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STUDY ON NANOPARTICLE SIZING USING FLUORESCENT POLARIZATION METHOD WITH DNA FLUORESCENT PROBE

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Abstract:

A fluorescent polarization method is well known for the detection of complementary base pairing of DNA in biological field. The fluorescent polarization method (FP) measures the rotational diffusion coefficient of Brownian motion of the fluorescent particle in the solution. The rotational diffusion coefficient is corresponding to inverse third power of diameter due to the Einstein Stokes Relation for nanoparticle as hard sphere.

We develop a novel rotational diffusion coefficient measurement method by using a fluorescent probe with DNA spacer, which is connected to particle. We investigate the relation between the gold nanoparticle and the fluorescent probe in order to verify the feasibility of the proposed method. The rotational diffusion coefficients of gold nanoparticles, whose diameters are from 5 nm to 20 nm, are evaluated by using the developed system. In this paper we describe the method of fluorescent polarization method by using fluorescent DNA probe (fl-DNA).

Keywords: nanoparticle sizing, fluorescent DNA probe, gold nanoparticle, fluorescent polarization, rotational diffusion coefficient, dynamic light scattering

1. INTRODUCTION

Controlling metal nanoparticle size and monitoring the aggregational state are important to the manufacturing process of functional nanostructure devices [1-5]. We suggest a particle sizing method based on the analysis of rotational Brownian motion. The average size of particles can be estimated from the rotational diffusion coefficient, which is a factor representing the rotational speed of Brownian motion. The rotational diffusion coefficient can be measured using a fluorescence polarization technique. The rotational diffusion coefficient of the fluorescent probe can be measured by analyzing the polarization direction of fluorescence emitted from the probe when a fluorescent probe is labeled on a particle.

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 [6-8].

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

where k_B is the Boltzmann constant, T is the temperature, η is the viscosity of solvent, and d is the particle diameter. Equation (1) shows that the rotational diffusion coefficient is proportional to the inverse of the third power of the particle diameter.

In this study, we developed a rotational diffusion coefficient measurement system and a fluorescent DNA probe (fl-DNA), that used to label the particle. A fluorescent probe is excited the polarized fluorescence signal, that reflect the sizes of labeled particles.

By using fluorescent polarization method, we investigate the relationship between the rotational diffusion coefficient of a fl-DNA connected to a metal nanoparticle. The rotational diffusion coefficient of the metal nanoparticle itself in order to verify the feasibility of the particle sizing by using fluorescent polarization methods [9-11].

2. ROTATIONAL DIFFUSION COEFFICIENT MEASUREMENT USING DNA PROBE

A standard coordinate system determined to evaluate fluorescence anisotropy is shown in Figure 1. $I_\parallel$ and $I_\perp$ are the parallel and perpendicular components of fluorescence intensity with respect to the polarization direction of the excitation light. Figure 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. 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

