Technical note

Maintaining optimum Raman spectrometer performance with the DXR3 SmartRaman+ Alignment & Calibration Tool

Author

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Industry/Application:

Maintatining optimal Raman performance and analysis results for all Raman applications

Products used:

Thermo Scientific™ DXR3 SmartRaman™+ Spectrometer

Goal:

Express the importance of easy automated alignment and calibration routines for maintaining peak Raman performance and reproducibility of Raman spectra

Key Words:

Raman spectroscopy, wavelength calibration, laser calibration, intensity correction

Key Benefits:

Maintaining maximum Raman performance and consistent analytical results

Introduction

Alignment and calibration of Raman spectrometers are essential for getting the best possible results. The Thermo Scientific™ DXR3 SmartRaman™+ Spectrometer Alignment and Calibration Tool and associated routines provide the means for both aligning and calibrating the Raman instrument in one easy to use package. Keeping the instrument well-aligned and properly calibrated produces consistent results for all types of applications, whether they involve quantitative analysis or identifying unknowns using library searches. Figure 1 shows the alignment and calibration tool installed on a DXR3 SmartRaman+ spectrometer. The tool is easy to install and remove, just like any other accessory, and contains everything required for aligning and calibrating the instrument in a single package. Once installed, instrument alignment and calibration routines are initiated in the software with a simple click.

Figure 1. DXR3 SmartRaman+ spectrometer with the Alignment & Calibration Tool installed.

Experimental

This note focuses on the alignment and calibration of the DXR3 SmartRaman+ spectrometer. An antacid pain relief tablet of a generic brand was purchased at a local drug store; it contained sodium bicarbonate, acetylsalicylic acid and citric acid as the major components. A polypropylene sample was obtained from commercial packaging. The samples were secured in place on the sampling accessory with tape so that the sample did not move when the sampling accessory and the alignment and calibration tool were exchanged.

Results

Alignment

Raman scattering is a relatively weak phenomenon, so it is important to collect as many of the Raman-scattered photons as possible. A properly aligned Raman spectrometer is important for optimizing results. Raman spectrometers are typically aligned at the time of manufacturing but small variations in alignment can be introduced over time as a result of temperature changes, vibrational stress, and wearing of components (especially moving parts).[1] Changing out components like lasers, gratings, and filters can also produce variations in the alignment. Periodic alignment to restore optimal performance is important but should also be easy, and require limited user interaction and expertise, to implement. Alignment is particularly important for Raman microscopes because both the visual and Raman paths must analyze the same precise area of the sample, but that is a separate topic that will not be discussed here.[2] For any Raman spectrometer, maintaining alignment is a key for optimum performance.

The DXR3 SmartRaman+ alignment and calibration tool includes a polystyrene reference sample, and during alignment the intensity of the 1001 cm^{-1} polystyrene peak is monitored while a laser steer mechanism moves the beam to maximize the peak intensity. The laser steer assembly uses a pair of sequential lenses in the beam path that are moved with respect to each other so they effectively introduce an angular deviation of the light passing through them, and thus can move the position of the beam to optimize the alignment.[3] Figure 2 shows an example of this type of laser steer assembly. These are very small adjustments and are merely fine tuning the alignment, but the process does produce a noticeable effect. Figure 3 shows the difference in intensities of Raman spectra of the same polypropylene sample collected before and after alignment. Running the alignment routine improved the intensity by more than 12%. While this increase in intensity might not be critical for all applications, it does help maintain more consistent results. Alignment also helps preserve a constant sampling point. When alignment changes, so can the sampling point on the sample. Figure 4 shows results from an inhomogeneous sample (an antacid tablet) where the aligned and unaligned spectra clearly are sampling different positions on the sample. Maintaining instrument alignment helps to keep the sampling point consistent.

Calibration

Raman spectra are usually plotted as a measure of Raman intensity versus Raman shift in wavenumbers or wavelength. It is common to calibrate the Raman shift axis, but fully calibrating the intensity axis is more involved. The DXR3 SmartRaman+ spectrometer provides tools for calibrating the x axis (wavenumbers) including a neon source and polystyrene wavelength standard. It also includes a white light source that is not a calibrated source but is used for normalization of intensities to improve comparisons of Raman spectra such as those collected with different lasers.

