Multiphoton-Excited Fluorescence of Silicon-Vacancy Color Centers in Diamond

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Silicon-vacancy color centers in nanodiamonds are promising as fluorescent labels for biological applications, with a narrow, non-bleaching emission line at 738 nm. Two-photon excitation of this fluorescence offers the possibility of low-background detection at significant tissue depth with high three-dimensional spatial resolution. We have measured the two-photon fluorescence cross section of a negatively-charged silicon vacancy (SiV−) in ion-implanted bulk diamond to be 0.74(19) × 10−50 cm4 s/photon at an excitation wavelength of 1040 nm. In comparison to the diamond nitrogen vacancy (NV) center, the expected detection threshold of a two-photon excited SiV center is more than an order of magnitude lower, largely due to its much narrower linewidth. We also present measurements of two- and three-photon excitation spectra, finding an increase in the two-photon cross section with decreasing wavelength, and discuss the physical interpretation of the spectra in the context of existing models of the SiV energy-level structure.

I. INTRODUCTION

Color centers in diamond have been the focus of intense interest in recent years. The nitrogen-vacancy (NV) color center in diamond has driven much of this interest, thanks to numerous promising applications including nano-scale magnetometry [1 2 3 4 5], NMR spectroscopy [6 7], quantum information [8 9 10 11 12 13], and use as fluorescent bio-labels [14 15]. More recently, the silicon-vacancy (SiV) color center in diamond, which has been known for over two decades [16 17 18], has generated increasing excitement [19 20 21 22] because of properties that are in some respects even more favorable than those of the nitrogen-vacancy defect, such as a narrow zero-phonon line (ZPL) [16] and weak phonon sidebands at room temperature [23]. This concentrated emission in a narrow ZPL allows detection of silicon-vacancy-containing diamonds at higher signal-to-noise ratios, and raises the possibility that silicon-vacancy-doped nanodiamonds bound to specific biomolecular targets could be detectable in the presence of high autofluorescence background, e.g. in deep and/or highly scattering biological tissue.

Specifically targeted nanoparticle probes that can be detected through millimeters of intervening tissue promise to be an enabling technology for minimally invasive in-vivo molecular imaging, with potential applications to biomarker discovery, studies of immune cell trafficking and circulating tumor cells, elucidation of molecular pathways in pre-clinical models, drug development, and possibly ultimately in clinical diagnosis. Towards these ends, various combinations of nanoparticle type and detection modality have been investigated, including superparamagnetic nanoparticles detected via MRI [24], fluorescence imaging of dyes [25], quantum dots [26 27], and nanodiamonds [28 29], and surface-enhanced Raman (SERS) particles [30 31].

Two-photon excited fluorescence imaging is particularly appealing for deep-tissue imaging because of its high spatial resolution, natural longitudinal sectioning, low background, and because the longer excitation wavelengths typically used allow enhanced penetration and lower phototoxicity in tissue [32]. These advantages have led to significant application of two-photon imaging in neuroscience [33], and will likely be important in other areas involving high spatial-resolution imaging through scattering tissue. While two-photon labels such as organic dyes and fluorescent proteins achieve very high brightness [34 35], SiVs are likely to offer distinct ad-
vantages thanks to their narrow emission bandwidth and lack of bleaching. A further unique feature of the silicon-vacancy color center is the circumstance that the excitation can be within the second near-IR transmission window of tissue (around 1040 nm) and the emission (around 738 nm) within the first transmission window (see, e.g., Ref. [30]). Song et al. [37] have previously observed a quadratic dependence of 830 nm-excited silicon vacancies coupled to the plasmon resonances of gold nano-ellipsoids, attributable to two-photon excitation. A quantitative measure of the strength of two-photon-excited fluorescence, however, has not previously been obtained for the SiV defect. Here we present the first report of the two-photon fluorescence cross section of the negatively-charged silicon vacancy (SiV−) color center in diamond. Based on the results, we evaluate the prospects of SiV nanodiamond as a contrast agent for in-vivo biological labeling applications, in particular for in-vivo imaging and deep-tissue imaging, where strong background fluorescence and low photon collection efficiency must be overcome.

II. BACKGROUND

The structure of the SiV− color center has been elucidated in recent years through a number of contributions [18, 19, 20, 22, 38]. The color center consists of a single silicon atom located midway between two adjacent vacant carbon lattice sites (vacancies), as shown in Fig. 1; this configuration possesses trigonal D3d symmetry. A level diagram tentatively suggested by Rogers et al. [19] is given in Fig. 1 showing ground and excited states relevant to the present work. Excitation of the defect from the 2Eg ground state to the 2En excited state can be achieved either directly or else indirectly via higher-lying levels such as the 2A1g state, combined with subsequent (presumed non-radiative) relaxation. The defect then returns to the ground state by emission of a 1.68 eV (738 nm) photon. An unusually large portion of the emitted radiation is in the narrow zero-phonon line, improving the signal-to-noise ratio with which the color center can be detected.

