Diffractive optical tweezers in the Fresnel regime

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Abstract: We demonstrate a flexible setup for holographic steering of laser tweezers in microscopy using a high resolution spatial light modulator (SLM). In contrast to other methods, hologram read-out is done in the off-axis Fresnel regime rather than in the typically used on-axis Fourier regime. The diffractive structure is calculated as a Fresnel hologram, such that after reflection at the SLM only the desired first diffraction order is guided to the input of an optical microscope, where it generates a tailored optical tweezers field. We demonstrate some advantageous features of this setup, i.e. undesired diffraction orders are suppressed, the optical traps can be easily steered in real-time by just "mouse-dragging" a hologram window at the SLM display, and a number of independently steerable optical traps can be generated simultaneously in a three-dimensional arrangement by displaying a corresponding number of adjacent hologram windows at the SLM screen.

OCIS codes: (140.7010) Trapping; (090.1760) Computer holography; (170.420) Optical confinement and manipulation.

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1. Introduction

Laser tweezers and related optical trapping methods have become valuable tools in various fields of applications in microscopy, e.g. for trapping or manipulating cells and cell components, for the measurement of interaction forces, or in the assembly of micro-structures. A detailed overview over the various applications is given in [1]. A recent advance in the flexibility of optical tweezers systems was achieved with commercially obtainable high resolution spatial light modulators (SLMs). Using the established methods of diffractive optics, they allow to to create optical traps in various shapes [2, 3, 4, 5], whole arrays of traps [6, 7, 8, 9, 10], or three-dimensional trapping structures [11, 12], which can be changed at video rate or even faster [7].

In most of these applications a laser beam is diffracted from a hologram which is displayed at a high resolution SLM. Then the diffracted beam is guided by some optical components to the inlet of a microscope, and is finally focused in the microscope object plane to form the pre-calculated optical field. Typically, SLM read-out is performed in the Fourier regime. There the SLM displays a phase pattern which basically corresponds to the Fourier transform of the desired light field distribution in the microscope object plane. If the SLM is then illuminated with a plane wave, a Fourier transform of the displayed phase pattern is projected into the object plane of the microscope, corresponding to the holographically generated optical tweezers.

However, there are some disadvantages of such a setup, resulting from interferences of the desired first order diffracted beam with other diffraction orders, mainly the zeroth order (i.e. the undiffracted beam) and the minus first diffraction order. These other diffraction orders result from a limited diffraction efficiency of SLMs. A major restriction is the so-called fill factor, i.e. the *active* area of each SLM pixel normalized by the *total* pixel area. The diffraction efficiency cannot exceed this fill-factor which can be rather low. Another constraint consists in the limited phase addressing capability of the SLM pixels. In order to obtain maximum diffraction efficiency in the desired first order, “blazed” phase holograms are required, i.e. each pixel of the SLM should be able to control the phase of a reflected (or transmitted) light wave in a continuously addressable range between 0 and 2π. Particularly in the near-infrared range the total achievable phase shift is often smaller, or in certain types of SLMs, phase addressing can be performed only binary, resulting in the appearance of undesired diffraction orders. In a typically used Fourier setup all of these diffracted beams are simultaneously focused in the microscope object plane. The zeroth order (i.e. the undiffracted light) focuses as an intense spot in the center of the microscope object plane, whereas the minus first order there produces an inverted copy of the calculated optical field. In the case of purely binary holograms, where the intensity of conjugate diffraction orders is always identical, this limits programming of optical tweezers fields to only symmetric geometries [7].

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In this paper we present an advanced optical setup which avoids these limitations and additionally increases the flexibility of diffractively steered laser tweezers. In optics such a setup is denoted as an off-axis Fresnel hologram, whereas typically used setups are using on-axis Fourier holograms.

2. Optical tweezers using Fresnel holograms

A sketch of our optical setup is displayed in Fig. 1.

