

Progress on Microfluidic Blood Cell Counting Techniques

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Highlights

The paper introduces three commonly used blood cell counting techniques, electrical impedance method, light scattering method and microscopic imaging method based on microfluidic chips, as well as their latest research progress.

Abstract

Accurately and efficiently detecting the quantity of blood cells is crucial in routine blood examination, as abnormal high or low numbers of blood cells are associated with the occurrence of various disorders. Due to inherent drawbacks, traditional blood cell analysis equipment cannot meet the demands of modern primary healthcare, particularly in terms of point-of-care testing. In recent years, the development of point-of-care testing blood cell counting equipment has been accelerated, thanks to the rapid advancement of microfluidic technology and the expanding research on blood cell counting using microfluidic chips. In this paper, we reviewed three blood cell counting methods based on microfluidic chips, electrical impedance, light scattering, and microscopic imaging, as well as the recent development and achievements in blood cell counting using microfluidic chips.

Keywords: Blood cell counting, microfluidic chip, electrical impedance method, light scattering method, microscopic imaging method

Introduction

The blood system serves important functions in the transportation of substances, maintenance of the internal environment homeostasis, and defensive protection. The blood primarily consists of plasma and blood cells. Numerous diseases that affect the human body can be reflected by changes of blood indicators. For instance, anemia is associated with an abnormally low number of red blood cells (RBCs), leukemia and liver cirrhosis are characterized by an abnormally high number of white blood cells (WBCs), and thrombosis is featured by an abnormally high number of platelets (PLTs) [1-6]. The blood routine is the most fundamental blood test item, determining the health of the blood by tracking changes in the quantity and distribution of blood cells. The RBC counts, WBC counts, WBC differential counts, hematocrit, and PLT are part of the indicators in the blood routine report, determined by a blood

cell analyzer. Blood cell analyzer has replaced all other options as a particular solution for routine blood testing due to its high accuracy. However, blood routine tests can only be conducted in hospitals due to the high cost, large size, and difficulty in maintenance of blood cell analyzers, making it inconvenient to meet the needs of point-of-care testing and other scenarios. Therefore, recent development of blood analysis equipment has been focusing on miniaturization and portability [7].

Microfluidics is an emerging technology that enables precise control of minute liquid volumes for biochemical analysis. In recent years, with the rise of microfluidic technology, microfluidic chips have become an important tool to support the development of miniaturization and portability of blood cell detection equipment due to their high bioaffinity, low sample consumption, quick reaction, and small size [8-11]. The principles of blood cell detection based on

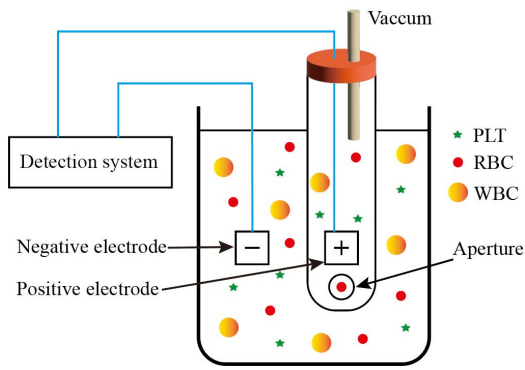


Figure 1. Schematic diagram of electrical impedance method. PLTs, platelets; RBC, red blood cells; WBC, white blood cells.

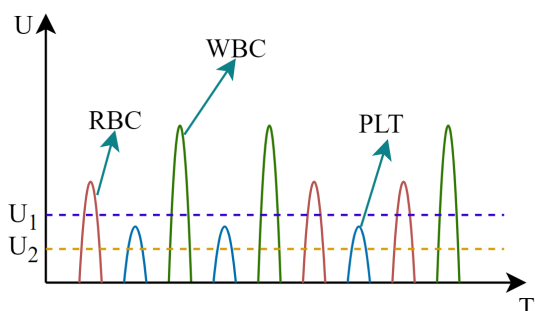


Figure 2. Principle of blood cell differential counting. PLT, platelet; RBC, red blood cells; WBC, white blood cells.

microfluidic chips include electrical impedance (Coulter's principle) and light scattering (flow cytometry principle), and the combination of the two [12, 13]. Additionally, differential counting of blood cells using image recognition has grown in popularity as a field of study with the rise of machine learning [14].

