Electronic Supplementary Material (ESI) for Lab on a Chip.

Self-aligned sequential lateral field non-uniformities over channel depth for high throughput dielectrophoretic cell deflection

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S1. Calculation of the Minimum Electric Field required for DEP translation in device geometry

The "Minimum E-Field" in fig 4 (d) (main text) is the minimum electric field gradient required to displace a cell in the +y direction significantly after one orifice, fig S1 (a). We define significant displacement as the case when two cells are just distinguishable, fig S1 (b). Two cells are considered distinguishable when they do not overlap in the x-y plane, i.e., the center-to-center distance between two cells must be larger than the diameter of the cells. The simulation in this work uses RBCs as the benchmark; therefore, the center-to-center distance was set to be 5μm.

COMSOL's electric current, laminar flow, and particle tracing modules were used to simulate the x and y displacement of a particle traveling across one orifice at different applied voltages (0 Vpp, 110 Vpp, 130 Vpp, and 150 Vpp), fig S1 (c), where particles are released sufficiently far from the orifices to avoid pDEP trapping. By comparing the initial and final positions of the particles in fig S1 (c), we determined a voltage applied across the channel must be greater than 130 Vpp to displace a particle by 5μm in the +y direction. The electric current module was used to calculate the electric field norm at the center of the orifice for each of the voltage cases and plotted as a function of y position starting from the posts in fig S1 (d). By tracing the 130 Vpp case, an electric field norm of 7.06E5 V/m was determined as the minimum electric field required to displace a particle by 5μm at the center of the sample channel.

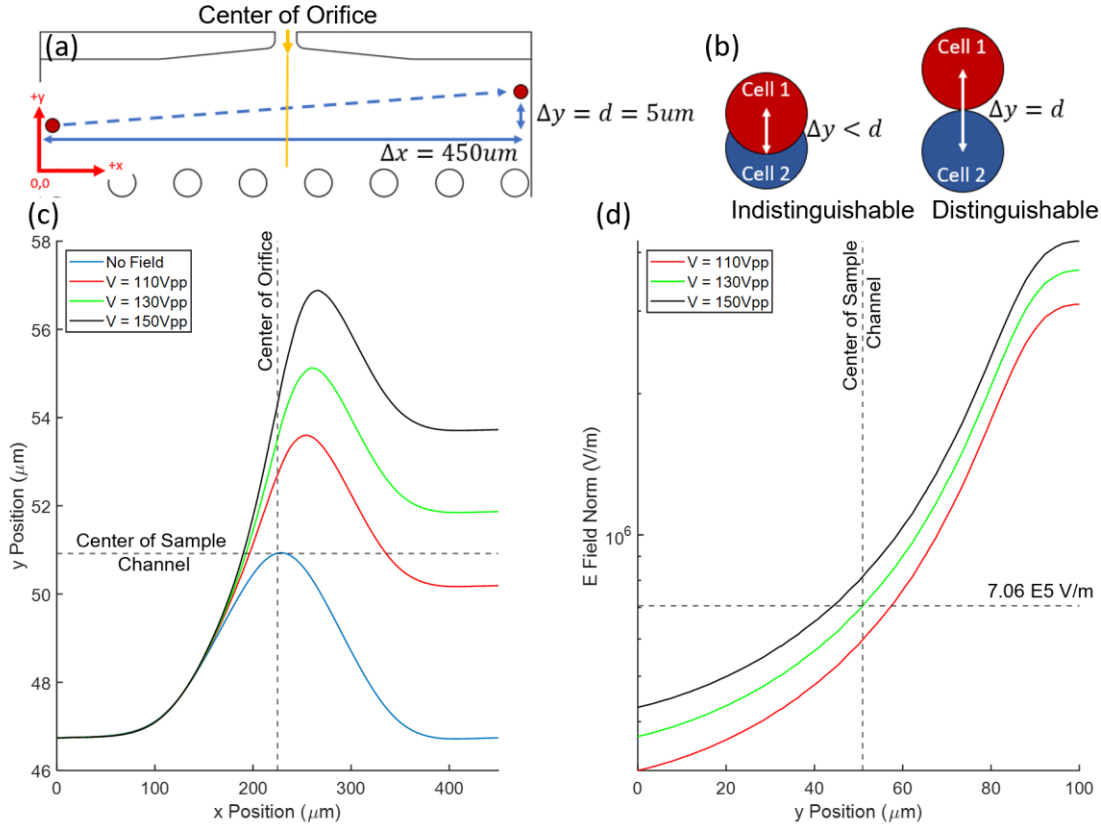


Fig S1. a, Minimum distance a particle must travers to be considered sufficiently displaced after traveling across 1 set of orifices. (b) Example of indistinguishable and distinguishable of two cells with current image processing techniques. (c) Particle's x and y displacement of a particle traveling across one orifice at different applied voltages (0 Vpp, 110 Vpp, 130 Vpp, and 150 Vpp). (d) Electric field norm at the center of the orifice (yellow line in (a)) for each voltage cases as a function of y position starting from the posts.

