Advantages of Holographic Optical Tweezers

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ABSTRACT

In the last decade optical tweezers became an important tool in microbiology. However the setup becomes very complex if more than one trap needs to be moved. Holographic tweezers offer a very simple and cost efficient way of manipulating several traps independently in all three dimensions with an accuracy of less than 100 nm. No mechanically moving parts are used therefore making them less vulnerable to vibration. They use computer-generated holograms (CGHs) written into a spatial light modulator (SLM) to control the position of each trap in space and to manipulate their shape. The ability to change the shape of the optical trap makes it possible to adapt the light field to a specific particle shape or in the case of force measurements to adjust the trapping potential. Furthermore the SLM can be used to correct for aberrations within the optical setup.

Keywords: optical tweezers, micro-manipulation, multiple traps, LCD, OASLM, light modulator, SLM, CGH, computer-generated holograms

1. INTRODUCTION

Stimulated by the work in the area of cooling atoms the first schemes of using light for trapping microscopic particles were used in the 1970s. In most of these experiments two laser beams were used to accomplish a stable trap. In 1986 Ashkin et al. realized a single beam gradient force optical trap and coined the name “optical tweezers”. The trapping of particles by a single beam is possible due to the gradient force caused by the refraction of the light. The gradient force pulls the particle in the direction of maximum intensity. If the light’s focus is above the particle it is drawn in a direction opposite to the incoming light’s propagation direction. It is therefore possible to get an equilibrium between the gradient force and the scattering force which pushes the particle along the light’s propagation direction. The amount of gradient force is determined by the amount of light that hits the particle under a high angle.

The possibility to trap particles with just one beam made an easy integration into microscopes possible. Using a microscope allowed to move the trapped particle by simply moving the microscope’s table (e.g.). An alternative way to accomplish the movement is the use of galvano mirrors. More sophisticated optical tweezers were built by using acousto-optical modulators (AOMs) in combination with mirrors. The mirrors are used for the coarse movement and the AOMs (one for each direction) for the fine movements.

Multiple traps which can be moved independently were achieved in several ways. AOMs can be used to scan the laser beam over the different trap locations and interrupt the beam in between. Fallman et al. built a setup which was able to move two traps independently in three-dimensions. It uses two light paths with a galvano mirror and a movable lens each. However this setup is difficult to upgrade to more than two traps. Optical tweezers were used in many microbiological experiments. A good survey is given for example in.

Holographic tweezers offer the possibility to create multiple traps which can be moved independently with a much less complicated setup. Furthermore new features are possible. In section 2 the basic principle of holographic tweezers is described. Section 3 deals with the advantages compared to traditional tweezers elaborating on e.g. multiple traps, aberration control and the precision of the position control . The article closes by concluding the advantages and giving some ideas for further development.

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2. HOLOGRAPHIC TWEEZERS

In 1991 He et al.\textsuperscript{7} introduced a static hologram in a optical tweezer setup for the first time. The hologram transformed the Gaussian-mode of the laser into a doughnut-shape mode realizing a ring of light with a dark centre. This allowed the trapping not only of transparent particles but also opaque ones in the dark centre. Instead of using static holograms our group used a liquid crystal device (LCD)\textsuperscript{7} as a spatial light modulator (SLM). This made a computer-controlled dynamical change of the hologram and consequently the generated light field possible.

2.1. Basic Setup

Figure 1 shows the basic setup of the holographic tweezers. A transparent LCD is illuminated by a laser. A personal computer (PC) is used to control the input to the LCD. It calculates the desired hologram, which is displayed on the LCD. The LCD is used as a phase modulator. The hologram is reconstructed in the sample plane by the microscope objective which serves as a Fourier lens creating the Fourier-transform of the hologram. The hologram reconstruction represents the optical trap. The trap can be moved by rotating the hologram or by changing its grating period respectively. In principle the hologram information can be renewed with the LCD refresh rate of about 30 Hz.

