

Leica SPEll confocal microscope

Short Manual



Switching ON sequence:

1. Turn on the Workstation under the bench (top, far right).



2. Turn on the Supply Unit - Laser box (big green switch first and then key, turn clockwise). Be careful not to pull the key out.



3. Switch on Microscope (if needed for BF). Green switch on left hand side of microscope.



4. Switch on EL6000 fluorescent lamp if using fluorescent samples. Shutter is always open and note position of attenuator. Can open this fully or close down if bright.



Wide-field fluorescence set-up:

1. Load slide onto microscope stage. Note that objectives move manually. Start with lowest x10 objective.

There are four long pass filters for wide-field fluorescence observation: **DAPI**, **FITC**, **Rhod** and **CY5**. These filters are changed manually using the fluorescence dial (see opposite). **Position 5** is for **BF** and confocal imaging (Scan).



2. Open **shutter** (left hand side of microscope: down is open) to allow excitation light to reach sample. Close **shutter** (up position = in) when not observing sample and when ready for confocal imaging.



3. When ready for confocal imaging, pull **lever from in to out** (top left of microscope) and make sure FL shutter closed and BF position (5) selected on filter turret.



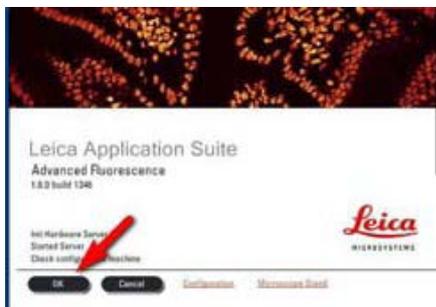
Confocal imaging:

Getting started with LAS software:

Log on as TCS user (password; Leicaspe2). Select LAS AF software.



Double click **LAS AF**. Wait for software to load.



Click on “**OK**” to start the initialization sequence.

Turn on lasers:

Select “**Configuration**” tab, then “**lasers**”. Select lasers lines that you require eg. 405 nm, 488 nm, 532, or 634 nm

Image acquisition:

Select the “**Acquire**” tab and “**Acquisition**” window. It will open in “**xyz**” mode and “**512x512**” pixel resolution at “**400 Hz**” (default settings). Details are displayed on a single line. (Note that the “**Zoom factor**” is **1.5**. This is the lowest factor to use.)

NB. Can pan in direction of arrows once zoom is greater than 1.5.

Beam Path settings:

1. Note the “**Beam Path**”. Select a fluorophore from the “**Leica Settings**” drop-down menu. This will set the defaults for the fluorophore you chose.