S2. Measurement of particle position using ImageJ

The function "find maxima" in ImageJ was used to measure the relative position of deflected RBCs from the orifice after DEP. An area after the last orifice was selected to be the measurement area, fig S2. The find maxima function may record artificial bright spots from the image; therefore, spots smaller than the expected RBC size are manually removed from the measured data points during analysis.

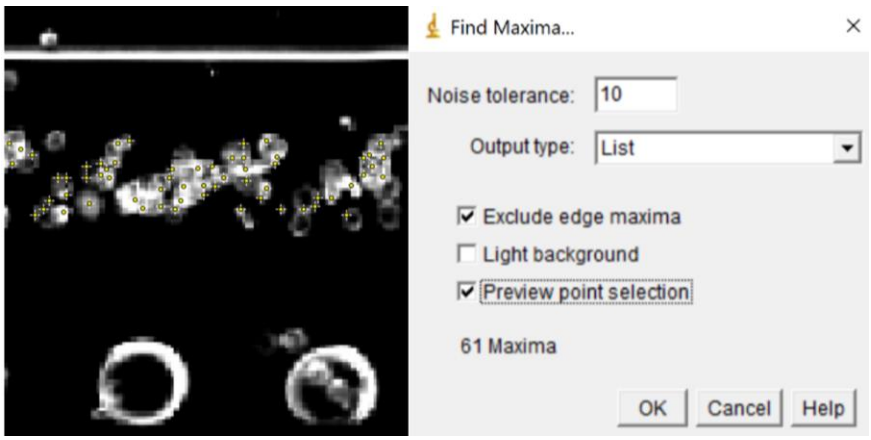


Fig S2. Example of the find maxima tool in ImageJ

S3. Membrane Capacitance of RBCs at Different Media Conductivities for Validating Device Performance

The membrane capacitance was calculated with Eq S1[1,2]

$$C_{mem} = \frac{\sigma_s}{\sqrt{2}\pi r f_{CO}} \qquad (1)$$

Eq S1. Membrane capacitance calculated from media conductivity, σ_s, radius of the particle, r, and crossover frequency f_{CO}.

Media Conductivity (µs/cm)	Membrane Capacitance (mF/m^2)
720	10.8
450	10.13
280	12.6

Table S1. Membrane Capacitance of healthy RBCs at different media conductivities with a mean of 11.13 mF/m^2 and a standard deviation of 1.28 mF/m^2. Membrane capacitance was calculated from equation Eq S1

S4. Statistical analysis on fRBCs and hRBCs deflection at different voltage frequencies

** fRBCs p>0.1

No Field	N = 1	N = 2	N = 3
N = 1		0.69	0.52
N = 2			0.22

40kHz	N = 1	N = 2	N = 3
N = 1		0.83	0.88
N = 2			0.68

200kHz	N = 1	N = 2	N = 3
N = 1		0.78	0.35
N = 2			0.2

** hRBCs p>0.1

No Field	N = 1	N = 2	N = 3
N = 1		0.76	0.78
N = 2			0.97

40kHz	N = 1	N = 2	N = 3
N = 1		0.43	0.77
N = 2			0.27

200kHz	N = 1	N = 2	N = 3
N = 1		0.27	0.97
N = 2			0.34

Table S2. T test comparing the particle positions of 3 individual frames of each experimental conditions (no field, 80V_{pp} at 40kHz, and 80 V_{pp} at 200kHz). All comparisons show a p value of greater than 0.1, indicating the measurement is reproducible at each condition for both the fixed and healthy RBCs.

