Further Development of the Laser Tweezers Technique for Biomedical Applications

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Abstract. The results of the experimental work aimed at a functional enhancement of the existing laser tweezers system by introducing a pixel-addressable liquid-crystal spatial light modulator (Holoeye HEO-1080P) are presented. The use of the modulator allows us to generate light fields of complicated structure including ones with the vortex component and control the objects positions in real time. The special software is developed to form an array of optical traps with the number of elements up to thirty two with the capability of individual or subgroup control. The method of a spatial separation of the modulator aperture is implemented. The ability to control the lateral power distribution of the light field as well as the value of its orbital moment brings new possibilities of a precise manipulation of microobjects including biological ones.

1. Introduction

Since the time of the laser tweezers introduction they are extensively used in biology to manipulate different biological objects. There are several trends in the studies on the application of the optical manipulation in biology and medicine carried out by several research groups (see e.g. reviews [1, 2] and the references therein). A large number of studies are focused on a quantitative evaluation of fundamental properties of various biological objects for example, nucleic acids, proteins, isolated organelles and whole cells (RBCs, white blood cells, etc.) with the use of optical traps. Changes of biological objects characteristics under the effects of various internal and external factors, as well as during some diseases are also studied (e.g. [3-6]). As a rule, the standard Gaussian single-beam traps or dual-traps are used for these studies.

Another line of the researches is represented by the works on further enhancements of the trapping technique itself, particularly maintaining 3D capture spatial stability [7, 8], possibility of the real-time process control, employment of the beams with dedicated complex configurations and demonstration of the applicability of the method to alive objects such as E coli [9], Saccharomyces cerevisiae [10] and other bacteria, RBCs [8, 11, 12], white blood cells.

In our previous studies [13, 14] we have demonstrated that the functional capabilities of the laser manipulators could be significantly enhanced owing to the use of beams with predetermined distributions of the intensity and angular momentum specifically so called spiral beams. Beams with the nonzero angular momentum were generated by using the phase and amplitude–phase masks. Such beams made it possible to rotate weakly absorbing particles and move them along a given trajectory.
Experimental results on the microobject motion along various trajectories (circle, triangle contour, self-intersecting curve, and Archimedes spiral) were presented. Liquid crystals spatial light modulators (LSLM) essentially expand the possibilities of the laser tweezers and are extensively used for optical trapping (e.g. [15, 16, 17]). In this study we further extend the LCSVLM-based tweezing technique. In particular, we have explored the formation of optical traps and their arrays of different types as well as vortex fields by means of the LCSVLM. Optical fields of the predetermined spatial structure are promising for the ordering microobjects into various spatial configurations, objects sorting and for the use as the optical shield. Vortical traps open up new possibilities for the control of microobjects motion, rotation and deformation. The advantages of the LCSVLM use for the formation of vortex traps are also demonstrated. Though our experiments were carried out with the latex spheres, we believe that the mechanical effects demonstrated here will be useful for biomedical technology applications as well.

2. The experimental setup

Figure 1 shows a typical scheme of the setup for the laser manipulations with the built-in spatial light modulator.

![Figure 1. The scheme of experimental setup.](image)

A diode-pumped frequency-doubled solid-state laser was used in our experiments as the light source. The laser wavelength was 0.53 µm and maximal output power was 500 mW. Along with the main radiation, a frequency component with \( \lambda = 1.06 \mu m \) was also present in the output spectrum. CMOS photodetectors are highly sensitive to this wavelength which may lead to strong flare lighting. To optically isolate the detector from a parasitic radiation, the green filter was inserted in the path of the beam. The filter was placed behind the beam expander to prevent the formation of the thermal lens in the body of the filter, which could occur due to the IR-radiation absorption by the filter. The expanded beam with a quasihomogeneous intensity distribution was directed on the spatial light modulator LCSVLM HOLO EYE 1080 with the number of controlled elements 1920×1080 and ability to specify 256 phase gradation within the range from 0 to 2\( \pi \).

To provide the adequate operation of the modulator, the optical scheme was arranged in such a way that the incident laser radiation angles differed from the right angles by less than 7°. The required phase distribution of the modulator was achieved with the aid of a separate computer via the DVI port of the video-card. The light field formed with the LCSVLM was directed into the objective of the
modernized microscope with a beam splitter. A dielectric mirror was used as the beam splitter, with the reflectance of 70% at the incident angles of 45° for radiation at $\lambda=0.53\ \mu m$. The mirror was made using 1 mm thick glass substrate. During the experiments on manipulation, the immersion micro-objective with a numerical aperture of 1.2 was used. Calibrated latex microspheres of various diameters suspended in water served as the objects for the laser trapping and manipulation.

