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

Supplementary Information

High-throughput Adjustable Deformability Cytometer Utilizing Elasto-inertial Focusing and Virtual Fluidic Channel

Zheng Zhou[1†], Chen Ni[1†], Zhixian Zhu[1], Yao Chen[1], Zhonghua Ni[1] and Nan Xiang[1*]

[1]School of Mechanical Engineering, and Jiangsu Key Laboratory for Design and Manufacture of Micro-Nano Biomedical Instruments, Southeast University, Nanjing, 211189, China.

†Zheng Zhou and Chen Ni contributed equally to this work.

***Corresponding Author**: Nan Xiang, E-mail: nan.xiang@seu.edu.cn.

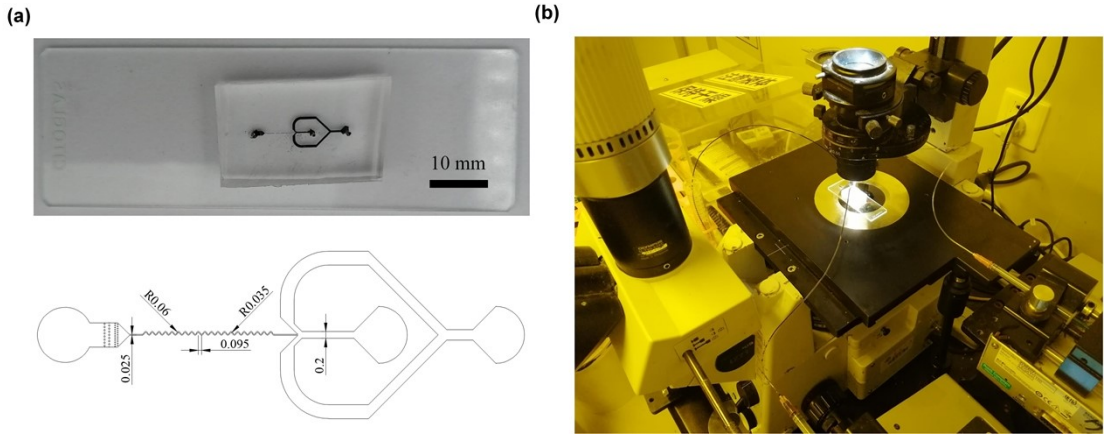

Figure S1. (a) Photograph and CAD drawing of our microfluidic chip. (b) Photograph of the system for deformability cytometry.

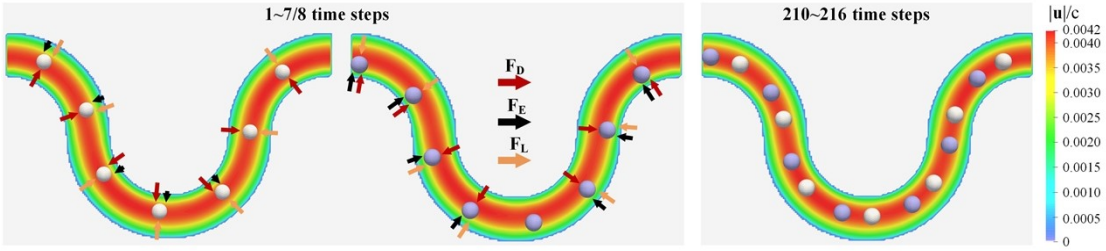

Figure S2. The force analysis of particles during the different simulation time steps. In the first few time steps of the simulation, the particles at different initial positions gradually migrated to the equilibrium position in the channel center under the combined action of F_D, F_L, and F_E. After a sufficient time step, the single-line focusing of the particles was achieved.

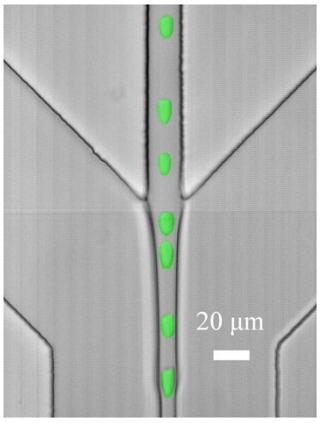

Figure S3. Stacked images illustrating elasto-inertial focusing of A549 cells flowing into the virtual fluidic channel. The flow rates of sheath and the sample were set to be 240 μL/min and 60 μL/min, respectively.

