Studies of the Anisotropic Diffusive Motion of Nanoparticles at the Solid-Liquid Interface Using Near-Field Fluorescence Correlation Spectroscopy

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ABSTRACT

Near-field fluorescence correlation spectroscopy measurements of 20 and 40 nm polystyrene nanoparticles were performed, using fiber probes with aperture diameters ranging from 100 to 250 nm. The experimental data were best fit by a two-dimensional diffusion model, implying that there was significant anisotropy associated with the movement of the particles, such that mobility normal to the wall was much lower than that parallel to the wall. Interestingly, the lateral diffusion coefficients were approximately ten times greater than predicted from the Stokes-Einstein relationship corrected by taking into account hydrodynamic drag forces due to particle-wall interactions. This discrepancy between experimental and theoretical results is possibly due to strong viscous forces as well as attractive forces at the interface.

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1. Introduction

Fluorescence correlation spectroscopy (FCS) is one of more powerful techniques available for elucidating the dynamic behavior of molecules and nanoparticles, and has been extensively applied in the field of biophysics.^{1,2} FCS enables the noninvasive study of biological samples with high temporal and spatial resolution. Measurements using this technique typically involve a confocal observation volume, with particles moving in and out as the result of Brownian motion, and this random motion in the small volume leads to fluctuations in fluorescence intensity. Through statistical analysis of these fluctuations using an autocorrelation function, FCS calculates the average residence time of particles in the observation volume, and this provides information concerning photophysical and photochemical reactions and mobility of the particles.³

Conventional FCS requires the sample to be prepared as a dilute solution, since the size of the observation volume is limited by optical diffraction. In the past, such dilution has restricted the application of FCS, especially in biological analysis. As an example, the observation volume in confocal microscopy is on the order of 0.2 fL and therefore, in order to isolate individual particles in solution, pM to nM concentrations are required. In many enzyme reactions, however, the interactions involved are weak and their dissociation constant is in the μM to mM range, thus it becomes necessary to reduce the detection volume using subdiffraction optical methods.^{4,5} To address this issue, we have recently developed a new FCS technique which employs a near-field optical fiber probe consisting of a metal-coated fiber tip with a small aperture, a device which is widely used in near-field scanning optical microscopy (NSOM).⁶

NSOM-FCS is a convenient analytical tool for the study of the Brownian motion of nanoparticles near a wall. The problems presented by viscous wall drag are important in the design of solution-based sensing and analytical systems, which are currently being scaled down from microscopic to nanoscopic dimensions. To date, the dynamics of nanoparticles near a wall have been studied via evanescent wave dynamic light scattering (EWDLS) based on

Figure 1. (Color online) Comparison of the relative observation depths of (a) evanescent wave dynamic light scattering and (b) near-field optical microscopy.

total internal reflection [Fig. 1(a)], in which there is a restricted observation depth from the wall, typically in the range of 150–400 nm based on the $1/e$ decay in the electric field intensity.^{7,8} Conversely, when NSOM-FCS is employed, the penetration depth from the aperture probe is much smaller, such that in the case of a 100 nm aperture the penetration depth can be reduced to 25 nm [Fig. 1(b)]. This allows for the analysis of particle mobility directly at the wall interface, since, at this extremely small distance, it is possible to unambiguously observe particle-wall interactions.

In this study, we conducted NSOM-FCS measurements of 20 and 40 nm polystyrene nanoparticles in aqueous dispersions using an NSOM fiber probe with aperture diameters ranging from 100 to 250 nm. We discuss the anisotropic diffusive motion of the particles resulting from particle-wall interactions and compare the anisotropic

Figure 2. (Color online) Experimental apparatus for fluorescence correlation spectroscopy based on near-field optical microscopy (NSOM-FCS).

diffusion constants derived from this work with those obtained using EWDLS with larger particles.

2. Experimental

Before our work,⁶ only one study reported the application of an NSOM fiber probe to FCS measurements, in which the NSOM probe was used solely to provide local illumination and fluorescence data were collected using a far-field-based technique.⁹ The limited application of NSOM probes has been due to their low light propagation efficiency as well their tendency to generate strong background noise signals due to autofluorescence and Raman scattering within the fiber core. However, by employing a carefully designed probe with much higher propagation efficiency,¹⁰ we have been able to conduct FCS measurements while operating in a hybrid illumination-collection mode, such that much smaller observation volumes are obtainable, as compared to the traditional illumination-only mode.⁶

The NSOM probes used in this study were made from a single-mode optical fiber with a 2 μm diameter core. A probe tip with a tapered structure was fabricated by chemical etching of the fiber with buffered hydrofluoric acid.¹¹ The probe was designed with a double-tapered structure to enhance the propagation efficiency of light by reducing the cut-off region of the waveguide. After coating the tip with gold by a sputtering process, a small aperture was created by forcing the tip apex against a sapphire substrate.¹² NSOM probes were produced in this manner with 100, 150, 210 and 250 nm diameter apertures.

