Development of multi-beam trapping and sorting techniques using microoptical elements

Sun, Yuyang

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**DEVELOPMENT OF MULTI-BEAM TRAPPING
AND SORTING TECHNIQUES USING
MICROOPTICAL ELEMENTS**

SUN YUYANG

School of Mechanical and Aerospace Engineering

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SUMMARY

The thesis provides a broad and systematic study on the micro-optical elements based multi-beam trapping and sorting techniques by means of theoretical formulations and experimental demonstrations. The optical sorting technique is not based on the tightly focused single-beam optical tweezers, but the periodical optical energy landscape. This all-optical sorting method does not rely on the fluorescent marker but on the intrinsic physical attributes such as geometrical size and refractive index. It is a passive continuous microfluidic sorting and requires no human interaction. The refractive and diffractive optical elements (DOE), including microlens arrays (MLA), Dammann gratings, holographic optical elements and chess-board gratings, are designed and fabricated to create array of optical traps. The multi-beam optical trapping and optical sorting have been demonstrated experimentally using MLA or DOE based lens projection trapping systems. The system requires minimum modification to traditional single-beam optical tweezers. All-optical on-chip optical sorting on periodical optical lattices is experimentally demonstrated using the Talbot effect and MLA respectively. It is further experimentally proved that the three-dimensional (3D) optical lattice generated by the modified Talbot effect can realize multi-level array trapping of particles. In addition, the 3D optical lattice is applied to the creation of 3D sub-micron sized periodical structures in polymer-dispersed liquid crystal (PDLC).

The 3D optical lattice created by the modified Talbot effect has been demonstrated for the first time to be capable of simultaneous multilevel trapping of particles arrays. Based on this secondary layer-by-layer self-imaging effect, optical trapping of silica and polystyrene colloidal particles at different planes in a microchamber are demonstrated experimentally. The method requires no lens projection and the trapping range in both the axial and lateral direction may reach hundreds of micrometers. In addition, the created 3D optical lattice was also employed to realize a convenient one-step fabrication of 3D sub-micron sized periodical structure in PDLC. The fabricated sol-gel microlens array is also capable of realizing on-chip multiple optical trapping and optical sorting. The integrated optical trapping system is composed of a micro chamber and a designed MLA fabricated on a glass substrate. The on-chip multi-beam optical trapping and locked-in transport of particles are experimentally demonstrated within the micro chamber.

The present research has successfully demonstrated the use of micro-optical elements and Talbot self-images effects for the generation of two-dimensional (2D) optical landscape and 3D optical lattices for the realization of 2D and 3D optical trapping and sorting of micro-particles.

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CHAPTER 1

INTRODUCTION

In this chapter, the introduction and motivation of this research work are provided. The objective of research is proposed and the scope of work is listed. Finally the organization of thesis covering each chapter is introduced.

1.1 Introduction

Within the last three decades, light has been widely employed to trap and manipulate microscopic objects. Most of today's optical manipulation methods derive from the single-beam optical trap, generally called as optical tweezers. The first optical tweezers was built by (Ashkin A. 1970) (Ashkin A. et al., 1986) and his coworkers at AT&T laboratories in the U.S.. The small biochemical particles or biological cells are trapped in the waist of a focused continuous wave laser beam due to the gradient force, resulting from a change in the momentum of light. The wavelength of the laser beam should be chosen from the region of optical transparency of the particle to avoid the absorption of light. Furthermore, by moving the focus of laser beam, the particle or cell trapped in the optical beam can be manipulated. Indeed optical tweezers is capable of trapping objects ranging from tens of nanometers to tens of micrometers (Svoboda K. et al., 1994) (Ke P.C. et al. 1999), and can exert forces exceeding 100pN (Ghislain L.P. et al. 1994) (Rohrbach A. et al. 2002) with resolutions as

fine as 100aN (Litvinov R.I. et al. 2002). Moreover, optical techniques offer a highly controllable driving mechanism which avoids any physical contact with the outside world. Hence, the optical tweezers enables the capture and trap of various types of small objects, including dielectric microspheres, colloidal gold, DNA and biological cells etc.

Today the applications of optical trapping are manifest in a wide range of applications in various branches of science and technology. For example, in physics, optical traps are used for accurately measuring the viscosity and surface effects within liquid media on a micron-sized scale (Bishop, A.I., 2004). In micro and nanoscale chemistry, individual droplets of chemical compounds can be manipulated and combined by optical traps. In biology and medicine, optical traps have been applied to modify the chromosomes of living cells (Berns, M.W., et al., 1998). Recently, optical induced micro-mechanical control has started to emerge into a strong field. It has been demonstrated that optical fields can also be used to arrange, guide or deflect particles individually or enbloc (Koss, B.A., et al., 2003). Such a technique opens up the possibility of the application of light in the key components (pump, mixer, sorting etc.) in microfluidic research.

1.2 Motivation for research

Based on the objects' fluorescent and light scattering characteristics, conventional fluorescence activated cell sorting (FACS) technique enables a high-speed, multi-parameter sorting of a mixed suspension of biological cells. However, the FACS suffers from the disadvantages like complexity in use, high cost, and inconsistency between object and equipment size. The idea of lab-on-a-chip (LOC) integrates several laboratory functions on a chip of centimeters or even smaller, which enables handling of extremely small amount of objects with high yield. The advantages of LOC include low fluid volumes consumption, safer handling, disposable, higher analysis and control speed etc. In the LOC field, most attentions are focused on minimization of analytical chemical methods, in particular cells sorting or separation. In recent years, there have been many reports focusing on the μ FACS. The μ FACS usually require two steps, the objects to be separated are first identified with inherent or external fluorescent marker at the detection region and then deflected with electrical forces (electrophoresis, dielectrophoresis, electroosmotic etc.) to the collection reservoir. Although the μ FACS technique is powerful and compatible with a microfluidic environment, it is still an 'active sorting' method, which means that the method requires the human interaction and not a one-step continuous sorting method. In this case, optical force provides another solution to microfluidic sorting with its non-invasive feature.

Optical tweezers have been demonstrated to be capable of manipulating objects of nanometers to micrometers with high resolution and non-invasive features. It is further demonstrated that the optical force can be used to deflect objects within a fluidic chamber. In these early works, the fact that using optical forces as the only driving force in the fluidic chamber greatly restrict the flow throughput of the sorting system. More recently, a periodical optical energy landscape is reported to be capable of deflecting and sorting micro-sized objects in the microfluidic chamber (Korda P.T., et al. 2002). The sorting principle lies in the competition between the microfluidic flow and exerted optical force caused by the whole potential landscape. This all-optical sorting method does not rely on the inherent fluorescent marker but on the intrinsic physical attributes like geometrical size and refractive index. It is in fact a passive continuous microfluidic sorting which requires no human interaction, and the method has relatively high throughput due to its 2D optical landscape. As a result, the all-optical sorting method has drawn great attention in the biophotonics field.

The all-optical sorting method employs an optical landscape rather than a single or double tightly focused laser beams. Among several typical optical landscapes types, the lattice is usually employed due to its theoretical exponential size selectivity. Hence, one of the key techniques of the all-optical sorting is the generation of an optical lattice on the sample plane, namely the multiple optical

traps. Although several results have been reported on the optical sorting based on the optical lattice created by multi-interference or computed hologram, there are no systematic experimental demonstration on the optical sorting based on the micro-fabricated diffractive elements and even the refractive optical elements. The fabricated optical elements may lead to development of the all-optical sorting technique towards the all-optical lab-on-chip, which is difficult to realize with the existing SLM and multi-interference techniques. Finally, the applications in all-optical sorting and the multiple optical tweezers also have the potential in creation of photonics crystal structures etc.

1.3 Research objective and scope of work

1.3.1 Main objective

The objective of this thesis is to fully investigate the all-optical sorting techniques based on the multiple optical traps created by fabricated microoptical elements. The fulfillment of this objective will entail a series of tasks, including

- (a) The theoretical analysis of optical sorting on periodical energy landscape.
- (b) Proposal and demonstration of a lens projection system based multiple optical tweezers for multiple trapping and sorting using fabricated refractive and diffractive microoptical elements respectively.

- (c) Proposal and demonstration of an on-chip optical multiple trapping and sorting system through the integrated microoptical elements and microchamber.

1.3.2 Scope of work

1. To quantitatively analyze the optical sorting on periodical landscape. To study the forces acting on the particles when they are driven through the optical landscape. To compare the size selectivity of sorting on different types of optical landscape.
2. To design MLA for generating optical lattice on sample plane for multiple trapping and sorting. To fabricate the MLA in photoresist through micro-lithography technique. To build a multiple optical tweezers experimental system using microscope and lens projection approach. To experimentally demonstrate the multiple optical trapping and optical sorting with the proposed system.
3. To design a computer generated hologram (CGH) for creating optical lattice for multiple trapping and sorting. To experimentally demonstrate the optical multiple trapping and sorting experiment with fabricated diffractive optical elements. Furthermore, to experimentally demonstrate the dynamic multiple

optical trapping with designed CGH displayed on SLM.

4. To propose and demonstrate solution to on-chip optical trapping and sorting.

To design and fabricate a microoptical element which is integrated with a microchamber. To experimentally prove the on-chip multiple optical trapping and sorting with the integrated chip. To provide a solution for simultaneous multi-level multiple optical trapping.

1.4 Organization of the thesis

Chapter 1 provides the introduction, motivation, objective of the project, as well as the contributions of the thesis.

Chapter 2 reviews the historical development of optical tweezers, and fully reviews the existing multiple optical trapping techniques, as well as the optical sorting techniques.

Chapter 3 builds the theoretical model for quantitative analysis on the relationship between the value of parameters and motion trajectory of particles. It is proved that the optical lattice has exponential size selectivity.

Chapter 4 designs and fabricates microlens array based lens projection system for optical trapping and optical sorting. The 45° separation angle is realized with a composite optical lattice.

Chapter 5 designs and fabricates binary-phase diffractive optical elements (Dammann grating) for generating multiple optical traps for array trapping and sorting. Multi-phase CGH is designed to generate dynamic multiple optical tweezers with SLM.

Chapter 6 proposes two approaches for realizing on-chip multiple optical trapping and optical sorting. The optical lattice is generated with the Talbot effect and sol-gel microlens array respectively. And the 3D on-chip optical trapping is realized through modified Talbot effect.

Chapter 7 draws a conclusion of the thesis, and summarizes the main contribution as well as the recommendation for future work.

CHAPTER 2

LITERATURE REVIEW

In this Chapter, a series of topics related to optical trapping and sorting will be reviewed, including the history of optical tweezers, the principle of optical trapping, the multiple-beam optical tweezers and optical sorting techniques. The development history of optical trapping in the past three decades will be reviewed. The reported techniques for multiple optical trapping, as well as the optical sorting will be reviewed in detail.

2.1 History of optical trap

The phenomenon of optical trapping is based on the transfer of momentum. It is known from the early physics that light radiation has linear and angular momentum. The deflection of light beam by an object gives rise to a change of the momentum of light. Since the total momentum in a closed system is conserved, the difference of momentum before and after the light deflection is transferred to the object. That is to say, the light could act radiation pressure and torques on physical objects. And the radiation pressure exerting on the object will give rise to an external force or torque, which is not easily to be detected. In the early 20 th century, the detection of radiation pressure and absorbing gases has already been demonstrated (Ashkin A. 2000). However, there are no practical applications using the light pressure forces caused by the incoherent sources at that time until the next half century. In 1960, the invention of the laser revolutionized lots of scientific fields, including the optical trapping and manipulation of particles in fluidics. Due to strong gradients and intensities of the laser beam, the forces of radiation pressure

become capable of trapping and manipulating micro objects with sizes ranging from nanometers to tens of micrometers.

A. Ashkin is known to be the founder of the field of optical trapping and optical manipulation (Grier D.G. 2003) (Dholakia K. 2002). The first optical trap was demonstrated by him at Bell Labs in 1970 (Ashkin A. 1970, 1997, 2000). He proved the acceleration of particles using a horizontal laser beam with the forces of radiation pressure from focused laser beam. Furthermore, he employed the dual counter-propagating laser beams to create a stable 3D trap. In 1971, Ashkin made another advance in the demonstration of optical levitation trap (optical fountain) in air and vacuum with a single vertically directed focused laser beam (Ashkin A. et al. 1971). In 1986, Ashkin and co-workers demonstrated the first stable 3D optical trap using the optical gradient force induced by a tightly focused laser beam (Ashkin A. et al. 1986). This optical trapping system is later called as ‘optical tweezers’, with which the micron-sized object can be held and manipulated in three dimensions with great ease and precision. In view of the great contribution of Ashkin to the field, his several milestones papers tracking the historic development of optical trapping technique will be reviewed in detail in the following sections.

2.1.1 First optical trap

The first optical trap was demonstrated by Ashkin at Bell Labs in 1970. He showed that the dynamics of micron-sized particles could be greatly influenced by the forces from radiation pressure (Ashkin A. 1970). It was experimentally

demonstrated that micron-sized particles could be accelerated, decelerated, and even stably trapped in water medium using mildly focused laser beam. According to Figure 2.1(a), when the horizontal laser beam was switched on, the particles were simultaneously drawn into the beam axis and accelerated in the light propagation direction. If the beam was blocked, the particles would go back to Brownian motion. A three-dimensional stable optical trap was further experimentally demonstrated with dual counter-propagating laser beam, as shown in Figure 2.1(b). It was observed that when one of the beams was blocked, the particle would accelerate in the propagation direction of remaining beam, and the stable optical trap would be established again when both the beams were restored.

Figure 2.1 First optical trap (a) Acceleration of high-index particles (b) Trapping of high-index particles in a potential well formed by two counter-propagation laser beam (Ashkin A. 1970)

The counter-propagating beam generates an equilibrium point where the scattering force by the left and right laser beams cancel. Consequently the loss due to the reflection at the beam surface is minimized, and the three dimensional trap is stable with high stiffness. The dual-beam optical trap

system can be vertical as well, and its main advantage lies in the fact that it will generate higher trapping forces for a given laser power and can be constructed with lower NA microscope objectives compared with the single beam optical tweezers. The main problem of this configuration lies in the lack of flexibility in alignment and steering.

2.1.2 Optical levitation

In 1971, A. Ashkin demonstrated the stable levitation of small transparent glass spheres in air and vacuum by forces of radiation pressure (Ashkin A. et al. 1971), and it is experimentally proved that a single vertically directed focused TEM₀₀ mode continuous wavelength laser beam of ~250 mW is sufficient to support stably a ~20 μm micrometer glass sphere. Optical levitation is suitable for lateral confinement and axial transport of particles. Figure 2.2 shows the configuration of a typical optical levitation. The laser beam is expanded and directed upwards through a reflecting mirror, subsequently mildly focused directly to the sample cell, therefore, the gravity play a more important role than in the previous horizontal optical trap. In the air/vacuum/liquid chamber, the scattering force pushed up the particle against the gravity from the bottom to higher planes. Finally the particle was suspending stably at a certain position in the chamber, where the forces balanced. Usually piezoelectric ceramic shaker is employed to shake the cell to overcome the adhesion forces between the particles and the bottom surface. The levitated particles may be manipulated freely though moving the incident laser beam.

Figure 2.2 Schematic of optical levitation by radiation pressure

2.1.3 Optical tweezers

In 1986, Ashkin and his co-workers demonstrated the first experimental observation of optical trapping of dielectric particles by a single-beam gradient force trap (Ashkin A. et al. 1986). The method holds true for the full spectrum of Mie and Rayleigh particles with diameters ranging from tens of nanometers to tens of micrometers. The experimental results demonstrated the existence of negative radiation pressure due to an axially oriented gradient. The optical tweezers make use of a strongly focused laser beam with a large axial gradient force, which dominated the axial stability. Whereas the axial stability of previous optical levitation trap depends on the balance of scattering force and gravity. For lots of applications with macroscopic particles, optical tweezers

were demonstrated to be advantageous to the optical levitation trap. Optical tweezers are capable of creating forces amount to thousands of mg (m is the mass, g is the acceleration of gravity), which is only restricted by the power of laser beam, however, the optical levitation traps dependent on the gravity could only generate forces of $\sim mg$. Consequently, optical tweezers are more suitable for the trapping of submicron particles compared to the levitation trap, especially when the Brownian motion dominates and gravity is relatively small. Additionally the optical tweezers was also demonstrated to have a higher tolerance of particle shape irregularities than the optical levitation trap.

Figure 2.3 shows the configuration of a single beam 3D optical trap, which is later well known as the optical tweezers. The 514.5 nm laser beam was focused tightly by a high numerical-aperture ($NA=1.25$) water-immersion microscope objective lens into a cell filled with water medium. For Rayleigh particles dispersed in water solution with appropriate concentration, the Brownian motion would introduce them to the trapping region. For the Mie particles, another assisting laser beam was employed to push up the particles from the bottom planes to the region near the focus of objective. Once the particle was approaching the focus point, it would be attracted into the optical trap. This configuration is now recognized as the first genuine optical tweezers.

Figure 2.3 Schematic of the first single-beam gradient force optical trap (Ashkin A. *et al.* 1986). *M* is microscope used to view the trapped particle; *S* is beam splitter *S*; *D* is a detector; *WI* is water-immersion ($NA=1.25$) microscope objective lens; *H* is an assisting laser beam to push up the particles to the region near focus of objective lens

2.1.4 Optical trapping of biological samples

Optical trapping technique was also demonstrated to be useful in biological field. A. Ashink and J. M. Dziedzic (Ashkin *et al.* 1987a) demonstrated the optical trapping and manipulation of viruses and bacteria in aqueous solution through single-beam gradient force optical trap. It was demonstrated that the individual tobacco mosaic viruses (rod-like protein with diameter of ~ 20 nm and length of ~ 300 nm) and arrays of aligned viruses were optically trapped and manipulated without obvious damage using ~ 120 milliwatts of argon laser power. The experimental result was regarded as the first rod-like molecule

being fully aligned by the optical field, and also the first optical manipulation of living cells. In order to further reduce the damage caused by laser, Ashkin proposed to employ infrared Nd:YAG laser with a wavelength of $1.06\ \mu\text{m}$ for optical trapping (Ashkin et al. 1987b). The reason is that the absorption of many molecules falls rapidly in the near infrared and the absorption of water is small as well. It was experimentally proven that the damage to bacteria greatly decreased. The authors observed that the E.coli bacteria and yeast cells would be reproduced right in the high intensity trap focus, which directly demonstrated that no apparent damage was caused. Several types of cells including pigmented red blood cells, green algae diatoms, amoebas and protozoans were also optically trapped by the infrared laser tweezers. Furthermore, it was demonstrated that a single selected bacterium could be separated from a collection of bacteria by the optical tweezers. Figure 2.4 shows the optical configuration for the manipulation and separation. A piece of fiber F with inner diameter of $\sim 15\ \mu\text{m}$ was attached to the ceiling of the sample chamber. It was demonstrated that the bacterium could be trapped and manipulated into the core of the hollow fiber. Subsequently the chamber lid was disclosed, washed, dried and then the liquid contents of the fiber were blown with a gentle air stream into another water filled vessel. It was also proved that bacterium and cell could be grabbed at their ends and be oriented or rotated freely by moving one trap with regard to the other.

Figure 2.4 Combined high resolution optical microscope and $1.06\ \mu\text{m}$ infrared optical trap for observation, manipulation and separation of bacteria and other organisms

(Ashkin A. et al. 1987)

2.2 Theoretical basis for optical trapping

Optical trap employs a strongly focused laser beam by an objective with high NA for trapping of objects (Block S.M. 1992). A particle located near the focus of a laser beam will be exerted a force caused by the transfer of momentum

from the scattering of incident photons. And the resulting optical forces are usually put into two categories, one is the scattering force in the direction of the light propagation and the other is a gradient force in the direction of the spatial light gradient. The scattering force will push the particle along the direction of the light propagation, and it plays a dominant part in the conventional traps like an optical levitation trap. The gradient trapping force is due to the interaction of the dipoles in the dielectric particle (induced by laser) with the inhomogeneous electric field at the focus. In order to achieve a stable 3D optical trap, the axial gradient force attracting the particle toward the focus must exceed the scattering force which pushes the particle away from the region. Hence, a steep gradient in the light is required, which could be generated by an objective with high NA. When the gradient and scattering forces reach a balance, the equilibrium position of the trapped particle is located slightly beyond the focal point.

2.2.1 Theory of optical trap

The size and shape of a particle have an impact on the force capability of the optical trap. The trapping objects can be grouped into two categories, Mie particles and Rayleigh particles. Mie particles denote those with sizes much larger than the wavelength of the trapping laser ($a \gg \lambda$), and the optical forces can be calculated through ray optics and conservation of linear momentum. Rayleigh particles are those with sizes much smaller than the wavelength of the trapping laser ($a \ll \lambda$), and the optical forces can be calculated by treating the particle as a point dipole. When the size of the trapped particle is comparable to the wavelength of trapping laser, neither ray-optics nor dipole model will hold

true. More complete electromagnetic models are required to accurately describe the trapping forces for those with particles with intermediate sizes ($0.1\lambda - 10\lambda$) (Almaas E. et al. 1995) (Zemanek et al., 2002) (Rohrbach et al. 2001, 2002).

2.2.1.1 Mie regime

For the Mie particles with $a \gg \lambda$, the shape has a great impact due to the light refraction and reflection, hence Mie particle are usually limited to spheres and ellipsoids. The calculation of the optical forces of Mie particles are based on the ray optics model. The refraction of incident light beam will result in a change of the linear momentum carried by light. According to Newton's third law, the particle will experience an equal and opposite momentum change. And the force exerting on the particle dependent on the value of momentum change is proportional to the intensity of the light. When the refractive index of the particle is higher than the surrounding medium, the optical force will follow the direction of the intensity gradient; on the contrary, the optical force will be in the opposite direction of the intensity gradient. The influence of light reflection is neglected because particle is regarded to be highly refractile and the difference of water/particle refractive index is small. In Figure 2.5(a), a single laser beam with Gaussian profile incident on a transparent dielectric particle offset from the propagation axis of light beam. As the particle is offset to the right of the center of the beam, it will refract more light from the right to the left; hence the particle will experience an equal and opposite force back towards the centre of the beam. Similarly, if the beam is tightly focused it is possible for the

particle to experience a levitation force towards the focus of the laser beam, as is shown in Figure 2.5(b).

Figure 2.5 Descriptions of optical forces with ray optics model (Dholakia K. et al. 2002)

In 1992, Ashkin provided a detailed calculation of the forces of single-beam gradient optical tweezers on micron-sized dielectric particles in the ray optics regime. The gradient force F_{gradient} and scattering force F_{scatter} are given by (Ashkin 1992),

$$F_{\text{gradient}} = \frac{\Delta P}{\Delta t} = n_m P Q_g / c = n_m P / c \left\{ R \sin 2\theta - \frac{T^2 [\sin(2\theta - 2\varepsilon) + R \sin 2\theta]}{1 + R^2 + 2R \cos 2\varepsilon} \right\} \quad (2.1)$$

$$F_{scatter} = \frac{\Delta P}{\Delta t} = n_m P Q_s / c = n_m P / c \left\{ 1 + R \cos 2\theta - \frac{T^2 [\cos(2\theta - 2\varepsilon) + R \cos 2\theta]}{1 + R^2 + 2R \cos 2\varepsilon} \right\} \quad (2.2)$$

where n_m, P, c, θ and ε are refractive index of the medium, laser power, speed of light, angle of incidence, and angle of refraction respectively. R and T are the Fresnel coefficients of reflection and refraction. Q is dimensionless angle-dependent factor, which is not the same for gradient and scattering force.

