Particle Sorting in Microfluidic Systems

Guiping Zhu and Nam-Trung Nguyen*

School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798

Abstract: Continuous particle sorting is one of the most important microfluidic applications. Fast and accurate sorting procedures can be performed using unique characteristics of microfluidics. Beside hydrodynamic means, provided by the flow itself, the application of an external field also contributes to sorting efficiency. Particle sorting has been realized in many effective ways. This paper systematically gives an overview on techniques used for particle sorting in microfluidics. The basic theories and fluid mechanics are presented for each approach. The implementation of the various sorting devices is discussed with their working mechanism, design and performance. Typical results from these applications are also presented. Based on the guidance from the existing technology, perspectives for future works are provided.

Keywords: Particle sorting, microfluidics, magnetic, DEP, optical, acoustic.

1. INTRODUCTION

In the late 20th century, micro electromechanical systems arising from silicon technology dominated the early development stage of microfluidics. Microfluidic devices have been widely investigated and developed due to their various applications. Medical diagnostics, chemical industry, drug discovery and proteomics are the main drivers for microfluidics. One of the most practical microfluidic applications is cellomics, which requires sampling, trapping, sorting, treatment and analysis of cells. Among these tasks, cell trapping and sorting are the key tasks. An effective particle sorting system is critical for medical diagnosis and biological applications. Various particle sorting systems have been proposed, reported, and applied in chemical, pharmaceutical industry, medical research and other research activities.

Centrifugal approach [1] is a conventional sorting method, which separates particles in bulk based on their different sizes and densities. In contrast, modern particle sorting devices are able to manipulate single particles. One of the earliest sorting techniques is flow cytometry [2] with optical detection. Positive control is applied to stained particles, while unstained particles are treated negatively. Flow cytometry is of tremendous importance for a variety of medical applications. However, due to the large scale and the high cost of flow cytometry, a number of other methods such as laser tweezers [3], dielectrophoresis (DEP) [4], acoustic traps [5], and magnetic tweezers [6] have been used for sorting of individual particles. Laser tweezer can offer good accuracy, thus is currently a standard tool for single particle manipulation. Despite the wide range of available forces, application of laser tweezers is limited due to the need of lasers and the large-scale external equipments. Magnetic sorting with fluorescence-activated particles is another popular sorting technique of single particles.

A number of experimental works on particle sorting have been carried out and reported. To facilitate and accelerate the development of this field, current research also emphasizes on developing numerical models for novel sorting concepts using computational fluid dynamics. This review attempts to summarize the different techniques for particle sorting covering basic theory, experiments and simulations. Sorting techniques are generally categorized as passive and active types. Passive techniques rely on hydrodynamic effects of the flow. The classification of active methods is based on the applied external fields. The performance of sorting devices is analyzed based on purity, recovery (yield), and throughput of the device.

2. PASSIVE SORTING

As mentioned above, sorting methods can be divided into two main categories: passive and active types. Passive methods attempt to change hydrodynamic behaviors of the particles by geometrically modifying the microchannels to achieve sorting. Currently, passive approaches have been developed based on different dynamic flow phenomena in microscale.

Flow-Based Sorting

Flow-based sorting is performed only by the flow pattern in the microchannels. One example of flow-based sorting is chromatography, which separates species in stationary and mobile phases. The common chromatography techniques are size exclusive and hydrodynamic chromatography. Particles are sorted in size exclusive chromatography based on their sizes. Particles of different sizes elute through a stationary phase at different rates. Several experimental works have been reported [7-8].

Hydrodynamic chromatography sorts particles in a capillary simply based on Brownian motion [9]. The average velocities of the particles depend on particle sizes. The larger particles have higher mean velocities than the smaller ones. Diffusion of the particles allows sorting, because the distributed distance increases as the square root of time. This phenomenon is called peak-broadening by diffusion. Hydrodynamic chromatography was first proposed by Small [10]. Both retention and diffusion effects of particles suspended in a fluid were discussed and investigated in early works on hydrodynamic sorting [11] [12]. Size distribution characteristic in the channels was studied [13]. Sorting by hydrodynamic chromatography has been achieved in microchannels [14-16]. Development of detection technology allowed the

*Address correspondence to this author at the School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798; Tel: (+65) 67904457; Fax: (+65) 67911859; E-mail: mntnguyen@ntu.edu.sg

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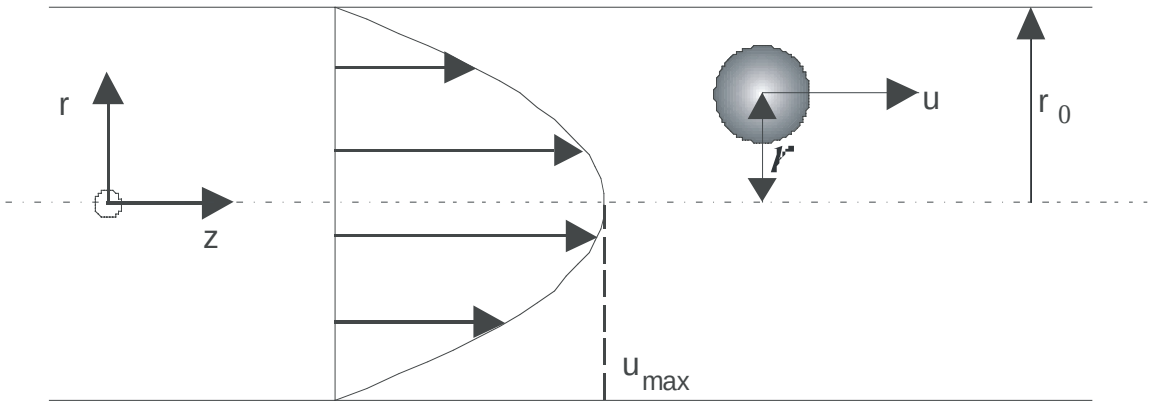

Fig. (1). Sorting particles by hydrodynamic chromatography [16].

study of the sorting mechanism. UV detection has been developed as the most popular technique in hydrodynamic chromatography, Fig. (**1**) [16-17].

The slow molecular diffusion is the main limiting factor of the sorting speed. Brownian ratchet arrays as shown in Fig. (**2**) were used, to further increase the sorting speed [18-20]. Size-based lateral displacement sorting is another elegant passive sorting concept that overcomes the problem of molecular diffusion, Fig. (**3**) [21]. Particles that are smaller than the lane width will follow the streamlines, and undertake a "zigzag mode" motion. While a particle with a radius larger than the width of the lane will behave in a different way. The periodic transport pattern is called the "displacement mode". Microspheres of 0.8, 0.9, and 1.0 micrometers in diameters were sorted in 40 seconds with a resolution of approximately 10 nm.

[22-23] demonstrated that particle-pinched effect due to sheath flow in microchannels is applicable for particle sorting. Retention of the particles was found to depend on another stream without particles. Sorting was achieved perpendicularly through an internal flow pattern. Multiple-branch microchannels [24] with different dimensions were arranged at the collection segment to allow sorting of particles with small diameters. Concentrating and sorting of the particles can be performed at the same time. [25] conducted a continuous and real time separation of blood plasma in a bifurcated microchannel structure.

Differential Inertial Focusing

The above-mentioned method requires a laminar flow leading to a relatively long sorting time. Thus, faster sorting methods commonly use differential inertial effects. Rotational flow based sorting was first introduced [26-27]. The flow is generated by anisotropic obstacles patterned only on the top wall of the flow section. The particle-obstacle interaction is called hydrophoretic ordering which diverts the large particles from their streamlines, leading to distinct flow paths for particles with the same size, Fig. (**4**). The sorting throughput based on hydrophoretic ordering is about 90 particles per second and 1.7×10^6 molecules per second. This throughput can be increased by using multiple channels. [28] utilized expansion channels to achieve higher speed without affecting the sorting quality.

