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Chapter 20

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Essential Practical Steps for MRI of the Kidney in Experimental Research

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Abstract

Magnetic resonance imaging (MRI) is an emerging method to obtain valuable functional and structural information about the kidney noninvasively. Before performing specialized MR measurements for probing tissue structure and function, some essential practical steps are needed, which are common for most applications. Here we describe in a step-by-step manner how to (1) achieve the double-oblique slice orientation coronal-to-the-kidney, (2) adapt the scan protocol for avoiding aortic flow artifacts and covering both kidneys, (3) perform localized shimming on the kidney, and (4) check perfusion in the large renal blood vessels using time-of-flight (TOF) angiography. The procedures are tailored to preclinical MRI but conceptionally are also applicable to human MRI.

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 explains the initial and essential MRI steps that precede specific functional and structural MR imaging techniques (T_1- and T_2^*-mapping, DWI, ASL, etc.), which are described in separate chapters.

Key words Magnetic resonance imaging (MRI), Kidney, Mice, Rats, Shimming, Time-of-flight (TOF) angiography

1 Introduction

Magnetic resonance imaging (MRI) is a state-of-the-art noninvasive method to obtain valuable functional and structural information about the kidney. The wide range of available contrast agent mechanisms and the corresponding MR techniques can enable a comprehensive characterization of the kidney. This includes morphology (e.g., kidney size), tissue microstructure (e.g., diffusion-weighted MRI, T1 mapping, elastography), and function (e.g., arterial spin labeling MRI for perfusion mapping or mapping of T_2^* for probing blood oxygenation).

Before carrying out these specialized MR measurements, some essential practical steps are required, which are common for most

applications. Here we describe in a step-by-step manner how to (1) achieve the double oblique slice orientation coronal-to-the-kidney, (2) adapt the scan protocol for avoiding aortic flow artifacts and covering both kidneys, (3) perform localized shimming on the kidney, and (4) check perfusion in the large renal blood vessels using time-of-flight (TOF) angiography. The procedures are tailored to preclinical MRI but are also conceptually applicable to human MRI.

This experimental protocol chapter is complemented by separate chapters describing the basic concepts, experimental protocol and data analysis of numerous renal MR techniques, 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 (e.g., Wistar, Sprague-Dawley, or Lewis) with a body mass of 250–350 g. Advice for adaptation to mice is given 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."

Monitoring several physiological parameters of the animal is essential to ensure the anesthesia works and while the animal is unconscious and does not feel pain, it is physiologically as close to the normal nonanesthetized state as possible. For an in-depth description of physiological monitoring please refer to the chapter by Kaucsar T et al. "Preparation and Monitoring of Small Animals in Renal MRI."

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 techniques described in this chapter are 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 is given where necessary. No special or additional hardware is required, except for the following:

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

This protocol requires only standard MR techniques that should be available on any commercial system. The shimming capabilities and procedure are specific to the MR hardware and each vendors software platform; in this protocol we focus on describing the shimming on a Bruker BioSpec system running ParaVision 6.0.1.

1. 2D spoiled gradient-echo sequence ("FLASH" on Bruker systems).
2. 2D spoiled gradient-echo sequence with flow compensation and angio-mode (only one slice excitation per TR) ("FcFLASH" on Bruker systems).
3. 2D fast spin echo sequence ("RARE" on Bruker systems).

3   Methods

3.1   MR Protocol Setup

The basic protocols described here are based on default protocols available in Bruker ParaVision, which have been adapted to the geometry of the rat abdomen. Although it is one of the aims of this book to also describe the reasons for the choice of parameters, we have refrained from doing so here because the choice of parameters is not crucial for these protocols. Here we present working protocols for rats on a Bruker 9.4 T system. For MRI at lower field strengths the reduced signal-to-noise ratio (SNR) may be compensated by signal averaging and/or reducing the spatial resolution. For MRI of mice a good starting point is to reduce the FOV used in the rat protocols to the smaller body size of the mouse while keeping the matrix size the same. In this way the relative resolution (voxels per animal/organ dimensions) is similar. Despite the smaller voxel size the SNR is often not much worse than for the rat protocol, because the smaller size of the mouse RF coil improves the SNR (e.g., mouse heart four-element surface coil vs rat heart four-element surface coil).

