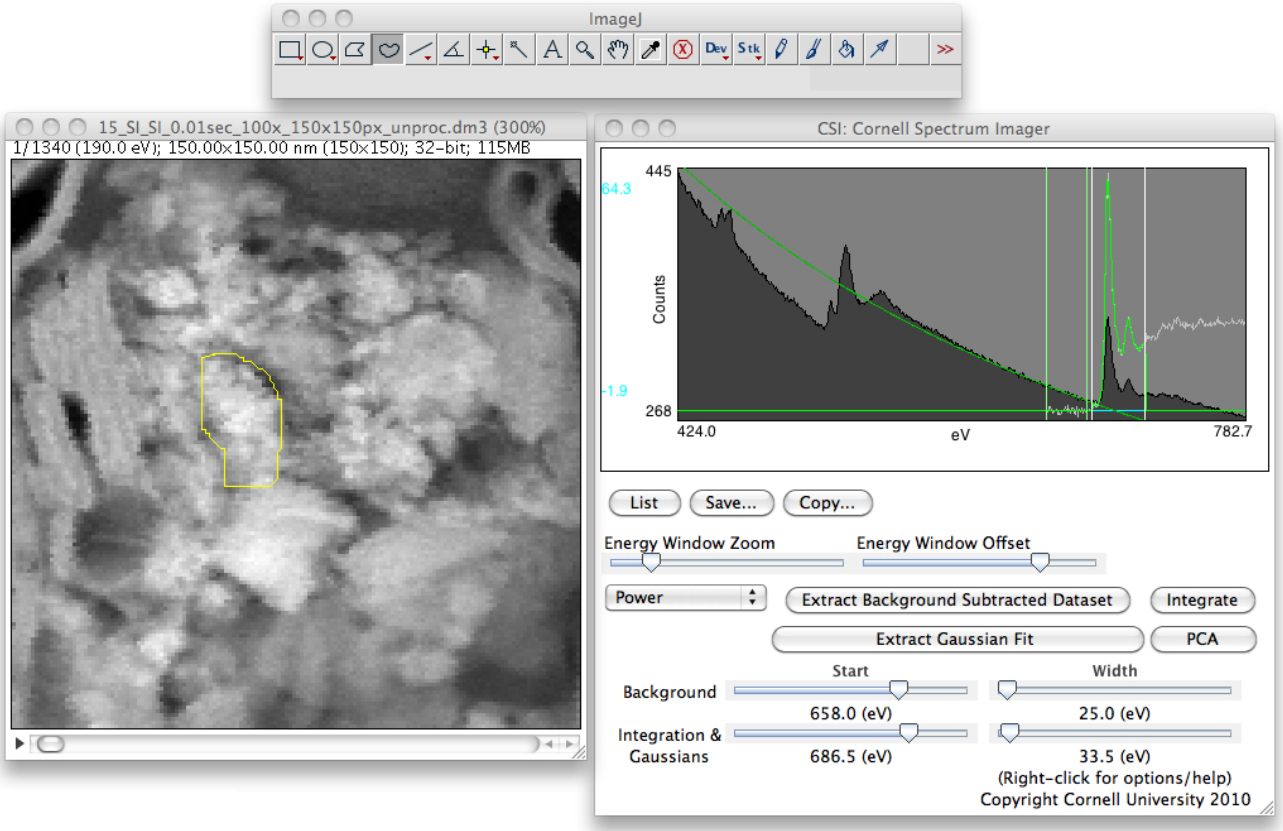
Cornell Spectrum Imager (CSI)

Open Source Spectrum Analysis with ImageJ

Tutorial



PARADIM

Electron Microscopy Summer School 2017

PARADIM
PLATFORM FOR THE ACCELERATED REALIZATION, ANALYSIS & DISCOVERY OF INTERFACE MATERIALS

Why CSI

Current Software

- Black box
- Expensive
- Steep learning curve

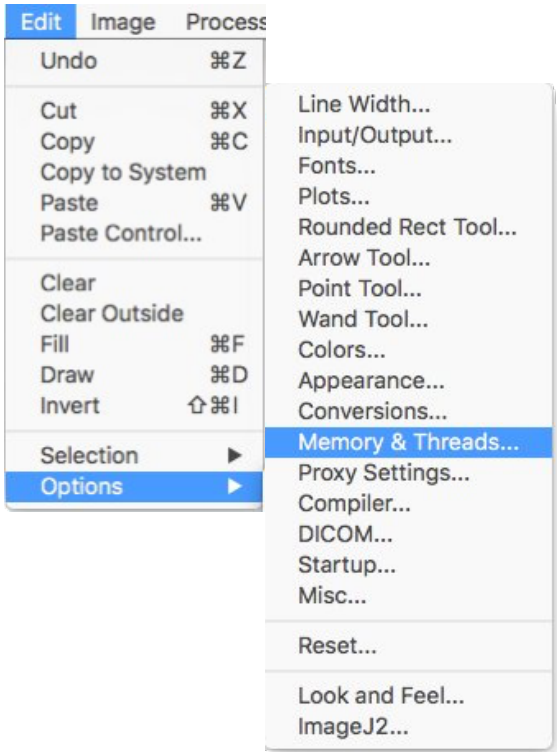
Cornell Spectrum Imager

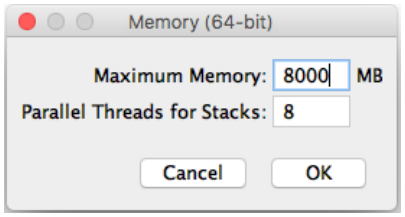
- Open Source
- Free
- User-friendly

Vs.

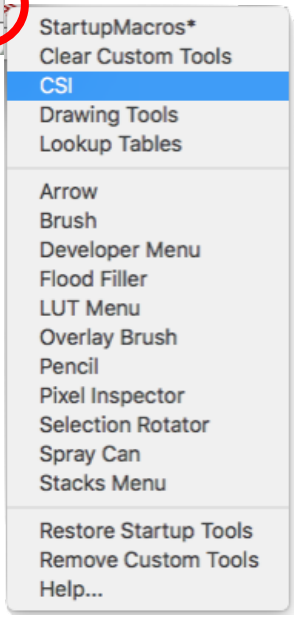
Setting Up ImageJ

Make sure ImageJ has access to enough memory (at least a 1024 MB). Close and relaunch the program.





Use the "More tools" menu to access the CSI plugins (can also access from the Plugins menus)



1. Dynamic Line Profiler

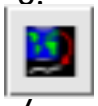
2. Open spectrum (`.tif`, `.dm3` or `.ser` files)

3. Subtract dark reference

4. Cornell Spectrum Imager

5. Extract Slice

6. Create RGB Composite



7. Map to line

Opening Spectrum Images

Download the spectrum image `SampleData_EELS_Map.tif` from the Box folder and open it with the CSI `Open spectrum` button.

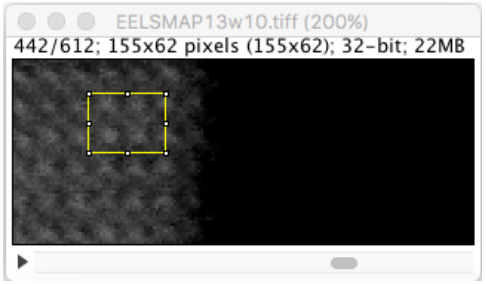
The display window is one energy slice of the SI. You can use the scroll bar at the bottom of the window to scroll through the full spectrum of energies.

Notice how particular areas of the map change contrast as you move past different elemental edges.

You can use the magnifying glass tool or the + and − keys to zoom in and out of the image.

