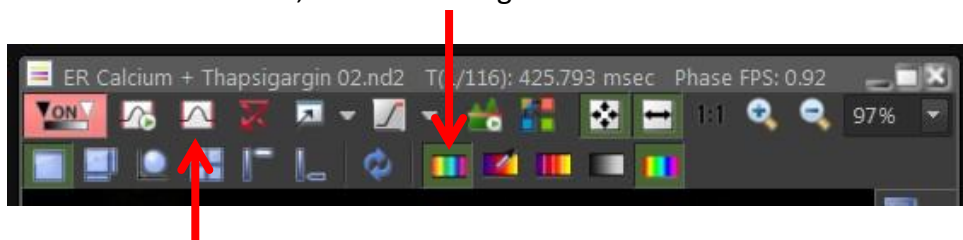# Spectral Data Analysis

1. Open a spectral dataset, like "ER Calcium + Thapsigargin 02.nd2"

2. The first image of the time series should be displayed in "true color" mode. If not, click on the True Color icon, above the image.


3. If the image appears dim, use the Auto Scale button to make it brighter. This just affects the LUT (not the data).

4. Right-click on the Elements desktop and select the Spectrum Profile option from the Visualization Controls. This will allow you to display spectral profiles in the image. As a default, the software will show you the spectral profile of the entire image (including background).
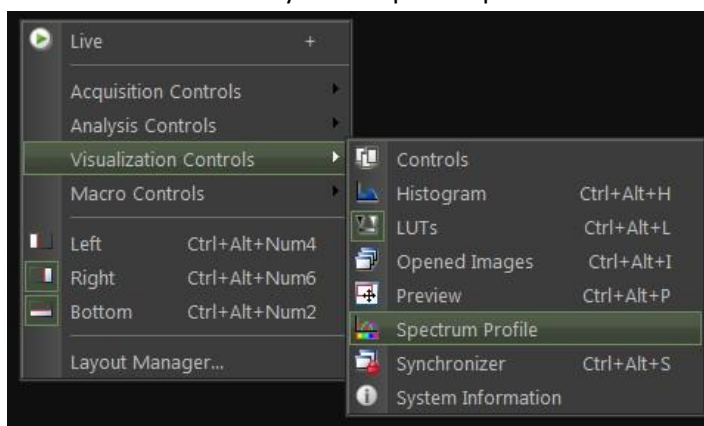

5. From the icons to the right of the image, select click on the small triangle next to the ROI button, and select the "Simple ROI Editor" from the menu.
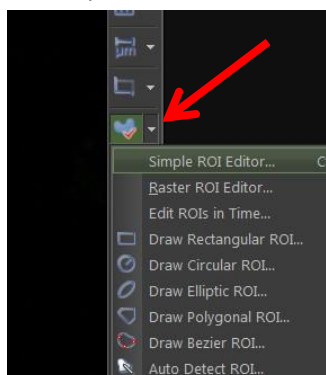
6. From the ROI Editor menu, you can select the desired type/shape of ROI, for example "Polygon".
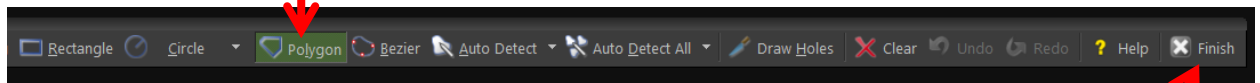

7. Draw one or more ROIs on the image. For polygons, click left to add a corner and click right to close and finish the polygon. Once you have completed drawing ROIs, left click on the "Finish" button on the ROI Editor.

8. For each ROI, the spectral profile will be displayed in the plot area in its corresponding color. You can now play through the time series and watch the plots change over time.

9. At any given timepoint, you can click the "X" key on the keyboard and select "Current Frame" to create a snapshot which can be saved as a TIFF (or other format). If you want to save a movie of the entire Time Series, click "All Frames" and save the result as an AVI.

