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QUANTUM SENSING USING NV CENTERS IN DIAMOND

by

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Abstract

This thesis is focused on understanding the interaction of nitrogen vacancy (NV) defect in diamond with its environment as well as its applications in nanotechnology and biophysics. First, we study the hyperfine interaction due to nearby ${}^{13}C$ carbon and ${}^{15}N$ nitrogen isotopes in the vicinity of the Level Anti-Crossing (LAC), we propose an experimental method at room temperature free of microwave, in which it is possible to find the sign of the Fermi contact term, using the coupling between nuclear spins 1/2 and electronic spin 1. For this, a three-dimensional coil system was constructed to study the fluorescence close to the LAC. Second we describe an experiment in order to use the NV center as a sensor for chemical reactions, in specific to measure the pH and relate it's macroscopic measurement with experiments like Ramsey interferometry and change in the spectrum, in order to observe changes on the charge state and T_2^* . Finally, we show a way to functionalize a nanodiamond hosting a NV defect, and use it as a biomarker for amyloid β , an Alzheimer desease related compound. We show that using this functionalized nanodiamonds might enable the development of longer experiments in biophysics due to the photostability of the NV defect compared to regular biomarkers that are widely used in biology. This works paves the way for performing unltrasensitive and reliable detection of cells involved in the pathogenesis of the Alzheimer desease.

Citations to previously published work

Large portion of chapter 4 have appeard in the following paper.

• Functionalization of stable fluorescent nanodiamonds towards reliable detection of Alzheimer biomarkers

Francisco Moralez-Zavala, Nathalie Casanova-Morales, <u>Raúl B. González</u>, América Chandia-Cristi, I. Alvizú, Victor Waselowski, Fanny Guzman, Simon Guerrero, Marisol Oyarzún-Olave, Cristián Rebolledo, Julien Armijo, Heman Bhuyan, Mario Favre, Alejandra R. Alvarez, Marcelo Kogan, and J. R. Maze. J Nanobiotechnology. 2018; **16**:60.

 Bayesian estimation for quantum sensing in the absence of single-shot detection Hossein T. Dinani, Dominic W. Berry, <u>Raul Gonzalez</u>, Jeronimo R. Maze, and Cristian Bonato

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Chapter 1

Introduction

1.1 Background

The control of quantum systems is an essential topic in physics due to its applications in several areas ranging from magnetometry and quantum information to biophysics. Multiple solid-state systems can be used for this purpose, each of which presents their challenges and opportunities. Among the solid state systems defects in diamond such as nitrogenvacancy has attracted growing attention. In particular, the Nitrogen-Vacancy (NV) defect in diamond, this defect corresponds to an impurity in a crystal diamond lattice is originated by replacing one lattice carbon atom with a nitrogen atom. The NV center has an electronic spin which can be optically read and manipulated at room temperature. Due to the energy gap between the conduction and valence band, these defects are in a stable configuration and behave like a trapped molecule. This is a significant advantage because the associated electronic wave function is localized and it can be studied and with confocal microscopy[1, 2, 3]. This has been proposed as a promising system for nanoscale sensing [4, 5, 6].

This opens numerous advantages for applications in, for example, biophysics, where drastic changes in the pH of the membranes of a cell, can disturb the state of biological components dramatically. As an example, there have been developments in using the NV defect as a biomarker to label biological structures such as cells or vesicles [7, 8]. Going even further, engineer onto functionalized nano diamonds that contain NV defects have been used to penetrate in a specific kind of cells, like prostate cancer cells or Alzheimer related cells (see

chapter 3), and using it as a marker.

1.1.1 Emergence of single emitters for metrology

Max Planck first proposed the concept of single-photons in 1900 [9] a true single-photon source was not created in isolation until 1974. This was achieved by utilizing a cascade transition within calcium atoms [10]. During the 21st-century defect centers in various solid-state materials have emerged [11], most notably diamond, silicon carbide [12] and boron nitride [13]. The most studied defect is the NV center in diamond that has been used as a source of single photons [14]. These sources along with molecules can use the strong confinement of light to enhance the emission of the NV centers. As well as NV centers and molecules, quantum dots (QDs) can emit single photons and can be constructed from the same semiconductor materials as the light-confining structures [15].

A new frontier of research is the development of non-classical light sources: sources that produce streams of photons with controllable quantum correlations. A central building block, in particular, is a single-photon emitter (SPE) a fundamental resource for many scalable quantum information technologies. There are more than 100 luminescent defects in diamond. A significant fraction has been analyzed in detail such that their charge and spin state is known under equilibrium conditions. Nitrogen-vacancy defects are most abundant in diamond since nitrogen is a prominent impurity in the material, nitrogen is a defect which either exists as a single substitutional impurity or in aggregated form.

1.2 Nitrogen vacancy center in Diamonds

The Nitrogen-Vacancy (NV) center in diamond has been studied since 1970s using different techniques to understand its physical properties based on optical measurements. The NV center is a point defect in a diamond with C_{3v} symmetry consisting of a substitutional nitrogen-vacancy pair orientated along the crystalline direction (figure 1). The center may be found as an ingrown product of the chemical vapor deposition (CVD) diamond synthesis process [43] or as a product of radiation damage and annealing [44] or ion implantation and annealing [42, 37, 41] in bulk and nanocrystalline diamond. The center is known to exist in a negative [18, 19] and neutral [20] charge states. The identifying features of NV⁻ and NV⁰ are their optical zero-phonon line (ZPL) at 637 nm and 575 nm, respectively (see figure 1.3), and associated vibrancy bands that extend from their ZPL to higher/lower energy in absorption/emission.



Figure 1.1: Basic structure of the NV center in diamond. A nitrogen atom (green) is substituted for a carbon atom (grey). Additionally one near-neighbor carbon atom is removed to create a vacancy, where actually the highest concentration of electron density resides (red). Basic structure of the NV centre, the nitrogen-vacancy defect in the carbon-matrix of diamond is formed by a substitutional Nitrogen atom (N) next to a lattice Vacancy (V).

In our case we are studying the NV⁻ wich is more dominant in natural diamond, and we will refer it as just NV center. This NV center has the advantage that we can optically read the spin-states associated to the defect and we can polarize it $m_s = 0$ to his fundamental state. With selective microwave radiation we can transfer the population between the spin states.

The orbitals are noted by the irreducible representation of the orbital symmetry (A_1, A_2, E) with spin degeneracy in the exponent. All three orbitals in the ground state transform according to a different irreducible representations of the C_{3v} group, which means that there are two non degenerate (A_1, A_2) and a double degenerate (E) level¹. A ZPL emission band associated with an A to E dipole transition and that his ground electronic state is a spin triplet ${}^{3}A_{2}[25, 28, 29, 30, 31]$. The ground state levels are not influenced by spin-orbit interaction, since either the state has no orbital $({}^{3}A_{2})$, or no spin momentum $({}^{1}E, {}^{1}A_{1})$. However the triplet is split by the spin-spin interactions, obtaining one S_{z} or $m_{s} = 0$ state with A_{1} character and two S_{x}, S_{y} or $m_{s} = \pm 1$ states with E character which are 2.87 GHz higher in energy together with the 637 nm ZPL, this zero-field ground-state splitting allows us to localize the defect as an NV center.

The S = 1 ground state structure has been well studied by many years through several experiments, but the excited state structure is not understood yet. Since the ZPL is associated with a $_{3}A_{2}$ to $_{3}E$ transition, there are six excited states which must be treated, corresponding to an $m_{s} = 0$ E level, and three levels A_{1} , A_{2} , and E with spin $m_{s} = \pm 1$ [23]. Additional perturbations, such as strain, may mix the levels and shift them. Furthermore, a metastable spin singlet A_{1} state is postulated to play an important role in the dynamics of the NV center under optical excitation [citeDraben]. In addition to this, there is a continuum of vibronic excited states which appear at higher frequencies in absorption and lower frequencies in emission. When the vibronic states are excited using above-band excitation ($\lambda = 532$ nm laser) phonon relaxation brings the NV center rapidly into one of the electronic excited states. The NV center then fluoresces primarily into the phonon sideband, which extends from 630-800nm. Even at low temperature, fluorescence in the ZPL represents only a small percentage of the light emitted [33].

¹This follows directly from group theory, since the dimensions of the irreducible representations give the degeneracy of energy levels. This degeneracy is not an orbital degeneracy.



