Diamond Quantum Devices in Biology

Yuzhou Wu, Fedor Jelezko, Martin B Plenio, and Tanja Weil

MINIREVIEW

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Yuzhou Wu*[a], Fedor Jelezko*[b], Martin B Plenio*[c] and Tanja Weil*[a]

Abstract: The currently available molecular imaging techniques capable of reaching atomic resolution are limited to low temperatures, vacuum conditions or large amounts of sample. The quantum sensors based on spin-dependent photoluminescence of nitrogen-vacancy (NV) centers in diamond offer great potential to achieve single molecule detection with atomic resolution under ambient conditions. Diamond nanoparticles could also be prepared with implanted NV centers thus achieving unique nanosensors to be able to traffic into living biological systems. Therefore, this technique might provide unprecedented access and insight into the structure and function of individual biomolecules under physiological conditions and able to observe biological processes down to the quantum level and with atomic resolution. The theory of diamond quantum sensors and the current developments from preparation to sensing techniques have been critically discussed in this review.

1. Introduction

Molecular scale imaging and structure determination technologies such as X-ray diffraction, NMR, electron microscopy or scanning tunneling microscopy play a pivotal role in the advancement of sciences. Most currently available techniques capable of reaching atomic resolution are limited to low temperatures, vacuum conditions or depend on the availability of crystals and/or large sample volumes. These requirements limit the potential impact especially in the area of life sciences, where single molecule optical spectroscopy and atomic force microscopy are the only methods able to gain insights into the molecular structure, although their resolution is not sufficient to detect single atoms.

Quantum sensors based on the spin-dependent photoluminescence of nitrogen-vacancy (NV) centers in diamond offer great potential for atomic resolution imaging[1]. The advent of diamond quantum sensing promises solving the longstanding goal of single molecule detection with atomic resolution under ambient conditions[1-2] and there is currently no other technique that would offer such features. Therefore, this technique would offer great impact for the understanding of biomolecules in their native environments as well as fundamental biological phenomena.

This minireview describes the new and rapidly evolving field of nanoscale quantum sensing based on NV centers in diamond and the potential applications for superresolution molecular imaging in living biological systems. The theoretical bases and most advanced techniques of diamond based quantum sensing are summarized. In particular, the chemical preparation of high quality diamond quantum devices and the modification of diamonds for sensing in a living biological environment will be introduced. Their great potential and challenges for biosensing under physiological conditions are especially highlighted.

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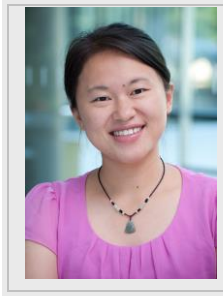

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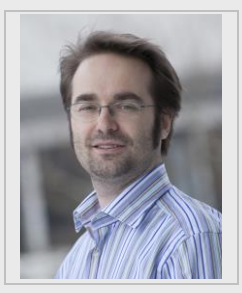

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2. The theory of diamond quantum sensing

A perfect diamond crystal is transparent to visible light because of a large optical bandgap of 5.48 eV corresponding to a wavelength of 226 nm. Despite this basic physical property, on rare occasions natural diamond is found to display vivid color. The source of this color can be traced back to plastic deformations of the diamond lattice or, more important for our purposes, substitutional and vacancy defects in the lattice structure that are capable of absorbing and emitting visible light. Hundreds of luminescent defects in diamond are currently known[3] and have been analyzed in sufficient detail to reveal their basic optical and electronic spin properties and in some occasions their chemical composition[4]. Impurity defects may originate from a wide range of elements including boron, nickel, silicon and, most commonly in diamond, nitrogen, whose color centers are of particular importance in the context of biological sensing applications.

2.1. Nitrogen vacancy center and other color centers in diamond.

The NV center[5] is a point defect in the diamond lattice that consists of a substitutional nitrogen atom that is directly adjacent to a lattice vacancy and which is oriented along the [111] crystalline direction (see Figure 1a). It is known to exist in several charge states including a neutral form, NV^0, and the negatively charged NV^-. While both forms are optically active, the optical and electronic properties of the NV^- are far better understood and appear to be more suitable for applications in sensing and quantum technologies.

The NV^- center possesses a sharp optical zero-phonon line at 637 nm (1.945 eV) and broad vibronic sidebands. Under illumination, it displays an additional, very weak zero-phonon line at 1042 nm (1.190 eV) supporting an electronic structure as shown in Figure 1b. It is noteworthy that the NV^- and other color centers do not bleach even when hosted in nanometer sized diamonds and subjected to intense illumination. Furthermore, their wavelengths are independent of the size of the host diamond down to sizes below 10 nm[6] (even around 2 nm[7] for silicon vacancy). These properties alone make fluorescent nanodiamonds (NDs) attractive biomarkers[8]. Importantly, both the ground and the excited state of the NV^- center display a zero field magnetic resonance at 2.88 GHz and 1.42 GHz, respectively, which occurs between the $m_s=0$ and the $m_s=\pm1$ magnetic states of an electronic spin triplet. This electron spin exhibits remarkably long coherence (T_2) and relaxation times (T_1), which can reach milliseconds in ultrapure diamond[9]. It is the combined availability of optical and electronic transitions that make the NV^- particularly suitable for a wide range of sensing applications. Crucially in this respect, even for single NV^- centers the electronic spin state can be detected by means of electron spin state dependent light scattering that discriminates between the $m_s=0$ and the $m_s=\pm1$ state[10] by making use of the concept of single molecule optically detected magnetic resonance (ODMR)[11]. The underlying principle of diamond quantum sensing and various applications will be described in sections 4.1 - 4.3. Interestingly, even at room temperature, the same underlying physics allows for the polarization of the electron spin triplet to the $m_s=0$ state in the space of a few microseconds by means of optical pumping. The resulting hyperpolarization of the electron spin may be transferred to nuclear spins[12] leading to potential applications in magnetic resonance imaging that will be described in section 4.4.

Despite the current emphasis of research on the NV^-, a multitude of color centers are known to exist, some of which display a similar combination of optical transitions, magnetic resonance from electronic spin states and spin dependent fluorescence. Recently, the silicon-vacancy (SiV^-) has been receiving growing attention in this respect. It has a strong zero-phonon line at 737 nm (1.68 eV) and an electronic spin-1/2 system whose structure has recently been elucidated in some detail to demonstrate its application potential[13].

