Development of in-situ evaluation system for monitoring the size of nanoparticles based on fluorescence polarization

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Nanotechnology research puts a great emphasis on the development of bottom-up technology to create functional nanostructure devices. The functions of the nanostructure are greatly influenced by the size and shape, and therefore it is important to monitor the growth of the structure. In this study, we are developing an in-situ evaluation system for monitoring the size of nanoparticles based on fluorescence polarization, aiming to control the growth of the nanoparticles by bottom-up methods at real-time. Fluorescence polarization is a technique used to estimate the size of macromolecular structure from the rotational diffusion constants, which can be obtained from the decorrelation of polarization in fluorescence of the fluorophore attached to the macromolecular structure.

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1. Introduction

Today, nanotechnology research puts a great emphasis on the development of bottom-up technology to create functional nanostructure devices. The bottom-up technology is one of the key technologies in future since the top-down technology such of photolithography hardly allows the large scale production of parts that are significantly smaller than 100 nm [1]. The commonly used bottom-up strategies for the production of nano-devices are wet-chemical production, biomolecule-directed nanoparticles organization, DNA-based nanoparticles aggregation, and etc. Metal nanoparticle with unique optical and electrical properties is one of the important building blocks for the bottom-up method. The functions of the nanostructures are not only greatly influenced by the overall structure, but also size and shape of this basic building block. Although the end-product can be evaluated with the current spectroscopy technology such as transmission electron microscopy, the in-situ evaluation technology is still limited. In order to enable precise fabrication of nanostructure devices by bottom-up methods, it is necessary to utilize real-time controlling, which is based on in-situ evaluation. The in-situ evaluation can also provide the better understandings of the fabrication mechanism, which allow further control of the fabrication method used.

In this study, we are developing an in-situ evaluation system for monitoring the size of nanoparticles based on fluorescence polarization, aiming to control the growth of the nanoparticles by bottom-up methods at real-time. The advantages of fluorescence polarization are its high sensitivity and fast responsivity, which make it suitable for in-situ real-time monitoring purpose. As the fundamental state of the development, our target is to measure the size of monodispersed nanoparticles ranging from a few nanometers to tens of nanometers, which function as the basic building block of the nano-devices.

2. Fluorescent Polarization

Fluorescence polarization is a technique for evaluating the size of a particle by analyzing the polarization condition of the fluorescence light emitted by the fluorescent molecule attached to the particle. As shown in Fig.1, particle with small size will have high degree of rotation due to Brownian motion, and the polarization plane of the light emitted will vary greatly from that of the excitation light. On the opposite, particle with bigger size will have low degree of rotation, and thus the light is emitted in the polarized plane almost same with the excitation light. The difference of the fluorescence polarization condition can be evaluated by fluorescence polarization P, as shown in the equation (1). $I_{\parallel}$ is the intensity of fluorescence component parallel to the incident polarized plane, while $I_{\perp}$ is the perpendicular component.

$$P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp}) \quad \text{-----(1)}$$

Steady-state excitation method is used in the current experiment setup, in which the fluorescence polarization P follows the basic equation of Perrin-Weber shown in equation (2). P_0 indicates the initial fluorescence polarization, θ the fluorescence rotational correlation time, τ the fluorescence lifetime.

$$\left(\frac{1}{P} - \frac{1}{3}\right) = \left(\frac{1}{P_0} - \frac{1}{3}\right) \cdot \left(1 + \frac{\tau}{\theta}\right) \quad \text{-----(2)}$$

To obtain the appropriate value with this steady-state method, the condition of $\theta \leqq \tau$ is required, so that sufficient information about the rotational motion of the particle can be obtained during one cycle of fluorescence emission.

3. Experiment Results and Discussions

3.1 Measurement of Dye

Alexa Fluor 488, which is similar to Fluorescein in chemical structure (the structure of both molecule is shown in the inset of Fig. 2), is measured. For simplification, Alexa Fluor 488 is approximated as a solid sphere, where the θ follows the equation (3). ϕ indicates the nanoparticle diameter, k_B the Boltzmann's constant, T the absolute temperature, η the solution viscosity.

$$\theta = \frac{\eta v}{k_B T} \quad , \quad \left(v = \frac{\pi \phi^3}{6} \right) \quad \text{-----(3)}$$

By using this approximation, equation (4) which is a linear equation of *1/P* versus *T/η*, can be derived. By plotting the data based on this equation, the initial polarization and the nanoparticle diameter can be obtained from the gradient and intercept of the graph.

$$\frac{1}{P} = \frac{(3-P_0)}{3P_0} \frac{6k_B\tau}{\pi\phi^3} \cdot \frac{T}{\eta} + \frac{1}{P_0} \quad \text{-----(4)}$$

In the experiment, Alexa Fluor 488 dissolved in the pH=8.0 TE buffer, with concentration of 1 μ M is used as the sample. The fluorescent polarization is then measured with increasing temperature. The measurement time is 30 ms, and 15 measurements are made for each case. The data plotted according to equation (4) is shown in Fig.2. The measured diameter of Alexa Fluor 488 is 1.11nm. This value is closed to the Fluorescein, which is estimated as 1.00nm [2]. This experiment shows sub-nano meter accuracy in measuring the nano material.

