7 µm AND 15 µm BEADS SORTING USING A MICROFLUIDIC PLATFORM

INTRODUCTION

Many research applications call for sorting and isolating cells from a heterogeneous cell mixture. This can be for purifying cell samples to improve efficiency in research applications and is a critical step in many diagnostic and therapeutic practices[1]. The expanding need to isolate rare cells such as circulating tumor cells (CTCs) from blood samples increases the demand for cell sorting devices. Cell detection is generally performed using optical methods such as FACS (Fluorescent Activated Cell Sorting). As it is an automated solution for cell sorting, it was considered a gold standard for decades. FACS has however, significant limitations, including the need for a large number (millions) of cells. This limits the volume of solutions to be sorted. A labeling step is required, and the high operating pressures required can affect cell function or viability. FACS systems are also cumbersome, expensive, and require experienced users. As an alternative, microfluidic spiral sorting devices allow one to passively sort cells with high throughput (>1.5 mL/min) without the need for a labeling step.

Why use microfluidics for cell sorting?

As opposed to conventional instrumentation, microfluidic devices are easy to use, smaller, versatile, and affordable. Microfluidic cell sorting can be combined with additional fluidic operations for complete lab-on-a-chip applications, as well as for diagnostic and therapeutic purposes. These devices make use of a wide range of techniques to sort cells with specific speeds and efficiencies. The possibility to easily tune the design of the microfluidic device allows for the sorting of cells of different sizes with throughput (flow rate) specific to the user's need.

Table 1: Advantages and disadvantages of cell sorting using FACS and microfluidics

	Standard FACS methods	Fluigent & *microfluidic ChipShop* solution
Control on experimental parameters	Medium	High
Throughput	Very high	High (>1,5 mL/min)
Automation	Yes	Possible
Pressure in use	High (can affect cells)	Controllable
Price	Very High	Medium
Device size	Bulky	Small

Cell sorting is based on determining a specific cell parameter that can differ from one cell type to another, such as cell size, shape, or density. The device is designed such that cells with different properties experience a different amount of force (that could originate from inertia, channel geometry, external source) while similar cells undergo equal forces. The differentiated cells are subsequently pushed into different streamlines and exit the chip from different outlets.

The advantages of passive cell sorting systems

Passive cell sorting systems do not require labeling steps nor additional external power (e.g. electrical field), which makes them attractive. Microfluidic devices can sort cells due to specific geometric designs. Channels with spiral shapes are used to separate particles according to their size based on the Dean forces.

The main benefit of this design is high throughput (>1.5 mL/min) without the need for sheath flow or sequential cell manipulation. Channel dimension, number of spirals, and diameter of the curvature influence the sorting effect. Flow rate is also a key parameter as the Dean and lift forces depend on it[2]. It is important to use well-designed microfluidic devices and excellent flow control for sorting experiments. Fluigent and *microfluidic ChipShop* validated an effective and commercial solution for cell sorting.

Our cell sorting platform consists of a cell sorter device from *microfluidic ChipShop* and flow controllers from Fluigent to maintain precise flow control. To demonstrate the separation of particle mixtures, a solution containing 7.5 µm and 15 µm diameter polystyrene particles labeled with FITC and TRITC fluorophores respectively, was used. The particle streams were viewed and captured separately using appropriate filter cubes.

MATERIALS AND METHODS

Material

Microfluidic flow controller

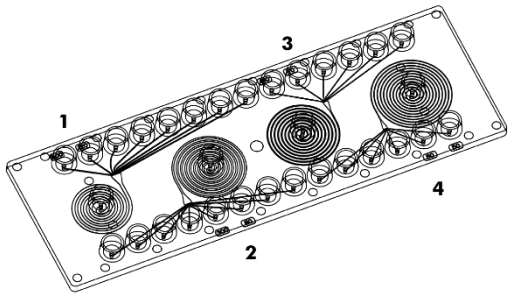
The Flow EZ is the most advanced flow controller for pressure-based fluid control. One Flow EZ with 7 bar is used in the system. It can be connected to a LINK for software control.

Flow sensor

The Flow Unit is a flow sensor that allows real-time flow rate measurement. By combining a Flow Unit with Flow EZ, it is possible to switch from pressure control to flow rate control. Flow Units L and XL are used depending on the targeted flow rate.

