

Tissue Microstructural Heterogeneity Revealed by MR Microscopy

Bibek Dhakal

`bibek.dhakal@vanderbilt.edu`

Vanderbilt University Institute of Imaging Science <https://orcid.org/0000-0003-3958-5029>

Benjamin M. Hardy

Vanderbilt University Institute of Imaging Science

Xiaoyu Jiang

Vanderbilt University Institute of Imaging Science

Adam W. Anderson

Vanderbilt University Institute of Imaging Science

Mark D. Does

Vanderbilt University Institute of Imaging Science

Junzhong Xu

Vanderbilt University Institute of Imaging Science

John C. Gore

Vanderbilt University Institute of Imaging Science

Research Article

Keywords: Magnetic resonance microscopy, High-field MRI, Microstructural heterogeneity, High-resolution, Relaxometry

Posted Date: August 27th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10819618/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Tissue Microstructural Heterogeneity Revealed by MR Microscopy

Bibek Dhakal^{1,2*}, Benjamin M. Hardy^{1,2}, Xiaoyu Jiang^{1,4}, Adam W. Anderson^{1,2,3}, Mark D. Does^{1,3}, Junzhong Xu^{1,2,3,4}, John C. Gore^{1,2,3,4}

1. *Vanderbilt University Institute of Imaging Science, Vanderbilt University Medical Center, Nashville, TN, USA*
2. *Department of Physics and Astronomy, Vanderbilt University, Nashville, TN, USA*
3. *Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA*
4. *Department of Radiology & Radiological Sciences, Vanderbilt University Medical Center, Nashville, TN, USA*

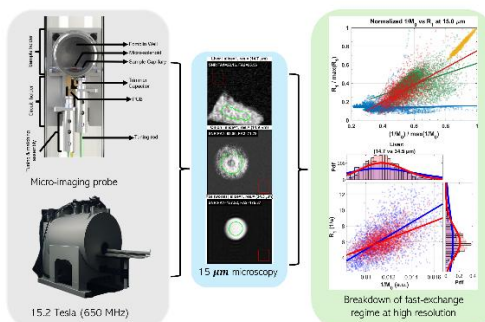
Corresponding author: Bibek Dhakal (email: bibek.dhakal@vanderbilt.edu)

Abstract

Magnetic resonance imaging (MRI) of biological tissues at typical millimeter-scale resolution depicts the values of signals averaged over voxels containing thousands to millions of cells, obscuring the heterogeneity of tissue properties at finer scales. MR microscopy (MRM) can reveal tissue microstructure at smaller scales, but quantitative relaxometry at micron-scale resolution is constrained by image acquisition times. We acquired high resolution maps of relaxation time T_1 and equilibrium magnetization M_0 from *ex vivo* mouse liver, spinal cord and onion root at $15\ \mu\text{m}$ isotropic resolution at 15.2 T, and quantified how the apparent heterogeneity across voxels changed with effective spatial resolution. Lower resolution images were generated by selected k -space truncations of the high-resolution data, enabling within-sample comparisons without changing acquisition conditions. Reducing resolution from $\sim 15\ \mu\text{m}$ to $\sim 35\ \mu\text{m}$ produced a marked contraction of the histograms of both the T_1 and M_0 distributions, whereas they showed minimal dependence on resolution in a uniform phantom. The variance of relaxation times at microscopic scale is a novel index of tissue microstructure. Simulations indicate that incomplete diffusion-dependent mixing within tissues can mediate the apparent relationship between $R_1 = 1/T_1$ and M_0 , consistent with a resolution-dependent departure from conventional fast-exchange assumptions about water in heterogeneous tissue.

Graphical Abstract:

Magnetic resonance microscopy at 15.2 T resolves voxel-scale variations in relaxation times (T_1) and equilibrium magnetization (M_0) in biological tissues. Reducing spatial resolution via controlled k -space truncation significantly contracts these parameter distributions in heterogeneous media, while uniform controls remain stable. This scale-dependent behavior suggests a resolution-dependent departure from conventional fast-exchange assumptions, establishing microscopic parameter variance as a novel index of tissue microstructure.



Tissue Microstructural Heterogeneity Revealed by MR Microscopy

B. Dhakal*, B. M. Hardy, X. Jiang, A. W. Anderson, M. D. Does, J. Xu, J. C. Gore

Wordcount: 5112

Keywords: Magnetic resonance microscopy, High-field MRI, Microstructural heterogeneity, High-resolution, Relaxometry

Funding: Chan Zuckerberg Initiative (GR005712) for deep tissue imaging.

Introduction

Magnetic resonance microscopy (MRM) refers to MRI performed at spatial resolutions below^{1,2} $\sim 100 \mu m$, and microscopic resolution has been demonstrated with isotropic voxel sizes down to a few micrometers in tissues^{3,4} and phantoms^{5,6}. At these scales, the number of contributing spins per voxel is relatively small, so achieving adequate signal-to-noise ratio (SNR) typically requires long acquisitions and/or repeated averaging. This challenge becomes especially acute for quantitative relaxometry, where multiple images are needed to estimate parameters such as T_1 , often making high-resolution mapping prohibitively time-consuming. Nonetheless, technical developments have been implemented to boost sensitivity and increase acquisition efficiency. For example, miniaturized RF coils (micro-coils) can substantially increase SNR for small samples and fields of view, and both planar microcoils^{3,6} and solenoid microcoils^{5,7–11} have been used in prior MRM studies. Additional gains can come from high static field strength, strong gradients^{9,12}, cryocoils^{13,14} and optimized acquisition and reconstruction strategies designed to reduce scan time for quantitative measurements. For practical high-resolution microscopic T_1 mapping, variable-flip angle spoiled gradient-echo methods (e.g.,

DESPOT1)¹⁵ are attractive because they can estimate voxel-wise T_1 and equilibrium magnetization M_0 from a small number of acquisitions.

The motivation for pursuing micron-scale resolution is that many tissue features vary on the micron to tens-of-microns range. For example, hepatocytes are $\sim 20 - 30 \mu m$ in diameter (with hepatic sinusoids $\sim 7 - 15 \mu m$)¹⁶, axon (fiber) diameters in cerebral white matter¹⁷ span roughly $\sim 0.16 - 9 \mu m$, renal cortical tubular lumens are typically tens of micrometers ($\sim 24 - 30 \mu m$ in rodents and $\sim 34 - 40 \mu m$ in humans)¹⁸. At resolutions coarser than these length scales, partial-volume averaging and imaging point-spread effects can suppress or distort microstructural heterogeneity, implying that “heterogeneity” becomes resolution-dependent rather than a purely intrinsic tissue property. For example, a $15 \mu m$ voxel may include only one or a few cells, whereas a $100 \mu m$ voxel averages the signal over 100s-1000s of cells, smoothing any intrinsic microstructural variations that may exist.

Relaxation in heterogeneous media involves water protons interacting with multiple sites, such as macromolecules, that promote relaxation, and by Brownian motion each proton may interact within a variety of environments in the time of a signal evolution. When different regions of a voxel have distinct relaxation properties, the composite signal will depend on the extent to which water samples and averages those components. In most practical applications of MRI, the observed signal is assumed to reflect a population-weighted average over microenvironments (the “fast-exchange” regime). A simple model of relaxation then relates the measured relaxation rate $R_1 = 1/T_1$ to the observable magnetization M_0 . At any instant a fraction f of the magnetization is assumed to be experiencing accelerated relaxation at rate R_{1b} (such as water within hydration shells around macromolecules) while the remaining fraction $(1 - f)$ relaxes by intrinsic dipolar interactions with rate R_{10} . The observed relaxation rate is then

$$R_1 = R_{10} + f(R_{1b} - R_{10})$$

f is proportional to the concentration $c = \frac{m}{M_0}$ of relaxation-promoting solute, where m is the amount in a total water content whose magnetization is M_0

$$R_1 - R_{10} = \frac{km}{M_0}(R_{1b} - R_{10})$$

where k denotes relaxivity. Note this can be extended to incorporate multiple pools i , each with its own characteristics but all in fast exchange so that

$$R_1 - R_{10} = \frac{1}{M_0} \sum_i k_i m_i (R_i - R_{10})$$

i.e. the observed relaxation rate varies inversely with the water content.

Note that in media characterized by relatively small fluctuations in water content, small increments in M_0 correspond to small decreases in relaxation rate. Writing $R_1 - R_{10} = \bar{R}_1 + \Delta R_1$ where $\bar{R}_1$ is the mean value and ΔR_1 is a small difference in relaxation rate, and $\sum_i k_i m_i (R_i - R_{10})$ as a fixed contribution Γ , if the deviation in water content is ΔM_0 then to first order, $\Delta R_1 = -\Gamma \Delta M_0 (\bar{M}_0)^{-2}$ (see Appendix A for detailed derivation).

Deviations from this model may be detected using, for example, multi-exponential fitting to signal decay (T_2) or recovery (T_1) curves, and these approaches work reasonably well if the relaxation characteristics of sub-components are sufficiently different, and the exchange between them is sufficiently slow, as demonstrated in foundational studies of frog nerve¹⁹, crayfish nerve cord²⁰, and human white matter *in vivo*²¹. However, fitting spin-lattice recovery to multiple components is notoriously difficult when there are small differences between populations and exchange is not sufficiently slow. Nonetheless, as voxel dimensions increase beyond the distance water molecules can explore over relevant timing intervals, averaging can become incomplete, and the apparent $R_1 - M_0$ relationship may change. For an apparent diffusion coefficient (D) of $\sim 2 \mu\text{m}^2/\text{ms}$ and $TR \approx 50 \text{ ms}$, the characteristic displacement ($\langle r \rangle = \sqrt{6Dt}$) is $\sim 24 \mu\text{m}$, comparable to $15 \mu\text{m}$ voxel dimension. This suggests that, at micro-scale resolution, intrinsic spatial variations in relaxation rate may not be fully averaged, and the relationship between R_1 and M_0 may be different across voxels compared to lower resolution.

In this work, we test this idea experimentally using 15.2 T MR microscopy and DESPOT1 mapping of heterogeneous *ex vivo* tissues and a uniform PBS control. By degrading spatial resolution from matched high-resolution acquisitions, we quantify how apparent T_1/M_0 heterogeneity and the $R_1 - M_0$ relationship change with voxel size. Complementary Bloch–Torrey simulations are used to separate the contributions of exchange, partial-volume averaging, and noise.

