Supporting information:

A Simple Spectral Method for Nanoplastic Identification and Characterisation

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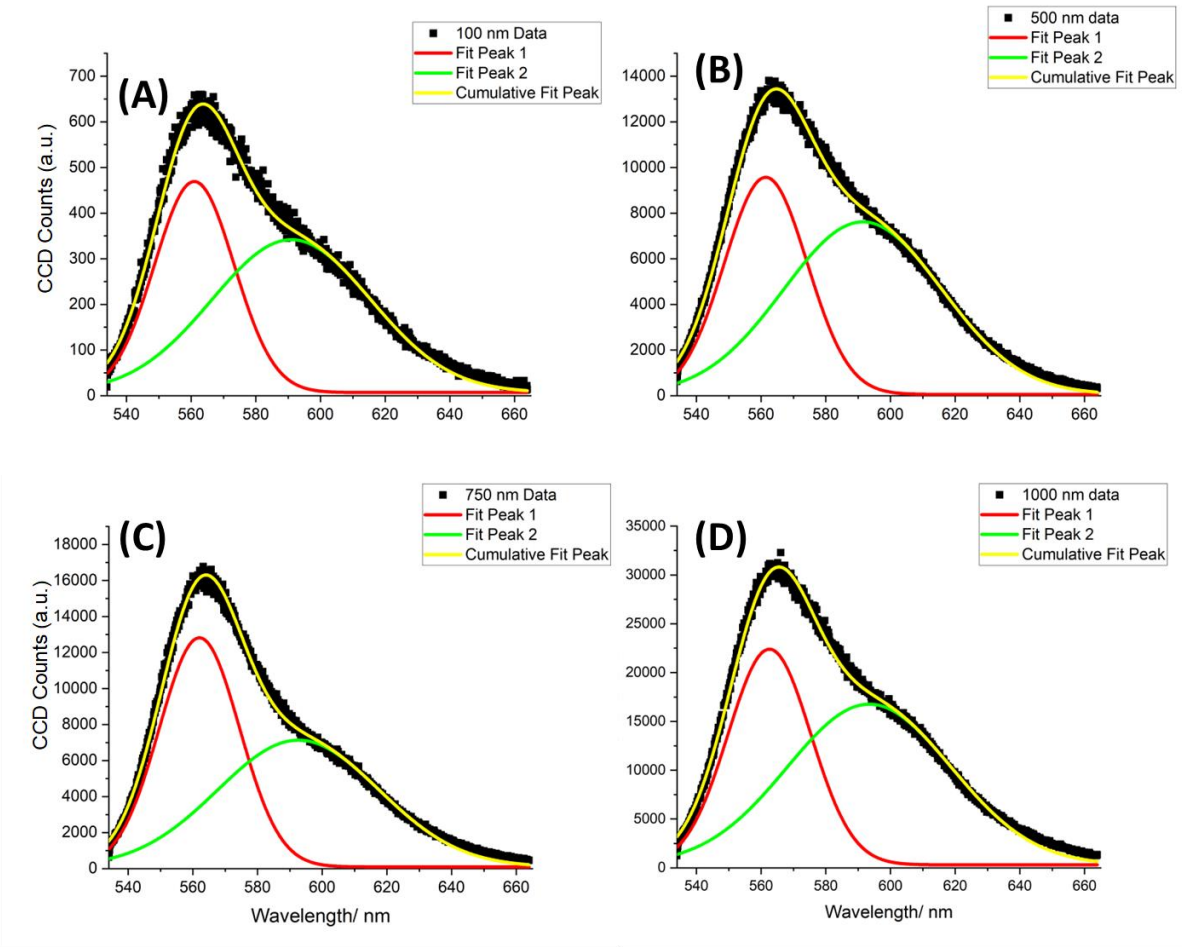


Figure S1: **Fluorescence emission spectra from different sized polystyrene (PS) micro/ nanospheres. (A) 100 nm (B) 500 nm (C) 750 nm (D) 1000 nm. Deconvolution analysis of each particle size is shown with peak maxima at 562 ± 1 nm and 595 ± 2 nm.**