Figure 1.2: NV center Electronic Structure: Energy-level diagram of NV where the green arrow represents the excitation process and the red arrow the fluorescence process, the dotted arrows indicate the non-radiative decay via metastable A_1 singlet state, ${}^{3}A_2$ denotes the electronic ground state and ${}^{3}E$ denotes the electronic excited state. The three spin sub-levels with $m_s = 0$ and $m_s = \pm 1$ at zero and non-zero magnetic field where D is the zero-field splitting and $\gamma_e B$ is the Zeeman splitting (γ_e is the gyromagnetic electron ratio of the NV center). We can also see the energy difference between the conduction band and the valence band which is 5.5 eV

1.2.1 NV charge state

As it was already pointed out the formation of negatively charged NV defects fabrication requires not only nitrogen bonded with the vacancy, but as well the association of an additional electron to it [40].

$$2N + V \to NV^- + N^+ \tag{1.1}$$

However, the proximity of the surface may affect the NV center charge state [60]. Indeed, it was already demonstrated that surface proximity provides a higher concentration of neutrally charged NV than negatively charged [38]. This may happen due to the presence of electronic acceptor layer nearby the surface, which is trapping the additional 6^{th} electrons



Figure 1.3: NV center photoluminescence spectrum, we can observe the ZPL associated to the NV^0 (575nm) and NV^- (637nm)

of nitrogen-vacancy centers needed to form NV^- . Indeed, bulk NV centers, which are far from the surface effects are stable and presented mostly in negative charge state. For this reason, the charge state of NV centers created at a low dose of implantation may be stronger affected by the proximity of the surface, due to a limited number of acceptors placed on it than at a high dose of implantation.



Figure 1.4: Schematic illustration for the two possible charge states for the NV, showing the ground and excited states of both

1.2.2 Shallow implanted NV centers

Most diamond contains natural NV centers due to natural inclusion of nitrogen impurities and vacancy defects. However, many applications require more exact, controlled NV densities, sometimes much higher than the natural abundance. NV centers can be implanted by several methods. For diamonds that have high abundance of nitrogen defects, the diamond can be irradiated with high-energy electrons or ions. The irradiation damages the diamond lattice, creating vacancies.



Figure 1.5: Schematic of irradiation of diamond by ¹⁵N and generation of NV centers.

In a first step, to create NV next to the surface we have to implant ${}^{15}N^+$ ions with different energies per atom into the diamond sample. The implanted nitrogen atoms have are subsequently converted to NV centers by annealing the sample at 800 °C under high vacuum, causing the vacancies to become mobile (see figure 1.5). When a vacancy encounters a nitrogen atom forms a NV center, and the vacancy is no longer mobile.

The overall density of NV centres can be carefully controlled by the irradiation energy



Figure 1.6: Simulations of the implantation using the SRIM software, the distribution of the nitrogen ions implanted inside the diamond lattice as a function of the incident kinetic energy of the ion.

dose, and the annealing temperature and time. Alternatively, bulk diamond grown by CVD is very high purity and contains low amount of nitrogen atom within the lattice. Using the results of Monte Carlo Simulations based on the SRIM/TRIM software [88], the depth of ion implantation was estimated to range from 11 nm to 2 nm accordingly (Figure 3.3). NV centers in bulk diamond are characterized with a perfectly stable photoluminescence even at room temperature. However implantation performed at low energy (below 2 keV) leads to the creation of NV centers in a close proximity of the surface. A factor to consider for the creation of shallow optical centers is the roughness of the surface. A surface with a rugosity of 10 nm (a standard for commercially available diamond samples) is not suitable for implantation at a depth of a few nm. The roughness enlarges the area and therefore strengthens the influence of the surface. Experience with NV centers shows that some of their properties are influenced by the proximity of the surface, depending on whether they are bulk or shallow (as in nanodiamonds). It has recently shown that for shallow implanted samples, the surface plays an essential role in the state of charge of NV centers. In particular, NV centers with a negative charge are produced mainly with an oxygen-terminated surface. [21-23]



Figure 1.7: SRIM simulations for the implantation ion energy in function of the depth

This thin layer of magnetic sensing NVs permits precise detection of magnetic fields located in very close proximity to the actual NV sensor. Higher NV densities have several advantages: Shorter integration times due to higher signal-to-noise ratio and enhanced sensitivity to magnetic fields. However, the nitrogen content in diamond increases with the implantation dosage due to the imperfect conversion efficiency from implanted nitrogen to NV. The sample that we are using to measure was implantated by Dr. Jan Meijer from the University of Leipzig, Institute of Experimental Physics. This diamond sample is 2mm long, 2 mm wide and 0.5 mm thick. As we have talked before, this diamond was implanted by means of an ionic implant and, as can be seen in 1.6, we are aware of the energy of implantation and the NV concentration per implantation zone. In figure 1.8 we can see a implantation spot and the single NV centers.



Figure 1.8: Photo-luminescence map of ensembles of NV centers (a) Implantation spot of 3keV (b) Zoom image generated by varying the energy of implantation. The photoluminescence (PL) is recorded with an optical excitation of 1 mW input power.

1.3 Thesis structure

This thesis describes three different experiments. The first chapter corresponds to the study of the fluorescence as a function of an external magnetic field applied on the NV center, to understand the possible nuclear couplings to use it as a qubit. Chapter 2 is an application for the NV center to sense chemical reactions, and Chapter 3 shows the functionalization of nanodiamonds with a particular peptide able to recognize amyloid β $(A\beta)$ plaques, which is believed to be a precursor of the Alzheimer disease.

Chapter 2

Magnetic field dependence of the fluorescence of nitrogen-vacancy centers in diamond

In atomic physics, the hyperfine structure refers to splittings and small shifts in the energy levels of atoms, molecules and ions, due to a magnetic interaction between the state of the nucleus and the state of the electron clouds. This is the result of the interaction between magnetic moments associated with electronic spin and nuclear spins.



Figure 2.1: (a) NV center physical and possible spin interactions (b) NV center spin bath, where orange arrow represent the nitrogen nuclear spin, blue arrows ^{12}C and the purple arrows represent the ^{13}C spins

In this chapter, we report the progress mode to study the hyperfine interaction between a nuclear spin 1/2 and an electronic spin 1 in the crystalline lattice of diamond at room temperature. The electron spin as we mentioned in chapter 1 is generated by the presence of the nitrogen-vacancy center defect (NV), and the nuclear spins are due to nearby ${}^{13}C$ carbon isotopes and ${}^{15}N$ nitrogen.

The spin associated to the NV center interacts with a bath of nuclear spins (${}^{13}C$ and ${}^{14}N$), but we will only consider the interaction between ${}^{14}N$ and ${}^{15}N$. So we consider single NV defects associated with native ${}^{14}N$ isotopes (99.6% abundance), corresponding to a nuclear spin I = 1, and 0.4% of ${}^{15}N$ with spin I = 1/2.

Towards this goal we built a 3-axis coil system in order to study the magnetic field dependence on the fluorescence.

2.1 Spin Hamiltonian and Optically detected magnetic resonance (ODMR)

The NV center, at room temperature, can be approximated by a structure of two triplets, one for the ground state $({}^{3}A_{2})$ and another excited state $({}^{3}E)$ connected with an optical transition [19]. The ground state has a zero-field splitting between the $m_s=0$ and the $m_s = \pm 1$ spin substates of 2.87 GHz. The ${}^{3}A_2 \leftrightarrow {}^{3}E$ transition has a zero-phonon line at 638 nm, and a phonon sideband that spans from 638 nm to about 460 nm in absorption. [20] Optical excitation of the NV center results in optical pumping into the $m_s=0$ substate due to an intersystem crossing through the singlet levels that preferentially transfers the $m_s = \pm 1$ states from the ³E excited state to the $m_s = 0$ substate in the ground ${}^{3}A_{2}$ state [35]. Moreover, the intersystem-crossing results in a higher fluorescence of the $m_s=0$ state than the $m_s=\pm 1$ states upon ${}^{3}A_2 \rightarrow {}^{3}E$ excitation, enabling optical spin read-out and detection of the ground-state spin transitions by optically detected magnetic resonance (ODMR). The intersystem-crossing to the metastable state not only allows for spin polarization, but also for optical readout of the electron spin. Due to the long lifetime of the metastable state (~ 300 ns), for $m_s=0$ more optical cycles can be undergone as compared to $m_s = \pm 1$ where the NV ends up in the metastable state. Therefore for a gated illumination, the fluorescence signal for the first 200-300 ns is significantly different (see figure 2.2). With a normalization for the steady state fluorescence a contrast of about 0.2between $m_s=0$ and $m_s=\pm 1$ is detectable. See reference [63] and [51] for a more detailed



description of the measurement process and improvement.

Figure 2.2: Avarage photon counts for different spin states. Life-time correlation for different spin states. Under laser excitation the photon counts for different spin states is detected. For $m_s=0$ more photons are detected in the first ~200 ns, since the NV is less unlikely to end up in the dark metastable state. Then the steady state is reached where the NV is polarized into the $m_s=0$ state. This can be used to normalize the fluorescence counts and make the measurements comparable.

