specific protein in the cell.
- We can use it in Crime Investigation, Educational Field, and Medical Field.



Pictures under Fluorescence Microscope

Electron Microscope

A type of microscope that uses an electron beam instead of light beam to illuminate a specimen and produce an enlarged image.

- The electron microscope uses electrostatic and electromagnetic lenses in forming the image, light microscope uses glass lenses to focus light on or through a specimen to form an image

- Some electron microscopes can magnify specimens up to 2 million times, while the best light microscopes are limited to magnifications of 2000 time.

Electron microscope

❖ The greater resolution and magnification of the electron microscope is due to the wavelength of an electron, being much smaller than that of a light photon.

❖ It provides a unique view of the interior details of the specimens.

❖ It is much useful in studying virus, chromosomes, and components of cell.

Types of electron microscope:

There are two main types of electron microscope:

1 -Transmission Electron Microscope (TEM): which detects electrons that pass through a very thin specimen.

2- Scanning electron microscope (SEM): which uses the electrons that are reflected on the surface region of a sample to create an image.

Parts of electron microscope:

1) Electron gun: is the source of illumination (to produce streams of electrons (electron beams)).

2) Condenser lens: (electromagnetic lens) there are two condenser lenses set one below the other directing the electrons on the object placed below it, the electrons are scattered according to the thickness and density of various parts of the specimen.

3) Object chamber: a place of sample, the object is placed below the second condenser lens in the focal length of the beam on the object chamber.

4) Objective lens: (another electromagnetic lens), below the object, gathers the scattered electrons from the object, and focuses them to form a real primary magnified image.

5) Projector lens: magnify the primary and project the final image on a screen.

6) Fluorescent screen or photographic plate: it displays the final image.

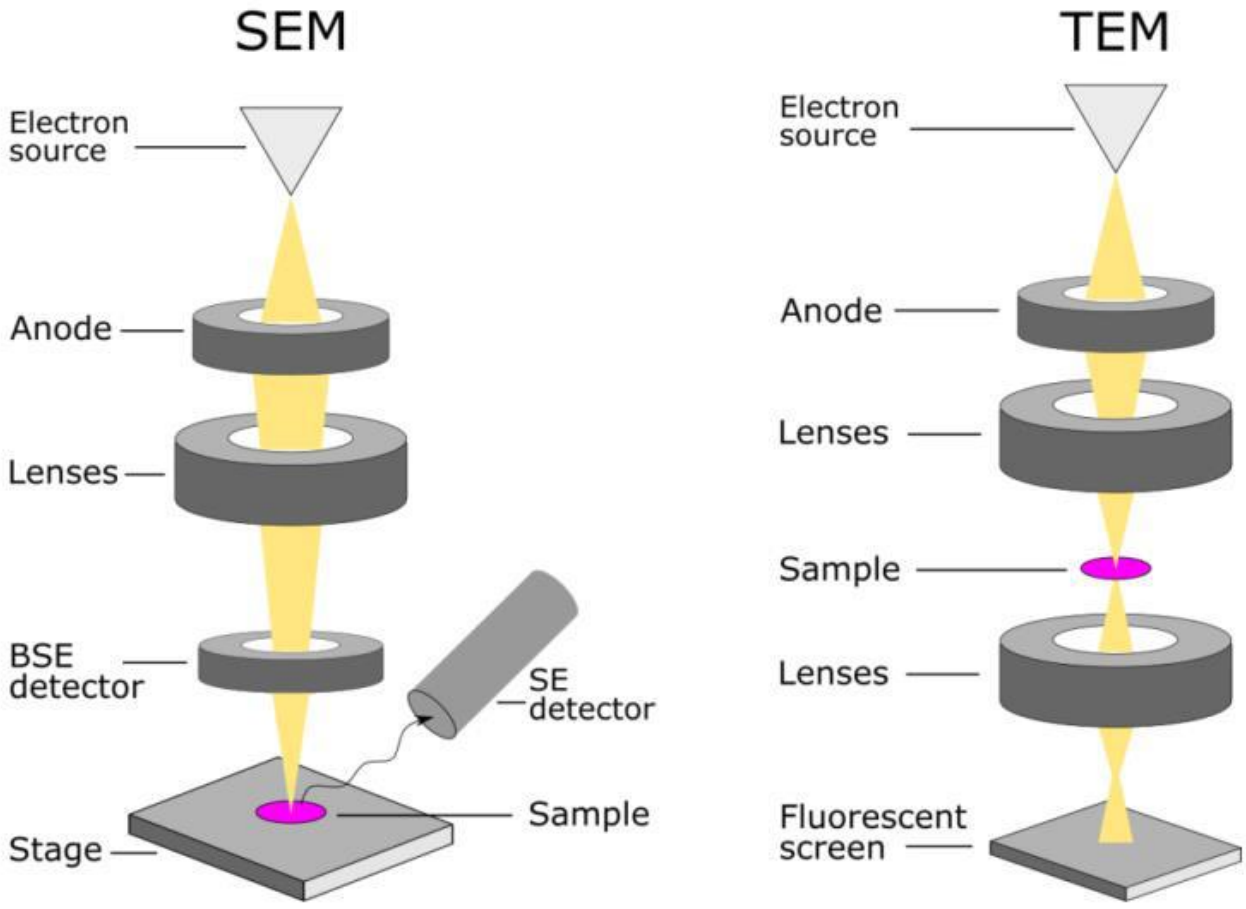
7) Vacuum stack: the electrons move only in the striated line in the vacuum.

