# Technical Note

# The Importance of Relative Intensity Correction of Raman Data and How to Utilize it for i-Raman Series Instruments in BWSpec



B&W Tek's basic Raman acquisition software is BWSpec. It has an easy-to-use interface for collecting basic Raman spectra with the ability to do spectral processing. One important aspect of collecting Raman data is correcting for the spectrometer's relative intensity, since the relative response for each Raman spectrometer is unique. BWSpec users are encouraged to apply this correction when collecting data in order to obtain more accurate spectra for their analysis.

## Relative Intensity: What is it and where does it come from?

Each spectrometer has its own, unique spectral sensitivities, which affect the relative peak intensities of the spectra they collect. These variations are the result of the quantum efficiency of the spectrometer's detector and the spectrometer's optical design and individual components (e.g.

lenses, grating, and probes).  In order to account for these variations, each spectrometer must be corrected for relative intensity.

Each probe also contributes its own unique variations that affect the relative peak intensities.  This is a result of the probe having its own set of optical components outside of the ones found within the spectrometer's main body.  That means any relative intensity correction of the spectrometer must factor in the probe as well.  When it comes to B&W Tek's portable Raman systems, different probes can be used with the same unit.  For example, there is the lab-grade Raman Trigger Probe, a process-grade Industrial Raman Probe (good for demanding manufacturing environments), as well as custom probes to fit any application.  In short, the correction for relative intensity of the spectrometer will need to be changed as one probe is exchanged for another.

## Spectral Variations

In some experiments, the ratio between two peaks is very important.  Correcting for the relative intensity corrects the differences in peak ratios.  Figure 1 shows two spectra of cyclohexane collected on an i-Raman® Plus 785 nm Raman system.  One of the spectra is relative intensity corrected (red) and the other is not (blue).  When looking at the two peaks: 801 cm$^{-1}$ and 1020 cm$^{-1}$ one can see that the ratio between these two peaks are different.  Using a spectral math function (I801 cm$^{-1}$ /I1020 cm$^{-1}$), you get a ratio of 4.45 for the uncorrected spectrum and 4.00 for the corrected version.  The difference between these ratios is quite significant.  It could lead to an incorrect conclusion for an experiment if the spectral data is not corrected for relative intensity.
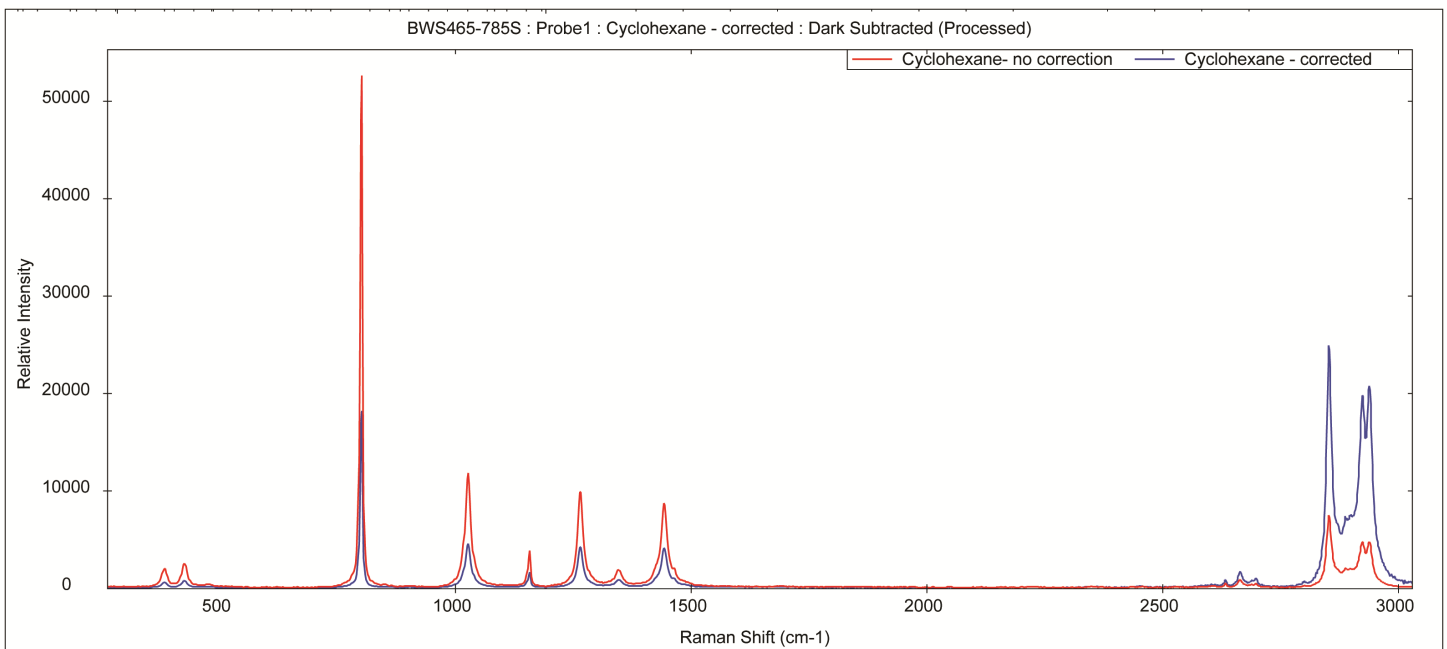


