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

Supporting information

An optimized PDMS microfluidic device for ultra-fast and high-throughput imaging flow cytometry

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S.1. The effect of the ratio of sample and sheath on vertical focusing under high velocity

Besides the velocity, extensive research has demonstrated that the ratio of sample and sheath can also significantly influence hydrodynamic focusing. To investigate the flow distribution of the sample under high ratios of sample and sheath at high velocity, we conducted experiments using the layouts shown in Figure 1d, the 44 mm long channel. Figure S1a presents the distribution of the sample (ink) under two velocities, 1 m/s and 10 m/s, and two ratios of sample and sheath: Qsample/Qsheath of 1/4 and 1/14. The results clearly indicate distinct differences in the sample distribution under these various conditions.

The photos of ink distributions can be converted to grayscale to investigate the ink distribution in both horizontal and vertical dimensions. In PDMS channels, high velocity can result in higher pressure, leading to larger deformation, which means the ink will have a higher distribution at high velocities (sample channel will have a higher height at higher velocity). As shown in Fig. S1b, the grayscale values of sample channel are lower at 10 m/s compared to 1 m/s, regardless of the ratio of sample and sheath.

In most research studies, a low ratio of sample and sheath has minimal effect on sample focusing or distribution, as reported by previous studies1,2. However, when the velocity is extremely high, the results can differ. In our research, the lowest velocity we investigated was 1 m/s, which is considered high in comparison to most research studies. At a velocity of 10 m/s and a ratio of Qsample/Qsheath = 1/14, we observed that the sample was not focused at the bottom and middle of the channel, but rather concentrated at the two corners at the bottom. This is illustrated in Figure S1c. When velocity is 1 m/s, the sample distributions under these two ratios of sample and sheath were similar, and the difference in grayscale values was primarily due to the different flow rates of the sample.

Specifically, when the velocity increased to 10 m/s and the ratio of Qsample/Qsheath was 1/4, the lowest grayscale values were found in the middle of the channel, indicating that the ink was distributed higher in the middle of the channel. However, with a ratio of Qsample/Qsheath of 1/14, the lowest grayscale values were located at the two sides of

the channel. In this case, the sample was divided and focused towards the two corners at the bottom of the channel.

These findings highlight the influence of velocity and the ratio of sample and sheath on sample distribution and focusing, particularly at high velocities where corner focusing effects become more prominent.

