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Not all fluorescent nanodiamonds are created equal: a comparative study

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Abstract

Fluorescent nanodiamonds (FNDs) are vital to many emerging nanotechnological applications from bio-imaging and sensing to quantum nanophotonics. Yet, understanding

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and engineering the properties of fluorescent defects in nanodiamonds remains challenging. We present the most comprehensive study to date, of the optical and physical properties of five different nanodiamond samples, in which fluorescent nitrogen-vacancy (NV) centers were created using different fabrication techniques. The FNDs' fluorescence spectra, lifetime and spin relaxation time (T_1) are investigated via single-particle confocal fluorescence microscopy and in ensemble measurements in-solution (T_1 excepted). Particle sizes and shapes are determined using scanning electron microscopy and correlated with the optical results. We use statistical tests to explore correlations between the properties of individual particles and also analyze average results to directly compare different fabrication techniques. Spectral unmixing is used to quantify the relative NV charge-state (NV^- and NV^0) contributions to the overall fluorescence.

We find and quantify a strong variation in the properties of individual particles within all analyzed samples and significant differences between the different particle types. This study is an important contribution towards understanding the properties of NV centers in nanodiamonds. It motivates new approaches to the improved engineering of NV-containing nanodiamonds for future applications.

Introduction

Fluorescent nanodiamonds (FNDs) have attracted significant attention in the scientific community over the past two decades.^[1–3] Today, FNDs are becoming increasingly important for technological applications from remote optical fiber-based sensing^[4] to single particle tracking^[5–9] and nanoscale sensing^[5,10–13] in biology. Despite the fact that many fluorescent color centers are known,^[14] the most studied and technologically most developed color center to date is the nitrogen-vacancy (NV) center. One reason for this is its unique

electronic structure that makes its fluorescence highly sensitive to magnetic and electric fields, temperature and pressure.^[15] Its fluorescence is also extremely photostable^[16] and emission from single NV centers has been used in a variety of quantum optics applications.^[17] Another reason for its dominance in the nanoparticle domain is the relatively high concentration of substitutional nitrogen defects (so-called P1 center) on the order of 100 ppm, that are incorporated during synthetic high-pressure high-temperature (HPHT) diamond growth. The presence of nitrogen makes the creation of NV color centers at concentrations on the order of 10 ppm via irradiation and annealing^[18] relatively simple, compared to other important color centers like the silicon vacancy center.^[19] Yet, the reliance on the incorporation of nitrogen impurities during the HPHT synthesis also means that their distribution and precise concentration in the diamond lattice cannot be controlled easily and indeed varies significantly across the various HPHT growth sectors.

The requirements with regards to the number, positioning and properties of NV centers in nanodiamond particles differ widely between different applications. For many quantum applications, a single NV defect in the geometrical center of a nanoparticle (NP), where the effects of magnetic noise from the surface is minimal, is highly desirable. For many nanoscale imaging and sensing applications, a strong fluorescence signal is critical and hence high NV concentrations required. Significant efforts have been made to optimize the creation of NV centers in nanodiamonds. In HPHT diamond, high concentrations of NV centers are generally created via irradiation with high energy particles for the creation of vacancies, followed by annealing that enables vacancies to diffuse and form NV centers with substitutional nitrogen. Particles used commonly today for irradiation include electrons^[20,21], protons^[22-23] and He⁺^[24,25]. Also, various annealing processes as well as different milling and chemical and physical purification protocols for the fabrication of pure diamond nanoparticles.^[26] To date very few studies have systematically compared the properties of nanodiamond particles fabricated using different processes or manufactured by different commercial suppliers. Sotoma et. al. have investigated the use of a range of ions (H⁺, He⁺,

Li⁺, N⁺) for the creation of NV centers in nanodiamonds with a focus on optimizing the irradiation doses.^[27] However, the variability of the properties of individual particles within a given batch and how these relate to ensemble properties of FNDs dispersed in water has not been systematically investigated. Yet, for many applications a thorough understanding of these parameters is critical for the development of emerging technologies based on fluorescent nanodiamonds.^[28]

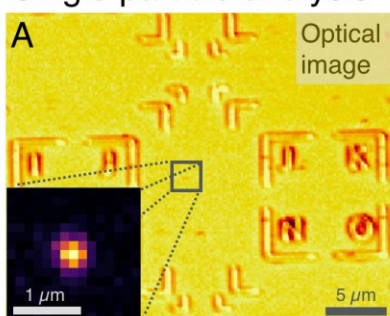
Here, we characterize the size, shape and optical properties of 205 individual FNDs across 5 different particle batches. Three different particle types were fabricated in-house from the same nanodiamond ‘raw’ material and were irradiated with electrons, protons and with a new approach where He²⁺ + Li⁺ particles are generated from ¹⁰B by neutron capture.^[29] In addition, FNDs from two commercial suppliers are also investigated. We determine each particle’s fluorescence brightness, lifetime and spectrum and T_1 spin relaxation time using confocal fluorescence microscopy as well as particle size and shape via scanning electron microscopy (SEM). Within each batch, we analyze the variation of properties between individual particles and systematically investigate correlations between the observed parameters. We also compare the properties of the different batches both based on single-particle results and based on ensemble results of the FNDs dispersed in water – the environment for many important applications.

Results

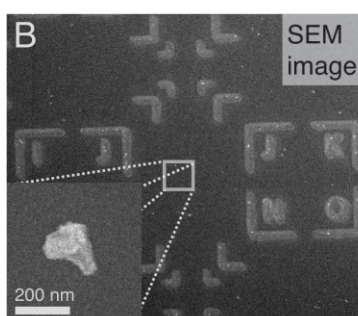
Figure 1 illustrates our experimental approach consisting of confocal fluorescence microscopy and SEM-based single-particle measurements and ensemble analysis of FNDs dispersed in water. Briefly, FNDs were spin-coated onto a marked silicon wafer substrate and a confocal reflection image was acquired (Figure 1A). Here, particles appear as slightly darker spots due to scattering of light. Identifying FNDs via reflection imaging prevents a particle selection bias based on fluorescence brightness. For each of the 5 samples, the

fluorescence from at least 100 nanodiamonds was imaged (Figure 1A, inset). Fluorescence spectra and a time resolved fluorescence decay traces were then recorded for each FND (excitation wavelength: λ_{ex} = 520 nm, 5 μ W, 20 ps pulse-width, 5 MHz repetition rate, see Experimental Section for details). Subsequently, SEM images of all spots were acquired (Figure 1B). 50% - 74% of all investigated spots contained more than one particle in the form of particle clusters. See Supporting Information (SI) Figure S1 and Table S1 for more information. Only the locations with individual particles (Figure 2B, inset) were further investigated and their spin relaxation times T_1 determined. Fluorescence spectra and time-resolved fluorescence decay traces were also acquired for all nanodiamond samples dispersed in water at a concentration of 0.1 mg mL⁻¹ (Figure 1C) using a custom-built fluorescence spectroscopy setup (see SI Figure S2 for details).

Single particle analysis

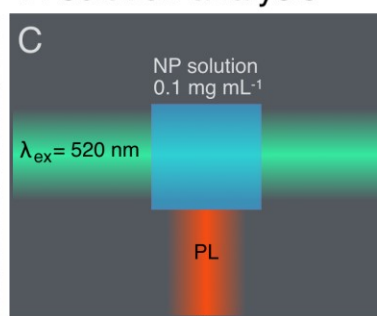


PL spectrum & lifetime
Spin relaxation T_1



Particle size
Particle shape

In-solution analysis



PL spectrum
PL lifetime

Figure 1. Schematic illustration of the experimental approach. A: Confocal reflection images of particles dispersed on a marked silicon wafer substrate were collected. The fluorescence from an individual particle located within a diffraction limited focal spot (inset) was analyzed in terms of its fluorescence spectrum, fluorescence lifetime and T_1 spin relaxation time. B: The same particle was imaged via scanning electron microscopy (SEM) to verify that a single particle is investigated (as opposed to a small aggregate of particles) and to determine particle size and shape. C: particles were also analyzed dispersed in water to obtain ensemble fluorescence spectra and fluorescence lifetime. The total number of individual particles investigated is 205 across 5 different particle types.

Important information and characteristics of the investigated nanodiamond samples are summarized in Table 1, including the abbreviations used throughout this manuscript. All FNDs were fabricated from HPHT bulk material. Samples fabricated in the Cigler lab were sourced from one commercial supplier and irradiated with electrons^[21,30] (e^-), protons^[22] (p^+) and with $He^{2+} + Li^+$ particles generated from ^{10}B by neutron capture (He^{2+}),^[29] The last, recently reported procedure is in principle different from the traditional irradiation methods based on exposure of nanodiamond layers to beams of accelerated particles. Here, the NDs are embedded in boron(III) oxide and placed in a neutron flux. Neutron-induced nuclear reaction on ^{10}B occurs homogeneously, generating *in situ* an isotropic flux of light ions ($He^{2+} + Li^+$) that uniformly irradiate the surrounding NDs. Because of the long penetration depth of neutrons into the material, favorably high cross section of ^{10}B for neutron capture, this approach can operate with extremely rapid irradiation times of only a few minutes per one batch, irradiating tens of grams of NDs.

After irradiation, the samples were annealed in an argon atmosphere. The diamond surface was oxidized chemically (e^- , p^+) or via annealing in air. See Experimental Section and SI for more information on irradiation and particle processing. The commercial particles were irradiated with electrons (e^-_{com}) and He^+ ions (He^+_{com}) and were also oxidized.^[31,32] Comparatively large particles were chosen to minimize the influence of the surface chemistry on the NV fluorescence properties. All particles show a strongly negative zeta potential well below -45 mV. Fourier transform infrared (FTIR) spectra of all samples show strong O-H and C=O absorption features at 1630 cm^{-1} and 1780 cm^{-1} , respectively, which are typical for oxidized nanodiamond surfaces^[33] (see SI Figure S3 for FTIR spectra).