2.2.1.2 Rayleigh regime

For particles smaller than the wavelength, the ray optical approach is unsatisfactory and it is better to consider the forces in terms of the electric field near the trapped particle. The dipole model is utilized to deal with the Rayleigh particles. The scattering forces arise from the absorption and retardation of light by the dipole. The scattering force acts along the light propagation direction and is proportional to the intensity of light; it is quantitatively described by,

$$F_{scat} = \frac{I_0 n_m}{c} \frac{128\pi^5 a^6}{3\lambda^4} \left(\frac{m^2 - 1}{m^2 + 2} \right)^2 \quad (2.3)$$

where I_0 is the intensity of light incident on the Rayleigh particle, a is the radius of the particle, n_m is the refractive index of the surround medium, n_p is the refractive index of the particle; m is the ratio of the refractive index of

particle to refractive index of medium (n_p/n_m), c is the speed of light in vacuum, λ is the wavelength of incident laser beam.

The Rayleigh particle also experiences another force in the direction of intensity gradient of the incident light and is proportional to the intensity gradient of light. The force results from the interaction between the induced dipole and the inhomogeneous field. The gradient force is described by,

$$F_{grad} = \frac{n_m^2 a^3}{2} \left(\frac{m^2 - 1}{m^2 + 2} \right)^2 \nabla I_0 \quad (2.4)$$

Rayleigh particle will experience the acceleration towards the region with higher light intensity and a stable trap will be formed when the gradient force balances the scattering force.

2.2.2 Typical optical trap setup

Optical trap is usually built by modifying a commercial microscope, which could provide the functions like imaging, 3D manipulation of chamber including samples and adjustment of the objective focus (Figure 2.6) (Visscher K. et al. 1996) (Smith S.P. et al. 1998). A single laser beam is introduced into the back aperture of microscope and focused by the objective into the trapping microchamber that contains samples suspending in aqueous (water is usually selected) solution. The trapping chamber is placed on a three-dimensional stage for the convenience of focusing and movement of sample after trapping. With

CCD camera and microscope, the whole trapping process can be observed.

Several main parts of optical trap are as follows.

Figure 2.6 Typical optical trapping systems

(1) A microscope (upright or inverted) with a high Numerical Aperture (NA) objective lens.

Optical trap is typically built by modifying a conventional upright or inverted microscope. The objective mounted on the microscope is employed to focus the trapping laser beam. And the parameters of objective like NA, transmittance, working distance and immersion medium play a significant role in the optical trapping experiment. Some typical specifications of objective are: NA 0.65-1.40,

magnification 40–100X. If a stable 3D optical trap is desirable, the objective with high NA (1.2-1.4) will be selected to generate a gradient force strong enough to overcome the scattering force. The most common objective adopts an oil immersion, however, the mismatch between the refractive index of water (trapping medium) and oil will give rise to great spherical aberrations. Moreover, the aberrations will restrict the axial trapping range in practice. The common solution to minimize the spherical aberration is to use the water immersion objective.

(2). A Continuous Wavelength (CW) trapping laser

Laser works as a basic component for optical trapping experiment. The output power and wavelength of the trapping laser will have a great impact on the practical optical trapping. As for the mode, usually Gaussian TEM₀₀ mode is adopted to produce the smallest beam waist and therefore achieve a high trapping efficiency. The output laser power determines the maximum optical force that could be generated. Generally the microparticle will experience the maximum trapping force on the order of 1 pN per 10 mW of power delivered to the sample plane (Svoboda et al. 1994). Hence, the necessary output power could be estimated if the trapping object and light efficiency of optical system are known. For the biological sample, a near-infrared laser wavelength is recommended to minimize the potential damage to the biological sample. The reason is that visible light is strongly absorbed by biological material, while far infrared light is absorbed by water. As a result, the near infrared trapping laser (700 - 1300 nm) is usually adopted for the biological application.

(3) A dichroic mirror

Dichroic mirror is inserted into the microscopic system to efficiently split the optical path of trapping laser beam and the illumination light. It will reflect the trapping laser beam into the microscope while transmitting the illumination light for observation of samples, with the result that the transmitting efficiency of trapping laser beam is obviously improved, and the influence of laser beam on illumination is minimized at the same time.

(4) Laser beam expander and beam steering

In order to generate a tighter, diffraction-limited spot, beam expander is used to magnify the laser beam size to fill the pupil of microscope objective. The beam expanders can be home-made or purchased from optics manufacturer. Beam steering system is included to give an extra degree of translational freedom in optical alignment. Usually beam steering system is made up of two plano-convex lenses, which constitute a simple 1:1 telescope arrangement to steer the laser spot. The lenses are placed apart the sum of their focal lengths, so that it produces parallel light of the same beam size as the incident light. By placing these lenses with their flat surfaces facing one another, spherical aberration will be reduced.

2.3 Review of multiple-beam optical tweezers

In the last decade, the requirement for simultaneous optical trapping and manipulation of the samples in the field of biology and biochemistry gave rise to the development of multiple-beam optical tweezers. Researchers have proposed various approaches to create multiple traps and even the tailored optical landscape, which mainly include time-sharing, holographic optical tweezers (HOT), generalized phase contrast (GPC), multi-beam interference, vertical-cavity surface-emitting laser (VCSEL) array based multiple traps, optical imaging fiber bundles trap etc. These popular techniques for generating multiple traps will be reviewed in the following paragraphs.

2.3.1 Multiple traps based on the time-sharing technique

The most direct approach to generate multiple optical traps is using a rapid scanning focused trapping laser beam (Sasaki K. et al. 1991) (Misawa H., et al. 1992) (Visscher et al. 1996) (Brouhard G.J. et al. 2003) (Vossen et al. 2004) (Kaputa D.S. et al. 2005) (Milne G. et al. 2007). When a laser beam is scanned rapidly enough between different trapping locations, time-sharing multiple optical traps are created to trap different particle independently at arbitrary locations in two-dimension. In the time-sharing multiple optical trapping system, the average potential energy has to be strong enough to overcome the diffusion acting on particles. The actual laser power acting on the trapped particle is dependent on duty ratio, which is the time that spent by the laser at each position relative to cycle time. If two objects are held then the duty ratio is

50 %, for four objects it falls to 25 % and so on. Consequently the number of trapped particles is limited by the scanning speed. As the precise and calibrated lateral motion of optical trap on the sample plane is required, beam steering is a key technique in trapping system. There are mainly three different beam-steering methods, scanning mirrors, acoustic-optical-deflector and electro-optical-deflector.

2.3.1.1 Scanning mirrors

Figure 2.7 shows a typical laser scanning system for creating multiplexed optical traps. The laser beam was reflected by a dichroic mirror and then focused into the sample plane with an objective lens. A computer controlled galvano mirror enabled the scanning of laser beam with designed patterns displayed on computer and the adjustment of scanning velocity. It was proved that the trapped particles formed a spatial pattern.

Figure 2.7 Optical configuration of the laser scanning system for multiple optical trapping (left) and the micrograph of trapped latex particles (Sasaki et al. 1991)

The galvano mirror's high stability and precision is attributed to the incorporation of feedback. The commercial systems usually operates at 1-2 kHz with step response times as short as $100 \mu s$. Although the temporal response of scanning mirror is relatively slow, it has the advantages like large deflection angle, low insertion loss etc.

2.3.1.2 Acoustic optic deflector (AOD)

The AOD consists of a transparent crystal inside which a diffraction grating is created by the density changes connected with the travel of an acoustic ultrasound wave. The grating period is determined by the wavelength of the acoustic wave in the crystal. The laser beam is deflected at specific angles and intensities that depend on the frequency and the amplitude of the sound wave in the crystal respectively. The laser beam is scanned quickly from point to point in the sample plane using the AOD to create arrays of optical traps with great control over the position of traps. The AOD can be integrated into a standard inverted microscopic trapping system, and multiple optical traps will be generated when the laser beam is deflected by the computer controlled AOD (Visscher et al. 1996) (Milne G. et al. 2007).

The time-sharing laser beam need to be scanned fast enough over the different locations to behave like it was trapped with a non-time-sharing optical trap, and roll-off frequency is given by (Vossen el al. 2004),

$$f_c = \frac{\kappa}{2\pi\gamma} \quad (2.5)$$

where viscous drag coefficient $\gamma = 6\pi\eta R$, κ is the trap stiffness, η is the viscosity and R is the radius of an isolated sphere. For frequencies above f_c , the sphere will show free diffusive behavior, while for frequencies below f_c the particle will be always confined within the optical trap. In Vossen's (2004) report, the roll-off frequency is 10 Hz, and array of optical traps were scanned at 96 Hz for trapping 400 spheres. The maximum deflection of the AOD is linearly related to the operating frequency range. For 1064nm, the maximum angle can reach a bit over 1° . The AOD has much higher switching frequency (can reach 200 kHz), and the response time is mainly limited by the ratio of the laser beam size to the speed of traveling sound inside crystal ($\sim 1.5 \mu\text{s} / \text{mm}$ laser diameter for T_eO_2 crystals). However, the response of AOD trapping system is usually attributed to other factors. An orthogonal configuration of AODs can permit both x and y deflections of optical trap, but this will reduce the diffraction efficiency from 80% to 60%. AOD's transmittance varies over the working range of drive frequencies, which may give rise to the change of stiffness of optical trap as it is moved. Another disadvantage of AOD is the systematic angular deviations from a linear response to the acoustic drive frequency. (Valentine et al. 2008)

2.3.1.3 Electro-optic deflector (EOD)

An EOD consists of a crystal inside which the refractive index can be altered by exerting an external electric field. The EOD can achieve the switching time of 100 ns while the deflections is ~ 20 mrad, which is good enough for the applications in optical trapping. The advantages of EOD trapping system also

includes low insertion loss and straightforward alignment, however the high cost and relatively small deflection range greatly limit the application of EOD in trapping instrument. Valentine et al. (2008) reported a single-beam optical trapping instrument employing twin EODs to steer the trap in the specimen plane. Compared with the traditional instrument using AODs, the light throughput is significantly improved and the deflection angle pointing errors are reduced.

2.3.2 Multiple traps based on interference patterns

The interferometric optical tweezers make use of interference fringes for the trapping and manipulation of micron-sized objects. The two-beam or three-beam interference pattern may enable the trapping of 1D or 2D array of objects transversely. Figure 2.8 shows the basic configuration for two-beam interferometric optical tweezers. The laser beam was first separated into two paths and then combined by lens L, with the result that the interference pattern was created at the back focal plane of objective lens MO1 (20x, NA=0.4). Subsequently the shrunk interference pattern (1/20) is projected onto the sample plane for multiple trapping in microfluidic chamber.

Figure 2.8 Optical configuration of interferometric optical tweezers

(Chiou A.E. et al. 1997)

Although the optical gradient of the interference pattern projected by 20x objective lens is strong enough to perform the multiple trapping transversely, the two-beam interference trapping system shown in Figure 2.8 is impossible to create stable 3D axis traps. In this case, Casaburi and colleagues (2005) proposed to add an additional counter-propagating beam to balance the radiation pressure generated by the two beams but did not contribute to the interference (Casaburi A. et al. 2005). Figure 2.9 shows the modified system for stable optical trapping axially. The Mach-Zender interferometer was employed, and a third beam was separated and its power could be continuously adjusted with a variable neutral density filter (VNDf) to balance the radiation pressure along axial direction.

Figure 2.9 Modified interferometric optical tweezers for stable optical trapping

(Casaburi A. et al. 2005)

In addition to the trapping of high refractive index particles, interferometric optical traps were also applied to the trapping and manipulation of low-index particles (MacDonald M.P. et al. 2001), and the method was proved to be more simple than generating an optical vortex or rotating a Gaussian beam. The basic principle of the low-index particles trapping in interference pattern is that the repulsion of low-index hollow spheres from the brightness of a focused light beam could conversely be thought of as an attraction to darkness. It was proved that the low-index hollow particles could be aligned and manipulated in adjacent dark fringes of interference pattern.

2.3.3 Holographic optical tweezers

HOT is regarded as one of the most popular multiple trapping techniques. It is to compute a certain hologram for modulating the amplitude or the phase of incident beam, with the result that an object pattern will be produced when illuminated by a standard laser beam. In order to reduce the loss of power efficiency, phase-only hologram is usually recommended, which can be calculated with iterative algorithms (Dufresne E.R. et al. 1998, 2001). Figure 2.10 provides a basic optical configuration of HOT. A collimated input laser beam was projected onto the phase-only diffractive optical elements with designed holograms, and subsequently passed through a telescope lens system before entering the back plane of an objective mounted on an inverted microscope. The plane B^* was conjugate to plane B and plane OP^* was conjugate to trapping plane, hence multiple beams passing through B^* all passed B and then constructed the multiple traps on the sample plane. The computed hologram and the trapped particle array were also shown in Figure 2.10 (bottom).

Figure 2.10 Optical configuration of holographic optical tweezers (Dufresne E.R. et al. 2001)

Dynamic optical trapping could be realized by combining spatial light modulator and HOT (Hayasaki Y. et al. 1999) (Liesener J. et al. 2000) (Melville H. et al. 2003) (Koss B.A. et al. 2003) (Grier D. 2003, 2006) (Ladavac K. et al. 2004) (Sinclair G. et al. 2004a, 2004b) (Leach J. et al. 2004). The SLM imposes a prescribed amount of phase modulation at each pixel in an array by varying the local path length. In the following several years after the invention of HOT, researchers developed many more interesting applications for the instrument. Koss and Grier developed an ‘optical peristalsis’ for rearranging mesoscopic objects in three dimensions using short repetitive sequences of holographic optical trapping patterns (Koss B.A. et al. 2003). With a modified iterative algorithm, Curtis and Grier developed a HOT system to a more complex degree,

the system was capable of totally 3D trapping of manipulation of particles in both lateral and axial direction and even generating an optical vortices array (Curtis J.E. et al, 2002). Sinclair and Padgett experimentally demonstrated the manipulation of objects into three-dimensional geometries within holographic optical tweezers through the hologram calculated by modified Gerchberg-Saxton (G-S) and direct binary search (DBS) algorithms (Gerchberg R.W. 1972) (Sinclair G., et al. 2004b), furthermore, Jordan reported the creation of permanent 3D structures of cells at predefined position within a gelatin matrix using HOT (Jordan P. et al. 2005).

2.3.4 Multiple optical traps based on the generalized-phase-contrast technique

The generalized phase contrast (GPC) technique originates from the Zernike phase-contrast technique, which made Zernike win the Nobel Prize in 1953 (Zernike F. 1955). The Zernike phase contrast technique suppose that the phase distribution at the input plane is restricted, then the input wavefront can be expressed by the first order of Taylor expansion as,

$$\exp(i\phi(x, y)) \approx 1 + i\phi(x, y) \quad (2.6)$$

However, if the restriction of the phase distribution of input is removed, the first order Taylor expansion will not be sufficient for describing the wavefront. In this case, terms of higher order should be considered so that the expansion follows,

$$\exp(i\phi(x, y)) \approx 1 + i\phi(x, y) - \frac{1}{2}\phi^2(x, y) - \frac{1}{6}i\phi^3(x, y) + \dots \quad (2.7)$$

In this equation, the contribution of different spatial items can not be separated from the supposedly focused light represented by the first term in this Taylor series expansion. In fact, all of the spatially varying terms can contribute to the strength of the on-axis focused light. In this case, Fourier analysis is employed for completely separating the on-axis and higher spatial frequency components.

$$\exp(i\phi(x, y)) = \left(\iint_{\Omega} dx dy \right)^{-1} \iint_{\Omega} \exp(i\phi(x, y)) dx dy + \text{"higher frequency items"} \quad (2.8)$$

In the Fourier decomposition, the first term is a complex valued constant connected with the on-axis focused light from a phase disturbance defined with the spatial region, and the second item is associated with the light scattered by spatially vary structures in the phase object. When doing the phase modulation, the GPC method provides additional optimization by encoding the phase distribution itself besides the filter parameters. (Glückstad J. et al. 2001).

Glückstad and his co-workers first proposed and experimentally demonstrated the multiple optical tweezers using GPC technique (Eriksen R.L. et al. 2002) (Rodrigo P.J. et al 2002) (Daria V.R. et al. 2003). They declared that the main advantage of using GPC is the direct correspondence between phase in the input plane and intensity at the output plane, so that the requirement of spatial bandwidth and the number of phase levels of SLM or phase elements were lower than the CGH method. The basic optical configuration of GPC optical

tweezers is shown in Figure 2.11. The laser beam was first collimated and expanded and then incident on a binary π phase mask with a 2mm aperture defined by an iris matching the diameter of the phase contrast filter, which was positioned at the Fourier plane of a 4-f filtering system. A high contrast binary intensity pattern will be created at the back focal plane of the lens L2, and the intensity is directly related to the phase pattern on the phase mask. Subsequently the intensity light was projected into an inverted microscope and focused by a high NA objective lens onto the sample plane forming a desirable multiple optical traps.

Figure 2.11 Optical configuration of GPC based multiple optical tweezers

(Eriksen R.L. et al. 2002)

By replacing the phase mask with an optically addressed phase-only SLM, Glückstad group demonstrated dynamic multiple optical trapping using GPC method. It was also proved that the GPC optical tweezers could be employed to create dynamic array of dark optical traps for simultaneous trapping and

arbitrary manipulation of multiple low-index particles (Daria V.R. et al. 2004). Furthermore, it was reported that spherical high-index and low-index particle could be simultaneously trapped and interactively manipulated by an array of confining by an optical potential energy landscape with top-hat and annular cross-sectional intensity profiles respectively (Rodrigo P J. et al. 2004).

2.3.5 Multiple optical traps based on the VCSEL

VCSEL array provided an integrated solution to realize multiple optical tweezers (Birkbeck A.L. et al. 2003) (Sumiyama F. et al. 2003) (Flynn R.A. et al. 2002). A schematic of VCSEL array trapping is shown in Figure 2.12(a). The VCSEL is an array of semiconductor laser sources fabricated with high density on a substrate. The maximum output power of each pixel of VCSEL array can reach several milliwatts, and it permits MHz phase modulation. The arbitrary intensity pattern for multiple trapping can be generated by independently controlling the emission intensity of individual pixels of VCSEL array. Moreover, no additional devices are required other than switching of the VCSEL and flexible trapping and manipulation of particles could be realized through controlling the spatial and temporal intensity distribution generated by the VCSEL array sources. In addition, VCSEL array is compatible with microoptical elements like Microlens array etc.

Figure 2.12 Schematic and experimental setup of optical VCSEL array based multiple trapping (Ogura Y. et al. 2001)

Figure 2.12(b) shows the experimental setup for multiple optical trapping using VCSEL array. The VCSEL array had 8×8 array pixels, and the maximum output power was about 3 mW. The microlens array was placed subsequently after the aperture of VCSEL array to increase the light efficiency. Each VCSEL in the array was modulated independently with an analog signal coming from the digital signal converted by a D/A converter. A long working distance 60x objective lens was employed to focus the beams array onto the sample plane for multiple optical trapping. The VCSEL array trapping system was experimentally demonstrated not only be suitable for the multiple trapping in transverse plane, but also could realize a stacking of multiple particles array, which had potential application in fabricating 3D microstructures like photonics crystal. It was reported that up to seven $10 \mu\text{m}$ polystyrene particles were stacked vertically by illuminated 2×2 beams (Sumiyama F. et al. 2003).

2.3.6 Multiple traps based on the imaging fiber

Imaging fiber bundle provides another interesting approach to realize multiple optical trapping and manipulation (Tam J.M. et al. 2004). The fiber bundle contains 50000 fibers within the bundle. Each illuminated individual fiber in the array propagates light to the end face of the bundle, which was designed to end in a lens element so that the light will be focused into an array of optical traps. The optical traps will enable the simultaneous trapping and manipulation of array of particles. Furthermore, the VCSEL array optical tweezers were applied to transport and manipulate array of red blood cells (Tam J.M. et al. 2006).

Figure 2.13 Schematic of imaging fiber bundle based multiple trapping system

(Tam J.M. et al. 2004)

As it is shown in figure 2.13, the imaging fiber bundle based multiple trapping system was built on an inverted microscope. The collimated TEM₀₀ laser beam

was coupled by an objective lens into the imaging fiber bundle, and subsequently focused by the lens element array at the end-face of imaging fiber bundle. The lens element could be fabricated in several ways, including chemically etching the step-index fiber bundle to create wells and deposit ball lenses into well at the end of individual fiber, fabricating a convex lens shape structure at the end face of individual fiber etc. Figure 2.13(c) shows the trapping of multiple $4.5\ \mu\text{m}$ silica particles with regard to the light spots array created by imaging fiber bundle. Furthermore, if a digital micromirror device (DMD) is integrated into the trapping system to generate a real-time user-defined pattern, with the result that select trapping or manipulation of objects becomes feasible (Tam J.M. et al. 2006).

2.3.7 Comparison of micromirror, AOD, EOD, VCSEL and CGH optical tweezers

In this section, a detailed comparison of AOD, EOD, micromirror and dynamic holography will be provided. Micro galvano mirror, AOD and EOD trapping are all based on the time-sharing technique. The advantages of galvano mirror system include high stability and precision, large deflection angle, low insertion loss, while the switching frequency is only about 1-2 kHz and the step response time is about $100\ \mu\text{s}$. The AOD has high switching frequency as much as 200 kHz (practically the response may be limited by other factor in system), and the maximum tilting angle of AODs can only reach about 1° . A cumbersome orthogonal configuration of AODs is required to permit both x and y deflections

of optical trap, with the result that the diffraction efficiency is reduced to 60%. AOD's transmittance varies over the working range of drive frequencies, which may give rise to the change of stiffness of optical trap as it is moved. Another disadvantage of AOD is the systematic angular deviations from a linear response to the acoustic drive frequency. The advantages of EOD trapping system also includes low insertion loss and straightforward alignment, however, the high cost and relatively small deflection range greatly limit the application of EOD in trapping instrument.

Dynamic holography trapping has advantages in generating complex trapping patterns, especially for large, real time trapping arrays. The SLM enabled CGH can be employed to easily realize the complex 3D (multi-level) trapping, which can be further extended to the application of 3D micro- and nano-fabrication. And the dynamic holography also has great potential in optical sorting and optical pumping, which plays an important role in the cutting edge lab-on-chip technique. The disadvantages of CGH trapping are relatively low power efficiency (40 - 50%), and the phase-hologram design requires complex computations before experiment, which may be a potential obstacle for its further clinical applications.

The VCSEL array trapping techniques are compact, individually addressable, potentially very inexpensive, and compatible with other optoelectronic functions that may be desirable for biological lab-on-a-chip devices. However, the VCSEL is not appropriate when dynamic multiple trapping is desirable. Intra-VCSEL array movement is not suitable for long distance continuous

transportation due to the limitations in resolution and pitch of current VCSEL arrays. In addition, the commercially manufactured VCSEL is not proper for large-scale multiple trapping of hundreds of objects.

Based on the above review, it is found that the selection of multiple optical trapping techniques is based on a series of factors such as speed, moving range, accuracy, scale, stability, complexity, cost and system arrangement flexibility and etc. Each multiple optical trapping technique will have both advantages and disadvantages, in other words, there will be no perfect approach, but only appropriate method for certain applications.

