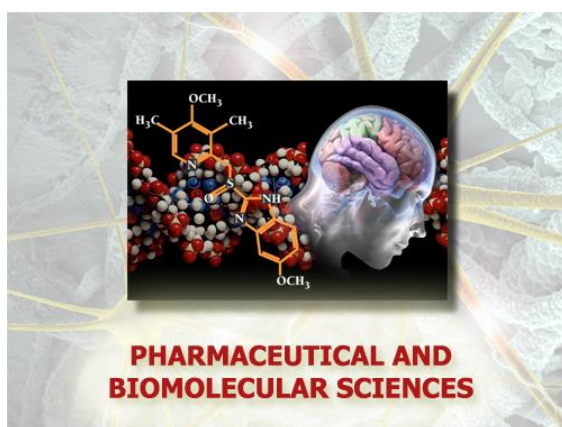


Università degli Studi di Torino



**Scuola di Dottorato in
Scienze della Natura e Tecnologie Innovative**

**Dottorato in
Scienze Farmaceutiche e Biomolecolari
(XXXIV ciclo)**



**Photoconversion of ensembles of NV centers
in nanostructured diamond: implications for
biosensing and polarization transfer
applications**

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TITOLO DELLA TESI: Photoconversion of ensembles of NV centers in nanostructured diamond: implications for biosensing and polarization transfer applications

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Abstract

Nitrogen-vacancy centers (NVs) in diamond have attracted considerable attention in recent years for sensing of magnetic and electric fields, and for hyperpolarization of nuclear spins. An increasing number of publications report the use of nanodiamonds as fluorescent probes for potential applications in materials science and biology. For these applications, dense ensembles of negatively NV centers (NV^-) and large surface areas would be highly desirable. However, dense ensembles of shallow NV^- are not easy to obtain, since surface effects tend to favor the less useful NV^0 .

In chapter 2, we investigate the charge and spin dynamics of NV centers in high-purity diamond samples implanted with nitrogen to create a shallow layer of defects. In some samples, we increased the exposed area by nanostructuring the diamond surface by plasma etching. Specifically, we explored the effects of laser illumination on spin-polarization and charge state of shallow NV centers in a power-density regime not studied before. We demonstrate that, under high-power laser illumination, shallow NV^0 can be efficiently photoconverted to NV^- centers, thus increasing their availability. We characterize thoroughly the properties of these defects with complementary techniques, including Raman spectroscopy, optically detected magnetic resonance (ODMR), and pump-and-probe fluorescence spectroscopy. Importantly, and quite surprisingly, we find that under this illumination regime the ensemble of excess NV^- centers created by photoconversion is stable over a timescale of milliseconds, thus providing a stable pool of shallow, magnetically active defects. We provide an explanation for this behavior based on established mechanisms of re-charging in the dark.

In chapter 3, we studied NV^- centers in nanodiamonds obtained by nanomilling, and in the native bulk material. Specifically, we investigated the effects of increasing laser power, as a means to increase the polarization and number of the magnetically active NV^- centers. We found by ODMR experiments that increasing laser power improves spin polarization of NV^- centers in bulk diamond, however, it can have a detrimental effect on nanodiamonds (NDs). This paradoxical behavior can be explained by accounting for the depletion of the NV^- pool by photoconversion to NV^0 , a process that is much more efficient in NDs than in the bulk. We also studied this phenomenon under experimental conditions typical of bioassays, and we found the same behavior in NDs internalized in macrophages in cell cultures. As an additional factor of novelty, we note that our study leverages a house-built wide-field microscope to map the magnetic properties of NVs at a sub-micrometric scale. Quite uniquely, this instrument makes it possible to account for the intrinsic heterogeneity in nanodiamonds behavior and to study the microenvironment in different cellular compartments.

In chapter 4, we anticipate that these results will have a significant impact on the development of diamond-based biosensors and in the hyperpolarization of nuclei in magnetic resonance applications involving fluorescent bulk and NDs. Indeed, our findings highlight the importance of surface effects on photoconversion and provide useful information on the adjustment and

optimization of experimental conditions, such as laser power. In conclusion, the increase in the exposed surface by nanostructuring is detrimental for shallow NV⁻, thus posing limitations to their applications (as we demonstrate in chapter 2 and 3). The results reported in this thesis have implications for the development of diamond based materials for sensing and hyperpolarization applications.

Chapter 1:

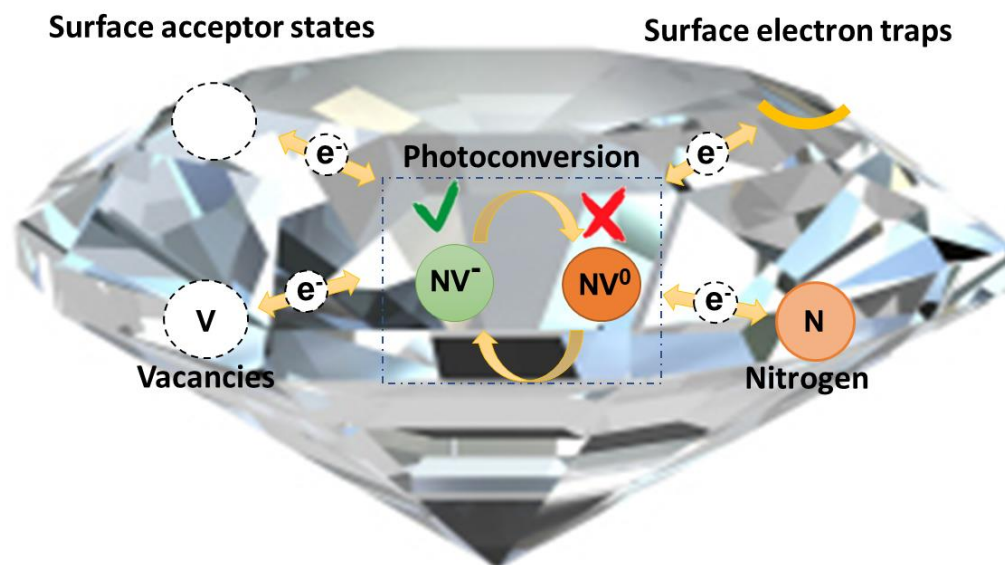
NV centers in diamond

Chapter 1: NV centers in diamond

Chapter summary

Nitrogen defects in diamond include Nitrogen-Vacancy centers (NV), which can be neutral (NV^0) or negatively charged (NV^-), and the paramagnetic P1 centers also known as single substitutional nitrogen atoms. NV^- can be used as probes for ultrasensitive detection of magnetic and electric fields, temperature, and nuclear spins at the nanoscale, with promising applications in biomedicine. Moreover, NV^- centers can be optically polarized with continuous irradiation of laser light at room temperature and at Earth's magnetic field and have been proposed as an alternative to Dynamic Nuclear Polarization (DNP) for the hyperpolarization of nuclear spins. These applications rely on ensembles of NV^- centers in the vicinity of the diamond surface. Laser light used to polarize NV^- centers and for readout can also induce charge switching between NV^- and its neutral form NV^0 . This charge photoconversion process depends on the experimental conditions and on the concentration of defects in the diamond such as vacancy complexes, surface acceptor states, surface electron traps, and local nitrogen centers. Therefore, in this chapter, we give an overview of the basic properties and electronic structure of NV centers, basic experiments using NVs, important parameters in the photoconversion process, the advantages and disadvantages of using nanodiamonds instead of bulk diamonds, diamond synthesis, and functionalization on the diamond surface to stabilize the shallow NV^- charge state.

Keywords: nitrogen-vacancy centers; diamond; polarization; nanodiamonds; bulk, photoionization; defects; shallow NV



1.1. Introduction

The unique properties of diamonds have been studied by researchers since a long time ago. The outstanding optical, electrical, chemical, mechanical, and thermal properties make diamonds a distinctive material. For instance, it has one of the highest hardness among all materials, high thermal conductivity, low thermal expansion coefficient, high electrical resistivity, high strength, broad optical transparency from ultraviolet to infrared, biologically compatible, resistant to chemical corrosion, etcetera [1].

Diamond can be enriched with color centers or crystallographic defects which can alter the spin and optical properties. For instance, as an ensemble of color centers have applications in optical magnetometry^[2,3] and quantum memory [4], while as a single color centers can be used in nano-thermometry [5], nano-magnetometry [6] or as an alternative to trapped ions and atoms for quantum computers and simulators [7–10]. This has led to the development of synthesis techniques to produce artificial diamonds with specific sizes and concentration defects that can fit the various applications. The most important color centers studied over the past two decades are the silicon-vacancy center (SiV) [11], the germanium-vacancy center (GeV) [12], the tin-vacancy center (SnV)[13], the NV center [14], and others [6,7].

Between all these color centers, the nitrogen-vacancy (NV) center has attracted interest as a promising system to perform robust, room temperature, single-photon experiments [17–20]. NV centers have a long electron spin coherence time and optical read-out properties make them suitable for quantum information [14,21] and magnetometry applications [22]. The NV center consists of a substitutional nitrogen atom next to a vacancy in the diamond lattice. It can exist in two forms, the neutral NV^0 and the negative NV^- charge state. We normally will refer to the negatively charged state NV^- since NV^0 does not present useful spin properties.

The negatively charged NV^- center can be used as probes for ultrasensitive detection of nuclear spins at the nanoscale, electric fields and magnetic fields, and temperature [5,23] [Fig. 1.1]. Under continuous laser irradiation, NV^- centers can be optically polarized at Earth's magnetic field and room temperature [17–20], thus paving the way for applications in biomedical assays [24–27], as optical magnetic imaging in living cells [28], optical magnetic detection of a single-neuron action potential [29] or intracellular thermometers [5]. Currently, NVs have some applications that are on the market such as microscopy [30,31] and for navigation and vehicle detection applications [32].

Moreover, NVs^- can transfer the polarization to external nuclei, to improve the sensitivity in nuclear magnetic resonance (NMR) [33–35]. Therefore, NVs have recently entered the group of hyperpolarization methods [36,37], which can change the magnetic properties of materials. Specifically, hyperpolarization is based on the transfer of polarization from a highly polarized electron system (such as an NV electron spin) to nuclear spins of a material far beyond thermal equilibrium conditions established by the Boltzmann distribution (see Chapter 4) [38].

Most of these applications rely on ensembles of shallow NV^- centers close to the diamond surface [39,40]. NV^- -enriched fluorescent nanodiamonds (NDs) are biocompatible and are promising candidates for such applications due to their large surface area [41] [Fig. 1.2]. Furthermore, it is possible to chemically functionalize the NDs surface to target specific proteins or cells [42,43]. However, the increase of surface area in the diamond sample inevitably affects NV^- charge stability since surface defects tends to favor the less useful NV^0 . Consequently, in this chapter, we give an overview of the electronic structure and basic experiments with NV centers, photoconversion process between the NV^- and NV^0 center, the pros, and cons of using NDs instead of bulk diamonds, functionalization of the surface, and synthesis strategies to stabilize the shallow NV^- .

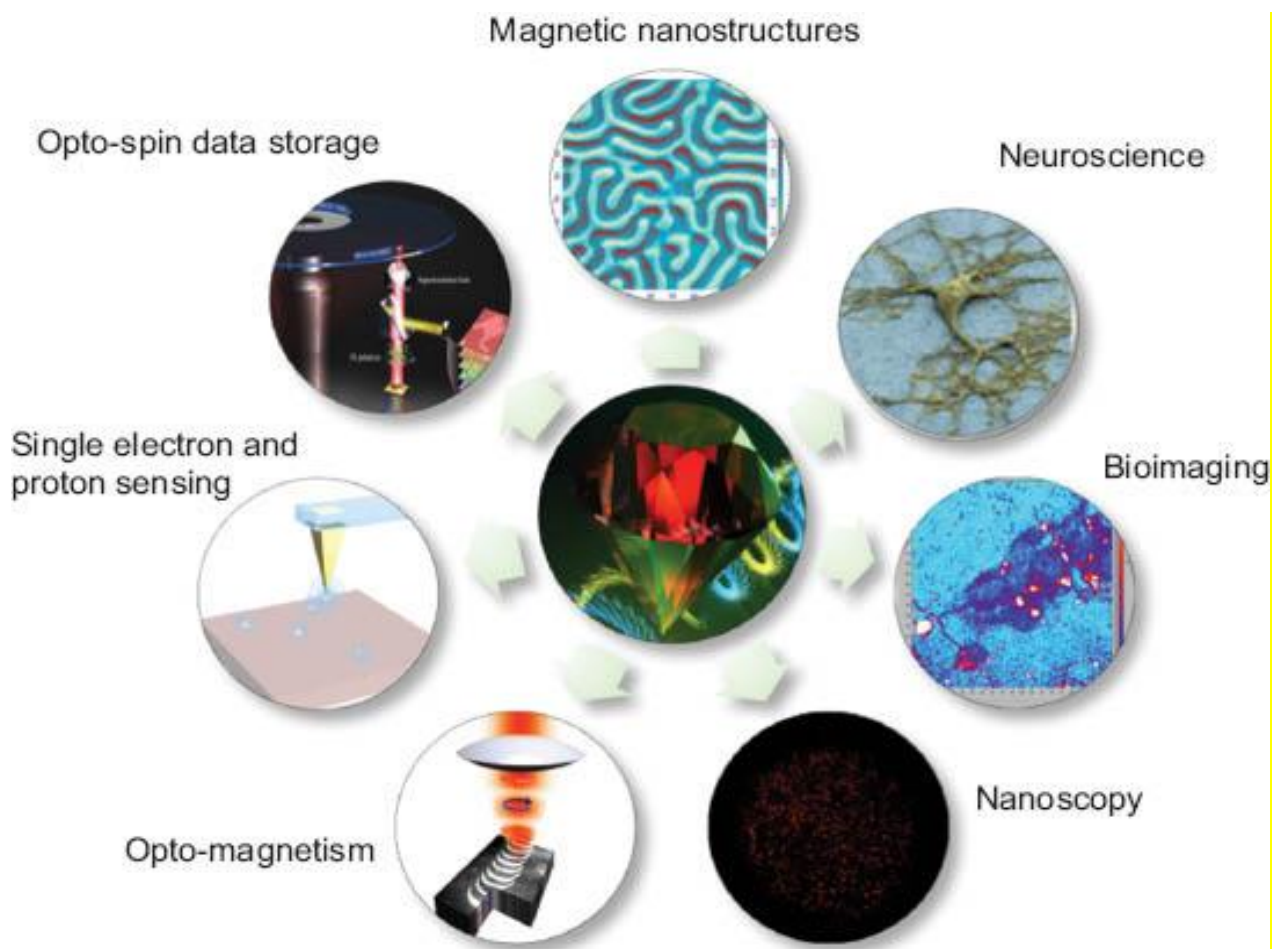


Figure 1.1 | Present and future applications of NV centers in diamond.

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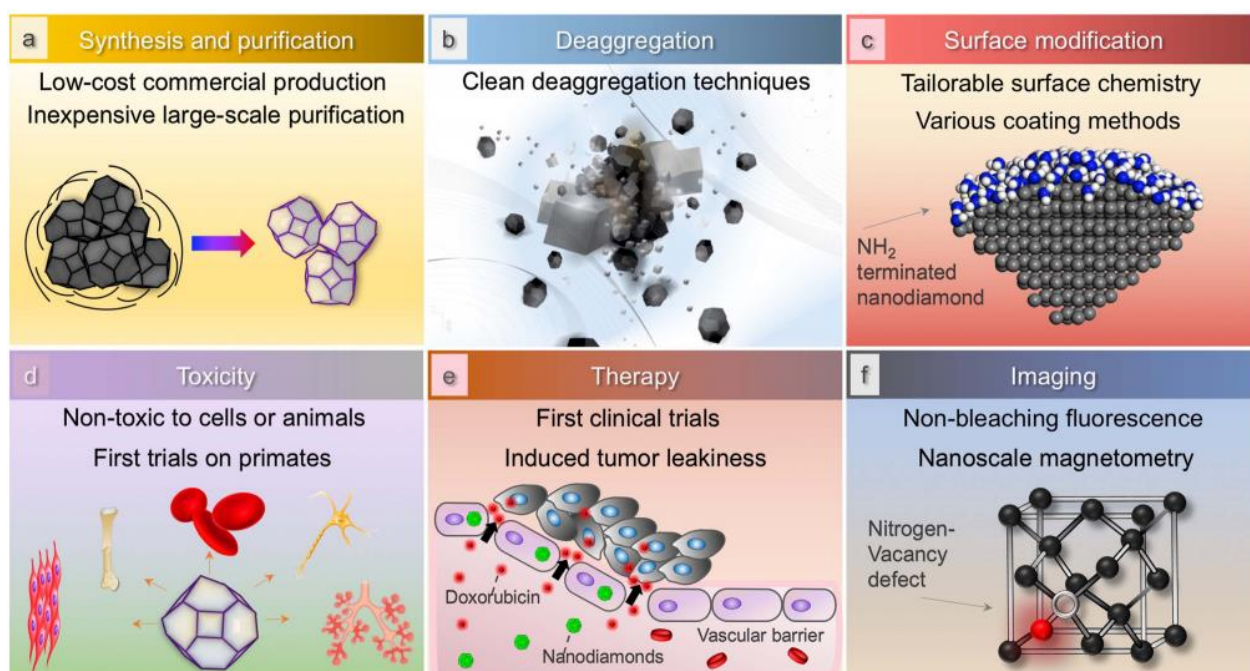


Figure 1.2 | Nanodiamond properties.

Steps towards nanodiamond theranostics: (a) synthesis and purification, (b) deaggregation, (c) surface modification, (d) toxicity evaluation, (e) nanodiamond-based therapy, (f) imaging using NV.

Panel e adapted with permission from Reference [44].

Panel f adapted with permission from Reference [45].

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1.2. NV centers

1.2.1. Electronic structure

The NV center is a point defect in the diamond lattice which consists of a substitutional nitrogen defect combined with an adjacent carbon vacancy [Fig. 1.3. b]. The NV is oriented along the [111] direction in the diamond crystal [46]. The diamond surface is chemically reactive, especially when the surface is functionalized. NV centers have remarkable photostability due to their uninterrupted continuous emission under continuous excitation. This NV photostability contrasts with a low photostability of widespread emitters such as organic fluorescent dyes that undergo irreversible conversion to a low quantum yield or non-emission state after a high number of cycles [17–20].

The NV center is a paramagnetic defect that can be in negative charge (NV^-), neutral charge (NV^0), and positive charge (NV^+). The NV^- and NV^0 charges are optically active, but the optical and electronic properties of the NV^- are more understood and appear to be better for applications in hyperpolarization, sensing, and quantum technologies. On the other hand, NV^0 and NV^+ are not magneto-optically active, so the experiments carried out in NV centers mainly refer to negative NV^- charge states [17–19].

The negatively charged NV^- center has an electronic structure involving six electrons. Three electrons come from the carbon atoms surrounding the vacancy, two electrons come from the nitrogen atom, and one electron comes from the diamond lattice. This last electron normally comes from a nitrogen donor. Four of these electrons form pairs, and the other two remaining unpaired electrons form the spin-triplet states in the ground energy level with electron spin quantum number $S = 1$ [17–20]. NV^- can be optically initialized and readout, which allows it to be used in different applications such as nano- [47] and micro-scale NMR [48], temperature sensing [49], magnetic-field [2], electric-field [50], pressure [51], quantum sensors [8], or in hyperpolarization applications [17–20,52]. When the NV^- is excited by a 532 nm green laser emits red fluorescence (600-800nm) without photoblinking or photobleaching [Fig. 1.4 a] [53]. These properties allow the application of NV-NDs as cell markers as a fluorescent probe for monitoring individual particles, with the additional advantage that NDs are non-cytotoxic and their surface can be functionalized [27].

Apart from NV centers, more than one hundred types of diamond defects have been identified [54]. Among all these defects, P1 centers or surface defects within the diamond are the most important for hyperpolarization experiments [Fig. 1.4. b] [46]. The natural abundance of ^{13}C is only 1.1% of the diamond lattice and therefore contains 98.9% spin-free ^{12}C , but it is possible to enrich the diamond up to 100% with ^{13}C nuclei. The ^{13}C has a nuclear spin ($I = 1/2$) and can be coupled to the NV^- center electronic spin ($S = 1$) through the dipolar-type hyperfine interaction. This interaction is stronger when the ^{13}C atom is in a neighbour position closer to the vacancy, where the electron density is higher [17–19]. The low concentration of ^{13}C in natural abundance (1.1 %), limits the sensitivity of NMR detection. Nevertheless, by dynamic nuclear polarization (DNP) is possible to hyperpolarize the ^{13}C nuclei and increase the ^{13}C signal by making it visible in NMR.

P1 defects (or N^0) is a paramagnetic impurity that occurs when a ^{14}N atom substitutes to a C atom. Nitrogen has five valence electrons: it forms covalent bonds with three of the neighbouring C atoms and the fourth sp^3 hybrid of the N atom is occupied by a lone pair, thus leaving a C atom with an unpaired electron [55,56]. P1 center has electron spin ($S = 1/2$) and nuclear spin ($I = 1/2$) [17–19].

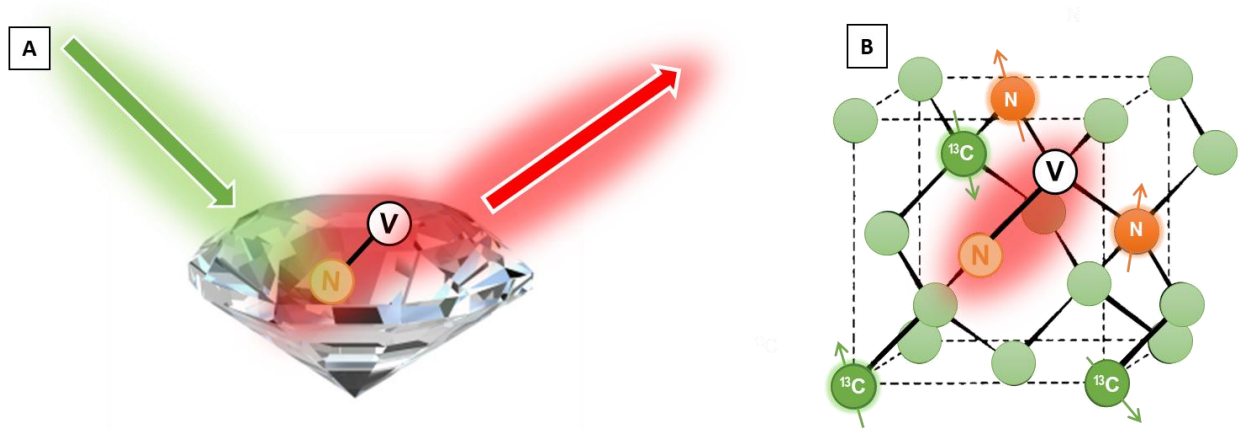


Figure 1.3. | NV center structure.

- Schematic representation of an NV center housed in a diamond that, when irradiated with a green laser, absorbs green light, and emits red fluorescence.
- Nitrogen vacancy (NV) color center surrounded by ^{13}C and single substitutional nitrogen atoms (P1 centers).

1.2.2. Energy level diagram

The energy level diagram of the NV center is shown in Fig 1.4 a. This diagram is divided into three electronic levels: 1) Ground state $|g\rangle$ with $^3A_2$ symmetry, 2) Excited state $|e\rangle$ with ^3E symmetry, and the metastable singlet state with two symmetry levels $^3A_2$ and $^3E_1$. When a 532 nm green laser irradiates the NV centers emits a red fluorescence signal (600-800 nm). It can also be excited over a wider range of wavelengths (458 – 640 nm) [17].

In the energy level diagram [Fig. 1.4 a], the ground $|g\rangle$ and excited $|e\rangle$ states are subdivided into three spin sublevels with spin triplet $S = 1$ due to the C_{3v} symmetry of the NV center. Specifically, due to the axial symmetry of the NV center, the two $|m_s = \pm 1\rangle$ states are degenerate with the same energy and the $|m_s = 0\rangle$ state is energetically lower. The difference in energy between the spin sublevels is called zero-field splitting (D_{zs}). Namely, the D_{gs} value of the ground state of $|g, m_s = \pm 1\rangle$ and $|g, m_s = 0\rangle$ is $D_{gs} = 2.87$ GHz and for the excited state is $D_{es} = 1.42$ GHz. The optical transitions are spin preserving, that is, when a green laser hits the diamond the population of electrons goes from $|g, m_s = 0\rangle$ to $|e, m_s = 0\rangle$ or from $|g, m_s = \pm 1\rangle$ to $|e, m_s = \pm 1\rangle$ through radiative decay [18].

During laser excitation, a part of the electron population that is in the $|e, m_s = \pm 1\rangle$ would decay via the metastable single state due to different cross-over rates, until finally reaching the $|g, m_s = 0\rangle$ state. This decay is a non-radiative channel, except for an infrared transition at 1042 nm. It is also known as intersystem crossing path (ISC) and is spin-selective that involves a spin-flip, which is the key to increasing the electron population of $|g, m_s = 0\rangle$ state. For instance, if

we irradiate bulk diamond with a 532 nm laser, the excited state lifetime of the NV^- and NV^0 for the bulk diamond is 12 ns and 21 ns [19] and for the NDs are 23 ns and 29 ns [57], respectively.

The electron population found in $|e, m_s = 0\rangle$ would also decay via metastable or intermediate singlet states but in a smaller proportion compared to $|e, m_s = \pm 1\rangle$. Particularly, an electron in $|e, m_s = \pm 1\rangle$ has a higher probability of decaying by $|s\rangle$ than $|e, m_s = 0\rangle$. On the flip side, when an electron is in $|e, m_s = 0\rangle$ it is most likely to decay to $|g, m_s = 0\rangle$ through the radiative decay [58]. Consequently, most of the electrons (70-80%) decay by the 637 nm the zero-phonon line (ZPL), that is, by the radiative triplet-triplet transitions and only a small percentage of electrons decay by the metastable singlet state. When using long laser pulses, the $|g, m_s = 0\rangle$ is more populated since the population will decay by the singlet $|s\rangle$ state after many cycles in the system. The larger the population of electrons in the $|g, m_s = 0\rangle$, the larger the spin polarization.

By simply irradiating with a green laser and monitoring with a photon counter detector how many photons are emitted by the NV centers, it is possible to determine the spin state of the NV center. When the laser is turned off, the system goes into equilibrium with the same electron population at the three levels in the ground state $|g, m_s = 0, \pm 1\rangle$ [17–19]. Measurements of the longitudinal relaxation time (T_1), which is the decay lifetime for the population of NVs initialized at one of the ground-state sublevels $|g, m_s = 0, \pm 1\rangle$, could be applied using an NV- probe to detect different T_1 in biological systems [59–61].

Applying a magnetic field, the $|m_s = 0, \pm 1\rangle$ states in the ground and excited states splits in opposite directions changing its energy [17–19]. The energy diagram of the ground and excited state triplet when a magnetic field is applied in the [111] direction of the NV axis is shown in Fig. 4b. The $|m_s = \pm 1\rangle$ and $|m_s = 0\rangle$ of the ground and excited states intersect at one point and are isoenergetic. This point is called the level anti-crossing (LAC) and it is where the coupling between the electron spin and the nuclear spin system occurs. In the ground state (GSLAC), the LAC occurs at ~ 1000 G, and in the excited state (ESLAC), this LAC occurs at ~ 500 G [Fig. 1.4 b]. The dependence on the magnetic field is the basis of magnetic sensing and hyperpolarization applications [18,19,62].

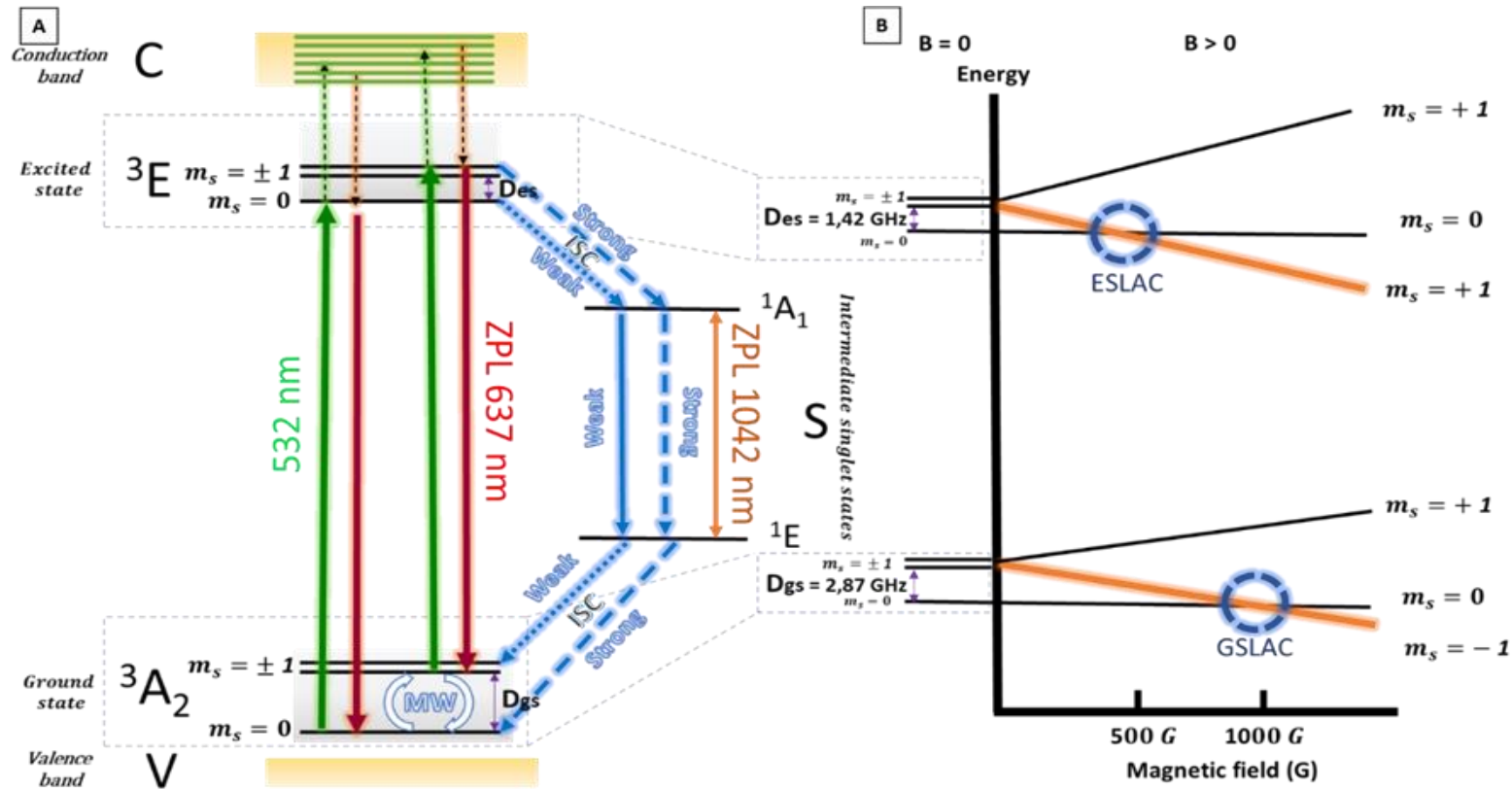


Figure 1.4 | Energy level structure of the NV⁻ center

- a) Energy level diagram of NV⁻ center where we can see the ground (³A₂ symmetry), excited (³E symmetry) and intermediate single states (with ¹A₁ and ¹E₁ symmetry levels). With the laser off, the NV center electron population is equally distributed in the ground state. When the 532 nm green laser is on, the population moves through the radiative spin conserving transitions from $|g, m_s = 0\rangle \leftrightarrow |e, m_s = 0\rangle$ or $|g, m_s = \pm 1\rangle \leftrightarrow |e, m_s = \pm 1\rangle$ (green line) and most of the population decays emitting red light at 637 nm (red line). A small part of the electron population that is in the excited state decays through the non-radiative non-spin conserving singlet state $|s\rangle$ along the path also known as the intersystem crossing path (ISC) until reaching the $|g, m_s = 0\rangle$ polarizing it. Within the excited state, an electron in $|e, m_s = \pm 1\rangle$ is more likely to decay non-radiatively to $|s\rangle$ than an electron in $|e, m_s = 0\rangle$, indicated as strong and weak non radiative transitions respectively, due to the different crossing over rates. An electron in the $|e, m_s = 0\rangle$ will have a better chance of decaying through the fast-radiative transition. To be more specific, the NV center can absorb light radiation at 500-540 nm and emit it at 600-800 nm.
- b) The energy diagram of the ground-state triplet (³A₂) and the excited-state triplet (³E₂) versus the magnetic field where the level anti-crossing (LAC) occurs at ~500 G in the excited state (ESLAC) and at ~ 1000 G in the ground state (GSLAC)

1.2.3. Basic experiments with NV centers

All the experiments carried out for the study of NVs are performed with one or more of the following sources [Fig. 1.5 a] [63]: laser, microwaves (MW) / radiofrequency (RF) [45,46], and a magnetic field produced by a permanent magnet or a coil. Among all the experiments, the most basic experiment is called optically detected magnetic resonance (ODMR) and is used to determine the sublevel structure of the NV ground state [46] [Fig 1.5 c]. Laser continuous irradiation polarizes the $|g, m_s = 0\rangle$ state through the ISC path, whereas a sweeping MW field transfer the population to the $|g, m_s = \pm 1\rangle$ state. When the MWs are resonant with the $|g, m_s = 0\rangle \leftrightarrow |g, m_s = \pm 1\rangle$ ground state transition, corresponding to the zero-field splitting $D_{gs} = 2.87$ GHz, the darker $|g, m_s = \pm 1\rangle$ states populate and a drop in the fluorescence (FL) is recorded [18]. The ODMR contrast provides a measure of spin polarization of the $|g, m_s = 0\rangle$.

Nonetheless, the ODMR contrast is reduced by the nuisance background signal from NV^0 fluorescence and is not modulated by MWs nor can it be removed completely by spectrum filtering. An increase in ODMR contrast may thus indicate an increment in the polarization of NV^- $|g, m_s = 0\rangle$ state or as a result of photoconversion ($NV^0 \rightarrow NV^-$), a gain in the number density of NV^- and consequently, a loss of NV^0 . The sequence of laser and MW irradiation to perform the ODMR experiments is described in Fig. 1.5 c and a similar sequence is used in many NV hyperpolarization experiments.

By way of example, Fig. 1.5 shows the FL and ODMR spectra and of an ensemble of NVs from a millimeter-scale bulk diamond. The sample has a concentration of NVs of ≈ 10 ppm, an enrichment of ^{13}C nuclei ranging from 5 to 10% ($\approx 5 \times 10^4 - 10^5$ ppm) depending on the position within the diamond lattice, and a concentration of P1 centers of ≈ 200 ppm. The FL spectrum of an ensemble of NV centers is shown in Fig. 1.5 b. Each NV charge state is characterized by the ZPL of the NV^0 at 575 nm and the NV^- at 638 nm. In addition, the phonon sidebands from the NV^0 at 620 nm and for the NV^- at 700 nm and both sidebands extending up to ≈ 850 nm, are observed.

Fig. 1.5 d shows the ODMR spectra from NV^- centers of the bulk diamond at zero-field magnetic field. The ODMR spectra shows two side resonances related to the hyperfine interaction between the NV^- spins and the ^{13}C nuclear spins (^{13}C sidebands, indicated by B_L and B_R and C_L and C_R). The spectra were acquired with 9mW laser power, and at different MW power levels (-30 to 5 dBm). Compared to previous reports, the ^{13}C sidebands are very prominent in the ODMR spectra, a consequence of the isotopic enrichment of diamond. The ^{13}C sidebands are separated from each other by ~ 130 MHz and by ~ 275 MHz [64–68]. We can also observe that the ODMR contrast increases with MW power, but also the linewidth of the resonances increases, reducing the ability to resolve the individual lines at the highest power levels.

Fig. 1.5 e and Fig. 1.5 f show the ODMR spectra at zero and non-zero magnetic field. In both ODMR spectra, the range of the MW frequency has been reduced to study the NV^- resonance (A_L and A_R). At zero field [Fig. 1.5 e], the FL drop centred at ~ 2.87 GHz splits in two bands because of the ^{15}N hyperfine coupling. When a magnetic field is applied along the [100] direction, these two NV resonances separate in the ODMR spectrum, hence, the degeneracy between the $|g, m_s = \pm 1\rangle$ levels is eliminated [Fig. 1.5 f]. The frequency separation between $|g, m_s = +1\rangle$ and $|g, m_s = -1\rangle$ is proportional to the applied field and is given by Zeeman factor of $2\gamma B$, where γ is the electron gyromagnetic ratio and B is the magnetic field applied to the diamond sample.

Magnetometry based on the measurement of magnetic field strength and orientation has been one of the main sensing applications of NVs. The sensing protocols relied on the measurement of the Zeeman splitting caused by an external magnetic field. The most basic method is the CW ODMR that permit to sense DC magnetic fields such as non-invasive detection of action potentials with single-neuron sensitivity [29], mapping of magnetic distributions with scanning probe magnetometers [69], or the magnetic imaging in living cells with sub-cellular spatial resolution [28,70]. NV magnetometers have been also able to measure localized ultraweak AC fields, attaining sensitivities around $pT\sqrt{Hz}$ at room temperature [71].

Furthermore, the NV centers can sense from nanometer to millimeter-scale volumes. Single NVs in NDs can observe the phenomena in the nanometer scale, as the magnetic field produced by a cell in a biomedical tissue [6,28]. Ensembles of NV's in a bulk diamond can detect the phenomena in a millimeter scale [72], which is one of the distinctive characteristics of NV quantum sensing. The four possible orientating of the NV- axis in the bulk permit to demonstrate vector imaging [73,74].

The separation between the two ODMR NV⁻ resonances enables the determination of the strength of the magnetic field [Fig. 1.6]. The ODMR spectra can be used to measure orientation tracking of NV-NDs tested on living HeLa cells [42], for magnetic measurements of magnetically immunolabeled breast cancer lines [75] or in magnetometry of magnetosomes in magnetotactic bacteria [28].

The NV⁻ magnetometry relaxation through the spin-relaxation time (T_1) measurement can probe the number of spins of the surrounding NV center [76,77] [Fig. 1.6]. A laser is used to create a nonequilibrium distribution of all spins accumulated in the $|g, m_s = 0\rangle$ state producing high fluorescence during the T_1 experiment (see upper right panel of Fig b). Then, during the dark time (in the absence of laser illumination) the NV interacts with the surrounding and the spins gradually return to equilibrium until they are equally distributed among the $|g, m_s = 0, \pm 1\rangle$ states, producing a decrease in fluorescence. Therefore, T_1 can be defined as the time required for the system to return to equilibrium, which is similar to conventional T_1 MRI. T_1 can be used as magnetic spin imaging tested on sections of gadolinium-labeled in dead HeLa cells [59] or detecting the concentration of gadolinium ions inside the bilayer of a simulated cell membrane [78]. Table 1.2 shows a summary of different sensing experiments in cells.

The NV⁻ ODMR resonance bands can also provide information about the temperature of the system [5,76,79] [Fig. 1.6]. For example, an increase in temperature produces a dilation of the diamond lattice, decreasing the spin-spin coupling between the two electrons of the NV⁻ center. This turns out that both NV⁻ resonances move at a lower frequency and increase their linewidth [Fig. 1.6]. Consequently, Kuckso et al. [5] were able to measure the temperature and detect temperatures variations down to 1.8 mK inside the human embryonic fibroblast cell by incubating NV-NDs and gold nanoparticles. Tsai et al. [80] measured the temperature within human embryonic kidney cells using NV-NDs conjugated to gold nanorods (GNRs) with near-infrared (NIR) lasers as the heating source

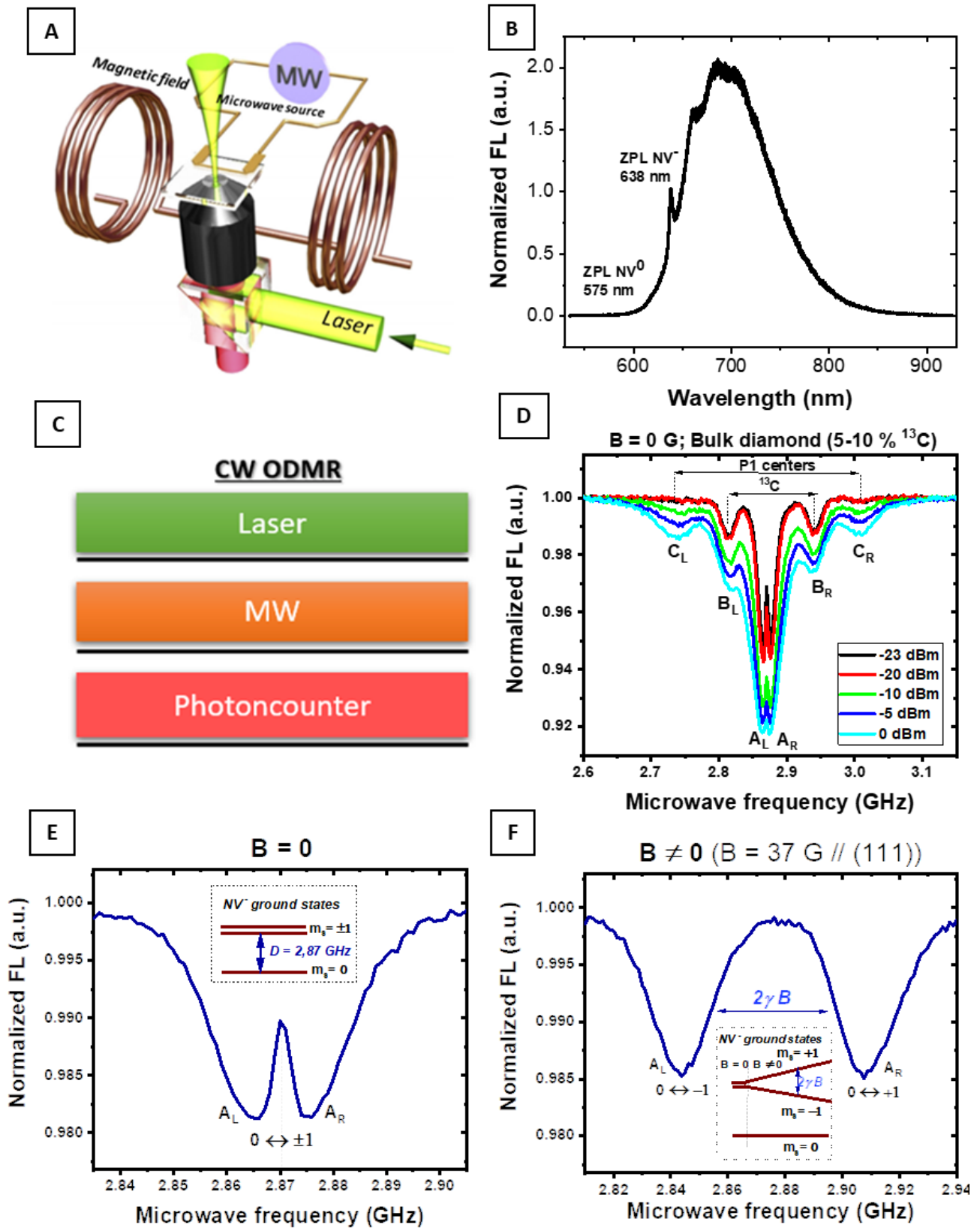


Figure 1.5 | Characteristics of a continuous-wave optically detected magnetic resonance (CW ODMR) spectra from an NV ensemble of a ¹³C-enriched crystal diamond.

