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FRET Between NV Centers In Diamond And Chlorophyll Molecules: A Novel Resource For Multimodal Sensing And Imaging In Plant Cells

Sebastian Westrich¹ | Nimba Oshnik¹ | Nina Thiele¹ | Nina Burmeister¹ | Yanis Abdedou¹ | Nikhita Khera¹ | Stefanie J. Müller-Schüssele²  | Elke Neu¹ 

¹Department of Physics and State Research Center OPTIMAS, RPTU University Kaiserslautern-Landau, Kaiserslautern, Germany | ²Department of Biology, RPTU University Kaiserslautern-Landau, Kaiserslautern, Germany

Correspondence: Elke Neu (nruuffing@rptu.de)

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ABSTRACT

This work demonstrates efficient Förster resonance energy transfer (FRET) between ensembles of shallow nitrogen-vacancy (NV) centers located 7 nm and 9 nm below a single-crystal diamond surface and a naturally occurring fluorophore, here a mixture of chlorophyll *a* and *b* molecules extracted from *Arabidopsis thaliana*. The broad fluorescence band of NV centers spectrally overlaps with the chlorophyll molecule's absorption, enabling FRET. Consequently, depositing a chlorophyll layer on the diamond surface reduces the NV fluorescence lifetime from approximately 14 ns to below 4 ns, indicating efficient FRET. Laser-induced photobleaching of chlorophyll restores the unquenched NV lifetime. NV centers located deeper within the diamond (40 and 72 nm) remain unaffected, confirming that the observed quenching originates from a short range FRET mechanism. The NV ensembles retain their optically detected magnetic resonance (ODMR) contrast while observing FRET, demonstrating preservation of their spin properties. Consequently, these proof-of-principle experiments demonstrate the feasibility of combining FRET-based distance measurements with magnetic sensing using optically readable spins.

1 | Introduction

In fluorescence resonance energy transfer often named Förster resonance energy transfer (FRET), two dipoles, referred to as donor and acceptor, exchange energy through a non-radiative process [1]. In this process, the donor dipole is excited by absorbing light and subsequently transfers its excitation energy to the acceptor. The acceptor either emits fluorescence or dissipates the energy non-radiatively. In both cases, FRET introduces an additional decay pathway for the donor, thereby reducing its excited-state lifetime and fluorescence intensity. There are two categories of FRET, namely homo-FRET, in which FRET occurs between two identical dipoles, e.g., between two identical dye

molecules, and hetero-FRET in which non-identical dipoles participate. In the latter case, FRET pairs are feasible in which one dipole is a molecule (dye, fluorescent protein, naturally occurring fluorophore) and the other one is in a solid (quantum dots, point defects in solids). FRET between different types of molecular fluorophores has become a standard resource to measure (intramolecular) distances with high resolution in the sense of a “spectroscopic ruler” [2–5].

Solid-state systems can be particularly stable electrical dipole emitters, making them valuable resources for sensing and imaging. A prominent candidate in this context is the nitrogen-vacancy (NV) center in diamond. Its long-term photostability and

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nanoscale size enables sensing and imaging with single emitters. In particular, single NV centers have been employed in scanning near-field imaging experiments based on FRET [6, 7]. NV centers additionally stand out as their fluorescence rate is related to their spin states. A change in the spin state induces a change in the fluorescence rate, thus making the spin states optically readable. Using this property of the NV centers, important measurements in the life sciences have been demonstrated, e.g., detection of paramagnetic free radicals in cells connected to neuro-degenerative diseases as well as stress and infections [8, 9]. Notably, NV centers in diamond nano-particles have also been shown to undergo hetero-FRET with various synthetic dyes as FRET partners [10–12]. In our own previous work, we demonstrated hetero-FRET between NV centers, implanted shallowly in a single crystal diamond, and excitons in 2D materials [13]. In this model system, we demonstrated that optical spin read-out remains feasible, while the NV centers undergo FRET. With these two prerequisites, we here investigate hetero-FRET between NV centers in a single crystal diamond and naturally occurring fluorophores, namely chlorophyll *a* and *b*, as a novel resource for multimodal sensing and imaging. In this work, we thus explore an approach that would require to introduce only a single foreign fluorophore, namely the NV center, into plant cells to establish multimodal sensing and imaging. Nanodiamonds containing NV centers can, in principle, be delivered into plant cells using established techniques such as gene guns [14, 15]. NV centers are particularly attractive as multimodal probes in the life sciences due to their biocompatibility and photostability, enabling long-term monitoring of intracellular processes without the limitations of photobleaching or toxicity associated with conventional organic dyes or quantum dots. Beyond their role as stable fluorescent markers, NV centers act as multimodal quantum sensors: they combine conventional fluorescence and near-field-based sensing approaches with spin-based techniques that can directly detect paramagnetic species like free radicals or transition metal ions [16, 17]. In photosynthetic organisms, reactive oxygen species and metal-containing protein complexes play a central role in electron transport and stress responses [18]. The ability to detect such paramagnetic species while simultaneously probing the spatial proximity to chlorophyll molecules via FRET opens new opportunities for investigating intracellular processes at the nanoscale. By combining the magnetic sensing capabilities of NV centers with the distance sensitivity provided by FRET, this platform offers a promising route for probing intracellular processes with high spatial resolution.

As a proof-of-principle, we build on our previous work [13] and employ a model system in which a dense layer of chlorophyll molecules from a plant extract is deposited onto the surface of single-crystal diamond via spin-coating from an ethanolic solution. In this case, we can control the depth of our NV centers via controlling the energy at which nitrogen ions are implanted into high-purity diamond which renders it feasible to investigate the FRET process in detail.

