Supplementary Material (ESI) for Lab on a Chip
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Electronic Supplementary Information (ESI)

Simultaneous counting of two subsets of leukocytes using fluorescent silica nanoparticles in a sheathless microchip flow cytometer

Table of Contents

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Electronic Supplementary Information Figure S-1

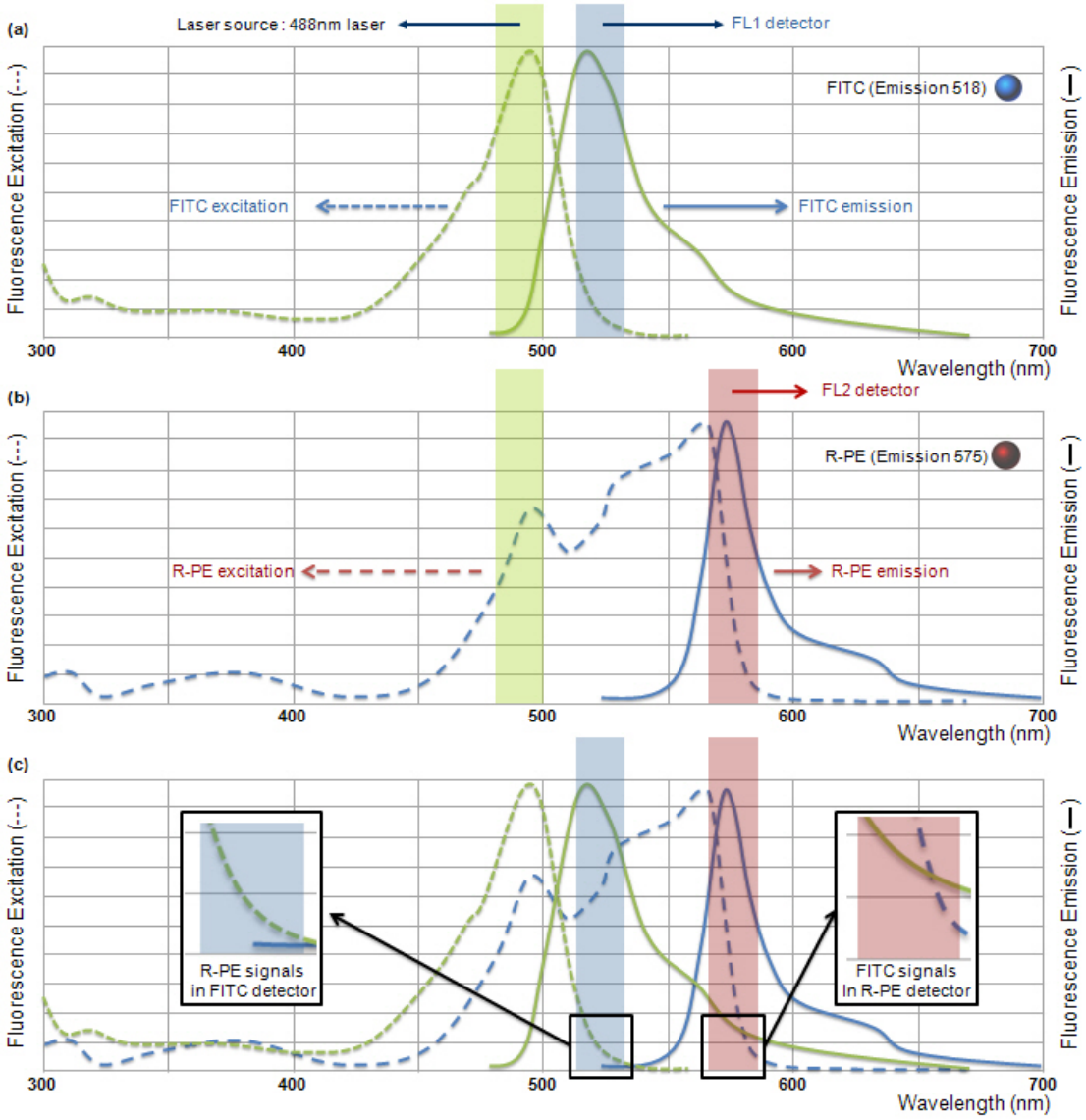

Fig. S-1. For flow cytometry analysis of two-parameter detection, the most common combinations of fluorescent dyes are FITC and R-PE. This is because both FITC and R-PE could be excited by a single light source such as a 488 nm blue laser but resulting in different emission spectra. FITC (a) and R-PE (b) have emission wavelength of 518 nm and 575 nm, respectively. Therefore, we can analyze two types of cells simultaneously with a single excitation source and two fluorescent channels (FL1, FL2). However, because most fluorescent dyes do not have a sharp emission peak, the inherent overlap of emission spectra from these fluorescent labels makes compensation a necessity. In the case of FITC and R-PE, spectral overlap between FITC and PE produces signals that are detected by both the FL1 and FL2 detectors (c). Therefore, the amount of FITC fluorescent signals