This paper discusses the above-mentioned three methods for differential counting of whole blood cells and provides an overview of the latest research progress in whole blood cell analysis utilizing microfluidic chips.

Electrical Impedance and Microfluidic Technology

Principle of electrical impedance method

During studying automatic blood detection, American scientists Wallace H. Coulter and Joseph R. Coulter Jr. came up with the electrical impedance method in 1956 [12, 15]. Therefore, this approach is also named the Coulter principle. **Figure 1** shows the schema of electrical impedance approach.

The diluted blood cell sample solution is centrifuged to ensure that the blood cells are equally

dispersed before pouring it into a non-conductive container. The blood cell sample suspension should be placed in an insulated test tube with an aperture. Then, positive and negative electrodes are placed on either side of the aperture, and power is provided by a continuous current source to create an electrically sensitive area. Due to the different conductivities of the blood cells and the solution, as the blood cells pass through the aperture under the influence of negative pressure, the voltage between the two electrodes will change, and a pulse signal will be generated [12, 16, 17]. The size of the identified particles directly affects the amplitude of the pulse signal. When non-metallic particles pass through the aperture, the voltage change can be described using Maxwell's electromagnetic field theory as [15, 18, 19]:

$$\Delta V = \frac{4\rho_e I d^3}{\pi D^4}$$

where ρ_e is the resistivity of the solution; I is the loop current; d is the diameter of the particle to be detected; D is the diameter of the aperture or the size of the electrically sensitive area. When measuring WBCs (diameter range 7-20 μm), the diameter of the aperture should be 70-100 μm , and when measuring RBCs (diameter range 6-9 μm) and PLTs (diameter range 1-8 μm), the diameter of the aperture should be about 50 μm .

Differential counting principle of blood cells

When the identified blood cells pass through the aperture, a pulse will be generated. However, the pulse amplitude is very small due to the low equivalent impedance of the cells, necessitating the amplification of the pulse signal. The amplified and shaped pulses make a voltage comparison in the threshold selection circuit, as shown in **Figure 2**. The pulse amplitude of RBC and WBC exceeds threshold U_1 in magnitude. When U_1 is used as the threshold, the number of RBCs + WBCs is counted. The amplitude of PLTs is more than U_2 's but less than U_1 's. When U_2 is used as the threshold, the total number of RBCs + WBCs + PLTs is enumerated.

In the human body, RBCs outnumber WBCs by a factor of over a thousand. All cells can be counted as RBCs when the threshold voltage is set to U_1 , and the quantity of WBCs can be ignored. When the threshold voltage is jumped to U_2 , the number of PLTs equals the second cell count minus the first cell count. The total number of the counted cells when the threshold

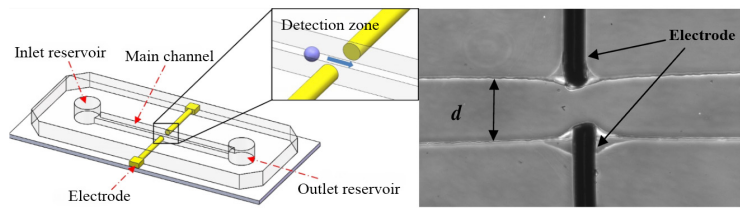


Figure 3. Microfluidic capacitive detection chip. This figure is cited from [22].

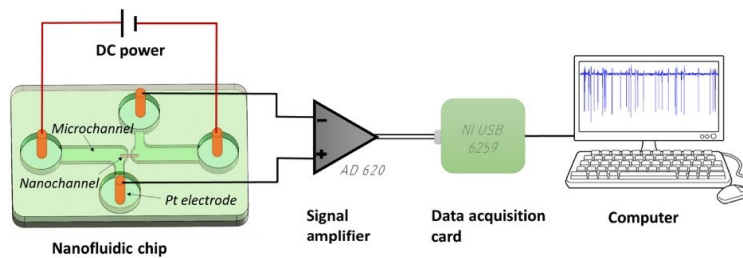


Figure 4. Schematic diagram of a microfluidic chip-based detection system by differential resistive pulse sensing. This figure is cited from [23]. DC, direct current; Pt, platinum.

voltage is U_2 represents the sum of WBC, RBC, and PLT counts. In order to perform the WBC counting procedure, the RBCs must first be dissolved with a hemolytic agent. After this, the result of voltage at U_1 is the quantity of WBCs.

