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Fluorescent Nanodiamonds for thermal biology

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ABSTRACT

Temperature measurement using fluorescent nanodiamonds is a highly reliable technique because it is largely unaffected by factors such as pH, ion concentration, viscosity, molecular interactions, or organic solvents. Using fluorescent nanodiamonds coated with photothermal polymers as quantum sensors, we measured thermal conductivity inside living cells. We found that intracellular thermal conductivity is about one-sixth that of water, which is much lower than previously assumed. This finding provides new insight into how heat spreads inside cells and has important implications for understanding cellular heat production in biomedical research.

Keywords: Fluorescent nanodiamond, Optically Detected Magnetic Resonance (ODMR), temperature measurement, thermal conductivity

1. INTRODUCTION

Measuring body temperature is very easy. By placing a thermometer under the armpit for only about ten seconds, we can quickly obtain a temperature reading. Human body temperature is usually maintained at around 37 °C, and even a change of just 1 °C can noticeably affect how we feel. In recent years, non-contact thermometers that detect infrared radiation from the skin have also become widely used.

However, measuring temperature inside a cell—the smallest unit of the human body—is far more challenging. Cells are only tens of micrometers in size, so temperature measurements inside them require sensors that are even smaller. In recent years, growing interest in the relationship between temperature and cellular function has led to the development of various intracellular temperature sensors, including polymers, proteins, small molecules, and semiconductor quantum dots [1]. Using these sensors, several studies have reported surprisingly large temperature differences within single cells, sometimes exceeding 1 °C, a range once thought to be physically impossible. Some studies have even suggested that mitochondrial temperatures may reach as high as 50 °C [2].

While many studies focus on measuring intracellular temperature itself, very few investigate how heat spreads inside cells. Heat generated within a cell moves through the cellular interior and ultimately contributes to the regulation of body temperature. Understanding how heat propagates within cells, and how it influences surrounding biomolecules and chemical reactions, is essential for clarifying the role of heat in biological processes [3].

For this reason, the main goal of our research is to measure cellular thermal conductivity. To achieve this, we have developed a new nanoscale thermal conductivity measurement system by combining quantum sensing with chemical techniques. In this paper, we first explain the operating principle of diamond-based quantum sensors and their functionalization through surface chemical modification. We then present recent results on the measurement of cellular thermal conductivity obtained using this approach.

2. DIAMOND QUANTUM SENSOR

Diamond nanoparticles are inorganic materials made mainly of carbon. Inside these particles, lattice defects called nitrogen-vacancy centers (NVCs) can be artificially introduced (Figure 1A). Because NVCs emit near-infrared fluorescence, nanodiamonds containing them are known as fluorescent nanodiamonds (FNDs) (Figure 1A) [4].

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Figure 1B illustrates the energy structure of an NVC [5]. Its ground state is split into two quantum spin states, $m_s = 0$ and $m_s = \pm 1$, due to zero-field splitting (D). Electrons occupy these states according to the Boltzmann distribution. When electrons in the $m_s = 0$ state are excited by light, they return directly to $m_s = 0$ while emitting fluorescence. In contrast, some electrons excited from the $m_s = \pm 1$ state relax to $m_s = 0$ through a non-radiative pathway, resulting in reduced fluorescence. As a result, light irradiation strongly polarizes electrons into the $m_s = 0$ state. Because of this mechanism, transitions between $m_s = 0$ and $m_s = \pm 1$ can be detected as changes in fluorescence intensity. This technique is known as optically detected magnetic resonance (ODMR). ODMR is highly sensitive to physical parameters in the vicinity of the NV center, and these changes appear directly in the fluorescence signal. Therefore, introducing FNDs into cells makes it possible to quantitatively measure various intracellular physical properties (Figure 1C) [6]. For this reason, FNDs are often referred to as quantum sensors.

Figure 2 shows a typical ODMR spectrum, which represents changes in fluorescence intensity as a function of microwave frequency. When the microwave frequency approaches D, transitions from $m_s = 0$ to $m_s = \pm 1$ occur, leading to a decrease in fluorescence. Changes in temperature cause a shift in the ODMR peak position, while magnetic fields cause the peak to split. In addition, interactions with magnetic molecules can be evaluated by measuring the spin relaxation time of the NV center [7]. ODMR-based measurements offer extremely high precision. In the temperature range relevant to this study, temperature changes of less than 0.1 °C have been reported to be detectable [8]. Moreover, because the NV center is enclosed within the rigid diamond lattice, temperature measurements are not affected by environmental factors such as pH, salt concentration, viscosity, or molecular interactions. This robustness makes fluorescent nanodiamonds particularly well suited for accurate temperature sensing in biological systems [9].

