Review

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Review

Fluorescent Nanodiamonds for Nanoscale Thermometry in Biology

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Abstract: Color centers in diamond and nanodiamonds can be utilized as quantum sensors for measuring various physical parameters, particularly magnetic and electric fields, as well as temperature. Due to its small size and possible surface functionalization, fluorescent nanodiamonds are attractive systems for biological and medical applications since they can be used for intracellular experiments. This review focuses on fluorescent nanodiamonds for thermometry with nanoscale spatial resolution for living systems. The current state of the art, possible further development, and potential limitations will be discussed here.

Keywords: fluorescent nanodiamonds; color center; nanoscale thermometry; temperature mapping; biological applications; living cells

1. Introduction

Temperature measurement within a single living cell is an important question for fundamental biological and medical sciences and industrial pharmacology applications. Nowadays, many fluorescent markers are applied to visualize temperature changes to study biological systems. This list contains fluorescent dye molecules, polymers, or proteins [1,2], quantum dots [3], and nanodiamonds with color centers [4,5]. Fluorescent markers are an attractive system due to the low invasive influence of such a method. Such optical detectors are typically based on temperature-dependent fluorescent properties, for instance, spectral shift, changes of intensity, or lifetime.

Organic fluorescent thermometers have the advantage of simple functionalization and addressed attachment processes. However, their main problem is that their optical properties are not only temperature response but depend on the environment, for example, the pH level [6] or viscosity [2]. It makes temperature measurements less reliable and environmentally unstable. Therefore, it is impossible to define whether the observed changes were provoked by temperature variation or other parameters.

Quantum dots are interesting optical marker systems since their fluorescent spectra can be efficiently designed by size variation. However, they also have some disadvantages, particularly for biological applications. First, they are toxic materials [7]. Therefore, cell poisoning can cause temperature changes during the measurements, leading to parasitic influence and cell death. Second, their fluorescent intensity, which is used for thermometry [8], also depends on the variation of the pH level [9].

Another essential point for biological study, especially for investigating cellular metabolism, is the observation of not only temperature changes but also the measurement of the absolute temperature. The organic fluorescent nanoparticles, as well as quantum dots, cannot provide it because their properties are also dependent on environmental conditions. Thus, it is impossible to provide a one-to-one correspondence between fluorescence and temperature. It is highly critical for biological systems due to many simultaneous chemical reactions going on all the time. One of the most discussed papers about the local temperature of mitochondria [10] claimed that it was observed to be 50°C. However, such measurements were done with the fluorescent probe MitoThermo Yellow without correcting the parasitic environment-related fluorescence changes and are still under discussion in the community.

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2. Fluorescent Nanodiamonds

The alternative to the already-named sensors is fluorescent nanodiamonds (ND) with various types of color centers [4,5]. One of the advantages of ND for biological study is that they are inert and nontoxic materials [11]. At the same time, the surface of ND can be chemically functionalized to make them more bioactive [12,13], for example, usable for drug delivery [14] or attachable to chosen intracellular parts [15]. Various diamond color centers offer different experimental protocols for temperature sensing (Figure 1). Currently, three mainly studied types of such optically active diamond defects are Nitrogen-Vacancy, Silicon-Vacancy, and Germanium-Vacancies. Each of them has its own advantages and disadvantages. Nevertheless, the most crucial reason to use ND for biological thermometry is the insensitivity of their fluorescent properties to the environment around diamond nanocrystals (pH level, ion concentrations, etc.). Next, ND with these three types of color centers will be deeply discussed.

