Master in Photonics

MASTER THESIS WORK

BACK FOCAL PLANE INTERFEROMETRY FOR HOLOGRAPHIC OPTICAL TRAPS

Jade Martínez Llinàs

Supervised by Dr. Mario Montes Usategui and Dra. Estela Martín Badosa
(UB)

Presented on date 7th september 2009

Registered at
Back Focal Plane Interferometry for Holographic Optical Traps

Jade Martínez Llinàs
Optical Trapping Lab, Grup de Biofotònica. Departament de Física Aplicada i Òptica. Universitat de Barcelona. Martí i Franquès 1, Barcelona 08028, Spain.

E-mail: somnajade@gmail.com

Abstract. Since its invention in 1986, optical trapping as a tool to manipulate microparticles non-invasively has found many applications in physics and biology. Force measurement on biomaterials is a potential application of optical tweezers which requires precise position detection. Back focal plane interferometry has become a commonly precise method for position detection, but it is limited to single trap calibration. However, the study of complex biological systems requires simultaneous multiple trap manipulation. Time-sharing a trap at kHz by acousto-optic-deflectors produces multiple independent tweezers, but limited to two dimensions. In this work, we aim to combine the versatility of digital holography for simultaneous multiple manipulation with the precision of back focal plane interferometry for force measurement. A holographic optical system set-up has been built and used to generate two traps with orthogonal polarizations: a fixed trap useful for measuring forces, and a holographic tweezer which allows dynamic manipulation. This method represents a step towards simultaneous manipulation with many holographic traps and precise position detection with only one trap.

Keywords: Optical trapping, back focal plane interferometry, acousto-optic-deflectors, digital holography, spatial light modulator.

1. Introduction

An optical tweezer uses a sharply focused laser beam to trap and manipulate particles ranging in size from tens of nanometers to tens of micrometers through the interaction of light with matter. Because photons carry both linear and angular momentum, they can exert forces as well as torques on matter [1]. The technique of optical trapping was studied back in the early 1970's by Ashkin, who proved that radiation forces could move dielectric microparticles in water and air, and developed a three-dimensional trap based on two counter-propagating laser beams [2]. 16 years later, working at Bell's laboratories, Ashkin et al. discovered that a single beam gradient force, by refraction, can hold a microscopic particle in three dimensions near the beam focus, and used laser trapping in several experiments, ranging from cooling and trapping atoms to manipulation of live bacteria and viruses [3]. Since then, optical tweezers have evolved and today find many applications in physics, biology and interdisciplinary areas such as physical chemistry or biophysics [4,5].

Depending on the particle's diameter (d), there are two theoretical frameworks by which the force on a sphere can be easily calculated: Rayleigh, when \( \lambda >> d \); and Mie, for \( d >> \lambda \); where \( \lambda \) is the wavelength of light in vacuum. In the Rayleigh regime, forces can be computed
considering the particle behaving as a dipole, while in Mie forces are obtained from Ray Optics [5]. Between these two limiting cases, neither of these approaches is strictly valid and the Generalized Lorenz-Mie Theory should be used. Unfortunately, most of the objects that are interesting in optical trapping fall in the intermediate case, and the exact theory is not practical to treat multi-particle systems. The particles trapped in our experiments were 1-5 μm sized, and the laser wavelength was 1064nm. Such a wavelength is convenient for trapping biological material, particularly in experiments in vivo, because it is placed in a window of relative transparency in the near infra-red region of the electromagnetic spectrum, therefore helping to limit damage or “opticution”. Although the condition for the Rayleigh regime is not fulfilled, the Rayleigh approximation is still applicable [6].

In Rayleigh, the radiation force can be broken down into two components: repulsive scattering, and gradient trapping in the direction of the spatial light gradient. For stable trapping, the gradient component must overcome the scattering contribution; this condition is accomplished with a high intensity gradient on the laser beam, produced by sharply focusing the laser light through a high numerical aperture (NA) objective lens. The trapped bead feels a harmonic potential near the trap centre, where the linear regime is valid for small displacements from the equilibrium position, and the trap can be calibrated as a hookean spring, that is, the trap stiffness or the linear ratio between force and displacement can be found [5].

