

Advances of biology sorter based on laminar flow

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Abstract. Now the biological sorters are widely applied in biology and medicine to separate specific sample. Each kind of the sorters are fitting different kind of situations and having a large difference between their cost, efficiency and other factor. In order to help researchers to find a suitable bio-sorters for their research, in this paper, four kind of passive bio-sorters that based on laminar flow were introduced, and their strengths and limitations were discussed. They share common strengths, such as simple design, low cost and so on. These makes them easy to be generalized and applied. Some possible improvements of the sorters were proposed, such as more detailed channels and external electromagnetic field to make the result more efficiency.

Keywords: biology sorter, laminar flow, H-Filter, Spiral channel sorter.

1. Introduction

In biology or medicine field, it necessary for researchers to separate cells from their original samples for more specific study. As a result, different types of sorters are developed; however, the current sorting technology is still facing to following challenges: Purity, efficiency, price, generality, and so on; for example, the dielectrophoretic sorter has relatively low efficiency, and small separating rate, about 90cells per minutes [1].

To solve these challenges, the sorter should be simple and efficient enough. One of the best solutions is laminar flow, because of its physical characteristics: "There is a gradient of velocity as you move from the stationary to the moving plate, and the liquid tends to move in layers with successively higher speed" (Encyclopædia Britannica, inc.) [2]. The isolation between layers of streams can prevents the mixture of results. So, many sorters utilize laminar flow to produce result with high purity in high sorting rate.

Improvement of biology sorters has proceeded for decades. Fluorescence activated cell sorting (FACS) is a representative platform for sorting cells or particles [3,4]. FACS detects the fluorescence signal of the object to sort desired objects in the sample with high specificity and high throughput manner [5]. Over the last decade, microfluidic sorting techniques can be categorized into two major types; Existence of labeling and external force is the basic of passive sorting and active sorting.

In this paper, some sorters which is based on laminar flow are listed, to provide researchers with some ideas if they are looking for sorters with these characteristics, or developing similar sorter.

2. Sorters based on laminar flow

2.1. Sorters utilizing Inertial and filtration

The principle of this technique was illustrated in 1961, however, it was applied to separate the cells until 2007. Another review published in 2009 indicate that it can be useful towards the high-throughput cell separation [6].

The structure diagram of the inertial and filtration-based sorter [7] is presented in Figure 1. In this sorter, the flow is laminar, and the Reynolds number is below 1 which can ensure the flow to be stable.

Moreover, the motion of flow is dominated by inertial force. The motion of cells will mainly be influenced by two kinds of forces: the shear-induced lift force and the wall-induced lift force. The magnitude of the two forces depends on the position, size, and shape of the cells. Hence, the cells are going to stay in a position where two forces are equal. Because the size and shape are factors influencing the magnitudes of the forces, the cells with different sizes and shapes will remain in distinctive positions. Hence, the cells are sorted in order from the random at the inlet. Finally, the cells follow the laminar flow to go through different outlets and we can gain the target cells in specific outlets [8].

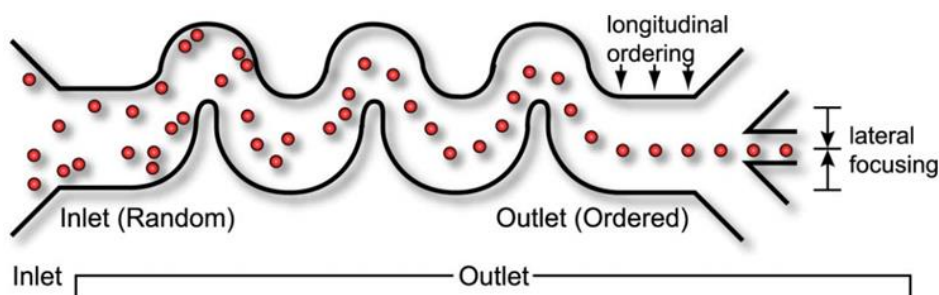


Figure 1. Structure diagram of inertial and filtration-based sorter [8]

This device has high efficiency. For example, the sorting efficiency of separating the neuroblastoma cells and glioma cells was 80%, and when separating the bacteria from human blood cells, the efficiency was 99%. The throughput of this device is about 106 cells/min. This figure is significantly higher than many other sorters. Because this device is totally based on hydrodynamic forces, the design can be very simple and easily integrated with other microfluidic devices.