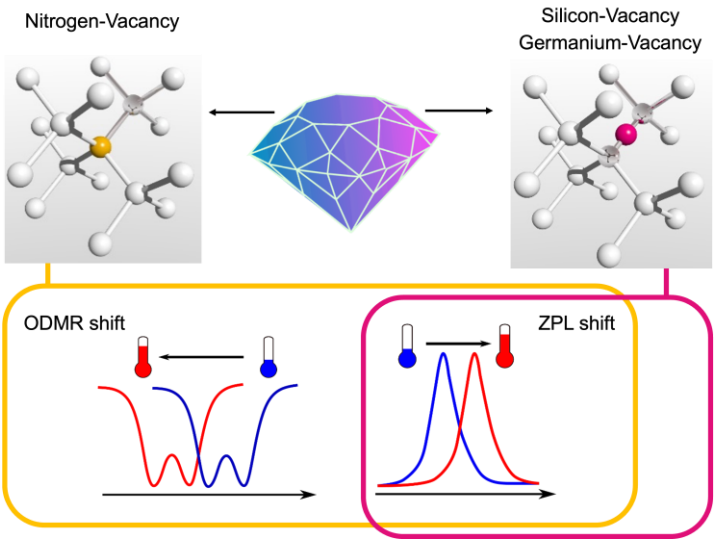

Figure 1. Crystal structures of diamond optically active defects: Nitrogen-, Silicon-, and Germanium-Vacancy centers. Temperature measurement protocols are based on the shift of optically detected magnetic resonance (ODMR) for the Nitrogen-Vacancy center only and on the shift of zero phonon line (ZPL) for all named defects.

2.1. Thermometry with Nitrogen-Vacancy centers

The most investigated and currently used diamond color center is a Nitrogen-Vacancy (NV) defect consisting of a substitutional nitrogen atom next to a missed carbon – vacancy. It can be found in two optically active charge states: neutral NV^0 and negative NV^-. Their spectra have zero-phonon lines (ZPL) at 575 and 637 nm, respectively, with broad phonon sidebands at room temperature [16]. Both NV charge states can be used for temperature sensing by observing the redshift of ZPL with a temperature increase [17–20]. Such a method is attractive due to the experimental simplicity since all operations and detection can be done purely optically. Many works focus on intracellular thermometry by detecting ZPL shift [17–20]. As one example, the work of the group from Jinan University can be chosen (Figure 2a) [20]. Here, ND with NV centers were used for temperature sensing in different types of cells, particularly 4T1, C127, and HeLa cells. The temperature distribution was presented over a single C127 cell (Figure 2b). It demonstrates the temperature gradient and indicates that the nuclear close area is 5°C warmer than the cell membrane region (Figure 2c).

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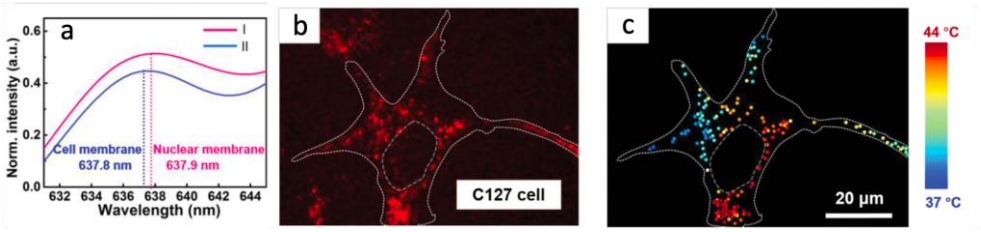

Figure 2. a) Two spectra of aggregated ND, where line I represents the area close to the cell nuclear and line II – cell membrane. b) Confocal image of fluorescent ND distributed inside a living C127 cell. c) Temperature mapping recalculated from the ZPL shift for observed ND in (b). Data from [20].