2.4 Review of optical sorting techniques

Microfluidic sorting is always a hot topic due to its significant role in biochemical field. Various popular sorting methods can be grouped into three categories, mechanical sorting, electrical sorting and optical sorting techniques. The trapping of biological particles mechanically on a chip poses great challenges due to the complex physical properties of biological particles (Andersson H. et al. 2003). The electrical trapping and sorting techniques mainly include electrophoresis, dielectrophoresis, electroosmotic etc, which is suitable for miniaturization due to the convenience of microscale generation and structuring of an electric field on microchips (Manzfs A. et al. 1994) (Fiedler S. et al. 1998) (Markx G. et al. 1994). Optical sorting techniques,

which will be reviewed here, provide another solution to realize the so call on-chip sorting and characterization. They include fluorescence activated cell sorting (FACS) (Fu A. et al. 1999), optoelectronic tweezers (Chiou P.Y. et al. 2005), optical chromatography (Hart, S.J. et al. 2001, 2003), and sorting on periodical optical energy landscape (Ladavac K. et al. 2004).

The FACS is grouped under the optical category is due to the fact that the marked particles of interests are identified using a laser to induces fluorescence and an optical photodetector to detect the fluorescence, although the transport forces are still dependent on electrokinetic, dielectrophoresis (DEP), hydrodynamic, and optical forces etc. FACS provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. FACS has been demonstrated to be powerful and compatible in a microfluidic system, however, it requires inherent or marking of the selected objects and requires detection and analysis stage, and accordingly FACS it is put into the category of “active sorting”.

The optoelectronic tweezers (OET) make use of dynamic optical image to create virtual electrodes for generating dielectrophoresis forces, which can be adopted for manipulation and sorting of microfluidic particles (Chiou P.Y. et al. 2005). The system posed low requirements on optical system, which mainly include an incoherent light source and a DMD. With direct imaging control, the

system was capable of realizing multiple complex functions like multiple parallel manipulation, complex virtual optical machine and even selective sorting of biological cells. The optical image driven dielectrophoresis technique permits high-resolution patterning of electric fields on a photoconductive surface for manipulating single particles. It is noted that optoelectronic tweezers has extremely large working area, which reaches $1.3 \times 1 \text{ mm}^2$. Making use of the dependence of the DEP forces on the properties of particles, OET technique was also capable of separate the biological cells with different sizes, membrane properties and internal conductivity. The OET sorting technique requires no external flow within microfluidics, but is limited only to the separation of large objects (several micrometers and above) only.

In optical chromatography, a laser beam is focused into the solution containing particles counter-flowing in a capillary. The particle is focused into the beam center and drifts at which point the radiation pressure is identical to the force induced by the liquid flow, resulting in separation of particles as a function of size. Optical chromatography technique has several advantages including sensitivity, resolution and time for separation and detection. However, the technique can not be applied to small particles and molecules. Furthermore, the number of the particles measured in optical chromatography is limited, generally decreasing the accuracy in the measurement. And the technique can not be applied to small particle or molecules (at least several micrometers). Due to differences in operation principle, optical chromatography may have its own application fields, such as single molecule detection and characterization,

measurements of immunological reaction rates or measurements of the microorganism's power etc.

Optical tweezers have been proved to be capable of manipulating objects of nanometers to micrometers with high resolution and non-invasive feature (Ashkin et al. 1986). Recently, optical landscape is reported to be capable of deflecting and sorting the objects in the microfluidic chamber (MacDonald et al. 2003) (Korda et al. 2002) (Ladavac et al. 2004). The sorting principle lies in the competition between the microfluidic flow and exerted optical force. When a group of microspheres are driven through a periodic potential energy landscape, their trajectories may be deflected from the original direction. And whether the trajectories are deflected or not are strongly dependent on the different physical attributes of microspheres, including size or refractive index. This all optical sorting method does not rely on the fluorescent marker but on the intrinsic physical attributes like geometrical size and refractive index. Hence, compared with FACS technique, all optical sorting on landscape is in fact a passive continuous microfluidic sorting which requires no human interaction. It is noted that all-optical sorting method employs an optical landscape rather than a single or double tightly focused laser beam. Accordingly, all optical sorting is possible to achieve very high throughput, as it would be relying on the response of the objects to an optical landscape that could take the form of an extended light pattern in two or three dimensions (Dholakia K. et al. 2006).

2.4.1 Fluorescent activated cell sorter (FACS)

Fluorescence-activated cell sorting (FACS) is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest. FACS is usually made up of several parts including light sources, optical system, flow chambers and sorting devices. Light sources are used to illuminate the particles or cells; optical systems direct the light path for illumination and collection of light scattered or emitted by the sample. Flow chambers maintain a stream of particles in a single file at a constant flow velocity to pass through the interrogation point where the excitation beam and the stream intersect. Sorting devices break up the jet of particles into charged droplets and deflect the pathway through a pair of high voltage plates. Photodetectors are placed at angular positions relative to the beam axis to detect emitted or scattered photos. Electronics then will capture and process the signals collected by photodetectors. In a sample run, as each cell passes through the point of interrogation, photo detectors collect its fluorescence and light scattering through optics. These photodetectors convert light signals into electrical signals and the computer analyzes and stores the data.

Contemporary FACS systems have evolved into high end instruments that use multiple high power lasers along with the beamsplitters, mirrors, filters,

photomultiplier tubes and signal-processing components and are capable of evaluating cells at extremely high rates up to 40000 particles per second. (Ibrahim, S.F., et al., 2003) However, they tend to be large, expensive bench top systems that require specially trained personnel for sample tagging and instrument operation and are therefore mostly found in core facilities. A miniaturized FACS has the potential of being a relatively cheap and portable cell interrogation tool with far-reaching applications in point of care diagnosis. Thus in the 1990's, several micromachining methods have been sought to replace the conventional flow chambers in FACS with micro fluidic devices (Fu, A. Y., et al., 2002) (Fu, A.Y. et al. 1999) that can potentially provide more accurate sorting and flexible design.

Fu, A.Y. et al. (1999) has constructed and demonstrated a microfabricated FACS device for sorting of objects using electrokinetic flow. The disposable sorting device was fabricated using soft lithography, which enabled the design of inexpensive and flexible miniaturized fluidic devices. The throughput of the device was about 20cells/s. The device was a silicone elastomer chip with three channels joined at a T-shaped junction. The sorting algorithm consisted of running the cells from the input channel to the waste channel until a cell's fluorescence is above a preset threshold, at which point the voltages are temporarily changed to divert the cell to the collection channel (Figure 2.13).

Figure 2.14 Schematic of the algorithms for the forward sorting and reverse sorting.

(Fu, A.Y., et al., 1999) The fluid flow is switched as fluorescence is detected so that red beads are sent directly to the collection channel. Fluid flow is established at a high rate from the input well to the waste channel. After event detection, the fluid flow is halted and reversed until the bead is detected a second time. It is then directed at a slower speed to the collection channel.

Wang M.M, et al. (2005) reported a FACS using fast all-optical control switch, in which optical force became a dominant force rather than the electrokinetic forces. In addition to the laser adopted for inducing the fluorescence, the other infrared laser worked as the optical switch aiming at directing the target objects to collection output. The approach confined the optical field to a critical junction of microfluidic chamber to fully use the power and the optical forces to provide an active binary switch for objects suspended in a microfluidic flow. The schematic of the device is shown in Figure 2.15. The objects were first aligned to the center of the microfluidic channel by flow focusing, and subsequently passed through the analysis region. If an object was identified as a target object, the optical switch would be activated and the focused laser spots would deflect the object to the target output channel. The laser spot would move at a speed matching the flow velocity and at a angle with regard to the flow to maximize the interaction time between the object and the laser. Finally,

the targeted cells would be directed by the laser force to the collection output while the rest flow out to the waste output channel.

*Figure 2.15 Schematic high throughput FACS using optical switch
(Wang M.M, et al. 2005)*

2.4.2 Particles separation by optical chromatography

Optical chromatography is a particles separation and characterization method first demonstrated by Imasaka T. et al. (1995) and Kaneta T. et al.(1997). The technique involves using a long focal length lens to mildly focus a laser beam into a fluid flow traveling in the opposite direction, as shown in Figure 2.15.

Microscopic particles in the fluid flow near the laser focal point are drawn toward the beam center and then accelerated in the direction of laser propagation. Trapped particles are pushed against the fluid flow and retained where the optical and fluidic forces acting on the particles are balanced. The distance particles travel from the focal point along the diverging laser beam, illustrated in Figure 2.16, is termed the retention distance (Z). Particles of larger size or higher refractive index experience greater optical pressure and are propelled farther than smaller or lower refractive index particles. This results in unique positions (separation) along the beam axis for particles of different size and composition (refractive index). Using this technique, it has been shown that particles can be separated not only by size, but also by refractive index, which is a physical property related to their chemical composition. (Hart, S.J. et al. 2001, 2003)

The typical optical chromatography system for particle separation was shown in Figure 2.17. The laser beams passes through the mirrors and is focused using a lens into the microfluidic channel. The entire optic and fluidic system is placed under a microscope for observation and a CCD camera is used for data collection. Fluid flow is generated using gravity feed between a raised fluid inlet and lower fluid output reservoir.

Figure 2.16 Microfluidic network design with laser beam super-imposed
(Hart, S.J., et al., 2004)

Figure 2.17 Optical chromatography system for particle separation
(Hart, S.J., et al., 2003)

2.4.3 Sorting by optoelectronic tweezers

Optoelectronic tweezers (OET) creatively combined optical image technique and dielectrophoresis using a multi-layer photoconductive device (Chiou P.Y. et al. 2005). The OET was first reported and demonstrated by Chiou and Wu from UC Berkeley. The technique employed optical image driven

dielectrophoresis permitting high-resolution patterning of electric fields on a photoconductive surface for manipulation of particles. The system posed low requirements on optical system, which mainly include an incoherent light source and a DMD. With direct imaging control, the system was capable of realizing multiple complex functions like multiple parallel manipulation, complex virtual optical machine and even selective sorting of biological cells. Figure 2.18 shows the basic configuration of the OET. The device included a upper transparent and conductive ITO glass and a low photoconductive surface including ITO glass and and n+ hydrogenated amorphous silicon (a-Si:H) layer, and the samples was included between these two surface which would be biased with a 10V ac signal during experiments. When the photoconductive layers were illuminated by projected light, the virtual electrodes were ‘switched on’ to produce electric fields, which would generate dielectrophoresis forces for manipulation.

Figure 2.18 Schematic of optoelectronic tweezers (Chiou P.Y. et al. 2005)

The particles would be attracted to or repelled from the illuminated area by DEP forces, and the direction was dependent on the physical properties of particles, the frequency of a.c. field as well as the dielectric properties of surface. Making use of the dependence of the DEP forces on the properties of particles, OET technique was capable of separate the biological cells with different sizes, membrane properties and internal conductivity. The OET sorting technique requires no external flow within microfluidics and is limited the separation of large objects only. Figure 2.19 shows the sorting results of live cells and dead cells with specially designed dynamic optical ring images. The membranes of live cell can maintain an ion concentration differential between intracellular and extracellular environments, whereas the dead cells can not. Consequently, the dielectric properties of live cell and dead cells are different. Positive DEP forces were acting on the live cells and pushed them to the bright areas, whereas the dead cells leaked out through the dark gap and not centered.

Figure 2.19 Separation of live cells and dead cells with OET using special dynamic images (Chiou P.Y. et al. 2005)

2.4.4 Sorting on periodical optical landscape

Optical sorting (or separation, as the name implies) involves the use of optical forces to select particles of interest, particularly in a microfluidic system where diffusion is too slow. The all-optical sorting on periodical landscape does not rely on the fluorescent marker but on the intrinsic physical attributes like geometrical size and refractive index. Accordingly, it is in fact a passive continuous microfluidic sorting which requires no human interaction. Different from the previous techniques, all-optical sorting method employs an optical landscape rather than a single or double tightly focused laser beam. And among several typical optical landscapes types, the lattice is usually employed due to its theoretical exponential size selectivity.

The optical sorting on periodical landscape was first experimentally demonstrated by MacDonald and Dholakia et al. (2003). While mesoscopic polarisable particle flows through an optical lattice, the path is significantly determined by the degree to which the particle interacting the optical lattice. And it is proven that two types of particles with different size, refractive index or even shape will interact in a different manner with such a lattice. Combining this selective interaction with a guiding mechanism has allowed us to achieve lateral separation of particles by all these properties simply by flowing them through an angled optical lattice. Practically, MacDonald and co-workers generate a three-dimensional optical lattice from a five-beam interference pattern to sort a mixed pool of particles flowing simultaneously in a microfluidic system. They show that some particles can be deflected from their

original trajectories, whereas others pass almost straight through the interference lattice. The schematic of optical sorting on periodical energy landscape is shown in Figure 2.20. The mixtures of two types of particles in chamber B were driven by hydrodynamic flow to pass through a periodical energy landscape. The forces exerted by optical landscape deflected the blue particles from original direction to follow the axis of optical lattice and made them to be collected at chamber C, while the red particles passed through the optical lattice without deflection and finally reached chamber D.

Figure 2.20 Schematic of optical sorting on periodical energy landscape

(MacDonald M.P., et al. 2003)

It is experimentally demonstrated that optical sorting is able to separate microscopic objects by sizes or refractive indices. A dense particle flow through this optical lattice could theoretically achieve exponential size selectivity. Figure 2.21 shows the optical sorting of a mixture of silica ($n=1.37$) and polymer ($n=1.58$) spheres of identical size passes through the optical lattice. It

is impressive that the sorting efficiency was measured to be 96% even for concentrated solutions flowing at $35\mu\text{m/s}$. Another impressive result is 45° angular separation of two groups of particles. In order to realize 45° angular separation, MacDonald moved beyond the locked-in microfluidic sorting (localized, isotropic traps array). They use light patterns that contain a tailored degree of ‘interconnectedness’ along the direction of desired channeling. Interlinks between the optical lattice sites are significant to deflecting particles into a desired trajectory. It is experimentally demonstrated that when a flow of mixed particles is passed through an optimized, selected particles are strongly deflected from their original trajectories (e.g., deflected at 45°).

Figure 2.21 Optical sorting by refractive indices on optical lattice (MacDonald M.P., et al. 2003) (a) silica/polymer mix, indicating the typical particle density. n , index of refraction. (b) Polymer trajectories are shown in red, and silica trajectories in black

The key factor in an optical sorting system is devising an optical scheme to create a multiple optical tweezers system. In addition to multi-interference

method, Grier group and Glückstad group also experimentally demonstrated microfluidic sorting on optical lattice generated with HOT (Ladavac K. et al. 2004) (Roichman Y. et al. 2007) and GPC (Perch-Nielsen I.R. et al. 2006) respectively. In the meanwhile, the investigations on the theoretical mechanism on the sorting on optical lattice were also reported (Gopinathan A. et al. 2004) (Pelton M. et al. 2004) (Lacasta A.M. et al. 2005).

2.5 New ideas in multiple optical trapping and sorting

In this thesis, the author fully investigates the all-optical sorting techniques based on the multiple optical traps created by fabricated microoptical elements. The fabricated microoptical elements include MLA, DOE, chess-board grating etc. Here two representative techniques are highlighted to compare with other dominant trapping and sorting techniques: one is the composite MLA multiple trapping and sorting, while the other is on-chip sorting using microoptical elements.

2.5.1 MLA based multiple trapping and sorting technique

In biochemistry and biotechnology field, there is an increasing requirement of the ability to trap, manipulate more than one objects at the same time. As single beam optical tweezers has been a standard instrument in many labs, it is desirable that there is an approach can easily modify the current single-beam

optical tweezers (including microscope) into a system capable of multiple optical trapping, and even sorting of two types objects in microfluidics. The modification should be low-cost, power-efficient, requiring no complex computation and having ability to realize arbitrary optical traps array. In this thesis, a new multiple optical trapping and sorting techniques based on fabricated MLA together with microscopic system is proposed to satisfy all of these requirements.

In MLA multiple trapping system, the fabricated MLA is employed to transform an incident laser beam into an optical lattice and subsequently projected by microscopic system to form optical traps array on sample plane. The MLA can be fabricated with mature microlithography together with reflow technique on glass substrate, consequently, it is cost-effective with those techniques requiring additional special device for generating multi-beam, such as AOD, EOD, and scanning mirror. Compared with AOD and EOD system, the MLA 2D trapping system is easy to align, and compatible with popular single beam optical tweezers microscopic system and the MLA system requires no complex computation like CGH design. Furthermore, the composite feature of fabricated of MLA makes it possible to create optical pattern with arbitrary distribution for trapping. The power efficiency is 100% theoretically, and 80% practically, which is obviously higher than CGH technique and AOD technique (2D). Of course, the MLA trapping also has its disadvantages. Not similar to scanning mirror, AOD, EOD and CGH, MLA trapping system is not easily to realize the dynamic trapping. Another disadvantage of MLA trapping system is attributed to the non-uniformity of optical lattice, which is mainly caused by the

Gaussian distribution of incident laser beam. Additionally, MLA system's application is limited to 2D trapping, that is to say, it seems not feasible for MLA to realize complex multi-level trapping as demonstrated by CGH.

The MLA generated optical lattice can realize continuous sorting of bi-disperse particles by sizes or refractive indices without external markers, and the throughput of optical sorting is between 20 ~ 30 particles per second, which is comparable with the multi-interference and GPC enabled optical sorting methods. Furthermore, the separation of two groups of particles with 45° separation angle is realized with composite optical lattice. Such separation angle is also achieved by MacDonald M.P. et Al. (2003) using multi-interference system.

2.5.2 On-chip optical trapping and sorting with Talbot array illuminator and MLA

Optical tweezers offers high precision for trapping particles, which sizes ranges from several nanometers to tens of micrometers; however, the trapping area is limited due to tight focusing requirement. In this thesis Talbot array illuminator (TAI) created by fabricated chessboard grating is proposed to realize on-chip multiple trapping without strong focusing laser beam. Different from those scanning mirror, AOD, CGH, VCSEL, GPC techniques, the Talbot trapping requires no lens projection system and objective, with the result that the working area of Talbot traps are large. The technique is capable of trapping

hundreds of particles within area of $200\text{ }\mu\text{m}$ by $200\text{ }\mu\text{m}$ or even larger. It is especially noted that for the Talbot trapping, there is no direct limit to the size of the working area. This is due to the fact that the chip's effective area with optical traps is only determined by the collimated laser beam's size, as long as enough laser power is maintained over the working area. When the technique is applied to continuous optical sorting, the high throughput (~ 100 particles/s) is possible provided the laser power is high enough for creating large area traps. Another unique feature of Talbot trapping is that the modified self-imaging effect is induced by a layer of trapped particles and it is subsequently used as a periodic grating structure to generate its own self-imaging pattern in 3D. Based on this secondary layer-by-layer self-imaging effect, optical trapping of silica and polystyrene colloidal particles at different planes in a microchamber can be realized. Final advantage of Talbot multiple trapping lies in its ability to be applied in fabrication of 3D periodical sub-microstructures. Despite of all of these advantages, it should also be recognized that the Talbot effect is not capable of generating complex non-periodical pattern for trapping.

Talbot optical traps can easily modified to an on-chip on sorting system. The chess-board grating can be adhered to the bottom surface of the microchamber. The optical chip is held by a high precision 3D microstage for adjusting the locations of the samples. Combined with a motorized stage, the system is complete enough to demonstrate the basic functions such as particles trapping and sorting, even if the PDMS microchamber and micropump are not employed. Such optical system is demonstrated to realize optical sorting of particles by sizes with fluid flow of $10\text{ }\mu\text{m}/\text{s}$.

In the thesis, MLA is also proposed to realize on-chip optical trapping. The particularly designed MLA could be utilized to directly create an optical lattice near the surface of element, without strongly focused laser beam. The MLA optical chip combining MLA on substrate on a microchamber is compact, efficient and easy to align. Compared with the Talbot multiple optical trapping technique, the MLA chip's advantage lies in its composite feature, which could generate optical lattice with arbitrary distribution.

CHAPTER 3

ANALYSIS OF OPTICAL SORTING ON PERIODICAL LANDSCAPE

In this chapter, a quantitative model is developed to analyze the optical sorting on a periodical optical energy landscape. It is demonstrated that the periodical optical lattice theoretically permits exponential size selectivity, and sorting particle sizes can range from hundreds of nanometers to several micrometers.

3.1 Introduction

The main idea of optical sorting is to non-invasively separate particles of different physical or geometrical parameters suspending in a flowing fluid by means of a specialized optical pattern. This sorting mechanism is based on the resultant deflection caused by optical gradient forces competing with a water-driven force in the laminar flow. Whether the particles will be deflected or not depends on physical properties and geometrical properties, such as refractive index, particle size, laser power, fluid flowing speed, force driving direction, and optical pattern (Korda P.T. et al. 2002) (Ladavac K. et al. 2004). That is to say, under the same condition except for the geometrical size, larger particles might be deflected with an angle while the smaller particles continue flowing in the original driving direction after passing through the optical potential landscape, thereby enabling the sorting of particles of different sizes. In this chapter, a simplified model for simulating the optical sorting on a 1D periodical optical lattice will be developed. The model provides a quantitative understanding of the effects of the various parameters which govern the result

of optical sorting process. The influence of temperature induced randomized thermal forces have been proved that to only degrade the sensitivity of particles selectivity in optical sorting, hence, they are neglected so as to simplify the model (Lacasta A.M. et al. 2005) (Pelton, M. et al. 2004).

3.2. Theoretical model for optical sorting

When a group of particles are driven passing through an optical energy landscape in microfluidic chamber, two types of forces including optical gradient force and a uniform dynamic driving force are working on the particles. As shown in Figure 3.1, the flow driving force is oriented at a fixed angle θ with respect to the optical potential well landscape axis in the horizontal direction. The flow driving force $\vec{F}_f$ can be resolved into two components, $\vec{F}_f \cos \theta$ and $\vec{F}_f \sin \theta$. To explain the mechanism of optical sorting, two particle sizes will be considered here – the small particles and the large particles. In the absence of optical landscape, the particles will flow faithfully along the straight lines in the θ direction. However, with the imposition of optical forces produced by an optical landscape, particles may detour from the original driving force direction. In Figure 3.1(a), the gradient force exerted on the larger particles is large enough to balance the component of driving force $\vec{F}_f \sin \theta$, the large particles flow in the x direction. On the contrary, the smaller particles will

remain flow in the original driving force direction (shown in Figure 3.1(b)). Thus, as long as one can quantitatively analyze the actual forces exerting on the particles when they pass through the optical landscape, the relevant physical parameters could be designed to realize the separation of two types of particles with different geometrical size or refractive indices.