Figure 1.5 | Characteristics of a continuous-wave optically detected magnetic resonance (CW ODMR) spectra from an NV ensemble of a ^{13}C -enriched crystal diamond.

- Schematic representation of the set-up for the study of NV centers in diamond with its main sources: laser, microwaves (MW), and a magnetic field. The wavelength of the laser will depend on the NV center type under study, that is, NV^- negatively charged or NV^0 or neutrally charged or both. The magnetic field can be created with a permanent magnet or a coil.
- Continuous-wave optically detected magnetic resonance (CW-ODMR) is a technique used to determine the sublevel structure of the ground state. With the laser turned off, the electron population of the NV-center is equally distributed in the ground state. When we turn on the laser, in this case, a green laser, we increase the population of $|g, m_s = 0\rangle$ due to the intersystem crossing path (ISC), polarizing it. The application of a MW resonant field with the $|g, m_s = 0\rangle \leftrightarrow |g, m_s = \pm 1\rangle$ transition at 2,87 GHz produces an increase in the population of the $|g, m_s = \pm 1\rangle$. With continued laser application, the population goes from $|g, m_s = \pm 1\rangle$ to $|e, m_s = \pm 1\rangle$. Then, the population of $|e, m_s = \pm 1\rangle$ can decay through the nonradiative decay via the ISC path to finally reach $|g, m_s = 0\rangle$. During the ISC path, there is a drop in the fluorescence intensity of the NV^- center at 2,87 GHz, which can reach a fluorescent contrast of 30%. The fluorescence intensity tells us the information about the spin state of the NV^- center that can be read optically.
- Photoluminescence spectrum of an ensemble of NV centers showing the NV^0 zero phonon line (ZPL) at 575 nm, the NV^- ZPL at 630 nm, and the NV^- vibrational sidebands in a range of 630-800nm.
- CW-ODMR at different MW powers of an NV ensemble. Here, the NV^- resonance (A_L and A_R) and the two types of side resonances can be observed, specifically the ^{13}C hyperfine interactions (B_L and B_R) and the simultaneous spin flips of NV^- and single substitutional nitrogen atoms, also known as P1 centers (C_L and C_R). Increasing the MW power broadens the ODMR line but also improves the contrast of all the bands. In this bulk diamond sample, we can notice the ^{13}C bands due to their enrichment of ^{13}C nuclei ranging between 5-10% and the P1 center bands due to their concentration of 200 ppm.
- CW-ODMR spectra at zero magnetic field. The drop in fluorescence corresponds to the energy difference between the spin sublevels of the ground state $|g, m_s = 0\rangle \leftrightarrow |g, m_s = \pm 1\rangle$ which is equivalent to zero-field splitting ($D = 2.87$ GHz).
- CW-ODMR spectra at non-zero magnetic field. The application of a magnetic field eliminates the degeneracy of the $|g, m_s = \pm 1\rangle$ allowing two different optical transitions ($|g, m_s = 0\rangle \leftrightarrow |g, m_s = +1\rangle$ or $|g, m_s = 0\rangle \leftrightarrow |g, m_s = -1\rangle$) separated by the Zeeman factor of $2\gamma b$, where γ is the electron gyromagnetic ratio.

All ODMR and fluorescence spectra belong to the NV ensemble of the same bulk diamond.

Panel a adapted with permission from Reference [63].

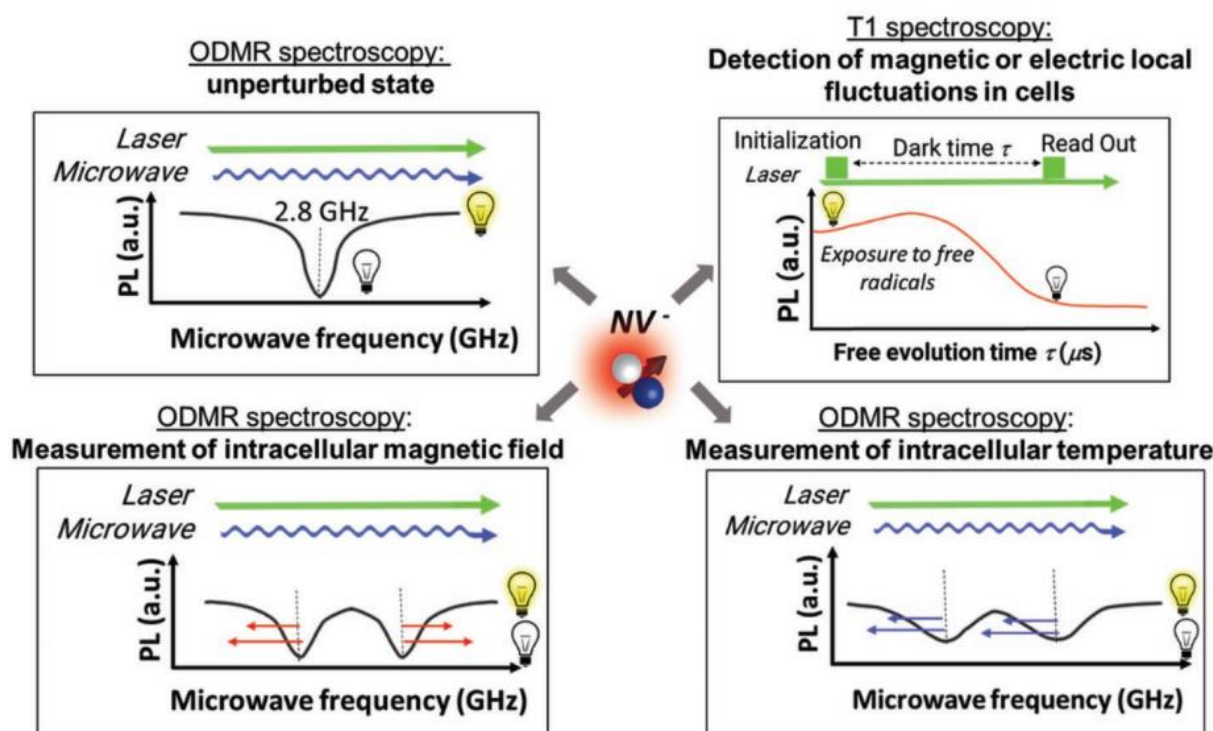


Figure 1.6 | ODMR applications

Use of ODMR spectroscopy to measure the intracellular magnetic field and temperature and T_1 relaxation time spectroscopy to measure the detection of magnetic or electric local fluctuations in cells. The position, width, and profile of the ODMR spectra bands depend on the physical properties of the surroundings such as magnetic field and temperature. On the flip side, T_1 spectroscopy is used for the detection of electric or magnetic fluctuations by measuring the time T_1 in which the distorted magnetic states return to equilibrium. The more the fluctuation noise, the shorter the T_1 .

Upper right panel a adapted with permission from Reference [81].

Other panels are adapted with permission from Reference [79].

Entire figure reprinted with permission from Reference [76].

References	Main results of biological interest	Physical parameters involved/sensitivity	Method used
McGuinness et al. [42]	Orientation tracking of FND tested on living HeLa cells	Orientation 1° (for 89 ms time resolution)	Optically detected magnetic resonance (ODMR)
Kucsko [5]	Nanometer-scale thermometry tested on living human embryonic fibroblasts	Temperature 44 mK (at 100 nm spat. res.)	ODMR, Ramsey fringes
Tsai [80]	Measuring nanoscale thermostability of cell membranes with gold–diamond nanohybrids tested on human embryonic kidney cells	Temperature 1.8 ± 0.3 mK	Shift of zero phonon line
Steinert [59]	Magnetic spin imaging tested on sections of gadolinium-labeled, dead HeLa cells	Magnetic labels 0.4×10^{-3} M Gd^{3+} (472 nm spat. res.)	T_1 measurement
Glenn [70]	Magnetic measurements of magnetically immunolabeled breast cancer lines (SKBR3, MCF7, MDA-MB-231)	Magnetic label, single cells, some microtesla sensitivity	ODMR
Le Sage [28]	Magnetometry of magnetosomes in magnetotactic bacteria	400 nm spat. res. Single magnetosomes, 100 μ T range	ODMR

Table 1.1 | ODMR and T_1 applications in cells

Summary of different sensing experiments in cells indicating the method used and the achieved sensitivity (spatial resolution is abbreviated with spat. res.).

Table adapted with permission from Reference [79].

1.3. Photoconversion

NV charge states are highly affected by the experimental conditions. NV centers can be converted into NV^0 or NV^- centers $NV^0 \leftrightarrow NV^-$ by applying laser, MW/RF sources, magnetic fields, chemical compounds, etcetera [39,82–84]. Other experimental parameters such as the temperature or the application of an electric field can vary the NV charge state. Furthermore, the characteristics of the diamond sample are of paramount importance, such as diamond size (bulk, micro or NDs, detonation NDs); concentration of defects in the diamond lattice such as NV^- and NV^0 centers, ^{13}C nuclei, P1 centers, surface trap states, vacancy complexes, surface acceptor states, surface electron traps, local nitrogen centers and so forth [Fig. 1.7]; distance from the NV centers to the diamond surface; surface functionalization; chemical solvents; paramagnetic species added to the diamonds and so on. Therefore, the variety of different parameters in the NV experiment is quite large.

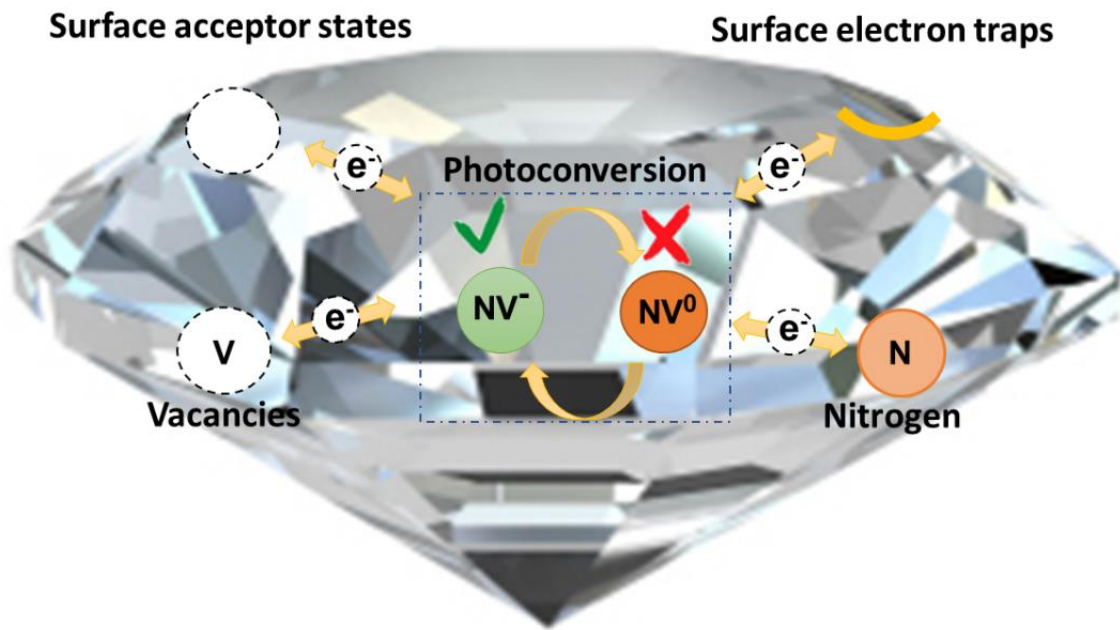


Figure 1.7 | Photo-induced ionization and recombination of NV^- .

NV charge state can be affected by the presence of defects such as vacancy complexes, surface acceptor states, surface electron traps, local nitrogen centers.

Figure reprinted with permission from Reference [125].

The energy level structures of the NV^0 and NV^- centers within the diamond band gap are exposed in Fig. 1.8 [54]. At the NV^- center, we can see the ground ($^3A_2$ symmetry) and excited triplet states ($3E$ symmetry), and intermediate single states (with $^1A_1$ and ^1E symmetry levels). At the NV^0 center, we observe the ground (^2E symmetry) and excited ($^2A_1$ symmetry) states [Fig. 1.8]. Specifically, the NV^- and the NV^0 center can absorb light radiation at 500–540 nm and emit it at 638–800 nm and 575–750 nm, respectively.

The laser wavelength depends on the NV charge state under study. Thus, NV^- center can be excited with an orange (624 nm) or red laser (637 nm) and NV^0 center with a blue laser (445–488 nm). Both

NV^- and the NV^0 center can be excited with a green laser (501-532 nm) [17]. Generally, a 532 nm green laser is used.

Laser irradiation can induce a charge-state photoconversion ($\text{NV}^0 \leftrightarrow \text{NV}^-$) between these defects [17], either by exciting an electron from the valence band to the NV^0 ground state ($\text{NV}^0 \rightarrow \text{NV}^-$ conversion) or by promoting an electron from the NV^- excited state into the conduction band ($\text{NV}^- \rightarrow \text{NV}^0$ conversion). When the laser is off during the dark time, there may be a charge-state conversion attributed to electron tunneling [17,85,86]. Through the tunneling effect, NV centers can be coupled to proximal acceptor or donor states, such as P1 centers, surface states, or vacancy complexes. The photoconversion $\text{NV}^0 \rightleftharpoons \text{NV}^-$ is simpler in the bulk diamond than in the NDs or close to the surfaces, therefore, we will refer to the straightforward situation.

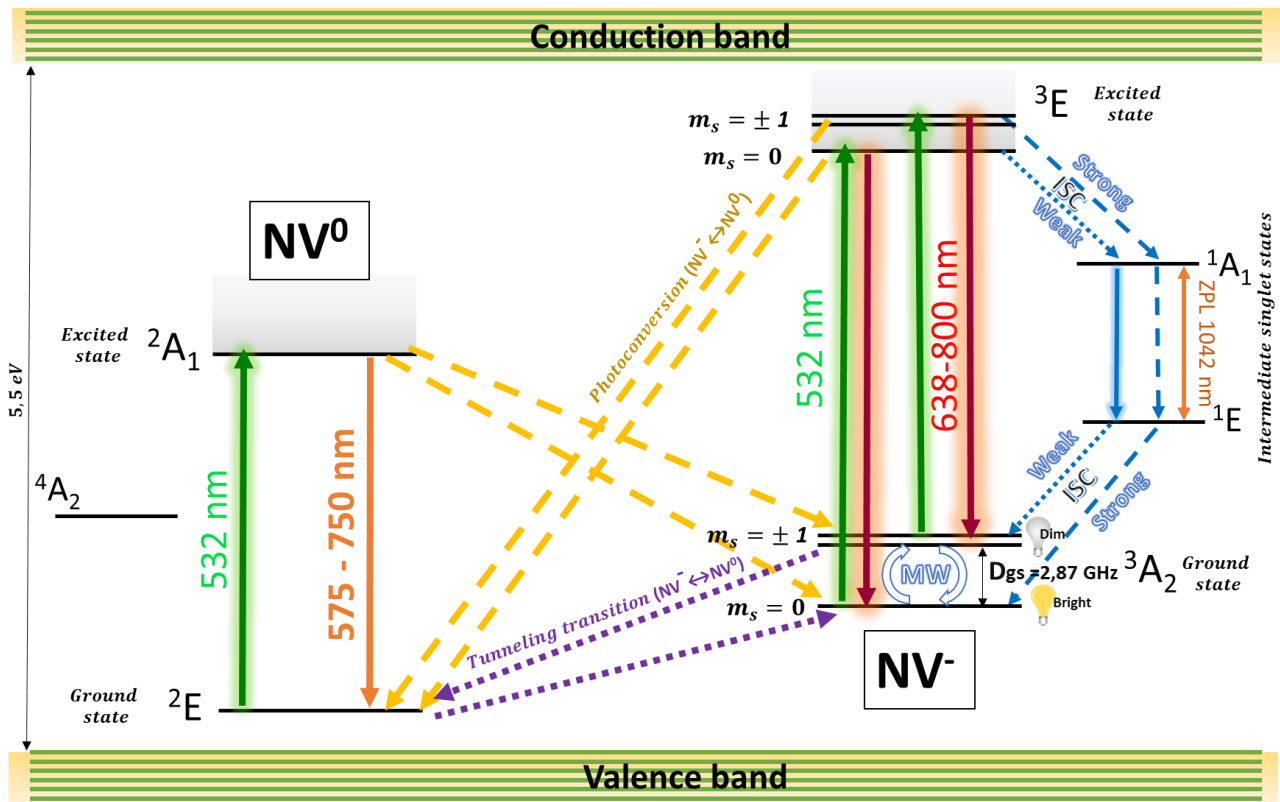


Figure 1.8 | Photo-induced ionization and recombination of NV^- .

Green arrows indicate optical excitation by a 532 nm laser. Red and orange arrows: radiative decay from NV^- and NV^0 centers, respectively. Blue arrows indicate the non-radiative pathway through the metastable state which leads to polarization of the $|g, m_s = 0\rangle$ NV^- ground state. Yellow and purple dotted arrows: suggest photoconversion and tunneling transition in the dark between the NV^- and NV^0 centers, respectively.

Aslam et al. [87] studied the photo-induced interconversion between the NV^- and NV^0 charges. They studied the dynamic ionization of single NV centers in bulk diamond at room temperature during the optical illumination and its dependence on wavelength and laser power excitation. The photochromism of the NV centers is shown schematically in Fig.1.9 a, where the conversion from ($\text{NV}^- \rightarrow \text{NV}^0$) and ($\text{NV}^0 \rightarrow \text{NV}^-$) is explained during the photoconversion process. Fig. 1.9 b shows the

real-time monitoring of the charge state that is used to analyze the ionization dynamics, where it is observed that the NV^- gives a high fluorescent signal reflecting high-count rates while the NV^0 gives a very low signal fluorescence that will result in low count rates [87].

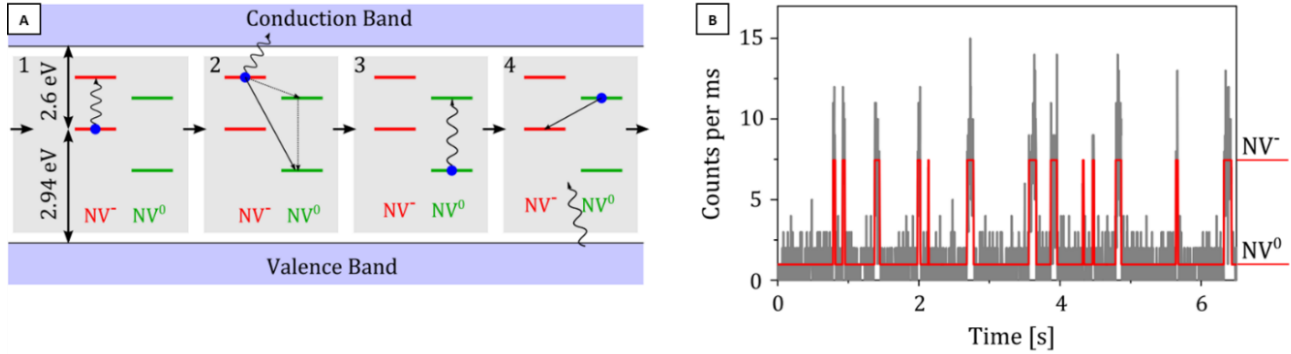


Figure 1.9 | Photo-induced ionization and recombination of NV^- .

- Schematic illustration of the laser photo-induced ionization and recombination of NV^- center, showing the ground and excited states of both NV^- and NV^0 . Ionization process ($\text{NV}^- \rightarrow \text{NV}^0$): 1 — NV^- is excited by one photon. 2— A second photon excites one electron from the excited state of NV^- into the conduction band, creating NV^0 . The NV will end up in the NV^0 ground state, either directly after the ionization or possibly via the excited state and then, fast decay. The recombination to the NV^- charge state is analogue to the ionization. Recombination ($\text{NV}^0 \rightarrow \text{NV}^-$): 3 — NV^0 is excited by one photon. 4 — An electron is captured from the valence band, forming NV^- . The NV will end up in the NV^- ground state. The figure shows that the ionization energy is 2.6 eV and the recombination energy is 2.94 eV. Both processes are induced by two photons and depend on the laser wavelength and its intensity and occur stochastically.
- Real-time monitoring of the fluorescence of a single NV under a 593 nm 1 μW -laser. a state of the NV with very low count rates which turns out to be NV^0 . The red line is the most likely fluorescence level of the NV. NV^- gives high fluorescence and high-count rates, while NV^0 provides low fluorescence and low count rates. The red line is the most likely fluorescence level of NV.

Figure reprinted with permission from Reference [87].

The nitrogen concentration in diamonds also plays an important role in the photoconversion $\text{NV}^0 \rightleftharpoons \text{NV}^-$. The conversion between NV^- and NV^0 is more straightforward within diamond crystals than for NDs or close to surfaces. Within diamond crystals that have a high or low concentration of nitrogen, there are mainly two different types of conversion mechanisms [88–90] [Fig. 1.9]:

- When the nitrogen concentration is high, the conversion is linearly dependent on the intensity. Here, the NV center traps an electron from a nearby nitrogen impurity by tunneling forming NV^- ($\text{NV}^- \rightarrow \text{NV}^0$).
- When the nitrogen concentration is low, the conversion is quadratically dependent on the excitation intensity. Here, we find the two photon-process where the electrons of the trap states are excited to the conduction band resulting in a reversible conversion between NV^- and NV^0 ($\text{NV}^0 \rightleftharpoons \text{NV}^-$) [88].

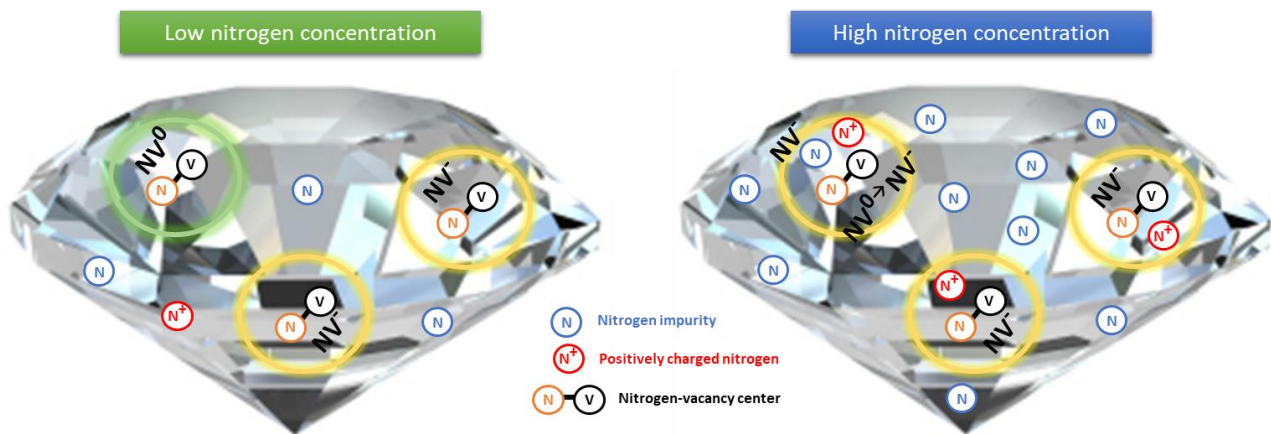


Figure 1.9 | Diamonds with a low and high concentration of nitrogen.

Diamonds with a low nitrogen concentration have a low correlation between the nitrogen that donates the electron and the position of the NV center and the NV^0 and NV^- charges can coexist in the diamond. At high nitrogen concentrations, there is a greater correlation between nitrogen and NV center position, consequently, nitrogen will donate an electron to the NV^0 center, increasing the concentration of NV^- center ($NV^0 \rightarrow NV^-$).

1.4. Important parameters for photoionization in diamond

In this section, we will describe the optical properties of diamonds that can affect photoionization. Consequently, we will discuss the main factors that can influence the production of NV centers in diamond particles, the properties of the diamond surface, the interconversion between NV^- and NV^0 charges, and the diamond size.

1.4.1. The synthesis of the diamond

The optical properties of NV centers in diamonds strongly depend on the process of their synthesis [91]. Bulk diamond crystals can be synthetically prepared by chemical vapor deposition (CVD) [92] or high-pressure high-temperature (HPHT) [93]. NDs can be produced such as by laser ablation [94,95], ion irradiation [96], detonation (DND) [91,97], CVD [98], the HPHT method [99,100] or by nanomilling the bulk material [100,101]. The most used method is the HPHT method since it produces the NV-enriched NDs more stable and fluorescent than other methods. These HPHT particles already contain substitutional nitrogen atoms and are usually created like this in a very brief way [52,102]:

- 1) To produce ND-NV, it is necessary to form vacancies in HPHT type Ib diamond powders (normally in microdiamonds). Thus, irradiating the diamond powders with high-energy particles, vacancies are produced.

- 2) By means of the high-temperature annealing ($> 700\text{ }^{\circ}\text{C}$), it causes the diffusion of these vacancies to the nitrogen, forming the NV centers.

These newly created diamond powders will have an inner diamond core (sp^3 carbon atoms) and an outer graphitic layer (sp^2 carbon atoms) with dangling bonds terminated with functional groups [76,103,104] [Fig. 1.10]. Then, these particles are milled to create the NDs, and the surface is oxidized to eliminate the graphical layer and activate the shallow NVs. Fig. 1.11 shows the main factors during the chemical synthesis of HPHT NV diamonds that can influence the final optical properties [105].

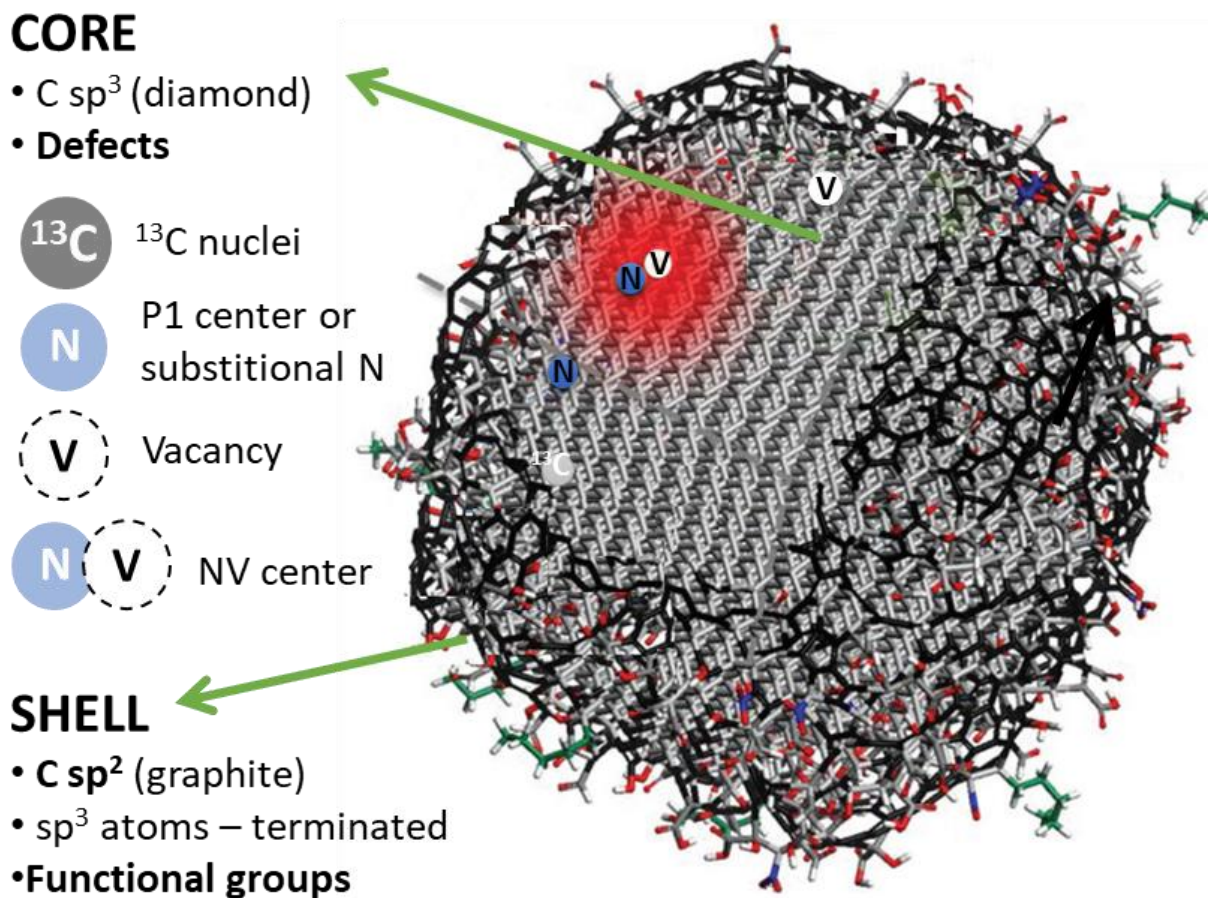


Figure 1.10 | Structure of an NV enriched nanodiamond.

Model of a single nanodiamond after the oxidative purification process. The diamond core (sp^3 carbon) is covered by a layer of surface functional groups to stabilize the particle. The sp^2 carbon (black) forms graphitic patches and chains. Most surface functional groups are terminated with oxygen-containing groups (oxygen atoms are in red, nitrogen in blue), hydrogen terminations (hydrogen atoms are in white), and hydrocarbon chains (green).

Figure adapted with permission from Reference [102].

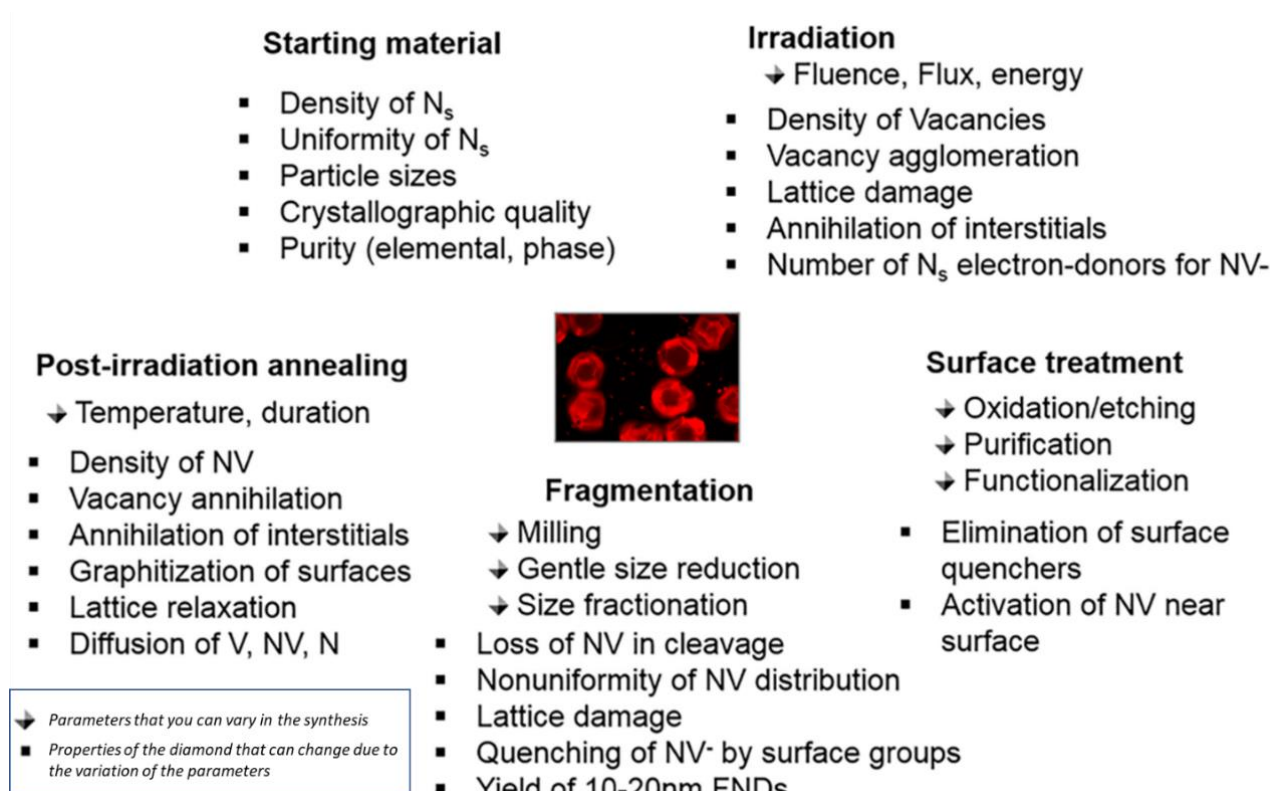


Figure 1.11 | Major factors influencing NV center production in diamond particles.

Summary of factors that can influence the production of diamond particles containing NV centers. Abbreviations; V, Vacancy; N_s , Substitutional nitrogen atom or P1 centers.

Figure adapted with permission from Reference [105].

1.4.2. Diamond surface

The diamond surface plays an important role in the stability of shallow NV centers when they are located close to the ND surface ($> 10\text{nm}$). The proximity of the NV⁻ to the diamond surface is of vital importance because the coupling strength between NV and external nuclear spins increases when the distance between both magnetic dipoles is reduced. The shallow NV⁻ stability can be increased, for example, by surface functionalization [106–110], reducing surfaces defects [111] such as the removal of the graphitic part on the ND surface [53,112–115], or creating layers of shallow NV⁻ centers [116,117], as described below:

- The functional groups of the diamond surface affect the NV emission, for example, the fluorine [110] and oxygen terminations [106,107] favour the NV⁻ centers, increasing the ND fluorescence and the hydrogen terminations [108] favour the NV⁰ centers, decreasing the ND fluorescence [108,109].
- Surface states and defects close and at the diamond surface can reduce the NV stability, increasing the number of NV⁰ centers. Thus, the nanomilling of a bulk diamond into NDs can

increase the surface area but unfortunately, it also increases the surface effects that tend to favour the NV^0 centers [111].

- The removal of the graphite layer (sp^2) after the process of acid cleaning and /or oxidative etching, increases the emission of the NV centers and consequently, the ND fluorescence. This is because the graphitic layer has electron acceptor states that can trap an electron from NV^- converting them to NV^0 ($NV^- \rightarrow NV^0$) [112,113].
- Blinking or luminescence intermittency can be interpreted as a photoinduced excitonic charge tunnelling from an NV center to a nearby charge acceptors reservoir. The elimination of the graphite part in an NV-ND system increased by 10% the percentage ratio of the blinking centers to the NV total number [53,114,115].

1.4.3. The size of the diamonds

The size of the diamond plays an important role in the NV optical properties [83,111]. Fig. 1.12 shows the advantages and disadvantages of using NDs compared to bulk diamonds [118]. NDs would be a good tool for biosensing, drug delivery, and hyperpolarization applications due to the larger surface area, however, NDs have a lower ^{13}C nuclei relaxation since they have a greater number of paramagnetic surface defects [119,120] and they exhibit a larger heterogeneity in the optical properties. In contrast, bulk diamonds are bigger and have a lower surface area [121].

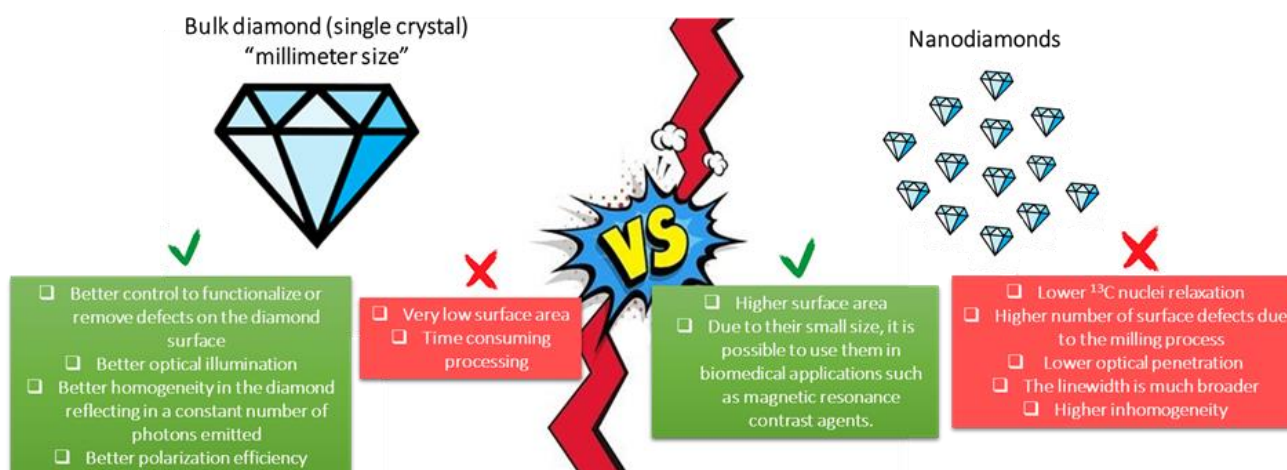


Figure 1.12 | Advantages and disadvantages of using nanodiamonds compared to millimeter-scale bulk diamond crystals.

Although NDs present more disadvantages than the bulk diamond, NDs present a larger surface area and smaller size makes them more suitable for hyperpolarization applications and biomedical applications, respectively.

The particle size of the diamond can also change the number of defects within the diamond. Various research groups [105,122] observed that the concentration of NV^- centers decreases when the

particle size decreases [Fig. 16] [105]. For example, it has been determined an NV⁻ concentration of 1 ppm for a 40 nm ND [122].

Shenderova et al. [123] analyzed the relative ratio of NV⁰ and NV⁻ center for a 40 nm diamond particle and determined that the concentration of NV⁰ was larger than NV⁻. Specifically, they found an average value of NV⁻ and NV⁰ centers equal to 6.46 and 9.11 emitters per ND, respectively. It is important to note that these 40 nm NDs were not subjected to a deep purification of the sp² carbon, that is, there could be a concentration of graphite on the ND surface resulting in a larger concentration of NV⁰. Another reason may be the great damage to the crystal lattice suffered during the nanomilling process. Thus, we can observe that controlling the concentration of NV centers in diamonds is a difficult challenge and further work is required in this field [123]. In Table 1.2 [105], we can observe that as the particle size decreases, the number of NV⁻ emitters per particle decreases, as well as the percentage of fluorescent particles in the sample. Currently, several research groups are trying to increase the number of NV centers in small-sized particles by using different techniques such as increasing the electron irradiation.

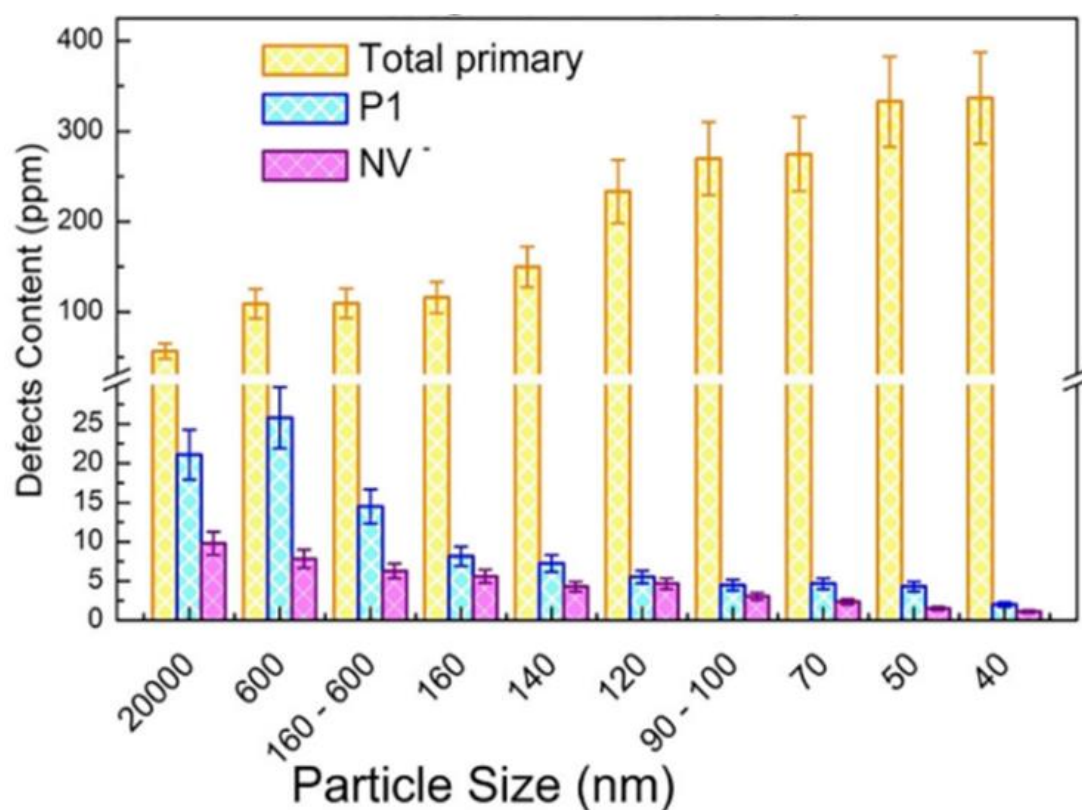


Figure 1.13 | Defect concentration as a function of diamond particle size.

Dependence of the content of some paramagnetic defects on average particle size: total primary defects ($S=1/2, 3/2$) (yellow columns), P1 centers (blue columns), NV⁻ centers (purple columns). It is observed that the smaller the ND particle size, the concentration of NV⁻ and P1 centers decrease.