For the NV^0 defects, ZPL shift observation is the single possible way of thermometry. It is not the most efficient technique since only around 4% of NV fluorescent come to ZPL [21]. Another measurement method is available for the NV^- center, and it is based on observing the optically detected magnetic resonance (ODMR) (Figure 1). NV^- defect has one extra electron from surrounding donors and, as a result, a spin equal to 1 with possible states $m_s=0, \pm1$. The spin-dependent fluorescence, explained by different relaxation processes for $m_s=0$ and $m_s=\pm1$, allows for the optical distinguishing of different spin states. The metastable state, which is probable for $m_s=\pm1$, is also responsible for spin conversion and polarization. Initially, ODRM, as well as spin relaxation times, were used for magnetic field detection, including the investigation of biological systems [22–26]. During such magnetometry experiments at different temperatures, it was observed by the group of D. Budker [27] that the positions of ODMR lines depend not only on the magnetic field due to the Zeeman effect (the split of ODRM) but also on temperature (the shift of the central ODMR frequency). The temperature increase provokes the shift of the central position of ODMR lines to the shorter microwave frequencies. Such ODMR shift appears linearly with temperature, which makes ND a simple handle system for absolute temperature sensing. Soon after this first experiment, two groups published simultaneous papers about thermometry with ND. The experiments provided in the Stuttgart group showed the sensitivity for ND-based sensors equal to 130 mK $Hz^{-1/2}$ for a sample with an average size of 50 nm [28]. The Harvard team demonstrated the first intracellular thermometry by NV^- containing ND with a size of 200 nm and achieved a sensitivity of 9 mK $Hz^{-1/2}$ [29]. The measured temperature increase was not biologically related but was made and controlled by adding gold nanoparticles to convert light into heat. It was proof-of-principle experiments and became the start of many following works [30–32].

Proof of the insensitivity to the environment of the thermometry ability of ND was demonstrated in the following work [31]. The group of scientists from Japan tested ND with NV^- centers for temperature sensing in different solvents, particularly in low-pH and high-pH solutions, high concentrations of Na^+ and Cl^- ions, glycerol with high viscosity, surface polymer coating, and ethanol as an organic solvent (Figure 3a). For all those environments, the ODMR shift (D) temperature dependence was measured (Figure 3b). The results clearly show that there is almost no influence from the surrounding solution parameters (ion composition, viscosity, etc.) on the temperature sensing ability of NV containing ND. It allows us to conclude that ND can be used inside cells as a robust thermometer without the need to correct the artificial negative influence of the environment.

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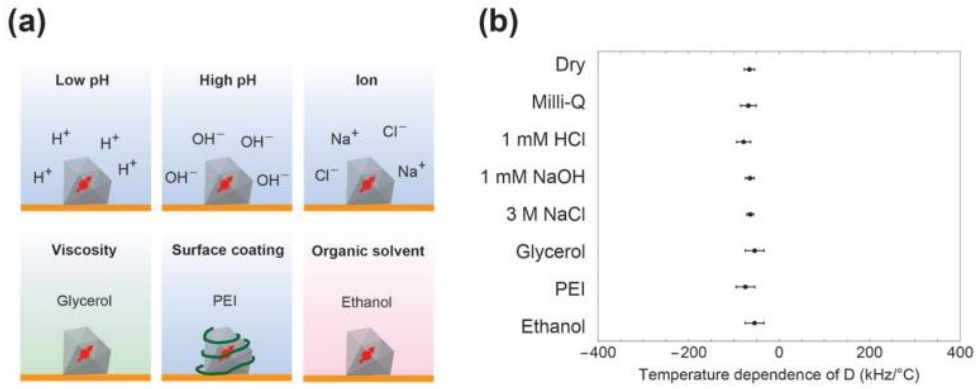

Figure 3. a) ND in various types of solvents (low-pH, high-pH, high ion concentrations, high viscosity, surface polymer coating, organic solvent) to evaluate the influence of the environment on NV-based thermometry. b) The temperature dependence of the ODMR shift (D) for different solvents. Data from [31].

Fluorescent ND can also be used for drug delivery and cancer treatment, as was demonstrated by many groups [14,33,34]. The extreme photostability of ND emission makes them attractive systems for theranostics applications, where long imaging and sensing ability are combined with therapeutic effect. One such utilization of ND with NV^- centers was demonstrated by the group of T. Weil [35], where fluorescent ND coated with nanogel (ND-NG) and additionally functionalized with indocyanine green molecules (ND-NG-ICG) were used to test cancer photo-treatment under infrared illumination with simultaneous intracellular thermometry (Figure 4a,b). Such measurements allow us to evaluate the saturation temperature for different ND concentrations in water (Figure 4c) and inside cells (Figure 4d). The demonstrated measurement monitored the local temperature at the start of cell apoptosis, which is essential for improving phototherapy protocols.

