Setting up Kohler Illumination

Dale A. Callaham
The Central Microscopy Facility
University of Massachusetts, Amherst, MA 01003 USA

Kohler illumination is an illumination technique that provides optimum resolution and contrast in a light microscope by aligning and focussing the illumination, and critically setting the apertures of the microsope to best match the objective lens Numerical Aperture.

1) **Place slide on the stage and bring to focus**. If the microscope is way out of alignment, this may require roughly aligning the illumination by centering the condenser, opening the condenser aperture diaphragm, opening the field diaphragm, and focusing the condenser to give initially reasonable illumination so you can see well enough to focus on the specimen.

2) **Stop down the Field Diaphragm.** This is the diaphram in the fixed part of the microscope, the part that bears a constant distance from the lamp source.

3) **Focus the Condenser**. Turn the focus knob on the condenser until the (edge) image of the Field Diaphragm is sharp - there may be a red fringe on one side of focus and blue on the other: go for the center between these.

4) **Open the Field Diaphragm to the edge of the field and center using the condenser centering controls**.

5) **Adjust the Condenser Aperture Diaphram.** This is the diaphram that is located in the Condenser unit that can be focused up and down. Two possibilites. From fully open, stop down until the image flare, if any just disappears. Or remove an ocular and look at the back of the objective. Close the CAD control until the diaphragm reduces the aperture to about 2/3 of full open.

Note: For critical work, it is necessary to touch up the alignment if obectives are switched, or even for large variations in focus plane in the sample.

You are finished.