

Introduction:

I. Compound Microscope

Biologists in numerous subdisciplines use microscopes: genetics, molecular biology, cell biology, evolution and ecology. The knowledge and the skills you develop today will be used and enhanced throughout this course and your career in biology. It is important, therefore, that you take the time to master these exercises thoroughly. There are many variations of the compound microscope (Fig. 1), but the principles underlying all of these instruments are the same. The microscope consists of a lens system, a controllable light source, and a geared mechanism for focusing the specimen by adjusting the distance between the lens system and the specimen or object observed.



Fig. 1. Olympus CX41 compound microscope.

Lens and magnification

The magnification achieved by a compound microscope is the result of two systems of lenses: the objectives, nearest the specimen, and the ocular, or eyepiece lens, which are at the upper end of the microscope.

To achieve different degrees of magnification, four objectives are provided on our microscopes. They are attached to a revolving nosepiece. The 10X is the shortest objective and has 10X inscribed on its side to designate its power of magnification. Two high-dry objectives are of intermediate length and will have a magnification of 20X or 40X. The longest objective is the oil immersion objective that will have a magnification of 100X.

To determine the total magnification of a specimen, it is necessary to multiply the

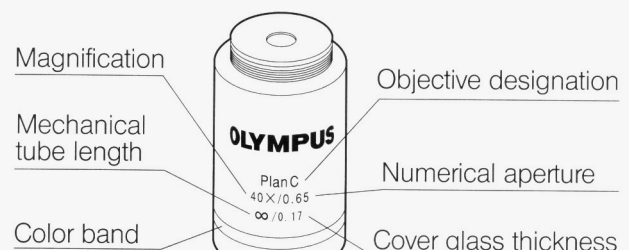


Fig. 2. Objective labeling.

magnification of the ocular lens by the magnification of the objective lens. The ocular magnification is inscribed on the top of the eye-lens mount (Fig. 2). If a specimen is viewed with the 40X objective, multiply 40 by 10 to get a total magnification of 400.

Focusing

For optimum viewing, adjust the eyepieces by following three steps. First, adjust the interpupillar distance (Fig. 3). While looking through the eyepieces, adjust for binocular vision until the left and right fields of view coincide completely.

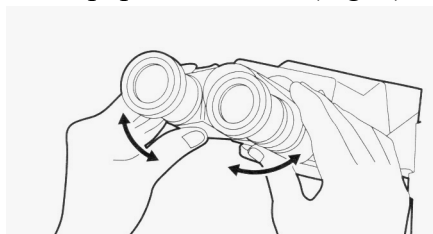


Fig. 3. Adjusting the interpupillar distance

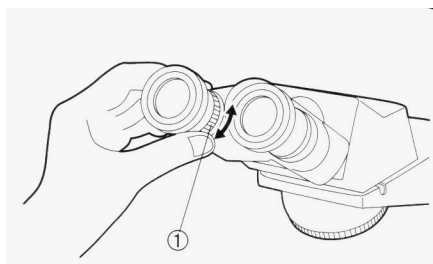


Fig. 4. Adjusting the diopter.

Record the value associated with the index dot so you can quickly make this adjustment in future labs. Second, adjust the diopter (Fig. 4). Looking through the right eyepiece with your right eye, rotate the coarse and fine adjustment knobs to bring the specimen into focus. Looking through the left eyepiece with your left eye, turn the diopter adjustment ring (1 in Fig. 4) on the specimen. Third, adjust the eyecups. If you wear eyeglasses, use the eyecups in the normal, folded-down position. This will prevent the eyeglasses from contacting and scratching the eyepieces. If you do not wear eyeglasses, extend the folded eyecups in the direction of the arrow (Fig. 5) for efficient use of the eyecups by preventing extraneous light from entering between the eyepieces and eyes.

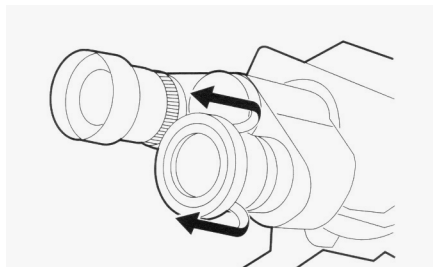


Fig. 5. Using the eyecups.

