Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information (SI)

Imprinted Gold 2D Nanoarray for Highly Sensitive and Convenient PSA Detection via Plasmon Excited Quantum Dots

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1. Simulation details and simulated plasmonic enhancement factors of gold nanostructures

The plasmonic filed simulations were performed with COMSOL software based on total field calculations, where a unit cell of a nanohole or a nanopillar array was composed of a single nanostructure embedded in a square box. Due to the geometrical symmetry, only a quarter of the unit cell was simulated with boundary conditions as shown in Figure S1(a).

In Figure S1(a), a normal incident plane wave from the wavelength of 400 nm to 900 nm was set up at the surface S0 (where a boundary pair condition was applied). A linearly polarized plane wave travelling in the - z direction was assumed to be x direction polarized. In order to mimic the entire array, the two boundaries perpendicular to x-axis were set as perfect electric conductors (PEC), and the other two boundaries perpendicular to y-axis were set as perfect magnetic conductors (PMC). The top (glass) and bottom (air/water) layers were defined as the perfect matched layer (PML) to absorb any scattered electromagnetic waves from the nanostructure. As the incident wave struck the structure, the reflected power and transmitted power were calculated through the surface integration of the power flow over the surfaces S1 and S2, respectively. The absorbed power was computed through the volume integration of the resistive heating in the gold film (highlighted in red in Figure S1(a)).

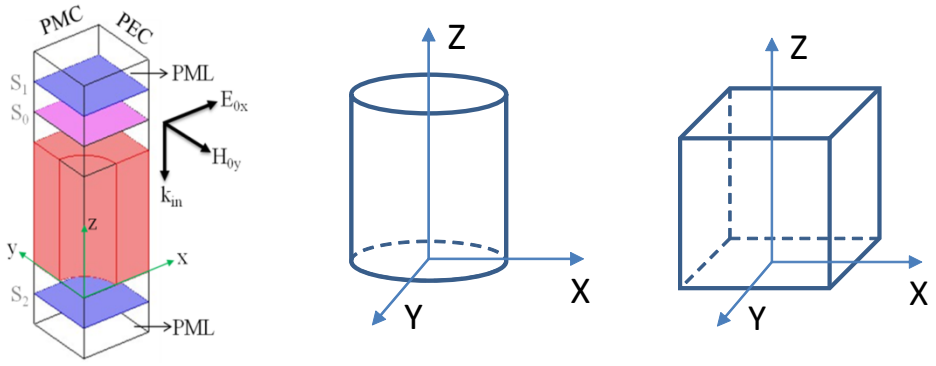
Different kinds of gold nanostructure arrays are simulated. The cross sections of the plasmonic field along different axes of the simulation coordinate systems are presented in Figure S1, and their plasmonic enhancement factors at the top and bottom rims (where the plasmonic field are strongest) are summarized in Table S1.

In Table S1, Since the QDs are excited at a central wavelength of 540 nm, the top and bottom rim enhancement factors at the wavelength of 540 nm are most important for the signal enhancement of QD excitations in our experiments.

Figure S1 also indicates that since 540 nm is not a strong plasmonic peak, the field intensity is not sensitive to the wavelength variation, by comparing the plasmonic intensities for the wavelengths of 530 and 540 nm for the gold nanoarrays.

Table S1. The simulated enhancement factors for various gold nanostructures of 50 nm thick.

Gold nanostructure shape	**Nanopillar Array** (320 nm pitch, 140 nm size)		**Nanohole Array** (400 nm pitch, 140 nm size)	
	Square	Circle	Square	Circle
Top rim @ 540 nm	**8.1**	**5.5**	**6.8**	**4.4**
Bottom rim @ 540 nm	19.5	19.3	16.1	10.7



(a) (b) (c)

