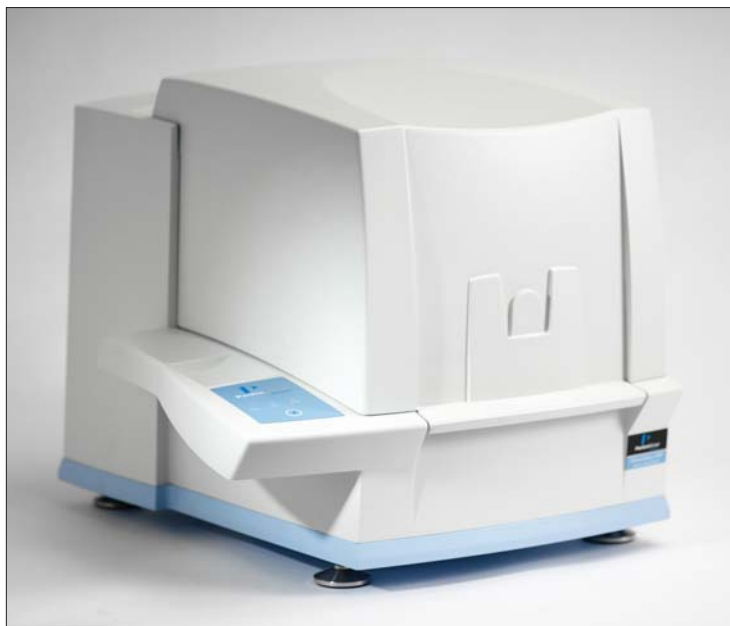


# Parameter Selection and Spectral Optimization Using the RamanStation 400



In modern dispersive Raman spectroscopy, good quality spectra can be obtained from the majority of typical samples. These spectra show sharp, well-defined peaks with, in most cases, a relatively flat, noise-free baseline. In some cases, however, laser-induced fluorescence of the sample may result in the spectrum having a sloping baseline. Other samples, typically those containing dark pigments or carbon black, can show adverse effects resulting from laser-induced sample heating. This is due to absorption of the incident laser energy and the Raman scattered energy from the sample and in extreme cases can lead to burning or deformation of the sample. The sample absorption reduces the Raman intensity and produces a “noisy” spectrum. While this does not prohibit the analysis of these types of samples, steps will need to be taken to prevent the degradation and to increase the signal to noise (S/N) ratio of the sample spectrum.

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Most Raman spectrometers have a wide array of scanning and processing parameters (such as laser power, number of scans, sample focus, baseline correction, noise smoothing, etc.) to give the analyst the maximum degree of flexibility in their analysis. Although it is important that the benefits accruing from these features are fully realized, choosing these parameters can be daunting for the experienced and inexperienced analyst alike. The RamanStation™ 400 has been designed so that these parameters are easily selected and, in many cases, are automatically optimized so that high quality spectra are readily obtained from both straightforward and more demanding samples.

This application note describes the Raman analysis of a non-typical sample which shows the effects of laser-induced decomposition and fluorescence. A simple analysis procedure is described which will give good quality, reproducible spectra from the vast majority of samples found in a typical laboratory.

A possibility in Raman spectroscopy is that the laser will burn the sample and prevent analysis. One parameter that can be varied to prevent the destruction of the sample is to reduce the power of the laser. Figure 1 shows a polymer sample before and after analysis. The sample has been pitted and photo-degraded.

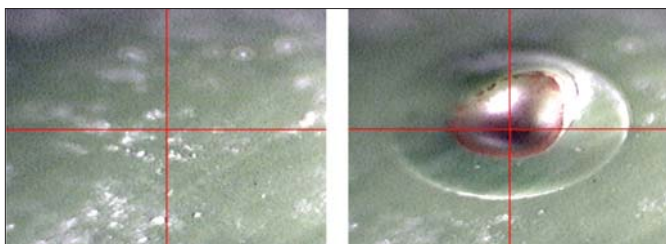


Figure 1. Photo-degradation of sample from the laser.

