Supplementary information

Inertial focusing with sub-micron resolution for separation of bacteria

Fabrication of high pressure tolerant chips

The chips were fabricated in silicon and were sealed with glass to allow visualization of the experiment. With small enough feature sizes, microchannels fabricated in these materials may stand hundreds of bars. This makes them well suited for our application, which according to our previous experience should require less than 200 bar.
Glass capillaries (Genetec; 150 and 75 µm, outer and inner diameter respectively) were glued with epoxy (Araldite) to the inlet and outlets to enable the connection to an HPLC pump (Waters, model 515).
A silicon wafer was dry etched into two depths, one for the inlets and outlets (~180 µm) and another for the functional channel (7.3 or 10 µm). In order to achieve this, two masks were used. They were both transferred initially to the wafer so that no photolithography step was needed after the first etch. First, 300 nm of oxide were grown in a 500 µm thick silicon wafer. 1 µm of photoresist was spin coated and the first mask (the one with the functional part) was transferred with UV lithography. The oxide was wet etched in buffered HF. The photoresist was removed with acetone and another 3 µm were spin coated. The second mask (with the inlets and outlets) was transferred in the same way. The photoresist served as the first mask for the deep dry etch. When 170 µm were reached, the photoresist was removed in acetone, leaving the oxide mask exposed and 7.3 or 10 µm were dry etched again. The oxide was removed in diluted HF. Then the Si wafer and a blank glass wafer (1.1 mm thick) were immersed in piranha solution for 15 min to clean and activate the surfaces. They were brought in contact and placed in an anodic bonder (380 °C and 1200 V) for 3 h. At this point, the two wafers were covalently bonded with high quality and the chips were diced with a diamond saw. (Only 0.8 mm of the glass was sawed. Then the bonded wafers were bent in order to crack them and separate the chips. This prevented the cooling water of the dicer from entering the channels and fill them with debris generated in the process). Finally, glass capillaries were inserted manually and glued with epoxy.

Strains

1) *Escherichia coli:* Eco galK::cat-J23101-dTomato (strain DA32838).
2) *Salmonella typhimurium:* Sty LT2 galK::cat-CP25-SYFP2_opt / pSIM5-tet (strain DA28403)
3) *Klebsiellla pneumoniae:* Kpn CAS55 galK::mScarlet (strain DA57581)

Tables

Table S1 Evaluation of the alignment of 1 and 2 µm particles in a microfluidic chip with 0.5 mm in *R* and cross section 10x20 µm (*HxW*). The outlets were collected in eppendorfs for several minutes while running the device at 70 bar (~100 µL/min). Outlet 1 is the closest to the inner wall.

		1 µm particles			2 µm particles		
	Volume collected (µL)	Particles/µl (Mean)	SD	% Total	Particles/µL (Mean)	SD	% Total
Outlet 1	123	19	3.1	0.8	150	24.3	98.1
Outlet 2	48	77	7.5	1.3	5	3.8	1.3
Outlet 3	62	4537	192.1	96.8	2	1.2	0.6
Outlet 4	61	47	6.6	1.0	0	0.0	0.0
Outlet 5	120	3	4.3	0.1	0	0.0	0.0

Table S2 Evaluation of the alignment of *E.coli* in a microfluidic chip with 0.5 mm in *R* and cross section 10x20 µm (*HxW*). The outlets were collected in eppendorfs for several minutes while running the device at 120 µL/min. Outlet 1 is the closest to the inner wall. *Sample from outlet 3 was diluted x1000 times before counting.

		Bacteria (*E. coli*)		
	Volume collected (µL)	Particles/µL (Mean)	SD	% Total
Outlet 1	115	498	120.5	0.1
Outlet 2	67	2334	209.7	0.2
Outlet 3	63	1137572*	47610.8	99.3
Outlet 4	58	3564	751.8	0.3
Outlet 5	111	1033	206.4	0.2

Table S3 Evaluation of the viability of the bacteria by culture on LB agar plates after passing through the microfluidic chips. The pressure was increased to 50, 100, 150 bar consecutively and decreased to 50 bar again. The samples were diluted a factor 10^5 and 100 µL of the dilution were seeded in LB plates. This was done in duplicates.

