

Supplementary Information

Monitoring plasmon coupling and SERS enhancement through *in situ* nanoparticle spacing modulation

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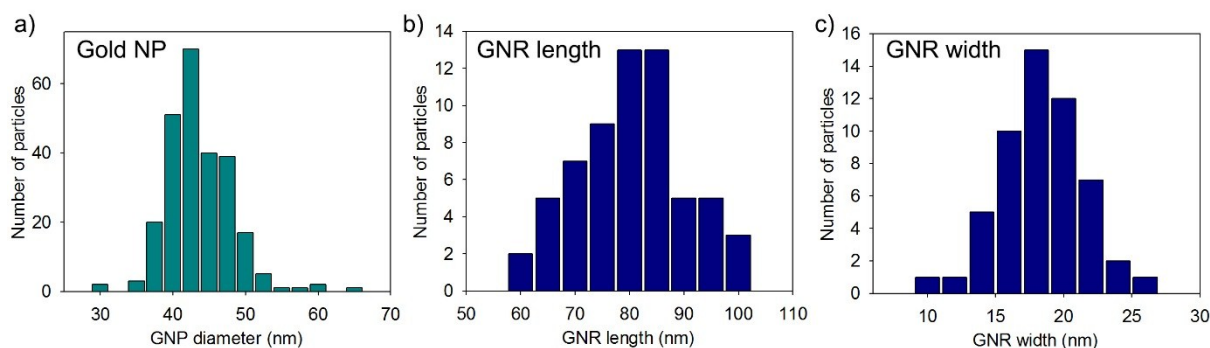
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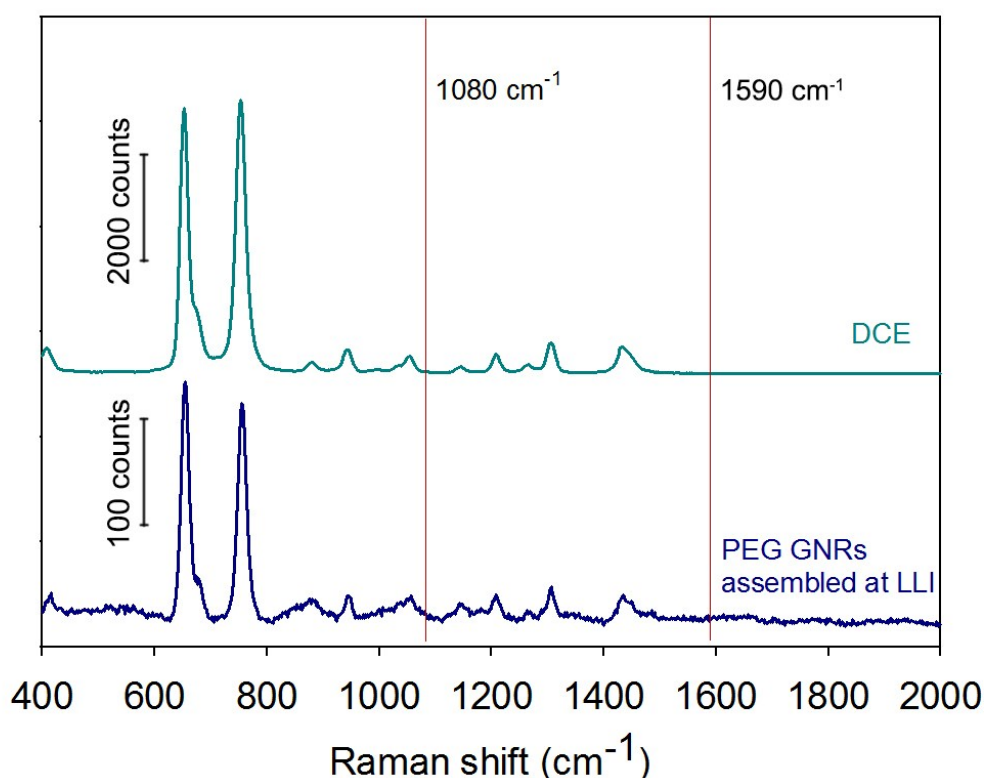
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The size distribution of gold NPs and GNRs were extracted from TEM images (SI Figure 1) yielding an average gold NP diameter of 42.3 ± 4.4 nm (determined from 252 NPs). The length and width of the GNRs were measured to be 77.6 ± 10.0 nm and 17.3 ± 3.0 nm respectively with an aspect ratio of 4.6 ± 1.1 (determined from 54 GNRs).



