TECHNICAL NOTE

RAMAN SPECTROSCOPY

Photo-Bleaching and Automatic Baseline Correction for Raman Spectroscopy

Introduction

The advent of the 785 nm laser has revolutionized Raman spectroscopy. Raman scattering is a relatively weak phenomenon and for many samples it competes with, and can be masked by, more intense fluorescence effects. In general, fluorescence is strongest when the sample is irradiated with typical green or red visible lasers, and is weakest (or entirely absent) when near-IR lasers are used. The 785 nm laser offers the analyst the advantages of efficient Raman scattering coupled with significantly reduced or removed fluorescence contamination in their spectra.

Even while using the 785 nm laser, there may still be some residual fluorescence contamination in the Raman spectrum of some samples. One 'cure' for removing this contamination is photo-bleaching.

Photo-bleaching

Photo-bleaching involves irradiating the sample of interest with intense light for a period of time. The light source for the photo-bleaching is simply the Raman spectrometer's laser. This irradiation often induces photolytic decomposition, breaking down the fluorescent molecules and reducing the fluorescent background.

Authors
Andrew Dennis
PerkinElmer Life and Analytical Sciences
1 Chlorine Gardens
Belfast, N. Ireland
BT9 5DJ

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precisely.

It should be noted, in many cases the molecule causing the fluorescence is an “impurity” in the main sample. Photo-bleaching “modifies” the sample by effectively removing the low level contaminant and leaving the species of interest unchanged. This contaminant is seldom, if ever, of interest. Figures 1 and 2 show the spectrum of an ink sample both before and after photo-bleaching.

A photo-bleaching option has been built into the PerkinElmer® Spectrum™ software and can be applied using any PerkinElmer Raman instrument at the click of a button.

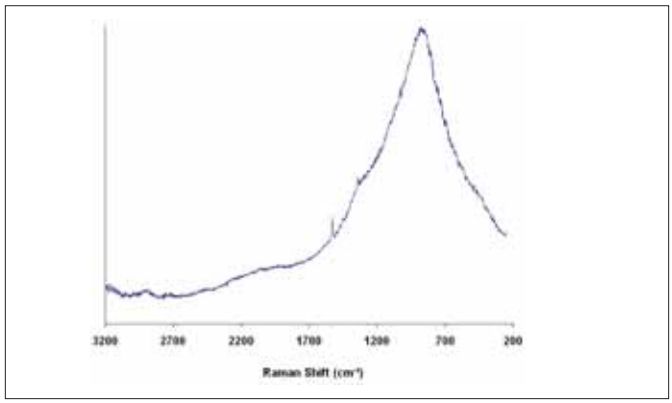

Figure 1. Highly fluorescent ‘Raman’ spectrum from an ink sample.

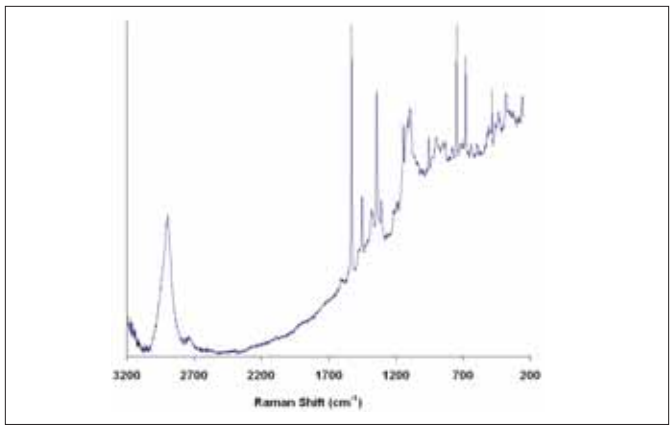

Figure 2. Two minutes of photo-bleaching radically reduces the intensity of the fluorescent background.

Automatic baseline correction

It is typical to have some residual fluorescence, sometimes referred to as cosmetic fluorescence. Cosmetic fluorescence does not necessarily mask spectral details, but the sloping baseline can make it more difficult to interpret the spectrum.

Automatic baseline correction, also included in the Spectrum software, can be used to remove this cosmetic fluorescence, resulting in a totally fluorescence-free spectrum (shown in Figure 3). The difference in spectral detail between the initial and final spectra can be considerable.

Conclusion

Photo-bleaching and automatic baseline correction are simple yet very powerful tools for Raman acquisition and interpretation. Both options are available in the Spectrum software.

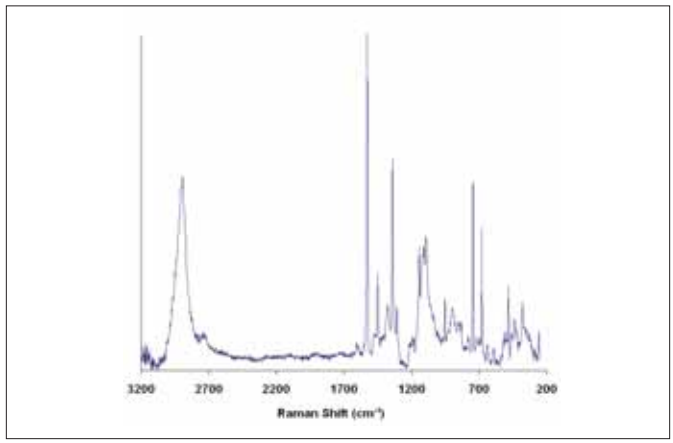

Figure 3. Automatic baseline correction removes residual fluorescent baseline, resulting in fluorescent-free Raman spectrum.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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