In the presence of a hyperfine interaction stronger than the inhomogeneous linewidth, nuclear spins in diamond such as those of ¹³C, ¹⁴N, and ¹⁵N, can be detected by ODMR. The spin Hamiltonian describing the ground state can be written as:

$$H_{nv} = D_{gs}S_z^2 + E(S_x^2 - S_y^2) + \gamma_e \mathbf{B} \cdot \mathbf{S} + \gamma_n \mathbf{B} \cdot \mathbf{I} + Q_N I_z^2 + \mathbf{S} \cdot \mathbf{A} \cdot \mathbf{I}$$
(2.1)

From this equation we know that $D_{gs} = 2.87$ GHz, wich is the zero-field-splitting due to the spin-spin interaction between unpaired electrons [20, 46]. In the presence of a external magnetic field along the NV axis, the spin transitions $m_s = \pm 1$ are split by the NV electron Zeeman splitting $m_s = \pm 1$, each one is shifted by $2\gamma_e \vec{B}$, where $\gamma_e = 2.8$ MHz/Gis the gyromagnetic ratio of the NV center. We also know that E is the transverse zerofield-splitting parameter [45, 46], $A_{\parallel} \approx -2.16$ MHz is the axial hyperfine constant [48] and $A_{\perp} \approx -2.7$ MHz the transverse hyperfine constant [47]. Additional structure due to the coupling of ¹⁴N nucleus is shown with $m_I = 0$ level separated from $m_I = \pm 1$ by $P \pm A_{\perp}$ $(Q_N \approx -4.95$ MHz, wich is the quadrupole splitting of the ¹⁴N. [48, 49]



Figure 2.3: Hyperfine interaction due to nearby ${}^{13}C$ carbon and ${}^{15}N$ nitrogen isotopes in the vicinity of the Level Anti-Crossing (LAC). Applying an external magnetic field by Zeeman effect we can separate the excited triplet and the triplet of the ground state, arriving at what is known as LAC. For the excited state it is abbreviated as ESLAC and for the ground state as GSLAC. This figure is a scheme of the hyperfine structure of the $m_s=0$ and $m_s=-1$ ground-state manifolds for a NV defect coupled to its ${}^{14}N$ (nuclear spin projection m_I)

2.1.1 Microwave excitation

In typical optically-detected magnetic resonance (ODMR) experiments, the microwave transitions are driven by linearly polarized fields, and individual transitions are selected by applying a magnetic field to lift the $m_s = \pm 1$ degeneracy. To avoid spin mixing, the magnetic field is usually applied along the quantization axis, which can be along any of the four $\langle 111 \rangle$ crystal axes. The optical pumping cycle of the NV center does not conserve spin angular momentum, leading to a spin-selective population of the ground triplet substates of the excited defects. Under continued optical illumination, the spin eventually polarizes into the $m_s = 0$ state. By tuning an applied microwave field in resonance with its transitions, the spin can also be readily manipulated. When the NV spin suffers an external microwave frequency which is resonant with one of the spin transitions $m_s = 0 \rightarrow m_s = \pm 1$, the population is redistributed between the two levels and the fluorescence level decreases.

Figure 2.4 presents the Electron spin resonance (ESR) spectra of an individual NV spin without and with magnetic field presence of the magnetic field. The continuous 532 nm laser excitation prepares the electron spin into the brighter $m_s = 0$ state by



Figure 2.4: (a) Electron spin resonance (ESR) without a external magentic field and (b) ESR with external magnetic field

an optical pumping process. When the microwave is applied to the NV center, and its frequency is resonant with one of the spin transitions $m_s = 0 \rightarrow m_s = 1$, the population is redistributed between the two levels, and the Photo-Luminescence (PL) decreases. A static magnetic B field induces the Zeeman effect of an electron spin, the resonance frequencies between $m_s = 0$ and $m_s = \pm 1$ will be separated into two. (Figure 2.4(b)) shows as an example of the PL versus the microwave frequency, with an applied magnetic field magnitude B = 11.48G. The two dips in PL from lower to higher microwave frequencies correspond to the resonances $m_s = 0 \rightarrow m_s = 1$ and $m_s = 0 \rightarrow m_s = +1$, respectively. Therefore, if we know the resonance frequency between the ground state $m_s = 0$ and $m_s = \pm 1$, it is possible to drive the population between these states as a function of the duration of a resonant pulse (frequency determined via ESR), obtaining oscillations known as Rabi oscillations. The interaction of a microwave field B_{MW} at frequency ν_{MW} can be described by an oscillatory term added to the Hamiltonian given by:

$$H_{MW} = \gamma_e B_{MW} S_x \cos(2\pi\nu_{MW}) \tag{2.2}$$

In matrix form the complete Hamiltonian is given by:

$$H_{MW} = \begin{pmatrix} D + \gamma_e B_z & \frac{1}{2}\cos(2\pi\nu_{MW}\tau)\gamma_e B_{MW} & 0\\ \frac{1}{2}\cos(2\pi\nu_{MW}\tau)\gamma_e B_{MW} & 0 & \frac{1}{2}\cos(2\pi\nu_{MW}\tau)\gamma_e B_{MW}\\ 0 & \frac{1}{2}\cos(2\pi\nu_{MW}\tau)\gamma_e B_{MW} & D - \gamma_e B_{MW} \end{pmatrix}$$
(2.3)

By transforming the Hamiltonian into the microwave frame by $\tilde{H} = U^{\dagger}HU - iU^{\dagger}\frac{\partial}{\partial t}U$ with $U = exp(\pm i2\pi\nu_{MW}S_z)$, assuming linear polarized light and applying the rotating wave approximation, the effective Hamiltonian is given by:

$$H_{MW} = \begin{pmatrix} D + \gamma_e B_z \pm \nu_{MW} & \frac{1}{2} \gamma_e B_{MW} & 0 \\ \frac{1}{2} \gamma_e B_{MW} & 0 & \frac{1}{2} \gamma_e B_{MW} \\ 0 & \frac{1}{2} \gamma_e B_{MW} & D + \gamma_e B_z \pm \nu_{MW} \end{pmatrix}$$
(2.4)

The case when $D + \gamma_e B_z \pm \nu_{MW} = 0$ gives us the resonance condition in order to drive transitions (see figure 2.5). We can call $\Omega_{MW} = \frac{1}{2}\gamma_e B_{MW}$ which will be the Rabi frequency that will rotate the population coherently. This frequency is proportional to the power $\propto \sqrt{I_{MW}}$, where I_{MW} is the microwave intensity [26]



Figure 2.5: Rabi oscillations: (a)Pulse sequence for Rabi oscillation (b) ESR spectra of a single NV center electron spin. PL signal of the color defect was recorded as a function of applied MW frequency in absence of magnetic field (c)

In order to investigate the evolution of a coherent superposition state ϕ_0 (created by a

 $\pi/2$ pulse), it is convenient to work in the microwave frame (equation 1.6). Since any spin manipulation is applied with a phase in regard to the microwave frame, the time evolution of a state $|\psi\rangle = \frac{|0\rangle + |1\rangle}{\sqrt{2}}$ is given by

$$\psi(t) = e^{-i2\pi \tilde{H}\tau} \psi_0 = e^{2\pi i (D + \gamma_e B_z - \nu_{MW})t} |\psi\rangle \frac{|0\rangle + |1\rangle}{\sqrt{2}} = \frac{1}{\sqrt{2}} (|0\rangle + e^{-i\phi} |1\rangle)$$
(2.5)

with $\phi = 2\pi (D + \gamma_e B_z - \nu_{MW}) \tau$ being the phase between the microwave and the corresponding eigenenergy (Larmor frequency). Since only population differences in $|0\rangle$ can be observed, the coherence is mapped by another $\pi/2$ pulse into a population difference. The observed signal S is then given by

$$S(\phi) = \sin^2(\frac{\phi}{2}) \tag{2.6}$$

This is only valid, as long the second microwave pulse stays in phase. If the second pulse is shifted by a phase ϕ_{mw} , the phase in equation 2.6 is given by $\phi = \phi_{evo} + \phi_{mw}$ This allows for example the determination of the sign of the accumulated phase [27]. In general, pulses in phase are denoted by a x (π_x) and pulses with a 90° phase difference are denoted by a y (π_y) . Another way to see it is reading the fluorescence. Since the states involved have different fluorescence, it is possible to detect population changes using optical reading. When the fluorescence is averaged, the probability P(t) of finding the spin in the $|0\rangle$ state (which corresponds to a probability (1 - P(t)) of finding the spin in the $|1\rangle$ state) is given by

$$P(t) = P(0) \frac{\Omega^2}{\Omega_{eff}^2} \sin^2(\Omega_{eff} t)$$
(2.7)

with Ω the Rabi frequency, $\Omega_{eff} = \sqrt{\delta^2 + \Omega^2}$ and $\delta = \omega_{1,0} - \omega$ the detuning between the transition frequency of the two levels $\omega_{1,0}$ and the microwave frequency ω .