SI Figure 1 Size distribution histograms for the diameter of gold NPs a) and the length b) and width c) of GNRs.

Control Raman spectra are shown in ESI Figure 2. The bottom spectrum is of a GNR film prepared without 4-mba (only PEG modification, PEG-GNR), displaying no peaks at 1080 and 1590 cm^{-1} where the 4-mba peaks reside. The Raman spectrum of pure DCE solvent (top) confirms the peaks present in the PEG-GNR film sample arise from the DCE solvent. As the 4-mba peaks are not occluded they are a good candidate for monitoring the Raman response of these films.



SI Figure 2 Raman spectrum of a film prepared at the LLI with GNRs only modified with PEG (bottom spectra). Raman spectrum of 1,2-dichloroethane. Vertical lines are drawn at 1080 and 1590 cm^{-1} to show where the dominant 4-mba peaks reside.

Calculation of enhancement factors

$$EF = \frac{ISERS \ N_{NRS}}{INRS \ N_{SERS}}$$

$$N_{NRS} = c_{NRS} H_{eff} A_{eff}$$

$$N_{SERS} = \mu_M \mu_s A_M A_{eff}$$

$$EF = \frac{ISERS \ c_{NRS} \ H_{eff}}{INRS \ \mu_M \ \mu_s \ A_M}$$

Where c_{NRS} is the number of molecules/m³;

As the density of bulk 4-mba is; $\rho = 1.489 \text{ g cm}^{-3}$

N_A : Avogadro's constant

M = molar mass of 4-mba; 154 g mol^{-1}

$$c_{NRS} = \frac{\rho N_A}{M} = 5.82 \times 10^{27} \text{ molecules m}^{-3}$$

H_{eff} is the height of the sampling volume, taken to be the beam height; $56 \text{ }\mu\text{m}$

A_{eff} is the area of the sampling volume, taken to be the beam diameter, which cancels out in the EF equation.

