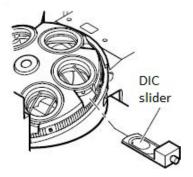


Imaging Facility Biotechnology Resource Center Cornell University BRC\_Imaging@cornell.edu

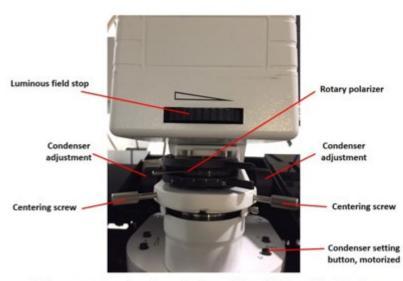
## Using Differential Interference Contrast, DIC (i880)

## Setting Up DIC

- 1. Select an objective suitable for DIC on the nosepiece.
- 2. Make sure objective is in load position. Rotate nosepiece clockwise towards the side so that it is out of the optical path. Take out empty slider.
- Insert the appropriate DIC slider into the slot on the nosepiece. Ensure that the DIC slider clicks into position. Return objective to optical path.



- 4. Place the specimen on the stage and focus using brightfield illumination.
- 5. Configure the luminous-field stop on the condenser for KÖHLER illumination.
  - a. Swivel the rotary polarizer on the transmitted light illuminator stand out of the optical path.
  - b. Close the luminous-field stop until it becomes visible in the field of view (not necessarily in focus).
  - c. Bring the edge of the luminous-field stop into focus by adjusting the height of the condenser.
  - d. Center the luminous-field stop using the centering screws, then, open until the edge of the stop just disappears from the field of view.



Microscope adjustments for setting Kohler illumination

 Select the appropriate DIC prism I, II or III on the condenser turret (button at right side of illuminator support). Check touchscreen monitor to confirm condenser setting. Options are PH2, PH3, BF, DICI, DICII, and DICIII.

The turret position on the condenser can also be configured for DIC from the graphical pop-up menu on the Locate tab on Zen.

7. Switch to the Acquisition tab to view DIC image (overlaid with fluorescence, if needed).



Condenser setup for DIC from Zen Locate tab

## Acquisition

- 1. Rotate the polarizer to the right until it clicks into position.
- 2. Check condenser setting on Touchscreen monitor and make sure it is still on the appropriate DIC setting.
- 3. Click on T-PMT in the Light Path. Checking the PMT, along with the detectors for fluorescence allows one to superimpose a multiple fluorescence image on a DIC image.
- 4. Scan Live and adjust the ChD gain in Channels tab. Decrease Offset to increases contrast.
- 5. If you can, adjust the knurled screw on the DIC slider (while scanning) for optimum contrast. Symmetrical adjustment of the DIC slider around its center position allows specimen details to be viewed in 3D as if raised or lowered.

## When you are done...

- 1. Bring objective down to load position, then rotate nosepiece clockwise to bring objective out of the optical path.
- 2. Take out DIC slider and replace with the empty slider.
- 3. Return DIC slider to its box in drawer.