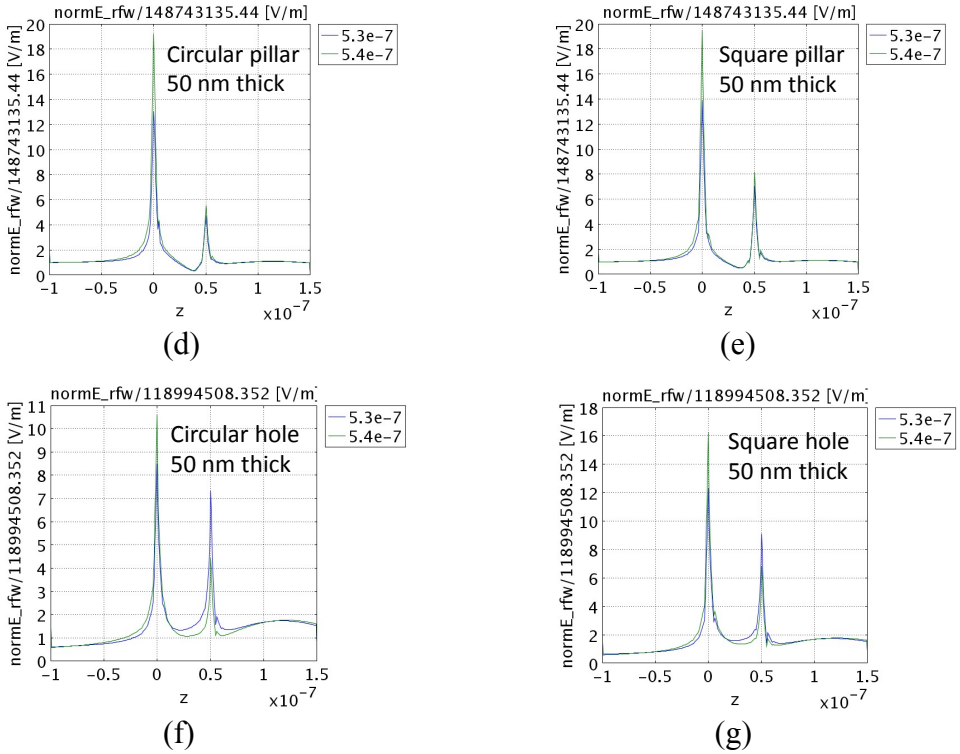


Figure S1. A quarter of the cell for plasmonic simulation (a) and simulated plasmonic enhancement factors at the top and bottom rims of the gold nanopillar array (pitch is 320 nm) and the nanohole array (pitch is 400 nm) at the wavelengths of 530 and 540 nm, respectively. The gold nanostructures have the diameter or square length of 140 nm, and the coordinate systems are plotted for (b) round and (c) square shapes of nanoarrays. (d)-(g) are the field enhancement factors for X = 70 nm, Y = 0, and Z varies from -100 nm to 150 nm, i.e., the left and right peaks stand for the enhancement factors at the bottom and top rims of the gold nanostructures.

2. The peak counts of the QD spectra on gold nanopillars

The bioassays on the gold nanopillars are tested with different integration time of 100, 50, and 20 ms, each test was conducted with a separate sensing chip. Figure S2 is the fluorescent spectra at the integration time of 50 ms. Peak counts of the spectra (extracted from the spectra plotted in Figures 3(b), 3(c) and Figure S2) are listed in Tables S2.

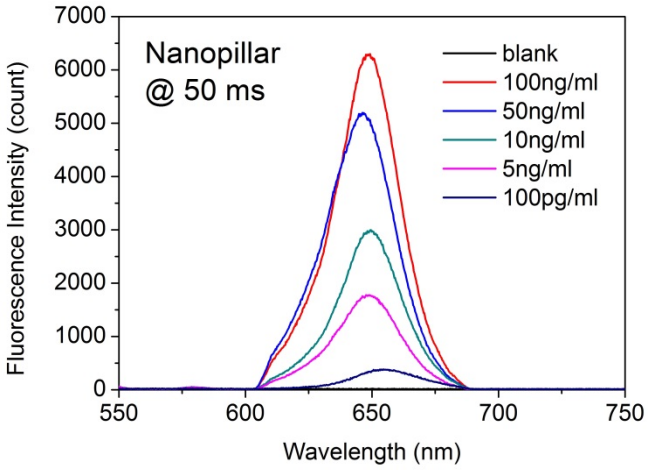


Figure S2. QD emission spectra versus PSA concentrations at the optical integration time of 50 ms, where the blank is the spectrum of the control chip with no PSA.