The two-photon fluorescence cross section σ2p for a single point fluorophore is defined by the relation

\[ \langle \Gamma \rangle = \sigma_{2p}(I^2), \]  

where \( \Gamma \) is the fluorescence photon emission rate, I is the excitation intensity at the fluorophore, conventionally measured in units of photon number per area per time, and angle brackets indicate time averaging over an interval significantly greater than the excited-state decay time. In practice, one measures not the total emission rate, but the quantity \( \Gamma_{\text{det}} \equiv \eta_{\text{det}}\langle \Gamma \rangle \), where the detection efficiency \( \eta_{\text{det}} \) includes the fraction of light collected by the microscope objective and the transmission of all optical components in the detection path.

Because a pulsed laser must typically be used to obtain appreciable two-photon-excited fluorescence, we must further relate the mean square intensity appearing in Eq. 1 to the more readily measurable mean intensity. Specifically, we consider excitation via a periodic pulse train with a temporal profile that is well approximated by a sum of gaussian-envelope pulses,

\[ I_0(t) = I_{\text{peak}} \sum_{n=-\infty}^{\infty} \exp(- (t - nT_{\text{rep}})^2 / \tau^2), \]  

where \( I_0 \) is the intensity at the laser focus, \( I_{\text{peak}} \) is the temporal peak intensity, \( T_{\text{rep}} \) is the repetition period, and \( \tau \) defines the pulse width. The time average of the intensity and the square intensity over a time long compared to the repetition period, neglecting overlap of distinct pulses, are readily calculated by integration, and obey the relation

\[ \langle I_0^2 \rangle = \langle I_0 \rangle^2 \frac{T_{\text{rep}}}{\tau \sqrt{2\pi}}. \]  

Moreover, the intensity \( I_0 \) at the laser focus is related to the total laser power by

\[ P = \int dx \, dy \, I(x, y) \equiv I_0 A_{\text{ex}}. \]  

Here \( A_{\text{ex}} \) is the area defined by the excitation point-spread function \( F_{\text{ex}}(x, y) \), i.e.,

\[ A_{\text{ex}} \equiv \int dx \, dy \, F_{\text{ex}}(x, y), \]  

with the unit-maximum point-spread function \( F_{\text{ex}}(x, y) \) in turn defined by the relation, \( I(x, y) = I_0 F_{\text{ex}}(x, y) \).

Thus for such a pulse train, the average detection rate of photons emitted by a single color center at the focus of the laser beam is given by

\[ \Gamma_{\text{det}, 2p} = \eta_{\text{det}} \sigma_{2p} \langle I_0^2 \rangle \]  

\[ = \eta_{\text{det}} \sigma_{2p} \langle I_0 \rangle^2 \frac{T_{\text{rep}}}{\tau \sqrt{2\pi}}, \]  

where the time-average intensity at the focus is given in terms of the time-average excitation power \( \langle P \rangle \) by \( \langle I_0 \rangle = \langle P \rangle / A_{\text{ex}} \). For an isolated color center, then, the two-photon cross section is related to measurable quantities by the equation

\[ \Gamma_{\text{det}, 2p} = \eta_{\text{det}} \sigma_{2p} \left( \frac{\langle P \rangle}{A_{\text{ex}}} \right) \frac{T_{\text{rep}}}{\tau \sqrt{2\pi}} S_P, \]  

where \( S_P \) is an empirically determined saturation factor (described more fully below) accounting for any deviation of the intensity dependence from the strictly quadratic dependence of Eq. 1.
III. EXPERIMENTAL APPARATUS

The experimental apparatus (illustrated in Fig. 2) consisted of a two-photon epifluorescence microscope to excite and detect silicon-vacancy color centers in a diamond sample. Excitation light was derived from a mode-locked 1040 nm ytterbium fiber laser (Menlo Orange) producing 144 fs (full-width at 1/e of maximum) pulses at a repetition rate of 100 MHz with an average power of around 300 mW. The excitation light was focused via a 40x, NA=0.75 air-spaced objective into a diamond sample mounted on a three-axis closed-loop translation stage (Newport VP-25XL and XPS controller). Two-photon-excited fluorescence from the sample was collected and collimated by the objective, reflected from a dichroic beam splitter, filtered by long-pass and band-pass filters, and refocused onto an sCMOS camera (Hamamatsu Orca Flash4.0) or alternatively free-space coupled into a spectrograph (Princeton Instruments Acton SP2500) for spectral analysis of the emitted light.

We employed samples consisting of bulk diamond implanted with silicon ions and annealed to produce silicon vacancies and minimize undesired defects. High-purity CVD-grown single-crystal diamond chips (< 5 ppb nitrogen, Element Six), were Si-ion implanted (Materials Diagnostics; Albany, NY), with the implantation energies chosen to achieve the desired depth profile, as calculated by the stopping range of ions in matter (SRIM) software [39]. The implantation surface was a (100) plane of the diamond crystal. Low-density samples for observation of single color centers were fabricated at an implantation density of $5 \times 10^9$ ions/cm$^2$ and ion energy of 3 MeV, while high-density samples for improved signal-to-noise measurement ratio were fabricated at implantation densities from $10^{12}$ ions/cm$^2$ to $10^{15}$ ions/cm$^2$ with a selection of energies between 500 keV and 3 MeV designed to produce approximately uniform SiV density over the first micrometer of subsurface crystal depth. After implantation, the samples were annealed in vacuum by ramping over 2 hrs to 400°C and holding for 4 hrs, then ramping over 2 hrs to 800°C and holding for 4 hrs, before ramping...
back to room temperature.

