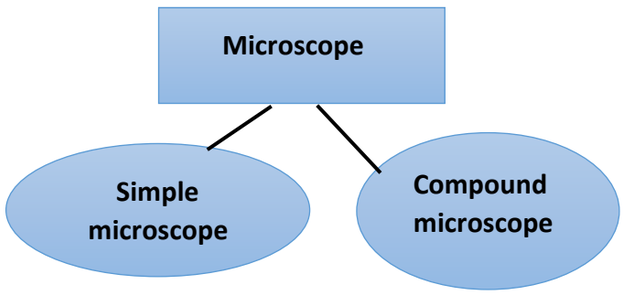
MICROSCOPY

A microscopy is an instrument used for viewing

Objects that are too small to be seen by naked eye.



1] Compound microscope:

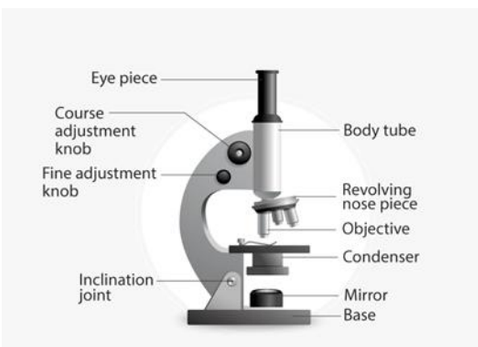


Fig.no.1 Compound microscope

Compound microscope is a high power of Microscope

That uses a compound lens system.

2] Simple microscope:

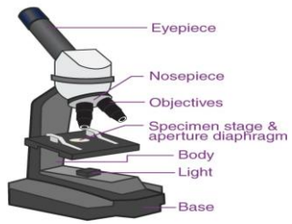
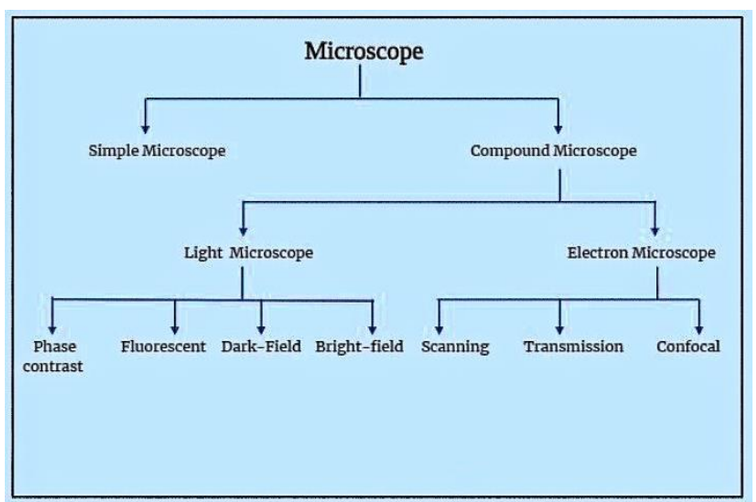


Fig.no.2 simple microscope

A simple microscope is a magnifying glasses that has

Double convex lens with short focal length.

Classification of Microscope:



Optical / Light Microscope-

1] Dark field microscope

- The microscopy which is forms bright images

against dark field microscopy .

-Many transparent and semi-transparent objective

Are not easily visible in bright field.

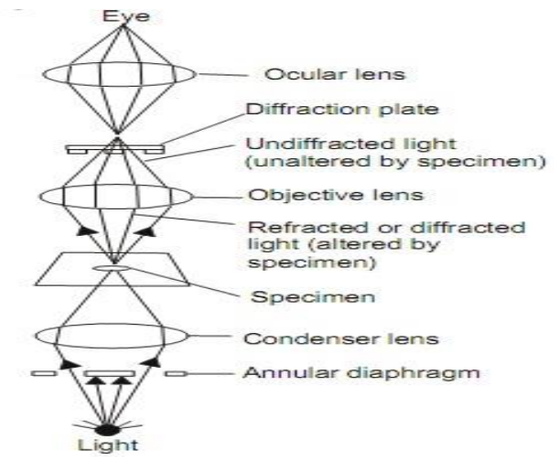


Fig no. 3 ray diagram

Principle:

A hollow cone of light focused on specimen in

Such way unreflected & unrefractedrays do not

Enter objective . the field surrounding specimens

Appears black while specimen itself brightly illuminatied

Requisites:

- Dark background condenser
- High intensities lamp
- Funnel stop which reduces numerical perture of objective to less than one.

