Supporting information

Label-free Resistance Cytometry at the Orifice of a Nanopipette

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S1 Method of Selecting Parameters from a Single Spike Signal

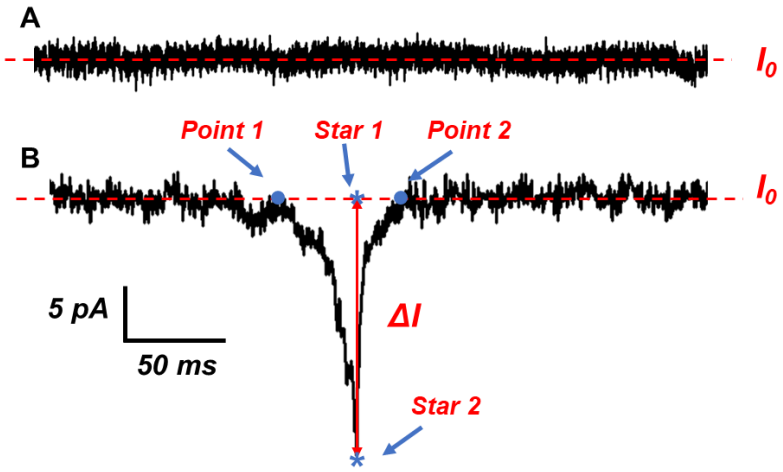

Figure S1. (A) Typical current-time traces with longer section of baseline (I_0) and (B) Typical single peak signal transient at high time resolution. The duration between point 1 and point 2 was defined as the dwell time (Δt) and the current differential between the star 1 and star 2 was defined as the current decrease (ΔI). Current decrease degree (CDD) was used to indicating the change of current, which was defined as a maximum decrease in current spike normalized to baseline current ($\Delta I/I_0$)

S2 In-situ Optical Imaging of Cell Motion at the Orifice of Nanopipette.

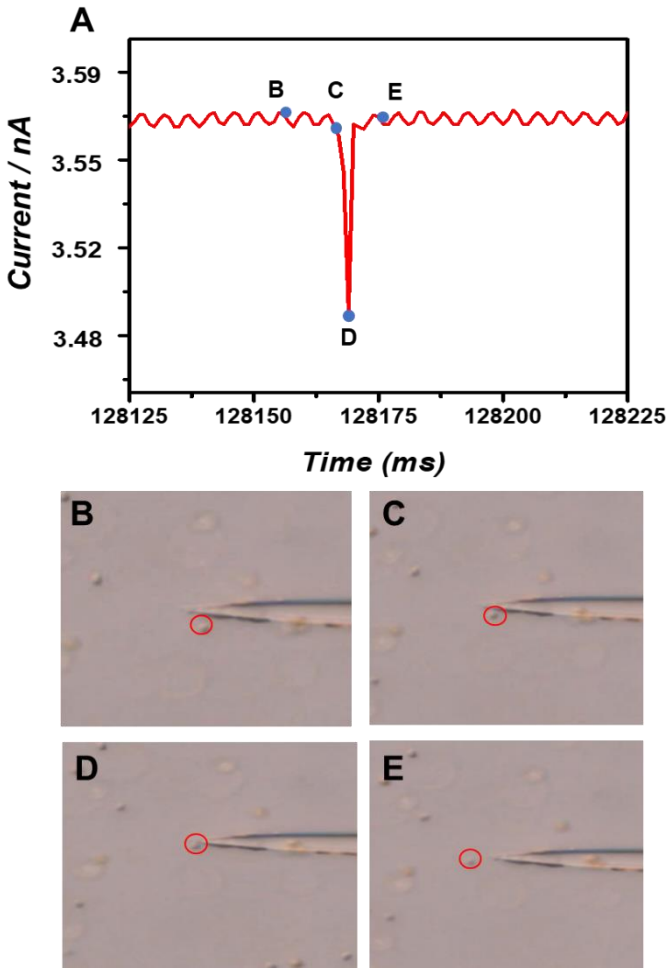

Figure S2. (A) Representative current transient recorded by a nanopipette with 200 nm diameter in 0.9% NaCl solution containing sheep RBCs. (B)~(E) Synchronous confocal laser scanning micrographs (CLSM) taken at the time indicated in (A) for the same cell. The concentration of sheep RBCs was 2 fM. The applied potential was 0.2 V.