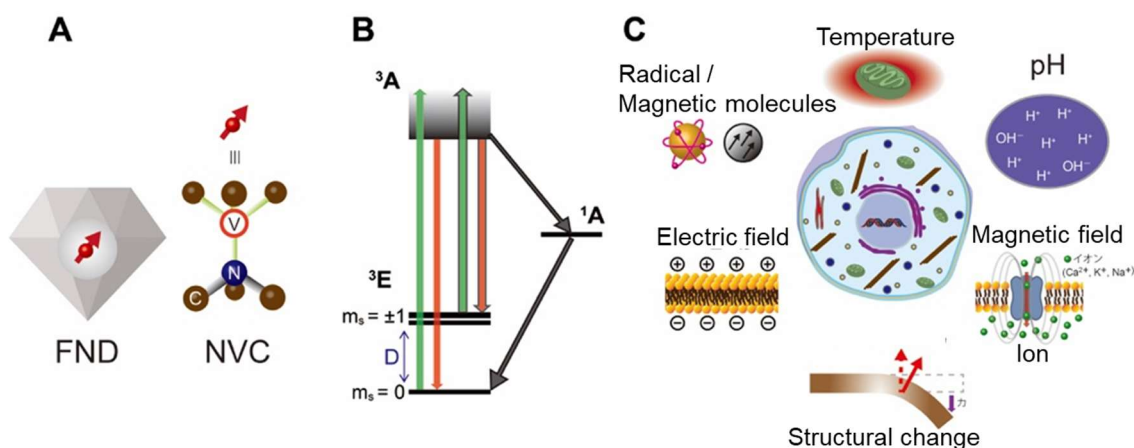


Figure 1. (A) Structure of FND and NVC. C: carbon, N: nitrogen, V: vacancy. (B) Energy diagram of NVC. (C) Example of expected cell sensing by FND.

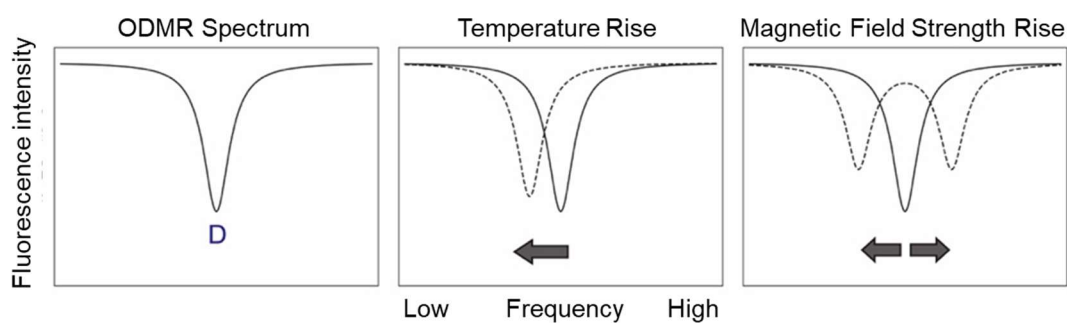


Figure 2. ODMR Spectrum and Its Response to Temperature and magnetic field.

As the temperature rises, the spectrum shifts toward the low-frequency side (left direction in the figure), and as the magnetic field strength increases, the splitting width of the spectrum becomes larger.

3. FUNCTIONALIZATION OF DIAMOND QUANTUM SENSORS

Fluorescent nanodiamonds (FNDs) have a robust diamond-based structure that allows additional functions to be introduced through surface coating with polymers and other molecules [10]. Figure 3 summarizes the main types of coatings used for FNDs.

Some coatings rely on non-specific interactions, such as electrostatic or hydrophobic interactions. Typical examples include protein coatings and polyethyleneimine (PEI) coatings. These methods are simple and can be achieved by merely mixing the coating material with FNDs. However, because the interactions are not covalent, the coatings are less stable than those formed by covalent bonding. Protein coatings can improve dispersibility and partially suppress non-specific protein adsorption, while PEI coatings can bind DNA and are therefore useful as platforms for cellular delivery.

In contrast, several covalent coating methods have been developed, including coatings based on polyglycerol, photopolymerizable lipids, silica, and polydopamine. These covalent coatings provide high structural stability, making them suitable for long-term imaging, sensing, and *in vivo* applications. Among them, photopolymerizable lipid coatings are particularly attractive because they are easy to prepare and highly stable. Their very thin coating layer, typically around 2–3 nm, makes them especially suitable for sensing applications.

