Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Biology

Nitrogen-Vacancy color center in diamond — emerging nanoscale applications in bioimaging and biosensing

Gopalakrishnan Balasubramanian[1,2], Andrii Lazariev[1], Sri Ranjini Arumugam[1] and De-wen Duan[1]

Nitrogen-Vacancy (NV) color center in diamond is a flourishing research area that, in recent years, has displayed remarkable progress. The system offers great potential for realizing futuristic applications in nanoscience, benefiting a range of fields from bioimaging to quantum-sensing. The ability to image single NV color centers in a nanodiamond and manipulate NV electron spin optically under ambient condition is the main driving force behind developments in nanoscale sensing and novel imaging techniques. In this article we discuss current status on the applications of fluorescent nanodiamonds (FND) for optical super resolution nanoscopy, magneto-optical (spin-assisted) sub-wavelength localization and imaging. We present emerging applications such as single molecule spin imaging, nanoscale imaging of biomagnetic fields, sensing molecular fluctuations and temperatures in live cellular environments. We summarize other current advances and future prospects of NV diamond for imaging and sensing pertaining to bio-medical applications.

Addresses
[1] Max-Planck Research Group Nanoscale Spin Imaging, Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany
[2] Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

Corresponding author: Balasubramanian, Gopalakrishnan (gbalasu@mpibpc.mpg.de)

Current Opinion in Chemical Biology 2014, **20**:69–77

This review comes from a themed issue on **Molecular imaging**

Edited by **Christian Eggeling** and **Mike Heilemann**

For a complete overview see the Issue and the Editorial

Available online 27th May 2014

http://dx.doi.org/10.1016/j.cbpa.2014.04.014

Introduction

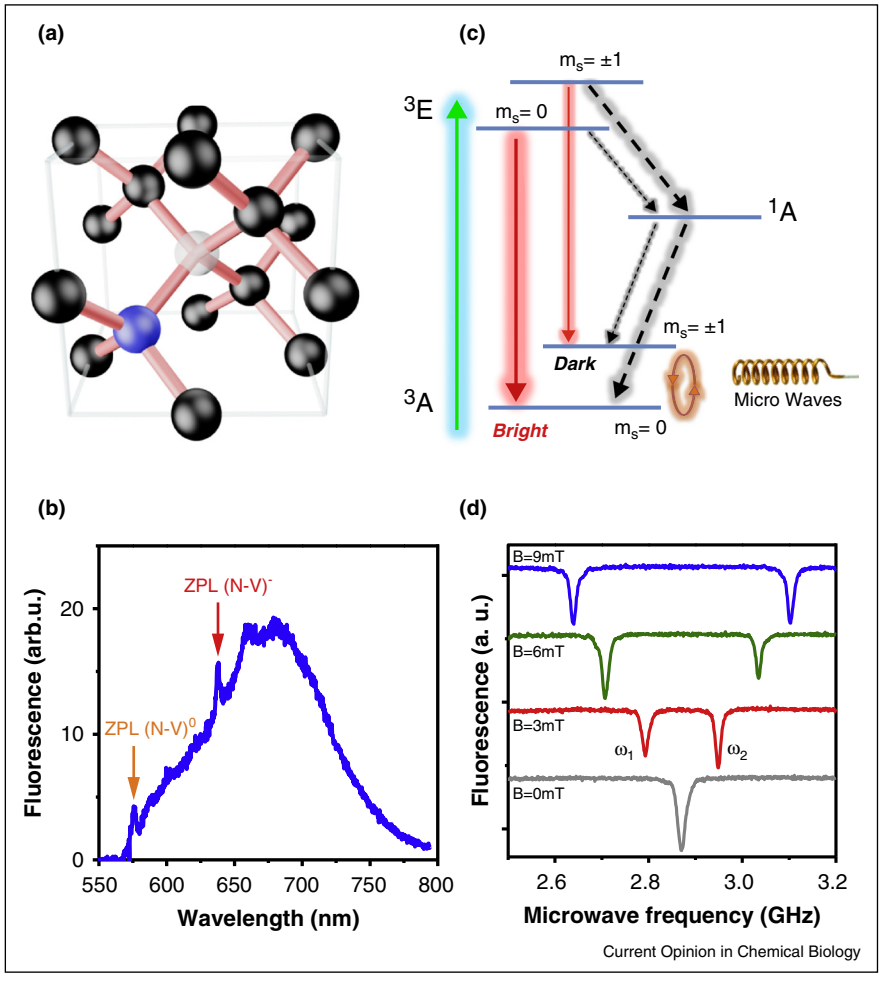
Nitrogen-Vacancy (NV) color centers in diamond is gaining significant interest because of its several unique properties and promising cutting edge applications. The applications range from nano-sized luminescent markers for novel light microscopy to 'spin-qubits' that are building blocks for quantum information technologies [1]. One of the main attractions of the NV-color centers for microscopy and imaging is their biocompatibility and functionality under ambient conditions. In addition, the NV center is an optically readable sensor that could measure variety of physical quantities with very high precision. During the last few years, several proof-of-principle demonstrations were shown on precision measurements of magnetic field, electric field, temperature, ion concentration and spin densities [2,3•,4••,5,6••]. Several diverse thrust areas are emerging on the NV center research front. In this article, we will focus on progress during the last three to four years that focuses on microscopy and imaging pertaining to bio-applications and analyze future prospects for emerging areas of NV center research.

Nitrogen-Vacancy defects in diamond

Lattice defects that form color centers are prevalent in a variety of crystalline diamond materials ranging from molecular sized diamonds present in asteroids [7] to chemically synthesized nano, micro and macro sized diamonds [8]. Nitrogen-Vacancy defects are crystalline imperfections in diamond lattice, comprising a nitrogen atom substituting a carbon lattice site that is bound to an adjacent vacant lattice site (Figure 1a). The NV defect in diamond is a luminescent color-center that absorbs in the wavelength range of 460–600 nm. The defect when excited by green light (532 nm) gives broad fluorescence emission in the near-infrared region with maximum centered on 680 nm (Figure 1b). A single NV defect center carries a negative charge and is usually denoted as NV^- or simply NV. While the neutral charge state is explicitly mentioned as NV^0 and has slightly blue shifted fluorescence emission with zero-phonon line at 575 nm.

