

Learning objective

- By the end of this lecture, the student of first year MBBS should be able to:
 1. Perform Focusing the slide under microscope for oil immersion lens, independently.

Microscope

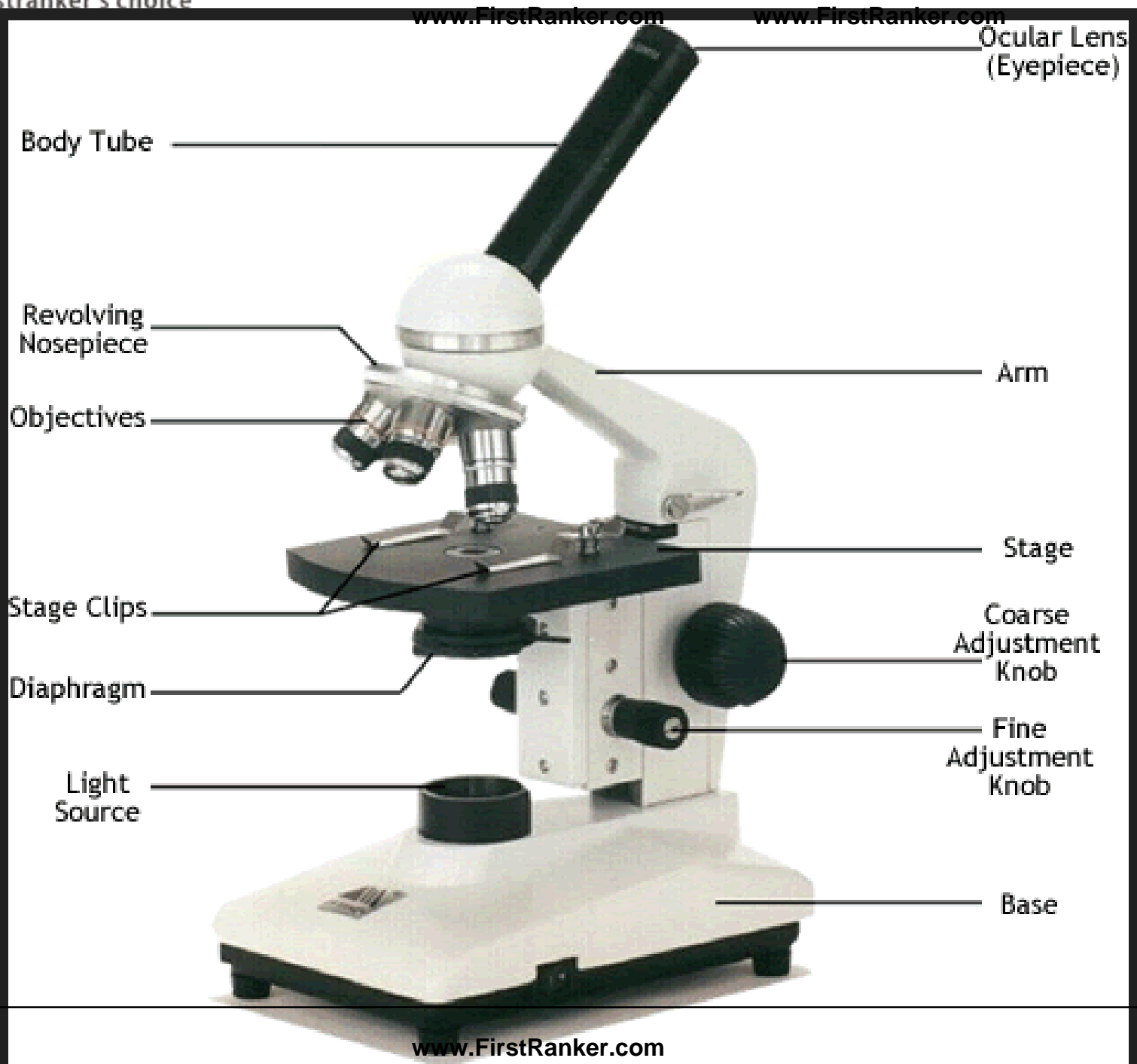
- It is an optical instrument by the help of which one can magnify and resolve microscopic structures.
- It is an instrument which is meant to see the minute objects which we are unable to see with naked eye.



