

Simple Reconstruction Algorithm for Shifted Excitation Raman Difference Spectroscopy

P. MATOUSEK,* M. TOWRIE, and A. W. PARKER

Central Laser Facility, CCLRC Rutherford Appleton Laboratory, Didcot, Oxfordshire, OX11 0QX, United Kingdom

Index Headings: Shifted excitation Raman difference spectroscopy; Resonance Raman spectroscopy; Fluorescence suppression.

INTRODUCTION

Raman spectroscopy is often hampered by high fluorescence backgrounds that can easily swamp the much weaker Raman signals. A common solution to avoid this is to use excitation wavelengths that do not excite fluorescence, for example, using near-infrared (NIR) sources that operate in a region where few molecular systems have electronic absorption bands. However, while this circumvents the fluorescence problem very effectively,^{1–3} it fails to take advantage of the unique features offered by the resonance Raman technique in terms of tremendous sensitivity and selectivity that are so beneficial for investigating complex heterogeneous samples such as those encountered in many biomedical applications.

One of the most widely used approaches for obtaining Raman data from molecular systems that give large fluorescence backgrounds is shifted excitation Raman difference spectroscopy^{4–6} (SERDS), a technique that is popular for its technical simplicity. The key benefit of SERDS is that it can effectively eliminate the large fluorescence backgrounds as well as other sources of random or systematic noise generated by, for example, the detector where the different sensitivity of individual pixels of the detector can produce systematic effects.⁵ Application of SERDS has permitted great sensitivity down to true photon shot levels.⁴

In its basic form, the SERDS approach relies on obtaining two Raman spectra using excitation wavelengths shifted by an amount comparable to the bandwidth of the measured Raman bands (typically 5–10 cm⁻¹). A similar but less effective result can also be achieved by shifting the spectrograph spectral window by this amount, and this can be preferred because of its instrumental simplicity.^{5,6} Having obtained two Raman spectra, S'_{λ} and $S'_{\lambda-\delta}$, these are subtracted from each other to produce a difference spectrum, $D = S'_{\lambda} - S'_{\lambda-\delta}$. This step results in the elimination of the invariant fluorescence signal that for the small change in excitation wavelength is unchanged. The invariance is a direct result of the fact that the majority of fluorescence is emitted from vibrationally relaxed states (Kasha's rule) and a small shift in the excitation wavelength does not perturb its spectral profile. In

contrast, the Raman spectrum *is* shifted by the corresponding amount δ , giving it a derivative-like appearance in the difference spectrum.

The final step in the SERDS methodology is the reconstruction of the Raman spectrum from the difference spectrum. This step can be cumbersome and is typically accomplished through band fitting^{4,5} or using deconvolution procedures.⁷ These reconstruction approaches have, however, their limitations. The fitting method requires a biased input and it produces uncertain results in the region where several Raman bands overlap. The deconvolution procedure is amenable to automatic reconstruction, but it can produce spectral artifacts or lead to the loss of spectral resolution for certain situations.⁷

Here we present an algorithm based on a recurrent formula that enables one to reconstruct the Raman spectrum directly from the raw SERDS difference spectrum using only a simple linear data manipulation.⁸ The only prerequisite for the applicability of this technique is the existence of a 'blank' region containing no Raman bands at one edge of the Raman spectrum. The width of this band-free region should ideally be at least five times the wavelength shift employed to collect the two SERDS spectra, i.e., typically 25–50 cm⁻¹. Such a region can often be found above the Raman fingerprint region within the 1700–2800 cm⁻¹ range.

The photon shot noise remains an ultimate limit of sensitivity that can only be overcome by deploying more powerful fluorescence suppression approaches that directly reject the fluorescence before it is detected. One of the most effective methods of this kind is the ultrafast temporal gating based on an optical Kerr shutter.^{9,10} Recently we have demonstrated the benefit of combining this approach with the SERDS method, enabling us to reach an unsurpassed level of signal to noise and to measure Raman signals 10⁶ times weaker than fluorescence background signals.¹¹ In this note, we use a raw SERDS spectrum taken from these measurements to demonstrate our new SERDS numerical reconstruction method and compare the outputs with conventional fitting techniques.

