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Supplementary Information

Section S1: Fabrication of PAA-coated magnetic nanoparticles for the creation of silica-coated magnetic microparticles

Materials

To synthesize of PAA-coated Fe_3O_4 nanoparticles, $FeCl_3$ (laboratory reagent grade, Fisher Scientific, USA), PAA (M_n = 1800, Aldrich), diethylene glycol (99%, Daejung), NH_4OH (28%, Daejung), and NaOH (98%, pellet, Daejung) were used.

Synthesis

A $FeCl_3$/DEG stock solution was prepared by dissolving 20 mmol $FeCl_3$ in 50 mL DEG and heating it at 120°C for 1 h under a nitrogen atmosphere. A NaOH/DEG stock solution was also prepared by dissolving 125 mmol NaOH in 50 mL of DEG and heating it at 120°C for 1h under a nitrogen atmosphere. Fe_3O_4 nanoparticles with tunable sizeswere synthesized using a high-temperature hydrolysis reaction with PAA as a surfactant. In a typical synthesis, a mixture of 288 mg PAA, 1 mL $FeCl_3$ stock solution and 15 mL DEG was heated to 220°C for 90 min under a nitrogen atmosphere with vigorous stirring to form a transparent, pale-yellow solution. Next, 1.8 mL NaOH/DEG stock solution was injected into the above solution, which turned black after approximately 2 min. The resulting mixture was further heated for 1 h to yield Fe_3O_4 colloids with a diameter of approximately 80 nm. The synthesized Fe_3O_4 colloids were first washed with a mixture of DI water and ethanol, then with pure water several times, and finally dispersed in 3 mL DI water.

FE-SEM and Bright field micoscope image

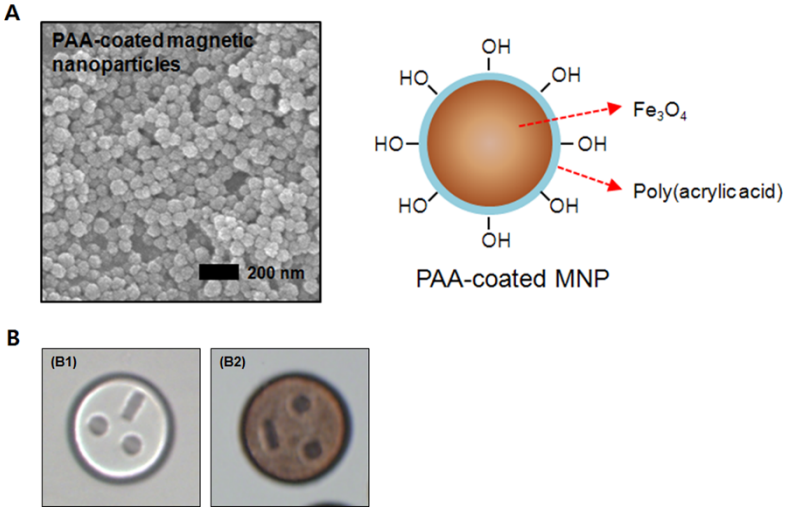

Figure S1.

(A) An FE-SEM image and a schematic illustration of PAA-coated magnetic nanoparticles.

(B) Bright-field images of a non-magnetic (B1) and a magnetic (B2) silica microparticle.

Mass magnetization

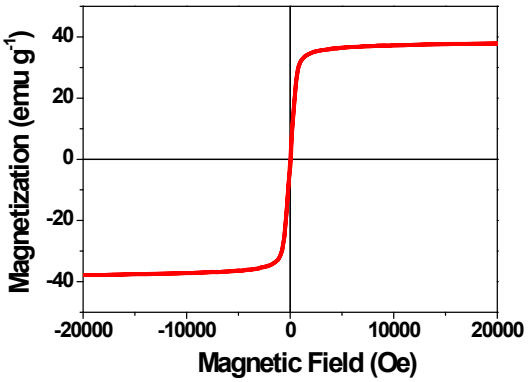

Figure S2. Mass magnetization curve of the Fe_3O_4 nanoparticles. The particles were super-paramagnetic at room temperature with no remnant magnetization.

