Spatial resolution in MRI is ultimately limited by the signal detection sensitivity of NMR, since resolution equal to \( \rho_{iso} \) in all three dimensions requires the detection of NMR signals from a volume \( \rho_{iso}^{3/2} \). With inductively detected NMR at room temperature, it has therefore proven difficult to achieve isotropic resolution better than \( \rho_{iso} = 3.0 \, \mu m \), even with radio-frequency microcoils, optimized samples, high magnetic fields, optimized pulse sequence methods, and data acquisition times around 60 h. Here we show that spatial resolution can be improved and data acquisition times can be reduced substantially by performing MRI measurements at 5 K and using dynamic nuclear polarization (DNP) to enhance sensitivity. We describe the experimental apparatus and methods, and we report images of test samples with \( \rho_{iso} = 2.6 \, \mu m \) and \( \rho_{iso} = 1.7 \, \mu m \), with signal-to-noise ratios greater than 15, acquired in 31.5 and 81.6 h, respectively. Image resolutions are verified by quantitative comparisons with simulations. These results establish a promising direction for high-resolution MRI of small samples. With further improvements in the experimental apparatus and in paramagnetic dopants for DNP, DNP-enhanced low-temperature MRI with \( \rho_{iso} < 1.0 \, \mu m \) is likely to become feasible, potentially enabling informative studies of structures within typical eukaryotic cells, cell clusters, and tissue samples.

Enhanced spatial resolution in magnetic resonance imaging by dynamic nuclear polarization at 5 K

Hsueh-Ying Chen, C. Blake Wilson, and Robert Tycko

Enhanced spatial resolution in magnetic resonance imaging by dynamic nuclear polarization at 5 K

MRI is a powerful and widely used technique for visualizing structures and characterizing other properties within organisms and heterogeneous materials. Spatial resolution in MRI can be limited by translational diffusion (1, 2) or by magnetic field gradient strengths, but, more generally, is limited by the signal-to-noise ratio (SNR) of the NMR signals from which MRI images are constructed. Due primarily to SNR considerations, inductively detected \(^1H\) MRI with isotropic spatial resolution \( \rho_{iso} \) better than \( \sim 3.0 \, \mu m \) has been difficult to achieve, even with the use of radio-frequency (RF) microcoils to improve NMR detection sensitivity, paramagnetic doping to increase signal-averaging rates, and optimization of MRI pulse sequences (3–5). Consequently, although studies of individual biological cells have been a goal since the early days of MRI (6–17), progress in this direction has not been rapid.

In principle, the SNR of NMR can be improved by cooling both the sample and the RF circuitry, with an expected \( T^{-3/2} \) dependence on temperature \( T \). If all other factors were unchanged, reducing \( T \) from 296 K to 5 K could increase the SNR by a factor of 455, allowing a reduction in \( \rho_{iso} \) by a factor of 455\(^{3/2} \) = 7.7. At low temperatures, dynamic nuclear polarization (DNP) based on the cross-effect mechanism (18, 19) could increase the SNR of \(^1H\) MRI by an additional factor of 100 or more, allowing a net reduction of \( \rho_{iso} \) by a factor greater than 30 relative to experiments at 296 K. In principle, MRI with submicron resolution seems possible, enabled by low temperatures and DNP (20).

However, various complications arise in practice when \(^1H\) MRI is performed at low temperatures with DNP. Most obviously, samples of interest become solids, implying that \(^1H\)–\(^1H\) dipole–dipole couplings are no longer averaged out by molecular motions. Pulse sequences that include homonuclear dipolar decoupling techniques (21–26) must be employed to overcome the severe NMR line broadening and signal losses during phase-encoding periods that would otherwise outweigh the SNR advantages described above. We have shown that inclusion of Lee–Goldburg (LG) decoupling (27) during phase encoding and pulsed spin locking (PSL) during NMR signal detection (28) allows \(^1H\) MRI images with \( \rho_{iso} = 2.8 \, \mu m \) to be obtained at \( T = 28 \) K without DNP (5, 29).

A second complication is that paramagnetic compounds must be added to the sample at relatively high concentrations to enable cross-effect DNP, typically nitroxide-based biradicals (30, 31) or triradicals (32, 33). Fluctuating hyperfine fields from these

Significance

Although MRI is a powerful method for visualizing features within organisms and materials, the relatively low signal-to-noise ratio (SNR) of the NMR signals that are used to construct an image makes MRI with isotropic spatial resolution below \( 3.0 \, \mu m \) impractical at room temperature. Here we show that SNR enhancements available from a combination of low temperatures and dynamic nuclear polarization allow MRI with \( 1.7-\mu m \) isotropic resolution. These results may enable informative MRI studies of eukaryotic cells, cell clusters, and small tissue samples.
compounds produce large reductions in transverse $^1$H spin relaxation times ($T_{2\text{H}}$), again leading to signal losses during phase-encoding and NMR detection periods that cancel out the signal gains from DNP. These signal losses can be minimized by operating at $T < 10$ K, where the flip-flop transitions among unpaired electron spins that produce short $T_{2\text{H}}$ values are largely suppressed because the electron spins become strongly polarized (34). A third complication, discussed further below, is that build-up times for nuclear spin polarization ($T_{\text{NMR}}$) are long at low temperatures, increasing the time between scans during MRI data acquisition.

Here we demonstrate that, despite the various complications, $^1$H MRI with DNP can be performed successfully at $T = 5$ K, thereby permitting substantial reductions in total image acquisition times and/or improvements in spatial resolution. Specifically, we report $^1$H MRI images of test samples with $\rho_{\text{iso}} = 2.6$ μm, acquired in 31.5 h, and with $\rho_{\text{iso}} = 1.7$ μm, acquired in 80.6 h. The latter value represents a reduction in voxel volume by a factor of 5.5 relative to the room temperature $^1$H MRI image with the highest isotropic resolution in the current literature ($\rho_{\text{iso}} = 3.0$ μm) (4).

Methods

**Apparatus and Samples.** MRI experiments were performed at 9.39 T with a Tecmag Redstone console, operating at a $^1$H NMR frequency of 399.2 MHz. Gradient current pulses up to 100 A were available from AE Techron model 7548 gradient amplifiers. As previously described (5, 29), the MRI apparatus was contained in a Janis Supertran ST-200 continuous flow cryostat (Fig. 1A), with liquid helium flow from a pressurized tank (170-mbar gauge pressure) being controlled manually by a needle valve in the helium transfer line (Cryo Industries model HTL-3123-2). The temperature of the copper cold finger block within the cryostat was below 4.5 K, indicated by a temperature sensor (Lake Shore Cryotronics, CX-1070) and controller (Stanford Research Systems model CTC100). The actual sample temperature was 5 K, determined from $^1$H NMR signal amplitudes without DNP. Liquid helium consumption during acquisition of MRI data was 1.2 l/h.