To date the most viable route to make fluorescent nano diamonds containing NV defects are by irradiation of nitrogen-rich (about 100 ppm) high pressure high temperature (HPHT) synthesized diamonds. Irradiation can be done either using high-energy electron beam or helium beam [9,10,11]. After irradiation, the crystals are annealed in a vacuum at 800°C. During this process the vacancies migrate, re-establishing the crystalline structure. Occasionally, when they encounter a nitrogen atom in the next lattice position they bind to form a stable NV defect. The NV enriched micrometer/sub-micrometer sized crystals are further reduced in size by ball-milling and subsequently sorted according to size using ultracentrifugation. Recently, a new

Figure 1



(a) The atomic structure of a single Nitrogen-Vacancy (NV) defect in diamond. Substituted Nitrogen atom (blue) bound to Vacancy site (white) in a diamond lattice (black). **(b)** Fluorescence emission from single NV defect showing zero-phonon-lines (ZPL) of NV^- and NV^0 characteristics. **(c)** Energy level structure of NV defect and the spin sublevels optical excitation 532 nm (green arrow), Fluorescence emission (red arrows 637–750 nm) non-radiative decay processes (black dashed lines) and orange lines spin transitions driven by MW fields. **(d)** Optically detected Magnetic resonance (ODMR) spectrum of single NV spin and the corresponding Zeeman effect on magnetic field dependence.

method has emerged that uses nitrogen ion implantation on high purity diamonds through an array of nanosized apertures to create NV centers. The patterned holes are masked and further treated by reactive ion etchings that create nanowhiskers. These whiskers are extracted to yield high purity nanodiamonds that have good spin coherence properties [12]. The method though scalable currently produces limited quantities of high quality NV nanodiamonds for specialized research applications.

Spin properties of NV center

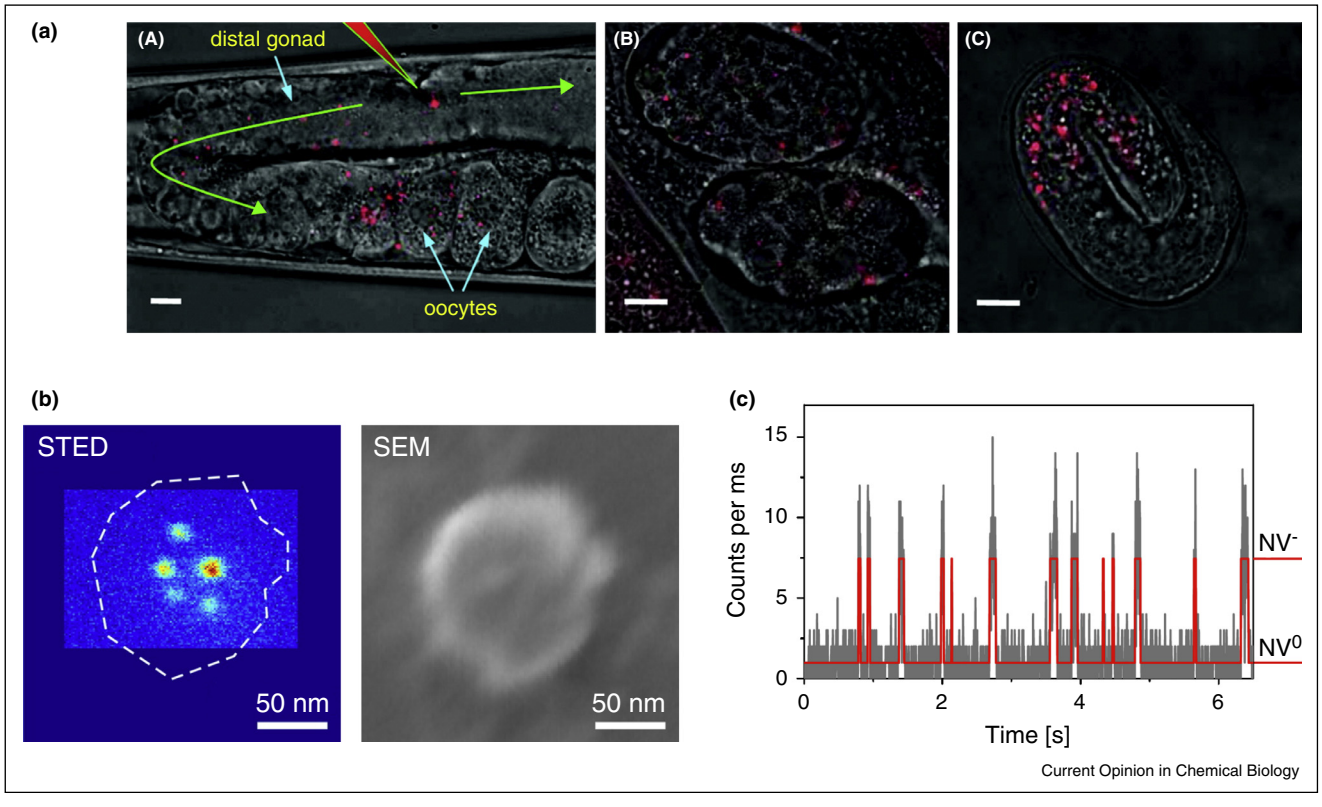
The NV defect has two unpaired electrons resulting in an integer spin (S = 1) system. The electrons spin levels manifests as triplet ground (^3A) and excited states (^3E). The ground state spin sublevels $m_s = \pm 1$ are degenerate at zero magnetic field and separated from $m_s = 0$ sublevel by 2870 MHz due to the interacting electrons. Optical transitions between ^3A and ^3E mostly conserves their spin states. The system from excited state (^3E) can occasionally cross into metastable singlet states (^1A) and this process is spin state dependent. The inter-system crossing (ISC) rate from the ^3E $m_s = \pm 1$ state into ^1A singlet state is higher than the transition rate from ^3E $m_s = 0$ into the singlet state. The singlet state then decays into ground state $m_s = 0$ spin sublevel by non-radiative processes. This results in electron spin polarization or optical pumping into the ground state $m_s = 0$ spin sublevel. When a resonant microwave (MW) field is applied, the electron spin undergoes

