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WHAT DIAMONDS CAN TELL US ABOUT BIOLOGY

INTRODUCTION

Diamonds are so popular they almost do not need an introduction. But beyond the sparkle diamond have also less known and to my opinion even more exciting properties. They are among the hardest materials on earth and are commonly used as abrasives as well as for drilling or cutting. Due to their unique optical properties they are used in all kinds of instruments as for example in infrared spectroscopy measuring cells or as optical elements. Due to their low electrical conductivity and high thermal conductivity, a rare combination of properties, they are also used in all kinds of electronic devices like transistors[1], sensors[2] or other applications like catalysts[3]. Many of these devices are outperformed by conventional devices (from other materials) at room temperature. However, under harsh conditions like high pressure, high temperature or high power applications, the diamond devices can shine, whereas the competition often does not function at all under these conditions[4].

Here I would like to focus on yet another unique property of diamond. The quantum information field first recognized that diamonds can contain very stable defects and their magneto-optical properties[5]. Since they are extremely photo stable and never bleach these defects in particles are investigated as biolabels[6, 7, 8]. They change their optical properties based on their magnetic surrounding. This means that the defects can convert a magnetic resonance signal into an optical signal. Since optical signals are much easier to detect (they are higher in energy and thus can be measured by photon counting) this technique reaches unprecedented sensitivity. In fact this method is so sensitive that the faint signal of a single electron[9] or of a few nuclear spins[10, 11, 12] can be detected. And since optical signals can also be located very accurately this technique achieves nanoscale resolution.

WHAT CAN BE MEASURED?

Being able to measure magnetic resonances accurately is extremely powerful. Since magnetic resonance signals are element specific (due to their gyromagnetic ratio), one can achieve chemical information[13]. In principle the method is able to detect anything that is measurable in conventional magnetic resonance spectroscopy. This means anything that has a non-zero spin. Until recently it was only possible to differentiate between elements. A recent major breakthrough in the field has been achieved which increases spectral resolution[14, 15]. This allows also resolving chemical shifts (differences in the chemical environment of an atom).

Apart from magnetic resonances themselves this technology opens up possibilities to detect several other properties. Fig. 1 shows an overview of the properties that can be measured, some examples, and the sensitivities that are typically achieved for these kinds of measurements.

Electric fields can be measured indirectly if they produce a magnetic field[20]. Temperature causes a shift in the magnetic resonance lines and is thus accessible, too[21]. If the magnetic resonance signal is known one can measure a microwave signal[22]. A strain in the material or pressure also alter the signal and thus is detectable[23].

Adding a coating, which contains a spin label and shrinks or swells in presence or absence of a certain stimulus, adds further possibilities. Rendler et al. applied this concept to the detection of pH changes/changes in redox potential inside cells.[24] Their sensing particles consisted of nanodiamonds which were coated with a polymer containing gadolinium. The polymer was designed to expand at low pH. As a result the gadolinium moves further away from the diamond and the signal goes down.

DIAMOND STARTING MATERIALS

Diamonds exist in various different forms which are synthesized by different routes and which have quite different properties. The most important diamond materials are shown in Fig. 2. Macroscopic pieces of bulk diamond can be produced by high pressure, high temperature (HPHT) synthesis, or CVD growth. HPHT diamonds are yellow and contain a substantial amount of nitrogen impurities. These are usually not useful for magnetometry. CVD diamonds can be produced and are commercially available in different quality/purity and with different crystal orientations. Electronic grade diamonds are most common for magnetometry. However, even higher quality material is also intensively researched. Material which contains as little ^{13}C as possible (instead of 1% natural abundance) is desired because ^{13}C has a non-zero spin and is thus picked up by magnetometry causing lower sensitivities[25]. Diamond plates in 100 orientation are the cheapest since they are easiest to produce. This is

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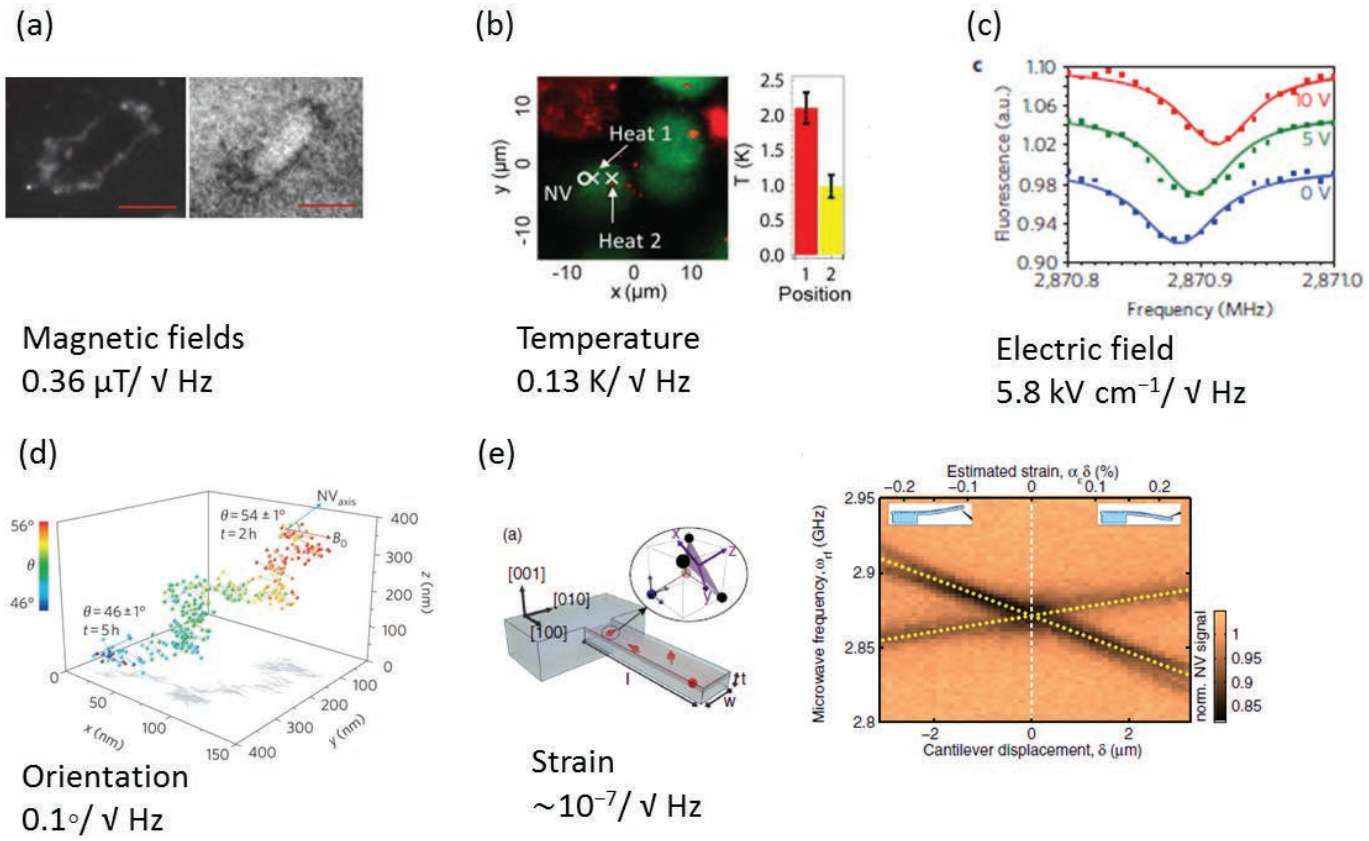

Fig. 1: Overview of properties that can be measured including example images and the achieved sensitivity. Sensitivity values for each quantity are from [31]. (a) The most popular quantity that has been measured is magnetic field. The image shows a control by fluorescence microscopy (left) and a so called T1 map of a cell section that is labelled with Gadolinium.[39] (b) shows cells which have ingested diamonds and gold particles. The gold particles are used to generate heat locally. The diamonds are used to measure the temperature at a given location.[16] (c) shows the electric fields measured by a diamond defect from a nearby electronic structure.[17] In (d) tracking of a diamond particles orientation is demonstrated[18] (e) shows a cantilever containing NV centers which was deformed. On the left side of the image is the measured signal in relation to the deformation and thus the expected strain in the material[19].

the case due to preferred growth in specific crystal orientations. However, other orientations are promising, too, since diamond defects have a preferred orientation with respect to the crystal in which they are most sensitive and it is even possible to produce defects in a preferred orientation[26, 27] (instead of statistical distribution over the 4 crystal orientations).

