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Title: **Use and Maintenance of the Leica DM4000B Microscope**

Version: **v2**

Author: **Peter Mullen**

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Valid to:	09/01/2024

SOP History		
Number	Date	Reason for Change
v1	10/01/2014	Original
V2	10/01/2019	Update

1.0 Purpose –

The purpose of this SOP is to outline the principles of the routine use of the Leica DM4000B Microscope 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 DM4000B Microscope within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of the Leica DM4000B Microscope within the SASoM to comply with this SOP.

4.0 Procedure –

The Leica DM4000B Microscope is capable of observing and photographing images using only **Transmitted Light (TL) with Bright Field (BF) illumination**. Illumination is provided by a 12V / 100W Halogen lamp (107/2 Lamp Housing; replacement bulb part number 500-974). Changing of the bulb is described in the manual (page 20). There is no capability for Incident Light (IL) and therefore any functions relating to this will be inactive. Likewise there is no Phase Contrast capability.

The microscope is semi-automatic in that it once an objective has been selected, it recalls all of the previously used settings and values for intensity (INT), aperture diaphragm (AP) and field diaphragm (FD). All of the values are updated each time the objective is changed. Values for INT, AP and FD can be changed individually at any point using the function buttons.

The microscope is fitted with a digital camera which is accessed by launching the 'LAS v4.2' software on the desktop.

Operation:

The main components of the microscope are outlined below:

3. Overview of the Instrument

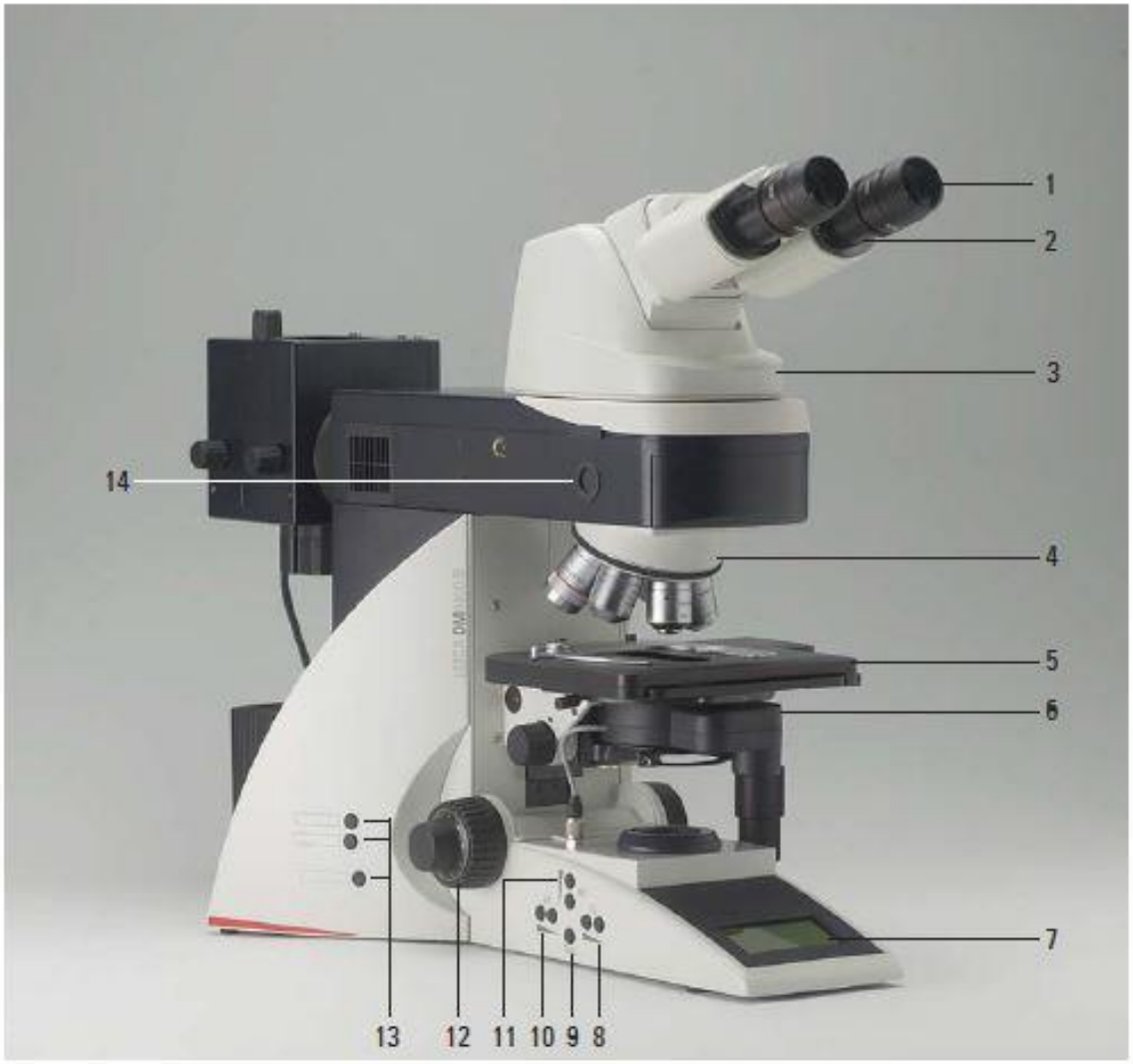


Fig. 1 Leica DM4000 M left side of the stand with AET22 advanced ergotube

1 Eyepiece
2 Eyepiece tube
3 Tube
4 Objective nosepiece with objectives
5 Specimen stage with specimen holder
6 Condenser
7 LC display
8 Function keys field diaphragm
9 Transmitted light/incident light switch
10 Function keys aperture diaphragm
11 Function keys: Light intensity
12 Focus dial with coarse and fine adjustment
13 Variable function keys (factory pre-assigned)
14 Lamp adjustment window

Fig. 2 Leica DM4000 B right side of the stand with Advanced Ergotube AET22

15 Lamp housing for incident light
16 Lamp housing for transmitted light
17 Transmitted light filter, optional
18 Transmitted light filter, optional
19 Variable function keys (factory pre-assigned)
20 X/Y coaxial drive, height adjustable
21 Focus fine adjustment
22 Motorized filter cube exchanger

Switching on the microscope:

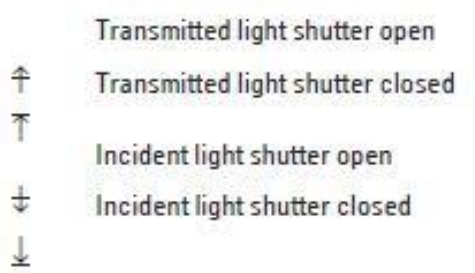
The microscope is switched on using the Power Switch on the REAR of the microscope. All motorised components then undergo an initialisation phase. After initialisation is complete, the display on the stand shows the current microscope settings.

Display:

The display shows the **current** microscope settings. The screen is for display purposes only (ie is not a touchscreen) and no adjustments can be made through the screen.

In the first **column**, pictograms indicate the type of information (ie contrast method, magnification, light intensity, diaphragms and light splitting for photo tubes).

1. Contrast Method: In **the first row** you will find an indication of the active light axis (transmitted light or incident light) of the current contrast method and the current filter cube. The shutter status is displayed for the transmitted light or incident light shutter.



2. Magnification; The display shows the current objective magnification along with the total magnification.

3. Light Intensity: The actual brightness setting is depicted graphically by a beam. The light intensity is also indicated in increments of 20 (course adjustment) or 255 (fine adjustment). The display can be toggled from ‘coarse’ to’ fine’ by pressing the light intensity buttons (on the left hand side) simultaneously.

4. Diaphragms: The values for the field diaphragm (FD) and the aperture diaphragm (AP) are indicated numerically.

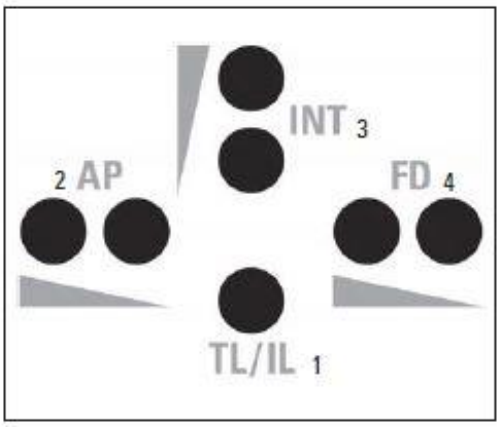
Function Keys:
There are a number of FUNCTION KEYS on the right and left hand side of the STAND. Some of these keys are defined and some of them are variable. The variable function keys have various meanings depending on the microscope configuration.

Defined Function Keys (on the left side of the Stand):

1. The TL/IL key (1) switches between transmitted light (TL) and incident light (IL). The last contrast method is restored. Since this microscope has only transmitted light capability, only the TL option will be available.

2. The INT (3) keys adjust the light intensity. Adjustments can be made in either large or small increments. Pressing both INT buttons at the same time toggles between coarse and fine setting. The display indicator changes accordingly.

3. The AP keys (for the aperture diaphragm) and FD keys (for the field diaphragm) are used to set each diaphragm.



Varaible Function keys on the right side of the Stand:
Since the functions of these keys can be changed according to user preferences, they will not be discussed in this SOP.

Diffuser (Diff) filter switch (bottom right hand side of stand):
The Diffuser filter switch moves forward to produce a sharper image and backwards for a more diffuse image. Leave in the forward position.

Daylight (DLF) Filter: switch (bottom right hand side of stand):
The daylight filter converts the yellow halogen image (forward position) to a cooler 'blue-white' colour (rear position) that is more pleasing to the eye. The filter switch should therefore be left in the rear position.

