

Product Note T29 12/15

## **BRAVO** **Efficient mitigation of** **fluorescence in Raman** **spectroscopy using SSE™**

For many years, Raman spectroscopy has been utilized as an important tool for materials characterization in the analytical laboratory. With new developments in laser technology and spectrometer design, handheld Raman spectrometers can now be designed and built for practical everyday use in a vast array of applications [1]. The BRAVO by Bruker takes handheld Raman spectroscopy to a new level, overcoming the limitations of previously available systems. These previous limitations included i.e., limited wavelength accuracy [2], non-safe laser usage, and fluorescence interference. The wavelength accuracy of the BRAVO is significantly better than competitive devices resulting in the highest data consistency. The BRAVO is also a Class 1M laser safe device. Lastly, the BRAVO incorporates new technology called Sequentially Shifted Excitation (SSE™) to mitigate fluorescence.

The general task of a handheld instrument is obvious – it is a spectrometer which can be employed anywhere with the highest degree of flexibility, where the sample in question does not need to be transferred to a laboratory for reliable and rapid identification. A requirement that goes hand-in-hand with the automation of measurements and data analysis in handheld devices is to achieve a maximum robustness with respect to factors affecting the reliability of results. This starts with minimizing the risk for operating errors with intelligent software that provides an intuitive guided workflow for acquiring and processing the data.



One of the most challenging aspects of Raman analysis is overcoming fluorescence interference. Fluorescence illumination is a highly efficient process that frequently yields a strong signal that can overwhelm the desired Raman spectrum. Additionally, a fluorescence background can limit reproducibility, which is required for conducting analyses in the materials identification and quantification process. The patented Sequentially Shifted Excitation (SSE™) method used by BRAVO detects and removes the fluorescence signal leaving only the desired Raman spectrum [3,4].

### **How does SSE™ work?**

In order to extract the Raman signal from an acquired spectrum, the BRAVO software must recognize and differentiate the Raman spectrum from other interfering signals (fluorescence) automatically. To make this possible the wavelength of the lasers used for excitation are sequentially shifted during the measurement. As a consequence, the position of the Raman signal on the detector is deliberately changed and the signals at constant wavelength, such as fluorescence, remain at the same position. The SSE™ algorithm extracts the “moving” signals which relate directly to the Raman signature (see Fig. 1) yielding the desired spectrum.

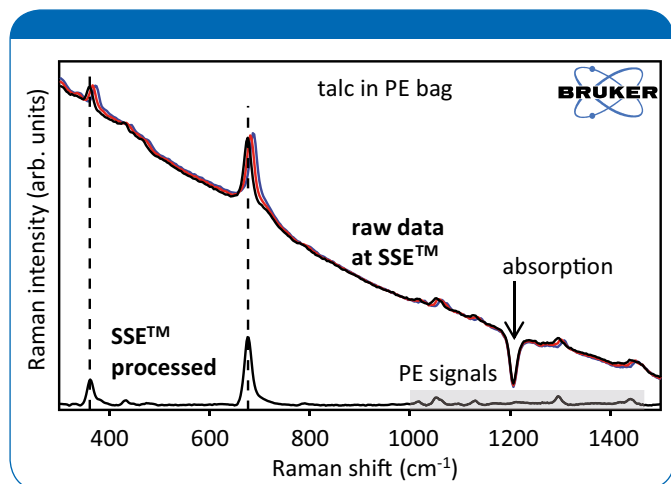


Figure 1: Raman spectra of talc in a PE bag at sequentially shifted excitation (raw data at SSE™) and after fluorescence mitigation (SSE™ processed). Raman lines of talc indicated by dashed lines are moving as the laser wavelength is changed. The SSE™ processed spectrum only contains the “moving” signals extracted from the three spectra.

### What are the benefits of SSE™?

One of the main advantages of SSE™ is that after the data is processed, the resulting spectrum is free of fluorescence and is most suitable for unambiguous identification. Without SSE™, a data pretreatment such as baseline correction would be necessary in order to attempt to remove the interfering fluorescence signal. The possible information loss because of a data pretreatment could yield invalid results [5].

The SSE™ method is most sensitive in retaining the intrinsic bandshape and positions of bands related to the material of interest. This allows not only the quick and accurate identification of unknown compounds, but also the characterization of contaminants and other trace compounds. Figure 2 shows an example where sodium alginate acquired with 785 nm excitation has an intense fluorescence background dominating the spectrum of interest (red spectrum). After application of the SSE™ technique with the BRAVO (blue spectrum), the resulting spectrum can be readily analyzed and the compound reliably identified.

Some handheld Raman devices employ 1064 nm lasers, because fluorescence is generally less common with NIR excitation. However, the Raman scattering efficiency decreases significantly as longer wavelength lasers are used. The resulting lower signal needs to be

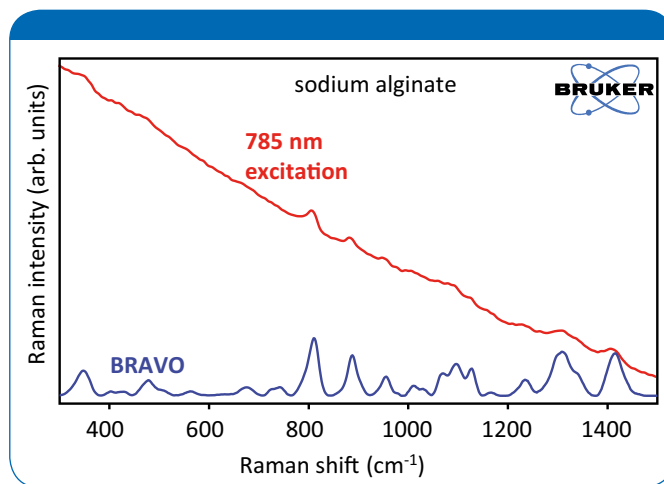


Figure 2: Comparison of Raman spectra of sodium alginate acquired using a conventional dispersive Raman instrument at 785 nm excitation (red) and BRAVO (blue).

compensated for with increased measuring time and/or higher laser power. Additionally, many samples undergo transient heating with 1064 nm excitation. SSE™ handles fluorescence while maintaining high sensitivity and under the safest conditions: The BRAVO is a class 1M system in all modes of operation.

Consequently, whenever fluorescence or background signals are present, SSE™ offers significant benefits for unambiguous material identification of the broadest range of materials.

### References

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- [3] J. B. Cooper et al., “Sequentially Shifted Excitation Raman Spectroscopy: Novel Algorithm and Instrumentation for Fluorescence-Free Raman Spectroscopy in Spectral Space”, *Appl. Spectrosc.* 67, 973 (2013)
- [4] J. B. Cooper et al., “Spatially compressed dual-wavelength excitation Raman spectrometer”, *Appl. Opt.* 53, 3333 (2014)
- [5] J. Kauffmann et al., “Spectral Preprocessing for Raman Library Searching”, *Amer. Pharm. Rev.* 14, (2011)