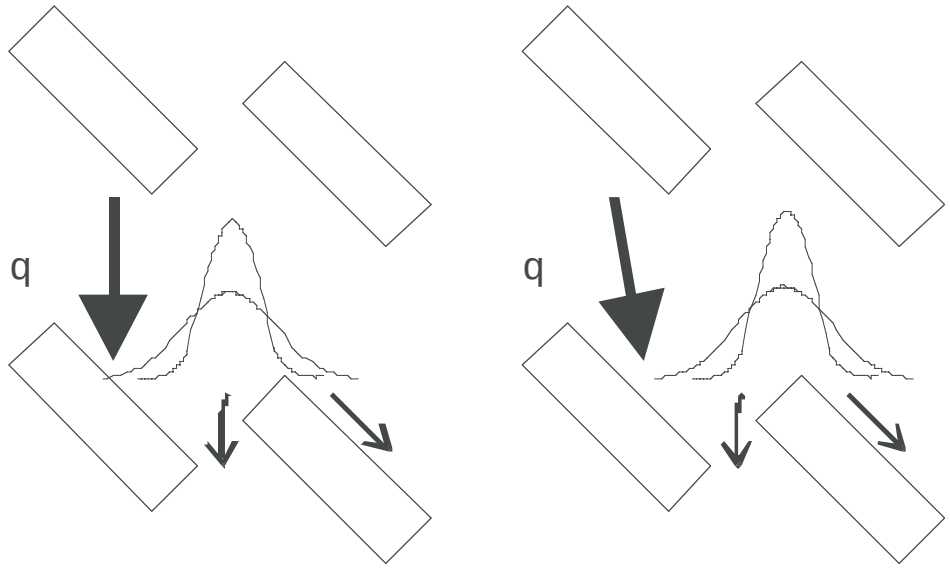

Fig. (2). Basic sorting principle of Brownian ratchet array with vertical or titled flow [20].

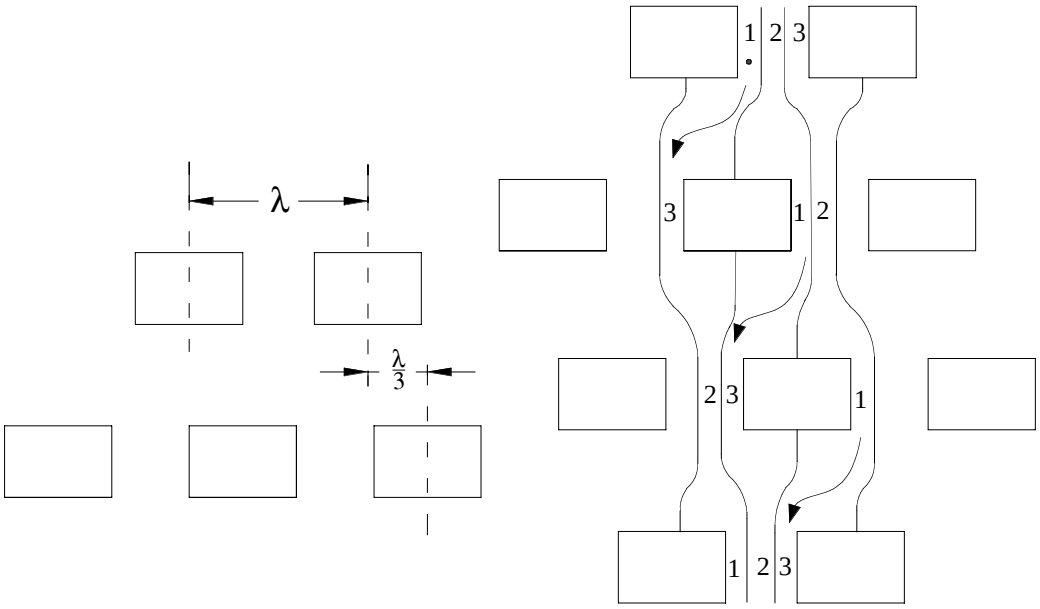

Fig. (3). Fluid streams in the zigzag mode [21].

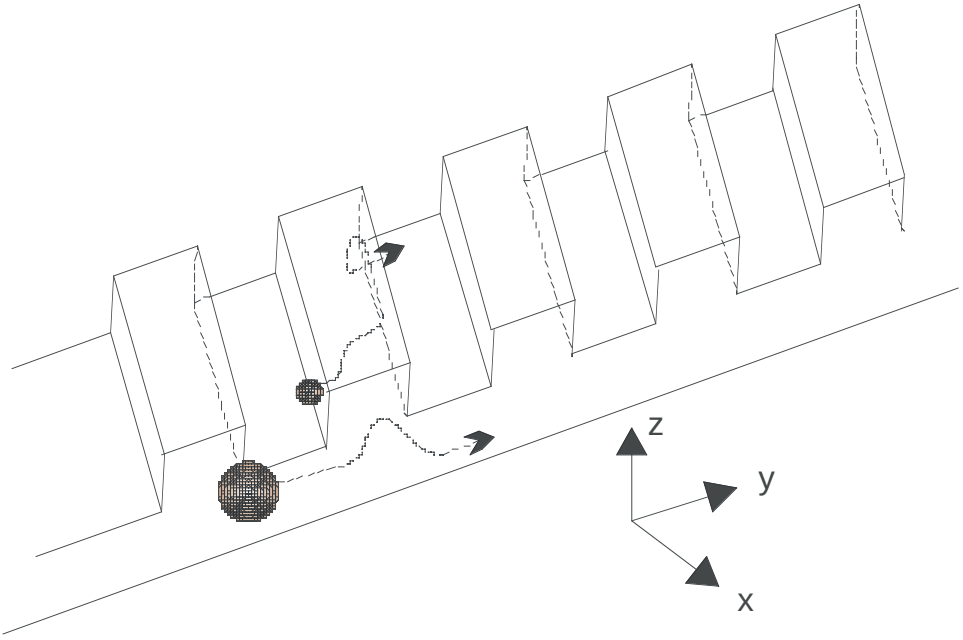

Fig. (4). Principle of hydrophoretic sorting [27].

The complexity of sorting systems has been increased, as the required processing rates have been rising gradually. Passive sorting methods were improved by modifying the channel geometry using spiral [29] or curved [30] shapes instead of the conventional straight design. Dean force caused by the spiral microchannel helps to sort the particles [31]. combine the tubular pinch effect, the centrifugal force and the Dean's vortex to control the migration direction of the particles and drives them to different outlets for collection. The induced cascading sorting in series is shown to be effective for high quality and throughputs. This method can separate particles based on size, with purities from 90 to 100% and high-volume throughputs of around 1 ml/min. Cascaded squeeze effect was further developed and improved by [32]. A single high voltage supply is applied to create electric fields of different intensities at different points of the microchips accompanied by a series of variable resistors. Sheath flows are thus generated to sort microparticles of different sizes. Microparticles of the same size are introduced into the channel to prove the ability of switching into the neighboring sheath flow by the squeezing effect, Fig. (**5**).

