Supplementary materials

Materials and Methods

Materials

SU8 2015, SU8 2050, and SU8 developer were purchased from Microchem. Perfluorooctyltrichlorosilane (PDMS), Tetraethoxyl silane (TEOS), Bovin serum albumin (BSA), (3-Aminopropyl)triethoxysilane (APTES) > 98%, Succinic anhydride (SA) > 99%, Polyurethane (PUA), 3-(Trimethoxysilyl)propyl methacrylate (TMSPA), Trimethylolpropane ethoxylate triacrylate (ETPTA), 2-Hydroxy-2-methylpropiophenone (Darocur), Triethylamine (TEA) > 99.5%, and Isopropyl alcohol (IPA), were purchased from Sigma Aldrich. Other chemicals and solvents including N-Hydroxysulfosuccinimide Sodium (NHS) salt 97%, Absolute ethanol, NN-Dimethylformamide (DMF), and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) 98+% were purchased from Alfa Aesar. Capture antibodies, Biotin-labelled polyclonal antibodies, and Antigens were purchased from either Millipore, eBioscience, or BD Bioscience. Streptavidin-linked phycoerythrin (SA-PE) was purchased from ProZyme. [2-(N-morpholino)ethanesulfonic acid] (MES) buffered saline pack was purchased from Thermo Scientific. Chemicals sensitive to moisture, such as NHS and EDC, was stored in desiccators. Other reagents were stored according to the manufacturer's recommendation.

Chip fabrication

For the PDMS chips, we designed FCG(Film Combine Glass) type film masks or chrome masks with Autocad and manufactured by Microtech. On 4 inch silicon wafers, for various SU8 pattern heights, we performed spin coating, soft baking, UV exposure, post-exposure baking was done as the manufacturer's guide. After developing, hard baking was done at 150°C for 10minutes. To reduce the surface energy, we coated the SU8 patterned wafer with perfluorooctyltrichlorosilane by dispensing few microliters of Perfluorooctyltrichlorosilane on slide glasses heated to 85°C and placing the wafer over the slideglass for 1 minute. PDMS (Sylgard 184, DowCorning) was mixed with curing agent at 10 : 1 w/w and poured into the SU-8 mold. After baking at 110° C for 30 min,the PDMS pattern was peeled off and attached to a slide glass by O2 plasma treatment (CUTE-MP, FemtoScience).

For PUA chip fabrication, we spread liquid PUA on a glass mold with a ramp with varying angle then UV cured the liquid into a polymer matrix. Then we assembled these triangular PUA blocks on the SU8 pattern of the original particle packing channel. Using this modified 3-dimensional pattern, we fabricated a series of PDMS molds and a final PUA channel that has a channel inlet height gradient.

For PS chip, we outsourced the injection molding to Quantamatrix using our Autocad design for the optimized gradient channel design obtained from PUA chip experiments.

Particle fabrication

We used the previously reported protocol[1]. Briefly, ETPTA was mixed with 10% w/w TMSPA and Darocur was added at 10% w/w of the ETPTA/TMSPA mixture. the ETPTA/TMSPA/Darocur mixture was poured on air plasma treated hydrophilic glass slides. Using masks for contact printing UV lithography using negative resist was performed. TEOS was used for silica coating. Amine groups were coupled to the particle surface using 0.095g/mL APTES solution (in absolute ethanol) and stirred at 25 °C for 2 hours. Bead were washed with absolute ethanol and dried at 110°C for 10 minutes. Terminal amine carboxylation was done by adding carboxylation solution (6mg SA, 8.4uL of 7.2M TEA in 1ml DMF) and stirring at 25°C for 2 hours. Beads were washed with DMF, absolute ethanol, and MES buffered saline. EDC/NHS-cross-linking solution (5mg NHS, 5mg EDC per 1ml MES) was then added and stirred at room temperature for 25minutes and washed with MES. Capture antibodies in MES (14ug/mL) was mixed with bead and stirred (1200rpm) at room temperature for 2 hours. After antibody cross-linking, the beads were blocked with BSA solution (1% BSA in PBS).