Methods

Micro-imaging Probe

A custom micro-solenoid transceiver radiofrequency (RF) circuit was fabricated using five turns of 0.3 mm thick copper wire mounted on an FR4 printed circuit board. The circuit integrated 1–10 pF trim capacitors, chip capacitors (33 pF), and SMB connectors. This assembly was encased in a 3D-printed housing, designed to be tuned and matched from outside the magnet bore using a tuning rod assembly consisting of brass springs, Garolite rods, a 3D-printed screw cap, and brass screws. To minimize T_2^* signal loss caused by susceptibility mismatches between the copper, glass, and sample, the housing incorporated a Fomblin-filled well, as illustrated in Figure 1. While tissue samples were sectioned for insertion into glass capillaries (Fisher Scientific, Cat. #: 22-362-566, OD: 1.5 mm; ID: 1.0 mm), the uniform PBS phantoms were scanned in a smaller quartz capillary (VitroCom, Cat. #: CV7087-Q-100, OD: 0.87 mm; ID: 0.7 mm). For tissue imaging, the remaining capillary volume was filled with Fomblin perfluoropolyether fluid (Solvay Solexis, Thorofare, NJ, USA). Each loaded capillary was inserted through access holes on either side of the Fomblin bath and secured with rubber stoppers, which also served to prevent fluid leakage. The bath was subsequently filled with Fomblin and sealed with a 3D-printed cap, featuring an O-ring to prevent fluid from spilling into the magnet bore. Comprehensive details regarding the design, characterization, and sample loading of this cost-effective micro-imaging probe are described in our previous work¹¹.

Sample Preparation

All animal protocols were approved by Vanderbilt's Institutional Animal Care and Use Committee (IACUC). A uniform phantom was prepared using a mixture of 1X PBS and 1.0 mM Gadolinium. For liver specimens, a 4-week-old male C57BL/6J mouse (Jackson Laboratory, Bar Harbor, ME, USA) was anesthetized with isoflurane and sacrificed via transcardial perfusion. The perfusion protocol consisted of an initial 1X PBS wash through the left ventricle, followed by a modified Karnovsky fixative solution composed of 1X PBS, 2.5% glutaraldehyde, 2% paraformaldehyde, and 1.0 mM Gadolinium contrast agent. Following the perfusion, the liver tissue was harvested and immersed in the fixative solution for one week. To remove excess fixative, the liver was then immersed in 1X PBS and 1.0 mM Gd for an additional week, with four changes of wash-solution during this period. Prior to imaging, the liver was sectioned using a sharp blade to produce thin sections with width smaller than the internal diameter (ID ~1 mm) of the glass capillaries (length ~40 mm). The capillaries were then filled with the 1X PBS and 1.0 mM Gd mixture for uniform phantom imaging or with Fomblin perfluoropolyether fluid (Solvay Solexis, Thorofare, NJ, USA) for tissue imaging. During tissue preparation, the sectioned liver samples were carefully submerged in Fomblin within the capillary environment.

For mouse spinal cord imaging, a protocol similar to that used for the liver was performed, as described in our previous work¹¹.

For the imaging of onion root tissue, fresh specimens were harvested and hydrated in a PBS solution for several hours. Prior to acquisition, the root was sectioned to a length of approximately 1 cm to ensure compatibility with the glass capillary and the micro-solenoid coil geometry.

Experimental Setup

After loading the sample and the sealing of the Fomblin bath, a fiber-optic thermoprobe was positioned adjacent to the bath to facilitate continuous monitoring of the coil temperature within the magnet bore. The sample loaded micro-imaging probe was then inserted into the bore of the 15.2 T scanner. To ensure mechanical stability and mitigate potential image artifacts arising from coil vibration, the assembly was secured to the exterior of the gradient coil using four sets of plastic screws.

Thermal monitoring was performed by connecting the thermoprobe to an ECG/T module (SA Instruments, Inc.), with real-time data tracked via PC-SAM software (SA Instruments, Inc.). To preserve a constant temperature environment within the magnet bore during high-field acquisition, an in-house developed fan was used to direct room-temperature air into the bore through a hose assembly. This active cooling system ensured that the internal bore temperature remained stable ($< 2^{\circ}\text{C}$ variation) throughout the duration of the imaging experiments.

MR Imaging

Imaging was carried out in a Bruker 15.2 T Avance III system (Bruker BioSpin, Billerica, MA, USA). The magnet has a horizontal bore with diameter of 11 cm and is equipped with gradient coil (Resonance Research, Inc) which has inner diameter of 6 cm and maximum strength of 1000 mT/m. ParaVision 6.0.1 software (Bruker BioSpin), was used to control the MR console.

A 3D fast low angle shot (3D FLASH) pulse sequence was used to acquire images at two optimal flip angles given by

$$\alpha = \cos^{-1} \left(\frac{\lambda^2 E_1 \pm (1 - E_1^2) \sqrt{1 - \lambda^2}}{1 - E_1^2 (1 - \lambda^2)} \right) \quad (1)$$

where $E_1 = \exp(-TR/T_1)$. The two flip angles are calculated with $\lambda = \frac{S_{\alpha_1}}{S_{\alpha_2}}$ taken to be 0.71 for maximum SNR¹⁵. A low-resolution T_1 measurement was first performed using a 2D spin-echo sequence by acquiring images at multiple repetition times while keeping the echo time fixed. The resulting T_1 estimate was then used to calculate the optimal flip angles (α_1, α_2) from equation (1). After determining the flip angles, 3D FLASH acquisitions were performed at α_1 and α_2 . Imaging parameters are provided below.

Imaging Parameters

Uniform Phantom

Two 3D-FLASH images were acquired with flip angles of 19° and 87°. The imaging parameters were: field of view (FOV) = $1.2 \times 0.6 \times 1.2 \text{ mm}^3$, image matrix = $80 \times 40 \times 80$, voxel size = $15 \times 15 \times 15 \text{ } \mu\text{m}^3$, repetition time (TR) = 65 ms, echo time (TE) = 3.72 ms, bandwidth (BW) = 25 kHz, and number of repetitions (Nrep) = 52. The scan time was 3 h 16 min per image.

Tissue Imaging

Liver Tissue: Two 3D-FLASH images were acquired with flip angles of 19° and 89°. The imaging parameters were: FOV = $1 \times 0.65 \times 0.5 \text{ mm}^3$, image matrix = $68 \times 42 \times 34$, voxel size = $14.7 \times 15.47 \times 14.70 \text{ } \mu\text{m}^3$, TR = 75 ms, TE = 4.06 ms, BW = 24 kHz, and Nrep = 256. The scan time was 7 h 36 min per image.

Spinal Cord: Two 3D-FLASH images were acquired with flip angles of 19° and 89°. The imaging parameters were: FOV = $6 \times 2 \times 2 \text{ mm}^3$, image matrix = $400 \times 134 \times 134$, voxel size = $15 \times 14.92 \times 14.92 \text{ } \mu\text{m}^3$, TR = 90 ms, TE = 5.12 ms, BW = 65 kHz, and Nrep = 64. The scan time was 28 h 43 min per image.

Onion Root: Two 3D-FLASH images were acquired with flip angles of 9° and 47°. The imaging parameters were: FOV = $2 \times 0.5 \times 2 \text{ mm}^3$, image matrix = $134 \times 34 \times 134$, voxel size = $14.92 \times 14.70 \times 14.92 \text{ } \mu\text{m}^3$, TR = 75 ms, TE = 4.78 ms, BW = 25 kHz, and Nrep = 64. The scan time was 6 h 4 min per image.

For all 3D excitations across the phantom and tissue samples, a slab-selective block RF pulse was used, with slab thickness equal to the FOV in the slab-encode direction, to confine excitation to the prescribed imaging volume and reduce through-slab aliasing that could bias DESPOT1 parameter estimates. All images were reconstructed using the REMMI-MATLAB toolbox (<https://github.com/remmi-toolbox/remmi-matlab>), and each repetition was registered and averaged in MATLAB.

Image Analysis

Once the two 3D SPGR datasets (flip angles α_1 and α_2) were acquired, ten ($n=10$) central transverse slices were selected from each volume for further analysis (see Figure 2). For each selected slice and for each flip angle, resolution was reduced by controlled k -space truncation. Specifically, the slice image $I(x, y)$ was Fourier transformed to k -space $K(u, v)$,

$$K(u, v) = \mathcal{F}\{I(x, y)\} \quad (2)$$

and then multiplied by a 2D window $W(u, v)$ that retains only a central square region of k -space (= a rectangular low-pass filter):

$$K'(u, v) = K(u, v)W(u, v) \quad (3)$$

For a rectangular window of side length N_c (in pixels), $W(u, v) = 1$ within the central $N_c \times N_c$ region of k -space (centered at the DC component) and $W(u, v) = 0$ elsewhere. The filtered k -space $K'(u, v)$ was kept in the original matrix size (i.e., high-frequency samples set to zero outside the central passband), and an inverse Fourier transform was applied to obtain a blurred image with the same matrix dimensions as the original high-resolution slice:

$$I'(x, y) = \mathcal{F}^{-1}\{K'(u, v)\} \quad (4)$$

By progressively reducing N_c , a series of matched low-resolution image pairs (α_1, α_2) was generated, spanning from the native $\sim 15 \mu m$ resolution to approximately twice that value ($\sim 35 \mu m$), while preserving the original image matrix via zero-filled reconstruction.

Next, signal and noise regions of interest (ROIs) were defined on the native-resolution reference slice of each dataset and applied to all ten analyzed slices and all resolution levels. For onion root and the PBS phantom, signal ROIs were auto-generated circular masks inset from the sample boundary by the expected blur margin at the coarsest resolution. For spinal cord, an interior gray-matter ROI was generated automatically by (i) detecting the cord as the largest connected foreground component, (ii) segmenting gray matter within the cord by intensity-based hysteresis thresholding of the bright GM butterfly relative to surrounding white matter, and (iii) retaining only the morphological GM core by a distance-transform inset from the GM-white-matter boundary (at least the worst-case blur margin) and intersection with a central disk, thereby excluding white matter and reducing partial-volume contamination under blurring. Liver used a manual freehand outline. Noise ROIs were user-placed rectangles in background voxels without signal. Voxel-wise DESPOT1 (Appendix B) fitting was performed within the signal ROI using a nonlinear least-squares estimator applied to the two-flip-angle SPGR signals to obtain T_1 and M_0 maps at each resolution. For each resolution level, the mean and standard deviation of T_1 and M_0 were computed across voxels (and aggregated across slices) (see Figure 3-7). Heterogeneity was also summarized using the coefficient of variation ($CV = SD/mean$) of voxel-wise T_1 and M_0 within the signal ROI, providing a scale-normalized dispersion metric for comparison across resolutions, parameters, and samples with different mean values. $R_1 (= 1/T_1)$ was calculated and scatter plots of R_1 versus $1/M_0$ were generated (Figure 3-6i, 8) for each resolution and fitted with a line to estimate the slope, intercept, and goodness of fit (R^2). This linear model is used as a descriptive summary of the dominant trend in the joint distribution and is most directly motivated under fast-exchange population averaging. It is not assumed to hold equally well at all

resolutions. Finally, the slope, intercept, and R^2 were summarized as functions of spatial resolution (Figure 3-6 i-l, 7).

