Supporting Information for

DNA-Mediated Size-Selective Nanoparticle Assembly for Multiplexed Surface Encoding

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Nanoparticle Synthesis

Single crystalline gold nanocubes were synthesized *via* the seed-mediated method described in detail by O'Brien *et al.*[1] Briefly, uniform, single crystalline spherical gold nanoparticles were synthesized *via* an iterative chemical refinement process. These nanoparticles were subsequently used as "seeds" to template the growth of cubes. Importantly, this synthetic procedure results in structurally uniform anisotropic nanoparticles produced with high yield. Figure S1 shows two representative Z-contrast scanning transmission electron microscope (STEM) images of cubes used in this work. The large cubes have an edge length of 86 ± 3 nm while the small cubes have an edge length of 63 ± 2 nm.

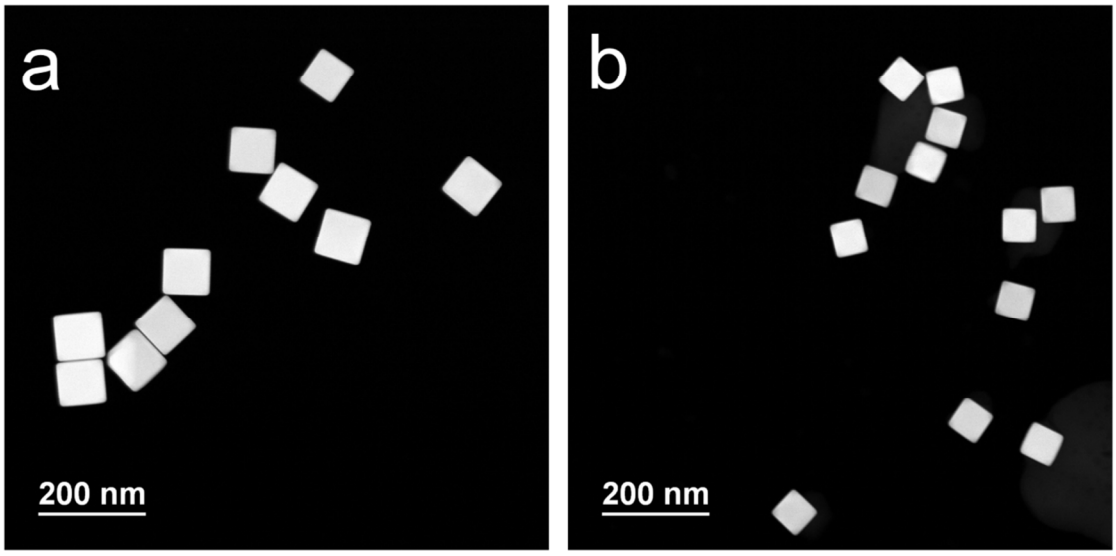

Figure S1. STEM images of gold nanocubes with an edge length of (a) 86 ± 3 nm (b) 63 ± 2 nm.

Substrate Fabrication

PMMA pores were fabricated on Au-coated Si substrates with electron beam lithography (EBL). First, a Si wafer was cleaned *via* an O_2 plasma at 50 W for 5 min. Following plasma cleaning, electron beam evaporation (Kurt J. Lesker Company) was used to deposit a 5 nm Cr adhesion layer, followed by a 100 nm Au layer onto the Si substrate. The wafer was then cut into smaller pieces (1.5 × 1.5 cm^2) and stored in vacuum desiccator. Substrates were then spin-coated with positive e-beam resist (poly(methyl methacrylate), PMMA, 495 A2, MicroChem) at 1000 rpm, resulting in an ~100 nm thick PMMA layer. The PMMA was baked at 200 ℃ for 60 s, followed by EBL using a FEI Quanta ESEM to define the sizes and positions of pores. We used an accelerating voltage of 30 kV with a dosage of 200-250 μC/cm^2 to pattern pores with 90 –

260 nm diameters. The substrates were developed in a methyl isobutyl ketone (MIBK) / isopropyl alcohol (IPA) 1:3 solution for 60 s, rinsed with IPA, and blown dry with N_2. After development, each substrate was cleaned *via* an O_2 plasma at 50 W for 1 min to remove potential PMMA residue at the bottom of pores, and cut into four smaller pieces to fit in 2 mL Eppendorf tubes, which was necessary for functionalization of the surface with DNA.

