Video-based calibration of holographic optical traps

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Abstract. Holographic optical tweezers give the possibility to dynamically generate multiple optical traps in arbitrary numbers and forms. This work investigates the implementation of the step response calibration method for a holographic optical tweezers system. The step response calibration is a promising method which requires the repeated subsequent generation and change of several trap positions for the calibration. It has not been undertaken yet using a holographic setup, despite the fact that using digital holography this necessary dynamic change is easier to achieve than with other setups used so far. We have implemented this method for multiple holographic traps calibration, and investigated new problems which arise due to the nature of the spatial light modulator included in the holographic setup. Some improvements to solve the drawbacks identified have been proposed and successfully implemented. Good results have been obtained for low trap stiffnesses between 0.5 to 3 pN/µm. The calibration of stronger traps was limited by the spatial light modulator response time.

Keywords: Optical traps, force calibration, digital holography, step response.

1. Introduction

The first milestone in the evolution of optical traps was done by Ashkin [1] in 1970. He demonstrated how small refractive particles (several nm to µm) could be confined in space with two counter propagating laser beams due to the radiation pressure and optical gradient forces. In 1986 Ashkin et al. [2] observed an optical trap generated by only one highly focused laser beam, which is today known as optical tweezers. It’s trapping force is based on the momentum exchange between the laser beam and the refractive trapped particle, and has typical values of 1-100 pN. The momentum exchange is a result of the scattering and reflecting of a laser beam by the particle. A high aperture objective is needed to focus the beam creating a stable optical tweezers. Usually the objective lens of a light microscope is utilized to focus the beam, because it gives the possibility to image the object plane with the trapped particles simultaneously.
Optical tweezers are applied in biological research, such as measuring the physical properties of DNA[3] and molecular motors[4]. In many applications the exact force which a trap exerts on a bead needs to be known. The force which actually acts at the trapped object is hard to obtain from theoretical calculations. Many properties such as the particle temperature are hard to estimate so a theoretical calculation of the trapping force is unreliable. Therefore optical tweezers need to be calibrated. A calibration can be done using several methods, where all have in common that the movement of a spherical bead with well known physical properties is evaluated. One group is based on the Brownian movement of a bead in a fixed trap centre, the other group is based on the movement of the bead when the position or amount of traps is changed in order to excite a defined particle movement. Investigating those movements we can

derive the trapping force at each point within the optical trap by different methods. If a bead has a distance of less than its diameter to the trap centre, the relation of distance and trapping force is approximately linear [5]. In this region, the proportional factor of trapping force and particle distance to the trap centre is called stiffness, analogue to the spring constant in Hooke's law.
Many applications make use of more than one optical tweezers at the same time trapping many particles simultaneously[6][7]. Multiple traps at arbitrary positions can be created with a combination of independent laser beams. Or a single laser beam can be split into multiple beams with different methods such as polarization and interferometry. However, with those methods the number of traps is either limited or their distribution is rather inflexible. Another sophisticated method is the high-speed beam deflection to switch the laser beam between a few trap positions at a high frequency. But this method is limited by the switch frequency of the laser beam, because the trapped particles may escape due to the Brownian movement while the laser beam supports the other traps. Also the cost for the necessary hardware of each method needs to be taken into account.
Holographic optical tweezers (HOT) is the most dynamic and flexible way to create an arbitrary amount of traps at any desired position. The laser beam which forms the optical trap can be described by a complex function (complex wave front), where the absolute amplitude represents the laser intensity and the complex phase the oscillation of the intensity.
In HOT setups the complex wave front of the laser beam is modulated in a way, that we find the desired intensity distribution of the laser beam in the object plane which corresponds to the desired optical trap. The coding information is called hologram and is usually displayed on a spatial light modulator (SLM). Because it is possible to change amplitude and phase of the wave front in the sample plane by just modulating the phase on the SLM and a subsequent optical Fourier transform, the amplitude of the wave front is preferred to remain unchanged in the SLM to save as much laser power as possible.
The hologram information can be calculated in different ways knowing the desired trap distribution. Simple trap distributions can be obtained exploiting Fourier transform properties to set the trap positions, and using approximations or random masks to combine several traps in one hologram. More complex distributions can be achieved simulating the light propagation in the setup. When the desired wave front in the object plane is known, it can be propagated backwards virtually so the hologram information is obtained.
Like any other type of multiple optical tweezers, HOT need to be calibrated. With video imaging an arbitrary amount of traps in the field of view can be calibrated at the same time. A calibration method which fits the demands of a holographic optical setup with multiple traps is the step response calibration method proposed by Simmons et al. [8]. The flexible and easy trap generation with holograms suits the needs of the step response method and makes it an interesting alternative for existing calibration methods. However, it has not been implemented for a holographic setup so far. This Master thesis deals with the implementation of the step response method for a holographic optical tweezers setup to investigate the limits of this method. Difficulties and problems which arise due to the limitations of the spatial light modulator used in the holographic optical tweezers setup are discussed and investigated.