Figure 3.1 Trajectories of particles passing through the optical landscape

(a) Larger particles are locked-in to the axis of 1D optical lattice

(b) Smaller particles flow along the original driving direction without deflection

The local optical intensity can be represented by optical intensity $I(\vec{r})$. An object's potential energy at r is not only determined by $I(\vec{r})$, but also by the object's response to it. Thus, the effective potential well can be calculated as the convolution of the optical intensity landscape $I(\vec{r})$ with a form factor that describes the object's interaction with the optical landscape. Generally, an object's form factor is nonzero only over a limited domain set by its size. The form factor expressed in separable Gaussian function is as follows (Pelton, M., et al., 2004),

$$f(\vec{r}) = \alpha a \exp\left(-\frac{r^2}{2a^2}\right) \quad (3.1)$$

where a is the radius of particle, α is the parameter describing the interaction between light and matter, and it is given by (Harada, Y., et al., 1996),

$$\alpha = 2\pi \frac{n_2}{c} \left(\frac{n_2^2 - n_1^2}{n_1^2 + 2n_2^2} \right) \quad (3.2)$$

where n_1 is the refractive index of the particles immersed in the liquid medium, n_2 is the refractive index of the liquid medium, c is the speed of light in vacuum thus, the effective potential energy is give by,

$$V(\vec{r}) = -I(\vec{r}) \bullet f(\vec{r}) = -\int f(\vec{x} - \vec{r}) I(\vec{x}) d^2x \quad (3.3)$$

Furthermore, the force caused by optical potential exerting on the particle is given by,

$$F_o = \vec{\nabla}(V(\vec{r})) = \vec{\nabla}[-(I(\vec{r}) \bullet f(\vec{r}))] \quad (3.4)$$

3.2.1 Linear Fringe Optical Pattern

In order to devise a practical optical landscape for achieving high size selectivity, let us consider a single linear fringe aligned to the x axis. The optical landscape can be described as a Gaussian profile of intrinsic width (radius of laser beam waist) ω ,

$$I(\vec{r}) = I_0 \exp\left(-\frac{y^2}{2\omega^2}\right) \quad (3.5)$$

According to equation (3.3), the effective potential well is given by,

$$V(\vec{r}) = 2\pi\alpha I_0 \frac{a^3\omega}{\sigma(a)} \exp\left(-\frac{y^2}{2\sigma^2(a)}\right) \quad (3.6)$$

Hence the gradient force in the y direction is,

$$F_o = 2\pi\alpha I_0 \frac{a^3\omega}{\sigma^3(a)} y \exp\left(-\frac{y^2}{2\sigma^2(a)}\right) \quad (3.7)$$

Based on the above equation, the locked-in condition in optical landscape can be expressed as follows,

$$F_f \sin \theta \leq \max[V(r)] = \max[-I(\vec{r}) \circ f(\vec{r})] \quad (3.8)$$

Obviously the optical gradient force in the y direction will reach a maximum

value when $y = \sigma(a)$. Based on equation (3.8), the condition for locked-in transport is as follows,

$$\sin \theta \leq \sin \theta_m = \frac{\max(F_o)}{F_f} = \frac{2\pi|\alpha|I_0a^3\omega}{\sqrt{e}F_f(a^2 + \omega^2)} \quad (3.9)$$

$$\theta_m = \arcsin\left(\frac{2\pi|\alpha|I_0a^3\omega}{\sqrt{e}F_f(a^2 + \omega^2)}\right) \quad (3.10)$$

where θ_m is the maximum locked-in angle. Equation (3.10) indicates that the maximum locked-in angle of driving force direction depends algebraically on the size of particle and linearly on other physical properties. This relationship can be adopted to separate particles of different size or refractive index.

3.2.2 One Dimensional (1D) Optical Lattice

In the further analysis, the attention is focused on a one dimensional periodically modulated fringe. The optical landscape is uniform outside a bounded region in the y direction and periodically modulated in the x direction. In the analysis, the optical landscape is assumed to be a linear line of Gaussian wells, that is, a one-dimensional optical lattice,

$$I(\vec{r}) = I_0 \exp\left(-\frac{r^2}{2\omega^2}\right) \quad (3.11)$$

After convolution with a Gaussian form factor, a single well of intrinsic width

ω takes the form,

$$V_1(\vec{r}) = -V_0 \exp\left(-\frac{r^2}{2\sigma^2}\right) \quad (3.12)$$

Hence the line with n Gaussian wells has the potential energy,

$$V(\vec{r}) = V_0(a) \sum_n \exp\left(-\frac{(r - nb\hat{x})^2}{2\sigma^2(a)}\right) \quad (3.13)$$

where $V_0 = 2\pi\alpha I_0 \frac{a^3\omega}{\sigma}$

The optical intensity maximum can be determined from the power of the single optical spot in the lattice,

$$P = \iint_s I_0 \exp\left(-\frac{r^2}{2\omega^2}\right) r dr = \int_0^{2\pi} \int_0^r I_0 \exp\left(-\frac{r^2}{2\omega^2}\right) r dr = 2\pi I_0 \omega^2 (1 - 1/\sqrt{e}) \quad (3.14)$$

$$I_0 = \frac{P}{2\pi(1 - 1/\sqrt{e})\omega^2} \quad (3.15)$$

Similar to the analysis for a linear fringe, the condition for locked-in state in the landscape can be expressed as follows,

$$\sin \theta \leq \sin \theta_m = \eta(a) \exp(-b^2/8\sigma^2) \quad (3.16)$$

where $\eta(a) = \frac{2}{\sqrt{e}} \frac{V_0}{\sigma F_0} = \frac{2}{3\pi\sqrt{e}} \frac{\alpha P}{\omega \xi u} \frac{a^2}{\sigma^2}$

Thus, the exponential size selectivity is proved by equation (3.16). That is to say, the line of Gaussian wells offers an exceptional exponential size selectivity for sorting of particles of different geometrical size. It has been demonstrated that in microfluidics the particles with larger size may be deflected and locked in to the x axis of lattice, while the smaller particles may continue to flow the original driving direction.

Linear fringe optical pattern and one-dimensional optical lattice composed of line of Gaussian wells have been discussed above. However, the analysis of two-dimensional energy landscape is more complex. The two dimensional optical landscape can be solved only if the potential can be written as a sum of modulations in the x and y direction. Pelton and Ladavac et al. (2004) showed that for two dimensional optical potential well landscape which can be separated into one dimensional terms, there is no qualitative difference between modulating in two directions and in one direction. Consequently, the following section will further discuss the locked-in condition for one dimensional optical lattice inclined at an angle to the driving force.

3.3 Analysis of Locked-in Condition for 1D lattice

According to equation (3.16), it can be found that θ_m will increase rapidly with the growth of particle size. However, Gopinathan and G.Grier et al. (2004)

proposed that there was another restriction for θ_m in practical microfluidic experiment due to physical reason. They suppose the effective direction of particles motion be φ , which is given by

$$\tan \varphi = q / p \quad (3.17)$$

where (p,q) are the smallest lattice indices satisfying,

$$|q - p \tan \theta| \leq \eta(1 + \tan^2 \theta)^{1/2} \quad (3.18)$$

Equation (3.18) provides another limit for driving direction angle θ except for θ_m , which demonstrates that the angle θ cannot increase infinitely with the growth of particles size, for the equation (3.19) offers another maximum limit. In other words, it implies that a large angle θ may result in no deterministic locked-in state (refers to the motion of particles is locked along the x axis of optical lattice). Figure 3.2 shows the relationship between relative intrinsic width M of optical spot ω/b and driving direction angle θ . When the value of ω/b is 0.4, the maximum angle is a little larger than 20° . Obviously the conclusion can be drawn that a larger intrinsic width of optical spot relative to spots interval optical will offer a larger maximum limit of θ , thus, the deflection angle is correspondingly increased. On the other hand, the following analysis will demonstrate that the particles sorting range will be influenced if the value of ω/b is too high, such as approaching the theoretical maximum value 0.5. As a result, in the practical experiment, it is recommended that a relative high value of ω/b is selected, usually about 0.3 ~ 0.4.

Figure 3.2 Relationship between relative intrinsic width of optical spot ω / b
and driving direction θ

In order to quantitatively analyze the various parameters' effects on marginal angle, the case where $n_p = 1.58$, $n_w = 1.33$, $\theta = 20^\circ$, $\xi = 10^{-3} \text{ kg/m}\cdot\text{s}$, $u = 30 \text{ }\mu\text{m/s}$, $P = 0.5 \text{ mW}$ was considered and from equation (3.2), we get $\alpha = 3.358 \times 10^{-9} \text{ s/m}$,

Consequently, according to equation (3.15),

$$\sin \theta_m = 7.204 \times 10^{-6} \frac{1}{w} \frac{a^2}{a^2 + w^2} \exp \left[-\frac{b^2}{8(a^2 + w^2)} \right] \quad (3.19)$$

Furthermore, for $w = 0.4b$ (proper for $\theta = 20^\circ$ according to Figure 3.2), $b = 1 \text{ }\mu\text{m}$, $a/b = k$, the locked-in condition is given by,

$$\sin \theta = \sin 20^\circ \leq \frac{7.204 \times 10^{-6}}{0.4b} \frac{k^2}{0.16 + k^2} \exp\left(-\frac{1}{1.28 + 8k^2}\right) \quad (3.20)$$

Figure 3.3 shows that particles with radius larger than about $0.13 \mu m$ will be deflected and locked-in to the x axis of the traps array under the defined conditions ($\theta = 20^\circ$, $w = 0.4b$, $b = 1 \mu m$, $P = 0.5 mW$).

Figure 3.3 The dependence of locked in deflection angle θ_m on particle radius a ,

when $\sin \theta = 0.342$, $w = 0.4b$, $b = 1 \mu m$, $P = 0.5 mW$

Figure 3.4 shows the dependence of locked in deflection angle θ_m on particle radius a under different optical spot power respectively (other conditions same as those in Figure 3.3). Obviously, the minimum particle size for the deflection will be smaller when the optical spot power is stronger. That is to say, the sorting range is expanded when the optical spot power is increased.

Figure 3.4 The dependence of locked in deflection angle θ_m on particle radius a when under different optical spot power respectively (other conditions same as Figure 3.3)

Figure 3.5 The dependence of locked in deflection angle θ_m on particle radius a at different fluid flow speed respectively (other conditions same as Figure 3.3)

Through increasing the optical power or reducing the fluid flow speed, the particles to be sorted can be as small as hundreds of nanometers if our model still hold true at this microscopic level. Furthermore, it is demonstrated that the model remains effective for particles as large as dozens of micrometers. Suppose $n_p = 1.58$, $n_w = 1.33$, $w = 0.2b$, $\theta = 10^\circ$ (according to Figure 3.1), $\xi = 10^{-3} \text{ kg/m}\cdot\text{s}$, $u = 20 \text{ }\mu\text{m/s}$, $P = 2 \text{ mW}$, then from equation (3.2), it is obtained that $\alpha = 3.358 \times 10^{-9} \text{ s/m}$, consequently, according to equation (3.16),

$$\sin \theta_m = 7.204 \times 10^{-6} \frac{1}{w} \frac{a^2}{a^2 + w^2} \exp\left[-\frac{b^2}{8(a^2 + w^2)}\right] \quad (3.21)$$

Furthermore, for $b = 20 \text{ }\mu\text{m}$, $a/b = k$, the locked-in condition is given by,

$$\sin \theta = \sin 10^\circ \leq \frac{7.204 \times 10^{-6}}{0.2b} \frac{k^2}{0.04 + k^2} \exp\left(-\frac{1}{0.32 + 8k^2}\right) \quad (3.22)$$

Figure 3.6 The dependence of locked in deflection angle θ_m on particle radius a ,

when $\sin \theta = 0.17$, $w = 0.2b$, $b = 20 \text{ }\mu\text{m}$, $P = 2 \text{ mW}$

From Figure 3.6, it is achieved that only those particles with radius larger than about $0.142b = 2.84 \mu m$ will be deflected and locked-in to the traps under the defined conditions. Thus it is proved that two bundles of particles could be separated: for particles larger than $2.84 \mu m$, there will be a 10° deflection with regards to the original driving direction; while for the other smaller than $2.84 \mu m$, there will be no difference of motion direction. To be concluded, through adjusting the optical power, fluid flow speed, driving force direction as well as the optical spot intrinsic width, the optical lattice enabled sorting particles by different sizes or refractive indices.

3.4 Conclusion of the Chapter

In this chapter, a quantitative model has been developed to analyze the optical sorting on a periodical optical energy landscape. It has been theoretically demonstrated that the 1D periodical optical lattice permits exponential size selectivity, and particle sizes can range from hundreds of nanometers to several micrometers. The optical sorting model provides a basic reference for selecting physical parameters (geometrical size of optical lattice, laser intensity, and flow velocity etc.) for optical separation of particles by different sizes or refractive indices.

CHAPTER 4

**MULTIPLE OPTICAL TRAPPING AND
SORTING USING MICROLENS ARRAY
PROJECTION SYSTEM**

In this Chapter, a multiple optical trapping and sorting system is proposed by employing MLA, which is used to transform an incident laser beam into an array of focal spots and subsequently projected by an external system to form an optical lattice pattern on the sample plane. The MLA is designed and fabricated according to the requirement of the objective optical lattice for trapping. It is experimentally demonstrated that the MLA was capable of creating an arbitrary optical lattice for multiple trapping of an array of particles. Continuous sorting of bi-disperse particles by sizes without external markers is experimentally realized, and the throughput of optical sorting is between 20 ~ 30 particles per second. Furthermore, the separation of two groups of particles with 45° separation angle is realized with composite optical lattice.

4.1 Introduction

For the optical lattice based optical sorting system, the generation of optical lattice plays a significant role. The existing approaches include holographic optical tweezers (Ladavac K. et al. 2004), multiple interference (MacDonald MP et al. 2003), and generalized phase contrast etc (Eriksen RL et al. 2002). In this Chapter, MLA is proposed to be inserted into a typical single-beam optical trapping system to realize a convenient, flexible and high-efficient all-optical continuous sorting system. Based on aperture division, refractive MLA is capable of focusing an incident plane wave into an array of focal spots.

Compared with the multiple-beam interference, the MLA provides a simple configuration and a minimum modification in existing optical microscopic system for microfluidic multiple trapping and sorting. Furthermore, the MLA has theoretical power conversion efficiency of nearly 100%, which is higher than the SLM and holographic diffractive element significantly. Additionally, the composite MLA enables to create an arbitrary optical pattern, which provides more flexibility in multiple trapping and sorting.

Various approaches has been proposed for the fabrication of refractive MLA, including thermal reflow (Popovic Z.D. et al. 1988) (Daly E. et al. 1990) (Yang H. et al. 2004), mass transport mechanisms in semiconductors (Swenson J.S. et al. 1995) (Ballen T.A. et al. 1999), direct writing technique (Kley E.B. 1997) (Gale M. et al. 1994), gray-scale lithography (Daschner W. 1996) (Peng, Q. et al. 2002), soft lithography (Kunnavakkam, M.V et al. 2003) (Yuan, X.-C. et al. 2005) etc. In this chapter, the thermal reflow technique (Sinzinger S et al. 2003) is employed to fabricate MLA in virtue of its features like cost-effective and ideal physical analog surface profile. The developed cylindrical island of photoresist is melted and then drawn by the surface tension into the shape of lens with smooth profile.

4.2 Generation of multiple optical traps by MLA

4.2.1 Design and fabrication of MLA for generation of optical traps array

Figure 4.1 shows the basic principle of how a laser beam can be used to produce an optical traps array using MLA and lens projection system. In spite of the relative simplicity of the principle, it is of necessity to determine the parameters of MLA to realize the required shape and size of the optical lattice on the sample plane and those of the lens projection system to enhance the performance of MLA projection system.

Figure 4.1 Principle of generation of optical traps array with MLA

According to Figure 4.1, MLA is employed for focusing an incident laser beam into an array of focus spots at the back focal plane of MLA, and subsequently

converted by a converging lens into different beamlets. Finally the objective will focus the incident beamlets into desirable optical lattice for trapping.

The relationship between the period of MLA and the spacing of traps array is described as (Merenda F. et al. 2007),

$$p_{trap} = p_{MLA} \times \frac{f_{obj}}{f_{lens}} \quad (4.1)$$

where p_{trap} , p_{MLA} is the period of traps array and MLA respectively, f_{obj} , f_{lens} is the focal length of objective lens and converging lens respectively, n_{water} , n_{air} is the refractive index of water and air respectively. Thermal reflow technique is employed for the fabrication of MLA. It provides a simplest way to fabricate a MLA with ideal analog surface, provided that the f-number (f/D) of microlens is in the range of 1 ~ 2.5 (Borrelli NF 1999). The ideal smooth surface is expected, because during the melting of photoresist the surface forces will control the shape and a segment of a sphere represents the minimum energy surface. The design and fabrication of the MLA with thermal reflow are provided as follows.

The radius of curvature of the spherical profile of microlens is given by,

$$r_c = \frac{h^2 + d_{MLA}^2 / 4}{2 \times h} \quad (4.2)$$

where the d_{MLA} is the aperture of microlens and h is the height of microlens, r_c is the radius of curvature of the spherical profile of microlens. And the focal length of the microlens is determined by,

$$f_{MLA} = \frac{r_c}{n_{pr} - 1} \quad (4.3)$$

where the n_{pr} is the refractive index of photoresist, hence, hence, the numerical aperture of microlens is given by,

$$NA_{MLA} = d_{MLA} / 2 \times f_{MLA} \quad (4.4)$$

It is reasonable to assume that the photoresist volume does not change during fabrication through reflow technique. The relationship between the coating thickness of photoresist and height of the resulting microlens array can be described by,

$$t = \frac{h}{6} \times (3 + 4 \times \frac{h^2}{d_{MLA}^2}) \quad (4.5)$$

As particles with sizes of $3 \mu m$ will be employed in the trapping experiment, the objective spatial period of traps array is designed to be $7 \sim 10 \mu m$. The aperture of single microlens is designed to be $d_{MLA} = 180 \mu m$ and the period of MLA is $p_{MLA} = 210 \mu m$. According to Equation 4.1, the projection lens with 50 mm is employed. For the reflow technique, it is noted that ideal spherical shape of the photoresist cap can only be achieved for specific ratios between the

diameter of microlens and the thickness of photoresist. The thickness of the coated resist should be larger than $5\ \mu m$ for lens with diameters larger than $10\ \mu m$ (Sinzing S et al. 2003). In the present system, the thickness of coating resist is designed to be $8\ \mu m$, and the height of microlens is calculated to be $15.5\ \mu m$ based on Equation 4.5. With the pre-designed values of $d_{MLA} = 180\ \mu m$ and equation 4.2, it is obtained that $r_c = 269\ \mu m$. If the photoresist (AZ P4620) with $n_{pr} = 1.69$ is employed, based on equation 4.3 and 4.4, the result is $f_{MLA} = 390\ \mu m$, $NA_{MLA} = 0.23$. Then the f-number (f/D) is 2.17, which is within the effect range of f-number ($1 \sim 2.5$) for reflow fabrication.

Figure 4.2 shows the steps of fabrication of MLA using the thermal reflow technique. A layer of photoresist is patterned through UV photolithography to form small cylinders on a substrate, and subsequently heated to temperature above the transition temperature of the photoresist. Due to the surface tension, the photoresist cylinders are transformed into spherical shapes. In actual fabrication process, thick positive photoresist AZ P4620 is employed for the fabrication of MLA. According to Figure 4.3 the resist is spin-coated on a pretreated $1.2\ mm$ thick glass substrate at a spin speed of 4000 rpm for 60 seconds to achieve the thickness of $8\ \mu m$. Before UV exposure, the photoresist on substrate has to be pre-baked, usually on a hotplate, to evaporate the solvent and to improve the adhesion between the film and the glass substrate. In the

pre-bake process, the photoresist is placed on a hotplate at 105°C for 180 s. Pattern transfer is realized by contact printing though a pre-designed mask. The sample is exposed to ultraviolet (UV) source with a peak emission wavelength of 365 nm for 45 seconds and the process was implemented on a Q 2001CT UV mask contact aligner (Quintel Corporation), which has an irradiance of 15mW/cm². After development in AZ developer for 90 s, the sample is rinsed briefly with demonized water to remove the unexposed areas to form the designed cylinder array patterns in the AZ P4620 photoresist on glass substrate. Subsequently, the patterned sample was heated to 160°C for 60 minutes in an oven, where the photoresist film was in fluid form. After the sample returned to the normal temperature, the desirable spherical shape of MLA was obtained after cooling down because of the surface tension.

Figure 4.2 Fabrication steps of microlens array using reflow technique

a. spin coating b. exposure c. developing d. heating and reflow

Figure 4.3 AZ P4620 photoresist film thickness (μm) vs. spin speed (rpm)

(Datasheet: AZ P4000 photoresist)

Figure 4.4 shows a micrograph of the fabricated MLA in photoresist. The diameter of individual microlens was $180\mu\text{m}$. Figure 4.5 shows the surface profile of MLA measured by surface profiler (Veeco Metrology). It is revealed that the smoothness and uniformity of MLA is satisfied. Figure 4.6 provides the optical lattice generated with MLA and a projection lens system.

Figure 4.4 Micrograph of the fabricated MLA (the scale bar is $100\mu\text{m}$)

Figure 4.5 Surface profile of the fabricated MLA

Figure 4.6 Optical spots array generated by MLA (captured by Ophir CCD profiler)

4.2.2 Multiple optical tweezers system with fabricated MLA

The multiple optical trapping system is built to combine the MLA with a typical upright microscope based single-beam optical tweezers. The schematic of MLA multiple optical trapping system is shown in Figure 4.7. The microfluidic chamber with a depth of $70\mu m$ is formed between two glass plates, which were separated by a spacer. An aqueous suspension of microparticles is sandwiched in the microchamber. The laser source is a continuous wave diode pumped solid state laser: Laser Quantum MPC 6000 (Ventus 532) at 532 nm with power up to 1.5 W . The output laser beam is collimated and linearly polarized. After collimation and expansion through two convex lenses (L1, L2) with focal length of 100 mm and 200 mm respectively, the laser beam is focused by the fabricated microlens array to form an array of focal spots pattern. With another convex lens L3 (focal length is 100 mm), the beam arrays are directed into the upright microscope (Carl-Zeiss Axiovert 25) equipped with a $100\times$ oil immersion objective lens (Zeiss, NA=1.25), and then two dimensional optical lattice is formed on the sample plane for sorting particles. A dichroic mirror is adopted to reflect the laser beam to enter the aperture of objective lens while allowing the illumination light and image of particles to pass through a charge-coupled device (CCD) camera connected to a PC, where the optical trapping can be observed dynamically.

Figure 4.7 Optical tweezers array system using MLA

Polymer particles ($n=1.59$) with diameters of $3.1\mu m$ are sandwiched in the microchamber for multiple trapping. When the laser beam is switched on with an output power of 400 mW , it is observed that regular array of particle are trapped at each individual light spot, as is shown in Figure 4.8. Furthermore, it is found that when the laser beam is switched off, the previous regular shape of trapped particles are destroyed in seconds.

Figure 4.8 Optically trapped regular array of $3.1\ \mu\text{m}$ particles with MLA system

(the scale bar is $5\ \mu\text{m}$)

One of the main advantages of MLA is attributed to its composite feature. The generated optical lattice is determined by the layout design of MLA. That is to say, arbitrary optical lattice could be generated on the sample plane with a designed composite MLA. Figure 4.9 shows a microfabricated composite MLA array with two axes. The individual microlens of composite MLA is designed to have the same geometrical and physical parameters as the one shown in figure 4.4, the diameter $d_{MLA} = 180\ \mu\text{m}$ and the height of lens is $h = 15.5\ \mu\text{m}$. Similarly, thermal reflow technique is used and the photo of fabricated MLA with two guiding axes is shown in Figure 4.10.

Figure 4.9 Fabricated MLA with two guiding axis

Figure 4.10 Particles with diameter of $3.1\ \mu\text{m}$ trapped corresponding to the light spot of composite optical lattice. The two guiding axes of lattice are highlighted.

4.3 Optical sorting using lens projection microscopic system

In optical sorting experiment, there should be a uniform flow driving mixture of particles through the fixed optical landscape to realize lateral separation of particles by sizes or refractive indices. The optical configuration for MLA sorting system is shown in Figure 4.11. The system is similar to the one employed for multiple optical trapping (Figure 4.7) except for replacing the manual sample stage with an electrically motorized 2D stage. In the experiment, the microparticles and fluid flow were realized through translation of the sample stage of the microscope in one direction while keeping the projected optical landscape stationary. Such a method is easy to implement and it also enables us to adjust the speed of particles flow conveniently. The optical pattern projected onto the sample channel was a 2D optical spots array ($30\mu m \times 30\mu m$) with its axis oriented at about 30° to the flowing direction.

