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

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12. A. Greengard, Y. Y. Schechner, and R. Piestun, “Depth from diffracted rotation,” Opt. Lett. **31**

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

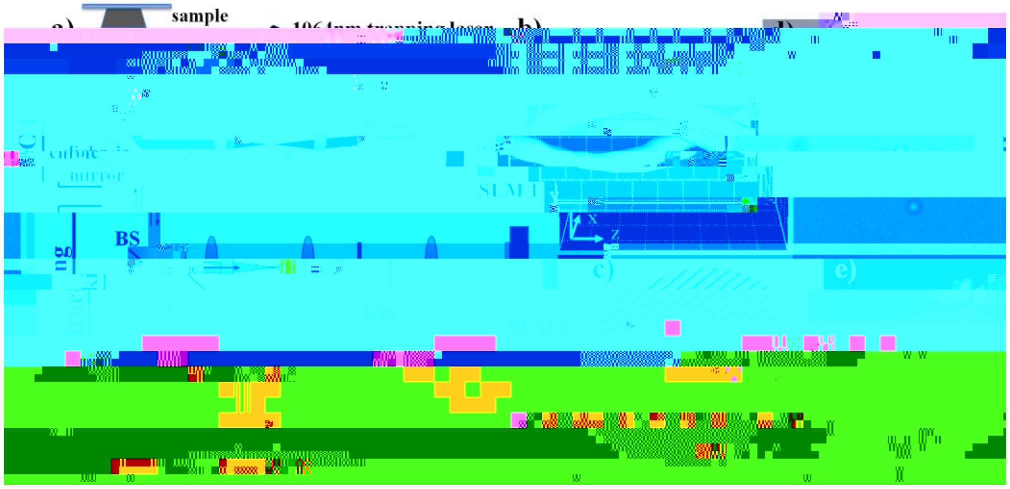

Fig. 1. (a) Schematic showing the experimental setup of the integrated Holographic Optical Tweezer (HOT) system (red beam) and DH-PSF system (green beam). The HOT uses a phase only SLM to phase modulate incoming light to create multiple optical traps in a volume. The DH-PSF has two lobes which rotate 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

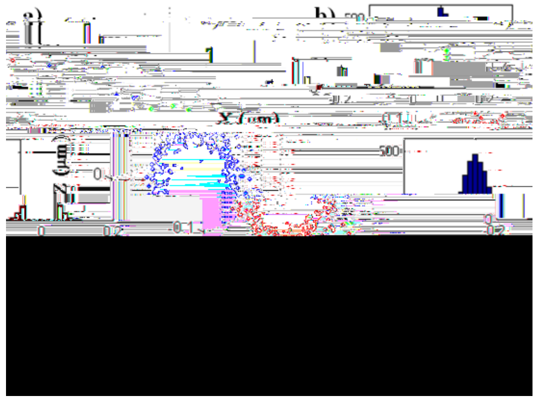

Fig. 2. (a) The random 3D thermal motion of an optically trapped 1.1 μm polystyrene bead in water tracked with the DH-PSF. (b) X, Y, and Z histograms of the particle position.

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.

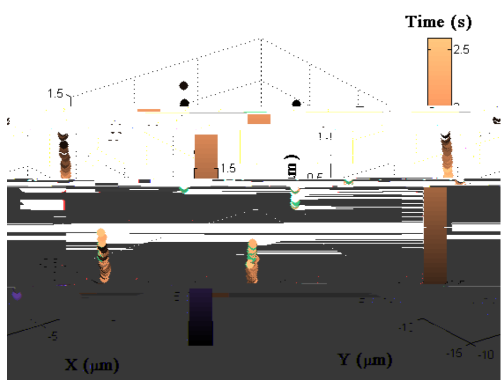

Fig. 3. 3D tracking of (f)50of

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: F6F

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