Panel a reprinted with permission from Reference [105].

Particle size (nm)	# NV ⁻ emitters/particle	Percent of fluorescent particles in sample
10	1–2	6
20	1–2	28
30	5–6	57
40	1–4	74
40 ^a	12–14	70

^aFrom higher electron irradiation batch.

Table 1.2. | Number of NV⁻ centers depending on particle size.

Single-particle atomic force microscopy (AFM) confocal analysis of several NV-NDs samples ranging in size from 10 to 40 nm. As the size of the ND particle increases, the number of NV⁻ emitters per particle increases, as well as the percentage of fluorescent particles in the sample.

Table reprinted with permission from Reference [105].

1.5. Summary points of chapter 1

1. The NV center consists of a substitutional nitrogen atom next to a vacancy in the diamond lattice. It can exist in two forms, the neutral NV^0 and negative NV^- charge state [Fig. 1.3]. We normally will refer to the NV^- since NV^0 does not present useful spin properties.
2. Under laser irradiation, NV^- centers can be optically polarized at Earth's magnetic field and room temperature [Fig. 1.4].
3. The negatively charged NV^- center can be used as probes for ultrasensitive detection of nuclear spins at the nanoscale, electric fields, magnetic fields, and temperature. NVs are also useful in quantum information, magnetometry, bioimaging, hyperpolarization, neuroscience, bioimaging, and biomedical applications [Fig.1.1]. Most of these applications rely on ensembles of shallow NV^- centers close to the diamond surface.
4. NV-enriched fluorescent nanodiamonds (NDs) are biocompatible and are promising candidates for such applications due to their large surface area. However, the increase of surface area in the diamond sample inevitably affects NV^- charge stability since surface defects tend to favour the less useful NV^0 .
5. Although NDs have disadvantages compared to bulk diamonds such as lower ^{13}C nuclei relaxation, larger number of surface defects, greater heterogeneity, or less optical penetration. On the contrary, NDs have a larger surface area, making them more appropriate for hyperpolarization and biomedical applications [Fig. 1.12].
6. Generally, all the experiments carried out for the study of NVs are performed with one or more of the following sources [Fig. 1.5 a]: laser, microwaves (MW) / radiofrequency (RF), and a magnetic field produced by a permanent magnet or a coil.
7. The most basic experiment is called optically detected magnetic resonance (ODMR) and is used to determine the sublevel structure of the NV ground state [Fig. 1.5 c,e,d and f].
8. Laser irradiation can induce a charge-state photoconversion ($NV^0 \leftrightarrow NV^-$) [Fig. 1.8 and 1.9]. The NV charge state can be affected by the presence of defects such as vacancy complexes, surface acceptor states, surface electron traps, or local nitrogen centers [Fig 1.7].
9. NDs contain many localized paramagnetic impurities such as P1 centers, charged vacancy sites, and dangling bonds. Moreover, they contain other impurities such as surface defects that can be detrimental for NV^- stability.
10. The functional groups of the diamond surface can affect the NV emission, for example, the oxygen terminations favour the NV^- centers increasing the ND fluorescence and the hydrogen terminations favour the NV^0 centers decreasing the ND fluorescence. It was also noted that the removal of the graphite layer (sp^2) after the process of acid cleaning and/or oxidative etching, increases the emission of the NV centers and therefore the ND fluorescence [Fig 1.11].
11. Several groups observed that the concentration of NV^- centers decrease when the particle size decreases [Fig. 1.13]. It was also noted that when the size of the ND particle increases, the number of NV^- emitters per particle increases as well as the percentage of fluorescent particles in the sample [Table 1.2].
12. The increase in the exposed surface by nanostructuring is detrimental for shallow NV^- , thus posing limitations to their applications. In the following chapters 2 and 3, we will explore the effects of laser intensity on the photoconversion process in bulk and NDs .

1.6. Reference of chapter 1

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Chapter 2:

Photoconversion of shallow NV centers in flat and nanostructured bulk diamond.

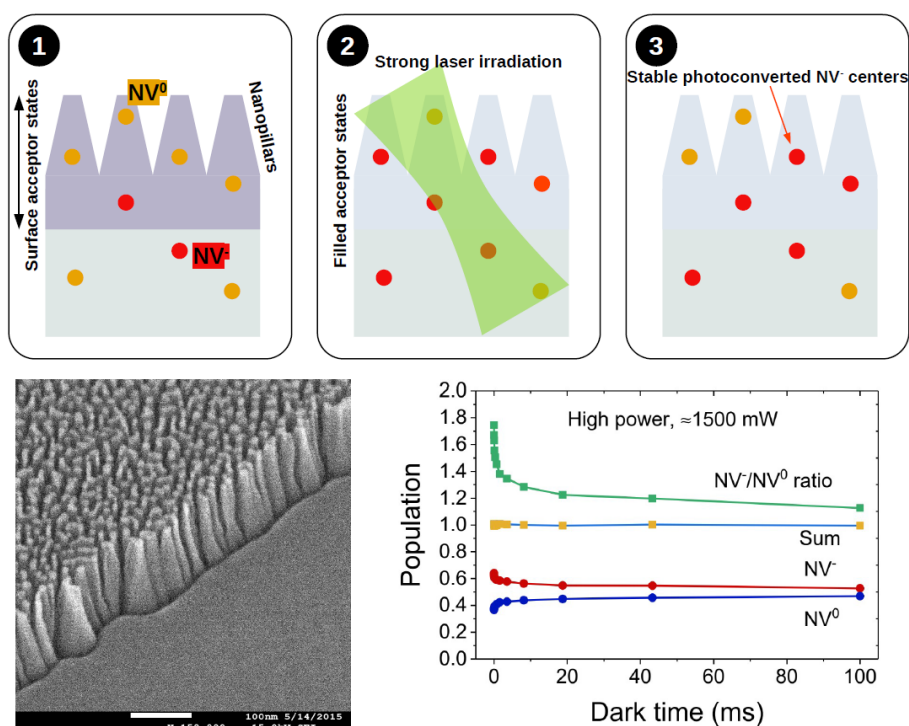
Chapter 2: Photoconversion of shallow NV centers in flat and nanostructured bulk diamond

Large portions of Chapter 2 have appeared in the following paper: Gorrini, F.; Dorigoni, C.; Olivares-Postigo, D.; Giri, R.; Aprà, P.; Picollo, F.; Bifone, A. Long-Lived Ensembles of Shallow NV – Centers in Flat and Nanostructured Diamonds by Photoconversion, *ACS Appl. Mater. Interfaces* **2021**, doi:10.1021/acsami.1c09825.

Chapter summary

We investigate the effect of green laser irradiation on nanometer-shallow NV centers, created in flat and nanostructured bulk diamond surfaces, from low to extremely high laser powers (up to $150\text{mW}/\mu\text{m}^2$). The properties of NV^0 and NV^- centers are explored separately, with a set of techniques: Raman spectroscopy, optically-detected-magnetic-resonance (ODMR), and charge-photoconversion fluorescence detection. We demonstrate that a high laser power enhances the NV-ensemble over time scales of tens of milliseconds, indicating a structural modification of the NVs environment. Our results might serve to increase the sensing abilities of NV ensembles in magnetometry or ^{13}C hyperpolarization schemes. In the end, as we will also demonstrate in chapter 3, the increase in the exposed surface by nanostructuring the diamond sample is detrimental for shallow NV, thus posing limitations to their applications.

Keywords: diamond, Nitrogen-Vacancy centers, NV^0 , photoconversion, nanostructures, surface effects, nanopillars.



2.1. Introduction

Negatively charged nitrogen-vacancy (NV^-) centers in diamonds enable the detection at the nanoscale of magnetic [1] and electric fields [2,3], interaction with nanoparticles and magnetic molecules [4–6] and mapping of temperature gradients [7,8]. Due to their biocompatibility, NV^- -enriched fluorescent nanodiamonds (NDs) have been used for sensing in biological systems [9–11]. Further, NV^- centers have been used in dynamic nuclear polarization (DNP) protocols for the hyperpolarization [12] of the ^{13}C nuclei in the diamond lattice [13–15].

In these applications, having a small distance between the NV^- spins and the target system is a significant constraint, as the coupling strength between magnetic dipoles reduces with increasing distance. The necessity of having NV^- centers close to the diamond surface either requires the use of nanodiamonds (see chapter 3) or specially engineered layers of shallow NV^- s a few nanometers from the diamond surface [16,17]. Unfortunately, defects close to the surface often favor the less useful neutral charge state (NV^0) at the expense of the NV^- , with a loss in the quality of fluorescence (FL) signal and polarization ability. The relative stability and interconversion between the neutral and negative charge states of the NV centers, depending on the environment and laser irradiation conditions, have been the subject of research in different work [18–22]. In addition, various attempts have been made to increase the charge state stability of the NV centers by application on an electric field [2], by doping of the diamond lattice [23] or by surface termination [24].

In this chapter, we show a simple and effective method to enhance the relative abundance of the NV^- s in an electronic-grade (EG) bulk diamond with an initial prevalence of the NV^0 centers by irradiating it at high laser powers. We demonstrate the effectiveness of this method with a set of experiments, from inspecting the $\text{NV}^- \rightarrow \text{NV}^0$ dynamics of charge conversion to fluorescence analyses and Raman spectroscopy. Importantly, we show that the $\text{NV}^0 \rightarrow \text{NV}^-$ ensemble charge conversion is stable over time scales much longer than the spin relaxation time (T_1) of the NV^- s. This observation suggested a structural modification of the defects surrounding the NVs and allowed a confident manipulation of the spin ensemble, a fundamental requirement in both magnetometry and polarization-transfer techniques. Finally, the proposed method is applied to a set of four diamonds, two flat and two nanostructured, with different energies of nitrogen implantation during the synthesis of NVs. We discuss the favoring conditions and the limiting factors for the NV^- enhancement and give an indication for the best sample design requirements.

2.2. Materials and methods

2.2.1. Fabrication of samples

We used four electronic-grade (EG), single-crystal bulk diamond plates, with an initial nitrogen concentration < 5 ppb (Element Six Technologies Ltd). Samples were 2.0×2.0 mm², 0.5 mm thick, with {100} face orientation. All EG samples were double-side polished, later two of these flat diamonds (F1, F2) were nanostructured (N1, N2) to increase the surface-to-volume ratio [Table 2.1].

Bulk diamond name	Surface type	Implantation energy (keV)
F1	Flat	20
F2	Flat	10
N1	Nanostructured	20
N2	Nanostructured	10

Table 2.1. | Type of surface and implantation energy of $^{15}\text{N}^+$ for the four electronic-grade samples.

The nanostructuring process (N1, N2) is outlined in Fig. 2.1. a-d. The nanostructuring in N1 and N2 was conducted via Inductively Coupled Plasma - Reactive Ion Etching (ICP-RIE) with oxygen gas O_2 (g), employing a mask of non-continuous gold (Au) layer [25]. Au was deposited by Direct Current (DC) sputtering (KS500 confocal sputtering system - Kenosistec Srl), with Ar (g) [Fig. 2.1 a]. Etching was carried out through ICP-RIE (SI500 - Sentech Instruments GmbH), applying 700W ICP power, 50 W plate power, and 40 sccm O_2 , at 1 Pa pressure, during 60 s [Fig. 2.1 b]. Subsequently, after the ICP-RIE procedure, Au was removed by a commercial Au etchant (Sigma Aldrich).

EG samples were implanted with ^{15}N (INNOVION Corp - San Jose, CA) at a fluence of 10^{13} cm⁻², at 7° angle from normal to avoid ion-channeling [Fig. 2.1 c] [26–28]. The ^{15}N implantation was carried out at two different energy conditions: the flat sample F2 and the nanostructured sample N2 were implanted at 10 keV, conversely, the F1 and N1 were implanted at 20 keV [Table 2.1]. Ion average ranges in the flat samples for the F2 sample are ≈ 15 nm, with straggling 5 nm, and for the F1, ≈ 27 nm with straggling 8.5 nm [29]. These values were calculated by SRIM (<http://www.srim.org>, SRIM-2013.00).

The four EG samples were annealed at 850 °C for 2 h in high vacuum conditions ($P = 10^{-6}$ mbar) to mobilize vacancies and create the NV pair [Fig. 2.1 d]. To prevent hydrocarbon contamination, we used a set-up consisting of a vacuum chamber complemented with a dry pumping system and a resistive heater. To maintain the EG sample and the surrounding black-body radiation in thermal equilibrium, we employed a tantalum box ($5 \times 5 \times 5$ mm³) as a heating element. We used a pyrometer pointed to the tantalum box through an optical window, to track externally the temperature. The cooling and heating rates were of 10 °C min⁻¹ to prevent thermal stresses in the diamond lattices. At

10 °C, interstitial nitrogen atoms and vacancies become mobile, but P1 centers are fixed in their position [30]. We employed a pyrometer pointed to the tantalum box through an optical window, to track externally the temperature. On average, we predict deeper NVs for the 20 keV than for 10 keV implantation energies. The implantation in the nanostructured diamonds takes place on an irregular surface thus, a more inhomogeneous distribution profile is assumed. Scanning Electron Microscopy (SEM) images showed a tight arrangement of nanostructures like-nanopillars over the diamond surface [Fig. 2.2], with a base of ≈ 50 nm and a height of ≈ 150 nm. The images were obtained before Au removal. Flat samples (F1, F2) were subject to the same implantation and annealing process as the nanostructured diamonds but conducted on a flat surface without nanopillars.

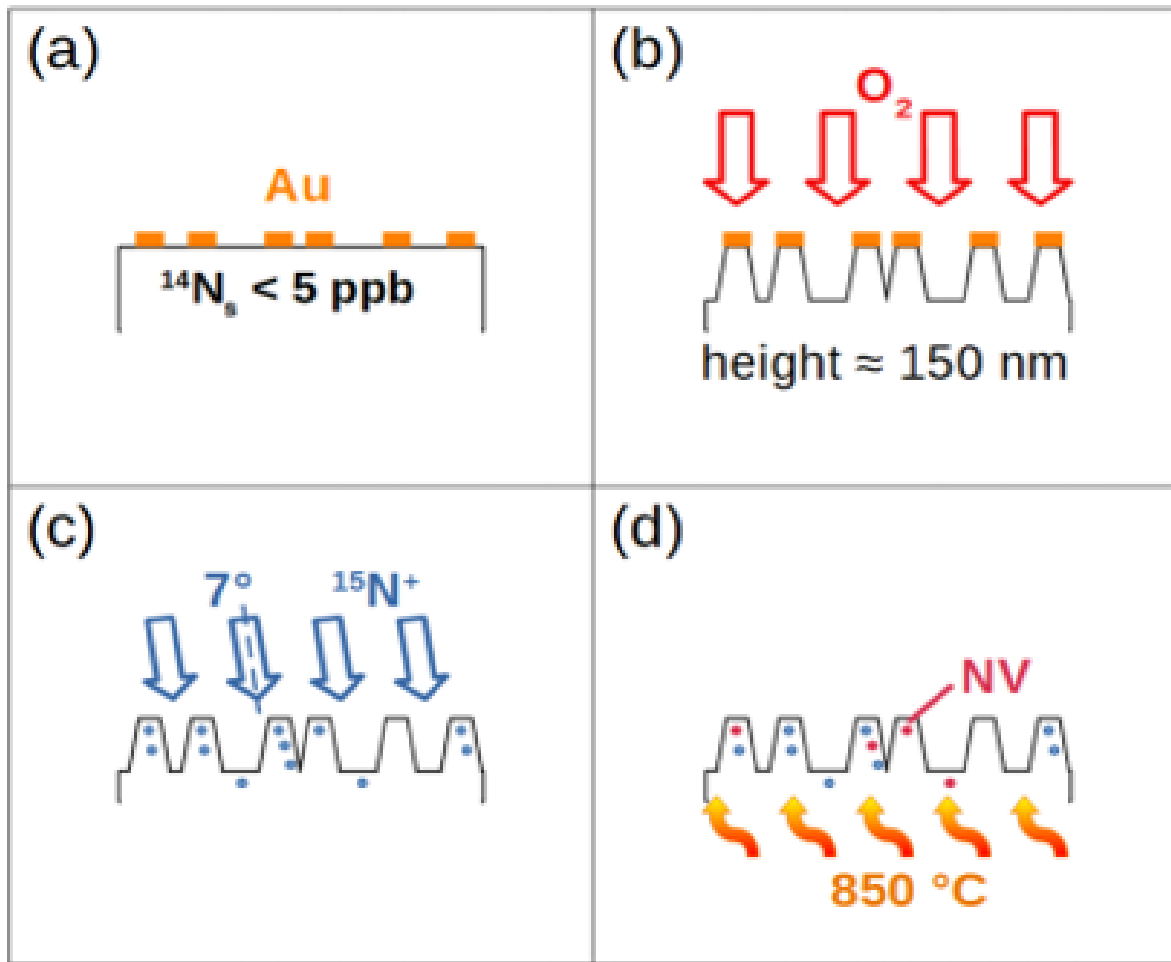


Figure 2.1 | Nanofabrication process and synthesis of shallow NV centers in nanostructured samples.

(a) Electronic grade diamond sample with a low concentration of nitrogen (< 5 ppb) was masked with gold (Au) by sputtering and then (b) etched by using oxygen O_2 reactive ion etching to fabricate nanopillar-like structures. After gold removal, (c) nitrogen was implanted. (d) Samples were annealed at 850°C for 2h in high vacuum conditions. Consequently, vacancies and interstitial nitrogen atoms become mobile in the diamond lattice, thus forming NV centers. Flat samples were implanted with ^{15}N ions and then annealed, without application of any etching procedure.

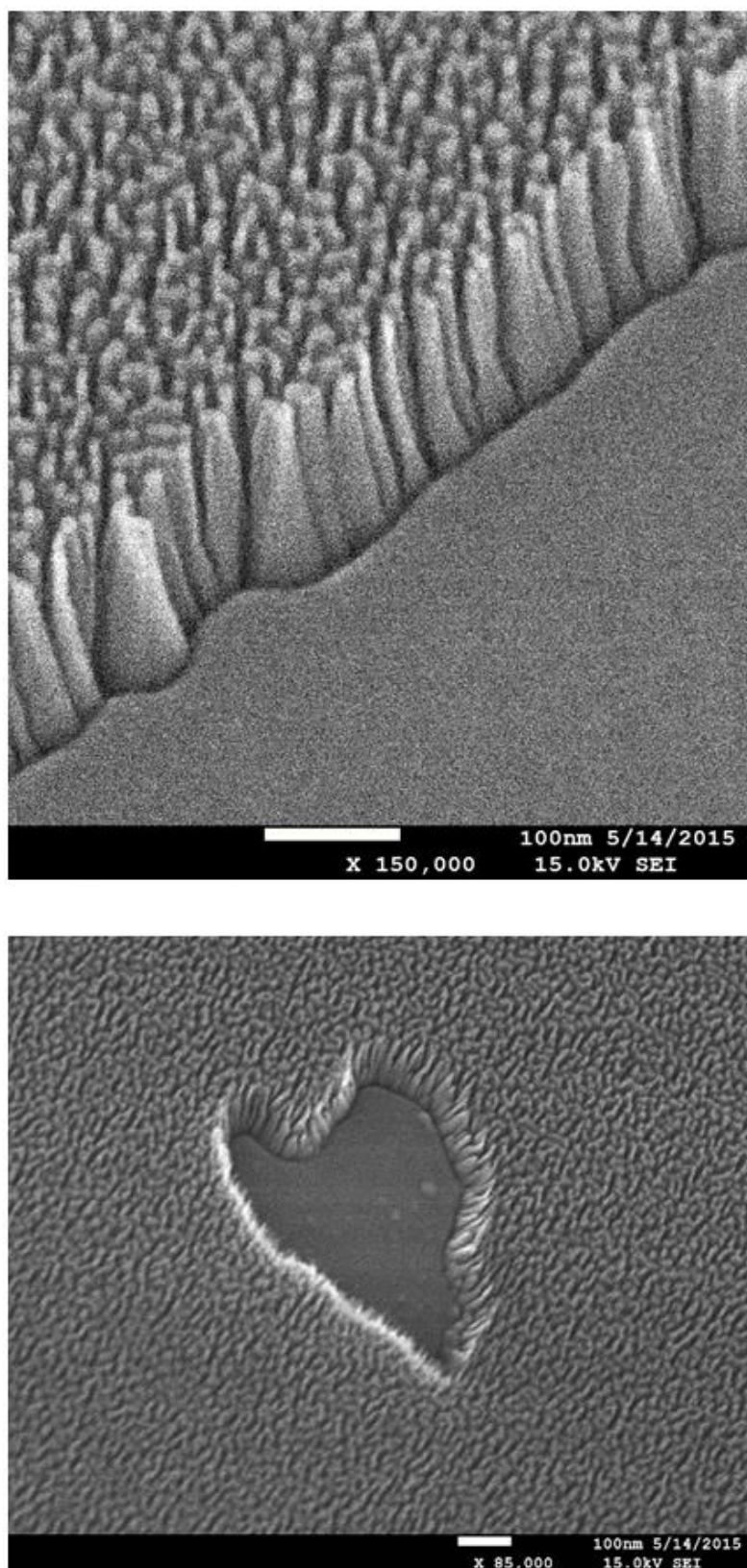


Figure 2.2 | SEM images showing the nanopillar-like structures in the nanostructured samples.

SEM images show ≈ 150 nm high nanopillar-like structures. The SEM image was taken between steps (b) and (c) from Fig. 2.2., with still some conductive gold on the nanopillar tips, to improve image quality.

2.2.2. Experimental setup

We used a home-built fluorescence microscope equipped with a high-power 532 nm green laser (5 W, Verdi, Coherent) and a 0.25 NA objective (Plan N, Olympus), and a microwave excitation [Fig. 2.3, 2.4, and 2.5]. We used the laser to initialize and read out the state of the system. The fluorescence is collected by the same objective and sent to a photon-counter module (SPCM-AQRH-14-FC Excelitas) (specs). Laser power can be opportunely varied from $\approx 1\text{mW}$ ($100\text{ }\mu\text{W}/\mu\text{m}^2$) to the maximum value of $\approx 1500\text{W}$ ($150\text{ mW}/\mu\text{m}^2$), depending on the experiment purpose, with a combination of absorptive filters. With this laser spot size, we estimated that we are simultaneously exciting 1000 NVs.

In nanostructures samples, laser power intensity should be downscaled by a factor of 2.5 - 3, since the irradiation occurs in an irregular surface, such as conical frustums or tilted side surfaces. This power reduction may play a role but cannot explain the important differences between nanostructures and flat samples (the laser power should be 2 orders of magnitude less to cause significant effects).

We collect FL through different spectral windows, with a proper combination of bandpass filters. These different spectral windows are meant to selectively emphasize the signal of the NV^- centers over the NV^0 centers, or vice versa. The first filter is a bandpass with a window from 550nm to 600nm (filter 550-600nm) to study exclusively the NV^0 . The second one is a long-pass with cut-on frequency at 750nm (filter 750nm+) to study the NV^- and have a NV^0 FL leakage. Microwaves are applied by a millimeter-sized copper loop placed below the EG sample. Moreover, the microwave lines comprised a microwaves generator (Keysight N5171B), an amplifier (ZHL-16W-43-S+).

For the spin and charge dynamics experiments, a high-power acousto-optic modulator (ISOMET 523C-6) was used to perform the pulsed sequences. Generally, to initialize the system, a variable preparation pulse was applied, followed by a read-out pulse ($t = 5\text{ }\mu\text{s}$). This read-out pulse provided good sensitivity while minimally perturbing the distribution of NV charge states even at the highest laser power employed (see appendix II of this chapter).

A three-axis Helmholtz coil (Micro Magnetics Inc.) provides a static magnetic field along any desired direction. In addition, a strong magnetic field of $\approx 750\text{ G}$ can be applied along the z-axis, parallel to a [100] crystallographic direction. Thus, the applied magnetic field has the same magnitude along the four possible orientations of the NV axes in the diamond lattice. This magnetic field is applied perpendicularly to the top diamond face, to quench the spin dynamics in favor of the charge dynamics

Full FL spectra of the NVs were acquired with a confocal microRaman setup (LabRam Aramis, Jobin-Yvon Horiba), equipped with a DPSS laser (532 nm), and an air-cooled multichannel CCD detector in the window range between 535 nm and 935 nm. Laser power density is attenuated by neutral filters by 0, 1 and 2 optical densities (OD): OD 0 ($41\text{ mW}/\mu\text{m}^2$), OD 1 ($4.1\text{ mW}/\mu\text{m}^2$) or OD 2 ($0.41\text{ mW}/\mu\text{m}^2$)

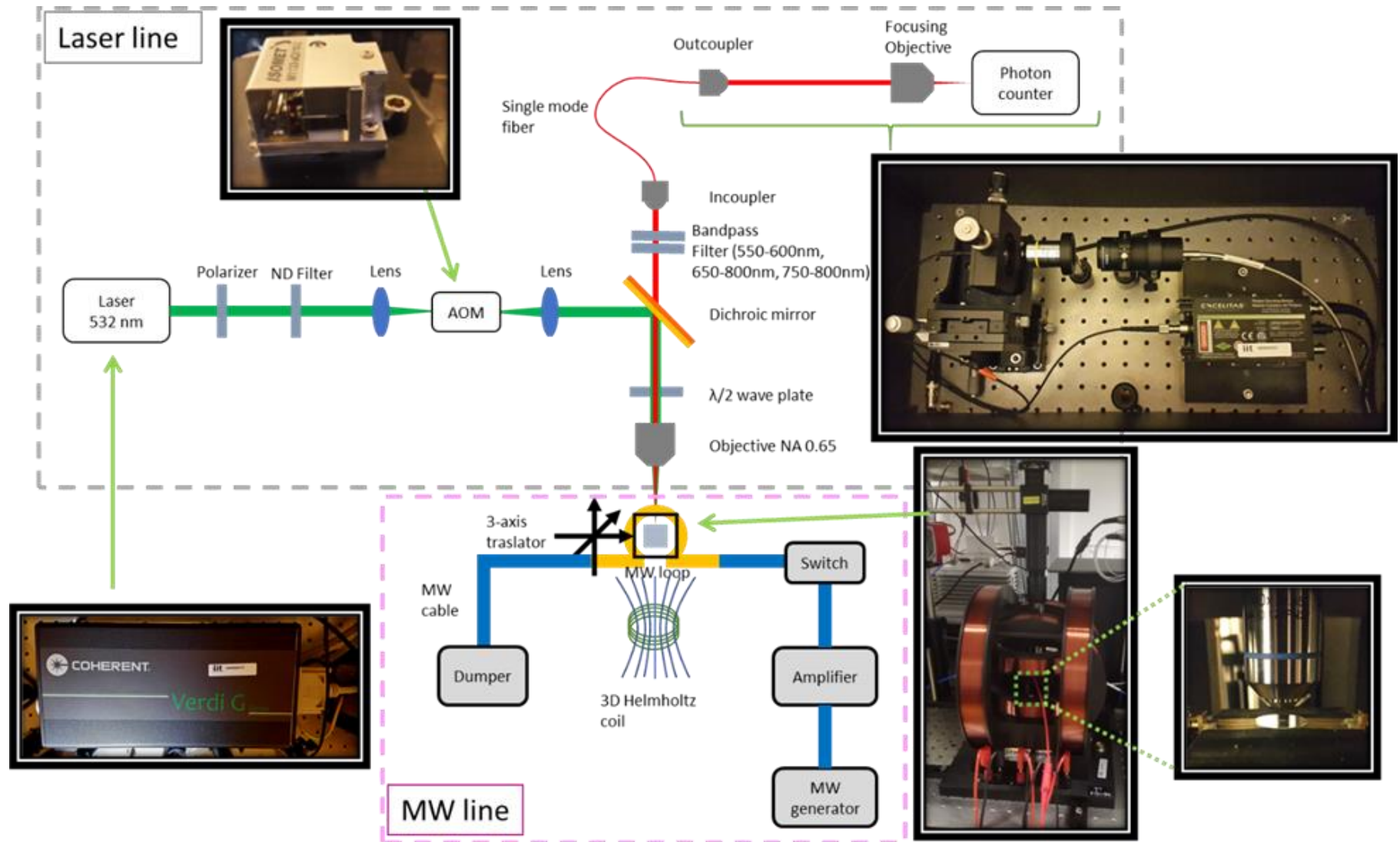


Figure 2.3 | Scheme of the ODMR set-up.

The experimental set-up is composed by a part containing the optical line (enclosed by the grey box) and another for the microwave line (pink box). The diamond sits on a loop, with the possibility of 3D motion and application of a magnetic field in different directions.



Figure 2.4 | Photo of the ODMR set-up.

ODMR set-up where the different optical parts and the microwave line of the previous diagram are shown.

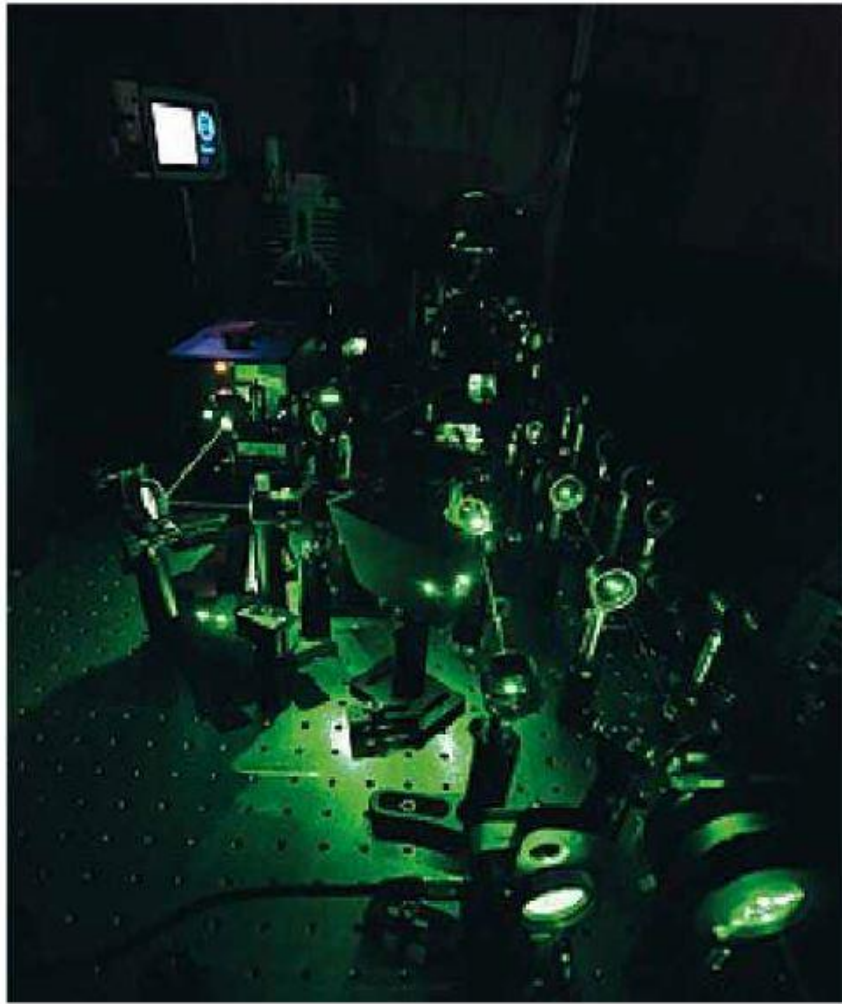


Figure 2.5 | Photo of the ODMR set-up during laser irradiation.



Figure 2.6. | Confocal microRaman setup (LabRam Aramis, Jobin-Yvon Horiba) used in this work.

2.3. Results

Fluorescence properties of NV centers

The whole fabrication process is meant to create shallow NV centers, which can exist in two charge states, the negative NV^- and the neutral NV^0 . The electronic structures of NV^- or NV^0 are represented in Fig. 2.7 a (explained in detail in Chapter 1, section 1.3) [31]. Each NV charge state is then characterized by its fluorescence (FL) spectrum, with zero-phonon lines (ZPL) at 575 nm and 637 nm for the NV^0 and NV^- , respectively, and a phonon sideband peaked around 620 nm for the NV^0 and 700 nm for the NV^- and extending up to ≈ 800 nm in both cases. The FL spectra from sample F2 was collected with a confocal microscope designed for Raman spectroscopy and show features of both NV^- and NV^0 centers, demonstrating the effectiveness of the fabrication process of NVs [Fig. 2.7 b]. Compared to the pristine diamond (black curve and the inset), the treated samples still show the first-order Raman peak of the diamond (sharp peak at ≈ 573 nm, corresponding to a Raman shift of 1332 cm^{-1}), while the second Raman order at 600-620 nm is almost undetectable. As the laser power intensity increases from $0.41 \text{ mW}/\mu\text{m}^2$ at optical density OD=2 to $41 \text{ mW}/\mu\text{m}^2$ at OD=0, the NV^- component grows with respect to the NV^0 component. This points out some degree of $\text{NV}^0 \rightarrow \text{NV}^-$ photoconversion at higher laser powers under continuous excitation (FL curves are normalized to 1 at 637 nm for a better comparison). At every value of laser intensity, or equivalently OD filters, two distinct behaviors from nanostructured and flat samples are registered [Fig. 2.7 c]. In particular, the overall FL from the two nanostructured samples is lower (not shown in the figure, where spectra are normalized for a better comparison), with a higher component in the NV^0 region (blue and red curves compared to yellow and green curves).

We summarize these observations in Fig. 2.7 d, similar to ref. [32]. The x-axis represents the integrated FL, containing the signal of both NV charge states, while the y-axis indicates the ratio between the areas under the NV^- and NV^0 curves. Three laser intensities were used for each sample with a choice of OD filters (0, 1, and 2). At a fixed laser power, the integrated FL is a qualitative indicator of the total number of NV centers initialized by the laser, irrespective of the charge state. The ratio between the NV^- / NV^0 areas accounts for the relative amount of the NV^- compared to the NV^0 . As already hinted by Fig. 2.7 b, this ratio increases with laser power, suggesting an $\text{NV}^0 \rightarrow \text{NV}^-$ photoconversion, namely as recharge. The different behavior of flat and nanostructured samples is also evident: N1 and N2 contain a lower amount of NV centers, indicated by the lower FL signal. Hence, nanostructured samples have a higher fraction of NV^0 than the flat ones.

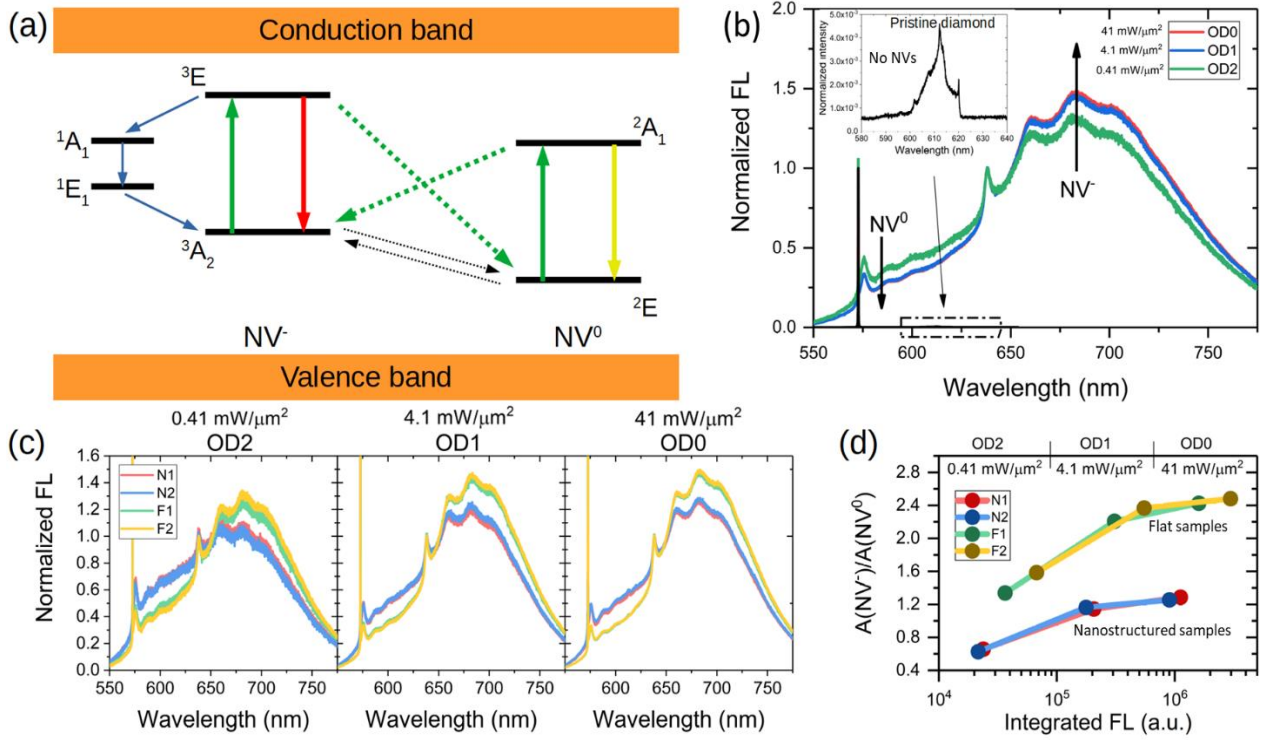


Figure 2.7 | Fluorescence properties of NV centers.

(a) Electronic structure of NV⁻ and NV⁰ centers explained in Chapter 1. Green arrows denote optical excitation and photoconversion (continuous and dotted arrows, respectively). Red and yellow arrows indicate radiative decay from NV⁻ and NV⁰ centers. Blue arrows represent the non-radiative pathway which produce polarization of the $|g, m_s = 0\rangle$ NV⁻ ground state. Black arrows indicate tunneling transitions in the dark between the NV⁻ and NV⁰ centers. (b) Both NV⁻ and NV⁰ FL spectra were detected in implanted samples. Emission from NV⁻s increases and NV⁰ decreases with laser power. (Inset, pristine diamond): no NV signal was detectable in non-implanted, non-annealed samples. (c) At all laser powers, nanostructures samples exhibit a lower FL than flat samples, with a larger component of NV⁰. d) Ratio of NV⁻ and NV⁰ FL area intensities as a function of total integrated FL and laser intensity. For all EG samples, the NV⁻/NV⁰ ratio increases with laser power. This ratio is larger in flat samples. No magnetic field was applied in these FL experiments.

Charge dynamics under laser pumping and charge recovery in the dark

The laser can induce charge conversion ($\text{NV}^0 \leftrightarrow \text{NV}^-$) and modifies the amount of NV⁻ inspected, the collected signal will generally consist of two parts, one coming from spin dynamic and the other from charge dynamic. Therefore, we need a protocol to decouple the charge and the spin dynamics. A viable method to investigate only the charge dynamics is the following: a strong magnetic field (B) is applied along the [100] axis, which means that the projection of B, is the same for the four possible orientations of the NV axes. The strong magnetic field mixes the spin states. It quenches the mechanism responsible for the $|g, m_s = 0\rangle$ polarization, leading to a reduction of the spin-related component in the FL [33–35]. Moreover, the observation of charge dynamics gives complementary

results whether one looks either at the NV^- signal or the NV^0 signal. Taking advantage of this freedom, we choose to collect only the NV^0 signal in the region 550-600 nm with a magnetic field of 750 G.

Fig. 2.8 depicts two pulse sequences adopted to assess the charge dynamics composed of an initialization laser pulse τ_L , a laser readout pulse and the dark time τ_D . In the first sequence [Fig. 2.8 a], a laser pulse (532 nm) of variable duration also known as initialization time (τ_L), (τ_L from 0.01 ms (10 μ s) to 100 ms) is focused on the diamond surface. This pulse induces photoionization ($NV^0 \rightarrow NV^-$), increases the NV^- signal, and decreases the NV^0 signal (orange curve in the sketch), which is recorded by the successive short detection laser pulse of 5 μ s. At the end of the sequence, a fixed time interval of dark time $\tau_D = 10$ ms with the laser off allows for charge relaxation increasing the NV^0 signal ($NV^- \rightarrow NV^0$), and after, the sequence is repeated. Points acquired after discrete initialization pulses for different laser power values are shown in Fig. 2.8 b (for sample F2). Fluorescence decreases according to a stretched-exponential law of the type [5]:

$$I(t) = I_{eq}[1 + Ce^{-(t/T_r)^n}] \quad (\text{Eq. 2.1})$$

where T_r represents a characteristic timescale for the photoconversion process under the laser pulse, n is the stretching factor and governs the dynamics of charge conversion, C is a parameter describing the fluorescence drop from the initial value to the equilibrium and I_{eq} is the FL value at equilibrium. All curves have been normalized by I_{eq} for a better comparison.

The second pulse sequence of Fig. 2.8 c is used to probe the dynamics of charges in the dark. Now the pulse sequence consists of a laser pulse of duration τ_L followed by a variable dark time τ_D (from 1 μ s to 10 ms) and a 5 μ s readout pulse. Again, only the FL of the NV^0 centers can be measured through the 550-600nm spectral window. As seen, the first initialization pulse decreases the number of NV^0 by photoconversion. During the dark time, a steady increase in the number of NV^0 centers occurs, as indicated by the fluorescence level registered by the readout pulse. The plausible explanation considers electrons tunneling from the negative NV^- to surface acceptors or vacancies. This mechanism has been previously observed and recently clarified [19,20]. The back-conversion in the dark from NV^0 to NV^- ($NV^0 \rightarrow NV^-$) depends on the length of the initialization pulse. As an example, Fig. 2.8 d shows the curves obtained with a lasers power of ≈ 625 mW and initialization times from 10 μ s to 100 ms for sample F2. Again, the curves can be fitted with a stretched exponential like:

$$I(t) = I'_{eq}[1 - Ae^{-(t/T'_r)^n}] \quad (\text{Eq. 2.2})$$

where I'_{eq} is the value of FL at long times, T'_r is a characteristic time for the charge conversion in the dark, the parameter A gives the initial FL value away from equilibrium, and n is the previous stretching coefficient. The curves have been fitted with common values of T'_r and m .

Equations 2.1 and 2.2 have the same form, but there are significant differences:

- The initial FL value is now lower than the equilibrium value, meaning that the parameter A is negative.
- The mechanism of charge conversion in the dark is not photoconversion, and T'_r is generally different from T_r .