To focus the

microscope, it is necessary to alter the distance between the slide and objective lens. Knobs on the side of the microscope accomplish this. On most instruments, the objective lens is stationary and the stage moves up and down. Use the coarse adjustment to cover large distances. For critical focusing, the fine adjustment knob is used. Care must be used when using coarse adjustment. Always begin with the lowest power objective in place and bring the stage to the highest position. While looking in the oculars, bring the stage down using coarse adjustment until the object is clear. Never bring the stage toward the objective using coarse adjustment. Slides and lenses can be easily broken if brought into contact.

The working distance of the lens is the distance between the lens and the slide when the specimen is seen in sharp focus.

Illumination

The preferred light source for a microscope is an incandescent bulb because its color, temperature, and intensity can be controlled and stabilized easily. Condensers consist of two or more lenses that focus light from the illumination source onto a specimen. The light of the condenser is adjustable using a substage knob. Image sharpness is affected considerably by the condenser position (see details in Figs. 6-9). For most work, the condenser will be kept close to the image. The iris diaphragm, located between the condenser and light source, controls the amount of light entering the condenser. If too much light is allowed to illuminate the specimen,

image contrast decreases and depth of field is reduced. Excessive illumination may actually burn out the image so that objects become difficult to differentiate. Unstained specimens are best observed with low illumination to increase contrast. Both the 20 X and 40 X objectives will have phase contrast, which provides a means to increase contrast of low-contrast or transparent specimens without use of stains.

Details on Centering and setting the aperture on the iris diaphragm

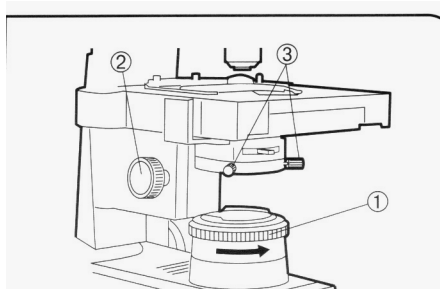


Fig. 6.

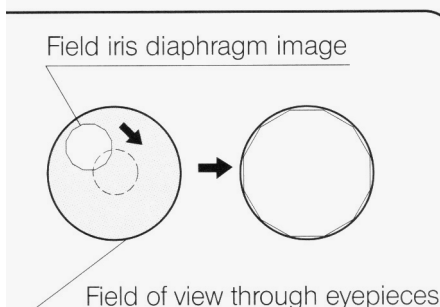


Fig. 7.

1. Centering the field iris diaphragm (Figs. 6,7)

- With the 10X objective engaged and the specimen brought into focus, turn the field iris diaphragm ring (1 in Fig. 6) counterclockwise to stop down the diaphragm to near its minimum size
- Turn the condenser height adjustment knob (2 in Fig. 6) to bring the field iris diaphragm image into focus
- Rotate the two auxiliary lens centering knobs (3 in Fig. 6) to adjust so that the field iris diaphragm image is centered in the eyepiece field of view
- To check centration, open the field iris diaphragm until its image touches the perimeter of the field of view. If the image is not precisely inscribed in the field of view, center again.
- When used for actual observation, open the field diaphragm until its image is slightly larger than the field of view.

2. Aperture iris diaphragm (Figs. 8,9)

- The aperture iris diaphragm determines the numerical aperture (1 in Fig. 9) of the illumination system. Matching the numerical aperture of the illumination system with that of the objective provides better image resolution and contrast, and also increases the depth of focus.
- Since the contrast of microscope specimens is ordinarily low, setting the condenser aperture iris diaphragm to between 70% and 80% of the N.A. of the objective in use is usually recommended. If necessary, adjust the ratio by removing the eyepieces and looking into the eyepiece sleeves while adjusting the aperture iris diaphragm knob until the image shown in Fig. 8 is seen (Fig. 9). Note: if the aperture iris diaphragm is set too small, image ghost may be observed.

