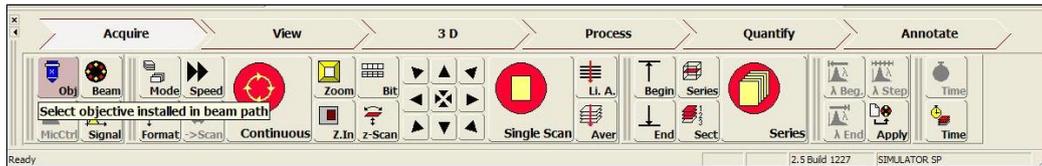


Leica TCS SP2 UV

Quick Start

After starting programme:

Note that the buttons at the bottom of the left-hand monitor (see below) are used to



set almost all of the experimental parameters; however, some parameters are directly accessible through the black knobs on the Control Panel (see below).



The assignment of each knob on the Control Panel is shown at the very bottom of the left-hand screen (see below; ignore labels on the Control Panel itself).



When the program starts an experiment will be automatically opened under 'Experiments' in the top left panel on the left-hand monitor (see right).

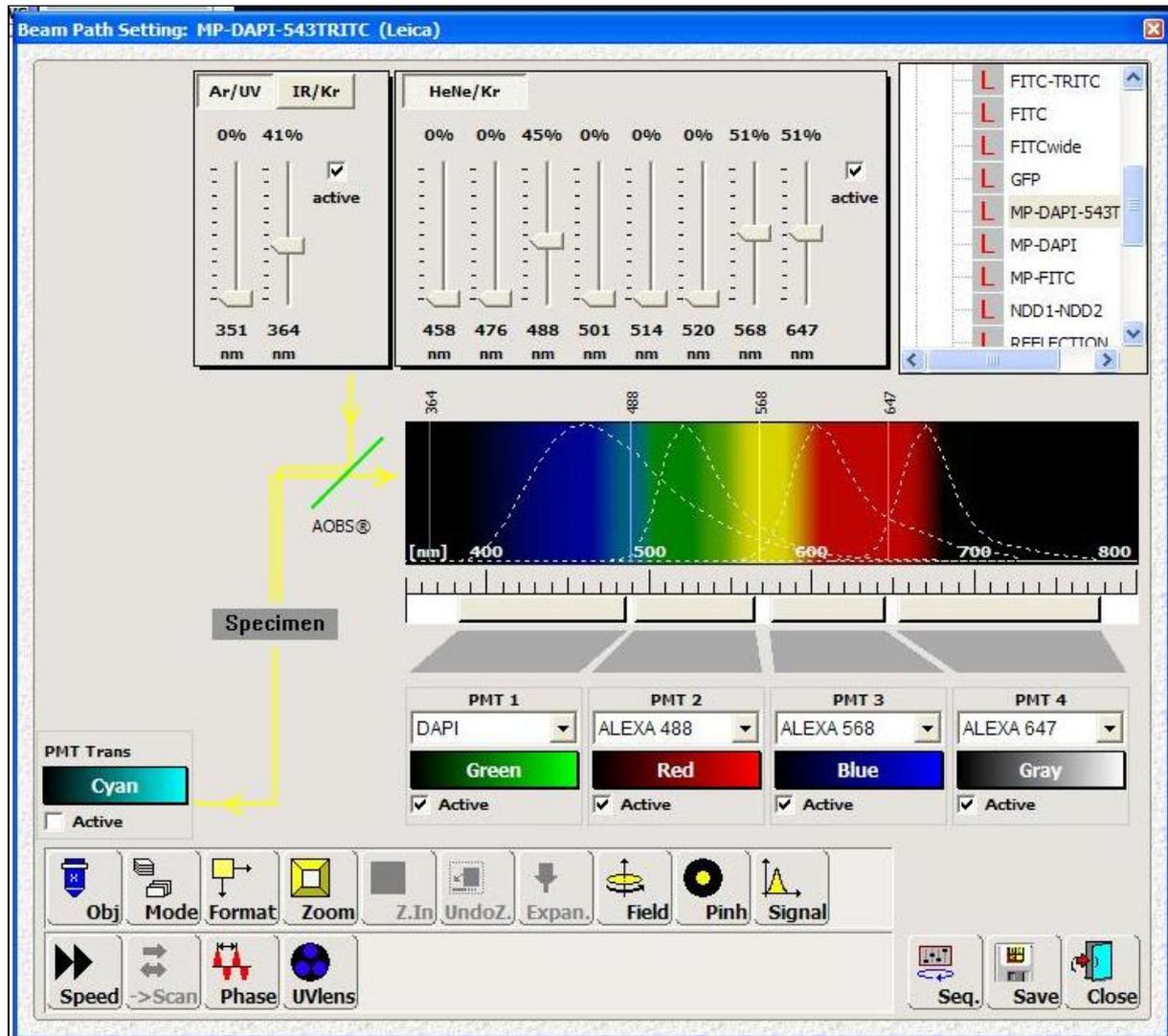
All images will be placed in this experiment until saved or deleted.



