Model-Based Optimization of Laser Excitation and Detection Improves Spectral Contrast in Non-Invasive Diffuse Raman Spectroscopy

Journal:	<i>Applied Spectroscopy</i>
Manuscript ID	ASP-21-0302.R2
Manuscript Type:	Submitted Manuscript
Date Submitted by the Author:	26-Nov-2021
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Manuscript Keywords:	Raman Spectroscopy, Diffuse Raman, Spatially Offset Raman Spectroscopy, Computational Modeling
Abstract:	Spatially offset Raman spectroscopy (SORS) is a powerful technique for subsurface molecular analysis of optically turbid samples. Numerical modelling of light propagation has been used to investigate opportunities for improving spectral contrast and signal to noise ratio when imaging regions of interest located 0 - 4.5 mm below the surface in polymer bulk material. Two- and three-dimensional modelling results demonstrate that when analysing a certain region of interest (ROI) of finite lateral dimensions below the sample surface, offsetting both the laser source and detector in opposite directions from the central point of the ROI can increase the spectral contrast as compared to conventional SORS approach where the detector or the laser source is maintained at the central point (centred SORS). The outlined modelling results have been validated experimentally using a bulk polymer sample with a trans-stilbene ROI (cylinder) below the sample surface. The results show that modelling of the spatial configurations of laser excitation and detection points can be used to optimise the instrument configuration to achieve significant improvements (up to 2.25-fold) in performance over the conventional centred SORS. Such optimal solutions can then be implemented, for example, using robust fibre-optics probes, moveable optics, or flexible spatial light modulator instruments for specific applications.

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Author Accepted Manuscript

Paper Type: *Submitted Manuscript*

Colour Figures in Print?: YES; colour figures in print, as well as online

Model-Based Optimization of Laser Excitation and Detection Improves Spectral Contrast in Noninvasive Diffuse Raman Spectroscopy

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Abstract **<TYPESET>Please do NOT place Graphical Abstract in typeset manuscript.**

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Spatially offset Raman spectroscopy (SORS) is a powerful technique for subsurface molecular analysis of optically turbid samples. Numerical modelling of light propagation has been used to investigate opportunities for improving spectral contrast and signal to noise ratio when imaging regions of interest located 0–4.5 mm below the surface in polymer bulk material. Two- and three-dimensional modelling results demonstrate that when analyzing a certain region of interest (ROI) of finite lateral dimensions below the sample surface, offsetting both the laser source and detector in opposite directions from the central point of the ROI can increase the spectral contrast as compared to conventional SORS approach where the detector or the laser source is maintained at the central point (centered SORS). The outlined modelling results have been validated experimentally using a bulk polymer sample with a trans-stilbene ROI (cylinder) below the sample surface. The results show that modelling of the spatial configurations of laser excitation and detection points can be used to optimize the instrument configuration to achieve significant improvements (up to 2.25-fold) in performance over the conventional centered SORS. Such optimal solutions can then be implemented, for example, using robust fiber optic probes,

moveable optics, or flexible spatial light modulator instruments for specific applications.

Keywords

Raman spectroscopy, diffuse Raman, spatially offset Raman spectroscopy, SORS, computational modeling

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Introduction

Raman spectroscopy is a noninvasive optical technique that conveys vibrational spectra of molecules by using excitation light and detection of inelastic scattered photons.¹ Raman spectroscopy has been combined with optical microscopes to obtain spatially resolved spectra by scanning the sample laterally or by scanning the laser beam across the sample surface.² When measuring from the surface of a sample (reflectance) or through a non-diffusely scattering sample (transmittance), Raman microscopy relies on direct imaging of the probed sample zones and can achieve diffraction limited spatial resolution. However, when attempting to obtain spatially resolved spectra from regions below the surface of an optical turbid (diffusely scattering) sample, often mainly diffusely scattered photons reach the detector, and the spatial resolution is degraded. There is also a reduction in signal contrast at greater depths as the contribution of diffuse photons increases relative to the ballistic (non-diffusely scattered) component of light.³ While several vibrational spectroscopy techniques can obtain sub-surface spectra in the depth range 0.1–200 μm in biological tissues (e.g., attenuated total reflectance infrared spectroscopy (ATR), photoacoustic IR spectroscopy, thermal emission decay Fourier transform infrared spectroscopy (TED FT-IR), single and multifocal confocal Raman microscopy, total internal reflection Raman spectroscopy, numerous biomedical applications require penetration depths of millimeters or centimetres.^{4–9}

Diffuse Raman spectroscopy techniques, operating in either space- or time-domain, have been developed to probe deeper below the surface of optically turbid samples, either by discriminating the diffusely scattered photons from the ballistic photons or by time-resolving the diffusely scattered component of light.^{10–12} Surface signal suppression can also be achieved with CW lasers by separating the excitation and detection points spatially in order to avoid the detection of ballistic photons. This spatial offset Raman spectroscopy (SORS) technique relies

on offsetting the excitation and detection points on the same surface.¹⁰ The excitation or detection zones can be arranged in a circle or semi-circle to maximize signal collection for a given spatial offset distance. In the case of conventional ring-collection SORS there is a single excitation point at the center of the ring formed by multiple collection points, which can be averaged to create a single spectrum for a give excitation-detection offset. Inverse SORS (ISORS) inverts this approach where a single detection point is placed at the center of a ring illumination.¹³

Multiple implementations of SORS techniques exist, including the classical approach of using fiber optic SORS probes based on central laser delivery and rings of detection fibres, use of axicon lenses or multimode optical fibers to create ring illumination for ISORS, using spatial light modulator to create patterns of detection and microscope defocusing techniques.^{13–20} SORS and its variants have been used for a broad range of applications, including medical diagnosis, regenerative medicine, pharmaceuticals, cultural heritage, food industry and security.^{21–33}

One of the main challenge in SORS is the reduction in signal contrast and intensity caused by the photon diffusion process. This effect is more pronounced when probing deeper under the sample, which requires larger offsets between excitation and collection, which increases the attenuation of light within a highly scattering medium. This means that signal from a SORS system can be weaker than in conventional Raman microspectroscopy. Increasing the power of the laser is often not a viable option because the power density is often limited by sample photodamage.