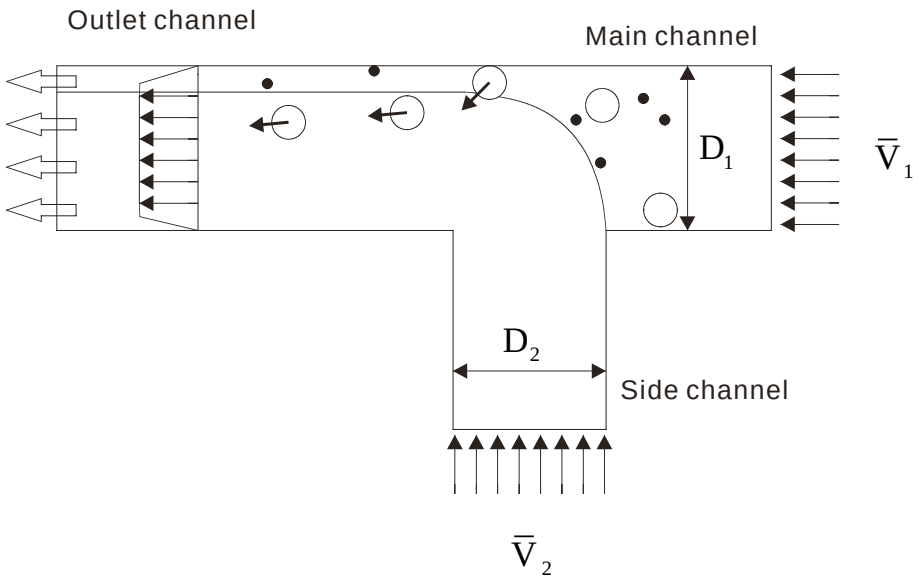

Fig. (5). Schematic diagram of sheath flow effect and the working principle [32].

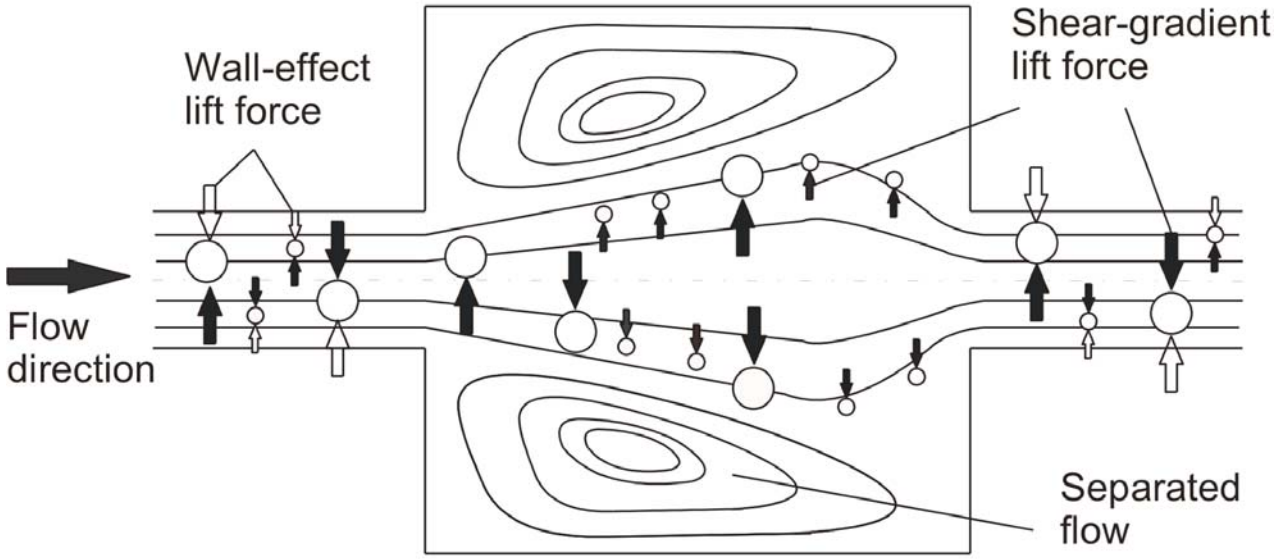

Fig. (6). Working principle of particle sorting by multiorifice flow fractionation (MOFF) [34].

[33] showed that splitting fluid flow from a main stream and recombining it dramatically enhances particle sorting efficiency. Based on the idea of geometric modification, the multi-orifice flow fractionation (MOFF) method for continuous size-based particle sorting was recently proposed [34]. A series of contraction/expansion microchannels was combined to form the structure, Fig. (**6**). In the multi orifice channels, sudden turns result in deflection and realigning of the particles.

The Reynolds number is a critical factor for the sorting process. Experiments indicated that large polymer particles (~15μm) were aligned along the centerline of the microchannel, whereas small particles (~7 μm) remained near the sidewalls at Reynolds number ranging from 63 to 91. The throughput of this multi-orifice channel system ranges approximately from 1×10^4 to 5×10^4 particles per second.

Hydrodynamic Switch

Conventional flow cytometry is based on the principle of hydrodynamic focusing with sheath flows. Flow cytometry utilizes optical detection to recognize and sort particles. Subsequent positive control is applied to the particles with fluorescent signal, while the rest is treated negatively.

Laser-induced heating to control the flow is widely used for hydrodynamic particle sorting systems. Fluorescently labeled target cells were detected with sensitive fluorescence microscopy. In the absence of a fluorescent signal, the collection channel is plugged while the specimens are pushed into the waste channels. In order to detect the target cells, laser beam is applied to plug the waste channel while the cells pass through into the reservoirs. [35] used solid-state lasers and PIN-based photodetectors to reduce the size and the volume of the flow cytometry system [35]. [36] used

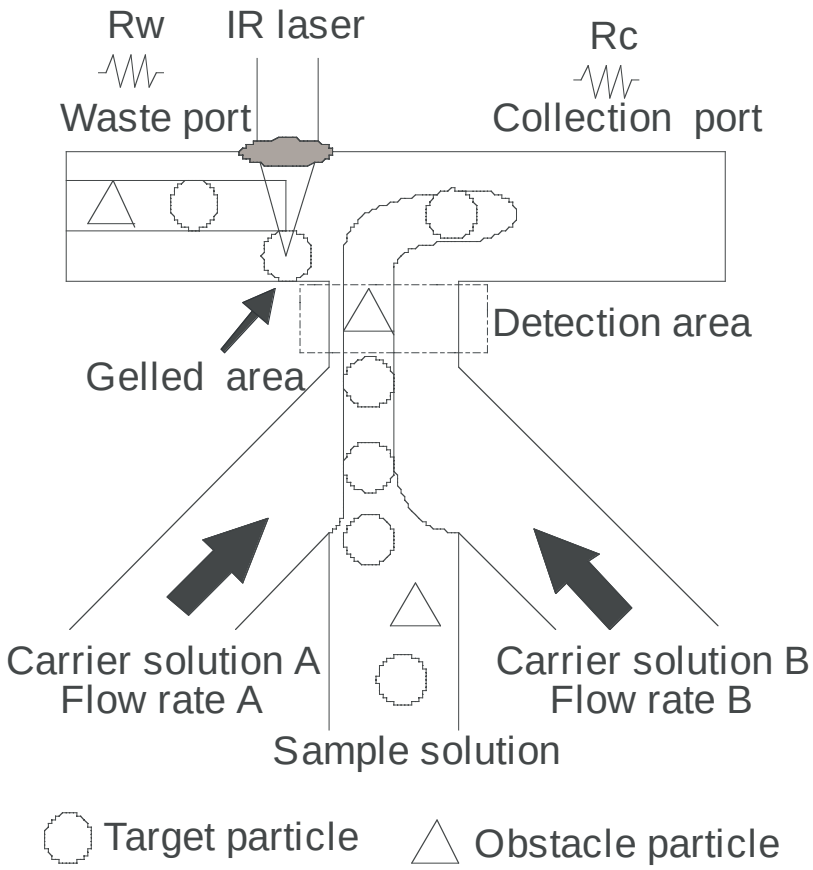

Fig. (7). Principle of the passive sorting with switching [37].

thermoreversible gelation polymer as the switching valve, Fig. (**7**). In a thermal switching system, sorting continuity is affected by the pressure fluctuation [37]. [38] integrated fluidic, mechanical, optic and electronic in a single system to improve the sorting performance. The performance can be optimized by controlling the pressure, the chamber length, and the entrance length, to improve sorting efficiency and to shorten the switching time.