$$r(t) = r_0 \exp\left(-\frac{t}{6D_r}\right) \tag{3}$$

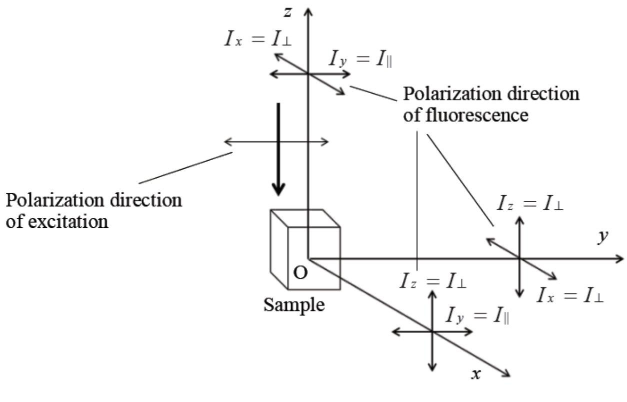

Fig. 1: Standard coordinate system

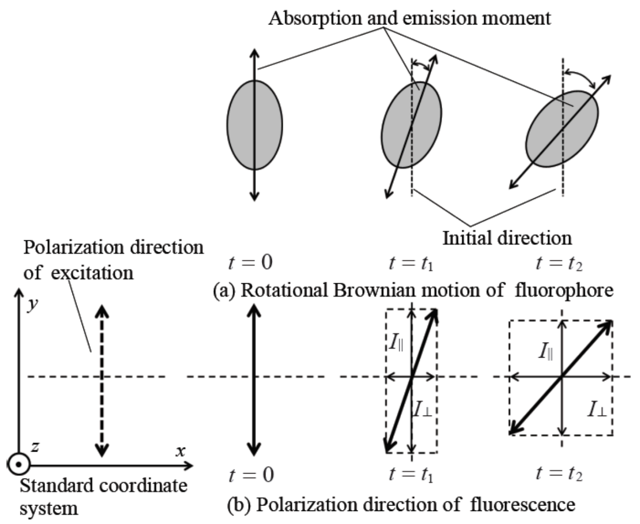

Fig. 2: Relationship between rotational Brownian motion and polarization direction of fluorescence

3. EXPERIMENTAL SETUP

3.1 Structure of the fluorescent probe

The experimental setup is shown in Figure 3. A linearly polarized laser with 488 nm wavelength is the excitation light. The symbol L means lens, M, mirror, and DM, dichroic mirror, respectively. The excitation light is modulated into a sinusoidal waveform when passed through an acousto-optic modulator (AOM). After the polarization direction of the excitation light is adjusted to be along the y-axis by a half-wave plate (1/2WP) and polarizer (P), the excitation light is focused onto the sample through the objective lens. The fluorescence emitted from the sample passes along the z-axis through the dichroic mirror (DM) and emission filter, while the reflected excitation light from the sample is blocked by these two components. The beam displacer divide the fluorescence to two orthogonal oriented polarization signal, that is $I_\parallel$ and $I_\perp$.

Both $I_\parallel$ and $I_\perp$ are evaluated by analyzing the brightness of the fluorescent spot in the image captured by the CCD camera. A sinusoidally modulated fluorescence signal is achieved by shifting the phase of the trigger signal of the image intensifier. The period of the trigger signal is synchronized with the period of modulation signal of the AOM. When the phase of the enhanced fluorescence signal is shifted. We scan the phase shift as much as 2 periods, that is from 0 degree to 720 degree, a sinusoidal modulation of the fluorescence signal is acquired as shown in Figure 4. Y_{DC} and Y_{AC} are obtained by modulated amplitude of polarized fluorescence emission and their DC intensity.

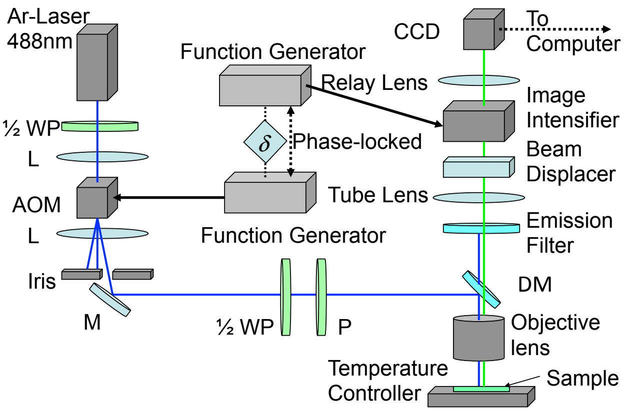

Fig.3 Experimental setup

$$Y_{DC} = \frac{I_{0\parallel}}{I_{0\perp}} \tag{4}$$

$$Y_{AC} = \frac{A_\parallel}{A_\perp} \tag{5}$$

We can calculate the rotational diffusion coefficient D_{r} based on Y_{DC} and Y_{AC} in fluorescence anisotropy imaging microscopy method (rFLIM) [10].