To retrieve the free dynamics of spin, which is dominated by the surroundings of the NV center, a sequence of microwave pulses of the form $\pi/2 - \tau - \pi/2$ is applied in a two

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level system and then the probability of retrieving the initial state is measured. If we polarize the system to the ground state $|m_s = 0\rangle$ and then apply a $\pi/2$ pulse, the state goes to a superposition of the form $|\psi\rangle = \frac{|0\rangle + |1\rangle}{\sqrt{2}}$. Later, this rotated state evolves freely for a time τ , making the $|1\rangle$ component of the superposition to acquire a phase. The last $\pi/2$ pulse will rotate evolved state to a $|0\rangle$ as shown in figure 2.6.



Figure 2.6: (a) Pulse sequence for Ramsey fringes (b) Typical Ramsey signal for a single NV center. (c) Fourier transform of the signal we can observe 3 peaks separated by 2.1 MHz approximately which corresponds to the splitting due to the presence of a nearby ${}^{14}N$.

The transition probability of finding the state in $|0\rangle$ is given by:

$$p(t) = \frac{1}{2} \left(1 - e^{-\left(\frac{t}{T_2^*}\right)^2} \sum_{j=1}^N a_j \cos(2\pi f_j t + \phi_j) \right)$$
(2.8)

where a_j is the amplitude of each frequency, f_j the frequencies corresponding to each transition, T_2^* the decoherence time and ϕ_j the phase of each frequency.

2.1.2 Spin properties

Profound aspects of fundamental and applied physics can be probed with a single quantum system in a rotating frame, including geometric phase and effective magnetic fields. The nitrogen-vacancy (NV) centre in diamond is an ideal solid-state qubit to study quantum physics in the rotating frame. In the case of NV centers, decoherence is produced due to hyperfine interaction between electronic spin and nuclear spins in the diamond structure(see figure 2.1) [21, 1]. This can be achieved by using standard quantum sensing protocols such as Rabi oscillations, Ramsey spectroscopy or spin echo [19].

As pointed out in the previous section the ground state is a spin triplet without spin orbit coupling. Thus the main influences on the electron spin are spin-spin interaction and Zeeman interaction. The general spin Hamiltonian H for a triplet system will be given in the basis $m_s=+1,0,1$ where m_s is the spin quantization along the z-direction. In this work usually the z-direction coincides with the NV axis, and the Hamiltonian is

$$H = D_{gs}S_z^2 + \underbrace{\gamma_e B_z}_{b_e}S_z + \underbrace{A_{\parallel}S_z I_z + A_{\perp}/2(S_{+}I_{-} + S_{-}I_{+})}_{\mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I}} + \underbrace{\gamma_n B_z}_{b_n}I_z \tag{2.9}$$

The transverse part A_{\perp} of the hyperfine coupling tensor **A** induces the spin flipflops. At the ground state level anti-crossing (GSLAC) only two electron spin levels are involved $(m_s=0,1)$ whereas the $m_s=1$ level is far detuned. Thus, we concentrate on a 4×4 Hamiltonian involving these states. Therefore if we write the two spins to describe the quantum states in order to form a complete basis for the Hilbert space will be: $\{-1,0\} \bigotimes \{\uparrow,\downarrow\} = \{|-1\uparrow\rangle, |-1\downarrow\rangle, |0\uparrow\rangle, |0\downarrow\rangle\}$. This corresponds to consider only the two closest levels of the electronic triplet and the electronic spin. The matrix representation this sub-Hamiltonian is

$$\widetilde{H} = \begin{pmatrix} -\frac{b_n}{2} & \cdot & \cdot & \cdot & \cdot \\ \cdot & \frac{b_n}{2} & \frac{A_{\perp}}{\sqrt{2}} & \cdot & \cdot \\ \cdot & \frac{A_{\perp}}{\sqrt{2}} & D_{gs} + b_e + \frac{-A_{\parallel} - b_n}{2} & \cdot \\ \cdot & \cdot & \cdot & D_{gs} + \frac{b_e + A_{\parallel} + b_n}{2} \end{pmatrix}$$
(2.10)

We want to put particular interest in the hyperfine interaction tensor \mathbf{A} . The hyperfine interaction between an electronic and a nuclear spin has two components, the traditional anisotropic component of dipolar character between the spins, and another isotropic component, which is known as the Fermi contact term. This last term is due to the fact that being spins of a different nature, the Pauli's principle of exclusion does not apply, allowing both spins to coincide in their state. The representation of each component from the hyperfine interaction tensor in the international system of units is of the form

$$A_{ij} = \frac{\mu_0}{4\pi} \frac{1}{2S} \int d^3 r n_s(\mathbf{r}) \gamma_e \gamma_n \hbar^2 \left[\left(\frac{8\pi}{3} \delta(r) \right) + \left(\frac{3x_i x_j}{r^5} - \frac{\delta_{ij}}{r^3} \right) \right]$$
(2.11)

The hyperfine tensor of nucleus I consists of the Fermi-contact term (first term in parenthesis) and the dipole-dipole term (second term in parenthesis). Where $n_s(\mathbf{r})$ is the spin density of the spin state S, γ_n is the nuclear gyromagnetic ratio, γ_e is the electronic gyromagnetic ratio, r_{en} the electron-nucleus distance and δ_{ij} is the Kronecker delta. The Fermi-contact term is proportional to the spin density localized at the position of the nucleus which is dominant compared to the dipole-dipole term. The ratio of the Fermi-contact and dipole-dipole terms characterizes the shape of the spin density. The total spin density, $n_s(\mathbf{r})$, can be defined as

$$n_s(\mathbf{r}) = n_{up}(\mathbf{r}) - n_{down}(\mathbf{r}) \tag{2.12}$$

Where $n_{up}(\mathbf{r})$ and $n_{down}(\mathbf{r})$ are the spin densities built from spin-up and spin-down electrons, respectively. It is important to understand that the spin density of electrons $n_s(\mathbf{r})$ originates from the nuclear spin, and it does not represent the tensor of spin evolution of electrons, but it indicates the allowed spatial distribution when a spin is up or down, which may be places other than space [88]. Two important things to distinguish are that: this amount may vary in sign, depending on the centering admitted by space at the origin of the nuclear spin and that the quantization of spin space is constant for a defined system, but it can change due to new perturbations.

Our work proposes a method to distinguish the sign from the contact term without the use of microwave radiation. These results could open the way for characterizing spins. the hyperfine tensor can be defined as

$$\mathbf{A} = a_c \mathbb{I}_3 + \begin{pmatrix} a_{xx} & a_{xy} & a_{xz} \\ a_{yx} & a_{yy} & a_{yz} \\ a_{zx} & a_{zy} & a_{zz} \end{pmatrix}$$
(2.13)

Where $a_c = (\pm)^2_3 \gamma_e \gamma_n \hbar^2 \mu_0$ are the Fermi contact terms and $a_{ij} = \gamma_e \gamma_n \hbar^2 \frac{\mu_0}{4\pi} \frac{3r_i r_j - \delta_{ij} r_{en}^2}{r_{en}^5}$ the dipolar terms. If we write the dipolar terms as a function of the angle of the nuclear spin with respect to the NV axis, we obtain the following:

$$\mathbf{A} = a_c \mathbb{I}_3 + a_d \begin{pmatrix} 1 - 3\sin^2\theta\cos^2\phi & -3\sin^2\theta\cos\phi\sin\phi & -3\sin\theta\cos\theta\sin\phi \\ -3\sin^2\theta\sin\phi\cos\phi & 1 - 3\sin^2\theta\sin^2\phi & -3\sin\theta\cos\theta\sin\phi \\ -3\sin\theta\cos\theta\cos\phi & -3\sin\theta\cos\theta\sin\phi & 1 - 3\cos^2\theta \end{pmatrix}$$
(2.14)

where $a_d(r_{en}) = \gamma_e \gamma_n \hbar^2 \frac{\mu_0}{4\pi} \frac{1}{r_{en}^5}$.

The contribution of s-like wave functions to the charge density has a large effect on the Fermi-contact term, but negligible effect on the dipole-dipole term since the s-like wave function has a maximum at the positions of the nuclei and is an even function. In contrast to this, the contribution of p-like wave functions to the charge density do not contribute to the Fermi-contact term, and has large effect on the dipole-dipole term since the p-like wave function has a node at the place of nuclei and is an odd function. Typically, the contribution of the dipole-dipole term is significant for the spin density built from well-localized dangling bonds.

The electronic spin interacts via hyperfine interaction with a spin bath composed of the adjacent ¹⁴N and the naturally occurring 1.1 % ¹³C isotopes which are randomly distributed in the diamond lattice, but we also have samples in which the isotope ¹⁵N was implanted to create NVs .



