# Chapter 23

# Experimental Protocol for MRI Mapping of the Blood Oxygenation-Sensitive Parameters $T_2$* and $T_2$ in the Kidney

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## Abstract

Renal hypoxia is generally accepted as a key pathophysiologic event in acute kidney injury of various origins, and has also been suggested to play a role in the development of chronic kidney disease. Here we describe a step-by-step experimental protocol for indirect monitoring of renal blood oxygenation in rodents via the deoxyhemoglobin sensitive MR parameters $T_2$* and $T_2$—a contrast mechanism known as the blood oxygenation level dependent (BOLD) effect. Since an absolute quantification of renal oxygenation from $T_2$*/$T_2$ remains challenging, the effects of controlled and standardized variations in the fraction of inspired oxygen are used for bench marking. This MRI method may be useful for investigating renal blood oxygenation of small rodents in vivo under various experimental (patho)physiological conditions.

This chapter is based upon work from the COST Action PARENCHIMA, a community-driven network funded by the European Cooperation in Science and Technology (COST) program of the European Union, which aims to improve the reproducibility and standardization of renal MRI biomarkers. This experimental protocol chapter is complemented by two separate chapters describing the basic concept and data analysis.

**Key words** Magnetic resonance imaging (MRI), Kidney, Mice, Rats, $T_2$, $T_2$*, BOLD

## 1 Introduction

Renal tissue oxygenation relies on a delicate balance between delivery of $O_2$, as determined by renal blood flow and arterial $O_2$ content, and consumption of $O_2$, which is predominantly determined by energy-dependent tubular reabsorption. Even under physiological conditions, tissue partial pressure of $O_2$ is low in the medulla. Hence renal tissue hypoxia may result from even a small detrimental imbalance between $O_2$ delivery and consumption. Based largely upon studies on animal models, intrarenal hypoxia is generally accepted as a key pathophysiologic event in acute kidney injury of various origins, and has also been suggested to promote its progression to chronic kidney disease CKD.

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The parametric mapping of the transverse relaxation times $T_2^*$ and $T_2$ (or relaxation rates $R_2^* = 1/T_2^*$ and $R_2 = 1/T_2$) has the potential to allow inferences about renal oxygenation, because both parameters are sensitive to blood oxygenation. The underlying mechanism is the inherent difference in the magnetic properties of oxygenated hemoglobin (diamagnetic) vs. deoxygenated hemoglobin (paramagnetic). The presence of deoxyhemoglobin in a voxel decreases both relaxation times, $T_2^*$ and $T_2$. Additional factors that influence this relationship between renal venous blood oxygenation and renal $T_2^*$ include the blood volume fraction, the hematocrit and the oxygen binding curve.

Here we describe quantitative mapping of the blood oxygenation sensitive parameter $T_2^*$ in the kidney of small rodents in a step-by-step experimental protocol. The rationale for the chosen acquisition parameters is given in generic terms, together with specific parameter examples.

Mapping of renal $T_2$ is described as an optional component of the experiment. This may serve two purposes. Firstly, $T_2^*$ includes the dynamic (irreversible) dephasing effects described by $T_2$ plus the additional effects that are due to static (reversible) dephasing effects described by $T_2'$. Hence, the additional measurement of $T_2$ permits calculation of $R_2' = R_2^* - R_2 = 1/T_2^* - 1/T_2$.

Secondly, blood oxygenation affects primarily $T_2^*$ (often referred to as blood oxygenation level dependent, BOLD) but also $T_2$ to a lesser extent. The $T_2$ effect is via water diffusion within the magnetic field gradient created by deoxyhemoglobin. This contrast mechanism results in $T_2$ being dominated by blood oxygenation effects in the microvasculature, which makes it a valuable complement to conventional $T_2^*$ measurements.

The effects of controlled and standardized variations in $T_2^*$ relaxation parameters in response to the fraction of inspired oxygen (hypoxia, hyperoxia) are used for benchmarking. This MRI method may be useful for investigating renal blood oxygenation of rodents in vivo under various experimental (patho)physiological conditions.

This experimental protocol chapter is complemented by two separate chapters describing the basic concept and data analysis, which are part of this book.

This chapter is part of the book Pohlmann A, Niendorf T (eds) (2020) Preclinical MRI of the Kidney—Methods and Protocols. Springer, New York.

## 2 Materials

### 2.1 Animals

These experimental protocols are tailored for rats (Wistar, Sprague-Dawley, or Lewis) with a body mass of 250–350 g. Advice for adaptation to mice is given in where necessary.

### 2.2 Lab Equipment

Anesthesia: For nonrecovery experiments urethane solution (Sigma-Aldrich, Steinheim, Germany; 20% in distilled water) can provide anesthesia for several hours with comparatively little side effects on renal physiology, which is an important issue. For an in-depth description and discussion of the anesthesia please refer to the chapter by Kaucsar T et al. "Preparation and Monitoring of Small Animals in Renal MRI."