*Figure 1: Cyclohexane uncorrected spectrum (red) and corrected spectrum (blue)*

There are numerous spectral variations that occur due to the spectrometer's sensitivities. One such variation is called the etaloning effect. Etaloning occurs when the laser strikes a component of the spectrometer that has two parallel surfaces and as a result constructive / destructive interference is created between those surfaces. This interference results in modulations of the spectral intensity. This is a common situation in interference filters used in Raman probes and back-thinned CCD sensors (working in the NIR region). Figure 2 is an uncorrected spectrum of a lapis lazuli stone (a deep blue semi-precious stone) collected on an i-Raman® Plus 785 nm Raman system. As you can see, there is a fringe pattern that runs along the spectrum. That pattern is the result of etaloning, and they are not real peaks in the spectrum. Figure 3 shows the same spectrum following relative intensity correction, where the pattern is removed and the spectrum looks a lot clearer.
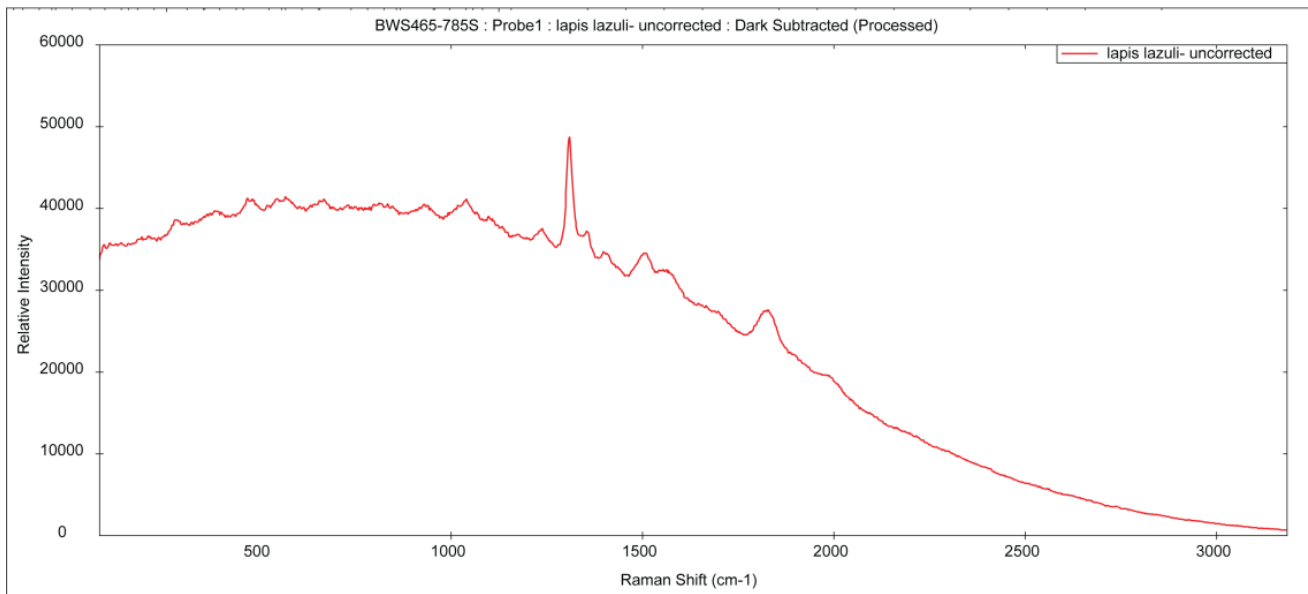


