Supplementary Material (ESI) for Lab on a Chip

Supporting Information

Enhanced separation of colloidal particles in AsPFF device with tilted sidewall and vertical focusing channels (t-AsPFF-v)

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Fabrication of AsPFF device with tilted wall and vertical focusing channels

For the preparation of tilted wall microfluidic devices, inclined photolithography technique was used to make master mold with inclined structure. Fig. S1(A) shows the schematic diagram of inclined photolithography procedure using general UV mask aligner (MDA-400M, MIDAS SYSTEM, Korea). The fabrication process is as follows. Coverslip (No. 1, #2935-246, Corning) was cleaned by using cleaning solution (5%, TICKOPUR R 36, DR. H. STAMM GmbH) and ultrasonic bath for 10 min. Cleaned coverslip was rinsed with DI water, and then it was heated up to 250°C on hotplate for 10 min for dehydration. SU-8 50 (MicroChem Corp., Newton, MA, USA) was spin coated on the coverslip with thickness of about 13 μm. The spin coating was performed at a rate of 1500 rpm for 30 sec and ramped up to 5000 rpm for 80 sec. The photoresist was soft baked on a 65 °C hot plate for 12 min and on a 95 °C it for 20 min. A piece of film combine glass photomask (FGC mask, Microtech, South Korea) was covered on the opposite side of resist coated coverslip for inclined back-side exposure. Back-side exposure was beneficial to avoid the UV reflection on the glass substrate, and the fabrication of more slanted structure, the edge of the structure between cured SU-8 and atmosphere was more oblique by UV refraction at the interface of SU-8 and air.[1] To adapt vertical focusing channels, the angle of particle aligned sidewall has to be below 90°. However it was difficult to reduce the angle of the fabricated structures on far side from the UV source because of reflection on the substrate.[2] Therefore we used backside exposure method and transparent glass coverslip substrate to make a master mold of slanted structure. Two or three drops of DI water or glycerol were used to remove the air gap between coverslip and the photomask.[3, 4] The backside of photoresist coated coverslip was exposed to UV with the i-line (365 nm) through a FGC mask for 15 sec, and baked on a hotplate at the temperature of 65 °C for 5 min and 100 °C for 13 min. The photoresist was developed in proplylene glycol methyl ether acetate (PGMEA, 6659-4400, Daejung Chemicals & Metals, Korea) for about 1-2 min at room temperature with mild agitation and rinsed with isopropyl alcohol (5035-4410, Daejung Chemicals & Metals, Korea). The angle of the master mold was found as ~50°.

The micro-channel patterned PDMS layer for microfluidic devices was fabricated by general soft lithography method. Sylgard 184 PDMS polymer and curing agent (Dow corning, Midland, MI, USA) was mixed in 10:1 weight ratio then cast on a fabricated inclined structure master mold. The PDMS was degassed in a vacuum chamber for 20 min before curing in a 60°C oven for 2 h. The cured PDMS layer was cut by a razor blade, peeled off from the master mold, and then holes were punched out for inlets and outlets. The dimension of the pinched segment was about 15 μm in width, 100 μm in length, and 13 μm in height.

Using general photolithography and soft lithography method, the PDMS based vertical focusing channel layer was fabricated. The fabricated channel width was 7 μm and the height was about 0.7 μm. The fabricated vertical focusing channel layer and inclined side wall AsPFF layer was treated with oxygen plasma (100 W, 0.2–1 mbar, 75 sec.; CUTE, Femto Science, Korea) and bonded together irreversibly.

