Inverse spatially offset Raman spectroscopy for deep spectroscopy of turbid media

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Introduction

One of the key goals of analytical sciences is the provision of a simple optical method for deep non-invasive spectroscopy of diffusely scattering media with high chemical specificity. Such a technique would be highly beneficial, for example, in disease diagnosis or in the identification of polymorphs in pharmaceutical tablets at depth and through capsules and tablet coatings. Raman spectroscopy has many key attributes for fulfilling this role such as high chemical specificity, instrumental simplicity and compatibility with water containing samples. A major feature preventing it presently from being applicable to deep layer probing in its conventional form is its excessive bias towards surface layers of probed turbid samples. This prevents the observation of weaker signals from deeper layers of probed media confining the applicability to only shallow layers.

Recently, a new approach, Spatially Offset Raman Spectroscopy [1] (SORS) for deep spectroscopy of tissue and powders has been developed through collaborative research. The principal of the SORS technique is based on collecting a set of Raman spectra from the surface regions of a sample that are at set distances, Ds, away from the point of illumination by the laser. Raman spectra obtained in this way exhibit a variation in relative intensities between the contributions from the surface and sub-surface layers. Such a set of spectra can be numerically processed to yield the pure Raman spectra of individual sub-layers. Since the first demonstration of the SORS concept [1] on powders, the technique has been used in numerous applications including the demonstration of Raman tomography in turbid media by Morris *et al.* [2], the Raman spectroscopy of bones non-invasively on cadavers and animal samples by Morris *et al.* [3] and the measurement of human bone in vivo under safe illumination conditions by our team [4].

SORS potential is, however, still yet to be fully realised as, in its most effective form based on optical fibres, it has several inherent characteristics that limit the obtainable signal-to-noise levels and consequently the accessible depths. A major limitation of the conventional fibre probe SORS concept stems from its reliance on the collection of multiple Raman spectra on different CCD tracks. These spectra are either scaled and subtracted from each other or processed using multivariate decomposition methods involving similar data manipulation steps to retrieve pure Raman spectra belonging to individual layers within the sample. The spectra collected in this way exhibit small distortions due to imaging imperfections present at some level with any spectrograph causing often major subtraction artefacts in processed spectra. This limits the sensitivity of the conventional SORS technique and consequently its capacity to recover weak Raman signals from deep layers of sample.

The above drawbacks can be eliminated by adopting a reverse delivery-collection geometry, inverse SORS. In this concept, Raman light is collected through a group of fibres tightly packed at the centre of the probe by binning all their signals on CCD chip into a single spectrum. The fibres can be randomly organised as is often the case with commercial fibre probes. The laser probe beam is brought onto the sample in the form of ring of a given radius centred at the collection zone, i.e. in reverse to conventional SORS (see Fig. 1). The artefact problems are absent as all Raman spectra are subject to the same imaging distortions and collected through the same set of CCD pixels. A similar ring-illumination approach was developed independently by Schulmerich *et al.* [5,6].

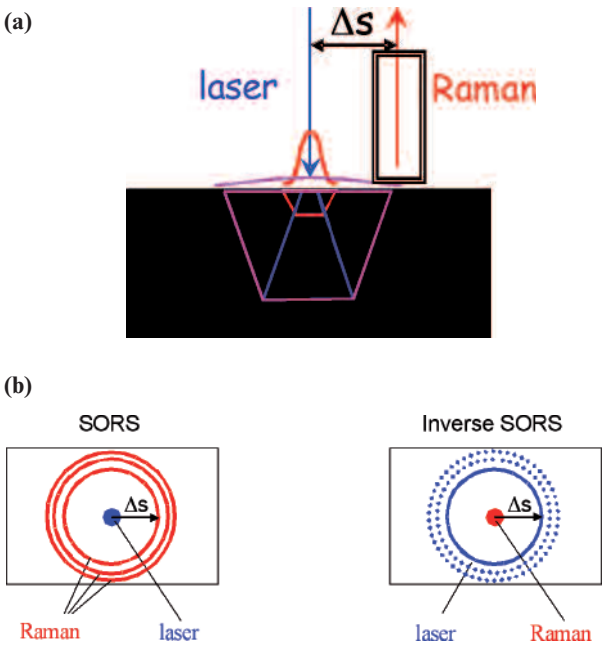

Figure 1: a) Principal of spatially offset Raman spectroscopy. b) Schematic diagram of conventional SORS and inverse SORS showing Raman collection and beam delivery geometries.