To interpret the experimental resolution dependence under controlled conditions, complementary 2D Bloch–Torrey simulations were performed. Heterogeneous mesoscopic phantoms were generated on a 30×30 grid ($\Delta x = 15 \mu m$) with spatially varying M_0 and relaxivity-like parameter k , and local R_1 assigned as $R_1 = R_{0,free} + k/M_0$. A uniform-null phantom served as a negative control. Three exchange/mixing regimes were compared (slow exchange, fast exchange by diffusion, and fast exchange by denser micro-domain geometry plus diffusion). SPGR signals were evolved with finite-difference Bloch–Torrey dynamics at two flip angles and fitted with the same DESPOT1 pipeline used experimentally. We then examined (i) native-resolution exchange effects with and without noise, (ii) noise-only behavior in the uniform-null phantom, (iii) resolution coarsening by Fourier k-space truncation (~ 15 – $35 \mu m$), and (iv) a joint SNR–resolution grid and one-factor sensitivity analysis. Full numerical parameters, figures, and implementation details are provided in Supplementary Information 1.

Results

Experiment results

Figures 3a,b show representative mouse liver image slices at high ($14.7 \mu m$) and low ($34.5 \mu m$) spatial resolution, with signal ROI (green) and noise ROI (red). Figure 3c–h summarizes how blurring affects the mean, standard deviation, and coefficient of variation (CV) of T_1 and M_0 within the signal ROI. From $14.7 \mu m$ to $34.5 \mu m$, mean T_1 changed by only 8.7%, whereas T_1 SD and CV decreased by 60.6% and 56.8%, respectively, indicating reduced relative heterogeneity rather than a global shift in mean T_1 . Figures 3f–h show the corresponding metrics for M_0 . Across the same resolution range, the mean M_0 changed by only 1.3%, whereas M_0 CV decreased by 31.9%, and the M_0 standard deviation decreased by 32.8%. As shown below, these changes are much larger than those in a uniform PBS phantom under the same resolution-degradation pipeline. Noise can contribute to apparent T_1 heterogeneity (and to part of the CV reduction as effective SNR increases with blurring), but it does not fully explain the tissue–phantom contrast or the resolution-dependent contraction of the joint $R_1 - 1/M_0$ distribution. These points are examined further using SNR–resolution simulations in Supplementary Information 1.

Figure 3i shows the R_1 vs. $1/M_0$ scatter plots at $14.7 \mu m$ and $34.5 \mu m$ along with the best-fit linear model at each resolution. With blurring, the joint distribution contracts, consistent with reduced voxel-scale variability in both parameters. This contraction is further illustrated by the marginal distributions in Figure 8a, where both R_1 and $1/M_0$ exhibit broader distributions at $14.7 \mu m$ than at $34.5 \mu m$. Figures 3j–l quantifies how the fitted linear relationship evolves with resolution: the slope magnitude changes by 35.2% from highest to lowest resolution, and the intercept changes by 93.9%. In addition, the goodness-of-fit (R^2) decreases from 0.43 to 0.33 with increasing blur. Error bars in all plots in Figure 3 represent slice-to-slice variations across the central 10 slices of the 3D liver volume.

Similar trends were observed in another cellular medium, Green onion root (Figure 4). Blurring reduced the T_1 CV by 33.9% (Figure 4c) and the M_0 CV by 5.3% (Figure 4f), while the mean T_1 and mean M_0 changed by $\leq 1\%$ (Figures 4d and 4g). The standard deviation of T_1 decreased by 34.6%, whereas the standard deviation of M_0 decreased by 5.4% (Figures 4e and 4h). Figure 4i shows the $R_1 - 1/M_0$ scatter plots at $14.9 \mu m$ and $35.1 \mu m$ with the corresponding linear fits, again demonstrating a visible contraction of the joint distribution with blurring. This is supported by Figure 8b: the R_1 distribution broadens at higher resolution, while the M_0 distribution shows comparatively little change with blurring. Consistent with the liver results, the fitted relationship also changes with resolution (Figures 4j–l). Across the resolution range, the fitted $R_1 - 1/M_0$ slope changes from approximately $+0.05$ to -0.05 (Figure 4j), reversing sign while the slope magnitude changes by only $\sim 16.5\%$ (Supplementary Table 2) while the intercept changes by $\sim 14\%$. The linear fit itself is weak ($R^2 \approx 0-0.03$): R^2 decreases slightly and then increases with blurring, and slice-to-slice variability becomes more pronounced at lower resolutions (Figure 4l). Accordingly, for onion root we emphasize the clearer resolution dependence of the T_1 CV/distribution width and the contraction of the joint scatter (Figures 4c–i, 8b) rather than the slope percentage alone.

Similarly, Figure 5 presents the same analysis in mouse spinal cord using an interior gray-matter ROI. Figures 5c–e show that blurring from $14.9 \mu m$ to $35.1 \mu m$ reduced the T_1 CV by 29.9% (and the T_1 SD by 30.2%) and the M_0 CV by 25.9% (and the M_0 SD by 25.9%), while mean T_1 and M_0 changed by $\leq 0.6\%$ (see also Figure 8c). Figure 5i shows the voxel-wise R_1 vs. $1/M_0$ relationship within spinal cord gray matter. Across Figures 5i–l, the fitted slope changed by only 4.2%, the intercept by 11.5%, and R^2 by 11.6%. Because the slope changed only modestly (4.2%), the clearer resolution dependence for spinal cord is the contraction of the T_1 and M_0 distributions (Figures 5c–h, 8c).

To demonstrate that these effects are driven by tissue heterogeneity rather than the effects of blurring of noise, the same analysis was performed in a uniform PBS phantom (Figure 6). In contrast to heterogeneous tissues, the CVs remain essentially unchanged with resolution: the T_1 CV changes by 3.6% and the M_0 CV changes by 0.8% across the blurring series (Figures 6c,f). The mean T_1 and mean M_0 are also highly stable, changing by $\leq 0.03\%$ across resolution (Figures 6d,g). Figure 6i shows the $R_1 - 1/M_0$ scatter at $15.0 \mu m$ and $35.3 \mu m$ with linear fits. Compared with liver and onion, the PBS data are well described by a straight line and show no marked contraction in the joint distribution with blurring; this is also evident from Figure 8d, where neither the R_1 nor M_0 marginal distributions change substantially with resolution. Across the full resolution range, the slope magnitude changes by 4.1% and the intercept changes by 7.9% (Figures 6j–k). The R^2 decreases modestly from ~ 0.95 to a minimum of ~ 0.91 ($\sim 3.5\%$ change) with increasing blur (Figure 6l).

Figure 7 summarizes the joint comparison across liver, onion, and PBS. Figures 7a–b show that T_1 CV and M_0 CV decrease with blurring in the heterogeneous tissues (liver and onion), whereas the corresponding metrics remain nearly constant in the uniform phantom. Figure 7c shows that the normalized $R_1 - 1/M_0$ slope decreases in magnitude

with blurring for heterogeneous tissues but remains comparatively stable for PBS across resolution. Finally, Figure 7d compares the normalized R_1-1/M_0 scatter at $15.0\ \mu\text{m}$, illustrating that heterogeneous tissues exhibit a broader and more structured joint distribution than the uniform phantom, consistent with underlying microstructural heterogeneity.

Figure 8 summarizes how reducing spatial resolution contracts the apparent joint ($R_1, 1/M_0$) heterogeneity in biologically heterogeneous samples, but not in a uniform phantom. In liver, spinal cord, and green onion root, the voxel-wise R_1-1/M_0 scatter at $\sim 15\ \mu\text{m}$ broadens into a visibly wider 2D cloud, and both marginal distributions (top: $1/M_0$; right: R_1) are correspondingly broader. After Fourier k -space cropping to $\sim 35\ \mu\text{m}$, the joint cloud collapses toward its central trend and the marginals narrow, indicating reduced apparent variability in both parameters (Figure 8a-c). In contrast, the PBS phantom (Figure 8d) retains a tight, nearly one-dimensional R_1-1/M_0 relationship across resolutions, and neither marginal distribution changes appreciably, consistent with minimal underlying microstructural heterogeneity.

Additionally, Supplementary Information 3 contains tabulated values for T_1 , M_0 , slope, intercept, and other derived parameters, along with their percentage changes between the highest and lowest resolution imaging maps across the aforementioned tissue and sample types.

Simulation results

Complementary Bloch–Torrey simulations (full methods and figures in Supplementary Information 1) reproduced the qualitative experimental pattern under controlled conditions. At native resolution, increasing mesoscopic exchange/mixing compacted the fitted $R_1 - 1/M_0$ distributions, whereas the slow-exchange regime retained the broadest scatter (Figure S2). In a uniform-null phantom, lowering SNR alone generated substantial apparent heterogeneity despite spatially constant ground truth (Figure S3). Resolution coarsening contracted fitted R_1 and M_0 distributions and changed the apparent $R_1 - 1/M_0$ slope in heterogeneous phantoms, with the largest native-to-coarse slope change in the slow-exchange regime. Conversely, the same coarsening procedure produced near-zero CV and negligible resolution trends in the uniform-null control (Figure S4). A joint SNR–resolution grid demonstrated that noise and partial-volume averaging are separable but interacting factors. Specifically, SNR shifts the baseline slope and CV, while coarsening produces additional native-to-coarse changes at a fixed SNR (Figure S5). Sensitivity analysis identified diffusion/mixing strength (D) and SNR as the dominant drivers of native-to-coarse slope change, whereas small random variability in baseline free-water R_1 had negligible effect (Figure S6). Together, these simulations indicate that the experimental contraction of parameter distributions with decreasing resolution in heterogeneous tissue, and the stability of uniform media, can be explained by partial-volume averaging of true spatial heterogeneity, exchange-regime-dependent mixing, and noise bias.

Discussion

This study shows that MR microscopy can resolve voxel-scale heterogeneity in T_1 (R_1) and M_0 that becomes substantially obscured as spatial resolution is reduced. At $15\ \mu\text{m}$ isotropic resolution, the voxelwise R_1-1/M_0 scatter is broad and both the M_0 and R_1 histograms are wide. Reducing resolution systematically narrows the M_0 and R_1 distributions and compresses the 2D R_1-1/M_0 scatter to a degree much greater than accountable by noise effects. Thus, there exist intrinsic sub-voxel fluctuations of MR properties that are not averaged by water diffusion, which averages only local (<diffusion distance) inhomogeneities.

A key interpretation is that these observations reflect a resolution-dependent departure from the conventional fast-exchange assumption. In microscopic images, as voxel dimensions approach the micron scale, each voxel becomes comparable to the characteristic diffusion length of water, allowing for complete averaging of environments within a voxel, but also allowing microstructural differences in R_1 and M_0 between different small voxels to emerge. In lower-resolution images of heterogeneous media, the measured signal is a superposition of multiple such contributions, each of which may be different, so the signal effectively reports a population-weighted average. When the heterogeneity is large enough, multiexponential decays or recovery should be detectable, but when variations are small the relaxation may still appear well fit by a single exponent. In practical terms, this framework explains why heterogeneity increases at $15\ \mu\text{m}$ and why apparent parameter distributions become narrower as resolution is degraded by spatial averaging.