DESCRIPTION AND DEMONSTRATION OF THE ALGORITHM

To demonstrate the technique we reconstructed a Raman spectrum from the test SERDS spectrum using three different methods, (1) a standard band-fitting reconstruction procedure described in Ref. 4, (2) a simple form of the proposed algorithm, and (3) a more comprehensive form of the algorithm including data interpolation. The results of the reconstruction process using these three methods are then compared.

The SERDS spectrum was obtained on our Kerr gated Raman spectroscopy apparatus^{9,10} under experimental conditions described in detail in Ref. 11. In brief, the Kerr gate consisting of a 2 mm long cell filled with CS₂ and two 41 × 41 mm Glan–Taylor polarizers was driven with 500 μJ (1 ps, 800 nm, 1 kHz) pulses generated in a regenerative amplifier system.¹² The gate was followed by a spectrometer (Spex, Triplemate, 600 grooves/mm) with a home-built filter stage by-pass option. The Raman scattered light was collected at 90° to the probe beam direction using a liquid nitrogen cooled, back-illuminated

Received 31 December 2004; accepted 4 March 2005.

* Author to whom correspondence should be sent. E-mail: P.Matousek@rl.ac.uk.

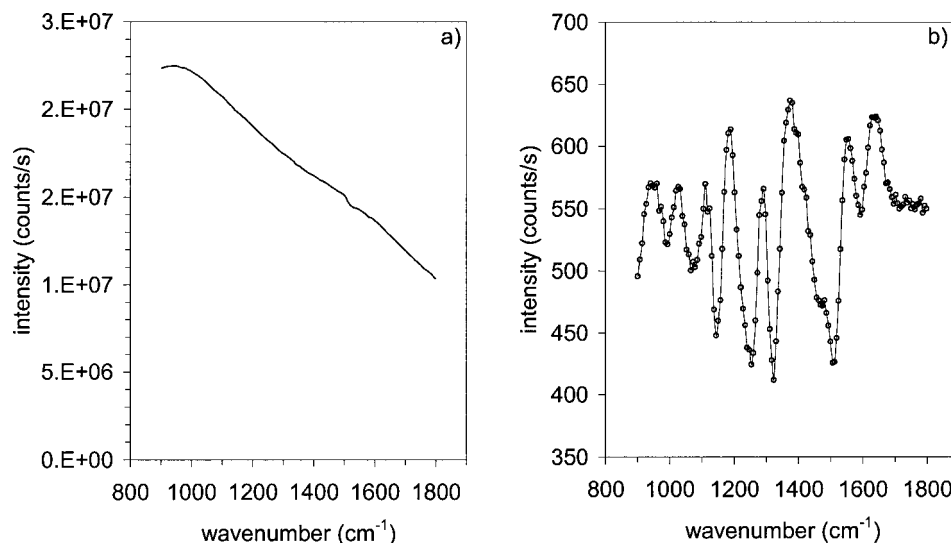


FIG. 1. Resonance Raman spectrum of Rh6G in methanol measured (a) without and (b) with the combined Kerr gate and SERDS method with 532 nm excitation wavelength using 1 ps laser pulses. The accumulation time was 800 s.

charge-coupled device (CCD) camera with an array of 2000×800 pixels (Jobin Yvon Ltd). The CCD was binned vertically across 400 pixels and horizontally across 5 pixels with one count corresponding to one photoelectron. The sample solution was re-circulated in an open liquid jet of 0.5 mm diameter. The probe at 532 nm (1 ps, 5 μ J, 1 kHz) was generated in an optical parametric generator (OPG)/amplifier (OPA)¹² and focused to an ~ 200 μ m diameter spot in the sample. The SERDS spectral shift of 28 cm^{-1} was accomplished by seeding different spectral components of dispersed OPG signal output into the final OPA stage using an optogalvanic mirror.¹¹ Raman spectra were collected by alternating every 10 s between the two shifted probe wavelengths with each cycle being synchronized with the CCD readout. The sample was rhodamine 6G (Rh6G) prepared at a concentration of 1×10^{-3} $\text{mol} \cdot \text{dm}^{-3}$ in methanol. Under these conditions the 532 nm Raman excitation beam was also inducing an extremely intense fluorescence emission with its maximum directly under the measured Raman spectrum.

