Supplementary Information (SI)

Double-Peak Signal Features in Microfluidic Impedance Flow Cytometry Enable Sensitive Measurement of Cell Membrane Capacitance

Karthik Mahesh[a*], Manoj M. Varma[a, b] and Prosenjit Sen[a]

[a]Centre for Nano Science and Engineering (CeNSE), Indian Institute of Science, Bangalore 560012, India
[b]Robert Bosch Centre for Cyber Physical Systems (RBCCPS), Indian Institute of Science, Bangalore 560012, India

*Correspondence to: karthikm@iisc.ac.in

SI Note 1: Calculation of Specific EDL Capacitance in Figure 5B

Parameters considered:
Permittivity of Free Space (ε_o) = 8.854×10^{-12} F/m
Relative Permittitivity of PBS (ε_r) = 80

Specific capacitance or capacitance/unit area (C) can be approximated as:

$$C = (\varepsilon_o \times \varepsilon_r)/d$$

Substituting EDL thickness d = 1 nm in the above equation, we get C = 0.708 F/m^2

Similarly, for d = 2 nm, C = 0.354 F/m^2

For d = 3 nm, C = 0.236 F/m^2

For d = 4 nm, C = 0.177 F/m^2

For d = 5 nm, C = 0.141 F/m^2

SI Note 2: Calculation of CMC used in Figure 7B and Figure 9

Parameters considered:
Permittivity of Free Space (ε_o) = 8.854×10^{-12} F/m
Thickness of Cell Membrane (d) = 7 nm

Cell Membrane Capacitance (CMC) per unit area is approximated as:

$$CMC = (\varepsilon_o \times \varepsilon_r)/d$$

Substituting membrane permittivity ε_r = 2, we get CMC = 2.53 mF/m^2

Similarly, for permittivity ε_r = 4, CMC = 5.06 mF/m^2

For ε_r = 6, CMC = 7.59 mF/m^2

For ε_r = 8, CMC = 10.11 mF/m^2

For ε_r = 10, CMC = 12.64 mF/m^2

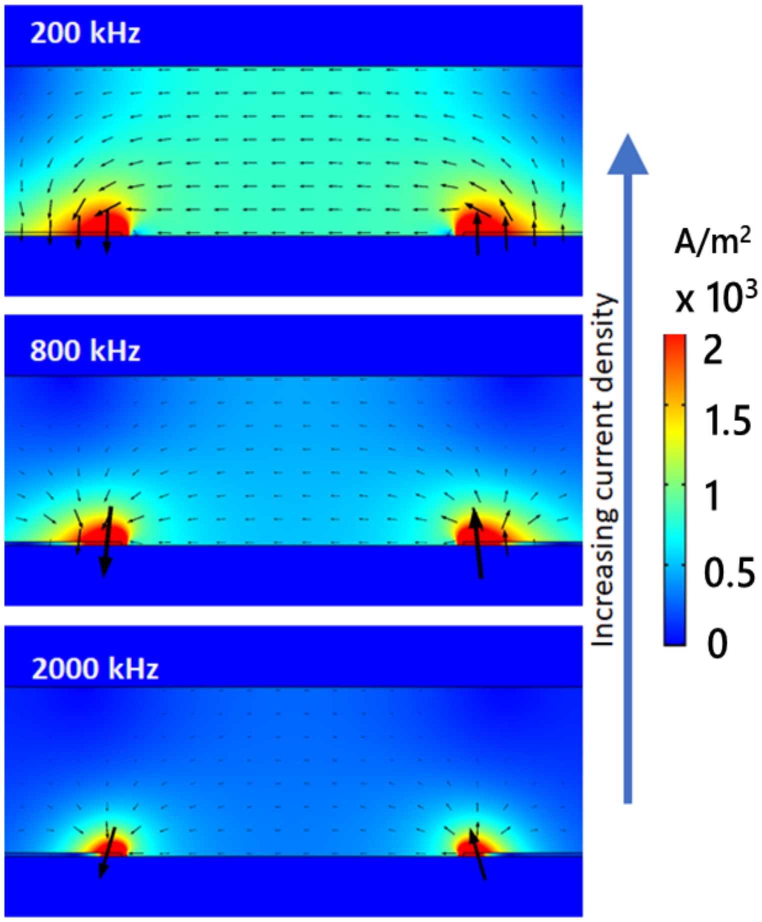


Figure SI-1: Colour map displays the imaginary component of the current density. Arrow shows the imaginary component of current. Identical colour map and arrow scaling has been used for all simulation results.