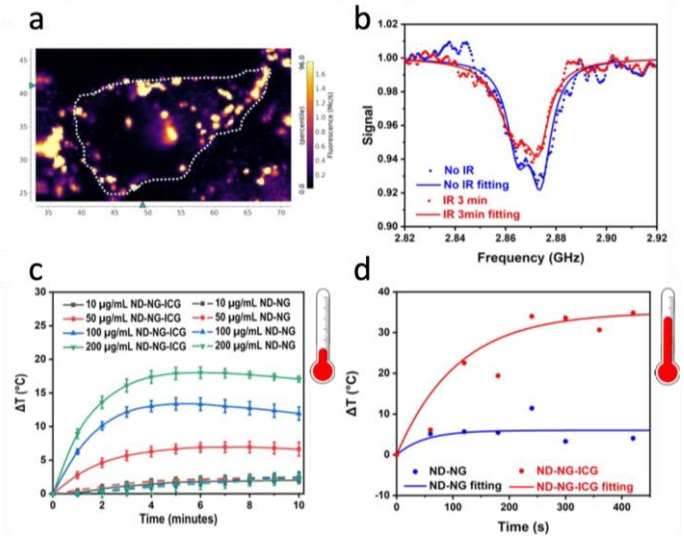

Figure 4. a) Confocal image of ND coating with indocyanine green molecules in a living HeLa cell (the dashed line is the cell border) after 4 h of incubation. The concentration of coated ND is 10 μg/mL. b) Selected ODMR spectra of coated ND with and without infrared irradiation (810 nm lamp; 0.35 W/cm^2). c) Thermal profiles of ND in water without (ND-NG) and with (ND-NG-ICG) indocyanine green with different concentrations under light irradiation (810 nm lamp; 0.35 W/cm^2). d) Intracellular thermometry and temperature saturation for NG-NG-ICG and ND-NG over 420 s under infrared irradiation (810 nm lamp; 0.35 W/cm2) for concentrations of 10 μg/mL. Data from [35].

Neuron thermometry also attracts great attention from the scientific community due to brain inflammations [36] and different neurological diseases [37–39], temperature-sensitive channels called

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transient receptor potential (TRP) channels [40], etc. Therefore, many groups worldwide try to apply fluorescent ND for neuron thermometry [41–43]. ND with NV centers were also proved to be used for thermometry in more complex living systems than just cells. For example, it was demonstrated that ND can be successfully used for measurements inside a living worm [43].

2.1. Thermometry with Silicon-Vacancy Centers

After the NV-based thermometry, Silicon-Vacancy centers (SiV) in diamond were used for temperature detection by observing the redshift of their ZPL [44]. SiV center consists of an interstitial silicon atom between two vacancies or a split vacancy. The main difference for SiV-based sensors is that the whole observation is only all-optical. In contrast to NV centers, the ZPL of SiV centers at 738 nm dominates the spectrum and contains approximately 70% of all emitted photons [45]. The near-infrared shifted fluorescence is also a significant advantage for investigating biological systems due to the near-infrared window in biological tissue [46]. All these make pure optical thermometry with SiV more efficient than NV (Figure 1). First temperature sensing experiments with SiV in 200 nm sized ND presented in [47] demonstrated the sensitivity of 521 mK $Hz^{-1/2}$. The red shift of ZPL with the temperature increase goes linear [44], which allows the recalculation of absolute temperature changes detected with SiV in ND. The first application of ND with SiV for intercellular thermometry was demonstrated in [47], where HeLa cells were incubated with ND coated with human serum albumin (Figure 5b,c). Another advantage of ND with SiV centers is that its narrow near-infrared fluorescence can be separated from those commonly used in biological research optical markers (Figure 5a), which makes dual imaging with simultaneous thermometry possible.

