Development of microfluidic flow cytometry capable of characterization of single-cell intrinsic structural and electrical parameters

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Abstract
Although single-cell intrinsic structural and electrical parameters (e.g. $D_c$ of cell diameter, $D_n$ of nuclear diameter, $\sigma_{cy}$ of cytoplasmic conductivity and $C_{sm}$ of specific membrane capacitance) are promising for cell-type classification, they cannot be obtained simultaneously due to structural limitations of previously reported flow cytometry. This paper presented a microfluidic flow cytometry made of a double T-type constriction channel plus a predefined fluorescence detection domain, capable of high-throughput characterizing single-cell $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ leveraging a home-developed impedance-fluorescence model. As a demonstration, the microfluidic platform quantified $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ from $\sim$10 000 individual cells of three well-established tumor cell lines of A549, SW620 and HeLa where successful rates of cell-type classification were estimated as 54.5 $\pm$ 1.3\% ($D_c$), 68.9 $\pm$ 6.8\% ($D_c + D_n$) and 84.8 $\pm$ 4.4\% ($D_c$, $D_n$, $\sigma_{cy}$ + $C_{sm}$) based on neural pattern recognition. Then $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ derived from $\sim$10 000 single cells of K562 vs Jurkat of leukemia and SACC-LM vs CAL 27 of oral tumor were quantified and compared, where successful rates of cell-type classification were estimated as 87.3\% (K562 vs Jurkat) and 79.5\% (SACC-LM vs CAL 27), respectively. In summary, the microfluidic platform reported in this study could quantify single-cell intrinsic structural and electrical parameters simultaneously, leading to significant increases in successful rates of cell-type classification.

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1. Introduction

Single-cell intrinsic structural and electrical parameters including cell diameter ($D_c$), nuclear diameter ($D_n$), cytoplasmic conductivity ($\sigma_{cy}$) and specific membrane capacitance ($C_{sm}$) have been closely related to both physiological and pathological progresses of blood cells [1–4], tumor cells [5–8] and stem cells [9–12] due to cellular heterogeneity [13]. In particular, based on single-cell structural and/or electrical parameters, hematology analyzers have been well established for both three-part and five-part differentials of leukocytes [14]. However, previously reported methods for single-cell analysis cannot achieve high-throughput obtain intrinsic structural and electrical parameters, which was introduced with comparison as follows.

As the golden approach of single-cell counting, initially the Coulter counter measured direct-current electric pulses of individual cells travelling through a small hole and calculated corresponding cell diameters of $D_c$, leading to the classification of leukocytes into mononuclear cells of lymphocytes and monocytes, and multinuclear cells of granulocytes [15–18]. With the inclusion of alternating-current components, the Coulter counter was capable of also measuring electrical properties of both membrane and cytoplasmic portions of single cells, to an extent, realizing the differential of leukocytes into mononuclear cells of lymphocytes and monocytes, and multinuclear cells of neutrophils, eosinophils and basophils [1, 19–21]. However, the Coulter counter cannot obtain the intrinsic structural marker of nuclei ($D_n$) and two intrinsic electrical markers of cytoplasmic conductivity ($\sigma_{cy}$) and specific membrane capacitance ($C_{sm}$) at the single-cell level, resulting in limited performances in cell-type classification.

In order to probe structural parameters of single nuclei, scattering flow cytometry was developed to capture multiple-angle scattered lights of a travelling single cell, where data of forward scattering was translated into $D_c$ and data of side scattering indicated nuclear granularities [2, 5, 6, 22, 23]. Although this approach can to an extent measure nuclear structures of single cells, it still could not collect intrinsic structural and electrical parameters of single cells such as $D_n$, $\sigma_{cy}$ and $C_{sm}$.

Aimed to measure intrinsic structural parameters of both $D_c$ and $D_n$, imaging flow cytometry was developed to capture fluorescent images of travelling single cells with both membrane and nuclear portions stained [3, 4, 7, 24, 25]. Although this approach could report critical structural parameters of single cells based on further image processing, it cannot at the same time collect intrinsic electrical parameters of single cells and thus cannot provide comprehensive evaluations of individual cells.

