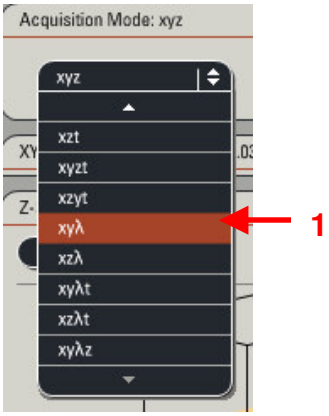
Confocal Application Notes
Vol. 4 May 2006

Lambda Scan

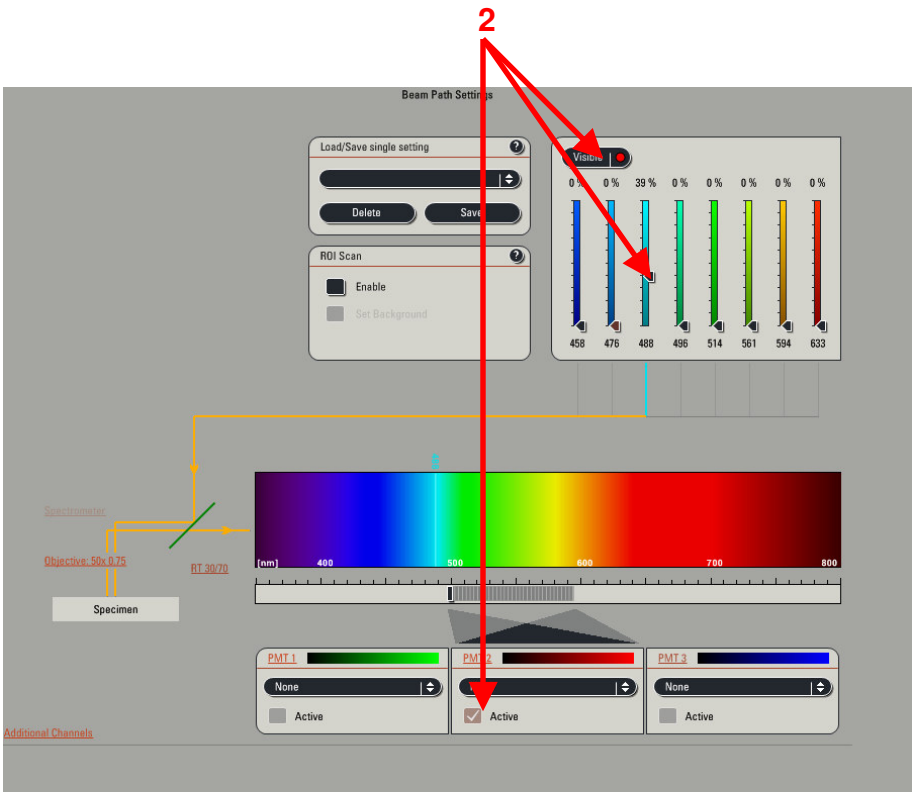
A lambda scan records a series of individual images within a user-defined wavelength range; each image will be detected at a specific emission wavelength. This can be used to measure the emission spectrum of new fluorochromes or to determine the emission maximum of a fluorescent dye in a specific sample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

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 sample exhibiting auto fluorescence.



1. In the **Acquisition Mode**, select a lambda mode such as xyλ, xzλ, xyλt, xzλy or xyλz. For the purpose of this application note, **xyλ** (1) was chosen.
The **λ-scan Range Properties** window will drop down.