DNA Synthesis and Purification

All oligonucleotides used in this work were synthesized on a BioAutomation MerMade 48 (MM48) automated oligonucleotide synthesizer using reagents purchased from Glen Research. After synthesis, all DNA was purified with reverse-phase high-performance liquid chromatography (HPLC, Agilent) followed by standard deprotection procedures. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was used to characterize purified oligonucleotides and confirm their molecular weight.

DNA Functionalization

The DNA sequences used in this work are shown in Table S1. In brief, we functionalized substrates and particles with orthogonal 3' thiolated DNA sequences, denoted as X and Y, respectively. Then, complementary linker DNA strands were hybridized to both the particles and substrate to provide rigidity to the DNA shell. Each linker strand possessed a short 5-base terminus designed to link the particles to the substrates through complementary DNA hybridization events. Importantly, the total number of nucleobases within each DNA sequence allows one to control the average distance between each layer with a resolution of 2.8 Å when in aqueous solution.[2]

Table S1. DNA sequences for cube assembly

Description	DNA Sequence (5′ to 3′)
Particle 'Y' anchor DNA	TCA ACT ATT CCT ACC TAC AAA AAA AAA A SH
Substrate 'X' anchor DNA	TCC ACT CAT ACT CAG CAA AAA AAA AAA A SH
Particle 'Y' linker	GTA GGT AGG AAT AGT TGA ATC TCT
Substrate 'X' linker	TTG CTG AGT ATG AGT GGA AAG AGA

Cubes were functionalized as described by Jones *et al.*[3] and O'Brien *et al.*[2] In brief, 3' alkylthiol-modified oligonucleotides were first treated with a 100 mM solution of dithiothreitol (DTT) in 170 mM sodium phosphate buffer (pH = 7.4), followed by purification on a Nap-5 size exclusion column (GE Life Sciences) to remove DTT. During this time, 1 mL aliquots of the cube solutions were centrifuged for 8 min at 6,000 - 10,000 rpm depending on the size of cubes, the supernatant was removed, and the

nanoparticles were resuspended in water. The cubes were then centrifuged a second time, the supernatant was removed, and then the purified DNA and water were immediately added to the pellet. Specifically, 5 μmol of thiolated DNA was added per 1 mL of original particle solution. The particle solution was then brought to 0.01 M sodium phosphate buffer (pH = 7.4) and 0.01 wt. % sodium dodecyl sulfate (SDS) in water. Stepwise addition of 2 M NaCl was carried out every half hour, such that the NaCl concentration was stepped through 0.05 M, 0.1 M, 0.2 M, 0.3 M, 0.4 M, and finally arrived at 0.5 M. Following this process, the nanoparticles were placed on a shaker at 1,000 rpm and left overnight to ensure a dense loading of oligonucleotides. After functionalization, the nanoparticle solutions were centrifuged three times to remove excess DNA. After each of the first two rounds of centrifugation, nanoparticles were resuspended in 0.01 wt. % SDS, and after the last centrifugation step, the particles were resuspended in 0.5 M NaCl, 0.01 M sodium phosphate buffer (pH = 7.4), and 0.01 wt. % SDS solution.

For the functionalization of substrate with DNA, the procedure was similar as described above. Specifically, 1 μmol of DTT-cleaved DNA in water was added to each substrate. However, instead of stepwise addition of NaCl, the substrates were brought to 1 M NaCl in one addition, and then shaken for 1 h at 1,000 rpm. Substrates were then rinsed three times with water and placed in a 0.5 M NaCl, 0.01 M sodium phosphate buffer (pH = 7.4), and 0.01 wt. % SDS solution.

