BASIC MICROSCOPY SETUP

by

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The subject of setting up the compound microscope for proper illumination is frequently misunderstood or not properly performed. The following procedure is used at CCI for the microscopy series of classes. For those of you who have been away from the microscope for some time, this procedure will help you review certain basic steps should you have occasion to use the microscope. Although this procedure is for the Olympus BH2 series microscope, the concepts are generic for all microscopes.

MICROSCOPE SETUP AND KOEHLER ILLUMINATION

I. Basic Microscope Setup

A. Binocular Adjustment

1. Turn on the light
2. Adjust the light intensity, it should not be higher than 6 volts on the LED scale.
   Make sure the eyepiece with the micrometer is in the right eyepiece tube. Align the positioning pin on the eyepiece with the slot in the tube and insert the eyepiece.
3. Focus on your specimen
4. Adjust the inter-pupillary distance for your best binocular vision.
5. Focus the right eyepiece (with the micrometer) to bring the eyepiece micrometer (figure 1) into focus by adjusting the knurled ring on the eyepiece.
6. Sharply focus on the specimen. The specimen and the micrometer should both be in sharp focus through the right eye lens.
7. Look into the left eyepiece, and with the left eye only, adjust the diopter adjustment ring on the left eye piece to bring the specimen into the sharpest focus.

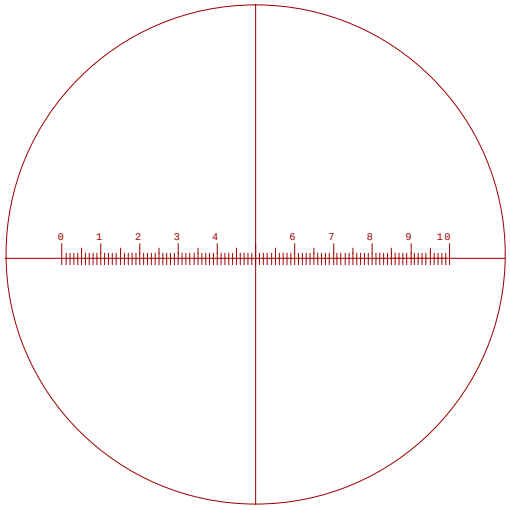
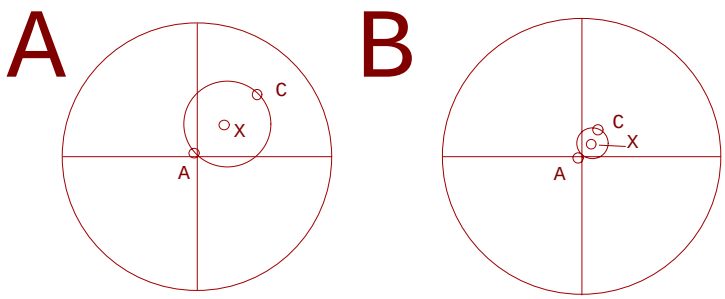


Figure 1

II. Stage Centering Procedure

1. Place a specimen on the stage and focus on it with the 40X objective. Be sure the 40 X is in a noncenterable position.
2. Select a point on the specimen and locate it under the cross hair. Note this point as point A (figure 2).
3. Rotate the specimen 180 degrees and this will be point C.
4. The center position is halfway between point A and point C at the X in figure 2. Use the centering wrench in the stage to move point "C" halfway to the cross hairs.
5. Repeat steps 2-4 until the stage is centered as much as possible.



Move "C" to "X" or "X" to the center of the cross hairs

Repeat until stage is centered: Move "C" to "X" or "X" to the center of the cross hairs

Figure 2

III. Objective Centering

1. Place a specimen on the stage and focus on it with the 10X objective.
2. Select a point on the specimen and locate it under the cross hair. Call this point A.
3. Rotate the specimen 180 degrees and this is point C.
4. The center position is halfway between point A and point C at the X in figure 2. Use the centering wrench in the stage to move point "C" halfway to the cross hairs.
5. Repeat the steps 2-4 until each of the objectives are centered as much as possible.

