Cell density detection based on a microfluidic chip with two electrode pairs

Yongliang Wang · Danni Chen · Xiaoliang Guo

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Abstract Cell density detection is usually the counting of cells in certain volume of liquid, which is an important process in biological and medical fields. The Coulter counting method is an important method for biological cell detection and counting. In this paper, a microfluidic chip based on two electrode pairs is designed, which uses the Coulter principle to detect the flow rate of liquid and count the cells, and then calculate the cell density. When the cell passes through the sensor channel formed by the electrode pair on the chip, the impedance will change between the electrodes. This phenomenon has been proved by experiments. The designed chip has the advantages of simple structure, small size and low manufacturing cost. The cell density detection method proposed in this article is of great significance to the research in the field of biological cell detection and development of related medical devices.

Keywords Two electrode pairs · Cell density · Impedance · Microfluidic

Introduction

In recent years, the medical field has developed rapidly. In the process of disease detection, it is particularly important to measure the density of cells in the blood (Hejazian et al. 2015). Traditionally, the cell density is obtained manually under a microscope (Jimbo et al. 2017; Minghao et al. 2020; Van de Geijn et al. 2016; Zeng et al. 2018; Zhang et al. 2020). The commonly used method of cell density is to calculate the number of cells in certain volume of liquid (Noor et al. 2018; Freitas et al. 2014; Pui et al. 2013; Smadi et al. 2019). This method of cell density detection requires cells to be uniformly distributed in the liquid, and then to count the cells in certain volume of sample (Alahmari et al. 2019; Drieschner et al. 2020; Falk et al. 2019; Tian et al. 2018). Commonly used methods for detecting cells are laser induced fluorescence method (Priesnitz et al. 2016; Gamarra et al. 2019; Qi et al. 2018; Riccio et al. 2019; Tamminga et al. 2016), image processing method (Imashiro et al. 2020; He et al. 2021; Yang et al. 2018; Safuan et al. 2018; Coakley et al. 2020; Ahn et al. 2018; Tran et al. 2019) and impedance method (Caselli et al. 2021; Hassan et al. 2014; Sobahi and Han 2020; Mansoorifar et al. 2019). Grishagin designed a cell counting program based on image processing, which obtains images of mammalian cell suspension in the hemocytometer component through a conventional optical microscope equipped with a web camera (Grishagin 2015). The algorithm is designed based on the ImageJ...
toolbox which can automatically count these cells. Due to the influence of cell aggregation and overlap, the number of cells detected by this method will be relatively low. Kim et al. designed an rWBC (residual white blood cell) counter based on optical devices (optical imaging sensors) to achieve high-throughput cell counting. The device has a complex structure resulting in the expensive cost to manufacture, and requires complex blood processing before detection (Kim et al. 2019). When the biological particles pass through the micropores, the resistance of the pores will increase because the insulating particles replace the conductive solution in the pores. This directly leads to a significant drop in the current passing through the hole, which is commonly referred to as the Coulter principle (Mansoorifar et al. 2019).

The early impedance flow cytometry (IFC) chips were based on the principle of Coulter counter using a DC supply. In 2002, Satake developed a silicon-based Coulter counter to detect polystyrene beads and red blood cells (RBCs) (Satake et al. 2002). Later, a four-channel parallel micro-Coulter counter was designed to simultaneously detect particles flowing through four sensing channels, and the device was capable of rapidly differentiating and counting micro-polymethylacrylate particles and Juniper pollen (Jagtiani and Zhe 2011). To avoid the polarization of metal electrodes, polyelectrolyte salt bridge-based electrodes (PSBEs) have successfully been fabricated by photopolymerization to distinguish RBCs and white blood cells (WBCs) in human blood (Kim et al. 2005). Afterwards, impedance micro-cytometry based on AC supply was established, with microelectrodes integrated into the walls of the microchannel instead of fabricating sensing electrodes at both sides of the aperture. In 2001, Morgan et al. (2007) fabricated two pairs of microelectrodes on the bottom of a microchannel and energized them with a voltage at one or more discrete frequencies. One pair is used for sensing the electric current fluctuation caused by a cell, whereas the other acts as a reference. This design can clearly detect the differentiation of beads, erythrocytes, and cells. Following that, a pair of parallel facing electrodes was proposed for impedance sensing and this electrodes design theoretically has a better performance because the electric field distribution is least divergent (Morgan et al. 2007). Then, a chip with this parallel facing electrodes is fabricated to measure the dielectric properties of RBCs (Chun et al. 2005). With these two types of electrodes: coplanar and parallel facing electrodes, microfluidic impedance cytometers have been used to analyze a wide variety of particles, human cell lines, phytoplankton, erythrocytes, and bead-labeled CD4 T lymphocytes.