transition from $m_s = 0$ to $m_s = \pm 1$ in the ground state (Figure 1c). This spin transition results in about 30% decrease of fluorescence emission intensity because from the excited state $m_s = \pm 1$ levels relatively prefer ISC transition ^3E–^1A non-radiative decay while the ($m_s = 0$) levels favors ^3E–^3A by fluorescence emission. This phenomenon of reading the spin state of electrons by fluorescence is called Optically Detected Magnetic Resonance (ODMR) [13]. The uniqueness of NV defect system in the ODMR process is observable at single spin level and in room temperature. External magnetic fields split the energy levels of spin sublevels as the manifestation of Zeeman effect on single spin (Figure 1d). MW field can also cause spin transition in the excited state ^3E manifold with a characteristic zero-field splitting 1440 MHz Both continuous wave (CW) and pulsed magnetic resonance protocols can be readily applied to probe and manipulate the spin. Several recent progresses in this direction have resulted in a wealth of information on the spin system and multitude of applications [14–17].

Nanodiamonds as fluorescent bio-markers

The prime attraction of diamond nanoparticles for biological applications is the robust photostability of the color center and its low cytotoxicity. Earlier Fu *et al.* and Neugart *et al.* demonstrated the first bio-application of fluorescent nanodiamond (FND) particles for single particle tracking in HeLa cells [18,19]. The major drive in this research area is because the defect center fluoresces in the near infrared (NIR) region without any photobleaching for an unlimited time. More recently Wu *et al.* have taken single particle tracking of fluorescent diamonds to the next level by monitoring stem cells transplanted in a mouse embryo and probed the location of the final delivery using fluorescence microscopy [20••]. In a related study Mohan *et al.* injected fluorescent nanodiamonds into the gonads of live *C. elegans* and observed the nanoparticles presence in their offspring. This reveals the ultimate cyto-compatibility of the nanodiamonds and the ability to optically track NV-containing nanodiamonds during long-term biological processes [21] (Figure 2a).

Figure 2



(a) Epifluorescence/DIC-merged images of *C. elegans* microinjected with FND particles. FND containing worm (A) its progeny at the early (B) and late (C) embryonic stages. **(b)** Stimulated Emission Depletion nanoscopy image of individual NV centers inside a single nanodiamond particle of size about 200 nm. STED of NV defects and Scanning Electron Microscopy images of the same diamond particle shown **(c)** Fluorescence emission time trace as ON/OFF bursts from a single NV defect when excitation of 590 nm is used. Adapted from Aslam *et al.* [27]. The figures (a) reprinted with permission from Mohan *et al.* [21]. Copyright 2010 American Chemical Society. (b) Reprinted with permission from Arroyo-Camejo *et al.* [24•]. Copyright 2013 American Chemical Society.

Super-resolution optical imaging

A research area gaining significant importance is sub-diffraction localization and super resolution imaging. Robust photostability of NV center is a compelling factor in realizing sub-diffraction localization of FNDs. Large stokes shifted fluorescence emission is very useful for realizing Stimulated Emission Depletion (STED) in NV containing FNDs [22,23]. Recently, Arroyo-Camejo *et al.* used STED nanoscopy to image individual NV defects within a single sub wavelength sized diamond particle with an optical resolution of 10 nm [24•] (Figure 2b). Till date the highest resolution achieved using STED microscopy is 2.4 ± 0.3 nm for a NV center contained in a diamond solid immersion lens (SIL) [25]. Han *et al.* and Aslam *et al.* carried out detailed analysis of the photo physics of NV centers with an interest to find optimal conditions for optical nanoscopy and efficient routes to address the NV^- charge state for quantum applications [26–28]. When optimal wavelength of 590 nm is used NV^- charge states get selectively excited. Two-photon ionization process causes single NV defect to stochastically switch between NV^- and NV^0 charge states. This tends to give a burst of photons with characteristic ON/OFF times depending on the incident wavelength and laser power. A typical time trace is shown (Figure 2c) and recently STORM type of localization microscopy has been demonstrated by Pfender *et al.* [27,29,30]. The broad emission and photostability of NV has found application as a donor in single molecule Förster resonance energy transfer (FRET). In an experiment Tisler *et al.* demonstrated efficient FRET between single NV defect and a single acceptor molecule (Black hole 3 and DY781) [31].