Figure 1. Basic setup for holographic tweezers. L1 and L2 is the beam expander, L3 and L4 is the telescope which fits the beam to the microscope objective, DS is the dichroic beam splitter and MO denotes the microscope objective. L5 is the tube lens and CF a colour filter.

In general the use of electrically addressed LCDs has the drawback of blocking part of the incoming laser power by the opaque driver electronics on the panel. To make things worse this pixelated mask resembles a cross-grating generating unwanted diffraction orders. In the Epson LCD we used in our experiments, 56% of the impinging laser power is lost due to the fill factor of 44%. The loss in unwanted diffraction orders plus the loss caused by absorption left only 15% of the power usable for the hologram. The Epson LCD we used was demounted from a commercial data projector. In order to be able to modulate the phase we removed the polariser foil from the panel. Since these panels are not indented for phase-modulation they barely ever have a modulation depth of $2\pi$. When a hologram is written into the display this also causes unwanted diffraction orders in addition to the ones mentioned above reducing the efficiency further to about 9.5%.
2.2. Improved Setup

The drawbacks mentioned in 2.1 gave reason to improve the setup depicted in Fig. 1. Inserting an optically addressed spatial light modulator (OASLM) at the location of the LCD one avoids the diffraction orders caused by the LCD’s pixel structure. This is because the OASLM is a non-pixelated device. However the OASLM needs to be addressed via an LCD which is imaged onto the OASLMs photosensitive area. The imaging needs to be accomplished in such a way that the LCD’s pixel information is lost whereas the hologram information is preserved. This can be done in two different ways. A simple approach is to image the LCD with a little defocus to smear out the pixel information. Alternatively a filter in the Fourier plane can be used.\footnote{~} Figure 2 shows the principle setup of such a device. In a distance of the focal length \( f \) after the lens \( L_2 \) the Fourier spectrum of the LCD appears. It has relatively narrow peaks caused by the LCD’s periodic pixelation. These peaks can be blocked by appropriate filters (P in Fig. 2) so that only the hologram information remains.

![Figure 2](image-url)

**Figure 2.** Improved setup for holographic tweezers. \( L_2 \) images the illuminated LCD onto the OASLM. This represents the path of the write-light. The LCD works in amplitude contrast, so a analyser A is needed. The pinhole P filters out the LCD’s pixel structure. The read-light comes from the \( \text{Ar}^+ \) laser which is reflected by a dichroic mirror inside the optically addressed spatial light modulator (OASLM). The other parts of the setup are described in Fig. 1.

Due to the homogeneous nature of the OASLM the losses caused by the LCD’s pixelation are reduced significantly. Furthermore the OASLM we used (Jenoptik SLM–O30) offers a near 2\( \pi \) modulation at a wavelength of 488 nm. Compared to the setup described in Sect. 2.1 about 53% of the incoming light goes into the desired diffraction order of the hologram.

3. ADVANTAGES OF HOLOGRAPHIC TWEEZERS

Using a SLM or combinations of SLMs like the one described in Sect. 2.2 offers substantial advantages compared to conventional optical tweezers. Some of these advantages shall be demonstrated in this section. The experiments were conducted with the setup shown in Fig. 2. Except that instead of eliminating the LCD’s pixel structure by Fourier–filtering we applied a slightly defocused imaging. An \( \text{Ar}^+ \)–Laser at 488 nm was used. This choice was guided by availability and the ability of the OASLM to modulate 488 nm with a modulation depth of 2\( \pi \). For biological applications a wavelength in the near infrared is certainly more desirable. The particles we trapped were 1 \( \mu \)m silica spheres (MicroMod GmbH, Rostock, Germany). These particles can serve as a model for biological cells.
3.1. Multiple Traps