Escherichia coli	Colonies (Mean)	SD	Cells/mL (x10^7)
Control	57	12,7	5,7
50 bar	49	1,4	4,9
100 bar	46	2,8	4,6
150 bar	48	2,8	4,8
50 bar (After)	47	4,2	4,7

Salmonella typhimurium	Colonies (Mean)	SD	Cells/mL (x10^7)
Control	9	1,4	0,9
50 bar	13	1,4	1,3
100 bar	18	11,3	1,8
150 bar	20	2,8	2,0
50 bar (After)	9	1,4	0,9

Klebsiella pneumoniae	Colonies (Mean)	SD	Cells/mL (x10^7)
Control	50	16,3	5,0
50 bar	46	2,8	4,6
100 bar	37	7,1	3,7
150 bar	48	4,9	4,8
50 bar (After)	37	7,1	3,7

Figures

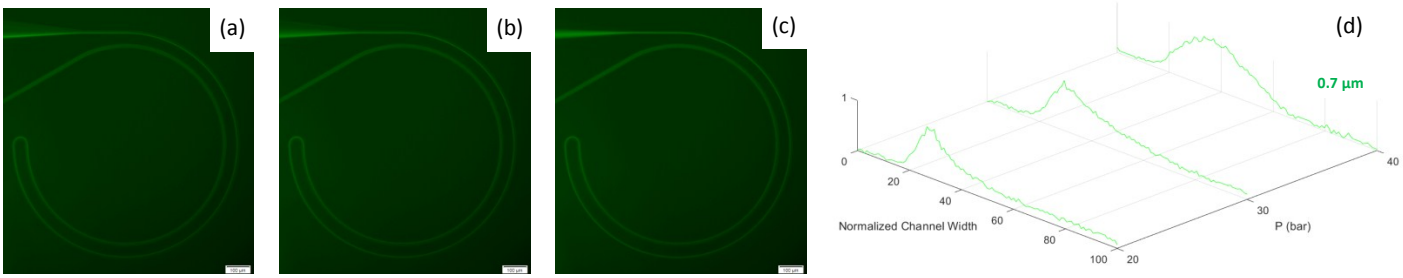

Figure S1 Performance with of 0.7 µm particles in a microfluidic chip with 0.5 mm in *R* and cross section 10x20 µm (*HxW*) at 20, 30 and 40 bar (30, 45 and 60 µL/min). (a-c) Raw images. (d) Intensity profiles. In the normalized channel width 0 and 100 represent the inner and outer wall respectively.