Figure 2.7: NV center (red) interacting with a nuclear spin(green)

2.2 Magnetic field dependence and alignment

We are interested in studying the couplings that arise from the hyperfine interaction between the spins. For this it is necessary to apply an external magnetic field in the direction of the quantization axis of the NV center (NV axis). The Zeeman interaction brings the energy of a polar level of the electronic spin (either $m_s=+1$ or $m_s=-1$) to the neutral level energy ($m_s = 0$), so that the energy differences are of the same order of the coupling energies. This situation occurs in the vicinity of what is known as anticrossing level of the NV center and allows us to make an important approximation, which corresponds to consider only the two closest levels of the electronic triplet. For this, we use a confocal set-up to monitor the fluorescence and we have built coils to apply magnetic fields on the 3 axes and a system to control a magnet.

If we analyze the hyperfine matrix as a function of the external field angle θ we can obtain the equation 2.14 form and if we assume $\phi = 0$, the matrix will have the next form:

$$\mathbf{A} = a_d \begin{pmatrix} 1 - 3\sin^2\theta & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 - 3\cos^2\theta \end{pmatrix}$$
(2.15)

For a theoretical analysis, we focus on the couplings that arise from the hyperfine in-

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teraction between the spins. For this, it is necessary to apply an external magnetic field in the direction of the quantization of the NV center (NV axis). Due to the Zeeman effect, the electronic spin splits proportional to $2g_eB_z$, whereby two transition lines appear in the ESR spectrum (see figure 2.4). If we apply a strong magnetic field, we could bring the electronic spin state $m_s=-1$ next to the $m_s=0$ state. This situation occurs in the vicinity of what is known as level anti-crossing (LAC) of the NV center.



Figure 2.8: Energy levels of the coupled NV center and a nuclear spin. We can see the two transitions, which are mixed by the magnetic field in x and y.

To study the possible transitions associated with a nearby nuclear spin we can simulate how the components of the external magnetic field influence the hyperfine interaction. We must find the axis of quantization of the NV center (NV axis), then apply a magnetic field in that direction with a value close to the LAC (510G) to finally vary the field in the x and y direction in order to observe changes in the fluorescence as function of the projections of the magnetic field.

The novelty of this is that we can study the interaction of a NV center with a nuclear spin without the use of microwaves, which opens many paths for the study of NV center and its use as a qubit. Since the vast majority of experiments use microwave radiation to study couplings to nuclear spins, we propose propose a way without the use of microwaves.

2.3 Fluorescence dependence at the level anti-crossing in the presence of a nuclear spin

NV centers with its strong and spin-dependent optical transitions allow single spin readout and exquisite coherent control, which is crucial for quantum information applications. In addition, due to weak coupling to acoustic phonons, NV electron spins show long coherence times.

The polarization of a single nuclear spin has been achieved by using either a combination of selective microwave excitation and controlled Larmor precession of the nuclear-spin state [11] or a level anticrossing (LAC) in the excited state [13]. Understanding the spin states and energy levels is of critical importance for optical control of NV centers in both the ground and excited states. Especially, the hyperfine interaction couples the electron spin and nuclear spin, thus the determination of hyperfine tensors of nearby nuclei is important for the creation of entanglement states and the decoherence processes [81,84]. While there is already addressed the hyperfine tensor of 14^N and 13^C isotopes in the ground state, the lack of the detailed study on ${}^{15}N$ hyperfine signal and the proximate ${}^{13}C$ isotopes in the excited state prohibits the understanding of the intriguing physical properties of this defect. Particularly, the sign of the ${}^{15}N$ hyperfine constant is controversially used to model the LAC effect [88].

As was mentioned in subsection 2.2 the inter-system crossing results in a higher fluorescence of the $m_s=0$ state than the $m_s=-1$ states upon ${}^{3}A_{2} \rightarrow {}^{3}E$ excitation, enabling optical spin read-out and detection of the ground-state spin transitions by ODMR. Therefore if our magnetic field is perfectly aligned with the NV axis, we could make small variations in different directions and study how the electronic spin dynamics is affected by hyperfine coupling to nuclear spinspopulations are distributed in the different substates separated by nuclear couplings. According to simulations carried out for the fluorescence as a function of the θ and ϕ , we know that in the GSLAC we obtain a peak of fluorescence as a function of the projection x of the applied field. This work was initiated by Hector Duarte (PhD Student).



Figure 2.9: Schematic representation for energy levels close to the ESLAC and GSLAC

At a given magnetic field, the degree of electron-nuclear spin state mix is determined by the energy separation between the electron and nuclear states and the hyperfine interaction (that is, the matrix elements connecting these states). This also determines the energy levels and the resulting transition frequencies between the two mixed states that are eigenstates of the perturbed Hamiltonian. In turn, the intensities of the various transitions depend on the populations of the mixed states, and both the frequencies and the relative intensities of allowed transitions can be measured in the ODMR spectra. The ODMR spectrum of the $0 \leftrightarrow -1$ is far away from the GSLAC. The only transition allowed is with the same nuclear spin projection ± 1 .

2.3.1 Nuclear spin-electron spin hyperfine interaction

Controlling single nuclear spins is challenging because the spins are immersed in a noisy environment, such as a surrounding bath of nuclear spins. The electron spin of an NV center is a powerful probe of its local magnetic environment [2,3,5,11]. If a single or a few nuclear spins are located particularly close to an NV center, the hyperfine interaction can well exceed the electron spin dephasing rate $1/T_2^*$ [12]. As the magnetic field along the NV axis is increased, two spin states start to reduce their energy difference without touching each other (level anti-crossing). We consider the fluorescence of the center as a

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Figure 2.10: Simulations for the fluorescence in dependence of the x component of the external magnetic field near the LAC: In the figures you can see the contribution of the constants A_N and the angular dependence of the near nuclear spin. As shown in the figure below right for an angle $\theta = \pi/4$ you can see two peaks of fluorescence which are associated with the two possible transitions $-1 \downarrow$ and $-1 \uparrow$

function of a small magnetic field perpendicular to the symmetry axis. Despite the long coherence time, fast nuclear spin manipulation is difficult as the small gyromagnetic ratio of the nuclear spin also isolates the spin from the control fields. Several experiments NV centers have shown that nuclear spins coupled to an NV center can exhibit Rabi oscillations that are significantly faster than the expected oscillations of a bare nuclear spin that is driven by the same AC magnetic field.

Close to the ESLAC, occurring at magnetic field around 510 G, the transverse hyperfine coupling induces electron-nuclear flip-flops, and consequently polarization transfer from electron to nuclear spins [18], this will cause flip-flops of the electronic spin from 0 to ± 1 and therefore will reduce the fluorescence intensity. Therefore, for a particular transverse magnetic field and hyperfine interaction, flip-flops do not take place and the electronic spin will no flip. This will result in maintaining the fluorescence intensity. Nearly perfect

nuclear polarization has been demonstrated in previous experiments for ^{14}N [19] or ^{15}N [48,49] composing the NV center, as well as for proximal ^{13}C [50,51].

Chapter 3

Functionalization of stable fluorescent nanodiamonds towards reliable detection of Alzheimer biomarkers

Fluorescent markers have revolutionized the field of bio-imaging by endowing to different biomolecules the ability to fluorescence. Stable and non-toxic fluorescent markers are gaining attention in molecular diagnostics as powerful tools for enabling long and reliable biological studies. Such markers should not only exhibit a large lifetime under several assay conditions and present no photo bleaching or blinking but also, permit their conjugation or functionalization as crucial step for numerous applications such as cellular traking, biomarker detection and drug delivery. Here, we report the functionalization of stable fluorescent markers based on nanodiamonds (NDs) with a bifunctional peptide, which is constructed by a cell penetrating peptide and a β -sheet breaker six amino acids long peptide that is able to recognize Amyloid- β (A β) aggregates, a biomarker for the Alzheimer disease (AD). Our results indicate that functionalized ND (fND) are not toxic and can be internalized in the assayed cells and allow the ultrasensitive detection of amyloid fibrils and amyloid aggregates in the brain of a mice with AD. Moreover, the fluorescence of fNDs is more stable than common color markers used to stain $A\beta$ such as Thioflavin T and FITC. These results pave the way for performing ultrasensitive and reliable detection of $A\beta$ aggregates involved in the pathogenesis of the Alzheimer disease.

3.1 Properties of fluorescent nanodiamonds

Diamond-based fluorescent markers use defect color centers as their active emitting part. Defects in the crystalline structures of diamond can lead to localized electronic states within the diamond band gap which is of the order of 5.6 eV [138]. Due to this large gap, non-defectors crystalline diamond exhibits fluorescence only if a very short wavelength laser is used. Also, when a defect is created, not only its ground state but also its first optically excited state might exist within this band gap. Therefore several different and stable optical defects can be hosted in the diamond matrix [139, 140]. A common color center is the nitrogen-vacancy (NV) center whose atomic structure is shown in figure 3.1 (a). They can be approximated as a two-level system that upon laser excitation at 532 nm shows a broad emission at around 700 nm (see figure 3.1 (b)).



