Setting up the Differential Interference Contrast (DIC) microscope

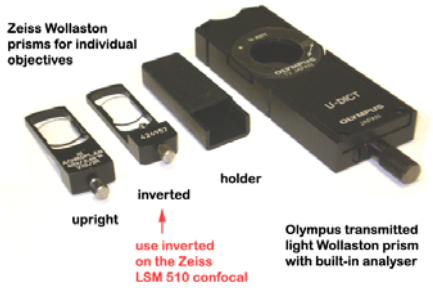
Set the microscope up, in proper adjustment for Köhler illumination for bright-field microscopy, using a well-stained specimen. Ensure that the condenser is set at the correct height, and is centered. If in doubt, refer to Oldfield (1994) or Bradbury & Evennett (1996). Without altering the focus, replace the stained specimen with the transparent specimen. Open the condenser aperture fully. Swing in a low power (10x or 20x) objective; the specimen will probably not be visible.

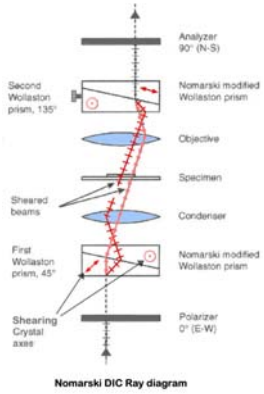
Locate the following components:

- Polariser filter
- Analyser filter
- Wollaston prism in the condenser – the beam splitter
- Wollaston prism for insertion above the objective – the beam combiner (sometimes called the DIC slider).

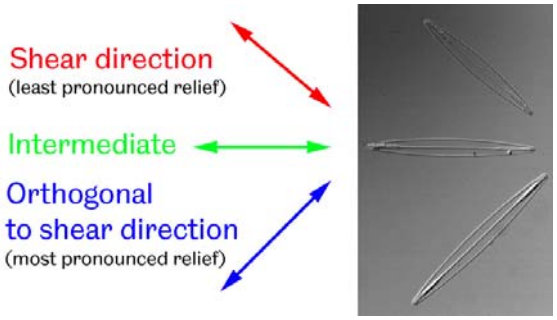
Cross the polariser and analyser filters in the optical path, so that the direction of polarization is at 90° to one another. The polariser, by convention, is oriented east-west and the analyser north-south. The polariser is placed between the illuminated field diaphragm and condenser, and the analyser between the objective and the eyepiece. The field of view should darken between crossed polars.

Swing in the Wollaston prism in the condenser into the optical path. This is often housed captive in a carousel within a 'universal' design of condenser which may also house phase annuli. The Wollaston prism located above the objective is usually free, and must be inserted when the microscope is set up for DIC, and removed afterwards. It has an adjusting screw, to move the DIC slider laterally. Remove an eyepiece and look down the microscope tube. Turn the adjustment knob on the DIC slider until a black band extends diagonally across the objective back focal plane. Replace the eyepiece, and make any final adjustment of the slider to give best DIC contrast.





Nomarski DIC Ray diagram



The DIC image has a 3-D quality, as if illuminated by a uni-directional angled light source. This is not a function of any three-dimensional relief or topography of the specimen, but rather the shows the gradient change in optical path length. The direction of the shadow cast reverses depending upon (a) the amount of shear from the Wollaston prism, and (b) the refractive index of the specimen relative to the surrounding medium. The axis of the optical shear is fixed, and image gradients should be aligned at right angles to the shear direction. Therefore changing the orientation of non-symmetrical specimens, by rotation on the microscope stage, will change the appearance of their images. Features orientated parallel to the direction of shear may well disappear in the image, but show maximum contrast when orientated at 90º to the shear direction.

If a satisfactory DIC image is not obtained, first check that the microscope is correctly set-up for Köhler illumination, and that the condenser is correctly centered, is set at the right height and the condenser diaphragm correctly set. Check that the polars are fully crossed, and that the correct Wollaston prisms, to match the NA of the objective, are in use. If this fails to remedy the situation, check that the orientation of the Wollaston prisms are at 45° relative to the polars, and that the Wollaston prism above the objective has been inserted the right way up (it may be necessary to insert it upside-down in an inverted microscope configuration). Finally specimens that are doubly-refracting (e.g. some tissue culture plastic) will give poor DIC images.

References

1. Bradbury, S. & Evennett, P.J. (1996) *Contrast Techniques in Light Microscopy*. Bios, Oxford. Royal Microscopical Handbook No. 34. ISBN 1-85996-085-5
2. Murphy, DB (2001) *Fundamentals of Light Microscopy and Electronic Imaging*. Chapter 10, Differential Interference Contrast (DIC) and Modulation Contrast Microscopy. pp 153-168. Wiley-Liss, NewYork. ISBN 0-471-25391-X
3. Oldfield, R. (1994) *Light Microscopy: An Illustrated Guide*. Wolfe Publishing, London. ISBN 0-7234-1876-4
4. Lasslett, A (2006) Principles and Applications of DIC Microscopy *Microscopy & Analysis* supplement Sept 2006 S9-S11. (See Jeremy for a copy of this PDF)