

## Leica DMRE, upright microscope

Checklist for use of Leica microscopes. Instructions from Smile staff is required in order to access the microscope. Per Brunmark, Martina Schneider 2017-06-16, v 2.0

### Starting up the instrument for brightfield microscopy

(Numbers) refer to separate illustration: "Quick reference- Leica Microscope".

1. Turn on the microscope: Power switch on (12)
2. Select lamp for transmitted light: Lamp selection (10), lower position V
3. Turn on computer & monitor. (Leica camera is turned on automatically)
4. Start image capture software: LAS v4.1 (shortcut on desktop)

### Basic settings

5. Choose an objective (8)- start at low magnification
6. Filter cube wheel (4) in position 4, "BF"
7. Wollaston prism turret (5): position BF.
8. Condenser wheel (6): position H.
9. Polarization filter (15) out
10. Camera/eyepiece beam splitter (14) in 50/50 position
11. Adjust light intensity (23)
12. Look in the oculars, adjust them to a comfortable inter-eye distance (3).
13. Focus (9) so you see sharply with your left eye. Then, adjust the right ocular to optimize focus for the right eye

### Optimize the image: Köhler adjustment

14. Select the objective with 10x magnification
15. Check that the condenser is in the light beam (19) and focus (9) on your specimen
16. Close the field aperture "F" (18)
17. Look in the microscope: you'll see that most of the field of view is black, except for the aperture, which should be in the exact center
18. If needed, center the aperture/condenser using the two symmetrically placed screws (21) on the condenser holder
19. Raise or lower (16) the condenser so that the edges of the aperture appear sharp
20. Open the field aperture (18), until its edges are almost at the edge of your field of view.
21. Finally, adjust the condenser aperture (17) to obtain optimal contrast and resolution. Look in the microscope while you adjust this!

**Note 1:** Do not close the condenser aperture (17) too much. This will give great contrast but drastically decrease resolution!

**Note 2:** For routine microscopy on a mechanically stable microscope, it is usually sufficient to adjust the Kohler setting once at the start of the session.

Available air objectives: **2,5x/0,07**, **10x/0,3**, **20x/0,5**, **40x/0,85**.

For Immersion objectives please see below.

## Microscopy with immersion objectives

1. For high magnification, the microscope has two immersion objectives: **63x/1,32** oil immersion, **100x/1,32** oil immersion.
2. Initially adjust the focus using an “air” objective.
3. Then add immersion oil and switch to immersion objective.
4. Make sure to clean objective properly after use: ethanol and tissues are available in the lab (20).

## Image acquisition in the LAS v 4.1 software

(Numbers) refer to separate illustration: “Quick reference- Software”.

1. Select the **No sequence** image mode (1) [button in upper left corner]
2. Select **Acquire** tab (7)
3. Select **Camera** tab (8)- You should see a live image on-screen.
4. Open **Image Format** (4) and select frame size for live image (**Live Format**), and for capture (**Captured Format**)
5. Open **Histogram** (5) and make sure you start with the full dynamic range (0-255) and the mid value in the middle.
6. In the **DFC500 window** (2), Areas of **over/under explosion** (13) can be visualized and **HDR** settings(12) activated.
7. Open **Exposure Adjust** (3) and adjust brightness with **Exposure**, and **Gain** = 1x.
8. Alternatively, use **Automatic exposure** (9) in **DFC500 window** (2)
9. Set White Balance by using one of the following methods:
  - (a) **Automatic White Balance** (10) in **DFC500 window** (2). Reset by right-clicking the button.
  - (b) Defining a ROI (region of interest): left-click and draw a rectangle over your image. A menu appears, choose **White Balance**. *Note: cannot be reset and must be tried out for each situation/staining/specimen. The auto-function usually gives a good starting point.*
10. Open **Histogram** (5) and adjust the lower and upper limits to increase the saturation and intensity of the image. Moving the mid value changes the overall hue of the image.
11. Open **Color** (6) if you want to fine-tune the overall hue and color balance. Click to position midpoint in the spectral wheel, and see the difference.
12. Opening the **Camera settings window** (11) allows for simultaneous manipulation of exposure, gain and histogram.
13. Click **Acquire image** [at the bottom]. Now you have captured the image!
14. You can access your saved image in the **Browser** tab (23) to rename and enter metadata (Leica employs an extended Tiff format).