Magneto-optical nanoscopy

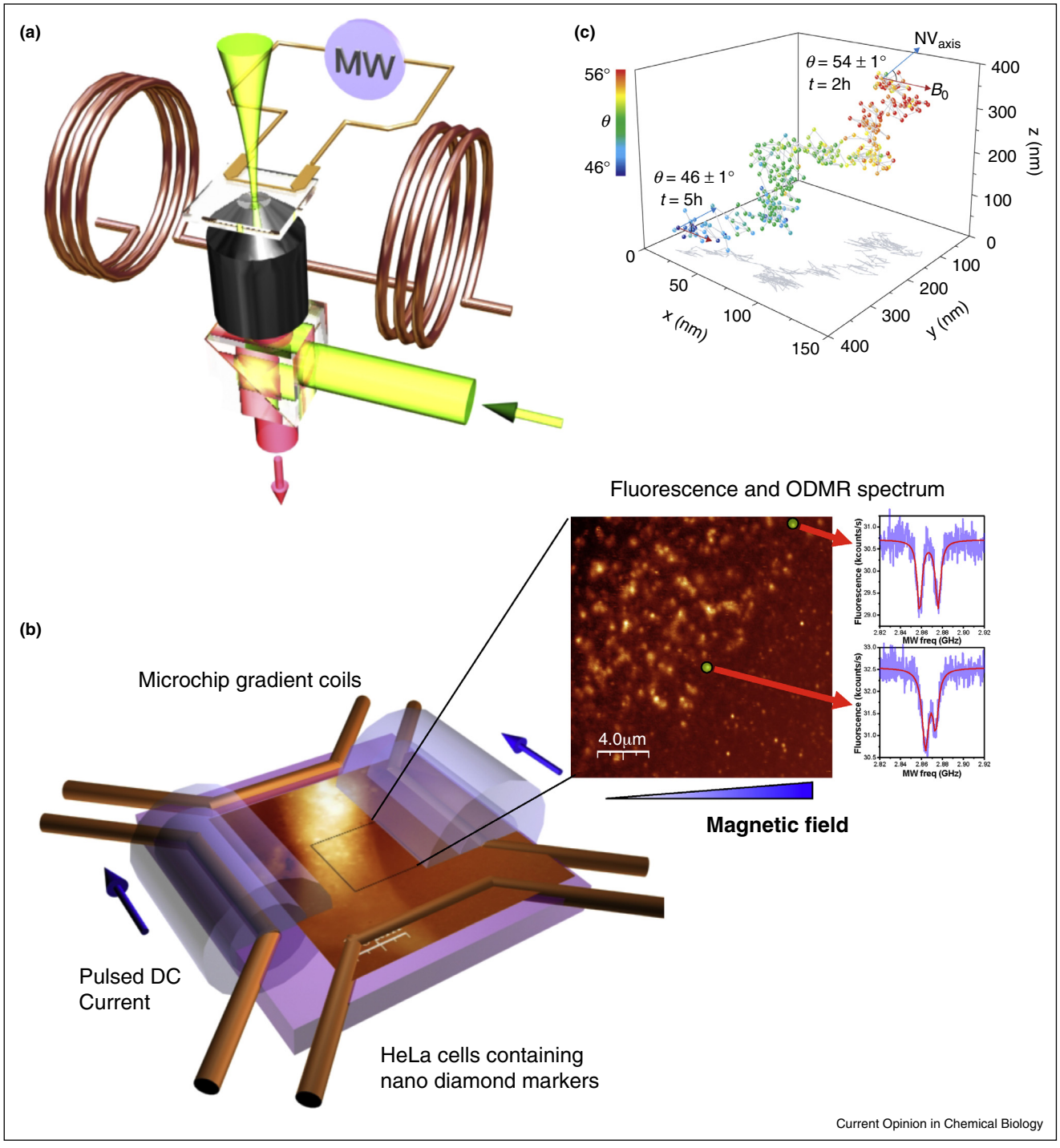
A unique method of subdiffraction localization/imaging using NV defects is microwave assisted magneto-optical nanoscopy that exploits the spin properties of the defect. The setup used for these experiments is a modified confocal fluorescence microscope. Additions are an antenna to apply microwave fields (MW) and pairs of coils for producing well-aligned magnetic fields. The basic principle is analogous to the magnetic resonance imaging (MRI) technique. In this well-known method magnetic field gradient is applied across the sample so that every spatial location (co-ordinate) is encoded into unique magnetic field values. This enables the spatial co-ordinates and orientation of the NV defect to be decoded from signatures of ODMR spectrum [32]. For example, when two NV defects are spatially separated and placed in magnetic field gradient, they will experience different magnetic field values. Hence their precise location can be estimated by measuring the Zeeman splitting of the spin sublevels that is unique at the location (Figure 1d). The resolution of this technique is given by the magnetic field gradient and the spin resonance line width of the NV defect. A micro coil type arrangement made using micro strip lines would impart field gradients of 10^{-3} Gauss/nm providing localization accuracy of about 7 nm over a 100 μm^2 field-of-view [32] (Figure 3a). Using magneto-optical means, McGuinness *et al.* demonstrated orientation tracking of FND particles inside HeLa cell by monitoring the symmetry of ODMR lines positions by appropriately binning the data collected for a period of few hours [33••] (Figure 3b). If the external magnetic field is aligned parallel to the NV axis the two ODMR lines arising from the transition $m_s = 0 - m_s = -1$ and $m_s = 0 - m_s = +1$ will appear at symmetric locations ($zfs - \omega_1$ and $zfs + \omega_1$) in frequency with respect to the zero-field-splitting (2870 MHz). The line positions will be asymmetric if the external magnetic field is oriented at an angle to the NV axis. Recently, two groups showed optical trapping of nanodiamonds in solution and simultaneously measured the ODMR spectrum [34•,35]. The setup is very similar to that shown in Figure 3a with an addition of 1064 nm laser beam used for trapping single nanodiamond. The technique readily offers potential to maneuver single FND to a required location inside a cellular environment and probe processes in real time by non-contact means.

Spin imaging microscopy with NV sensors

In a very simplistic view, single NV defect is a sensitive atomic-sized precision magnetic field sensor. An isolated electron spin produces a magnetic field of about 2 μT at a distance of 10 nm and a proton produces a field magnitude of approximately 2 nT at the same distance [36–38]. Employing pulsed magnetic resonance protocols Maze *et al.* and Balasubramanian *et al.* measured magnetic fields of μT and nT order using single NV in nanodiamonds [38] and in isotopically pure diamonds [2]. Recently, Staudacher *et al.* demonstrated external spin sensing using single NV defects close to the surface of the diamond (7 nm) [6••]. They measured signatures of hydrogen atoms from immersion oil and closely placed polymer film. The hydrogen spins in the molecule cause random magnetic fluctuations (spin noise) at their characteristic Larmor frequency. This magnetic noise signal from the surface at the location of the NV defect will be very weak. Thankfully the NV sensor is sensitive to a level that it is able to measure signals arising from hydrogen atoms contained in a voxel of 5 nm^3. In a parallel work Mamin *et al.* measured the dipole-dipole interaction between single NV spin and many ^1H spins by manipulating the proton spins with resonant radio frequency (RF) field [39].