2 | Modeling of the FRET Process

We first demonstrate that the NV–chlorophyll pair’s spectral properties allow efficient FRET: Figure 1a shows the broad

fluorescence band of the NV centers spanning 600–800 nm, with the zero-phonon line (ZPL) corresponding to direct optical transitions at 637 nm. This band spectrally overlaps with the maximum absorption of the chlorophyll mixture at 664 nm. While NV ensembles typically include both neutral (NV^0) and negative (NV^-) charge states, our detection path uses a 600 nm long-pass filter, which significantly suppresses the NV^0 zero-phonon line at 575 nm [19] and a substantial part of its associated phonon sideband. Moreover, the Tri-Acid treatment (sample preparation; see Section 6.2) of the diamond surface introduces oxygen-containing functional groups, which further stabilize the NV^- charge state, especially for shallow NV centers [20]. These considerations indicate that the observed FRET signal is dominated by the NV^- contribution. The Förster radius R_0 between the NV^- center and the chlorophyll mixture can be calculated using [10]:

$$R_0 = 0.211(\kappa^2 n^{-4} Q_D J(\lambda))^{1/6} \quad (1)$$

where, R_0 is the distance at which the FRET efficiency reaches 50%. $J(\lambda)$ denotes the spectral overlap between the measured emission spectrum of the NV^- center and the measured absorption spectrum of the chlorophyll solution. The factor κ describes the relative orientation of the donor and acceptor dipoles. Here, we assume a random orientation and thus $\kappa^2 = \frac{2}{3}$. The refractive index of the medium is given by n , with $n = 2.4$ for diamond. The parameter Q_D corresponds to the quantum efficiency of the donor in the absence of an acceptor. For NV^- centers shallowly implanted at 4.5 ± 1 nm and 8 ± 2 nm below the surface, previous work reported quantum efficiencies of 0.7 and 0.8, respectively [21]. From these parameters, we calculate the Förster radius for the NV^- -chlorophyll pair to be 3.6 ± 0.1 nm. This result indicates that FRET between the NV^- center and chlorophyll molecules is feasible for chlorophyll molecules placed on the diamond surface. For simplicity, we term NV^- only NV center in the following.

The expression in Equation (1) and the z^{-6} distance dependence for FRET between point dipoles describe the energy transfer rate for a single donor acceptor pair. In the present system, however, each NV center interacts not with an isolated, single acceptor but with a dense layer of chlorophyll molecules deposited on the diamond surface. We therefore approximate the chlorophyll layer as a continuous distribution of point-like acceptor dipoles with an effective surface density σ_{chl} . We cannot independently measure the value of σ_{chl} in our experiment and σ_{chl} may vary due to a locally inhomogeneous chlorophyll film (e.g. aggregation). Consequently, σ_{chl} enters the model only as an effective coupling parameter. This model reflects that each NV center interacts simultaneously with many chlorophyll molecules, which we effectively model as a continuous acceptor surface. In this geometry, the cumulative interaction with multiple individual acceptor dipoles modifies the distance dependence from the single-pair z^{-6} scaling to the behavior characteristic of a planar acceptor ensemble.

Following this line of argument, we neglect the finite thickness of the chlorophyll layer and restrict the integration to an effective 2D acceptor plane at the surface. The total non-radiative decay rate $\gamma_{\text{non-rad}}$ for a point donor interacting with

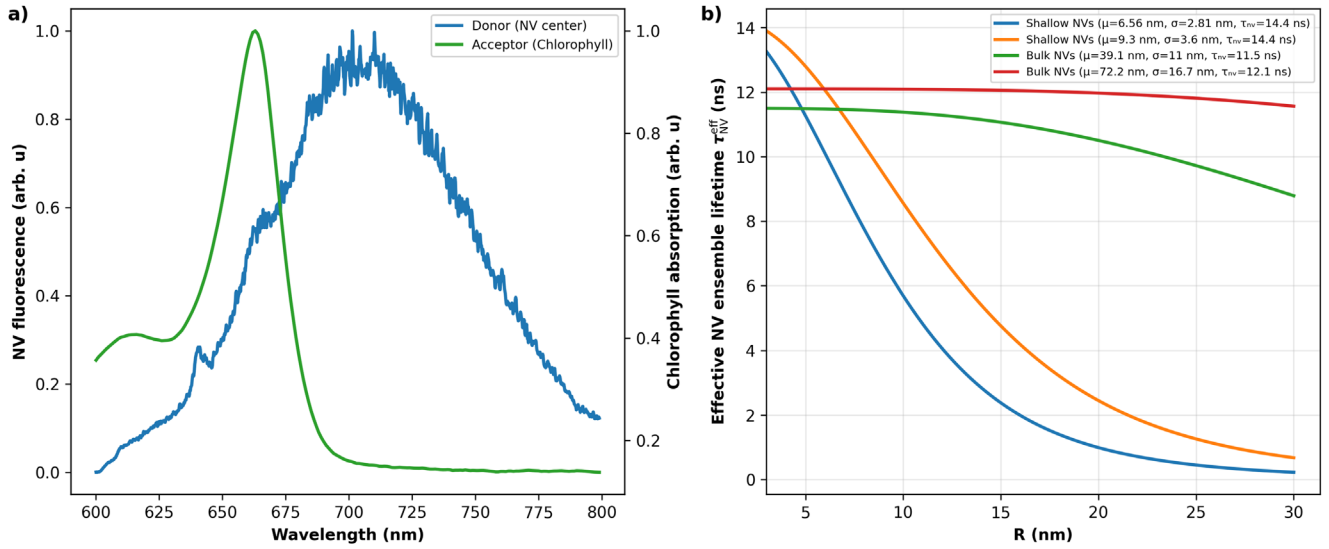


FIGURE 1 | (a) Spectral overlap between the NV center emission and the absorption spectrum of the chlorophyll sample. Both spectra were measured experimentally. (b) Simulated effective NV ensemble lifetime $\tau_{\text{NV}}^{\text{eff}}$ as a function of the effective coupling parameter R , calculated using the FRET model described in Equation (3).

