Supplementary Materials

Flow Characterization for different blood samples

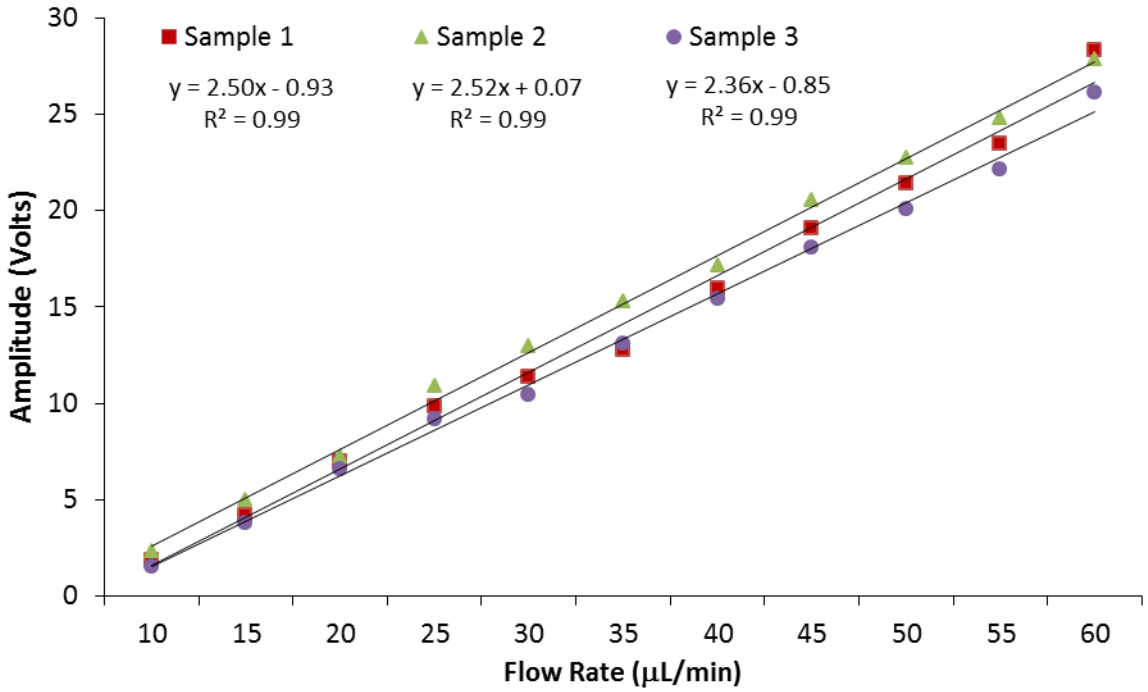

Fig. S1: Flow metering of the lysed blood in the measurement channel for different blood samples. The linear relationship between the amplitude increase of the baseline signal is plotted (i.e. V_2 or V_1 voltage measured with reference to ground) with increase in the flow rate for three different blood samples with hematocrit values of 43.9, 46.5 and 39.8 respectively.

Simulations

The microfluidic channel with electrodes and a biological cell flowing through it was also simulated in COMSOL 4.2b. Fig. S2a,b shows the cross-sectional view of the channel and surface plot of the electrical field lines when cell is placed far and near from the electrodes, respectively. Fig. S2c,d shows the corresponding electric potential surface plot of the channel. The change in the impedance can be measured as the change in output voltage and thus can be used to characterize the cell's position, cell's velocity, and the fluid flow rate.