Immunoassay

Immunoassay protocol was adapted from a previous report.[2] Particles were incubated with antigens (diluted in 0.1% BSA in PBS) and stirred at 4°C for 2 hours. After washing with washing solution (0.1% BSA, 0.02% Tween 20 in PBS), particles were incubated with 2μg/ml biotin-labelled anti-antigen polyclonal antibodies prepared in dilution solution (PBS solution containing 0.1% BSA) at 650 rpm, 25 °C for 1 hour and were washed with washing solution. Finally, particles were incubated with SA-PE solution (1 μg/ml SA-PE prepared in dilution solution) for 30 min and were washed rigorously with washing solution before imaging.

Assay readout strategy

We imaged the particles with Olympus fluorescence microscope (IX81 microscope, U-RFL-T fluorescence source) and used bright field with 40× magnification (Olympus UPlanFL N 4x/0.13na Objective) when imaging the entire FOV to measure particle assembly efficiency. When performing fluorescence-based immunoassay readouts and microparticle barcode decoding, we used 200x magnification (Olympus UPlanFL N 4x/0.13na Objective) with emission wavelength filtered at 575 nm–625 nm (U-MNIGA3 light filter) at exposure time of 0.1sec and/or 0.2sec. The immunoassay readouts from either the packing channel or 96wellplate wells were decoded using an in-built program from Quantamatrix Inc. From these decoded (i.e. code number and fluorescence intensity value) particles, we performed fluorescence intensity de-noising using the following three filtering strategies. We first decreased the mask size of each decoded particles from "100% of particle radius" to "40% of particle radius" so that only the fluorescence signals from the center 16% of the particle surface was used for measuring the intensity value. Second, we measured the average background (i.e. regions not occupied by particles) fluorescence intensity of the images and subtracted it from the particle's fluorescence intensity values. And Third, we heuristically removed low fluorescence intensity (<25 A.U.) particles that have more than one adjacent high fluorescence intensity (>100 A.U.) particles. This heuristic selection can in theory be automated by image processing algorithms.

For singleplex immunoassays (figure S4), standard curves were approximated to a 4 parameter logistic regression model using minipack.lm package in R. The LOD values were then calculated as the input antigen concentration that has fluorescence intensity equivalent to 2.5 times the average fluorescence intensity of blank particles (i.e. SNR=2.5).

COMSOL simulation

We used COMSOL Multiphysics 5.4 for our fluidic simulations. We used the 'particle tracing for fluid flow' module with no wall slip condition, extra-coarse meshing, and creeping normal inflow with velocity of 3.5mm/sec. Parameter sweeping was used for either i) sweeping the effects of the parameter (e.g. channel height or gradient angle variation) on particle movements or ii) determining the optimal diverging and converging channel design. We assumed solid-type particles with density value of 1200kg/m^3 and no surface charge. We released 5 particles per unit time (0.1sec) from the inlet wall for a total of 10 seconds (total 500 particles). We assumed steady state of the creeping flow that was solved before simulating particle tracing study. We placed a particle accumulator at designated channel walls; the vertical walls at the height-reducing region of 2 step channel, or the slanted top wall of the gradient channel (Fig S5c,d). These accumulators were placed to measure the particle-to-wall collision rate. Collison rate was measured as the number of particles contacting each mesh element per unit time (0.1sec). Notice that since one particle can contact multiple mesh element simultaneously, collision rate is not equivalent to 'the number of wall-colliding particles per unit time'.