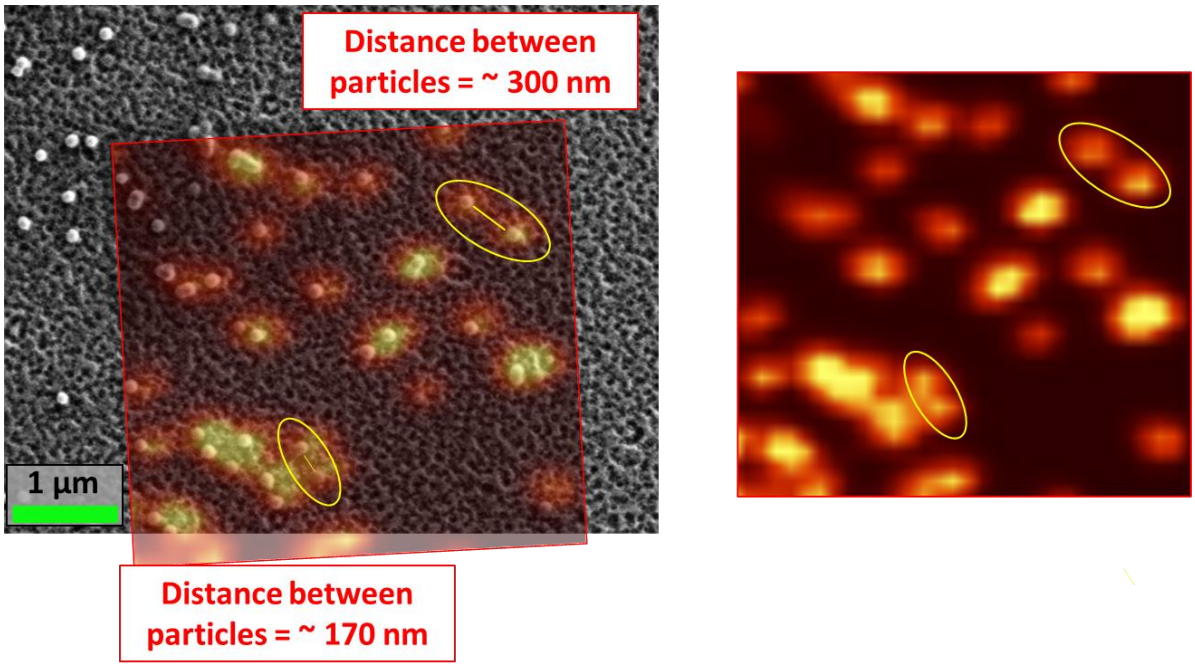
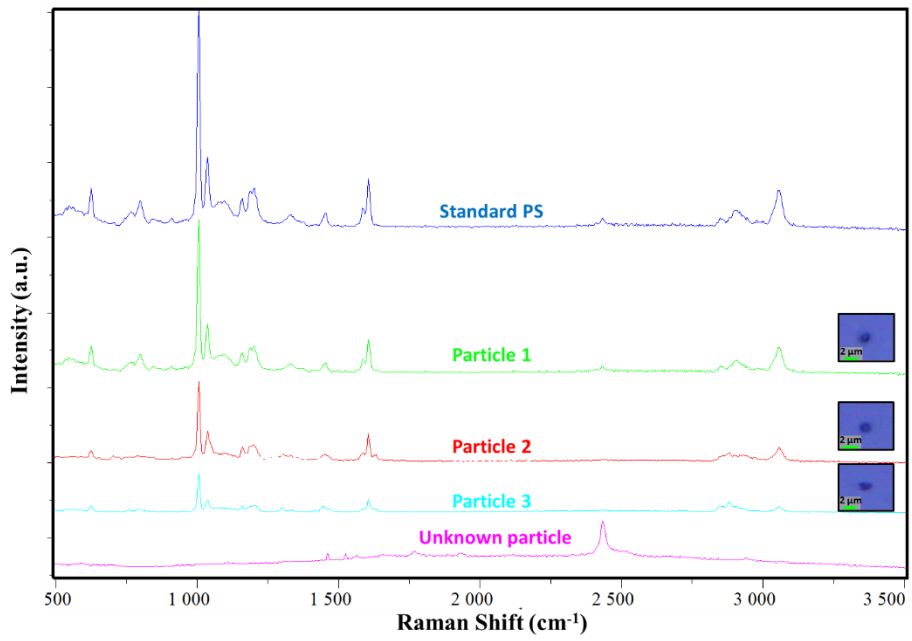


***Figure S2*: Showing fluorescence map of 100 nm PS nanoplastics (NPs) dropcast on quartz shadow mask superimposed on SEM image of same area. The sample provides examples in which NPs are separated by distances of ~ 300 and ~170 nm, with discrimination between the NPs still possible.**