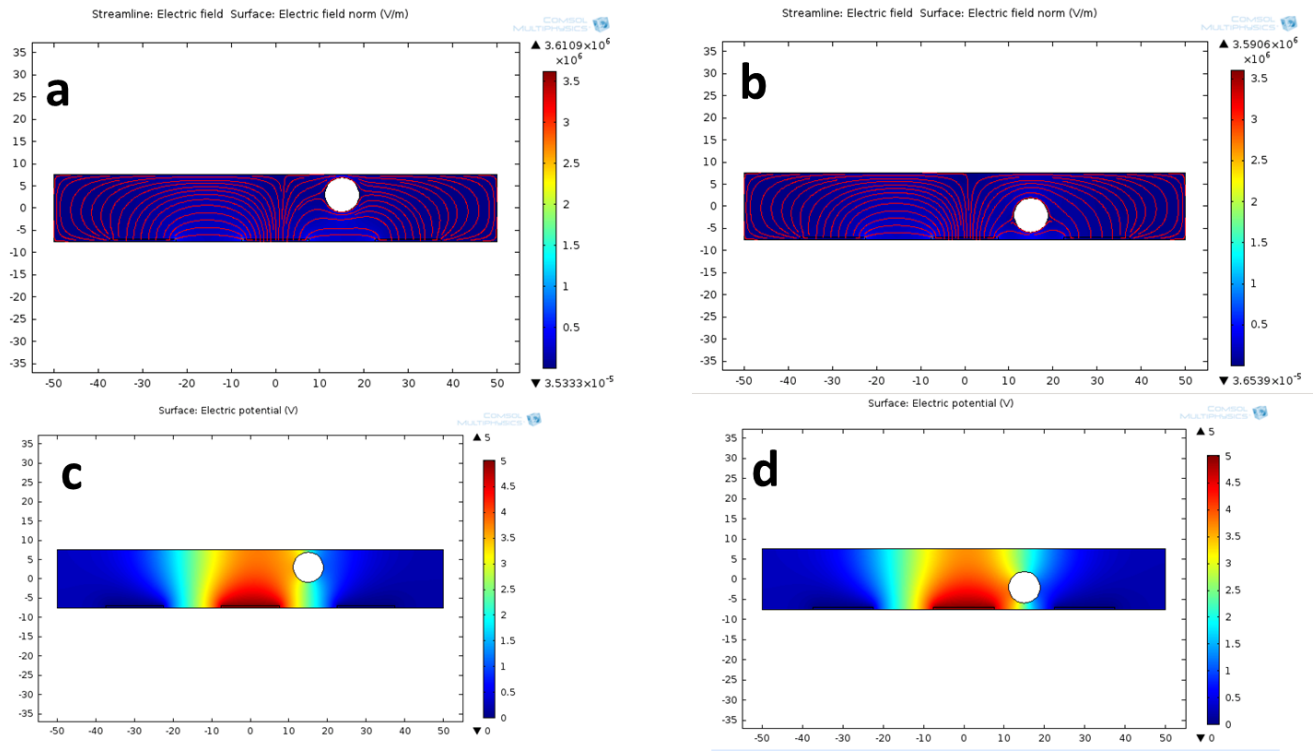

Fig. S2 (a) The simulation in COMSOL showing the normalized electrical field lines when the cell is near the top of the channel. (b) The simulation in COMSOL showing the normalized electrical field lines when the cell is near the bottom of the channel. (c) The simulation in COMSOL showing the electrical potential when the cell is near the top of the channel. (d) The simulation in COMSOL showing the electrical potential when the cell is near the bottom of the channel.