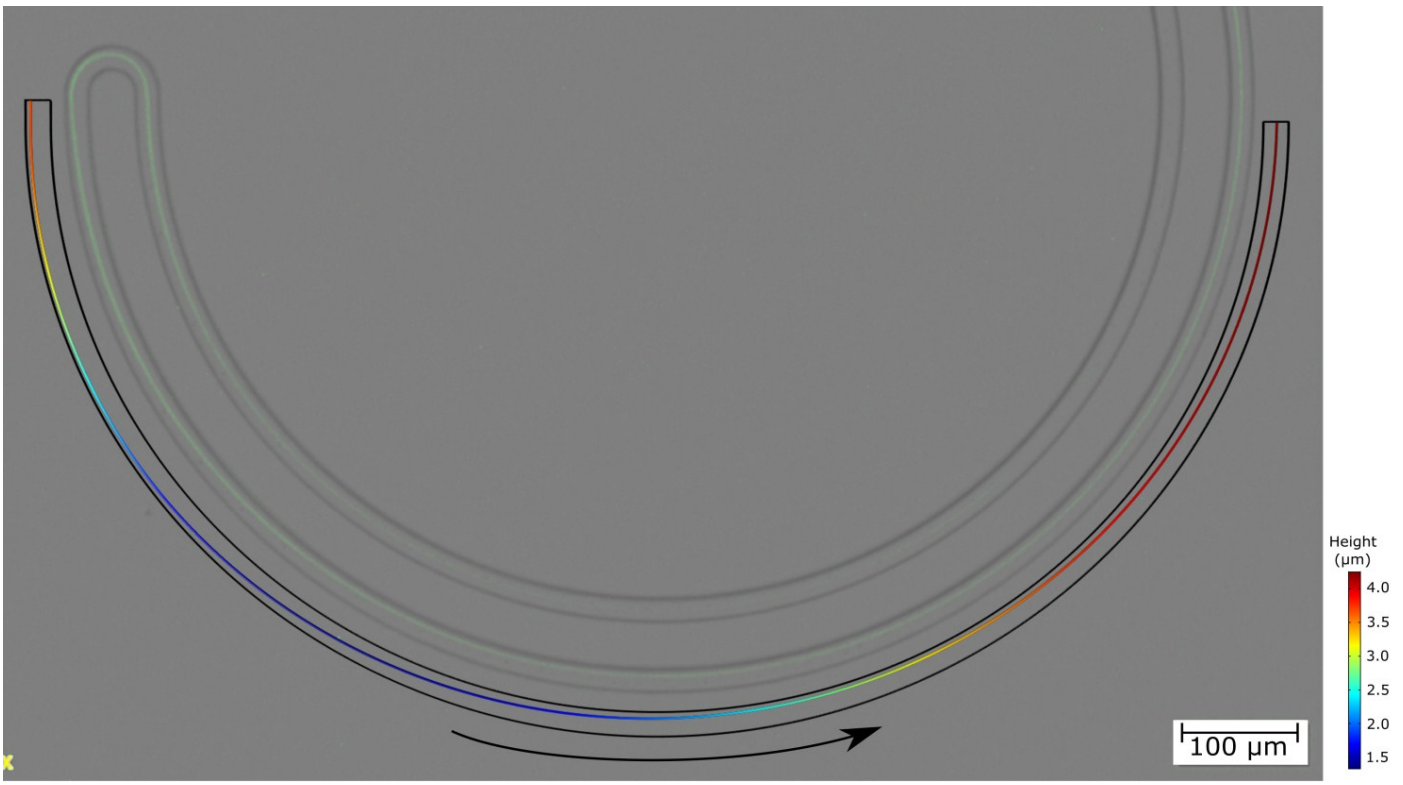

Figure S2 Similarity between the trajectory of 1 µm particles after the turn (at 90 bar – 135 µL/min) and an arbitrary streamline of the flow simulated in COMSOL. From top view, the particles initiate the migraition from the proximity of the outer wall, they pass by the equilibrium position and leave it behind to approach the inner wall, where they change direction towards the outer wall and finally reach the equilirbium position. This trajectory suggests that the equilibrium position it is closer to the center than the equilirbium perimeter where the shear gradient lift and the wall force cancell each other. The trajectory resembles the helicoidal movement of a stream line in curved channels.