Gases: $O_2$, $N_2$, and compressed air, as well as a gas-mixing system (FMI Föhr Medical Instruments GmbH, Seeheim-Ober Beerbach, Germany) to achieve required changes in the oxygen fraction of inspired gas mixture ($FiO_2$). Besides air, the following gas mixtures are required during the experiment: 10% $O_2$–90% $N_2$ for hypoxia and 100% $O_2$ for hyperoxia.

Device for $FiO_2$ monitoring in gas mixtures: for example Capnomac AGM-103 (Datex GE, Chalfont St Gils, UK).

### 2.3 MRI Hardware

The general hardware requirements for renal $^1$H MRI on mice and rats are described in the chapter by Ramos Delgado P et al. "Hardware Considerations for Preclinical Magnetic Resonance of the Kidney." The technique described in this chapter was tailored for a 9.4 T MR system (Biospec 94/20, Bruker Biospin, Ettlingen, Germany) but advice for adaptation to other field strengths and systems (e.g., 3 T Siemens Skyra or GE MR 750 human MR scanner using a wrist coil for signal reception or transmit-receive knee RF coil is given where necessary. No special or additional hardware is required, except for the following:

1. A physiological monitoring system that can track the respiration, and which is connected to the MR system such that it can be used to trigger the image acquisition.

### 2.4 MRI Techniques

Repeated measurements with a gradient-echo or spin-echo method and variable echo times (TE) would provide the most accurate $T_2$* and $T_2$ but require very long acquisition times. Here we describe fast multiecho MRI methods: multi-gradient-echo (MGE) for $T_2$* measurement and multi–spin-echo (also called "MSME") for $T_2$ assessment. They provide relaxation times that underestimate $T_2$* and overestimate $T_2$. With increasing echo number additional diffusion weighting is added due to the repeated magnetic field gradients (NB: this effect is much more pronounced in small animal systems because of the much stronger magnetic field gradients used compared to clinical systems). The superposition of stimulated echoes in multi–spin-echo imaging may lead to an overestimation of $T_2$. Although these biases depend on the acquisition parameters, they are fixed and reproducible for each protocol and hence acceptable in studies of relative differences/changes in $T_2$* and $T_2$, where precision is far more important than accuracy. If needed, more accurate $T_2$ values can be obtained from multiecho data using sophisticated postprocessing [1].

1. 2D multi-gradient-echo sequence for $T_2$*-mapping. This is a standard sequence that should be available on all MRI systems. (Bruker "MGE," Siemens "gre"; HE Healthcare 2D ME SPGR or ME FGRE) (*see* **Note 1**).
2. 2D multi-spin-echo sequence for $T_2$-mapping (optional; Bruker "MSME," Siemens "se_mc"; GE Healthcare "T2 Map" within the 2D ME FGRE family of sequences).
3. 3D double gradient echo sequence for acquiring a quantitative map of the static magnetic field $B_0$ (optional; Bruker "FieldMap"). The reconstruction performs a phase difference calculation, phase unwrapping, and a conversion to a frequency map. A similar sequence exists with Siemens ("AdjGreSeq"), however there is no reconstruction applied to the data. Choosing Magnitude and Phase under Reconstruction will save both Magnitude and Phase information, but no processing is performed to correct for the $B_0$ map. Similarly, for GE Healthcare clinical MR systems "IDEAL-IQ" allows for return of fat, water, $R_2$*, in-phase and out-of-phase water/fat images, but research mode is required to obtain magnitude, phase, and $B_0$ images.

## 3 Methods

### 3.1 MR Protocol Setup

#### 3.1.1 Multi-gradient-Echo Sequence for $T_2$*-Mapping

1. Load the 2D multi–gradient-echo sequence.
2. Set the shortest echo time (TE) and echo spacing (ΔTE) possible, under the condition that fat and water are in phase (*see* **Note 2**). The last TE should be close to the largest expected $T_2^{(*)}$ in the kidney multiplied by 1.5 (*see* **Note 3**). The aim is to acquire ten or more echo images. Fewer TEs or larger ΔTE are not advisable because during strong hypoxia the SNR in the kidney at high TEs can be so low that only a few data points above the noise level are available for the analysis. Consider increasing the acquisition bandwidth and using half Fourier acceleration to shorten the first TE and ΔTE (*see* **Note 4**).
3. Choose the shortest possible repetition time (TR) for good signal-to-noise per time (SNR/t) efficiency. TR will be limited by the length of the echo train and the number of slices you acquire.
4. Adapt the flip angle (FA) to the TR and $T_1$ in order to achieve the best possible SNR. Use the Ernst angle $\alpha_E = \arccos(\exp(-TR/T_1))$ as a good starting value. Then try a few smaller and larger FAs and determine the optimal FA experimentally by comparing the measured SNRs in a phantom with a $T_1$ comparable to that of the renal cortex (1–1.2 s at 3 T).

