Supplementary information for

A real-time simultaneous measurement on a microfluidic device for individual bacteria discrimination

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This file includes:

Supplementary Figures S-1 to S-4, derivation of theoretical equation, and Supplementary Videos S-1 and S-2

Derivation of theoretical equation

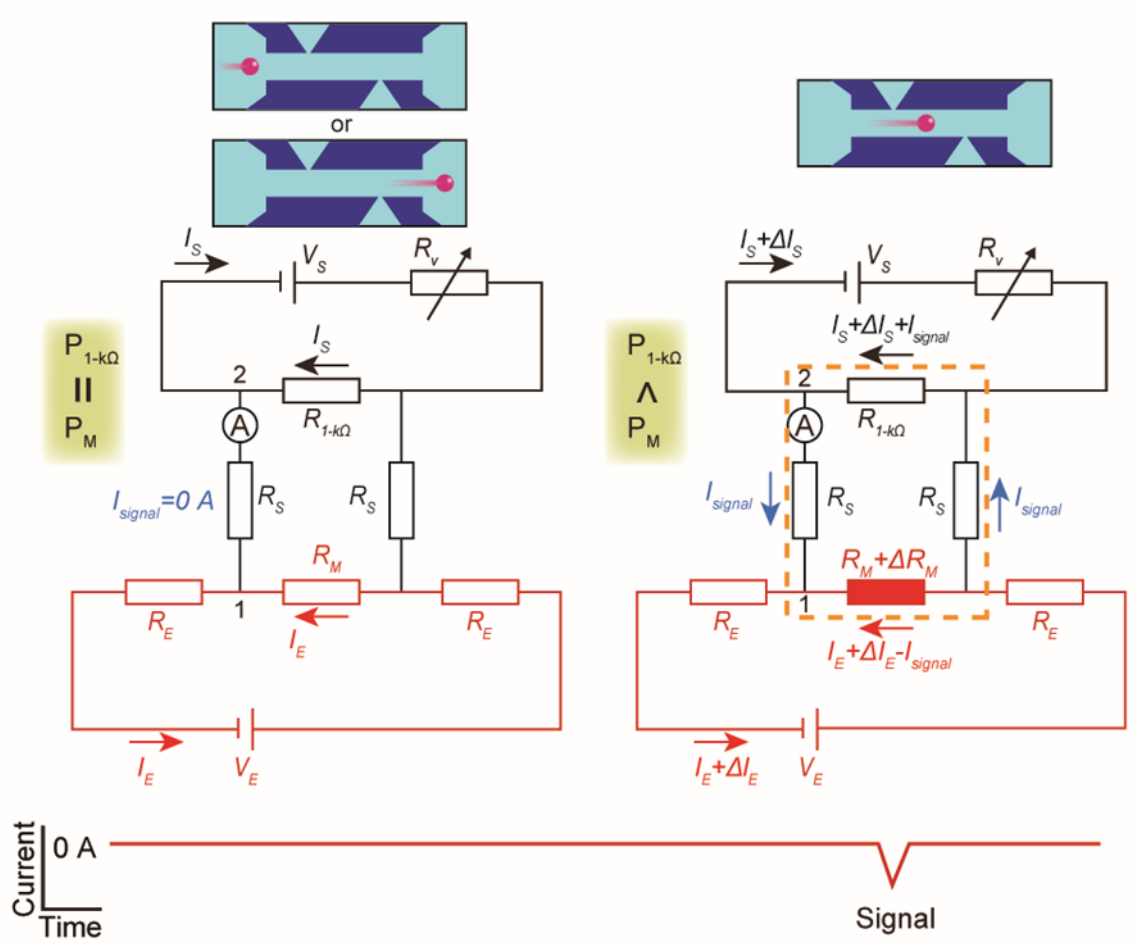

Figure S-1. Schematic illustrations of sample particle translocation, electric circuit diagrams, and schematic current value in the microfluidic bridge circuit for derivation of theoretical equation. The left side shows the situation before and after passage of a sample particle in the micropore. The right side shows the situation for the time the sample particle passed in the micropore. Red lines are for the electrophoresis circuit, and black lines are for the sensing circuit. The electrophoresis circuit has a voltage source (V_E) and resistors for the micropore (R_M) and electrophoresis channels (R_E). The sensing circuit have resistors for sensing channel (R_S), an Ampere meter (A), a voltage source (V_S), a variable resistor (R_v), and a 1-kΩ resistor ($R_{1\text{-k}\Omega}$). Red filled squares show increased resistance by sample introduction. Red and black arrows show flow directions of current in the electrophoresis circuit and the sensing circuit, respectively. Blue arrows show flow directions of current by deviation of potential differences between the electrophoresis and sensing circuits. The numbers "1" and "2" are the electrical potential of the micropore entrance and the electrical potential of the

cathode of the voltage source in the sensing circuit, respectively. $P_{1\text{-}k\Omega}$ and P_M are the potential differences of both ends for the 1-kΩ resistor and the micropore, respectively.

Our method uses two electrical circuits: an electrophoresis circuit and a sensing circuit. We derived a theoretical equation to calculate signal amplitudes, which are detected in this method, based on Ohm's law and Kirchhoff's law. By adjusting the electrical potential of the micropore entrance to the electrical potential of the cathode of the voltage source in the sensing circuit, we can get balanced state. In the balanced state, no current flows in the bridge channels (situation of the left-side drawing in Fig. S-1), and the following equations are formulated for each "isolated" circuit:

