Hidden depths? New techniques for sub-surface spectroscopy

Neil A. Macleod, Charlotte Eliasson[a] and Pavel Matousek*
Central Laser Facility, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Science and Innovation Campus, Didcot, Oxfordshire OX11 0QX, United Kingdom. E-mail: P.Matousek@rl.ac.uk

[a]present address: PeTek Oseberg South, Hydro, Sandsliveien 90, postboks 7190, 5020 Bergen, Norway

Introduction

A number of analytical applications in the area of security screening, medical diagnosis, drug authentication and quality control often require non-invasive probing of diffusely scattering (turbid) media in order to obtain chemical characterisation of deep-lying sample regions. Examples include non-invasive disease diagnosis, the detection of concealed explosives and illicit materials, the identification of counterfeit drugs and quality control applications in the pharmaceutical industry.

Raman spectroscopy holds particular promise in this area due to its inherently high chemical specificity [exceeding that of near infrared (NIR) absorption spectroscopy and comparable with mid-infrared and THz methods], the ability to probe samples in the presence of water (the Raman scattering cross-section of water is very low) and its high penetration depth into turbid non-absorbing or weakly absorbing samples. On the downside, the technique is restricted to samples that do not exhibit strong fluorescence emission although this problem can, in the majority of cases, be avoided by using NIR excitation.[1] Until recently, Raman techniques have generally been confined to applications involving surface layers of turbid media due to limitations imposed by the backscattering collection geometry common to the majority of commercial Raman probes.[1] In principle, confocal Raman microscopy can potentially resolve objects to depths of up to several hundred micrometres. Deeper layers cannot be readily resolved and, typically, are overwhelmed by Raman and fluorescence signals emanating from the surface layer.

Deep probing Raman techniques

Recent research efforts by Everall *et al.*[2] into time-resolved Raman photon migration in turbid media, in particular, have stimulated substantial progress in the area of deep probing of turbid samples and led to the development of effective spatial variants requiring only continuous wave laser beams. The methods rely on the diffuse component of light.[3] Two variants in particular have emerged as promising practical tools: Spatially Offset Raman Spectroscopy (SORS)[4] in which the Raman spectra of *individual sub-layers* within a complex multi-layered system are isolated and transmission Raman spectroscopy which provides information on *bulk* content[5] (see Figure 1).

SORS

The SORS method is based on the collection of Raman signals from spatial regions offset (Δs) from the point of illumination on the sample surface. The laterally offset spectra contain different relative contributions from sample layers located at different depths due to the wider spread of photons emerging from deeper layers on the sample surface. Consequently, SORS effectively suppresses interfering Raman and fluorescence signals from the surface layers and provides Raman spectra containing a varying degree of surface and sub-surface content at different spatial offsets; complete separation of the spectrum of each layer can be accomplished using numerical methods.[6] This is in contrast with conventional backscattering Raman spectroscopy, where the solitary Raman spectrum available is typically dominated by intense surface layer components.

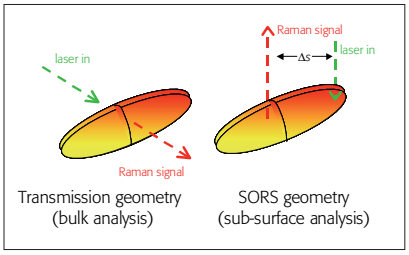

Figure 1. A schematic illustration of the transmission Raman (left) and SORS concepts (right).

Since the first experimental demonstration of the SORS concept on powders,[4] the technique has been used in numerous applications including the demonstration of Raman tomography in turbid media, non-invasive Raman spectroscopy of bones,[6] in the pharmaceutical industry[7] and in security screening applications.[8]

