Electronic Supplementary Material (ESI) for Lab on a Chip

Four-part leukocyte differential count based on sheathless microflow cytometer and fluorescent dye assay

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Supplemental Information

Coincidence error of counting leukocyte cells in the sheathless channel

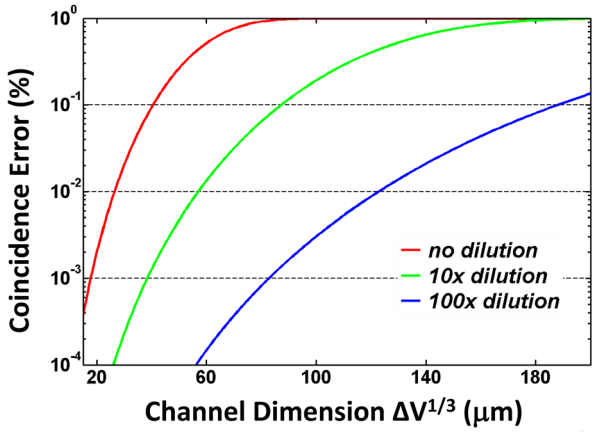

Fig. S1 The calculated coincidence error versus the detection channel dimension ($\Delta V^{1/3}$) for leukocyte count in the sheathless channel. ΔV is the volume of the detection channel. In this plot, a leukocyte concentration of 8×10^3 cells/μL is considered, and different levels of sample dilution are compared

The PDMS microfluidics device and its fabrication process

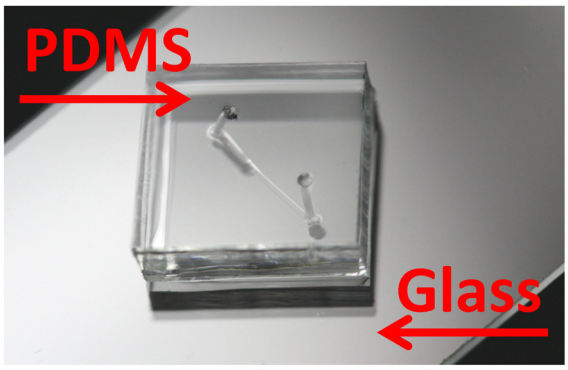

Fig. S2 Picture of the transparent microfluidic device fabricated by the PDMS soft lithography process.

A transparent microfluidic device, as shown in Fig.S2, was used to provide the sheathless channel for the leukocyte count test. This device was fabricated using a standard polydimethylsiloxane (PDMS) soft lithography process previously reported[22]. The fabrication process is briefly described here. First, the PDMS fluid was prepared by mixing

the part A and the part B of the Sylgard 184 kit (Dow Corning, USA) with a 10:1 ratio. It is then degassed and poured over a silicon mold with the patterns of the fluidic channels. Second, the PDMS was partially cured at 80°C for 30min to solidify, and then peeled off from the mold. The fluidic inlet and outlet were manually punched with a hypodermic needle (gauge 19, outer diameter approximately 1mm). Finally, the PDMS block was placed on a glass slide and the whole device was fully cured at 100°C for 2 hours to complete the process.

