Supporting Information

A Gold Nanohole Array Based Surface-Enhanced Raman Scattering Biosensor for Detection of Silver(I) and Mercury(II) in Human Saliva

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Figure S1 Optimization of operating condition for the SERS sensor. (a) Ionic strength optimization. The optimal ionic strength was found to be 30 mM; (b) Incubation time optimization with the optimal value of 16 min; (c) pH optimization. A neutral pH is preferred.
**Figure S2** SERS spectra corresponding to different levels of Ag(I) ions. The spectra were obtained from Au nanostar@MGITC@SiO$_2$ coupled with Au film at different concentrations of Ag$^+$ ions in the MOPS buffer.

**Figure S3** Selectivity test of the Ag(I) sensor against other metal ions. All metal ions were prepared with a concentration of 50 nM in 10 mM MOPS buffer solution containing 30 mM NaNO$_3$. The sensor shows excellent selectivity towards silver ions in the complex mixture solution.
Figure S4 Selectivity test of the Hg(II) sensor against other metal ions. All metal ions were prepared with a concentration of 50 nM in PBS buffer. The sensor shows excellent selectivity towards mercury ions in the complex mixture solution.

Figure S5 Simulated EM field distribution of a single Au nanostar. The peak SERS enhancement factor $(E/E_0)^4$ is calculated to be $1.4 \times 10^4$. $(E/E_0)^{0.25}$ was used to represent the EM field enhancement for visualization purposes.
Figure S6 Simulated EM field distributions for Au nanostar is coupled with Au film. This geometry led to a peak SERS enhancement factor \((E/E_0)^4\) of \(1.5 \times 10^5\). (a) A cross-section perpendicular to Au film with the cross-section cut from the center of the Au nanostar; (b) A cross-section parallel to the Au film with the cross-section cut from the center of the Au nanostar. \((E/E_0)^{0.25}\) was used to represent the EM field enhancement for visualization purposes.
Figure S7 Simulated EM field distributions. (a) FDTD simulation cell with four Au nanostars located at point A (the rim of a nanohole), point B (the gap center between two nanoholes), point C (the gap center between three nanoholes), and point D (the nanohole center); (b) simulated EM field distribution of Au nanostars on Au nanohole array at point A, B, C, and D. The simulations were conducted under y polarization with a 785 nm laser source. \((E/E_0)^{0.25}\) was used to represent the EM field enhancement for visualization purposes.

Table S1 SERS enhancement factors

| Maximum SERS enhancement factors \((E/E_0)^4\) of Au nanostar at different locations on Au nanohole array |
|---|---|---|---|
| A | B | C | D |
| \(4.5 \times 10^6\) | \(1.0 \times 10^6\) | \(5 \times 10^5\) | \(1.1 \times 10^5\) |