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Published version information

Citation: P Matousek. "Spatially Offset Raman Spectroscopy for non-invasive analysis of turbid samples." TrAC Trends in Analytical Chemistry, vol. 103 (2018): 209-214.

DOI: 10.1016/j.trac.2018.04.002

Spatially Offset Raman Spectroscopy for Non-invasive Analysis of Turbid Samples

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Abstract

Spatially Offset Raman Spectroscopy (SORS) and related methods for deep non-invasive Raman analysis of diffusely scattering samples have recently undergone major advances leading to the opening of a number of new applications across several fields. Here we review the latest instrumental concepts and outline related emerging applications. These include security screening, quality control of pharmaceutical and food products, characterisation of objects of art and diagnosis of bone disease and breast cancer.

Key words

Spatially offset Raman spectroscopy; non-invasive; security; bone; art; food; cancer; glucose; temperature; SERS

1. Introduction

In recent years, several forms of Raman spectroscopy for deep probing of diffusely scattering (turbid) samples have emerged. The methods have been shown to extend the penetration depth of conventional Raman spectroscopy in such media by up to two orders of magnitude [1].The concepts are based on photon diffusion in turbid media and are analogous to related approaches used in NIR absorption and fluorescence tomography [2, 3]. In this context Raman spectroscopy, however, provides much higher degree of chemical specificity. This along with its other distinct properties, which include compatibility with water, defines several emerging application areas. The disadvantages of Raman spectroscopy, on the other hand, include susceptibility to fluorescence and the general weakness of its signals compared with NIR absorption and fluorescence techniques.

In the core of the methods lies Spatially Offset Raman Spectroscopy (SORS) [4]. SORS early research was covered comprehensively in an earlier review paper [1]. Here we outline only its key principles and focus predominantly on most recent advances in this field. It should be noted that the account of this research is not exhaustive due to its large extent and only key illustrative approaches are chosen to outline and exemplify developing mainstream areas.

2. Principal Methods

2.1 Spatially Offset Raman Spectroscopy - SORS

The SORS concept is based on collecting Raman signal on sample surface from a region away from the laser illumination zone separated by 'spatially offset', Δs (see Fig 1). This geometry favours collecting more of the photons migrating through deeper zones of sample compared with conventional Raman spectroscopy where no spatial offset is used. The enhanced deep probing sensitivity stems from the fact that photons migrating from the laser illumination area

towards the collection zone near sample surface have a much higher likelihood of being lost away from the sample than photons migrating through deeper zones. The larger the spatial offset, Δs, the higher the penetration depth of collected Raman photons in statistical terms. Although the choice of the magnitude of the spatial offset has to be balanced with the fact that the absolute levels of signals generally diminish with increasing spatial offset [1].

These spatial properties can be used to retrieve pure Raman signatures of surface and subsurface layers in stratified samples. For example, for a two layer sample, two SORS spectra with different spatial offsets (e.g. zero and non-zero) would be acquired. The spectra are then subtracted one from the other with appropriately selected scaling factor to yield the pure Raman signature of the chosen layer, cancelling the components of the other. Analogous processing steps are required for a stratified sample with n-layers where n-SORS spectra obtained at different spatial offsets are required to yield the Raman spectra of individual layers. This process is comparable to solving simultaneous linear equations with n-unknown variables. Multivariate analysis can also be used to the same effect. The multivariate technique is beneficial in situations, for example, where the number of layers within the sample is unknown. Although much larger set of spectra with different spatial offsets is required [1].

The Raman signal is coupled to the detection system using either a bundle of optical fibres or free-space relayed using lenses. The detection of Raman signal is typically accomplished using a low f-number spectrograph with a high performance CCD camera.

There are several geometries in which SORS can be deployed; e.g. comprising a single spot laser illumination and Raman signal collection or a ring-shape illumination scheme or collection. For example, a ring-shape laser illumination geometry utilising Raman collection

through a central zone (‘inverse SORS’) is particularly well suited to medical applications [1] as the laser radiation is spread over an extended area permitting the delivery of larger laser power to the target in situations where laser intensities are constrained, e.g. due to laser safety considerations as in *in vivo* applications.

In general, SORS is not applicable with samples which are highly absorbing at the laser or Raman signal wavelengths because this restricts the photon diffusion process limiting achievable accessible depths. Samples with high level of fluorescence originating from the target sublayers can also be challenging although fluorescence from surface layers can be mitigated by SORS [1].

2.2 Micro-SORS

A combination of SORS and microscopy technique enables the analysis of very thin, micro-metre scale layers, e.g. layers of paint [5]. This specialist method is suitable, for example, for the non-invasive analysis of stratified samples in art, biology or in polymer studies [6,7,8].

2.3 SESORS

A combination of SORS with surface enhanced Raman spectroscopy (SERS) has been used to retrieve SERS signals from deep zones in diffusely scattering samples. The approach was first demonstrated by Stone *et al.* [9,10]. The method, named SESORS, is, however, not label free due to its requirement for the introduction of SERS nanoparticles or a SERS substrate into sample. The subsequent readout of signals is non-invasive though. The introduced nanoparticles can be functionalised to increase their selectivity and multiple reporting molecules can reside on a single nanoparticle permitting also a high level of multiplexing. The extremely strong enhancement of Raman signal associated with the SERS effect enables the

detection of analytes at very low levels which can be readily well beyond the limits of detection of conventional SORS.