an extended 2D point-like acceptor distribution is obtained by integrating the pairwise z^{-6} dipole-dipole interaction over the acceptor plane. This integration yields a z^{-4} distance dependence. Accordingly, the non-radiative decay rate $\gamma_{\text{non-rad}}$ is given by [6]:

$$\gamma_{\text{non-rad}} = \gamma_{\text{rad}} \frac{R^4}{z^4} \quad (2)$$

Here, z gives the distance between the donor and the acceptor plane, while R denotes an effective coupling parameter that depends on the Förster radius R_0 and the (in our case unknown) surface density of acceptor molecules σ_{Chl} . We denote by γ_{rad} the intrinsic decay rate of the NV centers and assume equal γ_{rad} for all NV centers in our ensemble. γ_{rad} is set equal to $(\tau_{\text{NV}})^{-1}$, where τ_{NV} is the lifetime measured for the different NV ensembles in the absence of FRET. This decay rate implicitly includes all intrinsic non-radiative decay channels present in the bare diamond that are independent of the FRET process. Equation (2) describes the distance dependence of the FRET induced non-radiative decay. As a result, NV centers located close to the surface experience stronger quenching, leading to shorter fluorescence lifetimes and reduced photon emission rates. However, in our experiment, the detected fluorescence originates from an ensemble of NV centers located at different depths z . In each confocal spot, we observe around 10 NV centers. This estimate is based on the ion implantation parameters and the associated NV creation yield (sample preparation; see Section 6.2). While the detection volume of a confocal microscope extends over several micrometers, the implanted NV ensembles are distributed in depth according to $D(z)$ with a mean and standard deviation on the nanometer scale, resulting in a total depth spread of less than 100 nm. Consequently, the measured fluorescence decay curves represent the added emission of all NV centers within the depth distribution $D(z)$. In principle, this spread in donor-acceptor distances should give rise to a multi-exponential fluorescence decay. Nevertheless, both previous reports [13] and our own measurements show that the NV ensemble decay can be well described by a

single exponential decay corresponding to an effective lifetime $\tau_{\text{NV}}^{\text{eff}}$.

To model the measured fluorescence decay of the NV ensemble, we consider the contribution of NV centers located at different depths z below the diamond surface. Each NV center exhibits a mono-exponential decay curve corresponding to its specific donor-acceptor distance. These decay curves are weighted by the NV's fluorescence intensity $I(z)$, since NV centers with quenched emission contribute less to the measured signal, and by the depth distribution $D(z)$, which accounts for the geometric distribution of NV centers in the sample. $D(z)$ arises from the ion implantation process and is well described by a Gaussian function characterized by a mean depth μ and a standard deviation σ . The overall fluorescence decay of the ensemble is then obtained by integrating over the NV depth profile, leading to

$$I(t)_{\text{Ensemble}} = \int_0^\infty D(z) I_0 \frac{\gamma_{\text{rad}}}{\gamma_{\text{rad}} + \gamma_{\text{non-rad}}(z)} \exp\left(-\frac{t}{\tau(z)}\right) dz \quad (3)$$

Using this model, the fluorescence decay of the NV ensemble is well described by a mono-exponential decay with an effective NV ensemble lifetime $\tau_{\text{NV}}^{\text{eff}}$, as demonstrated in our previous work [13]. We computed $\tau_{\text{NV}}^{\text{eff}}$ for the different depth distributions of our samples as a function of R in the range of $3 \text{ nm} < R < 30 \text{ nm}$. Figure 1b shows the corresponding curves.

We emphasize that R does not represent the Förster radius R_0 of a single donor-acceptor pair. Instead, it denotes an effective coupling length that parameterizes the interaction between one NV center and the planar ensemble of acceptor dipoles. This effective parameter incorporates the intrinsic Förster radius R_0 , the surface density of chlorophyll molecules σ_{Chl} , and the geometrical averaging over the NV depth distribution. Consequently, R should be regarded as an empirically determined effective quantity describing the collective donor-ensemble coupling strength rather than a microscopic single-pair FRET distance.

TABLE 1 | Nitrogen ion implantation parameters, associated depth distributions, and intrinsic NV lifetimes.

Energy [keV]	Fluence [cm^{-2}]	Depth [nm]	Uncertainty [nm]	Intrinsic NV lifetime [ns]
4	4×10^{11}	7	± 3	14.4 ± 0.4
6	3×10^{11}	9	± 4	14.4 ± 0.4
30	5×10^{10}	40	± 11	11.5 ± 0.5
60	3×10^{10}	72	± 20	12.0 ± 1.0