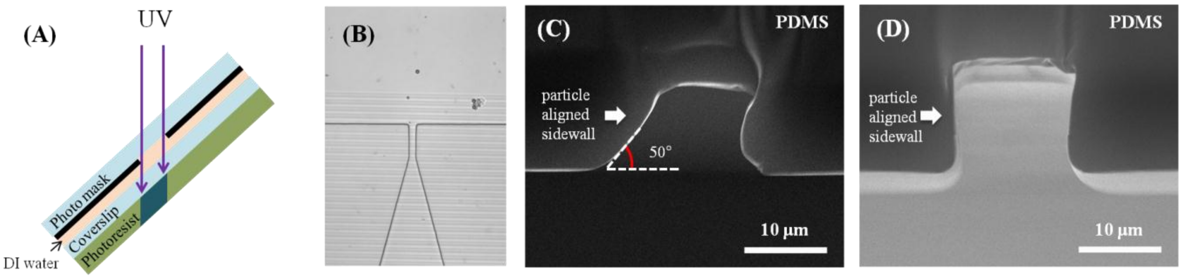


Figure S1. (A) Schematic illustration of fabrication of inclined wall master mold using back-side exposure method. (B) Optical microscopic image of tilted wall AsPFF device with vertical focusing channels. (C) Cross sectional SEM image of pinched segment in t-AsPFF and (D) n-AsPFF.

Separation of PS particles using tilted AsPFF device

Suspensions of polystyrene (PS) (17134, 17141, 18133, Polysciences, Inc., USA) with diameters of 3, 6, and 10 µm were purchased and diluted with DI water to 0.075, 0.5, and 1.5 % (v/v). Glycerol (20 wt%) and SDS (0.1 wt%) was added to prevent agglomeration or adhesion of PS particles. Diluted PS suspension and buffer solution without PS beads was injected into inlet 1 and inlet 2 using syringe pumps (KDS 210 and KDS 200, KD Scientific. New Hope, PA, USA). Flow rate for inlet 1 and inlet 2 were kept as 60 µL/h, and 1000 µL/h.

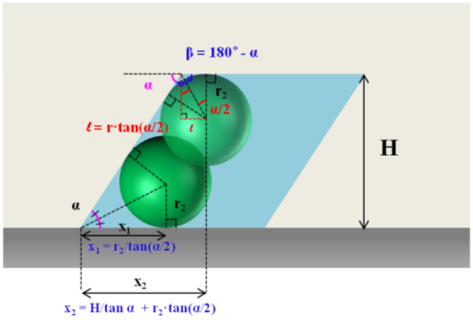
Image analysis for acquisition of particle position

In a broadened segment of the AsPFF device, the effluent PS particles were monitored by acquiring time-lapse images with inverted type microscope (Olympus IX51, Japan) equipped with CCD camera (Retiga 2000RV, QImaging, Canada). Acquired images were then analyzed using ImageJ (1.44a, http://rsb.info.nih.gov/ij, NIH, USA) to obtain information on the postion and the size of individual particle . For automated analysis of hundreds images, we used macros function of imageJ involving 'Threshold' and 'Analyzing particles'. For the background subtraction and contrast enhancement by standard deviation projection of bright field image stack, each image of bundle (image stacks, acquired a few hundred images at a regular time interval of 0.5 s) was differentiated by average intensity image of Z projection. This method helps to identify non-fluorescent particle by increased contrast and reduced background uneveness. The used macro is as follow.

```
name="type the file name";    //input(change) the file name in script
n=###;    // input(change) the number of images of stack in script
run("8-bit");
makeRectangle(0, 0, 1340, 1200);    //select a region for analysis
run("Crop");
run("Paste Control...");
setPasteMode("Difference");
selectWindow(name+".tif");
run("Z Project...", "start=1 stop=n projection=[Average Intensity]");
selectWindow("AVG_" +name+ ".tif");
run("Select All");
run("Copy");
selectWindow(name+".tif");
run("Select All");
for(i=0; i<n; i++)
{
run("Paste");
run("Next Slice [>]");
}    //background subtraction using "Average Intensity" of Z projection image
run("Threshold...");
setThreshold(30, 255);
waitForUser("Click OK to continue"); //wait for change proper value of threshold
run("Convert to Mask");
run("Fill Holes");
setAutoThreshold("Default");
run("Analyze Particles...", "size=4-2000 circularity=0.00-1.00 show=Nothing display exclude clear include record add stack");
String.copyResults(); //copy results to buffer memory
```

