Three-dimensional inertial focusing based impedance cytometer enabling high-accuracy characterization of electrical properties of tumor cells†

Chen Ni, Mingqi Yang, Shuai Yang, Zhixian Zhu, Yao Chen, Lin Jiang and Nan Xiang ○*

The differences in the cross-sectional positions of cells in the detection area have a severe negative impact on achieving accurate characterization of the impedance spectra of cells. Herein, we proposed a three-dimensional (3D) inertial focusing based impedance cytometer integrating sheath fluid compression and inertial focusing for the high-accuracy electrical characterization and identification of tumor cells. First, we studied the effects of the particle initial position and the sheath fluid compression on particle focusing. Then, the relationship of the particle height and the signal-to-noise ratio (SNR) of the impedance signal was explored. The results showed that efficient single-line focusing of 7–20 μm particles close to the electrodes was achieved and impedance signals with a high SNR and a low coefficient of variation (CV) were obtained. Finally, the electrical properties of three types of tumor cells (A549, MDA-MB-231, and UM-UC-3 cells) were accurately characterized. Machine learning algorithms were implemented to accurately identify tumor cells based on the amplitude and phase opacities at multiple frequencies. Compared with traditional two-dimensional (2D) inertial focusing, the identification accuracy of A549, MDA-MB-231, and UM-UC-3 cells using our 3D inertial focusing increased by 57.5%, 36.4% and 36.6%, respectively. The impedance cytometer enables the detection of cells with a wide size range without causing clogging and obtains high SNR signals, improving applicability to different complex biological samples and cell identification accuracy.

1. Introduction

Microfluidic impedance cytometers (MICs), as an emerging frontier for cell biophysical characterization, can realize the quantification and discrimination of cells in complex biological samples in a label-free and multi-parametric manner.1–3 By utilizing MICs, the electrical impedance spectra of cells at various frequencies spanning from 0.5 to 50 MHz can be obtained based on the obstruction of alternating current (AC) flow by single cells in the microchannel detection area.4 The multi-frequency spectra of single-cell impedance phenotypes can usually reflect cell characteristics such as the cell size (∼0.5 MHz), membrane morphology (2–10 MHz), cytoplasm and nucleus (≥10 MHz). Based on the analysis of single-cell impedance phenotypes, MICs have gained momentum in the identification of cell types,5–8 detection of differentiated cells,9 and analysis of cells treated with drugs.10–15

Because of the differences in the initial positions of cells entering the microchannel, it is common for cells to pass through the detection area at different cross-sectional positions, with multiple cells potentially traversing the detection area concurrently. These situations have a severe negative impact on accurately characterizing the impedance spectra of cells. To address these issues, numerous novel MICs have been reported with improvements in channel structures, electrode arrangements, signal calibration methods, etc. For example, Hou et al.16 achieved a stepped decrease in the channel height of the electrode detection area using standard soft lithography techniques, improving the signal-to-noise ratio (SNR) of detection signals of neutrophils. This method of reducing the microchannel height is adopted by many MIC platforms17–20 due to its simple and intuitive advantages. However, this method is only suitable for dealing with cells whose sizes are similar to the microchannel height, and thus the blockage of the microchannel by cells is
inevitable. Caselli et al.21 presented a five-electrode coplanar layout measuring the change of the differential current waveforms generated by particles passing through different cross-sectional positions. They also established the relationship between particle signals and particle trajectory heights, eliminating the positional dependence caused by the non-uniform electric field. However, the aforementioned method primarily accomplished the revision of the peak amplitude of low-frequency signals and could not achieve accurate characterization of multi-frequency electrical properties of single cells.

An ideal strategy is to take advantage of microfluidic technology to accomplish 3D focusing of cells, allowing cells to pass through the detection area one by one at a fixed cross-sectional position close to the electrode. Currently, passive microfluidic focusing techniques23,23 use fluid hydrodynamics to promote the lateral migration of cells with the advantages of low cost and simple operation, among which inertial focusing and elasto-inertial focusing are the most commonly used. For inertial focusing, a high aspect ratio microchannel combined with curved24–26 or contraction–expansion27 structures is usually used to eliminate multiple focusing equilibrium positions. However, the induced single focusing position in these channels is close to the side wall, which limits the placement of electrodes and complicates the chip manufacturing. For elasto-inertial focusing,28,29 although cells can be focused to the channel center in a simple channel, the sample processing throughput is low and the subsequent cell extraction and analysis may be compromised due to the addition of viscoelastic enhancers. In addition, 3D focusing based on multiple sheath fluids has also been attempted;30,31 however, its low sample processing throughput and the need to modify the flow rate ratio of the sheath fluid and sample fluid to adapt to the focusing of particles with different sizes are not conducive to the rapid detection of complex biological samples.