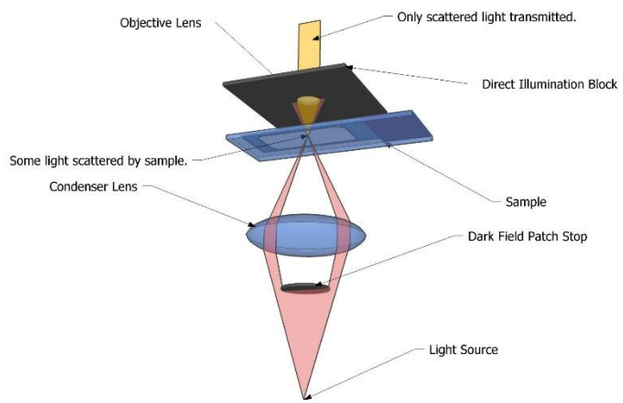


Fig no.4 Dark field microscope

Application:

- It is used for diagnosis of syphilis

Viewing bacterial algae & blood cells

a) Abbe condenser:

It is more commonly employed than other condenser because

it is suitable for objects that do not required highest

magnification to make them visible.

It may be employed either by inserting dark field stop below

Condenser oil substituting by dark field elements.

b) Parabolic condenser:

Parabolic condenser is designed to be used with oil immersion

Objective and intense source of light .

The specimen or object must be mounted in liquid or in

Centimeters and protected with cover slip.

The numerical aperture of objective must not be grater than

That of condenser.

C) Cardioid condenser:

It is best employed with strong arc lamp.

It designed for examination of colloid solution &suspension.

Advantages:

- It is ideal for viewing unstained transparent little absorbed objects.
- It is ideal study marine organisms such as diatoms , algae, planktons, etc.
- It is used for research on live bacterium mounted cells and tissues.
- It is used to examine external details like outer line , edges , grain, boundary, etc.

Disadvantages:

-The images are prone to degeneration and distortion.

-It needs an intense amount of light to work.

-If oil or water is used condenser then impossible to avoid air bubbles on slide

2] Phase contract microscopy

Fritz Zernike in 1953 has discovered this microscope.

-The basic construction of phase contrast microscopy is like a bright field microscopy expect type of condenser & phase plate.

-The condenser has a special diaphragm concentering annual

-The rays pass through objects in straight line are called as direct rays.

-The rays that are bend & slowed down to differences intensity of medium re call diffracted rays.

-Annular aperture in diaphragm placed in focal plane of sub-stage condenser control the illumination of object.

-Apparent brightness or darkness in image is proportional to square of amplitude of light waves.

-The image will be four times brighter or darker seen in bright field microscope . Hence it is possible visualizes micro-organisms without staining.

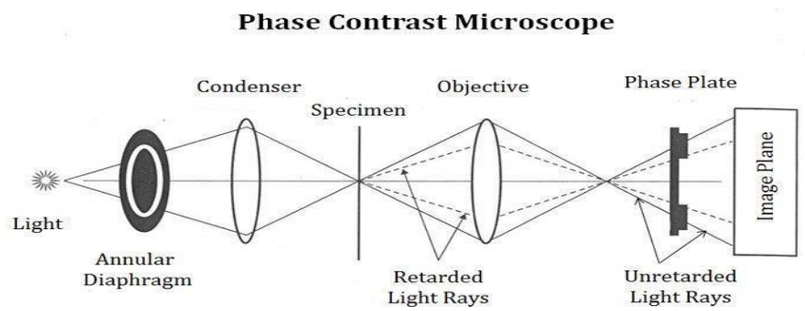


Fig no.5 phase contrast Microscope

Applications:

1) It enables visualization of living & unstained cells.
2) It helps to study cellular event like cell division, etc.
3) It helps to visualization of cellular moment.