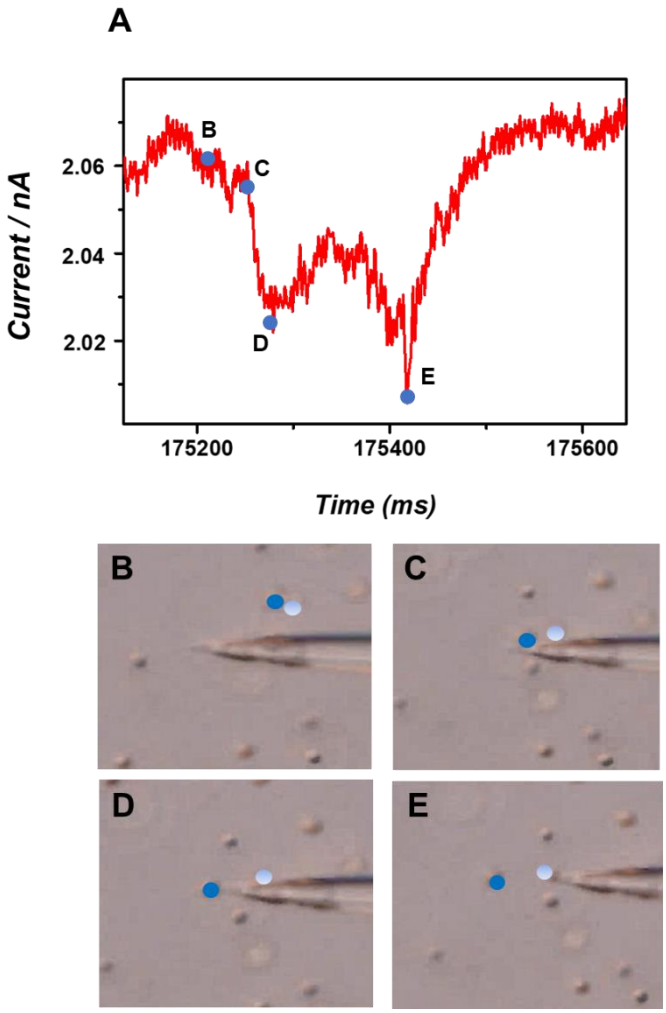

Figure S3. (A) Representative current transient recorded by a nanopipette with 200 nm diameter in 0.9% NaCl solution containing sheep RBCs. (B~E) Synchronous confocal laser scanning micrographs (CLSM) taken at the time indicated in the (A) for two different cells which almost arrived at the orifice of the nanopipette tip at the same time. The concentration of sheep RBCs was 5 fM. The applied potential was 0.2 V.

S3 Characterization of RBCs

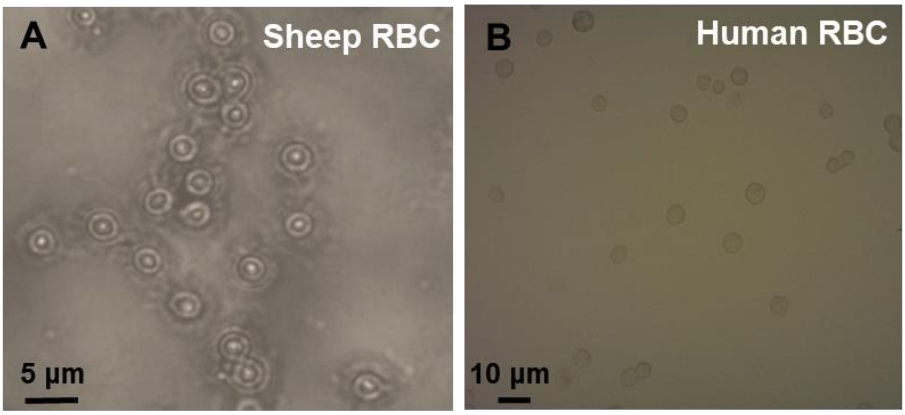

Figure S4. Optical images of sheep RBCs (A) and human RBCs (B).

S4 Typical *i-t* Traces of Human RBCs with Different Concentrations

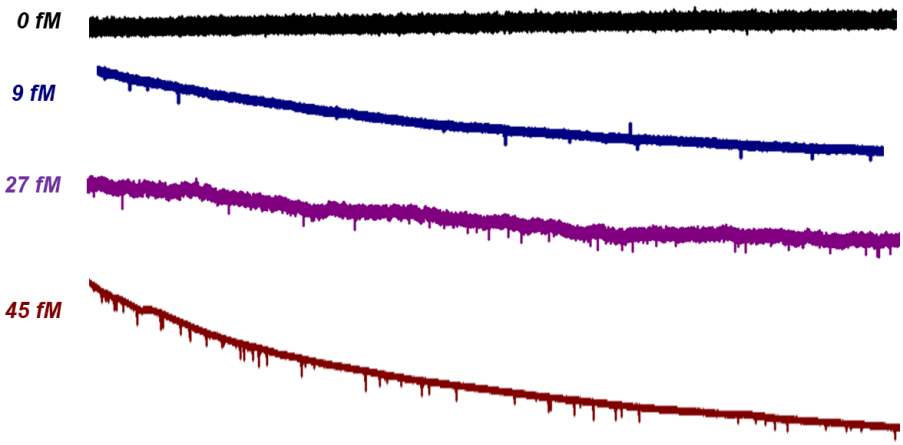

Figure S5. (A) Current transients obtained at nanopipette (*ca.* 200 nm in diameter) in 0.9% NaCl solution containing 0, 9, 27 and 45 fM human RBCs. The applied potential was 200 mV.

