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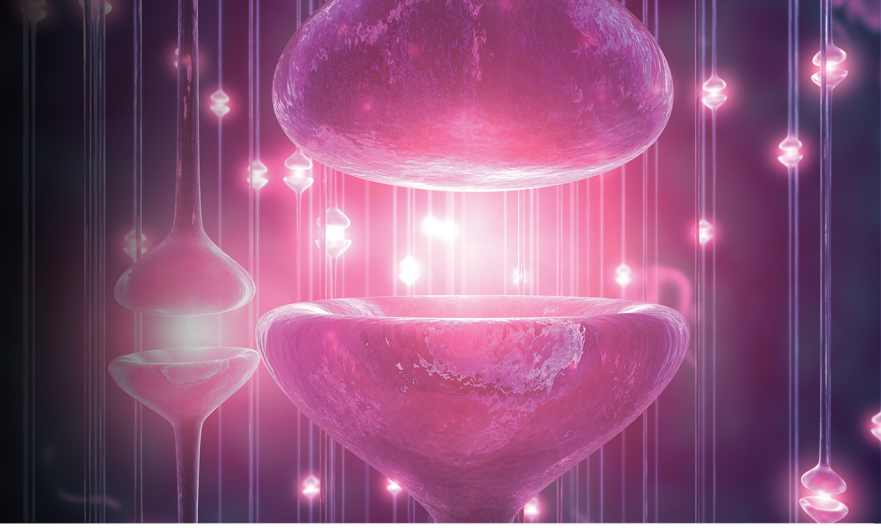
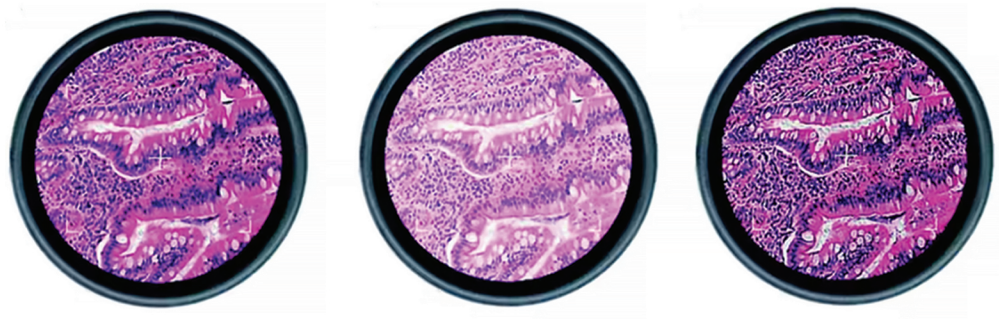


#LabHacks

A Six Step Guide to Setting Up Köhler Illumination

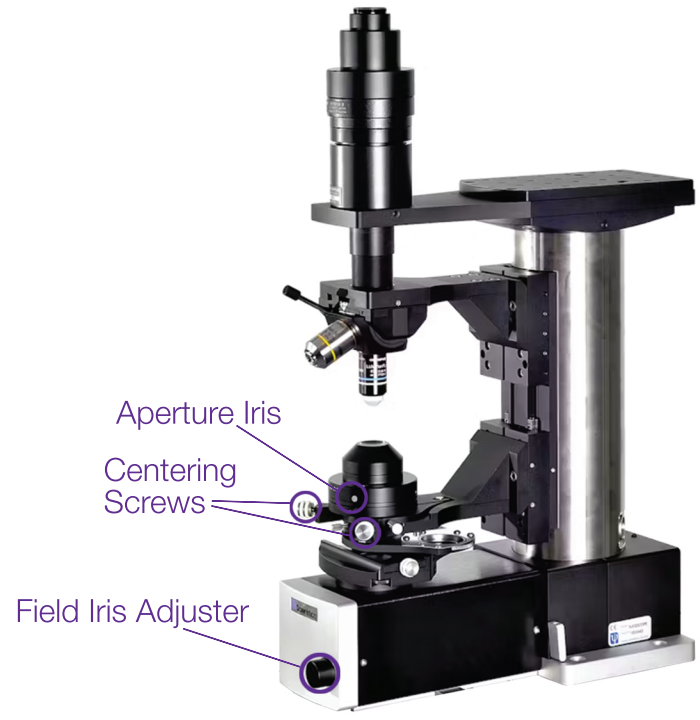
What is Köhler illumination?