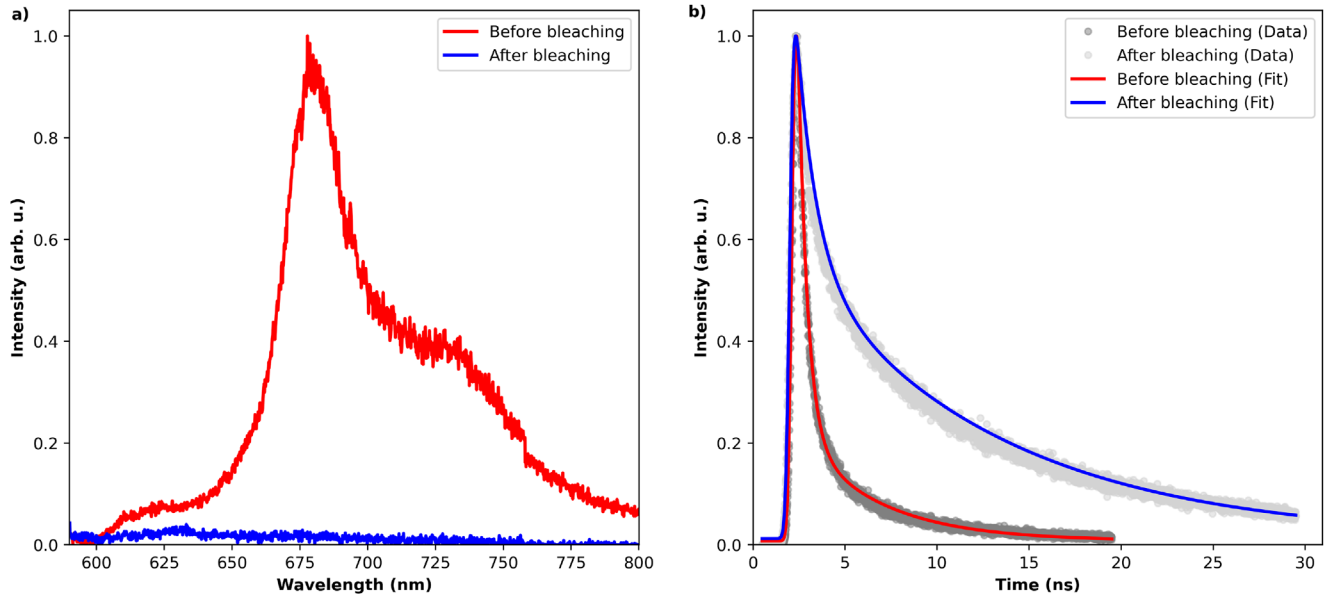


FIGURE 2 | (a) Emission spectra of the chlorophyll sample drop-cast onto a silicon substrate before and after bleaching. The fluorescence spans the spectral range from 650 to 800 nm with a maximum at 680 nm. The sample was excited using pulsed laser light at a wavelength of 520 nm. (b) Time-resolved fluorescence measurement of the NV-chlorophyll system. The data corresponds to an NV ensemble located at a depth of 9 ± 4 nm, excited with pulsed laser light ($\lambda = 520$ nm, pulse length ≈ 50 ps, repetition rate of 5.56 MHz). Bleaching of the chlorophyll layer leads to a recovery of the NV lifetime from 4.36 ± 0.03 ns to 11.01 ± 0.05 ns.

3 | Proof-of-Principle FRET

We first characterize the NV ensembles in four different depths (sample preparation; see Section 6.2), NV depths see Table 1, whereas we term the two ensembles at less than 10 nm depth as shallow. All lifetime measurements in this characterization step were carried out on the bare diamond samples before depositing a chlorophyll layer. Shallow NV centers exhibited an average lifetime of $\tau_{\text{NV}} = 14.4 \pm 0.4$ ns, whereas deeper NV centers showed slightly shorter lifetimes of $\tau_{\text{NV}} = 11.5 \pm 0.5$ ns at 40 ± 11 nm depth and $\tau_{\text{NV}} = 12 \pm 1$ ns at 70 ± 20 nm. The fluorescence emission of chlorophyll *a* and *b* spans the spectral range from 650 to 800 nm [22], which overlaps with the fluorescence band of NV centers. It is therefore not possible to spectrally separate NV and chlorophyll fluorescence. We obtained the fluorescence spectrum of our chlorophyll sample by drop casting the solution onto a silicon substrate to measure on a large quantity of chlorophyll. We observe a fluorescence maximum at 680 nm (Figure 2a), which is in accordance with literature values for chlorophyll *a* and *b* [23]. Moreover, we record the emission spectra of chlorophyll and NV centers in separate measurements: While chlorophyll is an efficient quencher, it exhibits only weak fluorescence under

green excitation and we thus need to work with larger quantities of chlorophyll for spectra acquisition than used in the FRET experiments. To determine the intrinsic fluorescence lifetime of the chlorophyll mixture, we use a chlorophyll layer on a silicon substrate and excite it using 520 nm laser light, ensuring direct comparability with the NV-chlorophyll experiments. We obtained an average fluorescence lifetime for chlorophyll of $\tau_{\text{Chl}} = 0.63 \pm 0.08$ ns, indicating that chlorophyll will act as an efficient acceptor and quencher. In addition, τ_{Chl} could be resolved in the measurements on the diamond substrate, where we found an average chlorophyll lifetime of $\tau_{\text{Chl}} = 0.6 \pm 0.1$ ns extracted from bi-exponential fitting of the decay curves. These results are in good agreement with previously reported in vivo lifetime measurements on green plants comparable to *Arabidopsis thaliana*, which yielded fluorescence lifetimes of $\tau_{\text{Chl}} = 0.6$ ns and $\tau_{\text{Chl}} = 0.7$ ns for chlorophyll *a* aggregates [24].