$$I_E = \frac{V_E}{2R_E + R_M} \qquad (S1)$$

$$I_S = \frac{V_S}{R_{1-k\Omega} + R_v} \qquad (S2)$$

where R_E, R_M, $R_{1\text{-}k\Omega}$, and R_v are the electrical resistances of electrophoresis channels, the micropore, the 1-kΩ resistor, and the variable resistor, respectively. V_E and V_S are the applied voltages for the electrophoresis and sensing circuits, respectively. I_E and I_S are currents in the electrophoresis and sensing circuits, respectively. Since potential differences between both ends of the micropore (R_M) and the 1-kΩ resistor ($R_{1\text{-}k\Omega}$) are the same in the balanced state, Eqs (S1) and (S2) are transformed as:

$$R_M \times I_E = R_{1-k\Omega} \times I_S \qquad (S3)$$

When a sample is passing through the micropore, the current in the electrophoresis circuit changes from I_E to $I_E+\Delta I_E$:

$$I_E + \Delta I_E = \frac{V_E}{2R_E + R_M + \Delta R_M} \qquad (S4)$$

where ΔR_M and ΔI_E are resistance change of the micropore and current change in the electrophoresis circuit by sample introduction, respectively. At this time, the potential difference between both ends of the micropore (P_M) is increased by the increasing resistance, and the signal (I_{signal}) flows to an Ampere meter to compensate for the potential difference between points 1and 2 (situation of the right-side drawing in Fig. S-1). At this time, the balanced state is lost. The following equation is the sum of the voltages of the circuit surrounded by the orange dotted line:

$$\left(I_E + \Delta I_E - I_{signal}\right)(R_M + \Delta R_M) = I_{signal}(2R_S + R_{1-k\Omega}) + (I_S + \Delta I_S)R_{1-k\Omega} \qquad (S5)$$

where R_S is the electrical resistances of the sensing channels. ΔI_S is current change in the sensing circuit by sample introduction to the micropore. The following equation is the expansion equation of Eq (S5).

$$I_E R_M + I_E \Delta R_M + \Delta I_E R_M + \Delta I_E \Delta R_M - I_S R_{1-k\Omega} - \Delta I_S R_{1-k\Omega} = I_{signal}(2R_S + R_{1-k\Omega} + R_M) + I_{signal}\Delta R_M \qquad (S6)$$

Since ΔI_E and I_{signal} are small enough compared with I_E and I_S, the fourth term on the left side ($\Delta I_E \Delta R_M$) and the second term on the right side ($I_{signal}\Delta R_M$) can be ignored, and Eq (S6) can be transformed by Eq (S3) as follows.