Supplementary figures

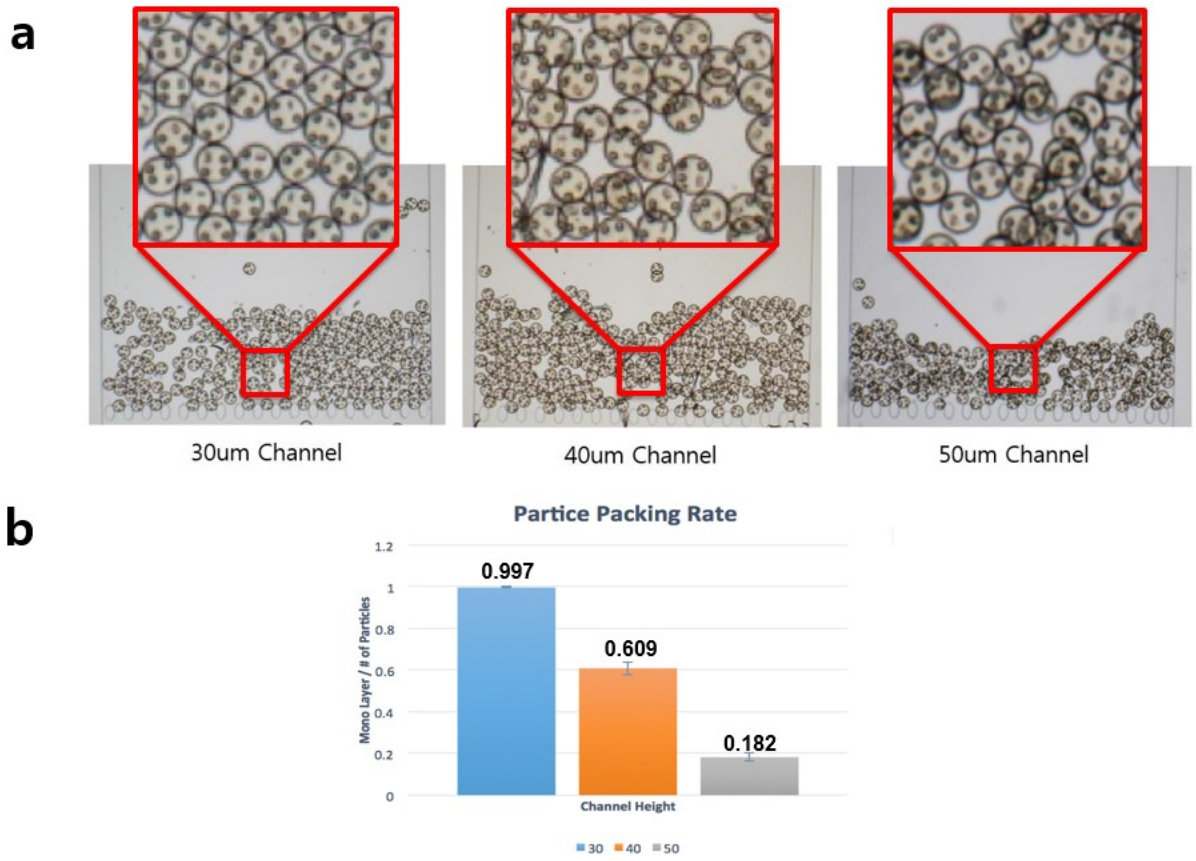


Figure S1. Channel height optimization of PDMS-based fluidic channel for monolayer spreading of microparticles. Particle packing PDMS channels with different channel heights (30μm, 40μm, 50μm) were injected with barcoded microparticles (diameter=100μm, thickness=~30μm) (a). Percentage of monolayer assembled microparticles among all microparticles were calculated (b).