2. Setting up the instrument parameters for your experiment.
Choose your laser line and activate your PMT (2).

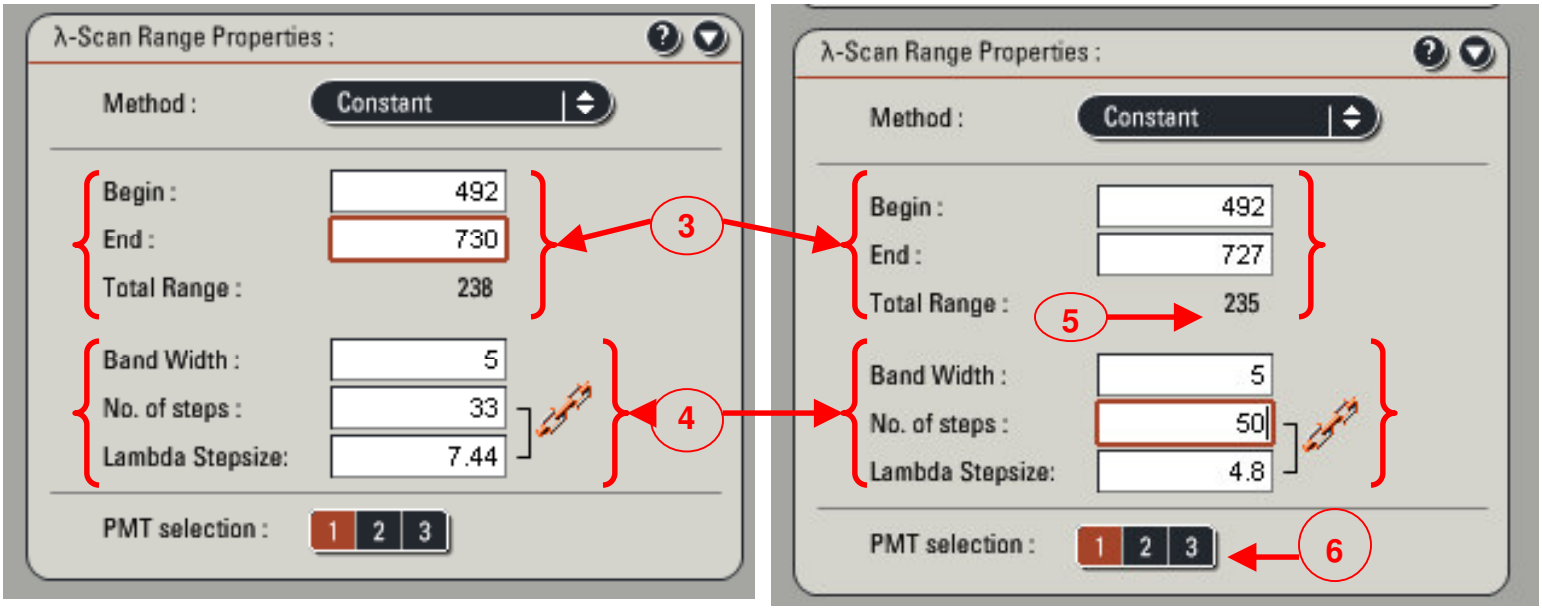


3. Set up the **Begin** point and **End** point of the lambda scan (3). In our example the detection window is set between 492 and 730 nm. The **total range** is then displaying 238 nm.

4. Still in the λ-Scan Range Properties window, choose your **Band Width**, and the **number of steps** (4)**.** The **Lambda stepsize** will be calculated automatically depending on the number of step and the total range of detection.

To cover the entire wavelength interval, the number of steps multiplied by 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 238 nm (730 - 493 nm) with a detection window of 5 nm, therefore we need a minimum of 48 steps to avoid any gaps. For this example, we will replace 33 in the No. of steps by 50 steps to be sure to overlap the detection bands and avoid gaps. The No. of steps will also change to adjust to the new Lambda Stepsize (5)