Table S2. QD emission spectral peaks with PSA bioassay on nanopillar array.

PSA (ng/ml)	100 ms (control chip count is 8)			50 ms (control chip count is 8)			Count difference between 50 and 100 ms	20 ms (control chip count is 17)		
	Peak wave-length	Peak count	Normal-ized to 100ng/ml PSA	Peak wave-length	Peak count	Normal-ized to 100ng/ml PSA		Peak wave-length	Peak count	Normal-ized to 100ng/ml PSA
100	648.17	**12873**	1	649.27	6298	1	6575	651.61	2834.5	1
50	647.07	10212	0.793	646.25	5197	0.825	5015	653.26	1279.5	0.451
10	648.99	8238	0.640	649.54	2994	0.475	5244	652.59	981	0.346
5	648.99	6340	0.493	648.99	1776	0.282	4564	652.32	526	0.186
1								650.40	163	0.058
0.1	652.56	1335	0.104	654.48	384	0.0610	951	654.78	77	0.027

3. QD emission intensity in each spectrum and image for gold nanopillar bioassay

The overall intensity of the QD emission in its spectra can be calculated. Assuming that the QD spectrum is a Gaussian distribution (http://en.wikipedia.org/wiki/Gaussian_function) of $f(x) = a\ exp[-x^2/(2c^2)]$, where a is the amplitude, and $c = FWHM/2.35482$. FWHM is the full width at half maximum, which is about 20 nm in our case. The integration of the QD's emission intensity is $ac \cdot \sqrt{2\pi} = 50a$, i.e., the spectral integrated fluorescent intensity is 50 times of the peak intensity of the QD emission.

It is interesting to calculate the light intensities as count per second (cps) in the spectra in Figures 3(b), 3(c) and S2, instead of showing the peak counts at 100 ms. For an actual light intensity *I* of the QD emission, the microscopic reading is *A lgI*, where A is a factor to turn the light intensity into counts. For an ideal situation where the noise is low compared with signal, light count difference between 50 and 100 ms is 2 times, i.e., *Alg2*, from which *A* can be obtained. The average count difference between 50 and 100 ms (when the curve looks linear at the PSA concentrations ≥ 5 ng/ml) is 5350 (Table S2), indicating *A*=17770.65. In logarithmic scale, the light count at 1 s equals to the light count at 100 ms plus *A*. Therefore the counts of the peak for 1 second integration are expected to be 30644, 27983, 26009, 24111, and 19106 for 100, 50, 10, 5 and 0.1 ng/m of PSA concentrations, equivalent to 1.53×10^6, 1.40×10^6, 1.30×10^6, 121×10^6 and 9.55×10^5 counts per second for each spectrum, respectively.

According to the value of *A*, the count differences at the QD emission peaks for different PSA concentrations can be converted into linear light intensity ratio by $R = 10^{count\ difference/A}$, so the linear light intensities for 50, 10, 5 and 0.1 ng/ml of PSA are 0.71, 0.55, 0.43 and 0.22 of that for 100 ng/ml of PSA, these linear intensities are the brightness of the images in Figure 3(a).

4. The peak counts of the QD spectra on gold nanoholes

The bioassays on the gold nanopholes are tested with different integration time of 100 and 20 ms, each test was conducted with a separate sensing chip. The peak counts of the spectra (extracted from the spectra plotted in Figures 4(b) and 4(c)) are listed in Tables S3 and S4.

Both the gold nanohole array and the gold film generate plasmon to enhance the QD emission, and the enhancement ratios of the QDs by the gold nanoholes and the gold film are listed in Tables S3 and S4. Longer optical integration time and higher PSA intensity render a higher ratio of the two, which is related to the nonlinearity of the gold nanohole QD bioassay.