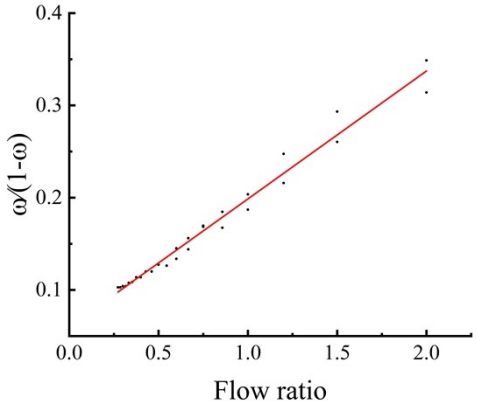

Figure S4. Ratio of relative widths of sample to sheath at different flow ratios (Q_{sa}/Q_{sh}). The relationship between $\omega/(1-\omega)$ and Q_{sa}/Q_{sh} could be described as: $\omega/(1-\omega) = 0.1387Q_{sa}/Q_{sh} + 0.5992$, with a high linear fit (R^2 = 0.9787). Q_{sa} and Q_{sh} are the flow rate of sample and sheath, respectively. ω is the relative virtual channel width and is equal to the ratio of the width of virtual fluidic channel to the width of solid channel. R^2 is the variance.

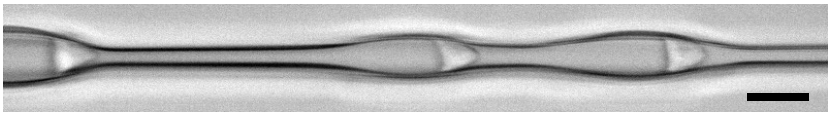

Figure S5. Multiple-cell image taken under a 1-μs exposure of the high-speed camera with the sheath and the sample at the flow rate of 240 μL/min and 60 μL/min, respectively. Scale bar is 20 μm.