Isolated single-SiV color centers were located in the low-density samples by raster scanning the sample in the plane transverse to the optical axis. Comparison between one-photon fluorescence microscopy of the SiVs with a large digital confocal “pinhole” and surface-reflection depth scans using the same 705 nm laser beam indicated that the color centers were within $\sim 1 \mu m$ of the diamond surface, as expected from simulations. A polarizer was placed in the imaging path following the dichroic mirror to allow analysis of the polarization direction of the emitted light, and a half-wave plate was mounted in a computer-controlled rotation stage immediately above the objective. As we observed no dependence of the emitted light intensity on the excitation polarization with the polarizer omitted, rotating the wave plate was considered equivalent to rotating the transmission axis of the polarizer, but with the advantage of holding the detected polarization axis constant throughout the downstream imaging system and spectrometer.

The power of the two-photon excitation beam was measured using a slide-format thermal power meter (Thorlabs S175C) placed in the position normally occupied by the sample. The excitation point spread functions for the one- and two-photon beams were measured by scanning a single color center through the laser focus and recording a photoluminescence (PL) map, as shown in Fig. 3. Detection point-spread functions were determined by centering a color center on the laser focus and recording a long exposure ($\sim 10$ s) on the camera using a long-pass filter and a narrow band-pass filter to reject any residual excitation light reflected by the dichroic beam splitter.

The pulse width of the two-photon excitation laser was measured by means of a home-built autocorrelator consisting of a scanning Michelson interferometer with a GaAsP photodiode at its output port; the GaAsP bandgap of 1.98 eV being larger than the single-photon energy at 1040 nm, the photodiode output was proportional to the square of the incident power. The quantity of glass traversed by the laser beam before impinging on the sample was sufficiently small to lead us to expect negligible dispersion and pulse broadening at the sample; this expectation was confirmed by the experimental introduction of a glass component of comparable thickness prior to the autocorrelator, which was not found to produce detectable broadening of the pulse. The repetition rate was separately measured on an oscilloscope using the laser’s synchronous radiofrequency output.

We further investigated the spectral dependence of the two-photon cross section using the signal output of a tunable optical parametric oscillator (Coherent Chameleon Compact OPO). The OPO signal wavelength was tuned under computer control over the range 1010 nm to 1550 nm, and fluorescence was detected at 738 nm. Making separate use of the remotely tunable titanium-sapphire pump laser allowed us to extend the excitation range down to 920 nm, limited by our dichroic beam-splitter. For these measurements, we employed an NA=0.95, 100 x objective corrected for use in the IR. The pulse width of the excitation laser was determined as a function of wavelength using the autocorrelator, substituting a silicon photodiode for the GaAsP detector for wavelengths beyond 1300 nm. The excitation power was determined by measuring it in transmission using a large-area thermal power meter placed directly beneath the diamond sample and underlying microscope slide. The analog output of the power meter was recorded via a data-acquisition system for each measurement, and a dark power-meter reading was also recorded at regular intervals during the measurement sequence by shuttering the excitation beam and waiting for the power meter reading to stabilize. A half-wave-plate in a computer-controlled rotator followed by a polarizing beam splitter allowed us to vary the excitation power for each wavelength. To focus the imaging system, we introduced a weak laser beam at 736 nm into the excitation path via a pellicle beam splitter; the imaging system was periodically focused on the diamond surface by maximizing the peak intensity of the reflected 736 nm beam on the camera with the pulsed excitation light shuttered. At all other times, the 736 nm laser beam was itself shuttered. For our excitation-spectroscopy measurements, a 50:50 beam-splitter was employed in place of the flipper mirror, allowing simultaneous measurement of the emission spectrum and the two-dimensional excitation beam profile.
TABLE I. Principal contributors to the error budget for the absolute measurement of SiV $\sigma_{2p}$ with excitation at 1040 nm and detection on the ZPL at 738 nm. The reported error in each quantity is an estimated single standard deviation. The total uncertainty is the sum in quadrature of the partial uncertainties. Symbols are as defined in the text.

| Measured Quantity | Value               | Partial Uncertainty (10$^{-2}$GM) |
|-------------------|---------------------|-----------------------------------|
| $\eta_{\text{det.}}$ | 4.04(35) $\times$ 10$^{-3}$ | 6.4                              |
| $\Gamma_{\text{det.,2p}}$ | 2.15(26) $\times$ 10$^{3}$ s$^{-1}$ | 8.9                              |
| $\tau$            | 72.0(3.5) fs         | 3.6                              |
| $A_{\text{ex.}}$  | 8.79(77) $\times$ 10$^{-13}$ m$^2$ | 12.9                             |
| $\langle P \rangle$ | 101.5(3.8) mW        | 5.5                              |
| $S_P$             | 0.355(20)            | 4.2                              |
| Total:            |                     | 10.0                             |

FIG. 6. Brightness distribution of individual SiV color centers obtained from sinusoidally fitting polarization-dependent spectrometer ZPL count rates. We have rejected from this distribution defects with nearly twice the median brightness or with much lower polarization contrast, presumed to correspond to double SiVs with (respectively) parallel or orthogonal projected orientations.

