## Interfacing spin-based quantum sensors with complex systems



### Qiushi Gu

Department of Physics University of Cambridge

This dissertation is submitted for the degree of Doctor of Philosophy

Christ's College

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### Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared and specified in the text. It does not exceed the prescribed word limit for the relevant Degree Committee. This thesis contains fewer than 60,000 words including appendices, tables and equations and has less than 150 figures.

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#### Abstract

**Title:** Interfacing spin-based quantum sensors with complex systems. **Author:** Qiushi Gu

Nitrogen-vacancy (NV) centres are naturally occurring colour centres in bulk diamond crystals that enable quantum sensing via optically active spin ground states. They have demonstrated high sensitivity in thermometry and magnetometry, reaching a temperature precision of  $5\text{mK}/\sqrt{\text{Hz}}$  and magnetic field sensitivity of a single nuclear spin. Due to their nanometer size and ability to be chemically functionalised, diamond nanocrystals (nanodiamonds) offer sensing in systems inaccessible with bulk diamonds. Of particular interest is magnetometry and thermometry in biological systems. This thesis explores interfacing nanodiamonds host NV centres with complex systems for decoherence spectroscopy, relaxometry and thermometry.

In Chapter 1, it is demonstrated that nanodiamonds can perform NMR experiments with nanoscale sensing volume. In this experiment a dynamical decoupling sequence is used to sense the stochastic and stationary magnetic field at the site of the NV created by the target nuclear spins. The spectral resolution of this technique is sufficient to distinguish between two different nuclear species <sup>1</sup>H and <sup>19</sup>F. Furthermore, a self-referenced calibration scheme is proposed and verified with experimental data to enhance the accuracy of concentration measurements, previously limited by the geometrical variability between nanodiamonds.

In Chapter 3, nanodiamonds are used to study triplet exciton diffusion in an organic semiconductor material. In this experiment, NV relaxometry is used to probe the diffusion process of optically generated triplet excitons. It was observed that the longitudinal relaxation time of NV ( $T_1$ ) is related to the optical excitation of the organic material and that the depolarisation rate saturates at large optical excitation power. A stochastic Liouville equation model is used to model the spin diffusion process.

In Chapter 4 and 5, nanodiamonds are used in thermometry to measure time-varying signal in biological cells. In this experiment, the theoretical temperature sensitivity is examined using Fisher information formalism and compared with experimental sensitivity. This forms the basis of optimal ODMR frequency sampling in situations where the ODMR lineshape fluctuates. It was also demonstrated that it is possible to simultaneously perform ODMR measurements and track single nanodiamonds, using an orbital tracking method. This enables a single nanodiamond to be measured over hours despite thermal motion. Simultaneously, the temperature can be determined with a sensitivity of  $1.5 \text{ K}/\sqrt{\text{Hz}}$  and the position with an accuracy of 7.7 nm. With these technical improvements the intracellular temperature variation in HeLa cells is measured when the cells are subjected to external temperature modulation or chemical stimuli.

Finally, despite the success of NV centres in diamond for room-temperature quantum sensing, in Chapter 6, we explore a novel two dimensional material, hexagonal boron nitride, that offers a more scalable platform for future quantum sensing applications. In this experiment, we identified stable spin-addressable single emitters at room temperature. ODMR experiments reveal the fine structure of the defect which was modelled as a spin triplet. Photokinetic properties are investigated with second order intensity correlation measurements and an optical level structure is proposed to provide a brand new understanding of this novel quantum sensing platform. This provides a basis for tackling the issues facing nanodiamond. I would like to dedicate this thesis to my parents, who encourage me to pursue my interest, and always support me to the best of their ability...

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- Optically detected magnetic resonance (ODMR) measurement displays two resonances at  $f_0 = (2848.34 \pm 0.08)$  MHz and  $f_1 = (2866.43 \pm 0.09)$  MHz. The measurement is performed by continuously measuring the PL while changing the microwave frequency (inset).

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2.1 Experimental setup for NMR experiment. The setup is a typical confocal microscope. The sample is mounted on a piezo stage (bottom left) to allow nanoscale movement of the sample. A 100x air objective with a numerical aperture of 0.9 is used. A CW laser with a wavelength of 532 nm is used for excitation and the fluorescence passes through a long-pass filter (LP550) at 550 nm is used to filter the scattered excitation light. The fluorescence is then coupled into a single-mode (SM) fibre, which as a pinhole. The photons are detected by an avalanche photodiode (APD). Pulsed MW is generated by an arbitrary waveform generator (AWG) and pulsed initialisation and readout are also controlled by the same AWG. Lateral scanning is achieved with a piezo mirror. DM550: dichroic mirror at 550 nm. A permanent magnet is used to provide a static magnetic field and is mounted on a two-axis rotation stage to align the magnetic orientation with the NV axis. A miniature translation stage is also mounted on the rotation stage to vary the applied magnetic field without changing its orientation.

- 2.2 Experimental configuration. **a**) shows nanodiamond on glass substrate covered by  $5 \mu m$  of a fluorine-rich liquid (PFPE), shown as a blue liquid environment on the figure. Microwave delivery is achieved with a loop antenna. The optical excitation is focused down from the liquid side. The surface of nanodiamonds is covered by a hydro-dense surface layer (dark blue). The nanodiamond used is typically 30 nm in diameter. **b**) shows the CPMG XY8-k sequence which also measures the NMR signal. The oscillating magnetic field generated by the nuclear spin bath (black curve) is frequency matched to the decoupling period between microwave  $\pi$ -pulses (yellow) and leads to a measurable phase of the quantum sensor. The eight-pulse subunit shown is repeated k times to form the complete sequence. **c**) shows a coherence measurement performed on one nanodiamond device using an XY8-k protocol with a constant  $\tau = 0.5 \,\mu$ s, showing a long  $T_2 = 35 \,\mu$ s.
- 2.3 Illustration of the NMR signal. **a**) shows the lineshape as a result of XY8k sequence which has a linewidth decreasing with the number of  $\pi$ -pulses applied. Illustration of contrast and CPMG XY8 decoherence measurement. As the spacing between  $\pi$ -pulses is varied, the intrinsic decoherence manifests as a decay in PL. **b**) shows this decay (dashed curve). An additional  $\pi$ -pulse is applied right before readout to create the contrast (lower branch of the dashed curve). When a NMR signal is present, the magnetic field due to nuclear dipole moment precession create a characteristic lineshape (solid curve).
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- Estimation of the geometry of the measured nanodiamond device using Monte 2.5 Carlo simulation. a) Left: Cross section of an example nanodiamond device procedurally generated by the Monte Carlo simulation, illustrating geometric parameters d (distance between the NV centre and the nanodiamond surface) and t (thickness of the hydrogen surface layer). We generate the nanodiamond shapes using a realistic model for surface roughness and place an NV centre within the each nanodiamond. Right: Eight examples of generated nanodiamond devices out of the 440 instances used for simulation. b) Histogram of the values of the geometric parameters extracted from the experimental data and Monte Carlo simulation. Each individual nanodiamond device shape generated by the simulation is plotted as a white dot, with coordinates indicating the values of t and d that are consistent with both measured data and the shape of that generated nanodiamond device. The single-parameter histogram for surface-layer thickness t (NV to surface distance d) is plotted as white bars on the vertical (horizontal) axis.
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2.6 Comparison of different calibration methods for the nanodiamond devices. **a**) Histogram of simulated analyte concentration measurements for different nanodiamond geometries, with surface-layer thickness with surface-layer thickness t held constant at t = 1 nm. Batch calibration shows large systematic errors, as measured by geometric standard deviation  $\sigma$ , due to uncontrolled geometry variation. Surface-enhanced calibration reduces systematic error by almost a factor of 10. **b**) We perform simulations similar to those shown in **a**), but allow surface-layer thickness t to vary between nanodiamonds in a log-normal distribution with geometric mean of  $\bar{t} = 1$  nm. When t has a large geometric standard deviation, this source of noise reduces the overall measurement accuracy.

- Illustration of the sensing of paramagnetic triplet excitons in DPH using a 3.1 NV spin sensor. a) Experiment configuration. Diamond materials (either bulk or nanocrystal, pale blue slab) are coated with DPH thin film (only carbon atoms are shown), where the photo-generated triplet excitons (blue arrows) causes a faster NV (red arrow) relaxation. b) is the Jablonski diagram showing optical transitions of DPH. The optical transitions upon excitation from the ground state  $S_0$  (purple upward arrow), the  $S_1$  population decays to the ground state (purple downward arrow) or undergoes singlet-fission to form triplet pairs (dashed arrow). Each triplet in the pair has a zero-field splitting (ZFS) with D = -2.4 GHz and E = 270 MHz [232]. The triplet to ground state transition is spin-forbidden (cross on the red dashed arrow) and thus only occurs slowly, giving phosphorescence. c) Molecular structure of DPH. d) the carbon atoms and carbon-carbon bonds of DPH in a unit cell of a orthorhombic DPH crystal. The orientation of the molecules are such that the intermolecular distance between molecules are greater along the c axis than along the other two axes.
- 3.2 Optical characterisation of DPH. a) is a wide-field image of DPH crystal on glass. The image is 200 µm wide. The surface texture and wrinkles are indication of the coverage of the material and the length scale of these ridges are on the order of a few micrometers. b) UV-Visible absorption spectrum of DPH on glass substrate for a few samples. The 400 nm peak corresponds to absorption peak of DPH. A general concern for organic semiconductors is that properties differ significantly between samples. The absorption spectrum does not vary significantly from sample to sample.
- 3.3 Experimental setup for relaxometry experiment. Optical excitation with a 532 nm laser and a 405 nm. A 375 nm LED provide wide-field illumination to the material. Fluorescence is collected beyond 650 nm. Both the 532 nm and 405 nm excitation beams are pulsed with an acousto-optic modulator (AOM). 10% of each laser beam is directed to a photodiode for monitoring the optical excitation power. The pulses and the MW for chirped and square-wave  $\pi$ -pulses are generated with an arbitrary waveform generator (AWG). An 1.45 NA oil objective is used. MW delivery is achieved with an on-chip co planar waveguide.

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3.8 Illustration of the triplet hopping process. a) Different oriented DPH molecule have different eigenstates and hopping between them lead to decoherence.
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- 3.9 Illustration of the stochastic Liouville equation (SLE) used to analyse the triplet diffusion process. The SF process starts with an occupied  $S_1$  state which forms a correlated triplet pair. The correlated pair can recombine via triplet fusion  $(k_{TF})$  to generate fluorescence or dissociate to form separated triplets which hop from one molecule to another. Once triplet excitons are well-separated spatially, they are unlikely to recombine via the correlated pair state.
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- 4.1 ODMR Lineshape and sensitivity dependence on laser and microwave powers for FND diamonds (ensemble NV). Each trace is averaged for 6 minutes in total. **a**) MW power dependence performed at laser power of  $18 \mu$ W with the colorbars showing the attenuation/amplification relative to 10 dBm delivered to the sample (measured at the SMA connector on the PCB). Increasing MW power increases the ODMR contrasts (blue to red transition). **b**) Laser power dependence at MW power of 5 dB attenuation in **a**) (15 dBm at the sample). The optical power measured at the sample plane has been indicated with colour bars. Panels **a**) and **b**) are plotted on the same vertical axes. **c**) Theoretical sensitivity extracted from experimental lineshape using Eq. A.1 based on the seven parameter fitting model (Eq. A.3).
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- 4.3 T-CPMG measurement on Single NV centres. a) The pulse sequence (right) involves performing π-pulses by driving two transitions (|0⟩ → |-1⟩ and |0⟩ → |+1⟩) while MW1 is detuned by Δ (left). The two transitions are driven by two separate microwave sources. b) and c) show the corresponding Rabi oscillations. d) shows the T-CPMG fringe as the delay τ is progressively changed.
  e) shows period of oscillation changing linearly with MW1 detuning in measurements like d). f) On-resonance T-CPMG with N=2 (12 π-pulses applied).
  g) T<sup>\*</sup><sub>2,T-CPMG</sub> as the number of π-pulse increases.
- Schematics of photolithographically fabricated structures on the substrate. a) 4.4 Picture of a fully assembled chip with PDMS channel attached. The green PCB is connected to the glass substrate, which is pattern with Ti-Au-Ti structures for temperature sensing, heating, and MW delivery. The PDMS-based microfluidic channel allows liquid to enter via the inlet and exit via the outlet. b) Optical microscope image. The yellow region is coated with gold (Ti-Au-Ti structure) while the dark background is the glass substrate. The central area circled by the dashed white line is the area used for cell culture and onchip temperature sensing and control. Inset: schematics of the pattern. CPW (green) refers to co-planar waveguides for MW delivery. RTD is a resistive temperature detector (cyan) for on-chip temperature detection. Heater is a resistive heating element (red) for on-chip heating and temperature control. c) Zoomed image of the central region. The dotted segment of the RTD is the active area. The CPW track pointed to by the black arrow is the signal line of the CPW.
- 4.5 Simulated magnetic field distribution in the CPW. **a**)  $B_1$  field in the normal direction with 25 dBm of incident MW power. The red area indicates magnetic field pointing out-of-the-plane. The magnitude is indicated by the color bar. The maximum  $\pi$ -pulse time is approximately 12 ns. **b**) shows the magnetic field vector around the central track of the CPW.
- 4.6 AC Magnetic Field along the CPW at the centre of the transparent area. **a**) A dry sample gives uniform magnetic field distribution, **b**) while a substrate with water above shows a reduction in magnetic field along the CPW. There is no clear periodicity of  $B_1$  beyond the area presented. **c**) The magnetic field normal to the plane along the black dotted line shown in **a**) and **b**) is plotted, where the blue curve corresponds to the dry substrate while the red to a substrate covered by water.

- 4.7 On-chip RTD sensor Characterisation. **a**) shows the linear dependence of relative resistance at temperature *T* referenced to resistance at 35 °C for sensor A. The gradient of the linear fit is shown for 5 sensors in the inset. The average value of the gradient is  $\eta = (2.44 \pm 0.12) \times 10^{-3}/$ °C, showing good consistency between sensors. The on-chip heaters use the on-chip RTD as a feedback to stabilise the temperature. **b**) shows the temperature measured by on-chip RTD (blue trace) when the temperatures are set at commanded steps. The voltage applied to the heaters are shown on the same plot (red trace). The achieved temperature is close to the commanded temperature within 16 mK (inset) and stabilises within less than 4 min.

- 4.10 Confocal Microscope for imaging NV centres. Laser Scanning Confocal Microscope. CL1/2/3/4 are collimation lenses. Note that CL1/2 are intentionally defocused to implement single particle tracking. L1/L2 are achromatic lenses forming the Keplerian telescope. DM532/DM605 are long-pass dichroic mirrors at 532 nm and 605 nm. LP550/LP650 are long-pass filters at 550 nm and 650 nm. SM: single-mode. BS: Non-polarising beamsplitter. AOM: acousto-optic modulator. APD: Avalanche Photodiode. PD: Photodiode. A linear polariser and a half-wave plate are placed along the excitation path to produce linearly polarised incident excitation (not shown).

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4.11 Theoretical point spread function (PSF). Panels a) and b) shows the excitation PSF in the XZ and XY plane. Panels c) and d) are the collection PSF, from an isotropically emitting single point emitter. Panels e) and f) are the point-by-point product of the excitation and collection and correspond to the PSF for a confocal microscope. The colormap shows the normalised intensity and is logarithmically scaled.
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4.13 Electronic setup illustration. The DAQ card is central to synchronising various hardwares. It sends voltage ramps to the MW generator for frequency modulation of the MW. It generates pulses to switch on and off the on-chip heater with a solid-state switch (green). The incoming photon counts from APD 2 and 3 are also counted and gated by the DAQ. In conjunction with the MCU tracker (discussed in chapter 5), two sinusoidal voltages are supplied to the galvo mirror to steer the galvo mirror for single particle tracking (blue). The MW generated from the MW source is connected to an RF switch (orange) and amplified by an external MW amplifier. The amplified MW is then sent to the CPW on the sample's substrate.

- 4.14 Timing illustration of Hardware-Timed ODMR and pulsed on-chip heating. A synchronisation 100 kHz clock (navy square wave, bottom box, labelled CLK) is supplied to the DAQ to trigger each photon count readout (pale purple bars, bottom box, labelled CTR 1&2). DAQ also switches voltage supplied to the MW source, modulating frequency of the MW source (magenta trace, MW FM). On a longer timescale (top of figure), DAQ uses this clock as a timebase to generate slower sinusoidal voltages with 90° phase difference to steer the galvo mirrors for orbital tracking (see Chapter 5) which is shown as the pale blue and pale red traces labelled X Pos and Y Pos. DAQ also generate pulses to switch the solid-state switch (maroon trace), which controls the current supplied to the on-chip heater. This configuration allows the ODMR data and tracking data to be simultaneously gathered. Note that the relative delays are only checked on an oscilloscope. The rise time (~ 20ns) and trigger delay (~ 20ns) of the DAQ device are small compared to the pulse duration (> 10 $\mu$ s) and the relative delays do not require dedicated calibration procedure. . . . .
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  b) is the averaged ODMR trace over the entire dataset gathered (with potential temperature induced broadening on the peaks of 1.4 MHz for temperature steps spanning 20 °C), showing a good fit with a double Lorentzian model. c) shows the linear relationship followed by the shift in ODMR central frequency and the temperature measured by the on-chip RTD, which gives a gradient of κ=-60.7(4) kHz K<sup>-1</sup>. d) shows the distribution of the difference (residuals) between the temperatures reported by the NV and the on-chip RTD. The histogram is fitted to a Gaussian distribution (blue), giving a spread of 0.6 K.
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5.3 Intracellular temperature measurement with FCCP treatment. a) The nanodiamond identified is localised in the cell as is also observed in the enlarged XY plot of the trajectory shown in b). The dotted white line indicates the boundary of the cell, identified via the difference in autofluorescence between the cytoplasm and the cell culture medium. The scale bar in a) has a length of 5 μm. c) The on-chip RTD registers a sharp change in temperature the moment FCCP is added. However this change is very small compared with the sensitivity of the NV sensor and is not registered. d) The nanodiamond thermometer reports a steady temperature until a rise is observed immediately after the addition of FCCP, and the temperature rise lasts till the end of the experiment. . . . . . . 119

- 5.5 Dynamic localisation accuracy. An artificial voltage generated according to Eq. 5.6 is sent to the galvo mirror to mimic a diffusing particle. a) shows the trajectory of such a diffusing particle. The tracker tracks the particle. b) shows the trajectory reported by the tracker and the commanded location of the particle in the x direction. The difference between the two gives the tracking accuracy, which is Gaussian distributed as shown in c). The spread of the Gaussian distribution gives the tracking accuracy and is dependent on diffusing rate. d) as the diffusion rate is varied where a minimum localisation accuracy is 7.7 nm achieved. e) A similar procedure in the z directions gives an best-case accuracy of 24 nm.
- 5.6 Velocity autocorrelation function (VACF) of single particle tracking. a) shows that VACF for the PC-based tracking approach and clearly there is non-trivial autocorrelation at short times ~ 0.5 s. b) is similar to panel a) but now with faster correction rate enabled by the fast feedback of a MCU-based tracker. When the particle is close to stationary, the intrinsic noise of the tracker leads to characteristic oscillations at the tracking frequency (104 Hz. As the particle diffusion rate increases, the VACF shows a transition from underdamped to overdamped oscillation, indicating a change of the proportional gain. c) Reducing the tracker's update frequency also reduces the characteristic frequency of the VACF.

- 5.7 Brownian motion trajectory. **a**) Three-dimensional trajectory of the diffusing particle. **b**) Dependence of MSD for trajectory in the x direction on delay  $\tau$ . The longtime behaviour is well-explained by a simple Brownian motion model where MSD  $\propto \tau$ . The short-term behaviour is limited by localisation error. **Inset**: velocity correlation function in x direction showing no clear correlation beyond the intrinsic correlation of the tracker. **c**) and **d**) display the corrections applied in x and z directions at each step, showing no clear deviation from Gaussian distribution.
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# Nomenclature

## **Roman Symbols**

- AOM acousto-optic modulator
- APD avalanche photodiode
- CPW co-planar waveguide
- CW continuous-wave
- DFT density functional theory
- ES excited state
- ESR electron spin resonance
- GS ground state
- HPHT high-pressure high-temperature
- HWHM half-width half-maximum
- ISC inter-system crossing
- MW microwave
- NMR nuclear magnetic resonance
- NV nitrogen vacancy
- ODMR optically detected magnetic resonance
- PL photoluminescence
- PSB phonon side band

SF	singlet-fission
SNR	signal-to-noise ratio
TA	transient absorption

ZPL zero-phonon line

# Chapter 1

# Introduction

# 1.1 Quantum sensing for biological and material research

The global pandemic of COVID-19 has inevitably marked 2020 as a special year in history. Significant attention has been paid to the development of vaccines in the two years following the outbreak. At the same time, various techniques for testing COVID patients have also been developed and employed. These in-vitro testing techniques, such as cellulose-based lateral flow immunoassays [74], epitomise the ever-growing needs for low-cost, fast, small and robust sensing techniques in response to the global health crisis and for biological and biomedical research in general.

In addition to the fast development of the healthcare industries, the digital revolution, which has transformed the world, relies on the shrinking nanoelectronic circuits. The burgeoning nanotechnology and nanoelectronics industries demand control and characterisation at the nanoscale, for example, the mapping of nanoscale currents and temperatures [101].

Quantum sensing offers the opportunity to address these technological challenges, providing unprecedented sensitivity and sensor-to-target distance, on a single-particle level. Quantum sensing leverages on the quantum physics processes, which feature one or more of the precise energy level quantisation, the coherence of quantum systems, and quantum entanglement, to enable sensing of miniature physics quantities [58]. Often such quantum processes take place at mesoscopic scales and are thus associated with nanoscale measurements.

One successful example of quantum sensing is the superconducting quantum interference devices (SQUID) [9] which are used in magnetocardiography (MCG) in hospitals and magnetometers for magnetization measurements. In addition, cold atoms [136], squeezed light [1] and nitrogen-vacancy (NV) centres in diamond have all been used to demonstrate quantum metrology.

Nitrogen-vacancy (NV) centres are naturally occurring atomic scale impurities in diamond

that have optically addressable single spins that can act as quantum sensors. NVs can be formed in either bulk diamond or diamond nanocrystals (nanodiamonds). NV centres in both forms share similar optical and spin properties. In comparison to the well-isolated optically trapped microkelvin cold atoms or the cryogenic SQUID, NV centres are protected by the diamond lattice and are capable of interfacing with room-temperature systems.

Nanodiamonds are nanocrystals of diamond and can also host NV centres. Contrary to bulk diamonds, nanodiamonds are low-cost, easy-to-process nanosensors, capable of probing closed structures such as a biological cell. Additionally, the ability to interface nanodiamonds with various materials motivates the application of nanodiamonds to quantum sensing and has led to an active area of research. The prominent examples are the use of nanodiamonds for probing magnetic field in magnetotactic bacteria [148], temperature in *C elegans* [49], nitrogen oxide species and redox reactions[188].

Nanodiamonds are not just a more convenient version of bulk diamonds but they can also bring new opportunities, and enable research in areas not possible by bulk diamonds. Nanodiamond-based sensors have been developed for paper-based HIV virus testing, because of its photostability and microwave (MW) modulated photoluminescence (PL) [166]. Optical tweezers are used to manipulate nanodiamond in microfluidic channels, making it possible for controlled manipulation in complex media [109].

Nonetheless, despite the extensive research already performed on NV centres in bulk diamond, challenges still remain in optimising sensitivity with nanodiamonds and in determining the full extent of their application in complex sensing environments. It is not immediately clear if the protocols typically used for NV in bulk diamond can be directly transferred to nanodiamonds. Furthermore, new challenges such as arbitrary NV axis orientation and fast translational diffusion in a cell emerge. The relevant techniques to address these issues deserve attention and further investigation.

In this context, this thesis explores using nanodiamonds for nanoscale sensing in three scenarios, presented chapter by chapter with increasing levels of complexity. This chapter starts with introducing the basic NV physics and coherent spin manipulations, which form the basis of NV sensing. The overview of the thesis will be left to the end of this chapter.

## **1.2** Diamond synthesis and NV optical properties

Diamond has a wide band gap of 5.47 eV. Naturally occurring NV centres have been extensively studied in the last century via electron spin resonance and optical spectroscopy. However, It was only in 1997 when Gruber *et al.*, observed single NV centres using confocal microscopy [98] that NV was recognised as a potential system for room-temperature single-spin quantum manipulation. Subsequently, coherent spin manipulation and coupling to proximal nuclear spins [47], and ultrafast dynamical control [83] were demonstrated. In fact, the superior quantum coherence property of NV centres at room temperature has aroused significant interest in quantum information processing, single photon emitters and quantum metrology.

For quantum metrology in particular, NV centres have been applied in areas such as DC and AC magnetic field [25, 198], temperature[171], electric field [31, 68], strain [237] and pressure sensing [66].

The advantage of long room-temperature coherence time, high chemical and optical stability and the ability to be optically initialised and read out, make bulk diamond compatible with other materials such as biological cells. In fact quantum sensing using NV centres has been demonstrated to give sub-millihertz spectral linewidth [198] and single-protein nuclear magnetic resonance (NMR) [156], paving the way for practical applications of NV centres for NMR applications. Nanodiamonds are non-cytotoxic [261] and readily uptaken by certain types of cells, with a high surface-to-volume ratio and chemically functionalisable surface [141], making them suitable candidates for nanoscale bio-sensors.

## **1.2.1** Formation of NV centres

Nitrogen-vacancy centres in diamonds are formed by replacing a carbon atom with a nitrogen atom and a neighbouring carbon atom with a vacancy (Fig. 1.1 **a**)). Such defects are naturally occurring in diamonds. The optical and spin level structures of the NV were elucidated with spectroscopy, electron spin resonance, optically detected magnetic resonance techniques and are presented in Fig. 1.1 **b**).

## **1.2.2** Nanodiamond synthesis

The fabrication of diamond for quantum sensing is an involved process which looks to enable desirable qualities such as nitrogen purity, isotopical purity, local strain inhomogeneity. Especially in shallow implanted NV centres and nanodiamonds, the surface qualities affect the NV coherence, charge state stability and thus surface chemistry is another focus of the synthesis process. Studies on NV centres for quantum sensing have largely been performed on single-crystal bulk diamond. Starting from diamond with nitrogen impurities, electron, proton, ion irradiation or laser writing creates vacancies which migrate to nitrogen impurities during the post-annealing process at 800 °C. Although nanodiamonds used in this work have been from commercially available sources, the various techniques for synthesising nanodiamonds are discussed here to guide the future efforts on synthesising diamond for quantum sensing.



Figure 1.1: Illustration of NV centre formation and optical transitions. **a**) Atomic structure of NV centres. NV centres are naturally occurring colour centres in diamond and are formed by a neighbouring nitrogen atom and a vacancy. The nitrogen-vacancy direction sets the intrinsic symmetry axis of the NV centre. **b**) shows the optical energy level structure. The ground state is a spin triplet with a zero-field splitting (ZFS) parameters D = 2.87 GHz and E = 0. In diamond nanocrystals the  $C_{3\nu}$  symmetry is often broken and gives nonzero E. NV centres can be non-resonantly excited and quickly decay non-radiatively from higher lying vibronic states to the lowest energy excited state.  $m_s = \pm 1$  states of  ${}^{3}E$  have a significant rate going into the  ${}^{1}A_1$  shelving state and subsequent radiative decay into the  ${}^{1}E$  state. The decay from  ${}^{1}E$  into  ${}^{3}A_2$  has a slight preference for  $|m_s = 0\rangle$ .

The two most commonly used methods for diamond production in industry and research are the high-pressure high-temperature (HPHT) method [4] and the chemical vapour deposition (CVD) method [270]. HPHT resembles the natural formation environment whereby carbon precursors are compressed to giga-Pascal pressure and heated to 1500 °C in an anvil cell. There is no clear best candidate for these carbon precursors and both organic [72] and inorganic [4] carbon-containing solid compounds have been used. The pressurisation and heating causes small crystalline domains to form and results in microdiamonds with various sizes. Non-diamond inpurities in this mixture are subsequently removed by acid cleaning and centrifugation. The resulting microdiamonds can be bead milled to result in nanodiamonds. Although there is no clear fundamental limit to the elemental purity, HPHT diamonds usually contain high nitrogen impurities.

CVD, on the other hand, is a technique that is conducted at a lower temperature (about 700 °C) and pressure (about 30 mbar) than HPHT. This synthesis process is unnatural because graphite, rather than diamond, is the stable allotrope in this condition. This process involves mixing a hydrocarbon precursor (typically  $CH_4$ ) with  $H_2$ . The hydrogen gas serves to quickly etch away the graphite formed. In CVD, hydrogen radicals and methyl radicals are important precursors to diamond growth. Thus hot-filament CVD has been used for large scale industrial processes, where thermal agitation facilitates radical production. Due to the temperature

tolerance of the filament material, this technique is slower compared to the plasma-enhanced CVD (PECVD) process, where a homoepitaxial growth rate of  $100 \,\mu m \,h^{-1}$  has recently been reported. Milling of bulk CVD diamond can result in diamond nanocrystals [105].

The milling process common to both top-down approaches leaves residual stress in the diamond material and also creates uncontrolled diamond geometry. The residual stress is believed to cause variations in zero-field NV ground-state resonances, which is detrimental in ensemble NV applications [237]. The aspect ratio of the individual single-NV nanodiamonds used in our experiments could vary between 1~5, with sharp corners resembling broken glass shards. Studies on other nanoparticles have suggested that high nanoparticle aspect ratios (~10) could increase the cytotoxicity level [154]. In addition, temperature rise and abrasion during repeated mechanical milling changes nanoparticle surface chemistry and introduces metallic impurities such as iron (steel beads) and zirconium (ZrO<sub>2</sub> beads), requiring subsequent chemical removal.

CVD procedure also enables direct synthesis of nanodiamonds from seeding nanodiamonds, typically from HPHT [170] or detonation ND sources. CVD allows nanodiamonds with very high elemental purity and very low residual stress to be formed, with a typical synthesis of  $10 \mu g h^{-1}$  rate.

The above mentioned processes are top-down approaches and nanodiamond can also be made by a bottom-up synthesis approach by detonating hydrocarbons, making single-digit nanometer-sized detonation nanodiamonds (DNDs). However as DNDs typically have smaller NV-to-surface distance and less controlled surface chemistry, they have poorer coherence [235] and are thus not discussed.

It is useful to note that the nanodiamonds used in the HeLa cell experiment in this work are about  $1 \mu g$  per experiment, equivalent to about  $10^9$  nanodiamonds each with a 50 nm radius. Thus any synthesis would need to be scalable for them to be useful for natural cellular uptake. Techniques such as nanopillar arrays would have limited applicability.

NVs can acquire or donate an electron from or to the conduction band of diamond and readily switch between the  $NV^0$  and  $NV^-$  charge states. The  $NV^-$  state has a spin triplet ground state and forms the basis of NV-based magnetometry and is often referred to simply as the NV centre. In subsequent discussions, NV will refer to  $NV^-$  unless stated otherwise.

Coherence property is crucial for a quantum sensor. NV centres embedded deeply in isotopically pure diamond have set the record (~1 s of  $T_2$  time) for NV coherence [24]. The coherence time decreases with natural abundance of carbon isotopes, and with more and more nitrogen impurities incorporated [102]. Yet it is not necessarily best to have a low nitrogen concentration. Despite various techniques such as electron, proton and He<sup>+</sup> irradiation for improving the NV yield [41] from implanted nitrogen atoms, the concentration of NV is generally positively correlated with concentration of nitrogen, thus an experimental compromise between coherence and yield needs to be made. Additionally, NV centres close to the surface, for example in shallow-implanted NV centres in bulk diamond, or in nanodiamonds, experience magnetic and electric noises due to dangling bonds, adsorbed impurities and charge traps on the diamond surface and are considerably less coherent than their bulk counterparts.

Nanodiamonds used in this work are commercially available from three suppliers, Adamas Nanotechnologies, FNDBiotech and NaBond. They are synthesised via the HPHT process. The HPHT nanodiamonds are type IB, containing ~10-100 ppm of nitrogen impurities. The nitrogen electrons and surface impurities are the dominating source of decoherence in these samples.

### **1.2.3** Optical level structures

Before discussing the optical properties and energy level structure of NV centres, a small excursion is here taken to examine how the energy level structures are labelled, to aid subsequent discussion. Group theory has been a very powerful tool in molecular spectroscopy for determining whether certain integral vanishes by symmetry considerations, and whether certain energy levels are degenerate.

To apply group theory to point defects, the symmetry of the defect is first determined by considering the local point symmetry. For example, NV centres are invariant under threefold rotation about its long axis, mirror image about a plane parallel to the long axis, and thus have a  $C_{3\nu}$  symmetry. The contributing atomic orbitals are the four  $sp^3$ -hybridised orbitals from three carbon atoms and one nitrogen atom, with a total of six electrons (three from carbon, 2 from nitrogen, and one acquired from the conduction band). The four constituting atomic orbitals can be linearly superimposed to obtain the symmetry-adapted molecular orbitals (MOs). As these orbitals will also follow the defect's symmetry, they can be labelled uniquely with the various irreducible representations (irreps) of the symmetry group. Each irrep corresponds to a conjugacy class in the group. In NV centres these MO's have symbols like  $a_1, a_2, e_x, e_y$ corresponding to irreps  $A_1, A_2, E$ , where the last irrep has a character of 2 and corresponds to two degenerate orbitals. These molecular orbitals can then be filled with electrons and form molecular orbitals like  $a_1^2 e^2$  which have an overall symmetry of  $A_2$  by multiplying the elements  $A_1 \times A_1 \times E \times E = A_2$ . In addition to the symmetry label, a spin multiplicity is also added and each state is now represented as  ${}^{2S+1}\Gamma$ . For example the ground state of NV centres is one of the three  ${}^{3}A_{2}$  states, which represent three triplet states following an overall  $A_{2}$  symmetry. Note that in principle the symmetry of the MO is not related to the spin multiplicity. The detailed energy structures can be found out by combining density functional theory (DFT) analysis [64] and optical spectroscopy but this is beyond the scope of this introduction.

Transitions	Rate $(\mu s^{-1})$
$({}^{3}E, m_{s} = \pm 1) \rightarrow {}^{1}A_{1}$	$50.7\pm4.4$
$({}^{3}E, m_{s}=0) \rightarrow {}^{1}A_{1}$	$6.4 \pm 2.3$
${}^{1}E \rightarrow ({}^{3}A_2, m_s = \pm 1)$	$0.6 \pm 0.3$
$^{1}E \rightarrow (^{3}A_{2}, m_{s}=0)$	$0.7\pm0.5$
$({}^{3}E, m_{s} = 0, \pm 1) \rightarrow ({}^{3}A_{2}, m_{s} = 0, \pm 1)$	$67.7 \pm 3.4$

Table 1.1: Intersystem crossing rates, and radiative transition rate for one NV (named NV C) measured in [233].

## **1.2.4** Optical transitions

The NV ground state is a triplet spin state belonging to the  $A_2$  representation class. The excited states are a set of two triplet states with *E* representation. Due to the Jahn-Teller effect, the two degenerate *E* excited states are degenerate at room temperature. An experimentally measured NV spectrum is presented in Fig. 1.2 **a**). The transition between the  ${}^{3}E$  and the  ${}^{3}A_2$  states has a zero-phonon line (ZPL) at 637 nm. Phonon side bands (PSBs) are observed following the emission of a phonon in the radiative decay from  ${}^{3}E$ . In Fig. 1.2 **a**), the broad feature at wavelength longer than 650 nm are the PSBs. The optical transition can be non-resonantly excited typically with a 532 nm laser. The 532 nm laser is also at the optimal wavelength to shift the steady-state equilibrium from the NV<sup>0</sup> charge state [19]. As both charge states contribute to the optical spectrum, their respective spectra adapted from [191] are shown in 1.2 **b**). The relative contribution varies between NV centres and typically exist in a 3:7 (NV<sup>0</sup>:NV<sup>-</sup>) equilibrium. In our experiments the NV<sup>0</sup> spectral region is suppressed by the optical fibre transmission (cut-off wavelength of 633 nm) used for connecting the confocal set-up to the spectrometer.

Intersystem crossing (ISC) is the key to the spin-dependent PL of NV centres. ISC describes the transitions between the S = 1 and the S = 0 submanifolds. The ISC transition rates shown in Fig. 1.1 b) (four dashed lines) are dependent on the spin state. These rates can be extracted based on modelling of magnetic field orientation dependent PL measurements [233] and example values are tabulated in Table 1.1. The  $m_s = \pm 1$  states are more likely to undergo ISC and transition into the singlet manifold, where a radiative transition at 1042 nm is observed. The ground-state in the singlet manifold <sup>1</sup>E decays into the  $m_s = 0$  triplet ground state preferentially. However the preference is almost negligible  $(0.6 \mu s^{-1} \text{ for } m_s = \pm 1 \text{ versus } 0.7 \mu s^{-1} \text{ for } m_s = 0)$  compared to the preference in the decay from <sup>3</sup>E to <sup>1</sup>A<sub>1</sub> (50.7  $\mu s^{-1}$  for  $m_s = \pm 1$  versus  $6.4 \mu s^{-1}$  for  $m_s = 0$ ). The spin-dependent ISC rates give rise to spin-dependent fluorescence and ground-state polarisation, which form the basis for high-fidelity spin initialisation and readout.



Figure 1.2: Optical emission spectrum of an NV centre. **a**) is an experimental spectrum measured with a single NV in nanodiamond in the set-up presented in Chapter 2, excited with 532 nm continuous-wave laser illumination. The emission shows the characteristic zero-phonon line (ZPL), indicated with a blue arrow. The broad feature between 650 nm and 800 nm with multiple peaks are the phonon side-bands (PSB). **b**) shows normalised spectra for NV<sup>-</sup> (crimson) and NV<sup>0</sup> (navy blue), adapted from [191]. The low apparent contribution of NV<sup>0</sup> in **a**) is potentially due to the higher attenuation of the optical fibre leading to the spectrometer below 633 nm.



Figure 1.3: Optical saturation curve of an NV centre. The  ${}^{3}E$  to  ${}^{3}A_{2}$  radiative transition can be optically saturated. The saturation power  $P_{sat} = (710 \pm 30) \,\mu\text{W}$  and saturation PL  $PL_{sat} = (30.3 \pm 0.8)$  kHz are dependent on the relative orientation of the NV and the experimental setup.

The transition rates are different between NV centres, potentially due to the local strain environments of the NV centers. These rates do not vary by more than the associated experimental errors as presented in Table 1.1. However, this variation does give rise to different optical contrasts between the  $m_s = \pm 1$  and  $m_s = 0$  states, which is typically 30%, i.e.  $PL(m_s = \pm 1)/PL(m_s = 0) \approx 70\%$  but can differ between emitters.

The  ${}^{3}E$  to  ${}^{3}A_{2}$  radiative transition can be optically saturated as expected of a single transition (Fig. 1.3). The saturation curve is described by

$$PL_0 = PL_{0,sat} \frac{s}{1+s},\tag{1.1}$$

where  $PL_0$  is the steady-state PL,  $PL_{0,sat}$  is the PL at saturation, *s* is the optical pumping parameter and is defined as  $s = P/P_{sat}$  where *P* is the actual excitation power. Note that although  $P_{sat}$  is denoted as the saturation power,  $PL_0 = PL_{0,sat}/2$  when  $P = P_{sat}$ . The transition saturates when  $P \rightarrow \infty$ . The saturation power and PL for each NV depend on the relative orientation between the NV axis and the optical polarisation, angles of the collection cone, in addition to the defect by defect variation.

The typical values of  $PL_0$  in the confocal set-up used for this work of single NV centers in nanodiamond is about 50 kcps. This is drastically different compared to the rates limited by the spontaneous decay rate of the radiative transition (~10 ns, ~100 Mcps). This gap can be remedied by improving the collection efficiency using higher-NA objectives, or more dedicated collection methods such as solid-immersion lens, as is done in Ref. [203], which reported 0.853 Mcps. In addition, the NV is in a constant dynamic equilibrium between NV<sup>0</sup> and NV<sup>-</sup> charge states under continuous illumination (70% of the time in NV<sup>-</sup>). A maximum count rate of 2.4 Mcps is reported for blinking emitters. The dark shelving state lifetime also limits the radiative transition rate. For example the  ${}^{1}E$  to  ${}^{3}A_{2}$  transition lifetime is ~300 ns [233] and thus with the branching into the dark shelving state, the observed count rate is reduced from the radiative lifetime limited photon count rate.

The NV orientation determines the angular emission pattern. The angular intensity distribution  $I(\hat{\mathbf{r}}) \propto |\mathbf{p_1} \cdot \hat{\mathbf{r}}|^2 + |\mathbf{p_2} \cdot \hat{\mathbf{r}}|^2$ , where  $\mathbf{p_1}$  and  $\mathbf{p_2}$  are the emission dipoles aligned with the NV's x and y direction (note that the NV axis is in the z direction and x and y are mutually orthogonal and also orthogonal to the NV axis).  $\hat{\mathbf{r}}$  is the emission direction. The collected photoluminescence is proportional to the integrated intensity over the collection angle of the objective. As such the  $PL_0$  depends on the orientation of the NV center and thus gives rise to significant differences between NV centers in different nanodiamonds, which are not purposedly aligned. In our confocal set-ups, the single NV emission intensity is approximately 20 kcps to 80 kcps. For bulk diamond, however, the emission intensity is more discrete, as NVs are aligned to four spatial directions, set by the diamond lattice.

#### **1.2.4.1** Statistics of emitted photons

NV emission is well described by a Poisson process. The number of photons measured from an emitter with photon emission rate, I, within a time  $\Delta t$  follows a Poisson distribution,

$$N \sim \text{Pois}(I\Delta t)$$
.

The mean is  $\langle N \rangle = I \Delta t$  and the variance is  $\langle N^2 \rangle - \langle N \rangle^2 = I \Delta t$ . This variance characterises the uncertainty in estimating the mean emission rate and is also known as the photon shot-noise.

The sensitivity of the NV sensors depend on the coupling between the external quantities (such as magnetic field) to the NV, and on how well this coupling can be extracted by measuring the spin state of the NV. For pulsed techniques, both photon shot-noise and projection noise contribute to the latter noise, which will be discussed in the following section.

Projection noise is associated with the quantum mechanical uncertainty principle where the  $\langle S_z^2 \rangle - \langle S_z \rangle^2 \neq 0$  unless the system is in one of  $S_z$ 's eigenstates. This variance decays with the number of measurements  $\propto N$ . For the readout schemes used in this work, however, the average number of photons per readout is low (0.002~0.03) and thus the photon shot-noise dominates over the projection noise. The exact sensitivity values will be discussed in the respective chapters.

## **1.3** NV ground state spin properties

The NV ground state (GS) is a spin-1 system with a good spin coherence property. The spin state can also be read out optically. This work centres around spin manipulation of the GS spin and thus the physical property of the GS spin is here discussed.

## 1.3.1 NV GS Hamiltonian

Extensive studies into diamond colour centres have been performed with electron spin resonance (ESR) techniques [78, 155]. The general form of a spin-triplet Hamiltonian is

$$H_{triplet} = \mathbf{S} \cdot \mathbf{D} \cdot \mathbf{S}$$

where **D** is the zero-field splitting (ZFS) tensor. As the ZFS tensor is a symmetric tensor, it can be diagonalised and completely characterised by the D and E parameters as

$$H_{NV} = D\left(S_z^2 - \frac{1}{3}S(S+1)\right) + E\left(S_x^2 - S_y^2\right),$$
(1.2)

where the  $S_x$ ,  $S_y$ ,  $S_z$  are the spin operators in the x, y, z directions which are principal axes of the D tensor and S = 1 is the spin quantum number. In general, the eigen-axes of the ZFS tensor are not aligned with the molecular axes, the crystal axes, nor the lab axes. However, in the special case of the NV, which possesses  $C_{3v}$  symmetry, one of the ZFS tensor's axes is aligned with the NV axis and is thus chosen to be the z direction.

In bulk diamond,  $E \approx 0$ , and the high-field basis,  $|m_s = 0\rangle$ ,  $|-1\rangle$ ,  $|+1\rangle$ , is an eigenbasis that diagonalises the Hamiltonian. The name 'high-field' is used since this basis describes the eigenstates when a strong magnetic field is applied along the z direction. The quantum states are labelled by,  $m_s$ , which are the eigenvalues of  $S_z$ . This is possible since  $S_z$  commutes with  $S_z^2$ . The corresponding energy differences are  $E_{+1} - E_0 = E_{-1} - E_0 = D$  as shown in Fig. 1.1 **b**).

In general (especially in nanodiamonds which have a high local strain)  $E \neq 0$ , the Hamiltonian of Eq. 1.2 needs to be diagonalised in the zero-field basis  $|T_x\rangle$ ,  $|T_y\rangle$ ,  $|T_z\rangle$ , giving energies,

 $E_x = \frac{1}{3}D - E, E_y = \frac{1}{3}D + E, E_z = -\frac{2}{3}D$ . The zero-field and high-field bases are related by

$$\begin{aligned} |T_z\rangle &= |0\rangle \\ |T_x\rangle &= \frac{1}{\sqrt{2}} \left(|-1\rangle + |+1\rangle\right) \\ |T_y\rangle &= \frac{i}{\sqrt{2}} \left(|-1\rangle - |+1\rangle\right). \end{aligned}$$

Since  $|-1\rangle \rightarrow |+1\rangle$  constitutes a double quantum transition and is usually not excited by MW, the observed MW transitions are at  $E_x - E_z = D - E$  and  $E_y - E_z = D + E$  respectively. The  $|T_{x,y}\rangle$  states are often called  $|+1\rangle$  or  $|-1\rangle$  states for convenience reasons, especially since they lift the originally degenerate  $|\pm 1\rangle$  similar to the result of Zeeman effect.

## **1.3.2** Coupling to magnetic field and temperature

Additional terms also appear in the NV Hamiltonian due to the NV's interaction with the lattice, or external fields. In particular, the Zeeman interaction with an external magnetic field contributes a term,

$$H_Z = \mu_B g \mathbf{B} \cdot \mathbf{S}$$

For a magnetic field applied along the NV axis and when E = 0, the eigenstates are the high-field eigenstates, with transitions  $|0\rangle \rightarrow |-1\rangle$  and  $|0\rangle \rightarrow |+1\rangle$  at  $D - \mu_B g B$  and  $D + \mu_B g B$  respectively. The electron's gyromagnetic ratio  $\gamma_e = \frac{\mu_B g}{h} \approx 2.8 \text{ MHz/G}$ .

With decreasing temperature, internal strain of the diamond lattice compresses the electron wavefunction, and thus increases the magnetic dipolar coupling between electrons. The corresponding term in the Hamiltonian is

$$H_{temperature} = D(T)S_z^2$$

## **1.3.3** Optically detected magnetic resonance (ODMR)

The GS spin transition can be observed by irradiating the defect with microwave frequency magnetic field (hereafter referred to as MW for simplicity) while monitoring the fluorescence. Figure 1.4 shows a typical ODMR trace. The ODMR spectrum consists of two Lorentzian peaks. Each peak corresponds to a GS spin transition, at energies  $D \pm E$ . The peaks have a negative contrast of typically 10%, and a typical half-width at half-maximum (HWHM) linewidth of 5 MHz. The exact linewidth and contrast depend on the interplay between MW coupling and optical excitation. For example Fig. 1.4 shows an ODMR contrast of 25% and 23% respectively. The ODMR contrast is ultimately limited by the optical contrast when the



Figure 1.4: Optically detected magnetic resonance (ODMR) measurement displays two resonances at  $f_0 = (2848.34 \pm 0.08)$  MHz and  $f_1 = (2866.43 \pm 0.09)$  MHz. The measurement is performed by continuously measuring the PL while changing the microwave frequency (inset).

system is completely in  $m_s = 0$  and in  $m_s = \pm 1$  states (~ 30%) and the linewidth is limited by the  $T_2^*$  time of the GS spin ( $\delta v = \frac{1}{\pi T_2^*} \sim 1 \text{ MHz}$ ).

Unlike for NV centres in bulk diamond, the ODMR for nanodiamonds show two resonances with a significant separation. The data shown in Fig. 1.4 displays a separation of 9 MHz, indicating a *E* parameter of 4.5 MHz (E = 0 for perfect  $C_{3\nu}$  symmetry in low strain diamond lattice produced for example by CVD method). This is commonly seen in NV centres in nanodiamond due to a strong strain-induced splitting ( $E \neq 0$ ). The central frequency also differs from D = 2.87 GHz by -12.6 MHz.

## **1.3.4** Coherent manipulation and dynamical decoupling

With access to the spin resonance, pulsed experiments can be performed to combine optical initialisation and readout with spin manipulation in between. A typical pulsed experiment starts with optical initialisation, which polarises the ground state. This leaves the ground-state initialised into the  $m_s = 0$  state for spin manipulation. After the spin manipulation, optical readout can be performed to read out the spin state using the fluorescence.

Continuous irritation of MW for a variable duration  $\tau$  produces Rabi oscillation fringes (Fig. 1.5). Rabi fringes are a result of coherent driving of the spin transition which is modelled as follows.



Figure 1.5: The ground-state spin can be coherently manipulated to produce the Rabi oscillation. The  $\pi$ -pulse time is  $(115.5 \pm 0.6)$  ns. The dashed line shows an exponential profile with characteristic decay time of  $(500 \pm 60)$  ns which approximates  $T_2^*$ . The optical contrast is close to 30%, dependent on the optical contrast between the  $m_s = 0$  and the  $m_s = -1$  states. The pulse sequence is at the top of the figure and the MW drive duration is varied to produce the Rabi fringe.

#### 1.3.4.1 Rabi

When the  $|0\rangle \rightarrow |+1\rangle$  and  $|0\rangle \rightarrow |-1\rangle$  are sufficiently well detuned, the GS spin can be approximated as a two level system. The Hamiltonian for Rabi drive for a two level system has the form

$$H=\frac{1}{2}\Delta\sigma_z+\Omega\cos(\omega t+\phi)\sigma_x,$$

where  $\sigma_x$  and  $\sigma_z$  are the Pauli's matrices. The MW is linearly polarised along an arbitrarily chosen direction *x* with an initial phase,  $\phi$ .  $\Delta$  is the splitting of the two-level system and  $\Omega = \mu_B g B_1$  is the diagonal element of the transition matrix.  $B_1$  is the amplitude of the MW frequency AC magnetic field. The Rabi rate characterises the rate of spin manipulation and increases with the MW field strength  $B_1$ . Thus in typical NV experiments, improving microwave delivery to the NV location improves the spin manipulation to rates faster than the intrinsic decoherence rate, thus enabling coherent spin manipulations.

In the interaction picture, the interaction Hamiltonian is

$$H_{int} = e^{iH_0t}\Omega\cos(\omega t + \phi)\sigma_x e^{-iH_0t} = \frac{1}{2}\Omega\left[\sigma_x\cos(\varepsilon t + \phi) - \sigma_y\sin(\varepsilon t + \phi)\right],$$

where  $\varepsilon = \omega - \Delta$  is the detuning between the driving field and the transition frequency of the two-level system. The rotating wave approximation (RWA) has been used here to eliminate sinusoidal terms with frequency  $\Delta + \omega$ . In fact, if instead of using a linearly polarised MW

drive, a circularly polarised drive is used, this approximation is not needed. Substituting in the Pauli's matrices the matrix representation of  $H_{int}$  is

$$H_{int} = rac{1}{2} \Omega \begin{bmatrix} 0 & e^{-i(arepsilon t+\phi)} \ e^{i(arepsilon t+\phi)} & 0 \end{bmatrix}.$$

The time-dependent wavefunction can be expanded as  $|\psi\rangle = c_0|0\rangle + c_1|1\rangle$  and using the Schrodinger's equation this is

$$i\frac{dc_i}{dt} = \sum_m H_{im} e^{iE_m t} c_m,$$

where the exponential factor is from the rotating frame transformation as applied to states. Expanding out this expression,

$$i\frac{dc_0}{dt} = \frac{1}{2}\Omega e^{-i(\varepsilon t + \phi)}c_1, i\frac{dc_1}{dt} = \frac{1}{2}\Omega e^{i(\varepsilon t + \phi)}c_0.$$

Combining these two equations results in

$$\begin{split} i \frac{d^2 c_0}{dt^2} &= \frac{1}{2} \Omega e^{-i\varepsilon t} e^{-i\phi} \left[ \frac{dc_1}{dt} - i\varepsilon c_1 \right] \\ &= \frac{1}{2} \Omega e^{-i\varepsilon t} e^{-i\phi} \left[ -i \frac{1}{2} \Omega e^{i(\varepsilon t + \phi)} e^{i\Delta t} c_0 \right] + \varepsilon \frac{dc_0}{dt} \\ &= -i \frac{1}{4} \Omega^2 c_0 + \varepsilon \frac{dc_0}{dt}, \end{split}$$

which then turns into a second order ODE,

$$\frac{d^2c_0}{dt^2} + i\varepsilon\frac{dc_0}{dt} + \frac{1}{4}\Omega^2 c_0 = 0,$$

and similarly,

$$\frac{d^2c_1}{dt^2} - i\varepsilon\frac{dc_1}{dt} + \frac{1}{4}\Omega^2 c_1 = 0.$$

Setting  $c_0(t=0) = 0, c_1(t=0) = 1$ , the solution is

$$c_0 = -\frac{i\Omega}{\gamma} e^{-i\varepsilon t/2 - i\phi} \sin \gamma t$$
  
$$c_1 = e^{+i\varepsilon t/2} \left[ \cos \gamma t - \frac{i\varepsilon}{\gamma} \sin \gamma t \right],$$

where

$$\gamma = \sqrt{arepsilon^2 + \Omega^2}$$

The probability of measuring the system in state  $|0\rangle$  and  $|1\rangle$  after time t is

$$P_0(t) = |c_0|^2 = \frac{\Omega^2}{\gamma^2} \sin^2(\gamma t/2),$$
  
$$P_1(t) = |c_1|^2 = 1 - \frac{\Omega^2}{\gamma^2} \sin^2(\gamma t/2).$$

Without detuning ( $\varepsilon = 0$ ),  $\gamma = \Omega$  and  $\Omega/\gamma = 1$ . If the number of photons collected is  $p_{0,\pm 1}$  if the system is in  $m_s = 0, \pm 1$ , and if the system starts from  $m_s = 0$ , then the observed PL is

$$PL(t) = p_0 P_1(t) + p_{-1} P_0(t)$$
  
=  $p_0 \cos^2(\Omega t/2) + p_{-1} \sin^2(\Omega t/2)$   
=  $\frac{p_0 + p_{-1}}{2} + \frac{p_0 - p_{-1}}{2} \cos \Omega t.$  (1.3)

#### 1.3.4.2 Bloch vector and Bloch sphere

For a quantum two-level system, a qubit, the density operator can be decomposed into a linear superposition of the identity matrix and Pauli matrices,  $\{\mathbf{I}, \sigma_x, \sigma_y, \sigma_z\}$ , as  $\rho = \frac{1}{2} \left(I - \overrightarrow{P} \cdot \overrightarrow{\sigma}\right)$ , where  $\overrightarrow{P} = (P_x, P_y, P_z)$  is the Bloch vector. For a pure state,  $|\overrightarrow{P}| = 1$  and this vector lies on the surface of a sphere. The expectation value  $\langle \sigma_z \rangle = \text{Tr}(\sigma_z \rho) = P_z$  and similarly for  $\sigma_x$  and  $\sigma_y$ . Without explicit proof, a Rabi rotation causes the Bloch vector to rotate about  $\overrightarrow{\omega} = (-\Omega \cos \phi, \Omega \sin \phi, \varepsilon)$ , where  $\Omega$  and  $\varepsilon$  are the Rabi frequency and the MW detuning.  $\phi$  is the initial phase of the MW, as defined in the previous section. When  $\varepsilon = 0$ , the Bloch vector rotates around an axis in the XY plane. The orientation is  $\Omega$  and thus at times of  $\Delta t = n\pi/2\Omega$ ,  $n = 1, 2, 3, \cdots$ , the Bloch vector has been rotated by  $n\pi/2\Omega$ . In particular,  $\pi/2$ -pulses and  $\pi$ -pulses are pulses with durations of  $T_{\frac{\pi}{2}} = \frac{\pi}{2\Omega}$  and  $T_{\pi} = \frac{\pi}{\Omega}$ . Figure 1.5 shows the characteristic Rabi fringe, which is fitted with (see Eq. 1.3)

$$PL = PL_0 \left[ 1 - Ce^{-\tau/T} \cos \Omega \tau \right].$$

The  $\pi$ -pulse time is deduced from the Rabi frequency,  $\Omega$ , to be  $(115.5 \pm 0.6)$  ns. Due to slowly fluctuating magnetic noise the envelop of the Rabi oscillation decays over the delay time  $\tau$ , giving a characteristic time, *T*, close (but not equal) to the  $T_2^*$  time.