Hyperbranched polyglycerol (HPG) coatings offer exceptionally high dispersibility compared with other coating types. They also strongly suppress non-specific adsorption and allow selective labeling of target molecules through surface activation [11]. Thanks to these properties, FNDs with such coatings have been applied in a wide range of studies, including stable dispersion under high-salt or extreme pH conditions, selective molecular labeling, single-molecule tracking of membrane proteins, drug delivery systems, detection of redox reactions, and monitoring of protein structural changes.

To effectively use FNDs for cellular sensing, it is essential to understand the characteristics of each coating and to select the most appropriate one for the intended application.

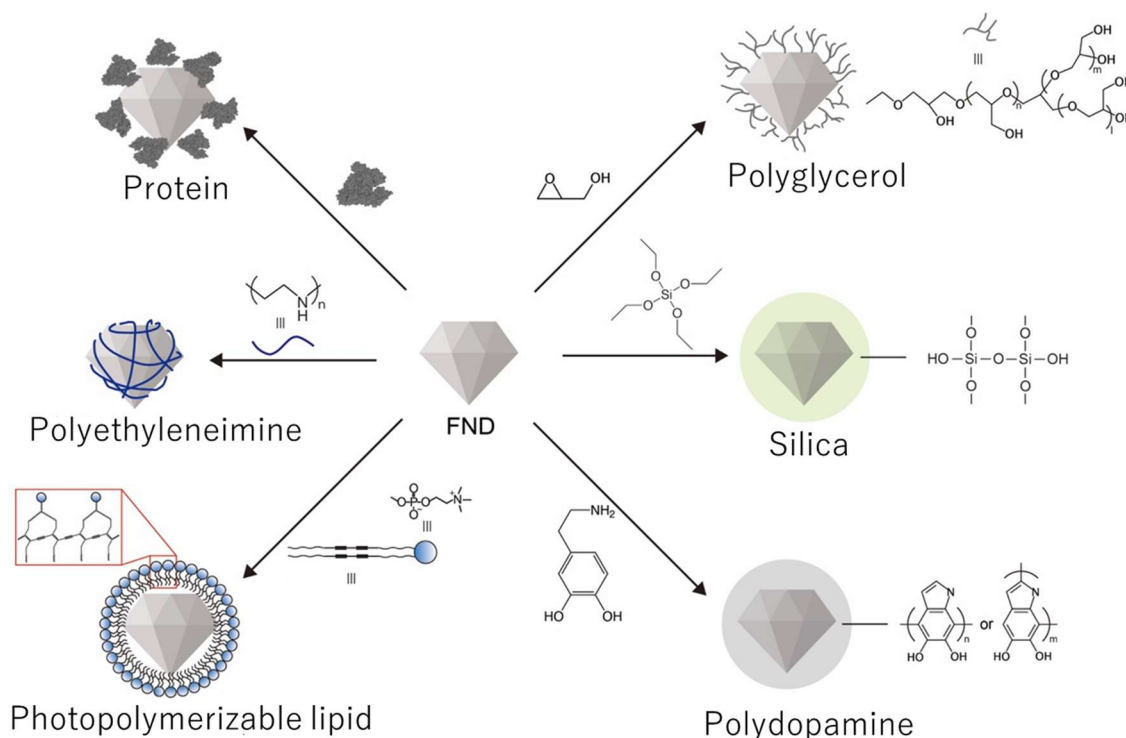


Figure 3. Surface Chemical Modification of FND

4. THERMAL CONDUCTIVITY MEASUREMENT OF CELLS USING HYBRID QUANTUM SENSORS

As described above, fluorescent nanodiamonds (FNDs) can serve as nanoscale sensors for cellular measurements because of their unique quantum properties. In addition, the chemically stable diamond structure allows their surface to be easily modified with biomolecules or polymers, greatly expanding their range of applications. Here, we introduce a method for measuring cellular thermal conductivity using FNDs coated with polydopamine (PDA).

4.1 PDA-FND Synthesis and Functional Evaluation as an Integrated Heater/Thermometer

Polydopamine (PDA) is a polymer formed by the polymerization of dopamine and is well known for its photothermal properties. When a fluorescent nanodiamond is coated with PDA (PDA-FND), the PDA layer generates heat upon light irradiation. This heat can then be detected by the nitrogen-vacancy center (NVC) inside the FND through changes in its fluorescence. In this way, PDA-FND functions as a hybrid system that combines a nanoscale heater and a thermometer in a single particle (Figure 4A).