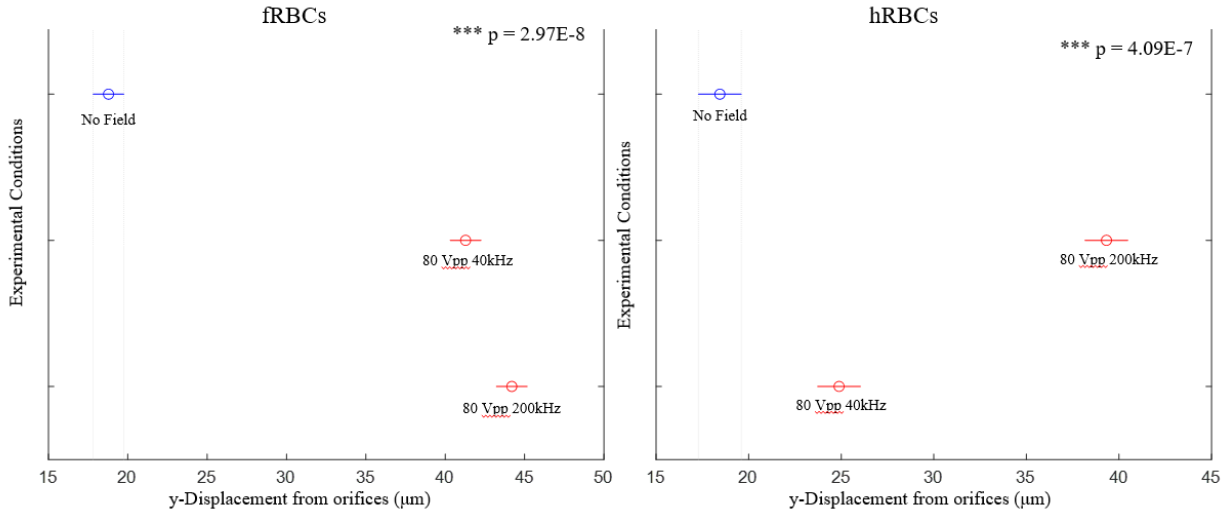


Fig S3. ANOVA comparing the mean deflected positions of each experimental conditions (no field, 80V_{pp} at 40kHz, and 80 V_{pp} at 200kHz) for fixed and healthy RBCs. The ANOVA shows each groups' means are significantly different from the other groups.

Mean Displacement (μm)

	40kHz	200kHz
hRBCs No Field	21	6.4
fRBCs No Field	22.5	25.4

P Values

	40kHz	200kHz
hRBCs No Field	7.1E-5	0.0033
fRBCs No Field	8.8E-5	5.7E-6

Table S3. Mean displacement in μm from their initial streamlines of hRBCs and fRBCs and their respective p values calculated from t tests.

S5. Dielectrophoresis Phenomena of Fixed and Healthy RBCs

Particle deflection by dielectrophoretic force is characterized by the polarizability of a particle in a non-uniform electric field[2] that is expressed as

$$\boldsymbol{F_{DEP}} = \mathbf{2}\pi\varepsilon_{\boldsymbol{m}}\boldsymbol{r^3}\boldsymbol{Re}(\boldsymbol{f_{cm}})(\boldsymbol{\nabla E^2}) \qquad (2)$$

where $\varepsilon_{\boldsymbol{m}}$ is the permittivity of the media, r is the radius of the particle, $\boldsymbol{Re}(\boldsymbol{f_{cm}})$ is the real part of the Clausius-Mossotti factor, and $\boldsymbol{\nabla E^2}$ is the Laplacian operator acting of the electric field squared. The equation[1] can be rewritten in terms of the crossover frequency ($\boldsymbol{f_{co}}$) as:

$$\boldsymbol{F_{DEP}} = \mathbf{2}\pi\varepsilon_{\boldsymbol{m}}\boldsymbol{r^3}\left(\frac{\boldsymbol{f^2}-\boldsymbol{f_{co}^2}}{\boldsymbol{f^2}+\mathbf{2}\boldsymbol{f_{co}^2}}\right)(\boldsymbol{\nabla E^2}) \qquad (3)$$

For frequencies, $\boldsymbol{f}$, below 1MHz, the crossover frequency can be approximated[1] as:

$$\boldsymbol{f_{co}} \approx \frac{\boldsymbol{\sigma_m}}{\sqrt{\mathbf{2}}\,\pi\, r\, \boldsymbol{C_{mem}}} \qquad (4)$$

where $\boldsymbol{\sigma_m}$ is the conductivity of the media and $\boldsymbol{C_{mem}}$ is the membrane capacitance of a certain cell type. Equation 4 shows cells with lower membrane capacitance will result in a higher cross over frequency which is especially useful when the cells are specifically modified to express a low membrane capacitance such as fixed RBCs.

