

## **Relief Contrast Adjustment**

- 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 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.



Back focal plane of objective



Slit plate



Rotate slit to overlay gradient objective



Final correct positioning

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