

MINI-MANUAL “HOW TO USE THE LEICA DM2500 MICROSCOPE”

Transmission light microscopy (BF, Ph, DF)

- Put the sample on the stage holder (12) and move the sample with the xy stage device (8) to the center of the field of view
- Open the transmission light switch 6 and adjust the intensity of the lamp (3; if necessary attenuate with neutral density filters 23)
- Choose an overview objective (13): 2.5 x for large objects overviews (for this objective remove the optional magnification lens in the condenser with switch 20) or 10x for average objects
- Focus (2)
- If necessary, move to a higher magnification (20x or 40x dry, or 100x immersion oil) and fine focus a bit.

Objective magnification	Type	Medium	Numerical aperture
2.5x	HCX FL PLAN	Dry	0.07
10x	HC PL Fluotar Ph1	Dry	0.30
20x	HC PL Fluotar Ph2	Dry	0.50
40x	HCX PL FLuotar Ph2	Dry	0.75
100x	HCX PL Fluotar Ph3	Oil	1.30

- Apply Koehler illumination using the field diaphragm (5) and the condenser height adjustment (4) (In general the centering should be already OK).
- For Bright field illumination turn the BF mark of the condenser disk towards the front. And give just a little bit of contrast with the condenser diaphragm (9; see also photo B).
- For phase contrast using the 10x objective turn the condenser disk to show Ph1 in front. For the 20x or 40x objective use the Ph2 position and for 100x magnification use the Ph3 setting. The aperture diaphragm of the condenser (9) should be set on PH-DF (see photo B).
- The DF is employed for dark field microscopy (works well for the 10x-20x and 40x only)
- Position 1 of the filter cube turret 19 (see detailed photo C) is reserved for transmission microscopy. Positions 2-5 are occupied by fluorescence filter cubes which cause a bias in the color of the images in transmission.

Capture images/time-lapse movies with the camera

- To capture images, ask assistance to have the Leica DCF420C installed on the C-mount of the DM2500. Get instructions on how to use the LAS software for image acquisition (fixed images, time-lapse or movies) and if needed use the dedicated manual about this software.
- Switch from direct eye vision to camera exposure by pulling rod 14 (and photo A). There is an option (ask assistance!) to adjust the angle of the (rectangular) frame captured by the camera. Note that only the central part of the field of view is imaged.

Fluorescence imaging

- For fluorescence microscopy, switch on the lamp (a HXP R 120W/45C Osram lamp) for excitation light by pressing switch 24 (fluo-lamp intensity 26 should be on low). Open the shutter 25. (See detailed views in photo D.
- Avoid to switch off the lamp for short time as the starting procedure is the most demanding step inferring on the life time of the lamp
- Choose the desired fluorescence filter (Pos 1 is reserved for transmission microscopy) on turret 19 (see also detailed photo C): 2 is for UV, 3 for blue , 4 for green and 5 for red excitation.

Excitation color (Example application)	Name	Position	Excitation filter	Dichroic mirror	Emission filter
			nm	nm	nm
Transmission (HV, Ph, DF)		1	---	---	---
Ultraviolet (DAPI, aniline blue)	A	2	BP 320-380	400	LP 425
Blue (Fluoresceine, Alexa 488)	I3	3	BP 450-490	510	LP 515
Green (Rhodamine, Alexa 561, Cy3)	N2.1	4	BP 515-560	580	LP 590
Red (Cy5)	Y5 ET	5	BP 590-650	660	BP700/75

- BP = bandpass filter
LP = longpass filter
- The aperture and focus are preset (usually OK already), but neutral density filters (22) may be inserted to attenuate the excitation intensity.
- The open-close shutter (21) for excitation light is operated manually (see insert photo E also).