3.1.1   Multislice Tripilot Scan (R01_TriPilotmulti)

1. Pulse sequence: 2D spoiled gradient-echo sequence (FLASH).
2. Contrast: TR = 126 ms, TE = 2 ms, flip angle = 90°, averages = 3.
3. Spatial resolution: in plane of $(234 \times 234) \, \mu m^2$; three packages of seven slices with 1.0 mm thickness, slice distances of 5.0/4.5/4.0 mm and hence slice gaps of 4.0/3.5/3.0 mm (axial/coronal/sagittal); FOV = $(60 \times 60) \, mm^2$; matrix size = 256×256 derived from a 85×128 acquisition matrix.

4. Acceleration: 2.0 in phase encoding and frequency encoding directions by zero-filling, 1.72 in frequency encoding direction by partial-Fourier.

5. Sequence: bandwidth = 89 kHz, trigger = off, fat suppression = off, flip-back = on, dummy scans = 10, motion suppression = on, duration: TA = 49 s.

3.1.2 Axial Multislice Pilot Scan

1. Pulse sequence: 2D fast spin echo sequence (RARE).

2. Contrast: TR = 540 ms, TE = 12 ms, effective TE = 24, flip angle = 90°, averages = 1.

3. Spatial resolution: in plane of (407×406) μm^2; eight slices of 1.0 mm thickness, a slice distance of 2.2 mm and hence a slice gap of 1.2 mm; FOV = (70×52) mm^2; matrix size = 172×128 identical to the 172×128 acquisition matrix.

4. Acceleration: RARE factor (echo train length or number of echoes per excitation) = 4.

5. Sequence: bandwidth = 63 kHz, trigger = on (per phase step), fat suppression = on, flip-back = on, dummy scans = 2, duration: TA = 17 s.

3.1.3 Coronal Pilot Scan

1. Pulse sequence: 2D fast spin echo sequence (RARE).

2. Contrast: TR = 540 ms, TE = 12 ms, effective TE = 24, flip angle = 90°, averages = 1.

3. Spatial resolution: in plane of (407×406) μm^2; one slice of 1.0 mm thickness; FOV = (70×52) mm^2; matrix size = 172×128 identical to the 172×128 acquisition matrix.

4. Acceleration: RARE factor = 4.

5. Sequence: bandwidth = 63 kHz, trigger = on (per phase step), fat suppression = on, flip-back = on, duration: TA = 17 s.

3.1.4 Abdominal Time-of-Flight (TOF) Angiography

1. Pulse sequence: Flow compensated 2D spoiled gradient-echo sequence (FcFLASH).

2. Contrast: TR = 13 ms, TE = 2.3 ms, flip angle = 80°, averages = 1.

3. Spatial resolution: in plane of (200×200) μm^2; nine slices of 1.4 mm thickness, a slice distance of 2.1 mm and hence a slice gap of 0.7 mm; FOV = (51.2×51.2) mm^2; matrix size = 256×256 derived from a 183×172 acquisition matrix.

4. Acceleration: 1.5 in phase encoding direction by zero-filling, 1.4 in frequency encoding direction by partial-Fourier.

5. Sequence: bandwidth = 75 kHz, angio mode = yes, trigger = off, fat suppression = off, duration: TA = 20 s.

3.1.5 Kidney
TOF-Angiography

1. Pulse sequence: Flow compensated 2D spoiled gradient-echo sequence (FcFLASH).

2. Contrast: TR = 7 ms, TE = 3 ms, flip angle = 80°, averages = 1.

3. Spatial resolution: in plane of (200×208) μm²; 15 slices of 1.0 mm thickness, a slice distance of 0.5 mm and hence a slice gap of -0.5 mm (overlapping); FOV = (60×40) mm²; matrix size = 300×192 derived from a 214×128 acquisition matrix.

