SORS – A new approach to subsurface probing of diffusely scattering media

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Introduction

Diffusely scattering media are frequently encountered in many analytical applications: examples include monitoring of food products, colloids, polymers, catalysts, powders in general, coating technology, dermatology applications involving the probing of tissue, and biochemical investigations of tissues, in particular bone, for medical investigations. Such samples are often highly heterogeneous and can be composed of various layers each having a different chemical make-up. A major goal for analytical sciences in this area is to provide a method capable of determining the chemical composition of these layers in a non-destructive way. Here we present a novel approach, enabling the retrieval of deep-layer Raman signals and their separation from those of the overlaying matrix in the spatial domain. The method is compatible with the use of cw lasers, an attribute important particularly for medical applications.

The proposed methodology is based on the collection of Raman spectra from regions spatially offset, by different amounts, from the point of incidence of the probe laser beam on the surface of a diffusely scattering sample. Crucially, such an array of spectra contains different relative Raman contributions from the surface and sub-surface layers. This is the key effect on which this technique is based. As the collection point is moved away from the probe launch point, the contribution of the spectrum from the top layer diminishes much faster than that from deeper layers, because Raman photons that are generated deeper within the sample are more likely to migrate laterally before they are emitted from the sample surface. By collecting a set of spectra at different distances from the launch point, and processing them using a multivariate data analysis technique, one can extract approximations to the pure Raman spectra of the individual layers. The proposed technique we call SORS (Spatially Offset Raman Spectroscopy) is akin to optical tomography using NIR absorbance[1] or fluorescence spectroscopy[2].

SORS can be deployed in two possible geometries: (i) point collection and (ii) collection from the circumference of (multiple) concentric circles symmetrically centred on the probe beam incidence point. The point-like collection geometry represents the simplest adoption of this concept. The concentric circle geometry is, however, superior as it yields much higher collection efficiency, but it requires the use of optical fibres to image the individual circles at different positions on the spectrometer slit.

A detailed account of this work can be found elsewhere[3].

Experimental

The probe wavelength was the 514 nm output from a cw argon ion laser with a power of 12 mW at the sample. The laser beam was focused to a diameter of 300 μm. The Raman light was collected in backscattering geometry using a f/1 collection lens, and imaged onto the spectrometer slit.

The sample was mounted on an x-y-z micro-positioning stage, and moved vertically, (together with the last turning prism placed in front of the collection lens, which reflects the probe beam arriving from below onto the sample, keeping the point of incidence on the sample fixed with respect to the sample). In this configuration, the spectrograph was always collecting light from a fixed region in space and the sample was scanned across this imaging zone, the pump beam incidence point remained fixed on the sample surface.

The test sample consisted of two-layers. The first layer was a 1 mm optical path cuvette of 1 cm width and ~ 4 cm height, with 300 μm custom made fused silica front and back windows, filled with PMMA (poly(methyl methacrylate)) spheres of ~20 μm diameter. This layer was followed by another cell of 2 mm optical path filled with trans-stilbene fine powder that was ground using a pestle and mortar.
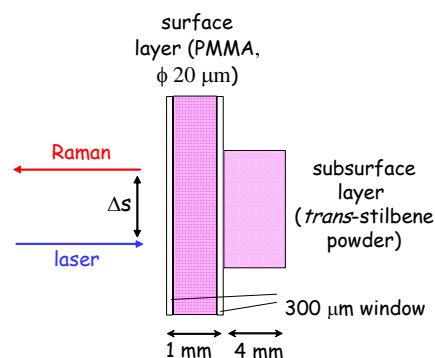

Figure 1. The principal of the technique and two-layer sample geometry used in the experiments.

Results

A set of Raman spectra measured with a varying degree of spatial offset is shown in Figure 2. For comparison, the Raman spectra of the pure layers measured in separate experiments are also displayed. The top spectrum is that of pure trans-stilbene and the bottom one that of pure PMMA. The spectrum measured with zero offset represents the Raman spectrum one would typically obtain using a conventional Raman instrument. It is evident that it contains an appreciable contribution from the top layer. The gradual separation of the PMMA and trans-stilbene signals is clearly accomplished using the SORS approach as one keeps increasing the lateral offset between the Raman collection point and the point of probe beam incidence, and is clearly observable in the raw data set presented. This is because, as the offset distance is increased, the signal intensity of the surface PMMA layer drops more than that of the lower layer, as predicted. At distances of >2 mm one reaches around an order of magnitude improvement in the ratio of the lower layer to the top layer Raman signals.

