Supporting Information

Potentiometric Multichannel Cytometer Microchip for High-throughput Microdispersion Analysis

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S1. Filter circuit design

While the voltage-applying electrodes maintained a constant potential drop of ~3 V across the sensing channel, the electric potential obtained by the sensing electrode was passed to a custom analog filter circuit (Figure S1). Specifically, a high-pass filter with a cut-off frequency of 0.5 Hz was used to filter the DC component. To amplify small electric potential variations, two operational amplifiers (total amplitude gain, ~1600) were used in combination with a low-pass filter with a cut-off frequency of 10 kHz. The filtered electric potential traces were then acquired using a data acquisition board (maximum sampling rate, ~400 kHz) at a sampling rate of 24 kHz for each channel. In the analysis of the detection throughput of a single channel, we used a low-pass filter with a cut-off frequency of 1 MHz instead of the one with a cut-off frequency of 10 kHz. The DAQ sampling rate was also set to 120 kHz in the detection throughput analysis.

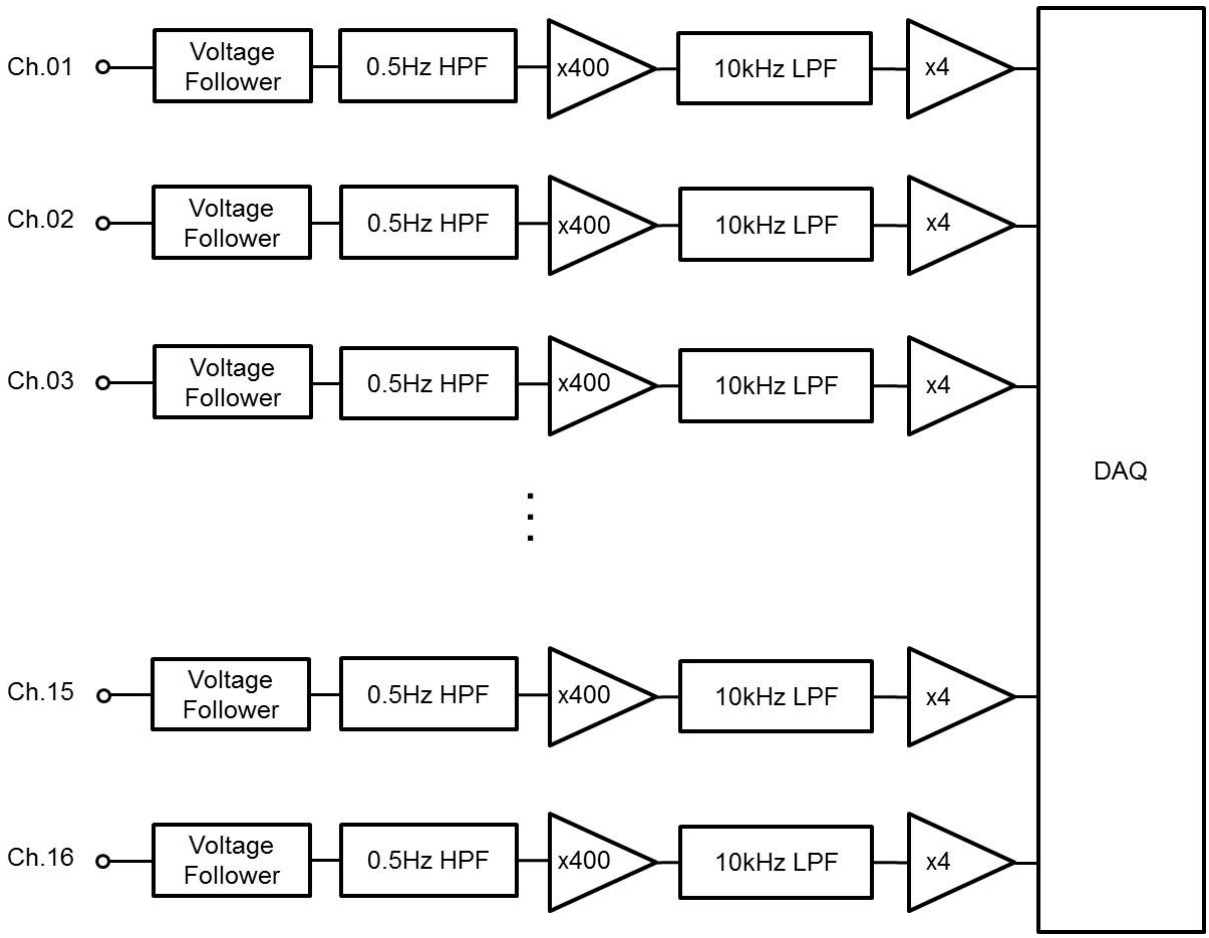

Figure S1. Block diagram of the analog filter circuit for parallelized analysis.

