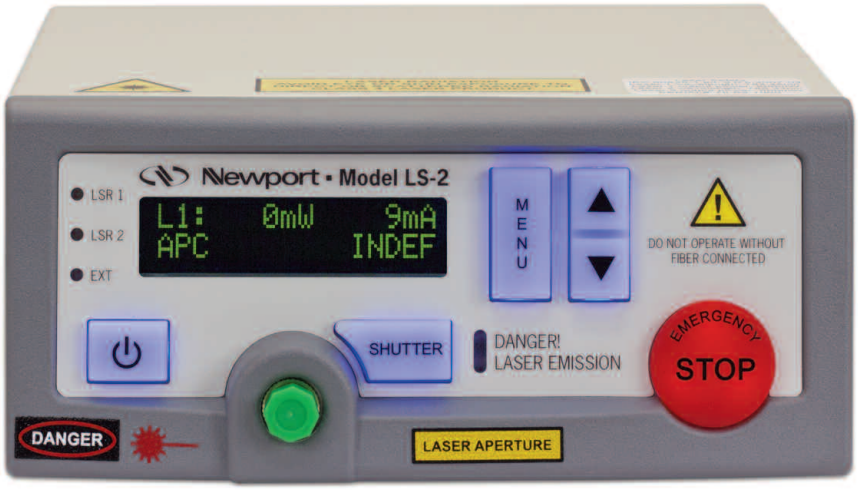
Application Note 52

Background Free Raman Spectroscopy using SERDS Technique with LS-2 Dual Wavelength Laser Source

Newport Corporation



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Introduction

Raman spectroscopy has been experiencing a period of growing interest for qualitative and quantitative analysis in a vast scope of applications pertinent to various industries, including pharmaceuticals [1-5], petrochemical [6-8] and law enforcement [9-13]. This growth is enabled by increase in the offering of affordable Raman instrumentation, some are portable and ruggedized for field use. Such increase in availability and portability is powered in turn by compact, high-performance wavelength-stabilized laser diodes. However, with widening of the scope of applications for Raman spectroscopy the challenge of sample fluorescence becomes more and more evident. Besides the natural fluorescence that many substances possess, there are issues of sample contamination with fluorescent compounds in the field that one must deal with in real life situations [9-10]. One example of that is fluorescent agents such as caffeine and flour regularly used in cutting illicit street drugs [10].

Although the long-wavelength, typically 1064 nm, Raman instruments have been considered the benchmark in dealing with fluorescent substances, working with that excitation wavelength presents a number of issues. First of all, as is well known, the Raman signal diminishes as the 4th power of laser wavelength, which means that it is ~ 3.4 times weaker with 1064 nm excitation as compared with commonly used 785 nm excitation. Generally it means that significantly longer collection times are necessary to collect as much Raman signal when using 1064 nm laser. Furthermore, the use of 1064 nm excitation laser requires using InGaAs detectors instead of silicon CCDs. In case of dispersive Raman systems these are InGaAs arrays, which typically have much higher thermal noise than that of a silicon CCD at the same temperature. As a result, InGaAs detector arrays for dispersive Raman instruments must always be cooled to lowest practical temperature to achieve satisfactory noise levels. Although cooling the detector is not a technical obstacle for laboratory instruments, it presents an obvious issue for portable, battery-operated Raman instrumentation, as it means more power consumption, larger battery and shorter battery life, in addition to increase in cost and complexity of the instrument.

For that reason a lot of attention has been paid over the years to other methods of combating fluorescence in Raman measurements, which can be separated into two classes: 1) the digital filtering methods; and 2) the physical methods of subtracting fluorescence contribution from the Raman signal, of which the Shifted Excitation Raman Difference Spectroscopy, or SERDS, is the prime example.

Shifted Excitation Raman Difference Spectroscopy

The underlying principle of the SERDS is the fact that Stokes and Anti-stokes components of the Raman scattering are frequency-shifted relative to the excitation wavelength and, therefore, are tied to it. Laser-induced fluorescence, on the other hand, is independent from the excitation wavelength and retains practically identical spectral shape when the excitation wavelength experiences a relatively small change (< 10 nm, for example). For that reason if two spectra are collected with a small shift in the excitation wavelength and subsequently subtracted, the resultant spectrum will be free from the fluorescence contribution, while retaining the Raman signal, albeit in a form of a difference spectrum (Fig. 1).