To investigate stability of the chlorophyll molecules in the film, we studied the temporal evolution of their fluorescence intensity under continuous-wave (cw) and pulsed laser excitation on both silicon and diamond substrates. Under cw excitation at 520 nm (laser power $280 \mu\text{W}$), the fluorescence intensity decreased to

approximately 10 % of its initial value within 15 min. Under pulsed excitation at an average laser power of approximately $6\ \mu\text{W}$, a reduction of the fluorescence count rate was also observed, but on a longer timescale of about 45 min. As shown in Figure 2a, the decay of the chlorophyll emission spectrum illustrates that the bleaching process is effective. Given that lifetime measurements involve an integration time of 300 s, this slow fluorescence reduction is negligible.

Based on this characterization, we established the following measurement protocol to probe FRET between NV centers and chlorophyll molecules. After spin-coating the chlorophyll layer onto the diamond surface (see Section 6.2), fluorescence decay curves were recorded using pulsed excitation at 520 nm (≈ 50 ps pulse length, 5 – 10 MHz repetition rate). Subsequently, the chlorophyll layer was photobleached under cw excitation (520 nm, 20 min, $280\ \mu\text{W}$), and the decay measurement was repeated under identical pulsed excitation conditions. This sequence was performed at several positions across the diamond surface.

The fluorescence signal, consisting of NV and chlorophyll contributions, was fitted using a two-component exponential model convolved with the Gaussian instrument response function (see Section 6.1):

$$I(t) = \frac{A_{\text{NV}}}{2} \exp\left(\frac{\sigma_{\text{IRF}}^2}{2\tau_{\text{NV}}^2} - \frac{t-t_0}{\tau_{\text{NV}}}\right) \left[1 + \operatorname{erf}\left(\frac{t-t_0 - \frac{\sigma_{\text{IRF}}^2}{\tau_{\text{NV}}}}{\sqrt{2}\sigma_{\text{IRF}}}\right)\right] + \frac{A_{\text{Chl}}}{2} \exp\left(\frac{\sigma_{\text{IRF}}^2}{2\tau_{\text{Chl}}^2} - \frac{t-t_0}{\tau_{\text{Chl}}}\right) \left[1 + \operatorname{erf}\left(\frac{t-t_0 - \frac{\sigma_{\text{IRF}}^2}{\tau_{\text{Chl}}}}{\sqrt{2}\sigma_{\text{IRF}}}\right)\right] + C \quad (4)$$

Here, τ_{Chl} and A_{Chl} describe the decay time and amplitude of the chlorophyll fluorescence, respectively, while τ_{NV} and A_{NV} correspond to the fluorescence lifetime and fluorescence amplitude of the NV ensemble. The parameter σ_{IRF} denotes the standard deviation of the Gaussian instrument response function. Figure 2b illustrates a representative measurement, showing a quenched NV-lifetime of $\tau_{\text{NV, before}} = 4.36 \pm 0.03$ ns and an NV-lifetime of $\tau_{\text{NV, after}} = 11.01 \pm 0.05$ ns after bleaching the chlorophyll layer.

We investigated four diamond samples with NV ensembles at different depths (see Table 1). Measurements were performed at several positions across the surface to account for local variations in chlorophyll coverage. For the NV ensemble with a depth distribution of 7 ± 3 nm, we observed an average NV lifetime of $\tau_{\text{NV, before}} = 3.3 \pm 0.5$ ns prior to bleaching. After bleaching the chlorophyll, the NV lifetime increased to $\tau_{\text{NV, after}} = 9.3 \pm 0.5$ ns. A similar trend was found for a second diamond sample with shallow NV centers at 9 ± 4 nm depth, where the lifetime increased from $\tau_{\text{NV, before}} = 3.8 \pm 0.6$ ns to $\tau_{\text{NV, after}} = 10.8 \pm 0.6$ ns. An overview of all measured NV lifetimes before and after chlorophyll bleaching is presented in Figure 3. In both shallow NV ensembles, chlorophyll acted as a strong acceptor and efficiently quenched the NV fluorescence lifetime. Extended laser irradiation bleached the chlorophyll layer and thereby restored the NV lifetime. However, the recovered lifetimes ($\tau_{\text{NV, after}} =$

9.3 ± 0.5 ns and 10.8 ± 0.6 ns) do not fully return to the intrinsic value of the untreated NV centers (14.4 ± 0.4 ns). We attribute this difference to incomplete bleaching of the chlorophyll layer, in which residual chlorophyll molecules remain on the surface and continue to introduce a weak non-radiative decay channel.

Furthermore, we employed our model of a point-like donor interacting with many individual acceptor dipoles that collectively form an effective acceptor plane for further analysis. The experimental fluorescence decay curves were fitted with a mono-exponential function to extract the effective NV ensemble lifetime $\tau_{\text{NV}}^{\text{eff}}$. To ensure consistency between the measured data and the model, the fitting range was restricted to the time interval dominated by the mono-exponential decay of the NV ensembles. Data from the first nanosecond were excluded, as this region is governed by chlorophyll fluorescence and the instrument response. The extracted effective NV ensemble lifetime was then inserted into Equation (3) to determine the effective coupling parameter R , which quantifies the donor–acceptor interaction strength. For the ensemble with a depth distribution of 7 ± 3 nm, we obtained $R = 13 \pm 2$ nm, whereas for the ensemble located at 9 ± 4 nm depth, we found $R = 17 \pm 2$ nm. It should be noted that the effective coupling parameter R obtained here does not correspond to the Förster radius R_0 of a single donor–acceptor pair. Instead, it represents a collective interaction length that incorporates the intrinsic R_0 , the surface density of acceptor molecules σ_{Chl} , and the depth distribution of the NV ensemble. Within the planar donor–acceptor geometry introduced in Section 2, where a point-like NV donor interacts with a 2D distribution of acceptor dipoles at the diamond surface, such an effective length scale naturally exceeds the single-pair Förster radius, as the cumulative contribution of many acceptors enhances the overall energy transfer rate. Reported interaction distances for comparable donor–surface-acceptor configurations lie in the range of approximately 1–40 nm [25], placing our extracted values well within the expected regime.

