Three-dimensional parallel particle manipulation and tracking by integrating holographic optical tweezers and engineered point spread functions

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Abstract: We demonstrate an integrated holographic optical tweezers system with double-helix point spread function (DH-PSF) imaging for high precision three-dimensional multi-particle tracking. The tweezers system allows for the creation and control of multiple optical traps in three-dimensions, while the DH-PSF allows for high precision, 3D, multiple-particle tracking in a wide field. The integrated system is suitable for particles emitting/scattering either coherent or incoherent light and is easily adaptable to existing holographic tweezers systems. We demonstrate simultaneous tracking of multiple micro-manipulated particles and perform quantitative estimation of the lateral and axial forces in an optical trap by measuring the fluid drag force exerted on the particles. The system is thus capable of unveiling complex 3D force landscapes that make it suitable for quantitative studies of interactions in colloidal systems, biological materials, and a variety of soft matter systems.

OCIS codes: (110.1758) Computational imaging; (110.6880) Three-dimensional image acquisition; (150.5670) Range finding; (350.4855) Optical tweezers and manipulation.

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1. Introduction

The use of optical forces to manipulate particles, molecules, and atoms has influenced fundamental physics, biophysics, and engineering [1–4]. The introduction of holographic optical tweezers (HOT) has further enabled the control of multiple optical traps in three dimensions (3D) [5,6]. As a consequence, new techniques are required to provide fast and accurate particle tracking for multiple optically trapped particles. Many traditional optical trap 3D tracking techniques utilize quadrant photodiodes [7,8], which cannot track multiple particles in a wide field of view. Other imaging methods, such as holography, are capable of tracking multiple particles in a volume [9]. However, holography requires, in principle, coherent illumination that produces speckle noise, suffers from crosstalk among particle sub-holograms, and requires high information content cameras, delivering poorer signal-to-noise performance. Stereo imaging concepts are an attractive alternative, but suffer from low localization precision in low signal to noise environments [10,11].

The system presented in this paper employs a PSF specifically designed for 3D localization and tracking named double-helix point spread function (DH-PSF) because of its

3D shape in the focal region [12–19]. The DH-PSF generates two lobes that trace out a double helix by rotating around the optical axis with image defocus [12–15]. As a particle moves away from focus in one direction, the lobes rotate clockwise while as the particle moves in the opposite direction, the lobes rotate counter-clockwise. Depth is encoded and can be determined through the rotation angle of the two lobes, while the lateral position can be estimated by calculating the centroid of the two lobes.

The inherent axial asymmetry exhibited by the DH-PSF allows for fine discrimination between axial positions. An information theoretic analysis based on Fisher information shows an improvement in depth estimation with respect to standard clear aperture imaging [12–14] and in three-dimensions with respect to other state of the art methods [19]. Experimental nanometer 3D precisions have been demonstrated in DH-PSF systems using scattering [14], fluorescent particles [13], quantum dots [20], and single molecules [16–18].

The DH-PSF system uses a simple optical setup and matched estimation algorithms to attain precise 3D localization for multiple particles in a wide field [13,14]. By integrating the DH tracking system with the HOT we demonstrate measurement of lateral and axial optical trap forces. The characterization of the traps in the lateral and axial directions enables quantitative understanding of interaction forces in multiple biological [21,22] and condensed matter systems [23–25] that are inherently parallel and 3D.

The main contributions presented in this report are: (1) A method to track in parallel multiple optically trapped particles (a) with the highest 3D localization precision per photons detected [19], (b) appropriate for particles emitting coherent or incoherent light; and (c) easy to adapt to existing HOT systems. (2) A system integration design including modifications to prior DH systems such as epi-illumination of scattering particles, higher speed cameras to detect fast moving particles, and dark-field DH detection. (3) A demonstration of the value of the system to unveil complex 3D force lansdscapes.