Particle	Diameter/ nm
1	400
2	190
3	220
4	250
5	200
6	90
7	250
8	130
9	200
10	300

***Table S1*:Full breakdown of particle sizes from PS NPs prepared by blending, dissolving and reprecipitating commercial PS pellets. Particle sizes from data in Fig 2C (left) and Fig 2D (right) from main text.**



Particle	HQI
1	0.91
2	0.85
3	0.75
4	0.80
5	0.82
6	0.85
7	0.90
8	0.83
9	0.78
10	0.80

***Figure S3*: Raman analysis of PS microplastics prepared by blending, dissolving and reprecipitating commercial PS pellets. Raman spectra for a standard PS particle, 3 analysed particles and an unknown particle are shown. Library searches were unable to return an adequate identification for the unknown particle. The images are of the 3 particles measured and the table displays the Hit Quality Index (HQI) for all measured particles.**

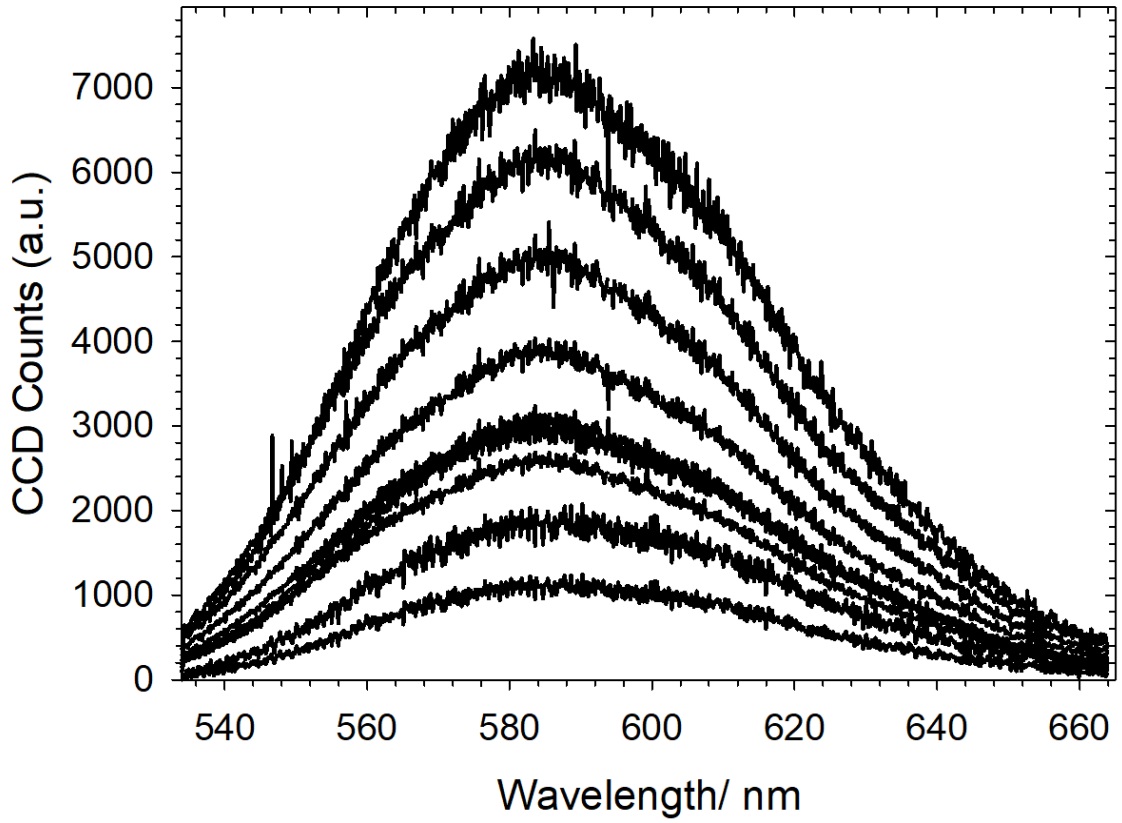


***Figure S4*: Collated fluorescence emission spectra from standard semi crystalline PET particles dyed using the standard protocol.**