Measuring spin noise and imaging the spin density from a biomolecule with nanoscale spatial resolution would readily give the three dimensional molecular structure. A schematic of such a molecular structure microscope is shown in Figure 4. The method is similar to the nanoscale scanning probe magnetometry using single NV demonstration by Balasubramanian *et al.* [40]. A

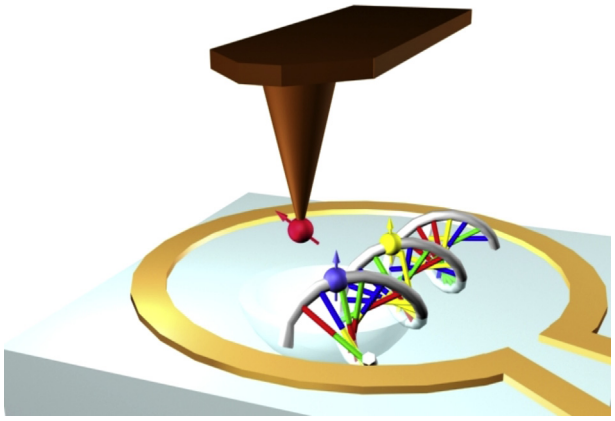
Figure 3



(a) Schematic representation of a setup used for Magneto-optical localization and tracking of FND in solution phase and in cellular environment. **(b)** HeLa cells endocytosed with FND particles are localized to sub-wavelength scales using microchip gradient coils. The fluorescence image of the endocytosed diamonds and ODMR of single NV containing particles are shown in the inset. **(c)** The time trace of orientation tracking of single FND particles by magneto-optical means. (c) reprinted by permission from McGuinness *et al.* [33••], copyright (2011).

single NV defect is placed at the apex of a cantilever and scanned at molecular scale proximities close to the target biomolecule to be imaged. A ferromagnetic tip provides the required magnetic field gradient that can select portions of proton spins. The NV defect is scanned over the sample with nanometer resolution

Figure 4



The schematic representation of a molecular structure microscope. The NV spin sensor is scanned relative to the biomolecule either in the manner shown above or in swapped configuration. Spin density can be effectively mapped at various locations to reconstruct the molecular structure of isolated biomolecules in three dimensions.

and maps the spin density of the sub-selected protons. This method has the potential to emerge as a new tool for structural biology to efficiently image three-dimensional structures of biomolecules in a single molecule level. This technique could offer an alternate method to image biomolecules that are hard to crystallize or that contain intrinsically disordered proteins.

In a recent experiment Grinolds *et al.* used this scheme in a scanning geometry and imaged the location of a single electron spin and dark-electron spin inside diamonds [41,42••]. The technique directly offers route to image nuclear spin clusters and protons in a biomolecule. This recent progress indicates the direction towards realizing a diamond based molecular structure microscope for three-dimensional imaging of isolated biomolecules.

Imaging bioprocesses with NV diamond

Another thrust area of NV center based bio-imaging is the use of arrays of sensors to image and measure bioprocesses in living cells. The sensors element used for this purpose is made from ultrapure chemical vapor deposition (CVD) grown diamond chips doped with N^+ ions to create near surface NV defects. Fluorescence emission from this dense array is imaged onto a sensitive CCD camera (charge-coupled device) readily giving a two-dimensional area sensor [43,44]. Selective optical and microwave manipulation is used to monitor the state of NV spin array thereby sensing the environment close to the surface. When a living cell is placed on top of the diamond chip the NV sensors placed beneath the surface senses bioprocesses and magnetic fluctuations pertaining to the cell. In a vivid demonstration, Le Sage *et al.* showed this by imaging a stray magnetic field from a magnetotactic bacteria [45••]. The bacteria assemble magnetic nanoparticles into magnetosomes along its body to help in navigating along geomagnetic fields. The array based NV imaging technique is rather unique in that it holds the potential to image live formation of magnetosomes in nanoscale and monitor the process even during cell division of these magnetotactic bacteria. In a related approach, Stienert *et al.* used the NV array based imaging technique to bring in contrast enhancement to nanoscale cellular features [46]. They selectively stained regions of a cell with Gd^+ magnetic contrast agents and placed a microtome of fixed cells on top of the diamond-imaging chip. The Gd^+ labeled regions of the single cell produce tiny magnetic fluctuations localized to nanoscale regions affecting the NV array. This provides good contrast in the magneto optical image of the cell and is able to resolve features at nanometer length scales. The technique has sufficient sensitivity that it offers the possibility to image magnetic fields from a single neuron or record firing patterns across neuronal circuits [47].

Sensing biomolecules with NV centers

Fluctuations on the nanoscale are fundamental bioprocesses that influence cellular transport and signaling. FND probes offer a new route to monitor these fluctuations on the nanoscale. In an diffusion sensing experiment Kaufmann *et al.*, attached some nanodiamonds containing a single NV to a glass slide and spread some Gd^+ spin markers labeled lipid molecules to form lipid bilayers [48•]. Magnetic noise signal sensed by the NV defect is related to the diffusion of the spin marker thus offering a nanoscale fluctuation-sensing probe. The basic idea could be extended to sense metal clusters and metallo-proteins. In a similar experiment, Ziem *et al.* used ensemble of NV defects to probe the presence of about 2000 Mn^+ ions and about 10 ferritin molecules in the proximity of NV defects [5]. In an elegant approach, Ermakova *et al.* used NV containing nanodiamonds functionalized with a few ferritin molecules and showed systematic dependence on the resulting spin properties [49]. The study unambiguously showed the sensing of a few biological molecules using single NV defects.