Microfluidic electrical impedance for blood cell counting

Microfluidic technology, also known as lab on a chip, emerged in the 1990s. It is a microanalysis technology using the complex microchannel structure and microfluidic control to facilitate the sequential passage of blood cells through designated fluid channel at a constant speed, enabling specific experimental analysis. The fluid shows laminar flow behavior when moving through a micron-scale channel, which may precisely control the speed of the fluid and greatly improve detection efficiency. The microfluidic chip also enables integration of small samples and multiple detection items. This means that microfluidic-based systems require less blood than traditional blood cell analyzers to perform a variety of detection tasks [20, 21].

Song et al. created the microfluidic capacitive sensor device with the comparatively straightforward liquid channel shown in **Figure 3** [22]. The main channel is 30 μm high and 2 cm long. At the left and right end, there are two liquid reservoirs. On both sides, a pair of copper wire electrodes with a diameter of 25 μm are integrated. The precise procedure entails fixing the copper wire in a specific location on the chip mold before pouring polydimethylsiloxane over

it. The copper wire is fixed on either side of the main channel after the Polydimethylsiloxane has fully solidified to create a capacitance detection area. The cell suspension should be injected into the liquid inlet using a syringe pump. The capacitance between the electrodes will change as the cells move through the capacitance detection area, and the subsequent processing circuit can then count the cells.

There are a few issues with this design. First, edge effect occurs due to the lack of precise alignment between the two electrodes and the uneven distribution of the electric field between them resulting from the relatively small size of the

electrodes. Second, copper wires are not the optimal materials for electrodes because of the possible chemical reactions in humid environments, although their chemical properties are comparatively stable in a dry environment.

As shown in **Figure 4**, Peng et al. designed a microfluidic chip-based particle detection system by differential resistive pulse sensing method [23-25]. The basic idea behind this system is to power the inlet and outlet of microfluidic chip with direct current, then set up a pair of platinum electrodes on either side of the main channel to serve as detection electrodes. The detection electrodes can provide a differential signal when blood cells travel through the nanochannel in the center. Through the data acquisition card, the differential signal is differentially amplified and then fed into the computer for signal processing and cell counting. The platinum electrode can be utilized for a longer period of time and has better physical and chemical consistency than copper electrodes. Differential processing is applied to the detection signal to reduce some of the noise and raise the signal-to-noise ratio. Additionally, it should be noted that the direct current power supply voltage should not be provided at an excessive level as this would result in electrolysis of the solution, faster particle movement, etc., which will lower the signal-to-noise ratio of the system.

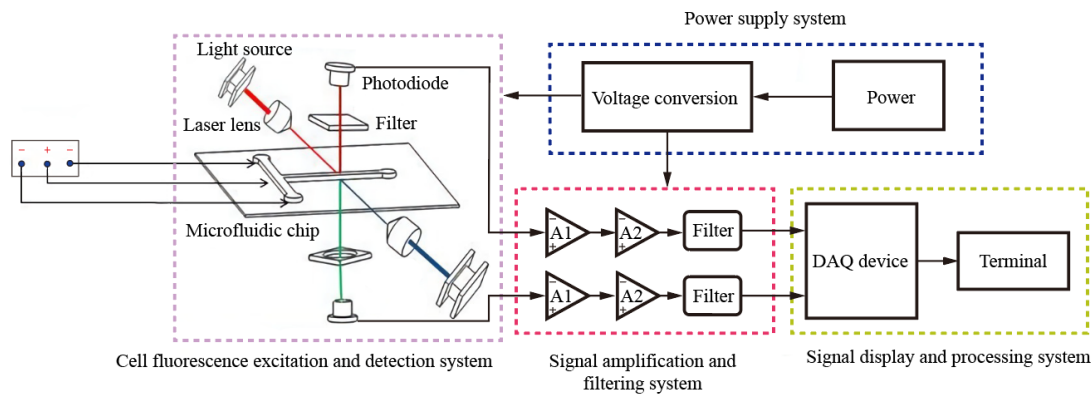


Figure 5. Schematic diagram of the microfluidic flow cytometry detection platform. DAQ, data acquisition.

