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A Portable Impedance Microflow Cytometer for Measuring Cellular Response to Hypoxia

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Abstract - This paper presents the development and testing of a low-cost (< $60), portable, electrical impedance based microflow cytometer for single cell analysis under controlled oxygen microenvironment. The system is based on an AD5933 impedance analyzer chip, a microfluidic chip, and an Arduino microcontroller operated by a custom Android application. A representative case study on human red blood cells (RBCs) affected by sickle cell disease is conducted to demonstrate the capability of the cytometry system. An equivalent circuit model of a suspended biological cell is used to interpret the electrical impedance of single flowing RBCs. RBCs exhibit decreased mean membrane capacitance by 24% upon hypoxia treatment while the mean cytoplasmic resistance remains consistent. RBCs affected by sickle cell disease exhibit decreased cytoplasmic resistance and increased membrane capacitance upon hypoxia treatment. Strong correlations are identified between the changes in the cells' subcellular electrical components and the hypoxia-induced cell sickling process. The results reported in this paper suggest that the developed method of testing demonstrates the potential application for low-cost screening technique for sickle cell disease and other diseases in the field and low-resource settings. The developed system and methodology can be extended to analyze cellular response to hypoxia in other cell types.

vi

Introduction

Hypoxia (deprivation of oxygen in the body) causes a variety of physiological changes in cells. Physiological responses to hypoxia due to high-altitude, deep-sea diving, or pathological responses have been extensively investigated at both the whole-body and single cell levels (Michiels, 2004; Storz, Scott, & Cheviron, 2010). Analysis of single-cell suspensions has become an important medical interest. Studies of cellular responses to hypoxia have provided insight in tumor pathology (Lewis & Murdoch, 2005), cancer treatment (Brown & Wilson, 2004), cardiovascular pathophysiology (Garvey, Taylor, & McNicholas, 2009), metabolism (Sarrafzadeh et al., 2010; Solaini, Baracca, Lenaz, & Sgarbi, 2010), and homeostatic mechanisms in mammalian cells (Semenza, 1999). A gold standard to measure the cellular response to hypoxic environments is flow cytometry. Flow cytometry analyzes single cells and measures levels of protein such as hypoxia-inducible factor 1-alpha (HIF1α) and BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) (Cheng, Kang, Zhang, & Yeh, 2007; Lee et al., 2017). This method provides high specificity via antibody-based immunostaining to target the proteins of interest, but also requires the analyzed cells being fixed and permeabilized.

Recently, electrical impedance-based flow cytometry has been demonstrated as an alternative method to the conventional optical approach for the analysis of single cells. It is inherently quantitative, non-invasive and label-free. Therefore eliminating the needs for fluorescence or biochemical labeling (Cheung et al., 2010). Electrical impedance-based flow cytometry of single cells has been demonstrated to discriminate healthy red blood cells (RBCs) from those affected by malaria (Bow et al., 2011; Du et al., 2013) or sickle cell disease (SCD) (Liu, Qiang, Alvarez, & Du, 2018). In the study of cellular response to hypoxia, microfluidics technology can be used to precisely control the gaseous microenvironment and allow simultaneous microscopy and impedance sensing of cells in flow and in stationary conditions (Liu, Qiang, Alvarez, & Du, 2019). However, these electrical impedance analyses were performed using large benchtop equipment. Miniaturization of electrical impedance-based flow cytometry systems can provide field-testing and point-of-care assay of single cells in response to hypoxia. Portable electrical impedance systems have been created for general bio-impedance purposes (Rottigni, Carminati, Ferrari, & Sampietro, 2011). Microcontrollers with peripheral devices for user control and observation have been used with the popular AD5933 integrated circuit (Devices, 2017) to create portable impedance sensors (Breniuc, David, & Haba, 2014; Simic, 2013). Further portability is

achieved when implementing wireless communication, such as Bluetooth, to separate data acquisition on the sensor from data processing on the personal computer (PC) (Al-Ali, Elwakil, Ahmad, & Maundy, 2017). Control of the sensor and visualization of the data can be managed using smart phone applications via wireless communication (Jiang, Sun, Venkatesh, & Hall, 2016; Talukder et al., 2017). Further research has led to wearable, impedimetric cytometry with results available via smart phone application (Furniturewalla, Chan, Sui, Ahuja, & Javanmard, 2018).

