Qualitative analysis of samples can be done quickly, but quantitative analysis will require additional sample considerations. For all EDS spectra, grounding your sample will affect the results. ==*Be sure that you have a path to ground!*== Prior to starting the EDS software you will want your sample to be in focus in the SEM and set to a WD of 10 mm (see SEM Operating Protocol). For quantitative analysis, you will want to have a smooth sample (< 1um roughness), homogeneous in the beam interaction area, and no charging. Finally, standards are required for best accuracy.

1. First, switch the com port switch to EDS (position B). This box is located to the right of the SEM monitor.
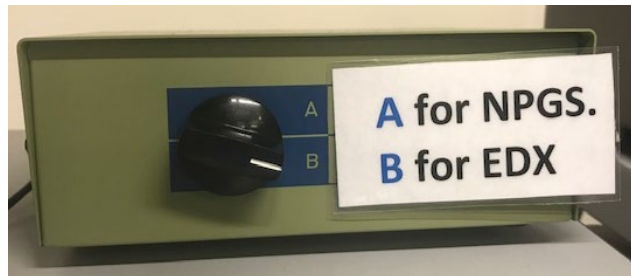
2. Open the EDS software located on the left monitor by clicking on the NSS icon.


3. The software will prompt you to create a new project folder. You must either choose or create a new green folder every measurement session. It is recommended that a new project folder be used for each imaging session for file organization.
    a. Locate your folder and create a new project file by clicking on the most left upper green folder icon.
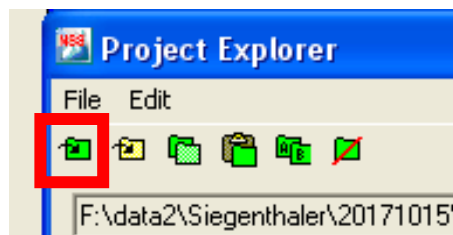

    b. Give the folder a meaningful and relevant name. You can always right click on the folder and select rename. Once done, select OK in the lower right corner.

<u>Note:</u> Make sure that the folder is green and selected. Folders that are not green cannot be used to save EDS data.
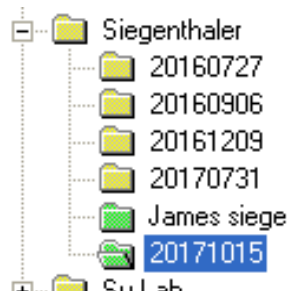

4. Once the folder is created the main window will appear. Choose what type of EDS spectra imaging mode is to be done. There are three options.
    a. Spectrum – Measures all elements in the image
    b. Point and Shoot – Measures selected spots
    c. Spectral Imaging – Maps the whole image for where different elements are located or concentrated.
    d. X-Ray Linescans – Allows you to draw a line on your sample and collect spectra along that line only.