Table S3. QD emission spectral peaks with PSA bioassay on nanoholes for 100 ms integration. (control chip count is 136)

PSA concentration (ng/ml)	100 ms, Inside the nanoholes			100 ms, Outside the nanoholes			Plasmonic enhancement ratio for inside and outside the nanoholes
	Peak wavelength	Peak count	Normalized to 100ng/ml PSA	Peak wavelength	Peak count	Normalized to 100ng/ml PSA inside nanoholes	
100	656.45	**18336.67**	1	656.73	6671	0.364	4.53
1	651.79	1233	0.0672	653.44	664	0.0362	1.08

Table S4. QD emission spectral peaks with PSA bioassay on nanoholes for 20 ms integration. (control chip count is 126)

PSA concentration (ng/ml)	20 ms, Inside the nanoholes			20 ms, Outside the nanoholes			Plasmonic enhancement ratio for inside and outside the nanoholes
	Peak wavelength	Peak count	Normalized to 100ng/ml PSA	Peak wavelength	Peak count	Normalized to 100ng/ml PSA inside nanoholes	
100	650.67	4149	1	647.93	630	0.152	1.58
50	650.67	1159	0.279	651.49	616	0.148	1.07
10	650.40	561	0.135	649.02	487	0.117	1.01

5. Surface and volume coverage of QDs excited by plasmon on gold nanopillar and nanoholes

The side-views of the gold nanopillars and gold nanoholes are illustrated in Figure S3, with P represents the pitch of the gold nanostructure array, and h represents their height. According to the simulations in Figure 1, only the adjacent area within a lateral distance of 15 nm to the top and bottom rims of a gold nanohole or nanopillar has strong plasmons. On the other hand, only the QDs have a vertical distance of at least 5 nm to the gold nanostructure surface can possibly be enhanced instead of being quenched by plasmons. Therefore, in Figure S3, only the red areas occupied with QDs will be enhanced by plasmons. Based on simulations in Figure 1, we roughly estimate $\Delta q = 5$ nm, $\Delta h = \Delta t = 15$ nm for calculating the ratios of the QDs being excited by the plasmons to the total surface or volume of the gold nanostructures.

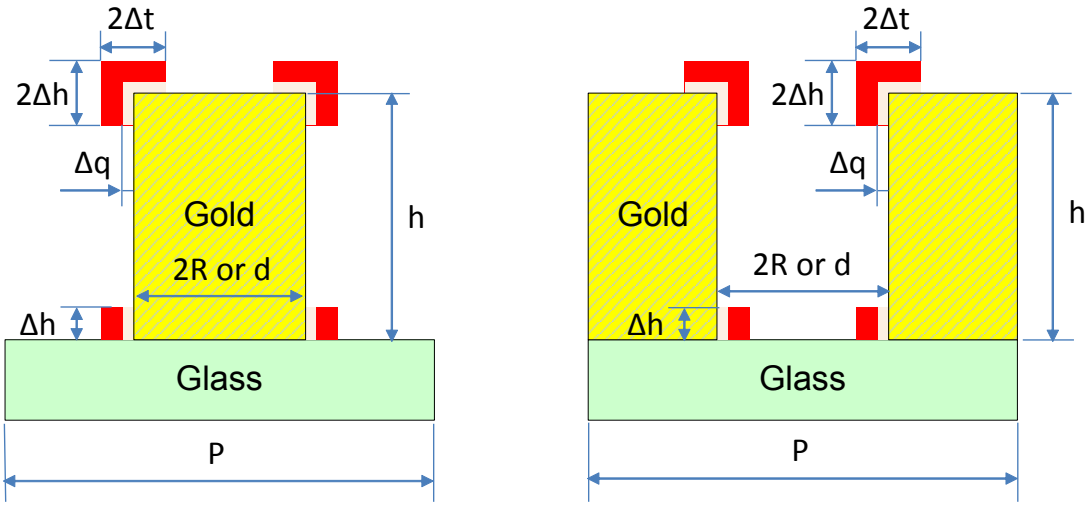


Figure S3. The surface and volume coverage of QDs under the plasmonic excitation compare with the overall surface and volume of the gold nanostructure. (a) is for gold nanopillars, and (b) is for gold nanoholes. The red parts are the QDs excited by plasmons.