2. Video-based Calibration methods

The force profile of an optical tweezers close to the trap centre behaves like the force exerted by a spring, and a trap parameter κ called "stiffness" is used to describe the force profile analogue to the spring constant in Hooke's law yielding $F_{trap} = -\kappa * x$. So the stiffness gives information about the force which is exerted by the trap on a particle at different distances to the trap. By calculations and simulations the trap stiffness can only be estimated roughly, so calibration measurements have to be done in order to calibrate the traps more precisely.
All common calibration methods are based on the tracking of the trapped particles, which means the position of the particle is measured over time. This can be done with video cameras, quadrant photo diodes (QPD) and other position sensitive detectors (PSD). From the movement of the bead the force profile can then be obtained in different ways. The most precise method, which is commonly used for calibration of single traps, is back-focal interferometry, in which an interference pattern between the direct laser light and the light scattered by the trapped

sample is read by a QPD or a PSD to derive the position information. However, this method does not allow multiple trap calibration as a superposed interference pattern of more than one trapped particle is obtained. With video cameras it is possible to simultaneously and independently track an arbitrary amount of particles in the field of view. For this reason, video based calibration is usually applied for multiple trap setups, although typical frame rates (30Hz – 2kHz) of video cameras are much slower than QPDs (>10 kHz) and therefore results are not so accurate. From the acquired videos the position of a particle in every frame can be obtained with tracking software. The movement can then be evaluated with the step response method to derive the traps stiffness.

2.1. Step response method

The step response method is based on the recording of trapping dynamics of beads. By recording the movement of a displaced bead to the centre of the trap, the stiffness can be derived from the kinetic equation[8]. Inside a liquid environment, the movement of a bead in the range of an optical tweezers is described by the power balance of inertial force, the velocity dependent friction force in the liquid and the position dependent trapping force yielding

$$m\frac{d^2x}{dt^2} + \gamma\frac{dx}{dt} + kx = 0\,,$$

where x is the distance of the bead centre to the trap centre, m is the mass of the bead, $\gamma = 6\pi\mu r$ the frictional force constant of the liquid environment with the bead radius r and the dynamic viscosity μ, and κ is the harmonic trap stiffness. For distances x smaller than the bead radius r the stiffness is constant [5][8]. For stiffnesses in the range of several pN/µm and bead radii of µm, the inertial force is negligible so the equation can be simplified to an overdamped harmonic oscillator

$$\gamma\frac{dx}{dt} + kx = 0 \quad \text{with the solution } x(t) = x_0 \exp\left(-\frac{t}{\tau}\right)$$

and $\tau=\kappa/\gamma$. . Hence the distance x between the attracted bead and the trap centre decreases exponentially. This equation holds for a constant trap stiffness i.e. a linear force profile, which is valid when the distance between bead and trap centre is less than half a bead diameter. From a measurement curve the parameter τ can be obtained with an exponential fit, and with the friction constant the stiffness $\kappa=\gamma/\tau$ is derived.

In practice, two different trap positions are needed for the calibration. A bead is trapped in a starting position to obtain the same starting point for subsequent measurements. Then the first trap is shut off and the second trap is turned on respectively so the position of the trap is changed to excite the exponential trapping movement of the bead.