Detonation nanodiamonds are historically the oldest and most common nanodiamond material. They received their name since they are created in a controlled explosion of carbon rich explosives. They are spherical and rather uniformly around 5 nm in size. If aggregation is not prevented they tend to form larger aggregates of around 100 nm. However they are usually not very clean, and apart from a few promising studies they are generally not considered useful for magnetometry applications. Larger diamonds are created via HPHT synthesis. Grinding down HPHT diamond material leads to diamonds, which are commercially available from sizes of 10 nm up to micron size. They are quite irregular in size and shape and have a flake like structure[28]. Grinding down high purity material[29] or cutting nanoparticles of a high quality material[30] leads to diamonds with highest coherence times and thus best sensitivity. However, they are rather expensive to produce, and especially the second have a very low yield.

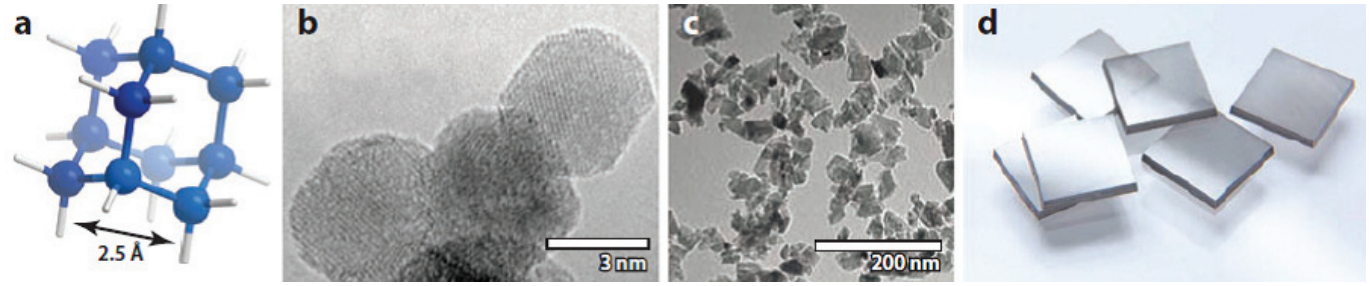

Fig. 2: Different diamond materials. Reprinted with permission from [31] (a) Adamantane, the smallest diamondoid. (b) Transmission electron micrograph of detonation nanodiamonds. (c) Scanning electron micrograph of diamond nanoparticles produced by mechanical grinding. (d) Millimeter-sized single-crystal diamond grown by chemical vapor deposition.

DIAMOND DEFECTS

Currently, there are over 700 color defects known in diamonds. So far, for most applications the most useful defect is the so-called nitrogen vacancy center. It consists of one nitrogen replacing a carbon atom in the lattice and a vacancy (a missing carbon) right next to it. Nitrogen vacancy centers appear naturally in diamonds, which contain nitrogen. But they can also be specifically engineered. In high purity diamond the nitrogen is brought in by implantation. When diamond is bombarded with ions, carbon atoms are also hit out from the lattice leaving vacancies behind[32]. When the material is heated to about 800 °C these vacancies become mobile. Since they are more stable next to a nitrogen atom they are trapped there. The resulting defect is perfectly photostable emitting a broad red peak above 600 nm when excited with green light.

In large efforts to find superior color centers scientists have started to implant almost the entire periodic table. The second most promising defects are silicon vacancy centers[33, 34]. These are of interest due to their relatively narrow emission line. However, they are even less bright than NV centers and only have promising magnetic properties at cryogenic temperatures. Recent interest has also been shown in germanium vacancy[35] and tin vacancy centers[36].

BASIC MEASURING SETUP

The basic diamond magnetometry setup is a confocal microscope with a few adaptations[37]. The optical setup can be divided in two parts, which are connected by glass fiber optics. Here it is important to choose a fiber, which is suitable for the laser wavelength in use (532nm). Fig. 3 shows a schematic representation of the first part where the green laser beam is generated. The setup consists of a Neodym YAG laser, which emits green light at 532 nm. This wavelength is suitable for exciting the NV center. Since the required laser powers are relatively low we can use comparably cheap standard equipment here. To tune laser intensities the beam is directed through a manually adjustable filter wheel. If pulsing is desired, the laser is directed through the path between (3) and (5) in Fig. 3. This path consists of a beam splitter cube, which directs the beam through an acoustooptical modulator, through a $\lambda/4$ plate and is then reflected back through the same aperture, which allows

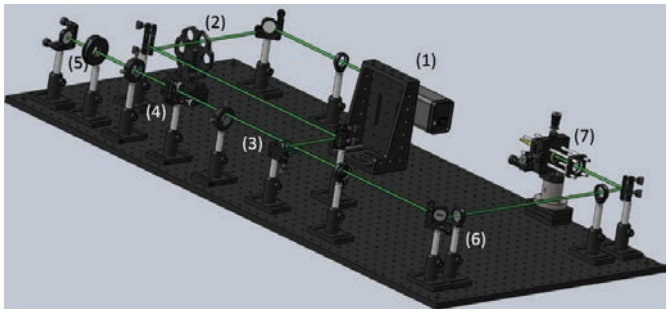

Fig. 3: First part of the optical setup: (1) laser, (2) filter wheel to tune laser intensity, (3) beam splitter cube, (4) Acoustooptical modulator which allows for laser pulsing, (5) $\lambda/4$ plate, (6) lenses increase beam size to ease fiber coupling, (7) coupling into a fiber.

fast and precise laser pulsing. Finally, the beam is coupled into a fiber and directed to the second part of the setup, which is shown in Fig. 4. There the laser is directed through a dichroic mirror (3), which divides the incoming laser light from the red fluorescence, which is emitted from the NV centers.

This is necessary to allow sensitive detection and avoid background signals from the incoming laser on the detector. The next element in the path is a scanning mirror (4) which allows addressing different parts of the sample. Different heights can be scanned by moving an objective with a piezo-stage. Here also other stages can be used but piezo stages show the desirable accuracy.

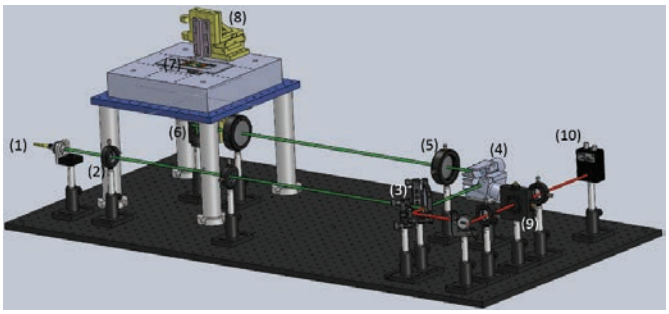

Fig. 4: Second part of the optical setup: (1) fiber port, (2) lenses to change beam size (3) dichroic mirror to direct only the NV fluorescence to the detector, (4) scanning mirror allows to move the laser to different locations on the sample, (5) lenses (6) objective that allows for focus scanning, (7) homemade microfabricated microwave stripline with sample on top (8) 3D stage for positioning a small permanent magnet close to the sample.