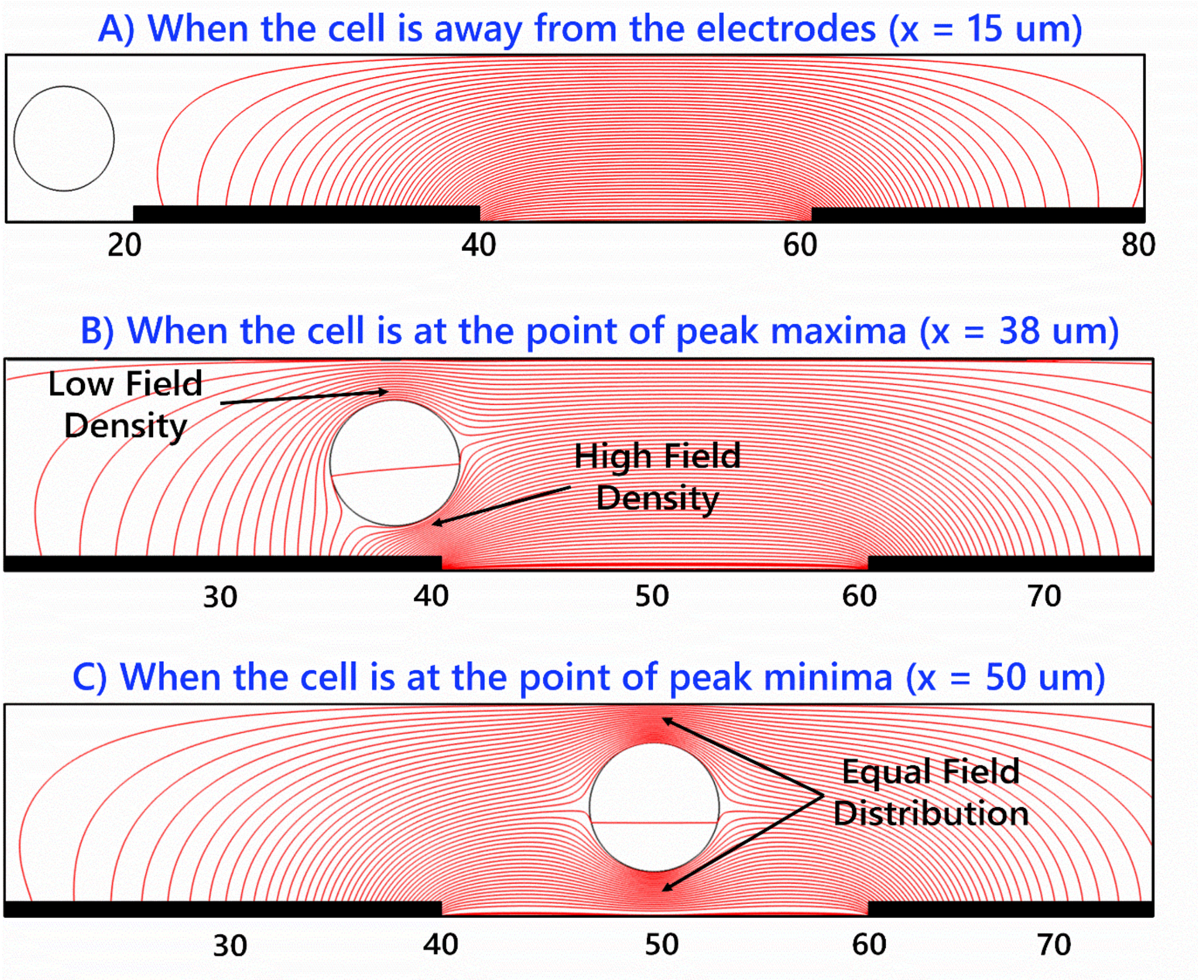


Figure SI-2: Variations in electric field distribution for a transiting cell at 800 kHz

