Supplementary information

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This file includes:
Figs. S1 to S6; Table S1; Discussion about time cost.

Supporting Figures

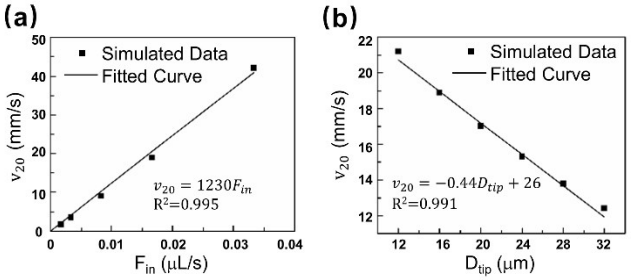

Figure S1. (a) The relationship between flow velocity at detection location (v_{20}) and the infusion volumetric flow rate (F_{in}), where the inner diameter of glass tip (D_{tip}) is set at 16 μm. (b) The relationship between v_{20} and D_{tip}, where F_{in} is set at 0.0167 μL/s. Note, since F_{in} is independent of D_{tip}, the relationship of the three variables can be written as $v_{20} = (kD_{tip} + b)F_{in}$, where k and b are constants. Combining with the two fitted equations in (a) and (b), it can be solved as $v_{20} = -26.4F_{in}D_{tip} + 1560F_{in}$.

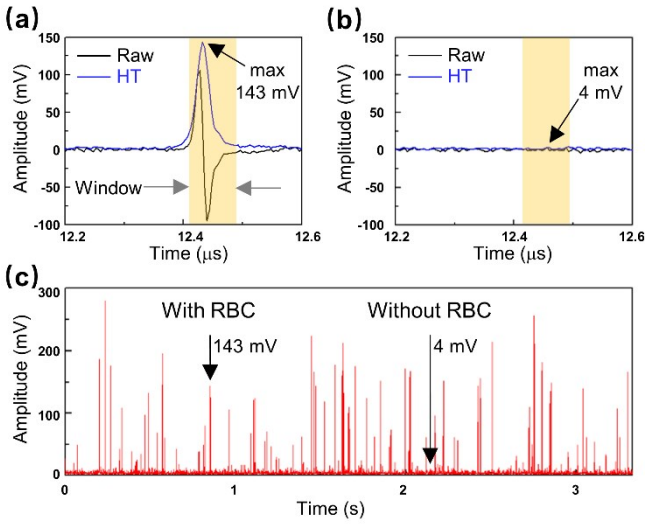

Figure S2. The raw and Hilbert transformed (HT) data acquired when (a) an RBC is passing through and (b) no RBC is passing through. The max amplitude values in the window are then extracted and used to generate the plot shown in (c), i.e., the temporal distribution of PA amplitudes. The plot shown in (c) is the same data for the case of 317 mOsm/L shown in Fig. 4(b).

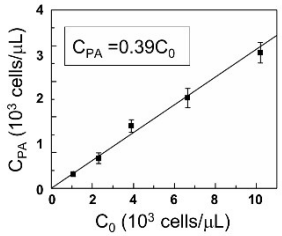

Figure S3. The Concentration measured by the PA method (C_{PA}) at different infused RBC concentrations (C_0). Each data point shown here were from three measurements.

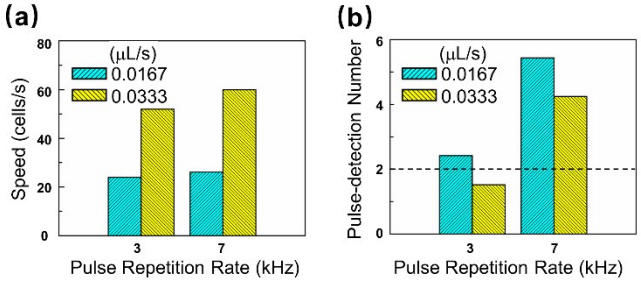

Figure S4. Comparison of RBC detection throughput as a function of pulse repetition rate (3 kHz and 7 kHz) and infusion volumetric flow rate (0.0167 μL/s and 0.0333 μL/s). (a) The RBC detection rate as functions of the pulse repetition rate and infusion volumetric flow rate; (b) the pulse-detection number as functions of the pulse repetition rate and infusion volumetric flow rate.

