J. Opt. A: Pure Appl. Opt. **11** (2009) 034010 (6pp) doi:10.1088/1464-4258/11/3/034010

Holographic phase contrast for dynamic multiple-beam optical tweezers

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Received 1 August 2008, accepted for publication 27 October 2008
Published 20 January 2009
Online at stacks.iop.org/JOptA/11/034010

Abstract
We propose and demonstrate holographic phase contrast (HPC) as a new method to transfer a spatial phase distribution of arbitrary shape into a corresponding intensity pattern. A powerful application of HPC is the use in optical tweezers to dynamically control multiple traps like arrays or even more complex trapping geometries. Due to the image plane nature of HPC no hologram calculation is required and hence real-time control of complex tweezers configurations is possible. The inherent optical amplification by HPC can improve the fundamental limit in trapping power in optical tweezers that are based on common spatial light modulators.

Keywords: multiple optical tweezers, phase contrast, holographic interferometry, photorefractive two-wave mixing

1. Introduction

The concept of single optical tweezers has been well known for more than twenty years. The immanent intensity gradient of a TEM_{00} laser beam is used to transfer a resulting force to dielectric particles with sizes from tens of nanometers to many micrometers [1]. Since single optical tweezers are relatively easy to implement and powerful in their applications, they have become established as important tools in biological and medical sciences [2].

However, single optical tweezers are limited to the manipulation of one object at a time. Consequently, there have been many concepts developed to allow for manipulation of two or more objects simultaneously. Probably the most obvious approach is taking two laser beams and coupling them into the same optical tweezers set-up [3]. If both beams are prepared separately before they are joined, both traps can be controlled independently. This configuration may be called spatial multiplexing. Another approach is time multiplexing of two or more traps [4]. Here, a single laser beam is deflected by a fast pivoting mirror such as a galvano- or piezomirror or by acousto-optic devices [5]. The beam is placed at the desired trapping position, kept there for a certain time and moved to the next position. Both concepts are subject to certain restrictions. Spatial multiplexing requires one beam per trap to be prepared and thus the effort scales with the number of traps. As a consequence, most optical tweezers realized on the basis of spatial multiplexing are limited to two independent traps. Temporal multiplexing, on the other hand, requires sharing the trapping time and laser power between all traps. Trap stiffness is thus significantly reduced.

Holographic optical tweezers are a very flexible way to create multiple traps [6]. A computer-calculated hologram is placed in the optical path and thereby read out by a reference wave. Commonly the hologram is positioned in a Fourier plane with respect to the trapping plane. The hologram can be designed such that in the trapping plane almost any arbitrary intensity distribution can be achieved. Multiple optical traps in this scenario are only a special case of possible complex trapping geometries. Strong optical tweezers require a high level of laser power in the trapping plane. Consequently, a high diffraction efficiency is mandatory and thus usually phase holograms are used. The required hologram can be produced, for example, by lithography techniques [6, 7]. A far more flexible way is dynamic holographic optical tweezers [8, 9], where the hologram is created by a computer addressable spatial light modulator (SLM). This allows changing trapping geometries without any changes in the optical set-up by just giving a new hologram on the SLM. A drawback of computer-generated holograms is that any local change in the trapping geometry requires the calculation of a completely new hologram. Hologram calculation time thus becomes a serious issue in real-time applications.

In principle, it is possible to simply image an amplitude mask or an amplitude SLM into the trapping plane to generate

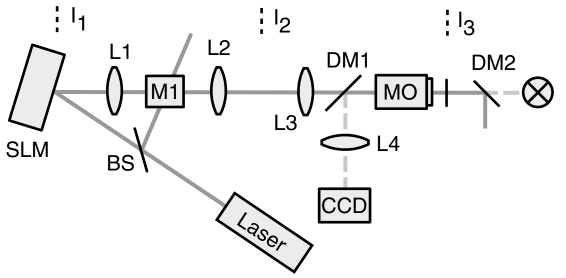

Figure 1. Basic principle of HPC optical tweezers. I_1, I_2 and I_3 are conjugate image planes. SLM, phase-only spatial light modulator; M1, holographic storage material; MO, microscope objective; L1–L4, imaging lenses; DM: dichroic mirrors; CCD, video camera.