To verify that the observed lifetime reduction is due to FRET, we performed the same measurements on NV ensembles located deeper in the diamond, where the donor–acceptor separation is substantially larger. These measurements were carried out on the same diamond sample, which contains two spatially separated regions implanted with different nitrogen ion energies, resulting in NV depth distributions of 40 ± 11 nm and 70 ± 16 nm. Based on our lifetime simulations, only a minor reduction in the NV ensemble lifetime is expected for these NV centers. In this case, we measured an average NV lifetime of $\tau_{\text{NV, before}} = 11.9 \pm 1.1$ ns prior to bleaching and $\tau_{\text{NV, after}} = 12.1 \pm 0.7$ ns after chlorophyll bleaching. These values agree with the intrinsic lifetimes of the respective NV centers measured prior to chlorophyll deposition (11.5 ± 0.5 ns at 40 nm and 12 ± 1 ns at 70 nm) and show no measurable change upon chlorophyll bleaching. This confirms that NV centers located tens of nanometers below the surface are unaffected, as expected for a short-range FRET mechanism.

4 | Spin Properties of NV Centers Interacting With Chlorophyll

Optically detected magnetic resonance (ODMR) forms the foundation of all NV-spin-based magnetic sensing techniques and directly enables applications such as magnetic imaging of living

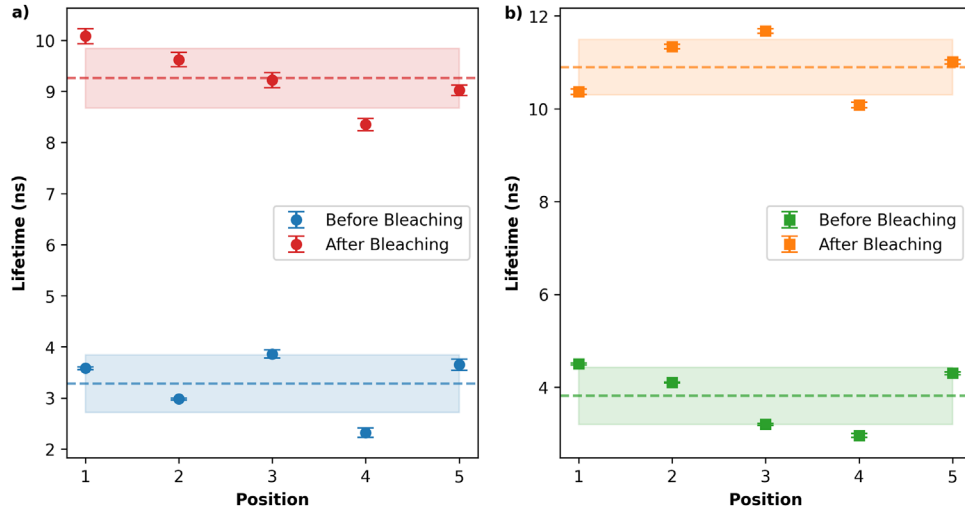


FIGURE 3 | NV fluorescence lifetimes before and after bleaching of the chlorophyll layer. The dashed lines indicate the mean NV lifetimes, and the shaded regions represent one standard deviation ($\pm 1\sigma$). (a) NV ensemble with a depth distribution of 7 ± 3 nm, showing an average NV lifetime of $\tau_{\text{NV, before}} = 3.3 \pm 0.5$ ns before bleaching and $\tau_{\text{NV, after}} = 9.3 \pm 0.5$ ns after bleaching of the chlorophyll layer. (b) NV ensemble with a depth distribution of 9 ± 4 nm, showing an increase in the average NV lifetime from $\tau_{\text{NV, before}} = 3.8 \pm 0.6$ ns to $\tau_{\text{NV, after}} = 10.8 \pm 0.6$ ns following chlorophyll bleaching. In both shallow NV ensembles, chlorophyll efficiently quenches the NV fluorescence, while bleaching restores the original NV lifetime.

cells [26]. The presence of a strong quencher, such as the chlorophyll layer at the diamond surface could, in principle, affect the spin-dependent optical readout of the NV center, thereby preventing ODMR and spin-based sensing. To analyze whether the presence of the chlorophyll layer compromises the spin-dependent optical readout, we investigate the electronic spin properties for the NV ensemble (depth: 9.3 ± 3.6 nm) under the chlorophyll layer using ODMR measurements. To ensure that chlorophyll does not bleach during the spin measurements, we monitored both the PL count rate and the NV lifetime before and after each ODMR experiment. Only datasets without significant changes in these parameters were included in the analysis. We used pulsed laser excitation when performing ODMR measurements. As in lifetime experiments, the detected fluorescence was filtered using a 600 nm long-pass filter. The applied microwave power was set to -10 dB at the source and subsequently amplified by 45 dB before being delivered to the sample. Without a chlorophyll layer on top of the diamond surface, we determine an ODMR contrast in the range of 10 to 12 % in the absence of an external magnetic field. In the presence of the chlorophyll layer, we observed an average ODMR contrast of 4 ± 2 %. Figure 4 shows a representative ODMR spectrum of the NV ensemble underneath the chlorophyll layer, with a contrast of 6 % and a full width at half maximum (FWHM) of 10 MHz. The reduced ODMR contrast arises from the additional chlorophyll fluorescence, which is not spin-dependent and therefore contributes a background signal. After the chlorophyll was bleached, the ODMR contrast recovered. Our ODMR measurements in the FRET case strongly suggest that spin-based sensing approaches can be combined with FRET-based approaches for NV centers.