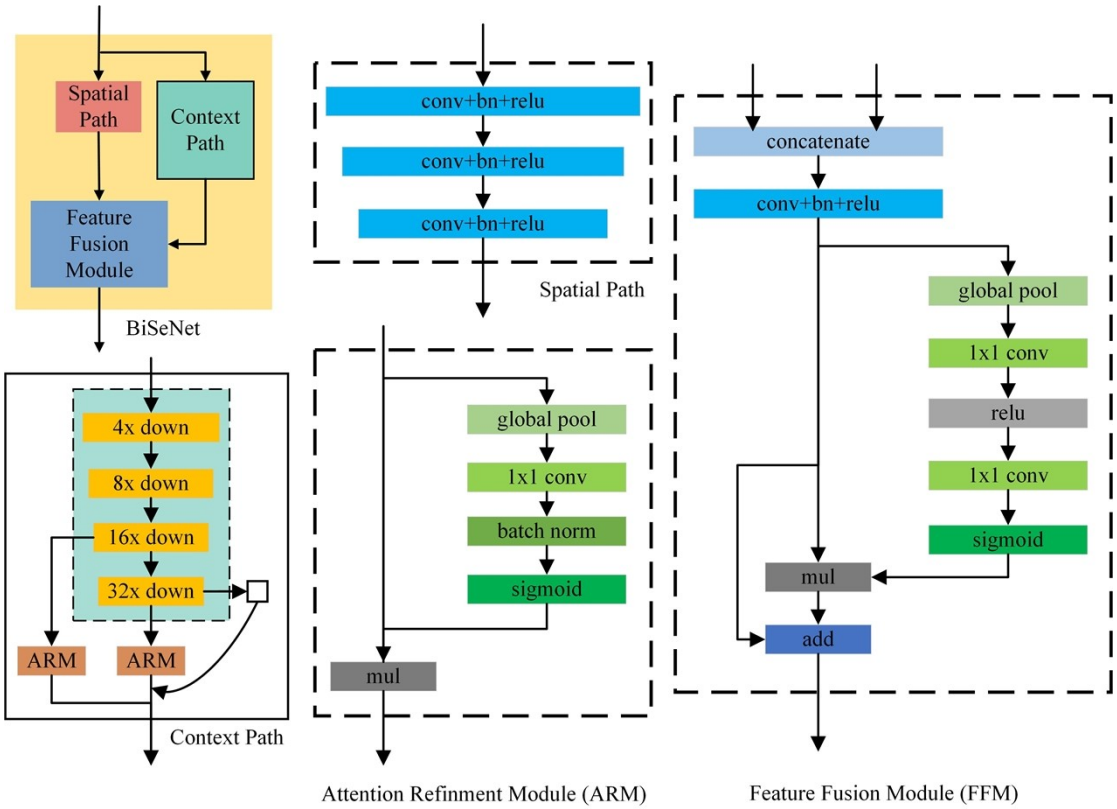

Figure S6. Detailed structure of spatial path, context path, attention refinement module (ARM), and feature fusion module (FFM) in the BiSeNet. In the dotted box of the context path, ResNet or MobileNet was called to enlarge the receptive field and extract multi-scale features.

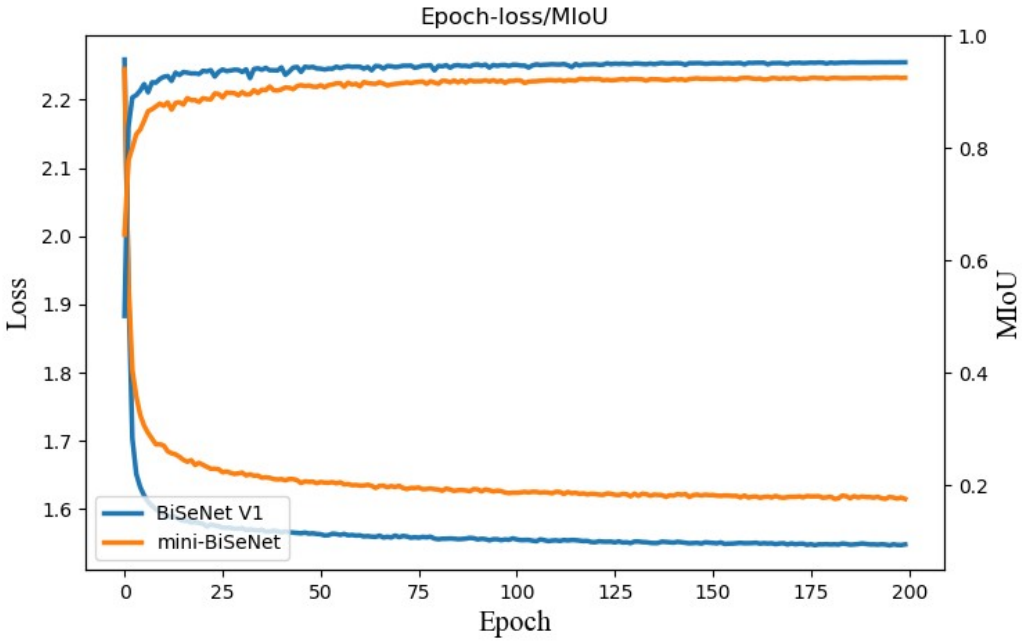

Figure S7. Epoch-Loss/MIoU curve of BiSeNet V1 and mini- BiSeNet. After 200 times of epoch, their loss was around 1.6.

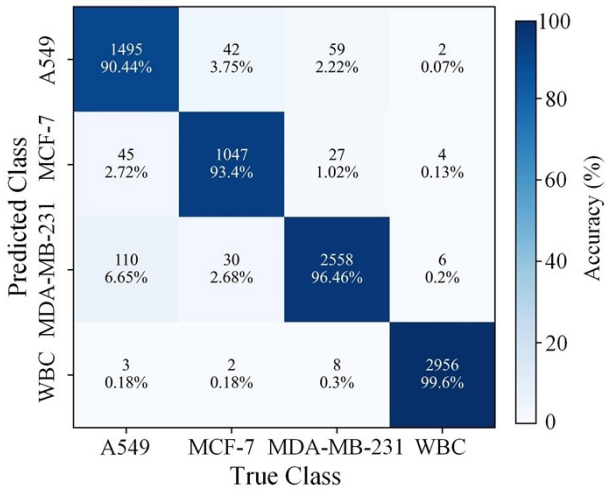

Figure S8. Confusion matrix of cell type identification based on size and deformation of cells captured in multiple frames.

