SUPPLEMENTARY INFORMATION

Well-Defined Assembly of Plasmonic Metal Nanoparticles by Dielectrophoresis for Highly Sensitive SERS-Active Substrates

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Fig. S1 Numerical analysis of the effect of the depth on the formation of a nonuniform electric field in the 5 μ m diameter. The spacing between the patterns was fixed at 5 μ m. Depth = (a) 1 μ m, (b) 3 μ m, (c) 10 μ m, and (d) 15 μ m.



Fig. S2 Numerical analysis of the effect of the depth on the formation of a nonuniform electric field in the 10 μ m diameter. The spacing between the patterns was fixed at 10 μ m. Depth = (a) 1 μ m, (b) 3 μ m, (c) 10 μ m, and (d) 15 μ m.



Fig. S3 Numerical analysis of the effect of the depth on the formation of a nonuniform electric field in the 15 μ m diameter. The spacing between the patterns was fixed at 5 μ m. Depth = (a) 1 μ m, (b) 3 μ m, (c) 10 μ m, and (d) 15 μ m.

Materials and Methods

Materials

Ethanol (99.9% purity) was purchased from Duksan General Sciences. Tetraethyl orthosilicate (TEOS, 98%), (3-aminopropyl)triethoxysilane (APTMS, 97%), Tetrakis(hydroxymethyl) phosphonium chloride solution (THPC, 80%), gold(III) chloride trihydrate (HAuCl4, \geq 99.9%), and polyvinylpyrrolidone (PVP, Mw: 10,000) were purchased from Sigma-Aldrich. Formaldehyde (\geq 35%) and isopropyl alcohol (IPA, 99.5%) were purchased from Samchun Chemical. Sodium hydroxide (NaOH; 98%) and potassium carbonate (K₂CO₃; 99%) were purchased from ACROS Organics. Sulfuric acid (H₂SO₄, 98% purity) and hydrogen peroxide (H₂O₂, 30% purity) were purchased from Daejung Chemicals. Malachite green isothiocyanate (MGITC) was purchased from SPI Supplies.

Ultraviolet-visible-near-infrared absorption spectra were obtained using a JASCO V-770 spectrophotometer. Field-emission scanning electron microscopy (FE-SEM) images were obtained using a Hitachi S-4300 FE-SEM instrument (SEMIONE, Hanyang University ERICA) at an accelerating voltage of 15 kV. Raman spectra and mapping images were acquired using confocal micro-Raman spectroscopy with a dark-field microscope (WEVE, HEDA). The following excitation sources were used: a 633 nm He–Ne gas laser (17 mW, Newport); (Integrated Optics). Raman scattering signals were collected using a charge-coupled device camera with a high-resolution grating (1200 grooves mm⁻¹) and measured by focusing a laser spot using a 100 × (NA 0.9) objective lens with a diffraction limit of \approx 200 nm. Spectra were obtained with exposure times of 1 s for verifying the performance of the SERS-active substrate

and for the competition immunoassay, respectively, both with a laser power of 0.4 mW after applying a 1% neutral density filter. For the back-ground subtraction, the mean difference equation was calculated using a window size of 13. The numerical simulations were calculated using COMSOL Multiphysics. Time-domain waveforms and signal characteristics were measured using a digital oscilloscope GDS-1102B (GWINSTEK, Digital Storage oscilloscope). Input signals for experiments were generated using a function generator MFG-2120 (GWINSTEK, Multi-Channel Function Generator).