Table 1. Samples investigated and a summary of important properties. See Experimental Section for more details about origin and product details of the commercial samples and processing parameters for the samples fabricated in the Cigler Lab. The dose of sample He^{2+} is given in displacements per atom (dpa). FNDs were dispersed in deionized water (0.1 mg mL^{-1}) for DLS and zeta potential measurements.

	Sample origin	Irradiation type	Irradiation energy	Fluence	DLS size [nm]	Zeta pot. [mV]
e ⁻	Cigler lab	e ⁻	16.6 MeV	$1.25 \times 10^{19} \text{ cm}^{-2}$	171	-51
p ⁺	Cigler lab	p ⁺	15.5 MeV	$5.88 \times 10^{16} \text{ cm}^{-2}$	179	-49
He ²⁺	Cigler lab	⁴ He ²⁺ + ⁷ Li ⁺ *	1.47 MeV (⁴ He ²⁺) 0.84 MeV (⁷ Li ⁺)	$6.6 \times 10^{18} \text{ cm}^{-3}$ #	199	-49
e _{com} ⁻	commercial 1	e ⁻	not provided	not provided	144	-57
He _{com} ⁺	commercial 2	He ⁺	not provided	not provided	166	-49

* generated simultaneously from ¹⁰B in a nuclear reactor

Due to isotropic and all-directional ion generation in the material, the fluence of ⁴He²⁺ and ⁷Li⁺ was calculated for a unit volume. The value given is the total fluence, which consists of $3.3 \times 10^{18} \text{ cm}^{-3}$ (⁴He²⁺) and $3.3 \times 10^{18} \text{ cm}^{-3}$ (⁷Li⁺).

Single particle analysis

A typical dataset obtained for each FND is shown in Figure 2. The brightness of each particle was determined as the integral over the entire spectrum, i.e. the total number of photocounts detected by the CCD camera of the spectrometer during the acquisition time of 30 seconds.

For each particle type, all single-particle spectra were analyzed using non-negative matrix factorization (NNMF)^[34] to determine the spectral shape of ‘pure’ NV⁰ and NV⁻ fluorescence and their relative contributions to each particle’s spectrum. The only a priori assumption is that the overall spectrum obtained for each particle (Figure 2A, black line) consists of two separate individual spectra (Figure 2A, yellow and red line).^[35] The ‘pure’ NV⁰ and NV⁻ spectra determined using this approach agree very well with reported spectra for both charge states.^[36,37] Even spectrally narrow features such as the zero-phonon lines at 575 nm (NV⁰) and 637 nm (NV⁻) are clearly visible in the ‘pure’ spectra. We also find that the ‘pure’

spectra determined using NNMF for the different particle types differ marginally (see SI Figure S4). From the relative contribution of the two charge states to the overall fluorescence, the *NV⁻ fraction* was calculated as $NV^{-}/(NV^{-} + NV^0)$. See SI for details on the NNMF analysis.

Time-resolved fluorescence decay traces (Figure 2B) were obtained by time-correlation of the pulsed laser excitation (at $t = 0$) and detection of fluorescence photons using an avalanche photodiode. The resulting fluorescence decay traces were fitted with a double exponential to determine the fluorescence lifetimes τ_1 and τ_2 . The *average lifetime* was also determined as the amplitude weighted average of τ_1 and τ_2 (see SI for a details).

To obtain a T_1 spin relaxation time from each individual nanodiamond, the NV spins were first initialized into the $m_s = 0$ ground state via a 3 μ s optical pulse.^[10] The spins were allowed to evolve for increasing times 't' before being readout using a second optical pulse. The T_1 time was determined by fitting a stretched exponential function to each relaxation curve, see Figure 2C. A stretched exponential function was used to fit the T_1 relaxation curves from the NV ensembles given there is a distribution of T_1 times from the individual NV⁻ centres within a nanodiamond.^[13,38,39]

SEM images of each particle were analyzed using ImageJ^[40] to determine the 2D area A covered by the particle, the major and minor axes of an ellipse fitted to this 2D area and the particle circularity. The particle's *aspect ratio* was calculated as the ratio of the major and minor axis of the fitted ellipse. The particle volume was estimated using $V = A \times \sqrt{A} / 2$. Atomic force microscopy images were acquired and correlated with SEM images to support our assumption that the particle z-height can be estimated by $\sqrt{A} / 2$ (see SI Figure S12 and S13). To enable comparison with dynamic light scattering results, the *nanoparticle diameter* d was calculated as the diameter of a sphere of equivalent volume: $= 2 \sqrt[3]{\frac{3}{4\pi} V}$. It is important to note that all results presented here remain valid irrespective of whether d , A or

V are used for analysis. See SI Figure S5 and Table S2 for details on image analysis and the calculation of all parameters.

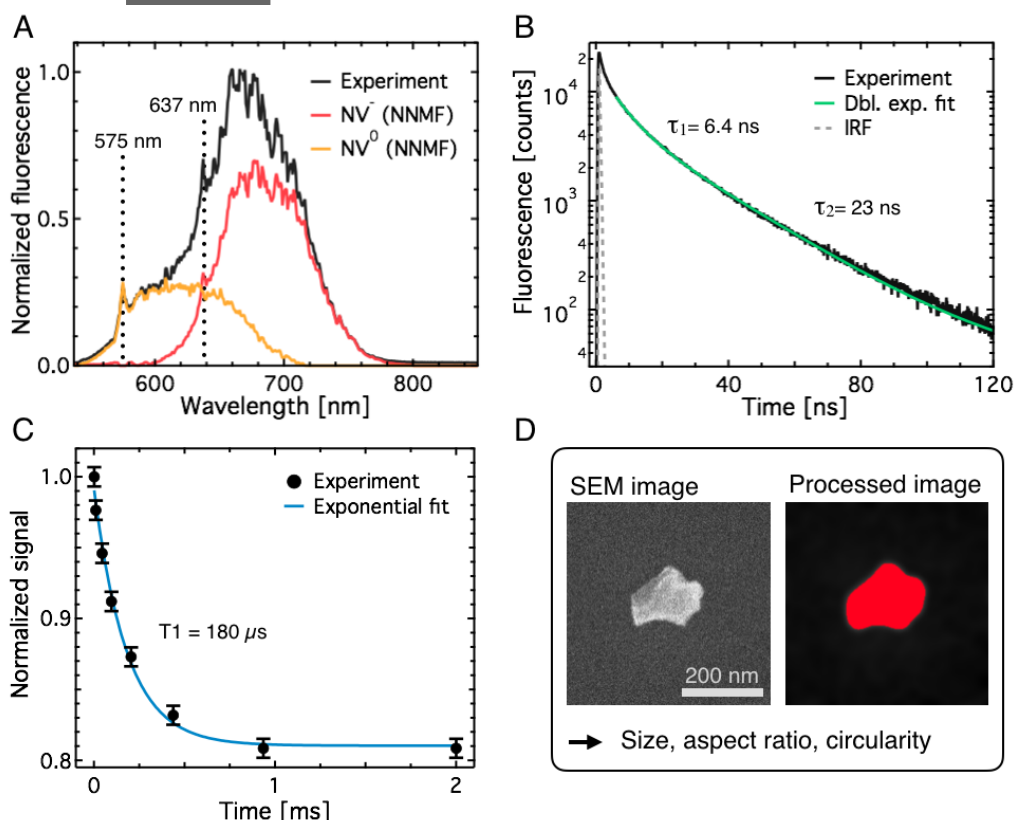


Figure 2. Exemplary dataset collected for each particle. A: PL spectrum. Spectral unmixing (non-negative matrix factorization, NNMF) is used to determine ‘pure’ NV^0 (yellow curve) and NV^+ spectra (red curve). B: Fluorescence decay. A double exponential fit (green linecurve is used to determine fluorescence lifetimes τ_1 and τ_2 . The instrument response function (IRF) is also shown (grey dashed curve). C: T_1 spin relaxation time is determined through a stretched exponential fit to the experimental data. D: raw and processed SEM image to determine NP particle size and shape.

Figure 3 shows typical SEM images of 25 particles from the p^+ sample to illustrate the significant variation in NP shapes and sizes present in all sample types. A wide range of particle shapes exist that exhibit anything from rounded features to sharp edges and strongly elongated structures. This is noteworthy as it sets HPHT nanodiamonds apart from the almost spherical 3-6 nm sized primary particles found in detonation nanodiamonds.^[1] It also

differentiates them from many other types of fluorescent nanomaterials, such as semiconductor quantum dots, polymer NPs or lanthanide-based upconversion NPs, all of which show highly regular or spherical shapes.^[41] Histograms of the particle sizes and aspect ratios of the particles investigated in the p^+ sample (50 particles in total) are plotted in Figure 3 B and C and show a wide distribution of both parameters.

