Lab Write-Up Week 8

Contrast Methods and Abbe Theory

Andrew Emerson

20 April 2023

Darkfield

Beads were imaged in brightfield and darkfield in order to show the effect of darkfield on image contrast.

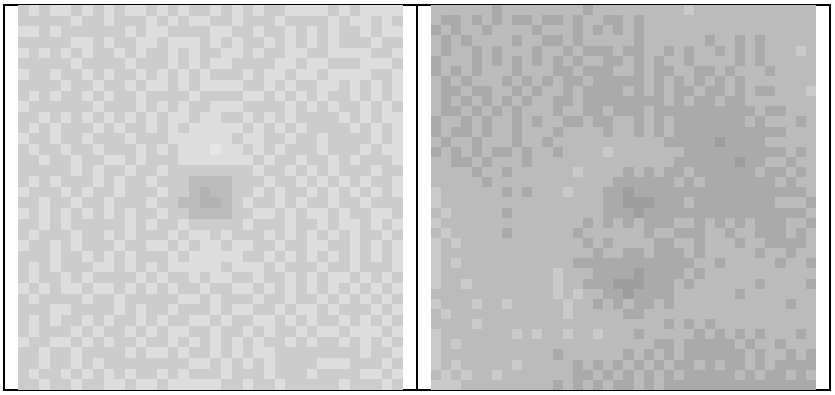

Figure 1. (left): image of 1.0um bead in brightfield. Exposure 0.8ms. (Right): image of 1.0um bead. Exposure 32ms.

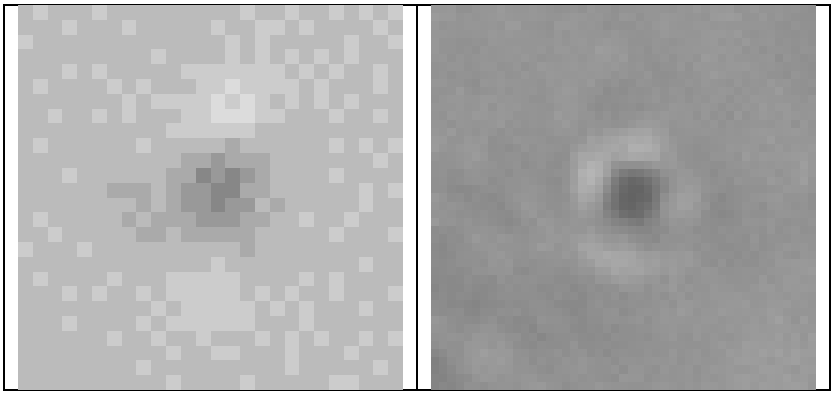

Figure 2. (Left): image of 0.5um bead in brightfield. (Right): image of 0.5um bead in darkfield. Exposure 14.6ms.

Bead Dia (um)	1	1	0.5	0.5
Imaging	brightfield	darkfield	brightfield	darkfield
I max	813	809	859	725
I min	634	633	527	398
I bead	634	633	529	398
I background	747	711	730	591
Contrast	0.124	0.122	0.240	0.291
S/B ratio	0.151	0.110	0.275	0.327

With the 0.5um bead in darkfield, the signal to background ratio increased by 16%. However, with the 1.0um bead the S/B ratio went down in darkfield. The reason for this seems to be the that the bead is not in proper focus or that ambient light in the room entered the system reducing darkfield contrast.

Aperture Stop and Resolution

Samples of fossilized diatoms, a member of algae with silica structures, were imaged with a variable condenser aperture

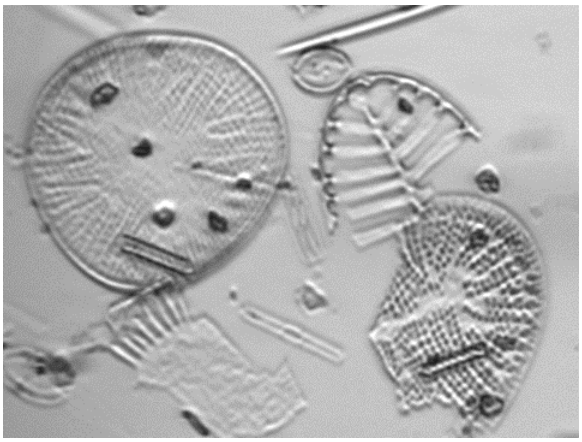

Figure 3. Diatom sample with incoherent illumination. Objective BPF diameter 7.5mm. Objective NA = Condenser NA = 0.15. Exposure 0.8 ms.