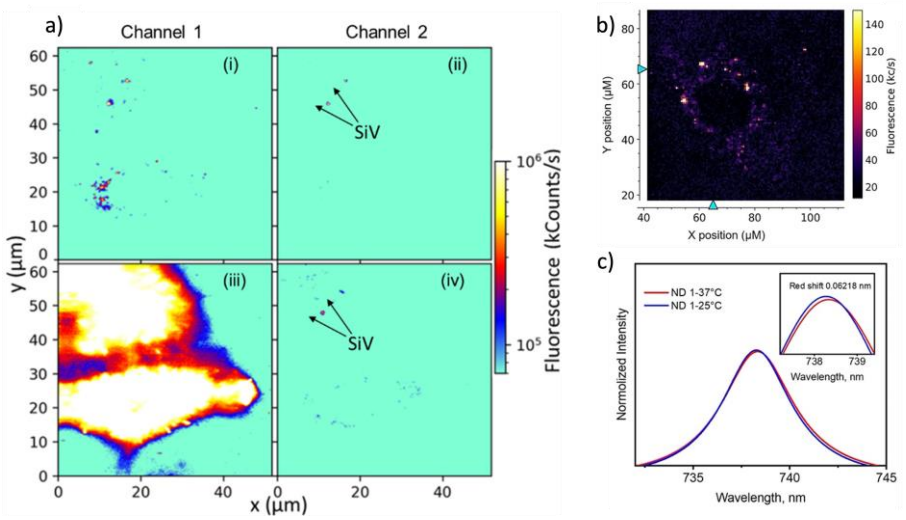

Figure 5. a) Confocal imaging of HeLa cells with SiV containing ND for dual imaging, where channel 1 collects all fluorescent with a wavelength higher than 560 nm, and channel 2 is selectively sensitive only to the SiV emission. (i) and (ii) cells with SiV Nd, (iii) and (iv) cells additionally marked with membrane dye. b) Confocal imaging of HeLa cells incubated with ND with SiV centers. c) Thermometry by the redshift of SiV ZPL measurement inside the living cell, where the cell incubator stabilized two temperatures. Data from [47].

The broad sensing applications for biological systems with SiV centers in ND were explored further. The group of I. Vlasov presented a series of works about temperature probes with SiV defects to investigate living cells [48,49]. Here, the alternative method was applied. Instead of incorporating ND with SiV centers inside a living cell, ND with SiV was placed at the end of the glass pipette (Figure 6, a) [48]. It allows the probe to be manipulated and controllably placed for temperature mapping (Figure 6b,c) [48]. This method was used to measure the temperature of the isolated mitochondria from the mouse brain [49]. These experiments demonstrated that the mitochondria temperature can experience a temperature difference of 4-22°C and reach an absolute maximum of 45°C.

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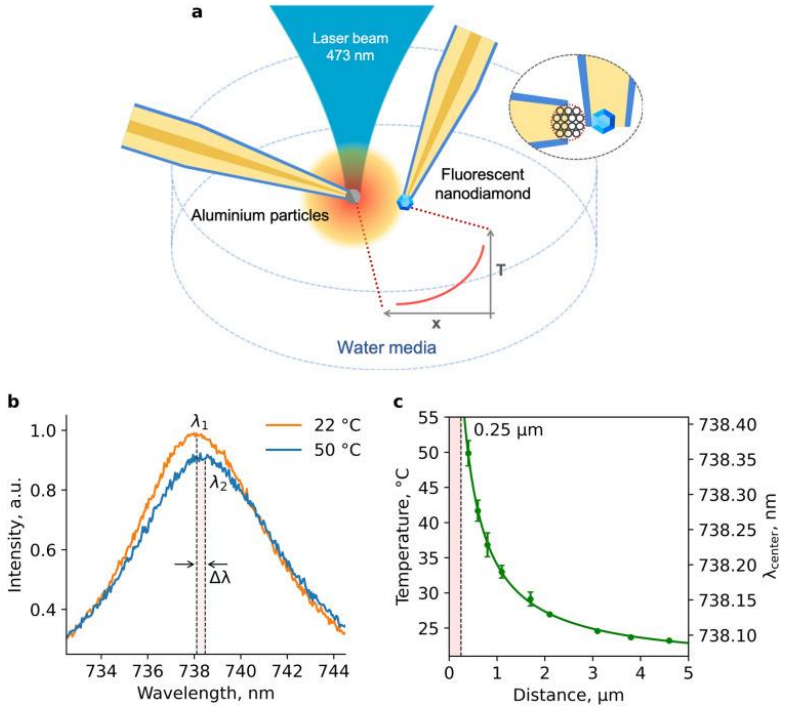