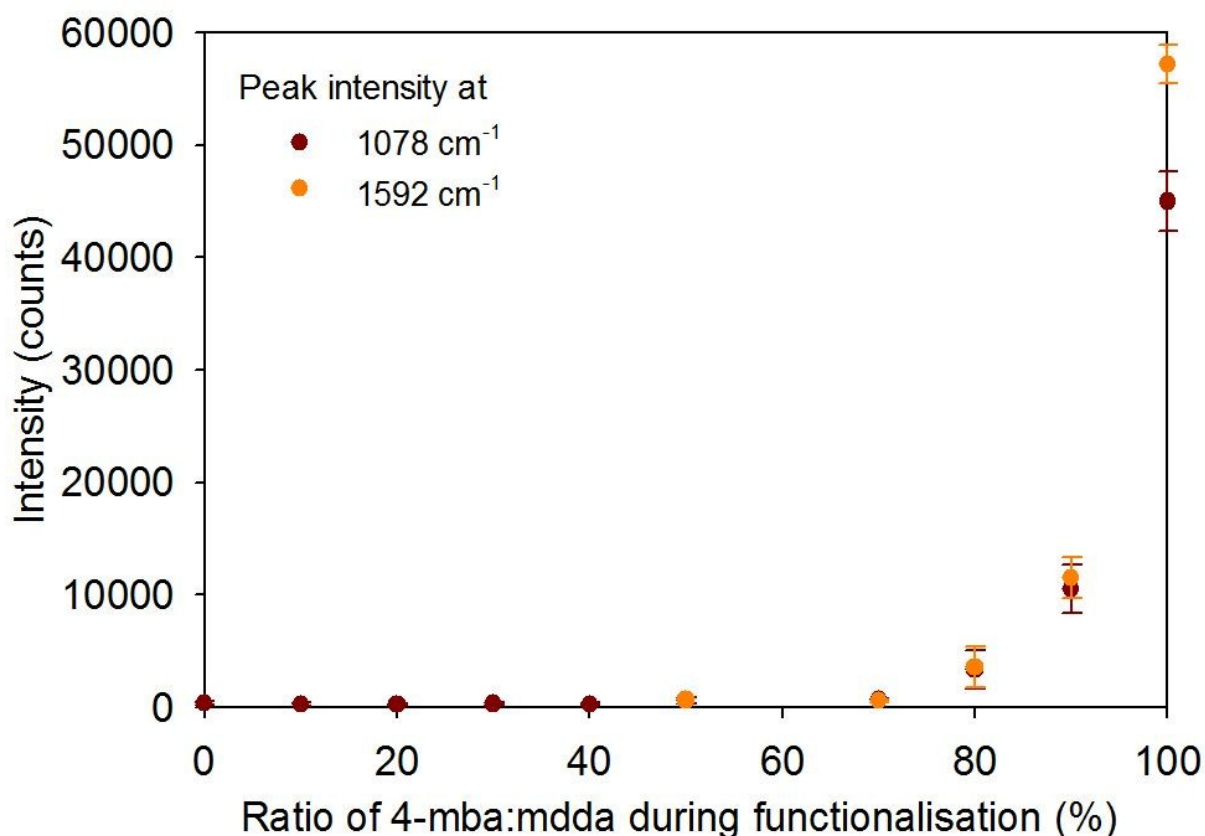
μ_M is the density of NPs in the film, for GNPs; $7.32 \times 10^{14} \text{ m}^{-2}$ and GNRs; $7.53 \times 10^{14} \text{ m}^{-2}$

μ_s is the surface density of 4-mba on an individual NP; assumed to cover 7 % of the NP surface in the case of spherical GNPs and 100 % of the NP surface in the case of GNRs.

A low density of 4-mba was used to modify the spherical GNP surface as a high surface density of mdda was required to maintain control over interparticle spacing and prevent aggregation. A calibration curve of the signal of 4-mba based on different ratios of 4-mba:mdda used to modify the GNPs are shown in ESI Figure 3. The ratio used to modify GNPs in this study was 80:20 4-mba:mdda. As the signal at 1079 cm^{-1} is approximately 7 % of the intensity of the layer made with 4-mba functionalised with 100 % 4-mba this is taken as an estimate of the surface density. Assuming the packing density of 4-mba on gold is 5.263 nm^{-2} .

A_M is the surface area of the individual NP, taken to be 5616 nm^2 for spherical GNPs (42.28 nm diameter), and 4620 nm^2 for GNRs (17 x 78 nm).

$$I_{NRS} = 368 \text{ counts}$$



SI Figure 3 Calibration curve of GNPs modified with different ratios of 4-mba:mdda. The Raman signal is obtained from aggregates formed from the GNPs when 1M NaCl is added.

