Supplementary Information

Magnetic spin imaging under ambient conditions with sub-cellular resolution

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Supplementary Figure S1. T1 relaxation of various environments. T1 relaxation is induced if the magnetic fluctuations of the environment match the NV transition, i.e. spectral density $S(\omega)$ with components $\sim$3 GHz for the low $B_0$ fields applied in this study. The diamond intrinsic NV relaxation $\Gamma_{1,\text{int}}$ was measured in an empty flow cell filled with air only and is very low due to the low phonon density. The presence of deionized water (dH$_2$O), varying pH or N$_2$-degased (aeration with pure N$_2$ for 5 min) caused only minor changes in $\Gamma_1$, while O$_2$-gased dH$_2$O (aeration with pure O$_2$ for 5 min, O$_2$ concentration $\sim$2mM), 1M MnCl$_2$ and 1M Gd$^{3+}$ induces significant $\Gamma_1$ relaxation. The T$_1$ effect of O$_2$ was in the same order of magnitude as $\sim$1.5 mM Gd$^{3+}$. The oxygen induced relaxation could be abrogated reversibly by aeration with N$_2$. Error bars, 1$\sigma$ statistical uncertainty.
**Supplementary Figure S2. Concentration dependent fluctuations of Gd^{3+}**. The combined spin dynamics subsumed by $f_{\text{Gd}}$ determine the width of the spectral density $S_{\text{Gd}}(\omega)$, cf. Supplementary Note 1. Shown here are the contributions to Gd$^{3+}$ fluctuations due to magnetic dipole-dipole interaction between Gd$^{3+}$ spins ($f_{\text{dipole}}$, red dotted line), interaction of the Gd$^{3+}$ ion with the ligands ($f_{\text{vib}}$, green dotted line), spatial diffusion of Gd$^{3+}$ ions ($f_{\text{trans}}$, yellow dotted line) and rotational diffusion of Gd$^{3+}$ ions ($f_{\text{rot}}$, blue dotted line), as well as their sum ($f_{\text{Gd}}$, black solid line).
Supplementary Figure S3. Sensitivities of relaxometric widefield detection. The minimum resolvable Gd\(^{3+}\) level for a single-\(\tau\) measurement (\(\tau_m=20\) s) was determined for \(T_2\) (red) and \(T_1\)-relaxometry (black) as a function of pixel binning with spatial lateral resolution given by \(\Delta x_y\) (see simulation details in SOM6). The diamond intrinsic relaxation was assumed to be \(T_{1,int}=1150\) ms and \(T_{2,int}=1.9\) µs and solid lines are fits to the data according to the shot noise limit. The transverse NV relaxation is caused by axial B\(_z\) fluctuations, where \(\Gamma_{2,Gd}\approx\langle B_z^2\rangle/2f_{Gd}\) \(^{21}\). Thus, both Gd\(^{3+}\) induced relaxation rates are about equal, i.e. \(\Gamma_{1,Gd}\approx\Gamma_{2,Gd}\approx 1\times 10^4\) s\(^{-1}\) for \(c_{Gd}=1\) M. The improved sensitivity of \(\Gamma_1\) is not attributed to \(S_{Gd}(\omega)\) with primary fluctuations in the GHz range, but is instead linked to the intrinsic noise sources \(\Gamma_{int}\). The dominant spin bath consisting largely of unconverted \(^{15}\)N and \(^{13}\)C induces a large \(\Gamma_{2,int}\) which can partially be suppressed by dynamical decoupling sequences like CPMG. This improves the signal to noise given by \(\Gamma_{Gd}/\Gamma_{int}\) and hence the sensitivity (see Table 1 in main text.