Because the interaction between cells can distort the result and low the efficiency [6], a highly concentrated solution should be avoided and only a dilute solution is suitable. Thus, they need to prepare an appropriate sample before using this device. As the size and shape can influence the forces, this device might not have the ability to separate these two kinds of cells if there is another kind of cell that has a similar size and shape with the target cell in the sample. For instance, the motile sperm and non-motile sperms are two cells with similar sizes and shapes.

2.2. H-Filter

The H-filter was invented to extract target particles in a fluid with other particles. The technique was further developed by Brenda and other people [9]. This kind of device is applied to separate the motile cells [6].

As shown in Figure 2, two inlets produce two flows and both of them are laminar flows, and the Reynolds number is lower than 1. These two flows will not mix together while flow in the diffusion channel, and in this process, because of the mobility of the motile cells, they can move from the mixed sample into the receiver solution. Then the motile cells can follow the receiver solution and be gathered. In order to avoid the velocity difference between two flows (According to Bernoulli principle, pressure is related to the velocity. Velocity difference mean pressure difference. It may provide an unexpected effect on the separation.), the gravitational force is used as a power supply to accelerate the fluid.

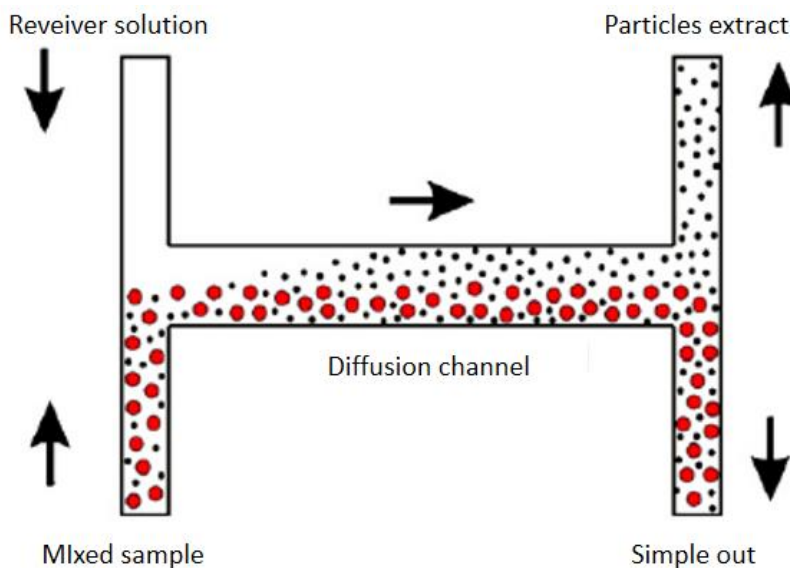


Figure 2. Structure diagram of H-filter

This device does not have a high efficiency. Although the separation can increase the purity of motile sperms from about 42% to over 95%, the separation rate of this device is still not high enough, causing relatively low efficiency.

As the motile cells are the only moving particles in the solution, the purity can be very high, so the result of separation is very accurate [6, 9]. This device does not require external power, so no other electronic devices such as motors are needed. Without these things, the structure can be designed very simply and the size of it can be very small.

The application of this kind of device is largely limited because it requires the target cell have the ability to move. The yield is limited. Its principle might be the reason for the low yield. While the cells are flowing in the diffusion channel, they can transfer to each section freely, which means that they can go back to their original section. If the diffusion channel is long enough, the number of the motile cells will finally reach equilibrium. The equilibrium is the highest yield that this device can reach.

