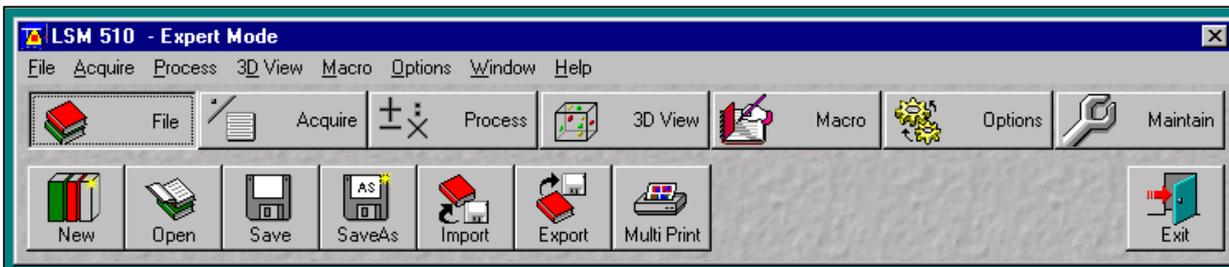# Zeiss LSM510 META

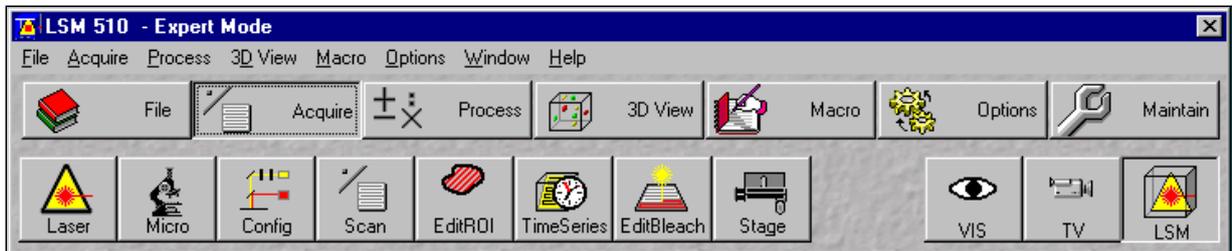## Quick Start

After entering Expert Mode:
The dialogue box below will appear on the left-hand monitor.

1) Click on the File button, choose New.

2) Navigate to your user directory on BMIFilestore (drive I), open the directory 'Zeiss LSM510' and create a new database. When the database opens minimise it.

3) Click on the Acquire button.

4) Click on the Laser button. The dialogue box on the right will open. If you are using the UV laser, switch Enterprise to Standby, after the 'screech' wait until it reads Ready then select On, leave at 50%. If you are using the Argon laser, switch Argon/2 to Standby, wait until it reads Ready and then click On, set current to 60%. If you are using the HeNe lasers turn HeNe1 and/or HeNe2 to On.
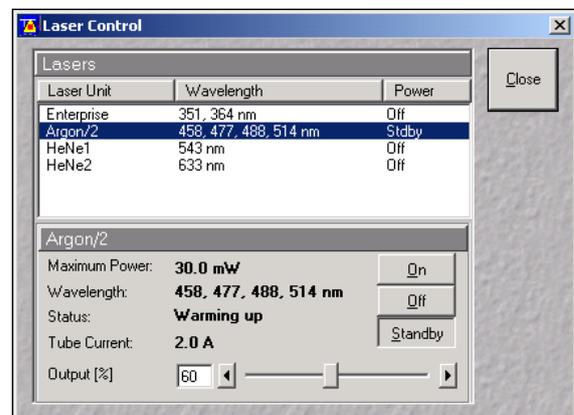
5) Click on the VIS button and place slide on stage.

6) Click on the Micro button. The dialogue box on the right will open. You can choose the objective, filter cube, *etc.* that you need by clicking on the appropriate icons.

7) Using brightfield or fluorescence optics, bring the sample into focus.