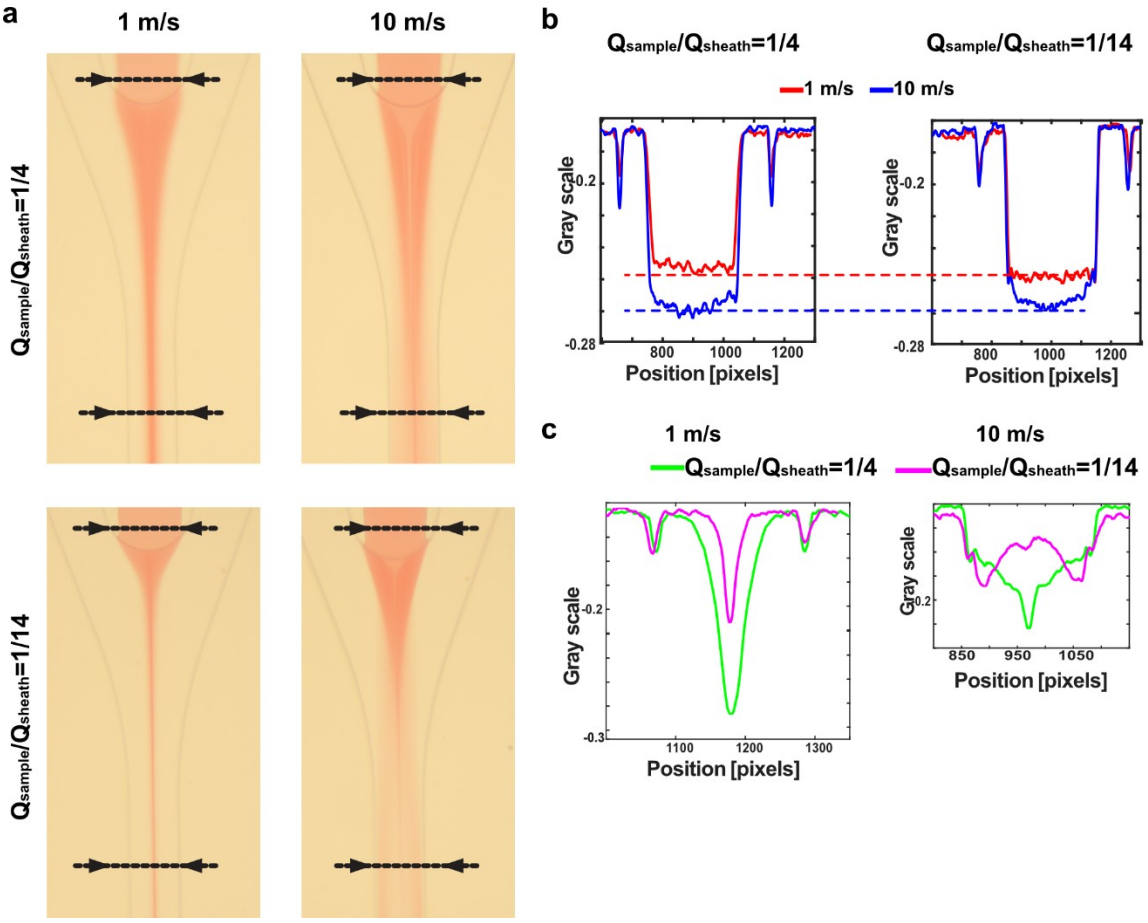

Figure S1. The sample distribution at velocities of 1 m/s and 10 m/s and the ratio of sample and sheath of 1/4 and 1/14; (a) The photos of ink distributions at four conditions; (b) The grayscale of sample channel at different conditions; (c) The grayscale of observation channel at different conditions.

S.2. Evaluation of wall deformation at high velocity

Fig.S2a resents the images of the channel walls, including walls at three positions with velocities of 1 m/s and 40 m/s. It is clear that the width and darkness of the wall is wider and deeper, respectively, at 40 m/s than at 1 m/s, which reflects large expansion of walls at a high velocity. The grayscale of walls in images are picked out to investigate the effect of velocity on wall deformation. As shown in Fig.S2b, four positions of the wall are concerned, from the sample channel (high pressure) to the observation channel (low pressure). The four small graphs represent the waveforms of walls at different velocities at four locations marked in Fig.S2a. Walls of sheath outer channel and junction have a tendency to move far from channel, due to the expansion of the channel at high velocity, and the grayscale of wall is low at high velocity. But this pheromone is not too clear for the wall of observation channel. Fig.S2c shows the evaluation of wall deformation using three parameters, width expansion rate of wall, the distance extension of a pair of walls, and the grayscale of wall. When velocity increases to 20 m/s, the width extension rate and distance extension of sheath channel and junction increase rapidly, and the grayscale of the wall decreases. Meanwhile, the observation channel shows a small difference between the beginning and the end. This is mainly due to the pressure distribution at high velocity; the observation channel is directly linked with the output chamber, and the local pressure is as low as the external environment. The high velocity in the narrow observation channel requires extremely high pressure at the beginning of the observation channel, which causes the high pressure in sheath channel and junction, resulting in large deformation of walls in these places.

The deformation of the observation channel wall is quite low, especially on the end side, ensuring that the velocity is quite similar to the expected value, which is related to the flow rate and channel cross-section. Then the imaged cells can reach the high expected velocity.

