Supporting information for

Efficient analysis of a small number of cancer cells at the single-cell level using electroactive double-well array

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Supplementary Methods

The Clausius-Mossotti (CM) factor for mammalian cells

When a dielectric particle is subjected to a non-uniform electric field, dielectrophoresis (DEP) force is exerted on the particle. The DEP force ($\mathbf{F}_{DEP}$) acting on the spherical cell of a radius (a) can be approximated by

$$\mathbf{F}_{DEP} = 2\pi\varepsilon_e a^3 \operatorname{Re}[K(2\pi f)]\nabla|E_\mathbf{e}|^2 \tag{1}$$

where ε_e and E_e are permittivity of suspending medium and applied electric field, respectively. Clausius-Mossotti (CM) factor, $K(2\pi f)$, which represent the relative permittivity between the cell and the suspending medium, for the spherical shell model[1] is

$$K(2\pi f) = \frac{\varepsilon_{cell}^* - \varepsilon_e^*}{\varepsilon_{cell}^* + 2\varepsilon_e^*}; \quad \varepsilon_{cell}^* = \varepsilon_m^* \frac{a^3(\varepsilon_i^* + 2\varepsilon_m^*) + 2(a - d_m)^3(\varepsilon_i^* - \varepsilon_m^*)}{a^3(\varepsilon_i^* + 2\varepsilon_m^*) - (a - d_m)^3(\varepsilon_i^* - \varepsilon_m^*)} \tag{2}$$

where $\varepsilon^* = \varepsilon + \dfrac{\sigma}{2\pi f}j$ is complex permittivity, and $j = (-1)^{1/2}$. σ, f and d_m are conductivity, frequency of the applied electric potential, and thickness of the cell membrane, respectively. Subscripts *cell* and *e* represent cell and the suspending medium, and *i* and *m* represent internal and membrane of the cell, respectively. Frequency dependences of the real part of the CM factor for various conductivity of the medium (**Supplementary Fig. S1**) were calculated with typical cell parameters of mammalian cells (**Supplementary Table**).

Supplementary Table. Typical cell parameters[2] used for the calculation.

		Unit	Internal (i)	Membrane (m)
Conductivity	σ	S m^{-1}	0.5	3×10^{-6}
Permittivity	ε	ε_0	50	8
Membrane thickness	d_m	m	8×10^{-9}	

Supplementary References

1. T. B. Jones, Electromechanics of particles, Cambridge University Press, Cambridge; New York, 1995.
2. G. Fuhr, H. Glasser, T. Muller and T. Schnelle, Biochim Biophys Acta, 1994, 1201, 353-360.