Figure 1.6: Bloch sphere illustration of the evolution of the Bloch vector when a spin-echo sequence is applied. **a**) The NV ground state is initialised to the  $m_s = 0$  state, corresponding to the north pole of the Bloch sphere. **b**) Upon application of a  $\frac{\pi}{2}$ -pulse, the Bloch vector is rotated to the equatorial plane about the x-axis. **c**) Due to slow magnetic fluctuations (slow compared to the timescale of a spin-echo sequence), the Bloch vector precesses by a different angle in the equatorial plane for each realisation of the system, represented by a distribution of Bloch vectors. **d**) A  $\pi$ -pulse around x flips the Bloch vectors and **e**) the same slowly fluctuating magnetic field causes Bloch vectors to be refocused. **f**) A final  $\frac{\pi}{2}$ -pulse or  $\left(-\frac{\pi}{2}\right)$ -pulse (not shown) rotates the refocused Bloch vector to either the south of the north pole to be measured by optical readout.

A  $\pi$ -pulse with a detuning,  $\varepsilon$ , or MW power fluctuations (thus a change in  $\Omega$ ) would lead to a lower fidelity of a  $\pi$ -pulse. Certain composite pulse sequences are designed to correct for pulse errors and will be discussed in Chapter 2.

#### 1.3.4.3 Dynamical decoupling

The spin coherence can be protected from environmental noise and prolonged using a dynamical decoupling sequence. In a central spin problem, the noise refers to any environmental fluctuations that can couple to the NV spin (magnetic or electric), which induces spin dephasing and loss of coherence [102]. Dynamical decoupling typically consists of sequence of MW pulses, with carefully designed phase and spacing to decouple the spin qubit. Thus it is expected that the sequence depends on the system under study. For the noise profile experienced by the NV centres in high nitrogen concentration diamonds, equally spaced pulses are typically used [249], which decouple the NV from the dominating noise [138] due to P1 centers (single nitrogen atoms).

The simplest equally spaced sequence is the spin-echo sequence (Fig. 1.7). To visualise the effect of a spin-echo pulse sequence, we use the Bloch sphere shown in Fig. 1.6. The NV ground state is initialised to the  $m_s = 0$  state, corresponding to the north pole of the Bloch sphere (Fig. 1.6a)). Upon application of a  $\frac{\pi}{2}$ -pulse, the Bloch vector is rotated to the equatorial plane about the x-axis (Fig. 1.6b)). In the presence of a slowly fluctuating magnetic field (slow compared to the timescale of the spin-echo sequence), approximately constant in magnitude over the duration of a spin-echo sequence, the Bloch vector precesses



Figure 1.7: The decoherence of the spin qubit is measured with a spin-echo pulse sequence (shown a the top of the figure), giving a  $T_2$  time of  $T_{2,SE} = (4.1 \pm 0.3) \,\mu$ s fitted with a stretch Gaussian with exponent  $\alpha = (2.5 \pm 0.6)$ .

by a different angle during time  $\tau$  in the equatorial plane for each realisation of the system, represented by a distribution of Bloch vectors in Fig. 1.6c). A  $\pi$ -pulse around x then flips the Bloch vectors (Fig. 1.6d)) and the same slowly fluctuating magnetic field causes Bloch vectors to be refocused during time  $\tau$  (Fig. 1.6e)). A final  $\frac{\pi}{2}$ -pulse or  $\left(-\frac{\pi}{2}\right)$ -pulse rotates the refocused Bloch vector to either the south of the north pole to be measured by optical readout **f**). Usually the same sequence is run twice, once with  $\frac{\pi}{2}$ -pulse and another with  $\left(-\frac{\pi}{2}\right)$ -pulse (not shown) just before readout. The two resulting PL traces can be subtracted to isolate spin-dependent signals. The resulting PL difference as a function of delay,  $\tau$ , is plotted in Fig. 1.7. The shape of the curve depend on the property of the noise is usually fitted with a stretched Gaussian of the form,  $\propto \exp\left[-\left(\frac{\tau}{T_2}\right)^{\alpha}\right]$ , where the parameter,  $\alpha$ , encapsulates the complex dynamics of the noise bath. For the presented nanodiamond,  $T_2^* = (500 \pm 60)$  ns while  $T_{2,SE} = (4.1 \pm 0.3) \,\mu$ s, prolonged by a factor of 8. This  $T_2$  is typical of NV centres in nanodiamonds.

### **1.3.5** Phase control

In the Bloch sphere representation, an applied resonant MW drive causes the Bloch vector to rotate around the vector  $\vec{\omega} = (-\Omega \cos \phi, \Omega \sin \phi, 0)$ , where  $\phi$  is the initial phase of the MW. The relative phase difference between two applied MW pulses is critical in pulse protocols



Figure 1.8: NV ground state depolarisation. After initial optical polarisation, the optical contrast decreases over a characteristic time of  $T_1$ . The resulting data is fit with an exponential decay and the corresponding  $T_1 = (77 \pm 6) \mu s$ . The sequence (top of figure) prepares NV in  $m_s = 0$  and read out its state after a delay of  $\tau$ . Optical contrast between  $m_s = 0$  and  $m_s = \pm 1$ is created by repeating the same sequence with a  $\pi$ -pulse just before readout.

utilising multi-axis control pulses.

In the example of a spin-echo pulse sequence, the three pulses are all applied along the same axis since the MW accumulates phase at the same rate as the qubit and thus there is no relative phase difference between the MW and the qubit. However, suppose at the start of the  $\pi$ -pulse an abrupt shift of MW phase is applied, such that the MW leads the qubit by a phase of  $\pi/2$ , the rotation axis is then changed by 90 degrees (for example from *x* to *y*). Thus by using hardware such as arbitrary waveform generators (AWGs) to directly generate an abrupt phase shift, or IQ-modulators to change the relative contribution from the in-phase and the quadrature contributions, it is possible to change the axis of the applied rotation in the Bloch sphere.

## **1.3.6** Depolarisation

Once the NV ground state is optically pumped and polarised, it is no longer in thermal equilibrium with its environment and starts to thermalise via interactions with the surrounding environment and depolarise. Unlike decoherence, the depolarisation process requires energy dissipation and the corresponding timescale is  $T_1$ . As shown in [124] for NV centres in bulk diamond this is dominated by a two-phonon Raman process and is therefore dependent on the occupancy of the phonon modes.  $T_1$  increases with decreasing temperature. Cross-relaxation is also shown at specific resonances when the NV energy is degenerate with another spin species (for example nitrogen electrons). In addition to the phonon-induced depolarisation, magnetic noise at the spin resonance frequencies also induces depolarisation. Therefore by measuring the change in  $T_1$ , it is possible to measure the magnetic field noise, and thus inferring properties of magnetic species that generate the magnetic noise. This forms the basis of the "relaxometry" technique to probe surface spin density [192, 214] or paramagnetic spin labels [132, 220]. For nanodiamonds, the close surface proximity leads to a decrease in  $T_1$  from its typical bulk value of 1 ms to 10 ms [124] to a range of 0.1 ms to 1 ms (Fig. 1.8). The sequence used to probe depolarisation is a simple initialisation and read out sequence. A  $\pi$ -pulse can be applied to remove spin-independent PL decay (see pulse sequence at the top of Fig. 1.8).

#### **1.3.7** Dephasing, decoherence and depolarisation timescales

Three timescales have been referenced so far and it is worth highlighting their differences compared to the classical NMR literature. In the classical NMR setting, an ensemble of nuclei are simultaneously addressed by external radio-frequency magnetic fields (RF). Due to the spatial inhomogeneity in the externally applied static magnetic field, each nucleus precesses at different Larmor frequencies and the ensemble averaged magnetization decreases in magnitude over time. However, this dephasing is only due to experimental implementation and is not an inherent dephasing of the system. Thus the  $T_2^*$  time is typically defined as the difference between decay time measured with spin-echo and measured with free induction decay (FID),

$$\frac{1}{T_2^*} = \frac{1}{\tau_{SE}} - \frac{1}{\tau_{FID}}$$

The  $T_2^*$  defined this way removes the contribution from ensemble averaging and is then limited by the intrinsic fluctuation over time experienced by each nuclear spin. Manipulation of a single spin does not suffer from this experimental limitation due to ensemble averaging. Instead the time-averaging procedure, as is typical for NV non-single-shot readout, limits  $T_2^*$ . Thus unlike in classical NMR, spin-echo in single-manipulation removes slow fluctuations in time. In NV literature,  $T_2^*$  is defined as the decay envelop of a Ramsey interferometry (or free induction decay) experiment, while  $T_{2,SE}$  is the decay envelop of a spin-echo experiment. Note that the  $T_2$  needs to be qualified with the corresponding sequence (for example  $T_{2,CPMG-XY8}$ ) as these can differ significantly between sequences.

The definition of the depolarisation timescale is defined similarly in classical NMR as the

decay envelop of a  $T_1$  measurement.

### **1.3.8** Optimising pulsed readout

The readout technique employed in this work is the "conventional" optical readout technique, which differs from the more sophisticated spin-to-charge (SCC) readout [108, 209] and near-IR readout [107]. In the conventional readout technique, the photons arrived between  $t_1$  and  $t_2$  times after the laser is switched on are counted. By changing the choices of the start time  $t_1$  and the readout duration  $\Delta t = t_2 - t_1$ , the readout efficiency can be optimised. Since the optical lifetime of the radiative transition is ~ 10ns, the quantity  $t_1$  is determined primarily by the hardware delays due to for example the response time of the acoustic optical modulator used to pulse the optical excitation.  $t_1$  has a typical value of 150 - 300 ns.

On the other hand  $\Delta t$  is limited by the NV centres' optical transition rates.  $t_1$  does not depend on optical power, while the optimal duration  $\Delta t$  does depend on optical excitation. Qualitatively, high optical excitation leads to shorter readout duration. For long  $\Delta t$ , the spin state information would be destroyed due to optical pumping. For short  $\Delta t$ , the total PL is small and gives large photon shot noise. Therefore the optical readout should find a compromise between the contrast (spin information) and the noise (photon shot noise).

To find the optimal readout durations, first denote the average PL rate at a time  $\tau$  after the laser is turned on given the spin state is  $m_s$  by  $I(\tau|m_s)$  and the integrated photon counts from  $\tau = t_1$  and  $\tau = t_2$  given  $m_s$  by  $F(m_s) = \int_{t_1}^{t_2} I(\tau|m_s) d\tau$ . Then define the signal-to-noise ratio (SNR) as

$$SNR(t_1, t_2) = \frac{F(0) - F(1)}{\sqrt{F(0) + F(1)}}.$$
(1.4)

Here the SNR characterises the ratio between the optical contrast and the shot noise. An ideal read-out duration should optimise the SNR, thus optimising the Fisher information (Sec. A.1.1). This is used instead of, for example, the more intuitive quantities such as the contrast, or the absolute photon counts.

The SNR can be measured with the sequence shown at the top of Fig. 1.9. The GS is first prepared in the  $m_s = 0$  state by optical pumping and is converted into the  $m_s = \pm 1$  by a  $\pi$ -pulse. A small APD readout time (50ns in this work) is used and by changing the delay,  $\tau$ , from the start of the readout laser to the start of the APD switch-on time,  $I(\tau|m_s = \pm 1)$  can be probed (Fig. 1.9 a) red trace). Alternatively, if the  $\pi$ -pulse is not applied,  $I(\tau|m_s = 0)$  is probed (Fig. 1.9 a) blue trace). By cumulatively summing  $I(\tau|m_s)$  to approximate  $F(m_s)$ , the SNR can be computed and is displayed in the colormap in Fig. 1.9 c). The optimal start time  $t_1$  and readout duration  $\Delta t$  corresponds to the maximum of SNR marked with a white cross in the figure. Note that the optimal SNR and maximum contrast (gray and black traces in



Figure 1.9: Illustration of readout pulse optimisation. The pulse sequence (top of figure) prepares the NV in  $m_s = 0$  and reads out its fluorescence after a laser illumination time of  $\tau$  for a duration of 50 ns. The same sequence is repeated by preparing the NV at  $m_s = -1$  using a  $\pi$ -pulse. In **a**),  $I(t|m_s = 0)$  (blue) and  $I(t|m_s = \pm 1)$  (red) are the PL a delay  $\tau$  after the laser is switched on. **b**) shows the contrast (black trace) which is optimal is at approximately 75 ns while SNR (gray trace) at optimal start time  $t_1 = 225$  ns as defined in Eq. 1.4 is peaked at approximately 150 ns. **c**) shows a colormap of  $SNR(t_1, t_2)$  and the optimal choices of the start time,  $t_1$  and the read time,  $t_2$  can be found, indicated as a white cross in **c**).

Fig. 1.9 b)) do not correspond to the same delay. The starting time,  $t_1$ , is hardware dependent and does not change for the same set-up. It is worth highlighting here that electrical signals travel at the speed of light in 50 $\Omega$ -matched BNC cables and a 3 m cable introduces a delay of 10 ns, typical of cables used in the lab. Thus for pulsed measurements requiring ~ 10 ns delay accuracy, such as  $t_1$ , it is necessary to calibrate this delay. Other instrumental errors such as AOM rise time (~ 100 ns) are also calibrated using the readout calibration sequence.

The readout duration,  $\Delta t$ , on the other hand, is an NV dynamics dependent quantity and should ideally be calibrated per optical excitation power per diamond. However, since every diamond has a different orientation and it is not practical to perform this calibration per diamond, for convenience  $\Delta t = 300$  ns are fixed across different experiments on the same set-up. This only limits the readout efficiency (SNR), but will not change the spin state information

extracted. Due to the way the data is taken, *I* is expressed as number of photons collected over 50 ns APD windows, with the pulse sequence repeated over one second. Thus the number of photons per readout is lost. This scales the SNR by a constant yet unrecorded factor and thus the exact value of the SNR is not comparable with other work.

## **1.4** Cellular uptake of nanodiamonds

Unlike small molecules, larger nanoparticles cannot diffuse across the plasma membrane. Some cells, such as the well-known cervical cancer cell line, HeLa, naturally uptake nanodiamonds via clathrin-mediated endocytosis [241].

The uptake observed in HeLa is not universal amongst cell types. In our experiments, MCF-7 and U2OS cells do not readily take up nanodiamonds when diamond concentrations between  $100 \,\mu g \,m L^{-1}$  and  $1 \,m g \,m L^{-1}$  are used (data not shown). For experiments presented in Chapter 5, HeLa cells have been used with a nanodiamond concentration between  $10 \,\mu g \,m L^{-1}$  to  $300 \,\mu g \,m L^{-1}$ . The detailed uptake protocol is discussed in Chapter 5.

Over the years nanoparticle delivery has been extensively studied for the purpose of drug delivery and techniques have been developed such as liposome assisted delivery [168] and surface coating via polyethyleimine (PEI) [275], which enable or enhance uptake.

Another class of methods such as electroporation were originally used for cell transfection (infecting cells with foreign genetic materials such as DNA and plasmids) and were effective for small charged molecules like DNA. This class of methods relies on transiently forming pores on the plasma membrane of cells which allow small particles to enter (see [221] for a review). Electroporation uses short but high-voltage pulses to transiently create a pore at the surface of the membrane and transfect negatively charged DNA molecules and have been extended to negatively charged nanoparticles. Optoporation is a method based on femto-second laser pulses which create local cavitation and disrupt the plasma membrane. Small particles and molecules present in the medium stochastically enter the cytoplasm. Acoustoporation uses ultrasonic waves to generate shear forces on the plasma membrane and thus forming pores for nanoparticle entry. Mechanical transfection uses small geometrical constraints in a microfluidic channel to shear apart the plasma membrane.

Finally, silicon nanowire has demonstrated high efficacy in nanodiamond delivery [142]. In this method, silicon nanowires are fabricated on a silicon substrate and nanodiamonds are attached to the nanowire via electrostatic forces by surface treating the silicon nanowire. Adherent cells are incubated on these substrates to allow nanowires to penetrate the plasma membrane and enter the cytoplasm. Upon trypsin detachment and subsequent washing, some nanodiamonds would detach from the silicon nanowire and remain in the cytoplasm.

Upon entering the cell, nanodiamonds are enclosed in the endosomal membranes which are later pierced open by geometrically sharper diamonds [53, 54] and escaping into the cytoplasm.

## **1.5** Thesis overview

This thesis explores the use nanodiamond quantum sensors to interface with other complex systems, and sensing signals of different nature.

Following the basic introduction in this chapter, the sensing of a **stationary** (**non-time-varying**), stochastic magnetic field generated by precessing nuclei is demonstrated in Chapter 2, achieving nanoscale nuclear magnetic resonance (NMR).

Furthermore, in Chapter 3, we apply NV sensing to photo-excited external triplet excitons in a singlet-fission material, where we show the sensing of **periodically generated** external paramagnetic species.

In addition to quantum sensing, NV centres have also been used as biomarkers in cells for their bright fluorescence and photo-stability. We aim to introduce nanodiamond sensors for temperature sensing in cells. Temperature represents an **uncontrolled**, **time-varying signal**, which imposes different challenges compared to the first two experiments. For this purpose, we first characterise temperature sensing in an abiotic environment and demonstrate its sensitivity in Chapter 4. Subsequently, in Chapter 5, nanodiamonds are introduced into HeLa cells to demonstrate simultaneous single-particle tracking for diffusion characterisation, and intracellular temperature sensing.

At the end of this thesis, Chapter 6 presents the recently found single defects in 2-dimensional layered van der Waals material, hexagonal boron nitride (hBN), for future applications as spin-photon interfaces and quantum sensors.

As each chapter revolves around very different techniques from sample preparation, measurement setup to data analysis, they will have an individual introduction detailing the respective techniques.

# Chapter 2

# Nanoscale nuclear magnetic resonance using NV centres in nanodiamonds

In this chapter, we explore the idea of using NV centres to achieve sensing of a stationary external magnetic signal, specifically the stochastic magnetic signal created by nuclei undergoing Larmor precession in the surrounding medium. The commonly used dynamical decoupling technique is used to demonstrate frequency selective sensing of an AC magnetic signal.

This work was done in collaboration with Jeffrey Holzgrafe. J.H. initiated the project idea. The set-up was originally built by Dr Jan Beitner, with control software written in Python by Josh Barnes and J.B.. J.H. was the main contributor to the work on surface-enhanced calibration technique. J.H. and Q.G. created XY8 sequences with phase control and performed all experiments, theory and analysis together with equal contribution.

## 2.1 Introduction

Nuclear magnetic resonance (NMR) techniques have transformed biology and chemistry. Conventional NMR is typically performed at strong external magnetic fields. The nuclear spins are thermally polarised at these strong magnetic fields and thus allow the spin polarisation to be detected by inductive sensing methods. This thermal polarisation arises from the very small imbalance in spin populations due to the Zeeman effect. A typical limit of detection (conventionally defined as SNR=3 for the NMR peak) is achieved around 5  $\mu$ M in 4 min for a 400 MHz <sup>1</sup>H spectrometer [273]. The high-field NMR spectrometers require the use of superconducting magnets and are bulky and expensive. Despite the strong field typically used, the thermal polarisation is still weak. For example, the thermal polarisation of <sup>1</sup>H in 7 T field is  $e^{-\gamma_H hB/k_BT} \sim 10^{-5}$ . Thus, conventional NMR requires large sample volume (mm),

degrading the spatial resolution in imaging applications. Various techniques are explored to address these issues. Dynamic nuclear polarisation improves the sample polarisation beyond the Boltzmann's distribution. Benchtop spectrometers use permanent magnets (1 T to 2 T) and integrated electronics in applications where space and cost are limited [40]. The conventional NMR spectrometers gives spectral linewidth approaching the limit of the intrinsic nuclear linewidth (< 0.5Hz) [212].

## 2.1.1 Spatial resolution

In the recent decade, nanoSQUID has enabled micro- to nanoscale NMR applications. Nevertheless these techniques have their own limitations. The nanoSQUID technique, which shares comparable nanoscale spatial resolution, relies on the use of superconducting quantum interference effect using metals such as aluminium and lead. which require cryogenic temperatures. Magnetic resonance force microscopy (MRFM) [259] and NV centres in bulk diamond have both demonstrated nanoscale detection volumes. In conventional NMR the sample volume (V) is large and thus the net thermal magnetization ( $\propto V$ ) is detected and is dominant over the stochastic fluctuation of nuclear spins ( $\propto \sqrt{V}$ ). For small sample volume however, the statistical polarisation is dominant. Thus the reduction of volume requires a new mode of detection.

As NVs detect the statistical polarisation of the spins instead of the usual magnetic dipolar coupling that depends on  $d^{-3}$ , the distance dependence is  $d^{-6}$  as NMR is in the "variance sensing" regime [58]. This strong distance dependence improves sensitivity rapidly for decreasing NV-nuclei distance, confining the sensing volume to a small shell around the nanodiamond. The room-temperature operation is an attractive feature of NV centre based NMR.

## 2.1.2 Spectral resolution

In conventional NMR, the Zeeman splitting due to an externally applied magnetic field is measured. The local magnetic field experienced by individual nucleus is 'shielded' by the distribution of local electrons, creating very small shifts in the Zeeman splitting, detected by NMR. This allows identification of nuclei in different bonding environments. This shift, called chemical shift, is usually measured with respect to the resonance frequency of the nucleus in a reference compound (for example CH<sub>3</sub>F for fluorine NMR) and is typically in the ppm level of the reference resonance frequency (1 ppm corresponds to 400 Hz in a 400 MHz spectrometer). Furthermore, coupling between neighbouring nuclei through chemical bonds ('J-'coupling) splits the NMR peak into multiple resonances, typically in the Hz level, independent of the external magnetic field. The spectral assignments of chemical shift and spin-spin coupling resonances allow precise deduction of the bonding in an unknown molecule.

Multiple experiments have demonstrated chemical shift level spectral resolution [20] using NV centres in diamond. The demonstration so far has been on model systems for benchmarking purposes. This drives the improvement towards single molecular NMR in biological systems or closed structure such as battery electrodes and biological cells. Nanodiamonds are a good candidate for such intracellular applications.

As shown by many experiments, nanodiamonds have poorer coherence [192, 214], in terms of  $T_2^*$ ,  $T_2$  and  $T_1$ , compared to bulk diamond. Experiments by Knowles *et al* demonstrates that high-pressure high-temperature (HPHT) nanodiamonds can still attain a coherence time long compared to spin-manipulation times ( $T_2 \sim 40 \,\mu s$ ,  $\pi$ -pulse time  $\sim 10 - 100 \,ns$ ), further enhanced by dynamical decoupling, to perform NMR experiments [138].

Therefore the work in this chapter explores the challenges in nanoscale NMR, using nanodiamonds instead of bulk diamonds.

# 2.2 Experimental implementation

## 2.2.1 Materials

The diamond used in this work is HPHT fabricated nanodiamonds with a nominal nitrogen concentration of 50 ppm. The sizes vary and are approximately 23 nm [138]. From atomic force microscopes of 12 nanodiamonds (containing single NVs, indicated by their ODMR), the size of nanodiamonds could vary significantly, ranging from 10 nm to 30 nm. This is a reasonable size as small diamonds (with single-digit sizes) are known to unstable due to surface charge-trap induced charge conversion and larger diamonds are statistically more likely to have more than one NVs. The nominal number of nitrogen atoms in this volume of diamond is about  $8 \times 10^3 \sim 72 \times 10^3$  per diamond. Thus, the expected NV center conversion ratio is about 0.1% and the NV concentration is in the ppb range.

Nanodiamonds suspended in ethanol are sonicated for ~100 min and are deposited on quartz substrate using a nebulizer to form well-dispersed ethanol droplets. This reduces clusters of nanodiamonds on the substrate. A thin layer of perfluoropolyether (PFPE), also known as Fomblin Y, is a long-chain polymer that contains high density of <sup>19</sup>F nuclei and is highly viscous to reduce NMR line broadening due to diffusion. The line broadening arises from the finite interaction time when the target molecule diffuses across the sensing volume of the NV. The PFPE is spin coated on the surface, resulting in an estimated thickness of 5  $\mu$ m, thus completely covering the nanodiamonds. In addition, the nanodiamond surface is typically covered by hydrogen atoms resulting from water vapour in air and organic solvents used during sample

processing.

In comparison to bulk diamonds NMR, nanodiamonds are able to access enclosed volumes such as biological cells and three-dimensional structures such as battery electrodes. They are commercially available and have a intrinsically smaller sensor to target distance with additional shallow implantation steps. Yet NMR using NV centres in nanodiamonds still presents a few technical challenges.

Firstly the spin coherence property is poor due to the close proximity to the surface. The recently identified nanodiamond [138] provides significantly better material property to host nanodiamonds with  $T_2 \approx 30 \,\mu$ s. The more recent work [260] also reports natural abundance <sup>13</sup>C nanodiamonds with a  $T_2$  time exceeding 400  $\mu$ s. With better material engineering and a good compromise between nanodiamond size and coherence property, it is in principle possible to further enhance the NMR sensitivity.

Secondly, each nanodiamond crystal is randomly oriented, thus the NV centre contained within is also randomly oriented. NMR experiment requires a strong external magnetic field to be aligned with the NV axis better than 1° to avoid PL quenching, whereby the ODMR contrast is diminished due to mixing of the  $m_S = 0$  and  $m_S = \pm 1$  states under a strong external magnetic field [233]. Such a well-aligned magnetic field also allows the NV to be used for an accurate measurement of the static external magnetic field, as the NV is only sensitive to longitudinal magnetic field in this regime. This issue is addressed with a dual-axis rotation stage, with the freedom to vary the orientation, while still capable of delivering magnetic field strength close to 200 mT.

## 2.2.2 Confocal microscope

The experimental setup used to perform the NMR experiment is a typical NV confocal microscope (Fig. 2.1). Optical excitation is achieved with a continuous-wave laser at 532 nm. the fluorescence passes through a long-pass filter (LP550) at 550 nm which is used to filter the scattered excitation light. The fluorescence is then coupled into a single-mode (SM) fibre, which acts as a pinhole. The photons are detected by an avalanche photodiode (APD).

A 100x air objective with a numerical aperture of 0.9 is used for maximum photon extraction in air. The sample is mounted on a piezo stage (bottom left) to allow nanoscale movement of the sample. The piezostage is a 'slip-stick' piezo stage such that the stage cannot be moved smoothly with a 10-nanometer positioning accuracy, which is important for confocal scanning. Instead, lateral scanning is achieved with a piezo mirror, which steers the laser beam across the back aperture of the objective. In this configuration, the photon collection efficiency is only optimal when the mirror is in its neutral position. Strategies such as a Keplerian telescope is often used to maximise the field of view (as shown in Chapter 4) yet by not using a telescope



Figure 2.1: Experimental setup for NMR experiment. The setup is a typical confocal microscope. The sample is mounted on a piezo stage (bottom left) to allow nanoscale movement of the sample. A 100x air objective with a numerical aperture of 0.9 is used. A CW laser with a wavelength of 532 nm is used for excitation and the fluorescence passes through a long-pass filter (LP550) at 550 nm is used to filter the scattered excitation light. The fluorescence is then coupled into a single-mode (SM) fibre, which as a pinhole. The photons are detected by an avalanche photodiode (APD). Pulsed MW is generated by an arbitrary waveform generator (AWG) and pulsed initialisation and readout are also controlled by the same AWG. Lateral scanning is achieved with a piezo mirror. DM550: dichroic mirror at 550 nm. A permanent magnet is used to provide a static magnetic field and is mounted on a two-axis rotation stage to align the magnetic orientation with the NV axis. A miniature translation stage is also mounted on the rotation stage to vary the applied magnetic field without changing its orientation.

system we avoid the loss and aberrations due to the two additional lenses (about 90% on each lens used in Chapter 4). In NV measurements with stationary particles, the piezo-stage is used to move the NV of interest to the center of the FOV.

Below the stage, a permanent magnet is used to provide a static magnetic field and is mounted on a two-axis rotation stage to align the magnetic orientation with the NV axis. A miniature translation stage is also mounted on the rotation stage to vary the applied magnetic field without changing its orientation.

To reduce detuning induced pulse errors, in particular that due to the hyperfine coupling to <sup>14</sup>N nuclear spins (S = 1), a static magnetic field of approximately 300 G is applied along the NV axis in our work, which allows the nitrogen-14 nuclear spins to be polarised when the electrons are optically cycled through the excited states [120].

The laser beam is split by a beamsplitter (90:10 R:T, BS2 at the center of Fig. 2.1) and the transmitted beam is measured with a photodiode. The laser power typically fluctuates by about 0.6% during the course of a few minutes and does not drift by more than this value over a day. This fluctuation is large compared to the PID stabilised laser power discussed in Chapter 4 but is sufficient in the pulse protocol we use, where a signal and reference measurement is taken every second, so that laser fluctuation is removed from the data (see discussion later in the chapter).

Pulsed MW is generated by an arbitrary waveform generator (AWG). The pulsed MW is delivered to the sample using a copper coil as illustrated in Fig. 2.2 **a**). A typical  $\pi$ -pulse time of 100 ns can be achieved with this coil. Pulsed optical initialisation and readout laser with an acouto-optic modulator (AOM) are also controlled by the same AWG. The APD output is also gated and the readout duration is optimised as discussed in Section 1.3.8 of Chapter 1.

## 2.2.3 Plain and reference measurements

The NV fluorescence could be influenced by laser fluctuation and sample drift caused by periodic temperature fluctuation and mechanical relaxation of the piezo-stage. The sequence as shown Fig. 2.2 b) shows the sequence termed 'plain' in this work ( $PL_{plain}$ ). To counteract any PL fluctuation, another sequence with an added  $\pi$ -pulse just before readout, termed 'ref' ( $PL_{ref}$ ), is run immediately after so that the PL difference ( $PL_{plain} - PL_{ref}$ ) is only related to spin dynamics. Experimentally, the plain sequence for one time delay  $\tau$  is run for 1 s followed by the ref sequence for another 1 s. The same pair of sequences are repeated for each delay  $\tau$ .



Figure 2.2: Experimental configuration. **a**) shows nanodiamond on glass substrate covered by 5 µm of a fluorine-rich liquid (PFPE), shown as a blue liquid environment on the figure. Microwave delivery is achieved with a loop antenna. The optical excitation is focused down from the liquid side. The surface of nanodiamonds is covered by a hydro-dense surface layer (dark blue). The nanodiamond used is typically 30 nm in diameter. **b**) shows the CPMG XY8k sequence which also measures the NMR signal. The oscillating magnetic field generated by the nuclear spin bath (black curve) is frequency matched to the decoupling period between microwave  $\pi$ -pulses (yellow) and leads to a measurable phase of the quantum sensor. The eight-pulse subunit shown is repeated k times to form the complete sequence. **c**) shows a coherence measurement performed on one nanodiamond device using an XY8-k protocol with a constant  $\tau = 0.5 \mu s$ , showing a long  $T_2 = 35 \mu s$ .

## 2.3 Theory of NV based NMR

In contrast to conventional NMR methods, nanoscale NMR relies on detecting the statistical fluctuation of the total magnetic moment of the detection volume, i.e.  $\langle B \rangle = 0$ ,  $\langle B^2 \rangle \neq 0$ .

To probe this magnetic field fluctuation, decoherence spectroscopy is used. In this method, an NV ground-state spin is protected with a dynamical decoupling sequence such as the Carr-Purcell-Meiboom-Boom (CPMG) XY-8 sequence. The NV ground state spin is first prepared into a superposition state  $|\psi_i\rangle = \frac{1}{\sqrt{2}} (|0\rangle + |-1\rangle)$  via continuous optical pumping followed by a  $\pi/2$ -pulse. Eight  $\pi$ -pulses about X and Y axes are applied, with a temporal separation of  $\tau$ . Finally another  $\pi/2$ -pulse is applied followed by optical readout. Under the application of a

XY-8 pulse sequence, the final state just before readout is

$$|\psi\rangle = rac{1}{\sqrt{2}} \left( |0\rangle + e^{-i\phi} |-1\rangle 
ight).$$

The phases accumulated is

$$\phi = \frac{\mu_B g}{\hbar} \int f(t) B_{\parallel}(t) dt, \qquad (2.1)$$

where f(t) is the window function and equals +1(-1) if even (odd) number of  $\pi$ -pulses have been applied. A final  $\pi/2$ -pulse is applied to map phase into population. The expectation value for a  $\sigma_z$  measurement is

$$\langle \cos \phi \rangle = \exp\left(-\frac{\left\langle \phi^2 \right\rangle}{2}\right),$$

where  $\langle \phi^2 \rangle$  is the ensemble average.  $B_{\parallel}(t)$  is the stochastic (classical) magnetic noise generated by random flip-flop of the surrounding nuclei along the axial direction of the NV. This noise is assumed to have a mean zero and to be stationary,

$$egin{aligned} &\langle B
angle = 0,\ &\langle B(t)B(t+ au)
angle = \langle B(0)B( au)
angle. \end{aligned}$$

Using this expression and Eq. 2.1,  $\langle \phi^2 \rangle$  can be linked to the spectral density of the magnetic noise,

$$S_B(\boldsymbol{\omega}) = \int dt' e^{-i\boldsymbol{\omega}t'} S_B(t') = \int dt' e^{-i\boldsymbol{\omega}t'} \left\langle B_{\parallel}(0) B_{\parallel}(t') \right\rangle,$$

as,
$$\begin{split} \left\langle \phi^{2} \right\rangle &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \left\langle \int dt dt' f(t) f(t') B_{\parallel}(t) B_{\parallel}(t') \right\rangle \\ &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \int dt dt' f(t) f(t') \left\langle B_{\parallel}(t) B_{\parallel}(t') \right\rangle \\ &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \int dt' f(t') \int S_{B}(t-t') f(t) dt \\ &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \int dt' \int d\omega f(\omega) e^{i\omega t'} \int S_{B}(t-t') f(t) dt \\ &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \int d\omega f(\omega) \int dt' e^{-i\omega t'} \int S_{B}(t-t') f(t) dt \\ &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \int d\omega f(\omega) f(-\omega) S_{B}(\omega) \\ &= \left(\frac{\mu_{B}g}{\hbar}\right)^{2} \int d\omega |f(\omega)|^{2} S_{B}(\omega), \end{split}$$

where the following Fourier transformation conventions are used,

$$g(\boldsymbol{\omega}) = \mathscr{F}(g(t)) = \int dt g(t) e^{-i\boldsymbol{\omega} t},$$
  

$$g(t) = \mathscr{F}^{-1}(g(\boldsymbol{\omega})) = \frac{1}{2\pi} \int d\boldsymbol{\omega} g(\boldsymbol{\omega}) e^{i\boldsymbol{\omega} t}.$$

For an XY8 sequence with k repeats, the window function is a convolution between a top-hat function with width  $8k\tau$  and a square wave with period  $2\tau$ . This gives a spectral window of,

$$f(\boldsymbol{\omega}) = \left[8k\tau \operatorname{sinc4}\boldsymbol{\omega}k\tau\right] \star \left[\sum_{l=-\infty}^{+\infty} \frac{4(-1)^l}{2\pi(2l+1)} \left[\delta(\boldsymbol{\omega} - \frac{(2l+1)\pi}{\tau})\right]\right] \\ = \sum_{l=-\infty}^{+\infty} \frac{4(-1)^l}{2\pi(2l+1)} \left[8k\tau \operatorname{sinc4}(\boldsymbol{\omega} - \frac{(2l+1)\pi}{\tau})k\tau\right] ,$$

as the linewidth  $\Delta \omega = \pi/2k\tau$  is typically much smaller than the frequency spacing  $\omega_{n+1} - \omega_n = \frac{2\pi}{\tau}$  and thus the cross terms are small,

$$|f(\boldsymbol{\omega})|^2 \approx \sum_{l=-\infty}^{+\infty} \left(\frac{2}{\pi(2l+1)}\right)^2 \left[ (8k\tau)^2 \operatorname{sinc}^2 \frac{8k\tau}{2} (\boldsymbol{\omega} - \frac{(2l+1)\pi}{\tau}) \right],$$

which is a sum of resonances with central frequencies  $\omega_n = \frac{n\pi}{\tau}$ , n = 1, 3, 5, ..., and linewidth (full width at first minima)  $\Delta \omega = \pi/2k\tau$ . The corresponding linear frequencies are  $v_n = \frac{n}{2\tau}$ , n = 1, 3, 5, ..., and  $\Delta v = 1/4k\tau$ . Note that when the pulses are ideal, the window function does not have a resonance below the fundamental frequency (n = 1). The spectral window is limited by the total phase acquisition time  $8k\tau$ . In addition, the relative linewidth  $\Delta v/v = 1/2kn$  is improved for higher resonances, albeit with smaller resonance amplitude



Figure 2.3: Illustration of the NMR signal. **a**) shows the lineshape as a result of XY8-k sequence which has a linewidth decreasing with the number of  $\pi$ -pulses applied. Illustration of contrast and CPMG XY8 decoherence measurement. As the spacing between  $\pi$ -pulses is varied, the intrinsic decoherence manifests as a decay in PL. **b**) shows this decay (dashed curve). An additional  $\pi$ -pulse is applied right before readout to create the contrast (lower branch of the dashed curve). When a NMR signal is present, the magnetic field due to nuclear dipole moment precession create a characteristic lineshape (solid curve).

 $\propto \frac{1}{n^2}$ . Assuming that the nuclear dephasing time is infinite,

$$S_B(\omega) = B_{RMS}^2 \delta(\omega - \omega_L).$$

[The nuclear spin bath has a simple dynamics containing limited autocorrelation. A more complex situation arises when the correlation does not vanish, for example due to diffusion, or intrabath dipolar interaction, and is discussed in the following chapter.]

Therefore,

$$\left\langle \phi^2 \right\rangle(\tau) = \frac{4}{\pi^2} (8k\tau)^2 \left(\frac{\mu_B g}{\hbar}\right)^2 B_{RMS}^2 \operatorname{sinc}^2 \left[\frac{8k\tau}{2} (\omega_L - \frac{\pi}{\tau})\right],$$

giving a contrast of (Fig. 2.3 a)),

$$C(\tau) = 1 - \exp\left\{-\underbrace{\frac{2}{\pi^2}(8k\tau)^2 \left(\frac{\mu_B g}{\hbar}\right)^2 B_{RMS}^2 \underbrace{\operatorname{sinc}^2 \frac{8k\tau}{2}(\omega_L - \frac{\pi}{\tau})}_{lineshape}}_{lineshape}\right\}.$$
 (2.2)

The contrast is defined so that C = 0 when  $B_{RMS} = 0$  and C = 1 when  $B_{RMS} \rightarrow \infty$ . There are two factors to the exponent. The contrast of the peak depends both on the total duration of

the pulse sequence and the strength of the nuclear magnetic field. The linewidth of the signal, however, depends only on the total duration of the pulse sequence.

The spectral window function  $|f(\omega)|^2$  determines the best linewidth achieved by the sensor, ignoring the broadening induced by the source. Note here that the exponent is in radians  $(\operatorname{rad} s^{-1})$  rather than revolutions (Hz).

The experimental data is fitted with (see Fig. 2.3)

$$PL_{plain}(\tau) - PL_{ref}(\tau) = PL_0C(\tau)\exp\left(-\tau/T_2\right),$$

where  $PL_0$ ,  $T_2$  are fitting parameters, representing the PL difference at  $\tau = 0$ , and the coherence time under the XY8-k DD sequence. Parameters contained in the contrast function  $C(\tau)$ , including  $\omega_L$ ,  $B_{RMS}^2$  are also fitting parameters, while *k* and other fundamental constants are fixed.

With reference to Fig. 2.3, when  $\tau \ll T_2$ , the exponential decay due to intrinsic decoherence is small over the linewidth of the NMR peak which can then be approximated as a Lorentzian peak. The pulse sequence has a total duration of  $\tau_{total} = \tau_I + \tau_R + 8k\tau \approx 8k\tau$ , where  $\tau_I$  and  $\tau_R$  account for initialisation and readout durations, while  $8k\tau$  is the spin manipulation time. The average PL counts when there is no signal is  $p \frac{\tau_R}{\tau_{total}} e^{-\tau_{total}/T_2}$ , where  $p e^{-\tau_{total}/T_2}$  is the rate of counts per readout duration and  $\frac{\tau_R}{\tau_{total}}$  is the proportion of readout time per measurement sequence. Thus the zero-signal shot-noise limited variance of PL is

$$\operatorname{var}(PL) = p \frac{\tau_R}{\tau_{total}} e^{-\tau_{total}/T_2}.$$

For a small signal,  $C(\tau) \propto (8k\tau)^2 = \tau_{total}^2$ . Thus the signal  $(PL \times C)$  to noise ratio is

$$SNR = C\sqrt{PL} \propto \tau_{total}^{3/2} e^{-\tau_{total}/2T_2}$$

This expression is maximum when  $\tau_{total} = 3T_2$ . The contrast (concentration) sensitivity is thus best at  $\tau_{total} = 3T_2$ . The frequency sensitivity depends both on the contrast and the linewidth and is approximately,

$$\delta f \propto \frac{\delta v}{C\sqrt{PL}} \propto \tau_{total}^{-5/2} e^{\tau_{total}/2T_2}$$

where  $\delta v \propto 1/\tau_{total}$  is the linewidth of the NMR peak. The frequency resolution is best when  $\tau_{total} = 5T_2$ .

As  $T_{2,XY-8}$  is approximately 40 µs, the linewidth limit is approximately 150 kHz. Additional methods such as quantum memory [20], correlational spectroscopy, can improve the frequency resolution to the level needed for detecting chemical shifts. Moreover, the sensi-

tivity can also improve with photon readout efficiency and fidelity, via for example repetitive readout method.

Finally as the window function shows a well-known 4*f* resonance when the pulses are of finite width, <sup>13</sup>C signal has been mistaken historically as the <sup>1</sup>H signal. This error is corrected by varying the phase of the last  $\pi/2$ -pulse to suppress the signal from carbon-13 while not degrading the signal from H-1.

## 2.4 **Results and discussion**

#### 2.4.1 Observing NMR due to two nuclear species

Figure 2.4 **a**) shows the single spectrum taken with XY8-10 sequence, where a total of 80  $\pi$ -pulses are applied per sequence. The peak at higher  $\tau$  (thus lower frequency given  $\tau = \frac{1}{2f_L}$ , highlighted in red) corresponds to <sup>19</sup>F. The other peak (blue) corresponds to <sup>1</sup>H. Pure PFPE itself contains no hydrogen atoms (Fig. 2.4**b**)), and we attribute this signal to a few-nm-thick hydrogen-dense layer on the surface of the nanodiamond, which is consistent with previous measurements on bulk diamond [59, 100, 180, 218]. The linewidths of both peaks are consistent with the instrumental bandwidth of the decoupling protocol (displayed as dashed lines, mostly overlapping with the actual lineshape), indicating that the intrinsic linewidth of the nuclear spin bath,  $1/T_2^*$ , is much narrower than the measurement's instrumental bandwidth. We extract the signal strength for each nuclear species by fitting data to the expected line shapes (Eq. 2.2) and obtain estimates of the magnetic field variance at the site of the NV generated by external nuclear species  $\langle B_{1H}^2 \rangle = 0.015(3) \mu T^2$  and  $\langle B_{19F}^2 \rangle = 0.019(1) \mu T^2$ , using Eq. 2.2.

#### 2.4.2 Confirming identity of nuclear species

To confirm these resonances indeed correspond to <sup>19</sup>F and <sup>1</sup>H, we perform spectrum measurement at various externally applied magnetic field,  $B_0$  and extract the resonance frequencies (Fig. 2.4 c)). The NMR resonance frequencies shift linearly with the applied magnetic field and the gyromagnetic ratios,  $\gamma$ , defined as

$$\gamma = f_L/B,$$

where  $f_L$  is the Larmor frequencies extracted from the NMR resonances, are 42.56(5) MHz T<sup>-1</sup> and 40.03(2) MHz T<sup>-1</sup> respectively for <sup>1</sup>H and <sup>19</sup>F peaks, consistent with commonly agreed values [88]. The magnetic field alignment to the NV axis is within 0.5° and thus the NV gives < 40 ppm uncertainty in the applied B field. As such the uncertainty in gyromagnetic ratios



Figure 2.4: Detection of NMR signals form multiple nuclear species using a nanodiamond. **a)** Example NMR spectrum measured with an XY8-10 dynamical decoupling sequence. Positions of each peak corresponds to  $\tau = \frac{1}{2f_L}$  with  $f_L$  is the Larmor frequency. The solid curves correspond to the fitted resonance contribution from <sup>1</sup>H (blue) and <sup>19</sup>F (red). The dashed curves indicate the window function of the dynamical decoupling sequence, mostly overlapping with the actual lineshape. **b)** shows the structure of the PFPE analyte. **c)** <sup>1</sup>H (blue) and <sup>19</sup>F (red) resonance frequencies extracted from NMR spectra measured as a function of the applied magnetic field magnitude  $B_0$ . Standard error bars (except the hydrogen measurement at 300 G) are smaller than the data markers. Gradients (indicated with dashed triangle) of linear fits (solid line) give the gyromagnetic ratio of the respective nuclear species which are consistent with literature values [88].

(0.12% and 500 ppm) is limited by the spectral resolution of the NMR spectra. This resolution is insufficient for identifying the chemical shift due to different chemical bonding environments of the fluorine or hydrogen nuclei, typically in the  $10 \sim 100$  ppm level. For example different fluorine atoms in Fomblin Y produce NMR shifts of  $-122 \sim -100$  ppm (see page 145, [127]). Using this results, we estimate that this nanodiamond NV NMR sensor has a spin concentration sensitivity of  $270 / \text{nm}^3 / \sqrt{\text{Hz}}$ , or  $450 \text{ M} / \sqrt{\text{Hz}}$  in Molar unit. This sensitivity corresponds to a detection time of less than 1 min for the PFPE ( $40 / \text{nm}^3$  spin concentration).

#### 2.4.3 Deducing nuclear spin concentration

To confirm that these devices achieve nanoscale sensing resolution, we used measured data to find the shortest path NV-to-surface distance d and thickness t of the hydrogen-dense surface layer, as illustrated in 2.5 a). This is achieved by integrating the contribution from each nuclear species over their volume,  $V_i$ :

$$\left\langle B_i^2 \right\rangle = \rho_i \left(\frac{3\mu_0\mu_i}{4\pi}\right)^2 \int_{V_i} \frac{\cos^2\theta \left(1-\cos^2\theta\right)}{r^6} dV \equiv \rho_i \left(\frac{3\mu_0\mu_i}{4\pi}\right)^2 \frac{S_i}{d^3},\tag{2.3}$$

where *i* denoting the identity of the nuclear species. *r* is the distance from the nuclear spin to the NV centre.  $\theta$  is the angle formed between the unit vector point from the NV to the nuclear spin, and the NV axis.  $\mu_0$  is the vacuum permeability and  $\mu_i$  is the magnetic moment of nuclear spin species *i*. The gyromagnetic ratio,  $\gamma$ , is related to the nuclear magnetic moment as

$$\gamma = \frac{g\mu_i}{h},$$

where  $\gamma$  is in units of Hz if the rest of the quantities are in SI units. This  $r^{-6}$  dependence leads to very sensitive dependence of the inferred concentration  $\rho_i$  on  $S_i/d^3$ . Here  $S_i$  is a dimensionless shape factor that quantifies the effect of the shape of the nanodiamond device on the measured signal strength.

#### 2.4.4 Calibration for concentration measurements

The surface hydrogen layer comes as a rescue. The surface hydrogen layer is equally affected by the geometrical variation between diamonds and by the depth variation between NV centres. Also since the spins closer to the NV constitutes a major contribution to  $S_i$ , the surface hydrogen layer can be used as a reference to reduce the uncertainty in the shape factor  $S_i$  and depth, d.

To verify this method, we generate variable nanodiamond shapes using a Monte Carlo simulation. Then we use the known value of fluorine density  $\rho_{19_F} = 40 \text{ spins nm}^{-3}$  and the commonly used value of hydrogen density  $\rho_{1_H} = 60 \text{ spins nm}^{-3}$  to infer the depth of the NV and the surface hydrogen layer thickness. The generation algorithm is designed to create nanodiamonds that correspond to transmission electron microscopy images of milled nanodiamonds similar in size to those used here.

The distribution of t and d values inferred for the generated nanodiamond shapes is a good estimate for the probability distribution of the true values of these parameters, including the systematic uncertainty caused by geometric variability. Figure 2.5 b) shows this distribution



Figure 2.5: Estimation of the geometry of the measured nanodiamond device using Monte Carlo simulation. **a)** Left: Cross section of an example nanodiamond device procedurally generated by the Monte Carlo simulation, illustrating geometric parameters d (distance between the NV centre and the nanodiamond surface) and t (thickness of the hydrogen surface layer). We generate the nanodiamond shapes using a realistic model for surface roughness and place an NV centre within the each nanodiamond. Right: Eight examples of generated nanodiamond devices out of the 440 instances used for simulation. **b)** Histogram of the values of the geometric parameters extracted from the experimental data and Monte Carlo simulation. Each individual nanodiamond device shape generated by the simulation is plotted as a white dot, with coordinates indicating the values of t and d that are consistent with both measured data and the shape of that generated nanodiamond device. The single-parameter histogram for surface-layer thickness t (NV to surface distance d) is plotted as white bars on the vertical (horizontal) axis.

for our measured device as a two-dimensional histogram of the extracted values t and d. Each white dot plots the values of t and d extracted for one of the generated nanodiamond shapes. We note that this distribution is robust to moderate changes in the algorithm we use to generate the nanodiamond shapes.

The distribution of geometric parameters in Fig. 2.5 b) shows that our device has a small NV centre depth,  $d \approx (13.2 \pm 1.6)$  nm, where the uncertainty is the standard deviation due to the geometric variability. A second device which we use to measure NMR signals has  $d \approx (11.4 \pm 1.3)$  nm confirms that these devices provide the high spatial resolution of NV centres close to the nanodiamond surface. We note that the uncertainty caused by the unknown nanodiamond shape is only four times larger than the uncertainty due to the signal-to-noise ratio in these measurements.

In actual sensing application, the concentration of the target analyte is unknown, and the geometrical shape and NV depth are also unknown. To investigate the conversion from the

measured magnetic field signal  $\langle B^2 \rangle$  and the actual spin density, we define the susceptibility,

$$\chi = \left\langle B^2 \right\rangle / 
ho$$
,

and investigate accurate measures of the  $\chi$ . It has been established in the previous discussion that without calibration the variability in  $\chi$  is large due to the  $r^{-6}$  distance dependence. Thus the NV sensor needs to be calibrated to reduce the geometrical variability. The most accurate method is to first calibrate the NV sensor in a known solution and then move the nanodiamond to the target solution by nanoscale manipulation. However tracking and manipulating nanoparticles are a challenging task. An alternative, yet less accurate method is to assume the variability between diamonds is small such that a subset of diamonds can be first calibrated to yield the ensemble mean,  $\langle \chi \rangle$  and use this on new nanodiamonds submerged in the target medium.

An alternative method we propose is to reference the target analyte spin density to the spin density of the surface hydrogen layer, which leads to less variation than does the geometrical variation. The existence of well-developed techniques for nanodiamond surface termination indicates that these requirements are feasible.

In the limit of a thin uniform surface layer, the surface-layer shape factor,  $S_s$ , and the analyte shape factor,  $S_a$ , can be approximated as

$$S_s \approx S'_s t/d$$
  
 $S_a \approx S_a(t=0)'$ 

where the first equation assumes that the shape factor can be locally approximated by the firstorder Taylor's expansion and  $S'_s$  is the derivative taken at the surface. The second equation assumes that the analyte shape factor can be approximated by not considering the thickness of the surface layer.

