Supporting information

Directional Fluorescence Emission Co-Enhanced by Localized and Propagating Surface Plasmons for Biosensing

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Interfacial molecular interactions. The amount of SA647 binding to the biotin modified surface at the concentration of 1 pM can be estimated according to the following reaction process.

$$A + B \underset{k_d}{\overset{k_a}{\rightleftarrows}} AB \tag{1}$$

where k_a and k_d are the association rate constant and dissociation rate constant of streptavidin and biotin interactions. Assuming the molecular interaction was limited by the affinity interaction, the reaction step can be expressed as the following equation:

$$[AB] = \frac{k_a[A][AB]_{max}}{k_a[A] + k_d}(1 - e^{-(k_a[A] + k_d)t}) \tag{2}$$

Where $[AB]_{max}$ = 4×10^{10} molecules/mm^2 calculated based on the assumption that each thiol molecule occupies 0.2 nm^2, and the thiol-PEG/thiol-bition=9:1, each streptavidin occupies about 25 nm^2 as the size of streptavidin is about 5.8 nm× 5.4 nm × 4.8 nm.[1] Assuming the molecular bound to the surface is proportional to the fluorescence signal changes, i.e., ΔF=G[AB], where G is a factor converting the number of molecules to the fluorescence signal. The dissociation rate constant is assumed to be k_d = 6.8×10^{-5} s^{-1} for the streptavidin and biotin affinity interaction.[2] Fitting the kinetic of 10 nM and 1 nM SA647 binding on flat Au film substrate, we can estimate the association rate constants of k_a =1×10^6 M^{-1} s^{-1}, respectively (Figure S3A).The value is lower than the reported association rate constant,[2] because of the fluorescence bleaching and partially mass transport limited diffusion. Based on Eq. (2), the amount of SA647 bound on the surface was estimated to [AB] =4333, 696, 73 and 7 molecules per 400×400 nm^2, after 20 min incubation of 1 nM, 100 pM, 10 pM and 1 pM SA647 on the flat Au film substrate, respectively. The corresponding fluorescence intensity changes are plotted in Figure S3B, in which the slope S=38.

Considering the laser spot of 4 mm^2, molecules binding on this area should be multiplied by a factor of 4 mm^2/0.16 μm^2 = 2.5×10^7. Thus the factor G=1.52×10^{-6} cps/molecule. On the AuNH substrate, the factor G=4.88×10^{-6} cps/molecule, assuming the amount of molecules bound on the AuNH substrate are the same as flat Au film.

The parameters of the AuNH array including pitch, radius and thickness of nanohole were indicated in Figure S1A. The simulation of the reflectance spectra of AuNH array were carried out for nanohole with radius R = 100 nm, thickness H_0 =200 nm and pitch (p) of 400 nm, 500 nm and 600 nm (Figure S1B), respectively. The LSP resonance at about 675 nm, 752nm and 852 nm were observed for nanohole pitch of 400 nm, 500 nm and 600 nm, respectively. It indicated that the pitch of nanohole array at about p = 400 nm can be employed for the excitation of Alexa-647 by further optimization of the nanohole radius and thickness. Figure S1C and D indicate the field intensity distribution on the surface of gold nanohole for radius from 50 nm to 190 nm and thickness from 90 nm to 400 nm. The radius of nanohole R=75 shows the highest field intensity at incident wavelength of λ = 647 nm. The detailed simulation can be found in our previous work.[3]