The RamanStation 400 allows the analyst to control the power of the laser continuously from 5-100% of the maximum laser power. The maximum laser power at the sample is 100 mW. The power was reduced by 50% and the sample re-run. Figure 2 shows that the sample was deformed but not degraded since there is no discoloration of the sample.

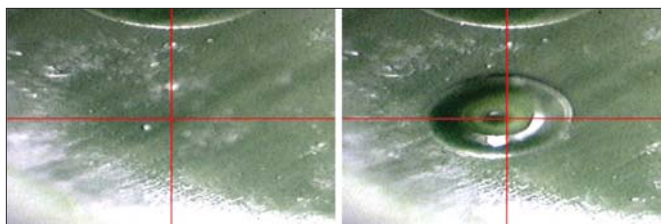


Figure 2. Photo deformation of sample from the laser.

Further reducing the power to 25% of maximum prevents any deformation of the sample (Figure 3).

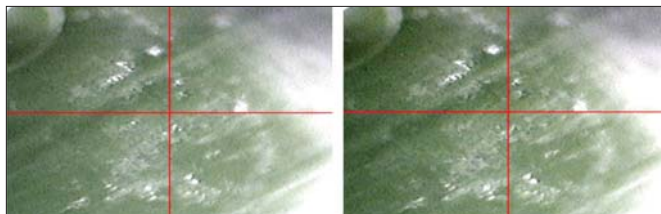


Figure 3. Effect of reducing the laser power on the sample to prevent degradation.

The intensity of a Raman spectrum is proportional to the intensity of the laser. Two ways of improving the S/N ratio are to increase the number of accumulations or to increase the length of the accumulations while keeping the laser power constant. Figure 5 and Table 1 show that doubling the number of scans effectively doubles the intensity and thus improves the S/N ratio. The maximum height is the peak height in intensity units from the zero baseline point to the maximum height. The corrected height is the height calculated from the tangential line drawn between two selected baseline points vertically to the maximum height (Figure 4).

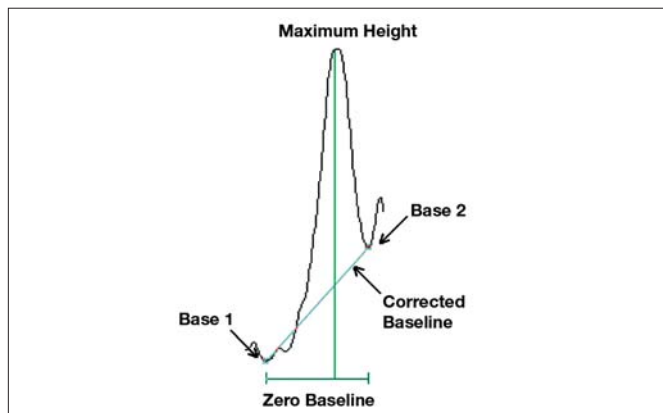


Figure 4. Method of determining maximum and corrected peak height values.

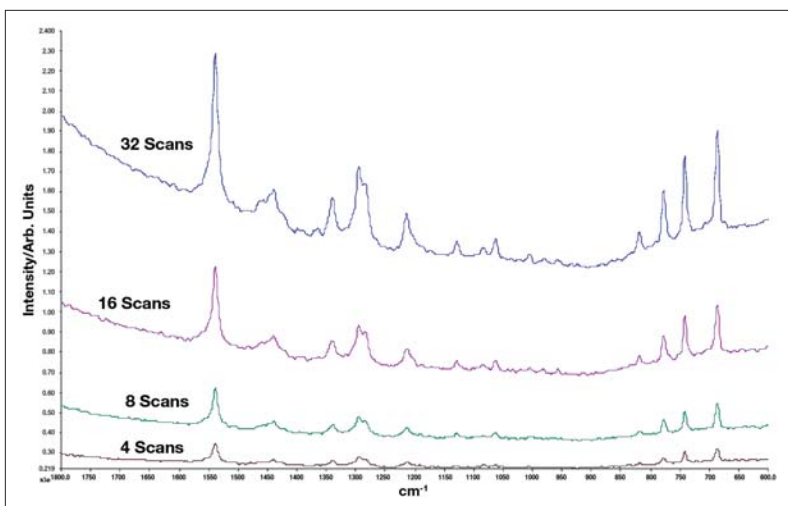


Figure 5. Effect of increasing the number of scans on peak intensity.