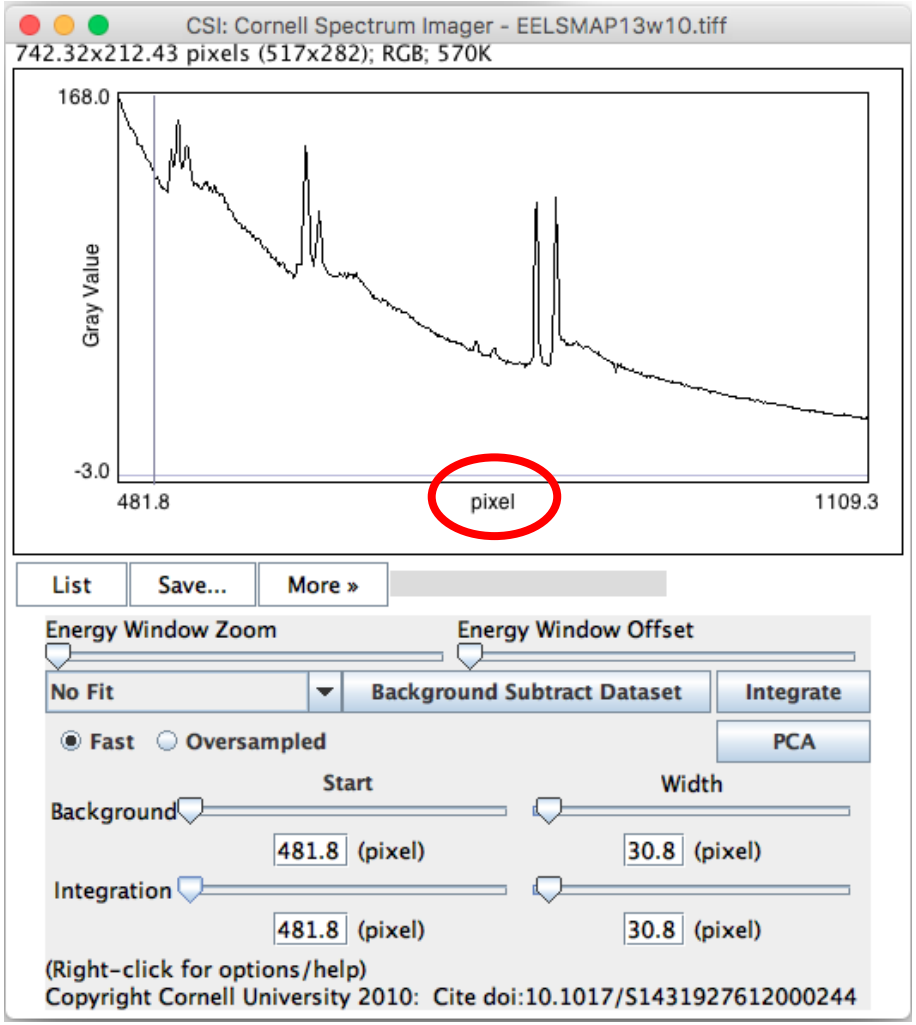
Loading Spectra

Launch the Spectrum Imager. You can use the drawing tools (rectangle, oval, polygon, freehand, or line) to select which area of the image will be summed into the spectrum.



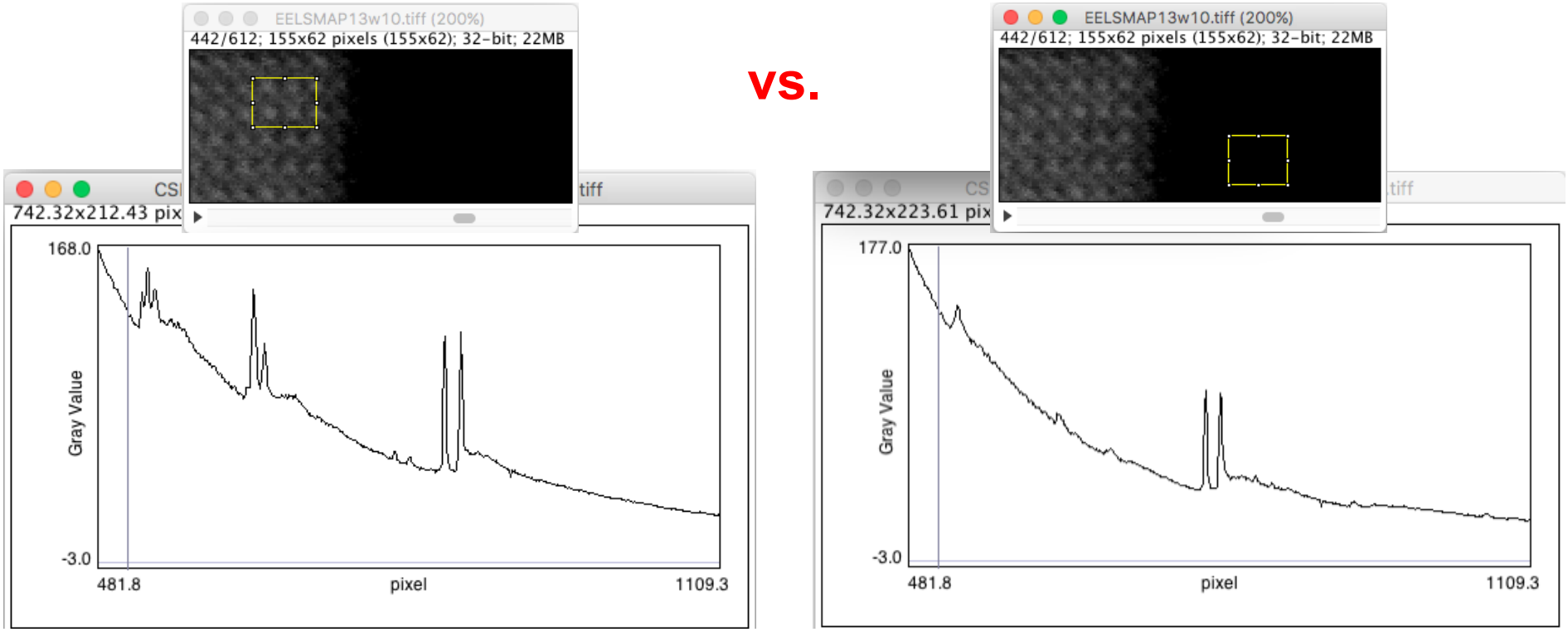
The spectra from each point in your selected area will be summed into the spectrum displayed in CSI. Make sure check the *y*-axis to be sure it's in energy (eV) rather than in pixels!

***Even if the axis label says "pixels", you can check that it's using really energy values by looking at the first channel. Here, the first channel is 480, so we know it must actually be in eV. You can change the label under `More… Axis options… x label`



