

Fluoro 1 – Olympus BX51

Quick-start Guide

Location: Room 4.022

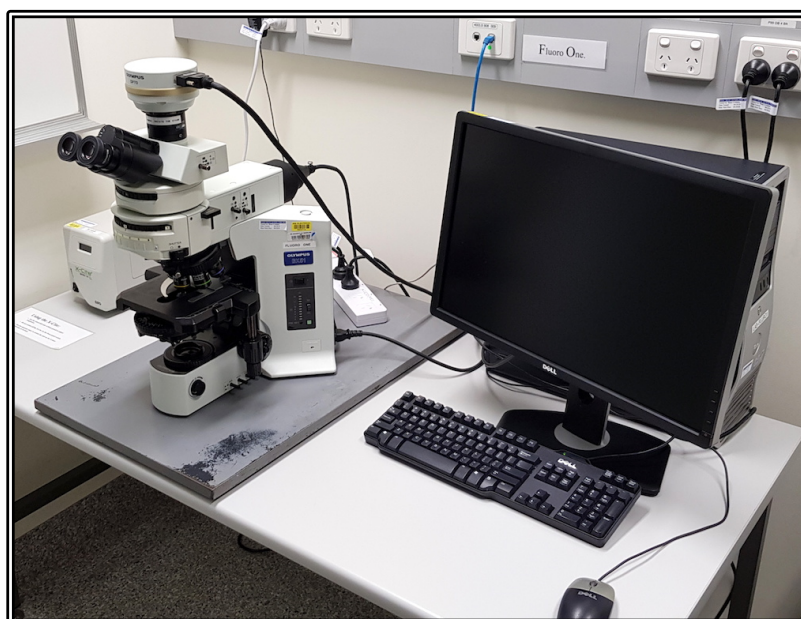
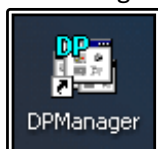
(Updated: 26/8/2019)

Acquisition Software:

DP Controller



DP Manager



Available Objective Lenses: 2x Air, 10x Air, 20x Air, 40x Oil, 60x Oil and 100x Oil

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.

Slide Cleaning and Preparation:

- Make sure your slide is scrupulously clean – all dust, old oil and mounting medium should be removed with a small amount of 70% Ethanol
- If you can use a hard setting mounting media please do so
- **DO NOT** use glitter nail polish to seal your slides as this will scatter the laser light
- Be sure to give your mounting media and sealant at least 8-hours to dry properly

Start-up Procedure

- Check the microscope is clean before you start doing anything else
- Rotate the **10x** objective lens into place
- Check the eyepieces to be sure that the Dioptre adjustment on both eyepieces are set to zero and that the eyepieces are a comfortable distance apart
- Log in to the computer using your IMB username and password
- If you are doing Fluorescence, click the **Lamp ON** Icon on the desktop
 - o If the lamp won't turn on, check the control box to the left of the scope – if the yellow light is on then you will need to wait for it to turn off before starting the lamp
- If you are doing Brightfield, turn on the brightfield lamp using the power switch on the right side of the microscope
 - o Ensure the **DIFF** and **LBD** filter switches are pushed in
 - o Push the **ND4** and /or **ND8** filters in if desired
 - o Set the bulb voltage to **9V** (adjust the brightness using the **ND** filters)
- Push the eyepieces/camera rod all the way in to send the light to the eyepieces
- Select a slide and ensure that it is clean – also be sure that any sealants have had time to set/harden, **DO NOT** bring wet slides to the microscope
- Place your slide on the stage and centre it below the 0x objective

Saving Your Data

Save your Data to C:\UserData 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.

Instructions on connecting network drives can be found on the help sheet at each microscope.

Light Path Settings

- Carefully bring your sample into focus using the 10x
- Now you can switch to whichever objective you would like to use
- Establish Köhler illumination – For Brightfield
 - o Close the **Field Stop** aperture and bring the image of the field stop into focus using the **Condenser Focus Wheel** (found below the stage but above the specimen focus)
 - o Centre the image of the field stop in the field of view using the sub stage condenser centring screws
 - o Open the field stop until it is just beyond the field of view
 - o Set the **Aperture Stop** to a value of three quarters of the N.A. of the objective ($0.75 \times A_N$)
- Setting Kohler for Fluorescence
 - o Pull the field stop rod out until the field iris diaphragm just shows up in the FoV; then push the fieldstop rod in until the iris just leaves the FoV
 - o Adjust the Aperture Stop until the image just becomes dimmer
 - o Select an appropriate filter position for your fluorescence image
 - 1 – DAPI; 2 – GFP; 3 – mCherry; 4 – YFP; 5 - CFP

Image Capture

- Pull the eyepiece/camera rod all the way out to send light to the camera
- Start **DP Manager** software
- Go to Menu > Image Capture > AutoSave Options – check to be sure that your images will not be saved to someone else’s folder, set up your own auto-save settings
- Start **DP Controller** Software and press the **Preview** button
- Select an image size of 1360 x 1024 which is fine for documentation and publication, select a larger size if you wish to use 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 select the objective you will be using from the drop-down menu
- Go to the colour tab and use the tool there to set white balance
- The **Scale** tab has tools to set the appearance of the scale bar – burn scale in image box must be ticked to save the scale bar in your image
- Bring the image into focus on the screen
- Click the **Capture** button to capture and image
- Save the image by going to Menu > File > Save in the DP Manager – Save your files to a local folder and transfer then to the Network storage at the end of the session

How to Oil a Slide

- Find your focus using a low-powered objective first (10x or 20x)
- Rotate the objective nosepiece to a half-way position between the objective you wish use
- **BE CAREFUL** not to get oil on an Air objective
- Place a small drop of oil on your sample and rotate the oil objective into place slowly
- To remove the slide simply reverse the procedure

Shut Down Procedure

- Gently clean any oil you may have used off of the objectives using the provided lens tissue - (please **DO NOT** Kim-Wipes as these may scratch the lens)
- Rotate the 10x objective back into place
- Turn the Brightfield lamp off if you have used it and turn the Fluorescent lamp off via the desktop icon
- Log out of the computer

Please remember that the optics on these microscopes are **very** delicate, please treat the microscopes gently and with respect. Think of them not at pieces of lab equipment but as **very, very expensive** lenses for very specialised cameras – a really nice lens for up close work for your camera can run around \$2000 and you wouldn’t grind pieces of glass into that, microscope lenses can cost much more than that and so why would you grind slides into them???

System Hardware Summary

Microscope Stand: Olympus BX51 Upright Microscope

Objectives:

Magnification	Type	N.A.	Immersion
2x	PlanApoN	0.08	Air
10x	UPlanSApo	0.40	Air
20x	UPlanSApo	0.75	Air
40x	UPlanApo Ph3	1.00	Oil
60x	PlanApoN	1.42	Oil
100x	UPlanSApo	1.40	Oil

Camera: Olympus DP70 CCD Camera

Fluorescent Filters:

Position	Excitation	Dichroic	Emission	Example Fluorophores
1	377/50	409LP	447/60	DAPI, Hoechst, Alexa-405
2	466/40	495LP	525/50	eGFP, Alexa-488, FITC
3	562/40	593LP	593/LP	mCherry, Alexa-568, tdTomato
4	438/24	458LP	483/32	eCFP, mCerulean
5	500/24	520LP	542/27	eYFP, mVenus