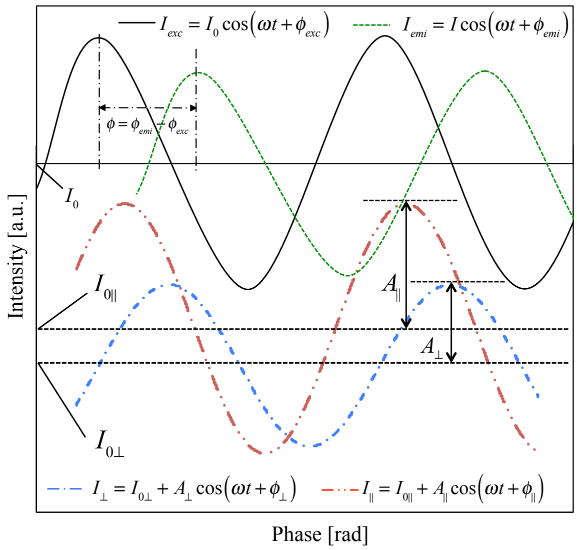

Fig. 4: Schematic of modulated fluorescence signal

3.2 Process to connect fl-DNA on nanoparticle

We arrange the fl-DNA on nanoparticle by forming thiol self-assembled monolayers (SAMS). Thiol SAMs are also important in the synthesis of gold nanoparticles, for stabilization of the nanostructures against aggregation and to control the cluster size by tuning the hydrocarbon chain length. We consider that the fl-DNA works as both probe to detect the rotational diffusion coefficient and coating to keep the size of gold nanoparticle statistic during the particle sizing procedure [12].

The DNA probe, that is DNA modified 3-thiol and 5-fluorophore, is named fl-DNA. The 3'thiol modified DNA can adsorbed on gold surfaces (Au (111)) in which intermolecular forces play a key role. It can also attach to the surface of the different metallic surfaces like Au such as Ag, Cu, Pd, Pt, Ni and Fe, and semiconductor surface such as GaAs and InP [12]. A thiol modified fl-DNA of three parts: (1) the sulfur headgroup, which forms a strong, covalent bond with the particle surface, (2) the DNA spacer (of variable length), which stabilizes the SAM through van der Waals interactions, and (3) the fluorophore, which can emitted the fluorescence signal.

In general, adsorption is performed in 10–1000 μM solutions of thiols. Initially physisorption step is started, and then followed by chemisorption of the molecules.

After physisorption, thiol molecules chemisorb on the Au(111) substrate through the S headgroup, forming a strong covalent bond, in a process that takes at least some minutes. During the process the thiol molecule loses the mercaptan H atom, transforming itself in a thiolate. We can write the adsorption process as follows

$$\mathrm{R_nSH + Au \rightarrow (R_nSH)_{phys}Au} \quad (6)$$

$$\mathrm{(R_nSH)_{phys}Au \rightarrow R_nS–Au + 1/2H_2} \quad (7)$$

where R means 3’ end of DNA spacer, and reactions in Eq. 6 and Eq. 7 correspond to thiol physisorption and chemisorption, respectively.

4. FUNDAMENTAL PROPERTY OF LABELING DNA PROBE

4.1 Structure of the fluorescent probe

In order to measure the rotational diffusion coefficient of the nanoparticles, we need the fl-DNA to label the particles due to quenching of the fluorophore. Fluorophores directly attached to a metal nanoparticle are quenched because of surface energy transfer (SET) from the fluorophore to the metal nanoparticle. The energy transfer efficiency is described by Eq. (6) [13].

$$\Phi_{EnT} = \frac{1}{1+(l/l_0)^4} \quad (6)$$

where l is the distance between the fluorophore and nanoparticle and l_0 is called the Forster distance, which is the distance at which energy transfer efficiency is 50 %.

The fl-DNA consists of the fluorophore and a spacer of double-strand DNA. We can control the length of the spacer based on the base number of DNA. Through a reaction of the metal nanoparticle with 3’thiol-modified and 5’fluorophore-modified DNA [14], the probe attaches itself to the surface of the metal particle, as shown in Figure 5. We used DNA of 23 bases as a spacer. Given the lengths of particle-thiol and DNA-fluorophore linkers (≈ 1.8 nm) the distance between fluorophore and metal particles is about 9.6 nm, which is longer than the Forster distance, 9 nm, calculated in the case of energy transfer from an organic fluorophore to a gold nanoparticle [13].