In this study we investigated the use of finite element modelling of light propagation in turbid materials to optimize the spatial configuration for laser excitation and Raman detection geometry at the sample surface to target a specific region of interest (ROI) beneath the surface. Work based on Monte Carlo have been previously used for improving resolution in conventional backscattering and transmission Raman spectroscopy.³⁴ Monte Carlo simulations have also been conducted to improve performance of SORS proposing a ring illumination and point collection geometry.^{35,36} Here we focus on optimizing the positions of excitation and detection points with the goal to enhance the signal originating from the ROI and minimize the photons outside this region (i.e., maximize the contrast between the region of interest and surrounding tissue). At the same time, increasing the number of excitation and collection points can increase the optical throughput while enabling to maintain the power density below the tissue safety thresholds, and

therefore can increase the signal to noise ratio. Here we compare computational results using NIRFAST software with experimental SORS data for layered polymer samples.^{37,38} Optimisation using modelling for diffuse Raman spectroscopy and 3D imaging in turbid samples has been reported^{3,39,40} although here our goal is uniquely to maximize the spectral contrast in the Raman spectrum from a particular ROI within the turbid matrix. This is especially important in situations where the ROI is of similar chemical make-up to that of the surrounding matrix, such as in bone grafts studies.

<AQ>Please confirm that ALL Greek characters appear in normal face (i.e., Roman, not italic). All variables represented by a Latin letter should be in italics. All constants and designators (i.e., λ_{max}) should be in normal (Roman) face. Vectors should be lowercase bold and matrices should be uppercase bold (both in normal, Roman, face).</AQ>

Materials and Methods

Modeling

The computational models are based on NIRFAST software as developed for modelling diffuse optical imaging and tomography.³⁸ NIRFAST utilizes a finite element method (FEM) that models photon propagation, the forward problem, using diffusion theory and then recovers the spatial distribution of the optical properties, the inverse problem has previously been used for diffuse Raman tomography of ex-vivo canine bone.³⁷ The focus of this study was the spectral contrast (C) defined as the ratio of the collected Raman photons generated in a particular region of interest (ROI) within the sample (S_{ROI}) relative to the collected Raman photons originated from outside the ROI (S_{Out}) and assuming the two zones are two chemical species yielding two Raman bands:

$$C = \frac{S_{ROI}}{S_{Out}} \quad (1)$$

Both two-dimensional (2D) and three-dimensional (3D) models were investigated. For the preliminary 2D computations a rectangular $41 \times 20 \text{ mm}^2$ sample was modelled with a circular-shaped ROI of 10 mm diameter. The ROI was set at depths from 0 to 10 mm in 1 mm increments, with reduced scattering coefficient $\mu_s'(785 \text{ nm})=1.065 \text{ mm}^{-1}$ and absorption coefficient $\mu_a(785 \text{ nm})=0.0181 \text{ mm}^{-1}$ for both the excitation and detection wavelengths at 785

nm (typical values for PMMA).³⁷ Each simulation was run twice for each depth, once with an effective Raman cross-section 1 in the ROI and 0 everywhere else, creating the values S_{ROI} , the other was inverted so the effective Raman cross-section was 0 in the ROI and 1 everywhere else, creating the values S_{Out} . These two values were then used to calculate the signal contrast using Eq. 1.

The model consisted of 41 laser excitation points equally spaced at one scattering distance inside the sample (below its illumination/collection surface) ($1/\mu_s' = 0.94$ mm) to allow accurate simulation of a diffuse isotropic source. For the 2D computation a source and a detector were placed in 1 mm steps from -20 mm to 20 mm and the combination of each source with each detector was computed.

Configurations for which the 2D modelling indicated an improved spectral contrast were further investigated using more realistic 3D models and then compared to experimental data. The 3D simulations were designed with the same dimensions and properties as the polymer samples with dimension $30 \times 30 \times 20$ mm³. The ROI was a 1.5 mm height cylinder of radius 3 mm placed at 1.5, 3.0, and 4.5 mm depths (measured from its center/top). Excitation and detection points were placed from -8 mm to $+8$ mm in 1 mm intervals. As in the 2D simulations the software places the sources one scattering distance below the sample surface. For all simulation, the scattering coefficients for the excitation wavelength were the same for all materials: $\mu_s'(785 \text{ nm}) = 1.065 \text{ mm}^{-1}$ and $\mu_a(785 \text{ nm}) = 0.0181 \text{ mm}^{-1}$. In order to allow the calculation of spectral contrast from the experimentally measured Raman spectra, we selected materials with Raman bands at different but similar wavelengths. The scattering properties at the Raman wavelength assigned to the ROI was $\mu_s'(897 \text{ nm}) = 0.98 \text{ mm}^{-1}$ and $\mu_a(897 \text{ nm}) = 0.0162 \text{ mm}^{-1}$. The scattering properties at the Raman wavelength assigned to the bulk material (outside ROI), was $\mu_s'(885 \text{ nm}) = 0.99 \text{ mm}^{-1}$ and $\mu_a(885 \text{ nm}) = 0.017 \text{ mm}^{-1}$.³⁸ This was to account for the fact that scattering coefficients are different over the wavelengths of the spectrum. It has been shown by Musca et al. that this difference in scattering coefficient is significant enough that it can be used to predict the depth of a ROI within a sample.^{42,43}

