Adjusting for Different Observation Methods

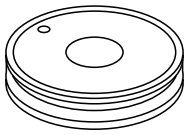
It's true that a well-maintained microscope will help ensure you obtain optimal images. However, it is also important to know how to properly adjust the microscope for your observation method.

The following guides are good references to keep on hand, especially if you do not use these methods on a regular basis.

Differential Interference Contrast (DIC) Adjustment—Transmitted Light Microscopy

1. Ensure that the microscope is properly adjusted for Köhler, set to brightfield illumination mode, and the aperture stop is fully opened.
2. Engage the polarizer into the light path.
3. Engage the analyzer into the light path.
   Turn the polarizer to achieve maximum image darkness (crossed polarized position). This works best without a sample.
4. Engage the nosepiece DIC prism slider positioned inside the objective nosepiece into the beam path (see Image 1), ensuring that no condenser prism is engaged.
   Note: On inverted microscopes, you need to place the DIC slider upside down.
5. Remove the eyepiece and watch the back focal plane of the objective lens (observation without eyepiece).
6. Rotate the DIC prism slider's turning knob until the black fringe is visible.
   The fringe should split the observed image of the back focal plane in half at 45° (see Image 2).
7. Reinsert the eyepiece.
8. Rotate the condenser turret to engage the dedicated prism matching the objective lens used.
9. Fine-tune the DIC prism slider's turning knob to optimize the contrast (positive or negative image topography).

DIC prism (large)



During installation, make sure that the white dot position on top of the prism matches the white dot position of the condenser turret insert. This ensures that the pin on the rear side of the prism fits the notch of the condenser turret's insert.

Note: If your objective has the BFP 1 marking on it, to get the best contrast, you need to use the prism position switch lever to move the prism to the appropriate height.

Image 1

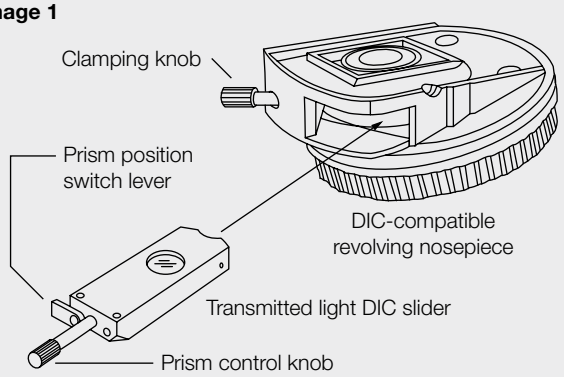
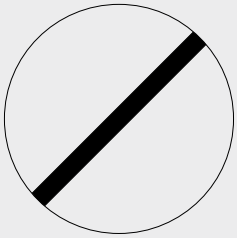


Image 2



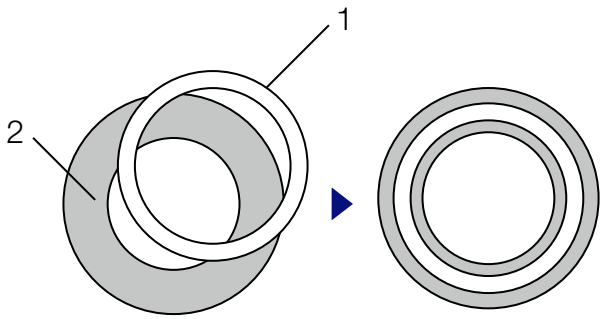
Phase Contrast Adjustment

1. Ensure that the microscope is properly adjusted for Köhler, set to brightfield illumination mode, and the aperture stop is fully opened.

2. For each phase objective, turn the condenser turret to engage the corresponding phase ring (see the inscription on the objective).

3. Remove the eyepiece and insert a centering telescope. Use the centering telescope's focus to bring the back focal plane image into focus so you get a pinsharp image of both the phase ring slit and the phase plate at the same focus position.

4. Turn the centering screws of the optical element to center the phase ring slit. The phase ring slit is perfectly centered when the bright ring slit image (see 1 in the image below) is concentrically inscribed in the dark image of the lens' phase ring (see 2 in the image below).



