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Low-Temperature Magnetic Resonance Imaging with 2.8 μm Isotropic Resolution

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Abstract

We demonstrate the feasibility of high-resolution ^1H magnetic resonance imaging (MRI) at low temperatures by obtaining an MRI image of 20 μm diameter glass beads in glycerol/water at 28 K with 2.8 μm isotropic resolution. The experiments use a recently-described MRI apparatus (*J. Magn. Reson.*, vol. 260, pp. 1–9, 2015) with minor modifications. The sample is contained within a radio-frequency microcoil with 150 μm inner diameter. Sensitivity is additionally enhanced by paramagnetic doping, optimization of the sample temperature, three-dimensional phase-encoding of k-space data, pulsed spin-lock detection of ^1H nuclear magnetic resonance signals, and spherical sampling of k-space. We verify that the actual image resolution is $2.7 \pm 0.3 \mu\text{m}$ by quantitative comparisons of experimental and calculated images. Our imaging approach is compatible with dynamic nuclear polarization, providing a path to significantly higher resolution in future experiments.

Graphical Abstract

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Keywords

magnetic resonance imaging; magnetic resonance microscopy; cryogenic NMR

1. Introduction

Although magnetic resonance imaging (MRI) is used primarily for anatomical and functional imaging of organs and organisms with centimeter-scale dimensions and with spatial resolution on the order of 1 mm, extension of MRI to smaller objects and to higher resolution (often called magnetic resonance microscopy) has been a long-standing goal [1–4]. Notable achievements include MRI of large cells [5–13], cell clusters [14], and tissue [15–20]. A series of experiments and technical developments by Pennington and coworkers [21–24] led to imaging of phantom samples with isotropic resolution below 3.7 μm [25]. Subsequently, isotropic resolution of 3.0 μm was achieved for phantom samples by Weiger *et al.* [26]

Higher spatial resolution has been achieved by unconventional magnetic resonance methods, including force-detected magnetic resonance [27–32] and optically-detected magnetic resonance based on diamond NV centers [33–35]. While these methods are certainly important, they are not directly competitive with conventional, inductively-detected MRI because they have limited capabilities for three-dimensional imaging of thick samples.

Spatial resolution in conventional MRI is limited primarily by the inherently low signal-to-noise ratio (SNR) of inductively-detected nuclear magnetic resonance (NMR). A 10 μm^3 volume of water contains fewer than 7×10^{11} ^1H spins, which are not readily detected at room temperature even with high-field magnets, radio-frequency (RF) microcoils, and optimized RF circuitry [2, 23, 36]. Therefore, it seems unlikely that MRI with isotropic resolution significantly better than 3.0 μm can be achieved at room temperature. In previous publications, we proposed that higher resolution can in principle be achieved at low temperatures [37, 38], where NMR signal strengths are enhanced by the larger spin polarizations at thermal equilibrium, where thermal noise is reduced, and where dynamic nuclear polarization (DNP) can provide further enhancements of nuclear spin polarizations [39–43].

Here we report the first demonstration of high resolution MRI at low temperatures. Using the MRI apparatus and pulse sequence method described in a recent publication [38], with minor modifications, we have obtained an image of glass beads in frozen glycerol/water at 28 K with nominal isotropic spatial resolution of 2.8 μm . The actual spatial resolution, based on the analysis described below, is 2.7 ± 0.3 μm . Thus, to our knowledge, the resolution of the image described below may be the highest reported to date for inductively-detected MRI at any temperature.

2. Materials and Methods

2.1. Sample and microcoil

The sample and radio-frequency (RF) microcoil are shown in Fig. 1a. Calibration-grade soda lime glass microspheres ($20 \pm 2 \mu\text{m}$ diameter, Cospheric LLC, catalog number S-SLGMS-2.5) were suspended in glycerol/water (1:1 w/w), which was doped with 4 mM dysprosium ethylenediamine tetraacetate (DyEDTA) by codissolving equimolar quantities of DyCl_3 and disodium EDTA in water. This sample was loaded into a fused silica capillary with 75 μm inner diameter and 150 μm outer diameter (Molex LLC) by injection with a syringe needle while plugging the opposite end with a 50 μm diameter glass fiber. Both ends of the capillary were sealed with Stycast epoxy (Lake Shore Cryotronics). A solenoid microcoil with 7.5 turns and 300 μm length was wound around the capillary by hand under an Olympus SZ61 microscope, using 20 μm bare copper wire (California Fine Wire Co.). The polyimide coating of the capillary was removed from the coil region prior to winding the coil. During the winding operation, the capillary was held in a home-made jig, shown in Fig. 1b. The microcoil was fixed to the capillary with transparent ethyl cyanoacrylate glue (Loctite 495). The spacing between turns of the microcoil was approximately equal to the wire diameter, because this configuration has been reported to provide optimal sensitivity [23]. The microcoil and sample volumes were approximately 5.3 nl and 1.3 nl, respectively. The two leads of the microcoil were soldered to thin copper strips that were connected to the rest of the radio-frequency (RF) circuit, which is depicted in Fig. 1c. The microcoil was placed as close to the center of the x, y and z magnetic field gradients as possible, based on visual inspection under the microscope (see below).