Raman Instrument

A schematic of the instrument is presented in Figure 1. The instrument used a digital micromirror device (DMD) in the Raman detection path to allow software selection of various configurations

of detection points.^{18,25} Two galvo scanners were used together to allow selection of different 2D excitation patterns on the surface of the sample. Laser wavelength was 785 nm with a power of 50 mW and an integration time of 250 seconds from the summation of five separate 50 second integrations. The DMD acts as a programmable slit as described in earlier work.²⁵

[Insert Figure 1]

The Raman light from the sample plane was focused onto a DMD before being collected by the spectrometer. The DMD works as a programable slit for the spectrometer in such a way as to allow for software selection of detection offsets.¹⁸ By moving the collection point on the DMD the collection point on the sample is moved from the central position by an amount that is dependent on the magnification of the system:

$$\Delta x = s \times \frac{f_0}{f_1} \quad (2)$$

where Δx is the offset at the sample surface, s is the distance of the collection point from the excitation point on the DMD, f_0 is the focal length of the objective lens, and f_1 is the focal length of the lens that focuses on the DMD. A magnification of one was selected in this study. It has been shown that various configurations of detection points are possible with the only constraint being that each detection point must be unique vertically to avoid the overlap of spectra on the CCD.¹⁹ The addition of the two scanning galvo mirrors allowed for fast changing the angle of the excitation laser in order to generate various laser patterns on the sample in a time-sharing mode (the laser pattern was created by scanning the laser beam during the acquisition time of the Raman photons by the CCD).

Samples

To validate the computational results obtained from the models, Raman spectroscopy measurements were performed on phantom samples made of polymer materials with optical scattering properties matching the values included in the NIRFAST models. The samples consisted of bulk PMMA (five 3 x 30 x 30 mm³ layers stacked on top of a 5 x 30 x 30 mm³ sheet) with a *trans*-stilbene inclusion representing the ROI. The *trans*-stilbene ROI was a 1.5 mm thick 6 mm diameter cylinder that could be placed inside a matching hole drilled in the PMMA layers.

The use of two separate materials allowed for different peaks to be used to identify the contribution from each material to the measured spectra. The *trans*-stilbene bands (1575 cm^{-1} and 1625 cm^{-1}) do not overlap with the PMMA band (1450 cm^{-1}). While in biological materials this it is not always the case that bands of interest are easily separable this approach has allowed us to accurately determine where the signal was being generated, and thus compare the experimental results with the modelling results.

Results and Discussion

2D Modelling

Although results from 2D modelling cannot be directly extrapolated to real SORS experimental data (signal intensity is related to 3D propagation of diffuse photons), the fast computation allowed us to search for approximate optimal signal contrast when placing the ROI at different depths using an exhaustive combinations of laser sources and detector placement. Figure 2 shows the optimal signal contrast obtained when using a single detector and combinations of laser sources in different SORS configurations, including the conventional “centered SORS” that uses a single laser source placed at the sample surface above the ROI and two detectors placed at equal distance on both sides of the laser source.

[Insert Figure 2]

The typical NIRFAST results in Fig. 2bii show that light scatters deepest at the midpoint of the source detector pair and the signal is described by a flattened arc extending between the excitation and detection points. This shape leads to a suppression of the Raman signal from the sample surface in favor for Raman photons excited in the ROI. Figure 2c shows that the signal contrast for all SORS configuration was similar to the contrast obtained in backscatter when the ROI was at a depth less than $\sim 2\text{ mm}$. When the depth increased beyond 2 mm , optimized SORS provided a higher spectral contrast compared to centered SORS: twofold higher at 5 mm depth and fourfold at 10 mm depth.

The improvements in signal contrast in optimized SORS compared to a simple centered SORS can be explained by comparing the positions of the laser sources and detectors corresponding to the optimal configurations (Figs. 2d and e). At 0 mm depth the optimal source and detector offset is 0 mm , which matches the expected result that backscatter Raman imaging is optimal for surface Raman spectroscopy. As the ROI depth increases the optimal offset

position of the source and the detector increases, as they are displaced in opposite sides of the ROI. However, the optimized SORS offsets simultaneously both the laser sources and detection points in opposite direction providing more efficient suppression of the surface/background signal compared to centered SORS (which forces either the detector or laser at the central point position). This effect is enhanced when the depth of the ROI increases. These computational results indicate that centered SORS configuration is not always optimal for a particular measurement condition and that optimization of spectral contrast may be possible.

3D modelling and experimental validation

Although the 2D modelling indicated that signal contrast can be optimized in SORS, the results cannot be used to determine quantitatively the improvements achievable in real experiments because light scattering occurs in 3D. Thus, 3D modelling was carried out guided by the information gathered from 2D modelling in order to calculate physically relevant values. The 3D models were then compared to experimental data to evaluate the accuracy of NIRFAST modelling to quantitatively estimate improvements in signal contrast for SORS. Figure 3 compares 3D NIRFAST results with experimental data obtained on PMMA bulk samples with a *trans*-stilbene ROI at 1.5 mm below the surface.

[Insert Figure 3]

The figure presents a cross section through the center of block and the defect in the XZ plane. Fig. 3a shows that the optimized configuration where both excitation sources and detector are offset in opposite direction at the sample surface. The centered SORS configuration used a single source and detector, the laser source being maintained at the midpoint position. Fig. 3b shows the spectral contrast calculated from the NIRFAST modelling at each detector position for both centered SORS and optimized SORS configurations. As expected, the centered SORS signal contrast shows a symmetrical distribution about the central position corresponding to the laser, with a maximum obtained when the detector offset was ± 3 mm. For optimized SORS the contrast increases when the detector is offset on the opposite direction to the laser source, producing an asymmetric response, with a maximum when the detector is offset at -1 mm and the laser source at $+3$ mm. The results also show that optimization provides a 1.15-fold improvement in signal contrast.