5 | Conclusion

In conclusion, we have demonstrated FRET between shallow NV centers in single-crystal diamond and a naturally occurring fluorophore, namely a mixture of chlorophyll *a* and *b* extracted

from *Arabidopsis thaliana*. The chlorophyll layer acts as a strong energy acceptor, leading to a significant reduction of the NV fluorescence lifetime ($\tau_{\text{NV}} < 4$ ns). Bleaching of the chlorophyll restores the unquenched NV lifetime. Importantly, the NV ensembles undergoing FRET retain their optically detected magnetic resonance, demonstrating that their spin properties remain intact during energy transfer. In contrast, NV centers located in the bulk diamond remain unaffected, consistent with the distance dependence of the FRET mechanism. Our experiments constitute a first step toward using NV centers as sensors in plant cells. While FRET could, e.g., determine the proximity to chlorophyll, NV spin measurements can help to detect radical species.

6 | Experimental Section

6.1 | Experimental Setup

A custom-built confocal scanning microscope (see ref. [27]) was employed to investigate the interaction between NV centers in single-crystal diamond (SCD) and a chlorophyll layer on the diamond surface. For lifetime measurements, we use a tunable pulsed laser (NKT EXW-12, 450–850 nm, pulse length ≈ 50 ps) equipped with a filter system (NKT SuperK Varia) as the excitation source. A diode laser at 520 nm (DL nSec, PE 520, Swabian Instruments) with a maximum output power of 40 mW was utilized to bleach chlorophyll molecules and to perform confocal fluorescence mapping. Excitation light was guided in a single-mode fiber, and a 600 nm short-pass filter located behind the fiber suppressed background contributions. Sample scanning was performed with a piezoelectric stage (ANSxyz100, Attocube Systems). Excitation and fluorescence light were separated using two dichroic mirrors with a cutoff wavelength of 600 nm and an additional 600 nm long-pass filter.

The collected fluorescence was directed either to a high-efficiency single-photon counting module (Excelitas

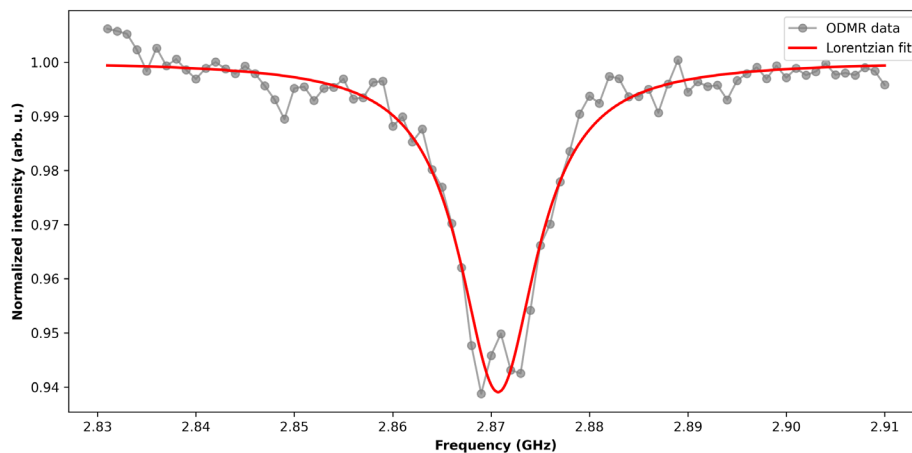


FIGURE 4 | Optically detected magnetic resonance spectrum of the quenched NV ensemble (depth: 9 ± 4 nm) under the chlorophyll layer in the absence of an external magnetic field. The measurement, performed with a microwave source set to -10 dBm and amplified by $+45$ dB demonstrates that NV centers maintain their spin-dependent fluorescence contrast even in the presence of efficient energy transfer, highlighting their potential as multifunctional quantum sensors.

SPCM-AQRH-14, quantum efficiency $\approx 69\%$ at 700 nm) or to a grating spectrometer (Acton Spectra Pro 2500) equipped with a Pixis 256OE CCD camera. A time-correlated single-photon counting module (PicoQuant PicoHarp 300) was used for time-correlated single-photon counting to measure the fluorescence decay curves. The respective sample was excited with pulsed laser light ($\lambda = 520$ nm, pulse length ≈ 50 ps, repetition rate of $5 - 10$ MHz). Since the repetition rates in our experiments are $5 - 10$ MHz while the detected photon count rates are in the kHz range, the probability of detecting more than one fluorescence photon per excitation cycle is negligible, and pile-up effects can be neglected. The instrument response function (IRF) of the setup was determined experimentally by detecting the reflection of the excitation laser pulses from a mirror surface. The resulting IRF is modeled by a Gaussian function with a full width at half maximum (FWHM) of 440 ps. Fluorescence decay curves were fitted with exponential decay functions convoluted with the IRF. For spin manipulation, the setup was equipped with a microwave source (TSG4100A, Tektronix) and a $+45$ dB amplifier (ZHL-16W-43+, Mini-Circuits), while the microwave drive was delivered to the NVs via a $50\ \mu\text{m}$ thick copper wire placed near the sample.

