

TECHNICAL NOTE



Advance of microfluidic constriction channel system of measuring single-cell cortical tension/specific capacitance of membrane and conductivity of cytoplasm

Yan Liu^{1,2} | Ke Wang³ | Xiaohao Sun⁴ | Deyong Chen^{1,2,5} | Junbo Wang^{1,2,5} | Jian Chen^{1,2,5}

¹State Key Laboratory of Transducer Technology (SKLTT), Aerospace Information Research Institute (AIR), Chinese Academy of Sciences (CAS), Beijing, China

²School of Electronic, Electrical and Communication Engineering (EECE), University of Chinese Academy of Sciences (UCAS), Beijing, China

³School of Electronic Engineering, Beijing University of Posts and Telecommunications, Beijing, China

⁴Department of Mechanical Engineering, University of Colorado Boulder, Boulder, Colorado, USA

⁵School of Future Technology, University of Chinese Academy of Sciences (UCAS), Beijing, China

Correspondence

Junbo Wang and Jian Chen, State Key Laboratory of Transducer Technology (SKLTT), Aerospace Information Research Institute (AIR), Chinese Academy of Sciences (CAS), Beijing, China.

Email: jbwang@mail.ie.ac.cn (J. W.) and chenjian@mail.ie.ac.cn (J. C.)

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Abstract

This paper reported a microfluidic platform which realized the characterization of inherent single-cell biomechanical and bioelectrical parameters simultaneously. Individual cells traveled through a constriction channel with deformation images and impedance variations captured and processed into cortical tension T_c , specific membrane capacitance C_{sm} , and cytoplasmic conductivity σ_{cy} based on an equivalent biophysical model. These properties of thousands of individual cells of K562, Jurkat, HL-60, HL-60 treated with paraformaldehyde (PA)/cytochalasin D (CD)/concanavalin A (ConA), granulocytes of Donor 1, Donor 2, and Donor 3 were quantified for the first time. Leveraging T_c , C_{sm} , and σ_{cy} , (1) high accuracies of classifying wild-type and processed HL-60 cells (e.g., 93.5% of PA treated vs. CD treated HL-60 cells) were realized, revealing the effectiveness of using these three biophysical parameters in cell-type classification; (2) low accuracies of classifying normal granulocytes from three donors (e.g., 56.4% of Donor 1 vs. 2), indicating comparable parameters for normal granulocytes. In conclusion, this platform can characterize single-cell T_c , C_{sm} , and σ_{cy} concurrently and quantify multiple parameters in single-cell analysis.

KEYWORDS

biosensor, cortical tension, cytoplasmic conductivity, microfluidic constriction channel, single-cell analysis, specific membrane capacitance

1 | INTRODUCTION

As key components of single-cell biophysical properties, biomechanical parameters mainly include Young's module, cytoplasmic viscosity, and cortical tension while bioelectrical parameters mainly include cytoplasmic conductivity and membrane capacitance [1]. Changes of single-cell biomechanical and/or bioelectrical

properties have been found in a variety of life processes and cell activities, including (1) abnormalities of white and red blood cells [2–9]; (2) tumor cells in evasion [10–12]; (3) differentiation and dedifferentiation of stem cells [13–16]. Thus, single-cell biophysical properties are widely applied to classify cell types and evaluate cell status, including diagnosing multiple diseases, like cancer, malaria, and sickle cell anemia [2, 17].

Though aforementioned biomechanical and bioelectrical parameters play key roles in both physiological and pathological processes, conventional measurement technologies (e.g., atomic force microscopy, optical stretcher, micropipette aspiration, patch clamping, electro-rotation, and dielectrophoresis) demonstrated limited performances in total numbers that can be processed and types of biophysical parameters that can be measured (i.e., ~50 cells from AFM [18], ~50 cells based on optical stretcher [19], ~30 cells based on micropipette aspiration [20], ~10 cells based on patch clamping [21], ~20 cells based on electro-rotation [22], and ~40 cells based on dielectrophoresis [23]).

Since the dimension of the microfabricated structure was comparable to the cell dimension, high-throughput measurements of single-cell inherent biophysical parameters were achieved by several microfluidic systems [24–27]. Recently, microfluidic platforms based on hydrodynamics technology or constriction channel technology were applied to characterize single-cell mechanical properties [28, 29]. For the sake of detection of single-cell mechanical properties, individual cells travel through and deform in the microfluidic channels with transit time or deformations captured due to fluid stresses and/or geometrical constrictions [30–34]. Using corresponding biomechanical models, these preliminary data of cell deformations or transit time were translated into inherent biomechanical properties of single cells including elastic modulus and cortical tension [35–41].

At the same time, microfluidic impedance flow cytometry has been employed to detect electrical properties of single cells by recording impedance signals when tested single cells travel rapidly through the microfluidic channel [42–48]. The intensity of impedance was decided by the electric properties (e.g., membrane capacitance, cytoplasm conductivity) and morphological properties (e.g., size, shape) of single-cell [49]. Using equivalent circuit models, these preliminary impedance amplitude and phase data were transformed into single-cell inherent bioelectrical properties (i.e., conductivity of cytoplasm and specific capacitance of membrane) [50–53].

Although, these approaches processed thousands of single cells, they only characterized a single biomechanical or bioelectrical property, but cannot simultaneously characterize two kinds of parameters, and thus their actual performances in the field of cell-type classification as well as cell-state assessment were restricted. Presently, characterization of single-cell biomechanical and bioelectrical properties with high-throughput was realized with deformability and impedance microfluidic flow cytometry in combination in which single cells deform through the microfluidic channel continuously under the function of pressure, hydrodynamic, and dielectrophoretic forces. In this process, corresponding deformation images and impedance varieties were captured, achieving accurate classification of normal breast epithelial cells (MCF-10A) and breast cancer cells (MDA-MB-231) [54, 55], multiple breast tumor cells (i.e., 95C, 95D, A549, H446, and H460) [56], wild-type, cytochalasin D treated, and concanavalin A treated blood cells (HL-60) [57], wild-type, cytochalasin B, and N-ethylmaleimide treated breast tumor cells (MCF-7) [58], normal, PMA-modified, fixed breast cancer cells (MCF-7), malaria-infected red blood cells [2] and LNCaP, LNCaP-C4-2, LNCaP-C4-2B, and PWR-1E cells [59]. Although characterization of single-cell bioelectrical and biomechanical properties

simultaneously was achieved, most of aforementioned methods could only obtain primitive indicators (i.e., transit time, deformation ratio, electrical amplitude, and phase changes), rather than inherent biophysical properties (e.g., cortical tension, membrane capacitance, and cytoplasmic conductivity) because of the lack of detective schemes and models, leading to poor results in distinguishing various types of cells.

In order to deal with this issue, this paper reports a microfluidic platform capable to measure single-cell inherent biophysical properties (e.g., cortical tension, specific membrane capacitance, and cytoplasmic conductivity) continuously and simultaneously according to a self-established equivalent biophysical model illustrating the relationship between (1) cell deformation and cortical tension, (2) impedance variations and membrane/cytoplasmic electrical components. Individual cell squeezed through the constriction channel (the width and depth of the cross section smaller than single cell diameter) in a quasi-stable state under aspiration where the deformation (front/rear portion curves of cell membrane) and impedance changes of single cells were recorded. According to the developed biophysical model for cell deforming through the microfluidic channel, these original cell deformation images and impedance variety signals were transferred into inherent biophysical properties of T_c , C_{sm} , and σ_{cy} . When the high-throughput characterization of bioelectrical or biomechanical properties of single cells individually is compared, the concurrent measurements of bioelectrical/biomechanical parameters of single cells up to several thousand by this microfluidic platform can lead to high accuracies of classifying cell types.