Take the ratio between the analyte and surface spin densities using Eq. 2.3,

$$ho_a = C \left(rac{4\pi}{3\mu_0}
ight)^{rac{1}{2}} rac{\langle B_a^2 
angle}{\mu_a^2} \left(rac{\mu_S^2}{\langle B_S^2 
angle}
ight),$$

where  $\mu_a$  and  $\mu_s$  are the magnetic moment for the analyte (<sup>19</sup>F) and the surface nuclei (<sup>1</sup>H).  $B_a$  and  $B_s$  are the magnetic field created by the analyte and surface nuclear spins.  $\langle \cdot \rangle$  denotes the ensemble average. The surface shape factor and depth dependence have been absorbed into the prefactor, *C*, such that

$$C = \frac{\left(\rho_S t S'_S\right)^{3/4}}{S_a},$$



Figure 2.6: Comparison of different calibration methods for the nanodiamond devices. **a**) Histogram of simulated analyte concentration measurements for different nanodiamond geometries, with surface-layer thickness with surface-layer thickness t held constant at t = 1 nm. Batch calibration shows large systematic errors, as measured by geometric standard deviation  $\sigma$ , due to uncontrolled geometry variation. Surface-enhanced calibration reduces systematic error by almost a factor of 10. **b**) We perform simulations similar to those shown in **a**), but allow surface-layer thickness t to vary between nanodiamonds in a log-normal distribution with geometric mean of  $\bar{t} = 1$  nm. When t has a large geometric standard deviation, this source of noise reduces the overall measurement accuracy.

which does not depend on *d* explicitly (although implicitly through the integration limits of  $S_S$  and  $S_a$ ). As nanodiamond shapes affect both  $S_S$  and  $S_a$ , they show a correlated variation for realisations of different nanodiamonds and thus their ratio partially removes the shape dependent variations. Finally any uncertainties in  $\rho_S$  would directly feed into the uncertainties in  $\rho_a$ . However as the surface spin density can be more accurately known (for example by well-controlled surface functionalisation, or with CVD nanodiamonds, which naturally have a hydrogen surface termination, or calibration with other nanodiamonds in the batch), this self-calibration method still reduces uncertainties due to geometrical variability.

To quantify the effectiveness of this method, the inferred fluorine spin density is simulated for 440 procedurally generated nanodiamonds. The generated diamond geometries are then used to compute the susceptibility  $\chi$ , which then converts the NMR signal to the analyte concentration.

Figure 2.6 a) shows the results of such a simulation in the ideal situation, where the surface-layer thickness is analyte concentration,  $\rho_a$ , follows a log-normal distribution the same t = 1 nm for every nanodiamond. The estimated with a geometric standard deviation of 224%, when using the batch calibration scheme, where one nanodiamond in the batch is calibrated and the same susceptibility is used across all nanodiamonds with shape variability in the batch. In contrast, our surface-enhanced calibration reduces the geometric standard deviation to only

24%. This accuracy using surface-enhanced calibration is comparable to that obtained with ratiometric dye indicator techniques for concentration sensing, without the need for extensive precalibration against environmental parameters, such as pH and temperature. To confirm that the surface-enhanced self-calibration scheme is robust to small variability in the surface-layer thickness, we repeat the simulation, but allow the thickness of the surface layer to vary between generated nanodiamonds in a log-normal distribution. Figure 2.6 b) shows that, even if the surface-layer thickness has a large geometric standard deviation of 50%, the surface-enhanced calibration still improves accuracy by fourfold over that of batch calibration. These results suggest that surface-enhanced self-calibration is advantageous and feasible for accurately measuring the local concentration of nuclear spins with nanoscale resolution.

## 2.5 Conclusion

Our results demonstrate that NV centres hosted inside small nanodiamonds can be used to detect NMR signal from multiple nuclear species in a sample volume of  $(19 \pm 5)$  nm<sup>3</sup>, containing about 1500 PFPE molecules. Such devices can be used to extend nanoscale NV NMR techniques to measurements inside cells. The surface enhanced calibration technique we propose here largely corrects the systematic errors caused by geometric variability of the nanodiamond devices and enables the decoding of the measured NMR signal.

Further improvements on readout techniques, such as nuclear-spin assisted readout, spinto-charge conversion, could further improve the readout fidelity and line width. Material engineering, on the other hand, could lead to better diamond coherence and thus narrower linewidth and higher sensitivity. The nanodiamond used in this work has a nominal nitrogen concentration of 50 ppm and the  $T_2$  time is still limited by the nitrogen electrons. It is therefore possible to improve coherence times by producing nanodiamonds from bulk materials with lower nitrogen concentrations, or with better nitrogen to NV conversion yield, via for example electron irradiation and/or post-annealing. Surface functionalisation methods would lead to a cleaner surface with less variability, thus allows accurate self-reference calibration of the nanodiamond sensors.

In future application of nanodiamond magnetometry in cellular systems, a few challenges need to be tackled. Firstly the intrinsic diffusion through the detection volume is potentially an issue. Nevertheless, detecting signals from a slowly diffusion object such as proteins anchored into the plasma membrane of the cells may not be an issue. Secondly, the measurement times are long compared with rotational diffusion and translational diffusion timescales of nanodiamonds in cells. This can be improved by improving readout efficiency using for example techniques such as repetitive readout, or continuous orientation tracking of the diamond with a moving magnet or a 3-axis electromagnet. Finally the sensitivity remains an issue in detecting millimolar concentrations. This can be improved by using better diamonds with better coherence.

# Chapter 3

# Detecting triplet excitons in a molecular semiconductor using an NV sensor

In the previous chapter, the quantum sensing of a stationary magnetic signal originated from the nuclear spin precession has been discussed. This signal of interest is of a known fixed frequency (Larmor's frequency) and is always present, thus making a frequency selective detection relatively straightforward to achieve. In this chapter, we interface the NV sensor with a bath of external paramagnetic species that create a broadband and transiently generated magnetic noise that can be detected by NV relaxometry. The synchronised material excitation and NV sensing is demonstrated. The paramagnetic species are optically generated triplet excitons in an organic photovoltaic material.

This work was done with guidance from Dr Paolo Andrich. Alan Bowman prepared and characterised the DPH sample. The diamond samples are prepared by Q.G. and P.A., and coated with DPH by A.B.. The NV relaxometry experiments were performed on the same setup as presented in Chapter 2 with major modifications involving oil objective NA matching, UV laser and UV LED excitation, relaxometry pulse sequences (all-optical and chirped) done by Q.G.. The theory for exciton transport and relaxometry are done by Q.G.. All experiments and data analysis were done by Q.G..

## 3.1 Introduction

Organic semiconductors (OSC) are promising candidates for photovoltaic cells as they are spin-processable and possess flexible chemical structures for tunable optical properties. Polymeric organic semiconductors have also been studied for molecular semiconductor spintronics devices [202]. Even naturally occurring small organic molecules play a very important role in

energy transduction and transfer, for example in photosynthesis.

Many molecular materials such as polyenes, acenes (pentacene for example), and carotenes, can undergo intersystem crossing (ISC) or singlet exciton fission (singlet fission, or SF) to form one or two triplet excitons [29]. Unlike in inorganic semiconductors, interaction between molecules in organic semiconductors, especially small organic molecules, is weak and thus electron delocalisation happens on the length scale of one or a few molecules. As such, optical excitation produces localised Frenkel excitons. Such excitons are in contrast to the Wannier-Mott excitons typically discussed in inorganic semiconductors where delocalisation can be over a few hundred nanometers.

In either of these two processes, triplet excitons are generated within a few nanoseconds of optical excitation. As triplet excitons are spin-forbidden to return to the ground state, they are good carriers for long range diffusion and subsequent energy transfer. Understanding their formation and transport on the nanoscale is informative for using them as solar energy down-conversion material to overcome the Shockley-Queisser limit [210].

#### **3.1.1** Characterisation methods for triplet excitons

As optical transitions and singlet-fission are ultrafast processes, many studies so far have focused on using optical spectroscopy methods, such as transient absorption (TA) and photoluminescence spectroscopies, to probe the dynamics of exciton generation process [245]. Despite the advance of TA microscopy, the commonly used TA spectroscopy is still a bulk technique that does not typically give spatially relevant information.

Fluorescence microscopy is also a commonly-used method and it probes the delayed luminescence from triplet excitons [5, 115]. The delayed fluorescence gives direct indication of local exciton density. This method is limited by the resolution of an optical microscope.

Drop casting and spin coating can create < 100 nm of OSC films. The triplet excitons can diffuse to the interface of one layer and transfer its energy to an acceptor material at the interface [70, 225]. This results in a change in phosphorescence or a photovoltage [169]. Such measurements have enabled precise determination of triplet diffusion length scales [159]. However the phosphorescence can be affected by factors including quenching, out coupling efficiency, and the specific analysis method used for extracting the diffusion length from the phosphorescence [165].

These optical techniques does not enable to direct detection of the spin of the triplets. The magnetic properties of triplet excitons, however, can be directly measured via transient electron spin resonance (ESR) technique, also commonly used in studying organic materials. ESR provides information about the lifetime, magnetic moments, orientation, spin structure of triplet excitons [157]. The sensitivity limit of ESR spectrometers requires a sample volume

of a few millimeters, and gives information on this length scale.

From these studies, a few aspects of the properties of triplet excitons emerge. Triplet excitons have a typical lifetime in the range 1  $\mu$ s to 100  $\mu$ s [5, 157, 245]. The diffusion is usually anisotropic, dependent on the crystal structure. The trajectories can fit well with a normal diffusion (Brownian motion), at a sufficiently long time after singlet fission. The diffusion length varies greatly and is within the range 0.1  $\mu$ m to 10  $\mu$ m with ~1  $\mu$ m commonly observed [165].

With the more recent microscopy methods, optical techniques give a higher, diffractionlimited spatial resolution but relies on the weak phosphorescence or delayed fluorescence due to triplet-triplet annihilation. The magnetic approach of using ESR is limited by spatial resolution. As such there is still gap in exploiting the magnetic property of the triplet excitons at the nanoscale to study their diffusion property.

#### **3.1.2** Detecting triplet exciton with NV sensors

The strong distance dependence of magnetic dipolar interaction  $(d^{-3}$  for magnetic field and  $d^{-6}$  for variance of magnetic field) provides a means for very localised detection with nanoscale spin sensors such as the NV. Experimental detection of triplet excitons in molecular semiconductors has been previously carried out using bulk diamond and pentacene-doped crystals to study the bulk triplet polarisation properties [264]. In this experiment single-crystal *p*-terphenyl is doped with a small concentration of pentacene- $d_{14}$  (0.05 mol%). At such small concentrations, pentacene molecules are well-isolated and triplets are generated only via the ISC pathway [157]. As these triplet excitons do not diffuse and are uniformly optically polarised, the magnetic dipole induced-field at the NV depends the distance as  $d^{-3}$  while the volume of contributing spins grows as  $d^3$ , cancelling each other. Distant excitons can also contribute the signal, effectively behaving as a macroscopic magnet with a size determined by the excitation volume.

However this study uses a diffraction-limited optical excitation volume, still large compared with the diffusion length of triplet excitons. To extend this experiment, we instead use a pure crystal of the singlet-fission material to generate the triplet excitons. As discussed in Section 1.3.6, the NV's spin depolarisation time,  $T_1$ , is dependent on the local magnetic noise and by measuring the spin relaxation it is possible to infer the property of the surrounding noise bath. We use this relaxometry-based technique to detect the magnetic noise created by these triplet excitons. From this perspective, optically excitable triplet formation is also a useful tool to provide a source of on-demand transient triplets for NV sensing experiments.

#### **3.1.3** Singlet exciton fission

The material system hosting triplet excitons used in this work is a singlet-fission material. Singlet-fission is an ultrafast process whereby an optically generated exciton with spin 0 and a neighbouring unexcited molecule interact to give two spin-1 excitons. The SF process goes through a correlated pair state, which eventually dissociates to form two triplet excitons, localised to two separate molecules. The correlated pair state is also called the (TT) state. Symbolically the SF process can be represented as

$$S_0 + S_1 \rightarrow (TT) \rightarrow T_1 + T_1$$

Although critical to the formation of triplets, the exact nature of the (TT) state depends on the material in discussion and in some cases is still not very well understood. The ultrafast dynamics of the SF process is typically characterised with TA spectroscopy, where the absorption spectrum of excited molecules are probed in a pump-probe configuration, on a femtosecond to nanosecond timescale [272]. Weiss *et al* observed the strong exchange correlation in TIPStetracene using high-field ODMR experiment [253]. The transition into the (TT) state takes place on the timescale of sub-nanoseconds and is fast compared to the temporal resolution of the NV centres. Thus the long-living triplet excitons contribute the major signal observed by the NV.

In this chapter we start with the experimental realisation of NV relaxometric sensing of triplet excitons. We discuss the results and model the observed result with stochastic Liouville equation.

## 3.2 Experimental

#### 3.2.1 Materials

For the generation of triplets, we use a pure crystal of diphenyl-1,3,5-hexatriene (DPH) which readily generates triplet excitons via singlet exciton fission [63, 243]. Triplet diffusion between molecules can take place. DPH consists of two phenyl groups and a six-carbon polyene backbone (Fig. 3.1 c)). In comparison to acene compounds, its high bandgap allows exploration of the UV part of the solar spectrum. Figure 3.1 b) shows the optical transitions for DPH. The main optical absorption is in the UV range and gives an emission around 391 nm [8], although this value could differ significantly depending on the local crystal structure and morphology [63]. The detailed optical absorption into higher lying states and the involvement of a vibronically coupled state to  $S_1$  have been characterised in previous literature



Figure 3.1: Illustration of the sensing of paramagnetic triplet excitons in DPH using a NV spin sensor. **a**) Experiment configuration. Diamond materials (either bulk or nanocrystal, pale blue slab) are coated with DPH thin film (only carbon atoms are shown), where the photogenerated triplet excitons (blue arrows) causes a faster NV (red arrow) relaxation. **b**) is the Jablonski diagram showing optical transitions of DPH. The optical transitions upon excitation from the ground state  $S_0$  (purple upward arrow), the  $S_1$  population decays to the ground state (purple downward arrow) or undergoes singlet-fission to form triplet pairs (dashed arrow). Each triplet in the pair has a zero-field splitting (ZFS) with D = -2.4 GHz and E = 270 MHz [232]. The triplet to ground state transition is spin-forbidden (cross on the red dashed arrow) and thus only occurs slowly, giving phosphorescence. **c**) Molecular structure of DPH. **d**) the carbon atoms and carbon-carbon bonds of DPH in a unit cell of a orthorhombic DPH crystal. The orientation of the molecules are such that the intermolecular distance between molecules are greater along the c axis than along the other two axes.

[77, 94, 190, 239, 256, 271]. The optical lifetime of  $S_1$  is approximately 5 ns in a crystal [63] as measured by a transient PL experiment.

The correlated triplet pair could either recombine (geminate recombination) to form  $S_1$ , giving photoluminescence, or dissociate to give a pair of triplets. The  $S_1$  state and the correlated triplet pair state are in a constant dynamic equilibrium. The generation of triplet excitons through singlet fission and triplet dissociation happen with a rate of ~ GHz [63, 243] and are fast compared to the NV sensing timescale.

Each triplet exciton generated is a S = 1 system akin to the NV ground state (see Chapter 1, Eq. 1.2) with ZFS parameters D = -2.4 GHz and E = 270 MHz [232, 243]. In general, near-field energy transfer can take place between molecules via the Dexter energy transfer mechanism, fluorescence resonance energy transfer, or the charge transfer mechanism. Given the intermolecular distance of DPH and the spin-forbidden decay into the ground state, Dexter energy transfer is the dominating mechanism [5, 165]. This energy transfer introduces random walk diffusion of triplet excitons. Separated triplets can also recombine via non-geminate recombination [27]. Various terms have used in the literature to refer to the recombination processes triplet pairs can undergo, including geminate and non-geminate triplet recombination

Diamond Name	Type of diamond	Type of NV	Solvent
Adamas	Nano, HPHT,	Single NV	Ethanol
FND	Nano, HPHT,	Ensemble	DI Water
	electron irradiated		
Bulk	Bulk, <sup>15</sup> N	Shallow	-
	enriched, CVD	implanted, Single	
	grown. 0.1 mm	NV, 10 nm	
	plate with a	nominal depth.	
	thickness of		
	100 µm.		

Table 3.1: List of diamond materials used in this chapter. Commercial nanodiamonds (Adamas, FND) are named after their suppliers. Adamas and FND nanodiamonds are suspended in deionised (DI) water when arrived. The single NV nanodiamonds (Adamas) are resuspended in ethanol and deposited with a nebulizer to improve particle dispersion and to prevent large droplets forming, which increases the probability of getting clumps of nanodiamonds. Bulk CVD diamonds implanted with shallow NV centres. The nominal depth is 10 nm.

[27], triplet fusion [204, 243] or triplet-triplet annihilation [242]. They are used interchangeably in this work.

The random diffusion allows the triplet exciton to sample different local ZFS if the molecules in the crystal lattice are oriented differently. There are three different relative orientations between DPH molecules [63, 243] (Fig. 3.1 d), where each pair of neighbouring molecules are of a different relative orientation). Such triplet diffusion process in small-molecule OSC via random-walk hopping is dominated by Dexter energy transfer [5]. Hopping between molecules of different orientations cause the triplet excitons to quickly lose spin coherence. The magnetic field created by the paramagnetic triplet excitons is therefore spectrally broadened, detectable with a relaxometry technique, in contrast to the double resonance technique used by Xie *et al* [264]. The decoherence induced by diffusion leads to a magnetic field fluctuation at the NV centre and can thus be sensed.

#### 3.2.1.1 Preparation

DPH crystal is prepared by drop casting onto a glass substrate. In this process, DPH powder dissolved in hexane is deposited by drop casting onto a glass substrate in an argon protected glovebox. The solvent is left to dry in the glovebox and the crystal is annealed on a 80 °C hotplate for 15 min. The sample is then covered with another glass coverslip and sealed with epoxy to prevent air oxidation. We verify its morphology in a wide-field microscope (Fig. 3.2 **a**)). The crystal shows micron-scale ridges but well covers the substrate. The optical



Figure 3.2: Optical characterisation of DPH. **a**) is a wide-field image of DPH crystal on glass. The image is  $200 \,\mu\text{m}$  wide. The surface texture and wrinkles are indication of the coverage of the material and the length scale of these ridges are on the order of a few micrometers. **b**) UV-Visible absorption spectrum of DPH on glass substrate for a few samples. The 400 nm peak corresponds to absorption peak of DPH. A general concern for organic semiconductors is that properties differ significantly between samples. The absorption spectrum does not vary significantly from sample to sample.

absorption is shown in Fig. 3.2 a). Significant absorption occurs at the wavelength range between 280 nm to 400 nm, (Fig. 3.2 b)) consistent with characterisation in other experiments [63, 215]. No significant variation is observed between different samples.

To probe nanometer-scale paramagnetic exciton species, the experiments presented in this chapter involve the use of three types of diamond samples listed in Table 3.1. The nanodiamond samples are first deposited using a nebulizer on a fused silica coverslip and left to dry in air. The bulk diamond sample is prepared by first spin coating a fused silica coverslip with SU-8 2000 photoresist and slowly warmed up from 90 °C to 150 °C at a rate of 1 °C min<sup>-1</sup> while having the diamond at the intended location. The face of the diamond with shallow-implanted NV centers are on the opposite side of the photoresist. The photoresist is cross-linked at the final temperature, bonds the diamond plate (see Table 3.1, bulk diamond, for details) and does not produce spurious fluorescence. The substrate so prepared undergoes the same DPH drop cast process to complete the sample preparation.

#### **3.2.2** Confocal microscopy

The setup used for these experiments (Fig. 3.3) is similar to the one used in Chapter 2 but is additionally equipped with a LED at 375 nm which serves as the excitation source for DPH. The LED illumination is not intentionally focused down the objective and provide a wide-field illumination on the sample plane. In some experiments, we alternatively used a 405 nm



Figure 3.3: Experimental setup for relaxometry experiment. Optical excitation with a 532 nm laser and a 405 nm. A 375 nm LED provide wide-field illumination to the material. Fluorescence is collected beyond 650 nm. Both the 532 nm and 405 nm excitation beams are pulsed with an acousto-optic modulator (AOM). 10% of each laser beam is directed to a photodiode for monitoring the optical excitation power. The pulses and the MW for chirped and square-wave  $\pi$ -pulses are generated with an arbitrary waveform generator (AWG). An 1.45 NA oil objective is used. MW delivery is achieved with an on-chip co planar waveguide.

diode laser, pulsed with an acousto-optic modulator (AOM) to excite DPH. A continuouswave (CW) 532 nm laser is used for exciting NV centres. By scanning with different excitation wavelength and collecting the fluorescence, areas covered by DPH material can be identified (Fig. 3.4). Figure 3.4 shows a comparison of the same field-of-view (FOV) in a confocal microscope when excited with a 405 nm **a**)) and a 532 nm **b**)) laser, while both use a collection long-pass filter of 650 nm. In the confocal image excited with 405 nm laser we could identify the boundary of the DPH material by the bright edges, due to possibly domain boundaries or material inhomogeneity. We used the 532 nm-excited confocal image to find nanodiamonds covered by DPH.

#### 3.2.3 Relaxometry

The relaxometry experiment measures the magnetic noise created by the spins of the decaying triplet excitons. In this Chapter, two relaxometry sequences are used. Due to the limitation



Figure 3.4: PL map of a sample with nanodiamonds containing NV centres, covered in DPH crystals. Fluorescence map of the same area obtained with **a**) 570 nW of 405 nm excitation and **b**) 5  $\mu$ W of 532 nm excitation. The scale bar is 5  $\mu$ m and is the same for both images. Image in **a**) clearly shows the boundary and coverage of the DPH material. Image **b**) indicates that the nanodiamonds are significantly brighter than the DPH material and can be clearly identified in confocal images.

	Relaxometry	$\pi$ -pulse strategy	DPH excitation
Adamas single	All-optical	-	375 nm LED
NV			
Bulk diamond	MW-based	Single frequency	375 nm LED
single NV			
FND ensemble	MW-based	Chirped with AE	405 nm laser
NV		model	(pulsed)

Table 3.2: Experimental protocols used for different diamond samples.

on material lifetime, different relaxometry and DPH excitation methods are used. These are tabulated in Table 3.2.

#### 3.2.3.1 Relaxometry sequences

The typical MW-based relaxometry technique is performed with the pulse sequence shown in Fig. 3.5 a) and also discussed in Chapter 1.3.6. In this protocol two sequences are run sequentially, one without and one with a microwave  $\pi$ -pulse just before readout to isolate spin-dependent signal from other PL fluctuations such as charge state conversion. These two measurements are named plain and reference respectively. The measurements are similar to the ones introduced in Chapter 2 Section 2.2.3 where a 'plain' and a 'reference' measurement are also taken for NMR protocols. The initialisation and readout pulses are 1 µs and 300 ns respectively. The plain sequence (each sequence is about 500 µs depending on the range of delays scanned) is first repeated for 1 s (about 2,000 sequences repeated) and the total photon counts during this time is collected. The reference sequence is repeated in the same manner for 1 s. The same pair of sequences are repeated for a range of delays and repeated for many sweeps (typically ~30 delays. 600 sweeps for single NV, and 120 sweeps for ensemble NV).

As the  $\pi$ -pulse requires additional characterisation and takes up material lifetime, measurements with single NV centres in Adamas nanodiamonds are run with the all-optical relaxometry technique (for example 3.7 a)) Such a technique uses two readouts; one is the conventional readout and the other readout measures photons after the NV has completely initialised. These two measurements are very closely spaced in time and cancel out any laser, or charge-state induced slow fluctuations. However, in the case where the initialisation pulse shifts the equilibrium of the charge state to mostly NV<sup>-</sup>, which gradually shifts toward NV<sup>0</sup> during the dark time, the all-optical technique will be measuring a spurious  $T_1$  time [188]. Thus MW manipulation is still preferred if possible. The corresponding sequence is presented, together with the data for clarity in Fig. 3.7 a).

For both MW-based and all-optical relaxometry, the difference between the plain and reference measurements at a delay  $\tau$ ,  $PL(\tau)$ , is fitted with

$$PL(\tau) = PL_0\left(1 - Ce^{-\tau/T_1}\right)$$

where  $PL_0$ , C,  $T_1$  are fitting parameters and the PL contrast is defined as  $PL(\tau)/PL_{ref}(\tau)$  and the normalised contrast as  $PL(\tau)/PL_0$ . The nonlinear least-square fitting, performed with the python scipy curve fitting algorithm, assumes equal errors on all data points, which, considering the small contrast and shot-noise limited PL measurements, applies to relaxometry data.

#### 3.2.3.2 Chirped pulses

In a single NV MW-based relaxometry experiment, a single-frequency  $\pi$ -pulse is typically used. However  $\pi$ -pulse fidelity deteriorates in ensemble NV samples where inhomogenous splitting is strong compared to spectral width of a  $\pi$ -pulse. Various techniques have been adopted to improve fidelity, such as quantum optimal control, Hamiltonian engineering, adiabatic pulses. Among these techniques, adiabatic transfer achieves good fidelity with relatively simple control sequences that do not require adaptation to each nanodiamond and is therefore chosen to improve relaxometry contrast.

Linear chirped pulses have been used previously [188] to enhance relaxometry contrast in ensemble NV centres in a chemical context. This replaces the single frequency  $\pi$ -pulse to improve the fidelity in an ensemble NV sample. The simple approach is to use a square amplitude and linear frequency sweep (3.5 b)) which is relatively easy to achieve experimentally. The more sophisticated and experimentally demanding Allen-Eberly model (3.5 c)) is



Figure 3.5: Relaxometry pulse sequence and chirped pulses. **a**) shows the four sequences used for chirped measurements. The sequence is run without (plain) or with (reference) MW  $\pi$ -pulse to create the spin-only optical contrast. For the ensemble NV measurements, a 405 nm is pulsed and switched on and off to isolate behaviour related to laser excitation, for both the plain and reference measurements (the -Sig and -noSig sequences). Microwave amplitude and frequency relationship in chirped pulses. **b**) a linear chirped pulse. **c**) Allen-Eberly chirped pulses.

also used in various context for its analytical simplicity and fidelity [92].

In this work, we used the chirped pulse with an amplitude, A, and frequency, f, following the AE model, which is described by

$$f = f_0 + \frac{R}{2} \tanh\left(\frac{t}{T}\right),$$
  
$$A = A_0 \operatorname{sech}\left(\frac{t}{T}\right),$$

where t is the time referenced to the centre of the pulse, R is the characteristic range of frequency sweep, T is the characteristic time,  $f_0$  is the centre of the frequency sweep and  $A_0$ is the maximum amplitude.  $A_0$  is set at the maximum achievable MW power to not induce heating.  $f_0$  is set at 2.87 GHz. R/T = 30 MHz  $\mu$ s<sup>-1</sup> and  $T = 2\mu$ s.

#### **3.2.3.3** Relaxometry measurement on ensemble NV centers with chirped pulse

The typical  $T_1$  measurement result with a single NV has been shown in Section 1.3.6. These are taken with a square  $\pi$ -pulse. A typical  $T_1$  trace, using ensemble NV centres, taken with a square  $\pi$ -pulse is shown in Fig. 3.6 a) and that with a chirped pulse is shown in b).

Despite giving the same  $T_1$  (34(1) µs and 35(1) µs), the pulse sequence with an adiabatic chirped pulse almost doubles the contrast (Fig. 3.6 c)) as more spins are addressed with the chirped pulse compared to the square pulse. A similar error in  $T_1$  is achieved with 200 s accumulation per data point for the chirped pulse, compared to 593 s for the square pulse measurements.



Figure 3.6: Comparison between  $T_1$  measurements with **a**) a square pulse and **b**) a chirped pulse following the AE model. The contrast is plotted in **c**) where the contrast for measurement with a chirped pulse is almost twice higher than the contrast for a square pulse **c**). Both sequences result in the same  $T_1$  values.

#### 3.2.3.4 Excitation of the DPH material

For the single NV experiments a 375 nm continuous LED excitation is used to excite the DPH material to generate paramagnetic triplet excitons. The CW LED excitation limits the accessible excitation power and also cannot be pulsed to take fast differential measurements to remove effects due to material degradation. Thus a 405 nm laser is used to replace the LED source and is switched on for 5  $\mu$ s. As a result a total of four measurements are taken. They are the plain and reference measurements with DPH excitation and without DPH excitation (named as plain-Sig, plain-noSig, ref-Sig, ref-noSig, respectively). The four pulse sequences are shown in Fig. 3.5 **a**) and the reference sequences are shown in Fig. 3.7 **g**), the plain measurements use the same sequences but without the  $\pi$ -pulse. The  $T_1$  with and without excitation are extracted from these four sets of measurements. Thus any optically induced charge-state switching [188], but unrelated to spin, would not be captured. The four sequences are repeated the same manner (in the order plain-noSig, ref-noSig, ref-Sig) as for the usual relaxometry measurements (1 s for each sequence).

## **3.3 Results and discussion**

#### **3.3.1** Exciting DPH induces faster NV ground state depolarisation

The relaxometry experiment first started with Adamas nanodiamond, with DPH excited by the 375 nm LED. Note that tests we performed show that 450 nm LED does not produce the extrinsic relaxation observed, consistent with the low absorption at this wavelength (Fig. 3.2 b)).

In these experiments the LED is constantly on during the relaxometry measurement with pulse sequence shown in Fig. 3.7 a)). Note that this is an all-optical measurement sequence, where no MW is applied to create the contrast. Figure 3.7 b) shows the change in all-optical NV  $T_1$  time when LED is turned off or on. The data is plotted by first fitting the data to an exponential and normalising the PL data. The gradient on the log-linear plot in Fig. 3.7 is  $-1/T_1$  and steeper lines have a smaller  $T_1$ . The NV relaxation time shows a change from  $T_1 = (169 \pm 26) \,\mu$ s (no excitation) to  $T_1 = (104 \pm 10) \,\mu$ s (with excitation). This observation suggests that external excitation of the DPH material causes the NV to depolarise faster, as manifested by the significant change in  $T_1$ .

Each measurement takes about 10 h to complete. Intense optical illumination used to probe the NV centres leads to change in material property of DPH, manifested as a rise in DPH PL. Within the lifetime of the DPH material, only two  $T_1$  measurements can be performed on single NV centres in Adamas nanodiamonds. Thus to verify this change in  $T_1$  is not due degradation of the material over time, optical excitation on DPH is first switched off and then switched on in the measurements shown in Fig. 3.7 c), where a change of  $T_1 = (175 \pm 40) \mu s$ (no excitation) to  $T_1 = (85 \pm 14) \mu s$  (with excitation). This confirms that the change is not due spurious changes related to the material degradation.

Using bulk diamond, which has a longer intrinsic  $T_1$  time and thus better sensitivity to extrinsic magnetic field fluctuations, we verify the optical power dependence. A total of 4 sets of experiments are obtained before the material degradation leads to extraneous PL. This measurement is performed using the 375 nm LED as in the previous section and contrast is created with square MW  $\pi$ -pulses. The sequence is shown in Fig. 3.7 d). The  $T_1$  measurements with no external excitation (blue in Fig. 3.7 e)) and with maximum external excitation are shown (red in Fig. 3.7 e)). As the LED power is increased, the  $T_1$  time of the NV centre decreases. The rate of depolarisation,  $1/T_1$  is shown in Fig. 3.7 f). The depolarisation rate increases with external optical pumping (Fig. 3.7 f)). To avoid any degradation induced erroneous signal, the measurement with no external excitation was performed once at the beginning and once at the end of the series of experiments, giving the same  $T_1$  result (the two overlapping data point at 0 excitation power in Fig. 3.7 f)).



Figure 3.7:  $T_1$  measurements performed on a series of diamond samples. **a**) shows the 'alloptical' relaxometry sequence, which is used to probe the  $T_1$  time of Adamas nanodiamond. The sequence consists of a plain (Plain) and a reference (Ref) readout (red boxes) which serves to cancel off any laser intensity fluctuations. The DPH material is excited by a 375 nm LED that is either constantly on or constantly off during the experiment. b) shows the comparison between  $T_1$  curve with DPH excitation (red) and without (blue) with the 'LED off' measurement performed first.  $T_1$  of the NV is significantly changed when the material is excited. c) shows the same measurement, on a different nanodiamond, but with the order of DPH excitation reversed. The 'LED on' measurement is performed first. The two  $T_1$  measurements yield a  $T_1$  time of  $T_1 = (175 \pm 40) \ \mu$ s (no excitation) which is changed to  $T_1 = (85 \pm 14) \ \mu$ s when LED is turned on. d) is the sequence used to probe  $T_1$  of NVs in a bulk diamond sample (blue slab) covered by DPH (pink slab). The sequence uses the same LED excitation, but a  $\pi$ -pulse is used to create spin-only contrast. The corresponding  $T_1$  measurements are shown in e), displaying significant  $T_1$  in response to DPH excitation. Additionally **f**) shows that this change is power-dependent. g) shows the pulsed 405 nm excitation used to transiently excite DPH, which uses a chirped pulse for the  $\pi$ -pulse. **h**) shows the  $T_1$  decrease with DPH excitation and the external depolarisation rate. The external depolarisation rate is defined as the inverse of change in  $T_1$  due to DPH excitation. This rate saturates with optical excitation power on DPH.

#### **3.3.2** Pulsed DPH excitation shows similar depolarisation

Despite these modifications, there is still only limited time window within which NV measurements can be performed due to DPH degradation. Thus to circumvent this issue, nanodiamonds containing ensemble NV centres are used to improve sensitivity while also reducing the amount of optical excitation power required. A chirped pulse is used in this experiment to improve contrast. The typical change in  $T_1$  is smaller (Fig. 3.7 h)) potentially due to the NV centres at the centre of the nanodiamonds are farther from the surface and thus show less change in  $T_1$ .

Figure. 3.7 i) shows the change in externally induced depolarisation rates as the 405 nm laser power is varied. In these experiments, the  $T_1$  time are differentially measured and thus allowing the variation of the intrinsic depolarisation rate to be excluded for each data point. The external depolarisation rate shows a saturation behaviour on the external excitation power, with a saturation power of 2.5 nW

#### **3.3.3** Understanding the power dependence of magnetic noise

The overall  $T_1$  time depends on the external magnetic noise as (see Appendix B section B.1 for derivation and discussion)

$$\frac{1}{T_1} - \frac{1}{T_{1,intrinsic}} \propto \gamma_e^2 \left\langle B_\perp^2 \right\rangle(\omega_0) \propto \rho, \qquad (3.1)$$

where the  $T_{1,intrinsic}$  is the  $T_1$  time measured without external excitation on DPH.  $\rho$  is the exciton density,  $\omega_0$  is the resonance frequency of the NV,  $\gamma_e$  is the electron gyromagnetic ratio, and  $\langle B_{\perp}^2 \rangle$  is the power spectrum of the transverse magnetic field fluctuation at the NV. The optical power dependence shown in Fig. 3.7 f) and Fig. 3.7 i) show that the externally induced depolarisation is dependent on the optical excitation power. The linear trend is consistent with a lower triplet density regime as discussed in Appendix B.3.4. The saturation behaviour could be consistent with the enhanced triplet-triplet fusion process at higher triplet densities. Although as the PL signal from DPH is not simultaneously measured, it is not straightforward to compare this with the optical signature.

#### **3.3.4** Spin bath modelling and relation to relaxometry results

To understand the origin of the reduced  $T_1$  time, the detailed spin dynamics during diffusion of the triplet is discussed and modelled.

Equation 3.1 shows that the power spectral density  $\langle B_{\perp}^2 \rangle(\omega_0)$  is the key quantity probed

by NV relaxometry. Thus magnetic noise with the wrong frequency coverage (for example a slowly fluctuating magnetic noise), longitudinal noise and narrow-band noise, would not lead to depolarisation. In fact as is seen in Ref. [264], the magnetic noise is present on the slow timescale measured by  $T_2$  decoherence spectroscopy. Thus by observing a change in  $T_1$  due to excitation of DPH, it could be inferred that the bath of triplet excitons creates magnetic noise at the NV resonance frequency. As the triplet exciton's zero-field transitions are not in resonance with the NV (2.67 GHz and 2.13 GHz), the noise at the NV resonance would be produced by broadening of these intrinsic resonances on the order of ~200 MHz.

#### 3.3.4.1 Sources of broadening

There are three established mechanisms for decoherence in OSC [201]. The first is the intrinsic decoherence as a result of local field fluctuations due to phonon coupling mediated by local spin-orbit coupling (SOC), local hyperfine interaction (HFI) or simply other magnetic noise. This is similar to the intrinsic decoherence of the NV centres and is typically in the range of µs as measured by pulsed ESR experiments [157]. The second mechanism, motional relaxation, describes the effects of exciton hopping motions as it moves through an environment with varying fine interaction, hyperfine interaction and local g-tensors, which can originate from for example a variation of molecular orientations throughout the course of the exciton's lifetime . This is akin to a stochastic fluctuation of magnetic field in the exciton's co-moving frame. The third is the Elliott-Yafet relaxation due spin-orbit coupling, which is not a major effect in the small-molecule OSC case. Other mechanisms [220], such as diffusion and intrabath dipolar coupling [248] would not create sufficient broadening.

Suppose in the triplet exciton's co-moving frame, the Hamiltonian is changed on a timescale of  $\tau_c$  and by a characteristic amount  $\Delta \omega$ , then the broadening can be classified into four regimes. Nuclear spins in NV NMR experiment presented in Chapter 2,  $\tau_c \Delta \omega \gg 1$  and approach the *rigid limit*. In the Gd<sup>3+</sup> measurements in a typical relaxometry experiment [220],  $\tau_c \Delta \omega \ll 1$  and approaches the *rapid fluctuation limit*. In organic semiconductors where hopping is the major contributor of decoherence,  $\tau_c \Delta \omega \sim 0.1$  is in the *slow-motion regime*, which requires numerical simulation using stochastic Liouville equation (SLE) to treat rigorously. As can be seen in Appendix B, both the rigid limit and the rapid fluctuation limit emerge from the SLE analysis as  $\tau_c$  is varied.

#### 3.3.4.2 Power spectral density modelling

Wakasa *et al.* [243] provides an estimate for the typical hopping rate ( $k_h = 1/\tau_c$ ) for DPH, using their magnetic PL measurements on powdered sample. In these experiments, the PL



Figure 3.8: Illustration of the triplet hopping process. **a**) Different oriented DPH molecule have different eigenstates and hopping between them lead to decoherence. **b**) is the simulated power spectrum of magnetisation in one direction in the NV's frame of reference. At low hopping rate, two resonances appear at the transition frequencies of the triplet. When the hopping rate is very high, the triplet samples the orientation of each molecule during diffusion and form a single peak at 2.13 GHz. At moderate hopping rate a broad peak is observed around ~2 GHz.

from DPH is measured while changing the external magnetic fields. We followed a similar procedure with our modelling and magnetic PL data to arrive at an estimate of  $k_h = 1.2 \text{ GHz}$ , which is comparable to a *D* of 2.4 GHz, consistent with the assumption that  $k_h \sim D$ .

Using the estimated hopping rate, the power fluctuation spectrum can be computed by modelling a single triplet exciton diffusion across the DPH material (see discussion in Appendix B Section B.3). Figure 3.8 a) shows the hopping between two molecules oriented in the herringbone configuration. The autocorrelation  $\langle S_y(t) S_y(0) \rangle$  can be Fourier transformed to result in the power spectrum of the total fluctuating magnetic moment show in Fig. 3.8 b). In the rigid limit (small  $k_h$ ), the triplet undergo similar precession as the nuclear spin species discussion in Chapter 2. The corresponding spectrum shows three peaks at the triplet spin transitions (540 MHz, 2.67 GHz and 2.13 GHz). At very high hopping rate however, the rapid hopping causes the transitions to the  $|T_y\rangle$  to be averaged out (Fig. 3.1 a)), leaving a single peak at 2.13 GHz. For intermediate hopping rate, the spectrum shows a broad peak around ~2 GHz.

It should however be noted that the measurements used to compare with the SLE modelling is on macroscopic DPH material and may only yield an estimate of  $k_h$  rather than a precise determination.



Figure 3.9: Illustration of the stochastic Liouville equation (SLE) used to analyse the triplet diffusion process. The SF process starts with an occupied  $S_1$  state which forms a correlated triplet pair. The correlated pair can recombine via triplet fusion ( $k_{TF}$ ) to generate fluorescence or dissociate to form separated triplets which hop from one molecule to another. Once triplet excitons are well-separated spatially, they are unlikely to recombine via the correlated pair state.

#### 3.3.4.3 Deducing the hopping rate from transient PL and magnetic PL experiments

In the previous section, we used the hopping rate  $k_h$ . To estimate this rate for our material, we use the available transient PL and magnetic PL experiments, following treatment in [243].

Wakasa et al, [243] used a more involved version of SLE to model the singlet-fission process, which is necessary for explaining the magnetic PL. The SLE consists of the spin Hamiltonian for triplet pairs (Fig. 3.9), the singlet fission and triplet fusion from a correlated triplet pair in their singlet state, the dissociation of the correlated triplet pair to a separated triplet pair, and one-dimensional hopping of a triplet exciton. The diffusing triplet excitons eventually become irreversibly free and can no longer diffuse back to undergo triplet fusion.

The result of the model is compared with transient PL experiment. Figure. 3.10 a) shows such an experiment taken from [63] where the PL is recorded as a function of time after a pulsed excitation. This measurement features a second, long timescale (at approximately 5 ns) due to triplet fusion. The transient PL gives the radiative transition rate and triplet and singlet fission rates [63].

The result of the model is also compared with magnetic PL experiment performed on our sample and is shown in Fig. 3.10 b). The magnetic PL data (Fig. 3.9 a)) is measured experimentally at 200  $\mu$ W and 1.4 mW with 405 nm excitation on two separate samples and the sample-to-sample variation is minimal. The measurement is taken with a UV-Vis spectrometer with a typical excitation beam diameter of 1 mm. The excitation power of 1.4 mW is thus comparable in areal intensity to 1.4 nW on the confocal set-up with the 405 nm excitation. The collected PL at different magnetic fields (*R*(*B*)) is normalised by the PL at zero applied magnetic field (*R*(0)) and plotted against the applied magnetic field. At magnetic field compa-



Figure 3.10: Comparison between simulated result from SLE and experimental results. **a**) Time-resolved PL (TRPL). **b**) magnetic field effect (MFE) on PL. The TRPL data was obtained from Dillon *et al.* [63]. Magnetic PL is measured with 200  $\mu$ W excitation at 405 nm. The model of the MFE data is done with Stochastic Liouville Equation (SLE).

Fitting parameters	Value
k <sub>rad</sub>	0.21 GHz
k <sub>SF</sub>	6.25 GHz
k <sub>TF</sub>	22 GHz
k <sub>dis</sub>	3.7 GHz
k <sub>h</sub>	1.2 GHz

Table 3.3: Fitting parameters extracted from SLE. The model is compared with transient PL and magnetic PL data.

rable to D (~0.08 mT), the normalised PL exhibits a decrease, due to less efficient conversion from the separated triplets back to the quintet state (4  $\rightarrow$  3 states in Fig. 3.9) and thus less triplet fusion. The MFE gives an indication of the rate of triplet hopping [243]. The fitting parameters are listed in Table 3.3, with fitting parameters  $k_{SF}$ ,  $k_{SF}$ ,  $k_{TF}$ ,  $k_{dis}$ ,  $k_h$ .

In addition to modelling the MFE data, the hopping mechanism can give predictions for  $T_2$  and  $T_2^*$  times of DPH which can then be directly compared with relevant experiments (though only low temperature experiments are available as of now). Similar modelling of  $T_2$  observations in ESR for other materials has been discussed in [23, 149]. Nevertheless, the zero-field spectral density can only be probed by zero-field ODMR experiments, which have not been carried out on DPH.

#### **3.3.5** Practical challenges in interfacing NV with photovoltaic materials

Interfacing NV centre experiments with photovoltaic materials is not without its challenges. Firstly, OSC photovoltaic materials are intended for absorbing solar radiation and are excited usually with light in the visible spectrum. The commonly used materials such as pentacene and TIPS-tetracene can be excited by the 532 nm laser used for NV experiments. We initially addressed this issue with a 635 nm excitation close to the NV ZPL. However the readout efficiency was significantly poorer than the 532 nm alternative. Subsequently we moved to materials with higher bandgap, such as anthracene and DPH and the latter proved to give only limited luminescence.

Secondly, the OSC materials are organic materials susceptible to local changes, for example when exposed to strong optical excitation, especially the laser power required for NV sensing. Stronger excitation power leads to stronger change of the material. This is possibly due to the local temperature variation of the material, or light-induced chemical reaction with residual water or oxygen in the encapsulated sample. This limits the duration of measurements, and also might lead to drift of the apparent  $T_1$  time independent of optical excitation. This can be avoided by using smaller laser power, and using ensemble NV centres to avoid prolonged strong optical excitation. However despite these efforts, the optically induced degradation still limited the amount of measurement we could obtain from a single sample.

Finally the preparation of organic materials was achieved with evaporation, drop cast or spin-coating whereby achieving a uniform coating on a substrate already coated with nanoparticles is difficult to verify. The complex interface between diamond surface and material interface, may also introduce unwanted local defects. We test this with various types of diamonds and nanodiamonds and show that they show similar behaviour.

### 3.4 Conclusion

In this chapter we demonstrated that the  $T_1$  relaxation process is enhanced by excitation of external triplet excitons. The magnetic noise created by these excitons requires a more involved SLE model to give rise to the moderate linewidth required to explain the NV relaxometry observation. This also suggests the triplet hopping plays a significant role in this material.

In addition, the extent of externally induced depolarisation initially increases with external optical excitation and saturates at high optical excitation. These observations are made with nanoscale sensing volume and is consistently observed across 3 types of nanodiamond and one type of bulk diamond sample. Differential measurements have also been carried out to clear the ambiguity of PL decay unrelated to the ground-state spin, or due to material degradation.

Nevertheless, as the NV measurements typically require a laser power much higher than the solar fluence used for the testing of organic photovoltaic cells, optically induced material degradation is still significant. This limits the number of measurements that can be performed on NV centre, thus limiting the number of corroborative measurements. It should also be pointed out that about only 5% of the NV centres measured in this work show a change in  $T_1$ , while the rest showed no significant decrease (or increase) in  $T_1$ . The absence of  $T_1$  change is possibly due to poor local DPH material coverage, or possibly large NV to surface distance. At present time, it is not straightforward to distinguish these possibilities. However, with a well controlled NV depth, for example by using a calibrated diamond nanopillar mounted on an AFM tip, it is possible to determine the NV-to-surface distance.

Further experiments could be performed by incrementally moving the DPH excitation location away from the NV location and monitoring the triplet excitons diffused to the nanodiamonds. This allows the triplet excitons that have diffused away from their original site of generation to be probed. Using an atomic force microscope (AFM) equipped with diamond tips [219], it would also be possible to probe new sample regions in the case of material degradation. An AFM provides sub-nanometer location resolution and will thus provide more spatial detail than the current optical methods. Furthermore, total internal reflection fluorescence (TIRF) microscopy restricts the optical excitation and collection to within  $\sim$  10 nm at the bulk diamond-DPH interface, such that the unintended excitation of the DPH material and spurious fluorescence can be minimized. More recently developed spin-to-charge conversion readout [108], near-IR assisted readout[107] are also potentially useful methods to relieve the restriction on the band gap of the material probed, to be able to cover materials more understood such as pentacene and tetracene.

# **Chapter 4**

## NV thermometry in abiotic environment

One unique application of diamond nanocrystals compared to their bulk counterpart is intracellular metrology. Nanodiamonds are capable of being uptaken by cells or small organisms without needing to puncture the membrane structure, thus preserving biological activity. Of particular interest, yet less investigated, is the nanoscale heat transfer and temperature distribution.

This chapter starts with introducing the applications driving the development of nanoscale thermometry and discusses previous efforts in developing NV-based thermometry. Nanoscale thermometry concerns the determination of temperature fluctuation and spatial differences in the nanoscale.

In particular, the different metrological protocols involving the ground-state (GS) spin are compared. In this work photolithographically deposited patterns are fabricated on the substrate to allow on-chip temperature sensing and control. These patterns form of a MW delivery circuitry, a resistive heating element and a resistive temperature detector and together they clear some of the uncertainties in heating effects from microwave and mixing. This is discussed prior to the hardware implementation of a confocal microscope for temperature sensing experiments. Finally the results on calibration and benchmarking the sensitivity and accuracy of the sensor are presented.

As thermometry is less well-studied, this is a necessary step to establish the reliability, sensitivity and accuracy of the sensor. We demonstrate with our experimental system that we can achieve a sensitivity of  $1.5 \text{ K}/\sqrt{\text{Hz}}$ , comparable to previous reports.

The project's current progress was a result of collaborative efforts with Dr Jack W. Hart and Louise Shanahan. All data presented in this chapter is Q.G.'s original contribution. Q.G. has redesigned an existing confocal microscope completely and written the control software based on adaptations of the python software presented in Chapter 2. Q.G. worked with L.S. on constructing the microscope. The design, COMSOL simulation, nanofabrication (photolithography, metal evaporation) and testing of the microfluidic chip are a result of Q.G.'s effort. Electronic control of the experiment, benchmarking temperature sensitivity, calibrating optical performances are done by Q.G.. All experiments and data analysis were done by Q.G..

## 4.1 Nanoscale thermometry

Nanoscale temperature mapping is particularly useful for microelectronic circuits which demands more and more densely packed electronics, for micro- and nanofluidics [267], nanoscale Seeback effects, and biological processes which is the focus of this chapter and the next. An excellent review by P. Childs [48] provides good introduction to associated technologies.

#### 4.1.1 Heat transfer at nanoscale

Unlike quantities such as total internal energy and entropy, temperature is a special quantity in classical thermodynamics because it is only defined for subsystems in thermal equilibrium. This equilibrium is typically guaranteed in macroscopic systems because such systems have small fluctuations about the equilibrium (typically  $\propto 1/\sqrt{N}$  with N being the total number of particles), and approach equilibrium fast (typically  $\propto 1/\sqrt{N}$ ). On the other hand, a small system such as NV centres, are well isolated from the surroundings such that they can be easily optically pumped out of thermal equilibrium and thermalise with the environment on a timescale of  $T_1$ . In nanothermometry in biological systems however, one faces such a situation of using microscopic sensors to probe a fluctuating quantity, whose fluctuation is comparable to the sensor's resolution. For example, a nanodiamond of diameter 50 nm in water has an intrinsic temperature fluctuation of  $13 \,\mu K / \sqrt{Hz}$  [224]. This fluctuation is typically a white noise beyond the thermalization time, typically on the pico-second scale [35], very fast compared to the typical timing resolution of nanothermometers. Thus even in theory, the temperature sensitivity of diamond cannot be improved indefinitely. Experimentally however, this sensitivity is still not within reach by the state-of-the-art nanodiamond based thermometers  $(13 \text{ mK}/\sqrt{\text{Hz}})$ silicon vacancy centre in nanodiamond [50]). A prediction of 33 mK is given by [6], however this seems to marginally violate the experimental evidence. The typical sensitivity achieved in NV nanothermometry in liquid medium is about 100 mK [49, 85] and is still above both predictions.

At room temperature, the peak black-body radiation (by Wien's displacement law) is at  $10 \,\mu\text{m}$  and on nanometer scale radiative heat transfer does not contribute to local temperature fluctuation. However, this treatment assumes the wavelength is much smaller than the feature size. When this breaks down, each molecule needs to be treated as fluctuating dipoles which
may interfere with other molecules and leads to larger or smaller heat flux compared to a perfect black body. As this leads to faster thermalization at the nanoscale, no further discussion is made on this.

# 4.1.2 Nanothermometry techniques

There are luminescent and non-luminescent nanothermometry techniques. Luminescent techniques rely on optical readout of temperature and enable remote sensing. Non-luminescent techniques are often contact techniques (although techniques such as photoacoustic imaging, are contact-free).

One such contact technique is the scanning thermal microscopy (SThM) technique which uses a small thermocouple, or resistance temperature detector (RTD) mounted on a scanning probe. This provides a contact sensor with nanoscale resolution. Such microscale thermocouples [186] and RTDs can also be deposited on sample surfaces with nanofabrication techniques, instead of mounting on scanning tips.

The non-luminescent techniques are often not live-cell compatible as they disrupt the plasma membrane of the cells and are thus not discussed here. The luminescent techniques are more applicable to biological cells and are discussed in Chapter. 5.

Diamond nanothermometry measurements using NV centres and silicon vacancy centres are examples of luminescent techniques and have been demonstrated in past experiments. The NV approach is discussed in detail in this chapter. Silicon vacancy centres in nanodiamonds have also been used for colorimetric thermometry and show three-orders-of-magnitude better sensitivity compared to the NV nanothermometers. Nevertheless, colorimetric measurements are susceptible to drawbacks common to colorimetric methods, which are discussed in detail in Section 5.1.2.

## 4.1.3 NV-based thermometry

NV centres in both bulk and nanodiamonds have been used to demonstrate temperature sensing. Most utilise the ground-state properties of the NV [2, 45]. The temperature sensitivity of NV centres is useful for nano- and microscale thermal mapping of integrated circuits [12, 82]. Temperature sensing using nanodiamonds brings the temperature sensitivity of NV in bulk diamond to enclosed structures such as cells and small organisms [49, 84, 142, 173].