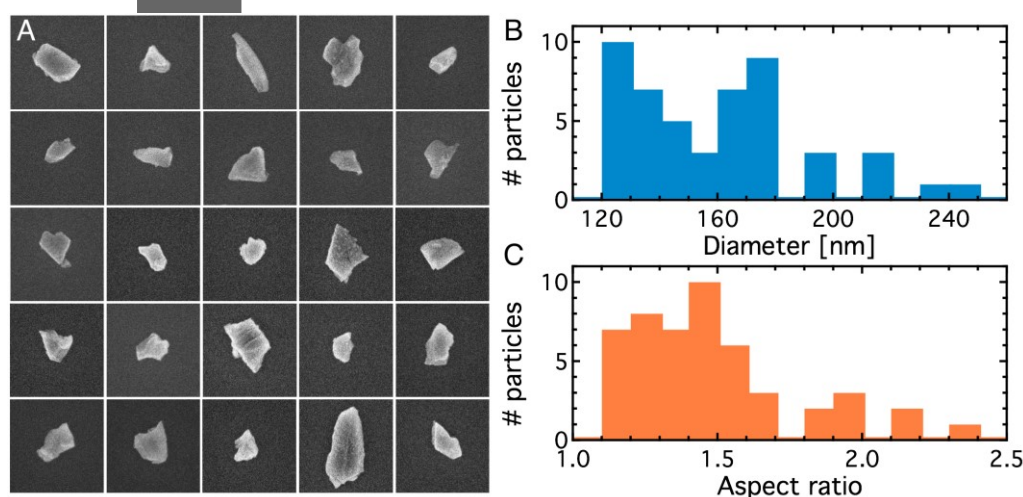


Figure 3. Illustration of the nanodiamond size and shape variability. Results for the p^+ sample (50 particles in total) are shown as an example. See SI for SEM images of all investigated individual particles of all samples. A: SEM images of 25 fluorescent nanodiamonds. The size of each square is 500 nm x 500 nm. B: Histogram of the particle size C: Histogram of the particle aspect ratios. All 50 particles investigated in the p^+ sample were used to create the histograms in B and C.

We analyze experimental results from the single particle experiments using two different approaches. The first one is based on statistical correlation to explore dependencies between different parameters (e.g. *PL brightness*, *NV fraction*, *NP diameter*...) for each sample (e.g. e^- , p^+ , ...) separately. These correlations are then also used to compare the different particle types. The second approach is to determine averages (arithmetic mean) of parameters like *PL brightness* and compare these for the different particle types.

To minimize implicit assumptions about the nature of potential correlations (e.g. linear correlation) between individual parameters, we use the Spearman rank correlation

coefficient r_s for this analysis.^[42] The absolute value of r_s is equal to one for every monotonic functional relationship between two parameters and zero if two parameters are not correlated at all. Hence, r_s will reveal any monotonic relationship between two parameters irrespective of the nature of this relationship – whether it is linear, exponential or a power law to name just a few examples. We assume our results to be statistically significant if r_s is greater than the critical value (test against null hypothesis) for a P-value of 0.05. See Experimental section and SI for details on the correlation analysis.

Figure 4 shows single-particle data for *PL brightness* as a function of *NP size* (A-E) and for *PL lifetime* as a function of *NV fraction* (F-J), where each black square is one single-particle measurement. Assuming a random distribution of NV centers in the diamond lattice it is expected and has been reported^[43] that the fluorescence brightness of a FND increases with particle size. We do find a positive correlation between these two parameters for three out of the five investigated particle types. However, this correlation is weak or moderate at best ($r_s=0.5$) and the samples p^+ and e^-_{com} show no significant correlation at all. This is despite the fact that the volume of individual FNDs varies by up to an order of magnitude for most samples. Many particles of about 100 nm in size are significantly ‘brighter’ than other particles that are more than 200 nm in size.

The trend observed for the samples e^- , p^+ and He^{2+} (where the exact processing parameters are known) may originate in the irradiation process itself: Both e^- and p^+ were irradiated in a macroscopic layer of nanoparticles on a solid target. The energy loss of protons is characterized by a Bragg curve, where significantly more vacancies are created in the region of the Bragg peak, which is positioned inside the particle layer.^[21,23,44] This effect is slightly less pronounced for electrons due to their higher penetration depth.^[21] In contrast, $He^{2+} + Li^+$ particles were generated in the whole volume of the material, irradiating the NDs isotropically.^[29] Hence, He^{2+} particles on the other hand were irradiated isotropically in solution. Hence, He^{2+} shows a much better correlation between FND size and fluorescence brightness than the other two samples. However, further investigations are required to

demonstrate which aspects of the source material and the fabrication process (see Discussion section) contribute to the observed lack of correlation.

Figure 4 F-J shows the average fluorescence lifetime of single particles as a function of their NV⁻ fraction. We find a moderate to very strong ($r_s = -0.87$) anti-correlation between these two parameters in all samples except p⁺. This means that a stronger NV⁰ contribution (relative to NV⁻) is correlated with a longer fluorescence lifetime. This is in agreement with recent studies reporting that the NV⁰ charge state exhibits a significantly longer lifetime than NV⁻.^[45]

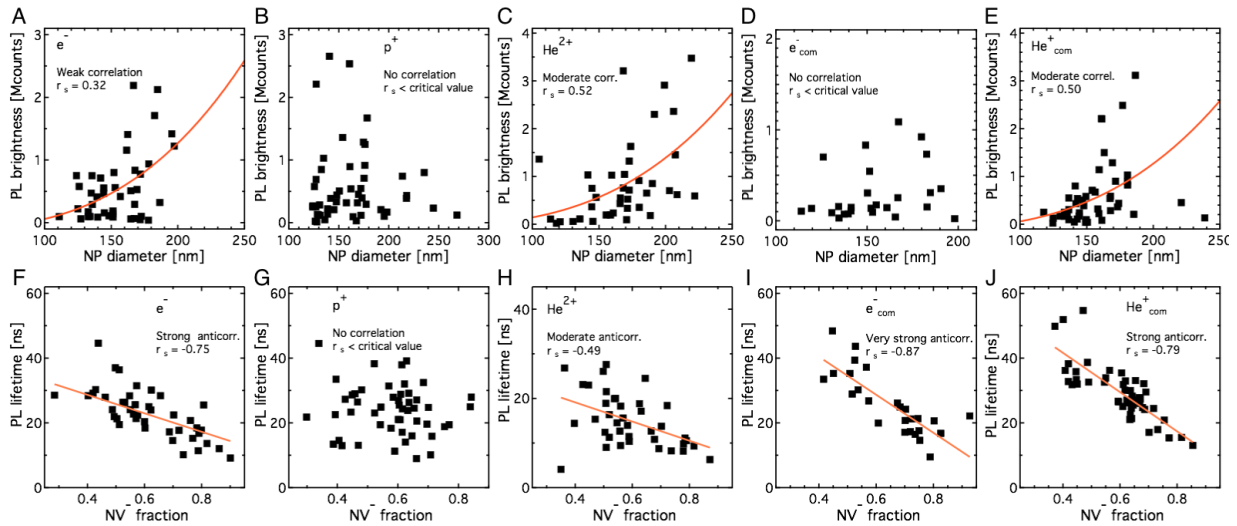


Figure 4. Single particle data for *PL brightness* as a function of *NP diameter* (A-E) and average *PL lifetime* (amplitude weighted average) as a function of *NV fraction* (F-J) for the different particle types as indicated in the graph. Each data point represents experimental results for one individual particle. Orange solid lines are fitted to the experimental data in cases where statistically relevant correlation was found. A-E: power law fit ($y = y_0 + Ax^3$, orange curve); F-J: linear fit (orange line). All fits are a guide to the eye only. The Spearman correlation coefficient r_s is also given in all cases where it is statistically significant.

We have investigated correlations between seven parameters in total. These parameters are *PL brightness*, *PL lifetime*, *NV fraction*, *NP diameter*, *NP aspect ratio (AR)*, *NP circularity* and *volume-normalized fluorescent brightness*. The result of these correlation tests is summarized in Figure 5 for the first 5 parameters. *NP circularity* and *volume normalized*

fluorescent brightness are omitted here to make results more readily accessible. Also, *NP circularity* and *volume-normalized brightness* are anti-correlated and correlated with *AR* and *NP brightness*, respectively. See SI Figure S6 for all results. Figure 5 shows all moderate ($|r_s|$ of 0.4 to 0.6) to very strong ($|r_s| > 0.8$) correlations between these parameters.

We find the correlation between the *fluorescence lifetime* and the *NV fraction* to be strongest and present in all samples except p^+ . The sample e^-_{com} exhibited the highest average *AR* of 1.68 and also the largest variation between individual particles with a standard deviation of 0.24. For this sample, we find a significant anti-correlation between the *NP aspect ratio* and a particle's brightness and fluorescence lifetime and a moderate positive correlation between *AR* and *NV fraction*. A higher *AR* results in a higher surface-to-volume ratio. Assuming that proximity of NV centers to the surface reduces or quenches their fluorescence, an anti-correlation between *PL brightness* and *AR* is reasonable. The strong negative correlation between PL lifetime and *AR* suggests that proximity to the surface also reduces the fluorescence lifetime of NV centers, possibly via the introduction of additional non-radiative decay pathways for the NV excited state.

We find no significant correlation between *volume normalized fluorescent brightness* and any other parameter. *NP circularity* shows a moderate positive correlation with the *fluorescence lifetime* for the e^-_{com} sample (see SI Figure S6). Since *NP aspect ratio* and *NP circularity* are intrinsically anti-correlated the same reasoning described above applies. We also find a moderate negative correlation between *NP brightness* and *NP circularity* for the He^{2+} sample. This is surprising as it suggests that the less circular particles tend to fluoresce brighter.