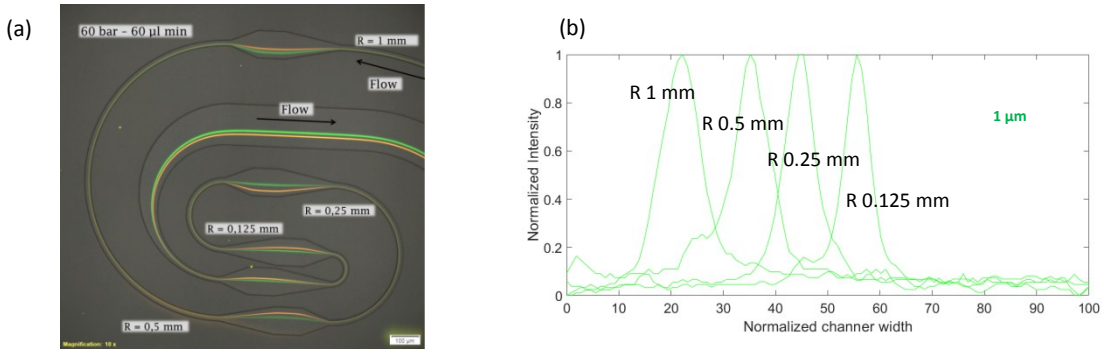

Figure S3 (a) Positions of equilibrium of 1 and 2 µm particles (green and red respectively) at different radii (1, 0.5, 0.25 and 0.125 mm) at 60 bar – 60 µL/min. (b) Intensity profile of 1 µm particles; from left to right, 1, 0.5, 0.25 and 0.125 mm in *R*. In the normalized channel width 0 and 100 represent the inner and outer wall respectively.

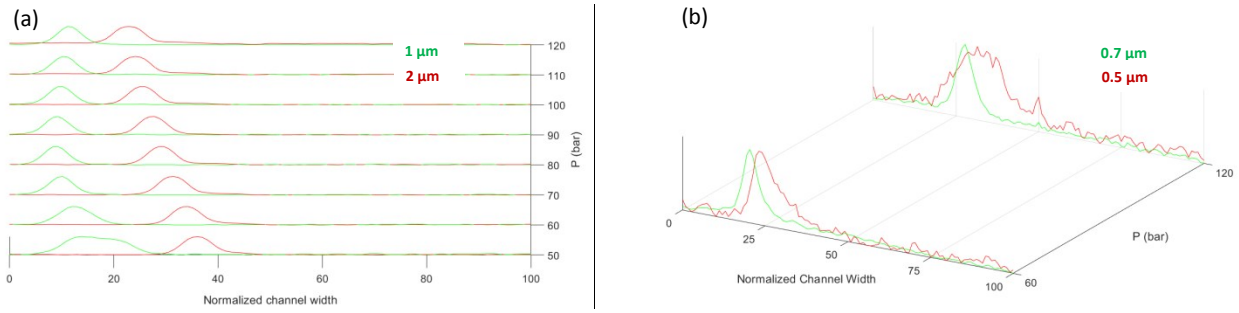

Figure S4 Intensity profiles in a microfluidic chip with 0.5 mm in *R* and cross section 7.3x20 µm (*HxW*). (a) 1 and 2 µm particles from 50 to 120 bar (37 to 90 µL/min). (b) 0.5 and 0.7 µm particles at 60 and 120 bar (45 and 90 µL/min). In the normalized channel width 0 and 100 represent the inner and outer wall respectively.

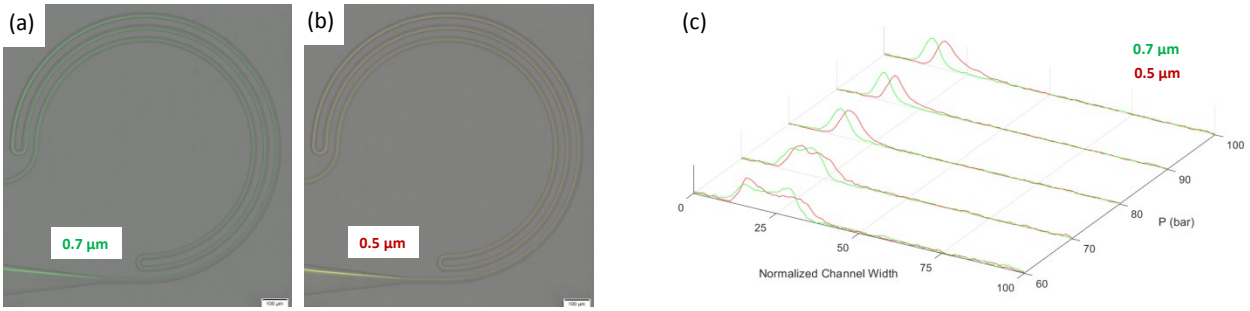

Figure S5 Performance of a microfluidic chip with three loops, cross section 7.3x20 µm (*HxW*) and 0.5 mm in *R* with (a) 0.7 and (b) 0.5 µm particles at 90 bar (45 µL/min). (c) Intensity plots 60-120 bar (30-60 µL/min). In the normalized channel width 0 and 100 represent the inner and outer wall respectively.