Relief Contrast Adjustment

1. Place a stained specimen (preferably a tissue thin section) on the stage and, using the 10X objective (with a modulator installed), align the microscope for proper Köhler illumination. The modulation contrast slit plate should be removed from the condenser for this operation. If the turret condenser has a position for brightfield illumination with an aperture diaphragm, rotate the turret to select this condenser.

2. View the modulator plate in the back focal plane of the objective, using a Bertrand lens, a phase telescope, or by removing an eyepiece and peering down the eye tube. Make certain that the sample is removed from the optical path or that it is moved to a clear area on the microscope slide (see Figure 1).

3. Select the slit aperture plate that corresponds to the 10X objective by moving the appropriate condenser (from the turret) into the optical path. There should be a set of adjustment screws or a lever that enables rotation and translation of the illuminating slit plate within the condenser.

4. Place the circular polarizing filter on the microscope light port beneath the condenser. Rotate this filter while observing the slit image through the Bertrand lens (or phase telescope). Observe that the angle of rotation influences the amount of light (brightness) passing through the polarizer portion of the slit (see Figure 2).

5. Translate the image of the slit so that the opened portion lacking a polarizer is superimposed over the gray region of the modulator plate as illustrated in Figure 3. The portion of the slit containing the polarizing material should be imaged in the clear portion of the modulator just to the right of the gray region.

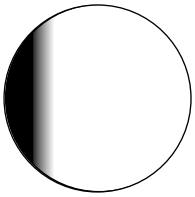
6. Rotate the circular polarizing filter and observe how the region of the slit containing the polarizing material appears and disappears. When the vibration plane of the circular polarizer is perpendicularly aligned with the vibration plane of the polarizer in the slit, the slit size is minimized and maximum contrast is obtained (see Figure 4).

7. Readjust the condenser position by refocusing the field diaphragm to achieve a sharp focus, and open the field iris diaphragm until it is just outside the field of view.

8. Rotate the specimen and/or the circular polarizer at the base of the microscope to achieve optimum contrast. These settings will vary from specimen to specimen.

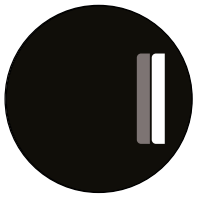
9. Repeat the above steps each time a different magnification is selected for viewing the specimen in modulation contrast.

Figure 1



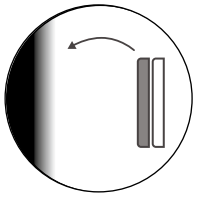
Back focal plane of objective

Figure 2



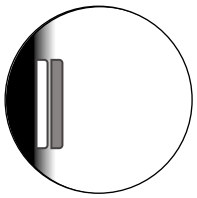
Slit plate

Figure 3



Rotate slit to overlay gradient objective

Figure 4



Final correct positioning

Polarization Adjustment

1. Ensure that the microscope is properly adjusted for Köhler illumination, and set it to brightfield mode.

2. Install the fixed or rotating polarizing element, ensuring that the polarizer is correctly secured into position, either permanently fixed into its holder or placed in the zero position. The polarizer transmission vibration axis must be set to the East-West orientation in this step.

3. Insert the analyzer into the microscope nosepiece or intermediate tube.

4. Rotate the analyzer until the transmission axes of the analyzer and polarizer are crossed at a 90-degree angle. The image should appear as a very dark cross in the field of view (see Figure 1).

5. The final step in polarized-light microscope alignment is to adjust the condenser aperture diaphragm so the bright outer regions of the polarization cross that are visible at the edge of the objective aperture are blocked (see Figure 2).

Figure 1

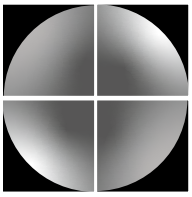
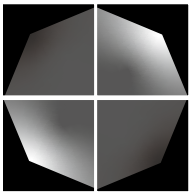


Figure 2



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