In this work, we present the development and testing of a portable, mobile app controlled, impedance-based flow cytometer for measuring cellular response to hypoxia. We demonstrate the capability of electrical impedance-based flow cytometry of individual RBCs as they are the most abundant type of cells and contain the oxygen carrier protein, hemoglobin (Hb), that is sensitive to the environmental oxygen tension. We show discrimination between normoxic and hypoxic states for the same population of RBCs. In SCD, the phenotypic responses of the mutation of normal HbA into HbS are the formation of rigid HbS fibers and resulting sickled shape as the HbS polymerize in response to hypoxia (Christoph, Hofrichter, & Eaton, 2005; Van Beers et al., 2014). Conventionally, SCD is diagnosed in labs via techniques such as hemoglobin electrophoresis (Billett, 1990), isoelectric focusing (Kanter, 2016), high-performance liquid chromatography (Colah et al., 2007), chromatographic immunoassay (Kanter et al., 2015), and density-based erythrocyte measurement (Kumar et al., 2014) to detect HbS. We demonstrate that this portable device is sensitive enough to discriminate between RBCs from healthy donor and SCD patients. The portable system can be readily used as a point-of-care diagnosis and monitoring tool of SCD. We present a novel method of cell membrane capacitance and cytoplasmic resistance from cell impedance utilizing a finite element model of the impedance sensing platform. The interpretation of the detailed sub-cellular changes associated with hypoxia treatment not only allows us to better understand the impedance changes in sub-cellular components associated with the cell sickling process (cell morphology change and hemoglobin phase transition from liquid to gel), but also hemoglobin affinity for oxygen in normal red cells. These analyses extended our preliminary, proof-of-the concept work demonstrating cell impedance as a label-free biophysical marker of sickle cell disease, as reported in our 2018 Sensors and Actuators B paper (Liu et al., 2018).

Materials and Methods

Design and fabrication of the microfluidic chip

The electrical impedance measurements of RBCs were carried out on a disposable microfluidic chip (Figure 1A). The microfluidic chip is a set of microchannels and two parallel Ti/Au electrodes (with 18 μm gap and 21 μm band width) deposited on a 0.7 mm thick glass. The microchannel consists two layers of polydimethylsiloxane (PDMS) channels: the top layer is a serpentine shape gas channel, and the bottom layer is a thin gas permeable membrane (150 μm thick) with a straight channel for cell suspension. PDMS channels were fabricated following a standard soft lithography method. The narrowest portion of the microchannel measures 20 μm wide and 3.4 μm deep. The microchannel depth takes into account common morphology of normal and sickle cells to minimize the distance between the cells and electrodes (Diez-Silva, Dao, Han, Lim, & Suresh, 2010; Kviatkovsky et al., 2017). Electrodes were deposited and patterned on a glass substrate using E-beam evaporation and standard photolithography techniques (Qiang, Liu, & Du, 2017). The double-layer microchannel and electrodes-glass substrate are bonded together using air plasma. Conductive wires were soldered to the electrodes to allow the cells to be measured by the portable impedance sensing system.

Portable Impedance-based Flow Cytometer System

Figure 1B illustrates the impedance sensing system as used during experimentation. The system is centered around the AD5933 impedance converter by Analog Devices, for its ability to output a sinusoidal wave with controlled frequency and voltage as well as calculating and providing the admittance of an unknown sample. A printed circuit board (PCB) was designed to connect directly to a commercially available Arduino UNO microcontroller. On the PCB, the AD5933 is connected to the Arduino UNO using the two-wire I2C communication protocol. The Arduino UNO is operated via an Android application through Bluetooth communication using an HC-06 Bluetooth Module. An example of the application interface displaying results is shown in Figure 1C. The Bluetooth module uses a two-wire serial connection to relay data to and from the Arduino UNO. The Android application allows the user to begin impedimetric scans over a set duration or until manually stopped. Scan results can be optionally plotted on phone and a text file containing the collected data can be distributed via email. The Arduino UNO and the AD5933

are connected to an HMC245A switch. The switch provides onboard calibration functionality for the AD5933 by establishing a multi output, single input signal path. An SD card reader is connected to the Arduino UNO using a 3-wire SPI connection. The SD card provides large storage capabilities to compensate the fast data throughput needed for flow cytometry in addition to providing an alternative method of data transfer to PCs for further analysis. Two female SMA connectors on the PCB provide connectivity to the soldered wires on the microfluidic chip.