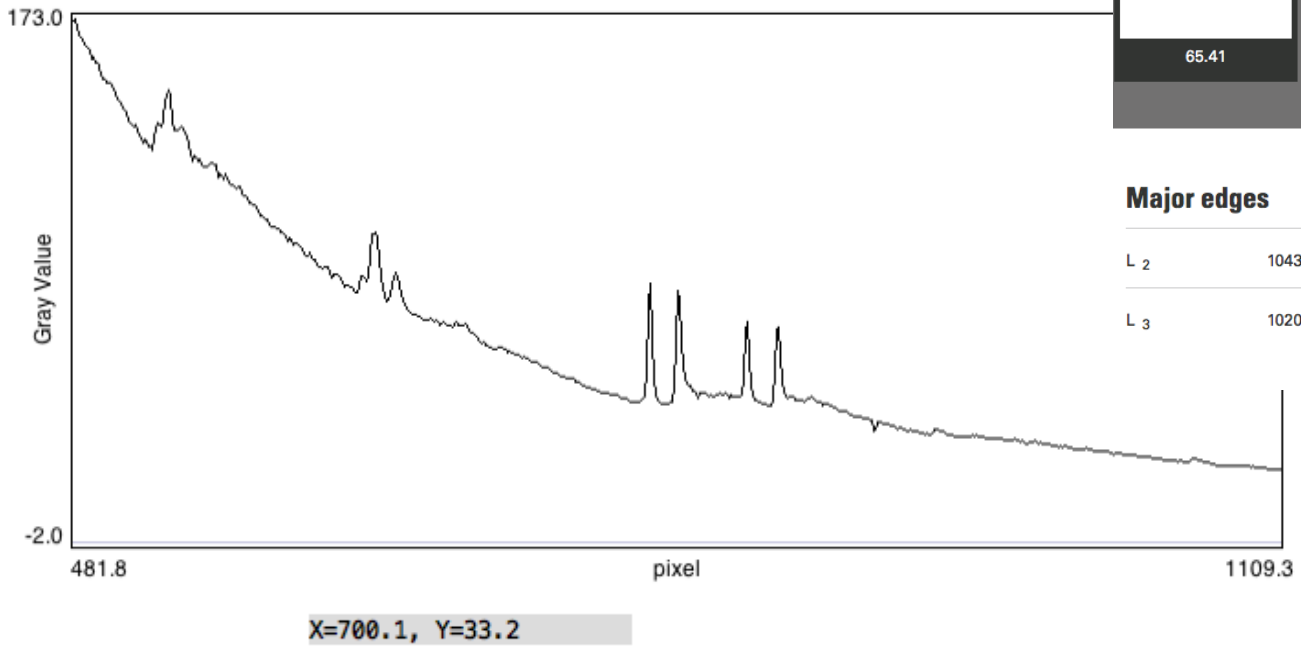
Dynamic Spectral Analysis

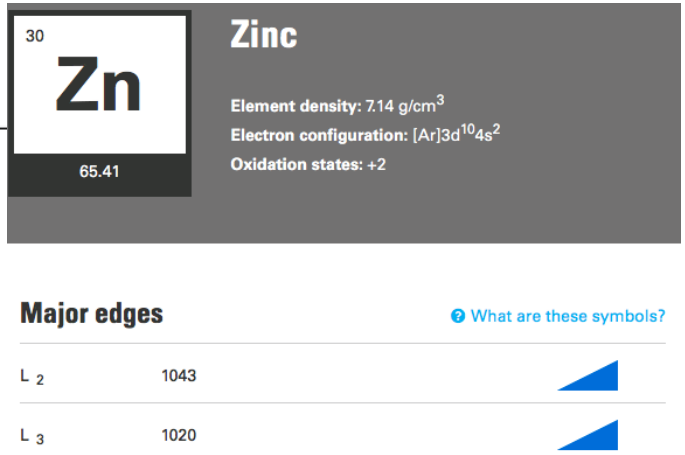
CSI dynamically updates the displayed spectrum: try changing the size and location of the selection area. A larger selection will generally improve the signal to noise ratio of a spectrum. Notice also that different elemental edges may emerge at different areas in the image.



Identifying Edges

Select a large region of the image so that you capture as many edges as possible. Try to identify the elements present in this sample. One helpful tool is the Gatan EELS Atlas (http://www.eels.info/atlas). You can use the periodic table to look up the edges for a known element or search by energy to identify composition.





Does this sample have zinc?

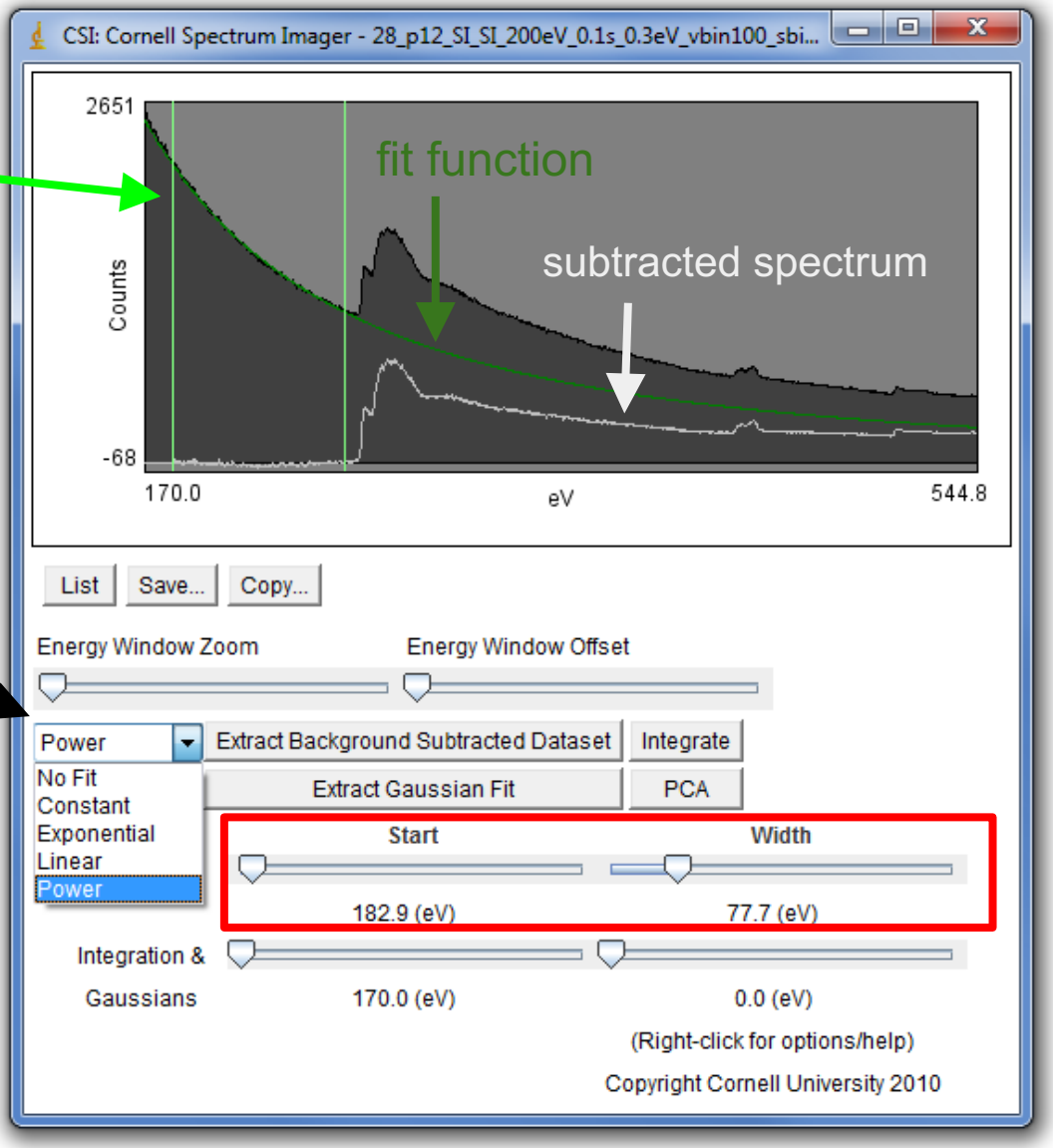
You can use the `Energy Window Zoom` and `Energy Window Offset` sliders to help you get a better view of specific edges (click on the sliders and use your arrow keys for fine control). Notice that as you move your mouse over the spectrum, the dynamic *X* and *Y* values displayed beneath the plot window read out exact energy and intensity values, respectively.

Background Fit & Subtraction

Use background window sliders to adjust the location and width of the background fit window (bright green vertical lines). ***You may need to change the color scheme in order to see the lines. Right-click somewhere on the spectrum, and use `Options -> Change the color scheme`… to find one that works.

Use drop down menu to select fit function; for most edges the standard power law background will work, but some care is needed in selecting integration and background fitting windows. For badly overlapping edges, sometimes a straight line works better. LCPL is a linear combination of power laws.