Owing to the long room-temperature coherence of the GS spin, precision quantum metrology techniques have been applied to improve the sensitivity, as demonstrated with the various pulsed protocols such as D-Ramsey [171], thermal-echo (TE), T-CPMG and T-CPMG-N sequences [236, 246]. The TE sequence is similar to spin-echo discussed in Chapter 1 and

<b>Mean</b> $ \kappa $ (kHz K <sup>-1</sup> )	Diamond	Calibration method	Protocol	Ref.
76(1)	Bulk	AD590 IC sensor	ODMR	[2]
84(4)	Bulk	-	ODMR	[45]
78.6(5)	Bulk/Nano (single)	Thermistor	D-Ramsey	[171]
60(10)	Nano (single)	-	ODMR	[183]
67.5 to 95.0	Nano (ensemble)	Objective Heater	ODMR	[86]

Table 4.1: Comparison of temperature dependence of *D*.  $\kappa = dD/dT$ . The calibration method is also listed as the linearity potentially affects the temperature dependence.

removes slowly fluctuating noise. T-CPMG sequence is similar to the conventional CPMG sequence discussed in Chapter 2 where faster fluctuating noise can be removed. These sequences originally use square  $\pi$ -pulses, which are challenging to implement when pulse errors are significant. Combination of these pulses with quantum optimal control to result in Cooperative D-Ramsey [139] are developed to form sequences more robust to pulse errors induced by nanodiamond rotation and diffusion.

### 4.1.3.1 Temperature-dependent Hamiltonian

The temperature dependence in NV centres arises from local strain due to thermal expansion of the diamond crystal lattice. The electron wavefunction of the NV centre is compressed when the diamond lattice contracts when temperature decreases, reducing the average distance between electrons. For the NV GS, the dominating zero-field splitting (ZFS) contribution is from spin-spin dipolar coupling (as opposed to spin-orbit coupling [67]), leading to a change in the ZFS parameters, D and E. Thermal expansion changes the D parameter [65, 67] but not the E parameter [2]. The resulting Hamiltonian is

$$H = D(T)S_{z}^{2} + E(S_{x}^{2} - S_{y}^{2}),$$

where the ZFS parameter *D* varies with temperature nonlinearly but can be linearized around room temperatures,

$$D(T) = D_0 + \kappa T. \tag{4.1}$$

The proportional constant  $\kappa$  varies between NVs and is listed in Table. 4.1. In fact these values not only differ between diamonds, but also between different methods of extracting them for the same sample [86], thus highlighting the need to calibrate each diamond to yield accurate results. Various measurement protocols are available to probe D(T) with high sensitivity and are discussed in Section 4.1.4.

The coupling to temperature  $\kappa$  is relatively small compared to, for example Zeeman cou-

pling, for commonly observed temperature  $(74 \text{ kHz K}^{-1})$  and magnetic fields  $(2.8 \text{ MHz G}^{-1})$ . As such, hybrid sensors combining hydrogels and ferromagnetic nanoparticles [274] convert temperature signals to magnetic signals which are in turn measured by the NV. In these sensors, the hydrogel shell contracts in response to a drop in surrounding temperature, bringing magnetic nanoparticles closer to the NV centres, effectively working as a transducer of temperature change to magnetic field change. Building on such concepts, magnetic nanoparticles can be engineered to exhibits temperature dependent magnetic phase transition, which leads to magnetization change as a result of temperature [247].

Besides the GS spin methods, the excited state (ES) splitting can also be used, albeit the sensitivity  $dD_{es}/dT$  is not improved [183]. Temperature also causes the optical transition (the ZPL) to shift, and forms the basis of all-optical thermometry [87, 183, 269].

Despite the many readout modalities, NV centre GS spin-based nanothermometry stands out among other protocols and other luminescent techniques, since fundamentally the spin transition is probed, not the optical fluorescence. By carefully designing the experimental protocol, changes in surrounding refractive index, background fluorescence, charge-state conversion and other confounding factors, do not affect the spin measurement.

### 4.1.4 Measurement protocols

Recognising the unique property of GS spin transitions, the experimental procedure to extract the temperature from GS spin transitions is now discussed. Continuous-wave (CW) ODMR is the more common method for its simplicity. More involved techniques designed for thermometry involves using the three ground states simultaneously and are also discussed.

#### 4.1.4.1 Continuous-wave ODMR

In this protocol, the NV fluorescence is continuously monitored while MW frequency is stepped through the two GS resonances. The photoluminescence forms two Lorentzian peaks described by the functional form (Fig. 4.1 a))

$$PL(f_i) = PL_0 \left\{ 1 - C_1 \frac{1}{1 + \left(\frac{f_i - (f_0 - \Delta)}{\Gamma_1}\right)^2} - C_2 \frac{1}{1 + \left(\frac{f_i - (f_0 + \Delta)}{\Gamma_2}\right)^2} \right\},$$
(4.2)

where  $C_1$  and  $\Gamma_1$  are the contrasts and half-width at half-maximum (HWHM) of Lorentzian peak 1 and similar definitions apply to  $C_2$  and  $\Gamma_2$  for peak 2.  $f_0$  is the mean and  $2\Delta$  is the separation between the resonance frequencies of the two Lorentzian peaks.  $PL_0$  is the offresonance PL. It is assumed that only the central frequency  $f_0$  decreases in response to a



Figure 4.1: ODMR Lineshape and sensitivity dependence on laser and microwave powers for FND diamonds (ensemble NV). Each trace is averaged for 6 minutes in total. **a**) MW power dependence performed at laser power of  $18 \,\mu\text{W}$  with the colorbars showing the attenuation/amplification relative to 10 dBm delivered to the sample (measured at the SMA connector on the PCB). Increasing MW power increases the ODMR contrasts (blue to red transition). **b**) Laser power dependence at MW power of 5 dB attenuation in **a**) (15 dBm at the sample). The optical power measured at the sample plane has been indicated with colour bars. Panels **a**) and **b**) are plotted on the same vertical axes. **c**) Theoretical sensitivity extracted from experimental lineshape using Eq. A.1 based on the seven parameter fitting model (Eq. A.3).

temperature rise and vice versa, but not the peak separation  $\Delta$ , widths  $\Gamma_{1,2}$ , and contrasts  $C_{1,2}$ . The *PL*<sub>0</sub> is also assumed to be fluctuating, due to diffusion of diamond or drift on the sample. Other functions have also been used to fit the data [211], see discussion in Appendix A.1.2.4.

Given this functional form, it is in principle possible to determine exactly the sensitivity expected. However, an intuition about how sensitivity is affected by various physical parameters can be gained by considering a single-peaked ODMR measurement. The resolution of the central frequency is [69, 199, 231]

$$\delta\omega \propto \frac{\sigma}{C\sqrt{PL_0}},\tag{4.3}$$

whereby  $\sigma$  is the linewidth of the Lorentzian peak, *C* is the contrast between PL on-resonance and off-resonance, and *PL*<sub>0</sub> is the off-resonance PL (in photon counts). Following this expression, it is worth noting that *N* identical NV sensors will improve the resolution by increasing the total PL, and thus  $\delta \omega \propto 1/\sqrt{N}$ . This is already described by the classical sensitivity limit, rather than the Heisenberg limit expected of a quantum sensor. In the sample used in this work, the linewidth  $\sigma$  also increases due to inhomogenous broadening and a balance can be achieved between the PL and the linewidth, both increase with number of NVs.

The proportional constant in Eq. 4.3 depends on the series of frequencies  $f_i$  used to map out the ODMR spectrum and is discussed in Appendix A.

The lineshape of NV ODMR depends on laser power and MW power through Eq. 4.3. Experimentally this is checked by measuring the ODMR spectra as a function of laser and MW powers. Full CW-ODMR spectra are taken with the protocol to be discussed later in Section 4.2.3 and 6 minutes of data is acquired for every pair of laser and MW powers to give plots in Fig. 4.1. A total of 90 (10 laser, 9 MW powers) pairs are gathered. Figure 4.1 **a**) shows individual ODMR traces corresponding to varying MW powers at a fixed laser power (9  $\mu$ W). The contrast increases with increasing MW power. Figure 4.1 **b**) shows the ODMR traces taken with varying laser powers at a fixed MW power (-25 dBm from the generator) where ODMR contrast decreases with laser power. Correspondingly, the ODMR based sensitivity also changes. Figure 4.1 **c**) shows the sensitivity based on the discussion in Section A.1.2.2.

 $PL_0$  in Eq. 4.3 depends on the laser power as described by Eq. 1.1 in Chapter 1.

The power broadening due to MW and optical pumping is [69]

$$\sigma = \frac{\Gamma_c^{\infty}}{2\pi} \sqrt{\left(\frac{s}{1+s}\right)^2 + \frac{\Omega_R^2}{\Gamma_p^{\infty} \Gamma_c^{\infty}}},\tag{4.4}$$

and the contrast is (assuming  $s > 10^{-2}$ , i.e. optical pumping is strong compared with MW drive),

$$C = \Theta \frac{\Omega_R^2 / \Gamma_p^{\infty} \Gamma_c^{\infty}}{\frac{\Omega_R^2}{\Gamma_p^{\infty} \Gamma_c^{\infty}} + \left(\frac{s}{1+s}\right)^2},\tag{4.5}$$

where  $\Gamma_c^{\infty} \approx 8 \times 10^7 s^{-1}$  and  $\Gamma_p^{\infty} \approx 5 \times 10^6 s^{-1}$  are NV system parameters determined from the radiative transition lifetime and shelving state lifetime.  $\Omega_R$  is the Rabi rate and *s* is the optical pumping parameter (see Eq. 1.1 in Chapter 1). Thus the contrast and linewidth increase with  $\Omega_R$  (Fig. 4.2 **a**), **b**)). In the parameter space explored in Fig. 4.2, the MW-induced power broadening dominates the linewidth, and thus Fig. 4.2 **d**) shows little change as laser power is varied. For single NV centres, contrast would first increase with laser power until optical pumping exceeds MW driving (which is not describe by Eq. 4.5, [43]) and then decrease at higher laser powers (described by Eq. 4.5). However, this is not observed in ensemble NV samples (Fig. 4.2 **c**)), possibly due to the random orientation of each constituent NV having a different peak laser power, thus resulting in a loss of the characteristic peak.

Increasing MW power increases sensitivity in the range presented and the optimal laser power is around  $100 \,\mu\text{W}$  (Fig. 4.1). This is not used for cytotoxicity reason and instead a power of  $2 \,\mu\text{W}$  to  $8 \,\mu\text{W}$  is used experimentally. This is in line with that used in a commercial confocal microscope. Note that this is the power measured after the objective as opposed to before the objective, and is thus the power experienced by the sample.



Figure 4.2: Dependence of ODMR contrast and linewidth on laser and MW power for FND diamonds (ensemble NV). **a**) The contrast increases with MW power. **b**) HWHM linewidths also increase with MW power. At low MW powers, the linewidth is limited by intrinsic inhomogenous broadening and is approximately 3.5 MHz. **c**) The ODMR contrast decreases with increasing optical power. However for reduced phototoxicity, the actual laser power used is approximately  $6 \mu W$ . **d**) The linewidth stays close to constant at high laser powers. But at low laser powers the linewidth increases with decreasing optical excitation. This is due to spin relaxation [85].

#### 4.1.4.2 Three-level system based techniques

CW ODMR is limited by laser power and MW-induced power broadening. The linewidth can be significantly reduced by utilising the coherence of NV centres to reach the  $T_2^*$ -limited linewidth, for example with a Ramsey interferometry technique. The Ramsey technique is in principle the best protocol for measuring slow energy level shifts given a two-level system. To find *D*, the two GS transitions are separately measured and averaged. This method is limited by the fast magnetic noise influencing both transitions, manifesting as a linewidth broadening characterised by  $T_2^*$ . However, this limit can be overcome by exploiting the third level in the NV GS.

Since only the ZFS parameter D needs to be measured, not frequency of each level, pulse protocols such as the D-Ramsey and T-CPMG have been reported to protect the GS spin from magnetic noise induced dephasing. The narrow linewidth which is in principle achievable with pulsed protocols would thus improve the temperature sensitivity. Eventually, the linewidth would be limited by the inhomogenous broadening of D, as is the case in the ensemble NV sample used in this work. Thus, barring the production of nanodiamonds with inhomogenous broadening in D less than the homogenous broadening limited by  $T_2^*$  as measured with a T-CPMG sequence, using this technique still requires single NV centres, or addressing one single NV centre in a sample containing few NV centres. This reduces the total count rate available.

#### Pulsed protocols and comparison with experiments

In this class of measurement schemes, low frequency axial magnetic noise, can be cancelled out to prolong the dephasing time. The pulse sequence is depicted in Fig. 4.3 **a**). In this sequence, the two transitions  $|0\rangle \rightarrow |-1\rangle$  and  $|0\rangle \rightarrow |+1\rangle$ , with resonance frequencies  $\omega_{0,1}/2\pi$ ,  $\omega_{0,2}/2\pi$ are separately driven with resonant microwave. Figure 4.3 **b**) and **c**) show the corresponding Rabi oscillation, driven by two separate MW sources. The simplest sequence is the D-Ramsey sequence [171], whereby after initialisation, the GS goes through pulse sequence,

$$\left(\frac{\pi}{2}\right)_{-1} \xrightarrow{\tau} (\pi)_{-1} (\pi)_{+1} (\pi)_{-1} \xrightarrow{\tau} \left(-\frac{\pi}{2}\right)_{+1},$$

followed by optical readout.  $(\pi)_{\pm 1}$  refers to a  $\pi$ -pulse applied to the  $|0\rangle$ ,  $|\pm 1\rangle$  qubit, driven with MW1/2. Under this sequence, the GS evolves as

$$\begin{array}{cccc} |0\rangle & \xrightarrow{\left(\frac{\pi}{2}\right)_{-1}} & & \frac{1}{\sqrt{2}} \left(|0\rangle + i \left|-1\rangle\right) \xrightarrow{\tau} & & \frac{1}{\sqrt{2}} \left(|0\rangle + i e^{i\delta\phi_{1}} \left|-1\rangle\right) \\ & \xrightarrow{\left(\pi\right)_{-1}} & & \frac{1}{\sqrt{2}} \left(\left|-1\rangle + e^{i\delta\phi_{1}} \left|0\rangle\right\right) \xrightarrow{\left(\pi\right)_{+1}} & & \frac{1}{\sqrt{2}} \left(\left|-1\rangle + e^{i\delta\phi_{1}} \left|+1\rangle\right) \\ & \xrightarrow{\left(\pi\right)_{-1}} & & \frac{1}{\sqrt{2}} \left(\left|0\rangle + e^{i\delta\phi_{1}} \left|+1\right\rangle\right) \xrightarrow{\tau} & & \frac{1}{\sqrt{2}} \left(\left|0\rangle + e^{i(\delta\phi_{1} + \delta\phi_{2})} \left|+1\right\rangle\right) \\ & \xrightarrow{\left(-\frac{\pi}{2}\right)_{+1}} & \frac{1}{\sqrt{2}} \left[\cos\left(\Delta\Phi/2\right) \left|0\right\rangle + \sin\left(\Delta\Phi/2\right) \left|+1\right\rangle\right] \end{array}$$

where  $\Delta \Phi = \delta \phi_1 + \delta \phi_2$ . The phases accumulated,  $\delta \phi_1$  and  $\delta \phi_2$ , are related to the magnetic field fluctuations  $B_z(t)$  and MW detuning,  $\delta \omega$ , as  $\delta \phi = \tau \delta \omega + m_S \mu_B g \int_0^{\tau} dt B_z(t)$  in the rotating frame. When there is no detuning, and the magnetic field fluctuation is very slow compared to the length of the pulse sequence,  $\delta \phi_1 = -\mu_B g B_z \tau$  and  $\delta \phi_2 = \mu_B g B_z \tau$  cancels out, and thus eliminates the effect of slow magnetic field fluctuations. When such static magnetic fields are not present, the phase accumulation is purely due to MW detuning,  $\delta \phi_1 + \delta \phi_2 = \tau (\omega_1 - \omega_{0,1} + \omega_2 - \omega_{0,2}) = \tau (\varepsilon_1 + \varepsilon_2)$ , where  $\varepsilon_{1,2}$  are the detunings for the two MW transitions. Thus when finally reading out the  $m_S = 0$  state as  $\tau$  is varied, a fringe will be produced resulting in  $PL \propto PL_0 [1 - A \cos[\tau (\varepsilon_1 + \varepsilon_2)]]$ . Suppose MW1 is artificially detuned by  $\delta f_1$ , the first maximum is observed at  $\tau = 1/\delta f_1$ . When expressed as a function of the total evolution time, T, ( $T = 2\tau$  for D-Ramsey),  $\delta f_1 = 1/2T$ . A similar argument can be applied to sequences with larger number of  $\pi$ -pulses [236] which gives the same dependence on the total evolution time.

The same principle can be applied to multiple triplets of  $\pi$ -pulses (Fig. 4.3 a)), with the same period extracted from the fringe (Fig. 4.3 d)).



Figure 4.3: T-CPMG measurement on Single NV centres. **a**) The pulse sequence (right) involves performing  $\pi$ -pulses by driving two transitions  $(|0\rangle \rightarrow |-1\rangle$  and  $|0\rangle \rightarrow |+1\rangle$ ) while MW1 is detuned by  $\Delta$  (left). The two transitions are driven by two separate microwave sources. **b**) and **c**) show the corresponding Rabi oscillations. **d**) shows the T-CPMG fringe as the delay  $\tau$  is progressively changed. **e**) shows period of oscillation changing linearly with MW1 detuning in measurements like **d**). **f**) On-resonance T-CPMG with N=2 (12  $\pi$ -pulses applied). **g**)  $T_{2,T-CPMG}^*$  as the number of  $\pi$ -pulse increases.

Similar to the observed  $T_2^*$  decoherence, the coherence of NV GS extracted using a T-CPMG-N sequence can be measured and the corresponding dephasing time (also called  $T_2^*$  here) is shown in (Fig. 4.3 g)). Unlike conventional CPMG dynamical decoupling, the T-CPMG-N sequence does not prolong the dephasing time with increasing number of pulses. In conventional CPMG, this is typically due to the noise spectrum having significant high-frequency components which cannot be removed by a CPMG sequence. In addition, even if T-CPMG does protect the qubit from slowly fluctuating axial magnetic noise, it does not protect against fluctuating electric noise which shifts *D*. Thus the electric noise may limit the  $T_{2,T-CPMG-N}^*$  time. Furthermore, increasing the number of pulse sequences also leads to an accumulation of pulse errors for the single-axis square  $\pi$ -pulses used in this protocol.

The susceptibility to electric noise can be reduced by applying an on-axis bias magnetic field [121]. With the current setting of the experiment, the narrowing in linewidth would not compensate for the loss in count rate (~100x). Technically with improved real-time magnetic field tracking and MW power adaptation to NV centre orientation, it is possible to use single NV centres to achieve the same temperature sensitivity, or even better, compared to CW ODMR technique. Also the added capability of orientation tracking of magnetic field and MW would allow multi-modal sensing using NV diamonds in applications such as nanoscale NMR. Nevertheless for the current application we refrain from this exciting yet more technically challenging route. This issue can also be circumvented by looking at nanodiamonds attached to intracellular structures such that they are less mobile (see discussion in the next chapter).

#### 4.1.4.3 Spectral hole burning

Even using CW method, we could gain in the temperature sensitivity by using MW spectral hole burning technique. CW spectral hole burning involves simultaneous driving both the  $|0\rangle \rightarrow |-1\rangle$  and  $|0\rangle \rightarrow |+1\rangle$  transitions [135]. As the GS population is conserved, when MW2 is on resonance with the  $|0\rangle \rightarrow |+1\rangle$  transition and MW1 is swept in frequency around the  $|0\rangle \rightarrow |-1\rangle$  transition frequency, a spectral "hole" is created with a narrower linewidth. This "hole" shifts with temperature with  $2\kappa$ . Both the narrower linewidth and doubled responsivity improve the temperature sensitivity. In our experiment, it was found that in an ensemble NV sample, one ODMR peak is completely removed when the other ODMR transition is saturated. This is due to the spread of D and E parameters such that the spectral hole is inhomogenously broadened and is no longer narrow as expected. As such this does not appear to be a viable method.

# 4.1.5 Metric for nanothermometer performance.

Having established that CW ODMR is currently the better method for nanothermometry, the criteria for benchmarking temperature sensitivity are listed here,

- Accuracy and traceability. The sensor under test must produce the same measurement (up to its quoted accuracy) at the same temperature to a known and "traceable" sensor over the temperature range of interest. "Traceable" sensors are available commercially and ensure that all traceable sensors give accurate readings.
- 2. Sensitivity. Sensitivity and resolution are used interchangeably in this work. The temperature resolution is defined as the smallest change in temperature detectable. This is usually shot-noise limited in NV measurements and is quoted in units of  $K/\sqrt{Hz}$ , as in magnetometry measurements. Thus in this work, sensitivity is defined to be the resolution (also in  $K/\sqrt{Hz}$ ).

In the following section, the methods for experimentally measuring these quantities are discussed, with particular focus on the sample preparation process during which on-chip temperature control is incorporated.

# 4.2 Experimental setup

## 4.2.1 Microwave delivery, environment temperature control

There are uncertainties in NV-based thermometry measurements from both intrinsic and extrinsic sources. The NV centres themselves have different temperature susceptibility,  $\kappa$ , requiring individual calibration per nanodiamond. Furthermore, the temperature in close proximity (1 mm) of the nanodiamond is very difficult to measure with conventional means. Past works have achieved this by placing the substrate on a temperature controlled platform for example in a cryostat [45], an oven [183], or objective heating collar [86]. This relies on the uniformity of temperature on the substrate. However, when probing local temperature of cells in a medium, it is important to measure and control local temperature to high spatial resolution (within a few millimeters). In addition, MW heating, local chemical mixing (enthalpy change due to mixing of two liquid at the same temperature) and temperature differences due to injection of fresh media, are all extrinsic uncertainties potentially leading to misinterpretation of thermometry results, which cannot be known and controlled with macroscopic methods.

To this end, a microscale RTD and a heater are deposited on the glass substrate (fused silica, diameter=25 mm, thickness=0.17 mm, Thorlabs), alongside the microwave delivery struc-



Figure 4.4: Schematics of photolithographically fabricated structures on the substrate. **a**) Picture of a fully assembled chip with PDMS channel attached. The green PCB is connected to the glass substrate, which is pattern with Ti-Au-Ti structures for temperature sensing, heating, and MW delivery. The PDMS-based microfluidic channel allows liquid to enter via the inlet and exit via the outlet. **b**) Optical microscope image. The yellow region is coated with gold (Ti-Au-Ti structure) while the dark background is the glass substrate. The central area circled by the dashed white line is the area used for cell culture and on-chip temperature sensing and control. Inset: schematics of the pattern. CPW (green) refers to co-planar waveguides for MW delivery. RTD is a resistive temperature detector (cyan) for on-chip temperature detection. Heater is a resistive heating element (red) for on-chip heating and temperature control. **c**) Zoomed image of the central region. The dotted segment of the RTD is the active area. The CPW track pointed to by the black arrow is the signal line of the CPW.

ture (Fig. 4.4). The pattern consists of gold (Au) layer (thickness=370 nm) sandwiched between two titanium (Ti) layers (thickness=7.8 nm each), while the top surface is coated with aluminium oxide (490 nm). The Ti-Au-Ti deposition is done by UV photolithography, thermal evaporation and lift-off. The aluminium oxide deposition is achieved with e-beam evaporation over a stainless steel shadow mask.

#### 4.2.1.1 Microwave delivery circuit

Microwave delivery is essential for manipulating NV GS spins. Getting high MW transmission at 2.87 GHz with good spatial homogeneity and with minimal heating on the sample allows high-fidelity spin manipulation and minimum thermal damage to biological cells. Various delivery schemes have been used in the literature, such as wire antenna, microstrip, co-planar waveguide (CPW) and loop-gap resonator (LGR).

A wired antenna is the simplest form of antenna where a straight wire [86, 138] or a looped wire [56] is held at close proximity to the sample. As these antennas are often made with a rigid wire, it is difficult to bring the wire to the sample closer than a few tens of micrometers. In addition as there is no ground plane in close proximity such a structure is lossy in MW frequencies. Photolithographically deposited wire antennas have also been demonstrated but



Figure 4.5: Simulated magnetic field distribution in the CPW. **a**)  $B_1$  field in the normal direction with 25 dBm of incident MW power. The red area indicates magnetic field pointing out-of-the-plane. The magnitude is indicated by the color bar. The maximum  $\pi$ -pulse time is approximately 12 ns. **b**) shows the magnetic field vector around the central track of the CPW.

given the complexity of the deposition procedure, better options such as CPWs and resonators are available.

An alternative to a wired antenna is to deposit conductive tracks on PCB or substrates to make a stripline [11]. In a stripline design, the bottom of the substrate is conductive (for example doped silicon) and the top of the substrate has conductive tracks. The tracks can be designed to impedance match the MW delivery circuitry. Impedance matching allows as much power to transmit as possible. PCB tracks can also be made inexpensively and in bulk.

Despite striplines being efficient at transmitting MW, they are not compatible with the insulating glass substrate used in biological imaging. CPWs are another device which have both the ground plane and the signal track on the same side of the sample. This design can also be impedance matched to the MW source to efficiently deliver MW to the sample region [125].

The above strategies are all relatively broadband and can deliver microwave efficiently within a bandwidth of a few gigahertz. Microwave resonators, on the other hand, are capable of achieving the same magnetic field strength with less driving power [26, 71, 240]. Common resonator designs applied to NV experiments include the loop-gap resonators (LGR) and the split ring resonators. The drawback of such a strategy is its narrow-band response. The narrow spectral response both limits the working MW range, and also makes the resonator susceptible to any fluctuation in dielectric permittivity. This can be mitigated in single-NV applications by tuning external magnetic fields to match the MW resonator's natural frequency, although this would not work with ensemble NV in nanodiamonds where an applied magnetic field in-

duces inhomogenous broadening and reduces ODMR contrast. It has also been demonstrated that such a resonator can be tuned post fabrication [71] or *in situ* [26]. This would allow a large number of potential specimens with a varied dielectric permittivity. Achieving a larger distance between the sample and the resonator allows the resonator to be fabricated on PCBs and greatly simplify the fabrication process. In applications where a large volume of samples need to be addressed, resonators are a more scalable option.

For experiments attempting to establish an effective measurement protocol, the resonator design lacks the versatility and the CPW design is chosen in favour of resonators. However the resonator design would be ideal for future applications.

The CPW design is numerically verified using COMSOL®Multiphysics. The computation uses the RF module and solves the Maxwell's equations, taking into consideration the current density in the thin layer of gold in the frequency domain. The CPW leading to the active area is designed to be 50  $\Omega$  matched. In the central area, the gap is fixed to guarantee a large imaging area while the width of the central signal patch is numerically optimised, to find a compromise between impedance matching and current density. The central track can be narrowed to increase current density and thus improve the local magnetic field strength between the track and ground. On the other hand, impedance matching requires a fixed ratio between the gap and the central track width, which favours a smaller gap between the ground and the signal. These two competing requirement results in an optimal design which is neither impedance matched, nor creates the highest field at the same current density, but is optimal when factoring in both design concerns. Figure 4.5 a) shows the magnetic field amplitude (out-of-plane component) in the CPW, assuming an input power of 25 dBm (5.5 V). The maximum magnetic field close to the central track is approximately 15 G, giving a Rabi rate of  $\Omega_R = \mu_e g B_1 \approx 42$  MHz, corresponding to a  $\pi$ -pulse time of 12 ns. Figure 4.5 b) shows the orientation of the AC magnetic field, which resembles the magnetic field produced by a current-carrying wire.

It is informative to compare between performance of the CPW with and without water, which absorbs MW and also changes the local dielectric permittivity. In this simulation a complex relative permittivity of  $\varepsilon_r = \varepsilon/\varepsilon_0 = 76.6 - 10.6i$  is used [86, 129]. Note there is no current flow between the CPW and the liquid in the channel due to surface aluminium oxide insulation.

When water is present above the substrate, MW amplitude is no longer uniform along the CPW (Fig. 4.6 a)), resulting in a loss of absolute magnitude.



Figure 4.6: AC Magnetic Field along the CPW at the centre of the transparent area. **a**) A dry sample gives uniform magnetic field distribution, **b**) while a substrate with water above shows a reduction in magnetic field along the CPW. There is no clear periodicity of  $B_1$  beyond the area presented. **c**) The magnetic field normal to the plane along the black dotted line shown in **a**) and **b**) is plotted, where the blue curve corresponds to the dry substrate while the red to a substrate covered by water.

#### 4.2.1.2 On-chip temperature sensing

To measure a temperature on the substrate, the NV sensor needs to be compared with a commercially available, calibrated sensor through a series of comparisons at a range of temperatures. There are three main types of commercially available macroscopic sensors including thermocouples, resistance temperature detectors (RTD) and thermistors. In the physiologically relevant temperature range (20 °C to 45 °C), thermocouples have the worst accuracy followed by RTD and then thermistors. On the other hand, both RTD and thermistors rely on resistance measurements and suffer from the well-known "self-heating" issue which could create a temperature rise.

Ensuring that the calibration and the test sensors are at the same temperature is important. This could be done with a water bath on hot plate, that is kept at a fixed temperature. However, the spatial inhomogeneity is an issue as the hot plate sensor and the PID controller sensor may not be at exactly the same temperature as the water bath, or the heating platform. For example the commonly used stage-top temperature control box (Okolab H201-K-Frame), measuring 3x10x16 cm heats from the top with a conductive indium doped titanium oxide glass reaches  $42 \,^{\circ}C$  at the top for a set temperature of  $37 \,^{\circ}C$  at the sample.

To resolve this issue, we used a box incubator measuring 70 cm each side to have a coarse temperature control in the proximity of the sample. The gradient is measured to be 1 K per 6 cm. A small RTD  $(1 \text{ mm} \times 5 \text{ mm})$  is used to calibrate the on-chip RTD. This way the sensor size is brought down from the incubator thermocouple (2.5 cm in size) to the gold RTD on glass which is 2 mm in size and 100 µm away from the measurement area. This sensor is



Figure 4.7: On-chip RTD sensor Characterisation. **a**) shows the linear dependence of relative resistance at temperature *T* referenced to resistance at 35 °C for sensor A. The gradient of the linear fit is shown for 5 sensors in the inset. The average value of the gradient is  $\eta = (2.44 \pm 0.12) \times 10^{-3}$ /°C, showing good consistency between sensors. The on-chip heaters use the on-chip RTD as a feedback to stabilise the temperature. **b**) shows the temperature measured by on-chip RTD (blue trace) when the temperatures are set at commanded steps. The voltage applied to the heaters are shown on the same plot (red trace). The achieved temperature is close to the commanded temperature within 16 mK (inset) and stabilises within less than 4 min.

then capable of truly measuring temperature environment in the neighbourhood of the area of interest.

The sensing element is a thin and narrow segment of gold approximately  $0.1 \text{ mm} \times 2.6 \text{ mm}$ in lateral size. The resistance of the RTD is measured with a four-point method using a Keithley 2200 sourcemeter, to eliminate any influence due to contact resistance. Typical resistance ranges from  $1.9 \Omega$  to  $2.2 \Omega$ . The resistance of bulk gold of the same dimension is  $1.47 \Omega$  and the generally higher resistance is possibly due to film inhomogeneity and local defects. The variation in resistance between different chips is due to the change in thickness between thermal evaporation and is not intentionally controlled as only the relative change in resistance is important for measuring a change in temperature. The RTD is calibrated at physiologically relevant temperatures 20 °C to 45 °C. The resistance is modelled as

$$R(T)/R(T_{ref}) = \eta (T - T_{ref}) + 1$$

which is motivated by the good linearity of temperature coefficient of the resistivity of gold (Fig. 4.7 a)). Experimentally,  $T_{ref}$  is taken to be the incubator temperature. The gradient of the linear fit of experimentally measured RTDs is  $\eta = (2.44 \pm 0.12) \times 10^{-3}/^{\circ}$ C. The error on



Figure 4.8: Sources of Temperature Change. **a**) At the beginning of the measurement, the wide field illumination is turned off, causing a temperature drop of  $2 \degree C$  over an hour. **b**) At t = 1.6 hrs, the empty channel is filled with water, causing a temperature drop of  $0.4 \degree C$ . When the channel is flushed with a fresh medium kept at room temperature, a transient drop is observed which lasts for less than 24 s. Finally, due to MW heating, temperature rises by  $0.5 \degree C$  when the channel is empty, and  $0.3 \degree C$  when the channel is filled.

the linearity is dominated by the temperature fluctuations during the calibration process on the order of 0.5  $^{\circ}$ C due to the opening and handling of the incubator door.

Various sources of temperature fluctuation could lead to inaccuracies in NV thermometry. Some are intrinsic to NV operation, for example MW induced heating, and some are related to microfluidics and imaging. To test these effects, a microfluidic channel is bonded to a glass substrate (Fig. 4.4 c)). The major contribution (Fig. 4.8, a)) to heat change is the LED used for wide field illumination. Furthermore pumping new media into a channel causes 0.4 °C drop. Fresh media kept at a lower temperature causes substrate temperature to transiently drop which recovers in less than 24 s. For experiments involving adding chemicals to a cell culture for example, it is important to either keep the media at the same temperature as the sample, or use a microscopic amount of medium. In our experiment, the typical volume added to a microfluidic channel is 10 µL which operates in this regime. Finally, MW causes a rise of  $0.3 \,^{\circ}$ C or  $0.5 \,^{\circ}$ C increase if the channel is filled or empty respectively. In fact, both the MW power and wide-field illumination can be kept unchanged during the measurement process, but temperature change due to the addition of chemicals, albeit tiny, is an effect often overlooked. The change in temperature can happen due to enthalpy change (mixing heat) or the chemical not being kept at exactly the same temperature. Given the spatial variation in temperature and the chemical being stored at a different location in the incubator, the latter was difficult to achieve in our system. Thus instead of attempting to keep the chemical close to 0.1 °C of the cell culture temperature, we calibrated this effect and use small amounts of chemicals to ensure a transient perturbation to the system undetected by the NV sensors.

#### Mechanisms of MW heating

There are two mechanisms for MW induced heating in this context [223], Joule heating and dielectric loss.

The first is the Joule heating in the gold metal trace due to MW current and electrical resistance of the metal or in conductivity of the cell culture medium (DMEM, to be discussed in Chapter 5). To calculate the Joule heating in the gold layer, COMSOL solves for the electric field at the surface and computes the current density at the gold surface by  $\mathbf{J} = \sigma \mathbf{E}$ . Assuming the gold layer thickness is small compared to the skin depth (5 µm at this frequency for gold), the volumetric heat loss is given by  $P = \frac{1}{2} \operatorname{Re}(\mathbf{J} \cdot \mathbf{E})$ , which is then integrated over the gold volume. Total resistive heat loss is  $P_{rl} = 37 \,\mu \text{W}$  for an input power of 25 dBm (316 mW).

The other is dielectric loss in the liquid medium above the glass due to the near-field electric field distribution of the MW which drives electric dipole flip-flop in water. Due to the lack of magnetic materials, magnetic field of MW does not contribute significantly to heating.

The conductive loss in a cell culture medium is non-negligible given its electrical conductivity of  $\sigma = 1.4$  S/m (corresponding to 7.4 cm penetration depth) [18]. The volumetric dielectric loss are given by  $P = \frac{1}{2}\omega\varepsilon_r''\varepsilon_0E^2$ . where  $\varepsilon_r'' = -10.6$  and  $\omega = 2\pi \times 2.87$  GHz. This assumes that the penetration depth is large compare to the typical spatial extent of the electric field (10 cm in water versus spatial confinement of  $\sim 100 \,\mu$ m). Mathematically, the total electromagnetic loss is  $P_e = \frac{1}{2}\omega\varepsilon_0 \left(\varepsilon_r'' - \frac{\sigma}{\omega\varepsilon_0}\right)E^2$  and the conductivity and relative permittivity are treated as one combined parameter in COMSOL. The total electromagnetic loss in a channel is  $P_e = 53.4$  mW (also at input MW power of 316 mW).

Thus dielectric and conductive loss in water is the major contribution to heat generation. Nevertheless this does not directly link to the temperature achieved for a dry versus a water-filled channel, as the temperature rise depends on both the heating power and the rate of heat loss which depends on sample geometry. As a comparison, the heaters generate 20 mW with 1 V of supplied voltage.

#### 4.2.1.3 On-chip temperature control

Temperature control is a well-studied area in microfluidics aiming at microscale polymerase chain reaction (PCR) for genetic marker identification [39]. Here we employed a similar technology by incorporating a gold-based microscale heating and sensing element. On top of this structure, a 500 nm thick layer of aluminium oxide is deposited and the thickness is



Figure 4.9: Simulated temperature distribution from electric current heating using the on-chip heater. **a**) electrical potential map on the heater. When an external voltage is applied, a gradual drop in voltage is expected along the resistive heating elements. **b**) temperature change after a step voltage is applied to the heater, showing a characteristic timescale of 10 s. The three locations (centre of CPW, centre of RTD, and 250 µm) share almost the same temperature response, indicating a negligible temperature differences between these locations. **c**) temperature distribution at equilibrium on the surface of a microfluidic channel filled with water. **d**) temperature distribution at equilibrium in PDMS. The pink isosurface corresponds  $\Delta T = 2 \text{ K}$ .

verified with a surface profiler (DekTak) for insulation against parasitic conductance from cell medium or other ionic solutions.

A current-based heating element is used to locally control the temperature on the substrate via Joule heating. This allows the temperature of the substrate to be quickly altered on the timescale of a few minutes. When a voltage,  $V_0$ , is applied across the contacts for each heater, the electrical potential uniformly drops across the serpentine resistive heating elements (Fig. 4.9 a)) and heats up the volume within the microfluidic channel within 10 s (Fig. 4.9 b)). In fact, the whole active area of the chip uniformly rises in temperature. Furthermore, as expected, the ends of the microfluidic channel are cooler as expected, as are the ends of the PDMS elastomer (Fig. 4.9 c), d)).

The temperature of a local volume of the fluid in the channel follows

$$C_{v}\frac{dT}{dt} = e\frac{V_{0}^{2}}{R_{heater}} - k\left(T - T_{0}\right),$$

where  $C_v$  is the (constant volume) heat capacity,  $R_{heater}$  is the resistance of the heater (typically 100  $\Omega$  for each heater), *e* is the duty cycle of the pulsed heating voltage,  $T_0$  is the room temperature and *k* is the rate of heat loss due to thermal conduction. These constants are not determined explicitly but the above differential equation is used to generate and tune a PID control algorithm for optimal control of the on-chip temperature, based on the temperature measured by the on-chip RTD. Although in principle a PI controller is needed for such first order systems, only proportional gain turns out to be necessary. The PID control stabilises the temperature to within 16 mK (Fig. 4.7 b) inset) with a stabilisation time of less than 4 min (Fig. 4.7 b) inset). This is limited by the frequency of temperature measurement (12 s).

The prepared sample is measured using a home-built confocal microscope, with a in-house control software based on the Python programming language. This section presents the technical details for optical and electronic aspects of the setup, and characterisation of the optical resolution of the system.

## 4.2.2 Confocal microscopy

The NV is interrogated with a laser scanning confocal microscopy setup to achieve high spatial resolution. As the spatial resolution and imaging characteristics of such a setup is particularly important for nanothermometry measurement, these are hereby discussed.

The confocal imaging part of the setup (Fig. 4.10) consists of an excitation arm and a collection arm. In the excitation arm, 532 nm optical excitation from a single-mode fibre (460HP, Thorlabs, mode field diameter= $3.5 \,\mu$ m) is collimated by an aspheric Lens (C280TMD-A, f=18.40 mm, Thorlabs) and overfills the back aperture of an oil immersion objective (Nikon



Figure 4.10: Confocal Microscope for imaging NV centres. Laser Scanning Confocal Microscope. CL1/2/3/4 are collimation lenses. Note that CL1/2 are intentionally defocused to implement single particle tracking. L1/L2 are achromatic lenses forming the Keplerian telescope. DM532/DM605 are long-pass dichroic mirrors at 532 nm and 605 nm. LP550/LP650 are long-pass filters at 550 nm and 650 nm. SM: single-mode. BS: Non-polarising beam-splitter. AOM: acousto-optic modulator. APD: Avalanche Photodiode. PD: Photodiode. A linear polariser and a half-wave plate are placed along the excitation path to produce linearly polarised incident excitation (not shown).

Quantity	Meaning	
n = 1.515	Refractive index (of immersion oil)	
N.A. = 1.45	Numerical aperture	
f = 2mm	Effective focal length of objective	
$M_{obj} = 100$	Magnification of objective (20 cm tube lens)	
$D = 2fN.A. \approx 5.8$ mm	Pupil diameter	
$M^2 \approx 1$	Beam quality factor	
$d = 0.82 \frac{\lambda}{N.A.} = 360 \mathrm{nm}$	$1/e^2$ diameter of Airy disc (@637 nm)	
	Mode field diameter of optical fibre	

Table 4.2: Parameters used in confocal design.

CFI Plan Apo Lambda 100X Oil MRD01905, 1.45 N.A.). The collection arm collects collimated fluorescence from the objective, filtered by a 532 nm dichroic mirror (Di02-R532-25x36, Semrock), and a 600 nm long-pass collection filter (FLEH600, Thorlabs), split into two collection pathways equally via a 50:50 plate beamsplitter (BSW29, Thorlabs), each focused via an achromatic lens (AC050-008-B-ML, f=7.5 mm) into another single-mode fibre (P3-630A-FC-2, Thorlabs, MFD=4.5 µm). The collection fibre is connected to a fibre-coupled avalanche photo diode (APD, SPCM-AQRH-14-FC Single Photon Counting Module, Exelitas Technologies). The collection fibre functions as a pinhole in a conventional laser scanning system, except the SM fibre couples to the first order Gaussian mode of the incoming light, unlike a sharp-edged pinhole. The collimation lens on each collection arm is defocused in opposite directions so that one arm collects at a higher plane and the other at lower plane than the laser focus. This is used for fast particle tracking in the longitudinal direction and is discussed in Chapter. 5. Common to both the excitation and collection arms are a Keplerian telescope lens pair and a scanning galvanometric (galvo) mirror (GVS002, Thorlabs). The expansion pair is formed of two achromatic doublets (AC508-300-AB-ML, f=30 cm, and AC508-080-AB-ML, f=8 cm, Thorlabs) which expands the beam by a factor of 3.75.

The choice of excitation and collection collimation lenses are crucial for achieving a high spatial resolution and collection efficiency. The beam waist diameter of a collimated Gaussian beam is given by

$$d = rac{4M^2\lambda f}{\pi[MFD]}.$$

The beam size is 13.35 mm for the excitation beam and 5.07 mm for the collection beam. The excitation beam overfills the objective to achieve a higher spatial resolution and the collection just underfills the objective (90% of Airy Disk  $1/e^2$  diameter) to maximise photon collection. For analytical simplicity, first assume that the lens is large enough so that the intensity profile



Figure 4.11: Theoretical point spread function (PSF). Panels **a**) and **b**) shows the excitation PSF in the XZ and XY plane. Panels **c**) and **d**) are the collection PSF, from an isotropically emitting single point emitter. Panels **e**) and **f**) are the point-by-point product of the excitation and collection and correspond to the PSF for a confocal microscope. The colormap shows the normalised intensity and is logarithmically scaled.

of the excitation beam after the objective is well approximated by a Gaussian beam,

$$I_{ex}(r,z) = I_0 \left(\frac{w_0}{w(z)}\right)^2 \exp\left(\frac{-2r^2}{w(z)^2}\right),$$

where *r* is radial distance from the optical axis and *z* the longitudinal distance from the focal spot. w(z) is the beam radius at each longitudinal distance *z* and  $w_0 = w(z = 0)$  is the focal spot size.

On the other hand, the collected photoluminescence depends on the overlap between the excitation and the collection optics' efficiency at different points in space. As a result the point spread function (PSF) is the point-by-point multiplication of the two individual PSF [250],

$$PSF = p_{ex} \times p_{collection}.$$

If the collection is Gaussian then the PSF is also Gaussian with width  $1/\sigma_{confocal}^2 = 1/\sigma_{ex}^2 + 1/\sigma_{em}^2$ , which gives the resolution enhancement of a laser scanning confocal com-



Figure 4.12: Point spread function measured experimentally. The colormaps show the PL intensity (linear scale) by taking xy confocal scans of a nanodiamond fixed on glass substrate at various axial locations. **a**) shows the lateral PSF. A x slice at the bottom indicates that the lateral  $(1/e^2)$  radius is 250 nm. **b**) shows the PSF in the XZ plane. The z slice on the right shows that the longitudinal radius  $(1/e^2)$  is 603 nm (not labelled). The two APD channels are intentionally defocused in longitudinal direction for single particle tracking (discussed in Chapter 5). The empirical PSF is not symmetrical in XZ plane, due to the change in refractive index between the glass substrate and air above.

pared to wide field imaging.

An isotropically emitting point source situated at point  $\overrightarrow{r}_0$  gives fluorescence proportional to the local excitation power. The total collected PL is

$$PL \propto \int d\overrightarrow{r} \delta(\overrightarrow{r} - \overrightarrow{r}_0) PSF(\overrightarrow{r}) = PSF(\overrightarrow{r}_0)$$

Thus by measuring the PL distribution of a point source, the PSF can be mapped out. The PSF provides an informative benchmark to compare with experimentally measured intensity distribution in contrast to the commonly used parameters such as the resolution based on Rayleigh criterion. The exact PSF needs to be numerically computed and is displayed in Fig. 4.11. The numerical computation is done with the python package psf, which implements the complex diffraction integral detailed in Ref. [189].

This diffraction integral computes the integral of the scalar optical wave amplitudes (with-

	Lateral (nm)		Longitudinal(nm)	
	Theory	Exp.	Theory	Exp.
Excitation (532 nm)	197	-	384	-
Emission (637 nm)	235	-	431	-
Confocal	154	250	294	603

Table 4.3: Comparison between theoretical and empirical point spread function widths.

out using the Gaussian approximation), as opposed to the vectorial model, where the optical polarisation is also considered. Especially for high-NA objectives where direction of propagation deviates significantly from the optical axis, summing the amplitude is no longer a good approximation of the local optical fields. In the simulation presented in [262], a incident beam with *x*-polarisation (perpendicular to the optical axis of the objective lens), the optical polarisation at the focal plane does not only contain *x*-polarisation, but also significant contributions from the *z*-direction and a small amount from the *y*-direction due to ray bendings. The use of high-NA objectives thus requires careful consideration in experiments concerning polarised photons. However in our application the emitter is isotropic due to randomly oriented NV centers in a nanoparticle and only the excitation intensity, not polarisation, is considered. In this setting, the intensity distribution given by the vectorial method does not deviate significantly from the scalar method in axial direction, and only slightly in the lateral direction (lateral resolution is worse in vectorial model by about 5% for a 1.4 N.A. oil immersion objective [3]). For this reason the scalar model is used as a crude approximation for understanding the optical system.

The theoretical and empirical PSF (Fig. 4.12) widths are tabulated in Table. 4.3.

There is a significant discrepancy between the experimental and the ideal PSF widths. The discrepancy can be attributed to the finite size of the nanodiamond used (50 nm), the abberation due to various optical components, the intended defocusing in the longitudinal direction and misalignment of the system. The experimentally measured resolution is comparable to a commercial system (Leica SP5) using objective of a similar NA (1.40).

Additionally, on the excitation arm, the beam is first passed through a linear polariser and then a half-wave plate (AHWP05M-600, Thorlabs) to deliver linearly polarised excitation light to the sample. After passing through the polariser, 10% of the beam is first split off to a photodiode for power monitoring and then the half-wave plate. This ensures that the power is constant and is independent of polarisation. The AOM diffraction efficiency into the first order (which is coupled to the set-up) is controlled via a high-bandwidth (100 kHz) PID controller (SIM900, Stanford Research Systems) feeding back on the photodiode laser power measurement for laser power stabilisation. The power stabilisation is critical, as any



Figure 4.13: Electronic setup illustration. The DAQ card is central to synchronising various hardwares. It sends voltage ramps to the MW generator for frequency modulation of the MW. It generates pulses to switch on and off the on-chip heater with a solid-state switch (green). The incoming photon counts from APD 2 and 3 are also counted and gated by the DAQ. In conjunction with the MCU tracker (discussed in chapter 5), two sinusoidal voltages are supplied to the galvo mirror to steer the galvo mirror for single particle tracking (blue). The MW generated from the MW source is connected to an RF switch (orange) and amplified by an external MW amplifier. The amplified MW is then sent to the CPW on the sample's substrate.

fluctuations on the timescale of orbital tracking ( $\sim 10 \text{ ms}$ , discussed in Chapter 5) will lead to position tracking noise.

## 4.2.3 Hardware-timed ODMR and temperature control

Conventional ODMR often steps MW frequencies slowly, partly because function generators have a limited switching time (~5 ms for the generator we use), and partly because fast scanning of frequencies is not required due to slow fluctuation of the environment (for example sample drift on the order of minutes to hours). To achieve a better temporal granularity and better rejection of slowly fluctuating PL noise, in NV temperature sensing experiment, it is necessary to scan ODMR frequencies on a microsecond time scale. An arbitrary waveform generator (AWG) with high sampling rate can generate MW frequencies in GHz range and be directly used for this purpose. Costs of such AWGs are often high and instead a combination of mixers and direct digital synthesizer (DDS) can be used with a conventional function generator to scan a few hundred MHz.

For this experiment, another implementation is to use the typical frequency modulation function of the more accessible conventional microwave source. The MW source modulates the output MW frequency based on an input analog voltage and maps the analog voltage range between -1 V and +1 V to MW frequencies 2.84 GHz and 2.90 GHz. An analog voltage is generated by the DAQ device and supplied to the MW source. The analog voltage is incremented (and returned to the minimum value after the last increment) every 10 µs, as shown in the 'MW FM' channel in Fig. 4.14. In the figure, an ODMR with 10 equally spaced points are shown (thus the ten steps in MW FM), but the number of frequency points depend on the experiments. The ODMR frequency sweep is synchronised with photon counting (Fig. 4.14 CTR1 and CTR2 shows the trigger for DAQ to read APD1 and ADP2 counters) so that a full ODMR spectrum can be gathered in a few ms. The 10 µs global hardware clock (Fig. 4.14 CLK) is supplied by a PulseBlasterESRPro pulse generator. Figure 4.13 X Pos and YPos channels show that the voltages sent to the XY galvo mirrors. These voltages set the XY galvo mirrors into oscillatory motion (at 50 Hz) and are synchronised with photon collection to enable simultaneous orbital tracking.

# 4.2.4 Data acquisition and post-processing

The hardware-timed ODMR (HT-ODMR) scans are acquired for 160 ms within a 200 ms duty cycle. The APD counters are gated such that they do not collect APD counts during the 40 ms off time (Fig. 4.13 APD CTR Gate). For a 200 point sampling strategy, a full ODMR scan is completed in 2 ms. 80 full scans of ODMR data can thus be taken in 160 ms.

During the 40 ms off time, the heater is switched on using a solid-state switch (Fig. 4.13 Heater) for 30 ms with 5 ms padding before and after. The solid-state switch has a switching rise and fall time of approximately 0.5 ms, much less than the padding. This avoids any magnetic field interfering with ODMR due the current supplied to the on-chip heater.

The number of photon counts acquired from both APD2 and APD3 (shown in Fig. 4.10) within 160 ms is readout  $(2 \times 200 \times 80$  numbers, for two APDs, with 200 frequency points taken sequentially, and then 80 full repeated scans), converted to counts per second (by dividing by 10 µs), summed to give a total counts from both APDs, and stored as  $80 \times 200$  numbers. Subsequently, each 80 rows of data (i.e. all data gathered within 160 ms time frame) is averaged (giving 200 numbers in counts per second) and stored as intermediate results for post-processing. Subsequent data processing does not use the finely timed raw data due to the long processing time. For a typical emitter giving 1 Mcps emission, in 10 µs readout time, each APD reads about 5 photon counts.



Figure 4.14: Timing illustration of Hardware-Timed ODMR and pulsed on-chip heating. A synchronisation 100 kHz clock (navy square wave, bottom box, labelled CLK) is supplied to the DAQ to trigger each photon count readout (pale purple bars, bottom box, labelled CTR 1&2). DAQ also switches voltage supplied to the MW source, modulating frequency of the MW source (magenta trace, MW FM). On a longer timescale (top of figure), DAQ uses this clock as a timebase to generate slower sinusoidal voltages with 90° phase difference to steer the galvo mirrors for orbital tracking (see Chapter 5) which is shown as the pale blue and pale red traces labelled X Pos and Y Pos. DAQ also generate pulses to switch the solid-state switch (maroon trace), which controls the current supplied to the on-chip heater. This configuration allows the ODMR data and tracking data to be simultaneously gathered. Note that the relative delays are only checked on an oscilloscope. The rise time (~ 20 ns) and trigger delay (~ 20 ns) of the DAQ device are small compared to the pulse duration (> 10  $\mu$ s) and the relative delays do not require dedicated calibration procedure.

# 4.3 Characterisation of thermometer sensitivity

Based on the aforementioned sample preparation and experimental setup, the performance of NV thermometer is characterised in a dry and well-controlled setting. The experimental sensitivity is characterised by stepping through temperatures of the substrate in 15 min time intervals and approximately 2 °C steps using the on-chip heater. This provides a fast and accurate method for calibration of nanodiamonds. Figure 4.15 **a**) shows the temperature stepping measured by the on-chip RTD as well as temperature extracted from a two-parameter fit of the ODMR (Fig. 4.15 **b**)). In the fitting procedure, all ODMR data gathered are averaged to give Fig. 4.15 **b**) (red dots). This provides a global estimate of the ODMR parameters other than the off-resonant PL and the central frequency.

The raw data gathered (with  $10 \,\mu s$  frequency switching) is too noisy to enable a fit with Eq. 4.2. Thus the intermediate data (taken within 160 ms) is first averaged and stored as chunks. We chose to average 320 ms of data to give an average per chunk and this choice is the minimum amount of averaging needed to enable a converged non-linear fit using the python curve fitting method curve\_fit. The data chunks can be further averaged and fitted (method 1, shown in red in the illustration below), or fitted first and then averaged (method 2, shown in blue in the illustration below), to reduce noise. These two procedures have different sensitivity implications.

$$\begin{array}{ccc} PL & \stackrel{fit}{\longrightarrow} & T_i \\ average \, (method \, 1) \downarrow & average \, (method \, 2) \downarrow \\ PL & \stackrel{fit}{\longrightarrow} & T \end{array}$$

Using method 2, the NV frequency shift is extracted and compared with the on-chip RTD data. They are then fitted with Eq. 4.1 (Fig. 4.15 c)) to extract  $\kappa$  and convert frequency shift to temperature.