Microfluidic device

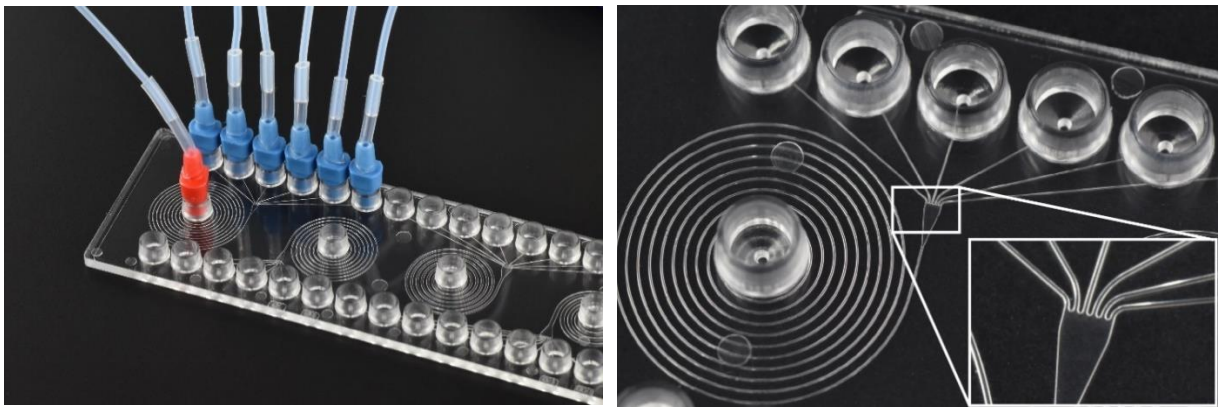
The spiral sorter chip Fluidic 382 features four individual sorting units. Each sorting unit possesses one inlet to introduce the fluid stream and six to eight outlets to collect sorted particle fractions. In- and outlets come in Mini Luer format and are interfaced with Mini Luer fluid connectors.



Picture heading: ***Schematic drawing of spiral sorter Fluidic 382 with sorting four units***

Sorting unit	Number of turns	Number of outlets	Channel With [µm]	Channel Depth [µm]	Channel Length [mm]
1	4	8	500	120	82
2	8	8	300	80	166
3	9	6	150	70	195
4	12	6	80	50	270

Structure 2 and 3 were used in this application note.



Picture heading: ***(left) Sorting unit 4 connected with tubing using Mini Luer fluid connectors (red: inlet; blue: outlets); (right) Close up of spiral sorting unit 4 with detailed view of the "sorting claw", feeding individual outlet channels.***

Reagents (micrometer beads)

Fluorescently labeled polystyrene particles purchased from Bangs Laboratories. 7.3 µm (+/- 0.53 µm) with red fluorescence and 15.25 µm (+/- 0.669 µm) with green fluorescence.

Optics

The surfaces are monitored using an inverted microscope (Eclipse Ti-U, Nikon) in bright field or in fluorescence mode. For the acquisition of the fluorescent beads, we use the software NIS-Elements with standard Nikon filter cubes for FITC and TRITC.

Protocol for micrometer beads size sorting

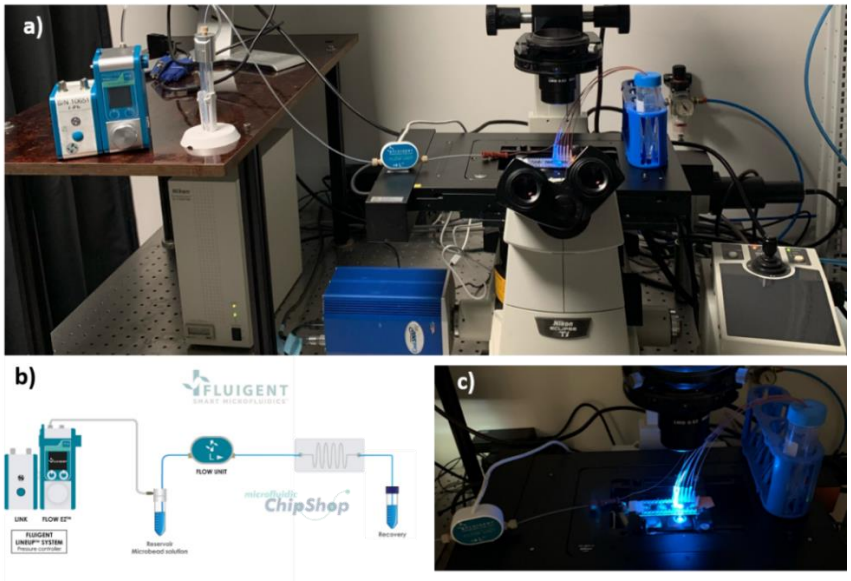


Figure 1: a) Complete system b) Schematic of the system c) Close up of the microfluidic chip