The sample, containing the diamonds, is located on top of a micro-fabricated (standard lift off lithography) microwave stripline. The stripline allows for microwave sweeping in order to find magnetic resonance line and perform pulse sequences. Instead of microfabricated striplines also more simple antennas are in use. These are made of a coiled or bent wire. However, generally better microwave transmission is achieved with striplines. Furthermore, microfabrication allows for more flexibility in the design. After excitation the defect emits red shifted fluorescence, which is directed back the same path until the dichroic mirror. There it is separated from reflected green light and enters the detection path (3)-(10). After passing a pinhole the light is detected by an avalanche photo-diode with single photon sensitivity. This is the most sensitive detector, which is currently available for this kind of detection. To reduce counts from reflected light the detection path is contained within a dark box (which for simplicity is not shown in the drawing.)

The basic principle (see Fig. 5) of a measurement is explained in the following. After excitation with a green laser the NV-center emits red photons. If the NV-center is in the $m_s = \pm 1$ substates of the triplet there is in addition to fluorescence an alternative nonradiative path to the ground state over a dark singlet state. As a result less red photons are emitted and a decreased fluorescence is observed. If microwave radiation is applied at the resonance frequency that equals the energy difference between the triplet sublevels (2.88 GHz at zero field) the $m_s = 0$ spin state can flip into the $m_s = \pm 1$ state. This effect can be observed as a drop in fluorescence. In presence of a magnetic field the $m_s = \pm 1$ states are no longer equal in energy

resulting in two resonance lines. Their distance is proportional to the field, which thus can be determined. In practice there are several options of how the diamond sensor can be implemented. These are described in more detail in the next section.

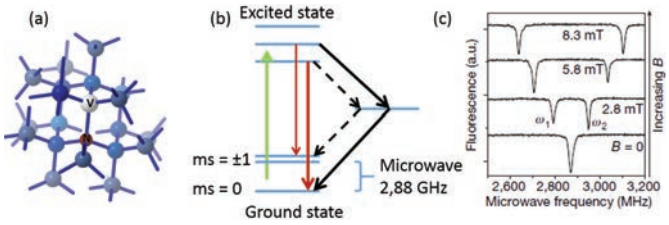

Fig. 5: Optomagnetical properties of the NV-center: (a) To form an NV center one carbon atom is replaced by a nitrogen next to a vacancy in the diamond lattice. (from [31]) (b) Simplified energy level diagram that explains the magnetic field dependence of the fluorescence. A detailed description can be found in box 1. (c) Optically detected resonance of an NV-center at different fields. At zero external field (B = 0), the +1 and the -1 state have the same energy. In the presence of an external magnetic field the energies of the two states differ resulting in two peaks (shown in (c) from [69]). While here the field comes from an external magnet in our experiments the field comes from free radicals close to the diamond defect.

BOX 1

Electronic structure of the NV^- centers

The NV-center consists of an N-atom next to a C-vacancy in diamond. A comprehensive review on the electronic structure can be found in [1]. Here we present a short summary. The fluorescent form of the NV-center used for sensoring applications is the anion NV^-. We can build a model for the electronic structure in the following steps: First, a carbon atom is removed from the diamond lattice, leaving a vacancy with four singly filled sp^3 orbitals of the neighboring carbon atoms pointing to the empty carbon site. Next, one of these carbon atoms is replaced by a nitrogen atom. By this, the singly filled sp^3 orbital at this site is replaced by the lone pair of nitrogen, and the symmetry is lowered to C_{3v}. Due to the larger electronegativity of nitrogen, the nitrogen lone pair orbital has the lowest energy of these four orbitals. The three other sp^3-orbitals form three molecular orbitals with symmetry species a_1 and e, respectively. In the NV^- anion, these are filled with 4 electrons. The a_1 orbital is lower in energy than the pair of e-orbitals, hence the occupation scheme with lowest energy is the open shell configuration $a_1^2\ e^2$, leading to the electronic states $^3A_2$, $^1A_1$, and ^1E. Following Hund's rule, the $^3A_2$ state is the ground state. Exciting one electron from the a_1 orbital to the e-orbitals yields the configuration $a_1^1\ e^3$ leading to the electronic states ^3E and ^1E.

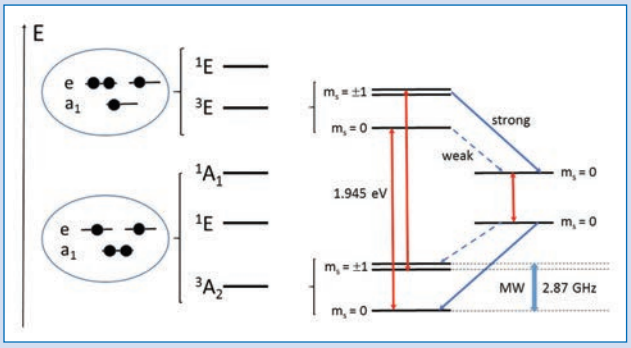

Figure A: electronic structure of the NV-anion center in diamond.

Figure A shows a sketch of the energy diagram. On the left side, the two lowest configurations $a_1^2\ e^2$ and $a_1^1\ e^3$ are represented by their orbital occupation scheme. The electrons are shown here as dots and not in the usual way as arrows in order to indicate that each configuration consists of the complete set of wavefunctions that can be made with these occupations and all spin combinations allowed by the Pauli principle. Forming the eigenfunctions of energy and spin within each configuration leads to the states $\{^3A_2, ^1A_1, ^1E\}$ and states $\{^3E, ^1E\}$, respectively. For the spectroscopic properties we restrict our discussion to the two lowest triplet and singlet states. The right side of figure A shows the energies of these two triplet states on an expanded scale, indicating the zero-field splitting of the triplet sublevels due to spin-spin interactions. Due to the C_{3v} symmetry the pair of $m_s = \pm 1$ states is degenerate in the absence of a magnetic field.

Symmetry- and spin-selection rules allow optical transitions only between the sublevels of the same m_s quantum number of the two triplet states. These transitions, that occur in absorption and emission, are indicated by the red arrows. On the other hand, transitions from the ground state to the singlet state are spin-forbidden, i.e., this state is metastable. This situation is quite opposite to the situation in "normal" organic compounds: There the ground state has singlet spin, optical excitations occur to higher singlet states, and the lowest triplet is a metastable state. The selection rules of spin-orbit coupling allow a nonradiative transition from $^3E(m_s = \pm 1)$ to $^1A_1(m_s = 0)$, and from $^1A_1(m_s = 0)$ to the $m_s = 0$ level of the $^3A_2$ ground state. However, spin-orbit coupling between $^3E(m_s = 0)$ and $^1A_1(m_s = 0)$ is weak. These transitions are shown by the green arrows in figure A. All population in the $^3A_2(m_s = 0)$ that is excited to $^3E(m_s = 0)$ returns quickly to the ground state by fluorescence, whereas some fraction of the excited population of $^3A_2(m_s = \pm 1)$ decays to the metastable singlet state and is trapped there for some time. As a consequence, the $m_s = \pm 1$ states have an apparently lower efficiency for fluorescence excitation than the $m_s = 0$ states. Excitation of the resonance between the triplet sublevels by microwave radiation in the 2.9 GHz range leads to population transfer between these levels and hence to a change of fluorescence intensity. Since single photons of fluorescence can be detected with high sensitivity, any change in the population or energy spacing of the triplet sublevels can be detected.

