Supplementary Information (SI) for Lab on a Chip.

Single-Cell Impedance Spectroscopy of Nucleated Cells

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Support Information

Double-shell model:

The cell complex permittivity can be modelled as a series of concentric shells as illustrated in Figure 1(a), showing the double-shell model applied to a nucleated cell. This model relates the dielectric parameters of each individual shell to the complex dielectric properties of the cell according to [1,2]:

$$\varepsilon_{cell}^{*} = \varepsilon_1^{*}\frac{2(1-\gamma_a)+(1+2\gamma_a)E_a}{(2+\gamma_a)+(1-\gamma_a)E_a}$$

The subscript "$_1$" indicates the cell membrane. The factor γ_a is given by $\gamma_a = (R_a/r_{cell})^3$, with r_{cell} the cell radius, d_1 is the thickness of the cell membrane and $R_a = r_{cell} - d_1$.

The parameter E_a is given by:

$$E_a = \frac{\varepsilon_2^{*}}{\varepsilon_1^{*}}\frac{2(1-\gamma_b)+(1+2\gamma_b)E_b}{(2+\gamma_b)+(1-\gamma_b)E_b}$$

Here the subscript "2" stands for cytoplasm, $\gamma_b = (R_b/R_a)^3$, with R_b the radius of the nucleus and

$$E_b = \frac{\varepsilon_3^{*}}{\varepsilon_2^{*}}\frac{2(1-\gamma_c)+(1+2\gamma_c)E_c}{(2+\gamma_c)+(1-\gamma_c)E_c}$$

The subscript "3" is the nuclear envelope, $\gamma_c = (R_c/R_b)^3$, with $R_c = R_b - d_3$ and d_3 the thickness of the nuclear envelope. Finally, E_c is:

$$E_c = \frac{\varepsilon_4^{*}}{\varepsilon_3^{*}}$$

with subscript "4" refers to the nucleoplasm.

	Double shell model	Single shell model
Cell parameter	Value	Value
Cell membrane thickness (d_1)	5 nm	5 nm
Cell nuclear envelope thickness (d_3)	20 nm	
Volume ratio of nucleus and cell	0.3	
Cell membrane conductivity (σ_1)	3.14×10^{-5} S/m	3.14×10^{-5} S/m
Cytoplasm permittivity (ε_2)	$60\varepsilon_0$	$60\varepsilon_0$
Nuclear envelope conductivity (σ_3)	3×10^{-3} S/m	
Nuclear envelope permittivity (ε_3)	$52\varepsilon_0$	
Nucleoplasm conductivity (σ_4)	0.82 S/m	
Nucleoplasm permittivity (ε_4)	$120\varepsilon_0$	

Table S1. Parameters used for the double-shell and single-shell model simulation in Figure 1 and Figure S1. Cell dielectric parameters are from [1].

