

## Koehler Illumination for the Marianas Axiovert 200M Microscope

Koehler illumination is an essential for optimal image quality in brightfield and DIC microscopy. The column of light entering the condenser is set to the correct diameter, focused to the same plane as the objective lens, aligned to the optical axis of the objective lens and, finally, the condenser NA is set to match the objective lens. The result is optimal contrast, even illumination across the field of view and improved resolution. The procedure sounds complicated, but takes longer to explain than to carry out.

All condenser controls are labeled in Figure 5 of the Slidebook Quick Reference Guide.

1. Place a familiar brightfield sample on the stage;
2. Set the microscope for brightfield microscopy, not DIC or fluorescence;
  - a. in the Focus Window, choose 'BFvis' filter in the Eyepieces filter set
3. Choose the objective you wish to use;
4. Turn on the halogen lamp
  - a. Slidebook: click on the 'Bright' button
5. Fully open the field stop diaphragm (lever at right of condenser, Figure 5);
6. Bring sample into focus with coarse and fine focus knobs, while using eyepieces;
7. Close field stop diaphragm most of the way while observing through eyepieces;
  - a. an octagonal field of illumination should appear as diaphragm closes
  - b. if you don't see the pattern, use condenser focus knobs to bring the edges of the diaphragm into focus
8. If the diaphragm image moves sideways as you close down the field stop, use the centering screws to center the light before closing the field completely;
9. When the field stop diaphragm is fully closed and in view, focus the condenser to bring the octagon shape of the diaphragm into sharp focus;
10. Center the field diaphragm with the left and right Condenser Centering Screws;
11. Open the field diaphragm until the octagon barely disappears from view at the edges of the field of view in the eyepieces
  - a. fine tune the centering, if needed
12. Koehler illumination is now set up for this objective. Changing the objective means repeating this process to set up the illumination properly.
13. Once this illumination is set up, image brightness is controlled by lamp voltage and camera exposure time;
  - a. do not adjust brightness by changing condenser focus or the diaphragms
14. Check focus in the eyepieces, if sample is a little blurry, adjust fine focus then close the condenser field stop again, adjust condenser focus then open the field stop to regain the field of view.
15. When switching to the camera, adjust lens focus, close the field stop diaphragm and adjust condenser focus then open and center the field stop until it is just outside the camera's field of view.