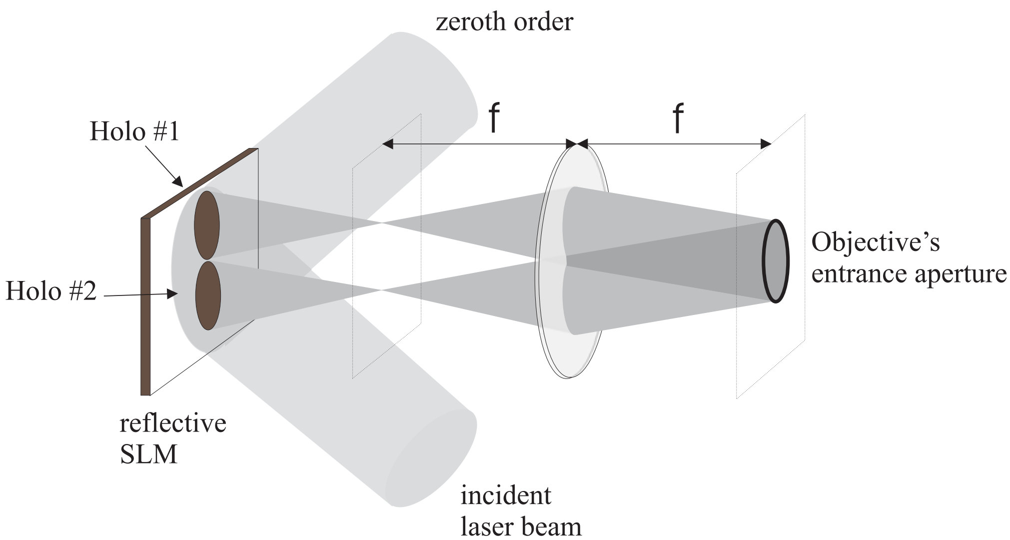

Fig. 1. Experimental setup for diffractive steering of optical tweezers. A high resolution (1920 x 1200 pixels) reflective spatial light modulator (SLM) is illuminated by an expanded collimated laser beam. At the SLM, a number of image windows displaying computer-designed off-axis holograms is presented. Only laser light diffracted from these holograms into the desired first order is guided by a lens to the rear input aperture of a microscope objective. There it is used to trap particles in different kinds of advanced optical traps.

For creating the optical trap we use a continuous wave Ytterbium fiber laser which emits a linearly polarized pure TEM_{00} mode at a wavelength of 1064 nm out of a single mode optical fiber, with up to 5 W adjustable power. Holographic steering of the beam is performed by reflecting it at a SLM, i.e. a liquid crystal phase modulator (Holoeye, LCR3000) with a pixel size of $10 \times 10 \mu\mathrm{m}^2$ and with a very high resolution of 1920 x 1200 individually addressable pixels. The SLM works like a computer monitor at 60 Hz video rate, i.e. it uses a second monitor output of a computer graphics card as its input driver, and it just displays a copy of the image at the computer monitor, however converted into a continuous phase pattern. At the display one can open several adjacent windows containing independent hologram patterns, which can be manipulated under mouse-control like ordinary windows on a computer monitor. An example screen-shot displaying six hologram windows, which produce four focused spots in different focal planes, a "cogwheel" mode (upper right window [17]) and a doughnut mode (upper middle window) is displayed in Fig. 2. On the SLM display the 8-bit gray values depicted at the computer monitor are presented as 8-bit phase values, controlling the phase of a reflected light wave. At our wavelength continuously adjustable phase shifts in a range between 0 and 1.5 π are obtained. Our maximal obtainable absolute diffraction efficiency, i.e. the ratio of light diffracted into the desired first order as compared to the incident light, is approximately

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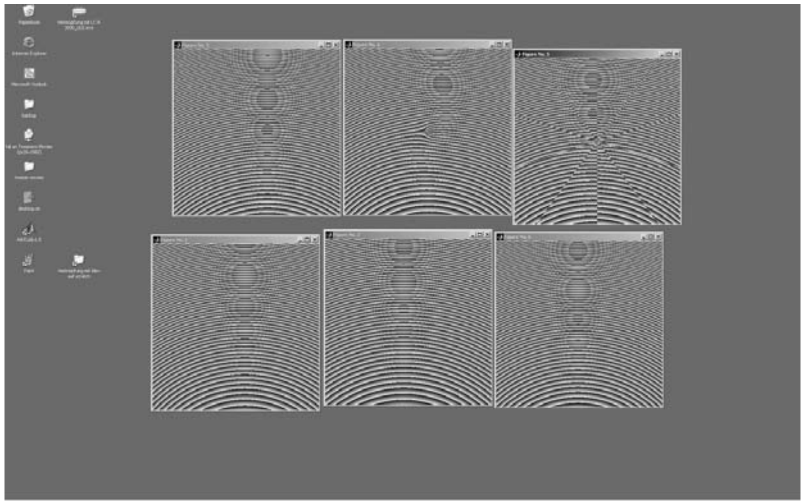