3. SORS Application Areas

Several application areas which are currently under developments are exemplified below.

3.1 Security screening

The current heightened terrorist threat underlines the importance of the availability of robust security screening techniques with high chemical specificity. As an example, the detection of liquid explosives at airports represents one such critical issue. Here SORS was shown to be able to play an important role for its high chemical specificity and ability to penetrate through non-metallic containers as demonstrated earlier by Eliasson *et al.* [11]. The technique of SORS was advanced and developed into a deployable device by a spin out company Cobalt Light Systems Ltd (now a part of Agilent Technologies) [12]. The device was shown to yield exceptionally low operational false alarm rates (<1 %) in comparison with alternative approaches [13]. At the time of writing around 500 of these SORS instruments are operational at 75 airports worldwide deployed to scan medical essentials taken on board of planes (e.g. baby milk or personal medicine) and duty free items in transfers. More recently, the company also developed the first handheld SORS device for applications in the field such as the detection of toxic industrial chemicals, flammable compounds, explosives, narcotics and chemical warfare agents [14].

In another research relevant to the detection of chemical warfare agents, Wilcox *et al.* demonstrated a potential of SORS to detect chemicals absorbed in porous concrete [15]. Additionally, Hopkins *et al.* shown the utility of using near-infrared excitation at 1064 nm with

InGaAs detection to screen unknown compounds through bottles to mitigate excessive fluorescence emission which can be present with some target compounds when using conventional SORS [16]. In a further development Cletus *et al.* demonstrated a combined time-resolved/SORS detection enabling the screening of explosive compounds through containers under ambient light [17] with the temporal gating effectively suppressing interference from ambient light. The combined time-resolved /SORS concept was also used in a stand-off detection of explosives which is yet another area of activity where SORS has been used by several teams demonstrating potential for explosive detection through containers at distances >10 m and under ambient light conditions [18, 19]. In this area Zachhuber *et al.* developed SORS for use with hyperspectral imaging demonstrating capability to detect explosives inside containers [20]. In this research, sodium chlorate was detected in a white plastic bottle from a distance of 10 m using pulsed excitation and time resolved detection with a gated CCD (5 ns gate width).

3.2 Pharmaceutical analysis

Another area which benefitted considerably from the emergence of SORS is pharmaceutical analysis. Here SORS enabled the analysis of unopened pharmaceutical products with potential, for example, for screening for counterfeit drugs in sealed bottles as demonstrated by Eliasson and Matousek [21]. Subsequently, Olds *et al.* showed that apart from chemical identity also quantity of active pharmaceutical ingredients in pharmaceutical formulations could be assessed using SORS through packaging [22].

A related SORS application, which is highly relevant to pharmaceutical manufacture, was developed by Bloomfield *et al.* [23]. The team demonstrated that SORS can perform raw material identification to confirm the identity of incoming raw materials against their bar code

labels through unopened packaging (see Fig. 2). This identity check is often a regulatory requirement in pharmaceutical manufacture and is, typically, performed invasively by opening the packaging and sampling the product in a special chemical booth. The process is laborious and resource intensive and can represent a major bottleneck in manufacture. SORS was shown to be able to perform this analysis within tens of seconds through unperturbed packaging directly *in situ*. A number of packaging materials viable with this method comprise translucent plastic sacks, paper sacks, plastic bottles and both coloured and clear glass bottles. Apart from high speed and reduced cost of analysis other added benefits include the prevention of the exposure of the product to ambient environment which could potentially degrade or contaminate it (e.g. due to the presence of moisture, oxygen or general contaminants).

The pharmaceutical analysis was also influenced by a related technique in the area of quality control of finished products. Here transmission Raman spectroscopy [24] permitting quantitative volumetric analysis of intact pharmaceutical tablets and capsules within seconds has proven to be highly beneficial. This field is also rapidly developing with a large body of research output generated in recent years. For a closer account of this activity the reader is referred to a recent review by Griffen *et al.* [25].

3.3 Food Analysis

The non-invasive subsurface analysis of intact samples is also beneficial in the field of food analysis. In this area, *Qin et al.* demonstrated the potential of SORS to quantify the lycopene content of intact tomatoes to monitor their ripening [26]. The team also developed hyperspectral SORS detection system for rapid, simultaneous acquisition of SORS spectra [27]. The concept was demonstrated to be effective in retrieving melamine signals through an overlayer of butter.

In another area Afseth *et al.* showed that SORS can be applied to monitoring the carotene content of intact salmon through skin in both qualitative and quantitative manner [28]. The study was performed *ex vivo*. The team also demonstrated the potential of monitoring iodine levels values (i.e. fatty acid unsaturation) through both light and dark parts of the salmon skin. The feasibility study paves the way for potential Raman analysis of intact salmon *in vivo*.

Ellis *et al.* used a hand held SORS device for the first time to detect multiple chemical markers suitable for use in monitoring the adulteration and counterfeiting of Scotch whisky, and other spirit drinks through bottles [29]. The team detected a total of 10 denaturants/additives in extremely low concentration levels without any contact with the sample and was able to discriminate between and within multiple well-known Scotch whisky brands. Ability to monitor methanol presence using SORS at concentrations well below the maximum human tolerable level was also demonstrated.

