

Procedure for the Use of Leica Microscope

I. Power on

1. Turn on camera power from the Hamamatsu Camera Controller module.
2. Turn on microscope power.
3. Turn on UV lamp power for fluorescence imaging.

Note: Never turn on UV lamp right after it has been turned off. Wait 30 minutes before turning the lamp back on.

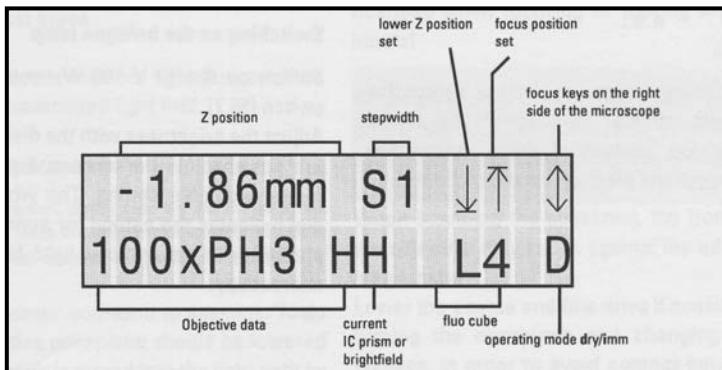
II. Setting Objective Operating Mode

Note: Click black objective buttons located on left side of microscope to switch between different magnification objectives.

Objectives	Mag.	NA(#)	WD (mm)	Immersion	Coverglass (mm)	IC prism
HC PL Fluotar 10x/0.30	10X	0.30	11	dry		
N PLAN L 20x/0.40 DRY Ph1	20X	0.40	1.9-3.2	dry	0 - 2	C
PI Fluotar L 40x/0.70	40X	0.70	0.41	dry	0.17	D
PI Fluotar L 100X/0.75	100X	0.75	4.7	Dry/oil		
HCX PL APO 40X/1.25-0.75 OIL	40X	1.25	0.1	oil	0.17	E
PL Fluotar L 100X/1.30 OIL	100X	1.30	0.1	oil	0.17	D

1. To choose between dry (**D**) and oil (**I**) objectives Push **↑↓** buttons on the microscope digital display **simultaneously**. '**Change Objective**' will flash on microscope display.
2. Push **lower** black objective toggle buttons located on left side of microscope base. This will switch microscope to **lower** numerical aperture objective turret.

IV. Microscope Display Settings



Step Focus Mode:

- ◇ S0 = 0.05 μm
- ◇ S1 = 0.1 μm
- ◇ S2 = 0.7 μm
- ◇ S3 = 1.5 μm

Objective operating mode

- ◇ D = Dry Objective Turret
- ◇ I = Oil Immersion Turret

III. Microscope Imaging

- **Fluorescence Imaging**

1. Make sure the condenser disc and turret for IC prisms are in position HF (brightfield).
2. Remove the condenser from optical path by tilting back condenser arm.
3. Toggle through fluorescence cubes until desired wavelength is selected. Each cube has a code which is displayed on the microscope display panel. The three available fluorescence cube wavelengths are:

Filter Cube	Fluorochrome	Excitation Range	Excitation Filter (nm)	Dichroic (nm)	Emission Filter (nm)
A	DAPI	UV	BP 340-380	400	LP 425
L4	FITC	Blue	BP 480/40	505	BP 527/30
N21	Rhodamine	Green	BP 546/12	565	BP 600/40

4. Focus onto specimen and adjust aperture diaphragm to desired contrast.

- **Phase Imaging**

1. Switch to 20x objective and focus on specimen.
2. Engage the condenser disk to the PH1 position.
3. Adjust the aperture diaphragm lever to the PH position.

Note: Only 20x objective is currently available for phase imaging

- **Differential Interference Contrast (DIC)**

1. Set Koehler illumination*.
2. Focus on the specimen using the 20x objective.
3. Remove specimen from light path.
4. Move polarizer into light path.
5. With the ICT/P engraving upright, push the analyzer into 2nd clickstop position in.
6. Rotate the polarizer and ensure that interference has occurred by observing a darkened field of view.
7. Return specimen into light path and focus.
8. Rotate objective turret to desired magnification.
9. Engage condenser disc (pos = 20, 40, 100oil) to correspond with the current objective magnification.
10. Select the correct objective-side prism (C, D, or E) in accordance with the current objective magnification.
11. Optimize contrast by adjusting the selected objective side prism turret to the right and left. Contrast can also be adjusted with the aperture diaphragm.
12. Color contrast can be achieved by turning over the polarizer so that “λ” faces up.

***Setting Koehler Illumination**

1. Select the 10x and focus on the specimen in bright field
2. Engage the condenser disc into the HF (brightfield) position.
3. Close the field diaphragm.
4. Adjust the height of the condenser until the edge of field diaphragm is sharply in focus.
5. Open the field diaphragm until it just disappears from the field of view.
6. Narrow the aperture diaphragm until the desired image contrast is obtained.

IV. Imaging Software (Openlab 3.5.1)

Turn on computer and open software AFTER camera and microscope are turned on in order for software to couple with equipment (not necessary for offline analysis).

• Image Capturing

1. Pull slide bar to adjust ocular prism to the 50/50 or Photo position.
2. Engage Video mode by selecting the **Video Camera** icon.
3. Adjust image brightness by increasing or decreasing **Exposure Time**.
4. Once desired image is obtained onscreen, click **Camera** icon to grab image.
5. Before applying color to image, change settings to 14-bit grayscale from **Depth** preferences in the **Image** menu.
6. If a fluorescence cube was used, click on the **Color Tool** icon and select the corresponding fluorescence cube color pallet to apply to image.

• Image Saving – Back up often!

1. Create a new folder with your name in the **Nanouser** directory.
2. Save images in the newly created folder using the **Save As** option.
3. If multiple images have been obtained, select all the images and use the **Save As Multiple** option.
4. Click the **Naming** button for additional options.
5. Check the **Save Selected Layers Only** box to ensure only desired images will be saved.

• Calibration

1. Double click on the **Calibration** tool function.
2. Click the right arrow button to open preset objective calibration options.
3. Select the calibration setting for the current objective magnification being used. The correct settings for 2x2 binning should read as indicated in the following table:

Magnification		Pixels	Microns
10x	X	100	131.5
	Y	100	131.5
20x	X	100	66.0
	Y	100	66.0
40x	X	100	33.0
	Y	100	33.0
100x	X	100	13.2
	Y	100	13.2

4. Click the **Calibrate** button.
5. Click and drag the ruler measurement tool to obtain a measurement reading.

- **Creating a scale bar**

1. Once the measurement reading is obtained, select the area-grabbing tool.
2. Click and drag a box along the ruler measurement.
3. Press the delete button to remove the highlighted box. Remaining should be a white void, approximately the same size as the ruler measurement.

IV. Power Off

1. Exit software and power down computer or put into sleep mode.
2. Turn off camera power supply.
3. Turn off microscope power supply
4. If fluorescence imaging was used, turn off UV lamp. Mark lamp time used on the time log sheet.
5. Sign out in Leica microscope log book and on Excel sheet.