Optical Manipulation of Objects and Biological Cells in Microfluidic Devices

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Abstract. In this paper, we review optical techniques used for micro-manipulation of small particles and cells in microfluidic devices. These techniques are based on the object's interaction with focused laser light (consequent forces of scattering and gradient). Inorganic objects including polystyrene spheres and organic objects including biological cells were manipulated and switched in and between fluidic channels using these forces that can typically be generated by vertical cavity surface emitting laser (VCSEL) arrays, with only a few mW optical powers. T-, Y-, and multi-layered X fluidic channel devices were fabricated by polydimethylsiloxane (PDMS) elastomer molding of channel structures over photolithographically defined patterns using a thick negative photoresist. We have also shown that this optical manipulation technique can be extended to smaller multiple objects by using an optically trapped particle as a handle, or an “optical handle”1. Ultimately, optical manipulation of small particles and biological cells could have applications in biomedical devices for drug discovery, cytometry and cell biology research.

Key Words. manipulation, optical tweezers, microfluidic devices

Introduction

In recent years, efforts have been focused on miniaturization of systems for biological and chemical analysis which resulted in a number of lab-on-a-chip technologies useful for applications in combinatorial chemistry (Paulus et al., 2000; Roberts et al., 1999), immunoassay analysis (Richardson et al., 2001), PCR amplification (Khandrina et al., 2000), DNA analysis (Buccholz et al., 2001), and implantable drug systems. Such devices, which generally utilize electrostatic, magnetic, and micro-mechanical forces, can significantly enhance the speed of analysis while utilizing ultra small sample and reagent volumes of biological samples, on the order of a few nanoliters. In the last decade, well practiced silicon microfabrication techniques have been the primary means of device fabrication. More recently, less expensive plastic micro-replication techniques, glass micromachining and PDMS (polydimethylsiloxane) molding techniques are being used (Xia et al., 1998). Microfluidic devices utilize electrostatic, magnetic and micro-mechanical forces for inducing the flow of fluids and biological media into the channels. New device technologies are being explored for the controlled manipulation of biological cells for applications in forming cellular arrays for biological research, cancer cell cytometry and drug discovery studies (Chu et al., 2000). Cell membranes are highly deformable and excessive shear stresses induced during fluid flow can cause rupture of the membrane or destruction of the cell. Depending on the specific application, high throughput cell handling might be required (such as in cell cytometry) which may require parallel manipulation of cells. In this paper, we present two techniques for handling objects in microfluidic devices, based on optical gradient and scattering forces. We show the use of an array of optical tweezers for parallel manipulation of objects in microfluidic systems. We demonstrated the feasibility of these techniques via experiments conducted using polystyrene microspheres manipulated in PDMS molded devices. We conclude that these techniques will benefit applications in cell characterization, sorting and array formation for cell based biological research.

Microfluidic Device Fabrication

The technique presented involves the application of optical tweezers to apply photonic pressure on organic and inorganic objects in fluidic channels, which requires that the fabricated devices be transparent. For fabricating the fluidic channels, we have used PDMS which is a silicone based and biocompatible elastomer material. A schematic of the fabrication process is shown in Figure 1.

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An inverse layout of the microchannel patterns is generated using conventional photolithography techniques with a thick negative UV photore sist (XP SU-8 25, MicroChem Corporation, Newton, MA) on silicon wafers (Xia et al., 1998). A PDMS elastomer kit (Sylgard 184, Dow Corning, Midland, MI) is used which consists of a prepolymer solution and curing agent that are mixed at a ratio of 10:1 and degassed in a vacuum desiccator. The prepolymer mixture is poured over the silicon master in a plastic petri dish. A transparency film is placed over the mixture and a weight is applied on top. The molding assembly is cured in an oven at 80°C for 3 hours and then for 12 hours at room temperature. After curing, the PDMS layer is peeled off from the silicon master and cleaned with a diluted HCl:DI water (1:5) solution (Figure 2). Inlet and outlet channels as fluidic interconnects are created by coring through the PDMS layer and placing plastic tubes within the cored channels. The soft PDMS layer easily seals around the inserted tubes. To seal the microfluidic channel structures, the patterned PDMS layer is bonded to a thin glass slide.