As already stated in the introductory section 1 there are, apart from the holographic tweezers, three methods for generating multiple traps which can be moved independently of each other. With two AOMs, one for the x- and one for the y-direction, one is able to scan the beam in the sample plane. However the deflection is often too small which makes an additional deflection mirror necessary. Furthermore a particle movement along the z-direction is not possible. A second approach requires one light path for each trap. In each path there are two mirrors responsible for the x- and y-movement of the trap and a movable lens, which accomplishes the movement along z. A third method uses generalised interference contrast to trap and move several particles in the plane. Using a holographic principle multiple traps can be generated by superposing the holographic information of each trap in the hologram. The information about the position of one trap – the grating period and rotation angle of its hologram – is added by complex addition to the other traps’ holograms to constitute a single hologram by taking the phase of the complex result. To move one trap its holographic information is changed and added again to the hologram of the other traps. In principle a very large number of traps can be generated this way. However there are several limiting factors. The limited spatial bandwidth given by the number of pixels available in spatial light modulators is one restriction. If the spatial bandwidth is too small for a given information the reconstruction will deteriorate. It is not possible to relate the number of pixels to the maximum displayable traps since for example a trap that has a large deflection consumes a higher spatial bandwidth due to the high grating period it has.

The fact that the light modulators we use are phase-mostly modulators reduces the quality of the reconstruction further. However for many holograms this effect is not very significant and the use of optimisation algorithms like IFTA or simulated annealing circumvents this problem. Despite these limitations the holographic approach to generate multiple traps is very successful. Figure 3 shows an example of trapping twelve 1 μm silica particles in a triangle shape. This was accomplished with the setup shown in 2. Compared to the setups which rely on one light path per trap this setup is extremely simple.

3.2. 3D-Manipulation

In we showed that it is also possible to move particles along the z-direction with this holographic approach. This is done by multiplying a Fresnel lens to the hologram of a trap to be moved along z. The additional phase

*the OASLM also has a limited resolution of about 40 lp/mm
term is given by

\[ \Phi(x, y) = \frac{2\pi}{\lambda} \left( \frac{x^2 + y^2}{2f} \right). \]  

(1)

Here \( \Phi \) denotes the phase, \( \lambda \) is the wavelength and the focal length is given by \( f \). The maximum shift in focal-length shall be approximated for the setup given in Fig. 2. With a pixel pitch of 42 \( \mu \)m and a wavelength of \( \lambda = 488 \) nm we get a maximum deflection angle of \( 1.410^{-3} \) rad. The telescope in our setup increases the angle to its eightfold. The microscope objective we use (Zeiss Achromplan 1.0 W) has a focal length of approximately 1.6 mm. This gives a maximum lateral deflection of \( d = 74 \) \( \mu \)m. Calculating where the marginal ray crosses the optical axis gives an estimate of maximum change of focal-length.

For our setup we calculated a maximum focal shift of about 179 \( \mu \)m. However the focus at this position would be very aberrated.

The sequence of images shown in Fig. 4 demonstrates the axial movement of a 1 \( \mu \)m silica particle. The moved particle is blurred because it moves out of the focal plane by approximately 10 \( \mu \)m.

![Figure 4](image)

**Figure 4.** Three dimensional trapping. The particle moves out of the focal plane and is blurred.

### 3.3. Light field engineering

A further important advantage shall be demonstrated in this section. Using holograms in combination with SLMs it is not only possible to dynamically control the position of the optical trap but also its shape. Reasons for doing so are manifold. Simpson et al.\(^7\) showed that doughnut–shaped light fields are able to trap high–index particles\(^4\) in the dark centre of the beam. Since trapping along \( z \) requires rays with a high angle of incidence doughnut beams are favourable. Compared to Gaussian beams doughnut beams have a better ratio of rays hitting a particle under a high angle than those hitting it centrally showing a more efficient trapping\(^7\). For biological applications this is important since the sample is damaged by a high power density. It might also be favourable to shape the light field in order to spare certain parts of a biological sample. More complex light fields can be generated by using optimisation algorithms like the iterative Fourier transform algorithm (IFTA), simulated annealing (SA) or genetic algorithms.

The optical tweezers are also used to measure pico-newton forces. If an external force tries to pull a particle out of the trap it is opposed by the traps restoring force. Since the force potential depends on the incident light field it should be possible to adaptively change the trapping potential by changing the incident light field distribution.