Experimental Protocol

The inlet to the gas channel is inserted with a standard 0.02 in. ID/0.06 in. OD microbore tubing and connected to a PC controlled switching valve to alternate between a high purity N_2 gas and a gas mixture of 5% CO_2, 17.5% O_2 and 77.5% N_2. This creates a deoxygenated (DeOxy) and an oxygenated (Oxy) condition for flow cytometry. For each patient sample, 5 µL of whole blood. was diluted into 1 mL of phosphate buffered saline (PBS). The cell channel was primed with PBS. Microliter samples of RBC suspension are injected into the suspension channel via the microbore tubing through 1.03 mm, 50 µL, Hamilton glass syringe. The flow rate of cell suspension is fixed at 100 pL/min using a Harvard Apparatus Pump 11 Pico Plus Elite syringe pump. The portable cytometer is operated to output a 2 V peak-to-peak (V_{pp}) sinusoidal signal at a frequency of 100 kHz and continuously measure data using the custom Android application. Microscope video is simultaneously recorded in order to validate impedimetric readings. Data is saved onto the SD card during the experiment and later analyzed by a custom MATLAB script.

Computational Analysis

Computation of medium impedance was conducted based on finite element modeling using the Electric Currents application mode of the COMSOL Multiphysics AC/DC Module, following the corresponding guidelines (https://doc.comsol.com/). The governing equations for computation are,

$$\nabla \cdot J = Q_{j,v}$$

$$J = \sigma E + j\omega D + J_e$$

$$E = -\nabla V$$

where **J** is current flux, **E** is the electric field, V is the electric potential, **D** is the electric displacement, $\mathbf{J_e}$ is an externally generated current density, and $Q_{i,v}$ is the current per volume.

The simulation was performed to take account capacitance-resistance computations with inductive response neglected. To reduce the memory requirement and computation time, the three-dimensional modeling for PBS impedance computation were simplified in the following aspects: a) the electrodes were modeled as boundaries (infinitely thin); b) the channel was symmetric and truncated on each side in the axis of length; c) the channel structure (PDMS) and substrate (glass) were modeled as boundaries with specific dielectric properties assigned. Figure 2 shows the computation domain and electrical potential boundaries, where one electrode was grounded, and the other electrode was defined as terminal with 1 V. The modeling domain consists of a rectangular channel and an ellipsoid with semiaxes of 4 μm, 4 μm and 1.25 μm in mimicking a typical cell volume of 84 *fL*. To compute reference PBS impedance, Z_{PBS}, when a cell is absent, ellipsoidal domain was assigned with dielectric properties of PBS. To compute PBS impedance, Z_{PBS*}, when a cell is present, ellipsoidal domain was subtracted from the calculation domain. The geometry of the calculation domain was created based on the information listed in Table 1.

The substrate of the channel was modeled by partitioning its bottom surface with two polygons of 20 μm in width and 20 μm in length. This enabled definitions of the boundary conditions for electrical potentials of the electrode surfaces and for electrical shielding from the glass substrate, respectively. The definition of electric shielding boundary condition follows,

$$n \cdot (J_1 - J_2) = -\nabla_t \cdot d_s ¿$$

where d_s is the surface thickness, σ is the sheet's electrical conductivity, ∇_t represents the tangential derivative around the shell, ϵ_0 and ϵ_r are the vacuum and relative permittivity respectively, and V represents the voltage potential at terminal 1.

The boundary conditions (BCs) for the two side surfaces perpendicular to x-axis were defined as electrical shielding with dielectric properties inherited from PBS. The remaining boundary conditions were defined as electrical shielding from the PDMS. Relative permittivity of PBS is assumed to be 78 at room temperature. The material properties of each material including electric conductivity and relative permittivity were defined and summarized in Table 2.

Tetrahedra meshing was utilized for the simulation. The total number of degrees of freedom of the computation domain was greater than 2.5 million. The computation was performed in two

steps, including a stationary source sweep followed by a frequency domain study at a fixed frequency, 100 kHz. The terminal current, *I* was obtained for impedance calculation, following relationship between terminal voltage and current,

$$Z = \frac{V}{I} = R + iX$$

where R is the resistance and X the reactance. It was found that the PBS response was primarily resistive.

Statistical Analysis

All data are expressed as mean ± SD. Statistical analyses of RBCs were performed with MATLAB. Mann Whitney test measurements of SS RBCs between Oxy and DeOxy conditions were used to generate the *p* values. Student t-test was used to generate the *p* values for normal RBCs. A *p*-value less than 0.05 is statistically significant. Sample distributions of normal RBCs and SS RBCs were fitted with normal function and Kernel density estimation, respectively.