The tissue-phantom comparisons support our finding that at micron scale resolution tissue exhibits variations not detectable with large voxels. For mouse liver, blurring from $14.7\ \mu\text{m}$ to $34.5\ \mu\text{m}$ reduced T_1 CV by 56.8% (and T_1 SD by 60.6%) while changing the mean T_1 by 8.7%; across the same range, M_0 CV decreased by 31.9% with only a 1.3% change in mean M_0 . Green onion root showed the same qualitative behavior: blurring reduced T_1 CV by 33.9%, while mean values changed by $\leq 1\%$, and the joint distribution contracted in the R_1-1/M_0 scatter. Similarly, in mouse spinal cord gray matter, T_1 CV contraction of 29.9 % and an M_0 CV contraction of 25.9 % was observed, with only a $\leq 0.6\%$ change in the mean T_1 and M_0 when the resolution was degraded from $14.9\ \mu\text{m}$ to $35.1\ \mu\text{m}$. In contrast, the uniform PBS phantom was largely invariant to resolution loss (e.g., T_1 CV change $< 3.6\%$ and M_0 CV change $< 0.8\%$), indicating that the contraction seen in tissue is not a consequence of the processing.

From these data, the R_1-1/M_0 relationship itself is scale-dependent. In liver, the fitted R_1-1/M_0 slope magnitude changed by 35.2% between highest and lowest resolution, and in onion root and spinal cord (gray matter), the slope changed by 16.5% and 4.2% respectively, whereas PBS showed only 4.1% change in slope magnitude. Therefore, the apparent R_1-1/M_0 coupling, or effective relaxivity of tissue solutes, estimated from voxelwise DESPOT1 maps is resolution dependent and linear fit parameters (slope/intercept) obtained at conventional resolution may not extrapolate to micron-scale

data (and vice versa). Instead, the apparent slope and intercept reflect the extent to which voxel-wise measurements preserve microstructural information.

A linear $R_1 - 1/M_0$ relation is the natural expectation under conventional fast-exchange averaging (Appendix A). At microscopic resolution, however, incomplete mixing can produce broad joint distributions whose detailed shape is not uniquely constrained by a two-parameter line fit. Accordingly, we use the linear fit primarily to track the direction and magnitude of resolution-dependent shifts in the joint cloud, while treating contraction of the T_1 and M_0 distributions (CV/SD and scatter width) as the more robust measure of apparent microstructural heterogeneity—especially when linearity is weak (e.g., onion root).

Bloch–Torrey simulations (Supplementary Information 1) further support this interpretation. Under controlled variation of exchange regime, Rician noise, and Fourier k -space coarsening, the simulations recovered the same qualitative pattern as the experiments: contraction of fitted T_1 and M_0 distributions with decreasing resolution in heterogeneous phantoms, but not in uniform-null controls. Although diffusion-mediated mixing during signal formation and post-acquisition k -space truncation can both compress the apparent $R_1 - 1/M_0$ distribution, their mechanisms are distinct. The former relies on physical inter-compartment exchange, whereas the latter depends on the spatial averaging of an already formed signal. Independent variation of SNR and resolution showed that noise and blur are separable but interacting sources of bias, and sensitivity analysis identified mesoscopic mixing strength and measurement noise as the dominant drivers of the apparent $R_1 - 1/M_0$ behavior. These results therefore support the view that apparent heterogeneity metrics and $R_1 - M_0$ coupling are emergent, scale-dependent readouts rather than fixed intrinsic constants. Detailed interpretation of the simulation modules is provided in Supplementary Information 1.

Appendix A and Supplementary Information 2 provide complementary analytical support: under population averaging, increasing voxel volume increases the number of mixed subunits, and the effective slope of R_1 v $1/M_0$ is predicted to decrease. This is consistent with the experimentally observed weakening of $R_1 - 1/M_0$ coupling under blurring. Micron-scale versus coarse-scale behavior can also be interpreted in terms of monoexponential model validity. At high resolution, each voxel samples a narrower subset of micro-environments, so fitted DESPOT1 T_1 values more closely reflect local behavior. With coarsening, each voxel contains a broader mixture of compartments with different R_1 , M_0 , and exchange conditions, so the underlying recovery is increasingly multi-component rather than strictly monoexponential. In that setting, two-angle DESPOT1 still returns a single fitted T_1 , but this value should be interpreted as an apparent T_1 (an effective parameter of the mixed signal) rather than a unique compartment-specific relaxation constant. Therefore, resolution-dependent changes in variance, slope, and intercept are expected consequences of both population averaging and changing mismatch between multi-component tissue behavior and a single-component fitting model.

Several limitations should be noted. First, DESPOT1 assumes monoexponential recovery; therefore, when voxels contain unresolved heterogeneous compartments, fitted T_1 values are apparent T_1 parameters rather than unique compartment-specific constants. DESPOT1 is also sensitive to flip-angle accuracy, and residual B_1 inhomogeneity could introduce spatial structure in estimated M_0 and T_1 . However, key comparisons are strengthened because low-resolution datasets were generated by controlled truncation of the same high-resolution acquisitions, so many acquisition biases are shared across the resolution series and phantom control. Magnetization transfer may also bias variable-flip-angle T_1 under some conditions²²; accordingly, this study emphasizes resolution-dependent trends in joint R_1 – M_0 behavior rather than absolute relaxometry accuracy.

Overall, these results show that fast exchange is not a binary assumption but a scale-dependent regime. As voxel size approaches microstructural length scales, apparent R_1 and M_0 distributions broaden and apparent R_1 – M_0 coupling changes in heterogeneous tissue, while uniform media remain comparatively stable. Micron-scale relaxometry therefore provides microstructural information that is averaged out at conventional resolution.

Acknowledgements

The authors thank Yue Zou and Jingping Xie for assistance with tissue preparation, Dan Colvin for support during MR imaging, and Gary Drake for technical support throughout this work.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- (1) Glover, P.; Mansfield, S. P. Limits to Magnetic Resonance Microscopy. *Rep. Prog. Phys.* **2002**, 65 (10), 1489–1511. <https://doi.org/10.1088/0034-4885/65/10/203>.
- (2) Benveniste, H.; Blackband, S. MR Microscopy and High Resolution Small Animal MRI: Applications in Neuroscience Research. *Prog. Neurobiol.* **2002**, 67 (5), 393–420. [https://doi.org/10.1016/S0301-0082\(02\)00020-5](https://doi.org/10.1016/S0301-0082(02)00020-5).
- (3) Flint, J. J.; Lee, C. H.; Hansen, B.; Fey, M.; Schmidig, D.; Bui, J. D.; King, M. A.; Vestergaard-Poulsen, P.; Blackband, S. J. Magnetic Resonance Microscopy of Mammalian Neurons. *NeuroImage* **2009**, 46 (4), 1037–1040. <https://doi.org/10.1016/j.neuroimage.2009.03.009>.

- (4) van Schadewijk, R.; Krug, J. R.; Shen, D.; Sankar Gupta, K. B. S.; Vergeldt, F. J.; Bisseling, T.; Webb, A. G.; Van As, H.; Velders, A. H.; de Groot, H. J. M.; Alia, A. Magnetic Resonance Microscopy at Cellular Resolution and Localised Spectroscopy of *Medicago Truncatula* at 22.3 Tesla. *Sci. Rep.* **2020**, *10* (1), 971. <https://doi.org/10.1038/s41598-020-57861-7>.
- (5) Ciobanu, L.; Seeber, D. A.; Pennington, C. H. 3D MR Microscopy with Resolution 3.7 Mm by 3.3 Mm by 3.3 Mm. *J. Magn. Reson.* **2002**, *158*, 178–182.
- (6) Weiger, M.; Schmidig, D.; Denoth, S.; Massin, C.; Vincent, F.; Schenkel, M.; Fey, M. NMR Microscopy with Isotropic Resolution of 3.0 Mm Using Dedicated Hardware and Optimized Methods. *Concepts Magn. Reson. Part B Magn. Reson. Eng.* **2008**, *33B* (2), 84–93. <https://doi.org/10.1002/cmr.b.20112>.
- (7) Seeber, D. A.; Cooper, R. L.; Ciobanu, L.; Pennington, C. H. Design and Testing of High Sensitivity Microreceiver Coil Apparatus for Nuclear Magnetic Resonance and Imaging. *Rev. Sci. Instrum.* **2001**, *72* (4), 2171–2179. <https://doi.org/10.1063/1.1359190>.
- (8) Hardy, B. M.; Zhu, Y.; Harkins, K. D.; Dhakal, B.; Martin, J. B.; Xie, J.; Xu, J.; Does, M. D.; Anderson, A. W.; Gore, J. C. Experimental Demonstration of Diffusion Limitations on Resolution and SNR in MR Microscopy. *J. Magn. Reson.* **2023**, *352*, 107479. <https://doi.org/10.1016/j.jmr.2023.107479>.
- (9) Hüfken, T.; Lobmeyer, T.; Gahr, B.; Bschorr, F.; Speidel, T.; Just, S.; Rasche, V. Magnetic Resonance Microscopy for Submillimeter Samples in a Horizontal MR Scanner. *Sci. Rep.* **2024**, *14* (1), 23583. <https://doi.org/10.1038/s41598-024-73271-5>.
- (10) van Schadewijk, R.; Krug, J. R.; Webb, A.; Van As, H.; Velders, A. H.; De Groot, H. J. M.; Alia, A. MRM Microcoil Performance Calibration and Usage Demonstrated on *Medicago Truncatula* Roots at 22 T. *J. Vis. Exp.* **2021**, *2021* (167), 1–20. <https://doi.org/10.3791/61266>.
- (11) Dhakal, B.; Hardy, B. M.; Anderson, A. W.; Does, M. D.; Xu, J.; Gore, J. C. A Practical Prescription for Magnetic Resonance Microscopy in a Horizontal Bore Magnet. *Npj Imaging* **2025**, *3* (1), 64. <https://doi.org/10.1038/s44303-025-00129-4>.
- (12) Seeber, D. A.; Hoftiezer, J. H.; Daniel, W. B.; Rutgers, M. A.; Pennington, C. H. Triaxial Magnetic Field Gradient System for Microcoil Magnetic Resonance Imaging. *Rev. Sci. Instrum.* **2000**, *71* (11), 4263–4272. <https://doi.org/10.1063/1.1313800>.
- (13) Ratering, D.; Baltes, C.; Nordmeyer-Massner, J.; Marek, D.; Rudin, M. Performance of a 200-MHz Cryogenic RF Probe Designed for MRI and MRS of the Murine Brain. *Magn. Reson. Med.* **2008**, *59* (6), 1440–1447. <https://doi.org/10.1002/mrm.21629>.
- (14) Hardy, B. M.; Drake, G.; Chai, S.; Dhakal, B.; Martin, J. B.; Xu, J.; Does, M. D.; Anderson, A. W.; Yan, X.; Gore, J. C. A Cryogenic Tune and Match Circuit for Magnetic Resonance Microscopy at 15.2T. *J. Magn. Reson. Open* **2024**, *18*, 100147. <https://doi.org/10.1016/j.jmro.2024.100147>.
- (15) Deoni, S. C. L.; Rutt, B. K.; Peters, T. M. Rapid Combined T_1 and T_2 Mapping Using Gradient Recalled Acquisition in the Steady State. *Magn. Reson. Med.* **2003**, *49* (3), 515–526. <https://doi.org/10.1002/mrm.10407>.
- (16) Pfeiffer, E.; Kegel, V.; Zeilinger, K.; Hengstler, J. G.; Nüssler, A. K.; Seehofer, D.; Damm, G. Featured Article: Isolation, Characterization, and Cultivation of Human