- Microscope is an instrument of magnification and resolution
- **Magnification** is the ability to make small objects seem larger, such as making a microscopic organism visible.
- **Resolution** is the ability to distinguish two objects from each other.

Components of microscope

- 1. Frame work which consist of Arm and base.
- 2. Mechanical stage.
- 3. Lens system in this system
 - (a) Nose piece which is movable and it holds the objective lenses.
 - (b) Head and body tube it holds the objective and ocular lens.
- 4. Coarse adjusting knob.
- 5. Fine adjusting knob.
- 6. Light source



Frame work of microscope

- The arm and base together make the frame work of microscope. The rest of the structures are attached to the base and arm.
- Base: is a disc shaped or U shaped support upon which the microscope rest.
- Arm: The arm is the curved ,upright structure which is attached to the base.

Stage of compound microscope

- The stage is a horizontal platform and contains a centrally located opening through which light can be reflected by the light source.
- It has two clips for holding a slide.
- The stage has two parts
- One where the slide is placed.
- Other part is the mechanical stage which has a measuring scale and knobs to move the slide and to record the previous position of the slide.



LENS SYSTEM

It consist of

- Condenser.
- Eye piece (Ocular lens).
- Objective lens.



Condenser

- Condenser is made up of two convex lenses. It condenses the light rays on the object.
- A movable lenses is located below the stage can be elevated or lowered using the control knob.
- Iris Diaphragm is attached to it, similar to shutter of a camera both condenser and iris diaphragm regulates the amount of light from the light source.
- When the condenser is elevated nearest the stage, the greater amount of light is directed towards the specimen when lowered less light falls on the specimen.
- Heavily stained slides for example blood smears require more light for observation then unstained specimens.
- The use of Oil immersion objective usually necessitates placing the condenser as close to specimen as possible and fully opening the diaphragm in order to provide the maximum amount of light.

Alignment of the Condenser Along the Microscope Optical Axis



Figure 1



Eye piece or Ocular lens

- The eye piece is the upper most part of the optical system.
- The magnification of the Eye piece is (10X).
- The real image produced by the objective lens is further magnified 10 times by the ocular lens.



Objective lenses

- There are three types of Objective lenses located on nose piece
- Low power objective(10X)---Yellow
- High power objective(40X)----Blue
- Oil Immersion objective(100X)---white
- Low power objective is used to initially focus the microscope for all type of specimens.
- It allows the rapid location of the specimen.
- It is the only objective that will not touch the slide when microscope is completely racked down.

Adjustment of Intensity of Illumination

General principle

- When we use low power(10X) Objective, a large area of field is visualized, so needs less illumination. When oil immersion lens(100X)is used, a very small area of field visualized, requiring highest illumination so that sufficient light reaches up to the eyepiece.

Means to increase the intensity of illumination

1.Aperture size of diaphragm

- Small size—less illumination.
- Big size---more illumination.