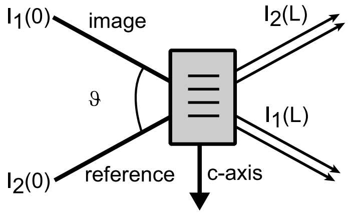

Figure 2. Sketch of holographic interferometry in a photorefractive medium. Two beams with intensities $I_1(0)$ and $I_2(0)$ are incident on the medium with an angle ϑ. $I_1(L)$ and $I_2(L)$ are the intensities after propagation of a distance L in the medium.

the desired intensity distribution. This obvious approach would eliminate the necessity for hologram calculations. However, typical trapping configurations require small areas of high intensity (the traps) and large areas of low intensity (the background). Consequently most laser power would be absorbed by the amplitude mask or the modulator. This results in a very low efficiency and requires lasers with a very high output power. Eriksen *et al* [10] proposed the use of a generalized phase contrast method for multiple-beam optical tweezers. This approach relies on a computer addressable phase-only SLM. The crucial point in the difference from dynamic holographic optical tweezers is that the SLM is not placed in a Fourier plane with respect to the trapping plane. Rather, the SLM is directly imaged into the trapping plane. In contrast to dynamic holographic optical tweezers, the transfer of the phase distribution on the SLM to a trapping intensity distribution is not done by optical Fourier transformation. Phase contrast [11] is utilized to perform this conversion. Image plane methods in general do not require any hologram calculation, but the desired trapping geometry is given directly on the SLM. Consequently, direct imaging methods are well suited for any real-time trapping task, with a time resolution only limited by the refresh rate of the SLM. Furthermore the concept is not limited to multiple-beam traps, but also complex trapping geometries can be generated easily.

Still there is a significant drawback with direct imaging methods. As in any tweezers set-up with an SLM, the SLM is the bottleneck if high trapping force and thus high laser power is required. Direct imaging approaches—as well as holographic optical tweezers—require that all laser power has to pass through the modulator. The maximal trapping power and the maximal number of traps are limited by the reflection or transmission coefficient, the diffraction efficiency and finally by the damage threshold of the SLM.

2. Concept of holographic phase contrast

In this paper we propose a new concept for generation of multiple optical tweezers. This approach relies on optical holography [12] and will be named *holographic phase contrast* (HPC). The principal set-up of HPC optical tweezers is shown in figure 1. A phase-only SLM is illuminated by a laser beam. The beam is then downsized and the SLM imaged by lenses L1 and L2 into an intermediate image plane I_2. At this plane, the conversion from the phase distribution on the SLM to an intensity distribution is already performed, as will be explained in detail. The image plane I_2 finally is imaged into the trapping and observation plane I_3 of the optical tweezers by the tube lens L3. Lens L4 acts as the microscope tube lens and images the observation plane I_3 onto a CCD camera. Two dichroic mirrors DM1 and DM2 are used to separate the optical paths of the tweezers and the imaging part of the microscope by their wavelengths [13].

The most important part of the set-up is the conversion from the phase distribution to an intensity distribution. This is done by holographic real-time interferometry in the material M1 [14]. The material can be a photorefractive crystal, a photorefractive polymer, a photosensitive polymer or any material which allows us to write and then read out a hologram and is self-developing [15]. A photorefractive material has the advantage of flexible writing, reading and erasure of holograms [16]. The principle of holographic real-time interferometry is illustrated in figure 2. Two beams with intensities $I_1(0)$ and $I_2(0)$ are overlaid within a suitable material. The two beams generate an interference pattern, which is stored as a refractive index hologram, for example by the photorefractive effect. The hologram now acts as a Bragg grating and diffracts parts of $I_1(0)$ and $I_2(0)$ in the direction of the other beam with a diffraction efficiency η. As a result, the complete wavefront of each beam is stored in the material and read out by the other beam. If the amplitudes of the incident beams are $\sqrt{I_1(0)}\mathrm{e}^{-\mathrm{i}\Psi_{10}}$ and $\sqrt{I_2(0)}\mathrm{e}^{-\mathrm{i}\Psi_{20}}$ with an intensity ratio $m = \frac{I_1(0)}{I_2(0)}$, the output intensities after the holographic medium are given by [17]

$$I_{1,2}(L) = (1-\eta)I_1(0) + \eta I_2(0) + 2\sqrt{\eta(1-\eta)I_1(0)I_2(0)}\cos\left(\Delta\Psi_0 \pm \frac{\pi}{2}\right). \quad (1)$$