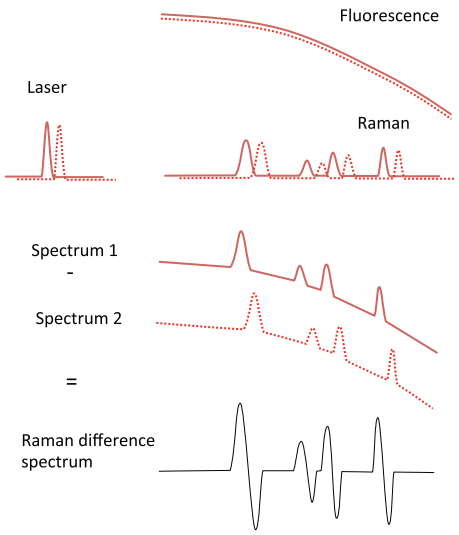

Figure 1. Illustration of the principle of SERDS.

For this ability of accurate subtraction of the fluorescence background the SERDS approach has long been considered attractive, effectively expanding the use of the conventional dispersive Raman instrumentation equipped with inexpensive and efficient CCD detectors to the classes of samples that exhibit strong fluorescence.

SERDS method was proposed and tested over time in various application areas generally with good success with respect to eliminating the fluorescence background and extracting the Raman signal from it. In majority of the studies conducted on SERDS large emphasis was put on the reconstruction of the Raman spectrum of the substance under study from the pseudo-derivative one obtained as a product of SERDS process. As a result, the often quoted drawbacks of SERDS method are in fact the deficiencies of the various reconstruction algorithms. At the same time it is a well-understood fact that reconstruction is not required for any of the numerical algorithms involved in substance identification, detection or quantitative measurements by means of Raman spectroscopy. When the need of spectrum reconstruction is removed, SERDS approach will deliver clearly superior performance to any of the numerical processing algorithms designed to eliminate the slowly varying fluorescence background.

Historically the SERDS method has been used in laboratories employing tunable-wavelength lasers. However, the use of this type of laser sources presents a significant challenge for portable Raman systems. Generally these lasers are much more costly than simple and efficient wavelength-stabilized laser diodes. But in addition to this there is an issue of the exact wavelength control over these lasers required to perform accurate qualitative and especially quantitative Raman analysis, as it is not fixed or stable, which requires constant active wavelength monitoring for accurate analysis. For these reasons we offer a comparatively much more simple and practical approach to performing SERDS analysis using two affordable wavelength-stabilized laser diodes operating at slightly offset wavelengths. The lasers are stabilized by use of the Volume Bragg Grating (VBG) technology and are very compact, efficient and inexpensive, making them well-suited for portable battery-operated Raman instrumentation.

Advantages of SERDS

The most important advantage of the SERDS method over the conventional methods of digital removal of the fluorescence background is accurate elimination of the fluorescence contribution, as opposed to approximation of such using a polynomial function, as is the case in baseline fitting, for example. This advantage is particularly obvious when the background fluorescence has some spectral features, but even in the situations when the fluorescence has a slowly varying smooth shape the advantages of SERDS are very apparent.

Using two fixed wavelength VBG-stabilized laser sources is a particularly simple and efficient method of implementation of SERDS. Such sources are now available in high brightness and high power at a variety of wavelengths, and are very reliable and stable in wavelength over the lifetime of a device. These lasers do not require constant wavelength monitoring as their wavelength is known and stable.

Availability of such sources in a wide range of spectrum, from the 400 nm to the near IR, is another convenient feature, which allows the end user the freedom of choice of the excitation wavelength. Since these laser sources are compact, they are very compatible with field-portable Raman instruments, which presents an important advantage, as the signal collection in the field is often not free from background light and the samples are often not pure, thus increasing the likelihood of encountering interference from the fluorescence of the contaminants.