Examples are magnetic fields produced by species containing electron spins like gadolinium ions, or even nuclear spins. They reduce the C_{3v} symmetry of the NV center, leading to a splitting of the $m_s = \pm 1$ levels, and hence to a splitting of the microwave resonance. A temperature or

pressure change also influences the zero-field splitting and can be detected. Whereas detection in the frequency domain (i.e. by scanning the microwave frequency) is most straightforward, more sensitive methods use time domain techniques (like the free induction decay of magnetic resonance) that yield also information about relaxation times.

[1] Doherty, M. W., et al. (2013). "The nitrogen-vacancy colour centre in diamond." *Phys. Rep.* **528**, 1-45.

MEASURING MODES

Depending on the application there are different ways of performing magnetometry. Fig. 6 gives an overview over the complementary techniques that are currently in use. The first choice is whether to use nanodiamonds or bulk. Bulk material with defects on the surface usually has superior defect properties and thus is ideal for the most demanding applications. These applications require nuclear spin detection and thus the highest possible sensitivities. Nanodiamonds on the other hand are much cheaper. Also, cleaning a bulk diamond after an experiment can be challenging. Especially some spin labels can be surprisingly hard to remove. Bulk diamond measurements are also limited to the surface of the diamond since the defects sense very locally (within some tens of nm). For measurements inside living cells or inside a bulk material for instance, nanodiamonds are the only option. If the choice is bulk diamond there are again several options. The simplest is to measure a sample which is on top of the surface. This is shown in Fig. 6 (c). The approach has been used for the most demanding applications which have been demonstrated so far. However, this approach has the disadvantage that one can only measure one sample at one position, and then the diamond has to be cleaned to recover the surface. A step further is the implementation of microfluidic channels in such a bulk diamond as shown in Fig. 6 (d). This approach allows flowing liquid samples over the surface. This has been utilized by Steinert et al. for detection of gadolinium, which is a common contrast agent for magnetic resonance imaging[39]. Fig. 6 (a) shows the most complex approach. Here the diamond is the tip of a scanning probe. This means that a scanning probe instrument is needed in addition to the optical equipment. In Fig. 6 (b) nanodiamonds are used instead which can for example be inserted into cells.

In addition to these different measuring modes, pulsing sequences are an extremely powerful tool to increase sensitiv-

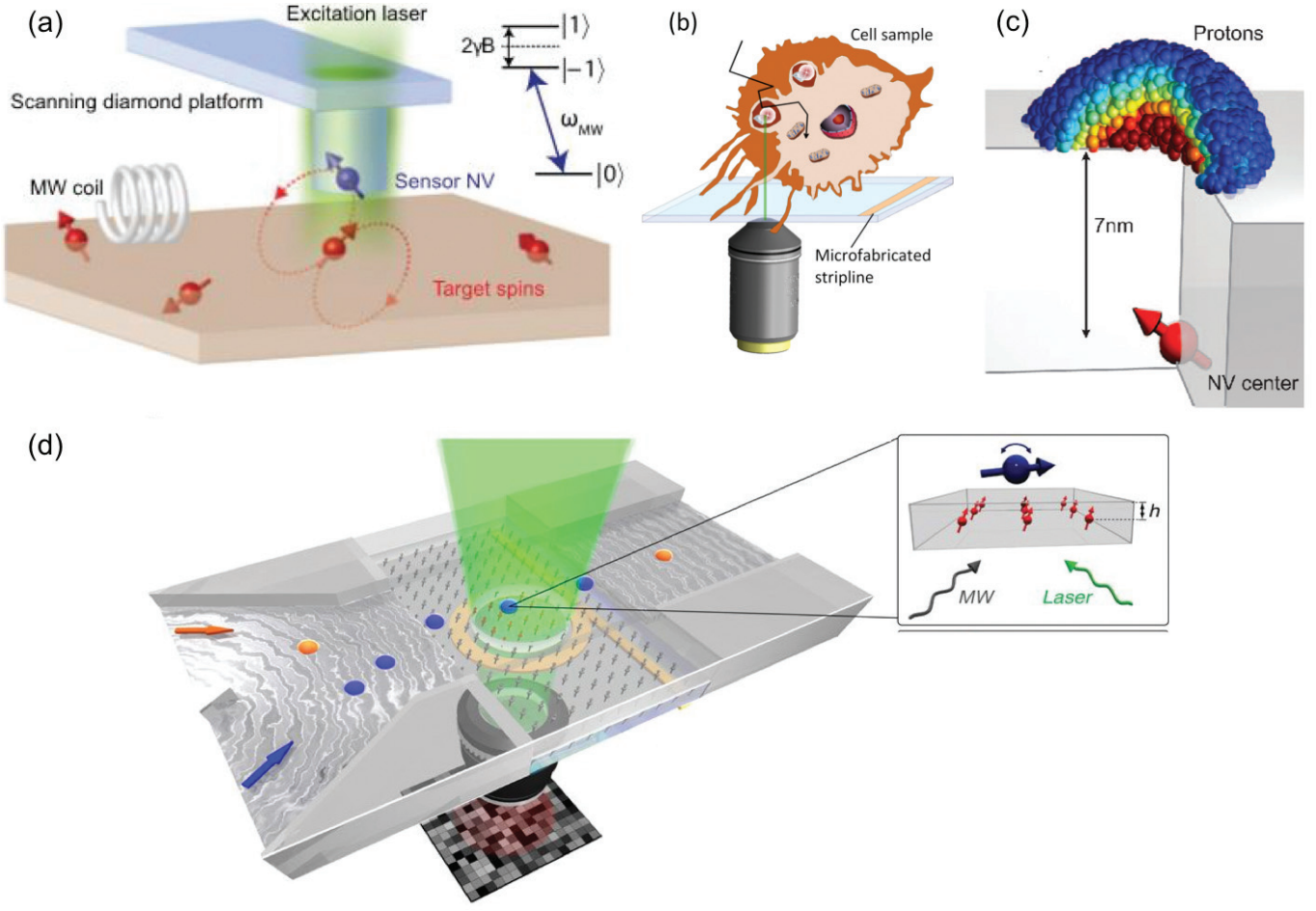

Fig. 6: Overview of different options for magnetometry: (a) Scanning magnetometer: the sample is scanned with a diamond cantilever, which contains the defect. Reprinted with permission from [38], (b) Free floating nanodiamonds are used for sensing. (for example in cells) (c) The sample is placed on the surface of a bulk diamond. Reprinted with permission from [11] (d) Microfluidic channels in diamond: similar to (c) but channels are etched into the material to contain the liquid sample. Reprinted with permission from [39].

ity and specificity. These can be combined with the measuring modes described here. In principle these are equivalent to the pulsing sequences that are in use in conventional magnetic resonance spectroscopy. There for instance ^{1}H NMR is sensitive to ^{1}H while ^{13}C NMR detects ^{13}C. These are not discussed in detail here. For further information we would like to refer to[46, 31].

BIOLOGICAL APPLICATIONS

Here I will shortly introduce a selection of biological applications, which are of most interest to the field, and discuss the progress that has already been made.

Structural biology: Probably the most demanding application that has been proposed for diamond magnetometry is to use it to determine protein structures[40]. The idea is to determine the position of hydrogen atoms via their nuclear spin. This is very attractive for several reasons. The most common technique for structural biology, x-ray crystallography, requires the protein to crystalize. However, many proteins (especially membrane proteins) do not crystalize despite enormous efforts. Alternative techniques, which circumvent crystallization, are conventional magnetic resonance spectroscopy as well as transmission electron microscopy. However, all of these techniques require at least thousands of identical proteins to reveal a protein structure. Diamond magnetometry works on a single protein. Since neither synthesis in high purity nor crystallization is required, a lot more proteins would be accessible. Apart from making structure determination a lot easier, this approach would also allow to determine heterogeneity. Such heterogeneity is lost during averaging in all the state of the art methods mentioned above. While this ambitious goal has not been achieved yet, several milestones have already been reached.