Here, η denotes the diffraction efficiency of the reference hologram stored in the holographic medium. $\Delta\Psi_0 = \Psi_{20} - \Psi_{10}$ is the phase difference between the incident beams. Hence a phase transfer function (PTF) can be given, which states the output intensity of one beam with the relative phase shift $\Delta\Psi_0$ as a parameter. The optimum contrast of the PTF function is given if the intensity ratio of the incident beams is chosen as [17]

$$m' = \frac{I_1'(0)}{I_2'(0)} = \frac{\eta}{1-\eta}. \quad (2)$$

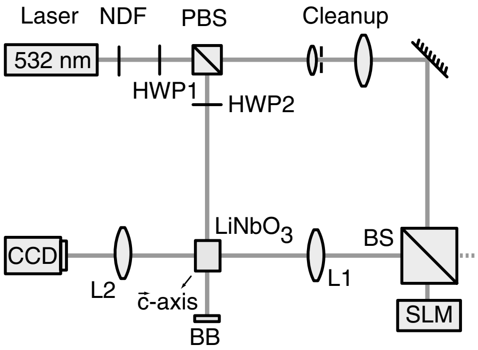

Figure 3. Experimental set-up of holographic phase contrast. L, imaging lenses; $LiNbO_3$, photorefractive crystal; (P)BS, (polarizing) beamsplitter; HWP, half-wave plate; NDF, neutral density filter; Cleanup, beam cleanup and expansion; BB, beam blocker; CCD, video camera.

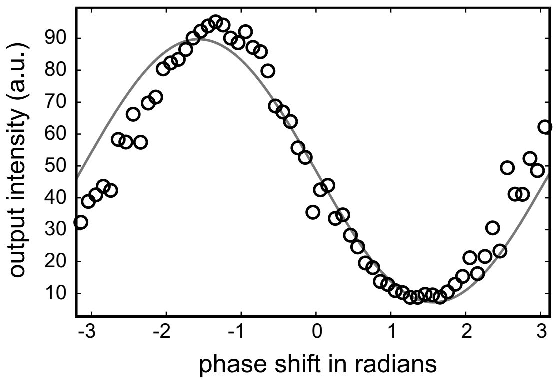

Figure 4. Experimentally determined PTF of the holographic phase contrast set-up (circles) and theoretical curve.

The PTF then has a $\sin^2(\frac{\Delta\Psi_0}{2} + \Phi)$ dependence (cf figure 4), where 2Φ defines a working point, i.e. an offset on the phase axis which can be chosen by an additionally introduced phase shift of one of the beams. It is important to note that there is no constraint on the absolute intensity of one beam at this point, but only the ratio is specified by (2). Therefore, the output intensity can be chosen as required without further restrictions.

3. Experimental demonstration

Figure 3 shows the experimental set-up we use to demonstrate the concept of HPC. We utilize a photorefractive 45°-cut $LiNbO_3$ crystal, since this material enables flexible writing and erasing of volume holograms. The SLM is a commercially available Hamamatsu X8267-16 phase-only modulator, which operates in reflection geometry. As the light source, a frequency-doubled, diode-pumped solid-state Nd:YAG laser, emitting at $\lambda = 532$ nm with an output power of $P = 100$ mW, is used. The combination of the half-wave plate (HWP1) and the polarizing beamsplitter (PBS) enables flexible adjustment of the intensity ratio m. With the neutral density filter (NDF), the total intensity can be set. This set-up allows us to investigate the basic properties of HPC. First, the PTF is determined.

The actual measurement can be divided into two steps. First, a reference hologram is stored. The time constant τ depends on the total intensity used. In our experiments, we use total laser powers of the order of $P = 10$ mW with a spot size on the photorefractive material of about $d = 1$ mm^2. The reference hologram is written for about $t = 5$ min. The set-up is now prepared for the second step, the determination of the PTF. For this purpose, one of the input beams is shifted in phase with respect to the other. Typically, the reference beam is shifted, for example with a piezoelectric mirror [18]. We utilize the convenient fact that a phase modulator is employed anyway, which can perform this task readily. The phase modulator is addressed with a homogeneous phase shift from 0 to 2π, in steps of 0.1 π radians. Figure 4 shows the measured phase transfer function.