Advantages:

1) It provides clear images of unstained cells.
2) It provides high contrast images of cells.
3) It cost is affordable.
4) It enhances prolong observation of living cells.

Disadvantages:

1) It produces bright halo surrounding image because of partial formation of direct & deviated rays.

2) It only effective to observers individual cells.

3] Bright field microscopy:

Bright-field microscopy (BF) is the simplest of all the

Optical microscopy illumination techniques. Sample

Illumination is transmitted white light, and contrast in

Living cells can be seen with bright-field microscopes.

The sample is caused by attenuation of the transmitted

Light in dense areas of the sample. Bright-field microscopy

Is the simplest of a range of techniques used for illumination

Of samples in light microscopes. The typical appearance of a bright-field

Microscopy image is a dark sample on a bright background.

Advantages:

1) Simplicity of setup with only basic equipment.

2) Living cells can be seen with bright-field microscope

Disadvantages:

1) Very low contrast of most biological samples.

2) Low apparatus optical resolution due to blur of out of focus material.

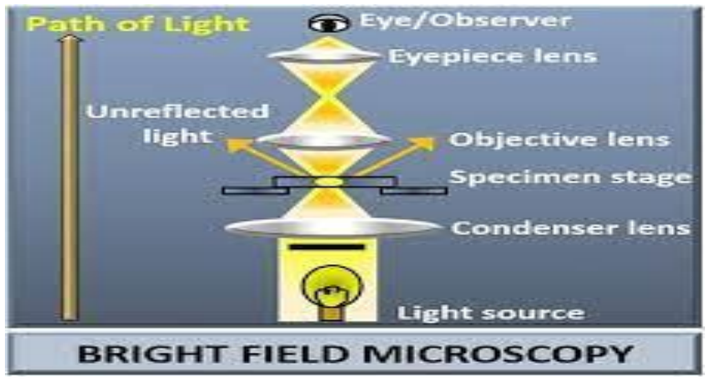


Fig no.6 Bright field microscopy

Fig.No..7 Steps in bright field microscopy

4] Fluorescence microscopy:

Fluorescence microscopy is a valuable toolbox to study

Cellular structures and dynamics spanning scales from

The single molecule to the live animal. The spatial resolution

That can be achieved with any light-based microscopy is

However limited to about 200 nm in the imaging plane

And >500 nm along the optical axis.

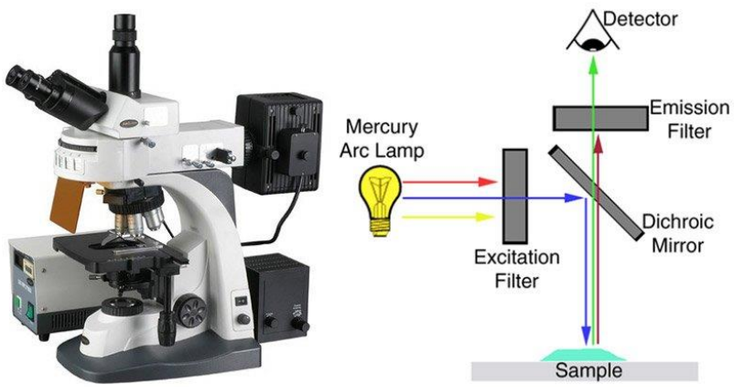


Fig no.8 Fluorescence microscopy

Principles:

Fluorescence microscopy is a technique whereby fluorescent substances

are Examined in **a** microscope It has a number of advantages

Applications

1) Imaging structural components of small specimen .
2) Imaging the genetic material within the cell.
3) Conducting viability studies on cell population .

Advantages:

1) Superior image clarity over Fluorescence microscopy
2) Can provide composites 3D images of samples
3) The sensitivity is high enough to detect as few 50 molecules per cubic micromete

Disadvantages:

1) Short life span of fluorophore.
2) Limitations associated with photostability .and loss of recognition capability
3) Biocompatibility issues due to local tissue trauma.

