Optical trapping and arrangement with reconfigurable “bottle” beam for digital holographic microscopy

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Abstract. The design and construction of optical tweezers based on uniaxial crystal anisotropy for generation of adjustable “bottle” beam trap carrying optical vortex with orbital angular momentum is considered. In coupling with digital holographic microscopy, optical trapping becomes a high precision instrument for visualization, shape definition and refractivity measurements of isolated microstructures and biological objects in-situ. The non-destructive and sterile non-contact tweezing of specimens or their parts in localized intensity minima of coherent vortex beam was performed with using of 200 mW semiconductor 532 nm trapping laser and LiNbO₃ crystal. Visualization and position control of trapped marine centric diatoms was performed by a lens-free axial digital holographic microscopy in liquid medium.

1. Introduction

One of the fundamental objects in the field of singular optics is an optical vortex, which has unique properties of maintaining continuous zero intensity on the phase uncertainty line and has ability to carry out its own orbital angular momentum, which is applicable in practice. These properties of singular beams are widely used in the interaction of the electromagnetic field with matter, in particular, high-resolution microscopy and in devices for trapping and manipulation of microparticles [1-3]. Optical traps have been used in biological and medical research, for example, in studying the mechanism of RNA polymerase movement during the synthesis of new DNA molecules [4]; the interaction of neutrophils with pathogens has been studied [5]. In [6] infrared optical tweezers were used to capture and manipulate red blood cells for cleaning of the blocked microvessel inside the subcutaneous capillaries of live mice, in such a way, this research significantly expanded the use of optical tweezers for studying the dynamics of living cells in animals. Special attention is paid to the laser tweezers and femtosecond scalpel manipulators for noncontact microsurgery laser-mediated polar body and biopsy of early mammalian embryos [7]. All mentioned above researches implement two-dimensional trapping in fluid media, thus the development of methods for three-dimensional manipulation in any surroundings become an actual task of modern research in optics and its application to microbiology, biophotonics and medicine.

Recently, optical tweezers with feedback have been used to apply arbitrary potentials to a colloidal particle. The feedback trap detects particle position, calculates the force based on the imposed “virtual potential” and shifts the centre of the trap to generate the desired force [8]. In 2018, it was demonstrated that with using of spatial light modulators it is possible to simultaneously form a large number of optical tweezers, and using them in a real time makes possible to perform various kinds of
manipulations with position of trapped particles or living cells [9] and to capture absorbing particles for position stabilization and orientation, also to control over several specimens simultaneously [10].

The searching for ways of forming optical structures carrying a stable three-dimensional tweezers is in focus of attention of many research groups. The using of singular beams as optical traps expands the range of their application to the gaseous medium. In such environments, the trapping becomes much more complicated in comparison to capturing in liquids, especially due to the uncompensated gravitational force and significant impact of forces associated with the thermal interaction of the captured particle with the gaseous medium. The advantages of optical trapping is in non-invasive, sterile interaction, with no special specimen preparation requirements, but using of conventional microscopy for visualization of trapped objects imposes restrictions and eliminates some of them.

In optical microscopy, the question of creation of compact imaging devices with noncontact trapping of studying object become a research challenge. Especially, in recent years, a growing number of publications indicates an interest to that question in biophotonics and medical applications due to the limitations of widely used conventional light microscopies with lens systems. Since most of the specimens are transparent, from the point of view of optics – a phase objects, it makes difficulties in applying of bright-field light microscopy or requires more sophisticated technological solutions based on confocal, dark-field, polarization or interference microscopy [11-13]. Such equipment has complex optical systems with lenses and polarization elements, prisms etc., which brings to the image, in addition to useful, also negative qualities: aberrations and wavefront distortions. Moreover, it takes up a lot of space and has special requirements for operation, which limits its using in research field, where the specimens has linear dimensions which doesn’t exceed a 100 μm or limited position in space. In particular, more effective and versatile analytical devices are needed when studying the behaviour of specimens in their natural environment, such as atmospheric aerosols [14], the dynamics of the movement of marine plankton [15], cellular processes [16], where it is not always possible to transfer them into the microscope stage [17].

In combination with optical trapping by “bottle” beams, which, in contrast to trapping with gradient forces, allow moving the specimen within a few centimetres, the using of conventional microscopy would be difficult for observation of objects due to the shallow depth of field and relatively small numerical aperture. In this case, the optimal solution is the choice of digital holography as a method of imaging [18]. Digital holographic microscopy is used as a non-destructive and contactless method and allow performing visualization with only one holographic image with numerically focus adjustment in a range much larger than a mechanical tuning in optical microscope.

Thereby, optical trapping in liquid and gaseous medium allows contact-free observation of particles under relevant conditions and provide access to manipulation over long period of observation time, while the digital in-line holographic microscopy provides height resolution maps of spatial particle positions. In this paper, we show the flexible ways to control “bottle” beam trap parameters by tilting of birefringent crystal which cause the dependence of the loss of a symmetric field pattern in the focusing region on the efficiency of the optical trap. By adjustment of crystal angular position, the shape of the trapping zone can be controlled with high accuracy. Visual observation of captured specimens was performed with in-line digital holographic lens-less microscopy.

