Optical Transmittance for Analysis of the Dynamics of
Self-Assembled Rotating Chains of Superparamagnetic
Micro- and Nano Beads in Solution

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Abstract. Optically monitoring biosensing procedures based on the dynamics of an aqueous solution containing functionalized superparamagnetic beads in the application of the external rotating magnetic field has been developed for a rapid, highly sensitive, and inexpensive bioassay. Typically, the dynamics of micrometer diameter beads is observed by conventional optical microscopes. For greater affinity to biomolecules, there is a demand which necessitates the use of nanometer sized superparamagnetic beads, comparable size to actual biomolecules. However, a limited amount of work for monitoring the dynamics of nanometer sized beads has been performed thus far due to the maximum resolution of microscopes. Here, we propose a novel protocol enabling us monitor the dynamics of nanometer-diameter beads via change in the optical transmittance.

1. Introduction

Biosensing protocols based on functionalized superparamagnetic beads (SPBs) have widely been studied for developing inexpensive, rapid, and highly-sensitive biodiagnosis of disorder [1] [2], even testing the use of illegal drag [3]. Several sorts of biosensing protocols
utilizing the functionalized SPBs have been introduced over past decades. The optical, magnetic, chemical, and mechanical properties depend mostly on the particle shape, size, composition, and more importantly the applied external magnetic field. Among many, an optical mean to measure the intensity of fluorescence to determine the biorecognition of interest has particularly been utilized [4]. However, the optical method requires expensive equipments, such as a fluorometer and a fluorescence microtiter plate reader, thus it is limited only in affluent research centers and hospitals [5]. For that reason, it finds difficult the protocol to become prevailed. In order to familiarize a biosensing protocol, it must be inexpensive, highly sensitive, and more importantly easy to use [6].

Many researchers have attempted to realize the requisites with using conventional optical microscopes. However, this mean has not been able to response to a recent trend due to its limitation, extremely challenging to observe magnetic beads smaller than intrinsic maximum resolution of a microscope [7]. The recent trend puts great focus on the use of the functionalized SPBs less than 300-nm-diameter comparable size to actual biomolecules for greater affinity to target biomolecules [8].

Recently, there has been considerable interest in magneto-optic devices which combine magnetic and optical phenomena. The optical transmittance of an aqueous solution containing SPBs under an applied external magnetic field is one of the essential areas of magneto-optical effects. Some reports show that the optical transmittance of the magnetic fluids can be modulated by varying the external magnetic field [9-12]. In addition to magnetic field effect, the wavelength effect on the transmission of the magnetic fluids was also reported [13]. Piecing together published reports, the optical transmittance is greatly dependent on the external magnetic field and its direction, the wavelength of the light, and the orientation of the magnetically induced superparamagnetic bead chains.

Here we show a potential application of a magneto optical method to optical biosensing protocols. Our biosensing new protocol have already been published elsewhere[14]. We need to more research of the effect of bead size, composition, and configuration on the transmittance of the light passing through a column filled with an aqueous solution containing a kind of superparamagnetic bead has been investigated as a function of the applied external magnetic field and frequency. The beads we used were 2.8-μm-diameter SPBs (consisting nanometer sized magnetic beads, such as Fe₃O₄, typically 5-10 nm in diameter, are embedded into polystyrene matrix) and 250-nm-diameter SPBs (composing of crosslinked nanometer sized magnetic beads with dextran). Our procedure enables to monitor the dynamics of sub-300-nm-diameter SPBs in application of the rotating external magnetic field, although the dynamics of such beads is not well observable under an optical microscope.
2. Experimental

The experiments were performed using two suspensions of super-paramagnetic particles obtained diluting 0.5% and 0.25% in weight suspensions supplied by dynabeads (M-270 carboxylic acid) and nanomag-D (09-02-252 carboxylic acid). The beads were diluted with deionized water to concentrations of M-270 = 1.0×10^7 particles/ml and 09-02-252 = 1.15×10^9 beads/ml, respectively. We conducted two different sets of experiments to observe dynamics of SPBs using the rotating external magnetic field. In the first set, we used a conventional optical microscope to directly monitor the dynamics of an aqueous solution containing SPBs subjected to the rotating magnetic field, illustrated in figure 1. The rotating external magnetic field was achieved by applying sinusoidal current to the two orthogonally aligned pairs of Helmholtz coils via a two channel function generator referenced to one another at a phase difference of 90 degrees shown figure 1. The function generator was used for controlling both the magnitude and frequency of the rotating external magnetic field. In the second set, we used an experimental setup depicted in figure 2 to observe the change in the optical transmittance due to the linearly aligned chains of SPBs following up the direction of the applied rotating external magnetic field. Unpolarized incident visible light produced by a tungsten halogen bulb was passed through the column filled with an aqueous solution containing SPBs, and the intensity of the transmitted light through the column was measured by a spectrum analyzer which was set across the column from the light source, shown in figure 2. The wavelength of 630 nm highly transmitted through aqueous solutions, and thus it was used for our analysis.