Figure 3.1: Emission properties of NV color centers in diamond. (a) Atomic configuration of NV color defect in diamond. One nitrogen (blue) and three carbons (green) are adjacent to a vacancy cite. The NV center can exist in two charge configurations, the neutrally charged NV^0 center and the negatively charged NV^- center. (b) Two level model of the electronic transitions of the NV^- color center. (c) Emission spectrum of nanodiamonds. The spectrums shows a zero phonon line at 575 nm of the NV^0 center and at 637 nm for the NV^- center. Both centers show a broad phonon sideband.

Ion implantation can produce defects with energies varying between few keV and MeV and posterior annealing at temperatures between 400 and 1200 °C [141, 142, 143]. In the case of color centers based on naturally abundant atoms, electron irradiation and annealing are used to mobilize vacancies [144, 145] in diamond to produce the right atomic configuration that leads to the fluorescent structures. Once the defect is created in the diamond matrix, its fluorescent properties are extremely stable.

In this work, we used a 35 nm diameter NDs acquired from Academia Sinica (brFND-35). Each ND contains on average about 15 nitrogen-vacancy color centers. For these particular NDs, NV exist in two different charges state: neutrally charged NVs (NV^0) and negatively charged NV (NV^-) with zero phonon line at 575 nm and 637 nm, respectively, under 532 nm laser excitation (see figure 3.1 (c)). We noted that the emission lines in the biological tissue window [146] and that the fluorescence of such defects do not present blinking or photobleaching provided they formed deeper than 2 nm from the surface [147, 148, 149].



Figure 3.2: Atomic force microscopy (AFM) image of brFND-35nm nanodiamonds: The image was taken at the AFM from our lab by Enrique Rodriguez. As we can observe, the NDs have different sizes and different shapes, but their great majority has an approximate diameter of 35 nm.

3.2 Functionalization of nanodiamonds

The functionalization process was carried out by Francisco Morales and Dr. Marcelo J. Kogan of the Department of Pharmacological and Toxicological Chemistry of the University of Chile. The NDs were functionalized with R7-CLPFFD peptide, a bi-functional peptide

that confers to ND's different charachteristics and functionalities. It is composed of two segments. The CLPFFD segment contains the native sequence of $A\beta$ and allows the molecule to recognize the aggregates of $A\beta$ [150, 151]. CLPFFD peptide is a modification of a peptide first designed by Soto et. al. [151] and has been used to stabilize, functionalize and enhance the brain arrival of gold nanoparticles [152, 153]. In the other side, the R7 segment is designed to promote the cell penetration as it has been shown that the R7 peptide allows to cross cell membranes [136]. Also the R7 peptide has a net charge of +7 allowing the electrostatic binding of the peptide to the negatively charged NDs surface (see figure 3.3 (a)) that contains carboxylate groups [154, 155]. Both peptides have been used separately for functionalizing nanoparticles and transfering their properties to the nanocargo [151, 153, 156]. In addition, it has been previously demonstrated that this peptide attached to gold nanospheres and gold nanorods selectively bind to $A\beta$ aggregates [152, 150, 157].



Figure 3.3: Functionalized nanodiamonds (fNDs). (a) Bi-functional peptide composed of a cell penetrating R7 peptide (blue dotted area) thath allows to their cargo to enhance their cellular up-take and a β -sheet breaker CLPFFD peptide (red dotted area) that recognizes toxic A β aggregates perent in AD. (b) Zeta potential (Zp), hydrodynamic diamater (HD) and Polydispersive index (PDI) of naked and functionalized NDs. (c) Illustration and properties of fNDs.

To evaluate the adsorption of the R7-CLPFFD peptide to the surface of NDs, Francisco Morales and Dr. Marcelo J. Kogan of the Department of Pharmacological and Toxicolog-

ical Chemistry of the University of Chile and Dr. Nathalie Cassanova of the Department of Physics of the Pontifical Catholic University measured the Zeta potential (Zp) and a hydrodyanmic diamater (HD) of the fNDs in aqueous solution as an indicator of the electrostatic coating of this bi-functional peptide. The Zp values after peptide adsorption increased from -29.7 ± 1.6 to $+29.1 \pm 4.0$ mV. This indicates that the nanocrystal was positively functionalized. The HD values increased from 74.3 ± 0.5 to 163.3 ± 2.0 nm, about twice the diamater of the bare nanocrystal. Both of these values confirm the functionalization. The Zp and HD parameters of the fNDs remained stable after several washouts. Moreover high-resolution transmission electron microscopy (HR-TEM) images of fNDs compared to bare NDs alos indicates the adsorption of the peptide (see figure 3.4). The electronic density gradient was observed to be denser at the center than in the periphery of the particles, indicating that NDs were sorrounded by peptides. The average patricle diameter was 199 ± 56 nm, larger than the bare NDs diameter. In the HR-TEM figure it is possible to appreciate that the sorroundings of the ND shows a thick layer of peptide (see figure 3.4). Lastly, the fluorescence spectrum of NDs did not show appreciable changes after the functinalization and severa washouts. These results support the successful adsorption of the R7-CLPFFD peptide onto the ND surface. In the following chapter we will evaluate the interaction of these R7-CLPFFD functionalized NDs with cells and their properties as color markers.



Figure 3.4: Structure of nanodiamonds, HR-TEM. Electron micrographs showing (A) naked nanodiamond and (B) functionalized nanodiamond.

3.3 Cell internalization and ultrasensitive detection of functionalized nanodiamonds

The positive R7 region of the R7-CLPFFD peptide endows the cell penetrating peptide (CPP) function allowing fNDs to cross the cell membrane and penetrate the cell [158]. To evaluate this, América Chandía and Dr. Alejandra Alvarez of the Department of Cell and Molecular Biology of the Pontifical Catholic Unversity, incubated fibroblast cells (30000 cell/ml) with 8 pM fNDs. After 6 hours the samples were washed and fixed to evaluate the fNDs internalization. The microtubules and cytoeskeleton components were immune stained using anti- β -tubulin antibody (1 : 1000) and a secondary antiboy conjugated with Alexa Fluor 488 (1 : 1000) for cell visualization puporses. The samples were observed in our home-built laboratory confocal microscope setup. This setup allowed us to use the NDs concentration as low as pM and even detect NDs containing single emitters due to the high sensitivity of the Avalanche photon detector (APD). Figure 3.5 (a) shows a common fibroblast cell image with fNDs. The two types of markers used in this experiment were observed in the same focal plane.

Two different rengions of interest (ROIs) of the sample show clearly distinguishable spectra: one corresponding to the Alexa Fluor 488 and the other corresponding to the fND (see figure 3.5 (c) and (b), respectively). Figure 3.5 (a) shows fNDs inside the perimeter of the cell whereas none was observed outside the cell (washed samples). Different focal planes were evaluated.

In addition, no fNDs were observed on top of the cell nucleus. These observations supports that fNDs are inside the cell. In the contrary, when non-functionalized NDs were used, the NDs fluorescnce intensity decreases by 60%. In order to evaluate the fluorescence stability of the fNDs, we compared it with the fluorescence of other color markers. First. fibroblast cells were stained with Alexa Fluor 555 and its fluorescence was monitored for 5 min under several excitation powers. We observed that their fluorescence intensity decreased over time at a rate of 0.8 Hz/mW (see figure 3.6). We also monitored the fluorescence of Alexa 488 and FITC (a common marker of A β aggregates) in figures 3.6 (c) and (d), respectively, under several laser power excitation. In contrast, the fluorescence of



Figure 3.5: Cell internalization of fNDs. (a) Composed image of fibroblast cell with Alexa 488 labeled tubulin excited under 488 nm laser illumination (green) and fNDs excited under 532 nm laser illumination(red). (b) Fluorescent spectrum of nanodiamonds showing the characteristic zero phonon lines at 637 and 575 nm. (c) Fluorescence spectrum of Alexa 488.

fNDs remained constant (see figure 3.5 (a) red circles). Therefore, dimaond based color centers are more stable than other color markers. Their fluorescence has no noticeable decay for days, months or even years under continuous laser excitation, enabling the study of longer experiments.

3.4 Binding of fNDs to $A\beta$ fibers

We performed *in vitro* experiments to evaluate the ability of fNDs to bind to $A\beta$ aggregates. $A\beta$ fibers were grown *in vitro* and the incubated with fNDs. The decoraton of fibers was evaluated by transmission electron microscopy (TEM). In figure 3.6 (a) is possible to appreciate the co-localization between fibers and fNDs. Almost no fNDs are observed in regions without fibers. This observation suggest that the CLPFFD region of the peptide is on the fNDs surface and thus allows its interaction with $A\beta$ fibers. Furthermore, in order to visualize the association between fNDs and $A\beta$ plaques, slices of brain tissues of AD transgenic mice, that over expressed $A\beta$, were incubated with fNDs. Then the slices were incubated with an antibody conjugated with Alexa 488 for the $A\beta$ plaques



Figure 3.6: Charachterization of the photo stability of diamond-based color marker and Alexa Fluor conjugateds. (a) Fluorescence traces under continuous 532 nm wavelength laser illumination of Alexa 555 (green markers) and fNDs containing nitrogen-vacancy color centers (red markers) for several laser powers. (b) Decay rate of Alexa 555 versus the excitation power. The fluorescence decreases its intensity at a rate of 0.8 Hz/mW whereas that of the fNDs remained steady. (c) Fluorescence intensity as a function of time of Alexa 488 and (d) of FITC.

visualization. In figure 3.6 (b, c, d and e) is possible to appreciate the association between both fluorescent labels. Therefore, fNDs can serve as fluorescent markers of A β aggregates with better photostability than other fluorescent markers. In fact, usually A β fibers are stained with Thioflavine T (ThT) [150, 159], a fluorescent marker which is known for its short lifetime when bound to amyloid fibers [160].