The sequence of microscopic images for the observation and analysis was captured and recorded with the 1.3 Mpixel CMOS camera (DCIM130) connected to the PC through the USB interface.

3. The formation of optical traps with the LC SLM and experiments on microobjects dynamic laser manipulation

The special software is developed to form an array of optical traps with the number of elements up to thirty two with the possibility of individual or subgroup control. The method of a spatial separation of the modulator aperture is implemented. The advantages of this method are the high diffraction efficiency and the complete independence of every element control. For each individual domain it is possible to specify its wedge, spherical and cylindrical components as well as a component corresponding to the axicon. Thus the traps in the shape of points, circles, line segments with individually controllable spatial characteristics or other arbitrary distributions are formed in the plane of manipulation. The examples of the phase distributions and the corresponding intensities in the plane of manipulation for 8 independent traps are presented in Figure 2. Three point traps, three traps in the form of rings and two traps in the form of the line segment (both vertical and horizontal) are shown here.

![Figure 2](image)

**Figure 2.** The formation of optical traps. The phase distributions (a) and the corresponding far field intensities (b).

It is possible to form single traps or an array of up to 32 traps of specified forms (points, circles or line segments). Thus the trapping of up to 32 objects can simultaneously be realized with the capabilities of an independent dynamic control of both a separate trap and of the traps group in whole or in part. It should be noted that the LC SLM are extensively used for the formation and dynamical motion of the group of points traps [15-17] and as a rule, the so-called holographic optical traps are formed with the LC SLM. In this study we demonstrate the capabilities of the manipulation with the complicated configuration traps, for example in the form of a circle and line segment (shown below) as well as the traps on the base of vortex fields (presented in Section 4).

The experiments on the dynamic laser manipulation of microobjects were carried out with the use of the experimental setup with the built-in LC SLM presented in Figure 1. The laser trapping of the 1.2 $\mu m$-diameter latex particles was realized with the use of the ring-shaped optical trap that subsequently changed its shape from a ring to an ellipse. The arrangement of captured particles is changed accordingly, as it is illustrated in Figure 3. One of the possible applications for this
manipulation is the deformation of biological objects, cells for instance. To achieve this effect, one should form the ellipse of a diameter comparable with the diameter of the cell.

Figure 3. The change of the trap form from ring to ellipse (Media 1). The laser power is 100 mW.

Figure 4 demonstrates the experiment by means of the optical trap in the form of a line segment. The latex spheres of 1.2 µm-diameter are used as laser manipulated objects. The line segment operates as the optical shield for particles since a pure area is formed in the fluid flow. The line segment optical trap can be applied for the separation of objects by their size due to the strong dependences of line segment trapping power on the objects size. The ratio of the trapping powers for 3.2 µm spheres and 1.2 µm spheres was evaluated. This ratio was 4.2 for the trap in the form of line segment and 1.4 for the point trap.

Figure 4. The laser manipulation with the use of the optical trap in the form of a line segment (Media 2). The laser power is 100 mW.

4. Experiments on manipulation with vortex fields
The vortex fields have non-zero orbital momentum and can be formed in the shape of curves. Owing to this they can be used to rotate the absorbing particles around the beam axis, to move them along a fixed trace and to apply non-uniform deformations. The experiments on a continuous rotation and control of the angular position of microobjects by means of the fields characterized by the non-zero angular momentum are carried out in many laboratories (e.g. see reviews [18, 19]). The advantageous feature of our experiments is the possibility to move the objects along the trajectories of widely various forms.

Our work is based on the use of the so-called spiral beams. These beams were for the first time discovered and developed by E. Abramochkin and V. Volostnikov [20, 21]. Basing on the spiral beams optics and Gerchberg-Saxton iterative algorithm, we have developed a new way of the phase masks computation [13]. We used the phase distribution of the corresponding spiral beam as the zero
approximation and therefore the vortex light fields could be formed by means of only the phase mask with the high efficiency of the energy conversion (about 80%). The use of only the phase masks is really important for the qualitative dynamic manipulation. The light fields in the shape of the boundary of a triangular, square, Archimedes spiral and other contours were formed with the use of diffractive optical elements on the dichromate gelatin. And the motion of micron-size objects along these trajectories was experimentally demonstrated. The motion was achieved without any mechanical shifts in the system of the laser tweezers, only by means of the transmission of the light beam momentum to the particles. Note, that the spatial distribution of the transverse component of the momentum is determined by spatial phase structure of the light field.

