PROJECT ON

ADVANCED MICROSCOPY

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MICROSCOPY

Microbiology is concerned with the study of microorganism-As microorganism are invisibles to naked eye, certain tools are required to study microorganisms. A microscope therefore is the most important tool for microbiologist which enables one to see microorganism.

Antony van leeuwenhoek was the first person to see microorganism, he made 250 microscopes during his life time which can produce 200-300 time larger image.

After leeuwenhoek many scientist contributed & suggested different modification in microscope.

There are various types of microscopes. The

1) Simple microscope :-

The microscope having only one magnifying lence is called simple microscope. The microscope discovered by Leeuwenhoek is the Example of simple microscope.

2) Compound microscope :-

The microscope having more than one magnifying lense is called compound microscope. The microscope which we use in lab is example of compound microscope.

- Microscope
  - Light microscope
    - Bright field microscope
    - Dark field microscope
    - phase contrast microscope
    - Fluoresence Microscope
  - Electron microscope.
    - Transmission electron microscope
    - Scanning electron microscope.

Defination, principle, construction, working and use of compound Bright field microscope :-

Difinition :- Microscope is an optical device / instrument consisting of a series of lenses and used for magnification of the image of object.

The microscope having more than one magnifying lenso called compound microscope and In this microscopy the micro- pic field is brightly lighted hence also called as bright field microscope

Principle :-

In compound microscope magnified image of object is first produced by objective lense and this image is Furthe enlarged by second lense to give still more highly magnified image. The total magnification obtained by microsope is the magnification obtained by its objective lense multiplied by magnification obtained by eyepiece.

Total magnification of microscope = magnification obtained by objective lense × magnification obtain by eyepiece.

Construction and working of microscope :-

Compound microscope is designed by using some mechanic and optical parts. Each part has its specific function.

1) Base or Foot :-

Foot or base supports to all parts of microscope It is heavy so resist vibration. Foot has various shapes particularly horse shoe shaped is most common.

2) Pillar :-

It is upward portion of base at which arm of microscope joined.

3) Inclination joint :-

It is point where are a pillars are joined. inclination joint is useful for adjusting inclined angle of microscope in favour of observer.

4) Arm :- Body of microscope is attached to arm and joined through pillar. Arm is useful for lifting the microscope.

5) Coarse adjustment screw :- It moves body tube up & down rapidly, so as to adjust the distance between the object and eye of observer

6) Fine adjustment screw :- It moves body-tube up & down very slowly & gives very accurate focusing of object.

) Body tube :- The body tube supports objective lense at it's lower end and eyepiece at it's upper end.

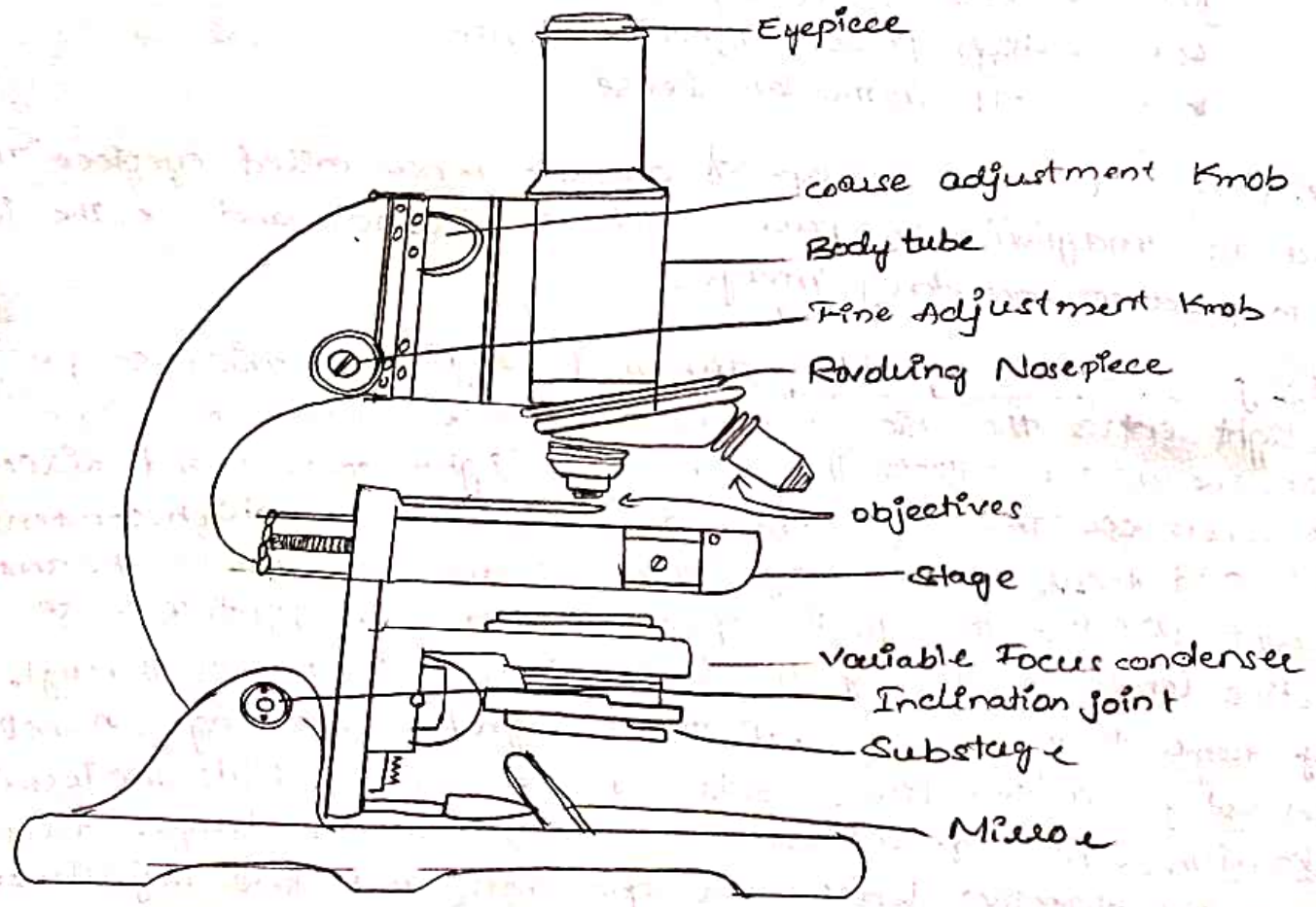
Draw tube :- Draw tube is attached to body tube. In draw tube eye piece at it's upper end. is placed loosely.

