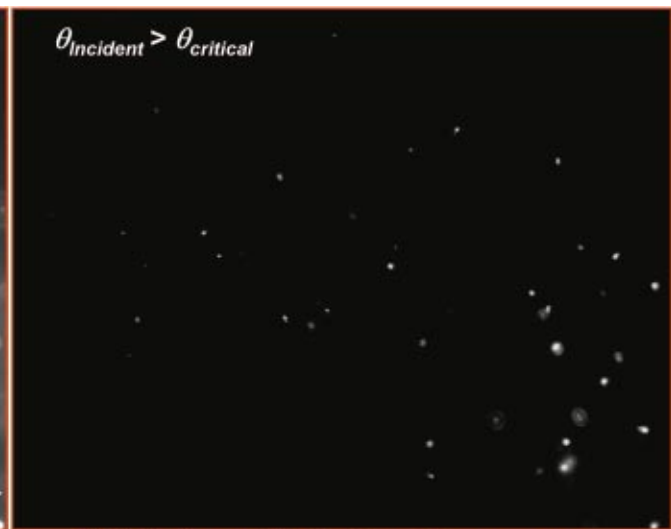
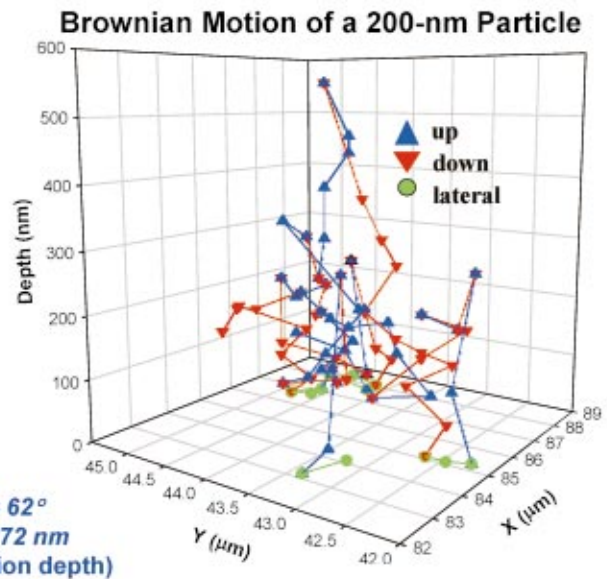
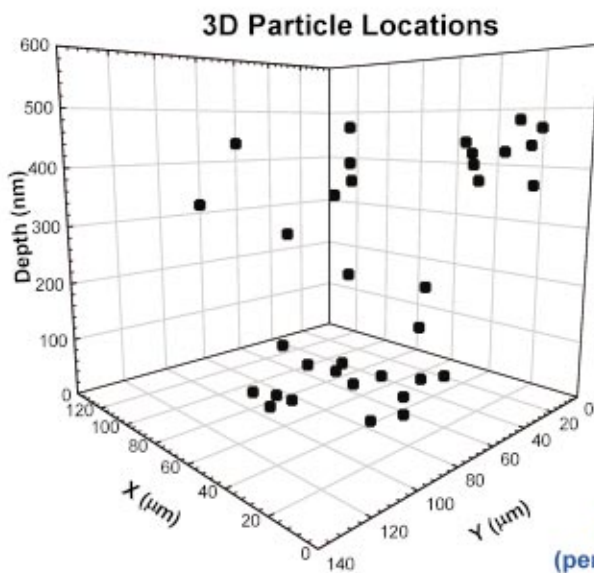


Regular Microscopy



Total Internal Reflection Microscopy



$\theta_i = 62^\circ$   
 $z_p = 272 \text{ nm}$   
 (penetration depth)

## Three-Dimensional Tracking of Nanoparticles Using R-TIRFM\* Technique

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The novel **Ratiometric-Total Internal Reflection Fluorescence Microscope\*** (R-TIRFM) imaging technique enables nano-particle tracking in the line-of-sight ( $z$ ) direction with a nanometer spatial resolution. The digital tracking of the particle image centers on the  $x$ - $y$  plane provides its lateral locations with the spatial resolution equivalent to the CCD pixel dimensions. An oil immersed TIRF lens (60X, NA=1.45) is used to track 200-nm fluorescent micro-sphere tracers illuminated by an argon-ion blue laser. The technique is the first of its kind to tag and track nano-particles with nanometer spatial resolutions in a full three-dimensional way. This work was partially sponsored by the NASA-Fluid Physics Program Grant No. 3-2712 and partially by the USDOE/Argonne National Laboratory Grant No. DE-FG02-04ER46101.