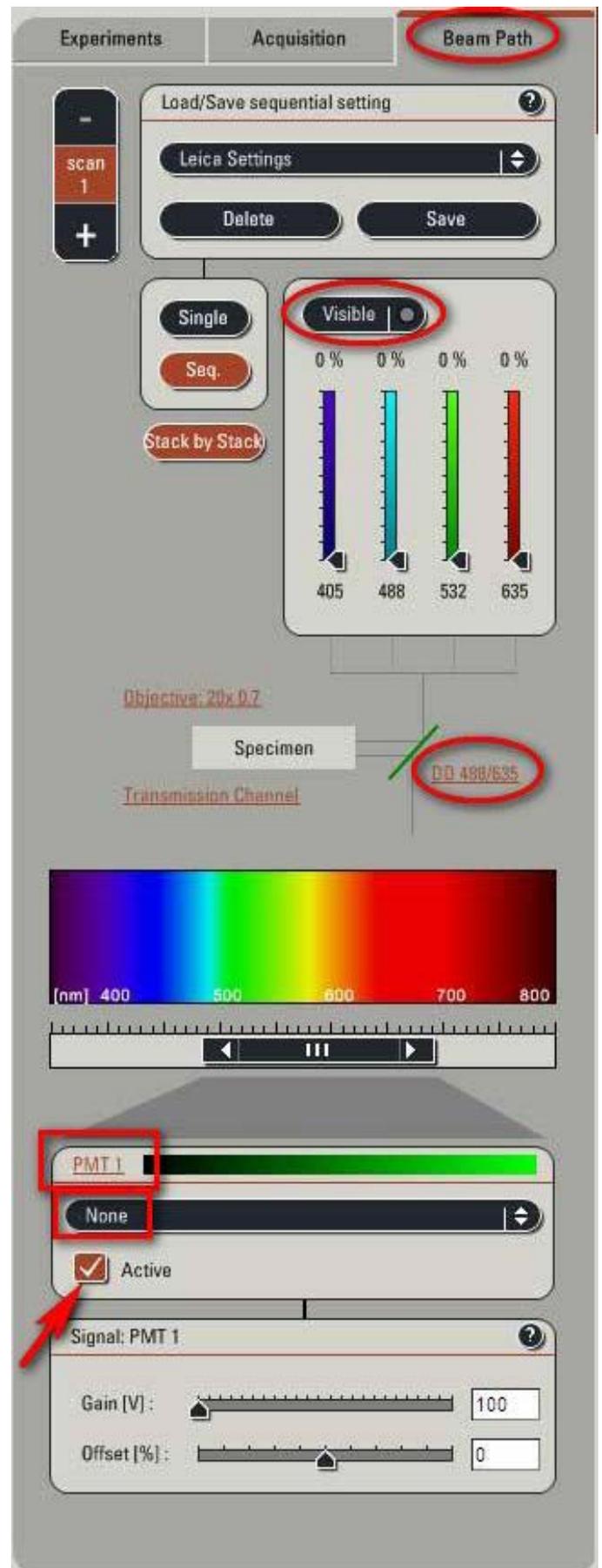
Alternatively, make up your own settings: click on “**Visible**” to activate the lasers and select the wavelength you wish to use by moving the slider. Start with 10-20% power.

If you select the laser using the slider, you will need to also select the proper dichroic filter and pseudo-color option on the “**PMT 1**” window.

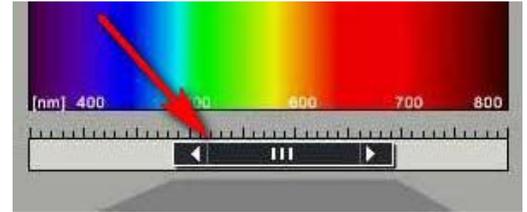
2. **IMPORTANT:** Manually select the appropriate objective

3. Activate the PMT by clicking on the “**Active**” check box in the “**PMT 1**” window.

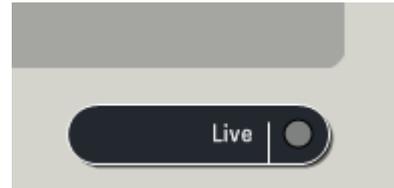
4. Click on the “**NONE/SETTINGS/MORE**” bar to open the drop-down menu and choose the fluorophore emission wavelength to assist in setting the PMT bar.



5. Adjust the PMT bar by clicking on it and moving it. Resize the bar by clicking on one edge and dragging it left or right. The slider can also be resized by double clicking on it and entering the values needed. (Note: the minimum wavelength distance from the excitation wavelength should be 10 nm longer).

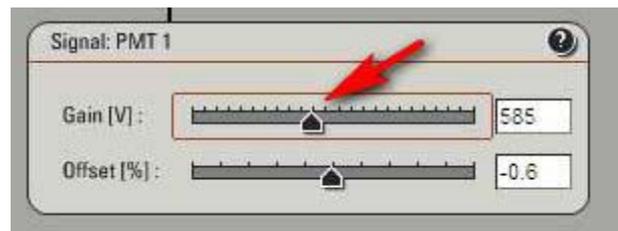


6. Click on the “Live” button at the bottom left of the screen or on the second from left button on the panelbox (see below).