The complete microflow cytometer system and its components

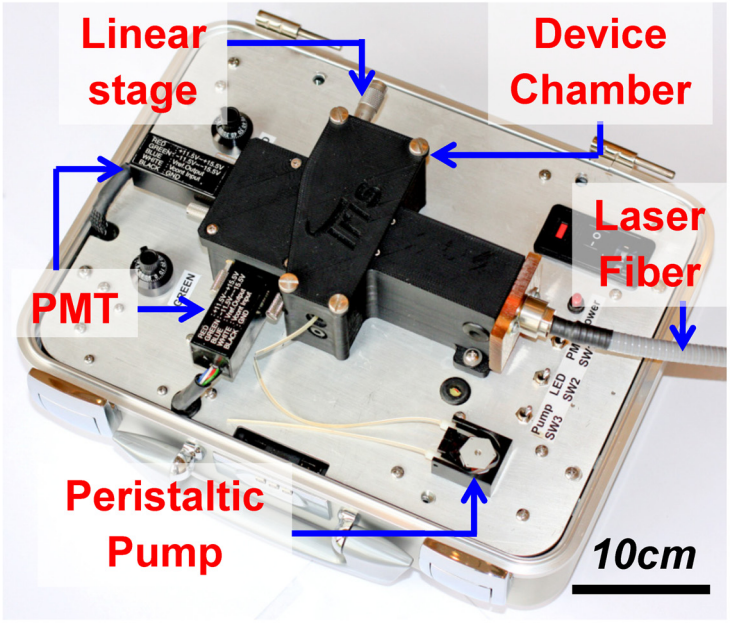

Fig. S3 Picture of the complete microflow cytometer system. The microfluidic device was enclosed inside the device chamber and shielded away from environment light.

The optical components are assembled inside a black housing, shielded away from environmental light. A solid-state laser module, FCD488 (488nm, 4mW, JDSU, USA), is used as the excitation source. The intensity of the laser beam has approximately a Gaussian profile. The focusing of the laser beam was adjusted by changing the distance between the condenser lens, which was mounted on a threading adapter, and the microfluidic device. The alignment between the laser beam and the sheathless channel was adjusted by moving the one-axis linear stage, where the microfluidic device was mounted. As the sheathless channel had a uniform cross section along its length, hence a one-axis linear stage was sufficient to achieve the alignment. The fluorescence intensities are measured by two photomultiplier tubes, H5784 (Hamamatsu, Japan), and recorded using a data acquisition module, USB-6211 (National Instrument, USA). A sampling frequency of 50kHz was used to record the data. A peristaltic pump P625 (Instech, USA) and the silicone tubing set P625/TS015S (Instech, USA) are used to drive blood sample to flow through the microfluidic device (flow rate 6μL/min). The measured data was readout through a USB port and recorded on a laptop computer.

The fluorescent intensities of leukocyte cells measured by microscope

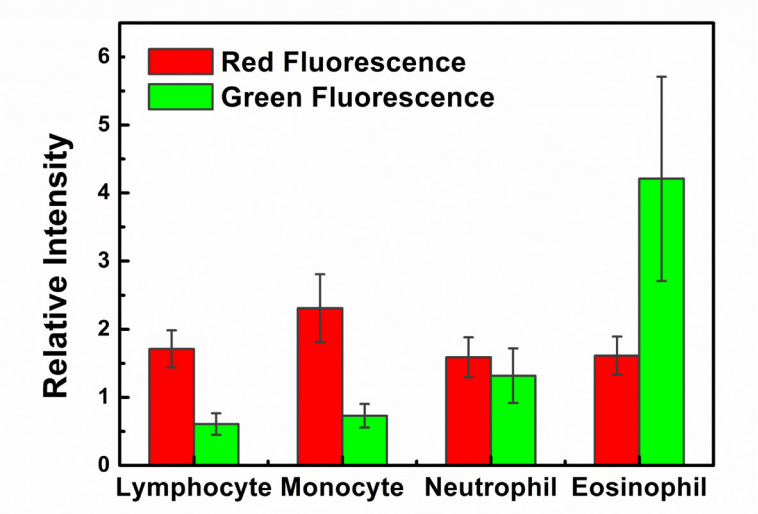

Fig. S4 The integrated fluorescence intensities measured from the microscope images of the leukocyte subtypes. The images were taken with Zeiss LSM510 microscope, and the intensities were quantified with the software, Zeiss AIM software. The intensities of the red fluorescence (>600nm) and the green fluorescence (515-545nm) were compared. The bar graph showed the averaged value from five cells of each subtype, and the error bar indicated the standard deviations