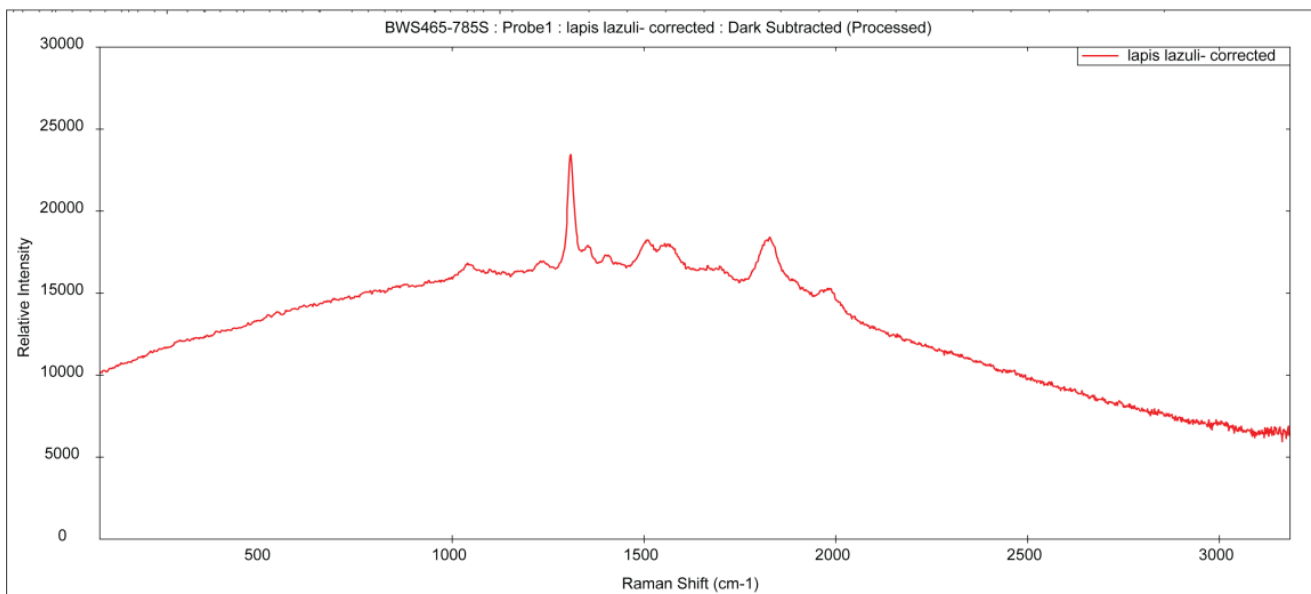*Figure 2: Raman Spectrum of Lapis Lazuli (uncorrected)*



*Figure 3: Raman Spectrum of Lapis Lazuli (corrected)*

# How is a spectrometer relative intensity corrected?

Now that we gone over some of the spectral issues that result from not correcting for relative intensity, we can go over how a spectrometer is relative intensity corrected. To start, a spectrometer is corrected by obtaining a certified material that will be analyzed with the Raman spectrometer that will be corrected. Originally, the certified material was a white light lamp (such as tungsten or tungsten-halogen lamps). The spectral output of the lamps was calibrated to a traceable irradiation source. In turn, the lamp was then used to calibrate the Raman spectrometer. However, there were some drawbacks with the lamps. First, the calibration of the lamps had a limited lifetime and often needed to be sent out for recalibration. Second, the lamps themselves were too big for most Raman experiments and posed their own difficulties in trying to interface them with the Raman experiment. Therefore, alternatives were desperately sought.



