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

Supplementary information

Integrated inertial-impedance cytometry for rapid label-free leukocyte isolation and profiling of neutrophil extracellular traps (NETs)

Chayakorn Petchakup[1], Hui Min Tay[1], King Ho Holden Li[1] and Han Wei Hou[1,2*]

1. School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Block N3, Singapore 639798.
Email: hwhou@ntu.edu.sg; Tel: +65 65923889

2. Lee Kong Chian School of Medicine, Nanyang Technological University, 11 Mandalay Road, Clinical Sciences Building Level 11, Singapore 308232.

Device fabrication

A double layer SU-8 mold of 20µm and 115µm height was fabricated using standard photolithography process. For the first layer, SU-8 2010 (Microchem) was spin-coated on a silicon wafer (Bonda Technology) and pre-baked at 65˚C for 1 min and 95˚C for 3.5 min. The coated wafer was then exposed to UV using mask aligner (MA6; Karl Suss) and post-baking was performed at 65˚C for 1 min and 95˚C for 4.5 min before developing with SU-8 developer (Microchem) for 3 min. To define the second layer of 115µm, SU-8 2100 (Microchem) was spin-coated on the same wafer at 2650rpm and pre-baked at 65˚C for 5 min and 95˚C for 22 min. The coated wafer was then exposed to UV using mask aligner and post-baking was performed at 65˚C for 5 min and 95˚C for 10 min before developing with SU-8 developer for 10 min. Then the wafer was hard baked at 120˚C for 30 min and left to cooldown to room temperature.

Before polydimethylsiloxane (PDMS, Dow Corning) casting, the patterned wafer was silanized with trichloro(1H,1H,2H,2H-perfluorooctyl)silane (Sigma Aldrich) overnight to ease PDMS releasing from the wafer. To cast PDMS device, a prepolymer and curing agent was mixed in a 10:1 ratio (w/w), vacuum degassed and poured over the fabricated SU-8 mold before curing at 80 °C for 2 h. The solidified PDMS slab was then gently peeled from the mold and a 1.5mm biopsy puncher (Harris Uni-Core™) was used to create the inlet and outlet holes. For the electrode fabrication, AZ9260 (Clariant) was used to define electrode patterns on a quartz wafer (Bonda Technology) prior metal deposition by sputtering of 25 nm chromium and 100 nm gold. The PDMS and electrode substrate were irreversibly bonded together by air plasma treatment using plasma cleaner (Harrick Plasma Cleaner) and heated on a hotplate at 100˚C for 1 h to ensure firm bonding.

Measurement setup

Three electrodes setup was employed for differential measurement scheme. The center electrode was energized with 2.5V at two different frequencies (0.3MHz and 1.72MHz) by a lock-in amplifier (HF2LI, Zurich instrument). Current responses were amplified and converted into voltage by transimpedance amplifiers (DHPCA-100, FEMTO®) with transimpedance gain of 10kV/A. Then magnitudes and phases at frequencies of interest were demodulated by lock-in amplifier at a sampling

rate of 230k sample/s. The data recording session were 3 min. The sequential data was processed to extract to peaks corresponding to single cell events using a custom-made program written in MATLAB (MathWorks).

Data presentation

2D scatter plots were based on cell size (µm) and opacity ($|Z_{HF}|/|Z_{LF}|$, ratio of impedance magnitude at 1.72MHz to 0.3MHz). The following equation was used to convert impedance magnitude at 0.3MHz ($|Z_{LF}|$) to electrical cell size[1].

$$Cell\ size = G|Z_{LF}|^{\frac{1}{3}}$$

Where G is a gain factor considering electronic setup. This parameter was calculated using impedance parameters from 10 µm monodisperse beads (reference) which were spiked into every samples.

To take in account of density of cell population, scatter plots were plotted in MATLAB using dscatter function from MATLAB Central File Exchange[2].

Fig S8A (left) shows impedance profile of sorted neutrophils from lysed blood spiked with 10µm beads. From the impedance profile, there were 4 apparent populations corresponding to 10µm beads and neutrophils. Beads have higher opacity than cells because cells exhibit a dielectric dispersion at high frequency. In this case, the two populations with opacity around ~1 were corresponding to 10µm beads (highlighted in red), whereas the rest populations with opacity around ~0.85 were corresponding to neutrophils (highlighted in green). The reason that there are two apparent populations in the impedance profile for each cell type is because when the particles flow inside microchannel, they can equilibrate to two different vertical positions owing to the inertial focusing effect (Fig S7A (right)). The particles close to the electrodes or far from the electrodes experienced electric field strength differently thereby affecting their measured impedance responses[1, 3, 4]. To simply data quantification of an impedance profile, density-based spatial clustering of applications with noise (DBSCAN) was used to extract a cell cluster with larger cell size (Fig S8B).