Being able to analyze fluorescent samples while using the preferred, i.e. shorter, excitation wavelength has the advantage of being able to use silicon CCD detectors, which have high sensitivity, lower thermal noise and low price tag associated with them.

Furthermore, SERDS can be implemented without any changes to the Raman probe and spectrometer hardware, making it simple and versatile for equipment manufacturers. When equipped with silicon CCD, even a portable Raman instrument will have higher resolution and faster signal processing times then even laboratory-size long-wavelength Raman instruments. Because the Raman signal is so much stronger at shorter wavelengths the signal collection times are often many times faster for 785 nm systems, for example, as compared with the 1064 nm system at similar laser illumination intensities.

As the signals for the two excitation wavelengths are collected in rapid succession, the SERDS method does not require collecting the background signal independently, as it is automatically corrected for it during the subtraction of the two collected spectra.

And finally, for majority of the practical applications the differential shape of the SERDS spectra is very convenient, as the spectra are naturally centered (i.e. have zero integral) and are nearly orthogonal for dissimilar substances. This feature makes them very fitting for all automatic signal processing algorithms, be it quantitative Raman, impurity detection or unknown substance recognition.

Experimental

The SERDS measurements are performed using a dual-laser SERDS laser source, LS-2, produced by Newport. The lasers operate at two closely spaced wavelengths, which in the case of the measurements shown here are 784.5 nm and 785.5 nm. The wavelength separation is selected to correspond to the approximate line width of the Raman lines of the substances under study. Note that the exact wavelength separation is not significant for the accuracy and practicality of SERDS, however, wavelength separation that is much smaller than the width of the Raman bands would result in increase in noise in SERDS spectra.

The LS-2 laser source delivers the output of either one of the two lasers to the output port via a fiber-optic switch. A fiber-optic cable is then attached to the output port of the LS-2 and coupled into a Raman probe or a bench-top Raman system (Fig. 2). The system is designed to accept a fiber-coupled input from a laser source, which is then collimated by an aspheric lens. After the lens there is a laser clean-up filter (a 3 nm bandpass filter) which serves to cut down the amplified spontaneous emission from the laser source as well as the Raman signal from the delivery fiber. Since the system is using larger aperture components and longer distances, lens relays are employed in order to properly guide the laser and, most importantly, signal light throughout the system. The first lens relay is comprised of lenses RL1 and RL2, which project the front focal plane of the laser collimating lens onto the front focal plane of the sample focusing/collection lens. The laser light is folded by a fold mirror and the first notch filter and then one more time by a pentaprism installed in a rotating

swivel mount. This is done in order to have the flexibility of pointing the laser down, up or horizontally, depending on the type of a sample being studied, without affecting the alignment of the system and, therefore, its collection efficiency.

The Raman signal is collected by the focusing/collection lens in the back-scattering geometry and then relayed by the lens pair RL2 and RL3 onto the front focal plane of the focusing lens that couples the Raman signal into the collection fiber. The signal passes through two notch filters that effectively eliminate the Rayleigh scattering from the sample. In order to have an ability to view the sample and the tested area, we have introduced a viewing camera, which creates an image of the sample using a small portion of collected light reflected by the beamsplitter. The image of the sample is created by the camera focusing lens that is positioned after the relay lens RL4. The system also employs a reticle that allows centering the laser light on the sample (not shown in Fig. 2).