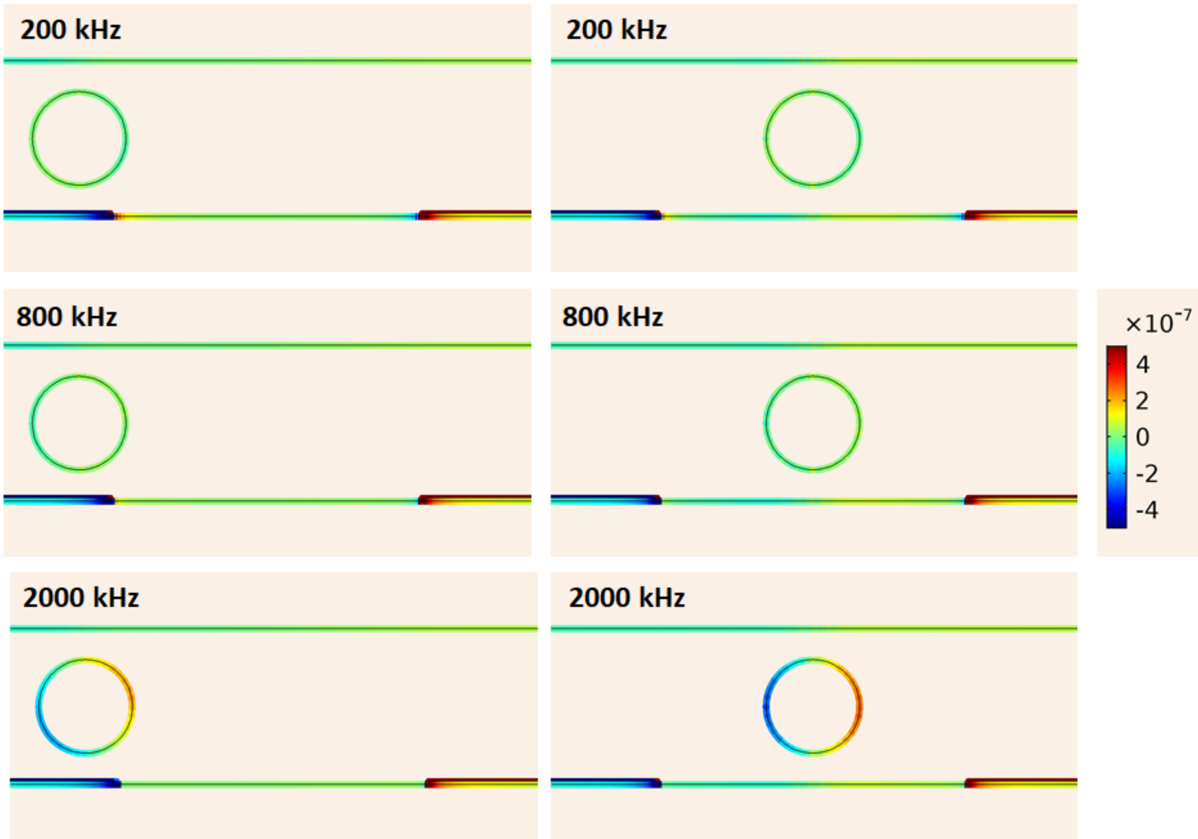


Figure SI-3: Surface charge density at different frequencies and cell positions

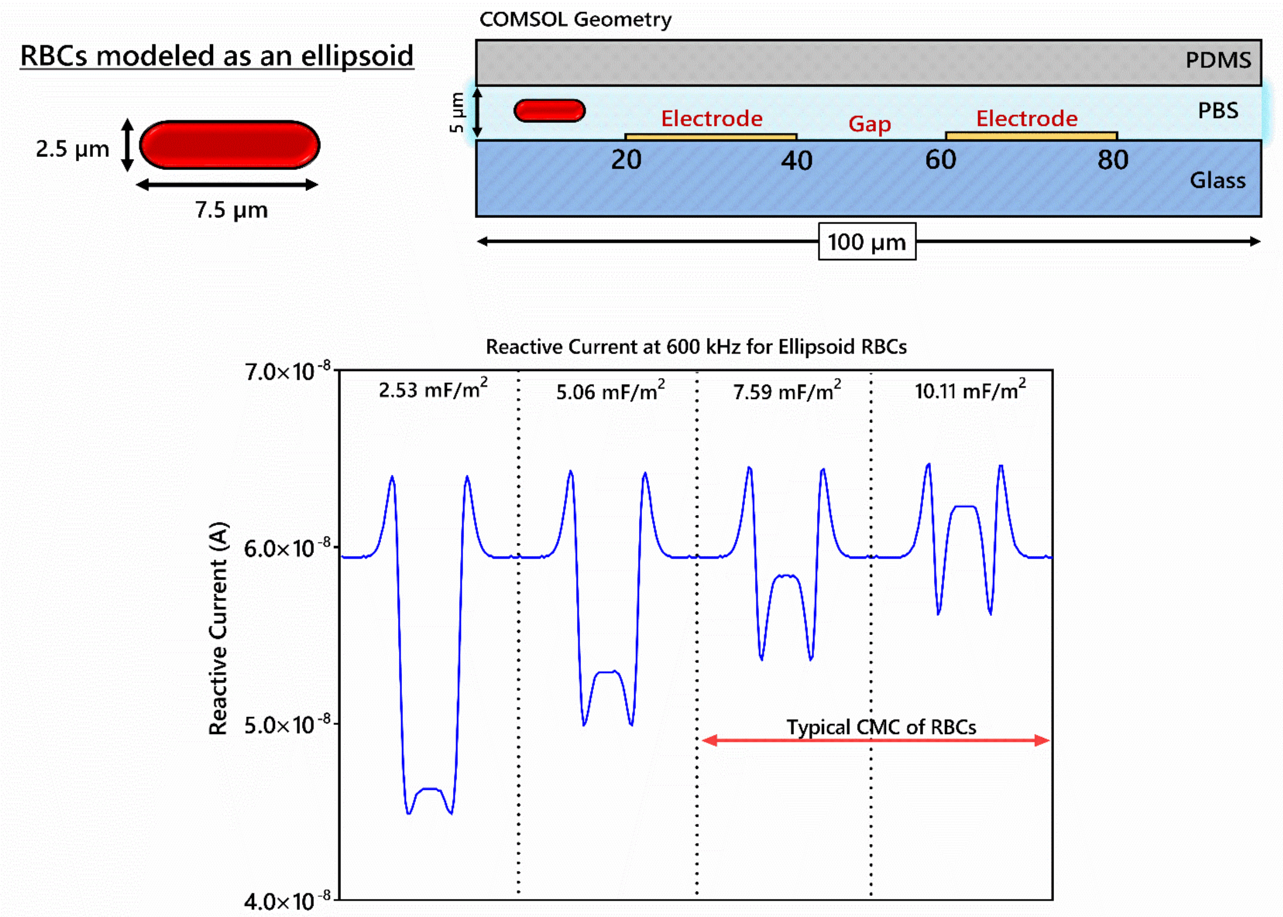


Figure SI-4: The double peak phenomenon is valid even when the RBCs are modelled as an ellipsoid (parameters same as Table 1). Additionally, the effect is observed in the typical CMC range of RBCs (8-10 mF/m^2).