However, due to a low phonon density in diamond\(^{27}\), \(\Gamma_{Gd}/\Gamma_{int}\) is much larger for \(T_1\) relaxometry, since \(\Gamma_{2,int}\gg\Gamma_{1,int}\). For a fixed integration time \(\tau_m\), the \(T_1\) scheme significantly outperforms \(T_2\)-relaxometry as the minimum resolvable concentration \(c_{Gd}^{min}\) is lowered by two orders of magnitude for \(T_1\)-relaxometry and our experimental settings.
Supplementary Figure S4. ESR spectrum of Gadovist solution. Gadovist solution was placed into a X-band EPR spectrometer (Bruker) and measured at $\nu_0=9.8$ GHz. The electron g-factor of Gd$^{3+}$ can be calculated according to $g_{Gd} = \frac{h \nu_0}{\mu_B B_0}$ yielding $g_{Gd} \approx 2.017$ for $B_0=3500$ G. Given the gyromagnetic ratio of $\gamma = g \mu_B / \hbar$ and $g_{Gd} \approx g_{_{NV}}$, then $\gamma_{Gd} \approx \gamma_{_{NV}}$. 
Supplementary Figure S5. Experimental spectral density of Gd$^{3+}$. For further explanation, cf. Supplementary Note 3. (A) Effective $S=1/2$ case allowing to determine individual relaxation rate chosen by the frequency of the resonant $\pi$-pulse. (B) Given a broad spectral density, a NV spin polarized into $|0\rangle$ can decay into both spin sublevels thus measuring the combined relaxation. (C) Experimental detection of $S_{\text{Gd}}(\omega)$ by probing $\Gamma_{1,\text{Gd}}$ of an isolated NV orientation with an aligned $B_0$. Tuning $B_0$ up to 13 mT allows the detection of a frequency range of 2.2-3.2GHz.
Supplementary Figure S6. Shot noise limited relaxometric detection. For further explanation, cf. Supplementary Note 4. (A) Fluorescent response of the NV sensor averaged over a ROI containing 250\times 250 pixels to water (c_{Gd}=0) and varying concentrations of Gd^{3+}. The detectable fluorescent change $dI$ upon a small change in $c_{Gd}$ allows distinguishing different Gd^{3+} levels. Error bars represent the standard deviation of the fluorescence signal acquired within $t_m=20$ s. (B) Shot noise scaling of experimental single-$\tau$ detection. For small changes in $c_{Gd}$, $dI$ can be approximated to be linear and constant. The improvement in sensitivity is given by the standard deviation $\sigma_m$ of the slope $dI/dc_{Gd}$ (black dots) which improves with the total measurement time $t_m$ according to the shot noise limit scaling $\sim k/\sqrt{t_m}$ (solid red line). Note that the expected sensitivity as a function of $t_m$ is invalid for a larger range of $c_{Gd}$ and thus a large range of $t_m$ as all important parameters change. This includes not only $\langle B^2 \rangle$, but also $f_{Gd}$, $S_{Gd}(\omega)$, $dI$, $\tau_{opt}$. 
Supplementary Figure S7. Data acquisition and variations of single-τ detection. For further explanation, cf. Supplementary Note 4. (A) Detection sequence. (B) Evolution of the fluorescent contrast δ of a single-τ detection and the shift of τ_{opt,contrast} to achieve a maximum δ for different Gd^{3+} concentrations.
Supplementary Figure S8. Relaxometric single-$\tau$ sensitivity. For further explanation, cf. Supplementary Note 4. (A) Minimum detectable Gd$^{3+}$ concentration within $t_m=20$ s as a function of the sample-sensor depth $h$ and the spatial resolution denoted by the lateral voxel dimension $\Delta r_{xy}$. Experimental values along the fixed implantation depth $h=6.7$ nm (black dots) are in good agreement to the simulation. (B) Detectable number of spins per detection voxel. The voxel size is governed via pixel binning affecting the lateral spatial resolution $\Delta r_{xy}$ while $\Delta r_z = 15$ nm is fixed by the Gd-NV interaction. Fewer spins per voxel can be detected by decreasing $h$ and/or the detection voxel equivalent to an increase in spatial resolution (WF: widefield detection, SIM: Structured illumination microscopy, STED: Stimulated emission depletion). Note that $T_{1\text{,int}}$ remains to be experimentally determined for shallow NV depths with $h<5$ nm and is assumed to be constant for the simulation. C) Evolution of the $T_{1\text{,Gd}}$ (blue) and the corresponding interrogation period $\tau_{\text{opt,contrast}}$ (red) and $\tau_{\text{opt,sensitivity}}$ (gray) for optimal contrast and sensitivity, respectively.
Supplementary Figure S9. ODMR spectrum of an NV ensemble. The Crystallography of diamond allows four distinct NV orientations which can be probed either simultaneously or individually. In zero field ($B_0 \sim 50 \mu T$), both spin sublevels $|\pm 1\rangle$ are degenerate due to the zero field splitting of the NV with $D$=2.87 GHz. This allows probing all NVs present with an ESR contrast of $R \sim 13 \%$. The application of a small static $B_0$ field aligned along one axis lifts the $|\pm 1\rangle$ degeneracy allowing to probe individual NV orientations, i.e. a single orientation aligned along $B_0$ (NV1 in this figure) or the three remaining orientations (NV2-4). The ESR contrast is lowered to $R \sim 4 \%$ and $R \sim 9 \%$ for a single and three orientations, respectively.
Supplementary Figure S10. Spatial resolution of widefield magnetometer. The information about the magnetic field is transmitted via fluorescence photons from the NV. For conventional widefield microscopy even an infinitesimal point is broadened by the optics, a function also termed Point-Spread-Function (PSF). Its Full-Width-Half-Maximum is a measure of the spatial resolution and is given by the Abbe limit for the lateral resolution to $\text{FWHM}_{xy} = \Delta r_{xy} = k \frac{\lambda_{em}}{NA}$, where $k$ is a resolution constant, $\lambda_{em}$ the emission wavelength of the NV and NA is the numerical aperture of the objective (1.49NA for our oil-objective). Given the mismatch in refractive index between the diamond sensor (n=2.4) and the oil/glass (n=1.5), the spatial resolution of the widefield-NV magnetometer is limited to $k\approx0.8$ (ideally $k=0.61$) with a resulting resolution of $\Delta r_{xy}\approx400\text{nm}$. The experimental verification is shown here. (A) CCD image of an isolated NV center embedded in a bulk diamond showing effectively the PSF of the optical system. (B) Line scan through PSF (see red line in (A)) demonstrating diffraction limited detection with a FWHM of 410 nm. (C) Single-τ image ($t_m=1\text{ min}$) of patterned Gd$^{3+}$ using laser-assisted photolithography. (D) Line scan through left Gd$^{3+}$ channel indicating diffraction limited magnetic imaging with a spatial resolution of 428 nm.
Supplementary Figure S11. Dependence of NV relaxation $\Gamma_{1,Gd}$ on the sample-sensor distance to Gd$^{3+}$ ions. The relaxation rate $\Gamma_{1,Gd}$ for increasing thicknesses of a layer of PMMA (Polymethylmethacrylat) was measured. The height of spin-coated PMMA was determined separately on a glass cover slip using an atomic force microscope (AFM).
Supplementary Note 1. Fluctuation sources and broadening effects of freely diffusing Gd$^{3+}$ ions

