

# Automatic Spectral Subtraction

## Automatic Spectral Subtraction

It is common to acquire Raman spectra of your species of interest and inadvertently have spectral features from the solvent/host/substrate contaminating the spectrum. This happens when working with solvated species (reaction monitoring), or when acquiring spectra from samples in a host material (tablet analysis) or samples placed on a substrate, (for example a multiwell plate).

Unlike with some other spectroscopic techniques, performing Raman spectral subtraction is not simply a 1:1 subtraction of two spectra.

Spectral subtraction must involve scaling the intensity of the spectrum that is to be removed (the subtrahend).

Result file = Sample file – (Subtrahend file x Subtraction factor).

Manual subtraction involves dynamically varying the subtraction factor, which increases/decreases the intensity of the subtrahend spectrum; this scaled subtrahend spectrum can be subtracted from the sample file in real time. By observing the result of the subtraction, the user can attempt to determine the optimum subtraction level. This technique is very subjective and requires significant user interaction.

When performing High Throughput Raman or acquiring Raman Chemical Images, it is easy to end up with tens of thousands of Raman spectra. Performing manually baseline-correcting these spectra is simply not an option. What is required is an automated baseline correction algorithm.

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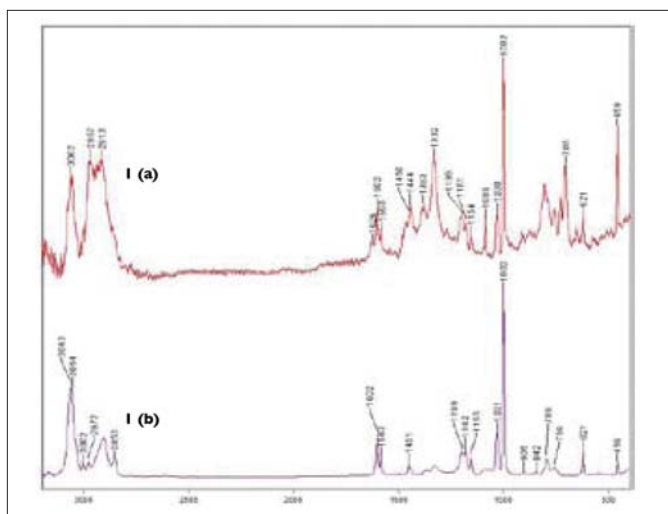


Figure 1. (a) shows Raman spectra acquired from a thin film (SERS) surface (b) shows a spectrum of polystyrene.

Figure 1(a) shows Raman spectra acquired from a thin film (SERS) surface, in a multiwell plate. In this example, all acquired spectra contained the spectrum of the analyte of interest, but were contaminated by a Raman spectrum from the multiwell plate material, in this case polystyrene. Figure 1(b) shows a Raman spectrum of polystyrene.

The PerkinElmer® Insight software offers automatic spectral subtraction using a technique known as the “Dwiggles” technique, initially described by Li and Banerjee in 1991<sup>(1,2)</sup>.

The Dwiggles technique does exactly the same thing as the manual subtraction technique, but is fully automated and non-subjective. It involves measuring the “complexity” of the sample spectrum, then subtracting the subtrahend from the sample spectrum using a subtraction factor of 1.

- If the resulting spectrum is more “complex” than the original, then the factor is too high, and the subtrahend was over-subtracted.
- If the resulting spectrum is less complex, then the resulting spectrum incorporates less of the subtrahend than the original spectrum.

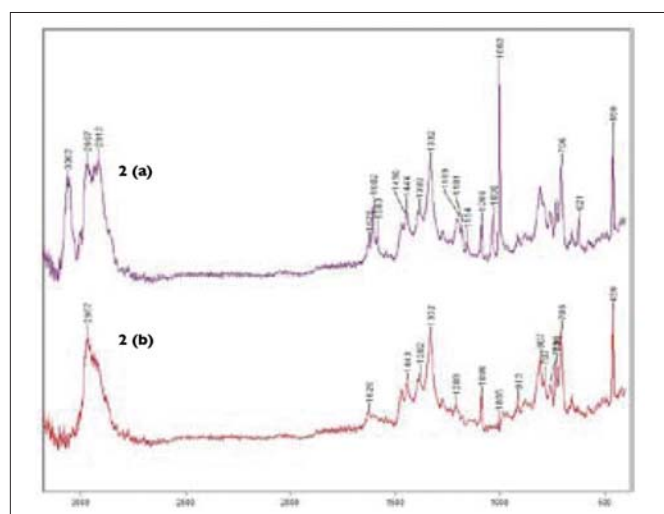


Figure 2. (a) shows Raman spectra acquired from a thin film (SERS) surface (b) is a result of spectrum 2(a) following subtraction of 1(b).

Using this information, the scaling factor can be increased or decreased; repeating this process many times can result in a “complexity minimum”. The complexity minimum is the spectrum that has the least complexity. This is achieved when the optimum amount of subtrahend has been removed.

This technique typically provides subtraction equivalent to or better than can be achieved manually; it is also highly reproducible and fully automated.

This auto spectral subtraction technique is incorporated into the Insight software, and can be applied in real time while chemical images or high throughput data are being collected or while a reaction is being monitored.

## References

1. K. Li and S. Banerjee, Appl. Spec. 45, 1047 (1991).
2. M.A. Friese and S. Banerjee, Appl. Spec. 46, 246 (1992).