The MRI apparatus consisted of a stack of sapphire plates containing gradient coils and the RF microcoil, with the design and dimensions reported previously (5, 29). The stack of sapphire plates was mounted on the cold finger block (Fig. 1B). Gradient coils were glued to the sapphire plates with Stycast epoxy. The RF microcoil was wound around a fused silica capillary tube with 105-μm outer diameter and 40-μm inner diameter (Molex/Polyimicro, TSP040105), using bare copper wire with 20-μm diameter, and was held in place with cyanoacrylate glue (Fig. 1C). Additional details of the cryostat and MRI apparatus are shown in SI Appendix, Fig. S1.

Prior to winding the microcoil, the capillary tube was loaded with monodisperse amorphous silica beads with 9.2-μm diameter (Cospheric, SiO2MS-2.0), suspended in partially deuterated glycerol/water (1:3.6 volume ratio of H2O, D2O, and d5-glycerol, 10 mM Tris buffer, pH ≈ 9) containing 12 mM 4-{[N,N-di-[2-succinyl,3-(TEMPO-4'-oxy)-propyl]-amino-TEMPO} (succinyl-DOTOPA) (33) as the paramagnetic dopant for DNP. Details of sample preparation and loading are given in SI Appendix, Supporting Methods and Fig. S2 A and B.

Gradient strengths were calibrated with one-dimensional (1D) images at room temperature and were found to be 0.385 T/m-A, 0.453 T/m-A, and 0.454 T/m-A in the $x$, $y$, and $z$ directions, respectively, equivalent to 16.4 Hz/μm-A, 19.3 Hz/μm-A, and 19.3 Hz/μm-A for $^1$H nuclei. The $x$ and $z$ directions are defined to be the directions of the capillary and the external magnetic field, respectively. Measurements at 5 K indicated that gradient strengths were reduced by ~9%, 14%, and 4%, respectively, presumably due to eddy currents. Gradient currents were adjusted accordingly.

A quasi-optical system, comprising wire grid polarizers, elliptical mirrors, and roof mirrors (Thomas Keating Ltd.), combined the outputs of two independent microwave sources (Virginia Diodes, Inc.) with perpendicular planes of linear polarization and with nominal output powers of 50 mW (source #1) and 30 mW (source #2). The combined outputs were focused on the entrance of a corrugated waveguide, which transmitted the microwaves to the cryostat (Fig. 1D and SI Appendix, Figs. S1 and S2C). After passing through a Teflon window at the lower end of the cryostat, the microwaves entered a tapered horn that was mounted on the bottom of the cryostat’s inner radiation shield. After the microwave beam diameter was reduced from 12 mm to 3.0 mm by the horn, microwaves were transmitted to the microcoil within the MRI apparatus by a tapered Teflon rod, with a final diameter of 1.8 mm (SI Appendix, Fig. S1J).

The output frequency of microwave source #1 could be modulated at 2.0 kHz with a symmetric triangular waveform. The output frequency of source #2 was variable but could not be modulated rapidly. The orientation of the cryostat relative to the microwave beam and the output frequencies of the two sources were adjusted to maximize DNP-enhanced $^1$H NMR signals, as shown in SI Appendix, Fig. S2 D and E and discussed further below. Images described below were
obtained with source #1 sweeping between 262.75 GHz and 263.05 GHz and source #2 set to 262.9 GHz.

MRI Pulse Sequence. We used the pulse sequence in Fig. 2A to acquire MRI data. The sequence began with a train of 64 RF pulses to destroy $^1$H spin polarization and suppress background signals (200-$\mu$s pulse lengths, 156-kHz RF amplitude, 1.0-ms delay between pulses). Microwaves were then applied for a period $\tau_{\text{DNP}}$, optimized as discussed below, followed by a 100-ms delay for reequilibration of electron spin polarization to maximize transverse $^1$H spin relaxation times ($\tau_{\text{en}}$). To create a field of view in the x direction (FOVx) that was experimentally feasible, as discussed below, slice selection in the x direction was performed by applying a train of eight $\pi$ pulses in the presence of a strong x gradient pulse (1.12 kHz/$\mu$m), with 50-$\mu$s delays for dephasing of transverse $^1$H polarization between pulses and with RF amplitudes alternating between 167 and 83 kHz.

In earlier work, we demonstrated an alternative approach to slice selection for $^1$H MRI of frozen samples, based on RF phase modulation during LG irradiation (35). In this alternative approach, signals outside the desired slice are suppressed in a difference image, obtained by subtracting data with different RF phase modulation from the original data.

**Fig. 2.** (A) Pulse sequence for DNP-enhanced MRI, as described in the text. (B) The $^1$H NMR signal at 5 K and $k_z = 0$, acquired with pulsed spin locking. (C) Signal after 11-Hz Lorentzian apodization and Fourier transformation. Spectrum in cyan is scaled up by a factor of 100 relative to the spectrum in blue to show the noise level. (D) Dependence of the NMR signal amplitude on $\tau_{\text{DNP}}$ from measurements with both microwave sources off (red points, scaled up by a factor of 32 relative to other data), with source #1 at 262.9 GHz and source #2 off (green points), with source #1 sweeping over a 0.3-GHz range around 262.9 GHz (cyan points) and source #2 off, and with source #1 sweeping and source #2 at 262.9 GHz (blue points). Color-coded curves are fits with the expression $S(t_{\text{DNP}}) = (S_0 - S_{\infty}) \left(1 - e^{-t_{\text{DNP}}/\tau_{\text{bu}}}ight) + S_{\infty}$, yielding the indicated best-fit values of the buildup time $\tau_{\text{bu}}$ and asymptotic signal $S_{\infty}$. (E) Dependence of the NMR signal amplitude on $\tau_{\text{LG}}$, fitted with the expression $S(t_{\text{LG}}) = S_1 e^{-t_{\text{LG}}/T_2} + S_2$, yielding best-fit values $T_2 = 688 \pm 4$ $\mu$s and $P = 1.57 \pm 0.02$. (3o f1 0
modulation from data without phase modulation. This approach could not be used in the current experiments because small signal fluctuations, apparently due to minor pulse sequence instabilities, prevented full cancellation of the large undesired signals in the difference data, leading to excess noise in the final images. With the slice selection method in Fig. 2A and SI Appendix, Fig. S3, undesired signals were directly suppressed in each scan, without requiring subtraction of signals in pairs of scans. Minor instabilities therefore did not contribute significant noise to the images.

