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Frequency Offset Raman Spectroscopy (FORS) for subsurface probing of highly scattering media

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Abstract: We present a new technique, Frequency Offset Raman Spectroscopy (FORS), for probing deep layer Raman spectra of diffusive media. It was demonstrated on a tissue mimicking phantom, and shows potential for *in vivo* applications.
OCIS codes: (170.5660) Raman spectroscopy; (170.5280) Photon migration; (290.0290) Scattering;

1. Introduction

Excellent chemical specificity to structural changes of molecules have made Raman spectroscopy a promising tool for probing biological tissues [1]. However, most often, Raman spectroscopy was limited to *ex vivo* sample analysis or *in vivo* analysis of the superficial layer with depths around few hundred microns [2]. The high scattering nature of biological tissues poses problems for deep tissue profiling with conventional Raman spectroscopy. However, the recent invention of Spatial Offset Raman Spectroscopy (SORS) [3] has brought major advancement in deep tissue probing.

SORS works on the principle of getting deeper information (bottom layer) of a medium by increasing the source-detector separation (d) that exploits a well-known spatial characteristic of light propagation in diffusing media. Though SORS has been a pioneer technique for deep material analysis, it suffers from low signal to noise ratio (SNR) at large source-detector separation and reduction in specificity due to changing the investigated volume upon increasing d.

We propose an alternative approach, named Frequency Offset Raman Spectroscopy (FORS), which achieves deep probing exploiting the spectral dependence of the optical properties (absorption and reduced scattering coefficients, μ_a and μ'_s, respectively) of the diffusive medium to be characterized.

Aim of our work is to demonstrate FORS as a novel technique to retrieve deep layer information. A dedicated tissue mimicking phantom with optical properties relevant to biological media was exploited to perform a comparison between SORS and FORS, and highlight the complementary nature and general relation between them. Finally, a novel FORS-SORS technique is proposed to harvest benefits of both techniques to enhance Raman detection from deeper layers.

1.1 Principle of SORS and FORS

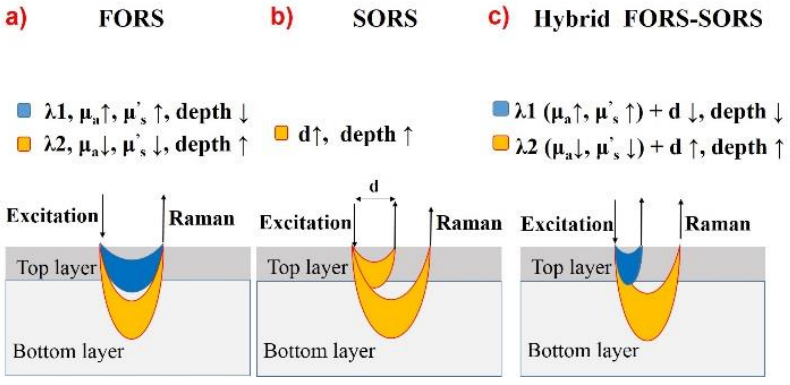

Fig. 1 The principles of FORS (a), SORS (b) and hybrid FORS-SORS (c) are shown.

The optical properties are intrinsic nature to a given specific medium, and vary with the frequency (wavelength) of light. The mean depth explored by the photons collected at a distance d from the injection point depends on the value assumed by the optical properties of the medium. Hence, depth probing can be achieved by exploiting the different values of the optical properties of the medium at different frequencies (Fig.1a). Thus, by performing Raman spectroscopy at different excitation frequencies it is possible to selectively probe different parts of medium, provided that different optical properties versus frequency are observed. Conversely, in SORS different depths are reached by increasing the source-detector distance (Fig.1b). Also, the combined SORS-FORS approach is possible (Fig.1c).