In the limit of a large number of magnetic spins, the distribution of the magnetic field follows a Gaussian according to the central limit theorem$^{42}$. Thus, all dynamics are described by the autocorrelation function given by

$$\langle (B(t)B(t+\tau)) \rangle = \langle B^2 \rangle \exp[-\tau/\tau_c] \cos(\omega_0 \tau),$$

where $\tau_c$ is the characteristic correlation time of the magnetic fluctuation with $f_{\text{Gd}}=1/\tau_c$ and $\omega_0$ represents the Larmor precession of the ion. The normalized spectral density $S(\omega)$ of a magnetic environment is then given by a Fourier transform of the above given autocorrelation function$^{43,44}$

$$S(\omega) = \frac{f_{\text{Gd}}}{2\pi} \left[ \frac{1}{f_{\text{Gd}}^2 + (\omega - \omega_0)^2} + \frac{1}{f_{\text{Gd}}^2 + (\omega + \omega_0)^2} \right],$$

which can be applied to any freely diffusing paramagnetic species. Evidently, a distinct $S(\omega)$ is expected depending on the dominating process and its fluctuation rate. In the following, the three parameters $\langle \rangle$, $\omega_0$ and $f_{\text{Gd}}$ are quantified in detail for the Gd$^{3+}$ case.