Figure 6. a) Schematics representation of the thermometry probe based on SiV containing ND. b) Photoluminescence spectra of the thermometer based on ZPL of SiV centers for two positions from the heater. c) The shifts of SiV ZPL as a function of the distance and temperature from the heat source. Data from [48].

2.1. Thermometry with Germanium-Vacancy Centers

Like SiV in diamond, there is a Germanium-Vacancy center (GeV). It has a similar crystal structure, and its spectrum also consists mainly of ZPL emission at 604 nm. Therefore, the experimental sensing protocol is based on observing the shift of ZPL (Figure 1), moving to the longer wavelengths with heating. The first demonstration of the GeV-based temperature sensor for a bulk diamond was made in the US-based research group [50]. In the last year, detonation ND containing GeV centers were recently tested for all-optical nanoscale thermometry [51]. There, the demonstrated sensitivity was at the level of 1 K $Hz^{-1/2.}$ (Figure 7). The obtained sensitivity is currently not as exciting as for NV or SiV centers. However, it is important to mention that, in general, detonation ND are much inferior in properties to CVD or HPHT nanocrystals, which were available for NV and SiV experiments earlier. Thus, these two types of nanodiamond synthesis attract a lot of attention and are actively used for the fabrication of GeV centers in ND [51–53] nowadays. Further improvement of the production of ND with GeV centers will increase the usability and sensitivity of that diamond defect.

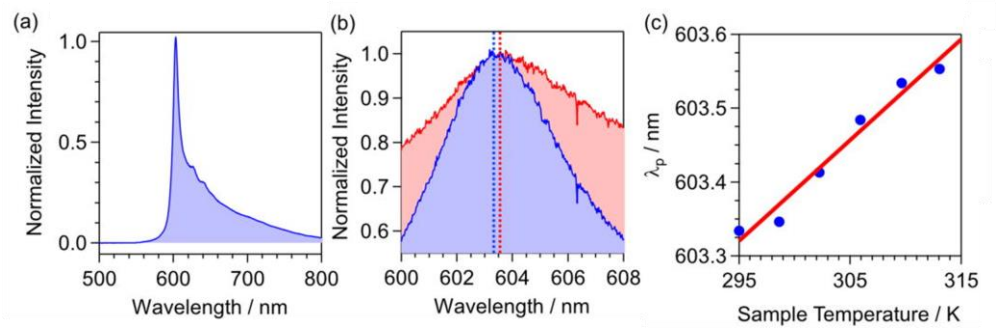

Figure 7. a) Photoluminescence spectrum of GeV in detonation ND at a temperature of 22.0 °C. b) The peak of the photoluminescence spectra of GeV centers was obtained at 22.0 °C (blue-filled) and 39.9 °C (red-filled). c) The shift of the ZPL position depends on the temperature. Data from [51].

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3. Discussion of Possible Limits

Fluorescent ND provide the outstanding possibility to measure temperature, especially intercellular temperature, independently from the environment [32], which is impossible for other currently used thermometers based on fluorescent nanoparticles [2,6,9]. The linear dependences of optical properties of various diamond color centers (ZPL or ODMR) allow for measuring absolute changes of variable temperature [20,27–29,44,50,51]. However, there is another important point related to the property of fluorescent ND that must be addressed for the complete picture. ND have a broad distribution in size, shape, and position of color centers inside. Any fabrication and subsequent separation of ND cannot provide a fully homologized sample. All that influences offsets for the central position of ODMR or intensity and broadening of ZPL that affect the detection of an absolute temperature.

