

How to Use the Leica Widefield Microscope

Several imaging methods are possible using the widefield microscope.

To use the microscope, select the required method and follow the protocol as described below.

Obtaining Bright Field Images

1. Manually rotate the objective turret to select the required magnification.
The following objectives are available
5x, 10x, 20x 40x, 100x oil
2. Place the sample on the sample stage. For optimal image quality you should ensure that there is only a coverslip or optical grade coverslip thickness tissue culture plastic between the sample and the objective.
3. Set the silver ring on the condenser to position 'H'
4. Switch on the halogen illumination using the switch on the bottom left hand side of the microscope.
5. Turn the wheel next to the switch to increase light intensity from the bulb. Start with this around the middle setting and change as necessary later to increase or decrease image brightness.
6. Focus upon your sample. If you cannot see anything down the eyepiece check that the lever labelled Vis, 50/50, photo on the LHS of the microscope, near the eyepiece is fully pushed in.
7. Check that you have proper Koehler Illumination (see the protocol below)
8. Adjust the aperture diaphragm until you have the desired contrast. This should only be done to change the contrast. Check that you have set this optimally (see the procedure below). Do not use this method to change the intensity of the image; this should be done by turning the wheel to change the intensity of the bulb.
9. If you want to record and save the images follow the procedure described at the end for capturing images by CCD.

Checking Koehler Illumination

When using the halogen lamp you should always ensure that you first have Koehler Illumination to allow you to obtain optimal image quality. This means that you achieve the best resolution by ONLY illuminating the viewed field of your specimen. This avoids excess illumination which would produce unnecessary scattered light. The light cone used to illuminate should be matched to the angular aperture of the objective used, allowing the full resolving power to be achieved.

N.B. This is only applicable when using the halogen lamp to illuminate the sample. It has no effect when using the mercury lamp which uses epifluorescence to illuminate from below

To check Koehler illumination:-

1. With the halogen lamp on, select the required objective and put the silver ring on the condenser to position 'H'

2. Open the diaphragm aperture by sliding the small black handle to the right. The handle is located on the condenser underneath the scale showing the numbers 2 to 8 and Ph. Go as far as the line indicating 9 on the scale but not as far as 'Ph'
 3. Partially close the field aperture by rotating the black collar at the top of the microscope labelled "open close" towards the closed direction. Do not close fully.
 4. Look down the eyepiece. You should be able to see the hexagonal outline of the edges of the field aperture. If you cannot, try closing the field aperture a bit more. If you still cannot see the edges turn the large silver knob on the left hand side of the condenser.
 5. Still looking down the microscope, turn the big silver knob on the left hand side of the condenser to move the condenser up and down until the edges of the aperture are in sharp focus.
 6. If the aperture is not in the centre of the field of view, turn the two smaller knobs at the front of the condenser to centralise it.
 7. Open the field aperture again until the corners just touch the edges of the field of view
 8. Check the centralisation again and adjust if necessary (see step 6)
 9. Open the field aperture a little more until the edges have just disappeared.
 10. You now have proper illumination.
- N.B. If you change objective you need to repeat this procedure

Optimising diaphragm aperture

1. Once you have set up Koehler illumination for the objective you wish to use, remove the right hand eyepiece from the holder. It should just slide out.
2. With the halogen lamp still on you will now see a bright central spot in the right hand tube.
3. Adjust the diaphragm aperture by adjusting the slider (see step 2 above) until this fills the central ring but is no bigger.
4. Replace the eyepiece. The aperture should now be optimal for contrast and resolution. If you wish to brighten or darken the image, you should now adjust the wheel that controls the halogen bulb.

Obtaining Phase Contrast Images

1. Manually rotate the objective turret to select the required magnification.
The following objectives are available
5x, 10x, 20x 40x, 100x oil
2. The objectives available have the following phase contrast requirements (as indicated on the objectives themselves).

