Nanoscale nuclear magnetic resonance with chemical resolution

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Nuclear magnetic resonance (NMR) spectroscopy is a key analytical technique in chemistry, biology, and medicine. However, conventional NMR spectroscopy requires an at least nanoliter-sized sample volume to achieve sufficient signal. We combined the use of a quantum memory and high magnetic fields with a dedicated quantum sensor based on nitrogen vacancy centers in diamond to achieve chemical shift resolution in $^{1}$H and $^{19}$F NMR spectroscopy of 20-zeptoliter sample volumes. We demonstrate the application of NMR pulse sequences to achieve homonuclear decoupling and spin diffusion measurements. The best measured NMR linewidth of a liquid sample was ~1 part per million, mainly limited by molecular diffusion. To mitigate the influence of diffusion, we performed high-resolution solid-state NMR by applying homonuclear decoupling and achieved a 20-fold narrowing of the NMR linewidth.

Nuclear magnetic resonance (NMR) spectroscopy is among the most widely used analytical techniques, with applications in materials science, biology, chemistry, and medicine (1–3). Its versatility stems from minimal invasiveness and chemical specificity. In addition, NMR underlies magnetic resonance imaging (MRI), one of the most important diagnostic tools in medicine (4–6). A drawback of NMR-related techniques is their relatively low sensitivity, which requires nanoliter-sized sample volumes (7). Given the increased importance of nanoscale analytics across all areas of science, and in particular in biology and medicine, increasing NMR sensitivity is highly desirable.

Various approaches to increase sensitivity include magnetic resonance force microscopy (8), superconducting quantum interference device (SQUID)-based MRI (9), optical detection of NMR (10, 11), and external high-quality-factor resonators for low field detection (12). Recently, the nitrogen vacancy (NV) center in diamond has demonstrated its outstanding capability as a NMR sensor (13–15). NMR detection of 0.1-zi samples with the NV center as well as imaging of nuclear spins has been demonstrated (16–18). Compared with conventional NMR, the number of nuclear spins required to generate a detectable signal is reduced by 12 orders of magnitude.

Full functionality of NMR requires high spectral resolution. The resonance frequency of every nuclear spin is determined by its neighboring nuclear spins (J-coupling) and the surrounding electron distribution. Specifically, the electron density at the nuclear spin location leads to modification of the experienced magnetic field. This so-called chemical shift is conventionally defined as a relative shift to the Larmor frequency of a reference molecule in parts per million (ppm): $\delta = (v_{\text{amp}} - v_{\text{ref}})/v_{\text{ref}}$. For example, a relative shift of $\delta = 0.8$ ppm in $^{1}$H NMR corresponds to an absolute frequency shift of $\delta v = 1$ Hz at 30 mT and $\delta v = 100$ Hz at 3 T. Consequently, NMR spectroscopy is preferably, but not exclusively, carried out at elevated fields in order to resolve such shifts (12), with resolution limited by the decoherence or dephasing times $T_{2}, T_{2}'$ of the sample spins. The spectral resolution of our nanoscale NMR method is challenged by additional factors. First, the spin relaxation time of the sensor electron spin $T_{\text{sensor}}$ limits the achievable phase acquisition time $T$ and hence spectral resolution (19). Second, the nanoscopic sample volume itself imposes further constraints. For liquid samples, molecular diffusion through the (5 nm)$^{3}$ large sample volume on time scales $T_{2}$ of a few tens of microseconds, as in (14), restricts the interaction time of the sample molecule with the sensor and hence limits the relative spectral resolution to

$$\Delta \delta = \frac{2}{T} \frac{1}{T_{2} + T_{B} + T_{\text{sensor}}}$$

Here, $\gamma$ is the corresponding nuclear spin gyromagnetic ratio, and $T$ limits the phase acquisition time. In previous NV nanoscale NMR studies, linewidths of 12,000 ppm have been achieved (19, 20), mainly limited by low magnetic fields, short $T_{\text{sensor}}$, and short $T_{B}$. By comparison, the chemical shift range of proton spins in organic molecules is ~13 ppm. Hence, distinguishing such protons and identifying and characterizing the related molecules typically requires a spectral resolution of 1 ppm (Fig. 1A), a factor 10,000 smaller than previously achieved. For NMR spectroscopy of molecules, it is therefore essential to increase the relative frequency resolution. We tackled this problem first by increasing the magnetic field to 3 T (21). At 3 T, the required phase acquisition time $T$ for resolving chemical shifts in a $^{1}$H NMR spectrum is reduced to a few milliseconds (Fig. 1A). The high magnetic field in our experiments is not required for thermal spin polarization because detection relies on statistical spin polarization (8, 13, 14). Second, the intrinsic nitrogen nuclear spin was included as a quantum memory (22, 23). At 3 T, the spin relaxation time of the memory spin ($T_{\text{mem}}$) lengths to ~260 s. With the use of this quantum memory, $T_{\text{sensor}}$ is replaced by the much longer $T_{\text{mem}}$ in Eq. 1, yielding a potential maximal spectral resolution of ~1 mHz or 10$^{-5}$ ppm at 3 T (Fig. 1A), which is comparable with conventional NMR. As a result, the achievable resolution is limited only by diffusion ($T_{2}$ of liquid samples) or by dipolar broadening, such as in the case of solid-state samples ($T_{2}$.)