2. Integrated HOT and DH-PSF system

DH-PSF tracking is attractive for integration with HOT because of its precision, parallelism, and simplicity. The DH-PSF creates a double image of a trapped particle due to the convolution between the two lobes and the trapped particle. The angular orientation of the two particle images contains information about its axial position. When the particle diameter is comparable to or less than the diffraction limit for the imaging wavelength, the two images do not overlap and position estimation is easier than for extended objects [12–14]. High-precision tracking of trapped particles enables measurement of the 3D picoNewton-level force landscape typical in optical traps. Furthermore, DH-PSF microscopy operates with coherent or incoherent illumination, in brightfield, darkfield, and fluorescence microscopy modes [15], making it compatible for use with a variety of particle-host systems.

The HOT system is implemented using a reflective, phase-only spatial light modulator (SLM, BNS P-512) and is built around an inverted microscope (IX 81, Olympus). A scheme of the system is shown in Fig. 1(a). The laser beam (CW Nd-YAG, 1064nm) is incident on the SLM which phase-modulates the beam front according to the trap configuration specified on the computer. The holographic pattern supplied to the SLM is refreshed at 15 Hz, thus allowing real time positioning of multiple traps. The telescope in 4f configuration changes the beam size to overfill the active area of the SLM and the back aperture of the objective lens. We use two oil immersion microscope objectives for the trapping experiments: 60X (NA ~1.42, ~70% transmission at 1064nm) and 100X (NA ~1.4, ~60% transmission at 1064nm).

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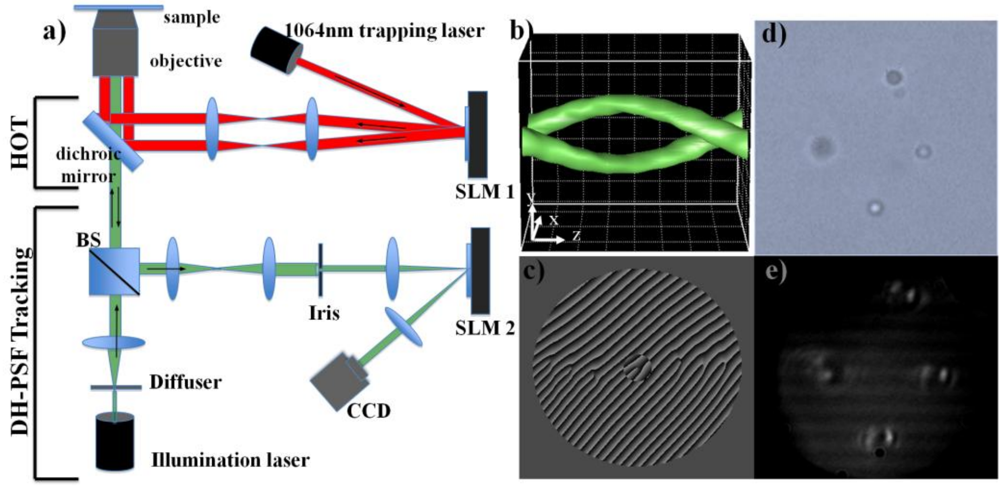

Fig. 1. (a) Schematic showing the experimental setup of the integrated Holographic Optical Tweezer (HOT) system (red beam) and DH-PSF system (green beam). The HOT uses a phase only SLM to phase modulate incoming light to create multiple optical traps in a volume. The DH-PSF has two lobes which rotate with defocus (b). The DH-PSF darkfield phase mask (c) is encoded on a second phase only SLM placed in a Fourier plane of the image to create the DH image used for tracking. The conventional brightfield image (d) and the corresponding off-axis darkfield DH-PSF image with the undiffracted on-axis light suppressed (e) are also shown. The two-lobed responses have different angular orientation for each particle, corresponding to specific axial positions, as can be seen by image defocus in the conventional image.

