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Direct monitoring of light mediated hyperthermia induced within mammalian tissues using surface enhanced spatially offset Raman spectroscopy (T-SESORS)†

Benjamin Gardner, ^a Pavel Matousek ^{*b} and Nick Stone ^{*a}

Here we demonstrate light mediated heating of nanoparticles confined deep inside mammalian tissue, whilst directly monitoring their temperature non-invasively using a form of deep Raman spectroscopy, T-SESORS. One of the main barriers to the introduction of photo-thermal therapies (PTT) has been recognised as the inability to directly monitor the local temperature deep within the tissue at the point of therapy. Here Au nanoparticles with a Raman reporter molecule (temperature reporters) are used in combination with Au nanoshells (heat mediators) to provide simultaneously heating under NIR illumination and direct spectroscopic monitoring of local temperature deep within mammalian tissues. The surface enhanced Raman signal was read out at the tissue surface using a transmission geometry in this example and the temperature of the tissue was ascertained from the anti-Stokes to Stokes Raman reporter. This approach opens the prospect of non-invasive hyperthermia treatments with direct temperature feedback from deep inside within tissue, where nanoparticles can be used to both provide localised heating and accurately monitor the local temperature.

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Introduction

The field of nanomaterials for photonics applications is rapidly expanding, with increasingly sophisticated materials being developed to monitor a plethora of physical and chemical properties, including but not limited to temperature,^{1,2} pH,^{3,4} redox,⁵ glucose,⁵ DNA⁶ and proteins such as neurotransmitters,⁷ with many of these being complex *in vivo* targets. One such active area is the development of advanced nanomaterials as therapeutics for the next generation of photothermal therapies for cancer treatment.^{8,9} The clinical need for such technology stems from the limitations with current photodynamic therapies (PDT), which are largely non-specific for cancer cells, depth limited and/or have various side effects including systemic light sensitivity.^{9–11} It is envisioned that nanomaterials would be selectively functionalised to not only specifically target cancer/tumours, utilising appropriate antibodies,^{12–17} but also to act simultaneously as a therapeutic agent. This is often considered in the form of photothermal or

magnetothermal heating. Furthermore, in the case explored here, a third critical characteristic is considered, where also the temperatures of the nanomaterial can be remotely monitored and controlled non-invasively, even utilising the same energy source as the heating. Potential nanomaterial mediated hyperthermia treatments require the temperature not only to be elevated, but also accurately monitored and maintained within relatively narrow therapeutic thermal windows (41–48 °C) for effective and selective treatments.⁹ However, until very recently non-invasive temperature sensing of tissue at depth was not possible^{1,2,8,18} representing one of the main barriers to the introduction of photo thermal therapies (PTT).⁹ Classically, Raman spectroscopy is seen as a technique limited to the sample surface in diffusely scattering samples such as biological tissues, with the sampling depth often limited to a few hundreds of micrometres. However, with the development of spatially offset Raman spectroscopy (SORS),¹⁹ the closely related inverse SORS,^{20,21} as well as resurgence of its variant, transmission Raman (TRS),^{22–25} this is no longer the case. These technical developments have radically improved the sampling depth by orders of magnitude demonstrating ability to access depths of several centimetres. The underpinning concept of these techniques constitutes the separation of the illumination and Raman collection zone on the surface of a scattering sample. This allows the contrast of the measured subsurface signal in terms of the surface signal to increase. This has led to a rapid expansion of the field with a variety of

^aBiomedical Physics, School of Physics and Astronomy, College of Engineering, Mathematics and Physical Sciences, University of Exeter, Exeter, EX4 4QL, UK. E-mail: n.stone@ex.ac.uk

^bCentral Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Oxford, OX11 0QX, UK

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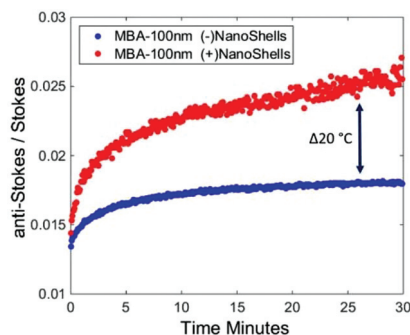