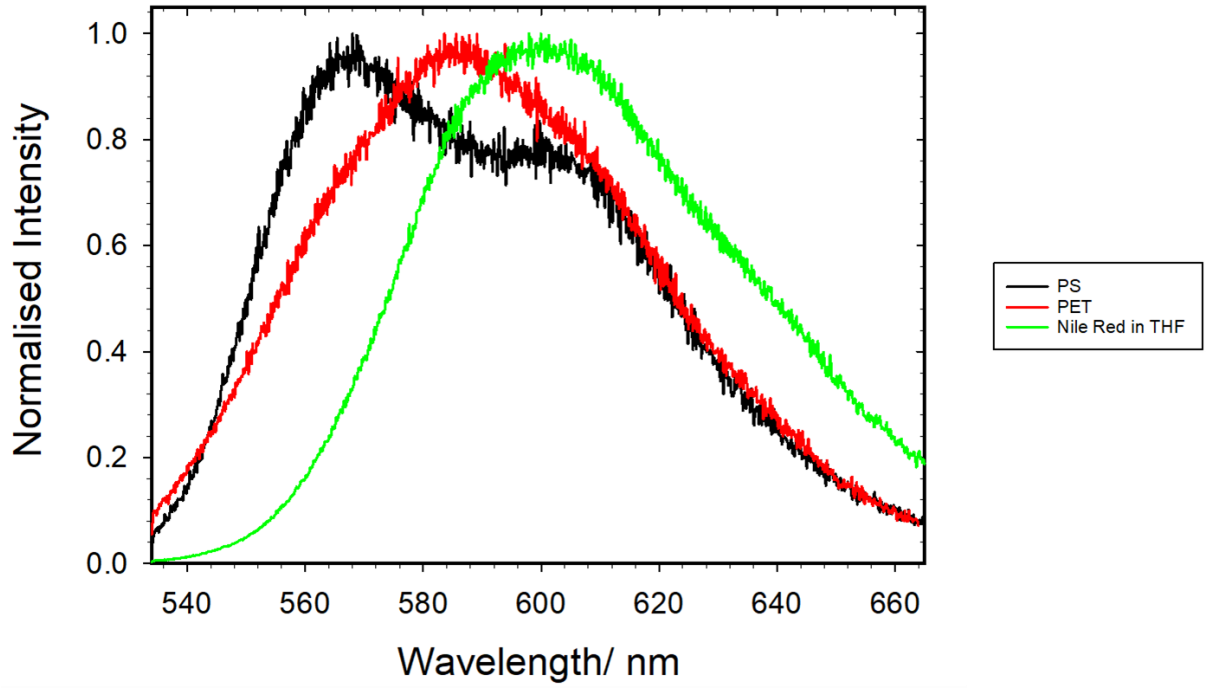
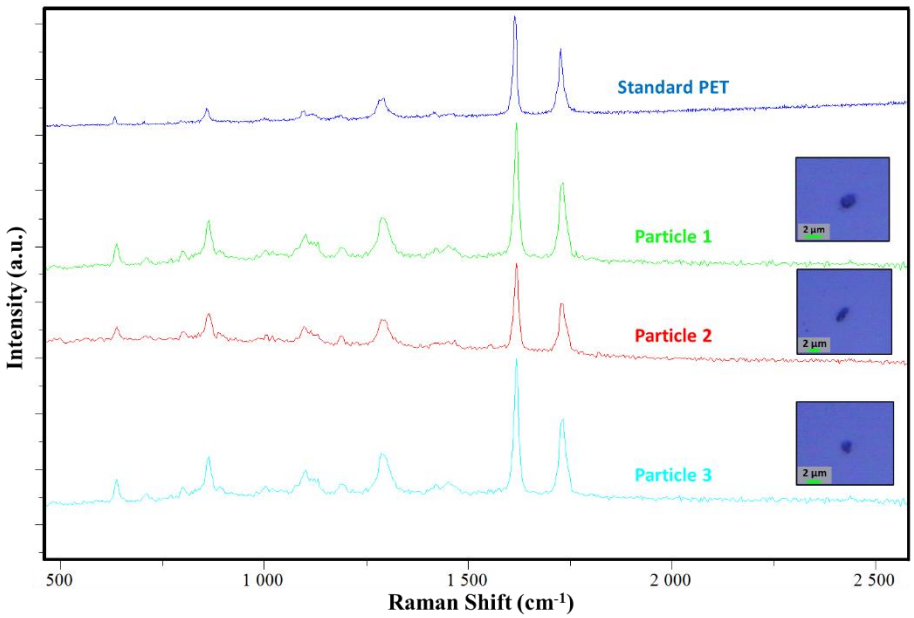


***Figure S5*: Normalised fluorescence emission spectra from individually NR dyed PS, PET NPS and NR dissolved in THF (10 µg/ mL) to demonstrate the solvatochromic property of NR.**