**When finished: close down microscope including all lamps.  
Put on the hood to protect the microscope from dust.**

## Starting up the instrument for fluorescence microscopy

1. Turn on the microscope: Power switch on (12)
2. Select lamp for incident light: Lamp selection (10) [upper position]
3. Turn on computer & monitor. (Leica camera is turned on automatically)
4. Start image capture software: LAS v4.1 (shortcut on desktop)

### Basic settings

5. Choose 20x objective to start with
6. Choose the appropriate filter in filter cube wheel (4)
7. Open fluorescence shutter (24) [push in]
8. If signal is low, push the camera/eyepiece splitter (14) into **vis** position
9. Make sure to close the fluorescence shutter (24) if you do not need fluorescence to avoid bleaching of your sample.

### Objectives

20x/0,5, 40x/0,85, 63x/0,75, 63x/1,32 oil immersion, 100x/1,32 oil immersion.

## Obtaining images with LAS v4.1. (Leica Application Suite)

*Monochrome pseudo color images -the professional option*

10. Select **Overlay** imaging mode (1) [upper left corner]
15. Select **Acquire** tab (7)
16. Select **Camera** tab (8)
17. Open **Image Format** (4)
18. Select frame size for live image (**Live Format**), and for capture (**Captured Format**), choose **Greyscale**
19. Open **Histogram** (5) and make sure you start with the full dynamic range (0-255) and the mid value in the middle.
20. Select the appropriate filter within the microscope's filter wheel (4)
21. Open the fluorescence shutter (24) [push inward].
22. To enhance a weak signal, choose **vis** position in beam splitter (14)
23. Select  $\lambda$  (*lambda*) tab (17)
24. Under **Channel Setup** (15) check the boxes of the filter(s) you will use, leave the other(s) unchecked.
25. Select the channel that corresponds to the current filter - see microscope filter wheel (4)
26. In **Exposure Adjust** (16), adjust brightness & contrast of live image with
  - (a) **Exposure** = exposure time - longer' gives brighter image but may bleach fluorescence
  - (b) **Gain** = amplification of signal -- higher gives brighter image but may introduce noise
  - (c) **Gamma** = non-linearity of intensity readout- 1.0 is the best starting point. (Changing Gamma effectively changes contrast – try it.)
27. Repeat filter selection and exposure adjustments for each fluorophore/channel. **Make sure that the selected channel in the software matches the filter on the microscope.**
28. Click **Acquire Overlay** (18)
29. ....and follow the on-screen instructions!
30. After the images are acquired, a new tab opens that allows adjustments

(You can choose between *Create a new sequence* or *Add to current sequence*. New sequence makes one new folder per overlay; with *Add to current sequence* you get all overlays in the same folder until you create a new sequence!)

You have now acquired one greyscale image per channel/fluorophore, and one overlay image where each channel has been assigned a pseudo color.

### **Adjusting the overlay image**

31. Select **Browse** tab (23) and  **$\lambda$**  tab (24)

32. Select one channel in **Overlay Image** (22)

33. In **Adjust Appearance** (20) you can adjust the channels pseudo color and the channels histogram to optimize intensity and contrast as well as the mixing intensity. Check the preview box (21) to follow the effects of your changes.

34. Try different methods for creating the overlay (19)

35. Repeat for the other channel(s)

36. Click **Create Overlay**

You have now created a new overlay image with optimal intensity & contrast. Note that the original data (greyscale & original overlay) have not been modified!

