

Gold nanoparticles for one step DNA extraction and real-time PCR of pathogens in a single chamber

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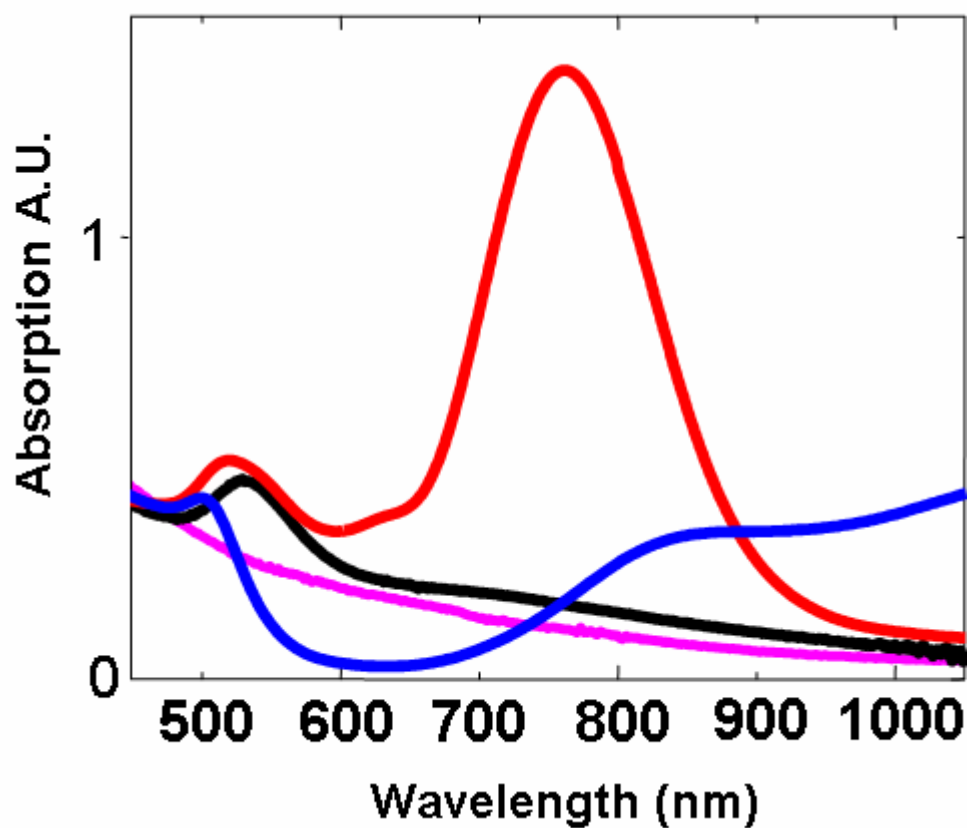


Figure S1. UV-Vis spectra for Au and silica coated nanoparticles. Black is for spherical Au nanoparticles (AR 1.3), red is for Au nanorod (AR 3), blue is for Au nanorod (AR 15), and purple is for SiO₂ coated Fe₂O₃ nanoparticles.

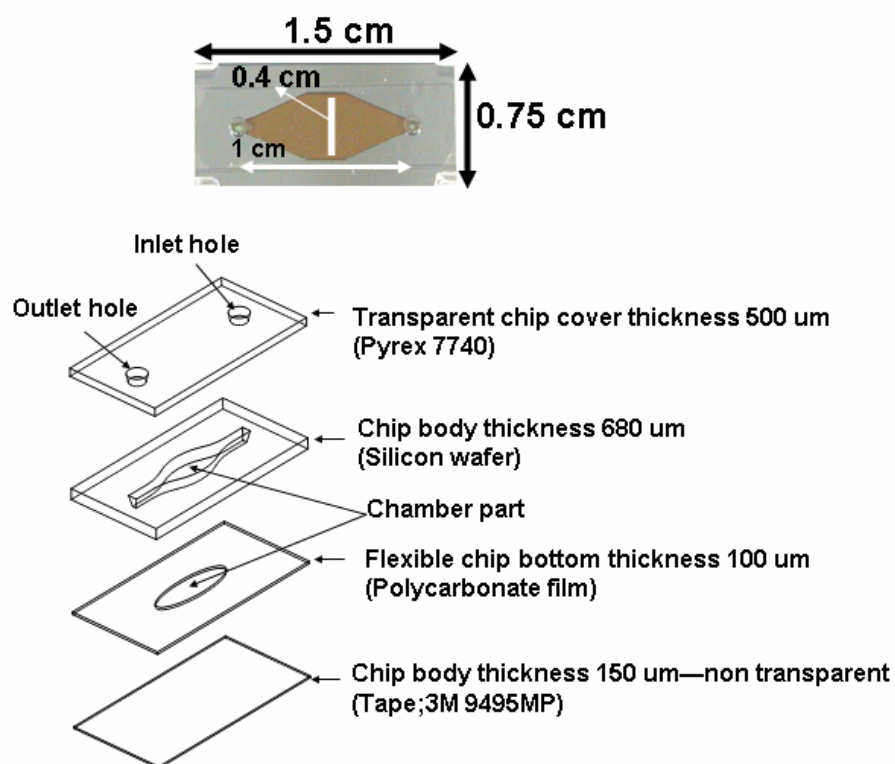


Figure S2. Microchip details.