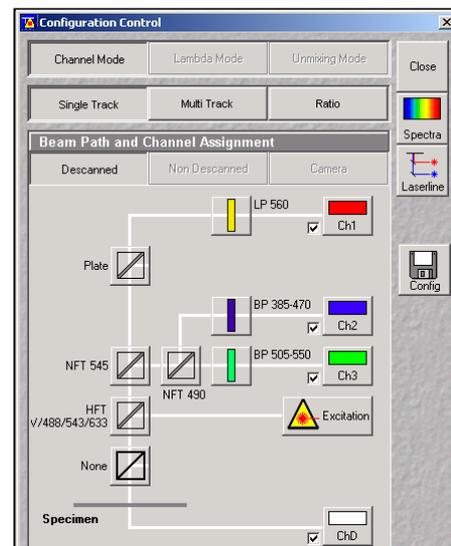8) Having found your sample close the Microscope Control dialogue box and click on the LSM button.
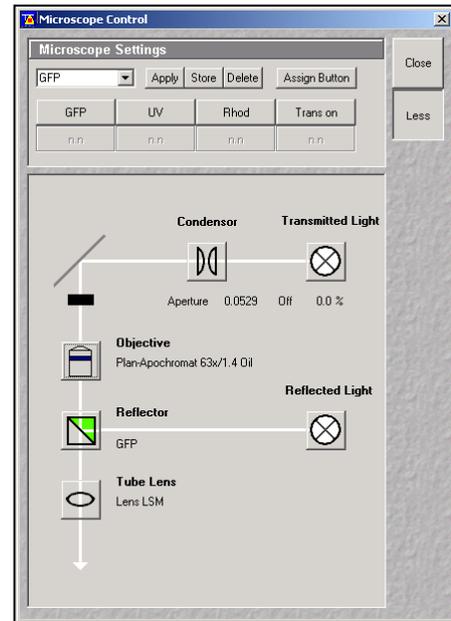
9) Click on the Config button. A dialogue box (right) will open in which you will choose the configuration needed for the fluorophore/s you are using.

Click on Channel Mode.

For a single fluorophore click on Single Track.

For multiple fluorophores click on Multi Track.

10) In the Configuration Control dialogue box click on Config.

A drop down menu (right) will appear from which you can choose the configuration you require (*e.g.*, CFP to image a sample labelled with Cyan Fluorescent Protein, or DAPI/FITC MT to image a sample labelled with DAPI and FITC).
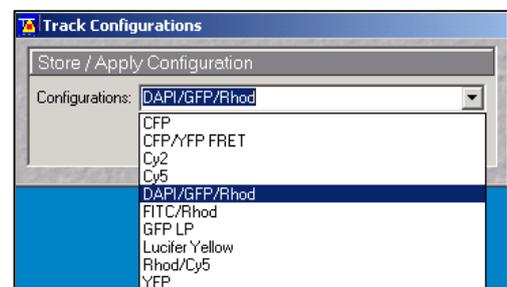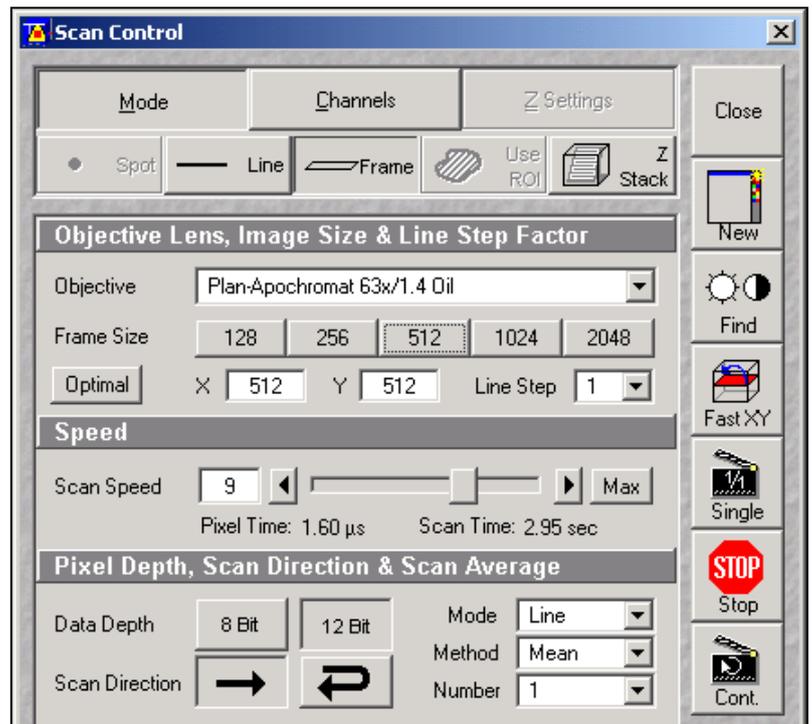
If using Single Track:

1) After choosing the particular configuration file you want to use, click on the Scan button.