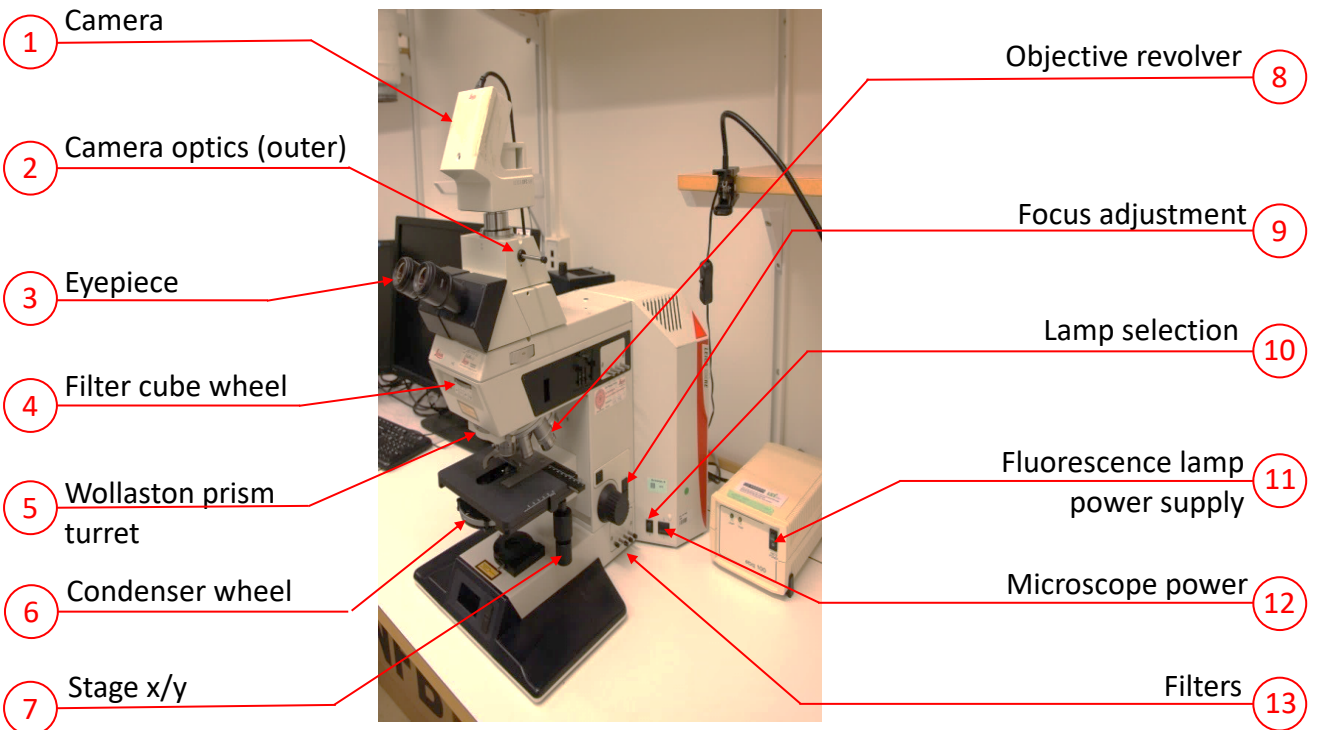
### **Image analysis**

In the **Analysis tab** (25), several measure tools (26) can be found: lines, points, counting etc.