2 | MATERIALS AND METHODS

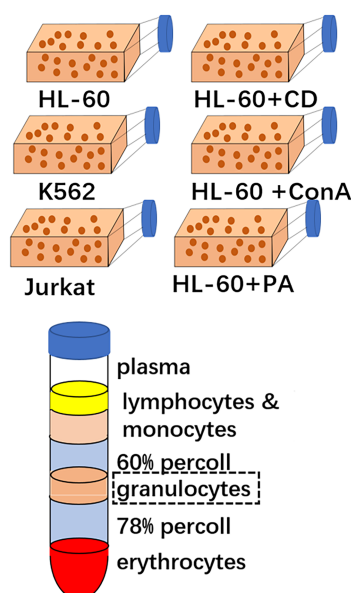
2.1 | Materials

HL-60 (human promyelocytic leukemia cell), K562 (human erythroleukemia lymphoblast), and Jurkat (human peripheral blood leukemic T cell) cell lines were bought from Biology and Medicine of Cell Resource of China. Cytochalasin D (CD) as a potent inhibitor of actin polymerization (Life Technologies, USA) and paraformaldehyde (PA) a common crosslinking fixative (Sigma-Aldrich, USA) were applied to destroy and crosslink cytoskeletons, respectively. Concanavalin A (ConA) as a tetrameric protein that can bind to glycoproteins on cell membrane surfaces (Life Technologies, USA) was applied to modulate membrane portions of HL-60 cells. The isolation of granulocytes from healthy volunteers' blood samples (with the statement of informed consent) was realized using Percoll (GE Healthcare, USA) according to gradient density centrifugation. Materials in manufacturing micro constriction channels involved photoresists of SU-8 obtained from the MicroChem, USA and AZ obtained from the AZ Electronic Materials, USA, as well as polydimethylsiloxane obtained from the Dow Corning, USA.

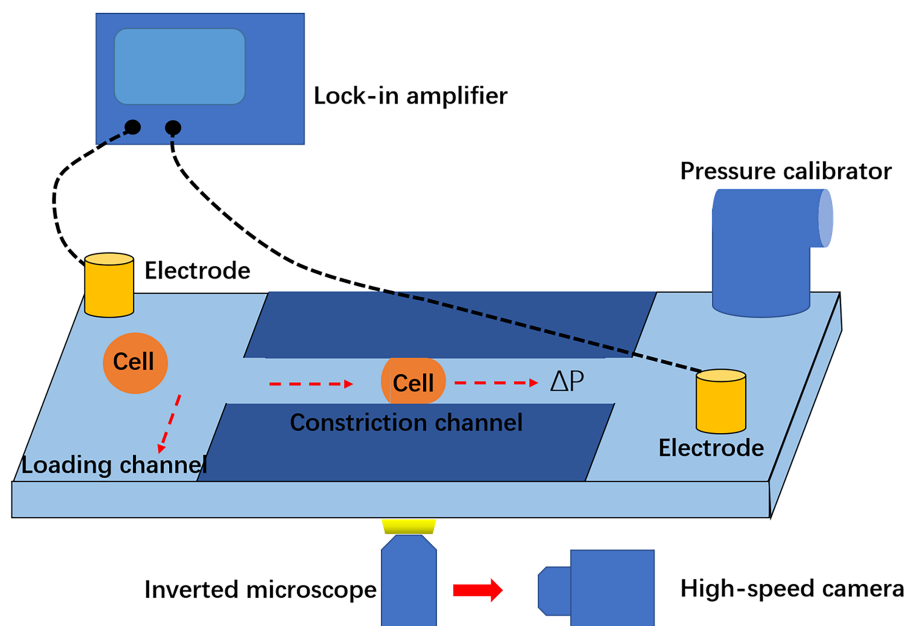
2.2 | Working flow chart

Figure 1 presents the schematics of this microfluidic system enabling the characterization of cortical tension T_c , specific membrane

(A) Cell preparation



(B) Device operation



(C) Data handling

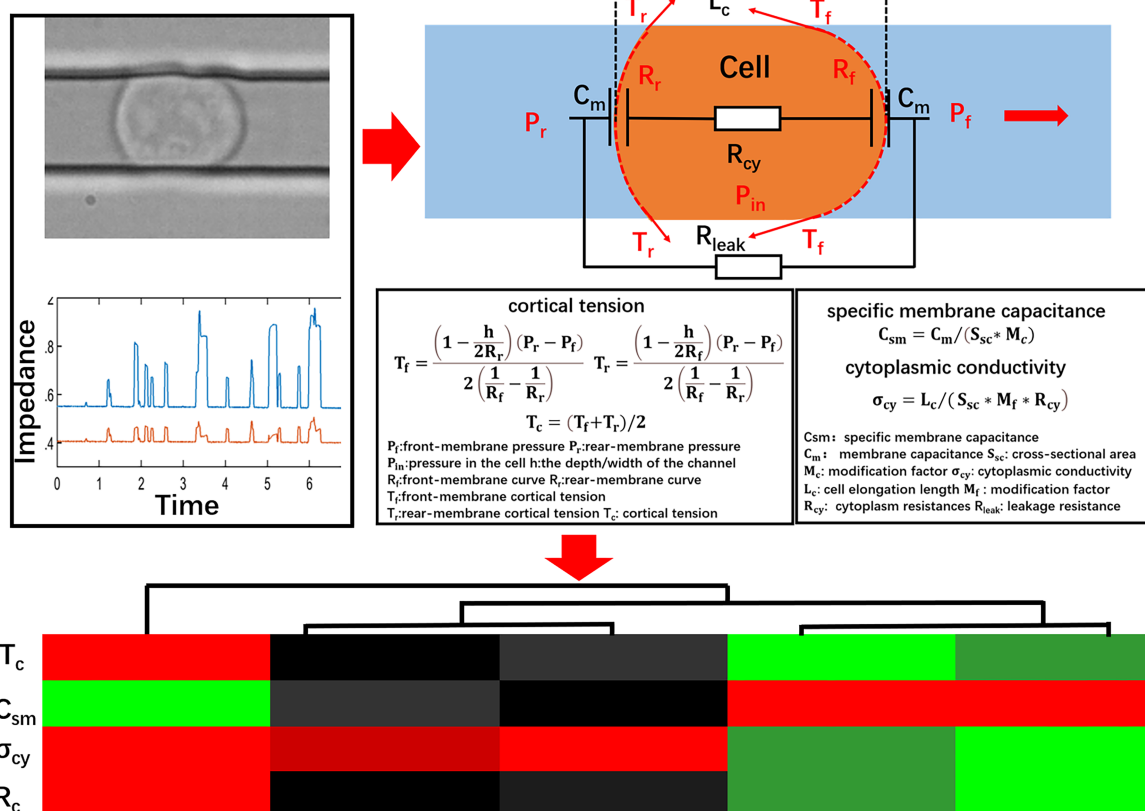


FIGURE 1 Flow chart of the developed micro-platform, measuring cellular T_c , C_{sm} , and σ_{cy} simultaneously based on an equivalent biophysical model of the constriction channel. Key steps include (A) cell treatment, (B) device manipulation, (C) data handling. In the step of device manipulation, individual cell is forced to deform by the constriction channel in a stable state. In this process, images of cell deformation and impedance variations were sampled by high-speed camera and impedance analyzer. Using a biophysical model illustrating the relationship between (1) cell deformation and cortical tension, (2) impedance variations and membrane/cytoplasmic electrical components, these original mechanical and electrical data were transformed into inherent biophysical properties of cortical tension, specific membrane capacitance, and cytoplasmic conductivity [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

capacitance C_{sm} , and cytoplasmic conductivity σ_{cy} of single cells simultaneously using a biophysical model of the constriction channel. This working flowchart is mainly composed of three steps, (a) cell treatment, (2) device manipulation, and (3) data handling. In preparing cells, besides commercially available cell lines of HL-60, K562, and Jurkat, for subtypes of HL-60 cells, wild-type counterparts were incubated with PA/cytochalasin D to change cytoskeletons or concanavalin A to regulate membrane parameters. In addition, the isolation of granulocytes from healthy volunteers' blood samples was realized using Percoll according to gradient density centrifugation (see Figure 1A).

In the second step, a pressure calibrator was connected to the outlet of the device. The prepared cell suspension was added to the inlet of the channel and aspirated into the constriction channel in a quasi-stable state under a predefined aspiration pressure. During the process, a light microscope combined with a high-speed camera was applied to capture cell images and an impedance analyzer was applied to capture electrical data of cells (see Figure 1B).

In the third step, radii of membrane curves of the front/rear portions (i.e., R_f and R_r) were acquired using image processing and curve fitting, enabling the quantification of the inherent biophysical parameter of T_c . Experimentally obtained preliminary impedance amplitudes and phases were translated to inherent bioelectrical parameters of C_{sm} and σ_{cy} . Neural pattern recognition was conducted to classify different cell types according to T_c , C_{sm} , σ_{cy} individually and in combination, followed by correlation coefficient and cluster analysis leveraging hierarchical clustering tools (see Figure 1C).