Figure 5 shows the SET efficiency against the distance l_0 between fluorophore based on Eq. (7) [15].

$$l_0 = \left(\frac{0.225 c^3 \Phi_{dye}}{\omega_{dye}{}^2 \omega_F k_F} \right)^{1/4} \quad (7)$$

where c is speed of light, Φ_{dye} is quantum efficiency of fluorophore, and ω_{dye} is its frequency of electron transition. ω_{F} is Fermi frequency and k_{F} is wave number vector of metal nanoparticle. Therefore, the energy transfer efficiency is below 50%, and sufficient fluorescence intensity can be obtained for performing measurements.

Based on the energy transfer calculation, DNA of 23 bases block at least 50 % of surface energy transfer to gold nanoparticle. We consider the fl-DNA with 23 base are bright enough as a fluorescent probe to evaluate the rotational diffusion coefficient. In addition, we guess the short and strength spacer has advantage to keep the simple rotational motion of gold nanoparticle with fl-DNA.

4.2 Number of DNA probe on nanoparticle

A number of DNA coated on nanoparticle surface can be determined based on the experiments. Hurst et. al. investigate coverage of DNA on gold nanoparticle [16]. They investigate the number of DNA loading on gold nanoparticle by varying the size of gold nanoparticle from 15 nm to 250 nm. The results shows the maximum density of DNA on gold nanoparticle keeps constant with changing the diameter of gold nanoparticles. The maximum density of DNA with 10 base length is described as 4.24×10^{10} /cm^2 for gold nanoparticle.

Based on the results, we estimate the number of DNA on gold nanoparticle with diameter of 0 - 25 nm with assumption as DNA loading density is fixed as 4.24×10^{10}/cm^2 on gold nanoparticle surface. Figure 7 shows the DNA loading as a function of nanoparticle sizes. As shown in Fig. 7, the maximum number of DNA on the particle of 8.2 nm in diameter is about 10. In the case of measuring 20 nm particle, it is considered that the number of fl-DNA is smaller than 60. We consider the number of the fl-DNA is small enough to evaluate the rotational diffusion coefficient of nanoparticle precisely.

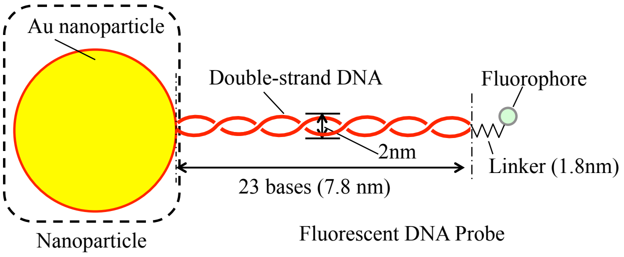

Fig. 5: Schematic of the fluorescent DNA probe

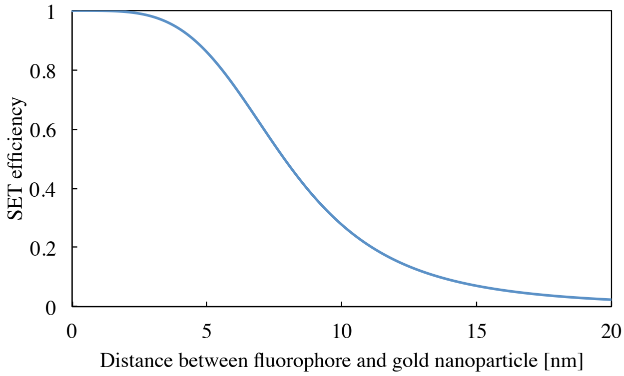

Fig. 6: Quenching of fl-DNA due to SET

5. CONFIRMATION OF DNA PROBE LABELLING

The absorbance peak of gold nanoparticle is known to be shifted to long wavelength range in the case of measuring the gold nanoparticle coating with dielectrics such as DNA [17]. We used the phenomena to check the coating of fl-DNA on gold nanoparticle. We prepare both the bare gold nanoparticle (8.2 nm in diameter) and the gold nanoparticle with fl-DNA. The absorbance peak of bare gold nanoparticle is appeared near 520 nm in the wavelength.