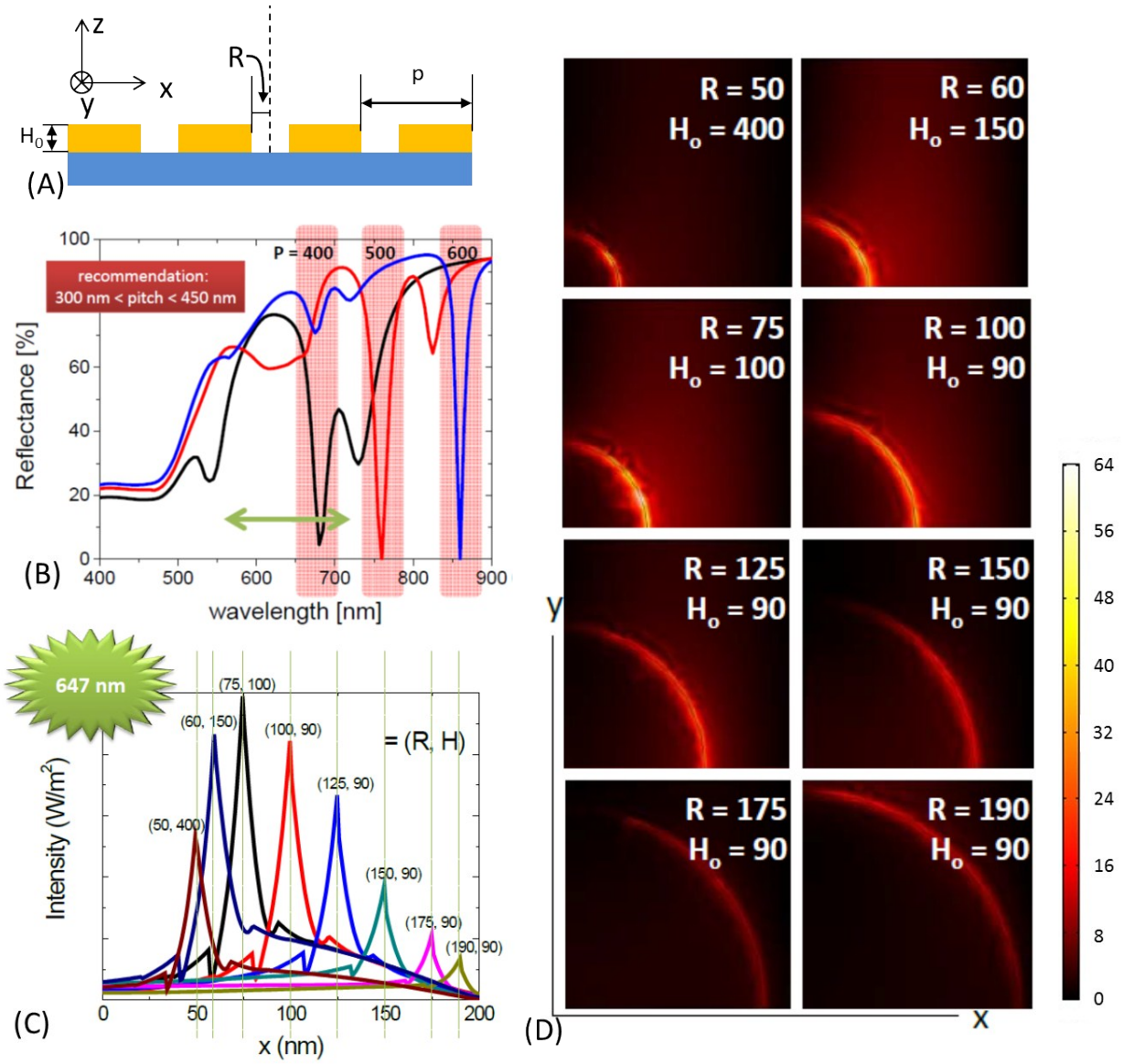

Figure S1. (A) The scheme of the AuNH array. (B) The simulated reflectance spectra of AuNH array with nanohole radius of R=100 nm, thickness H_0=200 nm and pitch of 400 nm, 500 nm and 600 nm, respectively. (C) The simulated intensity profile cross a nanohole at x axis with y=0, z=0 (i.e. at the surface of Au film).The maximal field intensity occurs at nanohole radius of R=75 nm at resonant wavelength of λ=647 nm. (D) The field distribution of Au nanohole with different radius and thickness.

Figure S2 shows the AFM image and line profile of AuNH array which indicates the hole pitch p = 400 nm, diameter d = 150 nm, and film thickness H_0 = 50 nm.

