MCB 3010 Lab THE COMPOUND MICROSCOPE

In microbiology, the microscope plays an important role allowing us to see tiny objects that are normally invisible to the naked eye. It is essential for the student to learn how to use the microscope in a skillful manner. Successful microscopy requires the student to:

a. **Be patient**.
b. **Know the basic principles of microscopy**.
c. **Have skill in the care of the microscope cleanliness. (see pages 4+5)**.
d. **Understand the nature of material observed and continue to be patient**.

For successful microscopy, it is necessary to understand how to control the variable microscopic factors. To do this the student needs to understand the relationships between **magnification, resolution,** and **contrast**.

PARTS OF THE MICROSCOPE **Refer to the Figure**

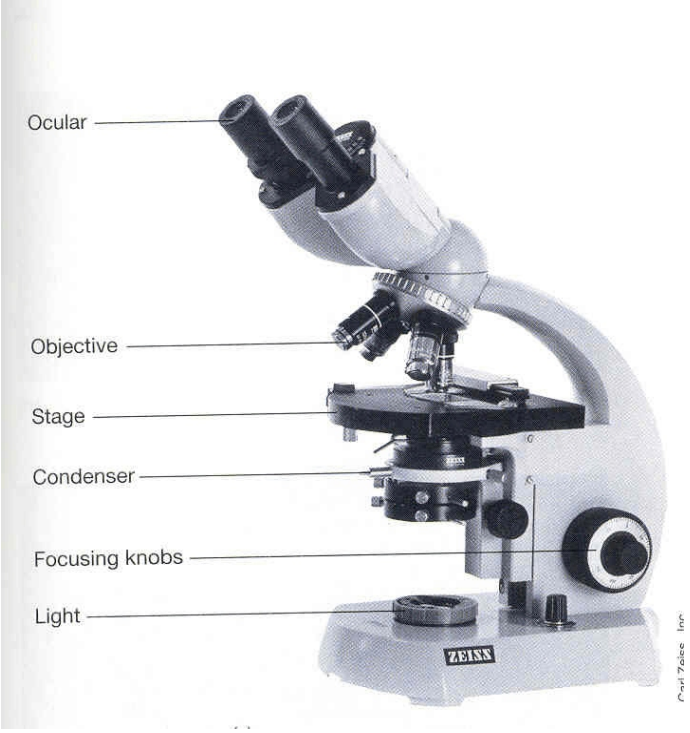
1. **Illuminator**. Most modern microscopes contain an built in base illuminator with a facility to vary the intensity of light by varying the voltage to the light as well as an iris diaphragm in the condenser. For optimal results, the proper amount of illumination should be obtained: fields that are too bright or too dim will not allow you to see the details of the preparation you are examining and can lead to eye-fatigue. Be sure you are familiar with both the iris diaphragm (mechanical) and the light intensity adjustment (electrical).

2. **Objective Lenses**. These are mounted on the revolving nosepiece. Each lens is marked with its Magnification/Numerical Aperture and Focal Distance. Be sure you can distinguish between these numbers. Most standard biology microscopes have 4X, 10X, 40X, and 100X oil immersion lenses. The first three are used with only air between the lens and the slide, the highest power lens (100X) is used with a drop of immersion oil between the lens and the slide. That is the light passing through the slide (material being viewed) passes through only oil, not air, as it is transmitted to the lens system.

The objective lenses must be kept clean. Use lens tissue or Kimwipes to clean the outer, exposed surfaces of the lenses. Although the oil immersion lens is designed to work in oil, the **oil must be removed after use and before you put the microscope away at the end of the period.** All the other lenses do not operate with oil on them and if oil gets on the air lenses it will ruin their mounts and surfaces; **oil must never contact the air lenses**.

3. **Ocular Lenses**. The ocular lenses usually magnify 10X. Thus the total magnification observed is the multiplication of the power of magnification of the ocular times the objective. For example an object magnified by the ocular and the 40X high-dry objective is viewed at 400

times its real size. Most ocular lenses can be moved back and forth to adjust to the interpupillary distance of the student. When first using the microscope, adjust the ocular lenses back and forth until a circular field is viewed with both eyes open. Additionally, many microscopes allow the ocular lenses to be adjusted up and down (mechanical tube length adjustment) and there is a scale alongside the tube. After adjusting the interpupillary distance, read the distance off the scale and adjust the tube length of the ocular lens to the same value. Now the ocular lenses are adjusted to your eyes.



4. **Condenser Lens**. Below the stage is the condenser lens. This focuses light onto the object and is not involved in the magnification. The focusing adjustment is a rack and pinion movement to permit vertical movement of the condenser. Clear images are obtained only when the condenser lens is in proper focus: when the cone of rays illuminating the object is equal to that observed by the objective lens. If you have a blurry object, it could well be that the condenser is out of focus...adjust both the iris diaphragm and condenser focus adjustments. With time, your patience will be rewarded by clear crisp images.

