Review Article

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From passive to active sorting in microfluidics: A review

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Abstract: Achieving high-efficiency sorting of microfluidics (such as cells, particles, droplets, etc.) has great significance in the fields of biology, chemistry, medical treatment, material synthesis, and drug development. This paper introduces the microfluidics sorting methods in recent years. The current research status and progress can be divided into the active sorting system and passive sorting system according to whether there is an external field. They can control the microfluidics by promoting more selective separation, so as to obtain higher resolution and selection rate. In this paper, the above methods are analyzed and discussed, and the future microfluidic sorting is prospected.

Keywords: Microfluidics, active sorting system, passive sorting system, microchannel

1 Introduction

Research based on cells and droplets has greatly increased the technical requirements for separating and purifying specific samples from complex samples [1, 2]. Classification and detection of cells according to their inherent characteristics are important goals directly related to biomedical and clinical applications [3, 4]. In recent years, the development of microfluidic technology has provided a new technology platform for the separation of cells and droplets. Micron-level particles can be sorted by the laminar distribution of microfluidics on the platform [5, 6]. When sev-

eral streams of fluid are in parallel, mixing does not occur, and material communication between the fluids relies on relatively slow and inefficient diffusion [7]. Molecular transfer between them must always consider diffusion [8]. Smaller size microfluidic devices can significantly reduce the number of samples and reagents used by several orders of magnitude [9, 10]. It also reduces the amount of biohazardous waste and greatly improves biological compatibility [4]. This property is particularly important in microfluidic instruments [11, 12]. The separation and screening of micro-scale objects have broad application prospects in the fields of biomedicine [9, 13–15], physical chemistry [16], and preparation of micro-nano materials [17, 18]. Therefore, microfluidic instruments can obtain finer, more stable chemical gradients than traditional experimental conditions. Smaller size microfluidic devices can reduce the amount of sample and reagent used by several orders of magnitude. The research of microfluidic chips is to further reduce the experimental equipment and processes. The research of microfluidic chip is to further reduce the experimental equipment and process. The integration and transportation of samples or reagents in the microchannel can be realized through micropumps and valves [13].

Therefore, how to achieve precise and high-throughput sorting of microfluidics is a crucial technical means. At present, there are many methods to improve the efficiency of sorting and shorten the time scale of sorting. The sorting technology based on microfluidic chips can be divided into active sorting and passive sorting. Active sorting system relies primarily on external fields to apply force to achieve microfluidic sorting (Including electrophoretic sorting [19–21], acoustophoresis sorting [22–24], magnetic sorting [25–28] and optical sorting [29, 30]). The passive sorting system mainly includes gravity inertia analysis [31, 32], micro-scale method [33–35], deterministic lateral displacement [36] and hydrodynamic fractionation [37, 38].

Among them, active sorting technology relies on external force field to classify microfluidics. Therefore, these sorting methods are often targeted and have special requirements on the properties of microfluidics (such as electrophoretic sorting requires microfluidics to have electrical properties [39]). Passive sorting technology mainly realizes

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particle sorting by using microchannels with complex structures. Therefore, the use of microfilters or well-designed microchannel structures can achieve high-efficiency and energy-saving microfluidic separation. In addition, active and passive sorting techniques can also be combined for particle sorting [40–42].

This review mainly investigates the latest research progress of microfluidic sorting systems from two perspectives: (1) based on active sorting system; (2) based on passive sorting system. Furthermore, the research progress of microfluidic sorting technology in recent years is explored, and it is hoped that it can help the development of microfluidic sorting technology.

2 Active sorting

2.1 Electrophoretic sorting

Electrophoretic sorting is the sorting of microfluidics by applying an electric field, which can be divided into a direct current (DC) electric field and an alternating current (AC) electric field. Among them, the DC electric field is a uniform electric field, and the droplet can be sorted due

to the existence of its surface charge [43]. The AC electric field is a non-uniform electric field, and the droplets do not need to have surface charges. Different from the DC field, AC electric field can polarize the droplet. Once the droplet is exposed to an AC field, the migration and orientation at the maximum electric field strength depends on the conductivity of the droplet itself, which is called dielectrophoresis (DEP), and the dielectrophoretic force depends on the size and characteristics of the fluid. A droplet with a higher permeability than a fluid is attracted to the field maximum, which is called positive DEP (pDEP) [44], and the opposite is negative DEP (nDEP) [45]. Dielectrophoresis has little effect on microfluidics and has been widely used.

