Supporting Information

Materials and Methods

All chemicals and small molecule Raman reporters were purchased from Sigma Aldrich unless otherwise stated. AuNPs with an average diameter of 83 nm were synthesized using a seeded method and left to stir overnight.[1] Briefly, gold seeds of 28 nm were synthesized using the citrate reduction method. Sodium tetrachloroaurate (III) dihydrate (681 µL, final concentration 0.254M) and sodium citrate trihydrate (528 µL, final concentration 0.171M) were added to 5.007 ml of 28 nm seeds and made up to 120 ml with dH_2O. The solution was left to stir overnight. NPs were characterized using extinction spectroscopy and had an LSPR of 555 nm. Scanning electron microscope (SEM) images were obtained (Fig.S1, Supporting Information) using a FEI Sirion 200 ultra-high resolution Schottky field emission scanning electron microscope with FEI software.

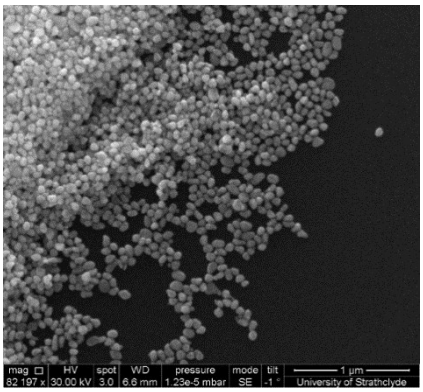

Figure S1 – SEM image of nanoparticles used in SESORS experiments.

Chalcogenpyrylium-based dyes were synthesized according to previously reported methods.[2,3,4] They are named according to the wavelength that they are resonant at. For example dye823 is resonant at 823 nm. Dyes 676, 823 and 959 were prepared by dissolving the solid in anhydrous N,N-Dimethylformamide (DMF, 99.8%) to produce a 1 mM stock. Subsequent dilutions were then carried out using DMF and dH_2O (50:50). Raman reporters 1,2-bis(4-pyridyl)ethylene (BPE) and 4,4-azopyridine (AZPY) were prepared by dissolving the solid in ethanol to produce a 10 mM stock. Subsequent dilutions were carried out using dH_2O. Dyes were characterized using extinction spectroscopy (Agilent Cary 60) to determine their λmax. BPE and AZPY are non-resonant Raman reporters.

Measurements were taken using a handheld Resolve instrument from Cobalt Light Systems (830 nm, average laser power 450 mW). All measurements were carried

out using a 2 s integration time, 5 accumulations and an 8 mm offset. The nose cone was fitted to use the instrument in a contact mode setting. The handheld instrument used here has a fixed exposure time, therefore it is noted that if longer acquisition times were used, the signal to noise ratio may have improved. The conventional Raman spectra for each of the five individual Raman reporters used in this work (dye 1-3, BPE and AZPY), can be seen in the Supporting Information (Figure S1). Measurements were carried out using 3 samples. Prior to dye addition, NPs were concentrated by centrifugation (1 mL aliquots, 5000 RPM, 10 mins) and resuspended in 500 µL of water. Investigation of the nanotags for SESORS applications was carried out by adding each reporter (3 uL, 300 µM) to 500 µL of NPs. The solution was then made up to 1 mL with dH_2O. A final dye concentration of 300 nM was used, thus by keeping the dye concentration as low as possible the benefit of using a Raman reporter which is in resonance with the laser was exploited.

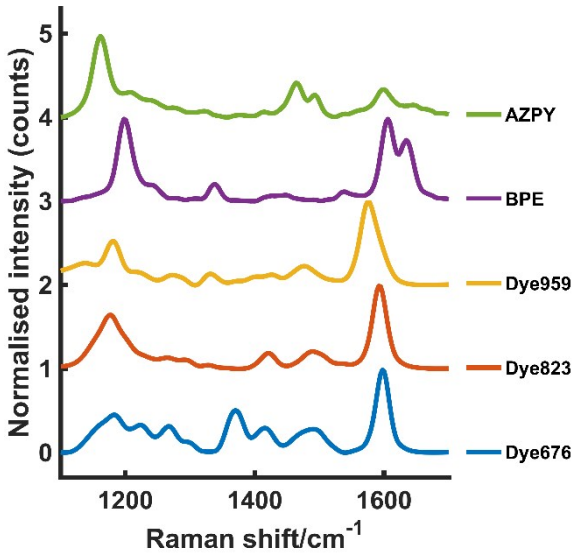

Figure S2. SERS spectra of the five Raman reporters used in this work. Dyes 676, 823 and 959 are chalcogen based reporters BPE and AZPY are commercially available. Spectra were obtained using the SORS instrument in a conventional Raman mode. Dyes 1 – 3 are resonant at 676, 823 and 959 nm respectively. BPE and AZPY are non-resonant molecules. All measurements were carried out using a 2 s integration time, 5 accumulations, 830 nm laser excitation wavelength.