Figure 4.16 a) displays the sensitivity dependence on averaging time using method 1. The sensitivity is proportional to  $1/\sqrt{\tau}$  for the first 20 s indicating the shot-noise limited range. PL is sensitive to noises such as laser fluctuation and emitter drift and thus sets the limit to this range. Method 2 takes advantage of the fast MW frequency modulation to remove influence due to PL fluctuations and the corresponding sensitivity analysis is shown in Fig. 4.16 b). This method of averaging can be characterised with the Allan deviation analysis discussed in the following section. The initial gradient of the Allan deviation curve is fitted with

$$\sigma = A\sqrt{t},$$



Figure 4.15: Temperature cycling with on-chip heater. **a**) shows the temperature readout from NV (pale red) and from the on-chip RTD (blue). A double Lorentzian fit is generated for PL data gathered over every 400 ms (320 ms actual data time, and 80 ms heating time) and each data point is an average over 51.2 s worth of such fits (128 fits) with errorbars given by the standard error of these fits. The substrate temperature is actively stepped using the on-chip heater. **b**) is the averaged ODMR trace over the entire dataset gathered (with potential temperature induced broadening on the peaks of 1.4 MHz for temperature steps spanning 20 °C), showing a good fit with a double Lorentzian model. **c**) shows the linear relationship followed by the shift in ODMR central frequency and the temperature measured by the on-chip RTD, which gives a gradient of  $\kappa$ =-60.7(4) kHz K<sup>-1</sup>. **d**) shows the distribution of the difference (residuals) between the temperatures reported by the NV and the on-chip RTD. The histogram is fitted to a Gaussian distribution (blue), giving a spread of 0.6 K.



Figure 4.16: Allan deviation analysis and accuracy analysis. **a**) dependence of standard deviation of NV temperature from true value on the number of ODMR scans averaged over. **b**) Allan deviation of temperature versus number of individual temperature measurement. The blue line indicates the theoretical sensitivity as predicted by the Cramér-Rao bound. **c**) accuracy of NV temperature measurements. As averaging time increases, the timing resolution decreases and leads to worse accuracy.

where  $\sigma$  is the Allan deviation (in temperature units), A is a fitting parameter and t is the accumulation time, shown in Fig. 4.16 b). This is done only in the region where  $\sigma \propto \sqrt{t}$  and A is the extracted temperature sensitivity. The fit is shown in Fig. 4.16 b).

## 4.3.1 Allan deviation

In the measurement of a fixed quantity, as common in physical measurements, the (statistical) error of measurements is quantified by the (unbiased estimation of the population) standard deviation of a series of identical measurements, assuming an independent and identically distributed (i.i.d) Gaussian distribution with mean  $\bar{T}$  and variance  $\sigma_N^2$ , denoted as  $T_i \sim \mathcal{N}(\bar{T}, \sigma_N^2)$ , each time,

$$\sigma_N^2 = \frac{1}{N-1} \sum_{i=1}^N (T_i - \bar{T})^2.$$

If each measurement is truly i.i.d, then the limit  $\lim_{N\to\infty} \sigma_N^2$  converges to the true and unique variance of the underlying Gaussian distribution. However, in the case where the underlying Gaussian changes with time due to noise in the system, and as measurements are often taken at different times, this limit may not converge to a unique value. In fact the variance depends on the time separation between two measurements, and the Allan deviation is a more informative measure. For a series of measurements sampled at  $\tau_0$  time intervals apart, the Allan variance is defined to be [7, 145, 244]

$$\sigma^{2}(\tau_{0}) = \frac{1}{2(N-1)} \sum_{i=1}^{N-1} (T_{i+1} - T_{i})^{2}.$$

The prefactor is defined such that if  $T_i \sim \mathcal{N}(\bar{T}, \sigma^2)$ ,  $\forall i$ , then  $T_{i+1} - T_i \sim \mathcal{N}(0, 2\sigma^2)$  and thus  $\sigma^2(\tau_0) = 2\sigma_N^2/2 = \sigma_N^2$ . Thus rather than using the variance about a mean value, Allan variance characterises the difference between two measurements separated by a fixed duration.

Neighbouring data points could be averaged to compute the variance at larger separations, such that,

$$\sigma^{2}(\tau) \equiv \sigma^{2}(n\tau_{0}) = \frac{1}{2(N-1)} \sum_{i=1}^{N-1} \left( \mathscr{T}_{i+1}^{(n)} - \mathscr{T}_{i}^{(n)} \right)^{2},$$

where  $\mathscr{T}_i^{(n)} = \frac{1}{n} \sum_{j=i}^{i+n-1} T_j$  is the mean of the neighbouring *n* points. As each  $\sigma^2(\tau)$  samples a different frequency of the noise spectrum, the Allan variance reflects the power spectrum of the underlying noise process. In particular, photon shot noise is a Gaussian noise with uniform spectrum, and the noise  $\sigma_{shot}^2 \propto 1/\tau$ . As such we expect  $\sigma^2(\tau) \propto 1/\tau$  to be a shot noise limited process, which is a fundamental limit of the measurement protocol we use.

Figure 4.16 b) shows the Allan deviation analysis of the same set of data averaged over a section of data where the temperature does not change. The corresponding measurement is shot-noise limited within the range of accumulation time presented. This indicates that Method 2 is the preferred method as it is more resilient to fluctuations in the measurement, as indicated by the longer shot-noise limited range.

## 4.3.2 Experimental sensitivity for ODMR and the Cramér-Rao bound

Having established that the measurement is shot noise limited, it is important to know if this is the best performance possible. In conventional ODMR, frequencies are often swept uniformly to map out the ODMR signal. However it is intuitively clear that data points close to the ODMR resonance carry more information than far-detuned data points. As such, by carefully choosing the more "informative" points, less data (thus integration time) is needed to reach the same sensitivity.

In biological applications where temporal resolution is important, various techniques have been employed, for example the 4-point method [49, 142], its 3- and 6-point variants [85] and a lock-in detection technique [199]. These techniques all rely on the intuition that a shift in central frequency gives the most shift in PL where the gradient  $dPL/df_0$  is largest.



Figure 4.17: Experimentally determined values of  $\kappa$  and sensitivity as the experimental design is changed. **a**) the  $\kappa$  value is dependent on which frequency points are sampled. The number indicates the number of uniformed spaced frequencies between 2.84 GHz to 2.90 GHz used during the ODMR measurement.  $\kappa$  does not depend on the accumulation time per ODMR scan. **b**) shows the experimentally determined sensitivity from Allan deviation analysis, as the number of uniformly spaced MW frequencies are sampled. Certain choices, for example the 4- and 6-point design, yield better sensitivities than the 40-point design. The theoretically best design (optical two-point design), however, does not perform significantly better then the 200point design (~2x), while the latter has the added benefit of capturing the linewidth variations.

#### 4.3.2.1 Optimal experimental design

A class of methods exist to quantify this information and to optimise the experimental data point selection. Each set of data points selected is a 'design', as used in a experimental design theories. A rigorous derivation supported by mathematical reasoning involves the use of Fisher Information formalism and Bayesian optimal experimental design which we employ in our experiment [50]. The detailed derivation is presented in Appendix A. Briefly, given a set of MW frequencies (a 'design') used for measuring the ODMR central frequency (the 'parameter') and the lineshape of the ODMR (the 'model'), the Cramér-Rao bound gives the minimum variance expected from a non-biased estimator of the parameters ( $PL_0$  and  $f_0$  for ODMR), and yield a sensitivity estimate.

The CR bound is plotted in both Fig. 4.16 **a**) and **b**) where the experimental sensitivity approaches the Cramér-Rao bound. More experimental sensitivities are extracted from other experimental designs (Fig. 4.17 **b**)). All experimentally measured sensitivities closely follow the CR bound, indicating that the noise profile in the system is close to the shot-noise assumption used to derive the CR bound.



Figure 4.18: Experimental and theoretical sensitivities for a total of 5 nanodiamond samples with various experimental designs. Data from the same nanodiamond share the same colour code. The best sensitivity achieved is  $1.5 \text{ K}/\sqrt{\text{Hz}}$ . The best theoretical performance of two-point designs are also indicated as crosses on the plot. The experimental sensitivity agrees very well with the theoretical sensitivity (dashed line showing when both perfectly agree).

The minimum number of sampling frequencies (the design) should be equal to the number of free parameters in the fitting function (see discussion in Sec A.1.2.4). In the ODMR case since the PL and the central frequency change during the measurement, at least two sampling points are needed. We could pick two points from a set of candidate points and compute the best choice of points giving the smallest CR bound. Such an optimal two-point design is also plotted in Fig. 4.17 b) and this bound is marginally better than the multi-point design. Although this seems a better choice, there are practical difficulties with a two-point optimal design. To use such a design, the lineshape needs to be determined *a priori* by measuring the full ODMR curve in order to choose these optimal designs. Furthermore, the design assumes that only the central frequency and the PL change and other lineshape parameters such as the contrast remain unchanged. These constant quantities may not stay unchanged for rotationally diffusing nanodiamonds. These changes however will not be detected with a two-point sampling strategy, thus causing apparent change in temperature measured by the NV. As such we reach a compromise between sensitivity and redundancy and work with 80-120 points.

In Fig. 4.17, the  $\kappa$  parameter is characterised for one nanodiamond. The values centred around 70 kHz K<sup>-1</sup> and is lower than the values usually used in literature (77 kHz K<sup>-1</sup> for bulk diamond and 74 kHz K<sup>-1</sup> for single NV in nanodiamonds). This difference is within the linearity error of the on-chip RTD. However the two sets of data corresponding to 4 and 6 equally spaced sampling points give smaller  $\kappa$  values. It is not clear what could have led to this difference. One hypothesis discussed in [86] is that the base and the tip of the Lorentzian shift differently and thus changing the design affects the  $\kappa$  value.

	This work	This work Fujiwara <i>et al</i>		
		[85, 86]		
MW Switching	10 µs	100 µs	100 µs	
Sampling	80~120 points	3, 4, 6 points	4 points	
Tracking	Simul. orbital	Grid search	Grid search	
	tracking			
Extracting	Nonlinear	Linear	Linear	
Central	least-square	extrapolation	extrapolation	
Frequency	Fitting			
Post Processing	Averaging	Moving	None	
		average/Kalman		
		filter (proposed)		
Calibration	On-chip RTD and	Objective collar	IR Laser local	
	heater	heater	heating	
MW Delivery	CPW on-chip	Linear wire	Omega loop	
		antenna	resonator	

Table 4.4: Comparison of sensitivity improvement techniques.

Finally the temperature stepping experiments are repeated over 5 nanodiamonds, with multiple sets of ODMR frequency points (i.e. different 'designs') for some of them. The compilation of the experimentally determined sensitivities are compiled against the corresponding theoretical sensitivity in Fig. 4.18. This demonstrates that the CR bound is a reliable tool for predicting the best sensitivity given a design. The best experimental sensitivity achieved is  $1.5 \text{ K}/\sqrt{\text{Hz}}$ .

#### **4.3.3** Comparison with other work

The approach used in this work is compared with the two existing intracellular temperature sensing work in Table. 4.4. In this work, instead of using the 3-, 4-, 6-point methods, 80-120 points are used to map out a full ODMR scan. This does not significantly reduce the sensitivity (Fig. 4.18), but does provide redundancy for confirming if the lineshape has changed over time, and allows switching to a full seven parameter fit with Eq. 4.2) during post-processing. Owing to this large number of data points, we employed the fastest frequency switching available to our hardware to be able to complete one ODMR scan within a few milliseconds. In addition, a nonlinear least square method is used to extract the central frequency, as it is known to be non-biased. Its iterative nature also allows data to be better fitted.

Furthermore, we specially designed fast on-chip temperature control, which is useful to measure temperatures within a very short distance from the nanodiamond. The small size of the on-chip RTD sensor also enables measurement of small amount of heat produced during,

for example, mixing.

Finally, as each diamond has a different frequency shift with temperature, calibrating each diamond is important. Choi *et al* [49] uses microscale IR laser heating modulated at 60 s period, while our approach uses an on-chip heater modulated on the scale of a few minutes. The IR method is preferred as it provides fast modulation. Nevertheless as will be discussed in the following chapter, our method allows investigation of the cell's response to external temperature stimuli.

# 4.4 Conclusion

In this chapter, a systematic calibration procedure is described to calibrate the sensitivity of the NV thermometer. The parameter  $\kappa \equiv dD/dT$  is shown to change between different diamonds, and even between different choices of MW frequencies. This highlights the importance of calibration per nanodiamond.

The concepts of "optimal experimental design", "Fisher Information" and "Cramér-Rao bound" are introduced as new tools to give theoretical prediction of the sensitivity for thermometry. Experimental results agree very well with the sensitivity predicted by the CRB. The CRB is thus a powerful tool to determine whether a set of ODMR measurement is shot-noise limited, which is the ultimate noise floor.

Finally, the experimental setup is characterised and its resolution is comparable to a commercial confocal system, with an optical PSF of 250 nm. This sets the optical resolution of the imaging system.

In the following chapter, the techniques presented in this chapter will be incorporated into the intracellular measurements and they will address issues such as translational diffusion in a intracellular measurement. These particular adaptation would turn out to be useful intracellular measurements.
# **Chapter 5**

# Intracellular nanoscale thermometry

This chapter discusses how NV measurements are made compatible with general cell biology protocols. Nanodiamonds undergo stochastic translational diffusion and a tracking protocol is developed for simultaneous ODMR and tracking. The nanoparticle's trajectory reflects the local rheological properties of the cell.

This chapter starts with discussing various nanothermometry techniques comparable to NV-based nanothermometry, and then moves on to discuss challenges facing a typical NV experiment as discussed in Chapter 4 when applied to an intracellular measurement context. The nanodiamond NV-based nanothermometers are used to study intracellular thermogenesis due to external chemical and physical stimuli. It was found that chemical uncouplers cause a temperature rise in HeLa cells by as much as 8 °C on the timescale of a few minutes, consistent with previous reports. Finally, we present the experimental realisation of single-particle tracking, which not only enables NV-based thermometry measurements in a complex diffusing medium, but also reveals the trajectories and the complex interaction between nanodiamonds and the internal of a cell.

The diamond uptake by cells have been previously verified by Dr Jack Hart who also provides the HeLa samples (discussed in Section 5.2.2) to enable all measurements. J.H. proposed the tracking algorithm and Q.G. implemented simultaneous tracking and ODMR. All experiments and data analysis were done by Q.G., with useful discussion with J.H. and L.S..

# 5.1 Introduction

## **5.1.1** Intracellular thermometry

Intracellular temperature measurement is both a useful tool for understanding intracellular respiratory processes, and also for cancer diagnosis [62]. A few important processes are intimately related to temperature variations. For example, the mitochondria, when at their full respiratory capacity, have been shown to elevate the local temperature by 10 °C [51]. Numerous reports have reported temperature rise in C. elegans [86] and cancer cells [61] over  $\sim 10$  mins, or a sharp rise 10 °C in one second [186], upon proton uncoupler treatment. As these findings are at odd with theoretical calculations [146] which predicts  $10^{-5}$  temperature variations in a biological cell. In fact, despite the cell being formed largely of water, its internal thermal conductivity can be as low as one sixth that of water [216], and drastically different from ensemble measurements of whole cells [144], thus displaying extreme spatial inhomogeneity. Other processes, such as neuronal activities accompanied by an influx of Ca<sup>2+</sup> ions, also cause cytosolic temperature changes [268] and could be probed with intracellular thermometry.

Recent experiments using fluorescence correlational spectroscopy (FCS) [178] on yeast have established that cells tune the ratio between glycogen and trehalose (a form of disaccharide) to vary cytosolic viscosity in response to external temperature change. This process, termed viscoadaptation, ensures that rates of diffusion-limited intracellular biochemical reactions are actively controlled, despite the temperature dependence of viscosity of water which is a major constituent of the cytoplasm. Nanodiamond thermometers can simultaneously probe local temperature and viscoelasticity, and are thus a promising candidate for the verification of the viscoadaptation process.

In addition, heat-shock response is a commonly observed and well-established series of biochemical reactions, whereby a cell up-regulates chaperone protein synthesis to reduce protein misfolding, thus increasing the chance of cell survival. Major efforts have been focusing on the transcriptional control aspect of this response on a timescale of tens of minutes to days. Despite this, knowledge on the immediate response of a cell to external temperature modulation is less well-understood, as exemplified by the only recently discovered viscoadaptation process and temperature induced  $Ca^{2+}$  ion influx [238]. With the ability to monitor NV response continuously for several hours, it is possible to study the temporal response of a cell reacting to external temperature stimuli.

Furthermore, understanding cellular thermogenesis also helps in cancer or degenerative disease diagnosis [55]. Artificially induced localised heating can cause C elegans to differentiate into different polarities; the head and tail of the worm is reversed with a reversed

temperature gradient. Photothermogenesis treatment (PTT) and magnetic hyperthermia [112], which are noninvasive methods for locally killing diseased tissues and cells, also requires understanding of nanometer scale heat distribution with intracellular noninvasive thermometers.

These discrepancies between the expected behaviour and the actual measurements (for temperature distribution, thermal conductivity and temperature-dependence of viscosity), as well as the useful new understanding and opportunities associated with temperature distribution and response, drive the interest of finding reliable and versatile intracellular thermometric tools with high spatial and temporal resolution, as well as stable and accurate performance.

To this end, techniques utilising various physical indicators have been developed in the literature (see [185, 267, 276] for reviews on microscale and nanoscale thermometry). Some of these techniques use a top-down approach, where macroscopic thermometers are engineered into smaller and smaller devices. A microscale thermocouple is a good example of such a device. A gold-lead based micro-thermocouple was used to probe the transient heat release due to proton uncoupling in mitochondria-dense abdominal ganglion cells [186], achieving 32 µs temporal resolution and 1 µm × 50 µm spatial resolution and 54 mK temperature resolution. On the other hand, a bottom-up approach is also employed to make small temperature-sensitive fluorescent molecules. Molecular dyes are small molecules that can be designed and synthesised with more versatility.

These techniques can be broadly classified into luminescence techniques which do not require invasive contacts and non-luminescence techniques which require the sensor to in direct mechanical contact with the measurement hardware. The contact techniques have been discussed in the previous chapter.

# 5.1.2 Advantages and limitation of various optical nanothermometry methods

Small molecular labels are spontaneously uptaken via diffusion or endocytosis. However larger particles, such as nanodiamonds or semiconductor quantum dots, are not universally uptaken by cells. Often these particles require long incubation time, or special intracellular delivery methods such as electroporation, liposome assisted delivery, polymer coating (for example via polyethylenimine) or micro-injection. In addition, the same delivery techniques may not work on all cell types and thus each cell type requires a separate uptake study. Specific fluorescent proteins can be expressed via genetic engineering techniques, useful for temperature mapping of a large number of cells.

Organic dye molecules photobleach over the course of the experiment due to optical excitation. For readout methods relying on temperature dependent PL, photobleaching contributes

Suscentible sources of noise	Sensing technique						
Susceptible sources of noise	Intensity	Lifetime	Ratiometric	Colorimetric	ODMR		
Local Concentration	$\checkmark$	×	×	×	×		
Refractive Index	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	×		
Autofluorescence	$\checkmark$	×	√/×	√/×	×		
Laser fluctuation	$\checkmark$	×	×	×	×		

Table 5.1: Relevant sources of noise contributing to each sensing technique. The entries with both a checkmark and a cross are techniques that may or may not be affected depending on if cell autofluorescence affects the spectral range they probe. A checkmark indicates the technique is prone to the corresponding source of noise.

to temperature-independent signal change (a systematic error). This also makes longtime monitoring of temperature difficult with the bare form of the dye. In addition, the fluorescence from small molecules are not only a function of temperature, but also of refractive index, viscosity, optical excitation power, pH, salt concentration and biochemical molecules such as proteins. Some of these parameters are not known *a priori* or may be hard to control or calibrate *in situ* and therefore introduce systematic error in the temperature measurements. Table 5.1 summarises the issues that need to be addressed for each type of sensor.

Techniques such as lanthanide-based nanoparticles and organic dyes have been developed for decades and their strengths and weaknesses highlight the valuable information accessible using nanothermometry. Moreover several issues with these nanothermometry techniques can be resolved with an NV-based method. The following list is by no means exhaustive, but highlights some of the challenges facing other techniques in the community. In addition to the uncertainties due to the sensor, the variation in behaviour between individual cells is still the biggest source of variations and yet due to the complexity of the cell, it is usually not straightforward to control the cells.

#### 5.1.2.1 Small organic molecules

Molecular thermometers based on aromatic compounds have made great advances in the past century, featuring rhodamine 101 and rhodamine B (RhB), fluorescein isothiocyanates and cyanine dyes. More recent work include endoplasmic reticulum (ER) targeting thermometry, where the ER thermo yellow dye successfully monitored the temporal evolution of ER temperature in response to changes in Ca<sup>2+</sup> concentration in HeLa cells [15]. The same group led by Chang also later developed a mitochondria targeting temperature sensor (Mito thermo yellow, MTY) [16]. Both of these sensors rely on the detection of changes ( $-3.9 \% ^{\circ}C^{-1}$  and  $-2.7 \% ^{\circ}C^{-1}$  decrease in PL for ER thermo yellow and MTY respectively) in fluorescence for

temperature measurement.

Fluorescent polymeric thermometers (FPT) [176] are another class of thermometers based on three constituent units; a thermosensitive unit, a hydrophilic unit and a fluorescent unit. At high temperature the thermosensitive unit is less hydrated and forms a globular structure around the fluorescent unit. The fluorescence lifetime is changed as a result of this change in local optical environment. The lifetime change can be detected by a time-correlated singlephoton counting (TCSPC) module typically used in a fluorescent lifetime imaging microscopy (FLIM) experiment. Although the fluorescence lifetime is unaffected by cell autofluorescence, it is affected by the local optical environment. A change in refractive index would result in a change in lifetime as described by the Strickler-Berg equation [222],

$$\tau_0 \propto \frac{1}{n^2}.$$

The refractive index, *n*, can vary from 1.365 to 1.60 between the cytoplasm and lysosomes of HeLa cells [99]. Thus, when applied to intracellular temperature mapping, where the lifetime is used to map temperature distribution, FPT may report a false temperature variation due to refractive index variations. The corresponding change in lifetime is 140%. A change of  $5 \,^{\circ}$ C would induce about the same change in lifetime. Thus this type of sensor would require additional modalities to calibrate the refractive index *in situ*.

#### 5.1.2.2 Lanthanide thermometers

Spin-spin dipolar interaction is the main contributor to the ground-state splitting in NV centres. The thermal polarisation of the ground is minimal since the 2.87 GHz splitting is small compared to  $k_BT \sim 6$  THz. However in systems containing heavy-element atoms such as lanthanides, the spin-orbit interaction dominates the ground-state splitting, making it possible to create two energy levels with a splitting comparable to the temperature range desired, and optically addressable. By probing the population in one state, or probing the ratio of populations in two states, at thermal equilibrium, the temperature can be calibrated against fluorescence, or inferred via the Boltzmann's distribution,

$$\ln\left(p_1/p_2\right) = -\Delta E/k_B T_2$$

where  $\Delta E = (E_1 - E_2)$  can be precisely known.

Lanthanide-based thermometers are such thermometers, with Europium (III) thenoyltrifluoroacetonate trihydrate (Eu-TTA) being a prominent example. Eu-TTA is a single centre luminescent thermometer and its fluorescence intensity decreases with temperature. By combining the Eu-TTA with a neighbouring fluorophore such as Rhodamine 101 [227], the intensity ratio between the two fluorophores can be used as a more robust thermometer against changes in local clustering, giving a ratiometric nanothermometer.

There are also two-centre lanthanide thermometers which monitor two transitions at different colours, giving a colorimetric indication of temperature. Di *et al.* [61] used the two emissions at 525 nm and 545 nm of europium and ytterium doped upconversion nanoparticles to monitor thermogenesis in HeLa cells.

Further techniques such as lifetime based and bandwidth based measurements are also possible and Brites *et al* [36] provides a very extensive review on this subject with a history dating back a few decades.

Lanthanide thermometers directly invoke the Boltzmann's distribution and thus are an example of primary thermometers. On the other hand, NV centres rely on the measurement of lattice expansion due temperature rise and are thus an example of secondary thermometers. Such thermometers need to be calibrated against a primary thermometer, which defines temperature.

### 5.1.2.3 Quantum dots

Cadmium telluride (CdTe), Cadmium selenide (CdSe) quantum dots are commercially available biomarkers and have been used to demonstrate the spatial inhomogeneity of thermogenesis in neuronal cells [228], chemically (Ca<sup>2+</sup> shock) and physically (cold shock) induced thermogenesis in NIH/3T3 cells [268]. The quantum confinement of electrons in a quantum dot allows them to emit in the visible range. The lattice expands with a temperature increase, reducing the extent of confinement, leading to a red-shifted emission. Yang *et al* [268] detected a shift of 0.105 nm<sup>o</sup>C<sup>-1</sup>.

#### 5.1.2.4 Nanodiamonds

Nanodiamonds stand uniquely among techniques discussed above as temperature is not directly read out from its fluorescence intensity alone, but via measuring a MW frequency dependent PL change, optically detected magnetic resonance (ODMR). The way temperature is measured using ODMR has been discussed in Chapter 4. NV ODMR-based nanothermometry is less prone to systematic errors due to local changes of viscosity, refractive index and autofluorescence. Being hosted by the diamond lattice, NV centres are also believed to be less prone to local pH and ionic strength [205]. As shown subsequently, they can also be multi-modal sensors, providing information about the local viscoelasticity through longtime single-particle tracking. With further development, the sensor can also potentially simultaneously measure the local magnetic field, enabling detection of, for example, free radicals.

However the inhomogeneity of the nanodiamond surface chemical environments and local strain environments [211] means that each NV has a different temperature dependence (see discussion in Chapter 4 and in [85]), and therefore require individual calibration.

# 5.2 Experimental

# 5.2.1 Optimising the experimental protocol

NV measurements are typically performed in a controlled dry lab environment, where laser exposure, microwave irradiation, sterility and measurement time are not limited (column 2 of Table 5.2). On the other hand, biological experiments are more challenging as these factors mentioned above need to be carefully controlled to maintain cell proliferation (see column 3 of Table 5.2). In this work we use a measurement protocol that is compatible with both ODMR and the bio-compatible experimental settings. Our approach is listed in column 4 of Table 5.2.

The experimental protocol adopted here combines techniques from traditional quantum NV experiment, microfluidics and biological experiments. In particular, we integrated a polydimethylsiloxane (PDMS) microfluidic channels into the typical CPW-on-glass substrate used for NV measurement (see Chapter 4). The chip is the exact one used in Chapter 5 except the addition of the PDMS channel and here we highlight a few features useful for biological experiments. To avoid issues with making an extensive number of fragile wirebonds, a compact and easy-to-attach PCB is used which enables fast substrate preparation. The resulting substrate can be sterilised using an autoclave and is thus bio-compatible. This substrate allows a biological sample to be ready for measurements within 5 minutes of the cells being removed from the incubator.

## 5.2.2 Cell preparation protocol

In this experiment, we use the cervical cancer cell line HeLa as a model organism for studying intracellular temperature changes. A table of chemical reagents used in this chapter is tabulated in Table 5.3. HeLa cells (ATCC CCL-2) are normally incubated in a cell incubator at 37 °C with 5% CO<sub>2</sub> and 95% air. HeLa cells are 'adherent' cells, i.e. they settle and attach to the bottom of the flask within hours. One day before an intracellular experiment, the cell-containing flask are treated with 10% trypsin for 5 min to detach the cells and diluted to a final number concentration of 560 kcells/mL with cell culture medium. The diluted cell suspension fills the PDMS channel attached to the glass substrate with the CPW already deposited, which

Experimental	Quantum optics	Biological	Our experiment
condition	experiment	experiment	
Temperature and	Room temperature	Elevated	Well controlled,
humidity	and dry samples	temperature 37 °C	thermally
		and wet sample	insulated
			chamber. Highly
			enclosed liquid
			PDMS channel
Laser and MW	Loose limits on	Stringent	Efficient MW
power	laser and	exposure limit to	delivery, limited
	microwave power	light and heat	laser power
Lab	Non-sterile	Sterile surfaces	Autoclavable
environments			substrate
Sample lifetime	One sample/few	Live samples to be	Compact device
	samples over	disposed daily	which allows fast
	months to years		docking and fast
			removal.
Auxiliary	MW delivery	Medium flow	Compact PCB and
structures	circuit required		PDMS system
Mechanical	ND not moving	Sample moving	Automated
stability of		due to Brownian	tracking
sample		motion	

Table 5.2: List of different experimental characteristics required for NV measurements in cells.

has been autoclaved and sealed in a sterile pouch. The substrate and channel assembly is kept in the cell incubator for a day (approximately 18 hours) for cells to reattach and grow.

The cell culture medium typically used is the Dulbecco's modified eagle medium (DMEM). Every 500 mL is mixed with 10% fetal bovine serum (FBS, 50 mL), Penicillin-streptomycin antibiotics (5 mL) and L-glutamine (5 mL). The DMEM as received has a pH indicating phenol red dye. We use the weak fluorescence from phenol red as a convenient method to distinguish the cell boundary in a NV experiment. The DMEM also contains a sufficient amount of glucose and pyruvate (see comments for DMEM in Table 5.3), both are key precursors for the respiration process.

Three hours before the experiment (15 hours after cell splitting), nanodiamonds resuspended in cell culture medium are injected into the channel and left to incubate with the cells until the beginning of the experiments. Some nanodiamonds are uptaken by cells however some excessive nanodiamonds remain in the suspension, or attached to the external of the cell membrane. These excessive nanodiamonds are washed off with cell culture medium just before the experiment to prevent confusion with intracellular nanodiamonds and the channel is refilled with cell culture medium. The substrate is then immediately brought to the measurement setup and mounted for measurement. Depending on intracellular nanodiamond concentration requirements, the nanodiamond suspension is usually adjusted to  $20 \,\mu g \,m L^{-1}$  to yield 1 to 2 nanodiamonds on average per cell.

The fixing procedure mentioned in Section 5.3.1 is a well-known process where paraformaldehyde (PFA) is used to crosslink the proteins in a cell. The typical fixing procedure in literature involves replacing the cell culture medium with 4% PFA in phosphate-buffered saline (PBS) for 5 min to 15 min. The PFA is subsequently washed off with PBS and kept in a mounting medium for use in microscopy. In our experiment however, the aim of fixing is to prevent biochemical reactions or intracellular transport influencing nanodiamonds measurements (for example local temperature variation introduced by the cell, or rotation of the nanodiamond). This provides a baseline measurement from a stationary diamond. Thus for the *in situ* measurements, the PFA is left in the microfluidic channel without being replaced by PBS as in the usual procedure. This reduces any mechanical perturbation to the system. Prolonged PFA exposure causes surface proteins to aggregate , however in our measurements we do not find any strong influence on the NV measurements.

# 5.2.3 Nanodiamonds

The nanodiamonds used in this chapter have been listed in Table 5.4. The nanodiamonds are used as received without any further surface modification. The two types of nanodiamonds are supplied by Adamas Nanotechnologies and FND Biotech and contain a large number of NV centres. The number of NV centres is around 100 for the FND diamond and 300 for the Adamas diamond. Experimentally it was found that nanodiamonds within the same suspension are of significantly different brightness (0.5 Mcps to 10 Mcps at the same laser power, for diffraction limited spots). We attribute this to the different extent of nanodiamond aggregation and intrinsic size distribution of unaggregated diamonds). Diamonds in the same suspension can also be of very different ODMR contrast (3%-14%) at the same measurement conditions and this is can be due to variations in NV orientation relative to the MW polarisation, and different extent of strain-induced inhomogenous broadening. These variations between diamonds in the same suspension are larger than the variations between suspensions from the two different suppliers. On average it is easier to find brighter Adamas nanodiamonds and this is possibly because of their larger nominal size. Both types of diamonds have been characterised and they do not differ significantly considering the variation between nanodiamonds in the same suspension. As a result the FND nanodiamonds are used in all work presented in this chapter. Experimentally the nanodiamonds are selected so that the PL is about 1 Mcps and

Chemical	Usage	Supplier	<b>Product number</b>	Lot/Batch	Comments
				number	
Fetal bovine serum		Sigma	F9665	0001655547	
(FBS)	Cell culture				
Dulbecco's modified	medium	Gibco	21969-035	2375274	4.5 gL <sup>-1</sup> D-Glucose, 0.11 gL <sup>-1</sup> Sodium Pyruvate
eagle medium (DMEM)					
Penicillin-Streptomycin		Sigma	P4333	0000129426	
L-Glutamine		Sigma	G7513	1	
Trypsin	Detach cells	Corning	25-052-CV	09819014	0.05% Trypsin, 0.53 mM EDTA
Paraformaldehyde	Fixation	Alfa Aesar	J61899	P25F500	
(PFA) in PBS					
FCCP	Proton	Sigma	SML2959	I	Received dissolved in DMSO and later diluted with
BAM15	uncoupiers	Sigma	SML1760	I	Powder then dissolved in DMSO
DMSO		Sigma	D2650	RNBK1495	Used in dissolving BAM15 and FCCP
		1. 5 7. Dagar	to the block		

Table 5.3:
Reagents
used fc
or bioch
nemical
reactions.

Diamond name	Type of diamond	Nominal size	Type of NV	Surface termina- tion	Solvent
Adamas	HPHT,	100 nm	Ensemble	Carboxylic	Cell
	3 ppm NV				culture
					medium
FND	HPHT,	50 nm	Ensemble	Carboxylic	Cell
	electron				culture
	irradiated				medium

Table 5.4: List of diamond used in this chapter. Nanodiamonds containing ensemble NV centres from Adamas Nanotechnologies and FND Biotech have been used. They are resuspended cell culture medium before introduced into the cell culture. No significant differences in uptake and ODMR property were found between these two types of nanodiamonds except that the Adamas nanodiamonds are brighter in general.

contrast within 8-14%.

# **5.3** Temperature measurements in HeLa cells

## **5.3.1** External temperature modulation on live and fixed cells

We test how external temperature modulation affects temperature measured and benchmark the sensor's performance.

In this experiment, the temperature of the substrate is stepped using the on-chip temperature control. Figure 5.1 **a**) and **b**) shows the NV reported temperature for the same diamond in the same cell before and after fixation with paraformaldehyde (PFA). For the fixed cell measurement the CRB predicts a sensitivity of  $2.827 \text{ K}/\sqrt{\text{Hz}}$  while the actual Allan deviation fit results in a shot-noise limited performance up to 100 s with sensitivity  $2.633(2) \text{ K}/\sqrt{\text{Hz}}$ . Since when the CRB is computed, the global fitting parameter is used, which includes the off-resonance PL, which fluctuates much more in cells due to diffusion and thus the small discrepancy (10%) between CRB and the actual sensitivity could be due to PL fluctuation giving the wrong estimate of input parameters to CRB. The increase in PL fluctuation is observed in all our experiments for fast-diffusing particles and this is attributed to the tracker making large correction steps (compared with the PSF size) due to larger particle diffusion rate. Note that this does not mean the PL fluctuation leads to more noise in the system since the ODMR is acquired at much faster sweep rate than the correction (2 ms compared to 200 ms) and thus the PL fluctuation does not directly feed into increased ODMR noise. Although, the estimation of the mean PL would be change over time as diffusion rate changes and thus the estimated PL



Figure 5.1: Intracellular temperature subjected to external temperature modulation. **a**) in a live cell. The blue trace is the temperature readout from the on-chip RTD and the red dots are the NV reported temperature reading. **b**) in the same cell with the same nanodiamond after the cell is fixed with PFA. The dark blue and the dark red are the RTD and NV temperature readouts.

parameter based on averaging the entire ODMR dataset is not a good representation for PL at each time. In fact, despite the cellular environment, the measurement is still photon shot-noise limited.

The temperature modulation experiment is noisier for live cells. We expect this to be due to the fast stochastic diffusion leading to fast nanodiamond tumbling.

# 5.3.2 BAM15 and FCCP both cause intracellular temperature to rise

To study the effect of heat generation due to external stimuli, we used uncouplers to enhance heat generation. Specifically BAM15 and FCCP are used. BAM15 (5-N,6-N-bis(2-fluorophenyl)-[1,2,5]oxadiazolo[3,4-b]pyrazine- 5,6-diamine) is a proton uncoupler which causes the established proton gradient across the inner membrane of mitochondria to be dissipated. FCCP (carbonyl cyanide- 4-(trifluoromethoxy)phenylhydrazon) has a similar function as BAM15 and is also a commonly used proton uncoupler. In comparison to FCCP, BAM15 is claimed to not disturb the proton gradient across the plasma membrane [186], critical for maintaining cell viability.

Proton uncoupling is a naturally occurring process in brown adipose tissue (BAT), which is present in human infants and in a small amount in adults. They contain membrane-bound uncoupling proteins UCP1 on the inner mitochondrial membrane which can facilitate proton leakage across the membrane. The UCP1 proteins are up-regulated in response to the need for



Figure 5.2: Intracellular temperature measurement with BAM treatment. a) Nanodiamond diffusion trajectory colour-coded with temperature reported by the NV centre. b) Trajectory is labelled in the confocal image of the cell, where the round dot indicates the beginning of the trajectory. A nanodiamond is chosen above the glass coverslip to ensure it is within the cytoplasm using a section scan in the XZ plane. c) shows an XZ confocal scan. The confocal image is taken with an inverted microscope and the objective is at the bottom of the glass. The dotted white lines in both **b**) and **c**) indicate the boundary of the cell, identified via the difference in autofluorescence between the cytoplasm and the cell culture medium. Scale bars are  $5 \,\mu\text{m}$ . d) The on-chip RTD registers a sharp change in temperature the moment BAM15 is added, which recovers in less than 12 s. The change is very small compared to the NV sensitivity and is thus not registered in panel e). Note that the temperature scale on d) is very small (~0.1 °C). The cell culture environment has a steady temperature within the measurement time frame, as measured by the on chip RTD d). e) The nanodiamond thermometer reports a steady temperature until a rise is observed approximately 3 min after the addition of BAM15, and the temperature rise lasts approximately 10 min. f) Throughout the measurement duration the nanodiamond continues to undergo local Brownian motion, thus leading to fluctuation in the total PL collected. The change over time is due to the nanodiamond particle stalling over the measurement period, which is discussed in Section 5.5.3.3.

thermogenesis. Although all work agree upon the heat generation due to proton uncoupling, it is not universally accepted that the dissipation of electrochemical potential [186] is the only source of heat. In response to the proton leakage, the respiratory chain favours forward reactions which are exothermic overall. Especially in nutrient rich medium and the enhanced respiration rate may also lead to increased thermogenesis [51]. This is further complicated by the differences in medium composition and oxygen availability between experiments. Despite the unclear origin of this heat production, proton uncouplers and calcium influx have been the two popular artificially induced biochemical thermogenesis processes. In this experiment we use FCCP and BAM15 and monitor the temporal evolution of NV reported intracellular temperature.

In this experiment, a cell of interest is first identified with wide-field illumination (widefield illumination is switched off after the identification) and then a nanodiamond in this cell is chosen by confocal imaging at a height approximately 1  $\mu$ m to 2  $\mu$ m away from the interface between the glass coverslip and cell culture medium. This interface can be easily identified by a XZ section scan which shows the interface as a bright edge due to sedimentation of nanodiamonds originally suspending in the cell-culture medium (Fig. 5.2 c)). Small area (4 µm square) time-lapse xy confocal images are then taken to confirm these NDs are slowly diffusing in the cells. Nanodiamonds typically diffuse much faster in the cell-culture medium, as seen in the motion-blurring in Fig. 5.2 b). This avoids the ambiguity of if the nanodiamond is internal or external to the cell. A more precise localisation of the diamond relative to the cell can be made with XYZ spatial scanning. Previously through this method we identified nanodiamonds that appears internalised in an XY scan (as it appears within the cell boundary identified with a membrane dye) but in fact is situated at a higher plane, adhering to the membrane of the cell (data not presented). In the ODMR experiment however the XY diffusion and XZ identification of cell boundary are used, to avoid prolonged laser exposure on the cell in a 3D scan. ODMR is started at this point. The sequence of steps after the identification of the cell on a wide-field illumination and before ODMR are performed within 5 min to avoid extraneous laser-induced phototoxicity to the cell.

Following the identification of a suitable diamond, an ODMR experiment is started to measure local temperature fluctuations. Subsequently a small amount of chemical stimuli (BAM15 or FCCP) is added to induce a temperature change in the cytoplasm. As the chemical stimuli are not held at exactly the same temperature as the glass substrate, their addition leads to a sharp temperature change which quickly recovers within a few seconds (Fig. 5.2 d)) as discussed in Sec. 4.2.1.2. It is difficult to distinguish the effect between heat generation due to chemical mixing from temperature differences between the substrate and the chemical [186]. The wide-field illumination and MW used for ODMR have both been shown to cause



Figure 5.3: Intracellular temperature measurement with FCCP treatment. **a**) The nanodiamond identified is localised in the cell as is also observed in the enlarged XY plot of the trajectory shown in **b**). The dotted white line indicates the boundary of the cell, identified via the difference in autofluorescence between the cytoplasm and the cell culture medium. The scale bar in **a**) has a length of  $5 \,\mu$ m. **c**) The on-chip RTD registers a sharp change in temperature the moment FCCP is added. However this change is very small compared with the sensitivity of the NV sensor and is not registered. **d**) The nanodiamond thermometer reports a steady temperature until a rise is observed immediately after the addition of FCCP, and the temperature rise lasts till the end of the experiment.

temperature rise (Section 4.2.1.2). Thus the wide-field illumination remained off and ODMR are not interrupted during a measurement, to avoid introducing changes in the sources of heat. The nanodiamond is monitored continuously and the temperature is extracted from the data post-processing using protocols discussed in Section 4.3.  $\kappa = 74 \text{ kHz}/^{\circ}\text{C}$  is used to convert an ODMR resonance frequency shift to a temperature change.

For the BAM15 experiments, BAM15 is first dissolved in DMSO and added through the opening of the PDMS channel using a pipette. Figure 5.2 e) displays the time trace of the temperature reported by the nanodiamond thermometer. Initially a steady temperature is measured with slight fluctuations. Approximately 3 min after the addition of BAM15 ( $10 \mu M$  final concentration), a temperature rise of  $3.5 \,^{\circ}$ C is observed, lasting for approximately  $10 \min$ , with maximum temperature appearing at 2.5 min after the rise. The cell then returns to its initial temperature.

A temperature rise of  $3.5 \,^{\circ}$ C is observed in another similar measurement involving BAM15, but the temperature rise is seen only 15 min after the addition of BAM15, with the maximum appearing at 9.5 min after the initial rise. Unlike in the previous experiment, a BAM15 concentration of  $30 \,\mu$ M is used, three times higher than in the previous experiment.

	Sensor	Chemical	$\Delta T_{max}/^{\circ}\mathrm{C}$	Latency	Duration	Conc.
This work		<b>BAM15</b>	3.5	3 min and	10 min and	10 µМ to
	NV	DAMIJ	5.5	15 min	>15 min	30 µм
		FCCP	6 and 8	20 min	>20 min	300 µм
[86]	NV	FCCP	4	18.4 min	49.4 min	30 µм
[61]	UCNP	FCCP	3	Immediate	30 min	10 µм
[186]	Micro- TC	BAM15	7.5	Immediate	5 s	10 µм
[137]	tsGFP	CCCP	-	Immediate	>7 min	10 µМ

Table 5.5: Comparison of influence of proton uncouplers measured by other thermometers. CCCP (carbonyl cyanide 3-chlorophenylhydrazone) is another type of proton uncoupler. ts-GFP stands for temperature sensitive green fluorescent protein. TC stands for thermocouple

To compare how FCCP affects intracellular heat generation, FCCP is used to repeat the same experiment as discussed above, with a final FCCP concentration of  $30 \,\mu$ M. Across two sets of experiments with FCCP, addition of FCCP induces a longtime rise in temperature (Fig. 5.3 d)). The rise takes place in 20 min with a magnitude of 6 °C and 8 °C respectively. This is in contrast to the BAM15 experiment where transient heat release and recovery are observed. Table 5.5 summarises the results and compares with other experimental work.

The variability in the temperature response highlights the need for more repeats of the same experiment for a statistically significant rise in temperature to be claimed and these experiments are underway.

As also observed by Fujiwara *et al* [84] and in this work, in dry environments for all types of nanodiamonds (not presented, NaBond single NV, Adamas and FND COOH terminated ensemble NV), the central ODMR frequency frequently shows a decrease over a period of a few hours at the laser power used. This only happens to nanodiamonds that have not been previous exposed to laser excitation and might be explained by surface chemical changes. As this also takes place in a dry sample where nanodiamonds have been immobilised, this change is unlikely due to rotational diffusion. Among the 10 nanodiamonds examined (all FND), 6 showed drifts (~0.1 MHz to 2 MHz) over ~10 min to 100 min, and 4 do not show significant drift. On the timescale of a biological experiment, this drift is effectively a linear background drift and in the two BAM15 experiments, this linear drift has been artificially removed by a linear fitting and subtraction. A baseline drift is not observed for the FCCP experiment and thus no correction was applied to these sets of data. There is potentially no correlation between the chemicals added and the baseline drift and this may just be an coincidence. This however highlights the issue of NV temperature measurements in situations where the baseline drift happens on comparable timescales as the real temperature variations.

# 5.4 Intracellular tracking and simultaneous location readout

## 5.4.1 Probing local viscoelasticity via single-particle tracking

The cell is not a homogeneous liquid environment but is instead a mix of macromolecules. These molecules create local molecular crowding and undergo active transport of intracellular materials by the close cooperation between molecular motors and cytoskeleton. Diffusion of various species in cells shed light on these complex local biophysical environments and reveal the underlying mechanism for intracellular transport.

Recent experiments have demonstrated that intracellular viscosity changes in response to temperature [178], cell cycle [114] and cell apoptosis [153]. Actively controlled protein aggregation, such as P-granules, also causes local domains in the cytoplasm to have higher local viscosity [73]. These results highlight that nanoscale viscosity plays an important role in controlling the cell's chemical reaction and reflecting the cell's status.

Among the successful techniques for measuring intracellular viscosity, single-molecule fluorescence correlational spectroscopy (FCS), fluorescence recovery after photobleaching (FRAP) and single-particle/single-molecule tracking (SPT/SMT) have been commonly used. FCS detects the average transit time of fluorescent labels across imaging volume in a confocal, or super-resolution microscopy imaging setup [206, 266]. FCS has been used in determining intracellular viscosity [178] recently in yeast. FRAP detects the recovery time of a photobleached region due to the back diffusion of non-bleached fluorophores. FRAP has in fact been combined with NV techniques to demonstrate measurement of diffusion constant of a supported lipid bilayer [132]. SPT and SMT use fluorescence imaging to track a large number of particles and infer the viscoelastic property of the surrounding based on their trajectories.

These methods rely on probing the passive diffusion and transport of the fluorescent molecules or nanoparticles. Active methods, such as atomic force microscopes [122] and optical tweezers [38] have also been used to study the viscoelastic behaviours in cells, by actively modulating the forces applied to nanoparticles or other macromolecules and observing the corresponding displacements.

Due to the slow morphological change of the cells, fast diffusion and intracellular transport, intracellular nanothermometry with NV centres in nanodiamonds inevitably require singleparticle tracking. Although this imposes significant challenges to thermometry, it also enables the simultaneous readout of the position information of the particle, and thus allows multimodal measurements of both temperature and nanoparticle transport.

In this work, we use the orbital tracking algorithm to simultaneously read out the position

and temperature information of single cells from the fluorescence of NV centres. Unlike the FCS or FRAP methods, which rely on the ensemble diffusion properties of fluorophores, nanodiamond-based single-particle tracking allows the trajectory of a single particle to be extracted. This makes it possible to detect, for example, the dwell and transport mode of molecular motors [153, 251]. It is possible to use techniques such as remote focusing and PSF engineering to achieve 3D tracking. In addition, techniques such as DNA-PAINT (DNAbased point accumulation for imaging in nanoscale topography) allows fluorophores to be replenished and potentially enable infinite imaging lifetime. The approach used in this work builds upon simple confocal microscopy and allows longer trajectories to be followed in three spatial dimensions. Thus the trajectories obtained enabled the observation of the temporal change in diffusion pattern of nanoparticles.

## 5.4.2 Experimental

# 5.4.3 Intracellular tracking

Translational and rotational diffusion of nanodiamonds in both cellular medium and cytosol [90] impose significant issues on typical NV experiments. Translational drift away from the focal spot of the confocal microscope leads to loss of PL. Rotational diffusion causes a reorientation of the NV axis, which changes the NV's coupling to both the incident microwave field and the optical excitation [79, 161]. These movements lead to a change in ODMR resonance frequency and lineshape. In addition, geometrical phase can accumulate due to physical reorientation of the NV axis. This has been demonstrated to lead to shortening of  $T_2^*$  when the reorientation is Brownian [84]. As such it is important, depending on the application, to actively compensate for this diffusion in 6 degrees of freedom.

#### 5.4.3.1 Comparison of existing single-particle tracking methods

Tracking algorithms are implemented in conventional fluorescent live-cell imaging to correct for translational diffusion [110, 207]. A tracking algorithm maintains a stable PL at the cost of reduced measurement time. Therefore, a good algorithm should ideally be fast, in addition to robust and accurate.

Post-acquisition image analysis is the most commonly used technique in single-particle tracking. In fact, it is also the only technique for SPT based on NV centres demonstrated so far [113]. This technique allows tracking of many particles (~100) in one frame and thus generate sufficient statistics very quickly. The timing resolution is typically based on the frame rate of the CCD or CMOS camera being used (~50 Hz or faster), or the scan rate of

a confocal scanner (~10 kHz line frequency for the faster resonant scanning mirrors). An image-based method can also include a large field-of-view, which allows tracking particles with high diffusion rate. However, one drawback is that tracking is only achieved in the lateral dimensions, and fast particles quickly diffuse into and out of the imaging plane. This limits the typical imaging time of one single particle to a few minutes. Techniques such as PSF engineering encodes axial location information into the shape of the lateral PSF and thus allows the axial location of the particles to be determined from 2D images. One such method uses the astigmatism of the imaging system to shape the PSF [130] and is widely used in the super-resolution technique 3D-STORM.

On the other hand, single-particle feedback tracking methods refocus on the emitter with real-time feedback control. This enables tracking in both the lateral and longitudinal directions. The tetrahedral detection method is a promising candidate as it uses four separate collection arms to collect from 4 spatially separate points, which can be used to collectively infer the location of the emitter [103, 151, 152, 254, 255]. Such a design does not require fast mechanical movements, beam-steering or change of focal length with electronic-tunable lens (ETL) and can thus be very fast. Various variants of orbital tracking method [14, 147, 251] are suitably fast, that can readily implemented on the typical confocal set-up without additional hardware and are used in this experiment with modifications.

Alternative methods that rely on nanoscale manipulation strategies, such as optical tweezers [91, 106, 109, 179, 194], anti-brownian electrokinetic (ABEL) traps [80, 133, 134] using nanodiamonds and magnetic tweezers, Atomic Force Microscope (AFM) and acoustic tweezers are also applicable to single particle tracking. However, techniques that do not rely on NV fluorescence lack specificity; subcellular components may be trapped along with nanodiamonds.

#### 5.4.3.2 Double-plane orbital tracking

In this work, the double-plane orbital tracking method is adopted (Fig. 5.4) [131]. In this scheme, two detectors are used, collecting photons from planes above and below the focal spot simultaneously (Fig. 5.4, left panel). The focal plane no longer needs to oscillate like conventional orbital tracking, thus avoiding mechanical coupling between the objective and the glass coverslip. It is worth noting that techniques such as remote focusing can map the sample space into another conjugate space, where the conjugate space can be imaged with an air objective [34]. This allows very fast mechanical oscillation without oil coupling.

While the laser spot executes circular orbits, 16 data points are taken with the following



Figure 5.4: Double-plane orbital tracking. Two photon detectors are used to collect photons from above and below the focal spot. The optical axis is indicated by the vertical red dotted line. The actual particle location is indicated by the yellow dot in left panel. The scanning mirror moves scanning focal spot in circular orbits (blue and read dashed lines). The orbiting planes are perpendicular to the optical axis and are indicated with blue and red shades. Right panel: the PL, as a function of orbiting angle, collected by the top and bottom photon detectors, corresponding to the particle location shown in the left panel. The top and bottom PL are summed to give corrections in xy, and subtracted to give correction in z.

coordinates relative to the previous location of the emitter before update,

$$(x_n, y_n, \pm z_n) = (R_{xy} \cos \theta_n, R_{xy} \sin \theta_n, \pm R_z)$$

where,

$$\theta_n = \frac{n\pi}{4} + \frac{1}{8}\pi, n = 0, 1, \cdots, 6, 7$$

and  $R_{xy} = 50$  nm. The radius  $R_{xy}$  is adjusted by changing the voltage sent to the scanning mirror.  $R_z$  is determined by first defocusing the respective collection arm's collimation lens to reach 70% of the peak PL, and measuring the PSF using a nanodiamond fixed on a coverslip. The axial separation of the PSF mapped by the two APDs is typically 200 nm, corresponding to the value of  $2R_z$ . The orbiting frequency is set at 50 Hz so that the integration time per point is 2.5 ms. The orbital tracking oscillation and corrections are run alongside the MW frequency sweeps (see Fig. 4.13 in Chapter 4). Data gathered is first averaged into consecutive 2.5 ms bins and post-processed as

$$PL_{xy}(x_n, y_n) = PL_{xy}(x_n, y_n, R_z) + PL_{xy}(x_n, y_n, -R_z),$$

$$PL_{t} = \sum_{n} PL_{xy}(x_{n}, y_{n}, R_{z}),$$
$$PL_{b} = \sum_{n} PL_{xy}(x_{n}, y_{n}, -R_{z}),$$

$$PL_{xy}(x_n, y_n, R_z) + PL_z$$

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where  $PL_{xy}$  is the PL data at different lateral locations (XY plane), and  $PL_t$  and  $PL_b$  are the total PL collected from the top and the bottom planes. The xy-plane data  $PL_{xy}$  is fit to (See Appendix C for discussion)

$$PL_{xy} = I_0 \left[ 1 - a\cos\theta_n + b\sin\theta_n \right],$$

to determine constants  $I_0, a, b$ . The predicted deviation is  $\delta_{xy} = \varepsilon_{xy}(a, b)$  with  $\varepsilon_{xy} = \frac{w_0^2}{R_{xy}}$  where  $w_0$  is the  $(1/e^2)$  radius of the lateral PSF.