Figure 3 shows the dependence of the absolute Raman intensities of the individual spectra on the spatial offset. The graph clearly demonstrates that as the Raman collection point is moved away from the probe illumination zone, i.e. the spatial offset is increased, the Raman signal from the bottom layer diminishes much more slowly than that from the top layer. This results in the overall relative Raman intensity ratio of the bottom over the top layer improving with the increased spatial offset.
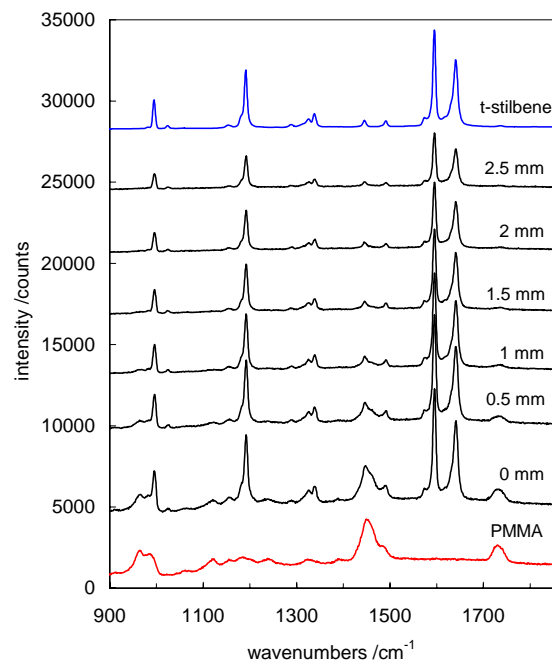

Figure 2. A set of spatially offset Raman spectra collected from a two-layer system consisting of 1 mm layer of PMMA spheres followed by a 2 mm-layer of trans-stilbene powder. The top and bottom spectra are those of pure trans-stilbene and pure PMMA layers, respectively. The acquisition time was 100 s for each spectrum.

To quantify the contrast improvement achievable on this sample by this approach, we have acquired a Raman spectrum with a longer acquisition time (1000 s) at an offset of 3.5 mm. By analysing the intensity of the residual PMMA component we established that the contrast of the lower layer was improved by a factor of 19 by rejecting the top layer spectral component. This result compares extremely favourably with the contrast improvement obtained using the temporal Kerr gating approach in our earlier work[4], where the attained contrast improvement was a factor of 5 (noise limited). Another striking observation is that the signal-to-noise obtained using the much more simple spatial gating method was substantially higher than that observed with the temporal gating approach. This is understandable as the spatial gating technique integrates all Raman components spread in the temporal domain, unlike in the temporal approach where the ultrafast gating results in a severe reduction of the collectable light, as discussed in our earlier work[4]. A downside of the SORS approach is, however, the fact that it does not have simultaneous fluorescence suppression capability as the Kerr gated approach.

Spectra collected by SORS are amenable to multivariate data analysis. Multivariate data reduction techniques provide an advantage when one desires a complete separation of the spectral features of the surface and sub-surface layers. These methods also provide a means of separating spectral features from layers that may have a moderate to high degree of spectral overlap, or where contributions of individual components to are not obtainable or known[3].

Conclusions

We have proposed and demonstrated a simple non-invasive methodology for the extraction of Raman spectra from a deep layer within a diffusely scattering sample. The technique can be used for the effective extraction of pure Raman spectra of top and underlying layers, without any prior knowledge of their chemical composition. The approach holds great promise for biomedical applications, where monitoring sub-surface tissue layers normally would require sectioning the superficial tissues, or many analytical applications such as catalysts, food, polymer research and dermatology applications.
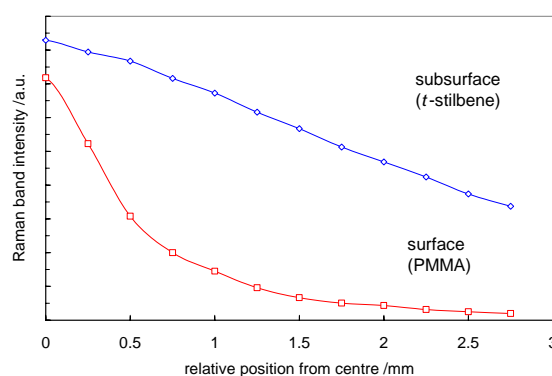

Figure 3. Intensity plots for PMMA (surface) and trans-stilbene (subsurface) Raman spectra.

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