Table 1. Peak Maximum Height Intensity as a Function of the Number of Scans.

Sample Name	Location	Max. Height	Corrected Height
32 Scans	1538.4	22917	7447 INT
16 Scans	1538.2	12316	3879 INT
8 Scans	1538.2	6219	1902 INT
4 Scans	1538.2	3470	964 INT

The second way is to increase the length of the accumulations while keeping the laser power constant. Figure 6 and Table 2 show that doubling the accumulation time effectively doubles the intensity and thus improves the S/N ratio.

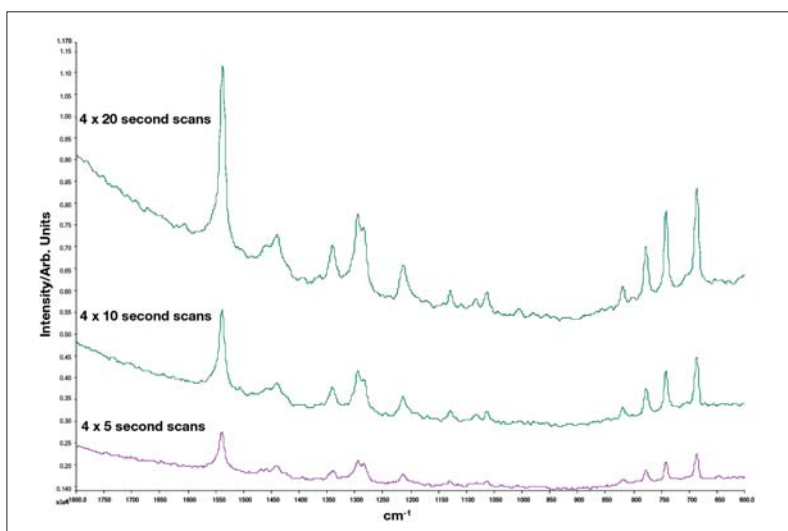


Figure 6. Effect of increasing the accumulation time on peak intensity.

Table 2. Peak Maximum Height Intensity as a Function of Scan Time.

Sample Name	Location	Max. Height	Corrected Height
4x20 second accumulation	1538.2	11044	4034 INT
4x10 second accumulation	1538.2	5555	1819 INT
4x5 second accumulation	1538.3	2760	887 INT

The main determinant on signal intensity is total exposure time. A sample was scanned 100 times for 2 seconds and then 2 times for 100 seconds. Figure 7 and Table 3 show that the sample intensities agreed to within 3%. The spectra have been offset for clarity.

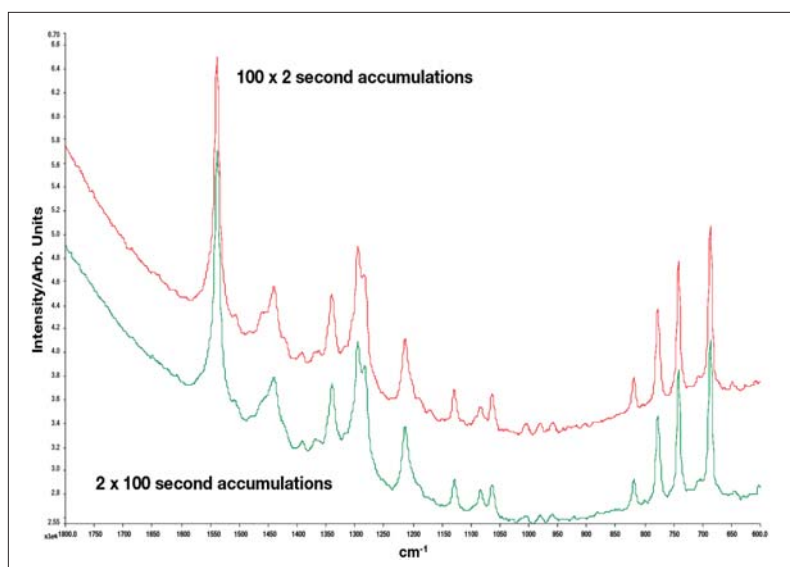


Figure 7. Comparison of increasing the number of scans versus longer scan accumulation times on signal intensity.