In order to increase the yield, we can add more outlets for the section of the receiver solution, as show in the Figure 3. A new outlet can lower the number of motile cells that can go back to mixed simple, thus the equilibrium can be distorted and increase the yield. This solution also has some disadvantage such as increase the cost and it will be less effective for the too many outlets.

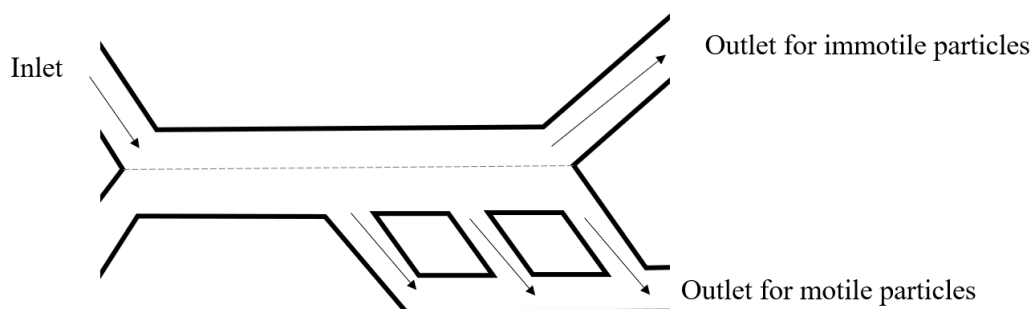


Figure 3. Structure diagram of the solution

2.3. Sorter based on Acoustic

Acoustic can be used to separate different types of particles in a suspension. Acoustic forces in a standing wave can be used to separate the lipid particles from erythrocytes in whole blood. The earlier reported separation channel (750 mm wide and 250 mm deep) was only allowed in a single wavelength standing wave, and using epoxy as interface [10].

The structure of the acoustic-based sorter shown in Figure 5. There is a channel and the end of the channel was divided into three outlets and the particles were collected via the side. The majority of the free medium particle exited through the central outlet.

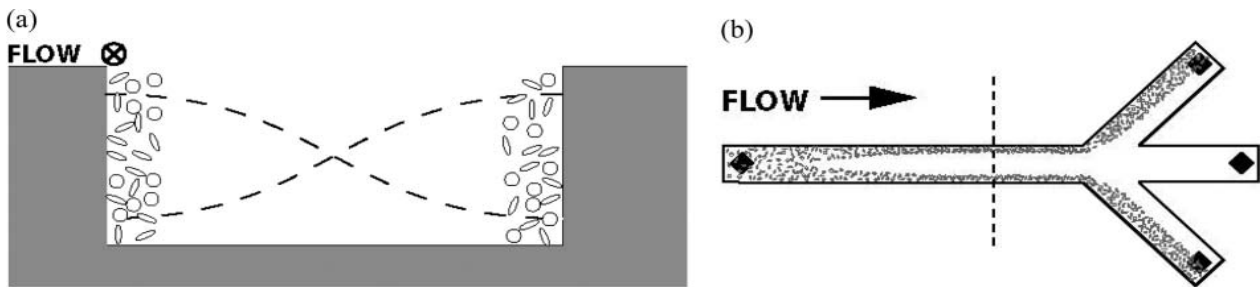


Figure 4. the structure of acoustic-based sorter: (a) particles positioned by the acoustic forces in the pressure anti-nodal plane of a standing wave, (b) top view of a continuous separation of particles.

Nilsson et al [10] improved a separation cavity in silicon by choosing a particular width of the channel to form a standing wave. Due to the nature of the laminar flow, the majority of particles are able to leave at their original place [10]. The force in the liquid will stand in the same direction by changing the pressure in the liquid medium. The direction and size of the force can be estimated by the acoustic force theory [11]. The size F_r and direction ϕ of the force can be estimated by the acoustic force theory, which are expressed as in Eq. (1)

$$F_r = - \left(\frac{\pi p_0^2 V_c \beta_w}{2\lambda} \right) \phi(\beta_c, \rho) \sin(2kx) \quad (1)$$

Where β_w is the compressibility of the medium, λ is the ultrasonic wavelength, p_0^2 is the square of the pressure amplitude, ϕ is a dimensionless constant, V_c is the volume of the particle, k is defined by $\frac{2\pi}{\lambda}$ and x is the distance from a pressure node, x is the distance from a pressure node.