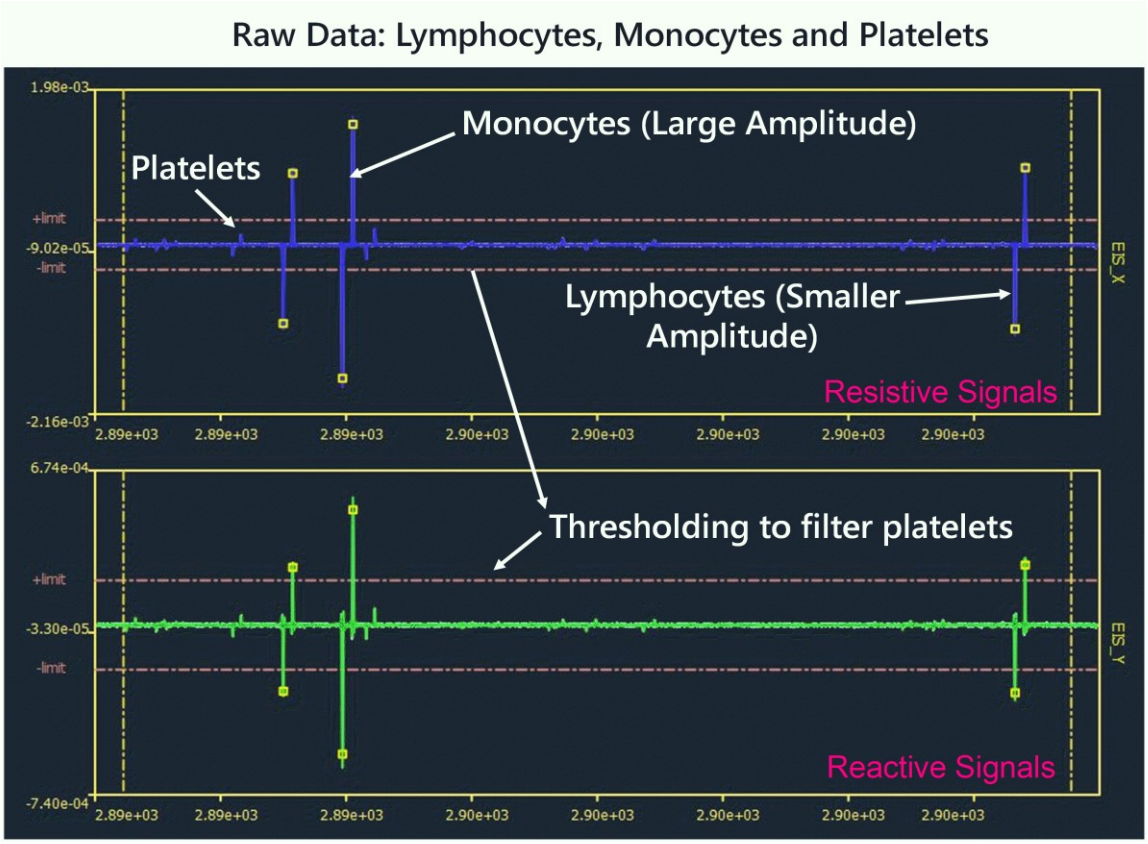


Figure SI-5: Captured raw data showing the resistive and reactive cell signals for platelets, lymphocytes, and monocytes

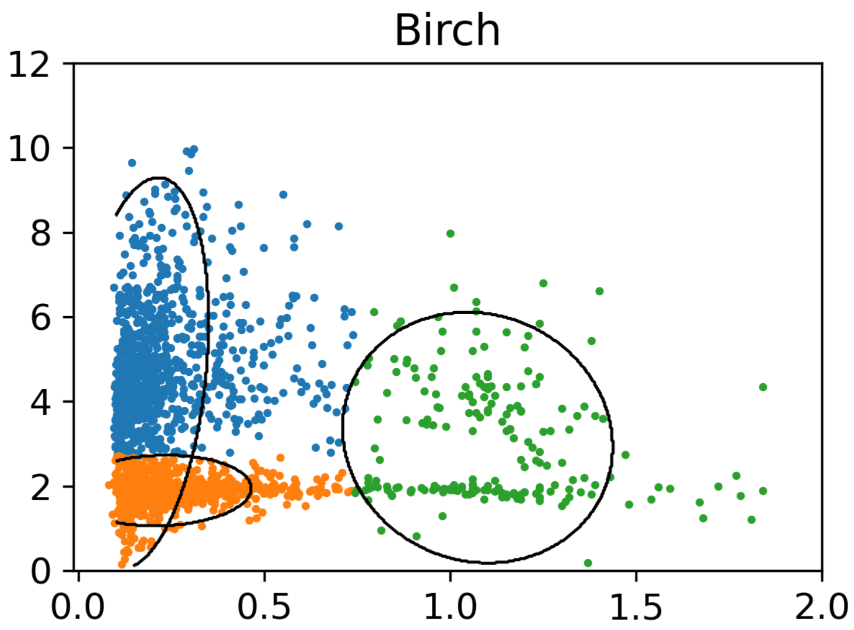


Figure SI-6: Birch clustering algorithm used to identify sub-populations of lymphocytes. X-axis is the amplitude of the resistive peaks; Y-axis is the peak ratio.

