# Electronic Supplementary Information

# Precise label-free leukocyte subpopulations separation using hybrid acoustic-optical method

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## SI Text

### Schematic of granulocyte sorting using acoustophoresis.

After the pre centrifugation process, mixture of granulocytes, red blood cells (RBCs) and platelets is conducted using acoustophoresis for the further separation. Generally, RBCs and platelets have much smaller diameter and different acoustic properties compared to granulocytes. In the SSAW field, platelet will be gathered in ANs, RBCs and granulocytes will be pushed to the PNs with different velocity, which have provide perfect separation basis using acoustophoresis. Fig.S1 shows the schematic of the acoustic sorting process, in the SSAW field region, granulocytes are pushed to PNs at much higher velocities, finally granulocytes are transported to the central outlet, while erythrocytes and platelets are transported to outside outlets. In experiment, syringes are used to inject samples through two outer inlets at the flow rate of 100 μl/h, while phosphate buffered solution is injected through the central inlet to push cell to side of the channel at the flow rate of 400 μl/h. Then the IDTs are applied signal at the frequency of 13.5 MHz, and the power is set to 650 mW. The PNs was adjusted to be the central of the channel.

### Experimental section of granulocyte sorting and viability test.

In this experiment, the channel in this experiment have three inlets and outlets, and the ratio between stealth flow and sample flow is set to 2:1, so that stealth flow will confine samples flowing along the side of channel. RF signal is applied at the power of 650 mW, the

frequency is 13.5 MHz. Fig. S2 shows the sorting process in the SSAW field, RBCs and granulocytes are stained with different fluorescent dye before mixture for the convenience of stain process. At the flow rate of 600 μl/h in total, RBCs have little motivation in the acoustic acting region, while granulocytes are attracted to the node which is in the center of channel. It can be easily comprehended that ARF is proportional to the cube of cell diameter, which means granulocytes are subject to more than eight times acoustic force than RBCs. The purity of cells in three outlets are shown in Fig. S3.

To assess the effect of the experiment on cell viability, we have conducted the test using trypan blue dye exclusion method as described in Stain and viability test section in the main text. The result is shown in Fig. S4.

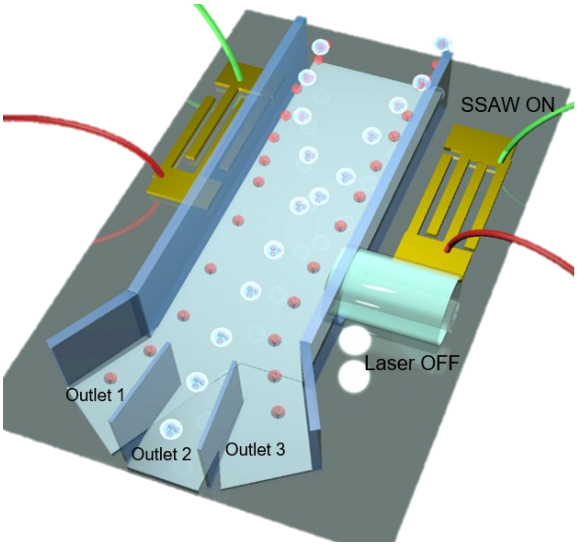


**Fig. S1** Schematic of the separation of granulocytes by acoustophoresis.

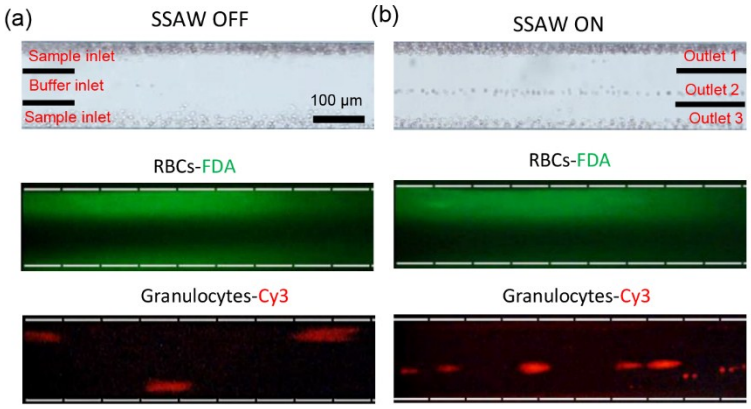


**Fig. S2** Bright field and fluorescence image of the sorting process of granulocyte from mixture. (a) When SSAW is off, all cells flow next to the two side wall. (b) While when SSAW is on, granulocytes with larger size are gathered to PNs, and flow to outlet 2. RBCs are stained with FDA and granulocytes are stained with Cy3.