Transmission Raman

In the transmission Raman concept, the laser beam is incident on the sample

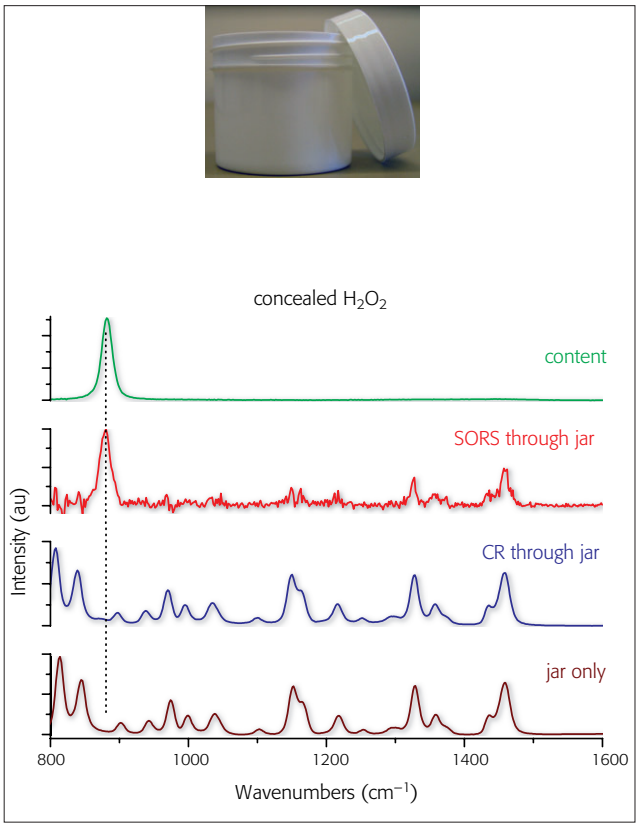

Figure 2. Detection of liquid explosives. Conventional backscattering Raman (CR) and processed SORS Raman spectra of a white plastic jar with a thickness of 1.2mm filled with H_2O_2 (30% aqueous solution). The bottom trace is the Raman spectrum of the empty jar itself and is essentially identical to the conventional Raman spectrum of the jar containing H_2O_2 with no obvious trace of the Raman signature of H_2O_2. The SORS spectrum, on the other hand, shows clearly the H_2O_2 Raman marker band. [Reprinted with permission from C. Eliasson, N.A. Macleod and P. Matousek, "Non-invasive Detection of Concealed Liquid Explosives using Raman Spectroscopy", *Anal. Chem.* 2007, in press. Copyright (2007) American Chemical Society.]

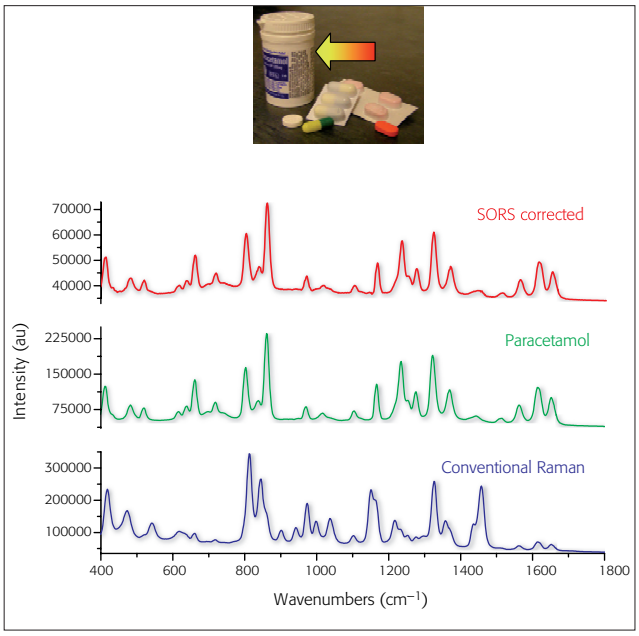

Figure 3. Detection of counterfeit drugs. Non-invasive Raman spectra of paracetamol tablets measured through a white, diffusely scattering 1.7mm thick plastic container. Conventional Raman and SORS raw data are shown together with the reference Raman spectrum of paracetamol. The processed SORS spectrum matches well that of paracetamol, while the conventional Raman spectrum is dominated by Raman signals originating from the bottle wall. [Adapted with permission from C. Eliasson and P. Matousek, *Anal. Chem.* **79,** 1696–1701 (2007). Copyright (2007) American Chemical Society.]

from one side and the Raman signal collected from the opposite side (Figure 1). This concept can be considered as a special case of SORS with the illumination and collection points situated at extreme displacement.[5] Although the transmission Raman technique was demonstrated in the very early days of Raman spectroscopy,[9] its benefits for the non-invasive probing of the bulk content of turbid samples have not been previously recognised. In particular, these include the removal of the so-called sub-sampling problem[10] (over-sensitivity to the surface layers of the probed medium) and the effective suppression of fluorescence components emanating from surface layers.[5,11]

The two techniques and their applications are reviewed in Reference 3. In this article we present a brief snapshot of potential applications in security and in the pharmaceutical industry.

Non-invasive detection of concealed explosives (SORS)

The recent heightened terrorist threat underlines the importance of robust security screening techniques with high chemical specificity. Recently, we have demonstrated the applicability of SORS to the detection of concealed liquid explosives through turbid plastic packaging.[8] In this work, hydrogen peroxide, a critical constituent of home-made explosives used recently in several terrorist bombings across Europe, was detected through various packaging materials. Figure 2 shows the results of probing a small white plastic jar containing H_2O_2 (30% aqueous solution). Such plastic jars are commonly used by travellers to transfer a smaller amount of moisturising cream or other products for the purposes of travel to save space and meet current travel regulations in carry-on luggage. The container is highly diffusely scattering and presented an insurmountable challenge to conventional Raman spectroscopy; *the signature Raman band of H_2O_2 at 876 cm^{-1} is completely overwhelmed by Raman signals originating from the container wall*. In contrast, SORS, after

"blind" automated data processing using spectra obtained at two different spatial offsets, clearly revealed the H_2O_2 Raman marker band. The experiments were performed using a continuous wave diode laser (830 nm, 250 mW); an acquisition time of 1 s was sufficient to obtain good quality spectra.