2) The dialogue box on the right will open.

3) By default the Mode and Frame buttons will be selected. Check that the correct Objective is still selected (NOTE: when you load a particular Config file it may move to a different objective than the one that you used to focus). Other items saved in the Config files include the Frame Size (probably best to start with 512x512, and then adjust appropriately), Scan Speed (7 is probably a good place to start, slower = better quality), Data Depth (*i.e.*, resolution; 8 bits = 256 grey levels, probably fine for most applications), Scan Direction (unidirectional), Scan Averaging (choose Line or Mean; 1 = no averaging).
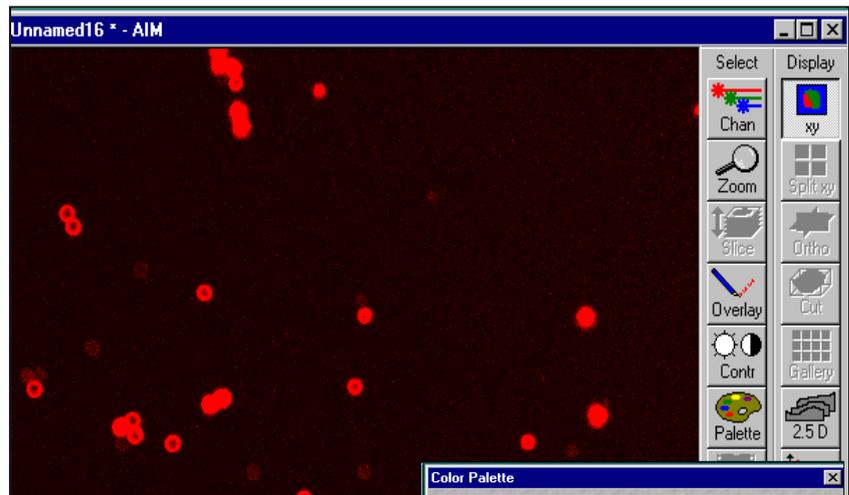
4) Then choose Channels. Set Pinhole (see right) to 1 Airy Unit by clicking on 1 (optimal signal:noise actually at 0.8 A.U., but signal may be low at this setting).

5) Click on New, and then Find. An image window will open on the right-hand monitor and the programme will attempt to find the best settings for the Detector Gain and Amplifier Offset.
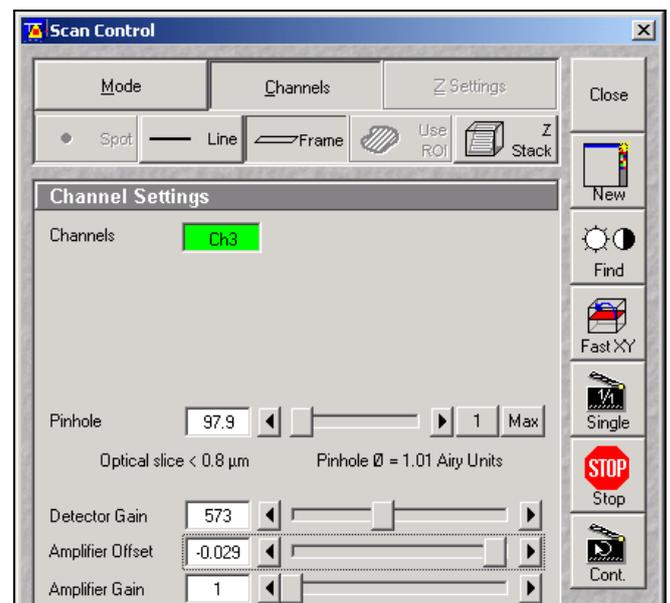
6) You can make minor adjustments to the Detector Gain and Amplifier Offset by using the slider controls. (Normally leave Amplifier Gain at 1.)

7) To make adjustments click on the Palette button found on the image window (see right). A dialogue box will open, choose Range Indicator. Increase the Amplifier Offset until all the blue pixels disappear, and then make it slightly positive. Reduce the Gain until the red pixels only just disappear. (Note: red indicates saturation, and blue indicates negative pixel values.)

