Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Design and Fabrication of Random Silver Films as substrate for SERS based Nano-Stress sensing of Proteins

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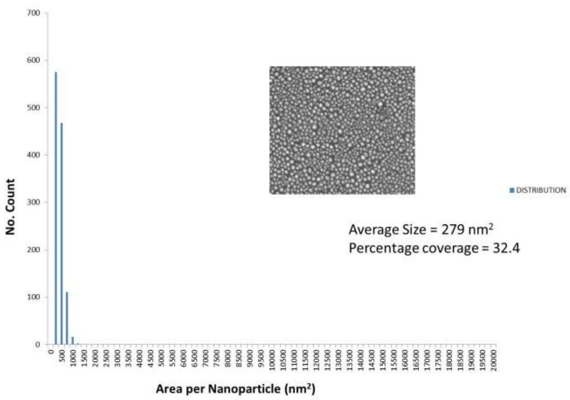
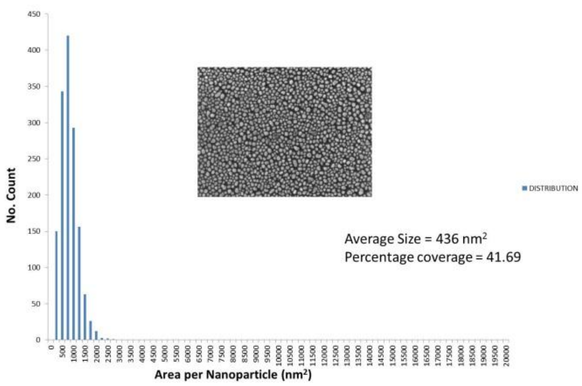
Grain size distribution data for the Random Silver film:

Grain size distribution data was collected from SEM image using "image J" software program. SEM data of Random Silver film with different mass average thickness from 3 nm to 15 nm were processed to collect the average grain size and also to monitor grain size distribution pattern. All the results were listed in the following slides.





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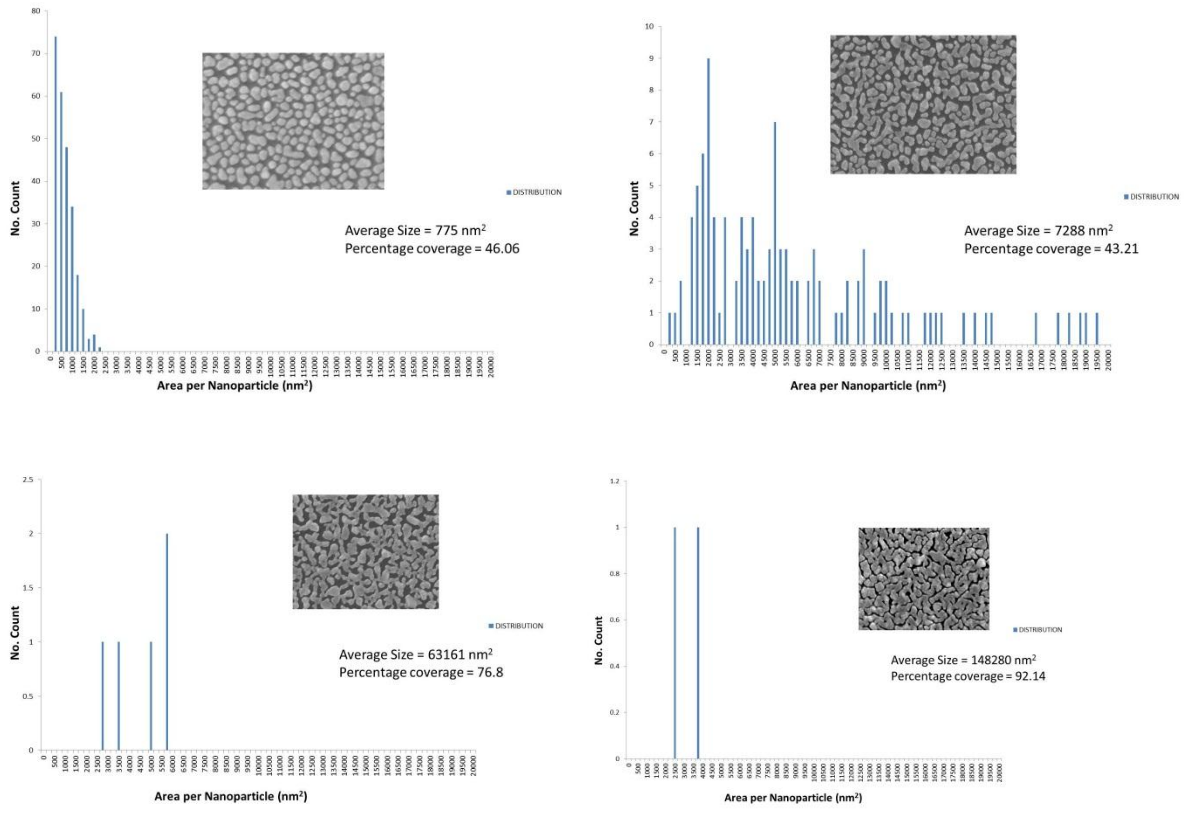