2.2. Low temperature MRI apparatus

Essential details of our MRI apparatus are given in a previous paper [38]. Briefly, the RF microcoil and three orthogonal gradient coils are assembled on a series of stacked, rectangular sapphire plates (31.5 mm $\times$ 6.10 mm $\times$ 1.52 mm), with machined grooves to accommodate the RF microcoil and the x and y gradients (O'Keefe Ceramics, Inc.). The stack of rectangular plates is mounted on a stack of five larger, square sapphire plates (31.5 mm $\times$ 31.5 mm $\times$ 5.08 mm each). The entire assembly is mounted on the temperature-controlled copper block of a Janis Supertran ST-200 continuous flow cryostat (Janis Research LLC), using 4–40 threaded brass rods that pass through holes in the sapphire plates and brass nuts to clamp the sapphire plates together. A thin coating of vacuum grease (Apiezon N) is applied to the sapphire surfaces to ensure good thermal contact.

The arrangement of sapphire plates in experiments described below differs somewhat from the arrangement in the previous paper [38] and is shown in Fig. S1b. Most importantly, plates that hold the upper and lower z gradient coils are swapped. The distance between the two z gradient coils is thereby reduced by approximately 1 mm.

A ceramic chip capacitor (10 pF, American Technical Ceramics, ATC100A1-0JPN150X) is soldered across the copper strips from the RF microcoil to increase its effective inductance (estimated to be 3 nH for the microcoil alone). A half-wavelength (24.8 cm long, 6.35 mm outer diameter) semi-rigid coaxial cable connects the microcoil and chip capacitor to

variable tuning and matching capacitors (Polyflon models, RP/VC3-6-05), which are mounted on a plate that is attached to the central stainless steel tube of the cryostat. Tuning and matching capacitors were modified for low temperature operation by reducing the diameters of their inner plungers, to prevent seizing due to thermal contraction of the outer Teflon dielectric. Tuning rods connect the variable capacitors to rotary feedthroughs at the top of the cryostat. During low-temperature operation, the interior of the cryostat is evacuated (HiCube 80 Eco pumping station, Pfeiffer Vacuum, Inc.), so that the entire RF circuit, sample, gradient coils, and other components are in high vacuum (approximately 0.2 mTorr). The stack of sapphire plates and the temperature-controlled copper block are contained within a radiation shield to reduce radiative heating from the outer stainless steel can of the cryostat. The temperature of the copper block is controlled by a Stanford Research Systems CTC100 unit, which reads the temperature from a Cernox sensor (Lake Shore Cryotronics, CX-1070) in the copper block and supplies current to heater coils in the cryostat. Liquid helium is supplied to the cryostat through a cryogenic transfer line (Cryo Industries of America, Inc.), which includes a needle valve to control helium flow. Liquid helium consumption at a nominal sample temperature of 28 K is approximately 0.55 l/h.

The cryostat inserts from above into the 89 mm room-temperature bore of a 9.38 T superconducting magnet (399.25 MHz ^1H NMR frequency). RF and gradient pulse sequences are applied and signals are acquired with a Tecmag Redstone spectrometer, connected to AE Techtron model 7548 gradient power amplifiers for the three gradient channels and an American Microwave Technologies RF power amplifier. Series-connected 1 Ω resistors are placed at the outputs of the three gradient amplifiers. LC low-pass filters inside the cryostat prevent RF pickup in the gradient cables from interfering with NMR signals. The spectrometer receiver noise figure was determined to be 1.9 dB, measured at the point where a coaxial cable to the receiver is connected to the RF circuit in the cryostat. At a nominal sample temperature of 28 K, the root-mean-squared noise in NMR signals from our cryostat is reduced by approximately 2.7 times relative to 300 K.

Gradient amplitudes (in Hz/ μm -A) were calibrated using 1D images of glycerol/water sample with known dimensions at room temperature as described in our previous work [38]. Calibrations at room temperature and at low temperatures showed no significant differences, indicating negligible effects on gradients from eddy currents (which might be expected to become more significant at low temperatures, where the conductivity of copper components of the cryostat and RF circuit increases).