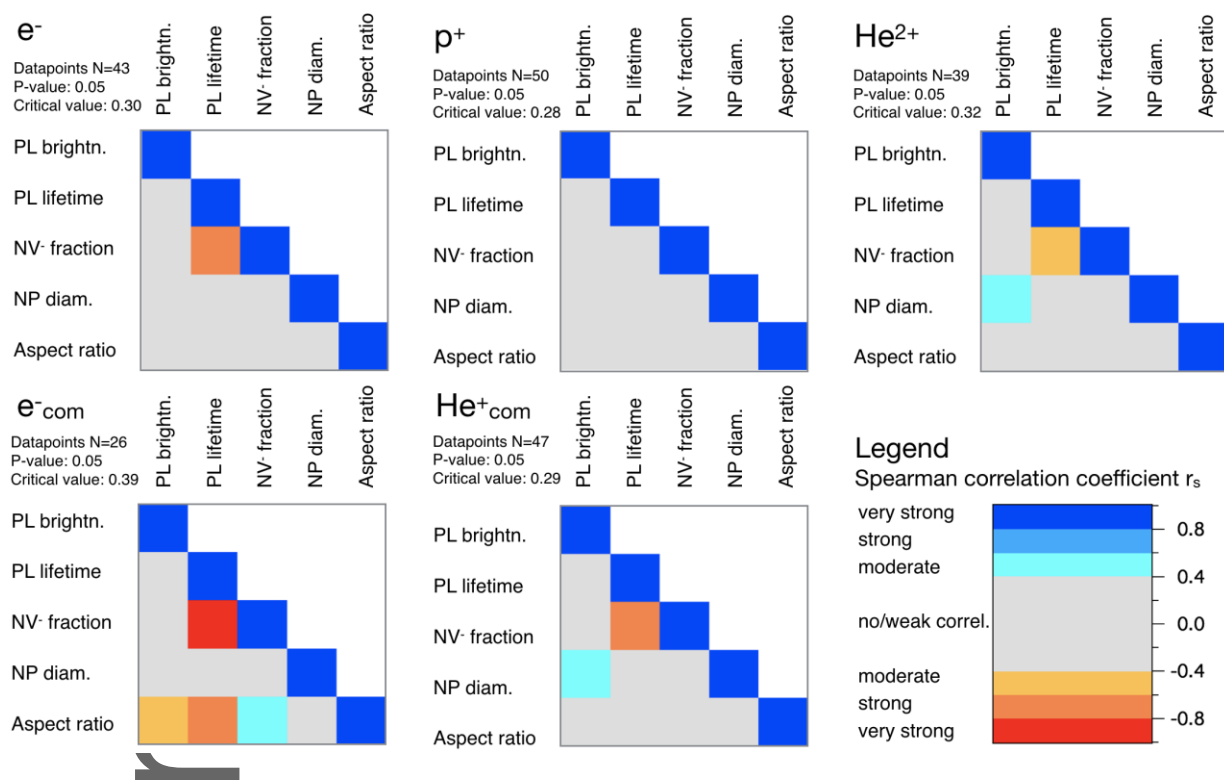


Figure 5. Spearman correlation matrices for each particle type. The Spearman correlation coefficient r_s is calculated and plotted for 5 parameters: *PL brightness*, *PL lifetime*, *NV fraction*, *NP diameter* and *NP aspect ratio*. Each parameter (trivially) shows a perfect positive correlation with itself (blue diagonal). Only the bottom left part of the matrix is calculated and the top right part omitted due to symmetry. The number of particles investigated (datapoints, N), P-value and critical value for r_s are also given for each dataset. Absolute r_s values below the critical value are not statistically significant. Therefore, absolute r_s values below 0.4 are plotted here as ‘no/weak correlation’, which only implies that our data is insufficient to demonstrate a correlation or shows a weak correlation.

We also calculated averages (arithmetic mean) of the single-particle results to be able to compare the properties of the different samples. Average values and the standard deviation for single-particle values (error bars) for *volume normalized PL brightness*, *PL lifetime*, *NV fraction*, T_1 spin relaxation time, *NP diameter* and *aspect ratio* are plotted for all 5 samples in Figure 6. The average ‘*volume normalized PL brightness*’ was calculated by normalizing each individual particle’s PL brightness by its volume and then the average value was determined. Despite this normalization, it remains clearly correlated with the average NP diameter. The brightest sample (He^{2+}) also contains the largest particles on average. This may point to a super-linear dependence of FNDs fluorescence brightness on their volume on

average. However, a determination of the exact relationship between NP size and its fluorescence brightness is beyond the scope of this study – in particular as the particle size range is limited and includes no particles less than 100 nm in size. Considering the large standard deviation, all samples show comparable fluorescence brightness. e^-_{com} shows a lower average brightness, but is also the sample with the smallest particles on average. The variation in brightness between individual particles (height of error bars in Figure 6A) is between 80% (e^-) and 130% (p^+) of the average value for all samples.

The average values for the *PL lifetime* τ_1 and τ_2 also don't significantly differ within standard deviations. The He^{2+} sample has the lowest average fluorescence lifetime. It is important to note that the fluorescence decay of all samples is well described by a double exponential decay, where the fast decay component (τ_1) is well below the value for NV centers in bulk diamond ($\sim 12 \text{ ns}^{[15]}$). The longer lifetime component (τ_2) is significantly higher than this value, with many particles exhibiting values above 40 ns.

The average *NV fraction* of samples is between 0.57 and 0.65. The difference in average values between samples is small compared to the difference between individual particles within each sample, which varies between below 0.5 to above 0.75 for some samples. This means that for some particles 3 out of 4 photons originate from the NV^- charge state (*NV fraction* = 0.75), while for other particles less than half do (4 out of 10 for *NV fraction* = 0.4). Technologically, this is highly relevant as only the emission from the NV^- charge state can be employed in most sensing applications and indeed all quantum sensing applications. More trivially, selecting the optimal combination of optical filters for any fluorescence microscope depends on the exact shape of the fluorescence to be collected. Given the spectrally distinct emission spectra of NV^0 and NV^- , knowledge of the *NV fraction* expected for a particular experiment becomes important to maximize the number of photons collected.

While the average T_1 time for most samples lies between 50 μs and 85 μs , the p^+ sample shows a roughly two-fold increase with an average T_1 of about 142 μs . This means that NV

electron spins polarized into the $m_s = 0$ ground state in the p^+ sample will retain their polarization on average about twice as long compared to most other samples. In general, the T_1 values reported here are in good qualitative agreement with literature values.^[13,46,47]

The p^+ sample was irradiated to a fluence on the order of 10^{16} cm^{-2} , which has been shown to be create the maximum amount of NV centers in a type 1b single crystal diamond, while higher fluences were found to create lattice damage that reduced NV PL.^[48] This is three orders of magnitude lower than the fluence used for the e^- sample. However, a direct comparison of fluences alone is insufficient to estimate the total lattice damage as protons for example create vacancies much more efficiently than electrons and far less efficiently than helium ions.^[27,49] Further studies are required to precisely quantify differences in lattice damage and compare them with simulations.

The He^{2+} sample contains the particles with the largest average *NP diameter* of 168 nm. e^-_{com} has the smallest particles with an average size of 154 nm, while the size of all other particles lies in-between these values. This is in qualitative agreement with DLS particle sizes (see Table 1), which show the same trend even though the largest particles show an even larger diameter (199 nm for He^{2+}) and the smallest particles a slightly smaller diameter (144 nm for e^-_{com}).

The average aspect ratio of all particles is between 1.4 and 1.5, with the exception of e^-_{com} , which shows an average value above 1.6. This shows that the vast majority of FNDs in all samples are significantly elongated.

We find that all particles – irrespective of their origin and the type of irradiation used – are photostable with the photostability within shot-noise of the photodetector. See SI Figure S7 for exemplary fluorescence photostability traces. Photoblinking was also not observed in any of the single-particle measurements.

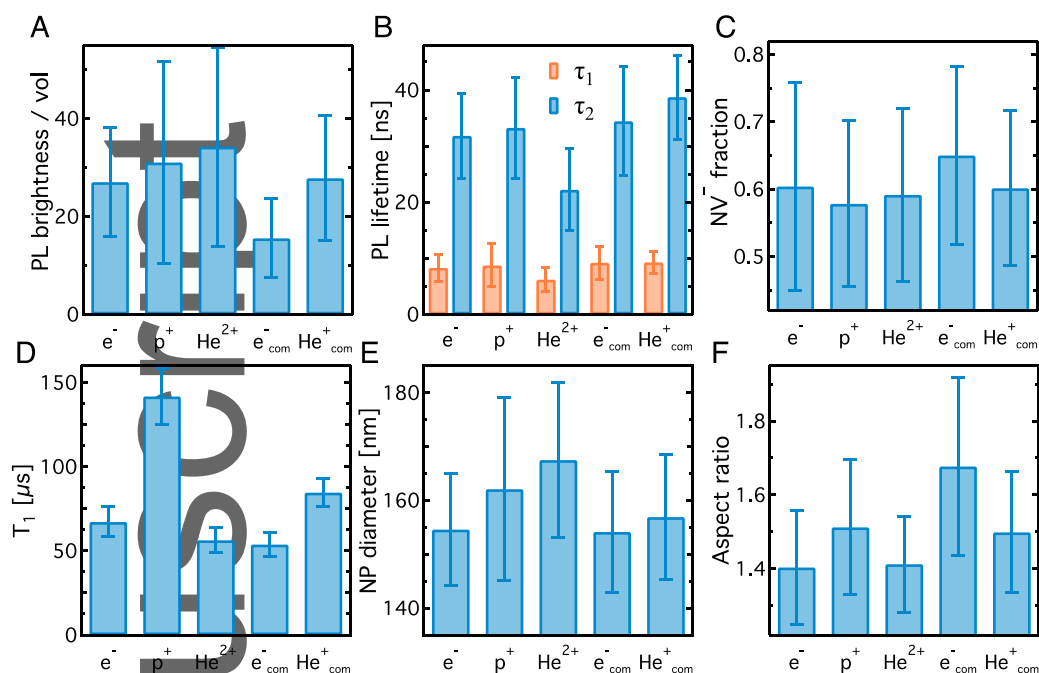


Figure 6. Average values (arithmetic mean) and their standard deviations (error bars) calculated from the single particle data obtained for each sample. A: *PL brightness* normalized by the particle volume (in arbitrary units), B: *PL lifetime*, C: *NV fraction*, D: T_1 spin relaxation time, E: *NP diameter*, F: *NP aspect ratio*.