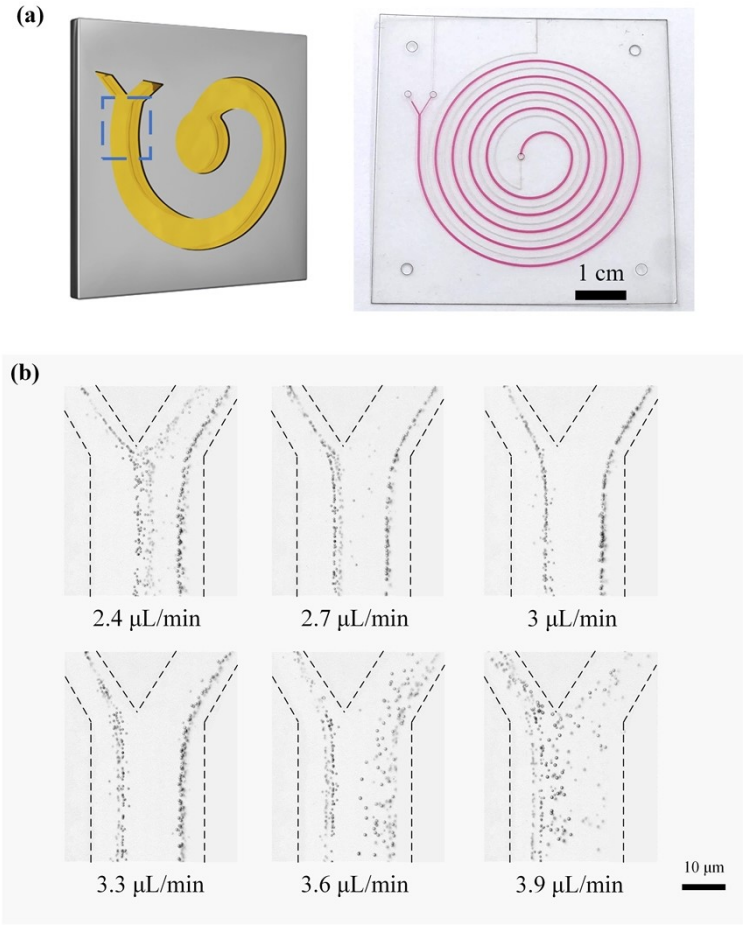

Figure S9. Photograph of our microfluidic sorter and particle separation performance. (a) Conceptual design and photograph of microfluidic sorter. (b) Separation performances of 10 μm and 15 μm particles at different flow rates near the trapezoidal spiral outlet.

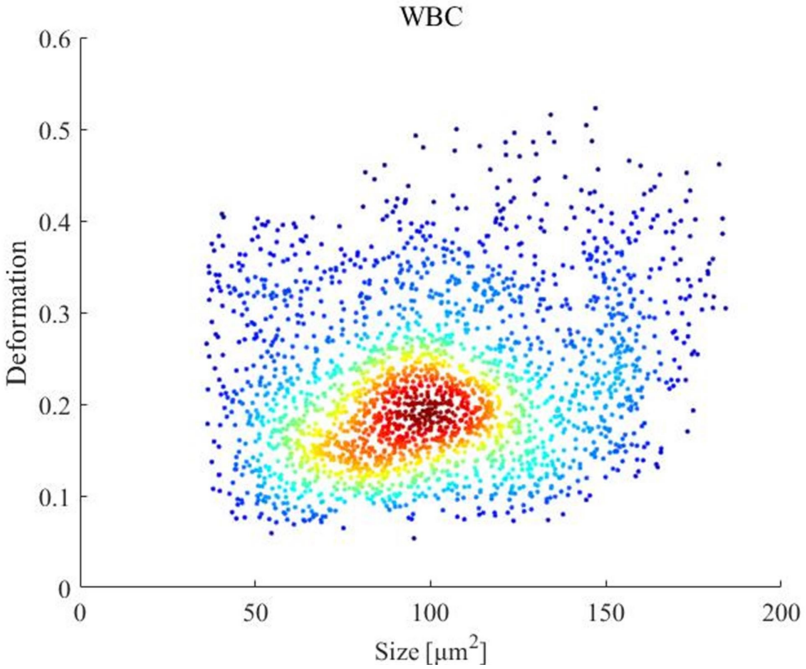

Figure S10. Scatter plots of deformation versus size of normal healthy WBCs as the control group of the clinical samples.