We used the NV center in diamond as a nanoscale NMR probe, optically detected with a confocal microscope. A high magnetic field along the NV center axis was provided by a 3-T superconducting vector magnet with a room-temperature bore. We conducted electron spin manipulation with a microwave (MW) antenna capable of operating at up to 90 GHz (Fig. 1B) (2D). We manipulated the nitrogen nuclear spin of the NV center acting as the memory spin via radio frequencies (RFs) applied by a copper wire that also excited the sample spins. The sensor-sample distance governs the strength of the detectable NMR signal and also defines the detection volume. Long sensor coherence times allow for large sensor-sample distances in the first place. To this end, we used an isotopically purified, high-quality type IIa diamond substrate to eliminate the deleterious effects of $^{13}$C-spins and paramagnetic defects (24).

Measuring NMR spectra with the nanoscale NMR probe was separated into three experimental steps (Fig. 1C): initialization, application of the NMR pulse sequence, and readout. During initialization, a snapshot of the sample spin statistical polarization was acquired for time $t/2$ by the electron spin sensor and stored on the nuclear spin memory via an entanglement-based algorithm (22, 23). During the phase acquisition time, different NMR pulse schemes can be applied to the sample spins (one-dimensional (1D), 2D, or higher-dimensional NMR sequences). In a concluding sequence of duration $t/2$, the effect of the sample spin NMR was detected and finally read out (Fig. 1C).

Different nuclear sample spin species (such as nitrogen, carbon, protons, and fluorine) were selectively addressed via resonant RF excitation.
Fig. 1. Nanoscale NMR sensor and its functionality. (A) Phase acquisition time $T$ required to resolve chemical shift and J-couplings for $^1$H NMR according to Eq. 1 at an applied magnetic field $B$ of 3 T (with $\gamma = \gamma_H = 2\pi \cdot 42.576$ MHz/T). The bottom light-blue arrow marks the phase acquisition time required to resolve different proton chemical shifts ($\Delta \delta < 13$ ppm). The top two arrows indicate the required phase acquisition time for resolving proton-proton and proton-carbon J-couplings ($J_{HH}$ and $J_{HP}$). The red line marks the maximum phase acquisition time (spectral resolution limit) imposed by the spin-lattice relaxation time of the memory spin. The gray vertical lines mark the resolution achieved in the present work. (B) Schematic representation of NV diamond–based nanoscale NMR probe setup. The probe and sample are located in the room-temperature (RT) bore of a 3-T superconducting vector magnet. The probe is optically excited, and its fluorescence response is detected via a confocal microscope. MW waveguides and RF wires provide the oscillating magnetic fields for spin manipulation. (Right) Zoomed-in view illustrating the nanoscale probe-sample arrangement. The nanoscale NMR probe consists of a NV electron spin quantum sensor in diamond with an intrinsic $^{15}$N nuclear spin quantum memory. It detects sample spins within the indicated detection volume. The probes used in this study had a distance to the sample ranging from 34 to 95 nm. (C) The nanoscale NMR probe detection scheme relies on in situ correlation of sample magnetization from the initialization and readout stages. During the phase acquisition time, arbitrary NMR pulse sequences alter sample spin magnetization (for example, Ramsey spectroscopy, magnetization decay measurement, homonuclear decoupling, or higher-dimensional spectroscopy). While the sensor detects, the memory preserves sample magnetization information for later correlation via efficient quantum algorithms. Hence, the memory lifetime determines spectral resolution [see (A)]. Quantum nondemolition measurement of the memory state yields measurement data bit by bit with high fidelity (details are provided in the supplementary materials).