Given the stochastic nature of the polarization, the Gd$^{3+}$ ions create a spatially isotropic magnetic field. However, the spherical symmetry is broken due to the sample-sensor distance of $h=6.7$ nm. Thus, the transverse and axial field strength field strength is given by $\langle B^2 \rangle = \langle B_x^2 \rangle + \langle B_y^2 \rangle \approx \langle B_z^2 \rangle$. For a given NV-Gd$^{3+}$ pair the dipolar interaction reads as

$$B_x^i = \frac{\mu_0 \mu_z^2}{4\pi h} g_{\text{NV}} g_{\text{Gd}} \frac{1}{r^3} \left[ S_x - 3 \sin(\Theta) \cos(\phi) (R \cdot \hat{S}) \right],$$

$$B_y^i = \frac{\mu_0 \mu_z^2}{4\pi h} g_{\text{NV}} g_{\text{Gd}} \frac{1}{r^3} \left[ S_y - 3 \sin(\Theta) \sin(\phi) (R \cdot \hat{S}) \right],$$

with $\hat{S}$ being the Gd$^{3+}$ spin vector and $R$ denoting the separation vector between the NV-Gd$^{3+}$ pair. Taking a trace over a purely mixed state with all possible random orientations and integrating the dipolar coupling in $x$ and $y$ from $-\infty$ to $+\infty$ and in $z$ from $h$, the mean implantation depth, to $\infty$ results in a field strength as given in the main text of $\langle B_{\text{Gd}}^2 \rangle = \langle B_x^2 \rangle + \langle B_y^2 \rangle + \langle B_z^2 \rangle = \frac{21+10^3 \pi^2 \varepsilon_{\text{Gd}} g_{\text{NV}}^2 g_{\text{Gd}}^2}{16h^3} \left( \frac{\mu_0 \mu_B}{4\pi} \gamma_{\text{NV}} \gamma_{\text{Gd}} \right)^2$, where $\gamma_{\text{NV}} \approx \gamma_{\text{Gd}}$ as detailed in Supplementary Fig. S4.

In addition to the distribution in the field amplitude, Gd$^{3+}$ itself exhibits distinct fluctuation sources causing a large distribution of magnetic frequencies. As statistical polarization dominates the Boltzmann polarization due to the small offset field $B_0 < 5$ mT, the few transiently polarized spins are subjected to an effective Larmor precession with $\omega_0 = \gamma B_0 < 140$ MHz. Given the isotropic nature of the statistical polarization it averages to zero, but this transient Larmor precession causes a peak in $S_{\text{Gd}}(\omega)$ for the transverse and longitudinal magnetic components. However, for the given case of freely diffusing spins, Gd$^{3+}$ is prone to various additional zero-mean fluctuations which cause a significant broadening of the Larmor peak. For the free spin diffusion, the total fluctuation rate $f_{\text{Gd}}$ determining the width of $S_{\text{Gd}}(\omega)$ can be decomposed into $\dot{f}_{\text{Gd}} = f_{\text{dipole}} + f_{\text{vib}} + f_{\text{trans}} + f_{\text{rot}}$. The dipolar coupling between a pair of Gd$^{3+}$ spins provokes spin flip-flop processes between Gd$^{3+}$ ions and depends on the distance to each other. Integrating the pairwise interaction between Gd$^{3+}$ pairs and averaging over the spatial distribution of Gd$^{3+}$ spins yields a concentration dependent fluctuation rate.
of $f_{\text{dipole}} = c_{\text{Gd}} \cdot 77 \text{ GHz M}^{-1}$. Another substantial broadening of magnetic fluctuations arises from the electron spin relaxation rate of Gd$^{3+}$ itself. As opposed to organic spin labels such as nitroxides, chelated metals tend to have much shorter lifetimes due to paramagnetic ions within the chelate of Gadobutrol. These paramagnetic ions cause a small fluctuating field which interacts with the central Gd$^{3+}$. This induces spin relaxation of the complex $S=7/2$ ion and for magnetic fields below $B_0<1 \text{ T}$ chelated Gd$^{3+}$ exhibits a constant value of $f_{\text{vib}} \sim 50 \text{ GHz}$. The motion of spins in aqueous solution also induces a fluctuating field. The fluctuation rate of the effective Gd$^{3+}$ field due to translational spatial diffusion is given by