Digital holography represents a major tool in optical trapping, since it enables one to implement diffractive optical elements that can be refreshed in real time, thus providing dynamic manipulation. Holographic optical tweezers (HOTs) can be implemented introducing a spatial light modulator (SLM) in the path of a laser beam prior to its focusing, in order to reshape dynamically the beam with computer-addressed phase-only holographic patterns written onto the SLM and removed at video frequency [7]; the modulated beam is focalized in the plane where the sample lies, and the resulting light pattern of traps at a given time depends on the hologram encoded on the modulator. HOTs show substantial advantages in comparison with other dynamic manipulating techniques; first, they allow multiple and simultaneous trapping of mesoscopic particles in three-dimensional structures [8, 9]; second, non-Gaussian modes such as Laguerre-Gaussian modes or Bessel beams can be generated; Bessel beams offer the capacity for extended guiding of particles that exceed the guiding distance of Gaussian beams [10]; while Laguerre-Gaussian modes produce optical vortices that carry angular momentum and can exert torques as well as forces on particles, with traps that are sensitive to the size of the particles [11]; finally, digital holography can be used to correct optical aberrations [12].

Optical traps provide a unique tool to trap and manipulate mesoscopic objects non-invasively, that is without mechanical contact, thus reducing damage to biological specimens; and to measure and exert piconewton forces on microwaved sizes particles [5]. A potential application of optical tweezers is optical force and torque measurements in single molecule experiments and molecular motors, which are required for the understanding of cellular processes such as replication and transcription of DNA [13].

On the other hand, sensitive position detection is essential for trap calibration and force measurements [5]. Back focal plane interferometry (BFPI) is a laser-based highly precise method for lateral position detection in the specimen plane [14]. As in polarization interferometry, in back focal plane detection the bead position becomes largely independent of the position of the trapped bead in the specimen plane because the same laser is used for trapping and position detection [5]; furthermore, while polarization interferometry provides only single lateral detection, in back focal plane interferometry, particle motion is monitored in both lateral directions with a quadrant photodiode (QPD) which captures the interference signal between scattered light from the particle and non-scattered light [14]. Compared with video based schemes, which are limited by video acquisition rates, computer speed, memory capacity, and ultimately to the number of recorded photons when high speed video tracking is implemented, a photodetector facilitates higher bandwidths because it can absorb higher
intensity of light [5]. Nevertheless, silicon photodetectors are bandwidth limited in the near infrared; QPDs become low-pass filter with $f_{3\text{dB}} \approx 8$-9kHz at 1064nm, while position sensitive detectors (PSDs) offer better temporal precision with $f_{3\text{dB}} \approx 150$kHz at 1064nm [14].

But back focal plane interferometry is limited to single trap calibration. Even though simultaneous multiple trap calibration is possible with video based methods, it is highly limited in position detection sensitivity [15]. However, manipulation with multiple traps is required in many experiments involving complex biological systems [1]. A quad-trap based on the splitting of a single laser into two orthogonally polarized beams and the time-sharing of one of the beams into three different positions with an acousto-optic deflector, has been demonstrated recently [16]; with such a multiplexed trap, Noom et al. were able to carry out experiments with two DNA molecules held between four beads used as handles in four independently movable optical traps [16]. Nevertheless, the set-up required for scanning the beam rapid enough to avoid particle diffusion and ensure efficient manipulation, is quite complex; another disadvantage of the multiplexed quad-trap is the lack of flexibility for manipulation, since acousto-optic scanning is limited to two dimensions. In this work, we follow Noom et al. to obtain, from the same laser, two traps with orthogonal polarizations. We replace the acousto-optic deflector by a spatial light modulator, with the aim to combine the versatility of digital holography with the precision of back focal plane interferometry. We generate a non-holographic trap useful for measuring forces, and a holographic tweezer convenient for dynamic manipulation.

