Supplementary Information for

LEUKOCYTE ANALYSIS AND DIFFERENTIATION USING HIGH SPEED MICROFLUIDIC SINGLE CELL IMPEDANCE CYTOMETRY

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S 1. Impedance analysis and Maxwell mixture theory

For a suspension of spherical particles (e.g. cells) in a medium, the equivalent complex permittivity of the mixture $\tilde{\varepsilon}_{mix}$ is given by:

$$\tilde{\varepsilon}_{mix} = \tilde{\varepsilon}_m \frac{1+2\Phi f_{CM}}{1-\Phi f_{CM}} \tag{1}$$

where $f_{CM} = \left(\frac{\tilde{\varepsilon}_p - \tilde{\varepsilon}_m}{\tilde{\varepsilon}_p + 2\tilde{\varepsilon}_m} \right)$ and $\tilde{\varepsilon}_m$ and $\tilde{\varepsilon}_p$ are the complex permittivity of the suspending medium and cell respectively. Φ is the volume fraction, the ratio of the volume of the cells to the detection volume. This model is based on the assumption that the particles are solid homogeneous objects. It can be extended to the case of a biological cell using the shell model, which treats the particle as a homogeneous object surrounded by a thin membrane. Now $\tilde{\varepsilon}_p$ is given by:

$$\tilde{\varepsilon}_p = \tilde{\varepsilon}_{mem} \frac{\left(\frac{R}{R-d} \right)^3 + 2\left(\frac{\tilde{\varepsilon}_i - \tilde{\varepsilon}_{mem}}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{mem}} \right)}{\left(\frac{R}{R-d} \right)^3 - \left(\frac{\tilde{\varepsilon}_i - \tilde{\varepsilon}_{mem}}{\tilde{\varepsilon}_i + 2\tilde{\varepsilon}_{mem}} \right)} \tag{2}$$

where $\tilde{\varepsilon}_{mem}$ and $\tilde{\varepsilon}_i$ are the complex permittivity of the cell membrane and cell inside (cytoplasm), respectively. *R* is the radius of the cell and *d* (*d*<<*R*) is the thickness of the membrane.

(a) Circuit parameters for a single shell object (cell)

For a cell held between two planar electrodes, and for low volume fraction (<10%) the individual electrical components can be represented as follows:

$$R_m = \frac{1}{\sigma_m (1 - 3\Phi/2) l \kappa} \qquad C_m = \varepsilon_\infty l \kappa$$

$$C_{mem} = \frac{9\Phi R C_{mem,0}}{4} \kappa l \qquad R_i = \frac{4\left(\frac{1}{2\sigma_m} + \frac{1}{\sigma_i}\right)}{9\Phi\kappa l}$$

with

$$\kappa = \frac{K(k)}{K'(k)} \qquad k = \tanh\left(\frac{\pi w}{2h}\right)$$

In this equation, κ is the geometrical cell constant for the impedance sensing region, solved using Schwarz-Christoffel mapping method that takes into account the non-uniform electric field. *h* is the height of the microfluidic channel, *w* the width and *l* is the electrode length. *K(k)* is the complete elliptic integral of the first kind, *K*'(*k*) is the complementary integral and *k* is the modulus of the elliptic function.

In these equations, the volume fraction Φ is given by (in ref [1], there is an error in this equation; the correct version is reproduced here):

$$\Phi = \frac{4}{3}\pi R^3 \frac{1}{wlh}$$

also

$$C_{mem,0} = \varepsilon_{mem} / d \qquad \varepsilon_\infty = \varepsilon_m \frac{2\varepsilon_m + \varepsilon_i - 2\Phi(\varepsilon_m - \varepsilon_i)}{2\varepsilon_m + \varepsilon_i + \Phi(\varepsilon_m - \varepsilon_i)}$$

where C_{mem} is the specific membrane capacitance (capacitance per unit area), d the thickness of the membrane, ε_∞ is the permittivity at infinite frequency, $\tilde{\varepsilon}_p$ and $\tilde{\varepsilon}_m$ are the complex permittivities of the particle and medium.