Results and Discussion

Electrical Impedance of Single Cells

The impedance response of individual RBCs on the portable device was validated by comparing it with the response of cells measured using the HF2IS Impedance Spectroscope by Zurich Instruments. Figure 3 displays the impedance of an RBC, in the form of bell-shaped curves, measured by the bench top HF2IS instrument and by our portable device. The curves' increase in impedance illustrates the change in impedance from measuring the PBS medium only to the medium with a cell passing through the pair of electrodes compared to the impedance of PBS medium only. A level 3 wavelet filter with BlockJS denoising in MATLAB was used to denoise the raw signal (gray color, Figure 3C) obtained from the portable device, which produces impedance measurement comparable to the commercial instrument.

Impedance of single cells are calculated utilizing the equivalent circuit models as shown in Figure 4. For the impedance of PBS medium only, (Z_{mo}¿, the equivalent circuit model (Figure 4A) consists of medium impedance, Z_{PBS} in series of an electric double layer impedance, simplified to a single variable, Z_{dlt}. The total impedance is,

$$Z_{mo} = Z_{dlt} + Z_{PBS}$$

where Z_{PBS} is obtained from a finite element simulation using COMSOL Multiphysics. The computed resistance and reactance values of PBS were $R_{PBS} = 1.92\times10^5$ Ω and $X_{PBS} = -1.77\times10^3$ Ω. These values were subtracted from the measured reference impedance (absence of cell) to determine the impedance of electrical double layer, $Z_{dlt} = R_{dlt} + iX_{dlt}$.

When a cell is present between the two electrodes, the total impedance measured, Z_{wc}, is expressed by,

$$Z_{wc} = Z_{dlt} + ¿¿$$

where $Z_{pbs*¿¿}$ is the impedance of PBS surrounding the cell, which can be calculated from finite element simulation with an ellipsoid shape subtracted from the computation domain. For simplification of data analysis, the value of Z_{PBS*} was assumed to be a constant, based on the void ellipsoid of volume of 84 *fL*. The computed resistance and reactance values of PBS were $R_{PBS*} = 2.09\times10^5$ Ω and $X_{PBS*} = -2.09\times10^3$ Ω. Variation in cell volume is likely to cause variation in the actual Z_{PBS*}. Such influence is minor. A relatively large variation in cell volume (e.g., when the ellipsoid volume increased to 100 *fL*, a 19% increase) leads to a minor variation in R_{PBS*} (1.2%) and X_{PBS*} (2.7%). This suggested that it is reasonable to use Z_{PBS*} computed from a standard cell volume (e.g. 84 *fL*) to extract single cell properties. The value of Z_{PBS*} together with the calculated Z_{dlt} were substituted into the equivalent circuit model to determine the single cell impedance,

$$Z_{cell} = ¿¿$$

The extracted single cell impedance, Z_{cell} is then analyzed by a custom MATLAB script to perform peak detection. Firstly, the peaks in the real part are identified. The corresponding indices are saved and used to extract the corresponding peak values in the imaginary part of Z_{cell}. Figure 5 shows the identified peaks in the real part and imaginary part of a representative impedance data.

Figure 6 shows the extracted single cell impedance from two normal blood samples (AA1 and AA2) and sickle cell samples from three SCD patients (SS1-SS3). In normal RBCs, the relative magnitude and relative phase resemble normal distributions with remarkable overlaps between Oxy and DeOxy conditions (Figures 6 AB). In SS RBCs, the relative magnitude and phase values were more widely distributed than normal cells, disregarding the level of oxygen tension. This was not surprising as SS RBCs are more heterogeneous in terms of cell volume and

other intrinsic properties, such as membrane damage and intracellular Hb variants. Additionally, SS samples show significant change in their distribution upon hypoxia treatment in terms of both magnitude and phase, where a relatively large fraction of cells exhibited a low magnitude and phase values (Figures 6 CD). Interestingly, the phase distribution of SS RBCs formed a weak bimodality upon hypoxia treatment. These observations could be largely attributed to the intracellular HbS polymerization and reduced cell volume along with cell sickling process.