Supplementary figures

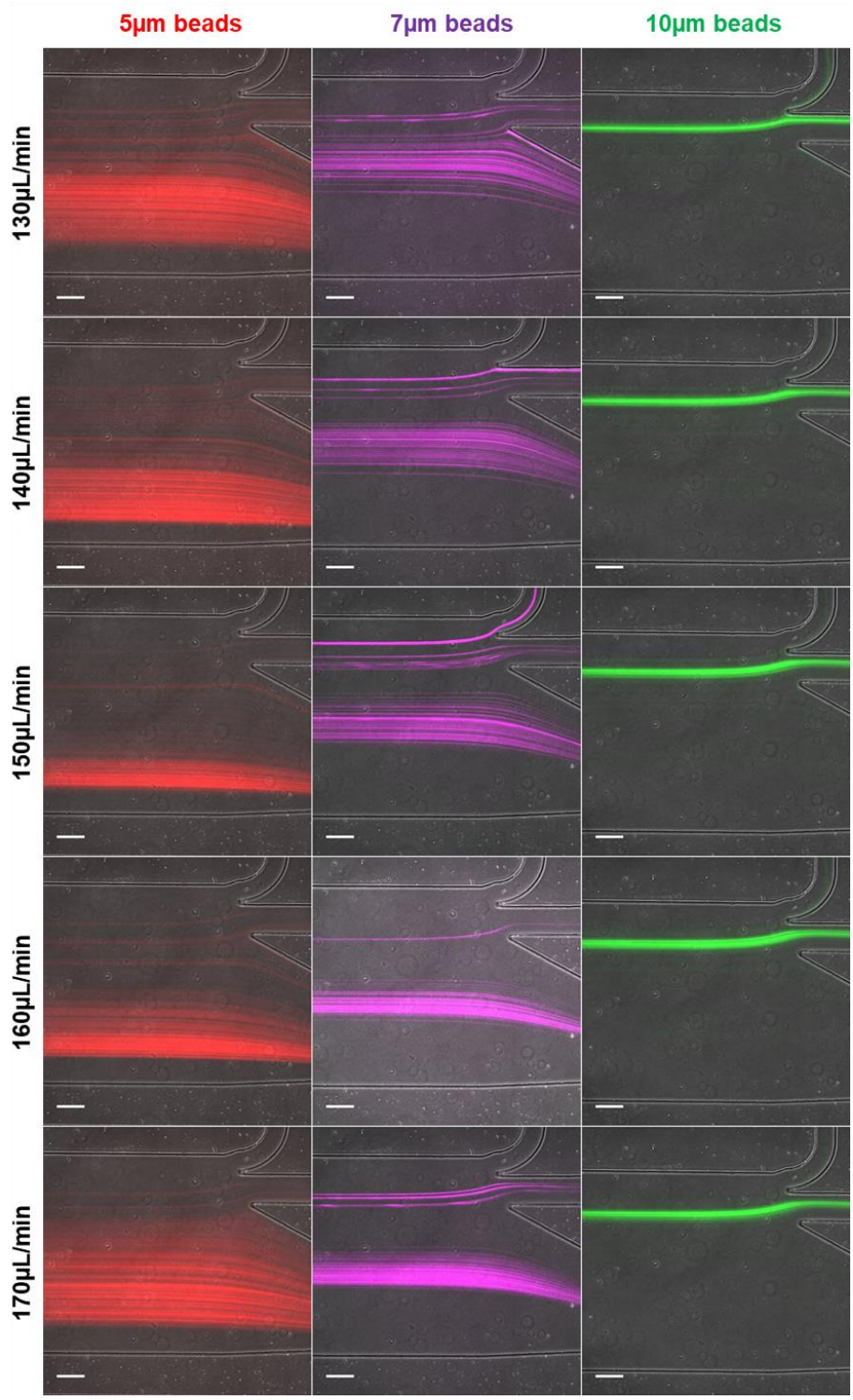

Fig. S1 Flow rate characterization: Fluorescent images showing different sized beads trajectory at sorting stage. Scale bar = 100 μm