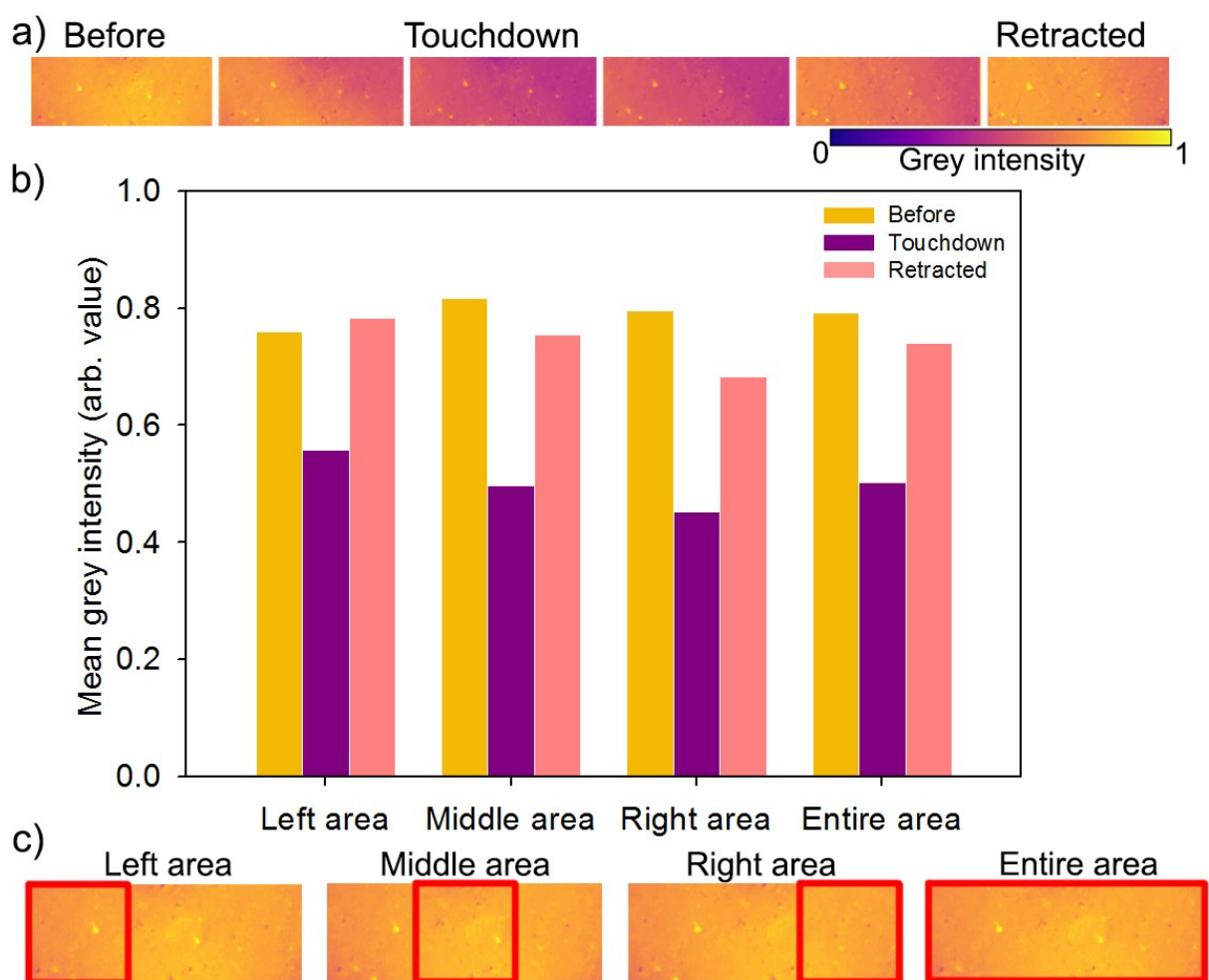
Analysis of colour change within microscopy images

Unfortunately we do not have microspectroscopy capabilities on our optical set up. With an incident light spot size of 2.8 – 4 mm and FOV of 1.33 mm we are unable to probe the spectra of the film on a few 100 μm scale. Instead, we perform a simple analysis on the grey intensity in the microscopy images to give us an indication of the optical changes within the film. To analyse the regeneration of the layer after flooding the film with water we have taken the set of microscopy images shown in Figure 2f where the pipette is lowered towards the interface, flooding the film with water and allowing the GNPs to move closer together (touchdown), and later retracted, allowing citrate ions to diffuse back to the layer and once again push the GNPs apart (retraction). Firstly, the image is changed to grey scale then overlaid with a colour profile for ease of viewing (ESI Figure 4a). The grey scale is normalised to an intensity of 1; for 1 we take the brightest pixels on the image (GNPs far apart) and 0 being the darkest (GNPs close together). The mean grey intensity of the image during the three stages (before, touchdown and retraction) is plotted in ESI Figure 4b.