Calibration of the microflow cytometer system with fluorescent beads

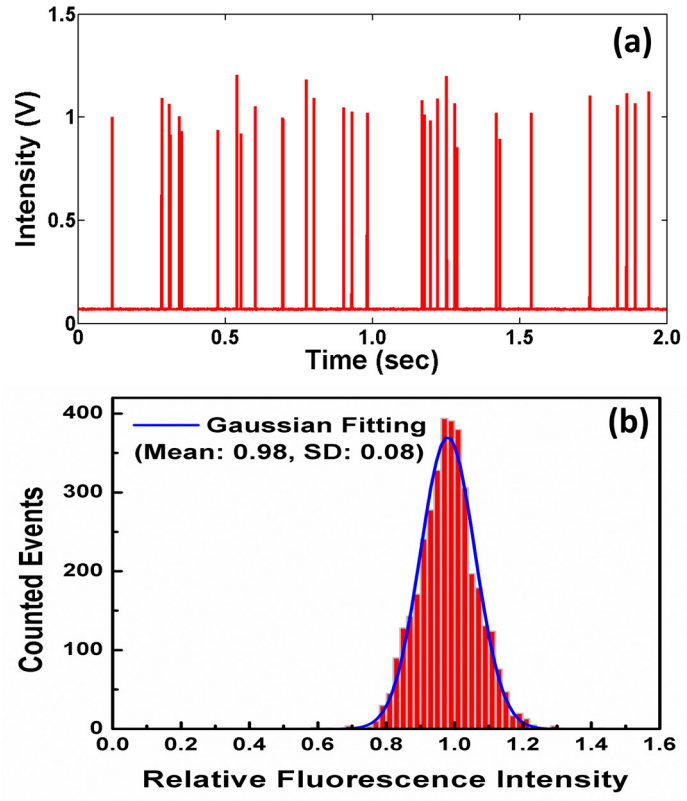

Fig. S5 (a) Measured time trace of the fluorescence signal from the beads sample. (b) The histogram analysis of the fluorescence intensities of the beads. The number of the counted bead events was plotted versus their fluorescence intensities. The data was fitted by a Gaussian distribution, showing a mean valve of 0.98 and a standard deviation of 0.08

Comparison of the two assays with and without BO21

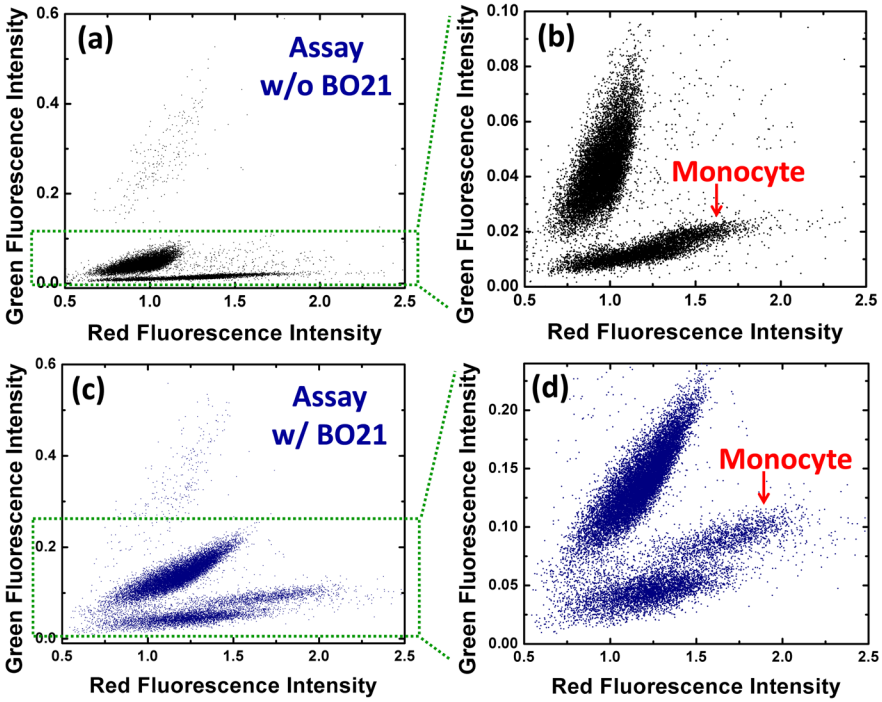

Fig. S6 Scatter plots comparing the results from the (a-b) previously reported assay (PI and FITC) and (c-d) the newly proposed assay (PI, FITC and BO21). Both assays were measured on the microflow cytometer of this study. (a) and (c) showed the full view of the measured scatter plots, whereas (b) and (d) showed the zoom-in view of the plots.