Impedance measurement of live biological cells is widely accepted as a label free, non-invasive and quantitative analytical method to assess cell status. This method is easy-to-use and flexible for device design and fabrication. The Coulter counter based on impedance measurement is a powerful tool for characterizing biological particles suspended in a liquid electrolyte environment (Guo et al. 2013), and has been widely used in the analysis of particles (Jagtiani et al. 2006), human cells (Wu et al. 2013), bacteria (Zheng et al. 2007), viruses (Zwicker 2010), DNA and other biological molecules (Bayley and Martin 2000; Steinbock et al. 2010).

In this paper, a microfluidic chip with two electrode pairs structure is designed based on Coulter principle, which can realize the absolute count of cells in the sample liquid. The single-electrode pair of the chip realizes cell counting, and the two electrode pairs realize the measurement of cells flow rate. Finally, we calculate the cell density in the detected liquid according to the structure parameters of the chip. The structure of this microfluidic chip is novel, and it is also innovative to detect the density of insulating particles.

Materials and methods

Theory and methods

When we apply an AC voltage to the electrode pair, the impedance between the electrodes will change because the cell replaces the liquid medium between the electrodes. Since the cell is a kind of insulating particle, when it passes through the electrodes, the impedance change will be significant. By capturing change of the impedance, it is possible to confirm the timing of the cell passing the detection electrode and the number of cells passing the detection electrodes in a period of time. As a non-invasive, label-free electrochemical method, impedance measurements can automatically provide sensitive and quantitative results. These advantages
make impedance measurements widely used methods to study cells, especially for live cell analysis and long-time live cell monitoring.

The microfluidic chip in this paper is composed of two electrode pairs and a microfluidic channel, and there is a micro-sensing channel in the middle of each pair of electrodes. When a cell passes through the micro-sensing channel, the impedance between the electrodes will change significantly. By continuously detecting the impedance signal of the electrode pair, the change of the impedance signal can be detected when certain quantity cells (n) pass, so as to realize the cell count. The distance (s) between the two electrode pairs is fixed. The average flow rate of the cell from one electrode pair to the other one is calculated by detecting the time difference (∆t) of the same cell flowing through the two electrode pairs. The average flow rate of the cell represents the average flow velocity of the liquid in this period of time (v). The width (d) and height (h) of the microchannel are known. The total time from the first cell passing through the first electrode pair to the last cell passing through the second electrode pair is denoted as t. According to the above principle, the cell density can be calculated. The formula for density (ρ) is as follows:

\[ \rho = \frac{n}{vdht} \]  

(1)

\( \bar{v} \) represents the average flow velocity of n cells, and as follows:

\[ \bar{v} = \sum_{i=1}^{n} v_i \]  

(2)

\( v_i \) represents the average flow velocity of each cell between the two electrode pairs, and as follows:

\[ v_i = \frac{s}{\Delta t_i} \]  

(3)

\( \Delta t_i \) represents the time difference between successive cells passing through two electrode pairs.