The system for cell sorting is shown in figure 1. The 7.3 µm and 15.25 µm fluorescent microbeads are mixed and diluted in DI water before testing to reach a concentration of ~1.10^5 beads/mL. The sample is homogenized using a vortex mixer. The Flow EZ is connected to the reservoir containing the mixture using a P-CAP. The reservoir is connected to the inlet of the microfluidic device using 1/32 and 1/16 outer diameter (OD) tubing of 500 µm inner diameter (ID). 1/32 and 1/16 tubing are connected using a MicroTight adapter. Tubing passes through the Flow Unit to control and monitor flow rate. Tubing is connected to the outlets of the microfluidic device to recover the beads. The flow rate is set using the Flow EZ. Here, sorting units 2 and 3 with flow rates of respectively 1.5 mL/min and 150 µL/min are used. The particle streams were viewed and captured separately using TRITC and FITC filter cubes. The individual images were superimposed to create a composite image to display the formation of two separate focused particle streams.

	Sorting unit 2	Sorting unit 3
Flow rate	1.5 mL/min	150 µL/min

Working principle of cell sorting using spiral channels

Particles that flow in a spiral microchannel with rectangular cross-section experience a combination of Dean drag forces and an inertial lift. Dean drag forces are a consequence of fluids experiencing centrifugal acceleration. This acceleration leads to the formation of rotating vortices known as Dean vortices in the top and bottom halves of the channel (figure 2 a)). The net lift force originates from shear-induced and wall-induced lift forces. Additional information can be found in some research papers[2,3]. The position at which particles of different sizes equilibrate depends on the ratio of lift and Dean drag forces. This results in several particle streams that are size-dependents, and that can be extracted by designing appropriate outlets (figure 2 b)).

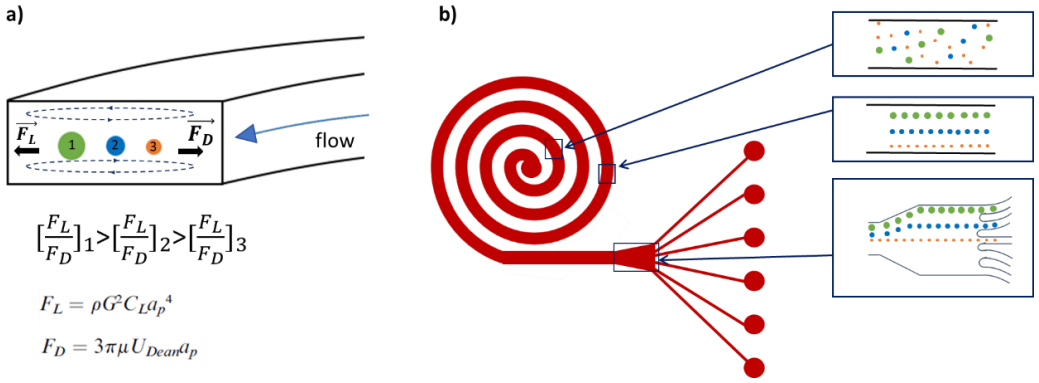


Figure 2: a) Channel cross-section and illustration of the effects of lift (F_L) and Dean forces (F_D). The position at which particles of different sizes equilibrate depends on the ratio F_L/F_D. More details about lift and Dean forces equations can be found in the literature[2] b) Schematic drawing of spiral sorter Fluidic 382. The randomly dispersed particles equilibrate at different equilibrium positions along the inner wall of the spiral microchannel.

RESULTS & DISCUSSION

As previously discussed, a homogeneous mixture of 7.3 µm and 15.25 µm fluorescent microbeads are injected into the central inlet of the microfluidic chip using flow rates of 1.5 mL/min for unit 2 and 150 µL/min for unit 3. Figure 3 a) and b) show composite images of the microfluidic channels in a part of the spiral and at the outlet of the chip using bright field, green, and red fluorescence. Figure 3 a) clearly indicates the formation of two distinct particle streams, confirming that 7.3 and 15 µm microbeads are well separated. In addition, as predicted[2], we observe the larger particles (green fluorescence, 15 µm) closer to the inner channel. We can observe in figure 3 b) that the green fluorescence particles are in great majority collected at the first and second outlets of the microfluidic device, while red fluorescence particles are collected at the fourth outlet of the same sorting unit. This result confirms that 7.3 µm and 15 µm particles are well separated and subsequently sorted by the microfluidic device with very high throughput.