The z correction is computed as

$$\delta_z = \frac{R-G}{1-GR}\varepsilon_z,$$

where,

$$R = \frac{PL_t - PL_b}{PL_t + PL_b}.$$

The factor G is an experimentally tuned constant. As G depends on the difference in collection efficiencies, beamsplitting ratio and the exact PSF, this constant is tuned just before each experiment to account for drifts in optics in the setup.

After data processing, the piezostage makes a correction of  $(\varepsilon_{xy}a, \varepsilon_{xy}b, \delta_z)$ .

This control algorithm can be linearised around the stable point a, b = 0, R = G, such that the position step is of the form,

$$u = P\hat{x},\tag{5.1}$$

where  $\hat{x}$  is (a, b, R-G) and *P* is a diagonal matrix with diagonal elements of  $[\varepsilon_{xy}, \varepsilon_{xy}, \varepsilon_z/(G^2-1)]$ . This abstracted proportional gain controller can give essential insights into its state space and dynamics, discussed in Section 5.4.3.6.

#### 5.4.3.3 Position estimation error

Each time this algorithm is run, it returns the predicted optimal location. Using a Fisher information approach, the photon shot noise contribution to this correction in  $\delta_{xy}$  can be estimated as

$$\Delta \delta_x = \Delta \delta_y = \varepsilon_{xy} \frac{1}{\sqrt{4I_0}},$$

where  $I_0$ , (in photon count units) is the PL collected at each orbiting sampling location.

For an emitter with PL rate of 2 Mcts/s,  $\varepsilon_{xy} = 338$  nm and when the dwell time per location of  $\Delta t = 20$  ms, the location uncertainty is 1 nm. The position estimation error is insignificant compared to typical translational diffusion with in the same time and the piezo actuator's position resolution ( $\sim \pm 2$  nm). In fact, for  $\Delta t > 1$  ms, the position estimation error is not a dominating factor. For  $\Delta t = 1.2$  ms, location uncertainty is 3 nm, which is still small.

The maximum particle diffusion rate is also a critical performance indicator. In this work and some others [147], the computation of correction is based on the control PC, which runs on non-realtime operating systems (RTOS) and thus typically has time jitters on the 10 ms timescale, limiting the correction rate to about 1 correction per 100 ms. This rate can be further improved by using real-time controllers as done in [251]. The prototype algorithm is first implemented on a PC with 200 ms correction interval. This limits the maximum diffusion constant to  $5000 \text{ nm}^2/\text{s}$ , which only constitutes a small subset of all nanodiamonds in a cell. A micro-controller unit (MCU) version of the same algorithm is then introduced to increase the update rate to a range between 26 Hz-417 Hz. In principle, 834 Hz is achievable but the current frequency range already allows tracking of the majority of nanodiamonds observed inside the cell. The working feedback time is 9.6 ms and allows tracking up to  $0.2 \,\mu\text{m}^2$ /second. Without a Kalman filter, there is a trade-off between maximum trackable diffusion rate and the steadystate noise. The position estimation error can be reduced with a Kalman filter, which takes into account historical locations, to update new particle locations [10, 80, 208]. As the MCU-based tracking was completed later in the project, it was only used in benchmarking experiments in glycerol. All other experiments presented in this chapter is based on the PC system unless specified otherwise.

Even faster applications would require beam-steering at sub-millisecond scale, and the galvanometric mirrors are not sufficient. Optoacoustic beam defectors devices are commonly used.

#### 5.4.3.4 Systematic errors in position tracking

The aforementioned orbital tracking technique assumes that the maximum intensity of the PSF corresponds to the location of the particle being tracked. This is true for an emitter at the top surface of the coverslip (also where the focal spot is) immersed in immersion oil, the design condition of the objective. However for particles in a medium at a finite distance above the coverslip, as is the case for diffusing particles in cells, the refractive index mismatch between the oil, glass and the cell culture medium ( $n \approx 1.515$  for immersion oil and glass, and n = 1.33 for cell culture medium) skews the axial PSF, such that the maximum intensity of the PSF deviates from the particle's real location. This deviation can be as large as -500 nm for a particle 10 µm above the coverslip (apparent location is 9.5 µm, see page 60 in [3]) and is nonlinear in the axial location. This leads to a systematic localisation error in the axial direction.

The index mismatch discussed so far is translationally invariant in transverse direction and thus only affects the axial PSF. Other abberations, for example coma, can also distort the lateral PSF.

In addition, the variation of refractive index in space due to the presence of the cell (even for an otherwise unabberated imaging system) would also cause distortions. The former can be corrected by a calibration with a known sample, while the latter would require techniques such as image-based method, or auxiliary wavefront measurement to separately determine the distortion of the PSF.

This is a problem yet addressed in the single-particle tracking literature (as in all work referenced in Section 5.4.3.1), but has been discussed in super-resolution imaging context [33, 126]. One class of method is to directly measure the wavefront phase information and correct for this distortion. The measurement could either be done with a well-known sample to calibrate the PSF at distances above the coverslip [3] or with wavefront sensors. An alternative class of method is an image-based method which iteratively optimises an image quality metric using, for example, the image sharpness and brightness as inputs.

Both classes of methods could in principle be to applied to our set-up to yield more accurate results. The results presented in this work deal only with the correlations in the correction of the trajectories, namely the MSD values. The MSD error due to optical abberation can be estimated as follows. For a particle at real positions  $z_1, z_2, \cdots$  and measured positions  $\tilde{z}_1, \tilde{z}_2, \cdots$ , the systematic errors due to PSF distortion are  $\delta z_1, \delta z_2, \cdots$ , with  $\delta z_i = z_i - \tilde{z}_i$ . Then the error in the MSD is

$$\delta MSD(\tau) = \frac{1}{N-\tau} \sum_{i=0}^{N-\tau} \left( \tilde{z}_{i+\tau} - \tilde{z}_i \right)^2 \left[ 1 + 2 \frac{\delta z_{i+\tau} - \delta z_i}{\tilde{z}_{i+\tau} - \tilde{z}_i} + \left( \frac{\delta z_{i+\tau} - \delta z_i}{\tilde{z}_{i+\tau} - \tilde{z}_i} \right)^2 \right]$$

Thus it can be seen that each contributing term in the MSD is reduced by an amount proportional to the DIFFERENCE in systematic errors (spatial gradient in systematic errors if  $z_{i+\tau} - z_i$  is small). The  $\delta z$ -z dependence is in general nonlinear and oscillatory for large NA objectives. To leading order, assume that the dependence is linear  $\delta z = kz$  where  $k \approx -0.05$  in light of 500 nm offset for  $z = 10 \,\mu$ m in the simulation results. Then the error in the MSD is about -10%. This is akin to a stage linearity calibration error, where the applied voltage to the piezostage is not proportional to the actual distance moved, or has uncertainty with the proportional constant. The stage linearity error for our system is about 1%. A similar error analysis would also apply to lateral directions. In this case the PSF is measured using nanodiamonds deposited on a coverslip. The PSF is symmetrical with no significant spherical distortion at locations farther away from the centre of the FOV ( $100 \,\mu$ m ×  $140 \,\mu$ m imaging area).

Considering the large variation (four orders-of-magnitude) in the viscosity constants measured (Fig. 5.12 a)), the systematic offsets are not the dominant source of error. Nevertheless, when analysing the data in Fig. 5.10 c) and d), the trajectory in the z direction alone could



Figure 5.5: Dynamic localisation accuracy. An artificial voltage generated according to Eq. 5.6 is sent to the galvo mirror to mimic a diffusing particle. **a**) shows the trajectory of such a diffusing particle. The tracker tracks the particle. **b**) shows the trajectory reported by the tracker and the commanded location of the particle in the x direction. The difference between the two gives the tracking accuracy, which is Gaussian distributed as shown in **c**). The spread of the Gaussian distribution gives the tracking accuracy and is dependent on diffusing rate. **d**) as the diffusion rate is varied where a minimum localisation accuracy is 7.7 nm achieved. **e**) A similar procedure in the z directions gives an best-case accuracy of 24 nm.

show a temporal profile because the particle moves to different heights. Thus the data in the xy directions are used to corroborate the temporal profile in z.

#### 5.4.3.5 Experimental tracking performance

To benchmark the performance of this tracker, an additional signal resembling a freely diffusing particle with diffusion constant D (see Eq. 5.8) is sent to the galvo mirror to mimic a diffusing particle. An ideal particle tracker will give the exact particle locations as controlled by the galvo. However as the correction is finite and there is error in the observation of the particle location, there is a difference between the tracker's location (Fig. 5.5 **a**) blue trace) and the actual particle's location (Fig. 5.5 **a**) red trace). This error is Gaussian distributed when there is no systematic over- or undercorrection (Fig. 5.5 **c**)) and the spread of this error sets the tracking accuracy. The traces of the laser spot location and simulated Brownian particle locations are shown in Fig. 5.5. The theoretical observation error is 0.8 nm for this particle (900kcts/s, 200 ms feedback time). The best total correction error is Gaussian distributed with standard deviation of 7.7 nm (Fig. 5.5 d)) dominated by the delayed correction ( $\sqrt{2D\Delta t} = 6.3$  nm). This could be explained by the diffusion during each correction step due to the finite photon accumulation time  $\Delta t$ ,

$$e_{total}^2 = 2D\Delta t + e_{observation}^2$$

where the first term is the error due to delayed correction and the second term is the observation error of 0.8 nm. A similar benchmarking performed in the z direction gives a tracking accuracy of 24 nm (Fig. 5.5 e)).

#### 5.4.3.6 Characterisation of the intrinsic velocity correlation of the tracker

Although obtainable from PSF measurements, in practice, the parameters  $w_0, R_{xy}, G, w_z, R_z$ are experimentally tuned to yield a good transient response. Inevitably this leads to overand undercorrection and thus multiple corrections are needed to reach the optimal position. The transient motion would manifest as a positive or negative velocity correlation, which in some cases would be confused with the intrinsic dynamics of the tracked particle. As limited work has been done to analyse the data produced by feedback-based tracking, it is particularly important to note this intrinsic nontrivial autocorrelation in the data.

The transient timescale can be characterised with a known diffusion with no intrinsic velocity autocorrelation, as used for tracker benchmarking in the previous section.

#### Stochastic difference equation for the tracker

To model the transient dynamics of the system under stochastic input [21] (observation noise due to photon shot noise,  $\varepsilon_{obv}$ , actuation noise,  $\varepsilon_{act}$ , and particle diffusion,  $\varepsilon_{par}$ ), consider the position of the focal spot x(t) and position of the particle  $\hat{x}(t)$  at the  $n^{th}$ tracking time step  $t = n\Delta T$ . The positions are updated according to

$$x(t+1) = x(t) - P[x(t) - \hat{x}(t) + \varepsilon_{obv}(t)] + \varepsilon_{act}(t)$$
$$\hat{x}(t+1) = \hat{x}(t) + \varepsilon_{par}(t), \qquad (5.2)$$

whereby the correction is written in a form of Eq. 5.1, and *P* is the proportional gain parameter. Defining a new variable, the velocity, as v(t) = x(t+1) - x(t). Then since

$$\begin{aligned} x(t+2) &= x(t+1) - P[x(t+1) - \hat{x}(t+1) + \varepsilon_{obv}(t+1)] + \varepsilon_{act}(t+1) \\ x(t+1) &= x(t) - P[x(t) - \hat{x}(t) + \varepsilon_{obv}(t)] + \varepsilon_{act}(t) \,, \end{aligned}$$

subtracting these two expressions and substituting in the difference equation for  $\hat{x}$  (Eq. 5.2) yields

$$v(t+1) = (1-P)v(t) + e(t), \qquad (5.3)$$

where  $e(t) = e_1(t) + e_2(t+1)$  with

$$e_{1}(t) = P[\varepsilon_{par}(t) + \varepsilon_{obv}(t)] - \varepsilon_{act}(t)$$
$$e_{2}(t+1) = -P\varepsilon_{obv}(t+1) + \varepsilon_{act}(t+1),$$

and the initial condition is such that  $v_0 \equiv v(t = 0) = 0$ . This is the stochastic difference equation governing the dynamics of the tracking system. The velocity at two different times, *t* and *s* where t > s, are related by

$$v(t) = (1-P)^{t-s}v(s) + \sum_{n=0}^{t-s-1} (1-P)^n e(t-n)$$
  
=  $\sum_{n=0}^{t-1} (1-P)^n e(t-n)$  (5.4)

At this point no assumption has been made on e. It is possible to assume  $e_1$  and  $e_2$  are uncorrelated with each other and with themselves except at the same time,

$$\langle e_i(t)e_j(s)\rangle = \delta_{ts}\langle e_ie_j\rangle, i, j = 1, 2,$$

where  $\delta_{ts} = 1$  when t = s and zero otherwise. The correlations  $\langle e_i e_j \rangle$  are assumed constant over time. One example where this assumption holds is when  $\varepsilon_{par}$ ,  $\varepsilon_{obv}$ ,  $\varepsilon_{act}$  are independently and equally distributed Gaussian distributions. The particle dynamics, captured by  $\varepsilon_{par}$ , could be more complex than this assumption and will be discussed later in this section. The velocity autocorrelation (using the second line of Eq. 5.4) of the focal spot is

$$\langle v(t)v(s) \rangle = \frac{(1-P)^{t-s}}{1-(1-P)^2} \left\{ \left\langle e_1^2 + e_2^2 \right\rangle + 2(1-P) \left\langle e_1 e_2 \right\rangle + (1-\delta_{ts}) \left[ (1-P)^{-1} - (1-P) \right] \left\langle e_1 e_2 \right\rangle \right\}.$$
(5.5)



Figure 5.6: Velocity autocorrelation function (VACF) of single particle tracking. **a**) shows that VACF for the PC-based tracking approach and clearly there is non-trivial autocorrelation at short times  $\sim 0.5$  s. **b**) is similar to panel **a**) but now with faster correction rate enabled by the fast feedback of a MCU-based tracker. When the particle is close to stationary, the intrinsic noise of the tracker leads to characteristic oscillations at the tracking frequency (104 Hz. As the particle diffusion rate increases, the VACF shows a transition from underdamped to overdamped oscillation, indicating a change of the proportional gain. **c**) Reducing the tracker's update frequency also reduces the characteristic frequency of the VACF.

By setting t = s to get  $\langle v^2(t) \rangle$  (assumed stationary), The velocity autocorrelation can be normalised as

$$\left\langle v(t+\tau)v(t) \right\rangle / \left\langle v^{2}(t) \right\rangle = (1-P)^{\tau} \left[ 1 + \frac{(1-\delta_{\tau 0}) \left[ (1-P)^{-1} - (1-P) \right] \left\langle e_{1}e_{2} \right\rangle}{\left\langle e_{1}^{2} + e_{2}^{2} \right\rangle + 2(1-P) \left\langle e_{1}e_{2} \right\rangle} \right]$$

When  $\tau \neq 0$ , this expression is proportional to  $(1 - P)^{\tau}$ . This is the discrete-time analogue of damped oscillations. When P > 1 (overcorrection), the system is underdamped such that each correction overshoots the correct value and is compensated by a backward correction in the following step. Similarly when P < 1 the tracker undercorrects and the trajectory is overdamped such that a few consecutive corrections are needed to catch up with the particle's movement (Fig. 5.6 **a**) and **b**)). The characteristic oscillation frequency is twice the update frequency (Fig. 5.6 **c**)). Without any correction, the trajectory with P < 1 would be mistaken with directed transport which has the characteristic positive velocity correlation.

The variance of the velocity can be obtained from 5.5,

$$\langle v^2(s) \rangle = \frac{1}{1 - (1 - P)^2} \left[ \langle e_1^2 + e_2^2 \rangle + 2(1 - P) \langle e_1 e_2 \rangle \right]$$
$$= \frac{1}{1 - (1 - P)^2} \left[ 2P(P^2 \langle \varepsilon_{obv}^2 \rangle + \langle \varepsilon_{act}^2 \rangle) + P^2 \langle \varepsilon_{par}^2 \rangle \right]$$

When the particle is not moving,  $\langle \varepsilon_{par}^2 \rangle = 0$ , the variance is dominated by the intrinsic noise of the tracker, and thus the random fluctuation around the particle is also controlled by the noise. When the particle is moving however, the variance contains both contributions from motion of the particle and the intrinsic observation and actuation errors. Especially, when  $\varepsilon_{par}(t) \sim \mathcal{N}(v, \sigma^2)$  while  $\varepsilon_{obv} = \varepsilon_{act} = 0$ , the particle undergoes persistent motion. In this case  $\langle e^2 \rangle = \frac{P^2}{1-(1-P)^2} (v^2 + \sigma^2)$ .

The feedback parameter *P* is not a fixed value. The derivation above assumes that the particle is at the centre of the orbit most of the time, i.e.  $x(t) - \hat{x}(t) \sim 0$ . When the particle's motion is faster than the tracker such that  $x(t) - \hat{x}(t) \sim R_{xy}$ , the effective proportional gain is less than that expected from the derivation. Thus, tracking could be underdamped when the particle is at rest while overdamped when the particle is diffusing.

This intrinsic correlation can be removed by averaging data in the neighbourhood of  $\sim 0.2$ s for the MCU-based tracker and  $\sim 1$ s for the PC-based tracker as is done in Ref. [251]. After averaging, the longtime correlation of the particle's motion can be considered.

# 5.5 Tracking results in HeLa cells

# 5.5.1 Modelling of stochastic translational diffusion in a complex medium

Diffusion of particles, such as small fluorophores, viruses and nanoparticles, is a complex process demonstrating spatial and temporal inhomogeneities. Single-particle tracking (SPT) data gives a timeseries of particle locations, which reflects the complex interaction between one particle and its complex surrounding.

The SPT trajectories are often noisy due to intrinsic stochastic diffusion processes and thus extracting information is difficult. Also, as the stochastic nature is intrinsic to the motion of the particle, it is unclear what parameters can be extracted from this very informative data. Camera-based methods have generated a wealth of single particle tracks and have enabled data processing algorithms to be proposed. It seems of foremost importance to answer two questions. Firstly, does the trajectory have homogeneous dynamics? Secondly, if the trajectory is homogeneous, what type of dynamics does it follow and what are the corresponding parameters?

The simplest approach is to use the mean squared displacement (MSD) and time lag to classify the motion as super-, sub- and normal diffusive. Brownian motion (BM) has classically been used to model particle diffusion in a purely viscous medium. A particle undergoing

BM in one-dimension has its trajectory, x(t), described by the difference equation,

$$x(t + \Delta t) = x(t) + \sigma \xi, \qquad (5.6)$$

where  $\xi$  is a random variable sampled from the standard normal distribution  $\xi \sim \mathcal{N}(0,1)$ . This descriptive relationship can be linked to the physical problem by solving the Langevin equation neglecting the inertial term,

$$0 = m\frac{d^2x}{dt^2} = \eta\frac{dx}{dt} + \zeta(t), \qquad (5.7)$$

where  $\zeta(t)$  is a stochastic force with zero-mean and finite, memory-less variance,

$$\langle \zeta(t) \rangle = 0, \langle \zeta(0)\zeta(t) \rangle = \zeta_0^2 \delta(t).$$

As  $\zeta$  is stochastic, the time-averaged MSD is a common quantity to compute. The MSD in 1D with a time lag of  $\tau$  is defined as

$$MSD_{1D}(\tau) = \frac{1}{t - \tau + 1} \sum_{t=0}^{t-\tau} [x(t) - x(t + \tau)]^2.$$

The kinetic relationship Eq. 5.6 gives MSD  $\propto \tau$  and the proportional constant is twice the diffusion constant in one-dimension,

$$MSD_{1D}(\tau) = 2D\tau. \tag{5.8}$$

The 3D MSD can be similarly defined,

$$MSD_{3D} = \frac{1}{t - \tau + 1} \sum_{t=0}^{t-\tau} [x(t) - x(t + \tau)]^2 + [y(t) - y(t + \tau)]^2 + [z(t) - z(t + \tau)]^2.$$

For a sphere diffusing in a purely viscous medium, the diffusion constant, D, can be related to the dynamic viscosity,  $\eta$ , by the Stokes-Einstein equation,

$$D=\frac{k_BT}{6\pi\eta r},$$

where r is the radius of the sphere. As the shape and size of nanodiamonds can have significant dispersion, the viscosity deduced from the diffusion constant would be highly variable.

# 5.5.2 Complexity of the cytoplasm and more involved models

The cytoplasm is not a uniform viscoelastic medium. Molecular crowding, binding and unbinding to organelles, and active trafficking along cytoskeletons are all factors influencing the motion of a nanoparticle

A conventional and simple method is to use the time-averaged mean-square-displacement (TA-MSD) and fit to the expression 5.8. However, the simple Brownian motion treatment is insufficient given the complexity that Eq. 5.7 fails to model. When more complex forces are involved, Eq. 5.7 needs to be modified (or changed into Fokker-Planck equation when the force is also location-dependent). As a result, the MSD dependence turns into MSD  $\propto \tau^{\alpha}$  and as opposed to BM, the diffusion is anomalous. The anomalous diffusion exponent ( $\alpha$ ) is sometimes extracted from MSD dependence. Using these features to characterise the trajectory may or may not be complete. For example the diffusion constant alone, does not reflect on whether or not the diffusion is sub- or super-diffusive. This descriptive method is problematic as it misses many important information such as longtime correlation, and also does not point to potential physical mechanisms that generate such trajectories.

#### 5.5.2.1 Temporal variation and correlation

These methods ignore the temporal information. However, a trajectory can change in dynamics (for example in diffusion constants, or Hurst exponent) as time progresses. These changes often correspond to a change in local environments, or interaction with cellular components. Hidden Markov modelling (HMM) [213], rolling window method, fitting to various subdiffusive models or more advanced schemes such as deep learning neural networks are such methods. They explore the longtime correlation of the trajectory.

A step further is to consider the short and longtime correlation in the data by using rolling window Fourier analysis, wavelet analysis [140] or the velocity autocorrelation function [217].

One popular approach is to map the trajectories to known stochastic models such as continuous-time random walk (CTRW), annealed transient time motion (ATTM), fractional BM (FBM) and many others. Each model then has its own set of parameters characterising the motion. This approach does not guarantee that the models available are exhaustive. However, this approach is also useful, for example, in the case of modelling viscoelastic medium with FBM [162]. In a viscoelastic medium, typically modelled with the generalised Stokes Einstein relation (GSER), the lack of a closed-form diffusive transport equation applicable to heterogeneous viscoelastic fluids demands alternative methods to generate trajectories of individual particles which can then be analysed to calculate desired statistics. The FBM model is such a method, which, although formulated phenomenologically, does capture the essential features

of viscoelastic medium and serves as a useful alternative.

There is still a gap between analysing SPT data and the type of data generated from our work. The majority of data analysis techniques in the literature concern data measured with imaged-based SPT, which are typically a few minutes long, and contain a few hundred particles per dataset. These trajectories typically exhibit a single or a few types of dynamics, with variation between tracks. A feedback-based tracking approach allows tracking of a single particle for a few hours. During this time, significant variations in the dynamics are observed. As such, the post-processing needs to accommodate for the temporal change but this is a problem not commonly treated in the literature.

#### 5.5.2.2 Inhomogenous diffusion dynamics

These methods assume a homogeneous diffusion process and ignore any subclasses of diffusion. Another class of methods characterises SPT data by various constituent diffusion parameters. Gaussian mixture models (GMM) is such a method.

HMM often refers to a model based on a Markov chain of order 1. The current state of the system only depends on the state of the previous step. It is highly effective in identifying the switching point between two diffusive states. However, this method does not take into account longtime correlation. As often the number of states are not determined *a priori*, it is necessary to use the Bayesian information criterion (BIC) to examine how many hidden states are needed to fit the data without overfitting. Furthermore, it is also possible to not assume the total number states by using the infinite HMM (iHMM) model [76]. Finally, a single set of transition probabilities are assumed for the entire trajectory and this does not account for changes with time.

Finally, combination the above approaches have been used to demonstrate classification and parameter inference on complex collections of trajectories.

Feature engineering and classification based on statistical tests or classical machine learning is one example. To justify that telomere motion follows FBM, Burnecki *et al* uses a set of 5 statistical tests [37]. Pinholt *et al* extract 13 numerical features from trajectories and classify the trajectories based on a linear discriminant analysis classifier [182].

#### 5.5.2.3 Combined methods and neural networks

Unlike classical machine learning techniques which use explicit rules for classification. Neural Networks extract features from the data without the experimenter designing fixed rules. Commonly used methods take experience from other applications, such as speech recognition. For such timeseries data, recurrent neural networks (RNN) take into account the relative order of the data. One RNN example is the long short-term memory (LSTM), which is used by Argun *et al* [17]. Similar to other neural network based methods, RNNs suffer from the lack of interpretability. Due to a change in how data is gathered or generated between the training and test sets, and the actual data set, a well-performing model may not perform as well, without a clear reason, and without warning. They are very difficult to apply to our case, where no previously established labelled data have become available.

As can be seen from the above discussion, extracting useful information from this data is difficult and there is still no consensus on an universal data processing algorithm. However, due to the lack of sufficient understanding of the noise in the data, machine learning methods involving neural nets is not applicable to our case. Among the feedback-based tracking literature, few have focused on classification and more focused on discriminating between different diffusion models such as between directed transport and diffusion [251]. Given this background, our analysis focuses on a two aspects, classification and assigning descriptive parameters to each classification.

# 5.5.3 Trajectory classification of measured nanodiamond trajectories

The 99 intracellular trajectories tracked revealed complex behaviours: simple Brownian motion, drift, confined Brownian motion and switching between mobile and stagnant regimes are observed.

HMM is used to first classify between stationary and non-stationary data. At this step, trajectories showing a single component is analysed with features such as the MSD and trajectories showing more complex multi-component behaviours are further analysed by fitting to a subdiffusive model.

#### 5.5.3.1 HMM segmentation

In a hidden Markov model (HMM), it is assumed that there exists a stochastic hidden variable, that cannot be known directly. Corresponding to different values of the hidden variable, the measured position will be drawn from different distributions. For example, a particle bound to the cytoskeleton will undergo confined Brownian motion, compared to free Brownian diffusion of an unbound particle. The hidden variable in this case corresponds to whether the particle is bound or not.

The HMM used in this work follows the approach in Ref [57] and assumes that the correction at each step follows a Gaussian distribution (also called the "emission"). The parameters of Gaussian distributions depend on if the particle is in state 1 or state 2. The probability of transition from state i to state j in a consecutive step is constant in time. These can be



Figure 5.7: Brownian motion trajectory. **a**) Three-dimensional trajectory of the diffusing particle. **b**) Dependence of MSD for trajectory in the x direction on delay  $\tau$ . The longtime behaviour is well-explained by a simple Brownian motion model where MSD  $\propto \tau$ . The shortterm behaviour is limited by localisation error. **Inset**: velocity correlation function in x direction showing no clear correlation beyond the intrinsic correlation of the tracker. **c**) and **d**) display the corrections applied in x and z directions at each step, showing no clear deviation from Gaussian distribution.

expressed as

$$\Delta r | i \sim \mathcal{N}(\boldsymbol{\mu}_i, \boldsymbol{\sigma}_i^2)$$
$$p(j|i) = p_{ij},$$

where i, j = 1, 2.  $\mu_i, \sigma_i^2$  are the respective mean and variance of the particle's correction step when the particle is in state *i* and  $p_{ij}$  is the (2 by 2) transition probability matrix. States 1 and 2 are arbitrarily defined states, but could be linked to physical boundedness of the particle. State 1 is constrained to have a mean of zero.

The number of hidden states,  $K^*$ , is chosen to be 2 in the above discussion but may not be known *a priori*. Thus when implementing the HMM, the number of hidden variables is varied and the resulting goodness-of-fit is compared to yield the best choice of  $K^*$ . The Akaike and Bayesian information criteria (AIC and BIC) are a common metric of choice, which describe the marginal gain in sum of residue squared (SSR) or the likelihood between models with different numbers of hidden states. With this criterion, we determine if a two component ( $K^* = 2$ ) HMM is preferred over a single component ( $K^* = 1$ ) HMM to explain the data. The data is also visually inspected afterward to verify the segmentation. For certain trajectories where tracking takes a few seconds to stabilise, the initial stabilisation phase is recognised as a second component and in this case segmentation is erroneous. The initial stabilisation phase is excluded and the remaining data is reclassified.

#### 5.5.3.2 Stationary trajectories

Figure 5.7 a) displays a simple single-component trajectory. This trajectory is stationary and its MSD linearly depends on the lag time  $\tau$ . In addition, the velocity autocorrelation function in x shows no correlation beyond the intrinsic correlation due to feedback tracking (Fig. 5.7 b) and inset). The small lag-time MSD is noisy as it approaches the localisation limit of about 10 nm (MSD  $\sim 10^{-4} \mu m^2$ ). Furthermore, the displacement steps in three directions (Fig. 5.7 c) and d), y not shown) perfectly follow a Gaussian distribution, suggesting that the trajectory is unlikely produced by models such as Lévy's walks. The particle gives a diffusion constant of  $(12.15 \pm 0.02) \text{ nm}^2/\text{s}$ .

Particles showing purely Brownian motion are common. However, as the cell contracts, some particles also drift together with the cell, displaying directed motion (Fig. 5.8). The speed is  $1.1 \text{ nm s}^{-1}$ , which is very slow compared to molecular motors. In fact, fast-moving particles are not captured by our measurement protocol. For a particle to be tracked, it will first have to be identified with confocal imaging and can not escape the focal volume before the tracking is started. This takes a few seconds (mainly PC overhead but can be reduced



Figure 5.8: Directed Motion trajectory. **a**) trajectory of the particle. **b**) MSD dependence showing clear quadratic dependence on lag time  $\tau$ . The short-time behaviour shows a combination of local Brownian motion and localisation error. **Inset**: no clear short-time correlation in velocity. **c**) and **d**) shows step displacements in x and z, well modelled by Gaussian distribution (indicated with dashed curve). Note that the frequency axis of the histogram is in a logarithmic scale.

in principle) which may limit the speed the observed particle and thus bias towards slower particles.

#### 5.5.3.3 Time-dependent trajectories

Trajectories showing switching between mobile and stagnant states are observed. Figure 5.9 **a**) shows such a trajectory. The particle is at first highly mobile but confined (confined region 1), transitions into a stagnant state and reverts back to the highly mobile confined state (confined region 2). Confined region 1 has a confinement radius of 280 nm. The transition from the mobile state to the stagnant state is clearly visible from the steps made by the tracker (Fig. 5.9 **c**)). Locally, the MSD shows saturation as lag time increases [197].

Finally, the most commonly observed trajectory involves an initially mobile particle gradually turning stationary. In the experiment performed, a mobile particle is first selected as to prevent monitoring particles external to the cell. Often the particle gradually slows down and becomes stationary over time. In one rare case the particle diffuses faster yet this case is only observed once and is thus not discussed here. Figure 5.10 **a**) displays such an orbit going



Figure 5.9: Confined motion. **a**) Trajectory in the xy plane, showing clear confinement in two regions. **b**) 3D trajectory. **c**) The particle switch between a highly mobile state (purple and red) and a stagnant state (pale red). **d**) within each confined area, the MSD shows the typical saturation behaviour with lag time.

gradually from the initial fast state to the final slow state in about 20 minutes.

The correction envelop gradually decreases over time (Fig. 5.10 c) d)), revealing the "ageing" effect. Although many different physical mechanisms can give rise to this effect, Continuous Time Random Walk (CTRW) is a minimal model that could explain the data. It is also noted that more complex models such as Lévy's Walks and Lévy's Flights also display ageing features [164]. In a CTRW model, instead of taking regular time steps as in Eq. 5.6, the time step is randomly drawn from an exponential distribution with probability density function given by [226]

$$P( au) = egin{cases} 0 & au < 1 \ eta au^{-1-eta} & au \geq 1 \end{cases},$$

where  $\beta > 0$  is the parameter characterising the distribution and the distribution normalises over the range  $\tau \in [0, +\infty)$ . The displacement is still updated as a BM at each (irregular) time step. CTRW gives (true only for  $\tau \ll t$ )

$$\delta^2(\tau,t) = |r(t+\tau) - r(t)|^2 \propto \frac{\tau}{t^{1-\beta}}.$$
(5.9)

The dependence on t gives rise to the ageing property of this model. The physical interpre-
tation of this model is that the particle could undergo constant binding and unbinding events with molecular motors on cytoskeletons such that they are transiently trapped with varying trapping strength. The particle returns to the state of normal diffusion once unbound. However this may not be the only mechanism giving rise to subdiffusive behaviour and in fact Tabei *et al* has demonstrated that a combination of models are necessary to accurately describe the physical data. For example, the assumption that the particle returns to normal diffusion when unbound may not hold when situated in a viscoelastic medium. In this case it may show longrange correlation, better described by a fractional Brownian motion model. The new scaling law for CTRW-fBM subordinate model is given by

$$\delta^2(\tau, t) \propto \frac{\tau^{1-\beta+2\beta H}}{t^{1-\beta}} = \tau^{\gamma} t^{\sigma}, \qquad (5.10)$$

where *H* is the Hurst's exponent characterising the extent of long-range correlation. When H = 0.5, a fBM corresponds to a normal diffusion.

To confirm this dependence, the MSD is plotted against the lag time  $\tau$  and measurement time *t*. The dependence on  $\tau$  is calculated as Eq. 5.9, but the *t*-dependence is calculated using Eq. 5.9 and then averaged over the neighbouring 100 data points to yield a better signal to noise. Figure 5.11 **a**) and **b**) show the  $\tau$  dependence with an exponent of  $\gamma = (0.812 \pm 0.004)$ and *t*-dependence with an exponent of  $\sigma = (-0.57 \pm 0.04)$ . The lag time of  $\tau = 1$  is chosen here to fulfil the requirements of Eqs. 5.9 and 5.10. The first exponent,  $\gamma$ , indicates that the spatial part of the the diffusion is not Brownian and thus unlikely governed purely by CTRW and is likely governed by the subordinate model. We can also deduce that  $\beta = 0.43$  and H = 0.28.

Finally there is no clear tendency for successive steps to be made in the same direction (Figure 5.11 c)). This is characterised by angles made by successive velocity vectors, defined as

$$\cos heta = rac{v(t+\Delta t) \cdot v(t)}{|v(t+\Delta t)| |v(t)|},$$

which suggests that the motion is not driven by active transport. The isotropic angular distribution is indicated on the plot, following the probability density function (PDF) of  $P(\theta)d\theta \sim \sin\theta d\theta$ . Note that this is not a uniform distribution in  $\theta$  alone ( $P(\theta) \neq const.$ ).

### 5.5.4 Discussion

Brownian motion describes the most commonly observed trajectories. In fact toward the end of a CTRW trajectory, the particle becomes close to BM. The experimentally observed trajec-



Figure 5.10: Transition from fast to slow diffusion. **a**) **b**) XY plane and 3D trajectory showing a gradual transition from fast (blue) to slow (red) motion. In **c**) and **d**) The corrections show a decaying envelop over time in X and in Z. The distribution also deviates from Gaussian (indicated with dashed curves) at the tail. Note that the histogram's frequency axis is in a logarithmic scale.

	Number of Particles
Brownian motion with drift	20
CTRW (Fast to slow transition)	15
Stationary	4
Confined	3
Slow to fast transition	3
Switching between mobile and stagnant states	2

Table 5.6: Number of intracellular trajectories of each category. A total of 47 nanodiamonds have been tracked. The most commonly observed trajectory is Brownian motion with drift (20). Time-dependent trajectories are also observed and among them fast to slow transition is the most common (15). Nevertheless due to localisation noise, stationary particles are difficult to distinguish from confined particles with a small confinement radius.



Figure 5.11: Dependence of MSD on measurement time (t) and lag time ( $\tau$ ). **a**) shows the MSD as a function of lag time  $\tau$  and **b**) on measurement time t. The MSD grows linearly as function of lag time on the log-log plot but the gradient is less than that expected for a Brownian motion (gradient of 1). This indicates subdiffusive behaviour. The MSD for a lag time of  $\tau = 1$  decays over time. The lag time of  $\tau = 1$  is chosen here to fulfil the requirements of Eqs. 5.9 and 5.10.

tories are often a combination of BM and drift, which could be well modelled by

$$MSD = 6D\tau + v^2\tau^2,$$

where the MSD is the 3D MSD.

The diffusion constants span the range  $10 \text{ nm}^2/\text{s}$  to  $10000 \text{ nm}^2/\text{s}$  (Fig. 5.12 a)). This distribution is biased due to the limit in the speed of particles trackable with PC-based tracking system. The speed of the net drift is very small compared to the typical speed of molecular motors (Fig. 5.12 b)). Experimentally, extremely mobile particles are rare. Many undergo slow diffusion, or permanently attach to intracellular structures. Thus, by choosing only particles that diffuse initially, there is unfortunately a bias. Furthermore, it has been reported that a 2 nm corona forms on the surface of nanoparticles once they are suspended in cell culture medium [104, 277]. The complex interaction between the surface functional groups and the surrounding environment implies that the motion of the particle is no longer a simple passive sample of the surrounding medium. However, if this surface coating is unchanged over time, a change of behaviour of the particle's motion over time would reflect on the change of the property of the environment.

In image-based SPT, tracks are usually not long enough to uncover the complete journey from the initial mobile state to its eventual attachment with an intracellular structure, such as the one presented in Fig. 5.10. For this particle, a 200 s track would unlikely reveal the the drop in  $\Delta x$  that takes place in ~ 1600 s, thus appearing similar to a BM.



Figure 5.12: Distribution of BM parameters. **a**) diffusion constants, D, span 3 orders of magnitude. **b**) The drift velocity is typically small.



Figure 5.13: Distribution of CTRW parameters for 15 trajectories. **a**) Scaling exponents of delay  $\tau$  and measurement time *t*. **b**) parameter  $\beta$  characterising the CTRW model. **c**) Hurst's exponent. **d**) timescales for transition from fast mobile state to slow stagnant scale.

The distribution of the scaling exponents  $\sigma$  and  $\gamma$  around the linear line suggests that CTRW is a major contributor to the subordinate model described by Eq. 5.10. In addition, the  $\beta$  parameters extracted from the scaling exponents distribute around 0.45, indicating a strong ageing behaviour. The Hurst exponent being close to 0.5 but also having a broad distribution, suggests that the local viscoelasticity can be significantly variable. Finally, the timescale for the the fast to slow transition can vary more than two orders of magnitudes, highlighting the need for longtime tracking to uncover the ageing behaviour.

We found that it is much more likely that a diffusing particle gradually stops diffusing (15 trajectories), than a particle initially slowly diffusing and turn into fast diffusion (3 trajectories). Once the particles stop diffusing, they would be in a confined or slow BM state. Thus the confined diffusion and BM states may be the final fate of a nanodiamond particle. This is consistent with a particle initially mobile diffusing quickly through the crowded cytosol, in the

process going through binding and unbinding events with the surrounding macro-molecules, eventually being captured and remaining stationary locally. This is in fact inline with the image-based SPT data, where nanodiamonds in the same cell show less diffusion after an hour.

Nanodiamonds have been claimed to be nanoscale sensors especially since in magnetometry measurements the detection volume is nanoscale. Yet in the above discussion it is apparent that diffusion causes nanodiamonds to sample a much larger volume than a stationary particle does. In NV sensing, there are multiple length scales at play. The first is the limit of optical resolution (limited by localisation accuracy) that is about 7.7 nm. The second is closest distance of approach between the sensor and the sensing target, that is limited by the size of the diamond, approximately 100 nm. The sensing volume is also sometimes used as the spatial resolution, which is very small in the case of magnetometry, about 20 nm. There is also the dynamic resolution, which is determined by the volume of space sampled by the diamond during the acquisition period due to diffusion, which can be large for a freely diffusing particle of the scale of 1 µm. Therefore as shown in this section, it is important, when claiming a nanoscale resolution, which quantity is of interest, as the actual volume sampled by one measurement integration period is limited by diffusion. Future techniques may evolve to enable selective binding between nanodiamond (for example through functionalisation with antibodies) and the sensing target and this would remove the ambiguity in the signal sensed by the nanodiamond.

# 5.6 Conclusion

In this chapter, we have discussed the challenge of performing the conventional NV experiments in a cell environment. In particular, the intracellular translational diffusion requires a fast tracking method. To resolve this issue we used a single particle tracking algorithm based on double-plane orbital tracking, to provide simultaneous tracking with ODMR acquisition. Later a dedicated tracking device is constructed in-house to enable tracking a particle as fast as  $0.2 \,\mu m^2/s$ . The particle trajectories measured in HeLa cells provide valuable information about their dynamics. These long trajectories are not possible with conventional image-based methods. The particle trajectories revealed that a large proportion of particles either undergo Brownian motion or transitions from a fast initial movement to a slow motion, showing the ageing behaviour.

Intracellular temperature measurements displays consistent change when the external temperature is modulated. In response to proton uncouplers (FCCP and BAM), cell temperature rises by as much 8 °C, with a duration greater 10 min. The simultaneous tracking and temperature readout capability can also be applied to studying other soft materials, to enable measurement of local temperature and viscosity. Such information could be useful for example in studying the glassy transition in soft materials where the bulk glassy transition differs from that under spatial confinement [13].

Furthermore, the ability of track particles has revealed that nanodiamonds gradually transition from a fast initial state to a slow stagnant final state. This behaviour might be dependent on the surface chemical bonding of the nanodiamonds. By functionalising the surface of the nanodiamonds differently the diffusion pattern could potentially reveal the affinity of various functional groups to organelles.

# Chapter 6

# Identification of single-spin colour centres in novel 2D materials for sensing applications

The successful techniques applied to NV centres in diamond, as discussed in previous chapters, have enabled high-fidelity quantum spin control, dynamic decoupling, efficient optical spin initialisation and readout. Quantum sensing with NV centres is now the most advanced technique for room-temperature contact quantum metrology. However, challenges with diamond-based technologies have emerged. These challenges include the difficulty in spatially controlling NV centre formation in bulk diamond, and the low light out-coupling efficiency from diamond.

A major challenge limiting the sensitivity of nanoscale sensing measurements is the sensorto-target distance. Shallow nitrogen ion implantation and delta doping have been developed for bulk diamond processing and nanodiamonds are also used for reduced sensor-to-target distance [263]. The delta-doping process involves introducing a layer of NVs toward the end of the CVD growth process. This only allows control over the depth, but not in the lateral dimensions. Ion implantation does allow control over lateral orientation with shadow masks and well-controlled ion beam energy and exposure [117]. In more recent studies, laser writing has also been shown to deterministically create vacancies in diamond which could subsequently form NV centres [46]. Diffraction limit restricts the use of this method for nanometer scale controlled activation.

In addition, bulk diamond substrate also reduces photon outcoupling efficiency due to the high refractive index of diamond. Solid immersion lenses [108]or lens in contact with diamond [44] are used to improve coupling into the collection optics.

Nanodiamonds address many of these challenges, including the issues with outcoupling ef-

ficiency and sensor-to-target distance. Yet the nanodiamond fabrication procedure also poses a few challenges. For biological applications in particular, the geometrical size and shape of the nanodiamonds produced by mechanically milling bulk diamond are difficult to control, often resulting in sizes spanning over an order of magnitude (0.1  $\mu$ m to 1  $\mu$ m for the Adamas nanodiamond used in Chapter 3). The milling process also introduces residual strain the material, causing inhomogenous broadening observed in Chapter 4. Nanodiamonds grown via CVD method potentially solve these issues.

Given these material limitations with diamond, it is important to explore new material platforms where the established quantum control techniques from diamond can be applied. In this chapter, we explore colour centres in a 2D wide-bandgap material where the reduced dimensionality enables higher spatial control of colour centre formation and higher light out-coupling efficiency. In addition, hBN can be produced on a wafer scale (large compared to diamond production). We report the first identification that these 2D colour centres are spin-active, showcasing hBN as a new material platform for quantum sensing. Therefore this chapter provides the basis for an outlook of using other material platforms to tackle issues facing nanodiamond quantum sensors.

In the work presented in this chapter, Q.G. contributed to the initial characterisation of the ensemble emitter sample which establishes the relevant parameters for observing ODMR of single-photon emitters in hBN. Q.G. discussed experiment design and data collection, and interpretation of data with Dr Hannah Stern, Dr John Jarman, and Simone Eizagirre-Barker, who constructed the confocal set-up and took the measurements. Q.G. subsequently individually built and tested the rate equation modelling and spin modelling presented in this chapter. The hBN samples were fabricated by a collaboration with Prof. Igor Aharanovich's and Prof. Hoe Tan's groups.

### 6.1 Introduction

In recent years, there has also been extensive search for colour centres in other materials, such as Silicon Carbide [52, 257], hexagonal boron nitride (hBN) [43, 75] and many others.

Emitters in layered van der Waals materials provide a building block to circumvent these few limitations, providing potentially single layer materials. They can also be stacked together to form heterostructures, as is demonstrated in transition metal dichalchogenides (TMDs) [167]. The reduced dimensionality of the material also allows easy access to the emitter for applying strain and electric fields. Strain and electric field tuning of the emitter's optical emission have been demonstrated [97, 172, 174, 184]. This tunability allows emitters to be integrated into devices and individually tuned. The technique of locally straining two-

dimensional layers [177] have also been applied to hBN to deterministically activate emitters by inducing local strains with nanopillars on the substrate [184]. The lack of optically dense substrates allow fluorescence to be more efficiently extracted.

The above properties have made emitters in 2D materials very promising for photonic devices. It remains a question if there are addressable spins in these systems. Spin addressable single defects provide a brand new opportunity for spin-based quantum sensing similar to that offered by NV centres.

### 6.1.1 Spin-addressable single defects

Various classes of defect emitters have been identified in hBN. However among them only a few have been shown to contain optically addressable spins. Ensemble studies have been carried out on singly charged vacancy centre,  $V_B^-$ , which is formed from a vacancy replacing a boron atom, and shows broad optical emission centred around 800 nm. They have been characterised with ODMR and display the characteristic ZFS for a spin-1 system, with D = 3.48 GHz and E = 50 MHz.  $V_B^-$  has a strong temperature dependence in the room temperature range of -623 kHz K<sup>-1</sup>, almost ten times higher than NV centres [95]. Coherent control in the form of a Rabi oscillation has also been demonstrated [96]. This study also investigated the coupling to surrounding nuclei and clearly resolved the hyperfine coupling. In addition, another class of carbon-related emitters have been reported by Mendelson *et al* display room temperature ODMR [163]. The density of the defects can be varied by changing the flux of the TEB precursor used in the study and at sufficiently density it would be possible to observe single emitters.

Exarhos *et al* reported magnetic field dependent anisotropy in PL for a class of single emitters in exfoliated hBN at room temperature [75] although the ODMR signature of such emitters has not been observed. It is thus difficult at this point to infer the spin structure of the defect. The only ODMR-active single emitters reported so far are by Chejanovsky *et al* which shows ODMR only at cryogenic temperature [43]. The three emitters report show a range of emissions from 700 nm to 800 nm. The emitter was concluded to be a spin-1/2 emitter and ODMR is not observed at room temperature.

The attractive features of van der Waals material, and the growing number of emitters identified, have driven the search for spin-addressable single emitters, especially ones at room temperature, which have not been discovered, but are crucial for sensing. Our work sets out to find such emitters.

### 6.1.2 Chapter overview

In this work, we confirm the observation of single emitters in hBN, which also display ODMR at room temperature.

The ODMR spectrum reveals a doublet structure previously not observed in ensemble measurements. In addition, magnetic field angle-dependence of ODMR are consistent with a  $S > \frac{1}{2}$  system with modest zero-field splitting (ZFS). In combination with observed laser power dependence of the ODMR contrast, an optical level structure is proposed to explain the  $g^{(2)}$  results. By tuning the transition rates, the level structure predicts the appearance of bipolar ODMR, appearance of a second bunching timescale associated with ODMR, as well as absence of ODMR.

# 6.2 Experimental setup

### 6.2.1 Materials

The hBN layer is first grown on copper by metal organic vapour phase epitaxy (MOVPE), using the same procedure reported by Mendelson *et al* [163], with a thickness of approximately 40 nm. The hBN sample is later transferred to a silicon wafer substrate. A photolithographically deposited microstrip on top the hBN layer is used to deliver MW to the sample. The sample is measured at room temperature.

### 6.2.2 Confocal

The confocal setup used to characterise the hBN material is different from those mentioned in previous sections. A schematic is shown in Fig. 6.1. This setup uses 532 nm continuous wave excitation and 550 nm long-pass collection. The collected fluorescence can be coupled to into an avalanche photodiode (APD) via a single-mode fibre, two separate APDs via a 50:50 single-mode fibre beamsplitter, or a spectrometer via a single-mode fibre. For confocal imaging and ODMR experiments, a single APD is used and the output pulses are directed to a National Instrument data acquisition card (DAQ) for photon counting. A scanning mirror and a 4f telescope system is used to achieve lateral confocal scanning on the sample.

For a second-order intensity correlation experiment, the two outputs from the fibre beamsplitter are sent to two separate APDs, which are then connected to a quTau time-tagger for time correlation studies.

### 6.2.3 **ODMR**

For ODMR experiments, microwave is delivered to the sample by using a microstrip that is photolithographically deposited on the hBN material.

The ODMR is performed by a lock-in technique where the MW is switched at 70 Hz rate, completing a on-off sequence in about 14 ms. This sequence is repeated 10 times for each frequency before moving on to the next MW frequency. The PL when MW is on (signal,  $PL_{sig}$ ) and when MW is off (reference,  $PL_{ref}$ ) are collected, giving the contrast as

$$C = \frac{PL_{sig} - PL_{ref}}{PL_{ref}}.$$

The lock-in method removes slowly varying common-mode noise due to laser fluctuation, charge-state fluctuation or setup drift.

Unlike in experiments all previous chapters where a in-house Python-based control software is used, this experiment is controlled by an open-source software, Qudi.

A neodymium permanent magnet is mounted on a linear motorised translation slide to apply a static magnetic field at the sample. These fields have been calibrated with a Teslameter (F71, Lakeshore) at the sample. The magnetic field is applied in the sample plane, perpendicular to the optical axis. The microstrip used to deliver the MW is such that the MW polarisation is out of the plane. The excitation laser is intrinsically linearly polarised but its polarisation is not intentionally controlled. For the angular dependence measurement, a small magnet is mounted on a rotation mount to apply magnetic field in other directions.

### 6.2.4 Second-order intensity correlation function

The second-order intensity correlation  $(g^{(2)})$  measurement is performed using the Hanbury Brown-Twiss (HBT) interferometer. The collected photons are first split via a fibre beamsplitter and directed to two separate APDs. The photon arrival times are tagged and post processed.

The second order correlation function,  $g^{(2)}(\tau)$ , can be used to infer the major optical structures of the single emitters.  $g^{(2)}(\tau)$  measures the normalised intensity correlation,

$$g^{(2)}( au) = rac{\langle I(t+ au)I(t)
angle}{ar{I}^2}$$

where the average denotes ensemble average and can be identified with time average for a stationary source.

Experimentally, this can measured by a Hanbury Brown-Twiss (HBT) interferometer. The second order autocorrelation can be computed from a "start-stop" type measurement where

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Figure 6.1: Experimental setup for hBN characterisation. The confocal microscope uses 532 nm optical excitation, and a long-pass filter at 550 nm is used to filter the scattered excitation light before the fluorescence is coupled into the collection avalanche photodiode (APD). For PL measurements the output from the APD is routed to A National Instrument data acquisition card (DAQ) for photon counting. The fluorescence can also be coupled to a spectrometer (not shown) for spectrum measurement or into a single-mode fibre beamsplitter into two APDs. The outputs from the two APDs are connected to a quTau timetagger for HBT measurements. MW is delivered from the MW generator to the sample using a microstrip. An externally applied static magnetic field is provided by a permanent magnet mounted on a motorised linear slide. L1/L2: lenses. DM550: long-pass dichroic mirror at 550 nm. CL1/2: collimation lenses. SM: single-mode.

one APD is used as a start trigger and another as a stop trigger. The distribution of time differences between start and stop results in the quantity  $K(\tau)$  which is the conditional probability density function of detecting second photon at time  $\tau$  after detecting the first photon (with no photon events in between),

$$K(\tau) = p(t = t_0 + \tau | t = t_0).$$

In comparison, in a continuous illumination experiment, where a stream of photon arrival times are recorded and correlated with each other, resulting in the quantity,

$$J(\tau) = rac{\langle I(t)I(t+ au)
angle}{ar{I}^2} \equiv g^{(2)}( au).$$

Here, photon arrival events in between t and  $t + \tau$  are permitted. For very short  $\tau$ , typically less than one photon arrival event occurs within this time on average and thus  $K(\tau) \approx J(\tau)$ . However these two quantities are different at longer intervals as multiple photon arrival events could have taken place. The intensity correlation is the probability of getting *n* photons within time  $\tau$  summed over *n* [81, 111],

$$J(\tau) = K(\tau) + K \star K + K \star K \star K + \dots,$$

where the  $n^{th}$  term gives the probability of getting exactly *n* photon emission events within time  $\tau$ . Even though  $K(\tau)$  is a common quantity measured experimentally, the  $J(\tau)$  function is more straightforward to relate to the atomic system as it is just the rate of photon emission after an emitter is excited from ground state (GS) for a duration  $\tau$ . This allows modelling  $g^{(2)}$  with a rate equation.  $g^{(2)}(\tau = 0) = 0$  is a unique feature of a single-photon source and single-photon emitters. In the atomic defect context such single-photon sources are due to a single atom that cannot emit two photons at the same time. Experimentally it is not possible to achieve this condition due to noise and equipment imperfection (see Appendix D) and thus  $g^{(2)}(\tau = 0) < 0.5$  is typically used for claiming single photon emission. Figure 6.4 **a**) for defect B. The antibunching dip is clearly observed.