2.3 | Cell treatment

Culture medium of RPMI-1640 (Biology and Medicine of Cell Resource of China, China) with 20% fetal bovine serum (Thermo Scientific, USA) was applied to suspend HL-60 cells. K562 and Jurkat cells were cultured with RPMI-1640 medium plus 10% fetal bovine serum in suspension. All cells were cultured in an incubator from Thermo Scientific, USA. For the construction of sub-cell types, (1) 0.1% PA was applied to wild-type HL-60 cells for 15 min to crosslink cytoskeletons; (2) 1 $\mu\text{g/mL}$ CD was applied to wild-type HL-60 cells for 30 min to destroy cytoskeletons; (3) 10 $\mu\text{g/mL}$ ConA was applied to wild-type HL-60 cells for 30 min to modify membranes. As to normal granulocytes, solutions of Percoll (78% and 60%), and peripheral blood samples were centrifuged for 30 min at a speed of 400 g and a temperature of 4°C.

2.4 | Design, manufacture, and manipulation

In this work, channels with constrictions were utilized to detect the deformation and impedance of the single cell and thus several key parameters were involved when designing the shape and dimension of the constriction channel: cross-sectional area and length. In the process of cell traveling through the constriction channel, the

membrane should tightly seal with channel walls to avoid leakage of electric field lines and thus the cross-sectional area should be smaller than the dimension of the measured cell to realize complete filling. On the contrary, the cells to be measured will undergo excessive deformation with too small cross-sectional area which could lead to changes in cortical tension. When considering these factors to constriction channel design, the cross-section area should be smaller than cells. Considering geometrical dimensions of HL-60 cells (10–12 μm in diameter), K562 and Jurkat cells (14–16 μm in diameter) as well as healthy granulocytes (8–10 μm in diameter), constriction channels of 10 $\mu\text{m} \times 10 \mu\text{m}$, 12 $\mu\text{m} \times 10 \mu\text{m}$, and 6 $\mu\text{m} \times 10 \mu\text{m}$ were chosen correspondingly.

In addition, for a cell within the channel, the pressure differences between the front/rear portion of membrane and surface tension can reach equilibrium, and thus the cell is in a steady state and travels through the constriction channel at a uniform speed. The decrease of the channel length can lead to unstable state of traveling cells and the increase of the channel length can enhance basal impedance amplitudes. When considering the factors mentioned above, the constriction channel with a length of 200 μm was used in this work.

The fabrication of the microfluidic platform was conducted leveraging well-established micromachining techniques, focusing on the molding of PDMS-based constriction channels from a master with SU-8. Key steps were composed of hard lithography of SU-8, soft lithography of PDMS and PDMS-Glass bonding. Similar fabrication procedures can be found in previous literatures [60].

In the step of device operation, the microfluidics-based channel was first filled of medium and added with cell suspensions. Negative pressures of 250 Pa (for HL-60, K562, and Jurkat cells) or 100 Pa (for granulocytes) provided by a pressure calibrator (PACE 5000 pressure calibrator, Druck, UK) were supplied to the outlet of the device. Images of cell deformation were sampled by the high-speed camera (M320S, Phantom, USA) at a speed of 1000 frames per second which was connected with a light microscope (IX71, Olympus, Japan). The dual-frequency sinusoidal voltage excitation signal (100 and 250 kHz) was supplied by a signal equipment consisting of an impedance analyzer (model 7270, Signal Recovery, USA) and a signal generator (WF1973, NF, Japan) and applied on the constriction channel to record impedance profiles of the single cell in traveling.

2.5 | Data processing

In order to quantify R_f and R_r for individual cells tested, key steps of image processing (e.g., background removal, thresholding as well as edge detection) were used to experimentally acquired images. Then R_f and R_r were translated into inherent biomechanical parameters of cortical tension for the front and rear membrane portions (T_f and T_r) using the home-developed equivalent biomechanical model which was deduced as follows.

More specifically, the relation among cortical tension T_f and T_r and the pressure difference between the front and rear of the cell ($P_r - P_f$), the depth/width of the channel h , the curves of the front/

rear membrane portion R_f and R_r were obtained based on the Young-Laplace equation as $P_{in} - P_f = 2T_f/R_f$ and $P_{in} - P_r = 2T_r/R_r$, respectively, where P_{in} is the pressure inside the cell. Due to the quasi-stable state of the deformed cell, the pressure difference between the front and rear portion of the cell ($P_r - P_f$) is offset by the friction force in a format as $(P_r - P_f)h^2 = 4flh$ where f represents the friction force per unit area, l represents the length of the region where the cell is in contact with the channel wall. In addition, the difference of T_c and T_f between the front and rear portions are balanced by the total friction leading to format as $Tr + fl = T_f$. Since the cells in motion are represented by the droplet model [61], the fluid in the constriction channel is regarded as a uniform pipe flow. The pressure difference between the front and rear portion of the cell is approximately expressed as $\Delta P = P_r - P_f = IP_a/L_c$, where L_c represents the length of the constriction channel and P_a represents the total pressure supplied to the outlet of the device. Finally, the averaged cortical tension T_c is obtained which is expressed as $T_c = (T_f + T_r)/2 = (((1 - h/2R_r)(P_r - P_f))/(2(1/R_f - 1/R_r))) + ((1 - h/2R_f)(P_r - P_f))/(2(1/R_f - 1/R_r)))/2 = (((1 - h/2R_r)\Delta P)/(2(1/R_f - 1/R_r))) + ((1 - h/2R_f)\Delta P)/(2(1/R_f - 1/R_r)))/2$.

The obtained raw impedance amplitudes and phases at 100 and 250 kHz were transformed into inherent bioelectrical properties of C_{sm} and σ_{cy} using the home-developed equivalent biophysical model as follows. Impedance data for single cells at 100 and 250 kHz were transferred to R_{leak} (a parameter for evaluating the sealing performance of cell and constriction channel inner wall), cytoplasmic resistance R_{cy} , and membrane capacitance C_m by curve fitting the dual frequency impedance amplitudes and phases with the equivalent circuit model shown in Figure 1C. Then, R_{cy} was processed into σ_{cy} using the equation: $\sigma_{cy} = L_{cell}/(S_c * M_f * R_{cy})$ where L_{cell} represents the length of deformed cell, S_c stands for the channel's cross-section area, and M_f is the correction coefficient obtained by simulation because of inhomogeneous distribution of electric field lines. Furthermore, C_m was processed into C_{sm} using the equation: $C_{sm} = C_m/S_c$.

In this work, the characterizations of various types of cells were realized with results expressed in scatter plots (data distribution and aggregation) and histograms (probability distribution within certain value intervals). Additionally, neural pattern recognition was used to distinguish cells including a two-layer (hidden and output layers) feed forward neural network with sigmoid hidden and softmax output neurons as well as a backpropagation algorithm (Neural Net Pattern Recognition App [62], MATLAB 2018b, MathWorks Corporation, USA). For the classifications of three individual cell groups (e.g., HL-60, K562, Jurkat; wild-type, PA treated, CD treated, ConA treated HL-60; granulocytes of Donor 1, Donor 2, Donor 3), the values of cortical tension, specific membrane capacitance, cytoplasmic conductivity were used as single or combined inputting datasets. For the classifications of nine cell groups together, the values of cortical tension, specific membrane capacitance, cytoplasmic conductivity, and radius were used as combined inputting datasets. The complete dataset was divided into 70% for training based on generated errors, 15% for validation to evaluate the generalization of the network, and 15% for testing to quantify the performance of the network after training and validation. The classification result was represented by a confusion

matrix, in which the value at the lower right corner represented the proportion of correctly classified samples to the total samples, which was termed as "accuracy." More specifically, when two samples were classified, an accuracy of 50% indicated that two samples cannot be differentiated at all. As the accuracy increased from 50% to 100%, two samples can be more and more differentiated. The accuracy of 100% suggested that two samples can be differentiated with 100% confidence. For the classifications of three individual cell groups (HL-60, K562, Jurkat; wild-type, PA treated, CD treated, ConA treated HL-60; granulocytes of Donor 1, Donor 2, Donor 3), the values of T_c , C_{sm} , and σ_{cy} were used as single or combined inputting parameters. For a simultaneous classification of all nine cell groups, the values of T_c , C_{sm} , σ_{cy} , and R_c were used as combined inputting parameters. As for the results, the neural pattern recognition produced confusion matrixes in which orange, green, and gray squares stand for false responses, true responses, and accuracies in total. To be more specific, the accuracies to classify two types and nine types of cells appeared in the squares in the lower right corner with black and green characters, respectively.