The SPBs we used were optically observable 2.8-μm-diameter SPBs and 250-nm-diameter SPBs, challenging to observe under conventional microscopes. The surface of both micro- and nano- sized SPBs is modified with carboxylic acid groups which help prevent SPBs from aggregating in an aqueous solution due to the static electric force. The transmitted light was guided to spectrometer by an optical fiber (Ocean Optics USB4000). The images were recorded with a CCD (a change-coupled device) video camera Sanyo VDC-3825 connected to a S-VHS VCR Panasonic AG 1975. We digitalized single frames on a computer for their later analysis.
3. Results and Discussion

Figure 1. A schematic diagram of experimental setups for monitoring the dynamics of micrometer-diameter SPBs under an optical microscope.

Figure 2. A schematic diagram of transmittance experimental setups utilizing two orthogonal pairs of Helmholtz coils. A column filled with an aqueous solution containing SPBs was located at the center of two pairs of Helmholtz coils. The incident beam passed through the aqueous solution and the intensity of transmitted light was detected by a spectrum analyzer.

Figure 3. Optical microscope images after 300 s, showing that the 2.8-μm-diameter in diameter SPBs formed column like chains along the direction of the external magnetic field, and their length varies with the magnitude of the external magnetic field. (a) $H_{\text{ext}} = 0$ Oe, (b) $H_{\text{ext}} = 3$ Oe, (c) $H_{\text{ext}} = 6$ Oe, (d) $H_{\text{ext}} = 9$ Oe, and (e) $H_{\text{ext}} = 12$ Oe, and (f) $H_{\text{ext}} = 15$ Oe.

Figure 3 shows optical microscope images of variation of the length of the linear aligned chain of the 2.8-μm-diameter SPBs with the magnitude of the applied external magnetic field.
at 0.01 Hz. The longer chains were achieved at higher magnitude of the external magnetic field. The result was also backed up by reciprocal square root of Mason number, ratio of viscous and magnetic forces acting on SPBs under the applied rotating external magnetic field [15, 16].

![Diagram of magnetic field and optical transmittance](image)

**Figure 4.** Variation of the optical transmittance of 2.8-μm-diameter super-paramagnetic particles with the angle between the external magnetic field up to 15 Oe and path of incident light. The light incident on the SPBs is scattered and the intensity of transmitted light depends on the angle (θ).

The figure 4 shows the variation of the optical transmittance as a function of the external magnetic field up to 15 Oe with angle (θ). The maximum and minimum optical transmittance for magnitude of the external magnetic occurred along parallel directions (θ = 180n degrees, n = 0, 1, 2…) and orthogonal directions (θ = 180n – 90 degrees, n = 1, 2…) with respect to the incident light, respectively. The intensity of transmitted light tended to be greater at higher magnetic field strength. Based on the optical images shown in figure 3, the length of the magnetically induced chain was greatly dependent on the magnitude of the external magnetic field. Thus, it might be thought that the change in optical transmittance was attributed to the length of the chain. As well as the magnitude of the magnetic field, the time, how long the magnetic beads experienced the applied magnetic field, affected the optical transmittance shown in figure 4. 2.8-μm-diameter SPBs are subdivided into polystyrene and magnetite in a ratio of 4 to 1. The approximated diameter of magnetite sphere inside the polystyrene could reach to 1.6-μm-diameter. Visible light with the wavelength of 630 nm, attempting to pass through the magnetite area, was most likely absorbed. Based on the fact, compared to the configuration of Figure 4 (a), the area of magnetic beads blocking the light increased in that of figure 4 (b). Therefore, when the magnetically induced linear chains were aligned parallel to the incident light as shown in figure 4 (a), the visible light went through more and it
achieved higher transmittance by geometrical shadowing effects [17, 18].

Figure 5. Variation of the optical transmittance of 250-nm-diameter super-paramagnetic particles with the angle between the external magnetic field up to 15 Oe and path of incident light. The light incident on the SBSs is scattered and the intensity of transmitted light depends on the angle (θ).