In this paper, we developed a 3D inertial focusing based microfluidic impedance cytometer for the high-accuracy electrical characterization and identification of tumor cells. By combining vertical sheath fluid compression and inertial focusing based on a symmetrical serpentine microchannel, single-line focusing of cells in close proximity to the electrodes was achieved. Cells passed through the detection area one by one at the same cross-sectional position, which improved the SNR and uniformity of cell detection signals. After systematically investigating the focusing trajectories of particles released at different initial positions and the compression effect of the sheath fluid, the single-line focusing of particles (diameters of 7–20 μm) was achieved, with a focusing efficiency greater than 96%. Additionally, the impact of particle positions along the channel height on the detection signal was investigated, proving that the impedance cytometer was capable of generating impedance signals with a high SNR and a low coefficient of variation (CV). Finally, the impedance cytometer was applied for the detection of multiple tumor cells (A549, MDA-MB-231, and UM-UC-3 cells) with a similar size distribution. After analyzing dielectric parameters (e.g., amplitude and phase opacity), machine learning algorithms were used to successfully achieve accurate identification of tumor cells. Compared with traditional two-dimensional (2D) inertial focusing, the identification accuracy of A549, MDA-MB-231, and UM-UC-3 cells using our 3D inertial focusing was improved by 57.5%, 36.4%, and 36.6%, respectively. Our impedance cytometer provides a method for rapid and high-accuracy electrical characterization and identification of cells.

2. Materials and methods

2.1 Working principle

Fig. 1a shows the workflow of the impedance cytometer for label-free and high-accuracy detection of tumor cells. When the sample is injected into the impedance cytometer, the randomly-distributed cells at the inlet will be focused into a train under the combined action of the sheath fluid and the fluid hydrodynamic forces induced by the symmetrical serpentine microchannel, which enables cells to traverse the detection area one by one. The detection area consists of an excitation electrode and a pair of detection electrodes. When cells pass over the electrodes, they will induce interference to the electric field between the electrodes, generating the response AC signals. The excitation signals of three different frequencies were applied to the central electrode. Then, the multi-frequency impedance signals (amplitude and phase shift) of a single cell were obtained by demodulating, differentiating, and amplifying the response AC signals on the electrodes on both sides. The amplitude and phase shift at three different frequencies were used to analyze the dielectric properties (such as amplitude opacity and phase opacity) of the single cells, achieving the accurate characterization and identification of cells. The 3D inertial focusing of cells was achieved by using a vertical-injection sheath fluid-based symmetrical serpentine microchannel. The principle of 3D inertial focusing is shown in Fig. 1b. Following the vertical injection of the sheath fluid, the position of cell distribution was compressed to the lower half of the channel. With the sample fluid and sheath fluid flowing into the symmetrical serpentine microchannel in the form of laminar flow, cells migrated towards the equilibrium position with zero net force under the combined action of the inertial lift force ($F_L$) caused by shear gradients and wall effects and the Dean drag force ($F_D$) induced by secondary flow. Under the periodically reversing secondary flow induced by the symmetrical serpentine microchannel, cells would not be carried away from the lower half of the channel. Therefore, cells were focused to the same equilibrium position close to the electrode and passed sequentially through the detection area (see Fig. 1c). Benefiting from this 3D inertial focusing, impedance signals with a high SNR and high uniformity were obtained (see Fig. 1d), contributing to the high-accuracy characterization and identification of cells.
2.2 Device design and fabrication

The impedance cytometer consists of a symmetrical serpentine microchannel and three gold electrodes. The symmetrical serpentine microchannel comprises 20 repeated serpentine units with an outer curve radius of 220 μm and an inner curve radius of 120 μm. The microchannel includes two inlets at its beginning for the injection of the sample fluid and sheath fluid. A straight channel with a width of 100 μm is situated at the end of the microchannel as the detection area and is connected to the outlet. The three electrodes have a width of 20 μm and are distributed in parallel in the detection area with a spacing of 20 μm. The microchannel height is standardized at 40 μm. The photographs of the microfluidic impedance chip is shown in Fig. S1.