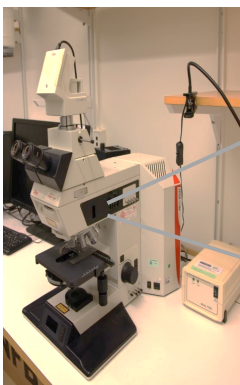
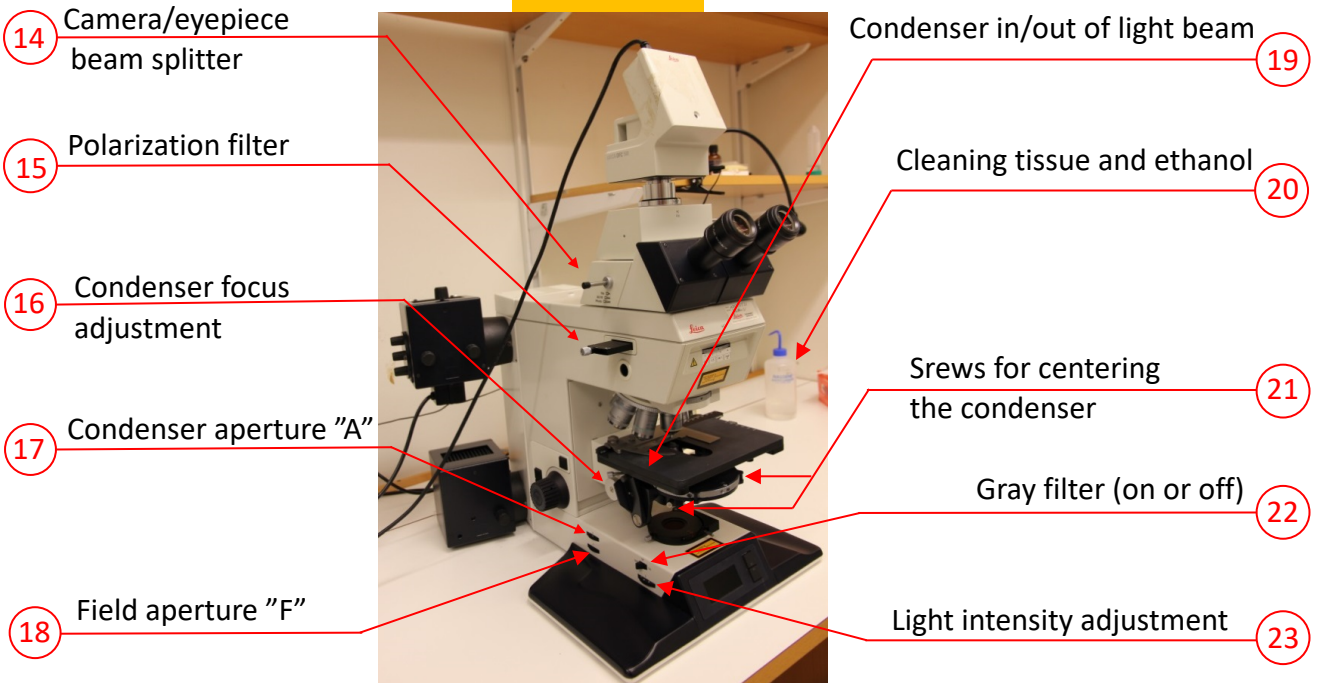
The properties for these tools can be adjusted under **Properties** (27). Create a report of your analysis with (28).

# Quick Reference – Leica Microscope

## (1) - (13)



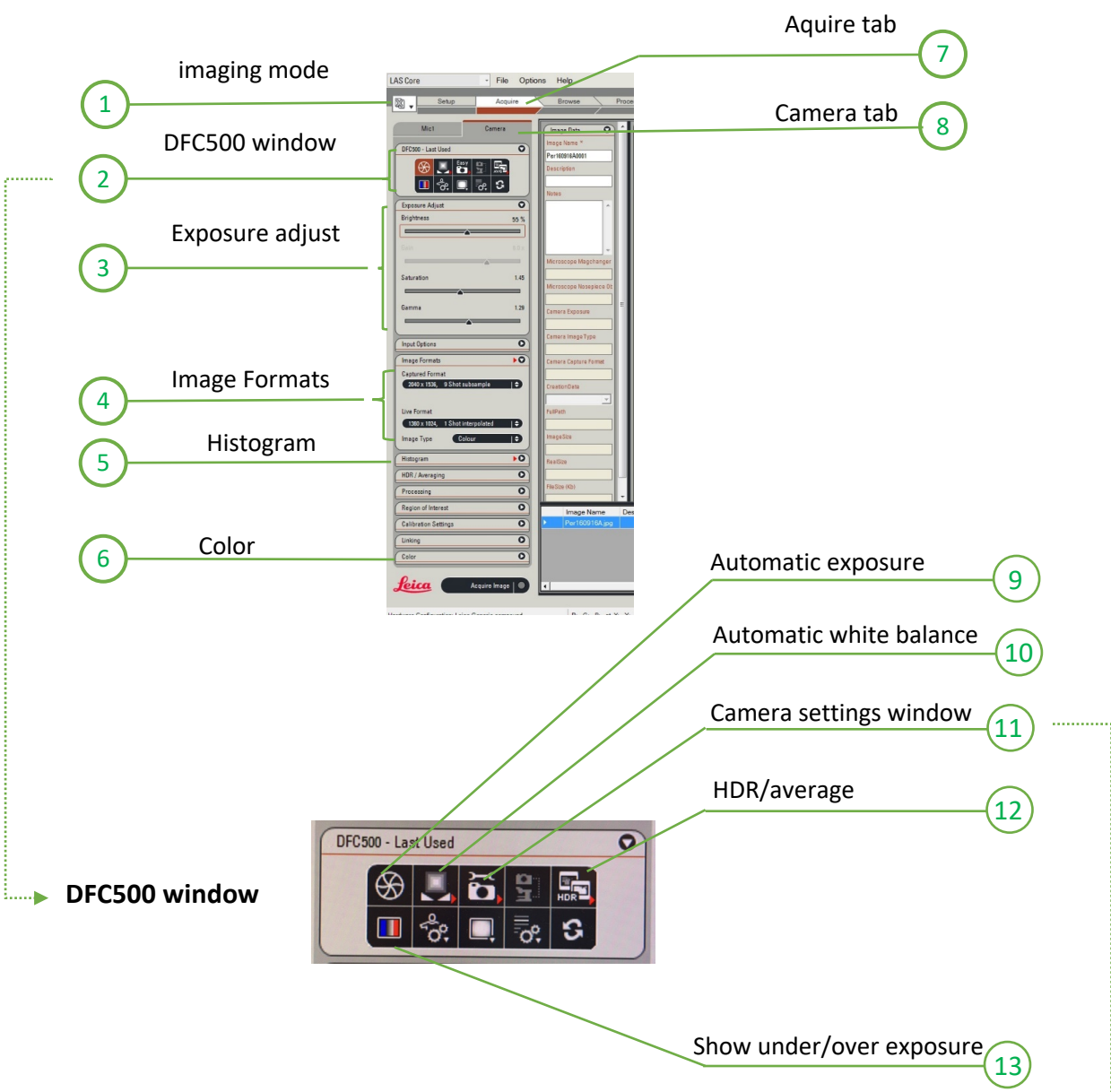
## (14) - (24)



