A novel technique for enhancing signal in conventional fluorescence and Raman Spectroscopy of diffusely scattering media

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Introduction

In a number of analytical applications of fluorescence and Raman spectroscopy involving diffusely scattering media, such as living tissue and pharmaceutical products, it is desirable to maximize the signal levels in order to improve the spectral quality, enhance sensitivity or reduce the acquisition times. When probing diffusely scattering media a major cause of photon loss occurs at the sample-to-air interface through which the laser radiation is coupled into the sample leading to inefficient generation of desirable signal. Here we demonstrate experimentally, a simple method of increasing the coupling of the laser radiation into the sample compatible with conventional collection schemes. The technique, see Figure 1, is applicable when the laser wavelength is not, or is only weakly, absorbed by the diffusely scattering samples, for example biological tissue and pharmaceutical tablets using near infrared excitation wavelengths. The technique was described in full in reference [1].

It has been shown that the photon loss at the sample-to-air interface can often represent the dominant loss mechanism of the laser radiation. [2,3] For opaque samples such as powders, over 90% of the incident laser radiation can be scattered backwards after travelling through just a few millimeters. [4] This photon loss can be dramatically reduced, as a means of enhancing useful signal (specifically fluorescence and Raman signals), by inserting a dichroic filter into the laser beam path in front of the sample as described in our earlier theoretical and experimental work. [5] In those studies, the benefits of such air-to-sample coupling optics were demonstrated using specialized variants of Raman spectroscopy (transmission Raman and spatially offset Raman spectroscopy (SORS)) which required the spatial separation of the laser deposition and the collection areas. [6,7,8] Since the majority of fluorescence and Raman spectrometers utilize a backscattering geometry where the illumination and collection areas are coincident, the current work widens the applicability of the enhancement concept and thus increases its usefulness and application areas.

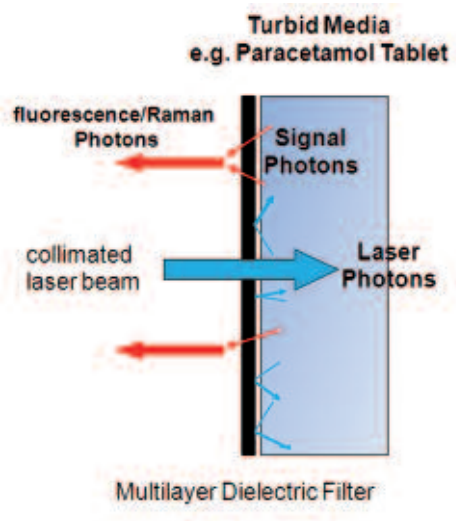

Figure 1. Schematic diagram of the principal of the enhancement technique.

The 'unidirectional mirror' concept

The enhancement technique relies on the fact that the transmission profiles of multilayer dielectric optical elements depend on the photon incidence angle; the spectral transmission window shifts to shorter wavelength when a photon is incident from an angle greater than zero. [9] The mechanism and the magnitude of this shift are discussed in reference [5]. If the filter is designed for high transmittance of the laser wavelength at normal incidence, then it can function as a unidirectional mirror; laser photons scattered from the sample surface with angles of incidence different

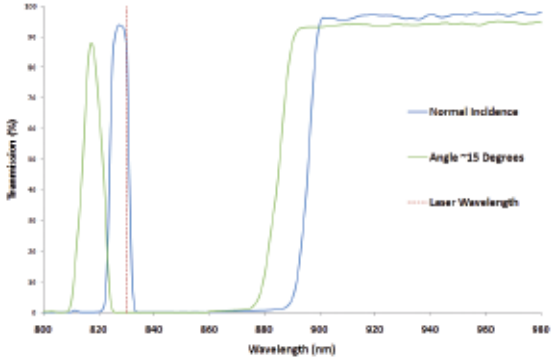

Figure 2. Transmission profile of filter used in Raman experiments, the dependence of the transmission on photon angle of incidence is shown.