Since the delivery of water to the surface is asymmetric within the microscopy images (water is directed to the right on the image), we perform analysis over divided regions on the image as well as the entire area as depicted in ESI Figure 4c. Overall, in ESI Figure 4b we observe a significant decrease in the intensity during pipette touchdown (GNPs come closer together) followed by an increase in intensity once the pipette is retracted (GNPs are pushed apart). For the middle, right and entire image analysis, the intensity does not reach back to its initial

intensity, suggesting that the NPs are closer together than when they started. However, the time between touchdown and retraction was only 5 s.

Regeneration depends on the time it takes for citrate to diffuse back into the layer. With more time, the layer may have been able to fully regenerate itself. In a separate study, to be published, we explore the reversibility of the NPs spacing in more detail. While we were able to achieve reversibility, there are signs that a small population of the NPs are indeed becoming irreversibly attached. The analysis of the left area of the image however, shows that the intensity slightly exceeds the original intensity after retraction of the pipette. Perhaps as the NPs are drawn closer together on the right hand side of the image where the water is dispensed, the NPs in the periphery adjust by positioning themselves farther apart, expanding into the space left by the compressed NPs.

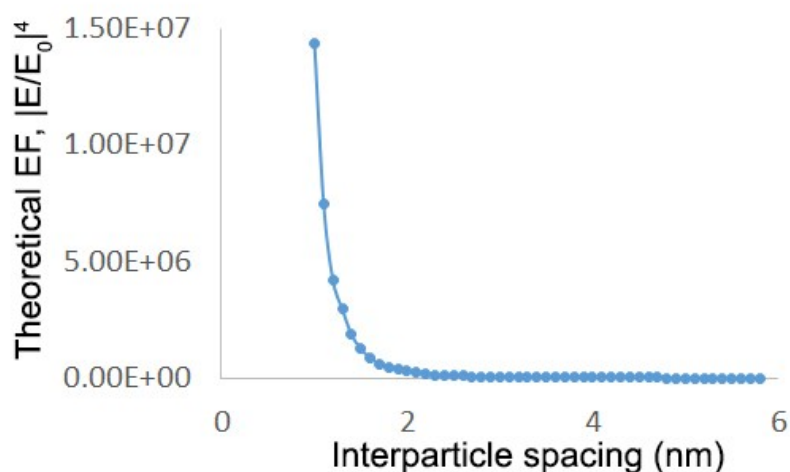


SI Figure 4 Colour mapping analysis of microscopy images displaying the regenerative properties of the film. a) From left to right the micropipette was initially positioned far above the surface (before) then brought close to the NP film (touchdown) and water injected just above the layer. The localised water environment dispels the citrate ions and allows the GNPs to move closer together. The pipette was finally retracted allowing citrate ions to diffuse back to the layer. b) Quantification of the grey intensity before, during touchdown and after tip retraction over several different areas on the microscopy images as highlighted in c).