For liquid-state samples, the time $T_2$ for diffusion through our nanoscale detection volume limits the achievable spectral resolution (19, 20, 25). In essence, diffusion changes the distribution of spatially inhomogeneous statistical polarization and thus diminishes the correlation between the sensor signal of the “init” and “read” parts (Fig. 1C). To mitigate this effect, one could change the viscosity of the host medium for the molecule under study. However, for high viscosity, NMR spectra broaden because of a lack of molecular mobility (19). As a result, a further parameter to optimize is the detection volume. With growing sample-sensor distance $d$, the spin signal per volume decreases, and the sensor acquires substantial spin signals from larger volumes (Fig. 2B). The diffusion time is thus prolonged as $T_2 \propto d^2$, where $D$ relates to the diffusion coefficient of the sample spins (supplementary materials) (25). To achieve a spectral resolution of 1 ppm for the $D$ of the substances we used (Fig. 3F), we require an effective sample volume of (20 nm)$^3$ corresponding to an NV depth of $d \approx 25$ nm (Fig. 2B). To reach sufficient signal strength $S$, we need to increase the total sensing time $t$ (Fig. 1C, “init” and “read”) according to

$$S = S_0 \left(1 - e^{-\frac{2\pi T_2^\text{eff}}{T}}\right) e^{-t/T_2^\text{max}}$$

where $\gamma$ is the gyromagnetic ratio of the sensor spin, $B_2^\text{eff}$ is the variance of the sample spin magnetic field at the sensor (the effect of statistical polarization), and $T_2^\text{max}$ is the coherence time of the sensor electron spin. $B_2^\text{eff}$, on the other hand, is proportional to $1/d^3$. The measured proton and fluorine signal strengths ($^1$H and $^{19}$F, respectively) are shown in Fig. 2C for different depths up to ~100 nm and varying total sensing time $t$ up to ~0.6 ms, corrected for the coherence decay of the sensor. The sample spin density $\rho$ and the sensor’s coherence time $T_2^\text{max}$ limit the practical sensor distance (supplementary materials). For the present case, because of the long $T_2^\text{max}$ (~200 ms), we were able to detect even diluted samples of proton and fluorine spins in principle up to a distance of 100 nm (supplementary materials).

For the present experiments, we chose highly viscous fluids: polybutadiene ([CH$_2$CH = CHCH$_3$]$_n$) with about $n = 90$ subunits per polymer strand in the case of $^1$H NMR, and perfluoropolyether (PFPE; CF$_2$O(-CF(CF$_3$)CF$_2$O)$_x$(-CF$_2$O)-) with about $x = 140$ and $y = 13$ subunits per polymer strand in the case of $^{19}$F NMR. The polymer strands are on the same length scale as our detection volume. Ramsey experiments for these two fluids are shown in Fig. 3, A and D. The data reveal multiple frequency oscillations (Fig. 3, A and D). In the case of PFPE, the fast Fourier transformed (FFT) data in Fig. 3B exhibit two peaks arising from fluorine spins with different chemical shifts, which match the reference NMR spectrum obtained with a conventional 400-MHz NMR spectrometer. The linewidth of the major peak is 1.3 ppm (Fig. 3C) for the NV nanoscale NMR. By comparison, conventional NMR yields a 10-fold narrower line. Furthermore, the FFT of the polybutadiene $^1$H NMR measurement in Fig. 3E shows two peaks, as expected (26). The proton chemical shift of the CH$_2$ groups (Fig. 3E, inset, chemical structure) is dominated by the sp$^3$ hybridized neighboring carbon atom and thus shows a chemical shift similar to alkanes (~1 to 2 ppm), whereas the chemical shift of protons in the CH group is dominated by the sp$^2$ hybridized neighboring...
Fig. 2. NV nanoscale NMR of multiple nuclear spin species. (A) Normalized signals from \(^{13}\)C (in diamond), \(^{1}H\) [in polyvinylidene fluoride (PVDF)], and \(^{19}\)F spins (in PVDF) spectrally separated via their differing gyromagnetic ratios. For \(^{13}\)C spins, the normalized signal can become larger than 1 because of coherent interaction with only a few close spins as opposed to an incoherent interaction with a \(^{1}H\) or \(^{19}\)F spin bath. On top of the three spectra, the measurement scheme is depicted (compare Fig. 1C and supplementary materials). (B) The circle and dark gray line show the dependence of measured and extrapolated diffusion-related NMR linewidth on sensor-sample distance as \(d^{-2}\) for polybutadiene (\(^{1}H\)) on diamond (\(2/\gamma B T D\) with \(B = 3\) T) (Eq. 1). Pale blue line indicates the deep-scaling of the detection volume (=\(0.49d^3\)), which contains half of the detected sample spins that contribute to the signal \(B_{\text{rms}}\). (C) Depth determination of nanoscale NMR probe. NMR contrast increases with sensing time \(t\) and depends on the sample spin density \(p\) and the distance \(d\) between the sample spins and the NV sensor (details available in the supplementary materials). Different samples are used for this purpose: polybutadiene oil (\(^{1}H\), dark red), cyanoacrylate solid glue (\(^{1}H\), light red), and PFPE oil (\(^{19}\)F, light and dark green). Vertical dashed lines indicate the required sensing time to reach 50% signal strength (horizontal dashed line) for each NV-sample combination.