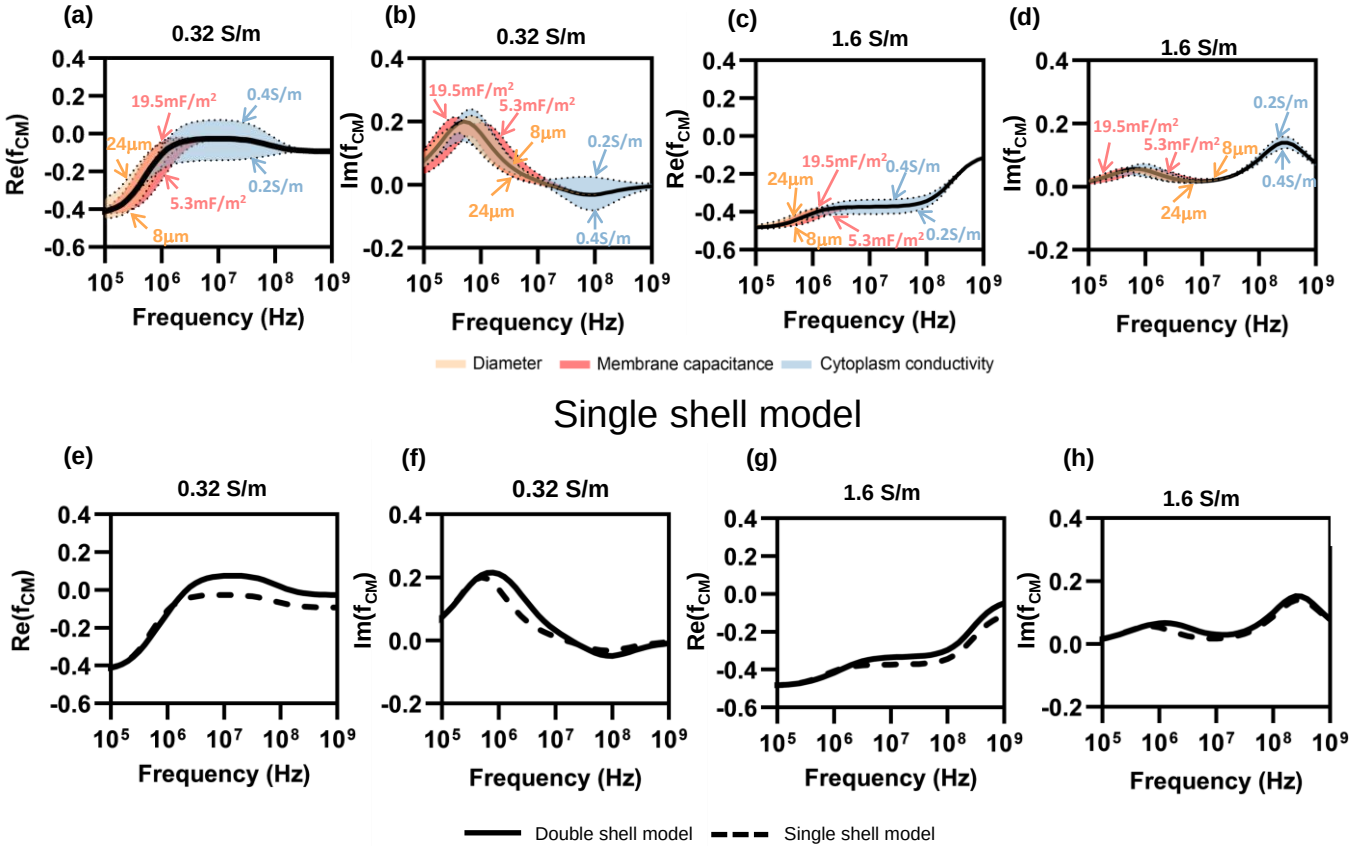


Figure S1. **(a)** and **(b)** are real part and imaginary part of normalised f_{CM}^{*} spectra in 0.32 S/m saline, calculated from single-shell model. **(c)** and **(d)** are real and imaginary parts in physiological saline with single-shell model. **(e)-(h)** Comparison of the double-shell model and single-shell model. Thes olid black lines are calculated curves for typical values of cell parameters: diameter = 12μm; membrane capacitance = 12.4 mF/m^2; cytoplasm conductivity = 0.3 S/m (for other parameters, see Table S1).

Electronic Circuit

The microfluidic impedance chip is driven with AC signals of variable frequency with inverted phases V_{in+} and V_{in-} (see Ref 12 main manuscript). The output from the chip is connected to a fully differential amplifier with high gain bandwidth. The output current is amplified in a symmetric common-mode feedback configuration, to give a differential signal (V_{out+} - V_{out-}). R_1 is the channel resistance and R_f the feedback resistor.

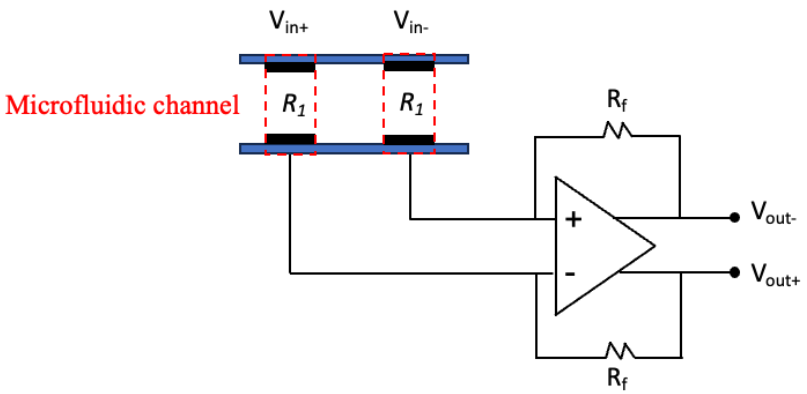


Figure S2. Circuit for a fully differential amplifier. Inputs V_{in+} and V_{in-} are inverted phases. The differential signal is amplified with common-mode feedback, giving outputs V_{out+} and V_{out-}.