Section S2: Silica coating of non-silane-grafted polymeric particles

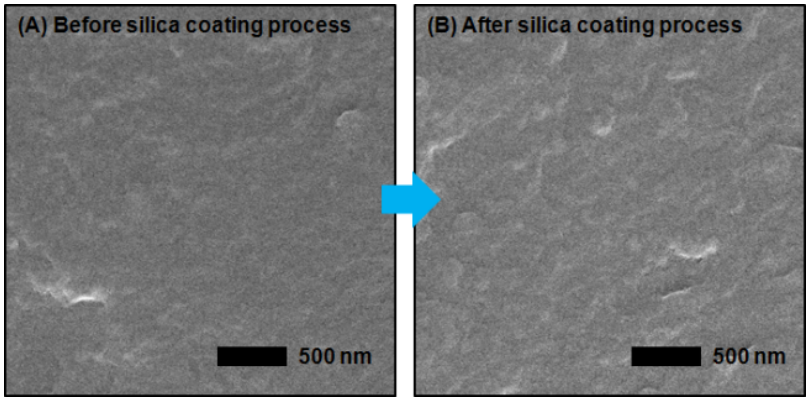

Figure S3. FE-SEM images of polymeric microparticles before (A) and after (B) the silica-coating process. The polymeric microparticles were generated without silane-grafting materials. Therefore, a silica layer was not formed on the surface of the polymeric microparticles after the silica coating process.

Section S3: FE-SEM images of silica-coated polymeric microparticle

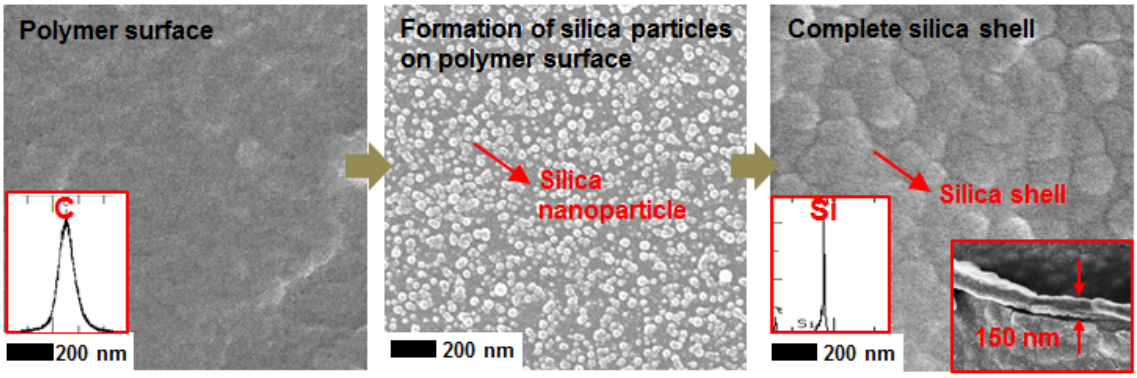

Figure S4. FE-SEM images showing silica shell growth on a copolymer microparticle with different silica coverage. Initially, the seed silica nanoparticles increase in size as the reaction proceeds. Eventually, the seed nanoparticles coalesce and form a continuous silica shell on the copolymer surface. The inset shows the thickness of the silica layer, which was approximately 150 nm.

Section S4: Oligonucleotide immobilization on an uncoated polymeric microparticle

Materials

To immobilize5′-amino-terminated DNA on a polymeric microparticle, sulfo-NHS, EDC, and 4-dimethylaminopyridine (DMAP, Alfa-Aesar, USA) were used.