- Hepatocytes and Non-Parenchymal Liver Cells. *Exp. Biol. Med.* **2015**, 240 (5), 645–656. <https://doi.org/10.1177/1535370214558025>.
- (17) Liewald, D.; Miller, R.; Logothetis, N.; Wagner, H.-J.; Schüz, A. Distribution of Axon Diameters in Cortical White Matter: An Electron-Microscopic Study on Three Human Brains and a Macaque. *Biol. Cybern.* **2014**, 108 (5), 541–557. <https://doi.org/10.1007/s00422-014-0626-2>.
 - (18) Morozov, D.; Parvin, N.; Charlton, J. R.; Bennett, K. M. Mapping Kidney Tubule Diameter Ex Vivo by Diffusion MRI. *Am. J. Physiol.-Ren. Physiol.* **2021**, 320 (5), F934–F946. <https://doi.org/10.1152/ajprenal.00369.2020>.
 - (19) Vasilescu, V.; Katona, E.; Simplăceanu, V.; Demco, D. Water Compartments in the Myelinated Nerve. III. Pulsed NMR Result. *Experientia* **1978**, 34 (11), 1443–1444. <https://doi.org/10.1007/BF01932339>.
 - (20) Menon, R. S.; Rusinko, M. S.; Allen, P. S. Proton Relaxation Studies of Water Compartmentalization in a Model Neurological System. *Magn. Reson. Med.* **1992**, 28 (2), 264–274. <https://doi.org/10.1002/mrm.1910280208>.
 - (21) Mackay, A.; Whittall, K.; Adler, J.; Li, D.; Paty, D.; Graeb, D. *In Vivo* Visualization of Myelin Water in Brain by Magnetic Resonance. *Magn. Reson. Med.* **1994**, 31 (6), 673–677. <https://doi.org/10.1002/mrm.1910310614>.
 - (22) Ou, X.; Gochberg, D. F. MT Effects and T_1 Quantification in Single-slice Spoiled Gradient Echo Imaging. *Magn. Reson. Med.* **2008**, 59 (4), 835–845. <https://doi.org/10.1002/mrm.21550>.

Figures

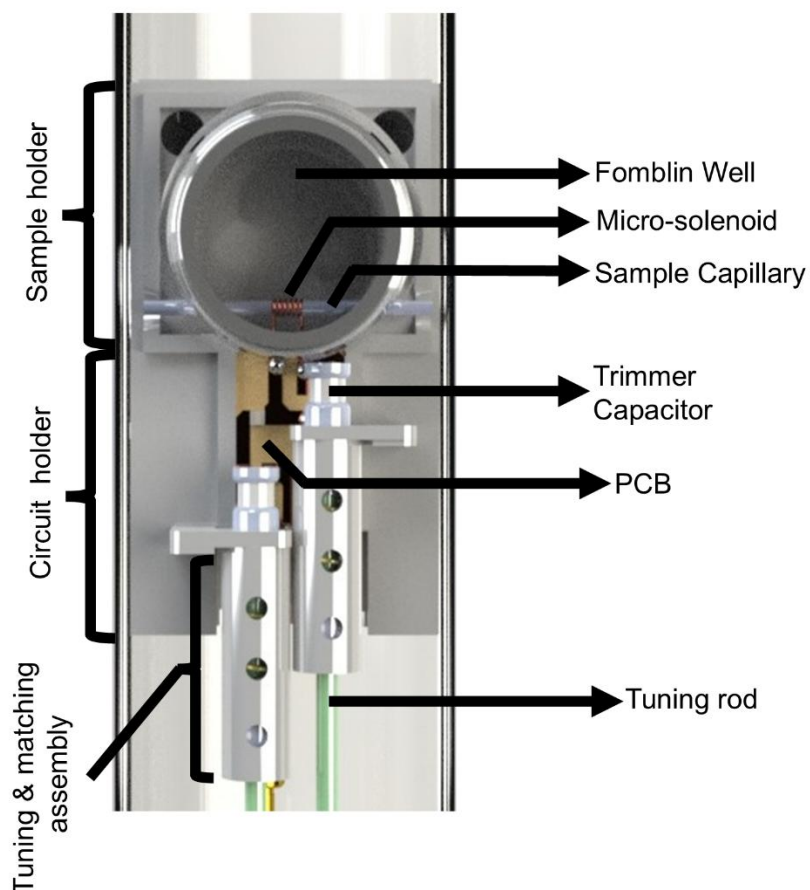


Figure 1. Custom-built micro-imaging probe for a 15.2 T NMR system. The probe comprises a sample holder with a Fomblin well for susceptibility matching and a micro-solenoid coil wound around a sample capillary. A separate circuit holder houses a printed circuit board (PCB) that integrates the trimmer and balance capacitors and interfaces to a 50 Ω transmission line via an SMB connector. Tuning rods couple to the trimmer capacitors through the tuning/matching assembly. Additional details on the probe design and micro-solenoid optimization are provided in our previous work¹¹.

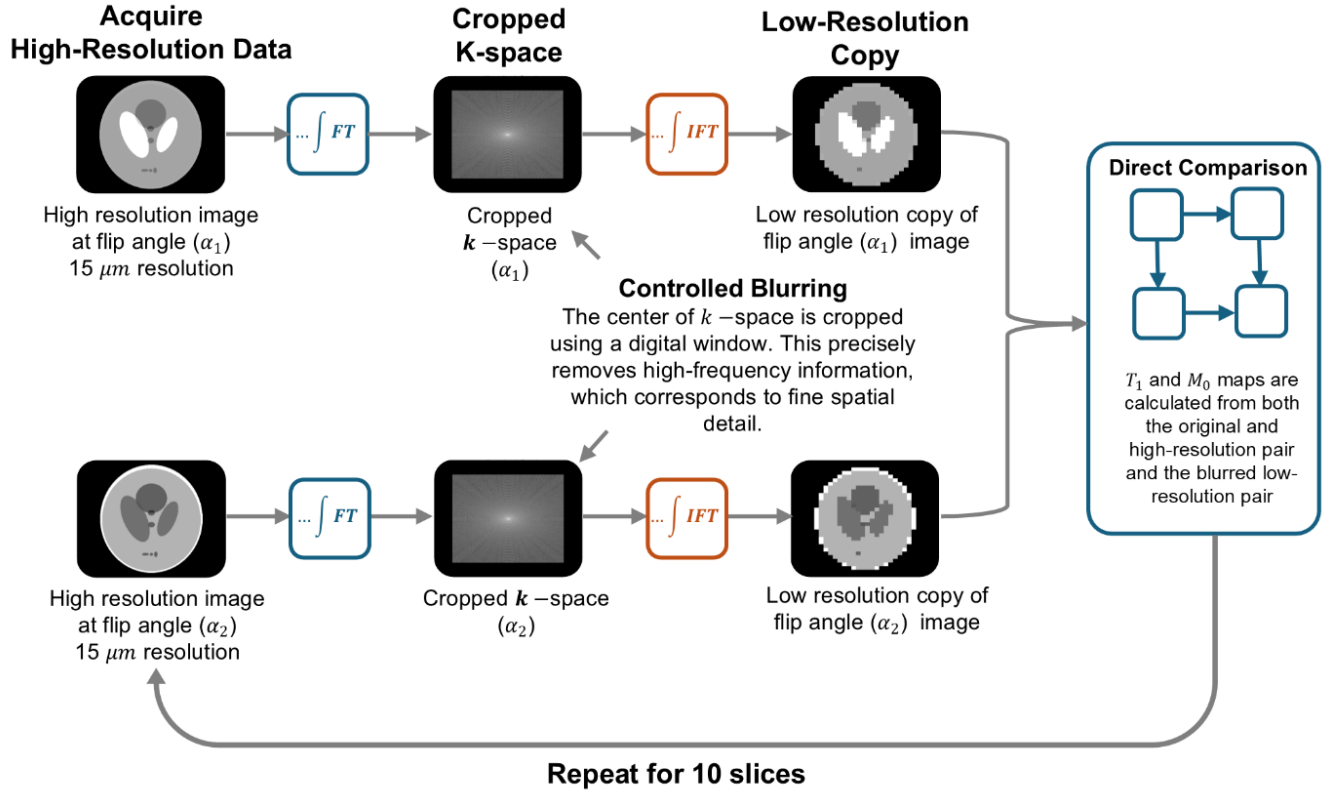


Figure 2. Resolution-controlled blurring pipeline for DESPOT1 analysis. Two SPGR flip angles (α_1 and α_2) are computed from Eq. (1). For each flip angle, the high-resolution image is Fourier transformed to k -space, centrally cropped using a rectangular low-pass window to remove high spatial frequencies, and inverse transformed to generate a matched low-resolution image. DESPOT1 fitting is then performed on the paired images to obtain T_1 and M_0 maps at the native (high) resolution and at the blurred (low) resolution, enabling direct evaluation of how spatial resolution alters the voxel-wise R_1 – M_0 relationship. This procedure is repeated across 10 slices.