To experimentally verify the modelling results, both configurations were applied to a PMMA bulk sample with a *trans*-stilbene (TS) ROI placed at 1.5 mm below the surface. Figure

3c shows that both spectra consist of Raman bands assigned to both PMMA (1450 cm^{-1}) and TS (doublet 1550 cm^{-1} and 1650 cm^{-1}). The results show that the intensity of the PMMA band at 1450 cm^{-1} in the centered spectrum is lower than in the SORS spectrum, indicating that optimized SORS has a higher contribution from the TS-ROI relative to the background PMMA. Figure 3d presents the calculated spectral contrast from both configurations which is the ratio of the area under the peaks of the TS bands (from 1550 cm^{-1} to 1675 cm^{-1}) divided by the area under the peak of the PMMA band (1400 cm^{-1} to 1500 cm^{-1}) when the offsets of the laser and detector was scanned across the field of view of the instrument (-8 mm to 6 mm). The spectral contrast curves obtained from the measured Raman spectra match the shapes of the curves obtained from the NIRFAST and indicate maxima at the similar laser/detector offsets. Furthermore, a 1.2-fold improvement in spectral contrast was obtained, in agreement with the 1.15 value predicted by NIRFAST.

Figure 4 presents a comparison between the experimental results and modelling when the TS ROI was inserted at 3 mm below the surface of the surface. Figure 4a suggests that the PMMA layer in between the ROI and surface has less contribution in optimized configuration than the centered SORS as there is more signal generated with spatial origins deeper within the sample and in the ROI specifically. In agreement with the modelling results, the intensity of the TS bands relative to PMMA bands is higher in the optimized SORS compared to the centered SORS spectra. Figure 4b shows that for SORS the spectral contrast has a symmetric shape when the detector is offset on both sides of the laser source with a maximum value when the detector offset was $\pm 3\text{ mm}$. The asymmetry in the optimized configurations spectral contrast is more pronounced, with a maximum at corresponding to -3 mm laser offset and $+4\text{ mm}$ detector offset. The modelling results indicate that the maximum improvement in signal contrast for optimized SORS compared to centered SORS is 1.55-fold. The measured spectra in Figure 4c confirm these results, indicating a maximum 1.5-fold improvement in spectral contrast SORS when the laser was offset at -3 mm and the detector at $+4\text{ mm}$.

[Insert Figure 4]

Figure 5 presents the comparison between the spectral contrast for optimized and centered SORS when the ROI was included at 4.5 mm below the surface, for both NIRFAST modelling and experiment. While the modelling results in Figure 5a confirm that the optimization leads to less contribution from the superficial PMMA layer, the experimental

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results in Figure 5c show that the intensity the TS bands relative to the PMMA are significantly higher in the optimized spectra compared to SORS.

[Insert Figure 5]

The calculated spectral contrast from NIRFAST indicates a similar trend as observed in Figs 3c and 4c, the results in Figure 5b suggest an improvement in signal contrast for optimized SORS 2.25-fold higher than for centered SORS. This maximum corresponds to -6 mm laser offset and +5 mm detector offset. The calculated value for spectral contrast from the measured spectra confirmed these results showing a twofold improvement for optimized versus centered SORS. However, the maximum was obtained when the laser was offset at -4 mm and detector offset +2 mm, which are smaller than the values predicted by NIRFAST. These values may be explained by the fact that the optical throughput of our instrument decreased for such large offsets because of the limited size of the optics.

3D Modelling of Multiple Excitation/Detection Points in a Single Measurement

The simulation and experimental validation results have shown that the placement of a single point excitation and single point detection can significantly affect spectral contrast in SORS. However, single point SORS requires higher excitation power than is safe in biomedical settings. It is typical to increase optical throughput by increasing the number of detection-excitation points. This can be achieved by using a fiber probe with a configuration where all the detectors encircle a single excitation point at given radius (circular SORS), or a ring illumination around a central detection point (ISORS). This approach creates symmetrical probes that have been shown to be highly efficient when used on layered samples. The biggest advantage of this methods such as ISORS is that it allows for an increased surface area of excitation. This means that much higher powers can be used while maintain biologically safe power density levels.

However, such configurations may not provide optimal signal contrast when used to investigate ROI of limited volume, particularly when the ROI is small enough to fit fully within the detection/excitation ring. If the ROI is placed along the axis of symmetry, with its center in line with the central excitation/detection point then the contribution from each fiber around the circumference will be the same. Once symmetry is broken and the ROI is no longer centered then each fiber will have a different contribution to the total signal. To investigate this, we simulated as ROI of interest 4.5 mm deep in the same configuration as for Figure 5. We