5. Set a high acquisition bandwidth (BW) to shorten ΔTE, while keeping an eye on the SNR, which decreases with the square root of BW. SNR may be increased with signal averaging. (*see* **Note 5**).
6. Enable fat saturation. On ultrahigh field systems this works well to avoid fat signal overlaying the kidney due to chemical shift. At lower clinical field strengths fat saturation is less efficient.
7. Enable the respiration trigger (per slice). This is essential to reduce motion artefacts, reduce motion blurring and unwanted intensity variations between the images acquired with different TEs.
8. Choose as phase-encoding direction the L-R direction and adapt the geometry so that the FOV in this direction includes the entire animal (approx. 40 mm).
9. Use frequency encoding in head-feet (rostral-caudal) direction to avoid severe aliasing. Adjust the FOV to your needs keeping in mind that in the frequency encoding direction the FOV can be smaller than the animal and a smaller FOV permits a smaller acquisition matrix, and in turn a shorter echo-spacing.
10. Use the lowest slice thickness the SNR allows, typically around 1.5 mm. The thinner the slices the better, as this reduces the unwanted $T_2^*$-effects of macroscopic field inhomogeneities associated with the large voxel size in slice direction. For adaptation of the geometry to mice *see* **Note 6**.
11. Use highest in-plane resolution that the SNR allows, typically between 100 and 200 μm. The higher the resolution the better, as this reduces the unwanted $T_2^*$-effects of macroscopic field inhomogeneities. Zero-filling in phase encoding direction can be helpful to speed up acquisition when monitoring of fast oxygenation changes is required. One may use half Fourier in the frequency encoding direction (asymmetric echo) to further shorten the first TE, if very short $T_2^*$ (<5 ms) can occur. Reducing the excitation pulse length to below 1 ms would then also help to shorten TE. For adaptation to mice *see* **Note 7**.
12. For examples of a specific parameter set please *see* **Notes 8–10**.

*3.1.2 Multi-spin-Echo Sequence for $T_2$-Mapping (Optional)*

1. Load the 2D multi–spin-echo sequence.
2. Use the same FOV, matrix size and slice thickness as for the multi–gradient-echo sequence.
3. Adapt the repetition time (TR) to the respiration: The effective TR will be given by the respiration trigger and will be the respiration interval, that is, one excitation per breath. Choose TR to be a little shorter (about 100 ms) than the average

respiration interval that is displayed on the physiological monitoring unit (*see* **Note 11**).

4. Set the number of echoes to 12 (*see* **Note 12**.).
5. Set the echo spacing (ΔTE) as small as possible (more echoes are effectively more data points for the analysis and reduce the error; consider that with increasing number of echoes also the SAR increases) and that the last TE is close to the largest expected $T_2$ in the kidney multiplied by 1.5 (*see* **Note 3**).
6. Due to the artificial increase in signal intensity of fat with a multi–spin-echo sequence, enable fat saturation. Note that the spectral fat saturation does not usually suppress the fat signal completely with some of residual fat signal overlaying the kidney due to chemical shift (Fig. 2).
7. Enable the respiration trigger (per slice).
8. For an example of a specific parameter set please *see* **Notes 13–15**.

#### *3.1.3 Field Map (Optional)*

1. Load the field map sequence.
2. Store the field mapping scan protocol with the default parameters when using a small animal system (*see* **Note 16**) or those adapted for a clinical system (*see* **Notes 17** and **18**). Make sure the 3D $B_0$ field-map has isotropic resolution and a FOV that includes the entire MR-visible part of the animal.

### *3.2 In Vivo Blood Oxygenation Sensitive Imaging*

#### *3.2.1 Animal Preparation*

1. Anesthetize the animal and transfer it to scanner. For more information on the choice and use of anesthesia please refer to the chapter by Kaucsar T et al. "Preparation and Monitoring of Small Animals in Renal MRI."
2. Start the temperature monitoring system, apply some surgical lubricant to the temperature probe and place it in the rectum of the animal.
3. Attach the respiration sensor (e.g., balloon) to the chest of the animal using adhesive tape. Start and setup the respiratory monitoring system. If necessary, adjust the position of the respiration sensor until the amplitude of the respiration trace is sufficiently large for the system to reliably detect the trigger points at the beginning of expiration.

#### *3.2.2 Scanner Adjustments and Anatomical Imaging*

1. Acquire a fast pilot scan to obtain images in the three orthogonal planes *x*, *y*, and *z*.
2. Acquire anatomical images in several oblique orientations to facilitate planning a coronal slice orientation with regard to the long axis of the kidney, as described in the chapter by Pohlmann A et al. "Essential Practical Steps for MRI of the Kidney in Experimental Research."