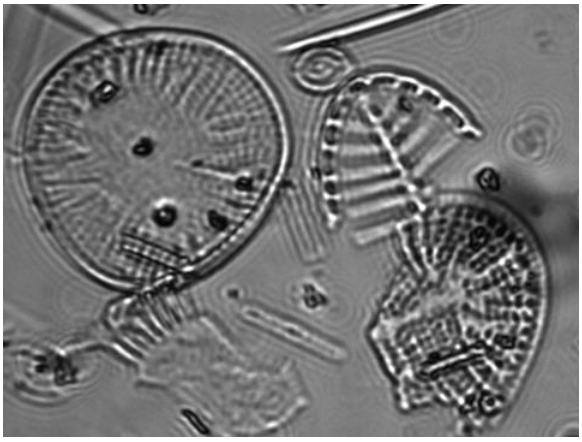

Figure 4. Diatom sample with coherent illumination. Objective BFP diameter 7.5mm. Objective NA 0.15. Condenser NA approx 0. Exposure 19ms.

With the aperture stop open in incoherent illumination the finer features can be seen better than in coherent illumination.

Aperture Stop and MTF

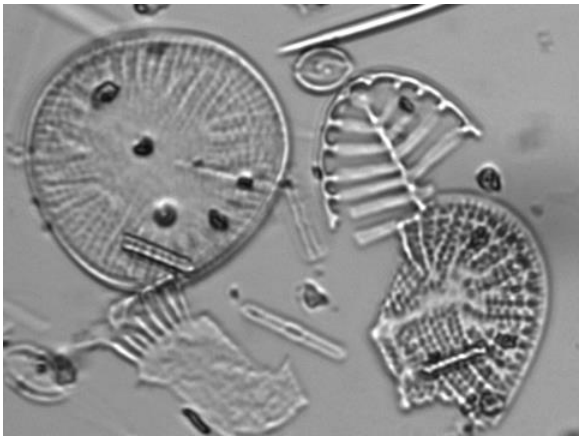

Figure 5. Diatom sample with partially coherent illumination. Objective NA = 0.15. Exposure 1.2ms. Condenser aperture adjusted to maximize contrast.

Adjusting the condenser aperture to maximize the contrast by balancing low and high spatial frequencies that appear in the image. With the aperture closed, the MTF acts as a low-pass filter, as such, fine features such as the edges of the cells are blurred. With the aperture stop open, the MTF becomes more linear, reaching to frequencies twice as great as coherent. In this mode very fine features can be images, but with increasingly lower contrast. The finest features visible in coherent illumination will have less contrast when viewed in incoherent illumination, but finer features no visible in coherent will be visible. By changing the aperture diameter, we modify the slope of the MTF such that we can choose to not image greater spatial frequencies, but image lower frequencies with greater contrast.

Closing the aperture is useful in situations whereas the spatial frequencies we want to image are greater the coherent limitation of $k = \frac{NA_{obj}}{\lambda}$. If the spatial frequencies are less than the coherent limitation, opening the aperture will only reduce contrast. If frequencies need to image are above the incoherent limitation of $k = \frac{NA_{obj}+NA_{condenser}}{\lambda}$, then opening the aperture will also not be effective.

Absorbing Sample

A tissue sample of a human prostate gland dyed with hematoxylin and eosin (H&E) stain.

