CELL/PARTICLE MANIPULATION AND COUNTING ULTILIZING OPTICAL TWEEZER AND DIGITAL IMAGE PROCESSING UNDER MICROFLUIDIC CONFIGURATION

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Abstract: This paper proposes a novel microfluidic system for cell/microparticle recognition and manipulation utilizing a digital image processing technique (DIP) controlled optical tweezer under microfluidic configuration. Cell/microparticle samples are firstly electrokinetically sorted in a microfluidic channel and pass through an image detection region. Digital image processing technique is used to count and recognize the cell/particle samples and then sends control signals to generate laser pulses to manipulate the target cell/particles optically. The optical tweezer system is capable of catching, moving and switching the target cells within the microfluidic channel. The trapping force of the optical tweezer is also demonstrated utilizing the relationship between Stocks-drag force of microparticles and the applied electroosmotic flow. The proposed system provides a simple but high-performance solution for microparticle manipulation in a microfluidic device.

Introduction

In the past decade, MEMS (Micro-Electro-Mechanical-Systems) applications have made a significant impact within the field of bio-analysis, e.g. in permitting the development of microfluidic systems for cell samples sorting, counting and manipulating, etc.[1, 2] Micro-fabricated devices are highly advantageous within bio-analysis applications since they require smaller sample and reagent volumes, facilitate a more rapid detection performance, and tend to be more efficient than conventional techniques. However, there is generally a requirement for integration of various kinds of function in a single chip such that the design and fabrication of the microchip device is complicate and cost ineffective.

Cell manipulation and separation technique is usually required for sorting specific cells in many bioanalytical applications. Traditionally, a large scale flow cytometry is adopted for this application. However, the system is bulky, expansive and not easy accessing. On contrast, Ashkin et al.[3] demonstrated trapping dielectric particles by using a single-beam gradient force in 1986. Optical tweezing scheme then became a powerful method for manipulating microparticles in cell scale. Recently, Enger et al.[4] combined the optical manipulation technique and microfluidic device to sort cells in a microfluidic channel. Cell was firstly captured using a focused laser beam and then was moved toward another direction. The through-put of their approach was limited since a single cell manipulation method was applied. Alternatively, cell sorting utilizing micromachined acoustic transducers[5], magnetic forces[6], and dielectrophoretic forces[7] have been successfully demonstrated. However, the fabrication of these approaches relies on an expensive thin-film deposition process to construct the metal electrode such that the procedure is complicated, time-consuming, and cost ineffective.

This study describes an integrated system for cell manipulation under microfluidic configuration. This approach includes an eletrokinetically focusing method, a DIP technique and a home-built optical tweezer for in-line cell/microparticle recognition and manipulation. Cell samples are continuously detected and recognized in microchannel. The target cells are then switched into another sample stream and sorted downstream. In addition, the catching force of the proposed optical tweezer is evaluated using a simple and novel Stork's drag method. The outcome of the proposed method can provide an easy way to continuously detect and sort cell sample in a small system and will give substantial impact in the field of bioanalytical applications.

Optical Tweezers Trapping Force Measurement

In order to evaluate the transverse trapping force of the optical tweezer system, a simple method utilizing electroosmotic flow induced viscous drag force of microparticles was proposed. Because of planar flow pattern of EOF flow in microfluidic channel, it is capable of generating stable Stork's drag force on the

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microparticle. The drag force acts on a microparticle, F_{drag}, can be determined by the Stoke's Law:

$$F_{drag} = 6\pi r n h \quad (1)$$

Where r is the particle radius, n is the flow velocity, and h is the dynamic viscosity of water (h = 1.025 x 10^{-3} N·sec/m^2).

On the other hand, the flow velocity of the electroosmotic flow for inducing the drag force is proportional to the applied electrical field and can be easily controlled. The electroosmotic flow velocity V_{eof} is given by

$$V_{eof} = m_{eo} E_{el} \quad (2)$$

Where m_{eo} is the electroosmotic mobility of the fluid and E_{el} is the applied electric field strength. During operation, one particle is firstly trapped by the focusing laser beam in a fluid within a microchannel, and then slowly increases the applied electric field strength to enhance the flow velocity of buffer liquid. When the particle escapes from the trapping point of laser beam, the applied velocity is so called "Max velocity". Since the trapping force of the optical tweezer is equal to the drag force acted on the particle, the trapping force of the proposed optical tweezer system can be evaluated by measuring the applied electric field.