Design and fabrication of the chip

The diagram of the designed chip is shown as Fig. 1. The chip utilizes ultraviolet lithography technology to form metal patterns and microchannel patterns on the substrate. The metal pattern is formed on the glass substrate using AZ-5214 (Micro-Chemicals, Ulm, Germany) photoresist. The technological process is shown in Fig. 2a. (1) After preheating the glass substrate on the heating plate at 110 °C for 120 s, remove the glass substrate and cool it to room temperature and fix it on the turntable of the spin coater; (2) Take 3 ml of AZ-5214 photoresist and pour it onto the glass surface. First, spin at 500 rpm for 30 s with acceleration of 100 rpm/s, and then spin at 1000 rpm for 10 s with acceleration of 200 rpm/s. Let it stand for 5 min; (3) Remove the glass substrate, place it on the heating plate and bake it at 110 °C for 90 s; (4) Expose the glass substrate with the photomask for electrodes via a photoetching machine at the exposure energy of 16.7 mJ/cm² for 8 s; (5) Immerse the exposed glass substrate in the AZ developer (Micro-Chemicals, Ulm, Germany) for 40 s; (6) After cleaning the glass substrate with deionized water for 30 s, blow it dry with filtered, pressurized nitrogen (do not leave water marks); (7) Post-baking the patterned glass substrate with a 70 °C heating plate for 120 s; (8) A layer of Cr (adhesion layer) is first plated on
the patterned glass surface with a sputtering apparatus, and then a layer of Au is plated; (9) Soak the metal-plated glass substrate in acetone, and use low-power ultrasonic to wash away the metal on the photoresist other than the metal patterning. Through this process, a metal electrode can be obtained, as shown in Fig. 3a. We adopt SU-8 2015 photoresist (MicroChem Corp, MA, USA) to obtain the convex microchannel pattern on the silicon wafer. According to the SU-8 2015 user manual, the exposure energy required for lithography of 50 mm thick pattern is 160 mJ/cm², the exposure energy of the lithography machine is 16.7 mJ/s cm², and the exposure time is calculated as 9.6 s. We place the photomask for microchannels directly over the wafer before exposure. The elastomeric material polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning, USA) mixture, with an adequate mix in the ratio of cross-linker/curing agent A:prepolymer B = 1:10, was degassed and poured onto the SU-8 molds. Then heat it with a 115 °C hot plate for 30 min to completely cure the PDMS. After separating the PDMS from the silicon wafer, a microchannel structure is formed, which is shown in Fig. 3b. Place the glass substrate with electrodes in

![Fig. 2](image1.png)  
**Fig. 2** Process flow for chip fabrication: **a** electrode pair manufacturing process; **b** micro flow channel manufacturing process

![Fig. 3](image2.png)  
**Fig. 3** Cell density detection chip process diagram: **a** glass layer with metal electrode pair; **b** PDMS layer with micro sensor channel; **c** bonding diagram of PDMS Microchannel and metal electrode pairs (alignment of bonded PDMS layer and glass substrate); **d** physical image of chip; **e** the structure diagram under the microscope of the chip
the PLASMA cleaning machine (Harrick, NY, USA) for surface treatment for 1 min, and then fix the glass substrate on the stage of the inverted microscope (Nikon Corp. Ti2-U, Japan), so that the microchannel structure on the PDMS can be accurately bonded to the glass sheet with metal electrodes. The complete chip is shown in Fig. 3c, the actual chip produced is shown in Fig. 3d, and the chip structure under the microscope is shown in Fig. 3e.

The structure parameters of the chip are as follows: the distance between the inlet of the chip and the outlet of the chip is 24 mm, the width (d) of the main channel is 200 mm, and the height (h) of the micro-channel is 50 mm according to the manufacturing process; Micro-sensing channel 1 and micro-sensing channel 2 have the same structural parameters. Their width is 30 mm, length is 15 mm, and the center distance (s) between the two electrode pairs is 6 mm; The structural parameters of the electrode pair 1 and the electrode pair 2 are the same, the distance between the positive and negative electrodes of the electrode is 1.1 mm, and the center distance between the electrode pair 1 and the electrode pair 2 is 6 mm.

Experiment setup

The experimental setup mainly includes an impedance analyzer (Made in Switzerland, HF2-DEV1285), an impedance analysis software (ziControl) on the PC and a microfluidic chip. The schematic diagram of the device configuration is shown in Fig. 4. This type of testing equipment can collect the impedance signals of two channels at the same time. Through the impedance analysis software, we can obtain the impedance signals of the two electrode pairs (electrode pair 1 and electrode pair 2) on the chip. The software can also store the impedance data of the two channels and time data corresponding to each impedance signal. In order to inject liquid into the microfluidic chip at a steady flow rate, we use a syringe pump (LongerPump, LSP01-2A) to inject the liquid mixed with cells into the chip.