Coherently with the results of Fig. 2.8 b, the initial value of FL decreases with longer initialization pulses, meaning that a substantial fraction of NV^0 is converted into NV^- upon laser irradiation (NV^0

→ NV⁻). In Fig. 2.8 d, the curves acquired after τ_L of 10 ms, 30 ms, and 100 ms are almost overlapped and agree with the observation that beyond 10 ms the photoconversion process saturates. Importantly, in all the experiments inspecting charge dynamics, we see no dependence on the duration of the readout pulse, from 0.5 μ s to 5 μ s, even at the highest laser powers.

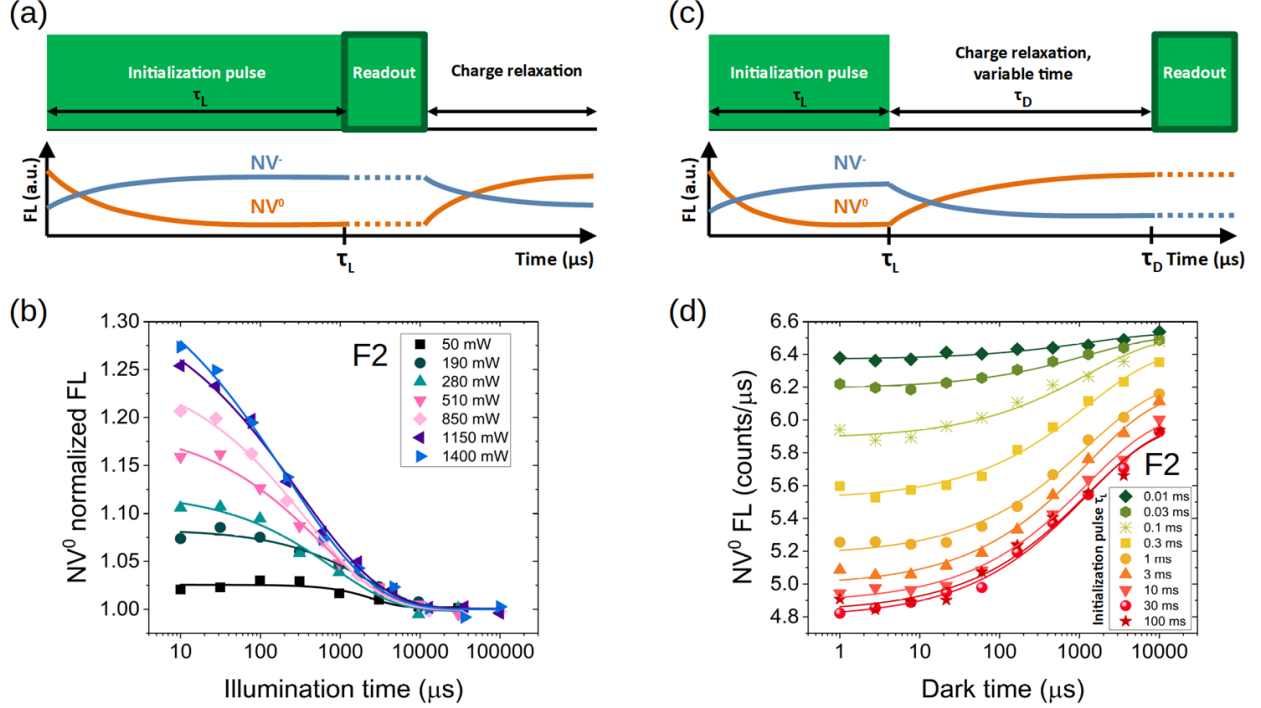


Figure 2.8 | Detection of charge dynamics using a magnetic field.

(a) Pulse sequence adopted to investigate charge dynamics under laser pumping, for variable irradiation pulse length τ_L . Laser-induced NV⁰ → NV⁻ photoconversion results in a decrease of the NV⁰ signal and an increase of the NV⁻. (b) Shows the decrease in NV⁰ FL for sample F2, as measured in the 550-600nm window, for different laser powers. (c) The pulse sequence is used to detect the charge recovery in the dark after a preparation pulse. (d) Recovery of NV⁰ FL for sample F2 at laser power 625 mW. The various curves correspond to different durations of the initialization pulse.

Parameters (C and T_r) describing NV⁰ → NV⁻ photoconversion under laser irradiation

The photoconversion data for all EG samples is shown in Fig. 2.7 a. We plotted $(1 + C)^{-1}$ from (Eq. 2.1) at different laser powers. C describes the ratio between the FL equilibrium and initial FL values. The flat samples exhibited a larger drop in NV⁰s (F1= 74 %; F2 = 81 %) than nanostructures samples (N1= 96 %; N2= 92%) due to photoconversion (NV⁰ → NV⁻) at the highest laser power. Hence, the sample F2 emerges as the most promising sample in its ability to develop a large amount of NV⁻ centers. The nanostructured samples presented a more limited variation. At high laser powers, the error bars on the x-axis were larger because the laser beam transmittance suffered a slight

deterioration (5-10% losses) during prolonged irradiation. Fig. 2.9 b shows the rate of photoconversion for the flat samples, where we plot the inverse of the photoconversion time $(T_r)^{-1}$ at different laser powers. $(T_r)^{-1}$ increases nearly linearly with laser power, with $(T_r)^{-1}$ (F1 sample) = 0.95 ± 0.07 and $(T_r)^{-1}$ (F2 sample) = 1 ± 0.07 . This almost linear dependence suggests a single-photon photoconversion $NV^0 \rightarrow NV^-$ mechanism. The nanostructured samples exhibited a large uncertainty on the $(T_r)^{-1}$ extracted values that prevent to extract conclusions.

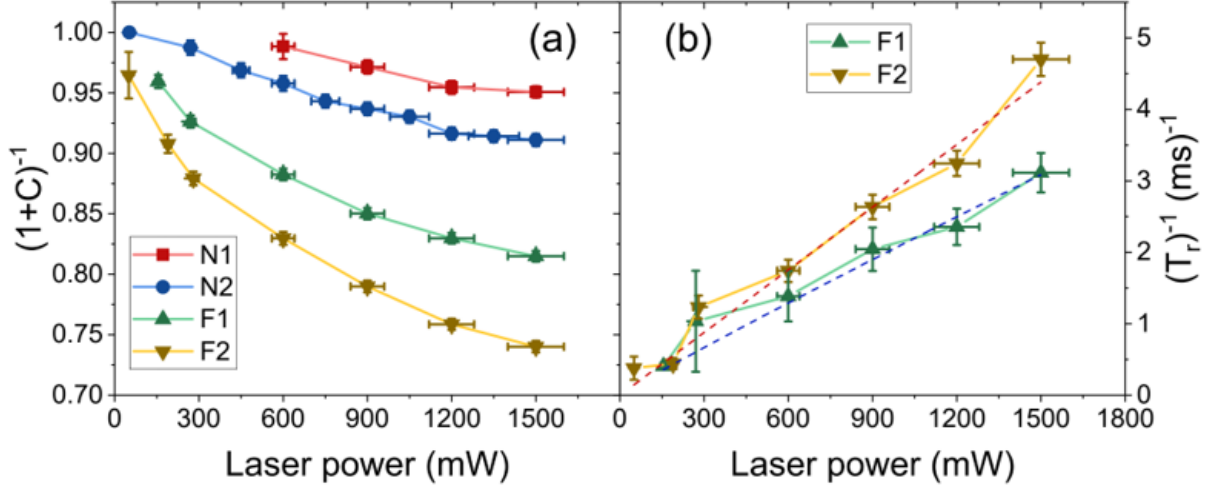


Figure 2.9 | Parameters describing $NV^0 \rightarrow NV^-$ photoconversion under laser irradiation

(a) The quantity $(1+C)^{-1}$ indicates the ratio between the equilibrium value of FL and the initial FL value, just after the initialization pulse (τ_i). The reduction is more consistent for the flat samples (down to 75% for sample F2). (b) The photoconversion rate $(T_r)^{-1}$ is nearly linear with laser power. It follows a power law with exponents for F1 of 0.95 ± 0.07 and for F2 of 1 ± 0.07 (red and blue dashed curves).

$[NV^-]/[NV^0]$ charge-state ratio after the laser pulse at high and low laser powers.

Fig. 2.10 a depicts the FL signal symmetry between the dynamics of the NV^- and NV^0 in the dark. We used the F2 sample at the highest available laser power (1500 mW), in the 550-600 nm and 750nm+ spectral windows dominated by NV^0 (blue curve) and NV^- (red curve) respectively. The experiment was conducted with and without a 750 G magnetic field (open and solid circles in Fig. 2.10 a, respectively). While the NV^- signal is influenced by both spin and charge dynamics, the NV^0 signal reflects strictly charge dynamics. Therefore, to precisely measure the effects of charge dynamics in NV^- , we applied a 750 G magnetic field to suppress the NV^- spin dynamics from NV^- . Fig. 2.10 b,c show the same pulse sequence experiment of Fig. 2.10 c with two additional spectral windows, a bandpass filter at 550 - 600 nm for the NV^0 centers, and a longpass filter from 750 nm for the NV^- centers with and without a magnetic field. Thus, we can estimate the charge state ratio at equilibrium, defined as $R = [NV^-]/[NV^0]$ [18] (see Appendix III).

The two assumptions to calculate the charge-state ratio R are:

- (i) All the NV centers are optically active.
- (ii) The sum of NV^- and NV^0 is always constant. This implies that the total amount of centers N_{tot} is constant, and a decrease in NV^0 s corresponds to an increase in NV^- s and vice versa.

Fig. 2.10 b,c shows the subdivision of the populations for the sample F2 between NV^- and NV^0 after the initialization laser pulse ($\tau_L = 10$ ms). In both low and high laser power cases, the initialization laser pulse produces an excess of NV^- s ($NV^0 \rightarrow NV^-$) that convert back into NV^0 s ($NV^- \rightarrow NV^0$) during the dark time. The black curves trace the ratio between NV^- and NV^0 from the initial value (right after laser irradiation) to the equilibrium value R as follows:

- (i) At low laser power (50 mW, corresponding to ≈ 5 mW/ μm^2), the dominant signal are the NV^0 s due to the ratio $R = [NV^-]/[NV^0] \approx 0.13/0.87 \approx 0.15$ after 100 ms.
- (ii) At high laser power (1500 mW, ≈ 150 mW/ μm^2), the ratio changes substantially to $R = [NV^-]/[NV^0] = 0.53/0.47 \approx 1.13$, and now, the NV^- s are the dominant state, with an approximately seven-fold increase ($R_{\text{at high laser power}}/R_{\text{at low laser power}} \approx 1.13/0.15 \approx 7.53$) compared to the previous low laser power ratio. The curve exhibits an initial faster decrease and then, a more slowly decaying component. Hence, the effect of an intense laser pulse is to create a large NV^-/NV^0 ratio ($R \approx 1.13$) stabilizing the NV^- s and sustaining them over tens or hundreds of milliseconds (see Discussion).

The dynamic of charges shows a stretched exponential behavior, with an initial fast decrease in the 0.01 - 1 ms timescale and then, and slowly decaying component in the 100 ms scale. From 1-10 ms, there is a combined effect of spin and charge dynamics and thus, an important overlap in FL. After 10 ms, the FL profile is dominated by charge dynamics. For example, in Fig. 2.10 a, we can observe that the difference between the two red curves (with and without magnetic field) from 1-10 ms is attributed to the spin dynamics. This difference vanishes after 10 ms demonstrating that is not influenced by the magnetic field, hence indicating that is exclusively dominated by charge dynamics.

The sample N2 presented a lower charge-state ratio ($R_{\text{sample N2}} = [NV^-]/[NV^0] \approx 0.1/0.9 \approx 0.11$) than the sample F2 ($R_{\text{sample F2}} \approx 1.13$) at the highest laser power (1500 mW). It means that the $NV^0 \rightarrow NV^-$ photoconversion is less efficient in nanostructure samples. They also exhibited a shorter lifetime (≈ 10 ms) of the NV^- photoconverted centers [see Fig. 2.16 in appendix IV].

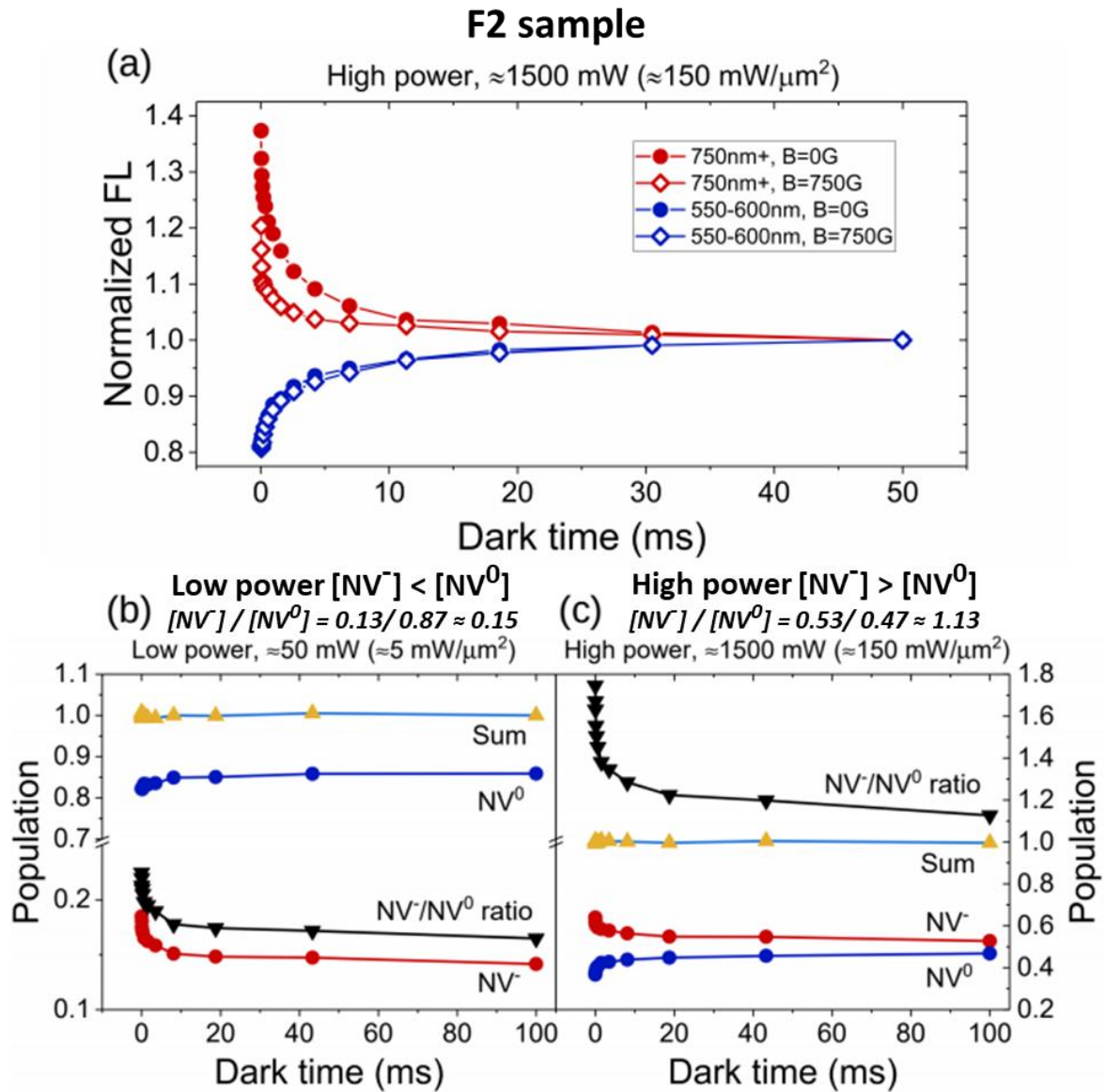


Figure 2.10 | Charge-state ratio after the initialization laser pulse.

(a) The effect on the FL of sample F2 under a strong magnetic field (750 G) at the highest available laser power (1500 mW). FL was collected in the 550-600nm (blue curves) and in the 750nm + window (red curves), with (empty symbols) and without magnetic field (solid symbols). The NV^0 centers (550-600nm filter) do not experience any change in the FL profile, regardless of the magnetic field applied. Conversely, the signal in the 750nm+ region decreases applying a 700 G magnetic field. This difference is due to the suppression of NV^- spin polarization by spin-states-mixing. Nevertheless, the difference disappears beyond $\approx 10 \text{ ms}$, because at longer dark times, the FL profile is dominated by charge dynamics. At (b) low and (c) high laser powers, the initialization laser pulse of 10 ms generates an excess of NV^- s (red curve) that photoconvert to NV^0 s (blue curve) in the dark time. NV^- and NV^0 populations are normalized such that their sum is 1 (azure-yellow-curve). The NV^-/NV^0 charge ratio R is represented by the black curve. After 100 ms, a near-equilibrium R (0.15) is achieved at low power (50 mW), while a greater and constant R (1.13) is noticed at high power irradiation (1500 mW).

ODMR contrast at different laser powers

The ODMR spectra from sample F2 at different laser powers (from 3 to 1500 mW) and fixed MW power is shown in Fig. 2.11 a. The ODMR contrast increases with laser power and permits to resolve the ^{13}C sidebands and the strain-split doublet [36]. We show that ODMR contrast increases with laser power for all samples [Fig. 2.11 b]. Flat samples showed a larger ODMR contrast ($\approx 8\text{-}9\%$) than nanostructured samples ($\approx 2\%$). The samples N1 and N2 exhibited a lower ODMR contrast since the NV^0 centers are the dominant charge, even at the highest powers.

The NV^0 signal leakage into the NV^- region has a relevant practical consequence on the ODMR contrast [37,38]. The FL is collected in the 750 nm+ window, where most of the signal comes from the NV^- centers, albeit with a small tail of the NV^0 emission band, which cannot be removed by spectral filtering. This tail, being insensitive to the MW field, provides an unwanted background noise. However, as the laser power increases, the NV^0 leaked component is reduced. Thus, pumping with an intense laser power has two consequences that can also increment the ODMR contrast:

- 1) It increases the number of NV^- centers and reduces the background noise from the NV^0 centers due to photoconversion ($\text{NV}^0 \rightarrow \text{NV}^-$).
- 2) It increases the $|g, m_s = 0\rangle$ NV^- spin polarization.

In sample F2, the ODMR contrast decrease at high laser powers (≈ 600 mW). This probably is due to saturation reflecting laser-induced repolarization related to insufficient MW power (see Discussion). In conclusion, we have shown the importance of using high-power laser irradiation to improve the availability and polarization of shallow NV^- . This effect is more prominent in flat samples than in the nanostructured ones.

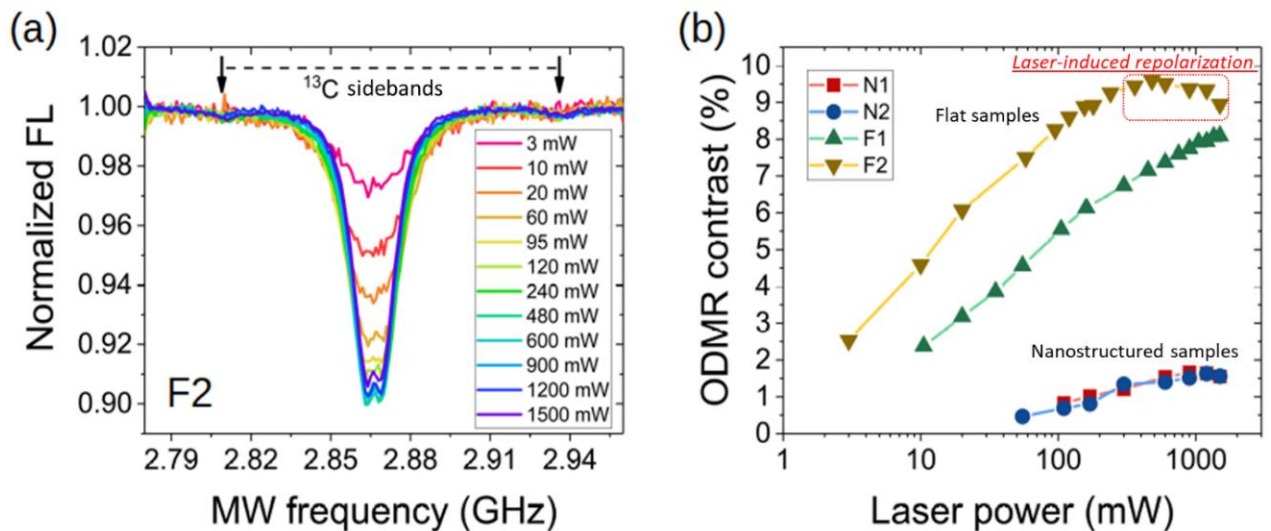


Figure 2.11 | Optically Detected Magnetic Resonance contrast at different laser powers.

(a) ODMR spectra as a function of laser power from flat sample F2. (b) ODMR contrast increases for all samples. The ODMR contrast is larger in the flat samples than in nanostructured ones.

2.4. Discussion

Laser power

The highest laser density power utilized in this chapter are higher than those commonly found in the bibliography. NVs are typically tested employing laser intensities in the range of few $\text{mW}/\mu\text{m}^2$ or less [21,39,40] and only infrequently approach $100 \text{ mW}/\mu\text{m}^2$. The utilization of this high-laser power is motivated by the absence of saturation of the NV optical transition [Fig. 2.15]. We tracked the FL emission with two filters: “ 550–600 nm ” and “ >750 nm ”. The FL signals with the > 750 nm filter exhibit hardly any signs of saturation (saturation laser power estimated at 8.5 W), whereas the 550–600 nm window displays a flexion only for the flat samples (estimated at 2.3 W). Therefore, we can assume that the highest laser power employed here was under the start of saturation. The saturation curves rely on whether on the diamond characteristics (defects density type) or an ensemble or a single NV center. Very different saturation intensities have been reported, varying from a few to thousands of $\text{mW}/\mu\text{m}^2$ [41–43].

Defects

The NV^0 centers constitute a considerable percentage of NVs for all EG samples. In the bulk, the existence of electron donors, like P1 centers, generates stability and formation of NV^- s. Close to the diamond surface [44–46], acceptor states and moities can reduce the stability of the NV^- , and NV^0 s may turn into the dominant NV charge state [47,48], with deleterious effects for polarization transfer and sensing applications. The samples were designed to have shallow NV centers (same as in preceding studies [49]) , and it is predictable to detect mainly NV^0 centers. In the case of nanostructured samples, the surface effects are aggravated by the large surface-to-volume ratio of structures like nanopillars. Hence, nanostructures samples have more NV^0 s than NV^- s, as shown in the Raman [Fig. 2.7 d], FL [Fig. 2.9 a], ODMR spectra [Fig. 2.11 b] and charge-state R ratio [Fig. 2.13]. Furthermore, the integrated FL of Fig. 2.7 d indicates that nanostructured samples possess fewer NV centers in total, independently of the NV charge state. This could be determined by less efficient ion implantation on the nanostructures or by the loss of nitrogen because of ion straggling.

Mechanism

Laser irradiations produce two effects simultaneously. It polarizes the $\text{NV}^- |g, m_s = 0\rangle$ spin state and photoconverts part of the NV^0 s into NV^- ($\text{NV}^0 \rightarrow \text{NV}^-$). When the laser is switched off, the $\text{NV}^- \rightarrow \text{NV}^0$ back-conversion occurs in the dark. Surprisingly in this work, the NV^- population persists during a long timescale (10 ms).

The following possible mechanism can qualitatively explain the $\text{NV}^0 \rightarrow \text{NV}^-$ photoconversion in our results: i) The laser pulse ionizes the substitutional nitrogen atoms (P1 centers) [50], ii) these electrons are released into the conduction band and iii) recombine with electron-trapping defects [51], including the NV^0 s, iv) thus forming NV^- s [52]. Recently, the Stark shift of optical transition has considered the phenomenon of redistribution of charges in a different light.

This mechanism could be explained by a single-photon ionization process ($\text{NV}^0 \rightarrow \text{NV}^-$) of P1 centers as a source of electrons, since there is an almost linear dependence of $(T_r)^{-1}$ [Fig. 2.9 b] and a decrease of NV^0 FL with laser power [Fig. 2.8 b]. It must be noted that the proposed mechanism is

different from the two-photon excitation of electrons from valence band [53] because it is represented by a quadratic dependence of $(T_r)^{-1}$ with laser power [21].

After the laser irradiation, a $NV^- \rightarrow NV^0$ conversion is observed in the dark time. The mechanism suggested is the electron tunneling which decisively depends on the NV environments. Tunneling to a neighboring trap can occur if the trap is empty [20]. A weak laser power only photoexcites few electrons, so the degree of $NV^0 \rightarrow NV^-$ conversion is low, and the filling of the traps is low. However, a strong laser pulse can photoexcite many electrons, converting a lot of NV^0 and filling many of the surrounding charge traps. If many charge traps are filled already, the NV^- centers are more stable, and the ionization process in the dark is slower. In other words, the NV^- s with empty traps in the surroundings can be depleted quickly forming NV^0 s (fast charge component in Fig. 2. 10 b and c), but the other NV^- s surrounded by filled traps are frozen in the negative charge state (see Fig. 2.8 c where the $[NV^-]/[NV^0]$ ratio persists during ~ 100 ms). Therefore, the charge reorganization operated by the intense laser pulse creates a state with an inverted ratio of charge states and a high abundance of NV^- s. For the practical purpose of manipulating NV^- spins, we can assume that the system is in a new equilibrium state between charges.

The surface effects compete with NV^0 to capture released electrons. Thus, the $NV^0 \rightarrow NV^-$ photoconversion is more efficient in the flat samples and less efficient in the nanostructured since the number of surface defects is large compared to the number of NVs. In our samples, the advantage of surface-to-volume ratio due to nanostructuring appears to be diminished by the less favorable charge dynamics. Possible additional chemical treatment of the diamond surface such as selective fluorination [24] or oxidation [54] may stabilize the NV^- s thus improving the yield of the nanostructure sample.

Comparison of our results with other diamond samples with nanopillars reported in the literature

McCloskey et al. 2020 [55], used a nanostructured sample with a nanopillar height and width size of ≈ 1000 nm. Our nanopillars are significantly smaller, with a nanopillar height of 150 nm and from a width of ≈ 50 μm at the base to 10 nm at the top. Hence, the effects of lateral surfaces are expected to be more important for nano-pillars and negligible for micro-pillars, where just a small number of NVs (the ones nearest to the sidewall) are affected. They observed [55] in their nanostructures samples a better spin properties, larger NV^-/NV^0 ratio and higher brightness compared to a bulk diamond reference. Further work would be needed to delineate the transition from nano- to micro-pillars in terms of the NV^-/NV^0 ratio and to provide a better understanding of whether shape and size can impact how photoexcited electrons recombine with defects, including NV^0 .

Stretched exponentials to fit the FL curves

We employed stretched exponentials to fit the FL curves to consider the heterogeneity present in the NV ensembles, therefore, we applied a superposition of many single exponential curves [5,56]. Miller et al. [57] and Tachiya et al. [58], proposed a model to address the dynamics of charge tunneling. They consider a spatially homogeneous distribution of charge acceptors and recently improved by assuming a discrete distribution [19]. The charge dynamics can be characterized by a functional dependence of the form

$$I(t) = I_{eq}(1 + C \exp\{\frac{-4\pi a^3 n}{3} [\ln(vt)]^3\}) \quad (\text{Eq. 2.3})$$

Here, a and v are parameters related to the NV^- center, defined as the attenuation length of the wavefunction and the tunneling frequency, respectively and, n is the density of trapping defects. Resolving this equation, we found $a = 0.1$ nm, $v = (0.8 \pm 0.1)$ MHz and, $n = 2\text{-}4$ ppm. After long laser pulses, the density n exhibits a small tendency to diminish, probably suggesting that photoexcited electrons have filled some charge traps. In the current model, further work would be required to understand the characteristics of n , for example, non-uniform values of n , v , and a in a defective sample. Nevertheless, we do not notice an important enhancement of the FL fit using the stretched exponential function of Eq. 2.3 instead of Eq. 2.1 for our data. We are mainly concerned with the initial out-of-equilibrium FL values of the curves in comparison to the equilibrium value. These values do not depend decisively on the selected fitting function and are adjusted during the initialization time by the laser power and pulse length. In this regard, the two stretched exponentials gave results that are in good agreement.

ODMR contrast can be affected by the leakage of the NV^0 FL component

The NV^- spectral region can be affected by the leakage of the NV^0 FL component and reduce the ODMR contrast [37,38]. The contrast for NV^- s is defined as $C_s = (I_{off} - I_{on})/I_{off}$, where I_{on} and I_{off} are the FL intensities with microwaves on- and off-resonance, respectively (see Chapter 1, section 1.2.3.). Generally, the FL intensity contains a nuisance signal from NV^0 -related component I_0 . Therefore, the contrast C_s should be scaled by a factor $I_{off}/(I_{off} + I_0)$. The intensity I_{off} (I_0) is the fraction f (f^0) of the total NV^- (NV^0) intensity. We used a 750nm+ long pass to study predominantly the NV^- signal and we normalized the FL intensity by the photon-counter efficiency. It is feasible to correlate these FL intensities to the charge-state ratio (R) by parameters involving to NV^- (NV^0) centers, including the excited state lifetime τ (τ^0) and the absorption cross sections σ (σ^0).

The ODMR contrast for the NV ensemble (C_{ens}) is:

$$C_{ens} = C_s \frac{R}{R+F} \quad (\text{Eq. 2.4})$$

where $F = \frac{f_0 \sigma_0 \tau_n}{f_n \sigma_n \tau_0}$ is a numerical factor that depends on detection conditions, for instance the choice of detectors and filters, and NV structural properties. On the contrary, R can change between samples. In shallow NV centers, the charge state is dominated by NV^0 , R is low and the ODMR contrast decrease. Conversely, the samples that have a high number of NV^- , R is much greater than F ($R > F$) and the correction is minimum.

We estimate $f^0/f \approx 0.42$ from the shape of FL for our set-up (see Appendix I). With the following data $\tau^- = 12$ ns, $\tau^0 = 21$ ns [59], $\sigma^- = 3.1 \times 10^{-17}$ cm² [60] and, $\sigma^0 = 1.8 \times 10^{-17}$ cm² [61], we obtain $F \approx 0.14$. Moreover, R changes substantially from low laser power ($R = 0.15$) to high power ($R = 1.13$). Using an indicative value of ODMR contrast ($C_s = 10\%$), we estimate at low power $C_{ens} = 5.2\%$ and at high laser power $C_{ens} = 8.9\%$. We also notice a tendency of C_{ens} and R to augment with laser power, according to the $\text{NV}^0 \rightarrow \text{NV}^-$ photoconversion. Theoretically, if the dependence of R on the laser power is known, C_s could be recovered from experimental values of the ODMR contrast. The suggested explanation describes the tendency of ODMR contrast from low to moderately high laser powers [Fig. 2.8 b], even though, at the highest power levels, the contrast shows a saturation or also

a small decrease (see, for instance, sample F2). Furthermore, it describes the remarkable difference between the nanostructured and the flat samples. The nanostructured samples have the NV^0 as the dominant charge state even at the highest laser powers, thus reflecting a lower ODMR contrast than the flat ones.

Concerning the saturation of the ODMR contrast at high laser power, the optimal contrast results from the combined laser and microwave spin initialization. If the laser power is very high, some degree of laser-induced repolarization may be expected since the MW field cannot compete with the laser irradiation in reverse the population levels of the NV ground state [62]. Despite adjusting the MW power to the highest available value, specifically, using a MW output ≈ 8 dBm and amplified by ≈ 35 dBm, the laser-induced repolarization effect may dominate. This reflects a saturation and a slight contrast reduction (see Fig. 6b where this contrast depletion is more pronounced in sample F2).

When the ODMR contrast increases, the sensitivity η improves and, the minimum magnetic field δB detectable in a time Δt augment, since $\delta B = \eta/\sqrt{\Delta t}$. The sensitivity in continuous-wave ODMR protocols is [63]:

$$\eta = \frac{4}{3\sqrt{3}} \frac{h}{g\mu_B} \frac{\Gamma}{C_{ens}\sqrt{r}} \quad (\text{Eq. 2.5})$$

where h is the Planck constant, $g \approx 2$, μ_B the Bohr magneton, Γ is the full-width-half-maximum of the Lorentzian, C_{ens} is the ODMR contrast of the NV ensemble given by Eq. 2.4 and r is the photodetection rate. The numerical factor in front ($4/3\sqrt{3}$) of Lorentzian function is applied to fit the NV resonance.

We find experimentally $r = 0.73 \times 10^6 \text{ s}^{-1}$ and $r = 17.8 \times 10^6 \text{ s}^{-1}$ at low (50 mW) and high power (1500 mW), respectively. These r values were normalized by limited photon counter efficiency of the detector. Taking values of $\Gamma = 10$ MHz, C_{ens} and R as before, we obtain $\eta = 6.2 \text{ } \mu\text{T}/\sqrt{\text{Hz}}$ at low power and $\eta = 730 \text{ nT}/\sqrt{\text{Hz}}$ at high laser power. Using pulsed-wave ODMR protocols [28], sensitivity could be improved and extended to the detection of alternating current (AC) magnetic fields [64,65]. Thus, high laser power improves magnetometry applications since it reflects an increase in the rate of photons detection, ODMR contrast, and R ratio. It is worth noting that the correction factor of Eq. 2.4 does not simply redefine ODMR contrast, but it is important to considered it in other pulsed sequences [Fig. 2.8 a and 2.8 c]. Certainly, laser power is constrained by the nature of the sample and available laser equipment. Accordingly, it should be approached a large number of NVs at expense of tighter focusing (consequently with lower laser intensity) for some applications such as widefield imaging [66], sensing [67] or hyperpolarization [12].

2.5. Appendix

2.5.1. Charge state fluorescence ratio and filtering of fluorescence signal

We used two different set-ups to collect the FL signals. We employed a Raman confocal microscope dotted with a spectroscopy to study the NV spectral emission and a home-built microscope equipped with a photon counter detector.

In the Raman set-up, the FL was collected at three different laser power (0.41, 4.1 and, 41 mW/ μm^2). As an example, we used the nanostructured sample N1 [Fig. 2.12]. We deconvolved the FL into the NV^0 and NV^- individual components (green and orange curves) using NV^0 FL and NV^- FL curves available in the bibliography and consequently, analysing the data with the LM algorithm. The experimental spectra precisely reproduce the sum of the both NV^0 and NV^- components. In Fig 2.7 d, NV curves were integrated and summed to quantify the integrated FL. On the contrary, the area ratio $A(\text{NV}^-) / A(\text{NV}^0)$ [Fig. 2.7 d] is proportional to the charge-state ratio R [Fig 2.10 b,c]. We find that the ratio increases with laser power from $0.41/0.59 \approx 0.69$ at 0.41 mW/ μm^2 , $0.45/0.55 \approx 0.82$ at 4.1 mW/ μm^2 to $0.58/0.42 \approx 1.38$ at 41 mW/ μm^2 .

With the home-built microscope, FL is collected with a combination of bandpass filters as shown in Fig. 2.13. The 550-600 nm (purple curve) and the 750 nm+ (blue curve) with peak transmittance of 80-90%, indicatively. The FL spectrum of the nanostructured sample N1 (black curve) was acquired with a confocal Raman setup at low power (0.41 mW/ μm^2). In Fig. 2.13, we take the FL spectrum of the nanostructured sample N1 (black curve) and decompose it in the NV^0 and the NV^- bands (green and red curves): the 550-600nm filter selects almost exclusively the NV^0 signal, but the 750nm+ filter selects both signals. Therefore, NV^- signal is always affected from NV^0 FL that cannot be entirely removed by filtering and produces an unwanted NV^0 background signal (compare Eq. 2.4). Nevertheless, the NV^-/NV^0 ratio improves [Fig. 2.7] and the NV^0 leakage is less important at high laser power.

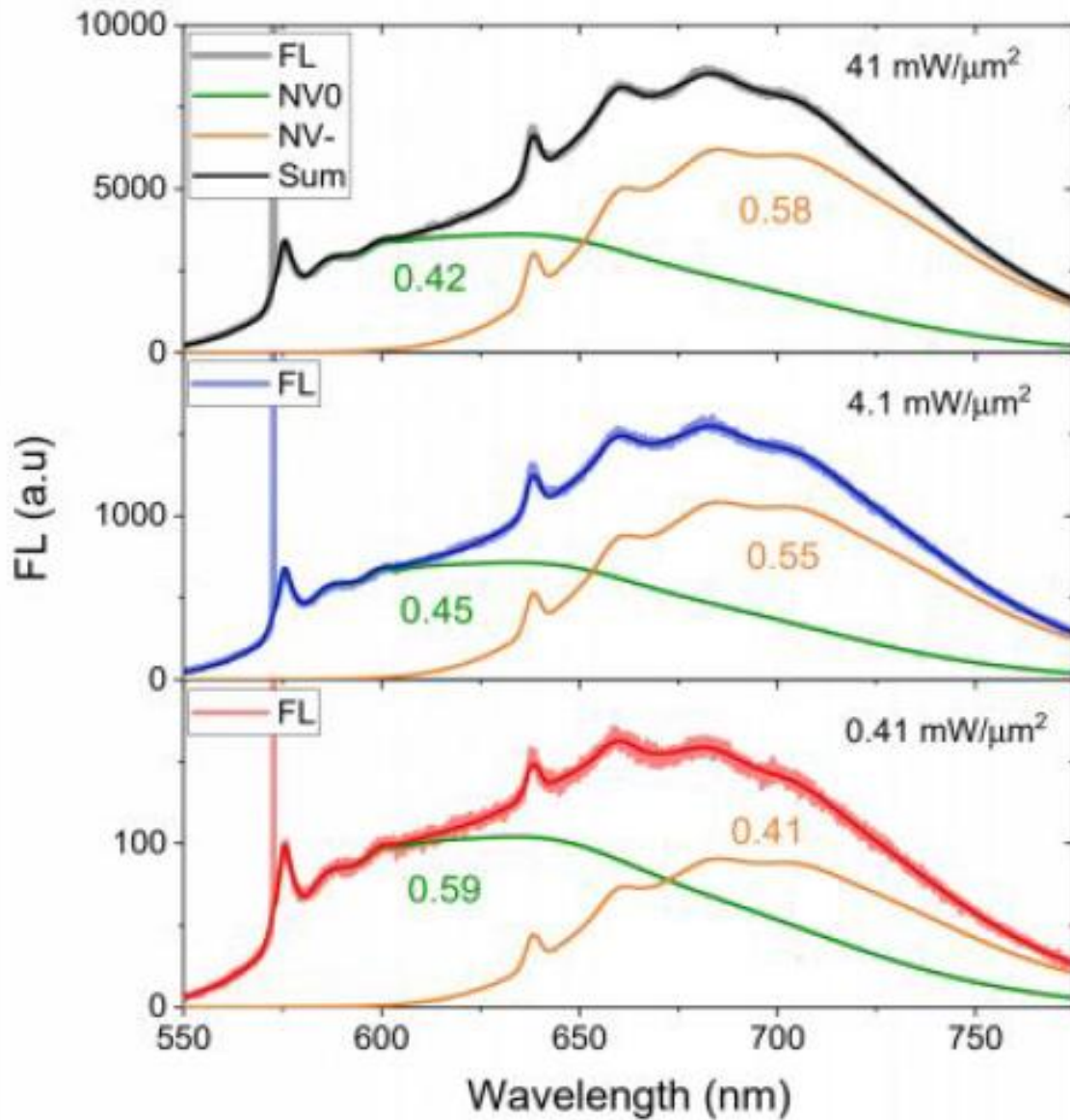


Figure 2.12 | Charge state fluorescence ratio for the nanostructured sample N1.

FL was collected at three different laser power (0.41, 4.1 and, 41 $\text{mW}/\mu\text{m}^2$). We deconvolved the FL into the NV^0 and NV^- individual components (green and orange curves). The area ratio $A(\text{NV}^-)/A(\text{NV}^0)$ is proportional to the charge-state ratio R . We find that $A(\text{NV}^-)/A(\text{NV}^0)$ increases with laser power from $0.41/0.59 \approx 0.69$ to $0.58/0.42 \approx 1.38$.

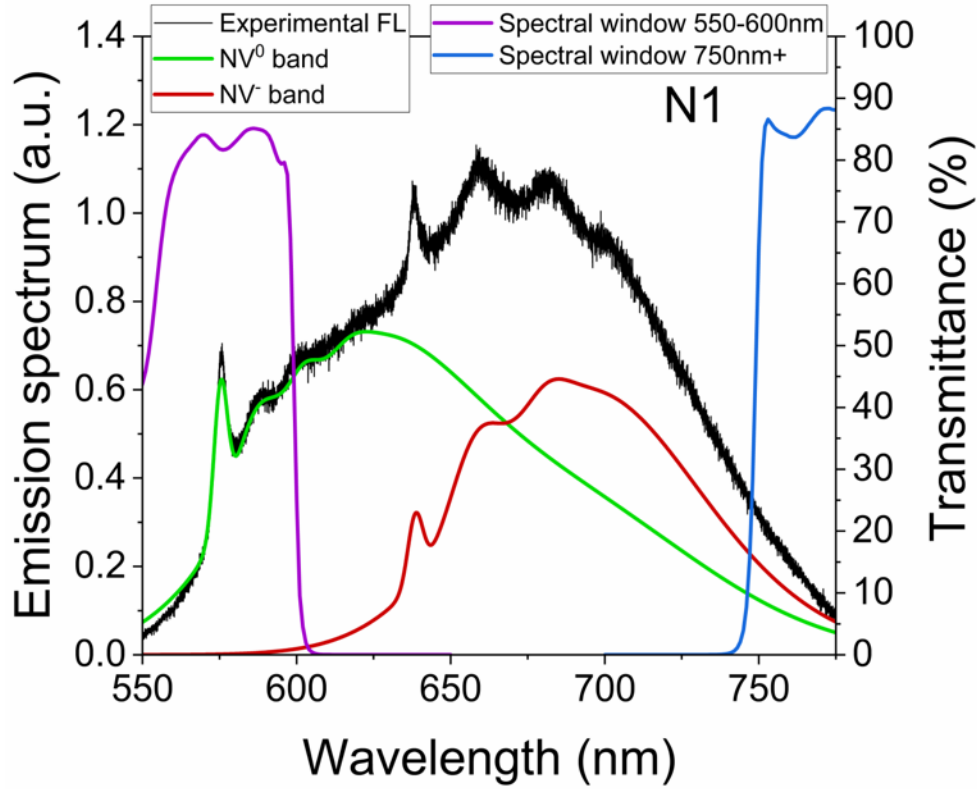


Figure 2.13 | Filtering of fluorescence for the nanostructured sample N1.