The microchannel was fabricated using standard soft lithography techniques. A wafer was spin-coated with a layer of 40 μm thick negative photoresist (SU-8 2050, MicroChem). After baking, exposing, and developing, a master mold with microchannel structures was obtained. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) was poured onto the master mold and then cured for two hours in an oven at 65 °C. PDMS blocks with a microchannel were obtained by demolding and cutting the cured PDMS. The electrodes were fabricated using etching technology. Briefly, gold-coated glass was spin-coated with a layer of positive photoresist (RZJ-304, Ruihong). After baking, exposing, and developing, a hard photoresist layer with an electrode shape was obtained. Then, the gold-coated glass was wet-etched to generate the electrodes. After being treated using an oxygen plasma cleaner (PDC-002, Harrick Plasma), the fabricated PDMS block with a microchannel and the glass plate with electrodes were bonded to obtain the impedance cytometer. To observe the particle trajectories in the channel height direction, the PDMS block with a microchannel was rotated by 90 degrees and vertically bonded to the glass plate. Prior to this, the PDMS block with a microchannel was bonded with a plain PDMS slab to ensure the sealing of the microchannel. The photograph of the fabricated chip is shown in Fig. S2.

2.3 Sample preparation

Five types of polystyrene particles (7 μm, 10 μm, 12 μm, 15 μm, and 20 μm, Thermo Fisher Scientific) were used to characterize the focusing and detection performance of the impedance cytometer. All types of particles were diluted with phosphate-buffered saline (PBS, Sigma-Aldrich) buffer to prepare the particle suspensions. In addition, 7 μm, 10 μm, and 15 μm particles were also diluted with a viscoelastic fluid for the elasto-inertial focusing. The viscoelastic fluid was prepared by adding hyaluronic acid (HA, Shanghai Future Industrial) with a molecular weight of 1650 kDa to PBS buffer at a concentration of 1000 ppm. Human lung cancer cells (A549), human breast cancer cells (MDA-MB-231), and human bladder cancer cells (UM-UC-3) were used to validate the biological application of the impedance cytometer. A549, MDA-MB-231, and UM-UC-3 cells were cultured in media (F-12k, L15, and MEM, Thermo Fisher Scientific) containing 10% fetal calf serum (Thermo Fisher Scientific) and 1%...
penicillin–streptomycin (Thermo Fisher Scientific) at 37 °C using an incubator (Forma 381, Thermo Fisher Scientific) containing 5% carbon dioxide, respectively. After incubation with 0.25% trypsin–EDTA solution (Thermo Fisher Scientific) for 3 min and centrifugation at 1000 rpm for 5 min, A549, MDA-MB-231, and UM-UC-3 cells were resuspended in PBS buffer to prepare cell suspensions, respectively.

2.4 Numerical simulation
To study the effect of the initial particle distribution on focusing positions, the lattice Boltzmann method (LBM) combined with the finite element method (FEM) and the immersed boundary method (IBM) was used to simulate particle focusing in the microchannel. The LBM–FEM–IBM can calculate the interaction between particles and fluids in real time and provide complete and reliable information on particle motion. More details on the LBM–FEM–IBM can be seen in our previous work. The numerical simulation of the co-flow of the sheath fluid and the sample fluid in the microchannel was performed using COMSOL Multiphysics 5.6. The visualization of sheath fluid compression was achieved by coupling the diluted species transport and laminar flow modules. The electric field distribution in the detection area was also simulated using COMSOL Multiphysics 5.6. The cell model was simplified into a sphere containing multiple layers of materials, including the cell membrane, cytoplasm, nuclear membrane, and nucleoplasm from the outside to the inside. By analyzing the electric field distribution under the interference of cells with different heights, the impact of the cell position on the impedance signal was studied.