6.2 | Diamond Sample Preparation

We employed three diamond samples with different NV depth distributions to investigate FRET between NV ensembles and the chlorophyll layer. The samples consist of high-purity, (100)-oriented, single-crystal diamonds (Element Six) of electronic grade quality ($[\text{N}] < 5$ ppb, $[\text{B}] < 1$ ppb). Two of the samples were polished by Almax easyLab and one by Delaware Diamond Knives, achieving a surface roughness of $R_a < 1$ nm. Following established approaches for creating shallow NV centers [28, 29], we first remove polishing-induced damage prior to nitrogen implantation. This is achieved by performing a stress-relief etch using inductively coupled plasma reactive ion etching (ICP-RIE, Oxford Instruments Plasmalab 100), which ensures NV centers in low-strain material. Nitrogen ions were implanted at multiple energies and fluences under an angle of 7° to suppress

ion channeling. Monte Carlo simulations performed with SRIM [30] were used to estimate the corresponding NV center depth distributions. The predicted implantation depths, along with the respective implantation parameters, are summarized in Table 1. The two shallow NV ensembles (< 10 nm) were realized on separate diamond samples, whereas the two deeper NV ensembles were created in different regions of a single diamond sample using distinct implantation energies. It should be noted that SRIM simulations typically underestimate experimentally measured implantation depths, as reported in prior studies [31].

After ion implantation, the diamonds were annealed at 800°C for 2 h in vacuum ($< 7.8 \times 10^{-7}$ mbar) using a custom-built furnace (Edwards T-Station 75, Tectra UHV-compatible Boralectric heater HTR-1001) to form NV centers [29]. To remove residual non-diamond carbon that may form during the annealing process, all diamonds were subsequently cleaned in a tri-acid mixture of nitric acid (65%), perchloric acid (70%), and sulfuric acid (96%) in a 1:1:1 ratio on a hot plate at 500°C for 1 h. This procedure was followed by sonication in acetone and isopropanol. The same cleaning sequence was also employed to remove chlorophyll residues in-between different measurement cycles. Assuming a typical creation yield on the order of 1% [32], the implantation parameters result in a sparse NV ensemble with less than 10 NV centers within our confocal microscope's focus. Given the axial resolution in the micron range, the entire NV distribution is effectively contained within the focal depth of our microscope.

6.3 | Chlorophyll Extraction

For the chlorophyll extraction from *Arabidopsis thaliana*, we followed a standard protocol. A volume of $500\ \mu\text{L}$ ethanol (95 % v/v) was added to $10 - 200$ mg of plant material in a 2 mL microcentrifuge tube. The samples were mixed for 5 min in a thermomixer at room temperature and subsequently centrifuged at $14\,000$ g for 5 min to remove remaining cell debris. A total of $400\ \mu\text{L}$ of the supernatant was then transferred to a fresh microcentrifuge tube. Subsequently, the chlorophyll concentration was determined by absorption spectroscopy. For this purpose, the optical density

TABLE 2 | Concentration of chlorophyll and carotenoids in *Arabidopsis thaliana*.

Sample	Dilution Factor	Chl a [$\mu\text{g/mL}$]	Chl b [$\mu\text{g/mL}$]	Total Chl [$\mu\text{g/mL}$]	Carotenoids [$\mu\text{g/mL}$]
<i>Arabidopsis thaliana</i>	10	64.63	30.88	95.50	12.67
<i>Arabidopsis thaliana</i>	100	59.84	46.17	106.01	7.49

(OD) of the supernatant was measured at excitation wavelengths of 664, 649, and 470 nm for two different dilution factors. We calculated the concentration of the respective molecules using the method described in [22], and the respective results are shown in Table 2.

The extracted chlorophyll solution was subsequently used for the preparation of thin films on diamond and silicon substrates.

6.4 | Spin Coating of Chlorophyll onto Diamond

The extracted chlorophyll solution was subsequently used for the preparation of thin films on the diamond substrates by spin coating, which was performed under ambient laboratory conditions. To optimize the process parameters, we first carried out test measurements on silicon substrates using 4 μL of the chlorophyll solution for each spin-coating process. The resulting film quality and surface coverage were then analyzed using atomic force microscopy. AFM [Park XE-70] measurements revealed that the chlorophyll layer is non-uniform and that the molecules tend to aggregate. To evaluate this behavior, we measured the surface roughness of the chlorophyll films. At low spin speeds (< 1000 rpm), the surface exhibited very high roughness values ($R_a > 100$ nm). In contrast, higher spin speeds (1500 – 3500 rpm) produced smoother films, with an average roughness of $R_a = 20 \pm 8$ nm. This aggregation behavior can be attributed to the molecular structure of chlorophylls *a* and *b*, which consist of a chlorine ring and a phytol side chain, a long-chain hydrocarbon. The phytol side chain promotes inter-molecular interactions that facilitate molecular clustering on the substrate surface, leading to the observed non-uniform films. In areas without agglomerates, we measured a film thickness of 14 ± 3 nm. For the deposition of the chlorophyll sample onto the diamond, 4 μL of solution was spin-coated at 2000 rpm for 1 min. In this case, no AFM roughness analysis was performed to not lose time for the optical measurements. To minimize external influences on the chlorophyll sample with respect to the lifetime measurements, it was necessary to perform the corresponding measurements immediately after coating. The quality and homogeneity of the deposited layer were verified using confocal microscopy. As already observed in preliminary tests with silicon substrates, the formation of agglomerates was also evident on the diamond surface. Nevertheless, chlorophyll molecules were present throughout the substrate. To ensure a reproducible donor-acceptor environment, all fluorescence lifetime measurements were performed at positions without visible agglomerates.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in Zenodo at the following URL/DOI: <https://doi.org/10.5281/zenodo.18185936>.

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