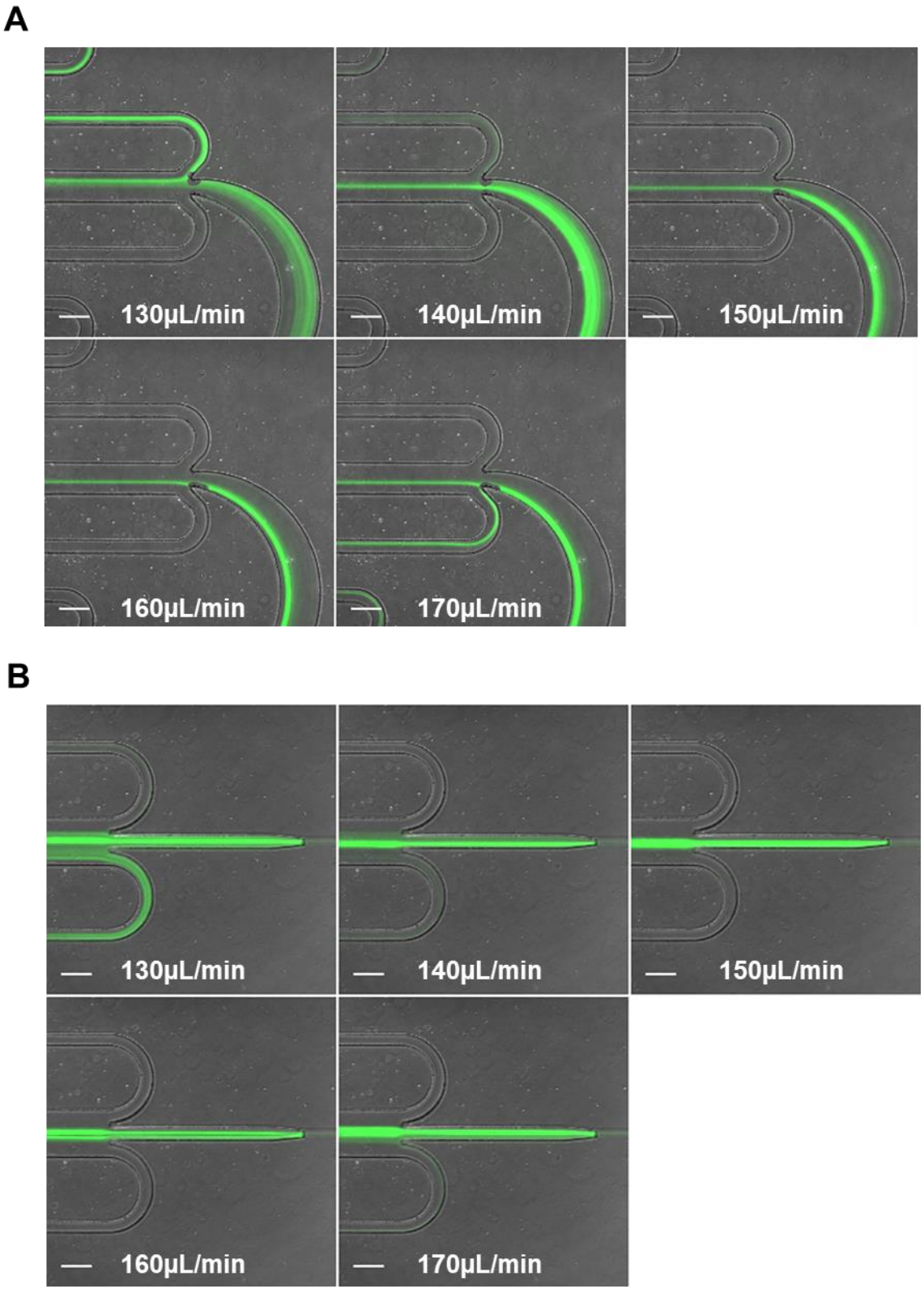

Fig. S2 Flow rate characterization 2: Fluorescent images showing 10µm bead trajectory at (A) FRR1 and (B) FRR2. Scale bar = 150 µm

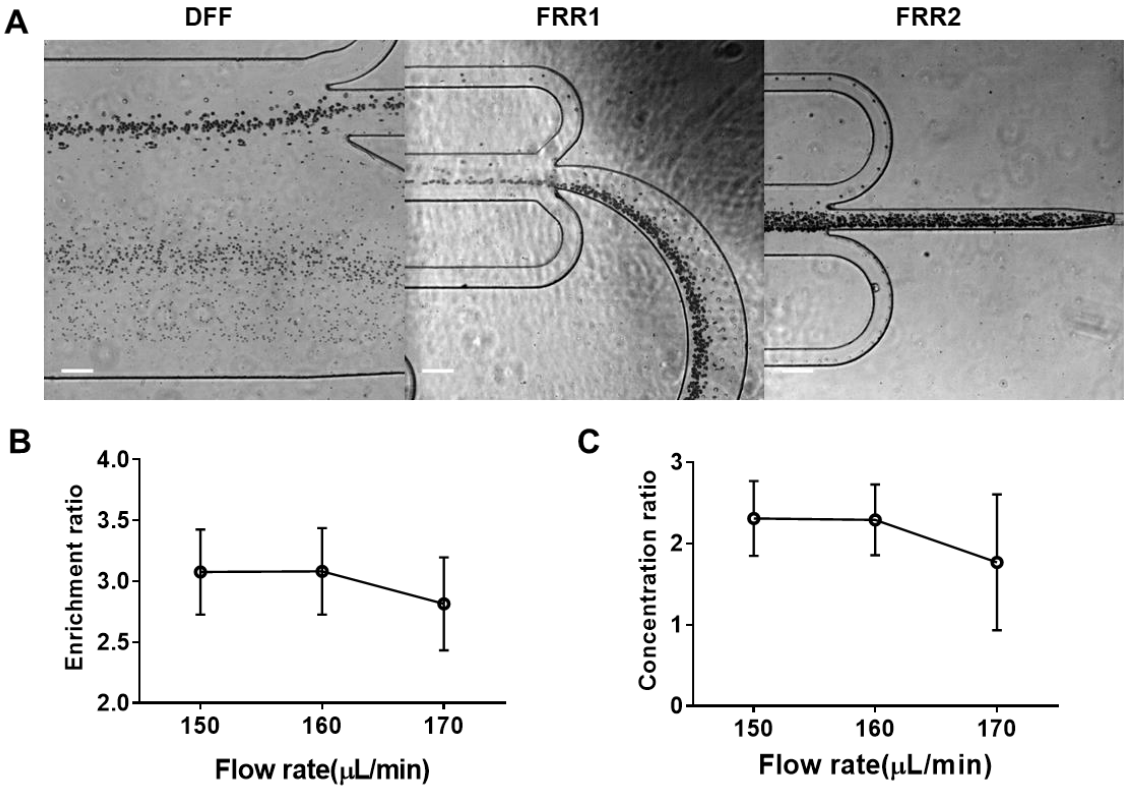

Fig. S3 Performance of 10 μm bead sorting from mixture of different sized beads (A) Representative stacked high speed images showing cell trajectory. Scale bar = 100 μm (B) Enrichment ratio and (C) concentration ratio of sorted 10 μm beads