Due to dimensional comparisons with biological cells, microfluidics was adopted to measure single-cell structural and electrical properties [8, 24]. More specifically, in microfluidic flow cytometry, single cells were flushed through detection regions where optical and electrical detections were realized [26–30], and the measured optical/electrical parameters were used for cell-type classification. However, in these previously developed microfluidic platforms, inherent structural markers such as nuclear diameter ($D_n$) and electrical markers such as cytoplasmic conductivity ($\sigma_{cy}$) and specific membrane capacitance ($C_{sm}$) cannot be quantified due to limitations in detection structures and lacks of equivalent optical/electrical models.

To address this issue, this paper presented a microfluidic flow cytometry made of a double-T-type constriction channel (cross-sectional area smaller than a cell) with a predefined fluorescence detection domain, enabling high-throughput measurements of single-cell $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$. More specifically, when single cells with fluorescence stained nuclei deformed through and properly filled the constriction channel, $D_c$, $\sigma_{cy}$ and $C_{sm}$ were derived from impedance signals while $D_n$ was derived from fluorescence signals leveraging a home-developed impedance-fluorescence model for the constriction channel.

In comparison to Coulter counters, scattering, imaging flow cytometry and previously reported microfluidic optical/electrical flow cytometry, the microfluidic platform reported in this paper could measure intrinsic structural and electrical parameters of single cells in a high-throughput manner, providing a more comprehensive evaluation of single cells.

2. Methodology

2.1. Material purchase

Cell types containing three well-established carcinoma cell lines of A549 (lung), SW620 (colon) and HeLa (cervix), leukaemia cell lines of K562 (chronic myeloid tumor) and Jurkat (acute T-cell tumor), and oral carcinoma cell types of SACC-LM (salivary) and CAL 27 (tongue) were bought from the National Experimental Cell Resource Sharing Service Platform (China).

Unless otherwise indicated, reagents for cell culture were bought from Life Technologies (USA), Fluorochrome Hoechst 33342 used for nuclear staining was bought from Sigma Aldrich (USA). Materials for the fabrication of microfluidic flow cytometry including photoresist of AZ serial, 184 silicone elastomer and SU 8–25 negative photoresist were purchased from AZ Electronic Materials (USA), Dow Corning (USA) and MicroChem (USA), respectively.
2.2. Cell preparation

All cells were cultured under the conditions of 37 °C and 5% CO₂. To be more specific, RPMI-1640 medium (10% foetal bovine serum, 1% penicillin and 1% streptomycin) was used to culture A549, SW620, SACC-LM, K562 and Jurkat cells. Meanwhile, DMEM medium (10% foetal bovine serum, 1% penicillin and 1% streptomycin) was used to culture HeLa and CAL 27 cells. For nuclear staining, living cells were collected by centrifugation and added with 10% fluorochrome Hoechst 33342 for an incubation of 5 min at 37 °C. After incubation, cells were washed for three times and collected by centrifugation at 4 °C, and then resuspended in phosphate buffer saline (PBS) containing 0.5% bovine serum albumin.

2.3. Working mechanism

Figure 1 shows the working mechanism of the microfluidic flow cytometry which was made of a double T-type constriction channel plus a predefined fluorescence detection domain, where a traveling cell with a fluorescence stained nucleus was squeezed through the constriction channel with varied electrical and optical signals detected by an impedance analyzer and a photomultiplier tube, respectively. (b) Preliminary electrical and optical signals of the traveling cell were divided into five domains (I–V) leveraging the impedance-fluorescence model for the constriction channel. (c) Corresponding time durations of \( t_{c1}, t_{c2} \) and \( t_{c3} \) from three domains of impedance (I, III and V) were obtained and translated into cell diameter of \( D_c \) while time durations of \( t_{m1}, t_{m2} \) and \( t_{m3} \) from three domains of fluorescence (II–IV) were acquired and translated into nuclear diameter of \( D_n \). Furthermore, impedance values (Amp₁ + Pha₁ and Amp₂ + Pha₂) from domain III of impedance were quantified and translated into cytoplasmic conductivity of \( \sigma \) and specific membrane of \( C_m \).

Figure 1(a) shows major components of the microfluidic flow cytometry for single-cell analysis including the aforementioned double T-type constriction channel for cell travelling, an impedance analyzer for electrical property measurements and a photomultiplier tube for optical property measurements. As to the double T-type constriction channel, it mainly included a major constriction channel for cell squeezing, two secondary constriction channels for electrical acquisition, and a fluorescence detection domain formed by a patterned zinc oxide window for fluorescent detection. In operation, a travelling cell with a fluorescence stained nucleus was squeezed through the major constriction channel where varied electrical and optical signals were detected by the impedance analyzer and the photomultiplier tube, simultaneously.