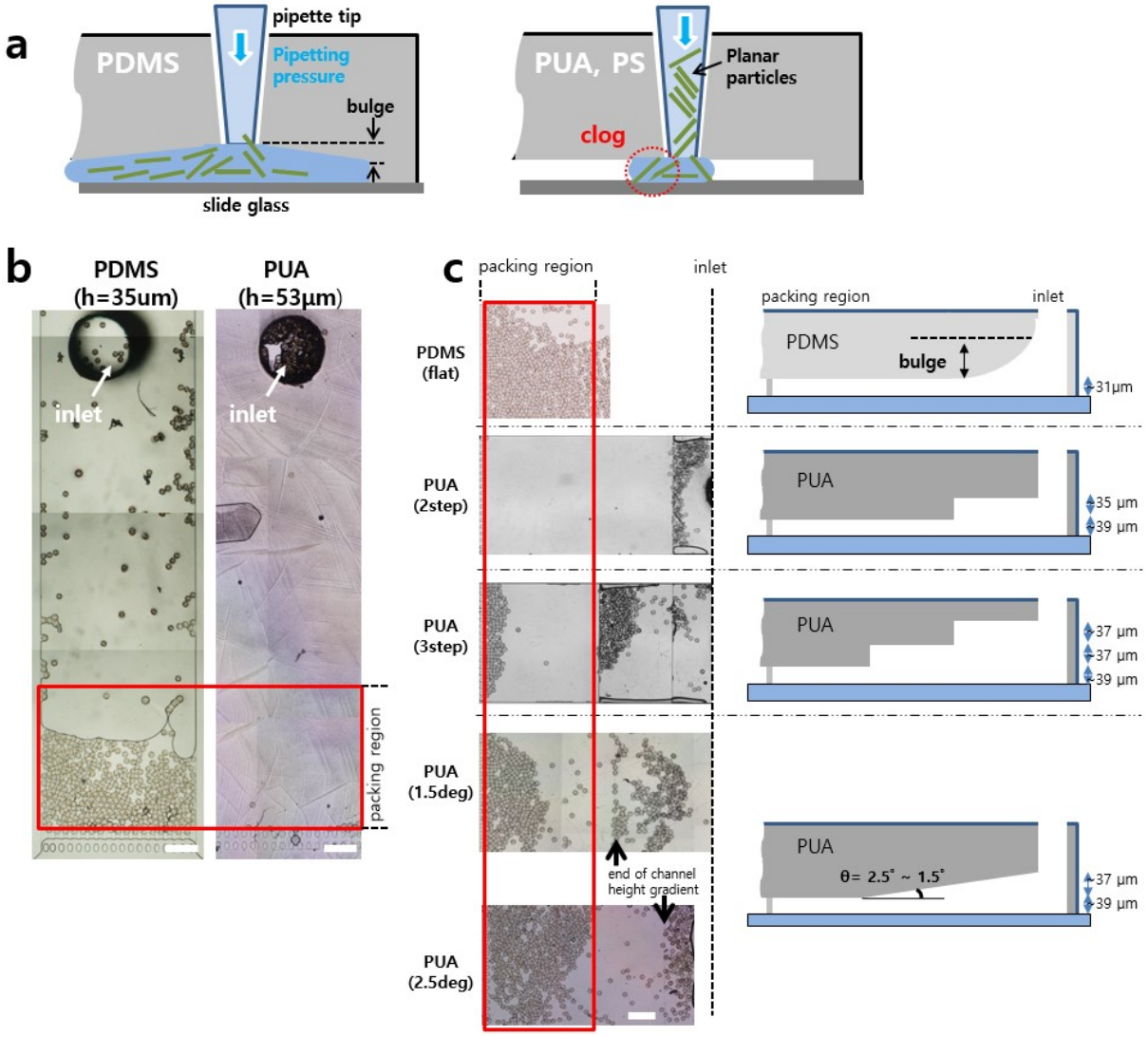


Figure S2. Design optimization of stiff PUA chip based particle packing channel. (a) Illustration of the effects of channel material stiffness on planar microparticle assembly by pipetting injection. (b) Comparing flat PDMS channel (channel height=35μm) with flat PUA channel (channel height=53μm) in particle injection performance. For the PUA channel case, most of the particles cannot enter the channel due to clogging at the inlet. (c) Particle packing trial results of different PUA-based particle packing channel designs compared to PDMS channel. Red squares indicate intended particle packing region. Scale bars are 600μm.