3. ACTIVE SORTING

Magnetic Actuation

Physics of Magnetic Cell Sorting

Magnetic forces are generated by the interactions between particles with magnetic properties and the external magnetic field. Since magnetic force is a volume-based force, the relatively small force in microscale makes it unfavorable for single particle trapping. In order to achieve tunability of the field strength, electromagnetic fields are preferred. For a small current coil, the magnetic force is defined as:

$$F_m = \left(I\ S\ n \cdot \nabla\right) B \tag{1}$$

where I is the current; S is surface area through the whole coil length; n represents unit vector of the coil surface; and B is the magnetic flux density. In a sorting system with particles subjected to the magnetic field, forces exerted on the particles are characterized by the magnetic force density f_m. With the assumptions that no magnetic monopoles exist, particles and the carrier fluid are incompressible, the magnetic force density is given by [39]:

$$f_m = -\frac{1}{2}\mu_0 H^2 \nabla \mu_m \tag{2}$$

Where μ_0, μ_m represents the magnetic permeability of free space and of the particle respectively; H is the magnetic field strength. In general, magnetic particle sorting can be categorized in two ways: directly using native magnetic susceptibility and indirectly using magnetic beads attached to the species to be sorted.

Magnetophoretic Mobility

Magnetic force was used in field-flow fractionation for sorting of the particles by applying external magnetic field perpendicular to the flow direction in the separation microchannel. Analytical solution of particle trajectories in microchannel has been well established [40].

Magnetic field is combined with flow field to create a force vector which moves the particles out of the stream lines [6]. Magnetophoretic mobility is a property used for describing kinetic characteristic of particles going through a channel filled with a viscous medium. Magnetophoresis is generated under the influence of an external magnetic field,

and can be measured by a particle tracking velocimetry. [41] described and discussed a method to determine magnetophoretic mobility of immunomagnetically labeled particles. Particle tracking velocimetry was used for the measurement.

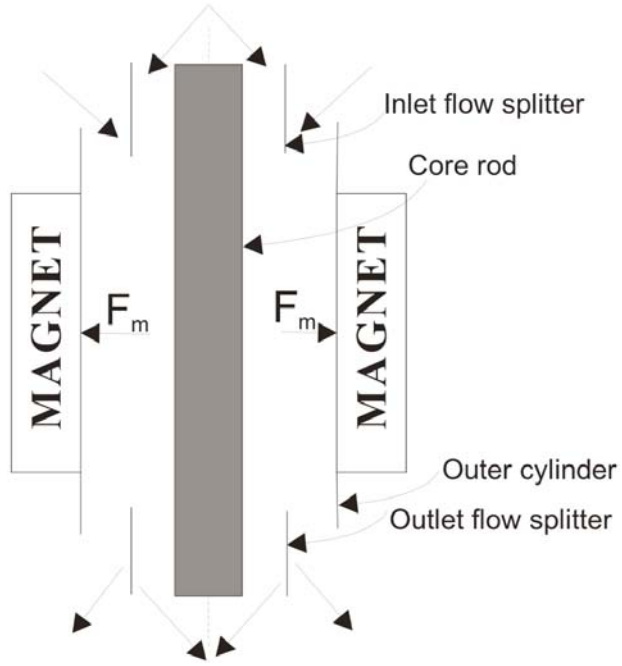

Fig. (8). Basic principle of particle sorting under magnetic force [44].

As a typical example, continuous particle sorting based on differences in magnetophoretic mobility was proposed [42-43] as the form of a quadrupole magnetic flow sorter. Oxygenated red blood cells are used to carry out experiments, and the quality of the magnetic separation is shown to be increased significantly. Particle magnetophoretic mobility was exploited as a linear function of the product of solution susceptibility and viscosity, Fig. (**8**) [44]. The working principle was described [45]. The separation device consists of two cylindrical flow splitters, an outer cylinder, and a coaxial solid rod as depicted in Fig. (**9**).

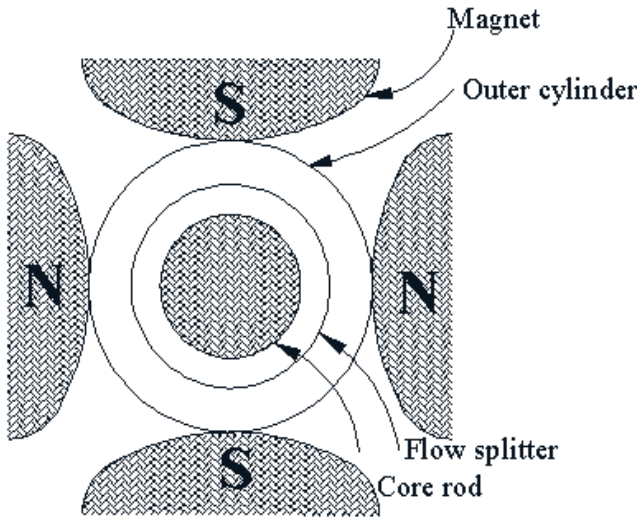

Fig. (9). Top view of the quadrupole sorter [45].

To improve the efficiency of quadrupole magnetic flow sorter, hydrodynamic parameters have to be optimized [46] achieved a high throughput of 3.29×10^5 cells per second and a high recovery rate of 0.89 of breast carcinoma cells.

Sorting by Native Susceptibility

Sorting by native susceptibility leaves particles largely unaffected, an asset for any later analysis. The paramagnetic properties of red blood cells make them possible to be isolated from any other diamagnetic material using a magnetic field. Native susceptibility method uses a small ferromagnetic stripe such as a nickel stripe. Thus stripe is magnetized by an external magnetic field. A magnetic field with high gradient is generated around the stripe where paramagnetic particles are trapped. It should be noted that different types of particles experience different magnetic forces and retained in different region of the magnetic field. Specific particles can be released from the system after external magnetic force is cut off. The high gradient magnetic separation [47] has been shown to be effective at separating red blood cells from whole blood. Magnetic changes in red blood cells have also been used to sort diseased cells and cells with congenital abnormalities.

Sorting by Attached Magnetic Beads

The use of magnetic beads coated with cell-specific antibodies to separate certain cell types is only about 15 years old, but has emerged recently as an affordable way of isolating rare cells. Once the magnetic beads are bound to the cells, a magnetic field gradient is all that is required to separate them from the bulk fluid. Magnetic beads with diameters ranging from 10 nm to 10 μm, are typically made of a mixture of polymer and iron-oxide particles, Fe_2O_3 and Fe_3O_4. Magnetic labeling can be achieved by binding magnetic beads to the particle surface or introducing microbeads into the particle. Until recently, several kinds of mixers for mixing magnetic beads and cells have been designed and proved to be effective for sorting purposes.

Binding capacity represents the ability of particles binding with magnetic beads. It is a commonly used technology allowing magnetically conjugated antibodies to bind which generate magnetophoretic mobility on target particles. Sorting was achieved and reported [48]. The relationship between antibody binding capacity and magnetic particle separation efficiency was investigated through calibration experiments. Recently, multi target magnetic activated particle sorting was proved to be possible by applying different magnetic beads with distinct saturation magnetization and size [49].