Fig. 3 Anti-Stokes/Stokes ratio of (1085 cm^{-1} ν_{12} benzene ring mode) temperature reporter Au-MBA nanoparticles in the absence (blue) and presence (red) of Au-NS with a peak absorbance at 800 nm while inserted in porcine tissue at a depth of 5 mm (with a total thickness of 10 mm) and illuminated with 1.2 W (λ_{ex} 830 nm). See ESI Fig. 2A & B† for corresponding raw Raman data.

temperature caused by the nanoshells as opposed to non-specific heating. This temperature increase is well within the range required to elevate natural temperature tissue *in vivo* to the required therapeutic window range (which is ideally 41–48 °C), demonstrating that either lower powers or treatment of deeper lesions is clearly viable.

Experimental

Temperature reporters were made up of 100 nm diameter gold citrate coated nanoparticles (0.05 mg ml^{-1}) (NanoComposix) λ_{max} 563 nm, labelled with mercaptobenzoic acid (Sigma Aldrich) (Au-MBA) using a previously described method.³⁰ In brief 100 μl of 1 mM MBA in EtOH was added to 1 ml of the nanoparticle suspension, the sample was mixed for 5 min before centrifugation at 3000 rpm for 10 min. For demonstration of photothermal heating, the Au-MBA nanoparticles were combined with 4 ml of (0.05 mg ml^{-1}) polyvinylpyrrolidone (PVP) coated gold nanoshells (Au-NS) λ_{max} 800 nm. Raman measurements were carried out on a homebuilt instrument, operating in transmission Raman geometry. In brief, a tuneable Ti:Sapphire laser (Newport, Spectra Physics) generated a laser beam at 830 nm. The beam was passed through a NIR acousto-optic tuneable filter (Gooch and Housego) to clean up spectrally the laser line. Following transmission through the sample the laser line was suppressed with two 830 nm notch filters (Kaiser) and the signal was collected using a deep depletion CCD camera (Andor iDus-420) which was coupled to a high throughput Raman spectrometer (Kaiser Holospec 1.8i) operating with a custom low dispersive holographic transmission grating (Kaiser). Raman spectra were collected for 5 s (10 accumulations of 0.5 s). Initial measurements testing the heating of the non-NIR absorbing temperature reporter NPs (Au-MBA) was undertaken by depositing them onto a CaF_2 slide and measuring the anti-Stokes/Stokes (AS/S) ratio and the surface temperature with an infrared temperature sensor (PyroMiniusb), as well as a thermal imaging camera

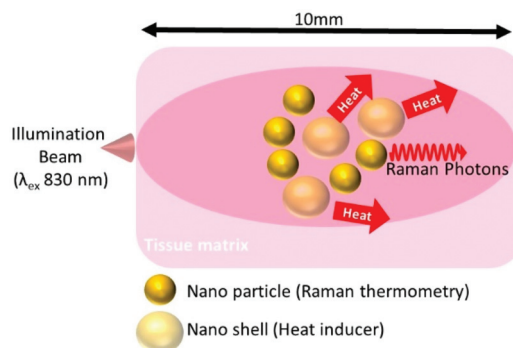


Fig. 4 Schematic of non-invasive Raman thermometry at depth in porcine tissue using labelled nanoparticles (Au-MBA) monitoring temperature changes induced by gold nanoshells (Au-NS).

(T430, FLIR). The illumination power at 830 nm was varied between 5 to 400 mW to explore the measured AS/S ratio *versus* power and its relationship with temperature. To monitor temperature induced by the NIR absorbing nano shells, the Au-MBA temperature reporter and the nanoshells were mixed together and deposited onto CaF_2 and left to dry. The mixture was monitored at three separate powers 250, 500 & 750 mW. The AS/S ratio of the reporter was monitored simultaneously with temperature monitors (infrared sensor, thermal imaging camera). To monitor temperature noninvasively at depth, the temperature reporter (Au-MBA) was deposited in the middle of 10 mm thick porcine tissue with and without nanoshells present (Fig. 4).