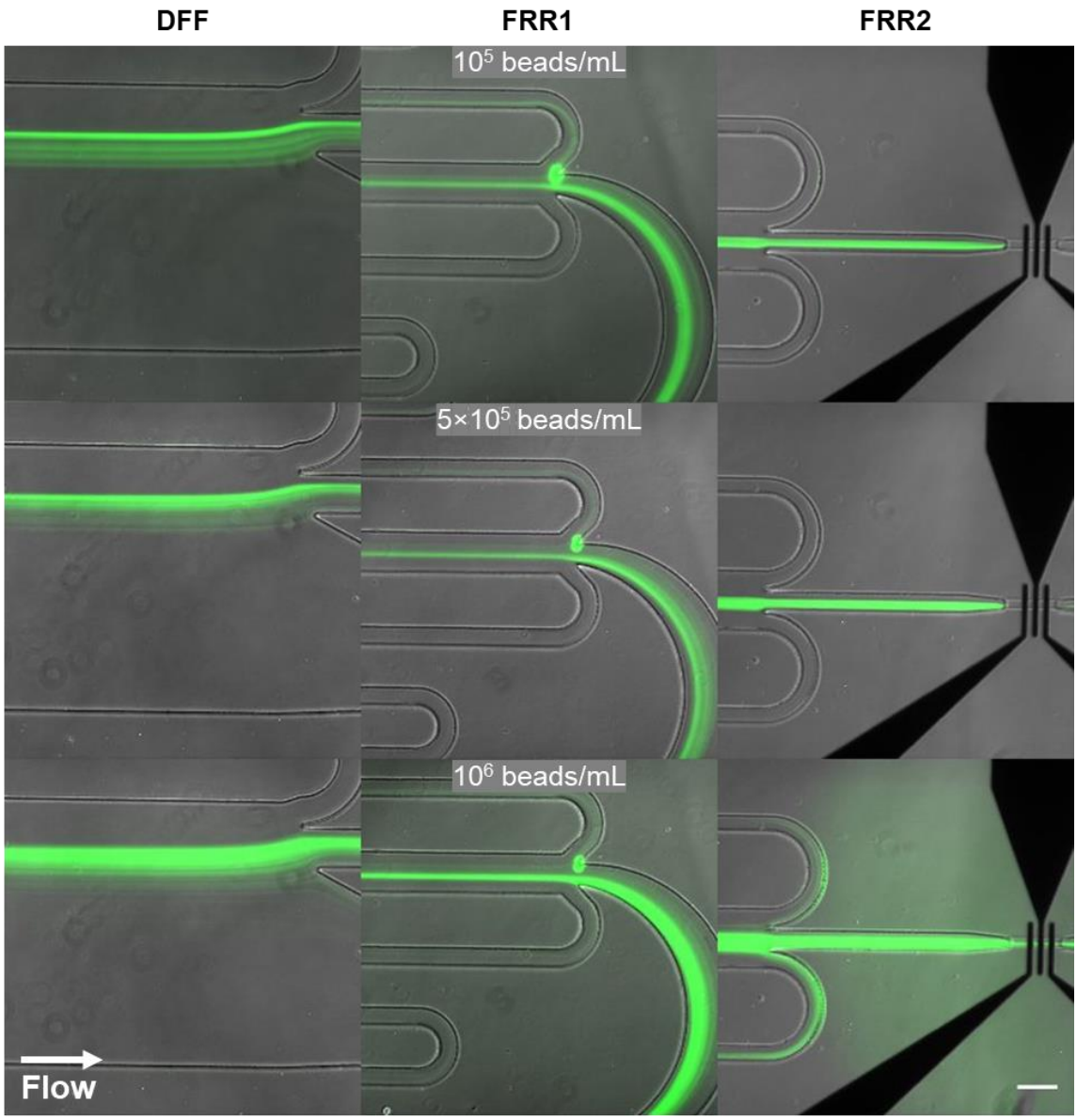

Fig. S4 Effect of concentration variation: Fluorescent images showing 10µm bead trajectory at different stages. Scale bar = 150 µm