2.3. Pulse sequence for 3D imaging

Fig. 2 shows the pulse sequence for three-dimensional (3D) ^1H MRI. To help suppress 1H NMR signals originating from material outside the microcoil, a single π_x pulse (the first striped rectangle in Fig. 2) is applied on alternating scans after the recycle delay period τ_{rec} , such that only ^1H magnetization near the center of the microcoil is fully inverted. Transverse magnetization dephases during the period $\tau_1 = 1.0$ ms. Frequency-switched Lee-Goldburg (FSLG) decoupling [38, 44, 45] is then applied during the constant-time phase-encoding period, beginning with a θ_x pulse [with $\theta = \pi/2 + \theta_m$, where $\theta_m = \cos^{-1}(1/\sqrt{3})$ is the “magic angle”] to prepare magnetization perpendicular to the rotating-frame Lee-Goldburg

effective fields in the yz plane. During subsequent +LG and -LG periods, RF pulses with length $\tau_{LG}/4$, phases y and $-y$, and frequency offsets $\pm \nu$ are applied, with RF field amplitudes of 226 kHz and $\nu = 160$ kHz so that the rotating-frame effective field is tilted from the z direction by angle θ_m . An on-resonance π_x pulse (180 kHz RF field amplitude) is applied in the middle of the FSLG period to refocus small NMR frequency variations due to static field inhomogeneities. At the end of the FSLG period, either a θ_{-x} pulse alone or the combination of a θ_{-x} pulse followed by a $\pi/2_{-y}$ pulse stores either the “real” component or the “imaginary” component of precessing magnetization (about the Lee-Goldburg effective field) as longitudinal magnetization. After allowing transverse magnetization to dephase during the period $\tau_2 = 1.0$ ms, NMR signals are excited by a $\pi/2$ pulse with phase ϕ and detected during a pulsed spin-locking (PSL) period [25, 46, 47], consisting of a train of 1.0 μ s pulses with flip angle α and phase y , separated by periods $\tau_3 = 4.2$ μ s. Phase cycling with $\phi = x, x, -x, -x$, alternating application of the initial π_x pulse, and coaddition of signals from four successive scans according to the pattern $+, -, +, -$ removes baseline offsets of the NMR signals and minimizes ring-down artifacts from the PSL pulses.

During the constant-time phase-encoding period, field gradient pulses are applied simultaneously in the x , y , and z directions, with lengths $\tau_{ph}/2$ and with opposite signs before and after the π_x refocusing pulse. Gradient pulses are switched off 30 μ s before the beginning of on-resonance π_x and θ_{-x} RF pulses, to ensure that gradient currents have fully dissipated before the RF pulses are applied.

In the sense of average Hamiltonian theory [48], Lee-Goldburg irradiation scales the strength of ^1H - ^1H magnetic dipole-dipole couplings by the factor of $\cos(\xi)$ where ξ is the angle between the rotating-frame effective field and the z direction. When a gradient pulse is applied, the NMR frequency at a displacement $\mathbf{r}$ from the center of the gradients (*i.e.*, from the point in space where gradient pulses produce no change in the z component of the magnetic field) is shifted from the nominal on-resonance frequency by the quantity $\gamma \mathbf{g} \cdot \mathbf{r}$, where $\mathbf{g} = (g_x, g_y, g_z)$ is the vector of field gradients in the three orthogonal directions and γ is the nuclear gyromagnetic ratio ($2\pi \times 42.577$ MHz/T for ^1H). The Lee-Goldburg field direction then becomes $\xi = \cos^{-1}[(1+\delta)/\sqrt{3+2\delta+\delta^2}]$, where $\delta = \gamma \mathbf{g} \cdot \mathbf{r}/\nu$. To lowest order in δ , the scaling factor becomes $2\delta/3$. Thus, as gradient amplitudes increase during the phase-encoding period, Lee-Goldburg irradiation becomes decreasingly effective in regions of the sample that are relatively far from the center of the gradients. In principle, this can lead to a lowering of spatial resolution (compared to the nominal resolution) in such regions. As shown in Fig. 3a, under our experimental conditions, maximum unscaled offsets greater than 20 kHz are potentially problematic, corresponding to $|\mathbf{r}| > 43$ μ m with maximum effective gradients of 270.6 Hz/ μ m. It is therefore desirable to position the sample and RF microcoil so that the center of the RF microcoil coincides with the center of gradients.

In practice, precise positioning of the RF microcoil is difficult. To compensate for imperfect positioning of the microcoil, we therefore replaced the Lee-Goldburg frequency offsets $\pm \nu$ with the quantities $\pm[\nu - \gamma(a_x g_x + a_y g_y + a_z g_z)]$ in the first half of the phase-encoding period and the quantities $\pm[\nu + \gamma(a_x g_x + a_y g_y + a_z g_z)]$ in the second half of the phase-encoding period. Lee-Goldburg offsets were therefore different in the four $\tau_{LG}/4$ periods in Fig. 2 and

different for different k -space points, with $\mathbf{k} = \gamma \mathbf{g} \tau_{\text{ph}}$. In effect, this shifts the center of the gradients by a_x , a_y and a_z . As shown in Figs. 3b–3d, values of a_x , a_y , and a_z were determined empirically in 1D imaging experiments, *e.g.*, by varying a_x in a 1D image in which only x gradient pulses were applied until the image was moved to the center of the 1D field of view (FOV). For the final high-resolution 3D image described below, the a_x and a_z values were 23.4 μm and $-44.5 \mu\text{m}$. The a_y value was set to zero.

