BASIC OBSERVATION PROCEDURES FOR COMPOUND MICROSCOPES

Good microscopy requires the adoption of work habits that facilitate observation,minimize fatigue and eyestrain, and protect the equipment from damage. Follow these steps each time you use the compound microscope.

Prepare the microscope

1. Use **both hands** to lift the microscope from its storage cabinet, and place it directly in front of you on the laboratory table. Carefully remove the dust cover and place it back in the storage cabinet so it is out of the work area. Unwrap one or two turns of the power cord and plug the microscope into a power outlet.

2. Switch the power switch to "I" (on) and adjust the brightness by turning the light intensity knob on the upper right side. Use the lowest intensity that provides good illumination for your specimen.

3. Engage the 4X objective: use the grooved ring at the top of the nosepiece to turn the objectives. **Never handle the objectives themselves**. Make sure that the nosepiece stops with an audible click.

Focus on your specimen

4. Pull back the spring clip prior to placing a specimen slide on the stage. Then gently release the spring clip so that it provides tension to hold your slide in place. When using a cover slip, always view a slide with the cover slip towards the objective lens (i.e. above the specimen, for the scopes in BI 454).

5. Turn the X-axis and Y-axis knobs (shown on the diagram) to move the specimen into light path.

6. Now, adjust the interpupillary distance of the eyepieces. With both eyes open, grasp the eyepiece base with the thumb and forefingers of each hand, and move the eyepieces slowly apart and together until you see a single image of the specimen.

7. It's a good habit to focus **away from the stage**: watch your 4X objective as you use the coarse adjustment knob to bring it close to your slide without touching it. Now, closing your left eye and looking through the right eyepiece with your right eye, turn the coarse adjustment knob to bring the specimen into focus. After obtaining approximate focus, use the fine adjustment knob to focus the image further.

*The objective lenses on your microscope are **parfocal**. This means that once you have focused on a specimen at 4X or 10X with the coarse adjustment, you should only need to use the fine focus adjustment for 40X and 100X because the specimen will already be in approximate focus.*

From time to time, you will find that the fine focus does not seem to work in focusing the image further. This may indicate that the fine focus has been wound to its maximum. "Unwind" by turning it 25 or so revolutions in the opposite direction. Re-focus with the coarse focus, and try again.

8. Looking through the left eyepiece with your left eye, turn the diopter adjustment ring on the left eyepiece until the image appears sharp and clear to your left eye.

Center and focus your condenser lens

Note: Steps 9-11 must be done for each objective. In other words, when you change objectives, you should recenter and focus the condensor lens for optimum viewing of the specimen.

9. **The condenser aligns and focuses light on the specimen.** Adjust the condenser lens so that it is centered with regard to your objective lens in the horizontal plane and focused with regard to your specimen in the vertical plane. Start with the 10X objective. Bring your specimen into focus. While looking through the eyepieces, turn the field

diaphragm counterclockwise until only a small circle of light is visible. Raise the condenser height adjustment knob to bring the field diaphragm image into focus: the edge of the circle of light should be sharp.

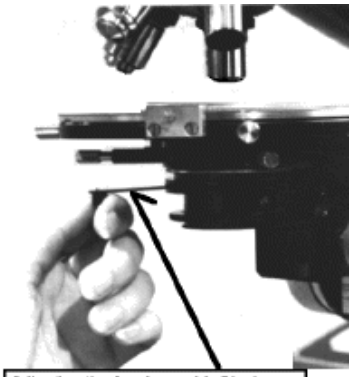
10. There are two small knobs on the front of the condenser, set at 45º, which are used to center it. Adjust the centering screws to insure that the circle of light is in the center of the field of view, and check your centering by opening the field diaphragm until its image touches the perimeter of the field of view. Does the image touch equally on all sides?

11. Open the field diaphragm until light just fills the field of view (i.e., do not fully open the diaphragm).

If you are having problems focusing, check the centering of your condenser lens for each objective.

