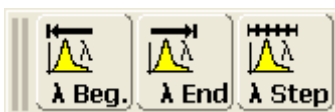


Confocal Application Notes

Vol. 2 July 2004



Lambda scan



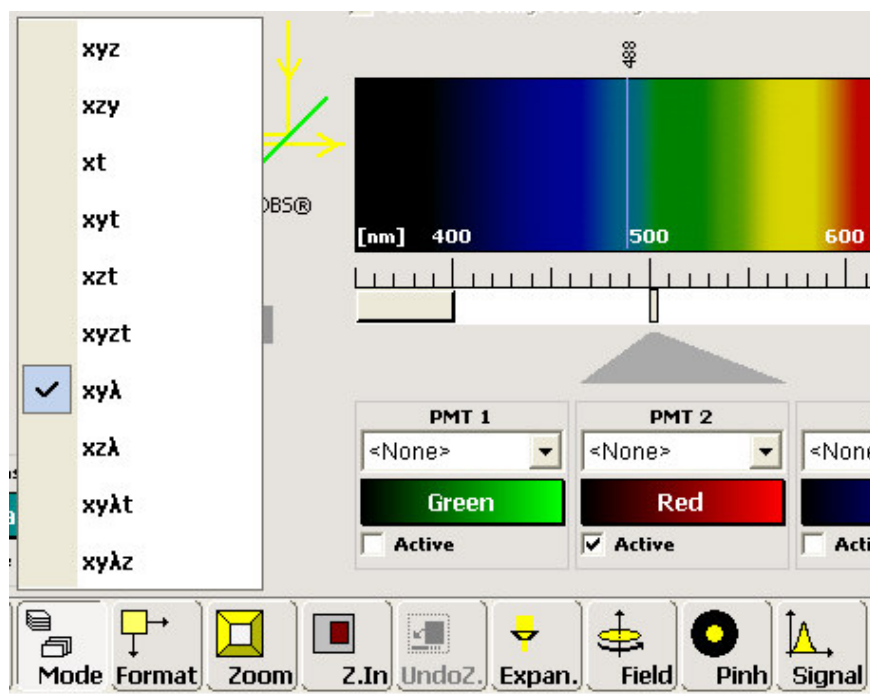
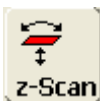
A lambda scan records a series of individual images within a user-defined wavelength range; each image will be detected at a specific emission wavelength. This can be used to measure the emission spectrum of new fluorochromes or to determine the emission maximum of a fluorescent dye in a specific sample to optimize detection.

The following protocol will help you to set up a single-channel lambda scan. In this example we will scan the emission spectrum of a fluorescent dye in a buffer solution over an interval from 500 nm to 650 nm with excitation at 488 nm.

1. Click on to the *Acquire* tab in LCS and open the beam path window.



2. Select a lambda scan mode such as $xy\lambda$, $xz\lambda$, $xy\lambda t$, or $xy\lambda z$. Please note that the $xz\lambda$ mode can only be used if *z-Galvo* has been selected for z-movement.



Confocal Application Notes

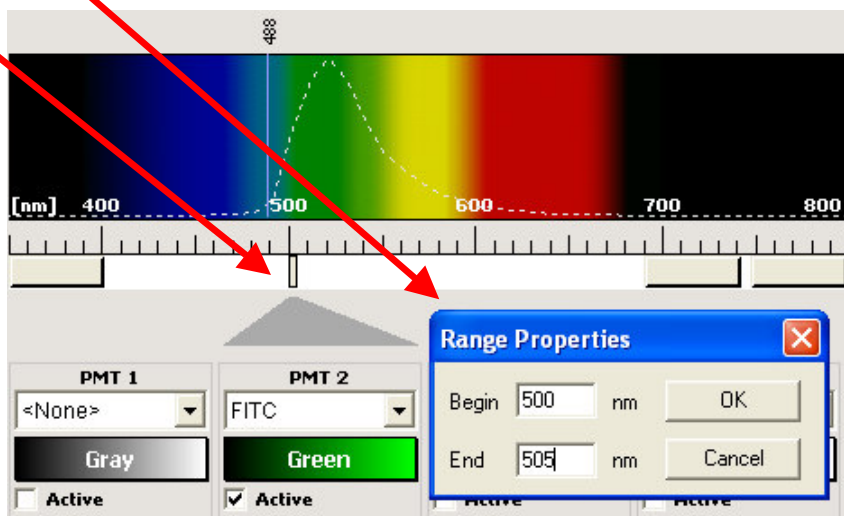
Vol. 2 July 2004



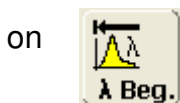
3. Setting up the instrument parameters for your experiment. In the beam path window choose the settings for your experiment, i.e. the laser line you would like to use, and activate a single PMT for detection. Move the PMT slider to the expected emission maximum, change the width of the slider to the value you will use in your experiment (see below) and adjust PMT gain and offset.