In inverse SORS, the spatial offsets can be varied simply by changing the radius of the ring illumination zone. This can be accomplished using an axicon optical element [7] positioned at different distances from the sample (see Figure 2). A single Raman spectrum with SORS spatial offset equal to the ring radius is then collected and averaged. An arbitrary number of spectra of different spatial offsets can thus be subsequently collected by simply altering the distance of the axicon from sample.

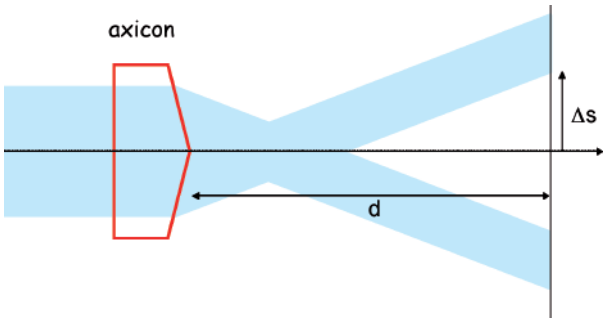

Figure 2: Schematic diagram depicting the principle of forming a ring shaped laser beam of adjustable radius using a conical lens (axicon).

Another important benefit is that the laser beam is delivered through an annulus rather than through a central spot enhancing the available illumination area and consequently higher laser powers can be employed. This is particularly beneficial in tissue in vivo spectroscopy when safe illumination intensity limits needs to be adhered to. A major advantage is also the ability to set an arbitrary spatial offset as well as their arbitrary number enabling the tailoring of the collection parameters to the sample. This is in contrast to conventional SORS fibre probes where spatial offsets are fixed as the tracks are built into the probe. Also the maximum number of tracks is limited in the conventional SORS by the number of fibres one can couple into the spectrograph slit.

This work is reported in detail in reference [8]. A review of SORS techniques and their applications is also presented in reference [9].

Experimental

The experiments were performed using a temperature stabilised diode laser for Raman spectroscopy operating at 827 nm with a laser beam power at the sample of 50 mW. The collimated beam of 3 mm diameter was passed through a UV fused silica axicon element with a cone angle $\alpha = 5°$ (DelMar Ventures) placed on a rail to permit the sample to axicon distance to be varied in the range 60 to 155 mm (corresponding to the spatial offsets from 0.9 to 7.9 mm). The zero radius (conventional Raman geometry) was realised by physically removing the axicon from the beam. For practical reasons, the transmitted part of the beam was incident on the sample at ~45° degrees away from normal incidence.

Raman light was collected in backscattering geometry 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 second lens, identical to the first one, was then used to image, with magnification 1:1, the sample interaction zone onto the front face of the annular fibre probe. The Raman light was propagated through the SORS annular fibre systems of length ~2 m to the linear fibre end oriented vertically and placed in the input image plane of a Kaiser Optical Technologies Holospec $f^{\#} = 1.4$ NIR spectrograph with its slit removed. Raman spectra were collected using a NIR back-illuminated deep-depletion TE cooled CCD camera (Andor Technology, DU420A-BR-DD, 1024 × 256 pixels).

The light collection end of the inverse SORS fibre probe was constructed with 61 fibres tightly packed at the centre of the probe although only 22 fibres within the centre of the probe could be coupled to the detector due to physical constraints stemming from the height of CCD chip. The individual fibres were made of silica with a core diameter of 220 µm. The fibre numerical aperture was 0.37. The bundle was custom made by CeramOptec Industries, Inc.