2. The “bottle” beam generation for optical trapping

Three-dimensional optical trapping can be performed using a “bottle” beams, a unique property of which is the maximum of the light intensity surrounds the central region which is “empty” from the light energy [19]. Once in such a beam, the absorbing particle is retained in its axial region, and under certain conditions cannot penetrate through the walls of bright light. Any shift of a particle leads to the heating of its part that is farther from the centre of the beam. The resulting pressure difference on the cold part of the particle closest to the centre of the beam and the hot peripheral returns the particle to a position of stable equilibrium inside the light beam with the “bottle” shape in sagittal section. Currently, great interest to singular optics is caused by studies of propagation and transformation of the laser beams structure after anisotropic crystals [19-22]. It arose due to the fact that anisotropic
medium makes it possible to form not only the arrays of phase and polarization singularities in the beam, but also to control their shape and mutual arrangement. It is known [23] that the structure of a circularly polarized Gaussian beam propagating along the optical axis of a crystal changes in such a way that an optical vortex with a double topological charge appears on its axis in its orthogonal polarized component.

One of the highly efficient methods for generating a bottle beam is the method, based on the use of a birefringent crystal. Gaussian beam passing along of crystal optical axis represents a superposition of ordinary and extraordinary beams having different radii of the wave front curvature [19]. When outgoing beam is focused by a lens located at a certain distance from the exit face of the crystal, three focus areas will be formed: the first focus corresponds to the ordinary beam, the second one to the extraordinary beam, hence, a zone between them corresponds to the field on the output face of the crystal. Such a structure has the form of a classic “bottle” beam with a minimum on the axis, evenly surrounded in all coordinates by a high intensity zone.

Let us consider the effect of focusing a Gaussian beam propagating at a small angle to the optical axis of a uniaxial crystal and the formation of a “bottle” beam. The distance between two foci will depend in this case on the orientation of the optical axis of the crystal and the direction of beam propagation \( \delta(\phi) = L \sin^2 \phi (n^2_e - n^2_o) / (n^2_e n_o) \). Positions of foci are given by \( z_o \) and \( z_e \) that corresponds to ordinary “o” and extraordinary “e” beams respectively. When the inclination angle of the Gaussian beam is changed with respect to the optical axis of the crystal, the patterns of the intensity and polarization distributions begin noticeably change [24]. The distance \( \delta \) between the two foci is not permanent and varies with the inclination. The calculated dependence of the distance between two foci on inclination angle of the crystal is illustrated in Figure 1.

![Figure 1. Computer modulated dependence of distance between foci \( \delta \) on angle \( \phi \) of tilt of optical axis of uniaxial crystal with respect to the beam axis.](image)

From analysis of the plotted curve, we may notice the dependence of the distance between two beam waists on the inclination angle of the crystal optical axis relatively to the z-axis. It can be seen that while angle increases, the distance \( \delta \) gradually decreased. When the angle is 5 degrees, ordinary and extraordinary beams are focusing in one plane. With further increasing of inclination angle, the beam waists change places with a further increase in the distance between each other. Moreover, when the inclination angle is more than three degrees, the axisymmetric picture is completely destroyed, therefore, such angles are not acceptable for rapping. Overall beam shape in sagittal section is shown in figure 2 (b) where two foci is separated by the distance \( \delta(\phi) \).

In our experiment we used diode laser with wavelength of \( \lambda = 532 \) nm, illuminating the uniaxial crystal of LiNbO\(_3\) with \( n_o = 2.2863 \), \( n_e = 2.2030 \). The crystal was mounted on \( \theta \) rotational plane (with step of rotation 0.35 deg) and initially aligned along the beam axis (\( \phi = 0 \) deg). Laser beam was focused by the lens \( L (f = 25 \mathrm{mm}) \) as illustrated in Figure 2 (a). The state of the intensity distribution in the beam waists can be changed by the second polarizer and a quarter-wave plate, cutting out orthogonally circularly polarized field components at the exit of the crystal. Microscopic objective MO\(_2\) focuses the beam in a cuvette filled with a suspension of specimens in water.
3. Digital holographic microscopy and image processing of trapped specimens

There are lot of modifications and optical schemes of digital holographic microscopes [25, 26], including combination with optical traps, where the trapping beam also served as an object beam for recording a hologram [27]. The advantage of using a single emitting source, in turn, limits the ability to control holographic recording, in particular, to change the wavelength of a light source, the optical scheme, etc. In addition, the using of such a scheme is impractical when large volume of media with specimens is recording on a digital hologram.

In this section, we demonstrate the possibility of using digital holographic microscopy with optical traps with discrete coherent light sources, which enables avoidance of mutual interference. For our task, we restrict ourselves to the axial recording scheme proposed by Gabor [28], built on a lens-less principle using a coherent radiation source and a pinhole [29-31].