- 1) Click on **MicCtrl** button, choose **User** if you are using brightfield optics to locate your sample; or if you need to use the Hg lamp to focus, choose **Visual**.
- 2) Choose objective by clicking on **Obj** button.
- 3) Place slide on stage and bring the sample into focus.

WARNING: If you use DIC, be sure to remove the half phase plate (ICT/P) and the lower polarizer (move silver wheel below objectives, on left hand side of microscope, to BF) before scanning.

- 4) Click on the **Beam** button. A dialogue box labelled **Beam Path Setting** (see below) will open.



- 5) Double click on required fluorophore settings from the list at top right; e.g., FITC-TRITC.

Note: The settings labelled with L are default files that cannot be modified. You can create your own personalized settings by modifying the default settings and then saving under a unique name. The latter will then be added to the list of user-defined settings (labelled U).

Generally,

6) Use this dialogue box to:

A) Activate (tick appropriate box), and set the laser power: using the sliders in the top, middle panel.

Note: Set power to zero for unwanted lines.

B) Select PMT (1-3; tick appropriate box), and adjust the wavelength range that is detected by the individual detectors by varying the width of the 3 grey bars beneath the spectrum

After adjusting settings, close box.

7) Choose bit depth (*i.e.*, resolution of the dynamic range) by clicking on **Bit** button (8 bits is fine for the majority of work).

8) The **Expan.** button is used to set the expansion of the laser beams (should be set to 6).

9) Use **Mode** button to choose type of experiment (*e.g.*, Z-stack = xyz, time course = xyt)

10) Use **Format** to set the size and resolution of the image (default = 512 pixels x 512 pixels).

*Note: smaller format = smaller files, but your choice will depend upon the size of your sample and the desired resolution. A good rule of thumb (unless using deconvolution) is that the pixel size should not be smaller than $0.25 \times \lambda_{em}$ (*e.g.*, for FITC: pixel size $\approx 130 \times 130 \text{ nm}$).*

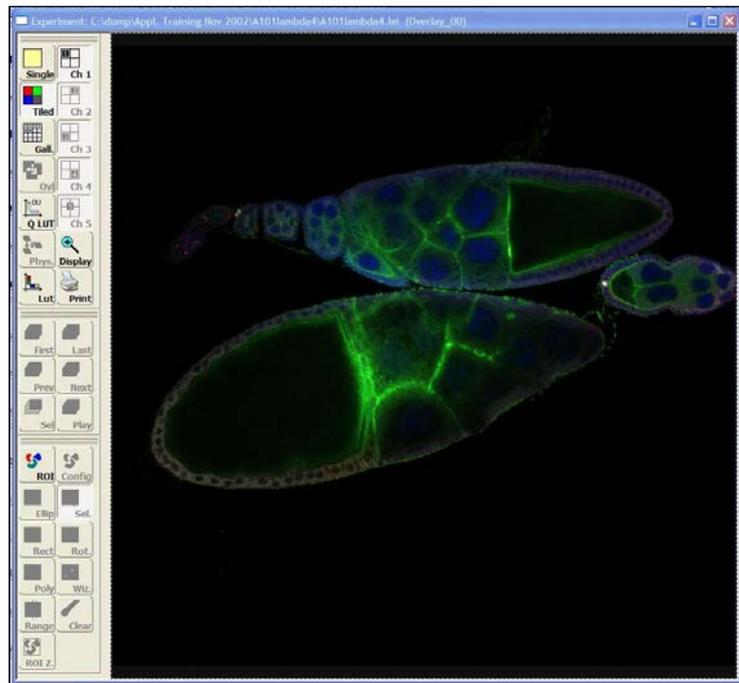
11) Use **Speed** to select the speed at which the laser is scanned over the sample (slower = more light collected = brighter image).

12) Use **Scan** button to switch between unidirectional or bi-directional scanning (default = uni-; *i.e.*, **_>**).

To make the final adjustments (PMT offsets, gains, Z-planes, *etc.*) you will now have to scan the sample.