FIG. 7. Power dependence of the observed two-photon-excited ZPL fluorescence signal from a single isolated silicon vacancy. Solid circles are measured data. The dashed line is a fit to pure quadratic dependence, while the solid line is the result of a curve fit including saturation.

IV. RESULTS

We determined the silicon-vacancy two-photon cross section $\sigma_{2p}$ via Eq. 8, with all terms apart from $\sigma_{2p}$ determined from experimentally measured quantities. In order to obtain reliable statistics on single-color-center emitters, we performed automated coarse, large-area scans of the transverse ($x$-$y$) position. Candidate SiVs were identified by means of the narrow ZPL at $\sim$ 738 nm (with a median width of 5.6 nm FWHM for our samples), as shown in Fig. 4. When the spectral power in a window surrounding the ZPL exceeded the background in this window by an empirically-determined threshold of 10%, the position of the candidate color center in the focus of the excitation beam was then optimized by sequential linear scans in the $x$, $y$, and $z$ directions. When this optimization routine terminated successfully (i.e., with an above-threshold brightness and at a position not on the boundary of the linear scans), the polarization contrast of the emitted light was then measured by recording spectra as a function of the wave-plate angle. Polarization curves for 162 distinct color centers were extracted from the raw spectrometer data by fitting each SiV ZPL spectrum to a lorentzian with a first-order polynomial background to obtain the area under the ZPL spectrum. Representative polarization curves are shown in Fig. 5. Individual SiVs may be aligned along any of four allowed axes (the unoriented body diagonals of the cubic diamond unit cell); the projection of the SiV axis on the plane transverse to the imaging axis can therefore be aligned along two orthogonal directions, which for our sample are parallel to the edges of the rectangular diamond chip and to the principal axes of the detection polarizer. Consequently we expect individual SiVs to fall into two classes, with orthogonal emitted polarizations. Indeed, we observe that
approximately half of the SiVs identified in a large scan have a maximum detection rate when the slow axis of the half wave plate is set to an angle close to 0°, and approximately half have a maximum at an angle around 45°, corresponding to emission polarizations at angles of 0° and 90° respectively. The polarization dependence and spectral width are consistent with those reported for the SiV defect in other studies [19, 40, 41, 42].

The total ZPL photon count rate for each SiV was then taken to be the peak-to-peak amplitude of a cosine-squared fit to the polarization curve. This count rate displayed a somewhat wide distribution, as shown in Fig. 6. We have confirmed via Monte-Carlo simulations that a distribution of approximately this width is expected due to fluctuations in the number of color centers contributing to the signal. Indeed, while the number of color centers contributing to a single measurement is close to one, the polarization curves in some cases receive non-negligible contributions from nearby color centers. Color centers with parallel dipole emission axes tend to enhance the amplitude of the polarization curve, while those with orthogonal dipole emission axes tend to reduce the contrast. The extreme cases of two color centers very close together with parallel or perpendicular emission axes can be excluded by rejecting candidate color centers whose polarization curves have exceptionally low contrast or exceptionally large amplitudes (specifically, amplitudes close to twice the typical single-SiV amplitude). After excluding color centers with low contrast (40 of the 162 candidate centers) or anomalously high amplitudes (20 of the candidate centers), the median peak-to-peak amplitude was taken as the value of the ZPL detection count rate. The uncertainty in this value was dominated by the uncertainty of the spectrometer intensity calibration, so that in practice excluding these centers had a negligible effect on our result.

The saturation factor $S_P$ was determined by varying the excitation power incident on a single SiV color center centered on the laser focus in three dimensions and recording the detected ZPL fluorescence. Results of one such measurement are shown in Fig. 7. These data were empirically well fit by the functional form

$$\Gamma(P) \propto S_P P^2 \frac{P^2}{1 + (P/P_{sat})^2}, \quad (9)$$

where $\Gamma$ is the detection rate at power $P$ and $P_{sat}$ is a time-averaged saturation power. For the measured beam shape, the fitted saturation power corresponds to a saturation intensity of $8.3 \times 10^9$ mW/cm², with a saturation count rate of $3.8 \times 10^5$ photons/s incident on the spectrometer camera. We point out that this count rate is limited in our system by the fact that a silicon vacancy can only emit photons every laser repetition period of ~10 ns, rather than every excited-state lifetime of ~1 ns. Taking this difference into account, our saturation count rate is comparable to the one-photon SiV saturation rate of $56 \times 10^5$ counts/s measured by Rogers et al. [43] for an air-spaced objective. From analysis of our saturation measurements, we determined the saturation factor at the power used for the cross section measurements to be $S_P = 0.36$. For a diamond sample containing many SiVs per excitation volume, the saturation behavior given by Eq. (9) is modified because color centers in the center of the beam experience a higher laser intensity and therefore saturate before those on the periphery. Assuming a gaussian laser intensity profile, the expected saturation behavior for a dense sample can be calculated analytically by averaging $\Gamma$ over spatial locations, the result being

$$\Gamma(P) \propto P_{sat}^2 \log \left\{ 1 + P^2 / P_{sat}^2 \right\}. \quad (10)$$