5. **Spectrum:** For basic EDS spectra of an image select Spectrum.
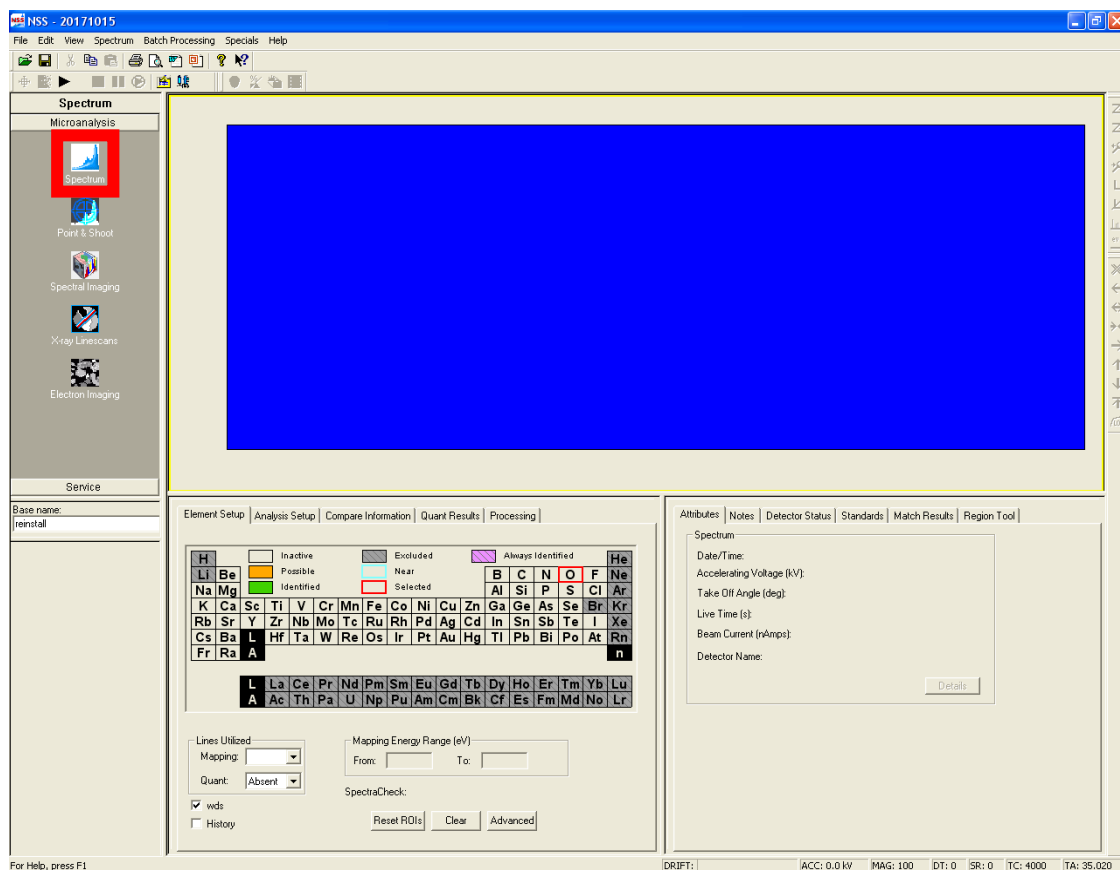
a. On the SEM adjust the HV to the desired setting. _Typically, 30 keV is used for survey or initial scanning._ Then the acceleration voltage should be decreased to 1.5 X the highest energy peak in your spectra. This is done so that you minimize the interaction volume but retaining enough energy in your beam to excite your peaks of interest.

    i. HV is directly related to sample penetration and imaging volume. EDS is a subsurface technique where we are probing up to 1-3µm below the sample surface. Reducing HV will reduce the penetration depth; however, the consequence of using a lower energy beam is that it will not be able to excite higher energy transitions.

https://www.phi.com/surface-analysis-techniques/aes.html
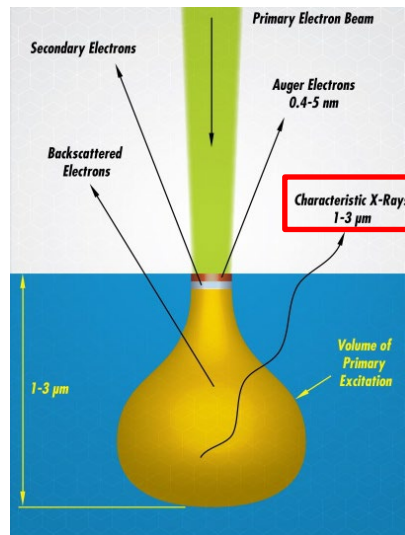
    ii. When adjusting HV it is important to step the HV one click at a time, then wait for the system to adjust, then step again. Note: Our SEM does not have enough current for EDS measurements at acceleration voltages below 10 keV.

    iii. Refocus the image to the best that it can be keeping WD at 10 mm.