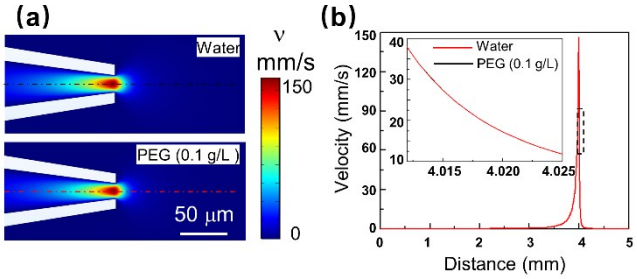

Figure S5. (a) Simulation of fluid flow near the tip of the tapered glass tube, with an infusion flow of 0.0167 μL/s, with water and a 0.1 g/L PEG solution used as medium; (b) fluid velocity profile along the dashed lines in (a) for water (black) and 0.1 g/L PEG solution (red); inset: enlarged velocity profile of the dashed rectangle in (b).

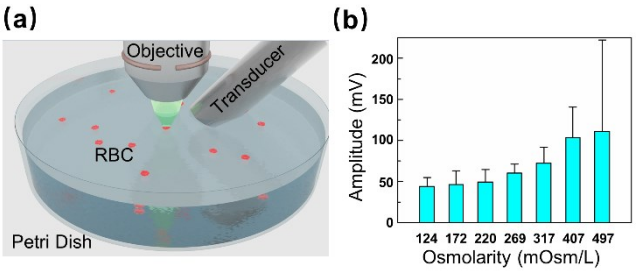

Fig. S6. (a) Illustration of the experimental setup of the PA signal measurement of RBCs lying on a substrate. The same laser source and the 10× objective lens as stated in the manuscript were used to irradiate RBCs; a transducer (Olympus V317) was used to detect the PA signal. (b) PA signals of RBCs in the environment of different osmolarities. 40 RBCs were measured in each group.

Table S1. The PA measured analysis throughput at different experimental groups of "Reference Concentration". Each experimental group of the RBC solution was analyzed 3 times by the PA system. The Reference concentration (C0) measurement error of the hemocytometer is ~12%.

	Analysis Throughput (cells/s)				Measured Concentration (Deduced from the PA analysis throughput)	Reference (Infused) Concentration (Measured by hemocytometer)
	1	2	3	Average ±STD	C_{PA} (cells/μL)	C_0 (cells/μL)
Exp. 1	7.3	7.0	5.5	6.6±0.8	1028±125	1080
Exp. 2	15.2	13.0	13.6	13.9±0.9	2164±140	2300
Exp. 3	27.3	31.2	28.8	29.1±1.6	4531±249	3900
Exp. 4	48.8	46.4	46.0	47.1±0.2	7333±31	6650
Exp. 5	64.8	62.1	63.3	63.4±1.1	9871±171	10200

Discussion about time cost

In general, pre-processing of the RBC samples is necessary in order to analyze the RBCs, including the proposed PA method and the analysis methods discussed in Table 1. For example, for RBC counting experiments, separation of RBCs from whole blood is required, which is a process that takes ~15 min. For clinical osmolarity measurement, plasma is required to be separated from whole blood, which is a process that also takes about 15 min. For the proposed PA method, besides the sample pre-processing (i.e., RBC separation from whole blood), the microfluidic system preparation (i.e., to infuse the buffer plus RBCs solution into the microfluidic chip) also takes ~15 min. Subsequently, the PA system is used to acquire signals from the RBCs for several minutes and the data is stored in a desk-top computer. Then, the data could be analyzed in about 30 seconds. On the other hand, the RBC analysis methods cited in Table 1 mainly utilize captured microscope images which contain cells' morphology and deforming information. Hence, relatively more sophisticated two-dimensional (or three-dimensional if color is also used as a parameter) time-series image processing algorithms and higher computational power are required compared with the proposed PA method where only the one-dimensional time-series signals are utilized for analysis. For example, in [16] the acquired microscope images were processed in Matlab. In [17], the acquired images were processed in ImageJ, and only a representative data for ~65 RBCs were discussed. For the image flow cytometry work [18], 100,000 images of samples were acquired, and then the morphological index were extracted to classify the RBCs.