3.4 Cultural Heritage

A number of analytical problems in cultural heritage require the non-invasive chemically specific analysis of thin layers such as layers of paint. Examples of relevant objects are panels, canvas and mural paintings, painted statues or other decorated objects. The analysis may be required for the purposes of art restoration or to learn artist's technique. The layers can often reside beyond the reach of conventional confocal Raman microscopy and as such cross sectional analysis may be the only effective method for retrieving the chemical information from such layers. Due to the high cultural value of these objects the cross sectional sampling is, however, highly undesirable and in some cases not even possible. The painted layers are, in general, highly turbid and typically only a few tens of micrometres thick and spread in multiple

stratigraphy. To tackle this issue, which is beyond the resolution capability of traditional SORS, the SORS technique was combined with microscopy by Conti *et al.* yielding a modality known as micro-SORS [5, 6]. A general review of the developed techniques in this area is given in [8]. The micro-SORS method was also shown to be beneficial outside cultural heritage area in the analysis of polymers, wheat seeds, and paper [7]. Recently, the technique was shown to also be capable of recovering hidden (e.g. overpainted) images in art [30]. To date, the main body of micro-SORS research was performed using laboratory, bench-top Raman microscopes. Very recently, a portable micro-SORS device was developed and demonstrated with an aim of providing in field analytical micro-SORS tool unlocking thus true potential of micro-SORS in this area as the majority of objects cannot be moved from their original location to specialist laboratory [31].

3.5 Medical Diagnosis

3.5.1 Non-invasive Bone Disease Diagnosis

There are also a number of SORS medical applications under intense development. SORS is investigated, for example, for its potential to diagnose non-invasively bone disorders such as osteoporosis, a potential identified through an early ex vivo research using conventional Raman spectroscopy by McCreadie [32]. A clinical need here stems from the deficiency of the current 'gold standard' method Dual-energy X-ray Absorptiometry (DXA) that is only 60-70% accurate in predicting osteoporotic fractures. This shortcoming is, in part, believed to stem from the inability of the technique to characterise the organic component of bone (collagen) that also plays an important role in providing mechanical properties of bone [1]. This issue could be addressed by SORS as it can sense non-invasively both the mineral and organic components of bone simultaneously as demonstrated, for example, by Schulmerich *et al.* [33].

Following this early research the technique has rapidly developed reaching yet higher accuracy and penetration depths [1].

Recently, Sowoidnich *et al.* performed a study to evaluate the sampling depths of SORS in bone samples for various special offsets. In this research thin, segmented sections of bones were stacked on top of each other with a thin test layer of polytetrafluoroethylene (PTFE) slice inserted into different depths into the stack [34]. The experiments, performed at 830 nm Raman excitation wavelength, revealed, for example, that a 7-mm spatial offset yields the maximum penetration depth of 3.7 mm inside the bone matrix.

SORS was further developed by Schulmerich *et al.* [35] who retrieved additionally also spatial information about the location and extent of an object within diffusely scattering matrix along with its chemical information, facilitating, in essence, a chemically specific tomographic capability with Raman spectroscopy. The research led to successful tomographic imaging of phantoms and a canine limb including skin and tissue up to thicknesses of 45 mm [36]. The concept was advanced by Srinivasan *et al.* [37] who developed image-guided Raman spectroscopy utilizing X-ray computed tomography images guiding the numerical recovery of Raman images [38] and further refined by Demers *et al.* [39].

Recently, Feng *et al.* used SORS to measure subcortical bone tissue and depth-resolved biochemical variability in intact, exposed murine bones and also applied the technique to study a mouse model of the bone disorder *osteogenesis imperfecta* [40]. The results suggest that SORS is more sensitive to disease-related biochemical differences in subcortical trabecular bone and marrow than conventional Raman spectroscopy. Furthermore, Liao *et al.* developed a fast method for setting spatial offsets in SORS of diverse shapes using a programmable digital

micro-mirror device (DMD) [41]. Several detection geometries, including annular and simultaneous multi-offset modalities were demonstrated. The method was applied to the non-destructive characterization of bone tissue engineering scaffolds opening prospects for analysing intact real-size 3D tissue engineering-constructs both *ex vivo* and *in vivo* [42].

3.5.2 Non-invasive Diagnosis of Breast Cancer

In the area of cancer screening, it was shown that a SORS related technique, Transmission Raman Spectroscopy (TRS), is well suited to the non-invasive detection and characterisation of cancer lesions in breast tissue *in vivo*. The early diagnosis of this disease would offer prospects for more conservative treatments and better patient outcomes [1]. For this reason, mammography screening is typically performed. If an abnormality is found, often present in a form of calcifications, a needle biopsy is carried out. This, in most cases, is unnecessary as no malignancy is found (in ~80 % of cases) [1]. TRS offers prospects for eliminating the needle biopsy step and associated patient recall to hospital by providing prospects for *in situ* non-invasive diagnosis in conjunction with mammography. This is based to great extent on findings of Haka *et al.* who recognised that the chemical content of micro-calcifications associated with benign and malignant calcifications are significantly different and that this difference is reflected in Raman spectra [43]. An early TRS research in this area achieved the penetration depths of 20 mm in porcine tissue at clinically relevant concentrations of calcifications [44]. Although this is around two orders of magnitude deeper than possible with conventional confocal Raman microscopy it is still about a half of the required penetration depth for clinical deployment [1]. Recently, the clinically relevant depth was achieved by Ghita *et al.* [45] by enhancing the detection rate of Raman photons by nearly two orders of magnitude. This was achieved, principally, through increasing the incident laser power permitted by larger illumination areas used (to >1 cm^2) and by boosting spectrograph collection efficiency through

increasing its slit width in conjunction with increasing grating dispersion (at the expense of reduced spectral range). These measures resulted in detecting calcifications in animal tissue at clinically relevant concentration range through 40 mm of phantom tissue (see Fig. 3). This research is now progressing to human *in vivo* stage.