Design and Fabrication

Figure 1 illustrates a schematic diagram of the working principle for the proposed optical sorter system. Cell samples are firstly focused electrokinetically and then pass through a DIP detecting area and the optical tweezer switching area. Target cells can be optically switched out of their original flow stream into the neighboring sheath flow. Sorted cell samples can flow in the laminar sheath flow stably and then colleted by reservoirs downstream.

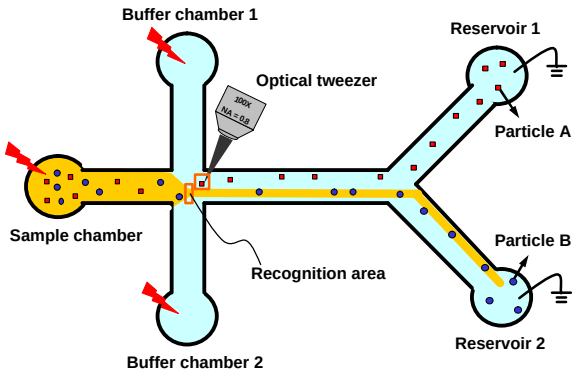

Fig. 1 A simplified schematic diagram for the working principle for cell/particle sorter.

Since the samples are to be electrokinetically driven in the present study, a silanol-group rich material such as glass is preferred as the substrate material. Fig. 2 presents a schematic illustration of the simple but reliable process developed for the fabrication of planar microfluidic channels on soda-lime glass substrates. The details of the fabrication process was presented in our previous work[8]. Initially, a thin layer of AZ 4620 photoresist was applied onto the glass substrate and then patterned using a standard photolithography process (Figure 2(a)). The patterned photoresist layer was hard baked and then used directly as a mask in the etching of the glass substrates in a commercially available buffered HF (buffered oxide etchant, J. T. Baker, USA) for 45 minutes (Figure 2(b)). The etched glass substrates were then immersed in a diluted KOH solution (KOH (45%):DI=1: 9, 80°C) to remove the photoresist layer (Figure 2(c)). Meanwhile, fluid via holes were drilled in the etched glass slide using a diamond drill-bit of diameter 1.5 mm (Figure 2(d)). Both glass substrates were cleaned in a boiling Piranha solution and then carefully aligned (Figure 2(e)). Finally, the substrates were fusion bonded in a sintering oven at a temperature of 580 °C for 10 minutes with an applied pressure of approximately 4.1 × 10^5 Nt/m^2. (Figure 2(f))

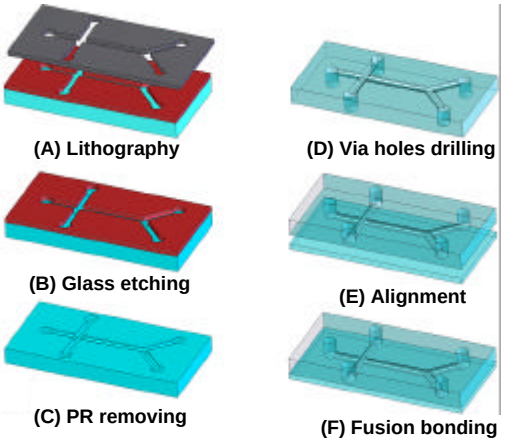

Fig. 2 Fabrication process of glass-based microfluidic chip.

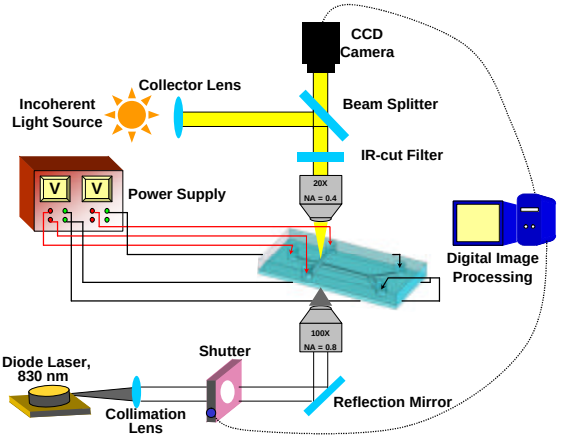

Fig. 3 A schematic diagram of the experimental system setup.