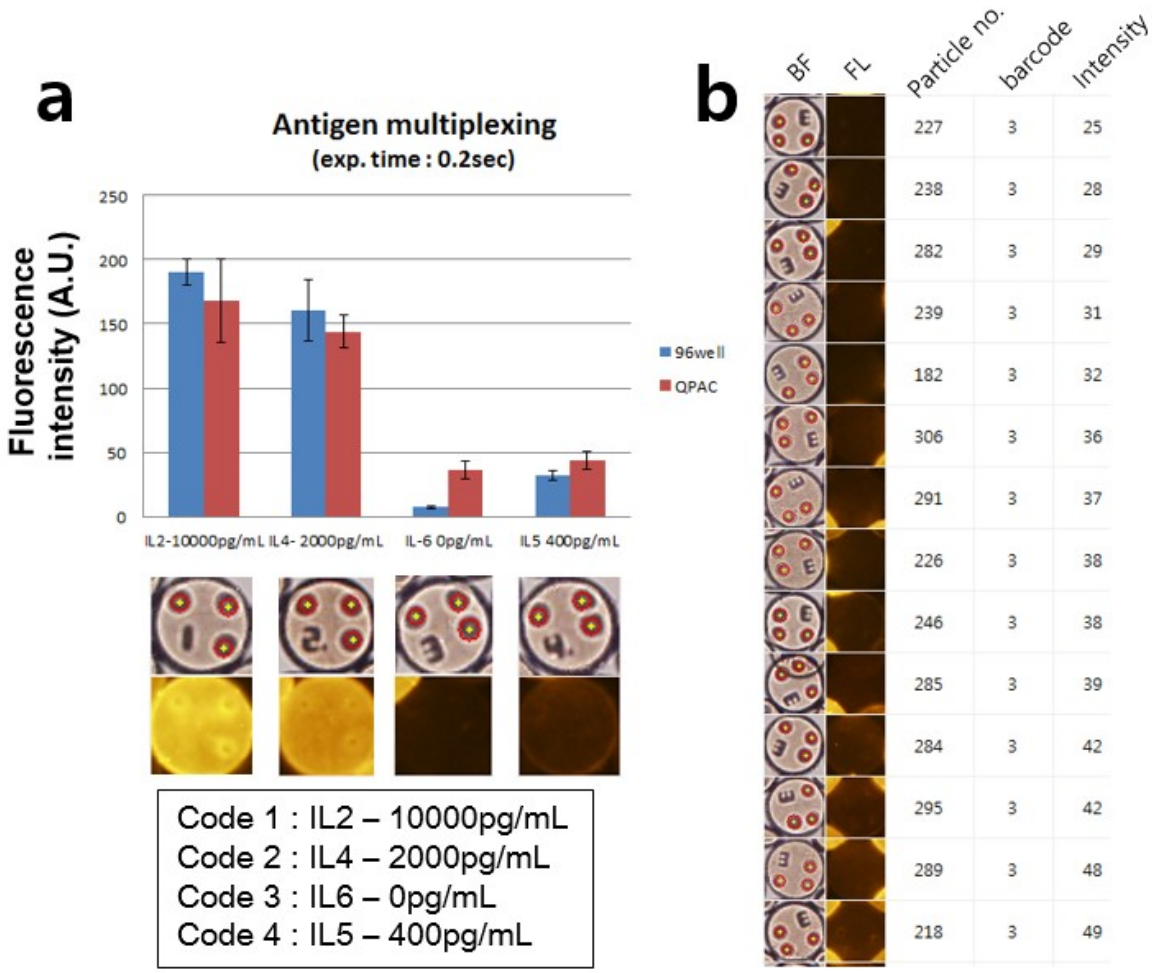
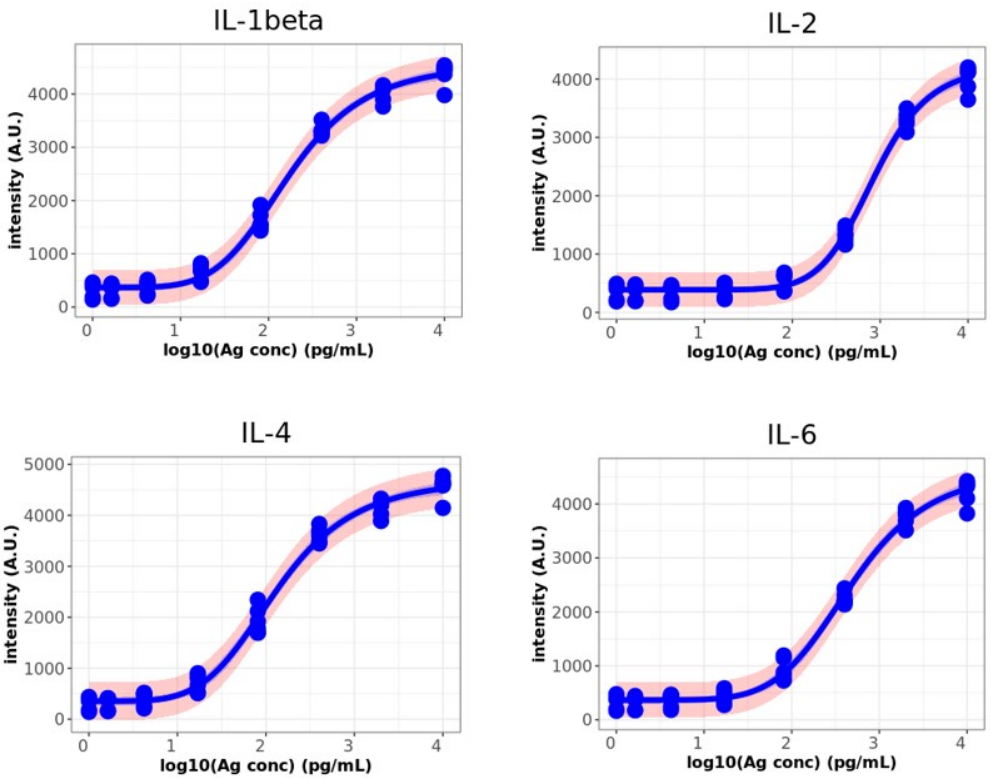