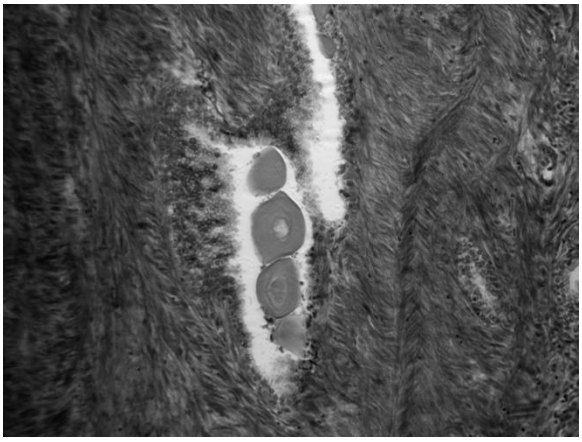

Figure 6. Human prostate gland. Brightfield. Objective NA = Condenser NA = 0.15. exposure 0.9ms.

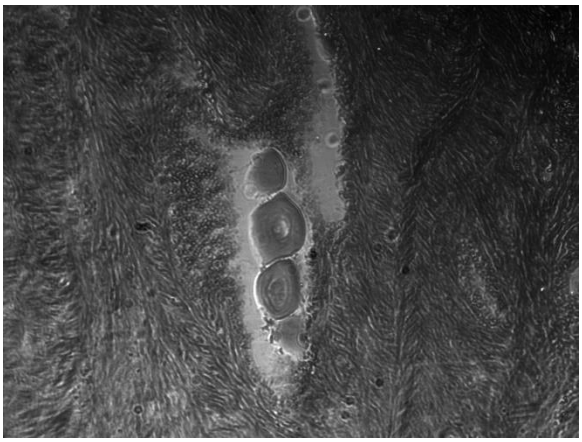

Figure 7. Human prostate gland. Darkfield. Objective NA = 0.15. Condenser NA approx 0. Exposure 40ms.

The tissue sample is appears to be a mostly flat sample, with parts of the sample being different colors. In brightfield, the different parts of the tissue appear to blend together. In darkfield, the edges between different parts of the tissue sample are bright.

The darkfield image of the tissue sample allows an easier understanding of the structure of the sample. With this sample, darkfield could arguably be considered the better image, but the improvement is not that large over the brightfield image. The brightfield image can be considered suitable for looking at the sample.

Phase Contrast

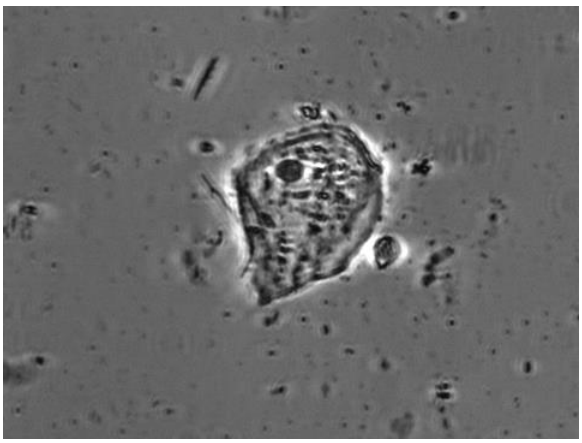

Figure 8. Phase Contrast image of a cheek epithelial cell.

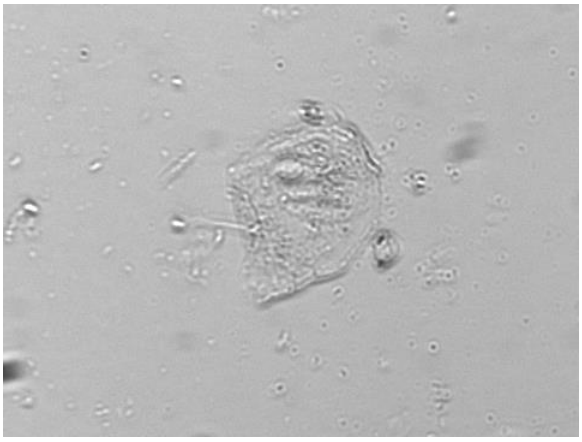

Figure 9. Cheek epithelial cell imaged in brightfield. Focus plane is same as in darkfield.