2.4. Spherical k -space sampling

To obtain a 3D image using a pure phase-encoding scheme, a four-dimensional measurement is performed to collect signals $F_R(\mathbf{k}, t)$ and $F_I(\mathbf{k}, t)$ (corresponding to real and imaginary magnetization components at the end of the phase-encoding period) at Cartesian grid points in the 3D k -space, where t is the time during NMR signal acquisition with PSL. In other words, the pulse sequence in Fig. 2 is repeated with a constant phase-encoding time τ_{ph} and with different combinations of x , y and z gradient amplitudes g_x , g_y , g_z . The maximum gradient pulse amplitude is dictated by the desired nominal resolution ρ_{nom} according to the expression $\rho_{\text{nom}} = (\gamma g_{\text{max}} \tau_{\text{ph}} / \pi)^{-1} = (k_{\text{max}} / \pi)^{-1}$. The field of view in each direction is dictated by the gradient amplitude increment δg in that direction according to $\text{FOV} = (\gamma \delta g \tau_{\text{ph}} / 2\pi)^{-1} = (\delta k / 2\pi)^{-1}$. In experiments described below, each gradient amplitude was varied between $-g_{\text{max}}$ and $g_{\text{max}} - \delta g$, with $\gamma g_{\text{max}} / 2\pi = 270.6 \text{ Hz}/\mu\text{m}$ and $\tau_{\text{ph}} = 660 \mu\text{s}$ to produce a data set with $\rho_{\text{nom}} = 2.8 \mu\text{m}$ in all three directions. k -space increments $(\delta k / 2\pi)$ in x , y , and z were 4.59 mm^{-1} , 10.5 mm^{-1} , and 10.5 mm^{-1} , producing a 3D FOV of $218 \mu\text{m} \times 95 \mu\text{m} \times 95 \mu\text{m}$. These gradient amplitude values include the scaling factor of $1/\sqrt{3}$ produced by Lee-Goldburg irradiation [44, 48].

If all three dimensions of k -space were sampled over their full ranges and independently of one another, corresponding to a cube in k -space, acquisition of a complete data set would require a total of $8(2g_{\text{max}})^3 / (\delta g_x \delta g_y \delta g_z)$ scans. The factor of 8 in this expression comes from the fact that $F_R(\mathbf{k}, t)$ and $F_I(\mathbf{k}, t)$ are acquired separately, each with the four-step phase cycle described above. For the current experimental conditions, this would be a total of 721,344 scans. To reduce this number, we did not acquire k -space points outside a sphere of radius k_{max} , *i.e.*, points for which $g_x^2 + g_y^2 + g_z^2 > g_{\text{max}}^2$. For these points, $F(\mathbf{k}, t)$ was set to zero. Sampling a sphere in k -space, rather than a cube, reduced the total number of scans for one complete data set to 377,184. As shown in Fig. S2, spherical k -space sampling affects the 3D point spread function of the image, but does not degrade the resolution significantly. We emphasize that k -space points were sampled on a Cartesian grid, but only within a sphere centered at the origin.

Data acquisition by the Tecmag spectrometer was controlled by an external Python script, which calculated a list of gradient amplitude parameters (*i.e.*, k -space points) for which data were collected. This list started with small $|\mathbf{k}|$ values and increased to $|\mathbf{k}| = k_{\text{max}}$, so that data could be previewed at low resolution as image acquisition proceeded.

2.5. Image processing

For each $\mathbf{k}$ point, $F_R(\mathbf{k}, t)$ and $F_I(\mathbf{k}, t)$ were Fourier transformed with respect to t after matched apodization (65 Hz Lorentzian line broadening), then phase corrected and baseline

corrected to yield frequency-domain signals $S_R(\mathbf{k}, \omega)$ and $S_I(\mathbf{k}, \omega)$. Signal amplitudes at $\omega = 0$, proportional to the area under the time-domain PSL signal, were used to construct the complex k-space signal $G(\mathbf{k}) = \text{Re}[S_R(\mathbf{k}, 0)] + i\text{Re}[S_I(\mathbf{k}, 0)]$. A third-order polynomial baseline correction was applied to $G(\mathbf{k})$ in the k_x dimension before Fourier transformation with respect to k_x . Linear baseline corrections were then applied in the k_y and k_z dimensions before Fourier transformation with respect to k_y and k_z . The k-space data were zero-filled in each dimension and Fourier transformed with respect to k_x , k_y , and k_z to yield the 3D image $I(\mathbf{r})$. The size of this image is $156 \times 68 \times 68$ and thus the digital resolution is $1.4 \mu\text{m}$.

The signal-to-noise ratio (SNR) of the image is defined as the average image intensity in regions containing water/glycerol divided by the standard deviation of image intensities in the background regions. For this purpose, signal and background intensities from several planes in the 3D image (Figs. S3 and S4) were extracted and used to calculate the SNR, with a customized script in ImageJ software (available at <https://imagej.nih.gov/ij/>).