The width of the detection window can be changed by double-clicking on the PMT slider bar. The *Range Properties* window will open; enter the values for *Begin* and *End*

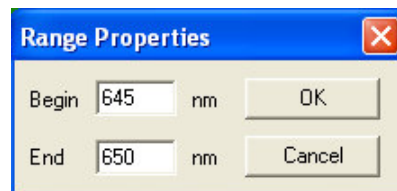
and press OK. The smallest possible width for the detection window is 5 nm. In our example we are going to set the width of the slider to 5 nm. The values for *Begin* and *End* are 500 and 505 nm, respectively.



4. After setting the range define the starting point of the lambda scan by clicking



5. In our experiment the scan should stop at 650 nm. Make sure that no other slider is located within the scan interval; otherwise LCS will display an error message to remind you.




Now double-click on the active PMT slider and set the detection window to 645 – 650 nm.


The width of the detection window has to be the same for begin and end of the scan interval.

Confocal Application Notes

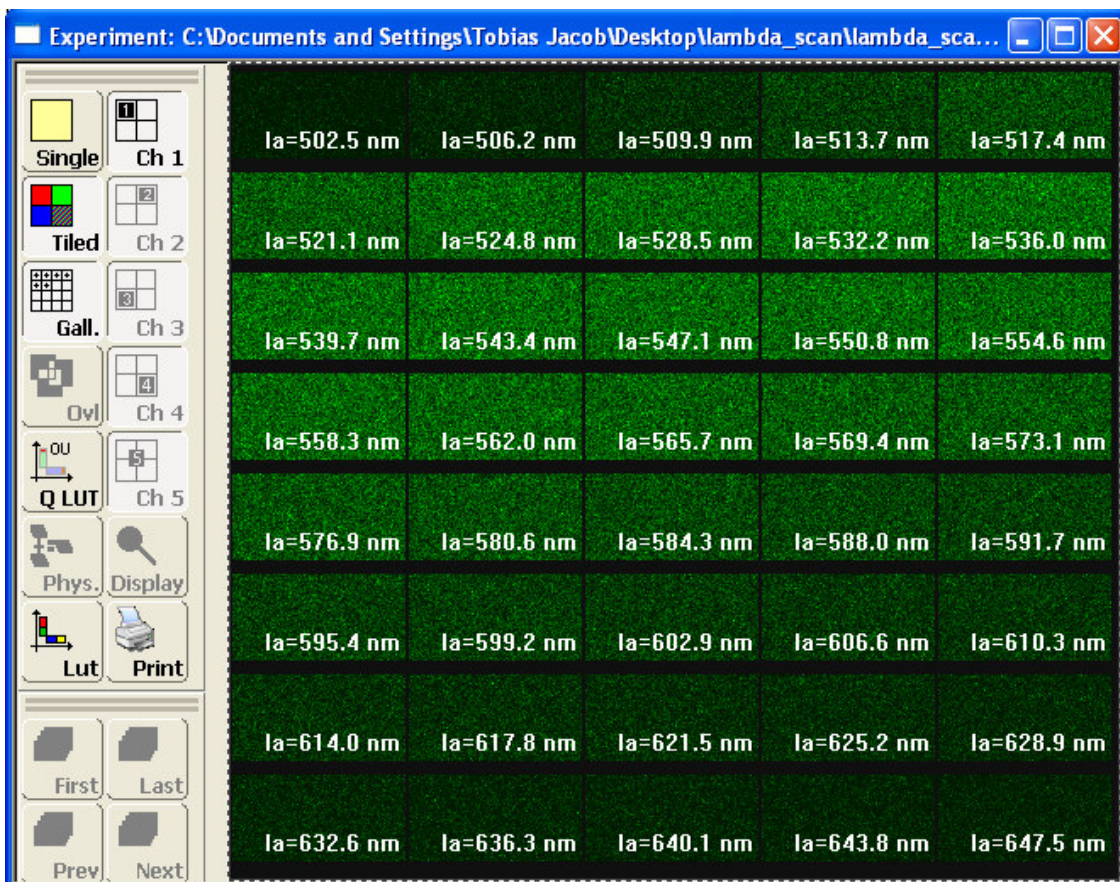
Vol. 2 July 2004



6. Now click on  to define the end point of the scan interval.

7. Select the number of steps by clicking on  To cover the entire wavelength interval the number of steps multiplied with the width of the detection slit should be at least the same or more as the total width of the interval. In our example we scan a 150 nm interval (500 – 650 nm) with a detection window of 5 nm, therefore we need a minimum of 30 steps to avoid any gaps. For this example we will record 40 steps.