2. Generation of two traps with orthogonal polarizations

2.1 The nematic liquid crystal spatial light modulator

Nematic liquid crystal is an intermediate condensed phase between crystalline solid and isotropic liquid, in which rod-like molecules with a preferred orientation (director axis) can move (tilting) [17]. On the one hand, when an electric field is applied, the nematic molecules tilt towards alignment with the field in order to minimize the free electrostatic energy. On the other hand, because liquid crystal is a birefringent material, light polarized parallel to the director axis feels a refractive index, called extraordinary index, while light polarized perpendicularly to the director axis experiences a different refraction index, known as ordinary refractive index. The ordinary refraction index does not depend on the applied electric field; however, the extraordinary refraction index it does. Due to different refraction indexes, each polarization mode travels a different optical path length, which is translated into a different phase retardation [18].

A spatial light modulator (SLM) is composed by a liquid crystal layer encapsulated between two glass plates. With an array of transparent conductive electrodes, the device can be electrically addressed, and spatially modulate a light beam with a different voltage applied on each electrode [18].

As a consequence of the above mentioned electro-optical properties of liquid crystals, when an electric field is applied between the two plates of a spatial light modulator, light polarized in the direction of the extraordinary refraction index changes its optical path length according to the electric signal, in other words, it can be phase modulated; by contrast, the phase of light polarized in the direction of the ordinary refraction index cannot be voltage-controlled.

2.2 The Holographic Optical Traps Set-Up

A HOT set-up (Fig. 1) based on an inverted microscope (Nikon Eclipse TE 2000-E) has been built and aligned. It incorporates a Hamamatsu X10468-03 reflective parallel-alligned nematic SLM, based on liquid crystal on silicon (LCOS) technology, to reshape the beam. A keplerian telescope (T1) expands the beam to overfill the effective area of the SLM (16 $\times$ 12 mm), and a
second telescope (T2) resizes the beam reflected from the SLM in order to match the entrance pupil (4.9 mm) of the objective.

The trapping light comes from a linearly polarized fibre laser from IPG Photonics (Ytterbium fibre laser YLM series), with a maximum output power of 5 W in continuous wave mode. This laser provides good pointing stability and low power fluctuations, two important conditions for trapping [5]; it delivers a 1064 nm TEM$_{00}$ Gaussian mode, which focuses on the smallest diameter beam waist, thus giving efficient harmonic trapping [5]. An auxiliary stage above the optical table contains the laser and its current supply in order to avoid noise and heat reaching the optical trap.

![Figure 1](image_url)

Figure 1. a) Layout of the experimental set-up. b) Expanded view showing beam conditioning for generation of HOTs. After passing through a half wave plate (HWP), which changes the polarization direction of the beam, a beam expander with focal lengths 30 mm (L1) and 130 mm (L2) resizes the diameter of the beam, a mirror re-conducts the light towards the SLM, where the phase is codified and the beam is reflected again. The second telescope is formed by lenses L3 and L4, with focal lengths 150 mm and 100 mm respectively. The last lens (L4) is mounted inside the microscope, in a cube containing a dichroic mirror tuned to the trapping wavelength. In order to be able to centre the position of the optical trap, L3 is mounted on a mechanical system designed for three dimensional micrometric movement, and monitored with a piezoelectric and connection to a computer with specific software.

The inverted microscope takes advantage of a dichroic mirror tuned to the trapping wavelength to reflect and couple the laser light into the optical path of the microscope and transmit the microscope illumination wavelengths (which is captured by a CCD camera). The laser beam is brought to the focus by the objective, producing the optical trap(s). The oil immersion objective (NA=1.3) determines the overall efficiency of the optical trapping system (stiffness versus input power). Forward scattered light is collected by the oil condenser (NA=1.4) and transmitted through a second dichroic mirror. The objective lens is used both to project optical traps focusing the laser beam onto the sample plane, and also to image the trapped particles onto a CCD camera (Qimaging QICAM 12-bit Mono Fast 1394).

Phase-only digital holograms are generated and imprinted onto the SLM with the Holotrap software [19] and transformed into spatial modulations of the reflected wave front. The hologram projected on the SLM acts as a phase grating that spatially modulates the reflected light, and its Fourier diffraction pattern is produced at the trapping plane by means of a lens. The phase shift is related to the tilting of the molecules in the liquid crystal, which is electrically addressed pixel by pixel using a complementary metal-oxide-semiconductor (CMOS) backplane
and a digital video interface (DVI) signal via a computer. Thanks to CMOS technology, almost all the area of the modulator is effective because the CMOS backplane avoids diffraction noise due to pixel structure since the electronics are not between pixels [20]. The SLM introduces optical aberrations due to lack of flatness of the surface where the beam is reflected; these aberrations are compensated for by adding an additional phase map.

