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Title: **Use and Maintenance of the Leica DM5500 B Microscope and Leica DFC550 Digital Camera**

Version: **v2**

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SOP History		
Number	Date	Reason for Change
v1	22/11/2013	Original
V2	22//11/2018	Update and change of author

1.0 Purpose –

The purpose of this SOP is to outline the principles of the routine use of the Leica DM5500 B fluorescent Microscope and the Leica DFC550 Digital Camera in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to routine use and maintenance of the Leica DM5500 B Microscope and the Leica DFC550 Digital Camera within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the Leica DM5500 B Microscope and the Leica DFC550 Digital Camera within the SASoM to comply with this SOP.

4.0 Procedure –

*****Users new to handling microscopes should undergo basic training in how to use a microscope. All users are required to be trained and signed off by supervisory personnel.*****

A booking sheet for this microscope is present next to the microscope (Name and group initials). Usage of the booking sheet will:

1. Avoid clashes with other users, and

2. Maximise efficient use of the microscope and its light bulb. If someone is using the microscope immediately after you, check with them that they are still planning to use it and leave switched on so as to avoid switching the bulb on and off if not necessary. If someone will not be using it after you within 15-30 min, switch off as normal.

The Leica DM5500 B microscope is an epifluorescent microscope, able to take bright-field and fluorescent images.

General care of the microscope and its features:

1. ALWAYS use lens tissue if lenses require to be cleaned.
    a. Dampen lens tissue with 70% ethanol or isopropanol.
    b. Gently wipe the lens of the objective without applying pressure. DO NOT rub!
    c. BOTH THE 40X AND 100X OBJECTIVES ARE OIL IMMERSION OBJECTIVES. USE ONLY LEICA OIL, APPLIED TO YOUR SLIDE, NOT THE LENS. TAKE CARE NOT TO BRING THE NON-OIL IMMERSION OBJECTIVES (5X, 10X, and 20X) INTO CONTACT WITH THE OIL. CLEAN AS IN a and b above. IN ADDITION, BOTH THE 40X AND 100X OBJECTIVES HAVE CUFF RINGS WHICH SHOULD BE UTILISED WHEN TURNING THE OBJECTIVES. THESE ARE USED BY PUSHING UP AND TURNING THE CUFF TO RETRACT OR RELEASE DEPENDING ON THE POSITION.
2. Take care not to damage the objective lenses under the stage.
    a. Always lower the objective turret using the coarse control before turning it to change the objective.
3. Always switch off the microscope after use.
    a. Cover the microscope when not in use.
    b. ALWAYS ensure the lamp is SWITCHED OFF before covering.

The microscope can be used with or without the computer. If using without the computer you will not be able to acquire images.

The microscope is equipped with three fluorescence filters able to image UV/DAPI, 488 (GFP/FITC), and 568 (including Texas Red/TRITC).

Image acquisition uses the digital camera (Leica DFC) and computer program (Leica LAS AF) to capture digital images.

Turning on the Leica system and Image Acquistion:

1. Turn on the microscope electronics control box before turning on the computer. Next, turn on the external light source on the microscope light box. (The microscope light source is LED rather a mercury lamp. Care should still be taken with this lamp to minimise damage and unnecessary usage of the light source. REMEMBER TO TURN OFF WHEN FINISHED.)

2. When using the computer, select the ‘LAS AF’ icon to begin the image acquisition software.

3. The electronic display module in front of the microscope should be illuminated.

Place the slide on the stage and gently bring the microscope objective into focus, using the desired magnification. **(Please refer to 'general care of microscope' above for details on the microscope objectives.)**

3a. The fluorescent light can be controlled through the electronic display module or the image acquisition software, both for on/off and for the selection of filter cubes. This is controlled by selecting the contrast method icon on the display module (2nd icon down on left side), or by selecting the filter on the image acquisition software under the 'acquire' menu. Select 'Fluo' followed by 'IL-Shutter' followed by selection of desired filter cube (A4 = UV, L5 = 488/GFP, TX2 = 562/Texas Red). By pushing the shutter on the left side of microscope near eyepiece all the way in, the image will be visible through the eyepieces. By pulling out the shutter all of the way, it can be viewed on the computer screen, when the computer screen icon 'Live' on bottom left of screen has been selected. Bring item into focus and take image if desired. To do so, ensure shutter is open, and select 'Start' on computer screen, near bottom left of screen next to 'Live' icon. An image will be acquired based on the filter cubes programmed into the acquisition software. Up to 3 cubes can be selected, with sequential overlaid images possible. The acquisition software will allow for the images to be automatically merged and/or left as single coloured images. Images are automatically saved as temporary files within one experiment file, allowing the user to continue image acquisition without losing previously acquired images. The user will be prompted at the end of the session to save the entire experiment file.

3b. Brightfield images can be acquired if the green microscope light has been turned on, using the power button located on the right-side of the microscope. Image acquisition follows the same protocol as above, but should be optimised for light conditions.

4. Focusing images:
   a. Move the slide using the X-Y control knobs at the right of the stage control
   b. Use the coarse control (outer part of the dial) to bring the objective NEAR the slide.
   c. Use the fine control (inner part of the dial) in order to optimise the focus.
   d. If necessary, adjust the dioptre at one of the eyepieces in order to compensate for differences in vision between eyes.

5. Image acquisition:

By pushing the shutter on the left side of microscope near eyepiece all the way in, the image will be visible through the eyepieces. By pulling out the shutter all of the way, it can be viewed on the computer screen, when the computer screen icon 'Live' on bottom left of screen has been selected. Bring item into focus and take image if desired. To do so, ensure shutter is open, and select 'Start' on computer screen, near bottom left of screen next to 'Live' icon. An image will be acquired based on the filter cubes programmed into the acquisition software. Up to 3 cubes can be selected, with sequential overlaid images possible. The acquisition software will allow for the images to be automatically merged and/or left as single coloured images. Images are automatically saved as temporary files within one experiment file, allowing the user to continue image acquisition without losing previously acquired images. The user will be prompted at the end of the session to save the entire experiment file.

5.0 Personal protection –
Howie coat should be worn at all times.

6.0 Training –
All users have to be trained before using the Instrument by a designated person.

7.0 Related documents –

7.1 Equipment manual - (Leica DM-5500,6000 Microscope - User manual (en,de))

7.2 Risk assessments – (RA-GEN-016-Microscopes)

8.0 Approval and sign off –

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Signature: Date:

Management Approval:

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Position: Laboratory Manager

Signature: Date:

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Name: Alex MacLellan

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