Fig. 3. Chemical shift resolved liquid-state NMR for \(^{1}H\) and \(^{19}\)F. (A) Ramsey measurement of \(^{19}\)F spins in PFPE (fomblin). We fit two exponentially decaying cosine functions with 2.1 and 9.8 kHz to the data (details available in the supplementary materials). The insets in (A), (D), and (F) show the measurement scheme (compare Fig. 1C and supplementary materials). (B) FFT power spectra of data (green line) and fit (bold light green line) from (A) together with the results of a conventional NMR spectrum (dark green line, 400-MHz NMR spectrometer) of our sample. Chemical structure of PFPE is shown. Pale green and blue areas under the curve mark signals of individual \(^{19}\)F atoms with different chemical shift. The corresponding positions within the PFPE molecule are highlighted by identical colors. For better visibility, conventional NMR and nano-NMR signals are enhanced by factors of 5 and 10 for the chemical shift range 130 to 160 ppm. (C) Enlarged view of the NMR resonance peak from (B) around 80 ppm. Here, the phase acquisition time \(\tau\) is increased to 5 ms, yielding a full width at half maximum (FWHM) of the NMR peak of 1.3 ppm (decay time 2.5 ms). Conventional NMR yields a FWHM that is an order of magnitude smaller. (D) Ramsey measurement of \(^{1}H\) spins in liquid polybutadiene. Measured data are fitted with a superposition of two cosine functions with 1.7 and 2.2 kHz (details available in the supplementary materials). (E) FFT power spectra of \(^{1}H\) spins in liquid polybutadiene. Chemical structure of polybutadiene reveals two subgroups: CH and CH\(_2\). The corresponding NMR peaks are detected with a FWHM of 1.3 and 1.4 ppm, respectively. Examples of conventional NMR spectra can be found in (26). The FFT of data and fit from (A) and (D) displayed in (B), (C), and (E) were all obtained by zero-filling and therefore represent interpolated FFT spectra. (F) Magnetization decay of \(^{1}H\) spins in liquid polybutadiene. An exponential decay fit function yields a decay time of \(T_D = 5.4 \pm 0.9\) ms owing to diffusion of sample molecules out of the detection volume.

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less than $\sqrt{2}$ in our experiments and will start scaling as $\propto \delta v^{-1/2}$ for $\tau \gg 8$ ms or $\delta v \ll 100$ Hz.

To enhance sensitivity, $T_{\text{read}}$ can be shortened by increasing the collection efficiency of NV fluorescence photons (29, 30). On the other hand, application of compressed sensing would reduce the overall measurement repetitions needed to reach a certain signal-to-noise ratio (31). Further enhancement can be achieved by incorporating more memory spins (proximal host $^{13}$C spins), which would allow the use of quantum algorithms such as quantum Fourier transform or quantum error correction, resulting in measurement speed-up and more robustness (32).

The discrepancy in spectral resolution of the present nanoscale NMR measurement and the measured bulk NMR linewidth can be overcome, for example, in liquid-state NMR by immobilizing sample molecules in nanocapsules such as liposomes, micelles, or polymer shells (33). Although such immobilization techniques might have an impact on molecular structures, they will prevent translational diffusion out of the detection volume and still allow averaging out of dipolar interactions through motional narrowing. However, for solid-state NMR the measured linewidth was limited by the finite pulse length in the homonuclear decoupling sequence. An optimized RF microstructure and higher RF power equipment would thus further improve resolution, enabling chemical structure analysis experiments. Additionally, in NV nanoscale NMR there is no need for a reference molecule because the host $^{13}$C spins can be used to determine the magnetic field very precisely (<0.03 ppm) (29).

This work paves the way for numerous applications of nanoscale NMR. For instance, imaging of sample spins' chemical shift $T_2^*$ and $T_1$ in cells with nanometer spatial resolution would open a promising contrast mechanism for optical microscopy.

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
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References (34–48)
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NMR on diamonds gets down to chemistry

Nuclear magnetic resonance (NMR) spectroscopy is immensely useful for chemical characterization, but it requires relatively large amounts of sample. Recent studies have leveraged nitrogen vacancy centers in diamond to detect NMR signals from samples of just a few cubic nanometers, but with low resolution. Aslam et al. optimized this technique to achieve a resolution of 1 part per million—sufficient to distinguish among alkyl, vinyl, and aryl protons in solution (see the Perspective by Bar-Gill and Retzker). They also demonstrated solid-state implementation and fluorine detection.

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