Wang *et al.* [46] developed an nDEP system with a set of fork-shaped alignment electrodes, which can provide repulsive forces on both sides of the microfluidic channel to allow tissue cells to pass through at a precise distance from the microfluidic channel wall. When the nDEP effect is added, due to the action of the electric field, the flow state of the polystyrene beads or cells in the microchannel changes and flows out from a specific outlet to achieve the purpose of sorting. Guo *et al.* [47] designed a micro-fluid sorting device for “droplet self-charging” phenomenon. The oil-in-water droplets produced by the flow focusing are in

Figure 1: (a) Photograph of the microfluidic device and electrodes, and an enlarged view of the microfluidic channel area. Adapted permission from [20]. Copyright 2019 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Schematic illustration of the device structure and its working principle showcased by particles (yellow and red) under positive- and negative-DEP. Adapted permission from [48]. Copyright 2020 the Royal Society of Chemistry.

the main flow channels with oppositely charged electrodes and across the channels that generate electric fields. When the charged droplets pass through this section, the different properties induced by the charge or switching between ions can achieve the sorting effect. Thomas *et al.* [20] used microelectrode array dielectrophoresis to flow through microfluidic channels to sort individual fluorescently labeled cells and particles. As shown in Figure 1a, a negative dielectric is used to create a “dielectrophoretic virtual channel” microfluidic channel extending along the center of the cell. The dielectrophoretic virtual channel is extended to any number of sorted outputs, and two alternative geometries are designed, simulated, and experimentally verified. By switching the polarity of the electrodes, the virtual channel can dynamically reconfigure the direct portions on different paths, and then classify the particles into two microfluidic streams that are streamed out from different outlets. The entire process is controlled by an automated system. Experimental results that the device is suitable for the separation of high-purity single particles. Based on a flow rate of 60 nl/min, the separation rate reaches 100% and the speed is up to 0.9 particles per second. In addition, it is also suitable for the separation of osteosarcoma and human bone marrow cells. However, the separation flux is small and cannot reach the flux of similar devices, and the electrode can be redesigned for improvement.

As shown in Figure 1b, Zhang *et al.* [48] demonstrated a new type of microfluidic nDEP sorting device, which is composed of 3D electrodes and a fluid structure integrated with silver-polydimethylsiloxane (PDMS) composite materials. The unique two-layer electrode forms a non-uniform electric field, which is convenient to capture the target cells drifting upward and improves the sorting efficiency. Experiments have shown that in the process of separating live/dead Hela cells, when the flow rate is 0.3 ml/h, the separation efficiency of live cells can reach 95.4%, and the purity can reach 85.6%. K. Tatsumi *et al.* [49] researched a device that manipulates and sorts particles by dielectric (DEP) force. This device is composed of electrode regions of different shapes. Trapezoidal and orbital electrodes can capture particles with nDEP in the central area of the electrode. Trapezoidal electrodes allow particles to be arranged at equal intervals in the direction of their flow, while flip-over electrodes can separate particles and combine these electrodes to improve particle separation efficiency.

2.2 Acoustrophoresis sorting

Acoustrophoresis means that the motion of an object changes with the change of sound pressure. In recent years,

the development of acoustic microfluidics has proposed a new research direction for the sorting of microfluidics. Acoustics can provide fast and accurate indications. Scattered sound waves were first applied to cell sorting by Johansson *et al.* and have since been followed by more and more scholars. Ding *et al.* [50] research shows that standing surface acoustic wave (SSAW) devices can be used as acoustic tweezers to manipulate cells and nematodes. Adjust the particle displacement by changing the pressure node of the target particle. As shown in Figure 2a, L. Ren *et al.* [51] designed an acoustic sorting system based on the SSAW method, which has the advantages of simplicity and high integration. At a flow rate of 2500 particles per second, the separation purity of the sample can reach more than 90%, and the separation efficiency can reach $94.9\% \pm 2.0\%$. The cell viability after sorting is basically unaffected, and it has excellent sorting performance in achieving high throughput and ensuring cell viability. Although its sorting speed is inferior to commercial fluorescence-activated droplet sorting (FADS), it has the advantages of small size, high biosecurity and biocompatibility. In Figure 2b, Chen *et al.* [52] designed a micro-flow cytometer based on SSAW, which can clearly identify two sets of different particles. The system can be used to focus and measure cells, showing a certain applicability to biological samples. In Figure 2c, Li *et al.* [23] designed a new type of FADS system that uses highly focused surface acoustic waves (HFSAW) with a width of approximately 50 μm to sort single cells and droplets. Acoustic waves are partially coupled in the microchannel, and the separation of cells and droplets is achieved by the micropillar waveguide structure between the channel and the interdigital transducer (IDT). The IDT in this sonic sorting device can be reused, and can achieve rapid separation of single particles. The separation purity is higher than 90% and the separation rate can reach 1 kHz. Moreover, the sorting range of the particles can be reduced to the sub-micron size, which greatly expands the demand for sorting sub-micron particles. However, sorting on the sub-micron scale is too dependent on the fluorescent signal and still has certain challenges.