In a related area, SORS is being developed for the detection of breast cancer margins during cancer removal surgery where it is important to strike a balance between removing enough tissue to ensure that no cancerous cells remain and minimising the amount of normal tissue removed for medical or aesthetic reasons. The first proof of concept experiments in this area was performed by Keller *et al.* [1, 46]. More recently the team demonstrated an automated 3D SORS scanner useable to assess the entire margins of a resected specimen within clinically feasible time at operating theatre [47].

3.5.3 SESORS applications

SESORS found its first practical application in the area of non-invasive glucose detection, demonstrated by Yuen *et al.* in rats *in vivo* [48]. In this application a SERS implant is first inserted under skin. Subsequent readout of the implant is then performed using SORS through skin non-invasively. Given the strength of SERS signals this technology holds considerable promise for deployment with low cost SORS readout units.

Recently, Asiala *et al.* compared the performance of SESORS with conventional SERS spectroscopy by recovering subsurface signals through tissue analogues of varied thicknesses for different SORS spatial offsets using hand-held devices [49]. The results confirmed that SESORS outperforms conventional SERS in terms of penetration depth. In this study, the SESORS signals could be recovered from depths of >6.75 mm.

SESORS is also investigated for its potential to detect low level bio-analytes in brain tissue through skull. The first proof-of-concept studies in this area were carried out by Sharma *et al.* [50]. Recently, the team succeeded in detecting neurotransmitters (melatonin, serotonin, and epinephrine) at various concentrations using SESORS in a brain tissue mimic through a cat skull [51].

SESORS involving freely floating nanoparticles inside body could also be used to detect cancer lesions at very early stages [1]. This prospect is yet to be explored *in vivo*. This area is held off by safety aspects of deploying freely floating nanoparticles in human body, a key issue still remaining to be addressed.

3.5.4 Blood Monitoring

Buckley *et al.* applied SORS to the monitoring of the quality of blood in transfusion bags without breaking sterility. The successful delivery of this application would permit the increase of blood available for transfusion by eliminating need for premature disposal of blood based solely on its date whilst ensuring its quality [52]. The team developed an effective form of micro-SORS that could be practised readily on an existing commercial confocal Raman microscope. The method yielded good quality blood SORS spectra through bags comparable to those obtained by conventional Raman means after decanting. It was shown that the retrieved spectra were dominated by features associated with hemoglobin.

3.6 Monitoring of Sample Physical Properties

Very recently, Gardner *et al.* demonstrated that apart from monitoring chemical properties of samples SORS and SESORS could be used to monitor their physical properties too, such as

temperature [53,54]. The basic approach relies on monitoring the ratio of corresponding Stokes and anti-Stokes Raman bands. The research showed that the technique can be used to monitor subsurface temperature of tissue at clinical relevant ranges and accuracies (see Fig. 4). The concept could open a host of new applications such as photo-thermal therapy with temperature feedback or subsurface monitoring of chemical or catalytic processes in manufacture quality and process control.

4. Conclusions

The recent advances in the spatially offset Raman spectroscopy technique led to opening of a range of novel applications across several fields. These include the security screening, quality control of pharmaceutical and food products, diagnosis of bone disease and breast cancer, photo-thermal therapy with temperature feedback, the identification of cancer margins, the detection of glucose levels and monitoring neurotransmitters in brain through skull. Some applications have already reached commercial readiness, such as the security screening and pharmaceutical analysis, whilst others, such as medical diagnoses, are still in research phase.

References

[1] P. Matousek, N. Stone, Development of deep subsurface Raman spectroscopy for medical diagnosis and disease monitoring, Chem. Soc. Rev. 45 (2016) 1794-1802.

[2] T. J. Pfefer, K. T. Schomacker, M. N. Ediger, N.S. Nishioka, Multiple-fiber probe design for fluorescence spectroscopy in tissue, Appl. Opt. 41 (2002) 4712-4721.

[3] L. Shi, R. Alfano, Deep Imaging in Tissue and Biomedical Materials, Pan Stanford Publishing Pte. Ltd, Singapore, 2017.

[4] P. Matousek, I. P. Clark, E. R. C. Draper, M. D. Morris, A. E. Goodship, N. Everall, M. Towrie, W. F. Finney, A. W. Parker, Subsurface probing in diffusely scattering media using

spatially offset Raman spectroscopy, Appl. Spectrosc. 59 (2005) 393-400.

[5] C. Conti, C. Colombo, M. Realini, G. Zerbi, P. Matousek, Subsurface Raman analysis of thin painted layers, Appl. Spectrosc. 68 (2014) 686-691.

[6] C. Conti, M. Realini, C. Colombo, P. Matousek, Subsurface analysis of painted sculptures and plasters using micrometre-scale spatially offset Raman spectroscopy (micro-SORS), J. Raman Spectrosc. 46 (2015) 476-482.

[7] C. Conti, M. Realini, C. Colombo, K. Sowoidnich, N.K. Afseth, M. Bertasa, A. Botteon, P. Matousek, Noninvasive analysis of thin turbid layers using microscale spatially offset Raman spectroscopy, Anal. Chem. 87 (2015) 5810-5815.