Preliminary electrical and optical signals of the travelling cell were divided into five domains (I–V) based on the impedance-fluorescence model for the constriction channel composed of five key geometrical parameters: cross sectional area \( S_m \) and length \( L_m \) of the major constriction channel, width of the secondary constriction channels \( W_c \), gap of the fluorescence detection domain \( L_f \) and cell elongation length \( L_c \) (see figure 1(b)). More specifically, for each impedance pulse, it was segmented into three time durations (e.g. cell entering (I), passing (III) and leaving (V)). For domain I and V, time durations of \( t_{c1} \) and \( t_{c3} \) with the same corresponding travelling distance of \( W_c + L_c \) were obtained. As to domain III, a time duration of \( t_{c2} \) with a corresponding travelling distance of \( L_m - L_c \) was obtained and corresponding electrical parameters of Amp₁ + Pha₁ and Amp₂ + Pha₂ were also acquired.

Meanwhile, for each fluorescence pulse, it was divided into three time durations (e.g. nuclear entering (II), passing (III) and leaving (IV)). For domain II and IV, time durations of \( t_{m1} \) and \( t_{m3} \) with the same corresponding travelling distance of \( D_n \) were obtained, and for domain III, time duration of \( t_{m2} \) with a corresponding travelling distance of \( L_n - D_n \) was acquired.

Figure 1(c) shows the translation of raw time and impedance data into single-cell intrinsic structural and electrical parameters of \( D_c, D_n, C_m \) and \( \sigma \). As to the quantification of \( D_c, L_c \) was first calculated under the assumptions of (a) a steady velocity for a travelling cell and (b) \( L_m > L_c \). More specifically, for domain I, III and V with time durations of \( t_{c1}, t_{c2} \) and \( t_{c3} \), the corresponding travelling distances were
\[ W_s + L_c, \quad L_m - L_c \quad \text{and} \quad W_s + L_c, \] respectively. Thus, \( L_c \) was obtained as follows:

\[
\frac{(W_s + L_c) + 2 \times (L_m - L_c) + (W_s + L_c)}{t_{c1} + 2 \times t_{c2} + t_{c3}} = \frac{(W_s + L_c) + (W_s + L_c)}{t_{c1} + t_{c3}} \quad (1a)
\]

\[
L_c = \frac{t_{c1} + t_{c3}}{t_{c1} + 2 \times t_{c2} + t_{c3}} (L_m + W_s) - W_s. \quad (1b)
\]

Then under the assumption that a cell was incompressible, the volume of the deformed cell in the constriction channel which was treated as a cuboid with an length of \( L_c \) and an cross-sectional area of \( S_m \) was equal with the volume of the cell in sphere with a diameter of \( D_c \). Thus, \( D_c \) was obtained as follows:

\[
D_c = 2 \times \sqrt[3]{3 \times S_m \cdot L_c/(4\pi)}. \quad (2)
\]

As to the quantification of \( D_m \), it was measured under the assumptions of (a) a steady velocity for a travelling cell and (b) \( L_g > D_m \). More specifically, for domain II, III and IV with time durations of \( t_{n1}, t_{n2} \) and \( t_{n3} \), the corresponding travelling distances were \( D_m, L_g - D_m \) and \( D_m \), respectively. Thus, \( D_m \) was obtained as follows:

\[
D_m = \frac{t_{n1} + t_{n3}}{t_{n1} + 2 \times t_{n2} + t_{n3}} L_g. \quad (3)
\]

As to the quantification of \( \sigma_{cy} \) and \( C_{sm} \), figure 1(c) shows key electrical components of a travelling cell within the major constriction channel including \( R_{cy} \) of cytoplasmic resistance and \( C_{m} \) of membrane capacitance which were obtained by electrical parameters of \( \text{Anmp}_1 + \text{Pha}_1 \) and \( \text{Anmp}_2 + \text{Pha}_2 \) for the travelling cell. \( R_{cy} \) and \( C_{m} \) as intermediate electrical parameters were further processed to \( \sigma_{cy} \) and \( C_{sm} \) when geometrical dimensions of the constriction channel were taken into consideration:

\[
\sigma_{cy} = L_c / (R_{cy} \cdot S_m) \quad (4)
\]

\[
C_{sm} = C_m / S_m. \quad (5)
\]

2.4. Device design and fabrication

For the microfluidic flow cytometry to function properly, key geometric parameters of the double T-type constriction channel including the major constriction channel’s cross sectional area (\( S_m \)) and length (\( L_m \)), fluorescence detection domain’s gap (\( L_g \)) and the secondary constriction channels’ cross sectional area (\( S_c \)) were designed carefully, as follows.