Cell parameter	Set parameter range	Initial value
Volume ratio of cell and bead	5~25 (5μm diameter bead), 2.6~8.6 (7μm diameter bead),	13.8 (5μm diameter bead), 5.6 (7μm diameter bead),
Volume ratio of nucleus and cell	0.3~0.8	0.6
Cell membrane permittivity ($\mathcal{E}_1$)	2~15ε_0	7ε_0
Cytoplasm conductivity (σ_2)	0.001~0.4 S/m	0.015 S/m
Cytoplasm permittivity ($\mathcal{E}_2$)	40~160ε_0	90ε_0
Nuclear envelope conductivity (σ_3)	10^{-4}~10^{-2} S/m	0.003 S/m
Nuclear envelope permittivity ($\mathcal{E}_3$)	1~200ε_0	30ε_0
Nucleoplasm conductivity (σ_4)	0.1~2 S/m	1.8 S/m
Nucleoplasm permittivity ($\mathcal{E}_4$)	20~150ε_0	70ε_0

Table S2. The initial values and range of cell parameters used for the "pattern-search" algorithm in MATLAB.

Chip Fabrication

The cytometry chip fabrication process has been previously described [3, 4]. Chips were fabricated using the process flow shown in Figure S3 below. The electrodes are made from a 200nm thick layer of Pt with a 20nm Ti adhesion layer, patterned by standard photolithography on 700µm thick 150mm diameter glass wafers. The microchannel is made using a thick photoresist, 30µm high and 40µm wide. Two wafers are bonded using a thermo-compression bonding process. The bonded wafer is diced to release individual chips, each 15mm x 15mm. Access holes are drilled using a laser. The chip is mounted in a custom PEEK holder with fluidic and electrical connections

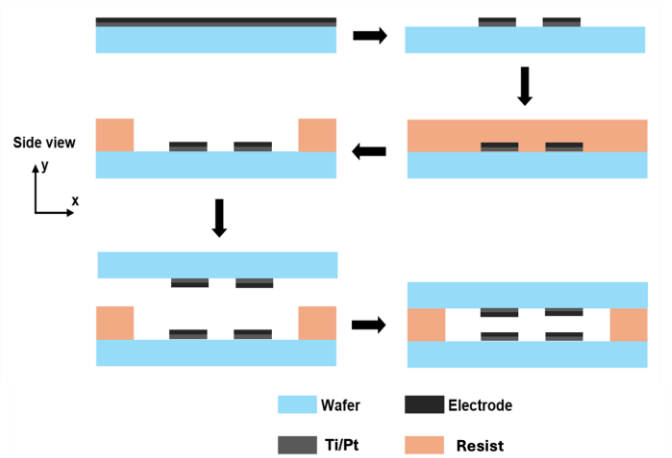


Figure S3. Microfluidic chip fabrication process flow. Electrodes are patterned on a wafer by photolithography and the microfluidic channel is created using Perminex resist*.

SNR (signal-noise-ratio) definition:

The impedance SNR is defined as the mean square ratio of the noise to particle impedance. The impedance signal is collected simultaneously at two frequencies, 18MHz and 15.9MHz. 18MHz is set as a fixed reference frequency and is used to gate the beads and cells. After gating the noise, beads and cells subpopulations (Figure S4), the SNR (for beads and cells) can be determined as follows:

$$SNR_{bead} = 10\times\log_{10}\left(\frac{mean\ square(beads)}{mean\ square(noise)}\right) = 10\times\log_{10}\left(\frac{23.4}{3.3}\right) = 8.5\ dB$$

$$SNR_{cell} = 10\times\log_{10}\left(\frac{mean\ square(cells)}{mean\ square(noise)}\right) = 10\times\log_{10}\left(\frac{45.9}{3.3}\right) = 11.4\ dB$$