Fluorescence shutter  
Outer= closed position

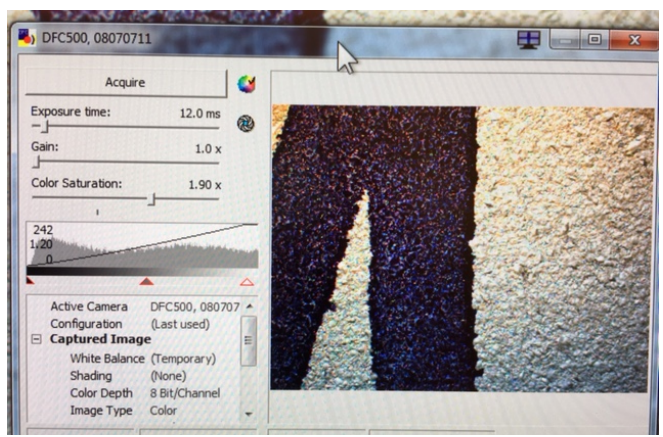
24

# Quick reference – Software (1) - (13)

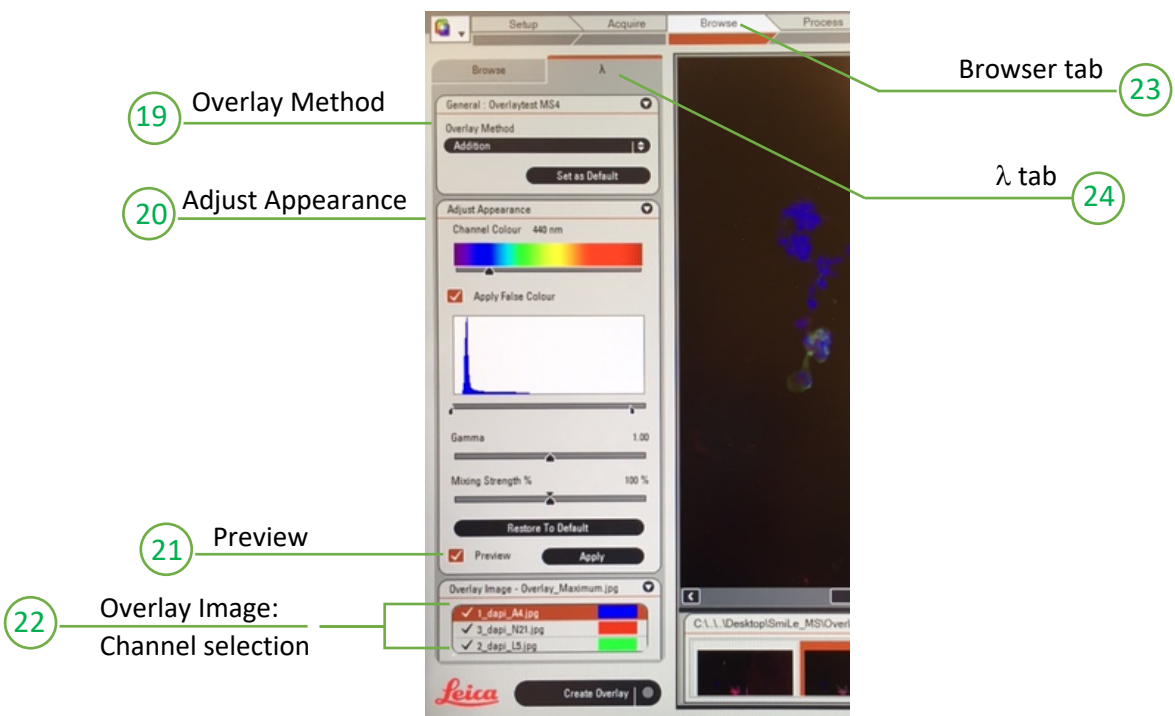
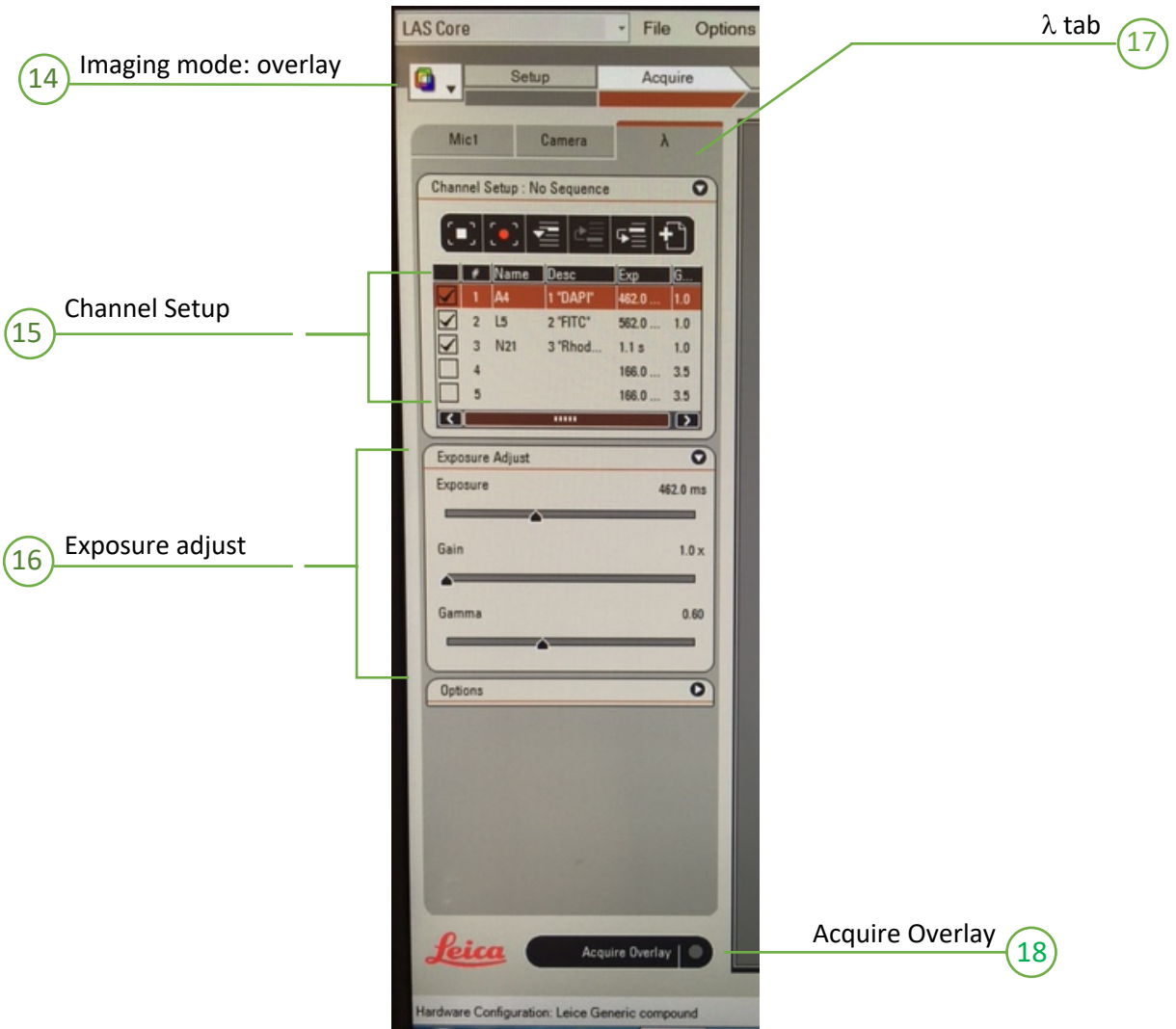