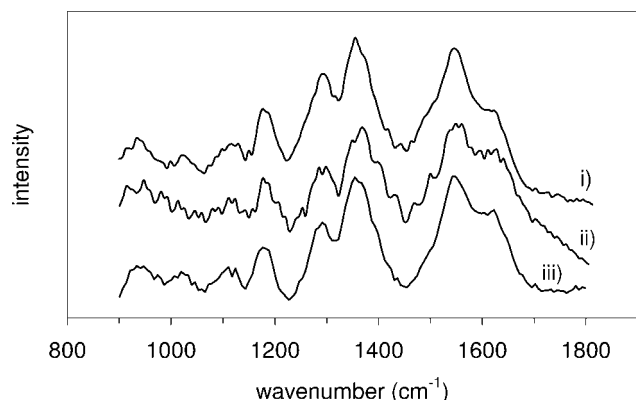


FIG. 2. The Rh6G spectrum reconstructed from the difference spectrum using three different methods: (i) numerical fitting (Method 1), (ii) a simple version of the proposed algorithm (Method 2), and (iii) an algorithm with data interpolation (Method 3). The spectra are offset for clarity.

The fluorescence intensity observed without using either the Kerr gate or SERDS methods was 20 000 000 counts/s strong (see Fig. 1a), i.e., overwhelmingly strong for the detection of any Raman signal. Although by applying the Kerr gate this background was reduced by three orders of magnitude, the resulting Raman signal-to-noise ratio (S/N) level was still too low to permit the clear detection of the Raman spectrum of Rh6G.¹¹ However, by deploying the SERDS methodology the level of S/N was improved by an additional factor of 10 above that obtained by using only the Kerr gate. This crucial step permitted the clean separation of SERDS signal from noise, as can be seen in Fig. 1b. The attained level of S/N was calculated to closely match the expected photon shot noise level born from the remaining fluorescence background after the Kerr gate. Three different reconstruction procedures were then applied to the observed SERDS spectrum as detailed below.

Method 1: Standard Approach Using Numerical Fitting. In line with a standard procedure, the difference SERDS spectrum shown in Fig. 1b was fitted to a sum of the following Lorentzian functions with purpose-written software¹³ based on the least squares fitting and utilizing a SERDS reconstruction method described in Ref. 4:

$$f(x) = \frac{wA}{2\pi[(x - x_0)^2 + (w/2)^2]} - \frac{wA}{2\pi[(x - x_0 + \delta)^2 + (w/2)^2]} \quad (1)$$

where w is the full width at half-maximum (FWHM), x is the wavenumber, x_0 is the peak center in wavenumbers, δ is the SERDS shift, and A is the band area.

The Raman spectrum reconstructed in this way is shown in Fig. 2. It is important to recognize that this procedure is subject to some uncertainty, particularly in the regions where several Raman bands overlap. This, with the fact that the method requires a biased input of initial fitting parameters, renders the approach unsuitable

for most automated applications. Typical computational time on a 1 GHz PC required to complete the iterative fitting of a spectrum with this complexity was in the region of tens of seconds.

Method 2: Proposed Linear Data Manipulation.

The proposed algorithm in its simplest form is based on performing a simple linear data manipulation on the difference spectrum array using the following recursion formula:

$$S_n = S_{n-\Delta} + D_n \quad (2)$$

where D_n and S_n are the intensities of the difference and reconstructed spectra, respectively, for the n th data point of the array. The wavenumber separation between the n th and $(n - \Delta)$ th data points corresponds to the downshift δ . As mentioned above, for this method to be applicable the difference spectrum must have a region free of Raman bands that is substantially wider than the SERDS shift δ . This condition enables the initial values of the S_n array, which are not known at the beginning of the recursion procedure, to be set to zero within this blank region. The spectral reconstruction must be initiated from the blank region of the spectrum moving towards the region containing Raman bands. In Methods 2 and 3 (below) we assume that the blank region is at lower array index values and this corresponds to higher wavenumbers. Obviously, the array can be reversed prior to the analysis if the opposite is the case.