2.6. Analysis of image resolution

We evaluated the actual image resolution by comparing the experimental 3D image $I(\mathbf{r})$ with calculated images $C(\mathbf{r}; \mathbf{r}_0, \beta)$ for a $20 \mu\text{m}$ glass sphere in glycerol/water that is centered at position $\mathbf{r}_0$ and “blurred” by an amount β . Blurred images were calculated according to

$$C(\mathbf{r}; \mathbf{r}_0, \beta) = \frac{1}{N} \sum_{j=1}^N \exp[-(\mathbf{r}_j - \mathbf{r}_0)^2 / \beta^2] W(\mathbf{r}; \mathbf{r}_j) \quad (1)$$

where $W(\mathbf{r}; \mathbf{r}_j)$ is an ideal image for a $20 \mu\text{m}$ glass sphere centered at position $\mathbf{r}_j$. $N = 1000$ ideal images were calculated on a 3D Cartesian grid of volume elements (voxels), with $1.4 \mu\text{m}$ grid spacings to match the experimental image and with randomly chosen positions $\mathbf{r}_j$. For each ideal image, $W(\mathbf{r}; \mathbf{r}_j)$ was set to 1.0 (or 0.0) for all voxels that lie entirely inside (or outside) a $10 \mu\text{m}$ radius from $\mathbf{r}_j$. For other voxels, $W(\mathbf{r}; \mathbf{r}_j)$ was set to the fraction of the voxel volume that lies inside this radius, approximated by dividing each voxel into $15 \times 15 \times 15$ subvoxels.

Comparisons between experimental (I) and calculated images (C) were based on the reduced chi-squared function, defined by

$$\chi_\nu^2(\mathbf{r}_0, \beta) = \frac{1}{\nu \sigma^2} \sum_{m=1}^M [I_m - \lambda(\beta, \mathbf{r}_0) C_m(\beta, \mathbf{r}_0)]^2 \quad (2)$$

where M is the number of voxels in the calculated image, σ^2 is the mean squared noise in the experimental image, $\nu = M - 4$ is the number of degrees of freedom, and

$\lambda(\beta, \mathbf{r}_0) = \sum_{m=1}^M I_m C_m(\beta, \mathbf{r}_0) / \sum_{m'=1}^M C_{m'}(\beta, \mathbf{r}_0)^2$ is the optimal scaling factor for the calculated image.

3. Results

3.1. Optimization of the signal-to-noise ratio

For a fixed total data acquisition time, a variety of factors affect the final image SNR, including the sample temperature, the paramagnetic doping of the sample, and the PSL and FSLG conditions. Paramagnetic doping reduces the ^1H spin-lattice relaxation time T_1 , which otherwise greatly exceeds 10 s for frozen glycerol/water. Based on earlier low-temperature NMR studies [49, 50], we chose to use DyEDTA as the dopant, because a relatively low concentration of DyEDTA produces T_1 values less than 1 s. Fig. 4a shows that T_1 has a minimum value of 0.3 s near 40 K for glycerol/water (1:1 w/w) with 4 mM DyEDTA. Fig. 4b shows that the PSL-detected ^1H NMR signal amplitude, measured with $\tau_{\text{rec}} \gg T_1$, is approximately inversely proportional to the nominal sample temperature T for $T > 25$ K. Below 25 K, the observed deviation from this Curie-law behavior is attributable to heat that is conducted to the sample through the wires that carry gradient pulses and the coaxial half-wavelength cable in the RF circuit, which leads to a discrepancy between the nominal and actual sample temperatures. Fig. 4c shows the temperature dependence of $\text{SNR}/T_1^{1/2}$, where SNR is the signal-to-noise ratio in the PSL-detected 1D spectrum (including temperature dependence of the noise). The maximum of $\text{SNR}/T_1^{1/2}$ occurs near $T = 28$ K.

With the pulse sequence in Fig. 2 and the image processing method described in Materials and Methods, the image SNR is proportional to the frequency-domain SNR in $S_R(\mathbf{k}=0, \omega)$ with matched apodization, which depends strongly on the PSL conditions. Important parameters include the PSL flip angle α , the average RF amplitude during the PSL period, and the fraction of time that signals are digitized during the PSL period. To optimize the SNR, we fixed the PSL pulse length at 1.0 μs , with 0.2 μs and 2.0 μs periods for unblanking and blanking of the ^1H RF power amplifier before and after each PSL pulse. Our Tecmag spectrometer digitizes signals at a 50 MHz rate and then applies downsampling and digital filtering to achieve a nominal dwell time t_d . Signals were digitized for one nominal dwell time before each unblanking period, and a total of 32,768 complex signal points were acquired (*i.e.*, 32,768 PSL pulses). Fig. 5a shows the dependence of the SNR in $S_R(\mathbf{k}=0, \omega)$ from a single scan (called SNR_1) on the PSL pulse amplitude for t_d equal to 2.0 μs , 4.0 μs , and 6.0 μs , corresponding to RF duty factors of 19.2%, 13.9%, and 10.9%, and to signal digitization duty factors of 38.5%, 55.6%, and 65.2%, respectively. The best results were obtained with a $t_d = 2.0$ μs and with an average RF amplitude near 33 kHz, corresponding to PSL pulses with 172 kHz amplitudes and $\alpha \approx 62^\circ$. From Fig. 5a, it is clear that the effectiveness of PSL is quite sensitive to α . Examples of time-domain PSL signals with $t_d = 2.0$ μs and different values of α are shown in Fig. 5b.