2.5 Experimental setup and data analysis
The movement of particles and cells within the microchannel was observed and photographed using an inverted microscope (IX71, Olympus) and a high-speed camera (Phantom V611, Vision Research). Two identical syringe pumps (Legato270, KD Scientific) were used to inject the sample fluid and sheath fluid, respectively. The electrodes were connected to an impedance analyzer (HF21S, Zurich Instruments), and the generated AC signal was amplified using a trans-impedance amplifier (HF2TA, Zurich Instruments). The discrete images were stacked to characterize particle/cell trajectories using the image processing software ImageJ (National Institutes of Health, USA). The detected impedance signals were processed using self-written Python scripts. The peaks of the multi-frequency amplitude and phase shift signals were extracted to calculate the amplitude opacity and phase opacity of single cells. The machine learning model was trained using a logistic regression (LR) algorithm with a sigmoid function. The data were divided into two parts. 70% of data were used for training the machine learning model and 30% were used for testing the accuracy of the model.

3. Results and discussion
3.1 Particle trajectories of 3D inertial focusing
The inertial focusing of particles in microchannels has been extensively investigated. For example, particles within a symmetrical serpentine microchannel are typically focused at two equilibrium positions in the vertical direction of the microchannel. We attempted to reduce the number of particle focusing equilibrium positions by adding vertical injection of the sheath fluid. However, the relationship between the equilibrium position and the initial cross-sectional positions when entering the microchannel is rarely reported. In order to answer the above question, we employed numerical simulation methods to demonstrate the co-flow of the sample fluid and sheath fluid and analyze particle focusing trajectories. Fig. 2a shows the flow of the sample fluid under the vertical compression of the sheath fluid. The flow rate ratio was set to 1 : 1. It was found that the sheath fluid applied an overall downward force on the sample fluid and then flowed downstream in a laminar flow with the sample liquid. It can be inferred that the particles would be distributed in the lower layer of the channel along with the sample fluid and enter the subsequent serpentine channel. Therefore, we tracked the position distribution of particles at different initial positions in the lower layer of the serpentine channel after focusing and their migration trajectories during the focusing process, as depicted in Fig. 2b and S3, respectively. Under the action of the inertial lift force and Dean drag force, the particles migrated to the equilibrium position in the lower layer of the channel instead of passing upward through the central plane, achieving 3D single-line focusing. The process of particles from dispersion to focusing can be seen in Video S1. The size independence of 3D single-line focusing was demonstrated by the position distribution of particles of different sizes (10 μm, 12.5 μm, and 15 μm) in the horizontal and vertical directions at initial positions and after focusing, as depicted in Fig. 2c and d. At the same time, the particle focusing in the designed microchannel was experimentally verified. To more intuitively demonstrate the 3D single-line focusing, the stacked particle trajectories in the vertical and horizontal directions were displayed simultaneously, as shown in Fig. 2e and f. Exactly consistent with the simulation results, the 10 μm and 15 μm particles were compressed to the lower layer of the channel via the sheath fluid, and finally realized 3D inertial focusing under the action of the inertial lift force and Dean drag force induced by the serpentine microchannel.

3.2 Characterization of focusing performances for particles with a wide size range
Next, to verify the focusing ability of the impedance cytometer on particles with a wide size range, we further expanded the particle size range to 7–20 μm, which encompassed the majority of cell sizes (such as red blood cells, white blood cells, circulating tumor cells, etc.). The focusing trajectories of 7–20 μm particles at different flow
rates are shown in Fig. 3a. The flow-rate ratio of the sample fluid and sheath fluid was set to 1:1 and the total flow rate was gradually increased from 100 μL min⁻¹ to 500 μL min⁻¹. Since the inertial lift force and Dean drag force are positively correlated with the flow rate and particle size, particles will be subjected to greater fluid hydrodynamic forces with the increase of the flow rate. However, as the flow rate increases, the mixing effect of the secondary flow will also become more pronounced. Therefore, the 7 μm particles underwent a series of focusing processes with the increase of the flow rate: band focusing under insufficient force, single-line focusing under force equilibrium, and double-line focusing under the disturbance of the mixing effect. Large-sized particles are subjected to greater fluid hydrodynamic forces and have a stronger anti-interference ability against mixing effects. Therefore, as the particle size increased, the focusing state of the particles gradually exhibited stable single-line focusing. The optimal total flow rate range to achieve single-line focusing of all tested particles was clearly found to be 200–300 μL min⁻¹.