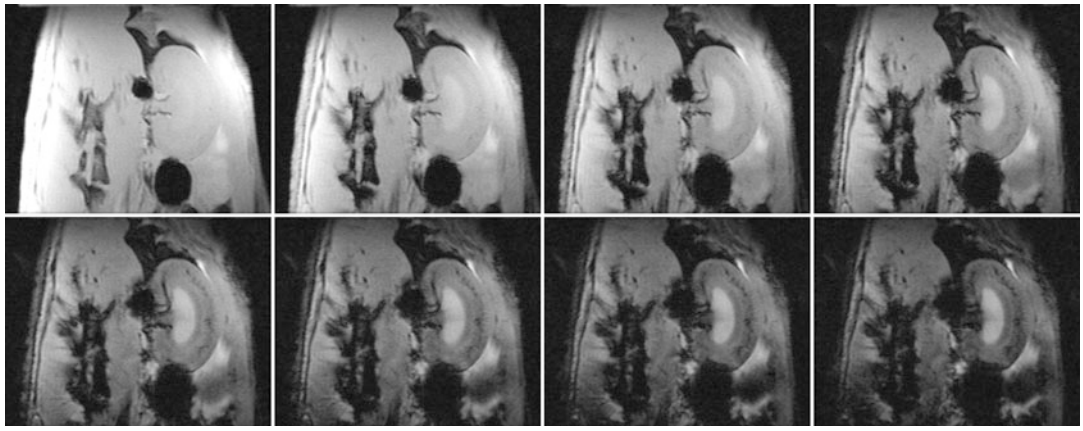

**Fig. 1** Series of 8 $T_2$*-weighted images of a healthy rat kidney acquired with a multi–gradient-echo sequence at 9.4 T. Images correspond to TE = 1.43, 3.57, 5.71, 7.85 ms (top row), TE = 9.99, 12.13, 14.27, 16.41 ms (bottom row)

3. Perform localized shimming on the kidney as described in the chapter by Pohlmann A et al. "Essential Practical Steps for MRI of the Kidney in Experimental Research" (*see* **Note 19**).
4. Acquire a 3D B0 field-map without adaptation of the geometry (optional; *see* **Note 20**).

*3.2.3 Baseline Condition*

1. Load the $T_2$*-mapping sequence, adapt the slice orientation to provide a coronal or axial view with respect to the kidney (in scanner coordinates this is double-oblique).
2. In the monitoring unit set the trigger delay so that the trigger starts at the beginning of the expiratory plateau (no chest or diaphragm motion) and the duration such that it covers the entire expiratory phase, that is, until just before inhalation starts (1/2 to 2/3 of breath-to-breath interval) (*see* **Note 11**).
3. Run the $T_2$*-mapping scan. Example images are shown in Fig. 1.
4. Load the $T_2$-mapping sequence, adapt the slice orientation to provide a coronal or axial view with respect to the kidney (in scanner coordinates this is double-oblique).
5. Adapt TR to be a little shorter (about 100 ms) than the average respiration interval that is displayed on the physiological monitoring unit.
6. In the monitoring unit set the trigger delay so that the trigger starts at the beginning of the expiratory plateau (no chest or diaphragm motion) and the duration to a short value, such as 10 ms (**Note 11**).
7. Run the $T_2$-mapping scan. Example images are shown in Fig. 2.

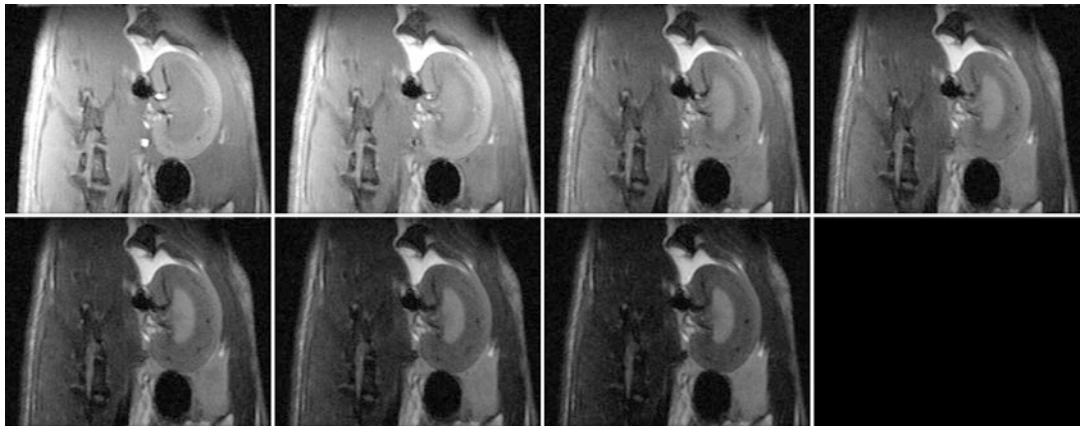

**Fig. 2** Series of 7 $T_2$-weighted images of a healthy rat kidney acquired with a 2D multi-spin-echo sequence at 9.4 T. Images correspond to TE = 10, 20, 30, 40 ms (top row) and TE = 50, 60, 70 ms (bottom row)