How does EM work?

Electrons are particles orbit around the atomic nucleus. When atoms of a metal are excited by the external heat energy, electrons started to fly off from the atom.
In the electron microscopes, a tungsten metal is heated with the help of a high- voltage current, electrons form a continuous stream, which is used like a light beam. The lenses used in EM are magnetic coils capable of focusing the electron beam on the specimen and illuminating it.

Work principle of EM:

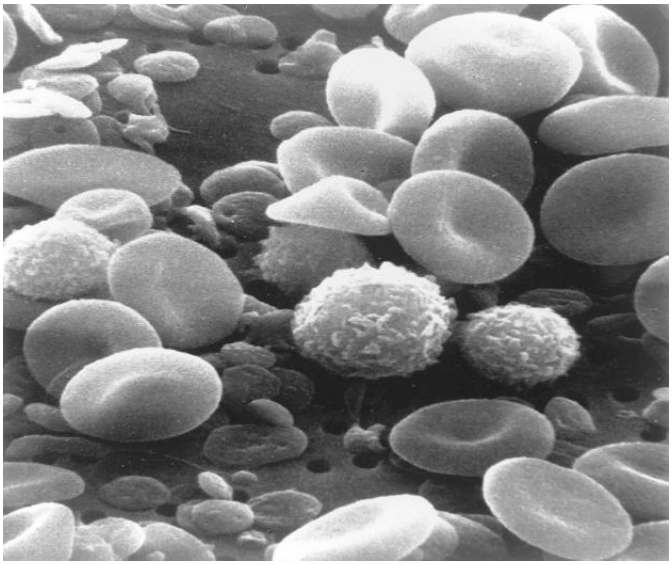
The electron beam falls on the sample, either it runs out, or it is reflected on its surface, and the image appears on a screen.