The complex impedance of the system $\tilde{Z}_{mix}$ becomes:

$$\tilde{Z}_{mix} = \frac{1}{j\omega\tilde{\varepsilon}_{mix} l\kappa}$$

The total impedance of the circuit (including the double layer) is:

$$\tilde{Z}_{mix\_DL} = \frac{2}{j\omega C_{DL}} + \frac{R_m(1 + j\omega R_i C_{mem})}{j\omega R_m C_{mem} + (1 + j\omega R_i C_{mem})(1 + j\omega R_m C_m)}$$

Typical values for the dielectric parameters for the cell:

$\varepsilon_o = 8.854\times10^{-12}$ Fm^{-1}, d = 5nm, $\varepsilon_m = 80\ \varepsilon_o$, $\sigma_m = 1.6$ Sm^{-1}, $\varepsilon_{mem} = 11.0\ \varepsilon_o$, $\sigma_{mem} = 10^{-8}$ Sm^{-1}, $\varepsilon_i = 60\ \varepsilon_o$, $\sigma_i = 0.6$ Sm^{-1} Typical geometric dimension for the microfluidic system are $w = h = l = 20$ μm. Actual values are obtained from measurement of individual chips.

(b) Circuit parameters for latex particle

Latex particles are solid homogenous dielectric objects. At low frequencies the dielectric properties are dominated by the surface conductance and at higher frequencies by the permittivity of the particle. The circuit elements in this case are cumbersome and not reproduced here. For full information see [2].

Dielectric parameters and used to obtain the fits to the impedance data shown in main text (figure 3) are taken from Yang et al [3], see Table S1

S 2. Cell handling

(a) Leukocyte purification and labelling:

Purified populations of T-lymphocytes ($CD3^+$ cells), monocytes ($CD14^+$ cells) and neutrophils ($CD16^+$ cells) were prepared using magnetic activated cell sorting (MACS, Miltenyi Biotec, Bisley, UK). Cell samples were labeled with fluorescently conjugated antibodies (and isotype matched controls) and analysed using a commercial flow cytometer (FACSAria, Becton Dickinson, Oxford, UK) to assess purity. All positive staining was compared to appropriate isotype controls and showed no non-specific labelling. Figure S2.1 shows the cell isolation and labelling protocol for the purified leukocyte sub-populations.

FACS scatter plots showing typical distributions for the samples of MACS purified neutrophils, monocytes and T-lymphocytes from human blood are shown. Figures S2.2 (a) – (d) show scatter plots of forward scatter (FSC) versus side scatter (SSC). Figures S2.2 (a) – (c) show MACS purified T-lymphocytes ($CD3^+$ cells, blue), monocytes ($CD14^+$ cells, red) and neutrophils ($CD16^+$ cells, green) respectively (colour coding is defined by the cell's fluorescent label). Figure S2.2 (d) shows a typical plot for a mixture of the three cell types following MACS purification and subsequent mixing of the purified populations.

Table S2 summarises the purities for each of the samples before and after the MACS purification step. Neutrophils harvested from the polymorphonuclear cell layer of the gradient had purities typically > 85 % (the main contaminating cells were erythrocytes). The leukocyte populations following MACS separation showed average purities of 90 % (87 – 92 %) for T-lymphocytes, 92 % (89 – 95 %) for monocytes and ~94 % (89 – 99 %) for neutrophils. The high purity of each of the three cell populations was essential for characterising the impedance properties of the leukocyte sub-populations.

Figure S2.4 shows the histograms for the low frequency impedance magnitude (|Z|@503kHz) and the opacity (|Z|@1.707MHz / |Z|@503kHz). Grey lines show the Gaussian fits to the three cell populations (T-lymphocytes, monocytes and neutrophils) and a population of 5.62 μm beads. The data corresponds to that of figure 4 in the main text.

(b) Whole blood

The whole blood experiments involved labelling the whole blood with anti-CD14-FITC and anti-CD16-Alexa700 and then lysing the erythrocytes using a saponin/formic acid lysis solution. Figure S2.3 outlines the protocol.