IV. Koehler Illumination

A. Condenser adjustment (see figure 3 for description of the microscope and its components parts)

1. Check the focus of a specimen with the 10X objective.
2. Open the aperture diaphragm on the condenser lens wide open.

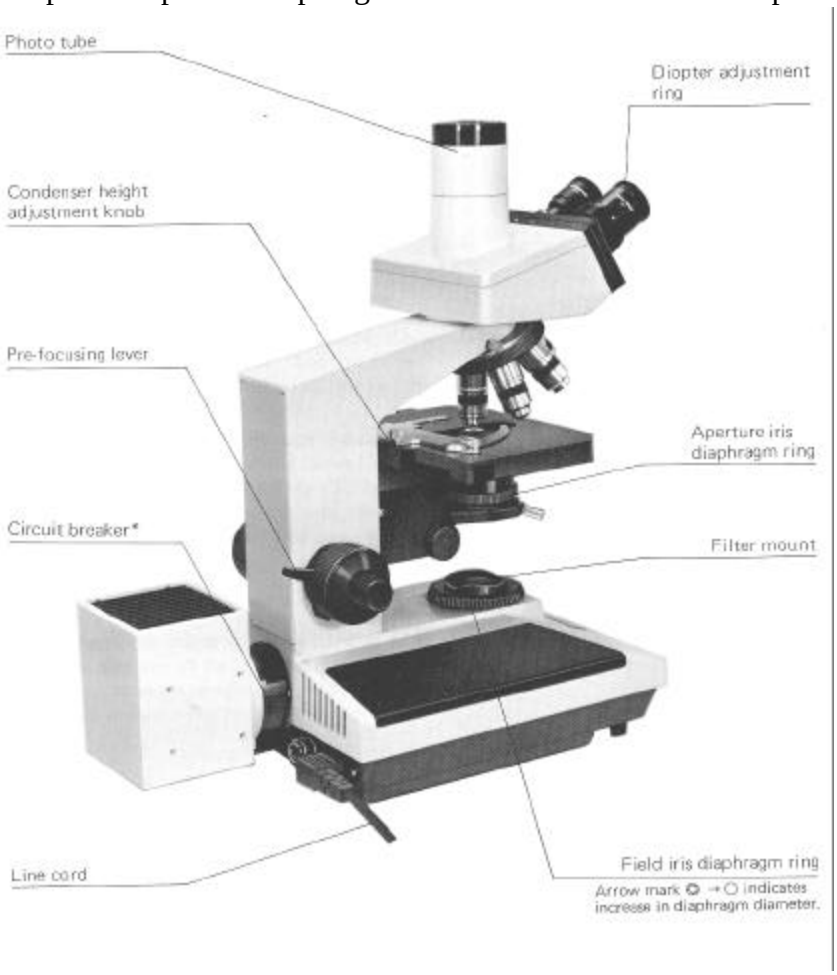


Figure 3

3. Make sure the top condenser lens is in position.
4. Close down the aperture lens of the field diaphragm.
5. Adjust the condenser height until the image of the field diaphragm (the edges of the diaphragm) are in sharp focus. (see figure 4). Reduce color fringes to a minimum.

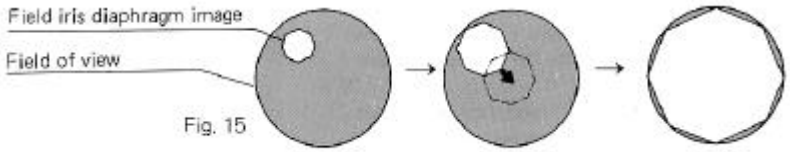


Figure 4

6. Center the image of the field diaphragm by using the centering screws on the condenser.

7. Gradually widen the field diaphragm ensuring that it remains centered. Open it to just outside the field of view.

V. Aperture Iris Diaphragm Adjustment

1. Adjust the aperture iris diaphragm to the numerical aperture of the objective lens by stopping it down.
2. Focus on the specimen.
3. Remove the right eyepiece.
4. Adjust the diaphragm until it leaves about 65 to 80% of the field of view. (figure 5).
5. This completes the Koehler illumination setup (figure 6)