The synthesis of PDA-FND is straightforward. Simply mixing FNDs with dopamine in a Tris-HCl buffer at pH 8.5 induces polymerization of dopamine on the FND surface [12]. An electron microscopy image of PDA-FND (Figure 4B) clearly shows a PDA layer coating the nanodiamond.

Both heat generation and temperature measurement are performed using a 532 nm laser. As temperature increases, the zero-field splitting parameter D decreases, causing the ODMR signal to shift toward lower frequencies at a rate of -0.07 MHz per degree Celsius. By measuring this frequency shift, the temperature rise caused by photothermal heating can be quantitatively estimated.

Figure 4C shows ODMR spectra recorded at different laser intensities. As the laser power increases, heat generation by the PDA layer becomes stronger, leading to a clear shift in the ODMR signal. Figure 4D converts these frequency shifts into temperature changes, demonstrating a distinct temperature increase in PDA-FND. In contrast, such heat generation is not observed for FNDs without the PDA coating, confirming that the heating effect originates from the PDA layer.

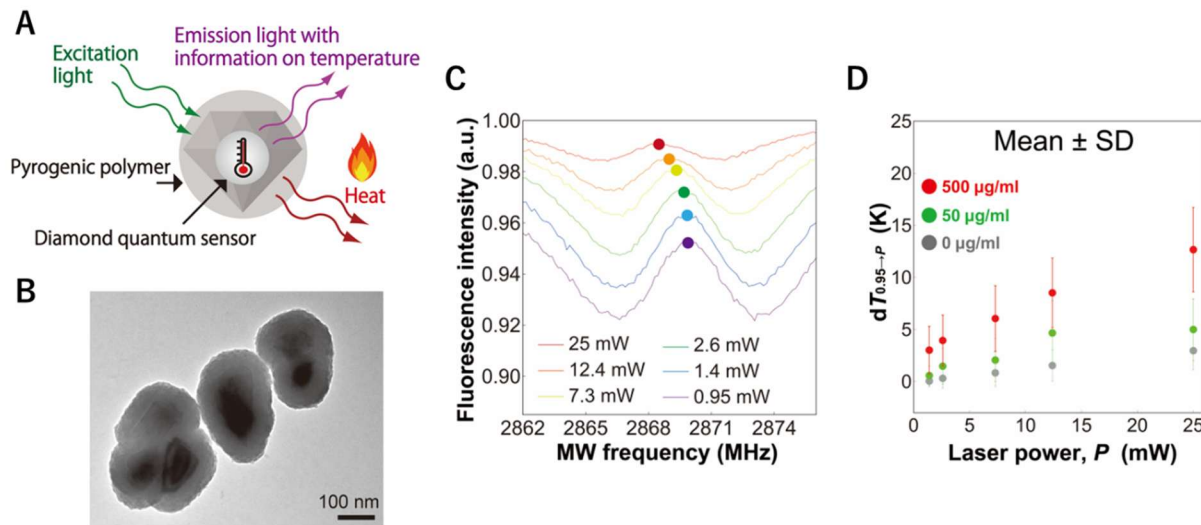


Figure 4. Integrated Polydopamine-Fluorescent Nanodiamond (PDA-FND) Heater/Thermometer

(A) Mechanism of PDA-FND functioning as an integrated heater/thermometer. (B) Electron micrograph of PDA-FND. (C) Change in ODMR signal versus laser light intensity. (D) Temperature rise of PDA-FND versus laser light intensity.

4.2 Measurement of Cellular Heat Conduction

Figure 5 illustrates the principle of measuring cellular thermal conductivity using PDA-coated fluorescent nanodiamonds (PDA-FNDs). When a PDA-FND is placed in an environment with high thermal conductivity, the heat generated by light irradiation of the PDA layer quickly diffuses into the surrounding medium. As a result, the temperature of the FND rises

only slightly. In contrast, in an environment with low thermal conductivity, heat diffusion is slower, leading to a larger temperature increase of the FND.

Therefore, by introducing PDA-FNDs into cells and measuring their temperature increase upon light irradiation, it is possible to quantitatively determine the thermal conductivity inside the cells.