After the slice selection period in Fig. 2A, the 3D phase-encoding period used LG irradiation to average out \(^1\)H-\(^1\)H dipole-dipole couplings that would otherwise cause rapid signal decay. A \(\pi\) pulse in the middle of the phase-encoding period focused gradient-independent frequency offsets. Effective LG decoupling field amplitudes were 250 kHz (204.1-kHz RF field with phase \(\gamma = -1\), 144.3- or -144.3-kHz carrier frequency offsets for “+LG” or “-LG” irradiation). Variations in LG precession frequencies due to RF inhomogeneity were refocused by alternating +LG and -LG periods, with equal length \(\tau_{PSL}/4\). The \(^1\)H polarization was prepared initially perpendicular to the +LG effective field by a pulse with phase \(-\pi\) and flip angle \(\theta = \pi/2 + \theta_m\), with \(\theta_m = \cos^{-1}(1/\sqrt{3})\). At the end of the phase-encoding period, polarization components that correspond to real or imaginary parts of the MRI data were stored as longitudinal polarization by applying either a single \(\theta\) pulse with phase \(\pi\) or a \(\theta\) pulse with phase \(\pi\) followed by a \(\pi/2\) pulse with phase \(\varphi\). To maximize sensitivity, NMR signals were detected in the \(\tau_{PSL}\) period with PSL, consisting of a \(\pi/2\) pulse followed by a train of 32,768 pulses with phase \(\gamma\) and flip angle \(\alpha\), with signal digitization after each pulse. To suppress ring-down and background signals, the phase of the \(\pi/2\) pulse and the receiver alternated between \(\pi\) and -\(\pi\) on successive scans. With a 1.0-\(\mu\)s pulse length and a 3.8-\(\mu\)s delay between pulses, \(\alpha\) was optimized at \(\sim 72^\circ\) as previously described (5). Under these conditions, \(^1\)H NMR signals at \(T = 5\) K decayed exponentially during the PSL period with a time constant of 29 ms, corresponding to an 11-Hz linewidth (Fig. 2B and C).

When strong gradients are applied, the angle between the effective LG field and the z axis of the usual rotating frame deviates from its ideal value in regions of the sample that are far from the center of the gradients, according to \(\theta_{LG} = \tan^{-1}(v_{LG}/(\Delta G + g \cdot \mathbf{r}))\) and \(\alpha_{LG} = \pi - \tan^{-1}(v_{LG}/(-\Delta G + g \cdot \mathbf{r}))\) for +LG and -LG periods, where \(v_{LG}\) and \(\Delta G\) are the magnitudes of the RF field and frequency offset, respectively, \(g\) is the gradient vector, and \(\mathbf{r}\) is the position within the sample relative to the center of the gradients. Therefore, the \(\theta\) pulse at the beginning of the phase-encoding period in Fig. 2A cannot prepare \(^1\)H polarization perpendicular to the effective LG field at all sample positions when gradients are applied. Moreover, polarization that is perpendicular to the effective field at the end of a +LG period is not entirely perpendicular to the effective field in a subsequent -LG period. These effects lead to nonnegligible polarization components from \(\mathbf{r} \neq 0\) that do not precess around the effective LG field (i.e., are spin locked along the effective field during the phase-encoding period) and consequently appear in images as strong artifacts near \(\mathbf{r} = 0\). As shown by simulations (SI Appendix, Fig. S4A), artifacts from spin-locked polarization can be eliminated by deliberately introducing nonzero rise and fall times for gradient pulses and by reducing gradient amplitudes close to zero around transitions between +LG and -LG periods. In this way, at least in principle, the \(\theta\) pulse can prepare all \(^1\)H polarization perpendicular to the effective LG field initially. As gradients turn on and effective LG field directions change, the polarization follows adiabatically and remains perpendicular to the effective field. In experiments, rise and fall times for gradient pulses were roughly 50 \(\mu\)s, determined by the properties of the gradient amplifiers (SI Appendix, Fig. S4B).

**Data Acquisition and Processing.** Points in \(k\) space were acquired in order of increasing \(|\mathbf{k}|\), which allowed preliminary images to be examined as data acquisition progressed. Data were stored as a set of 2D blocks in the Tecmag software, with \(^1\)H signals as a function of \(t_{PSL}\), in the direct dimension and 128 combinations of gradient amplitudes (representing various \(k\) vectors) in the indirect dimension. For each \(k\), real and imaginary parts of \(S(\mathbf{k})\) were acquired consecutively. Sixteen dummy scans were applied at the beginning of each 2D block, to suppress background \(^1\)H NMR signals from outside the sample. It was not possible to place the center of the sample precisely in the center of the gradients. Consequently, strong gradient pulses produced large \(^1\)H NMR frequency shifts even at the center of the sample, which reduced the effectiveness of LG decoupling. To compensate for inevitable errors in sample positioning, corrections proportional to the gradient amplitudes were applied to the values of \(\Delta G\) and -\(\Delta G\) in +LG and -LG periods as previously described (5). In addition, to compensate for minor drifts in RF amplitudes during data acquisition, the value of \(v_{LG}\) was optimized after each group of 10 2D blocks by maximizing the signal with all gradient amplitudes set to zero (SI Appendix, Fig. S5).

After exponential apodization and Fourier transformation with respect to \(t_{PSL}\), the amplitudes of the zero-frequency peaks in the resulting spectra were saved as the real or imaginary part of \(S(\mathbf{k})\). For images in Figs. 3 and 4, baseline...
corrections were applied in $k$ space as described below. Data were zero filled to 256 points in $k_x$ and to 128 points in $k_y$ and $k_z$ before Fourier transformation. Cyclic permutations were then applied to the images in $x$, $y$, and $z$ to bring the regions of greatest intensity to the center of the field of view.

**Results**

**Selection of Measurement Conditions.** Following standard definitions, the nominal resolution of an MRI image acquired with the pulse sequence in Fig. 2A is $\rho_{iso} = \pi/k_{max}$, where $k_{max} = 2\pi g_{max} t_{LG}$, with $g_{max}$ being the maximum average gradient amplitude (units of hertz per micrometer, assumed to be the same in $x$, $y$, and $z$) during the LG irradiation period $t_{LG}$.