In-solution analysis

To complement our single-particle-based analysis, we also investigated the fluorescence of FNDs suspended in water. Figure 7 shows fluorescence spectra, time-resolved fluorescence decay traces and extinction spectra for all samples in deionized water at a concentration of 0.1 mg mL^{-1} . For fluorescence experiments, the FND suspensions were excited at 520 nm (1 mW) with a pulsed (5 MHz repetition rate) laser beam weakly focused into the solution. The fluorescence was coupled into a fiber and analyzed with a spectrometer (Figure 7A) or avalanche photodiodes and a correlator card (Figure 7B). Extinction spectra were recorded using a commercial absorption spectrometer. See Experimental Section for more details on sample preparation and in-solution spectroscopy.

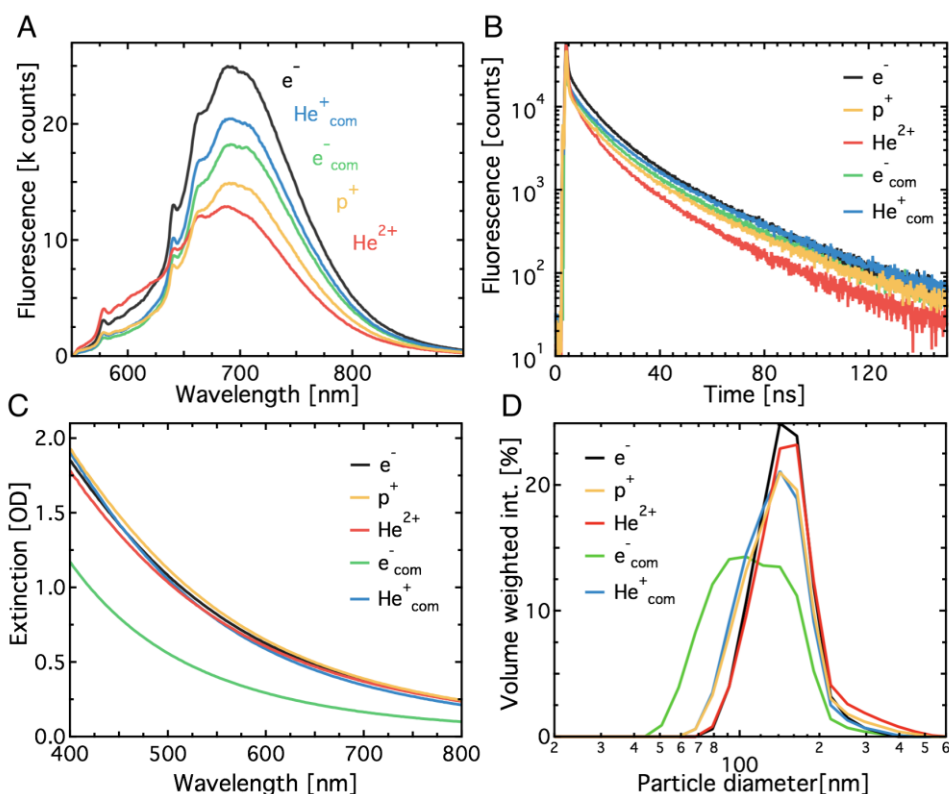


Figure 7. Optical properties of all FND samples dispersed in water (0.1 mg mL⁻¹). A: Fluorescence spectra, B: fluorescence decay traces, C: extinction spectra of the NP solutions and D: DLS size distributions.

A brightness comparison of FNDs of this size in solution is challenging since the collected fluorescence intensity is strongly affected by scattering of the excitation beam and the emitted photons. The extinction spectra (Figure 7C) are dominated by scattering as the absorption by e.g. NV centers is negligible in comparison. The spectra of all samples except e⁻_{com} are very similar and within a margin of 0.05 OD above 510 nm. e⁻_{com} scatters much less light. This can be explained by the fact that the particles' DLS size distribution (Figure 7D) is significantly shifted toward smaller particle sizes relative to all other particles. This makes a direct comparison of the fluorescence brightness between e⁻_{com} and other samples challenging.

The e⁻ sample shows the highest overall fluorescence, followed by He⁺_{com} and e⁻_{com} (also see Figure 8A). He²⁺ shows the lowest overall fluorescence. The He²⁺ sample also shows a

stronger NV^0 emission than all other samples, which is also evident in a significantly lower *NV fraction* of ~ 0.7 (Figure 8C) compared to values between 0.85 and 0.9 for all other samples. The zero-phonon lines of NV^0 (575 nm) and NV^- (637 nm) are clearly visible in all samples.

All samples show similar τ_2 fluorescence lifetimes of 21– 24 ns (Figures 7B and 8B) with only He^{2+} showing a value well below 20 ns. Similarly, all samples show a τ_1 above 6 ns, while He^{2+} 's τ_1 is 4.8 ns. Interestingly, the He^{2+} sample has a shorter lifetime and a lower *NV fraction* than the others samples. Assuming that NV^0 has a longer excited state lifetime than NV^- , the shorter lifetime can't be easily explained by the NV charge-state.

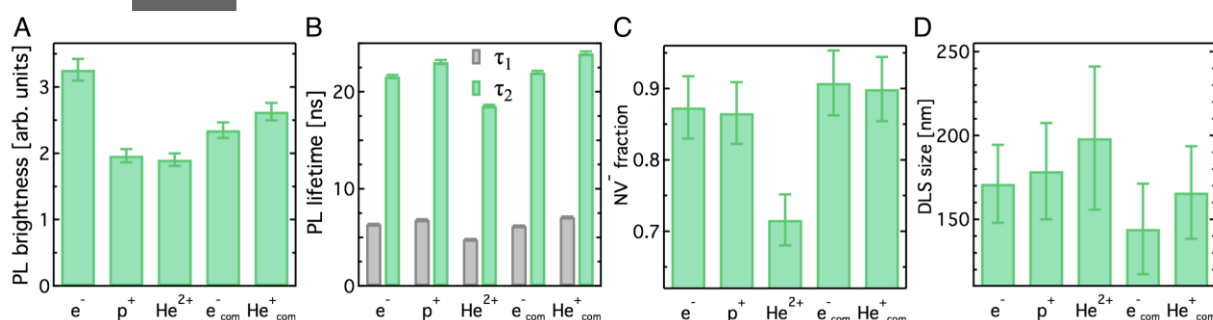


Figure 8. Comparison of the optical properties of HPHT NDs dispersed in water.

Care must be taken when comparing results obtained via confocal fluorescence microscopy of particles dispersed on a silicon substrate and those obtained from particles suspended in water. There are two major differences between these measurement approaches (besides the fact that one is based on single particle measurements while the other is an ensemble experiment). Firstly, the local dielectric environment of the particles is different. In the first case the particles are located at the interface between a low (air, $n = 1$) and high refractive index material (silicon, $n = 4.2$ @ 520 nm). In the second case, they are located in a

homogenous and constant refractive index medium (water, $n = 1.3$ @ 520 nm). The local refractive index is known to affect the fluorescence of NV centers in FNDs.^[50]

Secondly, the excitation intensities vary by at least three orders of magnitude. In our case, confocal excitation in a diffraction limited spot leads to local power densities on the order of 1 kW cm⁻², while power densities in solution are on the order of 1 W cm⁻² in a weakly focused beam. The excitation intensity is known to be an important parameter that impacts for example the NV⁻/NV⁰ charge-state dynamics^[36] and the coupling of NV centers to external magnetic fields.^[51] Nonetheless, a comparison is instructive. It highlights that the excitation intensity for example can strongly affect the fluorescence properties of the investigated nanodiamonds. While most commercial confocal fluorescence microscopes use excitation intensities similar to or above the ones used in our confocal experiments, commercial wide-field microscopes use significantly lower light intensities.^[16] This means that the fluorescence spectra of imaged nanodiamonds may significantly differ depending on the type of microscope employed.

Amongst the samples produced in the Cigler lab, He²⁺ shows the brightest fluorescence on average (both absolute and normalized for NP volume) in the single-particle experiments followed by p⁺ and e⁻. This order is reversed for the in-solution spectroscopy experiments. The fluorescence lifetime τ_2 of all samples in the single-particle experiments are in the range between 32 ns and 38 ns, while in-solution this range changes to 19 - 23 ns (see Table 2). Similarly, the average NV⁻ fraction of FNDs on a substrate is between 0.59 and 0.65 and between 0.85 and 0.90 for most samples in solution. Both the differences in τ_2 and in NV⁻ fraction are likely a result of charge-state conversion (also often referred to as 'ionization') from NV⁻ to NV⁰ under higher intensity illumination on the silicon substrate compared to in solution – even though weak confocal illumination was used here with a total beam power of 5 μ W (≈ 7 kW cm⁻¹).

