The Compound Microscope

Physiology Lab-1

October , 2022

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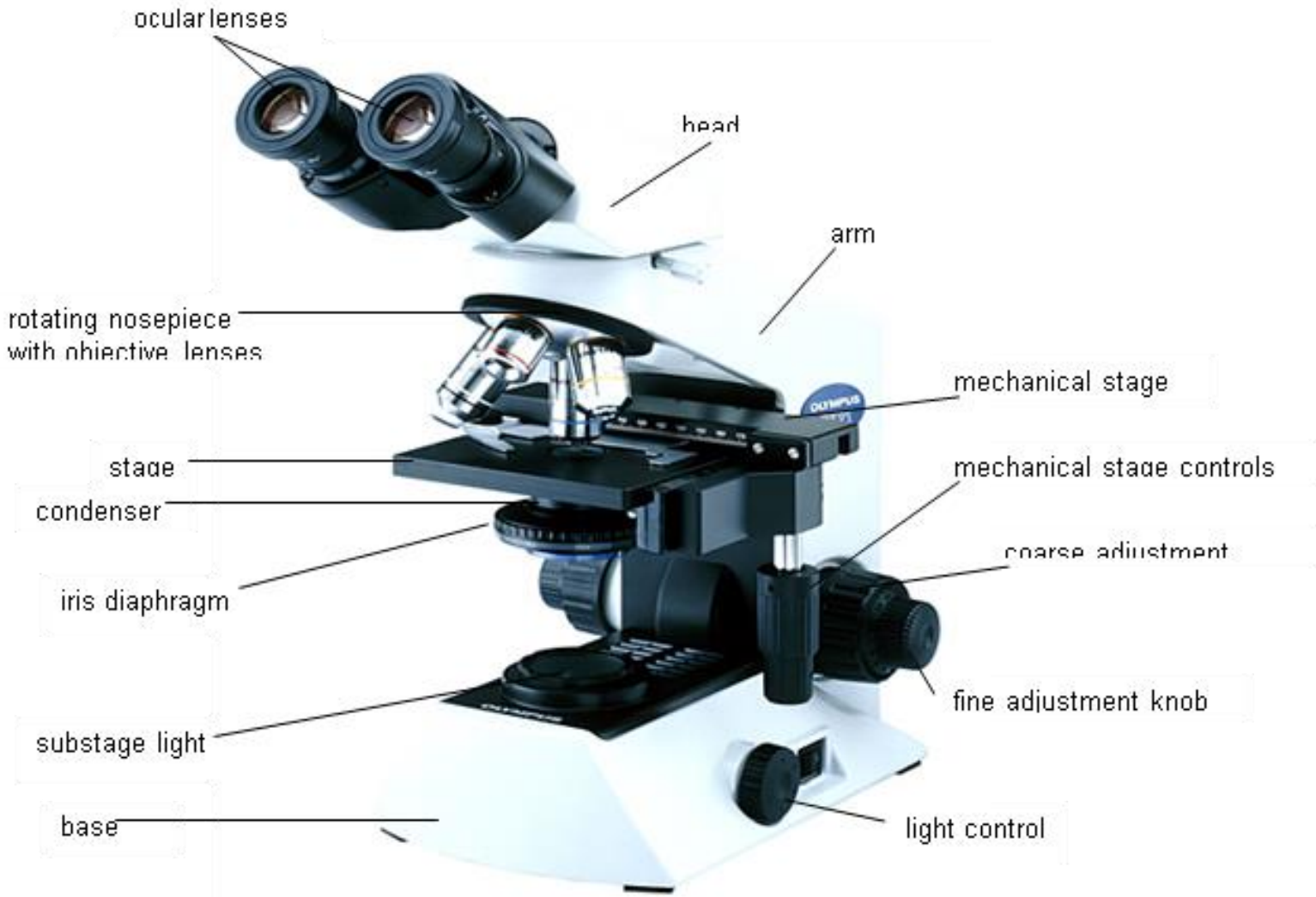
Background Information

- The microscope is one of the most commonly used instruments in the medical colleges and in clinical laboratories. Students of physiology use it in the study of morphology of blood cells and in counting their numbers.
- In this laboratory, you will be using a compound light microscope. Light microscopes use light and refraction (bending of light) to magnify small objects to view them better; the term compound means that two lenses are used (an ocular lens, or eyepiece, and an objective lens).