2.3 Magnetic sorting

Currently, we can use magnetic fields to control microfluidics. The main advantage of magnetic control is that it is easy to operate and can achieve wireless control of droplets. However, magnetrons have certain limitations. First, they can only classify the fluid system of magnetic materials. Another disadvantage of magnetic sorting is that the response speed is slow, and the switching frequency is usually about

Figure 2: (a) Schematically shows the integrated SSAW-based FACS. Adapted permission from [51]. Copyright 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (b) Schematic of the SSAW-based microfluidic cytometer. A microfluidic device is integrated with a LIF detection system. Adapted permission from [52]. Copyright 2013 the Royal Society of Chemistry. (c) Experimental setup of the fluorescence-activated droplet sorting (FADS) system. Adapted permission from [23]. Copyright 2019 American Chemical Society.

a few hertz or lower. Droplet magnetic manipulation can be categorized according to the type of magnet used, typically an electromagnet or permanent magnet. The electromagnet is simple to operate and its field strength can be adjusted with the applied current. However, when the current is too high, the electromagnet generates excessive heat, which may be harmful to the thermosensitive biological reaction. The performance of permanent magnets is generally superior to that of electromagnet microfluidic platforms. Since Surenjav *et al.* [53] introduced electromagnets into microfluidic platforms for droplet sorting in 2009, magnetic sorting has been used in large numbers. They use ferrofluids as a continuous phase to support magnetic sorting. When the ferrofluid system flows through the microchannels, they will split into different microfluidics ac-

cording to the flow conditions and channel geometry. By applying an uneven magnetic field on both sides of the microtube. The non-uniform magnetic field causes the ferromagnetic fluid to face a stronger magnetic field, causing the lower branch of the ferromagnetic fluid, thereby achieving sorting of the droplets. The droplets enter the device one by one to avoid interference, and in the vicinity of the orbit, the magnetic field gradient increases sharply, thereby attracting the droplets containing the magnetic beads into the orbit. Li *et al.* [54] use a permanent magnet magnetic fluid to manipulate the droplets, which is different from the above case in that the ferrofluid acts as a dispersed phase. By separating the water-based ferrofluid droplets at the T-junction and directing them to the bifurcation for sorting, based on this behavior, larger droplets can be sorted. Shi *et*

Figure 3: (a) Illustration of experimental steps of CTC capture. Adapted permission from [55]. Copyright 2017 the Royal Society of Chemistry. (b) Schematic illustration of the cell separation design. Adapted permission from [56]. Copyright 2012 American Chemical Society.

al. [55] designed a wavy-herringbone (wavy-HB) structured microfluidic device (Figure 3a), which captures circulating tumor cells (CTCs) by adding specific antibodies on the surface of magnetic particles (MPs). The coverage rate of MPs is 1.664 mg/h, the flow rate of the cell suspension is 540 ml/h, and the capture efficiency can reach $92\% \pm 2.8\%$. The cell regeneration ability after sorting is similar to the original cells, indicating that the device has almost no effect on cell activity. As shown in Figure 3b, scholars have designed a viable separation platform based on the microfluidic magnetophoretic cell separation platform, which can efficiently separate rare cell populations from whole blood samples [56], experiments show that the platform can directly isolate 50 MCF-7 cells from whole blood at a flux of 240 $\mu\text{l}/\text{min}$, with a collection efficiency greater than 85% and a purity exceeding 90%. This method can achieve the simultaneous sorting of two kinds of cells, and the efficiency reaches more than 96%. Compared with other magnetic separation methods, it is simple to operate and can perform simultaneous separation, but it still needs further improvement in terms of flux. Buryk-Iggers *et al.* [57] has developed a novel microfluidic reverse magnetic separation method that encapsulates cells inside droplets to isolate the cells from the magnetic continuity.