4. Acceleration: 1.5 in phase encoding direction by zero-filling, 1.4 in frequency encoding direction by partial-Fourier.

5. Sequence: bandwidth = 75 kHz, angio mode = yes, trigger = off, fat suppression = off, duration: TA = 14 s.

3.2 Animal Preparation

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".

Start the temperature monitoring system, apply some surgical lubricant to the temperature probe and place it in the rectum of the animal.

Attach the respiration sensor (e.g., balloon) to the abdomen 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.

3.3 Pilot Scans and Slice Positioning Coronal to tthe Kidney

3.3.1 Slice Planning: From Pilot Scan to the Coronal-to-the-Kidney Image

Here we describe how to acquire a single image slice in coronal-to-the-kidney orientation, which is the best approach if only one kidney is of interest. If both kidneys are of interest, each kidney may be imaged separately using the steps described below.

However, if the experimental conditions do not allow for imaging each kidney separately, then a compromise must be made. This could be the case if, for instance, the available scan time is limited: slice planning, shimming, and acquiring images twice may take too long. Also, the experimental procedure might demand acquisition of the images for both kidneys simultaneously (multislice scan), for example when monitoring dynamic physiological processes. In these cases shimming should be performed on a voxel enclosing both kidneys and the image slice package orientation should be chosen such that it depicts both kidneys in a view that is as close as possible to coronal-to-the-kidney orientation.

1. Start with acquiring a fast pilot scan to obtain images in the three orthogonal planes *x*, *y*, and *z*: load the *multislice tripilot* protocol and run it with the default slice geometry and the usual automatic adjustments (shimming and adjustments of the
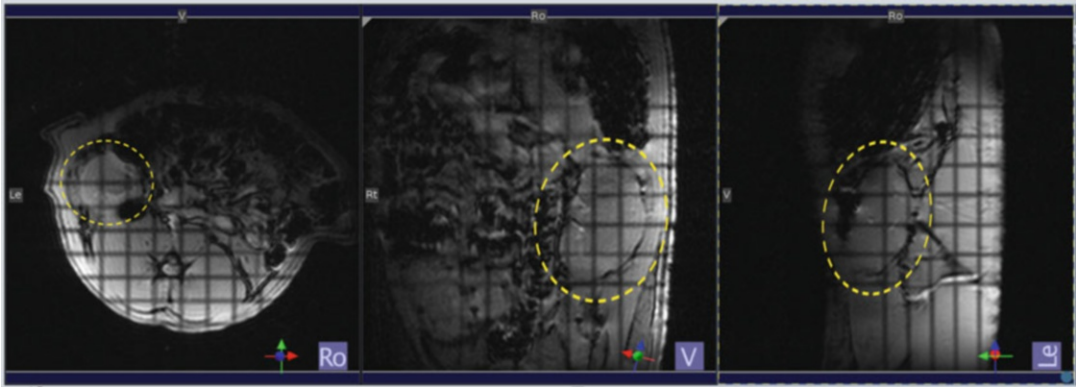
Fig. 1 Slice planning on a small animal MR system (here 9.4 T Bruker Biospec with ParaVision 6. Images obtained from the very first MR scan, a fast *multislice tripilot*: Shown are three selected image slices in strictly axial (left), coronal (center), and sagittal (right) orientation with respect to the scanner coordinates. The location of the left kidney is highlighted by the dashed yellow line. The dark stripes are saturation effects occurring at the intersection of the slices (*see* **Note 1**)
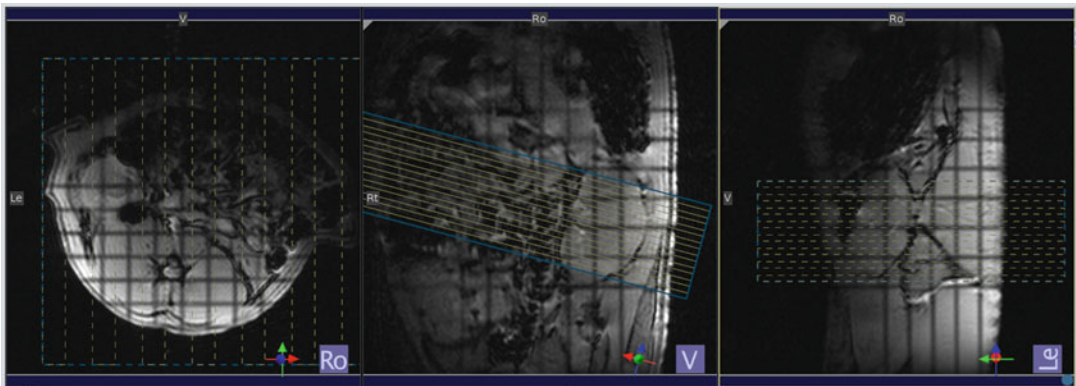