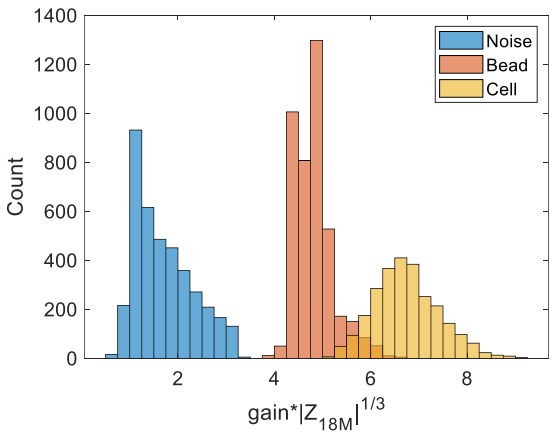


Figure S4. Histogram of Gain × $\sqrt[3]{|Z_{18MHz}|}$ for noise (blue), beads (orange) and cells (yellow).

252kHz	399kHz	631kHz	1MHz	1.6MHz	2.5MHz	3.9MHz	6.3MHz	10MHz
4795	4710	4497	4412	4225	4022	3646	3269	3119
16MHz	**25MHz**	**39MHz**	**63MHz**	**100MHz**	**158MHz**	**251MHz**	**398MHz**	**550MHz**
2690	2457	2677	3297	3857	3529	3531	2689	2971

Table S3. Number of cells for untreated group (0.32 S/m) at each frequency point in Figure 3.

0.32 S/m **Untreated** HL60 using population mean method			
	C_{mem}(mF/m^2)	R (μm)	σ_{cyt} (S/m)
Repeat 1	9.62	12.25	0.265
Repeat 2	9.14	12.00	0.334
Repeat3	9.07	12.47	0.283
mean	9.28	12.24	0.294
SD	0.30	0.24	0.036

0.32 S/m **Fixed** HL60 using population mean method			
	C_{mem}(mF/m^2)	R (μm)	σ_{cyt} (S/m)
Repeat 1	6.27	12.77	0.078
Repeat 2	6.32	12.62	0.074
Repeat 3	6.28	12.41	0.101
mean	6.29	12.60	0.084
SD	0.03	0.18	0.015

Table S4. Fit results for three repeat experiments using the population mean method for untreated and fixed cells suspended in 0.32 S/m saline. The results from "Repeat 2" are presented in the main text.

Single-shell Model Fit Example:

A comparison of the fit between the double-shell and single-shell models was done using the dataset for untreated HL60 cells suspended in 0.32 S/m saline (**Figure 3** and **Table 1** main text). In comparing the single shell fit, the parameter ranges and initial values for "Volume ratio of cell and bead", "Cell membrane permittivity (ε_1)", "Cytoplasm conductivity (σ_2)", "Cytoplasm permittivity (ε_2)" and "Cell membrane conductivity (σ_1)" were set to be the same as the double-shell model (**Table S2**). It is clear from Figure S5 that the fit is poor when using the single shell model for the same parameter set (R^2<0.9). Furthermore, the fitted parameters, (cytoplasm conductivity etc), are far from realistic values. This demonstrates that for nucleated cells such as HL60, the single-shell model cannot be used to fit the impedance data.

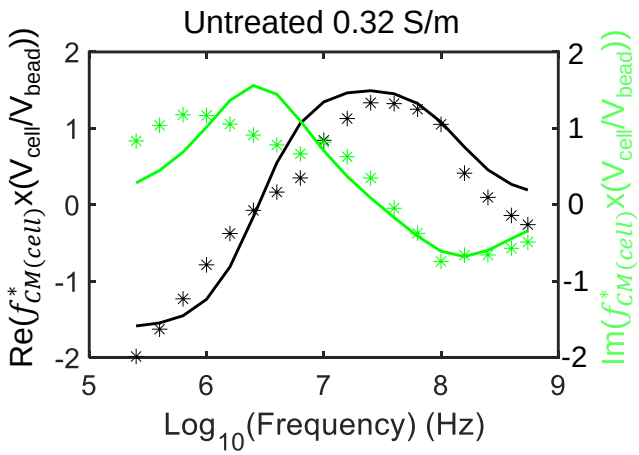


Figure S5. Normalised Clausius-Mossotti factor and a single-shell model fit for HL 60 cells. Black and green stars are real and imaginary parts of the data for cells suspended 0.32 S/m saline. The dataset is the same as used for the double-shell model fit in the main text. Black and green curves are fits for the real and imaginary parts.