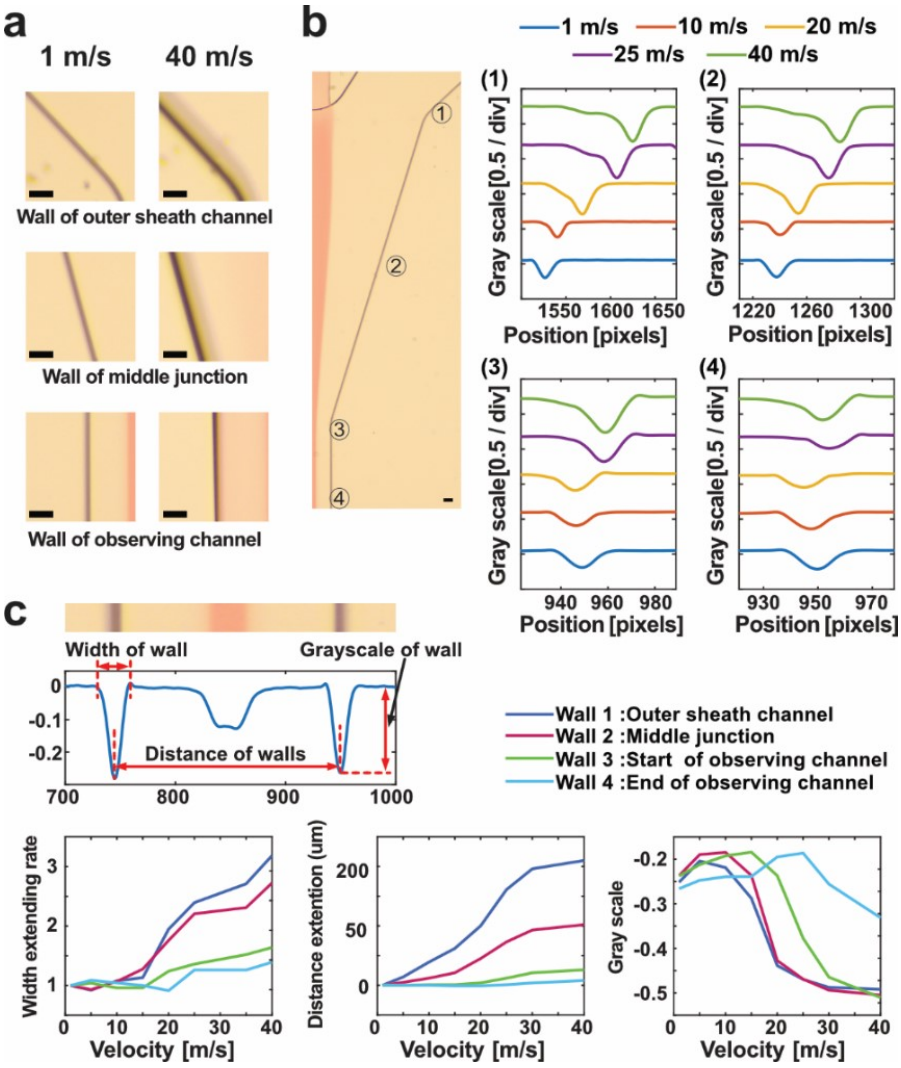

Figure S2. Wall deformation at different velocities. (a) Differences of channel walls at 1 m/s and 40 m/s (left), four positions of the wall used for observation and comparison (right), scale bar 25 μm; (b) Grayscale of channel walls of four positions at different velocities of 1 m/s, 10 m/s, 20 m/s, 25 m/s, and 40 m/s; (c) The evaluation on wall deformation, the extension of the waveform of the wall, distance of a pair of walls, and the grayscale of the waveform of wall over a range of velocities.

S.3. Syringe fixing

For most syringe pumps, syringes are always fixed by blocking part of the barrel flange. In our experiments, we found that the barrel flange of the syringe is easily deformed under high push forces of pumps at high flow rates. When barrel flange deforms, syringes can

be fixed at a certain place and fail to inject fluids into microchannels. To address this issue, we designed a syringe keeper by cutting a hole with a similar diameter of the syringe on a 2 mm thick stainless steel. The keeper can support the whole area of barrel flange of the syringe for large and well-distributed support forces, avoiding the deformation of barrel flange. The structure of our keeper and its installation are shown in Fig.S3.

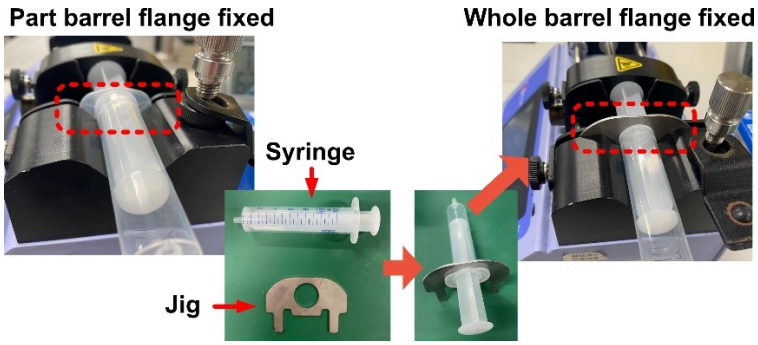

Figure S3. The syringe jig can support all area of barrel flange of the syringe, when syringe is fixed with the jig in a pump.

