Electronic Supplementary Material (ESI) for ChemComm.

Materials and Methods

All chemicals were purchased from Sigma Aldrich unless otherwise stated. AuNPs with an average diameter of 78 nm were synthesized using a seeded method. Au seeds with a 28 nm diameter were synthesised using the citrate reduction method. Large AuNPs were then grown from a solution of these seeds. 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 then left to stir overnight, allowing the NPs to grow to a size of 78 nm. The resulting colloid was characterized using extinction spectroscopy and a LSPR of 550 nm was observed.

Chalcogenpyrylium-based dyes were synthesized according to previously reported methods.[1,2,3] Dyes were characterized using extinction spectroscopy (Agilent Cary 60) to determine their λmax and are therefore named according to the wavelength that they are resonant at, e.g. dye810 is resonant at 810 nm. Stock solutions of each of the three dyes (1 mM) were prepared by dissolving the solid in anhydrous anhydrous N,N-Dimethylformamide (DMF, 99.8%). Subsequent dilutions were then carried out using a solution of DMF and dH_2O (50:50). Prior to dye addition, NPs were concentrated by centrifugation (1 mL aliquots, 5000 RPM, 10 mins) and resuspended in 500 µL of water. The Raman reporters were added to the nanoparticles and the solution made up to 1 ml with dH_2O. This produced SERRS nanotags with a final concertation of 300 nM.

SERRS and SESORRS measurements were taken using a handheld Resolve instrument from Cobalt Light Systems (830 nm, average laser power 450 mW). Measurements were carried out using 3 samples. All measurements were carried out using a 2 s integration time, 5 accumulations and an 8 mm offset. To use the instrument in a contact mode setting, the nose cone was fitted to the instrument, i.e. the nose cone was in contact with the tissue barrier and there was no space between the instrument and the tissue barrier.

MTS were used as a 3D breast cancer tumour model to demonstrate the clinical significance of SESORRS for in vivo multiplex applications. MCF7 human breast cancer cells were incubated overnight with 1 mL of each of the three nanotag solutions containing dye 810, 813 and 823. MCF7 cells were also incubated with a 1 mL triplex solution containing 33% of each of the three SERRS nanotags (13.7 pM of AuNPs), i.e the final number of NPs remained constant. Incubation resulted in the uptake and accumulation of the nanotags within the cancer cells. MTS were then grown from a suspension of these cells using a hanging drop technique over a period of 7 days at 37 °C and 5% CO2 in a humidified incubator to a size <1 mm.

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). Tissue experiments were performed using

either a quartz cuvette or by spotting the MTS models directly on to the tissue samples. The tissue layer containing the MTS was then overlaid with another section of tissue with a thickness of 10 mm. The experimental set up is described in Figure S1.

For measurements involving a cuvette, 350 µL of each NP-Dye solution (e.g. dye 813) was pipetted into a Suprasil quartz micro cuvette, path length 1 mm, chamber volume 350 µL. In keeping with the work involving MTS, tissue samples with a thickness of 10 mm were then placed in front of the cuvette. Therefore, both the MTS models and cuvettes containing each NP-Dye solution were obscured by 10 mm. 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 S2.