The SLM imposes some constraints on the computer-generated holograms that can be written onto it; these limitations are related to the digitalized structure of the device and the quantization of the phase [22]. The Hamamatsu SLM used is quite efficient; it is linearly pre-calibrated from factory and can introduce 256 different phase shifts ranging from 0 to $2\pi$ on the incident wavefront of the light beam at each 20 $\mu$m wide pixel in a $792 \times 600$ array; furthermore, it only modulates the phase of light without any change of intensity and rotation of polarization state [20]. Modulating only the phase and not the amplitude of the wavefront is in practice enough to produce any intensity pattern on the specimen plane and hence any desired design of traps; such beam-shaping phase gratings are commonly known as kinoforms and the related Fourier coefficients are assumed to carry the main information about the trap [21].

A half-wavelength plate (HWP) placed between the laser and the first telescope provides control over the polarization direction. By tilting 45º the HWP, maximum phase modulation is achieved because light is polarized with the director axis of the liquid crystal molecules. By contrast, a tilt of 0º corresponds to zero phase modulation. At an intermediate angle, part of the light is polarized with the extraordinary axis, and part with the ordinary one. As a result, at 22.5º, two traps with orthogonal polarizations are generated, a holographic or extraordinary trap whose position can be controlled by changing the hologram written onto the modulator thanks to the dependence of the extraordinary index with voltage, and a non-holographic or ordinary trap located at the centre.

2.3 Trap quality

With the HOTs set-up reported in section 2.2, we generated two traps with orthogonal polarizations (Fig. 2); we were able to trap 1 $\mu$m and 5 $\mu$m polystyrene beads (Sigma-Aldrich Company monodispersed microspheres) with both ordinary and extraordinary optical tweezers, to select the trap(s) generated on the sample plane and to manipulate beads with the holographic trap. As regards beam quality and optical aberration, the holographic trap is a Gaussian TEM00 mode thanks to aberration correction, but the central trap is affected by optical aberration that cannot be corrected for because light that generates the non-holographic trap is not modulated by the SLM.

Figure 2. a) Ordinary (up) and extraordinary (down) optical traps sharing 30 mW laser power on the sample plane. b) Imaging of two 1 $\mu$m beads trapped by two orthogonally polarized traps.

2.4 Harmonic approximation in Boltzmann statistics

So far we have demonstrated a set-up for generation of two traps with orthogonal polarizations and manipulation with a holographic trap. In order to check whether or not the ordinary trap can be calibrated, and thus be useful for force measurement, we apply video tracking and harmonic potential reconstruction in Boltzmann statistics.
On the one hand, for small displacements from the trap center, the radiation force exerted by an optical trap on a trapped bead scales linearly with the relative position between the trap center and the bead \((5)\); hence, the bead feels a harmonic potential that can be modeled in two dimensions by

\[
U(x, y) = \frac{1}{2} k_x (x - x_0)^2 + \frac{1}{2} k_y (y - y_0)^2, \tag{1}
\]

where \(k_x\) and \(k_y\) are the trap stiffness's in the lateral directions, and \((x_0, y_0)\) is the equilibrium position. On the other hand, according to Boltzmann statistics \((23)\) for a small particle in thermal equilibrium at temperature \(T\) within a potential \(U(x, y)\), the probability density of position \((x, y)\) is

\[
\rho(x, y) = C \exp\left(\frac{-U(x, y)}{k_B T}\right), \tag{2}
\]

where \(k_B\) is the Boltzmann constant and \(C\) is a normalization factor. Then, the optical potential can be reconstructed from a position histogram, \(h(x, y)\),

\[
U(x, y) = -k_B T \ln\left[h(x, y)\right] + C', \tag{3}
\]

where \(C'\) is an arbitrary constant, and the trap stiffness's can be estimated from standard deviations \((\sigma_x, \sigma_y)\) of Gaussian histograms taking into account the Equipartition theorem \([5]\):

\[
k_x = \frac{k_B T}{\sigma_x^2}, \quad k_y = \frac{k_B T}{\sigma_y^2}. \tag{4}
\]