The figure 5 shows the optical transmittance for 250 nm in diameter SPBs appeared to be similar to that for 2.8-μm-diameter SPBs, but had opposite behavior with respect to the incident light. The maximum and minimum optical transmittance for the external magnetic field occurred along orthogonal directions (θ = 180n – 90 degrees, n = 1, 2…) and parallel directions (θ = 180n degrees, n = 0, 1, 2…), respectively, shown in figure 5. The transmittance difference of maximum and minimum, ΔT, for an aqueous solution containing 250-nm-diameter SPBs was smaller than that for 2.8-μm-diameter magnetic beads solution. The conceivable reason would be the composition of SPBs we utilized: 2.8-μm-diameter beads are the polymer matrix in which tiny magnetite is embedded, and 250-nm-diameter magnetic beads which are composed of clustered magnetite (Fe₃O₄). The visible light is hardly absorbed by the transparent polymer, resulting in the high optical transmittance for 2.8-μm-diameter magnetic beads. Composition of 250-nm-diameter SPBs is considerably different: More than 80% are made of magnetite and the great number of small beads is crosslinked with dextran. Notice that when the wavelength of light is very similar to the size of single magnetic bead, it tends to strongly scatter forward, namely Mie scattering [19, 20]. When the orientation of the magnetic beads is orthogonal to the incident light as shown in figure 5 (b), it satisfies the condition and thus the wavelength of 630 nm transmitted well. On the other hand, when the configuration of bead chain became parallel to the incident light, the chain length exceeded the wavelength. The light would be blocked by the chain and thus there was almost no transmitted light in the light path. This resulted in making the optical
transmittance lowest, at the angle $\theta=180n – 90$ degrees, where $n$ is integer as depicted in figure 5. The difference of transmittance tendency under the rotating magnetic field for 2.8-µm-diameter and 250 nm in diameter magnetic beads would be mainly due to the dependence on the amount of light being scattered and absorbed by the beads. Thus, composition and size of magnetic beads could be the cause of the opposite behavior of the optical transmittance. The above statement is not yet good enough to explain why the lower optical transmittance was achieved at higher magnitude of the external magnetic field. Under the external magnetic field, fluctuations due to Brownian motion in magnetically induced chains gave rise to chain-chain interaction and the formation of thicker column like chains. Needless to say, the column like chains tended to have a bigger cross sectional area under the higher external magnetic field, resulting in increasing the blocking area of the incident light.

![Figure 6](image_url)

**Figure 6.** Variation of transmittance difference between maximum and minimum, $\Delta T$, as a function of frequency and the external magnetic field up to 15 Oe. (a) 2.8-µm-diameter SPBs and, (b) 250-nm-diameter SPBs.
Although there was a distinction of transmittance behavior between two kinds of beads, ΔT as a function of frequency made no difference between sorts of beads as shown in figure 6. Fig. 6 (a) and (b) shows after 300sec, the dependence of the maxima and minima of length of the optical transmittance of 2.8-μm-diameter and 250-nm-diameter beads were observed at for various rotation frequencies (f) and different external magnetic fields (Oe). Notably, as f was increased, ΔT decreased slowly and then rapidly decreased and eventually reach zero. These results show that the average length of chains begin to reduce with increasing f, and eventually the chains become dispersed singlet particles due to hydrodynamic forces which oppose the forces due to the applied magnetic torque. As the external magnetic field increased the length of the ΔT with chain structural length increased to comply with (see Fig 7), and transmittance fluctuations become an important component of the observed dynamic of rotating chain of magnetic particles. It is one of the greatest advantages in our method. ΔT as a function of frequency does not discriminate the size and composition of beads. As seen in the figure 6, ΔT was remarkable and remained almost constant until reaching to the frequency threshold. The frequency threshold was higher under the stronger external magnetic field. Once the frequency reached threshold, ΔT dramatically dropped and eventually reached to zero. At this moment, it was thought that none of magnetically induced chain could be remained as a linear chain. Based on the optical images in figure 7, the length of magnetically induced chains became shorter as frequency increased. Ideally, by knowing the frequency at ΔT = 0, we could estimate dipole-dipole interaction of the magnetic beads we used. A considerable application of this mean to bioassay would be to determine the biorecognition forces among biomolecules.
4. Conclusion

We have investigated the effect of the composition of magnetic bead on optical transmission of light passing through an aqueous solution containing the SPBs as a function of the external magnetic field and the orientation of magnetically induced bead chains. For the solution containing 2.8-μm-diameter SPBs, the transmission increased as the orientation of the chains became parallel to the direction of the incident light. On the contrary, the transmission declined for the aqueous solution containing 250-nm-diameter SPBs under the same condition. Despite the fact that transmission behaviors made distinction, when focusing on the difference between maximum and minimum transmission, ΔT, as function of frequency, we noticed that ΔT reached to zero at higher frequency. Based on results, our procedure enables to monitor the dynamics of sub-300-nm-diameter SPBs without using any expensive, difficult, and unwieldy experimental equipments, although the dynamics of such beads is not well observable under an optical microscope. Thus, our protocol has a great potential to apply into the bioassay field.
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