Cell culture

The experiment used HL60 cells (human promyelocytic leukemia cells), which were cultured in suspension in cell culture medium (RPMI 1640 + 10% FBS). We place the medium for culturing cells in a constant temperature (37 °C) incubator while maintaining the pH value of the incubator at 7.2–7.4 and the concentration of CO₂ at 5%. We dilute the HL60 cells solution after centrifuging and removing the cell suspension according to the requirements of the cell concentration in the experiment.
Results and discussion

Cells count and flow velocity measurement

Experiments were performed with 20 mm fluorescent microbeads (Tianjin Base Line Chrom Tech Research Centre) to verify the cell counting efficiency of the chip and the liquid flow rate measurement efficiency. After connecting the experimental device as shown in Fig. 4, set the injection speed of the syringe pump to 5 ml/min, set the impedance analyzer applying to the two electrode pairs to 1 Vpp and the frequency to 500 kHz. When the microbeads continuously pass through the two micro-sensing channels, electrode pair 1 and electrode pair 2 generate obvious impedance change signals, as shown in Fig. 5.

When 20 mm microbeads pass through the micro-sensing channel, they will cause impedance change. The width of the sensing channel will affect the detection results. For this, we make micro-sensing channels with different widths (25 mm, 30 mm, 35 mm, 40 mm) to optimize the parameter. Set the injection speed of the syringe pump to 5 ml/min, set the impedance analyzer applying to the two electrode pairs to 1 Vpp and the frequency to 500 kHz. The chip parameters are optimized according to the impedance change of the microbeads passing through the micro-sensing channels of different widths. As shown in Fig. 6, the change of impedance decreases as the width of the micro-sensing channel increases. However, when the width of the micro-sensing channel is 25 mm, the microbeads will block the micro-sensing channel with high probability. Therefore, the micro-sensing channel width of 30um is the best parameter for chip design.

Save the data collected by electrode pair 1 and electrode pair 2, and perform data processing to obtain the impedance value of the two electrode pairs and the time difference (Δt) between the microbeads passing through the two electrodes in turn. By detecting the peak value in the impedance data and the time data corresponding to the peak value, the liquid flow rate (v_l) is obtained according to formula (3).

HL60 cell experiments prove that the cell counting efficiency of the chip and the measurement efficiency

Fig. 5 Microbead resistance signal detection diagram

Fig. 6 The optimization analysis of the chip micro-sensing channel width

Fig. 7 HL60 resistance signal detection diagram
of the liquid flow rate are important. Impedance analyzer parameters remain unchanged and the injection pump speed is set to 5 ml/min. When the cell continuously passes through the two micro-sensing channels on the chip, the impedance was plotted vs. time in seconds shown in Fig. 7.

When the cell passes through the micro-sensing channel, the impedance change of the electrode is 70% of the 20 mm microbead but the signal can be detected. Because the diameter of HL60 cells ranges from 5 to 12 mm, it is generally smaller than the diameter of microbeads.

Algorithm

For the impedance data of the two channels mentioned above, the peak value of the impedance data and the time corresponding to the peak value are extracted through the multi-peak extraction algorithm, where the number of effective peak values is the number of detected microbeads/cells. The effective peaks are the obvious peaks we can see in the impedance diagram. We hope the computer can find them. Due to the drift of the impedance data, the peak value cannot be extracted directly by setting the threshold of the impedance peak value. Using the well-known zero-derivate method. Due to the noise, which is always there in real-life signals, accidental zero-crossings of the first derivative occur, yielding false detections. The typical solution is to smooth the curve with some low-pass filter, usually killing the original signal at the same time. The result is usually that the algorithm goes horribly wrong where it’s so obvious to the eye. But we realize that a peak is the highest point between “valleys”. What makes a peak is the fact that there are lower points around it. Besides, we require a difference of at least X (a value) between a peak and its surrounding in order to declare it as a peak (Li et al. 2021). Same goes with valleys. This paper uses this strategy to find the highest point: there are points below it on both sides around the highest point. At the same time, the detection time corresponding to the effective peak point is obtained.