6. Acquisition Settings must be set prior to collecting a spectrum within the NSS Software.

a. In the NSS software click on Edit>Acquisition Properties.

b. A window will appear for Acquisition Properties. Change both the max KeV and Time constant.
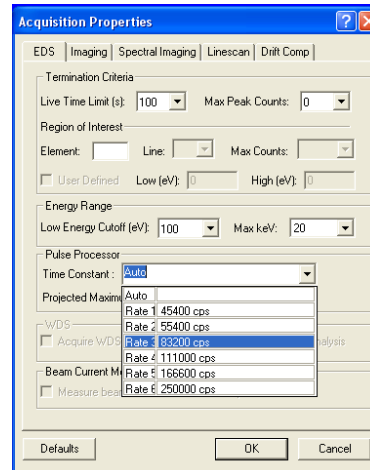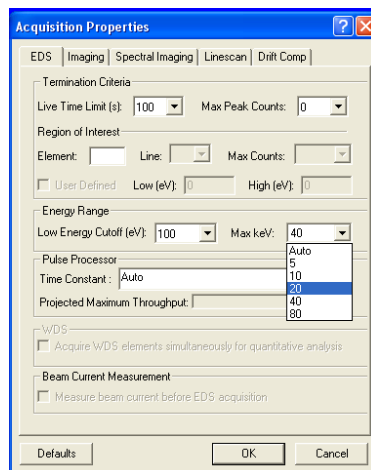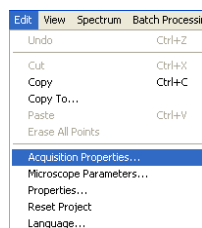
    c.   The max keV should be selected to match the HV from the SEM. Since the SEM was set earlier to 30kV for a survey scan, we will set the EDS to 40 kV. This setting determines the digital resolution of the X axis in your spectra (energy sampling).

    d.   The pulse processor rate should be chosen based on the throughput or resolution considerations of your experiment. *Typical rate used by most users is Rate 3*.

Note: The pulse processor removes noise through signal averaging. Adjusting the rate constant adjusts the duration of the process time of the detector. Changing the rate constant will change the time allocated to process each pulse (i.e. X-ray). Increasing the time given to the pulse processor to filter & shape the steps on the ramp will increase the accuracy (resolution) and lower the throughput.

| rate	cps	u sec	
1	45400	22	Highest Resolution
2	55400	18	
3	83200	12	
4	111000	9	
5	166600	6	
6	250000	4	Highest Throughput

    e.   Finally, the Live Time Limit is set to 100 seconds by default. Changing this value will enable you to have a longer or shorter collection time. You have the option to set the software so that the collection time depends on the peak counts for the maximum peak or select element peak.

    f.   Select Ok to exit.

7.   Adjust the spot size making it larger to make the dead time (DT) to be between 20 – 50. DT is the time when the detector is not collecting, but the system is still processing previously collected X-rays. Typically, users will work to achieve a DT = 20-30 for most experiments.
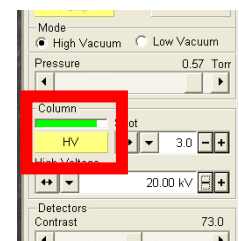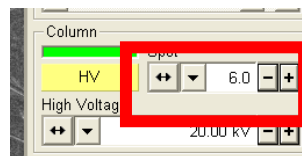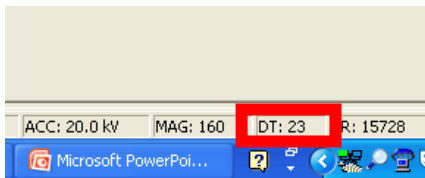

    a.   For best resolution & accuracy – Adjust count rate to achieve a DT percentage of approximately 20 with the longest pulse processor rate.

    b.   For maximum throughput, but lower resolution – Adjust count rate to achieve a DT percentage of approximately 50 with the shortest pulse processor rate.

Note: Once the spot size is 7.0, it can still be increased, but the spot size must be increased by manually inputting a number instead of using the arrows. The green HV bar may decrease but give it a minute and it should fill the column back up again. The SEM screen will probably be white, and that is fine because you can adjust brightness or use auto contrast to correct.