The comparative conventional SORS experiments presented here were performed using a two-track SORS fibre probe described in detail in reference [4]. In these measurements the laser power was around 80 mW at the sample and the laser beam was focused down to 0.2 mm diameter spot. The SORS probe had 7 and 26 fibres for the zero and 3 mm spatial offset tracks, respectively. The fibres had a core diameter of 200 µm and a numerical aperture of 0.37.

The demonstration experiments were performed with a standard paracetamol tablet placed against a wall of a white water drainage PVC pipe of 40 mm diameter and a wall thickness of 2 mm.

Results and Discussion

The comparison of the performance of conventional SORS and inverse SORS techniques in the recovery of a Raman signal from the sublayer in the presence of a dominant overlayer Raman signal is shown in Figure 3. The experiments were performed using a 2 mm thick white PVC pipe with a paracetamol tablet placed inside in contact with its wall. Raw spectra for two different spatial offsets using conventional SORS and the recovered pure paracetamol subsurface Raman obtained by scaled subtraction of the two spectra from each other eliminating the Raman band of the surface layer at 1450 cm^{-1} are shown in figure 3 a and b, respectively. Although the Raman spectrum of paracetamol has been recovered with sufficient quality required for determining its chemical identity the Raman spectrum is littered with large artefacts stemming from the imperfect subtraction of the surface PVC layer Raman spectrum. These types of artefacts are inherent to the conventional SORS methodology and originate from spectral distortions acquired within spectrograph as discussed earlier. Their appearance precludes more detailed analysis of Raman spectra for the presence of weak Raman features as well as the recovery of Raman spectra of very deeper layers.

In contrast, these artefacts are absent in the spectra obtained using inverse SORS as evident from figure 3d. The enhanced sensitivity of inverse SORS is particularly important in disease diagnosis where subtle Raman features are often the only indicators of the presence or absence of disease or in the monitoring of pharmaceutical products for the presence of polymorphs at low concentrations.

Conclusions

A new SORS concept, inverse SORS has been described. This technique is capable of providing distortion free Raman spectra and as such offers much higher sensitivity and penetrations depths than those available with conventional SORS. Its performance was demonstrated on a layered sample. It is foreseen that inverse SORS will find its applications in disease diagnosis, bone quality