2. Methods

2.1 Instrumentation and analysis

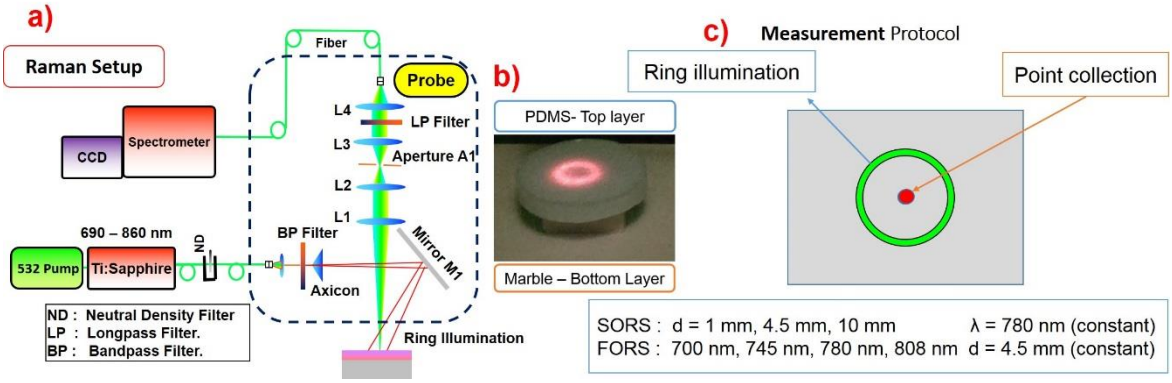

Fig. 2 (a) Raman setup, (b) two layer tissue mimicking phantom and (c) measurement protocol.

Fig. 2a shows the schematic of the experimental setup, designed to perform SORS and FORS measurements in a non-contact geometry, using multi-wavelength excitation (700 nm, 745 nm, 780 nm, 808 nm) by a Ti:Sapphire laser. A 100 µm optical fiber couples the laser light to a collimator in the probe using a 30 mm diameter cage system. An axicon lens is then exploited to create ring illumination, with an adjustable radius between 1 mm and 10 mm by translating the axicon. The collection system involves a set of four optical lenses (diameter 25 mm) with effective *f*-number *f*/2. A suitable long pass filter (715 nm, 750 nm, 785 nm, 808 nm) is placed between the lenses L3 and L4 to remove the excitation photons. L4 couples the Raman signal into a 1 mm optical fiber, which transfers the light to a spectrometer through a 200 µm slit followed by imaging into a cooled CCD camera to record Raman spectra.

The measurement protocol consists of two parts. At first, conventional SORS measurements were performed at 780 nm with multiple source-detector separations (d = 1 mm, 4.5 mm, 10 mm). These measurements validated our system with traditional SORS, and acted as a standard to compare with FORS results. Then, FORS measurements were performed at multiple excitations (700 nm, 745 nm, 780 nm, 808 nm) with constant source-detector separation (d = 4.5 mm). A schematic of the measurement geometry is shown in Fig. 2c. The two-layer tissue mimicking phantom is shown Fig. 2b: the top layer is made of polydimethylsiloxane (PDMS) and the bottom layer is marble.

The variation of the relative Raman intensity of bottom and top layers in the recorded spectra can be evaluated by calculating an enhancement factor, which is defined as follows for SORS (Equation 1) and FORS (Equation 2):

$$\eta_{SORS} = \frac{\frac{\mathbf{I(d)Bottom}}{\mathbf{I(d)Top}}}{\frac{\mathbf{I(d_0)Bottom}}{\mathbf{I(d_0)Top}}} \quad \text{(Eq.1)} \qquad \eta_{FORS} = \frac{\frac{\mathbf{I(\lambda)Bottom}}{\mathbf{I(\lambda)Top}}}{\frac{\mathbf{I(\lambda_0)Bottom}}{\mathbf{I(\lambda_0)Top}}} \quad \text{(Eq. 2)}$$

where d_0 and d are the extreme SORS contrast points, which in our case are 1 mm and 10 mm, respectively. Similarly, λ_0 and λ are the two extreme contrast points of FORS measurements and turn out to be 700 and 808 nm, respectively. To estimate the enhancement factor, we calculated the intensity values averaged over the peak for the desired peaks and used them in the above equations.