Figure 4.11 Optical sorting system based on MLA

A mixture of polymer particles ($n=1.59$) with diameters of $3.1\mu m$ and $1.5\mu m$ respectively were sandwiched in the microfluidic chamber with gap thickness of about $70\mu m$. Figure 4.12 shows the sequential images of motions of the mixed particles taken at $1/3$ second intervals. The diameter of the polymer spheres highlighted in square was $3.1\mu m$; while the smaller ones were $1.5\mu m$. With a laser power of 600 mW and with a flow speed of $30\mu m/s$, it was observed that there was a strong deflection for the $3.1\mu m$ particles when they passed through the optical landscape (the $3.1\mu m$ particle highlighted in square was deflected from the top of the image to bottom), whereas no obvious changes were observed of smaller ones.

*Figure 4.12 The sequential images of motions of mixed particles
taken with 1/3 s intervals*

In order to further clarify the separation process, the trajectories of particles motions are provided using the self-developed particles tracking software. The video of particles motions is first divided into consecutive frames. On each frame, the contour edges of the interested particles are recognized and then the central point of each particle contour is found using an algorithm written in Visual C++. By connecting and plotting the coordinates of the same particles on different frames, the trajectories are achieved. Figure 4.13 shows the differences between typical trajectories of $3.1\mu m$ and $1.5\mu m$ microparticles. The trajectories of $3.1\mu m$ particles (highlighted in red) have obvious deflection within optical landscape area, whereas the trajectories of $1.5\mu m$ particles are almost flat. Consequently, it is demonstrated that the mixture of particles in

microfluidics were laterally separated in a continuous way on periodical optical lattice generated by MLA.

Figure 4.13 Trajectories of two types of particles passing through the projected optical lattice. Trajectories shown in red are particles with radius of $3.1 \mu\text{m}$, and in black are $1.5 \mu\text{m}$.

Experimentally the moving velocity of particles is varied to evaluate the influence exerted by flowing velocity, under the same laser intensity of 600 mW . It is deduced that a flowing velocity at about $30 \mu\text{m/s}$ was appropriate for the system. Selecting a lower speed will lead to a jamming of the sorting system while a higher speed will cause increased number of larger particles to escape from the optical landscape. Typical throughput of the MLA sorting system can

be implemented at $20 \sim 30$ *particles/second* approximately. It is noted that this throughput value is higher than the optical chromatography method (Hart, S.J. et al. 2001, 2003) and it is comparable to the microfabricated fluorescence activated cell sorter and three-dimensional optical lattice sorting using multi interference reported by (MacDonald M.P., et al. 2003).

In order to characterize the influence of laser power on the sorting efficiency, the optical sorting experiments were also carried out for different laser power while keeping other parameters including flowing speed ($30\mu m/s$) unchanged. To quantify the sorting efficiency, the following definition has been proposed. The sorting efficiency was defined as the proportion of larger particles with obvious trajectory change minus the proportion of smaller particles being significantly changed in motion trajectories (only particles passing through the optical landscape were involved). In experiments, it was found that no obvious sorting phenomenon was observed until the laser power was increased to 300 *mW* and above. It is noted in Figure 4.14 that the sorting efficiency can be plotted as a function of laser power, which was in agreement with the theoretical analysis. The sorting efficiency can be achieved as high as 80% with a laser power of 700 *mW*. It is also seen in the figure that sorting efficiency reaches the maximum when laser power was 700 *mW*. This is because once the laser power is higher than 700 *mW* it would deflect a larger proportion of smaller particles, but without deflecting more particles with a bigger diameter.

Figure 4.14 Relationship between sorting efficiency and laser power

Larger lateral separation angle of the two groups of objects is usually desirable, because this will lead to less separation length in channel, and less ambiguity states in sorting process. It has been demonstrated that discrete optical traps array is not capable of deflecting particle to 45° angle, only if the output laser power is as large as 1.5 W. (MacDonald M.P. et al. 2004) (Roichman Y. et al. 2007). In order to further improve the separation angle in the optical sorting, a more deterministic form of channeling is needed rather than kinetic locked-in of hopping probabilities. The optical lattice with interconnected linkages should be employed. The individual optical spot of interconnected lattice is not discrete, and there are interlinks between optical lattice sites. It is desirable that the

object particles will be deflected along the linkage axis while passing through the interconnected optical lattice. A composite MLA is employed to generate the interconnected optical lattice. The angle between linkage axis of interconnected optical lattice and flow direction is about 45° . The mixture of $3.1\mu m$ and $1.5\mu m$ polymer microparticles were driven from right to left with a uniform speed of $10\mu m/s$ in the microchamber. And the typical throughput of optical sorting is about 10 particles per second. When the laser was switched on with an output power of 500 mW , it was observed that larger microparticles were strongly deflected to a large deflecting angle, while most of the smaller particles were hardly deflected. As a result, two types of particles with different sizes were laterally separated in a continuous way. Figure 4.15 shows the sequential snapshots extracted from the video recording the whole optical sorting process. Furthermore, the extracted trajectories of both types of particles are provided in Figure 4.16, indicating that the typical angle between the trajectories of $3.1\mu m$ and $1.5\mu m$ was about 45° . Consequently, it has been demonstrated that the composite MLA enabled interconnected pattern can achieve 45° separation angle.

Figure 4.15 The sequential images of motions of mixed particles taken with $2/3$ s intervals. The particles highlighted in square are $3.1 \mu\text{m}$ polymer microparticles

Figure 4.16 Trajectories of two types of particles passing through the optical lattice.

Trajectories shown in red are particles of $3.1 \mu\text{m}$, and in black are $1.5 \mu\text{m}$.

4.4 Conclusion

A simple, reliable and cost-effective method has been proposed for microfluidic multiple trapping and sorting using MLA. Compared with AOD and EOD, the MLA system requires no special expensive instrument to create optical lattice. The 2D MLA trapping system is easy to align, and compatible with popular single beam optical tweezers microscopic system the MLA system requires no complex computation like CGH design. The power efficiency of MLA is 100% theoretically, and 80% practically, which is obviously higher than CGH technique and AOD technique (2D). The composite feature of fabricated of MLA make it an effective approach to forming composite shape and size of the projected pattern. It has been experimentally demonstrated that the composite MLA based system is capable of continuously separating of bi-disperse suspensions by sizes with 45° angle in the microfluidic chamber. The disadvantage of MLA trapping and sorting system is that not easily to realize the dynamic trapping.

The MLA generated optical lattice can realize continuous sorting of bi-disperse particles by sizes or refractive indices without external markers, and the throughput of optical sorting is between 20 ~ 30 particles per second, which is comparable with the multi-interference and GPC enabled optical sorting methods. It has been experimentally demonstrated that the deflection of

particles occurs at 400 *mW* laser output, and the sorting efficiency can reach 80% with the output power of 700 *mW*.

CHAPTER 5

REALIZATION OF MULTIPLE OPTICAL TRAPPING AND SORTING WITH DIFFRACTIVE OPTICAL ELEMENTS

In this Chapter, diffractive optical elements are adopted for generating optical lattice for multiple optical trapping and optical sorting. Firstly, a binary-phase DOE Dammann grating is designed and fabricated as an array illuminator by direct design technique. It is experimentally demonstrated that the optical lattice created by Dammann grating is capable of array trapping and sorting particles by refractive indices. Secondly, phase-hologram is designed with iterative optimization technique, and realized with SLM to create a dynamic optical pattern. The created optical lattice is applied to the dynamic multiple optical trapping and continuous locked-in transport of microparticles.

5.1 Introduction

The diffractive optical elements provide another solution to generating optical landscape for optical trapping. And the DOE is capable of creating complex optical pattern with iterative optimization designs. The light spot uniformity of DOE array illuminator will not be influenced by the poor uniformity of input beam. The fabrication of binary DOE is compatible with general microlithography technique. Additionally, the DOE realized with SLM will have the ability to create a dynamic optical pattern. Based on all of these advantages, the diffractive optical elements are proposed to realize multiple optical trapping and optical sorting.

DOE can be designed by a number of direct, non-iterative techniques. It is designed in such a way that the light from the collimated input beam is distributed over M diffraction orders. For array illuminators, an array of equally bright diffraction orders is designed. One of the earliest DOE direct design technique is Dammann grating, which is a designed binary phase gratings for multiple imaging applications. The Dammann grating is 2D separable binary or multilevel phase grating that can be used to split an incident beam into an array of beam, and it is calculated in a direct way by Fourier series decomposition. Although the binary phase-grating has lower theoretical diffraction efficiency, it remains popular because of its compatibility with general microlithography fabrication techniques. Later on the iterative optimization algorithms become of interest. With suitable quantization schemes, these iterative algorithms can be better applied in the multi-level grating design.

In this chapter, the DOE is designed and fabricated to generate optical lattice for multiple trapping and sorting. The phase DOE is an element typically having multilevel microreliefs or continuous microreliefs with lateral feature size ranging from sub-micrometers to millimeters and vertical size of several micrometers. With the designed surface relief profile or optical function, the DOE has the ability to modulate the phase of incident light and transform it to a beam with desirable amplitude and phase function. The numerous methods for design of DOEs are mainly classified into two classes: analytical type and

numerical type. The phase profile of analytical type DOE like diffractive lens is usually described in an analytical form, while the phase function of numerical type DOE is more complex. In this chapter direct design technique and iterative optimization technique will be adopted to design Dammann grating and multi-level phase hologram respectively for creation of a desirable intensity pattern.

The DOE will be fabricated through microelectronic lithographic techniques and then integrated into a microscopic optical tweezers system to realize an array of optical traps. In addition to the fabrication method, liquid crystal SLM is employed as a phase modulator to realize a dynamic and reconfigurable optical traps array. The optical configuration, fabrication steps and multiple array trapping results will be provided in detail.

5.2 Generation of multiple optical traps by Dammann grating

5.2.1 Principle of using Dammann grating to create an array illuminator

Dammann grating, first proposed by Dammann and Gorlter (1971, 1977) is generally used to denote binary phase grating applied for multiple beam splitting. Combined with the traditional focus Fourier lens, this particular

phase-only grating is capable of converting an incident laser beam into a one or two dimensional array of equally intense light spots (Mait J.N. 1990) (Krackhardt U. 1989) (Walker S.J. et al. 1990). As shown in Figure 5.1, Dammann grating is placed in front of the focusing lens, while the image plane is the back focal plane of the lens. The grating splits the incoming laser beam into several diffraction orders, and then the following lens performs a Fourier transformation step, with the result of focusing several diffraction orders to a spots array. The grating is specifically designed such that the light spots are of the same intensity. Usually the grating of binary structure is adopted to reduce the difficulties in fabrication process. Being phase-only, Dammann grating has a relatively high diffraction efficiency compared with the amplitude grating, for no absorption losses will occur. Furthermore, the light spot uniformity will not be influenced by the poor uniformity of input beam because of the Fourier type of Dammann grating. This is the main advantage of Dammann grating method over the lens array based aperture division method.

Figure 5.1 Configuration for uniform array illumination using Dammann grating

5.2.2 Design of Dammann grating

Dammann grating is computed analytically in a direct way by Fourier series decomposition. The pattern of two-dimensional Dammann grating can be described mathematically with a transmission function $f(x, y)$ by two separable transmission function $f_a(x)$, $f_b(y)$ of x and y direction respectively,

$$f(x, y) = f_a(x) \cdot f_b(y) \quad (5.1)$$

In this case, the Fourier transform $F(m, n)$ of $f(x, y)$ can be separated as follows,

$$F(m, n) = F_a(m) \cdot F_b(n) \quad (5.2)$$

where $F_a(m)$, $F_b(n)$ are the one-dimensional transforms of $f(x)$ and $f(y)$ respectively.

Consequently, a regular two-dimensional array generator can be constructed by means of two crossed identical one-dimensional Dammann grating patterns. (Dammann, H., et al., 1971) In this case it is sufficient to consider a one-dimensional grating with a periodic p .

For simplicity, only one period of grating structure will be considered. As mentioned above, the Dammann grating to be designed is binary, that is to say, the grating's transmission has only two fixed value 1 and $\exp(i\varphi)$. Accordingly,

the power spectrum of grating is characterized completely by its N transition points $a_1, b_1, a_2, b_2, \dots, a_L, b_L$ (see Figure 5.2), where the phase changes occur. Thus, we focus on finding a set of transition points for which the corresponding diffraction intensities become equal. In the design, the positive and negative diffraction orders have the same values considering the symmetry requirements.

Figure 5.2 Phase profile of one period of Dammann grating

A Dammann grating with N pairs of transition points may have $2N+1$ diffraction orders $(-N, \dots, -1, 0, 1, \dots, N)$ or $2N(-N, \dots, -1, 1, \dots, N)$ apart from those higher orders which are of very low intensity and of no interest in the design.

Before the analysis of phase-only Dammann grating, the amplitude grating calculation is first introduced. In the design, it is assumed that the number of grating periods is infinite and the incident wave is of unit amplitude. Hence, the

complex transmission function of a grating unit cell can be expressed mathematically as:

$$f_a(x) = \sum_{l=1}^L \text{rect}\left(\frac{x - (b_l + a_l)/2}{b_l - a_l}\right) \quad (5.3)$$

For an infinite extension of the grating, $f_a(x)$ can be expanded as a Fourier series (Turunen, J., et al., 1988),

$$f_a(x) = \sum_{m=0}^{\infty} F_a(m) \exp(i2\pi mx) \quad (5.4)$$

where m indicates the diffraction order.

The Fourier coefficients are:

for $m = 0$,

$$F_a(m) = F_a(0) = \sum_{l=1}^L (b_l - a_l) \quad (5.5)$$

for $m \neq 0$,

$$F_a(m) = \frac{1}{2\pi m} [F_{aR}(m) + iF_{aI}(m)] \quad (5.6)$$

where

$$F_{aR}(m) = \sum_{l=1}^L [\sin(2\pi mb_l) - \sin(2\pi ma_l)] \quad (5.7)$$

$$F_{aI}(m) = \sum_{l=1}^L [\cos(2\pi mb_l) - \cos(2\pi ma_l)] \quad (5.8)$$

Thus the power spectrum for different diffraction orders of the grating is given by

$$P(m) = |F_a(m)|^2 \quad m = -\infty, \dots, \infty \quad (5.9)$$

In order to improve diffraction efficiency, phase-only Dammann grating is considered to replace the amplitude grating. In the design, suppose the complex amplitude transmittance are $\exp[i(\frac{\pi}{2} - \varphi)]$ and $\exp[i(\frac{\pi}{2} + \varphi)]$ respectively, then the complex transmission function for grating is achieved,

$$f_p(x) = f_a(x) \exp[-i(\frac{\pi}{2} - \varphi)] + [1 - f_a(x)] \exp[+i(\frac{\pi}{2} + \varphi)] = [2f_a(x) - 1] \sin \varphi + i \cos \varphi \quad (5.10)$$

the spectrum function is,

$$F_p(m) = \int_0^1 f_p(x) \exp(-i2\pi mx) dx = \int_0^1 \{[2f_a(x) - 1] \sin \varphi + i \cos \varphi\} \exp(-i2\pi mx) dx \quad (5.11)$$

for $m = 0$,

$$F_p(m) = F_p(0) = \int_0^1 \{[2f_a(x) - 1] \sin \varphi + i \cos \varphi\} dx = [2F_a(0) - 1] \sin \varphi + i \cos \varphi \quad (5.12)$$

for $m \neq 0$,

$$F_p(m) = [2 \int_0^1 f_a(x) \exp(-i2\pi mx) dx] \sin \varphi + \int_0^1 (\sin \varphi + i \cos \varphi) \exp(-i2\pi mx) dx \quad (5.13)$$

Considering the equation,

$$\int_0^1 \exp(-i2\pi mx) dx = 0 \quad (5.14)$$

Finally the spectrum becomes,

$$F_p(m) = 2 \sin \varphi F_a(m) \quad (5.15)$$

The phase grating power spectrum is in the same way as amplitude grating,

$$P(m) = |F_p(m)|^2 \quad (5.16)$$

Furthermore, according to Parseval's theory which states that the conservation of energy, we get,

$$\sum_{-\infty}^{\infty} |F_p(m)|^2 = \sum_{-\infty}^{\infty} I(m) = 1 \quad (5.17)$$

In my design, the equally bright output diffraction orders are desired. Hence, we obtain the relationship between the energy of the different diffraction orders,

$$|F_p(-N)|^2 = \dots = |F_p(-2)|^2 = |F_p(-1)|^2 = |F_p(0)|^2 = |F_p(1)|^2 = |F_p(2)|^2 = \dots = |F_p(N)|^2 \quad (5.18)$$

With the above equations, we are able to find a set of transition points $a_1, b_1, a_2, b_2, \dots, a_L, b_L$ for which the corresponding diffraction orders are of equal intensity. As the solution of L equations is a complex nonlinear

optimization problem for which no analytic procedure exists, various optimization numerical analysis methods including Newton-Raphson technique, simulated annealing and genetic algorithm, can be used to find a proper solution. Finally we can get the expected values of transition points.

After the calculation of the transition points, we can get one-dimensional diffraction efficiency,

$$\eta_a = \frac{\text{total energy of the expected equally bright orders}}{\text{total energy of the light passing through the grating}} = \frac{\sum_{-N}^N I(m)}{1} \quad (5.19)$$

In the same way we can obtain the value of η_b . Then the corresponding diffraction efficiency of the two-dimensional grating is,

$$\eta = \eta_a \times \eta_b \quad (5.20)$$

where η_a , η_b correspond to transmission function of x and y direction respectively.

Table 5.1 shows the calculation result for Dammann grating with up to 7 diffraction order, and Table 5.2 provides the total diffraction efficiency of the designed Dammann grating and the efficiency of each diffraction orders. Figure 5.3 compares the diffraction efficiency of one-dimensional, two-dimensional and theoretical upper bound diffraction efficiency of $\{0, \pi\}$ binary phase-only

Dammann grating (Krackhardt., et al., 1992). (In the figure, the 1D and 2D grating capable of generating 3 or 3*3 orders are computed with a phase difference of 2.008). Finally, Figure 5.4 displays the structure of a 5*5 binary Dammann grating.

Table 5.1 Transition points result for Dammann grating

N (Diffraction orders)	Phase difference	Transition points					
		t1	t2	t3	t4	t5	t6
3	2.008	0.00	0.15	0.74	0.96	×	×
4	π	0.00	0.09	0.36	0.73	×	×
5	π	0.00	0.04	0.39	0.65	×	×
6	π	0.00	0.08	0.10	0.44	0.64	0.77
7	π	0.00	0.26	0.28	0.46	0.70	0.92

Table 5.2 Diffraction efficiency and intensity of different orders of Dammann grating

N (Diffraction orders)	η_{1D} Diffraction Efficiency	Intensity						
		I(-3)	I(-2)	I(-1)	I(0)	I(1)	I(2)	I(3)
3	70.24%	×	×	0.2339	0.2386	0.2339	×	×
4	65.48%	×	0.1621	0.1653	×	0.1653	0.1621	×
5	77.04%	×	0.1575	0.1477	0.16	0.1575	0.1477	×
6	73.18%	0.1224	0.1208	0.1227	×	0.1227	0.1208	0.1224
7	71.38%	0.1019	0.1048	0.099	0.1024	0.099	0.1048	0.1019

Figure 5.3 Diffraction efficiency of Dammann grating (a) designed separable 2D Dammann grating (b) designed 1D Dammann grating (c) upper bound of diffraction efficiency for 1D $\{0, \pi\}$ binary phase-only Dammann grating

Figure 5.4 Calculation pattern of binary Dammann grating for generation of 5*5 spots

array ($t1=0$, $t2=0.04$, $t3=0.39$, $t4=0.65$)

5.2.3 Fabrication of Dammann grating

The fabrication techniques for DOEs include direct writing (Fujita T. et al. 1982) (Gale M.T. et al. 1993) (Bowen J.P. et al. 1997), photolithography (O'Shea D.C. et al. 2004) (Sheats J.R. et al. 1998) (Levinson H.J. 2001), interference exposure (Enger R.C. et al. 1983), near field holography (Tennant D.M. et al. 1994) (Suleski T.J. et al. 1999), direct machining (Ruckman J. et al. 1999) (Blough C.G. et al. 1997) (Behrmann G.P. et al. 1997), replication (Xia Y. et al. 1998) (Wilbur J.L. et al. 1994)(Zhao X. et al. 1996) (Lopez A. et al. 2001) etc. As the sizes and features of the fabrication of diffractive optical elements are similar to the manufacturing of semiconductor integrated circuits, photolithography is also widely applied for fabrication of DOE. The fabrication of Dammann grating for $5 * 5$ array illuminator using lithography is based on the design shown in Table 5.1 and Figure 5.4. As the phase grating will be directly fabricated on the transparency or glass substrate for use, its period and thickness of the binary phase grating need to be determined prior to the fabrication. In the design, the period of $100\text{ }\mu\text{m}$ is selected to obtain a relatively high diffraction angle for all of the orders, with the result that the optical system can be kept compact enough. In this case, the minimum feature size will be $8\text{ }\mu\text{m}$, which is easy for fabrication process using photolithography. The thickness for the binary Dammann grating with phase difference of π can be calculated by,

$$d = \frac{M}{2} \frac{\lambda}{n-1} \quad (5.21)$$

where n is the refractive index of the photoresist, λ denotes the wavelength of the laser beam, M is any odd number.

In the Dammann grating fabrication process, photoresist AZ 5214E is adopted. And the refractive index of the photoresist is about 1.61, according to its application note.. Then for laser beam with a wavelength of 532 nm, the required thickness of photoresist is approximate $1.31 \mu\text{m}$ ($M=3$). According to Figure 5.5, the $1.31 \mu\text{m}$ can be reached at spin speed of 4500 rpm.

Figure 5.5 AZ 5214E photoresist film thickness (μm) vs. spin speed (rpm)

(Datasheet AZ5214)

Although AZ 5214E is in fact a positive photoresist, it is almost exclusively used in the IR mode as a negative photoresist. The image reversal capability is

obtained by a special crosslinking agent in the photoresist formulation which becomes active at temperatures above 110°C. What is even more important, such phenomenon only occurs in exposed areas of the photoresist. The crosslinking agent together with exposed photoactive compound (PAC) leads to an almost insoluble (in developer) and no longer light sensitive substance, while the unexposed areas still behave like a normal unexposed positive photoresist. After a flood exposure (no mask required) this areas are dissolved in standard developer for positive photoresist, the crosslinked areas remain. The overall result is a negative image of the mask pattern.

In actual fabrication process (see Figure 5.6), photoresist AZ5214E was spin-coated on a pretreated 1.2 mm thick glass substrate at a spin speed of 4500 rpm for 30 seconds, with the result that an approximate 1.31 μm thickness of photoresist was reached. Before UV exposure, the photoresist on substrate has to be pre-baked, usually on a hotplate, to evaporate the solvent as well as to improve the adhesion of the film to the glass substrate. In the pre-bake process, the photoresist was placed on a hotplate at 105°C for 90 seconds. Pattern transfer was realized by contact printing though a pre-designed 5 inch binary chromium mask. The sample was exposed to ultraviolet (UV) source with a peak emission wavelength of 365nm for 2 seconds and the process was implemented on a Q 2001CT UV mask contact aligner (Quintel Corporation), which has an irradiance of 31mW/cm^2 . After exposure, the sample was put into

the oven to undergo the post-baking at 110°C for 8 minutes, which led to the fact that the special crosslinking agent in the resist formulation became active in the UV exposed area. The crosslinking agent together with exposed photoactive compound (PAC) in the AZ 5214E photoresist resulted in an almost insoluble (in developer) and no longer light sensitive substance; hence the exposed area would be kept after development. In the following, an additional 11 seconds flood UV exposure was exerted. Finally, the sample was developed in special AZ developer for 60 seconds and rinsed briefly with demonized water to remove the unexposed areas to form the designed patterns in the AZ 5214E photoresist on glass substrate.