Immobilization

Formation of amide bonds between the DNA amino groups and the carboxylic acid groups on the polymeric microparticles was mediated by sulfo-NHS activation. The intermediate product of the reaction between carboxylic acid and the sulfo-NHS is very labile and hydrolyzes quickly. Sulfo-NHS, EDC, and DMAP were dissolved in EtOH/H_2O. The carboxylic-acid-grafted polymeric microparticles were dipped into the prepared solution, and the mixture was agitated at 25°C for 8 h. After washing with DI water, amino-terminated DNA (in carbonate buffer, pH 9) was added to the sulfo-NHS-activated microparticles to carry out the coupling reaction. Following immobilization of the DNA onto the particles, they were washed with carbonate buffer and DI water to remove excess DNA.

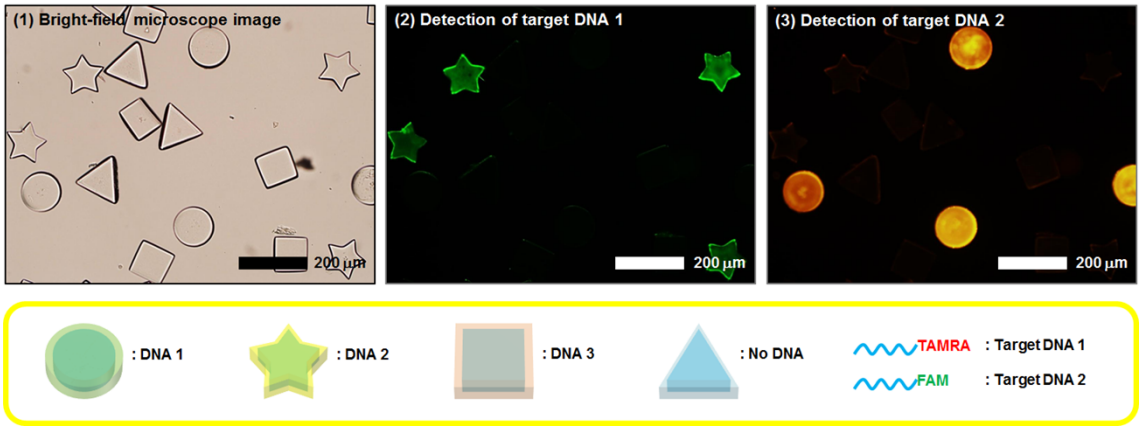

Figure S5. Attachment of oligonucleotides on the particles.

A multiplexed DNA hybridization assay using silica-coated microparticles. Green fluorescence signal was detected by hybridization reaction between probe DNA 1 and FAM-labeled target DNA 1. Redfluorescence signal was detected by hybridization between probe DNA 2 and TAMRA-labeled target DNA 2.

Section S5: Image processing and analysis program

We were able to greatly increase the number of available codes relative to conventional coding methods using a customized software program, in which we optimized a commonly widely used image-processing method for use with our system.

The program utilizes two images (i.e., bright-field and a fluorescent images), with the same coordinates, and determines the intensities for each particle in the image. We acquire location and code information from the bright-field image and signal intensity values from the fluorescence image. A two-step process is performed to gather information regarding particle location and code from the bright-field image. The first step is referred to as 'particle finding' and the second step is referred to as 'code identification'. In the particle finding step, a Hugh transformation is used for fast and accurate detection of circular shapes. To identify the code, a scoring method is used for the different code shapes. In the scoring method, the correlation between the original image and the template (in this case, the template is our designed code shape) can be thought of as the score of 1 pixel. After collecting the scores for all the pixels for all templates, we choose the particle code that gives the highest score. Once these two steps (particle finding and code identification) are complete, we measure the intensity values from the fluorescent images and sort them based on code using the information derived from the bright-field images. Fluorescence intensities are obtained by summing the brightness values of the pixels and dividing the sum by the number of pixels in each particle.

