3D Nanoparticle Trajectories by Orthogonal Tracking Microscopy

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In order to control directed self-assembly processes involving fluid-borne nanoparticles and patterned surfaces, we must be able to follow the detailed motions of nanoparticles in an environment where the potential varies in 3D. We have developed and quantitatively evaluated a new 3D tracking technique.

Conventional microscopes view a 3D volume as a 2D plane. Centroid-finding techniques permit the 2D coordinates of a nanoparticle moving in solution to be found with nanometer accuracy given enough signal-to-noise. However, getting the depth is more challenging. One recently developed strategy is to use angled micromirrors mounted within the microscope field of view to project a reflected side-on image of the volume of interest.[1] With this technique, termed orthogonal tracking microscopy,[2] we are able to resolve a particle's vertical position (Figures 1-3) using only computationally simple centroid-based image analysis[3].

We have performed the first quantitative study of orthogonal tracking microscopy. Using an EMCCD camera, we have tracked a 190 nm fluorescent polymer bead with precision < 20 nm in 3D in a movie acquired at 333 frames per second[2]. The high precision at high speed indicates that this technique may enable real-time feedback controlled nanoparticle assembly processes. One hurdle we have discovered, however, is that the introduction of angled micromirrors into the field of view of a high-numerical-aperture microscope appears to introduce a unique kind of aberration. If the angled mirror is steeper than the collection angle of the objective, the reflected image can be truncated asymmetrically, introducing bias into the depth measurement and reducing the accuracy of the technique. This bias appears pervasively in a variety of experimental conditions (Fig. 4); preliminary simulations based on scalar diffraction theory[4] indicate that the asymmetric angular aperture is the dominant effect. Measurements of complex objects, such as biological cells, will have to account for this aberration if orthogonal tracking is to be useful.

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[2]M.D. McMahon, A.J. Berglund, P.T. Carmichael, J.J. McClelland, J.A. Liddle, *ACS Nano*, to be published (2009).
[3]A.J. Berglund, M.D. McMahon, J.J. McClelland, J.A. Liddle, *Opt. Express* **16**, 14064 (2008).
[4]S. F. Gibson, F. Lanni, *J. Opt. Soc. Am. A* **8**, 1601 (1991).

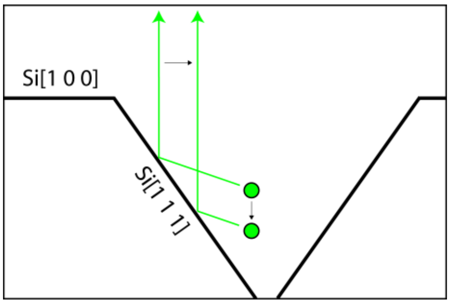

Figure 1. Orthogonal tracking cartoon. Vertical motion in the well is projected by reflection into lateral motion. A simple geometric correction is necessary because the (111) mirrors are oriented at 54.7° rather than 45° to the (100) wafer surface.

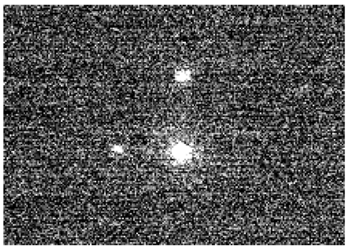

Figure 2. Movie frame showing direct image of a single 190 nm diameter polystyrene bead along with reflections from the upper and left faces of a well. There are two reflections because the particle is in a corner of a well.

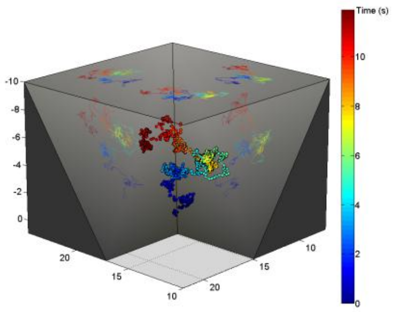

Figure 3. 3D trajectory obtained from centroid analysis of frames like Fig. 2 with a simple geometric conversion; cartoon well walls are a guide to the eye.

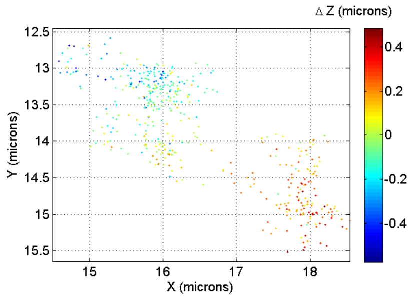

Figure 4. Experimental example of discrepancy between depth measurements from orthogonal well faces as a function of lateral particle position; we attribute the discrepancy to the angular aperture truncation effect.