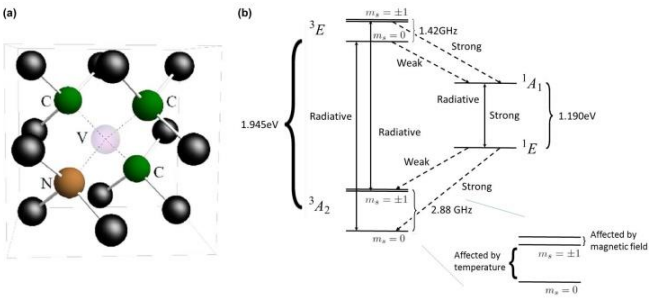

Figure 1. (a) The NV center in diamond - a substitutional nitrogen atom adjacent to a vacancy formed by a missing carbon atom. (b) The dangling bonds form an electronic spin-1 system with an optical transition at 1.945 eV. Optical and

infrared transition (solid arrows) and weak non-radiative transitions (dashed lines) allow for the observation of spin dependent fluorescence and optical electron spin polarization.

3. Preparation and functionalization of diamond quantum sensors

Diamond quantum sensors could be fabricated by implanting color centers in both bulk diamonds and diamond nanoparticles. Bulk diamond refers to diamond crystals with micrometer to millimeter dimensions, which are ideal for developing *in vitro* biosensing arrays. Their preparation is less challenging since color centers are normally stable if they are located more than 2nm below the surface. In comparison, nanoscale diamonds (nanodiamonds or NDs) are particularly attractive for biosensing applications, because they could serve as nanoprobes in solution that can be delivered into cells, tissue and living animals. However, it is much more challenging to prepare high quality NDs with stable color centers due to their very large surface to volume ratio. The color centers implanted in NDs can be easily perturbed by surface functionalities. These are however required to improve colloidal stability, biocompatibility as well as biofunctionality of the NDs to access biomedical applications. In this section, different methods for the preparation of both bulk diamonds and ND particles with color centers are briefly described and compared. Especially the challenges for producing high quality NDs and the functionalization of NDs for *in vivo* applications will be discussed.

3.1. Preparation of bulk diamond for quantum sensing.

Bulk diamond crystals could be prepared by several synthetic methods, including the High-Pressure High-Temperature (HPHT) growth and the Chemical Vapor Deposition (CVD) procedure (Figure 2). The HPHT growth is the major manufacturing method for synthetic diamond products and it offers a significant degree of control over the quality and geometry of the diamond obtained. Most diamonds produced by this method consists of small grains of type Ib (the classification of diamonds with isolated nitrogen impurity dispersed in the crystal) with dimensions from a few micrometers up to ~10 mm[14]. These diamonds often already contain substitutional nitrogen atoms (called P1-centers) from the solvent, metal and carbon source material, as well as from the residual gas left in the HPHT reactor. To produce lattice vacancies, these diamonds could be irradiated by high-energy particles such as electrons, protons, neutrons, ions and gamma particles. At temperatures of around 800 ℃, the substitutional nitrogen produces strain in the diamond lattice and efficiently captures moving vacancies, that then form the NV color center[15] (Figure 2c).

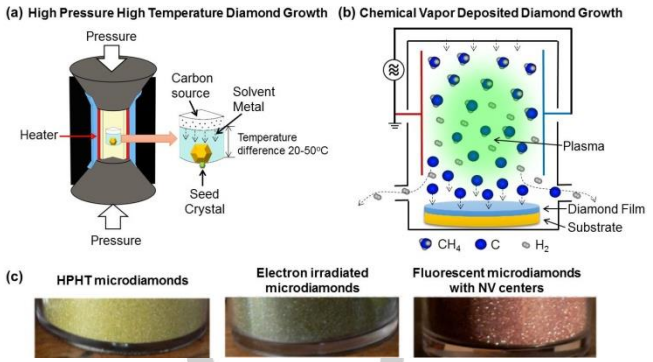

Figure 2. HPHT growth and CVD preparation of diamonds and creation of NV centers into diamonds.

Another popular method of growing synthetic diamond is Chemical Vapor Deposition (CVD)[16]. At low pressure (below atmospheric pressure), a mixture containing carbon-rich gases (typically 1 to 99 methane to hydrogen) is broken into fragments by plasma between two electrode and reassembles into a diamond film on a surface (Figure 2b). Therefore, the CVD diamonds are normally obtained as thin films. NV centers can be created during the CVD process by growth of diamond film with 0-0.1% N_2, 0.7% CH_4, 99.2% H_2 gas mixture[17]. The NV center concentration varies with the nitrogen doping levels. Therefore, even single defects can be created by optimizing the nitrogen ratio.

3.2. Preparation of nanodiamonds particles for quantum sensing

Nanodiamond particles could be produced by several methods, such as detonation[18], laser ablation[19], ion irradiation[20] and chlorination of carbides[21]. However, most of these methods produce polycrystalline NDs and ultra-nanocrystalline NDs (Figure 3) with only a few nanometers of crystalline regions, which are not suitable for quantum sensing applications. Ideal NDs for quantum sensing should contain very stable color centers in a highly crystalline diamond lattice, preferably with controllable numbers of color centers at spatially defined positions. Therefore, the NDs used for developing quantum sensors are normally monocrystalline and prepared mainly by high-energy ball milling of high-pressure high-temperature (HPHT) diamond microcrystals[6] and plasma assisted chemical vapour deposition (CVD)[22]. As explained in 3.1, HPHT growth could produce type Ib diamonds in a few micrometer diameters (>20–50 µm). By the mechanical milling step, these HPHT microdiamonds can be fabricated into NDs with high purity after acid cleaning (normally with highly oxidative acids such as 1:1:1 mixture of $HClO_4$, HNO_3 and H_2SO_4) and decontamination[21b]. By centrifugation purification, these NDs can be separated into different sizes from a few nanometers to tens of nanometers. Since HPHT synthetic diamonds already contain nitrogen defects (P1-centers), they can be used for producing stable and bright fluorescent NV-rich NDs. To create NV centers, the milled NDs can be embedded in graphite tablets to allow convenient irradiation and annealing

similar as for bulk diamonds. Subsequently, oxidation procedures such as acid treatment or thermal oxidation allow purification of NDs from graphite tablets to remove the surface contamination caused by annealing and to further increase the brightness of the NDs. With this method, the NV centers are normally stable after implanting, however, currently it is still challenging to render all the NDs fluorescent. The alternative strategy would be irradiation and annealing of HPHT microdiamonds and then milling into NDs[6], an approach which has been more widely used and which has even been commercialized by several companies[23]. For this method, some NV centers will be destroyed during the milling process and therefore, an extremely high concentration of NV centers is required in the microdiamond starting material, which is challenging to achieve.