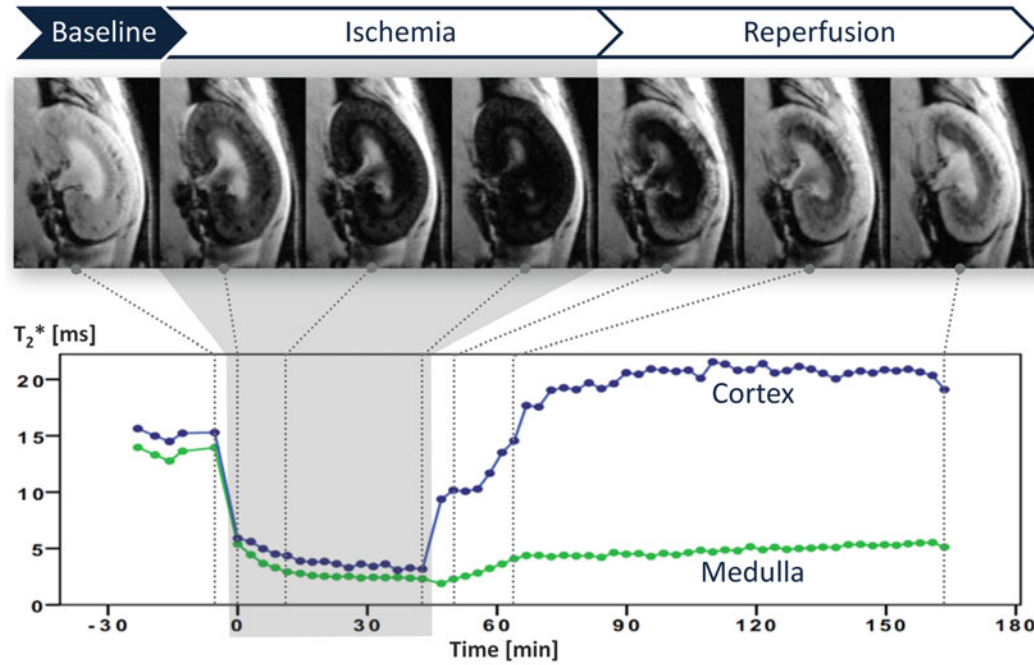


**Fig. 3** Demonstration of the contrast changes that can be expected in pathophysiological scenarios. $T_2$*-weighted images (from a 2D multi–gradient-echo acquisition, TE = 3.57 ms) of rat kidneys throughout an ischemia–reperfusion experiment, together with a plot of $T_2$* in regions-of-interest in renal cortex and medulla

A demonstration of the contrast changes that can be expected in pathophysiological scenarios is given in Fig. 3. Example parametric maps of $T_2$* and $T_2$ are shown in Fig. 4.