$$I_E \Delta R_M \approx I_{signal}(2R_S) + \left(I_{signal} + \Delta I_S\right)R_{1-k\Omega} + \left(I_{signal} + \Delta I_E\right)R_M \quad (S7)$$

The following equation is the sum of the voltages of the sensing circuit.

$$V_S = R_v(I_S + \Delta I_S) + R_{1-k\Omega}\left(I_S + \Delta I_S + I_{signal}\right) \quad (S8)$$

Eq (S8) can be transformed by Eq (S2) as follows.

$$\Delta I_S = \frac{-R_{1-k\Omega}}{R_v + R_{1-k\Omega}} \cdot I_{signal} \quad (S9)$$

Based on Eq (S9), the following approximate expression is obtained when R_v is large enough compared with $R_{1\text{-}k\Omega}$.

$$|\Delta I_S| \ll \left|I_{signal}\right| \quad (S10)$$

The following equation is the sum of the voltages of the red circuit.

$$V_E = (I_E + \Delta I_E)(2R_E + R_M) - I_{signal}R_M \quad (S11)$$

When ΔR_M is small enough compared with R_M, Eq (S11) can be transformed by Eq (S1) as follows.

$$\Delta I_E \approx \frac{R_M}{2R_E + R_M} \cdot I_{signal} \quad (S12)$$

Based on Eq (S12), the following approximate expression is obtained when R_M is small enough compared with $2R_E$.

$$|\Delta I_E| \ll \left|I_{signal}\right| \quad (S13)$$

Based on Eqs (S10) and (S13), Eq (S7) can be transformed as follows.

$$I_E \Delta R_M \approx I_{signal}(2R_S + R_{1-k\Omega} + R_M) \quad (S14)$$

Therefore, the detected current signals can be theoretically predicted by Eq (S8) (this is Eq (1) of the main text).

$$I_{signal} = \frac{\Delta R_M}{2R_S + R_{1-k\Omega} + R_M} \times \frac{V_E}{2R_E + R_M} \quad (S15)$$

Figure S-2

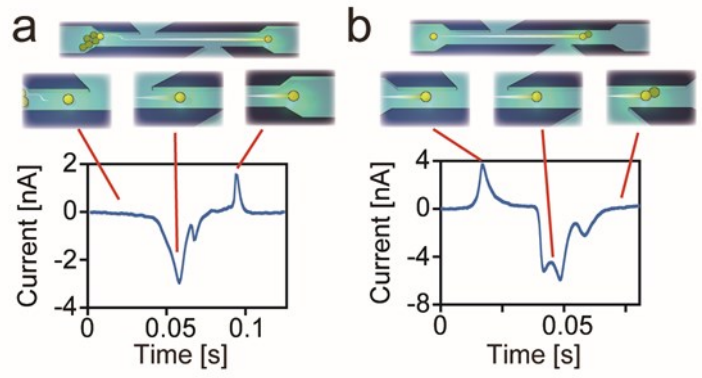

Figure S-2. Relationship between ionic current signal shape and behavior of sample around the micropore. (a) Ionic current signal when a particle clogging the entrance of guide-1 passed through the micropore (see Supplementary Video S-1). An early positive signal peak did not appear. (b) Ionic current signal when a particle clogged in gide-2 (see Supplementary Video S-2). A late positive signal peak did not appear.

