

Figure 4. Kohler illumination procedure

The purpose of Kohler Illumination is to adjust the field and condenser diaphragms to give uniform illumination of the field of view and to optimize contrast and resolution of a specimen by focusing and centering the light path.

Perform this procedure **daily** before using the microscope and after maintenance on the microscope.

1. Turn on the microscope and set the brightness control dial to a comfortable level.
2. Ensure that the condenser is positioned directly under the stage. If it is not, use the condenser knob to raise the condenser.
3. Rotate the 10X objective into position. Verify that the eyepiece diopter rings are set at the zero diopter setting.
4. Place the QC slide (a Wright stained blood smear) for Koehler on the microscope stage.
5. Adjust the interpupillary distance so that both right and left eye images merge into one.
6. Use the coarse knob to focus the 10X objective. Adjust with the fine focus.
7. Switch to the 40X objective and fine focus.
8. Switch back to the 10X objective.

Each eyepiece requires adjustment according to an individual's sight requirements. This is accomplished according to step 9.

9. Close the left eye. Use the right eye to look into the right piece. Focus the field of view using the right eyepiece diopter. Repeat the process for the left eye using the left eyepiece.
10. Close the field diaphragm down to the smallest opening with the field diaphragm ring.
11. Move the condenser up and down until the image of the field diaphragm is in sharp focus. This usually entails moving the condenser near the top.
12. Center the image of the field diaphragm by adjusting the two condenser centering screws on the condenser carrier.
13. Slowly open the field iris diaphragm until it just disappears from view.
14. Adjust fine focus for detail.
15. Open the condenser diaphragm completely.
16. Partially close the condenser diaphragm until the background darkens slightly to preference, or match the opening of the condenser diaphragm with the numerical aperture (N.A.) of the objective, usually achieved by reducing the diaphragm opening to 70 to 80% of the N.A. value.