

# Basic Widefield Training

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## Upright and Inverted microscopes

### Quick-start Guide

#### **Location:**

FL3 & FL5  
Room 6.033

FL1 & BF/DF  
Room 4.022

FL4  
Room 3.022  
(Updated: 19/6/2020)

#### **Acquisition**

#### **Software:**

NIS Elements F



**Be mindful of what you are doing at ALL times:** The microscope and the lenses are delicate and very expensive and should be treated with care and respect.

## Saving Your Data

Save your Data to C:\Data and create yourself a folder if it doesn't already exist. **DO NOT** save directly to the network, only transfer your files at the end of your session. Save images as a tagged image file (TIF) format. TIF's are lossless, while other file formats are either not portable (JPEG2000) or are lossy (JPEG). Thus we recommend that you save all your images as TIF files which is openable in all image software packages including FIJI/ImageJ.

## Connecting to Network attached Storage

- Data you acquire on this device should be backed up to a server for future retrieval and use.
- **UQ RDM**
  - Can be accessed locally (\\data.imb.uq.edu.au ) rather than the whole of UQ
  - For more IMB specific information see <https://bit.ly/2Na3eQv> or look at the sheet on the microscope table
  - Note connecting to UQ RDM uses UQ-SSO credentials (type `uq\username`)

## Slide Cleaning and Preparation

- Make sure your slide is scrupulously clean – all dust, old oil and mounting media should be removed with a small amount of 70% ethanol.
- If you can use a hard setting mounting media please do so and **DO NOT** use glitter nail polish to seal your slides.
- Be sure to give your mounting media and sealant at least 8 hours to dry properly. **Do not bring wet slides to the microscope.**

## Start-up Procedure

- Check the microscope to be sure it is clean. You are responsible for the condition of the microscope at the close of your session. If you find it dirty, make sure microscopy staff know about it; that is, email, phone us or by leaving feedback on the sheets provided.
- Log in to the computer using your IMB AD credential and password. This will power up the microscope and brightfield lamp
- If the microscope stand has not turned on, double click the **Scope On** icon on the desktop.
- Check the eyepiece dioptre settings. If you don't need to make an adjustment for your vision, set both to zero. Set the interpupillary distance for comfortable binocular viewing.
- If the brightfield lamp is too bright, use the knobs on the stand to adjust the brightness.

## Turn on the Fluorescence Lightsource

- The epifluorescence lamp will turn on when you double click on the **Lamp On** icon on the desktop.
  - If you have an Intensilight lamp attached to the microscope, the light on the front of the lamp housing should stop flashing after about *30 seconds*. If it does not, turn off the power, wait *1 minute*, and restart the lamp.
- The epifluorescence lamp has an internal shutter and neutral density (ND) filters. These are controlled via the switches on the front of the lamp housing.

- After 90 minutes, the automatic microscope control (AMC) unit will start to flash and beep. This signals that the epifluorescence lamp is about to be turned off.
  - If you wish to continue working, double-click the **Lamp On** icon on the desktop.
  - Otherwise, the lamps will be shut down and the AMC unit will enter cooling mode (red flashing light) and the lamps will not be able to be restarted for *5 minutes*.

## Light Path

- On the microscope stand, there will either be a knob or a slide to direct the light to either the eyepiece or the camera.



- If you are using FL4, there is a switch and a dial on the right-hand side of the microscope to control the light path
  - The dial has 4 positions (L, R, Aux & Eye); only L and eye are in use currently
  - The switch has two positions (Eye & Side)
    - For your focused sample to be visible at the:
      - **Eye Pieces** both the dial and the switch should = “Eye”
      - **Colour Camera** (brightfield) the dial = “Eye” & the switch = “side”
      - **Monochrome Camera** (fluorescence) the dial = “L”
- Objectives – always inspect and clean objectives both **before** and **after** a session
  - Dry (these **never** get oil): 4x; 10x; 20x; 40x
  - Oil-immersion (double check these to be *sure* that *Oil* is written on the side of the objective): 100x is the highest magnification.
  - Some objectives can also have correction collars to correct for differences in coverslip/plate bottom thickness. *If you are using slides with the recommended #1.5 glass coverslips ensure that the correction collar is set to 0.17.*
- Transmitted light (TL) Filters
  - Diffuser/IR pair – usually pushed in for DIFF.
  - Green filter (for phase contrast) – usually pulled out.
  - LBD filter –corrects the colour of the light source from yellow to blue; keep pushed in.
  - Neutral Density (ND) filter – push in if the image is too bright.
- Condenser Components
  - **A** for Brightfield
  - **PhL** for use with the 4x objective
  - **Ph1** for use with the 10x & 20x objectives

- **Ph2** for use with the 40x objective
- **Ph3** for use with the 100x objective
- The Emission filter wheel has six positions:
  - Blank – for brightfield and phase contrast imaging
  - UV – for blue fluorophores (e.g. DAPI)
  - C – for cyan fluorophores (e.g. ECFP, mTFP-1)
  - G – for green fluorophores (e.g. EGFP, Alexa Fluor 488, FITC)
  - Y – for yellow fluorophores (e.g. EYFP, mCitrine)
  - R – for red fluorophores (e.g. mCherry, TRITC).