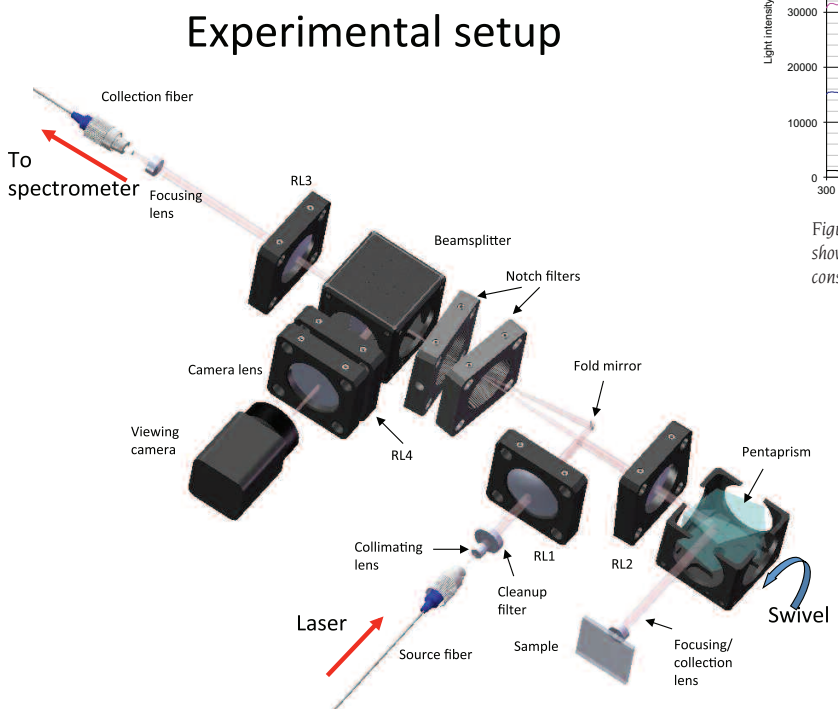

Figure 2. Schematic of the bench-top Raman system on which the measurements were taken. The lenses RL1 - RL4 are optical relay lenses projecting the front focal plane of the laser collimating lens onto that of the sample focusing/collection lens (RL1 and RL2 pair), and then to the front focal plane of the collection fiber focusing lens (RL2 and RL3 pair), as well as the observation camera arm (RL2 and RL4 pair). The pentaprism mounted in a swivel mount allows to point the focusing head of the system in any direction -- from facing down, to horizontally, to straight up, which is convenient when dealing with different type of samples (liquids in vials viewed from below, solid samples viewed from above, and liquids in flat wall vials as well as large objects, that are convenient to test with the head pointed horizontally.

The collection fiber delivers the signal to a bench-top system F/3 spectrometer with 30 cm focal length and 600 groves per millimeter diffraction grating. The spectrometer has adjustable slit and variable resolution. The detector was a cooled CCD with 1024x256 pixel array from Princeton Instruments. For SERDS analysis the spectra are collected sequentially with laser #1 and then with laser #2. In LS-2 laser source both lasers run continuously during the course of the experiment to assure best stability of the output power, whereas fiber-optic switch is used to cut off the light to the sample. The wavelengths of the lasers are stable to < 5 pm over the course of the day.

Measurements

In order to compare the performance of SERDS with conventional methods, such as baseline fitting, in a situation where high amount of fluorescence obscures the Raman signal, it is convenient to use a simple substance with strong and variable fluorescent background. For that purpose we have analyzed mixtures of dark rum, whose Raman spectrum

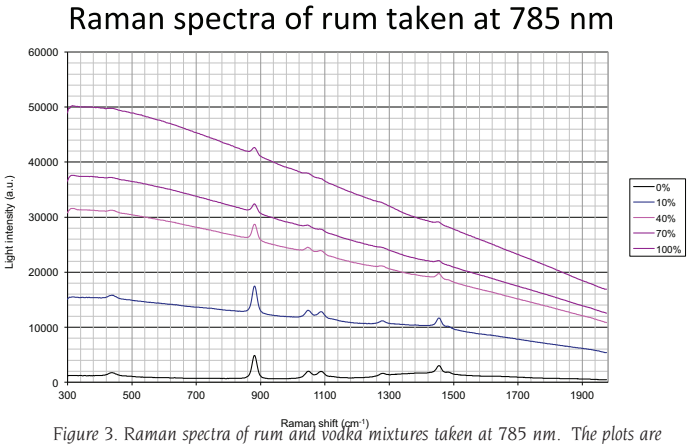

Figure 3. Raman spectra of rum and vodka mixtures taken at 785 nm. The plots are shown for different concentration of rum relative to vodka. The exposure time remained constant for all different concentrations.

is essentially that of pure ethanol, with vodka, which has about the same concentration of the analyte, i.e. ethanol in this case. Figure 3 shows raw Raman spectra of the dark rum collected with 785 nm. The spectra are shown before any baseline correction has been performed. As can be observed, the amount of fluorescence in the dark rum obscures the Raman signal nearly completely, when the 785 nm excitation is used.