5. Do not forget to select the **PMT** active during the lambda scan. In our example, the PMT1 was selected (6).



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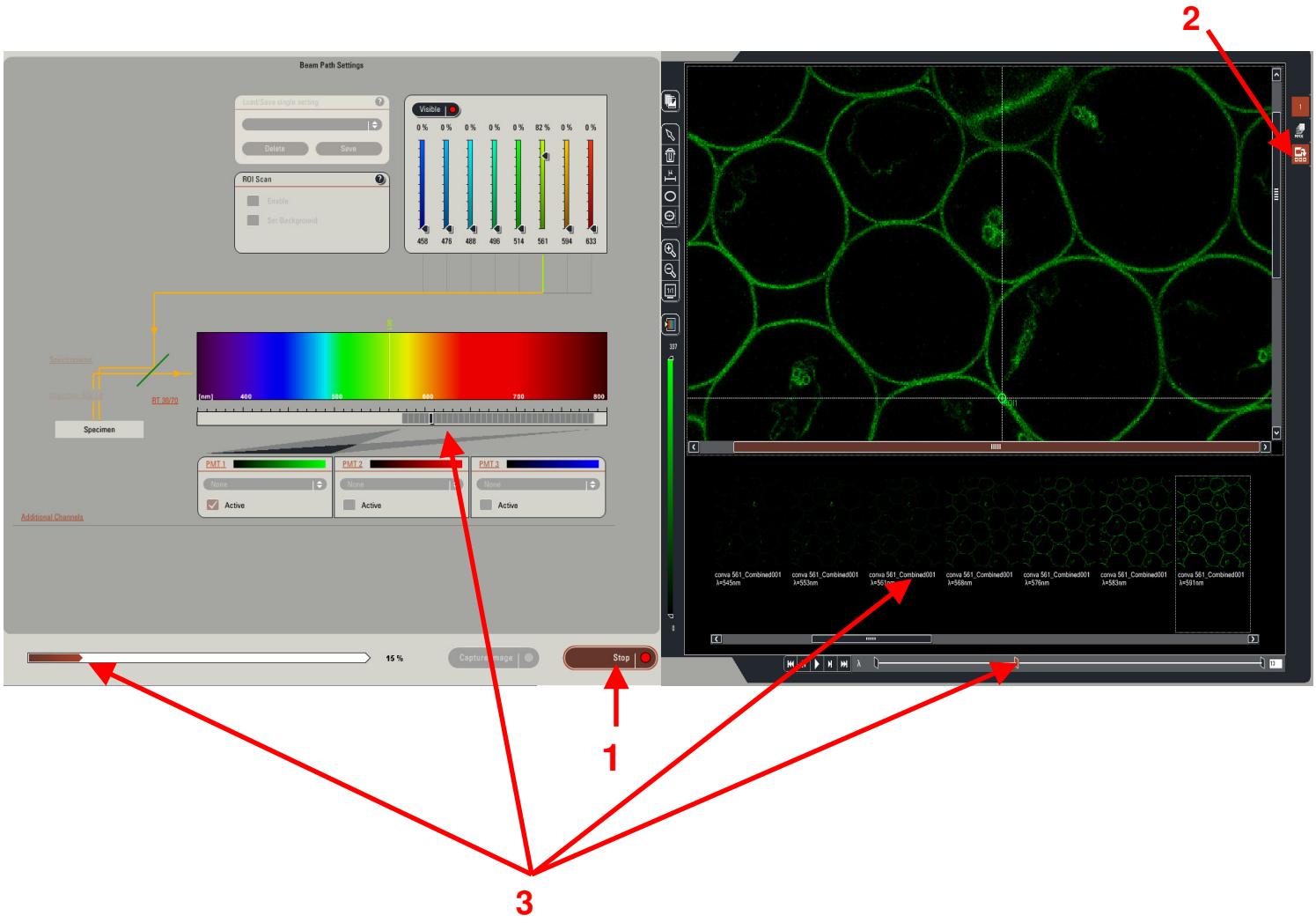
6. Press **Start** (1) to begin the acquisition. In our example, LASAF will now collect a series of 50 images over the wavelength interval from 492 to 730 nm. You can click on the Gallery tool button (2) in order to follow the scanning. The PMT slider and the image collection will allow you to follow the image sequence (3).



