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# Nanoparticle sizing method based on fluorescence anisotropy analysis

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ABSTRACT

Demand for applications of nanoparticles in electric architecture has been increasing. Nanoparticles provide new opportunities for improving circuit response. We discuss a novel method for evaluating nanoparticle sizes based on fluorescence anisotropy analysis. Particle size evaluation is possible through measurements of the rotational diffusion coefficient, which is sensitive to particle size. We develop a system for measuring rotational diffusion coefficients by using a fluorescent probe to label a particle. We report fundamental experiments that verify the feasibility of the proposed method. The rotational diffusion coefficients of gold nanoparticles with diameters ranging 6–20 nm were measured using the proposed method. The measured rotational diffusion coefficients decrease with increasing particle size. This finding indicates that nanoparticles smaller than 15 nm can be measured with fine resolution.



## 1. Introduction

Metal nanoparticles, which exhibit unique optical, electrical, and chemical properties, are key materials in the development of functional nanostructure devices [1–9]. Controlling particle size and monitoring the aggregational state are important to the manufacturing process of such devices because device functionality is determined by particle size and arrangement.

Generally, dynamic light scattering (DLS) can be used to evaluate particles in a solution without a drying process. DLS is a very useful technique, covering a wide range of particle sizes, from nanometers to micrometers. However, in the case of small nanoparticles, evaluation of particle sizes and aggregation becomes difficult. Analyzing small nanoparticles (<20 nm in diameter) within a solution, including aggregation, is difficult owing to the particle-size dependence of signal intensity [10]. Signal intensity in this case is proportional to the sixth power of particle size.

We suggest a particle sizing method based on the analysis of rotational Brownian motion. In the proposed method the range of particle sizes is narrow but the method is sensitive to the small nanoparticles. The rotational Brownian motions of nanoparticles depend on their sizes. The average size of particles can be evaluated from the rotational diffusion coefficient, which is a factor representing the rotational speed of Brownian motion, when the size distribution is determined to have a Gaussian profile. The rotational diffusion coefficient can be measured using a fluorescence polarization technique. By using a fluorescent probe to label a particle, the rotational diffusion coefficient of the fluorescent probe, which is related to the particle size, can be measured by analyzing the polarization direction of fluorescence emitted from the probe.

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The proposed technique is expected to be applied in quality control of the metal nano particle formation for manufacturing single-electron devices [4].

For particle size measurements, we propose a nanoparticle-sizing method based on this fluorescent polarization. We developed a rotational diffusion coefficient measurement system and a fluorescent probe used to label the particles. In this study, we verified the feasibility of the proposed method by performing fundamental experiments that investigated the rotational diffusion coefficient of a fluorescent DNA probe connected to gold nanoparticles.

When measuring the rotational diffusion coefficient of a rigid spherical particle that can emit fluorescence by itself, the rotational diffusion coefficient $D_r$, which is rotational diffusion coefficient of rigid sphere particle, can be described by the Debye–Stokes–Einstein equation [11–13].

$$D_r = \frac{k_B T}{6V\eta} = \frac{k_B T}{\pi d^3 \eta} \tag{1}$$

where $V$ is the volume, $k_B$ is the Boltzmann constant, $T$ is the temperature, $\eta$ is the solvent viscosity, and $d$ is the particle diameter. Eq. (1) shows that the rotational diffusion coefficient is proportional to the inverse of the third power of the particle diameter. The particle size can be calculated from Eq. (1), and $D_r$ is very sensitive to changes in particle size due to the inverse-cube relationship.

On the other hand, most metal nanoparticles are not fluorescent on their own. When measuring the rotational diffusion coefficient of metal nanoparticles, a fluorescent probe is used to label the nanoparticles for measuring the rotational diffusion coefficient. In this case, the measurement of nanoparticle rotational diffusion coefficients is indirect because the rotational diffusion coefficient of the fluorescent probe is not equal to the rotational diffusion coefficient of the metal nanoparticle.

In the proposed method, in order to establish the particle-sizing method based on the rotational diffusion coefficient measurements of the fluorescent probe, we use standard particle to define the scaling factor, which defines the relationship between the nanoparticle's diameter and the measured rotational diffusion coefficient of the fluorescent probe, that is attached to the nanoparticle. First, the standard curve for the fluorescent probe is generated by varying the standard particle's size. Next, the sizes of unknown particles are measured. In this study, we investigate the relationship between the rotational diffusion coefficient of a fluorescent probe connected to a metal nanoparticle and the rotational diffusion coefficient of the metal nanoparticle itself.

## 2. Nano particle sizing method

### 2.1. Rotational diffusion coefficient measurement using fluorescent DNA probe

A standard coordinate system determined to evaluate fluorescence anisotropy is shown in Fig. 1. The fluorescent probe is located at the origin and illuminated by excitation light, which travels along the $z$-axis. The probe emits

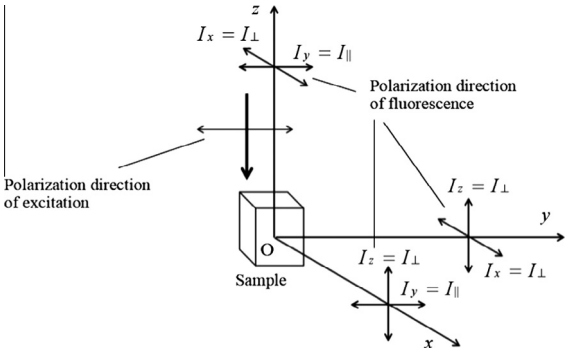


**Fig. 1.** Standard coordinate system.

fluorescence in all directions. $I_\parallel$ and $I_\perp$ are the parallel and perpendicular components of fluorescence intensity with respect to the polarization direction of the excitation light.

Fig. 2 shows the variation in the rotational motion of the fluorophore located on top of the fluorescent probe and the polarization direction of the fluorescence emitted from the fluorescent probe over time in the x–y plane. The fluorophore has absorption and emission moments and is excited by light with polarization parallel to the absorption moment.

After the fluorophore is excited, $I_\parallel$ decreases and $I_\perp$ increases over time owing to the rotational motion. The fluorescence anisotropy can be described by

$$r(t) = \frac{I_\parallel(t) - I_\perp(t)}{I_\parallel(t) + 2I_\perp(t)} \tag{2}$$

Assuming that the sample is a spherical rigid rotor, $r(t)$ is described by

$$r(t) = r_0 \exp\left(-\frac{t}{\theta}\right) \tag{3}$$

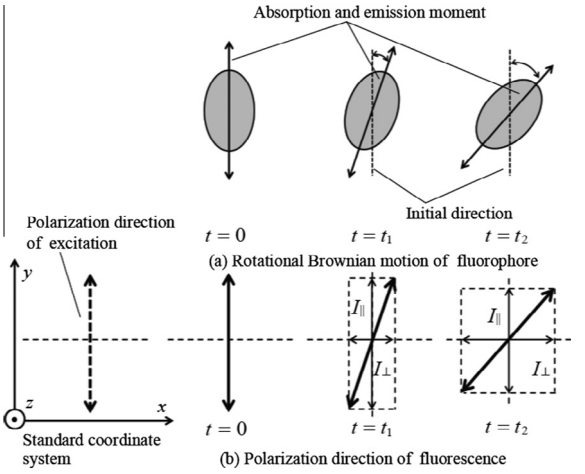


**Fig. 2.** Relationship between rotational Brownian motion of fluorophore and polarization direction of fluorescence ($0 < t_1 < t_2$).