Position and Intensity Modulations in Holographic Optical Traps Created by a Liquid Crystal Spatial Light Modulator

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Abstract: The addressing of the liquid crystals in spatial light modulators gives rise to temporal modulation of the phase pattern. Here we investigate the effect of this on the intensity and position of holographic optical traps. We use high-speed camera imaging of the laser spots back-reflected off the sample cover slip to determine the integrated pixel intensity and track the position of a laser spot. Using this method we find that the intensity modulation is 12% for three different trap configurations. We also find that for a single trap steered by 5 mrad (14.3 µm in the focal plane), the angular modulation is 0.35 µrad (1 nm), and that this modulation increases for increasing number of traps. These modulations need to be taken into account when using holographic optical traps for quantitative force measurements. ©2009 Optical Society of America

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1. Introduction

In holographic optical tweezers (HOTs) [1], the trapping laser beam is sent through or reflected off a so-called kinoform [2], a spatial pattern of local phase retardations that changes the wavefront of the laser beam. With the use of a spatial light modulator (SLM) to create the kinoform [3], phase retardations are created with a pixelated liquid crystal (LC) display, enabling real-time dynamic control over an array of optical traps; by choosing the appropriate phase patterns, an arbitrary number of traps can be moved in three dimensions. We have recently shown that HOT traps maintain constant trap stiffness when steered over distances of ~20 µm in a sample chamber, a key requirement for using HOTs for quantitative force measurements [4]. However, we also found temporal modulation of the trap positions and intensities. When used to probe dynamical systems, temporal stability of these optical traps is a critical issue. Here, we investigate more specifically the modulation of optical traps induced by the SLM.

Phase-only SLMs, such as the HoloEye 1080P used in this work, modulate only the phase, not the amplitude of the laser light. The LC mode used to obtain this is the so-called electrically controlled birefringence mode [5,6], in which the LC molecules tilt in the direction of an applied electric field, thereby changing the optical path length. The phase shift imposed on the laser light traveling through a pixel depends on the applied field for that pixel; for the HoloEye 1080P, 256 phase levels are available. However, the voltage of a pixel can only be ‘on’ or ‘off’, so different phase levels are achieved by applying sequences of binary bit planes encoding for these phase levels. In the time that the voltage of a pixel is ‘off’, though, the LCs can relax. LC relaxation can result in modulation of the kinoform on the SLM. Because of this, optical traps may show temporal variations in intensity and position with frequencies equal to the refresh rate, the addressing sequence rate, or higher harmonics of these [4]. The relaxation of the LC within a pixel depends on the phase level of that pixel and also, due to cross-talk, on the phase levels of neighboring pixels [5,6]. Therefore, the effect of LC addressing on optical traps will be different for every kinoform.

The HoloEye 1080P SLM, as used in our setup, is controlled as an external monitor with a refresh rate of 60 Hz. The addressing sequence has different settings: the so-called 22:6 setting, with an addressing frequency of 120 Hz and in which all 256 phase levels are available, and the faster 300 Hz 5:5 setting, in which only 192 phase levels can be used [5]. With faster addressing, the LCs have less time to relax and the modulations are therefore smaller [4].

In this paper we investigate in more detail the effect of these modulations on the trap intensity and position for a refresh rate of 300 Hz on our HoloEye SLM. We look at the effect of adding more traps, and of adding a trap in perpendicular direction. For this we image the back-reflection of the laser spots on a cover slip using a high-speed camera. From these images we determine the position and the intensity of the spots as a function of time.

2. Experimental methods

In our holographic optical tweezers setup (Fig. 1(a)), an infrared laser beam (Spectra Physics, J20-BL-106C, 4 Watt CW, 1064 nm) slightly overfills the SLM (HoloEye HEO 1080P, LCOS phase only, 1920×1080 pixels, pixel pitch 8µm). Two telescope lenses L1 and L2 (both f = 250 mm) place the SLM in a plane conjugate to the back focal plane of an infinity-corrected high-numerical aperture (NA) objective lens (Olympus UplanApo/IR, 60×, 1.2 NA,
water immersion). The modulated laser beam slightly overfills the back aperture of this lens. A high-speed camera (PCO, 1200 hs, 1280×1024 pixels, pixel size 12×12µm²) is used to image the trapping plane. For our camera image, 1 pixel corresponds to 53.1 nm in the focal plane of the objective. For more details on the HOT setup, please see [4].

Fig. 1. (a) Schematic of the holographic optical tweezers setup [4]. Kinoforms used to create (b) a single trap, (c) two orthogonal traps and (d) four traps. (For clarity, only 216×216 out of the 1080×1080 pixels of the kinoforms are shown.)

We created one trap by steering the laser beam over 5 mrad by applying a horizontal blazed grating kinoform with 80.6π total phase retardation (Fig. 1(b)), which in our setup corresponds to a displacement from the zero order of 40.3 × 0.354 = 14.3 µm in the focal plane [4]. This trap, positioned at (-14.3µm, 0), was present in all three trap configurations presented here and was the one we used for our intensity and position measurements. In the two-trap configuration the coordinates of the additional trap were (0, 23.5µm) (kinoform is shown in Fig. 1(c)). For the four traps, the additional 3 traps were randomly placed at (6.6µm, 23.5µm), (-16.2µm, -12.8µm) and (9.9µm, 18.8µm) (Fig. 1(d)). An asymmetric configuration was chosen to decrease the possibility of overlapping ghost traps [7].