$$f_{\text{trans}} = D_{\text{diff}} \left( \frac{3}{4r} \right)^2,$$

where $D_{\text{diff}}$ is the diffusion coefficient. With standard values at room temperature with $D_{\text{diff}} = 3 \cdot 10^{-9} \text{ m}^2 \text{s}^{-1}$ and $r=6.7 \text{ nm}$, the diffusional broadening can be approximated to $f_{\text{trans}} \sim 40 \text{ MHz}$. Furthermore, molecular rotation yields a fluctuation rate governed by Stoke’s law with

$$f_{\text{rot}} = \frac{k_B T}{8\pi d^3 \eta},$$

which can be approximated to $f_{\text{rot}} \sim 100 \text{ MHz}$. An overview of the total fluctuation of freely diffusing Gd$^{3+}$ ions in dependence on the concentration is shown in Supplementary Figure S2. Obviously, fluctuations originating from rotation and translational diffusion can be neglected given the dominating intrinsic vibronic relaxation $f_{\text{vib}}$ of Gd$^{3+}$ even at low $c_{\text{Gd}}$. With increasing $c_{\text{Gd}}$ $f_{\text{Gd}}$ and consequently $S_{\text{Gd}}(\omega)$ are dominated by the elevated fluctuation due to Gd$^{3+}$-Gd$^{3+}$ coupling. Although $\langle B^2 \rangle$ increases linearly with $c_{\text{Gd}}$ the power is then distributed over a wider frequency range, i.e. determined by $f_{\text{Gd}}$. Therefore, the concentration dependency of $\Gamma_{1,\text{Gd}}$ employing the NV sensor is not linear as reported in other studies, since not only the peripheral field $\langle B_{\text{Gd}}^2 \rangle$ of the mutually interacting Gd$^{3+}$ ions increases with $c_{\text{Gd}}$ but also $S_{\text{Gd}}(\omega)$ due to the elevated $f_{\text{dipole}}$. 


Supplementary Note 2. Dynamic Gd$^{3+}$-NV interaction

A complete description of the dynamic coupling between Gd$^{3+}$ spins and NV includes both transverse and longitudinal components acting on the elements of the NV Hamiltonian $\sigma_{xy}$ and $\sigma_z$, respectively. The NV dephasing is purely exponential as the magnetic fluctuations of the Gd$^{3+}$ ions are in the fast fluctuation regime $f_{\text{Gd}} \gg \langle B_{\text{rms}} \rangle^{21}$. Thus, the time evolution of the NV can be modeled by solving the density matrix $\rho(t)$ \cite{50}. In the interaction picture $\rho_I(t)$ is given by

$$\frac{d}{dt}\rho_I(t) = -i[H_{\text{env},I}(t),\rho_I(t)] - \Gamma_2[\rho_I(t) - \sigma_x \rho_I(t) \sigma_x],$$