12. **Finally, there is a lever which controls the aperture of the Condenser Iris. This improves contrast (difference between light & dark) especially at intermediate and high magnifications.** Using the 40X objective, focus the image. Experiment by altering the amount of light passing through the specimen by opening and closing the condenser iris diaphragm with the lever located above the centering screws. You will need to adjust the condenser iris diaphragm for each different specimen you observe, depending on the amount of contrast your observation requires.

*When actually observing your specimen, open the **condensor** (not field) iris diaphragm until its image just circumscribes the field of view. However, when greater contrast is desired, such as observation of unstained cells, turn up the light but close the aperture.*


Adjusting the Condenser Iris Diaphragm

Adjust the light intensity

13. Adjust the light intensity using the light intensity knob on the right side of the base. Generally, the higher the magnification, the higher the intensity of the light you will require. Always turn the light intensity knob fully down before switching off the power.

Storing the microscope

14. When you have finished using the microscope, lower the stage, gently remove the specimen from the stageclips. Lower the light intensity to its lowest setting, and switch off the power. Unplug the instrument and carefully wind the power cord either on the cord winder or on the base, replace the dust cover, and return the scope to its cabinet.

Parts Key for the CH30/CH40 Scope

Fig. 14
1. main power switch
2. light intensity knob

Fig. 15
1. field iris diaphragm dial

Fig. 16
1. coarse focus adjustment knob
2. fine focus adjustment knob

Fig. 17
1. Y-axis knob
2. X-axis knob

Fig. 18
Eyepiece base. Arrows show how to adjust the interpupillary distance.

Fig. 19
1. diopter adjustment ring

Fig. 20
1. field iris diaphragm dial
2. condenser height adjustment knob
3. condenser centering screws (these should be positioned in the front of the stage; if not, swivel to front)

Note that there are also small screws above the condenser centering screws. ***Do not turn these*** *or the condenser lens will fall out.*

Fig. 21
Field iris diaphragm image as seen by looking through ocular after centering and opening diaphragm.

Fig. 22.
For most specimens, set the condenser aperture iris diaphragm to 70% - 80% of the numerical aperture of the objective in use. When necessary, adjust the ratio by **asking your instructor** to remove the eyepiece and look into the eyepiece sleeve while adjusting the aperture iris diaphragm lever until this image is seen.

Fig. 23.
1. numerical aperture (matching this number to that of the objective provides better resolution and contrast).
2. aperture iris diaphragm lever (use to adjust aperture).