Particle	HQI
1	0.90
2	0.90
3	0.92
4	0.86
5	0.86
6	0.83
7	0.87
8	0.92
9	0.90
10	0.89

***Figure S6*: Raman analysis of PET microplastics prepared by reprecipitation from standard semi crystalline PET powder. Raman spectra for a standard PET particle, 3 analysed particles are shown. The images are of the 3 particles measured and the table displays the HQI for all measured particles.**

Fig. S7 show results from using the dyeing protocol to dye common additives (Stearic Acid and Behenamide) with NR compared with PS and PET. For each sample, a fluorescence measurement was taken on the presented particles (see insets) using identical imaging parameters. After a period of 1-2 minutes, identical measurements were taken on the same position of the particles to investigate photobleaching. Results show much lower fluorescence signals and faster quenching of additives compared with similar sized PS and PET plastics particles. NR background has been added to (A) and (B) to show similarity in emission profile between additive and NR.

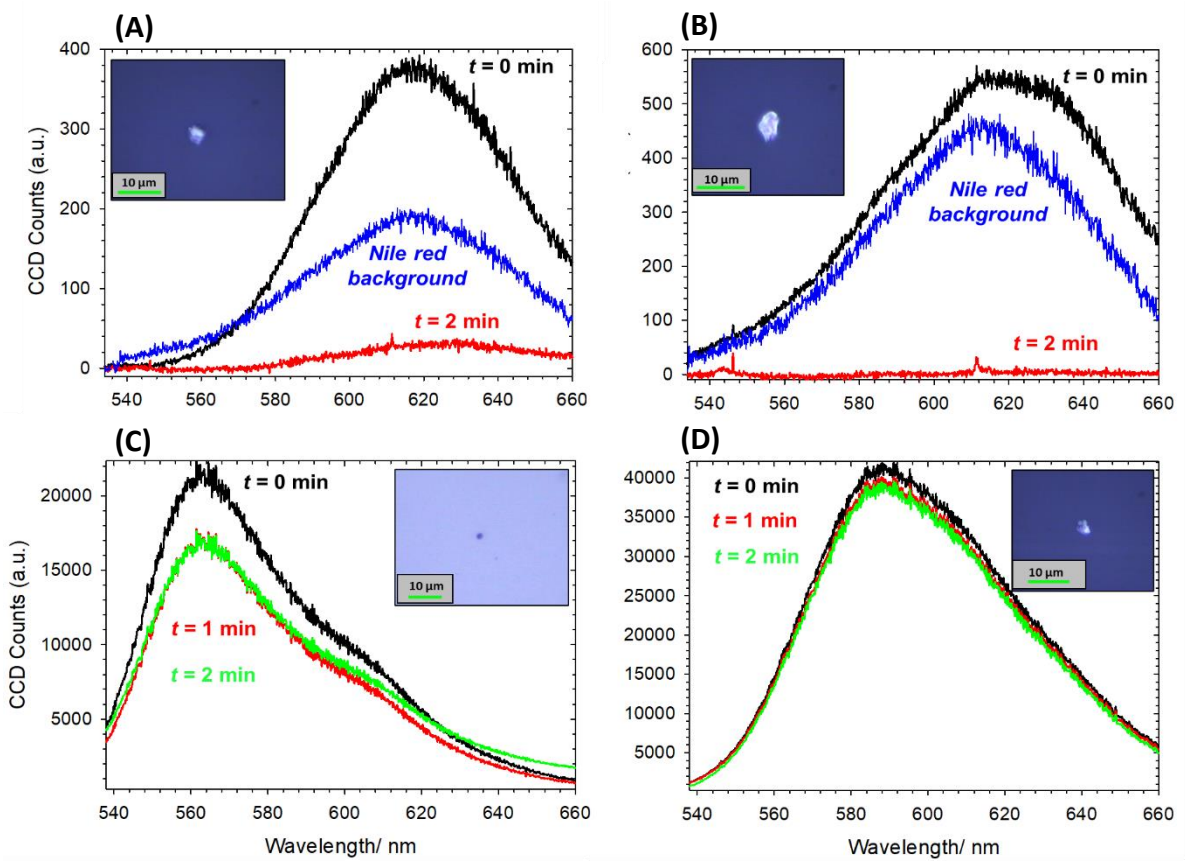


***Figure S7*: Fluorescence emission spectra of Stearic Acid (A), Behenamide (B), PS (C) and PET (D) and signal quenching over 1-2 minutes. Particle images are shown in the insets and NR background has been added to (A) and (B) to demonstrate similarity in emission profile between NR and stained additives.**