After functionalization of thiolated DNA strands, linker strands were hybridized to both the substrates and particles. To determine the appropriate number of linkers, the concentration of the cube solution was measured using UV-Vis.[1] Subsequently, 10,000 strands of linkers were added per cube. Substrates were incubated in a solution containing 0.5 μM linker. Both the substrates and the cube solutions were then heated to 55 ºC for 30 min, and then allowed to slowly cool to room temperature to ensure full hybridization between anchor and linker DNA sequences.

Nanocube Array Assembly

Following linker hybridization, the substrates were rinsed in 0.5 M NaCl, 0.01 M sodium phosphate buffer, and 0.01 wt. % SDS solution three times, while particles were used without further processing. For the assembly of two sizes of cubes, substrates were first placed in a large cube solution and shaken at 1,000 rpm for 1 h at 42 ºC. During this time, large cubes assembled preferentially into the wide pores and left the narrow pores empty. After assembly, the substrates were rigorously rinsed three times in 0.5 M NaCl, 0.01 M sodium phosphate buffer, and 0.01 wt. % SDS solutions to remove unbounded particles. Next, the same substrate was placed in a small cube solution and shaken at 1,000 rpm for 1 h at 42 ºC to fill the narrow pores with small cubes, and then rinsed with the same buffer three times. Then the substrates were immersed in 80% IPA in water (by volume) with 0.2 M ammonium acetate (AA) at 45 ºC for 30 min to fully remove the PMMA. After PMMA removal, the substrates were transferred back into 0.5 M NaCl,

0.01 M sodium phosphate buffer, and 0.01 wt. % SDS solutions for optical characterizations. After optical characterization, the substrates were rinsed three times with a solution of 80% IPA and 0.2 M AA, then blown dried with N_2. Figure S2 shows SEM images of large-scale cube arrays. Note that in addition to removing the PMMA, the alcohol reduces the solvent polarity around the DNA-assembled nanoparticle architectures, which condenses that DNA bonds between nanoparticles and allows structures to be transferred intact from the solution phase to the solid state.[4]

Figure S3 shows a pattern that encoded two images by arranging four types of pixels on a gold surface. Figure S4 - S6 show three additional examples of assembly of two sizes of cubes on the same substrate using different sized nanocubes. Additionally, this surface encoding method can be used to incorporate even more complex information into the metasurface. Figure S7 shows a metasurface design that combines two different sets of QR code into one pattern. Each QR code is 25 by 25 pixels, and we designed each pixel to have 10 by 10 nanoparticle arrays with a periodicity of 300 nm, therefore the pixel size is 3 μm. Figure S7a represents the design of multiplexed pattern for surface encoding. The red pattern is encoded with a website Uniform Resource Locator (URL) of the homepage of the Department of Materials Science and Engineering (MSE), while the green pattern is encoded with a website URL of the Department of Electrical Engineering & Computer Science (EECS). Each pixel in the red pattern consist of arrays of large pores for large cube assembly, while each pixel in the green pattern consists of arrays of small pores for small cube assembly. By overlapping these two patterns with each other, designed a metasurface that is capable of multiplexed encoding. When we read out the location of large cubes, we are able to reconstruct a QR code to reach the website of MSE, while reconstructing the location of small leads us to the EECS website. Figure S7b shows an optical image of the constructed metasurface, while Figure S7c shows an SEM image of the metasurface, showing the successful realization of such metasurface.