) Revolving Nose piece :- It holds various objective lenses to allow any of objective lense to bring quickly into position.

) Stage :- The object which is to be observe is placed on flat platform is called stage. The object is placed Exactly below the objective lense. At the centre of stage, there is a hole for light to pass upward from mirror through condenser.

) Mechanical stage :- It holds, Slide & moves it along stage slowly & Smoothly by rotation by two knobs for each direction.

) Iris diphragam :- It can be opened & closed by using a small projection as per the requirement of light. It controls rays of light passing through condenser & coming from mirror.



Compound microscope.

14) Condenser adjustment screw :- It is used to adjust light of conden along with iris diaphragam by moving up & down light is adjusted at point where image finds more clear.

B. Optical parts.

1) mirror :- Below the condenser & iris diaphragam mirror is adjust It is circular & loosely mounted so that it can be turned at any direction.

It reflects light from light source to object through diaphragm and condenser. There is plane mirror & concave mirror placed ba to back.

2) Condenser :- As name indicates, it condenses light into pencil shap cone & prevent escape of light rays. It also controls light in

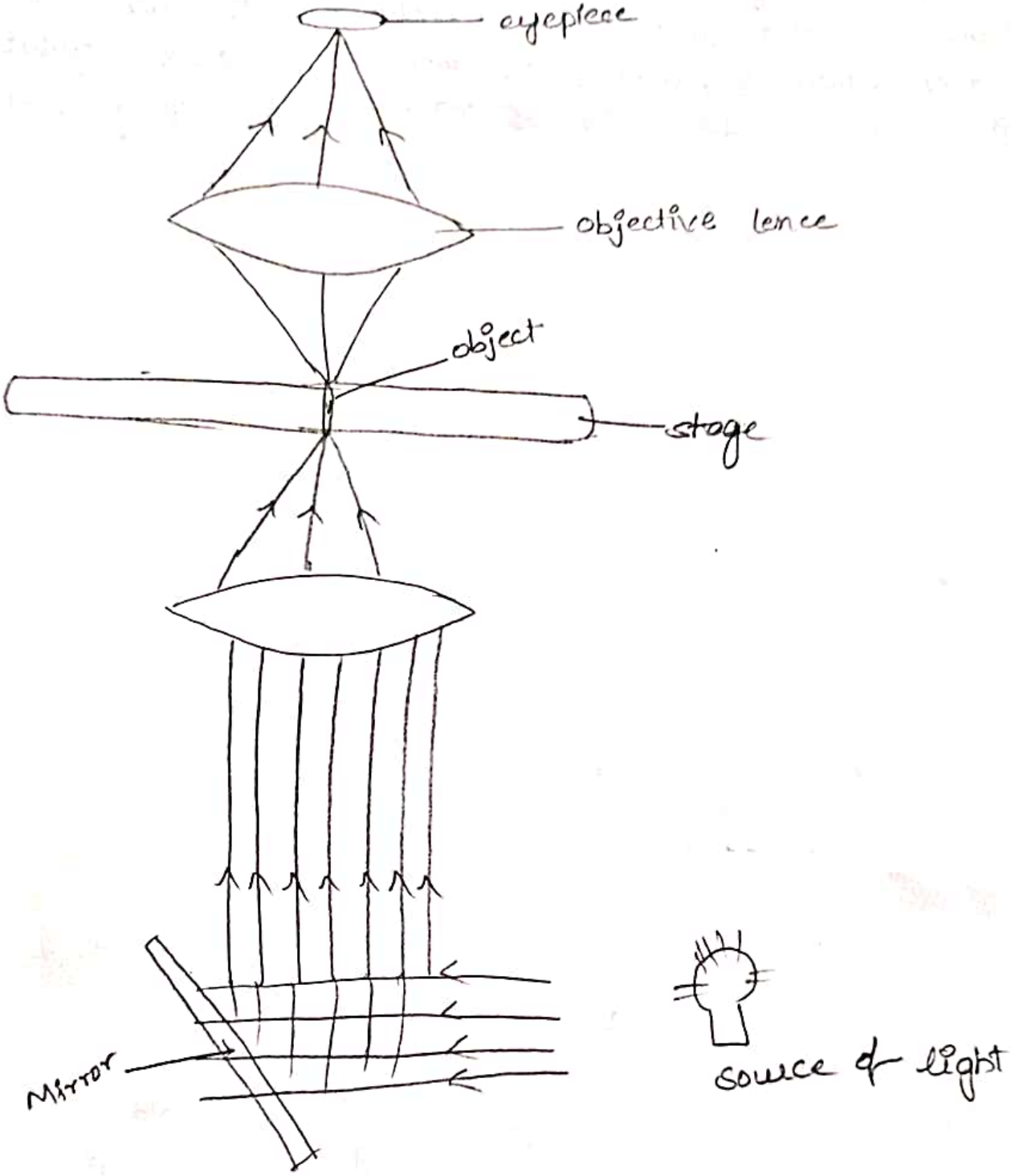
3) Objective lense :- As these lenses are present near the object it is called objective lense. It is of various magnification power such a

10X = lower power objective lense
40X = High power objective lense
100X = oil immersion lense.