being detected by the R-PE detection channel (FL2) and the amount of R-PE fluorescent signals being detected by FITC detection channel (FL1) should be compensated. To obtain only the R-PE signal, the amount of spectral overlap can be corrected by subtracting a percentage of the FITC signal from the total signal generated by the R-PE detection channel (FL2 detector). To make simultaneous measurements of multiple immune cell subsets, this compensation procedure should be performed before testing samples.

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Electronic Supplementary Information Figure S-2

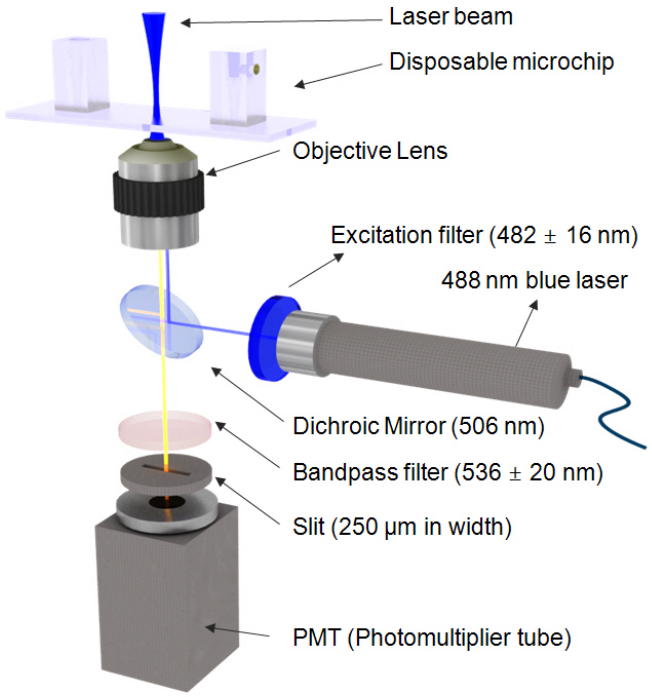

Fig. S-2. Schematic of the optical setup of the microchip flow cytometer

Electronic Supplementary Information Figure S-3

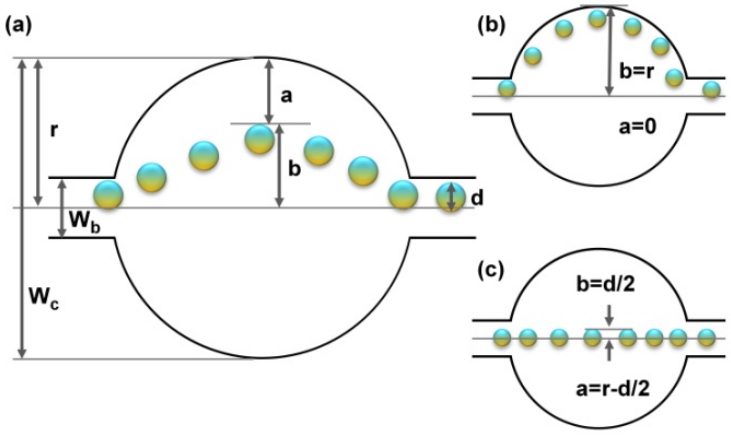

Fig. S-3. (a) Parameters for the analysis of particle focusing effect in the expansion channel area (i.e. detection area). *R* is the radius of the expansion channel and *d* is the diameter of a particle. The cell-free margins defined as *a* and *b* indicate the amount of particle deviation from the outer channel boundary and the centerline, respectively. (b) Conditions when particles are least focused. (c) Conditions when particles are perfectly focused and aligned into a single line.