Other observations made in single-particle measurements and in-solution are in good qualitative agreement. For example, the He^{2+} sample shows the shortest average fluorescence lifetime in both cases. Also, the ‘pure’ NV^0 and NV^- spectra obtained in both cases are very similar despite the fact that very different optical systems are used (see SI Figure S8).

Table 2. Comparison of the fluorescence lifetime τ_2 and NV fraction obtained on a silicon wafer substrate (average) and in solution. The errors given here for the ‘On substrate’ values are the standard deviations between single particle measurements. The errors associated with the lifetime fitting and NMF determination of NV fractions are negligible in comparison and omitted for clarity.

Sample	PL lifetime τ_2 [ns]		NV fraction	
	On substrate	In solution	On substrate	In solution
e^-	32 ± 7.6	22	0.60 ± 0.15	0.87
p^+	33 ± 9.0	23	0.58 ± 0.12	0.87
He^{2+}	22 ± 7.3	19	0.59 ± 0.13	0.72
e^-_{com}	34 ± 10	22	0.65 ± 0.13	0.90
He^+_{com}	39 ± 7.5	24	0.60 ± 0.11	0.90

Discussion

We find great variability in the optical and physical properties of single FNDs – irrespective of their origin and how they have been processed. The fluorescence brightness of individual FNDs is a good example. Assuming a random and homogenous distribution of NV centers on the ppm concentration scale across all particles from a given sample, one would expect

the fluorescence brightness of individual particles to be closely correlated with the particles' size: if the particle volume doubles, the particle hosts approximately twice the number of NV centers. Even though we find a correlation between fluorescence brightness and FND size, it is weak to moderate in some and not present at all in other samples (Figure 4A-E). There are several possible explanations for this observation:

1. Variations in the concentration of substitutional nitrogen. The presence of substitutional nitrogen is a prerequisite for the formation of NV centers and its distribution in the diamond lattice. It is well-known that the concentration of substitutional nitrogen in HPHT diamonds varies strongly between different growth sectors.^[52–54] These differences in the macroscopic starting material should translate to nanoparticles with nitrogen concentrations that vary accordingly. Furthermore, it has been shown that higher concentrations of substitutional nitrogen do not lead to higher NV densities.^[55,56] Other techniques such as chemical vapor deposition (CVD)-based growth are able to incorporate nitrogen more homogeneously into the diamond lattice and at relevant concentrations >10 ppm. This technique is not easily scalable, but may be an interesting alternative for several applications and to better understand the effect of nitrogen inhomogeneities.

2. Inhomogeneous irradiation. Depending on the exact process and sample geometry used to create vacancies via irradiation, the number of vacancies created in individual particles may vary widely.^[23] Hence, even in particles with identical nitrogen concentrations, the absence of vacancies can be the limiting factor for the formation of NV centers. Similarly, the creation of excessive numbers of vacancies locally can also prevent the creation of optically active NV centers.^[48]

3. Damage to the diamond lattice during milling. Milling process can take place at different stages of the FND fabrication process. Regardless of when it takes place, the milling process may damage the diamond lattice structure, resulting in a decrease in NV fluorescence from these particles.^[2]

4. Surface interactions. It is known that near-surface NV centers strongly interact with the diamond surface and the functional groups present there.^[46,57] Fluorescence from these centers can be quenched,^[58,59] or their fluorescence properties at least significantly altered due to this interaction.^[60] NV centers separated less than about 10 nm are generally assumed to interact with the surface.^[10] In our case about 35% of the volume of a particle would be within this range assuming a spherical particle with a diameter of 150 nm. For all other particle shapes this effect would be exacerbated by an increased surface-to-volume ratio, which is minimized for a sphere. If the surface chemistry or impurities (e.g. from milling) on individual particles varies significantly, this may explain the observed large variation in single particle properties. A strong NP surface-NV coupling in our samples would also suggest a strong correlation between NP aspect ratio and other parameters, which we only observe in one of the studied samples (e^-_{com}).

These effects are also possible explanations for the large variation in *PL lifetime* and *NV fraction* observed for individual particles (see error bars in Figure 6B and C). Surface proximity effects for example may also affect the rates of radiative and non-radiative decay of the NV center's excited state or the availability or mobility of electrons, impacting the charge-state dynamics.

Comparing the properties of the different samples with one another overall, several results stand out. The most pronounced difference is the long T_1 spin relaxation time of the p^+ sample. One major difference between the electron irradiated samples (e^- , e^-_{com}) and the p^+ sample is the irradiation dose. While the electron irradiated samples were exposed to a fluence on the order of 10^{19} electrons cm^{-2} , the p^+ sample is only irradiated with 10^{16} protons cm^{-2} . It is known that the number of vacancies created per proton is significantly higher than those created per electron under otherwise similar irradiation conditions.^[21] Nonetheless, the almost three orders of magnitude higher dose of the electron irradiation is likely to lead to the creation of significantly more vacancies and potentially other lattice damage compared to the proton irradiation. Di-vacancies and vacancy chains are known to be a source of magnetic

noise in diamond.^[61] Hence, a lower concentration of these defects may explain the considerably longer T_1 times observed for the p^+ sample.

Another difference between the samples is the relatively short fluorescence lifetime of He^{2+} , which we observe in single-particle measurements (Figure 6B) and in-solution (Figure 8B). In single particle experiments this short fluorescence lifetime does not coincide with a stronger NV^- contribution to the overall emission (i.e. high *NV⁻ fraction*) and to the contrary does coincide with a markedly stronger NV^0 contribution relative to all other samples in the solution-based experiments (i.e. lower *NV⁻ fraction*, Figure 8 B and C). Therefore, the difference in fluorescence lifetime between NV^0 and NV^- can't explain this result. Helium and lithium ions have been shown to very efficiently create vacancies in diamond. Hence, a possible explanation for the observed shortening of the PL lifetime is increased lattice damage in the He^{2+} sample compared to all other samples. However, low-temperature fluorescence spectroscopy and optically detected magnetic resonance experiments suggest that the local strain doesn't significantly differ in electron and $\text{He}^{2+} + \text{Li}^+$ irradiated samples.^[29]

Plakhotnik et. al. have recently reported a constant concentration of NV centers in FNDs of sizes between 40 nm and 150 nm and suggested a linear relationship between FND size and fluorescence brightness.^[43] This result is obtained by comparing the fluorescence brightness distribution of individual particles with a DLS size distribution and not – as reported here – by correlating an individual particle's brightness with its size. Hence, our results are not contradicting Plakhotnik et. al. as we also observe that on average, the larger particles are also the brighter ones (see Figure 6 A and E).

Our results are also in agreement with a report by Koenderik et. al.,^[62] which demonstrates that the PL quantum yield of individual NV centers in ~100 nm sized nanodiamonds varies greatly, resulting in a large variation of observed fluorescence brightnesses between particles of the same size.

Conclusion

We have analyzed 205 individual nanoparticles across five different samples using confocal fluorescence microscopy and SEM imaging. Our study is the most comprehensive analysis of FNDs produced using state of the art fabrication processes to date. Irrespective of the production method, we find that the fluorescence properties of individual particles, such as particle *PL brightness* and *NV fraction*, vary greatly within a given sample. Correlation of single particle results reveals that only few parameters show a significant correlation. We find the strongest correlation between *NV fraction* and *PL lifetime* across all samples except one (p^+). A particle's fluorescence brightness is at best moderately (He^{2+}) and often weakly or not at all correlated with its size. One sample (p^+) has a significantly longer T_1 spin relaxation time.

In-solution spectroscopy shows a much higher *NV fraction* and shorter PL lifetimes for all samples relative to results obtained on Si wafer substrates. This is mainly due to the lower excitation intensities in in-solution experiments and highlights the importance of the imaging conditions in general. In contrast to the single particle analysis, we find the He^{2+} sample to have a significantly lower *NV fraction* compared to all other samples.

Our study reveals some of the challenges associated with the properties of fluorescent nanodiamonds produced from HPHT diamond and also identifies gaps in our understanding of the NV center in nanodiamonds. It identifies opportunities to improve the properties of single fluorescent nanodiamonds, to develop a better understanding of the underlying physical mechanisms and to advance current nanofabrication technologies.

Experimental Section

Nanodiamond pretreatment

Nanodiamonds (NDs) were supplied by Microdiamant, Switzerland, (MSY 0-0.25, containing approximately 100–200 ppm of natural nitrogen impurities). The NDs were oxidized by air in a furnace (Thermolyne 21100 Tube Furnace, Barnstead Thermolyne Corporation, USA) calibrated with an external thermocouple (1009, Testo AG, UK) at 510 °C for 5 h and subsequently treated with a mixture of H₂SO₄ and HNO₃ (9:1) at 90 °C for 3 days. NDs were washed with water; 1 M NaOH; 1 M HCl; 5 times with water; lyophilized, and used for irradiations. The commercial FND samples e⁻_{com} (NDNV100nmHi, Adamas Nanotechnologies, USA) and He⁺_{com} (brFND-100, FND Biotech, Taiwan) were provided in deionized water (1 mg mL⁻¹) and used as received.

Electron irradiation^[21,30]

NDs (207 mg) were pressed in an aluminum target holder and irradiated with a 16.6 MeV electron beam extracted from the microtron MT25 for 21 hours (fluence 1.25×10¹⁹ cm⁻²).

Proton irradiation^[22]

NDs (355 mg) were pressed in an aluminum target holder and irradiated with a 15.5 MeV proton beam extracted from the isochronous cyclotron U-120M for 70 min (fluence 5.88×10¹⁶ cm⁻²).

