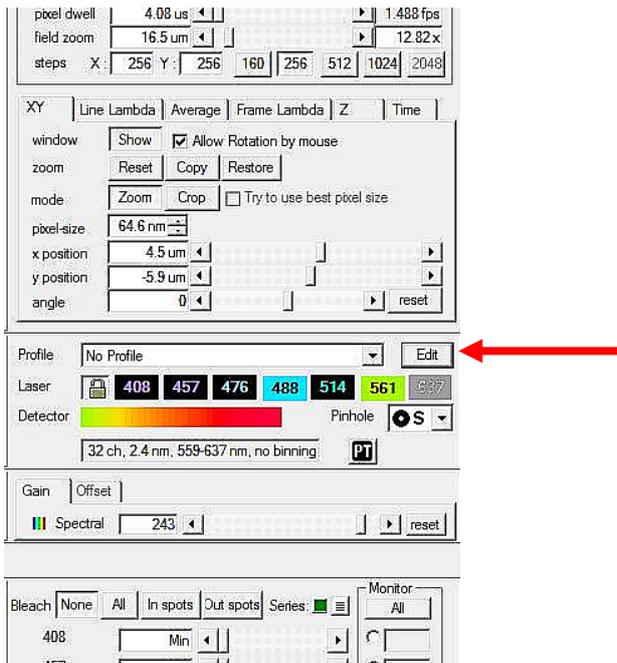# Spectral imaging on the Nikon C1Si confocal laser scanning microscope
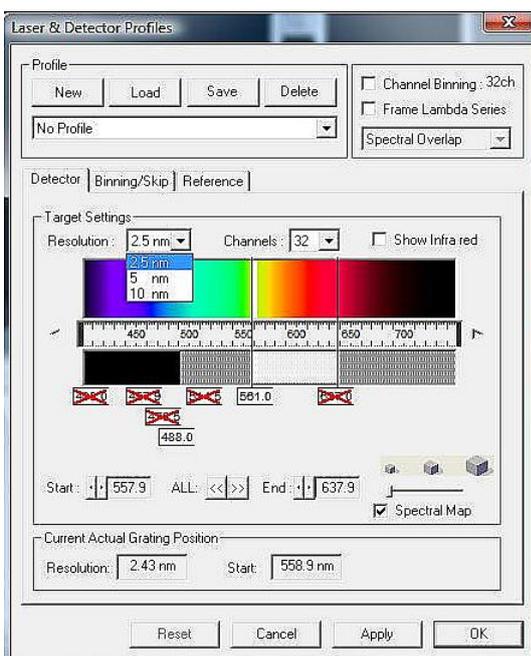
Turn the spectral switch at the left side of the microscope on 'Spectrum'. See red arrow on image right.

In spectral laser menu go to 'Edit'.

The following menu opens:

Select the required lasers and Resolution. The number of channels can be changed by changing the sliders in the spectral colour graph. Maximum number of channels is 32. The imaging setting has to be the same as the settings used to make the reference spectra. So save the settings as a profile. The width of the imaged spectrum will change if you change the Resolution (options are 10, 5 and 2.5 nm).

Set the laser output and the Gain for the spectral detectors.
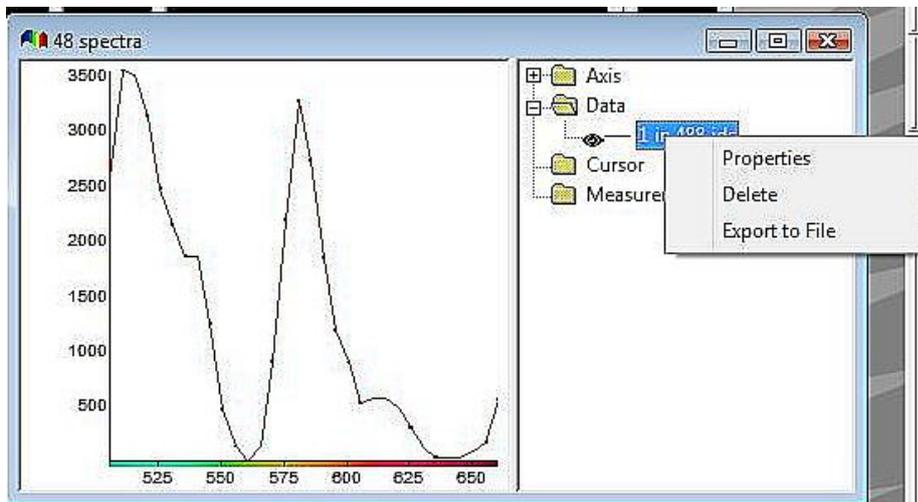
Set gain