4) Eye piece :- It is near the eye of observer hence called eyepiece It i of various magnification power such as 5X, 10X and 15X. The fund is to produce secondary image.

Working of microscope (Ray digram of Bright field microscope)

Light enters the microscope from a source. concave or plane mirror. The mirror collects the light from light sources and direct it to condencer. The light then goes to condencer which condense light and focus on the image. The iris diagram control the amount of light passing through the specimen and into objective lense. The objective lense magnifing the image before it passes through body tube to the eyepiece. Further magnifies the image. A mecha -nical stage allows Pieces control of moving the slide. The focusing mechanism consist of a course adjustment which changes distance between the objective lense and specimen and fine adjustment changes the distance slowly. The course adjustment is used to locate the specimen, the fine adjustment is used to bring it in sharp focus of specimen.



Working of microscope Ray Digram of microscope.

PHASE CONTRAST MICROSCOPE

Phase contrast microscopy is a method for controlling contrast in the image so that unstained living cells and cytological organelles within them become visible. In unstained preparation it is difficult to see organisms and its internal structure. This is because there is very little contrast in between object and surrounding field. This situation is almost like trying to see a fragment of ice in a glass of water.

Principle:

The objects having little difference in their refractive index could be made visible or more clear if this difference is enhanced by optical means. Enhancement of this difference to create a contrast is made by using phase shifting elements to change the phase of incident light rays.

Construction and working:

Ordinary light microscope can be equipped for phase contrast microscope with two modifications.

1) A special anular diaphragm.
2) Phase shifting disc.

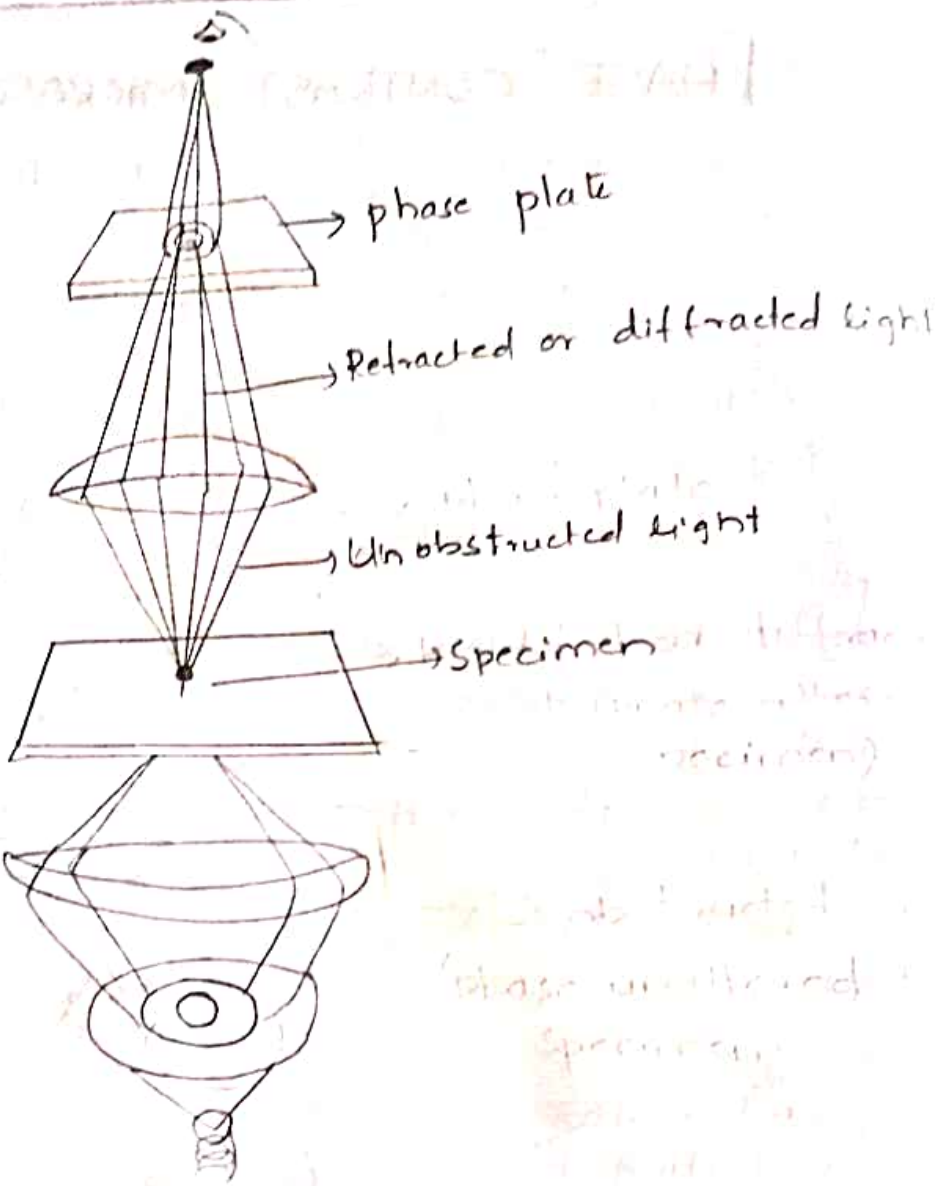
1) A special anular diaphragm: This diaphragm placed below the condenser. It permits only ring of light to pass upward through condenser and objective lense.