*Figure 4: Standard Reference Material 2241 for Raman Spectroscopy*

In cooperation with the ASTM Subcommittee of Raman Spectroscopy, the National Institute of Standards and Technology developed a suite of standard reference materials (SRMs). These SRMs are a series of glasses that are luminescent with Raman laser irradiation (Figure 4). The luminescent spectrum can be fit by an analytical expression (which represents the true shape of the glass) in order to create a spectrum that is free from relative interference from the spectrometer. BWSpec software's Relative Intensity Correction tool can create a ratio file by dividing the analytical expression (i.e. the true shape) of the glass by its uncorrected spectrum. This ratio correction file can be applied either automatically to spectra during acquisition or retroactively to previously acquired spectra. As an example, Figure 5 shows the Raman spectrum of the SRM 2241 standard measured on an i-Raman® Plus 785nm Raman system without intensity correction. Figure 6 shows the same spectrum when it has been intensity corrected with the ratio file. In the end, the relative-intensity correction yields Raman spectra that have a comparable response across all instruments using that specific laser (in this case 785nm).
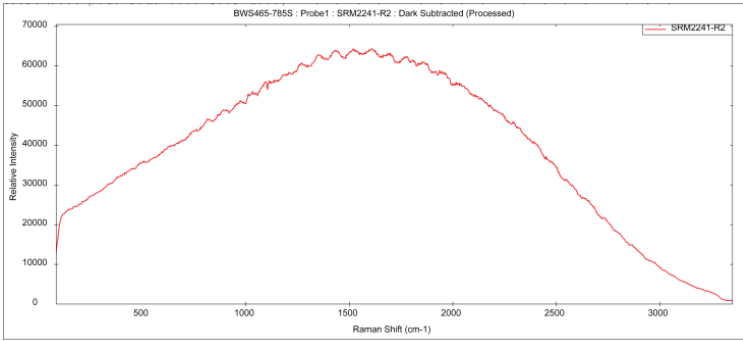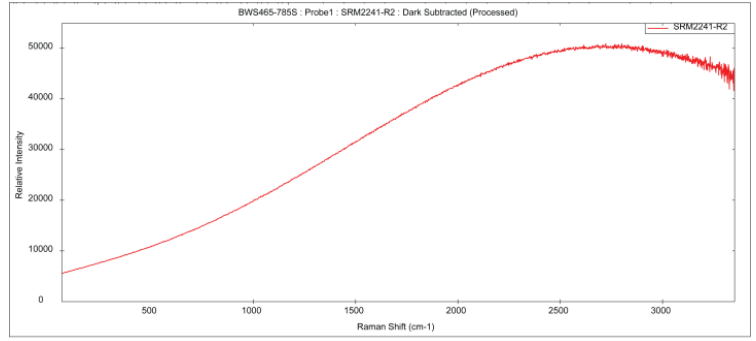
Figure 5: Raman Spectrum of SRM 2241 (uncorrected)



Figure 6: Raman Spectrum of SRM 2241 (Corrected)

# How do you apply the relative intensity correction within BWSpec?

Now that we know how the relative intensity is corrected, the question remains how do you apply it? B&W Tek's BWSpec software creates a ratio file that contains the information the software needs in order to apply the relative intensity correction. One can apply this ratio file in two different ways. The first is to set up the ratio file so that each scan you take, the relative intensity correction will automatically be applied to the spectrum. The second way is to apply the ratio file to a previous scan in which the spectrum was not corrected. This is a good way to apply the ratio file should you happen to forget to apply it ahead of time.

In order to set up the ratio file to apply to future spectra, click Setup in top menu bar. Then click Hardware Setup followed by clicking on the Probe Setup tab.
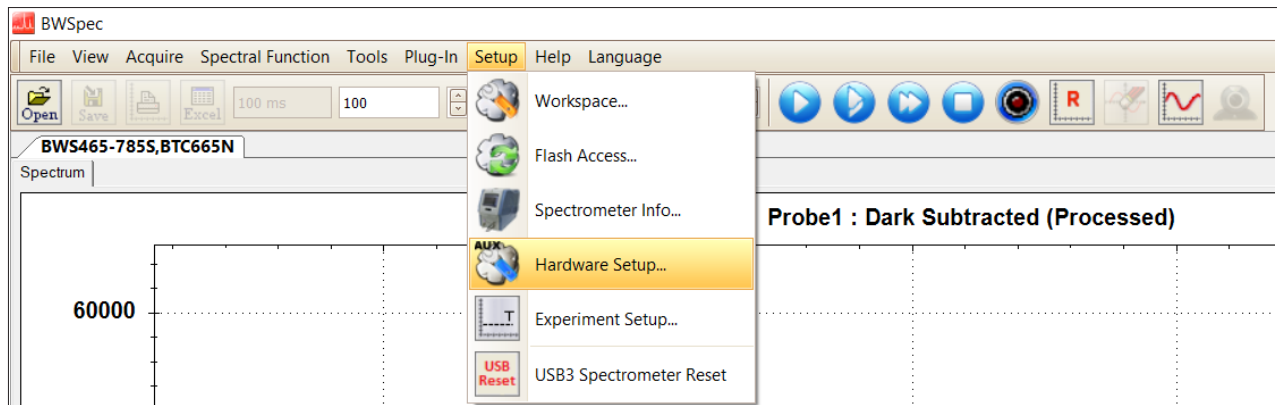