Fabrication of Gold-Coated Silica Nanoparticles

The restriction in dielectrophoresis is that small-sized particles are difficult to handle, and large-sized nanoparticles are difficult to synthesize uniformly. To address these issues, a seeded growth method was used to prepare gold-coated silica nanoparticles with a diameter of 800 nm [40]. Silica nanoparticles were synthesized according to a previously reported method [41]. Specifically, 1 g of silica nanoparticle powder was added to a round-bottom flask containing 100 mL of ethanol. The resulting powder was thoroughly dispersed using a sonicator. The flask was then connected to a reflux condenser and placed into a heating mantle with the temperature set to 70 °C. After 5 min, 0.9 mL of 3-aminopropyltrimethoxysilane (APTMS) was added to the mixture, which was refluxed with continuous stirring for 2 h. Following reflux, the reaction mixture was allowed to cool to room temperature with continued stirring. The mixture was then subjected to two rounds of centrifugation at 2000 rpm for 10 min using ethanol as the solvent; the precipitate was redispersed in 332 mL of ethanol. Next, this solution (0.5 mL) was stirred with 1 mL of a colloidal AuNP solution prepared as described in a previous study [40]. Subsequently, the solution was centrifuged again under the same conditions and the precipitate was then redispersed in 5 mL of deionized water (DIW). The colloidal gold nanoparticles deposited onto the surface of the silica particles acted as nucleation sites for subsequent gold growth. To promote the growth of gold on the silica surface, a solution of 3 mL of 25 mM HAuCl₄ was stirred into a solution of 0.5% K₂CO₃ for 30 min. A 4 mL aliquot of this mixture was then combined with 200 µL of the gold-seeded silica nanoparticle solution. The temperature of the solution increased to 50°C, after which 10 μ L of formaldehyde was added. The reaction was then allowed to proceed for 5 min. Finally, 3 mL of 0.1% polyvinylpyrrolidone (PVP) solution was added to the mixture to stabilize the particles [40,42].





Fig. S4 Synthesis and characterization of $SiO_2@Au$. (a) Schematic of the formation of gold nanoshells on the surface of silica nanoparticles. (b) SEM image of SiO_2 (b₁) and $SiO_2@Au(b_2)$ with a scale bar of 500 nm. EDX data of $SiO_2(b_3)$ and $SiO_2@Au(b_4)$ (c) UV–Visible spectrum of $SiO_2(red)$ and $SiO_2@Au(black)$; the particles are core–shell particle with a 75 nm thick gold layer formed on the surface of a silica nanoparticle with a diameter of 650 nm.



Fig. S5 (a) Raman spectra of substrate which particles arrayed by DEP obtained with 532, 633, 785nm wavelengths. (b) Schematics of the different substrates. Sample (1) is a substrate arranged in a pattern using DEP, sample (2) is a substrate with random gold particles on a gold substrate, sample (3) is a substrate with random gold particles on a Si wafer, and sample (4) is a gold substrate without gold particles.

Enhancement Factor Calculation

The enhancement factor (EF) in our study was calculated as following equation[51]:

Enhancement factor (EF) = $(I_{SERS} / N_{SERS}) / (I_{REF} / N_{REF})$

$$I_{SERS} = 2.667 \times 10^7$$
, $N_{SERS} = 3.602 \times 10^6$

 $I_{REF} = 376$

According to Brown & Doorn[52], the maximum surface coverage of MGITC molecules on a gold surface is 1 molecule per 1.2nm². Based on this value, we assumed the maximum contact area of a single MGITC molecule to be 1.2nm² and used this for the calculation of the EF value. Since this value represents an ideal maximum, the actual surface coverage in our research is expected to be equal to or lower than this estimate.

The diameter of the laser spot was calculated using the following formula:

Diameter of laser = 1.22 $\times \frac{\lambda}{NA} = 1.22 \times \frac{633nm}{0.9} = 858.06$ nm

 $N_{SERS} = (Surface of Substrate) / 1.2nm^2$

Surface of Substrate = Surface of laser spot + Surface of Particle

Surface of laser spot = $(858.06 \text{nm})^2 \times \pi = 2.313 \times 10^6 \text{ nm}^2$

Surface of particle = 4 × $(400 \text{nm})^2$ × π = 2.010 × 10⁶ nm²

Surface of N_{SERS} = $4.322 \times 10^6 \text{ nm}^2$

 $N_{SERS} = 3.602 \times 10^6$ molecules

$$N_{\text{REF}} = \frac{Density \cdot V_{laser} \cdot N_A}{M}$$

 $(V_{laser}: laser's confocal volume, N_A: Avogadro number, M: molecule weight(g/mol))$

$$N_{REF} = 2.574 \times 10^{-8}$$

Enhancement factor = 2.877×10^7

The enhancement factor of DEP arrayed SERS-active substrate is 2.877×10^7 .