Although Fig. 3a presents a perfect visualization of single-line focusing, it is difficult to derive the precise focusing efficiency of particles from the images. Therefore, we quantified the particle focusing efficiency by measuring the peak amplitude of the particle impedance signals. Since the focused particles passed through the detection area at the same cross-sectional position and generated identical impedance amplitudes (for particles of the same size), it was believed that the discrete peak amplitudes originated from the unfocused particles, as shown by the red circles in Fig. 3b. Therefore, the single-line focusing efficiency of particles at different flow-rate ratios could be characterized by statistically measuring the peak amplitude of particle impedance signals, as shown in Fig. 3c and d. It was found that the sheath fluid played a crucial role in single-line focusing of particles. In addition, appropriately increasing the flow rate ratio between the sheath fluid and the sample fluid might increase the stability and efficiency of particle focusing. Finally, 96.87% of 10 μm particles and 97.21% of 15 μm particles were effectively focused at the sample flow rate of 100 μL min⁻¹ and the sheath flow rate of 150 μL min⁻¹.

3.3 Detection with a high signal-to-noise ratio and low coefficient of variation

Due to the rapid attenuation of the electric field generated by the coplanar electrode along the microchannel height, the focusing position of the particles in the vertical direction had a severe impact on the impedance signals. To study the relationship between the particle position within the detection area and the impedance signal, the electric field distribution under the interference of particles with different heights was simulated numerically, as shown in Fig. 4a. The particle position in the x-axis was fixed in the middle of the
electrodes because the particle had the greatest obstruction to the current when it was located between the two electrodes. The voltage was applied to the middle electrode, and the feedback current was obtained from the ground electrodes on both sides. The magnitude of the differential current of the electrodes on both sides was representative of the impedance signal intensity of the particle. The differential current generated by the particles positioned at different heights is shown in Fig. 4b. It was found that the particle height was negatively correlated with the magnitude of the measured differential current. Therefore, to obtain a high SNR impedance signal, it is essential to regulate the particle positions within the detection region to be in close proximity to the electrode.

To quantify the advantages of our impedance cytometer, a comparison was made between the measured particle impedance signals obtained using three focusing methods. In Fig. 4c, the distributions of 10 μm particles within the detection area in the vertical direction are presented, and the measured impedance signals using the three focusing methods are provided. The peak amplitude distribution of the particle impedance signals is shown in Fig. 4d and S4†. Consistent with the above simulation results, the measured signal peak decreased as the particle height increased. The average peak amplitudes measured by elasto-inertial focusing, traditional 2D inertial focusing, and our 3D inertial focusing were 78.94, 117.23, and 186.66, respectively. The converted SNRs were 9.43 dB, 11.15 dB, and 13.17 dB, respectively. Compared with elasto-inertial focusing and traditional 2D inertial focusing, it was observed that the signal measured by our 3D inertial focusing exhibited a higher SNR (SNR = 13.17 dB) and a lower CV (CV = 0.079). In addition, 7 μm particles were also tested (see Fig. S5†). It was found that the signals of 7 μm particles at the channel center and in the vicinity of the upper wall were very weak and even obscured by the background noise when using elasto-inertial focusing or traditional 2D inertial focusing. In contrast, the SNR and CV of the 7 μm particle signal measured by our 3D inertial focusing were still at a high level (see Fig. S6†). In addition, for elasto-inertial focusing, the 7 μm particles showed a certain defocusing phenomenon, while the 15 μm particles showed double-line focusing in the top view (see Fig. S7†). The above results demonstrate that our impedance cytometer is capable of acquiring signals with a high SNR.
and high uniformity, allowing for the high-accuracy detection and analysis of particles with a wide size range.