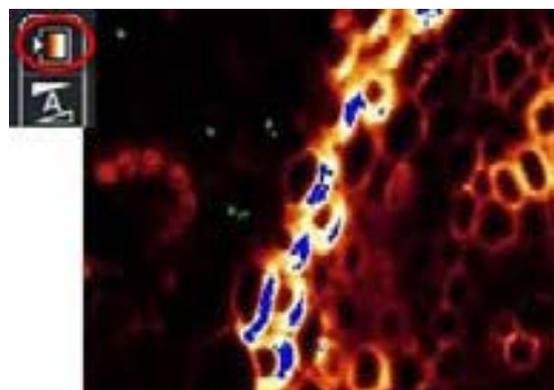


Optimising the image:

Increase the “Gain” until an image appears. This is done by using the “Signal PMT 1” slider, or clicking on the slider and turning the mouse wheel.



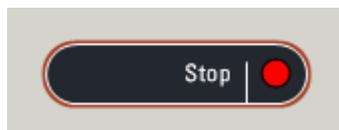
Adjust the gain and offset using the “QLUT” (Quick Look Up Table) to change the image to color as intensity values. Set the color values to mostly orange with some blue (saturation level) and some green (offset below which image will be black). If necessary, adjust the laser intensities and PMT bar position.



Note: A gain value lower than 400 V would mean that you can lower the laser power and go up the smart gain until about 900-1000 V). A smart gain between 1100-1250 would suggest going up on the laser power.

REMEMBER: By enhancing the laser power you will expose your sample to more laser exposition, hence your sample **will bleach faster**. On the other hand, enhancing the gain won't expose your sample to more laser exposition, and it will protect your sample from too much laser exposition. Thus, in order to protect your sample signal, it is better to first adjust the gain, and then if not enough signal is found, enhance the laser power percent.

Click the **“Stop”** button, and then click the **“Capture Image”** button to acquire an image.



When you have optimised each scan parameters, select **“Start”**.



Changing the quality of your image acquisition:

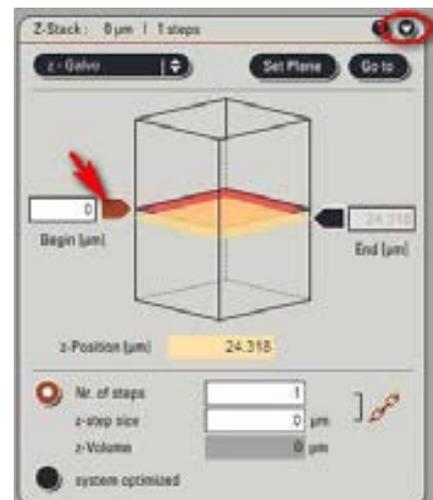
Averaging the Frame number can dramatically improve the quality of an acquired image. Under the **“acquire”** tab, change the number of frames to average. This will result in multiple scans where the resulting image is the average of the intensities.



Acquisition of a Z-Stack

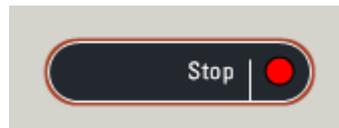
Under the **“Acquire”** and **“Acquisition”** tab, verify the mode is **“xyz”**.

Expand the **“z-stack”** band to show the full selection window.



Select the current focal plane (orange diamond) in the z-stack window. Click on “**Live**” mode and using the mouse wheel, focus to the top of the sample and click the left arrow “**begin point**” to set it. Then again using the mouse wheel, focus on the lowest plane of your sample and click the “**End point**” arrow to set it.

Select “**Stop**”.



Set the number of z-steps by either accepting the “**system optimized**” button, or selecting the number of steps, or the step size you prefer.

Click “**Start**” to begin the stack creation. It will end automatically.



NB. When using sequential scan in multi-channel imaging, select “**Stack by Stack**” for speed and deselect “Stack by Stack” if looking at co-localisation.