S2. Confirmation of biphasic potential traces under microbead translocations

To investigate the electric potential change induced by microbead translocations through a sensing channel, the microbead translocations were simultaneously monitored by electric potential recording and successive image acquisition using a high-speed camera (FASTCAM MC2, Photron) with an imaging rate of 1000 frames per second. Based on the comparison of two sets of synchronized measurement data, a single microbead translocation event was found to induce a single biphasic potential change at the sensing electrode (Figure S2).

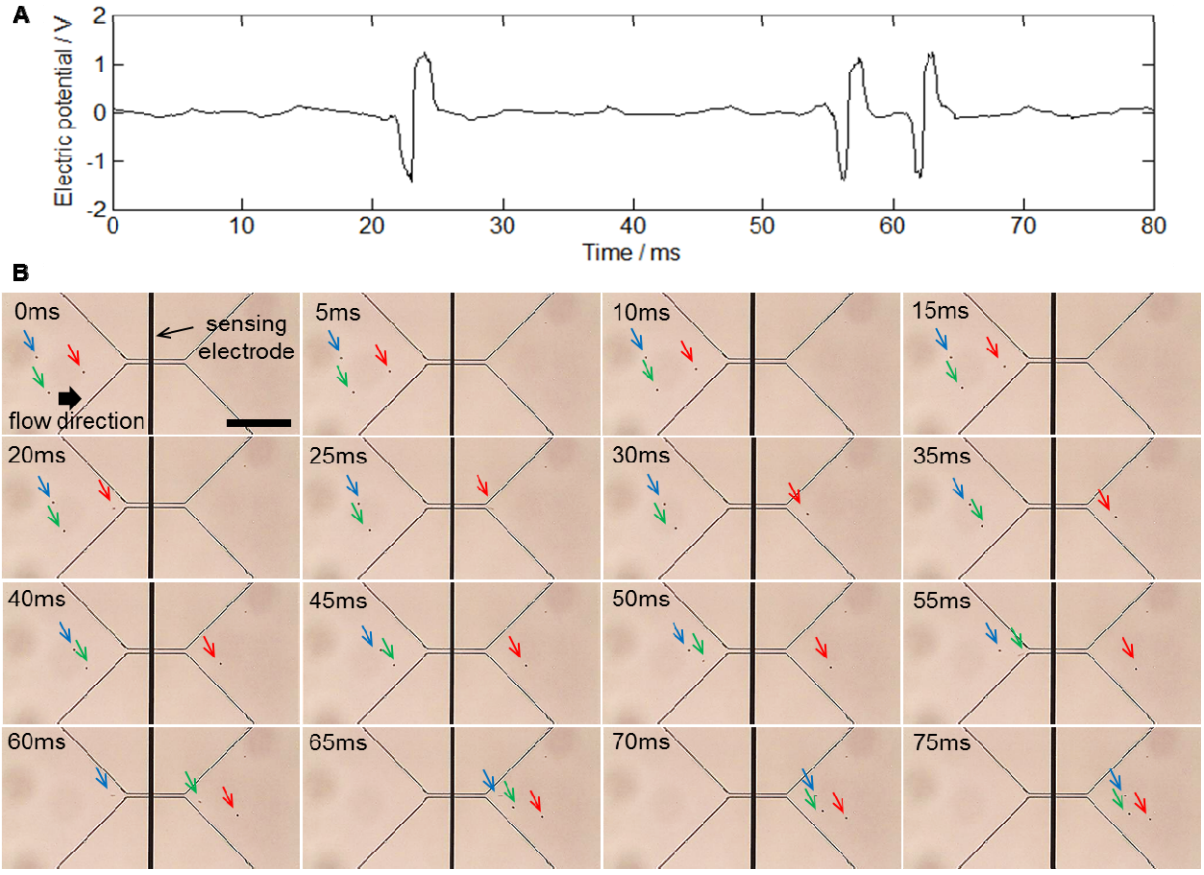

Figure S2. Comparison of electric potential trace with synchronized high-speed camera images. (A) Electric potential trace recorded at the sensing electrode. (B) A series of high-speed camera images. Colored arrows are used to indicate single microbeads. Scale bar indicates 100 μm.