The production of monocrystalline NDs can also be achieved by the chemical vapor deposition technique (CVD)[22], however, incorporation of nitrogen in small nanocrystals by this concept is very inefficient[24]. In comparison, the CVD method is more attractive for preparing Si-V containing NDs, since plasma etching of the silicon substrate on which the CVD diamonds are grown provides the source of the silicon. It is also convenient to introduce other defects by addition of alternative gases, such as trimethyl boron for boron defects[25].

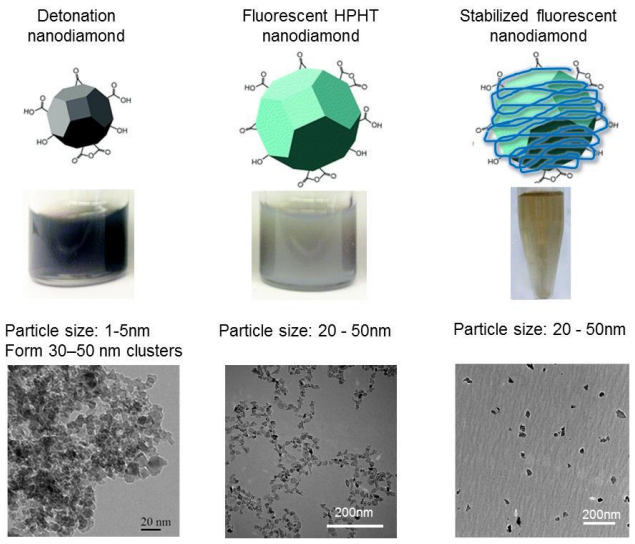

Figure 3. Comparison of detonation ultrananocrystalline ND, HPHT fluorescence NDs with NV centers and the fluorescence NDs after stabilization[26].

Although the current methods provide NDs of different sizes and color centers, it is still challenging to fulfil all the requirements for quantum sensing. Ideally, the NDs for quantum devices should have a controlled number of NV centers, and the stable NV centers should be at least 2nm below the surface. Therefore, NDs should have regular shapes with narrow size distributions. In some cases, when a single NV center as a spin probe is required, the diamond nanocrystal needs to have a controlled size and shape with the NV localized in the center. Therefore, new methods offering more controlled ND synthesis are still urgently needed. One potential method might be controlling the HPHT growth of diamond crystals as small as possible to directly obtain NDs with regular shape. In principle, if one could control the HPHT growth from a nitrogen containing molecular seed, it might be possible to produce NDs with only one NV center in the middle, which would be ideally suited for quantum sensing.

3.3. Functionalization of ND sensors for biological applications.

After fabrication of high quality NDs with NV centers, for biosensing applications it is crucial to achieve surface functionalization that improves colloidal stability in a biological environment. Due to the large relative surface area and the electrostatic interactions, biomolecules of interest could simply be absorbed at the surface of the NDs nonspecifically[27]. In first attempts, this early approach has facilitated the sensing of certain biomolecules. For instance, absorbing the protein ferritin on the ND surface has allowed non-invasive detection of iron levels inside the protein cage[27b]. In addition, different functional groups could also be introduced at the ND surface, facilitating covalent attachment of the biomolecules (Figure 4), which has been reviewed previously [8c, 28]. Such direct surface conjugation is currently the main method for ND biofunctionalization, and it has been applied successfully for conjugating antibodies[29], DNA[29], enzymes[30] and other functional proteins[31]. However, the raw, as-synthesized NDs easily form aggregates especially in biological media due their large surface to volume ratio, which makes it challenging to detect them as individual probes (Figure 3). Surface chemistry is also restricted by uncontrolled aggregation and even precipitation. Moreover, due to the limited number of surface functionalities, only very low densities of biomolecules could be attached to NDs by direct covalent surface modification.

To stabilize NDs in biological media and also to render functionalization more efficient and reproducible, biocompatible non-covalent coatings have been recognized. Initial studies have demonstrated that NDs with an absorbed layer of the plasma protein serum albumin already reveal improved stability facilitating the detection of a single particle probe inside living cells by stimulated emission depletion (STED) microscopy[32]. Along this line, different coating materials have already been investigated, including inorganic and polymeric materials (Figure 4). For instance, a silica shell has been grown at the ND surface, rendering the particle surface biocompatible, stable and readily functionalized through routine silica linking approaches[33]. The hydrophilic polymer polyethylene glycol (PEG) has been attached to the silica shell to further enhance colloidal stability over a broad pH range (pH 2-10) and even in 1M NaCl and cell culture medium[33a]. Alternatively, polymers could also be used as biocompatible ND coatings. For instance, PEG type polymers have also been directly coated onto NDs by covalent conjugation[34]. Since the PEG coating is known to be particularly biocompatible with low non-specific absorption, such NDs are found non-toxic for cells and tissues and are therefore promising for *in vivo* applications[34b, 34c]. However, PEG polymers do not

allow post-functionalization, which is a limitation of this coating method. Recently, a biopolymer coating strategy has been developed using a PEG hybrid polymer derived from native albumin[35]. NDs coated by these biopolymers are found to be perfectly stable in all tested biological buffers and also within a broad pH range (pH 2-8) without any aggregation. In addition, this protein derived biopolymer provides high numbers of orthogonal functional groups from the amino acid residues, which allows easy post modification of biomolecules (Figure 4).