The DH-PSF tracking system is implemented with an epi-illumination scheme through an imaging port of the microscope. The system illumination is provided by a laser source, in this case either 532 nm (diode) or 633 nm (HeNe). A diffuser placed in the optical path creates spatially incoherent illumination to reduce the effects of coherent interference. The DH-PSF is implemented by placing a phase mask via a second reflective phase only SLM (Holoeye HEO 1080 P) at a Fourier plane of the output image (Fig. 1(a)). A 3D plot of the PSF is shown in Fig. 1(b). An iris providing a field stop is placed in the image plane before the SLM. A blazed grating overlaid the phase mask to shift the modulated light off axis and separate it from the unmodulated light (Fig. 1(c)). The mask is designed with the blazed grating at the center of the mask oriented in the opposite direction to the rest of the mask. This directs low spatial frequency light in the opposite direction to reduce background light and create a darkfield image [15]. The DH-PSF image is captured by a CCD camera (Point Grey Research, Grasshopper). Figure 1 also shows images, conventional (Fig. 1(d)) and DH-PSF (Fig. 1(e)), of four particles manipulated and offset axially relative to one another using the holographic optical trapping system.

3. Experiments

3.1. Joint parallel trapping and localization

The 3D random thermal motion of a trapped particle was tracked first to demonstrate the integration of DH-PSF with optical tweezers. For this experiment we used a dispersion of polystyrene particles (diameter 1.1 microns) in water. The trap laser power at the optical trap was 14 mW. The real-time update of holograms was not used for generation of traps for this experiment, because only a single trap was needed. The CCD camera captured a total of 3000 frames at 40 frames per second. Figure 2 shows the estimated positions and histograms of the localization. The measured localization standard deviations were 26 nm in x, 31 nm in y, and 46 nm in z. The optical trap stiffness can be characterized with the standard deviation of the random thermal motion [8,26].

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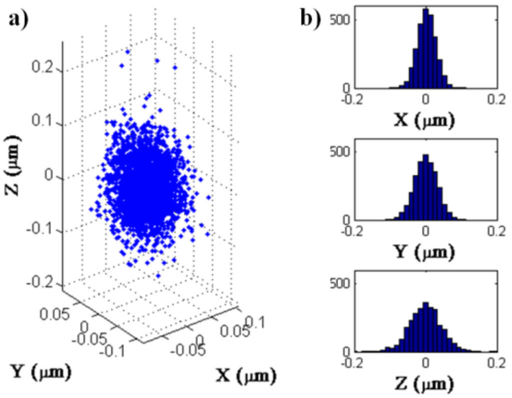

Fig. 2. (a) The random 3D thermal motion of an optically trapped 1.1 μm polystyrene bead in water tracked with the DH-PSF. (b) X, Y, and Z histograms of the particle position.

To demonstrate the ability of the DH-PSF system to track multiple trapped particles simultaneously, we utilized the HOT setup to trap four particles. These particles were separated laterally by between 6 and 8 microns. Using HOT, two of the four particles were moved 1.3 microns axially. As the particles moved axially the angular orientation of the DH-PSF lobes was measured. The axial position was determined by comparing the angular orientation of the double lobes to the calibration data. Figure 3 shows the positions of the particles in 3D as the traps were displaced in time. The two particles in the stationary traps experienced some axial movement during this time as well. As the hologram on the SLM changed to move the traps axially, the two stationary traps were affected and displaced by approximately half a micron. The DH-PSF imaging system implemented here and used in the following experiments had precision (1σ standard deviation) of 9 nm in X, 14 nm in Y, and 30 nm axially. This precision was obtained by 30 repeated localizations of a fixed particle and calculation of the standard deviation. While precision was limited in these experiments by the number of detected photons, background and readout noise, as well as inherent vibrations of the system, it can be brought down to a few nanometers in all three dimensions as needed [13,14,19]. This experiment demonstrates the ability and simplicity of using the DH-PSF to track multiple trapped particles.