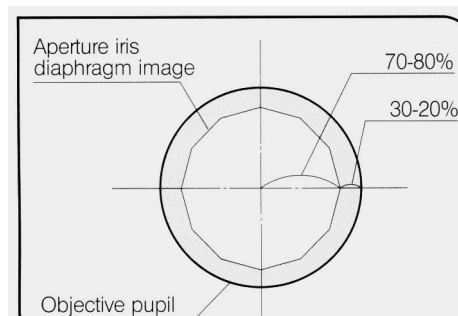


Fig. 8.

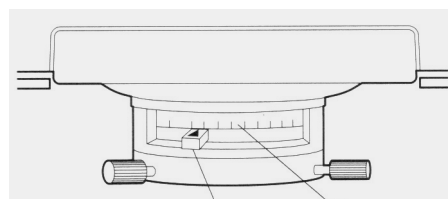


Fig. 9.

Use of the microscope for brightfield exposure: Terms listed in bold refer to controls illustrated in Figure 1.

1. If moving the microscope is necessary, grip the base firmly with one hand and the arm of the instrument with your other hand. Never pull or push the microscope across the bench. If it needs to be moved, carefully pick it up and move it.
2. Check the light source. Set the main switch to “|” (ON) using the **Main switch** and adjust the brightness with the **Light intensity knob**. Ask the Instructor for help if you are having problems. Notify the Instructor if the bulb fails to illuminate.
3. Check the lens: dust or oil on the lenses may impair your viewing. If the lenses seem to be smeared, please ask the Instructor for help. Improper cleaning can permanently damage the lenses. Use only dry lens tissue. Do not use any other type of paper or cloth. These will scratch the delicate surface of the lens.
4. Place the specimen on the stage using the **specimen holder** and **x-axis/y-axis knobs**. The specimen side of the slide should be facing up: move the slide until the material to be observed is illuminated by the light source.
5. Engage the 10X objective in the light path using the **revolving nosepiece**. Always begin with the lowest power objective to locate the specimen and then switch to greater magnification.
6. Bring the stage toward the objective using the **coarse/fine focus adjustment knobs**. Beginning with the stage all the way to the top allows you to use the coarse focus knobs to lower the stage until the object is in view. *Bring the stage toward the objective without looking can result in damage to the slide or objective.*
 - a. Adjust the interpupillary distance using the **binocular tube** (Fig. 3)
 - b. Adjust the diopter with using the **diopter adjustment ring** and **condenser height adjustment knob** (Fig. 4)
 - c. Adjust the light axis with the **auxiliary lens centering knob**
 - d. Adjust the aperture iris and field iris diaphragms (Figs. 6-9)
7. Engage the desired objective in the light path and bring the specimen in focus using the **revolving nosepiece** and **fine focus adjustment knobs** only.
8. Switch to higher magnification: **use only fine focus with higher power objectives.**
 - a. Adjust the brightness with the **light intensity knob**.
9. Oil Immersion (Fig. 10):
 - a. Locate the subject area using the 10X objective. Move from 20X to 40X centering the specimen in the viewing area.
 - b. Rotate the nosepiece between the 40X and 100X objectives. Place 1 drop of immersion oil on top of the cover glass
 - c. Rotate 100 X objective into oil and light path
 - d. Use fine adjustment knob to focus
 - e. Adjust condenser diaphragm to $\frac{3}{4}$ open
 - f. Adjust light intensity
 - g. **Avoid getting oil on other objectives**

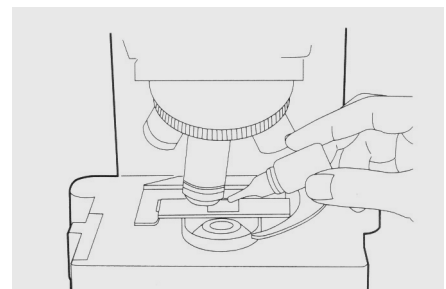


Fig. 10. Using oil immersion objective (100x)