The major constriction channel’s cross sectional area (\( S_m \)) has to be slightly smaller than the cross sectional area of the cell under measurement to ensure proper deformation of the cell to effectively block electric lines without damages for cell membrane and nuclear portions. When the diameters of the cells under measurement were estimated as \( \sim 15 \ \mu m \), \( S_m \) of 10 \( \mu m \times 10 \ \mu m \) was chosen in this study. As to the choice of the major constriction channel’s length (\( L_m \)), if \( L_m \) was too long, it could increase both basal impedance values without cells and cellular travelling durations, leading to compromised measurement sensitivities and throughputs. If \( L_m \) was too short, it could not meet the requirement of the impedance-fluorescence model, resulting in inaccurate measurements of intrinsic single-cell structural parameters. When these two factors were taken into consideration, \( L_m \) was set as 50 \( \mu m \) in this study. Similarly, since \( L_g \) cannot be too large or too small in this study, it was set as 25 \( \mu m \) under the consideration of \( \sim 10 \ \mu m \) of \( D_m \) and 10 \( \mu m \times 10 \ \mu m \) of \( S_m \). Note that the fluorescence detection domain was formed by patterned zinc oxide which was used to block the excitation light of 355 nm for Hoechst 33342 in nuclear staining.

Considering that the secondary constriction channels’ height was the same as that of the major constriction channel, the secondary constriction channels’ cross sectional area (\( S_c \)) was mainly determined by its width. \( S_c \) should be as small as possible so that travelling single cells can effectively seal without entering the secondary constriction channels. Meanwhile, the increase in \( S_c \) can decrease the impedance values of the constriction channels without cells. In addition, when resolutions of microfabrication were also considered, \( S_c \) was defined as 3 \( \mu m \times 10 \ \mu m \) in this paper.

The device fabrication consisted of three key steps, which were the fabrications of the double T-type constriction channel, the fluorescence detection domain and the bonding of these two components (see figure S1 (available online at stacks.iop.org/JMM/32/035007/mmedia)). More specifically, the microfabrication of the double T-type constriction channel included key steps of (a) exposure of AZ 5214, (b) development of AZ 5214, (c) deep reactive ion etching of silicon, (d) exposure of SU 8-25, (e) development of SU 8-25, (f) molding of Polydimethylsiloxane (PDMS), and (g) peeling of PDMS (see figure S1(a)).

Next, fabrication of the fluorescence detection domain included key steps of (a) exposure of AZ 1500, (b) development of AZ 1500, (c) sputtering of zinc oxide, (d) lift-off of zinc oxide, and (e) coating of PDMS (see figure S1(b)). Figure S1(c) shows the bonding of the fabricated double T-type constriction channel plus the predefined fluorescence detection domain enabled by an oxygen plasma treatment and figure S1(d) shows a fabricated microfluidic device with the double T-type constriction channel and the fluorescence detection domain highlighted.

2.5. Device operation

In operation, the microfabricated platform was first filled with PBS and the corresponding fluorescence detection domain was photobleached by a fluorescence microscope (IX73, Olympus, Japan). Then suspended cells stained with Hoechst 33342 (20 ~ 50 \( \mu l \), ~5 x 10^6 cells ml^{-1}) were aspirated through the constriction channel under a negative pressure of ~2 kPa (DPI-610, Druck, UK).

An impedance analyzer (MFLI 5 M, Zurich Instruments, CH) based on 40 and 100 kHz sinusoidal signals...
(root-mean-square voltage of 0.5 V, time constant of 25 μs) were connected with two secondary constriction channels using silver wires to measure impedance data of squeezing individual cells. Meanwhile, when the stained nuclei in single cells traveled through the fluorescence detection domain, it was excited with a metal halide lamp (X-Cite 120Q, Excititas, Canada) at a 100% output power with a bandpass filter (AT375/28x, Chroma, USA). A photomultiplier tube of H10723-20 (Hamamatsu, Japan) plus a bandpass filter of AT460/50 m (Chroma, USA) was used for fluorescent detection. The impedance profiles and fluorescence signals were further collected by a data acquisition card of USB-6349 (NI, USA) under a sampling rate of 100 kHz. Note that the inclusion of 40 and 100 kHz sinusoidal signals were based on a previous study where frequencies of single-cell impedance measurements were finely tuned [31].

