

Setting up Kohler Illumination on the Zeiss Axiovert Inverted Microscope

Koehler illumination¹ is an illumination technique that provides optimum resolution and contrast when using a bright-field light microscope. It does this by aligning and focussing the illumination light path, and critically setting the iris apertures of the microscope to best match the objective lens. This sheet is only for aligning the condenser on the microscope used with the **Zeiss 510 confocal**.

1) Place a slide on the stage and bring the specimen into focus.

If the microscope condenser is way out of alignment, you will have to roughly adjust the condenser height to give adequate illumination to focus on the specimen. There are two white tape indicators on the left side of the condenser that will be nearly aligned when the condenser is at the correct height.

2) Stop down the Field Diaphragm Iris so that you can see it in the field of view.

This is the horizontal diaphragm [silver metal] slider at the top right hand of the condenser [tucked in below the condenser head and the halogen lamp housing]. You slide the lever towards you to close [stop down] the iris, and away from you again to re-open the iris.

3) Focus the Condenser by adjusting its height.

Turn the large black focus knob on the left or right side of the condenser until the shadow edge of the Field Diaphragm Iris is in sharp focus when viewed down the microscope eyepieces. This iris edge may be blurred if your specimen is very thick, so get it as sharp in focus as possible.

4) Open the Field Diaphragm Iris to near the edge of the field of view and centre it to the middle, using the condenser centering controls.

The condenser centering controls are the two silver knurled knobs at the bottom front of the condenser. It's easier to adjust both simultaneously while looking down the microscope. Once you have centered the Field Diaphragm Iris open it to just beyond the field of view [so that you can't see it anymore]. You have now set up Kohler illumination.

5) Check and adjust the Condenser Aperture Diaphragm Iris².

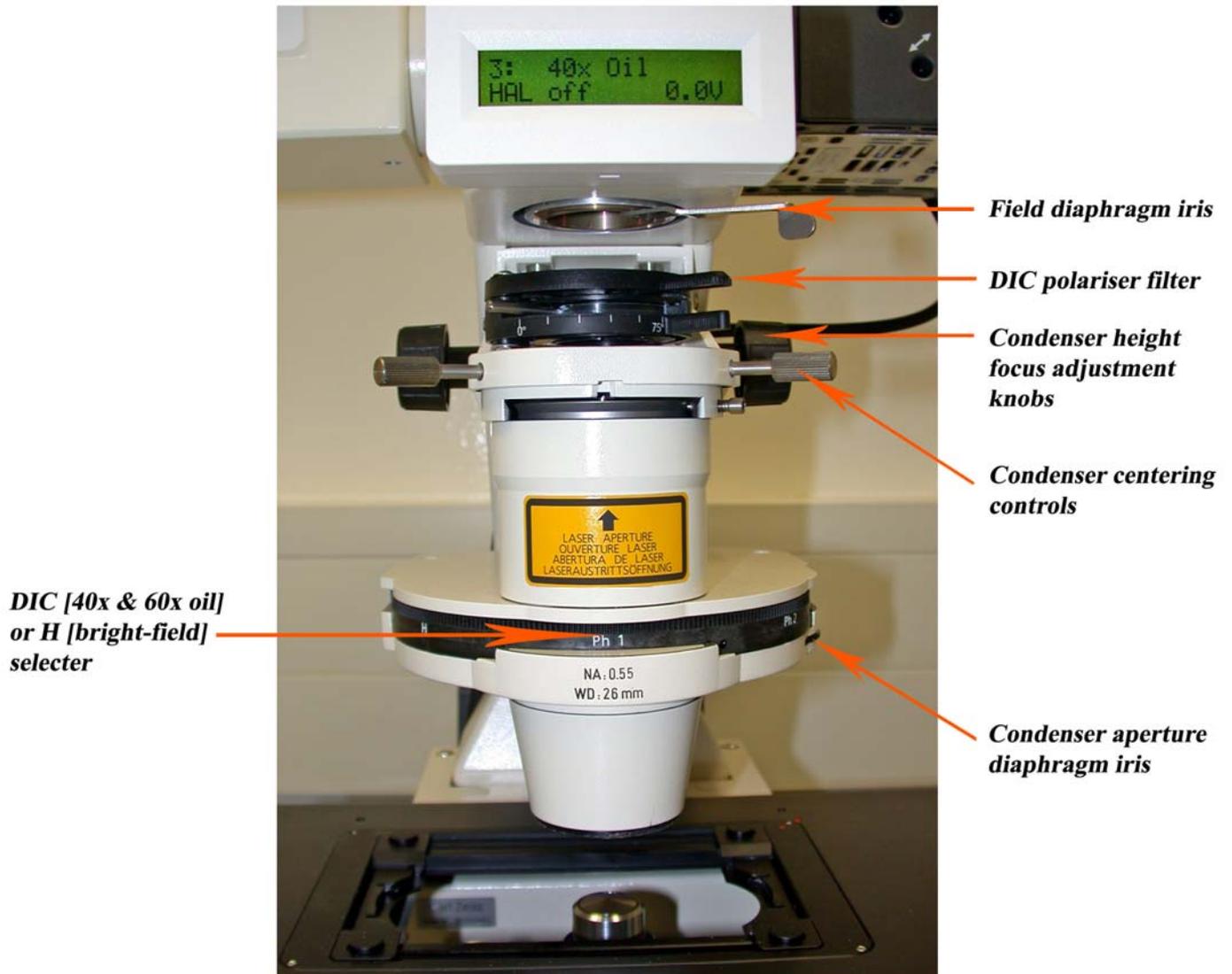
Generally unless you have a good reason not to, leave this iris [bottom right of condenser] fully open to 20% closed [optimal] to give the brightest illumination, particularly as many of the Zeiss objectives have DIC optics for contrast enhancement.

Note: For critical work, you should re-adjust the condenser alignment [Kohler illumination] when objectives are switched, or even when moving the focus through a very thick specimen.

¹ Note: Adjusting the condenser is only required when using the transmitted light halogen bulb, e.g. for bright-field or phase contrast photo-imaging. Adjustment of the condenser is irrelevant for epi-fluorescence microscopy with illumination via the mercury lamp [unless you are also taking a transmitted light phase contrast image as well as DAPI, FITC or TRITC images].

² Condenser Aperture Diaphragm Iris: This is the diaphragm that is located lower down in the Condenser unit, that also has an iris that can open or close [this one isn't the condenser field diaphragm iris that's just been adjusted for Kohler illumination]. If the condenser aperture diaphragm has been closed down it decreases the specimen's resolution and brightness, but increases its contrast and depth of field. Increases in the latter can bring in dust on the microscope internal optics producing an image that has dusty shadows all over the place, as well as improving specimen contrast.

The Zeiss Axiovert 200 Inverted Microscope Condenser



The view down the microscope when you close the field diaphragm iris

DIC contrast enhancement

When using DIC contrast enhancement with any of the objectives, you must select the ‘analyzer’ within the microscope filter wheel. Select it via the ‘Reflector’ button in ‘Microscope’ when using the confocal LSM software. This puts the polarising [analyzer] filter into the optical path. You must also push in the DIC polarising filter in the condenser [rotated & clicked to place] and adjust the DIC prism under the objective [using its small metal knurled knob] to get the desired effect.