Figure S3. 4plex immunoassay result without signal filtering. (a) The bar graph shows 4plex immunoassay result comparison between 96well plate-based assay and particle packing chip based assay (QPAC) without any intensity filtering. The middle panel is a collection of representative example images for each code. The values inside the lower panel box indicates the target antigen and the input antigen concentration for each barcode. (b) Example microparticle images and intensity values of code 3. It shows that even among microparticles with same barcode (same input antigen concentration) the resulting particle intensity correlates with fluorescence signals coming from adjacent microparticles.

a



b

LOD	IL-1b	IL-2	IL-4	IL-6
pg/mL	28.37	238.83	21.16	75.41

Figure S4. Standard curves and limit of detection measurements of microparticle-based single-plex immunoassays. Assay results were obtained using 96well readout not our particle packing chip. (a) Standard curves of planar microparticle based immunoassays for various cytokines. The light red shade region indicates the prediction interval. The blue line indicates the fitted function obtained by 4 parameter logistic regression. Blue dots are the individual values of observation. Number of observations was N=6 for each condition. b) Calculated LOD values. LOD values were calculated based on signal to noise ratio of 2.5. The noise values we used were calculated as the mean intensity of corresponding blank particles (0 pg/mL of antigen input).

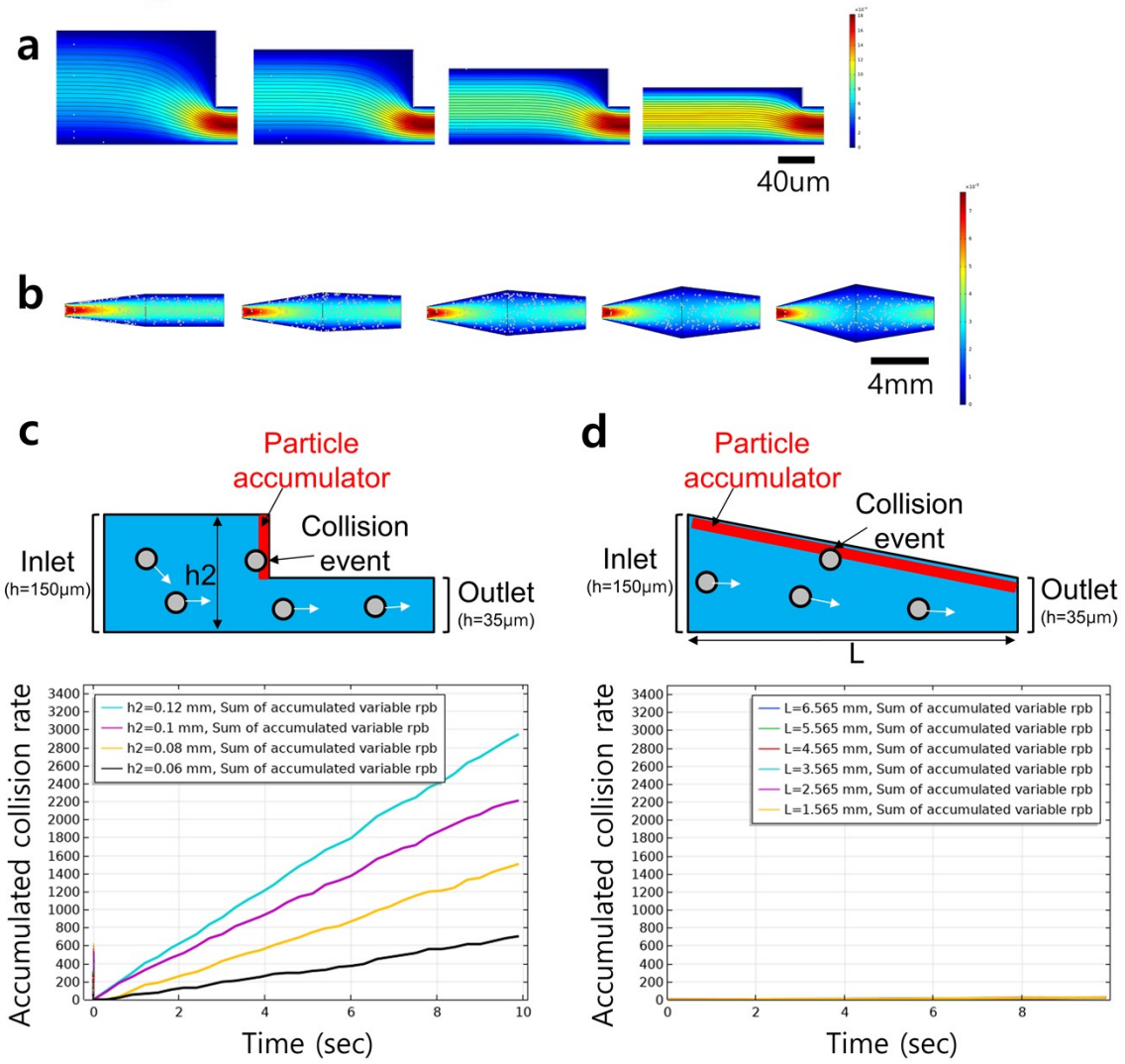