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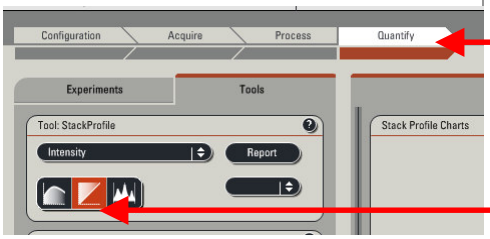
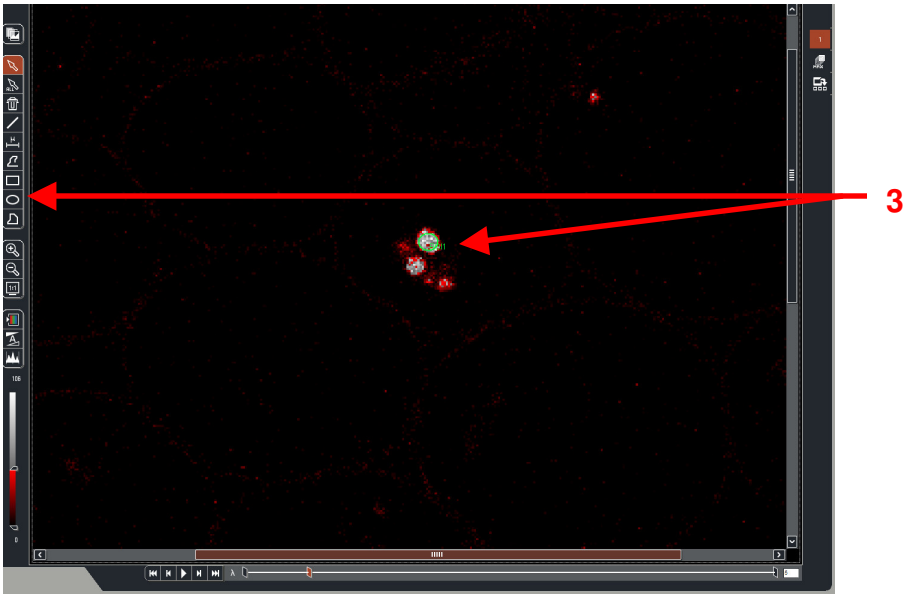
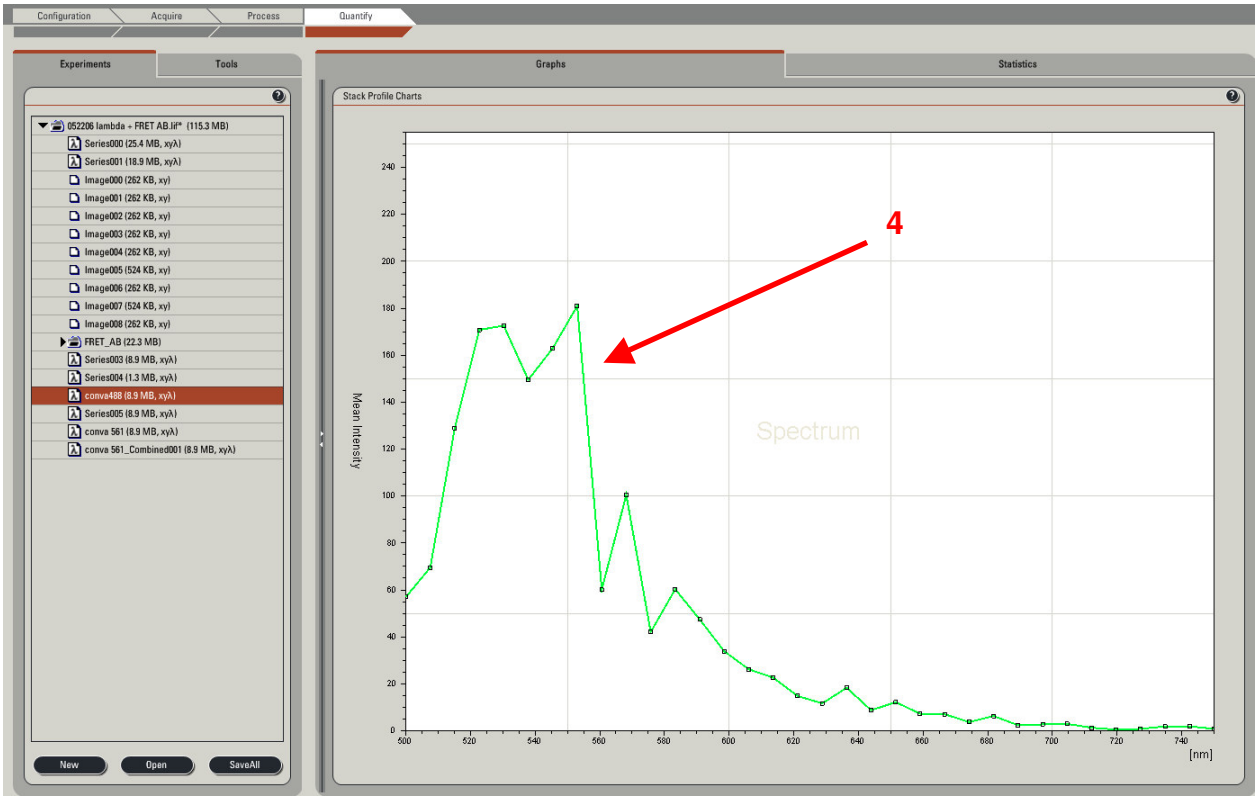
After the scan is finished you can analyze the data using the **Quantify** tool (**1**).
Chose the **StackProfile** tool (**2**).