Non-invasive detection of counterfeit drugs (SORS)

Another hot topic is the non-invasive detection of counterfeit drugs through plastic bottles and blister packs. These drugs present an increasingly severe threat to health and life in our society.[12] Recently we have demonstrated[7] that SORS can provide a chemical signature of the internal content through unopened plastic containers with higher sensitivity than that available with conventional Raman spectroscopy. This is demonstrated in Figure 3 for paracetamol tablets held inside white plastic pharmaceutical bottles. The conventional Raman approach, dominated by the Raman signal originating from the container wall, is clearly ineffective. In contrast, the SORS approach, following the scaled subtraction of two SORS spectra obtained at different spatial offsets, provides a clean Raman spectrum of the tablets held inside the bottle. The experiments were performed using a continuous diode laser (827 nm, 50 mW) with an acquisition time of 10 s. A practical use of SORS in the identification of "authentic" fake tablets was very recently demonstrated on anti-malarials by Ricci *et al.*[13] As the SORS concept can be easily incorporated into existing commercial hand-held Raman instruments it holds great promise for more accurate and sensitive identification of drugs at each stage of the supply chain.

Bulk probing of pharmaceutical products in quality control (transmission Raman)

In a number of pharmaceutical process analytical technology (PAT) applications it is essential to monitor the bulk content of pharmaceutical products. Ideally, this should be accomplished quickly and non-invasively with high chemical specificity.