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3 simulated a central excitation point with a ring of 16 detectors around the excitation point. We
4 calculated contrast with a ring centered on the ROI and with the ring off-center with respect to
5 the ROI. The centered ring used the optimal offset found for centered single point which was 8
6 mm radius. The source placement for the off-center SORS was chosen as the optimal source
7 detector pairing from Figure 5 with a source at -6 mm and the ring of detectors around this at a
8 distance of 11 mm.
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13 [Insert Figure 6]
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15 As expected from the earlier models, Fiber 1 in the off-axis configuration shows the best signal
16 contrast. As the fibres move around the circles however signal contrast reduces until it is worse
17 than the symmetric configurations. The signal contrast for each of the fibres for the sample
18 centered configuration is the same as expected. Since multiple points are used so that the signals
19 can be combined, we also showed the signal contrast for both configurations when the signal is
20 summed. In this case the sample centered SORS has approximately two times better signal
21 contrast than the off-axis SORS configuration.
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27 From this we can conclude that with a ring probe it is best practice to center the probe
28 over the ROI in order to maximize signal contrast, even though each individual fiber produces
29 less than the maximum possible contrast. In samples where maximizing optical throughput is
30 more important than signal contrast, such as in synthetic materials where the distinguishing
31 spectral bands may not overlap, this method continues to be a powerful tool.
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36 However, if the maximization of signal contrast is imperative then a more complex
37 solution for excitation and detection patterns may provide a better signal contrast and improve
38 optical throughput. Samples where spectral contrast is needed include those samples where the
39 difference between the spectra of the ROI and the bulk are very small. These configurations
40 would be highly specific to the morphology of the ROI and bulk material the first fiber (Fiber 1)
41 was the optimized configuration. Fibres 2 and 16 are adjacent to the optimized fiber and have
42 improved signal contrast compared to the centered SORS configurations. Fibres 3 and 15 have
43 less than half the signal contrast of centered SORS. The rest of the fibres has negligible signal
44 contrast. With further investigation the configurations of excitation and detection points may be
45 able to be developed to combine the signal contrast improvements of off-axis circular-centered
46 SORS with the signal to noise ratio benefits of the sample centered, circular centered SORS.
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55 NIRFAST can therefore be used as a tool for optimizing the configuration of the probe to
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maximize these quantities without the need for costly and timely prototyping and experimentation. Modelling could support the optimization of SORS instruments to improve signal contrast and in this way may facilitate shorter integration times and improve the feasibility of SORS for biomedical applications.

Conclusion

In this study we showed that NIRFAST modelling of light propagation in turbid materials can be used to predict important performance parameters in spatial offset Raman spectroscopy, such as spectral contrast. The modelling results were validated experimentally and showed that modelling can be used to determine optimal configurations of laser and detector points for SORS measurements. While such configurations can be implemented in Raman instruments based on spatial light modulators providing flexible software control of the laser–detection patterns, fiber optic bundles can be designed for specific applications and samples. The sample dimensions and scattering properties explored in this study are relevant to biomedical applications. Although off-center SORS based on a single laser point and detector may have insufficient optical throughput for biomedical applications, the use of numerical modelling for optimizing the SORS configurations could play a significant role in biomedical applications of SORS in the future.

Acknowledgments

This work was supported by the Engineering and Physical Sciences Research Council [grant number EP/L025620/1].

References
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1. D.W. Shipp, F. Sinjab, I. Notinger. Raman Spectroscopy: Techniques and Applications in the Life Sciences". Adv. Opt. Photonics. 2017. 9(2): 315–428.

2. M. Delhaye, P. Dhamelinourt. "Raman Microprobe and Microscope with Laser Excitation".
J. Raman Spectrosc. 1975. 3(1): 33–43.
3. B.B. Das, F. Liu, R.R. Alfano. "Time-Resolved Fluorescence and Photon Migration Studies in
Biomedical and Model Random Media". Rep. Prog. Phys. 1997. 60(2): 227.
4. K.A. Chan, F.H. Tay, G. Poulter, S.G. Kazarian. "Chemical Imaging with Variable Angles of
Incidence Using a Diamond Attenuated Total Reflection Accessory". Appl. Spec. 2008.
62(10): 1102–1107.
5. R.M. Dittmar, J.L. Chao, R.A. Palmer. "Photoacoustic Depth Profiling of Polymer Laminates
by Step-Scan Fourier Transform Infrared Spectroscopy". Appl. Spec. 1991. 45(7): 1104–
1110.
6. I. Notingher, R.E. Imhof, P. Xiao, F.C. Pascut. "Spectral Depth Profiling of Arbitrary Surfaces
by Thermal Emission Decay–Fourier Transform Infrared Spectroscopy". Appl. Spec.
2003. 57(12): 1494–1501.
7. N.J. Everall, J. Lapham, F. Adar, A. Whitley, et al. "Optimizing Depth Resolution in Confocal
Raman Microscopy: A Comparison of Metallurgical, Dry Corrected, and Oil Immersion
Objectives". Appl. Spec. 2007. 61(3): 251–259.
8. Z. Liao, F. Sinjab, H.M. Elsheikha, I. Notingher. "Optical Sectioning in Multifoci Raman
Hyperspectral Imaging". J. Raman Spectrosc. 2018. 49(10): 1660–1667.
9. D.A. Woods, C.D. Bain. "Total Internal Reflection Raman Spectroscopy". Analyst. 2012.
137(1): 35–48.
10. P. Matousek, I.P. Clark, E.R. Draper, M.D. Morris, et al. "Subsurface Probing in Diffusely
Scattering Media Using Spatially Offset Raman Spectroscopy". Appl. Spec. 2005. 59(4):
393–400.
11. P. Matousek, N.J. Everall, M. Towrie, A.W. Parker. "Depth Profiling in Diffusely Scattering
Media Using Raman Spectroscopy and Picosecond Kerr Gating". Appl. Spec. 2005.
59(2): 200–205.
12. C. Corden, P. Matousek, C. Conti, I. Notingher. "Sub-Surface Molecular Analysis and
Imaging in Turbid Media Using Time-Gated Raman Spectral Multiplexing". Appl. Spec.
2021. 75(2): 156–167.
13. S. Musca, C. Conti, N. Stone, P. Matousek. "Spatially Offset Raman Spectroscopy". Nat.
Rev. Methods Primers. 2021. 1(1): 1–16.