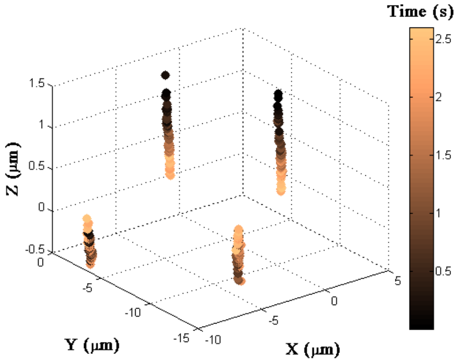

Fig. 3. 3D tracking of four optically trapped particles using the DH-PSF tracking system. The optical traps of the two particles in the background were moved ~1.3 μm axially over 2.5 seconds, while the foreground particle traps remained stationary. Changes in the hologram to move the background traps caused slight changes to the foreground traps, slightly affecting the foreground particles as well.

3.2 3D force landscape measurement

As mentioned previously, it is desirable to quantify the 3D optical force landscape applied to particles. Therefore, we explored the capability of the integrated system to measure the 3D components of the force acting on trapped particles using the drag force method [8,27]. In this method, the trapped particle is subject to a relative motion with respect to its surrounding fluid. The trapping force is determined by calculating the viscous drag force balancing it. Owing to low particle velocities and high medium viscosity, i.e., low Reynolds number regime, we neglect inertial forces acting on the particle. For a spherical particle in a known medium, the viscous drag force is found using Stokes drag formula: $F_d(x,y,z) = 6\pi R\eta V(x,y,z)$, where R is the radius of the particle, η is the medium viscosity, and $V(x,y,z)$ is the particle velocity [8].

In these experiments we removed the particle from the optical trap and allowed the particle to "fall" back into the trap, measuring the particle dynamics as a function of distance from the trap center [27]. More specifically, we trapped 1.1 micron diameter polystyrene beads in a glycerol-water mixture (84% glycerol by weight, n = 1.45, η = 50cP), with trap power of 29.6 mW at the sample. The HOT was utilized to move the trapped particle laterally 1.4 µm away from the trap center. In a second step, the holographic trap was removed via software, shifting all the trapping power to the zero order trap. As the particle moved towards the optical trap center, its 3D position was tracked with the DH-PSF subsystem.

Interestingly, and contrary to what could initially be expected, the particle did not stay on the transverse plane as it fell into the trap, but moved axially away from the objective as it moved towards the transverse center of the trap (Fig. 4(a)). Once near the origin the particle moved axially again, but now towards the objective until it reached the trap center. The large axial displacement could be caused, among other things, by the scattering force pushing the particle away from the axial center until it reached a stronger attracting gradient force which pulled it back towards the beam focus.

At every observed particle location the 3D forces were calculated with Stokes formula. Figure 4(a) shows the trajectory of the particle with ‘ + ’ indicating the particle position and the trap force experienced at that position represented by proportional line segments.