8. Choose what elements are to be identified by right clicking on the periodic table. If this is an exploratory search set all possible elements to "inactive". If something is to be identified and it is known in the sample, you can choose "always identified" to identify if an element is present. This step can also be done after acquisition of spectra.
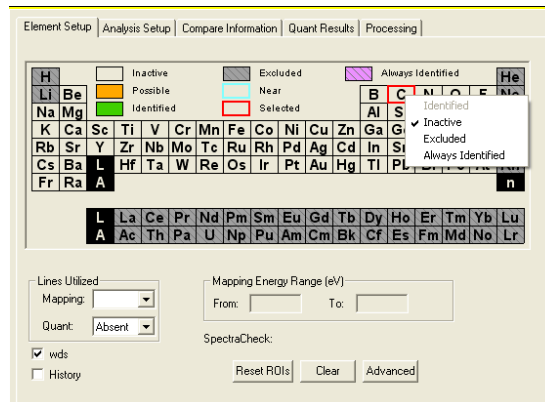

9. To start a spectrum acquisition, select the play button in the top left corner. Wait for the spectrum to collect noting the time remaining bar at the bottom of the screen.
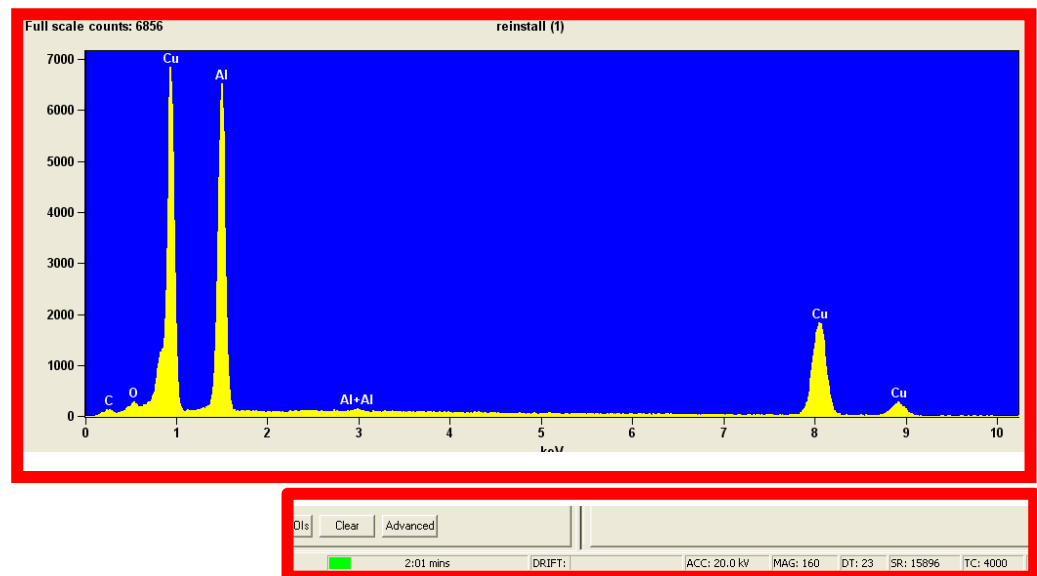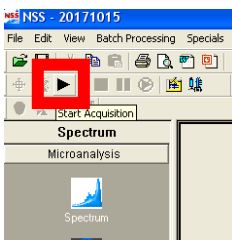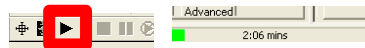