A fast trap change is essential for a correct measurement. Otherwise the bead might leave the well defined starting position due to its Brownian movement before the trap position is changed, so the bead might be attracted from a shifted starting point or escapes. For holographic optical tweezers, the response time of the SLM is a limiting parameter because the trap position is changed with the hologram. So far, research groups used hardware with a sufficient speed like acousto-optic modulators to change the trap within µs [8] [9], while typical relaxation times are in the ms range. The response time of liquid crystal SLMs has typical values of several ms. So additional investigations have to be done to investigate the limits of this setup.

The stiffnesses obtained by this method are compared with the result of the power spectrum method, which is a standard, very accurate and reliable calibration method and therefore used as a reference. Also in this method the only parameter we need to know is the friction constant γ, and for both the step response and power spectrum method the obtained stiffness is proportional to γ.

2.2. Power spectrum method

The power spectrum method[10] is based on the tracking of the Brownian movement of a bead, from which the stiffness in the trap can be derived. The force balance of a trapped bead with thermal energy is given by the Langevin equation of Brownian movement plus the trapping force yielding

$$m\ddot{x}(t) = -k_{trap}x - \gamma\dot{x}(t) + \sqrt{2k_B T\gamma}\,\zeta(t)\,,$$

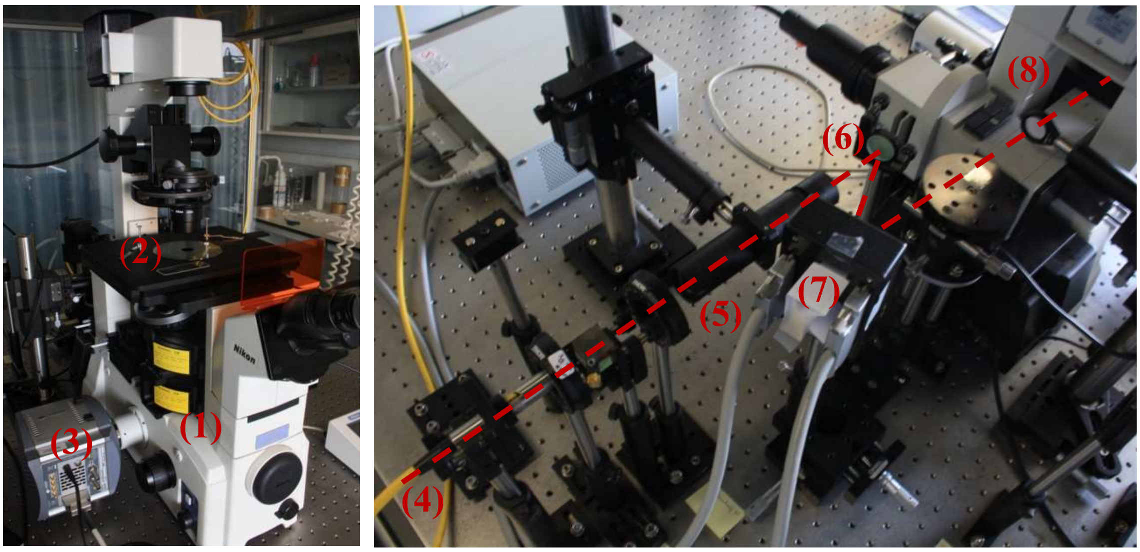
where m is the beads mass, γ the friction constant and ζ(t) the noise term. Solving the equation with a Fourier transform leads to the relation of power spectrum and trap stiffness, which is

$$p^{\exp}(f) = \frac{D^{\exp}/(2\pi^2)}{f_c^2 + f^2} \quad \text{with} \quad f_c = \frac{k_{trap}}{2\pi\gamma}.$$

For frequencies $f < f_c$ the power spectrum is nearly constant. If $f > f_c$ the spectrum shows an asymptotic behavior. In a logarithmic plot, both regions can be fit with a linear function to obtain the corner frequency f_c and hence the stiffness κ. High frequency information is needed from the measurement to obtain a reliable fit in the high frequency region. The sample rate r of the acquisition device is the limit for the high frequency information, and the Nyquist frequency f_{Nqst}=r/2 is the highest frequency information we can obtain from the measurement.
The same high speed camera as for the step response method is used to track the Brownian movement, and a MatLab software developed by Berg-Sorensen et al.[11] is used to obtain the corner frequency in order to calculate the trap stiffness.