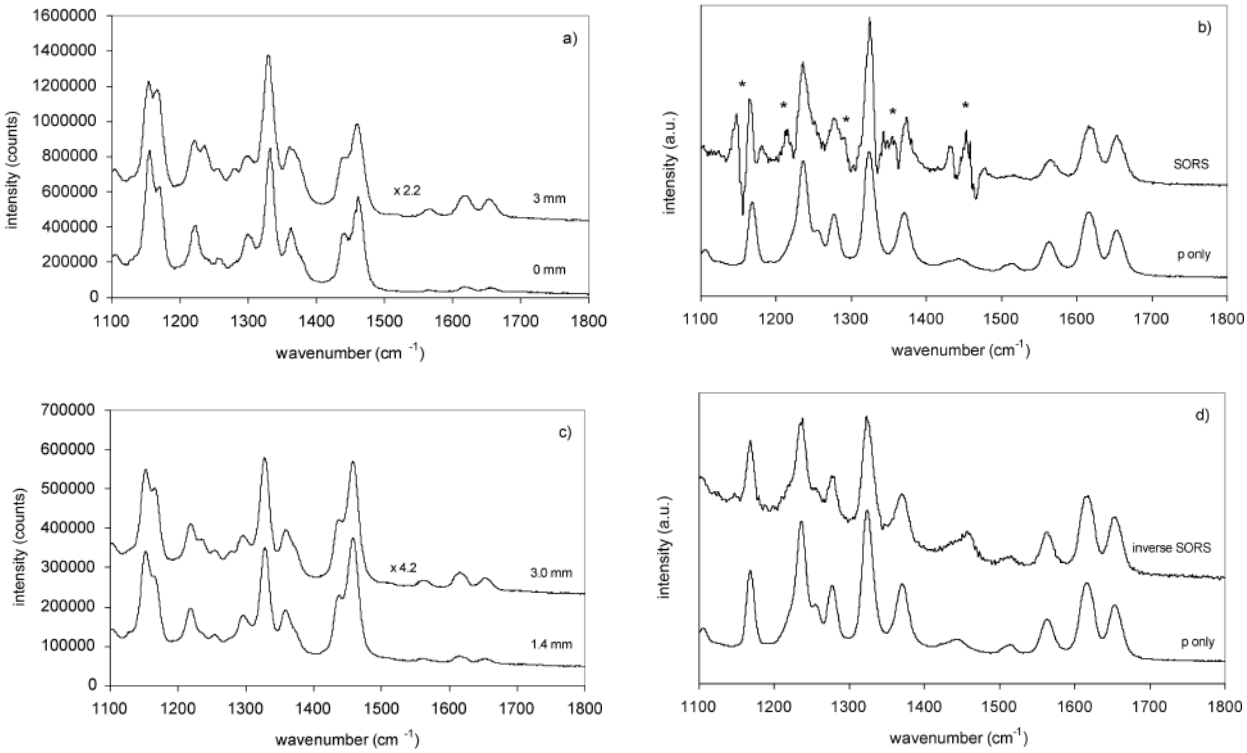

Figure 3. The comparison of the performance of (a,b) conventional SORS and (c,d) inverse SORS in the recovery of a subsurface Raman spectrum. The experiments were carried out using a paracetamol tablet placed behind a 2 mm thick white PVC wall. Raw Raman spectra obtained with two different spatial offsets are shown in the top frames. The recovered subsurface Raman spectra ('SORS' and 'inverse SORS') along with the pure Raman spectra of paracetamol ('p only') obtained separately are shown in the bottom frames. The acquisition times were 20 s for the conventional SORS and 10 s for the inverse SORS measurements. The spectra are offset for clarity. The spectrum recovered using inverse SORS is notable for the absence of subtraction artefacts present in the spectrum obtained using conventional SORS (marked with asterisk) and caused by spectrograph imaging imperfections.

assessment and industrial applications such as the probing of pharmaceutical products, paints in monitoring their adhesion to substrates or the determination of the presence of corrosion under paint, the screening of food products, the probing of powders through envelopes in homeland security applications, as well as in the research of polymer blends and filled materials.

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References

1. 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, *Appl. Spectrosc.* **59**, 393 (2005).
2. M. V. Schulmerich, W. F. Finney, R. A. Fredricks, M. D. Morris, *Appl. Spectrosc.* **60**, 109-114 (2006).
3. M. V. Schulmerich, W. F. Finney, V. Popescu, M. D. Morris, T. M. Vanasse and S. A. Goldstein, *Transcutaneous Raman spectroscopy of bone tissue using a non-confocal fiber optic array probe,* Proceedings of SPIE 6093, Biomedical Vibrational Spectroscopy III: Advances in Research and Industry, Anita Mahadevan-Jansen, Wolfgang H. Petrich, Editors, 609300 (2006).
4. P. Matousek, E. R. C. Draper, A. E. Goodship, I. P. Clark, K. L. Ronayne, A. W. Parker, *Appl. Spectrosc.* **60**, 758-763 (2006).
5. M. V. Schulmerich, K. A. Dooley, M. D. Morris, T. M. Vanasse, S. A. Goldstein, *Journal of Biomedical Optics* **11**, 060502 (2006).
6. M. V. Schulmerich, M. D. Morris, T. M. Vanasse, S. A. Goldstein, in *Proceedings of SPIE 6430, Advanced Biomedical and Clinical Diagnostic Systems V*, T. Vo-Dinh, W. S. Grundfest, D. A. Benaron, G. E. Cohn, R. Raghavachari, Eds. p. 643009 (2007).
7. B. Depret, P. Verkerk and D. Hennequin, *Opt. Commun.* **211**, 31 (2002).
8. P. Matousek, *Appl. Spectrosc.* **60**, 1341-1347 (2006).
9. P. Matousek, *'Review of Deep Non-invasive Raman Spectroscopy of Living Tissue and Powders', Chem. Soc. Rev.,* in press.