Untreated		*d* (μm)	C_{mem} (mF/m²)	σ_{cyt} (S/m)	Real part R^2	Imaginary part R^2
0.32 S/m	Single shell	10.5	4.63	1.32	0.867	0.744
	Double shell	12.0	9.14	0.33	0.995	0.985

Table S5 Fit outputs for cell diameter (d), membrane capacitance (C_{mem}) and cytoplasm conductivity (σ_{cyt}) for untreated HL60 cells suspended in 0.32 S/m saline using the single-shell and double-shell models. The results for the double-shell model are summarised in Table 1, main text.

Residual

In order to determine the frequency at which the f_{CM} is statistically significantly different between normal (N) and CytoD (CD) treated cells, we defined a simple metric - "residual" as shown below:

Residual real part: $errR = |\mathrm{Re}(CD\_f_i) - \mathrm{Re}(N\_f_i)|$;

Residual imaginary part: $errI = |\mathrm{Im}(CD\_f_i) - \mathrm{Im}(N\_f_i)|$

$$\text{Residual} = \frac{\sqrt{(errR)^2+(errI)^2}}{\sqrt{\mathrm{Re}(N\_f_i)^2+\mathrm{Im}(N\_f_i)^2}} \times 100\%$$

The largest residual is 25.6% at 6.3MHz.

Confidence Interval (CI) Evaluation:

The confidence interval (CI) of the parameters returned from the algorithm "patternsearch" was evaluated using bootstrap, widely used in non-linear fitting. The concept involves resampling the elements in the residual set with $\{r_i^*\}$ the difference between measured and fitted, given by

$$r_i^* = (f_{CM}^*)_{i(measure)} - (f_{CM}^*)_{i(fitted)}$$

where "*i*" stands for the *i*th frequency of the measurement. The new set of residuals $\{r_{i(resample)}^*\}$ is generated by resampling r_i^* with a replacement data. This is done by randomly generating a new set of *n* ($i \in 0 \sim n$) residuals, where each of *n* frequencies is one of the original residuals chosen with equal probability. Typically, some of the original residuals r_i^* can be chosen more than once, while some are not chosen at all.

The resampled residual $r_{i(resample)}^*$ is then added to its corresponding fitted response $(f_{CM}^*)_{i(fitted)}$, producing a new bootstrap dataset $\{(f_{CM}^*)_{i(bootstrap)}\}$, given by

$$(f_{CM}^*)_{i(bootstrap)} = (f_{CM}^*)_{i(fitted)} + r_{i(resample)}^*$$

The $\{(f_{CM}^*)_{i(bootstrap)}\}$ dataset is treated as an independent replicate experiment which is fitted to the model to calculate new estimates of model parameters giving new values of *d*, C_{mem} and σ_{cyt}.

For each group of cells, 1,000 new bootstrap datasets of the 18 frequency spectrum ("population-mean" method) and or 8 frequency spectrum ("single-cell" method) were generated and fitted using the "patternsearch" function 1,000 times. The initial values of the fitting parameters were set to the best-fit output. The 95% CI of *d*, C_{mem} and σ_{cyt} are given in the tables below.

CI for Table 1		Diameter (d, µm)	Membrane capacitance (C_{mem}, mF/m^2)	Cytoplasm conductivity (σ_{cyt}, S/m)
1.6 S/m	Untreated	[11.54, 11.64]	[10.00, 12.01]	[0.28, 0.34]
	Fixed	[11.61, 11.77]	[9.68, 13.10]	[0.28,0.37]
0.32 S/m	Untreated	[11.74, 12.27]	[8.48, 10.28]	[0.25,0.37]
	Fixed	[12.51, 12.75]	[5.63, 6.96]	[0.073, 0.081]
	CytoD	[11.56, 11.90]	[6.54, 7.78]	[0.32, 0.40]

Table S6. CI for Table 1 in main text.

CI for Table 2		Diameter (d, mm)	Membrane capacitance (C_{mem}, mF/m^2)	Cytoplasm conductivity (σ_{cyt}, S/m)
0.32 S/m	Untreated	[12.32, 12.66]	[9.74, 10.99]	[0.20,0.26]
	Fixed	[10.89, 11.62]	[4.55, 10.65]	[0.055, 0.078]
1.6 S/m	Untreated	[11.37, 11.48]	[9.52, 11.39]	[0.30, 0.42]
	Fixed	[11.55, 11.64]	[8.83, 9.92]	[0.38, 0.48]

Table S7. CI for Table 2 in main text.