3. Optical setup
Before the laser beam can be modulated in the SLM, it needs to be filtered and reshaped to fit the SLM display size. Afterwards a Fourier transform of the modulated wave front is performed by the optical setup to generate the desired trap distribution. An overview of the used optical setup is given in figure 1.

3.1. The laser beam
A Nd:YVO4 fibre laser with a wavelength of 1064nm is used as light source for the optical traps. A collimated beam exits the fibre end and is then expanded through telescope 1 to match its diameter to the SLM display size. From a mirror the beam is reflected to the SLM in a tilt angle to write the phase information in the light wave front. Telescope 2 resizes the beam diameter again to fit the input opening of the microscope objective. Then a dichroic mirror in the optical path between the camera and the microscope objective splits the laser beam and the visible light, reflecting the laser to the objective (which performs an optical Fourier transform) and the visible light in the camera to image and record the trapping movement and Brownian motion. The objective has a magnification factor of 100 and is part of a Nikon Eclipse TE2000E inverted microscope.



(a) (b)

Figure 1. Optical setup: (a) The Nikon Eclipse TE2000E microscope(1) with sample plane(2) and attached high speed camera(3). (b) Optical system with the fibre laser(4), telescope 1(5), mirror(6), SLM(7), and telescope 2(8).

3.2. Spatial light modulator

The SLM is a Hamamatsu X10468-03 liquid crystal on silicon (LCoS) chip with parallel-aligned nematic liquid crystal layer. It modulates only the phase of the beam with 256 gray values (8 bit) on 792x600 pixels, while the amplitude of the wave front remains constant. With no voltage applied, the liquid crystals axis is perpendicular to the incoming light and the phase of the beam experiences the maximum change. No phase change is observed with a maximum voltage applied, so the molecules are parallel to the light propagation. In between those voltages 256 gray values corresponding to 256 phase values can be displayed. A change in gray value corresponding to a phase change from 0 to 2π needs a time of 80ms (fall time). From 2π to 0 the SLM needs only 30ms (rise time), because the liquid crystals move much faster with an applied electric field than through mechanical relaxation.

3.3. High speed camera

The utilized high speed camera Andor X-4137 has a frame rate of about 1kHz if the region of interest (ROI) matches the area of a single trap calibration (ca. 30x30 pixel). By changing the ROI, frame rates between 500 and 1500Hz for a calibration are possible. The camera has a maximum resolution of 128x128 pixels with a pixel size of 24µm.

During the measurements, the cooling fan of the camera needs to be shut down because its mechanical vibration affects the particle movement.

4. Experiment and preliminary results

4.1. Micro-sphere samples

The beads used for both the step response method and the power spectrum are Polystyrene spheres of 2µm diameter, with a standard deviation of 0.05µm. Only the bead size is a necessary factor to know for the trap calibration, and in both methods the measurement result (τ, f_c) is proportional to the bead radius. So the standard deviation of the bead diameter will cause an uncertainty of less than 3% in the trap stiffness. The beads are diluted in distillted water and are confined between two glass cover slips. Table 1 shows the physical properties of the experiment which are needed to obtain the stiffness from the measurement, and typical measurement results for a trap stiffness of 1pN/µm.

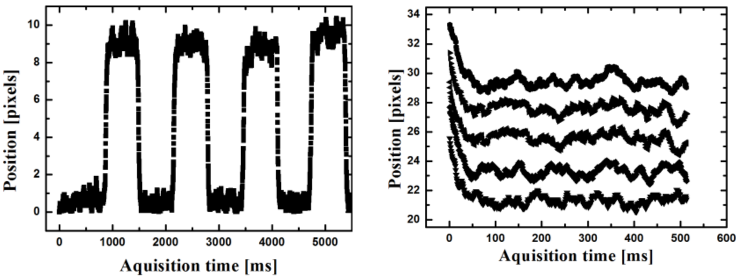
Table 1. Physical properties and typical values.