The depth experiments were carried out with 1.2 W illumination power at the tissue surface (a laser beam diameter of 1 mm) and the at depth temperature being monitored non-invasively throughout by the aforementioned temperature monitors. As has been reported in previous work^{1,18} a calibration point is required for temperature measurements at depth using Raman, which in this example was taken from time point one (at the onset of illumination), a reflection of the closest equilibrium temperature point, *i.e.* ambient temperature or body temperature for *in vivo* applications. This is due to the significant difference in wavelength of the anti-Stokes & Stokes photons $\sim 150 \text{ nm}$ in this case, the photons experience significant differences in absorption and scattering over the large photon pathlengths in scattering media (tissue) as well as being affected differently by the instrument response function; both effects changing the expected theoretical ratio-metric information.²⁵ The use of simple scaling factors derived from this first equilibrium temperature measurement eloquently solves this otherwise complex issue of calibrating the anti-Stokes/Stokes ratio to temperature (see ESI Fig. 3†). This is analogous to requiring emissivity information for thermal IR cameras to accurately report temperature.

Conclusions

This proof of concept demonstrates how deep Raman can not only be used to non-invasively monitor temperature at depth,



with chemical specificity if needed, but also be used as a tool to control the photothermal dose to the treatment zone. This can be achieved in real-time, to ensure optimum benefit is achieved and minimum damage to surrounding tissues. This is of increasing relevance for advancements in disease treatments such as cancer as, to date, one of the main barriers to the introduction of PTT has been recognised to be the inability to directly monitor the local temperature within the tissue.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- 1 B. Gardner, N. Stone and P. Matousek, *Faraday Discuss.*, 2016, **187**, 329–339.
- 2 E. A. Pozzi, A. B. Zrimsek, C. M. Lethiec, G. C. Schatz, M. C. Hersam and R. P. Van Duyne, *J. Phys. Chem. C*, 2015, **119**, 21116–21124.
- 3 L. E. Jamieson, V. L. Camus, P. O. Bagnaninchi, K. M. Fisher, G. D. Stewart, W. H. Nillon, D. B. McLaren, D. J. Harrison and C. J. Campbell, *Nanoscale*, 2016, **8**, 16710–16718.
- 4 X. S. Zheng, P. Hu, Y. Cui, C. Zong, J. M. Feng, X. Wang and B. Ren, *Anal. Chem.*, 2014, **86**, 12250–12257.
- 5 C. R. Yonzon, C. L. Haynes, X. Zhang, J. T. Walsh and R. P. Van Duyne, *Anal. Chem.*, 2004, **76**, 78–85.
- 6 S. Tian, O. Neumann, M. J. McClain, X. Yang, L. Zhou, C. Zhang, P. Nordlander and N. J. Halas, *Nano Lett.*, 2017, **17**, 5071–5077.
- 7 A. S. Moody, P. C. Baghernejad, K. R. Webb and B. Sharma, *Anal. Chem.*, 2017, **89**, 5688–5692.
- 8 J. Kneipp, *ACS Nano*, 2017, **11**, 1136–1141.