The field of view in direction $u$ is $FOV_u = 2\pi/\delta k_u$, where $\delta k_u$ is the increment of $k_u = 2\pi g_{u} t_{LG}$, so that $k_u = m_u \delta k_u$ and $-k_{max}/\delta k_u \leq m_u \leq (k_{max}/\delta k_u) - 1$ for integers $m_u$. In our experiments, we acquired data $S(k)$ within a sphere in $k$ space, including all points that satisfied $|k| \leq k_{max}$ and $-k_{max} \leq k_u \leq k_{max} - \delta k_u$, with $u = x, y, z$. If we had acquired all data within a cube in $k$ space, the number of complex data points would have been $(2k_{max})^3/(\delta k_x \delta k_y \delta k_z)$. With spherical $k$-space sampling, the number of complex data points is reduced by a factor of $\pi/6$. With four scans per $k$-space point (two scans each for the real and imaginary parts of $S(k)$), the total time to acquire one image is then

$$t_{total} = 16\pi k_{max}^3 t_{scan} / (3\delta k_x \delta k_y \delta k_z) = 2\pi (FOV_x \cdot FOV_y \cdot FOV_z t_{scan} / (3 \rho_{iso}^3))^3,$$

where $t_{scan}$ is the time per scan.

DNP-enhanced $^1$H NMR signals at 5 K are very large (compared to signals at room temperature) but build up slowly during $\tau_{DNP}$ on a 6.2-s time scale with optimized microwave irradiation (Fig. 2D). Although the SNR of the final image with a given $t_{total}$ would be maximized by using $t_{DNP} \approx 7.7$ s, $t_{total}$ would exceed 5 d if $FOV_x/\rho_{iso} \geq 30$ for $u = x, y, z$ and $t_{scan} = 8$ s. Hence, we used $t_{DNP} = 2.0$ s to accelerate data acquisition at the expense of SNR.

In terms of the SNR of the $k = 0$ data point, called $SNR_0$, assuming four scans per $k$-space point, and with the additional assumption that the total signal in the final image is divided equally among a fraction $f$ of the voxels, the SNR of the final image can be expressed as

$$SNR = (SNR_0 \sqrt{t_{total}/4t_{scan}}) / \left[\sqrt{2} f (FOV_x \cdot FOV_y \cdot FOV_z / \rho_{iso}^3)\right] = SNR_0 / \left[2 \sqrt{3} (FOV_x \cdot FOV_y \cdot FOV_z / \pi \rho_{iso}^3)\right].$$

$SNR_0$ decreases with increasing $t_{LG}$ as shown in Fig. 2E. From Fig. 2E, with $\tau_{DNP} = 2.0$ s and $\tau_{LG} = 600 \mu$s, $SNR_0 \approx 2,400$. Assuming $f \approx 0.3$, and with $FOV_x \cdot FOV_y \cdot FOV_z / \rho_{iso}^3 \approx 5 \times 10^6$, Eqs. 1 and 2 predict $SNR \approx 18$ for $t_{total} \approx 60$ h. In principle, with $\tau_{LG} = 600 \mu$s, our gradient system allowed values of $\rho_{iso}$ as small as $\sim 1.45 \mu$m with 100-A current pulses, including the scaling of effective gradient amplitudes by $1/\sqrt{3}$ under LG irradiation (5, 29) and reductions in $g_{max}$ due to the nonnegligible rise and fall times of gradient pulses.

**Image with 2.6-μm Isotropic Resolution.** Fig. 3 shows 2D planes perpendicular to $x$ and to $z$ from a 3D image of a sample of 9.2-μm silica beads in a capillary with 40.0-μm inner diameter, filled with partially protonated glycerol/water and doped with succinyl-DOTOPA triradicals as described above. Larger sets of 2D planes are shown in SI Appendix, Fig. S6. This image was acquired at 5 K with a total of 12,548 complex $k$-space points ($-21 \leq m_x \leq 20$, $-11 \leq m_y$, $m_z \leq 10$, with spherical sampling as described above), four scans per complex point, $\tau_{DNP} = 2.0$ s, and a total data acquisition time of 31.5 h (not including overhead for data transfer and reoptimization of RF amplitudes). Gradient increments were chosen to give equal resolution in $x$, $y$, and $z$, based on calibrations described above. Slice selection conditions were chosen to shift the region of nonzero image intensity away from $x = 0$ (to avoid artifacts discussed below) and to keep this region within $FOV_x$. 

PNAS 2022 Vol. 119 No. 22 e2201644119

https://doi.org/10.1073/pnas.2201644119
The actual fields of view in y and z were determined from the image itself by equating the number of points in the capillary diameter with 40 μm, resulting in \(FOV_y = FOV_z = 57.5 \mu m\) and implying a nominal spatial resolution of 2.6 μm. Assuming the same nominal spatial resolution in x, \(FOV_x = 109.8 \mu m\).

Silica spheres are clearly seen and clearly resolved from one another in Fig. 3. The actual SNR value is 16.6, defined as the ratio of the maximum image intensity within the capillary to the rms intensity in regions outside the capillary that contain only noise. It should be noted that \(\rho_{iso} = 2.6 \mu m\) is a small improvement over our previous results without DNP, in which \(\rho_{iso} = 2.8 \mu m\) was achieved at 28 K, using a microcoil with 150-μm inner diameter (5). The previous results required 208 h of measurements to produce SNR ≈ 11, with 0.50 s per scan and a Dy\(^{3+}\)-doped sample.

**Image with 1.7-μm Isotropic Resolution.** Fig. 4 shows 2D planes from a higher-resolution image of a similar sample, acquired at 5 K with a total of 32,102 complex k-space points \((-30 ≤ m_x ≤ 29, -16 ≤ m_y, m_z ≤ 15, \text{with spherical sampling})\), four scans per complex point, \(T_{DNP} = 2.0 s\), and a total data acquisition time of 80.6 h. Larger sets of 2D planes are shown in SI Appendix, Fig. S7. Again, gradient increments were chosen to give equal resolution in x, y, and z slice selection conditions were chosen to shift the region of nonzero image intensity away from \(x = 0\) and to keep this region within \(FOV_x\); k-space data were zero-filled to 256 × 128 × 128; and the real-space image was cyclically permuted to center the region of nonzero intensity. Maximum \(x\), \(y\), and \(z\) gradient currents were 88.0, 79.5, and 68.3 A, respectively. From the known capillary diameter of 40 μm, fields of view were calculated to be \(FOV_x = 52.3 \mu m\) and \(FOV_y = 54.6 \mu m\), implying a nominal spatial resolution of 1.7 μm. Assuming the same nominal spatial resolution in x, \(FOV_x = 100.2 \mu m\).