Light Scattering and Microfluidic Technology

Principle of light scattering method

Light scattering, also known as flow cytometry, is a cell analysis technique that has been used to measure cell volume since the middle of the 20th century [13]. After years of innovation and improvement by scientists, the flow cytometer has evolved into the mature instrument we have today.

An optical system, a data processing system, and a liquid flow system make up a standard flow cytometer. The sample liquid is wrapped in a sheath fluid by the liquid flow system, which also speeds up cell flow, arranges the cells of sample stream separately, and moves the cells sequentially through the detecting region. The optical system consists of an excitation light source and a number of beams shaping components, with the function to concentrate the light spot of excitation light source into a single point and direct it towards the cells in the sample stream for cell identification. The computer and software that make up the data processing system are primarily used to analyze detected signals to derive usable information.

The sheath fluid of flow cytometer accelerates the labeled cells as they go across the laser beam of the device one at a time. Light is dispersed and the fluorochrome is stimulated to emit fluorescence as cells move through the laser beam. Among them, the photodiode detects the forward small-angle scattered light (FSC) and converts it into an electrical signal; after the electrical signal has been processed, the information of cell volume is reflected. Due to the weak light intensity of the side scattered light and fluorescence signals, they are respectively received by photomultiplier tubes and turned into electrical signals after being filtered

and separated by the filter system. These two signals can each represent information that presents either inside the cell or on its surface [26, 27]. The computer receives these signals and processes them using certain algorithms. The processed data are then be depicted using statistical techniques and theories, and the outcomes are shown as graphs.

This conventional flow cytometer has a very quick detection speed and can quickly and precisely identify and evaluate the data from hundreds of cells. However, the widespread use of flow cytometers is still limited by a few drawbacks, including high cost, large volume, complex operation, and difficult maintenance [28].

Microfluidic light scattering for blood cell counting

The primary goal of designing a flow cytometer based on microfluidic chips is to overcome the drawbacks of the conventional flow cytometers and achieve downsizing and portability [29]. However, other peripheral devices are still too large to be employed for practical applications, and the present flow cytometric devices based on microfluidic chips are only downsized on the chip.

Huang et al. improved the existing microfluidic flow cytometer and designed a complete and truly portable flow cytometer, as shown in **Figure 5** [28]. The following are the innovations: Firstly, the electroosmotic and pressure driving modes are integrated to create the electroosmotic induced pressure flow, which replaces the conventional pressure driving and eliminates the requirement for a large negative pressure pump in the system [30]. Secondly, in order to obtain optimal detection results, the arrangement of each device is redesigned, and

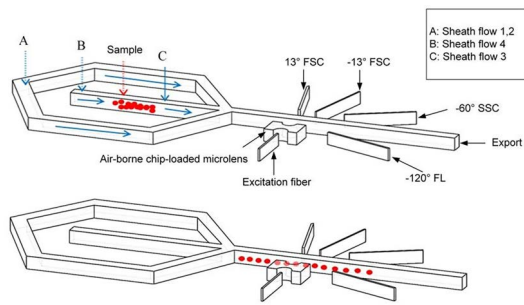


Figure 6. Schematic diagram of mFCM. This figure is cited from [31]. mFCM, microfluidic flow cytometer; FSC, forward small-angle scattered light; SSC, side scatter; FL, fluorescence

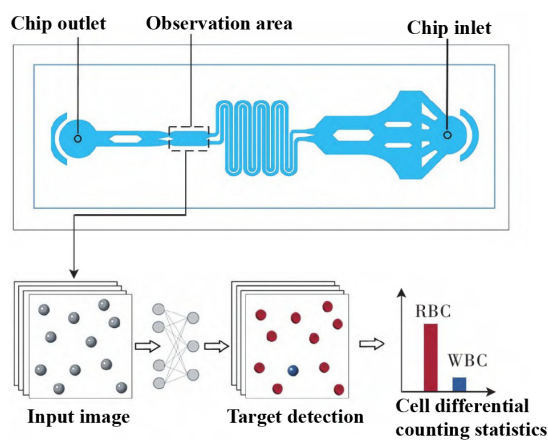


Figure 7. Microfluidic chip structure and detection process. This figure is adapted from [14]. RBC, red blood cell. WBC, white blood cell.

the laser light source is replaced with two laser diodes. Thirdly, a personal computer or smartphone is used as a terminal to receive and process signals. This allows the device to function independently in the system, enhancing portability and ease of maintenance.

