Supplementary Information for

Cancer marker-free enrichment and direct mutation detection in rare cancer cells by combining multi-property isolation and microfluidic concentration

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Dielectrophoresis

Dielectrophoresis (DEP) is a phenomenon in which a force is exerted on a dielectric particle when it is immersed in a non-uniform electric field. The DEP force ($\mathbf{F}_{DEP}$) induced on a spherical cell can be approximated by

$$\boldsymbol{F}_{DEP} = 2\pi\varepsilon_e a^3 \mathrm{Re}[K(2\pi f)]\nabla|E|^2, \quad (1)$$

$$K(2\pi f) = \frac{\varepsilon^*_{cell} - \varepsilon^*_e}{\varepsilon^*_{cell} + 2\varepsilon^*_e}, \quad (2)$$

where ε_e, a, f, and E are the permittivity of the medium, the radius of cell, the frequency, and the electric field strength, respectively. $\varepsilon^* = \varepsilon + \frac{\sigma}{2\pi f} j$ is the complex permittivity, where σ is the conductivity and $j = (-1)^{1/2}$, and subscripts *cell* and *e* represent cell and the medium. When the real part of the polarization factor, $\mathrm{Re}[K(2\pi f)]$, is larger than 0, particles are attracted toward the strong electric field (positive DEP, pDEP). Conversely, when $\mathrm{Re}[K(2\pi f)]$ is less than 0, particles are directed away from the strong electric field (negative DEP, nDEP). The real part of the polarization factor can be controlled by adjusting the conductivity of the external medium and the frequency of the applied electric fields.

The CTC-FIND method utilizes pDEP to trap fixed cells in the microfluidic device for the concentration of the cells. Since the fixation changes electrical property of the cells, DEP responses of live and fixed cancer cells with respect to the conductivity of external medium and the frequency of the applied electric field were investigated by using an interdigitated electrode (line: 92 μm; space: 8μm). The live cells showed pDEP responses (cells were trapped at the edge of the electrode) at a megahertz-order electric field with the conductivity of 160 mS/m as shown in the following table. However, the cells showed nDEP responses (cells were pushed away from the edge of the electrode) at a kilohertz-order electric field with the same conductivity of 160 mS/m.

Conductivity of medium	DEP response (live cell)			
	10 kHz	100 kHz	1 MHz	10 MHz
160 mS/m	Negative	Negative	Positive	Positive
17 mS/m	Negative	Positive	Positive	Positive

However, the cancer cells fixed with 1% paraformaldehyde showed nDEP responses with the conductivity of 160 mS/m. Further decreased conductivity of external medium was required to induce pDEP as shown in the following table.

Conductivity of medium	DEP response (fixed cell)			
	10 kHz	100 kHz	1 MHz	10 MHz
160 mS/m	Negative	Negative	Negative	Negative
17 mS/m	Negative	Negative	Negative	Negative
4.7 mS/m	Negative	Negative	Positive	Positive
1.7 mS/m	Positive	Positive	Positive	Positive
0.2 mS/m	Positive	Positive	Positive	Positive

The fixed cells showed pDEP responses at a megahertz-order electric field with the conductivity of 4.7 mS/m. The observed responses of fixed cells seem follow single-shell model[1] used for the prediction of DEP responses of live mammalian cells; nDEP at a low frequency and pDEP at a megahertz-order frequency. However, the required conductivity of external medium for paraformaldehyde-fixed cells was much lower compared to that for live mammalian cells to induce pDEP. Further investigation is required to clearly understand the electrical properties of fixed cells.

In the present study, a low-conductivity buffer (10mM HEPES, 0.1mM CaCl2, 59mM D-glucose, 236mM sucrose and 0.2% BSA, 4.2 mS/m) and 1MHz of electrical potential were used to trap fixed cells.

1. F. F. Becker, X. B. Wang, Y. Huang, R. Pethig, J. Vykoukal and P. R. Gascoyne, *Proceedings of the National Academy of Sciences of the United States of America*, 1995, 92, 860-864.

Supplementary Table 1. Detection conditions (primer sets, quenching probes and PCR conditions) for each genetic mutation analysis.

Detected genotypes	Forward primer sequence	Reverse primer sequence	Probe sequence	PCR condition
EGFR L858R	5'- AGG AAC GTA CTG GTG AAA ACA CCG C -3'	5'- GCC TCC TTC TGC ATG GTA TTC TTT CTC -3'	5'- TTG GCC CGC CCA AAA TC-(PB) -3'	95°C for 60 s, 10 cycles at 95°C for 2 s, and 56°C for 15 s, followed by 50 cycles at 95°C for 1 s and 58°C for 30 s
EGFR ex19del	5'- TCT CTC TGT CAT AGG GAC TC -3'	5'- GAA ACT CAC ATC GAG GAT TTC -3'	5'- (FL)-CCC GTC GCT ATC AAG GAA TTA AGA GAA GC -3'	95°C for 60 s, 10 cycles at 95°C for 2 s, and 56°C for 15 s, followed by 50 cycles at 95°C for 1 s and 58°C for 30 s
KRAS codon12, 13	5'- AAG GCC TGC TGA AAA TGA CTG -3'	5'- GGT CCT GCA CCA GTA ATA TGC A -3'	5'- (TAMRA)-CTC TTG CCT ACG CCA CCA GCT CCA ACT -3'	95°C for 60 s, 10 cycles at 95°C for 2 s, and 58°C for 15 s, followed by 50 cycles at 95°C for 1 s and 60°C for 15 s
PIK3CA G1633A	5'- GAA CAG CTC AAA GCA ATT TCT ACA CGA G -3'	5'- CAG AGA ATY* TCC ATT TTA GCA CTT ACY* TGT GAC -3'	5'- (TAMRA)-CTC TCT GAA ATC ACT AAG C -3'	95°C for 60 s, 10 cycles at 95°C for 2 s, and 56°C for 15 s, followed by 50 cycles at 95°C for 1 s and 58°C for 30 s

* : mixed oligonucleotide 〔Y=C/T〕

Supplementary Table 2. Sensitivity and reproducibility of spike-in experiments.

Test	Cell line (Genotype)	Expected count [cells in 8 mL]	Detection result		
			Cancer cell count [cells]	Remained leukocyte count [cells]	EGFR genotype
#1	NCI-H1650 (ex19del)	8 (S.D. ± 1)	2	98	ex19del
#2		8 (S.D. ± 1)	3	121	ex19del
#3		8 (S.D. ± 3)	2	25	ex19del
#4		8 (S.D. ± 3)	2	48	ex19del
#5		12 (S.D. ± 3)	2	30	ex19del
#6	NCI-H1975 (L858R)	11(S.D. ± 4)	5	80	L858R
#7		7 (S.D. ± 3)	4	129	L858R
#8		12 (S.D. ± 3)	9	79	L858R
#9		7 (S.D. ± 3)	1	165	L858R
#10		10 (S.D. ± 3)	3	91	L858R
Control	A549 (WT)	6 (S.D. ± 2)	4	199	WT
Mean residual nucleated cell [cells]				97	

To count the number of spiked cells (expected counts), the same volume of cell suspension used for the spike-in experiments was pipetted into each well of a 384-well plate and the images of each well were taken using a fluorescence microscope. The number of spiked cells was calculated from the average over three replicates.