2.4 Optical Sorting

Radiation forces generated by highly concentrated light have been applied to microfluidic sorting. The focused laser beam produces light scattering and gradient forces between cells due to different refractive indices. Gradient force can focus cells or droplets to produce optical tweezers. Sorting can be achieved by using a focused beam to apply different

radiant forces on the cells. Brzobohatý *et al.* [58] developed a fluorescence-based pulsed laser cell sorter that can achieve cell sorting. Some scholars have introduced a geometry that produces a "traction beam". Experiments showed that in addition to pulling particles, the two-dimensional motion and one-dimensional ordering can be manipulated by the polarization rotation of the linearly polarized incident beam. Xu *et al.* [59] demonstrated that the use of single-wavelength excited multi-step waveguide splitters in nanofluidic systems can achieve multi-level separation of dielectric nanoparticles. Under the excitation of optical force, nanoparticles of different sizes can be classified and derived from different ports. As shown in Figure 4a, Sun *et al.* [60] developed a large lensless chip trap (OTOC). When the particles pass through the optical lattice generated by OTOC, the flow direction of the particles is strongly deflected, this deflection is caused by the interaction of the optical gradient force with the external driving force in the laminar flow. When the laser output power is 800 mW, the mixed particles of two sizes are uniformly translated through the optical lattice at a speed of about 10 μm s. The particles with large sizes are obviously deflected, but the small particles have not deviated from the original direction of movement. Therefore, it is possible to sort mixed particles of different sizes. But the sorting efficiency is poor, and the input power of the laser also needs further study. The use of optics and fluid dynamics can realize the classification of nanoparticles (NPs), the laser beam separates the NPs restricted by hydrodynamic focus. Wu *et al.* [61] realized the sorting of NPs of different sizes as seen in Figure 4b. For the 50/100 nm NPs combination, the sorting purity is $\geq 92\%$, for the 100/200 nm group, the sorting purity is $\geq 86\%$, and the sorting throughput is 300 particles/min.

Figure 4: (a) Schematic of the OTOC system. Adapted permission from [60]. Copyright 2007 AIP Publishing. (b) Schematic of the sorting process of nanoparticles with different radii in fluid. Adapted permission from [61]. Copyright 2016 American Chemical Society. (c) Schematic of the trapping of a single Ag NP in the optical line near the PDMS surface. Adapted permission from [62]. Copyright 2018 American Chemical Society.

For the sorting of gold nanoparticles with a small size difference (50 nm and 70 nm), the throughput rate is lower than 100 particles/min. This has promoted the development of nanoparticle processing technology. Nan and Yan [62] designed an adjustable photofluidic potential well for nano-processing through the combination of optical phase gradient force and fluid resistance, which improved the resolution of the device (Figure 4c). When the laser power is

400 mW and the fluid velocity is about $175 \mu\text{m/s}$, the optical trap with adjustable phase gradient is used to sort 80 nm and 100 nm Au NPs. And through further simulation, it can be predicted that this device can achieve sub-50 nm NPs optical sorting with a super-resolution of 1 nm.

3 Passive sorting

3.1 Inertial

In addition to the above-mentioned methods of using an external pump for sorting, the separation of droplets can also be achieved depending on the fluid properties of the microfluidics and the geometry of the microchannels. Inertial force can deviate cells and particles from the original flow direction, Huh *et al.* [63] combined the fluid dynamics with the inertia of the droplets to separate the microorganisms and droplets. In this method, the gravity of the earth is used

to drive the flow of the fluid, and the positional deposition difference is induced by the microchannel. As shown in Figure 5a, this separation of hydrodynamic amplification can more effectively separate particles, so as to achieve the separation of small-size (less than $6\mu\text{m}$) microfluidics, and the separation purity of the sample can reach 99.9% in one minute. It is a quick and simple separation method. However, this gravity separation device is only suitable for the separation of particles with large size differences, and the separation flux is small, which is not suitable for the separation of batch samples. For other mixed particles, it is necessary to further expand the hydrodynamic

Figure 5: (a) Mass-dependent particle separation based on hydrodynamic amplification of sedimentation-driven particle separation in μ -SOHS. Adapted with permission from [63]. Copyright 2007 American Chemical Society. (b) Configuration of the microfluidic device containing either dimension-confined spiral channel. Adapted permission from [64]. Copyright 2017 the Royal Society of Chemistry. (c) Spiral channel chip with expansion structures. Adapted with permission from [65]. Copyright 2020 American Chemical Society.

effect and optimize the microchannel structure, such as adding parallel channels to achieve high throughput. Shen *et al.* [64] designed an inertial microfluidic system based on micro-spiral channels as seen in Figure 5b. Under high throughput (3 ml/min), the separation purity of samples is $\geq 96\%$. In addition, the device can be recycled for a long time (at least 4 hours) and has less damage to cells. Gou *et al.* [65] proposed an inertial microfluidic chip containing spiral channels with a periodic expansion structure as seen in Figure 5c. The experimental results show that when the flux is 750 $\mu\text{l}/\text{min}$, more than 99% of the target particles can be separated, and the purity of the target sample is as high as 86.12%.