S3. Electrorotation of monocytes

Samples of monocytes were isolated from whole blood using MACS separation (anti-CD14 conjugated MACS beads) as described above. The cell samples were split into two fractions: untreated and saponin/formic acid treated. The untreated cells were resuspended in low conductivity buffer (8.5% w/v sucrose, 0.1% w/v glucose, 0.2% w/v BSA, 0.1mM EDTA and KCl to adjust conductivity to 22.2 $mS.m^{-1}$) and electrorotation experiments were carried out. The other monocytes sample was resuspended in plasma (from the original blood sample) and exposed to the 6 second saponin/formic acid lysis and quench (as per whole blood experiments). These cells were then washed and resuspended in the same low conductivity buffer as the untreated monoctyes. Complete spectra for individual untreated and treated cells (12 untreated cells and 12 treated cells) measured for 10 points per decade in the range 10kHz - 40MHz. Typical spectra for both are shown in figure S3.1

The single shell model was used to calculate the electrical properties of cells. Non-linear least-square fitting was performed in Mathematica™. The boundary conditions for the fitting were taken from typical values for blood cells as found in the literature [3]. A summary of the results is shown in the following table S3.

S4. Calibration of the impedance cytometer

The impedance cytometer was calibrated to allow un-ambiguous size analysis of the saponin/formic acid treated whole blood. Three sizes of latex beads (5.82 μm, 7.18 μm and 9.52 μm) were mixed and suspended in the saponin/formic acid treated whole blood. The sample was run on the impedance micro-cytometer system under the same conditions as the whole blood experiments (data shown in main text figures 5 and 6). The data are shown in figure S4.1, where the means and standard deviations of the bead populations are plotted as beads size versus low frequency (503 kHz) signal magnitude. A second order polynomial is fitted to the bead data as a calibration curve. The mean signal amplitudes obtained from the whole blood data (main text) of figures 5 and 6 is then used with the calibration curve to estimate cell size for the lymphocytes, monocytes and neutrophils (data shown in Table 1 in the main text).

References

1. Morgan H., Sun T., Holmes D., Gawad S. and Green N.G. Single cell dielectric spectroscopy. J. Phys. D: Appl. Phys. 40 61–70, 2007.
2. Morgan H and Sun T. Equivalent circuit models for single particles and cells in suspension – a complete analysis. Submitted J. Phys. D Appl. Phys., 2009.
3. Yang J., Huang Y., Wang X-B, Becker F. F. and Gascoyne P. R. C., Differential analysis of human leukocytes by dielectrophoretic field-flow-fractionation. Biophys J, 78(5) 2680-2689, 2000.

Figures:

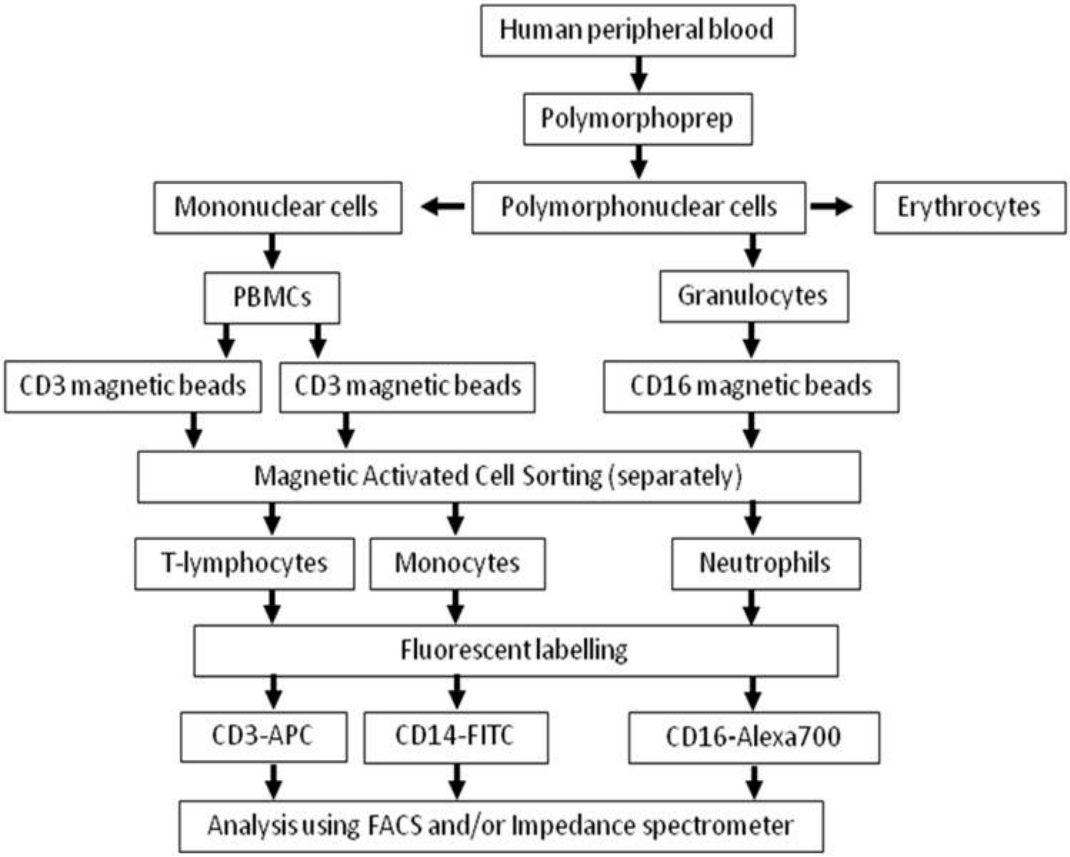

Figure S2.1. Cell purification and labelling protocol for purified leukocyte populations.

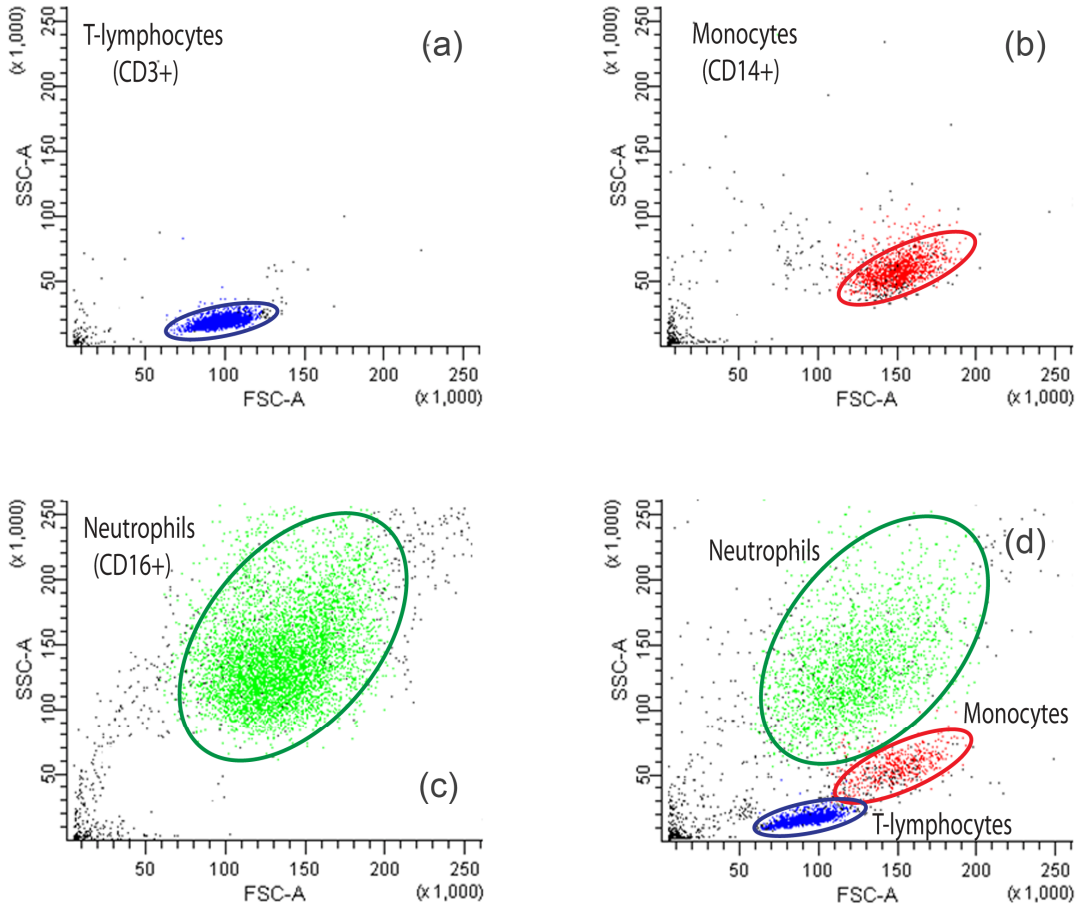

Figure S2.2. FACS scatter plots showing the (a) – (c) purified leukocyte populations following MACS separation and (d) the scatter plot following re-mixing of the purified populations.