The emission spectrum of N1 (black curve) is collected with the Raman confocal microscope and can be decomposed into NV^0 and NV^- components (green and red curves, respectively). Filter 550-600nm selects only the NV^0 component, but both components pass through the filter 750nm+. The leakage of NV^0 FL in the NV^- region decreases the contrast in ODMR and pulsed laser experiments. It is preferable to use a high laser power, as it increases the amount of NV's by photoconverting NV^0 s and reduces the NV^0 leakage.

2.5.2. Effect of the readout pulse

We selected a readout pulse of 5 μ s in most of the experiments. We also contemplated the risk that this readout pulse can produce photoconversion, particularly at the highest laser powers. We plotted the evolution of the FL signal in the dark from sample F1 [Fig. 2.14], using short (0.5 μ s, (green curve)) and long (5 μ s (purple curve)) readout pulses. We employed a 750nm+ filter at the highest laser power of 1.5 W and we did not apply a magnetic field (similarly to the red solid curve of Fig. 2.10 a). Not important differences were observed between the two curves. Nevertheless, the length of the readout pulse affects the acquisition time, because according to a Poissonian process, the photon-shot noise scales as the inverse square root of the acquisition time ($1/\sqrt{t}$) (or equivalently to the number of photons detected ($1/\sqrt{n \text{ photons}}$)). For example, the 0.5 μ s readout pulse was obtained after 220 repetitions, whereas with 5 μ s, only 50 repetitions are necessary. In

conclusion, we used a 5 μs readout pulse even at the highest laser power since ensures a high sensitivity without perturbing the NV charge-state distribution.

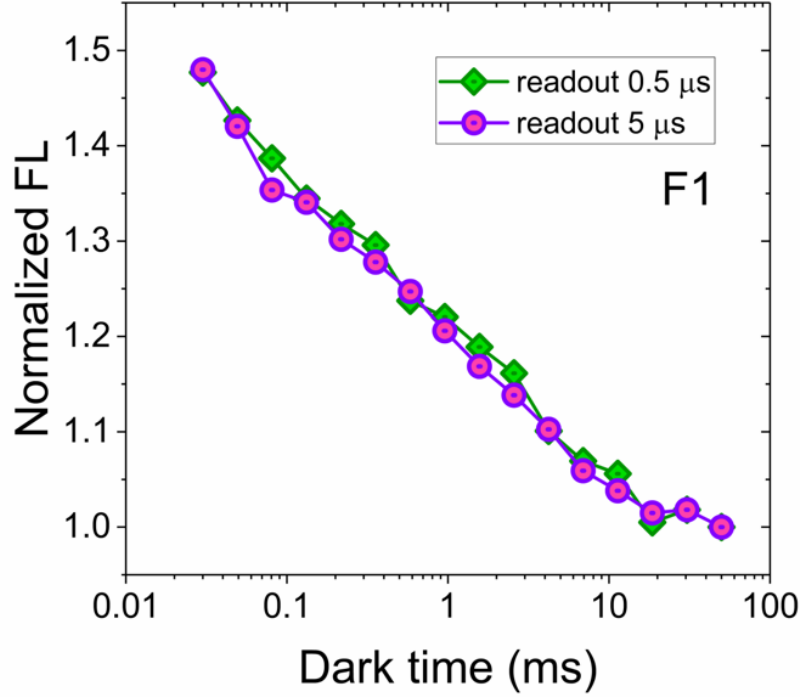


Figure 2.14 | Effect of the readout pulse in the flat sample F1.

Two readout pulses of 0.5 μs (green curve) and 5 μs (purple curve) were used to record the FL variation in the dark in a pulsed experiment where both charge and spin dynamics were investigated (no magnetic field applied, 750nm+ filter). Laser power was 1.5 W, the maximum available. Even under these conditions, the curves do not display any major difference.

2.5.3. Excited state saturation

The set-up is prepared to work with NV ensembles, with an areal density of $\approx 10^3 \mu\text{m}^{-2}$. Therefore, several thousands of NV centers can be excited in the laser spot at the same time. The saturation of the excited state was explored under these conditions. We used a continuous laser excitation and extracted the FL emission (in terms of millions of counts per second, "MC/s"). A strong magnetic field (>750 G) was also employed to quench the effects of spin on the emitted FL. After setting the laser power, the FL was recorded for a few seconds to permit charge dynamics to reach equilibrium. The FL values are represented in Fig. 2.15, for 550-600nm and 750nm+ spectral filtering (green and pink dots, respectively). Generally, the curves are fitted by a function of the type:

$$I(P) = I_0 \frac{P}{P+P_0} + I_{off} \frac{P}{P_0} \quad (\text{Eq. 2.6})$$

where I is the intensity of the FL and P is the laser power. The first term in Eq. 2.6 is the real saturation function, with P_0 being the saturation power and I_0 a scaling coefficient. The second term gives a linear offset to be subtracted. A reliable calculation of P_0 is a difficult task; we estimated it to be 2.3 W for NV^0 and 8.5 W of NV^- (corresponding to a saturation intensity of 230 mW/ μm^2 and 850 mW/ μm^2 , respectively, for a laser spot size of 10 μm).

Once again, we observe that nanostructured and flat samples vary in the values and in the shapes of the two curves. The nanostructured samples do not exhibit an onset of saturation in either curve, while for the flat samples the NV^0 curves start to flex. Here, we note that the effective laser intensity for the nanostructured samples might be lower by a factor of ≈ 3 compared to the flat samples, since of irradiation on tilted surfaces. Even by rescaling the laser power by the corresponding factor, the flat and nanostructured samples behave differently. Finally, this indicates that the laser power must be further increased to approach saturation in the nanostructured samples.

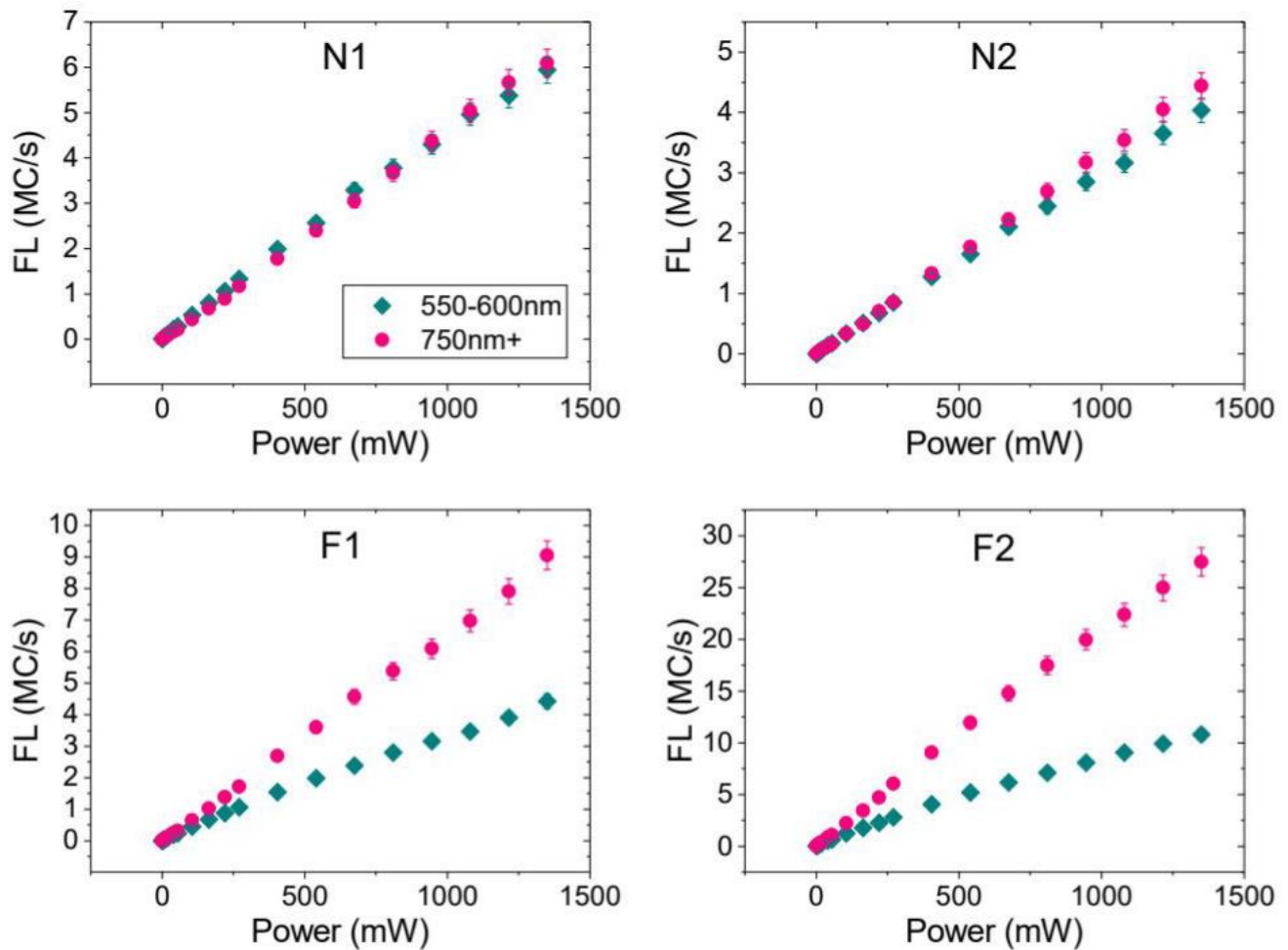


Figure 2.15 | Evolution of FL emission (in terms of million counts per second) with laser power

The nanostructured samples (N1 and N2) show only a small variation, whereas in the flat ones the photoconversion appears to be more solid.

2.5.4. Calculation of the charged state ratio

The charge-state ratio defined as $R=[NV^-]/[NV^0]$, is performed through the pulse sequence from Fig. 2.8 c. Here, we use a strong magnetic field (800 G) along the [100] direction to suppress the spin dynamics and, particularly, study the charges dynamics. In this experiment, we record FL through the 550-600 nm and the 750 nm+ to study the NV^0 and NV^- center, respectively. In fact, magnetic fields > 600 G can mix the ground spin states and suppress the mechanism of spin dynamics in control of the $|g, m_s = 0\rangle$ polarization, decreasing the spin-related component in the FL. The NV^0 and NV^- signals are fitted by stretched exponentials.

In the 550-600 nm filter, only NV^0 signal passes and the FL increases with time as:

$$I^0(t) = I_{eq}^0 [1 - \delta_0 e^{-(t/T_r)^n}] \quad (\text{Eq. 2.7})$$

Conversely, in the 750nm+ filter, an NV^0 leakage in the NV^- FL component is presented, and thus, the FL signal decreases with time as:

$$I(t) = k I_{eq}^0 [1 - \delta_0 e^{-(t/T_r)^n}] + I_{eq}^- [1 + \delta_- e^{-(t/T_r)^n}] \quad (\text{Eq. 2.8})$$

where I_{eq}^0 and I_{eq}^- are the levels of FL at equilibrium for the NV^0 and NV^- , respectively. n and T_r dominate the charge dynamic conversion, and δ_0, δ_- , indicate the initial out-of-equilibrium FL level of FL (they are both positive parameters). The NV^0 leakage is quantified by the number k (here $k \approx 0.19$) and it can be estimated from the spectral window transmittivity and, the FL profile. Knowing the number k from Eq. 2.8, we can recover the pure NV^- FL signal using Eq. 2.7. The laser pulses do not affect the total number of NV centers (N_{tot}), that is, the sum of the number of NV^- centers (N^-) and NV^0 centers (N^0). Consequently, the total number of NV centers (N^-) and NV^0 centers (N^0). Consequently, the total number of NV center is: $N^-(t) + N^0(t) = N_{tot} = \text{const}$. In all the calculations, the potential contribution of NV^+ is not considered since the concentration is very low. The fraction of NV^- centers is $\frac{N_{eq}^-}{N_{tot}} = \frac{\delta_0}{\delta_n + \delta_0}$ and NV^0 centers is $\frac{N_{eq}^0}{N_{tot}} = \frac{\delta_n}{\delta_n + \delta_0}$ over the total at equilibrium. Thus, the charge state ratio (R) is then: $R = \frac{N_{eq}^-}{N_{eq}^0} = \frac{\delta_0}{\delta_n}$. R is different from the FL ratio between the NV^- s and NV^0 s

The two NV centers have different relaxation times (τ) ($\tau^- = 12$ ns, $\tau^0 = 21$ ns) and absorption cross sections (σ) ($\sigma^- = 3.1 \times 10^{-17}$ cm², $\sigma^0 = 1.8 \times 10^{-17}$ cm²). The FL ratio should be scaled by a factor of $\approx F = \frac{f_0}{f_n} \frac{\sigma_0}{\sigma_n} \frac{\tau_n}{\tau_0}$, where f and f^0 depend on the detection condition and they are the fractions of the total emitted FL collected through the combination of filters. We cannot compare the ratio $R = \frac{N_{eq}^-}{N_{eq}^0}$ illustrated here and the FL ratio from Fig. 2.7 d and Fig. 2.12, because that FL ratio were performed without any magnetic field thus, containing a spin related component.

In Fig. 2.16, we collected the FL for the flat sample F2 and the nanostructured sample N1 at the highest available power of 1500 mW. We applied a strong magnetic field (750 G) to suppress the spin related

component and study the charge dynamics. FL was collected with (empty symbols) and without magnetic field (solid symbols) in the 550–600 nm window (blue curves) and in the >750 nm region (red curves). The magnetic field does not affect the NV^0 FL. The sample N2 exhibits a higher initial

value of NV^- FL since the initial FL contrast is predominantly related to the photoconverted charges. Specifically, the NV^- concentration is extremely low compared to sample F2 and the laser irradiation affects substantially the NV charge state.

Nevertheless, in sample F2, the dissimilarity between the FL curves with and without a magnetic field (red curves with the empty and solid points, respectively) is larger than in sample N2, suggesting a better spin polarization. The slope of the red curve is larger in sample N2 than F2, indicating a more effective $NV^- \rightarrow NV^0$ charge recombination in the dark [Fig S.4b]. The charge state ratio R is plotted for the samples F2 [Fig. S.4 c] and N2 [Fig. S.4 d] in the dark under a magnetic field of 700 G (calculations are described above in this section). For sample F2, the NV^-/NV^0 ratio goes from $R(1\text{ms}) \approx 0.70/0.30 \approx 2.33$, $R(10\text{ ms}) \approx 0.58/0.42 \approx 1.38$ to $R(100\text{ ms}) \approx 0.53/0.47 \approx 1.13$. In sample N2, NV^-/NV^0 ratio varies from $R(1\text{ms}) \approx 0.19/0.81 \approx 0.23$, $R(10\text{ ms}) \approx 0.13 / 0.87 \approx 0.15$ to $R(100\text{ ms}) \approx 0.10/0.90 \approx 0.11$.

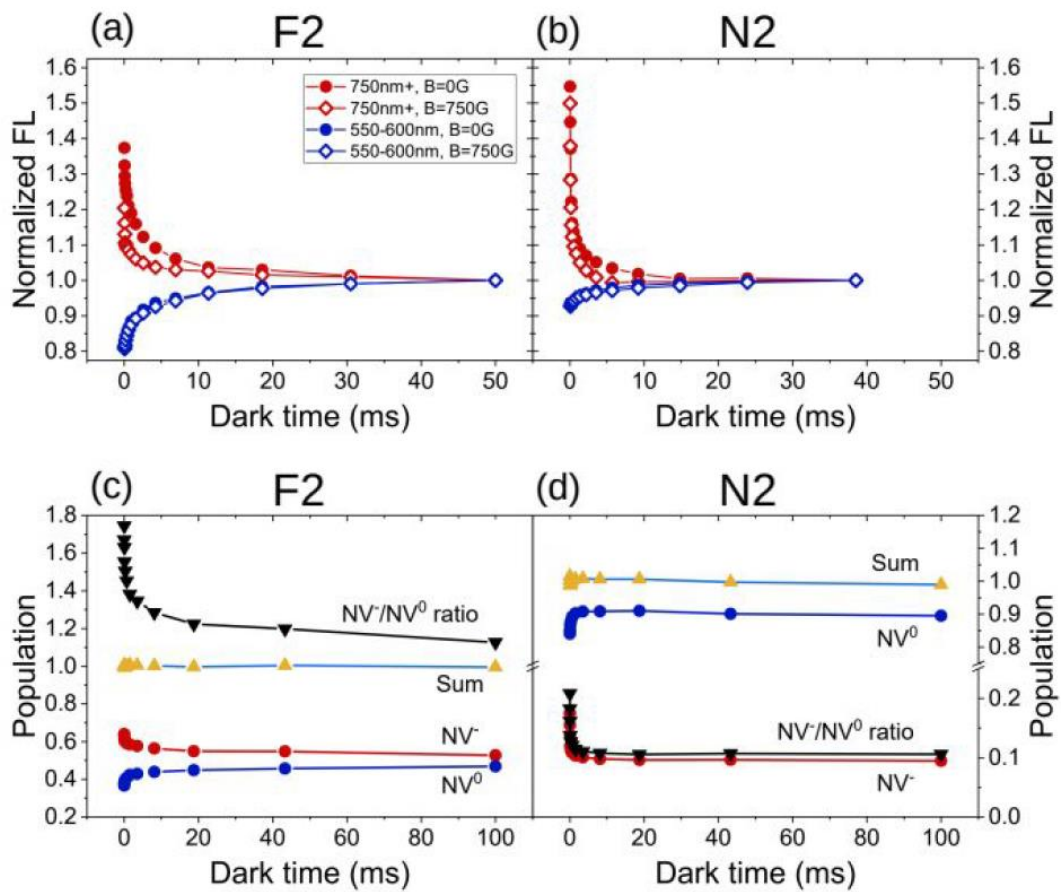


Figure 2.16 | Charge-state ratio for the flat sample F2 and the nanostructured sample N2.

(a,b) FL was collected in the $> 750\text{ nm}$ “ NV^- region” (red curves) and in the $550\text{--}600\text{ nm}$ “ NV^0 window” (blue curves) with (empty symbols) and without magnetic field (solid symbol). (c,d) At high laser power (1500 mW), the 10 ms laser pulse produces an excess of NV^- s (red curve) that photoconvert to NV^0 s (blue curve) in the dark time ($NV^- \rightarrow NV^0$). NV^- and NV^0 populations are normalized such that their sum is 1 (yellow-azure curve). The NV^-/NV^0 ratio (represented by the black curve) after 100 ms is $0.53/0.47 \approx 1.13$ for the flat sample F2 and $0.1/0.9 \approx 0.11$ for the nanostructured sample N2.

2.6. Summary points of chapter 2

1. We report the effects of green laser irradiation on ensembles of nanometer-shallow NV centers in flat and nanostructured diamond surfaces with nanopillars [Fig. 2.1 and 2.2].
2. We demonstrate that high laser power promotes photoconversion of NV^0 to NV^- centers ($NV^0 \rightarrow NV^-$), improving the availability and polarization of shallow NV^- defects. This effect is more effective in flat samples than nanostructured samples. Hence, flat samples have more NV^- s than NV^0 s, as shown in the Raman [Fig. 2.7 d], FL [Fig. 2.9 a], ODMR spectra [Fig. 2.11 b] and charge-state R ratio [Fig. 2.16].
3. Nanostructured samples have a larger amount of surface defects than flat ones. These defects tend to stabilize and favour the less useful NV^0 centers.
4. For all samples, the ODMR contrast increases with laser power because the laser irradiation: i) polarizes the NV^- spin and ii) increases the number of NV^- because of photoconversion ($NV^0 \rightarrow NV^-$). Flat samples showed a higher contrast than nanostructured ones [Fig. 2.11].
5. After switching off the laser, the excess of NV^- population is stable over a timescale of 100 ms, producing long-lived enrichment of shallow NV^- . The NV^-/NV^0 ratio is $0.53/0.47 \approx 1.13$ and $0.1/0.9 \approx 0.11$ for the flat F2 and nanostructure N2 sample at the highest available laser power (1500 mW) after 100 ms, respectively [Fig. 2.16].
6. Mechanism. A laser pulse ionizes the P1 centers, and electrons are released into the conduction band and recombine with electron-trapping defects including the NV^0 s forming NV^- s ($NV^0 \rightarrow NV^-$). The decrease of NV^0 FL is consistent with this mechanism [Fig. 2.8 b] and the nearly linear dependence of $(T_r)^{-1}$ with laser power [Fig. 2.9 b] suggests a single-photon ionization process.

Summary results of this chapter

Samples	Flat		Nanostructured	
	F1	F2	N1	N2
Implantation energy (KeV)	20	10	20	10
Raman. $A(NV^-)/(NV^0)$. From 0.41 to 41 mW/ μm^2	$\approx 1.2 \rightarrow \approx 2.4$		$\approx 0.6 \rightarrow \approx 1.2$	
$(T_r)^{-1}$	≈ 0.95	≈ 1		
NV^-/NV^0 ratio. At 1500 mW = 150 mW/ μm^2		$0.53/0.47 \approx 1.13$		$0.1/0.9 \approx 0.11$
Drop in FL (%)	≈ 74	≈ 81	≈ 96	≈ 92
ODMR contrast (%)	≈ 8	≈ 9	≈ 2	

* All the results shown in the table are approximated.

Useful information

Nanopillars have a height of 150 nm and a width narrowing from 50 nm at the base to 10 nm at the top

Magnetic field applied along the z-axis and perpendicular to the top diamond face: 750 G

Two filters were used: 550-600 nm and 750 nm+ to study the NV^0 and NV^- respectively.

Laser power used goes from 1 mW (0.1 mW/ μm^2) to 1500 mW (150 mW/ μm^2). Laser spot size: 10 μm^2 .

Saturation laser powers (P_0) are 2.3 W (230 mW/ μm^2) and 8.5 W (850 mW/ μm^2) for the NV^0 and NV^- respectively (see Appendix).

Initialization pulses used (ms) (see Fig. 3.6b): 0.01, 0.03, 0.1, 1, 3, 10, 30, 100. Finally, we selected 10 ms for the experiments.

2.7. References of chapter 2

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Chapter 3:

Photoconversion of NV centers in nanodiamonds: implications for biosensing and polarization transfer applications

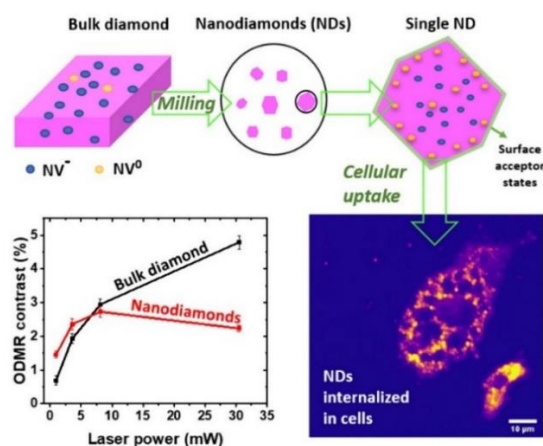
Chapter 3: Photoconversion of NV centers in nanodiamonds: implications for biosensing and polarization transfer applications

Large portions of Chapter 3 will appear in the following paper: D.Olivares-Postigo, F. Gorrini, R. Giri, A. Krueger and A. Bifone. Divergent effects of laser irradiation on ensembles of nitrogen-vacancy centers in bulk and nanodiamonds: Implications for biosensing, doi:10.33774/chemrxiv-2021-llrtb

Chapter summary

Dense ensembles of shallow NV^- in nanodiamonds (NDs) with large surface areas represent promising materials for biosensing, bioimaging, and hyperpolarization applications. However, surface acceptor states tend to favor the NV^0 centers. In this chapter, we study the Optically Detected Magnetic Resonance (ODMR) and the fluorescence properties of NV^- centers in ^{13}C enriched bulk diamond, and in NDs of various sizes obtained by nanomilling the native bulk diamond. Specifically, we investigate spin polarization through ODMR contrast of NV centers as a function of laser power at fixed microwave power, room temperature, and Earth's magnetic field. In bulk diamond, we find that increasing laser power increases ODMR contrast, consistent with a power-dependent increase in spin-polarization. Conversely, in NDs, we observe a decrease in ODMR contrast at higher laser powers. Thus, the $NV^- \rightarrow NV^0$ photoconversion process in NDs is more efficient than the bulk diamond, resulting in depletion of the NV^- pool. We also internalized NDs in macrophage cells and we found similar, albeit more variable results, under these experimental conditions, typical of a bioassay. Therefore, a wide-field fluorescence microscope was used to address the problem of the ND heterogeneous response due to the high tendency to aggregate. Our findings indicate a trade-off in the use of NDs for applications that require ensembles of shallow NV^- centers, and suggest that adjustment of experimental parameters, like laser power, can help find the optimal conditions for sensing and polarization transfer applications.

Keywords: nanodiamonds, nitrogen-vacancy centers, polarization, bulk diamond, spin dynamics, charge stability charge dynamics, cells, photoconversion, ^{13}C enriched



3.1. Introduction

Many sensing and hyperpolarization applications depend on ensembles of NV⁻ centers in the vicinity of the diamond surface [1,2] and thus, NV-rich, fluorescent NDs (NDs) are potential candidates [3–5]. NDs are biocompatible and can be internalized in living cells to probe the microenvironment in subcellular compartments [6,7]. They also possess a large surface area that can be functionalized to target specific proteins or cells [8–10]. ¹³C enrichment of NDs is desirable to improve magnetic resonance signal enhancement, quantum sensing, and hyperpolarization efficiency for contrast agents [11].

Increasing the surface area of the material by nanomilling can improve the exposure of shallow NVs to the external environment. Inevitably, nonetheless, it impacts NV⁻ charge stability [12], as surface effects are inclined to favor the NV⁰ charge state, which has no useful spin properties [13–15]. Therefore, NDs show a larger relative concentration of NV⁰ in NDs with respect to the bulk starting material.

Laser irradiation is employed to polarize and readout NV⁻s and can as well promote charge photoconversion between NV⁻ and NV⁰ ($\text{NV}^0 \leftrightarrow \text{NV}^-$) [1,16–21] [1,17–22]. Photoconversion relies on the presence of surface acceptor states and nitrogen defects, thus $\text{NV}^0 \rightarrow \text{NV}^-$ and $\text{NV}^- \rightarrow \text{NV}^0$ photoconversion pathways have been detected in various diamond samples [15,18,19]. In chapter 2, we have observed that the increase of laser power can importantly increase the number of shallow NV⁻ in nanostructured samples [23]. Nevertheless, it is not clear if a similar approach may be fruitful in NDs.

In this chapter, a highly fluorescent ¹³C-enriched HPHT bulk diamond was milled in 156 nm and 48 nm NDs [Fig. 3.1 a], subsequently, NDs were internalized in macrophage cells. We acquired ODMR and FL spectra at different laser powers for the bulk and for the NDs, to ascertain the effects of the nanofabrication process. Importantly, experiments were performed with a house-built wide-field microscope at a sub-micrometric spatial resolution to account for the intrinsic heterogeneity in NDs [Fig. 3.1 b and c]. This microscope also allowed us to study the effects of laser power in different cellular compartments. Our results emphasize the importance of surface effects on photoconversion and shed valuable insights into the refinement of experimental conditions for polarization transfer and biosensor applications using fluorescent NDs.

3.2. Materials

Samples: ^{13}C -enriched Bulk and 156 and 48 nm nano – diamonds.

Bulk ^{13}C -enriched diamond grown by the high-pressure, high-temperature (HPHT) technique was acquired from ElementSix. The concentration of NVs was estimated in the order of ≈ 10 ppm, and ^{13}C enrichment ranged from 5 to 10% ($\approx 5 \cdot 10^4 - 10^5$ ppm); the concentration of substitutional nitrogen (P1 centers) was approximately 200 ppm. In the bulk, spectroscopic measurements estimated the concentration of NV centers and P1 centers [1] and Element Six provided the concentration of ^{13}C .

The different ND sizes were acquired by attrition milling the bulk diamond following a procedure adjusted from [24]. The sample (62 mg) was placed in a stainless-steel milling cup, which was loaded to one-third of its height with 5 mm stainless steel milling balls. The bottom third of the milling cup was then loaded with isopropanol that had been dried using a molecular sieve. The sample was milled for 6 hours at 50 swings/s. After milling, the sample had many metallic impurities. To wash the diamond material, the sample was flushed out of the milling cup with distilled water into a 250 ml round bottom flask. The remaining steel balls were retired employing a magnet. Concentrated hydrochloric acid HCl (50 mL) was added to dissolve metallic impurities. The mixture was stirred overnight at room temperature. After settling, the supernatant was decanted and 50 ml 96% of sulfuric acid (H_2SO_4) was added. The resulting mixture was heated to 120 °C bath temperature without a reflux condenser to remove any remaining isopropanol ($\text{C}_3\text{H}_8\text{O}$).

After 1 hour, a reflux condenser was connected to the flask. Nitric acid 65% (HNO_3) (20 ml) was gradually added and the same time, monitoring the reaction mixture cautiously to avoid a violent reaction. It must be noted that any remaining isopropanol could strongly react with concentrated HNO_3 , thus it is essential to remove it cautiously before adding HNO_3 . The solution was stirred all night at 120 °C. After the solution cools to room temperature, the supernatant acid mixture was extracted by a pipette and the remaining solution was moved to centrifugation tubes. SEM images at different magnifications and the Raman spectra of the milled NDs before size separation by centrifugation, are shown in Fig. 3.2 and Fig. 3.3, respectively. To wash the diamond sample, centrifugation at 15000 rpm was applied over 1 hour to deposit the diamond material at the bottom.

The supernatant was extracted and replaced with distilled water. Using sonication, the diamond sample was dispersed. This operation was repeated until the supernatant had a neutral pH. After reaching the neutral pH, centrifugation was employed to separate the diamond particles by size. Two ND particle sizes were separated, and their size distribution was measured using dynamic light scattering (DLS), showing a D50 value (volume distribution) of 156 nm and 48 nm, respectively [Fig. 3.4].

Before the FL and ODMR experiments, NDs were suspended in water in glass vials. These NDs were sonicated and 10 μL of the sample were deposited either on a circular glass slide or in Ibides (Ibidi GmbH, Planegg/Martinsried, Germany) and allowed to dry. Moreover, NDs were incubated into macrophage cells following the method described below.

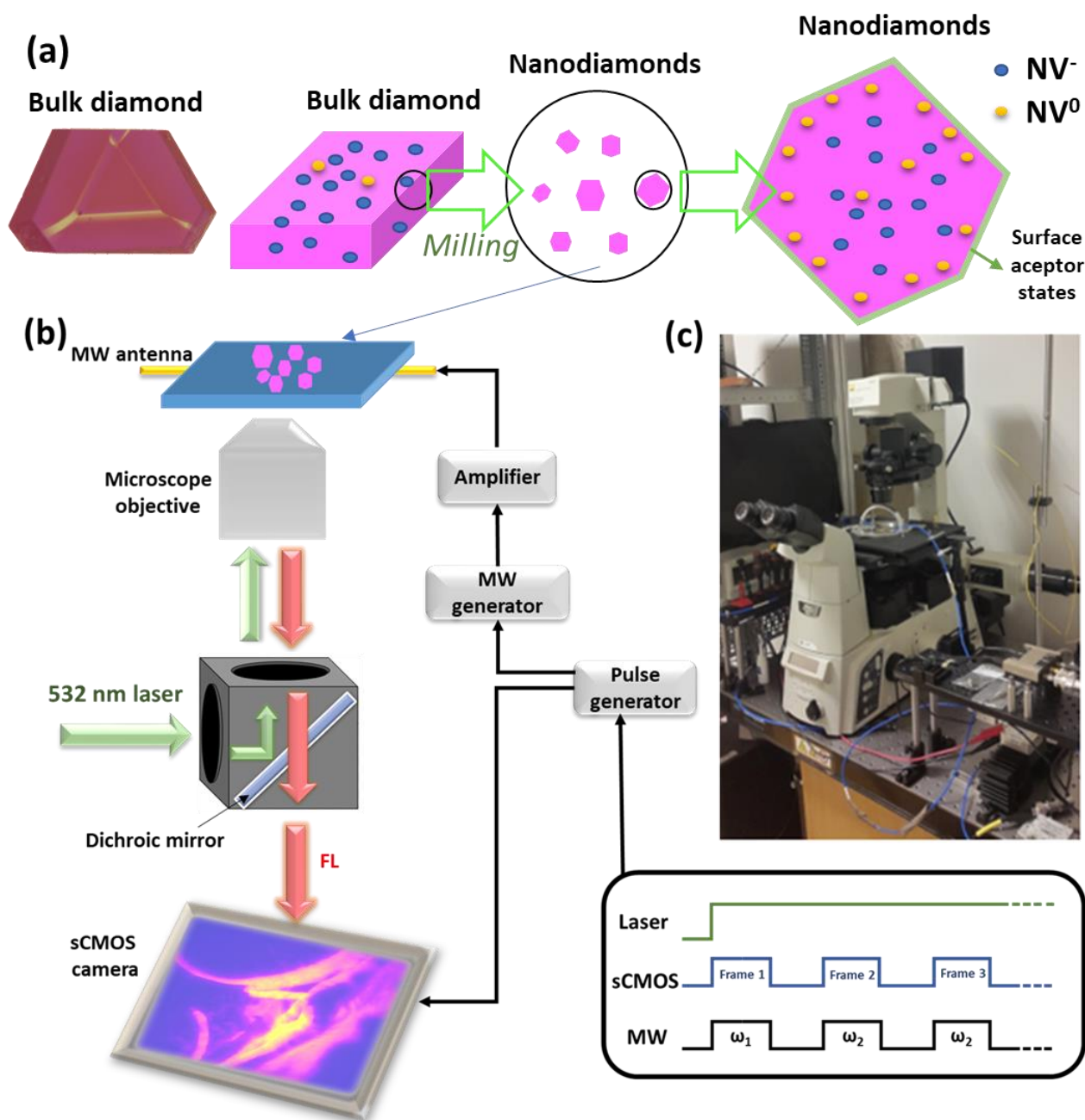


Figure 3.1. | Preparation of NDs and schematic of the wide-field ODMR set-up.

a) ¹³C-enriched fluorescent HPHT bulk diamond was milled to get two different sizes of NDs, 156 nm and 48 nm. *b)* Schematics of the inverted wide-field ODMR microscope. 532 nm green laser light is reflected onto the diamond sample. The fluorescence (FL) is collected by the objective through a dichroic mirror to the high-sensitivity CMOS camera. The image shows a current FL image of NDs deposited on a glass slide. MW delivery is obtained with an MW loop antenna. The panel shows the temporal diagram of the CW-ODMR experiment. The laser is kept ON during the whole measurement, while the camera detects the FL image synchronously with the MW irradiation. *c)* Photography of the Wide-field ODMR microscope set-up.

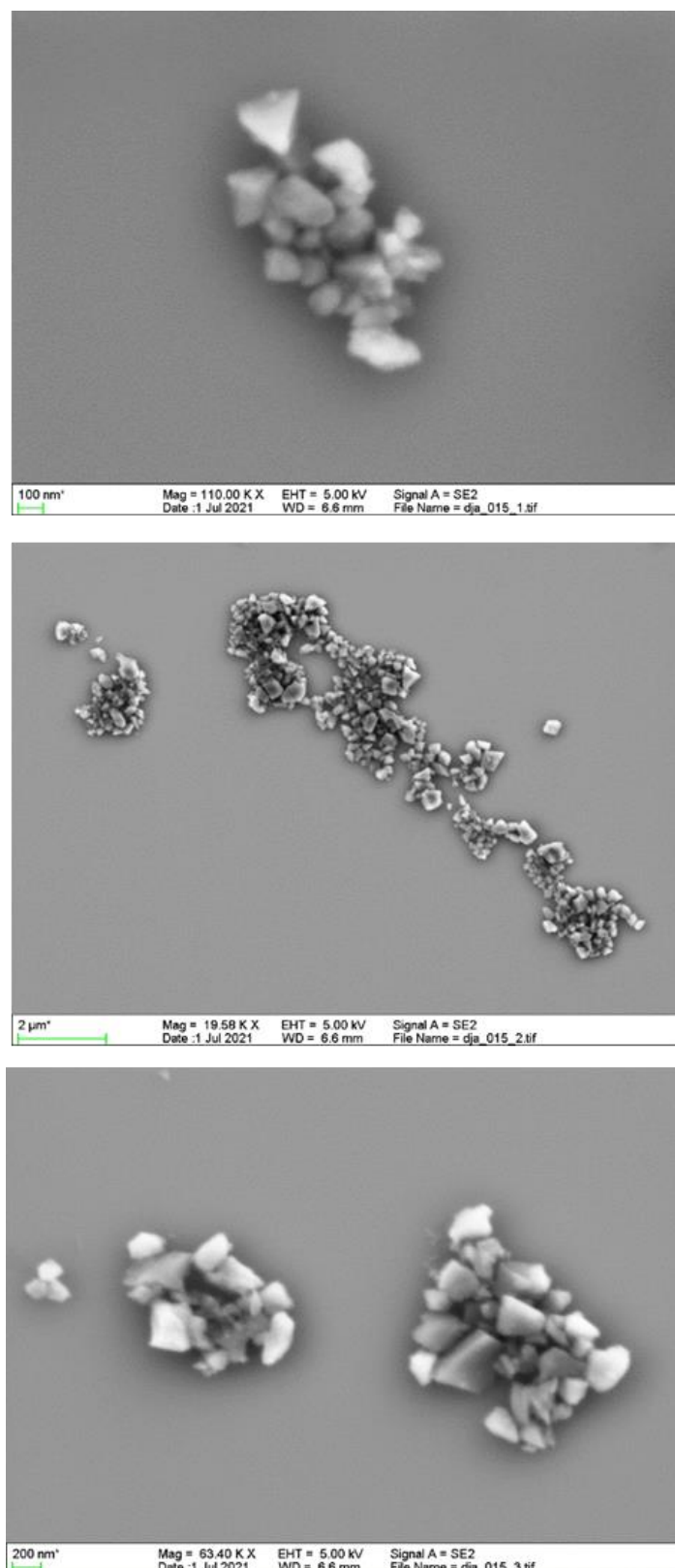


Figure 3.2. | SEM images of milled fluorescent ND before size separation by centrifugation and at different magnifications.

The samples were prepared by drop-coating on silicon substrate with an aqueous solution of the milled ND. Scale bars: 100 nm (first image), 2 μm (second image), 200 nm (third image).

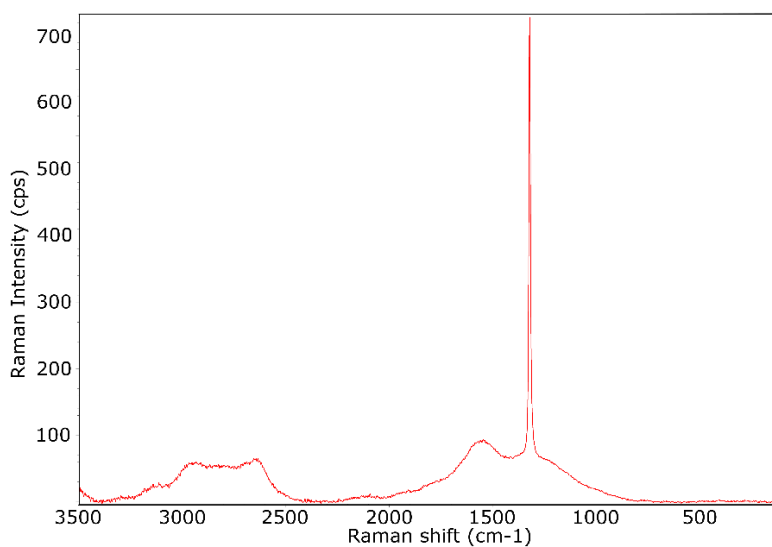


Figure 3.3. | Raman spectrum of milled NDs before size separation by centrifugation using laser excitation at a wavelength of 445 nm.

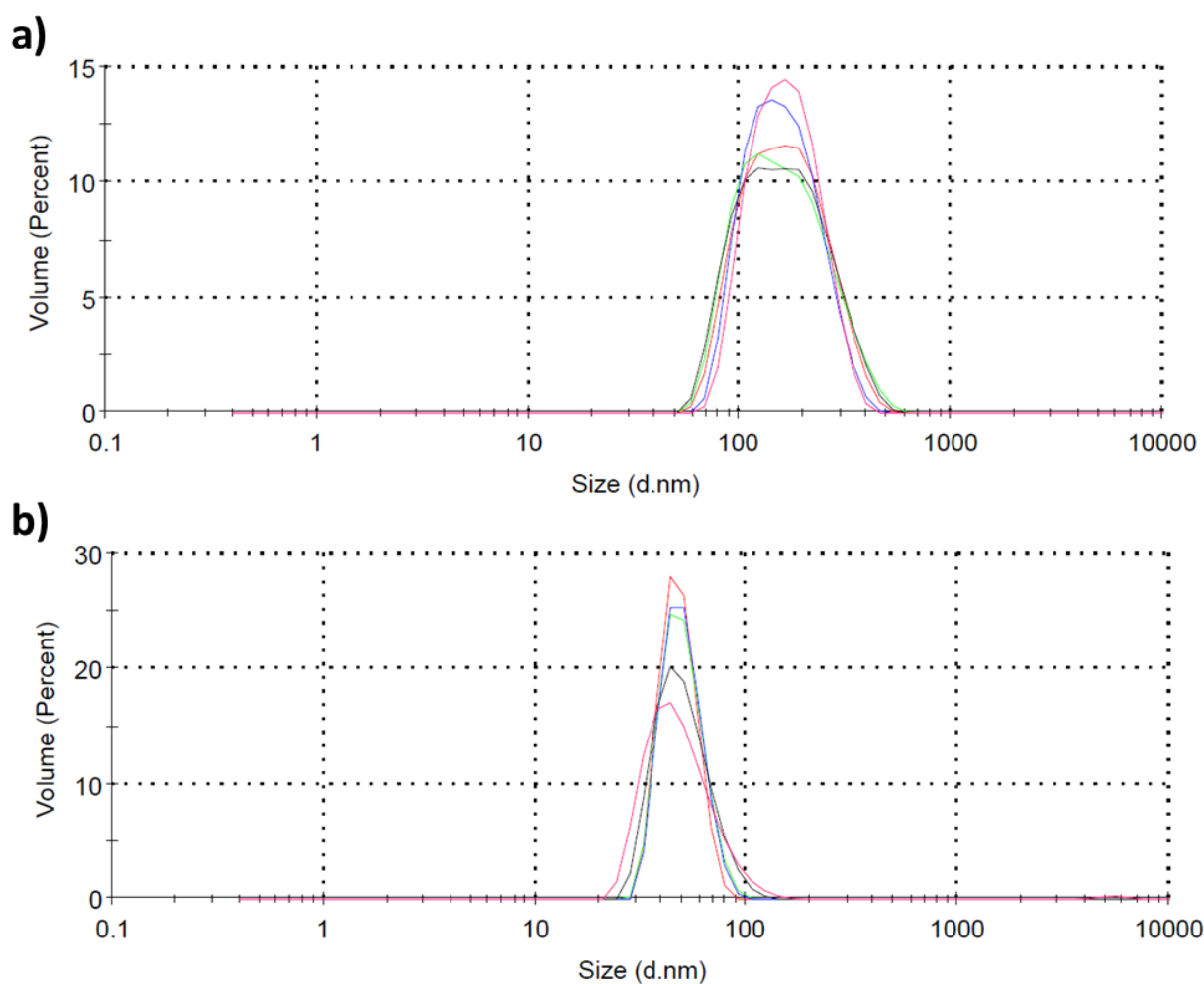


Figure 3.4. | Dynamic light scattering (DLS) of NDs.