3.2 Deterministic lateral displacement

Deterministic Lateral Displacement (DLD) sets up a series of periodically arranged obstacles, and uses the laminar flow characteristics of particles to follow through their center of mass, which can realize particle separation based on size when the fluid passes. As shown in Figure 6a, Liu *et al.* [66] used a DLD array to achieve rapid and label-free

separation of cancer cells. The efficiency of sorting at a speed of 2 ml/min is higher than 80%. The throughput can reach 10 ml/min with 5 parallel channels. Chien *et al.* [36] used a DLD device with a circular column to simulate the mesoscopic fluid dynamics of red blood cells (RBC), and then introduced the interaction between the cell behavior in the complex microfluidic flow and the classification capabilities of the device. Holm *et al.* [67] designed a simple and low-cost microfluidic separation system based on DLD (Figure 6). It is possible to separate the parasites from the blood. Thinning the equipment and adding parallel outlets can increase the sorting throughput from 1 nl/min to 1 $\mu\text{l}/\text{min}$. Tottori *et al.* [68] produced two DLD devices as seen in Figure 6c, one with a low aspect ratio straight rectangular channel for inertial focusing of particles, and the other with a high aspect ratio straight rectangular channel for focusing on the sidewall of particles. When the flow rate is 3 ml/min, the sorting efficiency of 13 and 7 μm magnetic beads are 99.4% and 96.4% respectively, and the corresponding purity can reach 95.9% and 99.5%. The first device is used in the cell sorting process, and the sorting efficiency reaches 99% when the flow rate is 5 ml/min.

3.3 Microfiltration

Crowley *et al.* [69] showed that a cross-flow microfilter device operated entirely under capillary action performs microfluidic separation of plasma in whole blood (Figure 7a). There is no obvious damage to the hemoglobin and red blood cells of the cells sorted by the flat microfilter. In addition, no cells and cell debris were observed in the filtrate. As shown in Figure 7b, Chen *et al.* [70] developed a microfluidic chip for separating plasma through stepwise filtration. The chip consists of a front-end cell capture structure and a back-end filter. Two types of filters are proposed: straight and square filters. In the chip with a linear filter, with a gap of 2 μm and a dilution factor of 10, the separation efficiency is low (only 20%). In the case of a gap of 1 μm and a dilution factor of 50, the separation efficiency is increased to 91%. In the chip with a square filter, with a 1 μm gap and a dilution factor of 20, the separation efficiency is close to 100%. Li *et al.* [71] designed and manufactured poly(dimethylsiloxane) (PDMS) microfiltration membrane (PMM) (Figure 7c), which can sort white blood cells from whole blood. When the sample throughput is 1 ml/h, the sorting chip can recover $27.4 \pm 4.9\%$ white blood cells with a purity of $93.5 \pm 0.5\%$. When sorting beads (3 microns and 11 microns), the sorting purity of 3 microns beads at a flux of 2 ml/h is $97.3 \pm 0.5\%$. The PMM can be used as a standard cell sorting component for upstream sample preparation in a highly integrated single-chip blood system in the future.

Figure 7: (a) Microporous membrane filtration of whole blood utilizing cross flow filtration. Adapted permission from [69]. Copyright 2005 the Royal Society of Chemistry. (b) Overview of DLD device. Adapted permission from [70]. Copyright 2014 Elsevier B.V. All rights reserved. (c) Schematic showing the PDMS microfluidic device structure and the principle of crossflow filtration. Adapted with permission from [71]. Copyright 2014 the Royal Society of Chemistry.

4 Conclusion

This article reviews the research progress and development status of microfluidic separation technology. According to the use of external force field, it is divided into active separation system and passive separation system. For the active classification system, four external electric field forces are described and discussed, namely electricity, sound, magnetism and light. For active sorting systems, the sorting methods are mainly discussed, such as inertia, deterministic lateral displacement and microfiltration. The microfluidic method consumes fewer samples and reagents, thus reducing biohazard waste and reducing the risk of contamination.

Although microfluidic devices provide encouraging advantages, there are still some technical barriers that need to be overcome. Due to the unity of channel size, many devices have poor throughput. Parallel microchannels can be set to effectively solve this problem. Secondly, due to the small size of the microfluidic chip, it is prone to blockage and shorten the service life of the chip. Third, the preparation process and cost of the microfluidic chip also limit the commercialization of the sorting technology. We hope that new technologies (such as 3D printing technology) can reduce the cost of chip preparation or modify methods to overcome this problem.

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