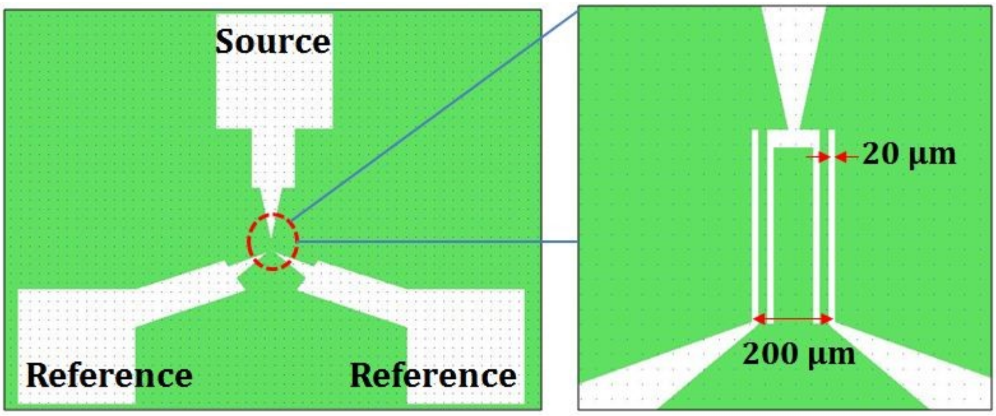


Figure SI-7: Layout of the microelectrodes used for all experiments

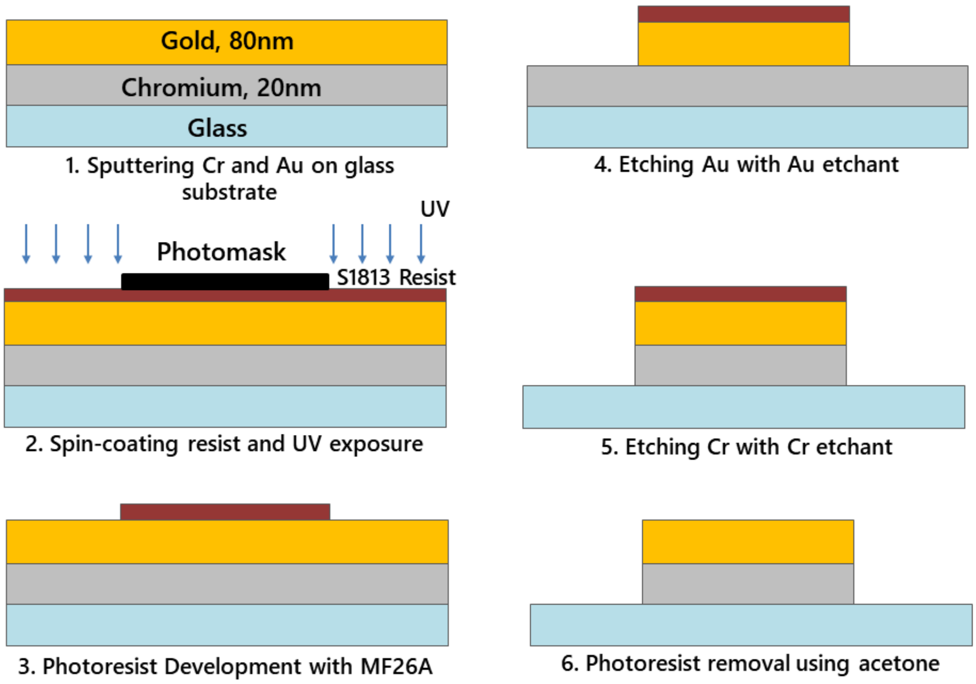


Figure SI-8: Process flow for microelectrode fabrication

Table SI-1: Process parameters for microfluidic device fabrication using SU8 2005 (for 5 μm device) and SU8-2015 (for 15 μm device)

Process	SU8 – 2005	SU8-2015
1) Spin-coating speed	1900 rpm, 30s	2200 rpm, 30s
2) Soft bake temperature	95 °C, 2 min	95 °C, 4 min
3) Exposure Dose	120 mJ/cm^2	140 mJ/cm^2
4) Post Exposure Bake (PEB) temperature	95 °C, 1 min	65 °C, 1 min 95 °C, 3 min
5) Development time (in SU8 Developer)	1 min	3 min
6) Hard Bake	250 °C, 10 min	250 °C, 10 min