In addition to the comparison between SERDS and numerical filtering methods mentioned above, we have performed long-wavelength Raman analysis with 1064 nm laser excitation as a benchmark comparison. Long-wavelength Raman has long been considered the preferred method for studying highly fluorescent compounds, so it was rather interesting to evaluate it side by side with SERDS performed at 785 nm. We have used a commercial 1064 nm laboratory dispersive Raman system for these experiments. The data were collected on the same samples, which were then processed by the same methodology, including baseline removal, to compare the results.

Figure 4 shows the spectra collected on the 1064 nm Raman

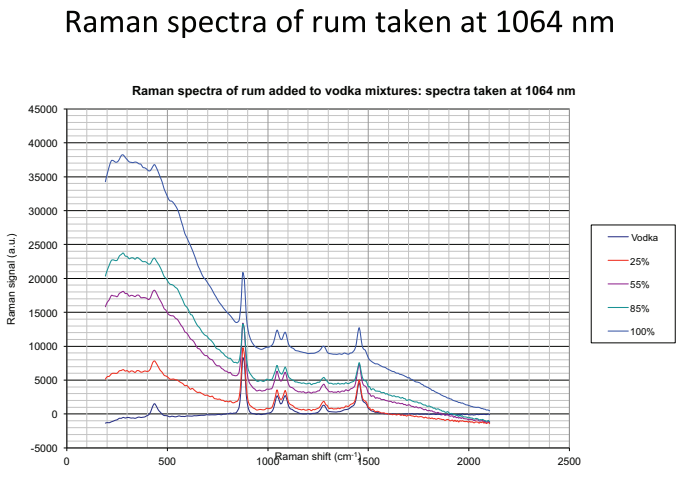

Figure 4. Raman spectra of the rum/vodka mixtures taken at 1064 nm. Spectra are shown prior to any corrections.

instrument. As expected, the long wavelength excitation produces much smaller amount of fluorescence, although fluorescence is still prominent even in that wavelength region, which makes the baseline removal a must even in this case in order to be able to perform substance recognition.

To keep the measurement conditions similar, all the experiments were performed at the same power on the sample (450 mW) for both the 785 nm and the 1064 nm systems. For better comparison the spectral resolution of the Spex spectrometer was also adjusted by opening of the slit to such width as to match the resolution of the 1064 nm Agility system (approximately 18 cm-1). The exposure time was always selected to keep the maximum of the signal just below the saturation level of the detector array. Therefore, when large amount of fluorescence was collected the exposure time was shortened accordingly.

When performing analysis of highly fluorescent samples, signal averaging should be employed to increase of the signal-to-noise ratio. Note that due to the shortening of the exposure time when fluorescence is present there can be a proportionately larger number of averages performed within the same total measurement time. Improvement in SNR with averaging is evidenced in the results shown in Fig. 5, where spectra of the dark rum collected with 785 nm excitation are shown for different number of averages and compared with library spectra of pure ethanol. The spectra are vertically offset for clarity. As is clearly obvious from the figure, the amount of noise is decreased significantly with averaging and becomes nearly negligible at 100 averages.

Note that the total acquisition time for 100 averages with 785 nm system was still under the time required for a single exposure of the 1064 nm system. This is due to smaller Raman scattering cross-section at longer wavelength, as well as to the difference between Si and InGaAs detectors, that the measurement times for the 1064 nm system are typically longer than those for the 785 nm systems (approximately by factor of 5 for the systems employed in this study). Therefore, an interesting comparison between the 785 nm and the1064 nm excitation wavelength would be the coefficient of determination, commonly referred to as R2, achieved within the comparable total collection times. It is calculated according to the following formula:

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(y_i - f_i)^2}{\sum_{i=1}^{N}(y_i - \bar{y})^2}, \qquad (1)$$

where y_i are the measured spectral intensities at pixel (or the spectral channel) number i, N is the total number of pixels in the detector (or the number of the spectral channels), fi is the spectral intensity of the library spectrum at the same point in spectrum, and $\bar{y}$ is the average of the measured spectrum.