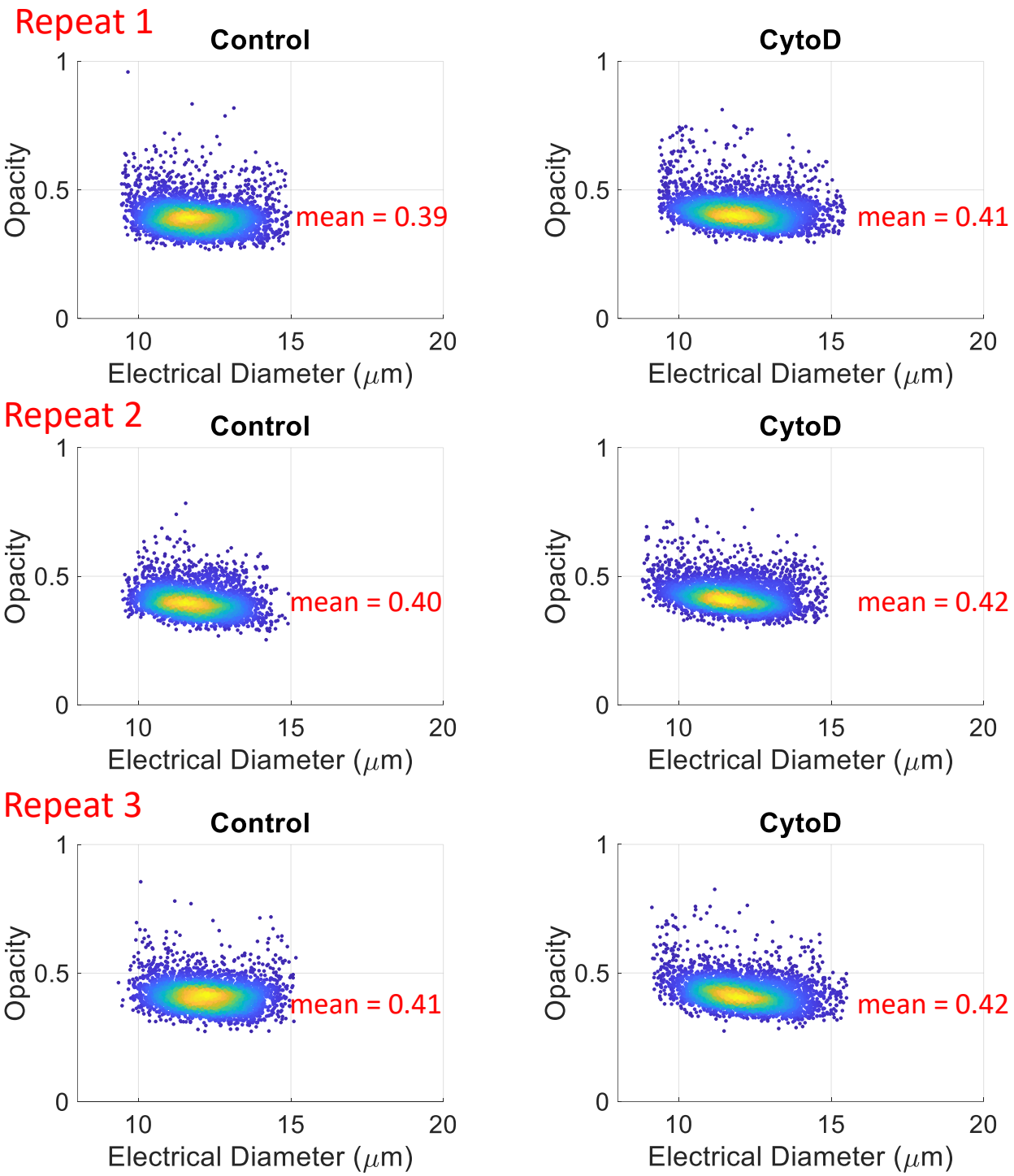


Figure S6. Scatter plots of electrical diameter ($\sqrt[3]{|Z_{150kHz}|}$) versus opacity ($|Z_{3MHz}|/|Z_{150kHz}|$) for control and CytoD treated cells. Data were collected on three different days with separate batches of cells.