5 x	Ph0
10x	Ph1
20x	Ph2
40x	Ph2
100x	Ph3
3. Place the sample on the sample stage. For optimal image quality you should ensure that there is only a coverslip or optical grade coverslip thickness tissue culture plastic between the sample and the objective

4. Switch on the halogen illumination using the switch on the bottom left hand side of the microscope.
5. Turn the wheel next to the switch to increase light intensity from the bulb. Start with this around the middle setting and change as necessary later to increase or decrease image brightness.
6. Focus on your sample
7. Check that you have Koehler illumination (see above for protocol)
8. Select the appropriate phase setting (Ph0 to Ph3) for your objective by turning the silver ring on the condenser to the correct Ph setting.
9. Open the diaphragm aperture to the Ph setting
10. Alter the brightness of the image as required by changing the halogen bulb intensity
11. If you want to record and save the images follow the procedure described at the end for capturing images by CCD.

Obtaining Fluorescence Images

1. Manually rotate the objective turret to select the required magnification.
The following objectives are available
5x, 10x, 20x 40x, 100x oil
2. Place the sample on the sample stage. For optimal image quality you should ensure that there is only a coverslip or optical grade coverslip thickness tissue culture plastic between the sample and the objective
3. Switch on the mercury lamp – i.e. box number 1
4. Switch on the orbit box – i.e. box number 3a
5. Open the shutter on the LHS of the microscope (labelled shutter) to allow the mercury lamp to illuminate the sample.
6. Turn down and then switch off the halogen lamp using the switch on LHS of microscope
7. Use the keypad and the grey wheel on the base of the microscope next to the shutter to select BOTH of the settings required for the fluorophore you wish to image.

Microscope	Keypad	Fluorophore
4	4	DAPI
4	5	FITC (narrow band)
4	6	TRITC/Cy5
2	7	FITC (Broad band)
3	7	TRITC (Broad band)
1	4	DAPI (Broad band)


8. Focus on the sample and move it to select the area required
9. If you want to record and save the images follow the procedure described at the end for capturing images by CCD.

Using the CCD camera to record and save images

1. Switch on the PC
2. Select your name from the list and log in. If you do not have log in, let me know and I can set one up for you.

3. Turn on the camera – i.e. box number 2
4. Open the Openlab 4.0 2 software
5. If you only see one window labelled “untitled” click on this window. If you now do not see a window named “video control” go to the windows option at the top of the screen and select video control from the palette list.
6. Set up the microscope and obtain an area that you wish to image using the relevant procedure from those described as above.
7. Ensure that the light path to the camera is open by pulling out the lever on the RHS of the microscope, labelled camera
8. In the image window click on the video camera icon to see a realtime image
9. In the software, set image size by selecting Image – Document size – Current video – resize
10. Use the video control window to set the exposure, gain and offset to obtain the required image.

This can be done by setting the gain to around the centre of the slide then clicking on the small button labelled AE to set the automatic exposure for this gain. Then set the offset to get a good dark background. The gain will amplify the signal but will also increase the noise, so if the image looks to be of a poor quality try reducing the gain and increasing the exposure time.

11. Drag out the bottom right hand corner of the image window to allow you to see the full FOV
12. Check your image is in focus for the camera
13. Click on the colour palette icon and select the correct fluorophore. The colour palettes have been set up to apply an intensity ramp to the image at a specific wavelength, so selecting the correct palette actually does more than change the colour of the image.
14. Click on the stills camera icon to capture a copy of the image. Each time you capture an image it is recorded as a separate layer in the window.
15. You can scroll through the layers by clicking on the up and down arrows but to properly manage the layers, delete unwanted layers or merge layers you need to do the following
 - a. Open the “untitled layers” window – this will show all the images you have captured
 - b. To delete an image – select it and drag it to the bin icon at the top of the window
 - c. To name a layer click on the small down arrow on the right hand side and choose “new name”
 - d. To merge layers, highlight all the layers you wish to merge by clicking on each layer while holding down the  key. Then go to the layers option from the top of the screen. Click on this and select Merge to RGB.
16. To save captured images delete all the layers that you do not require
17. Go to File – save as multiple – then change format to TIFF for publication and give a name. Each layer in the window will be given this name and a number which will increase incrementally.

Shutting down the system

1. Switch off all the boxes used and turn off the PC