Shi et al. have demonstrated magnetic resonance measurements from single proteins already[41]. However, they did not use the hydrogen signal but instead attached a spin label to the protein, which produces a much stronger signal than protons. In their pioneering study the authors detected MAD2 (mitotic arrest deficient-2) proteins (a protein which controls parts of the cell cycle) labeled with nitroxide spin labels. The sample was embedded in a polylysine layer placed on the diamond surface. The authors were able to detect the orientation of the spin label with respect to the diamond and thus the motion of the protein. Another achievement is the detection of ferritin with diamond magnetometry[42, 43]. There the iron core of the protein was measured instead of the proton signal. Although this is much less demanding than measuring proton signals, achieving this intermediate step is still a major milestone in the field.

Monitoring ion channel activity: Besides protein structures, which might be accessible for diamond magnetometry, there is also protein activity, which can be of interest. Particularly interesting are ion channels in the membrane of cells. They are enormously important, as they are a link between cells and their environment. They control transport through the cell membrane, react to stimuli, and much more. They are also the target of numerous drugs and thus of great interest for pharmacy. For diamond magnetometry they are also interesting for several pragmatic reasons. Influx of ions creates a current, which can be detected. Additionally, they are located on the surface of cells. Thus they are accessible from the outside. This has the advantage, that one can probe them from the surface with high quality bulk diamonds or even scanning probes and does not need to enter the cells. Although this has not yet been demonstrated, Hall et al. provide promising simulations, which predict that it should be possible to detect single ion channels[44].

Measuring in cells: Another ambitious goal of the diamond magnetometry community that is strongly driven by my own research group is intracellular sensing. Here especially free radicals are of interest since they are omnipresent whenever cells are under stress[45]. This is for example the case in all kinds of diseases including cancer, cardiovascular diseases or bacterial infections. But free radicals are also generated during the natural metabolism, apoptosis (=cell death) or the aging process. Apart from being relevant, free radicals are also interesting from a practical point of view. Radicals are free electron spins and thus have about a factor of 1000 higher magnetic moments than nuclear spins. Thus this application is technically less demanding than proton detection. Another idea is to measure metabolic activity by measuring temperature changes. Finally, also the diamond movement can give information about the state of the cell. Here it is particularly interesting that diamond defects have a distinct orientation, which can be determined. Thus, one has also access to the rotation of particles.

In order to be able to measure intracellular processes there are several hurdles that have to be overcome. In order to be able to measure signals from the cells interior, diamond particles need to enter the cell. How difficult this is depends strongly on the cell type. There are several cell types which readily take up diamond particles. Among those are for instance macrophages who's biological function is to eliminate particles[37]. But also several other cell types ingest nanodiamonds spontaneously[46, 47, 48]. This usually goes via endocytosis, and it has been shown that sharp diamond particles eventually escape the endosomes[49]. However, there are also several cells which do not ingest particles. To enter these cells my team and others have come up with several approaches. One option is to coat the diamond particles with positively charged peptides[50]. This works because diamonds are usually oxygen terminated which renders their surface electronegative. The cell surface is also electronegative due to negatively charged proteins in the membrane. A positively charged coating helps to overcome the electrostatic repulsion. Polymeric[51] or silica based[52] coatings have also been utilized successfully to promote uptake, but also to prevent aggregation.

Another strategy was used by Zhang et al. who attached folic acid to their particles to trigger uptake into HeLa cells via an endogenous receptor[53]. With this approach also some targeting effect is achieved as cancer cells have an increased uptake of folic acid.

A more radical approach, which has also proven to be effective even for cells with a rigid cell wall, is to permeabilise the cell wall temporarily[54]. This can be achieved by chemical ap-

proaches or by electroporation. Despite the invasiveness, the broad use of these techniques in gene transfection indicates that there are still viable cells left after the protocol and that cells can recover from this process. An even more invasive method is to inject diamond particles into the cells with a microneedle. The main limitation of this approach is that punching a needle through the cell membrane is not only very invasive, but is also limited to fairly large cells as, for instance, oocytes (egg cells)[55].

Another concern is whether diamond particles are toxic to the cells or influence the cell biology. Both of these cases would be bad news for intracellular sensing applications. Thus toxicity of diamond particles for all kinds of cell types and organisms has been excessively studied. Summarizing, no toxicity to any cells has ever been found. There are also a number of studies which investigate more subtle, non fatal influences of diamonds on the cell biology[56 - 60]. Also here diamonds have proven to be extraordinarily biocompatible.

Finally, it is also important that the diamond sensors retain their excellent magneto-optical properties. Given that in cells usually nanodiamonds are used, the nanoscale environment plays a crucial role in the sensing process. Generally, the best diamond defects in nanodiamonds are still about 2 orders of magnitude less sensitive than their counterparts in bulk diamonds. However, improving this fact is researched extensively. Even with sub-ideal diamond particles several impressive achievements in intracellular sensing have been made already. Particularly in the field of temperature sensing, which can work with somewhat larger nanodiamonds. When measuring a temperature, the distance between the sample and the defect is less critical since diamond conducts heat excellently and the distances are in the order of nm. Kucsko et al. achieved an impressive sensitivity to temperature and could detect changes of 1.8 mK within a cell[16]. However, it has to be noted that so far only temperature changes that were induced externally by a laser and no metabolic changes were sensed. But the proof of principle results are promising, and I am optimistic that there will be first measurements of metabolic activity soon. The biggest challenge for this field will probably be to proof that the observed effect is indeed due to a metabolic activity and not temperature fluctuations in the room or heating by the laser or microwave.

Following the movement of diamonds has already been successfully used to answer biological questions. Simpson et al. for instance were able to observe embryonal developments using nanodiamonds[61]. They followed the movement of diamonds inside the developing fruit fly embryo and could conclude movements of cells due to the development.

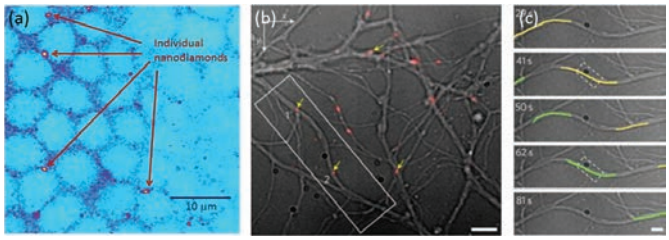

Fig. 7: Answering biological questions by tracking nanodiamond movement in cells: (a) shows nanodiamonds in a fruit fly (drosophila melanogaster) embryo. The diamonds were used to follow movements of cells during embryo development. (b) Nanodiamonds inside vesicles move up and down nerve cells. This vesicle movement, which can be divided based on its direction is further analyzed in (c) Reprinted with permission from [61, 62]

Haziza et al. analysed the movement of vesicles inside nerve cells[62]. They were able to follow the transport by having nanodiamonds inside these vesicles. The advantage of nanodiamonds for their study was that they do not bleach and thus enabled long-term tracking. They were able to detect abnormalities in the vesicle transport, which they could relate to genetic risk factors for brain-diseases.

Measuring action potentials from neurons: The human brain and its enormous complexity are deeply fascinating to many scientists. The diamond magnetometry community is not an exception. Several groups in the field aim to measure action potentials in neurons and eventually in the brain. These action potentials are charges which travel along a neuron when it is stimulated. For this application diamond magnetometry is particularly attractive since it is non-invasive and no electrical contacts are needed. Technically measuring an action potential is equivalent to measuring an electrical field (or the magnetic field caused by it). Hall et al. predicted that it should be possible to measure action potentials from single neurons with diamond magnetometry[63]. They also performed experiments with a nanowire in which they simulated action potentials.