Section S6: HPV genotyping using silica-coated polymeric microparticles

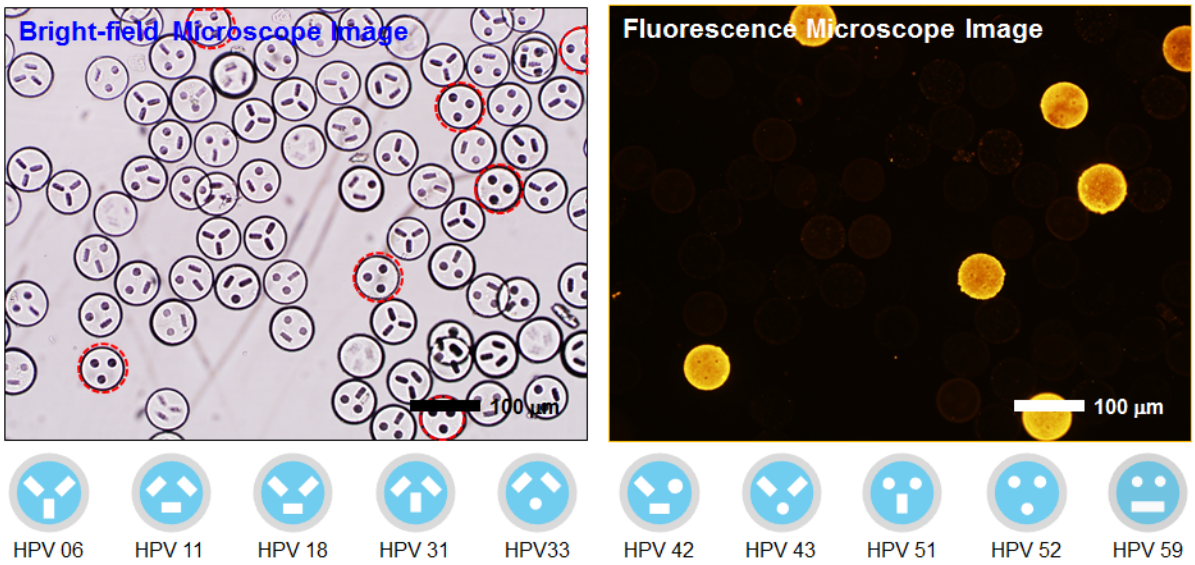

Figure S6. Bright-field and fluorescence microscope images demonstrating HPV 52 genotyping assay. Only particles with probes complementary to the target HPV 52 sequences showed strong fluorescence signal in the presence of other probe-bound particles.

Section S7: 20-plexed HPV genotyping using encoded magnetic silica microparaticles

This hybridization assay was performed HPV under same condition with 10-plex assay, but microplate shaking incubator (700rpm) at 37 °C.

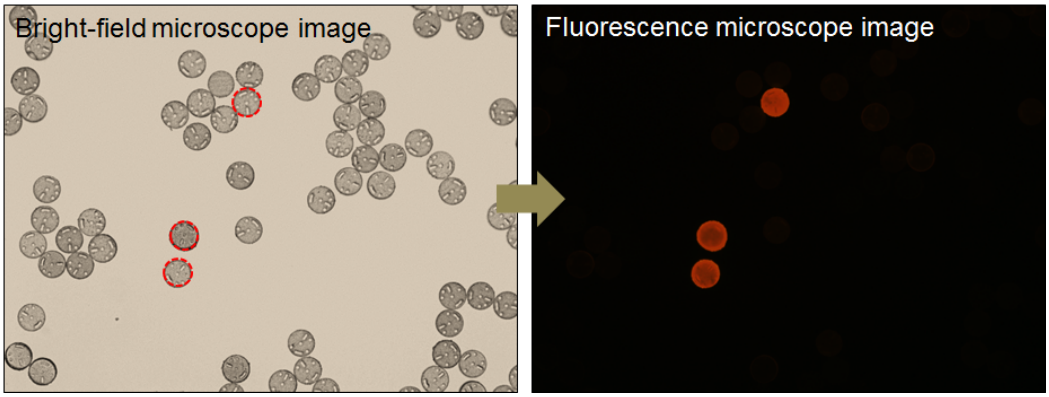

Figure S7. 20-plexed HPV genotyping using encoded magnetic silica microparticles. Bright-field and fluorescence microscope images after the hybridization assay. Only particles with probes complementary to the target sequences showed strong fluorescence in the presence of other probe-bound particles.