Figure 2. Example of a Laser Steer Assembly.

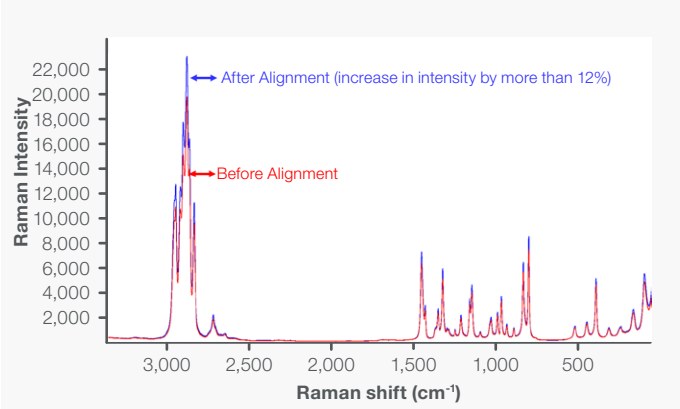

Figure 3. Raman spectra from a polypropylene sample. The Raman intensity increases after alignment.

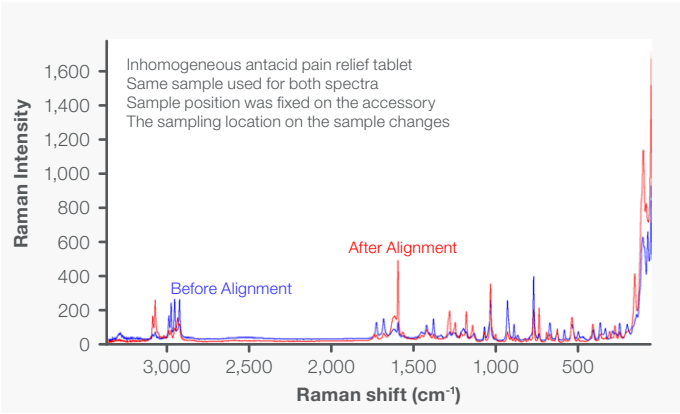

Figure 4. Spectra from an inhomogeneous antacid pain relief tablet that was fixed in place, so it did not move between collections. The sampling location moves during alignment to an optimal position.

	Known Component – Highest Match	Match Value	Position of Known Component in Top 10
Initial	Sodium Hydrogen Carbonate	72.78	1st
Shifted by +2 cm^{-1}	Sodium Hydrogen Carbonate	72.78	1st
Shifted by -2 cm^{-1}	Sodium Hydrogen Carbonate	72.78	1st
Shifted by +4 cm^{-1}	Sodium Hydrogen Carbonate	64.05	5th
Shifted by -4 cm^{-1}	Acetylsalicylic Acid	59.24	5th

Table 1. Initial and shifted spectra library searching results (match values are standard correlation).

	Components Identified	Cumulative Match Number
Initial	Sodium Hydrogen Carbonate	61.96
	Acetylsalicylic Acid	88.67
	Citric Acid	95.37
Shifted by +2 cm^{-1}	Sodium Hydrogen Carbonate	61.90
	Acetylsalicylic Acid	88.53
	Citric Acid	95.19
Shifted by -2 cm^{-1}	Sodium Hydrogen Carbonate	62.00
	Acetylsalicylic Acid	88.63
	Citric Acid	95.31
Shifted by +4 cm^{-1}	Wrong Matches	70.81
Shifted by -4 cm^{-1}	Wrong Matches	72.04

Table 2. Initial and shifted spectra multi-component search results. Match values are cumulative with each added component.

Wavenumber calibration

The x-axis of Raman spectra is typically expressed in Raman shift, which requires two pieces of information to determine accurately. It is necessary to know the actual wavelength of the Raman scattered light and the wavelength of the incident laser light. The laser wavelength is necessary because it sets the starting point for the shifted values.