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Electronic Supplementary Information Figure S-4

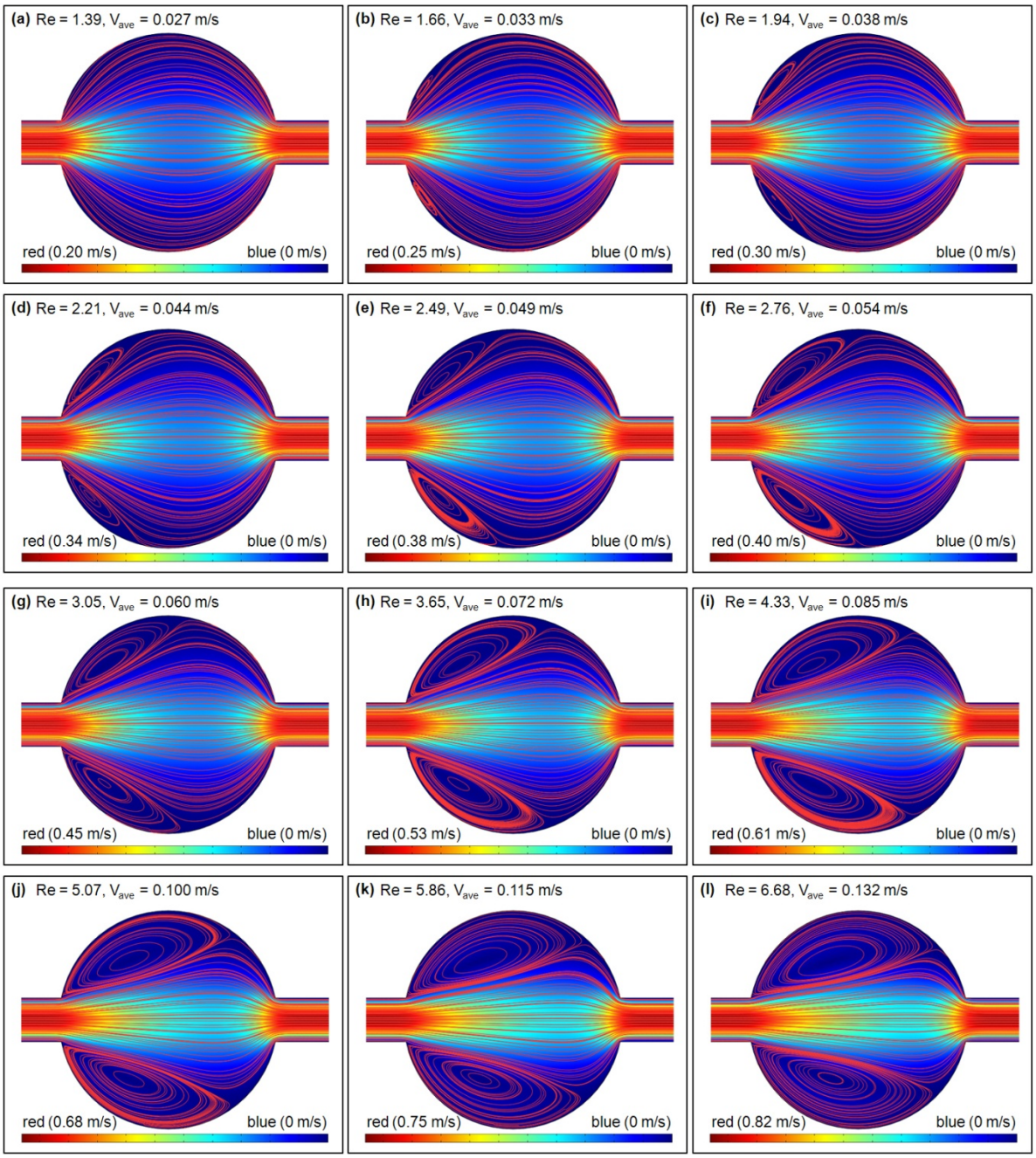

Fig. S-4. Numerical simulation results using COMSOL Multiphysics showing flow streamlines (shown in red color) formed in the expansion channel according to various *Re*. We applied *Re* ranging from 1.39 to 6.68 that was based on flow rates of 8.02-39.2 µl/min corresponding to an average flow velocity of 0.027-0.132 m/s. The dimension of expansion channel is 165 µm in width and 30 µm in height. The hydraulic diameter of the expansion channel is 50.8 µm. The separated flow in the expansion channel area expands as *Re* increases. The results also indicate the velocity field using a surface plot. As to velocity legend, blue color shows zero velocity and red color means maximum velocity. The simulation using 2D incompressible Navier-Stokes application mode in COMSOL Multiphysics was conducted under conditions of density of fluid of 1000 kg/m^3, dynamic viscosity of fluid of 0.001005 Pas, and pressure difference (507 Pa-2128Pa) between inlet and outlet.