Figure S-3

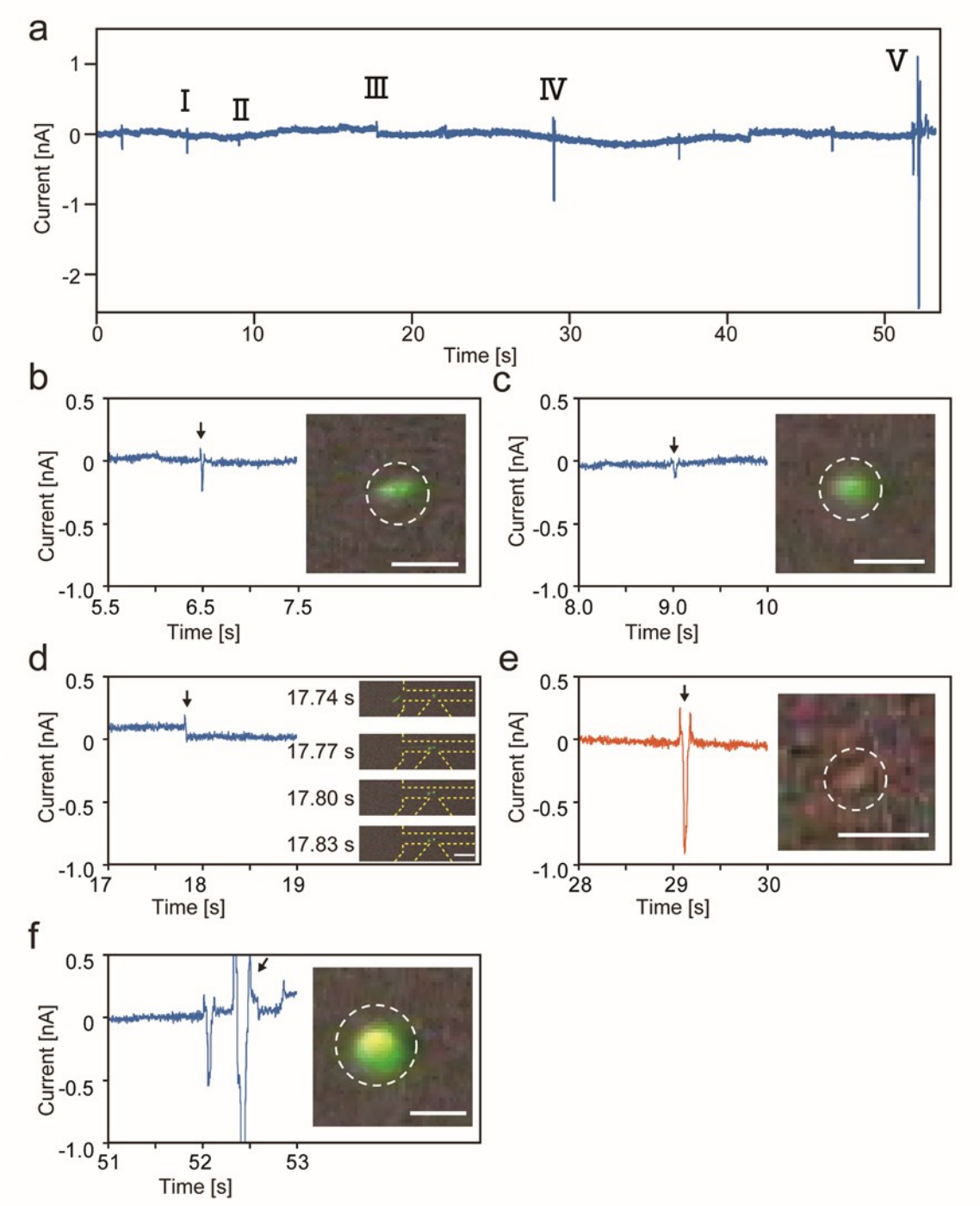

Figure S-3. Discrimination of solution mixture including bacteria sample and contaminants by the simultaneous ionic current sensing and fluorescence observation method in the micropore having width of 4.0 µm, height of 7.5 µm, and length of 50 µm. The solution mixture included *E. coli* (1.5 to 4.4 fL, green stained), *B. subtilis* (1.5 to 4.2 fL, orange stained), 0.75 µm polystyrene particles (about 0.22 fL, green fluorescent color), and 1.00 µm polystyrene particles (about 0.52 fL, green fluorescent color). The solution mixture was introduced into the micropore. (a) Raw

