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(CC- 6) : Unit -I, 1.2
Microscopy.

PRINCIPLES OF LIGHT MICROSCOPY

The light microscope is an instrument for visualizing fine detail of an object. It does this by creating a magnified image through the use of a series of glass lenses, which first focus a beam of light onto or through an object, and convex objective lenses to enlarge the image formed. In the majority of light microscopes, the image is viewed directly through binocular eyepieces that act as a secondary lens in the form of a magnifying glass to observe the projected image. Such instruments are termed 'compound microscopes,' and the total magnification is the sum of the objective magnification and the eyepiece magnification. The magnification range extends from ×10 to ×1000, with a resolving power of the order of 0.2 µm, depending on the type and numerical aperture (area available for passage of light) of the objective lenses.

For light microscopy, visible light is passed through the specimen and then through a series of lenses that bend the light in a manner that results in magnification of the organisms present in the specimen **(fig .A)**

IMPORTANT TERMS OF MICROSCOPY

- Magnification
- Working distance
- Resolving power
- Numerical aperture

1. Magnification

- Magnification is the enlargement of the image
- The degree of enlargement is called magnification or magnifying power of the instrument.
- The magnification is effected in two stages.
  - I. By the objective lens
  - II. By the eye-piece lens
    - Magnification of the microscope can be calculated by multiplying the magnification power of the objective by that of eye piece.
    - The magnifying ability is denoted by 'X' symbol

Generally used class microscope has following magnification-

	Magnification	Ocular lens	Total Magnification
Scanning	4X	10X	40X
Low Power	10X	10X	100X
High Power	40X	10X	400X

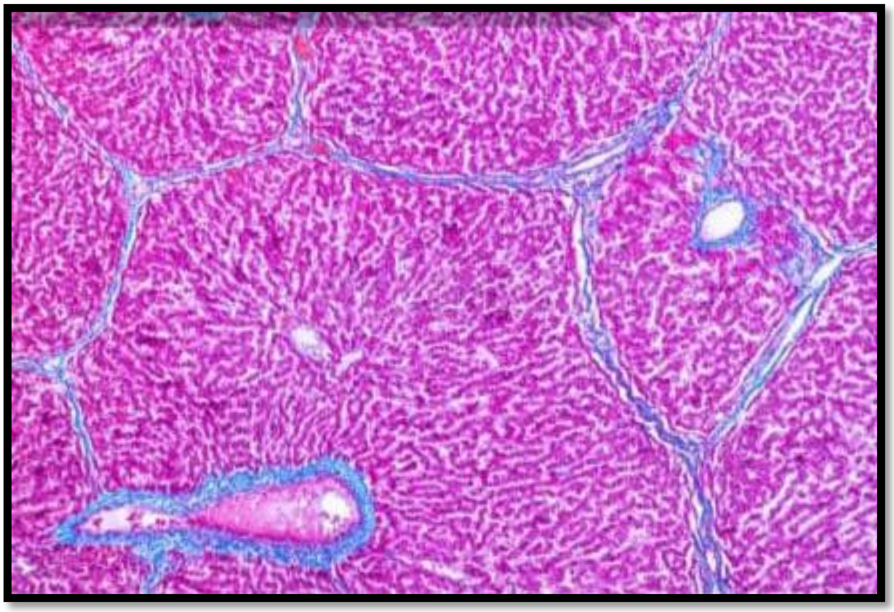


Fig A) : Liver tissue under light microscope.

2. Working distance

- Defined as the distance between the front lens of the objectibe & the object on the slide.
- Working distance easily determined by noting the number of complete turns of the microscope to raise the object from the surface of slide.

3. Resolving power

- It is defined as the ability to distinctly separate two small elements in the structure of an object that are a short distance apart.
- Resolving power is expressed quantitatively as the microscope's limit of resolution (LR).

4. **Numerical aperture (NA)**

- It sis defines as the simply diameter of the lens to its focal length.
- It is expressed mathematically as ..

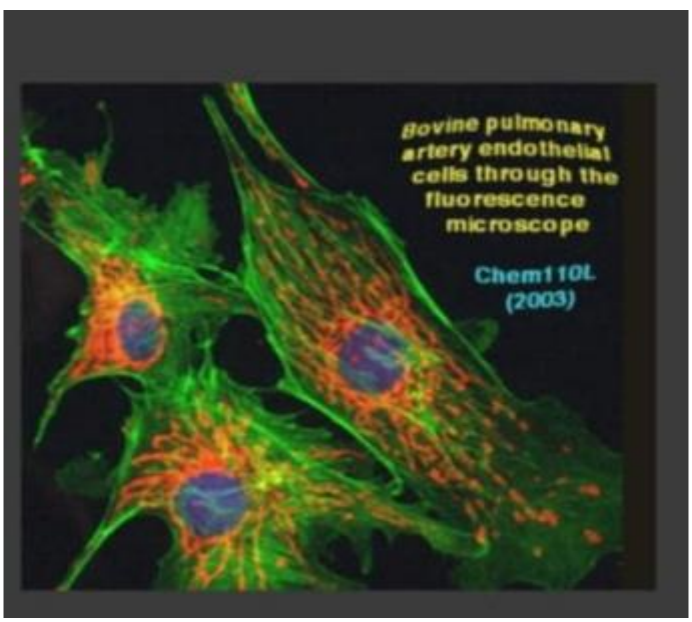
  NA = n sin Ø

  Where,

N = refractive index of the medium between object & objective

. Types of Light Microscopy :

A. Bright field microscopy.
B. Dark field microscopy.
C. Fluorescence microscopy.
D. Phase contrast microscopy.



Fig(B) cells under Fluorescence microscopy.