- 9 D. Jaque, L. Martínez Maestro, B. del Rosal, P. Haro-Gonzalez, A. Benayas, J. L. Plaza, E. Martín Rodríguez, J. García Solé, L. Martínez Maestro, B. del Rosal, P. Haro-Gonzalez, A. Benayas, J. L. Plaza, E. Martín Rodríguez and J. García Sole, *Nanoscale*, 2014, **6**, 9494–9530.
- 10 J. Dobson, G. F. de Queiroz and J. P. Golding, *Vet. J.*, 2018, **233**, 8–18.
- 11 M. B. Vrouenraets, G. W. Visser, G. B. Snow and G. A. van Dongen, *Basic principles, applications in oncology and improved selectivity of photodynamic therapy*, 2003, vol. 23.
- 12 S. Pahlow, K. Weber, J. Popp, B. R. Wood, K. Kochan, A. Rüther, D. Perez-Guaita, P. Heraud, N. Stone, A. Dudgeon, B. Gardner, R. Reddy, D. Mayerich and R. Bhargava, *Appl. Spectrosc.*, 2018, **72**(1_suppl), 52–84.
- 13 K. J. Yoon, H. K. Seo, H. Hwang, D. Pyo, I.-Y. Eom, J. H. Hahn and Y. M. Jung, *Bull. Korean Chem. Soc.*, 2010, **31**, 1215–1218.
- 14 S. Chen, C. Bao, C. Zhang, Y. Yang, K. Wang, B. V. Chikkaveeraiah, Z. Wang, X. Huang, F. Pan, K. Wang, X. Zhi, J. Ni, J. M. de la Fuente and J. Tian, *Nano Biomed. Eng.*, 2016, **8**, 315–328.
- 15 A. Samanta, K. K. Maiti, K.-S. Soh, X. Liao, M. Vendrell, U. S. Dinis, S.-W. Yun, R. Bhuvaneswari, H. Kim, S. Rautela, J. Chung, M. Olivo and Y.-T. Chang, *Angew. Chem., Int. Ed.*, 2011, **50**, 6089–6092.
- 16 Z. Chen, M.-F. Penet, S. Nimmagadda, C. Li, S. R. Banerjee, P. T. Winnard, D. Artemov, K. Glunde, M. G. Pomper and Z. M. Bhujwalla, *ACS Nano*, 2012, **6**, 7752–7762.
- 17 A. Oseledchik, C. Andreou, M. A. Wall and M. F. Kircher, *ACS Nano*, 2017, **11**, 1488–1497.
- 18 B. Gardner, P. Matousek and N. Stone, *Anal. Chem.*, 2016, **88**, 832–837.
- 19 P. Matousek, I. P. Clark, E. R. C. Draper, M. D. Morris, A. E. Goodship, N. Overall, M. Towrie, W. F. Finney and A. W. Parker, *Appl. Spectrosc.*, 2005, **59**, 393–400.
- 20 P. Matousek and N. Stone, *J. Biophotonics*, 2013, **6**, 7–19.
- 21 M. V. Schulmerich, K. A. Dooley, M. D. Morris, T. M. Vanasse and S. A. Goldstein, *J. Biomed. Opt.*, 2006, **11**, 060502.
- 22 M. M. Kerssens, P. Matousek, K. Rogers and N. Stone, *Analyst*, 2010, **135**, 3156.
- 23 M. Z. Vardaki, H. Sheridan, N. Stone and P. Matousek, *Appl. Spectrosc.*, 2017, **71**, 1849–1855.
- 24 N. Stone and P. Matousek, *Cancer Res.*, 2008, **68**, 4424–4430.
- 25 B. Gardner, N. Stone and P. Matousek, *Anal. Chem.*, 2017, **89**, 9730–9733.
- 26 H. N. Xie, R. Stevenson, N. Stone, A. Hernandez-Santana, K. Faulds and D. Graham, *Angew. Chem., Int. Ed.*, 2012, **51**, 8509–8511.
- 27 A. Williams, K. J. Flynn, Z. Xia and P. R. Dunstan, *J. Raman Spectrosc.*, 2016, **47**, 819–827.
- 28 R. A. Chipman, *OSA Handb., Opt. - Vol II*, 1995, pp. 22.1–22.35.
- 29 M. Nourhashemi, M. Mahmoudzadeh and F. Wallois, *Neurophotonics*, 2016, **3**, 015001.
- 30 A. Jaworska, L. E. Jamieson, K. Malek, C. J. Campbell, J. Choo, S. Chlopicki and M. Baranska, *Analyst*, 2014, **140**, 2321–2329.

