BF/DF Quick Reference Sheet

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First and foremost: be mindful of what you are doing at all times. The microscopes are delicate and must be treated as such.

SLIDE CLEANING AND PREPARATION

- Make sure your slide is *scrupulously* clean. All dust, old oil and mounting medium should be removed with a little 70% EtOH
- If you can use a mounting medium that sets hard, please do so.
- **Do not** use glitter nail polish to seal your slides as it scatters laser light.
- Be sure to give your slides and sealants at least eight hours to set.

STARTUP

- Check the microscope to be sure it is clean before you start doing anything else.
- Rotate the 10x objective into place.
- Check the oculars to be sure that the Dioptre adjustment on the left eyepiece is set to zero and that the eyepieces are a comfortable distance apart.
- Log in to the computer.
- Turn on the lamp using the power switch on the right side of the microscope body.
- Ensure that the DIFF and LBD filter switches are pushed in.
- Push the ND4 and/or ND8 filters in if desired.
- Set the bulb voltage to 9V (adjust the brightness using the ND filters).
- Push the eyepiece/camera rod all the way in to send the light to the eyepieces.
- Select a slide and ensure that it is scrupulously clean. Also, be sure that any sealants have had ample time to set and harden. Do not bring wet slides to the microscope.
- Place your slide on the stage and centre it below the 10x objective.

LIGHT PATH

- Carefully bring your sample into focus.
- Now you can switch to whichever objective you'd like to use.
- Establish Köhler illumination.
    - Close the **Field Stop** and bring the image of the Field Stop into focus using the **Condenser Focus Wheel** (found *below* the stage but *above* the Specimen Focus Wheel).
    - Centre the image of the Field Stop in the field of view using the sub-stage condenser centring screws.
    - Open the Field Stop until it is *just* beyond the field of view.
    - Set the **Aperture Stop** to a value of three-quarters of the numerical aperture of the objective ($0.75 \times A_N$, for the maths minded).
- Setting up Darkfield Illumination
    - Remove the brightfield condenser.
        - Fully lower the condenser.
        - Loosen the locking screw on the right side of the condenser.
        - Carefully slide the condenser forward until it clears the microscope.
    - Install the darkfield condenser.
        - Carefully slide the condenser into place below the microscope stage.

- Tighten the locking screw on the right side of the condenser.
- Make sure that the condenser turret is set to *O*.
- Establish Köhler illumination.
- Switch the condenser turret to *DF*.
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IMAGE CAPTURE

- Pull the eyepiece/camera rod all the way out to send the light to the camera.
- Start DP Manager.
- Go to Menu > Image Capture > AutoSave Options… and check to be sure that your images will not be saved to someone else's folder. You may set up your own autosave settings.
- Start DP Controller and press the Preview button.
- Select an Image Size of 1,360 × 1,024. This is fine for documentation and publication. Select a larger size if you want an image for a poster.
- Make sure the Sensitivity is set to ISO 200.
- Set the Exposure to Auto for brightfield or SFL-Auto for fluorescence. If you want more control over exposure, you can set Exposure to Manual and control the exposure time with the slider on the left of the Capture menu.
- If you want a scale bar, selext the Objective you will be using from the drop-down menu.
- Go to the Color tab and use the tools there to set the White Balance.
- The Scale tab has tools to set the appearance of the scale bar. The Burn Scale in Image box must be ticked to save the scale bar in your image.
- Bring the image into focus on the screen.S
- Click the Capture button to capture an image.
- Save the image by going to Menu > File > Save in DP Manager. Save your files to a local folder and transfer them to network storage at the end of your session.

WHEN YOU ARE DONE

- Rotate the 10x objective back into place.
- Turn the lamp off.
- Log out of the computer.

Finally, please remember that the optics on these microscopes are **very** delicate. Please treat the microscopes gently and with respect. Think of them not as pieces of lab equipment, but as **very, very expensive** lenses for very special cameras. A really nice lens for up-close work for your camera can run about $2,000 and you wouldn't grind pieces of glass into that, would you? Microscope lenses can cost much more than that, so why would you grind slides into them?