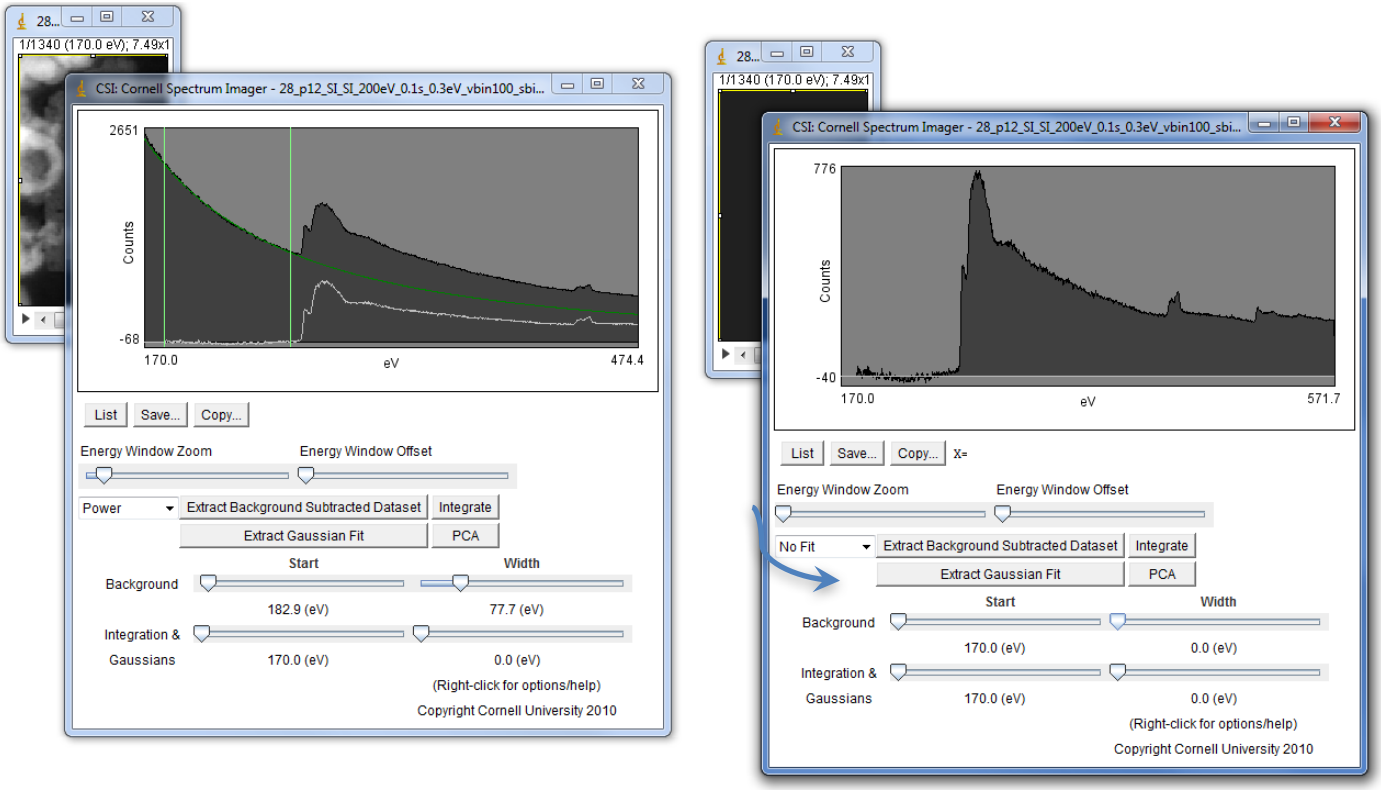
CSI shows the dynamically updated linear least squares fit (dark green) and subtracted spectrum (light gray).



Background Subtraction

Optional: Extract Background Subtracted Dataset

Use `Extract Background Subtracted Dataset` to produce a new spectrum map with selected background fit and subtraction performed on each spectrum/pixel. From here you can save the summed spectrum using `List`, `Save…` , or `More >> Copy` (see “Notes about file saving”)



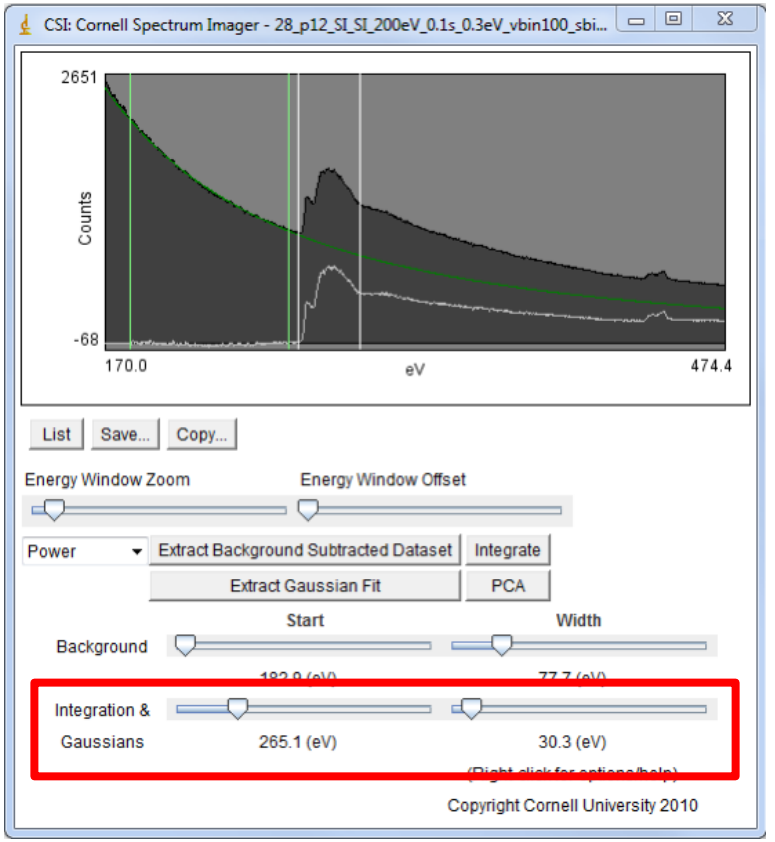
For elemental mapping, skip this step and proceed to integration.

Mapping by Integration

Use integration window sliders to adjust the location and width of the integration window (white lines). The integration window should just fit over the edge of interest: remember, you want to maximize the signal from this edge without introducing stray intensity by including extra noise.

Click the `Integrate` button to produce a background subtracted integrated density map. Try integration windows of different widths to see if you can optimize the contrast and minimize noise in your output map.

Is it better to include only the narrow, sharp part of the edge? Or to integrate out along the edge tails?