3. Results and Discussion

Absorption and scattering spectra of the PDMS top layer of the two-layer phantom are shown in Fig. 3a. The vertical lines represent the excitation wavelengths (700 nm, 745 nm, 780 nm, 808 nm) of FORS measurements. The effectiveness of FORS is maximum when the difference between optical properties (μ_a and μ'_s) at the excitation wavelengths is maximum. From Fig. 3a, at 700 nm, the phantom absorption is around 0.18 cm^{-1}, which is 3.75 times higher than that of absorption (0.048 cm^{-1}) at 808 nm. Similarly, the scattering at 700 nm is around 10 cm^{-1}, which is 1.3 stronger than at 808 nm. These features make it an ideal phantom for FORS measurements.

The results of the first part of the protocol (SORS) are shown in Fig. 4. From Fig. 4, it is clear that increasing source-detector separation d from 1 to 10 mm increases the Raman signal at 1087 cm^{-1} (*i.e.*, the carbonate peak of marble, bottom layer), and reduces the contribution from the top layer. The latter can be seen as a reduction in the Raman signal at 1260 and 1411 cm^{-1}, which correspond, respectively, to the symmetric and asymmetric stretching of -CH_3 vibrations present in the PDMS phantom. An enhancement factor of 2.6 is found between the peak of the top (1087 cm^{-1}) and bottom (1411 cm^{-1}) layers.

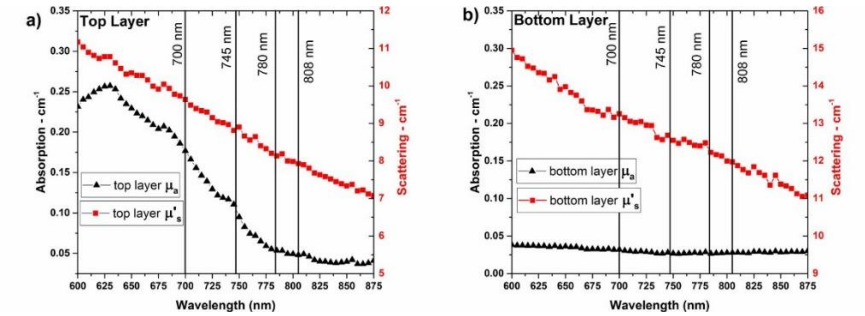

Fig. 3. Absorption and reduced scattering spectra of (a) top layer, (b) bottom layer.

The second part of the study was devoted to FORS. This was performed at four chosen excitation wavelengths (700 nm, 745 nm, 780 nm, 808 nm) on the same 2-layer phantom as SORS, in which the top layer shows a monotonous decrease in optical properties (μ_a and μ'_s) while moving spectrally from 700 to 808 nm. Similar to what observed with SORS upon changing source-detector separation, here varying contributions to Raman spectra from the top (peak at 1411 cm^{-1}) and bottom (1087 cm^{-1}) layers are seen by changing excitation wavelength. However, unlike SORS, here the changing contribution in spectra is due to changing optical properties rather than distance *d*. An enhancement factor of 2.8 was found for FORS.

It is clear from the above discussion that the SORS and FORS are complementary in natures. Interestingly, these two techniques (spatial and optical property dependence) can be combined to get maximum enhancement. This suggested us to propose the hybrid FORS-SORS approach to achieve enhanced probing of deep layers in a diffusive medium. As shown pictorially in Fig.1(c), hybrid FORS-SORS was performed at two extreme conditions. At first, a measurement was performed at short source-detector separation (d = 1 mm) at 700 nm: it enhanced the signal of the top layer by utilizing both spatial (short d) and optical properties (low μ_a and μ'_s). Similarly, the signal from the bottom layer is enhanced by performing measurement at 808 nm (high μ_a and μ'_s, *large* d = 10 mm).