Liver Tissue Image Analysis

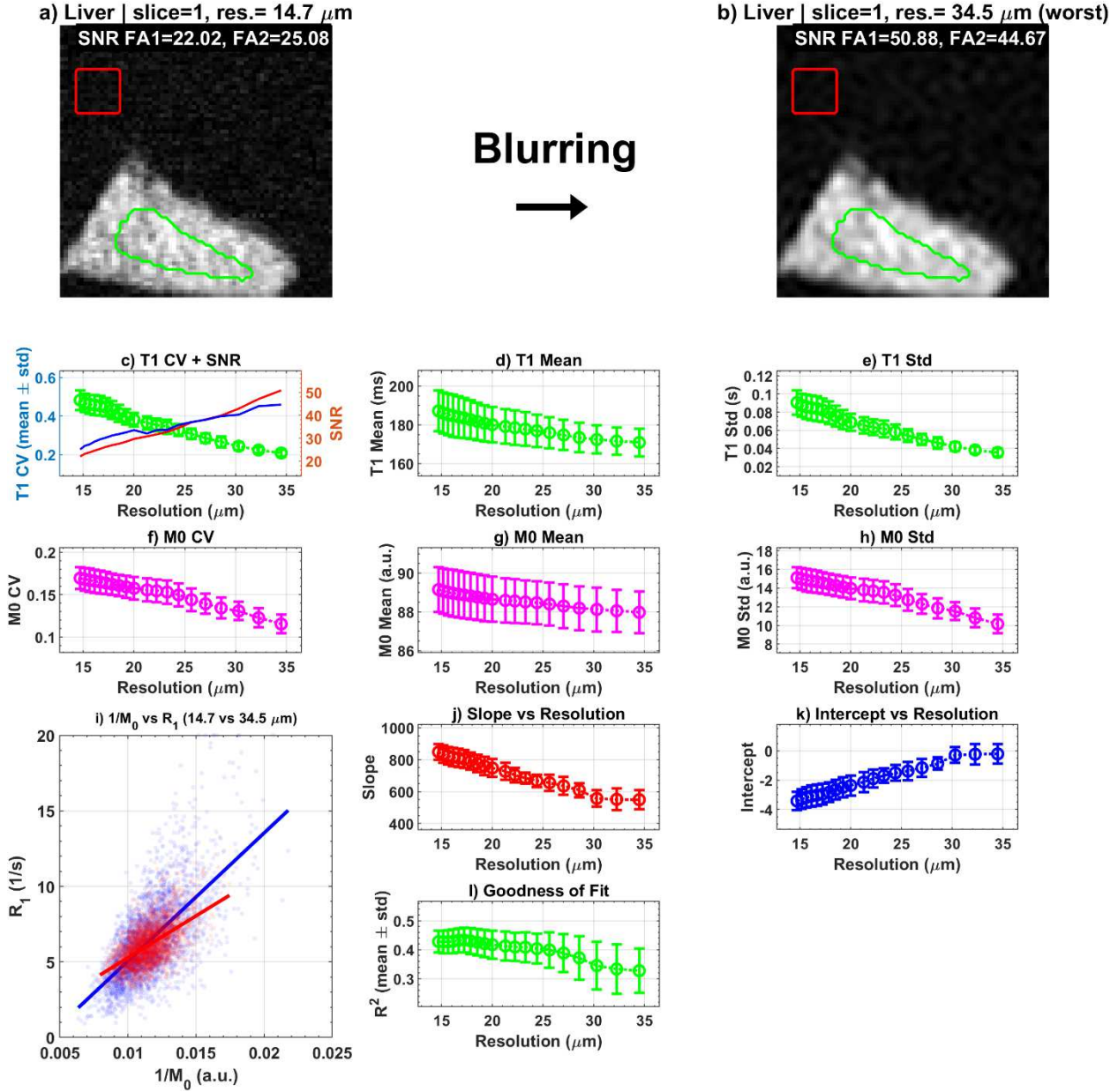


Figure 3. Liver tissue multi-slice resolution analysis and impact on the voxel wise R_1 - M_0 relationship. (a) Representative high-resolution liver SPGR image (slice 1; 14.7 μm) with signal ROI (green contour) and background noise ROI (red box) used for SNR estimation at both flip angles (α_1 (red), α_2 (blue)). (b) Corresponding blurred/low-resolution image (34.5 μm) generated by k -space cropping to mimic reduced spatial resolution. (c-h) multi-slice ($n = 10$) DESPOT1 summary metrics versus resolution (mean $\pm$ SD across slices): T_1 coefficient of variation (CV); SNR of the two flip angle images, mean T_1 , T_1 standard deviation, M_0 CV, mean M_0 , and M_0 standard deviation. (i) Voxel wise R_1 - $1/M_0$ scatter plots with linear fits shown for the highest resolution (blue, 14.7 μm) and the most blurred condition (red, 34.5 μm). (j-l) Linear model parameters and fit quality versus resolution (mean $\pm$ SD across slices): slope (R_1 per M_0), intercept, and coefficient of determination (R^2).

Onion Tissue Image Analysis

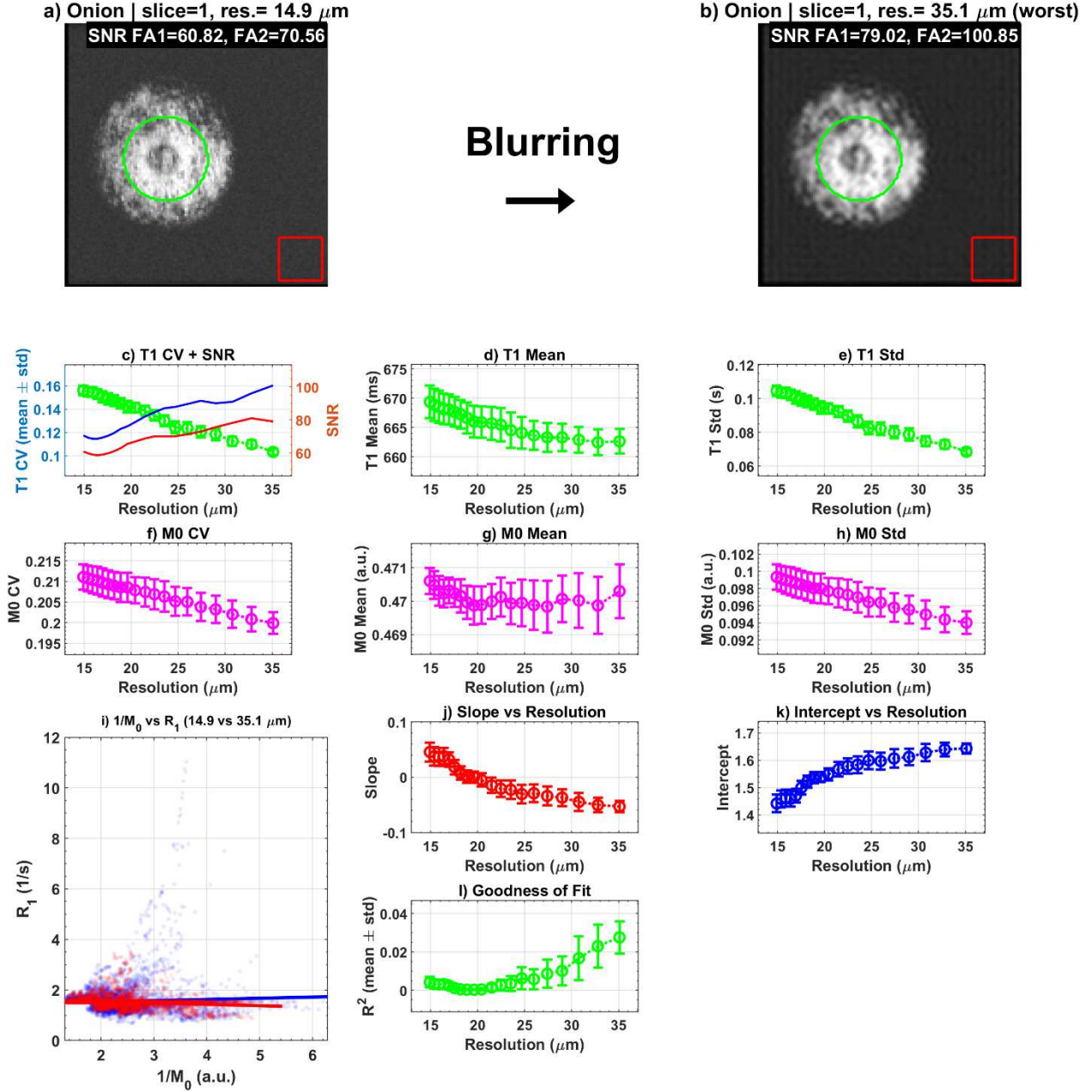
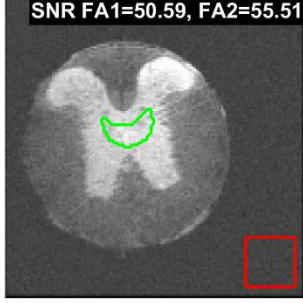


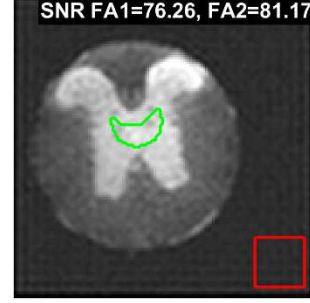
Figure 4. Onion root tissue multi-slice resolution analysis and impact on the voxel wise R_1 - M_0 relationship. (a) Representative high-resolution Onion SPGR image (slice 1; 14.9 μm) with signal ROI (green contour) and background noise ROI (red box) used for SNR estimation at both flip angles (α_1 (red), α_2 (blue)). (b) Corresponding blurred/low-resolution image (35.1 μm) generated by k -space cropping to mimic reduced spatial resolution. (c-h) multi-slice ($n = 10$) DESPOT1 summary metrics versus resolution (mean $\pm$ SD across slices): T_1 coefficient of variation (CV) ; SNR of the two flip angle images, mean T_1 , T_1 standard deviation, M_0 CV, mean M_0 , and M_0 standard deviation. (i) Voxel wise R_1 - $1/M_0$ scatter plots with linear fits shown for the highest resolution (blue, 14.9 μm) and the most blurred condition (red, 35.1 μm). (j-l) Linear model parameters and fit quality versus resolution (mean $\pm$ SD across slices): slope (R_1 per M_0), intercept, and coefficient of determination (R^2).