Figure 7: Setup => Hardware Setup

On the left hand side of the window, there is a list for all the probes currently set up in the software. Click the button entitled New at the bottom of that list. Type the name you would like to give the probe (it is suggested to name the probe "Probe_[spectrometer c-code]_[probe serial number]"). Underneath that, select the option to Select Ratio at HardDrive and then search for the ratio file on your computer or tablet. Once the ratio file is selected, the correction will be listed in the section titled Ratio3. Click on Done (the name will appear in the list at the right) and then click Close.
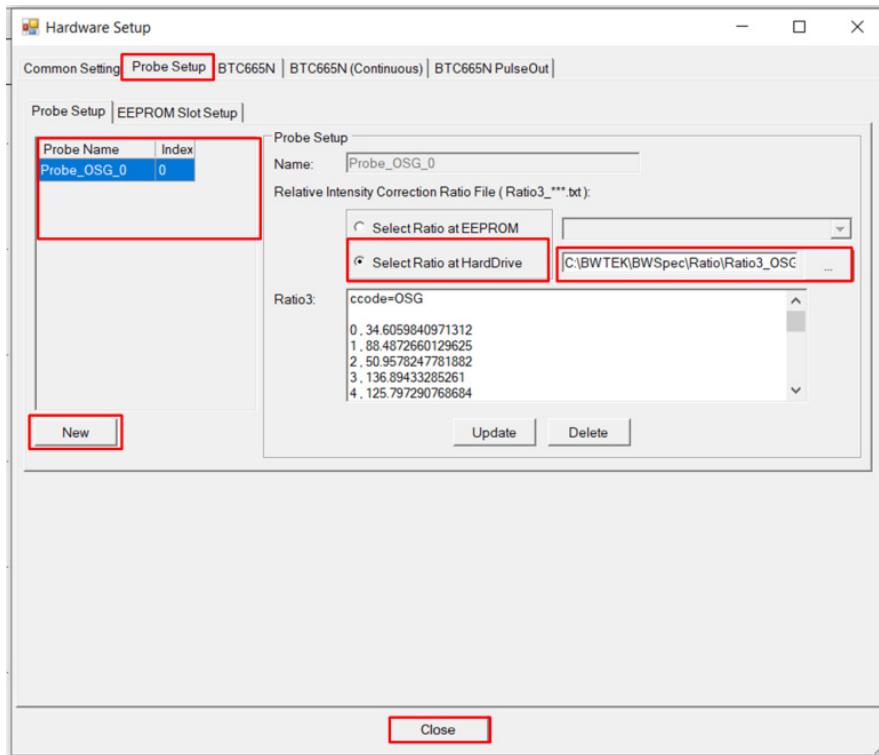
*Figure 8: Probe Setup highlighting areas of concern*

Now that the probe with the ratio file is set up, you need to enable the probe for use. In order to do that, click Setup in the top menu bar again. Then click Experimental Setup.
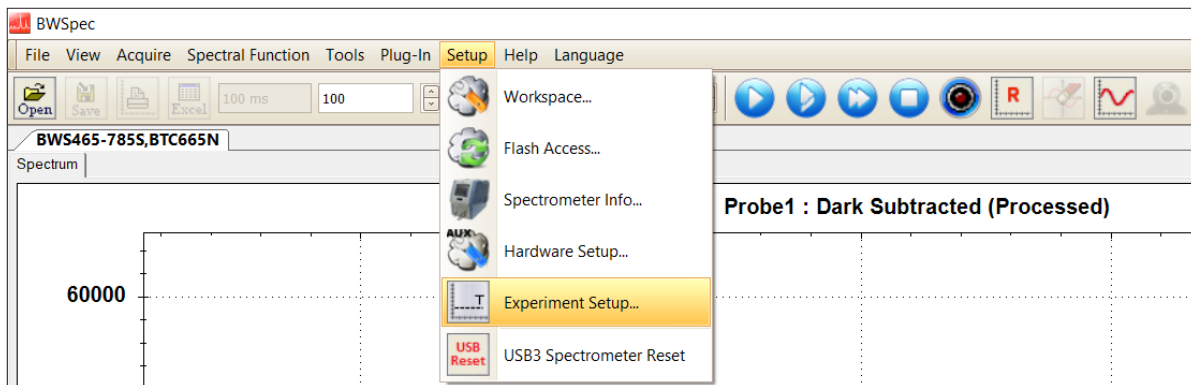