S.4. Imaging resolution

Fig.S4 shows the OTS image of line pairs of group 9 element 3 of the USAF-1951 resolution chart.

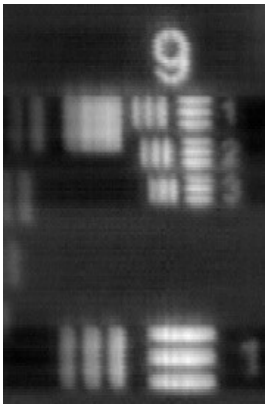

Figure S4. OTS image of the USAF-1951 resolution chart.

S.5. OTS images of whole blood cells

Whole blood sample is quite suitable for evaluation of image flow cytometry. As shown in Fig.5a, whole blood includes many kinds of cells with different phenotype.

Meanwhile, whole blood sample has a high concentration of cells which is good for test of throughput. High concentration of sample can increase the throughput directly. Fig.4b and Fig.5c are the OTS microscopy of 200-fold diluted whole blood sample and 40-fold diluted whole blood sample at the same velocity of 10 m/s, respectively. It is clear that, image of low dilution sample contains more cells. Image of 200-fold whole blood contain 9 cells and 27 cells. However, high concentration of sample is easy to make cells reunite and overlap, like marked in Fig.S5b and Fig.S5c, causing blur boundaries of cells in images.

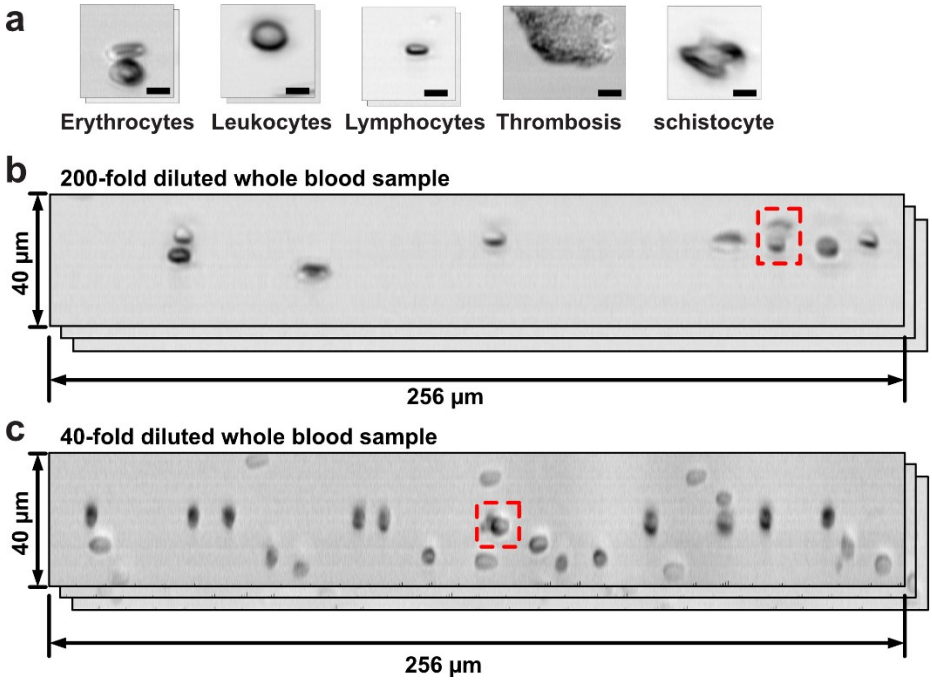

Figure S5. OTS images of whole blood sample; (a) OTS images of some typical blood cell, scale bar: 10 μm; (b) Long OTS image of 200-fold diluted whole blood sample, 256 μm wide and 40 μm high; (c) Long OTS image of 40-fold diluted whole blood sample, 256 μm wide and 40 μm high.

[1] Y.J. Chiu, S.H. Cho, Z. Mei, V. Lien, T.F. Wu, and Y.H. Lo, Lab Chip **13**, 1803 (2013).

[2] Y.M. Patel, S. Jain, A.K. Singh, K. Khare, S. Ahlawat, and S.S. Bahga, Biomicrofluidics **14**, 64110 (2020).