Fluoro1 and 2 Quick Start Guide

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1. Check the microscope to be sure it is clean before you start doing anything else.
2. Rotate the **10x** objective into place.
3. Check the objectives to be sure that the Dioptre adjustment on the left eyepiece is set to zero and that the eyepieces are a comfortable distance apart.
4. Log in to the computer.
5. If you'll be doing fluorescence, click the **Lamp On** icon on the desktop.
    - If the lamp won't turn on, check the control box to the left of the scope: if the yellow light is on, you'll need to wait for it to turn off before you can start the lamp.
6. If you'll be doing brightfield
    - turn on the brightfield 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)
7. If you're working on **Fluoro2** make sure the polarizer (below the condenser), the analyzer (on the right of the microscope between the nosepiece and the eyepieces), and the DIC prism (above the objective nosepiece) are pulled out and that the substage condenser turret is set to position **1**.
8. Push the eyepiece/camera rod all the way in to send the light to the eyepieces.
9. 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.**
10. Place your slide on the stage and centre it below the 10x objective.
11. Carefully bring your sample into focus.
12. Now you can switch to whichever objective you'd like to use.
13. Establish **Köhler illumination**.
    - Brightfield
        - 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).
    - Fluorescence
        - Pull the Field Stop rod out until the field iris diphragm just shows up in the field of view; then push the Field Stop rod in until the field iris diphragm just leaves the field of view.
        - Adjust the Aperture Stop until the image *just* becomes dimmer.
14. If you're doing fluorescence imaging, you'll need to select an appropriate filter
    - **NUA** – blue fluorophores (like DAPI)
    - **WIBA** – green fluorophores (like FITC or EGFP)
    - **WIGA** – orange fluorophores (like TRITC, Cy3, mRFP or Alexa Fluor 546)
    - **WIY** – red fluorophores (like mCherry or Alexa Fluor 594)
    - **CFP** – for cyan fluorescent proteins

15. 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.
    - If you are doing brightfield imaging, 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.
    - If you'd like to capture greyscale images rather than colour images, click the greyscale button in the tools at the top of the DP Controller window.
    - Bring the image into focus on the screen. If you are doing fluorescence imaging, you will need to do this for each colour you image to correct for chromatic aberration.
    - 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.
16. How to oil a slide
    - Find your focus using a low-powered objective (10x or 20x) first.
    - Rotate the objective nosepiece to a position between the oil immersion objective you wish to use and another oil immersion objective.
    - Place a *small* drop of oil on your sample.
    - Slowly rotate the objective you with to use into place.
    - To remove the slide, just reverse the procedure.
17. When you are done
    - Gently clean any oil you may have used off of the objectives using the lens tissues provided.
    - Rotate the 10x objective back into place.
    - Turn the brightfield lamp off (if you used it).
    - 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 or leave oil on them?