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Electronic Supplementary Information Figure S-5

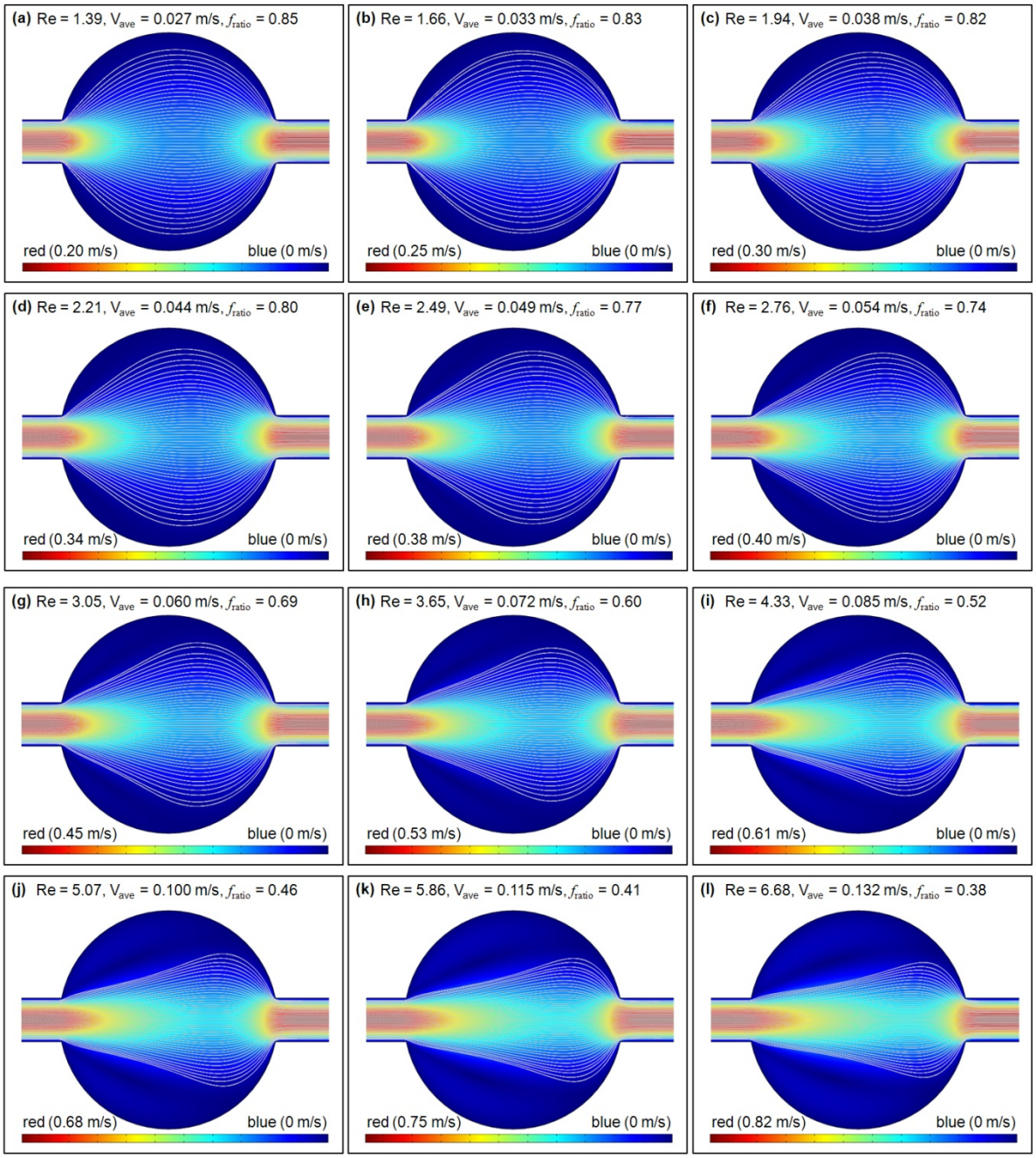

Fig. S-5. Numerical simulation results using COMSOL Multiphysics showing particle streak lines (shown in white color) according to various *Re*. The boundary conditions are same as the conditions of Fig. S-4. In order to simulate particle trajectories with the velocity field, a massless particle model in COMSOL Multiphysics was used. These results show that *Re* increases as f_{ratio} decreases, meaning higher focusing efficiency with increased *Re*.