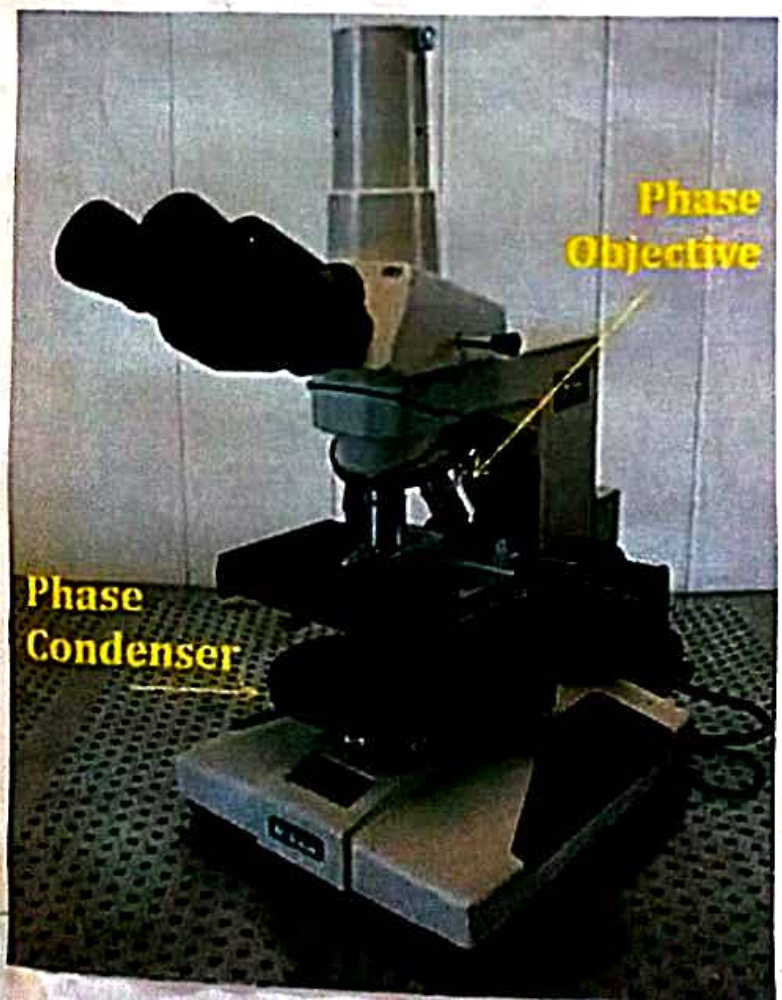
2) Phase shifting disc: Inside objective lense phase shifting disc is placed. This has ring of optical dielectric material on this dielectric material ring of light from anular diaphragm is focused. The dielectric material has property of retarding and advancing the wavelength of light passing through it and produce contrast between image and back ground.

Uses:

i) Small unstained specimens such as a living cell can be seen.
ii) It makes highly transparent objects more visible.
iii) Examining Intracellular components of living cells at relatively high resolution.

Ex: The dynamic motility of Mitochondria.


phase plate
Retracted or diffracted light
Unobstructed light
Specimen


Phase
Objective
Phase
Condenser

DARK FIELD MICROSCOPE

Principle: Microorganisms are transparent or semi transparent hence they scatter light rays which are not directly entering in the objective lense and becomes visible i.e., in dark field.

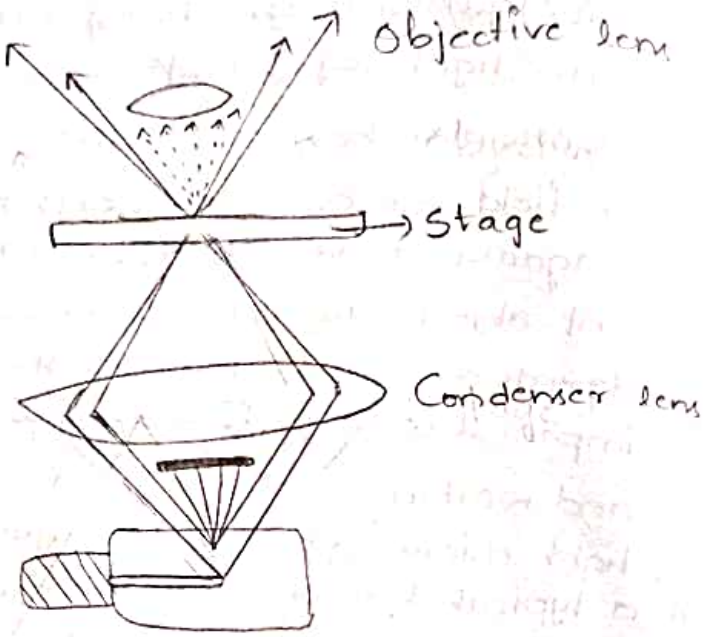
In the dark field microscope the object which is to be observed is illuminated against a dark backgraund. Many transparent or semi transparent objects are not readily visible in bright field. visibility is depends on contrast between the object and its backgraund and can be improved using a dark backgraund.

Construction and working:

Ordinary light microscope can be equiped for dark field micro-scope with a typical type of 'dark field stop' placed below the condenser. The dark field stop prevents the entry of rays of light the rays falling on the slide do not reach the eye. unless some object is present to reflect the light upward. The empty field appears dark. If transparent objects are placed on the slide there will be scattering of light by reflection and refraction. The scattered light will enter into objective lense and object will appear bright otherwise microscopic field is dark.

Uses:

i) Dark field microscope is particularly used for examination of unstained microorganisms suspended in field.
ii) For observing motility of micro-organisms by hanging drop technique.
iii) Viewing blood cells
iv) Viewing bacteria and different types of algae
v) Viewing other invertebrates.