10. **Point & Shoot spectrum:** Select Point & Shoot button on the left. An image must be read into the system. Click the acquire button on the top left, and see the image collected from the SEM. SEM will display external in the top right corner on the monitor. **_Note:_** _If the acquired image is black, make sure the switch box is on EDX and not NPGS._
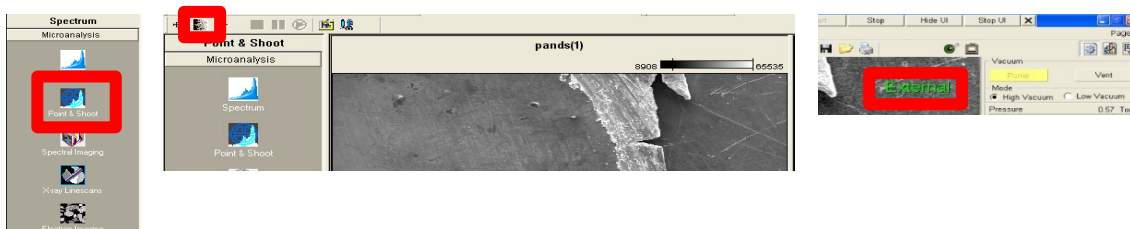
11. To choose where to collect an EDS spectrum, use the point tool. Using this tool, select where on the image a spectrum is to be taken. To delete a point, use the arrow tool, select point and press delete on the keyboard.   You can also draw squares, circles or custom areas depending on the tool selected.
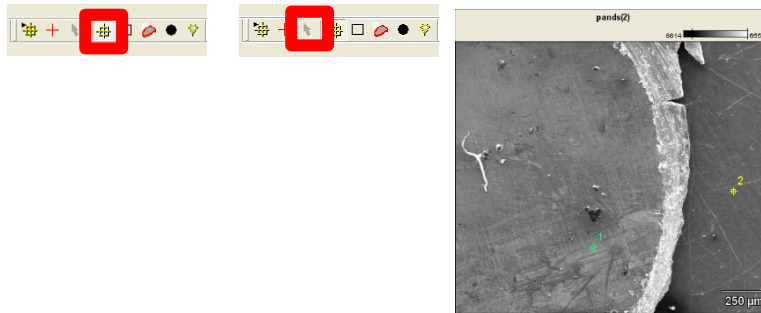
12. To begin collecting EDS spectra, ensure that the SEM has a large enough spot size by having a DT between 20 – 30, additionally that the rate has been changed to rate 3 as in step 6 (see previous discussion for tips on these settings). Select the "play" button. The EDX spectra will be collected for each point sequentially and automatically. Watch the time in the bottom bar.

13. To analyze the spectrum, use the arrow tool and select each point. Each point must be analyzed individually and exported. Jump to section 18 for data analysis.
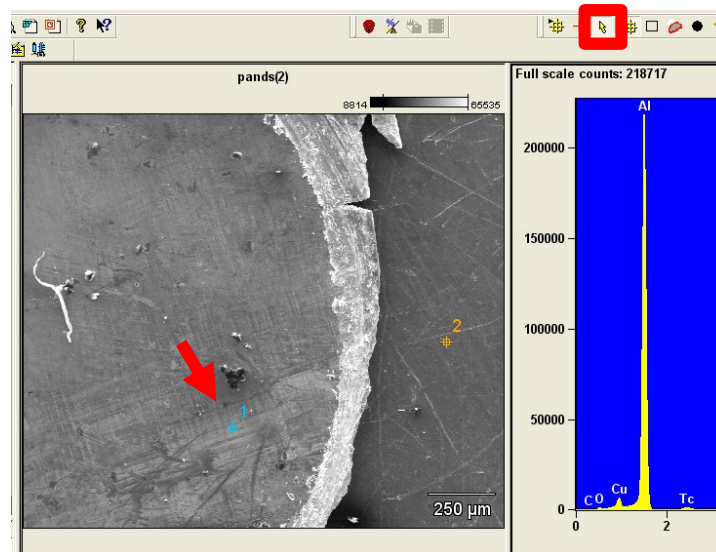

14. **Spectra Mapping:** To collect a spectral map of the sample, select Spectral Imaging. An SEM image must be read into the system. Click the acquire button, and see the image collected from the SEM. SEM will display external in the top right corner on the monitor. *Note: If the acquired image is black, make sure the switch box is on EDX and not NPGS.*

15. Change the acquisition properties under Edit>acquisition properties like in Spectrum and Point & Shoot to be rate 3, match the eV for the HV used on the SEM, and increase the spot size to reach a DT of 30-40. Then choose the Spectral Imaging tab. The resolution, frame time, number of frames will all influence the acquisition time. Choose the settings that will meet your needs. A 5 min acquisition can yield a low intensity overview map. A 60 min acquisition can yield a very detailed high intensity map. Generally for best spectral maps, you want to let it collect long enough that the contrast bar on top of your elements of intrest reaches 60-80. Once the settings are chosen, choose "play" to begin acquisition.