Supplementary Figures
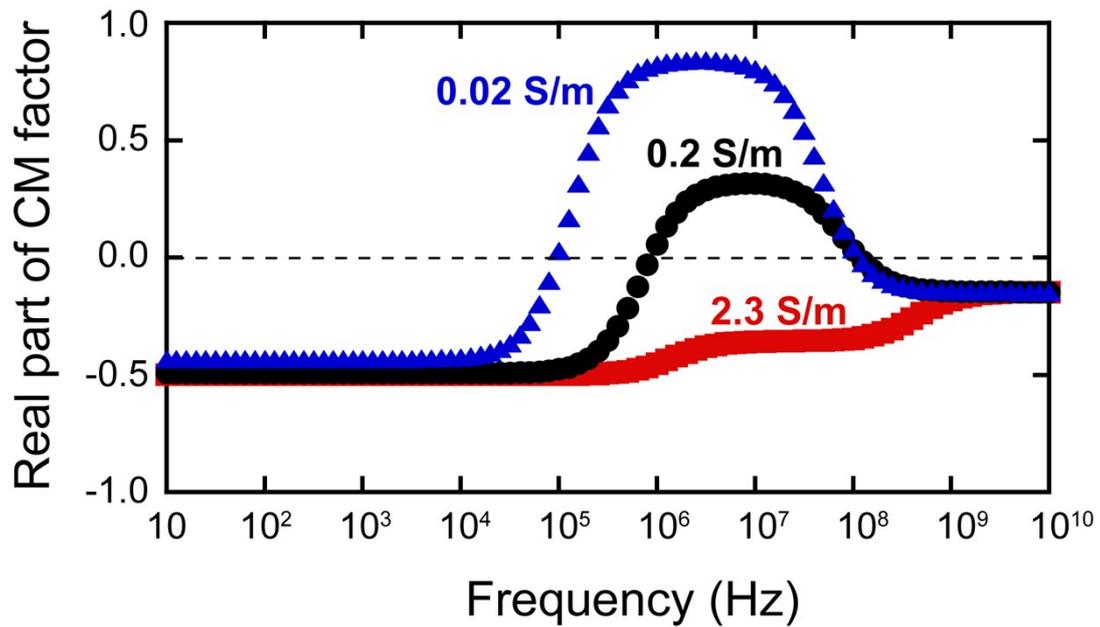
Supplementary Fig. S1. Frequency dependence of the real part of the CM factor for various conductivities of the medium. The real part of the CM factor showed negative values with the culture medium (2.3 S m^{-1}). When the conductivity of the medium decreased, the CM factor showed positive values with a megahertz-order electric field.
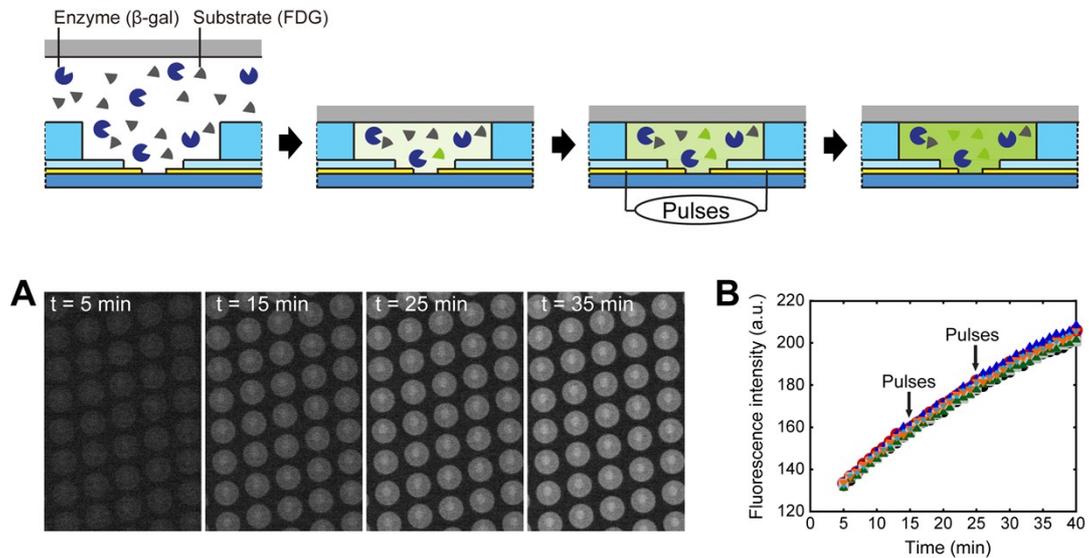
Supplementary Fig. S2. Recombinant β-galactosidase (β-gal) assay using an EdWA. To investigate a possible effect of the pulses on the β-gal activity, recombinant β-gal in the low conductivity buffer containing FDG was introduced into the device and confined into the EdWA by pressing on the PDMS membrane. Bipolar pulses used for the cell lysis were applied to the electrodes during the reaction. (A) Time-lapse images of the EdWA during incubation. The recombinant β-gal (10 nM) and FDG (1 mM) were mixed in a tube at t = 0 min and introduced into the device. (B) Kinetic traces of the average fluorescence intensity from each double-well, where bipolar pulses (Vpp = 100 V and f = 100 KHz for 10 ms) were applied to the electrodes at t = 15 min and 25 min. No significant changes in fluorescence kinetics were observed. The results indicated that effect of the pulses on β-gal activity was negligible.

Supplementary Movie Legend

Supplementary Movie. A representative PC3 cell trapping using an EdWA. PC3 cell suspension was introduced into the microfluidic channel at a flow rate of 2 μL min^{-1}. PC3 cells were trapped in the trap-wells that were patterned at the bottom of the reaction-wells by applying a sinusoidal electric potential (Vpp = 5V and f = 8 MHz) to the electrodes. Bright-field images were acquired at the start of the movie to verify position of the double-wells in the array. Subsequently, fluorescence images were acquired to monitor the single-cell trapping performance of the EdWA.