2.Position of condenser

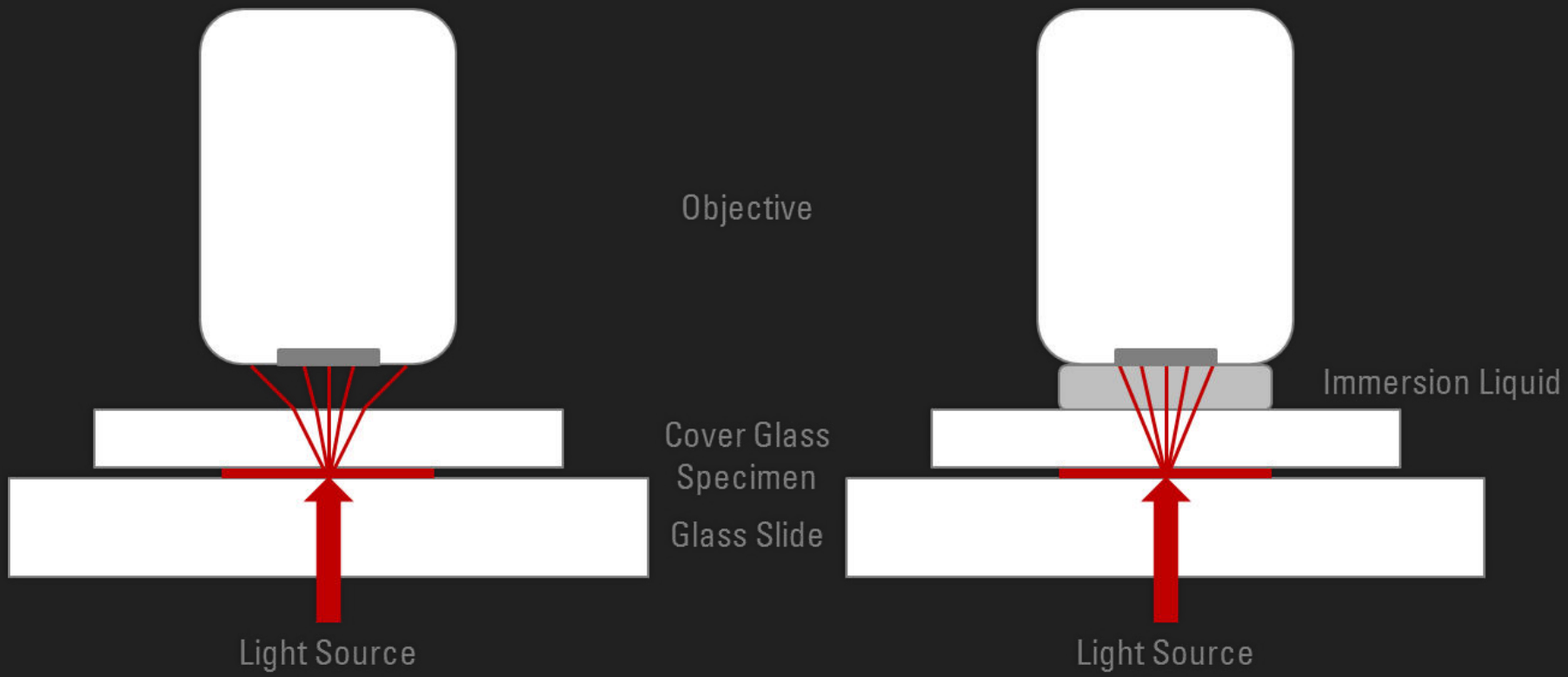
- Lower position—minimum illumination.
- Highest position—maximum illumination.

Objective lenses

- **Low power objective lens** has light aperture larger in diameter. While working on low power objective lens condenser is adjusted in lower position away from the stage Iris diaphragm is slightly open.
- **High power objective lens** diameter of light aperture is not too small nor so large. While using condenser should be adjusted in the middle nor to high nor to low and iris diaphragm is moderately opened.
- **Oil immersion objective lens** diameter of light aperture is pin pointed small. While using condenser should be adjusted high near to stage and iris diaphragm is fully opened.

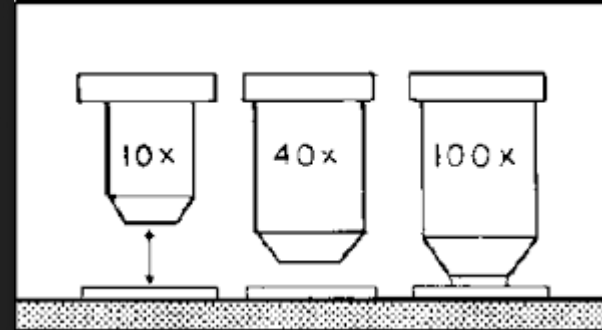
Use of Cedar wood oil or Liquid paraffin in oil immersion objective

- Refractive index of cedar wood oil or liquid paraffin is equal to that of glass, so it prevents the divergence of light rays and the image formed will be more clear when it is used with oil immersion lens.



Working distance

- The distance between specimen and the objective is called working distance.
- Decrease working distance is inversely proportional to magnification.
- Low power objective lens (10X) = 5mm.
- High power objective lens (40X) = 0.35-0.45mm
- Oil immersion lens (100X) = 0.1-0.3mm.