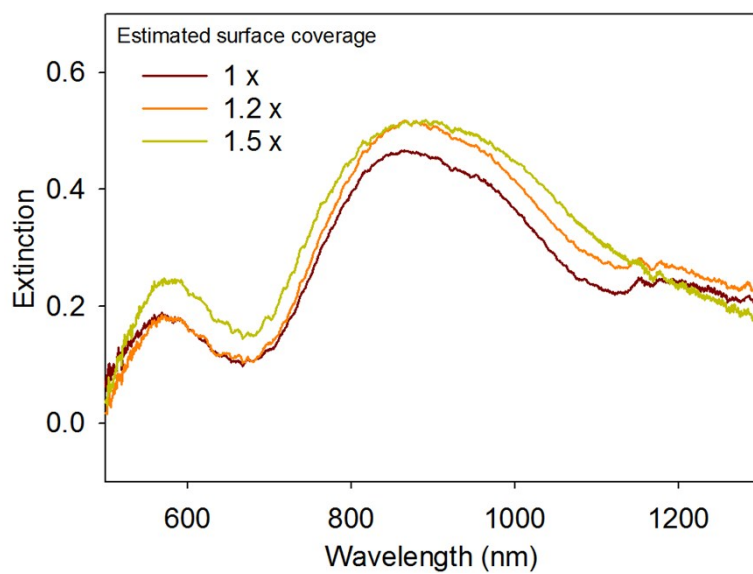
Numerical Simulations

The numerical simulations presented in this work were performed using CST Microwave Studio Suite®. The frequency domain FEM solver was used to obtaining the optical responses of nanoparticles dispersed in aqueous solution and when those nanoparticles self-assemble to form monolayer. For nanoparticles in solutions, where interparticle distance is significantly large to ignore interparticle coupling, optical extinction spectrum is calculated from a single nanoparticle. Open boundaries, emulating perfectly matched layers, were adopted in all directions so that incident light can pass the boundaries with minimal reflection. The simulations parameters for nanoparticles are based on the mean values of the dimensions measured from the experimental samples. The extinction spectrum of individual nanoparticles in water is calculated using the weighted average of the two spectra obtained for light polarization along length and width of the nanoparticle; with weight factors of 1/3 and 2/3, respectively.

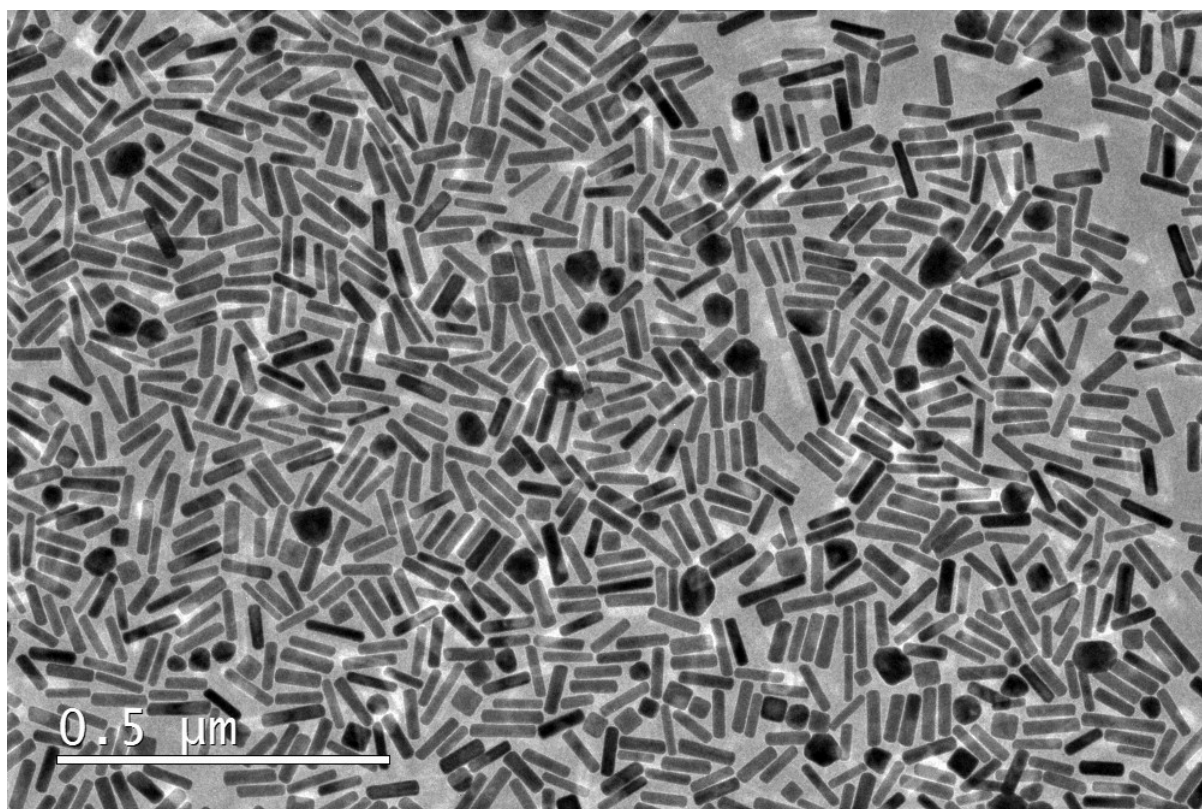
For nanoparticle monolayers, a unit cell is repeated in both lateral directions using periodic boundary conditions to model optical extinction properties of large superlattices under plane wave excitation. The dimensions of nanoparticles are taken from the mean values as measured experimentally. The periodicity parameters (along nanoparticles' length and width) in their two-dimensional array or monolayer are varied to theoretically calculate optical extinction spectrum as function of interparticle gap. The surrounding medium of the nanoparticles in monolayer is considered as water. In reality the nanoparticles may not be completely in the aqueous phase, but may prefer to settle towards the oil phase of the water/oil interface. In that case, the surrounding medium of nanoparticles will have a slightly larger permittivity than water. This would cause each extinction spectrum to undergo a red-shift. However, qualitatively the trends of these new estimates will still agree with the observed trends, and hence, we can safely explain the effects of interparticle gap on extinction peak and hot-spot formation with the simplifying assumption made above. The permittivity values of gold was taken from the literature.¹ Tetrahedral meshing with automatic mesh refinement was chosen to be fine enough for the frequency-domain simulations of the extinction spectra over the wavelength range of interest. To identify the hot-spots at different excitation laser wavelength, we calculated electric field distribution patterns along the plane passing through centres of the nanoparticles in a unit cell.



