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## **Supporting Information**

## A Simplified Sheathless Cell Separation Approach Using Combined Gravitational Sedimentation Based Prefocusing and Dielectrophoretic Separation

Tao Luo,<sup>a</sup> Lei Fan,<sup>a</sup> Yixiao Zeng,<sup>b</sup> Ya Liu,<sup>a</sup> Shuxun Chen,<sup>a</sup> Qiulin Tan,<sup>b</sup> Raymond H. W. Lam<sup>a</sup> and Dong Sun\*<sup>a</sup>

- a. Department of Mechanical and Biomedical Engineering, City University of Hong Kong, Kowloon 999077, Hong Kong, China.
- b. Key Laboratory of Instrumentation Science and Dynamic Measurement, Ministry of Education, North University of China, Taiyuan, China.



Fig. S1 Layout of the chip and simulated streamlines of the laminar flow in the down-stream of the chip.

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Cells and liquid		Density (g/ml)
Cells	Red blood cells <sup>1</sup>	1.10-1.12
	Monocytes <sup>2</sup>	1.067-1.077
	Lymphocytes <sup>2</sup>	1.073-1.077
	Platelets <sup>3</sup>	1.065-1.080
	Yeast cells <sup>4</sup>	1.10-1.12
Liquid	10XPBS <sup>5</sup>	1.063-1.072
	1XPBS <sup>6</sup>	1.00-1.01
	Cell culture media <sup>7</sup>	1.007
	Blood plasma <sup>8</sup>	1.025
	DEP buffer (8.5% (w/v) sucrose and 0.3% (w/v) dextrose)	1.016 (Measured)
	DI water	1.0



Fig. S2 Schematic of the chip fabrication process.



Fig. S3 Comparison between net inertial lift force  $F_L$  and gravitational sedimentation force G- $F_B$  at different flow rates. The net inertial lift force  $F_L$  can be simplified as  $FL = f_L \rho_f U^2 d^4 / D^2$ , where D is the diameter of the circular cross section of the tubing, d is the diameter of the cell,  $\rho_f$  is the density of the fluid, U is the average fluid velocity, and  $f_L$  is the lift coefficient, and can be approximated averagely as 0.5<sup>9</sup>. (A) Tubing diameter is set to 0.38 mm. (B) Tubing diameter is set to 0.72 mm.



Fig. S4 Measured size distribution of the cells used in this paper. Scale bar=100  $\mu m.$ 



Fig. S5 Schematics of the designed microfluidic chip with an inlet and a 360°-opened outlet for study gravitational sedimentation of cells in the tubing (Fig. 3). The outlet is a circular space sandwiched by two plates. The upper plate is a PDMS plate with pillars which have the diameter of 200-µm and the bottom plate is a glass slide. They are bonded together by the treatment of oxygen plasma. The pillars of the upper PDMS plate are the supports between two plates.

Devenentere	Cell Types			
Parameters	THP-1	OCI-AML3	Yeast	
Radius of the cell $r_1$ (µm)			3	
Permittivity of the cell wall $oldsymbol{arepsilon}_1$ ( $oldsymbol{arepsilon}_0$ )			60	
Conductivity of the cell wall ${f \sigma}_1$ (µS/cm)			140	
Inner radius of the cell wall $r_2$ (µm)	6.5	6.5	2.78	
Permittivity of the cell membrane ${f \epsilon}_2$ ( ${f \epsilon}_0$ )	6	6	6	
Conductivity of the cell membrane $\sigma_2$ (µS/cm)	0.0025	0.0025	0.0025	
Inner radius of the cell membrane $r_3$ (µm)	6.496	6.496	2.772	
Permittivity of the cytoplasm ${f \epsilon}_3$ ( ${f \epsilon}_0$ )	162	162	50	
Conductivity of the cytoplasm $\sigma_3$ (µS/cm)	6600	2900	2000	
$c_0 = 8.85 \times 10^{-12} (F/m)$				

Table S2 The size and dielectric parameters that were used to calculate the CM factor of THP-1, OCI-AML3 and yeast cells. The value of these parameters are obtained from literatures<sup>10–12</sup>.

 $\varepsilon_0 = 8.85 \times 10^{-12} (F/m)$  is the permittivity of free space



Fig. S6 Real part of the CM factor of THP-1, OCI-AML3 and yeast cells. For those three types of cells, Re [K( $\omega$ )]>0 at the working frequency of 5MHz, which means that all those cells experienced positive DEP force.



Fig. S7 Schematic of the chip design for increasing the throughput of DEP separation through extending the flow path in the DEP region.

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