

olympus bx-51 Im

TRANSMITTED LIGHT OPERATION

- 1 Turn power on with black switch on the right side of the base.
- 2 Rotate the objective turret to the 10x lens making sure that the stage is lowered enough for the lens to easily clear a slide.
- 3 Place a microscope slide on the stage.
- 4 Raise the condenser as high as it will go.
- 5 Fully open the field diaphragm and aperture diaphragm.
- 6 Move the specimen using the stage controls until the portion to be viewed is in the optical path.
- 7 Adjust the interpupillary distance of the eyepieces.
- 8 Locate sample and focus.
- 9 Align the condenser
 - a. Rotate condenser to brightfield.
 - b. Close the field diaphragm until visible.
 - c. Focus and center the condenser.
 - d. Adjust the field diaphragm so that it is just outside the field of view.
 - e. Set the condenser aperture diaphragm to about 75% of the NA printed on the objective lens (or remove an eyepiece and adjust the aperture diaphragm until it blocks the outer 25% of the view).
 - f. Switch to another lens if desired.
 - g. Refocus sample.
 - h. Check and adjust the field diaphragm if needed.
- 10 Rotate condenser to other settings (phase, DIC) as needed.
- 11 If using DIC, insert the analyzer, polarizer and prism into the light path. Select the proper DIC condenser setting for the objective. Rotate the knob on the prism as needed to adjust the shear (contrast).
- 12 Use caution when using the 60x and 100x oil lenses. Do not use too much oil. Remove oil with a cotton swab or lens tissue when done. **DO NOT GET OIL ON THE 4x, 10x, 20x, or 40x LENSES.** If you have, please let a CMRF staff member know immediately.

EPIFLUORESCENCE OPERATION

- 1 Make sure the computer and camera power supply are turned off and that the confocal microscope is not scanning.
- 2 Close the shutter.
- 3 Turn on the mercury lamp power supply (once this is done the computer can be turned on if needed and confocal scanning can be resumed).
- 4 Rotate the objective turret to the desired lens.
- 5 Move epifluorescence filter slider to desired setting:
 - 1 Open
 - 2 DAPI/UV
 - 3 FITC/GFP/Alexa 488/Cy2
 - 4 Rhodamines/RFP/Alexa 568/Cy3
 - 5 Cy5
 - 6 Epipolarization (for immuno gold) and DIC
- 6 Open the shutter and observe the sample.
- 7 When collecting images with the Spot camera exposure times may be several seconds. The time can be reduced by binning (in the Image Setup menu) at the cost of resolution.

SPOT CAMERA OPERATION

- 1 Align condenser to Koehler illumination as usual and center the region of interest. Try to have the light set to at least 6V, 9V being optimum.

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- 2 Turn on computer.
- 3 Start the Spot Advanced software (icon on desktop or in start menu).
- 4 Make sure the lever on the right side of the trinocular is pulled out (light to camera eyepieces).
- 5 Under Setup, click on Image Setup and select the proper image mode (brightfield, fluorescence, DIC, etc.).
 - a. Select Modify to customize settings if needed (usually not). These settings will affect the acquired image, not the live image.
- 6 Click on Live. After a few seconds, you should see an image. Focus as needed.
- 7 To acquire and image it is best to use camera icon on the toolbar. This will acquire an image using the settings from the Image Setup menu. You can also use the Snap command from the live window, but it will not take advantage of the Image Setup process settings, the images will not be optimal and the resolution will be reduced.
- 8 Once the image is grabbed, it can be annotated and/or adjusted. These choices can be found in the Edit menu. Measurements and scale bars can be added and burned into the image. Adjustments such as brightness and contrast are available, but are probably best to do these later in Photoshop or other imaging software.