A CW He-Ne laser with a wavelength of 632.8 nm was used as the illumination source and the background noise level associated with the apparatus was characterized by acquiring a spectrum from the NSOM fiber probe solely due to autofluorescence and Raman scattering. A dominant peak attributed to Raman scattering was observed, which exhibited a maximum at approximately 655 nm and grew considerably weaker above 690 nm.⁶ Based on this result, we chose to study fluorescent polystyrene beads with diameters of 20 and 40 nm and with excitation and emission maxima of 660 and 680 nm, respectively (Invitrogen F8783 and F8789). The bead surface is carboxylate modified.

The NSOM-FCS apparatus used in this work is shown in Fig. 2. He-Ne laser light was coupled to the fiber probe, the beads were illuminated with the laser light through the probe aperture and the resulting fluorescence emissions were collected through the same aperture, corresponding to the illumination-collection mode of NSOM. The fluorescence signal was separated by a beam splitter and detected by two photon-counting avalanche photodiodes (APDs), following which the two signals were converted to a cross-correlation function using a digital correlator (ALV Model

Figure 3. (Color online) Cross correlation functions of FCS measurements using the cleaved end of an optical fiber for 20 nm (blue) and 40 nm (red) beads. Solid curve fits were calculated using Eq. (1).

5000E). We used dual APDs to calculate the cross-correlation function to reduce any artifacts in the data due to afterpulses in the photodiodes.¹³

3. Results and Discussion

For reference purposes, we first acquired FCS measurements using the cleaved end of an optical fiber. Figure 3 shows the cross-correlation functions obtained in this manner for both 20 and 40 nm fluorescent beads. The cross-correlation function $G(\tau)$ in these plots has been normalized by its initial value $G(0)$. The results were analyzed using conventional FCS theory for three-dimensionally diffusing particles, and curves were fit to the experimental data using the equation

$$G_{3D}(\tau) = \frac{1}{N} \left(1 + \frac{\tau}{\tau_D} \right)^{-1} \left(1 + \frac{\tau}{K^2 \tau_D} \right)^{-1/2}, \quad (1)$$

where N is the average number of particles in the observation volume and K is the axial-to-lateral dimensional ratio of the observation volume. The lateral diffusion time, τ_D , is given by

$$\tau_D = \frac{w_0^2}{4D}, \quad (2)$$

where w_0 is the lateral radius of the observation volume (i.e., the mode field radius of the optical fiber) and D is the diffusion constant of the particles in water. D can be expressed as a function of the hydrodynamic radius of the particles, R , using the Stokes-Einstein formula

Figure 4. (Color online) Cross correlation functions of NSOM-FCS measurements for 20 nm (blue) and 40 nm (red) beads. Solid and dashed curve fits were calculated using Eqs. (1) and (4), based on three- and two-dimensional diffusion models, respectively.

$$D = \frac{kT}{6\pi\eta R}, \quad (3)$$

where k is the Boltzmann constant, T is the temperature, and η is the viscosity of water. The τ_D values for the 20 and 40 nm beads were calculated as 32 and 68 ms, respectively, using Eqs. (2) and (3), based on the actual values of the experimental parameters.

After these initial trials, we concentrated on experiments using NSOM fiber probes with small apertures. The cross-correlation functions obtained for 20 and 40 nm beads using a 250 nm diameter probe aperture are presented in Fig. 4. The results were analyzed using Eq. (1), assuming a value of 1 for K , based on the results of finite difference time domain (FDTD) calculations for the electric field distribution in the vicinity of the aperture.¹³ In contrast to the experiment using the cleaved end of the fiber, the curve fits to these data were not satisfactory, even when the value of K was allowed to vary over the range of 0.3 to 3. We therefore attempted to instead fit the experimental data using a two-dimensional diffusion model, as per Eq. (4).

$$G_{2D}(\tau) = \frac{1}{N} \left(1 + \frac{\tau}{\tau_D} \right)^{-1}. \quad (4)$$

As shown in Fig. 4, the curves resulting from the two-dimensional model more closely fit the experimental data. Although this result is somewhat unexpected, it may be at least partly explained in terms of the anisotropy of mobility of the particles caused by hydrodynamic interactions with a wall, such that the diffusion constant perpendicular to the aperture plane is much smaller than that parallel to it.⁸

We performed the same experiments for probes with aperture diameters of 100, 150 and 210 nm. Once again, the data acquired in these trials were best fit using the two-dimensional model for both particles sizes. Figure 5 plots the values of τ_D obtained by fitting experimental data with Eq. (4). The solid curves are theoretical estimates of τ_D calculated from Eq. (2), assuming that w_0 is equal to the aperture radius of the NSOM probe. For both particles sizes, the discrepancy between the experimental results and the theoretical predictions is significant, although the difference is more pronounced for the 40 nm particles. In addition to the anisotropy of mobility, these discrepancies might originate from hydrodynamic interactions of particles with the probe apex.

