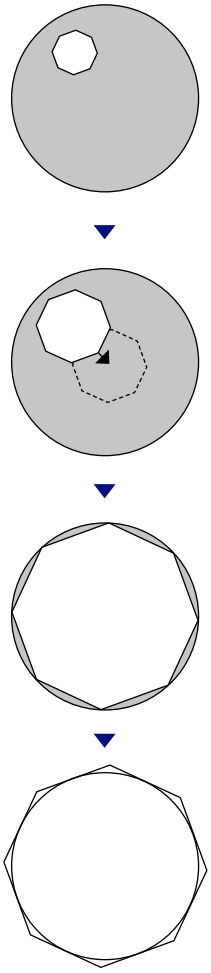
# Köhler Adjustment—Transmitted Light Microscopy

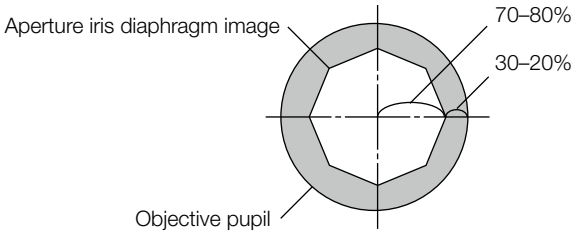
Another important procedure that should be included in your regular maintenance is checking the Köhler illumination. Köhler illumination is an optical alignment method that helps you produce an evenly illuminated visual field with excellent contrast and high sample detail, enabling you to exploit the microscope's full potential.

## Upright Microscopes

1. Turn on the microscope's illumination and set the microscope to brightfield mode.
2. Remove unnecessary contrast devices, such as DIC sliders, polarizers, or other optical elements.
3. Engage the 10X objective lens (if available), place the specimen on the microscope stage, and bring it into focus.
4. If available, engage the substage condenser front lens and move it closer to the specimen until it is at the approximate condenser working distance.
5. Close the field stop until its borders become visible when observing the focused microscopic image, and fine-tune the condenser height-adjustment knob to focus the field stop diaphragm image.
6. Using the condenser centering screws, center the field stop diaphragm image.
7. Slowly open the field stop, observing the ring as it approaches the border of the field of view and stopping as soon as the field stop ring disappears.
8. Watching the back focal plane of the objective (observation without eyepiece), open the aperture diaphragm until a considerable change in contrast is noticeable in the image (NA of condenser = 0.7 to 0.8 times the NA of the objective). When 70–80% of the image is bright (see the image on the bottom right), then reinsert the eyepiece.
9. Repeat steps 6 and 7 of this procedure each time you switch to another objective lens.

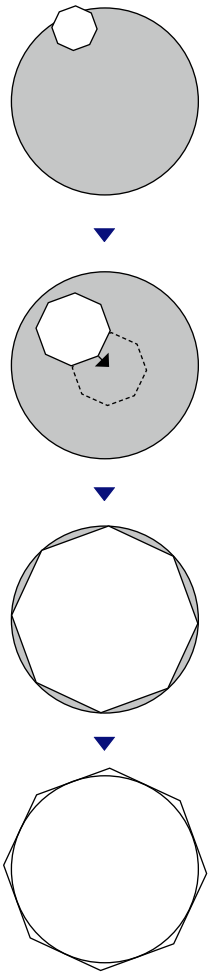


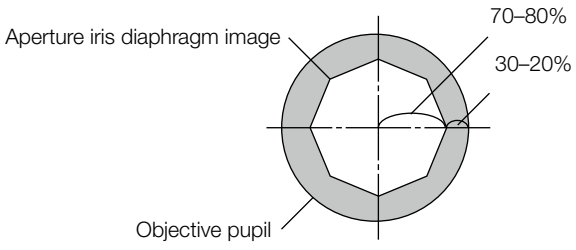
Tip: When using U-SC or UCD8(A) condensers in combination with objective lenses magnifying less than 10X, swing out the top lens of the condenser, open the aperture stop, and use the field stop as an aperture stop.



## Inverted Microscopes

1. Turn on the microscope's illumination and set the microscope to brightfield mode.
2. Remove unnecessary contrast devices, such as DIC sliders, polarizers, or other optical elements
3. Engage the 10X objective lens (if available), place the specimen on the stage, and bring it into focus.
4. Move the condenser front lens closer to the specimen until it is at the approximate condenser working distance position.
5. Close the field stop until its borders become visible when observing the focused microscopic image, and fine-tune the condenser height-adjustment knob to focus the field stop diaphragm image.
6. Using the condenser centering screws, center the field stop diaphragm image.
7. Slowly open the field stop, observing the ring as it approaches the border of the field of view and stopping as soon as the field stop ring disappears.
8. Watching the back focal plane of the objective (observation without eyepiece), open the aperture diaphragm until a considerable change in contrast is noticeable in the image (NA of condenser = 0.7 to 0.8 times NA objective). When 70–80% of the image is bright (see the image on the bottom right), then reinsert the eyepiece.
9. Repeat steps 6 and 7 of this procedure each time you switch to another objective lens.



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