The image SNR is also sensitive to signal decay during the constant-time phase-encoding period due to imperfect Lee-Goldburg decoupling. We optimized the Lee-Goldburg decoupling empirically at low temperatures by varying the RF amplitude during the +LG

and $-LG$ periods with τ_{LG} fixed at 160 kHz, using the pulse sequence in Fig. 2 with no gradient pulses. As shown in Figs. 5c–5e, at the optimal RF amplitude, the characteristic signal decay time was about 1.0 ms for DyEDTA-doped glycerol/water at 28 K. The optimal RF amplitude, calibrated by nutation experiments at low temperature with PSL detection, was 212 kHz, close to the ideal value of 226 kHz. Deviation from the ideal value is attributable to uncertainties in the RF amplitude calibration and to RF inhomogeneity, as well as imbalances between $+LG$ and $-LG$ periods.

During long imaging experiments, we reoptimized the Lee-Goldburg RF amplitude every 30 min, using a module within the Python script that controlled data acquisition. This module varied the RF amplitude and determined the value that produced the largest PSL-detected signal at $\mathbf{k} = 0$.

3.2. Estimated image measurement time

For an image of glass beads in glycerol/water obtained with the pulse sequence in Fig. 2, the SNR for one voxel (called SNR_v) is related to SNR_1 by the expression

$SNR_v = SNR_1 \sqrt{n/2/(N_v f)}$, where n is the total number of scans used to obtain the image, N_v is the number of voxels that contribute to the image, and f is the fraction of voxels that contain glycerol/water. In this expression, SNR_1 includes signal decay during the phase-encoding period and includes signal reduction by the factor $1 - \exp(-\tau_{rec}/T_1)$. The total measurement time is $n\tau_{rec}$, ignoring small contributions from τ_1 , τ_{LG} , τ_2 , and τ_3 periods in Fig. 2. According to data in Fig. 5, SNR_1 is approximately 200 with $\tau_{LG} = 720 \mu s$ and $\tau_{rec} = 0.5$ s at 28 K. The total sample volume that contributes to the image is about $6 \times 10^5 \mu m^3$, taking into account the fact that the strong sensitivity of FSLG to RF amplitude shown in Fig. 5e selects a volume that is about half the length of the RF microcoil. Then $N_v \approx 6 \times 10^5 / \rho_{nom}^3$, where the nominal image resolution ρ_{nom} is given in μm units. If $f \approx 0.7$, then an image with resolution ρ_{nom} and $SNR_v = 5$ requires $n \approx 2.2 \times 10^8 / \rho_{nom}^6$ scans. If $\rho_{nom} = 2.8 \mu m$, the image measurement time should be approximately 64 h.

3.3. MRI with nominal 2.8 μm isotropic resolution at 28 K

We acquired images of glass beads in DyEDTA-doped glycerol/water at 28 K, using $\tau_{rec} = 0.5$ s, $\tau_{ph} = 660 \mu s$, $\tau_{LG} = 720 \mu s$, and $k_{max}/2\pi = 0.179 \mu m^{-1}$ (2.8 μm nominal resolution in all dimensions), with eight scans per complex k -space point (four scans each for real and imaginary parts) and with $\delta k/2\pi = 4.59 \text{ mm}^{-1}$, 10.5 mm^{-1} , and 10.5 mm^{-1} in the x , y , and z directions ($218 \mu m \times 95 \mu m \times 95 \mu m$ field of view). With spherical sampling of k -space as described above, one complete image required 47,148 k -space points, or 52 h of measurements. In practice, the measurement time was further increased by a factor of 1.4, due to additional time required for data transfers from the Tecmag Redstone spectrometer to the computer that controls this spectrometer and for periodic re-optimization of the Lee-Goldburg RF amplitude as discussed above. Four complete images were recorded with identical conditions and were summed to produce the final image. Data were processed with zero-filling in k_x , k_y , and k_z dimensions, resulting in 1.4 μm digital resolution.

Examples of 2D planes from the 3D image are shown in Fig. 6. Full sets of 2D planes perpendicular to x , y , and z are shown in Figs. S3 and S4 of supporting information. The

SNR for voxels with the strongest signals is 11 ± 1.2 (one standard deviation), in good agreement with the estimate explained above. Image intensities change from maximum to minimum values within three digital points, consistent with $\rho_{\text{nom}} = 2.8 \mu\text{m}$. The glass beads appear clearly, with apparent diameters close to the expected $20 \mu\text{m}$ value. The inner wall of the capillary tube appears as a straight boundary along x in planes perpendicular to y and z (Figs. 6a, 6c, and S3), and the cross-section of the capillary tube appears round in planes perpendicular to x (Figs. 6b and S4), indicating negligible distortions from non-linearities in field gradients or other sources.