S5 Derivation of Equation 2

Equation 2 in the main text was derived as follows. Here, we consider RBCs as sphere particles with the same size to simplify the model. The transference number of a red blood cell (t_{RBC}) represents the relative flux of charged RBCs in the electrolyte solution.

$$t_{RBC}=\frac{|Z_{RBC}|\, D_{RBC}\, C_{RBC}}{\sum_i |Z_{RBC}|\, D_i\, C_i} \tag{S1}$$

The sum runs over all charged species in solution. The Nernst-Planck equation, equation S2, could be used to describe the fluxes of the ionic species

$$J_i=-D_i \nabla c_i-\frac{z_i F}{RT} D_i\, C_i \nabla \Phi+u\, C_i \tag{S2}$$

where, J_i, D_i, C_i and z_i are, respectively, the flux, diffusion coefficient, concentration and charge of species i. Φ and u are the local electric potential and fluid velocity, and F, R and T are the Faraday constant, the gas constant and the absolute temperature, respectively. In the present condition, this sum is completely dominated by the salt ions Na^+ and Cl^- since the RBCs concentration is femtomolar. Thus, we could simplify the equation (S2) to obtain equation (S3)

$$t_{RBC}=\frac{|Z_{RBC}|\, D_{RBC}\, C_{RBC}}{\left(D_{Na^+}+D_{Cl^-}\right) C_{NaCl}} \tag{S3}$$

t_{RBC} could also be obtained by the equation S4

$$t_{RBC}={i_{RBC}}/{i_{Total}} \tag{S4}$$

i_{Total} is the total current across the nanopipette (i.e., *I*). The current of RBCs can be converted to the flux of RBCs by equation S5.

$$j_{mig}={i_{RBC}}/{z_{RBC}F} \tag{S5}$$

Considering the relationship of mobility and diffusion coefficient as shown in equation S6

$$\mu_i={z_i D_i F}/{RT} \tag{S6}$$

One can estimate the flux of RBCs migrating to the nanopipette orifice per second by equation S7.

$$j_{mig} = \frac{iC_{RBC}}{FC_{NaCl}} \frac{\mu_{RBC}}{\mu_{Na^+} + \mu_{Cl^-}} \quad \text{(S7)}$$

The frequency of RBCs is obtained simply by multiplying by Avogadro's number.

$$f_{mig} = N_A j_{mig} = \frac{IC_{RBC}}{eC_{NaCl}} \frac{\mu_{RBC}}{\mu_{Na^+} + \mu_{Cl^-}} \quad \text{(S8)}$$

The diffusion coefficient of RBCs is approximately calculated by equation S9.

$$D = \frac{\kappa T}{6 \pi \eta r_{RBC}} \quad \text{(S9)}$$

Herein, for 3 μm-diameter sheep RBCs and 7 μm-diameter human RBCs, the mobility measured by DLS was *ca.* 9.226×10^{-9} $m^2v^{-1}s^{-1}$ and 7.638×10^{-9} $m^2v^{-1}s^{-1}$, respectively. In addition, $\mu_{Na^+} \approx 5.19\times10^{-8}$ $m^2v^{-1}s^{-1}$, $\mu_{Cl^-} \approx 7.79\times10^{-8}$ $m^2v^{-1}s^{-1}$. For the sheep RBCs and human RBCs, the diffusion coefficients were about 1.63×10^{-13} m^2 s^{-1} and 6.98×10^{-14} m^2 s^{-1}.

S6 Distribution Histograms of Different Measurements Number

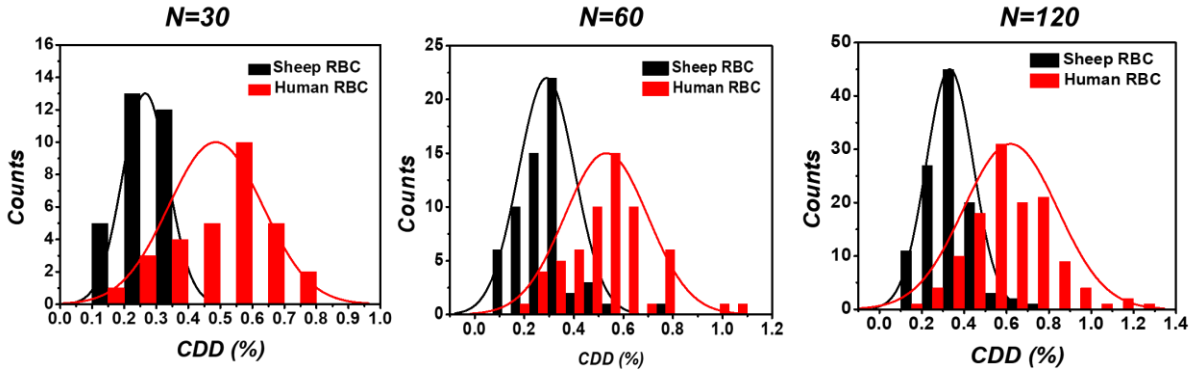

Figure S6. Distribution histograms of CDD based on different measurements number (from 30 to 120).