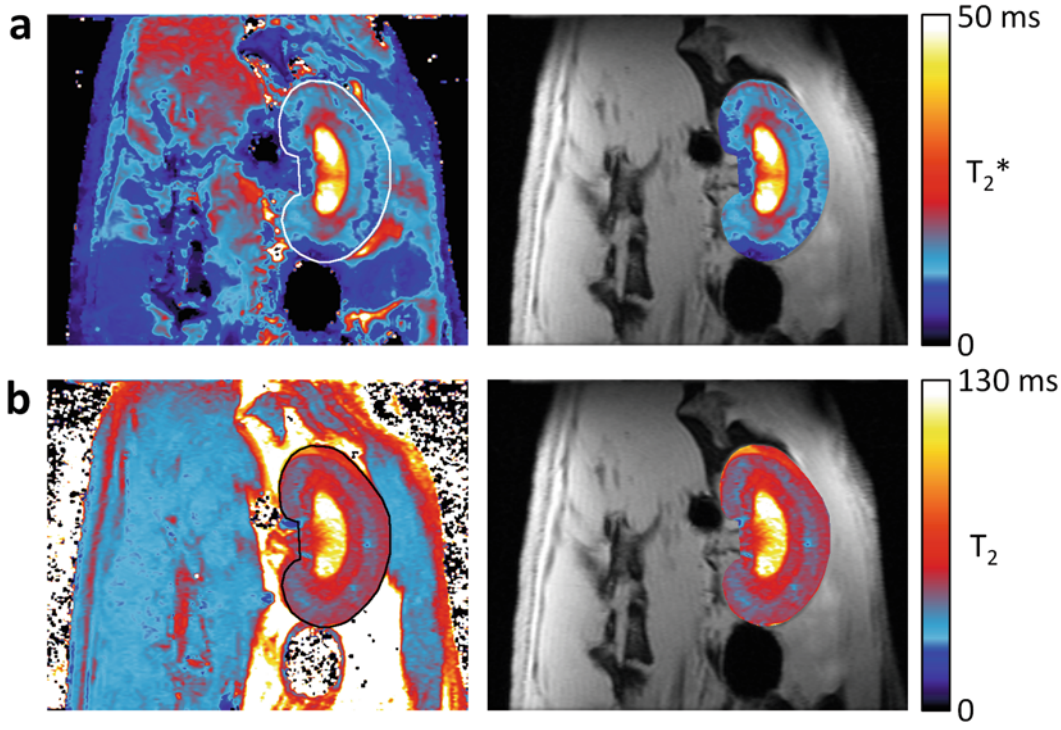


**Fig. 4** Example parametric maps of $T_2^*$ (**a**) and $T_2$ (**b**) acquired in a rat at 9.4 T. Shown are the entire map with the kidney outlined (left) as well as an overlay of the map onto an anatomical MR image (right). While the overlays are preferable because they focus on the relevant part and do not distract the eye, looking at the entire map can sometimes help to identify extra-renal sources of susceptibility artefacts, like here the bowel gas below the kidney and an implanted device near the hilus, which cast "shadows" onto the renal cortex. Beware to exclude from the ROI analysis any regions with overlaid fat signal due to chemical shift, here seen as a thin stripe at the rostral end of the kidney

*3.2.4 Hypoxia/Hyperoxia for Benchmarking (optional)*

1. Duplicate the two scans ($T_2^*$-mapping and $T_2$-mapping) and disable all adjustments (e.g., receiver gain, shimming) so that they are not changed from the previous scan.
2. Start hypoxia by changing the gas flowing through the respiratory mask to 10% $O_2$/90% $N_2$.
3. Three minutes after the start of hypoxia run the $T_2^*$-mapping and $T_2$-mapping scans. Hypoxia should not exceed 5 min in order to avoid long lasting effects.
4. Immediately after the two scans end, change the gas flowing through the respiratory mask back to air (21% $O_2$).
5. Wait for a recovery time of at least 5 min.
6. Start hyperoxia by changing the gas flowing through the respiratory mask to 100% $O_2$.
7. Two minutes after the start of hyperoxia run the $T_2^*$-mapping and $T_2$-mapping scans.

8. Immediately after the two scans end change the gas flowing through the respiratory mask back to air (21% $O_2$).
9. Wait for a recovery time of at least 5 min before conducting any further interventions.

#### 3.2.5 Noise Scan

We highly recommend acquiring one scan with the $T_2$*-mapping protocol and $T_2$-mapping protocol that only contains noise. This is the best way to estimate the noise standard deviation, which is needed for correcting the noise bias during the data analysis (*see* **Note 21**). Alternatively it is possible to estimate the noise standard deviation from an ROI in the "background" of the study images, but it is often difficult to find a suitable region outside the subject that is large enough and artefact free.

1. Create a copy of the $T_2$*-mapping protocol.
2. Open the protocol and in the parameter setting set either the flip angle to 0° degrees or the reference power for the flip angle calibration to 0 W.
3. Run the $T_2$*-mapping scan.
4. Repeat **steps 1–3** for the $T_2$-mapping protocol.

## 4 Notes

1. A 3D version of this sequence is also available, which allows for thinner slices with better SNR, but tends to be too slow for most in vivo applications.
2. The fat signal has a slightly different Lamor frequency ($\Delta f = 220$ Hz/Tesla) than the water signal. The faster precession of the fat protons means that with increasing time the fat and water signal fractions within a voxel are sometimes in phase (signals add up) and sometimes out of phase (signals subtract). This can lead to an additional unwanted variation in the signal along the exponential decay curve. This is mostly relevant for diseased kidneys with increased fat content (e.g., diabetes), but we recommend to generally take this into account, that is, also for healthy kidneys. The TEs at which fat and water are in phase depend on the field strength: $\mathrm{TE}\,[\mathrm{ms}] = n \cdot (6.7069/B_0[T])$.
3. Larger TEs are needed to accurately calculate longer $T_2^{(*)}$ under normal or hyperoxic conditions. We recommend using the largest expected $T_2^{(*)}$ in the kidney multiplied by 1.5 as the largest TE. Since the largest $T_2^{(*)}$ is typically in the inner medulla—if this region is not relevant to your research then use the largest $T_2^{(*)}$ in the cortex/outer medulla. With increasing TE the signal intensity decreases and may reach the noise floor at large TEs. This can introduce a noise bias during data

analysis. To account for this we recommend performing a retrospective noise correction (preferred) or exclude images acquired at larger TEs from the analysis. Both approaches are described in the chapter by Periquito JS et al. "Analysis Protocols for MRI Mapping of the Blood Oxygenation–Sensitive Parameters $T_2^*$ and $T_2$ in the Kidney."