The saturation behavior for a dense sample was also measured and fit using Eq. (10) and a consistent saturation intensity was obtained. Non-negligible saturation of the two-photon-excited fluorescence also changes the spatial profile of the PL map obtained by scanning the sample in the $x$-$y$ plane. At low intensity, the photoluminescence (PL) map is expected to have a width approximately $\sqrt{2}$ smaller than the actual excitation intensity distribution, assuming a near-gaussian beam profile, as a result of the quadratic dependence of scattering on intensity. For finite intensity, however, this factor is reduced; at the saturation factor $S_P = 0.36$ noted above, the width of the PL map is found numerically to be a factor of approximately 1.29 smaller than the actual beam width, but still reasonably well approximated by a gaussian profile. Analysis of the transverse PSF including this saturation behavior indicated a transverse excitation beam width approximately 20% larger than the diffraction-limited value.

Calibration of the detection efficiency of our imaging system was performed using light from a diode laser at 736 nm, with which we measured the reflection or transmission coefficients of each element in the imaging beam path. Intensity calibration of the spectrometer camera at the operating temperature of ~75°C was performed using the light from the same laser beam with separately calibrated neutral-density filters, and agreed with the manufacturer’s value to within approximately 12%. The collection efficiency of the objective (including Fresnel reflection from the diamond-air interface) was calculated using its numerical aperture and the known refractive index of diamond (see Appendix A).

Analysis of our measured data yielded a value of $\sigma_{2\mu,1040nm} = 0.74(19)$ GM for the two-photon fluorescence cross section of a single SiV at 1040 nm, where the Goeppert-Mayer (GM) is the unit of two-photon cross section, equal to $10^{-24}$ cm²/s/photon. The contributions of the various sources of uncertainty to our cross-section measurement are tabulated in Table 1. The uncertainty of each quantity has been estimated as the standard deviation of repeated measurements, where possible. The uncertainty in the intensity calibration of the spectrometer was limited by the uncertainty in the power of the calibrating laser beam; this in turn was estimated by comparing the measurements of two power meters after correcting for their wavelength dependence. The largest
V. DISCUSSION

A. Physical Interpretation of the Multiphoton Spectrum

The excitation spectrum of Fig. 8 possesses a number of interesting features that hint at the underlying physics of the SiV defect. First, the spectrum displays no visible peak at twice the one-photon ZPL wavelength (1476 nm). This is consistent with $D_{3d}$ symmetry of the SiV center, which does not permit a pure two-photon transition between the $^2E_u$ excited and $^2E_g$ ground states. Interaction with parity-odd phonons could still allow enhanced two-photon absorption near twice the ZPL wavelength, but our spectrum does not display any noticeable phonon sideband in the spectral region below 1476 nm. Our excitation spectrum also shows a considerable increase in two-photon-excited fluorescence at the short-wavelength end of the spectrum. A possible explanation is excitation to the continuum, but ab initio calculations[18] indicate that the depth of the SiV levels below the conduction band is too great to explain the observed increase. Alternative explanations of the increased two-photon-excited emission at shorter wavelengths are excitation via another, previously unknown bound level, most likely of even parity, or resonant enhancement of the two-photon absorption by the intermediate $^2E_u$ excited state. We also note a small bump in the two-photon excitation spectrum around 1200 nm, which may reflect direct two-photon excitation on the $^2E_g$ to the $^2A_{1g}$ transition; a transition between these levels would be two-photon allowed even in the absence of phonons. A thorough elucidation of the origins of the observed spectral dependence and that of the observed three-photon absorption, including a more detailed treatment of the role of symmetry, will require further investigation.

B. Prospects for Deep-Tissue Imaging

When detecting a fluorophore in the presence of a large background signal, such as autofluorescence from biological tissue, the practical detection limit for a given integration time is defined by the condition that the detected signal from the label must exceed the noise from the background. Since background light outside the spectral bandwidth of the signal of interest can in principle be rejected via spectral filtering, the relevant shot-noise-limited background noise is proportional to the square root of the emission line width. Thus the shot-noise-limited figure of merit $M$ for an optical label in deep-tissue molecular imaging is approximately given by the fluorescence cross section divided by the square root of the line width,