10. In the plot area, click on the small triangle next to Export, and select an export option. I typically use "Graph to Clipboard". Once you have selected this option, click on the "Export" button to save a copy of the plot.
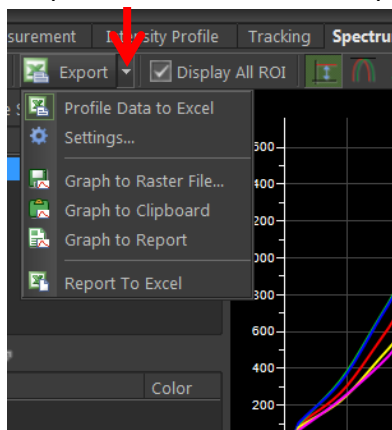

11. Next, you can paste it into "Paint" of a similar program and save it as a JPG, BMP, etc.

12. The next step is to "unmix" the spectral data. From the Image menu, select Spectral Unmixing Setting.

13. You can unmix data in a variety of different ways, based on (a) spectra from individually labeled control samples, (b) factory spectra, (c) spectra based on ROIs identified in the sample (only works if probes are physically separated), or (d) blind unmixing.
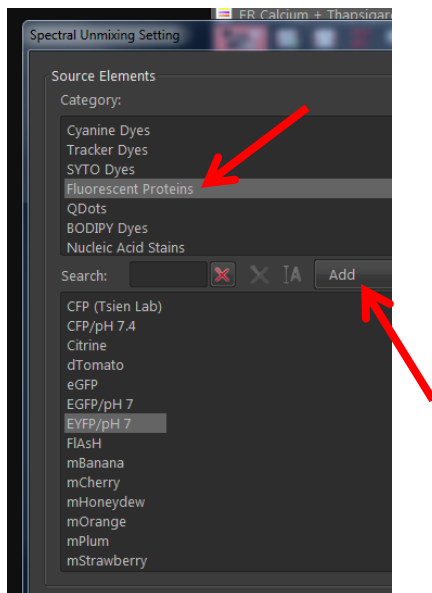

14. For your samples, I used factory spectra for CFP and YFP. Under "Category", select "Fluorescent Proteins". This should display a list of FP from which you can select. Click on CFP, and hit the "Add" button. Repeat for YFP.

15. The factory spectra for the selected probes will be displayed. You can change the colors in which they will be displayed in the final image by accessing the appropriate drop down menus next to the color indicator.
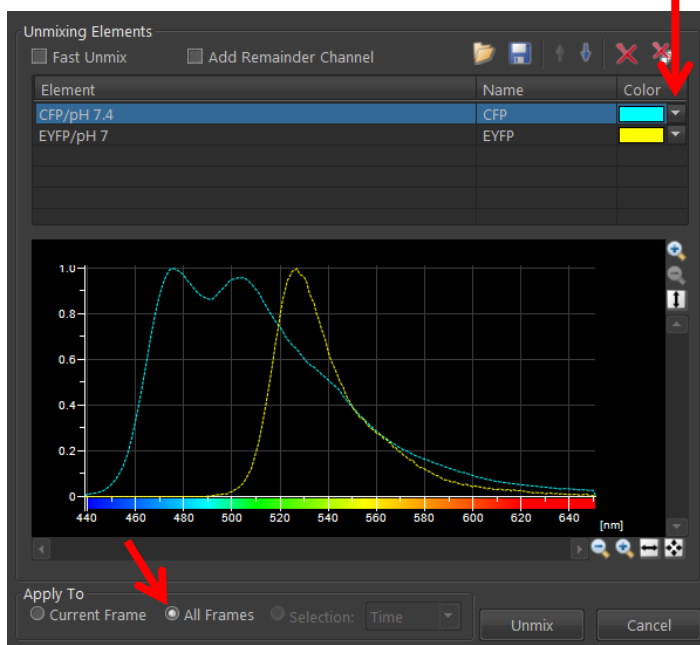
16. For comparison, it is nice to display the spectrum of one of your ROIs to the plot. Go back to the Category, and select "POIs". From the list of ROIs, select the desired ROI, and click Add. You should be able to see the CFP and YFP spectra "embedded" in the ROI spectrum.

17. To delete a single spectrum, highlight it in the "Element" list, and click on the large red X. To delete all displayed spectra, click on the icon showing a red X and multiple windows. Before unmixing, remove any ROI spectra displayed in the plot.
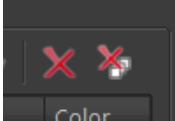

18. Select "All Frames" at the bottom of the menu, and click "Unmix". This will unmix the entire time series, based on the factory spectra for CFP and YFP.