where $H_{\text{env}}$ is the Hamiltonian representing the interaction of the NV with the environment. The transverse NV relaxation is governed by the diamond spin bath and longitudinal Gd$^{3+}$ fluctuations via $\Gamma_2 = \Gamma_{2,\text{int}} + \Gamma_{2,\text{Gd}}$, where $\Gamma_{2,\text{Gd}} = \langle b^2 \rangle / 2f_{\text{Gd}} \approx 10^4\ s^{-1}$ \cite{21}. Since no spin echo is applied for a $T_1$ based relaxometric detection, transverse relaxation can be approximated by $\Gamma_2 \approx \Gamma_{2,\text{int}}$ as $\Gamma_{2,\text{int}}$ is dominated by the short intrinsic decoherence rate $\Gamma_{2,\text{int}} \approx 1/\tau_{\text{int}} \approx 2 \cdot 10^7\ s^{-1}$. Solving the above given equation of motion for the time derivative of $\rho_{00}(t)$ then yields the probability of finding the NV in $|0\rangle$ as given in the main text by $P_0(\tau) = \frac{1}{6}(2 + e^{-\Gamma_2^+ \tau} + e^{-\Gamma_2^- \tau} + 2e^{-(\Gamma_2^+ + \Gamma_2^-) \tau})$. The individual decay rates $\Gamma_{1,\text{Gd}}^{\pm}$ of an NV polarized in $|0\rangle$ depend on the environmental spectral density $S(\omega)$ and are given by $\Gamma_{1,\text{Gd}}^{\pm} = \int \Gamma_{1,\text{Gd}}^{\pm}(\omega) S(\omega)d\omega$, where $\Gamma_{1,\text{Gd}}^{\pm} = \langle B^2 \rangle (\Gamma_{1,\text{Gd}}^0 + \delta^2 + \Gamma_{2,\text{Gd}})$ and $\delta$ is the relative detuning of $S(\omega)$ from the NV resonant transition. Given the broad spectral density of Gd$^{3+}$ and since $f_{\text{Gd}} \gg \omega_0$, the decay rates read as

$$\Gamma_{1,\text{Gd}}^{\pm} = f_{\text{Gd}} \langle B^2 \rangle \left[ \frac{1}{f_{\text{Gd}}^2 + D^2} + \frac{1}{f_{\text{Gd}}^2 + (D \pm 2\omega_0)^2} \right] \approx 2 \frac{f_{\text{Gd}} \langle B^2_{\text{Gd}} \rangle}{f_{\text{Gd}}^2 + D^2}.$$
**Supplementary Note 3. Spectral density mapping**

The T₁-relaxometric detection offers the capability to map the environmental magnetic spectral density $S(\omega)$ over a wide frequency range. As opposed to F₂, which is generally limited to the kHz-MHz (≈1/T₂), $F_1^{\pm}$ can in principal be tuned in a much wider range depending solely on $B_0$. The individual sensitivity windows $F_1^{\pm}$ act as a tunable band pass filter which can be tuned in its frequency due to the Zeeman shift. The latter causes a shift of the NV resonant transitions depending on the projection of static offset field $B_0$ onto the quantization axis z of the NV as given by

$$v_{NV,\pm 1} = D + \frac{m_1 g_{NV} \mu_B B_z}{\hbar} = (2870 \text{ T}^{-1} \pm 2.8 \cdot 10^4 B_z).$$