[8] P. Matousek, C. Conti, M. Realini, C. Colombo, Micro-scale spatially offset Raman spectroscopy for non-invasive subsurface analysis of turbid materials, Analyst 141 (2016) 731-739.

[9] N. Stone, K. Faulds, D. Graham, P. Matousek, Prospects of deep Raman spectroscopy for noninvasive detection of conjugated surface enhanced resonance Raman scattering nanoparticles buried within 25 mm of mammalian tissue, Anal. Chem. 82 (2010) 3969-3973.

[10] N. Stone, M. Kerssens, G.R. Lloyd, K. Faulds, D. Graham, P. Matousek, Surface enhanced spatially offset Raman spectroscopic (SESORS) imaging – the next dimension, Chem. Sci. 2 (2011) 776-780.

[11] C. Eliasson, N.A. Macleod, P. Matousek, Noninvasive detection of concealed liquid explosives using Raman spectroscopy, Anal. Chem. 79 (2007) 8185-8189.

[12] P.W. Loeffen, G. Maskall, S. Bonthron, M. Bloomfield, C. Tombling, P. Matousek, Spatially offset Raman spectroscopy (SORS) for liquid screening, Proc. of SPIE 8189 (2010) 81890C.

[13] P.W. Loeffen, G. Maskall, S. Bonthron, M. Bloomfield, C. Tombling, P. Matousek, The performance of Spatially Offset Raman Spectroscopy for liquid explosive detection, Proc. of SPIE 9995 (2016) 99950D.

[14] R.J. Stokes, M. Bailey, S. Bonthron, T. Stone, G. Maskall, O. Presly, E. Roy, C. Tombling, P.W. Loeffen, New capability for hazardous materials ID within sealed containers using a portable spatially offset Raman spectroscopy (SORS) device, Proc. of SPIE 9995 (2016) 999506.

[15] P. Wilcox, J. Guicheteau, S. Christesen, Spatially Offset Raman Spectroscopy for Chemical Detection in Porous Surfaces, AIP Conference Proceedings 1267 (2010) 551.

[16] R.J. Hopkins, S.H. Pelfrey, N.C. Shand, Short-wave infrared excited spatially offset Raman spectroscopy (SORS) for through-barrier detection, Analyst 137 (2012) 4408-4410.

[17] B. Cletus, W. Olds, E.L. Izake, S. Sundarajoo, P.M. Fredericks, E. Jaatinen, Combined time- and space-resolved Raman spectrometer for the non-invasive depth profiling of chemical hazards, Anal. Bioanal. Chem. 403 (2012) 255-263.

[18] B. Zachhuber, C. Gasser, E.t.H. Chrysostom, B. Lendl, Stand-off spatial offset Raman spectroscopy for the detection of concealed content in distant objects, Anal. Chem. 83 (2011) 9438-9424.

[19] E.L. Izake, B. Cletus, W. Olds, S. Sundarajoo, P.M. Fredericks, E. Jaatinen, Deep Raman spectroscopy for the non-invasive standoff detection of concealed chemical threat agents, Talanta 94 (2012) 342-347.

[20] B. Zachhuber, H. Östmark, T. Carlsson, Spatially offset hyperspectral stand-off Raman imaging for explosive detection inside containers, Proc. of SPIE 9073 (2014) 90730J.

[21] C. Eliasson, P. Matousek, Noninvasive authentication of pharmaceutical products through packaging using spatially offset Raman spectroscopy, Anal. Chem. 79 (2007) 1696-1701.

[22] W.J. Olds, S. Sundarajoo, M. Selby, B. Cletus, P.M. Fredericks, E.L. Izake, Noninvasive, quantitative analysis of drug mixtures in containers using spatially offset Raman spectroscopy (SORS) and multivariate statistical analysis, Appl. Spectrosc. 66 (2012) 530-537.

[23] M. Bloomfield, D. Andrews, P. Loeffen, C. Tombling, T. York, P. Matousek, Non-invasive identification of incoming raw pharmaceutical materials using Spatially Offset Raman Spectroscopy, J. Pharm. Biomed. Anal. 76 (2013) 65-59.

[24] B. Schrader. G. Bergmann, Die Intensität des Ramanspektrums polykristalliner Substanzen, Fresenius' Z. Anal. Chem. 225 (1967) 230-247.

[25] J.A. Griffen, A.W. Owen, D. Andrews, P. Matousek, Recent advances in pharmaceutical analysis using transmission Raman spectroscopy, Spectroscopy 32 (2017) 37-43.

[26] J. Qin, K. Chao, M.S. Kim, Nondestructive evaluation of internal maturity of tomatoes using spatially offset Raman spectroscopy, Postharvest Biol. Technol. 71 (2012) 21-31.

[27] J. Qin, M.S. Kim, W.F. Schmidt, B.-K. Cho, Y. Peng, K. Chao, A line-scan hyperspectral Raman system for spatially offset Raman spectroscopy, J. Raman Spectrosc. 47 (2016) 437-443.

[28] N.K. Afseth, M. Bloomfield, J.P. Wold, P. Matousek, A novel approach for subsurface through-skin analysis of salmon using spatially offset Raman spectroscopy (SORS), Appl. Spectrosc. 68 (2014) 255-262.