2.6. Data analysis

Raw impedance profiles were translated into intrinsic structural and electrical parameters (e.g. \( D_c \) of cell diameter, \( \sigma_{cy} \) of cytoplasmic conductivity and \( C_{sm} \) of specific membrane capacitance) based on the following two parts. First, \( D_c \) was quantified leveraging two steps: (a) kernel-based density estimation of preliminary impedance profiles at 100 kHz with binarization to locate individual pulses representing travelling single cells; (b) kernel-based density estimation and binarization to divide individual pulses into three domains of entering \((t_{n1})\), travelling \((t_{n2})\) and leaving \((t_{n3})\) the double T-type constriction channel, enabling the calculation of \( D_c \) (see figure 1(c) and equations (1a), (1b), (2)). Note that since peaks and valleys of preliminary impedance profiles at 100 kHz were more obvious than the counterparts at 40 kHz, 100 kHz impedance data were used in the calculation of \( D_c \).

After the quantification of \( D_c \), raw impedance profiles were further translated into \( \sigma_{cy} \) and \( C_{sm} \) leveraging four steps. (a) Weighted averages of the raw impedance profiles in the travelling domain within the double T-type constriction channel were processed into two-frequency amplitude values of \( \text{Amp}_1 + \text{Amp}_2 \) and phase values of \( \text{Pha}_1 + \text{Pha}_2 \). (b) Preliminary impedance parameters of \( \text{Amp}_1, \text{Amp}_2, \text{Pha}_1 \) and \( \text{Pha}_2 \) were translated into the equivalent impedance of the travelling cell at both 40 and 100 kHz, based on equivalent electrical components of the empty constriction channel (see figure 1(c)). (c) The equivalent impedance of the travelling cell was further decoupled into cytoplasmic resistance of \( R_{cy} \) and membrane capacitance of \( C_{sm} \) which were in series. (d) Based on equation (4) and (5)), when geometric parameters of the constriction channel were taken into consideration, \( R_{cy} \) and \( C_{sm} \) were translated into \( \sigma_{cy} \) and \( C_{sm} \).

Raw fluorescence profiles were translated into the intrinsic structural parameter (nuclear diameter of \( D_n \)) based on three steps: (a) median filtration of preliminary fluorescence profiles to obtain valid signals; (b) kernel-based density estimation and binarization of filtered signals to locate individual pulses representing traveling single nuclei of corresponding cells; and (c) curve fitting based on the least-square principle to divide individual pulses into three domains of entering \((t_{n1})\), travelling \((t_{n2})\) and leaving \((t_{n3})\) the fluorescence detection region, enabling the quantification of \( D_n \) (see figure 1(c) and equation (3)).

2.7. Statistical analysis

Multiple samples were measured and the results were expressed as mean ± standard deviation. In order to determine the level of differences, the student’s t-test was conducted where \( P \) value < 0.05 (*) was considered with statistically significant difference.

In addition, pattern recognition enabled by two-layer forward-feedback neural network (MATLAB 2016b, MathWorks, USA) was used to predict successful rates of cell-type classification. In this study, the results of two cell types with quantified single-cell values of \( D_c, D_n, \sigma_{cy} \) and \( C_{sm} \) were used as inputting matrix, which was divided into 70% for training, 15% for validation and 15% for testing. Note that for all the inputting data points, values of single-cell \( D_c, D_n, \sigma_{cy} \) and \( C_{sm} \) and specific cell types were clear. After training of 70% data and validation of 15% data, a ‘code function’ was generated to map single-cell values of \( D_c, D_n, \sigma_{cy} \) and \( C_{sm} \) with specific cell types. Then the obtained ‘code function’ was used to process 15% data points for testing and the results of cell types generated from the ‘code function’ were compared with the true cell types, producing a ‘successful rate’ as an indicator to determine the accuracy of cell-type classification. For an incoming cell with quantified \( D_c, D_n, \sigma_{cy} \) and \( C_{sm} \), these values were applied into the code function and the generated value of ‘0’ or ‘1’ indicated the specific cell type.