**Parallel Manipulation of Objects using Optical Tweezers**

Recently, optical tweezer arrays have been generated in several ways including the use of a rapid scan device, diffractive gratings, or a spatial light modulator (Sasaki et al., 1991; Dufrense et al., 1998; Reicharter et al., 1999). All of these techniques make use of a laser beam from a single high powered laser source that is temporally or spatially dividing optical spots that are generated. In the implementation of our optical tweezers arrays, we have chosen to use VCSELs (Kibar et al., 1999; Ozkan et al., 2001, 2002) where each laser in the array is focused and acts as an individual trapping and manipulation source, as depicted in Figure 3. The details of optical setup used here is given else where (Flynn et al., 2002; Wang et al., 2000). Precise uniformity or selective control over each trap can be achieved by appropriately modulating the current to each VCSEL. Such an array provides a potentially inexpensive and compact package, and the device substrate is compatible with other optoelectronic functions that might be desired in a bio-chip such as array detectors. A drawback of VCSELs as optical trapping devices is that they have a relatively low output power, and hence a lower trapping strength, compared to single high powered gas laser. We have demonstrated that this can be compensated for by altering the lasing mode of the VCSELs prior to use. This can be achieved by converting the spatial mode of a proton implanted VCSEL from a Hermite-Gaussian mode to a Laguerre-Gaussian mode through a simple post annealing process (Kibar et al., 1999). Laguerre modes are characterized by their rotational symmetry,

![Image of channel device](image1)

**Fig. 1.** A schematic of the process flow in fabricating PDMS devices.

![Image of channel device](image2)

**Fig. 2.** Microfabricated channel device with A. 40 μm wide, 20 μm deep T-shaped and B. Y-shaped channel structures.
and in higher orders, they can closely resemble the so-called “donut” mode. Shown on the right hand side of Figure 3 are the fundamental Gaussian mode emitted from a VCSEL and the high-order Laguerre mode. The energy of the emitted beam is moved to the outer edge of the aperture where, in an optical trap, photons have the greatest axial restoring force. Energy has been removed from the center of the beam thereby decreasing the detrimental scattering force that acts to push particles out of the trap (O’Neil et al., 2001).

An 850 nm Laguerre mode VCSEL array is used for optical trapping of live biological cells suspended in a buffer solution. Images captured by a CCD camera are shown in Figure 4, in which a $3 \times 3$ array of live yeast cells (about 1–2 μm diameter) and a $2 \times 2$ array of human red blood cells (with 5 μm diameter) are trapped, and translated simultaneously. The 3-dimensional capability of the trap is shown by translating along all axes. Brownian motion alone is insufficient to remove the cells from the trap. The strength of this trap is measured by first trapping and then translating the cells at increasingly higher speeds through the liquid media and observing the point at which the fluidic drag exceeded the optical trapping force. For very low Reynolds numbers ($\text{Re} \ll 1$), the inertial forces can be neglected and the drag force of a uniform sphere moving through a viscous fluid can be approximated by (Ashkin et al., 1970, 1992),

