# Köhler Illumination

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Köhler illumination[1] ensures uniform illumination and maximum microscopic resolution. Before beginning, adjust the interpupillary eyepiece distance and focus each eyepiece independently. The entire procedure is completed within seconds. The openings of the field diaphragm and substage condenser diaphragm are different for each magnification. For best results the microscope must be clean.

1. At 10x, select a high contrast object to focus on (eg, a superficial squamous cell nucleus), and center it within the field-of-view.
2. **Close** the field diaphragm to see whether its image is centered.
- Close the substage condenser aperture diaphragm more.

3. **Center** the image of the field diaphragm while using the dark surrounding area as a convenient guide. The cell focus should not change throughout this procedure.
- Close the field diaphragm more.

4. **Focus** the image of the field diaphragm iris leaves in the object plane by adjusting the height of the substage condenser. The halo will be red and blue; the intensity will vary with the substage condenser aperture opening. *After Step 6, it is OK to defocus dirt images under 10x.*

- If the image of the field diaphragm iris is ringed by a soft yellow halo, the substage condenser is too high and nearly touching the underside of the slide.

- Lowering the substage condenser slightly eventually produces a soft magenta halo, which appears more distinctive when using the 40× objective.

- Lowering the substage condenser even more produces a soft blue halo. The colors described in these 3 bullet points occur within a few millimeters. To the unaided eye, it is difficult to see that the substage condenser has been moved at all.

5. Open the field diaphragm until its image just disappears from view.
6. Adjust the substage condenser diaphragm until you see the best contrast. Closing it too far imparts a refractile quality to the cells due to diffraction. Opening it too far creates contrast-degrading glare. Follow the same 6-step procedure for each objective as needed. LM

1. Köhler A. A neues Beleuchtungsverfahren für microphotographische Zwecke. *Zwiss Mikr*. 1893;10:433-440.