3. Results and discussion

Constriction channels have been developed to measure electrical properties of individual cells where squeezing cells blocked electric lines dramatically, and the corresponding impedance variations were translated into intrinsic electrical parameters based on an equivalent electrical model. Developments of constriction channels witnessed structural improvements from straight [32] to crossing [33] and then double T-type [31] constriction channels, capable of quantifying single-cell structural and electrical parameters of \( D_c, \sigma_{cy} \) and \( C_{sm} \) in a high-throughput manner. Based on \( D_c, \sigma_{cy} \) and \( C_{sm} \), classifications of tumor cells with different malignant levels [32], stem cells with variously differentiation capabilities [34] and sub-group white blood cells [35] were demonstrated. However, previously reported constriction channels cannot measure biophysical properties of nuclear portions and thus cannot provide a comprehensive evaluation of biological cells.

3.1. Platform characterization

This study developed the microfluidic flow cytometry which was made of the double T-type constriction channel plus the predefined fluorescence detection domain and was capable
of high-throughput measurements of intrinsic structural and electrical parameters of $D_c, D_n$, $\sigma_r$ and $C_m$ for individual cells leveraging the home-developed impedance-fluorescence model. In the stage of platform characterization, figure 2 shows raw impedance profiles at 40 and 100 kHz, and fluorescence data with excitation at 355 nm and detection at 465 nm of individual A549 (a), SW620 (b) and HeLa (c) cells. Here electrical peaks in amplitude, valleys in phase and fluorescence peaks in intensity indicate a cell travelling in the double T-type constriction channel plus the predefined fluorescence detection domain. For each cell, impedance and fluorescence variations were processed to time duration parameters $t_{c1}, t_{c2}$ and $t_{c3}$ of impedance, $t_{n1}, t_{n2}$ and $t_{n3}$ of fluorescence as well as electrical impedance values of $\text{Amp}_1 + \text{Pha}_1$ and $\text{Amp}_2 + \text{Pha}_2$.

Figure 3 shows scatter plots of four vital intermediate indicators containing structural parameters of $r_{tc}$ and $r_{tn}$ for three well-established carcinoma cell lines of A549 (a, $N_{\text{cell}} = 12,089$), SW620 (b, $N_{\text{cell}} = 10,225$) and HeLa (c, $N_{\text{cell}} = 10,823$). More specifically, as indicators of $D_c$ and $D_n$, $r_{tc}$ and $r_{tn}$ were quantified as $0.46 \pm 0.10$ and $0.40 \pm 0.04$ for A549, $0.44 \pm 0.08$ and $0.40 \pm 0.09$ for SW620, $0.46 \pm 0.10$ and $0.39 \pm 0.09$ for HeLa cells, respectively. These results indicated the microfluidic system reported in this paper can (a) capture preliminary impedance and fluorescent signals of squeezing individual cells simultaneously; (b) extract key time and impedance parameters based on impedance and fluorescence pulses.
results indicated that these three cell types had comparable geometrical structures in term of cell and nuclear diameters.

Meanwhile, as indicators of $\sigma_{cy}$ and $C_{nm}$, $R_{c}$ and $C_{m}$ were calculated as 0.38 ± 0.28 MΩ and 2.13 ± 0.63 pF for A549, 0.32 ± 0.22 MΩ and 1.72 ± 0.51 pF for SW620, 0.40 ± 0.26 MΩ and 2.85 ± 0.81 pF for HeLa cells. Significant differences of $R_{c}$ and $C_{m}$ among A549, SW620 and HeLa cells were found, indicating that cells with similar geometrical parameters may be differentiated based on electrical properties.

Figure 4(a) shows scatter plots of four intrinsic structural and electrical parameters including $D_{c}$ vs $D_{n}$, $D_{c}$ vs $\sigma_{cy}$ and $D_{c}$ vs $C_{nm}$ for A549 ($N_{cell} = 12089$), SW620 ($N_{cell} = 10225$) and HeLa ($N_{cell} = 10823$), respectively. More specifically, structural parameters including $D_{c}$ and $D_{n}$ were quantified as 15.2 ± 1.4 $\mu$m and 10.5 ± 0.8 $\mu$m for A549 cells, 15.0 ± 1.2 $\mu$m and 10.2 ± 1.7 $\mu$m for SW620 cells, 15.1 ± 1.4 $\mu$m and 10.1 ± 1.7 $\mu$m for HeLa cells. For these three cell lines, comparable results of $D_{c}$ and $D_{n}$ were also derived from microscopic images, which were 15.2 ± 1.3 $\mu$m and 10.2 ± 1.6 $\mu$m for A549, 14.9 ± 1.6 $\mu$m and 10.0 ± 1.6 $\mu$m for SW620, 15.1 ± 1.9 $\mu$m and 9.9 ± 1.6 $\mu$m for HeLa. These results proved that the developed microfluidic system can obtain $D_{c}$ and $D_{n}$ from large populations of single cells with high accuracies.