The lateral movement of the bead within the traps was captured with the Qeye CCD, and video tracked. Histograms of the positions visited by the particle have been computed with Labview software \([15]\) and reconstructed with Matlab (Fig. 3a). The parabolic potential \((1)\) has been fitted to the Boltzmann potential \((3)\), taking experimental data from the histogram near the standard deviation. Typical results are shown in Fig. 3; although the ordinary trap profile is not a perfect Gaussian, the probability distribution is normal (with standard deviations \(\sigma_x = 19\)nm and \(\sigma_y = 33\)nm, comparable to standard deviations obtained for the holographic tweezer) and the optical potential is almost harmonic. Hence, the ordinary trap can be calibrated and used for measuring forces. Video capture and tracking with a CCD would provide a rough estimation of the trap stiffness's from analysis of histograms and expression \((4)\), but a QPD and calibration from power spectrum is preferred for high precision \((15)\).
3. Back Focal Plane Interferometry Analysis

3.1 Back Focal Plane Interferometry

This interferometric method is used for nanometric position detection of the trapped bead relative to the trap centre, by imaging the condenser back focal plane onto a position sensing device such as a QPD. Since positions at the sample plane and phases at the back focal plane (or at a conjugate plane) are related through a Fourier Transform, at the condenser back focal plane, the interference between light scattered by the particle and non-scattered laser light produces a pattern of rings that depends on the displacement of the particle from the trap center [14].

A QPD consists of four semiconductor quadrants able to detect intensity shifts (for instance, due to Brownian movement of a bead embedded in liquid), and additional electronics that convert current from quadrants into normalized X and Y signals proportional to the lateral positions; moreover, the total voltage provides the axial coordinate. Digital analysis of these signals can be used for three dimensional force calibration of a single trap [14].

3.2 Interferometric Detection Set-Up for orthogonally polarized dual tweezers

We have designed, built and aligned, a set-up (Fig. 4a) for back focal plane interferometric detection. The microscope condenser collects the light scattered by the trapped particle as well as non-scattered laser light. A dichroic mirror (DM1) transmits the illumination while reflects the laser light coming from the condenser. A linear polarizer (P) is placed before the relay lens in order to block one of the polarization components. As P blocks the part of light projected on the direction of absorption of the molecules forming the polarizer, it enables us to capture the interference pattern corresponding to only one of the traps and at the same time to maintain the other trap for manipulation. Interface to user is made through the Holotrap software and two CCD cameras (Fig. 2b) connected to the same computer: whereas the Qeye CCD detects the interference at the condenser back focal plane, the Qimaging CCD enables imaging the trapped particles as well as the optical traps. Typically, a QPD placed at the back focal plane of the condenser is employed for position detection. However, we have skipped the QPD in order to simplify the study to a qualitative analysis of interferometric patterns.

Figure 4. a) Set-up for back focal plane interferometric detection. The focal length of the relay lens (L), f = 40 mm, has been chosen to reduce the detection path and to produce a spot of 7 mm, which is smaller than the semiconductor region of the CCD used for detection. b) Camera ports.
3.3 Results from interferograms

In this section, we carry out a qualitative analysis of interferograms captured with the Qeye CCD. Selection of the traps (holographic, non-holographic or both) generated at the sample plane was achieved by changing the polarization direction of light, rotating the half wave plate 0º, 22.5º or 45º, as it is explained in section 2a. Before detection, the desired polarization component was blocked with the linear polarizer (see section 3a) at 0º, 45º or 90º.

First of all, the stuck bead method was applied to illustrate the effect of changing the relative position between a holographic optical trap and a stuck bead. The bead was fixed at the cover-slip and the trap was moved taking advantage of the monitored lens (L3 in Fig. 1b). As L3 is moved (Fig. 5), a position change of the trap at the sample plane induces a shift on the interference pattern at the condenser's back focal plane. This moving interferogram gives the relative position between trap and bead after integration by a QPD [14].

Figure 5. Different relative positions (a, b and c) between a HOT and a 5 µm diameter stuck bead at the sample plane; related interferograms (d, e and f respectively) at the condenser's back focal plane. Different colours (red-yellow-pink) indicate movement of the bead centre towards the edge.