The Pearson coefficient of correlation is a statistic which is applied to study the degree of linear relationship between samples. In this study, the Pearson coefficients of correlation among nine samples of cells (e.g., K562, Jurkat, HL-60, HL-60 treated with PA/CD/ConA, granulocytes of Donor 1, Donor 2, and Donor 3) were calculated using the Function of CORRCOEFF where values of single-cell parameters of T_c , C_{sm} , σ_{cy} , and R_c (radius of the cell, computed based on the calculated volume of the deformed cell within the constriction channel) in combination individual samples were used as inputting data in a matrix format (MATLAB 2018b, MathWorks Corporation, USA). The absolute value of the correlation coefficient ranges from 0 to 1 where an increase of the absolute value indicates an increase in correlation. Meanwhile, cluster analysis is a statistical analysis technology which groups a collection of data objects into relatively homogeneous groups. In this study, the hierarchical clustering method (Euclidean distance, complete linkage clustering) was used to classify aforementioned nine types of cells where values of single-cell parameters of T_c , C_{sm} , σ_{cy} , and R_c in combination were used as inputting data (MultiExperiment Viewer 4.9.0, J. Craig Venter Institute, USA). The results were represented as a cluster heat map where nearby cell types represented a close kinship while cell types far away from each other in the heat map represent a distant kinship. Note that red, black, and green represent a decreasing trend of the values of single-cell parameters of T_c , C_{sm} , σ_{cy} , and R_c without units.

3 | RESULTS AND DISCUSSION

Microfluidic constriction channels have been functioning as an enabling tool for the simultaneous characterization of inherent single-cell biomechanical and bioelectrical properties [56, 57, 63]. When single cells were deformed into the constriction channels, the degrees of cell deformation as a function of time were captured as preliminary biomechanical properties while when deformed single cells sealed the

walls of constriction channels to effectively block electrical lines, the varied impedances were measured as preliminary bioelectrical properties. Based on corresponding equivalent biophysical models, these raw biomechanical and bioelectrical properties can be translated into intrinsic biophysical properties (e.g., Young's modulus and specific membrane capacitance). However, due to limitations in detection structures and equivalent models, simultaneous measurements of three key biophysical parameters of T_c , C_{sm} , and σ_{cy} have not been reported. This study presented a "1"-shape constriction microchannel

with a corresponding biophysical model to illustrate the relationship between (1) cell deformation and cortical tension, (2) impedance variations and membrane/cytoplasmic electrical components. Based on this microfluidic platform, thousands of individual cells of K562, Jurkat, HL-60, HL-60 treated with PA/CD/ConA, granulocytes of Donor 1, Donor 2, and Donor 3 were processed with key results listed as follows (see supporting information Appendix S1 and S2).

Figure 2 shows time-lapsed microscopic images and two-frequency impedance profiles of individual cells of wild-type HL-60

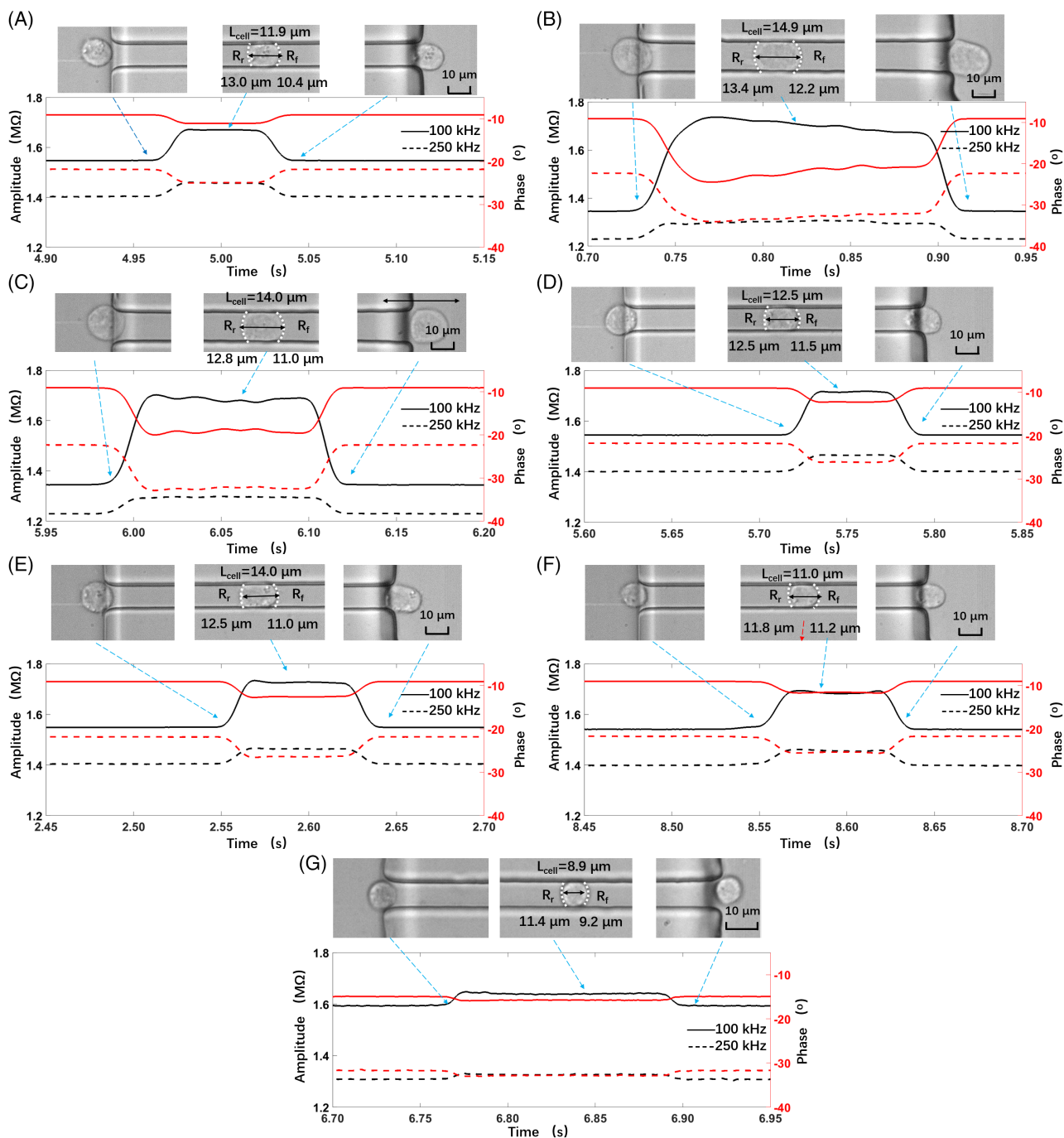


FIGURE 2 Time-lapsed microscopic images and impedance profiles of individual cells of HL-60 (A), K562 (B), Jurkat (C), HL-60 treated with PA (D)/CD (E)/ConA (F), and granulocyte (G) traveling through the constriction channel. Experimentally measured front/rear membrane curves (white dashed lines), two-frequency amplitude and phase profiles for these seven representative cells were included [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

(A), K562 (B), Jurkat (C), HL-60 treated with PA (D)/CD (E)/ConA (F), and normal granulocyte (G) traveling through the constriction channel. The white-dotted lines on the image represent curve-fitting results of front and rear portions of cell membrane within the constriction channel which were used to characterize single-cell mechanical properties. The amplitude and phase differences of the dual-frequency impedance when there is a cell filled in the constriction channel or not were obtained to characterize single-cell electrical properties. Based on original deformation and impedance results (see Figure 2), the passage time of a single cell in the constriction channel was estimated on the order of 0.1 s, so that the processing speed of the microfluidic system was ~ 1 cell per second. In order to increase the detection throughput, the performances of the high-speed camera should be improved. For example, EMCCD can be further used to reduce the exposure time while sCMOS can be integrated to increase the readout speed. With these improvements, the detection throughput of the developed microfluidic platform can be increased by one to two orders.

Figure 3A presents scatter plots of T_c versus R_c , C_{sm} versus R_c , σ_{cy} versus R_c and distribution diagrams of T_c , C_{sm} , σ_{cy} for HL-60, K562, and Jurkat cells. T_c , C_{sm} , and σ_{cy} were quantified as 1.25 ± 2.47 mN/m versus 2.18 ± 3.75 mN/m versus 1.67 ± 3.87 mN/m, 2.06 ± 0.50 $\mu\text{F}/\text{cm}^2$ versus 1.73 ± 0.41 $\mu\text{F}/\text{cm}^2$ versus 1.81 ± 0.49 $\mu\text{F}/\text{cm}^2$, 0.58 ± 0.13 S/m versus 0.62 ± 0.29 S/m versus 0.69 ± 0.14 S/m for HL-60 ($n_{\text{cell}} = 436$) versus K562 ($n_{\text{cell}} = 433$) versus Jurkat ($n_{\text{cell}} = 1045$) cells (see Table 1). The measured range of T_c for HL-60 cells was consistent with a previous report (1–10 mN/m [60]), suggesting that the inclusion of single-cell electrical phenotyping has no evident impact on single-cell mechanical performance. Meanwhile the measured ranges of C_{sm} and σ_{cy} for HL-60 cells was consistent with a previous report (~ 1 $\mu\text{F}/\text{cm}^2$ and ~ 1 S/m [56]), suggesting that the inclusion of single-cell mechanical phenotyping has no evident impact on electrical performance of cell membranes and cytoplasmic portions.