The inner wall of the capillary and the surfaces of the spheres are more sharply defined in Fig. 4 than in Fig. 3, consistent with higher spatial resolution. The actual SNR value of the image in Fig. 4 is 15.5. Compared with our previous results at 28 K without DNP (5), the SNR value is greater by a factor of 1.4, the data acquisition time is smaller by a factor of 2.6, and the voxel volume is smaller by a factor of 4.5.

**Baseline Corrections in k Space.** Images in Figs. 3 and 4 show minor artifacts near the origin (i.e., \(x = y = z = 0\)) due to low-frequency baseline distortions in the k-space data. In Fig. 3, the origin is shifted from image point (129, 65, 65) to image point (74, 69, 60) by the cyclic permutation described above. In Fig. 4, the origin is shifted to image point (199, 60, 97). Baseline distortions in k space arise from two sources. First, as gradient pulse amplitudes increase, the nonevolving, spin-locked component of nuclear spin polarization in the phase-encoding period varies due to changes in NMR frequency offsets that produce changes in the effective RF field direction during LG irradiation. Although gradient pulse shapes are modified to reduce spin locking along the effective field, as described above, RF inhomogeneity during \(\phi\) pulses and other pulse imperfections can still produce significant spinlocked components. This contribution is different for different gradient directions, and hence depends on both the magnitude and the direction of \(k\). Second, as data acquisition proceeds, slow drifts in RF amplitudes and temperature within the cryostat also cause the spin-locked component of nuclear spin polarization in the phase-encoding period to vary. Because our data were acquired in an “onion shell” manner in k space, proceeding monotonically from small \(|k|\) to large \(|k|\), this contribution to baseline distortions in k space is largely independent of the direction of \(k\).

The maximum amplitude of the spin-locked component is less than 5% of the total polarization, leading to baseline distortions that are less than 5% of the maximum NMR signal. Nonetheless, these distortions cause severe image artifacts if no correction is done (SI Appendix, Fig. S8A). To minimize these artifacts, k-space data were first symmetrized by replacing \(S(\mathbf{k})\) with \([S(\mathbf{k})+S(-\mathbf{k})]/2\). Next, functions proportional to \(k_x^2, k_y^2, k_z^2, k_x k_y, k_x k_z, k_y k_z\), and \(k_x^2 k_y^2\) were subtracted from the real part of \(S(\mathbf{k})\), and functions proportional to \(k_x, k_y, k_z\) were subtracted from the imaginary part of \(S(\mathbf{k})\), with coefficients that were optimized to minimize the total k-space signal power in the range 0.89 \(k_{max} ≤ |k| ≤ k_{max}\). These direction-dependent corrections reduced, but did not eliminate, the image artifacts (SI Appendix, Fig. S8B).

Finally, a direction-independent baseline-fitting function \(F(|\mathbf{k}|)\) was derived by calculating the average values of the real part of \(S(\mathbf{k})\) within each of 40 (Fig. 3) or 100 (Fig. 4) ranges of k-space radii, equally spaced in \(|k|^2\) from zero to \(k_{max}^2\), then performing cubic spline interpolation and smoothing of these average values as a function of \(|\mathbf{k}|\) (SI Appendix, Fig. S8 C and D). After subtraction of \(F(|\mathbf{k}|)\) from \(S(\mathbf{k})\), final images with only minor artifacts were obtained (Figs. 3 and 4 and SI Appendix, Fig. S8E).

**Verification of Image Resolution by Comparisons with Simulations.** Although the images in Figs. 3 and 4 have nominal isotropic spatial resolutions equal to 2.6 and 1.7 μm, respectively, the true resolution could be lower if the samples or gradient coils moved in response to strong gradient pulses, if NMR signals decreased over the many hours of data acquisition due to instrumental instabilities, or for other reasons. It is therefore important to assess the true resolution by comparing the experimental images with simulations. Two types of comparisons were performed, with results shown in Figs. 5 and 6. Additional details of image simulations are given in SI Appendix.

For Fig. 5, simulated images of silica spheres in glycerol/water were generated, using Gaussian point spread functions (i.e., Gaussian “blurring” of the spheres) with various values of \(\rho_{iso}\). According to the standard Sparrow criterion (36), \(\rho_{iso} = \Phi/\sqrt{2\ln 2}\) in this case, where \(\Phi\) is the full width at half maximum of the Gaussian function. The number of spheres and their approximate locations in the experimental images were first determined by running simulated annealing calculations to minimize the total squared deviation between experimental and simulated image intensities, using simulated images with \(\rho_{iso} = 1.0 \mu m\), a sphere diameter of 8.0 μm, a variable number of spheres, and variable coordinates for each sphere. Simulated annealing calculations used the standard Monte Carlo algorithm (37) to accept or reject changes in the number of spheres or their coordinates. The y and z coordinates were constrained to remain within the circular cross-section of the capillary. In a series of separate calculations, the x coordinates were constrained to remain within intervals of length 12.9 μm (30 points), starting at x points 95, 105, 115, 125, 135, or 145 (out of 256) for the image in Fig. 3, or within intervals of length 15.7 μm (40 points), starting at x points 90, 100, 110, or 120 for the image in Fig. 4. The overall scaling of intensities in the simulated images was optimized in each Monte Carlo step.

From multiple simulated annealing runs, coordinates for 36 spheres for the image in Fig. 3 and 32 spheres for the image in Fig. 4 were obtained. These coordinates were then used as initial conditions for the final simulated annealing calculations, in
which the number of spheres was kept constant, sphere diameters were set to 9.2 \( \mu \text{m} \), and spheres were constrained to remain within 3.0 \( \mu \text{m} \) of their initial positions. To prevent spheres from overlapping, repulsive potentials among all spheres, proportional to \( (r_u - r_j - 9.2 \mu \text{m})^2 \) for spheres \( u \) and \( v \) when \( r_u - r_j < 9.2 \mu \text{m} \), were included in the function that was minimized by the simulated annealing algorithm.