Total magnification

- The ocular lens and objective lens contribute to the total magnification of the specimen
- Ocular lens x objective lens=Total magnification.
- Low power objective $10 \times 10 = 100 \times$.
- High power objective $10 \times 40 = 400 \times$
- Oil immersion objective $10 \times 100 = 1000 \times$.

Procedure for focusing a specimen on microscope.

- **ALWAYS receive and handover microscope in Low power.**
- Turn on the light and rotate the nose piece to low power objective.
- While looking at the slide increase the working distance as far as possible.
- Place a prepared slide on the stage, secure it with the slide clamp and centre it using the knobs.
- While still observing from the side decrease the working distance as far as possible.

- Place your eyes over the oculars and begin initial focusing of the specimen by slowly increasing the working distance using the coarse adjustment knobs.
- After the specimen is in focus, adjust the amount of light using the condenser and iris diaphragm.
- Using the fine adjustment knob, focus to the sharpest possible image.

- With parfocality when the a sharp image is obtained with low power objective you will view the specimen with high power or oil immersion objective with little or no additional focusing.
- Parfocality is the property of microscope that once the object is focused sharply and working distance is adjusted if the lens is changed focus remains the same only fine adjustment is needed.

How to use oil immersion lens

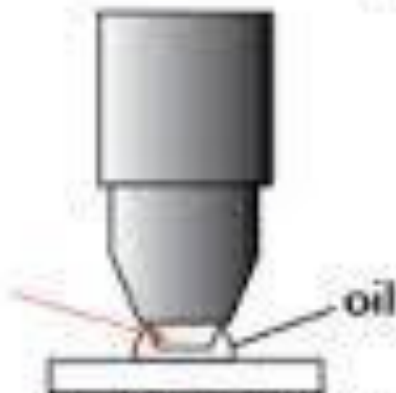
- Put a drop of cedar wood oil after placing the slide on the stage just over the area which has to be focused.



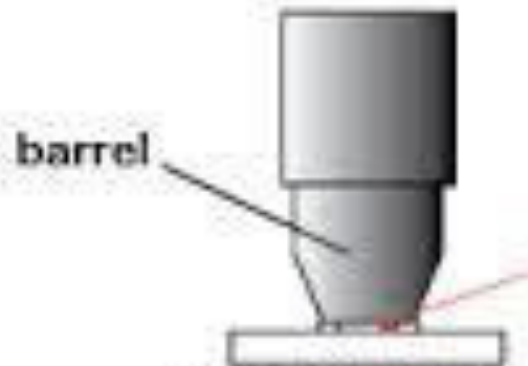
- Dip the oil immersion lens very carefully with the help of coarse adjustment screw by constantly looking from the side of microscope so that it will just dip in the oil without touching the slide.
- Do fine focusing with the help of fine adjustment screw by raising the objective till its view becomes very clear.



The retractable portion of the oil immersion (100X) lens (arrow) needs to just make contact with the slide but not be pushed up into the barrel of the lens.



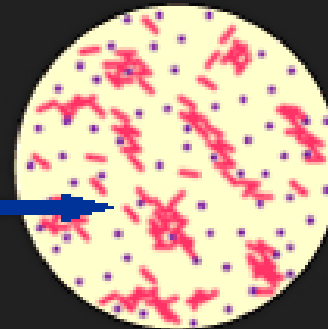
Lens is too high.
(not touching the slide)



Lens is too low.
(retractable lens is
pushed up into the barrel)