For the direction (ϕ) of the force can be calculate as in Eq. (2)

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

Where ρ_w and ρ_c are the densities of the medium and particles respectively, β_w and β_c are the compressibility of the medium and the particles, respectively.

If the channel diameters are small the laminar flow will be provided when the flow rate is low enough. Particles will be affected by the acoustic forces and move towards either the pressure node (Fig. 4a) or the pressure anti-nodes (Fig. 4b).

It is reported that the separation efficiency of polyamide spheres is almost 100%. It could remove more than 80% of the lipid particles and collect approximately 70% of the erythrocytes in one-third of the original fluid volume [12]

The new design (350 mm wide and 125 mm deep) allowed the system to use a half wavelength standing wave. In this way even the Reynolds number changes, it will still be laminar flow. Returning the blood to patients reducing the demand for blood it reduces transfusion-transmitted disease and immunologic reactions to blood. Ultrasound gel was used as an interface between the piezo ceramic element and the silicon. The advantage of not gluing the transducer to the chip was the possibility to reuse the same transducer several times. It is possible to reuse the same transducer several times [12].

The existing method uses the principle of centrifugal. However, the method has following shortcomings. Firstly, it cannot remove all of the lipids efficiently. Secondly, hemolysis will happen during the centrifugation process, Because the erythrocytes experience high gravitational

forces [13]. At last, the process is not continuous which makes a not suitable for many applications. The volume is separated once is not enough. It may be a question of the development of the method.

2.4. Spiral channel sorter with micro-obstacles

The spiral channel sorter with micro-obstacles sorter is spiral shaped and has eight layers from inside to outside. The channel structure is known as *dimension-confined spiral channel*, or D-channel, according to figure 6 below [14]. In each layer, there are many micro-obstacles, with different shape, which influences the streams. And, the detailed shapes of different obstacles are recorded in figure 7. Original sample enters the device via the inlet at the center of the device; after the separation, sample will be sorted into three isolated laminar flows. And, the result will leave the device via three different outlets.

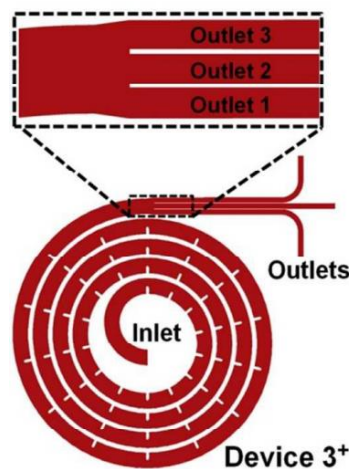


Figure 5. Structure of spiral channel sorter, channels are spiral to create dean drag force, and sample will be sorted into 3 outlets at the ends