Figure S1. Grain size distribution data was collected from SEM data of random silver film with various mass average thicknesses.

Calculation of Enhancement Factor:

The spatially-averaged enhancement factor, G, is determined by using the established method[1] of comparing the 2-Naphthalenethiol (NT) SERS intensity with a thin liquid layer of NT. G is expressed as

$$G = \left(\frac{I_{SERS}}{N_{SERS}}\right) / \left(\frac{I_{RAMAN}}{N_{BULK}}\right) \quad (1)$$

, where I_{Raman} and I_{SERS} are the intensity values at the scattering band of interest, i.e. at 1066 cm^{-1}, in bulk liquid Raman spectrum and SERS spectrum respectively. N_{Bulk} is the number of NT molecules in bulk solution contributing to the unenhanced Raman signal, while N_{SERS} is the number of NT molecules that are chemisorbed on the substrate and contributes to SERS signal. N_{Raman} and N_{Bulk} can be determined using the following equations:

$$N_{SERS} = A_{beam} R \mu \qquad (2)$$

$$N_{BULK} = A_{beam} H \rho \qquad (3)$$

,where A_{beam} is the area of laser beam, R is the area factor and μ is the packing density of NT molecules on the surface of substrate. H is the apparent height of the NT liquid layer emanating Raman signal and ρ is the molecular density of NT solution prepared.

Substituting Eq. (2) & (3) into Eq. (1), G can be rewritten as

$$G = \left(\frac{H\rho}{R\mu}\right)\left(\frac{I_{SERS}}{I_{RAMAN}}\right) \qquad (4)$$

Table S-1 lists the values of I_{Raman} and I_{SERS} of NT at Raman shift 1066 cm^{-1} measured from different substrates at normalized experimental settings with laser = 785 nm and integration time = 10 s

Description	**Intensity values 1066cm^{-1} (Counts)**
I_{Raman} of 100mM NT	2800
I_{SERS} from 7nm SSCF	29132842

The μ of NT was reported[2,3] to be 4.01×10^{-10} mol/cm^2 With our measurement settings, H, an instrumental parameter, was determined to be 16 μm. The I_{Raman} was measured from NT solution prepared at concentration of 100mM, and the corresponding ρ is 6.02×10^{19} $molecule/cm^3$. Simplifying Eq. 4, we obtain

$$G = \left(\frac{401}{R}\right)\left(\frac{I_{SERS}}{I_{RAMAN}}\right) \qquad (5)$$

R is an intrinsic parameter of substrate defining the effective SERS-active area as described in Eq. (6).

$$R = \left(\frac{S_{SERS}}{A_{beam}}\right) \quad (6)$$

, where S_{SERS} is surface area of SERS-active sites under laser illumination, and A_{beam} is the area of excitation beam. Using a 50× objective lens, the minimum beam diameter is approximately 1μm.

We have calculated the R factor based on the assumption that the nanoparticles in the SSCF is of spheroidal form. And one half of the spheroid is involved in the field enhancement. Then S_{SERS} can approximated as

$$S_{SERS} = S_{Spheroid}.A_{beam}.E_{ex}/A_{spheroid} \quad (7)$$

, where E_{ex} is the packing efficiency of spheroid which was determined to be ~0.68, $A_{spheroid}$ is projected area of a Spheroid and $S_{spheroid}$ is the surface area of a spheroid segment contributing to SERS. It can be defined as follows:

$$S_{spheroid} = 2\pi r_{spheroid} k \quad (8)$$

, where $r_{spheroid}$ is the radius of a spheroid and h is the height of the spheroid,

$$h = 2r_{spheroid} \quad (9)$$

based on our previous assumption, k=1/2h. Hence the equation 6 can be rewritten as,

$$R = \frac{0.5h}{h}.E_{ex} \quad (10)$$

Hence, R is determined to be 0.34. Substituting this value into Eq. (5), we obtain

$$G = 1179.\left(\frac{I_{SERS}}{I_{RAMAN}}\right) \quad (11)$$

Using the intensity values listed in Table S-1, the enhancement factors of SSCF substrates was calculated to be

$$G = 1.23 \times 10^7$$

Stability of the substrate:

We have examined the shelf-life of 7 nm substrate. To achieve this, we first exposed these substrates to PBS solution for a period of five consecutive days as shown below.