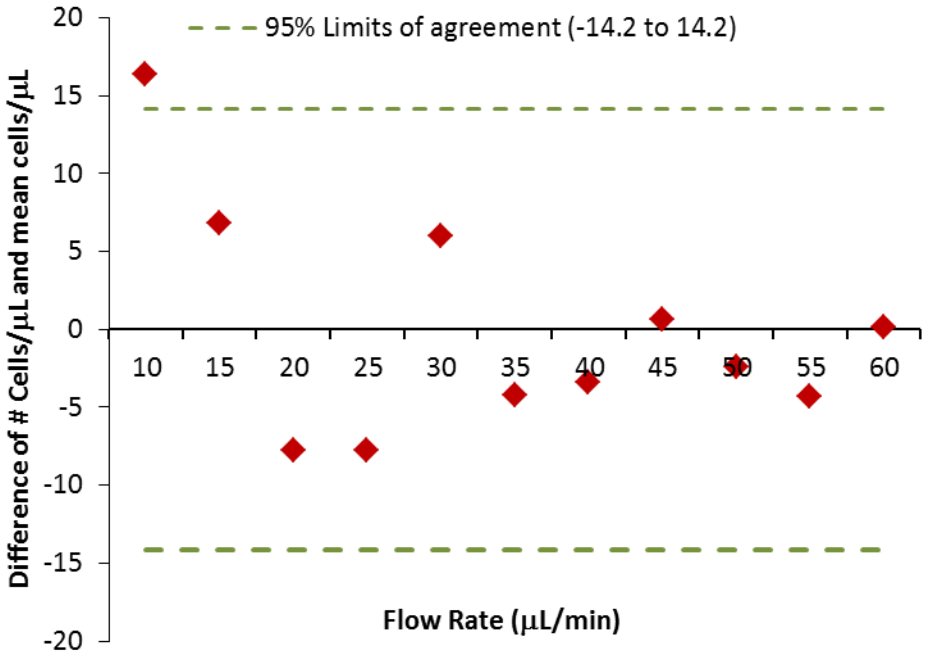

Fig. S3: The difference in between the cells/µL and the mean value of cells/µL (47 cells/µL) over the entire flow rate range vs. the flow rate. This plot shows the variation of the data and 95% limits of agreement.

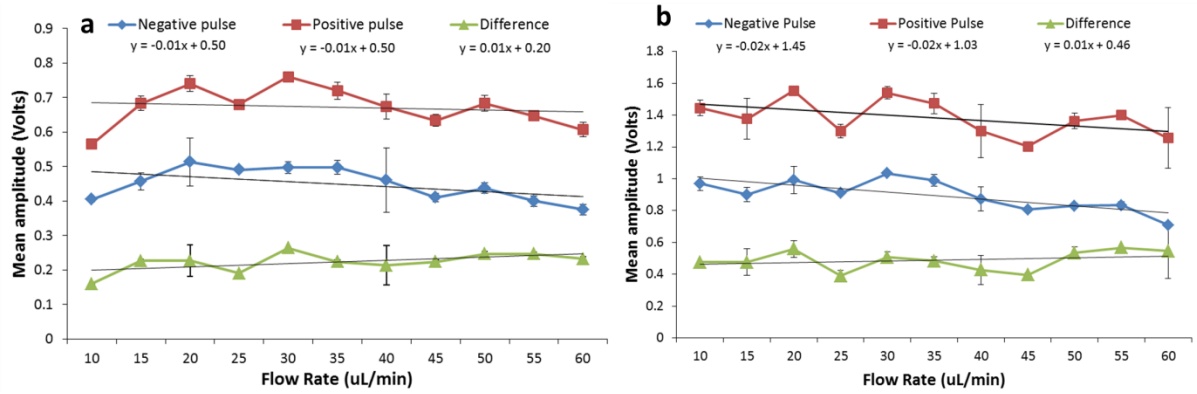

Fig. S4 The relationship between pulse amplitude for both positive pulse and negative pulse with varying flow rates. The difference between the positive pulse and negative pulse amplitudes is shown for (a) lymphocyte and (b) granulocyte/ monocyte. Error bars represents one standard deviation across 3 experiments.