As shown in Fig. 8, part of the peak data is extracted from a set of impedance data. Calculate the slope of each point on the curve. According to the change of slope (the slope of peak and valley points is zero), three peak points (P1, P2, P3) and four valley points (V1, V2, V3, V4) can be identified from the curve. The difference between the effective peak point P2 and the valley points V2 and V3 is much larger than the difference between the noise peak points P1 and P3 and the corresponding valley. Therefore, the effective peak point P2 is extracted as the target peak point, and it can be determined that P2 is the only peak point in the current data.

Microbeads experiment results

Through the microfluidic chip, as shown in the results of Fig. 9, the number of microbeads can be detected as 10, the detected flow velocity is in the range of 0.006 m/s to 0.011 m/s, and the average flow velocity ($\bar{v}$) is 0.0093 m/s. The total time (t) for all microbeads to flow through the detection chip is 8.47 s. From this, it can be calculated that the average density of microbeads in the liquid is $1.270 \times 10^4$ beads/ml. The experiment was repeated three times, and the average density was calculated to be $1.492 \times 10^4$ beads/ml. The original density of the microbeads is $1.62 \times 10^4$.
beads/ml. The relative error of the detection results is 7.9%.

Cell experiment results

Through the designed microfluidic chip, as shown in the results in Fig. 10, the number of cells detected is 11, the flow velocity detected at the same time is in the range of 0.0006 m/s to 0.0012 m/s, and the average flow velocity ($\bar{v}$) is 0.001 m/s. The total time ($t$) for all microbeads to flow through the detection chip is 40.65 s. From this, it can be calculated that the average cell density in the liquid is $2.710 \times 10^4$ cells/ml. The experiment was repeated three times, and the average density was calculated to be $2.477 \times 10^4$ cells/ml. The density of HL60 cells was obtained by artificial dilution and sampling and counting under the microscope. The detected cell density was $2.89 \times 10^4$ cells/ml. The relative error of the detected results was 14.3%.

The remaining HL60 cell solution after passage was taken as sample 1, the cell solution diluted by one time was taken as sample 2, and the solution diluted three times was taken as sample 3. The dilution process is as follows: 6 ml of cell solution is divided into three equal parts and placed in three test tubes, test tube 1 is left untreated, 2 ml of culture medium is added to test tube 2, and 4 ml of culture medium is added to test tube 3. Three samples were taken and counted under the microscope to obtain the original density of cells. The average value obtained by detecting the density of the cell solution three times in succession with the chip was used as the detection result. The obtained results and relative errors are shown in Fig. 11.

Result analysis

The detection of flow rate through this chip has certain limitations, which is requiring cells to pass through two electrodes in sequence. Once the cells overlap as getting through the electrodes, it will likely lead to missed detection, resulting in lower cell count and flow rate measurement results. However, by comparing the detection signals of microbeads and HL60 cells, it can be seen that when cells of different sizes
pass through the electrodes, the impedance peak signals are different. On the premise of ensuring that the particles can pass through the micro-sensing channel, the larger the diameter of the particles, the larger the impedance change. If the impedance change caused by the same kind of particles passing through the micro sensing channel is large, it can be considered that the particles are stuck together. Based on this conclusion, the algorithm can be improved later to solve the problem of missed detection caused by overlapping cells.

The detected cell density and microbead density are lower than the actual density because during the experiment, due to the slow injection speed of the syringe pump, microbeads and cells are easily deposited on the inner wall of the syringe and injection pipe, resulting in the detection result being lower than the actual density.

**Conclusion**

This paper designs a microfluidic chip based on Coulter principle that uses two electrode pairs to detect cell density. The chip adopts two electrode pairs and a micro-sensing channel structure to realize cell counting and liquid flow rate detection, hence obtain the cell density of the liquid to be measured. The microbead experiment and the HL60 cell experiment verified that the method can achieve cell density detection and the relative error of the detection results is within 15%.

The designed microfluidic chip can be used to assist biological cell experiments, helping researchers detect the current density of cultured cells. In addition, it can also be applied to cell counting in blood, and cell density detection. This technology will advance the development of the biomedical field.