14. P. Matousek. "Inverse Spatially Offset Raman Spectroscopy for Deep Noninvasive Probing of Turbid Media". *Appl. Spec.* 2006. 60(11): 1341–1347.
15. P. Matousek, E.R. Draper, A.E. Goodship, I.P. Clark, et al. "Noninvasive Raman Spectroscopy of Human Tissue in Vivo". *Appl. Spec.* 2006. 60(7): 758–763.
16. M.V. Schulmerich, K.A. Dooley, T.M. Vanasse, S.A. Goldstein, M.D. Morris. "Subsurface and Transcutaneous Raman Spectroscopy and Mapping Using Concentric Illumination Rings and Collection with a Circular Fiber-Optic Array". *Appl. Spec.* 2007. 61(7): 671–678.
17. K.M. Khan, S.B. Dutta, N. Kumar, A. Dalal, et al. "Inverse Spatially-Offset Raman Spectroscopy Using Optical Fibers: an Axicon Lens-Free Approach". *J. Biophotonics.* 2019. 12(11): E201900140.
18. Z. Liao, F. Sinjab, G. Gibson, M. Padgett, I. Notingher. "DMD-Based Software-Configurable Spatially-Offset Raman Spectroscopy for Spectral Depth-Profiling of Optically Turbid Samples". *Opt. Express.* 2016. 24(12): 12701–12712.
19. Z. Liao, F. Sinjab, A. Nommeots-Nomm, J. Jones, et al. "Feasibility of Spatially Offset Raman Spectroscopy for in Vitro and in Vivo Monitoring Mineralization of Bone Tissue Engineering Scaffolds". *Anal. Chem.* 2017. 89(1): 847–853.
20. C. Conti, M. Realini, C. Colombo, P. Matousek. "Comparison of Key Modalities of Micro-Scale Spatially Offset Raman Spectroscopy". *Analyst.* 2015. 140(24): 8127–8133.
21. K. Buckley, J.G. Kerns, J. Vinton, P.D. Gikas, et al. "Towards the in Vivo Prediction of Fragility Fractures with Raman Spectroscopy". *J. Raman Spectrosc.* 2015. 46(7): 610–618.
22. M.V. Schulmerich, J.H. Cole, J.M. Kreider, F. Esmonde-White, et al. "Transcutaneous Raman Spectroscopy of Murine Bone in Vivo". *Appl. Spec.* 2009. 63(3): 286–295.
23. G. Thomas, T.Q. Nguyen, I.J. Pence, B. Caldwell, et al. "Evaluating Feasibility of an Automated 3-Dimensional Scanner Using Raman Spectroscopy for Intraoperative Breast Margin Assessment". *Sci. Rep.* 2017. 7(1): 1–14.
24. F. Nicolson, M.F. Kircher, N. Stone, P. Matousek. "Spatially Offset Raman Spectroscopy for Biomedical Applications". *Chem. Soc. Rev.* 2021. 50(1): 556–568.

25. M. Dooley, A. Prasopthum, Z. Liao, F. Sinjab, et al. "Spatially-Offset Raman Spectroscopy for Monitoring Mineralization of Bone Tissue Engineering Scaffolds: Feasibility Study Based on Phantom Samples". *Biomed. Opt. Express*. 2019. 10(4): 1678–1690.
26. K.J. Ember, M.A. Hoeve, S.L. McAughtrie, M.S. Bergholt, et al. "Raman Spectroscopy and Regenerative Medicine: A Review". *NPJ Regener. Med*. 2017. 2(1): 1–10.
27. M.S. Bergholt, A. Serio, M.B. Albro. "Raman Spectroscopy: Guiding Light for the Extracellular Matrix". *Front. Bioeng. Biotechnol*. 2019. 7: 303.
28. M. Dooley, McLaren, J., Rose, F.R., I. Notingher. "Investigating the Feasibility of Spatially Offset Raman Spectroscopy for In-Vivo Monitoring of Bone Healing in Rat Calvarial Defect Models". *J. Biophotonics*. 2020. 13(10): E202000190.
29. C. Eliasson, P. Matousek. "Noninvasive Authentication of Pharmaceutical Products Through Packaging Using Spatially Offset Raman Spectroscopy". *Anal. Chem*. 2007. 79(4): 1696–1701.
30. W.J. Olds, S. Sundarajoo, M. Selby, B. Cletus, et al. "Noninvasive, Quantitative Analysis of Drug Mixtures in Containers Using Spatially Offset Raman Spectroscopy (SORS) and Multivariate Statistical Analysis". *Appl. Spec*. 2012. 66(5): 530–537.
31. C. Conti, A. Botteon, C. Colombo, D. Pinna, et al. "Advances in Raman Spectroscopy for the Non-Destructive Subsurface Analysis of Artworks: Micro-SORS". *J. Cult. Herit*. 2020. 43: 319–328.
32. J.D. Landry, P.J. Torley, E.W. Blanch. "Detection of Biomarkers Relating to Quality and Differentiation of Some Commercially Significant Whole Fish Using Spatially Off-Set Raman Spectroscopy". *Molecules*. 2020. 25(17): 3776.
33. W.J. Olds, E. Jaatinen, P. Fredericks, B. Cletus, et al. "Spatially Offset Raman Spectroscopy (SORS) for the Analysis and Detection of Packaged Pharmaceuticals and Concealed Drugs". *Forensic Sci. Int*. 2011. 212(1–3): 69–77.
34. S. Musca, P. Dey, M. Salimi, B. Gardner, et al. "Spatially Offset Raman Spectroscopy: How Deep?". *Anal. Chem*. 2021. 93(17): 6755–6762.
35. N.J. Everall, I. Priestnall, P. Dallin, J. Andrews, et al. "Measurement of Spatial Resolution and Sensitivity in Transmission and Backscattering Raman Spectroscopy of Opaque Samples: Impact on Pharmaceutical Quality Control and Raman Tomography". *Appl. Spec*. 2010. 64(5): 476–484.