The intensity modulation of a trap was determined from images of the focused laser light back-reflected off a sample cell cover slip by summing the intensities of all pixels in its vicinity. For the zero-order spot, the ghost trap and the background, a box of 88×88 pixels was used, while for the trap the box was 125×125 pixels.

To track the position of the trap, a home-written LabVIEW program was used for spatial correlation analysis of the camera images [4]. The algorithm first determines the correlation matrix of a template image with the current image. The 7×7 points around the maximum of this matrix are fit with a 2D parabolic function, giving the relative position of the template with sub-pixel resolution. This algorithm has been extensively tested for tracking beads of fixed shape and focus. Here, however, changes in the shape of the spot could reduce the spatial resolution of this tracking algorithm. This has not been investigated.

Fig. 2. (a) High-speed camera images recorded with 0.4 ms between frames. The time range is indicated in (b) by the red arrows. (b) Integrated pixel intensity for areas including the created single trap, the zero-order spot, the ghost trap, and the background. (c) Integrated pixel intensity for the spot in (a), measured for only this trap, with an additional trap, and with three additional traps present (see text for details on the positions). Average background is subtracted from all curves in (b) and (c); lines are a guide to the eye. Phases of plots in (c) are arbitrary, but in (b) are not.

3. Results

Figure 2(a) shows nine consecutive images of the single trap at (-14.3µm, 0), the zero-order spot, and a ghost trap at (-28.6µm, 0). The time between frames is 0.4 ms (frame rate 2500 Hz), so the 9 images cover ~1 cycle in the 300 Hz modulation. The integrated intensities from these (shown between the arrows) and other images are plotted in
position and intensity modulations. The amplitude of the position/angular modulation of a given trap depends on the
predicts a trapped particle variance of 6.4 nm
position modulation of the traps should be considered as angular modulation by the SLM, with distances dependent
earlier studies, trap modulation will be seen to underlie Brownian motion [7]. We should mention that these two
forms of modulation are not strictly coupled: particle variance in an optical trap depends on the shape and power of
the focused laser, while modulation by the SLM results from beam steering. Thus, the values presented here for
4. Discussion and conclusions
We have shown that the temporal modulation of traps arising from LC addressing in our SLM results in both
position and intensity modulations. The amplitude of the position/angular modulation of a given trap depends on the
number of additional HOT traps in a sample. This is not due to a change in intensity resulting from the creation of
additional traps; in separate experiments (data not shown), we found that the position modulation is not strongly
affected by laser power. Instead, it seems that the global change in kinoform required to introduce local traps affects
the modulation of each of these traps, and not in a straightforward manner. For example, even though the two traps
created in these studies are orthogonally located, it is obvious that the modulation in the x-direction is strongly
affected by the presence of a trap in the y-direction (Fig. 3). Factors such as the spatial frequency of the kinoform
may contribute, due to the implication of factors such as phase level [5] and cross-talk between neighboring pixels
[4] in determining relaxation times of each LC pixel. To gain a sense of the importance of this position modulation, the spatial modulation of a HOT trap can be compared to the expected particle variance within a fixed optical trap. The equipartition method \( \langle \frac{1}{2}k_B T \rangle = \frac{1}{2}k_B \langle x^2 \rangle \) predicts a trapped particle variance of 6.4 nm\(^2\) for a typical trap stiffness of \( \kappa = 1000 \text{N}/\mu\text{m} \). The largest modulation-induced variance of trap position observed here is smaller than this value, though only by \( \sim 3\times \). Thus, as found in our earlier studies, trap modulation will be seen to underlie Brownian motion [7]. We should mention that these two forms of modulation are not strictly coupled: particle variance in an optical trap depends on the shape and power of the focused laser, while modulation by the SLM results from beam steering. Thus, the values presented here for position modulation of the traps should be considered as angular modulation by the SLM, with distances dependent on the specific optical train. Further investigation is warranted into how trap locations and number of traps affects the temporal modulation of HOTs, though these preliminary studies indicate that the problem is complex.

Fig. 2(b). Here it can be seen that when the intensity of the trap decreases, the intensities of the zero-order spot
and the ghost trap increase; light is transferred from the desired laser spot to the zero-order and ghost trap.
In Fig. 2(c), the integrated intensity of the (-14.3µm, 0) spot is shown for all three trap configurations. The
modulation with respect to the average intensity is \( \sim 12\% \) for all configurations. As expected, the average intensity of
the trap decreases as the number of traps is increased (for a fixed input laser power). It is unclear why the
obtained intensity modulations are higher than estimated earlier for our setup by a different method (2%) [4]. We are
currently investigating the validity of our assumption that integrated pixel intensity scales with laser spot intensity.
In Fig. 3, the position of the trap is shown as a function of time for all three trap configurations. The position
modulations in both x- and y-direction are obvious, with the underlying 300 Hz modulation clearer in x than in y.
The amplitude of modulation increases for increasing number of traps, from \( \sim 2 \) nm for a single trap to \( \sim 4 \) nm (two
traps) and \( \sim 6 \) nm (four traps). The variances of the modulations are \( \sim 0.2 \) nm\(^2\), 0.7 nm\(^2\), and 2.3 nm\(^2\), respectively.

Fig. 3. (a) x-position and (b) y-position as a function of time of a trap located along the x-axis, for this single trap (top curves), and with one
additional trap on the y-axis (middle), or three additional randomly placed traps (bottom). Traces are offset for clarity, and have arbitrary phase.