Experimental set up

Pork loin tissue was obtained from a local butcher and cut into sections (roughly 3.5 cm inches x 4 cm with varying thicknesses). Pork was chosen as an analogue to human samples due its ability to mimic human tissue greater than that from avian species.[5] Tissue experiments were performed using either a quartz cuvette or by

spotting the NP solution directly on to the tissue samples. For measurements involving a cuvette, 350 µL of each NP-Dye solution was pipetted into a Suprasil quartz micro cuvette, path length 1 mm, chamber volume 350 µL. Tissue samples of varying thicknesses were then placed in front of the cuvette. The nose cone was brought into contact with the tissue samples, thus ensuring there was no space between the instrument and the tissue. The set up involving the cuvette is shown in Figure S3.

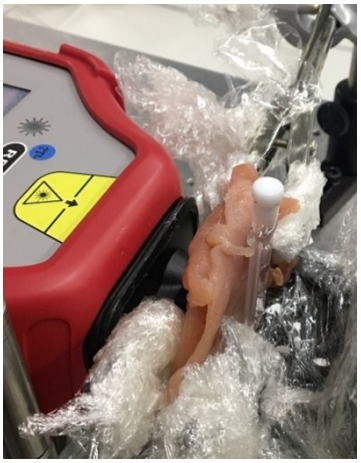

Figure S3. Experimental set-up using a handheld SORS spectrometer for the detection of nanotags through tissue. Nanotag solutions were held in a cuvette and the cuvette was placed behind tissue samples. The nose cone was brought into contact with the tissue ensuring there was no space between the tissue sample and the instrument.

Cell culture and nanotag delivery to cells

MCF7 human breast cancer cells were cultured in Rosewell Park Memorial Institute medium (RPMI) supplemented with 1% penicillin/streptomycin (10,000 units/mL), 1% fungizone, and 10% heat-inactivated foetal bovine serum (FBS). Cells were incubated at 37 °C and 5% CO_2 in a humidified incubator. Cells at a confluence of ca. 90% growing in a T75 flask were incubated overnight with 571 fM of AuNP (total of 7.092×10^{11} AuNP). The following day, cells were trypsinised and re-suspended in medium to give a concentration of ca. 2.4×10^6 cells cells/mL. Multicellular tumour spheroids (MTS) were grown using a hanging drop technique by pipetting 20 µL drops of this cell suspension onto the lid of a petri dish with ca. 12 mL of medium added to the dish. The lid was placed on the dish and MTS grew over a period of 9 days at 37 °C and 5% CO_2 in a humidified incubator. Medium was removed from the drops and replaced after 3 days.

For mapping experiments, the MTS models containing the SERRS nanotags were placed directly onto a section of tissue (Figure S4a) and left to equilibrate for 10 mins. Following this, 15 mm of tissue was then placed in on top of the tissue layer

containing the MTS models (Figure S4b). The two-layer sample was then brought into contact with the laser via the nose cone. The handheld SORS instrument was positioned above the tissue samples with the laser pointing down onto the tissue (Figure S4b). As shown, in comparison to previous work involving the cuvette, the tissue section was laid flat on the stage and the handheld instrument mounted above the sample. All measurements were performed using an 8 mm offset. This set up is more representative of an *in vivo* approach compared to that using the cuvette. An *x-y*-positioning stage was used to enable Raman mapping of the MTS containing SERRS nanotags through 15 mm of tissue. The SORS technique utilises the properties of photon diffusion in turbid media[6], thus when the scattered photons are returned to the collection probe they will undergo multiple scattering processes and are more likely to migrate laterally. This explains why there is larger area of maximum intensity (compared to actual size of the spheroids). The same principle of diffuse scattering also applies to the laser photons that must reach the MTS models in the first instance.