### How to use an oil objective

- Find your focus using a low-powered objective first (10x or 20x)
- Using the Z-position, move the objective away from the sample rotate the objective turret to the objective you need
- On an upright microscope, place a small drop of oil on your sample, on an inverted microscope, place a small drop of oil on the objective
- **BE CAREFUL** not to get oil on an air objective
- Change the Z position till the objective touches the oil. Look for the flash, then use fine focus.

### Setting up Köhler Illumination

- Select the 10x objective.
- Select an appropriate filter cube.
- Bring the sample into focus.
- Brightfield
  - Set the condenser turret to A (for Abbe).
  - Close the field stop and bring the image of the field stop (hexagon) into focus with sharp edges using the condenser focus wheel.
  - Centre the hexagon in the field of view using the 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.
- Fluorescence
  - Move the field stop rod in until the field stop just shows up in the field of view; then move the field stop rod out until the image of the field stop just leaves the field of view.
  - Adjust the aperture stop until the image just becomes dimmer.

### NIS Elements F software

- Start the NIS-F software via the icon on the desktop
  - If you are on FL4, select the camera you wish to use:
    - **Brightfield** (colour camera) choose the DS-Fi2-U3
    - **Fluorescence** (monochrome camera) choose the DS-QiMc-U3

- The software starts in **Live** view meaning that the software starts with the camera running and the image is displayed onscreen in real time. **Freeze** will stop the camera and display the last image.
- In the Docked Tools (on the right side of the window)
- Set the resolution for **Focus and Capture** to the highest settings
- Select your **exposure** mode:
  - If the **Auto Exposure** button is highlighted, auto exposure is active
  - If you are using **manual exposure**, set the Exposure time from the drop-down menu or enter a value in the exposure time field
- Set the **Gain** as low as possible while still giving an exposure time of < 1s. If you have an exposure time greater than 1s, then something is wrong and assess the setup
- Use the **Auto White Balance** to correct the colour balance of the camera – find a region in your sample in brightfield that should be clear and then click this button. This is definitely a good idea, even if you're doing fluorescence.
- This version of the software is not calibrated (meaning your images will **NOT** have the correct scale). Go to Scale and choose the 10x objective, then Camera > Calibrate > pixel size > enter the pixel size on the table, measured by the microscopy staff.
- To capture an image click the **Capture** button. Once the image has been captured, save your image using the save icon or Menu > File > Save.
- **Auto Save** will automatically save the image to the folder selected in File > Options > .
- **AE Area** – by clicking this button, a **green** region box allows you to modify the region being used to determine auto exposure settings.
- **Focus** – by clicking this button, a **red** region box helps you to focus the image using the focus bar at the bottom of the program.
- **Annotations** – clicking this button allows you to make annotations directly on your image.
- **Zoom** controls can be found in the upper right corner of the image frame.
- Red, green and blue channels can be combined into a single RGB image via Edit > File Merge.

### Moving between Brightfield imaging and Fluorescence imaging

- Turn the bright field light off
- Open the fluorescence shutter to illuminate your sample.
  - If you don't see light illuminating the sample, double click on the **Lamp On** icon on the desktop and wait for 2 minutes for the lamp to turn on and for the light to stabilize.
- Turn the emission turret so that the correct filter is in place for your fluorescent signal of interest.
- If you are using FL4, and was using the **Brightfield** (colour camera) the DS-Fi2-U3
  - Close down NIS-F software. Start the software again and choose the **Fluorescence** (monochrome camera) the DS-QiMc-U3
- Now focus on your sample again, you may find the focus has shifted.
- Adjust the exposure time to achieve good contrast in your image

## Shut Down Procedure

- Transfer your files off the microscope computer and onto your RDM.
- Log out of the computer. This will shut the microscope and lamps down automatically.
- Carefully clean the objectives and remove oil from the objective using lens tissues provided. Please DO NOT Kim-Wipes as these may scratch the lens. Think of them not as pieces of lab equipment but as a very specialised microscope with very expensive lenses which can cost much more than \$10k each so why would you grind slides and dirt into them?
- Wipe up any spills you may have made.
- Rotate the 10x objective back into place
- Log whether the microscopy session was good or if there were any incidents on the log sheet provided
- If you have any questions, contact microscopy staff