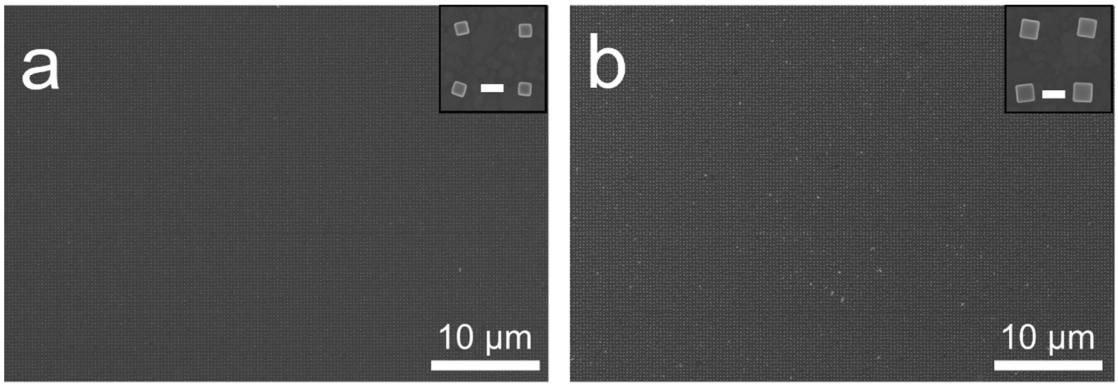

Figure S2. Large scale SEM images of assembled gold cube arrays with 300 nm periodicity: (a) cubes with 63 ± 2 nm edge length; (b) cubes with 86 ± 3 nm edge length. Inset scale bars: 100 nm.

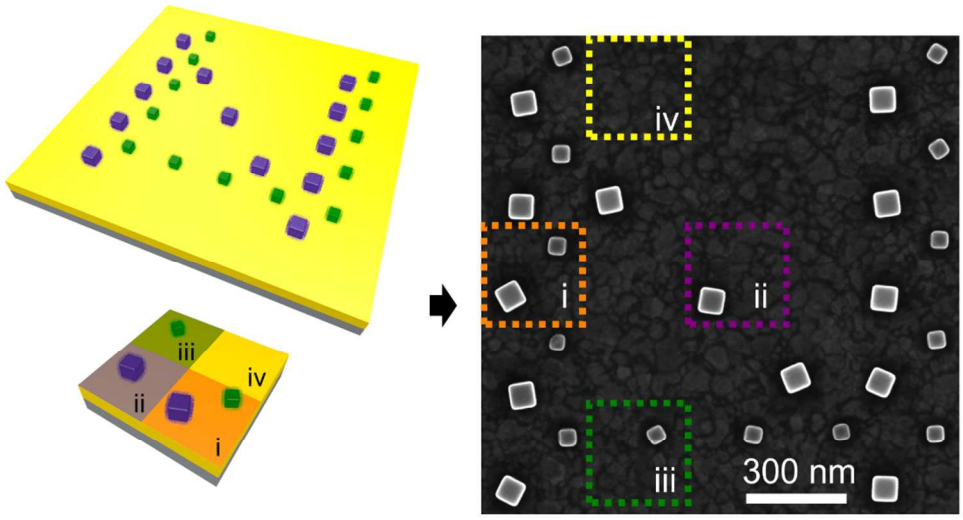

Figure S3. Experimental realization of four types of encoding pixels. These pixels were used to encode two sets of patterns on substrates with single particle resolution.

