Optical Tracking of Three-Dimensional Brownian Motion of Nanoparticles

C. K. Choi and K. D. Kihm

Abstract. Novel optical techniques are presented for three-dimensional tracking of nanoparticles; Optical Serial Sectioning Microscopy (OSSM) and Ratiometric Total Internal Reflection Fluorescent Microscopy (R-TIRFM). OSSM measures optically diffracted particle images, the so-called Point Spread Function (PSF), and determines the defocusing or line-of-sight location of the imaged particle measured from the focal plane. The line-of-sight Brownian motion detection using the OSSM technique is proposed in lieu of the more cumbersome two-dimensional Brownian motion tracking on the imaging plane as a potentially more effective tool to nonintrusively map the temperature fields for nanoparticle suspension fluids. On the other hand, R-TIRFM is presented to experimentally examine the classic theory on the near-wall hindered Brownian diffusive motion. An evanescent wave field from the total internal reflection of a 488-nm bandwidth of an argon-ion laser is used to provide a thin illumination field of an order of a few hundred nanometers from the wall. The experimental results show good agreement with the lateral hindrance theory, but show discrepancies from the normal hindrance theory. It is conjectured that the discrepancies can be attributed to the additional hindering effects, including electrostatic and electro-osmotic interactions between the negatively charged tracer particles and the glass surface.

Key Words: Optical Serial Sectioning Microscopy (OSSM), Ratiometric Total Internal Reflection Fluorescent Microscopy (R-TIRFM), Brownian Motion, Diffusion

1. Introduction

Single molecule detection (SMD) techniques to visualize the dynamic behavior and reaction kinetics of individual molecules in living cells have recently attracted a great deal of attention\(^1, 2\). The rapid development and progress of SMD techniques have ushered in a revolution in biological research. Stochastic characteristics of reactions of biological molecules, even if the reactions of biomolecules are initiated at the same time, cannot be synchronized as a result of which dynamic behaviors of individual molecules are averaged and hidden in the ensemble-averaged movements\(^3\). SMD is generally based on two key technologies, single molecule imaging and single molecule manipulation. But there are certain optical issues associated with the detection and subsequent tracking of single molecules. The size of individual molecules is on the order of nano-meters and so they are too small to be visualized by conventional optical microscopy. To overcome this problem, bio-molecules are labeled by fluorescence dyes and visualized using fluorescence microscopy.

The non-invasive nature of the fluorophores associated with the high sensitivity and contrast has made fluorescence microscopy a prominent tool in modern cell biology\(^4\). However, a significant drawback of light microscopy is dictated by the laws of diffraction. The limit of resolution that can be reached by optical techniques is directly proportional to the wavelength of incident light\(^5\). This diffraction limit originates from the fact that it is impossible to focus a beam of light to a spot smaller than approximately its wavelength. Therefore, there are two possible research areas as follows; one is to utilize this optical drawback of diffraction, and the other is to break this diffraction limit.