The PTF is the basis for volume holographic phase contrast. A phase distribution which generates the desired intensity pattern, that is the trapping geometry, is easily designed. The background is chosen such that its phase shift corresponds to a minimum in the PTF. The traps are chosen such that the phase shift corresponds to a maximum in the PTF. Figure 5(a) sketches the phase mask for a simple five-trap optical tweezers geometry. Figure 5(b) shows the corresponding trapping geometry and figure 5(c) an experimental result, respectively. It is clearly seen that the phase pattern is transferred in a corresponding intensity pattern. The background has a mean intensity of 66 gray values. The intensity spots which correspond to the desired

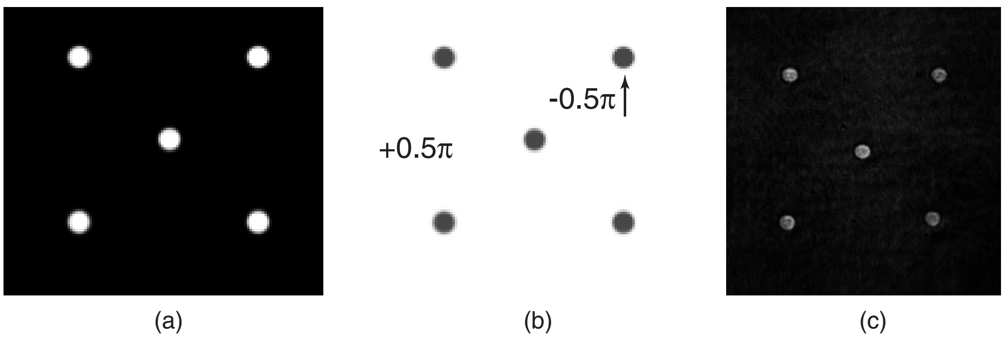

Figure 5. (a) Desired trapping geometry. (b) Corresponding phase mask, where the gray values indicate the relative phase (white: $+0.5\pi$ radians, black: -0.5π radians). (c) Resulting intensity distribution after conversion by holographic phase contrast.

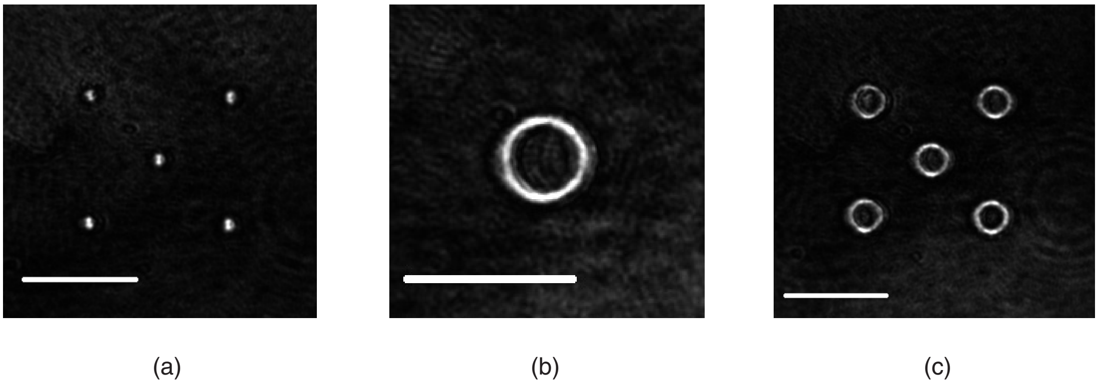

Figure 6. Examples of trapping geometries. Five single traps (a), a ring trap (b) and a cluster of smaller ring traps (c). The scale bar corresponds to 500 μm.

optical traps have a mean of 190 gray values. This implies a ratio of roughly 1:3 and means that a not negligible part of the incident laser power is not used for trapping. It is important to understand that this is not a conceptional problem of HPC. The PTF in figure 4 results in a ratio of better than 1:10, defining an approximate limit of the current experimental set-up. If lower values are desired, a better suited holographic material is required as will be discussed in detail in the next section. The theoretical limit to the ratio is zero, since the dark background is the result of destructive interference which obviously can be total if the interfering intensities are chosen to be equal.

The most obvious use of HPC is the generation of multiple traps (figure 6(a)). It is interesting to mention that there is no limit in principle—except for the SLM resolution—to the number of independent traps. This is due to the fact that energy is coupled into the traps from the reference beam. Other phase contrast methods require all intensity to pass the SLM and intensity is redistributed from dark to bright areas. As a consequence the intensity per trap reduces with increasing number of traps [19].

Figure 6(b) shows another example, a circular trap. This configuration can be used to confine high index objects to the ring or to enclose low index particles inside the circle. Furthermore, the ring can be filled with high index particles to enclose an object inside, which has an index of refraction very close to the surrounding medium. Of course it is easy to chose a different size for the circles and arrange an array to generate several of these traps. It has to be emphasized that these are only simple examples and any arbitrary trapping geometry can be realized.

