

Method for Laser Scanning DIC

12.9.96

I. DIC=Differential Interference Contrast (aka Nomarski, who invented the technique)

II. Visual Adjustments

- A. Focus objective on specimen using transmitted light.
- B. Minimize field aperture size (use knurled ring just below light source).
- C. Focus condensor until image of the octagonal field aperture is sharp.
Note: If the condensor is considerably out of alignment, the minimized field aperture will be beyond the field of view. To locate it, open the aperture, focus the condensor until the brightness is maximal, then reduce the aperture until it cuts across the field of view.
- D. Center field aperture with condensor alignment screws and enlarge to field size.
- E. Set condensor turret to DIC .5-1.3
- F. Ensure condensor aperture is maximized (position lever on condensor turret all the way to the left)
- G. Insert DIC prism beneath objective
 - 1. You need to remove the dust protective slider from the prism slot
 - 2. Use the prism that matches the mag/NA of the objective
 - 3. Insert the prism with the mag/NA specifications facing down
 - 4. Push the prism in until it locks with a click.
- H. Make sure the polarizer (beneath field aperture) is turned in and the lever is set to 0° (towards observer).
- I. Introduce analyzer (beneath reflector slider) into the optical path.
- J. Increase illumination intensity, re-focus specimen, and optimize contrast by turning adjustment screw on DIC prism (optimum will be slightly away from minimum intensity position).

III. LSM adjustments

- A. Reposition polarizer lever to 90° (toward laser)
- B. Switch to LSM mode as usual; select **T** as the detector.
- C. Increase laser attenuation (**AT**) to 100.
- D. Turn off overhead lights
- E. Click **Single**, verify laser operation, press **F9** to auto-contrast.
- F. Adjust **Contrast** and **Brightness** as necessary to achieve a preliminary usable image.
- G. Set **Zoom** to 1, click **Start**, reduce field aperture size until it is visible.
- H. Focus condensor until image of the octagonal field aperture is sharp.
- I. Open field aperture until it is beyond scanned area.
- J. Optimize contrast by turning adjustment screw on DIC prism.
- K. Reduce laser attenuation as necessary (shoot for a **Contrast** setting below 50).
- L. Use line averaging to collect the final image.

IV. Notes

- A. It is possible to collect the DIC image simultaneously with a fluorescence image using the overlay function (use the T detector as Channel 2)
- B. The 633nm (red) laser line seems to give the best contrast for DIC, but the resolution will be slightly lower.
- C. This mode of DIC is not confocal: there is no detector pinhole. However, the benefits of laser scanning microscopy (even illumination, illumination pinhole, and monochromatic high-intensity illumination) are still enjoyed.