The intensity distribution in the plane of the hologram can be described mathematically, using complex algebra. In this case, we calculate an intensity as a result of the interference of two waves is represented as follows [32]:

\[ I = |o_1 + o_2|^2 = |o_1|^2 + |o_2|^2 + o_1^*o_2 + o_1^*o_2^* = I_1 + I_2 + 2\sqrt{|I_1I_2|}\cos(\varphi_1 - \varphi_2) \]  

where \( o_1 \) and \( o_2 \) are the complex amplitudes of interfering waves, \( o(x, y) = o(x, y)\exp[-i\varphi(x, y)] \). \( o^* \) – complex conjugate, represent the initial phase of the wave. \( \varphi_1 \) and \( \varphi_2 \) represent the initial phase of the wave. Therefore, the optical intensity depends only on the phase difference.

In the process of recording a digital hologram, a specimen with a light scattering surface is located at a distance \( d \) from the CCD camera as shown in Figure 2 (a). To obtain the object wave of the test sample in digital holography, a numerical calculation of the optical field propagation in the form of a product from the hologram plane \( o_2(x, y)I(x, y) \) to the object plane \( (\xi, \eta) \) is used.

Restored Diffraction Field \( Q(\xi, \eta) \) in the image plane \( (\xi, \eta) \) at a distance \( d \) from the hologram plane can be represented in the paraxial approximation as follows [33-35]:

\[ Q(\xi, \eta) = \frac{1}{i\lambda d} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} o_2(x, y)I(x, y)dx dy \exp \left[ i \frac{\pi}{\lambda d} \left( (\xi - x)^2 + (\eta - y)^2 \right) \right] \exp \left( \frac{2\pi}{\lambda} d \right) dx dy \]  

Intensity \( I(x, y; d) \) and phase \( \varphi(x, y; d) \) of reconstructed images can be obtained from the complex field \( Q(\xi, \eta) \) calculated at a distance \( d \) using the following relations:

\[ I(x, y; d) = |Q(x, y)|^2, \quad \varphi(x, y; d) = \arg(Q(x, y)) \]
In digital holography the reconstruction of the object wave field is performed numerically by simulating diffraction process with using scalar diffraction theory. We used free open source software ImageJ with implemented angular spectrum approach for numeric reconstruction of digital holograms.

The principle of the in-line configuration is that the laser light, passing through sample, splits into the two optical fields: diffracted by the sample and reference beam, without phase perturbations. As depicted in Figure 3 (a), a modified digital holographic recording set-up carrying 532 nm diode laser Ls emits coherent beam into the input of pinhole of 25 µm in diameter. The chamber C with spacemen is located on the translation table. The set-up base BS enables rotation of a whole scheme around the chamber for adjustment of imaging plane. The hologram of rapped objects was recorded by CCD and after applied reconstruction, the image of specimen has been received as shown in Figure 3 (b, c).

![Figure 3 (a, b, c).](image)

(a) The sketch of rotational scanning holographic microscope: Ls is the 532 nm diode laser, PH – 25 mcm pinhole, C is the cuvette with the specimen, Dr is the motorised drive of rotational base stage BS and CCD – camera with 3 MPix resolution. Trapping laser beam is shown by red limes. The image of optically trapped objects represented as: digital hologram of marine centric diatoms in-situ (b) and their reconstructed image (c).

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The advantage of lens-less digital holography is an object can be placed close towards the CCD matrix, which increases the numerical aperture and improves resolution. For this purposes CCD is mounted on the movable along z-axis optical cage, in such a way we can change the distance between cuvette and imaging plane.

The experimental results of “bottle” beam trapping applied to marine centric diatoms is illustrated in Figure 4. Two frames sequentially depict the mutual positions of specimens before (Figure 4, a) and after trapping and spatial displacement (Figure 4, b). Red arrows show the direction of movement.

Figure 4 (a, b). (a) The numerically reconstructed digital hologram of three diatoms in water, trapped by “bottle” laser beam; the cropped frame to 50×50 mcm was extracted from original hologram with dimensions of 4.30×3.50 mm. The configuration of the traps can be changed using crystal adjustment and chosen particles had been displaced (b) by movement of the cuvette.

4. Conclusion
In our research, we present an analysis of the capture options of micron-sized objects by an optical trap with real-time tunable properties. Moreover, in addition to the implementation of optical trapping, optical manipulations in three coordinates together with digital holographic microscopy make it possible to study various isolated particles with great flexibility. Also, the loss of beam field symmetry in the tweezing region depends on the efficiency of an optical trap which may be used for selective...
trapping microparticles of different mass and shape. Digital holographic microscopy allows to scan the cuvette in various depths of space from only one shot and precisely define specimen localisation. Experiments are able to record the evolution of the trapped particles in their natural environment, their size and shape in real time.

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