In the final calculations, the \( \rho_{\text{iso}} \) value in the simulated images was varied from 1.7 \( \mu \text{m} \) to 3.5 \( \mu \text{m} \) for comparisons with the experimental image in Fig. 3, and from 0.8 \( \mu \text{m} \) to 2.6 \( \mu \text{m} \) for comparisons with the experimental image in Fig. 4. Four independent runs were performed with each value of \( \rho_{\text{iso}} \). The squared deviation between experimental and simulated images was minimized within two (for Fig. 3) or four (for Fig. 4) separate ranges of \( x \) values in separate runs. Fig. 5A shows examples of 2D planes from the final simulated images with various values of \( \rho_{\text{iso}} \). Fig. 5B shows the dependence of the minimized squared deviation on \( \rho_{\text{iso}} \) for the experimental image in Fig. 3. The optimal value of \( \rho_{\text{iso}} \) is about 2.3 \( \mu \text{m} \) to 2.7 \( \mu \text{m} \), consistent with the nominal resolution of 2.6 \( \mu \text{m} \) explained above. Fig. 5C shows the dependence of the minimized squared deviation on \( \rho_{\text{iso}} \) for the experimental image in Fig. 4. The optimal value of \( \rho_{\text{iso}} \) is about 1.6 \( \mu \text{m} \) to 1.9 \( \mu \text{m} \), consistent with the nominal resolution of 1.7 \( \mu \text{m} \) explained above. Thus, a quantitative comparison of simulated and experimental images, using a Gaussian point spread function in the simulations, indicates no discrepancy between true and nominal spatial resolution values.

In the second approach to assessing the true resolution, we calculated the \( k \)-space data \( S_{\text{sim}}(\mathbf{k}) \) that would produce simulated images with 9.2-\( \mu \text{m} \)-diameter silica spheres at positions determined in the final simulated annealing calculations described above. \( S_{\text{sim}}(\mathbf{k}) \) was truncated to include only the \( k \)-space points that were sampled in experimental measurements. Zero filling and Fourier transformation of \( S_{\text{sim}}(\mathbf{k}) \) resulted in simulated images that appeared quite similar to the experimental images within the range of \( x \) coordinates where spheres were located, as shown in Fig. 6 A and B. Note that simulated images in Fig. 6 A and B differ from simulated images in Fig. 5A because the point spread function in Fig. 6 A and B matches the experimental point spread function, which is not a Gaussian function. We then calculated average 2D \( k \)-space power spectra \( W_x(|\mathbf{k}|) \) and \( W_z(|\mathbf{k}|) \) for the experimental and simulated images in planes perpendicular to \( x \) and \( z \), as shown in Fig. 6 C–F. These power spectra are defined by

\[
W_x(|\mathbf{k}|) \propto \sum_{x=x_1}^{x_2} \left| FT_x\{S(k_x, k_y, k_z)\} \right|^2 \delta(|\mathbf{k}| - \sqrt{k_y^2 + k_z^2})
\]

\[
W_z(|\mathbf{k}|) \propto \sum_{z=z_1}^{z_2} \left| FT_z\{S(k_x, k_y, k_z)\} \right|^2 \delta(|\mathbf{k}| - \sqrt{k_x^2 + k_y^2})
\]

[3]
where $x_1 \leq x \leq x_2$ and $z_1 \leq z \leq z_2$ are the ranges of image points within which the power spectra are averaged, $FT_x$ and $FT_z$ represent Fourier transforms with respect to $x$ and $z$, and the $\delta$-functions select combinations of $k_x$ and $k_z$ or $k_y$ and $k_y$ with a given $|k|$. Values of $|k|$ are binned for the plots in Fig. 6 C–F. For calculations of $W_z(|k|)$ from $S_{z\text{sim}}(k)$, intensities in the simulated images were multiplied by a Gaussian function of $x$ to approximate the effects of slice selection in the experimental images.

Simulated power spectra in Fig. 6 (red squares) were multiplied by overall scaling factors to produce agreement with the experimental power spectra (blue circles) at small values of $|k|/k_{\text{max}}$. Importantly, good agreement at larger values is also observed in all cases until the $k$-space power from background noise in the experimental images (cyan triangles) becomes comparable to the “signal power” from real intensity variations from the silica spheres. The experimental power then exceeds the simulated power, because the experimental power is the sum of signal power and noise power.

Plots in Fig. 6C show that the experimental signal power is substantially greater than the noise power at all values of $|k|/k_{\text{max}}$. Together with the good agreement between experimental and simulated power spectra, this implies that the true resolution of the image in Fig. 3 must be essentially the same as the nominal resolution (at least in $yz$ planes). Plots in Fig. 6E show that the signal power exceeds the noise power when $|k|/k_{\text{max}} \leq 0.9$. This implies that the true resolution of the image in Fig. 4 is at least $(1.7/0.9) \mu m \approx 1.9 \mu m$. For $|k|/k_{\text{max}} > 0.9$, the power in the experimental image is dominated by the noise contribution and therefore exceeds the power in the simulated
image, because the \( k \)-space signal power from 9.2-\( \mu \)m silica spheres above \( |k|/k_{\text{max}} = 0.9 \) is inherently small.

**Effects of \( k \)-Space Truncation on Experimental and Simulated Images.** A surprising feature of the experimental image in Fig. 4 is the presence of bright spots in the centers of each silica sphere. This feature is also present in the simulated images in Fig. 6 A and B. As shown in SI Appendix, Fig. S9, strong peaks of positive intensity in the centers of the spheres result from truncation of \( S(k) \) when the value of \( k_{\text{max}} = \pi/\rho \) is such that the ratio of \( \rho \) to the sphere radius \( R \) is \( \sim \eta = 0.39 \pm 0.02 \). These peaks are due to “truncation wiggles” that extend inward from the surface of each sphere in all directions and combine constructively at the center. At other values of \( \rho_{\text{max}} \) the image intensity at the centers of the spheres is negative, for example, when \( \rho_{\text{iso}}/R = 0.565 \) as in Fig. 3. As also shown in SI Appendix, Fig. S9, the values of \( \rho_{\text{iso}}/R \) at which truncation of \( S(k) \) produces either positive or negative intensities at the centers of the spheres are different if the data are sampled within a cube (i.e., \( -k_{\text{max}} \leq k_x, k_y, k_z < k_{\text{max}} \)) rather than within a sphere. Different sampling methods in \( k \) space result in point spread functions with different symmetries in the final images.

**Discussion**

**Summary of Results and Comparison with Previous Work.** Images in Figs. 3 and 4 demonstrate that 3D MRI images can be obtained at low temperatures with signal enhancements from DNP. DNP-enhanced 1D images obtained at 30 K were reported in an earlier paper from our laboratory (35). As shown in Figs. 3 and 4, DNP-enhanced 3D images with SNR \( \sim 16 \) and with \( \rho_{\text{iso}} = 2.6 \mu \text{m} \) or \( 1.7 \mu \text{m} \) can be obtained at 5 K in 31.5 h or 80.6 h with our current apparatus and measurement conditions. Comparisons with simulations in Figs. 5 and 6 indicate good agreement between nominal and actual values of \( \rho_{\text{iso}} \). Bright spots in the centers of dark spheres in Fig. 4 are also an indication that the experimental point spread function matches expectations for \( \rho_{\text{iso}} \approx 1.7 \mu \text{m} \).