The  $g^{(2)}(\tau)$  can be further fitted with a sum of multiple exponential functions,

$$g^{(2)}(\tau) = 1 - \underbrace{ae^{-\tau/\tau_1}}_{antibunching} + \underbrace{be^{-\tau/\tau_2}}_{bunching 1} + \underbrace{ce^{-\tau/\tau_3}}_{bunching\cdots} + \cdots,$$
(6.1)

where *a* and  $\tau_1$  are the antibunching amplitude and timescale,  $b, c, \cdots$  and  $\tau_2, \tau_3, \cdots$  are the bunching amplitudes and timescales.

# 6.3 Results

### 6.3.1 Observation of room-temperature CW ODMR

To confirm if the same emitters can be observed as those reported in [163], we first characterised a sample with high defect density. A similarly prepared sample as in [163] has displayed ODMR and the optical emission has been associated with carbon impurities. Scratches and wrinkles are common on the surface, possibly due to the transfer and processing.

Figure 6.2 **a**) shows a representative confocal map for the ensemble form of the defects, with small variations in local PL intensity. The PL does not saturate with increasing laser at the range probed (Fig. 6.2 **b**)). The optical spectrum shows a broad feature peaked at around 650 nm. Unlike the single emitters, zero-phonon line (ZPL) is hard to identify. Additionally the ensemble emitters are bleached after a few hours of exposure the 532 nm optical excitation.

Figure 6.2 d) is a confocal map for a sample with low density of defects where single diffraction limited emitters are identified. The emitter circled, named Defect A, shows a clear optical saturation (Fig. 6.2 e)). The saturation curve is typical of single emitters (see Chapter 1, Section 1.2.4) and is fitted with Eq. 1.1, reproduced here,

$$PL_0 = PL_{0,sat} \frac{s}{1+s}.$$

The fitted saturation power is  $P_{sat}^{optical} = 70(2) \,\mu\text{W}$  with a saturated PL of  $I_{sat} = 37.9(3) \,\text{kHz}$ . In sharp contrast to the ensemble case, the spectrum displays clear features with phonon side bands (PSBs) with 180 meV energy separation. The ZPL centres around 590 nm but varies greatly from emitter to emitter (535 nm to 670 nm).

Continuous-wave (CW) ODMR is next performed on these emitters with an externally applied static magnetic field. Figure 6.3 a) illustrates the setup configuration and Fig. 6.3 b) shows a wide field microscope image of the microstrip used to deliver MW to the sample.

Contrary to NV centres where every defect displays an ODMR with negative contrast and similar MW saturated optical contrast, we observed ODMR with both positive (Fig. 6.3 c) red points) and negative ODMR contrast (Fig. 6.3 c) yellow points), with a range of contrast from as high as 35% to as low as not distinguishable from noise (data not shown). The single emitters show significantly higher CW ODMR contrast compared to the ensemble emitters, which typically gives 0.2% of MW saturated contrast (Fig. 6.3 c) gray points). Both the ensemble and the single emitters show saturation with MW power (Fig. 6.3 d)) as typically observed in NV systems [69]. The MW saturated linewidths are however similar between the ODMR spectra presented in Fig. 6.3 c) and are ~35 MHz. The difference between the ensemble emitters' ODMR and single emitter's ODMR spectra could arise from the cancellation



Figure 6.2: Optical characterisation of ensemble and single emitters. **a**) and **d**) are confocal PL maps of ensemble and single emitters. **e**) The single emitters showed clear laser power saturation while **b**) the ensemble emitters do not within the experimental laser power range. **c**) The ensemble emission spans a broad spectrum and **f**) the single emitters show well resolved peaks in optical emission.

between defects with positive and negative ODMR contrasts, in combination with the large number of ODMR-inactive emitters. It however is unlikely due to inhomogeneous broadening of ensemble defects.

The ODMR resonance frequency increases linearly with applied magnetic field strength, giving a g-factor of 1.98(3) (Fig. 6.3 e) inset). This is typical of atomic defects in system without significant spin-orbit coupling. The linewidth does not significantly change with magnetic field. Due the frequency dependence transmission of the microstrip used for MW delivery, the ODMR contrast appears to change with magnetic field. As such we map out the saturation curve similar to those in Fig. 6.3 d) at a few representative MW frequencies and indicate the contrast at MW saturation as crosses in Fig. 6.3 e). The ODMR contrast remains constant going above 700 MHz but decreases toward lower magnetic fields.

Among the >400 defects studied, a total of 27 defects displayed ODMR with >1% optical contrast at a static magnetic field of 25 mT, corresponding to a free-electron Zeeman splitting of 700 MHz. The overall yield is 6.8 %.

# 6.3.2 Bunching dynamics and appearance of an additional bunching component

Second-order correlation spectra are taken for a series of emitters and are used to select single photon emitters. Figure 6.4 a) is an example of the  $g^{(2)}(\tau)$  taken for defect B. The data is first normalised with data at very large  $\tau$ , and then fitted with a tri-exponential function (a three-timescale version of Eq. 6.1)

$$g^{(2)}(\tau) = 1 - \underbrace{ae^{-\tau/\tau_{ab}}}_{antibunching} + \underbrace{be^{-\tau/\tau_b}}_{bunching} + \underbrace{ce^{-\tau/\tau_{b(additional)}}}_{bunching additional}, \tag{6.2}$$

where  $\tau_{ab}$ ,  $\tau_b$ ,  $\tau_{b(additional)}$  are the antibunching, bunching and an additional bunching timescales. *a*,*b*,*c* are the respective amplitudes.  $g^{(2)}(\tau = 0) = (0.35 \pm 0.06) < 0.5$  is calculated from the fit.

It was observed (Fig. 6.4 **a**) and **b**)) that defects showing ODMR can only be fitted well with two bunching timescales ( $\tau_b$  and  $\tau_{b(additional)}$ ), while defects that do not show ODMR can be fit sufficiently well by only one bunching timescale ( $\tau_b$ ). When defects showing no ODMR are fitted with two bunching timescales, there third timescale has a significant fitting error such that  $\sigma_{b(add)}/\tau_{b(additional)} > 1$  (Fig. 6.4 **c**)).

Furthermore, the antibunching timescales are similar between emitters (Fig. 6.4 d)). However unlike NV centres where only one bunching timescale is observed and the bunching timescale is approximately constant across emitters [30], the bunching timescales span at least



Figure 6.3: ODMR measurements and dependence on magnetic field. **a**) is an illustration of the confocal microscope set-up used to perform ODMR, showing a microstrip for MW delivery. **b**) An optical microscope image of the microstrip. The hBN material covers the region imaged uniformly. **c**) hBN single emitters show a bipolar behaviour in ODMR contrast where some emitters display a positive ODMR contrast (red points) while some others a negative ODMR contrast (blue points). In either case, the contrast at saturation is significantly stronger than that for the ensemble emitters (gray points). **d**) MW power saturation curve of the ODMR contrast. **e**) Magnetic field dependence of the ODMR resonance. The colored traces show individual ODMR spectra taken at various magnetic fields at a constant MW power. The crosses show the MW saturated ODMR contrast. Inset: the resonance frequency linearly depends on the applied external magnetic field, as would be expected for a constant g-factor, determined to be 1.98(3).  $g=1.98 \pm 0.02$ .



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Figure 6.4: Comparison of  $g^{(2)}$  between **a**) ODMR-active and **b**) inactive emitters. **c**) ODMR-inactive defects have  $g^{(2)}$  well-fitted by a bi-exponential fit while ODMR-active defects require a tri-exponential fit. When ODMR-inactive defects are fitted with a tri-exponential, the fitting error on the additional timescale,  $\sigma_{b(add)}$ , is large compared to its absolute value,  $\tau_{b(additional)}$ . **d**) The antibunching time are similar between defects, while as shown in **e**) and **f**) the bunching timescale and the additional bunching timescale can span at least two orders of magnitude.

two orders of magnitude between emitters (Fig. 6.4 e) and f)).

(The following data is from experiments conducted by H.S., J.J, S.E and the modelling is conducted and tested by Q.G.)

#### 6.3.2.1 Rate equation modelling

To further infer the optical transitions of the defect, we use the rate equation approach to model the  $g^{(2)}$  data. This formulation does not take into account any coherent transitions between energy levels, which is typically true in atomic defects, which are driven non-resonantly and typically couple to phonons and quickly lose optical coherence.

In a simple system where the *i*<sup>th</sup> level has a occupancy of  $n_i$ , such that  $\sum_i n_i = 1$ , the rate equation can be modelled as [43, 75, 143, 170, 233]

$$\frac{dn_i}{dt} = \sum_j k_{ij} n_j - \sum_m k_{mi} n_i, \tag{6.3}$$

where the first term accounts for rate of population arriving at the  $i^{th}$  state and the second term for population leaving state  $n_i$ .  $k_{ij}$  is the rate of population leaving state j and arriving at state i, while  $k_{mi}$  is the rate of leaving state i and arriving at state m.

The rate equation can be further represented as a matrix equation,

$$\frac{dn}{dt} = -\Lambda n,\tag{6.4}$$

with  $\Lambda$  is a matrix of rate constants. This rate equation has the formal solution,

$$n(t) = Ae^{-\Lambda t}n_0, \tag{6.5}$$

which can in turn be solved as,

$$n(t) = \sum_{i} A_i e^{-\lambda_i t} u_i = \sum_{j} u_{ij} A_j e^{-\lambda_j t}, \qquad (6.6)$$

where  $\lambda_i$  are the eigenvalues of  $\Lambda$  and  $u_i$  is the normalised column eigenvector corresponding to eigenvalue  $\lambda_i$ . This can be compared with experimental data by fitting the experimental data with the following function (assuming three timescales, as will be discussed later),

$$g^{(2)}(\tau) = 1 - ae^{-\gamma_1\tau} + be^{-\gamma_2\tau} + ce^{-\gamma_3\tau}, \tag{6.7}$$

and extract the amplitudes a, b, c and timescales  $\gamma_1, \gamma_2, \gamma_3$ .

In Eq. 6.6,  $A_i$  is an undetermined coefficient.  $u_{ij}$  is a matrix formed of eigenvectors  $u_{ij} = [u_1, u_1, \dots, u_N]$ . For a closed system  $\sum_j \Lambda_{ij} \equiv 0$ , and thus at least one of  $\lambda_i$  will vanish, thus making possible the inhomogeneous boundary condition,

$$\sum_{i} n_i(t) = 1, \forall t \in [0, \infty).$$
(6.8)

The corresponding term in Eq. 6.6 gives the steady state population distribution. The matrix,  $u_{ij}$  is orthonormal and invertible, and thus the vector  $A_i$  can be unique determined from the initial value n(t = 0) by,

$$A_i = \sum_j (u^{-1})_{ij} n_j (t=0) = \sum_j u_{ji} n_j (t=0),$$
(6.9)

where the last step uses the property of an orthonormal matrix  $u^T = u^{-1}$ . Specifically, provided that  $\Lambda$  only has one eigenvalue of zero (let it be  $\lambda_M$ ), the steady-state population tends to one

distribution regardless of the initial condition,

$$A_M = \frac{1}{\sum_i u_{iM}}, n_i(t \to \infty) = \frac{u_{iM}}{\sum_i u_{iM}}.$$
(6.10)

One caveat is that  $\Lambda$  may give complex eigenvalues when population can cycle between more than two states for example  $A \to B \to C \to A$ . Although these cases still give longtime equilibrium population distribution they would give rise to  $g^{(2)}(\tau)$  that is hard to observe experimentally and is thus not considered.

In the case where the ground state is a singlet (S = 0, and spin multiplicity is one),  $n_{GS}(t = 0) = 1$ . When the ground state is a triplet (S = 1, spin multiplicity of three), continuous optical pumping may partially polarise the ground state, such that  $n_i(t = 0) \neq n_i(t \rightarrow \infty)$  even for  $i \in GS$ . Upon measurement of a photon, it can be inferred that the system was originally in one of the radiative excited states (ESs) j, and is currently in one of the ground states i. Thus the probability of the emitter in state i given a photon has been detected is [75]

$$n_{i}(t=0) = P(i|photon) = \sum_{j \in ES} P(i|j)P(j|photon)$$
$$= \sum_{j \in ES} \frac{k_{ij}}{\left(\sum_{m \in GS} k_{mj}\right)} \frac{n_{j}(t \to \infty)}{\left(\sum_{k \in ES} n_{k}(t \to \infty)\right)},$$
(6.11)

where the first factor describes the branching ratio - the proportion of decay into GS i among all decays from ES j - and the second factor describes the proportion of state j among all ES population. With the following redefinition,

$$P_{ij} = \begin{cases} \frac{k_{ij}}{(\sum_{j} n_j(t \to \infty))(\sum_{i} k_{ij})} & i \in GS, j \in ES \\ 0 & otherwise \end{cases},$$
(6.12)

the explicit sum over only the ES or GS in Eq. 6.11 can be presented as

$$n_i(t=0) = \sum_{j=0}^N P_{ij} n_j(t \to \infty) = \sum_{j=0}^N P_{ij} \frac{u_{jM}}{\sum_k u_{kM}}$$
,

where the last step uses Eq. 6.10. This expression allows numerical computation of the initial condition, given the rate equation 6.3 and the branching ratio Eq.6.12.

### 6.3.2.2 Proposed optical level structure

For three defects we recorded a series of  $g^{(2)}$  measurements as a function of multiple laser powers. This data could be modelled by a five-level kinetic model, consistent with similar models previously proposed for other hBN spin defects [43, 75]. This model allows the ODMR behaviour to also be explained.

The level structure for this model is shown in Fig. 6.5 a). It includes a spin-triplet GS (states 1 and 4) and ES (states 2 and 5) and a spin-singlet shelving state (state 3). The  $m_s = +1$  and  $m_s = -1$  spin sublevels are treated as one state in the model to simplify computation. The radiative transitions ( $k_{54}$  and  $k_{21}$ ) are spin-preserving and have spin- and laser power-independent rates. The transition rate from GS to ES ( $k_{12}$  and  $k_{45}$ ) is proportional to the laser power. Intersystem crossing rates, ( $k_{23}$  and  $k_{53}$ ), and de-shelving rates ( $k_{34}$  and  $k_{31}$ ), are spin-dependent. We find that they must be also laser-power dependent to model the quadratic dependence of the bunching rates on laser power (Fig. 6.5 d)) and a linear power dependence is assumed,  $k_{31}, k_{32}, k_{34}, k_{35} \propto s$ .

This five-level model is motivated by the observation of three timescales in the  $g^{(2)}$  data and observation of ODMR. It is similar to the NV centre optical level structure. The ISC rates are proportional to the optical pumping parameters, s, ( $s = P/P_{sat,opt}$ ). Power-dependent deshelving rates and ISC rates were also proposed in other systems [160, 170] and may hint at other higher-lying states not included in the model. Nevertheless, one undesirable feature of this power dependent rates is that if the initialisation is switched off while there is still population in the shelving state, this population will remain in the shelving state and thus the atom would remain in an energetically less favourable state.

The above discussion gives the following rate matrix,

$$\Lambda = \begin{bmatrix} -k_{12} - k_{MW} & k_{21} & k_{31} & k_{MW} & 0\\ k_{12} & -k_{21} - k_{23} & k_{32} & 0 & 0\\ 0 & k_{23} & -(k_{34} + k_{31} + k_{32} + k_{35}) & 0 & k_{53}\\ k_{MW} & 0 & k_{34} & -k_{45} - k_{MW} & k_{54}\\ 0 & 0 & k_{35} & k_{45} & -k_{54} - k_{53} \end{bmatrix}.$$
(6.13)

The rate  $k_{MW}$  is a phenomenological constant added to describe the effect of MW-induced spin transitions and is set to zero unless the ODMR contrast is modelled. A more sophisticated Linblad's master equation approach can be used to account for the coherent driving and decoherence of the spin transitions. This is not done here for simplicity and thus the linewidth and contrast need to be phenomenologically added subsequently.



Figure 6.5: Proposed optical level structure and rate equation modelling. **a**) The proposed optical level structure and transition rates. This model allows fitting of the parameters extracted from  $g^{(2)}$  experiments, including **c**) the antibunching rate, **d**) the two bunching rates for ODMR active defect and **e**) the respective amplitudes. The presence of ODMR and the sign of the contrast depend critically on the balance between the ISC rates. As illustrated in **b**), when  $k_{34} = k_{31}$ , tuning the ratio between  $k_{43}$  and  $k_{53}$  always results non-positive ODMR contrast (red curve and top inset). When  $k_{34} \ll k_{31}$ , tuning the ratio between  $k_{43}$  and  $k_{53}$  allows a smooth transition from positive to negative ODMR contrasts.

Radiative		ISC		
$k_{21} = k_{54}$	0.38 GHz	$k_{23}/s$	69.5 MHz	
$k_{12}/s = k_{45}/s$	0.076 GHz	$k_{53}/s$	3.47 MHz	
		$k_{34}/s$	421 kHz	
		$k_{31}/s$	2.25 MHz	

Table 6.1: Parameters used to fit transition rates for defect B. The rates proportional to the optical pumping parameter *s* are first divided by *s*.

To facilitate comparison with the model, Eq. 6.2 is converted into

$$g^{(2)}(\tau) = 1 - \underbrace{ae^{-\gamma_1 \tau}}_{antibunching} + \underbrace{be^{-\gamma_2 \tau}}_{bunching 1} + \underbrace{ce^{-\gamma_3 \tau}}_{bunching 2}, \qquad (6.14)$$

where  $\gamma_1 = 1/\tau_1$  and similarly for  $\gamma_2$  and  $\gamma_3$ . The parameters a, b, c share the same definition at in Eq. 6.2. This is the same equation as Eq. 6.2 but is written this way so that the rates  $\gamma_{1,2,3}$ have the same units as the rates  $k_{ij}$ .

The transition rates from the three-level model (6 in total) are tuned manually to achieve a set of rates that allow all 6 parameters ( $\gamma_{1,2,3}$  and a, b, c) in the  $g^{(2)}$  model function Eq. 6.14 to fit the experimental  $g^{(2)}$  laser power dependence (Fig. 6.5 c)-e)). In the figure below the fits of the model are shown alongside the antibunching and bunching rates and amplitudes measured for defect C, as a function of optical pumping parameter *s*. The fitting parameters are tabulated in Table 6.1.

By adding a MW induced transition rate  $k_{MW}$ , the laser power dependence of the MW saturated ODMR contrast is compared against the model and yield a good agreement (Fig. 6.5 **f**)).

The timescales in  $g^{(2)}$  reflect the typical timescales in the rate matrix (Eq. 6.13). The broad range of bunching timescales (Fig. 6.4 e) and f)) thus hint at the origin of the variability in ODMR being the intricate balance between various rates used in the rate matrix Eq. 6.13. By tuning the six parameters involved, it is possible to recreate the positive and negative ODMR contrasts, the laser power dependence of the ODMR contrast.

The optical contrast of ODMR is as a result of competition between the ground-state optical pumping, and the spin-dependent photoluminescence. Provided  $k_{43} = k_{23}$ , a larger  $k_{34}$ would polarise the system into state 4. On other hand, provided  $k_{31} = k_{34}$ , a larger  $k_{23}$  would lead to an optically dark state 2. Thus the emitter would show negative ODMR if optical pumping polarises the ground-state into the bright state (for example in NV centres in diamond) and would show positive ODMR otherwise.

In Fig. 6.4 e) and f), we present the wide range of bunching rates measured for our defects

that do and do not show ODMR. Below we give evidence for the tunability of these rates in hBN defects leading to the positive and negative ODMR that we observe. We find that the ODMR contrast and sign predicted by the model is highly sensitive to the balance of transition rates (Fig. 6.5 b)). We use the model to compare the effect on the ODMR contrast when the shelving and de-shelving rates are tuned. In Fig.6.5 b), when the ratio of  $k_{23}/k_{53}$ , the two intersystem rates that convert population from the excited state to the meta-stable state (see fig inset), is varied between 0.01 and 100, the ODMR contrast always remain negative. In this simulation the two de-shelving rates ( $k_{31}$  and  $k_{34}$ ) are the same. However, when the deshelving rates are asymmetrical ( $k_{34} \ll k_{31}$ ), the contrast can be positive or negative depending on  $k_{23}/k_{53}$ . This model also shows how defects do not show ODMR when the shelving and de-shelving rates are symmetrical.

### 6.3.3 Fine structure revealed by ODMR

#### 6.3.3.1 Observation of fine structure

The aforementioned ODMR experiments have been performed with high MW power beyond saturation to result in a single ODMR peak. To reveal any sub-linewidth structures in the ODMR spectrum, we operate at low MW driving conditions to avoid power broadening. The MW power is  $\sim P_{sat,MW}$  as extracted from a saturation measurement similar to that in Fig. 6.3 d). Most defects (~80%) display a doublet ODMR (three examples are present in Fig. 6.6 c), d) and g)), while for others no splitting is resolved, which we treat as singlets (Fig. 6.6 e), f) and h)). The singlets could either correspond to a single spin transition, or two transitions showing below-resolution doublet splittings. The doublet feature is unchanged with the magnetic field at 7 mT, 14 mT, 25 mT and 89 mT (Fig. 6.6 a)) and both peaks increase linearly with increasing magnetic field (Fig. 6.6 b)). The lower field (below 100 MHz splitting) behaviour is complex, confounded by the low ODMR contrast at low field, and inaccuracies in field calibration. The data is thus not very well understood and thus not presented here.

For doublet defects, we fit the ODMR spectra with two independent Lorentzian, as would be expected for two homogeneous broadened ODMR [69]. The peak separation for defect A is ~30 MHz with a single Lorentzian linewidth (FWHM) of ~20 MHz. The splitting ranges from 19 MHz to 50 MHz with a mean of 34(8) MHz.

(The following modelling is based on experiments taken by H.S., J.J, and S.E., with modelling and testing by Q.G.)



Figure 6.6: Fine structure revealed by ODMR. **a**) defect A displays a double ODMR at both low and high fields and **b**) both peaks shift with magnetic field linearly. Both singlet and doublet defects have been observed **c**)-**h**) in our experiments. A doublet ODMR could be explained by zero-field splitting of **i**) a spin-1 or **j**) a spin- $\frac{3}{2}$  systems.

### 6.3.3.2 Modelling fine structure

ODMR provides a simple method to deduce the spin level structure of a spin manifold. The experimental observation suggests that the MW addressable states have a small splitting. The zero-field energy splitting can often be attributed to zero-field splitting (ZFS) due to spin-orbit interaction (SOI) and spin-spin interaction of magnetic dipolar origin, and electron-nuclear interaction (Hyperfine Interaction, HFI). Another possibility is that the two peaks correspond to transitions in two separate manifolds, but it is unclear how such a scenario would result in constant splitting with magnetic field.

The SOI is challenging to treat theoretically especially when the *g*-factor does not significantly deviate from 2. We thus consider the possibility of HFI and ZFS.

**Hyperfine coupling** The hyperfine interaction (HFI) is fundamentally due to the magnetic dipolar coupling between a nucleus and an electron spin. The general form of the Hamiltonian of a spin- $\frac{1}{2}$  ground state with HFI is

$$H = H_Z + H_D + H_{HFI}.$$

The HFI term is given by

$$H_{HFI} = S \cdot A \cdot I,$$

where A is the  $(3 \times 3)$  hyperfine tensor consisting of an isotropic and an anisotropic part,

$$A = A_{iso}I + T,$$

with  $A_{iso} = \rho_S \cdot \frac{2}{3} \frac{\mu_0}{\hbar} g_e \mu_B g_n \mu_n |\psi_0(0)|^2$  given by the Fermi contact interaction, and,

$$T_{ij} = \frac{\mu_0}{4\pi\hbar} g_e \mu_B g_n \mu_n \left\langle \psi_0 \left| \frac{3r_i r_j - \delta_{ij} r^2}{r^5} \right| \psi_0 \right\rangle,$$

which reflects the non-contact magnetic dipolar interaction. The anisotropic coupling tensor is traceless,

$$\operatorname{Tr}(T) = \sum_{i} T_{ii} \propto \left\langle \frac{3\sum_{i} r_{i}^{2} - 3r^{2}}{r^{5}} \right\rangle = 0.$$

It is also clear that the hyperfine tensor is real-symmetric and thus can be diagonalised in its eigen-bases. The HF interaction can be reduced (in its eigenbasis) to

$$H_{HFI} = A_z S_z I_z + A_x S_x I_x + A_y S_y I_y.$$

Nucleus	spin, I	Abundance
<sup>10</sup> B	3	20%
<sup>11</sup> B	$\frac{3}{2}$	80%
<sup>14</sup> N	1	99.6%
<sup>15</sup> N	$\frac{1}{2}$	0.4%
$^{12}C$	Ō	98.9%
<sup>13</sup> C	$\frac{1}{2}$	1.1%

Table 6.2: Spin isotopes present in hBN.

Unlike in NV centres where the system symmetry dictates that  $A_x = A_y = A_{\perp}$ , it is not possible to further reduce this HF tensor without more assumptions.

At experimentally relevant magnetic field, the Zeeman interaction dominates over the HFI and thus sets the quantisation axis. As a result the electron and nuclear spin operators can be replaced by  $m_s S_z$  and  $m_I I_z$  respectively, reducing the HFI Hamiltonian to [252]

$$H_{HFI} = A'_z m_I m_s S_z I_Z,$$

where  $A'_{z} = \hat{u} \cdot A \cdot \hat{u}$  with  $\hat{u}$  being the unit vector in the direction of the magnetic field.

For a spin-1/2 nucleus, this leads to two transitions  $|\pm \frac{1}{2}, \pm \frac{1}{2}\rangle \rightarrow |-\frac{1}{2}, \pm \frac{1}{2}\rangle$  and  $|\pm \frac{1}{2}, -\frac{1}{2}\rangle \rightarrow |-\frac{1}{2}, \pm \frac{1}{2}\rangle$  with the states labelled as  $|m_s, m_I\rangle$ . The two transitions have energies  $\mu_B gB \pm \frac{1}{2}A'_z$ , giving a splitting of  $A'_Z$ . For nuclei with higher total spin, there would be (2I + 1) transitions. As only a doublet is observed experimentally, only a spin- $\frac{1}{2}$  nucleus can give rise to such a doublet splitting. The nuclear species in carbon-doped hBN are listed in Table 6.2. The intrinsic <sup>15</sup>N is present with a very small percentage (0.4 disproportional to the doublet we observed in ODMR. Since every other nuclear species in hBN host lattice has  $I > \frac{1}{2}$ , it is unlikely any would give rise to a doublet in ODMR. The externally doped <sup>13</sup>C with a nuclear spin of  $\frac{1}{2}$  only has an abundance of 1.1%, inconsistent with the observed percentage of doublet ODMR (~ 5%). It is therefore unlikely the splitting is due to HFI.

**Spin-orbit coupling** In NV centres, the ground-state ZFS is mainly due to spin-spin magnetic dipolar coupling. The first-order contribution from the spin-orbit coupling of the form  $\sim \sum_j s_j \cdot \Omega_j$  where  $s_j$  is the electron spin operator for the constituent electron j and  $\Omega_j$  is the corresponding orbital operator

$$(\Omega_j)_k = \frac{1}{2}m^2c^2\left[\nabla V(r_j) \times p_j\right]_k,$$

where V is the local electric potential, p is the momentum operator, m is the electron mass and c is the speed of light.

The SOI creates the energy difference between states of different *S* ( ${}^{3}A_{2}$  and  ${}^{1}E$  states) but does not contribute to the ZFS due to symmetry considerations [150]. In this derivation, since both  $s_{j}$  and  $\Omega_{j}$  transform as axial vectors, the first order energy correction can be deduced with a group theory approach by examining terms like  $\langle \phi | \Omega_{j} | \phi \rangle \langle v | s_{j} | v \rangle$  where  $| \phi \rangle$  and  $| v \rangle$ are orbital and spin parts of the ground-state single-electron wavefunction. For NV centres in particular, this first order energy correction vanishes and thus the spin-orbit coupling only contributes to second order.

For the particular defect, it is unclear if spin-orbit interaction would lead to any splittings detectable by ODMR, without knowledge of the exact defect symmetry, charge state and energy ordering. Further experimental evidence combined with ab initial simulation need to be explored to answer this question.

**ZFS model due to spin-spin interaction** A simple model to explain the experimentally observed ODMR spectrum is a  $S \ge 1$  system with the following Hamiltonian,

$$H = H_{ZFS} + H_Z + H_D, \tag{6.15}$$

where the zero-field splitting and the Zeeman terms bear resemblance to the NV Hamiltonian (Chapter 1 Eq. 1.2).  $H_D$  is the microwave driving term. The ZFS term is expressed in the frame (molecular frame) formed from principal axes of the ZFS tensor and the Zeeman term is expressed as

$$H_{ZFS} = DS_z^2 + E(S_x^2 - S_y^2)$$
$$H_Z = \mu_B g \mathbf{B} \cdot \mathbf{S}.$$

The microwave creates a AC magnetic field  $W(t) = W_0 \cos \omega_D t$ , which contributes a driving term  $H_D$ ,

$$H_D = \mu_B g S \cdot W(t).$$

The model here is by no means exhaustive, as a general Hamiltonian of a spin S > 1/2 system requires higher order terms such as  $S_z^4$ . Such higher order terms are often represented with the extended Stevens operators (ESO) in the form  $H = \sum_{k=2,4,6} \sum_{q=-k}^{k} B_k^q \hat{O}_k^q(S)$  [118]. For a simple model to explain the data, higher order terms are not considered here. S = 1 and  $S = \frac{3}{2}$  systems are common among single defects such as NV centres in diamond, silicon vacancy centres in SiC, excitons in organic systems such as pentacene and DPH, VB<sup>-</sup> defect

in hBN, to name a few.

When the MW drive is sufficiently weak compared with the ZFS or the Zeeman terms, the driving term can be treated as a time-dependent perturbation and the transition probability  $\Gamma_{i\rightarrow f}$  due to the drive for CW ODMR can be computed with Fermi's golden rule,

$$\Gamma_{i \to f} \propto |\langle i | W \cdot S | f \rangle|^2 \delta \left( \omega_D - |E_i - E_j| \right)$$

where  $|i\rangle$  and  $|f\rangle$  are the initial and final states, and are eigenstates solve from the time-independent Hamiltonian,

$$H = H_{ZFS} + H_Z. \tag{6.16}$$

Apart from the spin transition probability, the ODMR contrast is also affected by the optical contrast between the two states, which is not included in the model. In fact in a spin-3/2 system in Silicon Vacancy centres in SiC, the  $\left|-\frac{1}{2}\right\rangle \rightarrow \left|+\frac{1}{2}\right\rangle$  transition are spin-allowed transition but is not observed in ODMR because of the lack of optical contrast. A more involved model with Lindblad's equation can account for both the spin transition and the optical transition [93].

To determine which spin model is applicable, it is necessary to measure each contributing Hamiltonian due to ZFS and HFI, which consists of tensors that could have in general 6 (ZFS) or 9 (HFI or g-aniostropy) independent components. The lab frame in which the experiments are carried out, the principal axes of the local site symmetry of the defect, and the principal axes of the g, A, D-tensors may not align. As such determining each tensor, if their principal axes do not align, generally requires sweeping the magnetic field in 2-4 planes [158, 200]. This is not permitted in our current sample where emitters tend to bleach after a few weeks of measurements. Thus to fit the model, we assume one tensor to be the dominating tensor and determine if the model is consistent with experimental data.

In principle the ODMR spectrum due to  $S \ge 1$  ZFS as magnetic field is changed in direction can be numerically simulated. However analytical approximation exists at high field where the Zeeman term dominates and sets the quantisation axis [119, 252]. The ZFS term in the Hamiltonian (see eq. 6.15) can be treated as perturbations.

The Zeeman term contributes only to zeroth order,

$$E^{(0)}(m_s) = \langle m_s | H_Z | m_s \rangle = \mu_B g B m_s.$$

To derive the first-order perturbation due to the ZFS, the following form of the ZFS Hamilto-

nian can be used,

$$H_{ZFS} = (u \cdot D \cdot u)S_z^2 + \frac{1}{4}D_0(S_+S_- + S_-S_+) + \frac{1}{2}D_2^*(S_zS_+ + S_+S_z) + \frac{1}{2}D_1(S_zS_- + S_-S_z) + \frac{1}{4}D_2^*S_+^2 + \frac{1}{4}D_2S_-^2, \quad (6.17)$$

with,

$$D_0 = -(u \cdot D \cdot u)$$
  

$$D_1 = (u \cdot D \cdot i) + i(u \cdot D \cdot j)$$
  

$$D_2 = (i \cdot D \cdot i) - (j \cdot D \cdot j) + 2i(i \cdot D \cdot j)$$

where *u* is the unit vector u = B/|B| and *i*, *j*, *u* together form a right-handed orthonormal basis. To first order,

$$\Delta E^{(1)}(m_s) = \langle m_s | H_{ZFS} | m_s \rangle = -\frac{1}{2} \left( \hat{u} \cdot D \cdot \hat{u} \right) \left[ S(S+1) - 3m_s^2 \right]$$

where, conveniently, only  $\langle m_s | S_z^2 | m_s \rangle$  and  $\langle m_s | S_+S_- + S_-S_+ | m_s \rangle$  terms in Eq. 6.17 contribute to the first order energy correction. For S = 1, this equation gives two transitions at  $\left| \Delta E^{(1)}(m_s = +1) - \Delta E^{(1)}(m_s = 0) \right| = \left| \frac{1}{2} (\hat{u} \cdot D \cdot \hat{u}) + \mu_B g B \right|$  and  $\left| \Delta E^{(1)}(m_s = -1) - \Delta E^{(1)}(m_s = 0) \right| = \left| \frac{1}{2} (\hat{u} \cdot D \cdot \hat{u}) - \mu_B g B \right|$ . A doublet feature with separation  $\Delta = 3 (\hat{u} \cdot D \cdot \hat{u})$  is resulted. For S = 3/2, two transitions at  $\left| \Delta E^{(1)}(m_s = +3/2) - \Delta E^{(1)}(m_s = +1/2) \right| = \left| 3 (\hat{u} \cdot D \cdot \hat{u}) + \mu_B g B \right|$  and  $\left| \Delta E^{(1)}(m_s = -3/2) - \Delta E^{(1)}(m_s = -1/2) \right| = \left| 3 (\hat{u} \cdot D \cdot \hat{u}) - \mu_B g B \right|$  would be observed with a doublet separation of  $\Delta = 6 (\hat{u} \cdot D \cdot \hat{u})$ .

# 6.3.4 Angular dependence of ODMR fine structure on external magnetic field

#### 6.3.4.1 Modelling angular dependence

The above modelling shows that a constant splitting,  $\Delta$ , would be observed at high field. Figure. 6.6 i) and j) show the magnetic field dependence of ODMR predicted by a spin-1 model and a spin-3/2 model based on numerically solving for the eigenvalues of Eq. 6.16 respectively. The bright blue feature is the simulated ODMR peak positions as a function of magnetic field, showing two lines having a constant separation,  $\Delta$ , at different magnetic fields. This well predicts the magnetic field dependence observed in Fig. 6.6 b).

If the magnetic field is swept in a plane perpendicular to the normal  $\hat{n} = (n_{x_i}, n_y, n_z)$ , then

the splitting is

$$\Delta = 3 \left( D_x u_x^2 + D_y u_y^2 + D_z u_z^2 \right)$$
  
s.t.  $n_x u_x + n_y u_y + n_z u_z = 0$ ,  
and  $u_x^2 + u_y^2 + u_z^2 = 1$ 

where

$$D_x = E + \frac{1}{3}D$$
$$D_y = -E + \frac{1}{3}D,$$
$$D_z = -\frac{2}{3}D$$

are the eigenvalues of the ZFS tensor. In the special case when the magnetic field is swept around the principal axes, the corresponding splittings are

$$\begin{split} \Delta_z &= -D + 3E\cos 2\theta, \\ \Delta_x &= -\frac{1}{2} \left( 3E + D \right) - \frac{1}{2} \left( 3E + 3D \right) \cos 2\theta, \\ \Delta_y &= \frac{1}{2} \left( 3E + D \right) - \frac{1}{2} \left( 3E + 3D \right) \cos 2\theta, \end{split}$$

where  $\theta = 0$  aligns along x, y and z respectively. For rotations sweeping an arbitrarily oriented plane, the high-field splitting is of the general form,

$$\Delta = \alpha + \beta \cos 2\theta + \gamma \sin 2\theta \equiv \alpha + \rho \cos \left[2(\theta - \theta_0)\right].$$

This has a periodicity of 180°. However since experimentally the absolute value of the splitting is observed, the periodicity may turn out to be 90°. At angle  $(\theta - \theta_0) = \frac{1}{2}\cos^{-1}(\alpha/\rho)$  the ODMR turns from a doublet to a singlet.

To numerically simulate effect of the orientation of the magnetic field on ODMR splitting, the orientations of magnetic field and MW polarisation also need to be accounted for. Experimentally the microwave is delivered by a microstrip which gives a linear out-of-plane MW polarisation. The magnetic field can be applied in a range of directions in the lab frame. In the lab frame, the magnetic field vector,  $B_L$ , are measured and the MW polarisation,  $W_L$ , is assumed to be out of the sample plane. In the molecular frame, these vectors have new components,  $B_M$  and  $W_M$ , according to the transformation,

$$B_M = RB_L, W_M = RW_L, \tag{6.18}$$

where  $R = R(\phi, \theta, \psi)$  is the rotation matrix relating to vectors in the lab frame, to vectors in the molecular frame, corresponding to the set of (intrinsic) Euler's angles following the *zxz* convention.

The angular dependence is compared against experimental observation. Figure 6.7shows the ODMR results for two separate defects as the magnetic field is swept in-plane and out-ofplane. For defect A (Fig. 6.7 **a**)), a doublet transitions into a singlet when the magnetic field is moved from in the sample plane (red) to out of the sample plane (orange). In addition, for defect E (Fig. 6.7 **d**)), the ODMR peak separation goes through a transition from a doublet to a singlet. The results are fitted to transition frequencies predicted by Eq. 6.7, with magnetic field orientation accounted for by Eq. 6.18.

In the fitting procedure, the lab frame magnetic field orientations are calibrated and known (coloured arrows in Fig. 6.7 c) and f)). The orientation of the principal axes of the defect, described by the three Euler's angles, is not known. The ZFS parameters D and E are also unknown, thus giving 5 free parameters ( $\phi, \theta, \psi, D, E$ ). It was found that the ZFS parameters D and E can be fixed to D = 25 MHz, E = 5 MHz across different emitters and still fit the ODMR results observed (Fig. 6.7 a) and d)). The ODMR spectra predicted by the model for various angle swept in the xz plane (for defect A, Fig. 6.7 b)) and in the yz plane (for defect E, Fig. 6.7 e)) are shown and the corresponding measured angles are shown as dashed lines, with the same colour code as data in Fig. 6.7 a) and d).

From the fitted orientations, the z direction of the defect A is along the optical axis. However defect E is not orientated in any special orientations. Contrary to common beliefs, the hBN layer is not entirely flat; certain areas are wrinkled. This would explain the arbitrary orientation of defect E. In addition to the two emitters presented in Fig. 6.7, 6 other emitters have also been fitted to this model and yield good agreement. These 8 emitters constitute all angular dependence data we collected.

# 6.4 Discussion

### 6.4.1 Relating to *ab initial* predictions of defect structure

Finally, we review the *ab initial* predictions in existing literature to find potential candidates consistent with the observed spectrum, optical structure and spin transitions. In deducing NV centre structures, *ab initial* results provide valuable insights into defect energy level structure, symmetry and their response to external strain, magnetic and electric fields [64, 67]. Various flavours of Density Functional Theory (DFT) are used for this purpose. DFT models predict the relative ordering of molecular orbitals, their energy separation, phonon energy, the ZFS



Figure 6.7: ODMR angular dependence. The below-saturation ODMR spectra are presented for **a**) defect A and **d**) defect E. For defect A, the ODMR is taken at two magnetic field orientations, along the z axis (red) and in the x-y plane (orange) parallel to the sample. The orientation of the magnetic fields relative the MW magnetic field and sample plane are illustrated by the coloured arrows in **c**). **b**) shows the ODMR predicted by a spin-1 model as the angle swept out by magnetic field in the xz plane. The corresponding slices shown in **a**) are indicated by dashed lines in **b**). A similar measurement are shown for defect E and the magnetic field is swept in a plane normal to the optical axis (x-axis). A constant ZFS parameters, D = 25 MHz, E = 5 MHz, have been used for both defects.

and HFI parameters. Although DFT provides valuable insights, it fundamentally solves for the ground state and misses important properties of the excited states, such as excitonic effects [89].

Prior to result published by Chejanovsky *et al*, and Mendelson *et al*, carbon-related defect have attracted significant attention [195, 196, 230], narrowing down from more general class of carbon containing emitters to emitters that show optical transitions in the visible range. New discussions arose from the experimental observation of carbon emitters in the visible range, with a paramagnetic ground state. The small ZFS and spin- $\frac{1}{2}$  characteristics reported by [43] lead to discussion of defects containing one, two and three substitutional carbon atoms without any vacancies [22].

Work by Jara et al [123] and Sajid et al [195] modelled data by Mendelson et al [163].

Auburger et al modelled data by Chejanovsky et al [43].

All DFT work rules out single neutral carbon substitutional defect based on its high ZPL resulted from only a single defect state in the bandgap. Both neutral, positively and negatively charged carbon dimers resulted from neighbouring carbon atoms ( $C_N C_B^{-,0,+}$ ) are ruled out based on the large ZPL in [22, 123, 230]. However carbon dimer formed from more distant pairs of carbon may decrease the ZPL to a suitable range [22]. Nevertheless, this work focused on  $S = \frac{1}{2}$  systems and thus did not discuss possibility of a spin-1 system and its level structure.

Furthermore, systems formed of three [22, 93, 123] and four connected carbon atoms [123] have also been investigated .  $C_2C_N^0$  has  $E_{ZPL} = 1.62 \text{ eV}$  with  $C_2C_B^0$  having a similar energy [123]. The charged forms of these trimer has  $E_{ZPL} > 3 \text{ eV}$  and therefore have also been ruled out. Although, Auberger *et al* predicts lower ZPL for  $C_2C_B^0$ [22]. Golami *et al* [93] studied in detail a  $C_2C_N$  model. In order to see ODMR this model requires a transition with 2.6 eV to be excited, which might be possible via a two-photon absorption process. Nevertheless this model has a doublet ground state and the ZFS for the quartet shelving state has not been calculated for meaningful comparison. Neutral and charged tetramers have too large or too small ( $E_{ZPL} < 1 \text{ eV}$ ) and are both ruled out [123].

Finally, the  $C_N V_B$  and  $C_B V_N$  are very promising candidates.  $C_N V_B$  is predicted to have a triplet ground state, while  $C_B V_N$  is predicted to have a Singlet GS, with an energy uncertainty that may also give rise to a Triplet GS [195, 196]. The D parameter is predicted to lie within GHz range, thus incompatible with the observation. Despite this concern, Sajid *et al.* still assigned  $C_B V_N$  as the origin of defects observed in [163].

Among the probable models,  $C_B V_N^-$  was adopted by the authors to explain their emitters. This defect has a ground state quartet,  ${}^4A_2$ , and the radiative transition is dominated by  $a_1(\sigma) \rightarrow b_2(\sigma)$ , polarised in-plane, and is in principle not detected by the collection optics. Additionally, as the ground state is formed from  $a_1(\sigma)$ ,  $b_1(\pi)$ ,  $b_2(\sigma)$ , the sigma bond contribution would give significant overlap with the nuclei, thus significant hyperfine splitting, which was not observed either in this report, or in Mendelson *et al.* Furthermore, as the  $C_B V_N^-$  is localised to within 3 local bonds, the short distances between electrons also gave rise to strong spin-spin interaction, and thus a strong ZFS, which is observed in similar systems like NV centres, or predicted by other DFT calculations [196], thus making defects like  $C_B V_N$  or  $C_N V_B$  unlikely.

In summary, the carbon-vacancy species such as  $C_N V_B$  and  $C_B V_N$  are promising candidates, although their ZFS are all in the GHz range. On the other hand, the carbon trimers  $(C_2C_B, C_2C_N)$ , or charged form of carbon-vacancy defects  $(C_N V_B^-)$ , can also give  $S = \frac{3}{2}$  spin manifold but more work is required to give predictions for the ZFS. The results discussed above have been summarised in Table 6.3.

Atomic Structure	Comments	ZPL	Ground State	ODMR active state	Ref
C <sub>N</sub>	Single Transition in Bandgap	-	-	-	[22, 123, 196, 230]
C <sub>B</sub>	Single Transition in Bandgap	-	-	-	[22, 123, 230]
C <sub>B</sub> V <sub>N</sub>	D=8.6 GHz	1.44 eV[230] 2.08 eV [196] 1.74 eV [163] 1.75 eV [195]	${}^{1}A_{1}$	<i>S</i> = 1	[163, 195, 196, 230]
C <sub>N</sub> V <sub>B</sub>	D=7.15 GHz	1.93 eV[230] 1.58 eV[196] 1.40 eV [163]	${}^{3}A_{2}$	S = 1	[163, 196, 230]
C <sub>B</sub> N <sub>B</sub> V <sub>N</sub>		1.00 eV[230]	-	_	[230]
C <sub>N</sub> V <sub>B</sub> <sup>-</sup>	-	1.72 eV [163]	${}^{4}A_{2}$	$S = \frac{3}{2}$	[163]
$C_2^+$	-	1.814 eV to 2.229 eV [22]	-	$S = \frac{1}{2}$	[22, 123]
$C_2C_B$	-	1.360 eV [22]	${}^{4}A_{2}$	$S = \frac{3}{2}$	[22, 123]
C <sub>2</sub> C <sub>N</sub>	-	1.623 eV [22] 1.62 eV [123]	${}^{4}A_{2}$	$S = \frac{3}{2}$	[22, 123]
C <sub>4</sub>	-	-	-	$S = \frac{1}{2}$	[123]
Double Defects	Two oppositely charged monomers	-	-	_	[123]
C <sub>2</sub> C <sub>N</sub>	-	1.6 eV [93]	$^{2}A_{2}$	$S = \frac{1}{2}$	[93]

Table 6.3: List of DFT calculations performed on defects containing only carbon impurities. The zero-phonon line is a typical parameter presented in DFT studies. Not all studies give prediction for the ZFS parameters. Those do are listed in the table.

$R_0$ (symbolic)	Value (Å)	$\frac{\mu_0}{4\pi\hbar}(g_e\mu_B)^2/R_0^3~(2\pi imes \mathrm{MHz})$
-	1	8,283
a	1.446	2,739
2a	2.892	343
4a	5.784	43
b	6.667	28
2b	13.334	3.5

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Table 6.4: Prefactor for two magnetically dipolar-coupled electrons. a is the in-plane bond length and b is the interlayer bond length for hBN. The numbers used for  $R_0$  are the common inter-atomic distances in hBN.

### 6.4.2 Magnitude of ZFS

The origin of the ZFS due to spin-spin interaction is from the magnetic dipolar interaction between electrons present in  $S \ge 1$  systems. For a pair of electrons, the spin-spin interaction parameters can be calculated as

$$D_{ij} = \frac{\mu_0}{4\pi\hbar} (g_e \mu_B)^2 \int \frac{3r_i r_j - \delta_{ij} r^2}{r^5} n(r_1, r_2) dr_1^3 dr_2^3, \tag{6.19}$$

where the joint electron density  $n(r_1, r_2) = |\psi(r_1, r_2)|^2$ ,  $r = |r_1 - r_2|$ . The pre-factor is tabulated in Table 6.4 for common dipolar interaction geometries. The integral in Eq. 6.19 is of dimension  $r^{-3}$  and thus the ZFS parameter  $D = \frac{\mu_0}{4\pi\hbar} (g_e \mu_B)^2 \frac{1}{R_0^3} \Gamma$ , where the characteristic distance  $R_0$  is used to non-dimensionalise the integral. The values of the prefactor for typical distances in the hBN lattice has been tabulated in Table 6.4. If the  $\Gamma \sim 1$ , the two electrons need to be ~5 Å away to achieve the observed ZFS. This is very distant in the in-plane direction, but is approximately the interlayer distance in hBN.

Equation 6.19 cannot be evaluated easily without access to the charge density distribution, which a DFT-based model could predict. Qualitatively, since the electron wavefunction affects the averaged distance between constituent electrons, such systems would have a zerofield splitting parameter that can be tuned by compressing the electron wavefunction via electric field, local strain, or temperature induced host material expansion, which would serve as proofs for non spin half system.

### 6.5 Conclusion

In this study we confirmed the observation of spin-addressable single emitters in hexagonal boron nitride at room temperature. The hBN defects presented in this chapter initially seem to
display a wide variability in ODMR contrast,  $g^{(2)}$  and ODMR splitting. However as discussed, this variability in fact can be captured by a simple 5-level optical energy structure where the transition rates are variable from defect to defect. This explains the observation of the range of ODMR contrast observed going from positive to negative, and the large proportion of defects not showing ODMR. The observation of both doublet and singlet ODMR could be explained by the differences in orientation of defects. Thus despite the highly variable observations, our results and analysis show that they may arise from a single type of defect.

Building on the kinetic and spin models, we found that the presence of ODMR is correlated with the appearance of a new bunching timescale, which can be used to screen ODMR-active defects. The exceptional variability of hBN optical transition rates may reflect the tunability of the defect energy levels in this 2D system via local strain. This could open routes to a new room-temperature spin-photon interface where spin readout can be reversibly and easily tuned for use in sensing applications. The prevalence of nuclear spins would also potentially enable memory-assisted quantum networks.

This new class of emitters have very small zero-field splitting and display ODMR at least up to 4 GHz (~142 mT). NV centres on the other hand has large ZFS. Although this gives NV the ability for vector magnetometry, it also limits the strength of magnetic field measurements, which is crucial in the trending nanoscale scanning NV magnetometry on magnetic materials [219]. In addition, unlike NV centres in diamond, the optical lifetime of this class of hBN emitters is 10 times shorter, thus allowing a faster optical readout.

In our study, we have not been able to infer the atomic structure of the defect. The comparison with DFT-based theories have also been limited by the lack of work on predicting the zero-field splitting parameters to be compared with experiments. Furthermore, more pulsed experiments such as spin-echo would reveal the nuclear environments.

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# **Appendix A**

## **Fisher information formalism**

Many experimental data presented in the main text require regression against a known model. Thus the property of curve fitting and how well the fitting parameters can be fitted from a set of noisy data needs to be thoroughly investigated.

Fisher information describes the information content of a given experimental design with respect to a model function. The Fisher Information theory provides a way to estimate the lower bound without addressing the details of how this bound is achieved, thus encourages exploration of the best possible fitting method.

In this section the Fisher information, nonlinear least square fitting and its biasedness are discussed.

#### A.1 Cramér-Rao Bound

The Cramer-Rao Bound is the best fitting error achievable with a given experimental design. Specifically, given a set of independent variables  $w = [w_1, w_2, ..., w_i]$  and a parametric functional model  $f(w, \theta)$ , with  $\theta \in \mathbb{R}^n$  being the parameter vector, any unbiased estimator,  $\varepsilon_{\theta}$ , of the parameters  $\theta$ , based on the outcome of measurements,  $c_i$ , at these independent variables, can achieve at best a covariance matrix of (in the positive-definite sense)

$$\operatorname{Cov}(\hat{\boldsymbol{\theta}}) \geq I^{-1}(\boldsymbol{\theta}).$$

where  $I(\theta)$  is the Fisher Information matrix, defined as

$$I(\theta) = \mathbf{E}\left[\left(\frac{\partial}{\partial\theta}\ln p_{\theta}(w)\right)^{T}\left(\frac{\partial}{\partial\theta}\ln p_{\theta}(w)\right)\right],$$

with  $p_{\theta}(w)$  being the probability of observing  $c_i$  at locations  $w_i$  given parameters  $\theta$ , and E the expectation value.  $\frac{\partial f}{\partial \theta} \equiv \theta \cdot \nabla f = \begin{bmatrix} \frac{\partial f}{\partial \theta_1}, & \frac{\partial f}{\partial \theta_2}, & \cdots, & \frac{\partial f}{\partial \theta_{N-1}}, & \frac{\partial f}{\partial \theta_N} \end{bmatrix}$  is a N-dimentional vector, formed form partial derivatives with respect to each component of the N-dimentional vector  $\theta = \begin{bmatrix} \theta_1, & \theta_2, & \cdots, & \theta_{N-1}, & \theta_N \end{bmatrix}$ . A different form of this theorem is

$$(\hat{\boldsymbol{ heta}} - \boldsymbol{ heta}) 
ightarrow \mathcal{N} \left( 0, I^{-1}(\boldsymbol{ heta}) 
ight),$$

i.e. the error in the estimation of  $\theta$  is (multi-dimensionally) normally distributed with a mean of zero (unbiasedness) and variance equal to the Cramer-Rao lower bound. The set *w* is the experimental design, and the set  $w_{opt}$  which gives the "optimal" covariance matrix is the optimal design. Different criteria of "optimality" exists; for example the *D*-optimality condition seeks to minimise the determinant of the covariance matrix by

$$w_{opt} = \underset{w}{\operatorname{argmin}} \left| I(\theta)^{-1} \right|$$

In addition, as the parameter  $\theta$  are often also inferred experimentally, they are best described by a statistical distribution rather than concrete values. As such the optimal design should take into account the variability of these parameters. Multiple approaches exist. For example the *maximin* criteria in a *D*-optimal design [60],

$$w_{opt-maximin} = \underset{w}{\operatorname{argminmax}} \left| I(\theta)^{-1} \right|,$$

finds a design that minimises the worst-case D-optimal design.

It is not immediately true that the Fisher information matrix is invertible. In fact  $I(\theta)$  can have a rank ranging from 1 (as  $I(\theta)$  is not trivial) to the full-rank. When  $I(\theta)$  is not invertible, the asymptotic normal distribution limit will not hold and in special circumstances a lower bound can still be derived [193]. However these cases can be avoided by choosing w carefully. Also experimentally, redundancy is preferred as a check on the self-consistency of the experimental noise model.

The covariance is dependent on the specific choice of design and on the functional model. Applied to case of ODMR measurements, we wish to use a Lorentzian model, and measurements at MW frequencies  $f_k$ , to infer the NV ground state transition frequency  $f_0$ . This problem is treated in detail in [42, 175, 187, 258] for super-resolution imaging and is here adapted to sampling a Lorentzian lineshape.