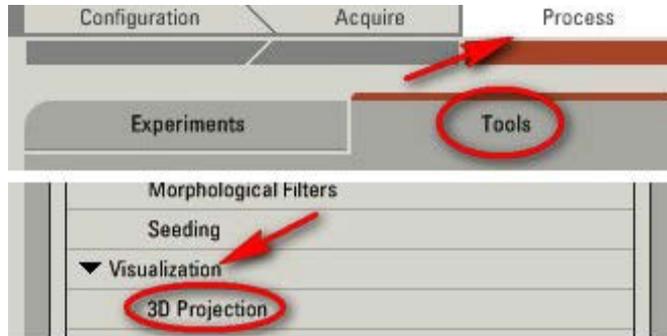
REMEMBER to deselect “begin” and “end” arrows and put No. of steps as “1” if do not want to re-do a z-stack.

Saving Images

The stack will be saved under the “**Experiment**” tab where it may be renamed (right mouse click and select “**rename**”) and saved by selecting the “**Save all**” button. All images are saved as created as .lif (**Leica Image Format**) files. Be sensible about your file names and create new experiments as needed.

Creating a 3-Dimensional Projection:

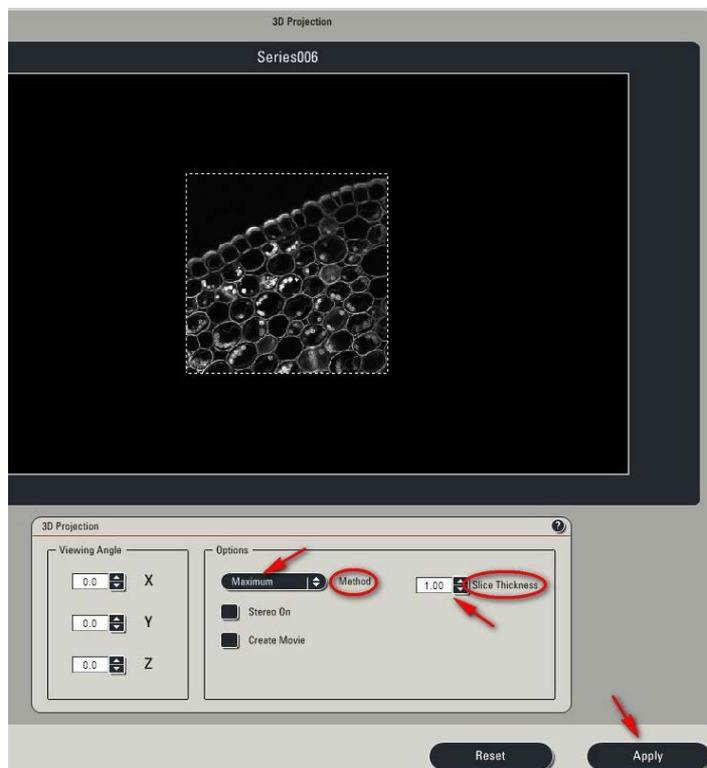
After acquiring a z-stack the images may be processed into a 3-D projection directly or using the 3D projection tool.



Without animation:

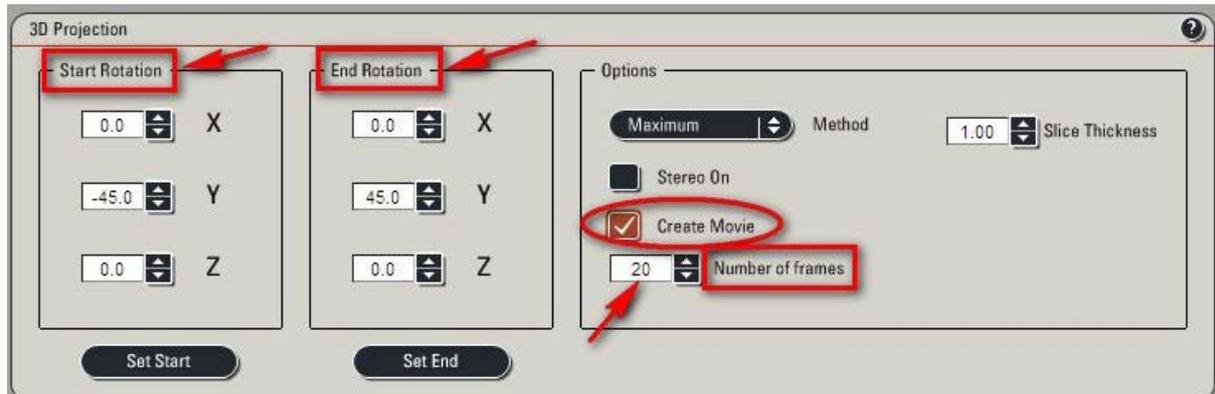
Select the stack to use under the “Experiment” tab. Click on “Process” > “Tools” > “Visualization” > “3D Projection”.