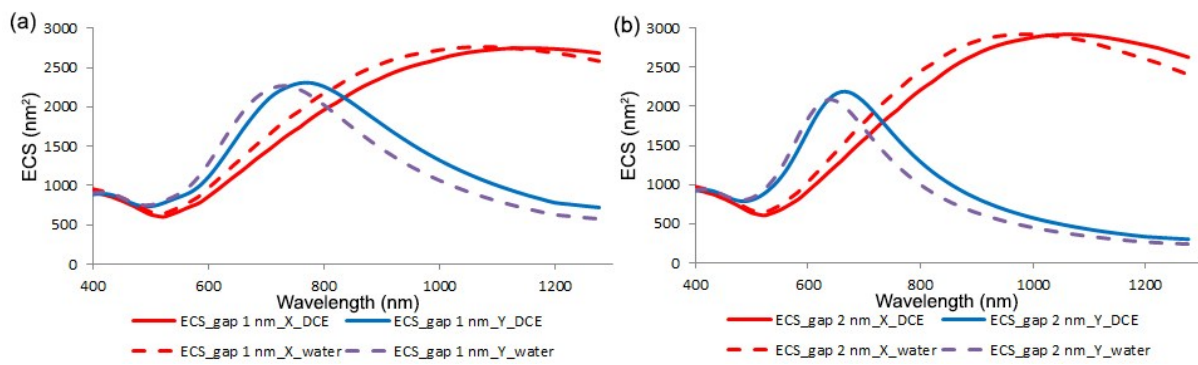
SI Figure 5 Plot of theoretical EF for spherical GNPs as interparticle spacing is varied.



SI Figure 6 Extinction spectra of GNR films prepared at an estimated surface coverage of 1x, 1.2x and 1.5x.



SI Figure 7 TEM image of GNRs assembled at 1.5 x estimated surface coverage. Extracted onto a TEM grid by pushing the grid horizontally through the film at the LLI then dried.



SI Figure 8 Theoretical simulation of GNR extinction spectra when GNRs are sitting fully in the water phase and fully in the DCE phase for 1 nm interparticle spacing (a) and 2 nm interparticle spacing (b).

1. P. B. Johnson and R. W. Christy, *Physical Review B*, 1972, **6**, 4370-4379.