Hydrodynamic forces on cells

As the cells pass through the measurement channel, hydrodynamic forces are being exerted on the cells. Sedimentation forces the cells downward and could eventually get the particle to settle at the bottom of the channel. The sedimentation force is proportional to *g*, the difference in density between particle and medium, and the volume of the particle, as given by Equation S1. Fig. S2a compares the hydrodynamic force on erythrocytes, platelets, lymphocytes, granulocytes and monocytes. For a typical leukocyte, the average value is above 100 fN. Fig. S2b gives the sedimentation velocity, the velocity with which the cells settle down in the measurement channel with different cell types. The different cell types with their radius and densities are listed in Table S1. The density of the medium is assumed to be 1010 kg/m^3.

$$F_{sedi} = \frac{4}{3}\pi R^3 \left(\rho_p - \rho_m\right) g \qquad \text{(S1)}$$

Table S1: Different cell types with their physical properties.[21-23]

Cell Type	Radius (μm)	Density (kg/m^3)	Cell mass (p g)
Platelet	1.5	1070	15.1
Erythrocyte	3.1	1099.6	137.22
Lymphocyte	3.9	1075	267.11
Monocyte	4.63	1065	442.77
Granulocyte	4.71	1077	471.37

The cells suspended in the fluid also experience a hydrodynamic force which depends on the relative velocity of the fluid with respect to the particle, viscosity, and size of the particle as given by Equation S2.[19, 20]

$$F_{HD-Drag} \approx 6\pi kR\eta\left(\upsilon_m - \upsilon_p\right) \tag{S2}$$

Assuming a parabolic laminar flow profile with U as flow rate in μL/min,

$$\upsilon = 6\langle\upsilon\rangle\frac{x}{h}\left(1-\frac{x}{h}\right) \tag{S3}$$

$$\langle\upsilon\rangle = \frac{U}{wh} \tag{S4}$$

The dynamic viscosity of the medium is 1.33 mPa.s. Fig. S2c shows the hydrodynamic drag force exerted on different cells with different position from the channel wall and shows a parabolic profile. The flow rate is kept constant at 20 μL/min. The typical value of hydrodynamic force for a lymphocyte is around 70 nN, almost one million times greater than sedimentation force. Hydrodynamic lifting force tries to lift the particle in the suspension fluid and depends on the square of the size of the particle and co-efficient of viscosity.[12,13]

$$F_{HD-lift} \approx 0.153R^2\eta\frac{1}{\left(x-R\right)}\frac{d\upsilon_m}{dx}\bigg|_{x=0} \tag{S5}$$

Fig. S2d shows the hydrodynamic lift force on the cells as the flow through the measurement channel. It is also shown with respect to the cell position from the cell wall and it decreases exponentially as the cell moves away from the bottom of the channel wall. The typical value for a lymphocyte is 0.5 fN at the center of the channel. Comparing all forces, hydrodynamic force is the most dominant one.

The pH value of the blood is about 7.4. However, in our device after lysing the erythrocytes, the pH of the fluid changes, however the quenching buffer is then infused to maintain the pH/osmolarity of the solution. The isoelectric point of the PDMS decreases when it is treated with the oxygen plasma. We believe that this change in the isoelectric point doesn't affect the hydrodynamic forces including sedimentation force, hydrodynamic drag and lift forces. However, change in the isoelectric point would change the electro-osmotic characteristics in the cell measurement channel, and would result in changing the complex electrical permittivity of the medium, thereby changing the dielectrophoresis force being exerted on the cells. However, in our case, the most dominant force is the hydrodynamic drag force.