Characterization of Subcellular Changes in Response to Hypoxia

To characterize the contributions from the subcellular components, i.e., membrane and cytoplasm upon hypoxia to the changes in single cell electrical impedance (Z_{cell}), the membrane capacitance and internal resistance were calculated for each cell using the well-established equivalent circuit model of biological cells, where the cell membrane capacitance and the cytoplasmic resistance are electrically parallel. The real part of the impedance is assumed to reflect the internal resistance, R_i, of the cell and the imaginary part is assumed to reflect the membrane capacitance. Value of membrane capacitance is then calculated by

$$C_{mem} = \frac{1}{2\pi f \cdot \Im\left(Z_{cell}\right)}$$

where f is the frequency used for the impedimetric measurement.

Figure 7 shows the distribution of single cell analysis. Similar to the impedance results, cytoplasmic resistance and membrane capacitance of normal RBCs follow normal distribution. Their corresponding variations in cytoplasmic resistance and membrane capacitance transitioning from an Oxy to DeOxy state are less as compared to SS RBCs. Table 3 summarizes the mean and standard deviation results for both normal and SS RBCs under Oxy and DeOxy conditions. In normal RBCs, there was no noticeable change in the mean cytoplasmic resistance while the mean membrane capacitance decreased by 7% upon hypoxia treatment. In SS RBCs, the cytoplasmic resistance decreased by 24%, while the membrane capacitance increased by 3.5 times upon hypoxia treatment. It should be noted that upon hypoxia treatment, more than 20% of SS RBCs showed a membrane capacitance greater than 95 pF while the remaining fraction of cells (24.2 ± 19.8 pF) showed a distribution resembling that under Oxy condition.

The changes recorded from normoxia to hypoxia vary from sample to sample, but the collective trends observed when grouping samples provide a means of understanding the

measured data. We attempt to understand the changes in cellular internal resistance and membrane capacitance by correlating these values to the physical changes occurring during cell sickling. Generally, variations in subcellular electric components may be interpreted using a simple relationship between resistance, electrical resistivity (ρ), length (L) and cross-sectional area (A) of a specimen using the following equation

$$R = \frac{\rho L}{A}$$

The lipid bilayer of a cell membrane is typically viewed as a parallel-plate capacitor. Capacitance of a standard parallel-plate capacitor can be defined as a function of a specimen's dielectric permittivity (ϵ), cross-sectional area (A), and distance between the plates (d),

$$C = \frac{\epsilon A}{d}$$

These relations, when applied to biological cells, describe the cell's cross-sectional area as directly proportional to the membrane capacitance and inversely proportional to the internal resistance.

During cell sickling, the cross-sectional area of a sickle cell subjected to hypoxia can be reduced significantly if the cell takes on its classical sickle shape. Normal RBCs are not expected to exhibit changes to their morphology as much as sickle cells, therefore the cross-sectional area of these cells should remain fairly consistent. This is supported by the results in Table 3 and Figures 6AB that normal RBCs had an equal mean of cytoplasmic resistance while SS RBCs showed a 7% decrease upon hypoxia treatment. In addition, comparing to normal RBCs, the internal resistance of SS RBCs showed a significantly wider distribution regardless of oxygen tension value, indicating the marked heterogeneities in intracellular Hb concentration, the content of polymerized HbS and the consequent effective subcellular resistance to current. On the other hand, the increase in the mean SS RBC membrane capacitance was largely attributed to a fraction of cells of remarkably high capacitance values (Figure 7D). The significant changes in both subcellular electric components for SS RBCs were associated with the polymerization process of intracellular HbS, different from the mechanism for normal RBCs.

Conclusion

Impedance-based flow cytometry is a label-free, non-invasive method for cell analysis. We have utilized this technique in our portable, mobile app-controlled device in tandem with our PDMS based microfluidic chip to perform an impedimetric analysis of cell samples under normoxia and hypoxia conditions. Our microfluidic chip design was optimized to reduce the variability in position as cells traverse the electrodes. Each cell had its cytoplasm resistance and membrane capacitance characterized. The experimental results are positively correlated with mathematical models to provide probable explanations to observed trends. There is potential for utilizing this work for as a foundation for a diagnosis method for SCD. The changes in the single cell electrical impedance can serve as a potential biophysical marker for SCD. The developed single cell analysis system can be extended to study cellular response to hypoxia for other cell types. A notable limitation of the current methodology is the use of lumped parameters to characterize the cell morphological change in addition to influencing factors such as the cells distance from the electrodes. However, future work may lead us to explore the extreme heterogeneities in sickle cells and quantify the effect of influencing factors through a combination of experimental characterization and numerical simulation.

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Table 1. Geometry parameters for impedance computation.