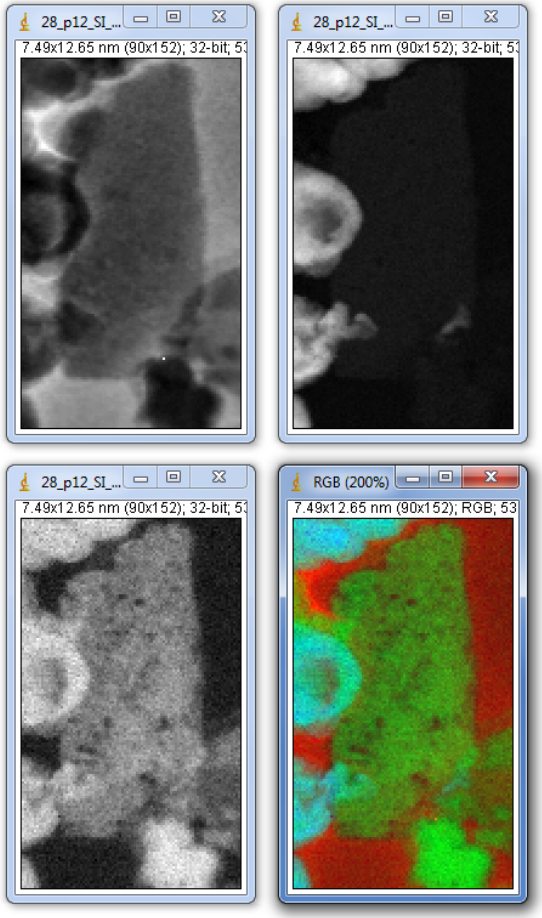


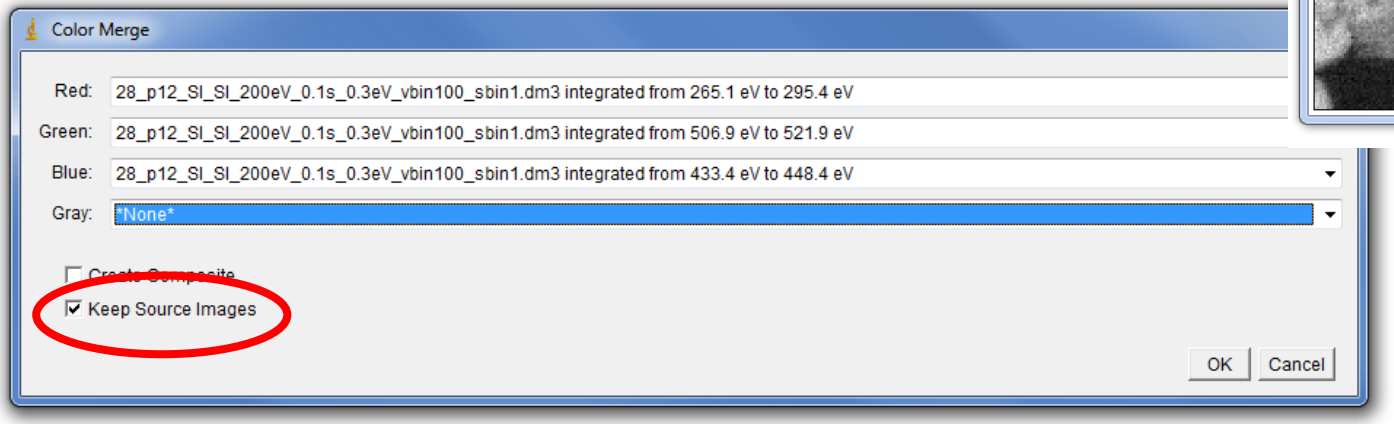
Making an RGB Composite

Make maps for several different edges. You can rename them as you like, but it's good practice to keep all the important information about which background and integration windows were used for each

Use the `RGB Composite` tool to combine multiple maps. Assign each map to a different color with the drop-down menus. Newer versions of CSI have cyan/magenta/yellow in addition to traditional RGB -- you might want to try out a few different color combinations to see which one gives you the best result.

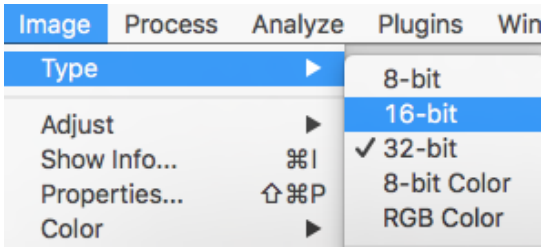
***Make sure you ☑`Keep source images`! Otherwise the .tif of your original maps will get eaten by the RGB and you have to re-create or re-open them every time!





Notes about file saving

Map images are automatically output as 32-bit .tif files. For most presentation formats, you'll want something with a little less depth. You can convert the images to 16-bit tiffs from the `Image/Type` menu, but note that once you convert *down* a bit size, you won't be able to convert back up. Alternatively, you can use the `File/Save As…` menu to output your images in your favorite format (.jpeg, .png, …)



You can access the actual spectrum data from the `List`, `Save…` , and `More >> Copy` menus underneath the spectrum in the CSI window. All three will give you an array consisting of X0 = energy loss (eV) and the intensities of Y0 = the original spectrum, Y1 = zero, Y2 = the fit function, and Y3 = the background subtracted spectrum. The data will be output as a .csv file or you can copy and paste into Excel, Numbers, etc.

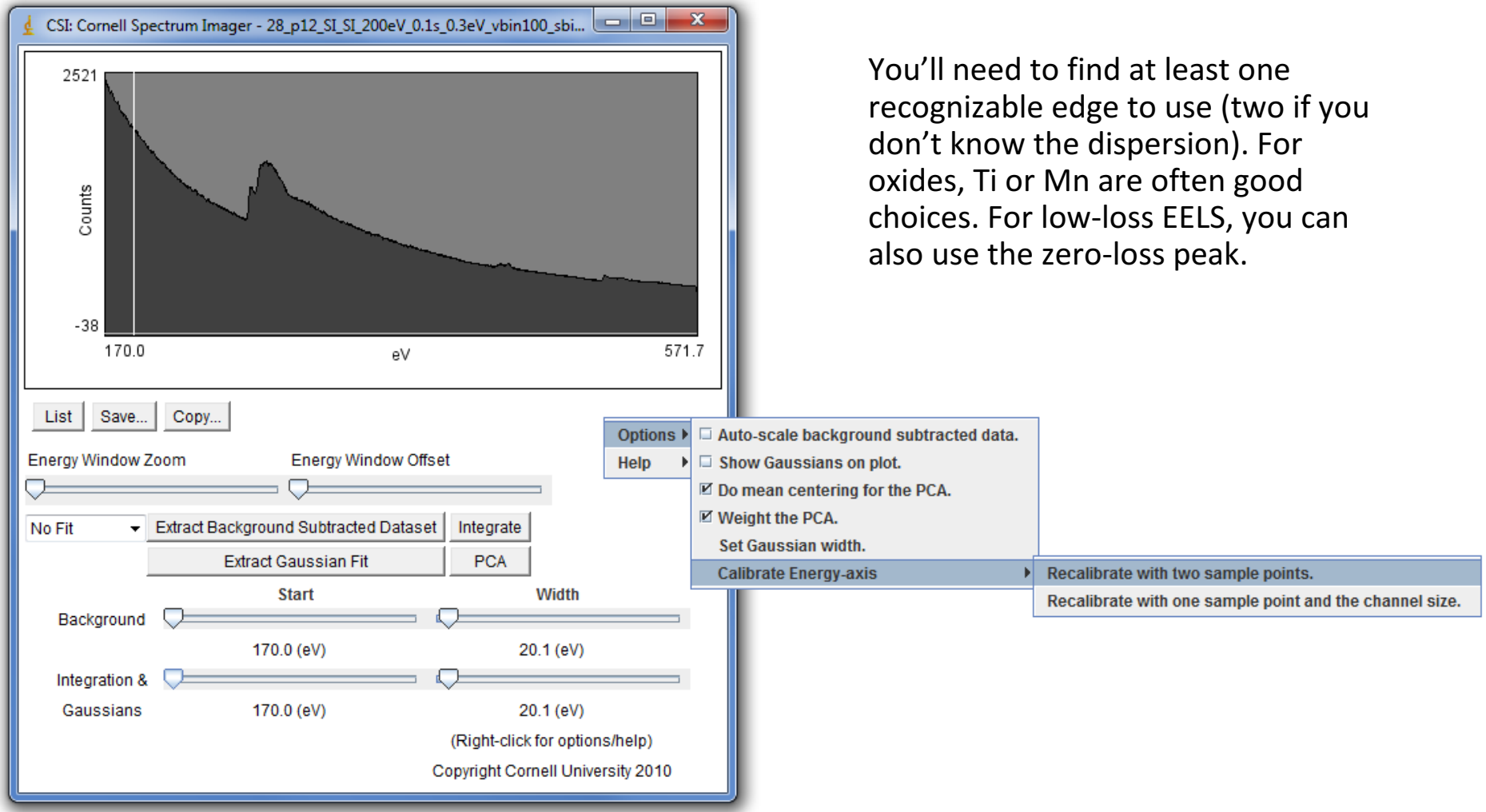
Plot Values

X0	Y0	Y1	Y2	Y3
801.192	51.947	0	40.158	11.7898
802.219	51.145	0	39.974	11.1717
803.246	49.485	0	39.790	9.6947
804.273	49.222	0	39.608	9.6134
805.300	48.790	0	39.428	9.3624
806.327	47.159	0	39.248	7.9108
807.354	48.666	0	39.069	9.5968
808.380	48.729	0	38.891	9.8379

More activities

Recalibrating Energy Axis

If you open an SI with the `Open spectrum` tool, CSI should automatically calibrate the energy axis from the metadata of the file. In case the energy calibration is somehow lost or missing, you can always recalibrate the energy axis by right-clicking to access `Options/Calibrate…`



You'll need to find at least one recognizable edge to use (two if you don't know the dispersion). For oxides, Ti or Mn are often good choices. For low-loss EELS, you can also use the zero-loss peak.