36. P. Matousek, M.D. Morris, N.J. Everall, I.P. Clark, et al. "Numerical Simulations of Subsurface Probing in Diffusely Scattering Media Using Spatially Offset Raman Spectroscopy". *Appl. Spec.* 2005. 59(12): 1485–1492.
37. S. Srinivasan, M.V. Schulmerich, J.H. Cole, K.A. Dooley, et al. "Image-Guided Raman Spectroscopic Recovery of Canine Cortical Bone Contrast In Situ". *Opt. Express*. 2008. 16(16): 12190–12200.
38. M. Doulgerakis-Kontoudis, A.T. Eggebrecht, S. Wojtkiewicz, J.P. Culver, H. Dehghani. "Toward Real-Time Diffuse Optical Tomography: Accelerating Light Propagation Modeling Employing Parallel Computing on GPU and CPU". *J. Biomed. Opt.* 2017. 22(12): 125001.
39. H. Dehghani, M.E. Eames, P.K. Yalavarthy, S.C. Davis, et al. "Near Infrared Optical Tomography Using NIRFAST: Algorithm for Numerical Model and Image Reconstruction". *Comm. Numer. Meth. Eng.* 2009. 25(6): 711–732.
40. M.V. Schulmerich, J.H. Cole, K.A. Dooley, M.D. Morris, et al. "Noninvasive Raman Tomographic Imaging of Canine Bone Tissue". *J. Biomed. Opt.* 2008. 13(2): 020506.
41. H.Y. Wu, A. Filer, I. Styles, H. Dehghani. "Development of a Multi-Wavelength Diffuse Optical Tomography System for Early Diagnosis of Rheumatoid Arthritis: Simulation, Phantoms and Healthy Human Studies". *Biomed. Opt. Express*. 2016. 7(11): 4769–4786.
42. S. Musca, P. Dey, T.A. Tabish, F. Palombo, et al. "Spatially Offset and Transmission Raman Spectroscopy for Determination of Depth of Inclusion in Turbid Matrix". *Anal. Chem.* 2019. 91(14): 8994–9000.
43. J.L. Ferretti., R.F. Capozza, N. Mondelo, J.R. Zanchetta. "Interrelationships Between Densitometric, Geometric, and Mechanical Properties of Rat Femora: Inferences Concerning Mechanical Regulation of Bone Modeling". *J. Bone Miner. Res.* 1993. 8(11): 1389–1396.

Captions

Figure 1. Schematic of the Raman instrument. DCM: dichroic mirror; f_0 : objective lens that focuses the laser on the sample surface and collects the signal from the sample; f_1 : lens that focuses the collected Raman light on the DMD; f_2 : lens that collects/collimates the Raman light from the DMD at the selected spatial offsets; f_3 : lens that focuses the Raman light into the

spectrometer. The spectrometer has a 4 mm wide slit.

Figure 2. Two-dimensional NIRFAST modelling of SORS. (a) Schematic of the sample geometry: triangles represent laser sources (blue triangle represents the laser source at the center of the sample, cross: detector; white area: ROI; black area: bulk material. (b) Typical 2D NIRFAST results presenting the origin and intensity of the Raman signal calculated for an optimal configuration in three configurations. (i) Backscatter Raman (conventional Raman, i.e., zero spatial offset), (ii) single excitation and detection points both offset, and (iii) a single central excitation point with two symmetric detection point (arrows indicate the position of the laser source and detector). (c) The optimal spectral contrast computed by NIRFAST for various source/detector configurations depending on the ROI depth. (d) The optimal configurations for signal contrast when placing the ROI at different depths for a single source and detector. (e) The optimal configurations for a centered excitation with two detectors for signal contrast when placing the ROI at different depths. (f) The optimal configurations for a centered detection point with two excitation points for signal contrast when placing the ROI at different depths.

Figure 3. Comparison between 3D NIRFAST modelling and experimental SORS when ROI is 1.5 mm below the sample surface. (a) The spatial distribution of the origin of signal for optimized SORS and centered SORS. The blue line outlines the ROI; The figure presents a cross section through the center of block in the XZ plane. (b) Calculated signal contrast from NIRFAST modelling blue: centered SORS with 0 mm offset laser source, and red: optimal SORS when the source was at -1 mm from center. The colored bar above and below the lines are the 5% error bounds. (c) The Raman spectra for both configurations compared to reference PMMA and trans-stilbene spectra; the red optimized spectrum corresponds to -1 mm offset source and a 3 mm offset detector, and the blue centered SORS spectrum corresponds to 0 mm offset source and a 3 mm offset detector. (d) Calculated signal contrast from the measured optimized SORS (-1 mm source offset) and SORS (0 mm source offset) spectra with detectors from -8 to +6 mm. For (b) and (d), the signal contrast is normalized so that the optimal single contrast from centered SORS is equal to unity; laser source: blue/red triangle; detector: black cross).

Figure 4. (a) The spatial distribution of the origin of signal for optimized SORS and centered SORS. The blue line outlines the ROI. (b) Calculated signal contrast from 3D NIRFAST modelling blue: SORS with 0 mm offset laser source, and red: optimal SORS when the source was at -1 mm from center. The colored bar above and below the lines are the 5% error bounds. (c) The Raman spectra for both configurations compared to reference PMMA and trans-stilbene spectra: red optimized spectrum corresponds to -3 mm offset source and a 3 mm offset detector, and the blue SORS spectrum corresponds to 0 mm offset source and a 5 mm offset detector. (d) Calculated signal contrast from the measured optimized SORS (-1 mm source offset) and SORS (0 mm source offset) spectra with detectors from -7 to $+7$ mm. For (b) and (d), the signal contrast is normalized so that the optimal single contrast from centered SORS is equal to unity; laser source: blue/red triangle; detector: black cross).