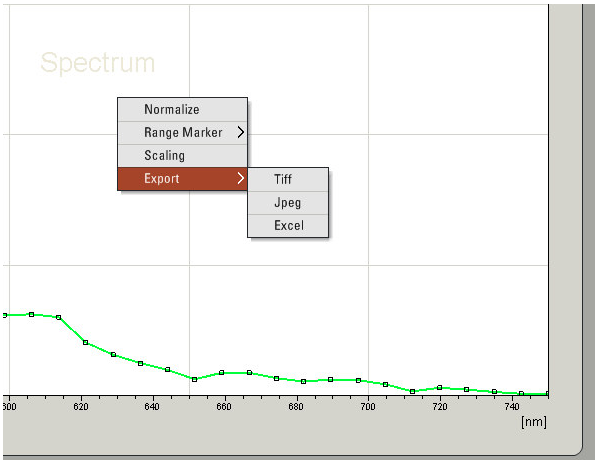


Draw a region of interest (**ROI**) (**3**) into your lambda Series. The **Stack Profile Chart** will then automatically appear (**4**).

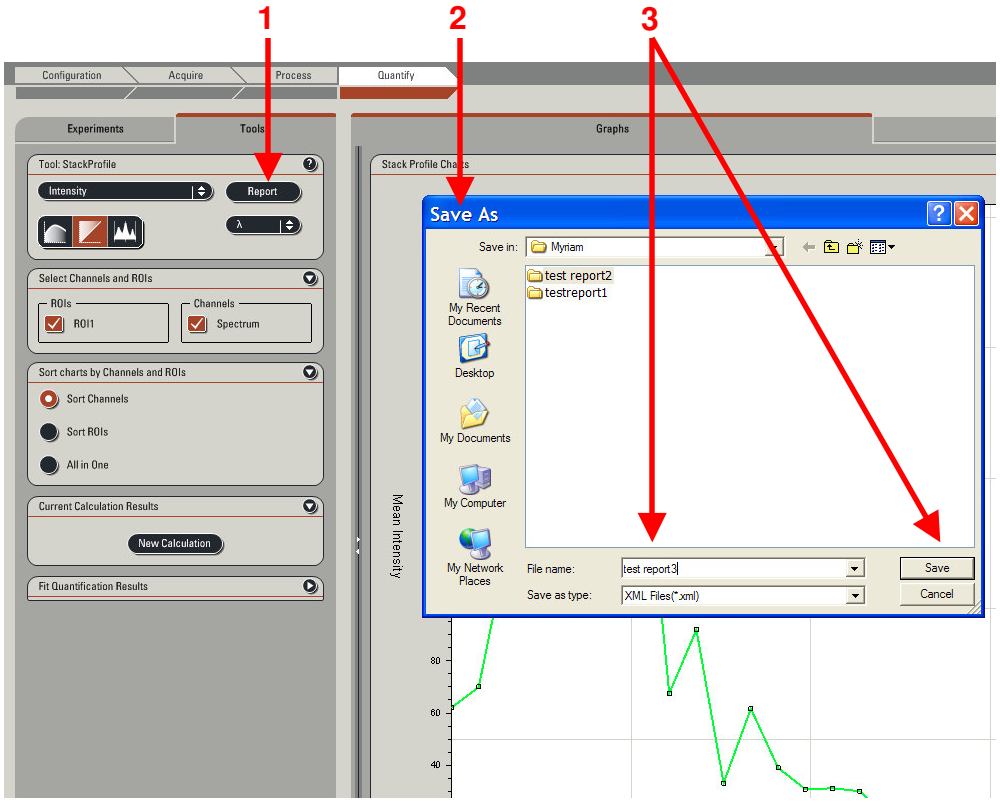




7. To export the graph right-click into the graph window and select ***Export*** and chose between the different formats proposed (**Tiff**, **Jpeg**, or **Excel**).



8. A complete report of your experiment is available by clicking on the **Report** tag (**1**). A **Save As** (**2**) window will open and you will be asked to name your report (**3**) and then **Save**.