Particle	Diameter/ nm
1	170
2	130
3	230
4	200
5	110
6	120
7	60
8	100
9	110
10	70
11	90
12	100
13	190
14	220
15	90
16	340
17	150
18	270
19	160
20	260
21	90
22	100
23	140
24	160

***Table S2*: Full breakdown of particle sizes from prepared re-precipitated PET NPs. Particle sizes from data in Fig 3 from main text.**

In **Fig. 4** in the main text, eighteen particles were located within the 10 x 10 um scanned area. There were two instances in which the fluorescence signal was seen to merge as a result of the NPs being exceptionally close to each other (~100 nm and ~150 nm for locations 5 and 7, respectively). **Figs S6** and **S7** present an attempted analysis to use deconvolution to estimate the locations of the individual NPs in locations 5 and 7, respectively. The fluorescence maps were broken down into pixels with each pixel providing an individual fluorescence spectrum. For **Fig S6**, six spectra are displayed providing examples of pixels that are directly over NPs, in between the NPs and surrounding the NPs. Pixels that are surrounding the NPs (Pixels 1 and 11) show clear reductions in intensity compared to pixels directly on top and between, and in the case of Pixel 1 a clear difference in the deconvolution analysis and shape of the spectra. The analysed pixel in between the two particles (Pixel 5) shows no observable differences in terms of shape of the spectrum, deconvolution analysis or intensities.