For T_c , values of K562 cells with two peaking ranges of 0.5–2.0 mN/m and 4.0–16.0 mN/m were higher than Jurkat cells with a peaking range of 0.5–2.0 mN/m and HL-60 cells with a peaking range of 0.25–1.0 mN/m. For C_{sm} , values of HL-60 cells with a peaking range of 2.00–2.50 $\mu\text{F}/\text{cm}^2$ were higher than K562 and Jurkat cells with a peaking range of 1.75–2.25 $\mu\text{F}/\text{cm}^2$. For σ_{cy} , values of K562 cells with a peaking range of 0.65–75 S/m were higher than Jurkat cells with a peaking range of 0.60–0.70 S/m and HL-60 cells with a peaking range of 0.55–0.65 S/m.

Figure 3B shows the results of the classification accuracies of HL-60 versus K562 versus Jurkat cells using single-cell inherent biophysical properties of T_c , C_{sm} , σ_{cy} only and in combination (see Table 2). More specifically, since T_c of K562 cells was higher than Jurkat and HL-60 cells, neural pattern recognition produced higher accuracies to classify K562 versus Jurkat (56.6%) and K562 versus HL-60 (58.5%) than Jurkat versus HL-60 (53.2%). Meanwhile, since C_{sm} of HL-60 cells was higher than K562 and Jurkat cells, neural pattern recognition produced higher accuracies to classify HL-60 versus K562 (68.8%) and HL-60 versus Jurkat (65.3%) than K562 versus Jurkat (56.0%). In addition, since σ_{cy} of K562 cells was higher than Jurkat and HL-60

cells, neural pattern recognition produced higher accuracies to classify K562 versus Jurkat (67.3%) and K562 versus HL-60 (71.1%) than HL-60 versus Jurkat (52.8%). Compared with the classification of cell types using single parameters of T_c , C_{sm} , or σ_{cy} , when multiple inherent properties were applied in combination, the accuracies were significantly improved to 74.5% (HL-60 vs. K562), 69.3% (K562 vs. Jurkat), and 66.5% (HL-60 vs. Jurkat). These results showed the fact that characterizing multiple biophysical properties plays an important role to classify cell types and evaluate cell status.

Figure 4A presents scatter plots of T_c versus R_c , C_{sm} versus R_c , σ_{cy} versus R_c and distribution diagrams of T_c , C_{sm} , σ_{cy} for HL-60 cells processed with PA/CD/ConA. T_c , C_{sm} , and σ_{cy} of HL-60 cells processed with PA ($n_{\text{cell}} = 360$) versus HL-60 cells processed with CD ($n_{\text{cell}} = 571$) versus HL-60 cells processed with ConA ($n_{\text{cell}} = 395$) were quantified as 1.65 ± 4.15 mN/m versus 0.67 ± 0.75 mN/m versus 1.42 ± 3.72 mN/m, 1.90 ± 0.61 $\mu\text{F}/\text{cm}^2$ versus 2.49 ± 1.30 $\mu\text{F}/\text{cm}^2$ versus 2.34 ± 0.49 $\mu\text{F}/\text{cm}^2$, 0.37 ± 0.10 S/m versus 0.70 ± 0.30 S/m versus 0.58 ± 0.10 S/m (see Table 1).

Compared with wild-type counterparts, HL-60 cells treated with PA demonstrated higher T_c (1.65 ± 4.15 vs. 1.25 ± 2.47 mN/m; 0.19 vs. 0.17 mN/m of first quartile; 0.45 vs. 0.37 mN/m of median; 1.43 vs. 1.12 mN/m of third quartile) with a peaking range of 0.4–1.6 mN/m, lower specific membrane capacitance with a peaking range of 1.5–2.0 $\mu\text{F}/\text{cm}^2$ and lower cytoplasmic conductivity with a peaking range of 0.3–0.4 S/m. On the contrary, HL-60 cells treated with CD demonstrated lower T_c (0.67 ± 0.75 vs. 1.25 ± 2.47 mN/m; 0.16 vs. 0.17 mN/m of first quartile; 0.36 vs. 0.37 mN/m of median; 0.96 vs. 1.12 mN/m of third quartile) with a peaking range of 0.25–1.0 mN/m, higher specific membrane capacitance with a peaking range of 2.5–3.0 $\mu\text{F}/\text{cm}^2$ and higher cytoplasmic conductivity with a peaking range of 0.6–0.7 S/m. These results suggested that PA treatments crosslinked both membrane and cytoplasmic proteins while CD treatments destroyed cellular cytoskeletons. Meanwhile, HL-60 cells treated with ConA demonstrated higher T_c with a peaking range of 0.25–1.0 mN/m and higher specific membrane capacitance with a peaking range of 2.5–3.0 $\mu\text{F}/\text{cm}^2$ but basically the same cytoplasmic conductivity with a peaking range of 0.55–0.65 S/m comparing with HL-60 cells. These results suggested that ConA modified the cell membrane portion only but had no effect on cytoskeletons.

Figure 4B shows the results of the classification accuracies of HL-60 cells treated with PA versus CD versus ConA using inherent biophysical indicators of T_c , C_{sm} , σ_{cy} only and in combination (see Table 2). More specifically, since T_c of HL-60 cells treated with CD was lower than the cells treated with ConA and PA, neural pattern recognition produced higher accuracies to classify CD treated versus ConA treated (54.4%) and CD treated versus PA treated (56.9%) than CD treated versus PA treated HL-60 (52.9%). Meanwhile, since C_{sm} of PA treated HL-60 cells was lower than ConA treated and CD treated HL-60 cells, neural pattern recognition produced higher accuracies to classify PA treated versus ConA treated (71.8%) and PA treated versus CD treated (64.4%) than ConA treated versus CD treated HL-60 (58.2%). In addition, since σ_{cy} of PA treated HL-60 cells was lower than ConA treated and CD treated HL-60 cells, neural pattern