Images of a histology tissue section showing from left to right, situation with a non-aligned condenser, the process of aligning the condenser, and a proper aligned system - notice how the contrast and resolution in the image are improved after the condenser has been aligned. Image Credit Michael W Davidson, University of Florida

Köhler illumination is a technique used in microscopy to achieve optimal contrast and resolution by focusing and centering the light path, ensuring even illumination across the field of view.

Sophisticated and well-equipped microscopes fail to yield quality images because of incorrect use of the light source. Illumination of a specimen should be bright, glare-free, and evenly distributed throughout the field of view to produce high-quality images. Köhler illumination is critical for any observation method (ie DIC).

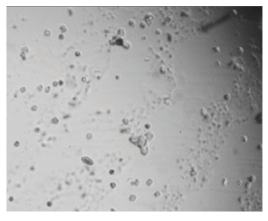


To use this technique you need a condenser that's movable in Z for focus and able to be centred.

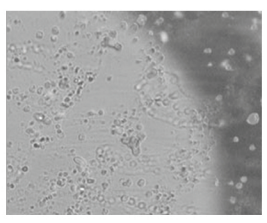
Steps to set up Köhler illumination

Focusing the condensor

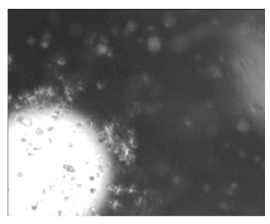
1. Place a thin specimen onto the stage, making sure it is properly centered and secured. Focus using a low magnification (4x or 10x) objective, ensuring the condenser front lens is about 0.5cm from the bottom of the coverslip for an optimal starting position.



2. Using the field iris diaphragm control, located on the front surface of the substage optics, close the diaphragm right down while looking at your monitor. You will see a dark circle encroaching on the screen. If dark circle does not fall within your field of view, you may need to use the two silver adjusting screws on the condenser arm to centre your condenser.

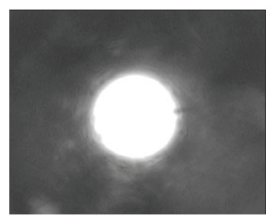


3. Move the condenser up or down until the edge of the dark circle (the iris blades) appears in sharp focus on the monitor. Note- depending on the working distance of the condenser you may be in a close proximity to the sample.

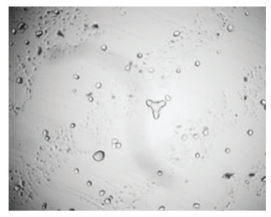


Centering the condensor

4. Use the two silver adjusting screws on the condenser arm to center the multi-edged shape. Turn the screws until the shape appears in the center of the field of view. Opening the diaphragm near the edge of the field of view can aid in this process.

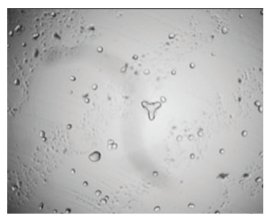


5. After focusing and centering the condenser, open the diaphragm just beyond the field of view. While the condenser stays centered with different objectives, adjust the field iris diaphragm to the edge of the field of view for each magnification.



Adjusting the aperture iris

6. Locate the aperture iris control, typically a lever on the condenser. Ensure the condenser is focused and centered, then close the iris to occupy roughly the outer 20% of the field. This enhances contrast for easier observation, but avoid closing it too much as it can reduce resolution significantly.*



Top Tip : A more accurate way of adjusting the aperture iris is to note the numerical aperture (or NA) on the objective, then set the NA on the condenser to 20% less. For example, with a 40x objective with a numerical aperture value of 0.65, set the graduation on the condenser to 20% less, approximately 0.5.