Fig. 2 Images obtained from the fast *multislice tripilot* showing the planning of the *axial multislice pilot scan*: The slice package should be rotated such that it is axial to the kidney in the coronal *multislice tripilot* images (center)

base frequency, the RF pulse reference power, and the receiver gain). Example images are shown in Fig. 1 (*see* **Note 1**).

2. Keep a record of the reference power in the lab notebook for later reference. Comparing the reference power to those used in previous studies with the same setup is very helpful for early identifying possible technical issues in an in-vivo study.

3. Load the *axial multislice pilot scan*, adapt the slice package position and orientation such that it is centered on the kidney and axial to the kidney (not to the animal's body) (Fig. 2).

4. In the monitoring unit set the trigger delay so that the trigger starts at the beginning of the expiratory plateau and the
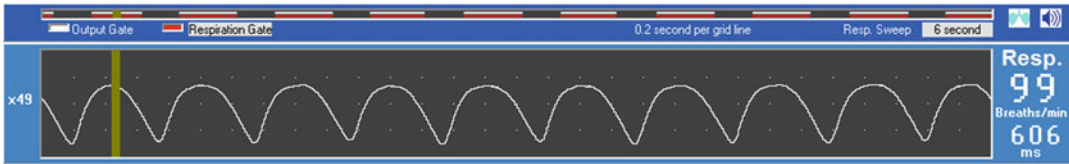
Fig. 3 Setup of the respiratory triggering in the physiological monitoring system. The respiratory gate is defined such that it spans across the expiratory phase and avoids the inspiratory motion. The above trace is for a urethane anesthetized rat. With isoflurane anesthesia the respiratory rate is lower and a long expiratory plateau is typical
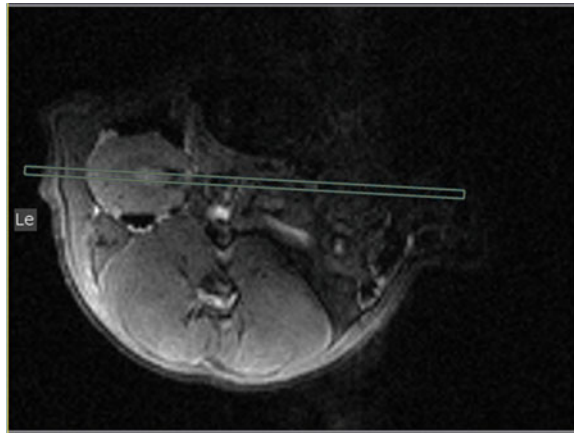

Fig. 4 Image obtained from the fast *axial multislice pilot scan* showing the planning of the *coronal pilot scan*: The slice should be perpendicular to the *axial multislice pilot scan* and rotated such that it runs through the center of the kidney

duration such that it covers the entire expiratory phase (Fig. 3, *see* **Note 2**).

5. If you use a RARE protocol like described here, adjust the TR so that it is longer that the respiratory trigger window but shorter than the current respiration period. For the example shown in Fig. 3, TR should be set to a value within the range 400–570 ms, in order to lie safely between 370 ms (respiratory trigger window) and 606 ms (respiration period). Run the scan. An example image slice is shown in Fig. 4.