Note: Frame time is the time to collect per each frame. Number of frames is the number of passes that the system will scan over over the sample area. The acqustion time is the multiplication of these two parameters. You can also set the number of frames to infinity which enables you to manually stop aquisiton once you have reached your desired elemental contrast.
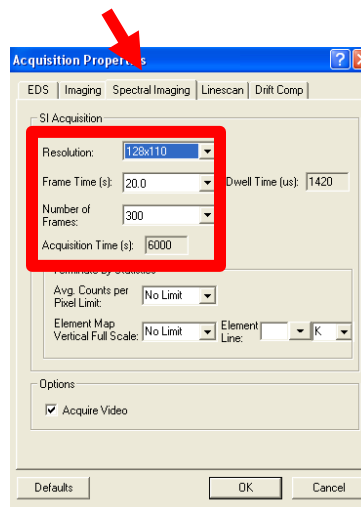
16. As the acquisition continues, elemental maps will be extracted from the image. These will increase in signal and detail as more data is collected. Watch the time bar at the bottom, and it will count down until the acquisition is finished.
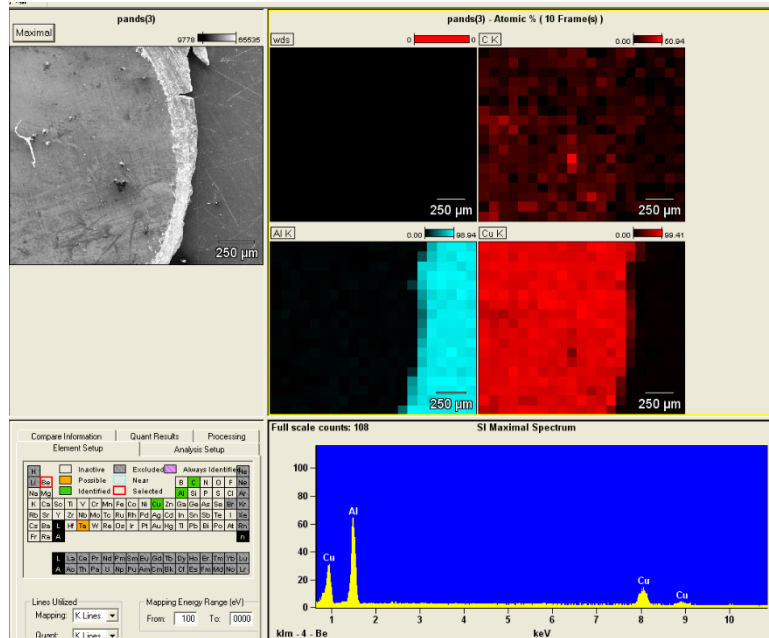
17. Once complete, using the spectrum, identify what elements are present and not. To re-extract the maps based off the identified elements choose the extract button (looks like a little window). Follow Step 18 for data export and identification.
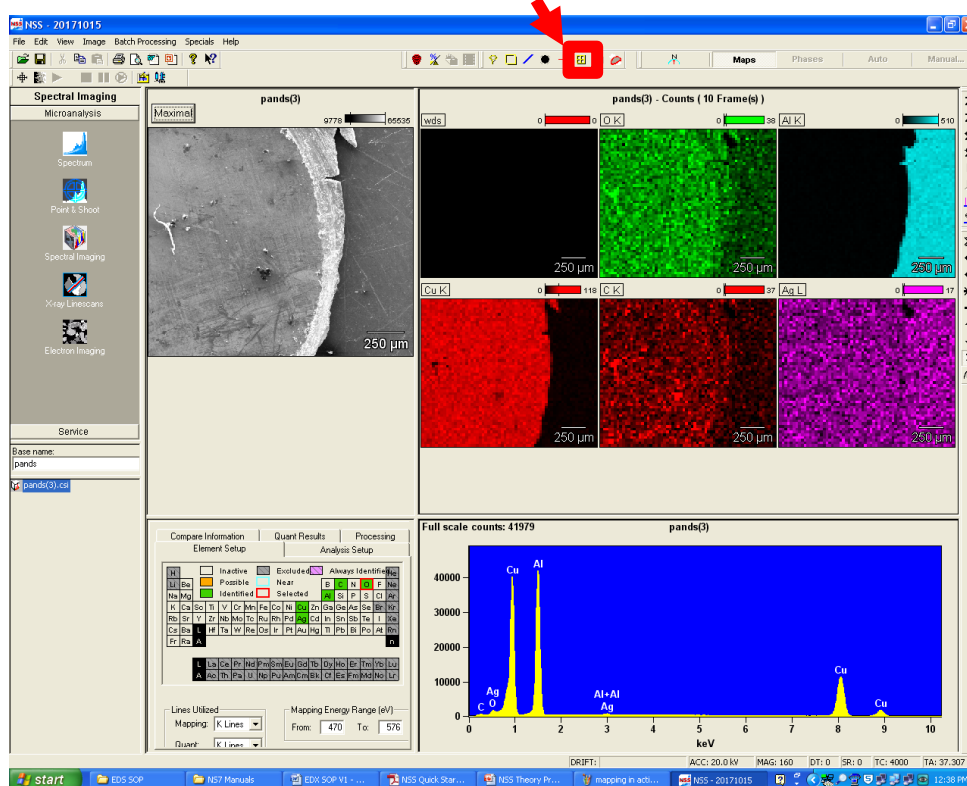
18. **Data Analysis:** Following the spectrum acquisition, confirmation/determination and identification of the elements must be done. These are brief guidelines to get started on data analysis. This is a powerful technique and this guide is only meant to show you the basics. Please ask about further analysis of your spectra to meet your experimental needs.