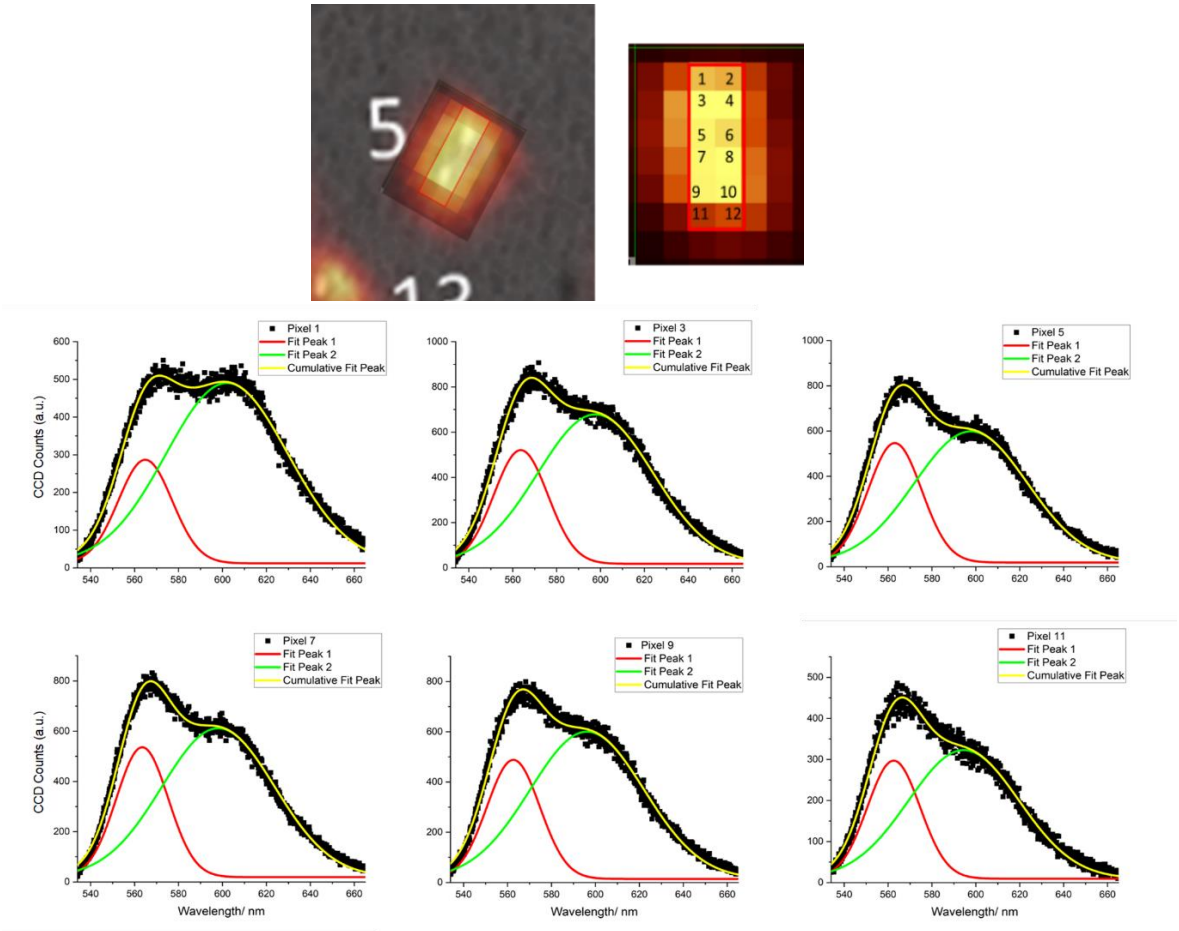


***Figure S8*: Analysis of fluorescence mapping data to try and locate individual NPs through deconvolution. Fluorescence map superimposed on SEM data are shown in top left image, with two NPs being identified. Top right image shows individual pixels from the same mapping region. 12 pixels are present in the red box and the individual fluorescence emission spectra of pixels 1, 3, 5, 7, 9 and 11 are shown, all of which have been subject to deconvolution analysis.**

Fig. S7 shows the same analysis for location 7. In this example, the orientation of the particles requires analysis of pixels 1, 6, 7, 10, 11 and 16 to present data that represents regions in which there are NPs, region in between the two NPs and surrounding areas. As above, the only differences that can be observed are when considering a pixel that provides an area close to but surrounding a NP (Pixel 1). Considering the other analysed pixels, it is clear that the intensities, shapes and deconvolution analysis is all very similar. In this respect, using deconvolution analysis to estimate the locations of the of the individual NPs when located in very close (≤ 150 nm) to each other is challenging using fluorescence mapping alone.