Although the capillary tube was filled with glycerol/water, only a $150 \mu\text{m}$ long portion of the sample, approximately half the length of the RF microcoil, appears in the image. We attribute this effect to rapid decay of signals from regions of the sample near the ends of the microcoil during the phase-encoding period, due the sensitivity of FSLG to RF field amplitude (Fig. 5e). Even within the visible portion of the sample, variations of the RF field amplitude along the x direction produce variations in image intensities (Fig. S3).

A band of low image intensity at $x \approx 105 \mu\text{m}$ (Figs. 6a and 6c) is an artifact attributable to polynomial baseline correction of $G(\mathbf{k})$ in the k_x direction before Fourier transformation with respect to k_x . This baseline correction was required to minimize the circular “ringing” artifact that is evident in Figs. 6c, S3, and S4. The ringing artifact is attributable to nuclear spin magnetization that is parallel to the Lee-Goldburg effective fields during the phase-encoding period in Fig. 2, and that consequently does not evolve with $\mathbf{k}$ in the ideally expected manner. Although the flip angle θ in Fig. 2 was adjusted to place the spin magnetization perpendicular to the Lee-Goldburg effective fields, perfect elimination of parallel magnetization components was not possible, especially for large values of $|\mathbf{k}|$, where the directions of the Lee-Goldburg effective fields vary with position within the sample.

3.3. Quantitative analysis of actual image resolution

We analyzed image regions around individual glass beads to determine the actual resolution of the experimental image. In principle, the actual resolution ρ_{act} may differ from ρ_{nom} if the MRI apparatus is not mechanically stable (*e.g.*, if the gradient coils or RF microcoil move when gradient pulses are applied), if signal decay during the constant-time phase-encoding period varies with gradient pulse amplitude as discussed above, or possibly for other reasons. As illustrated in Fig. 7a, selected regions of the experimental image were compared with calculated images of $20 \mu\text{m}$ diameter spheres with different image resolutions, represented by blurring factors β in Eq. (1). Each region was represented by three 2D planes, limited by the presence of nearby beads. The ten beads that were analyzed are indicated in Figs. S3 and S4. To quantify the similarity between experimental and calculated images, reduced chi-squared values $\chi_{\nu}^2(\mathbf{r}_0, \beta)$ were calculated as functions of the sphere center position $\mathbf{r}_0$ and β using Eq. (2). For each of the ten beads, the value β_{min} at the global minimum of $\chi_{\nu}^2(\mathbf{r}_0, \beta)$ was used to produce an estimate of the actual image resolution according to $\rho_{\text{act}} = \sqrt{2}\beta_{\text{min}}$. This definition of ρ_{act} corresponds to the Sparrow criterion for distinguishing two points in an image [51] when the point-spread functions are Gaussian functions of the form $\exp[(x-x_0)^2/\beta^2]$.

Fig. 7b shows the dependence of χ_v^2 on β , minimizing over for each choice of β , for one of the selected beads (bead number 3 in Fig. S3). In this case, $\beta_{\min} = 1.8 \pm 0.3 \mu\text{m}$, using the range of β values for which χ_v^2 is within 15% of its minimum value to define the error limits on $\beta_{\min}$ (± 1 standard deviation). Fig. 7c shows values of ρ_{act} estimated from image regions around each of the ten beads. The average value of ρ_{act} was found to be $2.7 \mu\text{m}$ (with $0.3 \mu\text{m}$ standard error) in good agreement with the nominal resolution determined by the pulse sequence parameters and gradient pulse amplitudes.

4. Discussion

Experiments described above demonstrate the feasibility of high resolution ^1H MRI at low temperatures. The $2.8 \mu\text{m}$ isotropic resolution achieved in these experiments is slightly higher than isotropic resolutions reported in previous experiments at room temperature by Pennington and coworkers [25] and by Weiger *et al.* [26]. Compared with experiments at room temperature, imaging at low temperatures results in an approximate 30-fold enhancement of the inherent SNR, due to larger nuclear spin polarizations at thermal equilibrium and lower thermal noise. The more rapid signal decay during the PSL detection period (by a factor of approximately 40), the smaller duty factor for signal digitization during the PSL detection period (by a factor of approximately two) and the larger signal loss in the phase-encoding period (by a factor of approximately two) counteract this SNR enhancement, resulting in a net gain by a factor of approximately $30 \times (40 \times 2)^{-0.5} \times 0.5 \approx 1.7$. All other things being equal, this net gain in SNR would permit an enhancement in isotropic image resolution by a factor of 1.2. In practice, differences in microcoil diameter, RF circuit efficiency, and external magnetic field strength also contribute to differences in SNR, resolution, and total data acquisition time between our low temperature experiments and previous room temperature experiments.