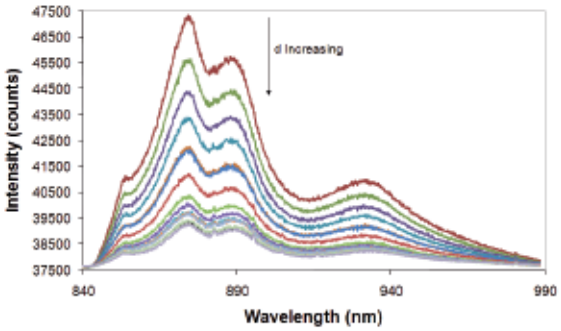

Figure 3. Fluorescence spectra of a paracetamol tablet recorded with varying separations between filter and surface.

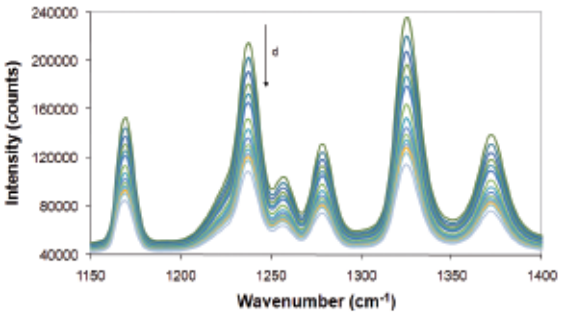

Figure 4. Raman spectra of a paracetamol tablet recorded with varying separations between filter and surface.

from normal will be reflected back into the sample with high efficiency. By choosing a suitable dichroic filter, photons shifted to longer wavelength (due to fluorescence or Raman scattering) are able to pass through the filter and onto the detection system (see Figure 2) whilst the Rayleigh photons are reflected back into the sample. In addition, broadband mirrors can be used at the other sample-air interfaces to minimize the loss of photons (at all wavelengths) and further enhance the detected signal.

As discussed in our earlier work [5] the dichroic filter in the conventional geometry constrains the usable Raman spectral range to above a certain threshold wavenumber value (set in our experiments at ~900 cm^{-1}). This limitation stems from the requirement for positioning the filter spectral edge at a sufficiently long wavelength (relative to the laser line) to act effectively as a mirror for a significant portion of laser photons emerging from the sample at non-normal incidence. Such photons will experience a blue-shifted spectral profile of the enhancing dielectric mirror. No such constraint exists on the spectral profile for the transmission geometry. [5] For fluorescence spectroscopy this is a lesser issue as a similar threshold value would not significantly affect the fluorescence profile which typically exhibit much larger red shifts from the laser excitation wavelength.

Although conventional mirrors have long been used to redirect transmitted laser light back into the sample as a way of increasing the intensity of Raman signal [10] and to reduce photon loss near the laser radiation coupling zone [11] such elements do not prevent the photon loss at what is often the most critical area, the delivery zone of laser radiation into the sample. This loss becomes more marked in applications where safety or other limits prevent the laser radiation from being concentrated onto a small area. Examples include the illumination of human skin or applications in explosive powder environments in the pharmaceutical industry. The solution presented here is fully compatible with the defocused laser beams used in such conditions.

Experimental section

For fluorescence measurements, the probe laser was a green HeNe laser (543.5 nm, 0.40 mW, 1.1 mm beam diameter) equipped with a 10 nm bandpass filter centered at the laser wavelength to purify the spectrum. For the Raman measurements, the laser was a continuous near infra-red diode laser (Process Instruments, model PI-ECL-830-300-FS, 830 nm, 250 mW, 1.0 mm beam diameter). The laser beam was directed through a prism and a dichroic filter (Semrock BrightLine® single-band filter (525 nm)) for fluorescence and an Iridian Spectral Technologies custom made filter (with a transmission peak at the lasing wavelength and with high transmission above 900 nm (corresponding to a Raman shift of 930 cm^{-1})) for Raman) onto the sample (see Figure 2). The dichroic filter was mounted on a translation stage in order to precisely control its distance from the sample.