Nanoscale fluorescence thermometry using NV defects

Though the atomic sized NV defect is hosted inside a rigid diamond lattice, temperature has an effect on the crystal parameters. Temperature change causes perturbations in the local strain governing the Hamiltonian of the NV spin. In a study, Acosta *et al.* measured the temperature dependence of the NV spins magnetic resonance spectrum and found that the zero-field-splitting (zfs) parameter shifts by -74 kHz/k upon temperature change [50]. Recently, three

groups independently came up with experiments demonstrating nanoscale optical thermometry using NV defects in bulk diamonds and nanocrystals. In one method, Kucsko *et al.* measured the cw-ESR spectrum collected from about 500 NV defects contained in a FND particle and estimated the zfs [4••]. In this experiment Kucsko *et al.* demonstrated the first use of NV nano-thermometry to accurately report temperature gradients across micrometer distances inside a living embryonic human fibroblast cells. In a parallel work Neumann *et al.* and Toyli *et al.* measured precise temperature using pulsed magnetic resonance protocols on single NV defects in nanodiamond and bulk diamond crystals [51,52]. The nanosized diamond sensor offers spatial resolution of nanometer range while the measured temperature sensitivity is in the order of mK. The optically readable thermometer has a high precision and a wide range of operability from near zero to hundreds of degrees Kelvin. The ability to remotely measure temperature has biomedical applications for monitoring bioprocesses and optimized cancer therapy.

Conclusion

This article is a compendium of recent advances and current status of NV based methods focusing on bio-imaging and bio-sensing applications. It is evident that the most crucial factor for wide spread biological applications of FND is chemical functionalization. Standard protocols fall short of delivering chemical flexibility and uniform surface coverage thus limiting direct usage of FND as standard fluorophores. The progress in chemical functionalization indicates that viable processes are attainable in the near future. Novel applications are expanding and many of the NV based proof-of-principle experiments are in the next stage for real world applications. These exciting possibilities further stimulate researchers in discovering new sensing methods and efficient schemes to transform the test phase NV diamond based imaging/sensing research to standard analytical technique for everyday applications.

Acknowledgements

We thank S. Tayob for critical reading of the manuscript. We would like to thank Prof. S.W. Hell for valuable discussions and Prof. F. Jelezko and Prof. J. Wrachtrup for some works done at 3rd Physikalisches Institute, Universität Stuttgart. This work was supported by the Cluster of Excellence and DFG Research Center for Nanoscale Microscopy and Molecular Physiology of the Brain. Funding from the Volkswagen stiftung and the Max-Planck Society is greatly acknowledged.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ladd TD, Jelezko F, Laflamme R, Nakamura Y, Monroe C, O'Brien JL: **Quantum computers**. *Nature* 2010, **464**:45-53.

2. Balasubramanian G, Neumann P, Twitchen D, Markham M, Kolesov R, Mizuochi N, Isoya J, Achard J, Beck J, Tissler J *et al.*: **Ultralong spin coherence time in isotopically engineered diamond**. *Nat Mater* 2009, **8**:383-387.

3. • Dolde F, Fedder H, Doherty MW, Nöbauer T, Rempp F, Balasubramanian G, Wolf T, Reinhard F, Hollenberg LCL, Jelezko F *et al.*: **Electric-field sensing using single diamond spins**. *Nat Phys* 2011, **7**:459-463.
This paper experimentally demonstrate high precision electric field sensing with nanoscale resolution using single NV defects.

4. •• Kucsko G, Maurer PC, Yao NY, Kubo M, Noh HJ, Lo PK, Park H, Lukin MD: **Nanometre-scale thermometry in a living cell**. *Nature* 2013, **500**:54-58.
This work reports NV-nanodiamond based precision sensing of temperature and temperature gradients by optical means with nanoscale spatial resolution.

5. Ziem FC, Götz NS, Zappe A, Steinert S, Wrachtrup J: **Highly sensitive detection of physiological spins in a microfluidic device**. *Nano Lett* 2013, **13**:4093-4098.

6. •• Staudacher T, Shi F, Pezzagna S, Meijer J, Du J, Meriles CA, Reinhard F, Wrachtrup J: **Nuclear magnetic resonance spectroscopy on a (5-nanometer)3 sample volume**. *Science* 2013, **339**:561-563.
This paper experimentally demonstrate how a single NV defect can be used for sensing Hydrogen spins from a polymer in an active volume of 5 nm^3.

7. Vlasov II, Shiryaev AA, Rendler T, Steinert S, Lee SY, Antonov D, Voros M, Jelezko F, Fisenko AV, Semjonova LF *et al.*: **Molecular-sized fluorescent nanodiamonds**. *Nat Nanotechnol* 2014, **9**:54-58.

8. Markham ML, Dodson JM, Scarsbrook GA, Twitchen DJ, Balasubramanian G, Jelezko F, Wrachtrup J: **CVD diamond for spintronics**. *Diamond Relat Mater* 2011, **20**:134-139.

9. Boudou JP, Tisler J, Reuter R, Thorel A, Curmi PA, Jelezko F, Wrachtrup J: **Fluorescent nanodiamonds derived from HPHT with a size of less than 10 nm**. *Diamond Relat Mater* 2013, **37**:80-86.

10. Chang YR, Lee HY, Chen K, Chang CC, Tsai DS, Fu CC, Lim TS, Tzeng YK, Fang CY, Han CC *et al.*: **Mass production and dynamic imaging of fluorescent nanodiamonds**. *Nat Nanotechnol* 2008, **3**:284-288.

11. Su LJ, Fang CY, Chang YT, Chen KM, Yu YC, Hsu JH, Chang HC: **Creation of high density ensembles of nitrogen-vacancy centers in nitrogen-rich type Ib nanodiamonds**. *Nanotechnology* 2013:24.