The highest resolution in \( ^1\text{H} \) MRI near 300 K was obtained by Weiger et al. (4), who reported \( \rho_{\text{iso}} = 3.0 \mu \text{m} \) and SNR \( \sim 43 \) for an image of glass fiber spheres in \( \text{Cu}^{3+}\)-doped \( \text{H}_2\text{O} \), acquired at 18.8 T in 58 h with 0.10 s per scan. Somewhat earlier, Ciobanu et al. (3) reported an image of polymer beads in \( \text{Cu}^{2+} \) at 9.0 T with a somewhat larger value of \( \rho_{\text{iso}} \) (3). The discrepancy factor of \( \sim 40 \) between ideal and real SNR values is attributable to nonideal behavior of solenoidal coils as their diameters decreases below 1 mm (39, 40), additional noise from resistance in other RF circuit elements, reduction in the real value of \( \eta \) due to RF fields outside the RF coil, signal loss from transverse spin relaxation during the MRI pulse sequence, and a factor of \( 2^{1/2} \) from the need for separate acquisition of the real and imaginary parts of \( k \)-space data.

Compared with MRI images at 300 K with similar values of \( \eta, V, t_{\text{total}} \), and \( \rho_{\text{total}} \) DNP-enhanced MRI images at 5 K could, in principle, have greater SNR by a factor of \( 10^{-5} \text{NMR} = |w_{9900}/w_{9500}|^{1/2} / (300/5)^{1/2} \), where \( w_{9900}/w_{9500} \approx 0.1 \) (due to the slower buildup of polarization at 5 K compared with spin-lattice relaxation in paramagnetically doped water at 300 K), and \( w_{9900}/w_{9500} \approx 0.1 \), this expression predicts improvements in SNR by a factor of \( \sim 4,000 \). The predicted improvement should be reduced by factors of 2.4 from signal decay during \( \tau_{\text{LG}} \) 1.5 from the fact that signals were sampled during only 42% of \( \tau_{\text{PSL}} \) 3.6 from our use of a suboptimal value of \( \tau_{\text{DNP}} \) \( \sim 4 \) from our use of a standard preamplifier in the NMR spectrometer’s receiver with a noise temperature around 80 K, and \( \sim 10 \) from our use of partially protonated glycerol/water. With these corrections, the predicted improvement in SNR becomes a factor of \( \sim 8 \). In fact, we have achieved an improvement by a factor of 5 to 20 relative to previous micrometer-scale MRI experiments at room temperature (3, 4).

This analysis indicates several directions for further improvements: 1) Lower sample temperatures may lead to slower signal decays during \( \tau_{\text{LG}} \) and \( \tau_{\text{PSL}} \) (34), resulting in larger signals; 2) a cryogenic preamplifier may result in substantially lower noise levels; 3) paramagnetic dopants that are more efficient at very low temperatures may allow smaller values of \( \tau_{\text{DNP}} \) and provide larger values of \( \epsilon_{\text{DNP}} \). More efficient dopants may also allow higher protonation levels. A further increase in SNR by a net factor of 5 would permit \( \rho_{\text{iso}} = 1.0 \mu \text{m} \).

**Potential Applications.** With further improvements as outlined above, we believe it will become possible to apply DNP-enhanced, low-temperature MRI to real biological samples. One goal is to perform MRI on individual eukaryotic cells with sufficient resolution to visualize the internal structures of membrane-enclosed organelles, as well as phase-separated entities known as “membraneless organelles” (41). Obviously, a great deal is already known about the internal organization of eukaryotic cells from well-established methods of optical microscopy, fluorescence microscopy, and electron microscopy.
Nonetheless, because contrast mechanisms in MRI are different from contrast mechanisms in other forms of microscopy, it seems possible that MRI will provide information that is not otherwise accessible. In DNP-enhanced $^1$H MRI, contrast may be generated from variations in nuclear spin relaxation properties, or variations in $^1$H dipole–dipole coupling strengths, in addition to variations in local $^1$H densities. Double-resonance techniques may also be used to produce contrast, for example, by taking advantage of dipole–dipole couplings between $^{31}$P and $^1$H couplings to select $^1$H NMR signals from regions with higher or lower $^{31}$P concentrations. DNP-enhanced MRI may also be useful for characterizing the arrangement of different cell types within cell clusters, such as the α, β, and 6 cells within pancreatic islets (42). Applications to small tissue samples, such as samples from biopsies, may be possible and useful. DNP-enhanced MRI studies of small organisms that are commonly used as model systems in biology, or portions thereof, such as Caenorhabditis elegans worms and Drosophila melanogaster brains, may also prove to be fruitful. Extensive exploratory studies are needed before the true feasibility and scientific significance of these applications can be assessed.

Data Availability. Raw and processed data for Figs. 3 and 4, as well as Fortran programs for data processing and for simulations in Figs. 5 and 6, are available at https://doi.org/10.17632/vk344gdnwp.1.

ACKNOWLEDGMENTS. This work was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases, NIH. We thank Dr. Kent R. Thuber for helpful discussions and Dr. Wai-Ming Yau for synthesizing succinyl-DOTOPA.