*Figure 9: Hardware => Experiment Setup*

Under the Acquisition section of the window, select the Probe. Under the Spectral Processing section, check Enable Relative Intensity Correction. Finally, in the upper right hand corner of the window, check Enable Experiment. Save the configuration and then click close. Now the ratio file is ready to be applied to all future scans.
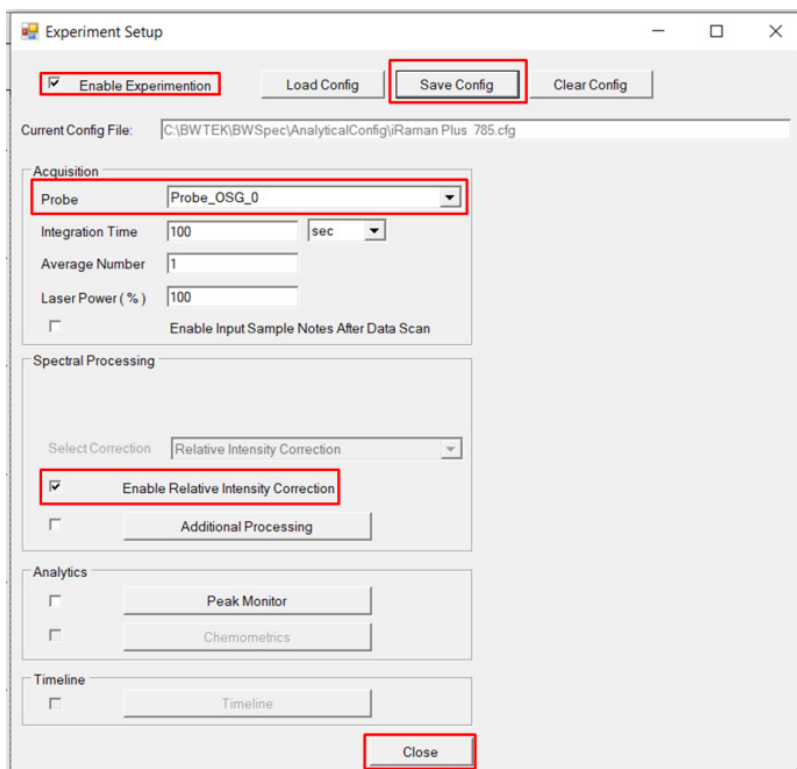
*Figure 10: Experiment Setup highlighting areas of concern*

If you had saved a spectrum in BWSpec's TXT or TXTR format and did not apply relative intensity correction when you acquired a spectrum, the ratio file can be applied retroactively using the Batch Reprocess function. How to achieve this depends on the file format. For TXT files, you need to first open up the spectrum that you wish to be relative intensity corrected. Check the file (Batch Reprocess function applies to checked spectra only). Make sure that you are viewing the spectrum as the Dark Subtracted (not the Raw Data). Click Spectral Function in the top menu bar. Then click Batch Reprocess. Under the Spectral Processing section, click on the Ratio button and search for the ratio file on your computer / tablet. Once the ratio file has been selected, check Enable Relative Intensity Correction. For TXTR format, the ratio file info is saved within the data file provided the spectrum was collected with an Experiment where the probe relative intensity correction was saved, and you simply check the Enable Relative Intensity Correction. In the upper right hand corner of the window, check Enable Batch Process. Save the Configuration and then click Do (in some versions the Do button is accessed at the Experiment Batch Panel tab under Spectrometer Control Panel). Your spectrum will then be relative intensity corrected. At this point, you will need to save the corrected spectrum in order for the correction to remain permanent.

# References

1. Choquette, S.J., Etz, E.S., Hurst, W.S., Blackburn, D.H., and Leigh, S.D. Relative Intensity Correction of Raman Spectrometers: NIST SRMs 2241 Through 2243 for 785 nm, 532 nm, and 488nm / 514.5 nm Excitation. Applied Spectroscopy. 61 (2) 117 – 129, 2007. Retrieved from https://www.s-a-s.org/