In the spectral reconstruction process, we rely on the fact that by starting the reconstruction from the blank spectral region we are able to identify the first pixel in the difference spectrum that exhibits a rise due to a Raman peak, and its intensity then originates solely from the intensity of the spectrum S'_{λ} , which is 'closer' to the blank region than the downshifted spectrum $S'_{\lambda-\delta}$ that is still 'blank' at this point. Therefore, the intensity of this pixel is directly equal to the corresponding intensity within the reconstructed spectrum. Also, it is equal to the intensity of the pixel within the $S'_{\lambda-\delta}$ spectrum downshifted by Δ and consequently we know how much to add to the difference spectrum D at the downshifted point to compensate for the effect of the subtraction of the shifted spectrum $S'_{\lambda-\delta}$.

The applicability of Method 2 is, however, limited to cases where the SERDS shift δ is approximately equal to some integer multiple of inter-pixel separation within the spectrum array. If a reasonable match cannot be found then an approach combining this routine with linear interpolation between pixels is required, and this method, while slightly more complex, can be easily implemented and is described below as Method 3. (An alternative possible approach is to increase the number of data points within the array (e.g., 10 or 100 times) using a linear interpolation until an acceptable match can be found and then use Method 2.) As Method 3 comprises a data interpolation step, it is also essential that there are no large variations of Raman spectral intensities on the scale of data point separation. This condition can be easily satisfied by appropriate choice of spectrometer dispersion. Method 3 is also well suited to a situation in Raman spectroscopy where the Raman spectrum is not linear across a CCD, for example, due to conventional spectrographs giving linear dispersion in wavelength and non-

linear dispersion in relative wavenumbers. This may result in the variation of inter-pixel wavenumber separation to a degree that cannot be tolerated by Method 2. In this instance, the SERDS spectrum can still be processed using Method 3 if the inter-pixel separation between any two neighboring data points can be assumed to have approximately a linear dependence on wavenumbers in the interpolation process. However, it should be noted that our instrument did not exhibit any meaningful nonlinearity in the detection region.

Method 3: Proposed Linear Data Manipulation with Interpolation. A more rigorous approach applicable even to cases where no good match between some integer multiple of the inter-pixel separation and the shift δ cannot be found involves the refinement of Method 2 by adding a data interpolation step to the algorithm. Let us assume that the spectral array D containing N spectral values consists of two columns: wavenumbers and corresponding intensities, $[w_n, D_n]$. The process is initiated as in Method 2 from the blank region. To calculate the n th point in the spectrum the routine performs the following steps:

- (1) The routine finds a pair of points within the D array with indices x and $x + 1$ for which the following logical operation is true:

$$(w_x > w_n + \delta) \quad \text{AND} \quad (w_{x+1} \leq w_n + \delta)$$

- (2) It then calculates the value $S_{n-\Delta}$ by the linear interpolation between the data points S_x and S_{x+1} :

$$S_{n-\Delta} = (w_n + \delta - w_x) \cdot (S_{x+1} - S_x) / (w_{x+1} - w_x) + S_x$$

- (3) The routine then calculates the S_n value: $S_n = S_{n-\Delta} + D_n$.
- (4) The recursion algorithm then repeats the above for the next data point in the array, $n + 1$.

The above can be recast into a computer code compatible single formula:

$$S_n = D_n + \sum_{x=1}^{N-1} \left\{ \left[\frac{(w_n + \delta - w_x)(S_{x+1} - S_x)}{(w_{x+1} - w_x)} + S_x \right] \times [(w_x > w_n + \delta) \text{ AND } (w_{x+1} \leq w_n + \delta)] \right\} \quad (3)$$

where the last term in the square brackets is a logical operation adopting value 1 if TRUE and 0 if FALSE.

DISCUSSION

Figure 2 shows the resonance Raman spectrum of Rh6G reconstructed from the difference spectrum shown in Fig. 1b using the three described methods for treating the SERDS data. Notice that all the methods yielded satisfactory results, although Method 2 produced spectra with moderately higher noise-like distortions. This is believed to be brought about by the fact that no perfect match could be found between the shift δ and some integer multiple of inter-pixel wavenumber separation. By inspecting the two results obtained using Method 1, which because of its curve fitting nature does not add any extra statistical noise to the SERDS spectrum, and Method 3, which exhibits a broadly similar level of noise,

it is concluded that no substantial increase in the level of noise (above a factor of 3) arises from using Method 3. This is a consequence of the fact that Method 3, unlike Method 2, does not rely on the perfect match between SERDS shift δ and the inter-pixel wavenumber separation but instead it always uses a data interpolation procedure to correct for any arising discrepancies that could lead to incorrect data reconstruction. However, due to the fact that each data point in the reconstructed Raman spectrum is calculated from two different points within the SERDS spectrum (see Eq. 3), each having uncorrelated photon shot noise, the resulting reconstructed spectrum is expected to contain a higher level of noise by a factor of $\sqrt{2}$. This prevents reaching the true photon shot-noise level with Methods 2 and 3. This mild increase in noise could not have been detected in our spectra.