Note: Element peaks can overlap with other elements. You should check the peak energies with supplied Thermo X-ray energy sliders.

    a. The auto identification button is a great place to start. The software will identify peaks based off library standards. This is a start and not a finish. Peaks must be confirmed based on the user's knowledge of what is present or most likely to be present in the sample.


    b. The system is already calibrated, and it is the user's job to correctly identify if the software is auto identifying the peaks correctly. **Note:** We assume that there is NO sample charging which will shift peak energies.

        Tools to help identify are:

        i. Energy Cursor
            1. Allows user to drag a cursor around on the spectra and the cursor location highlights inside the periodic table below the possible elements that would have a peak at the cursor location.
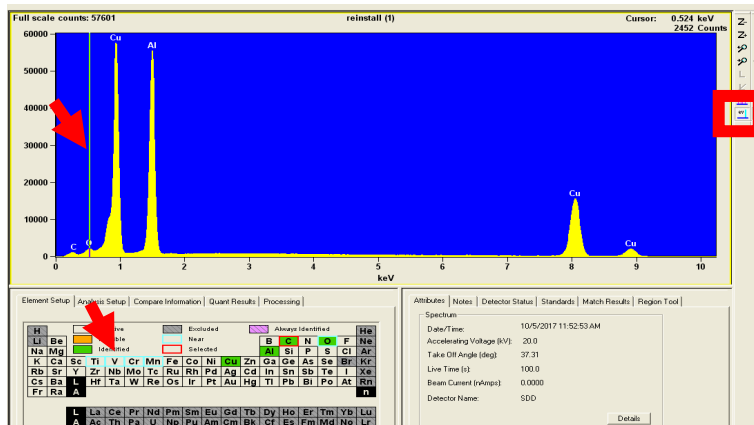
ii.   KLM Line Cursor: Allows users to pick elements on the periodic table and the software highlights, with proportional energy lines, where on the graph that element would appear if present in the spectra.
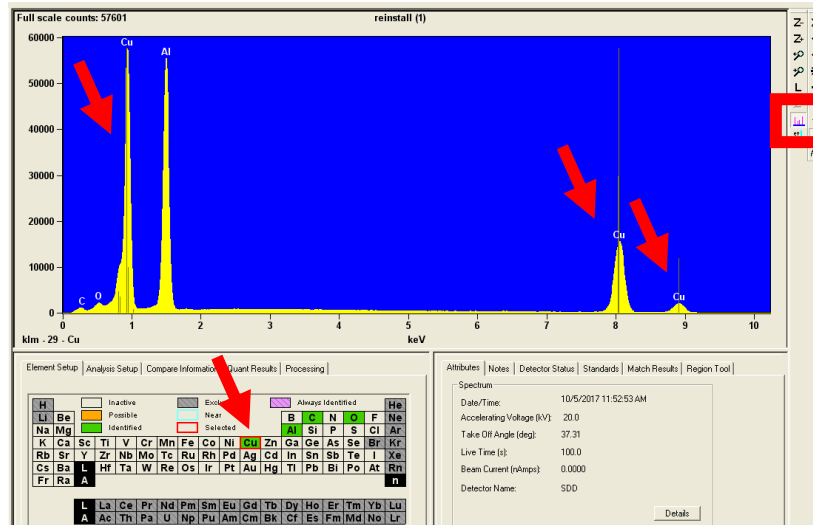
c.   Based on the system tools and your knowledge of the sample, identify elements on the periodic table by right clicking. Identified elements are green.