Recalibrating Energy Axis (cont.)

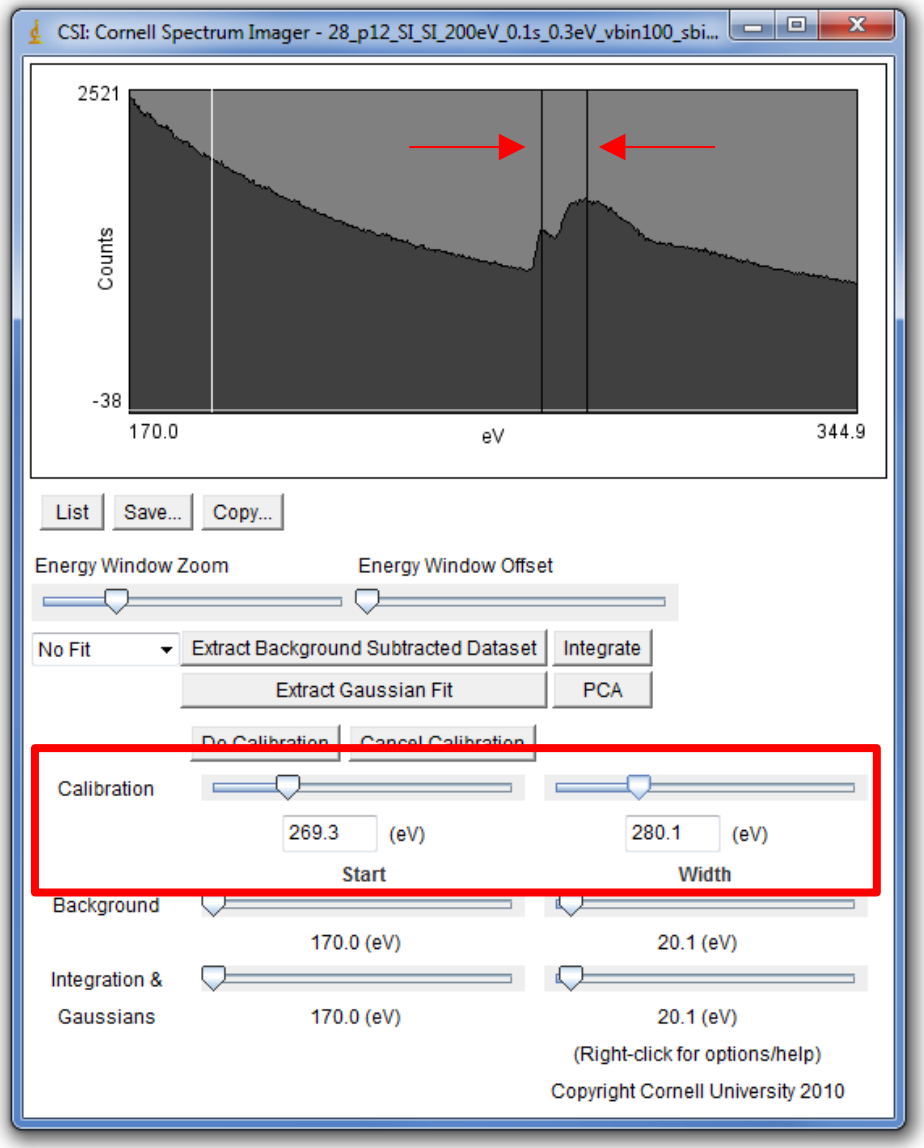
Close all your windows and re-open the SI by **drag-and-drop**, NOT with the `Open spectrum` tool. Notice the *y*-axis is now truly given in pixels rather than energy.

Try to recalibrate the energy axis from one of the edges you identified earlier:

Use calibration sliders to move black lines to known energy feature(s).

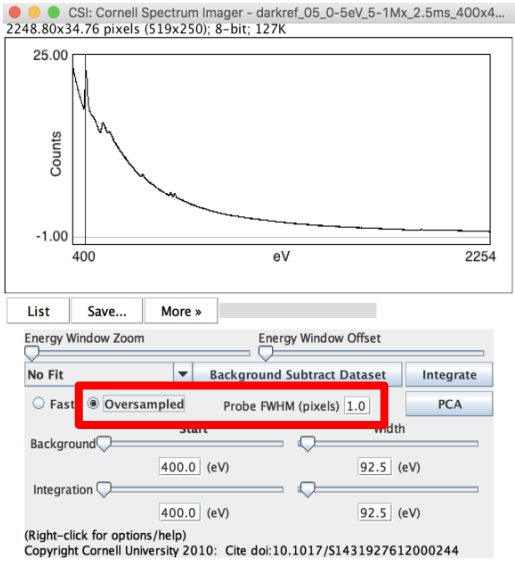
Enter known energy values/channel size into text boxes.

Click `Do Calibration` when done.



Local Background Averaging

Sometimes you can further improve the quality of a map by calculating the background fit for a specific pixel from an average of its neighbors. CSI can perform LBA on integration maps with the `Oversampled` option. The `Probe FWHM` sets the radius of neighboring pixels that will be included in the LBA: generally, using a larger px radius will yield a nicer map, but you must be careful about introducing artefacts to your data. If the background across a window changes abruptly, like at the edge of a nanoparticle or other sharp interface, overzealous LBA can introduce "ringing" effects.



Download the files `SampleData_EELS_Pt3Co_nanoparticle_N-edge.dm3` and `SampleData_EELS_Pt3Co_nanoparticle_M-edge.dm3` from the Box folder and open them with CSI. Using the `Spectrum imager`, notice that the two edges have very different backgrounds (try using the `freehand` select tool to trace the particle). Create some maps of the N-edge using a variety of LBA FWHMs from 0 to 20. ***Large LBA windows will take much longer for CSI to process, so keep in mind the memory capabilities of your computer.

Try similar oversampling on the M edge. Do you get the same ringing effects?

The file is an image of a Pt_3Co nanoparticle on a carbon support, so the true background should be nearly uniform and around zero. Notice that using LBA with a few-px FWHM can produce a significant improvement to no LBA. Notice also that using a very large FWHM creates artefacts near the edges of the nanoparticle that could be mistaken for a core/shell structure.

For a full description of this and other data processing techniques, see Cueva, et al. (2012) https://doi.org/10.1017/S1431927612000244

Other artefacts

Explore the other data set `SampleData_EELS_LuFe2O4.dm3` in the same way. This is a .dm3 file as it was output by a Gatan instrument. You can load it into ImageJ using the same `Open spectrum` button in the CSI menu that you used for a 3D tiff (if you drag-and-drop, you'll have to recalibrate the energy again, which can be tricky if you don't already know which edges you have).