Objective lens
Stage
Condenser lens

FLUORESCENT MICROSCOPE

Principle:-

Fluorescent is the property of specific substance such substances emits rays having wavelength of different value from that of incident rays. Substances having this property is called fluorescent substance.

Advantage of this property of substance is taken in fluorescent microscope. objects which are not seen by normal microscope with visible light rays are made with visible by staining them with fluorescent stain. Fluorescent stain above U.V rays emit light rays of visible wavelength and thus object becomes visible, which otherwise could become non-visible.

Construction and working:-

Fluorescent microscope can be made by following modification in simple light microscope.

1) U.V lamp:-

As a source of UV light, UV lamp is used

2) Reflector:-

It reflects the U.V rays upward in the diaphragm condenser.

3) Collecter:-

It collects UV rays.

4) Excitation filter:-

U.V lamp when emits rays of different wavelength which is not neccessary, in such condition excitation filter, filters out all unwanted rays & only U.V rays having specific wavelength are allowed to pass through filter

5) Barrier Filter:-

It is placed in body tube which prevents the U.V rays coming to the eye & protect the eye from U.V rays.

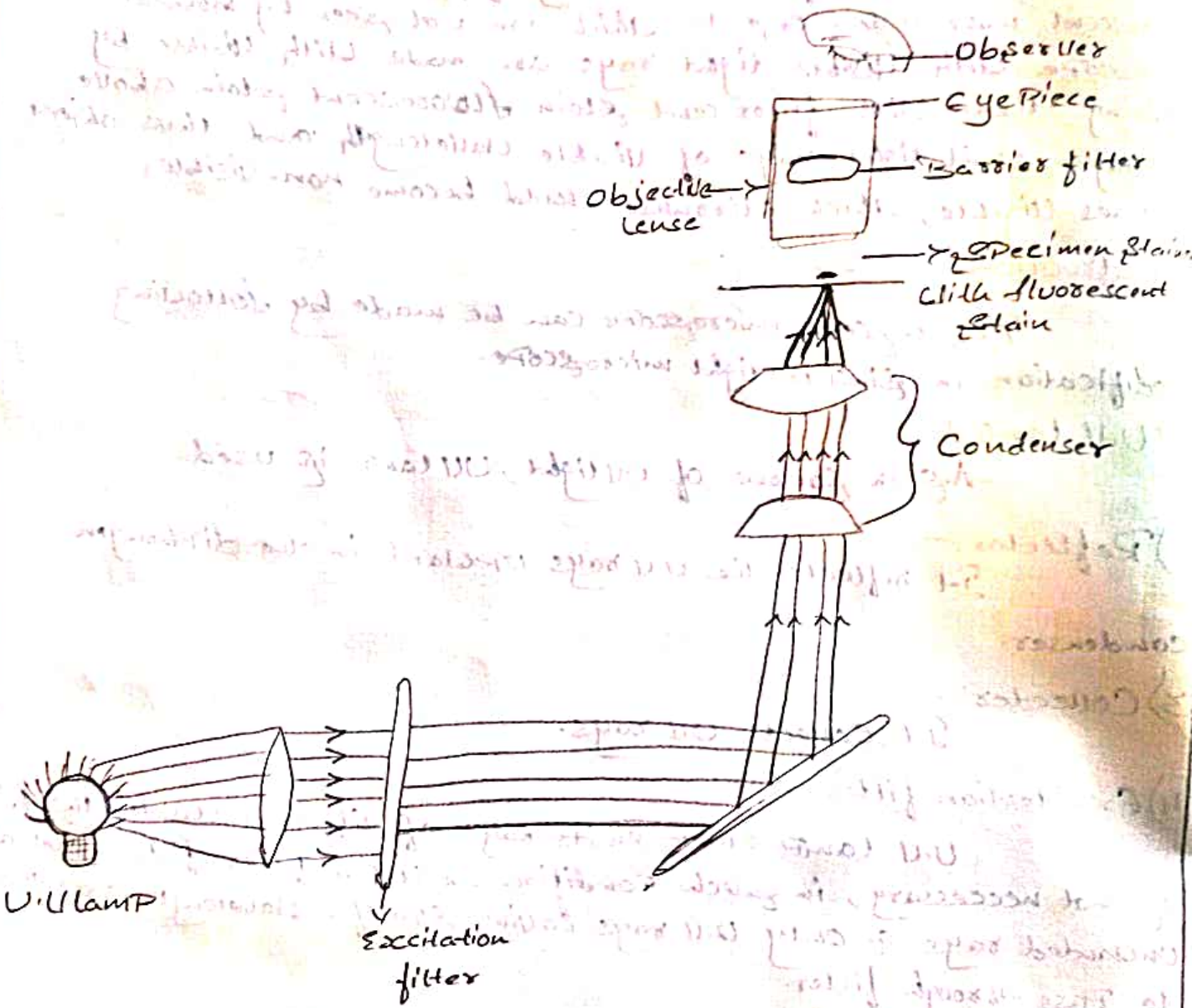
6) Fluoresent stain:-

Microorganisms that are stained with fluorescent dye/ stain will appear luminous object when observed by light microscope with U.V illumination This is not possible by ordinary stain.

Uses:-

=> Most important application of fluorescent microscope is for detection of tubercle bacilli, Fluorescent stain has a strong affinity towards tubercle bacilli

=> This microscope is also useful for fluorescent antibody technique which is used for identification of either antigen/antibody.