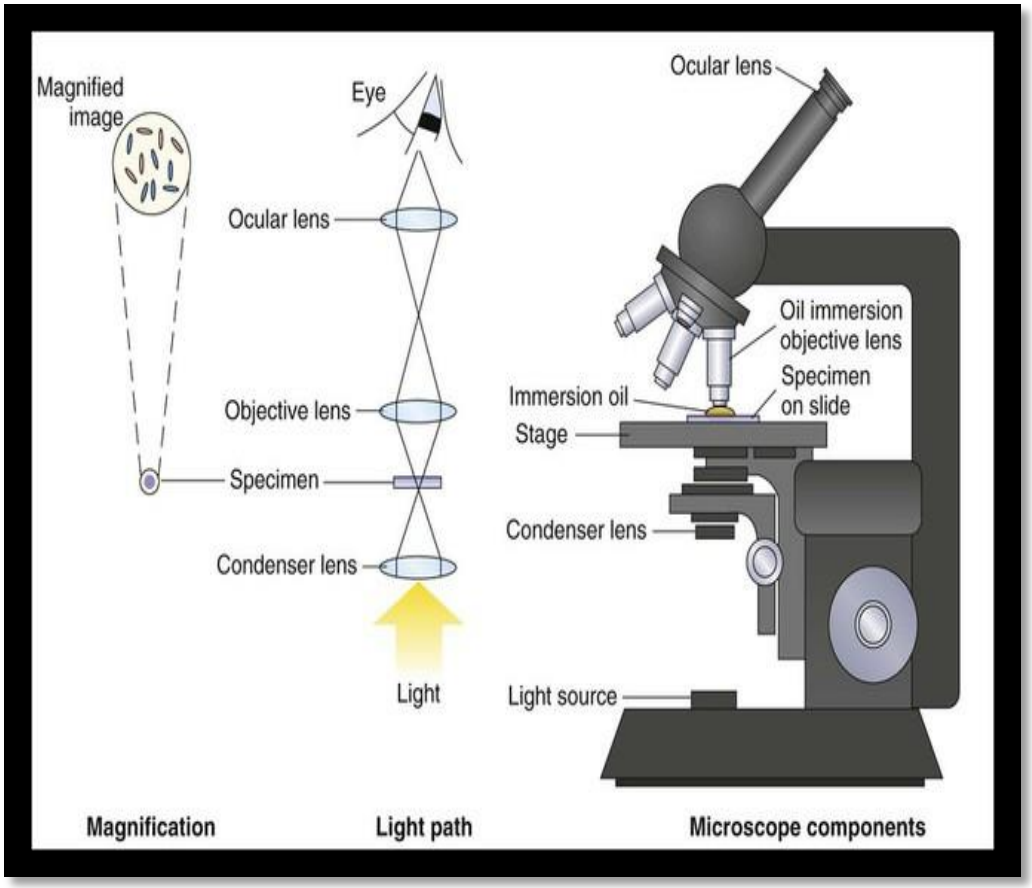


FIG (C) Principles of light microscopy.

The basic principle of light microscope is shown in the (fig : B)

Light is produced from either an internal or external light source and passes through the iris diaphragm, a hole variable size which controls the amount of light reaching the specimen.

- ❖ The light then passes through the condenser which focuses the light onto the specimen.

- The slide is held on the stage at 90 degrees to the path of light which next travels through the specimen.
- The objective lens magnifies the image of the specimen before the light travels through the barrel of the microscope.
- The light finally passes through the eye pieces lens and into the viewer's eye which sends impulses to the brain which in turn interprets the image.another and from background material and debris.

APPLICATION OF LIGHT MICROSCOPY :

- Light microscopes play an important role in many research laboratories.
- Biologists use microscopes to observe objects and details at a cellular level to learn more about the building blocks of all organisms.
- Microscopes are also used to observe real time movement in cells and organisms.
- Lastly, microscopes are used in biology to study diseases like cancer and AIDS to help diagnose the disease in patients and to help find a cure for them.
- Microscopes are used when studying light and optics to learn how light refracts through converging lenses and how a combination of different lenses with varying focal lengths affects the properties of the image.
- Microscopes are used in schools throughout north America as part of the technology section of the curriculum.
- Microscopes provide students with an understanding of real cells and their supporting structures.
- Also, microscopes provide students who are inclined towards the medical field a more intense look at the career choice and develop basic skills.
- Lastly, microscopes are used in forensics to help solve many crimes.
- Often times, there will be human evidences left on the crime scene.

- This allows forensic scientists to examine the evidence under a microscope and match the results with a database to find the culprit.
- Mineralogists also use light microscopy, typically with a special preparation of a sample called thin section.
- As the name implies, thin section are very thin slices of a rock. The sample needs to be thin enough for light to travel through from the light source to the user's eye.
- The thin section will allow the shape of different crystal grains to be seen.
- Light microscopes are very much an 'easy to learn, hard to master' type of equipment.
- The microscope can be used with different techniques, like epifluorescence and phase contrast.