Importantly, the resolution of low temperature MRI can be further improved by DNP [37]. In principle, DNP can enhance the SNR by a factor of 100 or more, leading to an improvement in isotropic resolution by factor of four or more. In practice, the extent of this improvement will depend on several factors, including the efficiency of microwave transmission through the RF microcoil to the sample, the build-up time for DNP-enhanced nuclear spin polarization, and the effects of DNP dopants on signal decay during PSL and phase-encoding periods. These factors are currently under investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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High sensitivity at low temperatures leads to high resolution MRI

Lee-Goldburg decoupling leads to manageable gradient requirements

Pulsed spin-lock detection and use of a microcoil further enhance sensitivity

Nominal 2.8 μm resolution verified by quantitative comparison with simulated images

Figure 1.

(a) Microscope photograph of the RF microcoil and MRI sample, consisting of a capillary tube containing glass beads immersed in glycerol/water, doped with DyEDTA. (b) Photograph of the jig used to hold the capillary tube during the microcoil winding operation. Yellow arrow points to the capillary tube. (c) RF circuit diagram, with L_0 being the microcoil inductance, C_0 being a ceramic chip capacitor that is soldered across the microcoil leads, and C_M and C_T being variable matching and tuning capacitors, which are contained within the cryostat and connected to the microcoil by a half-wavelength coaxial cable. Elements in red are the transmit/receive network of the spectrometer, located outside the cryostat.

Figure 2.

Pulse sequence for 3D phase-encoded MRI, including ^1H RF pulses and gradient pulses. In the current experiments, delays τ_1 and τ_2 for dephasing of transverse ^1H magnetization are 1.0 ms. Spin-lock pulses with flip angle α are separated by delays τ_3 during which signals are digitized. The Lee-Goldburg preparation pulse θ has flip angle 144.7° . FSLG and phase-encoding periods are τ_{LG} and τ_{ph} , respectively. The recycle delay is τ_{rec} . The first π pulse is applied only on odd-numbered scans, for background suppression. The first $\pi/2$ pulse is applied only for acquisition of the imaginary part of phase-encoded signals. Other parameters are described in the text.

Figure 3.

(a) Dependence of the ^1H NMR signal amplitude on the NMR frequency offset during FSLG decoupling. Data were acquired at 28 K using the pulse sequence in Fig. 2, with $\tau_{\text{LG}} = 720$ μs , $\tau_{\text{ph}} = 660$ μs , and $\gamma = 160$ kHz. A DyEDTA-doped glycerol/water sample without glass beads was used. For these data, field pulses with increasing amplitude were applied with the z gradient channel of the MRI apparatus, after configuring the z gradient coils in the MRI head to produce a uniform magnetic field at the sample, rather than a field gradient. (b,c,d) 1D images of the DyEDTA-doped glycerol/water sample with glass beads along x , z , and y directions, respectively. Red images were obtained without compensation for imperfect centering of the sample within the gradient coils. In panels b and c, images in blue were obtained with non-zero values of a_x and a_z (see text), adjusted to center the images in the 1D field of view.

Figure 4.

(a) Temperature dependence of the ^1H spin-lattice relaxation time T_1 for glycerol/water with 4 mM DyEDTA. (b) Temperature dependence of the total ^1H NMR signal, detected with PSL. Dashed line is an ideal $1/T$ dependence. (c) Temperature dependence of the ratio of the single-scan ^1H NMR SNR to $T_1^{1/2}$, showing that optimal sensitivity in a fixed measurement time occurs near 30 K.

Figure 5.

(a) The signal-to-noise ratio in one scan at the center point in the PSL-detected ^1H spectrum obtained with various PSL pulse amplitudes and three different RF duty factors (○: 19.2%, □: 13.9%, ×: 10.9%). No gradient pulses and no FSLG decoupling were applied. The sample was DyEDTA-doped glycerol/water at 28 K. (b) Time-domain PSL signals acquired at 19.2% RF duty factor with indicated values of the PSL flip angle. (c) Grayscale map showing the PSL signal at different Lee-Goldburg RF amplitudes and pulse lengths, with $\omega_{\text{RF}} = 160$ kHz. (d) Dependence of signal on τ_{LG} , with 211 kHz RF amplitude, corresponding to the dashed horizontal line in panel c. (e) Dependence of signal on RF amplitude, with $\tau_{LG} = 728$ μs , corresponding to the vertical dashed line in panel c.

Figure 6.

(a–c) 2D planes from the 3D ^1H MRI image of glass beads in DyEDTA-doped glycerol/water at 28 K. 1D slices at color-coded positions are shown to the right of each 2D plane. Purple double-headed arrows span 20 μm distances, corresponding to the diameter of one glass bead. Dotted yellow line in each 2D plane indicates the position of one of the other 2D planes.

Figure 7.

(a) Example of a region from the experimental 3D ^1H MRI image that was used to evaluate the image resolution, and examples of calculated images with the indicated blurring factors β . (b) Example of the dependence of the reduced chi-squared function on β , as defined in Eq. 2. The actual image resolution is taken to be $\sqrt{2}\beta_{\min}$, where $\beta_{\min}$ is the value of β that minimizes the reduced chi-squared function. (c) Image resolutions estimated from ten independent image regions, as shown in Figs. S3 and S4.