The first step involves establishing a relationship between the detector (i.e., pixels of a CCD) and the wavelengths of light. This calibration of the spectrometer usually involves using a standard with known emission wavelengths. The DXR3 SmartRaman+ spectrometer uses neon emissions because these are known with a great deal of accuracy, and also because there are multiple peaks across the spectral ranges of interest. Using multiple points across the spectral range helps account for any non-linearity effects. A single point does not allow for addressing non-linearity and thus is inherently less effective.[4] A neon bulb is integrated into the alignment and calibration tool, and this calibration is part of the automated software routine. There is also a second neon source inside the instrument that gives the user the option to periodically run a wavelength calibration without having to remove the sample or sampling accessory.

The calibration of the laser wavelength is usually done using a wavelength standard. In the case of the DXR3 SmartRaman+ spectrometer, a polystyrene standard is incorporated in the alignment and calibration tool. Polystyrene is one of the materials recommended by ASTM to use as a wavelength standard.[5]

The peak positions for polystyrene are well known, so as long as the spectrometer has been calibrated it is then possible to calculate the laser wavelength. Laser wavelengths can shift for a variety of reasons and thus it is necessary to verify the actual laser wavelength to accurately express the Raman scatter as shifted values.[4]

Accurate Raman shifts are important for both qualitative and quantitative measurements. Variation in the peak positions can result in lower match values and incorrect identification when searching against libraries. Figure 5 shows Raman spectra from an antacid pain relief formulation containing sodium hydrogen carbonate, acetylsalicylic acid, and citric acid where the spectra are shifted by +/- 2 cm^{-1} and +/- 4 cm^{-1}. Performing a standard library search on the spectra from the mixture, it was possible to ascertain one of the known components when the spectra were off by 2 cm^{-1}, but when the peaks were off by 4 cm^{-1} the top match was not one of the three known components and there was a drop in match value (standard correlation library match value) for the known component (see Table 1). This means that if the components were not known it would be difficult to identify even one component correctly with the peaks off by 4 cm^{-1}. Similar results are observed with multicomponent search, where the search routine looks for the best combination of components. Just as with the single result search, the multicomponent search fails if the peak positions are off by more than 3 cm^{-1} from the library spectra (see Table 2). In a previous technical note,[6] it was reported that variations in peak positions can have significant effects on the results from a partial least square (PLS) chemometric method. Small shifts of 1 cm^{-1} or smaller had relatively small effects but when peak positions were off by 4 cm^{-1} it resulted in up to a 26.5% difference. This illustrates why x-axis (wavenumber) calibrations are so important for Raman spectroscopy.

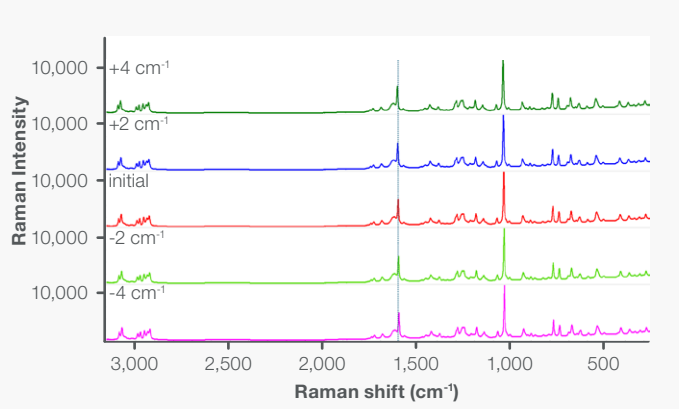

Figure 5. Spectra from the antacid pain relief tablet with shifts of +2 cm^{-1}, -2 cm^{-1}, +4 cm^{-1}, and -4 cm^{-1} in the peak positions.