Fig. 2. Examples for holograms displayed at the SLM for producing a “cogwheel” beam, a “doughnut” beam, and four single optical traps in different focal planes. The holograms are directly displayed as pictures on the computer screen, and can there be moved by mouse-dragging. The positions of the corresponding optical traps in the object plane of the microscope follow these mouse movements instantaneously.

40%. It is limited by the restricted phase addressing range, furthermore by an approximately 20% absorption of the liquid crystal material and the reflector behind the display, a fill factor of 90%, specular reflection from the cover glass plate of the SLM (approximately 8%), and probably by electronic phase noise superposed over the mean phase value of each pixel. Using a diffraction efficiency of 40% for holographic optical tweezers usually results in a disturbance of the pre-calculated light field by undesired diffraction orders.

In our case we avoid the appearance of other diffraction orders by hologram read-out in the Fresnel regime. For computing such a hologram, we first calculate the phase pattern $P_{Fourier}$ for the reconstruction of our desired tweezers field (see e.g. [12]). Algorithms for this calculation are based on the “kinoform” principle [13], or on adaptive iterative algorithms (e.g. [10]). First, the two-dimensional Fourier transform of the finally desired field distribution is calculated. Then the amplitude of each pixel of this complex array is normalized to one, keeping just the complex phase angle. Finally, the phase angle is truncated modulo 2π. As a result one gets a “conventional Fourier hologram” $P_{Fourier}$, i.e. if this pattern is displayed as a phase modulation (i.e. $\sim \exp(iP_{Fourier})$) at a suitable spatial phase modulator and reconstructed with a plane wave, the desired field distribution is holographically reconstructed on-axis in the far-field, or in the focal plane of a succeeding lens. These so-called Fourier holograms are typically used in holographic optical tweezers setups.

Such a hologram can be transformed into an off-axis Fresnel hologram $P_{Fresnel}$ with some advantageous features explained later. Technically this is done by mathematically superposing a focussing lens phase term and, additionally, an inclined plane term to the calculated hologram. For this purpose the original Fourier hologram $\exp(iP_{Fourier})$ is transformed as:

$$\exp(iP_{Fresnel}) = \exp(i[P_{Fourier} + \pi(x^2+y^2)/f_F\lambda + (G_x x + G_y y)]\mathrm{modulo}2\pi) \qquad (1)$$

There, λ is the laser wavelength, x and y are the coordinates of the SLM plane (measured from the center of the SLM), f_F is a free parameter defining the focal length of the Fresnel lens term, and G_x and G_y are components of a grating vector $\vec{G}$ which determines the direction and the magnitude of the off-axis diffraction angle. The final operator "modulo 2π" reduces the phase range which has to be addressable by the SLM to an interval between 0 and 2π, without changing the diffraction efficiency, similar to the mode of operation of a blazed grating. If such a hologram is read out with a plane wave, then the focal length parameter f_F defines the distance from the display where a real image of the calculated tweezers field is reconstructed in the first diffraction order. In Fig. 1 this is indicated for the simple case of two adjacent holograms which are both calculated to reproduce a single focussed point, i.e. the holograms consist only of a Fresnel lens term superposed by an inclined plane term. In the focal plane, the zeroth order (i.e. the specular reflection of the plane wave from the SLM display) remains a plane wave, whereas the minus first diffraction order (not indicated in the Figure) is diverging. The direction of the beams diffracted in the first diffraction orders, and their diffraction angles $\pm\theta$ are selectable by the grating vector components G_x and G_y of the additionally superposed inclined plane term, i.e. for small diffraction angles we get $\theta \approx \pm \mid \vec{G} \mid \lambda/2\pi$.