#### A.1.1 Application to readout duration in a pulsed experiment

In a typical pulsed experiment, the NV spin state is read out optically. In this procedure, the laser is turned on (for about 1 microsecond) and the photoluminescence (PL) as a function of optical illumination shows a time dependence, as shown in Fig. 1.9 **a**). The initial photons are more informative than the later photons, as the NV is optically polarised with continuous illumination. With the hardware used in this work, it is possible to adjust the start and stop of the APD window, so that only the most informative photons are captured, leaving out the not informative photons, so as to avoid photon shot-noise.

The NV ground state, which can be either in  $m_S = 0$  or  $m_S = \pm 1$  states at the collapse of spin-state superposition during readout. The NV state thus follows a Bernoulli distribution, with parameter,  $1 - \theta$ ,

$$\psi \sim \operatorname{Ber}(1-\theta)$$

where  $\psi$  is a random variable such that  $\psi = 0$  denotes  $m_S = 0$  and  $\psi = 1$  denotes  $m_S = \pm 1$ . The Bernoulli distribution implies the probability of NV in  $m_S = 0$  is given by  $p(m_S = 0) = \theta$ and in  $m_S = \pm 1$  given by  $p(m_S = \pm 1) = (1 - \theta)$ . The task of a readout pulse is to extract the parameter  $\theta$  so as to uncover the spin state right before readout.

The count rate at any delay after laser onset,  $\tau$ , can be experimentally measured as described Fig. 1.9 a), and is denoted by  $\tilde{f}(\tau|\psi)$ . The curves presented in Fig. 1.9 a), correspond to  $\tilde{f}(\tau|\psi=0)$  (blue) and  $\tilde{f}(\tau|\psi=1)$  (red), respectively.

For NV centres with conventional readout procedure, the photon counts per readout is far less than one. Therefore many repeats of the same experiment is needed. Each time the spin state is collapsed, there is a chance of  $\psi = 0$  with probability  $\theta$  or  $\psi = 1$  with probability  $(1 - \theta)$ . After many repeats of these measurements, the final expected PL at delay  $\tau$  is  $f(\tau|\theta) = \sum_{\psi=0,1} \tilde{f}(\tau|\psi)p(\psi)$ . Denoting  $c_0(\tau) = \tilde{f}(\tau|\psi=0)$  and  $c_{\pm 1}(\tau) = \tilde{f}(\tau|\psi=1)$ , then

$$f(\tau|\theta) \equiv c_0(\tau)\theta + c_{\pm 1}(\tau)(1-\theta).$$

This quantity would not be necessary should we be able to to single-shot readout. Unless in a dedicated calibration experiments where the APD window is shifted relative to the laser in time, this function cannot be measured conveniently with our hardware as the photon arrival times are not lost. Instead all photons arrived within the start and stop times, denoted  $t_1$  and  $t_2$ is integrated. Denote the PL obtained during this time by

$$F(t_1, t_2|\theta) = C_0(t_1, t_2)\theta + C_{\pm 1}(t_1, t_2)(1-\theta),$$

where  $C_0(t_1, t_2) = \int_{t_1}^{t_2} c_0(\tau) d\tau$  is the integrated count per readout if the NV is in  $m_S = 0$  state

and similarly for  $C_{\pm 1}$ . For conciseness, denote  $F(\theta) = F(t_1, t_2 | \theta)$ .

To estimate  $\theta$  in a pulsed experiment, a set of values  $F(\theta)$  is measured as photon counts. So far the randomness in  $F(\theta)$  purely comes from the projection noise due to the collapse of spin wavefunctions. In addition, it is assumed that the measured photon counts  $y_i$  follows Poisson statistics (photon shot-noise),

$$y_i \sim \text{Pois}(F(\theta)).$$

whereby the logarithmic probability of measuring photon counts  $y_i$  is  $\ln p(y_i) = y_i \ln F(\theta) - F(\theta) - \ln y_i!$ . Note that only the first two terms depend on the parameter  $\theta$ .

The Fisher information in this data, with respect to  $\theta$ , is

$$I(\theta) = \mathbf{E}\left[y_i^2\right] \left[\frac{1}{F(\theta)} \frac{\partial F}{\partial \theta}\right]^2 - 2\mathbf{E}[y_i] \frac{1}{F(\theta)} \left(\frac{\partial F}{\partial \theta}\right)^2 + \left(\frac{\partial F}{\partial \theta}\right)^2.$$

Using properties of Poisson distribution,  $E(y_i) = F(\theta)$  and  $E(y_i^2) = F(\theta) + F(\theta)^2$ ,

$$I(\theta) = \frac{1}{F(\theta)} \left(\frac{\partial F}{\partial \theta}\right)^2 = \frac{\left[F(0) - F(1)\right]^2}{F(\theta)},$$

where  $\frac{\partial F}{\partial \theta} = C_0(t_1, t_2) - C_{\pm 1}(t_1, t_2) = F(0) - F(1)$  has been used.

Thus to extract maximum information,  $t_1$  and  $t_2$  can be varied to maximise  $I(\theta) = I(t_1, t_2, \theta)$ for different values of  $\theta$ . However since  $\theta$  can take on different values, this would be difficult in practice. In principle, a Bayesian experimental design technique can be used by real-time optimising  $t_1$  and  $t_2$  as  $\theta$  is updated and more precisely known.

From a more simplistic approach, considering the maximum difference between  $F(\theta)$  for  $\theta$  going from 0 to 1 is about 30%, the value  $F(\theta = \frac{1}{2}) = \frac{1}{2}[F(0) + F(1)]$  can be used to approximate  $F(\theta)$ ,

$$rac{1}{\sigma} \leq \sqrt{I( heta)} pprox rac{\sqrt{2} [F(0) - F(1)]}{\sqrt{F(0) + F(1)}}.$$

Intuitively, the numerator is the 'signal' measuring the difference in PL, while the denominator is the 'noise' measuring the photon shot-noise. It is for this reason in the main text, the SNR is defined as  $\begin{bmatrix} E(0) & E(1) \end{bmatrix}$ 

$$SNR(t_1, t_2) \equiv \frac{[F(0) - F(1)]}{\sqrt{F(0) + F(1)}} \propto \sqrt{I(t_1, t_2, \theta)},$$

and is maximised with experimentally measured values of  $f(\tau|\theta)$ . Note that in the above derivation, *F* is the photon counts per readout. The SNR would still be proportional to  $\sqrt{I}$  should *F* be the photon counts per time, if a fixed number (*N*) of readouts are performed per
unit time. In the main text, F should be interpreted as the photon counts per second and SNR is correspondingly scaled by  $\sqrt{N}$ .

The discussion so far is restricted to the case where only the integrated counts  $F(t_1, t_2 | \theta)$  can be measured. Potentially better information extraction can be achieved when  $f(\tau | \theta)$  can be measured with time-to-digital conversion (often called time-tagging) of incoming photons. This however is not achieved with the current hardware and is thus not discussed.

## A.1.2 Application to ODMR experiments

With a Lorentzian lineshape, the PL is given by

$$PL = \Lambda_0 L_\theta \left( f \right),$$

where  $\Lambda_0$  is the cumulative off-resonance PL (in number of photon counts), and  $L_{\theta}$  is the lineshape function (parameterised by  $\theta$ ) and is equal to one when MW drives off-resonantly. Suppose a set of PL measurement is taken at frequencies  $\{f_i, i = 0, 1, 2, \dots, k\}$  with PL values of  $\{c_i, i = 0, 1, 2, \dots, k\}$ , to infer the central transition frequency  $f_0$ , the probability of getting measurement  $c_i$  is given by the Poisson distribution with mean,  $\Lambda_0 L_{\theta,i}$ , as

$$p(c_i|\boldsymbol{\theta}) = \frac{\left(\Lambda_0 L_{\boldsymbol{\theta},i}\right)^{c_i} e^{-\Lambda_0 L_{\boldsymbol{\theta},i}}}{c_i!},$$

$$\ln p(c_i|\theta) = c_i \ln L_{\theta,i} - \Lambda_0 L_{\theta,i} + const,$$

where *const* refers to terms not explicitly dependent on  $\theta$ . As each measurement is independent, the joint probability of getting this set of measurements is

$$p(c|\theta) = \prod_{i=0}^{k} p(c_i|\theta).$$

To compute the Fisher information matrix we first find

$$\Gamma \equiv \frac{\partial}{\partial \theta} \ln p(c|\theta) = \sum_{i=0}^{k} \left[ c_i \frac{L'_{\theta,i}}{L_{\theta,i}} - \Lambda_0 L'_{\theta,i} \right] = \sum_{i=0}^{k} \left( c_i - \Lambda_0 L_{\theta,i} \right) \frac{L'_{\theta,i}}{L_{\theta,i}},$$
$$E\left[\Gamma^2\right] = \sum_{i,j} \left[ \left( \frac{L'_{\theta,i}}{L_{\theta,i}} \right)^T \left( \frac{L'_{\theta,j}}{L_{\theta,j}} \right) E\left[ \left( c_i - \Lambda_0 L_{\theta,i} \right) \left( c_j - \Lambda_0 L_{\theta,j} \right) \right] \right],$$

with,  $L'_{\theta,i} = \frac{\partial L(f_i)}{\partial \theta}$ . E  $\left[ \left( c_i - \Lambda_0 L_{\theta,i} \right) \left( c_j - \Lambda_0 L_{\theta,j} \right) \right]$  is just the covariance matrix between measurements at different frequencies. As  $c_i$  and  $c_j$  are independent Poisson distributed random variables, with means of  $\Lambda_0 L_{\theta,i}$  and  $\Lambda_0 L_{\theta,j}$ , then

$$\mathbf{E}\left[\left(c_{i}-\Lambda_{0}L_{\theta,i}\right)\left(c_{j}-\Lambda_{0}L_{\theta,j}\right)\right] = \operatorname{Var}\left[c_{i}\right]\delta_{ij} = \Lambda_{0}L_{\theta,i}\delta_{ij},$$

and thus,

$$\mathbf{E}\left[\Gamma^{2}\right] = \sum_{i=0}^{k} \frac{\Lambda_{0} L_{\theta,i}^{2}}{L_{\theta,i}}.$$
(A.1)

In fact this is true for a general lineshape with a Poisson distributed noise process. Also as each term in the sum is non-negative, including more data points in the design will not decrease the total Fisher Information. And

$$\operatorname{Cov}(\hat{\theta}) \ge \frac{1}{\Lambda_0} \left[ \sum_{i=0}^k \frac{L_{\theta,i}'^2}{L_{\theta,i}} \right]^{-1},$$
(A.2)

where  $L_{\theta,i}^{\prime^2}$  should be interpreted as the vector outer product  $L_{\theta,i}^{\prime^T}L_{\theta,i}^{\prime}$ .

#### A.1.2.1 Single Lorentzian lineshape

For a single Lorentzian ODMR lineshape,

$$L_{\theta,i} = 1 - C \frac{1}{1 + \left(\frac{f_i - f_0}{\Gamma}\right)^2},$$

where C is the ODMR contrast,  $f_0$  is the resonance frequency and  $\Gamma$  is the half-width at halfmaximum (HWHM), and parameter set  $\theta = (f_0)$ ,

$$L_{\theta,i}' = \frac{\partial L_{\theta,i}}{\partial f_0} = \frac{2C}{\Gamma} \left(\frac{f_i - f_0}{\Gamma}\right) \frac{1}{\left[1 + \left(\frac{f_i - f_0}{\Gamma}\right)^2\right]^2},$$
$$I(\theta) = \sum_{i=0}^k \Lambda_0 \left(\frac{2C}{\Gamma}\right)^2 \frac{x_i^2}{\left(1 + x_i^2 - C\right)\left(1 + x_i^2\right)^3},$$

where  $x_i = \frac{f_i - f_0}{\Gamma}$ . The frequency resolution is,

$$\delta f = \frac{\Gamma}{2C\sqrt{\Lambda_0}} \frac{1}{\sqrt{\sum_{i=0}^k \frac{x_i^2}{\left(1+x_i^2-C\right)\left(1+x_i^2\right)^3}}}$$

The best sensitivity is achieved with only one measurement is by taking a measurement at x which maximises the denominator. For C << 1 this corresponds to  $x = \frac{1}{\sqrt{3}}$ , giving  $\delta f = \frac{\Gamma}{C\sqrt{\Lambda_0}}\frac{8}{3\sqrt{3}}$ , which agrees with results from Dreau *et al* [69].

#### A.1.2.2 Double Lorentzian lineshape

For double Lorentzian fitting,

$$L_{\theta,i} = 1 - C_1 \frac{1}{1 + \left(\frac{f_i - (f_0 - \Delta)}{\Gamma_1}\right)^2} - C_2 \frac{1}{1 + \left(\frac{f_i - (f_0 + \Delta)}{\Gamma_2}\right)^2},$$
 (A.3)

where  $C_1/C_2$  and  $\Gamma_1/\Gamma_2$  are the contrasts and HWHM of Lorentzian peak 1 and 2.  $f_0$  is the mean and  $2\Delta$  is the separation between the resonance frequencies of the two Lorentzian peaks. It is assumed that only the central frequency  $f_0$  is changed in response to temperature change, but not the peak separation  $\Delta$ , width  $\Gamma_{1,2}$ , and contrasts  $C_{1,2}$ . The exact calculation is less trackable and can be done numerically by substituting Eq. A.3 into Eq. A.2, while considering the given design  $\{f_i, i = 0, 1, 2, \dots, k\}$ . In particular, the experimentally measured sensitivity can be compared with the theoretical sensitivity.

#### A.1.2.3 Gaussian noise on photon counts

For situations where the mean photon count is affected by or noise process such as laser fluctuations, system drift etc, the mean rate would follow a noise distribution, then the above equation needs to be modified slightly,

$$\mathbf{E}\left[\Gamma^{2}\right] = \sum_{i,j} \left[ \left( \frac{\left(\Lambda_{0}L_{\theta,i}\right)'}{\Lambda_{0}L_{\theta,i}} \right)^{T} \left( \frac{\left(\Lambda_{0}L_{\theta,i}\right)'}{\Lambda_{0}L_{\theta,i}} \right) \mathbf{E}\left[ \left(c_{i} - \Lambda_{0}L_{\theta,i}\right) \left(c_{j} - \Lambda_{0}L_{\theta,j}\right) \right] \right],$$

where  $\theta = (\Lambda_0, f_0, \Delta, \cdots)$  is the new parameter vector. The expectation value requires knowledge of the exact noise process the mean photon counts undergoes. For a Gaussian distributed noise process  $\lambda \sim \mathcal{N}(\Lambda_0 L_{\theta,i}, \sigma^2)$ , where  $\sigma$  is the standard deviation of photon counts, the joint probability distribution of  $c_i$  is,

$$p(c_i) = p(c_i|\lambda)p(\lambda) = \int d\lambda \frac{\lambda^{c_i}e^{-\lambda}}{c_i!} \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(\lambda-\Lambda_0 L_{\theta,i})^2}{2\sigma^2}},$$

which could be approximated as a Gaussian probability if  $\Lambda_0 \gg 1$  as

$$c_i \sim \mathcal{N}(\Lambda_0 L_{\theta,i}, \sigma^2 + \Lambda_0 L_{\theta,i}),$$

$$\mathbf{E}\left[\Gamma^{2}\right] = \sum_{i,j} \left[ \left( \frac{\left(\Lambda_{0}L_{\theta,i}\right)'}{\Lambda_{0}L_{\theta,i}} \right)^{T} \left( \frac{\left(\Lambda_{0}L_{\theta,i}\right)'}{\Lambda_{0}L_{\theta,i}} \right) \mathbf{E}\left[ \left(c_{i} - \Lambda_{0}L_{\theta,i}\right) \left(c_{j} - \Lambda_{0}L_{\theta,j}\right) \right] \right],$$
$$\mathbf{E}\left[\Gamma^{2}\right] = \sum_{i} \left[ \left( \frac{\left(\Lambda_{0}L_{\theta,i}\right)'}{\Lambda_{0}L_{\theta,i}} \right)^{2} \left(\sigma^{2} + \Lambda_{0}L_{\theta,i}\right) \right].$$

The corresponding Cramer-Rao bound is

$$\operatorname{Cov}(\hat{ heta}) \geq \left[\sum_{i} \left( rac{\left( \Lambda_0 L_{ heta,i} 
ight)'}{\Lambda_0 L_{ heta,i}} 
ight)^2 \left( \sigma^2 + \Lambda_0 L_{ heta,i} 
ight) 
ight]^{-1},$$

where the standard deviation  $\sigma$  might depend on total acquisition time, and similarly the photon counts  $\Lambda_0$ . This dependence can be checked experimentally by Allan deviation analysis.

#### A.1.2.4 Discussion

From these comparison, it can be seen that,

- 1. The Fisher Information only increases with more data points taken.
- 2. Due to the matrix inversion procedure, the marginal gain in fitting uncertainty does not increase trivially with the Fisher Information gain from each point, i.e. the additional improvement in sensitivity depends on which other data points are also taken.
- 3. For models with more than one parameter, the Fisher information matrix is not invertible (rank 1) unless there are as many (sufficiently different) data points as there are unknown parameters. The "sufficiently different" requirement prevents invertible but "ill-conditioned" Fisher matrix which would not be invertible numerically.

4. Additional parameters in the fitting leads to more uncertainty in frequency estimation as more terms contribute to the sum in eq. A.1.

Based on these observations, the strategy to improve sensitivity are,

- 1. Eliminate fitting parameters using physical understanding of the system to fix unknown parameters, or by eliminating parameters that do not introduce significant variations in the measured data.
- 2. Optimise the experimental design.
- 3. Use at least the same number of data points as there are free parameters.

To reduce the number of parameters in the data, a data-based approach could be applied to the covariance matrix and only retain principal components that can explain a significant variation in the data. This approach maps the parameter space  $\Theta$  to a smaller subspace (a *shrinkage*) based on correlation in the parameters. Principal Component Analysis and Partial Least Squares Regression are examples of such an approach. However, the covariance matrix may not represent the true variations in the fitting parameters. In fact the nonlinear Least Square regression method increases correlation between fitting parameters despite them being independent to start with [128].

Another approach is based on physical reasoning. For example, the NV ODMR linewidth, contrasts and splitting between the double Lorentzian peaks are in principle not directly dependent on temperature change and can therefore be treated as fixed parameters. In this situation, only the central frequency and off-resonance PL are to be estimated.

Finally this method is still based on a model function known *a priori*, which is not necessarily a "complete" or "efficient" representation of the actual lineshape and neither is the central frequency extracted from this fitting models. In fact a parametric approach as used here is only one way to extract the temperature data. Any method that links PL to temperature change is sufficient. For example a single Gaussian, linear extrapolation have been used [49, 86, 142, 211].

## A.1.3 Application to orbital tracking

In 5.4.3.3, the position estimation of orbital tracking is quoted and here is the derivation based on Fisher Information formalism. In orbital tracking, Poisson distributed photon data is fitted using

$$PL_{xy} = I_0 \left[ 1 - a\cos\theta_n + b\sin\theta_n \right],$$

where  $I_0$  is the total number of photon counts collected per  $\frac{1}{4}\pi$  angle. Using Eq. A.1,

$$E\left[\Gamma^{2}\right] = I_{0} \begin{bmatrix} \sum_{n} \cos^{2} \theta_{n} & 0\\ 0 & \sum_{n} \sin^{2} \theta_{n} \end{bmatrix}.$$
  
Since  $\sum_{n} \cos^{2} \theta_{n} = \sum_{n=0}^{7} \cos^{2} \left(\frac{1}{4}n\pi + \frac{1}{8}\pi\right) = 4$  and  $\sum_{n} \sin^{2} \theta_{n} = 4$ ,  
 $\Delta a, \Delta b \ge \frac{1}{\sqrt{4I_{0}}}.$  (A.4)

## **Appendix B**

## **Triplet exciton and NV relaxometry**

As NV centers sense the power spectrum of the magnetic noise generated by external spins, it is necessary to understand the fluctuation of the bath. This is relatively simple to achieve for simple spins such as nuclear spins which has very long  $T_2^*$  [180] or contrasting agents such as Gd<sup>3+</sup> where the  $T_2^*$  is very short [132, 220]. To compare with the NV measurements, the autocorrelation function ( $\langle B(0)B(\tau)\rangle$ ), or the power spectrum ( $\mathscr{F}\{\langle B(0)B(\tau)\rangle\}$ ) are of interest. This is related to the  $T_2^*$  which could be probed by transient pulsed EPR measurements. This measurement is however not usually studied for DPH. This appendix chapter aims to estimate these quantity based on measurements including the Magnetic PL, ODMR, and transient PL measurements.

As discussed in the main text, the dynamics of a triplet exciton is usually in the slow-fluctuation limit, such that the full SLE modelling is required. Solving SLE would give the steady-state density matrix of a triplet exciton,  $\rho(t)$ , which then allows computation of the two time correlation such as  $\langle S_x(0)S_x(t)\rangle$ , thus allowing computation of the power spectrum. We also discuss how external spins influence  $T_1$  time of the NV.

## **B.1** NV Relaxometry

Experimentally, the depolarisation time of the NV is measured by preparing the NV in the  $|-1\rangle$  state or the  $|0\rangle$  and measure the time  $(T_1)$  taken to reach thermal state. In the Redfield's model for depolarisation,

$$\frac{1}{T_1} = \frac{1}{T_{1,0}} + \gamma^2 S_{\perp}(\omega_0)$$
(B.1)

where the first term on the right describe intrinsic depolarisation of the NV and the last term describes external sources of magnetic noise at the NV transition frequency that depolarises the NV.  $S_{\perp}(\omega)$  is the magnetic field spectral density at the location of the NV, contributed by

all triplet excitons and is defined as the Fourier transform of the autocorrelation function of the magnetic field operator B perpendicular to the NV axis,

$$S_{\perp}(\omega) = \int \langle B_{\perp}(0)B_{\perp}(t) \rangle e^{-i\omega t} dt$$

where the ensemble average  $\langle \cdot \rangle$  is taken with respect to either repeated measurements of *B* by a single NV centre or one measurement by spatially distributed identical NV centres such as an ensemble NV system.

If the noise bath is formed of simple spin- $\frac{1}{2}$  nuclei, there is a technique to treat correlators. Setting up a Cartesian coordinate with z axis aligned with the NV axis, the correlator can be defined as [180]

$$\langle B_z(0)B_z(t)\rangle = \sum_j D_j^2 \left[9f^{x,x} \left((u_x^j u_z^j)^2 + (u_y^j u_z^j)^2\right) + f^{z,z}\right],$$

with  $D_j$  being the magnetic field created by each spin. The goal is to find the correlators  $f^{\alpha,\alpha}$ . And for simple systems where the Hamiltonian is not time-dependent and thermal energy much larger than the intrinsic energy scale of the spin- $\frac{1}{2}$ ,

$$f^{\alpha,\alpha}(I,\omega) = \int_{-\infty}^{+\infty} \langle S_{\alpha}(t) S_{\alpha}(0) \rangle e^{-i\omega t} dt = \frac{2\pi}{\mathrm{Tr}(\mathbf{I})} \sum_{n,m} |\langle n_z | \hat{S_{\alpha}} | m_z \rangle|^2 \delta\left(\frac{E_m - E_n}{\hbar} - \omega\right).$$

To derive this relation, we first work in the Heisenberg picture where  $\rho$  is not time-dependent but the operators  $S_{\alpha}(t)$  are time-dependent. As a result the correlation can be expressed as

$$\langle S_{\alpha}(t)S_{\alpha}(0)\rangle = \operatorname{Tr}\left(\rho e^{i\hat{H}t/\hbar}S_{\alpha}(0)e^{-i\hat{H}t/\hbar}S_{\alpha}(0)\right).$$

Using the closure relation of the energy eigen-basis  $\mathbf{I} = \sum_{n} |n\rangle \langle n|$  and the circular permutation property of the trace operation, this can be Fourier transformed to give the  $\delta$ -function. The sum n,m is taken over all possible quantum states for the spin- $\frac{1}{2}$ . Applying this to a triplet with S = 1,

$$\hat{S}_z T_+ = T_-, \hat{S}_z T_- = T_+, \hat{S}_z T_0 = T_0,$$

$$f^{z,z}(S,\omega) = \frac{2\pi}{3} \left[ \delta(\omega) + \delta\left(\frac{E_+ - E_-}{\hbar} - \omega\right) + \delta\left(\frac{E_+ - E_-}{\hbar} + \omega\right) \right],$$

and  $\langle T_+|S_x|T_0\rangle = \langle T_0|S_x|T_+\rangle = 1$ , to give

$$f^{x,x}(S,\omega) = \frac{2\pi}{3} \left[ \delta \left( \frac{E_+ - E_0}{\hbar} - \omega \right) + \delta \left( \frac{E_+ - E_0}{\hbar} + \omega \right) \right]$$

$$\langle T_0|S_y|T_-\rangle = -\langle T_-|S_y|T_0\rangle = i$$
, to give  
$$f^{y,y}(S,\omega) = \frac{2\pi}{3} \left[ \delta\left(\frac{E_- - E_0}{\hbar} - \omega\right) + \delta\left(\frac{E_- - E_0}{\hbar} + \omega\right) \right]$$

The  $\delta$ -functions need to be replaced by Lorentzian functions with unit integral and finite width. The linewidth is determined by  $T_2^*$ . Combining with Eq. B.1, the externally induced depolarisation is

$$\frac{1}{T_{1,external}} = -\gamma^2 \left( \langle B_x B_x \rangle(\omega_0) + \langle B_y B_y \rangle(\omega_0) \right)$$

## **B.2** Stochastic Liouville equation

Triplet excitons as they traverse the crystal structure induces motional relaxation. In particular in OSC formed of small molecules based on Stochastic Liouville Equation (SLE) is documented in [229, 243]. Ultimately the aim is find how the total magnetization of the material is correlated in time.

There are various forms of SLE and this work follows the line of work by Wakasa *et al.* [243], who have applied SLE to successfully describe Magnetic Field Effect (MFE) in DPH and various other DPH derivatives. This also serves as a verification for our final numerical model.

SLE has the following form

$$\frac{d\rho}{dt} = -\frac{i}{\hbar}[\hat{H},\rho] + \hat{K}\rho,$$

where  $\hat{H}$  is time independent and describe dynamics of the system. The  $\hat{K}$  superoperator includes terms like hopping, singlet-fission, triplet-fusion, molecular libration, trapping of triplets etc. The density operator  $\rho(n,t)$  represents the spatial and spin state of a pair of triplet excitons. Nine spatial locations were considered, the first two (site 1 and 2) corresponds to singlet (S = 0) states  $S_0$  and  $S_1$ . Site 3 corresponds to a pair of triplets (S = 0, 1, 2), site 4~9 correspond to S = 1 triplet.

$$\frac{d\rho(1,t)}{dt} = k_f \rho(2,t) 
\frac{d\rho(2,t)}{dt} = -(k_f + k_{SF})\rho(2,t) + k_{TF}Q_{SS}\rho(3,t)Q_{SS} 
\frac{d\rho(3,t)}{dt} = -\frac{i}{\hbar}[H(3),\rho(3,t)] + k_{SF}\rho(2,t) - \frac{k_{TF}}{2}(Q_{SS}\rho(3,t) + \rho(3,t)Q_{SS}) - k_{dis}\rho(3,t) + k_h\rho(4,t) 
\frac{d\rho(4,t)}{dt} = -\frac{i}{\hbar}[H(4),\rho(4,t)] + k_{dis}\rho(3,t) - 2k_h\rho(4,t) + k_h\rho(5,t) 
... 
$$\frac{d\rho(N,t)}{dt} = -\frac{i}{\hbar}[H(N),\rho(N,t)] + k_h\rho(N-1,t) - 2k_h\rho(N,t) + k_h\rho(N+1,t) 
... 
$$\frac{d\rho(9,t)}{dt} = -\frac{i}{\hbar}[H(9),\rho(9,t)] + k_h\rho(8,t),$$
(B.2)$$$$

where the constants  $k_h$ ,  $k_{dis}$ ,  $k_{trap}$ ,  $k_{SF}$ ,  $k_{TF}$  are fitting parameters,  $k_f$  was taken from measurements by Dillion *et al.* [63]. The number of sites (9 in this case) is usually chosen such that increase it further does not affect the result (see [229]) yet it is unclear how this was chosen in this paper. The site-dependent Hamiltonian will be discussed in detail next.

## **B.2.1** Orientation effect of Hamiltonian

The Hamiltonian accounts for Zero-field splitting (ZFS) of the two triplets residing on separate molecules, Zeeman effect, and exchange coupling between strongly coupled excitons,

$$H = H^a + H^b + H^{ab}$$

where

$$H^{a} = \mathbf{S}^{\mathbf{a}} \cdot \mathbf{D}^{\mathbf{a}}(\Omega^{a}) \cdot \mathbf{S}^{\mathbf{a}} + \mu_{b} g \mathbf{S}^{\mathbf{a}} \cdot \mathbf{g}^{\mathbf{a}}(\Omega^{a}) \cdot \mathbf{B}$$

is the Hamiltonian for triplet a and the Hamiltonian for triplet b has a similar form except a different orientation  $\Omega^b$ . The exchange coupling term is

$$H^{ab} = -2J\mathbf{S}^{\mathbf{a}} \cdot \mathbf{S}^{\mathbf{b}}.$$

The relative orientation of these two triplets have to been considered very carefully.

Further explanation on this point is found in the supplementary to the comment of [181], which points out and clears some of the common confusions with molecular orientation. Sup-

pose that there are two set of basis one aligned with the lab frame and also with molecule A's intrinsic molecular axis,  $\{|x\rangle, |y\rangle, |z\rangle\} \equiv \{|x\rangle_A, |y\rangle_A, |z\rangle_A\}$ . The other is aligned with the rotated molecule  $\{|x'\rangle, |y'\rangle, |z'\rangle\}$ . We can represent the Hamiltonian of molecule B by a matrix with respect to the basis  $\{|x\rangle_B, |y\rangle_B, |z\rangle_B\} \equiv \{|x\rangle_A, |y\rangle_A, |z\rangle_A\}$  rather than the primed basis. This way all states are represented with respect to the lab frame. The Hamiltonian in this representation is formed by rotating the diagonal Hamiltonian  $H_A$  as,  $H_B = R^T H_A R$  where R is a series of rotation matrix following the ZXZ convention. As long as the same set of axes are chosen for both triplets, the Singlet state for the triplet pair will not change.

In principle, the axes with respect to which  $H^a$  is represented is arbitrary and are usually chosen with respect to the molecular axes x, y, z which are also the eigen-axes of  $H^a$ . However, when the total spin operator  $S^a + S^b$  is concerned, a fixed axis much be chosen to give the correct result. Once this basis is chosen, the Hamiltonian for either triplets needs to be rotated by the correct rotation matrix R. There are three configurations of a pair of triplets and they are detailed in [116].

#### **B.2.1.1** Singlet character

Wakasa *et al.* [243] provides some more information to verify if our Hamiltonian gives the correct result. One such information is a plot of singlet character as a function of applied magnetic field and molecular orientation relations.  $|C_S^l|^2 = |\langle \psi_l | S \rangle|^2$  gives the singlet character of state  $\psi_i$  and these percentages sum to 1.

We start off with the lab frame with respect to which the singlet state  $|S\rangle$  is defined. As both molecules are rotated, their eigenstates as represented by the lab frame basis are changed. This rotation changes the percentage of triplet character.  $C_S^l$  depends on the molecular orientation.

For the slip parallel configuration, our results agree with Wakasa *et al*'s result. However there is a significant difference between our results for the Herringbone structure. In particular, the state with eigenfrequency  $-6.67 \times 10^{10}$  rad s<sup>-1</sup> is said to have a singlet character of 51% yet any eigenstates corresponding to a non-degenerate eigenvalue will have a maximum of 33% singlet.

### **B.2.2** Molecular libration

Another source of decoherence is molecular libration. The molecular libration theory considers how molecular motion (phonons) induces transition between different molecular states. The relaxation superoperator is therefore a products of a transition probability (Lorentzian in transition frequency) and two molecular coordinate matrices ( $Q \sim S_{x,y,z}^2$ ). As molecular vibra-

tions are typically in the range  $10^{-11} - 10^{-12}$ s  $\ll 1/D$ , the variation in the Lorentzian factor does not contribute much to the resulting spectrum. On the other hand, the extent of molecular fluctuation, controlled by the coupling factor  $A^{\alpha}$  controls the spin-lattice relaxation time more strongly. This factor was suggested to be around  $10^{-2}$  for a  $10^{0}$  of gyration. Yet we needed a value between 10-50 to bring across SLR sufficient to match the data in Wakasa *et al* [243]. In the work by Yago *et al.* [265], the same parameters for rubrene was used as opposed to the DPH value. This already gives about a factor of 10 difference. After further investigation, we deem this effect unimportant for our present case.

Although a larger libration coupling strength  $A^{\alpha}$  reduces the MFE which is wanted, the value quickly goes to 100 times larger and it is unlikely the values actually used in [243]. In contrast, as they claimed the molecules are rotated about x, y, z by  $\pm 10^{\circ}$ , the MFE reduction might be due to enhanced decoherence due to molecular orientation differences.

## **B.2.3** Magnetic field effect

Checking if the simulation agrees with magnetic field effect measurement is the first step to confirming the validity of the simulation. We run simulation for the three explicitly declared molecular orientations and list the result here. The magnetic field effect to refers to structure of total singlet transition PL changing as applied magnetic field changes. To compare to literature, the PL from a powdered sample is simulated by setting t = 1s and total PL,

$$r(B) = \operatorname{Tr}[\rho(1, t \to \infty)Q_{SS}]$$
$$R(B) = \frac{r(B)}{r(0)}$$

The powdered sample is taken into account by averaging over all orientations. See Appendix for details.

#### **B.2.3.1** Dip position

The dip position at low field is controlled by the ZFS of the triplet ground state. This is at around 38mT and 47mT when the magnetic field is aligned with *x* or the *y* axes. Any other orientation will result in peaks in between these two values.

### B.2.3.2 Dip depth

The dip depth is controlled by both the singlet character of each eigenstates  $C_S^l$  and the kinetic parameters most strongly by the three parameters  $k_{TF}$ ,  $k_{SF}$  and  $k_{rad}$ . These parameters are model-dependent and as the models used by different authors are different, these values differ significantly.

## **B.3** Motional relaxation of a single triplet

The above discussion has enabled extraction of the hopping rate  $k_h$  based on the magnetic field effect. When the triplet has formed and hop between molecules, a simpler model can be used to compute the autocorrelation function.

## **B.3.1** Deriving the correlation Function

The two-time correlation function can be computed either in the time domain with the Quantum Regression Theorem, or in the frequency domain with Fourier transform.

## **B.3.2** Frequency domain method

The two time correlation function can be expressed as

$$\langle A(t)B(0)\rangle = \langle A(t)U(t,0)\{B\rho(0)\}\rangle$$
  
=  $\langle A(t)e^{t(-iH^{\times}/\hbar+K)}\{B\rho(0)\}\rangle,$ 

The Fourier transform of this is

$$S(\boldsymbol{\omega}) = \int \langle A(t)B(0) \rangle e^{i\boldsymbol{\omega} t} dt$$
  
=  $\langle A| \int U(t,0)e^{i\boldsymbol{\omega} t} dt | B\boldsymbol{\rho}(0) \rangle$   
=  $\langle A| \int e^{t(-iH^{\times}/\hbar + K + i\boldsymbol{\omega} t)} dt | B\boldsymbol{\rho}(0) \rangle$   
=  $\langle A| [iH^{\times}/\hbar + K - i\boldsymbol{\omega}]^{-1} | B\boldsymbol{\rho}(0) \rangle$  (B.3)

Here although the density matrix is written as one single matrix, and seems not readily applicable to the SLE with stepwise hopping, we can actually write  $\rho$  as  $\rho(\Omega, t)$  where  $\Omega$  denotes the spatial part of the wavefunction. Different hopping sites can be seen as the rotational eigenbasis. The trace should be taken over all spatial directions,

$$S(\boldsymbol{\omega}) = \sum_{\Omega} \langle A | U(\boldsymbol{\omega})^{-1} | B \boldsymbol{\rho}(\Omega | 0) \rangle = \sum_{\Omega} \operatorname{Tr} \left[ A U(\boldsymbol{\omega})^{-1} \{ B \boldsymbol{\rho}(\Omega | 0) \} \right].$$

## **B.3.3** Triplet SLE

Once the  $k_h$  parameter is extracted from Eq. B.2, we use this parameter to simulate a singlet triplet hopping between two different oriented DPH molecules, as is done in EPR literature [28].

Suppose a triplet exciton hopes between two molecules with different molecular orientation, we can represent the SLE as

$$\frac{d\rho_1}{dt} = -\frac{i}{\hbar}[H_1,\rho_1] - k_h\rho_1 + k_h\rho_2 \tag{B.4}$$

$$\frac{d\rho_2}{dt} = -\frac{i}{\hbar}[H_2,\rho_2] - k_h\rho_2 + k_h\rho_1 \tag{B.5}$$

where the Hamiltonian consists of appropriately rotated ZFS of the form,  $H_i = \mathbf{S} \cdot \mathbf{D}(\Omega_i) \cdot \mathbf{S}$ .

We start off with a thermal state,

$$\rho_1 = \rho_2 = rac{1}{6} \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}.$$

This state has  $\langle \mathbf{S}^{\mathbf{i}} \rangle = tr(\rho_i \mathbf{S}) = 0$ , implying zero polarisation in any direction. The two-time correlation function of  $S_y$  on molecule A is however given by  $\langle S_y(t+\tau)S_y(t) \rangle = tr[\rho_1(t)]e^{-\tau/T_2^*}\cos(\omega_y\tau)$ , where the frequency  $\omega$  is dependent on the operator. Also in this model, as the population oscillates between molecular A and molecule B,  $\rho_1(t)$  also depends on time and has a frequency of the hopping rate  $k_h$ . The two-time correlation function of the total  $S_y$  is a sum of the contribution from molecule A and molecule B which is  $\frac{2}{3}e^{-\tau/T_2^*}\sum_i \cos(\omega_i\tau)$  which is then time-translation invariant and  $\langle S_y(0)^2 \rangle = \frac{1}{3}S(S+1)$ , takes into account any correlation between the two molecules.

When there is no difference between orientations of the two molecules, there is no loss of coherence. The hopping rate can be varied to see the effect of motional averaging using Eq. B.3 and the result is shown in Fig. 3.8 in the main text.

## **B.3.4** Combining SLE and NV relaxometry

The transverse magnetic field experienced by the NV is

$$B_{\perp}^2 = \rho \left(\frac{\mu_0 \mu_B g}{4\pi}\right)^2 \frac{1}{h^3} \times \Gamma,$$

where the function  $\Gamma$  was the non-dimensionalised quantity capturing the spectral density and geometrical integral over the entire volume of contributing triplet excitons.

The density of exciton is typically estimated with the absorption coefficient  $\alpha_{\lambda}$ , the incident light intensity *I*, and area of the beam spot *A*, steady-state occupancy of the *S*<sub>1</sub> state, as follows,  $\rho = \frac{\alpha_{\lambda}I\lambda}{hcA} \times \beta$ . No attempt is made to further estimate this quantity as the local absorption is not known exactly, but the key characteristics is the externally induced depolarisation rate is proportional to the exciton concentration, which at low excitation is linear in the optical power.

## **B.4** Numerical solution

The SLE equation is solved numerically in Python programming language, using packages including numpy and scipy. All computations are done in the basis of individual triplets  $\{|T_{+1}\rangle, |T_0\rangle, |T_{-1}\rangle\} \otimes \{|T_{+1}\rangle, |T_0\rangle, |T_{-1}\rangle\}$  which is fixed with respect to the lab frame. Alternative bases are the "high-field" basis labelled by  $|S, m_s\rangle$  and the "zero-field" basis formed by tensor product or the *x*, *y*, *z* eigenbasis of individual molecules, giving terms like  $|x\rangle|y\rangle$ .

### **B.4.1** Representation in matrices

The SLE is a group of simultaneous equation with superoperators and matrices. This has to be converted into the "Liouville" space in order to computed numerically. This conversion is done by identifying  $\{\mathbf{e}_i \otimes \mathbf{e}_j | \mathbf{e}_i, \mathbf{e}_j \in \mathcal{L}_d, i, j = 1, 2, ..., d\}$  with basis of the Liouville space  $\{\mathbf{e}_{(i,j)} | \mathbf{e}_{(i,j)} \in \mathcal{L}_{d \times d}\}$ . The same rule of matrix multiplication is preserved in this mapping.

In this conversion,  $\rho_{ij}$ , the density matrices are converted into Liouville supervectors  $\rho_{i\times9+j}$ , which are formed by filling a  $81 \times 1 = 9 \times 9$  vector with rows of  $\rho_{ij}$ . The superoperators  $\hat{H} = [\hat{H}, \cdot]$  and  $\hat{K}$  get converted into Liouville superoperators by noting

$$[A\rho]_i \equiv [L\{A\}\rho]_i = (A \otimes I)_{ij}\rho_j,$$

and

$$[\boldsymbol{\rho} A]_i \equiv [R\{A\}\boldsymbol{\rho}]_i = (I \otimes A^{\dagger})_{ij}\boldsymbol{\rho}_j.$$

After conversion,  $\rho$  becomes a 729-element vector with each 81 elements representing the density operator for one site. The superoperators are represented as  $729 \times 729$  matrices. These objects are based on 128-bit complex numbers native to numpy package.

After these conversions, the ordinary differential matrix equations is converted into

$$rac{d\hat{
ho}}{dt} = \left[-rac{i}{\hbar}\hat{H} + \hat{K}
ight]\hat{
ho} \equiv \mathscr{L}\hat{
ho}\,.$$

Various approaches have been attempted to solve the SLE equation in this form and surprisingly only two worked. The first is the usual finite difference method, which perform poorly beyond 10ns. The second is a direct exponential inversion, which although takes significant computation power, yields satisfactory results for large times after computational optimisation. There are various other methods to solve this equation numerically, for example Laplace transform, and finite-difference integration of the equation.

As  $\mathscr{L}$  is not explicitly time-dependent, this can be solved by numerically compute the time evolution (super)operator,  $U = \exp(-t\mathscr{L})$ , which then acts on the initial density operator  $\rho_0$  to give,  $\rho(t)$ , the density matrix at time t. An exponentiation procedure of the 729 × 729 square matrix takes 0.8 seconds to complete.

## **B.5** Discussion

From this calculation, we come to a conclusion that the power spectrum forms from resonances with line widths depending on the hopping rate. This gives the signal for a relaxometry measurement.

## **Appendix C**

## **Orbital tracking correction algorithm**

## C.0.1 xy correction

For a Gaussian point spread function (PSF), the collected PL in the lateral directions for the top and the bottom orbits are

$$I_{1} = I_{10} \exp\left[-2\frac{(x-\delta_{x})^{2} + (y-\delta_{y})^{2}}{w_{xy}^{2}}\right] f(z-R_{z}),$$
  
$$I_{2} = I_{20} \exp\left[-2\frac{(x-\delta_{x})^{2} + (y-\delta_{y})^{2}}{w_{xy}^{2}}\right] f(z+R_{z}),$$

where  $w_{xy}$  is the characteristic radius of the PSF,  $\delta_{x,y}$  are the particle deviation from the centre of the orbit in the x and y directions, x, y are the deviation of the collection spot from the centre of the orbit,  $I_{10,20}$  are the maximum PL for each orbit. The z-dependent factors,  $f(z \pm R_z)$ , account for relative displacement between the top and the bottom plane from the excitation focal spot. During data post-processing, the intensity collected from the top and the bottom planes are summed to calculate the correction in the lateral directions, and the summed total intensity is

$$I(x,y) \equiv I_1 + I_2 = I_0 \exp\left[-2\frac{(x-\delta_x)^2 + (y-\delta_y)^2}{w_{xy}^2}\right],$$

where the z-dependence, and differences in  $I_{10,20}$  have been lumped into one parameter  $I_0$ , such that,  $I_0 = I_{10}f(z - R_z) + I_{20}f(z + R_z)$ . This can be linearised around small values of  $\delta_{x,y}$ and ignore terms in  $\delta_{x,y}^2$ ,

$$I(x,y) \approx I_0 \exp\left[-2\frac{x^2 + y^2}{w_{xy}^2}\right] \left[1 + \frac{4x}{w_{xy}^2}\delta_x + \frac{4y}{w_{xy}^2}\delta_y\right].$$

Since the orbit is circular  $x^2 + y^2 = R_{xy}^2$  and  $x_n = R_{xy} \cos \theta_n$ ,  $y_n = R_{xy} \sin \theta_n$ ,

$$I_n \approx I'_0 \left[ 1 + \frac{4R_{xy}}{w_{xy}^2} \delta_x \cos \theta_n + \frac{4R_{xy}}{w_{xy}^2} \delta_y \sin \theta_n \right] \equiv I'_0 \left[ 1 + a \cos \theta_n + b \sin \theta_n \right]$$

where  $I'_0 = I_0 \exp\left[-2\frac{R_{xy}^2}{w_{xy}^2}\right]$ . Fitting this expression gives the offset of the particle,

$$\delta_x = \varepsilon_{xy}a, \delta_y = \varepsilon_{xy}b, \tag{C.1}$$

where  $\varepsilon_{xy} = \frac{w_{xy}^2}{4R_{xy}}$ .

The linearisation procedure dictates that the correction is only accurate when  $\delta_{x,y}$  are small compared to  $R_{xy}$ . In the extreme case when  $\delta \to \infty$ , no PL is measured and thus the correction given by the algorithm vanishes. Thus the actual correction is a decreasing function of  $\delta_{x,y}$ . This implies that the feedback parameter  $\varepsilon_{xy}$  needs to be increased if on average  $|\delta|$  is large, otherwise, as shown in Fig. 5.6 the correction algorithm transitions from overcorrection to undercorrection when the mean value of  $|\delta|$  increases.

The PSF is assumed to be perfectly circular for the correction to always centred on the particle. Imperfections in the PSF could lead to elongated PSF, or asymmetrical PSF. In the former case, a modulation at twice the frequency would be resulted, thus not affecting the correction. In the latter case, correction algorithm will centre to where the PL is maximum, thus offsetting from the actual particle location by a fixed displacement.

### C.0.2 z correction

For correction in the longitudinal direction, the total PL from the top and bottom planes are separately summed, resulting in two intensities  $PL_t$  and  $PL_b$ . As a function of the emitter's position *z*, the PL collected from the top and the bottom planes are approximated by a Gaussian function

$$PL_t = PL_{t0} \exp\left[-\frac{2(z-R_z)^2}{w_z^2}\right],$$

and

$$PL_b = PL_{b0} \exp\left[-\frac{2(z+R_z)^2}{w_z^2}\right].$$

When the particle is slightly deviated from the centre between the top and bottom orbits,

 $z = \delta_z$ , and the quantity

$$R \equiv \frac{PL_b - PL_t}{PL_b + PL_t} \approx \frac{I_{b0} \exp\left(-\frac{4R_z\delta}{w_z^2}\right) - I_{t0} \exp\left(+\frac{4R_z\delta}{w_z^2}\right)}{I_{b0} \exp\left(-\frac{4R_z\delta}{w_z^2}\right) + I_{t0} \exp\left(+\frac{4R_z\delta}{w_z^2}\right)} \approx \frac{G - \delta_z/\varepsilon_z}{1 - G\delta_z/\varepsilon_z}$$

where  $G = \frac{I_{b0} - I_{t0}}{I_{b0} + I_{t0}}$  is a setup-dependent parameter,  $I_{b0} = PL_{b0} \exp\left(-\frac{2R_z^2}{w_z^2}\right)$  and similarly for  $I_{t0}$ .  $\varepsilon_z = \frac{w_z^2}{4R_z}$ . The correction is thus

$$\delta_z = \frac{R-G}{RG-1}\varepsilon_z.$$

Experimentally G is set at one value  $G_0$ .  $\varepsilon_z$  is fixed and R is obtained from measurements. Thus when change in  $R \approx G_0 + \delta R$ ,

$$\delta_z \approx \frac{\varepsilon_z}{G^2 - 1} \delta R,$$
 (C.2)

which takes the form of a linear feedback like in Eq. C.1. Thus the general correction algorithm is of the form,

$$\delta = Pu$$
,

where  $\delta = (\delta_x, \delta_y, \delta_z)$ ,  $u = (a, b, \delta R)$  and *P* is a diagonal matrix with diagonal elements  $P = \text{diag}\left\{\left(\varepsilon_{xy}, \varepsilon_{xy}, \frac{\varepsilon_z}{G^2-1}\right)\right\}$ . As the *P* matrix is fixed before performing the experiment, this is analogous to a controller with only proportional gain and thus may introduce the over- or under-correction problem as discussed in the main text Chapter 5 Section 5.4.3.6.

## **Appendix D**

# Corrections of $g^{(2)}( au)$ data

Background fluorescence, the timing jitter on both APDs of the HBT interferometer, and finite bin size during data post-processing are among the common sources of error in a typical  $g^{(2)}(\tau)$  data. Assuming an experiment model (see Eq. 6.1) of  $g^{(2)}(\tau) = 1 - \beta e^{-\gamma_1 |\tau|} + \alpha e^{-\gamma_2 |\tau|}$ with the assumption  $\gamma_1 \gg \gamma_2$  and  $1/\gamma_1$  is comparable to APD timing jitter and bin size, the effect of the three sources of corrections on the parameters in the model is listed in Table. D.1.

## **D.0.1 Background correction**

Background fluorescence is anything that does not have an intrinsic temporal correlation, or correlation with the single photon source, such that the correction can be applied as [30]

$$g_{exp}^{(2)}(\tau) = g^{(2)}(\tau)\rho^2 + (1-\rho^2),$$

where  $\rho = \frac{S}{S+B}$  is the ratio of single photon source in the total collected fluorescence. The background is typically measured on a spot away from the emitter to give an estimate of *B* [75].

	$\gamma_1$	<b>Y</b> 2	a	b
Background	×	×	$\checkmark$	$\checkmark$
Jitters	$\checkmark$	×	$\checkmark$	×
Bin size	$\checkmark$	×	$\checkmark$	×

Table D.1: Influence of the three types of corrections on parameters extracted from experimentally fitted  $g^{(2)}(\tau)$ .

## D.0.2 Jitter

The timing jitter of the APDs can be incorporated by convoluting an ideal  $g^{(2)}$  with the instrument response function (IRF)  $f(\tau)$  as [32]

$$g_{exp}^{(2)}(\tau) = \int_{-\infty}^{+\infty} f(\tau - t) g^{(2)}(t) dt$$

For a typical Gaussian IRF,

$$f(t) = \frac{1}{\sqrt{2\pi\sigma^2}}e^{-t^2/2\sigma^2},$$

where  $\sigma$  is the typical time jitter due to the APD.  $f(\tau)$  is small beyond the characteristic timescale  $\sigma$  and thus this correction affects mainly the short-time feature with  $\tau \sim \sigma$ , serving to broaden the anti-bunching dip and decreases the extent of anti-bunching (increasing  $g^{(2)}(\tau = 0)$  to above zero). The experimental  $g^{(2)}$  can be fitted with

$$g_{exp}^{(2)}(\tau) = 1 - \frac{1}{2}\beta e^{\gamma_1^2 \sigma^2} \left[ [1 + \operatorname{erf}(a)] e^{-\gamma_1 \tau} + [1 - \operatorname{erf}(b)] e^{+\gamma_1 \tau} \right] + \alpha e^{-\gamma_2 \tau},$$

where  $a = \frac{\tau - \gamma \sigma^2}{\sqrt{2}\sigma}$ ,  $b = \frac{\tau + \gamma \sigma^2}{\sqrt{2}\sigma}$ , with erf being the error function. This may not sum to 0 at  $\tau = 0$  as this may not be possible due to background fluorescence.

Specifically for an antibunching peak  $g^{(2)}(\tau) = 1 - \beta e^{-\gamma_1 \tau}$ ,

$$g_{exp}^{(2)}(\tau=0) \approx 1 - e^{\gamma_1^2 \sigma^2} \left[ 1 - \beta \operatorname{erf}\left(\frac{\gamma_1}{\sqrt{2}}\sigma\right) \right] + \alpha.$$

This can be computed numerically and the result is shown in Fig. D.1a and Fig. D.1c. Thus experimentally observing  $g^{(2)}(\tau = 0) = 0$  within the statistical error could be difficult depending on the timescale of radiative decay and the ratio of signal in the total fluorescence.

## **D.0.3** Finite bin size

In addition, "accidental coincidences" may arise due to the finite binning size of a  $g^{(2)}(\tau)$  measurement [234]. In this case there is an additional window function to be applied,

$$f_{bin}(t) = egin{cases} 1/ au_b & |t| \leq au_b/2 \ 0 & otherwise \end{cases},$$



(a) Convolution of Instrument Response Function (IRF), and binning window function with an ideal  $g^{(2)}$  function.







(b) Effect of instrument timing jitter on  $g^{(2)}(\tau = 0)$ .



(d)  $g^{(2)}(\tau = 0)$  as a function of ideal  $g^{(2)}$  parameters, given the IRF and jitter of the set-up.

Figure D.1: Corrections to experimental  $g^{(2)}$ .

leading to,

$$g_{exp}^{(2)}( au) pprox egin{cases} 1 - rac{2eta}{\gamma_{1} au_{b}} \left[ 1 - e^{-\gamma_{1} au_{b}/2}\cosh\gamma_{1} au 
ight] & | au| \leq rac{ au_{b}}{2} \ 1 - rac{2eta}{\gamma_{1} au_{b}} \left[ e^{-\gamma_{1}| au|}\sinhrac{\gamma_{1} au_{b}}{2} 
ight] & | au| > rac{ au_{b}}{2}, \end{cases}$$

which only contributes significantly when  $\gamma_1 \tau_b \gg 0$ . The convolution can be numerically computed and shown in Fig. D.1a and Fig. D.1c.

Experimentally each APD has a timing jitter of 350 ps (thus 495 ps in total) and the bin size near  $\tau = 0$  is chosen to be 386 ps. These collectively gives a minimum  $g^{(2)}(\tau = 0) = 0.237$  for even the most ideal single photon source.