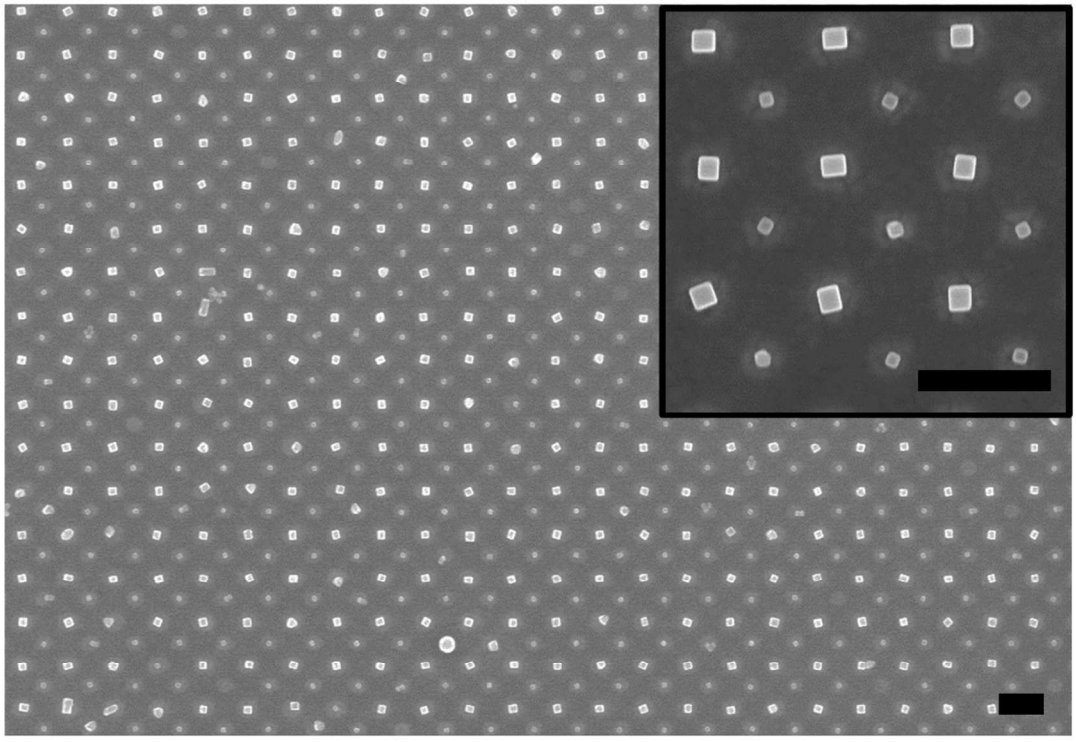

Figure S4. Assembly of cubes with 86 and 46 nm edge lengths. The yield for the 86 nm cubes is 97%, while the yield for the 46 nm cubes is 92 %. Scale bars: 500 nm.

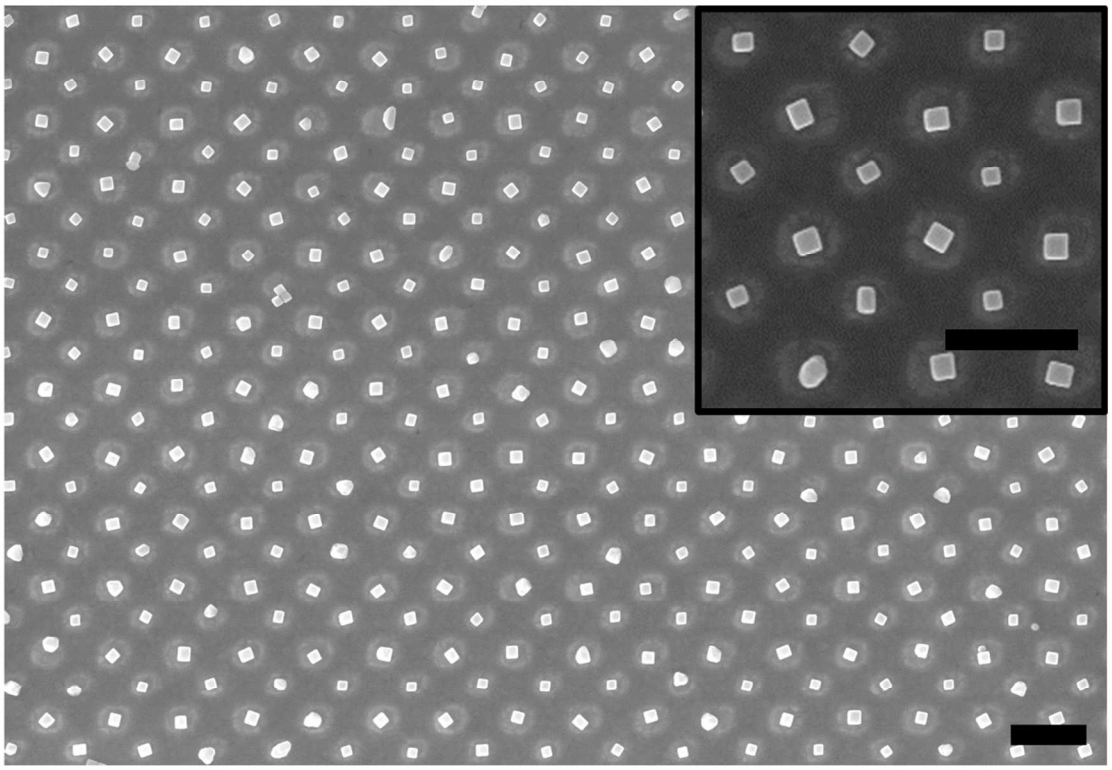

Figure S5. Assembly of cubes with 86 and 63 nm edge lengths. The yield for the 86 nm cubes is 95%, while the yield for the 63 nm cubes is 90 %. Scale bars: 500 nm.