12. Trusheim ME, Li L, Laraoui A, Chen EH, Bakhru H, Schröder T, Gaathon O, Meriles CA, Englund D: **Scalable fabrication of high purity diamond nanocrystals with long-spin-coherence nitrogen vacancy centers**. *Nano Lett* 2013, **14**:32-36.

13. Jelezko F, Gaebel T, Popa I, Gruber A, Wrachtrup J: **Observation of coherent oscillations in a single electron spin**. *Phys Rev Lett* 2004, **92**:764011-764014.

14. London P, Scheuer J, Cai JM, Schwarz I, Retzker A, Plenio MB, Katagiri M, Teraji T, Koizumi S, Isoya J *et al.*: **Detecting and polarizing nuclear spins with double resonance on a single electron spin**. *Phys Rev Lett* 2013:111.

15. Taminiau TH, Wagenaar JJT, Van Der Sar T, Jelezko F, Dobrovitski VV, Hanson R: **Detection and control of individual nuclear spins using a weakly coupled electron spin**. *Phys Rev Lett* 2012:109.

16. Dobrovitski VV, Fuchs GD, Falk AL, Santori C, Awschalom DD: **Quantum control over single spins in diamond**. *Annu Rev Condens Matter Phys* 2013, **4**:23-50.

17. Bar-Gill N, Pham LM, Jarmola A, Budker D, Walsworth RL: **Solid-state electronic spin coherence time approaching one second**. *Nat Commun* 2013:4.

18. Fu CC, Lee HY, Chen K, Lim TS, Wu HY, Lin PK, Wei PK, Tsao PH, Chang HC, Fann W: **Characterization and application of single**

fluorescent nanodiamonds as cellular biomarkers. *Proc Natl Acad Sci U S A* 2007, **104**:727-732.

19. Neugart F, Zappe A, Jelezko F, Tietz C, Boudou JP, Krueger A, Wrachtrup J: **Dynamics of diamond nanoparticles in solution and cells**. *Nano Lett* 2007, **7**:3588-3591.

20. •• Wu TJ, Tzeng YK, Chang WW, Cheng CA, Kuo Y, Chien CH, Chang HC, Yu J: **Tracking the engraftment and regenerative capabilities of transplanted lung stem cells using fluorescent nanodiamonds**. *Nat Nanotechnol* 2013, **8**:682-689.
This experimental work shows grafting of FND particles to stem cells and demonstrate cytocompatiblity and ability to target certain regions in an organism.

21. Mohan N, Chen CS, Hsieh HH, Wu YC, Chang HC: **In vivo imaging and toxicity assessments of fluorescent nanodiamonds in *Caenorhabditis elegans***. *Nano Lett* 2010, **10**:3692-3699.

22. Rittweger E, Han KY, Irvine SE, Eggeling C, Hell SW: **STED microscopy reveals crystal colour centres with nanometric resolution**. *Nat Photon* 2009, **3**:144-147.

23. Han KY, Kim SK, Eggeling C, Hell SW: **Metastable dark states enable ground state depletion microscopy of nitrogen vacancy centers in diamond with diffraction-unlimited resolution**. *Nano Lett* 2010, **10**:3199-3203.

24. • Arroyo-Camejo S, Adam MP, Besbes M, Hugonin JP, Jacques V, Greffet JJ, Roch JF, Hell SW, Treussart F: **Stimulated emission depletion microscopy resolves individual nitrogen vacancy centers in diamond nanocrystals**. *ACS Nano* 2013, **7**:10912-10919.
This work shows ability to perform sub-diffraction imaging of NV defects inside a single FND partcile using STED micrsocopy.

25. Wildanger D, Patton BR, Schill H, Marseglia L, Hadden JP, Knauer S, Schönle A, Rarity JG, O'Brien JL, Hell SW *et al.*: **Solid immersion facilitates fluorescence microscopy with nanometer resolution and sub-Ångström emitter localization**. *Adv Mater* 2012, **24**:OP309-OP313.

26. Han KY, Wildanger D, Rittweger E, Meijer J, Pezzagna S, Hell SW, Eggeling C: **Dark state photophysics of nitrogen-vacancy centres in diamond**. *New J Phys* 2012:14.

27. Aslam N, Waldherr G, Neumann P, Jelezko F, Wrachtrup J: **Photo-induced ionization dynamics of the nitrogen vacancy defect in diamond investigated by single-shot charge state detection**. *New J Phys* 2013:15.

28. Beha K, Batalov A, Manson NB, Bratschitsch R, Leitenstorfer A: **Optimum photoluminescence excitation and recharging cycle of single nitrogen-vacancy centers in ultrapure diamond**. *Phys Rev Lett* 2012:109.

29. Gu M, Cao Y, Castelletto S, Kouskousis B, Li X: **Super-resolving single nitrogen vacancy centers within single nanodiamonds using a localization microscope**. *Opt Express* 2013, **21**:17639-17646.

30. Pfender M, Aslam N, Waldherr G, Wrachtrup J: **Single spin stochastic optical reconstruction microscopy.ArXiv e-prints**. 2014:1520.

31. Tisler J, Reuter R, Lämmle A, Jelezko F, Balasubramanian G, Hemmer PR, Reinhard F, Wrachtrup J: **Highly efficient FRET from a single nitrogen-vacancy center in nanodiamonds to a single organic molecule**. *ACS Nano* 2011, **5**:7893-7898.

32. Shin C, Kim C, Kolesov R, Balasubramanian G, Jelezko F, Wrachtrup J, Hemmer PR: **Sub-optical resolution of single spins using magnetic resonance imaging at room temperature in diamond**. *J Luminesc* 2010, **130**:1635-1645.