Magnetic beads attached chromosomes were effectively separated with Chinese hamster *x* human hybrid cells [50]. Sorting by native susceptibility was compared with sorting *via* magnetic beads [51]. Magnetically labeled cells were attracted to the stripes and tended to follow the stripe direction, while unlabeled cells did not interact with the stripes and follow the direction of the fluid flow. The greatest challenge with the reported design is preventing magnetically labeled cells from permanently sticking to the stripes. Based on a stable expressed surface marker, magnetic cell sorting

was shown to be practical for purification of all kinds of particles or cells, such as differentiated embryonic stem cells [52-54]. Successfully separated mouse macrophages and human ovarian cancer cells.

Active Magnetic Method

Electromagnetic actuation in the form of an electromagnetic solenoid coil was first used [55] for particle sorting. Electromagnets are suitable for integration in a microfluidic system such as lab on a chip for immunoassay-based biochemical detection. [56] reported a magnetic bead-based biochemical detection system. One of the main disadvantages of active sorting using electromagnets is the generation of Joule heating which could be harmful for the sorted species. Cooling measures have to be considered [57]. Microfabricated electromagnets have been used for both magnetic manipulation and separation in lab-on-a-chip systems [58-59].

Passive Magnetic Method

Passive sorting is usually carried out in a large field with a gradient across the microchannel. The requirement of an external magnetic field will be reduced. [60] reported a microfluidic separator using an external magnetic field with a flux density of 50 mT. To improve manipulation ability, integrated current traps were used to generate large and highly localized field gradients in the device.

Particles such as DNA or protein are sensitive to heat and strong chemicals. [61-63] proposed devices with arrays of soft magnetic elements combined with hydrodynamic focusing [61-63] to prevent excessive heating through the electromagnets. These designs are also suitable for systems handling a large amount of magnetic beads.

Dielectrophoretic Actuation

Dielectrophoresis (DEP) has been extensively studied and reported as an effective way for particles manipulation, trapping, separation or sorting. DEP mainly relies on an electric field to manipulate the particles remaining or moving towards a specific direction. Early works on dielectrophoretic phenomena were investigated and reported [64]. The fundamental theory was established through analyzing dielectrophoretic force exerted on a neutral object by a non-uniform electric field. The particles exposed to a non-uniform electric field would undergo polarization, and differences in the induced particle dielectric properties enable manipulation of particles suspended in a fluid. A dielectric generally contains polar material with random dipole directions. When an external electric field is applied, both particles and fluidic medium will be polarized by orienting the dipole moments of the polar molecules in a direction. As a result, surface charges accumulate at the interface between particles and fluid. Additional electric field is generated acting to distort the original electric field, Fig. (**10**).

The interaction between the surface charges and the electric field results in a net force on the particle due to the non-uniformity of the electric field. A number of research works emphasized on calculating the force on the particles. With the assumption that surface charges are small enough to be treated as a dipole with the same direction as the electric field, the DEP force is determined as:

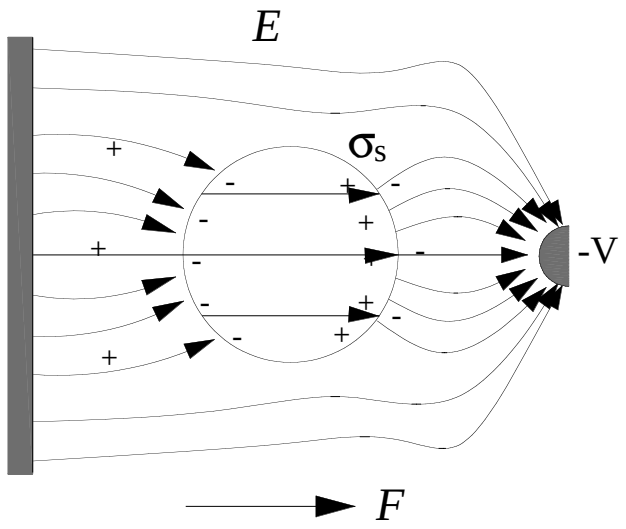

Fig. (10). Interfacial polarization and dielectrophoresis.

$$\vec{F} = \int_v \left(\vec{P} \cdot \nabla\right) \vec{E} dv \tag{3}$$

Where $\vec{P}$ is the dipole moment, $\vec{E}$ is the magnitude of external electric field. DEP force on a small spherical particle is calculated as:

$$\vec{F} = 2\pi a^3 \varepsilon_m K \nabla E_0^2 \tag{4}$$

Where $\vec{F}$ is the DEP force exerted along direction of the electric field, and a is the radius of spherical particle. Clausius-Mossotti factor K is defined as

$$K = \frac{\left(\varepsilon_p - \varepsilon_m\right)}{\left(\varepsilon_p + 2\varepsilon_m\right)} \tag{5}$$

Where ε_p and ε_m represent permittivity of the particle and fluid, respectively. The moving direction of the particle is determined by the notation of K. If $K > 0$, the particle will move towards regions with high electric strength. Otherwise, the particle will move to the opposite direction. Applying an electric field with different frequencies, magnitude and direction of the DEP force can be controlled for effective sorting.

Integrated microelectrodes or external electric fields are needed to utilize DEP. Integrated electrodes are preferred in most applications because of its ability to generate multiple and independently activated electric fields at different locations in the system. Furthermore, electrodes can be placed in the microfluidic system to generate a larger field gradient. Electrode shapes are significant for field generation and thus the sorting efficiency. Electrodes can have the form of stripes or planar surfaces; and their geometries could be either curved or straight. Pohl and Pollock [65] described theoretically the impact of electrode shapes and recommended optimized electrode shapes as well as their applications. Besides the shape, the electrode arrangement also plays an important role. Electrodes should also be well arranged in order to make full use of dielectrophoretic forces.

In conventional DEP-based sorting systems, alternating current (AC) electric field is used because of the frequency dependency of the dielectric properties. Cumings and Singh [66] first used DEP forces generated by spatially non-uniform direct current (DC) electric field in a particle sorting system. The preliminary purpose of DC field was the elimination of electrolysis of the cells being handled. DC-DEP is not sensitive to change of microchannel surface, and makes fabrication much easier for nonmetallic components in the system. [67] proposed a DC electric field in which DEP particle sorting and electrokinetic particle transport can be achieved simultaneously. An array of insulating posts has been used as electrodes [4, 68] for separation and concentration of bacteria mixtures.

Field-Flow Fractionation

DEP has been combined with field-flow fractionation (FFF). The device proposed [69] is one of the examples of DEP field-flow fractionation. The basic sorting capability was demonstrated [70] in a field-flow fractionation device with DEP, Fig. (**11**). [71] reported theoretical model for the relationship between different parameters of the separation process. In these devices, separation can be achieved by applying a relatively small AC voltage (<10 V p-p) with a typical frequency of 1 kHz.

In the review on dielectrophoretic particle sorting by Gascoyne and Vykoukal [72], FFF has been further classified into three main categories: normal, steric, and hyperlayer types. In normal FFF, thermal diffusion of the particles has to be considered. Besides DEP force, particle sedimentation force and the hydrodynamic lift force also act on the particle. Thus in steric FFF, the combination of the three forces makes the particles move at a lower speed along one side of the sorting chamber. In hyperlayer FFF, particles are driven to an equilibrium height where the sedimentation and levitation forces are balanced. Particles possessing different densities or dielectric properties are levitated to different characteristic heights in flow-velocity profile and attain different velocities in the chamber, and are thereby fractionated. A typical hyperlayer FFF system was developed [73] to separate erythrocytes and latex beads. Kim and Soh [74] recently reported a technique called MAG-DEP-FFF which also separates magnetic antibody-labeled particles. This technique can rapidly and accurately sort multiple particles to achieve a purity of over 95%.