data of ionic current sensing. Signals I to V were defined. (b) Expanded view and fluorescence image of signal I. Volume was calculated as 0.54 fL by I_{signal}. Based on volume and fluorescent color, this signal was caused by introduction of 1.00 μm green fluorescent particle. (c) Expanded view and fluorescence image of signal II. Volume was calculated as 0.21 fL by I_{signal}. Based on volume and fluorescent color, this signal was caused by introduction of 0.75 μm green fluorescent particle. (d) Expanded view and fluorescence image of signal III; scale bars. Based on fluorescence observation, this signal was caused by clogging of green fluorescent particle in the micropore. (e) Expanded view and fluorescence image of signal IV; scale bars. Volume was calculated as 1.58 fL by I_{signal}. Based on volume and fluorescent color, this signal was caused by introduction of *B. subtilis*. (f) Expanded view and fluorescence image of signal V; scale bars. Volume was calculated as 5.20 fL by I_{signal}. Based on volume and fluorescent color, this signal was caused by introduction of aggregated green fluorescent particles. Scale bars of all images are 10 μm.

Figure S-4

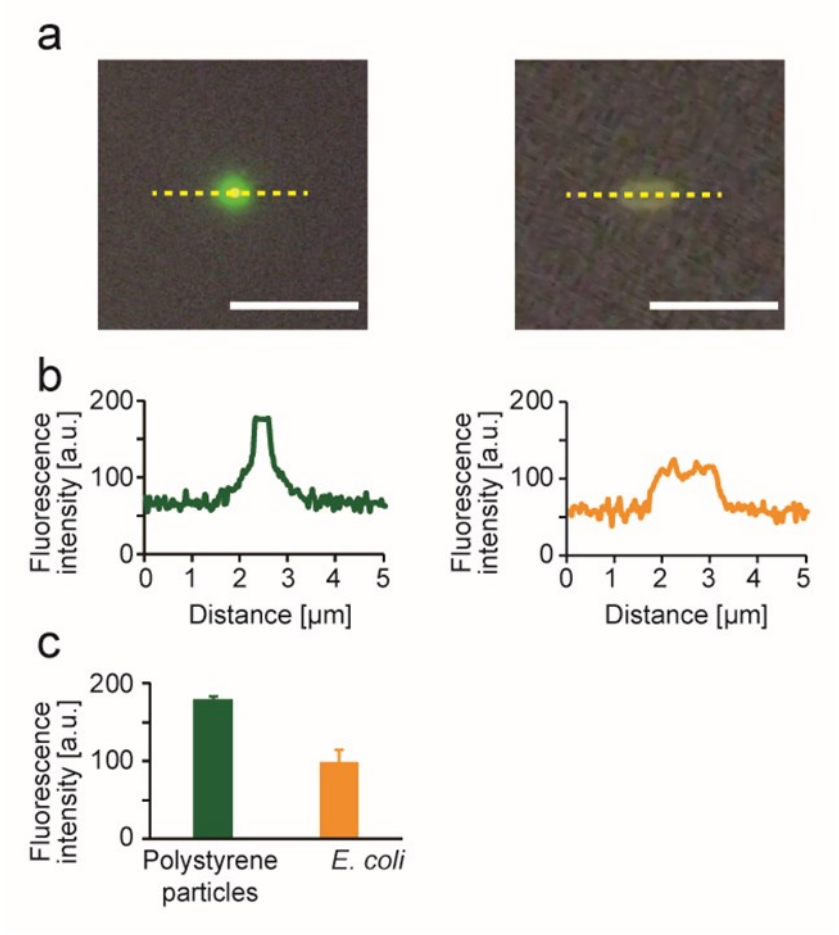

Figure S-4 Comparison of fluorescence intensity between *E. coli* and polystyrene particles which have same fluorescence wavelength. (a) Fluorescence images of polystyrene particle (left) and *E. coli* (right); scale bar, 2 μm. (b) Fluorescence intensity of yellow dashed line of each image. (c) Comparison of fluorescence intensity. Error bars show the standard deviation for a series of measurements (N = 100).

Supplementary Video S-1

Supplementary Video S-1. Fluorescence observation of a 3.10 μm particle clogging the entrance of guide-1 passed through the micropore. Time of video is 4 s. (AVI; 14.7 MB)

Supplementary Video S-2. Fluorescence observation when a 3.10 μm particle clogged in gide-2. Time of video is 4 s. (AVI; 14.7 MB)