Emitted photons were collected by a collimating lens (50 mm diameter and a focal length of 60 mm) and imaged by a second lens, identical to the first, onto the front face of a fiber probe consisting of 22 active optical fibers. In this arrangement the signal is collected from a ~1.2 mm diameter circle on the sample surface. For fluorescence measurements, an edge filter (Semrock) was used to remove all radiation with a wavelength shorter than 900 nm while a notch filter (Kaiser Optical Systems) was used to remove the laser line scatter in the Raman measurements. The fiber bundle length was ~2 m and, at the output end, the fibers were arranged in a linear shape orientated vertically and placed in the input image plane of a spectrograph equipped with a NIR back-illuminated deep-depletion CCD camera. The spectra are not corrected for the variation of the filter and detection system's sensitivity across the spectral range. Spectra were obtained by varying the distance between the sample and the dielectric mirror.

In Raman measurements the sample was a standard 3.9 mm thick paracetamol tablet. In fluorescence studies, the same tablet was used with a thin coating layer of fluorescent ink applied to its surface using a green highlighter pen to enhance fluorescence emission in the 900-1000 nm region covered by the detection system. Additional Raman experiments were performed on a 4 mm thick block of plastic (Teflon) with the dichroic mirror at the illumination site and a second mirror (Aluminum UV protected mirror, Reflectivity >90%; 400 nm-10.0 µm, Thorlabs) at the back end of the slab. Raman spectra were taken with no filters present, with the filter at the illumination zone, with the mirror at the back of the sample and finally with both filter and mirror.

Results and discussion

Fluorescence and Raman spectra of a paracetamol tablet are shown in Figures 3 and 4 for different distance of the enhancing dichroic filter from the sample; the observed signal decreases rapidly with increasing distance (d) between sample and filter. The apparent structure in the fluorescence spectra is an artifact of the transmission curve of the filter used in the 900-1000 nm range. The maximum enhancement is achieved when the filter is in contact with the sample.

By dividing the peak height of the most enhanced spectrum by the peak height of the least intense spectrum we calculated that at zero separation between the sample and the filter, the signal is enhanced by a factor of 6 (Figure 3) for fluorescence and by a factor of 3 for Raman (Figure 4). The enhanced signals were stable with no signs of any undue intensity fluctuations and the enhancement factors were reproducible upon successive approaches of the filter to the sample.

In some cases, further enhancement can be obtained by the incorporating an additional broadband mirror at the "transmitted side" of the sample. For 4 mm thick Teflon slabs, a single dichroic filter at the illumination zone resulted in an enhancement of the Raman signal by a factor of 1.7. A single broadband mirror at the transmitted side gave an enhancement of 1.5 and the combination of the two gave an enhancement factor of 2.8 (Figure 5). These results suggest that, using suitably selected filters and mirrors, a photon 'trap' could be constructed which would be capable of increasing the Raman signal by a significant and useful amount. The precise enhancement factors achievable in each application will vary depending on the experimental parameters and the scattering/absorption properties of the probed sample. The efficiency of coupling of laser radiation into turbid media using enhancing elements as a function of sample properties was also discussed in our earlier theoretical study. [12]

In order to achieve an equivalent enhancement using other means it would be necessary to increase laser power, collection time, detector sensitivity and/or collection system/spectrograph throughput [13] by similar factors. However, these alternative measures may not be straightforward (or even possible) to implement since many of these parameters are often already optimised.

Conclusions

A concept for the enhancement of fluorescence and Raman signals from turbid media using a conventional backscattering geometry has been demonstrated. A substantial enhancement in observed signal was achieved using a custom made dielectric bandpass filter inserted into the proximity of the laser illumination spot. Additional enhancements were achieved by inserting broad-band mirrors at the other sample to air interfaces. The enhancement contributes to improve signal or permits shortening of applied laser power or acquisition times.

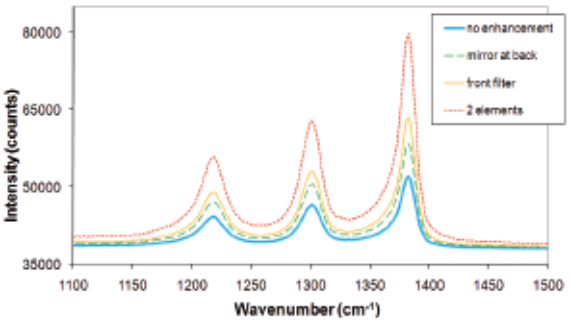

Figure 5. Raman spectra of a 4 mm thick slab of Teflon with different filters.

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