Barry et al. finally demonstrated this on single neurons from marine worm and squid[64]. The authors were even able to measure action potentials from the outside of optically opaque marine worms for extended periods. They did not observe any adverse effects on the animals. In their ground-breaking article they achieved single neuron sensitivity. Another simulation even predicts imaging of brain slices[65]. However, we still await a practical realization.

DISCUSSION AND CONCLUSIONS

Summarizing, diamond magnetometry is an extremely powerful and potentially useful tool. However, several obstacles still have to be overcome to reveal its full potential. Since diamond defects for sensing are close to the surface their nanoscale environment plays a critical role. If for instance dangling bonds are close by the defect performance is strongly deteriorated[66]. As a result the amazing performance is only achieved by very few defects. Thus hunting for defects that are good enough can be a tedious process. This is especially critical for single defects in combination with highly demanding applications. Ensemble based sensing averages out these differences in defect quality and thus is way more reproducible. However, the overall defect quality is also worse for ensembles[67, 68]. Better control over the surface chemistry and thus the nanoscale environment would certainly benefit this field and is researched excessively[69]. Producing a “good” (biological) sample, for example a membrane with ion channels that are functioning, can be very tricky, too (especially in physics labs, which are typically not equipped for handling biological samples and keeping them alive). Thus it often takes many years until an interesting proposal or theoreti-

cal simulation is practically realized. Another issue, which limits the use of diamond magnetometry, is the reach of the sensors. This is a plus for spatial resolution since the sensor only feels the signal if the sample is within a few nanometers or a few tens of nanometers maximum. However, the requirement to be very close to the diamond defect also limits the applicability. A way around this issue has been proposed by the Budker group who placed a diamond defect at the end of a fiber needle, which can be inserted into a sample[70]. The hope is to use this device for medical applications eventually. Another hurdle, which I am expecting to be solved within the next few years, is that the equipment is so far still homebuilt. However, commercial and user-friendly instruments are already developed. While availability of diamond was still a major issue a few years ago this issue has already improved tremendously. Several suppliers provide all kinds of diamond bulk or nanodiamonds. I am expecting that also higher quality diamond material will become available commercially in the future.

REFERENCES

[1] Matsumoto, T., Kato, H., Oyama, K., Makino, T., Ogura, M., Takeuchi, D., Inokuma, T., Tokuda, N. and Yamasaki, S., Inversion channel diamond metal-oxide-semiconductor field-effect transistor with normally off characteristics. *Scientific reports*, **6**, 31585. (2016).

[2] Garrido, J.A., Härtl, A., Kuch, S., Stutzmann, M., Williams, O.A. and Jackmann, R.B., p H sensors based on hydrogenated diamond surfaces. *Applied Physics Letters*, **86**, 073504. (2005).

[3] Zhu, D., Zhang, L., Ruther, R.E. and Hamers, R.J., Photo-illuminated diamond as a solid-state source of solvated electrons in water for nitrogen reduction. *Nature materials*, **12**, p.836. (2013).

[4] Wort, C.J. and Balmer, R.S., Diamond as an electronic material. *Materials Today*, **11**, pp.22-28. (2008).

[5] Gruber, A., Dräbenstedt, A., Tietz, C., Fleury, L., Wrachtrup, J. and Von Borczyskowski, C., Scanning confocal optical microscopy and magnetic resonance on single defect centers. *Science*, **276**, pp.2012-2014. (1997).

[6] Hemelaar, S.R., de Boer, P., Chipaux, M., Zuidema, W., Hamoh, T., Martinez, F.P., Nagl, A., Hoogenboom, J.P., Giepmans, B.N.G. and Schirhagl, R., Nanodiamonds as multi-purpose labels for microscopy. *Scientific reports*, **7**, p.720. (2017).

[7] Chao, J.I., Perevedentseva, E., Chung, P.H., Liu, K.K., Cheng, C.Y., Chang, C.C. and Cheng, C.L., Nanometer-sized diamond particle as a probe for biolabeling. *Biophysical journal*, **93**, pp.2199-2208. (2007).

[8] Mohan, N., Tzeng, Y.K., Yang, L., Chen, Y.Y., Hui, Y.Y., Fang, C.Y. and Chang, H.C., Sub-20-nm Fluorescent Nanodiamonds as Photostable Biolabels and Fluorescence Resonance Energy Transfer Donors. *Advanced materials*, **22**, pp.843-847. (2010).

[9] Grinolds, M.S., Hong, S., Maletinsky, P., Luan, L., Lukin, M.D., Walsworth, R.L. and Yacoby, A., Nanoscale magnetic imaging of a single electron spin under ambient conditions. *Nature Physics*, **9**, p.215. (2013).

[10] Zhao, N., Hu, J.L., Ho, S.W., Wan, J.T. and Liu, R.B.,. Atomic-scale magnetometry of distant nuclear spin clusters via nitrogen-vacancy spin in diamond. *Nature nanotechnology*, **6**, p.242. (201).

[11] Staudacher, T., Shi, F., Pezzagna, S., Meijer, J., Du, J., Meriles, C.A., Reinhard, F. and Wrachtrup, J., Nuclear magnetic resonance spectroscopy on a (5-nanometer) 3 sample volume. *Science*, **339**, pp.561-563. (2013).

[12] Mamin, H.J., Kim, M., Sherwood, M.H., Rettner, C.T., Ohno, K., Awschalom, D.D. and Rugar, D., 2013. Nanoscale nuclear magnetic resonance with a nitrogen-vacancy spin sensor. *Science*, **339**, pp.557-560.

[13] Loretz, M., Rosskopf, T. and Degen, C.L., Radio-frequency magnetometry using a single electron spin. *Physical review letters*, **110**, p.017602. (2013).

[14] Boss, J.M., Cujia, K.S., Zopes, J. and Degen, C.L., Quantum sensing with arbitrary frequency resolution. *Science*, **356**, pp.837-840. (2017).

[15] Glenn, D.R., Bucher, D.B., Lee, J., Lukin, M.D., Park, H. and Walsworth, R.L., High-resolution magnetic resonance spectroscopy using a solid-state spin sensor. *Nature*, **555**, p.351. (2018).

[16] Kucsko, G., Maurer, P.C., Yao, N.Y., Kubo, M.I.C.H.A.E.L., Noh, H.J., Lo, P.K., Park, H. and Lukin, M.D., Nanometre-scale thermometry in a living cell. *Nature*, **500**, p.54. (2013).

[17] Dolde, F., Fedder, H., Doherty, M.W., Nöbauer, T., Rempp, F., Balasubramanian, G., Wolf, T., Reinhard, F., Hollenberg, L.C., Jelezko, F. and Wrachtrup, J., Electric-field sensing using single diamond spins. *Nature Physics*, **7**, p.459. (2011).

[18] McGuinness, L.P., Yan, Y., Stacey, A., Simpson, D.A., Hall, L.T., Maclaurin, D., Prawer, S., Mulvaney, P., Wrachtrup, J., Caruso, F. and Scholten, R.E., Quantum measurement and orientation tracking of fluorescent nanodiamonds inside living cells. *Nature nanotechnology*, **6**, p.358. (2011).

[19] Teissier, J., Barfuss, A., Appel, P., Neu, E. and Maletinsky, P., Strain coupling of a nitrogen-vacancy center spin to a diamond mechanical oscillator. *Physical review letters*, **113**, p.020503. (2014).

[20] Van Oort, E. and Glasbeek, M., Electric-field-induced modulation of spin echoes of NV centers in diamond. *Chemical Physics Letters*, **168**, pp.529-532. (1990).

[21] Acosta, V.M., Bauch, E., Ledbetter, M.P., Waxman, A., Bouchard, L.S. and Budker, D., Temperature dependence of the nitrogen-vacancy magnetic resonance in diamond. *Physical review letters*, **104**, p.070801. 2010.

