

The Compound Microscope

We will be using microscopes extensively throughout General Biology. This exercise is designed as either an introduction to or a review of microscopy.

Parts of the Microscope and their Function

Requirements

It is crucial for you to preview this material before coming to your discussion section. Upon completing this section, you should be able to name the parts of the microscope and know their function. At your desk you will find an Olympus Binocular Microscope (specimen viewed through both eyes). The following is a description of the parts and function of a compound microscope (refer to Fig. 1).

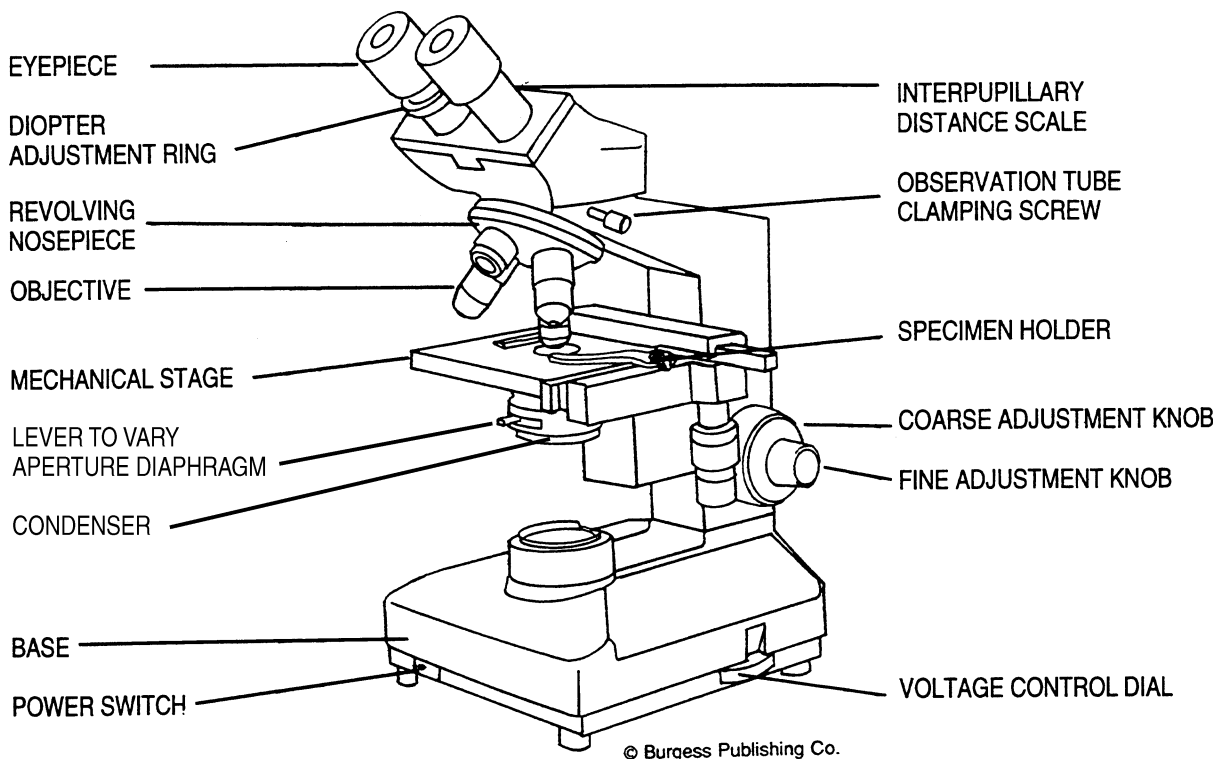


Fig. 1
The Binocular Compound Microscope.

Stage

This is the platform on which material (i.e., the “object”) is placed to be examined. The object is usually mounted on a glass slide, covered by a “cover slip” of thin glass (*we use disposable plastic cover slips in Biology 1*), and placed in the center of the stage over the aperture through which light enters from underneath. Stage clips, or a mechanical stage, are used to hold the slide in place. The specimen can be centered with the “mechanical stage adjustment knob.”

Objectives

The objectives contain lenses, which magnify the object being examined. The name “objective” indicates the position of the lens directly above the “object.” The binocular microscope has 4x, 10x, 40x and 100x objectives. The 100x objective must only be used with a drop of immersion oil between it and the slide. We rarely use this objective in Biology 1.

Revolving Nosepiece

The nosepiece, located at the lower end of the body tube, holds the objectives. Turn it gently by means of the objectives. There is a faint click when an objective is in proper alignment.

Ocular

The ocular, or “eyepiece,” also contains lenses. It fits into the top of the body tube and gives a secondary magnification of the image formed by the objective. The eyepieces of most microscopes have a magnification of 10. The total magnification is calculated by multiplying the objective and ocular magnifications. In the binocular microscope the eyepiece on the right will in most cases contain a micrometer, i.e., a measuring ruler with 100 subdivisions. In some cases the eyepiece may also contain a small hair, which will appear as a black line and which can be used as a pointer. If the microscope is tilted too much, the eyepiece may fall out and break.