Spatial Particle Separation by Frequency Effect

Particles can be sorted once they are identified by subjecting them to differential forces. Depending on the above-mentioned theoretical expression, variation of electric field frequency has a significant impact on electric force and thus moving direction of the particles. For binary sorting, it is possible to apply a frequency between the respective cross-overs of the different particles. The two types of particles will be driving towards opposite directions. Obviously, particles with significant difference in electric properties can be sorted effectively [75]. Using this technique,[76] separated malignant human breast cancer epithelial cells (MCF 7) from healthy breast cells (MCF 10A). A flow rate of 290 μm/s and a voltage of 48MHz-8 V_{PP} were used in the DEP separation system. Experimental results showed that MCF 7 and MCF 10A cells were separated with a maximum efficiency of 86.67% and 98.73%, respectively.

Optical Actuation

Optical actuation was used in particle sorting because of its simplicity. In the early development stage, a single beam was used to exert optical forces on particles. Fig. (**4**) shows the concept of optical force based on the scattering principle. The motion of the particle is determined by the combination of the axial gradient force and the gravitational force.

Optical scattering and momentum transfer to large particles are shown in Fig. (**12**). However, for small biological particles, the optical forces are calculated from the expression [77]:

$$F_{scat} = \frac{I_0}{c}\frac{128\pi^5 r^6}{3\lambda^4}\left(\frac{m^2-1}{m^2+2}\right)^2 n_b \tag{6}$$

$$F_{grad} = -\frac{n_b}{2}\alpha\nabla E^2 = -\frac{{n_b}^3 r^3}{2}\left(\frac{m^2-1}{m^2+2}\right)\nabla E^2 \tag{7}$$

where I_0 and λ are the intensity and wavelength of the light; r is the radius of the particle; and m is the effective index; n_b indicates index of medium surrounding the particles. Obviously, the force can be increased through decreasing beam angle of the scattered light. This flexibility allows

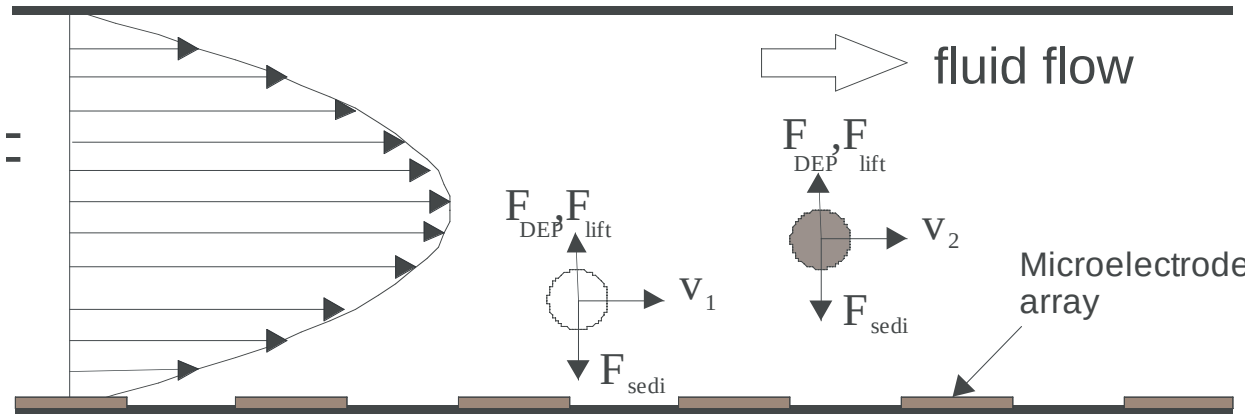

Fig. (11). Sorting particles through applying microelectrode array [70, 72].

manipulating particles with different sizes in a single device. Optical forces may only be on the order of few piconewtons, but sufficiently large for microscale applications. It is difficult to obtain optical forces when the radius of the particle is comparable to the wavelength of the light [78]. Experimental verification on small particles was achieved [79] with good agreement with theoretical expectations.

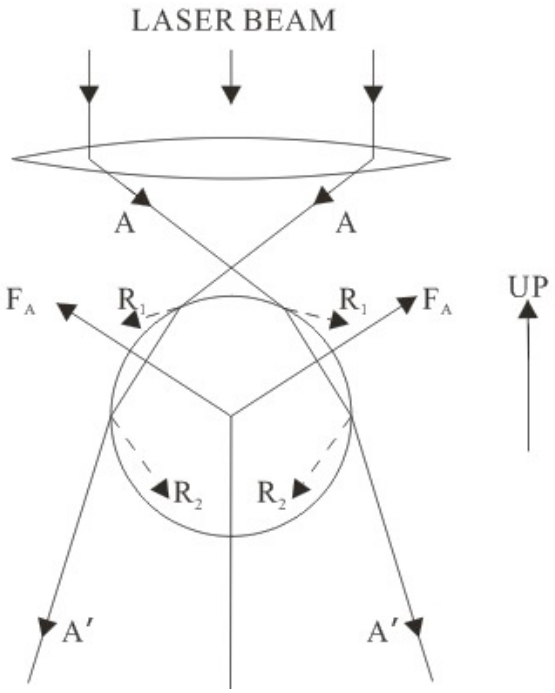

Fig. (12). Ray optics of a spherical particle trapped by the single-beam gradient force [77].

Optical tweezers, originally called single-beam gradient force trap, is the most popular technology for particle sorting. Both observation and identification of optical trap have been reported using a single laser beam. Computer-generated holograms [80] displayed real time beam shaping during the operation. Therefore, axial or lateral position of the trapped particles could be controlled, Fig. (**13**). Visual identification of particles and subsequently sorting can be achieved in optical tweezers in conjunction with direct microscopic analysis [81-82].

There are several approaches for triggering particle switching. The most common method was the introduction of fluorescent particles. A microfabricated fluorescence-activated cell sorter ($\mu FACS$) is usually implemented with a commercial microscope to provide precise signal detection and real-time high-speed imaging. Once a fluorescence-labeled particle passing through the analysis region, the fluorescence is excited by the laser. The resulting optical forces cause the deflection of the fluorescent particles and make them flow to the target channel. Particles without optical deflection enter the waste reservoir. The typical configuration of an active sorting system is shown in Fig. (**14**).

The concept of fluorescence tagged particles was first proposed [83], who demonstrated detection and deflection of several particle types using a focused beam. Besides optical force, hydrodynamic, electrokinetic, electroosmotic, DEP and magnetic forces can also be used as the actuation sources for particle switching. The emitted fluorescent signals from the particles are detected by a photo detector, and then processed by a special electronics [84]. Decision on switching is made based on the processing result of the particle signal. The particle is driven towards the selected collecting branch.

