Week 4 Lab: Introduction to Phase/DIC/Darkfield

Introduction:

Phase contrast, differential interference contrast (DIC), and darkfield are all methodologies that enhance the contrast of transparent specimens so that they can be viewed. Both phase contrast and DIC use the fact that cells and their surroundings have different refractive indices, and therefore the optical path length (OPL) of the light that passes through the cells is different than the OPL of the surrounding media. You can then exploit this difference to create different patterns of interference to create contrast.

If you permeabilized and fixed cells in in a mounting media that matches glass, would you be able to use DIC microscopy? How about phase contrast?

We will also have the Fourier demo scope set up to further explore the relationship between the information present at the front and back focal planes of the microscope.

Samples:

Cheek cells
Diatoms
Stentor
Other prepared samples

Protocol:

1. Identify the parts of the microscope used for phase contrast and differential interference contrast microscopy. Are all the lenses on your microscope capable of DIC and phase contrast? If not, which lenses can do which contrasting technique? What part of the lens allows it to do do Phase and/or DIC? Do you have a dark-field condenser? If so, what NA is it?
    a. *Phase Contrast:* There should be a phase ring in the illumination path and in the detection path of the microscope; where are they located?
    b. *DIC:* Similarly, there should be illumination and detection polarizers and illumination and detection Wollaston prisms; where are they located?

Phase Contrast

2. Set up your microscope for Kohler illumination in brightfield as you did in the previous lab. You will not be able to get good DIC or phase without it. Put on a diatom slide or other sample easy to find in brightfield.

3. Now switch to a phase objective, observing first without the illumination phase ring in place. Then rotate the illumination phase ring in place and observe how the contrast changes.
4. Looking at the back focal plane, verify that the illumination and detection phase rings are aligned.
5. If the phase rings are movable, move them out of alignment and observe how the contrast changes as they are misaligned.

DIC

6. Now set up DIC. Switch to a DIC objective, and make sure both Wollaston prisms are out of the light path. If the polarizers are adjustable, adjust them for maximum extinction. Now reinsert the Wollaston prisms and examine the resulting image. Adjust the Wollaston prism or the illumination polarizer (de Senarmont DIC) to get the best image.
7. Now that you've successfully set up phase contrast and DIC, try a more difficult sample to image: human cheek cells. These are easily obtained by scraping the inside of your cheek with the wooden end of a cotton swab or other blunt object. They can then be spread on a slide and a coverslip placed on top. They're very hard to see in brightfield alone, so you should first find them in phase contrast or DIC. Once you've found some cells, image them in brightfield, phase contrast, and DIC to see how the images differ using these imaging techniques. See what organelles you can identify and how they appear using these different contrasting techniques.
8. Look at the cheek cells at high resolution 60 or 100x, 1.4NA objective. There are very small ridges on the cell surface that can be seen in high-resolution DIC.
9. If you can, capture some images in each contrasting technique and do line scans across features to observe, quantitatively, how the contrast is generated.
10. If you can rotate your sample, investigate the directional dependence of DIC microscopy.
11. If a thick sample is available, investigate the optical sectioning abilities of each modality. Which gives the best 3D imaging?

Dark Field

12. Dark-Field Microscopy. Cheek cells also make a good dark-field microscopy specimen. If you have a dark-field condenser, set it up. What is its numerical aperture? Find a lens that you can use with it and take some images of your

cheek cells. If you don't have a dark-field condenser, you can approximate dark-field illumination by finding a phase contrast ring with an NA higher than that of a low-NA objective. This will produce a cone of illumination not captured by the objective. Examine both the cheek cells and empty areas of the slide under dark-field.

References

1. Wikipedia Differential Interference Contrast Microscopy http://en.wikipedia.org/wiki/Differential_interference_contrast_microscopy
2. Wikipedia Phase Contrast Microscopy http://en.wikipedia.org/wiki/Phase_contrast_microscopy
3. Wikipeida Dark Field Microscopy http://en.wikipedia.org/wiki/Dark_field_microscopy
4. Frits Zernike, "How I discovered Phase Contrast": http://www.nobelprize.org/nobel_prizes/physics/laureates/1953/zernike-lecture.pdf