Aim:

To study the parts of a compound microscope and to view the hemocytometer slides under microscope.

Parts of the Compound Microscope:



- Base: Supports the microscope.

- Substage light: Located in the base, the light passes directly upward through the microscope.

- Stage: The platform the slide rests on while being viewed. The stage has a hole in it to permit light to pass through both it and the specimen. The mechanical stage permits precise movement of the specimen.

- Condenser: Concentrates the light on the specimen. The condenser has a height-adjustment knob that raises and lowers the condenser to vary light delivery. Generally, the best position for the condenser is close to the inferior surface of the stage.

- Iris diaphragm dial: Dial attached to the condenser that regulates the amount of light passing through the condenser. The iris diaphragm permits the best possible contrast when viewing the specimen.

- Coarse adjustment knob: Used to focus on the specimen when on 4x or 10x.

- Fine adjustment knob: Used for precise focusing once coarse focusing has been completed. Use only this knob when on 40x or 100x.

- Head or body tube: Supports the objective lens system, and the ocular lenses .

- Arm: Vertical portion of the microscope connecting the base and the head.

- Ocular (or eyepiece): There are two lenses at the superior end of the head, through which observations are made. An ocular lens has a magnification of 10x.

- Nose piece: Has four objective lenses and permits sequential positioning of these lenses over the light beam passing through the hole in the stage. Use the nose piece to change the objective lenses.

- Objective lenses: Adjustable lens system that permits the use of a scanning lens, a low-power lens, a high-power lens, or an oil immersion lens. The objective lenses have different magnifying and resolving powers.

Objective Lenses:

1. The shortest lens is the scanning lens, This objective, a very low power lens, magnifies the image 4 times (4x). It is used for scanning a much larger area on the slide.
2. The low power lens is 10x.
3. The high-power objective lens is 40x.
4. The oil immersion objective lens is usually the longest of the objective lenses and has a magnifying power of 100x.

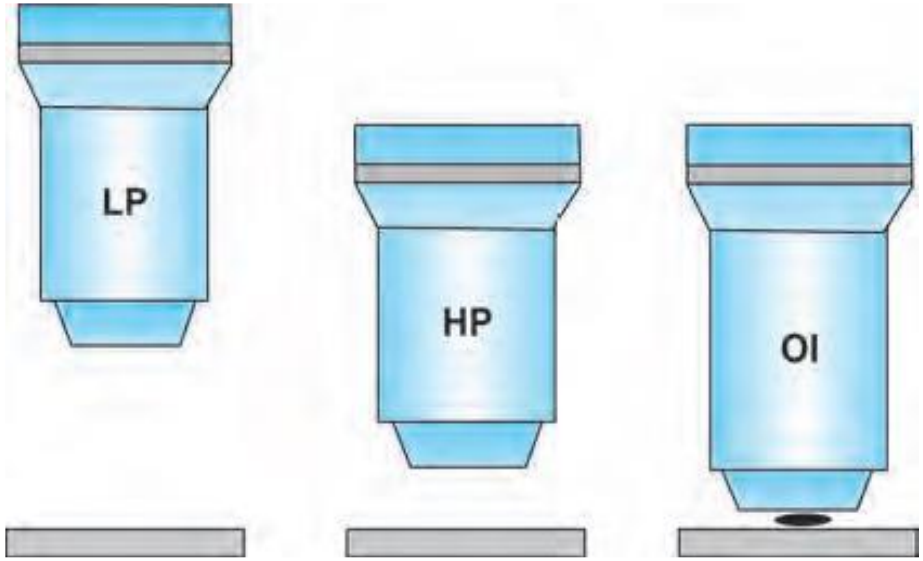
Total Magnification of the Compound Microscope

- The microscope is designed to magnify specimens. The objective lens magnifies the specimen to produce a real image that is projected to the ocular. This real image is magnified by the ocular lens to produce the virtual image seen by your eye.