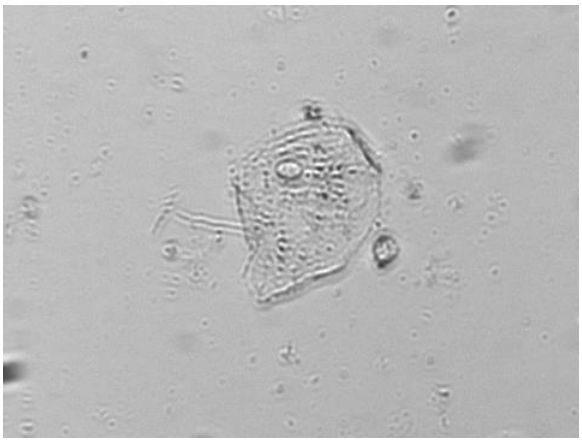

Figure 10. Cheek epithelial cell imaged in brightfield. Focused properly.

In Phase-Contrast imaging, the nucleus, and other features inside of the cell are much more visible than it is in brightfield. Bacteria outside of the cell is also much more visible than in brightfield. When the illumination is changed from Phase Contrast to brightfield, the image is not in the best focus as it

appears to be in Figure 10. This is an effect of brightfield with transparent samples, where imaging slightly out of focus as done in Figure 10 introduces Phase Contrast effects which make the cell more visible.

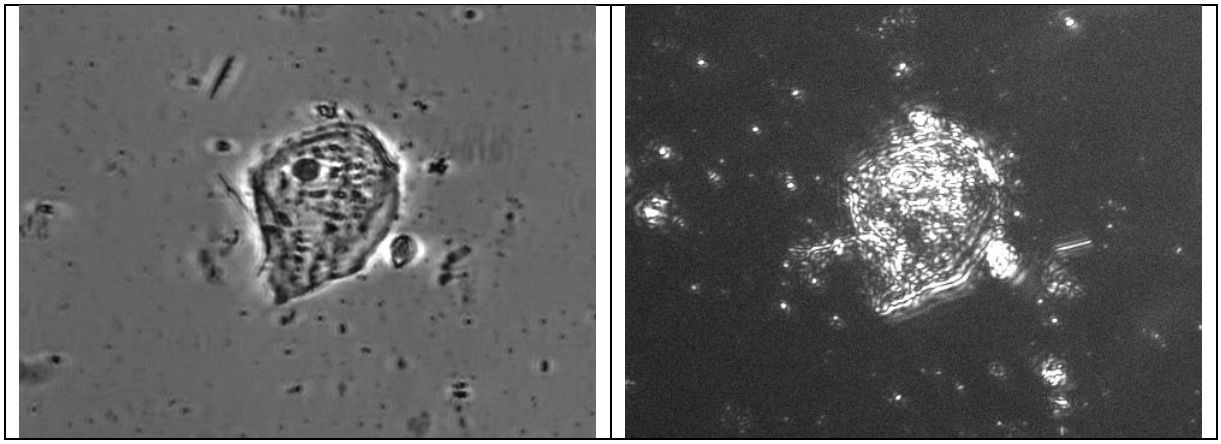

Figure 11. (Left): Phase Contrast image of cell. (Right): Darkfield image of cell.

The darkfield image of the cell is not good for looking at the cell. Due to the nature of darkfield imaging, sample emissions brighter and darker than the average emission appear bright in darkfield; in effect doubling spatial frequencies in the image. This makes the image hard to interpret, and the nucleus of the cell is unidentifiable in darkfield.

Figure 12. Close up of lower right section of cell and nearby bacteria. Halos common in Phase Contrast are visible around the cells.

Due to the setup of the optical system, the phase contrast filter is blocked along the central vertical axis. As such, the halos only appear on the left and right of cells. The halos are caused by frequencies also passing through the phase ring, instead of only the undeflected illumination light. The low frequency features are thus also subtracted from the image, causing the characteristic halos of phase contrast.