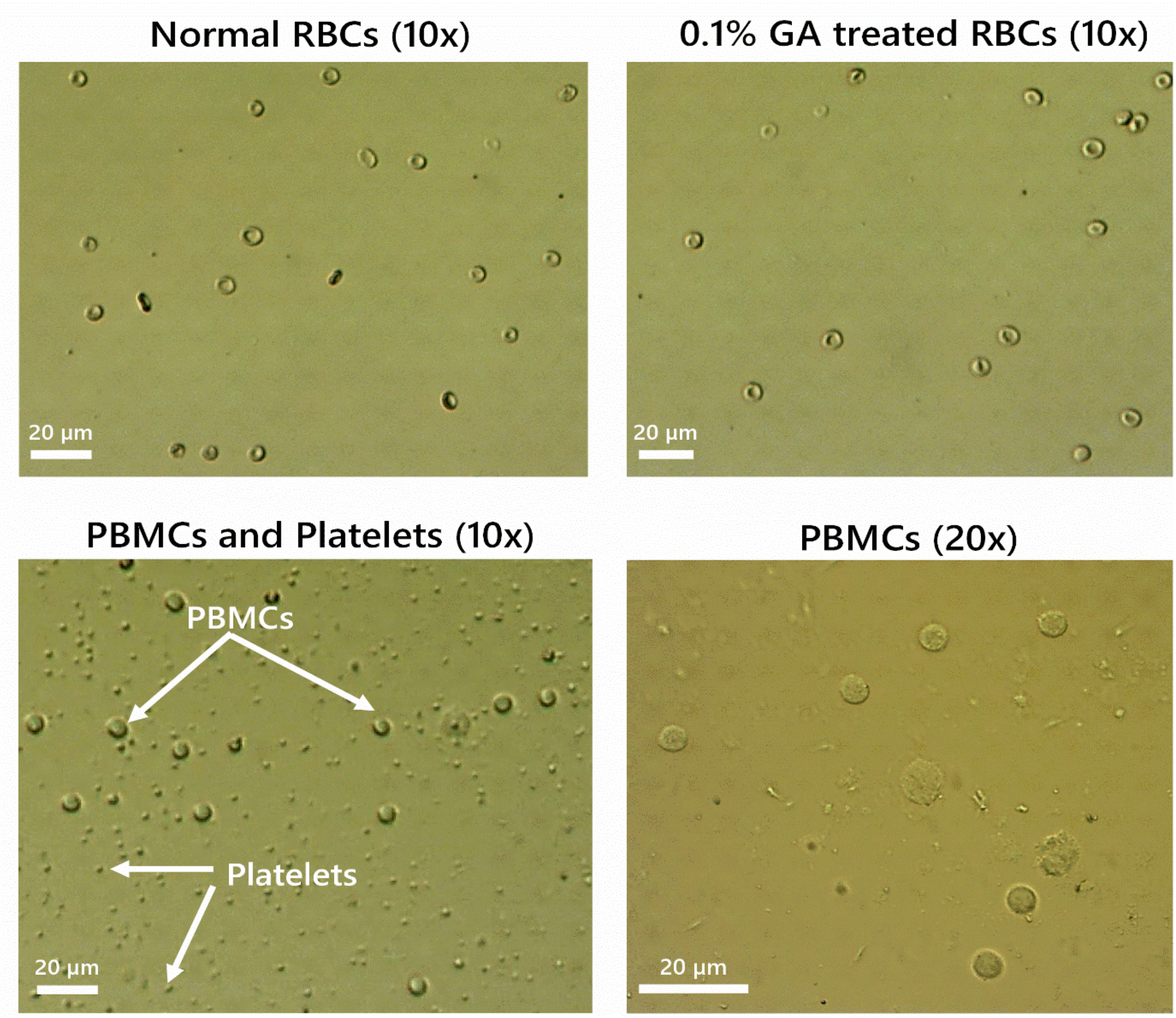
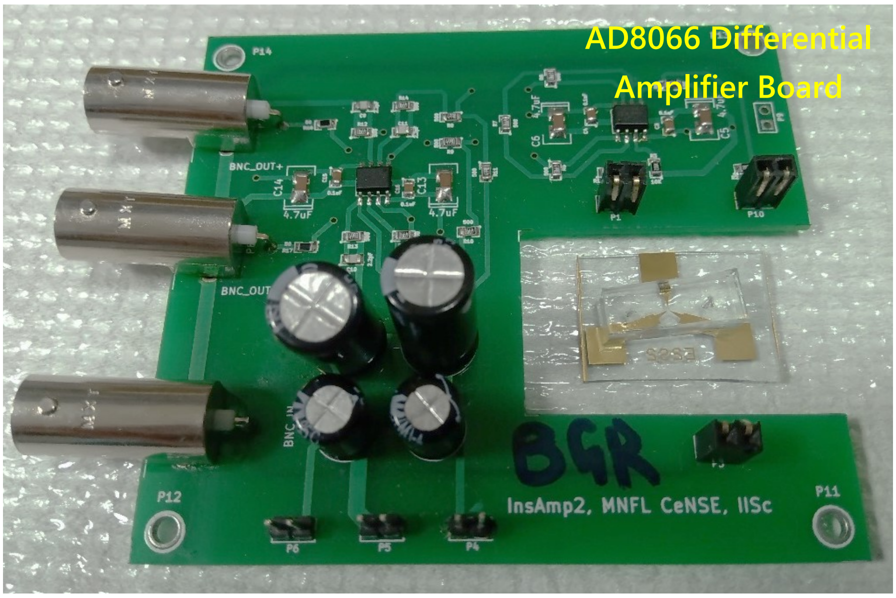


Figure SI-9: Microscope Images of RBCs, 0.1% glutaraldehyde (GA) treated RBCs, PBMCs (lymphocytes + monocytes) and accompanying platelet contamination