[29] D.I. Ellis, R. Eccles, Y. Xu, J. Griffen, H. Muhamadali, P. Matousek, I. Goodall, R. Goodacre, Through-container, extremely low concentration detection of multiple chemical markers of counterfeit alcohol using a handheld SORS device, Sci. Rep. 7 (2017) 12082.

[30] A. Botteon, C. Conti, M. Realini, C. Colombo, P. Matousek, Discovering hidden painted images: Subsurface imaging using microscale spatially offset Raman spectroscopy, Anal. Chem. 89 (2017) 792-798.

[31] M. Realini, C. Conti, A. Botteon, C. Colombo, P. Matousek, Development of a full micro-scale spatially offset Raman spectroscopy prototype as a portable analytical tool, Analyst 142 (2017) 351-355.

[32] B.R. McCreadie, M.D. Morris, T. Chen, D.S. Rao, W.F. Finney, E. Widjaja, S.A. Goldstein, Bone tissue compositional differences in women with and without osteoporotic fracture, Bone 39 (2006) 1190-1195.

[33] M.V. Schulmerich, W.F. Finney, V. Popescu, M.D. Morris, T.M. Vanasse, S.A. Goldstein, Transcutaneous Raman spectroscopy of bone tissue using a non-confocal fiber optic array probe, Proc. of SPIE 6093(2006) 60930O.

[34] K. Sowoidnich, J. Churchwell, K. Buckley, A.E. Goodship, A.W. Parker, P. Matousek, Photon migration of Raman signal in bone as measured with spatially offset Raman spectroscopy, J. Raman Spectrosc. 47 (2016) 240-247.

[35] M.V. Schulmerich, W.F. Finney, R.A. Fredricks, M. D. Morris, Subsurface Raman spectroscopy and mapping using a globally illuminated non-confocal fiber-optic array probe in the presence of Raman photon migration, Appl. Spectrosc. 60 (2006) 109-114.

[36] M.V. Schulmerich, J.H. Cole, K.A. Dooley, M.D. Morris, J.M. Kreider, S.A. Goldstein, S. Srinivasan, B.W. Pogue, Noninvasive Raman tomographic imaging of canine bone tissue, J. Biomed. Optics 13 (2008) 020506.

[37] S. Srinivasan, M. Schulmerich, J.H. Cole, K.A. Dooley, J.M. Kreider, B.W. Pogue, M.D. Morris, S.A. Goldstein, Image-guided Raman spectroscopic recovery of canine cortical bone contrast in situ, Opt. Express 16 (2008) 12190-12200.

[38] C.M. Carpenter, B.W. Pogue, S. Jiang, H. Dehghani, X. Wang, K.D. Paulsen, W.A. Wells, J. Forero, C. Kogel, J.B. Weaver, S.P. Poplack, P.A. Kaufman, Image-guided optical spectroscopy provides molecular-specific information in vivo: MRI-guided spectroscopy of breast cancer hemoglobin, water, and scatterer size, Opt. Lett. 32 (2007) 933-935.

[39] J.L.H. Demers, S.C. Davis, B.W. Pogue, M.D. Morris, Multichannel diffuse optical Raman tomography for bone characterization in vivo: a phantom study, Biomed. Opt. Express 3 (2012) 2299-2305.

[40] G. Feng, M. Ochoa, J.R. Maher JR, H.A. Awad, A.J. Berger, Sensitivity of spatially offset Raman spectroscopy (SORS) to subcortical bone tissue, J. Biophotonics 10 (2017) 990-996.

[41] 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 24 (2016) 12701-12712.

[42] Z. Liao, F. Sinjab, A. Nommeots-Nomm, J. Jones, L. Ruiz-Cantu, J. Yang, F. Rose, I. Notingher, Feasibility of Spatially Offset Raman Spectroscopy for in Vitro and in Vivo Monitoring Mineralization of Bone Tissue Engineering Scaffolds, Anal. Chem. 89 (2017) 847-853.

[43] A.S. Haka, K.E. Shafer-Peltier, M. Fitzmaurice, J. Crowe, R.R. Dasari, M.S. Feld, Identifying microcalcifications in benign and malignant breast lesions by probing differences in their chemical composition using Raman spectroscopy, Cancer Res. 62 (2002) 5375-5380.

[44] N. Stone, P. Matousek, Advanced transmission Raman spectroscopy: A promising tool for breast disease diagnosis, Cancer Res. 68 (2008) 4424-4430.

[45] Ghita A, Matousek P, Stone N, High sensitivity non-invasive detection of calcifications deep inside biological tissue using transmission Raman spectroscopy, J. Biophotonics. (2017) doi: 10.1002/jbio.201600260. in press.

[46] M.D. Keller, S.K. Majumder, A. Mahadevan-Jansen, Spatially offset Raman spectroscopy of layered soft tissues, Opt. Lett. 34 (2009) 926-928.

[47] G. Thomas, T.-Q. Nguyen, I. J. Pence, B. Caldwell, M. E. O'Connor, J. Giltnane, M. E. Sanders, A. Grau, I. Meszoely, M. Hooks, M. C. Kelley, A. Mahadevan-Jansen, Evaluating feasibility of an automated 3-dimensional scanner using Raman spectroscopy for

intraoperative breast margin assessment, Sci. Rep. (2017) DOI:10.1038/s41598-017-13237-y . in press.

[48] K. Ma, J.M. Yuen, N.C. Shah, J.T. Walsh, M.R. Glucksberg, R.P. Van Duyne, In vivo, transcutaneous glucose sensing using surface-enhanced spatially offset Raman spectroscopy: multiple rats, improved hypoglycemic accuracy, low incident power, and continuous monitoring for greater than 17 days, Anal. Chem. 83 (2011) 9146-9152.