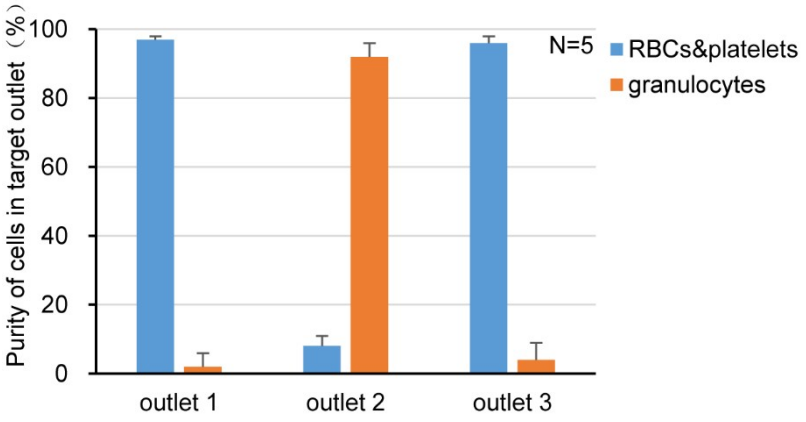


**Fig. S3** The purity of granulocytes and RBCs & platelets. Error bar represent standard deviation of result from five tests.

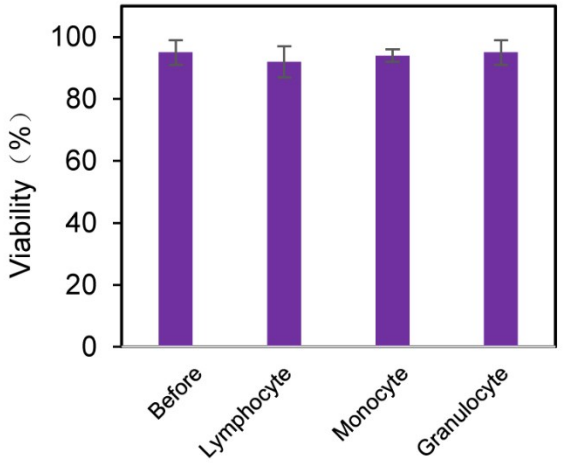


**Fig. S4** The viability test of cells before and after separation. Error bar represent standard deviation of results from four tests.

**Table S1.** Comparison of laser power intensity and expose times observed to cause damages to cells in different studies.

| Ref. | Cell Type | λ (nm) | Exposure time (sec) | Power density ($W/cm^2$) | Damage Characterization |
|---|---|---|---|---|---|
| 42 | NC37 lymphoblasts | 1064 | 30 | $2.7\times10^7$ | DNA damage |
| | | 760 | 30 | $1.3\times10^7$ | |
| 40 | Sperm | 1064 | 120 | $6.8\times10^7$ | Cell viability |
| Hybrid acoustic-optical method | leukocytes | 1064 | 0.1 | $2.14\times10^5$ | Cell viability |

**Table S2.** Parameters used in the numerical simulations.

| **Properties** | **Symbol** | **Value** |
|---|---|---|
| Buffer density | $\rho_w$ | 997 |
| Buffer compressibility | $\beta_w$ | $4.5e^{-10}$ |
| Cell density | $\rho_c$ | 1050 |
| Cell compressibility | $\beta_c$ | $2.25e^{-10}$ |
| Acoustic pressure | $P_0$ | $1.1e^5$ |
| Buffer viscosity | $\eta$ | $0.801e^{-3}$ |