ELECTRON MICROSCOPE :-

Electron microscope was first constructed by Ernst Ruska and max knoll in 1931, Resolving Power of light microscope is 200nm(0.2u), when it is required to observe viruses or Parts of cell with diameter less than 200nm, it is necessary to use the electron microscope. As the resolving power of electron microscope is 0.2nm. Therefore, it makes possible to observe very Fine details.

Principle:- In electron beam high speed of electrons are used instead of visible. For the Passage of electrons, the tube of electron microscope is evacuated. The electron beam is focussed with electromagnetic lenses. Electron microscope has high resolving Power because Electron beam has a very small wavelength about 0.005nm on one lakh time shorter then the wavelength of visible light object forms image in electron microscope because its solid content scatters electron and so casts a shadow in electron beam. Electrons are diffracted from the way of Propogation of Magnetic field and that circular magnetic field focus electron beam and forms image.

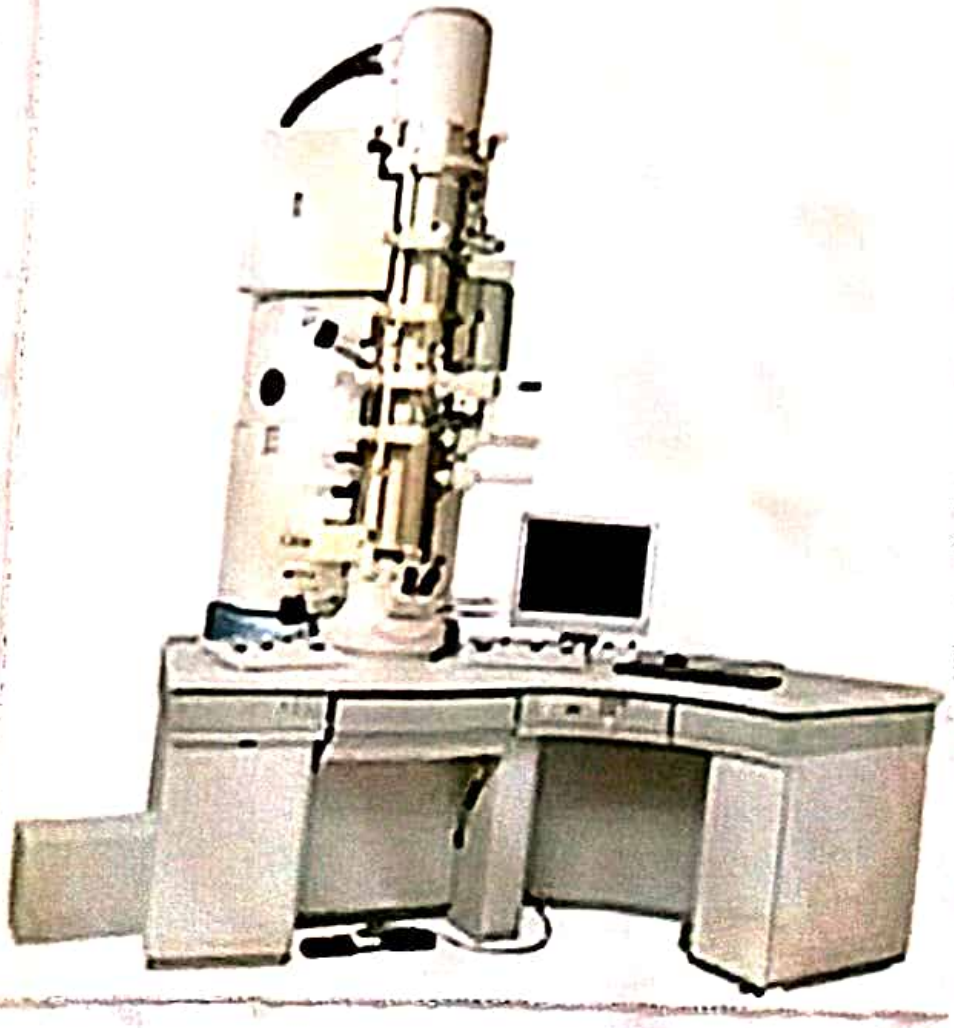
Construction:- Electron Microscope consists of tubular column at the top of which source of electron called electron gun is mounted. Election gun emitts electron from a hot tungston filament. Tungston filament act as cathod. Below this filament cathod shield is placed in its centre anode is placed with a small hole. A high voltage is Applied b/w cathode and Anode to accelerate the emitted electron