Electron microscopy-

Max Knoll & Ernest Ruska in 1931, developed the first electron microscope. It is highest resolution & magnification . The electron microscope works on principle similar to that of light microscope.

1] Transmission electron microscopy:

- Beam of electron is projected from electron gun and it is passed through series of electromagnetic lenses .

- They get scattered and Transmitted through objecive and pass through objecive lens which magnifies image of objects.

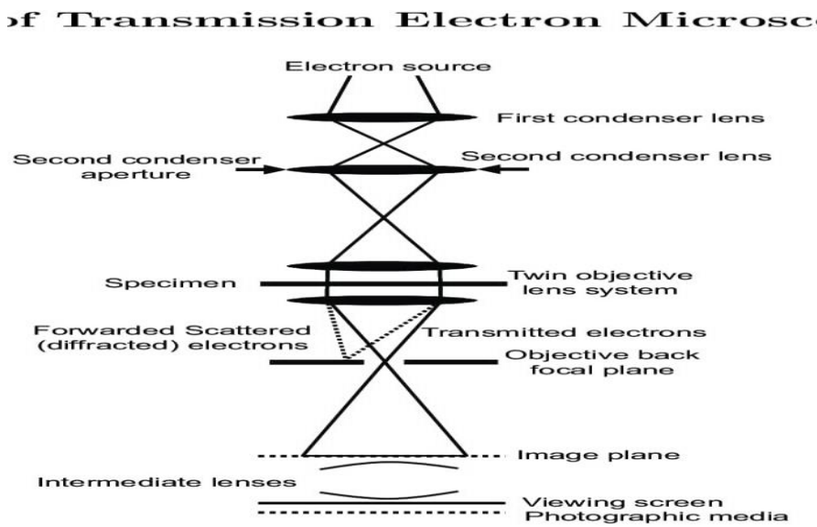


Fig No.9 Transmission electron microscopy.

2] Scanning electron microscopy

-The scanning electron microscope was built by Van Ardene in 1938

-SEM is microscopy technique that produces image sample by scanning with focused beam of electron

-The electron interact with atoms in sample and various signal that contains information about sample surface topography and composition

- SEM gives three dimensional view of an object.

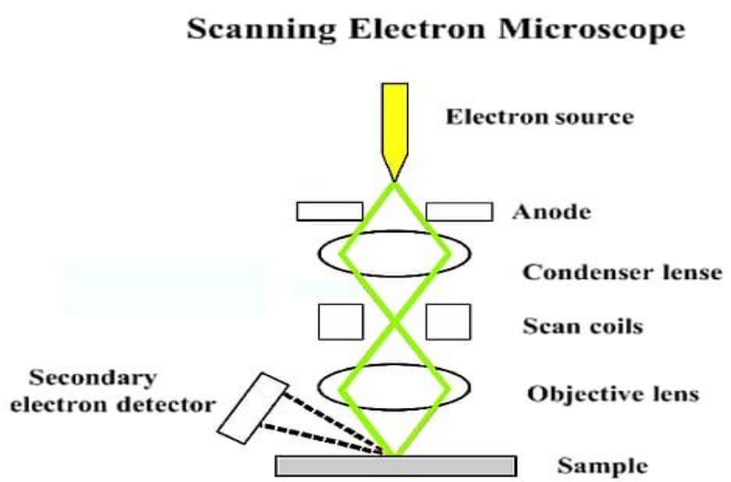


Fig no. 10 Scanning electron microscopy.

Limitations of electron microscopy:

1) Numerical aperture of electron microscopy lenses is very small
2) Drying process may be some morphological specimen being examined in chamber that is under very high vacuum.

References:

1] Dr. Chandrakant Kokare, Pharmaceutical Microbiology, Nirali Prakashan , 12th edition, Page no. 5.1-5.12

2] Gerard J. Tortora, Pharmaceutical Microbiology, 5th edition, page no 13-20

3] Vijay Tahilyani, Pharmaceutical Microbiology, text book, page no 107-125.

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