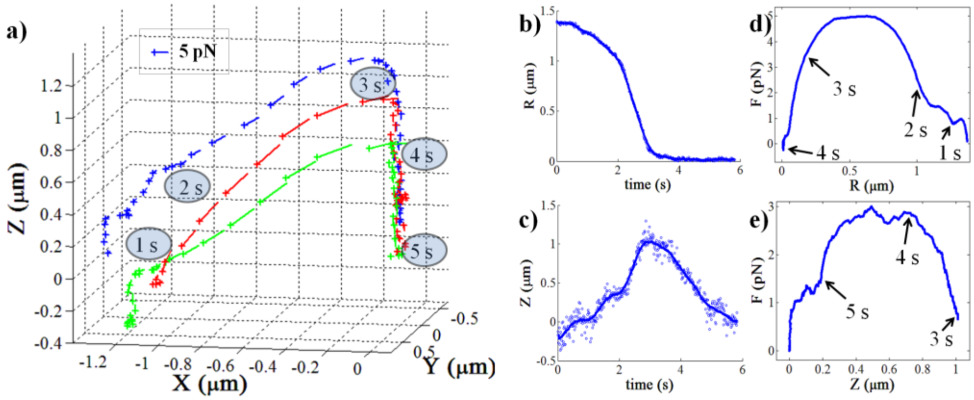

Fig. 4. (a) 3D tracking of a particle displaced laterally from an optical trap. The ‘ + ’ indicates the particle position, while the line segments are proportional to the force vectors at the given position. In this experiment the particles do not stay in the lateral plane as they move into the optical trap, but move axially as well. Plots (b,c) show, respectively, the transverse and axial position of the particle as it moved into the optical trap. The circles indicate the measured position. The line indicates the averaged position used for calculating forces. Plots (d,e) show the transverse and axial forces of the optical trap calculated using Stokes Law. The arrows indicate at which particular time the force was experienced.

We separated the transverse and axial forces experienced by the particle during a single run (Fig. 4(d) and Fig. 4(e)). The transverse and axial positions as a function of time (Fig. 4(b) and Fig. 4(c)) were used to calculate the particle velocity and in turn the trapping force. The positions used to calculate the force were smoothed using a sliding average of 40 points. For the axial force calculation we used tracking data after the particle began moving towards the trap center (after 3s). The calculated force indicates a maximum transverse force of 5 pN and a maximum axial force of 3 pN.

4. Discussion

The integration of HOT with DH-PSF tracking expands the utility and understanding of the 3D optical trapping of multiple particles. For example, the 3D tracking of trapped particles in Fig. 3 reveals unintended motion of trapped (presumed) stationary particles when the other particles in the sample are moved by the HOT system. The nanoscale 3D tracking capability could potentially be used to introduce feedback to a compensation mechanism for modifying the holograms. Furthermore, the high axial precision of DH-PSF tracking is ideal for revealing detailed quantitative information about the force landscape associated with particle tracking. The example provided in Fig. 4 shows the complex 3D nature of optical forces exerted on a colloidal particle with realistic trapping conditions, where the gradient and scattering forces are combined with the complex effects of spherical and other aberrations. Hence, the technique is useful for characterizing the limitations of holographic tweezers. Further, the use of DH-PSF is not restricted to HOT systems, but can be used in conjunction with other trapping and manipulation approaches as well. It should be emphasized that the SLM can be replaced by a fixed DH dielectric phase mask with a two-fold advantage in terms of efficiency and simplicity [28]

The integrated HOT and DH-PSF system could be a valuable tool in the study of 3D many-body interactions between colloidal particles and topological defects in soft matter, cells in bacterial biofilm communities, and various other mesoscopic biological and materials systems. It allows for more robust use of multiple trapped colloidal particles as handles for manipulation at micrometer and nanometer scales, especially with 3D interactions (for example, due to the interactions with confining glass plates). The ability to perform parallel and precise measurement of axial and 3D forces could help establish laser tweezers as a delicate tool for the measurement of pico- and femto-Newton forces, mechanical properties of polymers, and line tension of topological defects in soft matter.

5. Conclusions

We demonstrated parallel 3D tracking of holographic optically trapped particles using a DH-PSF. The DH-PSF tracking system provided high precision 3D position estimates. We estimated the particle position by simply measuring the angular orientation and centroid of the two DH-PSF lobes. The integrated system proved useful to measure complex 3D force landscapes generated by an optical trap, thus making the system suitable for a quantitative study of interactions in colloidal systems, biological materials, and a variety of soft matter systems. This approach may be further expanded to track orientations of optically-manipulated micro- and nano-sized particles with anisotropic shapes [29,30]. It can be combined with various 3D optical and nonlinear optical imaging approaches, such as confocal fluorescence microscopy, coherent anti-Stokes Raman scattering microscopy, multiphoton excitation fluorescence and multiple harmonic generation microscopies, so that the spatial tracking of optically manipulated particles can be correlated with 3D maps of composition and structure of complex soft matter and biological systems.

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