Parameter	Value (μm)	Description
d	3.4	†Channel depth
w	20	†Channel width
b	21	†Electrode band
g	18	†Electrode gap
r	4	‡Semi-axes of ellipsoid in the x-y plan
t	1.25	Semi-axis of ellipsoid in the z axis

† In simulation of a large cell with volume of 100 *fL*, the semi-axis of the ellipsoid in the x-axis increased to 4.76 μm.

‡ The measurements were obtained from ZETA 20 3D optical profiler.

Table 2. Material properties for the computation

Material	Electric conductivity, σ	Relative permittivity, ε_r	Surface thickness, d_s
PBS†	1.6 S/m	78	5 mm
PDMS	0.25×10^{-13} S/m	2.75	3 mm (side), 0.15 mm (top)
Glass	1×10^{-15} S/m	4.2	0.7 mm

†Specifics from manufacturer.

Table 3. Electrical impedance and single cell electrical properties of normal and SS RBCs. AA-Oxy (n = 236), AA-DeOxy (n = 295), SS-Oxy (n = 293), SS-DeOxy (n = 222).

Sample	Δ|Z| (Ω)		ΔΘ (deg)		R_i (MΩ)		C_{mem} (pF)	
	Oxy	DeOxy	Oxy	DeOxy	Oxy	DeOxy	Oxy	DeOxy
AA	1082.3 ± 557.2	1286.5 ± 511.8	0.2 ± 0.1	0.2 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	53.6 ± 36.2	40.8 ± 21.4
SS	2594.5 ± 901.4	1787.0 ± 800.2	0.3 ± 0.1	0.2 ± 0.1	2.8 ± 0.2	2.6 ± 0.2	14.6 ± 9.3	66.1 ± 106.4

Figure 1. Device overview of the portable impedance-based flow cytometer prototype. (A) A double layer PDMS device bonded to a glass substrate patterned with Ti/Au electrodes. The electrodes provide connectivity to the portable device. The top view shows the intersection of the gas channel over the point of measurement between the electrodes. The cross-sectional view depicts the gas exchange between two channels to induce cell sickling. (B) The flow chart of the portable device consisting of all major components used and how information moves between the components. (C) The Android application used to control the portable device operated to continuously scan for a designated length of time and produce a graph of the results.

Figure 2. Computational domain and boundary conditions for finite element modeling of PBS impedance.

Figure 3. (A) Representative microscopic image of RBCs under hypoxia travelling through the microfluidic channel to be measured between two microelectrodes. (B) The impedance magnitude response of a single cell passing through the microelectrode pair using the HF2IS. (C) The impedance magnitude response of a single cell passing through the microelectrode pair using the portable device. The gray signal is the raw response recorded. The blue signal is result of passing the raw response through a level 3 wavelet filter with BlockJS denoising in MATLAB.

Figure 4. (A) The impedance circuit model when a cell is not present. Z_{dlt} refers to the total double layer impedance. Z_{PBS} represents the impedance of the PBS when no cell is being measured. (B) An impedance circuit model of an RBC in the PBS medium between two electrodes. Z_{dl} represents the double layer impedance where the electrodes meet the PBS. $Z_{PBS*¿¿}$ and Z_{cell} are the impedance of the PBS while a cell is present in the channel and the measured RBC, respectfully.

Figure 5. (A) A MATLAB algorithm is used to identify fourteen peaks from the real part of the calculated cell impedance results obtained from the portable device using a minimum threshold.

(B) The corresponding imaginary values were obtained by matching the time coordinate from the real plot.

Figure 6. (A) Histogram depicting the relative magnitude of the impedance for detected normal cells in Oxy and DeOxy conditions. The data includes samples AA1 and AA2. (B) The corresponding relative phase of the impedance for detected normal cells from A. (C) The relative magnitude of the impedance for detected sickle cells in Oxy and DeOxy conditions. The data includes samples SS1, SS2, and SS3. (D) The corresponding relative phase of the impedance for detected sickle cells in C. *** represents $p < 0.001$.

Figure 7. (A) This graph depicts the calculated internal resistance for detected normal cells in Oxy and DeOxy conditions. The data includes samples AA1 and AA2. (B) The calculated membrane capacitance for detected normal cells from A. (C) The calculated internal resistance for detected sickle cells in Oxy and DeOxy conditions. The data includes samples SS1, SS2, and SS3. (D) The calculated membrane capacitance for detected sickle cells from C. *** represents $p < 0.001$.