Figure S5. COMSOL simulation of particle tracing for optimization of gradient channel design. (a) side view of step channel height variation (0.06, 0.08, 0.1, 0.12mm) simulation. Color code indicates flow velocity. (b) Top view of diverging-converging channel width variation (maximum width=2~5mm) simulation. (c) Simulation result of accumulated collision rate of particles at channel walls in step channels. The four plots, from top to bottom, are from step channels with initial channel height (h2) of 0.12mm, 0.10mm, 0.08mm and 0.06mm, respectively. (d) Simulation result of accumulated collision rate of particles at channel walls in gradient channels with different channel height gradient angles of 0.5~3° which was controlled by changing the entire channel length L. For (c) and (d), COMSOL creeping flow and particle tracing module was used. To measure the particles colliding with the channel wall of interest, we placed particle accumulators and used sticky wall condition. This means that among the particles moving along the fluidic flow, those that contact the accumulator become instantly static and are counted as collision event. These accumulators were placed to measure the particle-to-wall collision rate. Collison rate (indicated in the figure as 'rpb') was measured as the number of particles contacting each mesh element per unit time (0.1sec). Accumulated collision rate is simply the time integral of collision rates. Notice that since one particle can contact multiple mesh element simultaneously, collision rate is not equivalent to 'the number of wall-colliding particles per unit time'.

