Enhancement of signal in transmission Raman spectroscopy of turbid media

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Introduction

A number of analytical applications require the probing of turbid media with high chemical specificity. The primary target is the composition of the sample; information that is vital in, for example, the analysis of pharmaceutical tablets to identify the presence of undesired polymorphs. Ideally, information should be obtained non-destructively, non-invasively and rapidly. Practical applications include cancer diagnosis and security screening.

Raman spectroscopy holds a great potential in this area due to its high chemical specificity and compatibility with water, a feature crucially important in biomedical applications. To date Raman has been used predominantly in the backscattering collection mode for its instrumental simplicity and ease of use, often in conjunction with confocal microscopy. The drawback of this configuration is that it exhibits a relatively small penetration depth. In living tissue, for example, the maximum accessible depth is typically only several hundred micrometers.

Recently, several new methods based on Raman spectroscopy capable of probing diffusely scattering samples to much greater depths have been developed through access to the LSF's Ultrafast Spectroscopy Laboratory. In addition to enriching the portfolio of available Raman techniques, these developments have opened up a host of potential new applications [1]. Particular promise is shown by Transmission Raman Spectroscopy [2,3,4,5] which has proved to be extremely effective in probing the bulk content of non-absorbing or weakly absorbing pharmaceutical and biological samples at depths well beyond the reach of conventional approaches.

The transmission concept has been applied to the non-invasive probing of the bulk content of pharmaceutical tablets and capsules [2] where it effectively suppresses both the Raman and fluorescence components emanating from the capsule shell [3]. A similar suppression effect has also been utilised in the probing of calcifications in breast tissue phantoms through a 16 mm slab of chicken tissue [6].

In general, spontaneous Raman spectroscopy suffers from low photon flux; the achievement of sufficient signal-to-noise ratios at acceptable acquisition times often requires the use of high throughput detection systems and relatively high average laser powers. This is particularly the case for weakly scattering Raman media or in the presence of intense fluorescence backgrounds, which can severely reduce Raman signal-to-noise ratios. Consequently, any measures leading to improved absolute Raman signal intensities, often even at fractional levels, are highly desirable and project directly into improved Raman signal-to-noise (S/N) ratios.

In this work, a novel method for enhancing Raman signals using a multilayer dielectric element placed in the proximity of a probed turbid sample is presented. It is shown that such a simple passive modification of the experimental setup can yield a significant increase in detectable Raman signals leading to enhanced signal-to-noise ratios, sensitivity and, in the case of transmission Raman, an increased penetration depth. The details of this

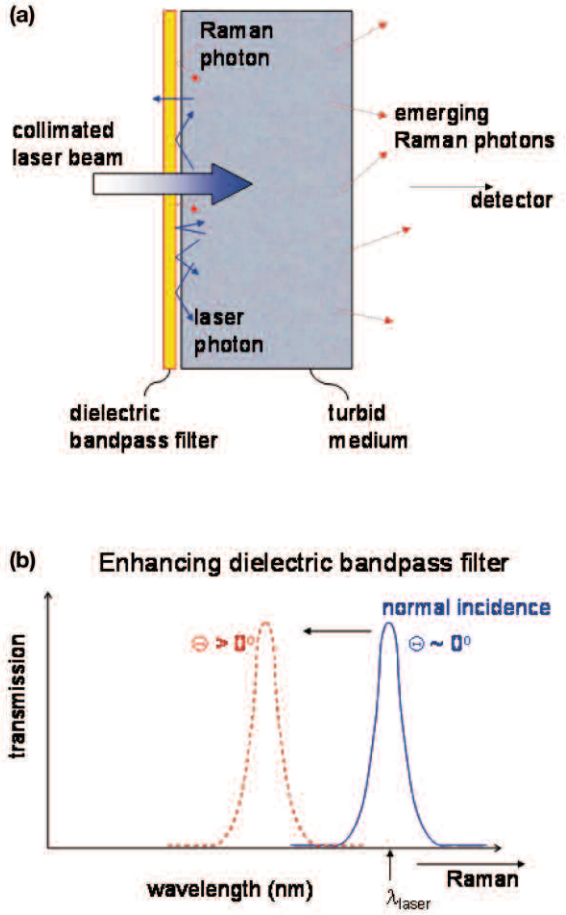

Figure 1. a) Dielectric bandpass filter used for the enhancement of signals in transmission Raman spectroscopy. b) Schematic illustration of the shift of the design wavelength with the angle of incidence for a bandpass dielectric filter.

work including numerical simulations and additional experimental results are presented in reference [7].

Raman signal enhancing dielectric elements

The principal role of the multilayer dielectric coupling element is the prevention of the large loss of laser photons from the medium at the critical point of entry of the laser beam into the sample. This is where the dominant photon loss mechanism occurs; a significant portion of the laser photons injected into the medium re-emerge from the sample before they can build any appreciable amount of Raman signal.

In general, the spectral transmission or reflection profiles of dielectric optical elements such as reflectors and filters exhibit a strong dependence on the angle of photon incidence with the spectral profile shifting to higher frequency (shorter wavelength) with increasing deviation away from normal incidence. Crucially, the fraction of the beam energy which is not transmitted by this filter is instead reflected as losses by absorption within these elements are negligible.

The central transmission wavelength of a dielectric bandpass filter will consequently shift to the blue part of the spectrum, i.e. away from the Stokes spectral region, upon an increase of the photon angle of incidence away from normal incidence. If the filter is designed to transmit collimated light at normal incidence, then this element can be utilised in transmission Raman spectroscopy to transmit a semi-collimated laser beam impacting on it at normal incidence at one side whilst, at the other side, reflecting the majority of laser and Raman photons emerging from the sample in random directions back into the medium.