Although the signal intensity in this example is essentially the same, whether 100x2 second scans or 2x100 second scans are collected, there are other considerations that need to be taken into account when selecting the optimum sequence of number and duration of scan times.

- 1) Almost all Raman spectrometers have filters to eliminate the effects of cosmic rays reaching the detector. However, there is still the possibility of a rogue cosmic ray reaching the detector and generating a random spike in the spectrum. These spikes can be detected and eliminated from the resultant final spectrum if more than one scan is used in the collection of the data.
- 2) For a strong Raman scattering sample or one that shows a high degree of fluorescence, there is a danger that the CCD will become saturated with photons if the exposure time is too long. This saturation does not damage the detector but is clearly undesirable and should be protected against. For some samples, this detector saturation can occur in less than one second whereas for weak Raman scattering samples, the scan times can be tens of minutes before saturation occurs. Once the detector is read, the detector signal reverts to zero. This means that for strong Raman scattering samples it is advisable to accumulate many short scans whereas for a weak Raman scattering sample, a few longer scans may be the best option.
- 3) Every time the detector is read, spectral noise is generated, the magnitude of which is independent of the

Table 3. Effect of Exposure Time Versus the Number of Scans on Peak Intensity.

Sample Name	Location	Max. Height	Corrected Height
2 Second 100 scans	1538.1	64956	21414 INT
100 Second 2 scans	1538.0	57089	20762 INT

signal size. This read noise is cumulative and where possible, should be kept to a minimum particularly for weakly scattering samples. This is another reason why for such weakly scattering samples it is best to collect a few longer scans rather than many short scans.

Trying to determine the optimum combination of number-of-scans and scan-times for each type of sample can be difficult to do for the inexperienced Raman user. The RamanStation 400 (and the RamanFlex™ and RamanMicro™ range of spectrometers) has two modes of data collection. For those analysts who know the best scanning conditions for a particular sample or who want to compare spectra collected under the same conditions, there is a manual option to specify the number and duration of the scans.

There is also an automatic mode where the analyst simply enters the total accumulation time for the analysis and the system will calculate the best combination of number-of-scans and scan-times to give the optimum spectrum. It does this by opening the laser shutter and monitoring the signal reaching the detector for a short time. If the analyst selects, say, 30 seconds then this might result in 60 x 0.5 second scans or 2 x 15 second scans (or any combination in between) to give the best result. This function takes into account the intensity of the Raman signal, detector saturation, and background fluorescence. There will always be at least two scans collected in order to minimize any unwanted effect caused by cosmic rays.

Finally, the sampling depth for the particular sample should be optimized. Visually aligning the sample optically does not mean that the best Raman signal will be obtained. The RamanStation 400 has an in-built Raman focus feature that will optimize the sample depth of analysis to give the strongest Raman signal. Figure 8 compares spectra from the same sample where visual and Raman focusing has been used. In one case, the surface of the sample was brought into visual focus prior to collecting the spectrum while in the second case, the automatic Raman focus was used.

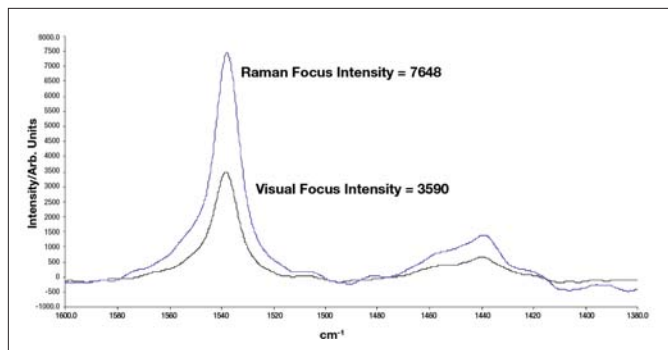


Figure 8. Effect of Raman focusing on peak intensity.