Meanwhile, electrical parameters including $\sigma_{cy}$ and $C_{nm}$ were calculated as 0.53 ± 0.17 S m$^{-1}$ and 2.64 ± 0.78 $\mu$F cm$^{-2}$ for A549 cells, 0.62 ± 0.19 S m$^{-1}$ and 2.13 ± 0.63 $\mu$F cm$^{-2}$ for SW620 cells, 0.49 ± 0.16 S m$^{-1}$ and 3.54 ± 1.00 $\mu$F cm$^{-2}$ for HeLa cells. These results fell within the ranges of $\sigma_{cy}$ (∼1 S m$^{-1}$) and $C_{nm}$ (∼1 $\mu$F cm$^{-2}$) obtained by conventional tools such as electrorotation and patch clamping with limited throughputs [36]. Comparable results of $\sigma_{cy}$ and $C_{nm}$ were also derived from the double T-type constriction channel without the fluorescence detection domain, which were 0.49 ± 0.07 S m$^{-1}$ and 2.46 ± 0.55 $\mu$F cm$^{-2}$ for A549, 0.52 ± 0.07 S m$^{-1}$ and 2.00 ± 0.51 $\mu$F cm$^{-2}$ for SW620, 0.51 ± 0.07 S m$^{-1}$ and 3.28 ± 0.65 $\mu$F cm$^{-2}$ for HeLa cells [31]. These results indicated that the step of nuclear staining had negligible effects on intrinsic electrical parameters of single cells. In addition, there were significant differences of $\sigma_{cy}$ and $C_{nm}$ among A549, SW620 and HeLa cells, indicating that single cells with similar structural parameters may be differentiated based on electrical parameters.

Figure 4(b) shows confusion matrices of A549 vs SW620, A549 vs HeLa, and SW620 vs HeLa under the input parameters of $D_{c}$ only, $D_{c} + D_{n}$, $D_{c}$, $D_{n}$, $\sigma_{cy}$ and $C_{nm}$ together where the numbers in the bottom right-hand boxes of the matrices indicated successful rates of cell-type classification.

Figure 4. (a) Scatter plots of four intrinsic structural and electrical parameters of single cells including $D_{c}$ vs $D_{n}$, $D_{c}$ vs $\sigma_{cy}$ and $D_{c}$ vs $C_{nm}$ for A549 ($N_{cell} = 12089$), SW620 ($N_{cell} = 10225$) and HeLa ($N_{cell} = 10823$). (b) Confusion matrices of A549 vs SW620, A549 vs HeLa, and SW620 vs HeLa under the input parameters of $D_{c}$ only, $D_{c} + D_{n}$, and $D_{c}$, $D_{n}$, $\sigma_{cy}$ and $C_{nm}$ together. Note that the numbers in the
Figure 5. (a) Scatter plots of four intrinsic structural and electrical parameters of single cells including $D_c$ vs $D_n$, $D_c$ vs $\sigma_{cy}$ and $D_c$ vs $C_{sm}$ for leukemia cell lines of K562 ($N_{cell} = 14,083$) and Jurkat ($N_{cell} = 11,377$). (b) Confusion matrixes of K562 vs Jurkat cells under $D_c$ only, $D_c + D_n$, and $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ together. Note that the numbers in the bottom right-hand boxes of the matrixes indicated successful rates of cell-type classification.

3.2. Platform demonstration

As a demonstration, the microfluidic system in this paper was first used to classify leukemia cell lines of K562 and Jurkat. Preliminary electrical and optical profiles, and processed data of $r_{tc}$, $r_{tn}$, $R_{cy}$ and $C_m$ for K562 and Jurkat cells were shown in figures S2 and S3. Final results of intrinsic structural and electrical parameters of $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ of K562 ($N_{cell} = 14,083$) vs Jurkat ($N_{cell} = 11,377$) were shown in figure 5(a), which were $15.0 \pm 1.0$ vs $14.7 \pm 1.4 \mu m$, $11.6 \pm 1.6$ vs $10.7 \pm 1.3 \mu m$, $0.70 \pm 0.15$ vs $0.48 \pm 0.13$ S m$^{-1}$ and $2.12 \pm 0.34$ vs $1.78 \pm 0.44$ $\mu F$ cm$^{-2}$, respectively. These results indicated that single cells with comparable structural parameters ($D_c$ and $D_n$) may be differentiated based on variations in electrical parameters ($\sigma_{cy}$ and $C_{sm}$).