4. Sensing with diamonds – methods and applications

A unique optical access to a long living ground state spin is a key element for magnetic sensing at nanoscale described in this section. Single NV centers are particularly interesting compared to other magnetic field based sensing techniques (like magnetic resonance force microscopy) due to the extremely weak perturbation since a single NV center carries only weak magnetic moments (similar to a magnetic moment of a single electron spin). In addition, the absence of bleaching of fluorescence allows using color centers for sensing applications *via* fluorescence resonance energy transfer (FRET)

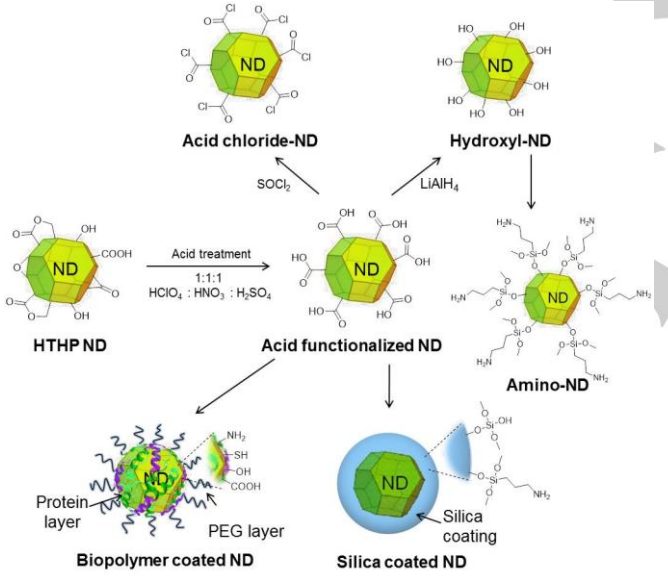

Figure 4. Functionalization of NDs by direct chemical modification and biocompatible coating. The original prepared HTHP NDs have uncontrolled functional groups on the surface and acid treatment would yield NDs with carboxylic acid functionalized NDs. Such carboxylic acid groups on NDs could be subsequently derived to acid chloride, hydroxyl and amino groups[8c, 28] that facilitate further chemical modifications with biomolecules. The acid treated NDs could also be coated by silica [33] or positively charged biopolymers[35] to alter the surface properties of NDs.

4.1. Optical sensing with diamonds

Optical techniques developed during the past two decades nowadays provide ultimate sensitivity of detection reaching single fluorophores[36]. Fluorescence markers even offer ultrasensitive imaging beyond the limits imposed by the diffraction of light[37]. However superresolution imaging techniques often suffer from limited photostability of the fluorophores. Stimulated emission depletion (STED) microscopy routinely reaches resolution of 20-50 nm[38], but higher resolving powers require high intensity of depletion beams leading to fast photobleaching of the fluorescence markers. Other superresolution imaging methods are based on the analysis of blinking events of single chromophores, which also requires high photostability (i.e. absence of irreversible bleaching) for achieving high resolution[39]. That´s why color centers in diamond with their extreme photostability are very promising probes for superresolution microscopy when perturbation due to the relatively large size of nanodiamonds is not crucial. Initial experiments performed with NV centers in bulk diamond demonstrated resolution down to 6 nm[40]. More recently, STED microscopy was applied to NV doped diamond nanocrystals[41] and up to 10 nm resolution was demonstrated [42].

Another very powerful optical technique for sensing at the nanoscale is fluorescence resonance energy transfer (FRET). FRET sensing is based on the sharp distance dependence of energy transfer between individual chromophores. The unique photostability of color centers offers new opportunities for improving FRET-based sensing techniques. It was shown that single NV centers embedded in 20 nm diamond nanocrystals allow detecting single molecules absorbed at the nanodiamond surface with high efficiency[43]. Although the dynamic range of FRET microscopy is limited, this drawback can be overcome by scanning probe FRET techniques where a donor e.g. the NV center in a diamond nanocrystal is attached to the tip of an atomic force microscope[44].

4.2. Detection of external electron spins using NV centers

Nanometer-sized diamonds containing NV centers are promising nanosensors in biological environments due to their biocompatibility, bright fluorescence, and high magnetic sensitivity at ambient conditions. The triplet ground state of the NV center can be employed as nanoscale magnetometer[1, 45]. Owing to the localized nature of the NV defect, it could be considered as a point magnetic dipole, whose energy is modified by the presence of external spins. Such changes of energy (recorded as shift of spectral lines or perturbances of the spin echo or enhanced relaxation of NV centers) can be accessed using optically detected magnetic resonance on NV center spins. The detection of external electron spins using such a diamond magnetometer[46] suggested many opportunities for studying biomolecules even in their cellular surrounding. Fast fluctuating external spins have been measured *via* enhanced relaxation of NV centers. In this case, noise from external spins is recorded as a signal (noise or Decoherence microscopy)[47].

Typically, the goal in sensing schemes is the reduction of noise to gain optimal access to a coherent signal. In Decoherence microscopy, noise can also constitute the signal. The sensitivity

of this technique can reach the ultimate limit down to the detection of a single paramagnetic ion[48]. It also can be extended to the detection of biomolecules e.g. when paramagnetic ions are attached to proteins[49]. The detection of single electron spins can also be applied to proteins, where spins are natively present and carry a particular function, for example in magnetic proteins such as ferritin[27b, 50].

The detection of ferritin (proteins carrying iron in many organisms) using magnetic noise induced by the inner paramagnetic iron as a contrast mechanism has been accomplished with sensitivities reaching the single molecule detection threshold[27b, 50]. A significant reduction of both coherence (T_2) and relaxation time (T_1) due to the presence of ferritin on the surface of NDs was obtained (Figure 5). Thus, nanoscopic magnetic field sensors based on single defects in diamonds pave the way towards a novel sensing technology that may potentially even resolve important biological processes such as electron transfer in cellular environments. The remarkable sensitivity paired with the non-invasive character of the detection process may also provide access to quantum mechanical properties at the biological level, e.g. in electron transfer and radical pair dynamics, that are typically hidden in ensemble and highly invasive measurement schemes[51].