6. Load the *coronal pilot scan* protocol and adjust the geometry: the slice should be perpendicular to the *axial multislice pilot scan* and rotated such that it runs through the center of the kidney (Fig. 4). The frequency encoding direction should be rostral–caudal in order to avoid aliasing. Run the scan. An example image is shown in Fig. 5.

Fig. 5 Image obtained from the *coronal pilot scan* showing the planning of the *sagittal pilot scan*: The slice should be perpendicular to the *coronal pilot scan* and rotated such that it runs along the long axis of the kidney

7. To acquire a *sagittal pilot scan*, duplicate the previous scan protocol and adapt the slice orientation: The slice should be perpendicular to the *coronal pilot scan* and rotated such that it runs along the long axis of the kidney (Fig. 5). Run the scan. An example image is shown in Fig. 6.

8. For the *coronal-to-the-kidney scan*, duplicate the *coronal pilot scan* protocol and fine-adjust the slice orientation: The slice should be rotated such that it runs along the long axis of the kidney in the *sagittal pilot scan* (Fig. 6). Run the scan. An example of the final image acquired in double-oblique *coronal-to-the-kidney* orientation is shown in Fig. 7.

3.3.2 Transferring the Coronal-to-the-Kidney Geometry to Other Scans

1. Start from the pilot scan with coronal-to-the-kidney geometry (Fig. 7).

2. From this protocol copy the slice orientation (*see* **Note 3**) to the first of your special scans (e.g., DWI, T_2*-mapping). Pay attention <u>not</u> to copy the entire slice package geometry, because this typically would include the FOV, slice thickness, and number of slices, which are usually different between the pilot scans and functional scans.

3. To illustrate how a typical result should look like, Fig. 8 shows an example of a coronal-to-the-kidney T_2-weighted image from a multispin echo scan.
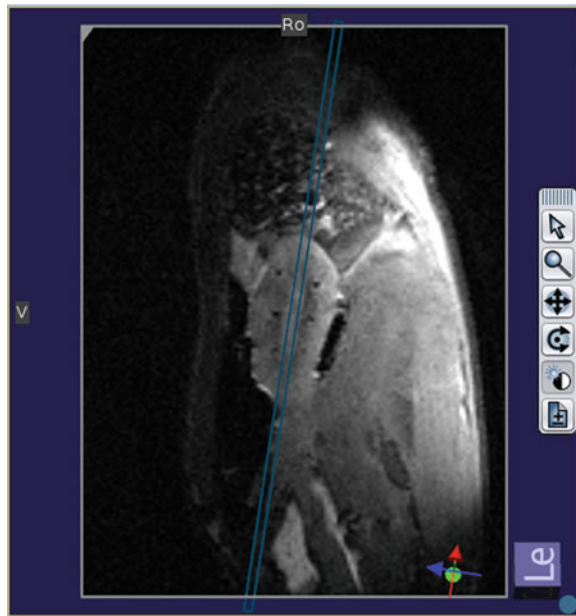
Fig. 6 Image obtained from the *sagittal pilot scan* showing the planning of the *coronal-to-the-kidney scan*: The slice should be rotated such that it runs along the long axis of the kidney
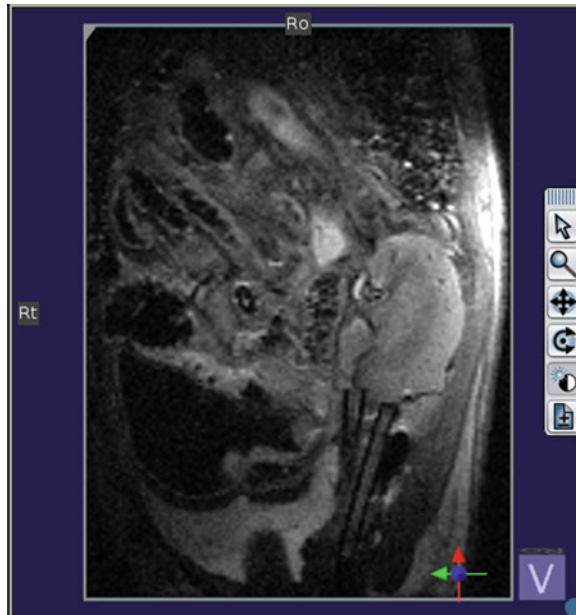