S3. Effect of sample loss on microbead concentration

To determine the effect of sample loss on the concentration estimation, we investigated the microfluidic channel after measurements were performed for ~20 min. From the inspection of the microscopy images, we found that few microbeads were settled or adhered to the channel wall (Figure S3). Therefore, we did not consider the effect of sample loss in the estimation of microbead concentrations.

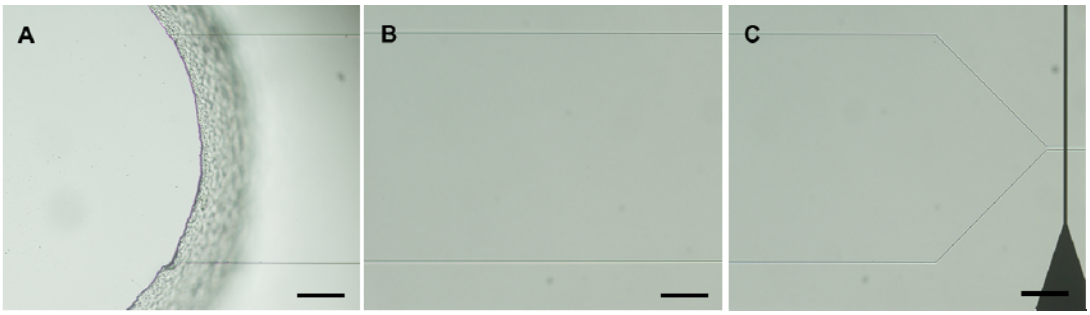

Figure S3. Microscopy images of a microfluidic channel. All images were captured after the measurement was completed: (A) near fluid inlet, (B) at the middle of the channel, and (C) near a sensing channel. Scale bars indicate 100 μm.

S4. Development of 16-channel potentiometric cytometer for parallelized analysis

By assembling and bonding two glass substrates—one with a microfluidic channel and the other with an electrode pattern—a single-channel or multichannel device was fabricated (See "Materials and Methods - Microchip Fabrication" section in this article for details). Figure S4 shows a photograph of a 16-channel device in which 16 microfluidic channels share a fluid inlet (or outlet). A block of thermally cured polydimethylsiloxane with a through-hole is bonded onto the microchip for tube-to-microchip coupling.

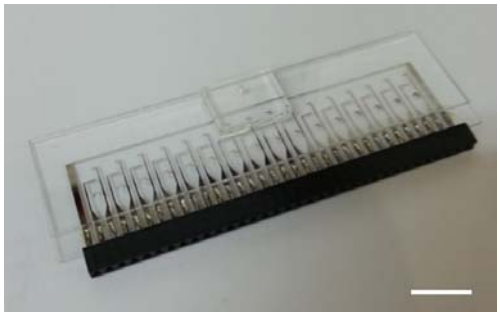

Figure S4. Photograph of a 16-channel cytometer microchip. Scale bar indicates 10 mm.

S5. Crosstalk analysis of parallelized measurement data

By using a potentiometric multichannel cytometer, 16 electric potential traces were obtained simultaneously in a single measurement of microbead translocations. To investigate the interchannel signal crosstalk, cross-correlation analyses were conducted for all detection channel combinations ($_{16}C_2 \sim 120$). The cross-correlation coefficient of two arbitrary potential traces ($X(t)$ and $Y(t)$) is obtained as follows:

$$r_{X,Y}(t_1,t_2) = \frac{C_{X,Y}(t_1,t_2)}{\sqrt{C_X(t_1,t_1)}\sqrt{C_Y(t_2,t_2)}}$$

where $C_{X,Y}(t_1, t_2)$ is the cross-covariance of $X(t)$ and $Y(t)$, and $C_X(t_1, t_2)$ and $C_Y(t_1, t_2)$ are the autocovariances of $X(t)$ and $Y(t)$, respectively.[R1] Here, we considered the potential traces as jointly stationary random processes (i.e., $t_2 = t_1 - \tau$, where τ is a time shift). No distinct correlation was observed for any of the combinations ($|r| < 0.13$, Figure 5D) including adjacent (Figure S5) and nonadjacent channels.

