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ILLUMINATION

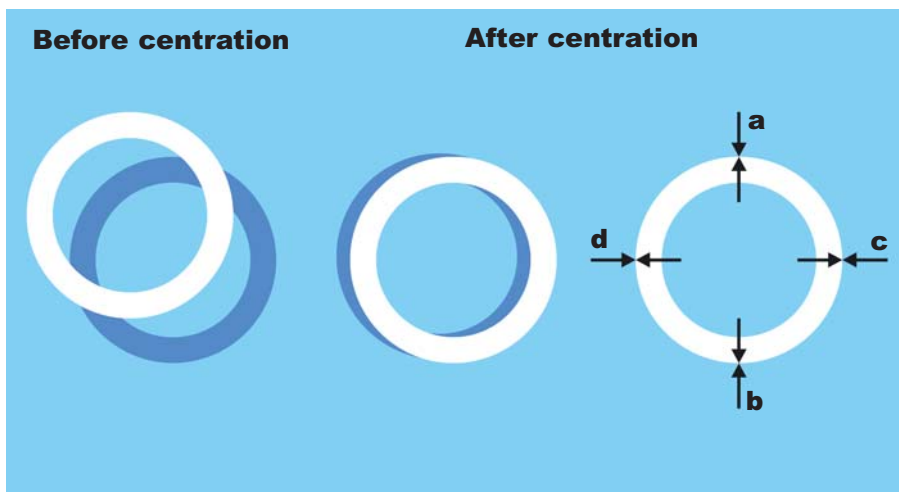
Phase Contrast



SETTING UP PHASE CONTRAST

Requirements

- Phase contrast condenser with relevant annuli (“ring slits”)
- Phase contrast objectives to match
- Centring telescope or Bertrand lens
- Green filter (optional)
- Unstained specimen, e.g. cheek cells



Setting up

1. First check that Koehler illumination is set up, using a stained specimen if necessary.
2. Focus on the unstained specimen in brightfield using a phase contrast objective. Closing the aperture stop may help visualise the specimen.
3. Bring the relevant phase annulus into the light path. This is usually done by rotating part of the condenser, or by moving a slider in the condenser. Check that the annulus matches the objective, e.g. “PH1” or “10X”. Open the aperture stop.
4. Either remove an eyepiece and install the phase telescope in its place, or bring the built-in Bertrand lens into play. Focus the device to provide a sharp image of the circles.
5. Locate the phase centration screws on the condenser (not the condenser’s centring screws) which may be permanently fitted to the back of the condenser (they will need to be pushed in to operate them) or stored separately, perhaps in the microscope frame.
6. Rotate the phase centration screws while observing through the telescope. Move the bright circle (the light from the annulus) until it aligns exactly with the grey circle (the phase plate in the objective). Note that if the circles are different in size you have selected the wrong annulus for that objective.
7. Repeat the centration process for each phase objective in use.
8. Remove the telescope and replace the eyepiece or take the Bertrand lens out of the light path.

Other points

- A green filter will help give a sharp image.
- Phase contrast objectives are identified with the engraving Ph, PL, Phaco or similar lettering. Olympus also uses green engraving on objectives.
- Cheek cells make good specimens for setting up. Scrape out some cells using, e.g. a coffee stirrer, and mount them in saliva on a slide and coverslip.
- The illumination will need to be increased for phase contrast, so take care to reduce it again when viewing in brightfield.
- Many phase condensers have a dark-field position. Take the opportunity to see what the images look like. Notice how well edges and particles (such as bubbles, dust and the edge of the coverslip) show up.