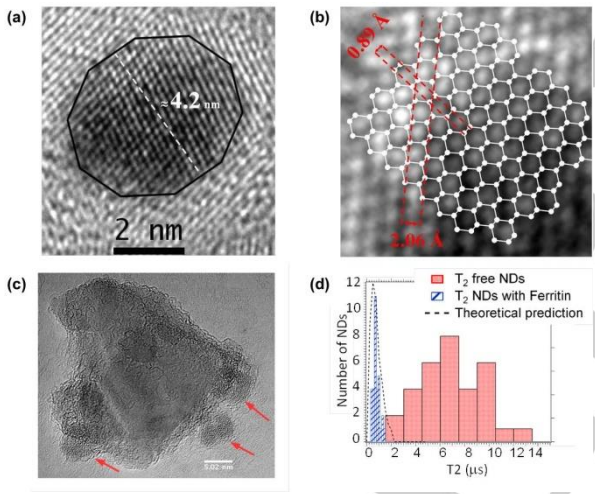

Figure 5. Detection of iron containing ferritin using NV center in ND with single protein sensitivity. (a) High resolution transmission electron microscopy (HRTEM) image of ND. (b) The carbon lattice of ND according to HRTEM image. (c) HRTEM image of ferritin absorbed on ND (ferritins are indicated by red arrows). (d) Detection of ferritin by the significant reduction of coherence time (T_2).

4.3. Single molecule nuclear magnetic resonance (NMR) spectroscopy with diamond spins

Nuclear magnetic resonance is one of the most powerful imaging techniques in life sciences. Highly informative NMR spectroscopy allows reconstruction of the structure of proteins *via* unraveling interactions between nuclear spins inside complex molecules[52]. The sensitivity of conventional NMR techniques (based on induction detection) is limited to large spin ensembles. Therefore, there is a growing interest in the development of new NMR detection methods. NV-based magnetometry is one of the most promising avenues in this research field.

A single nuclear magnetic moment is weak and is approximately 1000 times weaker compared to that of an electron. However, when brought into close proximity to the NV center (a few nanometers), small spin ensembles can produce fields in the order of microtesla which allows for the detection and localization of such spins[53]. The main challenge of NV-based NMR experiments is to place NV centers close to the molecule of interest and retain long coherence time. Note that the coherence time of NV defects in the presence of noise originating from parasitic spins located at the diamond interface can be improved by orders of magnitude using high order spin echoes[54]. Careful design of such echo sequences allows for the realization of a situation when a significant part of the noise is cancelled but a narrow spectral channel for sensing remains open. Recently, the detection of NMR in 100 nm^3 volume of liquid sample placed at the diamond surface[55] and in a microfluidic device with below 500 nm spatial resolution[56] was achieved. For solid samples with long coherence time of nuclear spins (Si^{29} in quartz and nuclear spins associated with hydrogen bound to diamond interface), sensitivity sufficient for the detection of a single nuclear spin within a few seconds of measurement time was reached[57] (Figure 6).

In order to apply NV-based NMR technique for biomolecule structure elucidation, the spectral resolution of NV magnetometers needs to be improved to detect dipolar couplings and chemical shifts. Such an improvement can be achieved on the one hand by the development of new measurement protocols similar to two dimensional NMR [58] and on the other hand by improving the coherence time of NV centers close to the interface. New techniques allowing removal of magnetic noise using new protocols that have been developed in the context of quantum computation (such as quantum error correction) are very promising in this respect[59].

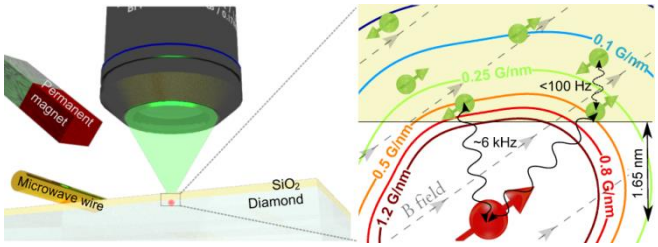

Figure 6. NMR of ^{29}Si nuclei with a strongly coupled sensor. (a) Schematic of the experimental setup. Dilute, unpolarized nuclear spins in a silica layer interact with an electronic spin in a (100)-surface diamond, which is readout with optical microscopy. (b) Schematic of strong coupling regime. A shallow NV center in diamond (2 nm from the surface) couples to nearby ^{29}Si nuclei in a silica layer, due to hyperfine interaction. The contour lines show the strength of the effective magnetic gradient experienced by the nuclear spins. Reproduced from reference[57] with permission, copyright Nature Publisher Group.

4.4. Hyperpolarisation of diamonds for molecular imaging

Magnetic resonance imaging (MRI) is one of the most important imaging techniques in medicine. Owing to the long wavelength of radiofrequency photons, it does not suffer from scattering allowing non-invasive imaging of tissues. The main drawback of MRI is a low sensitivity, which in part is related to low polarization of nuclear spins at low temperature. Recently it was demonstrated that the sensitivity of MRI can be improved by using dynamic nuclear spin polarization (DNP) of NDs[60] (Figure 7).

The key element of this scheme is optical pumping allowing polarization of the electron spins of NV centers on a microsecond timescale (hyperpolarization). Polarization can then be transferred to nuclear spins using well established NMR protocols such as Hartmann-Hahn match[12], solid effect[61] or energy match between electron and nuclear spins *via* level anticrossing[62]. The polarization of NDs represents a more challenging task compared to bulk diamond owing to disordered orientation of NV centers, but advanced DNP protocols addressing this issues were developed recently[60b].

The main advantage of NV defects for dynamic nuclear spin polarization is related to the optical pumping, which allowsuse of the same NV center to polarize multiple nuclear spins on a short timescale[12]. In addition, experiments can be performed in low field and room temperature without loss of performance. Hyperpolarized diamond nanoparticles show long (more than a minute) relaxation time[63] and in bulk diamond relaxation on the hours scale was measured, which is crucial for MRI. Furthermore, NV defects can also be used to polarize external nuclear spins for the case when they are located close to the diamond surface[64] and may permit the construction of flow channels, in which highly polarized molecules may be obtained at volumes sufficient for NMR detection[65].