In all the previous spectra shown in this application note, there is a sloping baseline due to some sample fluorescence. In some samples, this is a major problem which must be overcome prior to spectral interpretation; in other cases, it is merely a cosmetic effect. When required or to further enhance the appearance of the spectrum, a baseline correction algorithm can be applied to the spectrum to remove or minimize this baseline effect (Figure 9). This correction can be made automatically as the sample is being scanned or applied after the spectrum has been collected.

A final point of consideration is calibration verification. In order to compare spectra, the instrument needs to be calibrated and subsequently validated for wavelength accuracy and peak intensities. The RamanStation 400 has validation procedures in the software to accomplish this task. This is extremely important if spectra from different instruments are going to be compared or if reference libraries (used in spectral compare or spectral search) are being generated. Search algorithms use peak position, peak intensity and peak height ratios to identify compounds.

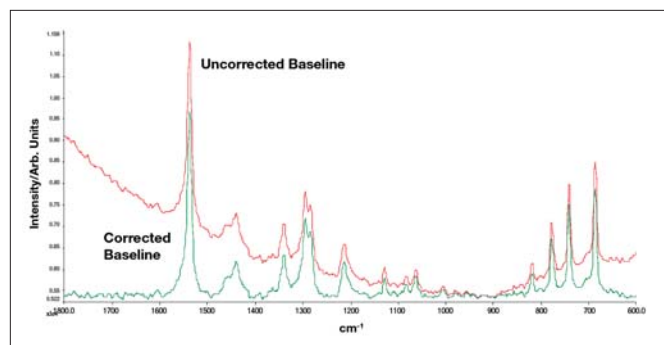


Figure 9. Effect of automatic baseline correction.

Wavelength calibration can be verified using a polystyrene standard or other appropriate standard. Intensity calibration is somewhat more complex. In order to intensity-correct an instrument so that the peak heights are consistent between runs (intra-instrument variation) and subsequently when comparing data from different instruments (inter-instrument variation), calibration must be done at all monochromator positions. This is virtually impossible to do on conventional instruments that use a moving grating. A unique feature of the RamanStation 400 is that it uses an Echelle grating that has no moving parts. Therefore it can be calibrated by using NIST 2241 intensity calibration standard.

There are many scanning and software parameters which can affect the final quality of a Raman spectrum. These range from control over the laser power, through scanning sequences to software algorithms such as baseline correction. Optimizing these to give the best quality spectrum can be time consuming for the experienced spectroscopist, and daunting for the inexperienced spectroscopist.

A good guideline for acquiring a high quality spectrum can be listed as follows:

- 1) Select in the software the number of background scans to be equal to the number of sample scans.
- 2) Use the visual and Raman monitoring functions to optimize the Raman signal.
- 3) Enter a suitable analysis time (20 seconds) into the automatic scan option and allow the instrument to calculate the best combination of scan duration and number of scans. A minimum of two scans will be selected.

- 4) After scanning, use the visible monitor option to check that there has been no sample degradation or burning of the sample. If any such degradation has occurred, the laser power should be reduced, a fresh area of sample selected and Steps 1-4 repeated.
- 5) Optional: if the sample is precious, delicate or irreplaceable then it is often advisable to start with a low laser power and gradually increase it to obtain the maximum signal while ensuring no damage to the sample. This routine is strongly advised when using a Raman microscope where the laser power density can be very high and focused on a small area of the sample.
- 6) If a better S/N ratio for the spectrum is required then the number of scans should be increased while maintaining the scan duration as calculated in 2).
- 7) At this stage, any post-run data processing such as baseline correction should be applied to the spectrum. The Spectrum™ software is designed so that the original spectrum, any intermediate spectra and the final spectrum are saved independently and a full audit trail of these manipulations is recorded.

The RamanStation 400 has a range of features designed to make the above decision making easier or even automatic. The continuous control of the laser power allied with the real-time visual display of the sample means that it is straightforward to select the optimum laser power while maintaining sample integrity. The ability of the system to automatically select the best sequence of number of scans and scan times for any particular analysis combined with automatic Raman focusing ensures that even the inexperienced user selects the optimum scanning conditions. Further enhancements to the final quality of the spectrum can be achieved using automated software processes such as baseline correction.

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