These results might constitude a powerful method for studying the formation of $A\beta$ aggregates in the evolution of the AD. It has been shown that fNDs have been internalized to fibroblast cells, resulting in no fNDs in the region between the cells. At the same time, this bi-functional ND peptide conjugation can serve as a biodetector of extracellular $A\beta$ fibrils once these nanosystems attach to $A\beta$ aggregates thanks to its CLPFFD peptide region and the ability to cross biological barriers. The observation of the charachteristic emission spectra of ND fluorescence in the extracellular region might serve as an indicatior of the presence of $A\beta$ fibrils. The remarkable fluorescent stability of the ND emission would allow for reliable biodetection of the $A\beta$ for long experimental conditions and therefore the opportunity to track the progression of the disease.

Chapter 4

Conclusions

Nitrogen-vacancy centers in diamond are stable quantum systems that may be carefully manipulated to provide valuable information about local magnetic fields. The energy structure of these defects allows for careful manipulation with both optical and microwave frequencies, while specific decay channels enable easy optical pumping of the $m_s = 0$ ground state.

We can conclude for chapter 2 that we can study the hyperfine interaction without the use of microwave, which opens a path of possibilities for experiments. In addition we built a coil system which allows us to control the field in each axis and close to the anti-crossing level For chapter 3 we have shown that the surface of nanodiamonds containing stable fluorescent color centers can be functionalized to perform multiple tasks without damaging the stability of their fluorescence. This remarkable stability allows for the detection of NDs with concentrations as low as a picomolar using confocal microscopy. In particular, the presented peptide functionalization might allow for reliable and long-term experiments on the formation or presence of the A β aggregates. Our results indicate that functionalized ND (fND) are not toxic and can be internalized in the assayed cells and allow the ultrasensitive detection of amyloid fibrils and amyloid aggregates in the brain of a mice with AD. Moreover, the fluorescence of fNDs is more stable than common color markers used to stain A β such as Thioflavin T and FITC. These results pave the way for performing ultrasensitive and reliable detection of A β aggregates involved in the AD.

Appendix A

Supplemental material for chapter 2

A.1 Hamiltonian between electron-spin 1 and Nuclear-spin 1/2 in diamond

As I mentioned before the Hamiltonian of interaction for the NV center can be described as:

$$H = D_{gs}S_z^2 + \underbrace{\gamma_e B_z}_{b_e}S_z + \underbrace{A_{\parallel}S_z I_z + A_{\perp}/2(S_{+}I_{-} + S_{-}I_{+})}_{\mathbf{S}\cdot\mathbf{A}\cdot\mathbf{I}} + \underbrace{\gamma_n B_z}_{b_n}I_z \tag{A.1}$$

Where the hyperfine interaction between an electronic and a nuclear spin has two components, the traditional anisotropic component of dipolar character between the spins, and another isotropic component, which is known as the Fermi contact term. This last term is due to the fact that being spins of a different nature, the Pauli's principle of exclusion does not apply, allowing both spins to coincide in their state. The representation of each component from the hyperfine interaction tensor in the international system of units is of the form:

$$A_{ij} = \frac{\mu_0}{4\pi} \frac{1}{2S} \int d^3 r n_s(\mathbf{r}) \gamma_e \gamma_n \hbar^2 \left[\left(\frac{8\pi}{3} \delta(r) \right) + \left(\frac{3x_i x_j}{r^5} - \frac{\delta_{ij}}{r^3} \right) \right]$$
(A.2)

The ratio of the Fermi-contact and dipole-dipole terms characterizes the shape of the spin density. The total spin density, $n_s(\mathbf{r})$, can be defined as:

$$n_s(\mathbf{r}) = n_{up}(\mathbf{r}) - n_{down}(\mathbf{r}) \tag{A.3}$$

$$\mathbf{A} = a_c \mathbb{I}_3 + \begin{pmatrix} a_{xx} & a_{xy} & a_{xz} \\ a_{yx} & a_{yy} & a_{yz} \\ a_{zx} & a_{zy} & a_{zz} \end{pmatrix}$$
(A.4)

To achieve the reconstruction of the hiperfine matrix, we approach the site with this:

$$\mathbf{S} \cdot \mathbf{A} \cdot \mathbf{I} = \begin{pmatrix} \frac{a_c + a_d (3\cos^2 - 1)}{2} & \frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta & \frac{3a_d}{2\sqrt{2}} e^{-i\phi} \sin\theta\cos\theta & \frac{3a_d}{2\sqrt{2}} e^{-2i\phi} \sin^2\theta & 0 & 0 \\ \frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & -\frac{a_c + a_d (3\cos^2 - 1)}{2} & a_c - \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2\sqrt{2}} e^{-i\phi} \sin\theta\cos\theta & 0 & 0 \\ \frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & a_c - \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{-i\phi} \sin\theta\cos\theta & \frac{3a_d}{2\sqrt{2}} e^{-2i\phi} \sin^2\theta \\ \frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin^2\theta & -\frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & 0 & 0 & a_c - \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & a_c - \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{a_c + a_d (3\cos^2 - 1)}{2} & -\frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{2i\phi} \sin^2\theta & -\frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & \frac{a_c - a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{a_c + a_d (3\cos^2 - 1)}{2} & -\frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{2i\phi} \sin^2\theta & -\frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{a_c + a_d (3\cos^2 - 1)}{2} & -\frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{2i\phi} \sin^2\theta & -\frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{a_c + a_d (3\cos^2 - 1)}{2} & -\frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{2i\phi} \sin^2\theta & -\frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{-i\phi} \sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}} e^{2i\phi} \sin^2\theta & -\frac{3a_d}{2\sqrt{2}} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2} e^{i\phi} \sin\theta\cos\theta & \frac{a_c + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d + a_d (3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d + a_d (3\cos^2 - 1)}{2$$

If we rewrite the Hamiltonian including all the terms we obtain the following:

$$\mathbf{H} = \begin{pmatrix} b_e + b_n + A_{zz}/2 & \Lambda & \Delta + b_\perp & \Gamma & 0 & 0 \\ \Lambda & b_e - b_n - A_{zz}/2 & a_c + \Gamma & -\Delta + b_\perp & 0 & 0 \\ \Delta + b_\perp & a_c + \Gamma & b_n/2 & 0 & \frac{3a_d}{2\sqrt{2}}e^{-i\phi}\sin\theta\cos\theta & \frac{3a_d}{2\sqrt{2}}e^{-2i\phi}\sin^2\theta \\ \Gamma & -\Delta + b_\perp & 0 & -b_n/2 & a_c - \frac{a_c + a_d(3\cos^2 - 1)}{2\sqrt{2}} & -\frac{3a_d}{2}e^{-i\phi}\sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}}e^{i\phi}\sin\theta\cos\theta & a_c - \frac{a_c + a_d(3\cos^2 - 1)}{2\sqrt{2}} & -b_e - b_n - A_{zz}/2 & -\frac{3a_d}{2}e^{-i\phi}\sin\theta\cos\theta \\ 0 & 0 & \frac{3a_d}{2\sqrt{2}}e^{2i\phi}\sin^2\theta & -\frac{3a_d}{2\sqrt{2}}e^{i\phi}\sin\theta\cos\theta & -\Lambda & -b_n + b_n - A_{zz}/2 \end{pmatrix}$$
(A.6)

$$\mathbf{H} = \begin{pmatrix} b_e + b_n + A_{zz}/2 & \Lambda & \Delta & \Gamma \\ \Lambda & b_e - b_n - A_{zz}/2 & a_c + \Gamma & -\Delta \\ \Delta + b_\perp & a_c + \Gamma & b_n/2 & 0 \\ \Gamma & -\Delta + b_\perp & 0 & -b_n/2 \end{pmatrix}$$
(A.7)

Where:

$$a_d(r_{en}) = \gamma_e \gamma_n \hbar^2 \frac{\mu_0}{4\pi} \frac{1}{r_{en}^5}$$
$$a_c = (\pm) \frac{2}{3} \gamma_e \gamma_n \hbar^2 \mu_0$$
$$b_e = D + \gamma_e B_z$$
$$b_n = \gamma_n B_z$$
$$A_{zz} = a_c + a_d (3\cos^2 \theta - 1))$$
$$\Gamma = \Delta \tan \theta$$
$$\Delta = \frac{3a_d}{2\sqrt{2}} \sin \theta \cos \theta$$
$$\Lambda = \sqrt{2}\Delta$$
$$\epsilon = \frac{1}{\sqrt{1 + (\frac{\gamma_e}{\gamma_n} \frac{\Delta}{D - a_c/2})^2}}$$

Using only the Zeeman effect approximation, we find that degeneration occurs when two

auto-energies are zero at the same time, leaving us to solve a fourth order equation for magnetic fields. This equation is simplified considerably the approximation of gyromagnetic ratios, where $\gamma_n/\gamma_e \sim 10^{-3} \sim 0$, thus $\epsilon \to 1$.