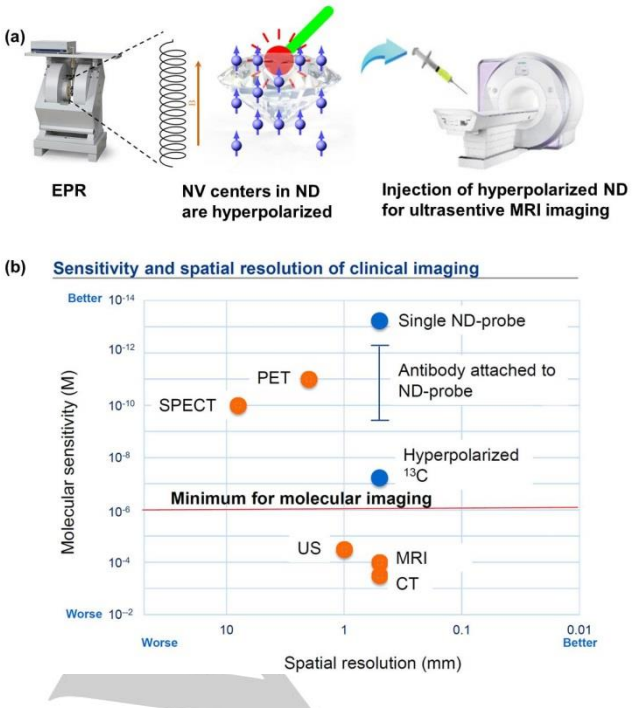

Figure 7. Hyperpolarization of NV centers in ND for enhanced MRI. (a) Illustration of the process of hyperpolarized NV centers for enhanced MRI imaging. (b) Comparison of the sensitivity and spatial resolution of clinical imaging techniques and hyperpolarization MRI imaging. PET stands for positron emission tomography; SPECT stands for single-photon emission computed tomography; US stands for ultrasound; CT stands for computerized tomography. Hyperpolarized ND probe would allow the same spatial resolution of MRI, but with extremely high sensitivity for molecular imaging with existing MRI systems.

4.5 Self-assembled nanodiamond structures

The realization of scalable arrangements of NV centers in diamond remains a key challenge on the way towards efficient quantum information processing, quantum simulation and quantum sensing applications. Although technologies based on implanting NV-centers in bulk diamond crystals or hybrid devices have been developed, they are limited by the achievable spatial resolution and by the intricate technological complexities involved in achieving scalability. A novel approach for creating an arrangement of NV-centers is based on the self-assembling capabilities of biological systems and their beneficial nanometer spatial resolution has been proposed and demonstrated[66]. The feasibility of interconnecting NDs with biological structures presents the formation of small ND complexes using an SP1 (Stable Protein 1) protein variant and first steps towards the formation of regular arrays of NDs on SP1 arrays[66] (Figure 8). As an important result towards the creation of ordered ND structures, the formation of numerous dimers and trimers along with larger ordered structures such as a seven ND hexagon has been achieved.

Going beyond 1D and 2D periodic patterns, the method of DNA-Origami, based on folding a large single-stranded DNA molecule directed by staple strands, enables the creation of more and complex, highly controllable structures, ranging from aperiodic arrays to real three dimensional architectures[67]. Based on selective Watson-Crick base-pairings, precise 2D or 3D DNA nanostructures have designed and manufactured. Composite assemblies based on DNA nanostructures offer an ideal platform to study the distance and orientation-dependent electronic and optical properties of fluorophores and nanoparticles. Until now, low colloidal stability even of coated NDs at high ionic strengths has limited their conjugation to DNA nanostructures. Recently, a biopolymer-based coating has been reported that efficiently encapsulates NDs and imparts high colloidal stability even at high ionic strength required for DNA conjugation (see section 3.3). Biotin-functionalized and perfectly dispersed nanodiamonds have been realized that were self-assembled in predefined 1D, 2D and 3D geometries[68] based on the tight interaction of biotin and streptavidin. Such assemblies offer unique opportunities for accomplishing self-assembled spin lattices or plasmon-enhanced spin sensors as well as improved fluorescent labelling for bioimaging.

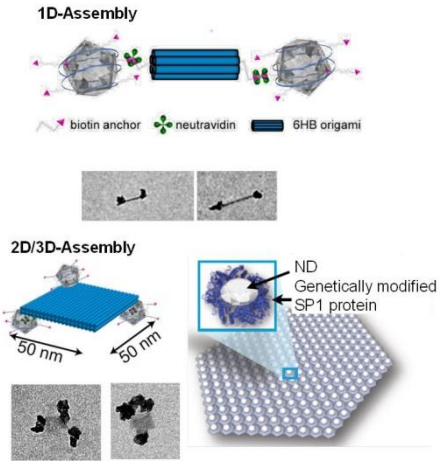

Figure 8. Self-assembly of NDs on a DNA origami template and a protein template (SP1).

5. Diamond sensing in a living biological system – progress and challenges

Diamond based sensing for studying various biological questions such as the structure and dynamics of proteins in a cell represents an emerging area. Beyond the advantages of traditional optical imaging, NDs could be applied in living biological systems as probes for detecting the tiniest magnetic fields, electron spins and temperature with nanoscopic and even atomic precision[1, 69] using the techniques discussed in section 4. Therefore, applying such diamond magnetic field sensors for biological studies will provide a potentially revolutionary tool to elucidate the structure and dynamics of biomolecules in a biological environment. To achieve these ultimate goals, there are still several major challenges that need to be addressed. In this section, the current progress of NV-diamond materials for biological applications will be briefly discussed highlightingthe future potential and challenges.