Binocular Observation Tube

The binocular tube allows adjustment of the two oculars for the particular spacing of your eyes. The left ocular should be set at zero unless your eyes require a correction. The binocular tube can be rotated on the stand allowing the microscope to be used with the stage directly in front of you or with the focusing knobs in front of you.

Light Source

The light source is built into the base of the microscope. There is an on/off switch and a sliding control which regulates light intensity. ***Always return the sliding control to zero before turning on the lamp to prevent premature lamp burn-out.*** Avoiding high lamp intensity will also prolong lamp life.

Condenser

The condenser contains another set of lenses and is located below the stage. It focuses light coming from the lamp onto the material being studied. Notice the adjustment knob by which the condenser may be raised and lowered. The condenser at first should be adjusted so that it is at its uppermost position, very close to the stage.

Iris (Aperture) Diaphragm

The iris (aperture) diaphragm consists of metal plates and functions much like the iris of your eyes. It is built into the substage condenser. A lever at the side of the condenser regulates the size of the diaphragm opening and thus the amount of light which enters the microscope. Each time you examine a slide, the diaphragm opening should be readjusted to balance contrast, resolution and brightness. Beginning students usually overlook the use of the iris diaphragm and condenser and therefore get poor images.

Focusing Mechanism

Coarse Adjustment

The stage is moved rapidly up or down by means of the large coarse adjustment knob. THE COARSE ADJUSTMENT SHOULD BE USED ONLY WITH THE LOW AND INTERMEDIATE POWER OBJECTIVES, NEVER WITH THE HIGH POWER OBJECTIVE! An automatic prefocusing lever incorporated into the coarse adjustment prevents running the specimen into the objective.

Fine Adjustment

Fine adjustments of the level of the stage or body tube can be made with the smaller knob lateral to or beneath the coarse adjustment. After initial focusing with the coarse adjustment, sharp focus is obtained by using the fine adjustment.

Care of the Microscope

The microscope is a delicate and expensive instrument. Always carry the microscope with two hands, one hand on the arm and the other supporting the base.

The lenses of the ocular, objectives, and condenser should be cleaned before and after using the microscope. Use only the lens paper provided as other materials will scratch the lenses.

If reagents are spilled on any part of the microscope they should be cleaned away immediately. If immersion oil is used, care should be taken to remove all traces of oil from the objectives, using lens paper and xylene. Do not use ethanol to clean the lenses.

Before putting away the microscope, return the light intensity sliding control to zero and turn off the lamp. Return the low power objective to center position and remove the slide from the stage. Wind up the electrical cord and cover the microscope with a plastic cover. **Return the microscope to the correct cabinet (note number on back of microscope).**

Setting Up the Microscope

Requirements

At the end of this exercise, you should be able to set up the microscope: 1) to obtain optimal illumination (“critical illumination”), 2) to bring an object into focus at intermediate and high magnifications, and 3) to estimate the size of microscopic objects by use of an eyepiece micrometer which you have calibrated.

Critical Illumination in 5 Steps

- 1) Your GSI will demonstrate the condenser lens and how the iris diaphragm functions. You are now ready to set up the microscope for critical illumination. First, gently close the iris diaphragm as far as it will go, and turn the condenser knob until the condenser is at its uppermost position.
- 2) Next, turn the nosepiece to the intermediate (10x) objective and lower the lens to within 1 cm of the stage, watching from the side.
- 3) Then turn the lamp to the lowest setting at which you can see the light.
- 4) Center on your stage a slide of a piece of yellow nylon parachute cloth or Kimwipe. Bring the fibers into focus with the coarse and fine adjustment knobs. If the knobs are hard to turn, you may have to release the “prefocusing lever” which is a safety device to prevent you from mashing the specimen with the lens. Now lower the condenser slowly until you see granularity come into focus superimposed on the nylon. You are seeing the granularity of the ground glass diffuser covering the light source. Now raise the condenser slightly until the granularity of the diffuser just becomes no longer visible. This will be your optimal condenser setting.
- 5) Setting the iris diaphragm: The last step is to familiarize yourself with the iris diaphragm, which is still at its most closed setting. Open and close the iris slowly, watching the changing quality of the image of the nylon fibers. When the iris is full closed, contrast is maximum and the chance for “false lines” is the greatest, but the “washout” of the image takes over, due to imperfections in the condenser. Find a position, slightly opened, where the image is clear, bright, detailed and sharp. This is your optimal iris setting. You will have to change this setting with different magnifications and with the various specimens you view. You have now achieved “critical illumination.”

Intermediate and High Magnification

With the fibers in focus with the 10x objective, look at the perimeter of the illuminated area. The circular area seen with a particular objective is called the “actual field” of view. The size of the actual field will decrease directly in relation to increasing magnification. To determine the diameter of the actual field of view we are using the parachute fibers as a ruler. The distance between the center of one fiber to the center of the next fiber is 100μ (micron), which is equal to 0.1 mm. Manipulate the stage such that the center of one parachute fiber aligns with the left edge of the field of view. Now measure the diameter of the actual field of view by counting the number of fibers in view. For example, as in Fig. 2, if there were 6 fibers (center to center) between the left edge and the right edge of the field of view, this would represent 500μ ($5 \times 100\mu = 500\mu$).