The deflection by optical forces is preferred due to its fast response, high throughput, purity, and recovery of live cells. Sorting with optical deflection was demonstrated to be suitable for living biological cells [85-86]. Bacteria and viruses could be trapped by a focused laser beam. Mammalian cells were sorted by optical force switching within a relatively short time. In order to overcome the limitation of single-cell sorting, multi-spot detection was obtained through placing optofluidic waveguide along the microfluidic channel [87]. Recently, microfluidics, optics, and acoustics were integrated in the same device to improve the performance of fluores-

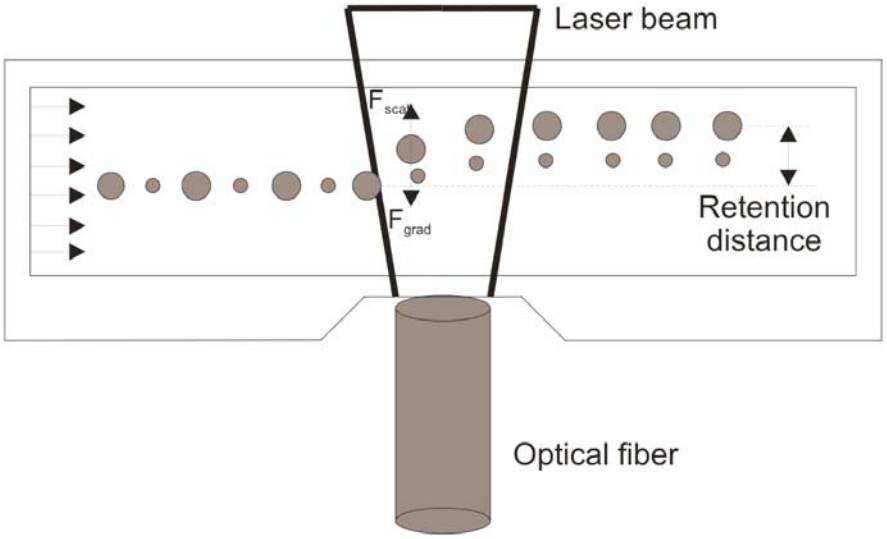

Fig. (13). Schematic of the optical particle sorting.

cence activated sorting. Sorting of both particles and biological cells was performed with a throughput higher than 1000 cells per second.

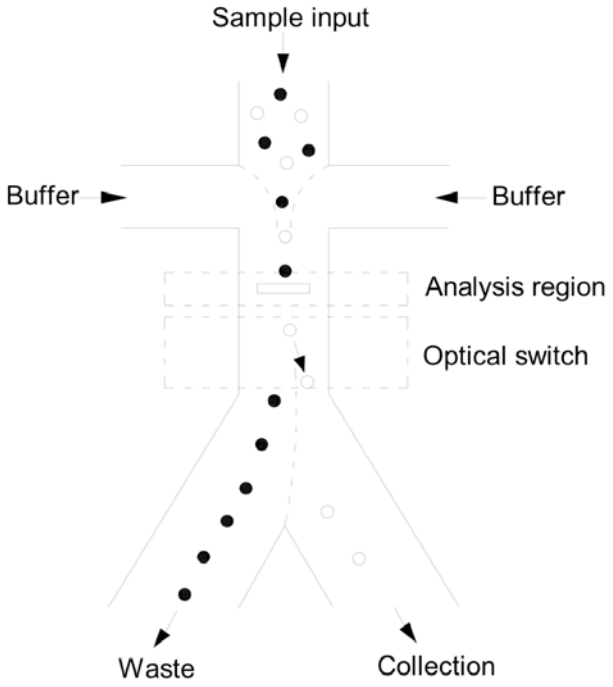

Fig. (14). Schematic representation of an active sorting system [86].

Moderation of laser intensity was indicated to be suitable for changing the optical force. The deviated distance was determined by the titling angle of the trajectory and time taken by the light to reach the target particle [88]. In addition, particles of different physical properties were able to be detected by Raman spectroscopy. A computer-aided algorithm is needed for identification and discrimination from the data collected by Raman spectroscopy. Laser tweezers combined with Raman spectroscopy were used for biological cells [89] and bacteria [90-91]. Digital image processing (DIP) has the advantage of full field evaluation without complex electronics [92]. DIP was used in particle sorting systems to identify and trace particles in microchannels. Automatic switching can be achieved with a feedback signal from DIP.

Multiple focusing points were required if several particles are manipulated simultaneously. In this case, spatial light modulator (SLM) is inserted into the path of the laser, and divides the laser into multiple beams [93]. Intensity of the laser is affected by the SLM. The phase of the laser can also be modulated if necessary. Recently, active particle sorting with optical switching can reach a yield rate up to 90% [92].

When particles are handled without switching, passive optical sorting can be achieved based on intrinsic properties of the particles. The properties for sorting are size, shape and refractive index. Thus, passive sorting can usually be applicable for sample with homogenous size (such as blood) rather than those with random dimension variation. For this purpose, complex intensity configuration of the laser beam is required instead of a single-beam trapping laser. Optical pattern has been created by different means such as holographic tweezer [94], microlens array [95], and acousto-optical modulator [96].

Optical chromatography is one of the earliest methods of passive optical particle sorting. As particles passing through a microfluidic channel, radiation pressure force acts on them due to optical scattering. The mutual effect of radiation pressure and viscous force determines the motion of the particles. [97] reported a model for the radiation force as a function of particle size and laser power. This model was later verified by experimental results [98].

In contrast to active sorting, passive sorting is feasible without fluid flow. In a static fluid, optical pattern can be designed to be dynamic to cover the entire area of interest. The interaction between Stokes force and optical force can be established thanks to the compensating impact of moving optical element [99]. Obviously, passive optical sorting is more flexible because of its ability to handle tag-free particles. However, active sorting is better established and is proved to be safe for biological cells.

Acoustic Actuation

Acoustic forces have been used for particle sorting offering low-cost, simple designs with high throughput. The application of acoustic standing wave combined with microtechnology opens up a new trend for particle sorting in microfluidic systems. Recently, [100] gave a review on particle sorting based on acoustic actuation. The acoustic technology is attractive as all types of particles can be sorted in a non-contact manner.

Studies of acoustic forces started with the research on acoustic radiation pressure. Doinikov [101] derived a general expression to calculate the acoustic radiation pressure in a viscous fluid. In order to improve the original theory of acoustic radiation pressure, two limiting cases were considered: acoustic wavelength is much larger than the radius of the particle and the opposite case. Expressions for radiation pressure were determined for both cases. The latter case is useful for particle sorting analysis as the particles are usually much smaller than the acoustic wavelength.

Based on the calculation of acoustic radiation pressure, direct radiation force (DRF) can be easily obtained. The direct radiation force drives suspended particles and concentrates them. The difference in acoustic potential energy induces the generation of radiation force. Radiation force is the combined effect of both primary force and secondary force. The primary radiation force (PRF) is the strongest acoustic force acted on suspended particles and can be determined as [5, 100, 102]:

$$F_r = -\left(\frac{\pi p_0^2 V_c \beta_w}{2\lambda}\right) \cdot \phi(\beta, \rho) \cdot \sin(2kx) \tag{8}$$

where p_0 is the acoustic pressure amplitude, V_0 is the volume of the particle, ϕ represents the acoustic contrast factor, which is related to particle density (ρ_c), particle compressibility (β_c), and corresponding properties of the medium (ρ_w, β_w) as:

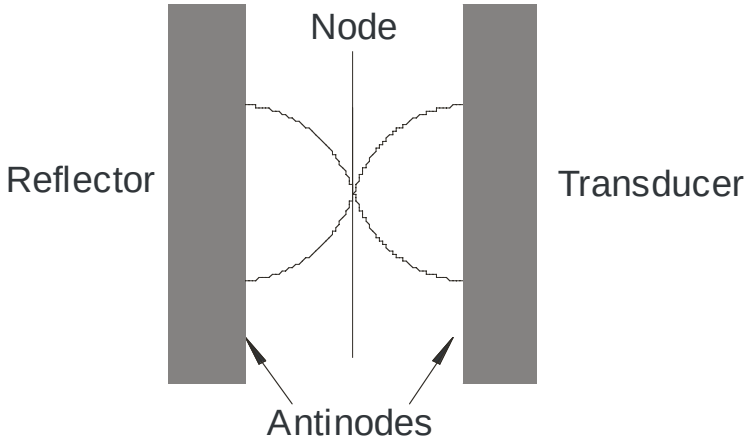

Fig. (15). Scheme of separation chamber having one-half wave-length spacing between piezoelectric transducer and reflector [102].

$$\phi(\beta,\rho)=\frac{5\rho_c-2\rho_w}{2\rho_c+\rho_w}-\frac{\beta_c}{\beta_w} \quad (9)$$

The secondary forces are usually much smaller, and can only influence particles in a short distance.