5.1. Biocompatibility of diamond materials

Diamonds are generally considered stable, bioinert and biocompatible[70] due to their highly stable lattice. However, the biosafety of diamond nanoparticles for *in vitro* and *in vivo* applications still needs to be carefully investigated due to their potential “nanotoxicity”, i.e. toxicity induced by their nano-size. Until now, many *in vitro* cytotoxicity studies suggest that, in comparison to other nanomaterials such as carbon nanotubes, semiconductor or metal particles, NDs represent the most biocompatible materials with no cytoxicity to different cell lines[71]. Long time observation of NDs in cell culture also shows that NDs do not cause chromosomal or genetic damage and they equally divided into daughter cells without affecting cell division and differentiation[72]. Intensive *in vivo* studies in mice and *Caenorhabditis elegans* also revealed promising biocompatibility without any liver toxicity, systemic inflammation, or deleterious effects to the offspring[73]. However, recently some potential risks of NDs have been pointed out, such as the occurrence of increased total reactive oxygen species in human hepatocytes [74], causing slight DNA damage in embryonic stem cells[75] and long-time accumulation in liver, spleen and lung with slow excretion rate[76]. NDs as any other non-endogenous material might elicit side effects *in vitro* and *in vivo* depending on the concentrations applied. However, based on the *in vitro* and *in vivo* studies reported until now, NDs are still among the most biocompatible nanoparticles with promising biosafety profile. In addition, NDs of different sizes, surface modifications and administration routes will most likely also have a different biodistribution and potential side effects. Therefore, biocompatibility of NDs requires careful assessment when designing new *in vivo* ND probes. Improving the quality of ND samples by optimizing the diameters, narrowing the size distributions, enhancing the colloidal stability under *in vivo* conditions, as well as functionalization of the ND surface will provide NDs optimized for *in vitro* and *in vivo* applications.

5.2. NDs for drug delivery

The current applications of NDs in living biological systems are mainly focused on drug delivery. NDs as drug delivery carriers offer many advantages such as their detection by fluorescence imaging as well as surface functionalization facilitating different drug loading and cell targeting strategies. The first generation of ND-drug delivery systems have been prepared by simply absorbing mainly lipophilic drug molecules onto the ND surface.[77] These ND-drug complexes composed of about 4-6 nm primary detonation ND particles cluster in solution into 100-200 nm aggregates, which can substantially absorb drug molecules and significantly enhance their blood circulation half-life and tumor retention.[77-78] Different anti-cancer drugs have been delivered by this approach[77, 79] and progress has been made to address targeted drug delivery, controlled drug release, intracellular tracking and evading chemoresistance[34b, 80]. However, it is highly challenging to prepare stable and well-defined nanoparticles with narrow size dispersion by this method, which leads to the risk of non-specific interactions with plasma proteins and unpredictable side effects[81]. Second generation ND transporter have been designed by surface modification of single ND particles with stabilizing polymers[82]. Drug molecules[29, 83] such as the anti-cancer drug doxorubicin (DOX)[84] and platinum complexes[85] have been covalently conjugated to these NDs, some even offering stimulus-responsive drug release[83b, 84-85]. Among these covalent ND modification approaches, the introduction of a polyglycerol shell [84-85] significantly increases ND stability and offers great prospects especially for the delivery of hydrophilic drugs, e.g. platinum complexes, since no agglomeration in mammalian cells was observed. However, achieving high colloidal stability after loading hydrophobic drugs still remains a key challenge[27a]. Recently we have investigated drug delivery with the albumin biopolymer coated nanodiamonds since they have revealed excellent stability under biological conditions as introduced in section 3.3. Drug molecules have been covalently conjugated to the albumin backbone *via* an acid labile linker. Even after a high loading of hydrophobic drug molecules, the resulting ND-drug conjugates reveal high solubility

in bio-fluids without observable aggregations. The preliminary in ovo data with breast cancer xenografts on Chick Chorioallantoic Membrane (CAM) model also indicated the enhanced tumor therapy effect[35]. All these attempts show that surface coating and modification of NDs could be an efficient strategy to optimize NDs as efficient drug transporters.

5.3. Potential and challenges of diamond sensing in biological system

The biosafety evaluation of NDs and their drug delivery studies support the promising potential of using NDs in living biological systems. In addition, the unique and powerful quantum sensing technique based on NV centers has already been discussed above. Combining these advantages, NDs offer great potential to be developed as the smallest sensing probes for detecting the tiniest magnetic fields and electron spins *in vitro* and eventually *in vivo*, and may serve as noninvasive nanosensors for studying the structure and biological reactions inside living organisms. Although this field is just emerging, several successful examples have already been demonstrated that underline the unique potential of ND quantum sensors. Studies have shown that the magnetic resonance of individual fluorescent NDs can be optically detected inside living human Hela cells, and their location, orientation, spin levels and spin coherence times can be measured with nanoscale precision[80d]. The energy of spin levels of individual NV centers served as fingerprints, allowing identification and tracking of ND particles with identical fluorescence. Furthermore, monitoring decoherence rates in response to changes in the local environment may provide unique insights into intracellular processes. One successful example is using ND-based sensitive nanoscale thermometry by measuring changes of the spin coherent time of the NV center in response to a local temperature. In the absence of an external magnetic field, the precise value of the transition frequency has a temperature dependence, which was used to report the local temperature changes (see Figure 1 and Figure 10). With this technique it is possible to directly measure the local temperature in living cells at length scales as short as 200 nm[69]. Other examples include the potential for measuring minute forces in cells and proteins employing analogous principles[86].

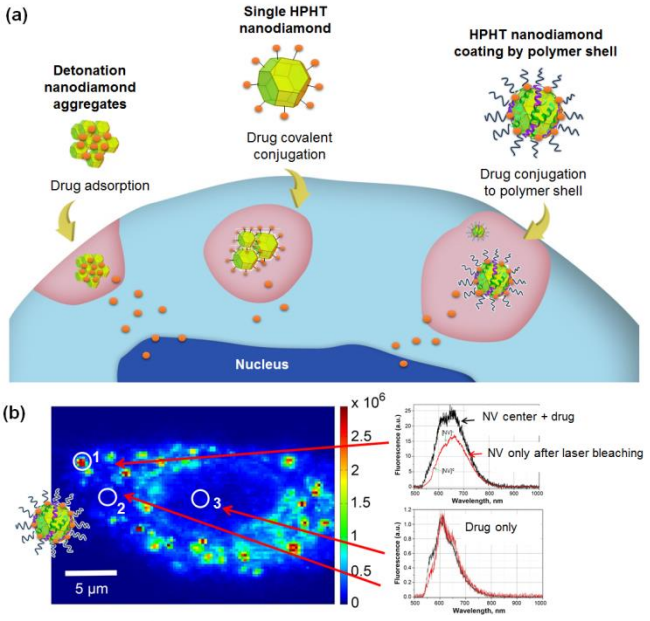

Figure 9. Illustration of ND based drug delivery systems. (a) Comparison of different ND based drug delivery strategies. Detonation NDs form aggregates that could absorb drug molecules and also allow uncontrolled drug release[27a, 77-78, 79b]. Non-coated HPHT ND with covalently linked drugs often revealing aggregations inside biological systems[29, 83a]. Polymer coated HPHT ND allow loading of drug molecules on polymer shell and stimuli responsive drug release[34c, 35]. (b) Confocal fluorescence microscopy imaging of doxorubicin (DOX, an anti-cancer drug) loaded on biopolymer coated HPHT NDs inside living cells[35]. Circle 1 highlights the representative emission from cell vehicles where overlap of florescence spectra from NV center and DOX drug can be observed. After laser bleaching, DOX emission can be quenched and non-bleaching fluorescence from NV center could be clearly recorded. Circle 2 and 3 represent the fluorescence spectra from cytosol and nucleus where only DOX emission can be observed indicating drug release from ND carriers.