**Author contributions** YW and XG: contributed to the study conception and design. Material preparation, data collection and analysis were performed by YW and DC. The first draft of the manuscript was written by YW and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Declarations**

**Conflict of interest** The authors have no relevant financial interests in the manuscript and no other potential conflicts of interest to disclose.
References

Ahn D, Lee J, Park T, Moon S (2018) Human-level blood cell counting on lens-free shadow images exploiting deep neural networks. Analyst 143(22):5380–5387

Alahmari SS, Goldgof D, Hall L, Phoulady HA, Patel RH, Mouton PR (2019) Automated cell counts on tissue sections by deep learning and unbiased stereology. J Chem Neuroanat 96:94–101

Bayley H, Martin CR (2000) Resistive-pulse sensing from microbes to molecules. Chem Rev 100(7):2575–2594

Caselli F, Ninno AD, Businario RRL, Bisegna P (2021) A bayesian approach for coincidence resolution in microfluidic impedance cytometry. IEEE Trans Biomed Eng 68(1):340–349

Chun H, Chung TD, Kim HC (2005) Cytometry and velocimetry on a microfluidic chip using polyelectrolytic salt bridges. Anal Chem 77(8):2490–2495

Coakley A, Orlowski TJ, Muhlbaier A, Moy L, Speiser JJ (2020) A comparison of imaging software and conventional cell counting in determining melanocyte density in photodamaged control sample and melanoma in situ biopsies. J Cutan Pathol 47(8):675–680

Drieschner T, Ostertag E, Boldorini B, Lorenz A, Rebner K (2020) Direct optical detection of cell density and viability of mammalian cells by means of UV/VIS spectroscopy. Anal Bioanal Chem 412(14):3359–3371

Falk T, Mai D, Bensch R, Çiçek Ö, Abdulkadir A, Marракхи Y, Böhm A, Deubner J, Jäckel Z, Seiwald K, Dovzhenko A (2019) U-Net: deep learning for cell counting, detection, and morphometry. Nat Methods 16(1):67

Freitas AI, Vasconcelos C, Vilanova M, Cerca N (2014) Optimization of an automatic counting system for the quantification of Staphylococcus epidermidis cells in biofilms. J Basic Microbiol 54(7):750–757

Gamarra M, Zurek E, Escalante HJ, Hurtado L, San-Juan-Neves D, Freitas AI, Vasconcelos C, Vilanova M, Cerca N (2014) Microdialysis and automated cell counting in determining microfluidic devices. Analyst 139(10):3391–3392

Grishagin IV (2015) Automatic cell counting with ImageJ. IET Nanobiotechnol 9(5):69–79

Hejazian M, Li W, Nguyen NT (2015) Lab on a chip for continuous-flow magnetic cell separation. Lab Chip 14(8):1469–1476

Hejazian M, Li W, Nguyen NT (2015) Automated cell counts on tissue sections by deep learning and unbiased stereology. J Chem Neuroanat 96:94–101

Bayley H, Martin CR (2000) Resistive-pulse sensing from microbes to molecules. Chem Rev 100(7):2575–2594

Caselli F, Ninno AD, Businario RRL, Bisegna P (2021) A bayesian approach for coincidence resolution in microfluidic impedance cytometry. IEEE Trans Biomed Eng 68(1):340–349

Chun H, Chung TD, Kim HC (2005) Cytometry and velocimetry on a microfluidic chip using polyelectrolytic salt bridges. Anal Chem 77(8):2490–2495

Coakley A, Orlowski TJ, Muhlbaier A, Moy L, Speiser JJ (2020) A comparison of imaging software and conventional cell counting in determining melanocyte density in photodamaged control sample and melanoma in situ biopsies. J Cutan Pathol 47(8):675–680

Drieschner T, Ostertag E, Boldorini B, Lorenz A, Rebner K (2020) Direct optical detection of cell density and viability of mammalian cells by means of UV/VIS spectroscopy. Anal Bioanal Chem 412(14):3359–3371