$$M = \frac{\sigma_{\text{fluc}}}{\sqrt{\gamma}}$$

(11)
Using this figure of merit, we can predict the relative performance of similar types of labels for deep-tissue imaging. For instance, two-photon excitation of NV centers in diamond were measured by Wee et al. [44] to have a two-photon absorption cross section of 0.45 GM, (corresponding to a two-photon fluorescence cross section of around 0.32 GM), and the NV emission linewidth is approximately 100 nm. Our measured SiV ZPL line width is 5.6 nm; Neu et al. [45] have observed room-temperature line widths as small as 0.7 nm in SiV-containing nanodiamonds grown on iridium, though such narrow linewidths have not to our knowledge been observed in bulk diamond or in other nanodiamond samples. Consequently, our measurements suggest that the figure of merit of a SiV defect label, with a two-photon cross section of 0.74 GM at 1040 nm and line widths of 0.7 nm to 5 nm, is between 10 and 30 times larger than that of an NV defect, pointing to a bright future for silicon-vacancy-doped diamond two-photon labels in applications where their photo- and chemical stability are of key importance. Our spectral measurements indicate that the figure of merit can be enhanced further by tuning the excitation laser toward 900 nm. Furthermore, the narrowness of the SiV ZPL offers greater potential for spectral multiplexing in combination with labels such as the recently-discovered narrow-line germanium vacancy defects [46] or the large number of other known defects in diamond [17 48].

Also important in an evaluation of the prospects of SiV defect-containing diamond labels is an assessment of the ease of producing nanoparticle labels containing a high density of color centers. We have studied the achievable density of SiV color centers by systematically varying the implantation dose and measuring the SiV one-photon fluorescence, as shown in Fig. 6. Our measurements show a maximum of around $n_{\text{SiV}} = 3 \times 10^{11} \text{cm}^{-3}$ in the density of silicon vacancies produced as a function of the implantation ion dose, as shown in Fig. 9. Assuming based on our SRIM simulation results that these vacancies are quasi-uniformly distributed over a depth of 1 $\mu$m, the three-dimensional number density is then $n_{\text{SiV}} \approx 3 \times 10^{15} \text{cm}^{-3}$, or 17 ppb. This density is substantially (~ 10$^3$ times) lower than that achieved for NV centers [49]. However, work by d’Hanens-Johansson et al. [50] has shown densities of neutrally-charged SiV centers with densities around 10$^{17}$ cm$^{-3}$, while Vlasov et al. [61] have shown that silicon vacancies can exist stably in nanodiamonds as small as 2 nm in diameter, corresponding to a single-nanodiamond density of over 10$^{20}$ cm$^{-3}$. Based on these observations, we believe that much higher SiV densities are likely to be achievable following additional optimization of the growth or implantation and annealing steps, allowing realization of bright two-photon SiV nanodiamond labels.

VI. CONCLUSION

We have measured the two-photon cross section of the negatively charged silicon-vacancy color center in diamond at an excitation wavelength of 1040 nm. Our results yield an absolute determination of the two-photon silicon-vacancy cross section at 1040 nm equal to approximately 0.74 GM. We have measured the wavelength dependence of the two-photon fluorescence cross section, finding a significant increase of the cross section for the shortest wavelengths measured. Finally, we have assessed two-photon-interrogated SiV-containing diamonds as a bright non-bleaching biological label for deep tissue imaging, and found its expected performance significantly exceeds that of NV centers in diamond, provided SiV nanodiamonds can be produced with defect densities comparable to NV nanodiamonds. Our result shows that two-photon-excited SiV-nanodiamond labels offer an outstanding combination of brightness, narrow-line emission, and photostability. These characteristics promise improved sensitivity, greater detection depth, and longer interrogation times in long-term cell tracking and in-vivo molecular-imaging experiments, with significant applications to many topics of fundamental biological and biomedical interest, including drug efficacy testing, studies of circulating tumor cells, and immune cell trafficking.
where the maximum collection angle is defined by 
the calculated collection efficiency is the same, whether 
the numerical aperture of the objective. As shown below, 
the transmission coefficients from diamond into air 
by the color center that enters the objective as follows.

We compute the fraction of the total fluorescence emitted 
for emission with these polarizations are, respectively, 
and P polarization at the interface. Thus the amplitudes 
θ

from diamond into air 

η

NA of 0.75, implying an angle 

θ

θ

and the polar axis normal to the diamond-air interface. 
The two polarization axes are ̂φ = − sin φ ̂x + cos φ ̂y and 
̂θ = cos θ cos φ ̂x + cos θ sin φ ̂y − sin θ ̂z, corresponding to S 
and P polarization at the interface. Thus the amplitudes 
for emission with these polarizations are, respectively,

and 

where  is a proportionality constant, and where we have 
used the fact that ̂n is orthogonal to both polarization 
unit vectors. The proportionality constant is determined 
to be  = √3/8π by normalizing to unit probability. 
Thus the total collection efficiency is given by 

θ

θ

\begin{align}
\eta_{\text{coll.}} &= \frac{\sum_i \int dΩ \frac{dF}{dΩ_i} T_i}{\sum_i \int dΩ \frac{dF}{dΩ_i}}, 
\text{(A1)}
\end{align}

where  is the Fresnel transmission probability at the 
diamond-air interface for polarization i, and the integration 
in the numerator is over the solid angle defined by 
the numerical aperture of the objective. As shown below, 
the calculated collection efficiency is the same, whether 
we assume isotropic emission or dipole emission from a 
dipole oriented along the (111) direction of the diamond 
lattice.