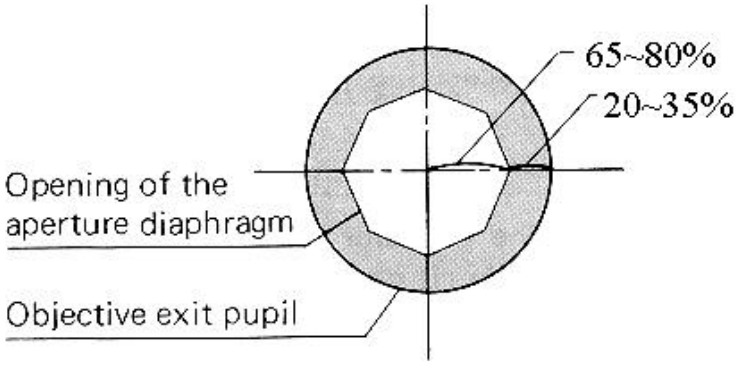


Figure 5

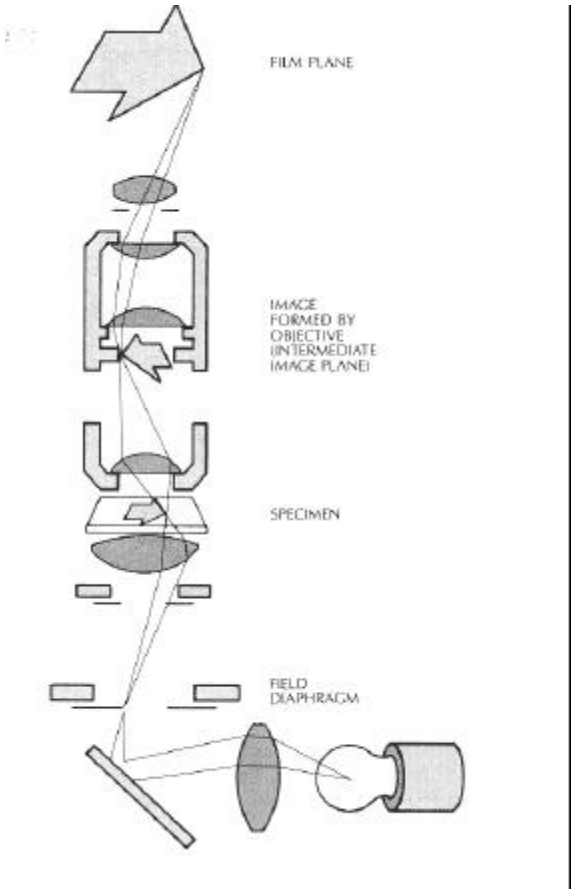


Figure 6

VI. Miscellaneous Hints

A. For observation at 4X, swivel the top condenser lens out of the light path.

VII. Optional Focus Adjustment Options Adjustment

A. Tension Adjustment

1. One can adjust the tensioning of the coarse focus knob by rotating the tension adjustment ring (see figure 7)

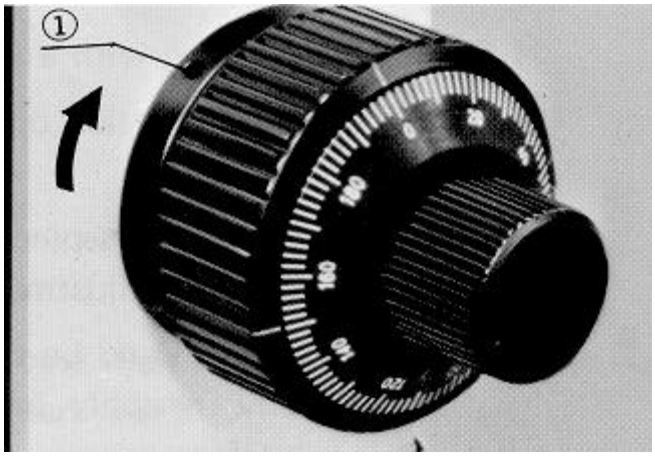


figure 7

B. Prefocus Lock

1. After coarse focus has been made on a specimen, one can lock the lever to prevent the objective from making contact with the specimen. This does not affect the adjustment of the fine focus.(figure 6)

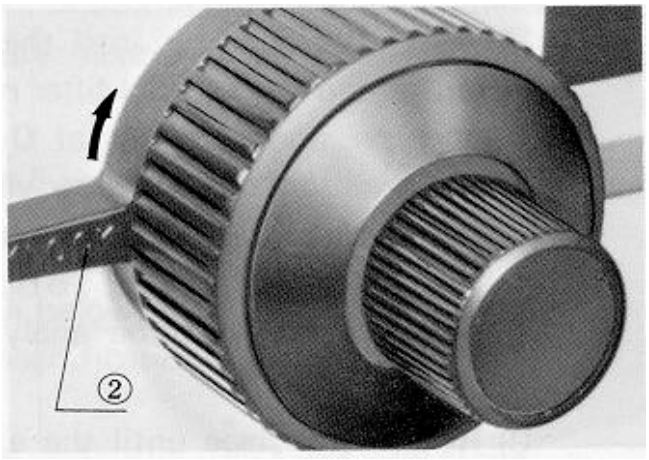


Figure 8