19. The software will now display an unmixed dataset with CFP and YFP assigned to their separate channels. You'll probably need to click on the Auto Scale button (see step 3) to bring up the brightness. Display either CFP or YFP or both (All).
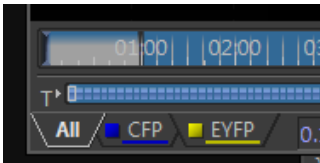

20. To display a Time Plot for CFP and YFP, right-click on the Elements desktop and select Time Measurement from the Analysis Controls.
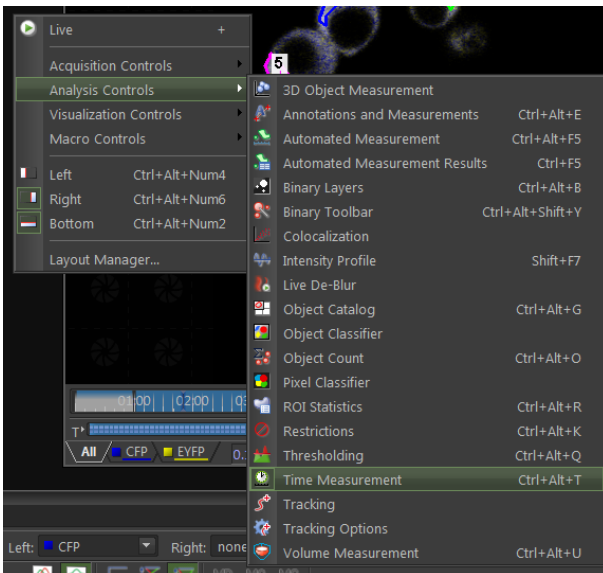
21. You can draw new ROIs on this image, or simply copy-and-paste the ROIs from the raw, unmixed dataset.

22. Once you have added ROIs, click on the Measure button in the Time Measurement window.
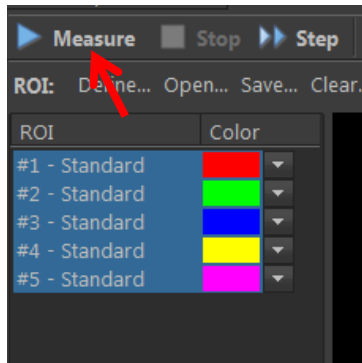
23. You have a lot of different options to change the plot display. I like to display the plots for CFP and YFP separately, as shown below.
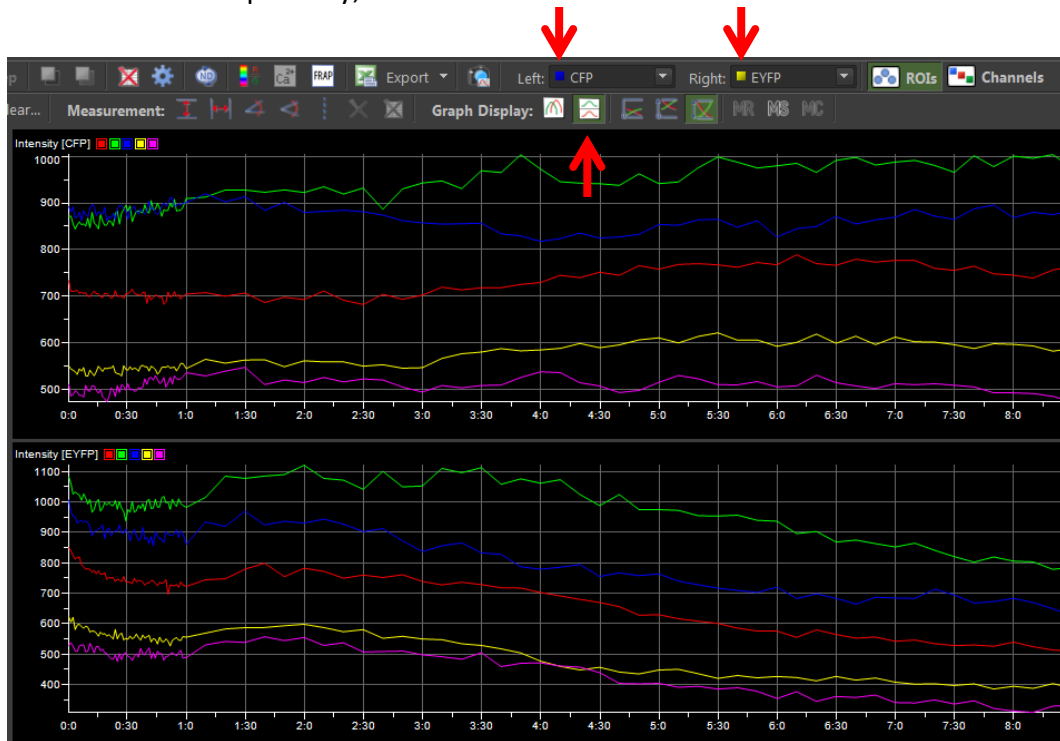
24. At this point, you can again make TIFFs AVIs of the images, and JPGs or BMPs of the plot.