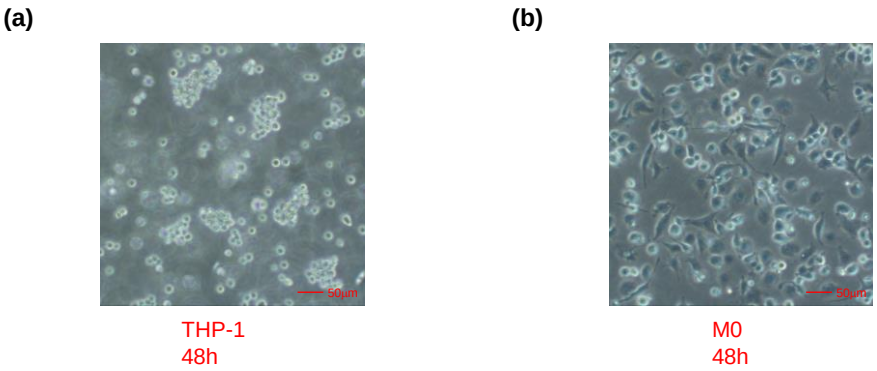
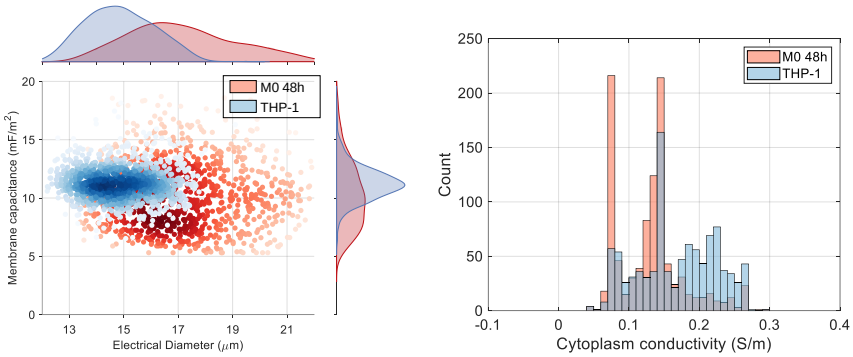


Figure S7 Images of **(a)** THP-1 and **(b)** M0 cells in the culture flasks.

Repeat 2



Repeat 3

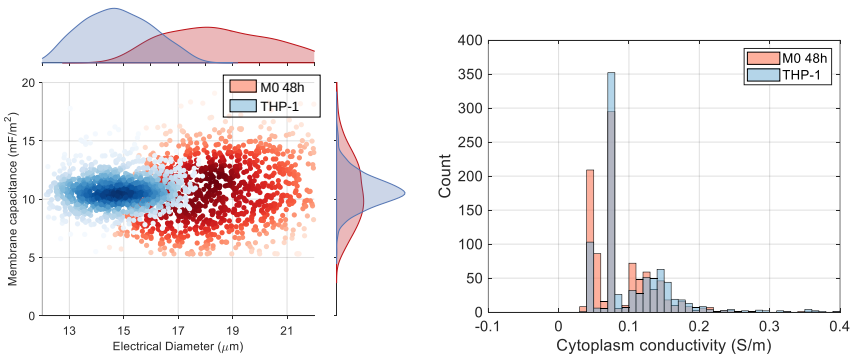


Figure S8. Data for repeats 2 and 3 for THP-1 and differentiated M0 cells.

References

[1] Mansoorifar, A., et al., *Accuracy of the Maxwell–Wagner and the Bruggeman–Hanai mixture models for single cell dielectric spectroscopy.* IET Nanobiotechnology, 2017. **11**(7): p. 874-882.

[2] Irimajiri, A., T. Hanai, and A. Inouye, *A dielectric theory of "multi-stratified shell" model with its application to a lymphoma cell.* Journal of theoretical biology, 1979. 78(2): p. 251-269.

[3] Spencer, D., Elliott, G., and Morgan, H., *A sheath-less combined optical and impedance micro-cytometer.* Lab Chip, 2014. 14(16): p. 3064-73.

[4] Spencer, D., Hollis, V., and Morgan, H., *Microfluidic impedance cytometry of tumour cells in blood*. Biomicrofluidics, 2014. 8(6): p. 064124