1. Calculation for Isotropic Emission

If the color center emits isotropic, unpolarized radiation, 
the collection efficiency simplifies to 

\begin{align}
\eta_{\text{coll.}} &= \frac{1}{4} \int_0^{\theta_{\text{max}}} \sin \theta d\theta \left\{ T_S(\theta) + T_P(\theta) \right\}, 
\text{(A2)}
\end{align}

where the maximum collection angle is defined by 
the numerical aperture of the collection objective, i.e., 
n sin θ_{\text{max}} = NA, n is the index of refraction of diamond, 
and the transmission coefficients from diamond into air 
for S and P polarization are

\begin{align}
T_S &= \frac{n \cos \theta - \sqrt{1 - n^2 \sin^2 \theta}}{n \cos \theta + \sqrt{1 - n^2 \sin^2 \theta}} 
\text{(A3)}
\end{align}

and

\begin{align}
T_P &= \frac{n \sqrt{1 - n^2 \sin^2 \theta} - \cos \theta}{n \sqrt{1 - n^2 \sin^2 \theta} + \cos \theta}. 
\text{(A4)}
\end{align}

Eq. [A2] can be integrated numerically, using a diamond 
dielectric constant of  = 2.42 and a numerical aperture 
NA of 0.75, implying an angle θ_{\text{max}} ≈ 0.315 rad., result-
ing in a collection efficiency η_{\text{coll.}} ≈ 0.0203.

2. Calculation for Dipole Emission

The dipole emission amplitude along direction ̂n due to 
a dipole oscillating along axis ̂p is proportional to ̂n × (̂n × 
̂p) = ̂n (̂n · ̂p) − ̂p. Here we take ̂p = \frac{1}{\sqrt{3}}(̂x + ̂y + ̂z), with 
the origin of spherical co-ordinates at the color center 
and the polar axis normal to the diamond-air interface. 
The two polarization axes are ̂φ = − sin φ ̂x + cos φ ̂y and 
̂θ = cos θ cos φ ̂x + cos θ sin φ ̂y − sin θ ̂z, corresponding to S 
and P polarization at the interface. Thus the amplitudes 
for emission with these polarizations are, respectively,

\begin{align}
A_S &= -\alpha \hat{\phi} \cdot \hat{p} = -\frac{\alpha}{\sqrt{3}} \left( \cos \phi - \sin \phi \right) 
\text{(A5)}
\end{align}

and

\begin{align}
A_P &= -\alpha \hat{\theta} \cdot \hat{p} = -\frac{\alpha}{\sqrt{3}} \left( \cos \theta (\cos \phi + \sin \phi) - \sin \theta \right) 
\text{(A6)}
\end{align}

Simplifying the integral for η_{\text{coll.}, dipole}, we obtain

\begin{align}
\eta_{\text{coll.}, \text{dipole}} &= \frac{1}{8\pi} \int_0^{\theta_{\text{max}}} \sin \theta d\theta \int_0^{2\pi} d\phi 
\left\{ |A_S|^2 T_S(\theta) + |A_P|^2 T_P(\theta) \right\}. 
\text{(A7)}
\end{align}

Noting that the transmission coefficients depend only on 
θ, we can perform the φ integral; terms in the integrand
The electric field at the output port of the Michelson interferometer is then

\[ E = E_0' e^{-i\omega_0 t} e^{-t^2/2\tau^2} + E_0 e^{i\Delta \phi} e^{-i\omega_0 t} e^{-(t-2\Delta x/c)^2/2\tau^2}, \]  

where \( \Delta \phi \) incorporates the phase difference \( 2k\Delta x \) due to the mirror displacement \( \Delta x \) as well as any static phase difference between the two paths. The intensity corresponding to this output field is then of the form

\[
I = I_0 e^{-t^2/\tau^2} + e^{-(t-2\Delta x/c)^2/2\tau^2} + \ldots
\]

\[
2 \cos(\Delta \phi) e^{-t^2/2\tau^2} e^{-(t-2\Delta x/c)^2/2\tau^2}
\]

\[
= I_0 e^{-t^2/\tau^2} \left( 1 + e^{4t\Delta x/c^2 - 4\Delta x^2/c^2 + \ldots} \right)
\]

\[
+ 2 \cos(\Delta \phi) e^{2i\Delta x/c^2 - 2\Delta x^2/c^2 + \ldots}
\]  

The total signal detected at the photodiode is proportional by design to the square of the time-averaged intensity, i.e., to the time-integral of the \( I^2 \). Performing this integration yields a photodiode signal \( S \), given by

\[
S = S_0 \left( 1 + e^{-2\Delta x^2/c^2\tau^2} + 4 \cos \Delta \phi e^{-3\Delta x^2/2c^2\tau^2} \ldots \right)
\]

\[
+ 2 \cos^2(\Delta \phi) e^{-2\Delta x^2/c^2\tau^2} \right).
\]  