$$F_D \propto 6\pi\eta\upsilon a; \quad \text{Drag force on a sphere,}$$

where, $\eta$ is the fluid viscosity, $\upsilon$ is the sphere velocity, and $a$ is the sphere radius. During trapping force calculations, we assumed the drag force on the sphere to be equal to the optical trapping force. Therefore, for a 10 μm diameter polystyrene microsphere (Bangs Laboratories) and a VCSEL driving current of 18 mA, a maximum drag speed of 6.4 μm/sec is observed, corresponding to a lateral trapping force of 0.6 picoNewtons. Smaller live murine fibroblast cells (< 5 μm) are also shown to be trapped by the VCSEL tweezers, however the strength of the trap is considerably less due to the lower dielectric constant and irregular structure (not a perfect sphere as compared to polystyrene microspheres) of cells (trapping force is predicted as about 0.1 picoNewtons). This limitation is resolved by combining the powers of several VCSEL beams within the large array into a single spot and thus created a higher intensity of light to trap live cells. Recently, we successfully manipulate 20 μm size murine
primary hepatocyte via binning of a $2 \times 2$ VCSEL beam to a single spot (results are not shown here).

**Optical Switching of Objects in Fluidic Channels**

Arrays of optical tweezers can be used for manipulating and directing objects, or “switching”, in microfluidic systems for a variety of applications. For demonstrating this methodology, we have developed an optical switching system in microfluidic channel devices whose concept is illustrated in Figure 5. Biological cells or polystyrene microspheres are transferred into a T-shaped fluidic channel as shown. It is desired that each sample should be sequentially identified through selective fluorescence or by measuring the dielectric constant of the cells. Next, the cells are directed into one of the two branches of the T-channel, depending on its type. Sorting or cytometry is achieved at the junction of the channel by capturing the sample in an optical trap and then drawing it either to the left or to the right of the main channel. Fluidic flow should be controlled such that turbulence or recirculation is prevented at the junction region. This way, the movement of the cell will follow the fluid flow towards the closest branch of the junction. At the end, sorted samples may be collected in a reservoir or recycled for further iterations.

Figure 6 shows a sequence of images of a polystyrene microsphere manipulated and directed in an optical microfluidic switch. T-channels (40 μm wide, 20 μm deep) are fabricated in PDMS based silicone elastomer. Gold electrodes are inserted into the reservoirs at the end of each channel to induce electro-osmosis for enabling fluid flow. These microfluidic devices are placed in an optical tweezers setup based on an 850 nm diode laser. Fluid flow is initiated by applying a DC bias between the electrodes. Polystyrene microspheres flowing down the channels from the top are captured in the optical trap and then translated manually to either side of the main channel (both cases are shown in Figure 6). Since the fluid flow in microchannels is laminar in nature, the microspheres will continue to flow through the nearest side channel when they are released from the trap. Flow rate of fluid is measured about 10 μm/sec. During this experiment 850 nm diode laser is used as light source with the input power of 27 mW. Finally, the microspheres are collected in exit reservoirs. Fluidic rate can be varied by changing the applied DC voltage and to achieve strong enough trapping at the given flow rate, the
intensity of input light beam can also be adjusted accordingly. This is a powerful technique for applications such as cell sorting and cellular array formation. Furthermore, transparent PDMS devices would enable optical spectroscopy or detection creating a pathway for other possibilities.

A second type of microfluidic switching system has been demonstrated based on the scattering force of an optical beam instead of the attractive gradient force in the optical tweezers arrangement. In essence, when the optical beam is less tightly focused in an optical trap, then the scattering force (equation (2)) will dominate over the gradient force (equation (3)). Below equations are valid in the Rayleigh regime where object size is much smaller than the wavelength of the light. Here, in our experiments, we operate at the Mie regime where the object size is larger than 5 μm. In this regime, to evaluate the individual trapping forces ray optics analysis can be applied. In this analysis, incident rays are traced through the sphere through an infinite number of reflections and then are summed. Due to complexity of this analysis, Rayleigh approach is commonly used even for the objects in larger sizes than the wavelength of the input light to estimate the resultant trapping force (Ashkin et al., 1970, 1992; Harada et al., 1996).

\[ F_{\text{sc}} \propto \frac{ma^6}{\varepsilon_k^2 I_0}; \quad \text{Scattering force,} \]

\[ F_{\text{gr}} \propto ma^3 \nabla I_0; \quad \text{Gradient force,} \]

where, \( a \) is the radius of the sphere, \( I_0 \) is the intensity of input beam, \( \nabla I_0 \) is the intensity gradient of input beam, and \( \lambda \) is the wavelength of input beam. Notice that while gradient force is proportional with the gradient of the input beam, scattering force is relative with the magnitude of the input intensity.