Assuming a $B_0$-invariant spectral density $S(\omega)$, both transitions $F_1^{\pm}$ can be employed to measure the relaxometric NV response $\Gamma_{1,Gd}^{\pm}$ at distinct frequencies. Two distinct techniques are possible to detect the frequency dependent relaxation. i) If $F_1^{\pm}$ are to be measured individually, i.e. because $S(\omega)$ varies, the NV needs to be prepared in either $|\pm 1\rangle$ or $|0\rangle$ state. Due to spin forbidden transitions between $|\pm 1\rangle$ sublevels, the NV relaxes only to $|0\rangle$ allowing to probe the individual rate, i.e. $\Gamma_{1,Gd}$ as shown in Supplementary Fig. S5A. ii) $S(\omega)$ can be measured by probing the $|0\rangle$ polarization (Supplementary Fig. S5B). Assuming a $S(\omega)$ covering both sensitivity windows $F_1^{\pm}$, this scheme measures the combined decay given by $\Gamma_{1,Gd} = \Gamma_{1,Gd}^- + \Gamma_{1,Gd}^+$ since $|0\rangle$ simultaneously decays into $|\pm 1\rangle$. In the case of Gd³⁺, $S_{Gd}(\omega)$ is very broad (>50 GHz) and largely unaffected by $B_0$ as $S_{Gd}(\omega)$ is dominated by the coupling strength $f_{dipole}$ and the energetic relaxation $f_{vib}$. As $S_{Gd}(\omega)$ equally covers both sensitivity windows $F_1^{\pm}$ for the applied 50μT<$B_0$<13mT, the scheme shown in Supplementary Fig. S5B can be applied to experimentally map $S_{Gd}(\omega)$. As demonstrated in Supplementary Fig. S5C, within the detectable frequency range $S_{Gd}(\omega)$ matches the theoretical curve with a high Gd³⁺ induced relaxation up to >50GHz.
Supplementary Note 4. Sensitivity estimation

The single-\(\tau\) detection allows discriminating magnetic noise, in this paper originating from Gd\(^{3+}\), from water based on a two-point fluorescent measurement. Thus, the fluorescent signal \(\delta I\) of the magnetometer changes upon small changes in \(\delta c_{\text{Gd}}\) as demonstrated in Supplementary Fig. S6A. Given the averaging of multiple independent measurements within the total measurement time \(t_{\text{m}}\), the uncertainty in the measured data points \(\sigma_{\text{m}}\) is determined by its standard deviation which is dictated by shot noise of the detected photons. The shot noise scaling of the magnetometer sensitivity \(k\) can thus be verified experimentally by

\[
c_{\text{Gd}}^{\text{min}} \approx \frac{k}{\sqrt{t_{\text{m}}}} \approx \frac{\sigma_{\text{m}}}{dI}
\]

where \(dI\) is the maximum slope of the signal change. Fitting the experimental data for increasing number of repetitions, i.e. images, reveals shot noise limited relaxometric detection (Supplementary Fig. S6B).

A quantitative statement about the magnetometer performance within the traditional context of some \(T/\sqrt{\text{Hz}}\) is not possible. This is because the detected relaxation rate \(\Gamma_{\text{env}}\) depends not only on the square of the magnetic field fluctuations \(\langle B^2 \rangle\), since \(c_{\text{Gd}}\) also affects \(f_{\text{Gd}}\) and the spectral density \(S(\omega)\). This in turn affects the detectable signal change \(\delta I\) as well as \(\tau_{\text{opt}}\) (Supplementary Fig. S7B). In order to solve the multiparameter space and to determine the sensitivity over a wide range, we fully simulated the single-\(\tau\) measurement. Since the fluorescent measurement obeys the shot noise limit the minimum detectable concentration can be determined for a given detection voxel, i.e. spatial resolution or number of binned pixels. It is possible to distinguish between water and a certain Gd\(^{3+}\) concentration, when the number of detected photons \(n_{\text{ph}}\) is larger than the shot noise of the achievable fluorescent contrast \(\delta I\). Thus, the minimum resolvable \(\delta I\) is given by

\[
\delta I_{\text{min}} \geq \frac{1}{\sqrt{n_{\text{ph}}}}
\]

where \(n_{\text{ph}}\) depends on the number of fluorescent readouts \(N\) within total acquisition time \(t_{\text{m}}\) and the binned voxel size. As illustrated in Supplementary Fig. S7B, for the wide field detection \(t_{\text{m}}\) is determined by

\[
t_{\text{m}} = B[(t_{\text{Laser}} + t_{\text{wait}} + \tau)^N + t_{\text{CCD}}],
\]

where \(t_{\text{Laser}} = 800\) ns is the optimized length of the laser readout/re-polarization of the NV, \(t_{\text{wait}}\) is the waiting interval allowing to relax NVs trapped in the metastable state to relax to \(|0\rangle\) and \(\tau\) is the free interrogation period. Multiple repetitions \(N\) are averaged within a single CCD frame which is subsequently readout within \(t_{\text{CCD}} = 40\) ms. For the applied laser power, the CCD saturates for high \(N\), thus an image \(B\) is readout after \(N=25000\).