Falk T, Mai D, Bensch R, Çiçek Ö, Abdulkadir A, Marракхи Y, Böhm A, Deubner J, Jäckel Z, Seiwald K, Dovzhenko A (2019) U-Net: deep learning for cell counting, detection, and morphometry. Nat Methods 16(1):67

Freitas AI, Vasconcelos C, Vilanova M, Cerca N (2014) Optimization of an automatic counting system for the quantification of Staphylococcus epidermidis cells in biofilms. J Basic Microbiol 54(7):750–757

Gamarra M, Zurek E, Escalante HJ, Hurtado L, San-Juan-Neves D, Freitas AI, Vasconcelos C, Vilanova M, Cerca N (2014) Microdialysis and automated cell counting in determining microfluidic devices. Analyst 139(10):3391–3392

Grishagin IV (2015) Automatic cell counting with ImageJ. IET Nanobiotechnol 9(5):69–79

Noor AM, Masuda T, Lei W, Horio K, Miyata Y, Namatame M, Hayase Y, Saito TI, Araî F (2018) A microfluidic chip for capturing, imaging and counting CD3+ T-lymphocytes and CD19+ B-lymphocytes from whole blood. Sens Actuators B 276:107–113

Priesnitz C, Spoerber S, Garg R, Orsini M, Noor F (2016) Fluorescence based cell counting in collagen monolayer cultures of primary hepatocytes. Cytotechnology 68(4):1647–1653

Put TS, Yu C, Wong CC, Nadipalli R, Rahman A (2013) High density CMOS electrode array for high-throughput and automated cell counting. Sens Actuators B 181(3):842–849

Qi Y, Lu X, Feng Q, Fan W, Liu C (2018) An enzyme-free MicroRNA assay based on fluorescence counting of click chemical ligation-illuminated magnetic nanoparticles with total internal reflection fluorescence microscopy. ACS Sensors 3(12):2667–2674

Riccio D, Brancati N, Frucci M, Gragnaniello D (2019) A new unsupervised approach for segmenting and counting cells in high-throughput microscopy image sets. IEEE J Biomed Health Inform 23(1):437–448

Safuan SNM, Tomari MRM, Zakaria WNW (2018) White blood cell (WBC) counting analysis in blood smear images using various color segmentation methods. Meas Sci Technol 116:543–555

Satake D, Ebi H, Oku N, Matsuda K, Takao H, Ashiki M, Ishida M (2002) A sensor for blood cell counter using MEMS technology. Sens Actuators B 83(1–3):77–81

Jagtiani AV, Zhe J, Hu J, Carletta J (2006) Detection and counting of micro-scale particles and pollen using a multi-aperture Coulter counter. Meas Sci Technol 17:1706–1714

Jimbo HC, Ngongo SI, Mbassi A, Andjiga NG (2017) Novel quantitative approach for predicting mRNA/protein counts in living cells. Appl Math 8:1128–1139

Kim S K, Kim J H, Kim K P (2007) Continuous low-voltage dc electroporation on a microfluidic chip with polyelectrolytic salt bridges. Analytical Chemistry 79(20):7761–7766

Kim B, Shin S, Lee Y, Um C, You D, Un H, Choi S (2019) High-throughput residual white blood cell counter enabled by microfluidic cell enrichment and reagent-containing patch integration. Sens Actuators B 283:549–555

Li J, Guo G, Duan F et al (2021) A novel self-adaptive, multiplex detection algorithm for blade tip clearance measurement based on a capacitive probe. Meas Sci Technol 32(8):1–10

Mansoorifar A, Koklu A, Beskok A (2019) Quantification of cell death using an impedance-based microfluidic device. Anal Chem 91(6):4140–4148

Minghao Z, Lingui G, Peihua Z, Zhixin C, Xinqi D (2020) Improvement of cell counting method for Neubauer counting chamber. J Clin Lab Anal 34(1):23024

Morgan H, Sun T, Green NG, Gawad S (2007) Analytical field and sensitivity analysis for two microfluidic impedance cytometer designs. IET Nanobiotechnol 1(5):69–79