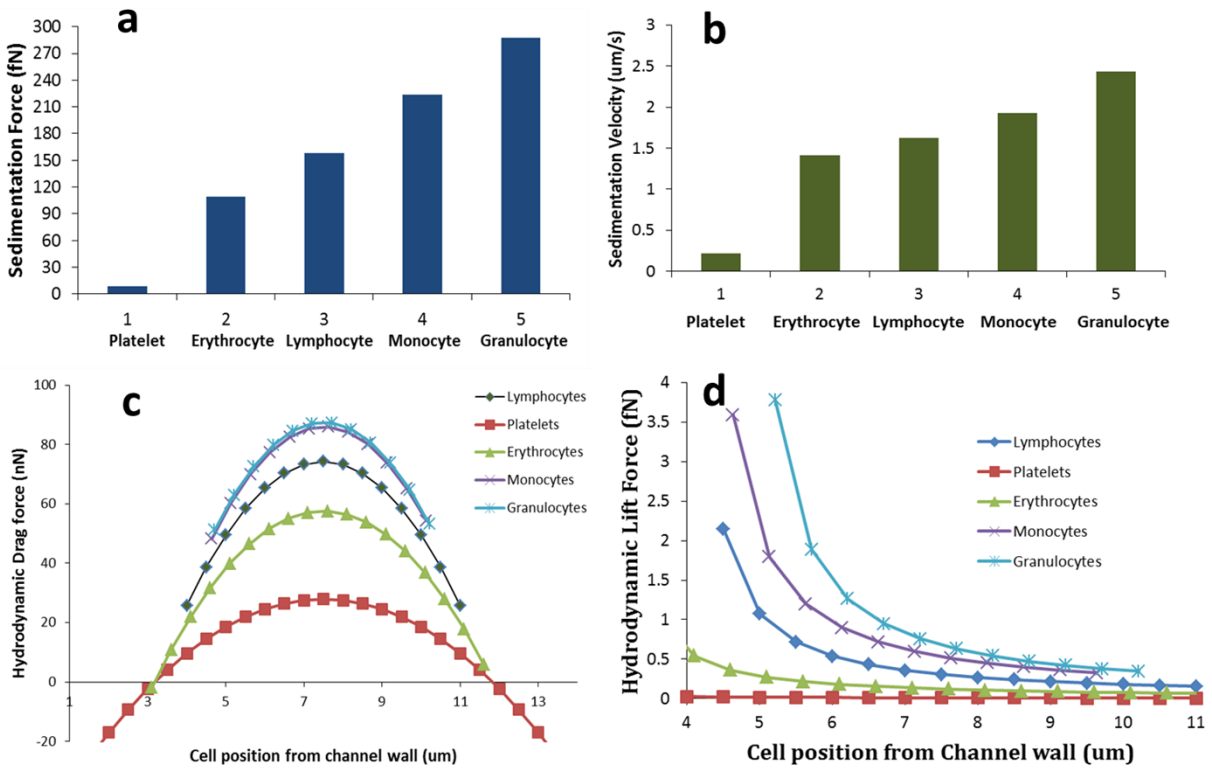

Fig. S5 Hydrodynamic forces on the cells as they pass through the measurement channel. (a) Shows the sedimentation force for different blood cells. It acts downward and its typical value for a leukocyte is around 100 fN. (b) Show the sedimentation velocity for different cell types. (c) Shows hydrodynamic drag force for blood cells which shows a parabolic profile and dependent of the position from the channel wall. It is computed for the flow rate of 20μL/min. (d) Hydrodynamic lift force tries to life the cells to the top of the channel, which is also dependent on

cell position from wall. The order of the forces in magnitude is F_hydro>>>F_sedi>F_lift. The sedimentation force is almost 100 times greater than lift force. The drag force is 10^5 times greater than sedimentation force.

Our simulation model of the cell for measuring impedance and hydrodynamic forces is not detailed. Normally, for determining the dielectric properties of the cell, multi-shell cell model[24] has been used in which cell membrane, cytoplasm, cell wall, all are represented by different shells, as they have different conductivities and permitivities. This multi-shell model would be more effective in measuring the dielectrophoresis force more accurately. However for calculating the hydrodynamic forces, a single shell model can also serve as fair representation of the cell.

References

21 J. Yang, Y. Huang, X. Wang, X. Wang, F.F. Becker, and P.R. C. Gascoyne, *Biophysical Journal*, 1999, **76**, 3307.

22 J. Zhuang, W. Ren, Y. Jing and J.F. Kolb, *IEEE Transactions on Dielectrics and Electrical Insulation,* 2012, **19***,* 609.

23 M. Egger, and E. Donath, *Biophysical Journal*, 1995, **68**, 364.

24 G.H. Markx, and C.L. Davey, *Enzyme and Microbial Technology,* 1999, **25**, 161–171.