Figure 8 shows the comparison of the absorbance spectra of both bare gold nanoparticle and DNA functionalized gold nanoparticle. The spectra of gold nanoparticle with fl-DNA are shifted to the long wavelength as compared to that of bare gold nanoparticle. The peak shift has same tendency when the dielectrics is coated on the gold nanoparticle. From above results, we can confirm the labeling fl-DNA on nanoparticle surface.

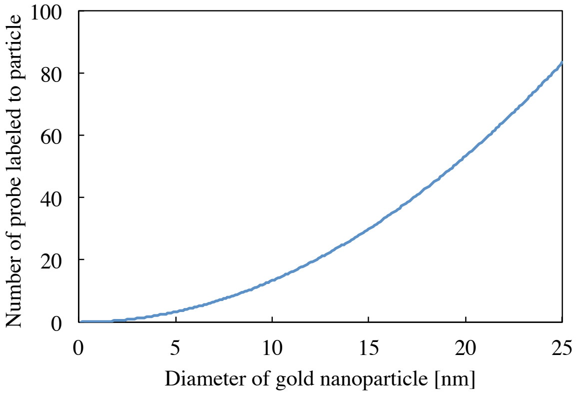

Fig.7 Number of fl-DNA on the gold nanoparticle

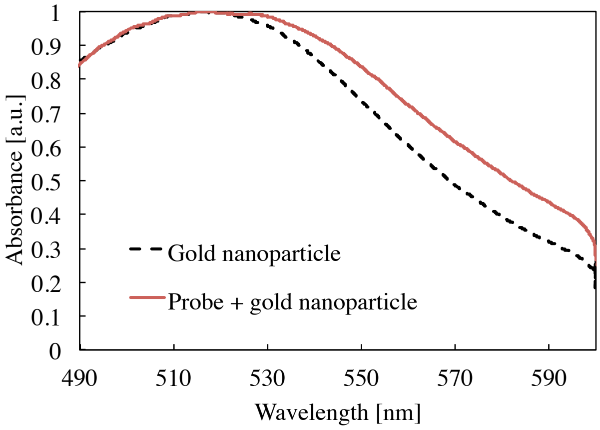

Fig.8 Absorbance spectra of gold nanoparticle

6. COMPARIZON OF ROTATIONAL DIFFUSION COEFFICINET

We measured rotational diffusion coefficients of gold nanoparticles with diameter of 8.2 nm. Figure 9 shows the TEM image. The shapes of the particle are sphere. And figure 10 show the typical distribution of gold nanoparticle of the sample. The distribution can be approximated Gaussian distribution. Fig. 11 also shows the particle diameter distribution by using dynamic light scattering method. As shown in Fig. 10 and Fig. 11, the distribution of particle diameter is narrow, that is from 5nm to 15 nm, and the value of the peak diameter is near 8 nm.

We prepare two samples. One is the fl-DNA probe with 23 base lengths. The other is the bare gold nanoparticle labeled with fl-DNA. The rotational diffusion coefficients are evaluated by varying the temperature of solvent by using the measurement system as shown in Fig. 3.

The temperature of solvents was maintained at 293 K, 298 K, 303 K, 308 K, 313 K, and the modulation frequency was set to 60 MHz, and the values were averaged from 10 experimental trials. The rotational diffusion coefficients of the fluorescent DNA probes connected to gold nanoparticles are shown in Figure 12. The horizontal axis shows the T/η, which shows particle mobility in solvent. The vertical axis shows the rotational diffusion coefficient. The error bars in the graph show the standard deviation of measurements.

The rotational diffusion coefficient is sensitive to the mobility of nanoparticle in this condition as shown in Fig. 12. It is considered that the gap of the rotational diffusion coefficient between fl-DNA and fl-DNA with gold nanoparticle has the information of the sizes of the gold nanoparticles.

D_r linearly increases with T/η in the range under 308 K for both the fl-DNA probe and fl-DNA probe connected to gold nanoparticle. The relation was fitted by Eq. 1.