Now we can rewrite the Hamiltonian using this aproximation:

$$\mathbf{H} = \begin{pmatrix} b_e + b_n + A_{zz}/2 & 0 & 0 & 0\\ 0 & b_e - b_n - A_{zz}/2 & a_c + \Gamma & 0\\ 0 & a_c + \Gamma & b_n/2 & 0\\ 0 & 0 & 0 & -b_n/2 \end{pmatrix}$$
(A.8)

To perform the fluorescence simulations *Hector Duarte* used the "Master Equation" which is used to describe the temporal evolution of a system, which can be modeled as a probabilistic combination of states at any given moment. The switching between the states is determined by a transition speed matrix. These equations are a set of differential

equations in time of the probabilities that the system occupies in each of the different states. For this a density operator is defined and the von Neumann [161, 162] equation is solved. This equation describes how a density operator evolves in time $(i\hbar \frac{\partial \rho}{\partial t} = [H, \rho])$, density matrices make it much easier to describe the process that involves entanglement with other systems and calculate its consequences.

Thus, the condition for the magnetic field perpendicular B_{\perp} , as a solution for degenerate

auto-energies is equal to zero remains as:

$$\mathbf{H} = \begin{pmatrix} b_e + A_{zz}/2 & \Lambda\sqrt{2} & \Delta + b_{\perp} & \Gamma \\ \Lambda\sqrt{2} & b_e - A_{zz}/2 & a_c + \Gamma & -\Delta \\ \Delta + b_{\perp} & a_c + \Gamma & 0 & 0 \\ \Gamma & -\Delta + b_{\perp} & 0 & 0 \end{pmatrix}$$
(A.9)

Where $b_{\perp} = \frac{1}{2\sqrt{2}} \gamma_e B_{\perp}$, thus the condition comes from demanding the degeneration of two of the Hamiltonian's autoenergies, and this is achieved, if and only if, there is:

1. A perpendicular magnetic field

2. A positive Fermi contact term (approximately)

Both demands are related with the value that the perpendicular magnetic field must take for there to be degeneration. This value is: $\gamma_e B_{\perp} = \sqrt{3a_d(2a_c + a_d)\sin^2\theta}$. Note that, $a_d > 0$ is always a term related to dipolar interaction, $\sin^2\theta > 0$ it's never negative. and $a_c > 0$ or <0 undefined, this contact term allows or does not exist, a perpendicular magnetic field that achieves degeneration, by means of the condition $a_c > -a_d/2$ (this is the true condition). The size of the contact term is usually much greater than the dipolar, in this case, we say that if:

 $a_c > 0 \rightarrow$ degeneration, which would be measurable.

 $a_c < 0 \rightarrow$ No degeneration, we would never see the degeneration experimentally

$$\mathbf{A} = A_N \begin{pmatrix} 1 - 3\sin^2\theta & 0 & 0\\ 0 & 1 & 0\\ 0 & 0 & 1 - 3\cos^2\theta \end{pmatrix}$$
(A.10)

Where A_N are all the constant contributions due to the Fermi contact en the dipolar terms.

A.2 Coil system

To build the coil system and determine the number of turns required in each coil we use the Biot-Savart law, which tells us the following:

$$d\vec{\mathbf{B}} = \frac{\mu_0}{4\pi} \frac{I d\vec{\mathbf{s}} \times \hat{\mathbf{r}}}{r^2} \tag{A.11}$$

From there, we can use the law to derive the expression for magnetic field.

In our case we are only interested in the z component since we must calculate the number of turns that the coil will have for the field that we want to generate, therefore integrating the equation A.11 in a loop $(0 \rightarrow 2\pi)$ we obtain:

$$B_z = \frac{\mu_0 I R^2}{2} \frac{1}{(R^2 + z^2)^{3/2}}$$
(A.12)

Therefore we know how the magnetic field behaves at a certain distance from the center of a loop. By a other hand to calculate the number of loops that are necessary to generate a given field we use the law of Ampere which tells us that the magnetic field inside a solenoid with N loops is:

$$\oint \vec{\mathbf{B}} \cdot d\vec{\mathbf{l}} = \frac{\mu_0 N I}{L} \tag{A.13}$$

Where L the length of the coil and I the current. With this we combine both laws and Ohms law $(I = V/R = \frac{V}{\rho_{cu}L_{wire}/(\pi r_{wire}^2)})$ to know what the dimensions of our coils should be, the number of turns in each, the diameter of the cable and the length necessary to obtain the necessary turns. Since our voltage source (Rhode & Schwarz HMP2030) has limits for the current we want to apply (0 - 32V/0 - 5A).

In order to build a good system of coils in the 3 axes, simulations were performed (figure) to have a prior notion of the magnetic field that could be generated.

The system consists of 5 coils, 2 for the x-y axes and one for the z axis, which will be located on the edge of the objective lens (see figure). As you can see in the figure, the field between the two coils is approximately 200.6 G and the edge of the coil z is 712 G, with a current of 4.8 A



Figure A.1: Magnetic field simulation in function of distance, number of loops and current



Figure A.2: Coil system design, the configuration shows 2 coils for xy-axis and one coil for the z-axis



Figure A.3: Coil system image. Each coils has 1200 turns the

Appendix B

Supplemental material for chapter 3

B.1 Experimental set-up

B.1.1 Confocal microscope

According to the figure B.1 we can see that the fluorescence produced by the excitation, then passes trough a dichroic mirror (532 nm BrightLine, laser dichroic beam splitter) which only reflects green light. Both beams (green laser and fluorescence) were collimated to obtain a good resolution. The excitation beam is focused onto the sample with an oil-immersion-lens (Nikon Plan 100x NA:1.25) the photons emitted by the defect are directed to a black box (to avoid light from other sources) and counted through an avalanche photodetector (APD: Perkin Elmer SPCMAQRH-14-FC). Before the fluorescence passes trough the dichroic mirror we mounted a magnetic mirror which is directed to a spectrometer (QE Pro Spectrometer, OceanOptics) this allows us to read the spectrum of the defect at the same time that we are measuring the fluorescence.

To obtain the fluorescence range of the NV center we set a long pass filter of 600nm and a notch filter before the APD, this is to avoid other wavelength that we are not interested to locate the NV centers we used a high precision piezoelectric (Modular Piezo-Controller E501, Physik Instrumente(PI)GmbH&Co.KG) with which the sample can be scanned by the target moves in XYZ.



Figure B.1: A green laser and a blue laser is coupled to an optical fiber, both are focused to a telescope collimator, then the beams are directed to a mountable dichroic mirror (for 488 nm and 532 nm for the green laser) which reflects the beam toward to the oil-immersion-lens. This lens focuses the beam onto the sample which is attached to the piezoelectric (so that we can make a XY scanning). Then sample emmittes fluorescence back trough the oil-immersion-lens which passes trough a magnetic mirror splitter (coupled to the spectrometer) and then trough the dichroic mirror. For collecting the flourescence we have a second telescope and collimator so we know that we are collecting all the possible photons, before being couples we have a 630 nm filter and a notch filter

B.1.2 Microwave generator

We can apply different microwave pulse sequences to manipulate the electron spin associated to the NV center and rotate this spin producing transitions in the sub-state $m_s = \pm 1$. Furthermore, continuous wave spectroscopy provide us the measurement of the energy levels. For generating microwaves we have a signal generator (Rohde & Schwarz SMF100A Signal Generator), which can produce frequencies between 100 kHz and 22 GHz, these signals are sent to a switch, so that we can turn on/off the signal, which is driven by a pulse generator (PulseBlaster ESR 500MHz) and its connected to a computer where we can control the pulse that we want to send. The signal is amplified and then is connected to a low loss cable, this microwave signal passes through a 25 μ m diameter copper wire which passes trough the sample and in the other side is attenuated by a 50 Ω resistance (see figure B.2)



Figure B.2: Microwave generator set-up: We can see the signal generator which we can turn on/off sending TTL (Transistor Transistor Logic) signals from the PulseBlaster.

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