Signal enhancing reflective enclosures or reflective cavities have been used with turbid samples in the past, with both elastically scattered light [8] as well as with Raman spectroscopy [5]. However, since these 'cages' comprised only simple reflective mirrors they could not be located in the laser interaction zone, thus the critical point of entry of the laser beam into the medium, where the majority of photon loss occurs, is left untreated.

Experimental section

The Raman spectra were measured using a home-built Raman apparatus modified to operate in the transmission Raman geometry [7]. The probe beam was generated using an attenuated 115 mW temperature stabilised diode laser for Raman spectroscopy operating at 827 nm (Micro Laser Systems, Inc, L4 830S-115-TE). The laser power at the sample was 50 mW and the laser spot diameter was ~4 mm. The beam was spectrally purified by removing any residual amplified spontaneous emission components from its spectrum using two 830 nm bandpass filters (Semrock). These were slightly tilted to optimise their throughput for the 827 nm laser wavelength. The beam was polarised horizontally at the sample.

Raman light was collected from the opposite side of the sample using a 50 mm diameter lens with a focal length of 60 mm. The scattered light was collimated and passed through a 50 mm diameter holographic notch filter (830 nm, Kaiser Optical Systems, Inc) to suppress the elastically scattered component of light. The filter was also slightly tilted to optimise the suppression at 827 nm. A second lens, identical to the first, was used to image, with magnification 1:1, the sample collection zone onto the front face of a fibre probe made of 22 active optical fibres. The individual fibres were made of silica with a core diameter of 220 μm, a doped silica cladding diameter of 240 μm and a polyimide coating of 265 μm diameter. The fibre numerical aperture was 0.37. The bundle was custom made by CeramOptec Industries, Inc, the bundle length was ~2 m and at the output end the fibres were arranged into a linear shape [9] oriented vertically and placed in the input image plane of a Kaiser Optical Technologies Holospec 1.8i NIR spectrograph. Raman spectra were collected using a NIR back-illuminated deep-depletion thermoelectrically cooled CCD camera (Andor Technology, DU420A-BR-DD, 1024 × 256 pixels) by binning the entire chip vertically.

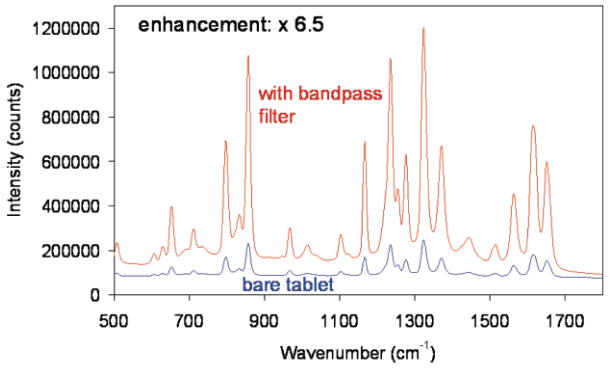

Figure 2. The experimental demonstration of the concept for the transmission Raman geometry on a standard paracetamol tablet. The Raman spectra are those of a tablet with ('with bandpass filter') and without ('bare tablet') a bandpass filter placed over the laser illumination area in contact with the sample. The spectra are offset for clarity. The acquisition times were 10 s in both cases.

The dielectric optical element placed over the laser beam deposition area on the sample was a 25 mm diameter Semrock bandpass filter centred at 830 nm with bandwidth of 3.2 nm (LL01-830-25, MaxLine Laser-line Filter) and transmission at the central wavelength >90 %. The slight mismatch between the laser wavelength (827 nm) and the filter wavelength was compensated by introducing a small tilt to the incident beam at sample. Although the mismatch somewhat reduced the effectiveness of the element a substantial enhancement of the Raman signal was still present.

Results and discussion

A basic experimental demonstration of the enhancement concept has been carried out in the transmission Raman geometry on a standard paracetamol tablet of diameter 12.8 mm and a thickness of 3.8 mm. The measurement was performed using a tablet with and without the enhancing filter. The results of the measurement are shown in figure 2. The data are presented in raw, unprocessed form. A substantial enhancement (~6.5) is observed upon the insertion of the filter into the proximity of the tablet.

Importantly, the observed enhancement exhibits good reproducibility upon subsequent multiple approaches of the filter to the tablet and no signal fluctuation was observed when the filter was in place. In addition, the signal was enhanced uniformly across the entire spectrum as would be expected for a linear enhancement process. This is important in analytical applications involving complex analytes where the spectral pattern serves as means of identifying and quantifying the relative concentrations of the individual components.

The application of the filter to Spatially Offset Raman spectroscopy [1] is discussed in reference [10]. Further work [11] details the use of the same concept for the enhancement of laser radiation in turbid media. Our present efforts focus on the extension of the applicability of this concept to conventional backscattering Raman and fluorescence spectroscopy.

Conclusions

A concept for the enhancement of Raman spectroscopic signal from turbid media has been described. It is shown that a substantial enhancement can be obtained using optical elements (such as a dielectric bandpass filter) inserted into the proximity of the turbid medium. The concept is readily applicable to transmission Raman spectroscopy in particular. Potential applications which could benefit from enhanced signal and/or reduced acquisition times include non-invasive disease diagnosis and quality control of pharmaceutical products.

The concept is also applicable in an analogous manner to other types of analytical methods such as fluorescence spectroscopy of turbid media and other general applications where the coupling of a laser beam into a diffusely scattering medium is of importance.

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