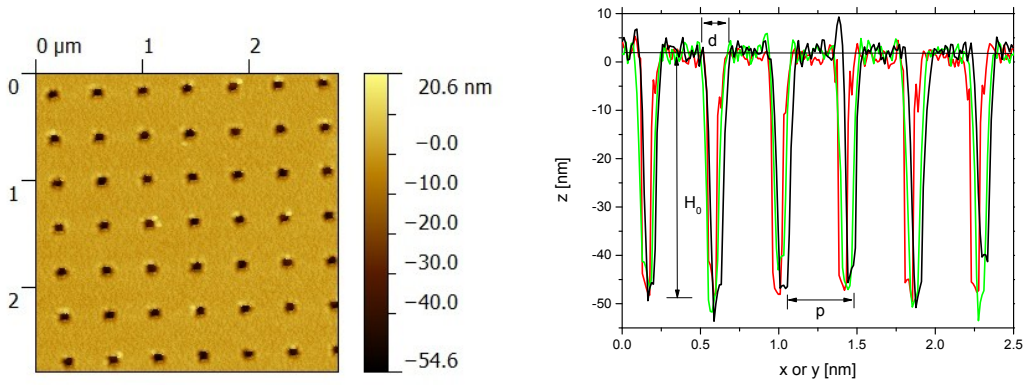

Figure S2. The AFM image and line profile of AuNH array.

Figure S3A shows the fluorescence intensity kinetics for the affinity binding of SA647 on the biotinlyted flat Au surface. The red curves are the fitting curves based on exponential method: $y = A_1 - A_2 e^{-kt}$. Based on the fitting and Eq. (3), one can estimate the k_a =1×10^6 M^{-1} s^{-1}, assuming k_d = 6.8×10^{-5} s^{-1}. Figure S3B indicates the sensor sensitivity for the PSP on flat Au film and LSP-PSP co-excitation on AuNH array substrates. The co-excitation of LSP and PSP on AuNH array shows about 3 fold higher sensitivity as compared with that measured on flat Au film.

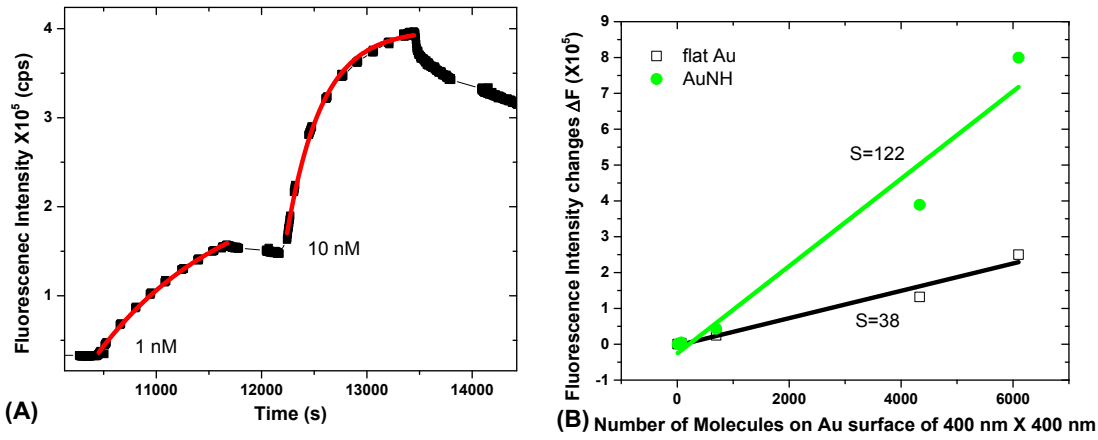

Figure S3. (A) The fluorescence kinetics and fittings for the binding of 1 nM and 10 nM SA647 on flat Au film. (B) The fluorescence intensity changes as a function of the number of molecules on 400 nm × 400 nm surface area of the flat Au film and AuNH substrates.

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2. C. E. Chivers, E. Crozat, C. Chu, V. T. Moy, D. J. Sherratt and M. Howarth, *Nat. Methods*, 2010, **7**, 391-U376.
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