Fig. 7 Final image acquired in double-oblique *coronal-to-the-kidney* orientation (The black tubular structures caudal to the kidney are implanted probes that are not relevant in this context)
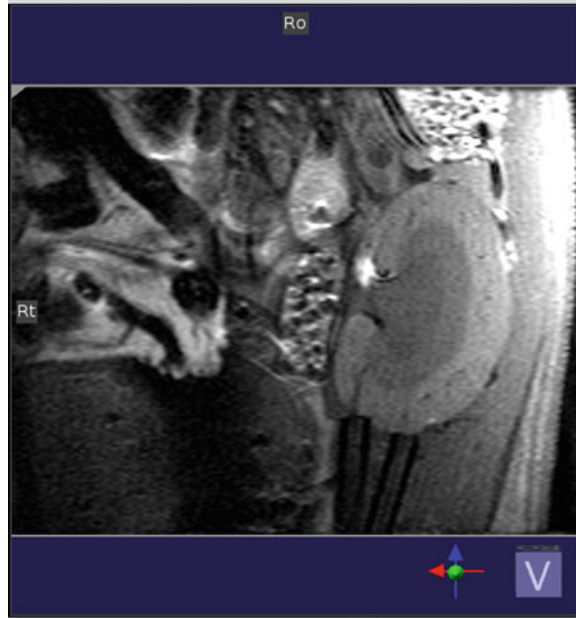
Fig. 8 T$_2$-weighted image acquired in double-oblique *coronal-to-the-kidney* orientation

3.3.3 Reducing Aortic Flow Artifacts (Optional)

The coronal-to-the-kidney slice orientation will naturally vary from animal to animal and of course between the right and left kidney. Sometimes the aorta lies within the image slice, which can result in unwanted flow artifacts. These artifacts can be reduced using a saturation slice, where a block of repeated excitation and spoiling is applied just before the imaging block in order to suppress the signal:

1. In the protocol setup look for the field-of-view saturation option (e.g., "FOV sat") and enable it. This will add a saturation slice with default geometry.

2. Load the *abdominal TOF-angiography* scan. Copy the slice orientation (*see* **Note 3**) from the *axial multislice pilot* and run the scan. An example image is shown in Fig. 9.

3. Load the following images to the viewer: the *axial pilot* scan, *coronal-to-the-kidney* scan, and the *abdominal TOF-angiography* scan (Fig. 9).

4. In the image viewer enable the display of saturation slices (gray in Fig. 9).

5. Adjust the saturation slice thickness and orientation: place it in approximately sagittal orientation and rotate it such that it covers the aorta without intersecting the kidney(!).
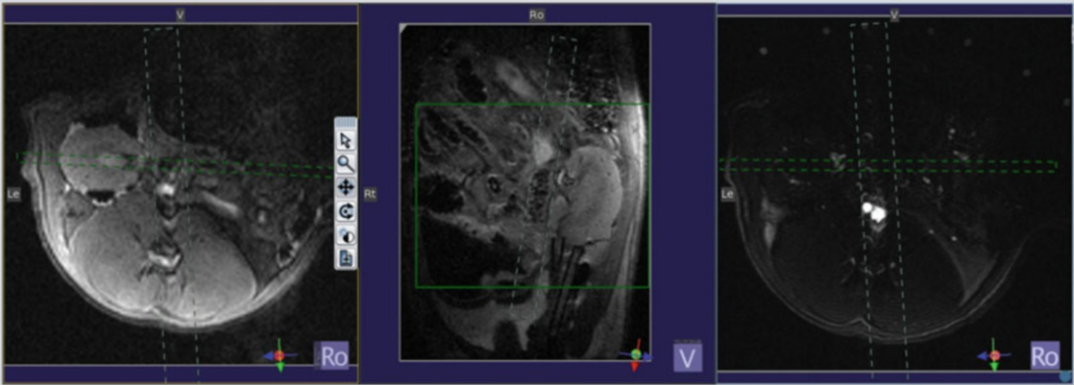
Fig. 9 Images obtained from the *axial pilot* scan (left), *coronal-to-the-kidney* scan (center), and an *abdominal TOF-angiography* scan (right) showing the planning of an optional *FOV saturation slice* (gray dashed lines). The saturation slice has the purpose of reducing possible flow artifacts from the aorta, and hence should be placed in approximately sagittal orientation and rotated such that it covers the aorta without intersecting the kidney. Please note that in this example the aorta was not within the kidney image slice, but, nevertheless, placing a saturation slice was performed as part of our standard operating procedure (SOP)