[49] S.M. Asiala, N.C. Shand, K. Faulds, D. Graham, Surface-enhanced, spatially offset Raman spectroscopy (SESORS) in tissue analogues, ACS Appl. Mater. Interfaces 9 (2017) 25488–25494.

[50] B. Sharma, K. Ma, M.R. Glucksberg, R.P. Van Duyne, Seeing through bone with surface-enhanced spatially offset Raman spectroscopy, J. Am. Chem. Soc. 135 (2013) 17290-17293.

[51] A.S. Moody, P.C. Baghernejad, K.R. Webb, B. Sharma, Surface enhanced spatially offset Raman spectroscopy detection of neurochemicals through the skull, Anal. Chem. 89 (2017) 5688-5692.

[52] K. Buckley, C.G. Atkins, D. Chen, H.G. Schulze, D.V. Devine, M.W. Blades, R.F. Turner, Non-invasive spectroscopy of transfusable red blood cells stored inside sealed plastic blood-bags, Analyst 141 (2016) 1678-1685.

[53] B. Gardner, P. Matousek, N. Stone, Temperature spatially offset Raman spectroscopy (T-SORS): Subsurface chemically specific measurement of temperature in turbid media using anti-Stokes spatially offset Raman spectroscopy, Anal. Chem. 88 (2016) 832-837.

[54] B. Gardner, N. Stone, P. Matousek, Non-invasive chemically specific measurement of subsurface temperature in biological tissues using surface-enhanced spatially offset Raman spectroscopy, Faraday Discuss. 187 (2016) 329-339.

Figure Captions

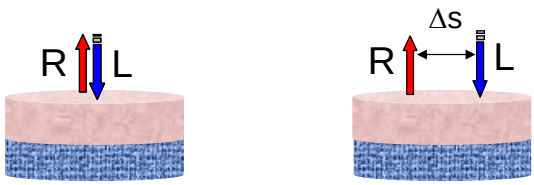
Figure 1: Schematics of conventional and SORS sampling geometries.

Figure 2: SORS and conventional Raman spectra of active pharmaceutical ingredient (API) measured through opaque polypropylene container. The reference spectra of the two polymorphic form of API are also shown. The spectra are offset for clarity. Reprinted from Ref. 23. Copyright (2013), with permission from Elsevier.

Figure 3: Results of TRS measurements of hydroxyapatite (HAP) powder inside 40 mm thick block of tissue at different concentrations of HAP: reference spectrum of HAP and difference spectra. The marker HAP peak is highlighted. Reproduced from Ref: 45 with permission from John Wiley and Sons.

Figure 4: Partial Least Squares (PLS) model of heating nanoparticles buried in 10 mm porcine tissue (25 - 42 ^{o}C). (a) The fit of the model response and the measured temperature. (b) Predicted temperatures vs. the measured temperature of the gold nanoparticles. Reproduced from Ref: 54 with permission from The Royal Society of Chemistry.

