Imaging Response of Optical Microscopes Containing Angled Micromirrors

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Abstract

We describe the aberrations induced by introducing micromirrors into the object space of a microscope. These play a critical role in determining the accuracy of recent three-dimensional particle tracking methods based on such devices.

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Most techniques for tracking the motion of single particles in optical microscopy only provide the two-dimensional, in-plane position of a particle. Nevertheless, the nanometer-scale position information achievable through such experiments has been used in diverse applications, for example to measure inter-particle colloidal forces,[1] to monitor intracellular transport in living neurons,[2] and to characterize the mechanical dynamics of molecular motors.[3] The extension of traditional particle tracking methods to achieve full three-dimensional position resolution would greatly increase the utility of these methods for studying more complex systems without restriction to planar geometries. A variety of techniques have been developed for achieving nanometer resolution in three-dimensional particle tracking, including astigmatic imaging,[4] off-focus imaging,[5] and point spread function engineering.[6] In each of these methods, three-dimensional information is encoded in the changing shape of the point spread function as a particle moves in the axial direction.

Recently, several groups have developed a different approach to three-dimensional particle tracking in which angled micromirrors are introduced into the object space of a microscope in order to provide a reflected image in a plane nearly perpendicular to the focal plane.[7–9] It has also been suggested that full three-dimensional images could be reconstructed using such micromirrors.[7] The principle of these techniques is to leverage the very high lateral (xy) resolution of a diffraction-limited optical system to obtain simultaneous high resolution in a (nearly) perpendicular reflected plane (for example, yz). Using readily available two-dimensional particle-tracking algorithms,[10] the full three-dimensional position of a particle can be obtained from the simultaneous xy and yz information obtained in such a configuration using only the law of reflection and the (known) angle of the micromirror face.

Indeed, we recently used such a method to achieve three-dimensional localization of fluorescent nanoparticles with sub-20 nm repeatability within a 3 ms camera integration time, giving a three-dimensional dynamic position resolution of approximately 1 nm/$\sqrt{\text{Hz}}$.[9] However, a detailed analysis of our experimental data revealed a systematic error that could not be detected simply by analysis of repeated measurements. We used pyramidal micromirror wells (PMWs) etched into silicon. Depending on particle location, PMWs often provide two (and up to four) simultaneous reflected images of a freely diffusing particle, so that the z position of a single particle can in many cases be determined in more than one way. Comparing results, we found a systematic error in the
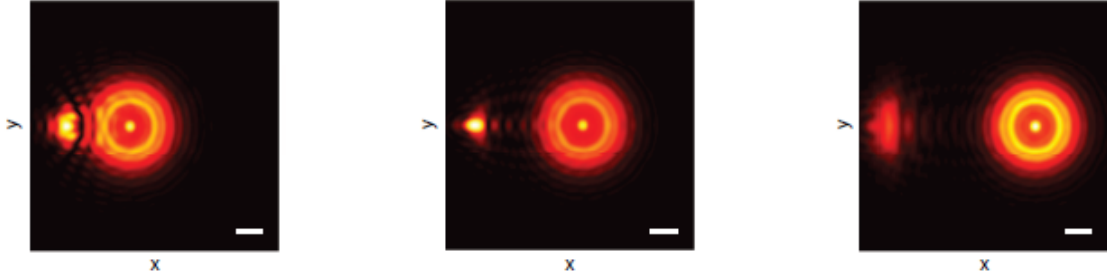
Figure 1: Calculated images of a particle next to an angled micromirror as the lateral (xy) position is varied. The direct image (toward the right side of each frame) is aberrated because it is out of focus but has a symmetric profile that does not alter its centroid position. The reflected image (toward the left of each frame), whose centroid is used to calculate the z position, is heavily aberrated. The scale bar spans 1 μm. The contrast has been nonlinearly enhanced. Simulation details: We modeled a 40X/NA 0.9 air objective, with the particle and micromirror in water. z corresponds to the distance from the cover glass ($z = 0$), with the particle at $z = -5$ μm and the Gaussian focus of the optical system at $z = -8$ μm. The micromirror begins at $z = -2$ μm, intersects the optic axis at $z = -9$ μm, and ends at $z = -100$ μm. The coverglass thickness was taken to be 160 μm, 10 μm less than the design value of 170 μm.

z position determined from simultaneous reflections from different mirror faces. This systematic effect is both subtle and insidious, potentially limiting the utility of micromirror-based orthogonal imaging devices.