In summary, we conclude that Method 3, linear data manipulation with data interpolation, represents the most convenient route for reconstructing SERDS spectra when its deployment conditions can be satisfied as it offers wide applicability, is simple, produces good signal to noise, and, most importantly, does not involve any ambiguity associated with formulating the initial fitting conditions as is the case with Method 1. As a result, it can be performed automatically in real time. Computational time required to accomplish the calculations by Method 2 and 3 was less than 1 s using a PC with a 1 GHz processor.

Although the example analyzed here used a combined Kerr gated and SERDS approach, Methods 2 and 3 are applicable in exactly the same way to conventional SERDS spectra collected without a Kerr gate and using a pulsed or continuous wave (cw) laser.

In the reconstructions shown in Fig. 2, a small residual curved background present in the reconstructed spectra was removed by fitting a second-order polynomial function to the baseline and subtracting the resulting curve. This background is believed to originate from small pointing changes associated with the way in which we

changed wavelength by δ in the setup used to acquire the spectra.¹¹

An example of the computer code for the reconstruction of the SERDS spectra by Method 3 in Mathematica 4.0 (Wolfram Research) can be found in Ref. 8.

CONCLUSION

We have described a simple algorithm for reconstructing Raman spectra from SERDS spectra. The linear routine is based on a simple recurrent formula applicable to spectra with no Raman bands at one edge of the spectrum. This region should be wider than 5 times the shift of the SERDS. No additional bias input is required. The method is applicable to automated real-time applications and as such is highly suited to manufacturing and process control.

1. B. Schrader, A. Hoffmann, and S. Keller, *Spectrochim. Acta, Part A* **47**, 1135 (1991).
2. M. Fujiwara, H. Hamaguchi, and M. Tasumi, *Appl. Spectrosc.* **40**, 137 (1986).
3. D. B. Chase, *J. Am. Chem. Soc.* **108**, 7485 (1986).
4. A. P. Shreve, N. J. Cherepy, and R. A. Mathies, *Appl. Spectrosc.* **46**, 707 (1992).
5. S. E. J. Bell, E. S. O. Bourguignon, and A. Dennis, *Analyst (Cambridge, U.K.)* **123**, 1729 (1998).
6. P. A. Mosier-Boss, S. H. Lieberman, and R. Newbery, *Appl. Spectrosc.* **49**, 630 (1995).
7. J. Zhao, M. M. Carraba, and F. S. Allen, *Appl. Spectrosc.* **56**, 834 (2002).
8. P. Matousek, M. Towrie, and A. W. Parker, "Fluorescence Suppression in Resonance Raman Spectroscopy using Combined Kerr Gated and SERDS Technique and Automated Spectrum Reconstruction", Annual Report of the Central Laser Facility, RAL-TR-2002-013 (2002), p. 195.
9. P. Matousek, M. Towrie, A. Stanley, and A. W. Parker, *Appl. Spectrosc.* **53**, 1485 (1999).
10. P. Matousek, M. Towrie, C. Ma, W. M. Kwok, D. Phillips, W. T. Toner, and A. W. Parker, *J. Raman Spectrosc.* **32**, 983 (2001).
11. P. Matousek, M. Towrie, and A. W. Parker, *J. Raman Spectrosc.* **33**, 238 (2002).
12. M. Towrie, A. W. Parker, W. Shaikh, and P. Matousek, *Meas. Sci. Technol.* **9**, 816 (1998).
13. P. Matousek, A. W. Parker, W. T. Toner, and M. Towrie, "Numerical Analysis of Raman Spectra", Annual Report of the Central Laser Facility, RAL-93-031 (1993).