The use of LC SLM HOLO EYE 1080 in the system of the laser tweezers opens up the new capabilities and advantages for the dynamical laser manipulation. The experiments with vortex fields formed by means of the LC SLM were carried out.

The frames illustrating the particles movement along two concentric rings formed with the vortex axicons are shown in Figure 5. The phase distribution needed for the forming of the respective intensity distribution is presented in Figure 6. One can see that the particles movement along the ring-shape trajectory is realized due to the transmission of the light beam momentum to the particles, and this is consistent with the phase distribution of the formed vortex fields (Figure 6.). Since the topological charges of the inner and exterior rings have the opposite signs (in other words, the vortex twists of the light fields are oriented in opposite directions for the inner and exterior rings), the particles move in opposite directions along the inner and exterior rings. Going back to Figure 3 you can see that the particles were trapped within the maximal intensity region, while the movement along the trajectory was not observed during that experiment since the used light field was not vortex.

**Figure 5.** The particles movement along the inner and exterior rings of the vortex optical traps according to the phase distribution (for example, the particle “a” moves clockwise and the particle “b” moves counterclockwise) (Media 3).

**Figure 6.** The phase distribution.

The motion of microobjects of various diameters (0.46 µm, 1.2 µm, 3.2 µm) along the Archimedes spiral was carried out. The light field was formed by means of the LC SLM. The laser power was 140 mW, Archimedes spiral’s radius was 8.2 µm and light line width (for half the intensity value) was about 1 µm. The results of the angular velocity motion (ω) dependence (at the second spiral turn) on the particles size (D) are presented in Table 1.
Table 1. The dependence of the angular velocity of the motion (ω) on the particles size (D)

| D, µm | ω, degree /second |
|-------|-------------------|
| 0.46  | 25±3              |
| 1.2   | 37±4              |
| 3.2   | 27±2              |

It is seen from the table that the maximal angular velocity of the motion was observed for the spheres of 1.2 µm size. This fact could be explained by the following. For the particles of the diameter less than the beam width the amount of the light beam angular momentum transmitted to the particles increases as square of the particle diameter (D²) due to the increase of the sectional area of microspheres. At the same time the force of the viscous drag increases proportionally to the diameter (D). Thus the increase of the microspheres diameter to the size close to the spiral turn width results in the increase of the microspheres angular velocity. For the case of the particles with the diameter more than the width of the light line forming the spiral, a different dependence is observed. The particle sectional area intercepting the light field increases approximately as the first degree of the particle diameter (D). Therefore the particles of this size and more have to move with the equal velocities. A certain decrease of the velocity with the increase of their diameters observed within the given size range could be explained by the increase of the friction force on the substrate. This force is proportional to the mass of the particle i.e. to the third degree of the diameter, D³. Besides, the microobjects oscillations in the transverse plane were observed during their motion along the trajectory. It is the result of the Brownian motion effect due to the thermal motion of liquid molecules, since the particles are very small and the experiments were carried out at the room temperature. The influence of the Brownian motion is especially appreciable for the particles of the submicron size.

It should be noted that generally the rate of movement depends on the geometrical parameters of particles, their refraction indexes and the environment, as well as the laser power and light field spatial structure. The comparison of the experiments with the use of the dichromate gelatin masks and those with the use of the LC SLM demonstrates that in the case of the light fields formation by means of the LC SLM it is possible to reach the increase of the particle velocity (about by 3 times) due to the adaptable capabilities to form the light field thoroughly.

The advantages of the use of multielement modulators in laser manipulation are the capability of the fast load of the phase distribution on LC SLM array. It is resulted in the abilities of the fast change of the motion direction and dynamical control of the trajectory form. In Figure 7 the change of the motion direction of the 1.2 µm diameter latex spheres with the load of the phase distribution with direct and invert phase on LC SLM array is presented. Also the change of the form trajectory of the particle’s motion from Archimedes spiral to triangle form was realized.
5. Conclusion

Several methods for generating various traps and dynamic control of the manipulation process are presented in this study. The use of the vortex fields in the form of curves makes it possible to move the micro-objects along these curves. The light field formation with the help of the phase LC SLM permits to produce a universal set-up capable of a dynamic change of the traps types, including a single-point trap, point traps arrays, vortex traps in the form of curves. The results obtained can be used for a further development of new technologies in biophotonics and micromechanics.

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6. References

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