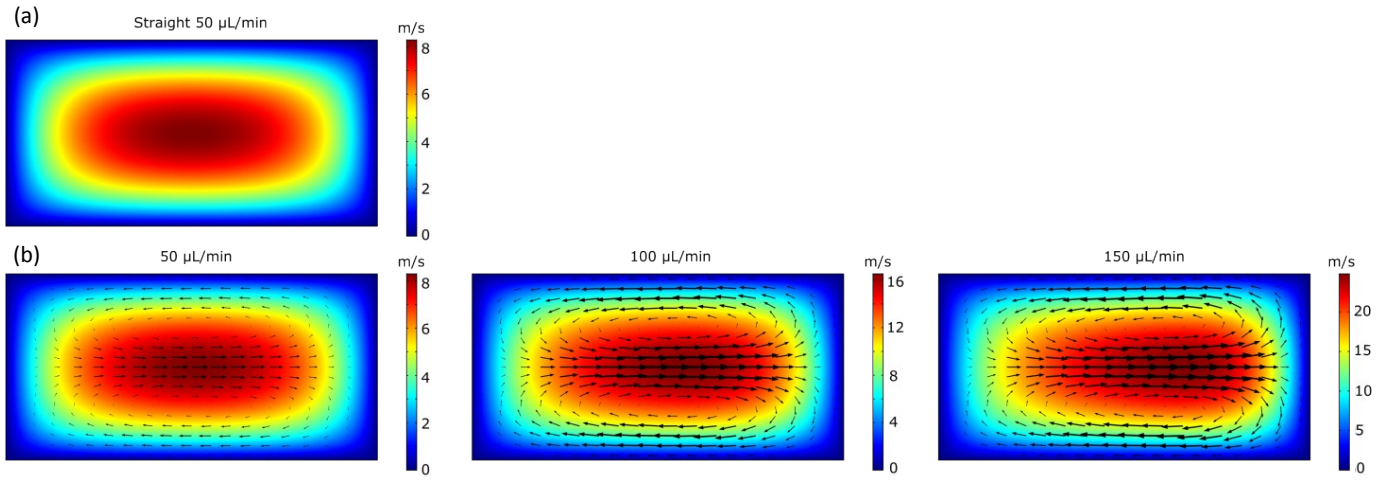

Figure S6 COMSOL simulations of flow profiles (main flow and secondary flow) in a microchannel with cross section 10x20 µm (*HxW*). (a) Straight microchannel and (b) curved microchannel with 0.5 mm in radius. *The arrows of the secondary flow were scaled to improve the visibility (a factor 1/2 and 1/4 for 100 and 150 µL/min, relatively to the 50 µL/min image).