Experimental

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Figure 3 presents a schematic diagram of the proposed system. This system is mainly composed of two microscopes with a confocally alignment. An 100X objective lens (NA=0.8, Olympus, Japan) is adopted for coupling the laser light from an IR laser diode (830 nm, SDL-2364-L2, JDS Uniphase, USA) to form the optical force gradient. The IR laser diode is connected to a 10X collimator lens via an integrated optic fiber with a SMA connector. One another 20X objective lens (NA=0.4, Olympus, Japan) is used for observation and image collection purposes. Images are acquired using a CCD camera (DXC-190, Sony, Japan) with a high-speed image acquisition interface (DVD PKB, V-gear, Taiwan). Since this system is driven by electrokinetic pumping, a high voltage power supply (P-3500, Major science, Taiwan) was used for sample driving and a buffer solution of 10 mM sodium borate (pH=9.2, Showa, Japan) was used as the sample fluids. In stead of using living cell sample, microparticles of 10-μm and 12-μm polystyrene (PS) beads (Duke Scientific Corp., CA, USA) are tested in this study. The diameter and physical properties of the chosen polystyrene bead are closed to the properties of red blood cells such that PS bead is a good constituent for the living cells.

Figure 4 shows the developed graphical user interface (GUI) constructed using MATLAB® software for real-time DIP recognition purpose. An user-defined area is adopted in the microchannel for particle sample recognition and number counting simultaneously. The recognition protocols can be defined either particle size, color or even the shape of the cell/particle. DIP system counts and recognizes the cell/particle samples and then sends control signals to generate a laser pulses to manipulate the target cells/microparticles optically.

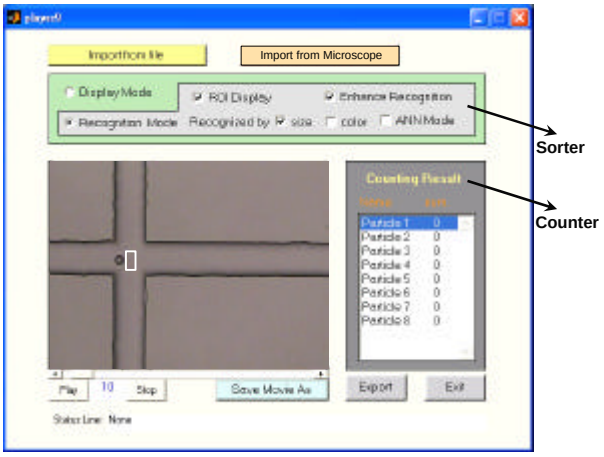

Fig. 4 The developed graphical user interface (GUI) for real-time digital image processing.

Results

Figure 5 (A) shows the relationship between the maximum EOF velocity and the corresponding applied laser power for microparticle capturing. The transverse trapping force on the microparticle can be determined by Eq. 1. The relationship of the corresponding generated force gradient versus the applied laser power is presents in Fig. 5 (B). Results show that the trapping force is direct proportional to the applied laser power. In addition, a larger particle receives larger trapping force than a smaller particle. Results also indicated that a laser power of 100 mW is large enough for changing the direction of a moving cell/particle with the flow speed of 80 μm/s in the microchannel. Furthermore, in order to prevent the cell/particle from the damage of the focus laser beam, a small laser power is preferred. In this study, a laser power of around 100 mW is used for the cell switching purpose.

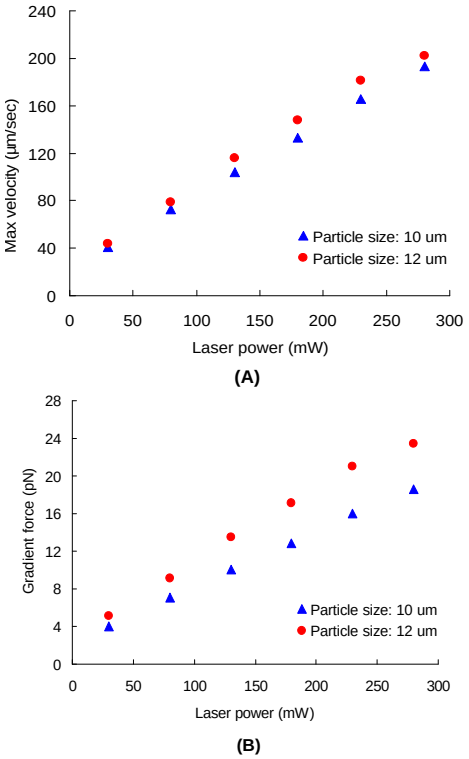

Fig. 5 The relationship between the applied laser power versus the maximum EOF velocity and the corresponding generated force gradient.