Figure 1



conventional Raman

SORS

Figure 2

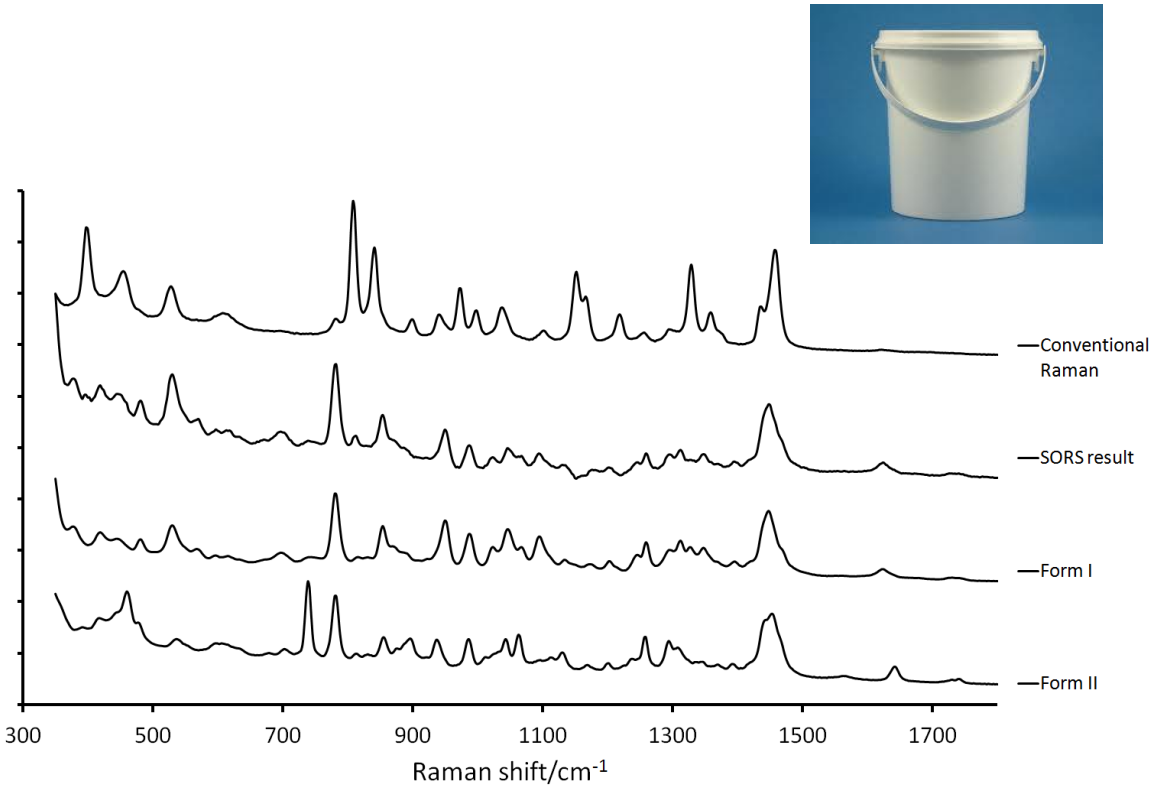

Conventional Raman
SORS result
Form I
Form II
300
500
700
900
1100
1300
1500
1700
Raman shift/cm-1

Figure 3

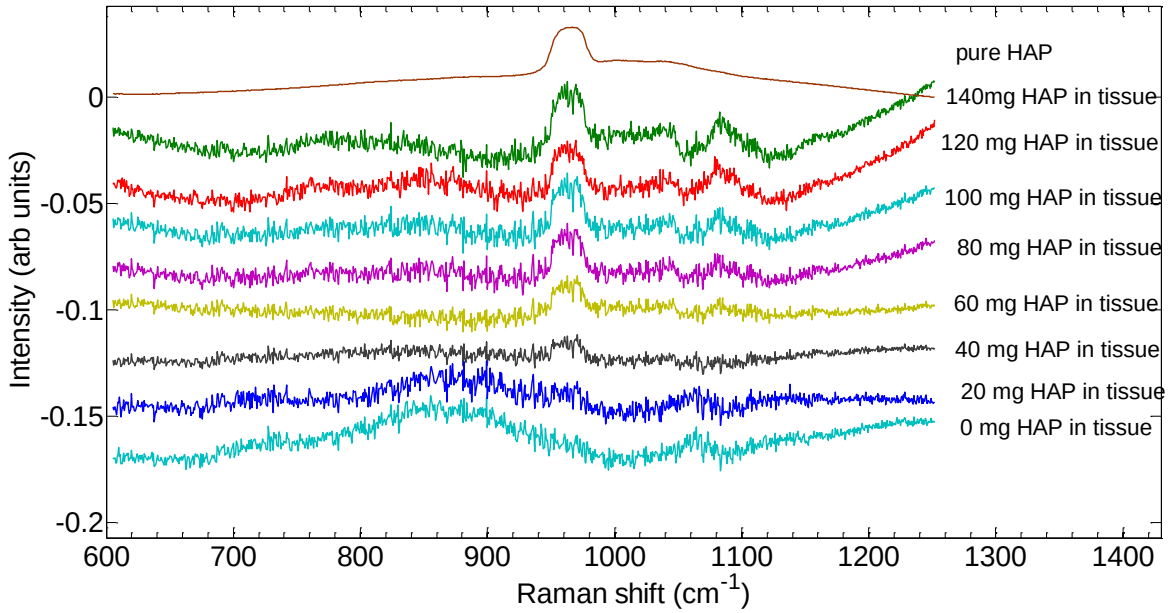
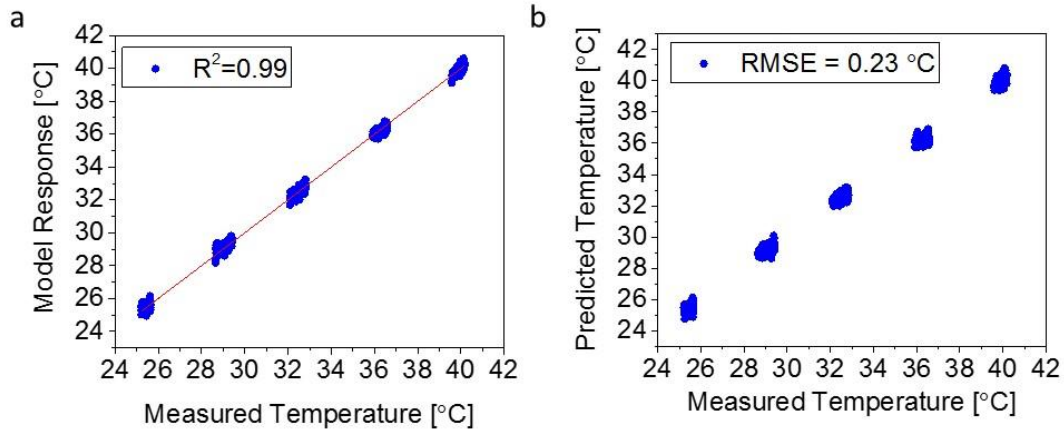

pure HAP
140mg HAP in tissue
120 mg HAP in tissue
100 mg HAP in tissue
80 mg HAP in tissue
60 mg HAP in tissue
40 mg HAP in tissue
20 mg HAP in tissue
0 mg HAP in tissue
Intensity (arb units)
0
-0.05
-0.1
-0.15
-0.2
600
700
800
900
1000
1100
1200
1300
1400
Raman shift (cm-1)

Figure 4


a
Model Response [°C]
R²=0.99
Measured Temperature [°C]
b
Predicted Temperature [°C]
RMSE = 0.23 °C
Measured Temperature [°C]