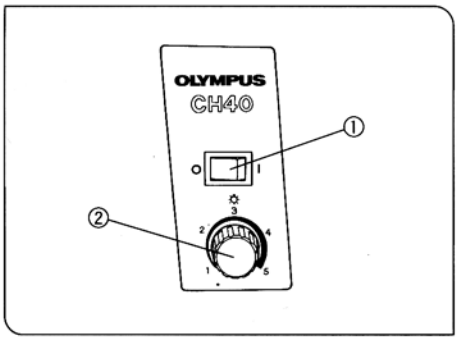


Fig. 14

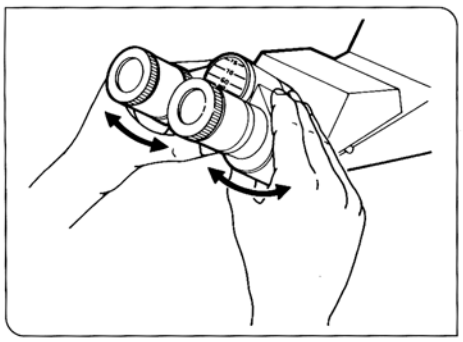


Fig. 18

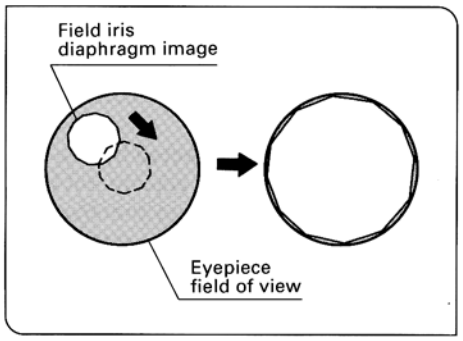


Fig. 21

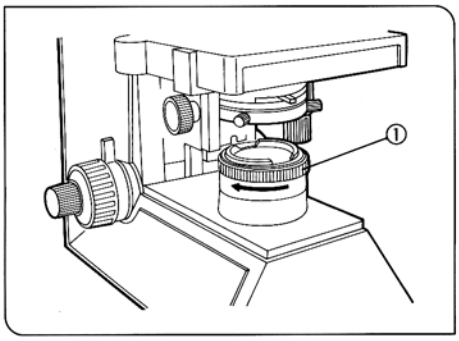


Fig. 15

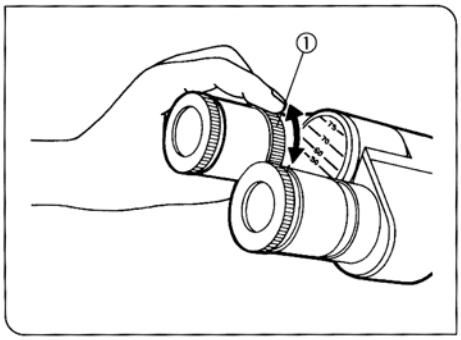


Fig. 19

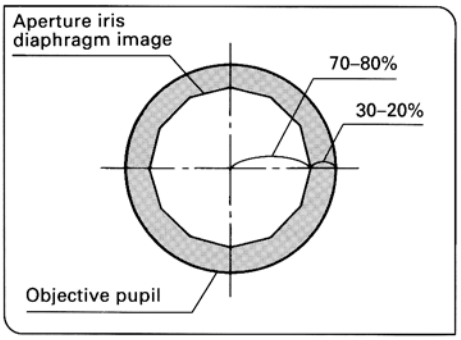


Fig. 22

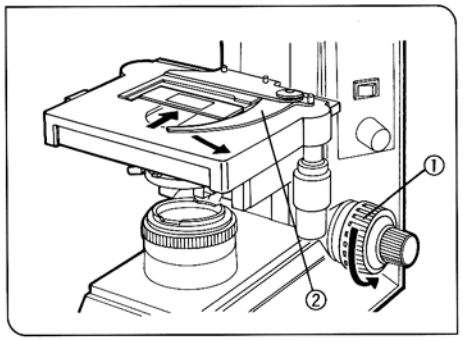


Fig. 16

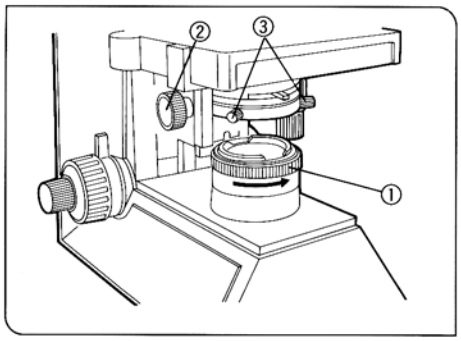


Fig. 20

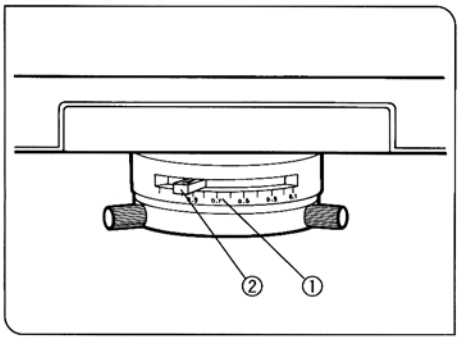


Fig. 23

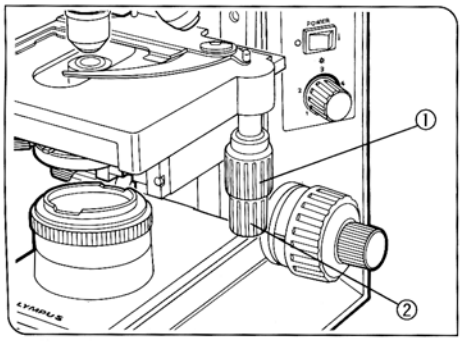
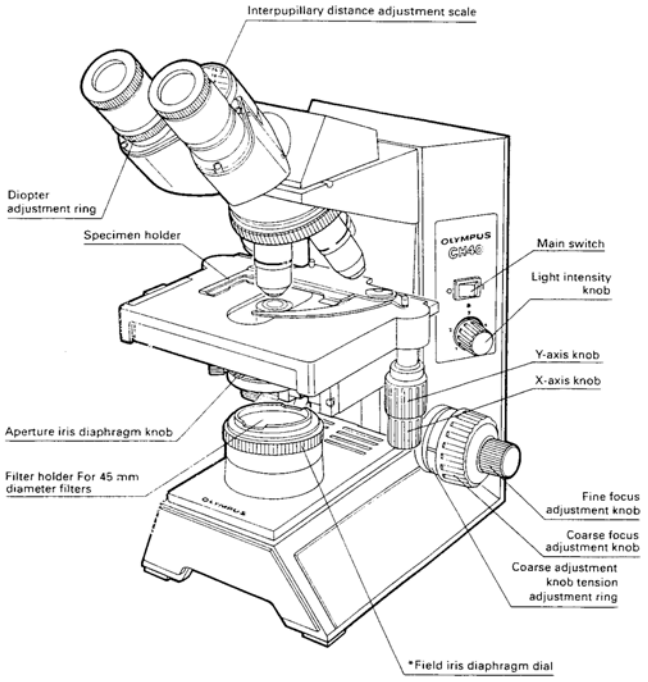


Fig. 17



HOW TO USE THE OIL IMMERSION OBJECTIVE

1. Focus on the specimen with the 10X objective. (why 10X and not 40?? If you focus with 40 X with typical slides you should just rotate the 100X objective into place. I think doing step 4 is dangerous for the microscopes-although necessary for the gridded slides with the bars around edge.)
2. Place a small drop of immersion oil onto the specimen at the area to be observed.
3. Rotate the 100X objective into position without getting the 40X objective in the oil.
4. While observing from one side of the stage, use the fine focus knob to slowly raise the stage until you see the meniscus of the oil on the specimen come in contact with the tip of the 100X objective. Now go to the eyepieces and observe as you finish focusing with the fine focus knob. You can make small lateral slide movements with the immersion oil on the slide.

Since any bubbles in the oil will impair the image, make sure that the oil is free of bubbles. To check for bubbles, ***ask your instructor*** *to remove the eyepiece and fully open the field and aperture iris diaphragms, then look at the exit pupil of the objective inside the observation tube. The pupil should appear round and bright. To remove bubbles, rock the nosepiece slightly to move the oil immersion objective back and forth a few times.*

5. When you have completed your observations using the immersion oil, slowly lower the stage with the coarse focus knob until the 100X lens is out of contact with the oil and rotate the objective right or left so a shorter lens, the 4X clicks into position. DO NOT rotate the 40X objective through the oil!!!
6. Remove the slide and wipe the excess oil off the slide and the tip of the 100X objective.

HOW TO CLEAN LENSES

Wipe any dirty lenses clean by pulling a lens tissue (not Kimwipe) across the surface while applying light finger pressure. If the lens is still dirty, wet the lens tissue with a drop of lens cleaning solution, rub gently, and dry by pulling a dry lens tissue across the surface.

It is often sufficient to wipe the oil immersion lens with a dry lens tissue. A thin film of oil can be left on the lens without impairing focus. Using too much xylene (a solvent found in lens cleaner) can dissolve the lens adhesive.