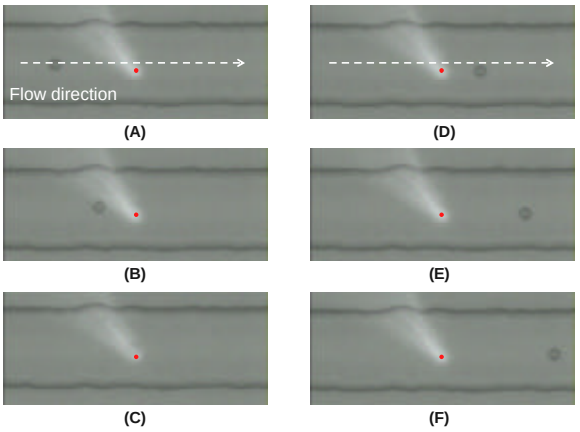

Fig. 6 Continuous images of the flow path of a microparticle changed by the optical tweezer system.

Figure 6 presents the continuous images of the optical tweezer system successfully changes the flow

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path of a 10-μm polystyrene microsphere with flow speed of 180 μm/sec. The dash line represents the original flow path of the particle and the dot illustrates the active point of the laser tweezer. The result indicates that a 220 mW output power is large enough to shift the microsphere 10-μm away from its original flow path. It is clearly observed that the microparticle was pulled by the optical gradient and moved away from its original flow path about half of the particle size. This result confirms that the flow direction of a moving particle can be changed in the microchannel without stopping the speed of the particle. Therefore, sorting microparticles continuously in the microchannel can be achieved with this approach only if we can switch the microparticles into the sheath flow.

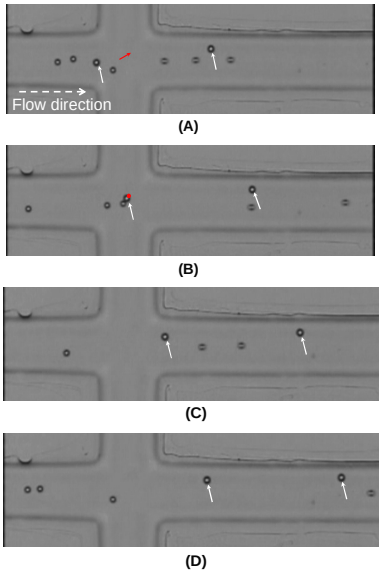

Fig. 7 Continuous images of sorting 12-μm PS beads out of a sample mixture composed of 10 and 12-μm PS beads. Note that the 10-μm PS beads flow in the focused sample stream located at the center of the microchannel and the 12-μm PS beads were switched into the neighboring sheath stream and flowed stably to the downstream.

Figure 7 shows the continuous images of the proposed system for sorting of 12-μm PS beads out of a 10-μm PS bead mixture. Note that the applied electric fields for the sample stream and sheath flow were 120 V/cm and 130 V/cm, respectively. The focus width of the cell flow stream was around 12 μm. The white arrow indicates the particle of 12-μm PS bead and the red arrow means the flow path of the microparticle attracted by the laser gradient. The added spot in fig. 7b illustrates the active area of the optical tweezer. In this experiment, the sample flow rate is 97 μm/sec and the output power of the laser beam is 105 mW. In this figure, it is clear observed that the 12-μm PS beads were successfully switched from the center flow steam into the neighboring sheath flow and stably flowed in the sheath flow stream. The current result confirms the feasibility of the proposed integrated system to manipulate and sort microparticle in microfluidic channels.

Conclusions

This study has successfully integrated an optical tweezer, digital image processing techniques and a microfluidic chip into a fully system for cell/microparticle sorting, manipulation and counting. Thus, we have also proposed a novel method to measure the trapping force of optical tweezer. The integrated system has successfully sorted different microparticle in diameter with 10-μm and 12-μm, respectively. The proposed system is feasible of high-throughput catching, moving, manipulation and sorting specific microparticles/cells within a mixed sample and results in a simple solution for cell/microparticle manipulation in the field of micro-total-analysis-systems.

Acknowledgments

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