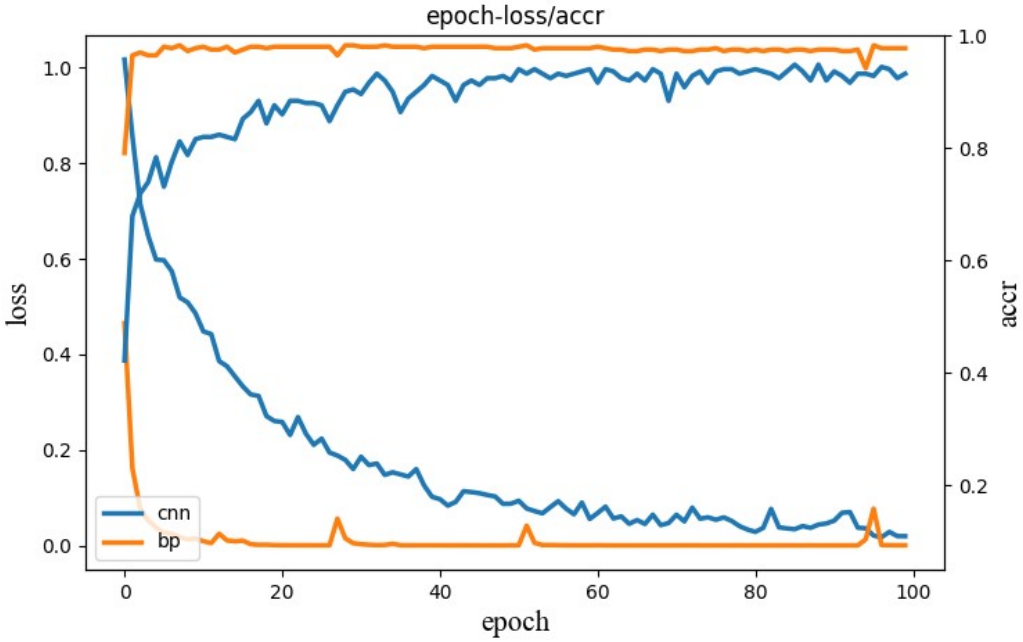

Figure S11. Epoch-Loss/Accuracy curve of CNN and BP neural network. After 100 times of epoch, BP neural network driven by multiple deformation parameters had the accuracy of ~ 98.21% and the loss of ~ 0.0002, while the accuracy and loss of CNN were 94.77% and ~0.02, respectively.

Table S1. Parameters on cell size and deformation.

Parameters	Equation
Roundness (Rd_1)	$Rd_1 = 2(\pi A)^{1/2}/C$
Roundness (Rd_2)	$Rd_2 = 4\pi A/C^2$
Aspect ratio (Lr_1)	$Lr_1 = (y - x)/(y + x)$
Aspect ratio (Lr_2)	$Lr_2 = x/y$
Aspect ratio of circle (Ar_1)	$Ar_1 = (b - a)/(b + a)$
Aspect ratio of circle (Ar_2)	$Ar_2 = a/b$
Area ratio (Cr_1)	$Cr_1 = A/\pi b^2$
Area ratio (Cr_2)	$Cr_2 = A/\pi a^2$

where A and C are the area of cell size and the cell diameter, respectively. x and y are the length and width of the outer rectangle of the cell contour, respectively. a and b are the length of the short axis and the long axis of the cell contour, respectively.

Table S2. Structure of BP neural network.

Layer	Parameters	Activate function
Fc1	n*32	leakRelu
Fc2	32*64	leakRelu
Fc3	64*128	leakRelu
Dropout	0.5	-
Fc4	128*4	-