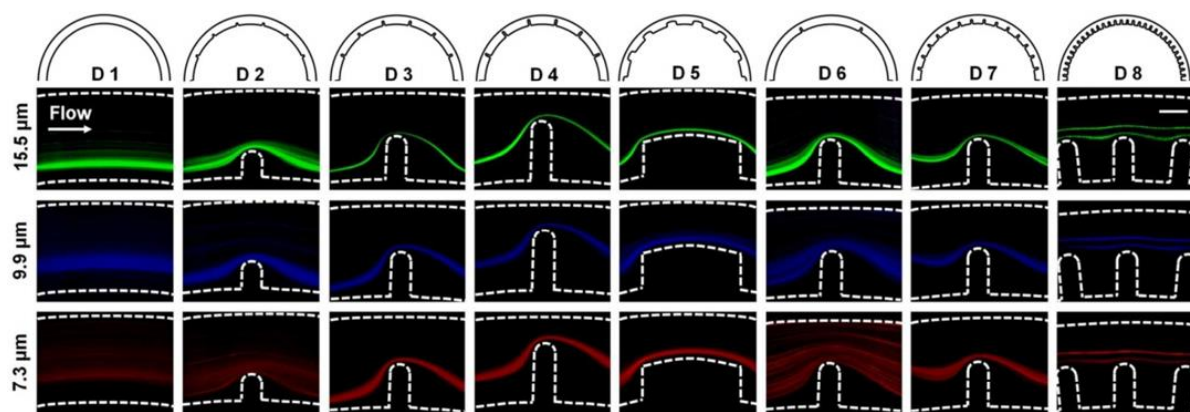


Figure 6. Micro-obstacles in different layers, and their influences on particles with different sizes.

In the channel of the devices, the buoyancy and gravity cancel each other out, so the dominant force to the cell are: net inertial lift force (F_L) and dean drag force (F_D) For the net inertial lift force can be calculated as in Eq. (3)

$$F_L = \frac{\rho U^2 a^4}{D_h^2} f_L \quad (3)$$

Where ρ is fluid density, U is max velocity in the channel, a is particle diameter, h is height of the pipe, w is the width, and f_L is lift constant.

Especially for D_h , hydraulic diameter can be calculated as in Eq. (4)

$$D_h = \frac{2hw}{h+w} \quad (4)$$

For the dean drag force can be calculated as in Eq. (5):

$$F_D = 3\pi\mu \times 1.8 \times 10^4 \left(\text{Re} \sqrt{\frac{D_h}{2R}} \right)^{1.63} a \quad (5)$$

Where R is the curvature radius of the channel path.

The ratio $\frac{F_L}{F_D}$ decides the vertical location of particles, the higher ratio represents particles are closer to the inner wall of the device. This ratio is mainly decided by the size of the particles: $\frac{F_L}{F_D} \propto a^3$

In sum up, the particles in the sample with larger diameter will locate at the inner wall, and the smaller one will locate at the outer wall in isolated laminar streams.

In the whole sorting process, the Reynold number in the channel should be small enough to guarantee that streams in the channel are all laminar flows, so F_D is small enough and sorted streams are isolated from each other.

The device is highly accurate because it can make sure 100% particle focusing, and produce result with high purity, about 98.4% to target cells.

In addition, the sorting rate of this device is about 3ml/min, which is one of the fastest sorters.

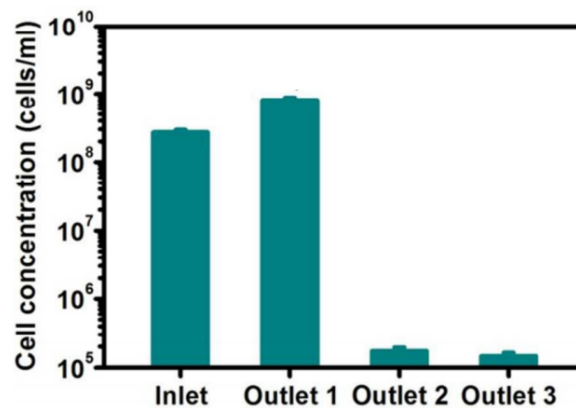


Figure 7. This chart reflects the cell concentration in the sample before and after passing the sorter.

Spiral channel sorter can be used to sort various cells from their original samples because the location of cells is depended on their sizes. And, the device can produce the result more than 95% for separating MCF-7, Hela, K562 cells/. Besides, the structure is relatively simple, because it doesn't require sheath flow.

Indeed, the device based on the size of the particles have simple structure and fine generality. However, it cannot deal with the sample with similar-sized particles. In the channels, the manufacture of micro-obstacles might be a challenge as well, because of its extremely small size and different shape. So, the manufacture might require precise machines, which is not apply to some developing regions well.

3. Conclusion

In conclusion, some sorters based on laminar flow are collected and analysed. Laminar flow is used in these methods for one main purpose: isolating particles from other particles, caused by the unique physical feature of it.