Property	Value
Bead radius r	1 µm
Dynamic viscosity of water (25°C) μ	9*10e-4 Pa*s
Friction constant γ	1,6964e-8 Pa*s
Trap stiffness κ	1 pN/µm
Relaxation time constant (step response) τ	16,96 ms
Corner frequency (power spectrum) f_c	10,66 Hz

4.2 Particle tracking and data-processing

The particles positions (pixel coordinates in the video) are obtained with additional software (Video Spot Tracker v.6.04) from the acquired video. A nice feature of the step response method is that we can directly derive the parameters of the exponential decay from the pixel values without knowing the pixel to length relation. Since the frame rate r of the high speed camera is very constant, we can estimate the time increment dt between 2 subsequent frames with $dt = 1/r$. With the tracked positions and the time increment we have all the necessary information to derive the stiffness from the measurement results.

In one video, subsequent measurements for the same trap are recorded to reduce the noise of Brownian motion by averaging several measured curves. A self developed MatLab program was used for the data processing. Figure 2 illustrates the steps of the data analysing. First, the software reads and plots the full measurement curve to evaluate the fitting range manually. This is necessary in case a part of the movement is not in the linear range of the power profile. Then the software separates the trapping movements according to the selected calibration distance and finally creates an averaged curve by summing up all single curves. The fit of the averaged curves delivers the step response time τ, and the stiffness κ is obtained with the dynamic viscosity γ from the equation κ=γ/τ.



(a) (b) (c)

Figure 2. Post-processing of the acquired data. (a) The full measurement sequence with subsequent trapping movements, (b) separated trapping movements with exponential decay, (c) averaged curve.

4.3 Holograms

In preliminary investigations of the step response method [8][9] very fast devices like acousto-optic modulators are utilized to change the trap position within micro seconds. In the holographic setup we use a rather slow SLM to display holograms which modulate the laser beam wave front to form the holographic traps.

The holograms are calculated in MatLab and LabView using the Fourier shift theorem. So the laser beam is modulated by a linear phase to shift the position of the beam. A perfectly linear relation of the SLM gray values from 0 to 255 and the corresponding phase modulation of 0 to 2π is assumed in the calculation. In reality, the relation is not perfectly linear, so a non-linearity correction is done to improve the quality of the traps. Also the aberration of the SLM has to be taken into account by adding a negative phase image of the aberration to the hologram. In our setup the effect of the aberration was already minimized by an optimized optical configuration, so no aberration image was finally added to the holograms.

Multiple traps are obtained by mixing holograms with the random mask method. A random mask separates the available SLM pixels in as many groups as holograms have to be displayed. The separation is randomly chosen to avoid side effects in the object plane which occur when the pixels are distributed symmetrically. To generate a multiple trap, first the single holograms for each trap are calculated and then the holograms are put together in the random mask, using only a part of each original hologram.

4.4. Laser intensity response on hologram change

A correct measurement can only be done provided the trap position changes fast enough. While the hologram is changed in the SLM display, a mixture of both the actual and the new hologram is displayed, and as a result of that a mixture of both traps is found in the object plane. If the change is not fast enough, the bead might reach the centre of the new trap before the new hologram is fully displayed. In this case the new trap is not fully generated and is therefore weaker, so the measurement will deliver a lower stiffness with respect to the real stiffness of the fully generated trap.

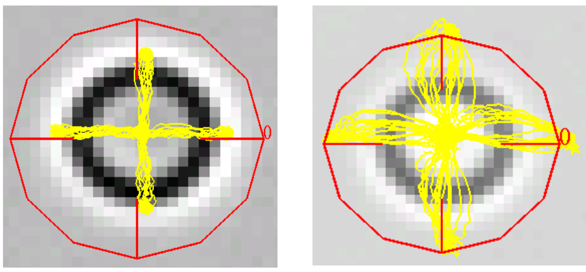
This problem is completely new since the step response method has not been investigated with a holographic setup so far. The following chapter describes how the slow hologram change limits the step response method, and how improvements can be made.