To do this:

- 13) Click on **MicCtrl** and select **Scan**.
- 14) Click on **Continuous** button - an image/s will appear in the right-hand monitor (see right; one image per channel).
- 15) Click on the image you want to adjust.
- 16) Click on **QLUT** button on the left side of the right-hand monitor (see right).



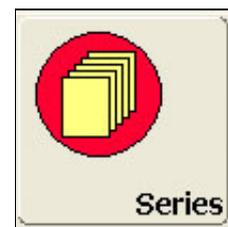
- 17) Iteratively adjust the gain and offset using the **Smart Gain** and **Smart Offset** knobs on the Control Panel (see "After starting programme" on first page). Adjust the offset until there is a sprinkling of green pixels, and the gain until there is a sprinkling of blue pixels. Then adjust focus with **Z POS** knob, on the Control Panel, to make sure the brightest part of the sample is in focus. Readjust **Smart Gain** and **Smart Offset** if necessary.
- 18) Repeat this procedure for any other channels.
- 19) Click on **QLUT** (colour will return to the appropriate LUT for the sample; e.g., green for FITC).
- 20) Then click the **Stop** button.

For a single image:

- 21) Click the **Single Scan** button. A single image will be added to the active experiment (see upper left panel).

For a time series:

- 22) Click on the LARGE **Series** button(see right). Then click on the



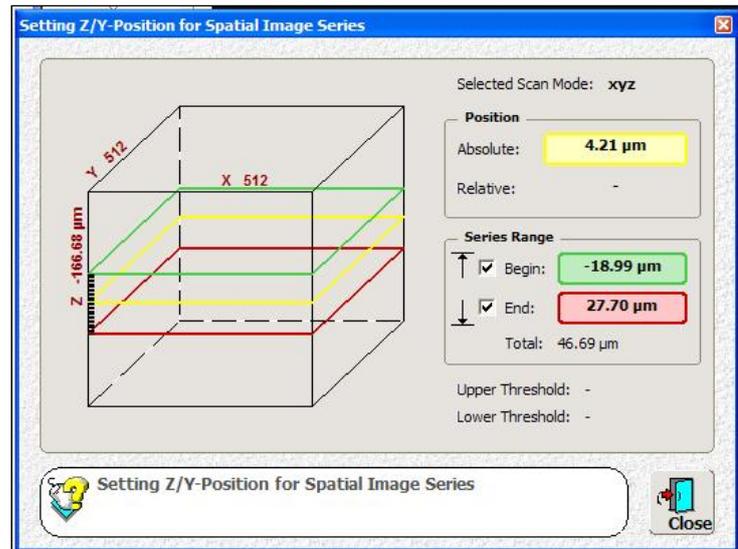
[Stop](#) button to bring the series to an end. A multi-image series will be added to the active experiment (see upper left panel).

For a Z-stack:

- 23) Click on [SMALL Series](#) button (see right). A dialogue box will open ([Setting Z/Y-Position for Spatial Image Series](#), below) that outlines the Z positions.



- 23) After clicking [Continuous](#), move up and down through the sample using the **Z POS** knob on the Control Panel (note the variation of the focal position shown in the dialogue box).



- 22) Having decided where you want to begin the scan, move to this position (e.g., bottom of the stack) and click on the [Begin](#) button (the starting position will be ticked in the dialogue box).
- 24) Repeat for the end position (e.g., move to top of stack), clicking on the [End](#) button (the ending position will now be ticked in the dialogue box). Click on [Stop](#) to finish.
- 25) Click on the [Sect](#) button to determine the number of, or distance between, sections.

Note: The programme will calculate an optimum number of sections based upon the objective and pinhole. You can override this by either selecting a number of sections from the list, or choosing [Others](#) and setting the [Step size](#) by inserting the desired distance and then clicking the [Calculate](#) button to the right of the #Sections box (or vice versa by entering the number of sections).

- 26) After setting up the Z-stack you can review and edit the settings by clicking on the [SMALL Series](#) button.

- 27) Click on the LARGE **Series** button to acquire the stack. Click on the **Stop** button to end acquisition. A multi-image series will be added to the active experiment (see upper left panel).
- 28) Click on the **Save** button (see below) to save an individual experiment (the one that is active). Click on **All** button to save all experiments in the window.



*Note: Even if you haven't saved an experiment, you can start another by clicking on the **New** button. A new experiment will appear in the experiments list below the previous experiment that has not yet been saved (the latter will not be overwritten).*