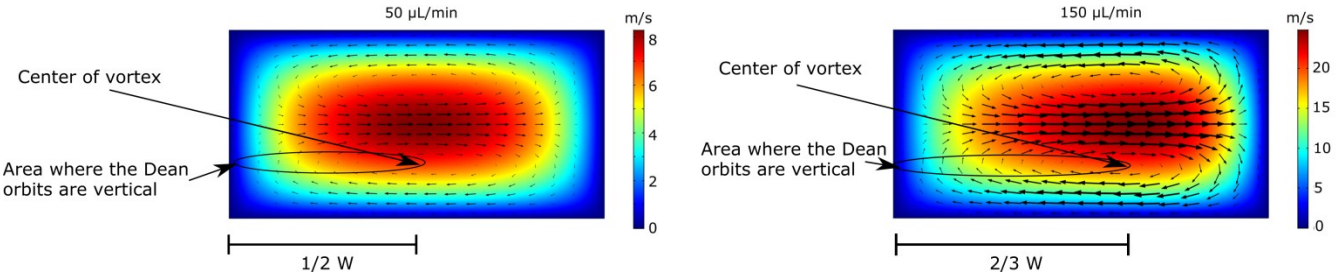

Figure S7 COMSOL simulations of flow profiles at 50 and 150 µL/min with indications of the areas where the Dean drag opposes the Lift force. As the flow rate increases the flow profile deforms; 50 µL/min keeps the main flow shape of a straight channel while 150 µL/min is deformed by the large secondary flow. It can be seen how the area where the Dean orbits are vertical exceeds W/2 for the deformed flow, thus making it possible for particles to align past W/2.

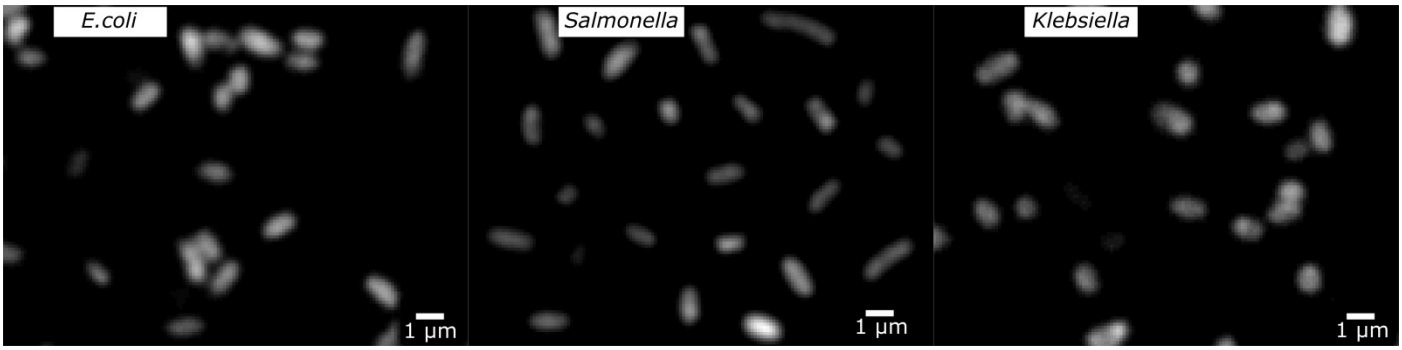

Figure S8 Fluorescent images of the bacteria (grown in agar plates) few minutes after being diluted in sterile deionized water.

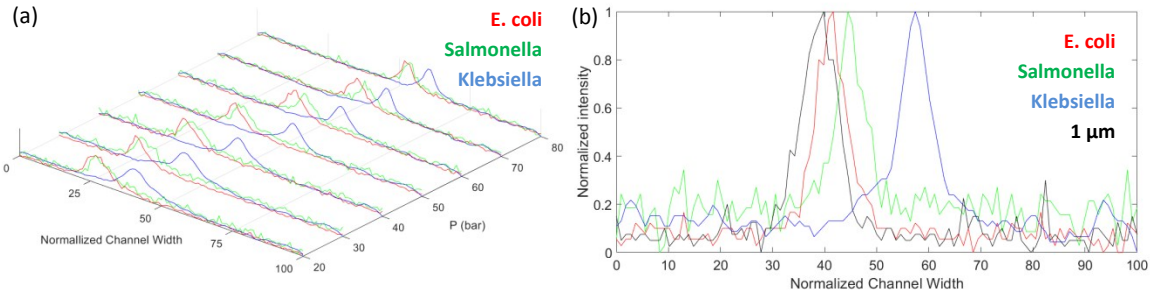

Figure S9 (a) Intensity profiles in a microchannel with 0.5 mm in *R* and cross section 10x20 µm (*HxW*) with bacteria at 20 to 80 bar (30 to 120 µL/min). (b) Intensity profile of the bacteria and 1 µm particles at 60 bar (90 µL/min). In the normalized chennel width 0 represents the inner wall and 100 the out wall.