Setting up the phase-contrast 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) phase-contrast objective; the specimen will probably not be visible. Insert the correct annulus; an indication of the appropriate annulus is usually marked on the barrel of the objective in green script (e.g. Ph2).

Remove an eyepiece and insert a centring-telescope (sometimes called a 'phase-telescope'), or insert a Bertrand lens system into the optical path to image the back focal plane of the objective through the eyepieces. On some microscopes (e.g. Zeiss Axioplan) the Bertrand lens may be held in a captive slider between the objective and eyepiece(s) labelled 'Ph'. Whichever device is used, focus on the phase plate within the objective. The image of the annulus in the condenser (conjugate with the objective's phase plate) will also be in focus.

Using the centring adjustments provided for the annuli, and without disturbing the normal centration of the condenser itself, superimpose the image of the condenser annulus precisely over that of the objective phase ring (Fig. 2A). The centring screws used for this superimposition (usually set at 90° or 120° on the condenser housing) are not those used for Köhler illumination. They are either captive on the condenser, or may be recessed hexagonal screws at the rear of the condenser, requiring an Allen key for adjustment. If in doubt on this point, refer to the manufacturer's instructions. Once adjusted, the annuli in the condenser should remain centered over a lengthy period; it should not be necessary to carry out this adjustment each time the microscope is used. Remove the centring-telescope and replace the eyepiece, or remove the Bertrand lens. For an inverted microscope the alignment procedure is usually the same. Some types (e.g. Leitz Diavert) have annuli which are inserted in holders rather than being held within a rotating turret in the condenser.

Although in practice the phase-contrast system works over the full spectrum of white light, it must necessarily be manufactured for illumination of one wavelength, generally selected as 550 nm. This is chosen because the eye is most sensitive to green light and objectives are therefore best corrected for spherical aberration at this wavelength. For optimum contrast, therefore, a green filter should be used in the illuminating light path. If a satisfactory phase-contrast image is not obtained (Fig 2B), first check that the microscope is correctly set-up for Köhler illumination, and that the condenser is correctly centered and set at the right height. If this fails to remedy the situation, check that the image of the annulus is of the correct size and its image is precisely superimposed over the phase ring.

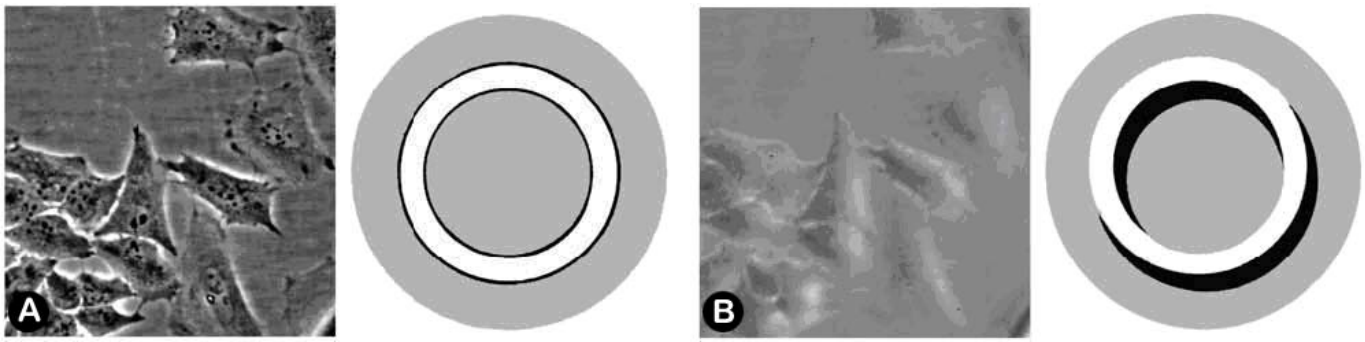

Image BFP objective Image BFP objective

A. Phase plate and objective correctly set up. **B. Incorrect, off-centred, set up.**

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. Oldfield, R. (1994) *Light Microscopy: An Illustrated Guide*. Wolfe Publishing, London. ISBN 0-7234-1876-4

3. Sanderson, J.B. (1999) *Phase Contrast Microscopy*. Encyclopedia of Life Sciences. http://www.els.net