Calculation of particle distribution at pinched segment

Width of Gaussian distribution curve is estimated as 4 times the standard deviation of the curve in general chromatogram[4]. Thus the standard deviation is calculated from the maximum displacement of position in the inclined sidewall pinched segment (x_2-x_1). When the particles are aligned at the lowest left corner, the position is r/tan(α/2). While the particles are aligned at the highest left corner, the position is H/tan α + r·tan(α/2). Therefore difference of positions is re-arranged for (H-2r)/tan α by double angle formula, and the standard deviation is a quarter of it.



$$4 \cdot s_2 = x_2 - x_1$$

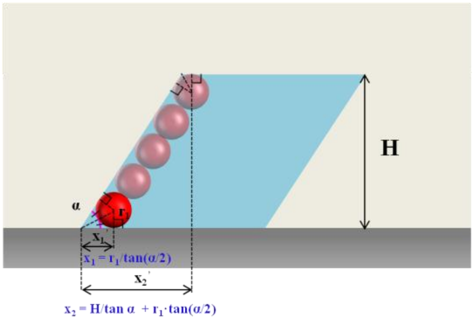
$$s_2 = \frac{1}{4}\left[\frac{H}{\tan\alpha} + r_2 \cdot \tan(\alpha/2) - \frac{r_2}{\tan(\alpha/2)}\right]$$

$$= \frac{1}{4}\left[\frac{H}{\tan\alpha} + \frac{r_2\{\tan^2(\alpha/2)-1\}}{\tan(\alpha/2)}\right]$$

$$= \frac{1}{4}\left[\frac{H}{\tan\alpha} - \frac{2r_2\{1-\tan^2(\alpha/2)\}}{2\tan(\alpha/2)}\right]$$

$$= \frac{1}{4}\left[\frac{H}{\tan\alpha} - \frac{2r_2}{\tan\alpha}\right]$$

$$= \frac{1}{4}\left[\frac{H-2r_2}{\tan\alpha}\right]$$



$$4 \cdot s_1 = x_2' - x_1'$$

$$s_1 = \frac{1}{4}\left[\frac{H-2r_1}{\tan\alpha}\right]$$

$$\text{if } r_2 > r_1 \rightarrow s_1 > s_2$$

Figure S2. Calculation of standard deviation of the particle distribution.

Separated particle position and effluent distribution

Table S1. Effluent particle peak position (X) and standard deviation of effluent particle distribution (s) at broadened segment.

		Sample		
		3 μm PS	6 μm PS	10 μm PS
n-AsPFF	X	64.7	137.8	186.1
	s	32.1	17.5	10.0
t-AsPFF	X	116.1	233.4	314.2
	s	52.6	24.1	33.1
t-AsPFF-v	X	88.8	160.7	381.0
	s	41.1	5.7	4.9

Table S2. Separation distance (ΔX) and separation resolution (R) at broadened segment.

		Sample		
		3 & 6 μm PS	6 & 10 μm PS	3 & 10 μm PS
n-AsPFF	ΔX	73.1	48.3	121.4
	R	0.7	0.9	1.4
t-AsPFF	ΔX	117.3	80.8	198.1
	R	0.8	0.7	1.2
t-AsPFF-v	ΔX	71.9	220.3	292.2
	R	0.8	10.4	3.2

Supplementary References

1 H. Sato, S. Ito, K. Tajima, N. Orimoto and S. Shoji, *Sensors and Acuators A*, 2005, **119**, 365-371.
2 M. Han, W. Lee, S.-K. Lee and S. S. Lee, *Sensors and Acuators A*, 2004, **111**, 14-20.
3 H. Sato, D. Yagyu, S. Ito and S. Shoji, *Sensors and Acuators A*, 2006, **128**, 183-190.
4 K.-Y. Hung, H.-T. Hu and F.-G. Tseng, *J. Micromech. Microeng.*, 2004, **14**, 975-983.
5 D. C. Harris, Exploring Chemical Analysis, W. H. Freeman and Company, New York, 3rd edn., 2005, ch. 21, pp. 450.