### 3.4 High-accuracy electrical characterization and identification of tumor cells based on the 3D inertial focusing impedance cytometer

After verifying the excellent detection performance, our impedance cytometer was used for the electrical characterization and identification of tumor cells. Three different tumor cells (A549, MDA-MB-231, and UM-UC-3 cells) were sequentially injected into the impedance cytometer. Under the combined action of the sheath fluid and inertial effect, all three types of cells were focused to a single equilibrium position close to the electrodes and passed through the detection area one by one (see Fig. 5a). Fig. 5b shows the impedance amplitude of cells at three different frequencies (0.5, 2, and 12 MHz). The dielectric properties of single cells were characterized by extracting three peaks of the impedance amplitude produced by the cells. First, the peak amplitude of 0.5 MHz was used to obtain the number and size characteristics of cells. The number of cells was expressed by the peak number detected. The diameter of the cell was calculated by calculating

$$D_{\text{cell}} = \frac{K}{\sqrt{|Z_0.5\,\text{MHz}|+C}}$$

(\text{where } |Z|_{0.5\,\text{MHz}} \text{ is the peak amplitude of the cell at 0.5 MHz, and the parameters } K \text{ and } C \text{ can be obtained by linear fitting data}).^28$$

A strict linear relationship between the peak amplitude and particle diameter is shown in Fig. 5c. It was found that there was a large overlapping area in the size distribution of the three types of cells, especially between A549 and UM-UC-3 cells. Therefore, the accurate identification of cells cannot be achieved based on size differences.

Next, amplitude opacity was calculated to further investigate differences in dielectric properties between tumor cells. The 1st amplitude opacity distribution and the 2nd amplitude opacity distribution were obtained by calculating $|Z|_{2\,\text{MHz}}/|Z|_{0.5\,\text{MHz}}$ and $|Z|_{12\,\text{MHz}}/|Z|_{0.5\,\text{MHz}}$. The distribution of the 1st amplitude opacity of the three different tumor cells is illustrated in Fig. 5d. UM-UC-3 cells exhibited significantly larger 1st amplitude opacity than A549 and MDA-MB-231 cells. In addition, due to the relatively small size, the scatter plot of the 1st amplitude opacity versus the size of UM-UC-3 was distributed in the upper left corner, which allowed UM-UC-3 to be well distinguished from A549 and MDA-MB-231 cells, especially MDA-MB-231 cells (see the black line in Fig. 5e). Regrettably, a large overlapping region persisted in the 1st amplitude opacity of A549 and MDA-MB-231 cells. For the 2nd amplitude opacity (see Fig. 5f and g), although the overall distribution trend of the three cells was similar to the 1st amplitude opacity, the difference between A549 and MDA-MB-231 cells decreased, whereas the difference between A549 and UM-UC-3 cells as well as the difference between MDA-MB-231 and UM-UC-3 cells increased. This implied that it was easier to identify A549 and MDA-MB-231 using the 2nd amplitude opacity. In addition, we also analyzed the impedance signals of cells under traditional 2D inertial focusing. The distributions of cell size and 1st amplitude opacity under traditional 2D inertial focusing are illustrated...
in Fig. S9 and S10.† Due to the attenuation of the electric field in height, cells focused on the upper side of the channel were measured to have a smaller electrical size. And due to the heterogeneity of the cell size, large cells on the upper side and small cells on the lower side might have the same signal peak amplitude. This results in a multimodal distribution of cell diameters (see Fig. S9†), rendering it impossible to measure their actual diameter. Furthermore, the high-frequency current might be unable to traverse the cell membrane of small cells focused on the upper layer of the channel, resulting in abnormal amplitude opacity (see the red box in Fig. S10†). Compared with traditional 2D inertial focusing, our 3D inertial focusing provided a guarantee for the high-accuracy characterization of the electrical properties of single cells.

When cells pass through an AC electric field, alterations in both the amplitude and phase of the impedance signal occur. Therefore, we also extracted the peaks of the phase shift (θ) produced by the cells at three frequencies. The distributions of 1st and 2nd phase opacities were determined through the calculations of θ_{2 MHz}/θ_{0.5 MHz} and θ_{12 MHz}/θ_{0.5 MHz}, respectively. The phase opacities of the three tumor cells are shown in Fig. 6a and b. The 1st and 2nd phase opacities of UM-UC-3 cells were significantly different
from those of MDA-MB-231 cells, but they were both covered within the phase opacity range of A549 cells. Therefore, compared with A549 cells, UM-UC-3 cells were easier to identify from MDA-MB-231 cells. In addition, neither the 1st phase opacity nor the 2nd phase opacity achieved effective differentiation between A549 and MDA-MB-231 cells.