4. Renal $T_2$* may be quite short at ultrahigh field strengths and in particular under hypoxia, which is what one wishes to detect. One needs to consider the shortest renal $T_2$* that could occur during experiment; under extreme conditions this could be only a few milliseconds, so several TEs around that value are needed.
5. When establishing the MR technique you need to define an SNR acceptance threshold for the image with the shortest TE. The aim is to have at least three (better five or more) number of echoes with an SNR > 5. This threshold will depend on the expected $T_2^{(*)}$ values, which in turn depends on parameters like the magnetic field strength, shim quality, and tissue properties (pathology). Example: for a rat imaged at 9.4 T using a 4-element rat heart array receive surface coil together with a volume resonator for excitation in combination with interventions leading to strong hypoxia, an SNR > 60 was needed.
6. For mice reduce the FOV to the body width and keep the matrix size the same. The relative resolution is then identical and the SNR should also be similar, because the smaller mouse RF coil gives better SNR, for example, mouse heart four-element surface coil vs rat heart four-element surface coil.
7. A good starting point is to use the same relative resolution as for rats. For this, reduce the FOV to the mouse body width and keep the matrix size the same.
8. *Example parameters for $T_2$* mapping of a 300 g rat at 9.4 T (Bruker small animal system)*: TR = 50 ms; flip angle = 16°; pulse length 1.0 ms; pulse bandwidth 5.4 kHz; receiver bandwidth = 109 kHz; number of echoes = 12; first echo = 2.14 ms; echo spacing 2.14 ms; TE = 2.14, 4.28, 6.42, 8.56, 10.7, 12.84, 14.98, 17.12, 19.26, 21.40, 23.54, 25.68 ms; averages = 4; slice orientation = coronal to kidney; frequency encoding = head-feet; FOV = (38.2 × 48.5) mm; matrix size = 169 × 115 zero-filled to 169 × 215; resolution = (0.226 × 0.421) mm; 1–3 slices with 1.4 mm thickness; fat suppression = on; respiration trigger = per slice; acquisition time = 40–60 s (with triggering under urethane anaesthesia).
9. *Example parameters for $T_2$* mapping of a 30 g mouse at 4.7 T (Agilent small animal system)*: TR = 350 ms; flip angle = 30°; receiver bandwidth = 100 kHz; number of echoes = 32; first

echo = 2.0 ms; echo spacing 2.4 ms; TE = 2.00, 4.40, 6.80, 9.20, 11.60, 14.00, 16.40, 18.80, 21.20, 23.60, 26.00, …, 76.40 ms; averages = 4; slice orientation = coronal to kidney; frequency encoding = head-feet; FOV = (30 × 30) mm; matrix size = 128 × 128; resolution = (0.230 × 0.230) mm; 1 slice with 1.0 mm thickness; fat suppression = on; respiration trigger = on; acquisition time = 3.5–9.0 min (with triggering under isoflurane anaesthesia).

10. *Example parameters for $T_2$* mapping of a 300 g rat at 3.0 T (Siemens Skyra$^{fit}$, a clinical system)*: Animal position: Right decubitus; Coil: Knee; TR = 69 ms; flip angle = 30°; receiver bandwidth = 320 Hz/pixel; number of echoes = 12; first echo = 3.56 ms; echo spacing 3.43 ms; TE = 3.56, 6.99, 10.42, 13.85, 17.28, 20.71, 24.14, 27.57, 31.00, 34.43, 37.86, 41.29 ms; averages = 20; slice orientation = axial; frequency encoding = anterior-posterior; FOV = (120 × 60) mm; matrix size = 256 × 128; resolution = (0.470 × 0.470) mm; 3 slices with 2.0 mm thickness; fat suppression = on; respiration trigger = off; acquisition time ~ 3 min. If one desires in-phase echoes, use first echo = 4.92 and echo spacing = 4.92 ms. If no specific animal holder is used, it is preferable to position the animals in left of right decubitus position to keep the bowels away from the kidneys to mitigate susceptibility artifacts.
11. You must monitor the respiration continuously throughout the entire experiment and if necessary adapt the TR accordingly.
12. Acquiring more echoes with smaller echo spacing will be beneficial because it improves the fitting, but keep in mind the SAR associated with sending many 180° RF pulses in a short time could heat up the tissue. This will usually not be detectable via a rectal temperature probe, but measurements with a temperature probe placed in the abdomen next to the kidney showed that significant temperature increases are possible with a multi–spin-echo sequence.
13. *Example parameters for $T_2$ mapping of a 300 g rat at 9.4 T (Bruker small animal system)*: TR = [respiration interval] − 100 ms; receiver bandwidth = 50 kHz; number of echoes = 12; first echo = 10.0 ms; echo spacing 10.0 ms; TE = 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120 ms; averages = 1; slice orientation = coronal to kidney; frequency encoding = head-feet; FOV = (38.2 × 48.5) mm; matrix size = 169 × 115 zero-filled to 169 × 215; resolution = (0.226 × 0.421) mm; 1 slice with 1.4 mm thickness; fat suppression = on; respiration trigger = per slice; acquisition time = 55–75 s (with triggering under urethane anaesthesia).