It is noted that the application of laminar flow can bring benefits such as simpler structure and higher purity. On the other hand, utilization of laminar flow will be limited by many environmental

influences, and only applied in some specific circumstances. For instance, the gravity and the size of particles are crucial limitation for these types of sorters.

To achieve the goal of high generality on size of particles and environmental forces, more improvements have to be done, and these improvements may also cause the sorter harder to manufacture. Some potential solutions can be:

- More detailed channels in the sorter for producing more isolated streams with different Reynold number.
- To against the environmental force such as the gravity, electromagnetic can be applied on some cells.

References

- [1] Shen, Y., Yalikun, Y., Tanaka, Y. (2018) Recent advances in microfluidic cell sorting systems. <https://doi.org/10.1016/j.snb.2018.11.025>.
- [2] Encyclopædia Britannica, inc. (n.d.). (2018) Laminar flow. <https://www.britannica.com/science/laminar-flow>.
- [3] Herzenberg, L.A., Parks, D., Sahaf, B., Perez, O., Roederer, M., Herzenberg, L.A. (2002) The history and future of the fluorescence activated cell sorter and flow cytometry, A view from Stanford. *Clin. Chem.* 48: 1819 – 1827.
- [4] Hulett, H.R., Bonner, W.A., Barrett, J., Herzenberg, L.A. (1969) Cell sorting: automated separation of mammalian cells as a function of intracellular fluorescence. *Science* 166: 747 – 749.
- [5] Gossett, D.R., Weaver, W.M., Mach, A.J., Hur, S.C., Tse, H.T.K., Lee, W., Amini, H., Di Carlo, D. (2010) Label-free cell separation and sorting in microfluidic systems. *Anal. Bioanal. Chem.* 397: 3249 – 3267.
- [6] Bhagat, A. A. S., Bow, H., Hou, H. W., Tan, S. J., Han, J., & Lim, C. T. (2010) Microfluidics for Cell separation. <https://link.springer.com/article/10.1007/s11517-010-0611-4>.
- [7] Carlo, D. D., Irimia, D., Tompkins, R. G., & Toner, M. (2007) Continuous inertial focusing, ordering, and separation of particles in microchannels. *PNAS*. <https://www.pnas.org/content/104/48/18892>.
- [8] Carlo, D. D. (2009) Inertial microfluidics. *Lab on a Chip*. <https://pubs.rsc.org/en/content/articlelanding/2009/lc/b912547g>.
- [9] Cho, B. S., Schuster, T. G., Zhu, X., Chang, D., Smith, G. D., & Takayama, S. (2003) Passively driven integrated microfluidic system for separation of motile sperm. *ACS Publications*. <https://pubs.acs.org/doi/10.1021/ac020579e>.
- [10] Nilsson, A., Petersson, F., Jonsson, H., and Laurell, T. (2004) *Lab Chip* 4: 131 – 135.
- [11] Nyborg, W. L. (1978) in *Ultrasound: Its Applications in Medicine and Biology*, ed. F. J. Fry, Elsevier, New York, Part 1, pp. 1 – 76.
- [12] Petersson, F., Nilsson, A., Holm, C., Jönsson, H., & Laurell, T. (2004) Separation of lipids from blood utilizing ultrasonic standing waves in microfluidic channels. <https://pubs.rsc.org/en/content/articlelanding/2004/an/b409139f>.
- [13] Klodell, C. T., Richardson, J. D., Berdamini, T. M. and D. A. Spain, *Am. Surg.* (2001) 67(1): 44 - 47.
- [14] Shen, S., Tian, C., Li, T., Xu, J., Chen, S.-W., Tu, Q., Yuan, M.-S., Liu, W., & Wang, J. (2017) *Spiral microchannel with ordered micro-obstacles for continuous and highly-efficient particle separation*. *Lab on a Chip*. <https://pubs.rsc.org/en/content/articlelanding/2017/lc/c71c00691h>.