S7 Distribution of Dwell Time at Different pH

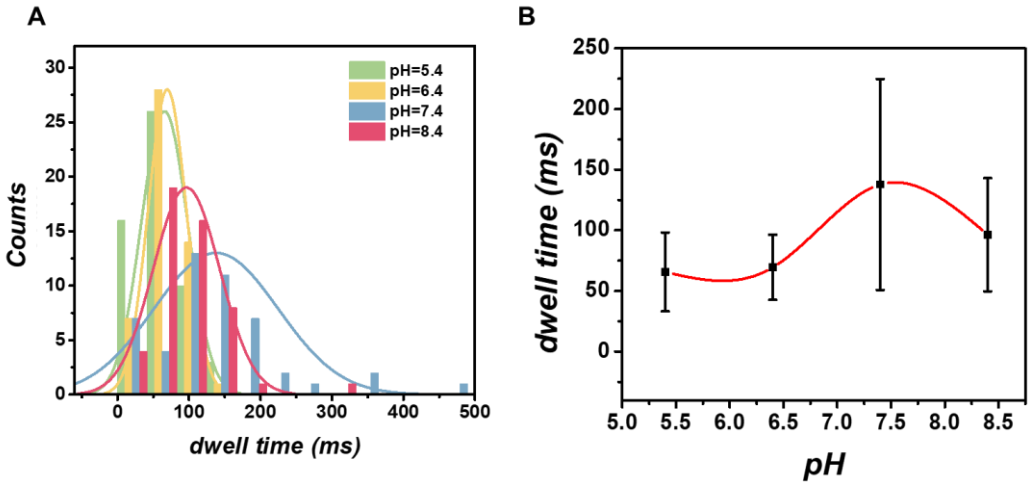

Figure S7. Distribution of dwell time at different pH.

S8 Distribution of Dwell Time under Different Osmotic Pressure

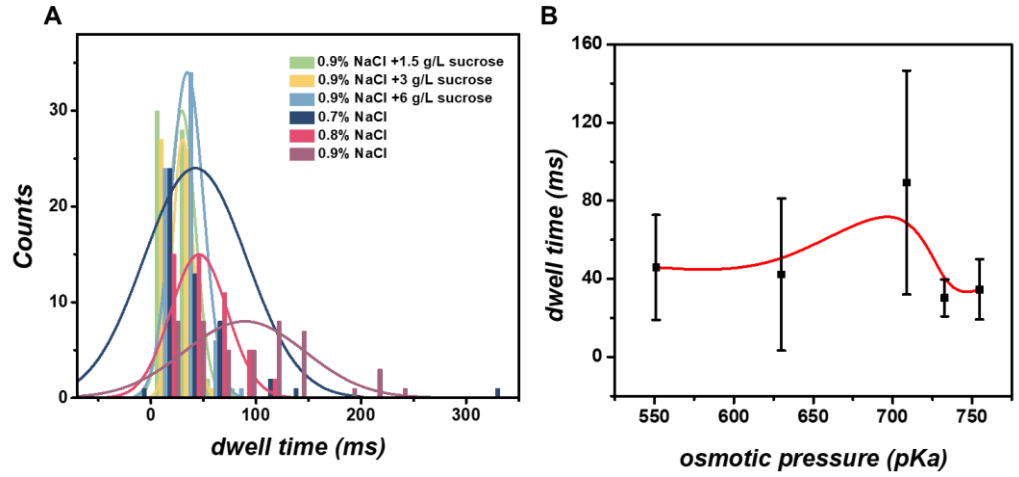

Figure S8. Distribution of dwell time under different osmotic pressure.

S9 The values of osmotic pressure corresponding to different kinds of saline

Table S1. The values of osmotic pressure corresponding to different kinds of saline. The values of osmotic pressure were calculated by J.H.van't Hoff formula: Π=cRT

Concentration of saline	0.7% saline	0.8% saline	0.9% saline	0.9% saline + 3g/L sucrose	0.9% saline + 6g/L sucrose
Value of osmotic pressure (kPa)	551.4	630.1	708.9	732.8	754.8