8) Adjustment can be carried out while scanning (click Cont. button); or if you wish to minimise photodamage then adjust and click Single, iteratively).

9) After final adjustments, click Single to obtain your image. Click on File and choose SaveAs.

10) Give the image a name and it will then be saved to the database you opened at the beginning of the session. (Note: the image can now be safely closed.)

If using <u>Multi Track</u>:

Follow steps 1-3 as described for Single Track recordings (see above).

1) Choose Channels in the Scan Control dialogue box (see right). Select the channel with the longest wavelength by clicking on the appropriately-coloured button. Set the Pinhole to 1 Airy Unit. Take a note of the size of the Optical slice (to the left of the slider beneath the pinhole size), then select all other channels in turn, and adjust the pinhole until the Optical slice thickness is the same for all channels. (Note: the shorter the wavelength, the larger the pinhole size in Airy Units.)
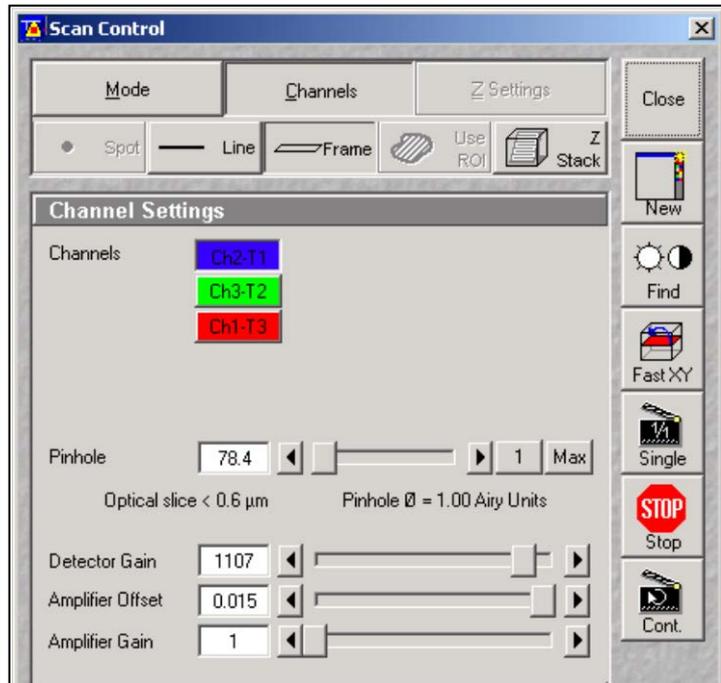
2) You can then optimize the Detector Gain and Amplifier Offset as described in step 5 for Single Track recordings (see above). However, in order to make minor adjustments to individual channels (see steps 6-9, above) you have to deselect the unwanted channels in the Configuration Control dialogue box (see right).
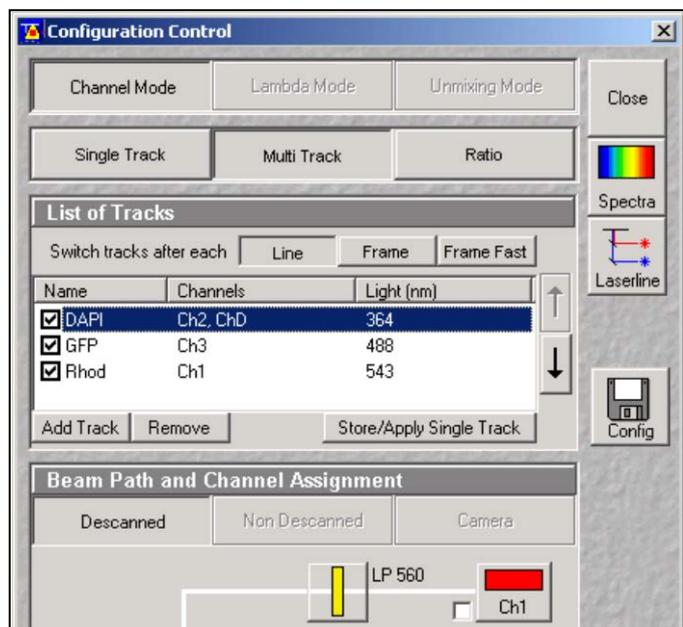
3) Under List of Tracks make sure only one channel is selected (tick box to left, under Name). Then adjust Detector Gain and Amplifier Offset for this channel as described for Single Track (steps 6-9, above). Select other channels in turn, and repeat.