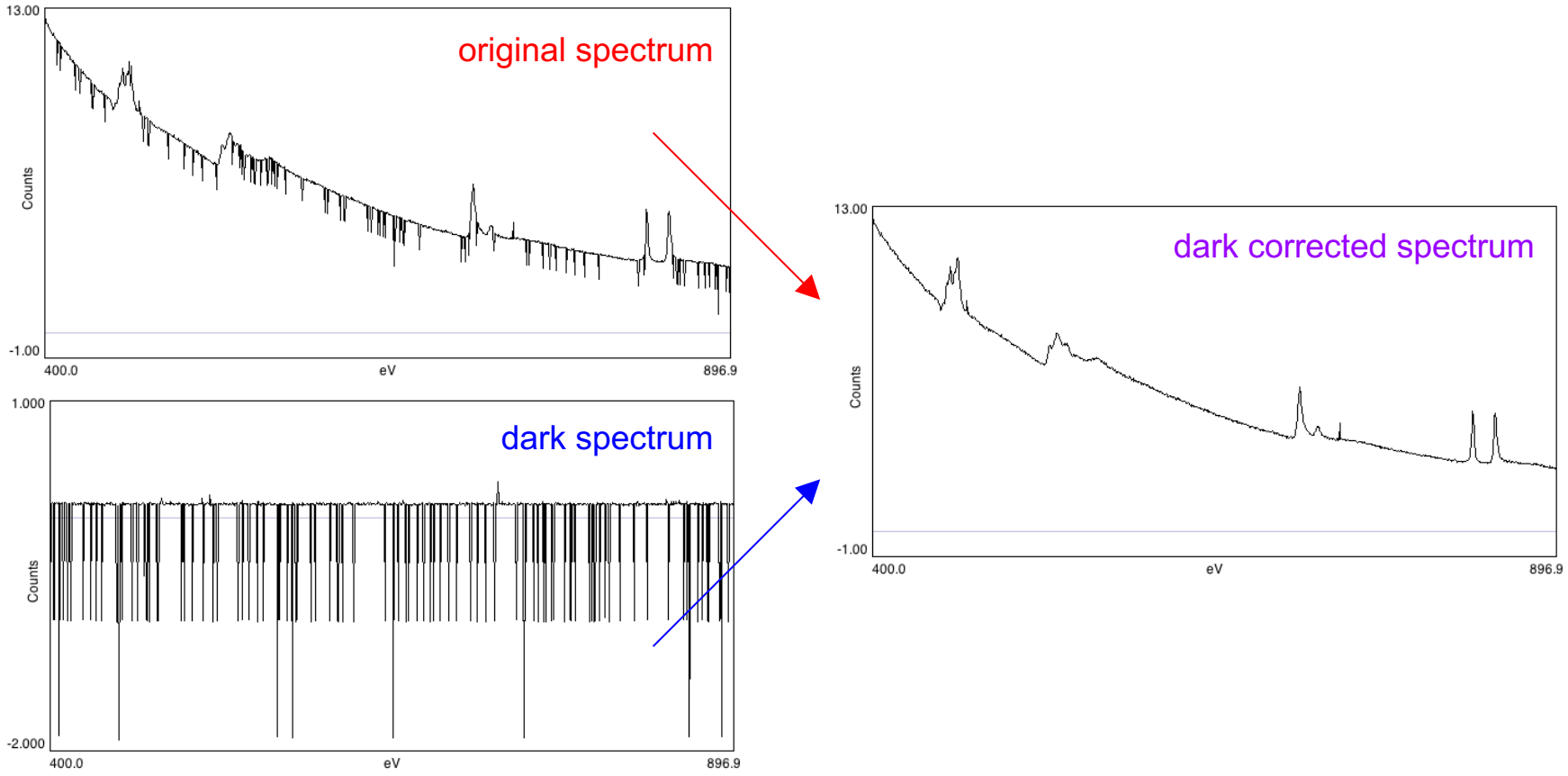
How can you affect the output maps by change the size or location of the background fit window? The integration window? What other artefacts can be introduced from careless data processing?

Visit the EELS Atlas and look closely at the energies given for Fe. Think about the other elements in this sample and their edges: do you foresee any challenges? What can you do to work around them?

Other tools, tricks, and techniques

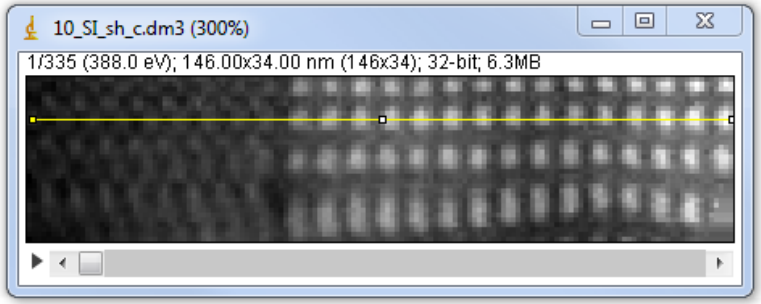
Dark-reference Subtraction

Some data collection software has dark-reference subtraction built in. For data that is not already dark-corrected, however, dark reference subtraction is a simple but very important step. In addition to the regular spectrum image, collect another SI without any signal on the detector. The `Subtract dark reference tool` will use the dark SI to correct your original SI, outputting a new 3D tiff file on which you can perform all of the usual analysis.

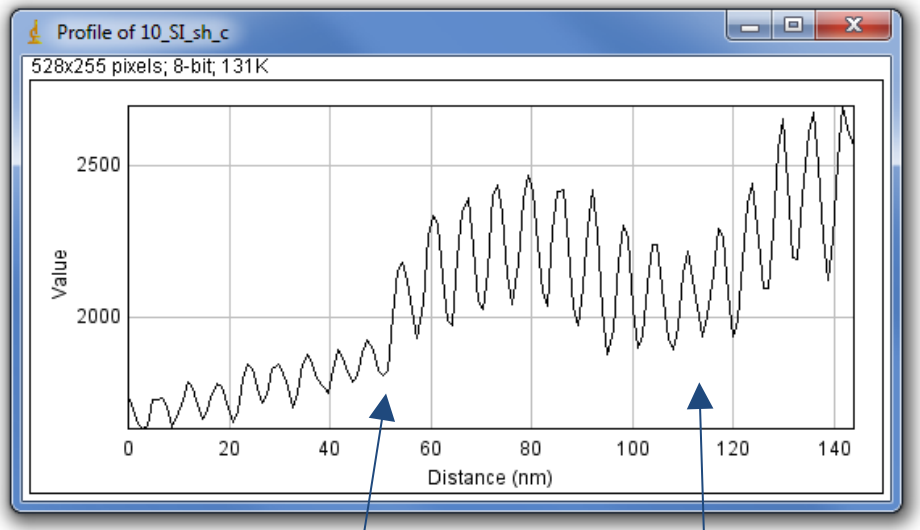


Line Profiler

Create a profile of relative intensity along a line. Double click on the `Line profile` button to change the line width. Wider lines improve signal to noise by summing across the width of the line.



Notice how the jumps in intensity across the atomic columns can help identify features like interfaces or diffuse superlattice layers.