Figure 5.6 Fabrication steps of Dammann grating

A Dammann grating for generation of a 5*5 optical spots array was fabricated through the above steps. The image taken by a scanning electron microscope

(JEOL SEM- JSM5300) is shown in Figure 5.7

(a)

(b)

*Figure 5.7 SEM photo of fabricated Dammann grating
(a) zoom-out view (b) zoom-in view*

5.2.4 Multiple optical tweezers system with fabricated Dammann grating

The Dammann grating is included in a lens projection based microscopic system to generate multiple optical traps on the sample plane, as is shown in Fig 5.8. The lens projection system is constructed to convert the multiple optical beams into an optical lattice. The laser source is the Laser Quantum MPC 6000 (Ventus) at 532 nm with an output power up to 1.5 W and the output beam is linearly polarized with a diameter of about 2 mm. The laser beam is collimated and expanded three times by a telescope system composed of two converging lens with focal length of 100 mm and 300 mm respectively. The collimated beam incident on the fabricated Dammann grating and an optical spots array is formed at the back focal plane of subsequent converging lens. The two converging lens with focal length of 200 mm after the grating constitutes a telescope system and make the plane of Dammann grating conjugate to the plane of back aperture of the objective. Hence, the light beams passing through the grating will all pass through the aperture of the objective and then focused into a desirable optical traps array on the sample plane. The oil immersion objective lens (100x Carl-Zeiss) is mounted on the upright microscope (Axiostar, Carl-Zeiss). The motion of the particles is captured by a CCD camera and displayed on the screen of monitor. A dichroic mirror is adopted to reflect the laser beam to enter the aperture of objective lens while allowing the illumination light and image of trapping of particles to pass through a

charge-coupled device (CCD) camera connected to a PC, where the particles motions are real-time observed.

Figure 5.8 Optical configuration of multiple optical trapping system enabled with fabricated Dammann grating

The microchamber is a sandwiched structure created with two glass slide separated by a spacer of $70\ \mu\text{m}$ thick. As the density of latex beads is higher than the medium, the samples will sediment into a monolayer at about several micrometers above the bottom surface of the microchamber. Figure 5.9 shows the micrograph of the pattern of optical traps array. When the laser is switched on with an output of 500 mW, it is observed that the latex beads with diameters of $3.1\ \mu\text{m}$ are trapped in a regular array, whose axis is rotated 20° with

regard to the horizontal direction. Figure 5.10 shows the photo of particles regularly trapped corresponding to the individual light spot of optical array.

Figure 5.9 Optical spots array generated by Dammmann grating (taken on the sample plane in microfluidics) (the scale bar is 10 μm)

Figure 5.10 Regular array of trapped particles (the scale bar is 10 μm)

It has been theoretically proved in Chapter 3 that the optical lattice not only separate particles by sizes, but also able to sort by the refractive indices. As the

MLA enabled optical sorting system has been demonstrated to be capable of sorting by sizes in Chapter 4, the objective of the following experiment using Dammann grating is to realize a lateral separation of the trajectories of two types of particles with the same diameters but different refractive indices. A mixture of polymer particles ($n=1.59$) and silica particles ($n=1.42$) with same diameters of $3.1\text{ }\mu\text{m}$ were sandwiched in the microfluidic chamber with thickness of about $70\text{ }\mu\text{m}$. In the experiment, the microparticles and fluids flow were realized through translation of the sample stage of the microscope in one direction while keeping the projected optical landscape stationary and the driving velocity of particles flow could be adjusted conveniently. As it is shown in Figure 5.11, sequential images of motions of the mixed particles were taken with $1/3$ second intervals. With a laser power of 800 mW and with a flow speed of $15\text{ }\mu\text{m/s}$, it was observed that there was a strong deflection for the $3.1\text{ }\mu\text{m}$ polymer particles (two of them highlighted in square) when they passed through the optical landscape (the $3.1\text{ }\mu\text{m}$ particle highlighted in square was deflected from the top of the image to bottom), whereas no obvious changes were observed for the trajectories of the silica particles. Consequently two types of particles with different refractive indices were laterally separated in a continuous way. The throughput of Dammann grating sorting experiments is ~ 20 particles/s and the sorting efficiency is $\sim 70\%$ with laser output of 700 mW . It is noted that the laser power required for the Dammann grating sorting system is obviously larger than that is required for the MLA sorting system. For MLA

system, when the output power of laser reached 400mW, there is occurrence of locked-in transport of 3.1 μm polymer particles, whereas the Dammann grating system needs 600~700mW to achieve the similar result. Such differences are mainly attributed to the power-efficiency between MLA and Dammann grating. Theoretically the MLA has 100% power efficiency compared to ~60% of designed 2D 5*5 orders Dammann grating. Consequently, higher power is needed to achieve higher sorting efficiency.

Figure 5.11 The sequential images of motions of mixed particles

taken with 1/3 s intervals

5.3 Multiple optical traps enabled by CGH

5.3.1 Design of DOE with iterative optimized algorithms

The popular iterative optimization algorithms (Fienup J.R. 1980) (Jennison B.K. et al. 1989) are grouped into two categories. The first group performs a single pixel change in the DOE at each iteration and analyzes their effects on the reconstruction, including direct binary search algorithm (DBS) (Seldowilz M.A. et al. 1987) (Clark M. 1996), simulated annealing (Yoshikawa N. et al. 1994) (Kim M.S. et al. 1990), iterative discrete on-axis algorithm (IDO) (Stack J.D. et al. 1992) etc. And the second category group performs one or more global transformations at each iteration, and employs forward and backward projectors, including G-S algorithm, Yang-Gu algorithm (Yang G. et al. 1994) etc. The design of DOE is based on a phase retrieval algorithm, which belongs to the IFTA group. IFTA performs one or two global transformations in each iteration with forward and backward propagators, and the result is exerted different constraints defined by the certain application. G-S Algorithm is a popular IFTA optimization program and the flowchart of G-S algorithm is shown in Figure 5.12 (Gerchberg R.W. et al. 1972). A denotes the amplitude of illuminating beam and a random initial phase φ_0 is chosen. The complex amplitude of incident beam is integrally transformed (FFT) into an image with $E_{out}(n) = A_{out}(n)\exp(i\varphi_{out}(n))$, where n is the iteration times. Then the intensity-intensity constraint is exerted on this computed image to form a new

complex amplitude $\overline{E_{out}}(n)$,

$$\overline{E_{out}}(n) = A_{object} \exp(i\varphi_{out}(n)), \quad A_{obj} > 0 \quad (5.21)$$

where A_{obj} is the desirable amplitude in image plane and α is a coefficient to be chosen. This new complex amplitude $\overline{E_{out}}(n)$ is subsequently inverse transformed (FFT⁻¹) to $\overline{E_{in}}(n) = \overline{A_{out}}(n) \exp(i\overline{\varphi_{in}}(n))$. Finally $\overline{\varphi_{in}}(n)$ is quantized and used as the phase of $E_{in}(n)$ in a new iteration. And the quantization is described by,

$$\begin{aligned} \varphi &\in [0, 2\pi) \\ \varphi_q &= \text{floor}[\varphi \times \text{level} / (2\pi)] \times 2\pi / \text{level} \end{aligned} \quad (5.22)$$

The iteration will repeat until the root mean square error (RMS-E) reaches a desirable predefined value (e.g. 0.001). When all of the iterations are over, the computed phase-only hologram has $\varphi_{in} = \varphi_{in}(n)$. The RMS-E is a parameter describing how close the computed reconstruction image close to the object reconstruction image and it is usually defined as,

$$RMS-E = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} |A_{out}(\xi, \eta) - A_{obj}(\xi, \eta)|^2 d\xi d\eta \quad (5.23)$$

In some cases, the RMS-E is computed within the desirable object area ($A_{obj} > 0$), then equation (5.23) is changed into,

$$RMS-E = \iint_{A_{obj} > 0} |A_{out}(\xi, \eta) - A_{obj}(\xi, \eta)|^2 d\xi d\eta \quad (5.24)$$

Figure 5.12 Flowchart of the G-S algorithm

Based on the G-S phase retrieval algorithm and standard Fourier optical setup, a 2D DOE is designed to generate an 5×5 array of optical spots with uniform intensity. The DOE is assumed to be illuminated by a plane light wave, and the profile of incident light beam is sampled with 256×256 pixels. The wavelength of laser beam is $\lambda = 0.532 \mu m$. Usually the IFTA algorithm will converge after approximate 20 iterations, here 50 iterations are run to ensure a relative low RMS-E. Figure 5.13 shows the computed phase-only hologram for converting the input plane wave into an optical spots array, and this DOE includes 128 quantization phase steps. As it is shown in Figure 5.14, the RMS-E of G-S algorithm decrease rapidly in initial several iterations, however, there

are no signification reduction in the following iteration. The final RMS-E is 0.1345, 0.1133, 0.1042, 0.0962 after 10, 20, 30, 50 iterations respectively. And the diffraction efficiency of computed phase hologram is 0.9804 after 50 iterations. Figure 5.15 further discover the computed 2D (left) and 1D (right) intensity distribution in the frequency plane at different iteration times, $k=10, 20, 30, 50$. The 1D result is extracted from the corresponding 2D result to illustrate the uniformity of object patten at certain iterations.

Figure 5.13 The computed phase-only hologram for generation of 5×5 spots array using G-S algorithm

Figure 5.14 RMS-E vs iteration times (k) of G-S algorithm

Figure 5.15 Simulation results of 2D and 1D intensity distribution of
object pattern with G-S

It has been demonstrated that the convergence of G-S algorithm features a stagnation effect. In order to increase the convergence rate, some modifications to the algorithm are introduced. One of those modified G-S algorithms is called as adaptive-additive (A-A) algorithm. As it is shown in Figure 5.16, adaptive-additive (A-A) algorithm is another IFTA optimization algorithm, and there is only a small difference in the replace form between A-A and G-S algorithm. In the replacement of G-S algorithm (highlighted in red in Figure 5.12), the amplitude $A_{out}(n)$ of nth iteration step is replaced by the desirable amplitude A_{object} to form a new complex amplitude $\overline{E_{out}}(n)$. That is to say, the amplitude $A_{out}(n)$ of Fourier transform is totally removed in the G-S algorithm. Whereas in each iteration of A-A algorithm, after the FFT of initial

complex input complex amplitude, the $A_{out}(n)$ will not be directly replaced with A_{object} , but with a linear combination with different weights of A_{object} and $A_{out}(n)$. (highlighted in red in Figure 5.16)

$$\overline{E}_{out}(n) = \begin{cases} [\alpha A_{object} + (1-\alpha)A_{out}(n)] \exp(i\varphi_{out}(n)), & A_{obj} > 0 \\ A_{out}(n), & \text{else} \end{cases} \quad (5.26)$$

where A_{obj} is the desirable amplitude in image plane and α is a coefficient to be chosen.

Figure 5.16 Flowchart of the A-A algorithm (highlighted region is the only difference from G-S algorithm)

Using the same parameters as G-S algorithm, a new phase-only hologram with 128 phase levels is computed through modified A-A algorithm ($\alpha = 1$), as it is shown in Figure 5.17. According to Figure 5.18, the RMS-E of A-A algorithm does not drops as rapid as that of G-S in the first several iterations, however, the stagnation problem of G-S is partly overcome. The final RMS-E is 0.1455, 0.1055, 0.0864, 0.0663 after 10, 20, 30, 50 iterations respectively. And the diffraction efficiency of computed phase hologram is 0.8766 after 50 iterations. Figure 5.19 provides the computed 2D (left) and 1D (right) intensity distribution in the frequency plane at different iteration times, $k=10, 20, 30, 50$. The 1D result is extracted from the corresponding 2D result to illustrate the uniformity of object pattern at certain iterations. Finally, Figure 5.20 shows how the RMS-E of AA is dependent on the weight α with iterations of 10, 20 and 50 respectively. It is found that the RMS-E is always reduced with the increase of α . In detail, more iterations times like $k=50$, is required to minimize the RMS-E with small α , whereas $k=20$ is enough to obtain a relatively low RMS-E with α approaching 1.

Figure 5.17 The computed phase-only hologram for generation of 5×5 spots array
using A-A ($\alpha = 1$) algorithm

Figure 5.18 RMS-E vs iteration times(k) of A-A algorithm ($\alpha = 1$)

Figure 5.19 Simulation results of 2D (left) and 1D (right) intensity distribution of object pattern with A-A algorithm

Figure 5.20 Relationship between RMS-E and α of A-A algorithm using different iteration time. Green curve with star is for $k=10$; Red curve with square is for $k=20$; blue curve with circle is for $k=50$.

Although A-A algorithm partly solves the converging stagnation problem of G-S algorithm and reduces the MSE-E, it has two disadvantages. The first is the slow convergence rate at the initial several iterations, because the A-A algorithm requires more iterations times to realize a desirable RMS-E. The second disadvantage is that the diffraction efficiency of A-A is reduced to 0.8766, which is about 10% smaller than that of G-S. In this case, a third algorithm GS-AA which combines the merits of both G-S and A-A algorithm is used for DOE design. In the GS-AA algorithm, the first 10 iterations are implemented following the GS algorithm with the replacement of Equation

(5.21); while the remaining 40 iterations are implemented with the replacement Equation (5.26) used in A-A algorithm. Figure 5.21 shows the comparison of these three IFTA algorithms. It is found that the RMS-E of GS-AA algorithm drops at a same speed as GS algorithm, and there is no further stagnation in the rest iteration. In detail, the RMS-E is 0.1348, 0.0529, 0.0454, 0.0431 after 10, 20, 30, 50 iterations respectively. Furthermore, the diffraction efficiency of GS-AA reaches 0.9224 which is a compromise between the GS and AA algorithm.

Figure 5.21 The dependence of RMS-E on iteration times (k) using

(1) G-S algorithm (2) A-A algorithm (3) GS-AA hybrid algorithm

Phase holograms are usually realized through various fabrication techniques or with reconfigurable SLM. Thus, it is necessary to quantize the hologram. It is

noted that the more quantized levels, the higher diffraction efficiency and lower RMS-E will be achieved. Low number of quantized level will result in the loss of useful phase information, whereas large number of quantized level will improve the fabrication complexity. Accordingly, there must be some trade-off between keeping phase information and decrease of quantized phase levels. On the one hand a relatively large quantized level like 128 (should be smaller than the maximum step enabled by SLM) or even higher is selected for SLM; on the other hand 4-16 levels are often employed for those DOE requiring practical fabrication. Figure 5.22 describes the relationship between RMS-E and quantized phase levels for GS, AA, hybrid GS-AA algorithm respectively.

Figure 5.22 Relationship between RMS-E and quantized phase levels for GS, AA, hybrid GS-AA algorithms respectively

5.3.2 Spatial light modulator

In the first part of this chapter, a binary phase DOE, Dammann grating is fabricated through binary transmission lithographic mask. In order to further improve the diffraction efficiency of phase DOEs, the DOE with more quantized phase levels may be tried. The usual microfabrication techniques for fabrication of multi-level DOEs include laser writing, e-beam writing, gray-scale lithography etc. However, these fabrication techniques is time-consuming and enable only restricted numbers of levels. To this ends, SLM is introduced to realize a maximum phase steps in a convenient way. In addition, SLM has the advantages like reconfigurable and dynamic control ability.

The SLM is a device that exerts some form of spatially varying modulation on a beam of light. There are mainly two types of SLMs, electrically written SLMs and optically written SLMs. For the electrically written SLM, the information is carried by electrical signal, which will be sent as the input to system and control the spatial distribution of absorption or phase shift. For the optically written SLM, the information to be sent to an SLM is stored in the form of an optical image.

Figure 5.23 Internal structure of PPM X8267 produced by Hamamatsu

Currently there are various commercial SLMs available for different application like beam shaping, optical imaging etc. One of the popular modules is the Programmable Phase Modulator (PPM) produced by Hamamatsu. The internal structure of this PPM is shown in Figure 5.23. The PPM is an electrically addressed phase modulator using an optically addressed Parallel Aligned Nematic Liquid Crystal Spatial Light Modulator (PAL-SLM), which is efficiently coupled with an electrical signal input type LCD through lens and also incorporating a write-in LD module. Conventional optically-addressed modulators have difficulties in computer control, while electrically-addressed modulators will create unwanted diffraction light caused by the pixel structure in the electrical signal input section, and the light utilization efficiency is low due to its small fill factor. The PPM X8267 series makes use of an LCD to simplify the computer control problems and adopts an optical system to

eliminate the unwanted diffraction light originating from the LCD pixel structure. Furthermore, the PPM offers large phase modulation characteristics, as it is shown in Figure 5.24. In the phase range between 0 and 2π , the PPM is capable of providing as many as approximate 200 levels, making it competitive and attractive in the application of realizing diffractive optical elements. Table 5.3 shows the relevant technical parameters of PPM X8267-11 by Hamamatsu.

Figure 5.24 Transfer characteristics for PPM X8267-11

Table 5.3 Technical parameters of PPM X8267-11

Parameter	PPM X8267 series	Unit
Input signal	XGA	
Number of control pixels	Approx. 59,000	Pixels
Effective image area	20×20 (Four corners rounded)	Mm
Phase modulation level	$> 2.6 \pi$	Radians
Maximum display spatial resolution	19	Lp/mm
Response time	Rise<60, fall<100	Ms
Suitable wavelength range	500 – 600	Nm

5.3.3 Optical system using SLM for multiple trapping and sorting

Optical system based on SLM for multiple trapping is shown in Fig 5.25. The laser source is the Laser Quantum MPC 6000 (Ventus) at 532 nm with an output power up to 1.5 W and the output beam is linearly polarized. The laser beam with a diameter of about 2mm passed is collimated and expanded 4 times by a telescope system composed of two converging lens with focal lengths of 200 mm and 800 mm respectively to fit the active areas of SLM. The half-wave plate within the optical path is to change the direction linear polarization to

match the phase mode of SLM. The SLM is used as a reflective mode without employing the beam-splitter to avoid a high loss of light energy. The computed phase-hologram is display on the screen of PC and transmitted to the SLM, hence, once the collimated beam incident on the SLM with a small angle, the designed optical intensity pattern will be formed at the back focal plane of subsequent converging lens. If a video is played on the screen of PC, a dynamic optical pattern will also be expected to display at the designed plane. The two converging lens after the grating constitutes a telescope system and make the plane of SLM conjugate to the plane of back aperture of the objective. The aperture is adopted to stop the unnecessary diffraction order. Hence, the second lens converge the light beam into the aperture of the objective and then focused into the desirable optical traps array on the sample plane. The oil immersion objective lens (100x Carl-Zeiss) is mounted on the upright microscope (Axiostar, Carl-Zeiss). The motion of the particles is captured by a CCD camera and displayed on the screen of monitor. A dichroic mirror is adopted to reflect the laser beam to enter the aperture of objective lens while allowing the illumination light and image of trapping of particles to pass through a charge-coupled device (CCD) camera connected to a PC, where the particles motions are real-time observed.

Figure 5.25 Optical system based on SLM for multiple trapping

In order to demonstrate the dynamic multiple optical trapping ability of the SLM system, a video comprising two holograms is designed. The two desirable object patterns are shown in Figure 5.26(2). The design of CGH is based on the above hybrid GS-AA algorithm, in which the profile of incident light beam is sampled with 256×256 pixels and the phase levels is 128. The computed phase hologram shown in Figure 5.26(1) is combined to form a .gif video file, in which the hologram will be played repeatedly with interval of 5 seconds. When the video is played and the output laser power is 300mW, it is observed that the 2×2 array of particle will be dynamically trapped following the generated optical patterns by phase hologram. The two representative snapshots of the motion of trapped polymer particles particles ($n=1.59$) with diameters of

3.1 μm are provided in Figure 5.26(3).

Figure 5.26 (1) Phase hologram designed with hybrid GS-AA algorithm (2) Object patterns (3) Snapshots of the dynamically trapped particles according to the above corresponding optical pattern

The SLM based optical system is also applied to the locked-in transport of 5 μm silica particles ($n=1.42$) with same diameters of 5 μm were sandwiched in the microfluidic chamber with thickness of about 70 μm . The basic conditions for this experiment is the same as those applied for Dammann grating based optical sorting technique. As it is shown in Figure 5.27, sequential images of motions of the mixed particles were taken with 1 second intervals.

With a laser power of 800 mW and with a flow speed of $20\text{ }\mu\text{m/s}$, it was observed that there was a strong deflection for the $3.1\text{ }\mu\text{m}$ polymer particles (two of them highlighted in square) when they passed through the optical landscape, the $5\text{ }\mu\text{m}$ silica particle highlighted in square was obviously deflected and locked-into the axis of optical lattice from the top of the image to bottom. The throughput of the system is around 20 particles/s. Figure 5.27 (c) shows the difference of original flow direction and deflection direction.

Figure 5.27 Locked-in transport of $5\text{ }\mu\text{m}$ silica particles, the scale bar represents

$10\text{ }\mu\text{m}$. The interval between each snapshot is 1s.

5.4 Conclusion

The CGH is usually realized with diffractive optical element DOE. In the chapter, DOEs are designed with two totally different approaches, direct design technique (Dammann grating) and IFTA. Both techniques have their own

advantages and disadvantages, which can be compared in different areas, including fabrication complexity, diffraction efficiency, computation complexity and flexibility. As for fabrication, the binary Dammann grating is more compatible with general UV exposure based microlithography fabrication techniques, whereas the fabrication of multi-level DOE will have greater fabrication error and more complex fabrication process like e-beam direct writing etc.. The design of Dammann grating is calculated analytically in a simple and direct way by Fourier series decomposition; however, the theoretical efficiency diffraction efficiency for design 2D separable binary phase Dammann grating is only about 60%, whereas the multi-level DOE designed by IFTA method can theoretically exceed 90%. The application of Dammann grating is limited to array illuminator or simple beam-shaping applications. The DOE is capable of creating complex patterns, and special designed hologram encoded in the SLM can realize dynamic optical trapping and even the 3D multi-level optical trapping.

DOE provides a solution as a tradeoff between the reduced phase and spatial resolution; hence it is more compatible with the common fabrication techniques than those refractive optical elements like MLA. And the uniformity of optical lattice is not affected by the non-uniformity profile of the incident beam. Experimentally the fabricated Dammann grating is employed to create the optical lattice for multiple optical trapping and sorting two types of particle

with different refractive indices (same diameters of $3.1\ \mu\text{m}$), polymer particles ($n=1.59$) and silica particles ($n=1.42$). The throughput of Dammann grating sorting experiments is ~ 20 particles/s and the sorting efficiency is $\sim 70\%$. It is experimentally proved that the Dammann grating needs 700 mW to achieve the sorting, while the MLA system only needs 400 mW-500 mW to get the similar result. Such differences are mainly attributed to the power-efficiency between MLA and Dammann grating. Theoretically the MLA has 100% power efficiency compared to $\sim 60\%$ of designed 2D 5×5 orders Dammann grating.