Spinal cord Tissue Image Analysis

a) Spinal cord | slice=1, res.= 14.9 μm



b) Spinal cord | slice=1, res.= 35.1 μm (worst)



Blurring

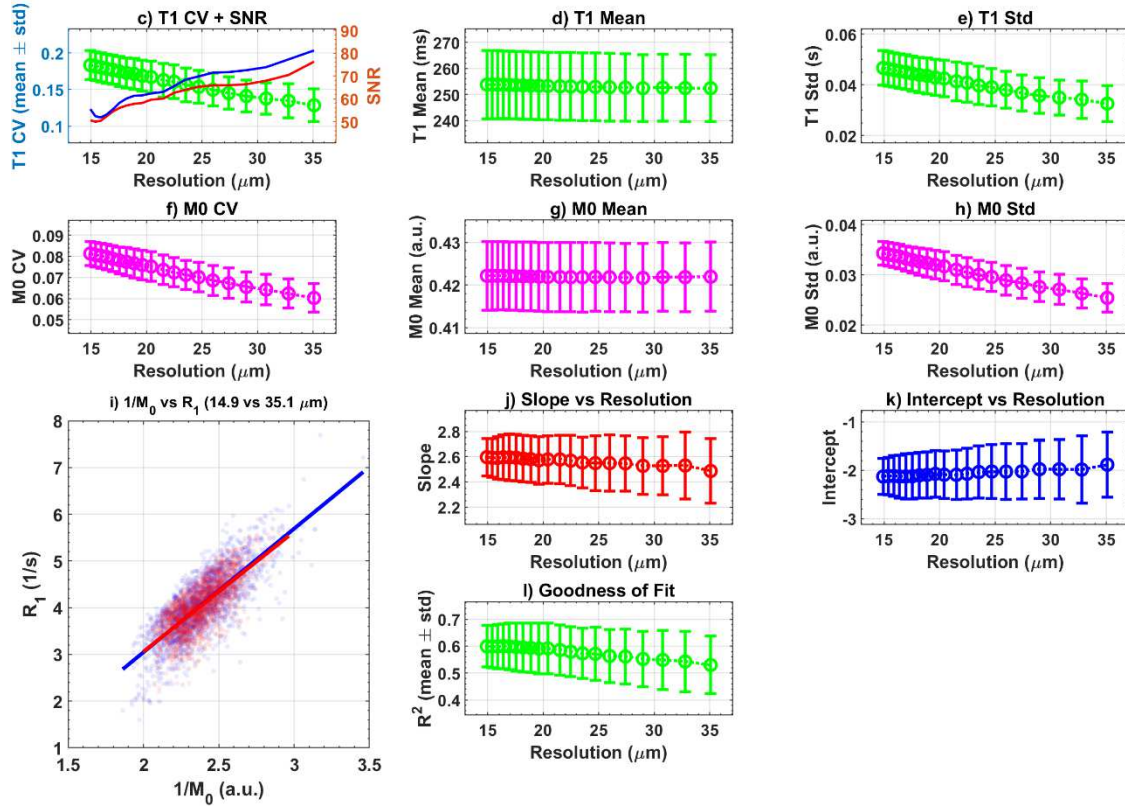


Figure 5. Spinal cord tissue multi-slice resolution analysis and impact on the voxel wise R_1 - M_0 relationship. (a) Representative high-resolution spinal cord SPGR image (slice 1; 14.9 μm) with signal ROI (green contour) and background noise ROI (red box) used for SNR estimation at both flip angles (α_1 (red), α_2 (blue)). (b) Corresponding blurred/low-resolution image (35.1 μm) generated by k -space cropping to mimic reduced spatial resolution. (c-h) multi-slice ($n = 10$) DESPOT1 summary metrics versus resolution (mean $\pm$ SD across slices): T_1 coefficient of variation (CV); SNR of the two flip angle images, mean T_1 , T_1 standard deviation, M_0 CV, mean M_0 , and M_0 standard deviation. (i) Voxel wise R_1 - $1/M_0$ scatter plots with linear fits shown for the highest resolution (blue, 14.9 μm) and the most blurred condition (red, 35.1 μm). (j-l) Linear model parameters and fit quality versus resolution (mean $\pm$ SD across slices): slope (R_1 per M_0), intercept, and coefficient of determination (R^2).

PBS Tissue Image Analysis

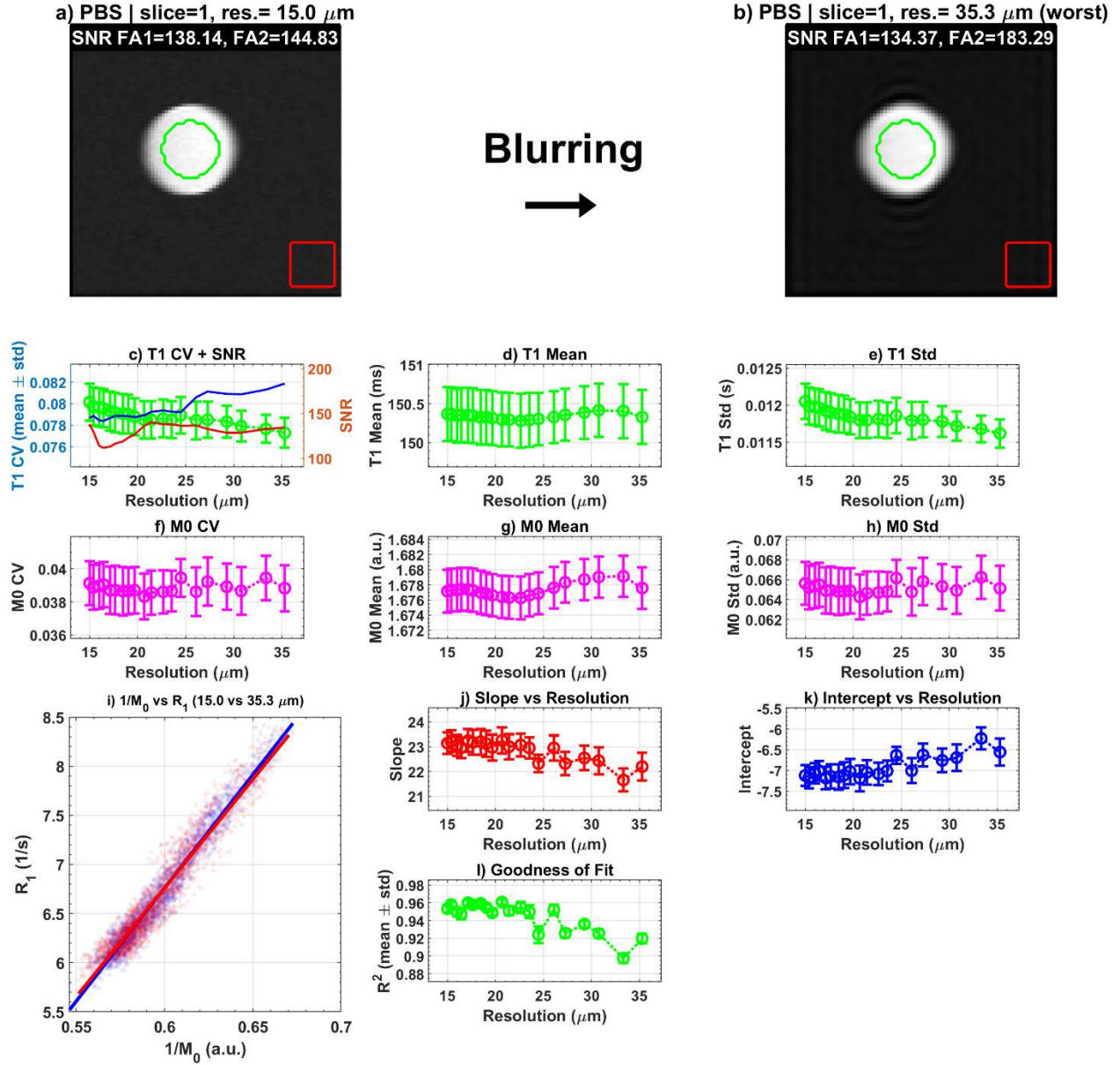


Figure 6. Uniform PBS phantom multi-slice resolution analysis and impact on the voxel-wise $R_1 - M_0$ relationship. (a) Representative high-resolution uniform pbs phantom SPGR image (slice 1; 15 μm) with signal ROI (green contour) and background noise ROI (red box) used for SNR estimation at both flip angles (α_1 (red), α_2 (blue)). (b) Corresponding blurred/low-resolution image (35.3 μm) generated by k -space cropping to mimic reduced spatial resolution. (c-h) multi-slice ($n = 10$) DESPOT1 summary metrics versus resolution (mean $\pm$ SD across slices): T_1 coefficient of variation (CV); SNR of the two flip angle images, mean T_1 , T_1 standard deviation, M_0 CV, mean M_0 , and M_0 standard deviation. (i) Voxel wise R_1 - $1/M_0$ scatter plots with linear fits shown for the highest resolution (blue, 15 μm) and the most blurred condition (red, 35.3 μm). (j-l) Linear model parameters and fit quality versus resolution (mean $\pm$ SD across slices): slope (R_1 per M_0), intercept, and coefficient of determination (R^2).