The basic concept of sorting particles by acoustic field and laminar flow was exploded by Johnson and Feke [102]. The flow direction is perpendicular to the acoustic force. As indicated in Fig. (**15**), pressure nodes are generated if the transducer is energized at a given frequency and voltage. Suspended particles are driven into pressure nodes or pressure antinodes. An approximate model on particles suspended in acoustic chamber was established by balancing acoustic force, fluid flow force, viscous drag, and gravitational force. Validating experiments were performed with red polystyrene particles of 170 μm in radius and blues ones of 100 μm in radius. The main stream entered the channels at a speed of 31.4 ml / min or 17.8 ml / min , while the feed stream joined at 5.6 ml / min or 3.2 ml / min . Frequency and voltage applied to the piezoelectric transducer is 250 kHz and 20-35 Vpp, respectively.

With a theory and model of acoustic particle sorting, design optimization of the sorting devices can be carried out. [103] proposed a simple design for generating ultrasonic standing wave. The standing wave was generated by a piezoelectric ceramic plate bonded on the back of the sorting chip. This design allows a larger contact area for transmitting the acoustic energy into the chip. A cross type device was tested with 750 μm wide and 250 μm channels, Fig. (**16a**). A separation efficiency of approximately 90% was achieved. The impacts of voltage, concentration of sample, and flow rate on the separation efficiency were investigated. A 45°-structure as shown in Fig. (**16b**) was adopted in order to eliminate the stagnation when the particles turn around the 90°corner. Fig. (**17**) [104-105]reported a device for discriminating erythrocytes from lipid particles using laminar flow and acoustic standing wave forces. A parallel separation

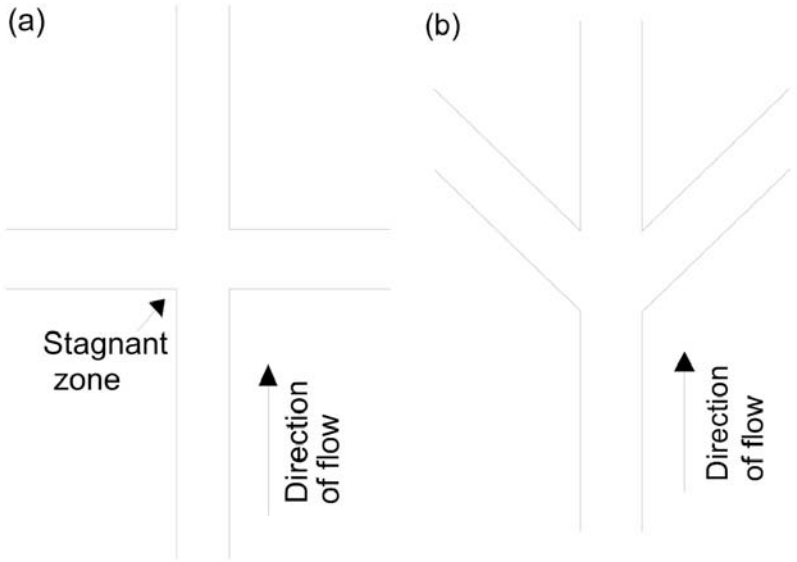

Fig. (16). (**a**). Cross-type structure with a two band formation. (**b**). 45°-structure with a two band formation [103].

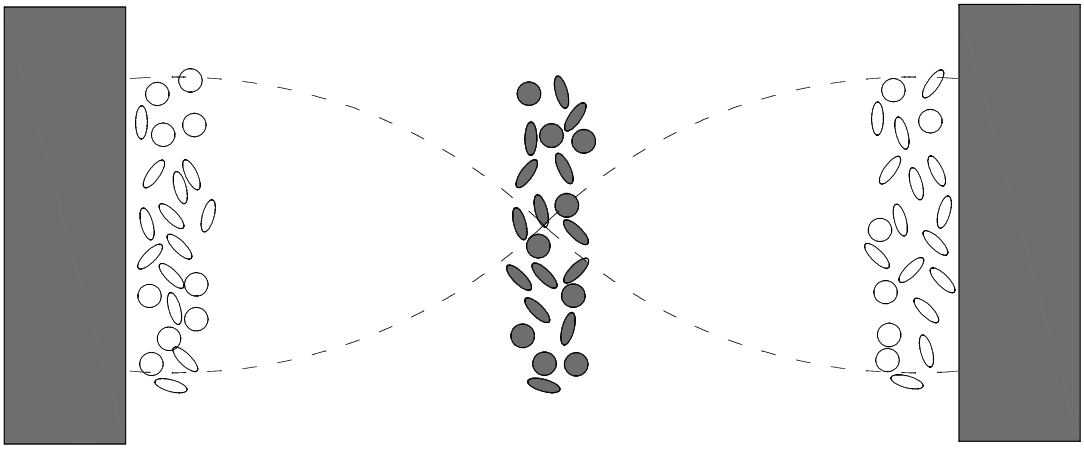

Fig. (17). Two particle types positioned, by the acoustic forces, in the pressure nodal and anti-nodal planes of a standing wave [105].

channels and multiple outlets can be used for large-volume processing and to increase the throughput of the sorting device [106].

In the early development stage, acoustic standing wave was used for particle filtration [107-109]. Different configurations such as plane and tubular transducer were used for optimizing the performance of acoustic resonators [110]. The filtration efficiency was between 60 and 85%. Hawkes and Petersson [111-112] utilized acoustic standing wave in liquid washing by continuous field-flow fractionation. When the fluid flows through the channel, acoustic standing wave works as the switching mechanism for the particles. To explore future clinical relevance, the device was tested with biological particles [113]. Tests conducted on cells before and after the sorting process showed that the devices are safe for medical applications [1, 114].

4. CONCLUSIONS

This paper reviews different sorting concepts in microfluidics. Both passive and active sorters were proved to be effective. Passive sorting devices commonly have a simple structure and produce sample with high purity without consuming extra energy. However, a large device footprint is required due to the relatively long flow channels. Compared to active methods, passive sorting is more time consuming. Active sorting can be performed in various forms with or without flow. A variety of microfluidic devices has been developed to manipulate and sort particles and cells. These devices rely on different concepts of force generation. Each of these methods has its own advantages and disadvantages. A particular concept can be chosen depending on the requirements of the sorting sample and the subsequent handling steps. The performance of sorting devices is evaluated based on the throughput, the recovery rate and the purity. However, most published works emphasize the throughput as an important performance indicator. Besides a high throughput, future developments may aim at the high recovery rate and high purity. For applications dealing with cells, a safe sorting method is needed. The ability of fabrication and integration of the sorting device is another critical factor. Robust, simple and continuous sorting has been proven to be feasible in numerous published works. Today, research efforts are still focused on the development of cheaper, simpler and safer sorting technologies.

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