[22] Chipaux, M., Toraille, L., Larat, C., Morvan, L., Pezzagna, S., Meijer, J. and Debuisschert, T., Wide bandwidth instantaneous radio frequency spectrum analyzer based on nitrogen vacancy centers in diamond. *Applied Physics Letters*, **107**, p.233502. (2015).

[23] Maze, J.R., Gali, A., Togan, E., Chu, Y., Trifonov, A., Kaxiras, E. and Lukin, M.D., Properties of nitrogen-vacancy centers in diamond: the group theoretic approach. *New Journal of Physics*, **13**, p.025025. (2011).

[24] Rendler, T., Neburkova, J., Zemek, O., Kotek, J., Zappe, A., Chu, Z., Cigler, P. and Wrachtrup, J., Optical imaging of localized chemical events using programmable diamond quantum nanosensors. *Nature communications*, **8**, p.14701. (2017).

[25] Balasubramanian, G., Neumann, P., Twitchen, D., Markham, M., Kolesov, R., Mizuochi, N., Isoya, J., Achard, J., Beck, J., Tissler, J. and Jacques, V., Ultralong spin coherence time in isotopically engineered diamond. *Nature materials*, **8**, p.383. (2009).

[26] Ishiwata, H., Nakajima, M., Tahara, K., Ozawa, H., Iwasaki, T. and Hatano, M., Perfectly aligned shallow ensemble nitrogen-vacancy centers in (111) diamond. *Applied Physics Letters*, **111**, p.043103. (2017).

[27] Lesik, M., Plays, T., Tallaire, A., Achard, J., Brinza, O., William, L., Chipaux, M., Toraille, L., Debuisschert, T., Gicquel, A. and Roch, J.F., Preferential orientation of NV defects in CVD diamond films grown on (113)-oriented substrates. *Diamond and Related Materials*, **56**, pp.47-53. (2015).

[28] Ong, S.Y., Chipaux, M., Nagl, A. and Schirhagl, R., Shape and crystallographic orientation of nanodiamonds for quantum sensing. *Physical Chemistry Chemical Physics*, **19**, pp.10748-10752. (2017).

[29] Boudou, J.P., Curmi, P.A., Jelezko, F., Wrachtrup, J., Aubert, P., Sennour, M., Balasubramanian, G., Reuter, R., Thorel, A. and Gaffet, E., High yield fabrication of fluorescent nanodiamonds. *Nanotechnology*, **20**, p.235602. 2009.

[30] Trusheim, M.E., Li, L., Laraoui, A., Chen, E.H., Bakhru, H., Schröder, T., Gaathon, O., Meriles, C.A. and Englund, D., Scalable fabrication of high purity diamond nanocrystals with long-spin-coherence nitrogen vacancy centers. *Nano letters*, **14**, pp.32-36. (2013).

[31] Schirhagl, R., Chang, K., Loretz, M. and Degen, C.L., Nitrogen-vacancy centers in diamond: nanoscale sensors for physics and biology. *Annual review of physical chemistry*, **65**, pp.83-105. (2014).

[32] Ofori-Okai, B.K., Pezzagna, S., Chang, K., Loretz, M., Schirhagl, R., Tao, Y., Moores, B.A., Groot-Berning, K., Meijer, J. and Degen, C.L., Spin properties of very shallow nitrogen vacancy defects in diamond. *Physical Review B*, **86**, p.081406. (2012).

[33] Müller, T., Hepp, C., Pingault, B., Neu, E., Gsell, S., Schreck, M., Sternschulte, H., Steinmüller-Nethl, D., Becher, C. and Atatüre, M., Optical signatures of silicon-vacancy spins in diamond. *Nature communications*, **5**, p.3328. (2014.)

[34] Hepp, C., Müller, T., Waselowski, V., Becker, J.N., Pingault, B., Sternschulte, H., Steinmüller-Nethl, D., Gali, A., Maze, J.R., Atatüre, M. and Becher, C., Electronic structure of the silicon vacancy color center in diamond. *Physical Review Letters*, **112**, p.036405. (2014).

[35] Iwasaki, T., Ishibashi, F., Miyamoto, Y., Doi, Y., Kobayashi, S., Miyazaki, T., Tahara, K., Jahnke, K.D., Rogers, L.J., Naydenov, B. and Jelezko, F., Germanium-vacancy single color centers in diamond. *Scientific reports*, **5**, p.12882. (2015).

[36] Iwasaki, T., Miyamoto, Y., Taniguchi, T., Siyushev, P., Metsch, M.H., Jelezko, F. and Hatano, M., Tin-vacancy quantum emitters in diamond. *Physical review letters*, **119**, p.253601. (2017).

[37] Hemelaar, S.R., de Boer, P., Chipaux, M., Zuidema, W., Hamoh, T., Martinez, F.P., Nagl, A., Hoogenboom, J.P., Giepmans, B.N.G. and Schirhagl, R., Nanodiamonds as multi-purpose labels for microscopy. *Scientific reports*, **7**, p.720. 2017.

[38] Grinolds, M.S., Hong, S., Maletinsky, P., Luan, L., Lukin, M.D., Walsworth, R.L. and Yacoby, A., Nanoscale magnetic imaging of a single electron spin under ambient conditions. *Nature Physics*, **9**, p.215. (2013).

[39] Steinert, S., Ziem, F., Hall, L.T., Zappe, A., Schweikert, M., Götz, N., Aird, A., Balasubramanian, G., Hollenberg, L. and Wrachtrup, J., Magnetic spin imaging under ambient conditions with sub-cellular resolution. *Nature communications*, **4**, p.1607. (2013).

[40] Kost, M., Cai, J. and Plenio, M.B., Resolving single molecule structures with nitrogen-vacancy centers in diamond. *Scientific reports*, **5**, p.11007. (2015).

[41] Shi, F., Zhang, Q., Wang, P., Sun, H., Wang, J., Rong, X., Chen, M., Ju, C., Reinhard, F., Chen, H. and Wrachtrup, J., Single-protein spin resonance spectroscopy under ambient conditions. *Science*, **347**, pp.1135-1138. 2015.

[42] Ermakova, A., Pramanik, G., Cai, J.M., Algara-Siller, G., Kaiser, U., Weil, T., Tzeng, Y.K., Chang, H.C., McGuinness, L.P., Plenio, M.B. and Naydenov, B., Detection of a few metallo-protein molecules using color centers in nanodiamonds. *Nano letters*, **13**, pp.3305-3309. 2013.

[43] Schäfer-Nolte, E., Schlipf, L., Ternes, M., Reinhard, F., Kern, K. and Wrachtrup, J., Tracking temperature-dependent relaxation times of ferritin nanomagnets with a wideband quantum spectrometer. *Physical review letters*, **113**, p.217204. 2014.

[44] Hall, L.T., Hill, C.D., Cole, J.H., Städler, B., Caruso, F., Mulvaney, P., Wrachtrup, J. and Hollenberg, L.C., Monitoring ion-channel function in real time through quantum decoherence. *Proceedings of the National Academy of Sciences*, **107**, pp.18777-18782. (2010).

[45] Sigaeva, A., Hamoh, T., Perona, F. and Schirhagl, R., Fluorescent nanodiamonds: potential free radical detectors in live cells. *Free Radical Biology and Medicine*, **120**, p.S87. (2018).

[46] Chipaux, M., van der Laan, K.J., Hemelaar, S.R., Hasani, M., Zheng, T. and Schirhagl, R., Nanodiamonds and Their Applications in Cells. *Small*, p.1704263. (2018).