As demonstrated in Supplementary Fig. S7B, all parameters affecting \(n_{\text{ph}} \propto N \cdot t_{\text{Laser}}\) and the duty cycle of the detection governed by the chosen \(\tau\) vary upon different \(c_{\text{Gd}}\). In order to find the minimum detectable \(c_{\text{Gd}}^{\text{min}}\) within \(t_{\text{m}} = 20\) s, the achievable contrast \(\delta I\) for varying \(\tau\) is then a function of \(N\) applicable within \(t_{\text{m}}\) and the chosen spatial resolution \(\Delta r_{\text{xy}}\), i.e. number of pixels, given by
\[ \delta l_{\text{min}}(\tau) = \frac{1}{\sqrt{N \cdot t_{\text{Laser}} \cdot \hat{n}_{\text{pixel}} \cdot \Delta r_{xy}^2/(115\text{nm})^2}}, \]

where \( \hat{n}_{\text{pixel}} \) is the fluorescence count rate originating from the detection voxel of the NV sensor, i.e. \( 2 \cdot 10^6 \text{ s}^{-1} \text{p}^{-1} \) for a single pixel \((115 \text{ nm})^2\) containing 15 NV centers each with an average countrate of 125 kcps. The maximum sensitivity for a given detection voxel is then given by the minimum concentration resolvable for \( \delta l_{\text{min}}(\tau) \), i.e. by matching it to Supplementary Fig. S7B. As demonstrated in Supplementary Fig. S8A, lowering the spatial resolution, for instance by increasing the voxel size \( \Delta r_{xy} \) due to a binning of more CCD pixels, elevates \( n_{ph} \) and subsequently also the magnetometer’s sensitivity as smaller \( \delta l \) can be resolved within a given acquisition time \( t_m \).

Interestingly, the optimal interrogation period \( \tau \) is not necessarily at the maximum contrast \( \tau_{\text{opt}} \) as demonstrated in Supplementary Fig. S8B (given \( \tau \) and \( T_1 \) times correspond to the concentrations shown in Supplementary Fig. S8A). As smaller detection voxels and larger sample-sensor distances \( h \) lower the sensitivity, higher \( c_{Gd}^{\text{min}} \) are required thus prominently decreasing \( T_{1,Gd} \) (blue plane). While the maximum fluorescent contrast \( \delta l \) would be achieved at \( \tau_{\text{opt,contrast}} \) illustrated by the red plane, a maximum sensitivity within \( t_m \) is accomplished by applying shorter \( \tau_{\text{opt,sensitivity}} \) (gray plane). Under the given experimental setup the ideal interrogation time is in the order of \( \tau \sim T_{1,Gd}/2 \) for \( 0 \leq c_{Gd} < 10 \text{mM} \) and approaches \( T_{1,Gd} \) for higher \( c_{Gd} \) (corresponding to smaller detection voxels). The discrepancy to \( \tau_{\text{opt,contrast}} \) is plausible taking the duty cycle of the detection into account. Low \( c_{Gd} \) exhibit a comparatively long \( T_{1,Gd} \) which significantly prolongs the interrogation period \( \tau \) and thus lowers \( n_{ph} \) since fewer repetitions \( N \) can be conducted within \( t_m \). As \( \delta l(\tau) \) is a very broad function for low \( c_{Gd} \) (Supplementary Fig. S7B), the reduced \( \delta l \) for lower \( \tau \) is compensated by the elevated \( n_{ph} \).
Supplementary References.