The R^2 coefficient allows to evaluate the relative quality of the Raman analysis under the conditions of the experiment. It provides a measure of the magnitude of the residual between the measured spectrum and the library spectrum of a

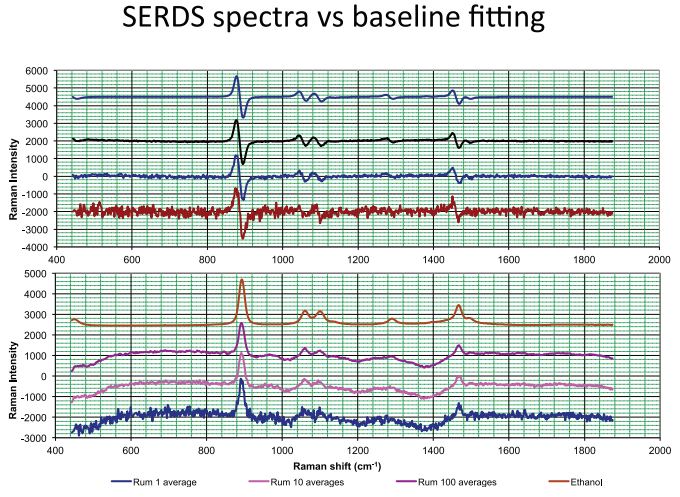

Figure 5. SERDS spectra of rum (top) and spectra of rum obtained by the conventional method of baseline fitting (bottom) compared with that of pure ethanol. The spectra were obtained with different number of averages and have been normalized and offset for clarity. Different order polynomial functions were tested for the baseline removal and the best results are shown.

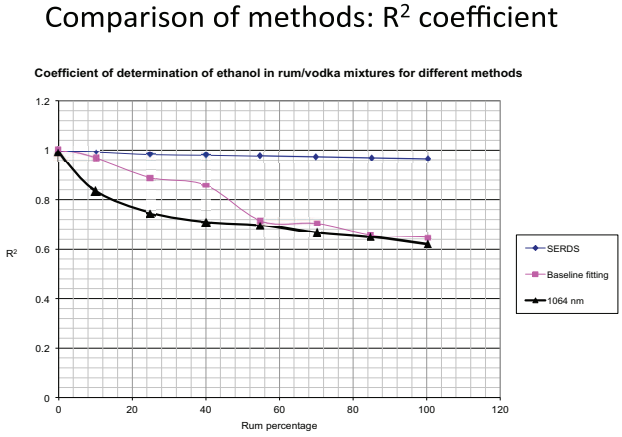

Figure 6. Comparison of R2 for identification of ethanol in rum/vodka mixtures obtained by SERDS at 785 nm, conventional Raman with baseline subtraction method at 785 nm, and 1064 nm Raman with baseline subtraction. In the case of 785 nm measurements the maximum exposure time was selected to bring the signal close to the CCD saturation. Different number of averages was taken with the rule that the total measurement time remained the same as for pure vodka while the fluorescence increased and the exposure time decreased.

compound. Note that the R^2 is sensitive to any component of the residual -- be it the shot noise or other spectral features.

Figure 6 compares the R^2 values for the ethanol in the dark rum mixtures obtained via SERDS, baseline fitting, and long-wavelength Raman with baseline fitting. The R^2 values are shown as a function of relative concentration of rum in the mixture with vodka. As is clearly obvious from the figure, the R^2 deteriorates quickly for the baseline fitting method and gets noticeably worse even when measured on the 1064 nm system, even though baseline fitting was applied in that case as well.

Due to the fact that SERDS spectra are automatically free from any background, have zero integral over the spectral window and nearly perfect derivative shape they are extremely convenient for performing automated Raman analysis, as they turn out to be nearly orthogonal for majority of the substances of interest to Raman analysis. A case in point is quantitative Raman analysis, which can be illustrated on relative concentration measurements in binary mixtures, e.g. of methanol and ethanol, performed in the presence of a strong fluorescence background. In this experiment a SERDS spectrum of an unknown mixture was compared with SERDS spectra from a library of a few dozen of pure components, the two most likely components contained in the mixtures were identified and then relative concentration of the two was determined. Note that no multivariate calibration methods (e.g. partial least square regression, PLSR) were employed, i.e. the system was not trained on the known mixtures – rather all samples were treated as completely unknown.