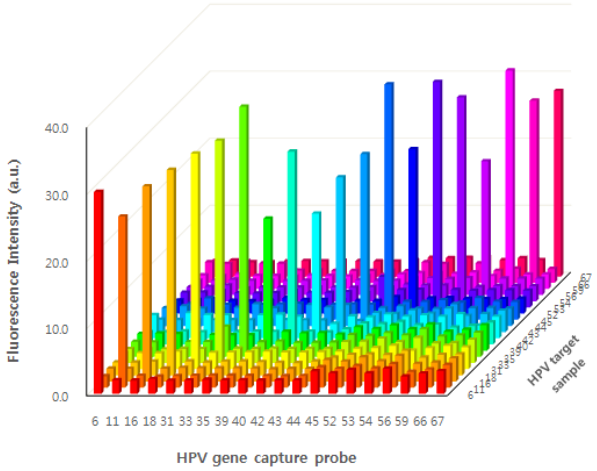

Figure S8. The graph shows measured fluorescence intensity of all particles in an obtained fluorescence image. 10^3 copies of target sequence were efficiently detected using our encoded silica microparticles. The bars indicate the arithmetic mean of the fluorescence intensity from all type-specific particles in an analyzed image, and the error bars indicate the standard error of the measurements.

Section S8: Experimental

Materials: 1:1 volumetric ratio of ETPTA (M_n = 428, Aldrich, USA) and TMSPA (92 %, Aldrich) were used as alkoxysilane-grafted photocurable resin, and 10 wt% of 2-hydroxy-2-methylpropiophenone (97 %, Aldrich) as photoinitiator. During the silica coating process, the precursor particles were reacted in a solution of tetraethyl orthosilicate (TEOS, 98 %, Aldrich), ethanol (99 %, Daejung, South Korea) and ammonium hydroxide (25~28 %, Daejung). APTES (98 %, Aldrich), and DMF (99.8 %, Sigma, USA) were used for surface functionalization. Sulfo-NHS and EDC were purchased from Alfa-Aesar (USA) for bioconjugation. 5′-Amine modified HPV type-specific oligonucleotide probes (HPV 6, 11, 18, 31, 33, 42, 43, 51, 52, and 59), plasmids containing the L1 sequences, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes, and a PnE genotyping kit were purchased from YBT (South Korea). Streptavidin-R-phycoerythrin (Prozyme, USA) was used to obtain fluorescent signals.

Silica coating: Silica-coated microparticles were prepared using a modified version of the Stöber method. Approximately 10,000 alkoxysilane-grafted polymeric microparticles were dipped into a solution of deionized (DI) water (3 mL), ethanol (20 mL), and NH_4OH (28 %, 1 mL). TEOS (0.1 mL) was injected into the solution until the total amount of TEOS reached 1 mL. The silica-coated particles were then washed 5 times with ethanol.

Immobilization of oligonucleotide probes on encoded silica microparticles: Approximately 3×10^4 carboxylated microparticles were activated with 50 mM EDC/sulfo-NHS in MES buffer solution (pH 6) for 15 min. Amino-terminated probes (2 μM) were added to the microparticle solution. After incubation for 1 h, the microparticles were washed with phosphate-buffered saline (PBS, 0.02 % Tween 20) and PnE washing buffer 2 containing

sodium dodecyl sulfate (SDS). Finally, the microparticles were stored in 100 μL TE buffer at 4°C.