- The total magnification of any specimen being viewed is equal to the power of the ocular lens multiplied by the power of the objective lens. If the ocular lens magnifies 10x and the objective lens magnifies 50x, the total magnification is 500x (10 x 50).

The working distance:

- is the distance between the objective and the slide under study. This distance decreases with increasing magnification. It is 8–13 mm in LP, 1–3 mm in HP, and 0.5–1.5 mm in OI lenses respectively. Note that the OI lens has to be immersed in a drop of oil.

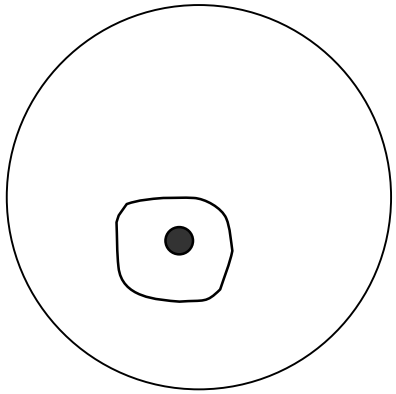


Size of the microscope field:

- The size of the microscope field decreases with increasing magnification.
- This information will allow you to make an estimate of the size of the objects you view in any field. For example, if you have calculated the field diameter to be 4 mm and the object being observed extends across half this diameter , you can estimate the length of the object to be approximately 2 mm.
- Microscopic specimens are usually measured in micrometers (µm) and millimeters (mm), both units of the metric system.
- Use the following formula to obtain the total field of view diameter:

  (F.O.V = Field Number / Total Magnification)

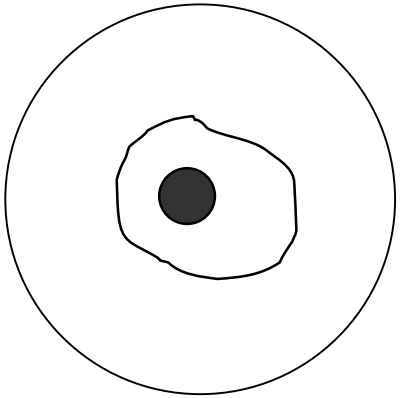
- Multiply your Answer by 1000 to convert to Micrometer.
- Determine (guesstimate) the percent of the diameter of the field of view is occupied by the object (image).
- Object Size = (Percent/100) X diameter of field of view



Object seen in low-power field:

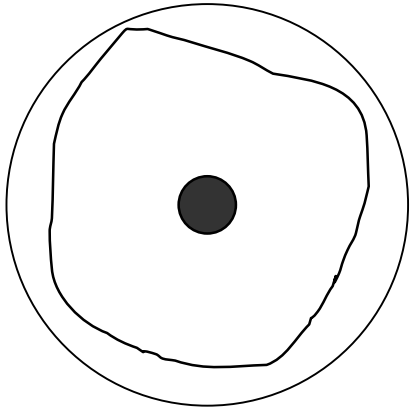
Approximate length: $\frac{22}{10x10} * 1000 = 220$ µm

$\frac{33.5}{100} * 220 = 73.7$ µm



Object seen in high-power field:

Approximate length: _____ µm



Object seen in oil immersion field:

Approximate length: _____ µm

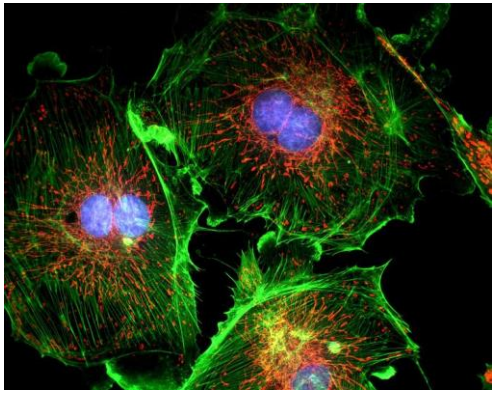
PRECAUTIONS WHILE USING THE COMPOUND MICROSCOPE