Focusing:

There is a focus knob on the left side of the microscope for 'course' and 'fine' adjustment (see 2 and 3 below). On the right hand side of the microscope there is also a large focus knob which is exclusively for fine focusing.

Rotating the stage:

The stage can be rotated from 0° – 110° by first loosening the 'fastening screw (1), rotating the stage to the desired position, and then re-tightening the fastening screw.

Fig. 59 Revolving object stage
1 Clamping screw
2 Fine focusing
3 Coarse focusing

Adjusting the viewing distance:

The viewing distance of the eyepieces can be adjusted by moving the two eyepieces closer together or further apart. The eyepieces can also be tilted up / down to adjust the height.

Fig. 60 Tube setting
↔ Personal eyebase settings
1 Motorized tube connection

Objectives:

The microscope is currently equipped with objectives of 1.6x, 2.5x, 5x, 10x, 20x and 40x magnification. There is NO OIL IMMERSION lens on this microscope.

The objectives must be manually moved into the light path, making sure that the turret locks into place. When you rotate the objective into position, the microscope automatically recognizes (i) the selected contrast method, (ii) the optimal settings for field and aperture diaphragm, and (iii) the optimal condenser setting. The objective magnification and the total magnification appear in the display panel.

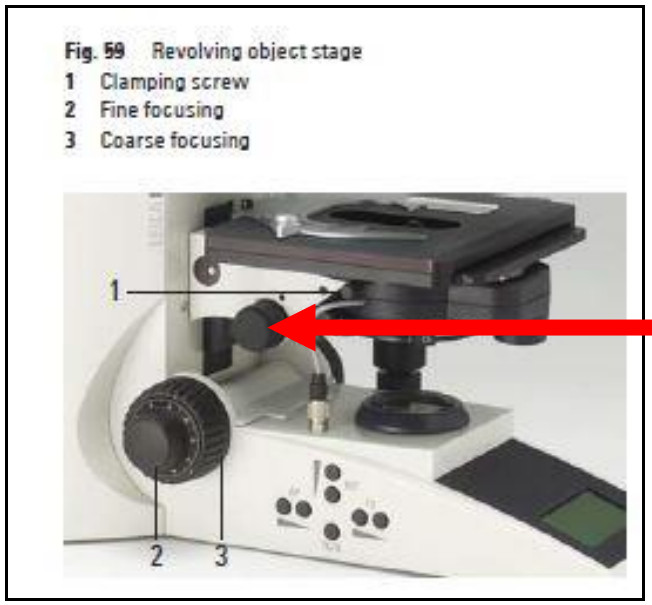
Always start with the smallest level of magnification.

Beam Splitting for camera use:

Beam splitting is set at 50:50 between the eyepiece and the camera and therefore no adjustment is necessary if the camera is switched on / off.

Operation:

1. Switch on the microscope using the power switch at the rear of the stand.
2. Select an objective with lower magnification (eg 2.5x)
3. Transmitted Light (TL) and Bright Field (BF) illumination will be automatically selected as there are no other options available.
4. Place the specimen / slide on the stage and secure with the spring arm.
5. Focus on the specimen using the coarse and fine adjustment knobs.
6. Adjust the light intensity using the INT buttons
7. The Field Diaphragm (FD) is set up on the 10x objective for Kohler illumination. To do this,
    a. Select the 10x magnification and focus on a slide.
    b. Close the FD using the defined function keys on the left of the stand until the edge of the diaphragm appears in the Field of View (FOV).
    c. If the edges are not sharp, raise or lower the condenser using the knobs on either side (red arrow below) until it becomes sharp.
    d. If the diaphragm is not centred, adjust by rotating the small grub screws on either side using the red-handled allan key (see illustration below).
    e. Open the slightly FD until it just disappears from the FOV.



Condenser Adjustment knob

8. The Aperture Diaphragm (AD) should be set individually for each objective. This is best achieved by,

    a. Remove and eyepiece and look down the tube- you should see the AD at the bottom of the tube.
    b. Open / Close the AD using the defined function keys on the left of the stand until it is about 70-80% open. This will be different for each objective but the settings will be remembered (unless somebody changes them).
    c. Repeat this for each of the objectives.
    d. Replace the eyepiece.

9. Default values for Field Diaphragm (FD) and Aperture Diaphragm (AD) are as follows:

	AP	FD
1.6x	17	27
2.5x	20	21
5x	32	10
10x	12	30
20x	19	21
40x	26	15

10. SWITCH OFF the microscope after use.

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

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

7.0 Related documents –

7.1 Leica DM4000B Instruction Manual

7.1 Risk assessments –RA/GEN/016 (Microscopes)

8.0 Approval and sign off –

Author:

Name: Peter Mullen

Position: Research Fellow

Signature: Date:

Management Approval:

Name:

Position: Laboratory Manger

Signature: Date:

QA release by:

Name: Alex MacLellan

Position: QA Manager

Signature: Date:

STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

NAME	POSITION HELD	SIGNATURE	DATE