He²⁺ + Li⁺ irradiation^[29]

The mixture of NDs (500 mg) and H₃¹⁰BO₃ (2.0 g; Katchem, Czech Republic) was transferred to a synthetic corundum crucible and heated to 600 °C for 5 min in a vertical furnace (Thermolyne 21100 tube) to dehydrate the boric acid. The temperature was then increased to 700 °C, the melt was homogenized by stirring, left to cool to room temperature and pulverized in a ball mill. The final melt consisting of boron(III) oxide and NDs contained 33 wt.% NDs and 22% ¹⁰B was sealed in a quartz glass, sodium-free capillaries (inner diameter, 1.5 mm; height of the melt, 11 mm), inserted in an aluminum container, and

irradiated for 20 min in a vertical water-cooled ($\sim 45\text{ }^{\circ}\text{C}$) channel H8 positioned in the Be reflector of the LVR-15 nuclear reactor. The neutron fluence rates were $2\times 10^{13}\text{ cm}^{-2}\text{ s}^{-1}$, $1\times 10^{13}\text{ cm}^{-2}\text{ s}^{-1}$, and $7\times 10^{12}\text{ cm}^{-2}\text{ s}^{-1}$ for thermal, epithermal, and fast neutrons, respectively.

After irradiation, the capillaries were opened and left overnight in a vial with 6 M NaOH at $60\text{ }^{\circ}\text{C}$ to dissolve boron(III) oxide. The residue adhering to quartz glass was released in an ultrasonic bath. Supernatant was washed gradually with 6 M NaOH; water; 1 M HCl; and 5 times with H_2O . Possible quartz glass splinters were separated by sedimentation. The supernatant was treated with concentrated HF for 12 h; washed with H_2O ; 1 M HCl; 5 times with H_2O ; and lyophilized.

Annealing and oxidation of NDs

The proton-irradiated sample was annealed at $700\text{ }^{\circ}\text{C}$ for 2 h. The electron and $[\text{He}^{2+} + \text{Li}^+]$ -irradiated samples were annealed at $900\text{ }^{\circ}\text{C}$ for 1 h in an argon atmosphere followed by air oxidation at $510\text{ }^{\circ}\text{C}$ for 4 h at normal pressure. All the samples were further oxidized in a mixture of conc. H_2SO_4 and HNO_3 (9:1) at $80\text{ }^{\circ}\text{C}$ for 3 days and washed with twice H_2O ; 1M NaOH; 1M HCl; five times with H_2O ; and lyophilized.

Sample preparation

Marked silicon wafer substrates were prepared using a focused ion beam (Scios Dualbeam FIBSEM, FEI, USA). The substrates were cleaned with ethanol, followed by plasma cleaning (Ar/ozone, 50 W, 2 min.). All nanoparticle samples suspended in deionized water ($20\text{ }\mu\text{L}$, 0.05 mg mL^{-1}) were then spin coated onto the silicon wafer substrate at 2500 rpm for 1 minute, followed by 4000 rpm for 30 s to remove remaining solution at the sample edge. For in-solution spectroscopy, all nanoparticles were used suspended in deionized water (0.1 mg mL^{-1}) in an optical glass cuvette (18FL, FireflySci, Inc., USA).

Fluorescence imaging and spectroscopy

A custom-built scanning confocal fluorescence microscope with a high numerical aperture objective lens (100x magnification, N.A.= 0.9) operating at room temperature was used. A tuneable and pulsed (20 ps pulse-width) white light laser (SuperK Extreme, NKT Photonics, Denmark) was used as a light source. Avalanche photo diodes (SPCM-AQRH-14, Excelitas Technologies, USA) were employed for imaging and a spectrometer (Princeton Instruments, SpectraPro with a PIXIS CCD camera) to obtain fluorescence spectra. A correlator card (TimeHarp 260, Picoquant, Germany) was used to obtain time-resolved direct fluorescence decay traces. The fluorescence signal was separated from the excitation beam ($\lambda_{\text{ex}} = 520$ nm) using a 532 nm dichroic and long-pass optical filter (both Semrock, USA). Images were acquired using a piezo scanning stage (PI nanoXYZ, Physik Instrumente, Germany). In-solution spectroscopy was performed using the equipment described above in the configuration described in SI Figure S2.

T_1 spin relaxation measurements

T_1 measurements were performed on a custom-built inverted confocal fluorescence microscope (Nikon Ti-U, Nikon, Japan), with a 100× 1.3 NA oil-immersion objective (Nikon, Japan). Optical excitation was provided by a laser at 532 nm (Verdi, Coherent Scientific, Australia). The power density of the optical excitation was $\sim 10 \mu\text{W} \mu\text{m}^{-2}$. The laser was modulated via an acousto-optic modulator (Crystal Technology, USA) and controlled by a PulseBlaster card (Spincore Technologies, USA) to implement the T_1 measurement sequence. The NV fluorescence was filtered using a long pass filter at 560 nm in combination with a bandpass filter from 650 to 750 nm (Semrock, USA). The filtered

fluorescence was detected using a single photon counting avalanche photodiode and time tagged using multiple-event time digitizer (MCS6A, FAST ComTec GmbH, Germany).

SEM imaging and data analysis

SEM images were acquired with a FEI Verios XHR (FEI, USA) at 3 kV using a beam current of 25 pA and analyzed using ImageJ as described in detail in the SI. All data analysis was carried out using IgorPro (Wavemetrics, USA). Details on the correlation analysis and the statistical tests can be found in the SI.

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References

- [1] V. N. Mochalin, O. Shenderova, D. Ho, Y. Gogotsi, *Nat. Nanotechnol.* **2012**, 7, 11.
- [2] J. Arnault, *Nanodiamonds: Advanced Material Analysis, Properties and Applications*; Elsevier Inc., 2017.
- [3] V. N. Mochalin, K. Turcheniuk, *Nanotechnology* **2017**, 28, 252001.
- [4] Y. Ruan, D. A. Simpson, J. Jeske, H. Ebendorff-Heidepriem, D. W. M. Lau, H. Ji, B. C. Johnson, T. Ohshima, S. V. Afshar, L. Hollenberg, A. D. Greentree, T. M. Monro, B. C. Gibson, *Sci. Rep.* **2018**, 8, 1268.
- [5] S. Claveau, J. R. Bertrand, F. Treussart, *Micromachines* **2018**, 9, 247.
- [6] W. W.-W. Hsiao, Y. Y. Hui, P.-C. Tsai, H.-C. Chang, *Acc. Chem. Res.* **2016**, 49, 400.
- [7] Y. Y. Hui, W. W. W. Hsiao, S. Haziza, M. Simonneau, F. Treussart, H. C. Chang, *Curr. Opin. Solid State Mater. Sci.* **2016**, 21, 35.
- [8] S. Haziza, N. Mohan, Y. Loe-Mie, A.-M. Lepagnol-Bestel, S. Massou, M. Adam, X. L. Le, J. Viard, C. Plancon, R. Daudin, P. Koebel, E. Dorard, C. Rose, F. Hsieh, C. Wu,

- B. Potier, Y. Herault, C. Sala, A. Corvin, B. Allinquant, H. Chang, F. Treussart, M. Simonneau, *Nat. Nanotechnol.* **2017**, *12*, 322.
- [9] L. P. McGuinness, Y. Yan, A. Stacey, D. A. Simpson, L. T. Hall, D. Maclaurin, S. Praver, P. Mulvaney, J. Wrachtrup, F. Caruso, R. E. Scholten, L. C. L. Hollenberg, *Nat. Nanotechnol.* **2011**, *6*, 358.
- [10] R. Schirhagl, K. Chang, M. Loretz, C. L. Degen, *Annu. Rev. Phys. Chem.* **2014**, *65*, 83.
- [11] V. Petrakova, V. Benson, M. Buncek, A. Fiserova, M. Ledvina, J. Stursa, P. Cigler, M. Nesladek, *Nanoscale* **2016**, *8*, 12002.
- [12] D. A. Simpson, E. Morrisroe, J. M. McCoey, A. H. Lombard, D. C. Mendis, F. Treussart, L. T. Hall, S. Petrou, L. C. L. Hollenberg, *ACS Nano* **2017**, *11*, 12077.
- [13] S. Kaufmann, D. A. Simpson, L. T. Hall, V. Perunicic, P. Senn, S. Steinert, L. P. McGuinness, B. C. Johnson, T. Ohshima, F. Caruso, J. Wrachtrup, R. E. Scholten, P. Mulvaney, L. Hollenberg, *Proc. Natl. Acad. Sci.* **2013**, *110*, 10894.
- [14] A. M. Zaitsev, *Phys. Rev. B* **2000**, *61*, 12909.
- [15] M. W. Doherty, N. B. Manson, P. Delaney, F. Jelezko, J. Wrachtrup, L. C. L. Hollenberg, *Phys. Rep.* **2013**, *528*, 1.
- [16] P. Reineck, A. Francis, A. Orth, D. W. M. Lau, R. D. V. Nixon-Luke, I. Das Rastogi, W. A. W. Razali, L. M. Parker, V. K. A. Sreenivasan, L. J. Brown, B. C. Gibson, *Adv. Opt. Mater.* **2016**, *4*, 1549.
- [17] I. Aharonovich, A. D. Greentree, S. Praver, *Nat. Photonics* **2011**, *5*, 397.
- [18] V. M. Acosta, E. Bauch, M. P. Ledbetter, C. Santori, K. M. C. Fu, P. E. Barclay, R. G. Beausoleil, H. Linget, J. F. Roch, F. Treussart, S. Chemerisov, W. Gawlik, D. Budker, *Phys. Rev. B* **2009**, *80*, 115202.