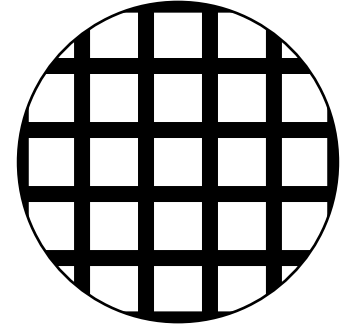


Fig. 2
“Actual field” of view.

Repeat the measurement with the 40x objective and complete Table 1.

Table 1

<u>Objective Magnification</u>	<u>Ocular Magnification</u>	<u>*Total Magnification</u>	<u>Field Diameter</u>
10x			
40x			

*Total magnification = (ocular magnification) x (objective magnification)

Calibrating the Measuring Eyepiece

Your right or left eyepiece should contain a “micrometer,” a ruler which you will have to calibrate twice, once for the 10x and once for the 40x objective. With the 10x in place, estimate the distance between the closest black lines by comparing their separation to the known space between fibers of parachute cloth. Since the actual distance between fibers is 0.1mm (100μ), divide the number of micrometer lines into 0.1mm to obtain the actual distance measured. For example, if 10 marks fit between two fibers, each mark measures 0.01mm ($0.1\text{mm}/10$), or 10μ at that particular magnification. Record your measurements for both the 10x objective and the 40x objective in Table 2.

Table 2

<u>Objective</u>	<u>Distance between marks</u>
10x	
40x	

You have now calibrated your measuring eyepiece for intermediate and high magnification. Use this calibration to measure the size of objects in the field during laboratory exercises. See Figures 3 and 4 for some comparative scales encountered in biology.

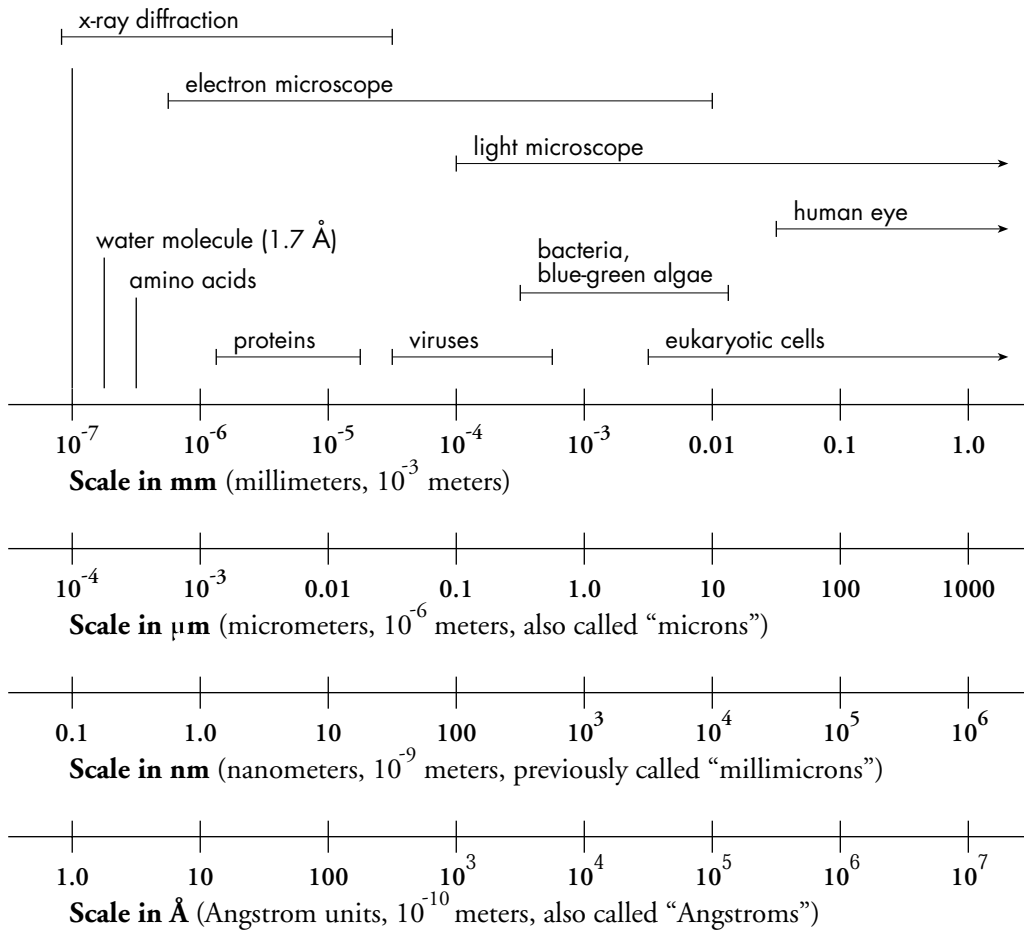


Fig. 3

Limits of resolution for various instruments. In cell biology, the micrometer and nanometer scales are the most useful. In chemistry, the nanometer and angstrom scales are important. Be familiar with these scales.