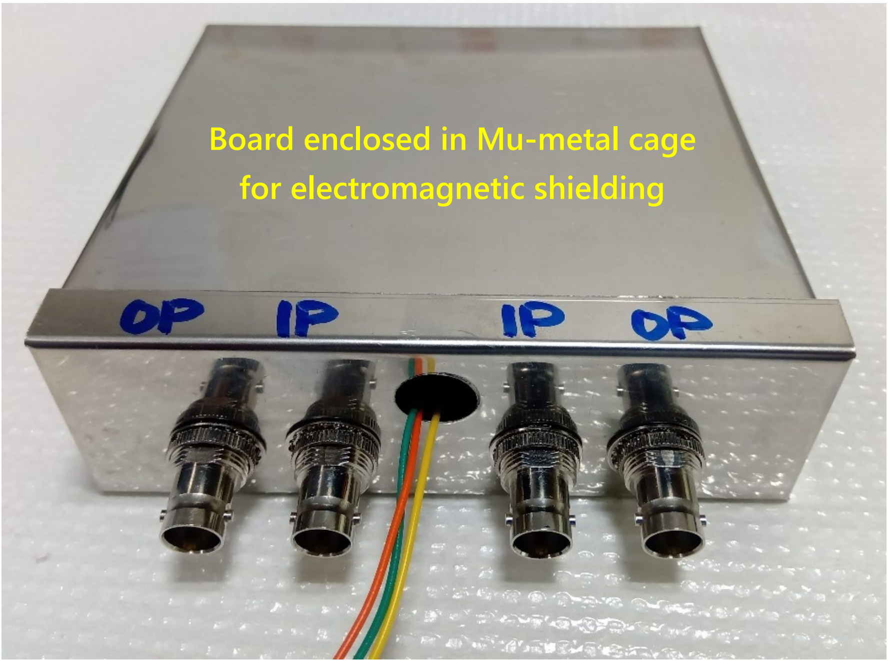


Figure SI-10: Top: The low-noise instrumentation amplifier built with AD8066 along with the fabricated microfluidic device for scale; Bottom: Custom built Mu-metal cage for electromagnetic shielding

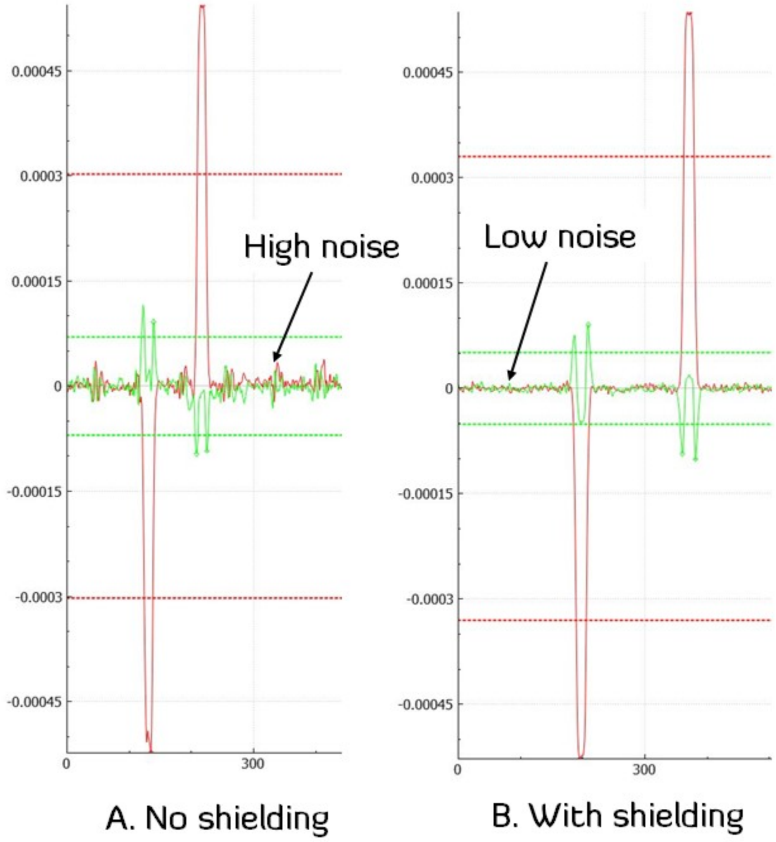


Figure SI-11: Comparison of signals for a single RBC A) before EMI shielding and B) with EMI shielding

Details of Supplementary Videos

SI Video 1: Effect of frequency on electric field lines when the cell is above the electrodes and at the point of peak maxima (position = 38 μm)

SI Video 2: Effect of frequency on electric field lines when the cell is in the electrode gap and at the point of peak minima (position = 50 μm)

SI Video 3: Effect of % area occupied by the cell (in the channel cross-section) on electric field lines

SI Video 4: Reactive current density plot for ellipsoid RBC model with CMC 7.59 mF/m^2 at 600 kHz

SI Video 5: Reactive current density plot for ellipsoid RBC model with CMC 10.11 mF/m^2 at 600 kHz

SI Video 6: Reactive current density plot for double-shelled lymphocyte model at 600 kHz