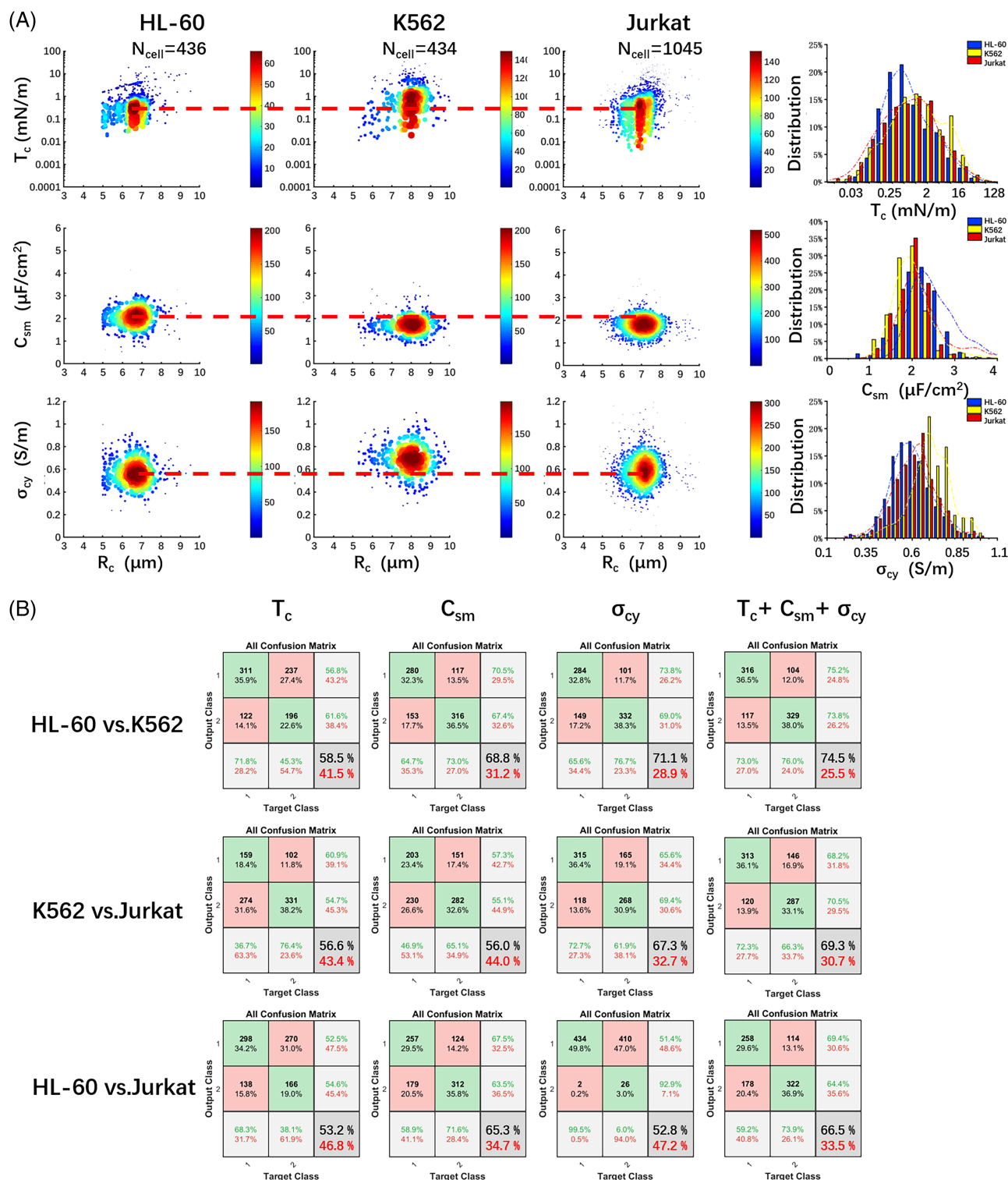


FIGURE 3 Scatter plots and histograms of T_c versus R_c , C_{sm} versus R_c , and σ_{cy} versus R_c for HL-60, K562, and Jurkat cells (A); Confusion matrices of classifying cell types of HL-60 versus K562, K562 versus Jurkat, and HL-60 versus Jurkat using single-cell parameters of T_c , C_{sm} , σ_{cy} only and in combination (B) [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

recognition produced higher accuracies to classify PA treated versus ConA treated (84.0%) and PA treated versus CD treated (93.1%) than ConA treated versus CD treated HL-60 (64.1%). Comparing with cell-type classification using single parameters of T_c , C_{sm} , or σ_{cy} , when

multiple inherent properties were applied in combination, the accuracies were significantly improved to 93.5% (CD treated vs. PA treated HL-60), 76.7% (CD treated vs. ConA treated HL-60), and 90.3% (ConA treated vs. PA treated HL-60). These results demonstrated that

TABLE 1 A summary of single-cell cortical tension, specific membrane capacitance, and cytoplasmic conductivity of K562, Jurkat, HL-60, HL-60 treated with PA/CD/ConA, granulocytes of Donor 1, Donor 2, and Donor 3

	Cortical tension (mN/m)	Specific membrane capacitance ($\mu\text{F}/\text{cm}^2$)	Cytoplasmic conductivity (S/m)	Cell radius (μm)	Cell number
HL-60	1.25 ± 2.47	2.06 ± 0.50	0.58 ± 0.13	6.61 ± 0.82	436
K562	2.18 ± 3.75	1.73 ± 0.41	0.69 ± 0.14	7.88 ± 0.95	433
Jurkat	1.67 ± 3.87	1.81 ± 0.49	0.62 ± 0.29	7.07 ± 0.82	1045
HL-60 treated with PA	1.65 ± 4.15	1.90 ± 0.61	0.37 ± 0.10	6.50 ± 0.76	360
HL-60 treated with CD	0.67 ± 0.75	2.49 ± 1.30	0.70 ± 0.30	6.71 ± 0.83	571
HL-60 treated with ConA	1.42 ± 3.72	2.34 ± 0.49	0.58 ± 0.10	6.25 ± 0.95	395
granulocytes of Donor 1	0.13 ± 0.23	3.30 ± 0.63	0.44 ± 0.06	4.82 ± 0.40	481
granulocytes of Donor 2	0.14 ± 0.23	3.14 ± 0.13	0.46 ± 0.08	4.86 ± 0.43	748
granulocytes of Donor 3	0.13 ± 0.14	3.15 ± 1.04	0.44 ± 0.09	4.75 ± 0.76	644

TABLE 2 Neural network-based accuracies of classifying K562, Jurkat, wild-type HL-60, HL-60 treated with PA/CD/ConA, granulocytes of Donor 1, Donor 2, and Donor 3 leveraging inherent biophysical properties of single cells: Cortical tension, specific membrane capacitance, and cytoplasmic conductivity only and in combination

Accuracies based on different parameters	Cortical tension	Specific membrane capacitance	Cytoplasmic conductivity	Cortical tension-specific membrane capacitance cytoplasmic conductivity in combination
HL-60 versus K562	58.5%	68.8%	71.1%	74.5%
K562 versus Jurkat	56.6%	56.0%	67.3%	69.3%
HL-60 versus Jurkat	53.2%	65.3%	52.8%	66.5%
HL-60 treated with PA versus CD	56.9%	64.4%	93.1%	93.5%
HL-60 treated with CD versus ConA	54.4%	58.2%	64.1%	76.7%
HL-60 treated with PA versus ConA	52.9%	71.8%	84.0%	90.3%
Granulocytes of Donor 1 versus Donor 2	50.0%	50.8%	54.6%	56.4%
Granulocytes of Donor 2 versus Donor 3	50.5%	50.1%	50.2%	51.4%
Granulocytes of Donor 1 versus Donor 3	51.0%	50.9%	50.0%	51.2%

charactering multiple biophysical properties can improve the performance to classify cell types and evaluate cell status.

Figure 5A presents scatter plots of T_c versus R_c , C_{sm} versus R_c , σ_{cy} versus R_c and distribution plots of T_c , C_{sm} , σ_{cy} for Donor 1, 2, and 3. Granulocytes from Donor 1 ($n_{\text{cell}} = 481$) versus Donor 2 ($n_{\text{cell}} = 748$) versus Donor 3 ($n_{\text{cell}} = 644$) were measured and the corresponding values were 0.13 ± 0.23 mN/m versus 0.14 ± 0.23 mN/m versus 0.13 ± 0.14 mN/m of T_c , 3.30 ± 0.63 $\mu\text{F}/\text{cm}^2$ versus 3.14 ± 0.13 $\mu\text{F}/\text{cm}^2$ versus 3.15 ± 1.04 $\mu\text{F}/\text{cm}^2$ of C_{sm} , 0.44 ± 0.06 S/m versus 0.46 ± 0.08 S/m versus 0.44 ± 0.09 S/m of σ_{cy} . Similar numbers of T_c , C_{sm} , and σ_{cy} derived from three donors were found, which suggested that there may exist a range of T_c (e.g., 0.1–0.2 mN/m), C_{sm} (e.g., 3.0–3.5 $\mu\text{F}/\text{cm}^2$), σ_{cy} (e.g., 0.4–0.5 S/m) for normal granulocytes (see Table 1).

Figure 5B shows confusion matrix for the classification accuracies of granulocytes from three healthy donors based on single-cell inherent biophysical parameters of T_c , C_{sm} , σ_{cy} only and in combination (see

Table 2). Although all three inherent biophysical parameters were used, low accuracies of 56.4% (Donor 1 vs. Donor 2), 51.4% (Donor 2 vs. Donor 3), and 51.2% (Donor 1 vs. Donor 3) were obtained to classify granulocytes from three healthy donors. These results further indicated comparable values of T_c , C_{sm} , and σ_{cy} for normal granulocytes.

Finally, a comprehensive analysis of multiple types of cells including K562, Jurkat, HL-60, HL-60 treated with PA/CD/ConA, granulocytes from healthy donors was carried out using neural pattern recognition, correlation, and cluster analysis. Figure 6A shows confusion matrix for the classification accuracies of aforementioned nine groups of cells based on inherent biophysical parameters of T_c , C_{sm} , σ_{cy} , and R_c of single cells. For the successful classification of K562 cells, the biggest error came from Jurkat cells (1.7%) and vice versa, indicating that these two types of cells can be clustered into one group, which was consistent with the fact that both K562 and Jurkat cells belong to cell lines of lymphoma.