In order to compare the results obtained by SERDS with what conventional Raman analysis would predict, the fluorescence background was removed via numerical differentiation and then similar analysis was carried out as in the case of SERDS (i.e. the spectra of the mixtures were compared with the

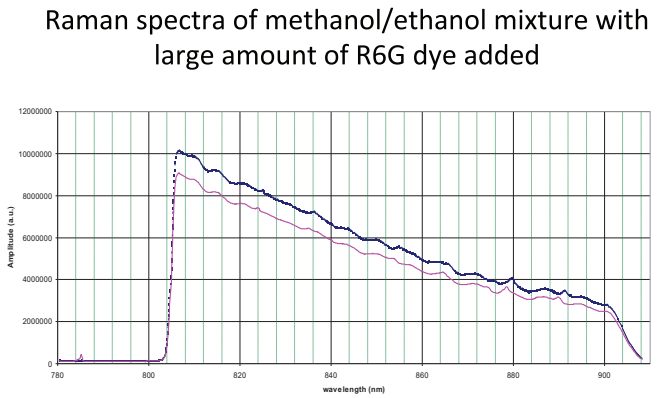

Figure 7. Uncorrected Raman spectra of a methanol/ethanol mixture with R6G dye added to create fluorescence background. The spectra were taken with the two lasers with different wavelength. This amount of fluorescence was added to the mixtures for quantitative determination of the relative concentration of the components.

library spectra). The Raman spectra were collected on a series of solutions with different concentration of methanol in mixture with ethanol. All solutions contained large amount of R6G dye to simulate strong interference from fluorescence. The raw Raman spectra of one of these solutions are shown in

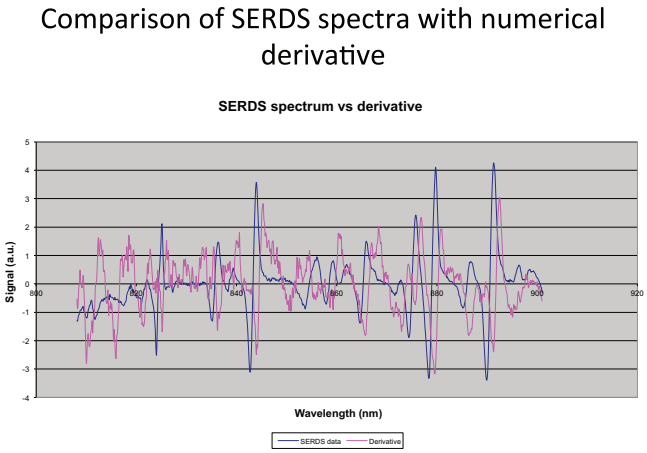

Figure 8. SERDS spectra of the methanol/ethanol mixture obtained using the spectra shown in Fig. 8 (blue line) and the derivative spectrum obtained with the same date numerically (magenta line). The difference in SNR of the two spectra is obvious.

Fig. 7. Large amount of fluorescence blocks the Raman features nearly completely, and the peaks that are resolvable are actually those of the R6G dye. Figure 8 shows SERDS spectrum produced from these Raman spectra and also a spectrum obtained via numerical differentiation. As can be plainly observed, SERDS spectrum is much less noisy than the one obtained numerically.

Figure 9 shows the predicted concentrations of methanol in the mixtures obtained via SERDS and the numerical differentiation. As one can see, even though both methods give reasonably accurate prediction of the methanol concentration when it is present in large proportions, the picture is quite different when lower proportion of the