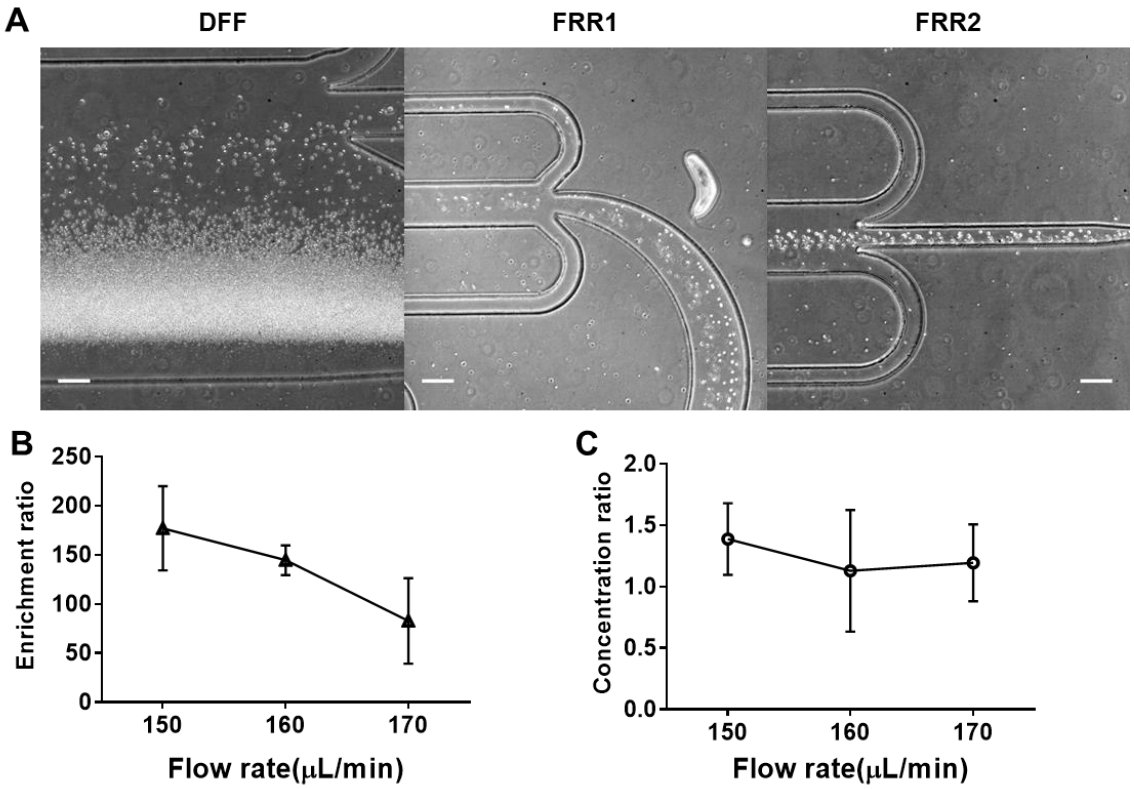

Fig. S5 Performance of neutrophil sorting from diluted blood (500×) (A) Representative stacked high speed images showing cell trajectory. Scale bar = 100 μm. (B) The enrichment ratio and (C) concentration ratio of isolated neutrophils (n = 4).

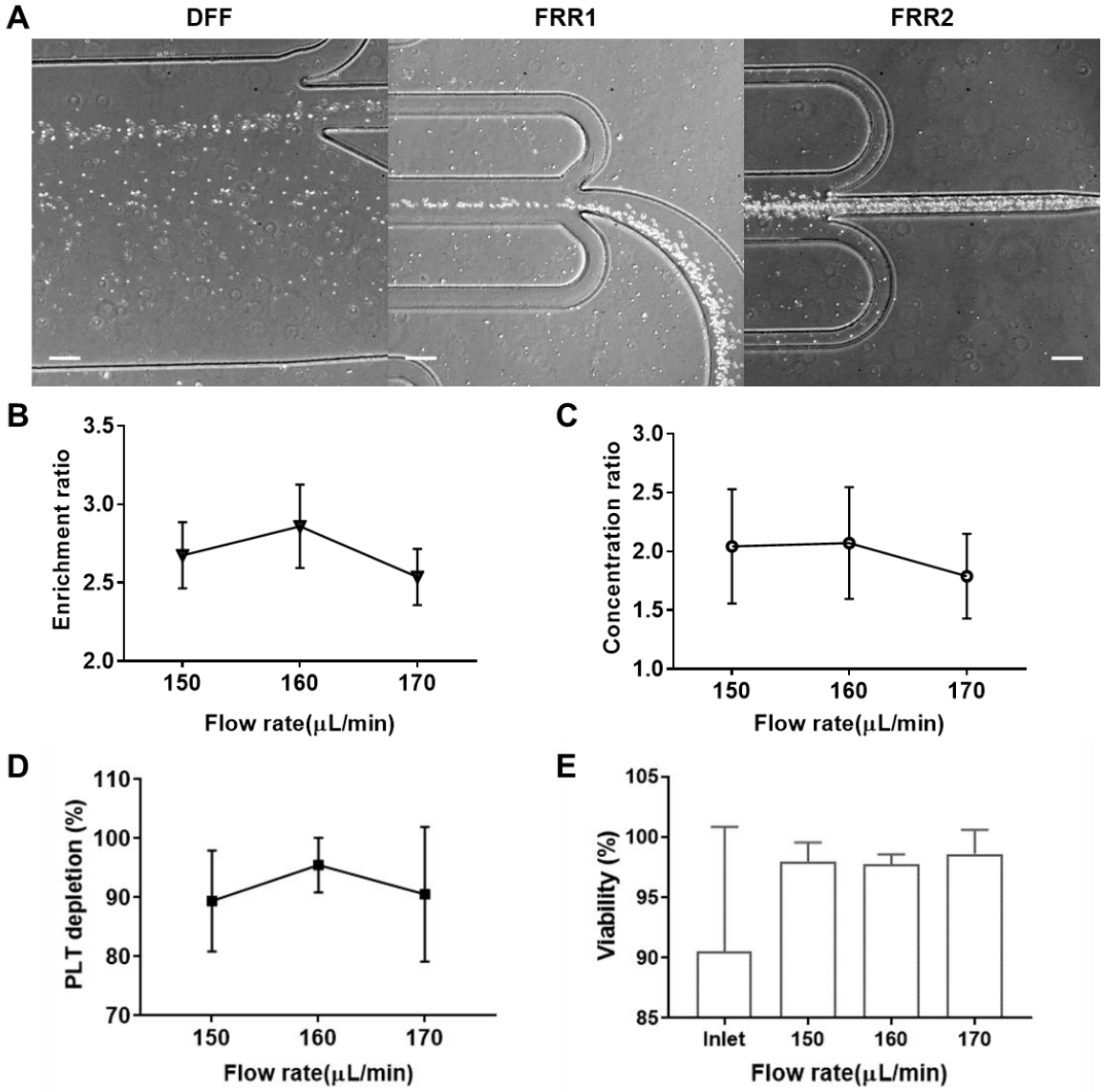

Fig. S6 Performance of neutrophil sorting from lysed blood (A) Representative stacked high speed images showing cell trajectory. Scale bar = 100 μm (B) The enrichment ratio and (C) concentration ratio of sorted neutrophils (n = 4) (D) Platelet depletion (n = 2) (E) Cell viability (n = 2)