Figure 5. Comparison between 3D NIRFAST modelling and experimental optimized/centered SORS when ROI is 4.5 mm below the sample surface. (a) The spatial distribution of the origin of signal for both configurations. The blue line outlines the ROI. (b) Calculated signal contrast from 3D NIRFAST modelling blue: SORS with 0 mm offset laser source, and red: optimal SORS when the source was at -6 mm from center. The colored bar above and below the lines are the 5% error bounds. (c) The Raman spectra for optimized SORS and centered SORS compared to reference PMMA and trans-stilbene spectra: red optimized spectrum corresponds to -4 mm offset source and a 3 mm offset detector, and the blue centered SORS spectrum corresponds to 0 mm offset source and a 6 mm offset detector. (d) Calculated signal contrast from the measured optimized SORS (-1 mm source offset) and centered SORS (0 mm source offset) spectra with detectors from -9 to $+5$ mm. For (b) and (d), the signal contrast is normalized so that the optimal single contrast from centered SORS is equal to unity; laser source: blue/red triangle; detector: black cross).

Figure 6. (a) The spatial distribution of the origin of signal for centered SORS. The blue line outlines the ROI, each grey cross represents a detector that is numbered 1–16 and the blue triangle is the central excitation point. The image shows a cross section across the XY plane. (b) The spatial distribution of the origin of signal for off-axis positioned SORS. The blue line outlines the ROI, each grey cross represents a detector that is numbered 1–16 and the red triangle

outlined in black is the central excitation point. (c) A bar chart showing the spectra contrast for each of the detectors. The blue chart shows the spectral contrast for the centered SORS and the red shows the spectral contrast for the off-axis SORS.

Schematic of the Raman instrument. DCM - dichroic mirror, f_0 - objective lens that focuses the laser on the sample surface and collects the signal from the sample, f_1 - lens that focuses the collected Raman light on the DMD, f_2 - lens that collects/collimates the Raman light from the DMD at the selected spatial offsets, f_3 - lens that focuses the Raman light into the spectrometer. The spectrometer has a 4 mm wide slit.

183x80mm (150 x 150 DPI)

181x189mm (150 x 150 DPI)

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Comparison between 3D NIRFAST modelling and experimental SORS when ROI is 1.5 mm below the sample surface. a) The spatial distribution of the origin of signal for optimised SORS and centred SORS. The blue line outlines the ROI; The figure presents a cross section through the centre of block in the XZ plane. b) Calculated signal contrast from NIRFAST modelling blue: centred SORS with 0 mm offset laser source, and red: optimal SORS when the source was at -1 mm from centre. The coloured bar above and below the lines are the 5% error bounds; c) The Raman spectra for both configurations compared to reference PMMA and trans-stilbene spectra: red optimised spectrum corresponds to -1 mm offset source and a 3 mm offset detector, and the blue centred SORS spectrum corresponds to 0 mm offset source and a 3 mm offset detector; d) Calculated signal contrast from the measured optimised SORS (-1 mm source offset) and SORS (0 mm source offset) spectra with detectors from -8 to +6 mm. For b) and d) the signal contrast is normalised so that the optimal single contrast from centred SORS is equal to unity; laser source - blue/red triangle; detector - black cross).

175x191mm (300 x 300 DPI)

a) The spatial distribution of the origin of signal for optimised SORS and centred SORS. The blue line outlines the ROI; b) Calculated signal contrast from 3D NIRFAST modelling blue: SORS with 0 mm offset laser source, and red: optimal SORS when the source was at -1 mm from centre. The coloured bar above and below the lines are the 5% error bounds; c) The Raman spectra for both configurations compared to reference PMMA and trans-stilbene spectra: red optimised spectrum corresponds to -3 mm offset source and a 3 mm offset detector, and the blue SORS spectrum corresponds to 0 mm offset source and a 5 mm offset detector; d) Calculated signal contrast from the measured optimised SORS (-1 mm source offset) and SORS (0 mm source offset) spectra with detectors from -7 to +7 mm. For b) and d) the signal contrast is normalised so that the optimal single contrast from centred SORS is equal to unity; laser source – blue/red triangle; detector – black cross).

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Comparison between 3D NIRFAST modelling and experimental optimised/centred SORS when ROI is 4.5 mm below the sample surface. a) The spatial distribution of the origin of signal for both configurations. The blue line outlines the ROI; b) Calculated signal contrast from 3D NIRFAST modelling blue: SORS with 0 mm offset laser source, and red: optimal SORS when the source was at -6 mm from centre. The coloured bar above and below the lines are the 5% error bounds; c) The Raman spectra for optimised SORS and centred SORS compared to reference PMMA and trans-stilbene spectra: red optimised spectrum corresponds to -4 mm offset source and a 3 mm offset detector, and the blue centred SORS spectrum corresponds to 0 mm offset source and a 6 mm offset detector; d) Calculated signal contrast from the measured optimised SORS (-1 mm source offset) and centred SORS (0 mm source offset) spectra with detectors from -9 to +5 mm. For b) and d) the signal contrast is normalised so that the optimal single contrast from centred SORS is equal to unity; laser source – blue/red triangle; detector - black cross).

165x181mm (300 x 300 DPI)

a) The spatial distribution of the origin of signal for centred SORS. The blue line outlines the ROI, each grey cross represents a detector that is numbered 1-16 and the blue triangle is the central excitation point. The image shows a cross section across the XY plane. b) The spatial distribution of the origin of signal for off-axis positioned SORS. The blue line outlines the ROI, each grey cross represents a detector that is numbered 1-16 and the red triangle outlined in black is the central excitation point. c) A bar chart showing the spectra contrast for each of the detectors. The blue chart shows the spectral contrast for the centred SORS and the red shows the spectral contrast for the off-axis SORS.

243x229mm (150 x 150 DPI)

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