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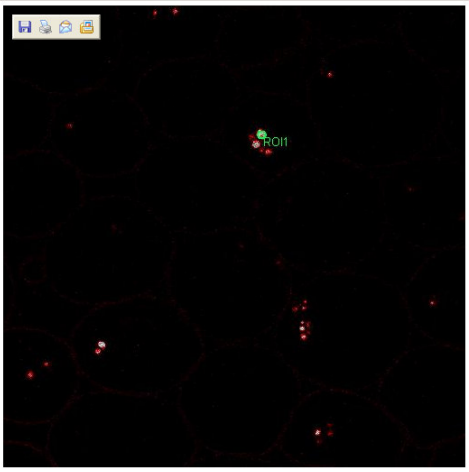
The report will be in an .xml format.

D:\users\Myriam\test report2\test report2.xml

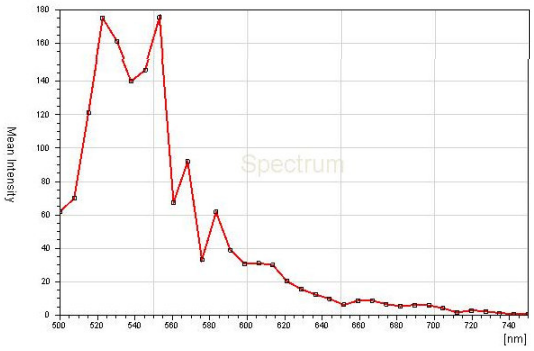
Report: **test report2** - created on 5/30/2006 2:36:35 PM

Legend informations

Images



conva488



Spectrum

Spectrum

	ROI1
MeanValue	45.79
PixelCount	1768
PixelSum	80.97 * 10E+3
Length	250.00 nm
Frame Count	34
Variance	3161.39
Standard Deviation	56.23
Average Deviation	1538.71
Max Amplitude	175.62
Max Position	553.03 nm
Min Amplitude	653.85 * 10E-3
Min Position	742.42 nm
Center Of Mass Pos.	553.11 nm

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