One of the first reports about such inhomogeneity of ND was presented in [54] regarding the initial splitting and shift of the ODRM line for NV centers in commercial ND. The samples were evaluated in a shielded environment to exclude external influence. Figure 8 shows the distributions of such ODMR shifts (a) and splits (b). It indicates that the absolute temperate determination from a single nanodiamond measurement can be problematic due to the natural variation of nanocrystal properties. A similar point was addressed in [32], where ND were tested in different solutions (Figure 3b). Minor variations of the temperature dependence of ODMR shift and, more importantly, its statistical distribution also demonstrated that absolute temperature can hardly be found from a single spot measurement. However, it does not influence critically the observation of temperature changes monitored with a single nanocrystal. In such experiments, we are interested in the difference between two ODMR positions at two temperatures, and initially, its shift is canceling during calculations.

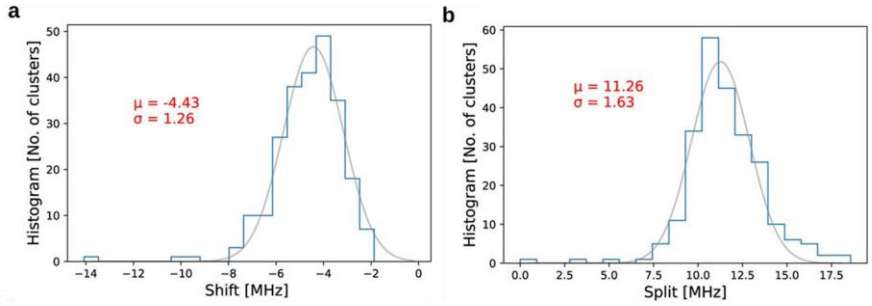

Figure 8. a) The distribution of the ODMR shift for ND over 247 nanocrystals, where the axial strain is determined as ~4.43 MHz. b) The distribution of the ODMR split for ND over 247 nanocrystals with the mean value of transverse strain at 11.26 MHz. Data from [54].

Similar inhomogeneity in the initial position and broadening of ZPL was observed for SiV centers in ND [47]. Here, the positions of ZPL were observed for temperatures ranging from 25°C to 35°C with a step of 2.5 °C, where the cell incubator stabilized the temperature of the solution (water). Two groups of ND with SiV can be selected: One demonstrates a good theoretically predicted shift of ZPL with the temperature increase. The second one has a low match with the theoretical calculation for SiV in bulk crystal. Therefore, additional investigation of ND is essential for reliable absolute thermometry.

4. Conclusions

Fluorescent ND provide a unique opportunity for nanoscale thermometry with high sensitivity and nanoscale spatial resolution and are insensitive to the environment. The non-toxic properties of nanodiamonds and different chemical functionalization methods allow for their application to biological study, where their sensing ability can be combined with theranostics applications. Nevertheless, NV centers recommended themselves as an outstanding system for biologically oriented magnetometry; effective thermometry based on them requires the application of an external microwave field that might bring limitations for intracellular use. Notably, the external microwave

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field can provoke a slight parasitic temperature increase, which can be crucial for biochemical reactions and cell biology. In the case of experiments with 3D tissue, deep measurements can be problematic since they will require high power of an external microwave field.

On the other hand, diamond color centers like SiV and GeV offer a fully optical measurement technique. Such an observation method is devoid of microwave-related problems and is highly promising for biological applications. At the same time, ND with color centers give the possibility of fast temperature mapping of living cells. The further development of such methods, together with the improvement of ND functionalization for addressed intracellular attachment, will provide a breakthrough method for cell biology and pharmacology, especially in combination with organ- and body-on-chip systems.

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Conflicts of Interest: The author declares no conflicts of interest.

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