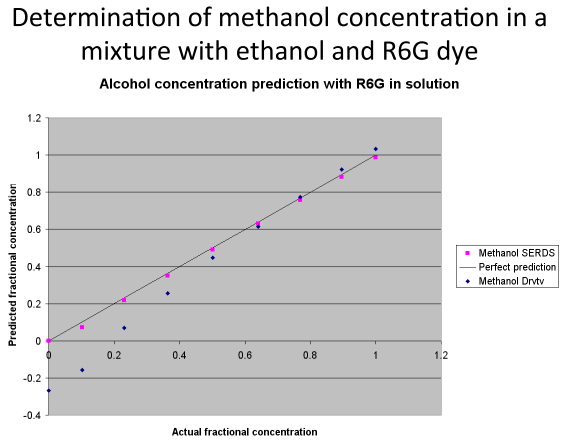

Figure 9. Comparison of quantitative determination of concentration of methanol relative to ethanol in a binary mixture with R6G dye added to create fluorescence background as shown in Fig. 8. The concentration was determined using SERDS and numerical differentiation to remove slowly varying fluorescence background. The straight line shows the ideal values.

methanol is present in the mixture. The conventional method starts loosing accuracy at about 20 – 25% methanol content and then fails completely at concentrations lower than 10%. SERDS, on the other hand, predicts accurate concentrations

even at 5% methanol content in the mixture.

Besides the advantages of SERDS illustrated above, there are some substances which produce strong fluorescence when measured on a long-wavelength Raman instrument. For example, Fig. 10 shows Raman spectra of emerald collected at 785 nm and 1064 nm. As is evident from this result, the amount of fluorescence is not reduced by moving to the 1064 nm excitation. The SERDS method, on the other hand, produces very clear and identifiable spectrum, as is shown in the same figure.

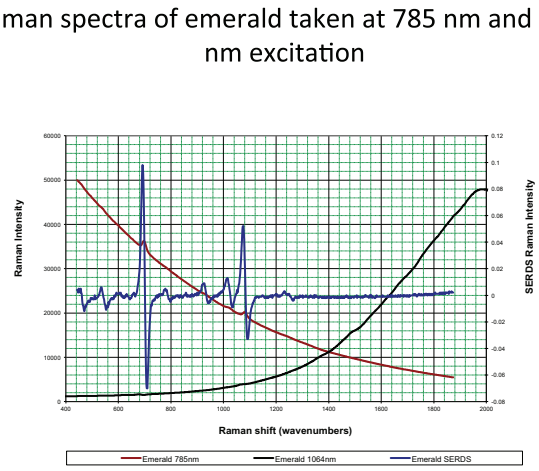

Figure 10. Raman spectra of emerald taken at 785 nm and 1064 nm. SERDS spectrum of emerald is also shown to demonstrate the clarity of spectral features resolved by this method.

Summary and conclusions

As is evident from these results, the SERDS method offers distinct advantages for Raman analysis in most situations. Its advantage in comparison with the digital filtering methods is primarily in accurate elimination of the fluorescence, whereas the latter methods allow only approximate removal of the fluorescent background and only in the situations where it is varying very slowly. Even in the examples considered here, where the fluorescence has no identifiable spectral features, the residual left after the baseline removal deteriorates the R2 significantly.

In comparison with 785 nm excitation, the 1064 nm Raman system usually produces much smaller amount of fluorescence, as is the case for the dark rum. For that reason it has traditionally been used as the benchmark in Raman analysis. However, as the side-by-side comparison with SERDS in similar experimental conditions shows, SERDS can produce not only comparable but superior in quality analysis even when strong fluorescence interference is present. In the case under study, the advantages of stronger Raman signal and the better detector enjoyed by the 785 nm system resulted in nearly the same R^2 for the highly fluorescent rum in about the same total acquisition time. Furthermore, as the example of Raman analysis of a specimen of emerald demonstrates, the long-wavelength excitation is not the ideal solution for all cases, because some of the substances will exhibit fluorescence even with 1064 nm excitation.

Due to much stronger signal at shorter wavelengths and better sensitivity and noise of the CCD arrays the total analysis time in SERDS measurements is at worst comparable to that of a 1064 nm system. And the SNR of SERDS measurements can be improved in a straightforward manner by simply increasing the number of averages for a strongly fluorescent sample.

Overall, the drawbacks assigned to SERDS historically are almost entirely connected with the reconstruction of the difference spectra. When no reconstruction is performed, SERDS produces superior results in all circumstances, as compared with conventional Raman.

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