If, on the other hand, an external magnetic field is applied in the biological sample, the spin coherence time of the NV center is then dependent on the magnetic environment, which enables magnetic imaging of proteins in vitro[27b] and even inside living cells. As the first example, the magnetosomes produced in living magnetotactic bacteria could be imaged and analyzed using an optically detected magnetic field imaging array consisting of a nanometre-scale layer of NV centers implanted at the surface of a diamond chip[87]. In addition, as discussed in section 4.2, NV centers could also detect electron spins in a local biological environment. If such an electron spin label was introduced to the biomolecule of interest, this technique would also allow detection and analysis of this biomolecule *via* NV centers in a living system with single molecule sensitivity. In addition, the single molecular NMR technique described in section 4.3 might eventually also allow the detection of nuclear spins thus facilitating deeper understanding of cellular reactions in real time even in living cells. The recognition of nuclear spins represents a greater challenge than electron spin detection due to the higher noise of nuclear spins in a biological system as well as the weak nuclear spin magnetic moment that requires very close proximity to NV centers. However, promising solutions are already under substantial

investigation as discussed in section 4.3. Furthermore, the hyperpolarization technique using NV centers is envisioned to achieve ultra-high sensitive MRI as described in section 4.5. These studies demonstrated the viability of controlled NV centers as single spin probes for nanoscale magnetometry in biological systems, thus opening up a host of new possibilities for quantum-based imaging in the life sciences.

It should be stressed that a variety of obstacles have to be overcome before these ideas can be brought to full fruition. The sensitivity of the NV sensor to minute signals also implies that it is easily perturbed by the wide variety of noise sources such as nuclear and electron spins, charges and radicals in random motion that are intrinsically present in biological systems. Furthermore, the nanodiamond sensor is not stationary and will move and rotate thus leading to randomization of the signals. Solutions for these challenges are currently being developed in physics laboratories (see section 4) but will certainly need to be adapted and transferred to biological environments to allow for their successful application initially *in vitro* and finally *in vivo*. While certainly challenging, current research has not identified fundamental limitations in this direction. Hence it can be expected that ND sensing application in biology will be realized. Then their performance relative to established technologies can be assessed in detail and application areas in which they outperform current technologies can be identified to open the door to a new mode for observation of biological phenomena.

6. Conclusions

Detection and analysis of the structure, dynamics and interactions of single molecules under native conditions with high sensitivity and nanometer spatial resolution represents an outstanding challenge with strong impact on modern science and technology. The NV center in diamond is a unique tool that offers (1) non-bleaching fluorescence measurement required for superresolution fluorescence imaging, (2) electron spin and nuclear spin detection with great potential for atomic resolution under ambient condition, as well as (3) hyperpolarization by easy optical pumping to enhance MRI sensitivity. In addition, diamond nanoparticles down to 2 nm could be implanted with stable NV centers which would allow nanoscale sensing at the single molecule level. Particularly, nanodiamonds could be functionalized with biomolecules and trafficked into biological systems with promising biocompatibility. Therefore, the quantum sensing techniques with NV center could be transferred into cells and would allow nanoscale sensing even inside a living cell environment. The theoretical and quantum optical studies have already demonstrated the power of these quantum sensing techniques in vitro. Some pioneering studies have also shown the great potential for nanoscale sensing inside living cells. We envision that the manifold opportunities of ND based quantum sensing techniques will become a pioneering imaging tool for the broad field of life science research and provide unprecedented access and insight into the structure, dynamics and function of individual bio-molecules under physiological conditions.

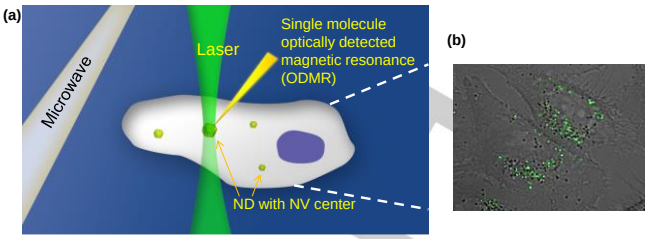

Figure 10. Illustration of ND quantum sensing in living cells. (a) The experimental setup for ND quantum sensing inside living cells. (b) Confocal fluorescence microscopy image of NDs with NV centers inside living cells (green dots).

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Keywords: NV diamond • quantum sensor • hyperpolarization • drug delivery • single molecule magnetic resonance

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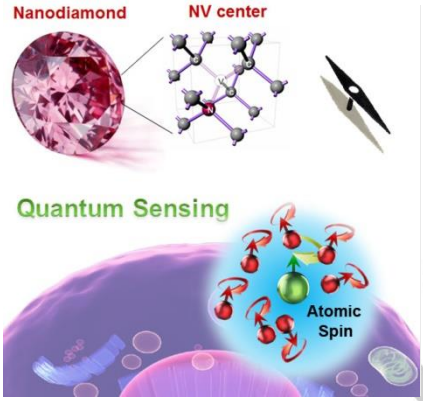
MINIREVIEW

Quantum sensors based on the spin-dependent photoluminescence of nitrogen-vacancy (NV) centers in diamond offer atomic resolution imaging at ambient conditions, which might evolve as a revolutionary technique for the understanding of biomolecules in their native environment. The recent development of this technique and its potential and challenges are critically discussed.



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