3.3.4 Multislice and Covering Both Kidneys (Optional)

1. To set up a multislice protocol, simply follow the steps for a single slice and subsequently change the number of slices to the desired number. This should be an odd number (3,5,7, . . .), so that the original single slice remains in the center.

2. For acquiring coronal-to-the-kidney images from both kidneys with the same scan protocol add a second slice package. This allows you to acquire two slice packages with arbitrary orientation, one coronal to the left kidney and one coronal to the right kidney.

3. First plan the geometry of the slice package for one kidney and afterward repeat the same steps for the other kidney.

4. Pay attention to where the two slice packages intersect. In the rare case when the slices of the right and left kidney cross within the kidney, adjust the slice orientation slightly to move the intersection to outside of the kidney. This is important, because otherwise the saturation effect would lead to a dark stripe artifact in the kidney. Alternatively, split the protocol into two, one for each kidney. This will permit acquiring images in the desired orientations without artifacts; however, it also doubles the scan time.

3.4 Localized Shimming

Shimming is particularly important for T_2^*-mapping, 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 intersubject comparisons. But a good shim is also relevant for other imaging techniques, such diffusion weighted imaging

(DWI) or perfusion measurements with arterial-spin-labeling (ASL), because they are commonly based on an echo-planar-imaging (EPI) sequence due to its fast imaging speed. EPI is prone to magnetic susceptibility artifacts, which increase with the number of echoes acquired after each excitation. Hence, this can particularly become a problem when the number of segments is small or one in the case of single-shot acquisition. These artifacts appear as T_2^* induced signal loss (or even signal voids) in areas with very high B_0 gradients, image distortion due to the inherently low phase encoding bandwidth, and off-resonance effects caused by frequency dispersions. Therefore, EPI is in kidney regions adjacent to bowels or in close proximity to skin/fat/muscle boundaries is particularly challenging. To this end, it is often beneficial not to use the default standard shim (usually on the entire field-of-view), but to perform additional shimming tailored to the kidney.

Ideally, shimming is performed on a voxel enclosing only the kidney of interest. When a good shim is crucial, the shim volume may be reduced even further to a volume that is smaller than the kidney and just a little thicker than the slice package (*see* **Note 4**). This is the procedure we describe in the following.

However, if both kidneys are of interest and the available scan time or experimental procedure does not allow for imaging each kidney separately, then shimming should be performed instead on a voxel enclosing both kidneys.

Field map-based shimming has become the standard shimming method on preclinical and clinical MR systems. It consists of (1) the manual definition of a shim volume, (2) the acquisition of a 3D B_0 field map (usually with a dual-echo gradient-echo method), and (3) the calculation of the shim currents needed to make the B_0 field within the shim volume as homogeneous as possible. This is the approach we recommend and describe in the following step-by-step instructions. Yet, in our experience, simple iterative linear (first-order) shimming on the selected volume is usually sufficient.