Finally, machine learning was used to achieve the identification of A549, MDA-MB-231, and UM-UC-3 cells. The cell diameter, two amplitude opacities, and two phase opacities were divided into three parameter groups to train three different machine learning models. Fig. 6c shows the confusion matrix of the machine learning results trained based on parameter group 1 of cell diameter, 1st amplitude opacity, and 1st phase opacity. In the identification of two types of tumor cells between A549 and MDA-MB-231 cells, the identification accuracy of A549 and MDA-MB-231 cells was only 69.6% and 65.0% due to their large overlapping regions (see Fig. 5d and 6a). In contrast, a better identification was achieved between UM-UC-3 cells and A549 cells (>88%) as well as between UM-UC-3 cells and MDA-MB-231 cells (>91.2%). Based on parameter group 2 of cell diameter, 2nd amplitude opacity and 2nd phase opacity, the identification results of two types of cells are shown in Fig. 6d. As in the previous analysis, the 2nd amplitude opacity enhanced the identification of A549 and MDA-MB-231, but at the expense of a diminished accuracy in identifying UM-UC-3 cells. By merging parameter group 1 and parameter group 2 into parameter group 3, the trained machine learning model was used for identification between the three types of tumor cells. The identification accuracy of A549, MDA-MB-231, and UM-UC-3 cells was 74.8%, 71.9%, and 90.4%, respectively (see Fig. 6e). Compared with the training results using cell impedance parameters measured under traditional 2D inertial focusing (see Fig. S11†), the identification accuracy of A549, MDA-MB-231, and UM-UC-3 cells using our 3D inertial focusing was improved by 57.5%, 36.4%, and 36.6%, respectively. Due to the fact that analyzing cell differences based only on amplitude opacity and phase

Fig. 6  (a and b) Boxplot of the 1st (a) and 2nd (b) phase opacities for A549, MDA-MB-231, and UM-UC-3 cells. (c–e) Confusion matrix of machine learning results using three different parameter groups. (c) Parameter group 1 of cell diameter, 1st amplitude opacity, and 1st phase opacity. (d) Parameter group 2 of cell diameter, 2nd amplitude opacity, and 2nd phase opacity. (e) Parameter group 3 of cell diameter, 1st amplitude opacity, 1st phase opacity, 2nd amplitude opacity, and 2nd phase opacity (* and *** indicate p values less than 0.05 and 0.001).
opacity is a dimensionality reduction of the original data, searching for other parameters that can reflect the dielectric properties of cells based on the original impedance signal of cells will help further improve the accuracy of cell identification. The impedance cytometer provides a powerful tool for subsequent high-accuracy characterization of cell dielectric properties and identification of cells with a wide size range.

4. Conclusions

In this work, a 3D inertial focusing based impedance cytometer was developed by combining sheath fluid compression and symmetrical serpentine microchannel based inertial focusing for high-accuracy electrical characterization and identification of tumor cells. Through numerical simulation of the co-flow of the sheath fluid and sample fluid and particle focusing trajectories, the mechanism of 3D inertial focusing was first analyzed. Then, the efficient focusing of differently-sized particles in the size range of 7 to 20 μm was experimentally demonstrated. The ability of the impedance cytometer to obtain high SNR and low CV impedance signals was verified using both numerical and experimental methods. Finally, we tested the impedance detection of A549, MDA-MB-231, and UM-UC-3 cells. To correctly identify cell types, machine learning techniques were used based on calculated cell electrical parameters. The machine learning results trained based on the cell diameter, 1st amplitude opacity, 1st phase opacity, 2nd amplitude opacity, and 2nd phase opacity showed that 74.8% of A549 cells, 71.9% of MDA-MB-231 cells, and 90.4% of UM-UC-3 cells were correctly predicted. The impedance cytometer enables identification of tumor cells with similar size distribution without causing clogging. Benefiting from the ability to acquire high SNR and low CV impedance signals, the impedance cytometer has great potential for counting, high-accuracy electrical characterization, and identification of cells in complex biological samples.

Data availability

All relevant data are within the manuscript and its additional files.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research work is supported by the National Natural Science Foundation of China (52375562, 51875103, and 81727801), the Natural Science Foundation of Jiangsu Province (BK20190064), the ‘333’ Project of Jiangsu Province, and the Jiangsu Graduate Innovative Research Program (KYCX23_0228).

References