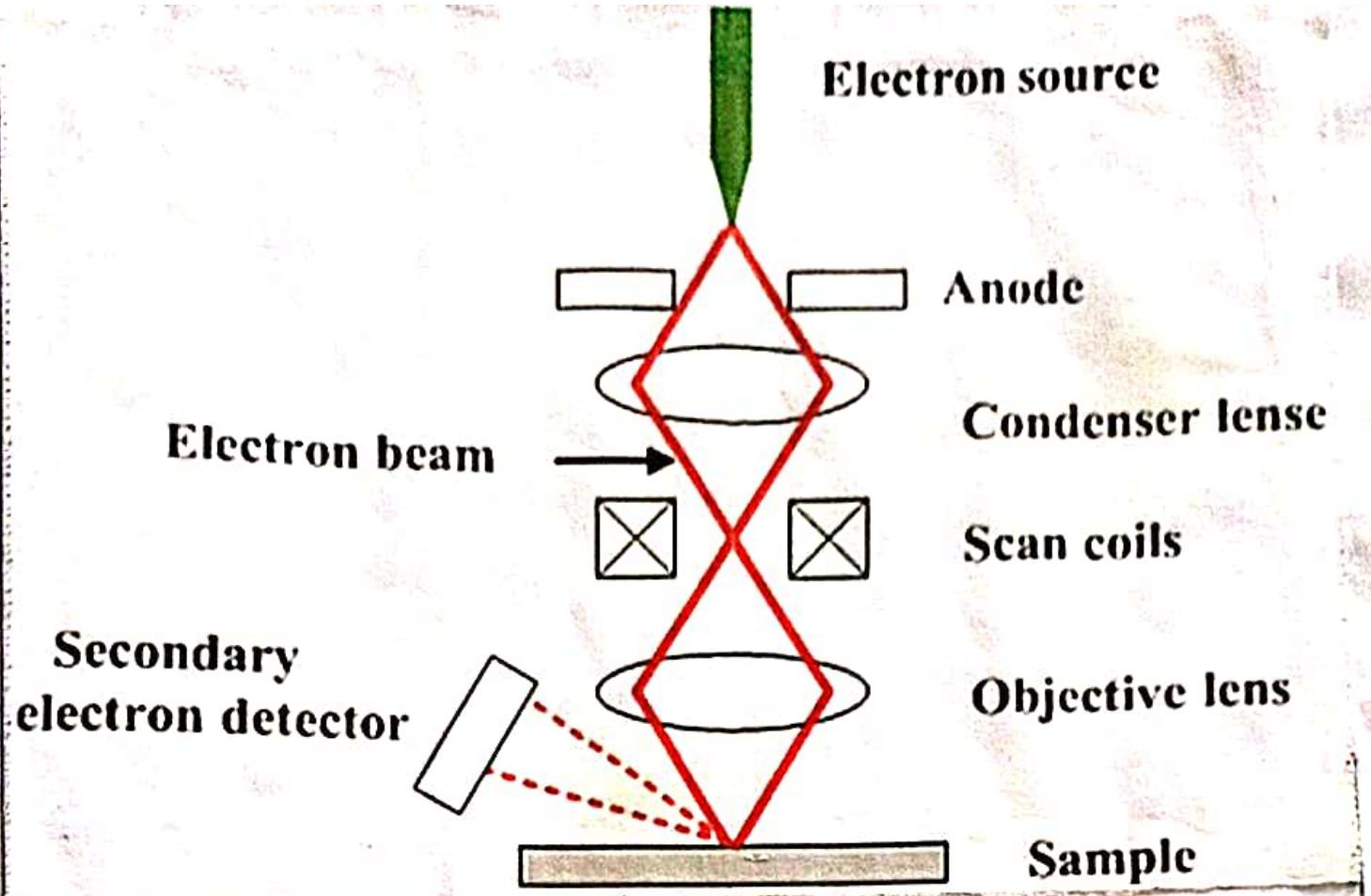
A narrow beam of electrons passes with high speed through the hole in the anode downwards in column in microscope.

Focussing and Magnification is achieved with a series

of lense which are electromagnates producing magnetic field. This is magnetic field condenser lense system which Focuses the beam of electron on specimen by concentrating the beam of the scattered electron pass through small objective aperture and are focused by objective lense to form a real Primary Image.

Two projector lenses which have the function of eyepiece further magnifiese the Primary image to 1000 times. The final image is formed on fluroscent screen at lower end of column and can be observed through glass window. This image can be taken on Photographic plate on camera below it.