Noor AM, Masuda T, Lei W, Horio K, Miyata Y, Namatame M, Hayase Y, Saito TI, Araî F (2018) A microfluidic chip for capturing, imaging and counting CD3+ T-lymphocytes and CD19+ B-lymphocytes from whole blood. Sens Actuators B 276:107–113

Priesnitz C, Spoerber S, Garg R, Orsini M, Noor F (2016) Fluorescence based cell counting in collagen monolayer cultures of primary hepatocytes. Cytotechnology 68(4):1647–1653

Put TS, Yu C, Wong CC, Nadipalli R, Rahman A (2013) High density CMOS electrode array for high-throughput and automated cell counting. Sens Actuators B 181(3):842–849

Qi Y, Lu X, Feng Q, Fan W, Liu C (2018) An enzyme-free MicroRNA assay based on fluorescence counting of click chemical ligation-illuminated magnetic nanoparticles with total internal reflection fluorescence microscopy. ACS Sensors 3(12):2667–2674

Riccio D, Brancati N, Frucci M, Gragnaniello D (2019) A new unsupervised approach for segmenting and counting cells in high-throughput microscopy image sets. IEEE J Biomed Health Inform 23(1):437–448

Safuan SNM, Tomari MRM, Zakaria WNW (2018) White blood cell (WBC) counting analysis in blood smear images using various color segmentation methods. Meas Sci Technol 116:543–555

Satake D, Ebi H, Oku N, Matsuda K, Takao H, Ashiki M, Ishida M (2002) A sensor for blood cell counter using MEMS technology. Sens Actuators B 83(1–3):77–81

Springer
Smadi OA, Al-Momani TD, Abdallat RG, Awad SI (2019) Automated identification and counting of proliferating mesenchymal stem cells in bone callus. Int J Comput Vis Robot 9(1):1–13

Sobahi N, Han A (2020) High-throughput and label-free multi-outlet cell counting using a single pair of impedance electrodes. Biosens Bioelectron 166:112458

Steinbock LJ, Otto O, Chimerel C, Gornall J, Keyser UF (2010) Detecting DNA folding with nanocapillaries. Nano Lett 10(7):2493–2497

Tammenga GG, Paulitsch-Fuchs AH, Jansen GJ, Euverink G (2016) Different binarization processes validated against manual counts of fluorescent bacterial cells. Microbiol Methods 128:118–124

Tian Y, Chen X, Liang Z, Li D, Xiong Y, Xiong P, Guan Y, Hou S, Hu Y, Chen S (2018) Microfluidic dielectrophoresis device for trapping, counting and detecting *Shewanella oneidensis* at the cell level. Biosens Bioelectron 99:416–423

Tran MV, Susumu K, Medintz IL, Algar WR (2019) Supraparticle assemblies of magnetic nanoparticles and quantum dots for selective cell isolation and counting on a smartphone-based imaging platform. Anal Chem 91(18):11963–11971

van de Geijn G-JM, van Gent M, van Pul-Bom N, Beunis MH, van Tilburg AJP, Njo TL (2016) A new flow cytometric method for differential cell counting in ascitic fluid. Cytom B 90(6):506–511

Wu Y, Han X, Benson JD, Almasri M (2013) Erratum to: micromachined Coulter counter for dynamic impedance study of time sensitive cells. Biomed Microdevices 15(2):381

Yang B, Chen B, He M, Yin X, Xu C, Hu B (2018) Aptamer-based dual-functional probe for rapid and specific counting and imaging of MCF-7 cells. Anal Chem 90(3):2355–2361

Zeng Y, Ke J, Jie L, Liu J, Li S (2018) A low cost and portable smartphone microscopic device for cell counting. Sens Actuators A 274:57–63

Zhang H, Ding W, Li S, Ya S, Qiu B (2020) On-chip analysis of magnetically labeled cells with integrated cell sorting and counting techniques. Talanta 220(315):121351

Zeng S, Nandra MS, Tai YC (2007) Human blood cell sensing with platinum black electroplated impedance sensor. In: Proceedings of the Nano/Micro Engineered and Molecular Systems, 2007 NEMS ’07 2nd IEEE International Conference

Zwicker JI (2010) Impedance-based flow cytometry for the measurement of microparticles. Semin Thromb Hemost 36(8):819–823

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