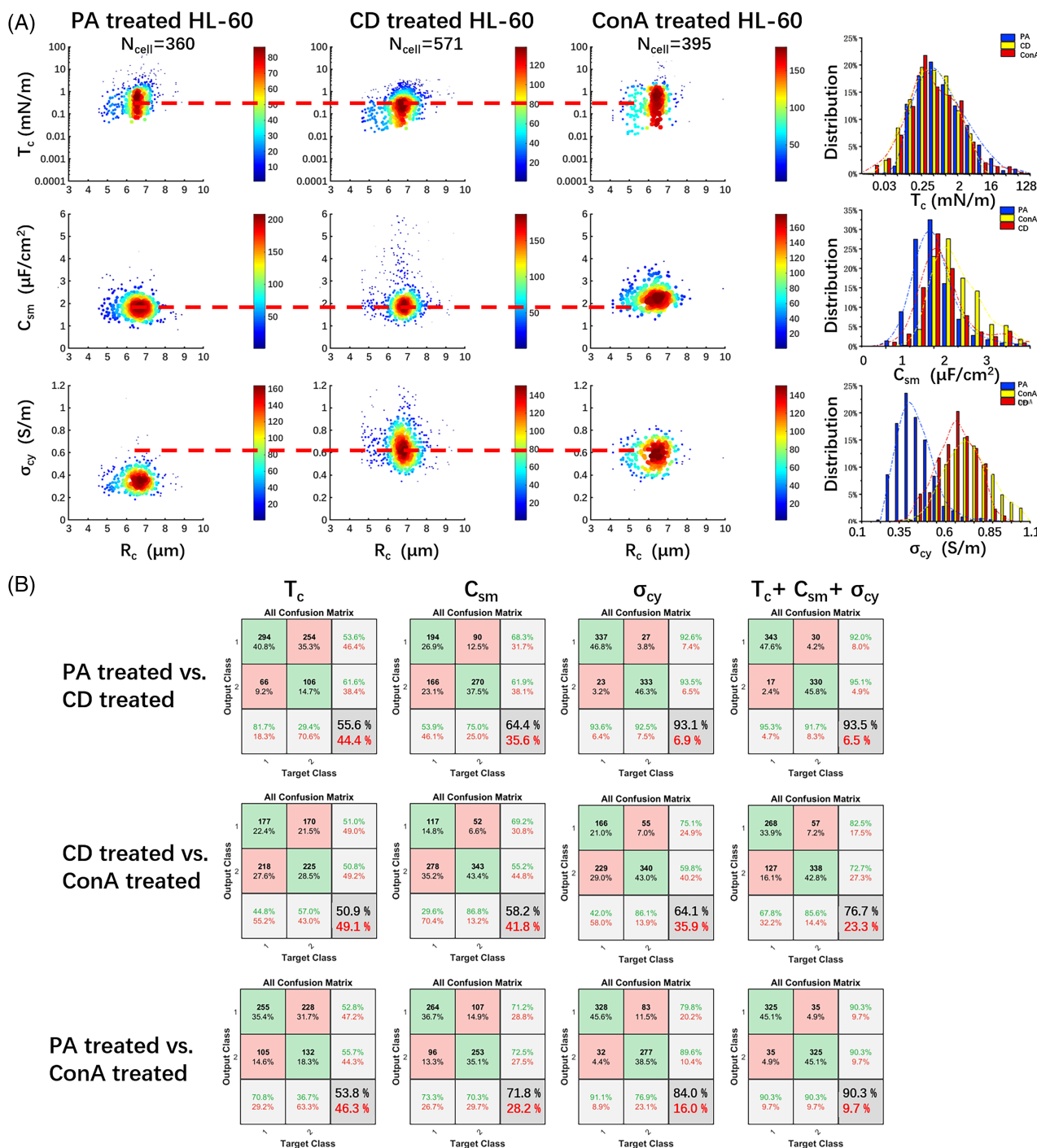


FIGURE 4 Scatter plots and histograms of T_c versus R_c , C_{sm} versus R_c , and σ_{cy} versus R_c for subtype cells of HL-60 processed with PA/CD/ConA (A); Confusion matrices of classifying cell types of HL-60 treated with PA versus CD, CD versus ConA, and PA versus ConA using T_c , C_{sm} , σ_{cy} only and in combination (B) [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

As to wild-type, PA treated, CD treated, and ConA treated HL-60 cells, they can be clustered into one group because the biggest errors of classifying a sub-cell type of HL-60 cells came from the accumulated effects of the other three sub-cell types (i.e., 1.1% for wild-type HL-60, 1.4% for HL-60 treated with PA, 1.0% for HL-60 treated with CD, and 4.3% for HL-60 treated with ConA). As to granulocytes from three healthy donors, they can be clustered into one group because the

biggest errors of classifying granulocytes from one donor came from the accumulated effects of granulocytes from the other two donors (i.e., 4.0% for granulocytes from Donor 1, 3.9% for granulocytes from Donor 2, and 2.9% for granulocytes from Donor 3).

Figure 6B shows the absolute value of Pearson coefficients of correlation of aforementioned nine groups of cells based on inherent biophysical parameters of T_c , C_{sm} , σ_{cy} , and R_c of single cells. From the

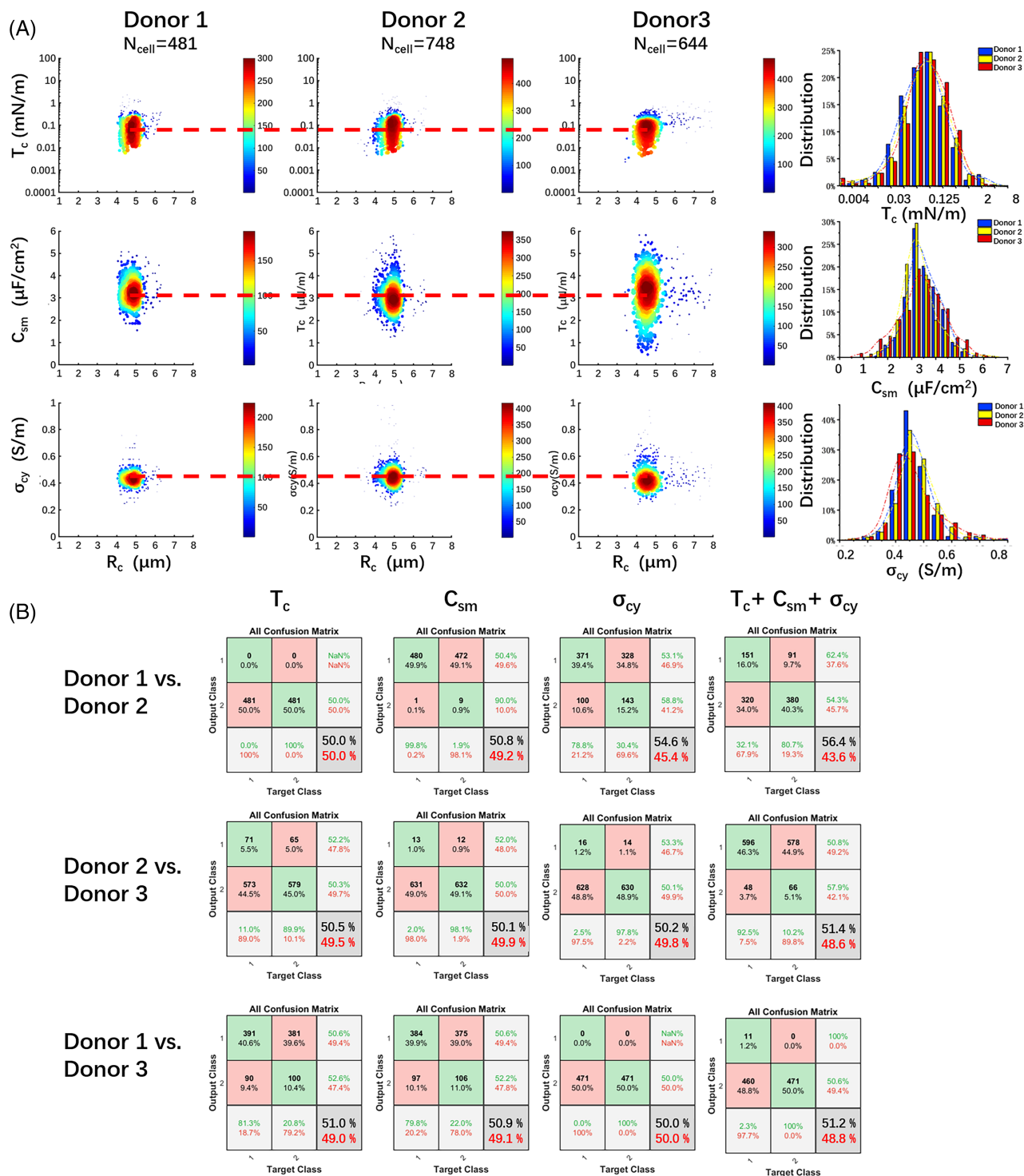


FIGURE 5 Scatter plots and histograms of T_c versus R_c , C_{sm} versus R_c , and σ_{cy} versus R_c for granulocytes of Donor 1, Donor 2, and Donor 3 (A); Confusion matrices of classifying cell types of granulocytes from Donor 1 versus Donor 2, Donor 2 versus Donor 3, and Donor 1 versus Donor 3 based on single-cell parameters of T_c , C_{sm} , σ_{cy} only and in combination (B) [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/terms-and-conditions)]

results, high correlation coefficients among granulocytes from three healthy donors were located as 0.96 for Donor 1 versus Donor 2, 0.93 for Donor 2 versus Donor 3, and 0.93 for Donor 1 versus Donor 3. These results indicated that the inherent biophysical properties of granulocytes in healthy humans were comparable.

