Single channel layer, single sheath-flow inlet microfluidic flow cytometer with three-dimensional hydrodynamic focusing

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Supporting Information

Fig. S1 The numerical simulation results with the particle tracing for sample flow (blue) and sheath flow (red) with different flow rate combinations. Figure S1(a) shows the simulation results when the sample/sheath flow rate ratio is set to be 1:1, and the total flow rate of 100 μ l/min. The particle trajectory in the expansion chamber indicates that the sample flow contacts the sidewalls of the expansion chamber right after flowing in the chamber. In this case, the sample flow is pushed to the bottom floor to form the top/bottom two-layered flow pattern. Figure S1(b)shows the simulation results when the total flow rate increased to 500 μ l/min with the same flow rate ratio of 1:1.The simulation results show that the sample flow is encircled by sheath flow and 3D hydrodynamically focused.



Fig. S2 (a) Cross-sectional view of the simulated vertical velocity contours for the device with 2.0 and 3.0 mm-diameter sheath flow inlets under the same flow conditions (total flow rate = $300 \,\mu$ /min, and sample/sheath flow rate ratio = 1:2). The results show that the device with a smaller (2.0 mm) sheath flow inlet diameter possesses higher downward velocity (momentum) compared to the device with a larger (3.0 mm) sheath flow inlet diameter. The simulation results show that the 3D hydrodynamic focusing can only be achieved using the device with larger downward momentum (2.0 mm-diameter sheath flow inlet); even another one (3.0 mm-diameter sheath flow inlet) has more lateral space for the sheath flow to flow underneath the sample flow. (b) Flow direction velocity profiles for a device with a 2.0 mm-diameter sheath flow inlet with two different flow conditions. One is total flow rate of 300 μ /min and sample/sheath flow rate ratio of 1:5; another one is total flow of 500 μ /min and sample/sheath flow rate ratio of 1:1. Both cases have the same sheath flow downward velocity (momentum), since the device geometries and the sheath flow rates (250 µl/min) are the same; however, they have different sample flow forward momentum. The result shows only the device with higher sample flow forward momentum can achieve 3D hydrodynamic focusing.



Fig. S3 Simulated particle tracing profiles for devices with various sheath flow inlet diameters, including: 1.0, 1.5, and 2.0 mm. The results show that the sheath flow has sufficient lateral space to flow underneath and encircle the sample flow well only in the device with 2.0 mm-diameter sheath flow inlet. Figure S3(b) shows the concentration profiles right after the expansion chambers for the devices. The concentration profiles show the 3D hydrodynamic focusing can be accomplished with the 2 mm-diameter sheath flow inlet. In contrast, the sample and sheath flows form top-bottom two-layered flow in the device with the 1.0 mm-diameter sheath flow inlet. The results demonstrate that the sheath flow inlet (expansion chamber) diameter plays an important role to provide enough lateral space for sheath flow to flow underneath the sample flow for 3D hydrodynamic focusing formation.



Fig. S4 Comparison of concentration profiles with two flow rate conditions and various bottom layer thicknesses. (a) Concentration profiles for the device with various bottom layer thicknesses (0, 600, and 4000 μ m) when the total flow rate is 100 μ l/min and sample/sheath flow rate ratio is 1:1. The results show no 3D hydrodynamic focusing in any case. (b) Concentration profiles for the devices with the same bottom layer thicknesses when the total flow rate is 500 μ l/min and sample/sheath flow rate is 500 μ l/min and sample/sheath flow rate ratio is 1:1. The results demonstrated 3D hydrodynamic focusing is achieved with the depth of the expansion chamber larger than 600 μ m. The results suggest the thickness of the bottom layer (i.e. depth of the expansion chamber) has limited affect to promote 3D hydrodynamic focusing when the thickness is larger than 600mm. Furthermore, the larger bottom layer thickness is undesired for the optical detection experiment setup when a high numerical aperture (NA) objective is exploited to achieve better optical detection sensitivity.