S6. Impedance Analysis of the Collected nDEP and pDEP Fractionated Cells

Impedance cytometry was used to compare the pDEP and nDEP collected fractions following DEP separation. A schematic of the measurement process is shown in Fig S4 (a) with data on healthy (blue) and fixed (red) RBCs in 1X PBS to act as the control in Fig S4 (b), (c), and (d). For analyzing the collected fractions, the pDEP and nDEP collected samples were resuspended in 1X PBS, along with 10 μm polystyrene beads to enable signal normalization. The samples were passed through a microchannel with facing electrodes, such that an impedance analyzer can measure the cell characteristics, as shown in Fig S4-S6. The procedure to gate impedance signals from standard polystyrene beads for normalization is shown in Fig. S6. Since Fig 8 (d) (main text) shows the same opacity as the data obtained on non-DEP manipulated cells in Fig S4 (d) it is likely that the predominant population in the nDEP collected fraction are healthy RBCs; the pDEP collected fraction are fixed RBCs, based on membrane capacitance. Similarly, the invariance of impedance signals before and after DEP collection, based on impedance phase, electrical diameter, and opacity metrics indicates the maintenance of cell viability during DEP separation.

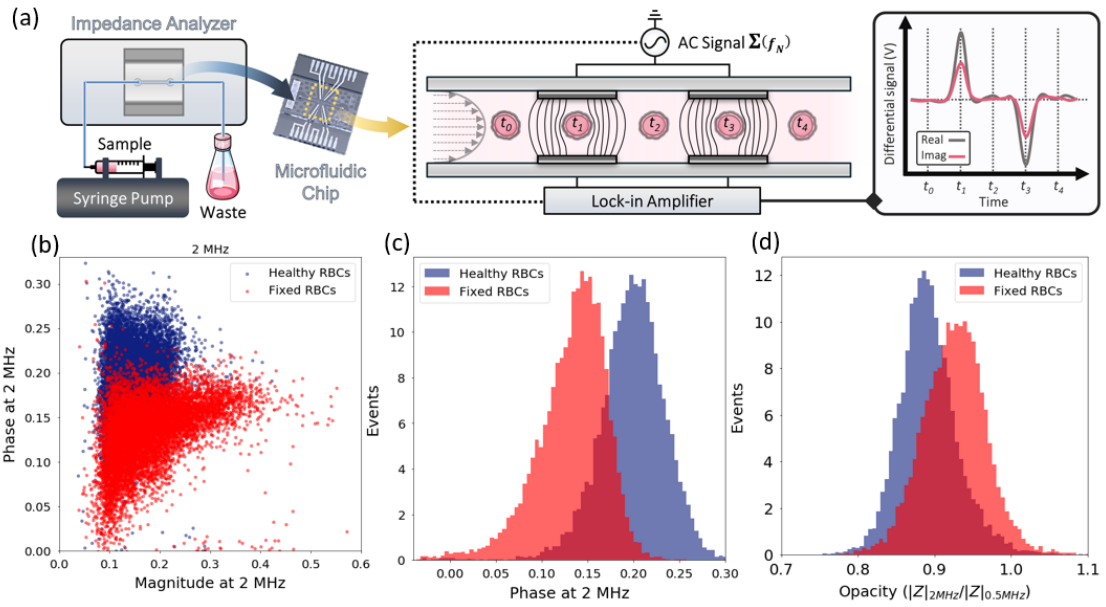


Fig S4. Schematic of the impedance cytometry set up (a), impedance phase and magnitude (b), phase comparison (c), and opacity (d) of healthy (blue) and fixed (red) RBCs in 1X PBS at 2 MHz.