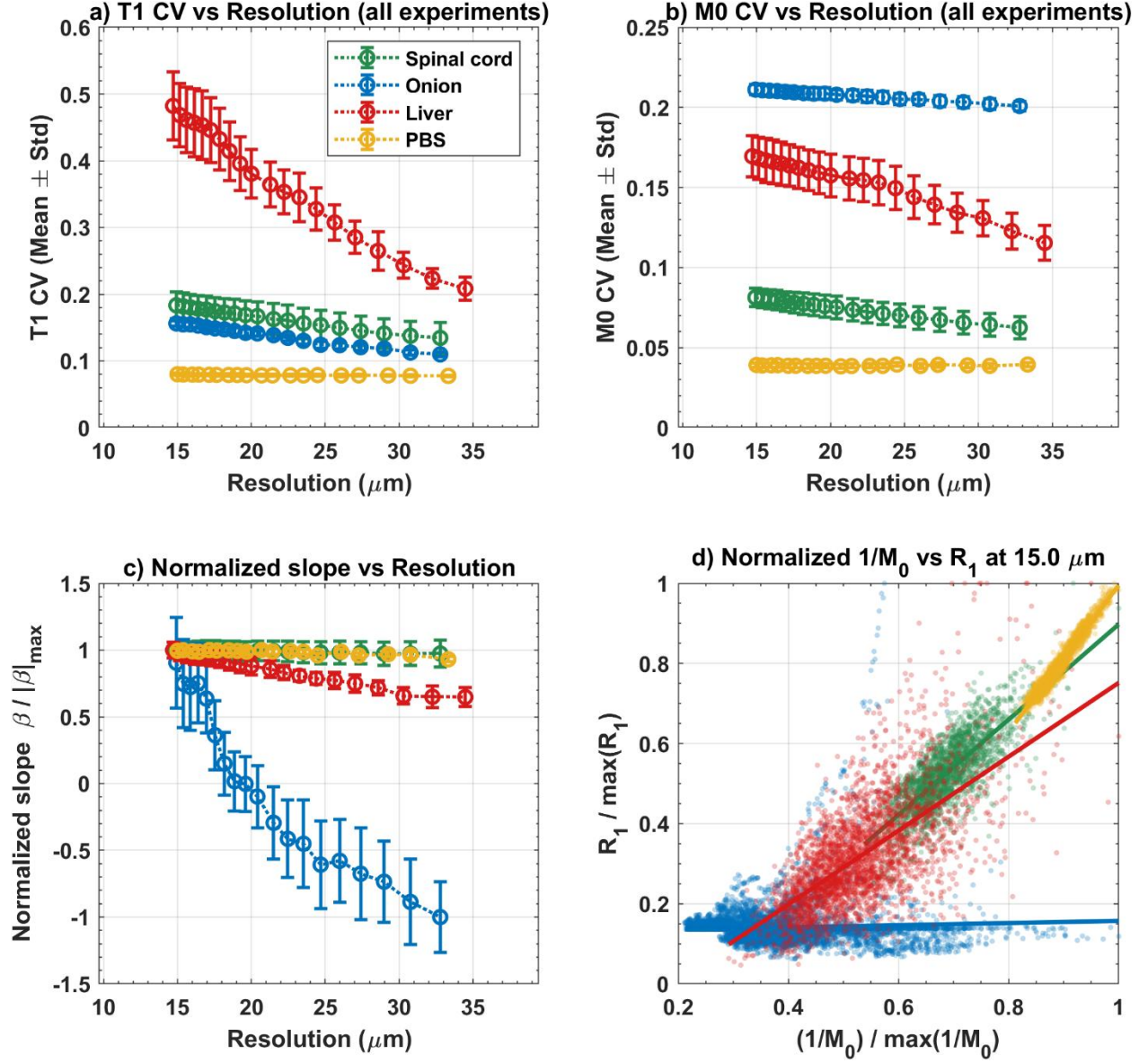


Figure 7. Comparison of heterogeneous tissues and a uniform phantom across spatial resolution. (a) T_1 coefficient of variation (CV) versus resolution for spinal cord (green), onion (blue), liver (red), and PBS phantom (yellow), reported as mean $\pm$ SD across slices. (b) Corresponding M_0 CV versus resolution (mean $\pm$ SD across slices). (c) Resolution dependence of the fitted $R_1 - 1/M_0$ slope (β) after normalization by each experiment's maximum absolute slope ($\beta/|\beta|_{\max}$); error bars denote slice-to-slice variability (mean $\pm$ SD). (d) Normalized voxel-wise $R_1 - M_0$ scatter at $\sim 15 \mu m$, plotted as $M_0/\max(M_0)$ versus $R_1/\max(R_1)$ to enable cross-sample comparison; overlaid lines indicate the corresponding linear fits for each sample type.

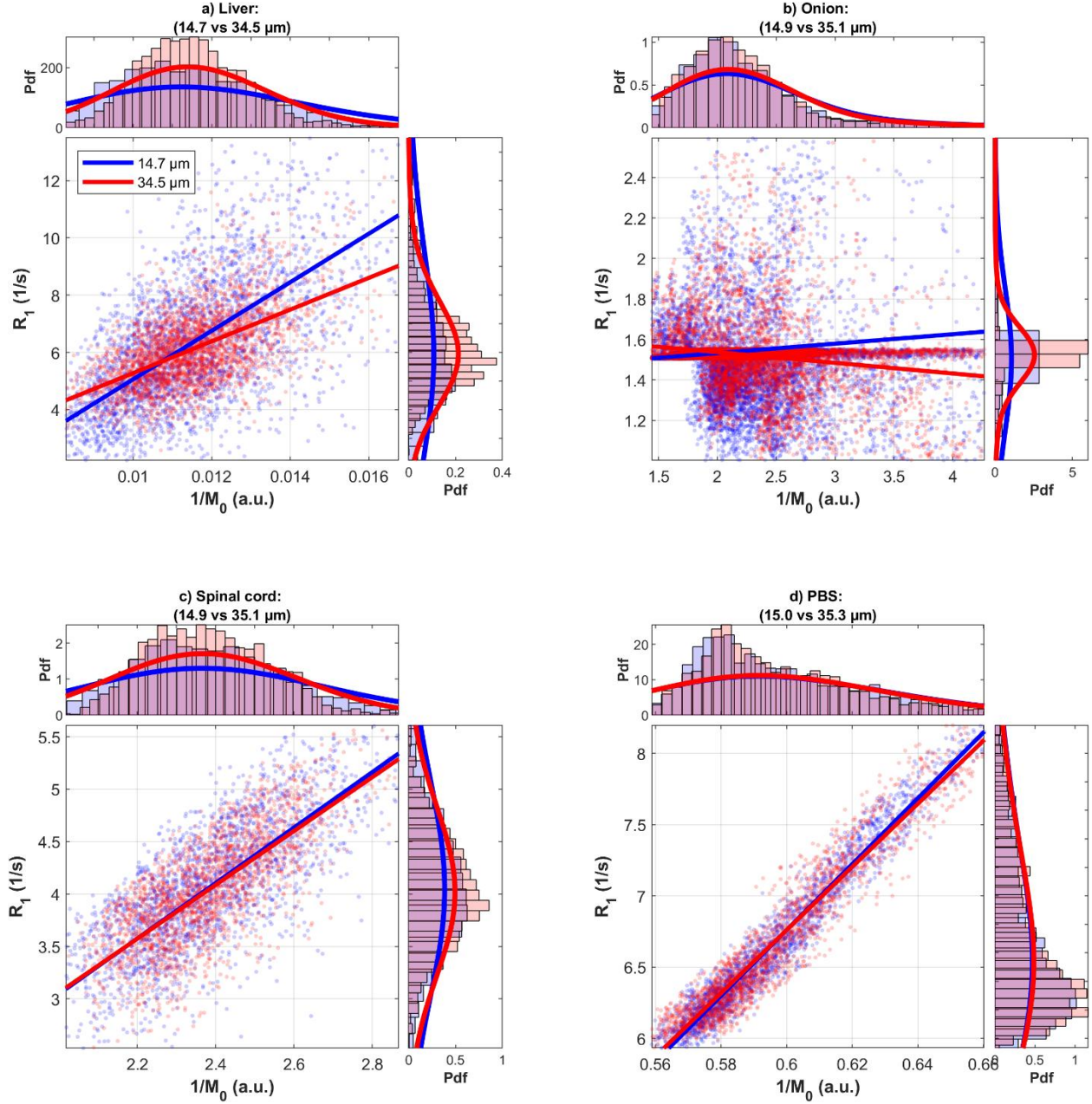


Figure 8. Resolution-dependent R_1 - $1/M_0$ relationship in heterogeneous tissues and uniform pbs phantom. (a) Liver: voxel-wise scatter of R_1 versus M_0 at high resolution (blue, 14.7 μm) and after Fourier k-space cropping to low resolution (red, 34.5 μm). Solid lines show the corresponding linear fits. The dashed box indicates the region magnified at right, where the joint scatter is shown with marginal distributions (top: M_0 ; right: R_1) for each resolution. (b) Green onion: same analysis (14.9 μm vs 35.1 μm), illustrating reduced apparent heterogeneity and a weaker dependence of R_1 on M_0 after blurring. (c) Spinal cord tissue: same analysis (14.9 μm vs 35.1 μm) (d) Uniform phantom (PBS): same analysis (15.0 μm vs 35.3 μm); the tight, nearly one-dimensional R_1 - $1/M_0$ cloud and its persistence across resolutions indicate a primarily systematic/global relationship rather than microstructural heterogeneity.

Appendix A

As stated in the introduction, the observed relaxation rate is

$$R_1 = (1 - f)R_{10} + fR_{1b} \quad (A1)$$

$$R_1 = R_{10} + f(R_{1b} - R_{10}) \quad (A2)$$

Where f is proportional to the concentration $c = m/M_0$ of relaxation-promoting solute, where m is the amount in a total water content whose magnetization is M_0 .

$$R_1 - R_{10} = \frac{km}{M_0}(R_{1b} - R_{10}) \quad (A3)$$

Where k denotes relaxivity. This can be extended to incorporate multiple pools i , each with own characteristics but all in fast exchange so that

$$R_1 - R_{10} = \sum_i \frac{k_i m_i}{M_0} (R_{1b} - R_{10}) \quad (A4)$$

Now, let us define

$$\Gamma = \sum_i k_i m_i (R_{1b} - R_{10}) \quad (A5)$$

Thus, equation A4 becomes:

$$R_1 - R_{10} = \Gamma / M_0 \quad (A6)$$

Now, assume the water content fluctuates slightly around the mean value:

$$M_0 = \overline{M_0} + \Delta M_0 \quad (A7)$$

Where $\overline{M_0}$ is mean water content and ΔM_0 is a small perturbation.

Substituting equation (A7) in A6,

$$R_1 - R_{10} = \frac{\Gamma}{\overline{M_0} + \Delta M_0} \quad (A8)$$

Equation A8 can be rewritten as:

$$R_1 - R_{10} = \Gamma \overline{M_0}^{-1} \left(1 + \frac{\Delta M_0}{\overline{M_0}} \right)^{-1} \quad (A9)$$

For small perturbations,

$$\left| \frac{\Delta M_0}{\overline{M_0}} \right| \ll 1$$

Using Taylor's expansion

$$R_1 - R_{10} \approx \Gamma \overline{M_0}^{-1} \left(1 - \frac{\Delta M_0}{\overline{M_0}} \right) \quad (A10)$$

$$R_1 - R_{10} \approx \Gamma \overline{M_0}^{-1} - \Gamma \Delta M_0 \overline{M_0}^{-2} \quad (A11)$$

Let's define mean relaxation rate as

$$\overline{R_1} - R_{10} = \Gamma \overline{M_0}^{-1} \quad (A12)$$

Then the total relaxation rate can be written as:

$$R_1 = \overline{R_1} + \Delta R_1 \quad (A13)$$

Substituting A12 and A13 in A11, we get:

$$\text{after cancellation,} \quad (\overline{R_1} + \Delta R_1) - R_{10} = (\overline{R_1} - R_{10}) - \Gamma \Delta M_0 \overline{M_0}^{-2} \quad (A14)$$

$$\Delta R_1 = -\Gamma \Delta M_0 \overline{M_0}^{-2} \quad (A15)$$

Appendix B

DESPOT1¹⁵ technique has two advantages: (1) only two images are required which significantly reduces the scan time (2) SPGR sequences have faster readouts compared to inversion recovery-based sequences which makes the acquisition of the images faster. The idea is based on linearizing the signal equation of SPGR sequence. The signal in a SPGR sequence depends upon equilibrium longitudinal magnetization, repetition time, TR , longitudinal relaxation time, T_1 , and flip angle, α_1 :

$$S_{SPGR} = \frac{M_0(1 - E_1) \sin(\alpha)}{1 - E_1 \cos(\alpha)} \quad (B1)$$

Where $E_1 = \exp(-TR/T_1)$. Linearizing the equation in the form of a straight-line equation $y = mx + c$, we get:

$$S_{SPGR}/\sin(\alpha) = \exp(-TR/T_1) [S_{SPGR}/\tan(\alpha)] + M_0 [1 - \exp(-TR/T_1)] \quad (B2)$$

Since the TR is fixed, the two α s and signal at these flip angles are known, the slope and intercept can be estimated using linear regression, and thus, T_1 and M_0 :

$$T_1 = -TR/\ln(m) \quad (B3)$$

$$M_0 = c/(1 - m) \quad (B4)$$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformationFileTissueMicrostructuralHeterogeneityRevealedbyMRMicroscopy.docx](#)