The system can accurately identify fluorescent particles of 10 μm , 5 μm , 2 μm , and 900 nm with nanoscale accuracy.

Peng et al. designed a microfluidic flow cytometer for WBC analysis (Figure 6) [31]. By using three beams of sheath fluid (shown as A, B, and C in the figure) to focus the sample stream, a three dimensional hydrodynamic focusing system is created. By adjusting the flow velocities of the sheath fluid and sample fluid, blood cells can be focused both horizontally and vertically to the center of the microchannel. Additionally, a microfluidic chip with an incorporated air microlens is used to concentrate the excitation light source, along with several micron-level optical fibers for transmitting scattered light and fluorescence excitation data.

The design has reached the commercial standard for the detection of FSC, but the detection of side scattered light and fluorescence still needs to be improved, and the design of excitation light source and optical path also needs further optimization.

Microscopic Imaging and Microfluidic Technology

Cell morphology can be indirectly measured by cell analysis techniques based on electrical impedance or optical scattering. The two methods share the issue that the cells are easily overlapping, which causes the superimposition of detected signals, decreasing the accuracy of the results. Additionally, because both approaches detect cells one at a time, it is challenging to achieve high system throughput.

Directly detecting cell morphology is possible with the use of image recognition-based cell analysis. In this way, blood cell images under a microscope can be instantly converted into digital images using microscopes and computers, which can quickly process a classification count or other quantitative analysis [32, 33].

In the traditional manual method of blood cell counting, diluted blood is placed in a hemocytometer, observed under a microscope, and the number of blood cells in a specified volume is manually counted. This number is then converted into the number of blood cells per liter of blood. This approach is not only inaccurate, but also inefficient. Imaging-based microfluidic blood cell counting techniques have gained popularity in recent years. They are affordable and highly efficient, allowing for the quick detection of a large number of samples [34, 35].

Du et al. proposed a method for differential counting of whole blood cells based on microfluidics and dynamic microscopic imaging (Figure 7) [14]. The entrance of the microfluidic chip adopts a decentralized design to achieve a higher test throughput. RBCs and WBCs in the detection area are classified and counted after being combined with the target detection algorithm (YOLO V4 model) in deep learning. The photos in the data set include a wide variety of cell types, with each image containing a random number of cells ranging from 1 to 15, which are used for training the model. The image resolution is unified to 416x416. The training set includes a total of 8,000 photos (including 4,000 RBC images and 4,000 WBC images). The verification set and test set both include 1,000 photos (both including 500 RBC

Table 1. Comparison of the advantages and disadvantages of the three methods

Method	Advantages	Disadvantages
Impedance method	Simple structure, easy integration	Low flux, easy to clog
Light scattering method	Multi-parameter measurement, high accuracy	Low flux, complex structure
Microscopic imaging method	High throughput, high detection speed	Complex structure, difficulty in integration