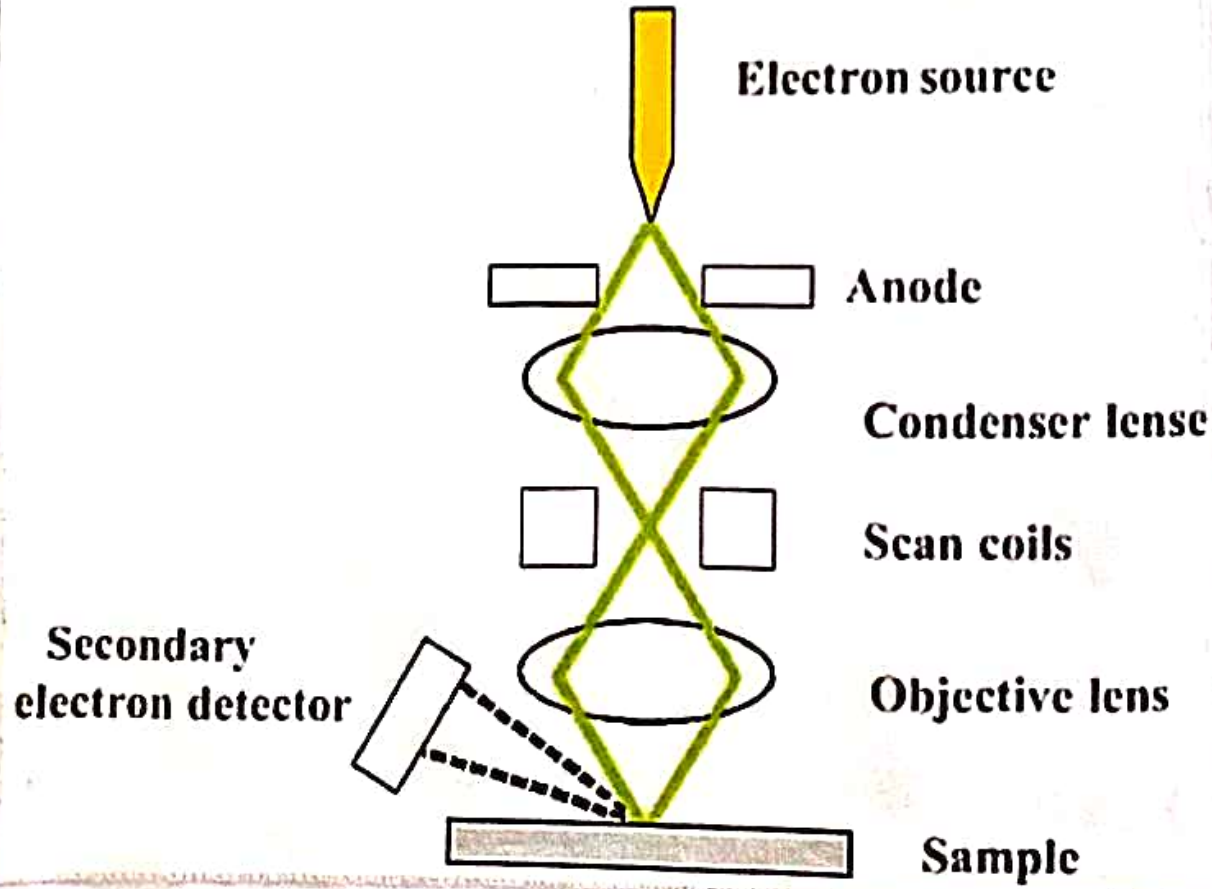




Types of Electron Microscopes:-

1) Scanning Electron Microscope:- In a scanning Electron microscope (or) SEM, a beam of electrons scans the surface of a sample. The electrons interact with the material in a way that triggers the emission of secondary electrons. The secondary electrons are captured by a detector, which forms an image of the surface of the sample. The direction of the emission of the secondary electrons depends on the orientation of the features of the surface. There, the image formed will reflect the characteristic feature of the region of the surface that was exposed to the electron beam.

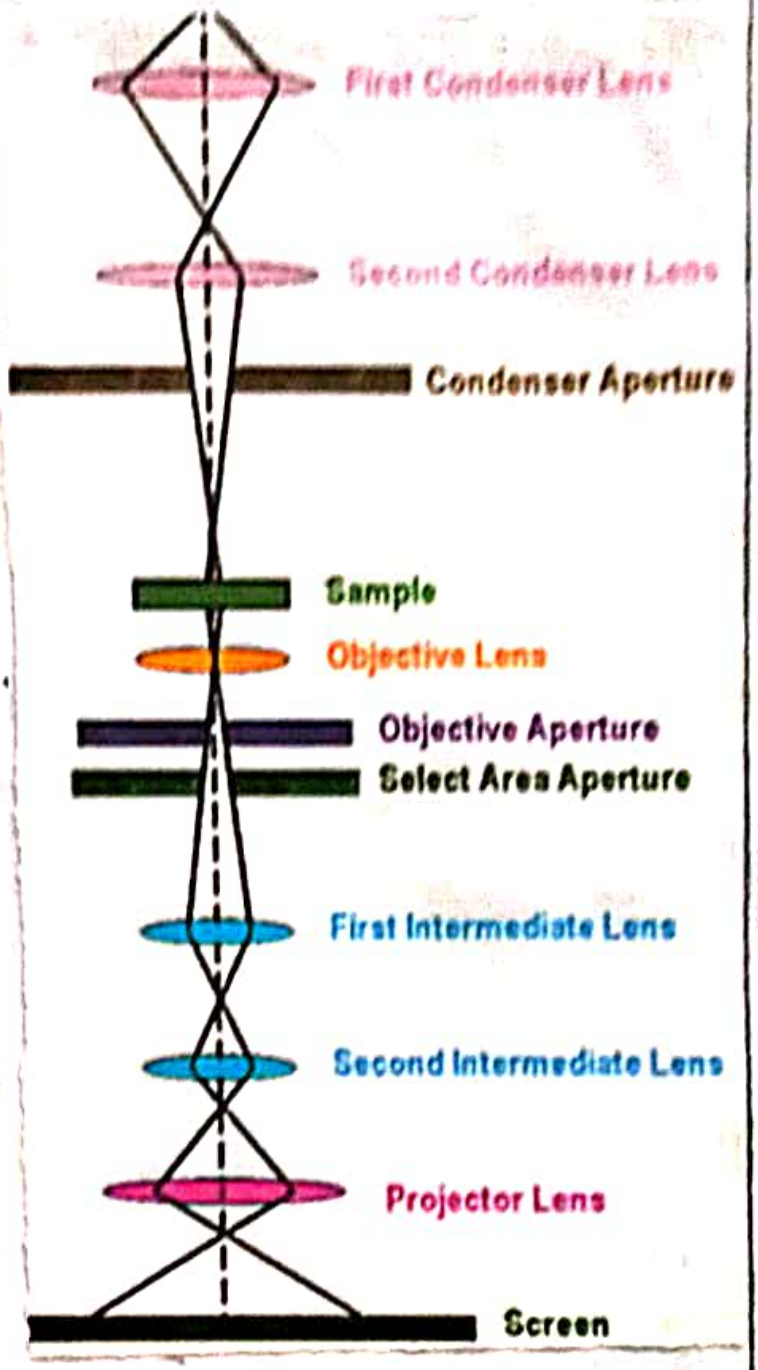
Scanning Electron Microscope



Transmission Electron Microscope:

In TEM, a beam of electrons hits a very thin sample. The electrons are transmitted through the sample. After the sample, the electrons hit a fluorescence screen that forms an image with the electrons that were transmitted. You can better understand this process by imagining how a movie projector works.

In a projector, you have a film that has the negative image that will be projected. The Projector shines white light on the negative and the light transmitted forms the image contained in the negative.



Limitations of electron Microscope:

→ Specimen being examined must be dry because it is in a chamber i.e., under complete vaccume. Therefore cells cannot be observe in metabolically active condition.

Uses:

1) To study in detail internal structure of Bacterial cells.
2) To observe morphological characters of Viruses, thin section of Bacterial cells can be observed under electron microscope.