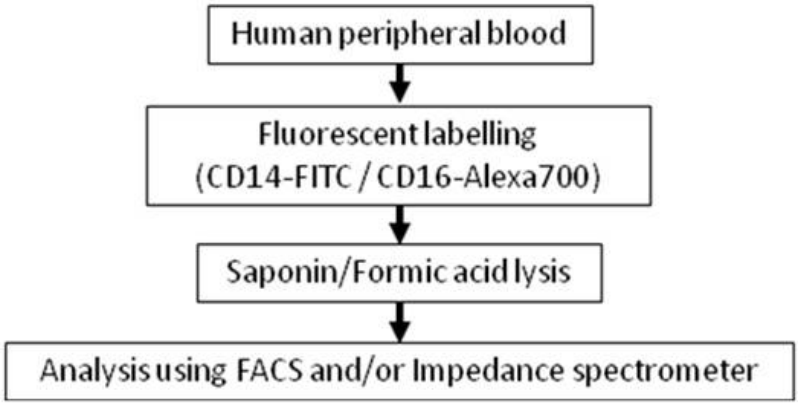

Figure S2.3. Cell labelling and lysis protocol for purified leukocyte populations.

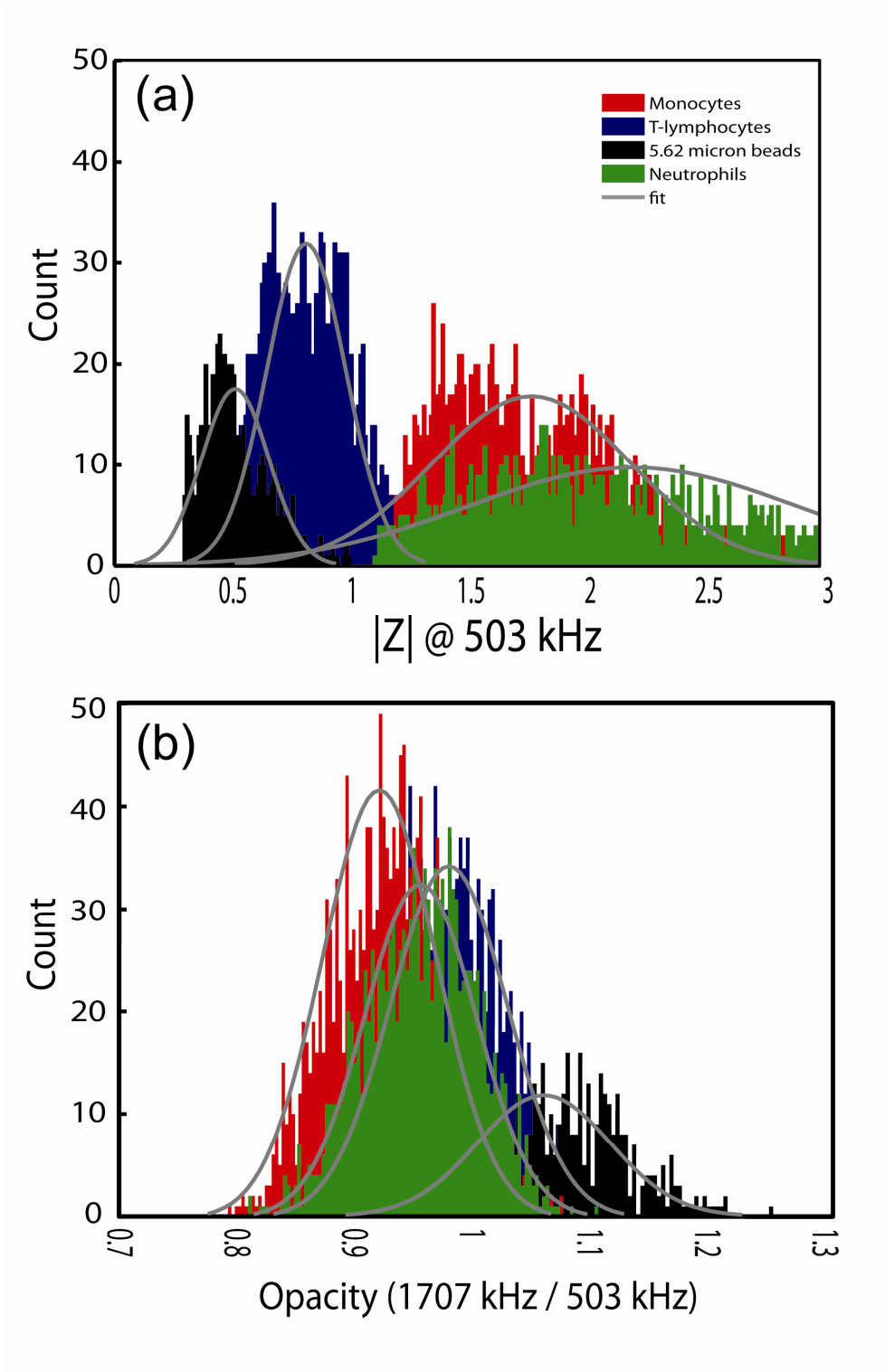

Figure S2.4. Histograms for the (a) low frequency impedance magnitude (|Z|@503kHz) and (b) opacity (|Z|@1.707MHz / |Z|@503kHz) of the purified cell