5. **Coarse and Fine Adjustment Wheels**. These are used to raise and lower the body tube or stage, depending upon the manufacture of the microscope. The coarse adjustment is used to first bring the object into approximate focus starting first with the stage as close as possible to the objective lens **without touching**. Then move the coarse adjustment so that the stage moves away from the lens until the object is in relative focus. If this is always done in this way, there is

no possibility that the lens gets jammed into the slide....damaging both. After the object is in relative focus, it can then be brought into sharp, critical focus with the fine adjustment knob.

MAGNIFICATION AND RESOLVING DISTANCE

It is relatively easy to think of the microscope in terms of magnification; the importance of which is without dispute. However, the importance of magnification is meaningless without a clear crisp image and **resolution** is a measure of clarity. Resolution is defined by **resolving distance.** Resolving distance is the smallest distance between two points that allows the observer to see those points as distinctly separate and more importantly to see small objects. The most limiting factor in obtaining good resolution is the wavelength of light used for illumination. Bear in mind that the bacteriologist observes objects whose own dimensions are of the same order of magnitude as the wavelength of light. Blue light (360 nm to 420 nm) permits greater resolution than red light (650 nm to 800 nm).

Sometimes it is possible to magnify an image beyond the smallest resolving distance of the lens system; this is termed "empty magnification" because although the image is magnified, it is not distinct but blurry and can not be seen as well as if it were magnified to a lesser amount within the resolving distance of the objective.

Another factor that determines resolving distance is the refractive index of the medium through which the light rays pass. The refractive index of glass is 1.52 compared to air (N = 1.00). Light rays passing through a glass slide, using the high-dry lens, will pass through air and be bent before reaching the objective lens. Less bending will occur with water than air and even less with oil. The maximal angular aperture of the lens (the angle of greatest divergence of light rays that the objective lens can collect) will not be realized with air. With an oil immersion lens, the air space between the glass slide and the lens is replaced with oil that has a refractive index very close to that of glass. These factors are combined in the **Numerical Aperture** of the lens. The numerical aperture of a lens is defined as:

$$\text{Numerical Aperture} = N (\sin a)$$

where "N" is the refractive index of the material between the object and the objective lens and "a" is one half of the angular aperture of the objective lens. If a high-dry lens has an angular aperture of 111°48'(sin a = 0.828), its numerical aperture working in air (N = 1.00) will be:

$$\text{N.A.} = 1.00 \times 0.828 = 0.83$$

If that same lens could be used in oil (most likely it couldn't), it would have a numerical aperture of:

$$\text{N.A.} = 1.52 \times 0.828 = 1.26$$

assuming a refractive index of 1.52 for the oil. These calculations are important because of their relationship to the resolving distance.

The formula for **Resolving Distance** (RD) is:

$$R.D. = wavelength/(2\ N.A.)$$

The reason the numerical aperture is multiplied by two is that two numerical apertures are involved: that of the objective lens and that of the condenser. When the condenser is in perfect focus it has the numerical aperture of the objective. The denominator of the Resolving Distance equation is really the numerical aperture of the objective plus the numerical aperture of the condenser. If one assumes that "average blue light" is being used (wavelength = 400 nm), using an oil lens of numerical aperture of 1.25 will allow you to resolve distinctly objects as small as the resolving distance:

$$R.D. = 400\ nm/(2 \times 1.25) = 160\ nm \text{ or } 0.16\ \mu m$$

This means that under the most ideal conditions, this lens is capable of distinguishing two objects as separate if they are 0.16 μm or greater apart. If the two objects are less than 0.16 μm apart, say 0.10 μm, then they will be blurred together at the point where they are 0.10 μm apart. Most importantly, objects >0.16 μm should be clearly visible.

CALIBRATION OF THE OCULAR MICROMETER

The ocular micrometer is a glass disc with evenly inscribed markings located in the ocular lens (rotate the ocular lens and the micrometer should rotate). The actual distance between these lines is unimportant as long as they are exact. The micrometer is used to measure objects being magnified by the objective. The distance between the inscribed lines must be determined (calibrated) for each lens. To do this, place a stage micrometer on the stage and focus using the low power lens. The lines on the stage micrometer are accurately determined and marked on each individual micrometer. Count the number of ocular micrometer lines per each division on the stage micrometer and calculate the distance between the smallest divisions of the ocular micrometer. Repeat this for each objective lens and record these calibrations for each lens. The ocular micrometer may now be used to measure objects magnified by the microscope.

RULES FOR USE AND CARE OR THE MICROSCOPE

1. Use **only lens paper or Kimwipes** to clean the optical parts of the microscope. Do not use paper towels, lab coat tails, handkerchiefs or other such to clean the lenses.

2. **Never immerse the 10X or 40X lenses in oil**.

3. When done each day, **wipe off oil from the 100X oil immersion lens**.

4. When done each day, clean stage, condenser lens, the other objective lenses and ocular lenses.

5. **Do not attempt to clean the inside** of the microscope or a lens.

6. **Keep the microscope upright** when taking or returning the microscope to the cabinet.

7. When the microscope is put away, the lowest power lens should be in place.

YOU ARE RESPONSIBLE FOR THE WELL BEING OF YOUR MICROSCOPE