3.2 Dependency of Fluorescence Polarization P to the Size of Gold Nanoparticles

Gold nanoparticles with 3 different sizes, 1.4 nm, 5 nm and 10 nm are evaluated with fluorescence polarization method. In order to prevent the energy transfer from the excited flurophore to the metal core, sample shown in Fig.3 is used. Streptavidin is used as the spacer between gold nanoparticle and the Alexa Fluor 488 fluorophore, which isolated the fluorophore from interacting with the metal core. The sample is dissolved in pH=8.0 TE buffer, to the concentration of 500 nM. The fluorescein polarization P is then measured with increasing temperature. The measurement time is 30 ms, and 15 measurements are made for each case. The results are shown in Fig.4, where the measured P value is corresponding with the theoretical approximation. As the temperature increases, the Brownian motion of the nanoparticles increases and lead to the decrease of P value. For each temperature, the larger particle will have larger P value. This is because larger particle which has lower degree of rotation causes lower degree of depolarization, and therefore larger P value is shown (refer to equation (1)).

Although qualitatively changed according to size can be shown in this case, the exact diameter of the sample cannot be obtained by using equation (2) and the approximation of solid sphere, due to two reasons. Firstly, the θ of 5 nm and 10 nm samples is larger than the τ, which causes insufficient information. Secondly, the simplification into solid sphere cannot be fully applied. Fundamental mathematical model for this case will be investigated.

4. Conclusions

In-situ monitoring setup applying fluorescence polarization method is constructed. Size of Alexa Fluor 488 dye is measured and sub-nano meter accuracy is shown. Also, qualitative change according to size is observed through the measurements of gold nanoparticles with 3 different sizes.

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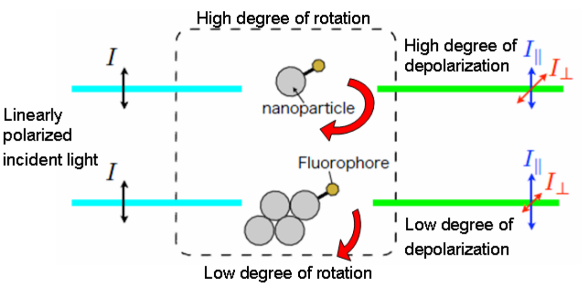

Fig.1 Principle of fluorescence polarization

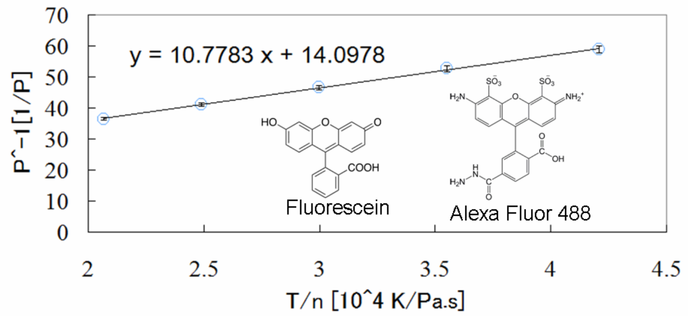

Fig.2 Measurements of Alexa Fluor 488 dye

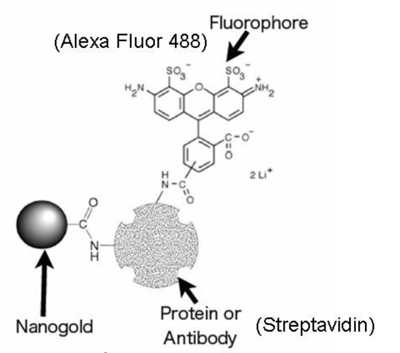

Fig.3 Structure of gold nanoparticles used in experiment

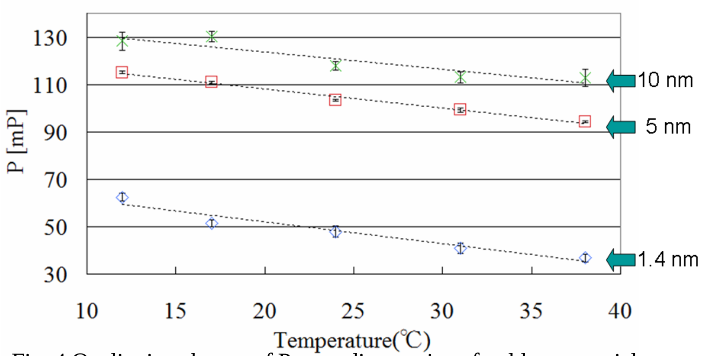

Fig. 4 Qualitative change of P according to size of gold nanoparticle