populations. Grey lines show the Gaussian fits to the three cell populations 5.62 µm beads. Data corresponds to that of figure 4 in the main text.

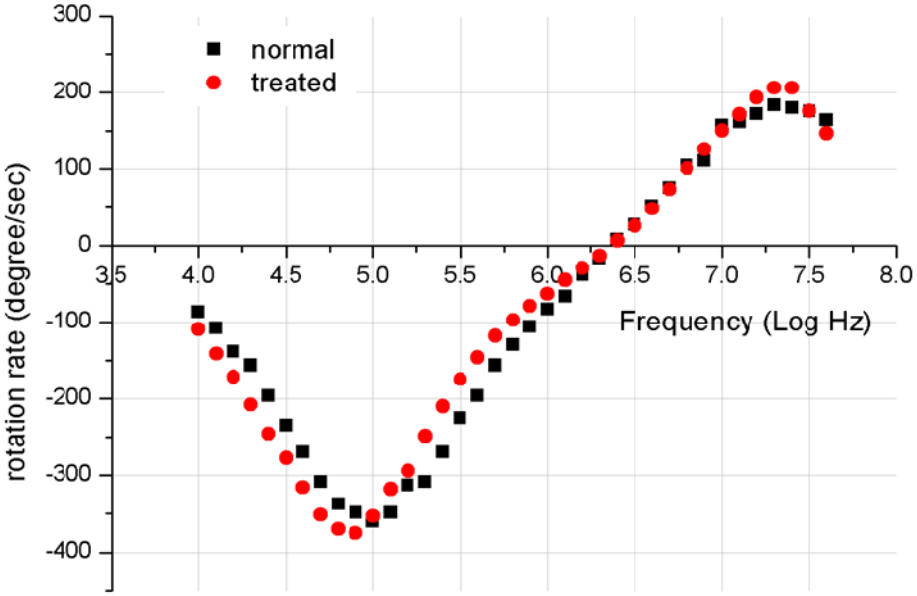

Figure S3.1. Electrorotation spectra for (a) untreated (squares) and (b) saponin/formic acid lysis treated (circles) monocytes.