Do NOT change the “X, Y, or Z” planes if you want a simple projection.



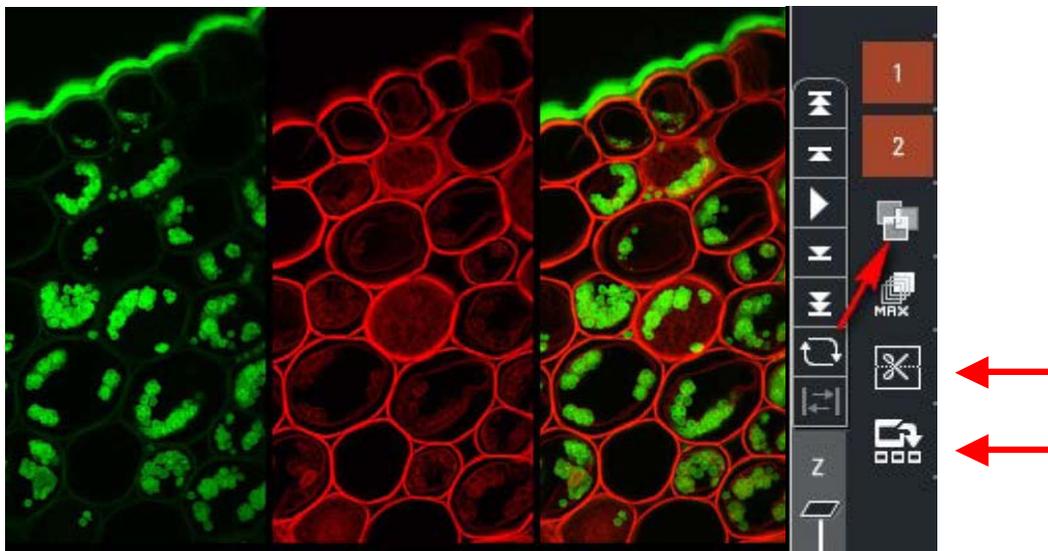
Click “Apply”.

With animation:



Click **“Create a Movie”**. Enter the **“Start Rotation”** angle (in degrees) corresponding to the start view of the movie and click **“Set Start”**. Enter the **“End Rotation”** angle corresponding to the end view of the movie and click on **“Set End”**. Enter the **“Number of Frames”** desired for the rotation. Note that the higher the number of frames, the slower the rotation speed. Click on **“Apply”**.

The 3D movie may be viewed through the **“Acquire” > “Experiment”** tab. Layers may be turned on or off as can the overlay view.



Note: Double clicking on a view will toggle between full frame and multiple frame options. Note the 3D-orthogonal view and the gallery view

Switching OFF sequence: This is the reverse of turning on. Check to see if another user is coming on to the system.

1. Save all images. Shut down software: **File > Exit.**
2. Log hours on EL6000 fluorescent lamp and then switch off.
3. Turn off Microscope (if halogen lamp was on)
4. Turn off Supply unit - Laser box (turn key anti-clockwise to "0" and then turn off big green switch).
5. Shutdown computer.