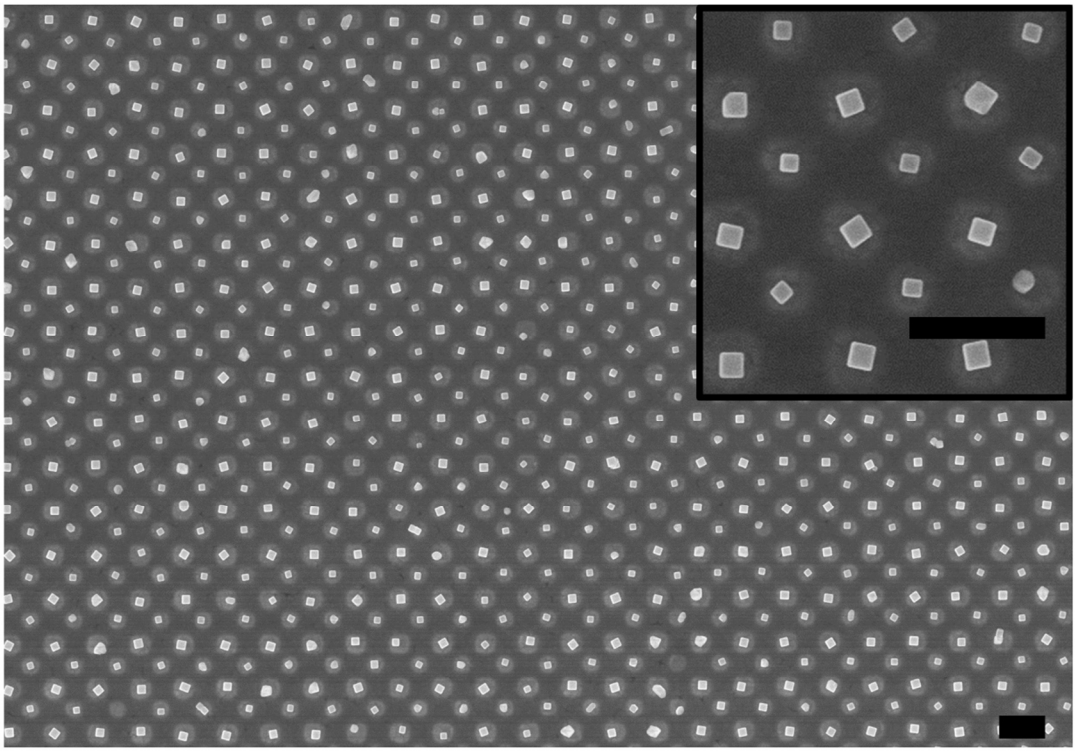

Figure S6. Assembly of cubes with 94 and 46 nm edge lengths. The yield for the 94 nm cubes is 87%, while the yield for the 46 nm cubes is 96 %. Scale bars: 500 nm.