At the same time, relatively low correlation coefficients between wild-type and processed HL-60 cells were calculated as 0.56 for HL-60 with/without CD processing, 0.29 for HL-60 with/without PA processing, and 0.33 for HL-60 with/without ConA processing. These results indicated that the treatments of

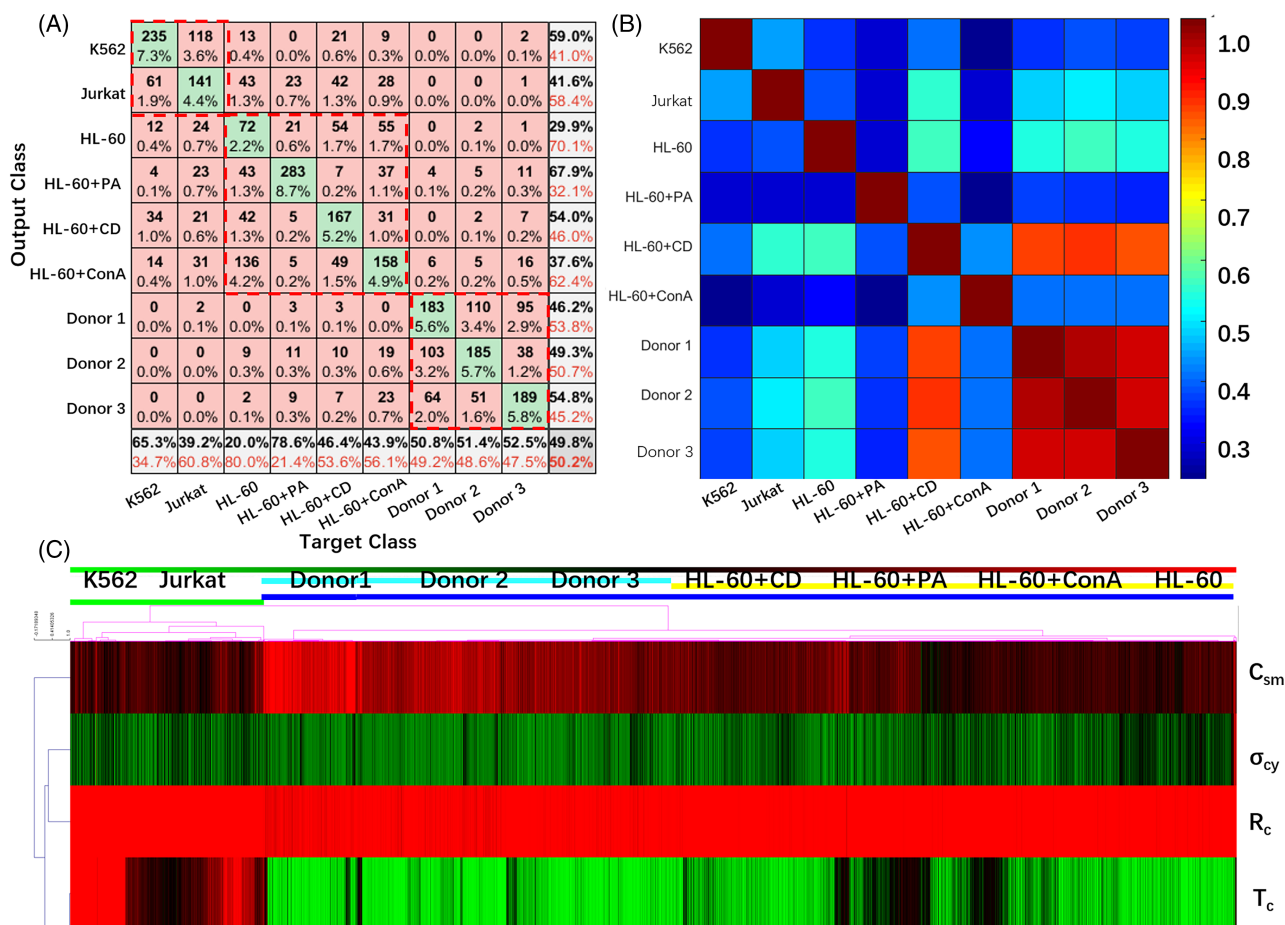


FIGURE 6 Confusion matrix (A), the absolute values of Pearson coefficient of correlation (B), and cluster analysis (C) of nine groups of white blood cells (e.g., K562, Jurkat, HL-60, HL-60 treated with PA/CD/ConA, granulocytes of Donor 1, Donor 2, and Donor 3) using single-cell properties of T_c , C_{sm} , σ_{cy} , and R_c in combination [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com/doi/10.1002/cyto.a.24517)]

HL-60 cells can significantly modulate inherent biophysical properties.

Figure 6C shows cluster analysis of nine groups of white blood cells (e.g., K562, Jurkat, HL-60, HL-60 treated with PA/CD/ConA, granulocytes of Donor 1, Donor 2, and Donor 3) using single-cell properties of T_c , C_{sm} , σ_{cy} , and R_c in combination. These results indicated that these nine groups of white blood cells can be first clustered into two groups, which were K562 and Jurkat cells as group 1, wild-type and treated HL-60 cells as well as granulocytes from three donors as group 2. These results of cell-type clustering were consistent with the fact that HL60 and granulocytes were similar while Jurkat and K562 were far away from neutrophils.

Furthermore, wild-type and treated HL-60 cells as well as granulocytes from three donors were clustered into two groups, which were wild-type and treated HL-60 cells as group 1, normal granulocytes from three healthy donors as group 2. These results of cell-type clustering were identical to the truth that HL-60 cells belong to a cell line of derived from granulocytes and further indicated that these inherent biophysical parameters of T_c , C_{sm} , σ_{cy} , and R_c can efficiently classify different cell types.

To sum up, in this study, the developed microfluidic system was proved to quantify single-cell cortical tension, specific membrane capacitance, and cytoplasmic conductivity from thousands of cells. According to these inherent biophysical properties, (1) ~70% classifications of HL-60 cells versus K562 cells versus Jurkat cells were realized; (2) ~90% classifications of wild-type versus treated HL-60 cells were realized; (3) estimated ranges including 0.1–0.2 mN/m for T_c , 3.0–3.5 $\mu\text{F}/\text{cm}^2$ for C_{sm} , and 0.4–0.5 S/m for σ_{cy} of healthy granulocytes were found.

Technically, further studies may pay attention to the measurements of more types of biophysical parameters of single cells with the purpose to obtain higher accuracies in the field of cell-type classification. From the perspective of applications, the microfluidic system can be further applied in characterization of leucocytes from different patients for disease diagnosis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Yan Liu, Deyong Chen, Junbo Wang, and Jian Chen. **Data curation:** Yan Liu, Ke Wang, and Xiaohao Sun. **Formal analysis:** Yan Liu, Ke Wang, Xiaohao Sun, Junbo Wang, and Jian Chen. **Funding acquisition:** Ke Wang, Deyong Chen, Junbo Wang, and Jian Chen. **Investigation:** Yan Liu, Ke Wang, Xiaohao Sun, and Jian Chen. **Methodology:** Yan Liu. **Project administration:** Deyong Chen, Junbo Wang, and Jian Chen. **Resources:** Deyong Chen, Junbo Wang, and Jian Chen. **Software:** Yan Liu, Ke Wang, and Xiaohao Sun. **Validation:** Yan Liu. **Visualization:** Yan Liu. **Writing—original draft:** Yan Liu, Ke Wang, Xiaohao Sun, Junbo Wang, and Jian Chen. **Writing—review and editing:** Yan Liu and Jian Chen. All authors have read and agreed to the published version of the manuscript.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/cyto.a.24517>.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Xiaohao Sun  <https://orcid.org/0000-0002-5888-1600>

Jian Chen  <https://orcid.org/0000-0003-4612-3279>

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SUPPORTING INFORMATION

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