[47] Perevedentseva, E., Hong, S.F., Huang, K.J., Chiang, I.T., Lee, C.Y., Tseng, Y.T. and Cheng, C.L., Nanodiamond internalization in cells and the cell uptake mechanism. *Journal of nanoparticle research*, **15**, p.1834. 2013.

[48] Faklaris, O., Joshi, V., Irinopoulou, T., Tauc, P., Sennour, M., Girard, H., Gesset, C., Arnault, J.C., Thorel, A., Boudou, J.P. and Curmi, P.A., Photoluminescent diamond nanoparticles for cell labeling: study of the uptake mechanism in mammalian cells. *ACS nano*, **3**, pp.3955-3962. 2009.

[49] Chu, Z., Zhang, S., Zhang, B., Zhang, C., Fang, C.Y., Rehor, I., Cigler, P., Chang, H.C., Lin, G., Liu, R. and Li, Q., Unambiguous observation of shape effects on cellular fate of nanoparticles. *Scientific reports*, **4**, p.4495. (2014).

[50] Zheng, T., Perona Martínez, F., Storm, I.M., Rombouts, W., Sprakel, J., Schirhagl, R. and De Vries, R., Recombinant Protein Polymers for Colloidal Stabilization and Improvement of Cellular Uptake of Diamond Nanosensors. *Analytical chemistry*, **89**, pp.12812-12820. (2017).

[51] Rehor, I., Mackova, H., Filippov, S.K., Kucka, J., Proks, V., Slegerova, J., Turner, S., Van Tendeloo, G., Ledvina, M., Hruby, M. and Cigler, P., Fluorescent nanodiamonds with bioorthogonally reactive protein-resistant polymeric coatings. *ChemPlusChem*, **79**, pp.21-24. (2014).

[52] Rehor, I., Slegerova, J., Kucka, J., Proks, V., Petrakova, V., Adam, M.P., Treussart, F., Turner, S., Bals, S., Sacha, P. and Ledvina, M., Fluorescent nanodiamonds embedded in biocompatible translucent shells. *Small*, **10**, pp.1106-1115. (2014).

[53] Zhang, B., Li, Y., Fang, C.Y., Chang, C.C., Chen, C.S., Chen, Y.Y. and Chang, H.C., Receptor-mediated cellular uptake of folate-conjugated fluorescent nanodiamonds: A combined ensemble and single-particle study. *Small*, **5**, pp.2716-2721. (2009).

[54] Hemelaar, S.R., Laan, K.J., Hinterding, S.R., Koot, M.V., Ellermann, E., Perona-Martinez, F.P., Roig, D., Hommelet, S., Novarina, D., Takahashi, H. and Chang, M., Generally Applicable Transformation Protocols for Fluorescent Nanodiamond Internalization into Cells. *Scientific Reports*, **7**, p.5862. 2017.

[55] Lin, Y.C., Wu, K.T., Lin, Z.R., Perevedentseva, E., Karmenyan, A., Lin, M.D. and Cheng, C.L., Nanodiamond for biolabelling and toxicity evaluation in the zebrafish embryo in vivo. *Journal of biophotonics*, **9**, pp.827-836. (2016).

[56] Thomas, V., Halloran, B.A., Ambalavanan, N., Catledge, S.A. and Vohra, Y.K., In vitro studies on the effect of particle size on macrophage responses to nanodiamond wear debris. *Acta biomaterialia*, **8**, pp.1939-1947. 2012.

[57] Zakrzewska, K.E., Samluk, A., Wierzbicki, M., Jaworski, S., Kutwin, M., Sawosz, E., Chwalibog, A., Pijanowska, D.G. and Pluta, K.D., Analysis of the cytotoxicity of carbon-based nanoparticles, diamond and graphite, in human glioblastoma and hepatoma cell lines. *PloS one*, **10**, p.e0122579. (2015).

[58] Hemelaar, S.R., Saspaanithy, B., L'Hommelet, S.R., Perona Martinez, F.P., van der Laan, K.J. and Schirhagl, R., The Response of HeLa Cells to Fluorescent NanoDiamond Uptake. *Sensors*, **18**, p.355. (2018).

[59] Li, J., Zhu, Y., Li, W., Zhang, X., Peng, Y. and Huang, Q., Nanodiamonds as intracellular transporters of chemotherapeutic drug. *Biomaterials*, **31**, pp.8410-8418. (2010).

[60] Schrand, A.M., Huang, H., Carlson, C., Schlager, J.J., Ōsawa, E., Hussain, S.M. and Dai, L., Are diamond nanoparticles cytotoxic?. *The journal of physical chemistry B*, **111**, pp.2-7. 2007.

[61] Simpson, D.A., Thompson, A.J., Kowarsky, M., Zeeshan, N.F., Barson, M.S., Hall, L.T., Yan, Y., Kaufmann, S., Johnson, B.C., Ohshima, T. and Caruso, F., In vivo imaging and tracking of individual nanodiamonds in drosophila melanogaster embryos. *Biomedical optics express*, **5**, pp.1250-1261. (2014).

[62] Haziza, S., Mohan, N., Loe-Mie, Y., Lepagnol-Bestel, A.M., Massou, S., Adam, M.P., Le, X.L., Viard, J., Plancon, C., Daudin, R. and Koebel, P., Fluorescent nanodiamond tracking reveals intraneuronal transport abnormalities induced by brain-disease-related genetic risk factors. *Nature nanotechnology*, **12**, p.322. (2017).

[63] Hall, L.T., Beart, G.C.G., Thomas, E.A., Simpson, D.A., McGuinness, L.P., Cole, J.H., Manton, J.H., Scholten, R.E., Jelezko, F., Wrachtrup, J. and Petrou, S., High spatial and temporal resolution wide-field imaging of neuron activity using quantum NV-diamond. *Scientific reports*, **2**, p.401. (2012).

[64] Barry, J.F., Turner, M.J., Schloss, J.M., Glenn, D.R., Song, Y., Lukin, M.D., Park, H. and Walsworth, R.L., Optical magnetic detection of single-neuron action potentials using quantum defects in diamond. *Proceedings of the National Academy of Sciences*, **113**, pp.14133-14138. (2016).

[65] Karadas, M., Wojciechowski, A.M., Huck, A., Dalby, N.O., Andersen, U.L. and Thielscher, A., Feasibility and resolution limits of opto-magnetic imaging of neural network activity in brain slices using color centers in diamond. *Scientific reports*, **8**, p.4503. (2018).

[66] Rosskopf, T., Dussaux, A., Ohashi, K., Loretz, M., Schirhagl, R., Watanabe, H., Shikata, S., Itoh, K.M. and Degen, C.L., Investigation of surface magnetic noise by shallow spins in diamond. *Physical review letters*, **112**, p.147602. (2014).

[67] Tetienne, J.P., de Gille, R.W., Broadway, D.A., Teraji, T., Lillie, S.E., McCoey, J.M., Dontschuk, N., Hall, L.T., Stacey, A., Simpson, D.A. and Hollenberg, L.C.L., Spin properties of dense near-surface ensembles of nitrogen-vacancy centers in diamond. *Physical Review B*, **97**, p.085402. (2018).

[68] Stanwix, P.L., Pham, L.M., Maze, J.R., Le Sage, D., Yeung, T.K., Cappellaro, P., Hemmer, P.R., Yacoby, A., Lukin, M.D. and Walsworth, R.L., Coherence of nitrogen-vacancy electronic spin ensembles in diamond. *Physical Review B*, **82**, p.201201. (2010).

[69] Nagl, A., Hemelaar, S.R. and Schirhagl, R., Improving surface and defect center chemistry of fluorescent nanodiamonds for imaging purposes – a review. *Analytical and bioanalytical chemistry*, **407**, pp.7521-7536. (2015).

[70] Personal communication with Arne Wickenbrock