Set laser output
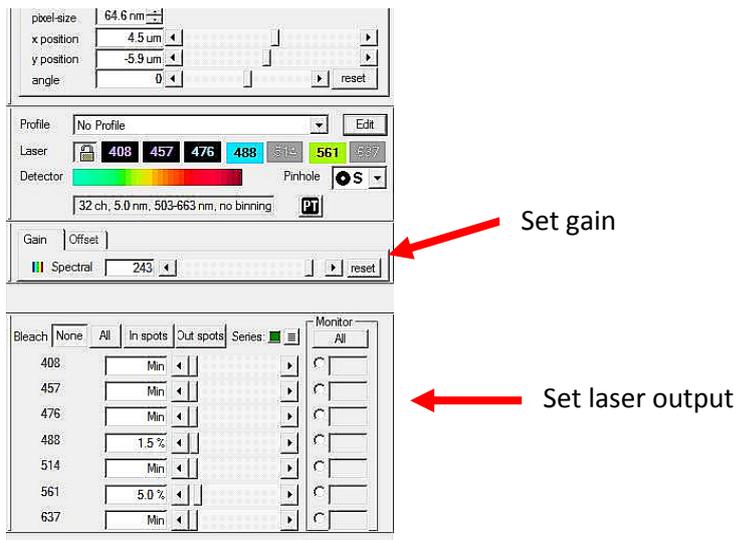
Take images from the individual fluorochromes to save in the spectral database.

Make a ROI of your signal. Click 'Show spectra in spot' icon.

This will result in a window displaying the spectra. Click on the Data tab in the right hand menu and right mouse click on the item. This will bring up a menu that allows you to 'Export to file' as *.txt file.

You can do this for each fluorochrome.

Take an image of your sample with mixed fluorochromes. Go to:
 Spectral > Unmix > Unmix Spectral Image.
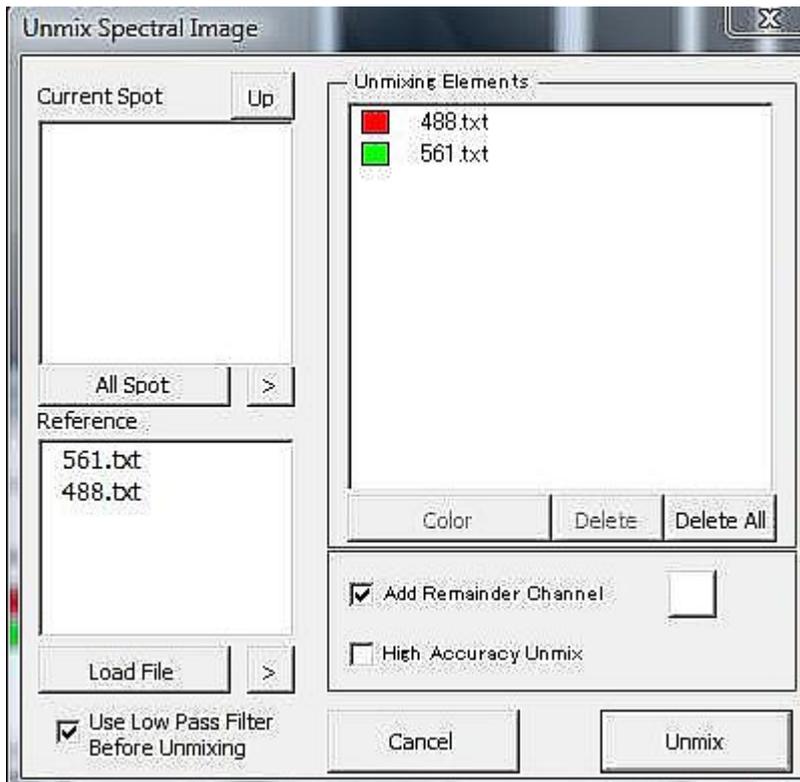
Load the spectral data txt file via Load File. It will appear in the right hand panel with a coloured box. You can change this colour by double click on it. Items with this spectrum will get the attached colour in the results file.

Select Unmix and the signal in your image will be spectrally separated according to the databases selected. The resulting image might look like:
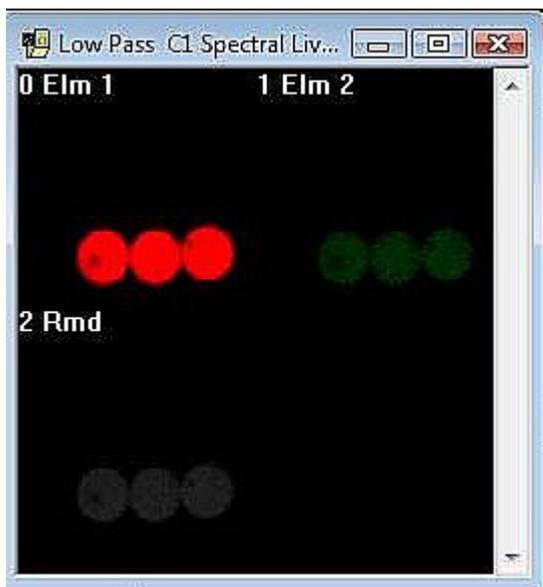
0 Elm 1 = channel 1
1 Elm 2 = channel2
2 Rmd = a rest channel. This channel will be displayed if some of the signal cannot be fitted into the database spectra provided.

Save your results file.

You also have the option to do live unmixing under:
 Spectral > Unmix > Spectral Live Unmix.

Also here you have to load the different spectra you want to use.