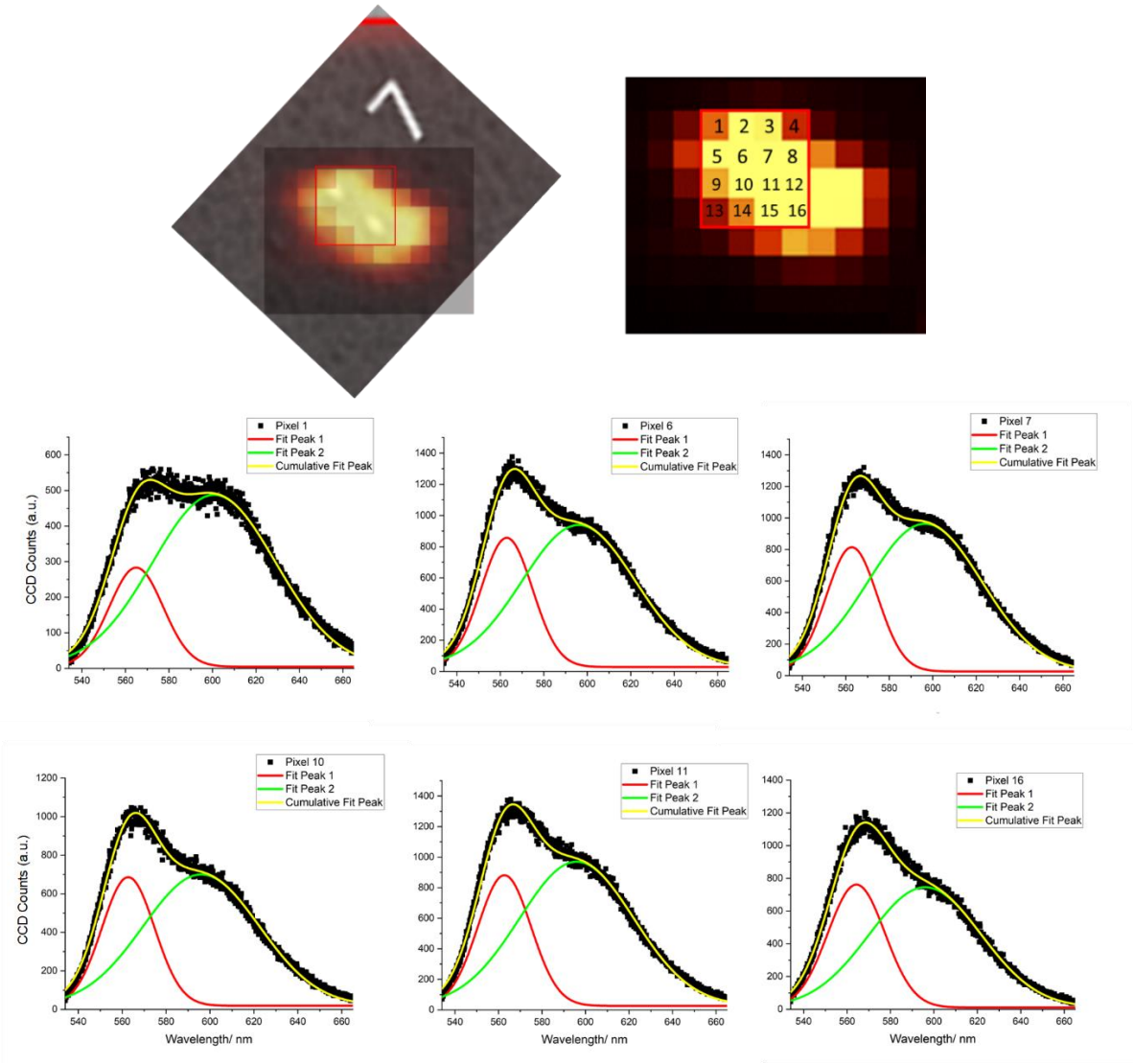


***Figure S9*: Analysis of fluorescence mapping data to try and locate individual NPs through deconvolution. Fluorescence map superimposed on SEM data are shown in top left image, with two NPs being identified. Top right image shows individual pixels from the same mapping region. 16 pixels are present in the red box and the individual fluorescence emission spectra of pixels 1, 6, 7, 10, 11 and 16 are shown, all of which have been subject to deconvolution analysis.**

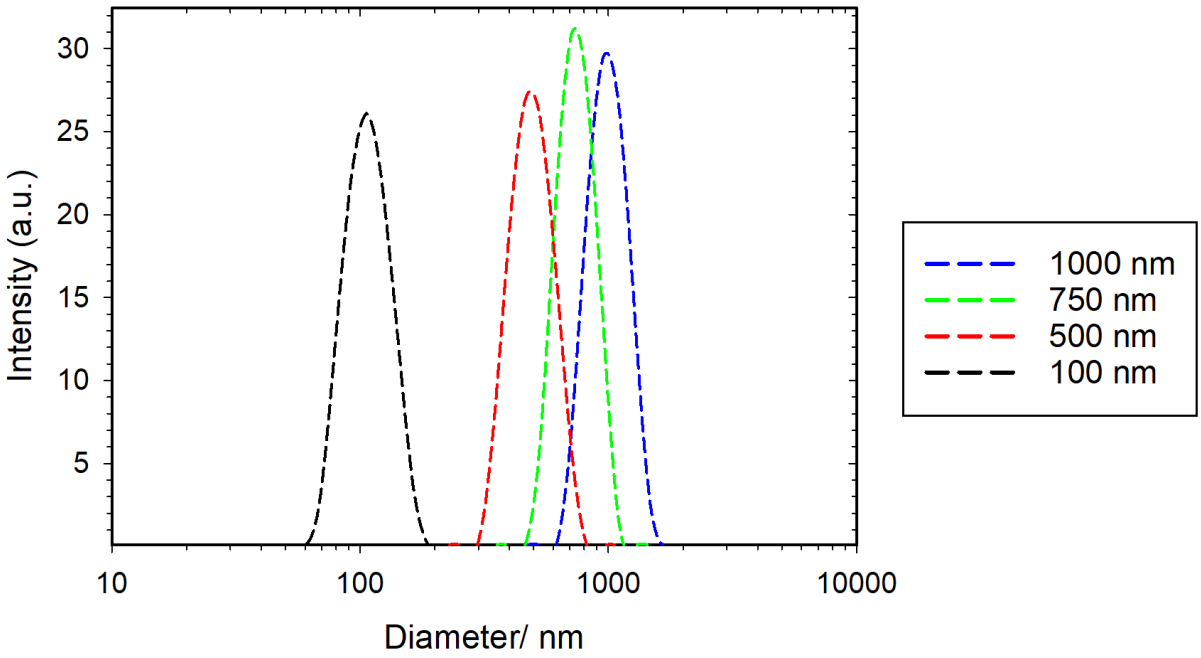


***Figure S10*: Dynamic light scattering data for PS nanospheres. Each particle size was measured at a concentration of 0.1 wt% in DI water.**