4. The optimal holographic material

Photorefractive $LiNbO_3$ is suited for the proof-of-principle of the HPC concept, due to its high flexibility. A hologram can be written easily and erased afterward. However, in optical tweezers' applications a material is desired in which a hologram can be written and afterward read out without erasure. Many different concepts are suitable. For example, a wide range of fixing techniques [20, 21] allow us to preserve a hologram written in photorefractive materials. Another option, which is attractive in view of commercial production, is the use of self-developing photosensitive polymers [22]. These materials allow us to write a hologram which develops itself or by using short homogeneous illumination and can be read out without any losses afterward. It is very convenient that the requirements for the optimal material for HPC are identical to the requirements of low-cost, write-once read-many (WORM) holographic media, which currently are investigated intensively due to their importance for the consumer market. As a result, there is already a good choice of suitable materials available, which probably will increase even more.

With the optimal volume holographic material, HPC will be very easy to use. The material is simply placed in the optical path without the need for very accurate alignment, as is required, for example, for the phase plate in other phase contrast set-ups. The signal beam is then overlaid inside the material with the reference beam and the reference hologram is written while the SLM is addressed with a homogeneous relative phase shift of 0. This has to be done only once, before the tweezers are used for the very first time. After that the tweezers can be used without any further modifications in the set-up.

The optical quality of the created trapping geometry is essential for high-fidelity optical tweezers. A comprehensive review of the optical properties of holographic media and their influences on the image quality is outside the scope of this paper and can be found in the literature, e.g. [23]. In summary, there are materials with excellent optical qualities available which enable almost aberration-free trapping geometries with very high image quality.

5. Performance considerations

SLM-based concepts for generation of multiple-beam optical tweezers can be assorted in holographic and image plane methods. Both concepts usually rely on phase-only spatial light modulators in order to minimize absorption losses by the SLM and exploit the major part of the laser power. Holographic optical tweezers generally require relatively time-consuming calculations to generate the phase distribution that corresponds to a desired trapping geometry. In image plane methods such as HPC or generalized phase contrast [10] the SLM generates a phase distribution which is transferred by a phase contrast technique into an intensity distribution. This intensity distribution corresponds to the final trapping geometry and is imaged into the trapping plane of the optical tweezers. HPC shares the principal advantages of other image plane methods in comparison to holographic optical tweezers [24]. In particular, there are no time-consuming calculations required to generate a specific trapping geometry. Any desired geometry can be created in real-time, neglecting response times of the controller unit and the SLM. In this context, it is not a contradiction that writing the reference hologram in HPC may take a longer time—depending on the material and laser power—of the order of seconds to minutes. The reference hologram is written only once before the tweezers' set-up is used the first time. After that, the reference hologram is merely read out, which happens instantaneously and does not add any delay in the phase contrast process.

There is one significant difference between HPC and other image plane or holographic methods. While most methods require all laser power to pass through the spatial light modulator, in HPC the laser power which is used to trap objects has to pass through the modulator only partially. This difference addresses one of the main limitations of all modulator-based optical tweezers concepts. The modulator usually is the bottleneck if high trapping forces and a larger number of traps are required, because its damage threshold is an unavoidable limit to the maximal laser power. With HPC a part of the laser power is passed by the modulator. This advantage scales with the number of traps. In the case of only a few traps, HPC will perform similar to other image plane methods. However, with increasing numbers of traps, the available power per trap in other approaches diminishes, because laser power is redistributed from areas without traps to areas with traps. This is less efficient the more traps are desired [19]. The available power per trap in HPC does not scale with the number of traps. The maximum power is constant, in the case of one as well as in the case of hundreds of traps, since laser power is coupled to the trap by the reference beam. The homogeneity of the intensity distribution between different traps of the same intentional force depends solely on the homogeneity of the SLM illumination. In particular, no ghost traps or traps with varying trapping force occur, as is often the case in holographic optical tweezers [25].

6. Conclusions

We have demonstrated holographic phase contrast, a promising method to generate multiple optical tweezers, dynamically and in real time. HPC shares the basic advantages of other image plane concepts and addresses the fundamental drawback of any optical tweezers approach which involves spatial light modulators. SLM always are the bottleneck, especially if many optical traps with a good trapping force are desired. HPC uses two-beam interference and thus allows one to pass a significant part of the laser power by the SLM, thereby avoiding the bottleneck.

Acknowledgments

The authors would like to thank the Deutsche Forschungsgemeinschaft for the financial support of this project in the frame of the priority program SPP 1147 and the German–Chinese transregional research project TRR61.

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