1. E. W. McFarland, Time-independent point-spread function for NMR microscopy. Magn. Reson. Imaging 10, 269-279 (1992).
2. W. B. Hyslop, P. C. Lauterbur, Effects of restricted diffusion on microscopic NMR imaging. J. Magn. Reson. 94, 501-510 (1991).
3. L. Gobanu, D. A. Seeber, C. H. Pennington, 3D MRI microscopy with resolution 3.7 microm by 3.3 microm by 3.3 microm. J. Magn. Reson. 158, 178-182 (2002).
4. M. Weiger et al., NMR microscopy with isotropic resolution of 0.3 μm using dedicated hardware and optimized methods. Concepts Magn. Reson. Part B 6 338, 84-93 (2008).
5. H. Y. Chen, R. Tycko, Low-temperature magnetic resonance imaging with 2.8 μm isotropic resolution. J. Magn. Reson. 287, 47-55 (2018).
6. J. B. Agyau, S. J. Blackband, J. Scheininger, M. A. Mattingly, M. Hintemann, Nuclear magnetic resonance imaging of a single cell. Nature 322, 190-191 (1986).
7. R. W. Bowtell, G. D. Brown, P. M. Glover, M. McClure, P. Marsfield, Resolution of cellular structures by NMR microscopy at 11.7 T. Philos. Trans. R. Soc. Lond. Ser. A Math. Phys. Eng. Sci. 333, 457-467 (1990).
8. G. A. Johnson et al., Histology by magnetic resonance microscopy. Magn. Reson. O. 9, 1-30 (1993).
9. E. W. Hsu, N. R. Aiken, S. J. Blackband, Nuclear magnetic resonance microscopy of single neurons under hypothalamic perturbation. Am. J. Physiol. 271, C1985-C1990 (1996).
10. S. C. Grant et al., NMR spectroscopy of single neurons. Magn. Reson. Med. 44, 19-22 (2000).
11. R. A. Wind et al., An integrated confocal and magnetic resonance microscopy for cellular research. J. Magn. Reson. 147, 371-377 (2000).
12. L. Gobanu, C. H. Pennington, 20 micron-scale MRI of single biological cells. Solid State Nucl. Magn. Reson. 25, 139-147 (2004).
13. A. Pura, T. Neuberger, A. G. Webb, Simultaneous NMR microimaging of multiple single-cell samples. Concepts Magn. Reson. Part B 28B, 7-14 (2004).
14. S. C. Lee et al., In vivo magnetic resonance microscopy of differentiation in Xenopus laevis embryos from the first cleavage onwards. Differentiation 75, 84-92 (2007).
15. J. J. Flint et al., Cellular-level diffusion tensor microscopy and fiber tracking in mammalian nervous tissue with direct histological correlation. Neuroimage 52, 556-561 (2010).
16. N. Banax et al., Microscale-based MR phase imaging and mammalian enhanced microscopy of glial tumor neovessels with direct optical correlation. Magn. Reson. Med. 68, 86-97 (2012).
17. J. J. Flint, K. Menon, B. Hansen, J. Forder, S. J. Blackband, Visualization of live, mammalian neurons during Kainate-infusion using magnetic resonance microscopy. Neuroimage 219, 116997 (2020).
18. T. R. Cover, C. P. Slichter, Experimental verification of the Overhauser nuclear polarization effect. Phys. Rev. 102, 975-980 (1956).
19. C. T. Farrar, D. A. Hall, G. J. Gerfen, S. J. Inati, R. G. Griffin, Mechanism of dynamic nuclear polarization in high magnetic fields. J. Chem. Phys. 114, 4922-4929 (2001).
20. K. R. Thuber, R. Tycko, Prospects for sub-micron solid state nuclear magnetic resonance imaging with low-temperature dynamic nuclear polarization. Phys. Chem. Chem. Phys. 12, 5779-5785 (2010).
21. G. C. Chingas, J. B. Miller, A. N. Garroway, NMR images of solids. J. Magn. Reson. 66, 530-535 (1986).
22. D. G. Cory, J. B. Miller, A. N. Garroway, Time-suspension multiple-pulse sequences: Applications to solid-state imaging. J. Magn. Reson. 90, 205-213 (1990).
23. H. M. Cho, C. J. Lee, D. N. Shykred, D. P. Wientekamp, Nutation sequences for magnetic resonance imaging in solids. Phys. Rev. Lett. 55, 1923-1926 (1985).
24. F. Delucia, N. Lugen, S. Motta, G. Cammissa, B. Maraviglia, Full-bandwidth parameter distribution in line-narrowing solid-state imaging. J. Magn. Reson. A 115, 1-6 (1995).
25. F. Wengard, B. Blumich, H. W. Spiess, Application of nuclear magnetic resonance magic sandwich echo imaging to solid polymers. Solid State Nucl. Magn. Reson. 3, 59-66 (1994).
26. M. R. Tarasek, D. J. Goldfarb, J. G. Kempfi, Enhancing time-suspension sequences for the measurement of weak perturbations. J. Magn. Reson. 209, 233-243 (2011).
27. M. Lee, W. I. Goldberg, Nuclear magnetic resonance line narrowing by a rotating RF field. Phys. Rev. 140, 1261-1271 (1965).
28. E. D. Ostooff, J. S. Waugh, Multiple spin echoes and spin locking in solids. Phys. Rev. Let. 16, 1097-1098 (1966).
29. E. Moore, R. Tycko, Micron-scale magnetic resonance imaging of both liquids and solids. J. Magn. Reson. 260, 1-9 (2015).
30. K. N. Hu, H. H. Yu, T. M. Swager, R. G. Griffin, Dynamic nuclear polarization with biradicals. J. Am. Chem. Soc. 126, 10844-10845 (2004).
31. C. Sauvée et al., Highly efficient, water-soluble polishing agents for dynamic nuclear polarization at high frequency. Angew. Chem. Int. Ed. Engl. 52, 10859-10863 (2013).
32. W. M. Yau, K. R. Thuber, R. Tycko, Synthesis and evaluation of nitroxide-based oligoradicals for low-temperature dynamic nuclear polarization in solid state NMR. J. Magn. Reson. 244, 98-110 (2014).
33. W. M. Yau, J. Jeon, R. Tycko, Succinyl-DOTOPA: An effective triradical dopant for low-temperature dynamic nuclear polarization with high solubility in aqueous solvent mixtures at neutral pH. J. Magn. Reson. 311, 106672 (2020).
34. H. Y. Chen, R. Tycko, Temperature-dependent nuclear spin relaxation due to paramagnetic dopants below 30 K. Relevance to DNP-enhanced magnetic resonance imaging. J. Phys. Chem. B 122, 11733-11742 (2018).
35. H. Y. Chen, R. Tycko, Slice selection in low-temperature, DNP-enhanced magnetic resonance imaging by low-Goldberg spin-locking and phase modulation. J. Magn. Reson. 313, 106715 (2020).
36. C. M. Sparrow, On spectroscopic resolving power. Astrophys. J. 44, 76-86 (1916).
37. N. Metropolis, S. Ulam, The Monte Carlo method. J. Am. Stat. Assoc. 44, 335-341 (1949).
38. A. Abragam, Principles of Nuclear Magnetism (Oxford University Press, New York, 1961), pp. 71-84.
39. T. L. Peck, R. L. Magin, P. C. Lauterbur, Design and analysis of microcils for NMR microscopy. J. Magn. Reson. B 108, 114-124 (1995).
40. D. A. Seeber, R. L. Cooper, L. Gobanu, C. H. Pennington, Design and testing of high sensitivity microwave coil apparatus for nuclear magnetic resonance and imaging. Rev. Sci. Instrum. 72, 2175-2179 (2001).
41. E. Games, J. Shorter, The molecular language of membraneless organelles. J. Biol. Chem. 294, 7115-7127 (2019).
42. D. Bosco et al., Unique arrangement of α- and β-cells in human islets of Langerhans. Diabetes 59, 1202-1210 (2010).