The upper envelope of the oscillatory curve given by this expression as a function of \( \Delta x \) is

\[
S_+ = S_0 \left( 1 + 3e^{-2\Delta x^2/c^2\tau^2} + 4e^{-3\Delta x^2/2c^2\tau^2} \right),
\]  

while the lower envelope is

\[
S_- = S_0 \left( 1 + 3e^{-2\Delta x^2/c^2\tau^2} - 4e^{-3\Delta x^2/2c^2\tau^2} \right).
\]  

Consequently the difference envelope is given by a pure Gaussian,

\[
\Delta S \equiv S_+ - S_- = 8S_0 e^{-3\Delta x^2/2c^2\tau^2}.
\]  

The spatial period of the oscillatory waveform enclosed by these envelopes is \( \lambda/2 \), corresponding to a relative pulse time delay of \( \lambda/2c \).

When this signal is recorded on an oscilloscope as a function of time, the position is assumed to be a linear function of time, \( \Delta x = at \). Thus the temporal period \( T \) of the oscilloscope trace is given by \( T = \lambda/2\alpha \), or \( \alpha = \lambda/2T \). The 1/e half-width \( t_{1/e} \) of a gaussian fit to \( \Delta S \) occurs when

\[
3 \alpha^2 t_{1/e}^2 / 2c^2 \tau^2 = 1
\]

or

\[
\tau = \sqrt{\frac{3}{8}} \frac{\lambda}{cT}.
\]

The difference envelope obtained from an autocorrelator measurement on our 1040 nm laser and a gaussian fit to the difference envelope are shown in Fig. 10.
Appendix C: Measurement in Dense Sample

If the color centers being whose fluorescence is to be collected are present on a surface with a certain area number density $n_{2d}(x, y)$, and the excitation laser beam has a transverse spatial profile given by the function $I(x, y) \equiv I_0 F_{ex}(x, y)$, with a spatial maximum $I_0$ at the center of the excitation beam, then the observed signal is given by

$$
\Gamma_{\text{det}, 2p} = \sigma_{2p} \int dx \, dy \, n_{2d}(x, y) \langle I^2(x, y) \rangle,
$$

where $\eta \equiv \int dx \, dy \, I^2(x, y)$ is the spatially-dependent detection efficiency, and $G$ is the detection point spread function (PSF) of the microscope, equal to the convolution of the confocal pinhole aperture function and the imaging point-spread function at the detection wavelength. To avoid ambiguity, we choose $G$ to be a unit-maximum function and to include the finite on-axis transmission of the pinhole $\eta_{\text{pinhole}}$ as one of several factors contributing to $\eta_{\text{det}}$. In other words,

$$
\eta_{\text{pinhole}} G(x, y) = \int dx' \, dy' \, G_0(x' - x, y' - y) H(x', y'),
$$

where $G_0$ is the PSF in the absence of any pinhole. Simplifying under the assumption that the fluorophore density is uniform, we obtain

$$
\Gamma_{\text{det}, 2p} = \sigma_{2p} \eta_{\text{det}} \langle I_0^2 \rangle n_{2d} A_2,
$$

where $A_2$ is an effective area defined by the spatial overlap of the detection point spread function and the square of the excitation point spread function as

$$
A_2 \equiv \int dx \, dy \, G^2(x, y) F_{ex}^2(x, y).
$$

The time average square intensity for a train of gaussian-envelope pulses is related to the average intensity by Eq. [3]. Hence, for such a pulse train, the average detection rate is given by

$$
\Gamma_{\text{det}, 2p} = \eta_{\text{det}} \sigma_{2p} \langle I_0^2 \rangle n_{2d} A_2 \frac{T_{\text{rep}}}{\tau \sqrt{2\pi}},
$$

where $A_3$ is the PSF in the absence of any pinhole. Simplerly, the under the assumption that the fluorophore density is uniform, we obtain

$$
\Gamma_{\text{det}, 2p} = \sigma_{2p} \eta_{\text{det}} \langle I_0^2 \rangle A_2 \frac{T_{\text{rep}}}{\tau \sqrt{2\pi}}.
$$

The total excitation power measured by a power meter is related to the intensity at the focus by Eq. [4]. Thus the detected signal is given in terms of the average excitation laser power by

$$
\Gamma_{\text{det}, 3p} = \eta_{\text{det}} \sigma_{3p} n_{2d} A_3 \frac{\langle P \rangle}{A_{\text{ex}}} \frac{T_{\text{rep}}^2}{\tau^2 \sqrt{3}}.
$$

For three-photon excitation, the calculation is very similar and results in the relation

$$
\Gamma_{\text{det}, 3p} = \eta_{\text{det}} \sigma_{3p} n_{2d} A_3 \frac{\langle P \rangle}{A_{\text{ex}}} \frac{T_{\text{rep}}^2}{\pi \sqrt{3}}.
$$

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