Hybridization assay: HPV positive products were prepared by PCR, and the initial copy number of each type was 10^4 before PCR. 15 μL of biotin-labeled HPV PCR product were added to a mixture of 2 μL microparticle (~1000 microparticles) and 15 μL of 2× hybridization buffer. A thermocycler was then used to hybridize the biotin-labeled HPV sequences with the of probes on the microparticles. In particular, the hybridization mixture was denatured at 95 °C for 10 min and then incubated at 37 °C for 40 min. After hybridization, the microparticles were washed 3 times with PBS (0.02 % Tween 20) and resuspended in 100 μL of streptavidin-R-phycoerythrin. Following 15 min incubation at room temperature, the microparticles were washed 3 times with washing buffer. Finally, for the image analysis, the microparticles were transferred to a 96-well plate, and PBS was added to each well to a final volume of 100 μL.

Maskless lithography: Encoded polymeric microparticles were generated by photopolymerization using a spatial light modulator (S2). An optical microscope (IX71, Olympus, Japan), UV light source (200W, mercury-xenon lamp, Hamamatsu, Japan), and digital mirror device (DMD, Texas Instruments, USA) were aligned to expose a UV pattern. A 10× magnification microscope lens with a numerical aperture (N.A.) of 0.28 was used to project the computer-controlled image pattern onto the MEMS spatial light modulator (SLM) to create a final object plane with a demagnification factor of approximately 8.9; as the pitch of the micromirror array was 13.68 μm in the SLM plane, the pixel size in the object plane was approximately 1.54 × 1.54 μm^2. The optical projection system and imaging optics shared the same objective lens, and the light paths were separated with a beam splitter. With this

system, we were able to reliably synthesize encoded polymeric microparticles with single-pixel resolution (~1.54 × 1.54 μm^2) and an exposure time of 0.25 s.

Surface functionalization: To introduce amine groups onto their surfaces, the silica-coated microparticles were treated with a 5 % (v/v) APTES anhydrous ethanol solution at 25 °C for 12 h with agitation. After the microparticles were washed with ethanol, they were then baked at 110 °C for 1 h. Introduction of amine groups was confirmed using the Kaiser ninhydrin test, which yielded a dark blue color. [34] Amine-functionalized microparticles were added to the DMF solution containing succinic anhydride (50 mM) and tris-acetate-EDEA (TAE, 55 mM). Finally, the mixture was shaken for 2 h at 25 °C and washed with DMF and ethanol. Amine conversion was confirmed by loss of the blue color in the Kaiser ninhydrin test.

Amplification of sequences from HPV types: Amplification of the viral L1 open reading frame (ORF) fromeach HPV sequence was conducted using a thermocycler (MyCycler, Bio-Rad, USA). The PnE genotyping kit was used for amplification. As a positive PCR control, a GAPDH sequence was also amplifiedusing the same reaction mixture. An initial 5-min denaturation step at 95 °C was followed by 40 cycles of amplification, which consisted of denaturation step at 95 °C for 1 min, an annealing step at 55 °C for 1 min, and an elongation step at 72 °C for 1 min; a final elongation step at 72 °C was performed for 7 min. The amounts and sizes of the amplified products were analyzed by electrophoresis.

Labeling of the target sequences: Following amplification, labeling PCRs were performed to label the complementary probe sequences with biotin-14-dCTP. After the purification process, 10 μL amplicon was labeled with biotin-14-dCTP, and the mixture was brought to a final volume of 20 μL. An initial 5-min denaturation step at 94 °C was followed by 35-cycles of

amplification step, which consisted of a denaturation step at 94 °C for 30 s, an annealing step at 55 °C for 1 min, and an elongation step at 72 °C for 2 min.

Preparation of HPV 18 from HeLa cells: HeLa cells (CCL-2, ATCC, Manassas, VA, USA) encoding HPV 18 were cultured under a 5 % CO_2 atmosphere in RPMI 1640 medium (Biowest, UK) containing 10 % fetal calf serum (FBS) and 1 % antibiotics. Genomic DNA extraction was performed using a G-DEX Genomic DNA Extraction Kit (Intron Biotechnology, South Korea), according to the manufacturer's instruction.