After that, we investigated on the detection from two traps with the same polarization. With the HOTs set-up, we can generate many traps, but it is not possible to calibrate more than one HOT by BFPI because all traps have the same (extraordinary) polarization and thus information about each trap is not separable by the polarizer. A bead in a single trap (Fig. 5, 6d) produces the expected Schmidt's pattern of rings at the condenser's back focal plane [14]. However, information about the relative positions between trap and bead is lost when a second HOT is added, as it is shown in Fig. 6e and 6f; instead of a ring pattern, the interference between the two spherical waves is composed by Young fringes, where the phase period depends on the distance between the two traps (orientation of fringes is arbitrary on the figures, as it depends on camera rotation). Similarly, when two beads are simultaneously trapped by two HOTs (Fig. 7), the pattern of rings predicted by Gittes and Schmidt for a single trap is not detected.

Figure 6. A single HOT centred on a 5 µm diameter stuck bead (a) generates a ring interferogram (d) with a central dark spot. Addition of a second HOT (b) on the sample plane produces a pattern of interference (e) dominated by Young fringes. The phase period in (f) is different because the second HOT (c) is generated on a different position at the sample plane.
Nevertheless, with the half wave plate permitting laser light with both ordinary and extraordinary polarization modes passing through, two traps with orthogonal polarizations on the sample plane (Fig 8a, 8e) enable independent detection. This result is demonstrated in Fig. 8. For a trapped bead in the trap generated by light with the same polarization than the polarizer, we identify a ring interference (8b, 8f), as in 6a). In 8c) and 8g), a mix of Young fringes and Schmidt rings is observed because both ordinary and extraordinary modes are detected. Finally, when light detected comes from the optical trap without bead, as in 8d) and 8h), we find the uniform light pattern predicted from Fourier Optics for a point source.

Two traps with orthogonal polarizations are expected to be independent from each other because the laser provides linearly polarized light and the half wave plate only changes the direction of polarization. Furthermore, as the same laser is used for the two traps, the laser power in each trap should be the same when the half wave plate is at the intermediate angle (22.5°). In practice, however, it is not possible to isolate completely the two polarizations: some depolarization (10:100) has been measured before the polarizer. Sources of polarization cross-talk are the polarizer itself, dichroic mirrors and the high numerical aperture of the objective. Because polarization cross-talk increase with the number of traps, there is a limit in the number of HOTs useful for manipulation.
4. Conclusions and future work

We have built a HOT set-up to generate two optical tweezers with orthogonal polarizations, taking advantage of the property of birefringence of nematic liquid crystals. Since each trap feels a harmonic potential near its trap focus, both traps can be calibrated, that is, a linear relationship between displacement from the trap centre and applied force can be found.

We have shown a novel technique for force calibration in a dual trap. As the method combines high precision of back focal plane interferometry with versatility of holographic optical tweezers, it represents a powerful tool for manipulation in molecular biology experiments. The next step would be to obtain the stiffness of each trap with a PSD or a QPD.

The use of a spatial light modulator and a single laser reduces costs and facilitates the alignment because both traps are generated from the same beam. An advantage of using digital holography for multiple trap generation instead of acousto-optic deflectors is the possibility to simultaneously create many holographic traps. However, as intensity is shared from the same laser, there is a limit on the number of holographic traps that can be used for manipulation. In the future, we expect to investigate this limit.

Acknowledgements

I dedicate this memory to my parents and my sister. Thanks to my sister, who has been always passionate in biophysics, I had the pleasure of finding out photonics as an exciting new research area. Without the aptitudes I have acquired from my parents, I would not have been able to start doing research. I thank my mother for her effort over many years in supporting my education and that of my sister.

I would like to express my gratitude to all the professors of the master in photonics and many other researchers who have generously shared with me their knowledge, ideas and expertise. Among them, I am specially grateful to Morgan Mitchell, Mario Napolitano and Marco Koschorreck.

This work would not have been completed without the financial support of UB. I am indebted to my advisors Dr. Mario Montes Usategui and Dra. Estela Martin Badosa, as well as to my laboratory mate Josep Mas Soler, for his help and encouragement throughout the course of this work.

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