4.4.1 Direct hologram change

The time which is necessary for a trap change is directly connected to the response time of the SLM. If we change from one hologram to another, the slow fall time (80ms) of the Hamamatsu LCoS display is the significant parameter because several pixels will change from the maximum to the minimum gray value and vice versa. An exponential decay parameter τ of 80ms in the

trapping movement corresponds to a stiffness of 0.24 pN/µm. Approximating we need at least half of the bead movement for the fit to reduce the noise, a stiffness of around 0.24 pN/µm would be the maximum stiffness obtainable with this setup. For higher stiffnesses the hologram change is not fast enough, so a lower stiffness would be measured.
In practice the limit is higher, because the bead will not start to move in the same moment when the liquid crystals of the SLM start to move. First, the new trap needs to exceed the old traps force in order to attract the bead. Also not all gray values change from the maximum to the minimum, so a big part of the trap is built up faster than the maximum fall time. With this additional time the limit is above 0.24 pN/µm.

An improvement can be made by simply increasing the calibration distance. The starting position of the bead will not be in the linear region anymore, but the part of the movement in the non-linear region can be simply cut out with the MatLab software, so only the movement in the linear region is used for the fit.
Figure 3 compares the trapping movement of a bead from different starting positions. The yellow line in figure 3(a) corresponds to a bead trajectory with a trap translation of 1µm with the spherical bead in the middle of the picture. Four different starting positions are used, so direction dependencies of the trap stiffness can be investigated. As expected, with a bead diameter of 2µm the maxima of the trajectories touch the beads edge which confirms that the bead moves only within the linear region of the force profile. An increased trap distance of 1,7µm results in the trajectory of 3(b). Here we can observe that the trajectories pass the beads edge and leave the linear region of the traps power profile. The difference in the quality of the movements is obvious. In the case of a calibration from 1µm the trajectories are very straight and reproducible. With an increasing distance the trajectories get curved and more random, or the bead simply escapes the trap and vanishes from the field of view. Measurements at distances of more than 1,4µm lead to unreasonable results for the trap stiffness, so the distance of 1,4µm is the maximum distance where we can obtain a useful trajectory.

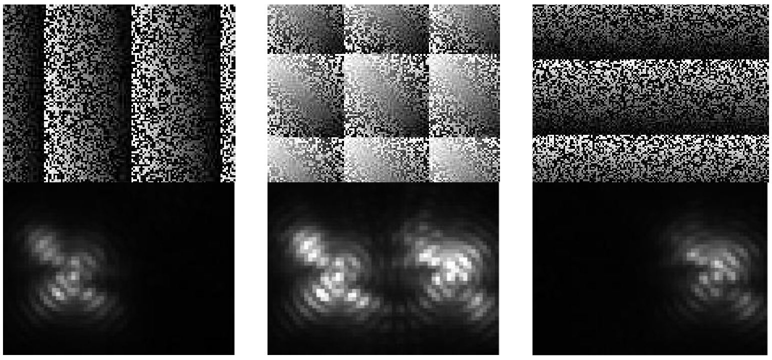


(a) (b)

Figure 3. Trapping trajectories of a bead (yellow lines): (a) the starting position for the bead has a distance of half a bead diameter (1µm) to the trap centre. When the hologram changes, the bead is directly fully trapped by the new trap and moves on a straight trajectory to the 4 starting points. (b) If the starting position is more than a bead diameter away (here 1.7µm), the bead follows a curved trajectory because it is trapped from outside.