## Camera settings window (11)

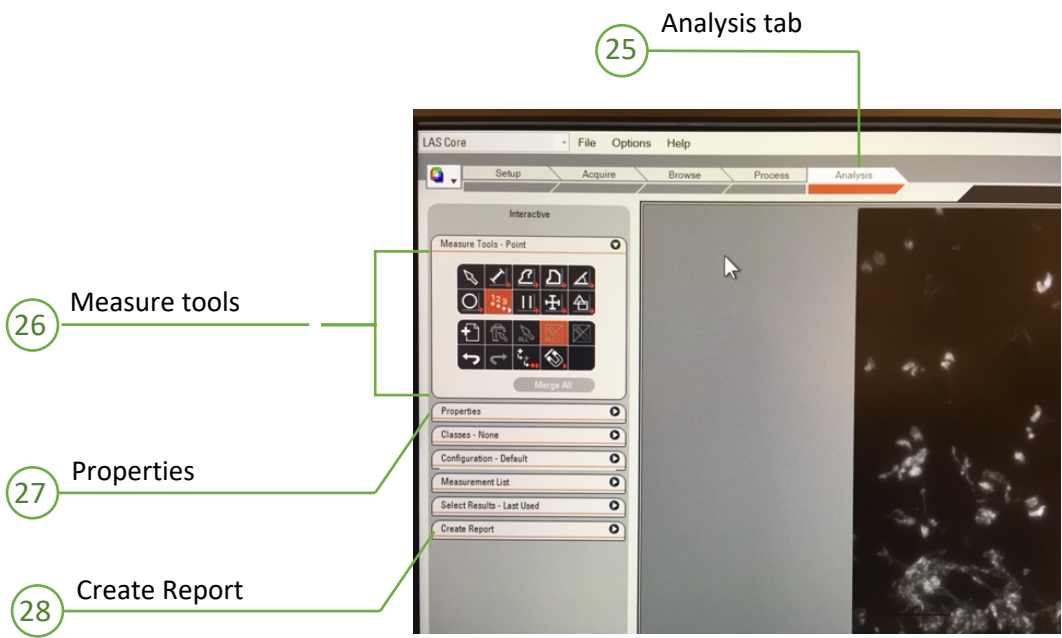
Adjust  
*Exposure, gain* and  
 manipulate  
*histogram*  
 simultaneously



# Quick reference – Software (14) - (24)



# Quick reference – Software (24) - (28)





# Quick Reference - Fluorescence

## Filters



**1 “DAPI”** (A4) for **blue** fluorescence:  
excitation 340-380 nm  
detection 450-490 nm  
e.g. DAPI, Hoechst 33482

**2 “FITC”** (L5) for **green** fluorescence:  
excitation 460-500 nm  
detection 512-542 nm  
e.g. Cy2, Alexa488, GFP

**3 “Rhodamine”** (N2I) for **red** fluorescence:  
excitation 515-560 nm  
detection >590 nm  
e.g. Cy3, Alexa546.  
(Texas Red, Alexa563 and Alexa 94 usually OK, too)

## Optimizing the light path for weak signals

Camera/eyepiece beam splitter (14)

**Vis:** all light to eyepiece

**Photo:** all light to camera -> better pictures

**50/50:** light is split between eyes and camera