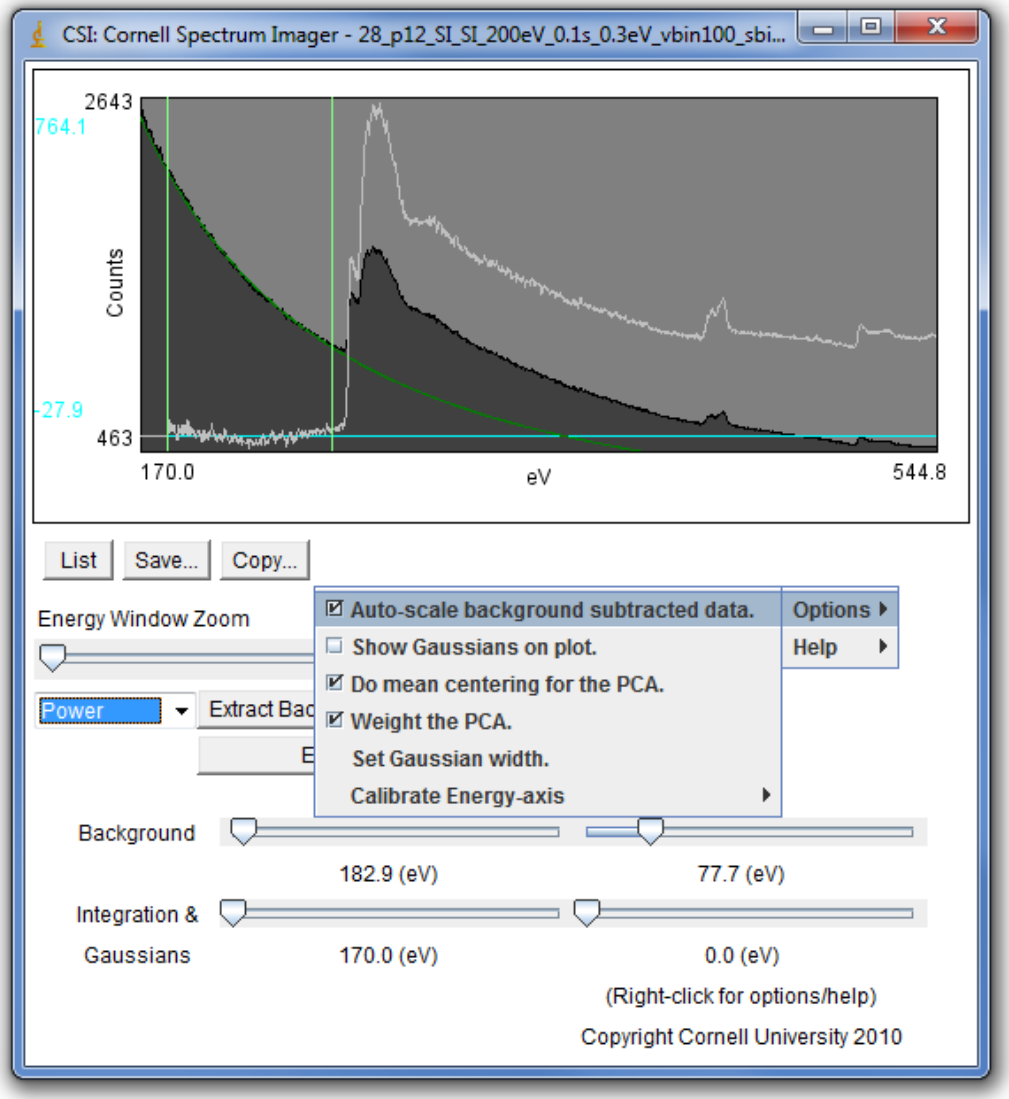
interface

diffuse superlattice layer?

Auto-Scaling Spectra

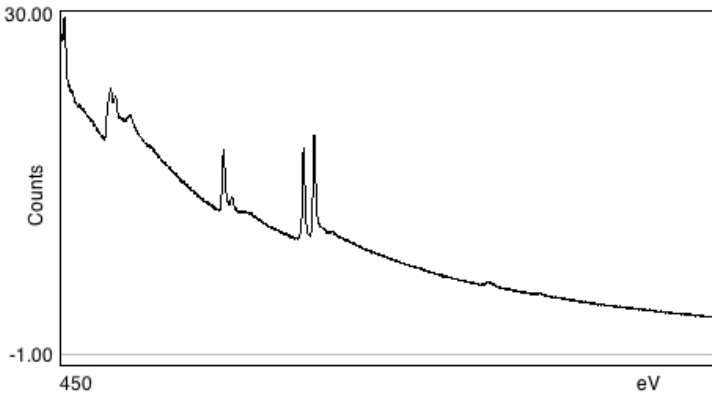
Scale Subtracted Data

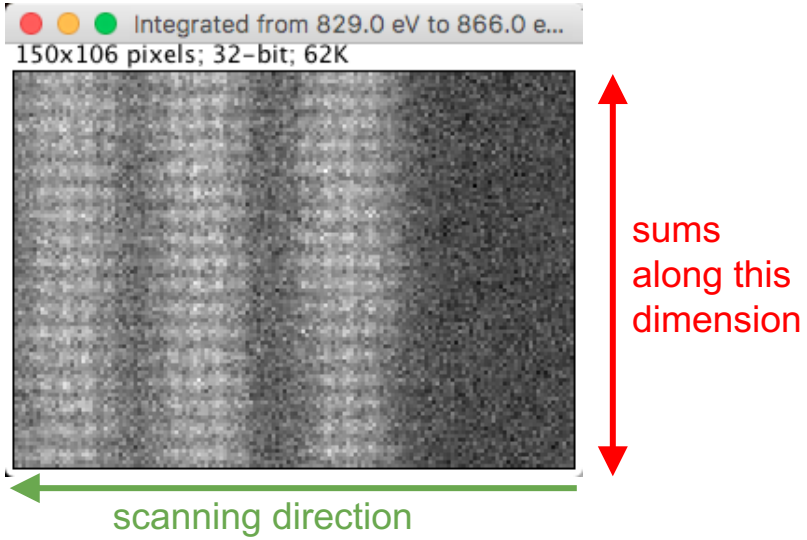
- Use the right-click options menu to enable auto-scaling.
- Zero line and alternate counts scale are shown in cyan.



Map to Line

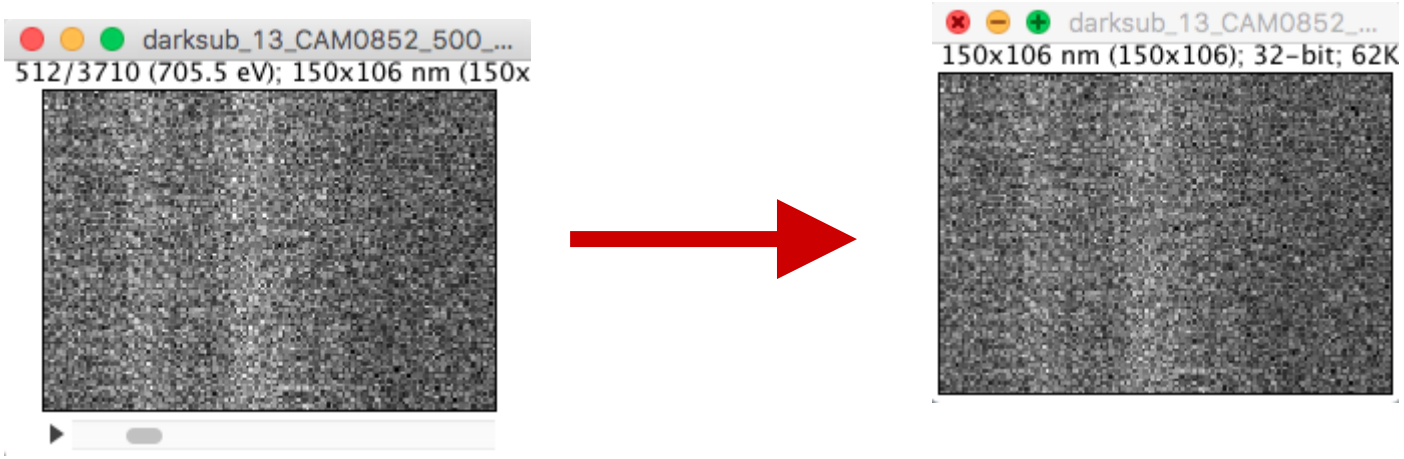
Project the SI along the one spatial dimension (default is the scanning direction) to create a 2D image with energy along one axis. This can be a great way to pick out interfaces or layers along one dimension.





Extract Slices

Quickly generate an integration map for the single channel currently selected in the SI window.



3D SI file

2D .tif image of 705.5 eV