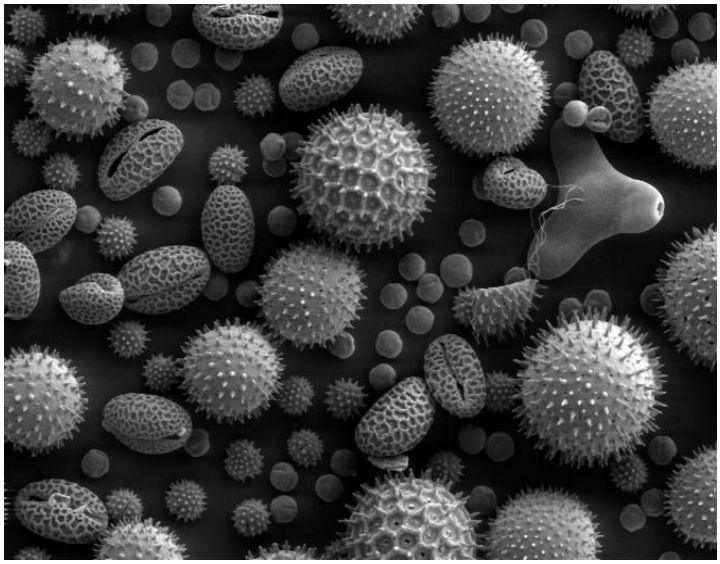


Work principle of EM

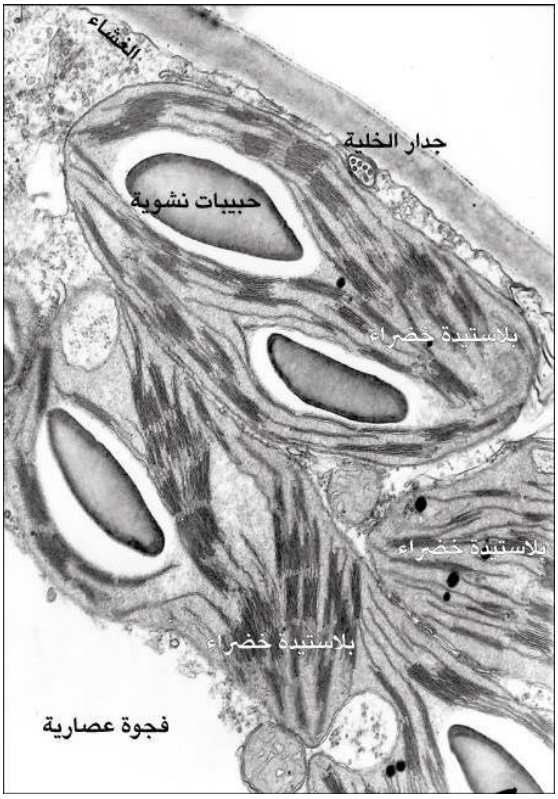
Comparison between light and electron microscope:

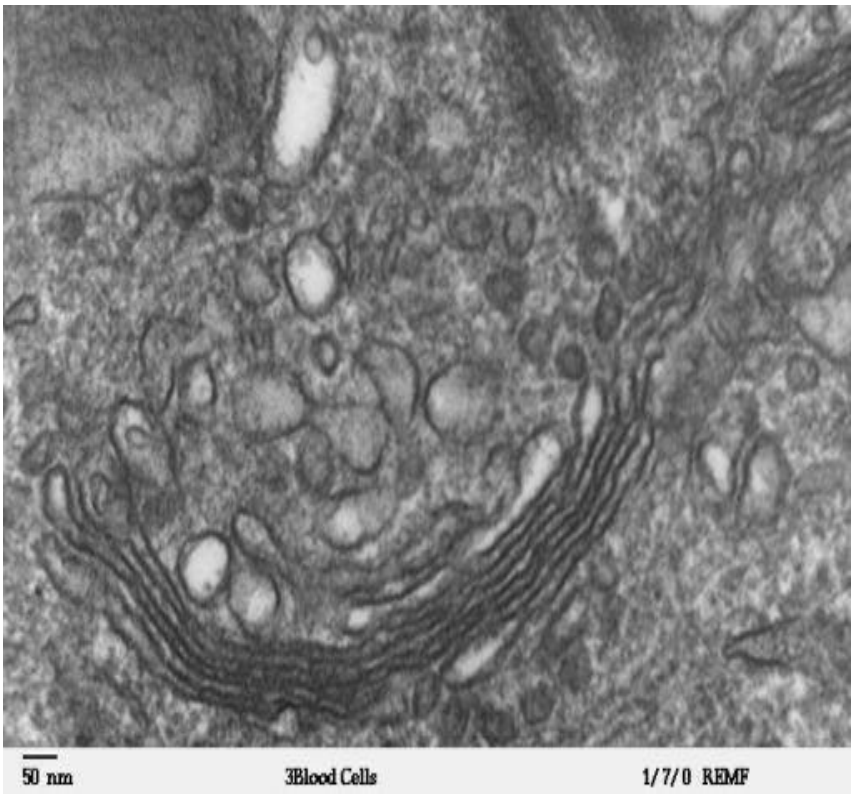
Light microscope	Electron microscope
Uses light rays to illuminate specimens**.**	Uses a beam of electrons to view specimens
Lenses are made of glass.	Lenses are made of electromagnets.
Lower resolving power.	Higher resolving power.
Low magnification.	High magnification.
Cheap to buy.	Very expensive to buy.
Images are viewed by the eyes through the eyepiece.	Images are viewed on a photographic plate or zinc sulphate fluorescent screen.
Not used under a vacuum.	used under a high vacuum.
Simple to use.	Users need technical skills.
Small size.	Large size.





Pictures under SEM





Pictures under TEM