In our setup, an intermediate lens is placed such that its front focal plane coincides with the focal plane of the Fresnel lens displayed at the SLM, whereas its rear focal plane matches the entrance aperture of the microscope objective at its rear focal plane. Consequently, in the rear focal plane of the intermediate lens, a Fourier transform of the desired tweezers field is reconstructed as a real image (in this case again a plane wave), which is finally converted by the objective lens to the desired optical tweezers field in the object plane of the microscope.

This setup straightforwardly allows to suppress the undesired negative and zeroth diffraction orders, by choosing a sufficiently steep inclined plane term, such that the individual diffraction orders are separated far enough so that only the desired first diffraction order is coupled through the intermediate lens to the input of the optical microscope. Even if some residual light from the undesired minus first and zeroth order might be captured by the aperture of the intermediate lens, this does not disturb the optical tweezers field in the microscope object plane, since there only the first order diffracted beam is focused, whereas all other orders are defocussed.

Fig. 1 also indicates that beams diffracted by the two adjacent holograms displayed at the same SLM both can be fully coupled through the microscope aperture, although incident at different angles. The reason basically is that the intermediate lens performs a Fourier transform of the light field between its front and rear focal plane, which is shift invariant, i.e. lateral shifts in one Fourier plane do not convert to lateral shifts in the other Fourier plane, but rather to a linear phase offset, corresponding to a directional change of the beam. Thus the two beams indicated in the figure focus at different positions in the microscope object plane. The beam diameter D at the microscope inlet depends linearly on the diameter H of the hologram displayed at the SLM, and on the focal length f of the intermediate lens, i.e. $D \approx fH/f_F$. Thus the setup can be optimized for a certain hologram window size, to be completely coupled into the rear aperture of the microscope objective. In our case we chose $f_F = f = 30$ cm, such that beams diffracted from holograms with a diameter of 400 pixels (corresponding to H=4 mm) are fully coupled through the 5 mm rear input aperture of the microscope objective.

If such a hologram window is shifted in the SLM plane by Δr (e.g. by just "mouse-dragging" the window to another position at the screen), this results in a change of the incidence angle $\Delta\vartheta$ of the diffracted beam at the rear microscope aperture, i.e. $\Delta\vartheta \approx \Delta r/f$. This change in the incidence angle finally converts into a lateral change Δd of the position of the tweezers field in the microscope object plane, given by: $\Delta d \approx \Delta\vartheta\, f_{Obj} = \Delta r\, f_{Obj}/f$, where f_{Obj} is the effective focal length of the microscope objective. In our case we use an oil immersion objective with a magnification of 100 and a numerical aperture of N.A.=1.3, and an effective rear focal length of $f_{Obj} = 1.27$ mm, such that a shift of the hologram window by $\Delta r = 1$ mm (100 pixels) translates into a lateral shift of approximately 4.2 microns in the microscope object plane. Consequently,

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if a 400 × 400 pixel hologram window is shifted across the longer edge of the 1920 × 1200 pixel display, i.e. over a distance of 1520 pixels (15 mm), the position of the reconstructed optical field in the microscope object plane changes by 65 microns, corresponding to almost the whole field of view imaged by our CCD microscope camera. This gives a very convenient tool for flexible movement of the optical trap without re-calculating the holograms. Since the SLM screen displays a copy of a computer monitor image, it is possible to move the optical trap in the microscope object plane in real time (at video rate) by "mouse-dragging" the corresponding hologram window at the computer monitor. Using the whole size of the computer screen allows displacements of the optical tweezers over the whole field of view of the microscope. Such an experiment, demonstrating arbitrary movements of two beads (with diameters of 10 μm and 7 μm, respectively) in different focal planes is demonstrated in an included mpeg-movie.