DLS of the colloidal fraction of milled NDs centrifuged at a) 3000 rpm and b) 15000 rpm for 1 hour. The D50 value of the volume size distribution is a) 156 nm and b) 48 nm, respectively.

Cell line: Murine macrophage cells

Murine (RAW 264.7) cell line was acquired from American Type Culture Collection (ATCC LGC Standards, Sesto San Giovanni, Italy) and cultured in DMEM supplemented with 10% (v/v) of FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO₂.

In vitro nanodiamonds (NDs) uptake experiments

For uptake experiments, RAW 264.7 cells were seeded in an Ibidi at a density of 3×10^4 cells/ well and incubated at 37° C for 24 h, to allow them to adhere to the slide surface. The incubation of NDs in cells (both sizes 156 nm and 48 nm) was done for 24 h at 37 °C in a humidified atmosphere with 5% CO₂. At the end of the ND incubation, cells were washed 3 times with PBS and fixed in 4% PAF at room temperature for 15 minutes.

3.3. Methods

Confocal microscopy

Cells were rinsed twice with phosphate-buffered saline (PBS) and permeabilized with 0.1 % Triton in PBS for 10 minutes. Actin filaments were stained with phalloidin fluorescein isothiocyanate (FITC) (Sigma) for 30 minutes at room temperature. After washing twice with PBS, cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Coverslips were mounted with a glycerol/water solution (1/1, v/v). Observations were conducted under confocal microscopy (Leica TCS SP5 imaging system) equipped with an argon-ion and a 561 nm DPSS laser [Fig. 3.5]. Cells were imaged using an HCX PL APO 63×/1.4 NA oil immersion objective. NDs were excited by 561 nm laser, while the emission was collected in the 570–760 nm spectral range. Phalloidin was imaged using a 458 nm laser and the emission was collected in the 498–560 nm range. DAPI was imaged using a 405 nm laser and the emission was collected in the 415–498 nm range. Image analysis was performed using ImageJ software.

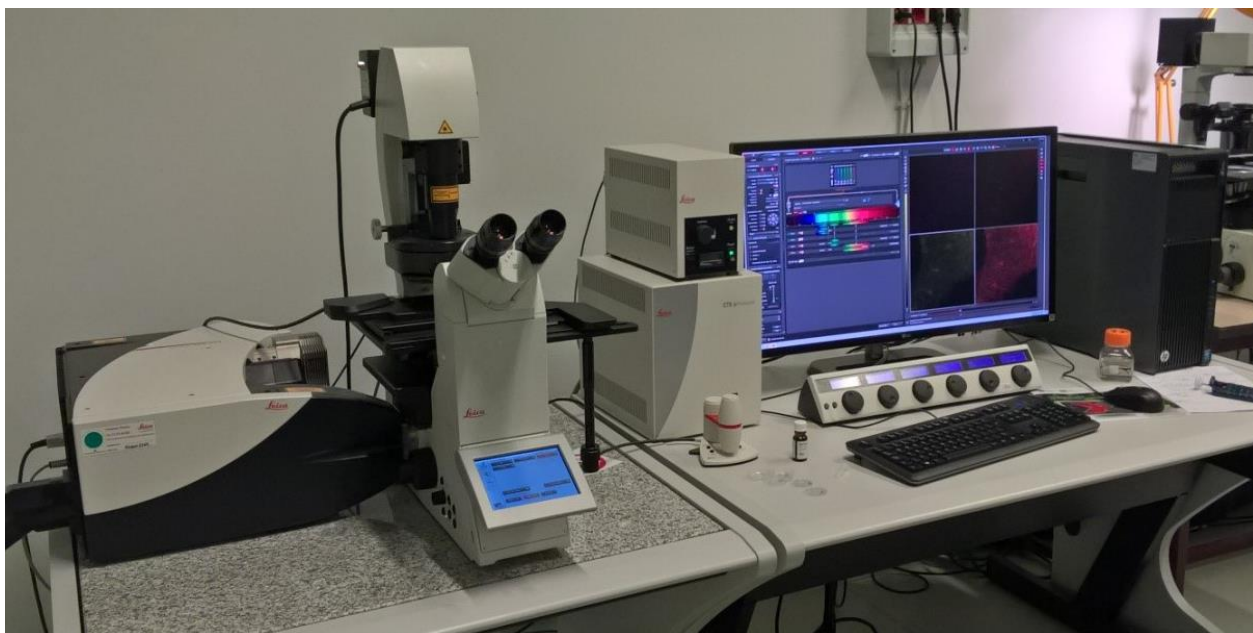


Figure 3.5. | Confocal microscope (Leica TCS SP5 imaging system) used in this work.

Wide-field ODMR microscope

Wide-field ODMR imaging was accomplished with a modified Nikon Ti-E inverted wide-field microscope [Fig. 3.1 b,c and Fig. 3.6 a,c] provided with a high-sensitivity CMOS camera (Hamamatsu ORCA-Flash4.0 V2) and a microwave channel source [25]. A 532 nm continuous-wave laser (Model: CNI laser mod. MGL-III-532/50mW) delivered on the sample a laser power of ~30 mW via a 40X (working distance (WD) of 0.66 mm and NA=0.75) refractive objective.

By inserting neutral density filters on the laser path, the laser power could be adjusted. We utilized a custom-made dichroic beam splitter, to prevent backscattering of laser light. FL from NV⁻ and NV⁰ contribution signals are collected in a spectral window ranging from 590 to 800 nm.

The microwave (MW) field source was a WindFreak MW generator (SynthHD v1.4 54MHz-13.6GHz) and the signal was amplified by a Mini-Circuits ZVE-3W-83+ 2W RF amplifier. The MW irradiation was delivered to the sample with an MW single loop antenna terminated with a high-power MW damper.

The temporal sequence of the experiment, both the camera acquisition and RF delivery, was controlled by a SpinCore 100 MHz TTL generator (Model: TTL: PB12-100-4K). Finally, the image acquisition was processed with the Nikon NIS-Elements Advanced Research software and analyzed with Fiji software.

The home-built instrument used for these experiments makes it possible to extract ODMR spectra pixelwise with sub-micrometric spatial resolution, thus enabling the analysis of heterogeneously distributed samples. Indeed, the deposition procedure can result in a non-uniform distribution of NDs, with region-dependent concentration and size of aggregates. NDs uptake from cells is also inhomogeneous, and cells with varying amounts and clustering of NDs inside were observed.

ODMR technique description

This modified wide-field microscope was used to perform spatially-resolved Continuous Wave ODMR (CW-ODMR), a technique to determine the sublevel structure of the ground state. Different laser power levels in the range of 1-30 mW are used for this experiment. MW frequency is swept in the 2.75-3.00 GHz region at a fixed power output of 15 dBm to detect ¹³C sidebands and NV⁻ central resonances. The sequence of laser and MW irradiation to perform the ODMR experiments is described in the box of Fig. 3.1b.

Full FL spectra of the NVs were acquired with a confocal microRaman setup (LabRam Aramis, Jobin-Yvon Horiba) (already described in chapter 2, section 2.2).

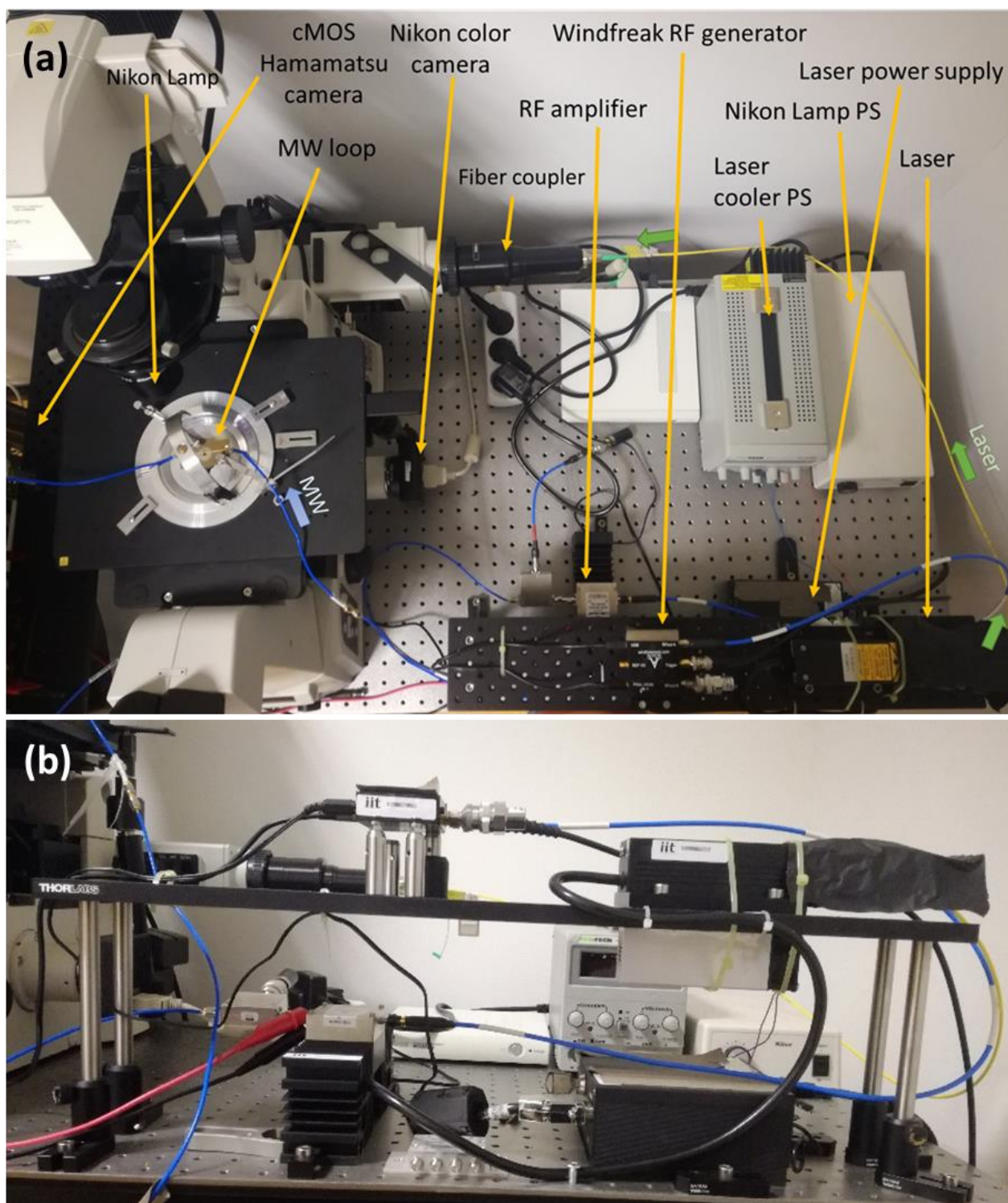


Figure 3.6. | Photos of the modified inverted wide-field ODMR microscope where the different parts are indicated a) from above and b) from the side.

The green arrows indicate the direction of the 532 nm laser beam, which passes through the fiber until it reaches the MW loop and irradiates the diamond sample from below using different microscope objectives. The blue arrow points the direction of the MWs along the blue wire from the Windfreak RF generator through the RF amplifier to the MW loop and ends at the MW dumper. The Hamamatsu camera was used in the experiments. PS ~ Power supply

3.4. Results and discussion

Fluorescence (FL) spectra from the bulk diamond, the 156 nm, and the 48 nm NDs are shown in Fig. 3.7 a,b,c. For all samples, we can distinguish the zero-phonon lines (ZPL) peaked at 575 nm for the NV^0 and 638 nm for the NV^- , and a phonon sideband around 620 nm for the NV^0 and 700 nm for the NV^- , extending up to ≈ 850 nm in both charge states. FL spectra are normalized to the NV^- ZPL for comparison. Importantly, FL spectra from NDs show a much larger component from the NV^0 region with respect to the bulk diamond (depicted as black and red curves in the spectra, respectively). This means that surface effects present on the NDs favor the NV^0 centers [19,26]. Specifically, NDs have a higher concentration of surface effects due to the nanomilling, thus increasing the number of NV^0 centers.

The ODMR spectra of the bulk diamond and NDs at four different laser powers (1, 3.6, and 8.1 and 30.5 mW) at fixed MW power of 15 dBm perform with the wide-field microscope are shown in Fig. 3.7 d,e,f. We can differentiate the NV^- central lines (A_L and A_R) and two ^{13}C side bands (B_L and B_R). The ^{13}C bands are related to the hyperfine interaction between the NV^- spins and the ^{13}C nuclear spins and are separated from each other by ~ 130 MHz [11,27–30]. For all samples, increasing laser power at fixed MW power (+15 dBm), the linewidth of the ODMR resonance bands decreases [31], and the signal-to-noise (SNR) ratio increases, thus improving the resolution of the NV^- strain-split doublet (central lines A_L and A_R). Any detectable temperature effect on the position of the ODMR resonance that could be caused by MW absorption or laser power was not detected [32] because the position of the resonance bands remained fixed.

In bulk diamond, the ODMR contrast increases with laser power, conversely, in both NDs sizes, the ODMR contrast increases until 8,1 mW and decreases onwards [Fig. 3.7 d,e,f]. The bulk presents a monotonic increase in ODMR contrast reflecting an increase of the NV^- polarization with laser power. Conversely, NDs exhibit a non-monotonic behavior. At the highest laser power, the ODMR contrast decrease, and therefore, the NV^- polarization is reduced. This interesting effect due to the higher contributions of NV^0 centers in the NDs [Fig. 3.7 b,c], was systematically observed for both ND sizes in different ROIs and sample regions [Fig.3.8 b,c].

The leakage of the NV^0 FL component in the NV^- FL region can reduce the ODMR contrast [see Chapter 1, section 1.3.]. This NV^0 reduction factor of the ODMR contrast is larger in NDs than in the bulk. Most of the NV centers are negative in the bulk and under stronger laser irradiation these NVs remain like NV^- . This results in a higher NV^- spin initialization and thus, improving ODMR contrast with a NV^0 FL contribution (I_0) practically negligible.

Contrarily, NDs have a higher relative number of NV^0 centers (Fig. 3.7 a,b,c). Accordingly, $NV^- \rightarrow NV^0$ photoconversion may be more efficient in NDs under strong laser irradiation, owing to surface effects that can trap a photoexcited electron from NV^- . Consequently, this increases the NV^0 concentration and produces a reduction in the ODMR contrast that competes with NV^- polarization and reflecting, a non-monotonic behavior [Fig. 3.8 b,c].

In principle, the larger surface area of NDs favors exposure of shallow NV^- centers to the external environment, with potential advantages for sensing and polarization transfer applications. However, surface effects can also have detrimental effects, as they favor depletion of the NV^- pool through

photoconversion to NV^0 ($NV^- \rightarrow NV^0$) and set a limit to the laser power that can be used to prepare the NV^- spin states. Additional chemical treatment such as fluorination or selective oxidation (see chapter 1, section 1.4) of the ND surface may stabilize NV^- centers and improve ODMR contrast at higher laser powers.

It should be mentioned that the microwave field cannot compete in inverting the population levels in the NV ground state when the laser power is extremely high. This could lead to some degree of laser-induced repolarization resulting in the saturation and in an ODMR contrast reduction. Here, the composition of the NDs and the bulk diamond is the same and both were irradiated with the same laser power density and MW power conditions, therefore, by exclusion, the reduction in ODMR contrast observed in NDs is due to photoconversion ($NV^- \rightarrow NV^0$).

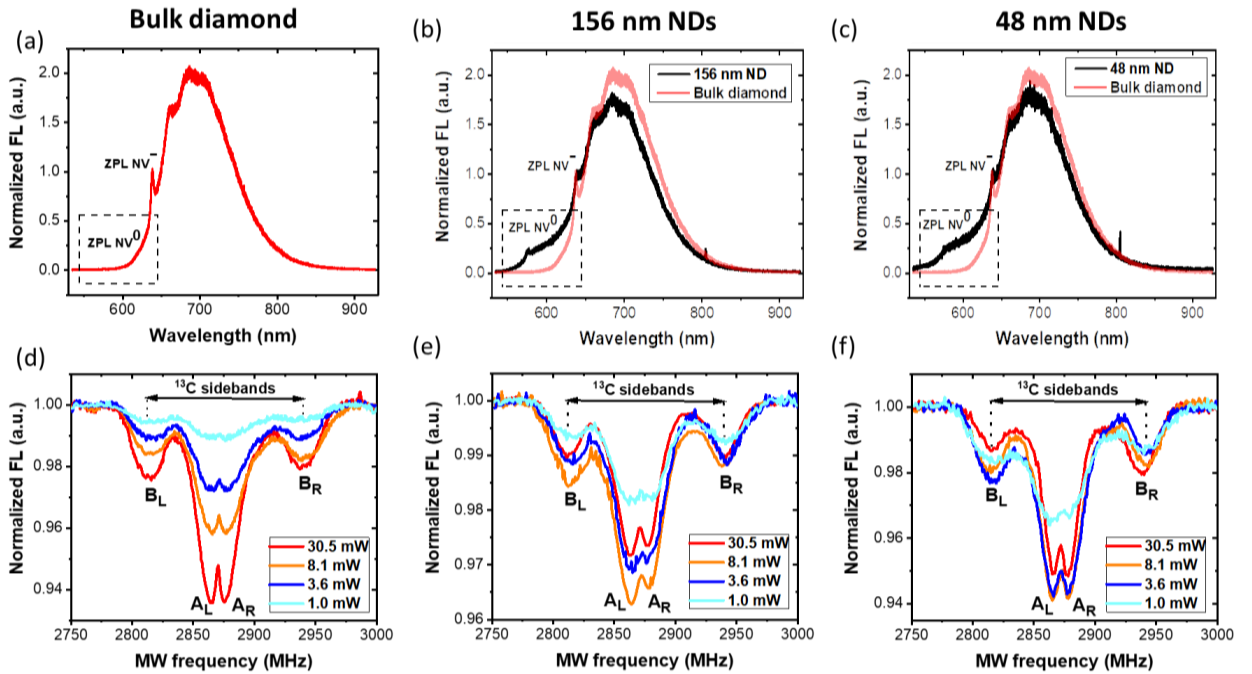


Figure 3.7. | Fluorescence and ODMR spectra.

Fluorescence (FL) and ODMR spectra for the (a,d) bulk diamond, (b,e) 156 nm and (c,f) 59 nm NDs, respectively. (a,b,c) Both NV^0 and NV^- FL spectra are characterized by ZPL lines at 575 nm (NV^0) and 638 nm (NV^-). NDs have a larger NV^0 component than bulk diamond due to ND surface effects that favor NV^0 centers. In the bulk, the NV^0 band is practically undetectable. (d,e,f) ODMR spectra at different laser power with fixed MW power. In the ODMR spectra, the NV^- central lines (A_L and A_R) provide a measure of the spin polarization of the $|g, m_s = 0\rangle$ ground state. The MW range was set to 2.75-3 GHz to show the NV^- central lines and the ^{13}C sidebands (B_L and B_R). In bulk diamond, ODMR contrast increases with increasing laser power. Conversely, in NDs, the ODMR contrast increases up to 8.1 mW and then, decreases at higher laser powers.

Generally, NDs have a heterogeneous optical response [Fig. 3.8 b,c] unlike the bulk that is homogeneous [Fig. 3.8 a]. This heterogeneous behavior from NDs in aggregation and concentration has been attributed to ND size distribution, different NV initialization efficiency in ND aggregates,

and inter-aggregate ND interactions [see appendix]. To avoid difficulties, we used a wide-field ODMR microscope to study different ROIs both bulk and NDs. [16,33–35]. The different ROIs are characterized by FL levels [Inset Fig. 3.8] and ODMR contrast. The 156 nm NDs presented a lower variability than 48 nm NDs. Despite the ND region and ROI, the ODMR contrast curve exhibited the same trend. Contrarily, the bulk diamond showed a contrast curve uniform in different ROIs throughout the whole sample.

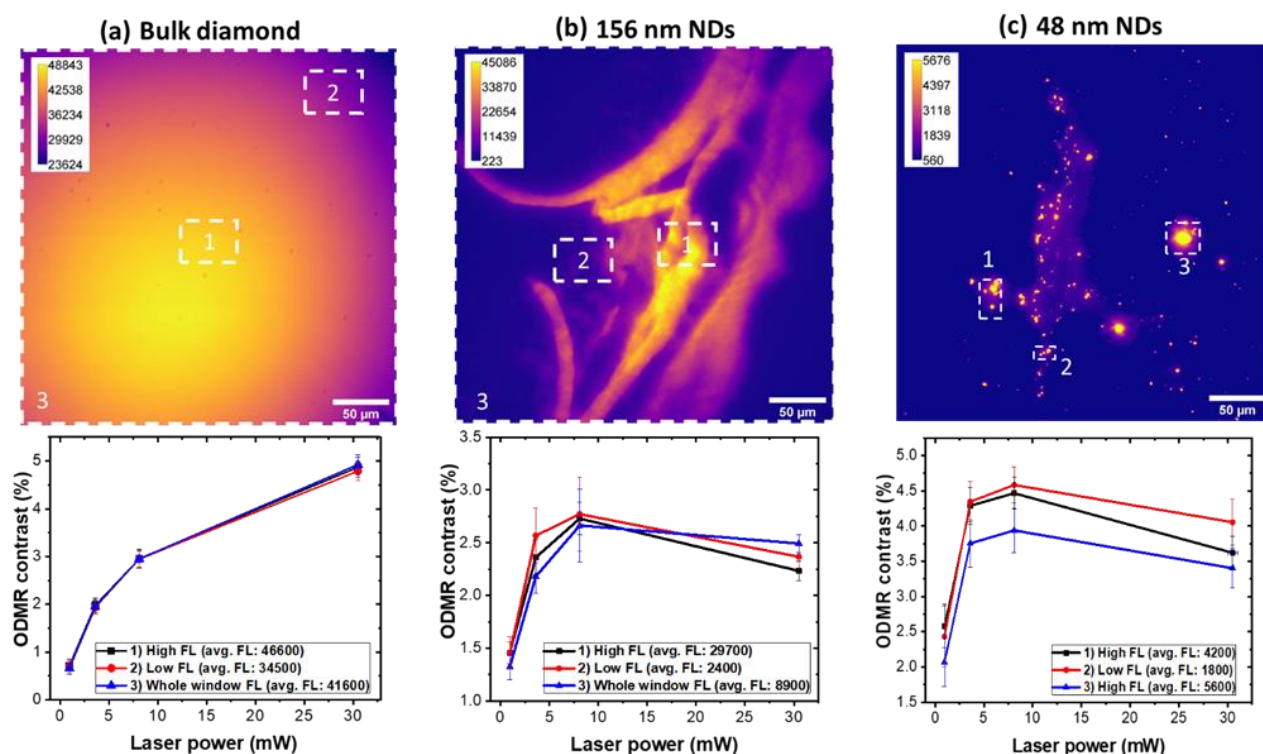


Figure 3.8. | Comparison of ODMR contrast at different laser powers for bulk diamond and NDs.

ODMR curves are extracted from three different regions of interest (ROIs) containing the bright spots that indicate the presence of NV centers (see wide-field images). As laser power increases, in bulk diamond, the ODMR contrast increases, consistently with larger NV⁻ polarization levels (a). Conversely, in NDs of both sizes, the ODMR contrast increases up to 8.1 mW and decreases at higher laser powers, resulting in a non-monotonic behavior (b,c). The homogeneity of the bulk diamond is reflected in the uniform and constant values of ODMR contrast are shown in the various ROIs with different FL levels (inset). In contrast, NDs show non-uniform aggregation and deposition, resulting in a region dependent ODMR contrast. 156 nm NDs were less inhomogeneous than the 48 nm NDs. The laser power dependence of the ODMR signal was similar in all the ROIs extracted, for both 156 nm and 48 nm NDs sizes.

Spin and charge dynamics of NV centers vary depending on the ND microenvironment. Therefore, we internalized both 156 nm and 48 nm NDs in macrophage (RAW 264.7) cells to study the above effects (see Methods) [Fig. 3.9]. The confocal images displayed the NDs (orange), incubated in the macrophages, cell nuclei (blue), and the actine filaments from the cytoplasm (green). After the ND incubation in cells, the sample was subject to a rinsing method to remove the non-internalized NDs outside the cell. The concentration of the 156 nm NDs in suspension is larger than the 48 nm NDs, resulting in a greater internalization of the larger NDs. Thus, only the results of 156 nm NDs are reported in the following section.

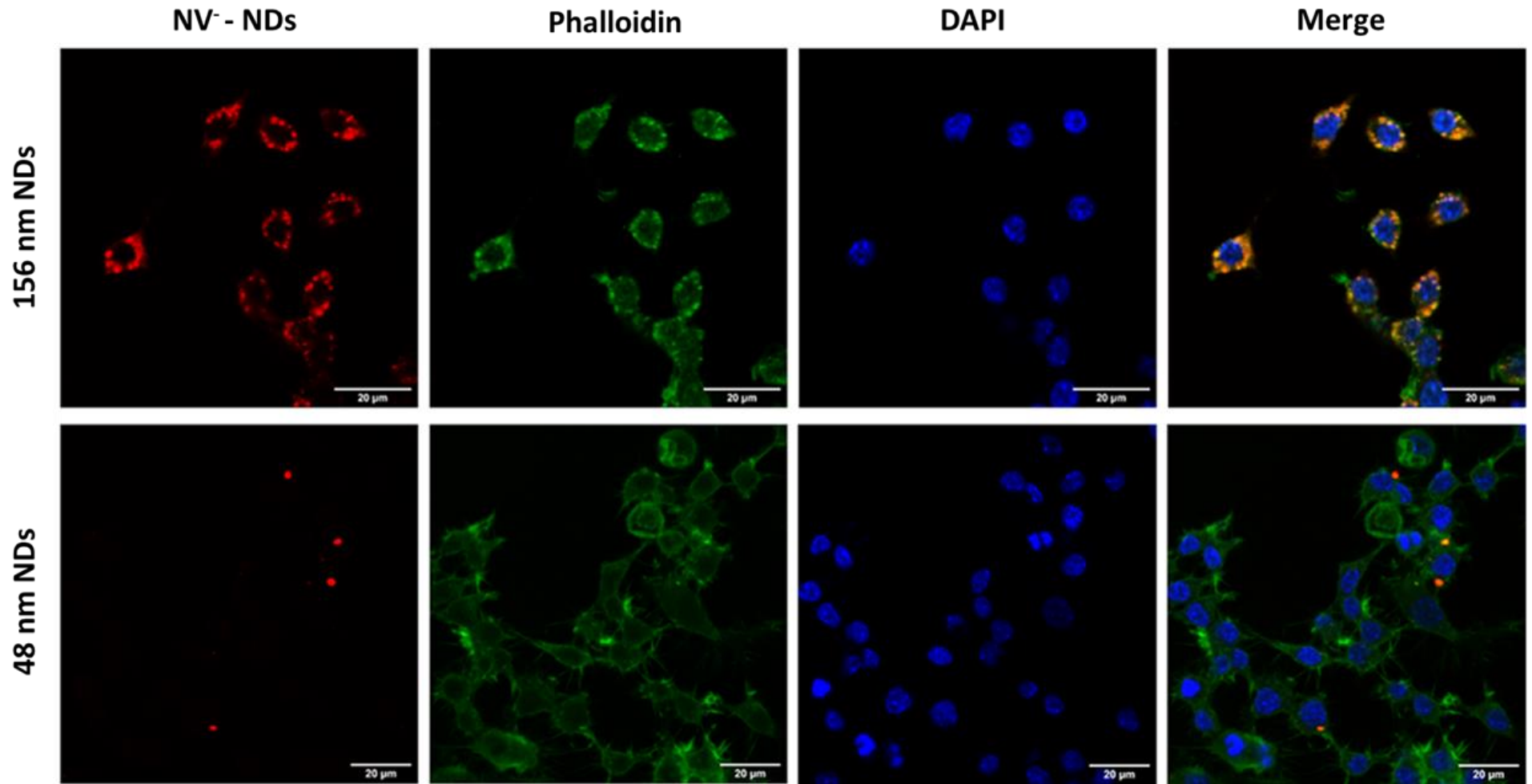


Figure 3.9. | Confocal Microscopy images of RAW 264.7 macrophage cells incubated during 24 h with 156 nm and 48 nm NDs (red) and stained with Phalloidin (green).

Cell nuclei were counterstained with DAPI (blue). The merge figure demonstrates the incubation of the NDs (orange) within the cells. Cellular uptake of the 156 nm NDs is much higher than that of the 48 nm NDs. Scale bars= 20 μ m.

FL images were taken to evaluate the different amount of internalization of NDs in different cells. To this end, we extracted the ODMR contrast from ROIs of different sizes and locations [Fig. 3.10]. ODMRs curves were obtained from single-cell ROIs ($\sim 6 \times 6 \mu\text{m}$) [Fig. 3.10 b] or a cell-aggregates ROI (from $\sim 40 \times 40 \mu\text{m}$ to $\sim 100 \times 100 \mu\text{m}$) [Fig. 5c]. Comparing with the non-internalized NDs [Fig. 3.8 d,e,f] it is still possible to resolve the NV^- central lines and ^{13}C sidebands, despite the line broadening in the resonance bands. Also in this case, as we increase the laser power, the linewidth of the resonances bands decreases whereas the SNR increase, thus augmenting the resolution of the NV^- strain split doublet (central lines A_L and A_R). We also do not observe any temperature-related effect like a shift of NV^- central lines, despite a possible MW absorption from the water in the cells or in the biological environment.

ODMR contrast curves at different lasers are shown in Fig. 3.10 d. The ODMR trend is qualitatively similar to the bare NDs Fig. 3.8 b and 3.8 c, and at the highest laser power, we can observe a wider variety on the contrast evolution such as a plateau-like behavior, a slight increase, or a small reduction. To explain these results, we hypothesize that this variety may be due to different pH values at the cellular compartments, for instance, the cytoplasm has a $\text{pH} \approx 7.2$ and the lysosomes a $\text{pH} \approx 5$. According to previous works, pH can affect the functional groups of the ND oxidized surface such as esters, ketones, carboxylic acids, alcohols, etcetera. which can change the NV shallow stability. At $\text{pH} \approx 7.2$, carboxylic acids can be protonated more than under physiological conditions as a result, this may modify the charge state of nearby NVs. However, it is necessary further investigation to prove this hypothesis.

We also studied the heterogeneity of the optical properties of NDs internalized in a cell in greater detail [Fig. 3.10 e,f], with the help of a simple faster procedure. Here, we acquired two FL images, with MW on- and off-resonance and we take the difference between both images. Subsequently, we normalize pixelwise the resulting image to make an ODMR contrast mapping [Fig. 3.10 g] where an average 4.5 % ODMR contrast is calculated. Parts with a low (blue) and high (red) ODMR contrast can also be calculated from ND inside the cell. NDs cannot access easily to the cell compartments (e.g. nuclei), for that, the ODMR contrast is low. We selected a 1% contrast threshold in the ODMR mapping to minimize the level of noise fluctuations that could affect the ODMR signal since this mapping is based on the ratio between FL levels and not on the absolute FL values.

The ODMR mapping paves the way to sense the temperature [3,36] and magnetic fields inside cells. Specifically, the ODMR contrast mapping can be useful for magnetic sensing applications [5,9,37]. For example, an increase in the ODMR contrast improves sensitivity and the minimum detectable magnetic field. Moreover, the contrast mapping can detect the presence of paramagnetic agents inside cells because of fluctuating magnetic noise in the gigahertz range [20,38].

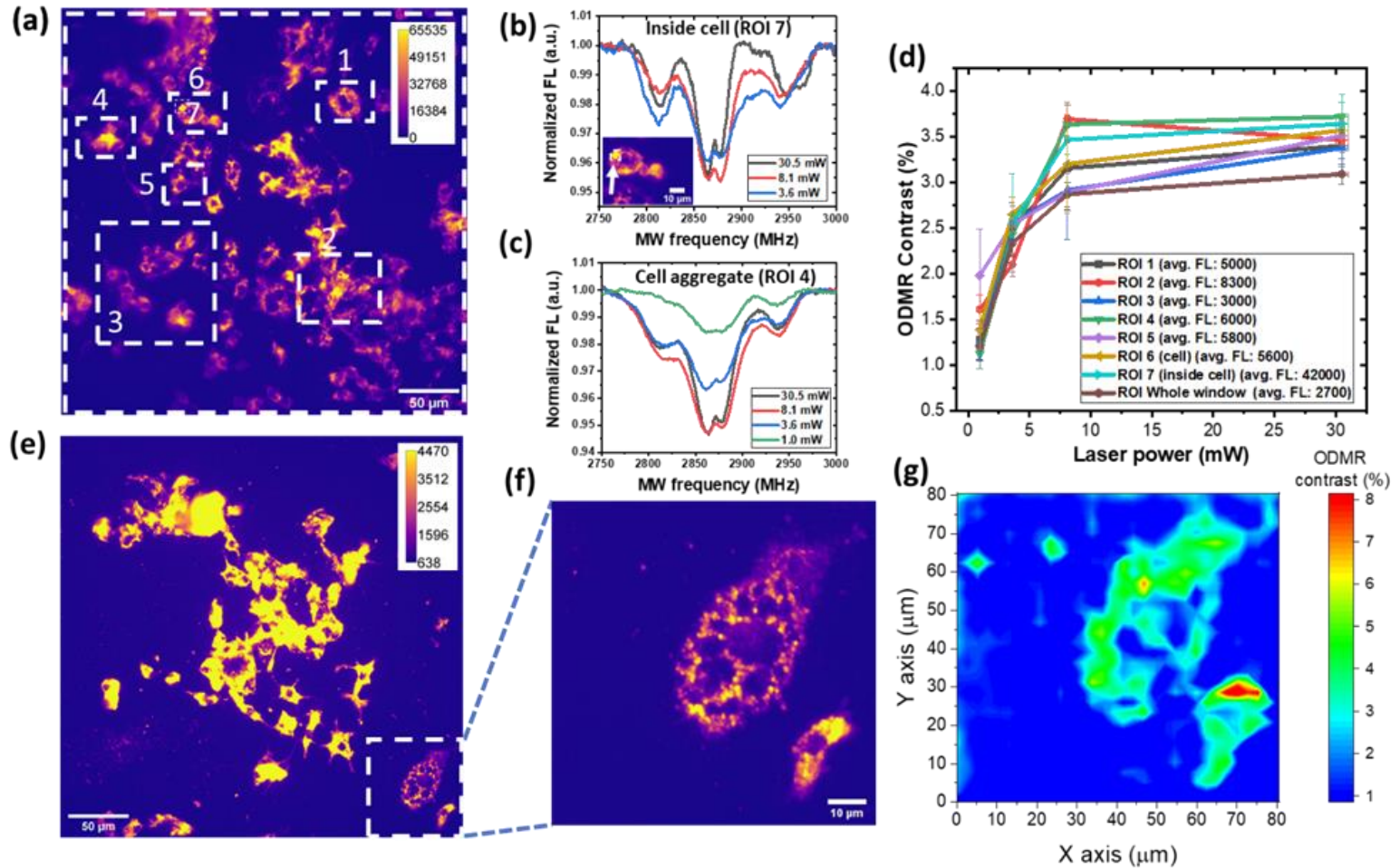


Figure 3.10. | ODMR contrast comparison of NDs internalized in cells.

(a) FL image of 156 nm NDs internalized in macrophage cells. ODMR spectra showing the NV⁻ central lines (A_L and A_R) and the ^{13}C sidebands (B_L and B_R) at different laser power with fixed MW power inside a cell are shown in (b), and in (c) for a cellular aggregate. (d) ODMR curves from different ROIs of various FL intensities. ODMR contrast steadily increases to 8.1 mW, while at higher laser powers it manifests a diversity of contrast behaviors. Like the NDs outside cells, NDs incubated within cells also show inhomogeneous aggregation and concentration, reflecting in a region dependent different ODMR contrast. (e) FL image of 156 nm NDs incubated in cells. (f) ROI that shows a cell with its nucleus and various cell compartments (g) ODMR contrast mapping within the cells. ODMR contrast differs depending on the cell zone under study, with an average contrast value of ~4.5%.

Heterogeneity of nanodiamonds

The variation of ODMR contrast values observed in distinct aggregates of NDs might be due to their disparate NV⁻ fraction (NV⁻/NV⁰ ratio). Moreover, there might be other different factors that could explain this phenomenon, such as those reported in individual ND particles [33,34]:

1. Creation of ND aggregates. When NDs are deposited and dried on the glass circular slide, NDs tend to create aggregates of different sizes, shapes with rounded or sharp edges, elongation, and surface that show different values of FL and ODMR contrast (see wide-field images of Fig. 3.8). It has been reported by A. Khalid et al. [39] [REF] that by diminishing the diamond size, the excited state lifetime increases, thus, lessening the FL emission rate of the radiative decay. This improves the non-radiative decay by the intersystem crossing (ISC) path and increases the population of electrons in the $|g, m_s = 0\rangle$, and consequently, augment the ODMR contrast.

On the other hand, detonation NDs (particles diameter $\sim 1-5$ nm) can form aggregates of ultra-small particles [40]. Resulting in a lower average refractive index than the nanocrystals, thus, they exhibit an increase in lifetime and consequently, a diminution in the ODMR contrast. In other words, ND aggregates with different sizes and refractive index can lead to various levels of ODMR contrast on the same sample. Differently, this contrast variation of the ND cluster might be also due to the intrinsic damage suffered by the diamond lattice during the nano-milling process.

2. Surface interaction between ND clusters. The oxidation of the ND surface can lead to oxygenated functional groups [41,42] (alcohols, ketones, esters, etcetera.) interact with shallow NV centers separated by less than 10 nm from the surface, thus varying their FL properties. Furthermore, functional groups from the clusters might interact with each other varying the NV spin properties and consequently, the ODMR contrast values.

3. ND size. The reduction of the diamond size decreases the number of NV⁻ and P1 centers and augments the primary defects (mostly negative vacancies V⁻ and P1 centers, as well as a small amount of dangling bonds) diminishing their FL brightness [16]. The FL brightness and the ODMR contrast are not entirely correlated. Different FL values are observed depending on the ND cluster under study within the same image. For example, 156 nm NDs manifest a larger average FL than 48 nm NDs, but this does not mean that the contrast ODMR values should be higher in the 156 nm NDs.

To summarize, within the same ND sample, we can find different size aggregates of NDs. For instance, bigger aggregates would have a larger concentration of NV⁻ and P1 centers and fewer primary defects. In addition to this factor, the functional groups of each cluster might interact with each other by changing the stability of shallow NVs and reflecting disparate ODMR contrast values and FL brightness. Despite these factors, in NDs, a similar ODMR trend with laser power was observed in all ROIs regardless of their heterogeneity.

3.5. Summary points of chapter 3

1. Different dependencies of ODMR contrast on laser power are observed in bulk diamond and in NDs obtained by nanomilling of the bulk material [Fig. 3.1 a].
2. In bulk, the ODMR contrast increases with laser power [Fig. 3.8 a]; conversely, in NDs, the ODMR contrast increases until 8,1 mW and decreases at stronger powers [Fig. 3.8 b,c].
3. This surprising ODMR contrast reduction may be to a higher relative number of NV^0 centers in NDs than the bulk [Fig. 3.7 a,b,c]. In NDs, $NV^- \rightarrow NV^0$ photoconversion may be more efficient under strong laser irradiation, owing to surface effects, that can trap a photoexcited electron from NV^- , increasing the number of NV^0 s.
4. NDs have a heterogeneous optical response [Fig. 3.8 b,c], unlike the bulk that is homogeneous [Fig. 3.8 a]. To address this problem of inhomogeneity in NDs, we have used a modified ODMR wide-field microscope [Fig. 3.1 b,c].
5. NDs internalized in cells [Fig 3.9 and Fig 3.10 a,e,f] showed a qualitatively similar ODMR contrast trend [Fig 3.10 d] as in the bare NDs [Fig 3.8 b,c]. At the highest laser power, ODMR contrast values were more variable suggesting that the different pH cellular environment or ND surface interactions may have a role in the dynamics of NV charges [Fig 3.10 d].
6. The effects reported here highlight a trade-off using NDs at high laser powers for polarization transfer and sensing applications since NDs have larger surface interactions or surface states that can be detrimental for NV^- charge stability.