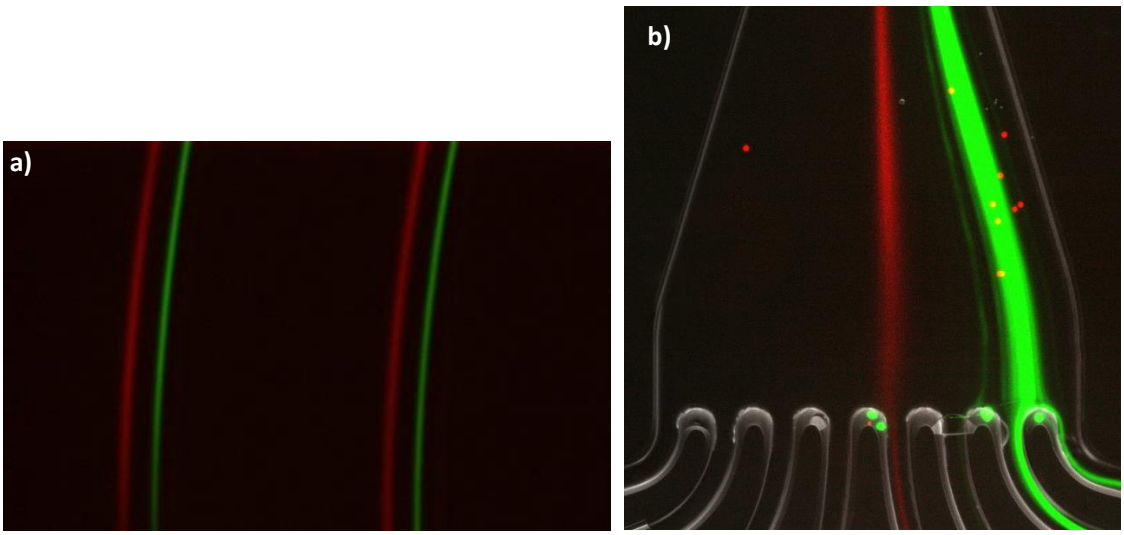


Figure 3: Composite images illustrating the position of the 15 µm (green fluorescence), and 7.3 µm (red fluorescence) diameter particles in the channel section a) prior to the outlet, and b) at the outlet of unit 2 of the microfluidic device. Flow rate: 1.5 mL/min.

It can also be of interest to sort cells using lower flow rates, as some cell types can be affected by the shear stresses generated by high flow rates. We repeated the experiment using sorting unit 3 of the Fluidic 382, which has smaller channel dimensions compared to unit 2. Figure 4 shows composite images of the microfluidic channel in a part of the spiral of the channel of unit 3. We clearly observe two well-separated streamlines, confirming that 7.3 and 15 µm microbeads are also well separated at 150 µL/min using the appropriate combination between channel geometry and flow rate.

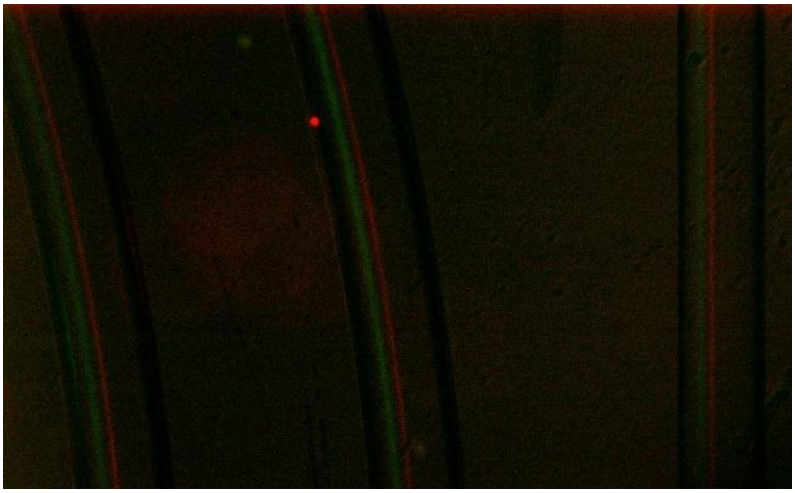


Figure 4: Composite images illustrating the position of the 15 µm (green fluorescence), and 7.3 µm (red fluorescence) diameter particles in the channel section prior to the outlet of unit 3 of the microfluidic device. Flow rate: 150 µL/min.

CONCLUSION

In this application note, we introduced a commercially-available microfluidic system to perform passive size separation of a microparticle mixture. The system includes a spiral-shaped microfluidic device from *microfluidic ChipShop* and pressure-based flow controllers from Fluigent. Tuning Dean and lift forces induced by spiral microchannels allow to obtain distinct particle streams and subsequently sort particles according to their sizes. Beads with diameters of 15 and 7.3 µm were successfully sorted using flow rates of 150 µL/min and 1.5 mL/min. We provide an easy-to-use, versatile, and cost-effective solution for particle size sorting.

REFERENCES

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