Furthermore, with this setup it is possible to display a number of adjacent hologram windows at the SLM screen which reconstruct their corresponding tweezers fields at adjacent positions in the microscope object plane. As an example, the light intensity distribution in the microscope object plane produced by the six holograms of Fig. 2 is displayed in Fig. 3. The image was

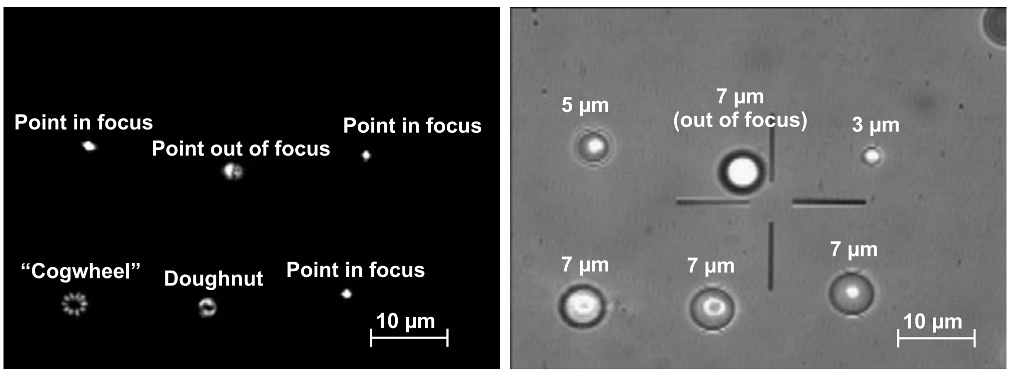

Fig. 3. Left: CCD image of the light intensity distribution in the object plane of the microscope, generated by the six holograms displayed in Fig. 2. The lower left "cogwheel" shaped intensity distribution results from a superposition of two counter-propagating doughnut modes with a helicity of 5, the ring-shaped intensity distribution (lower, middle) corresponds to an optical doughnut beam generated by the upper middle hologram in Fig. 2, whereas the other holograms each reconstruct a single optical focus at a different position. Right: Six micro beads (diameters indicated in the figure) trapped simultaneously in the 6 light traps generated by the 6 holograms. The upper middle bead is trapped in another focal plane than the other beads to demonstrate the feasibility of 3-dimensional steering of the holographic tweezers. A mpeg-movie which demonstrates movement of two beads in different focal planes by mouse-dragging the corresponding hologram windows at the computer monitor in attached in "beads.mpg" (2.2 MBytes).

taken with a CCD microscope camera and displays the reflection of the laser beams at the glass coverslip of the object chamber. The lower middle light field corresponds to a doughnut mode with a helical pitch index [14] of 5 generated by the upper middle hologram window of Fig. 2. The lower left image shows a "cogwheel"-shaped intensity distribution which is generated by the hologram at the upper right edge of Fig. 2. Such a laser mode corresponds to a coherent on-axis superposition of two counter-rotating doughnut modes (with a helicity of 5). The other holograms reconstruct spots focussed in different focal planes, each capable of trapping a single bead in the corresponding position. Note that the zeroth diffraction order, which often appears as an intense spot in the center of the field of view (e.g. in [2]) is completely suppressed, and that no contributions from the minus first order (which would result in "ghost-images" appearing at

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a point symmetric position with respect to the center of the image) are obtained.

The right side of the figure shows 6 beads with different diameters (indicated in the figure), trapped in the 6 corresponding light fields. The beads were trapped stably with the same trapping force constant as obtained in single beam optical tweezers of the same light intensity. The upper middle bead is trapped in another focal plane than the other beads, indicated by its slightly out-of-focus appearance, in order to demonstrated the 3-dimensional trapping capability of the setup. At a total laser power of 2 W illuminating the complete SLM display, each bead could be moved at an arbitrary path across the whole field of view by "mouse-dragging" the corresponding hologram window across the area of the SLM display. The speed was only limited by the monitor refresh rate, i.e. dragging of a bead across a distance of 60 microns (corresponding to the field of view) could be achieved in about half a second. Using a size of 400 x 400 pixels for each hologram window it was possible to display up to 12 independent hologram windows. Each of these windows could contain a different hologram, i.e. each individual hologram could display a whole array of focused spots, or it could create a special mode of light like a doughnut mode of a certain helicity, or a Bessel beam.