5-5 Focusing Adjustment Knobs

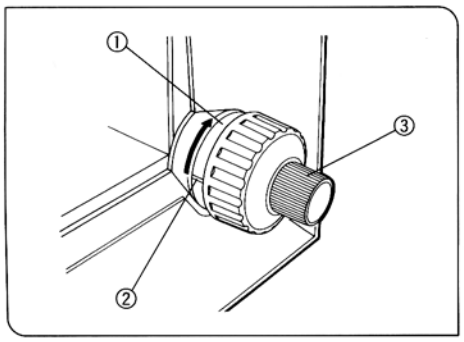


Fig. 24

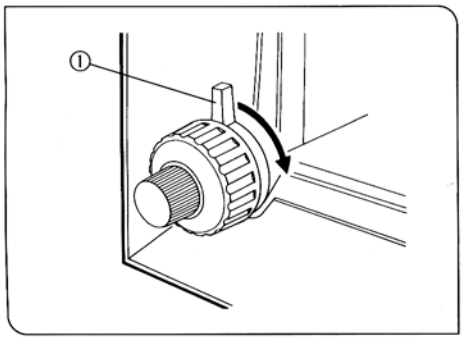


Fig. 25

1 Adjusting the Coarse Adjustment Knob Tension (Fig. 24)

1. The coarse adjustment knob tension is preadjusted for easy use. However, if desired, one can change the tension using the tension adjustment ring ①. Applying a flat-bladed screwdriver to any of the grooves ② on the circumference of the ring and turning the ring in the direction of the arrow increases tension, and vice versa.
2. The tension is too low if the stage drops by itself or focus is quickly lost after adjustment with the fine adjustment knob ③. In this case, turn the ring ① in the direction of the arrow to increase tension.

2 Pre-focusing Lever (Fig. 25)

The pre-focusing lever ensures that the objective does not come in contact with the specimen and simplifies focusing. After focusing on the specimen with the coarse adjustment knob, turn this lever ① clockwise in the direction of the arrow to set a lower limit on coarse adjustment movement.

◎ Focusing with the fine adjustment knob is not affected by this pre-focusing lever. Accordingly, after using the coarse adjustment knob to lower the stage for changing specimens or applying immersion oil (see Section 5-6), refocusing is easily accomplished by rotating the coarse adjustment knob to reach the pre-focusing position, then making fine adjustments with the fine adjustment knob.

★ When not required, leave the pre-focusing lever unlocked.

5-6 Immersion Objectives

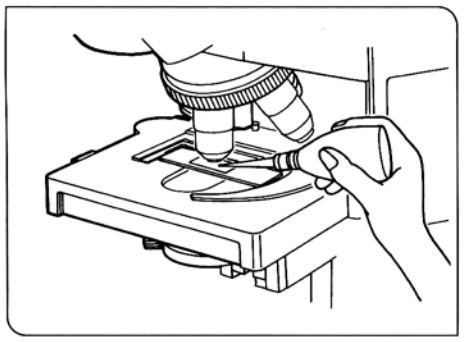


Fig. 26

1 Use of Immersion Objectives (Fig. 26)

1. Focus on the specimen with a low power objective.
2. Place a drop of immersion oil (provided) onto the specimen at the area to be observed.
3. Turn the revolving nosepiece to engage the immersion objective, then focus using the fine adjustment knob.

   ★ Since any bubbles in the oil will impair the image, make sure that the oil is free of bubbles.

   a. To check for bubbles, remove the eyepiece and fully open the field and aperture iris diaphragms, then look at the exit pupil of the objective inside the observation tube. (The pupil should appear round and bright.)

   b. To remove bubbles, rock the nosepiece slightly to move the oil immersion objective back and forth a few times.

◎ If the condenser marking shows a numerical aperture (NA) of 1.0 or more, the number applies only when oil is present between the slide glass and the top surface of the condenser. When oil is not present, the NA is about 0.9.

4. After use, remove oil from the objective front lens by wiping with gauze slightly moistened with an ether (7 parts) / alcohol (3 parts) mixture or with xylene.

   ★ Using too much xylene can dissolve the lens adhesive.