4.4.2. Indirect hologram change

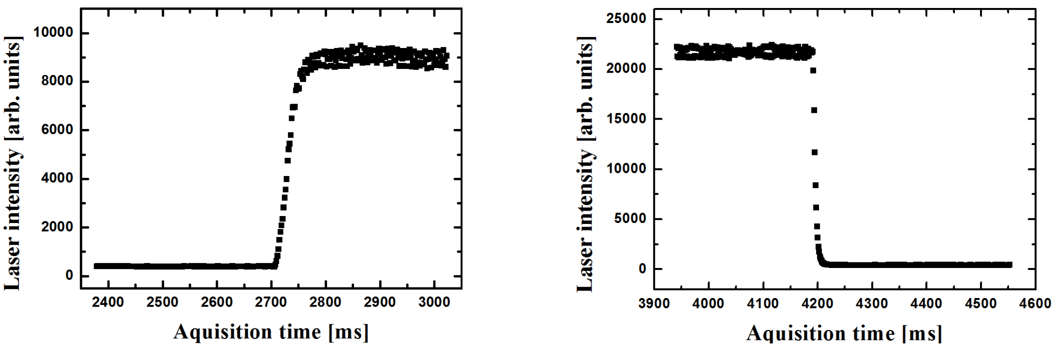
The rise time of the SLM is 30ms, so it is a factor of 2.5 faster than the fall time. We can exploit this fact by not directly replacing one hologram by another, but displaying both holograms at the same time with the random mask method and overwriting one of them by the fast writable value (gray value 0) within 30ms. This is possible because once a bead is trapped, it will not change its position to a neighbouring similar strong trap if the traps are separated by more than a bead radius. Figure 4 illustrates the hologram change sequence and the resulting traps. First the hologram of the helping trap is displayed to fix the bead in the start position. Then a intermediate hologram with the information of both traps is displayed to generate the new trap without moving the bead from the start position. In the last step the pixels of the SLM containing the information of the old hologram are fast overwritten by the gray value 0, so only the new trap remains.



(a) (b) (c)

Figure 4. Developed hologram sequence for a faster trap position change: (a) Half of the SLM pixels display the hologram of the helping trap, the other half has the gray value 0 (black), (b) The 0 values are overwritten with the hologram of the new trap, so both traps are active, (c) All pixels of the helping trap are overwritten with the value 0, so only the second trap, which has to be calibrated, remains.

A comparison of the laser intensity variation during a direct and indirect hologram change is made in figure 5. Graph 5(a) corresponds to the indirect hologram change and shows the increasing laser intensity at the new trap position as a function of time. It needs about 25ms until the trap reached 90% of the trap intensity. In the indirect hologram change we already have the new trap built up, so the time it needs to erase the helping trap is significant. Graph 5(b) shows the decreasing intensity of the helping trap over time. It reduces to 10% of the maximum power in about 13ms. This corresponds to a twice as fast change of the trap.



(a) (b)

Figure 5. Measured laser intensity on the trap positions while the hologram is changed: (a) shows the intensity for the direct hologram change. In about 25ms the intensity at the new trap position rises from 10% to 90%. In (b) the intensity at the old trap position during the indirect change is shown. Here the intensity of the first trap decreases from 90% to 10% within 13ms, which means the trap changes faster by a factor of two.

5. Results and Discussion

The discussed improvements of the step response method in section 4.2 can be proved by comparing the results of different trap calibrations. A comparison is made between different calibration distances and the direct/indirect hologram change. The power spectrum method is used as reference.

5.1. Direct hologram change

A direct hologram change is the easiest way to switch the trap position. Only two subsequent holograms are displayed. It is very convenient to choose 1μm as trap distance, because the bead will be directly in the linear region of the power profile where the exponential decay of the distance holds. So the whole measured movement can be used to fit the exponential decay parameter τ. Also a measurement at 1.4μm was done to investigate the improvement of a optimized calibration distance. Figure 6 shows the results of the step response method with the direct hologram change at a calibration distance of 1μm (red line) and 1.4μm (green line). The obtained stiffnesses are plotted as a function of the reference stiffness (black line). As expected, the stiffnesses coincide with the reference values for low stiffnesses, and then the difference increases because the trap is not changed fast enough. We can clearly see that a calibration distance of 1.4µm delivers significant better results than the calibration distance of 1µm. This proofs the assumption that the bead moves too fast for high stiffnesses, so the hologram change is not fast enough to generate the new trap completely, and therefore the measured stiffness is lower. The bead needs more time to reach the trap centre, and this additional time is provided by the increased distance the bead has to move until it reaches the trap centre.