It was also possible to project two optical traps at the same position, although the corresponding holograms at the SLM screen did not overlap. This was possible by superposing the individual holograms with different inclined plane terms [see Eq.(1)], i.e. with different grating vectors $\vec{G}$.Using this method it is for example possible to grab a small object at different positions with different optical tweezers. It is also straightforward to trap different beads at different axial positions (i.e. at different axial distances from the objective lens) by superposing the corresponding holograms with lens terms [see Eq.(1)] of different focal lengths f_F. Thus a number of beads can be trapped in a three-dimensional arrangement, and moved in each plane individually.

Finally, we can create different types of doughnut modes with different pitch angles and with different signs of their helical pitches simultaneously using different holograms. These doughnut modes transfer orbital angular momentum (i.e. a bead which touches a doughnut mode moves tangentially along the doughnut ring) with a magnitude and rotational direction depending on the magnitude and the sign of the helical pitch index, respectively. If such a set of counter-propagating doughnut rings is arranged in an adequate way (such that adjacent counter-propagating doughnuts are almost in contact with each other), they can act as passive optical pumps or "conveyor belts" for the micro beads. Similar experiments have been demonstrated in [15], however, there all of these so-called optical vortices were calculated within one single hologram, which limits flexible rearrangement of the individual vortex positions, since for each modification of a single doughnut mode the whole hologram has to be recalculated.

3. Discussion

We demonstrated an easily implementable setup which has a few significant advantageous features. First, in contrast to typical Fourier holograms, the off-axis Fresnel setup suppresses all undesired diffraction orders, both by separating their directions from the desired first order using an off-axis term, and by defocussing them using a Fresnel lens term with a relatively high refraction power. Whereas beam separation by an inclined plane term is also possible in an (off-axis) Fourier setup, the defocussing and subsequent "dilution" of all undesired diffraction orders is a unique property of the Fresnel setup, exploiting the fact that there the beam divergence of all different diffraction orders (including the zeroth order) is also different. With this method it should be possible to even use only binary addressable light modulators, like the newly developed high speed ferroelectric liquid crystal displays [7], to produce holograms of arbitrary (particularly asymmetric) structures or rotationally directed doughnut modes.

Second, in contrast to a Fourier geometry the Fresnel setup allows to move individual optical tweezers fields by just “mouse-dragging” the corresponding hologram windows (or a whole cluster of grouped hologram windows) at a computer monitor in real time, i.e. without recomputing any hologram. Thus rearranging of microscopic particles trapped in the object plane is as easy as rearranging a corresponding number of hologram windows with a “mouse” on a computer screen.

Third, in contrast to Fourier holograms the Fresnel geometry allows to generate multiple optical traps by just displaying a corresponding number of holograms in other adjacent hologram windows at the SLM monitor. Using the high resolution of advanced SLM displays it is presently possible to display up to 12 independent hologram windows at the same time, all of them displaying individually calculated holograms out of a “hologram-toolbox”. For example, the individual holograms can be tailored to produce doughnut beams of different helicity, Bessel beams [16] or arbitrary 3-dimensional light field distributions. All of these holograms can be additionally computed such that they focus in different planes, thus allowing to construct 3-dimensional arrangements of optical traps. Changing some parameters of the setup it is also possible to increase the number of individual hologram windows (e.g. the diameter of each hologram can be cut in half if the focal length of the intermediate lens is doubled) at the cost of a lower light intensity diffracted by each individual hologram. However, modern fiber lasers have excessive power (up to 100 W emerging from a single mode fiber) such that intensity problems can be compensated. A disadvantage of cutting the SLM display into a large number of individual hologram windows is, however, that the area in the microscope object plane, which can be addressed by each individual hologram, also shrinks. In the limit of a very large number of correspondingly small hologram windows, the reconstructed optical field resembles the image displayed at the SLM. In this limit our setup passes over into a direct projection method. In related experiments such a projection system has already been demonstrated [3, 4]. There, the phase image displayed at the SLM was directly projected into the microscope object plane using a phase contrast setup. While this arrangement offers the same flexibility in redistributing a tweezers array by “mouse-dragging” of individual image pixels, it does not provide the additional advantages of holography, as e.g. to project doughnut modes, or to produce 3-dimensional trapping arrays in the microscope object chamber.

These advantages in combination with the easy implementation of off-axis Fresnel holography increase the flexibility of holographic optical tweezers systems and promise a significant improvement for many of their practical applications.

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