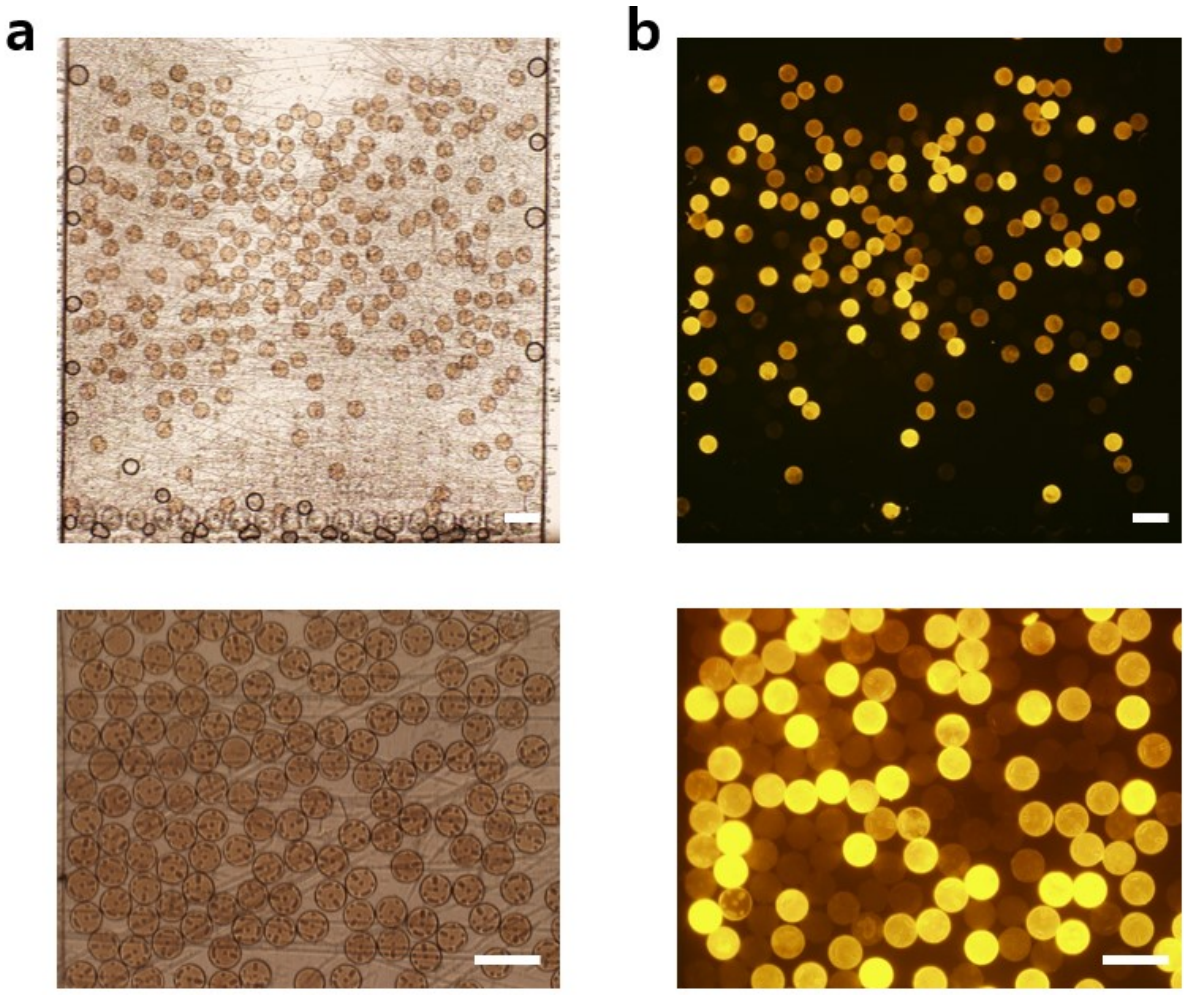


Figure S6. Example bright field (a) and fluorescence (b) images obtained from PS chips. Upper panel images were taken with 40x magnification and the lower panel images were taken with 200x magnification. As seen in the bright field images, the channel surface has multiple scratch-like patterns that could potentially prohibit particle decoding performance. Scale bars are 200μm.

Supplementary references

1 L. N. Kim, M. Kim, K. Jung, H. J. Bae, J. Jang, Y. Jung, J. Kim and S. Kwon, *Chemical Communications*, 2015, **51**, 12130–12133.

2 J. Kim, S. Bae, S. Song, K. Chung and S. Kwon, *Biomicrofluidics*, 2015, **9**, 044109.