Several IFTA phase-retrieval programs including G-S, A-A and hybrid GS-AA algorithms are compared, and the hybrid GS-AA is finally adopted for the design of phase hologram due to its smaller RMS-E and more rapid convergence rate. The designed phase-hologram is realized through SLM and together with holographic microscopic optical system, and an arbitrary optical pattern could be created in the sample plane. Experimentally dynamic multiple optical trapping is demonstrated with a video combining designed hologram displayed on the PC connected to SLM. And continuous locked-in transport of microparticles with the generated optical lattice is also experimentally demonstrated with throughput of ~ 20 particle/s.

CHAPTER 6

STUDY ON THE ON-CHIP MULTIPLE

OPTICAL TRAPPING AND SORTING

TECHNIQUES

In this chapter, Talbot self-imaging and MLA are proposed to realize on-chip multi-beam optical trapping and sorting of micro-particles respectively. The chessboard grating is fabricated to create a longitudinal periodical optical lattice. The 2D on-chip multiple optical trapping within microchamber is experimentally demonstrated. Furthermore, the multi-level array trapping of particles is realized using a modified Talbot effect. And the on-chip continuous sorting of particles by sizes on the optical lattice enabled by Talbot effect is experimentally demonstrated. Additionally, the 3D optical lattice generated by Talbot self-imaging is applied to the creation of 3D sub-micron sized periodical structure in PDLC. A MLA based on-chip trapping and sorting system is proposed. The sol-gel MLA capable of focusing incident laser beam into optical lattice within microchamber is designed and fabricated. The array trapping of particles, as well as the locked-in transport of particles on the created optical landscape are experimentally demonstrated.

6.1 Introduction

The idea of lab-on-a-chip (LOC) integrates several laboratory functions on a chip of centimeters or even smaller, which enables handling of extremely small amount of objects with high yield. The advantages of LOC include low fluid volumes consumption, safer handling, disposable, higher analysis and control speed etc. In LOC field, the cells and particle separation technique plays a significant role.

In Chapter 4 and Chapter 5, multiple optical traps are created with refractive and diffractive optical elements based lens projection microscopic systems. In order to realize on-chip trapping and sorting of particles or biological sample, it is of necessity to develop a more compact system which is capable of on-chip generating optical lattice without lens projection system. To this ends, two approaches being proposed in this Chapter, one is based on the Talbot self imaging effect and the other uses the small aperture microlens array.

The Talbot effect created optical lattice is able to realize on-chip multiple optical trapping and sorting within microchamber. Furthermore, a modified self-imaging effect will be proved to be capable of optical trapping and array manipulating in multiple-planes simultaneously. The proposed method requires no lens projection and the trapping range in both axial and lateral direction may reach hundreds of micrometers. It is also proved the 3D longitudinal periodical lattice can be applied to the creation of 3D sub-micron sized periodical structure in PDLC.

The MLA provides an alternative solution to on-chip trapping and sorting. The integrated chip is composed of a fabricated MLA and a sealed microchamber. Sol-gel microlens array with short focal length is designed and fabricated to create optical lattice in the microchamber without lens projection system. The generated optical lattice is experimentally demonstrated to be capable of on-chip optical trapping and locked-in transport of particles in microfluidics.

6.2 Development of Talbot self-imaging based on-chip optical trapping and optical sorting technique

6.2.1 Introduction of Talbot effect

The creation of massive multiple beams is based on the Talbot self-imaging of periodic structures. The Talbot effect is a longitudinally periodic self-imaging phenomenon of a transversely periodic structure (Talbot H.F. 1836) (Lohmann A.W. et al. 1988, 1990, 1992) (Da X.-Y. 1992) (Kolodziejczyk A. et al. 2004). When an infinite periodic grating (period is p) located at the $z=0$ plane is illuminated by a unit amplitude coherent plane wave with wavelength of λ , the scalar light field at the emergent plane behind the grating can be described by the Fourier series expansion of the structure,

$$U_0(x) = \sum_{(m)} C_m \cdot \exp(2\pi i m x / p) \quad (m=0, \pm 1, \pm 2 \dots) \quad (6.1)$$

where C_m is m th Fourier coefficient, representing the complex amplitude of the grating spectrum. Then the wave field in the $z=Z$ plane is given by

$$U_z(x) = \sum_{(m)} C_m \cdot \exp(-i 2\pi m^3 \frac{z\lambda}{2d^2}) \cdot \exp(i 2\pi m x / p) \quad (m=0, \pm 1, \pm 2 \dots) \quad (6.2)$$

According to Equation 6.2, the self-image of the grating will occur repeatedly at distances of integer multiples of Talbot distance ($z = N z_T = N \times 2p^2 / \lambda$), as shown schematically in Figure 6.1.

Figure 6.1 Schematic of Talbot self-imaging effect

Figure 6.2 describes the distribution of light intensity field after the emergent plane of periodical grating along the propagation direction. Except for the self-images at Talbot distance, there also exist several fractional Talbot imaging planes within a period. For example, it can be derived from Equation 6.2 that after a lateral shift of $z_T/2$ an image of the grating will be reconstructed but with a lateral shift of half period.

Furthermore, for a periodic phase grating, there are more fractional Talbot planes when certain phase difference and duty cycle are satisfied. However, the attention in this chapter will be focused on the self-images at integer times of Talbot distance for multiple trapping and optical sorting.

Figure 6.2 Simulation of the light distribution after the emergent plane of microstructure along the light propagation direction

The method based on Talbot effect to generate optical lattice is called as Talbot array illuminator (TAI) (Klaus W. et al. 1998) (Da X-Y. et al. 1996). For TAI, more high frequency information is kept in this lensless imaging method. The TAI is in fact formed by multi-interference of multiple diffraction orders. Therefore, the created lateral profile of light spot in the optical lattice is fairly steep. Figure 6.3 shows the 3D intensity profile of Talbot images created by a 2D chess-board grating. Furthermore, comparison of the steepness of three different methods is shown in Figure 6.4. It is noted that the steepness is much higher than the two-beam interference and even a bit higher than the four beam interference (a pair of counter-propagating beam).

Figure 6.3 Schematic of Talbot self-imaging effect

Figure 6.4 The 2D intensity profile of (1) TAI with blue line (2) four-beam interference in red (3) two-beam interference in green

6.2.2 Design and fabrication of chess-board grating with electron-beam lithography

The size of individual optical spot of optical lattice is designed to have a comparable size to the particle size for stable trapping. As the sizes of samples for trapping and sorting are in the range of 3 ~5 μm and collimated laser beam will be employed, a chessboard-like 2D binary amplitude optical grating with period of 10 μm and a duty cycle of 50% is designed to produce an interconnected optical lattice, as it is shown in Figure 6.5. Direct binary electron-beam write technique is employed for the fabrication of the grating. Experimentally a converted JEOL-6600 SEM with ELPHY plus software was used to fabricate the amplitude chessboard structure, and MMA e-beam resist is employed in the fabrication. The dependence of the coating film thickness on the spin speed is shown in Figure 6.6. In electron-beam lithography, accelerating voltage of 20 KeV and beam current of 200 pA were employed respectively. MMA resist is spin-coated on a pretreated indium-tin-oxide coated glass substrate at a spin speed of 4000 rpm for 60 seconds, resulted in a thickness of 470 nm resist layer. Before exposure, the spun resist on substrate was pre-baked on a hotplate to 150°C for 90 seconds. The sample was then developed in the developer (MIBK: IPA = 1:3) for 150 seconds and rinsed with IPA for 60 seconds. Subsequently a metal layer of Ti/Au (30nm/80nm) was deposited. Finally the resist was removed from the surface through a lift-off process in acetone. A SEM photo of the fabricated amplitude grating is given in Figure 6.7.

Figure 6.5 Schematic of amplitude chess-board grating.

The period of grating is $10\text{ }\mu\text{m}$, and each square is $5\text{ }\mu\text{m} \times 5\text{ }\mu\text{m}$

Figure 6.6 Relationship between the coating film (MMA) thickness and spin speed

Figure 6.7 SEM photo of EBL fabricated 2D chess-board grating

6.2.3 On-chip multiple optical trapping using Talbot image

The optical system for on-chip optical trapping is called as optical-traps-on-a-chip (OTOC). The OTOC is designed to be composed of three parts: laser source, integrated optical chip and monitoring system. The optical chip integrates the fabricated chessboard grating for lattice generation and the microchamber for trapping experiment; hence on-chip trapping can be realized with the external laser beam. The schematic of OTOC is shown in Figure 6.8. The laser source is a continuous wave 1 W Yb fiber laser (IPG Photonics) at 1070 nm. The laser beam is collimated and linearly polarized with a diameter of about 1.6 mm. The beam is subsequently reduced and collimated to about 200 μm by a telescope system comprising two convex lenses (L1, L2) with focal length of 200mm and 25mm respectively. Then the beam will directly incident on the optical chip without any projection lenses. The OTOC comprises a two-dimensional (2D) chessboard-like optical grating and a

microfluidic chamber. The microfluidic chamber with a depth of $70\ \mu\text{m}$ was formed between two glass plates, which were separated by a spacer. The chessboard grating is adhered to the bottom surface of the microchamber, and the optical chip is held by a high precision 3D microstage for adjusting the locations of the samples. Spatial period of the chessboard grating was $10\ \mu\text{m}$, corresponding to a calculated Talbot distance of $70\ \mu\text{m}$ in water. At the mid-way of the half Talbot distance, the self-image was laterally shifted by half of a period. In this case, only one layer of self-image will work as the optical lattice for multiple trapping. A $100\times$ long working distance objective lens is employed to magnify the image of samples. The OTOC system is different from the lens projection system, in that uses an objective lens for observation only. A dichroic mirror is employed to avoid the strong laser beam entering the CCD camera while permitting the passing of white illuminant light for real-time monitoring of particles motions on a PC.

Figure 6.8 Optical configuration of optical-traps-on-a-chip (OTOC)

Figure 6.9 shows micrographs of self-image pattern in microfluidics with maximum and minimum image contrast within one period captured by a CCD camera. Since higher diffraction orders diverge too fast to form an image when the distance becomes greater, the self-imaging effect will then disappear after a distance along the propagation direction. For a grating with a period of less than $10\text{ }\mu\text{m}$ in our system, practically the Talbot effect can only exist within several millimeters or even less from the emergent plane along the propagation direction.

Figure 6.9 Micrograph of the laser pattern at different planes within one period

(a) maximum image contrast (b) minimum image contrast.

In the present inverted microscopic system without an objective lens, the scattering force acting on a particle is a dominant force that is much stronger than the gradient force exerted by the optical spots array at different planes. This observation has been validated by the trapping experiments. When the laser was switched on (each spot is approximate $1.5 \pm 0.5\text{mW}$), we observed

that all the particles were trapped regularly and levitated close to the top plane of the microchamber, which was about 60–70 μm higher than the emergent plane of the grating. Once the laser was switched off, all the particles fell down to the original bottom plane and became randomly located. Figure 6.10 presents a micrograph of large-scale trapped array of 3.1 μm polymer microparticles. It is observed that all of the particles in the field are trapped regularly corresponding to the light spots of lattice. It is further demonstrated hundreds of optical traps in a regular square area of $200 \times 200 \mu\text{m}^2$ simultaneously. It is especially noted that for the OTOC there is no direct limit to the size of the working area. This is due to the fact that the chip's effective area with optical traps is only determined by the collimated laser beam's size, as long as enough laser power is maintained over the working area.

Figure 6.10 Micrograph of the trapped large array of 3.1 μm polymer particles.

6.2.4 Transport of trapped particles array using fluctuating Talbot images

Optical tweezers will confine the high-index particles to locations of maximum light intensity. The dynamic optical tweezers enabled by SLM has been demonstrated to be able to interactively arrange and transport particles. By contrast, there are also reports on the transport of particles without adaptive control. The asymmetry optical landscape is demonstrated to be able to transport or even sort the mixed cells (Paterson L. et al. 2005). When the symmetry of optical potential energy landscape is broken, it will give rise to the motion of previously trapped particle. This asymmetry of the system can be easily accomplished by adding a tilt to the light beam or other methods. In order to realize the deterministic transport of particles, Koss and Grier (2003) reported an efficient approach for transporting particles using short repetitive sequences of holographic optical trapping patterns. The designed trap forming hologram replaced with one another through the SLM. The created optical landscape is symmetrical and no adaptive control is needed.

Now the Talbot effect is proposed to perform the transport of particles efficiently and deterministically. The Talbot effect based multiple trapping system can be modified to create a deterministic fluctuating optical lattice without the SLM. The fluctuating symmetrical optical lattice is generated by moving the fabricated chessboard grating at certain frequency. Such dynamic optical lattice can efficiently transport an array of spheres without human interaction and requires no computation for CGH. Figure 6.11 illustrates the principle of using three discrete sampled states in the transport. In Figure 6.11a,

periodical optical traps are represented by Gaussian potential wells, while the optical traps shown in Figure 6.11b and 6.11c has a half-period lateral shift respectively. When the optical traps begin to shift from left to right at certain velocity, the trapped particles will be “dragged” by the traps to locations shown in Figure 6.11b and then Figure 6.11c.

Figure 6.11 Principle of transport of particles array with fluctuating optical lattice

In the optical configuration modification scheme of Talbot transporting system, the chessboard grating is separated from the microchamber, that is to say, there is relative motion of the grating corresponding to the fixed microchamber. The uniform moving grating will modulate the incident laser beam into a dynamic optical lattice, whose frequency is dependent on the moving speed. Experimentally the Talbot grating is mounted on a motorized stage so that it will move smoothly in the horizontal or vertical direction, while the position of laser beam (no involving grating here) is fixed. In this case, it is capable of creating a dynamic optical lattice with regard to the fixed microchamber.

Consequently, the trapped array of spheres will be translated following the corresponding spots in the optical lattice provided that the optical gradient force is strong enough. In order to improve the optical force, the diameter of the incident beam is further minimized. The four snapshots of the guiding of spheres array are shown in Figure 6.12. The diameter of the silica sphere is $4.7\mu\text{m}$, and the guiding velocity is $6\mu\text{m}/\text{s}$.

Figure 6.12 Translation of array of spheres array with moving optical lattice

6.2.5 Simultaneous on-chip trapping of microparticles in multiple planes

In spite of the axial periodicity of self-images, it is noted that the existing approach employing the self-imaging effect does not support optical trapping in multiple planes due to reasons below. Firstly, in an inverted microscopic system, radiation pressure will dominate the net forces along the propagation direction so that particles can only be confined as a 2D array and they will be pushed upwards to the top surface in the microchamber. In this case, physically separate chambers may be required to confine particles to different vertical planes. Secondly, in spite of the restoring ability of Talbot effect, when the

density of particles reaches certain amount but not full, the self-imaging intensity pattern will no longer be formed due to the non-uniform diffraction and scattering of the trapped particles. Hence, array trapping of particles will not occur in 3D. However, if particles are fully trapped at the first plane near the chessboard as a self-assembled grating structure, the self-images of the grating will be tailored to continue without being destroyed in propagation. By virtue of this fact, a layer-by-layer self-imaging of the trapped particles was employed to generate a 3D optical lattice for trapping in a cascaded way.

The principle of array trapping in multiple planes is depicted in Figure 6.13. When the collimated laser beam incidents on the amplitude chessboard grating, the self-images occur periodically along the beam propagation direction until encountering the particles. In a microfluidic medium, particles on the first plane are trapped in an array (particles array 1) following the self-imaging pattern of the chessboard structure. When the size of the ‘particle lens’ is only several micrometers, the trapped particles array may be described as a 2D microparticle tailored phase-only grating, for the diffraction effect dominates. It can be proved with angular spectrum method that the 2D grating composed of trapped particles does not change the axial period and the quality of the self-images, provided that the particles plane is located around the Talbot planes or fractional Talbot planes. Hence, along the axial direction, particles on the second plane can be trapped in an array (particles array 2) tailored by self-imaging of the trapped particles on the first plane. The array of particles at this plane may shift half of the period when the condition of half Talbot distance is met. Subsequently, another group of particles (particle array 3) will be trapped

following the optical lattice created by the self-imaging of particles in the second plane.

Figure 6.13 Principle of trapping of particles in multiple planes using modified self-imaging. The depth of each separate microfluidic chamber is comparable to half of the Talbot distance. The particles array 2 shifts half of the period laterally with regard to particles 1 and 3.

In order to prove the feasibility of proposed scheme of multiple-level trapping, a numerical simulation model is built. The aim of the model is to investigate the change of the light field when a propagating light is diffracted and scattered by a spherical particle, and also to further study the impact on the longitudinal propagating Talbot images by the regularly trapped particles array or randomly trapped particles. It is known that when a spherical homogeneous microparticle

is placed in a uniform plane wave, a rigorous solution of the scattering field could be described by the Lorentz-Mie solution of the Maxwell equations, which is relatively complex and for the numerical simulation. Consequently, a simplified model is adopted here to compute the light intensities scattered in the forward direction by a spherical microparticle.

In this simplified model, the scattering process is fully described by the diffraction theory, and the trapped particle is regarded as an opaque disk. The discussion is restricted to scalar diffraction theory and assuming that the incident laser beam is a plane wave. According to the propagation of the angular spectrum of the plane wave, the amplitude function of the (x, y, z_0) plane parallel to the $(x, y, 0)$ can be described by (Goodman J.W. 1996),

$$U(x, y, z_0) = \iint A(f_x, f_y; 0) \times \exp\left[j \frac{2\pi z_0}{\lambda} \sqrt{1 - \lambda^2 (f_x^2 + f_y^2)}\right] \times \exp[j2\pi(f_x x + f_y y)] df_x df_y \quad (6.3)$$

where λ is the wavelength of the light, f_x, f_y are spatial frequencies, $A(f_x, f_y; 0)$ is the angular spectrum of the plane $(x, y, 0)$. In the simulation, the period of the amplitude chessboard grating is assumed to be $10\mu\text{m}$, although the period should be calculated as $p = 10 \times (\sqrt{2}/2) = 7.07\mu\text{m}$; the wavelength of the laser is $1.06\mu\text{m}$, and the propagation medium is air. Then the Talbot distance is computed to be $z_T = 2p^2 / \lambda = 94.3\mu\text{m}$. When the light beam propagates in the

air without obstacles, the Talbot images and fractional Talbot images will occur periodically, as it is shown in Figure 6.14.

Figure 6.14 Simulation of the light distribution after the emergent plane of fabricated 2D chessboard grating along the light propagation direction in the air

The aim of simulation is to study the light propagation when the particles are diffracted by the regularly trapped particle array. Hence, it is assumed that the particles are regularly trapped as an array at a plane with distance of $160\mu\text{m}$ from the emergent plane of grating. The numerical simulation is then performed through angular spectrum method, and the result is shown in Figure 6.15. The result demonstrates that the trapped particles array will give rise to a secondary modified series of self-images. It is further proved that the grating composed of trapped particles does not change the axial period of the self imaging except for

introducing a tiny delay of wavefront along the propagation direction. It is noted that the contrast of intensity decreases to some extent compared with the original Talbot images, and the contrast of the self-images are dependent the distance between particles trapping plane the last self-image of the chessboard grating before this plane.

Figure 6.15 A secondary series self-imaged caused by the diffraction of 'trapped particles grating'.

The optical system same as OTOC configuration shown in Figure 6.8 is employed for simultaneous multi-level optical trapping of particles. In the experiment, a microfluidic chamber with a depth of $70\text{ }\mu\text{m}$ was constructed between two coverglasses (0#) separated by a spacer, and the same chessboard grating with period $10\text{ }\mu\text{m}$ was used. Initially the mixture of silica ($5.72\text{ }\mu\text{m}$)

and polystyrene ($4.97\ \mu\text{m}$, dyed red) particles was suspending at the same plane which is about several micrometers above the bottom surface of chamber. When the laser was switched on with a power of 700 mW, it was observed that in seconds polystyrene particles were levitated to the higher plane near the top surface of the chamber due to the radiation pressure, while there were no obvious changes for the vertical locations of silica particles. In other words, two groups of particles were vertically separated into two planes with the radiation pressure. Figure 6.16 shows 2 snapshots of simultaneous multi-level trapping of particles. The silica particles at the first plane were much dense so that they were optically trapped as an array with regard to the individual light spot, as shown in Figure 6.16 (a). When the observation plane was levitated to the polystyrene particles near the top surface of chamber, it was found that the self-image has been shifted for a distance of half of a period. That is to say, the amplitude profiles of images at two planes were reversed. Figure 6.16 (b) shows that the particles were stably trapped corresponding to the light spots of modified Talbot plane.

In order to further demonstrate that our result is attributed to the modified Talbot effect of trapped particles grating rather than the initial Talbot effect produced by the fabricated chessboard grating, we repeated trapping experiments under the same condition except for some decrease of the density of silica particles. It was observed that the Talbot images were destroyed when the silica particles at the first plane were not dense enough to form a regular integral grating; hence, multiple trapping at different level was impossible to realize. As a result, we demonstrated that the result of trapping of multi-levels

of particles were implemented initially by the fabricated grating and then continued by the modified Talbot effect of the subsequent trapped particle grating layers.

Figure 6.16 Micrograph of the particles simultaneously trapped at multiple levels. The trapping of multilevel particles occurs at the highlighted region. (a) Trapping of array of polystyrene particles at lower level induced by the self-imaging effect of amplitude chessboard grating. (b) Trapping of silica particles (highlighted in blue squares) at higher level corresponding to the light spots of optical lattice.

6.2.6 On-chip optical sorting using Talbot self-imaging effect

The OTOC is also explored to realize a so-called locked-in transport of particles along the interconnected axis formed by the adjacent diagonal spots in an on-chip optofluidic channel. When microparticles pass through the optical lattice created by the OTOC, a strong deflection of particles can be observed while deviating from the original flowing direction. Consequently, it is feasible to

apply this effect to sort mixed microparticles with different sizes or refractive indices.

In the optical sorting experiment using OTOC, a mixture of silica particles with diameters of $4.74\text{ }\mu\text{m}$ and $1.85\text{ }\mu\text{m}$ respectively is tested for sorting. The transport of particles' flow is implemented by the motorized stage, which translates at a constant speed ranging from several to hundreds of micrometers per second. When the laser is switched on with an output power of 800 mW, the particles are translated uniformly at about $10\text{ }\mu\text{m/s}$ passing through the optical lattice. Figure 6.17 shows the consecutive snapshots of the optical sorting. In the figure, it shows 8 particles of $4.74\text{ }\mu\text{m}$ diameter highlighted with squares, were deflected while being translated through the optical lattice. In the experiment, it was observed that majority of the $4.74\text{ }\mu\text{m}$ diameter particles were deflected from the original driving direction while the smaller particles are almost un-deflected. The particles' was observed to deflect along the diagonal of the grating, because there was a strong interconnection between the adjacent spots produced by the chessboard-like grating. In order to further describe the deflection of $4.74\text{ }\mu\text{m}$ silica spheres, their typical trajectory is extracted, as it is shown in Figure 6.18.

Figure 6.17 Snapshots of microspheres sorting while passing through the created optical landscape. The interval between the snapshots is 2s.