14. *Example parameters for $T_2$ mapping of a 30 g mouse at 4.7 T (Agilent small animal system)*: TR = [respiration interval] -100 ms; receiver bandwidth = 50 kHz; number of echoes = 12; first echo = 10.0 ms; echo spacing 10.0 ms; TE = 10, ..., 120 ms; averages = 1; slice orientation = coronal to kidney; frequency encoding = head-feet; FOV = (30 × 30) mm; matrix size = 128 × 128; resolution = (0.230 × 0.230) mm; 1 slice with 2.0 mm thickness; fat suppression = on; respiration trigger = on; acquisition time = 2–4 min (with triggering under isoflurane anaesthesia).
15. *Example parameters for $T_2$ mapping of a 300 g rat at 3.0 T (Siemens Skyra$^{fit}$, a clinical system)*: Animal position: Right decubitus; Coil: Knee; TR = [respiration interval] − 500 ms; receiver bandwidth = 399 Hz/pixel; number of echoes = 12; first echo = 10.0 ms; echo spacing 10.0 ms; TE = 10, ..., 120 ms; averages = 2; slice orientation = axial; frequency encoding = left-right; FOV = (120 × 60) mm; matrix size = 256 × 128; resolution = (0.470 × 0.470) mm; 1 slice with 2.0 mm thickness; fat suppression = on; respiration trigger = off; acquisition time ~2 min. If no specific animal holder is used, it is preferable to position the animals in left of right decubitus position to keep the bowels away from the kidneys to mitigate susceptibility artifacts.
16. *Example parameters for field mapping of a 300 g rat at 9.4 T (Bruker small animal system)*: use the vendors default protocol *AnyObject > AnyRegion > Adjustments > ADJ_B0MAP*. TR = 20 ms; flip angle = 30°; first echo = 1.60 ms; echo spacing = 3.57 ms; fat/water in-phase = on; slice orientation = main orientations (no angles) and offset = 0; FOV = (58 × 58 × 58) mm; matrix size = 64 × 64 × 64; resolution = (0.906 × 0.906 × 0.906) mm; respiration trigger = off; acquisition time = 1–2 min.
17. *Example parameters for field mapping of a 300 g rat at 3.0 T (Siemens Skyra$^{fit}$, a clinical system)*: The default setup uses the BODY Coil. TR = 20 ms; flip angle = 15°; in-phase echo times, first echo = 4.78 ms, and second echo = 7.17 ms; slice orientation = main orientations (no angles) and offset = 0; FOV = (350 × 350) mm; matrix size = 96 × 96; resolution = (3.6 × 3.6 × 8.0) mm; GRAPPA factor = 2; respiration trigger = off; acquisition time = 17 s.
18. *Example parameters for field mapping of a 300 g rat at 4.7 T (Agilent small animal system)*: TR = 34 ms; flip angle = 30°; number of echoes = 8; first echo = 4.23 ms; echo spacing 0.4 ms; averages = 8; slice orientation = coronal to kidney; frequency encoding = head-feet; FOV = (30 × 30) mm; matrix size = 128 × 128; resolution = (0.230 × 0.230) mm;

1 slice with 2.0 mm thickness; fat suppression = on; respiration trigger = on; acquisition time = 7.0–11.0 min (with triggering under isoflurane anaesthesia).

19. Shimming is particularly important, since macroscopic magnetic field inhomogeneities shorten $T_2$*, but provide no tissue specific information—rather they overshadow the microscopic $T_2$* effects of interest and hinder quantitative intra- and inter-subject comparisons. Shimming should be performed on a voxel enclosing only the kidney using either the default iterative shimming method or the Mapshim technique (recommended).
20. This serves to keep a record of the $B_0$ influence on the measured $T_2$*. It allows explanation of unusually small $T_2$* due to a bad shim. It may also be used later during the prost-processing for calculating a corrected $T_2$* by removing the influence $B_0$ inhomogeneities.
21. During the data analysis care must be taken to avoid noise biasing the mapping of $T_2$* and $T_2$. With increasing echo time the signal intensity in the images approaches more and more the noise floor, where the signal is so small that it is not detectable anymore. However, even though only noise is detected the signal is still above 0, because of the Ricean nature of the noise (negative values are "flipped" to the positive side). This means that the shape of the exponential curve will not fit well, as it expects the signal to decay toward 0. Adding an offset parameter to the fitted curve does not solve the problem because an offset will affect the signal intensities of all echoes, but the noise bias only affects those echoes where the true signal is very small. Instead a noise bias correction should be performed, which is described in the chapter by Periquito JS et al. "Analysis Protocols for MRI Mapping of the Blood Oxygenation–Sensitive Parameters $T_2$* and $T_2$ in the Kidney." Please note that you may not need to perform such a noise correction if all echo images are well above the noise floor.

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