8. Press *Series* to start the acquisition. LCS will now collect a series of 40 images over the wavelength interval from 500 nm to 650 nm.

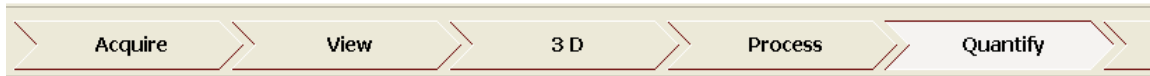


Confocal Application Notes

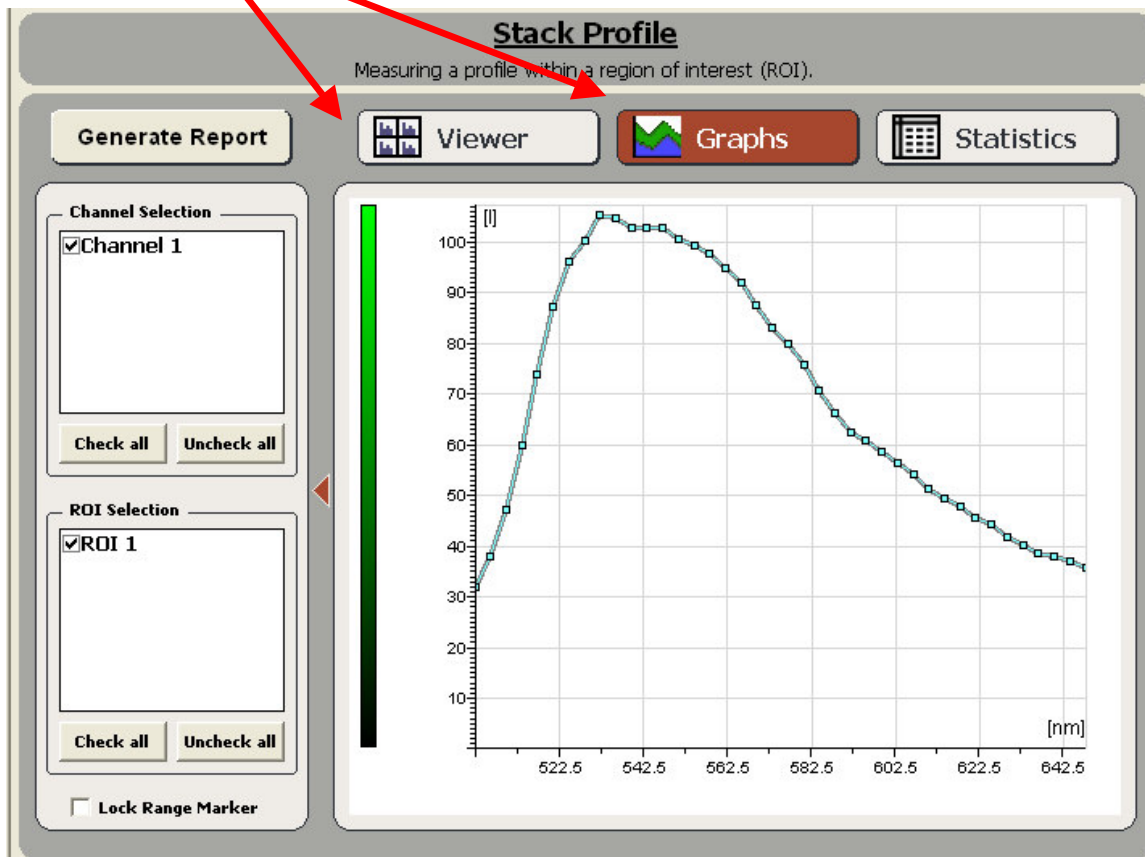
Vol. 2 July 2004



After the scan is finished you can analyze the data using the *Stack profile* tool from the *Quantify* bin.



In the *Viewer* window you can now select a ROI where you would like to collect data. Select *Graph* to view a plot of your data.



Confocal Application Notes

Vol. 2 July 2004



To export the graph right-click into the graph window and select *Export* from the menu. You can now save the data as a .txt-file that can be imported into Excel.

