

Koehler Illumination Alignment

Nearly all modern microscopes incorporate Koehler illumination because it provides superior control over the light rays during brightfield microscopy and thus ensures the best resolution and contrast. Koehler illumination is also essential for the implementation of the following advanced contrasting techniques: Phase contrast, Varel contrast, Hoffman modulation, PlasDIC and DIC. Prior to setting up and aligning the advanced contrasting components, the microscope's Koehler illumination must be aligned.



Step 1: Determine which objective will be used most for your detailed work. This will be the target objective for aligning the Koehler illumination.



NOTE: For publication images, the Koehler illumination should be set for each objective

used to photograph specimens.

Step 2: Place a sample slide on the stage and bring it into focus with the selected target objective. (it may be helpful to start by focusing with a lower magnification objective and then stepping up to the

target objective).

Step 3: Close down the field diaphragm. (It may be out of focus).





Step 4: Adjust the condenser focus knob until the edge of the field diaphragm comes into sharp focus.



NOTE: You may have to adjust the opening of the field diaphragm to get an edge into the field of view (This condenser focus point tends to be near the specimen end of the condenser focus travel).

Step 5: Adjust the condenser centering knobs until the field iris is in the center of the field of view. Gradually open the field iris and make final tweaks of the centration with the field iris at the very edge of the field of



view. Then open the field iris just outside the field of view.

Step 6: Adjust the condenser aperture iris to match the NA (numerical aperture) indicated on the objective. **NOTE:** The aperture iris can also be stopped down to 60% of



the NA setting (trading image resolution for contrast). An alternative way to set the condenser aperture is to remove the eyepiece and look down the eye tube at the back of the objective and adjust the Aperture diaphragm until approximately 90% of the area is illuminated (a setting that visually looks very near the edge of the objective but is not quite fully to the edge).



"Koehler Illumination Alignment" written by Philip Merlo. Copyright 2012 by SPOT Imaging Solutions. Used by permission only.