For gold nanopillars, if they are round with a radius of R, the surface coverage of the gold area with QDs excited by plasmons is:

$$S_{pr} = \frac{\left[(R + \Delta q)^2 - (R - \Delta t)^2\right] + 2(R + \Delta q) \cdot (2\Delta h + \Delta q)}{R^2 + 2R \cdot h} \tag{1}$$

The volume coverage of the round nanopillars with QDs excited by plasmons is:

$$V_{pr} = \frac{\left[(R + \Delta t)^2 - (R - \Delta t)^2\right] \cdot (\Delta h - \Delta q) + \left[(R + \Delta t)^2 - (R + \Delta q)^2\right] \cdot (2\Delta h + \Delta q)}{R^2 \cdot h} \tag{2}$$

If the gold nanopillars are in square with a length of d, the surface coverage of the gold area with QDs excited by plasmons is:

$$S_{ps} = \frac{\left[(d + 2\Delta q)^2 - (d - 2\Delta t)^2\right] + 4(d + 2\Delta q) \cdot (2\Delta h + \Delta q)}{d^2 + 4d \cdot h} \tag{3}$$

The volume coverage of the square nanopillars with QDs excited by plasmons is:

$$V_{ps} = \frac{\left[(d + 2\Delta t)^2 - (d - 2\Delta t)^2\right] \cdot (\Delta h - \Delta q) + \left[(d + 2\Delta t)^2 - (d + 2\Delta q)^2\right] \cdot (2\Delta h + \Delta q)}{d^2 \cdot h} \tag{4}$$

For gold nanoholes, if they are round with a radius of R, the surface coverage of the gold area with QDs excited by plasmons is:

$$S_{hr} = \frac{\left[\pi(R + \Delta t)^2 - \pi(R - \Delta q)^2\right] + 2\pi(R - \Delta q) \cdot (2\Delta h + \Delta q)}{P^2 - \pi R^2 + 2\pi R \cdot h} \tag{5}$$

The volume coverage of the round nanoholes with QDs excited by plasmons is:

$$V_{hr} = \frac{\left[\pi(R+\Delta t)^2 - \pi(R-\Delta t)^2\right] \cdot (\Delta h - \Delta q) + \left[\pi(R-\Delta q)^2 - \pi(R-\Delta t)^2\right] \cdot (2\Delta h + \Delta q)}{\left(P^2 - \pi R^2\right) \cdot h} \tag{6}$$

If the gold nanoholes are in square with a length of d, the surface coverage of the gold area with QDs excited by plasmons is:

$$S_{hs} = \frac{\left[(d+2\Delta t)^2 - (d-2\Delta q)^2\right] + 4(d-2\Delta q) \cdot (2\Delta h + \Delta q)}{P^2 - d^2 + 4d \cdot h} \tag{7}$$

The volume coverage of the square nanoholes with QDs excited by plasmons is:

$$V_{hs} = \frac{\left[(d+2\Delta t)^2 - (d-2\Delta t)^2\right] \cdot (\Delta h - \Delta q) + \left[(d-2\Delta q)^2 - (d-2\Delta t)^2\right] \cdot (2\Delta h + \Delta q)}{\left(P^2 - d^2\right) \cdot h} \tag{8}$$

According to the equations (1)-(8), the surface and volume coverage of the QDs excited by plasmons are calculated in Table S5.

Table S5. Surface and volume coverage of QDs excited by plasmons on gold nanopillars and nanoholes. (h = 50 nm, 2R or d = 140 nm, Δq = 5 nm, Δt = Δh = 15 nm, nanopillars are with P = 320 nm, nanoholes are with P = 400 nm)

Nanostructure	Gold nanopillars		Gold nanoholes		Coverage ratio of nanopillars/nanoholes	
Shape	Round	Square	Round	Square	Round	Square
Surface coverage	65.97%		14.24%	17.93%	4.63	3.68
Volume coverage	40.00%		3.65%	4.79%	10.96	8.36