4) After final adjustments, make sure all channels are selected in the Configuration Control dialogue box. Then click Single in the Scan Control dialogue box to obtain your image, click on File and choose SaveAs.
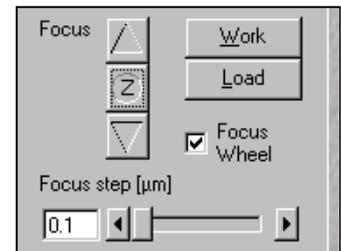
To obtain a Z-stack:

1) Focus on approximately the middle of your sample, and optimize image acquisition parameters as described above.
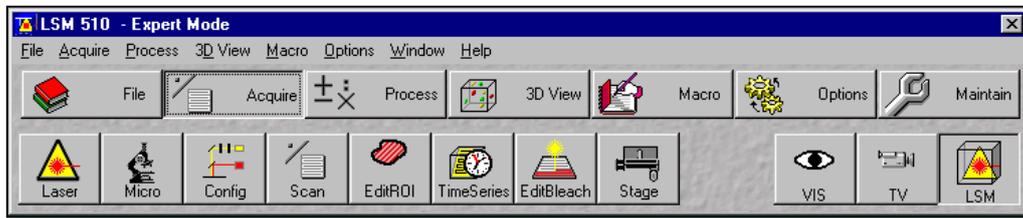

2) Make sure Acquire is selected then click on Stage.

3) In the dialogue box that opens (see right), set the Z level to 0 by clicking on the Z between the two arrowheads under Focus.

4) Return to the Scan Control dialogue box and choose 'Z Stack'.

5) Click on Mark First/Last, and then click on Fast XY. Focus on bottom of specimen and click Mark First, move focus to top of specimen and click Mark Last, click Stop. This sets the limits of the Z-stack acquisition.
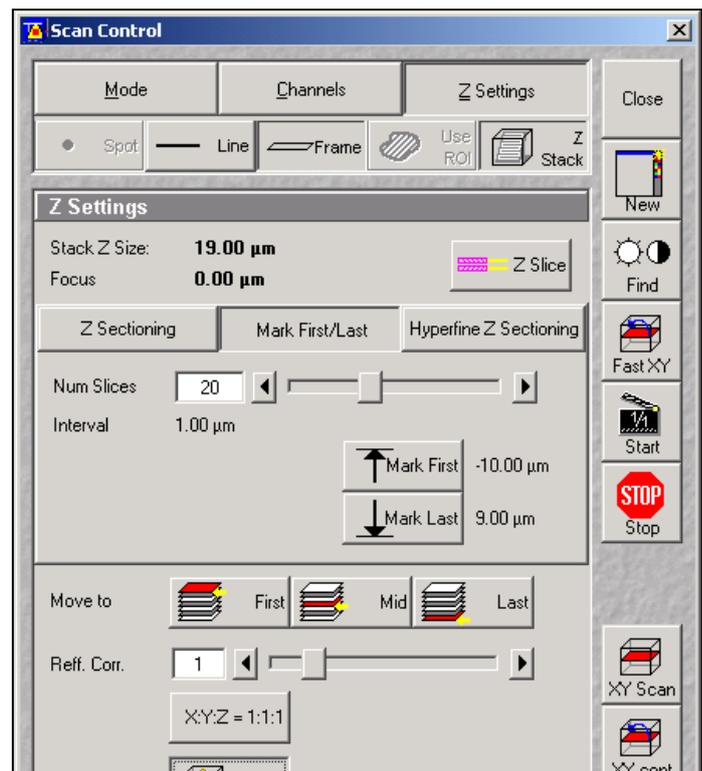
6) To set the distance between sections, click on Z Slice. The Optical Slice dialogue box will open (see below right).

7) Select Optimal Interval and close.

8) The Z settings (Num slices and Interval) in the Scan Control dialogue box will now have been up-dated to the appropriate values. Click 'Start' and a Z-stack will be acquired. After acquisition, to add the file to your database, choose File and SaveAs.