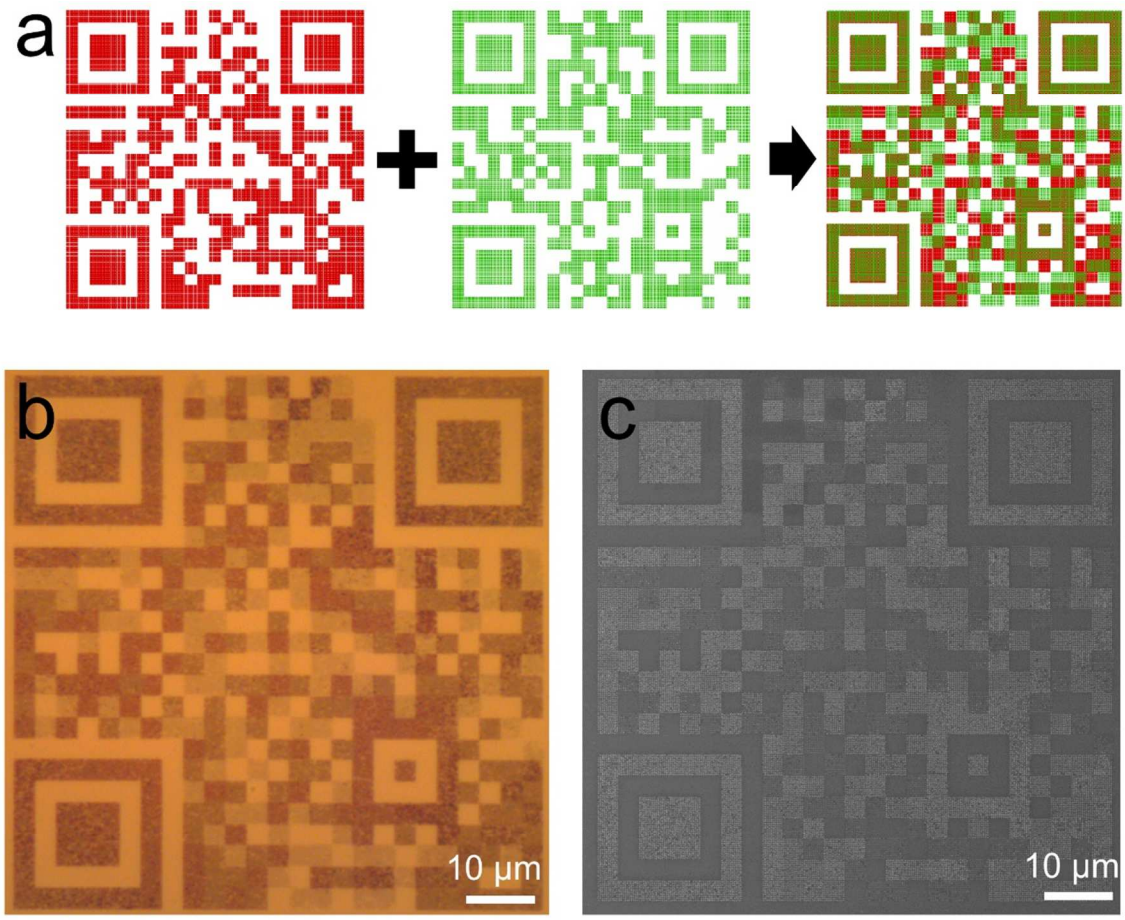

Figure S7. The design of a metasurface with a multiplexed QR code. (a) The design of the pattern for the metasurface. The red pattern represents the URL of the MSE homepage, while the green pattern represents that for the EECS homepage. The pixels where both colors overlap will have two sizes of pores within one pixel. (b) An optical image of the metasurface. The intensity difference from the optical image where cubes with different sizes are located is clearly visible. (c) An SEM image of the same sample. Using SEM, we can confirm the location of each individual particle.

Optical Measurements

Single reflection spectra and mapping was acquired using a Nikon inverted microscope coupled into a 303-mm-focal-length spectrometer containing an Andor Newton electron multiplication charge-coupled device (EM-CCD) detector. A broadband Halogen light was used as the source. For mapping, the x-y piezo stage was computer controlled and the step size was set to 500 nm over a 4 μm x 4 μm region. In the final measurements, all data was taken from an area equivalent to ~479 x 479 nm region using both pixel binning and a computer controlled physical slit before the spectrometer.

FDTD Simulations

2D-electromagnetic wave numerical calculations were performed using the finite-difference time-domain simulation software package Lumerical™. The simulation region was 300 x 300 x 2000 nm in XYZ, respectively, using perfectly matched layers (PML) and anti-symmetric boundary conditions perpendicular to the electric field polarization. A mesh override was set over the Au cubes to 1 nm x 1 nm x 1 nm with a 10 nm buffer representing the gap distance. Refractive indices were taken from literature for Si[5] and Au[6] while the DNA and background indices were set to a constant 1.35.

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