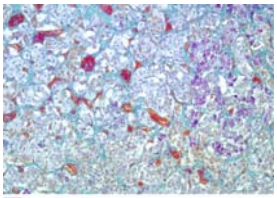
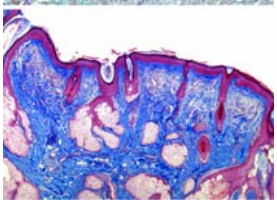
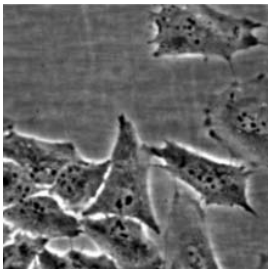
Brief Set-up Protocols for Bright-field, Phase-contrast, DIC and Fluorescence microscopy





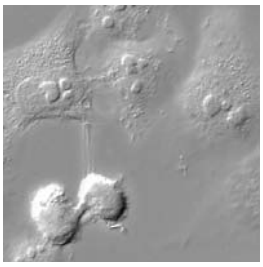
Köhler illumination for transmitted-light bright-field microscopy

1. Turn on the light, swing in a 10x objective,
2. Open fully both the field and condenser diaphragms.
3. Insert a slide, coverslip towards the objective, and focus an image of the specimen. Do not run past the point of focus and smash the slide!
4. Raise the condenser to its limit, close down the field diaphragm to a pinhole. Focus sharply.
5. Open up the field diaphragm, centre the condenser.
6. Remove an eyepiece and adjust the condenser diaphragm to just under-fill the back focal plane of the objective (about 80% diameter).
7. Adjust the eyepieces. If the binocular has one adjustable dioptre focusing control, set up Köhler illumination with the fixed eyepiece. Adjust the variable control for the other eye whilst relaxed and viewing the image.
8. For two adjustable dioptre focusing controls, first focus the microscope with high magnification, change to the lowest magnification objective, and adjust the eyepiece dioptre controls without changing focus.
9. Change both field and condenser diaphragm settings when changing objective.



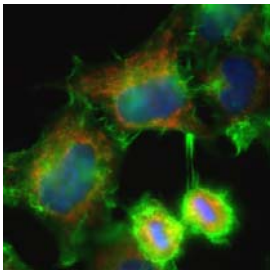
Phase-contrast

1. Adjust microscope for Köhler illumination; use a stained sample if needs be.
2. Ensure the condenser is set to the correct height and centered. Open fully the illuminating iris diaphragm on the condenser.
3. Insert the transparent specimen on the stage.
4. Select the desired phase-contrast objective.
5. Swing in the correct sized annulus to match the objective in use.
6. Check the back focal plane of the objective and, if needs be, centre the phase annulus in the condenser.
7. Insert the (optional) green filter, if desired.



Differential Interference Contrast (DIC)

1. Adjust microscope for Köhler illumination; use a stained sample if needs be.
2. Change the stained slide for an unstained one, to be examined by DIC.
3. Insert the polariser and analyzer. Check that they are crossed to give maximum extinction.
4. Insert the beam-splitting Wollaston prism at 45º to the two permitted vibration directions of the crossed polars, between the polariser and the condenser.
5. Insert the beam-combining Nomarski Wollaston prism at 45º to the crossed polars. It is either located as a matched slider above each objective, or as a common slider in the instrument body.
6. Adjust the beam-combining Wollaston prism, or rotate one of the polars to give the most informative contrast.
7. Insert a full-wave plate (also called a 'one lambda' or red sensitive tint plate) at any suitable position between the two Wollaston prisms to give colour contrast, if required.



Fluorescence Microscopy

1. Use a stained or phase-contrast sample to locate the focal plane, if needs be.
2. Switch off the transmitted-light illumination.
3. Check that the proper set of excitation and barrier filters have been selected or that the correct dichroic mirror filter block is inserted in the beam path.
4. Open the epi-fluorescence illumination shutter. Insert any neutral density filters to reduce photobleaching.
5. Adjust the illuminated field diaphragm, and centre as needed. If fitted, adjust the condenser aperture diaphragm as for Köhler illumination.

Remember that because fluorescence microscopy uses an epi-illuminator, the controls for the illuminated field diaphragm and the illuminating aperture diaphragm (condenser control) are reversed from their positions on a transmitted-light stand. On the epi-illuminator, the illuminating aperture diaphragm (condenser control) is nearer the lamp source, and the IFD (field diaphragm) closer to the condenser/objective.