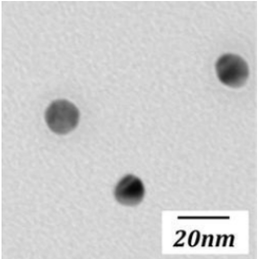

Fig. 9 TEM image of gold nanoparticle of 8.2 nm

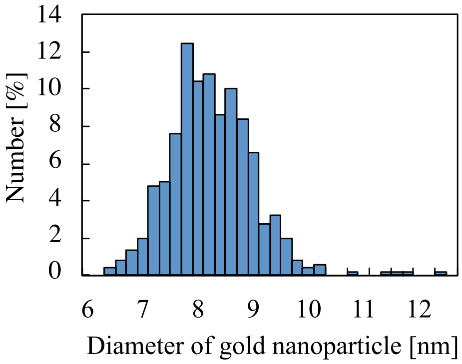

Fig. 10 Histogram of particle diameter distribution for 8.2 nm particles by using TEM

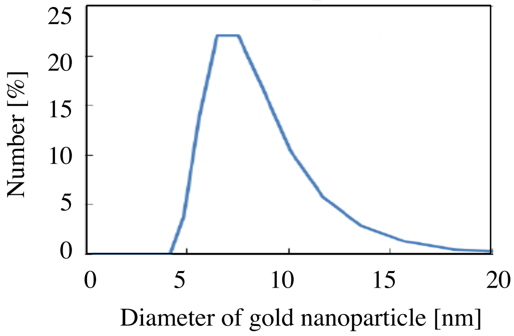

Fig. 11 Particle diameter distribution for 8.2 nm particles by using dynamic light scattering (DLS)

However, the nonlinearity is appeared in the range over 313 K. We have to investigate the reason of the nonlinear change over 313 K range. We have two assumptions. One is simply the decrease of viscosity of solvent. The other is the disaggregation of DNA in high temperature range.

From above results, we confirm the feasibility to evaluate the rotational diffusion coefficient for nanoparticle, whose size is smaller than 10 nm, in the range from 293 K to 313K. Based on the linear relation between D_r and T/η.

However the rotational diffusion coefficient, which is measured by using the proposed method, is not for the gold nanoparticle itself but the fl-DNA, which connected with gold nanoparticle. Thus we need to confirm the relation between the rotational diffusion coefficient of gold nanoparticle and that of fl-DNA connected to the particle.

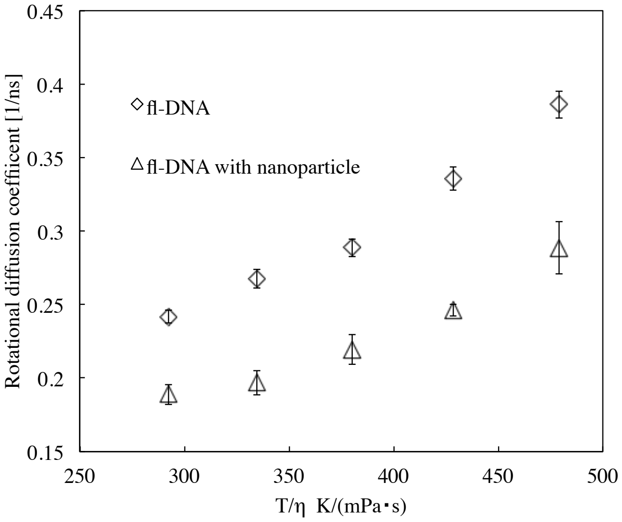

Fig.12 Rotational diffusion coefficient by varying T/η

7. CONCLUSION

In order to verify the feasibility of the proposed nanoparticle sizing method, fundamental experiments were performed. The rotational diffusion coefficient of the fluorescent DNA probe (fl-DNA) was measured precisely using the developed system.

We measured rotational diffusion coefficients of gold nanoparticles with diameter of 8.2 nm. The rotational diffusion coefficients decrease with the mobility of fl-DNA. It indicates that nanoparticles with diameters smaller than 15 nm can be evaluated by using the proposed method.

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