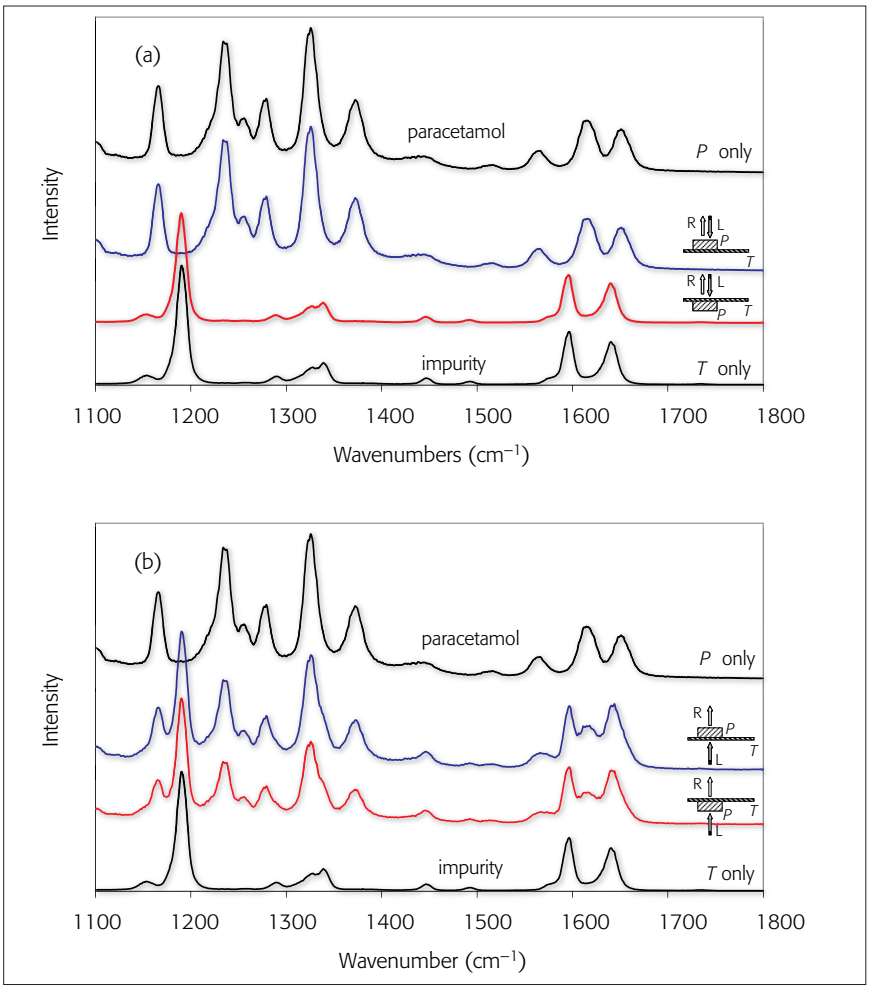

Figure 4. Removal of sub-sampling problem in quality control of pharmaceutical tablets. The Raman spectra obtained from a two-layer sample (a paracetamol tablet and a 2 mm thick *trans*-stilbene "impurity" layer) using (a) conventional backscattering geometry and (b) transmission geometry. The measurements are performed at two sample orientations, with paracetamol at the top and bottom of the *trans*-stilbene cell as indicated. The top and bottom traces are reference spectra of paracetamol and *trans*-stilbene, respectively, obtained in separate experiments. The transmission Raman spectra are notable for the absence of the sub-sampling problem, i.e. over-sensitivity to the surface layers. Legend: *P*: paracetamol, *T*: *trans*-stilbene, R: Raman light, L: laser beam. (Reprinted with permission from P. Matousek and A.W. Parker, *Appl. Spectrosc.* **60,** 1353–1357 (2006). Copyright (2006) The Society for Applied Spectroscopy.)

Although NIR absorption spectroscopy is widely used in this area it provides insufficient chemical specificity in a number of applications. The conventional Raman approach, on the other hand, suffers from the sub-sampling problem, which precludes the characterisation of deep sample components. Research in this area has recently demonstrated the removal of this effect with the transmission Raman approach,[5] thus widening the prospects for the quantification of the bulk content of undisturbed tablets and capsules.

The experimental demonstration of the elimination of the sub-sampling problem was performed on a standard paracetamol tablet of 3.9 mm thickness with a simulated impurity layer of a 2 mm thickness of *trans*-stilbene powder. The impurity layer was located either at the front or the back of the tablet; conventional Raman spectroscopy is beset by the sub-sampling problem and yields only the Raman signature of the surface layer in both possible orientations [Figure 4(a)]. In contrast, the transmission geometry [Figure 4(b)] provides a Raman spec-

trum comprising of a mixture of the tablet and the "'impurity"; the similarity of the spectra in both sample orientations is expected for a technique that does not suffer from the sub-sampling issue. The experiments were performed using a continuous wave diode laser (827 nm, 80 mW) and acquisition times ranging from 0.2 s to 10 s.

The transmission Raman geometry appears to be very well suited to the requirements of pharmaceutical production lines, underlining the potential of this method to displace NIR absorption spectroscopy in applications where higher chemical specificity is desired. Further studies are needed to establish the technique's sensitivity limits and validate its potential to provide sufficiently accurate quantitative information on the composition of the probed sample. We are presently working on establishing these points in collaboration with major pharmaceutical companies. Our preliminary data indicate that the quantification of active pharmaceutical ingredients with errors comparable to established methods (e.g. 1–2% relative error) using the transmission Raman approach is indeed feasible.

Conclusions

The advent of SORS and the renaissance of transmission Raman spectroscopy have stimulated the development of numerous new analytical methods for probing tissue and powders at previously inaccessible depths. Many new exciting practical applications are already looming on the horizon including the diagnosis of bone disease, breast cancer detection, accurate quality control and authentication of pharmaceutical products, as well as the detection of powder and liquid explosives through packaging.

References

1. M.J. Pelletier, *Analytical Applications of Raman Spectroscopy*. Blackwell Science, Oxford (1999).
2. N. Everall, T. Hahn, P. Matousek, A.W. Parker and M. Towrie, *Appl. Spectrosc.* **55,** 1701 (2001).
3. P. Matousek, *Chem. Soc. Rev.* **36,** 1292 (2007).
4. 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).
5. P. Matousek and A.W. Parker, *Appl. Spectrosc.* **60,** 1353 (2006).
6. M.V. Schulmerich, K.A. Dooley, T.M. Vanasse, S.A. Goldstein and M.D. Morris, *Appl. Spectrosc.* **61,** 671 (2007).
7. C. Eliasson and P. Matousek, *Anal. Chem.* **79,** 1696 (2007).
8. C. Eliasson, N.A. Macleod and P. Matousek, "Non-invasive Detection of Concealed Liquid Explosives using Laser Spectroscopy", *Anal. Chem.* (2007), in press.
9. B. Schrader and G. Bergmann, *Zeitschrift fur Analytische Chemie Fresenius* **225,** 230 (1967).
10. J. Johansson, S. Pettersson and S. Folestad, *J. Pharmaceut. Biomed.* **39,** 510 (2005).
11. P. Matousek and A.W. Parker, *J. Raman Spectrosc.* **38,** 563 (2007).
12. R. Mukhopadhyay, *Anal. Chem.* **79,** 2622 (2007).
13. C. Ricci, C. Eliasson, N.A. Macleod, P. Newton, P. Matousek and S.G. Kazarian, *Anal. Bioanal. Chem.* (2007), in press.