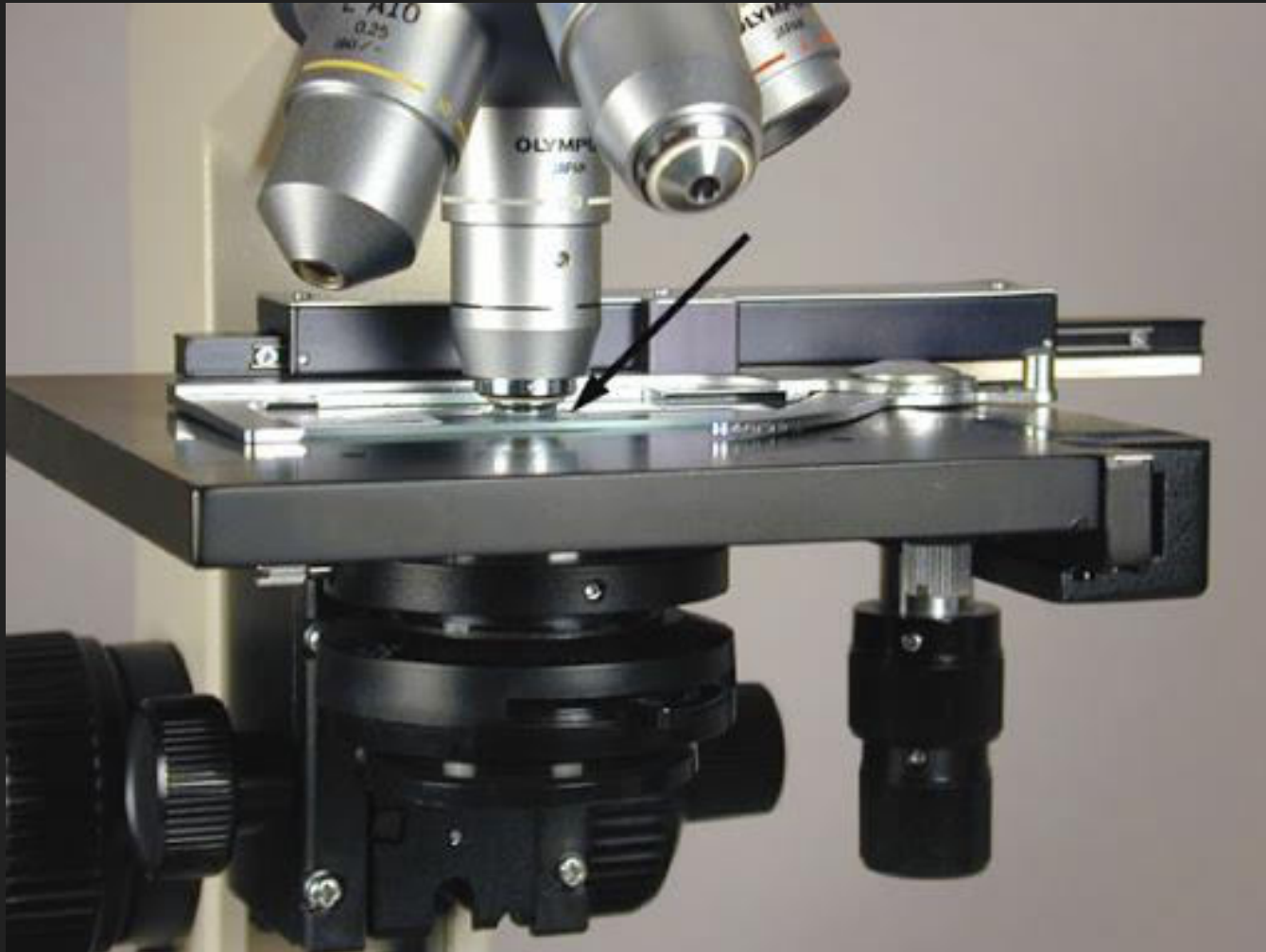
Lens is just right.
(just touching the slide
but not pushed up
into the barrel)