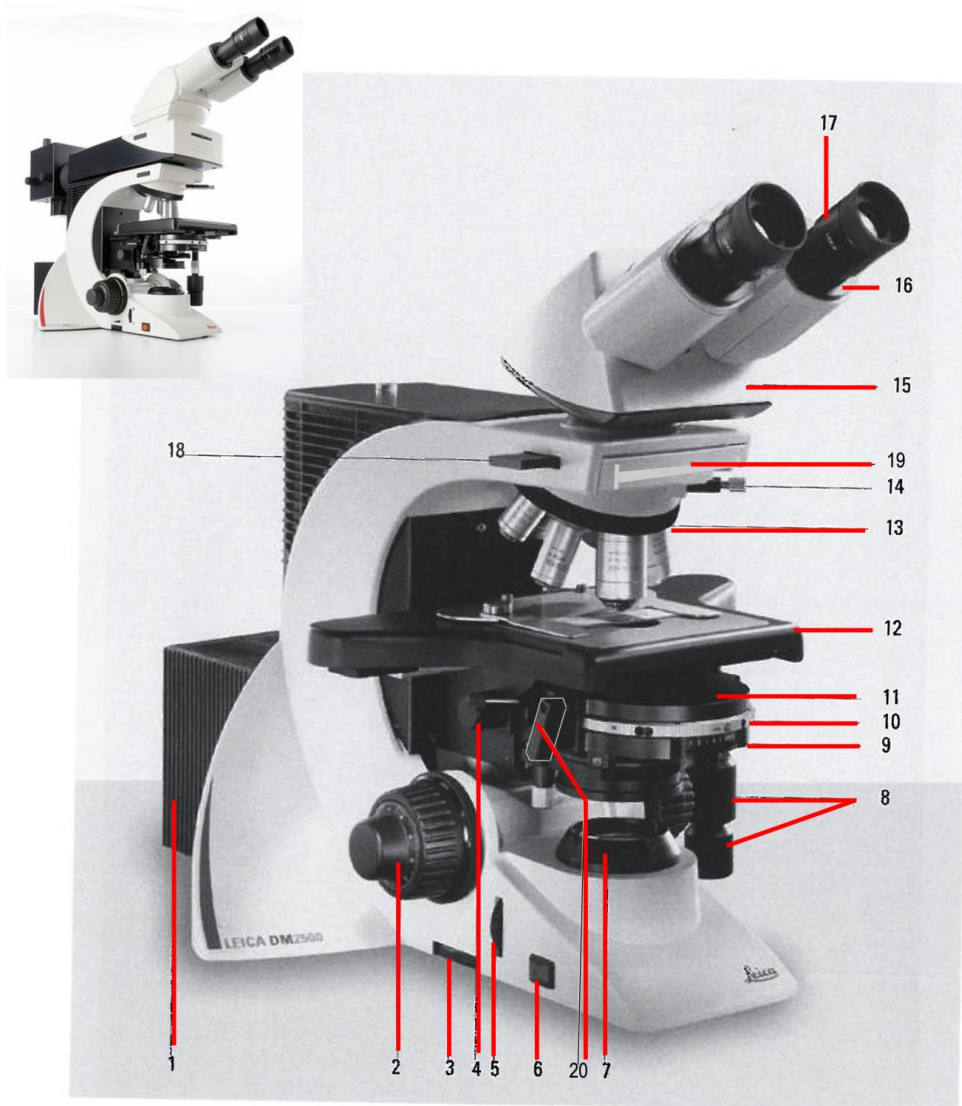


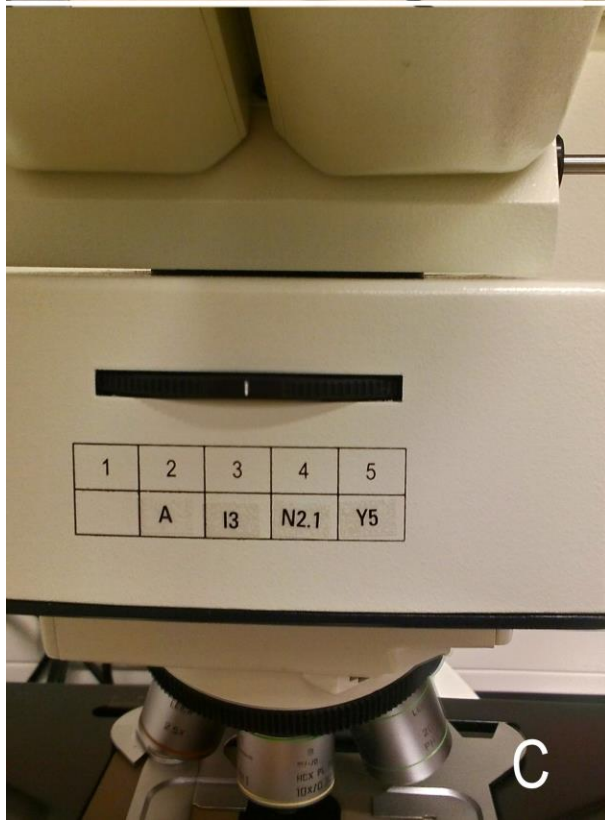
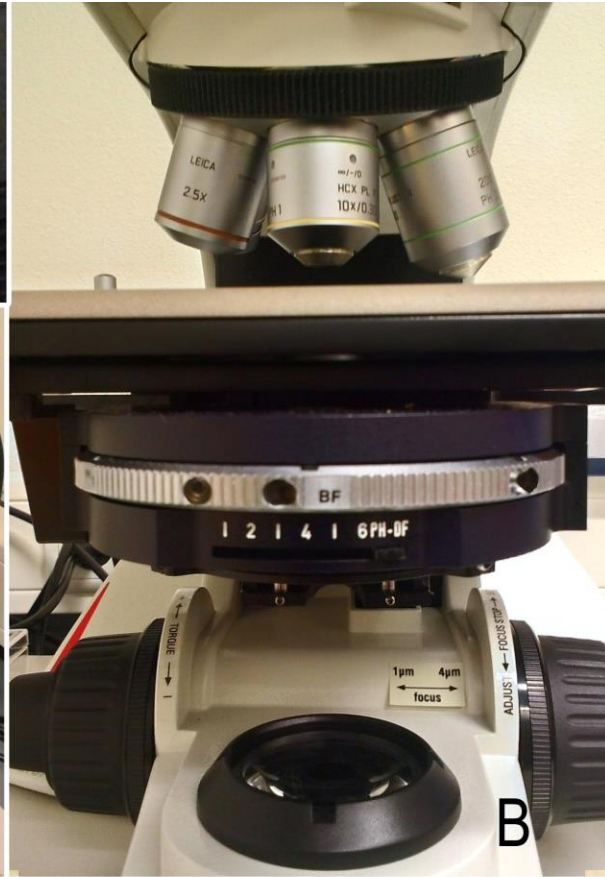
Fig. 3a Left side of the stand Leica DM2500

- 1 Lamp housing
- 2 Coarse and fine focusing
- 3 Brightness control
- 4 Condenser height adjustment
- 5 Field diaphragm adjustment
- 6 On/Off switch
- 7 Field diaphragm
- 8 Coaxial pinion for x/y stage movement
- 9 Aperture diaphragm

- 10 Condenser disk
 - 11 Condenser
 - 12 Specimen stage with specimen holder
 - 13 Objective turret with objectives (2.5x, 10x, 20x, 40x, 100x immersion oil)
 - 14 Objective prism slide
 - 15 Tube
 - 16 Eyepiece tube
 - 17 Eyepiece
 - 18 Analyzer
 - 19 Wheel for fluorescence filters (1 trans, 2 UV, exc. 3 Blue, 4 Green, 5 Red)
 - 20 Switch to remove the additional lens when using the 2.5x objective
- Options BF Bright field
Ph1, Ph2, Ph3 phase-contrast: choose combination objective
DF for dark-field

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General Instrumentation. Science Faculty Radboud University Nijmegen. For internal use only.
 Assistance: e.pierson@science.ru.nl