3.6. References of chapter 3

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Chapter 4:

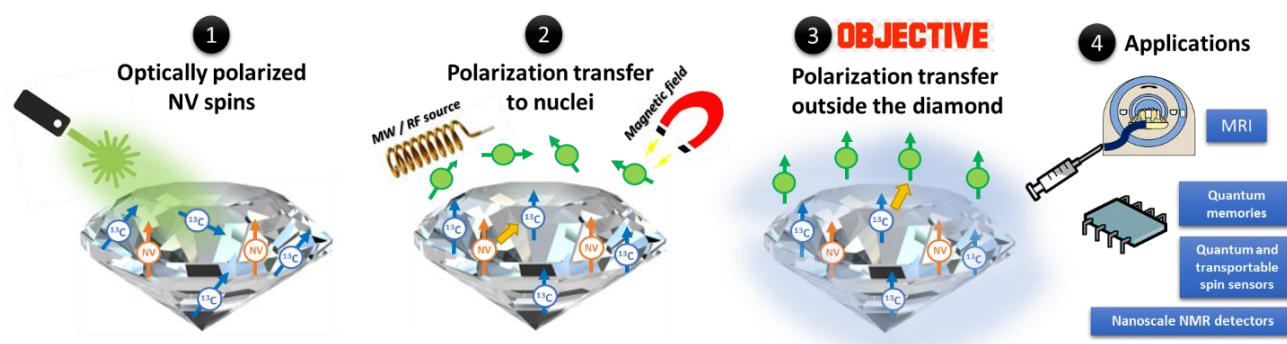
**Future prospective:
Overview of optical
nuclear polarization by
NV centers in diamond**

Chapter 4: Future prospective: Overview of optical nuclear polarization by NV centers in diamond

Chapter summary

NV centers have been used in dynamic nuclear polarization (DNP) protocols for the hyperpolarization of the ^{13}C nuclei in the diamond lattice (ref). Significant works are underway to transfer polarization from shallow NVs to molecules absorbed at the diamond surface, allowing hyperpolarization of high-sensitivity tracers for biomedical MRI. Therefore, the need to have shallow NV centers require an effective $\text{NV}^0 \rightarrow \text{NV}^-$ photoconversion and the use of specially engineered layers of NVs close to the diamond surface (see chapter 2) or the use of nanodiamonds (see chapter 3). Among all the hyperpolarization techniques, NV centers in diamond are presented as an inexpensive alternative since its spin can be optically polarized at room temperature and transfer the polarization to other nuclei using low magnetic fields and no chemical compounds. In this chapter, we describe theoretically polarization mechanisms, how to transfer the polarization within the diamond using similar samples as shown in the previous chapters, how to polarize nuclear spins of an external sample that is in contact with the diamond surface, experimental set-ups, and spin diffusion dynamics.

Keywords: hyperpolarization; nitrogen-vacancy centers; diamond; polarization; dynamic nuclear polarization; optical nuclear polarization; nanodiamonds; bulk



4.1. Introduction

Nuclear magnetic resonance (NMR) is an effective technique for the use of chemical analysis or medical diagnosis. However, the low sensitivity of NMR has limited its applications due to the low level of polarization of nuclear spins that follow a Boltzmann distribution. To improve this low signal-to-noise ratio (SNR) and increase the NMR sensitivity, it is necessary to use low temperatures (T) and high magnetic fields (B) according to the Boltzmann distribution (population difference $\propto e^{-\mu B/kT}$) which are a great impairment in NMR. Consequently, it is possible to use different mechanisms that can generate hyperpolarized nuclear systems, and improve NMR sensitivity through several orders of magnitude [1–4] for magnetic resonance imaging (MRI) applications [Fig. 4.1].

A wide variety of nuclei have already been polarized using different hyperpolarization techniques such as ^1H , ^3He , ^7Li , ^{13}C , ^{15}N , ^{19}F , ^{31}P , ^{83}Kr , and $^{129}\text{Xe}/^{131}\text{Xe}$ [2,3]. Apart from brute-force polarization (BFP) where hyperpolarization of the target sample is achieved at high magnetic fields and/or at low temperatures [5], the polarization transfer can be from electrons or molecules [Fig 4.2]. Within the methods that use molecules, quantum rotor induced polarization (QRIP) [6] and parahydrogen techniques such as Parahydrogen Induced Polarization (PHIP) [7] and signal amplification by reversible exchange (SABRE) [8] can be found. PHIP is based on the spin-conserved hydrogenation of an unsaturated substrate. PHIP has become a promising hyperpolarization technique using SABRE protocols. The disadvantages of PHIP are the presence of metal complexes as catalysts at the end of the solution and the need to de-hydrogenate the precursor of the hyperpolarized target product.

In the methods that use electrons as a source of nuclear spin order, we find spin-exchange optical pumping of noble gases (SEOP), chemically induced dynamic nuclear polarization (CIDNP), dynamic nuclear polarization (DNP), and optical nuclear polarization (ONP) [Fig. 4.2]. SEOP is based on spin-exchange collision between noble gases nuclear spins and alkali metals [9]. Noble gases such as Xe^{129} are hyperpolarized, which can be used in the study of the human lungs by MRI or porous structures. CIDNP is the nuclear spin recombination of a radical pair mediated by spin-orbit coupling [10]. In DNP, an unpaired electron transfers the polarization to nuclei via microwave irradiation close to the electron paramagnetic resonance transition frequency. DNP uses cryogenic temperatures ($\sim 1\text{K}$), high magnetic fields ($> 3\text{T}$), and microwave irradiation. Within DNP, we can find magic-angle-spinning (MAS) DNP for solid samples [11] and dissolution DNP (dDNP) for liquid samples [12]. DNP has emerged as a good solution to obtain high levels of hyperpolarization in surrounding nuclei. However, the use of preclinical, and clinical diffusion of this technique has been hampered by cost, the cryogenic temperatures, the long time needed for the polarization cycle, or the technical complexity such as a shuttling system, microwave instrumentation, or freeze-thaw cycle.

The need to find inexpensive room-temperatures hyperpolarizer platforms with low magnetic fields has promulgated the possibility of using optical nuclear polarization (ONP) with NV defect in diamonds as a new alternative [13–16]. NV enriched diamonds can be optically polarized with

the continuous irradiation of a laser at room temperature and low magnetic fields, and therefore could be used as an alternative to DNP agents for NMR/MRI applications. The NV electron spin exhibits remarkably relaxation times (T_1) and long coherence (T_2), which can reach milliseconds [17].

The use of the NV spins to polarize external nuclear spins has been done by two methods:

1. NV ensembles relay the polarization to the ^{13}C nuclei within the diamond and it is possible to detect this ^{13}C hyperpolarized NMR signal [18]. The mechanism is also known as the solid effect and it is based on Landau-Zener (LZ) transitions in which the energy levels of the NV^- electron spin are hyperfine coupled to the ^{13}C nuclear spins [19–21]. This polarization transfer can also occur through the LAC mechanism when there is an energy matching of the NV electron and nuclear spins of interest [22–25].
Next, ^{13}C nuclei are used as a relay medium as they can store polarization for long periods and can transfer it to the nuclei of the target sample. A system formed by ^{13}C and substitutional nitrogen atoms (P1 centers) has also been proposed since both defects are in a much higher concentration than NVs [26,27]. The polarization transfer from ^{13}C nuclei to external nuclear spins has not been determined yet [Fig. 4.3].
2. Ensembles or single NVs are being studied to directly polarize external nuclei. Single NVs have directly polarized small-scale external nuclear spins, unfortunately, due to the tiny, hyperpolarized volume, they cannot be used in many applications [28–36].

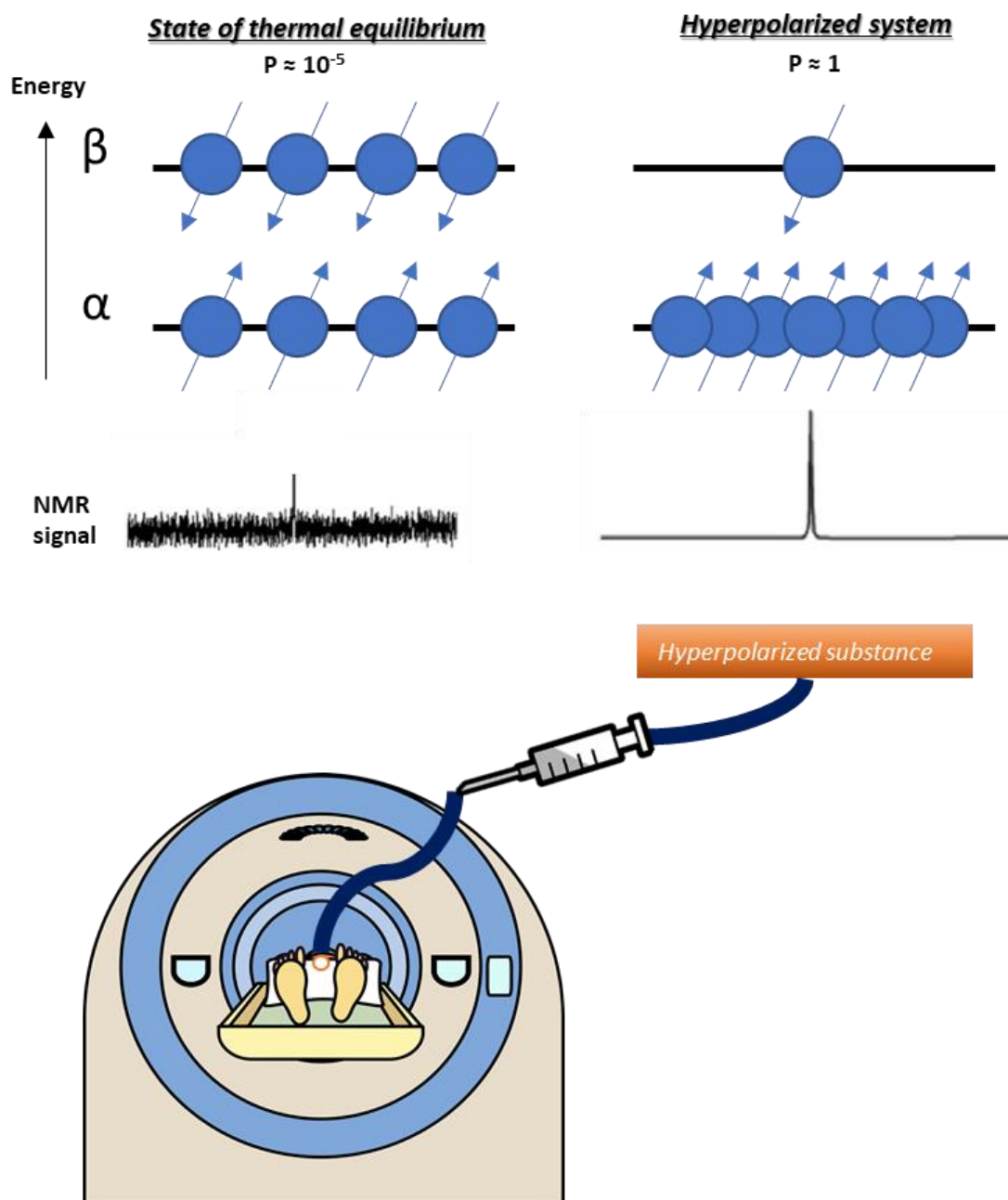


Figure 4.1 | Principle of hyperpolarization

In conventional nuclear magnetic resonance (NMR), only $1/100,000$ of the nuclear magnetic spin dipoles contribute to the signal as described by Boltzmann statistics at 3T. Using hyperpolarized systems, this fraction can be increased with a factor of $10^4 - 10^5$. The hyperpolarized substance could be injected into a patient and improve sensitivity in medical imaging applications such as MRI among many more applications.

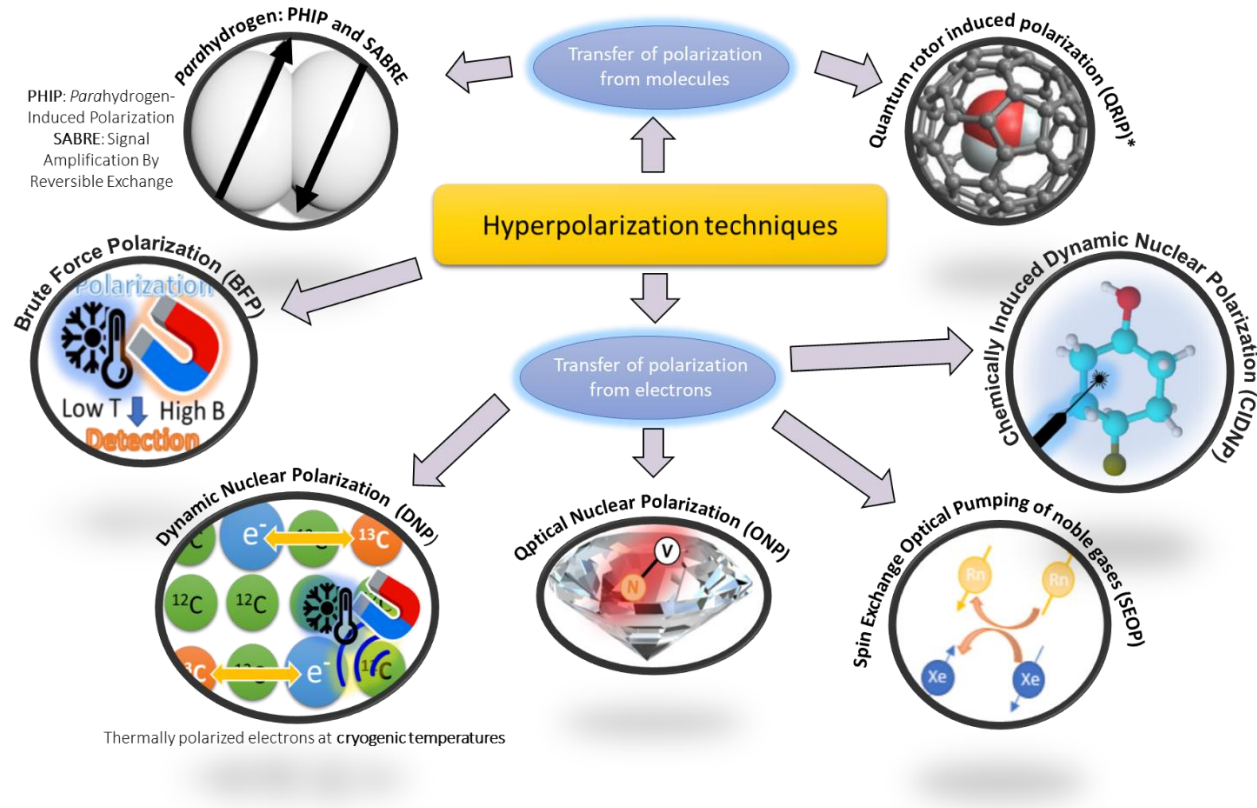


Figure 4.2 | Hyperpolarization techniques.

If the transfer of polarization is from molecules, we can find the Quantum rotor induced polarization (QRIP) and the Parahydrogen techniques such as Parahydrogen Induced Polarization (PHIP) and Signal Amplification By Reversible Exchange (SABRE). If the source of polarization is the electrons, we have Dynamic nuclear polarization (DNP) and its variations such as magic-angle-spinning (MAS) DNP and dissolution DNP (dDNP), Chemically Induced Dynamic Nuclear Polarization (CIDNP), and its variation photo-DNP. Furthermore, in this group, we find the Spin Exchange Optical Pumping of noble gases (SEOP) or the Optical Nuclear Polarization (ONP) which use NV centers. We would have the Brute Force Polarization (BFP) outside these groups.

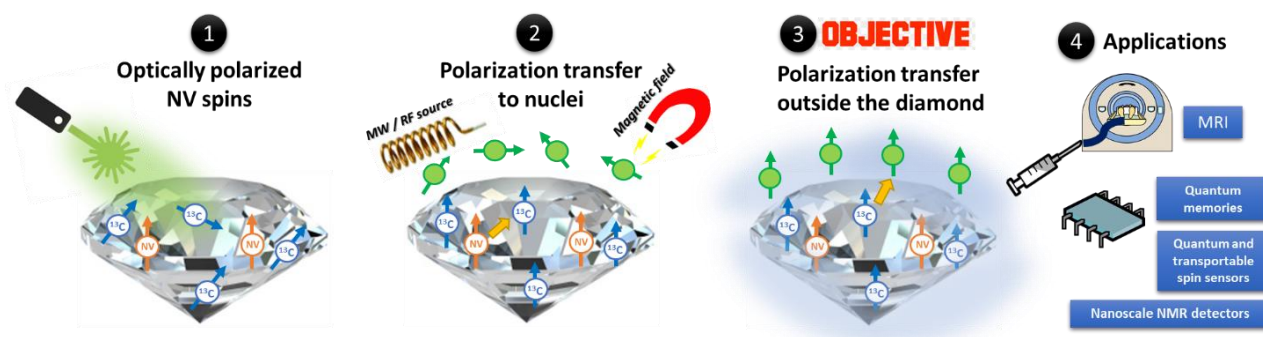


Figure 4.3 | Diagram of the transfer of spin polarization inside and outside the diamond.

First, the diamond is irradiated with a laser to polarize the NV centers. Second, a microwave or radiofrequency source and a magnetic field are used to transfer the polarization from the NV centers to the nuclei of interest, in this case, ^{13}C nuclei. Third, the objective that has not yet been achieved, is to transfer the polarization from ^{13}C nuclei to nuclei located outside the diamond corresponding to a liquid, gas, or solid sample. Later, this polarized material, if it is a liquid or a gas, can be injected into a patient to improve MRI sensitivity, or it can be used as a nanoscale NMR detector where the diamond can polarize substances to make them visible in NMR, in quantum memories at high field, can be used as a transportable spin sensor where the hyperpolarized nanodiamond is encapsulated for example in a cell or vesicle, as highly-coherent quantum sensors, etcetera. The polarization transfer is indicated by a yellow arrow in the drawing. Specifically, the transfer of spin alignment from NV to ^{13}C inside the diamond or from ^{13}C to nuclei outside the diamond has been noted. Other hyperpolarization protocols have also been designed where the ^{13}C nuclei could be replaced by the P1 centers to transfer the polarization outside.

4.2. Polarization transfer from NV center to nuclei

The NV center can be prepared in an almost perfect state of the electron spin polarization by means of the optical excitation of a laser. The high magnetic purity of the diamond lattice in which the NV center is located and its long spin-lattice relaxation time (T_1), allows its spin polarization at room temperatures. This feature makes NV centers in diamonds an interesting tool to be used as either spintronic devices or a polarization source for NMR experiments. The laser irradiation can pump the population to the $|g, m_s = 0\rangle$ state and create an electron spin polarization close to ~100% due to the ISC path [37,38].

The transfer of polarization from NV spins to ^{13}C nuclei within the diamond has already been demonstrated by different research groups [19–27,39–47] [Fig. 4.3]. The ^{13}C nuclear spins act as a stocker and propagator of spin polarization and it is possible to measure the corresponding ^{13}C NMR signal in the diamond using a conventional NMR coil. Unfortunately, the spin polarization of the NV center is not accompanied by nuclear polarization due to the mismatch between the NV electron and the nuclear resonance frequencies.

Electrons and nuclei can exchange polarization under certain conditions within the diamond. For example, near ESLAC at ~50 mT, an almost total polarization of the ^{15}N nuclei (nitrogen adjacent to the vacancy that forms the NV center) has been detected. The polarization of the ^{14}N [25] and the ^{13}C nuclei adjacent to the NV center have also been observed [24]. The spin polarization of the ^{15}N and ^{14}N nuclei strongly depends on the magnetic field since the hyperfine interaction with these nuclei is isotropic in the excited state [48,49].

The ^{13}C hyperfine couplings are generally anisotropic in the ground state. The ^{13}C polarization varies depending on the position of the ^{13}C nuclei to the NV center and other defects in the diamond lattice [50,51]. H. Wang et al. [24] showed that the magnetic field applied to this anisotropic hyperfine interaction produces electron-nuclear mixing resulting in nuclear polarization. Consequently, an almost total ^{13}C polarization of a first shell ^{13}C ensemble coupled to NV centers has been observed near the GSLAC (~102,5mT) [23]. The GSLAC method [52] is less used than the ESLAC [Fig. 1.4 from chapter 1]. This could be because the GSLAC requires high stability of the magnetic field since small changes in the magnetic field can stop the transfer of polarization from NV electron spin to the target nuclear spins.

Different set-ups have been suggested to transfer the spin polarization from NVs to ^{13}C nuclei [Fig. 4.4]. G. Alvarez et al. [46] proposed a set-up to hyperpolarize and detect ^{13}C nuclei at high field in an NMR apparatus [Fig. 4.4 a, b, and c]. Specifically, the hyperpolarization of the ^{13}C -bulk diamond occurs through the combination of laser, MW, and a low magnetic field-aligned to one of the NV orientations during 30 s. Then, the diamond sample is released (<1s) to the 4.7 T NMR apparatus where the ^{13}C signal is detected employing a spin-echo sequence [Fig. 4.4 c]. The cycle is then continuously repeated to improve the signal averaging, that is, the sample is hyperpolarized and is measured again in the NMR apparatus [46].

J.King et al. [47] combined the ONP part and the NMR detection in the same set-up [Fig. 4.4 d]. The ^{13}C signal was enhanced by 6% (or $\sim 170,000$ over thermal equilibrium) and was detected in situ with an NMR probe. The sample shuttling or a precise diamond orientation were not necessary, except in orientations near the magic angle where the $|g, m_s = 0\rangle$ and $|g, m_s = \pm 1\rangle$ states are in an equal mix. For the ^{13}C hyperpolarization, the sample was continuously irradiated with a 532nm laser, MW fields at 8.900 and 14.600 MHz, and a magnetic field of 420mT [47]. For the measurement of the ^{13}C NMR signal, they used a Magritek Kea2 spectrometer to extend the frequency range of the signature NMR spectrometer line [53]. Later, they used a similar set-up to study the ^{13}C hyperpolarization in ^{13}C enriched diamonds [41].

E. Scott et al. [39] designed a probe to produce ^{13}C hyperpolarization and measure the signal at 7.05 T NMR [Fig. 4.4 e]. The ^{13}C hyperpolarization protocol is based first on the elimination of the thermal ^{13}C polarization by saturating the nuclear transitions with a series of fifty 90° pulses separated by 10 ms of dephasing time. Next, the diamond crystal is irradiated by a 532 nm laser, followed by a 90° pulse and then, the acquisition. The ^{13}C signal is enhanced up to 2.5% at 17 °C and 0.125% at 290K. Furthermore, the positive or negative sign and the maximum value of the ^{13}C polarization signal depend on the orientation of the crystal to the external magnetic field and the electric field vector of the laser. In addition, they reported that increasing the defect concentration, increases the rate at which the ^{13}C polarization is generated and decreases the ^{13}C polarization percentage.

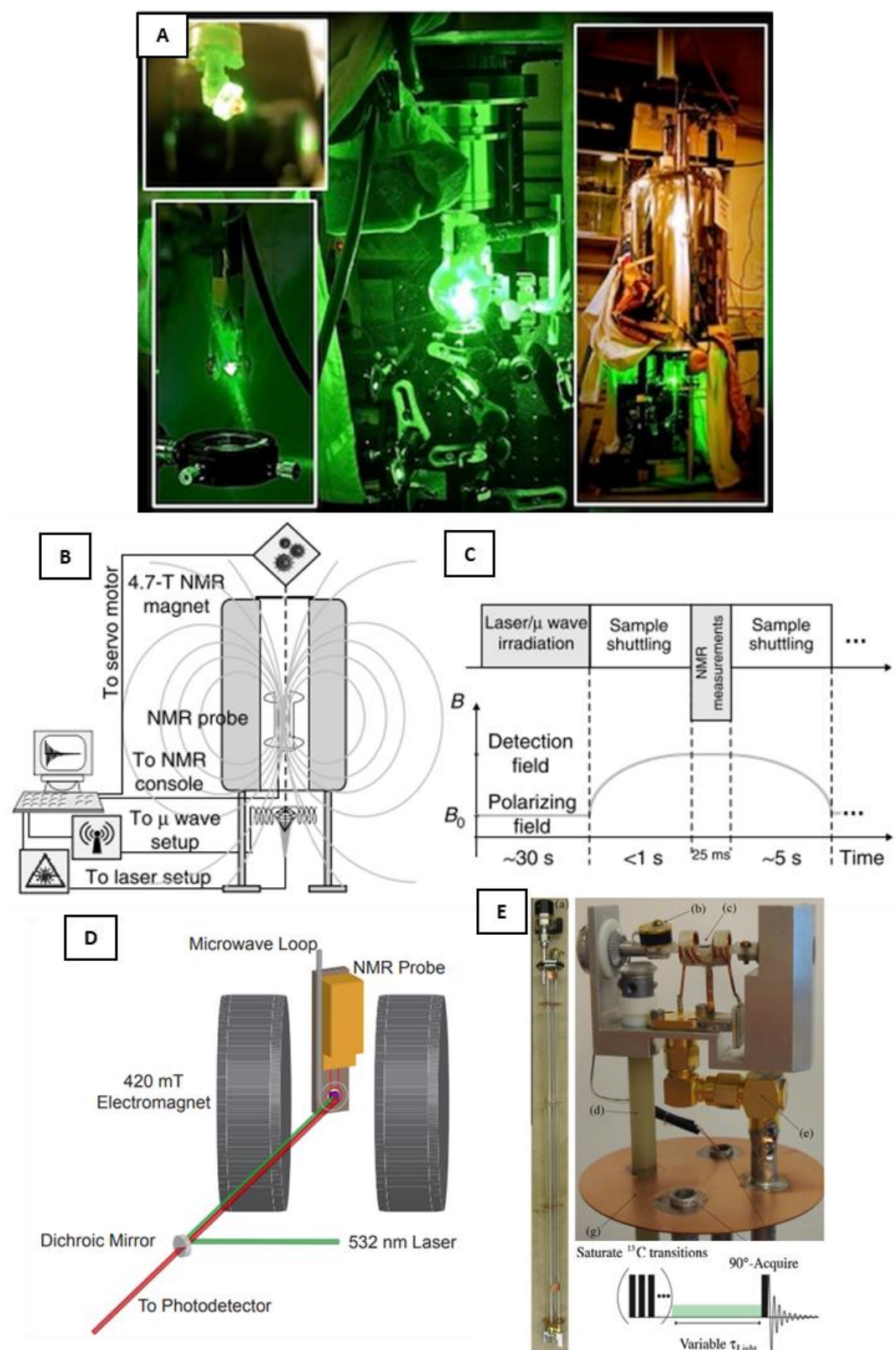


Figure 4.4 | Different set-ups for the hyperpolarization of NV centers.

Figure 4.4 | Different setups for the hyperpolarization of NV centers.

- a) Photo of the Opto-NMR setup using NVs to transfer the polarization to the ^{13}C nuclei at room temperature for the subsequent ^{13}C NMR detection.
- b) Diagram of the Opto-NMR set-up above and c) detection sequence used in these experiments. First, during the polarization transfer phase to hyperpolarize the ^{13}C nuclei, the entire single-crystal diamond is irradiated with laser light and MWs at a low magnetic field underneath the NMR magnet. The hyperpolarized diamond is then shuttled in less than 1 s from the hyperpolarization part into a 4.7-T NMR magnet to directly detect its macroscopic ^{13}C magnetization signal via a spin-echo sequence. It is observed that the orientation of the magnetic field with respect to the NV orientation can change the ^{13}C nuclei hyperpolarization.
- d) The set-up generates ^{13}C nuclear spin polarization in diamonds by using microwave-driven ONP from NVs. The ^{13}C bulk nuclear spin polarization of 6% (~170,000 over thermal equilibrium) is achieved at arbitrary orientations of the NV $^-$ defects since the process generally depends on the matching of the MW frequency at a given transition rather than at a specific strength or orientation of the magnetic field. The setup combines Optically Detected Magnetic Resonance (ODMR), Optical Nuclear Polarization (ONP), and NMR detection. An unfocused 532 nm laser beam provides optical pumping and induces red/near-infrared (NIR) sample fluorescence which is read out using a photodetector. A tuned NMR probe provides radiofrequency (RF) irradiation and detection while a microwave loop provides microwave irradiation.
- e) The set-up is equipped with a homebuilt non-resonant transmission probe ([a] inside the photo) that is positioned inside the NMR cryostat. A laser from outside irradiates the diamond that is placed on the sample holder [c]. The orientation of the sample (adjusted with a goniometer) is observed to change the maximum ^{13}C polarization. In the hyperpolarization protocol, they initially used a series of fifty 90° pulses separated by 10 ms of dephasing time to remove the ^{13}C thermal polarization from the bulk diamond. Consequently, the diamond is irradiated with a 532 nm laser followed by a 90° pulse to then acquire the ^{13}C NMR spectrum. They obtained a ^{13}C polarization up to 2.5% at 17 °C and 0.125% at 290K. The temperature was produced by a cryostat and controlled by a temperature sensor [b].

Panels a, b, c were reprinted with permission from Reference [46].

Panels d adapted with permission from Reference [22].

Panels e reprinted with permission from Reference [39].

To have an overview of this field in recent years, the most important articles in this field are listed below in chronological order [Tables 4.1, 4.2, and 4.3] (following the structure of the tables in K.Jeong's article [18]). In table 4.3, the research articles published by the Pines and Meriles groups are described. They have been among the research groups that have been able to make the most progress in the investigation of hyperpolarization with NV centers latterly.

Within the tables, the main transfer mechanisms from the NVs to the nuclear spins, primarily ^{13}C or ^{14}N are ESLAC, GSLAC, working near ESLAC conditions, by MWs, by radiofrequency (RF) or MW / RF. The polarized and detected nuclei are ^{13}C (mainly), ^{14}N , or the ^{14}N - ^{13}C pair. The single/bulk section refers to the number of the detected nucleus, that is, whether it is a single nucleus or an ensemble of nuclei. The detection method section describes how the corresponding nucleus has been detected, for example, high field means that generally an NMR apparatus has been used to measure the signal of the nucleus studied [54]. In the table, ODMR means that the signal from the nuclei has been obtained by ODMR protocol and then this signal has been digitalized with computer programs to obtain the NMR signal of the nucleus studied. The sample section indicates the type of enrichment of the diamond sample, most of them have a ^{13}C enrichment or natural abundance of ^{13}C (1.1%). The temperature section describes the temperature at which the hyperpolarization of the nucleus is performed, mostly at room temperature (RT).

Table 4.1, 4.2, and 4.3 | Summary of the most important studies in the transfer of polarization from NV center to nuclei (^{13}C , ^{14}N) within the diamond.

Panels a reprinted with permission from Reference [22].

Panels b reprinted with permission from Reference [24].

Panels c reprinted with permission from Reference [27].

Panels d reprinted with permission from Reference [46].

Panels e reprinted with permission from Reference [39].

Panels f reprinted with permission from Reference [40].

Panels g reprinted with permission from Reference [41].

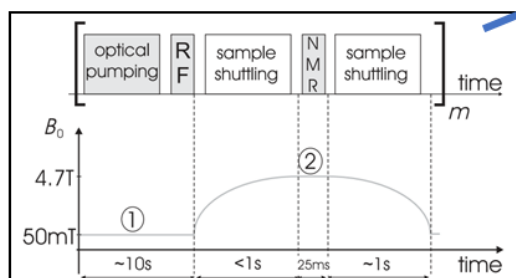
Panels h reprinted with permission from Reference [42].

Panels i reprinted with permission from Reference [20].

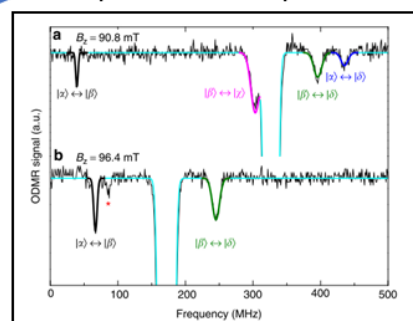
Panels j reprinted with permission from Reference [21].

Panels k reprinted with permission from Reference [43].

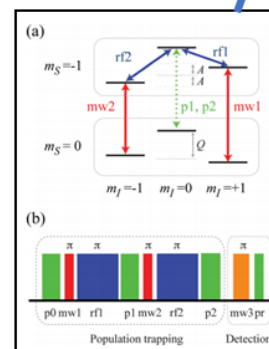
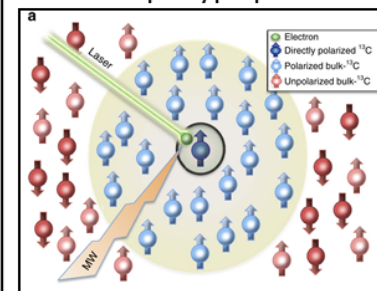
1	Title	Optical polarization of ^{13}C nuclei in diamond through nitrogen vacancy centers	Optical polarization of nuclear ensembles in diamond	Bulk Nuclear Polarization Enhanced at Room Temperature by Optical Pumping	Sensitive magnetic control of ensemble nuclear spin hyperpolarization in diamond	Recursive polarization of nuclear spins in diamond at arbitrary magnetic fields	Local and bulk ^{13}C hyperpolarization in nitrogen-vacancy-centred diamonds at variable fields and orientations
Reference and year	[45](2010)	[22](2013)	[23](2013)	[24](2013)	[27](2014)	[46](2015)	
Mechanism	Dipole-field fluctuations	ESLAC	ESLAC	GSLAC	MW/RF	MW	
Nuclei	^{13}C	^{14}N , ^{13}C	^{13}C	^{13}C	^{14}N	^{13}C	
Detection method	High field	ODMR	High field	ODMR	ODMR	High field (4,7T NMR)	
Single/bulk	Bulk	Single	Bulk	Single	Single	Bulk	
Temperature	Low	Low	RT	RT	RT	RT	
Sample	Natural	Natural	Natural	100% ^{13}C	Natural	10% ^{13}C	
Extra info	^{13}C nudei are polarized into the higher-energy Zeeman state with a bulk-average polarization up to 5.2%, although local polarization may be higher. The kinetics of polarization are temperature independent and occur within 5 min. Fluctuations in the dipolar field of the NV-center spin bath are identified as the mechanism by which nuclear-spin transitions are induced near defect centers. Polarization is then transported to the bulk material via spin diffusion, which accounts for the observed kinetics of polarization.	They polarize 90% of the ^{14}N nuclear spins within the NV centers, and 70% of the proximal ^{13}C nuclear spins. The temperature dependence of nuclear polarization was measured.	Experiments begin with laser pumping of the electronic spins in a 50 mT axial field facilitating polarization transfer to the nuclear spins. (Nuclear spins could also be manipulated at his low field by applying resonant RF pulses). The sample is then shuttled from the polarizing field (1) to the “sweet spot” of a high-field magnet (2), where ^{13}C spins are subject to a pulsed NMR detection. The diamond sample returns to the polarizing field for a repeated pumping and further signal averaging.	Close to the ground-state level anti-crossing (LAC) condition of the NV electron spins, ^{13}C nuclei in the first shell are polarized in a pattern that depends sensitively upon the magnetic field. Based on the anisotropy of the hyperfine coupling and of the optical polarization mechanism, they predict and observe a reversal of the nuclear spin polarization with only a few millitesla change in the magnetic field.	They build two complementary schemes: a) Called “spin exchange” : concatenates optical excitation with selective microwave (MW) and radio-frequency (RF) pulses to ex-change the initial NV and nitrogen spin states. b) Called “population trapping”: uses a similar pulse sequence to sequentially drag the NV-^{14}N system into a target polarized state. Interestingly, this class of schemes promises some advantages when compared to Level anti-crossing (LAC) and Hartman-Hahn (HH) based methods, particularly when nuclear polarization at high magnetic field is required.	They report an approach for achieving efficient electron-^{13}C spin-alignment transfers, compatible with a broad range of magnetic field strengths and field orientations with respect to the diamond crystal. This versatility results from combining coherent microwave- and incoherent laser-induced transitions between selected energy states of the coupled electron–nuclear spin manifold. ^{13}C-detected nuclear magnetic resonance experiments demonstrate that this hyperpolarization can be transferred via first-shell or via distant ^{13}Cs throughout the nuclear bulk ensemble. The method is based in the electron–nucleus hyperfine (HF) interaction imposed by the spin-1 nature of the NV center by MW irradiation.	

A. Sequence used to hyperpolarize the ^{13}C nuclei

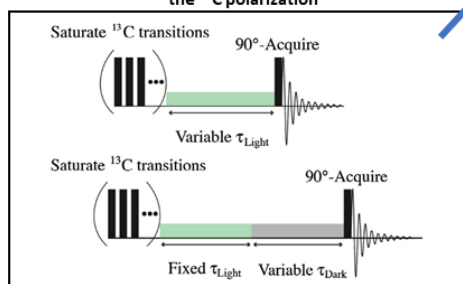
B. Representative ODMR spectra near LAC



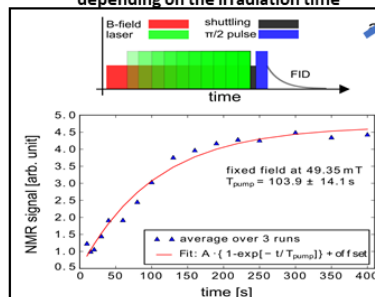
C. The two schemes

D. MW-driven ^{13}C polarization derived from optically pumped NVs

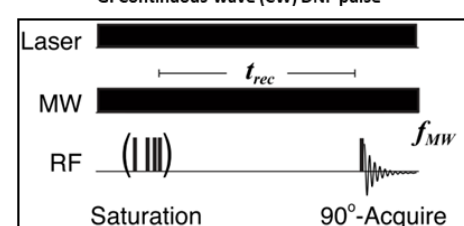
2	Title	Room-temperature in situ nuclear spin hyperpolarization from optically pumped nitrogen vacancy centres in diamond	The phenomenology of optically pumped ^{13}C NMR in diamond at 7.05 T: Room temperature polarization, orientation dependence, and the effect of defect concentration on polarization dynamics	Dynamic polarization of single nuclear spins by optical pumping of NV color centers in diamond at room temperature	Room temperature bulk diamond ^{13}C hyperpolarisation – Strong evidence for a complex four spin coupling	Optically-pumped dynamic nuclear hyperpolarization in ^{13}C enriched diamond	Dynamically Polarizing Spin Register of N-VCenters in Diamond Using Chopped Laser Pulses
	Reference and year	[47] (2015)	[39] (2016)	[25] (2016)	[40] (2017)	[41] (2017)	[42] (2019)
	Mechanism	MW	High magnetic field and a green laser beam	ESLAC	Cross polarisation between a NV carbon-13 system and a nearby P1 center (50 mT)	MW/RF; 472 mT	Double-resonance DNP scheme based on chopped laser pulses in an arbitrary magnetic field using MW/RF
	Nuclei	^{13}C	^{13}C	^{15}N , ^{13}C	^{13}C	^{13}C	^{14}N , ^{13}C
	Detection method	Middle field (Kea 2 spectrometer)	High field (7.05 T NMR)	ODMR	High field (7.05 T NMR)	Middle field (Kea 2 spectrometer)	ODMR
	Single/bulk	Bulk	Bulk	Single	Bulk	Bulk	Bulk
	Temperature	RT	Low/RT	RT	RT	RT	RT
	Sample	Natural	Natural	Natural	Natural	^{13}C enriched diamonds: 1, 10, 25, 50, 100%	Natural
	Extra info	They report a bulk, room-temperature hyperpolarization of ^{13}C nuclear spins observed via high-field magnetic resonance. The technique harnesses the high optically induced spin polarization of diamond nitrogen vacancy centres at room temperature in combination with dynamic nuclear polarization. They observe bulk nuclear spin polarization of 6%, an enhancement of $\sim 170,000$ over thermal equilibrium. The signal of the hyperpolarized spins was detected in situ with a standard nuclear magnetic resonance probe without the need for sample shuttling or precise crystal orientation.	Room temperature optical illumination of NV single crystal diamonds with a 532 nm laser produces ^{13}C polarization enhancements up to 200 times greater than that of the thermal equilibrium value at 7.05 T. They report high field NV-mediated ^{13}C polarization as a function of the number and type (NV- and P1) of defects in commercially available diamonds. Surprisingly, both positive and negative ^{13}C polarizations are observed depending on the orientation of the crystal with respect to the external magnetic field and the electric field vector of the optical illumination	They report a versatile method to polarize single nuclear spins in diamond, based on optical pumping of a single nitrogen-vacancy (NV) defect and mediated by a level anticrossing in its excited state. A nuclear-spin polarization higher than 98% is achieved at room temperature for the ^{15}N nuclear spin associated with the NV center, corresponding to μK effective nuclear-spin temperature. They then show simultaneous initialization of two nuclear spins in the vicinity of a NV defect.	Their data suggest that the observed effect can not be allocated to the hyperfine coupling in ESLAC but is much more likely related to a cross polarisation between a NV carbon-13 system and a nearby P1 centre. The dominant polarisation pattern can be explained by and attributed to cross relaxation between dipolar coupled NV and ^{14}N -P1 centres in good agreement with the theoretical simulation. In that picture the P1 defect acts as mediator for the nuclear polarisation effect much more efficient than the ESLAC.	They investigate ^{13}C nuclear spin hyperpolarization from NV centers in isotopically enriched diamonds with ^{13}C concentrations up to 100%. ^{13}C enrichment leads to hyperfine structure of the nitrogen vacancy electron spin resonance spectrum and as a result the spectrum of dynamic nuclear polarization. They show that strongly-coupled ^{13}C spins in the first shell surrounding a NV center generate resolved hyperfine splittings, but do not act as an intermediary in the transfer of hyperpolarization of bulk nuclear spins.	They propose and demonstrate a magnetic-field-independent, deterministic, and highly efficient polarization scheme by introducing chopped laser pulses into the double-resonance initialization method. With this method, they demonstrate the initialization of a single-nuclear-spin system approaching 98.1% and a ^{14}N - ^{13}C double-nuclear-spin system approaching 96.8% at room temperature in a magnetic field below 10G. The initialization is limited by a nuclear-spin depolarization effect due to chopped laser excitation.