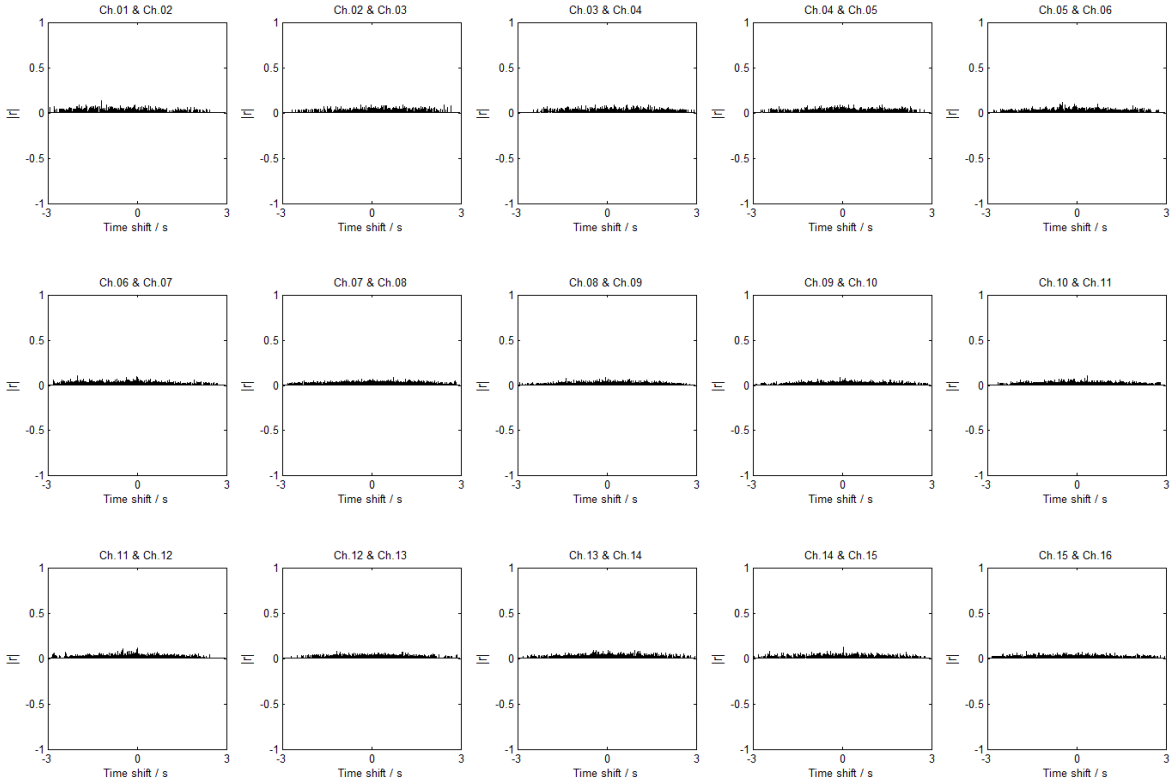

Figure S5. Cross correlation analysis of adjacent channel combinations using 16 electric potential traces obtained in a single measurement of microbead translocations. Microbeads with a diameter of 2.58 μm were used.

S6. Detection throughput of parallelized device

The detection throughput of a multichannel device can be simply determined by the multiplication of the detection throughput of a single detection channel unit with the number of detection units in the device. To experimentally determine the detection throughput of a single detection channel unit, we obtained a distribution of microbead (diameter, ~2.58 μm) transit times at various flow rates ranging from 10 to 500 μL h^{-1} (Figure S6). From the cut-off transit time at which the normalized electric potential change was obtained to be 70% of its original level, we determined the experimental detection throughput of a single detection channel unit as ~3000 s^{-1}. The total detection throughput of a parallelized 16-channel device is therefore obtained as ~48000 s^{-1}.

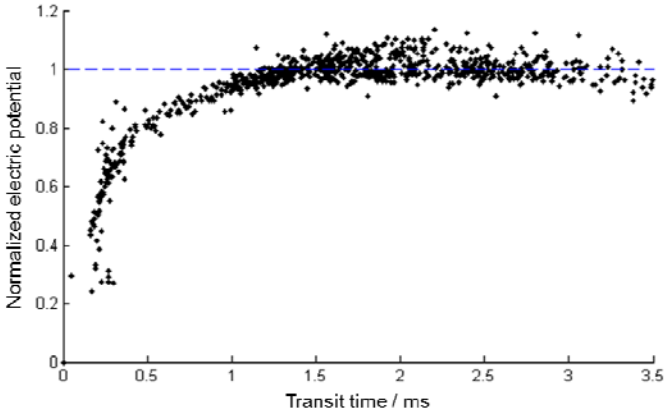

Figure S6. Transit time distribution of microbead translocations at various flow rates.

Reference

[R1] Leon-Garcia, A. *Probability and random processes for electrical engineering*, *2nd Ed.*; Addison-Wesley, 1994.