The dynamics of colloidal spheres in motion near a wall has been intensively studied, both theoretically and experimentally. During Brownian motion of a particle in the vicinity of a wall, the fluid surrounding the particle will undergo hydrodynamic interactions with the interface and so the particle experiences a larger drag force than in bulk form. Brenner calculated the diffusion coefficient of a particle of diameter ϕ_p moving perpendicularly to the wall under Stokes viscous force as

Figure 5. (Color online) Plots of τ_D obtained by fitting NSOM-FCS cross correlation functions with Eq. (4) as a function of aperture diameter. Solid curves are theoretical estimates of τ_D calculated from Eq. (2).

Figure 6. Diffusion coefficients D_n and D_t (normalized by D_0) as a function of relative distance from a wall, as calculated from Eqs. (5) and (6).

$$D_n = D_0/\lambda_n,$$

in which

$$\lambda_n = \frac{4 \sinh \alpha}{3} \sum_{n=1}^{\infty} \frac{n(n+1)}{(2n-1)(2n+3)} \times \left[\frac{2 \sinh[(2n+1)\alpha] + (2n+1) \sinh[2\alpha]}{(2 \sinh[(n+1/2)\alpha])^2 - ((2n+1) \sinh \alpha)^2} - 1 \right], \quad (5)$$

where $\zeta = \phi_p/2z$, $\alpha = \cosh^{-1} \zeta$ and z is the distance between the particle center and the wall.¹⁴ In the case of motion parallel to the wall, the expression may be approximated by

$$D_t = D_0/\lambda_t,$$

where

$$\lambda_t = \left[1 - \frac{9}{16} \zeta + \frac{1}{8} \zeta^3 - \frac{45}{256} \zeta^4 - \frac{1}{16} \zeta^5 \right]^{-1}. \quad (6)$$

Figure 6 displays plots of the calculated diffusion coefficients D_n and D_t , normalized by D_0 , as a function of z/ϕ_p . For perpendicular motion, D_n approaches 0 as the particle draws closer to the wall, while D_t becomes approximately one third the diffusion coefficient in the bulk. The anisotropic nature of the relationship between D_n and D_t may be the cause of the two-dimensional diffusion behavior noted above. The value of τ_D obtained by fitting the data via Eq. (4) corresponds to the lateral diffusion time related to the tangential diffusion coefficient D_t , as in Eq. (7) below.

Figure 7. (Color online) Comparison of tangential diffusion coefficients D_t obtained from NSOM-FCS measurements with those from EWDLS using 170 nm particles in Ref. 8.

$$\tau_D = \frac{w_0^2}{4D_t} \quad (7)$$

Figure 7 allows a comparison of the tangential diffusion coefficients obtained from our experimental data with results from EWDLS measurements using larger particles with ϕ_p of 170 nm.⁸ This figure plots normalized tangential diffusion coefficients as a function of $\xi = 2d/\phi_p$, where d is the penetration depth of the evanescent field based on $1/e$ decay of the electric field amplitude. For the evanescent field generated in the vicinity of aperture, we approximated d as the aperture radius based on the FDTD simulation and the bulk diffusion coefficient D_0 was calculated using Eq. (3). The values of D_t/D_0 for the 20 and 40 nm particles change almost linearly with ξ , but are much smaller than the values obtained for the 170 nm particles. Although both our measurements and the EWDLS work were performed over the same range of ξ , our smallest particle size (20 nm) is about one tenth of that used in the EWDLS work (170 nm), and therefore the distance between the smaller particles in our trials and the wall will also be proportionately smaller. At such small distance the electro-osmotic interaction can hinder the diffusive motion of nanoparticles. The glass part at the probe apex is directly exposed to the aqueous solution [see Fig. 1(b)]. The glass surface is negatively charged and the zeta potential is estimated to be ~ -60 mV at pH 7.0.¹⁵ The carboxylate-modified bead is also negatively charged with zeta potential of ~ -50 mV¹⁵ and therefore repulsively interacts with the glass surface. This interaction can cause a reduction of the diffusion coefficients beyond the theoretical prediction. Further experimental studies, such as measurements with solvents other than water or with

surface modified particles, will be required to further elucidate the nature of these interactions.

4. Conclusions

We performed near-field FCS measurements of nanoparticles, using specially designed fiber probes with aperture diameters ranging from 100 to 250 nm. We found that the experimental data were best fit using a two-dimensional motion model, implying that diffusivity normal to the wall is much smaller than that parallel to the wall. The values of measured lateral diffusivity were also much smaller than both theoretical predictions and values obtained using larger particles. This significant discrepancy from diffusion in the bulk material was partially explained by hydrodynamic interactions with the probe apex. Thus, although NSOM-FCS measurements allowed the visualization of the dynamics of nano-objects precisely at the solid-liquid interface, further work will be necessary to determine the mechanism involved in the anomalous behavior observed during this study.

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