Note: Semi-quantification of the results can tell approximate elemental composition of the sample. This quantification analysis does a comparison of your experimental data to a synthetic spectrum calculated from reference spectra for the elements identified. It does not correct for the sample roughness, sample matrix or instrument yield variations. The accuracy (the closeness of agreement between an observed value and an accepted reference value - 95% analysis) of this measurement is generally:
±1% for polished bulk target, pure standards on site
±2% for polished bulk target, standards collected on another SEM and then corrected for the geometry and settings of the present microscope (NSS terms this "without standards").
±5% for particles and rough surfaces "without standards"

19. Select the quantify button from the top bar and see the results in the quant results tab by the periodic table.

20. A data report can be made in either Word or a PowerPoint (PPT is the preferred method). The graph will be represented in the output file as shown in the NSS software, so it is important to fix the representation (ie. scale, labels, KLM line and, energy cursor tool) of it prior to exporting to the report. Be patient while it exports, *its XP and slow*. You can populate many different spectra and data into the same PowerPoint. Just reselect the PPT for every spectrum you want added. It will autosave to your project folder and append to the presentation, so don't close the PPT file.
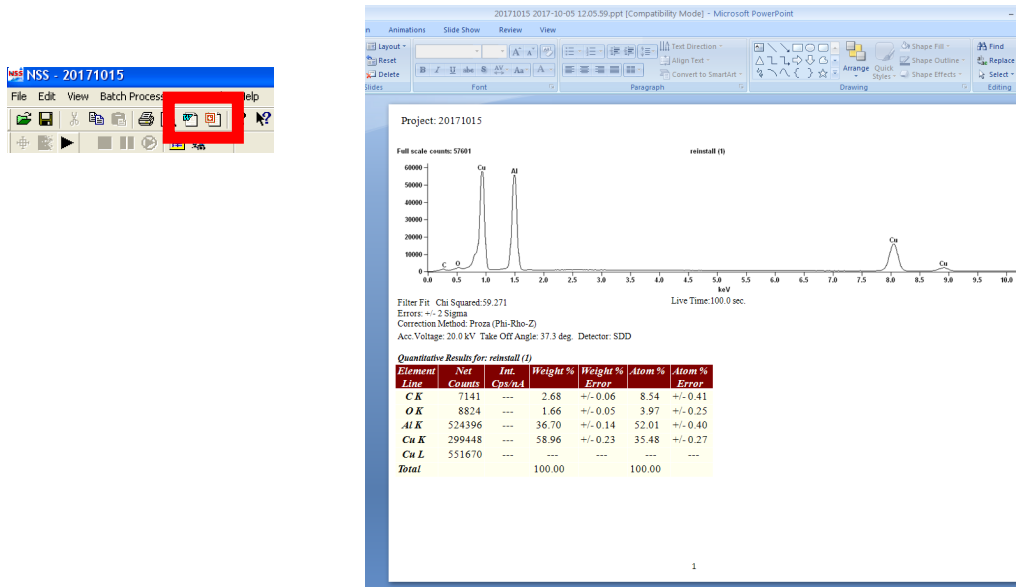
21. Data can be additionally opened with a text editing software such as Notepad, or Notepad++. Just right click on the spectra file and select "open with". The data will be represented in a 2-column format. Scroll down in the file to find the start of the XY data. This can then be replotted within any graphing software such as MS Excel or Origin. Please note that the peak labels will not be present and must be manually added.