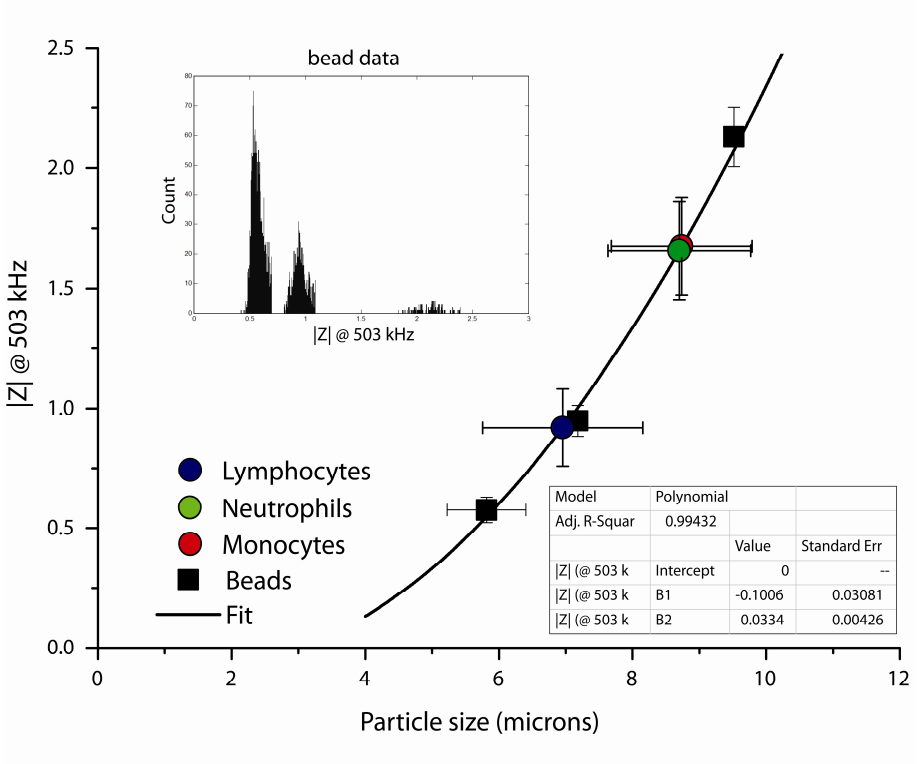

Figure S4.1 Calibration data for impedance micro-cytometer. Three sizes of latex beads (plotted as black squares) were run on the system. A calibration curve was calculated and used to determine the size of the cell sub-populations following saponin/formic acid treatment of whole blood: lymphocytes (blue circle), monocytes (red circle) and neutrophils (green circle).

Tables:

Table S1. Mean size and dielectric parameters of cells used to calculate fits to the data shown in Figure 3; parameters taken from Yang et al [18]. Also shown (final column) are the mean experimentally determined sizes for the cells, calculated from the low frequency impedance data following saponin/formic acid treatment (data from figure 6). The cells sizes extrapolated from the data of figure 6 compare favourably with published values.

	Diameter (μm)	Membrane Capacitance (mF/m^2)	Cytoplasm conductivity (mS/m)	Cytoplasm permittivity	Mean size (Saponin treated whole blood).
T-lymphocyte	6.58±0.7	10.5 ± 3.1	0.65±0.15	154.4±39.9	6.96±1.2
Monocyte	9.26±0.72	15.3 ± 4.3	0.56±0.1	126.8±35.2	8.74±1.05
Neutrophil (Granulocyte)	9.42±0.46	11 ± 3.2	0.6±0.13	150.9±39.3	8.7±1.07

Table S2. Purity of leukocyte sub-populations before (i.e in whole blood) and after MACS separation as assessed by FACS.

Cell type	Typical Purity (whole blood)	Purity (post MACS)
T-lymphocytes ($CD3^+$)	20 - 45 %	90 ± 4 %
Monocytes ($CD14^+$)	3 - 11 %	92 ± 3 %
Neutrophils ($CD16^+$)	40 - 75%	94 ± 6 %

Table S3. Electrical properties of untreated and saponin/formic acid treated monocytes. Values are the mean and standard deviation obtained by fitting individual spectra with the single shell model.

Cell type	diameter (μm)	ε_{Cyt} (F/m^2)	σ_{Cyt} (S/m)	C_{Mem} (F/m^2)
untreated monocytes	8.6±0.6	130±20	0.32±0.07	0.016±0.002
treated monocytes	8.8±0.6	90±10	0.36±0.07	0.019±0.003
monocytes (Yang et al. [2])	9.2±1	130±40	0.6±01	0.015±0.004