

## **Leica Confocal Instructions (rm. 341H)**

Startup Procedure (pg 67 User Manual):

1. Switch on the PC at the control panel
2. Make sure the microscope is on - if it is not on, activate the toggle switch
3. Log on to the computer and let the system boot completely
4. Turn on the scanner on the control panel – the windows status bar must show the “SP5 Scanner Gigabit Interface” is enabled. The scanner must be left to warm up for at least 1 minute prior to starting the Leica software.
5. Switch on the lasers on the control panel
6. Activate the detachable key switch
7. Turn on the Hg lamp
8. Start the LAS AF software by double-clicking the program icon
9. Initialize the system
10. Only initialize the stage if you have not mounted your sample and after ensuring the objective is retracted

### **Focusing your sample**

1. Mount your sample on the stage with the coverslip facing down
2. When using the 63x oil objective, use 1 drop of oil and clean the objective after use with lens paper. Fold the paper several times and wipe uni-directionally over the lens surface.
3. Using the xyz manipulator and the eyepiece, focus your sample using either transmitted or fluorescent light. The TL/IL button on the left side of the scope will switch between the two. Fluorescent specimens can be focused using either the I3 or the N2,1 filter cube buttons on the front panel of the microscope. The shutter button opens and closes the shutter.

### **Data Acquisition**

1. Open the LAS AF software and go to Configuration

2. Click on Laser, set the laser power to 30% and click on the boxes for the required lasers. NOTE only turn on the laser you are going to use.
3. Go back to Acquire. Set your excitation line and emission range, or choose one of the preloaded settings and ensure the relevant PMTs are active. If you are using your own settings, it generally suffices to set the start of the emission band 3-5 nm to the red side of the excitation line (to avoid interference from reflected light). Make sure you have an appropriate beam splitter selected for your excitation line.
4. Click on the objective button and select the objective that is being used by the microscope
5. Choose the pixel resolution to be used – use 512 x 512 or smaller for fast, lower resolution work and 1024 x 1024 for higher resolution work. A scan speed of 400Hz (the system will scan 400 lines in one second) is a reasonable starting point.
6. Click the “Live” button to start scanning – you should be able to see the laser scanning over your sample.
7. Your image, once optimized, will be visible on the right hand monitor. If you are using multiple PMT channels, each image should be optimized separately by clicking in the image box and activating that channel.
8. Use the “smart gain” and “smart offset” knobs to optimize your image. The “gain” adjusts the intensity (brightness) of the PMT channel in the image and the “offset” adjusts the black level (contrast). The “glow over/under” pseudocolouring (color bar at left side of image) is useful to adjust the brightness so that the maximum dynamic range of the PMT is made available. Pixels which are saturating the PMT are colored blue and pixels which have a value of 0 are green. The idea is to set the PMT gain so that the brightest pixels are just slightly under being saturated, and the offset such that the darkest pixels are just above a value of 0.
9. The “zoom” button can be used to go to the required zoom level.
10. Image averaging is a method of improving the signal-to-noise ratio of an image. Averaging by line permits averaging during a live (continuous) scan; averaging

by frame (scanning a number of times and averaging the brightness of each pixel across scans) permits the automatic switching of settings between channels during a sequential scan.

11. To acquire an image at any stage, stop the Live scanning and hit Capture Image.

The color of the image can be adjusted using the color bar on the lhs of the image.

### **Z-Stack**

12. Change from z-galvo to z-wide.
13. To capture a z-stack, set the upper and lower limits by marking the location of the top and bottom of the specimen while in the “live” mode. Enter the number of steps to be captured between the two positions – the thickness of each slice will then be shown (an average setting would capture between 1 and 5 sections per micron in z). Begin acquisition of the z-series by clicking the “start” button on the bottom right of the left monitor.

### **Saving Data**

14. Switch from the acquisition to the experiment window. You should see a series of images and/or series under Experiment 1. These are all the images you have captured and/or series you have taken since you started the software. The experiment itself and also any of the images can be renamed by right-clicking on the name and choosing “rename experiment as ...”. Once you have the image correctly named, you can right-click on the name and “export as tiff, jpeg etc”. You will be given the opportunity to choose your folder and save the data there. The computer is not networked (and is unlikely to be at any stage) so it is recommended that you bring a USB key to transport your data.

### **Shut down procedure (pg 137 User Manual):**

1. In the configuration window of the software, bring the laser power down to zero
2. Save your image data

3. Close the LAS AF software
4. Shut down the computer
5. Switch off the lasers with the key switch
6. Turn off the switches on the control panel for the PC and the scanner
7. The external fan of the Ar laser will switch off automatically after several (about 10) minutes. Now turn off the switch for the lasers on the control panel. It is very important not to turn the switch off before the fan has switched off.
8. Turn off the microscope and the Hg lamp.

#### Usable VIS/UV Lasers

Laser Type	Wavelength (nm)	Max. power at laser output (mW)	Max power in focal plane (mW)	Pulse duration
Ar	458, 488, 514	<200	<30	CW
HeNe	543	<1.5	<0.5	CW
HeNe	633	<15	<4	CW

#### Objectives on the Leica Confocal in 341H

Magnification	Numerical Aperture	Dry or oil immersion
63x	0.7	D
63x	1.4	O
40x	0.85	D
10x	0.3	D