E. Pulse sequences for rise (top) and decay (bottom) of the ^{13}C polarization

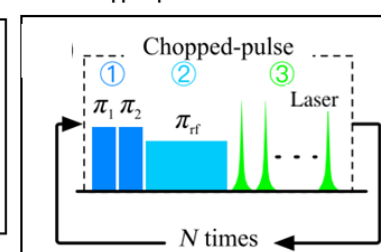
F. Time behavior of the hyperpolarisation effect depending on the irradiation time



G. Continuous-wave (CW) DNP pulse

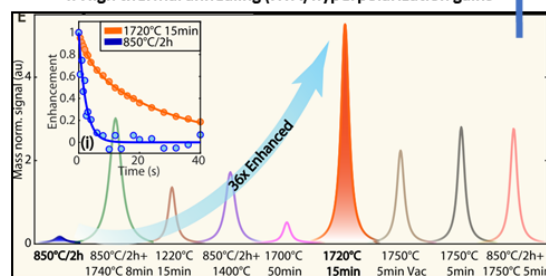
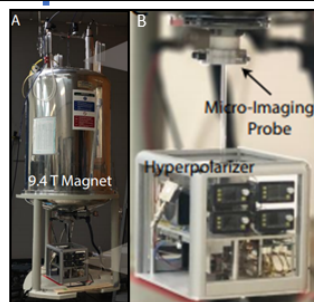


H. Chopped-pulse double-resonance DNP

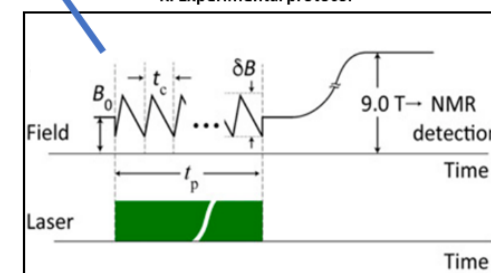


3	Reserach group	Pines group			Meriles group	
Title	Orientation-independent room temperature optical ^{13}C hyperpolarization in powdered diamond	High temperature annealing enhanced diamond ^{13}C hyperpolarization at room temperature	High contrast dual-mode optical and ^{13}C magnetic resonance imaging in diamond particles	Carbon-13 dynamic nuclear polarization in diamond via a microwave-free integrated cross effect	Multi-spin-assisted optical pumping of bulk ^{13}C nuclear spin polarization in diamond	Optically pumped spin polarization as a probe of many-body thermalization
Reference and year	[19] (2018)	[60] (2019)	[59] (2019)	[43] (2019)	[44] (2017)	[26] (2020)
Mechanism	Laser (520 nm) with a chirped microwave irradiation at low field (Bpol ~38mT) to transfer polarization from the NV spin to the ^{13}C nuclei			Green laser and Bpol = ~46-56 mT Working near the ESLAC condition—where the P1 Zeeman splitting matches one of the NV spin transitions.	Green laser and Bpol ~52 mT (close to ESLAC). P1-driven model: cross polarization of ^{13}C spins coupled to P1 centers.	Green laser and Bpol ~52 mT (close to ESLAC). Study of the ^{13}C spin diffusion (^{13}C -P1-P1- ^{13}C) with RF excitation.
Nuclei	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C	^{13}C
Detection method	High field (NMR 7T)	High field (NMR 7T)	High field (microcoil in a 9.4T NMR)	High field (NMR 9T)	High field (NMR 9T)	High field (NMR 9T)
Single/bulk	Microdiamonds or Nanodiamonds both dry and suspended in liquid	Bulk (Microdiamonds)	Bulk (NDs)	Bulk	Bulk	Bulk
Temperature	RT	RT	RT	RT	RT	RT
Sample	Natural and different ^{13}C enriched diamonds (1, 3, 10, 25, 100% ^{13}C)	Different diamond particles with different electron irradiation and annealing conditions	Natural	Natural	Natural	Natural
Extra info	They combine the use of continuous optical illumination and a MW sweep over a broad frequency range. They use powdered diamond with which they attain bulk ^{13}C spin polarization in excess of 0.25% under ambient conditions. Remarkably, their technique acts efficiently on diamond crystals of all orientations and polarizes nuclear spins with a sign that depends exclusively on the direction of the microwave sweep. This scientific article is the basis for the following articles in the Pines group.	They discover that diamond annealing at elevated temperatures close to 172 °C have remarkable effects on the hyperpolarization levels, enhancing them by upto 36-fold over materials annealed through conventional means. They unravel the intriguing material origins of these gains, and demonstrate they arise from a simultaneous improvement in NV- electron relaxation time and coherence time, as well as the reduction of paramagnetic content, and an increase in ^{13}C relaxation lifetimes.	For MR imaging, they employ dynamic nuclear polarization (DNP) at 38mT to enhance the ^{13}C polarization, and a microcoil in a 9.4T magnet for imaging. The same optical excitation polarizes (initializes to $m_s=0$) the NV electron spins, and microwave (MW) sweeps across the NV ESR spectrum drive Landau-Zener dynamics that transfers polarization to the ^{13}C nuclei in a fully orientation-independent manner.	They demonstrate efficient MW-free ^{13}C DNP through the use of consecutive magnetic field sweeps and continuous optical excitation. The amplitude and sign of the polarization can be controlled by adjusting the low-to-high and high-to-low magnetic field sweep rates in each cycle so that one is much faster than the other. By comparing the ^{13}C DNP response for different crystal orientations, they show that the process is robust to magnetic field/NV misalignment, a feature that makes the present technique suitable to diamond powders and settings where the field is heterogeneous.	They focus on the dynamics near 51 mT, where a fortuitous combination of energy matching conditions between electron and nuclear spin levels gives rise to alternative polarization transfer channels. By monitoring the ^{13}C spin polarization as a function of the applied magnetic field, they show ^{13}C spin pumping takes place via a multi-spin cross relaxation process involving the NV- spin and the electronic and nuclear spins of neighboring P1 centers. Further, they find that this mechanism is insensitive to the crystal orientation relative to the magnetic field.	They investigate spin pumping of engineered color centers. They focus on low-abundance, strongly hyperfine-coupled nuclei, whose on the observable bulk ^{13}C magnetic resonance signal. Unexpectedly, they find good thermal contact throughout role in the polarization transport they expose through the integrated impact of variable radio-frequency excitation the nuclear spin bath, virtually independent of the hyperfine coupling strength, which they attribute to effective carbon-carbon interactions mediated by the electronicspin ensemble.

I. High thermal annealing (HTA) hyperpolarization gains

J. 9.4T-Bruker DRX System with a micro-imaging probe (^1H / ^{13}C) used to acquire MR images (a) coupled to a portable polarizer device (b).

K. Experimental protocol



4.3. Outlook and polarization transfer outside the diamond

The hyperpolarization technique with NV centers is very promising but it is practically new and is still further behind in high levels of hyperpolarization and possible applications compared to other techniques such as DNP and PHIP. Another disadvantage is that NV centers are confined within the diamond, which makes the contact area to be much smaller than in other hyperpolarization techniques which are performed on molecular scales like CIDNP, DNP, SEOP or PHIP. However, in the last few years, methods based in the absence of relaxation processes (see Appendix 4.5), have shown that single and small ensembles of NVs can hyperpolarize multiple external nuclear spins successfully. Although its applications are now challenging, the prospect of realizing a non-invasive, versatile, and cheaper hyperpolarization platform that can improve the results of current techniques justifies the research of its great potential as discussed below [Fig. 4.5 a and b].

Tetienne et al. [55] theoretically model the polarization process from NV centers to external samples and evaluate its performance in different scenarios [Fig. 4.5 d]. They found theoretically that a suitable diamond geometry that maximizes the diamond-sample contact area could reach higher levels of nuclear spin polarization of 10% over macroscopic sample volumes ($> \mu\text{L}$). They also explore the possibility of using the NV local polarization enhancements in nano / micro-NMR experiments. In micro-NMR systems, hyperpolarized signals can be enhanced by 1-2 orders of magnitude. It can be improved by performing a micro-structuring of the sample/substrate interface, such as adding a layer of shallow NV centers to further hyperpolarize the sample. In this way, we can improve the recently developed NV-based micro-NMR which could lead to the creation of portable NMR spectrometers. However, they did not find any improvement using the theoretical nano-NMR model where the detection of statistical polarization does not provide a higher SNR ratio. In Fig. 10d, they show the concept of NV-based hyperpolarization, where the diamond crystals (blue regions) contain the NV spins (blue arrows) which are in contact with the sample (orange region) that contains the nuclear spins (orange arrows). Here, the laser polarizes the NV spins, and consequently, NVs transfer the polarization to the nuclear spins of the sample of interest utilizing MW irradiation at a given magnetic field [55].

The Meriles group [56] theoretically investigated a route where near-surface NV centers could hyperpolarize a liquid or gas that is in contact with the NDs [Fig. 4.5 e]. They studied how hyperpolarization depends on the depth of the NV centers and the surface roughness of the NDs. Therefore, they theoretically designed a "diamond spin pump" [Fig. 4.5 e]. The microfluidic diamond chip contains a diamond crystal that contain shallow NV centers, which is placed in the channel of the chip. The diamond crystal is irradiated with a laser that polarizes the $|g, m_s = 0\rangle$ of the NV center. In addition, a dc magnetic field (B) placed in the direction of the NV axis is used to break the degeneracy between the $|g, m_s = \pm 1\rangle$ ground states. The target gas or liquid molecules pass through the microfluidic channel, which is in contact with the diamond crystal [Fig. 10f]. A fraction of these molecules is temporarily adsorbed on the diamond surface,

producing dipolar magnetic interactions with the NV centers. The interaction between NVs and molecules is constantly changing since the molecules are in contact with the diamond surface for a certain period (correlation time). Thus, it would be possible to hyperpolarize these molecules for a sufficient time and volume to improve the sensitivity of MRI or NMR detection [56].

The Meriles group [43] has also studied the possibility of using ancillary electron spins to improve the NV-sample coupling and thus reduce the distance between the NV and the sample [Fig. 4.5 g]. The paper proposes a MW-free theoretical model (NV – P1 – ^1H) where the NV spin can transfer the spin polarization to the P1 center, and the latter hyperpolarize protons (^1H) on the diamond surface [Fig. 4.5 g]. Specifically, NV is coupled to the P1 center through a dipole interaction and thus, the P1 center interacts with the ^1H spin on the diamond surface through a dipolar-type hyperfine coupled. The theoretical mechanism is based on the Landau-Zener (LZ) dynamics induced by partially non-adiabatic magnetic field sweeps through the set of avoided crossing from nearly matched energy differences of the individual NV and P1 spins without using any MW source at room temperature. This model is robust to any NV orientation disorder due to an external magnetic field, which makes it applicable to diamond powders such as NDs and is insensitive to fluctuations in the system such as laser heating [43].

The Ajoy and Pines group has proposed to use ^{13}C nuclei as a polarization transmitter since they are at a higher concentration than the NV centers. They have also envisioned another ND polarizer using the technique described in section 3.2. [Fig. 4.5 c] [19]. NV centers are optically polarized and transfer the spin alignment to the ^{13}C nuclei and these in turn, to the nuclei of a frozen liquid by spin diffusion aided by the large ND surface area, which increases the contact surface with the liquid. Right after, the sample is thawed so that it can be measured in the NMR apparatus, thus enhancing the sensitivity and chemical shift resolution of the NMR experiments [19].

Large surface area, NV-rich, fluorescent nanodiamonds (NDs) are promising to transfer the polarization from shallow NVs to the external nuclei. However, NDs could present a disordered orientation of the NV centers compared to the bulk diamonds, where the NV centers have the ordered orientations. Recently, this problem has already been overcome through LZ transitions (see table 4 describing Pines and Ajoy works) [19–21,57–60]. Single NVs can polarize multiple nuclear spins in a very short time, for instance, a single NV could polarize many ^{13}C nuclei in minutes to hours, which would be limited by the longitudinal relaxation time (T_1) of ^{13}C in diamond [55]. Consequently, the ^{13}C nuclei which have a long lifetime could transfer the spin alignment to the external spin nuclei.

It is crucially important to have the suitable diamond depending on the experiment. In a standard diamond sample, there are three main spin reservoirs ordered from lowest to highest concentration: NV centers, P1 centers, and ^{13}C nuclei. P1 centers are typically 10-100 times higher than NV centers. Then, a standard sample would have the following concentrations: [NV] - 1ppm, [P1] -10-100 ppm and [^{13}C] -10000x ppm, where x is the ^{13}C lattice enrichment level [61]. Thus, a typical sample with a ^{13}C natural enrichment (1.1%), would have 10000 times more ^{13}C nuclei than NV centers.

To transfer the polarization from the ^{13}C nuclei within the diamond to the external nuclei, the target spins should have a much shorter T_1 than the ^{13}C . An ensemble of NV centers could polarize a larger number of these ^{13}C nuclear spins than a single NV. NDs could be chemically modified with a 100% enriched ^{13}C layer (free of paramagnetic defects) that can transfer the polarization to other nuclei outside the diamond. Particularly, $T_1(^{13}\text{C}) \sim 1\text{h}$ has been observed in $2\text{ }\mu\text{m}$ ND in a 3T NMR [62].

We have designed the ideal diamond for the protocol used by the Pines group [19,20,57,58,60,61,63–65], which uses the ^{13}C nuclei as a polarization transmitter [Fig. 4.6]. The ND would be functionalized with chemical groups for use in drug delivery. These functional groups would have 100% enriched ^{13}C nuclei to increase the spin diffusion of the polarization since the ^{13}C nuclei of the layer have a maximum diffusion range to polarize external nuclei such as a liquid or gas. Thereby, this liquid or gas could be injected to perform ultrasensitive MRI imaging, improving the current low sensitivity of MRI in a greater proportion than using only single NVs.

It is worth mentioning that ^{13}C nuclear spins have a long T_1 that allows to accumulate polarization. Specifically, in diamonds type Ib and Ia, a $T_1(^{13}\text{C})$ ranging from 1.4 to 36 h in a 4.7T-NMR at room temperature has been measured [66]. Nevertheless, $T_1(^{13}\text{C})$ is mainly restricted by their interactions with the dipole-coupled electronic spin bath of P1 centers, and in a less proportion due to its lower concentration with NV centers. For this reason, it is necessary to make a layer of 100% enriched ^{13}C nuclei that is free of paramagnetic defects, but the greatest difficulty lies in making an epitaxial growth of a pure ^{13}C layer on the ND surface layer since these two layers have different facets [67]. Another possibility would be to create a 100% ^{12}C enriched layer between the previous NV enriched ND layer and the ^{13}C enriched layer, to protect the NV spin properties from the surface magnetic noise that can induce decoherence [68].

The use of single-multiple probes suitable for DNP hyperpolarization has been based on small organic molecules with a ^{13}C labeling of the carboxylic position due to their long T_1 [69]. The impediment that hyperpolarized MRI presents, is the time available to perform the MRI imaging, since the polarization of the nuclear spins can decay rapidly. Therefore, the use of ^{13}C enriched molecular probes whose ^{13}C nuclei have a large T_1 (20-70s) [70], allows the observation of rapid enzymatic activity or fast metabolic pathways. Although it has not yet reached the results of the fluorodeoxyglucose positron emission tomography (FDG-PET) imaging technique, where the time window to operate can reach $\sim 2\text{h}$ [71,72].

The 2 – 10 nm NDs have shown the highest biocompatibility of carbon nanomaterials [73]. The possibility of functionalizing the ND surface to target specific parts of the human body [74] and the large achievable polarization due to the inherent long T_1 of ^{13}C spins in diamond at room temperature [75], make NDs one of the most promising novel agents for hyperpolarized MRI.

Besides the hyperpolarization methods that use optically polarized NV centers, there are other mechanisms using diamonds. For example, DNP methods based on MW irradiation of unpaired electrons from paramagnetic defects in the ND surface (such as P1 centers, charged vacancy sites, and dangling bonds) to hyperpolarize nuclear spins like ^{13}C [76] and ^1H [77,78]. For instance,

Waddington et al. [78] used NDs suspended in water, where electron spins from the paramagnetic impurities on the ND surface can interact with ^1H nuclei of the water. In the DNP mechanism by MW irradiation when the electronic paramagnetic resonance (EPR) transition is driven, electron spin polarization from paramagnetic impurities is transferred to ^1H nuclei via the Overhauser effect [78]. It has also been possible through DNP, the transfer of polarization from P1 centers to ^{13}C nuclear spins inside [79] and outside the diamond lattice [76]. On the other hand, there are hyperpolarization methods based on attaching magnetic elements to the ND surface as Mn [80] or Gd complexes [81], or only Gd [82,83]. Other methods involve the insertion of Fe atoms directly into the ND lattice (Fe-NDs) for in vitro [84] and in vivo [85] MRI contrast enhancement.

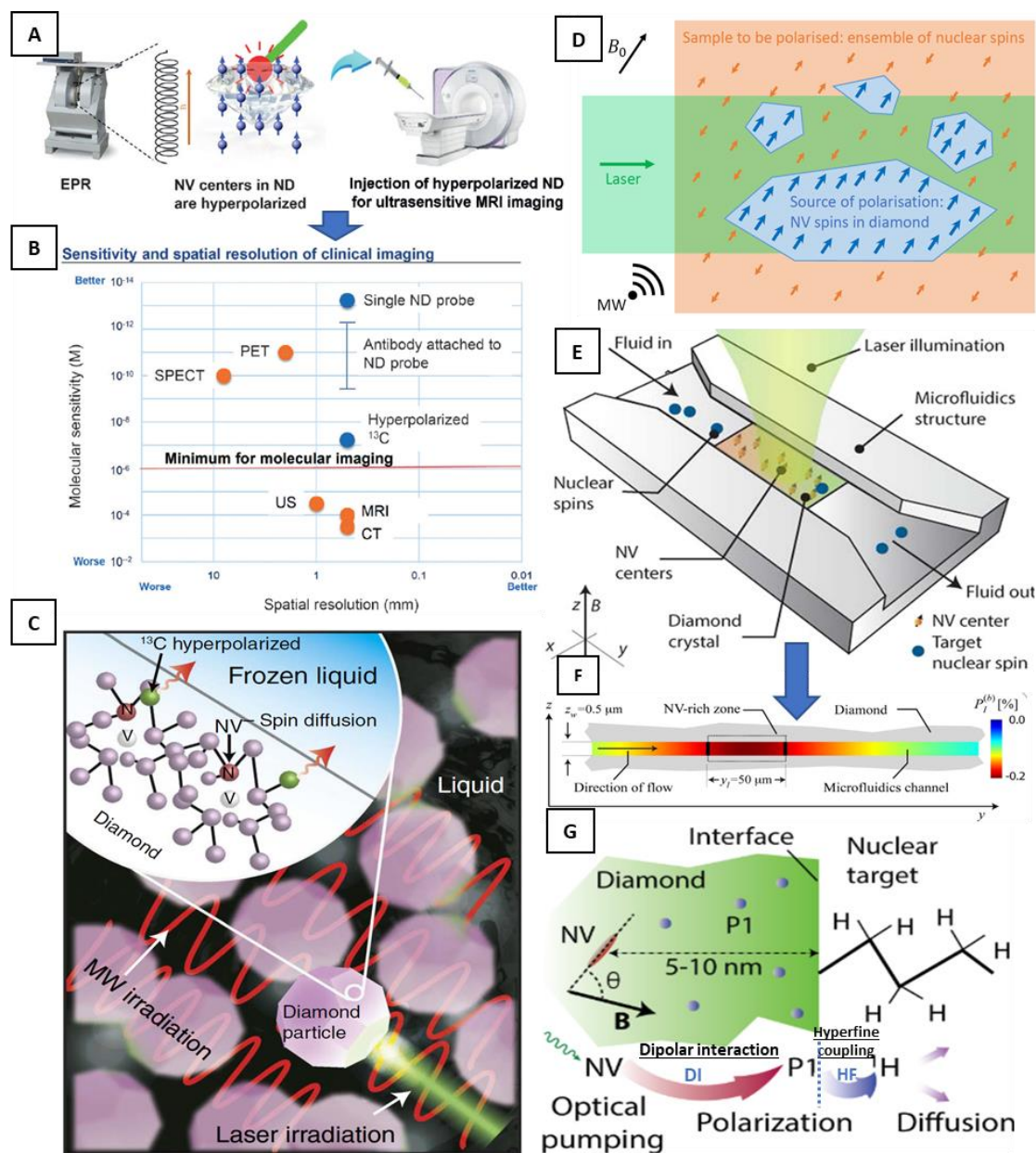


Figure 4.5 | Polarization transfer outside the diamond.

- Simple scheme of the hyperpolarization of NV centers in NDs to improve the current MRI imaging.
- Comparison of the molecular sensitivity and spatial resolution of clinical imaging techniques (orange dots) and hyperpolarization MRI (blue dots). Hyperpolarized ND probes could allow the same spatial resolution as MRI but offer extremely high sensitivity for molecular imaging. SPECT: single-photon emission computed tomography; PET: positron emission tomography; US: ultrasound; CT: computerized tomography.
- Envisioned ND polarizer by Pines group. NV centers are optically polarized by laser and transfer the spin polarization to the ^{13}C nuclei within the ND, which has a large surface

area. In this case, these ^{13}C nuclei of the diamond transfer the spin alignment to the ^{13}C nuclei of another target liquid sample. The hyperpolarization process is done in a frozen liquid to improve spin diffusion as in DNP. Afterward, the target sample is thawed, and the hyperpolarized NMR signal is measured, which would have a high chemical shift resolution and a high signal-to-noise ratio.

- d) Schematic illustrating the concept of NV-based hyperpolarization by Tetienne et al. Diamond crystals (blue regions) containing NV spins (blue arrows) are in contact with a sample (orange region) containing nuclear spins (orange arrows). The NV spins are polarised by laser excitation and this spin polarization is transferred to the nuclear spins by microwave (MW) irradiation. The quantization axis is set by the external magnetic field, of strength B_0 .
- e) Schematics of a microfluidic diamond chip by Meriles group. The fluid circulates through a microfluidic channel, which is directed to the surface of a diamond crystal engineered to host shallow NV centers. Laser illumination and MW pulses are used to define a steady-state NV polarization. permanent magnetic field B is applied along the z -axis to the diamond crystal surface and assumed to coincide with the NV symmetry axis to break the degeneracy between the $|g, m_s = \pm 1\rangle$.
- f) Model of the polarization of fluids from the previous microfluidics device. The microfluidic channel directs the target liquid or gas and puts it in contact with walls that contain optically polarized diamonds (NV-rich zone) and thus, the target sample is hyperpolarized. The color map is the calculated steady-state spatial distribution of the proton polarization of a solvent throughout the microfluidic channel.
- g) Normally, the NV and the target outer nuclear spins outside the diamond (in this case protons) are separated by at least 5-10 nm, leading to comparatively weaker couplings with protons on the surface. The P1 centers can thus serve as proxies to mediate polarization transfer to the protons since the P1 centers are closer to the NVs and are between 10-100 more in concentration than the NVs. Therefore, in this MW-free theoretical model using magnetic field sweeps and a laser, the NV spins transfer the polarization to the P1 centers, which have a dipole interaction (NV-P1) between them, and later, the P1 centers transmit the spin alignment to the protons via a dipole-type hyperfine coupling.

Panels a, b reprinted with permission from Reference [86].

Panel c reprinted with permission from Reference [19].

Panel d reprinted with permission from Reference [55].

Panels e,f reprinted with permission from Reference [56].

Panels g adapted with permission from Reference [43].

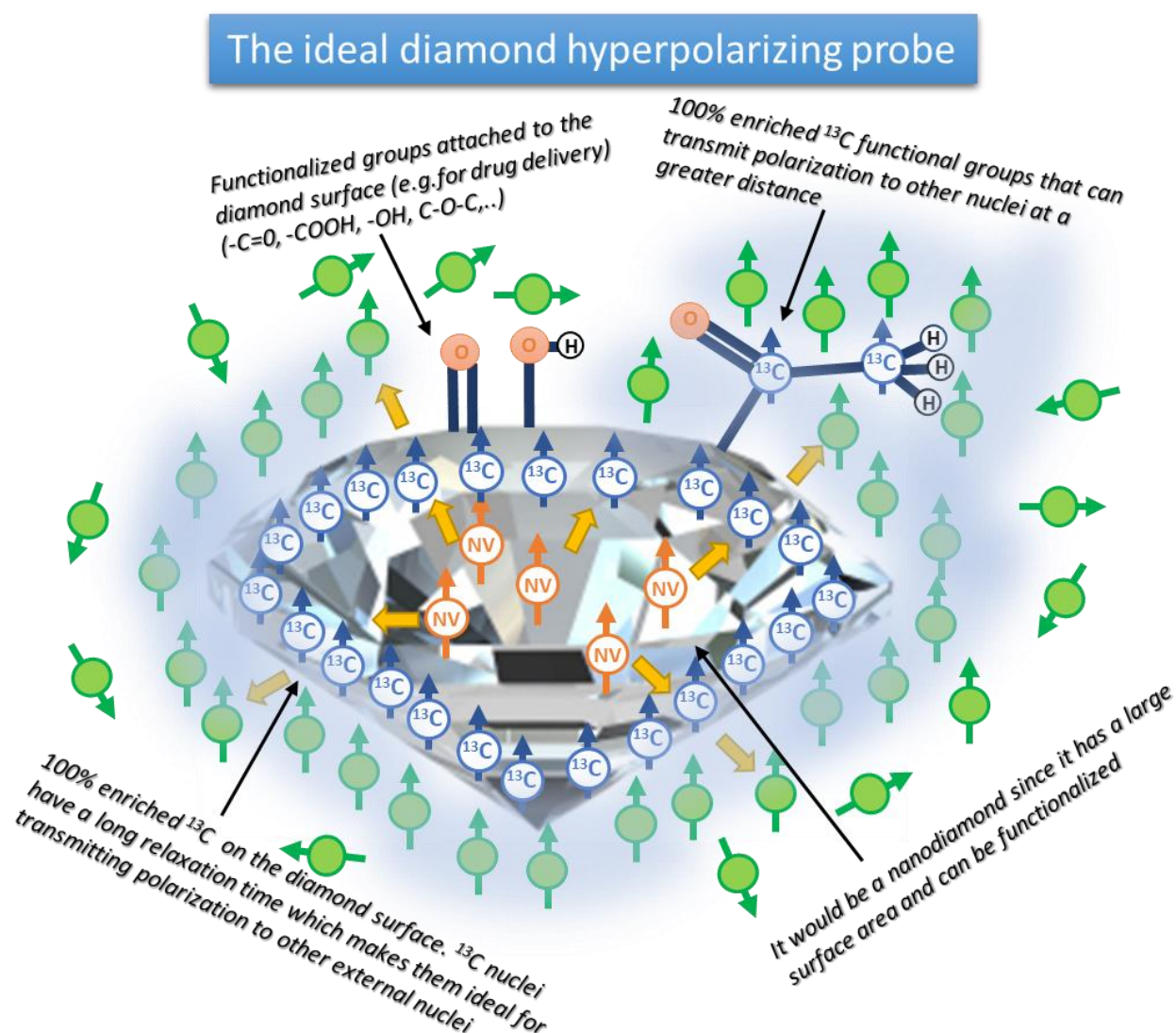


Figure 4.6 | The ideal diamond hyperpolarizing probe for polarizing other materials.

The ideal sample would be a nanodiamond since it has a large surface area and can be functionalized with different chemical groups on the surface to be used for drug delivery. The ND would have a high concentration of NV centers, therefore, NVs could transfer polarization to a 100% enriched ^{13}C nuclei layer at the surface, whose ^{13}C nuclei have a long T_1 that allows them to accumulate a great spin polarization. The ^{13}C layer should not have any paramagnetic defects (such as NV or P1 centers) that due to interactions with the ^{13}C nuclei can be detrimental to the T_1 (^{13}C) and can create polarization sinks. Thus, the ^{13}C nuclei within the diamond could transfer the polarization by cross-polarization or the Overhauser effect to other nuclei of another substance at a certain distance. The ^{13}C spin diffusion has a maximum range, which can be improved by attaching ^{13}C functional groups that can relay polarization at a greater distance. The polarization transfer in the figure (NV - ^{13}C or ^{13}C - other nuclei (green spins)) has been marked with yellow arrows.

4.4. Appendix

I – Classification of hyperpolarization methods using NV centers in relation to the existence or absence of relaxation processes.

We can classify the NV hyperpolarization methods in process with and without relaxation processes [Fig. 4.9]. The methods based on relaxation processes are the ones described previously in the tables, where mainly the NV spin transfers the polarization to the ^{13}C nuclei, and this relays it to the nucleus of the target analyte [19–27,39–47].

In the methods based on the absence of relaxation processes, the NV spin and the external target spin nuclei undergo a coherent polarization transfer (flip-flop) directly. Here, methods such as cross-relaxation induced polarization (CRIP) and Nuclear spin Orientation Via Electron spin Locking (NOVEL) can be found. CRIP is based on the use of an external magnetic field that tunes the NV $^-$ ground-state spin transition frequency into the resonance of the target nuclear spins, without the need to use a MW / RF source [28]. NOVEL is based on the electron-nuclear double resonance at Hartmann-Hahn condition [29–32].

There are also protocols based on pulsed manipulation of NV electron spins such as PulsePol which combines fast polarization transfer with remarkable robustness [33], refocused NOVEL (rNOVEL) [34], or dynamic decoupling protocols such as the polarization transfer by Carr-Purcell-Meiboom-Gill protocol (POLCPMG) [35]. The low polarization that these single NVs or small NV ensembles produce using the above methods cannot be directly measured via a conventional NMR coil to obtain an NMR signal and can only be detected using the same NV center. Therefore, the polarization readout via polarization inversion (PROPI) method has been proposed [36], where it is possible to measure the spin polarization from the NV center to the nearby ^{13}C nuclear spins. All the above sequences use MW pulses, except CRIP.

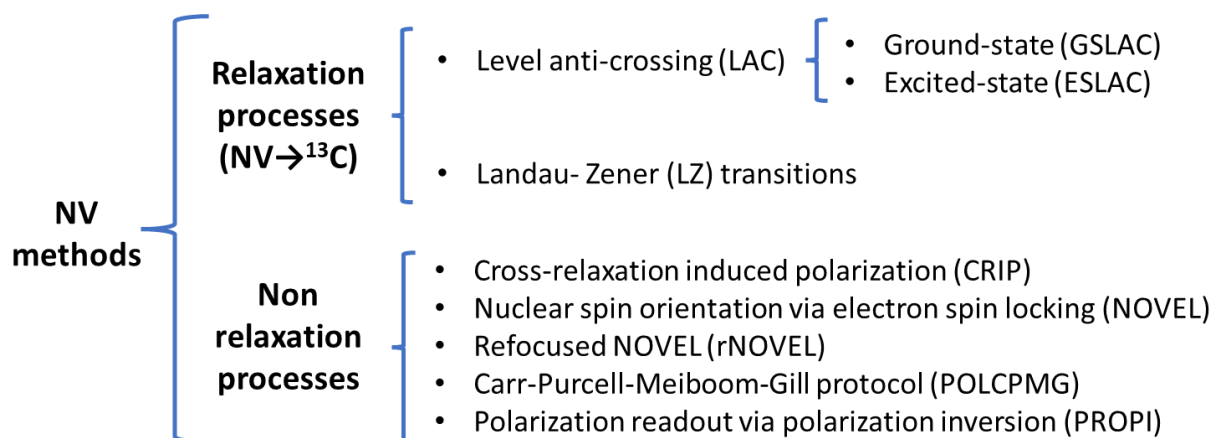


Figure 4.9. | Hyperpolarization methods using NV centers in diamonds classified by the existence or absence of relaxation processes.

4.5. Summary points of chapter 4

1. The need to find inexpensive room temperature hyperpolarizing platforms with low magnetic fields and the use of biocompatible chemicals has prompted research on the possibility of using NV centers in diamonds as a new alternative.
2. Spin polarization can be transferred from the NV electron spins to the nuclear spins inside the diamond by different methods such as: (1) through the level anti-crossing (LAC) that occur between the energy matching of the electron and nuclear spins. This LAC occurs at 500 G for the ground state (GSLAC) and 1000 G for the excited state (ESLAC); (2) solid effect; (3) Hartmann – Hahn matching. Other groups have successfully attempted to transfer the spin polarization from an ensemble of NV-electron spin to the ^{13}C nuclear spins hyperfine coupled inside the diamond via Landau-Zener transitions.
3. Hyperpolarization of ND powders could be used as biocompatible ^{13}C markers in magnetic resonance; as a nanoscale NMR detector where the diamond can polarize substances to make them visible in NMR or using them for drug delivery; in quantum memories at high field; as a transportable spin sensor where the hyperpolarized NDs is encapsulated for example in a cell or vesicle; as a highly-coherent quantum sensor or if this polarized material is a liquid or a gas, can be injected into a patient to improve MRI sensitivity. Therefore, the versatility offered by NV centers also allows the possibility of being used in the food safety industry, medical imaging, environmental tests, etcetera.
4. Recently, it has been demonstrated that a single NV center can hyperpolarize multiple external molecular spins by nuclear spin orientation via electron spin locking (NOVEL) and cross-relaxation (CP). Now, several groups are trying to use NV ensembles to transfer the polarization to other atoms that are in higher concentration than the NVs such as the ^{13}C nuclei and P1 centers within the diamond. These defects could be used as polarization transmitters to hyperpolarize another sample and thus enhance the NMR/MRI signal.

4.6. Bibliography of chapter 4

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General conclusions

Nitrogen-Vacancy (NV) centers in diamond are attracting increasing attention for several applications, including sensing of magnetic and electric fields at the nanoscale, or polarization transfer to nuclei, as a means to increase the sensitivity of NMR detection (see chapter 1). To this end, negatively charged NV centers (NV^-) in the vicinity of the diamond surface are critically important. However, surface effects tend to favor and stabilize the less useful neutral form, NV^0 , thus reducing the availability of shallow NV^- centers. This has likely limited sensitivity in magnetometry applications of ensembles of NVs and has hampered the possibility of transferring polarization from optically polarized NV^- to nuclei outside the diamond lattice.

In chapter 2, we studied the effects of laser irradiation on ensembles of shallow NVs in flat and nanostructured diamond surfaces as a function of laser power. We characterized the spin and charge dynamics properties of NV^- and NV^0 using fluorescence (FL) spectroscopy, optically detected magnetic resonance ODMR, and charge-photoconversion detection as a function of laser power. We observed that the $\text{NV}^0 \rightarrow \text{NV}^-$ photoconversion is promoted at high laser powers. After switching off the laser, the shallow NV^- population is stable over a millisecond timescale. The beneficial effect of strong laser power is less marked in nanostructures samples.

In chapter 3, we studied the FL properties and ODMR spectra of ensembles of NV^- centers in ^{13}C enriched bulk diamond, and in NDs obtained by nanomilling the native material. In bulk diamond, the ODMR contrast increased with laser power, consistent with an increase in spin polarization. On the contrary, in NDs, the ODMR contrast decrease at higher laser powers. Therefore, $\text{NV}^- \rightarrow \text{NV}^0$ photoconversion process in NDs is more efficient than the bulk, reflecting a decrease in the number of NV^- centers. We also observed this detrimental photoconversion phenomenon in NDs internalized in macrophage cells. These results are consistent with the ones obtained in Chapter 2.

To sum up, the diamond nanostructuring increases the surface area but can also reduce the stability of shallow NV^- , putting limitations for magnetometry, biosensing, and hyperpolarization applications (see chapter 4). These findings have important bearings on applications of ensembles of shallow NV^- centers and pave the way to the development of more effective protocols to improve sensitivity and efficiency of polarization transfer at the diamond surface.

Appendix

Publications:

- 1) D.Olivares-Postigo, F. Gorrini, R. Giri, A. Krueger and A. Bifone. Divergent effects of laser irradiation on ensembles of nitrogen-vacancy centers in bulk and nanodiamonds: Implications for biosensing, doi: 10.33774/chemrxiv-2021-llrtb
- 2) F. Gorrini, C. Dorigoni, D. Olivares-Postigo, R. Giri, P. Aprà, F. Picollo, A. Bifone, Long-Lived Ensembles of Shallow NV – Centers in Flat and Nanostructured Diamonds by Photoconversion. ACS Appl. Mater. Interfaces 2021, doi:10.1021/acsami.1c09825
- 3) D.Olivares-Postigo, F. Gorrini and A. Bifone. Review: Optical nuclear polarization of nitrogen-vacancy centers in diamond (Under revision).

Citations to Previously Published Works on the thesis:

Large portions of Chapter 2 have appeared in the following paper:

D.Olivares-Postigo, F. Gorrini, R. Giri, A. Krueger and A. Bifone. Divergent effects of laser irradiation on ensembles of nitrogen-vacancy centers in bulk and nanodiamonds: Implications for biosensing, doi: 10.33774/chemrxiv-2021-llrtb

Large portions of Chapter 3 have appeared in the following paper:

F. Gorrini, C. Dorigoni, D. Olivares-Postigo, R. Giri, P. Aprà, F. Picollo, A. Bifone, Long-Lived Ensembles of Shallow NV – Centers in Flat and Nanostructured Diamonds by Photoconversion. ACS Appl. Mater. Interfaces 2021, doi:10.1021/acsami.1c09825

Large portions of Chapter 4 have appeared in the following paper:

D.Olivares-Postigo, F. Gorrini and A. Bifone. Review: Optical nuclear polarization of nitrogen-vacancy centers in diamond (Under revision).

Poster presentations at congresses:

- 1) D.Olivares-Postigo, F. Gorrini and A. Bifone, Applications of the NV centers in diamonds. *st Conference on Zero and Ultra Low Field (ZULF) NMR*, 1st–4th September **2020**, Online. (Winning poster).

Oral presentations at congresses:

- 1) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone. Study of Nitrogen Vacancy centers in nanodiamonds to transfer polarization to an external liquid. *1st ZULF ITN congress*, 26th– 31st November **2018**, Lyon, France.
- 2) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone. Hyperpolarization of external nuclear spins with NV centers in nanostructured diamond. *2nd ZULF ITN congress*, 18th– 23th March, **2019**, Mainz, Germany.
- 3) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone, Hyperpolarization of ¹³C nuclei through optical stimulation of NV centers in diamond, *3rd ZULF ITN congress*, 23th– 27th September **2019**, York, United Kingdom.
- 4) D.Olivares-Postigo, F. Gorrini and A. Bifone, Applications of the NV centers in diamonds, *ZULF NMR virtual congress*, 1st–4th September **2020**, Lyon, France (Winning poster).
- 5) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone, Optical nuclear polarization of NV centers in diamond, *4th ZULF ITN virtual congress*, 6th– 9th April **2021**, Barcelona, Spain.
- 6) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone, Hyperpolarization of ¹³C nuclei through optical stimulation of NV centers in diamond, *5th ZULF ITN virtual congress*, 27th– 30th September **2021**, Turin, Italy.

PhD annual presentations at the University of Turin in the PhD days:

- 1) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone. Hyperpolarization of ¹³C nuclei through optical stimulation of NV centers in diamond, November **2019**, Turin, Italy.
- 2) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone. Study of Nitrogen Vacancy centers in nanodiamonds to transfer polarization to an external liquid, November **2020**, Turin, Italy (online).
- 3) D.Olivares-Postigo, F. Gorrini, R. Giri and A. Bifone. Optical nuclear polarization with nitrogen Vacancy centers in bulk and nano-diamonds, October **2021**, Turin, Italy (online).

PhD courses held at the University of Turin:

- 1) Advances in Pharmaceutical Technology, March and July **2020**.
- 2) Advances in Pharmacology, May - June **2020**.
- 3) Advances in Phytochemistry, July **2020**.
- 4) Molecular imaging, May **2021**.

- 5) Green and sustainable chemical processes, June **2021**.
- 6) Biosensors, September **2021**.

Other courses carried out in other institutions:

- 1) Leadership and Management skills course for Early career researchers, *hfp consulting*, 7th– 23rd November **2018**, Heidelberg, Germany (online).
- 2) Training course in Academic Entrepreneurship and Intellectual Property, Jagiellonian University Center for Technology Transfer, 2nd and 13th September 2021, Krakow, Poland (online).
- 3) Career development for young scientists, *hfp consulting*, 7th– 23^{rs} November **2018**, Heidelberg, Germany (online).

Acronyms and abbreviations

Abbreviations

NMR - Nuclear Magnetic Resonance

MRI - Magnetic Resonance Imaging

ssNMR – Solid state Nuclear Magnetic Resonance

DNP - Dynamic Nuclear Polarization

MAS - Magic-angle-spinning

ODMR – Optically Detected Magnetic Resonance

ESR - Electron Spin Resonance

RF - Radiofrequency

MW – Microwave

NIR - Near-infrared

CW - Continuous Wave

FID - Free Induction Decay

NV – Nitrogen-Vacancy Defect

NV⁻ – Negatively Charged Nitrogen-Vacancy Defect

NV⁰ – Neutral Charged Nitrogen-Vacancy Defect

NV⁺ – Positive Charged Nitrogen-Vacancy Defect

P1 - Substitutional nitrogen centers

LAC - Level Anti-Crossing

ESLAC - Excited State Level Anti-Crossing

GSLAC - Ground State Level Anti-Crossing

LZ - Landau-Zener

HH - Hartmann – Hahn

ISC – Intersystem Crossing path

HPTP - High Pressure High Temperature

CVD - Chemical Vapor Deposition

NDs – Nanodiamonds

CW-ODMR – Continuous wave - optically detected magnetic resonance

ODMR – Optically detected magnetic resonance

FL – Fluorescence

ROI – Region of interest
MW – Microwave
ZPL - Zero-phonon line
 D_{zs} - Zero-field splitting
RT – Room temperature
B - External static field
 γ - Electron gyromagnetic ratio
 ω_L - Nuclear Larmor frequency
 B_0 - Component of the static external field oriented parallel to the z-axis
SNR - Signal-to-Noise Ratio
AOM – Acousto-Optic modulator
T - Tesla
K – Kelvin
Hz – Hertz
J – Joule
W- Watt

Hyperpolarization techniques

QRIP - Quantum rotor induced polarization
PHIP - Parahydrogen Induced Polarization
SABRE - Signal Amplification By Reversible Exchange
DNP – Dynamic Nuclear Polarization
CIDNP - Chemically Induced Dynamic Nuclear Polarization
dDNP - Dissolution DNP
SEOP - Spin Exchange Optical Pumping of noble gases
ONP - Optical Nuclear Polarization
BFP - Brute Force Polarization

Hyperpolarization protocols with NV centers

CRIP - Cross-Relaxation Induced Polarization
NOVEL - Nuclear spin Orientation Via Electron spin Locking
rNOVEL - Refocused NOVEL
POLCPMG - Polarization transfer by Carr-Purcell-Meiboom-Gill protocol

PROPI - Polarization Readout via Polarization Inversion

Time constants

T_1 - Spin lattice relaxation time constant

T_2 - Spin-spin relaxation time constant

Spin Operators and Quantum Numbers

$\hat{S}$ - Spin lattice relaxation time constant (Electron spin or general spin angular momentum operator
($= \hat{S}_x + S_y + S_z$)

I - Nuclear spin angular momentum operator ($= I_x + I_y + I_z$)

I - Nuclear spin quantum number

S - Electron spin or general spin quantum number

m_s - Quantum number for the z – component of s

Diamond samples

CVD – Chemical vapor deposition

HPHT - high-pressure, high-temperature

DND – Detonation nanodiamonds

Biological components

FBS - Fetal bovine serum

PBS - Phosphate buffered saline

FITC - Fluorescein isothiocyanate

DAPI - 4',6-diamidino-2-phenylindole