Figure 6.18 Extracted trajectories of the $4.74\ \mu\text{m}$ silica spheres deflected from the original horizontal flow direction

6.2.7 Fabrication of 3D sub-micron sized periodical structure

In addition to realize multiple optical trapping, the Talbot effect can also be employed to fabricate a sub-micron sized periodical structure. PDLC material adopted will be employed in the fabrication. It is a relatively new class of materials that have potential applications ranging from switchable windows to projection display (Domash L.H. et al. 2003). The LC/prepolymer mixture syrup adopted for the fabrication of the sub-micron-sized structure includes 48% monomer, trimethylolpropane triacrylate (TMPTA); 8% cross-linking monomer, *N*-vinylpyrrolidinone (NVP); 8% surfactant, octanoic acid (OA); 1.2% coinitiator, *N*-phenylglycine (NPG); and 0.8% photoinitiator, rose bengal (RB); which are all from Sigma-Aldrich. In pre-polymer/LC mixture, the LC concentration was about 34 wt %. The LC used was E7 (Merck) with an ordinary refractive index of $n_o = 1.521$ and a birefringence of $\Delta n = 0.225$. The details of material preparation processes can be found elsewhere (Liu Y.J. et al. 2006, 2007). The mixture was injected into a LC cell by capillary action at room temperature. The cell gap was $30\mu m$. The refractive index of prepolymer syrup used here is $n_s = 1.489$ and the refractive index of cure film is $n_p = 1.522$. Then the effective index of the mixture of LC/prepolymer is calculated to be 1.5. As it is shown in Figure 6.19, the Ar^+ laser beam with a wavelength of 514.5 nm is expanded and incident on the fabrication chessboard grating and the Talbot images will be created subsequently after the emergent plane of the grating. The square chessboard grating has a horizontal/vertical period of $1.4\mu m$. The mixture syrup of LC/prepolymer is sandwiched between two cover-glass substrate with a space of $30\mu m$. And the

sandwich cell is held with a 3D stage and kept in close contact with the surface of chessboard grating. The simulation result of the light distribution after the emergent plane of microstructure along the light propagation direction in the medium of LC/prepolymer mixture syrup is shown in Figure 6.20.

Figure 6.19 Optical setup for fabrication of 3D photonic crystal enabled by Talbot effect

Figure 6.20 Simulation of the light distribution after the emergent plane of microstructure along the light propagation direction within the medium of LC/prepolymer mixture syrup

In the fabrication experiment, the output of laser beam is 300 mW and the exposure time is 2 mins. When the mixture of LC/prepolymer mixture is exposed to the diffractive intensity pattern caused by the chessboard grating, the prepolymer will be polymerized, which result in the redistribution of LC and polymer. The polymerization will give rise to two separate regions, pure polymer region and polymer-rich region. The pure polymer regions contain no LC, while the polymer-rich region contains both LC and polymer. And the polymer is regarded to have 5% volume shrinkage during the polymerization. As a result, the structure of 3D periodical microstructure will be formed within the cell corresponding to the diffraction pattern of chessboard grating in the Fresnel region.

Figure 6.21 shows the two micrographs (different layer) of the cross-section of fabricated 3D structure, which are obtained through an optical microscope. And the Figure 6.19 (a) and (b) represents the cross-section micrograph of sub-micron structure at different axial planes. The observation is fulfilled through continuous adjusting the height of focal plane of the objective to find the images with high contrast. It is observed that the image (b) has a half period horizontal/vertical shift with regard to image (a), which indicates that the images are obtained at two planes with difference of odd times of half Talbot distance. After the development of the polymerized mixture syrup in ethanol, the LC is removed and the surface morphology is characterized by an atomic force microscopy (AFM) image, as it is shown in Figure 6.22. When an output beam of He-Ne laser at 532 nm incidents on the fabricated square microstructure, a diffraction pattern is created, as it is shown in Figure 6.23.

From the inside to outside of the figure, it is the 0, ± 1 , ± 2 diffraction order respectively. In addition to the fabrication of square periodical microstructure, Talbot effect is also demonstrated to be capable of fabricating a sub-micron sized hexagonal microstructure by means of an electron-beam fabricated mask with hexagonal lattice. Similarly, Figure 6.24 and Figure 6.25 show the AFM image of the surface morphology of fabricated and diffraction pattern of the fabricated hexagonal PDLC microstructures respectively.

Figure 6.21 Two micrographs (different layer) of the cross-section of fabricated 3D periodical microstructure. The scale bar represents 1 μm .

Figure 6.22 AFM image of the surface morphology of fabricated square periodical microstructure (LC has been removed with ethanol)

Figure 6.23 Diffraction pattern of the fabricated square PDLC periodical microstructure (before development)

Figure 6.24 AFM image of the surface morphology of fabricated hexagonal periodical microstructure (LC has been removed with ethanol)

Figure 6.25 Diffraction pattern of the fabricated hexagonal PDLC periodical microstructure (before development)

6.3 Development of MLA based on-chip optical trapping and optical sorting technique

6.3.1 Principle MLA enabled on-chip optical trapping and sorting

It is known that MLA has the ability to focus optical spot of nearly uniform intensity. In Chapter 4 the array of focus points generated by MLA is projected into objective lens for multiple trapping and sorting. In order to realize a more compact optical trapping system, Here an integrated optical chip incorporating MLA and microchamber is proposed for multiple trapping and sorting of particles. The particularly designed MLA could be utilized to directly create an optical lattice near the surface of element, without including the optical projection system. The MLA optical chip is compact, efficient and easy to align. The schematic of integrated optical chip is shown in Figure 6.26. The

integrated optical trapping system is composed of a microchamber and a designed MLA fabricated on a glass substrate. The incident laser beam will be focused by the MLA into a 2D light spots array within the microchamber. The sample in the microchannel includes two types of particles of different sizes or refractive indices. If MLA parameters and channel thickness are appropriately designed, with the pump driving force coming from the inlet, the particles will flow and be sorted while passing through the optical potential well landscape. After sorting, the separated samples will be driven to the outlet and collected respectively.

Figure 6.26 Schematic of on-chip optical trapping using MLA

6.3.2 Design and Fabrication of microlens array

The complicated photolithography and etching processes are required in the conventional fabrication of microlens array (MLA). Furthermore, it is difficult to obtain uniformity and homogeneity of MLA. In the proposed optical sorting system, an inorganic-organic hybrid $\text{SiO}_2/\text{ZrO}_2$ sol-gel material is introduced in the fabrication of MLA. The sol-gel material used in the fabrication of MLA offers advantages such as simple fabrication, low cost, highly tolerable optical properties, good mechanical strength, high chemical and environmental durability and no production of toxic gases. Sol-gel MLA can be used as an end product, for it can be fabricated in a single step without etching.

The hybrid $\text{SiO}_2/\text{ZrO}_2$ sol-gel material is in-house synthesized (He M. et al. 2003, 2004). The fabrication of sol-gel MLA is realized by direct laser writing technique. A direct laser writing system with a lateral resolution of $3\mu\text{m}$ using He-Cd UV laser at 325 nm (maximum output power is 200 mW) is employed. The laser beam is guided into a microscope and then focused with objective lens onto the sample, and the intensity of laser beam is controlled by an acoustic optical modulator (AOM). And the calibrated relationship between $\text{SiO}_2/\text{ZrO}_2$ sol-gel film thickness and the AOM value is shown in Figure 6.27. It is indicated that the thickness has a linear relationship with AOM value when the thickness is within the range of $0 \sim 2.3\mu\text{m}$. This linear characteristic proves the feasibility of fabricating MLA using direct UV laser writing technique.

Figure 6.27 Measured calibration curve showing the dependence of the SiO₂/ZrO₂ sol-gel film thickness on the AOM value.

The sol-gel material was synthesized at the beginning and regarded as a negative photoresist. It was then spin-coated at 2500 rpm for 35 s onto a slice glass substrate to achieve a thickness of $0.9\mu\text{m}$ and followed by pre-bake on a hotplate at 80°C for 3min. After development in acetone for 12s, the desirable MLA structures will be formed in the sol-gel thin film. Figure 6.28 shows the SEM photo of the fabricated MLA.

Figure 6.28 SEM photo of fabricated MLA

The diameter of individual microlens in the refractive MLA is designed to be $22\ \mu\text{m}$. By means of design calculations, we can obtain the focal length of the fabricated MLA. The radius of curvature(R) and focal length (f) of the microlens are given by,

$$R = \frac{D^2 + 4h^2}{8h} \quad (6.4)$$

$$n_2 R = (n_1 - n_2) f \quad (6.5)$$

where h is the “sag” of the lens, n_1 is the refractive index of the fabrication material for MLA (1.42 for the above used $\text{SiO}_2 / \text{ZrO}_2$ sol-gel material), n_2 is the refractive index of water for microfluidic trapping and sorting. According to the equation (6.4) and (6.5), the focal length of the fabricated MLA is $161.1\ \mu\text{m}$ in the air and $1000\ \mu\text{m}$ in the water.

Figure 6.29 Schematic of individual microlens

6.3.3 On-chip optical trapping and sorting experiment

In the optical trapping and sorting experiment the microfluidic chamber with a depth of 70 μm is formed between two glass plates, which were separated by a spacer. An aqueous suspension of microparticles is sandwiched in the microchamber. In the system, a continuous wave laser with 1 W maximum at 1070 nm wavelength is employed. The laser beam is collimated before being focused by the MLA on the chip. Two dichroic mirrors are positioned along the optical path. Dichroic mirror is a specially coated mirror which only reflects a certain spectrum of the light and allows the remaining spectrum to pass through. According to the wavelength of laser, the mirror produced by Thorlabs Inc. (BB1-E03), is used to reflect only the 750-1100 nm range. Consequently, the laser beam in the system will be reflected, while most of the illumination light at visible range will pass through the mirror. The bottom dichroic mirror in the system is added to reduce the amount of infrared laser beam entering the CCD camera. Otherwise, the view on the monitor will be too bright to see the motion of microparticles. The dichroic mirror at the top is to help reflect the infrared more efficiently without affecting the passage of illumination light. The magnification factor of the objective lens should be selected according to the size of particles and the optical spot size, and 100x is adopted in our system for viewing the micron-sized particles. Furthermore, long working distance (13mm) objective lens is adopted to be suitable for a large range of the thickness of microchamber. To make the particles and optical spots display clearly on the monitor, a high intensity fiber source is employed as the illumination source.

In order to achieve the highest power efficiency of the trapping system, it is significant to make the focal plane of MLA within the space microchamber. To this end, the microchamber and fabricated MLA are held at two respective 3D microstage, which enable precise control of the air gap between the planes. In the present inverted microscopic system without an objective lens, the scattering force acting on a particle is a dominant force that is much stronger than the gradient force exerted by the optical spots array at different planes. When the laser was switched on with an output power of 300mW, it was observed that all particles were levitated and trapped regularly corresponding to each individual light spot of optical lattice, as is shown in Figure 6.30. Furthermore, when the laser was switched off, in seconds all the particles fell down to the original bottom plane and became randomly located.

Figure 6.30 On-chip multiple optical trapping of 3.1 μm polymer particles

Subsequently, the experiment of locked-in transport of particles in the chamber is conducted. As the continuous transport requires an additional driving force to create a flow of particles with uniform velocity, the microchamber including samples is held by a motorized stage. Figure 6.31 shows sequential images of motions of the mixed particles taken at 0.5 second intervals. With a laser power of 400 mW and with a flow velocity of $10 \mu\text{m/s}$, it was observed that there was a strong deflection for the $3.1 \mu\text{m}$ polymer particles (two of them highlighted in square) when they passed through the optical landscape, the $3.1 \mu\text{m}$ polystyrene particles highlighted in square was obviously deflected and locked-into the axis of optical lattice from the top of the image to bottom. Figure 6.32 shows the abstract of trajectories of particles to demonstrate the continuous locked-in transport caused by the optical lattice.

*Figure 6.31 The sequential images of motions of mixed particles
taken with 0.5 s intervals*

Figure 6.32 Trajectories of the $3.1\ \mu\text{m}$ polystyrene particles being locked-in transported

6.4 Conclusion

The on-chip multi-beam optical trapping and sorting system is studied and developed. The all-optical compact system is demonstrated to realize on-chip multi-beam trapping and sorting. The Talbot effect generated optical lattice has been applied to the on-chip multiple optical trapping and separation of mixture of microparticles by sizes within the microchamber, and the translation of array of particles has been proved with moving optical lattice. Different from those scanning mirror, AOD, CGH, VCSEL, GPC techniques, the Talbot trapping requires no lens projection system and objective, with the result that the working area of Talbot traps is large. The technique is capable of trapping

hundreds of particles within area of $200\mu m$ by $200\mu m$ or even larger. It is especially noted that for the Talbot trapping, there is no direct limit to the size of the working area. As more high frequency information is kept, the created lateral profile of light spot in the optical lattice generated by Talbot self-imaging is steeper than multi-interference technique. Based on the secondary layer-by-layer self-imaging effect, optical trapping of silica and polystyrene colloidal particles at different planes in a microchamber are demonstrated experimentally. The spacing between two layers is $\sim 60 \mu m$. The method requires no lens projection and the trapping range in both axial and lateral direction may reach hundreds of micrometers. Compared with the CGH enabled multi-level trapping technique, the proposed method is free from complex computation in hologram design and has a larger working area. In addition, the created 3D optical lattice is also employed to realize a convenient one-step fabrication of 3D sub-micron sized periodical structure in PDLC. The sorting of $4.74\mu m$ and $1.85\mu m$ silica spheres are experimentally demonstrated with flow speed of $10\mu m / s$, and the sorting efficiency reaches 85%.

The fabricated sol-gel microlens array is also capable of realizing on-chip multiple optical trapping and optical sorting. The integrated optical trapping system is composed of a microchamber and a designed MLA fabricated on a glass substrate. The MLA is capable of creating on-chip optical lattice with arbitrary distribution for trapping. The on-chip multi-beam optical trapping and locked-in transport of particles are experimentally demonstrated with laser power of 400mW and flow speed of $10\mu m / s$.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

The thesis has provided a broad and systematic study on the microoptical elements based multi-beam trapping and sorting techniques by means of theoretical formulations and experiment demonstrations. The continuous optical sorting technique is not based on the tightly focused single-beam optical tweezers, but the periodical optical energy landscape. This all-optical sorting method does not rely on the fluorescent marker but on the intrinsic physical attributes like geometrical size and refractive index. And the sizes of the particles to be sorted can range from hundreds of nanometers to micrometers. It is a passive continuous microfluidic sorting which requires no human interaction. The refractive and diffractive optical elements, including microlens array, Dammann grating, holographic optical elements and chess-board grating, are designed and fabricated to create array of optical traps respectively. The multi-beam optical trapping and optical sorting are experimentally proved using MLA or DOE based lens projection trapping system. The system requires minimum modification to traditional single-beam optical tweezers. All-optical on-chip optical sorting on periodical optical lattice are experimentally demonstrated using Talbot effect and MLA respectively, such system requires no additional lens projection system for generating optical lattice, and accordingly, it has great potential in the cutting edge LOC field. It is further experimentally proved that the 3D optical lattice generated by modified Talbot effect. In addition, the 3D optical lattice is applied to the creation of 3D sub-micron sized PDLC.

Firstly, the mechanism of optical sorting is studied quantitatively, and the result implied that the technique has the ability to separate bi-disperse particles by sizes or refractive indices. The optical sorting on periodical optical lattice is theoretically demonstrated to have exponential size selectivity and is capable of separating objects with sizes ranging from hundreds of nanometers to tens of micrometers.

Secondly, the refractive and diffractive optical elements are designed and fabricated to insert into lens projection microscopic system for multi-beam trapping and sorting. The composite microlens array fabricated by thermal reflow technique is demonstrated to be capable of creating optical lattice with arbitrary layout distribution for multi-beam optical trapping and sorting by sizes. It is the first time to demonstrate large deflection angle optical (45 degree) sorting using composite MLA based lens projection system. The advantages include cost-effective (no additional expensive equipment for create multi-beams), power-efficient (theoretically 100%) and is compatible with standard single beam optical tweezers. Two types of DOE are designed and employed to create optical lattice for trapping and sorting. The binary-phase DOE Dammann grating is designed as array illuminator by direct design technique and the created optical lattice is experimentally applied to the multiple trapping and sorting particles by refractive indices. The throughput of MLA sorting system for separation of $3.1\ \mu\text{m}$ and $1.5\ \mu\text{m}$ polymer particles is 20-30 particles/s with output laser power of 700 mW, and 80% sorting efficiency is achieved. As for the Dammann grating sorting of polymer particles ($n=1.59$) and silica particles ($n=1.42$) with same diameters of $3.1\ \mu\text{m}$, 700mW laser power output only

achieves ~ 20 particles/s and sorting efficiency of $\sim 70\%$. The IFTA phase-retrieval programs, hybrid GS-AA is adopted for the design of phase hologram. The designed phase-hologram is realized through SLM and together with holographic microscopic optical system. Dynamic multiple optical trapping of $3.1\ \mu\text{m}$ polymer particles is demonstrated. And locked-in transport of particles is experimentally demonstrated using $5\ \mu\text{m}$ silica particle, with result throughput of ~ 20 particles/s.

Thirdly, the on-chip multi-beam optical trapping and sorting system is studied and developed to pave the way for the cutting-edge lab-on-chip sample separation technique. The all-optical compact system is enabled by two solutions, Talbot effect and MLA respectively. Both solutions for all-optical sorting are proposed and demonstrated for the first time. The 3D optical lattice created by the Talbot self imaging is investigated with numerical simulation. The Talbot effect generated optical lattice has been applied to the on-chip multiple optical trapping and separation of mixture of microparticles by sizes within the microchamber, and the translation of array of particles has been proved with moving optical lattice. Different from those scanning mirror, AOD, CGH, VCSEL, GPC techniques, the Talbot trapping requires no lens projection system and objective, with the result that the working area of Talbot traps are large. The technique is capable of trapping hundreds of particles within area of $200\ \mu\text{m}$ by $200\ \mu\text{m}$ or even larger. It is especially noted that for the Talbot trapping, there is no direct limit to the size of the working area. The sorting of $4.74\ \mu\text{m}$ and $1.85\ \mu\text{m}$ silica spheres are experimentally demonstrated with flow speed of $10\ \mu\text{m}/\text{s}$, and the sorting efficiency reaches 85%. As more high

frequency information is kept, the created lateral profile of light spot in the optical lattice generated by Talbot self-imaging is steeper than multi-interference technique. Additionally the 3D optical lattice created by modified Talbot effect has been demonstrated for the first time to be capable of simultaneous multilevel trapping of particles array. Based on this secondary layer-by-layer self-imaging effect, optical trapping of silica and polystyrene colloidal particles at different planes in a microchamber are demonstrated experimentally, with the spacing between two trapping layers is $\sim 60\ \mu m$. In addition, the created 3D optical lattice was also employed to realize a convenient one-step fabrication of 3D sub-micron sized periodical structure in PDLC. The fabricated sol-gel microlens array is also capable of realizing on-chip multiple optical trapping and optical sorting. The integrated optical trapping system is composed of a microchamber and a designed MLA fabricated on a glass substrate. The on-chip multi-beam optical trapping and locked-in transport of particles are experimentally demonstrated within the microchamber.

7.2 Main contributions of the thesis

Quantitative model for sorting on optical lattice

The quantitative model is built for analyzing the optical sorting on a periodical optical energy landscape. Two types of optical patterns are compared. It is theoretically demonstrated that the 1D periodical optical lattice permits the exponential size selectivity. The model provides a reference for selecting physical parameter (geometrical size of optical lattice, laser intensity, and flow velocity etc.) for optical separation of particles by different sizes or refractive indices.

Fabricated microoptical elements for multi-beam optical trapping and sorting

The thesis provides systematic investigation on using microoptical optical elements for creating multiple optical traps. The microoptical element ranges from refractive optical element (MLA) to diffractive elements (Dammann grating etc.) and chessboard grating, which is capable of creating optical landscape respectively meeting different requirements. All of the microoptical elements employed in the optical system are self-fabricated. The fabrication techniques employed include photolithography, reflow, e-beam direct writing, and the material used includes photoresist, sol-gel, e-beam resist etc, according to the design requirement.

It is experimentally demonstrated for the first time that the composite MLA is capable of creating the arbitrary optical lattice for multiple trapping and sorting of particles. Continuous sorting of bi-disperse particles by sizes with throughput of optical sorting is between 20 ~ 30 particles per second, which is higher than the optical chromatography method (Hart, S.J. et al. 2001, 2003) and comparable to the microfabricated fluorescence activated cell sorter. Enabled by the composite optical lattice, the separation of two groups of particles with 45° lateral separation angle is realized, which reaches the same result as the optical lattice generated by multi interference reported by MacDonald M.P. (2003)

The binary-phase DOE Dammann grating is designed as array illuminator by direct design technique and the element is fabricated through UV exposure based microlithography technique. The Dammann grating optical sorting system is experimentally demonstrated to be capable of separating particles by refractive indices. The IFTA phase-retrieval programs, hybrid GS-AA is adopted for the design of phase hologram. The designed phase-hologram is realized through SLM and together with holographic microscopic optical system. Experimentally dynamic multiple optical trapping is demonstrated with a video combining deigned hologram displayed on the PC connected to SLM. And the locked in transport of particles are also realized with the optical lattice generated by the computed hologram.

On-chip created optical lattice for trapping, sorting and fabrication

One of the most significant contributions of this thesis is the demonstration of the scheme of on-chip all-optical trapping and sorting using without lens projection system. The Talbot self-imaging and on-chip MLA are proposed for the first time for on-chip creating optical lattice. Both methods are based on the idea of eliminating the lens projection system and integrating the passive optical lattice creating element and microchamber on a single chip. Such system is compact and easy to align. Firstly, the fabricated chessboard grating adopts the Talbot self imaging to creates optical lattice within the microchamber for 2D array trapping and continuously. The optical lattice is further applied to the translation of array of particles has been proved with moving optical lattice. And the 3D optical lattice created by modified Talbot effect has been demonstrated for the first time to be capable of simultaneous multilevel trapping of particles array. Secondly, the other optical chip integrating MLA and microchamber is designed and fabricated for the on-chip creation of optical lattice, which is experimentally proved to be capable of array trapping and locked-in transport of the particles.

The Talbot self imaging created 3D optical lattice is also applied to the single-step creation of 3D sub-micron sized periodical structure in polymer-dispersed liquid crystal (PDLC), and this approach may have potential application in the fabrication of photonics crystals. The fabrication is enabled by a single chessboard grating and has advantages like high-efficiency, convenient, large-area, easy alignment.

7.3 Recommendations for future work

The quantitative model based on 1D optical lattice has been developed to analyze the optical sorting on a periodical optical energy landscape. Although the 1D optical lattice has been proved to have no big differences from the situation of sorting on separable 2D lattice, the present model is still hard to totally describe the sorting on complex non-separable 2D optical lattice, and the randomized thermal forces are not included to describe the sorting process more precisely. The future work on the model may include providing a more integral optical sorting model involving all of the key impacting factors.

The MLA has been proved to be capable of sorting two groups of particles by sizes in the study using lens projection system and on-chip scheme respectively. As the composite MLA permits the generation of optical lattice with arbitrary distribution, the further work may take the advantage of this feature to create specially designed optical pattern for separation of poly-disperse suspensions. One of the possible optical lattice design is the lattice with two cascaded deflection axes to realize optical sorting of a tri-disperse suspension with different refractive indices and sizes. The cascaded optical lattice with different axes will give rise to different maximum locked-in angle, with the result of the sorting of three types of particles mixtures.

The study has successfully demonstrated the on-chip trapping and sorting by means of Talbot self-images and on-chip microlens array. The demonstrated integrated optical chip in this thesis is a simplified edition combining the

chessboard grating/microlens array and the cover glass microfluidic chamber. In order to develop a more practical lab-on-chip for sample separation, in the future it is necessary to develop a more complex system integrating PDMS microchamber, microoptical elements as well as the micropump. As the ultimate objective of the optical trapping and sorting method should be the practical application in the separation and analysis of biological samples; future work should include the experiments on the biological cell samples.

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