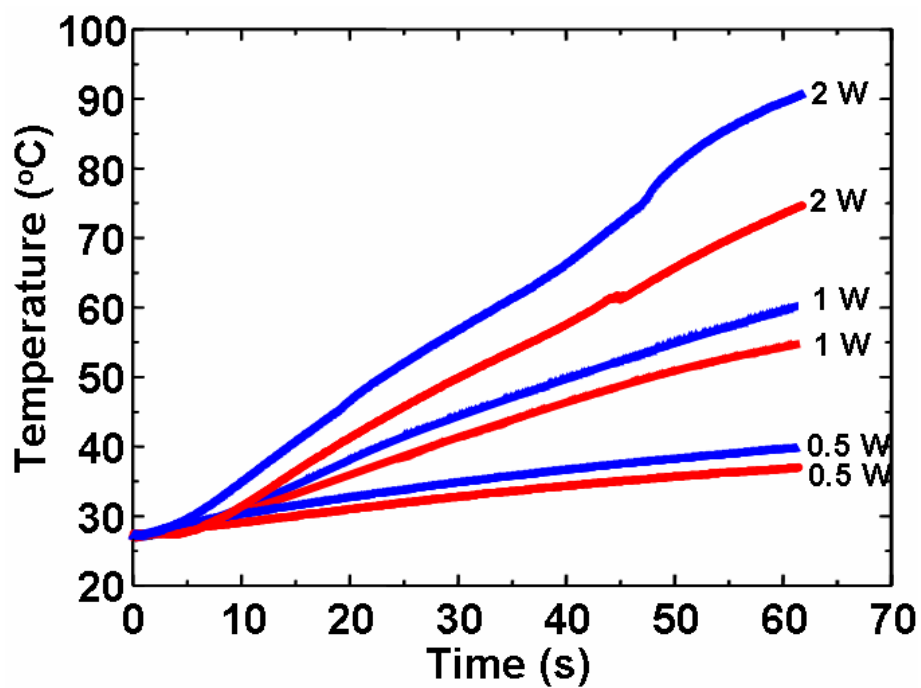


Figure S3. Laser power effects on the optothermal property of Au rods, blue for short rod (AR 3), and red for long rod (AR 15), concentration was set 8 mg/ml.

Methods

Au rod synthesis

Au nanorods were synthesized as described previously.¹⁸ Briefly, a 20 mL aqueous solution containing 2.5×10^{-4} M HAuCl₄ and 2.5×10^{-4} M tri-sodium citrate was prepared in a conical flask. Next, 0.6 ml of ice cold 0.1 M NaBH₄ solution was added to the solution all at once while stirring. The solution turned pink immediately after adding NaBH₄, indicating particle formation. The particles in this solution were used as seeds within 2-5 h after preparation. In a clean test tube, 10 ml of growth solution, containing 2.5×10^{-4} M HAuCl₄ and 0.1 M cetyltrimethylammonium bromide (CTAB), was mixed with 0.05 ml of 0.1 M freshly prepared ascorbic acid solution. Next, 0.025 ml of the 3.5 nm seed solution was added. No further stirring or agitation was done. Within 5-10 min, the solution color changed to reddish brown. The rod aspect ratio can be tailored using different CTAB concentrations and aging times.

SiO₂ coated Fe₂O₃ synthesis

The uniform SiO₂ coated Fe₂O₃ nanoparticles were prepared using monodispersed γ -Fe₂O₃ nanocrystals (average *dia.*~12.5 nm), which had been synthesized by the thermal decomposition of iron pentacarbonyl precursor in the presence of an oleic acid stabilizer and octyl ether. The SiO₂ coating of the γ -Fe₂O₃ nanoparticles was performed through the formation of water-in-cyclohexane reverse microemulsion. Specifically, for a 20 nm-thick SiO₂ shell, polyoxyethylene(5)nonylphenyl ether (0.56 mM, Igepal CO-520, containing 50 mol % hydrophilic group) was dispersed in cyclohexane (4.2 ml) by sonication. Then 300 μ l of Fe₂O₃ solution (0.8 mg/ml of cyclohexane) was added. The resulting mixture was vortexed, and ammonium hydroxide (29.4 %, 35 μ l) was added to form a transparent, brown solution of reverse microemulsion. Next, tetraethylorthosilicate (20 μ l, TEOS) was added, and the reaction was continued for 42 h at room temperature. By adding methanol into the reaction solution, SiO₂ coated Fe₂O₃ nanoparticles were precipitated and collected by a magnet. Finally the nanoparticles were then washed using methanol and transferred into ethanol.

Real time PCR experimental

Real-time PCR assays were performed with a primer set (forward:5'-YCCA KACTCCTACGGGAGGC - 3' and Reverse : 5'-GTATTACCGCRRCTGCTGGCAC - 3') to amplify a region of 16S ribosomal RNA of the *E. coli* genome using a GenSpector[®] TMC-1000 instrument (Samsung Advanced Institute of Technology, Korea). PCR was performed in a silicon-glass-bonded chip with a total volume of 8 μ l of reaction mixture containing LightCycler[®] FastStart Reaction Mix SYBR Green I and LightCycler[®] FastStart Enzyme (Roche Diagnostics), 250 nM of forward and reverse primers (Bioneer, Korea), 5 mM MgCl₂ and PCR grade water. After loading, the PCR chip was heated at 95 °C for 10 min to activate the hot-start enzyme and to predenature the DNA templates. This was followed by 30 cycles of a denaturation step at 95 °C for 5 sec, an annealing step at 55 °C for 10 sec, and an extension step at 72 °C for 15 sec. When

the *E. coli* BL21 cells were added, PCR was performed without heating at 95 °C for 10 min to avoid the boiling lysis of bacterial cells and DNA extraction by heating. After amplification was completed, a melting curve analysis was performed by slow heating (0.1 °C/s) the sample from 65 °C to 95 °C. The total time required for the 30 cycles of amplification and the melting analysis was about 35 min. Following the PCR reaction, the amplified DNA was analyzed by Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).