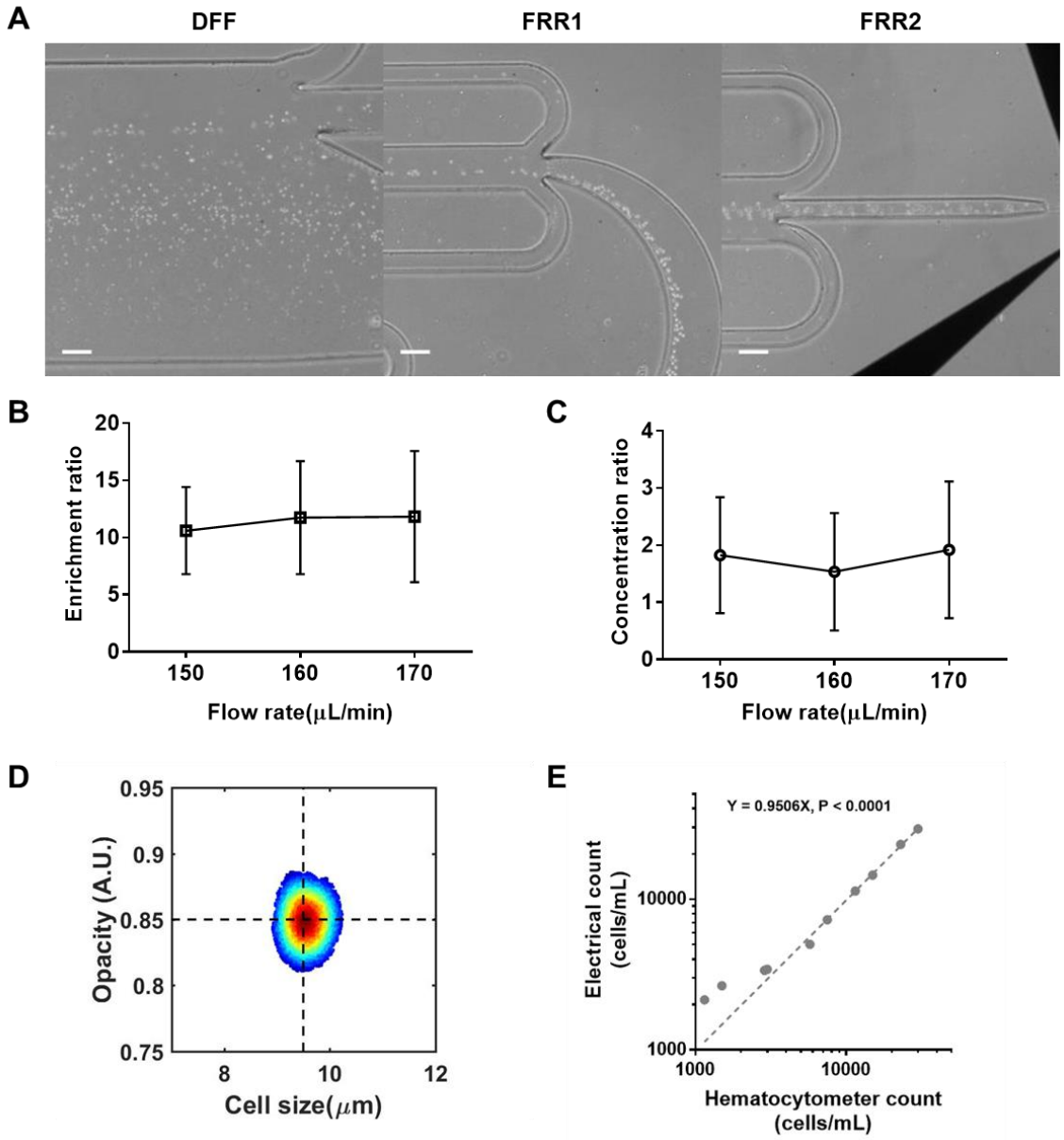

Fig. S7 Performance of monocyte sorting from PBMCs (A) Representative stacked high speed images showing cell trajectory. Scale bar = 100 μm (B) The enrichment ratio and (C) concentration ratio of sorted monocytes (n = 4) (D) Impedance profiling of monocytes sorted from PBMCs with the dotted lines indicating the mean of the population and (E) monocyte counting comparison between integrated platform and hematocytometer