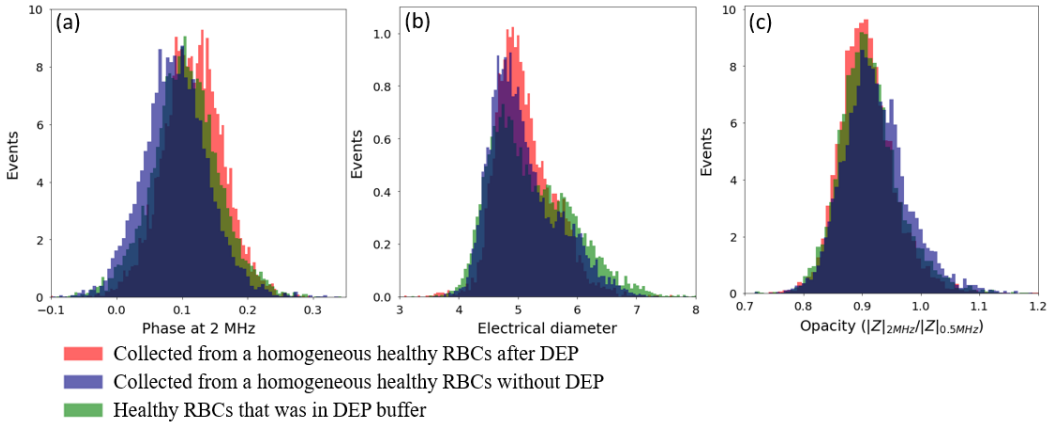
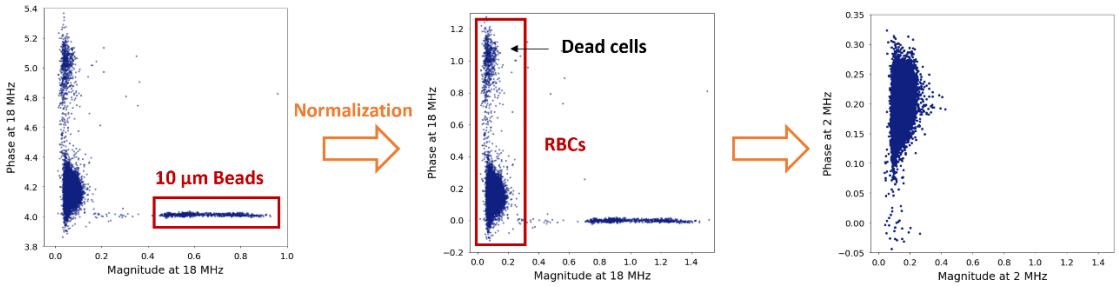


Fig S5. The metrics of impedance phase at 2 MHz (a), electrical diameter (b) and opacity at 2 MHz (c) are used to compare the RBC sample in so called DEP buffer (green), in the DEP buffer without DEP collection (blue) and in their DEP buffer after DEP collection (red).



Scatter plot of impedance Magnitude *versus* impedance Phase at reference frequency 18 MHz is used to normalize the data against the 10 μm polystyrene beads. After normalizing the data, a gate is then generated to include the cells. This gate is used for all the measurements at prob frequencies.

Fig. S6: Impedance Data Processing and normalization

S7. Comsol Simulation Parameters and Properties

Parameters and Properties	Values
Operation Parameters	
V_{AC} Input	150 V_{pp}
Frequency (nDEP)	40 kHz
Frequency (pDEP)	500 kHz
Normal Inflow Velocity	$3 * 10^4\ \mu m/s$
Fluid Properties*	
Fluid Medium Conductivity (nDEP)	$700\ \mu S/cm$
Fluid Medium Conductivity (pDEP)	$50\ \mu S/cm$
Fluid Density	$1000\ kg/m^3$
Fluid Dynamic Density	$1 * 10^{-3}\ Pa * s$
Particle Properties[3,4]	
Particle Diameter (RBCs)	$5\ \mu m$
Particle Diameter (Platelets)	$1.8\ \mu m$
Shell Thickness (RBCs)	$9\ nm$
Shell Thickness (Platelets)	$8\ nm$
Shell Electrical Conductivity (RBCs)	$1 * 10^{-6}\ S/m$
Shell Electrical Conductivity (Platelets)	$1 * 10^{-6}\ S/m$
Particle Conductivity (RBCs)	$0.31\ S/m$
Particle Conductivity (Platelets)	$0.25\ S/m$
Shell Relative Permittivity (RBCs)	4.44
Shell Relative Permittivity (Platelets)	59
Particle Relative Permittivity (RBCs)	50
Particle Relative Permittivity (Platelets)	80

*Fluid properties are from COMSOL built-in materials library

References

1. P. R. C. Gascoyne, S. Shim, J. Noshari, F. F. Becker and K. Stemke-Hale, *Electrophoresis*, 2013, **34**, 1042–1050.
2. P. R. C. Gascoyne and S. Shim, *Cancers (Basel)*, 2014, **6**, 545–579.
3. S. Park, Y. Zhang, T.-H. Wang and S. Yang, *Lab Chip*, 2011, **11**, 2893.
4. M. Egger and E. Donath, *Biophys J*, 1995, **68**, 364–372.