Here, a 3-dimensional PDMS structure with two layers and a cross-level junction at the intersection of the microfluidic channels of the two layers is fabricated for this purpose. A less tightly focused optical beam impinging from the top or the bottom of the device transfers the microspheres from one level to another.

Operation of the cross-junction switch is illustrated in Figure 7 and images from an experiment are shown in Figure 8. Microspheres flowing in the lower channel are directed to either output channel by modulation of the optical beam. It could also be possible to generate a more versatile switching system by having two concurrently focused beams illuminating from both top and bottom of the device. The scattering force switch has a distinct advantage over the gradient force switch in that it does not require a spatial translation of the optical beam. In addition, low numerical aperture optics can be used in scattering force switches which minimizes optical design requirements.

The Optical Handle

An optical trap can also be used as a handle to grab an object which in turn could be used to grab onto other objects. This phenomenon is relatively new and is illustrated in Figure 9, in which a microsphere flowing in a 100 μm channel is trapped and used as a handle to collect other microspheres and direct them inside the channel. A 5 μm diameter polystyrene microsphere (Bangs Laboratories) is trapped first (B) and then used as a handle to trap and manipulate several 2 μm diameter microspheres (C–E). The assembly of microspheres remained stable as it is moved inside the 100 μm channel. The trapping beam is defocused (F) and the objects are released from the trap. Next, the beam is refocused (G) and the assembly is reformed and further translated (H–J). Our observations suggest the 3-dimensional nature of the optical trapping. Smaller

![Fig. 7. The scattering force from an optical beam transfers an object between two fluidic channels at different levels. When the switch is off, the object flows with the fluid stream down the channel.](image-url)
**Fig. 8.** Particle switching using optical scattering force: Fluid is drawn through two overlapping channels at a constant rate. At the intersection of the two channels a 5 μm microsphere will either remain in its original channel or be pushed by an optical beam into the opposite channel.

**Fig. 9.** Demonstration of an optical handle (a center particle) to grab and manipulate other objects in a fluidic channel.
objects are collected in 3-dimensional format. By moving the focal point during the imaging up and down along z-direction, we were able to resolve other trapped small spheres above and below the center sphere.

We believe that the attraction between particles is due both to fringing optical fields, indicating the formation of a wide optical trap due to refracting beams from the first object, and due to the electrostatic attraction via polarization of the dielectric microspheres. More detailed studies are underway to predict the effects of each parameter to grasp smaller objects around a center particle. 3-dimensional nature of trapping force suggests formation of 3-dimensional close-packed structures. Finally, optical handles could be useful for the pick and place of multiple objects for biological platforms and for optically assisted self assembly processes.

Summary and Conclusion

In this paper, optical manipulation of objects in microfluidic devices is discussed. Optical manipulation has the advantages of non-contact and contamination free manipulation of biological species on fluidic microchips. Polystyrene microspheres and live biological cells were manipulated by photonic momentum transfer using optical forces. Arrays of optical traps generated in parallel by VCSEL arrays provide a low cost yet powerful approach to manipulate objects in a network of microfluidic channels. Such trap arrays can be used for parallel manipulation of multiple objects, for optical switching at fluidic channel intersections either via the gradient force or the scattering force for a number of applications. When optical manipulation of live large cells requires higher power optical traps, the combined optical power of several VCSELs within an array can be used. It is conceivable that VCSEL driven optical manipulation of objects could be integrated into the same device for future applications in fabricating active cellular arrays, for toxicity analysis, fast cell cytometry and drug discovery applications.

References