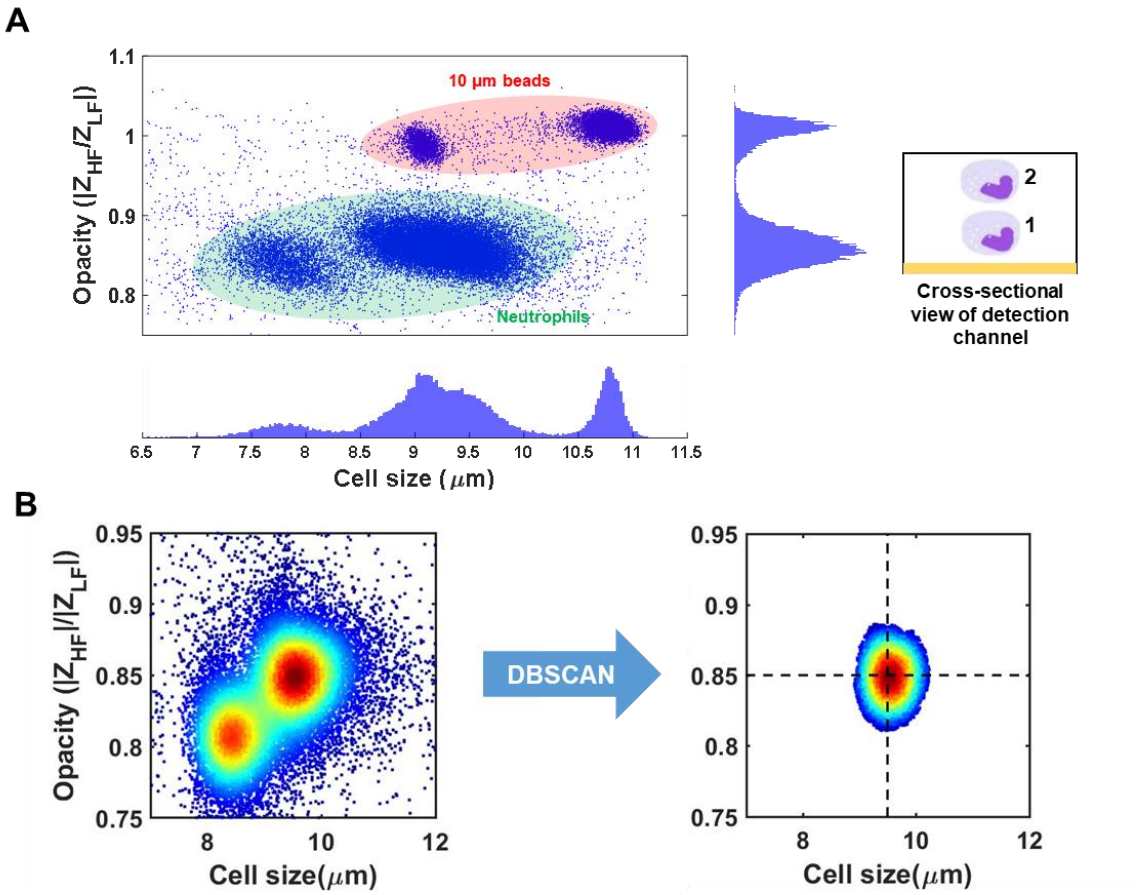

Fig. S8 (A) Impedance profiles of 10µm beads spiked blood sample showing 2 distinct populations owning to inertial focusing effect which focus target cells to two different equilibrium positions. (B) Data processing to acquire a cluster with larger cell size by Density-Based Spatial Clustering of Applications with Noise (DBSCAN)