- [19] E. Neu, D. Steinmetz, J. Riedrich-Möller, S. Gsell, M. Fischer, M. Schreck, C. Becher, *New J. Phys.* **2011**, *13*, 025012.
- [20] J.-P. Boudou, P. A. Curmi, F. Jelezko, J. Wrachtrup, P. Aubert, M. Sennour, G. Balasubramanian, R. Reuter, A. Thorel, E. Gaffet, *Nanotechnology* **2009**, *20*, 235602.
- [21] Z. Remes, J. Micova, P. Krist, D. Chvátal, R. Effenberg, M. Nesladek, *Phys. Status Solidi Appl. Mater. Sci.* **2015**, *212*, 2519.
- [22] J. Havlik, V. Petrakova, I. Rehor, V. Petrak, M. Gulka, J. Stursa, J. Kucka, J. Ralis, T. Rendler, S.-Y. Lee, R. Reuter, J. Wrachtrup, M. Ledvina, M. Nesladek, P. Cigler, *Nanoscale* **2013**, *5*, 3208.
- [23] J. Stursa, J. Havlik, V. Petrakova, M. Gulka, J. Ralis, V. Zach, Z. Pulec, V. Stepan, S. Abbasi, M. Ledvina, M. Nesladek, F. Treussart, P. Cigler, *Carbon*. **2016**, *96*, 812.
- [24] Y.-R. Chang, H.-Y. Lee, K. Chen, C.-C. Chang, D.-S. Tsai, C.-C. Fu, T.-S. Lim, Y.-K. Tzeng, C.-Y. Fang, C.-C. Han, H.-C. Chang, W. Fann, *Nat. Nanotechnol.* **2008**, *3*, 284.
- [25] R. Mahfouz, D. L. Floyd, W. Peng, J. T. Choy, M. Loncar, O. M. Bakr, *Nanoscale* **2013**, *5*, 11776.
- [26] O. A. Shenderova, G. E. McGuire, *Biointerphases* **2015**, *10*, 030802.
- [27] S. Sotoma, Y. Yoshinari, R. Igarashi, A. Yamazaki, S. H. Yoshimura, H. Tochio, M. Shirakawa, Y. Harada, *Diam. Relat. Mater.* **2014**, *49*, 33.
- [28] A. Nagl, S. R. Hemelaar, R. Schirhagl, *Anal. Bioanal. Chem.* **2015**, *407*, 7521.
- [29] J. Havlik, V. Petrakova, J. Kucka, H. Rabova, D. Panek, V. Stepan, Z. Zlamalova Cilova, P. Reineck, J. Stursa, J. Kucera, M. Hubry, P. Cigler, *Nat. Commun.* **2018**, DOI: 10.1038/s41467.

- [30] T. Rendler, J. Neburkova, O. Zemek, J. Kotek, A. Zappe, Z. Chu, P. Cigler, J. Wrachtrup, *Nat. Commun.* **2017**, *8*, 14701.
- [31] Adamas Nanotechnologies. <http://adamasnano.com>; Accessed Sept. 2018.
- [32] FND Biotech. <http://www.fndbiotech.com>; Accessed Sept. 2018.
- [33] P. Reineck, D. W. M. Lau, E. R. Wilson, K. Fox, M. R. Field, C. Deeleepojananan, V. N. Mochalin, B. C. Gibson, *ACS Nano* **2017**, *11*, 10924.
- [34] A. Orth, R. N. Ghosh, E. R. Wilson, T. Doughney, H. Brown, P. Reineck, J. G. Thompson, B. C. Gibson, *Biomed. Opt. Express* **2018**, *9*, 2943.
- [35] M. W. Berry, M. Browne, A. N. Langville, V. P. Pauca, R. J. Plemmons, *Comput. Stat. Data Anal.* **2007**, *52*, 155.
- [36] N. Aslam, G. Waldherr, P. Neumann, F. Jelezko, J. Wrachtrup, *New J. Phys.* **2013**, *15*, 013064.
- [37] J. Jeske, D. W. M. Lau, X. Vidal, L. P. McGuinness, P. Reineck, B. C. Johnson, M. W. Doherty, J. C. McCallum, S. Onoda, F. Jelezko, T. Ohshima, T. Volz, J. H. Cole, B. C. Gibson, A. D. Greentree, *Nat. Commun.* **2017**, *8*, 1.
- [38] S. Steinert, F. Ziem, L. T. Hall, A. Zappe, M. Schweikert, N. Götz, A. Aird, G. Balasubramanian, L. Hollenberg, J. Wrachtrup, *Nat. Commun.* **2013**, *4*, 1607.
- [39] J. P. Tetienne, A. Lombard, D. A. Simpson, C. Ritchie, J. Lu, P. Mulvaney, L. C. Hollenberg, *Nano Lett.* **2016**, *16*, 326.
- [40] C. A. Schneider, W. S. Rasband, K. W. Eliceiri, *Nat. Methods* **2012**, *9*, 671.
- [41] P. Reineck, B. C. Gibson, *Adv. Opt. Mater.* **2017**, *5*, 1600446.
- [42] M. M. Mukaka, *Malawi Med. J.* **2012**, *24*, 69.

- [43] T. Plakhotnik, H. Aman, *Diam. Relat. Mater.* **2018**, *82*, 87.
- [44] O. Faklaris, J. Botsoa, T. Sauvage, J. F. Roch, F. Treussart, *Diam. Relat. Mater.* **2010**, *19*, 988.
- [45] J. Storteboom, P. Dolan, S. Castelletto, X. Li, M. Gu, *Opt. Express* **2015**, *23*, 11327.
- [46] R. G. Ryan, A. Stacey, K. M. O. Donnell, T. Ohshima, B. C. Johnson, L. C. L. Hollenberg, P. Mulvaney, D. A. Simpson, *ACS Appl. Mater. Interfaces* **2018**, *10*, 13143.
- [47] J. Tetienne, T. Hingant, L. Rondin, A. Cavaill, L. Mayer, G. Dantelle, T. Gacoin, J. Wrachtrup, J. Roch, V. Jacques, *Phys. Rev. B* **2013**, *87*, 235436.
- [48] J. Botsoa, T. Sauvage, M.-P. Adam, P. Desgardin, E. Leoni, B. Courtois, F. Treussart, M.-F. Barthe, *Phys. Rev. B* **2011**, *84*, 125209.
- [49] B. Campbell, A. Mainwood, *Phys. Status Solidi* **2000**, *181*, 99.
- [50] A. Khalid, K. Chung, R. Rajasekharan, D. W. M. Lau, T. J. Karle, B. C. Gibson, S. Tomljenovic-Hanic, *Sci. Rep.* **2015**, *5*, 11179.
- [51] M. Capelli, P. Reineck, D. W. M. Lau, A. Orth, J. Jeske, M. W. Doherty, T. Ohshima, A. D. Greentree, B. C. Gibson, *Nanoscale* **2017**, *9*, 9299.
- [52] Y. V Babich, B. N. Feigelson, *Inorg. Mater.* **2006**, *42*, 971.
- [53] I. Kiflawi, H. Kanda, S. C. Lawson, *Diam. Relat. Mater.* **2002**, *11*, 204.
- [54] M. Capelli, A. H. Heffernan, T. Ohshima, H. Abe, J. Jeske, A. Hope, A. D. Greentree, P. Reineck, B. C. Gibson, *Carbon*, **2019**, *143*, 714.
- [55] L.-J. Su, C.-Y. Fang, Y.-T. Chang, K.-M. Chen, Y.-C. Yu, J.-H. Hsu, H.-C. Chang, *Nanotechnology* **2013**, *24*, 315702.

- [56] K. V. Bogdanov, M. V. Zhukovskaya, V. Y. Osipov, E. V. Ushakova, M. A. Baranov, K. Takai, A. Rampersaud, A. V. Baranov, *APL Mater.* **2018**, 6, 086104.
- [57] M. V. Hauf, B. Grotz, B. Naydenov, M. Dankerl, S. Pezzagna, J. Meijer, F. Jelezko, J. Wrachtrup, M. Stutzmann, F. Reinhard, J. A. Garrido, *Phys. Rev. B* **2011**, 83, 081304.
- [58] V. Petráková, A. Taylor, I. Kratochvílová, F. Fendrych, J. Vacík, J. Kučka, J. Štursa, P. Cígler, M. Ledvina, A. Fišerová, P. Kneppo, M. Nesládek, *Adv. Funct. Mater.* **2012**, 22, 812.
- [59] V. Petráková, M. Nesládek, A. Taylor, F. Fendrych, P. Cígler, M. Ledvina, J. Vacík, J. Štursa, J. Kučka, *Phys. Status Solidi Appl. Mater. Sci.* **2011**, 208, 2051.
- [60] J. Havlik, H. Raabova, M. Gulka, V. Petrakova, M. Krecmarova, V. Masek, P. Lousa, J. Stursa, H. G. Boyen, M. Nesladek, P. Cigler, *Adv. Funct. Mater.* **2016**, 26, 4134.
- [61] F. F. De Oliveira, D. Antonov, Y. Wang, P. Neumann, S. A. Momenzadeh, T. Häußermann, A. Pasquarelli, A. Denisenko, J. Wrachtrup, *Nat. Commun.* **2017**, 8, 15409.
- [62] A. Mohtashami, A. F. Koenderink, *New J. Phys.* **2013**, 15, 043017.

TOC

We analyze and correlate the fluorescence properties and size and shape of 205 individual nanodiamonds from five different samples. The samples include commercially available particles and materials made in-house using different irradiation techniques. We find a strong variation in the properties of individual particles within all samples and also significant differences between different particle types.