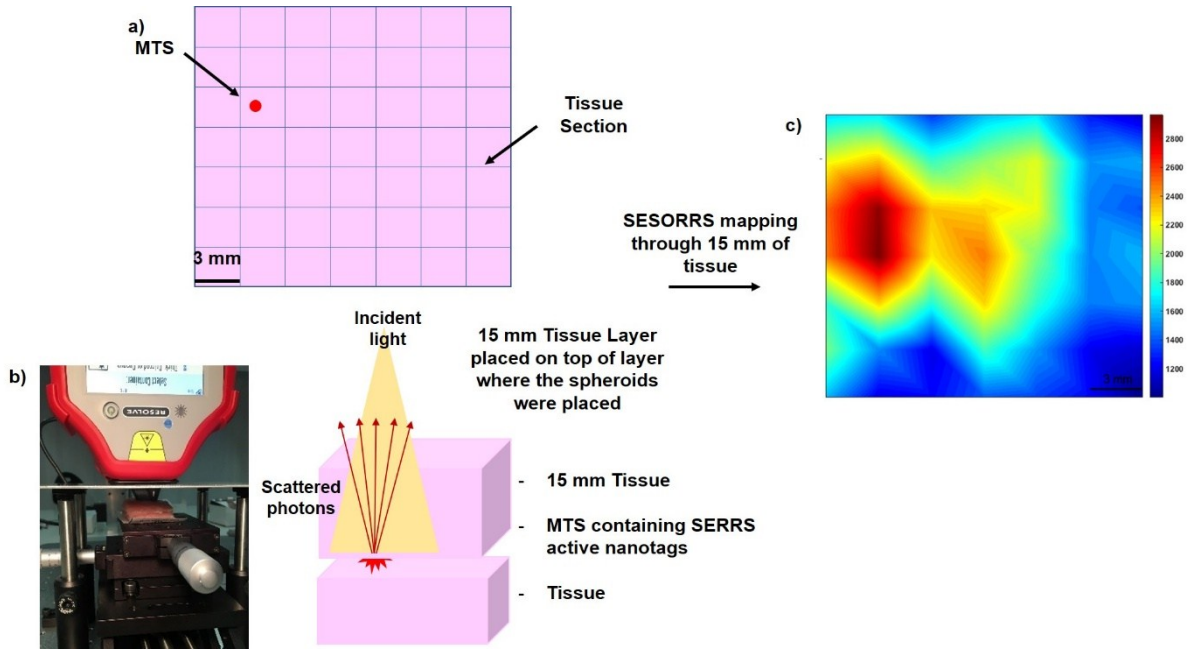

Figure S4 – Correlation of the MTS position on the underlying tissue layer (a,b) to the heat intensity observed in the false colour 2D heat map (c). MTS were placed onto a section of tissue (a). A 15 mm section of porcine tissue was then placed on top of the tissue layer upon which the MTS models were positioned. The experimental set-up involved mounting the instrument above the tissue samples. The sample was then brought into contact with the laser via the nose cone (b). Detection of SERRS nanotags through 15 mm of tissue was measured in a 7 x 7 grid, pixel size 3 mm. All spectra were collected at an 8 mm offset. In the region where the MTS models were present, the largest SERRS intensity is observed (c). The map was constructed using the peak intensity at 1178 cm^{-1}. Measurements were carried out using an xy translational stage in step sizes of 3 mm to create an image of 7 x 7 pixels. This shows the tracking of MTS models through 15 mm of tissue. All measurements were carried out using a 2 s integration time, 5 accumulations, 830 nm laser excitation wavelength.

Data processing

All spectra were processed using Matlab software (Version 2017a, The MathWorks, Natrick, MA, USA). Preprocessing involved truncating and baselining the spectra. A scaled subtraction was also applied in some instances. Briefly spectra were truncated and baselined using a high order polynomial which removed the fluorescent background. Polynomials were chosen on their ability to remove as much of the fluorescent background under the spectrum as possible. Following this, the surface spectrum (i.e. the signal detected at the zero position) was removed from the subsurface spectrum (i.e. signal detected at the offset position) using a scaled subtraction. Data was subsequently smoothed using Savitzky-Golay filtering.

Mapping experiments were performed using an x-y positioning stage to enable Raman mapping of the SERRS nanotags through 15 mm of tissue. All measurements were performed using a 8 mm offset. Spectra were truncated, baselined and smoothed using Savitzky-Golay filtering before the intensity at 1178 cm^{-1} at each of the 3 mm steps was plotted as a combination surface/contour false colour 2D heat map.

A scaled subtraction was applied (Figure S5) which aims to show the presence of the analyte obscured by the barrier. The scaled subtraction removed the spectral contribution from the tissue and revealed the peaks at 1178 cm^{-1} and 1592 cm^{-1}, characteristic of resonant dye 823. This further demonstrates the detection of dye 823 through 25 mm of tissue using SESORRS in a back-scattering configuration.

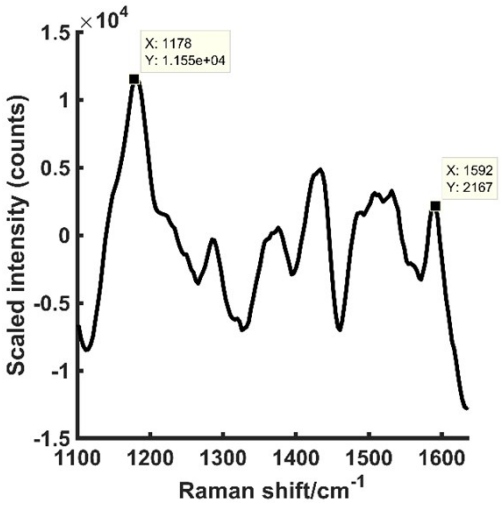

Figure S5. The tracking of dye 823 nanotag solution through 25 mm of tissue following a scaled subtraction. The peaks at 1178 cm-1 and 1592 cm-1 are revealed. This approach removes signal collected at the zero position (tissue) from that collected at the 8 mm offset (tissue and dye). All measurements were carried out using a 2 s integration time, 5 accumulations, 830 nm laser excitation wavelength.

References

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