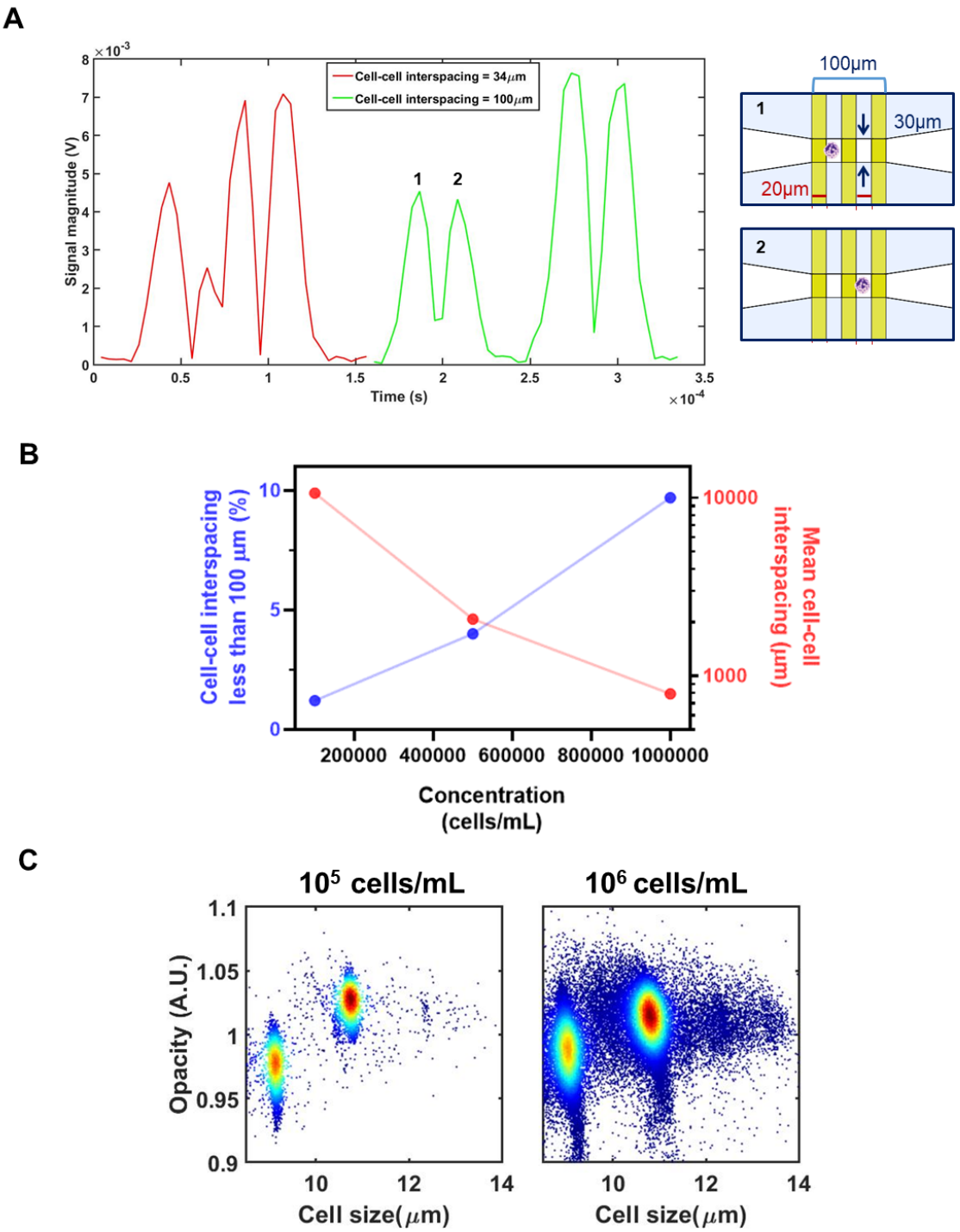

Figure S9 Characterization of sample concentrations. 2 (A) Cropped signals with different cell-cell interspacing 1 and 2 indicates single cell event when it is in between 1st electrode pair and 2nd electrode pair respectively. (B) Quantification of cell-cell interspacing. (C) Impedance profiles of different bead concentration.

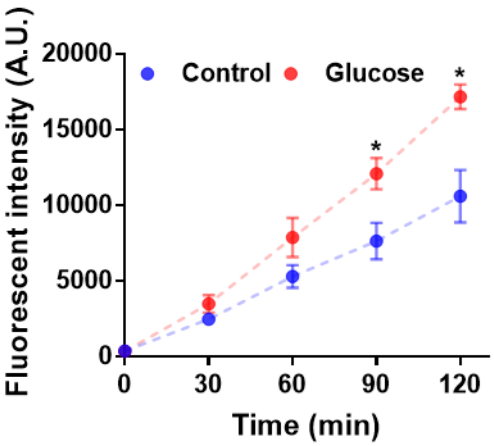

Fig S10 Fluorescent intensity of Sytox green for healthy neutrophils and glucose treated neutrophils undergoing NETosis (n = 4) Bars were represented as mean ± s.e.m. *P ≤ 0.05

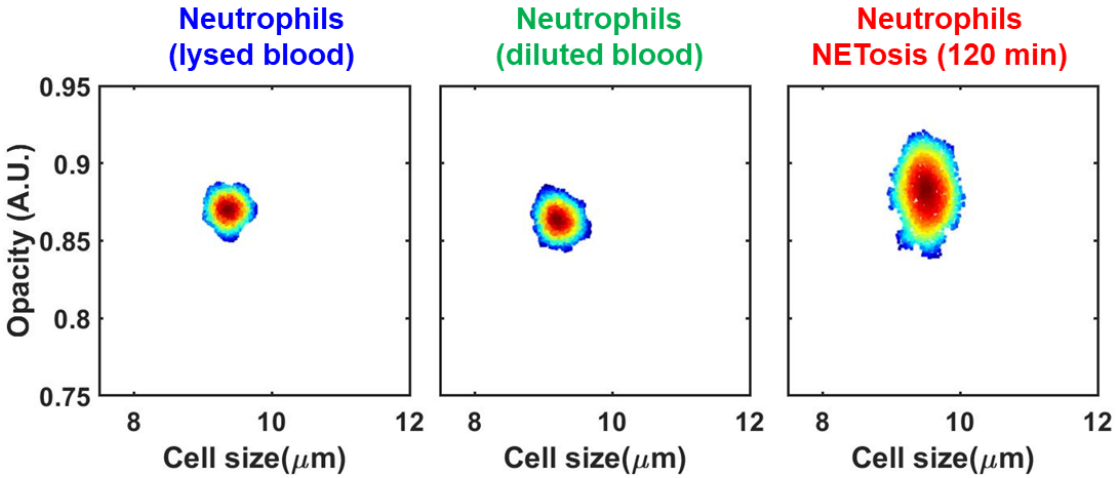

Fig S11 NETosis from whole blood Impedance profiles of neutrophils from lysed blood (left) and diluted blood (center) and NETosis neutrophils stimulated in diluted blood (right)

References

1. *A. De Ninno, V. Errico, F. R. Bertani, L. Businaro, P. Bisegna and F. Caselli, Lab on a Chip, 2017, DOI: 10.1039/C6LC01516F.*
2. *R. Henson, MATLAB Central File Exchange, 2016.*
3. *D. Spencer and H. Morgan, Lab on a Chip, 2011, **11**, 1234-1239.*
4. *D. Spencer, F. Caselli, P. Bisegna and H. Morgan, Lab on a Chip, 2016, DOI: 10.1039/C6LC00339G.*