

Bright-field/Dark-field System

Quick-start Guide

Location: Room 4.022

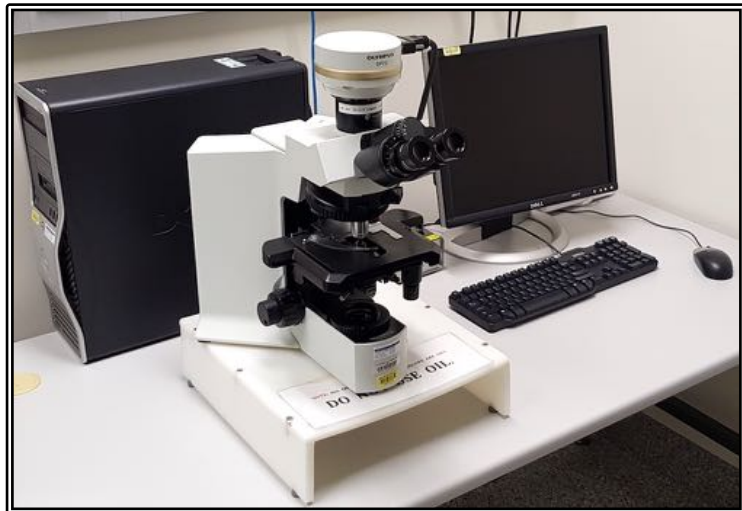
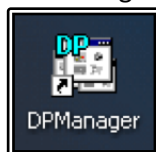
(Updated: 31/8/2021)

Acquisition Software:

DP Controller



DP Manager



Available Objective Lenses: 2x Air, 4x Air, 10x Air, 20x Air, 40x Air

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
- 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 required to adjust light intensity
- 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 10x 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.

Connecting to Network attached Storage

- Data you acquire on this device should be backed up to a server for future retrieval and use.
- The IMB has access to a number of servers both internally managed, as well as UQ-wide resources, think carefully where you would like to store your data.
- Internal IMB Server address which will be UNAVAILABLE by end of 2020, include:
 - **IMB Share**
 - Backed up
 - Group shared server
 - \imbshare.imb.uq.edu.au
 - **IMB Transfers**
 - Not backed up
 - Deleted every 7 days
 - \transfers.imb.uq.edu.au
 - **Objective Server**
 - Microscopy only
 - Not backed up
 - \objective.imb.uq.edu.au
- UQ-wide resources:
 - **UQ RDM**
 - Project based shared drive
 - Can be cached locally (\data.imb.uq.edu.au)
 - For more information see rdm.uq.edu.au
 - Note this uses UQ-SSO credentials (type `uq\username`)

Light Path Settings

- Carefully bring your sample into focus
- Now you can switch to whichever objective you would like to use
- Establish Köhler illumination
 - 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 up darkfield illumination
 - o 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
 - o Install the darkfield condenser
 - Carefully slide the condenser into place below the microscope stage
 - Tighten the locking screw on the right-hand side of the condenser
 - Make sure that the condenser turret is set to 0
 - Establish Köhler illumination
 - Switch the condenser turret to DF

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
- 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

Shut Down Procedure

- Rotate the 10x objective back into place
- Turn the lamp off
- 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 as 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	PlanN	0.06	Air
4x	UPlanApo	0.16	Air
10x	PlanN	0.25	Air
20x	UPlanSApo	0.75	Air
40x	UPlanSApo	0.90	Air

Camera: Olympus DP70 CCD Camera