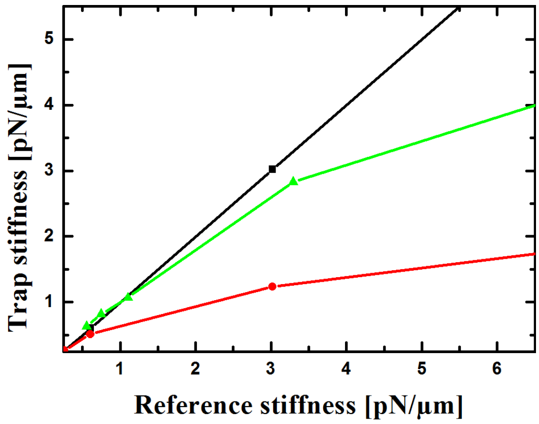


Figure 6. Calibration with the step response method. The obtained stiffness is improved by changing the calibration distance from 1µm(red line) to 1.4µm(green line). Further increases of the distance result in unreasonable values because the trajectories become curved and random.

5.2 Indirect hologram change

The indirect hologram change was developed to directly decrease the time which is necessary to switch the traps. So the laser intensity at the trap position rises faster and the measurement is more precise. A measurement with the indirect hologram change was not possible for a calibration distance of only 1µm. Because both traps are generated at the same time in the intermediate step, the bead moves to a equilibrium position in the middle of both traps. The recorded trapping movement from this short distance was not sufficient for the fit because of the high noise from the Brownian movement. At a calibration distance of 1.4µm the bead hardly moved from its defined starting position, so a calibration was possible.

Figure 7 compares the stiffnesses obtained with the direct (red line) and indirect hologram change (green line) at a calibration distance of 1.4µm. We can clearly see that the new measurement has more precise stiffnesses with respect to the reference values of the power spectrum (black line). Here, the relative error of the stiffnesses up to 3pN/µm is only about 6% and therefore usable for low stiffness calibrations.

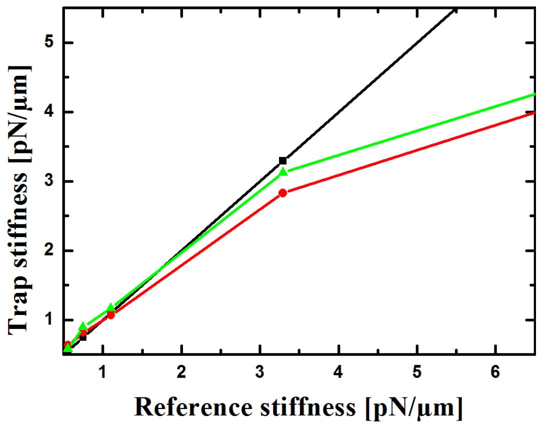


Figure 7. Obtained stiffnesses of the step response method at a trap distance of 1.4µm with the direct hologram change(red line) and the indirect hologram change (green line). The faster trap change of the indirect hologram change improves the accuracy of the measurement.

The results of the enhanced measurements with the indirect hologram change and the optimized calibration distance proofs that the response time of the SLM is a limiting factor in the holographic setup.
Modifying subsequent holograms for a faster hologram change, and increasing the calibration distance to gain more time improved the results for low trap stiffnesses up to 3pN/µm significantly.

6. Conclusion
The step response method is an easy way to implement a calibration technique for multiple optical traps. In this work, the method was for the first time implemented in a holographic optical tweezers system. The characteristic limitation of the holographic setup, which is the slow SLM response time, was investigated and significant improvements were developed. With the available holographic optical tweezers setup, optical traps with stiffnesses up to 3pN/µm were successfully calibrated at low relative errors of around 6% with respect to the power spectrum method.
For a further improvement of the method, optimized holograms for a faster trap change can be investigated. An interesting attempt could be the restricted phase change (RPC) algorithm proposed by Mattias Goksör et al.[12], which is based on the Gerchberg-Saxton algorithm and allows the hologram values to change in a confined range only. This could lead to a faster direct hologram change and more precise values for higher stiffnesses.

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