33. •• McGuinness LP, Yan Y, Stacey A, Simpson DA, Hall LT, Maclaurin D, Prawer S, Mulvaney P, Wrachtrup J, Caruso F *et al.*: **Quantum measurement and orientation tracking of fluorescent nanodiamonds inside living cells**. *Nat Nanotechnol* 2011, **6**:358-363.
This work reports a method to perform orientation tracking and nanoscale single particle tracking using FND inside a live cell.

34. • Geiselmann M, Juan ML, Renger J, Say JM, Brown LJ, Javier García De Abajo F, Koppens F, Quidant R: **Three-dimensional optical manipulation of a single electron spin**. *Nat Nanotechnol* 2013, **8**:175-179.
This paper experimentally show optical trapping of FND partciles and ability to perform ODMR was demonstrated.

35. Horowitz VR, Alemán BJ, Christle DJ, Cleland AN, Awschalom DD: **Electron spin resonance of nitrogen-vacancy centers in optically trapped nanodiamonds**. *Proc Natl Acad Sci U S A* 2012, **109**:13493-13497.

36. Taylor JM, Cappellaro P, Childress L, Jiang L, Budker D, Hemmer PR, Yacoby A, Walsworth R, Lukin MD: **High-sensitivity diamond magnetometer with nanoscale resolution**. *Nat Phys* 2008, **4**:810-816.

37. Degen CL: **Scanning magnetic field microscope with a diamond single-spin sensor**. *Appl Phys Lett* 2008, **92**.

38. Maze JR, Stanwix PL, Hodges JS, Hong S, Taylor JM, Cappellaro P, Jiang L, Dutt MVG, Togan E, Zibrov AS *et al.*: **Nanoscale magnetic sensing with an individual electronic spin in diamond**. *Nature* 2008, **455**:644-647.

39. Mamin HJ, Kim M, Sherwood MH, Rettner CT, Ohno K, Awschalom DD, Rugar D: **Nanoscale nuclear magnetic resonance with a nitrogen-vacancy spin sensor**. *Science* 2013, **339**:557-560.

40. Balasubramanian G, Chan IY, Kolesov R, Al-Hmoud M, Tisler J, Shin C, Kim C, Wojcik A, Hemmer PR, Krueger A *et al.*: **Nanoscale imaging magnetometry with diamond spins under ambient conditions**. *Nature* 2008, **455**:648-651.

41. Grinolds MS, Hong S, Maletinsky P, Luan L, Lukin MD, Walsworth RL, Yacoby A: **Nanoscale magnetic imaging of a single electron spin under ambient conditions**. *Nat Phys* 2013, **9**:215-219.

42. •• Grinolds MS, Warner M, De Greve K, Dovzhenko Y, Thiel L, Walsworth RL, Hong S, Maletinsky P, Yacoby A: **Sub-nanometer resolution in three-dimensional magnetic-resonance imaging of individual dark spins**. *ArXiv e-prints*. 2014:1401-2674.
This work reports imaging dark-spins inside diamond using single NV defects in a scanning geometry.

43. Toyli DM, Weis CD, Fuchs GD, Schenkel T, Awschalom DD: **Chip-scale nanofabrication of single spins and spin arrays in diamond**. *Nano Lett* 2010, **10**:3168-3172.

44. Steinert S, Dolde F, Neumann P, Aird A, Naydenov B, Balasubramanian G, Jelezko F, Wrachtrup J: **High sensitivity magnetic imaging using an array of spins in diamond**. *Rev Sci Instrum* 2010:81.

45. •• Le Sage D, Arai K, Glenn DR, Devience SJ, Pham LM, Rahn-Lee L, Lukin MD, Yacoby A, Komeili A, Walsworth RL: **Optical magnetic imaging of living cells**. *Nature* 2013, **496**:486-489.
This article demonstrates wide area magnetic field imaging using NV defects, some magnetic features from a living magnetotactic bacteria.

46. Steinert S, Ziem F, Hall LT, Zappe A, Schweikert M, Götz N, Aird A, Balasubramanian G, Hollenberg L, Wrachtrup J: **Magnetic spin imaging under ambient conditions with sub-cellular resolution**. *Nat Commun* 2013:4.

47. Hall LT, Beart GCG, Thomas EA, Simpson DA, McGuinness LP, Cole JH, Manton JH, Scholten RE, Jelezko F, Wrachtrup J *et al.*: **High spatial and temporal resolution wide-field imaging of neuron activity using quantum NV-diamond**. *Sci Rep* 2012:2.

48. • Kaufmann S, Simpson DA, Hall LT, Perunicic V, Senn P, Steinert S, McGuinness LP, Johnson BC, Ohshima T, Caruso F *et al.*: **Detection of atomic spin labels in a lipid bilayer using a single-spin nanodiamond probe**. *Proc Natl Acad Sci U S A* 2013, **110**:10894-10898.
This paper reports sensing few Gd ions labelled lipid bilayers using fluctuations in single NV spins properties.

49. Ermakova A, Pramanik G, Cai JM, Algara-Siller G, Kaiser U, Weil T, Tzeng YK, Chang HC, McGuinness LP, Plenio MB *et al.*: **Detection of a few metallo-protein molecules using color centers in nanodiamonds**. *Nano Lett* 2013, **13**:3305-3309.

50. Acosta VM, Bauch E, Ledbetter MP, Waxman A, Bouchard LS, Budker D: **Temperature dependence of the nitrogen-vacancy magnetic resonance in diamond**. *Phys Rev Lett* 2010:104.

51. Neumann P, Jakobi I, Dolde F, Burk C, Reuter R, Waldherr G, Honert J, Wolf T, Brunner A, Shim JH *et al.*: **High-precision nanoscale temperature sensing using single defects in diamond**. *Nano Lett* 2013, **13**:2738-2742.

52. Toyli DM, De Las Casas CF, Christle DJ, Dobrovitski VV, Awschalom DD: **Fluorescence thermometry enhanced by the quantum coherence of single spins in diamond**. *Proc Natl Acad Sci U S A* 2013, **110**:8417-8421.