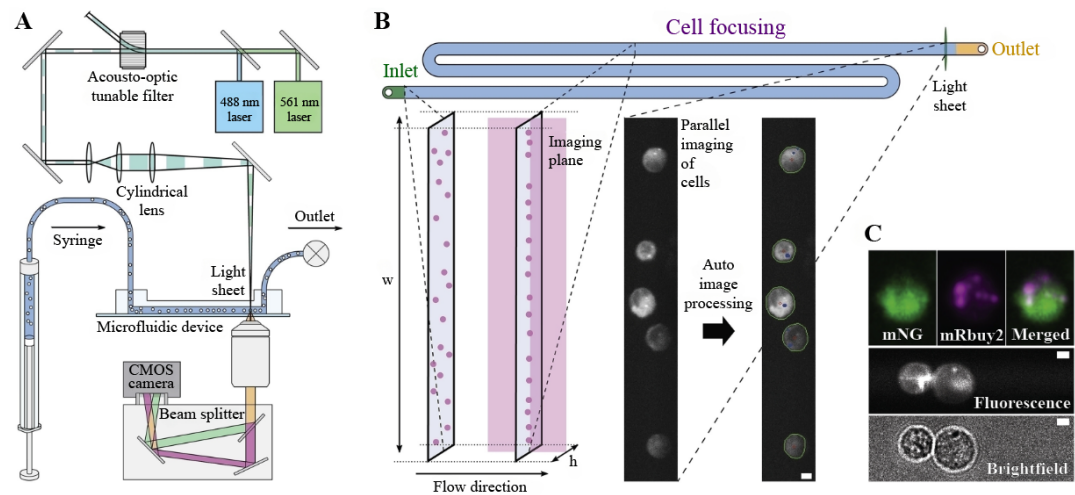


Figure 8. Schematic diagram of imaging flow cytometry detection. (A) The imaging flow cytometry platform integrates stroboscopic multi-color light sheet illumination, microfluidic cell focusing system, a dual-color beam splitter, and a CMOS camera; (B) Top view of the microfluidic channel used to position the cells in the imaging plane. The microfluidic device consists of a straight and high-aspect ratio channel with one inlet and one outlet. Through the use of a viscoelastic carrier fluid, cells can be precisely focused in the center plane of the channel. Cells are imaged upstream of the outlet using stroboscopic light sheet illumination, with image processing being used for cell identification and spot foci counting; (C) Representative dual-color fluorescence images of 293T cells expressing mNeonGreen (left, top) and mRuby2 (middle, top) tagged proteins. The corresponding merged image is shown in the top right panel. Images in the lower two panels show the simultaneous acquisition of fluorescence and bright-field images at high-throughput. Scale bars, 5 mm. This figure is adapted from [36]. CMOS, complementary metal oxide semiconductor.

images and 500 WBC images).

The RBC and WBC recognition accuracy of this method in actual blood samples reaches 99.25%, essentially consistent with the indicators of healthy adults in clinical blood routine reports.

Gregor et al. designed a high-throughput, multi-parameter imaging flow cytometer, as shown in **Figure 8** [36]. Combined with stroboscopic illumination technology, it can perform unambiguous fluorescence detection at ultra-high analytical throughput, and subcellular analysis of cells down to 500 nm.

At a magnification of 15 times, the imaging system achieves its maximum throughput, about 20,000 cells per second, and the effective pixel size is 0.43 μm . At the same effective pixel size, the best commercial imaging flow cytometers have a throughput of only 2,000 cells per

second.

Comparison of the advantages and disadvantages of the three methods

Table 1 shows a summary of the advantages and disadvantages of the three microfluidic chip-based blood cell counting techniques, electrical impedance method, light scattering method, and microscopic imaging method.

Although the electrical impedance method-based microfluidic blood cell counting device has a relatively simple structure and is easy to incorporate, its disadvantages include low throughput, a sluggish detection speed, and easy clogging. The microfluidic blood cell counting device based on the light scattering method can detect multiple parameters of the cells by measuring fluorescence and scattered light at different angles, with high accuracy. However, it needs to integrate a variety of optical compo-

nents with complicated structure, and similar to the electrical impedance method, it also requires one-by-one detection in a single cell, resulting in a limited throughput. Multiple cells can be measured simultaneously by the microfluidic blood cell counting device based on the microscopic imaging technique, which has the advantages of high throughput and quick detection speed, but it requires artificial intelligence that is currently underutilized in the embedded field. Additionally, the accuracy of current machine learning models in challenging real-world settings remains unknown, and therefore, this approach has not been considered thus far.

Conclusion

The paper covers three blood cell counting techniques, the electrical impedance method, the light scattering method, and the microscopic imaging method. Traditional blood cell analyzers are limited in their use in primary medical care and are unable to satisfy people's daily needs due to the drawbacks of their sizes, high costs, and difficulty in maintenances. On the other hand, microfluidic chip-based blood cell counting apparatus is inexpensive and portable, which makes up for the drawbacks of conventional blood cell counting equipment and can inspire the creation of portable and miniaturized equipment. Perhaps blood tests will no longer require a dedicated visit to the hospital and can be completed at home using smaller blood analysis devices in the future.

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