Figure 5(b) shows confusion matrixes of K562 vs Jurkat cells under the input parameters of $D_c$ only, $D_c + D_n$, $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ in combination where the numbers in the bottom right-hand boxes of the matrixes indicated successful rates of classifying cell types. The successful rates of cell-type classification of K562 vs Jurkat were estimated as 63.4% ($D_c$), 70.2% ($D_c$ and $D_n$) and 87.3% ($D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$). These results of cell-type classification were due to the fact that these two leukemia cell lines demonstrated comparable structural parameters of $D_c$ and $D_n$ with less than 10% differences and significant differences in $\sigma_{cy}$ with a $\sim 40\%$ difference and $C_{sm}$ with a $\sim 20\%$ difference. Thus the combination of intrinsic structural and electrical parameters of single cells including $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ can be used to effectively classify leukemia cell lines of K562 and Jurkat.

Similarly, oral carcinoma cell lines of SACC-LM and CAL 27 were characterized and compared in this study, where corresponding raw and intermediate data were also shown in figures S2 and S3. The final results of intrinsic structural and electrical parameters of $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ of SACC-LM ($N_{cell} = 13,326$) vs CAL 27 ($N_{cell} = 11,218$) were shown in figure 6(a), which were $15.7 \pm 1.4$ vs $14.5 \pm 1.2 \mu m$, $10.4 \pm 1.4$ vs $9.2 \pm 1.3 \mu m$, $0.51 \pm 0.13$ vs $0.58 \pm 0.13$ S m$^{-1}$ and $2.73 \pm 0.69$ vs $2.80 \pm 0.63$ $\mu F$ cm$^{-2}$, respectively.

The successful rates of cell-type classification of SACC-LM vs CAL 27 were shown in figure 6(b), which were estimated as 67.8% ($D_c$), 73.7% ($D_c$ and $D_n$) and 79.5% ($D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$). Different from leukemia cell lines of K562 and Jurkat, these two oral carcinoma cell lines demonstrated
limited differences (\sim 10\%) in both structural and electrical parameters. However, the combination of intrinsic structural and electrical parameters of single cells including $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ can improve the successful rates of differentiating SACC-LM from CAL 27.

Furthermore, for the comparisons of K562 vs Jurkat cells and SACC-LM vs CAL 27 cells, the inclusion of the intrinsic structural parameter of $D_n$ contributed to the increases of the classification rate from 85.1\% (K562 vs Jurkat) and 74.0\% (SACC-LM vs CAL 27) based on $D_c$, $\sigma_{cy}$ and $C_{sm}$ derived from the previously reported double T-type constriction channel \cite{31} to 87.3\% (K562 vs Jurkat) and 79.5\% (SACC-LM vs CAL 27) based on $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ derived from the double T-type constriction channel plus the predefined fluorescence detection domain reported in this study. Note that since K562 vs Jurkat and SACC-LM vs CAL 27 have comparable structural parameters of cell and nuclear diameters, the inclusion of $D_n$ was observed to demonstrate a limited effect on cell-type classification. If two cell lines with significant differences in geometrical parameters were compared, the intrinsic structural parameter of $D_n$ may function as an enabling parameter in cell-type classification.

4. Conclusions

In summary, this paper presented the microfluidic flow cytometry made of the double T-type constriction channel plus the predefined fluorescence detection domain, capable of high-throughput measurements of single-cell intrinsic structural parameters of cell diameter ($D_c$) and nuclear diameter ($D_n$), and electrical markers of cytoplasmic conductivity ($\sigma_{cy}$) and specific membrane capacitance ($C_{sm}$). As to platform characterization, this microfluidic flow cytometry was demonstrated to quantify $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ from three well-established carcinoma cell lines with high accuracies. As for platform demonstration, $D_c$, $D_n$, $\sigma_{cy}$ and $C_{sm}$ from leukemia and oral tumors were measured, demonstrating higher successful rates of cell-type classification when these four parameters were used compared to the results using individual biophysical markers only.
Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

The authors declare no conflicts of interest.

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