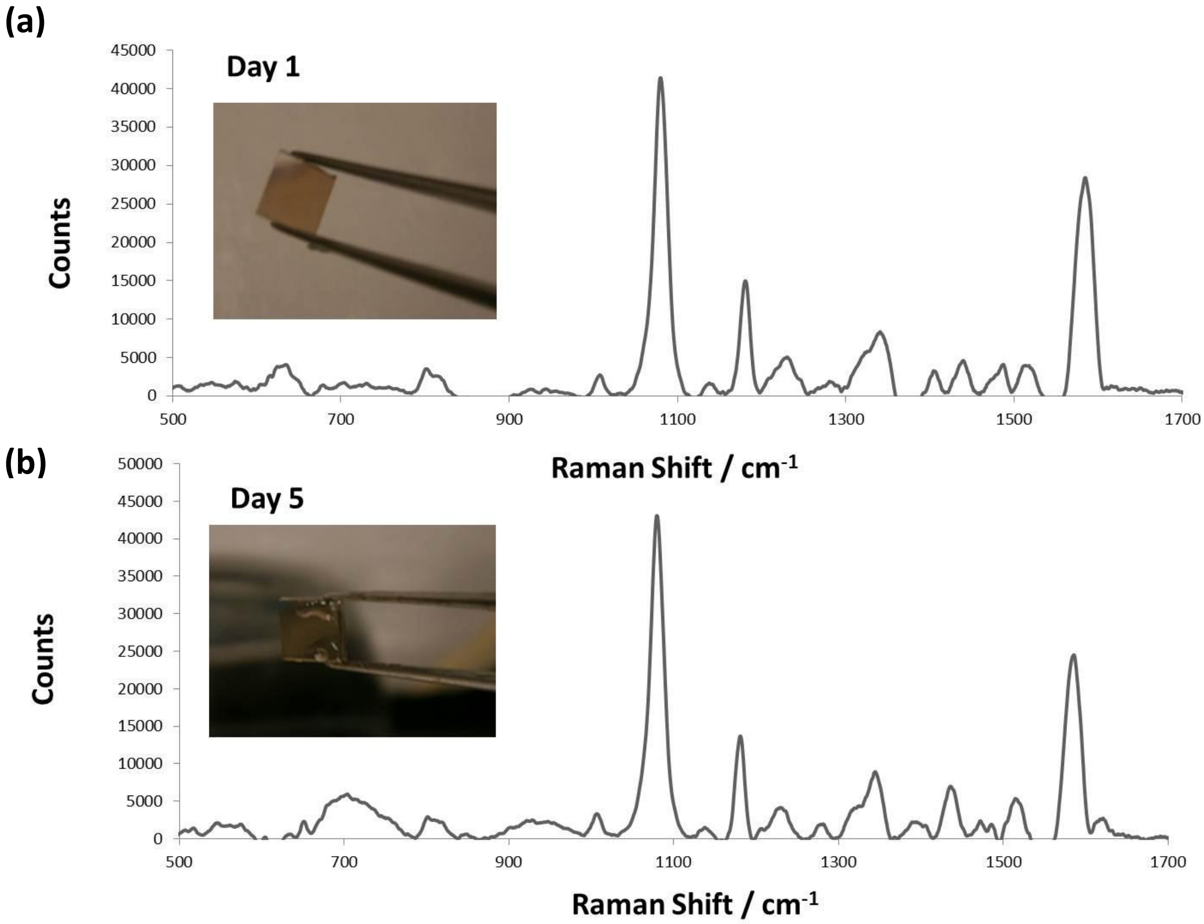

Figure S2. Images and SERS spectra of ATP functionalized Ag substrate (a) after incubation in PBS for 1 day (b) after incubation in PBS for 5 days.

1. W. B. Cai, B. Ren, X. Q. Li, C. X. She, F. M. Liu, X. W. Cai and Z. Q. Tian. *Surface Science*, 1998, **406**, 9-22.

2. R. R. Kolega and J. B. Schlenoff, *Langmuir* 1998, **14**, 5469-5478.

3. C. Y. Fu, K. W. Kho, U. S. Dinish, Z. Y. Koh and M. Olivo, *J. Raman Spectrosc.* 2012, **43**, 977-985.