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Application of fluorescent diamond nanoparticles to bio-imaging

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1. Introduction

Nitrogen vacancy centers (NVCs) in nanodiamonds have recently been attracting much attention as a new fluorescent probe. NVC is an atomic-sized defect paired with a nitrogen atom and the adjacent vacancy of a carbon atom in the diamond lattice. It is known that fluorescence from NVC-containing nanodiamonds is markedly stable in contrast to organic and semi-conducting fluorescent probes commonly used in cell and *in vivo* observations. A notable property is that the fluorescence from NVC is dependent on a spin degree of freedom in the ground states, and the fluorescence intensity can be manipulated by microwave (MW) irradiations in optically detected magnetic resonance (ODMR). By using this property that the fluorescence intensity of NVC, but not that of other sources, changes upon resonant MW irradiation at 2.87 GHz, we reported a selective imaging method to completely eliminate extraneous fluorescence in real-time microscope observations, and demonstrated successful observations of nanodiamonds in a living HeLa cell, *Caenorhabditis elegans*, and a mouse[1).]

Furthermore, the NVC has also been attracted as an atomic-sized sensor to detect not only magnetic field but also electric field and temperature in the nanoscale region[2)]. Another pressing need in measuring dynamics in biology is the creation of an efficient tool to quantitatively detect rotational motions of an object *in vivo*. Here, we introduce observation of the rotary motion that we used diamond nanoparticles including NV^- for as a fluorescent probe.

2. Results

There is an equivalent of four Nitrogen-Vacancy (N-V) orientations within diamond crystals. When an external magnetic field is applied, the resonance frequency of NV^- (2.87 GHz) shifts to two different frequencies: a low and a high frequency centered on 2.87 GHz. As a result, the ODMR spectrum shows two dips. In addition, the resonance frequency varies depending on the angle between the magnetic field and the N-V orientation. Accordingly, when NV- of four orientations is present within one diamond nanoparticle, the ODMR spectrum will show a maximum of 8 dips. In circumstances where the external magnetic field has been fixed, the shape of the ODMR spectrum will change in accordance to the rotational motion of the diamond nanoparticles. In other words, it is possible to determine the orientation of the diamond nanoparticles by analysis of the ODMR spectrum. By tracking the temporal variation in the orientation of particles, it is also possible to observe their rotational motion. We measured every 10 min, over the timespan of 40 min, the ODMR spectrum of 200-nm diamond nanoparticles that had been adsorbed on the intestine wall of nematode, estimating the movement of diamond nanoparticles by fitting. The results obtained by the comparison of the movement in a normal nematode, a dead nematode, and a mutant nematode with poor intestine movement showed that the diamond nanoparticles displayed active angular variation in the normal nematode, whereas the movement of diamond nanoparticles in the variant nematode was sluggish, and there was no movement at all in the dead nematode.

3. Conclusions

Here, we introduce the observation of the rotary movement using diamond nanoparticles, including NV^- as fluorescent probes. This is a unique and unconventional method of measurement that is achieved through a combination of spin operative techniques, using fluorescence detection technology and magnetic resonance. It is also possible to measure the movement of protein molecules by combining them with diamond nanoparticles.

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References

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