For these devices to realize their potential for nanometer-resolution tracking and imaging in three dimensions, systematic errors must be understood and alleviated. Here, we explain the physical origin of systematic errors in PMW imaging and confirm our explanation by calculating the image of a point particle in close proximity to an angled reflective surface. We show that systematic errors arise due to the presence of an angled micromirror in the object space, which occludes both reflected rays and direct rays in an angle- and position-dependent fashion. This angular truncation of collected light results in a lateral distortion of the reflected image of a particle that depends on the full geometry of the problem, including the particle's position and proximity to the mirror surface. To calculate these effects, we closely follow the method of Gibson and Lanni,[11] which is based on a simple ray-tracing model to calculate wavefront aberrations within a scalar diffraction theory.[12] Accounting for the additional geometric constraints introduced by the micromirror, we have developed an extended scalar model that captures much of the complexity of the PMW imaging system and successfully predicts the systematic error between the apparent position of a particle's reflection and the expectation from simpler geometric considerations.

As shown in Fig. 1, calculated images of a particle and its reflection show rather exotic aberrations resulting from the position-dependent truncation of large-angle rays that would otherwise be captured by the high numerical aperture optics. As a result of these aberrations, the apparent positions of the direct and reflected images of a single particle differ from the geometric prediction of the law of reflection. To quantitatively evaluate this effect, we calculated the diffracted image of a particle as its position is scanned in the xz plane, next to a surface with 26 % reflectivity inclined at $\theta_{mirror} = 54.7°$; these parameters correspond to Refs.[7,9] in which micromirrors are formed along the {1 1 1} crystal plane of silicon. The position of the direct and reflected images were then determined through standard particle tracking methods and the z-position of the particle was reconstructed from the geometrical prediction. The resulting errors are shown as a function of x and z in Fig. 2. If an error in the reconstructed z position does not depend on the particle's position, then it can be neglected as a simple offset term. However, as shown in the figure, diffractive aberrations lead to a variation of the error of approximately 600 nm over the 2 μm$\times$2 μm scan. The calculated values compare favorably with the 100 nm variation observed over approximately 500 nm travel in our experimental results.

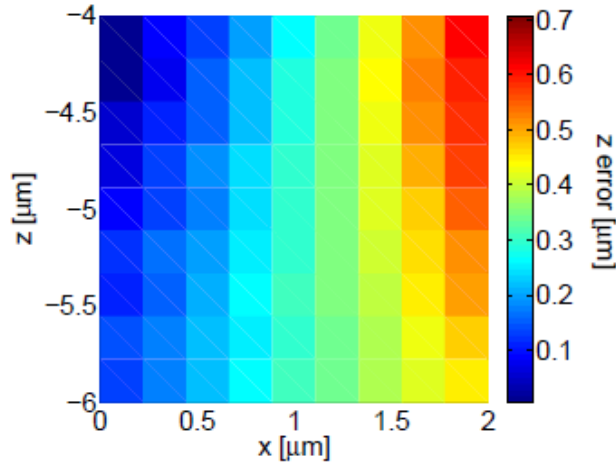Figure 2: Calculated errors in the reconstructed z position (colorbar) as a function of the particle's x and z position. Simulation parameters are the same as in Fig.1.

In summary, we have shown that a relatively simple extension of Gibson and Lanni's scalar diffraction model accounts for systematic errors in three-dimensional particle tracking using angled micromirrors in a high numerical aperture optical system. We find good qualitative agreement with our experimental results.[9] An extension of these calculations to include polarization-dependent effects within a vector diffraction theory could be accomplished with established theories.[13,14] The scalar theory developed here, or an extended vector calculation, may be sufficient to compute a reliable three-dimensional point-spread function for use in deconvolution microscopy.[15] This would enable researchers to exploit the additional z information gained through the inclusion of micromirrors within the sample volume to reconstruct complete three-dimensional images of complicated specimens.

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