Köhler Alignment

Köhler alignment should be performed every time you change your sample or the microscope objective. With proper alignment, transmitted light imaging will require lower intensity illumination light and the images will show better contrast. Note the double image and the poor colour reproduction due to mis-alignment in the images below.

1. Choose the desired objective lens.
   It is often best to start with 10x and then fine tune the alignment on higher magnification lenses.

2. Place a sample on the microscope and focus on it.

3. Close the Field Diaphragm.

4. Move the Condenser Focus Knob so that the field diaphragm is in focus in the eyepieces.

5. Use the centering screws on the condenser to position the field diaphragm in the centre of the field of view.

6. Open the field diaphragm so that it appears to just touches the edges of the field of view.

7. Use the centering screws to fine tune the alignment so that the field diaphragm in the centre of the field of view.
8. Open up the **field** diaphragm until it is just slightly larger than the field of view.

9. **The condenser aperture should be set to \(~80\%\) of the NA for the objective lens.** This can be done using the aperture scale on the condenser aperture slider. It can also be done by eye. You can remove the eyepieces and look down the oculars and set the aperture visually to that it is filling \(~80\%\) of the back aperture of the objective lens. For example, for a 0.5 NA lens you would set the aperture to 0.4.

***** Köhler alignment should be performed **EVERY TIME** you change the microscope objective. *****