- The microscope must always be handled properly. You must observe the following rules for its transport, cleaning, use, and storage:
- 1- Transport in an upright position with one hand on the arm and the other supporting the base. Set it down carefully at your work station. Do not drag it across the table.
- 2- Use only special lens paper to clean the lenses. Clean all lenses before and after use.
- 3- Always begin the focusing process with the 4x or 10x objective lens in position, changing to the higher-power lenses as necessary.
- 4- The coarse adjustment knob may be used with the 4x or 10x lens, but use only the fine adjustment with 40x or 100x.
- 5- Adjust lighting appropriately. Turn off the light when not in use.
- 6- Always use a cover slip with temporary (wet mount) preparations.
- 7- When you put the microscope away, remove the slide from the stage, and rotate the lowest-power objective lens into position. Wrap the cord around the clips on the back, not around the base.
- 8- Never remove or loosen any parts from the microscope.

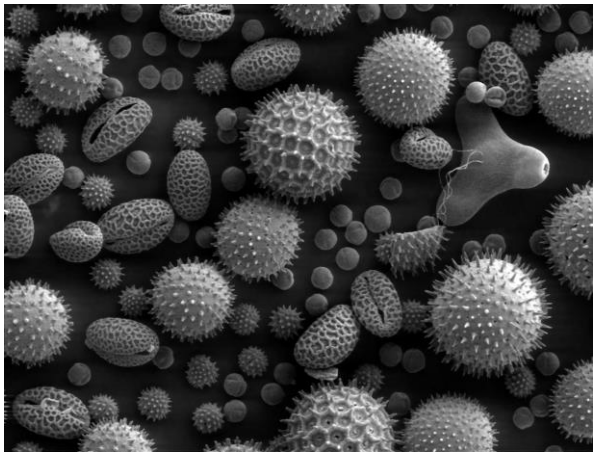
Other Types of Microscope

- 1. The dissection microscope, is light illuminated. The image that appears is three dimensional. It is used for dissection to get a better look at the larger specimen. You cannot see individual cells because it has a low magnification.

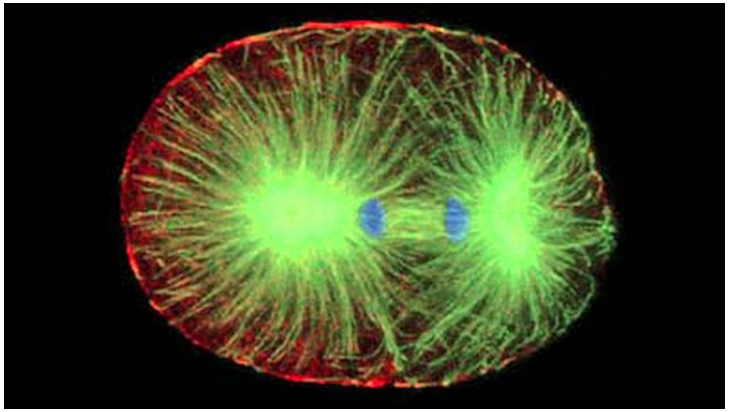
- 2. The fluorescence microscope that uses a fluorescent dye to stain tissues, which are studied under the microscope.



- 3. The scanning electron microscope SEM uses electron illumination. The image is seen in 3-D. It has high magnification up to several hundred thousand times and high resolution. The pictures are in black and white.



- 4. The confocal microscope this microscope uses a laser light. It enables the reconstruction of three-dimensional structures from the obtained images.



SUMMARY CHART	Scanning	Low Power	High Power	Oil Immersion
Magnification of objective lens	x	x	x	x
Total magnification	x	x	x	x
Working distance	mm	mm	mm	mm
Detail observed (draw or describe)				
Field size (diameter)	mm mm	mm mm	mm mm	mm mm