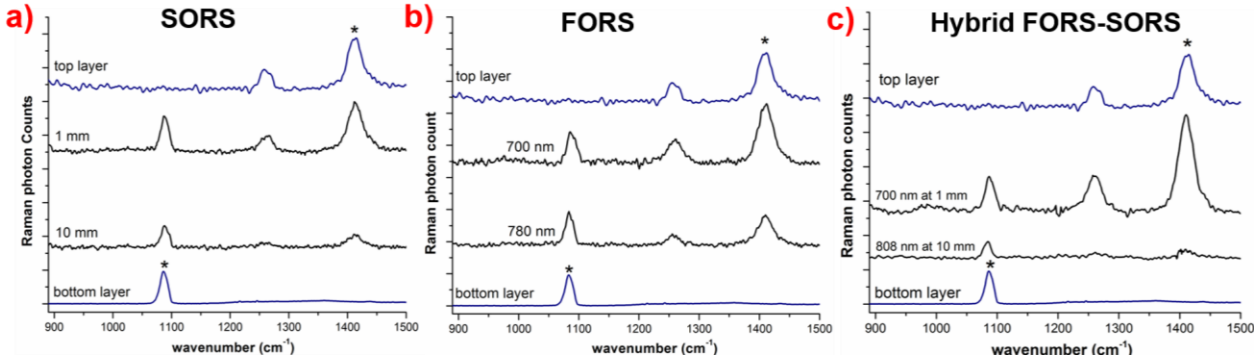

Fig. 4. Raman spectra of the 2-layer phantom acquired by (a) SORS at different source-detector separation, (b) FORS at different excitation wavelengths, (c) hybrid FORS-SORS exploiting advantage of both FORS and SORS.

4. Conclusions

We propose Frequency Offset Raman Spectroscopy (FORS) for deep probing of diffusive media. It was successfully demonstrated on tissue mimicking phantom, obtaining an enhancement factor of 2.8 and 2.6 for FORS and SORS respectively. A hybrid FORS-SORS technique that exploits the advantages of both approaches is also proposed, yielding an enhancement factor of 6.0. With advancement in Raman spectroscopy of diffusive media [4][5] we expect the proposed techniques to have great potential for *in vivo* applications.

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6. References

[1] R. Petry, M. Schmitt, and J. Popp, "Raman Spectroscopy - A Prospective Tool in the Life Sciences," *ChemPhysChem*, vol. 4, no. 1, pp. 14–30, 2003.

[2] P. J. Caspers, G. W. Lucassen, and G. J. Puppels, "Combined in vivo confocal Raman spectroscopy and confocal microscopy of human skin.," *Biophys. J.*, vol. 85, no. 1, pp. 572–80, 2003.

[3] P. Matousek, I. P. Clark, E. R. C. Draper, M. D. Morris, A. E. Goodship, N. Everall, M. Towrie, W. F. Finney, and A. W. Parker, "Subsurface Probing in Diffusely Scattering Media Using Spatially Offset Raman Spectroscopy," vol. 59, no. 4, pp. 393–400, 2005.

[4] S. Konugolu Venkata Sekar, S. Mosca, A. Farina, F. Martelli, P. Taroni, G. Valentini, R. Cubeddu, and A. Pifferi, "Frequency offset Raman spectroscopy (FORS) for depth probing of diffusive media," *Opt. Express*, vol. 25, no. 5, pp. 4585–4597, Mar. 2017.

[5] F. Martelli, T. Binzoni, S. Konugolu Venkata Sekar, A. Farina, S. Cavalieri, and A. Pifferi, "Time-domain Raman analytical forward solvers," *Opt. Express*, vol. 24, no. 18, p. 20382, 2016.