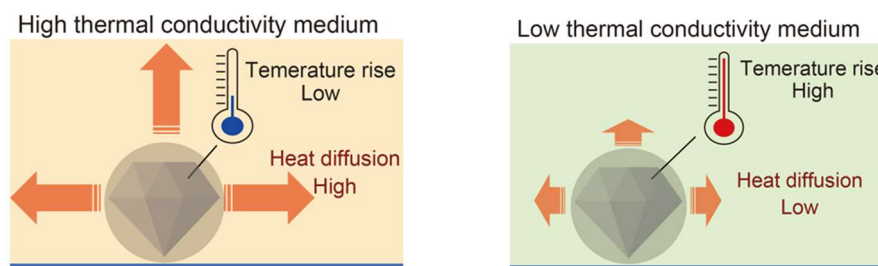


Figure 5. Thermal Conductivity Measurement Using PDA-FND

In this experiment, we first tested PDA-coated fluorescent nanodiamonds (PDA-FNDs) in air, water, and mineral oil—materials whose thermal conductivity values (κ) are well established—to confirm whether PDA-FNDs can accurately measure thermal conductivity (Table 1).

A key challenge in this validation was measurement error caused by variations in the size and shape of individual FND particles. Although commercially available FNDs with a nominal diameter of 100 nm were used, the particles are not perfectly uniform. Larger FNDs are coated with more PDA and therefore generate more heat under light irradiation, whereas smaller particles generate less heat. As a result, the amount of heat generated varies from particle to particle.

To distinguish this particle-dependent variation from differences arising from the surrounding environment (i.e., thermal conductivity), we collected a sufficiently large number of measurements and performed statistical analysis. This approach allowed us to reliably extract the effect of thermal conductivity.

The results showed that the temperature increases measured in air, water, and mineral oil closely matched the values predicted from their known thermal conductivities. These findings demonstrate that PDA-FNDs can accurately measure thermal conductivity and validate the reliability of this measurement principle.

Table 1. Thermal Conductivity Measurement Experiment Results and Calculated Values.

	Temperature Rise (Experiment 1) [°C]	Temperature Rise (Experiment 2) [°C]	Thermal Conductivity (Literature Value) [W/m·K]	Temperature Rise (Calculated Value) [°C]
Air	5.6 ± 1.1	5.7 ± 0.8	0.026	5.32
Water	0.63 ± 0.5	0.70 ± 0.3	0.61	0.84
Mineral oil	1.9 ± 0.6	1.7 ± 0.4	0.135	1.76
Cell	3.0 ± 1.0 (HeLa)	2.9 ± 0.8 (MCF-7)	0.11 (This study)	3.00

PDA-coated fluorescent nanodiamonds (PDA-FNDs) were then introduced into HeLa cells, derived from human cervical carcinoma, and MCF-7 cells, derived from human breast carcinoma, to measure the temperature increase inside the cells. For the MCF-7 experiments, a newly prepared PDA-FND sample (Experiment 2) was used instead of reusing the sample applied in the HeLa cell measurements (Experiment 1). In each case, cellular measurements were performed as independent experiments together with reference measurements in air, water, and mineral oil.

The results showed intracellular temperature increases of 3.0 ± 1.0 °C in HeLa cells and 2.9 ± 1.0 °C in MCF-7 cells. From these temperature rises, the intracellular thermal conductivity was estimated to be approximately 0.11 W/m·K for both cell types. This value is significantly lower than the thermal conductivity of water and represents the first experimental

demonstration of this difference [13]. Notably, this thermal conductivity is close to that of lipids and proteins, which typically range from 0.1 to 0.2 W/m·K.

5. CONCLUSIONS

We have developed a new nanoscale sensor that combines a diamond-based quantum sensor with polydopamine, integrating a heater and a thermometer into a single particle. This system enables direct measurement of thermal conductivity at the nanoscale. Using this approach, we discovered that the thermal conductivity inside cells, which had long been assumed to be similar to that of water, is actually about one-sixth of the value for water.

Although confocal microscopy has confirmed that fluorescent nanodiamonds are present inside cells, their precise localization within specific cellular structures remains unclear. In future studies, we plan to apply advanced surface chemical modification techniques to PDA-coated fluorescent nanodiamonds. By targeting specific intracellular structures, such as mitochondria or the nucleus, we aim to measure thermal conductivity at distinct locations within the cell. This will help uncover the mechanisms responsible for the unusually low thermal conductivity observed in cells.

To further improve measurement accuracy, we will also work toward preparing spherical fluorescent nanodiamonds with uniform particle sizes, thereby reducing errors caused by variations in particle size and shape. We expect that the thermal conductivity measurement system developed in this study, based on chemical technologies, will bridge quantum science and life science and open a new avenue for the quantitative analysis of physical properties at the intracellular nanoscale.

Use this section to identify people who have aided the authors in accomplishing the work presented and to acknowledge sources of funding. Include grant numbers and the full name of the funding body.

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