Intensity correction

Raman is a single beam technique and does not readily provide a way to compensate for instrument response the way absorbance measurements such as FTIR or UV/Vis can. Numerous factors contribute to Raman intensities. These include the laser, the focus on the sample, various optical components such as filters and gratings, and the quantum efficiency of the detector. Each of these elements can cause instrument-dependent variations in the measured Raman intensities. Absolute photometric intensity calibrations are complicated and are rarely undertaken, but a simpler approach sometimes employed is to generate a relative instrument response function to produce spectra with consistent relative peak intensities. The two main approaches to generating these types of instrument response functions have been either by using calibrated white light sources or certified NIST standard reference materials.[7] While effective for generating relative instrument response curves, each of these methods has issues. There are experimental issues with positioning and use of calibrated white light sources, while the NIST standards have limited and variable availability.

The DXR3 SmartRaman+ spectrometer includes a white light source in the alignment and calibration tool. However, this white light source is not intensity-calibrated with a known standard. While the light provides some functions similar to a calibrated instrument response curve, it should not be considered an intensity calibration. A white light spectrum is collected using the source in the tool and along with a calculated blackbody emission curve these are used to generate a normalized instrument response curve. This functionality was never intended as a full intensity calibration but is mainly to compensate for differences in the quantum efficiency of the CCD when using different laser sources. This allows for better comparison of spectra collected using different lasers, and it allows for using common spectral libraries rather than ones dedicated to a specific laser wavelength. This effect is illustrated in Figure 6 where a spectrum of polypropylene is shown with and without a white light correction. The spectrum was collected using a 785 nm laser, and when using this type of laser, all silicon CCD detectors exhibit lower sensitivity (lower quantum efficiencies) at the higher shifted region (longer wavelengths) resulting in the relatively lower peak intensities. When a library search is carried out using a commercial library the match value for the white light corrected spectrum (96.01) is much better than the uncorrected spectrum (70.49). Using the white light correction makes it possible to use commercial spectral libraries to identify unknown materials without having to generate laser wavelength-specific libraries. While this white light correction compensates for some instrument-dependent intensity differences and it may help to compensate for some instrument-to-instrument variations, it is not intended to fully address those issues. Additional considerations will be required to normalize Raman intensities between instruments.

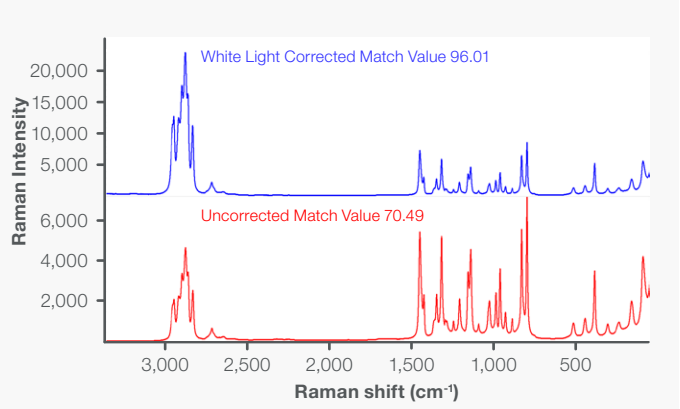

Figure 6. Spectra from the polypropylene sample with and without a white light correction. The match value from searching against a commercial library for the white light corrected spectrum was 96.01 where it was only 70.49 for the uncorrected spectrum.

Summary and conclusions

- Alignment maintains peak instrument performance and consistent results.
- Wavelength calibration uses the emission lines of a neon bulb inside the alignment tool to account for non-linear dispersion and determining absolute wavelengths.
- Laser calibration uses a polystyrene sample inside the alignment tool to accurately determine the laser wavelength which is necessary for defining Raman shifts.
- White light correction uses a white light source to generate an instrument correction curve to account for the relative intensity variations resulting from instrument components and configurations differences.

The alignment and calibration tool for the DXR3 Smart Raman+ spectrometer contains all the components needed to optimize instrument alignment and calibrate the Raman shift scale. All operations are automated and initiated with a simple software button. It requires no additional user expertise or any additional tools or parts. Together, this provides enhanced performance, improving any Raman application.

References

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