\(^4\)particles with refractive index higher than their surroundings
3.4. Precise Control

The holographic tweezers are able to move particles in three dimensions completely without any mechanically moving parts. This feature allows high reproducibility as well as high accuracy. The position of the trap is controlled by the periodicity of the blazed grating written into the SLM. However, since the LCD is pixelated and the number of independently addressable phase levels is 8 to 16 rather than 256\(^1\), the blazed grating in the LCD is deviated from the desired one. To investigate the limit in position accuracy we simulated this effect, assuming eight independently addressable grey levels. The smallest deflection of the trap (relative to the central zeroth diffraction order) is caused by a single sawtooth. This sawtooth is approximated by one pixel at the edge of the panel set to the minimal grey value. Increasing the deflection does not cause any deflection until a second pixel can be set to the minimal grey value. The position of the trap in the approximated case was calculated using a discrete Fourier transform. By increasing the desired deflection and comparing it with the maximum of the resulting Fourier transform we received the curve displayed in Fig. 5.

![Figure 5](image)

**Figure 5.** Effects of pixelation and limited number of addressable phase levels.

It can be seen that for small deflections the difference between the desired deflection and the one obtained with the LCD is the biggest. It amounts to 18 nm at a desired deflection of 30 nm.

3.5. Aberration Control

Introducing a light modulator into the optical setup opens up the possibility to correct the setup for aberrations. This was done using a method described in\(^2\). The SLM displays two small apertures – similar to Young’s experiment – which move over the SLMs area probing the wavefront. A camera is positioned in the focal plane of a lens detecting the interference fringes caused by the two apertures. The position of the fringe pattern is a measure of the relative phase difference of the wavefront at the two sampled positions. By combining the phase differences of the sampled positions it is possible to get the phase distribution of the wavefront. The aberrations occurring in the wavefront can now be eliminated by writing the conjugate phase into the SLM. We demonstrated the application of this correction method for the holographic tweezers in the following experiment. The microscope objective was replaced by a lens with a focal length of 600 mm. Using the microscope objective the interference fringes would not have been resolved by the camera. It was assumed that the microscope objective is highly corrected and does not need to be accounted for. The wavefront was sampled on a 12\(\times\)10 grid and fitted with a Zernike–fit. The result of the correction is shown in Fig. 6. A correction is necessary partly because of the introduction of the SLM the optical quality of which often does not meet the high optical standards of microscopes.

\(^1\)the characteristic curve maps the 256 grey levels onto a reduced number of phase levels
Figure 6. Two traps without correction hologram (left). These same traps with correction (right). The zeroth order is the top right trap.

In our experiments it proved significant to correct for the aberrations. Only with the aberrations corrected it was possible to trap a particle at low laser powers.

4. CONCLUSION AND OUTLOOK
The holographic tweezers investigated in this article represent a very versatile tool in the field of micro-manipulation. They are able to trap many particles and move them independently of each other in three dimensions. Yet, their setup is less complex compared to traditional optical tweezers. Since the movement is accomplished purely by changing the hologram in the light modulator, they do not require any mechanically moving parts. In combination with the precise computer-controlled change of the trap position by the light modulator this leads to high accuracy and reproducibility of the trap deflection making an automated manipulation possible. It was shown that the accuracy of the light modulator controlled deflection is less than 20 nm. The absence of high-precision actuators also gives the possibility of cost saving. In addition to its abilities in terms of moving particles the holographic tweezers are able to change the light distribution of the trapping light. This makes it possible to adapt the light field to the particle’s shape or certain experimental constraints. Furthermore the introduction of a light modulator to the setup enables us to compensate for aberrations. At this stage our setup works at 488 nm. This is not very suitable for biological samples. Using a light modulator designed for 800 nm we will modify our setup in this respect. The aberration correction was realized only up to the microscope objective. This shall be extended in the future to be able to correct the entire setup including the sample space.

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