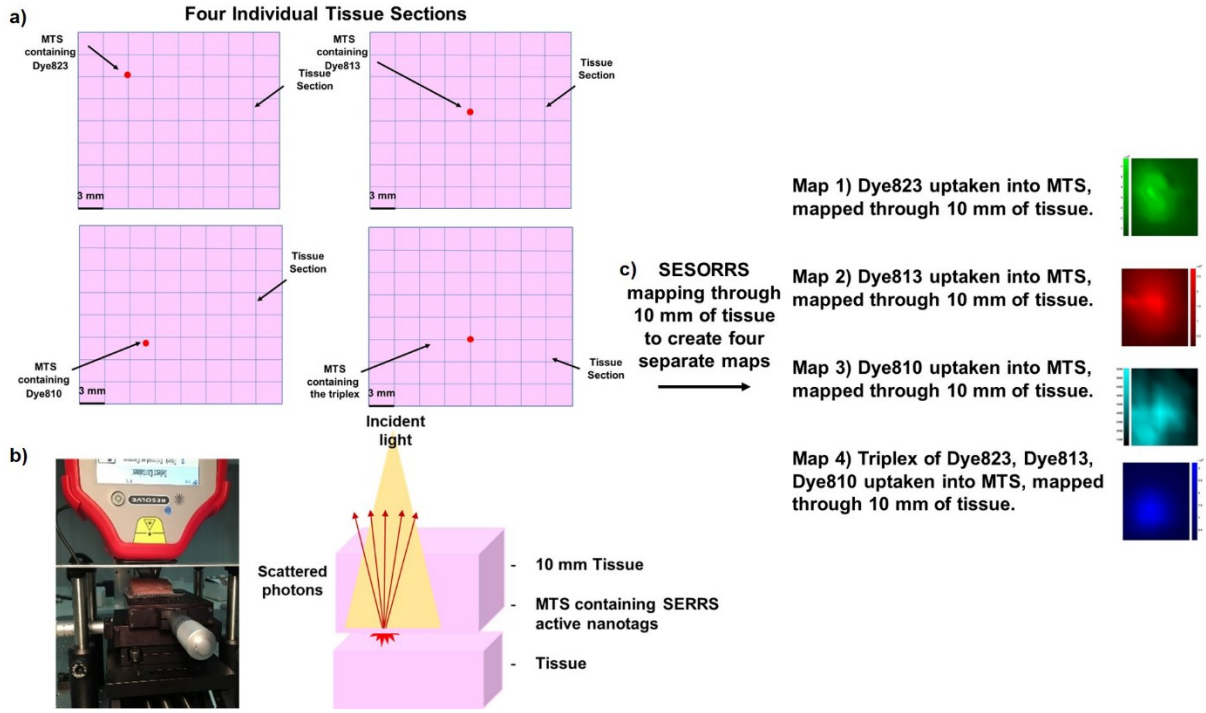

Figure 1 – MTS containing either the single nanotags (dye823, dye813 and dye810) or the triplex of the three SERRS nanotags were placed onto four separate sections of tissue (a). A 10 mm section of porcine tissue was then placed on top of each of the tissue layers upon which the MTS models were positioned. The experimental set-up involved mounting the instrument above the tissue samples. The samples were then brought into contact with the laser via the nose cone (b). Detection of each of the four MTS models containing the varying SERRS nanotags (single nanotags or triplex) through 10 mm of tissue was measured in a 8 x 8 grid, pixel size 3 mm. This created four individual SERRS heat intensity maps (c). 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.

Figure S2 - 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 to ensure there was no space between the tissue sample and the instrument.

Data processing

All spectra were processed using Matlab software (Version 2017a, The MathWorks, Natrick, MA, USA). Preprocessing involved truncating and baselining the spectra. Mapping experiments were performed using a x-y positioning stage to enable Raman mapping of the SERRS nanotags through 15 mm of tissue. All measurements were performed using an 8 mm offset. Spectra were truncated, baselined and smoothed using Savitzky-Golay filtering before the intensity at 1178 cm^{-1} (dye823) 1181 cm^{-1} (dye813), 1185 cm^{-1} (dye810) and 1181 cm^{-1} (triplex) was plotted as a combination surface/contour false colour 2D heat map.

Principal component analysis (PCA) was applied to develop a multiplexed imaging technique. The solution multiplex was performed by collecting15 spectra (5 replicates, 3 samples) through 10 mm of tissue of each of the four nanotag solutions, i.e. dye823, dye813, dye810 and the triplex containing the three dyes. PCA was performed on spectra obtained at an offset of 8 mm through 10 mm of tissue. The same was applied for the multiplex detection of MTS models through 10 mm of tissue. 15 spectra were collected at the point where the MTS models containing each of the four nanotag solutions, i.e. dye823, dye813, dye810 and the triplex containing the three dyes. The spectra collected at an 8 mm offset through 10 mm of tissue was then used to perform PCA. Pre-processing involved truncating and scaling the spectra, before applying the first order derivative coupled with Savitzky-Golay smoothing. The first order derivative was used in PCA to remove slight variances in the background which were found to affect the resulting zero order PCA scores plots.[5]

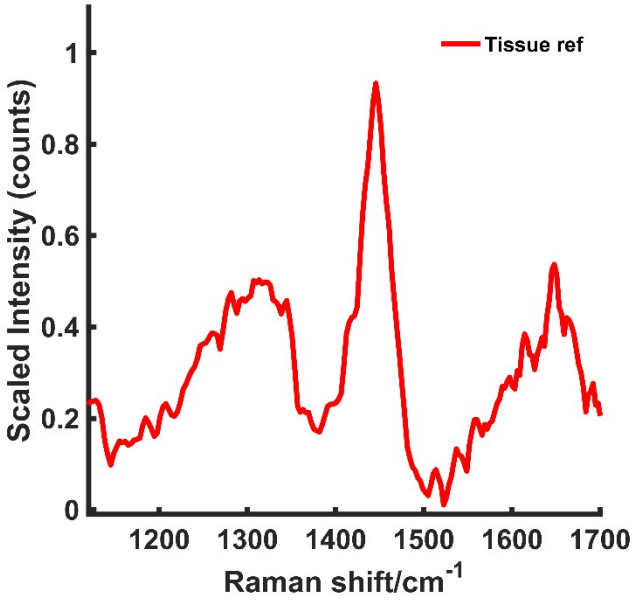

Figure S3 – 10 mm tissue reference spectra collected at an 8 mm offset. The nose cone was brought into contact with the tissue to ensure there was no space between the tissue sample and the instrument. Measurements were carried out using a 2 s integration time, 5 accumulations, 830 nm laser excitation wavelength.

References

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