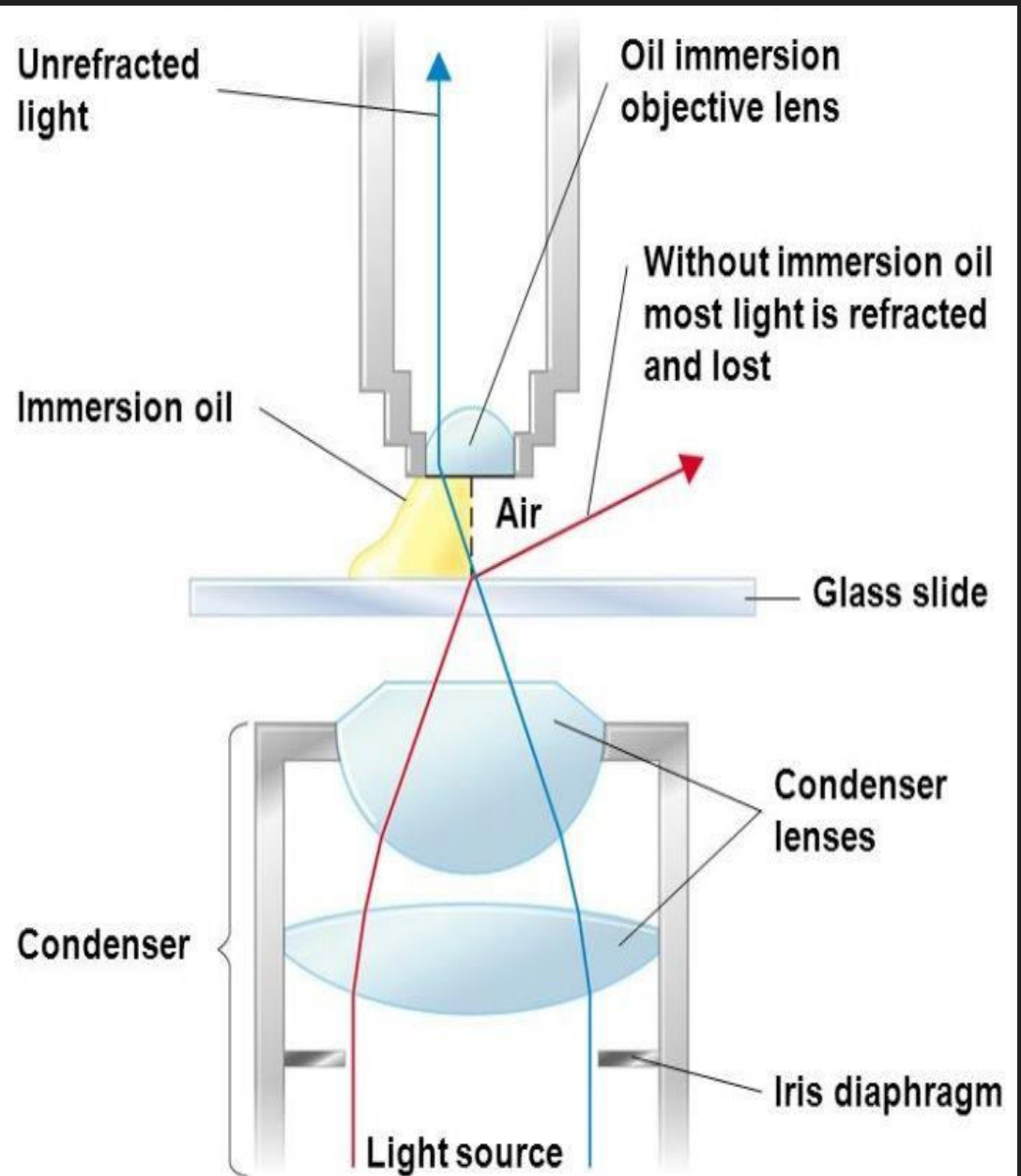


Care of microscope

- Always during transport of microscope hold it in upright position with one hand underneath the base and other hold the arm.
- Keep the microscope at least 6 inch away from the edge of the table.
- Donot remove any part of the microscope yourself.
- Never touch the lens with fingers.
- Do not allow the liquids for example alcohol, water to come in contact with microscope.
- Do not use dry cotton to clean the lens.Xylene with soft cloth should be used for this purpose.
- Do not use spirit to clean lenses since it may dissolve the fixing material of the lens.

- For wet specimen use cover slip.
- Always increase the distance between stage and slide maximum while changing the slide.
- Whenever microscope is not in use switch off the light.
- After the work is finished switch the low power in place again.





Task

- Draw the diagram in your practical copy
- Write the steps of procedure
- Answer the questions given in copy
- Get the practical checked by the teacher.

JUST KIDDING