The main limiting factor for achieving a good shim is the capability of the shim coil set. For first-order (linear) shimming no additional shim coils are needed, since the standard $x/y/z$ gradient coils used for imaging can double as shim coils. Higher-order shimming (nonlinear spatial harmonics) requires additional coils, typically one for each spatial harmonic (newer designs are more efficient), that is, a minimum of five separate coils are employed for second-order field correction. Therefore, it is usually impossible to correct complex and small B_0 inhomogeneities caused by for example by bowel gas, invasive measurement probes, or trapped air bubbles (from surgery). Considering the very small size of a rat and mouse kidney, in practice the shimming cannot compensate much more than a linear B_0 gradient within the rodent kidney.
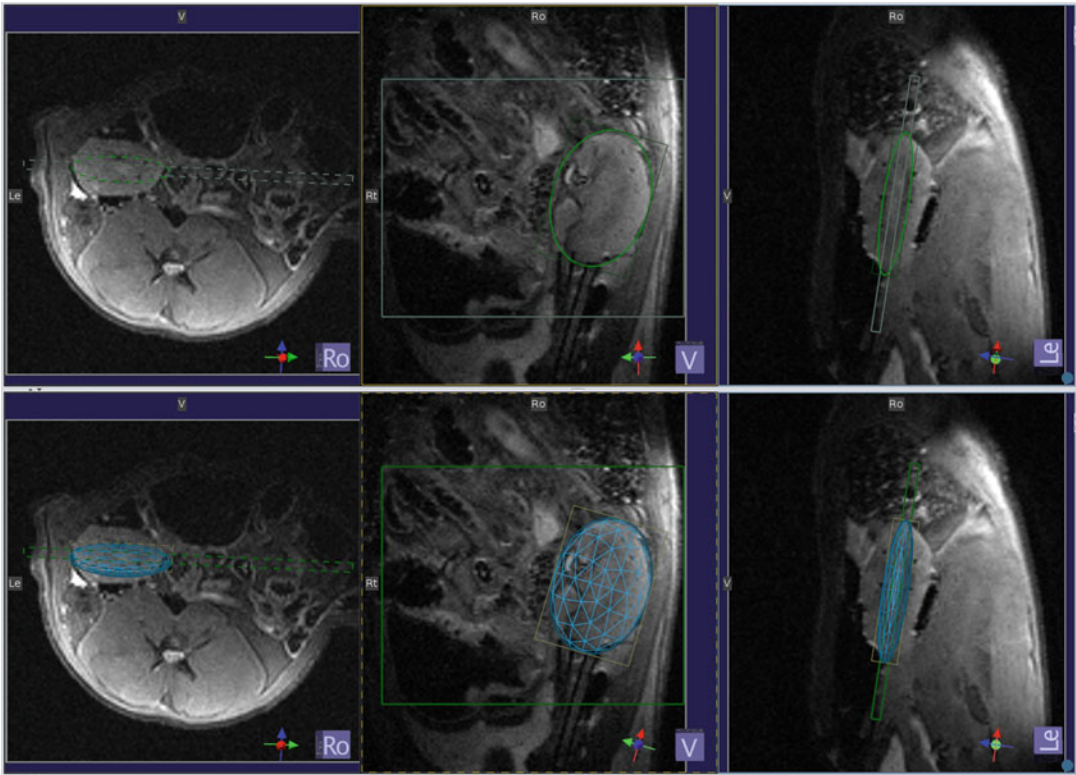
Fig. 10 Shimming on a rat kidney on a small animal MR system (here 9.4 T Bruker Biospec with ParaVision 6). Images obtained from the *axial pilot* scan (left), *coronal-to-the-kidney* scan (center), and an *sagittal pilot* scan (right) showing the planning of the double-oblique *shim volume*. The shim volume tightly encloses the kidney in "coronal" view and is about twice as thick as the imaging slice. Top panel: green outline of the ellipsoid shim volume. Bottom panel: ellipsoid shim volume shown as blue 2D grid

3.4.1 Planning the Shim Volume (Bruker ParaVision)

1. Load into the viewer the images obtained from the *axial pilot* scan, *coronal-to-the-kidney* scan, and *sagittal pilot* scan, as shown in Fig. 10.

2. Open the scan protocol for which the shimming should be performed and go to the *Adjustment* section of the protocol.

3. In the *Adjustment > Automatic Shim* tab select *Mapshim*.

4. In the *Adjustment > Automatic Shim* tab select *Default Shim Volume*, then deselect it again. You now have a shim volume geometry that is identical to the slice geometry.

5. In the *Adjustment > Automatic Shim* tab select a cuboid or better the *ellipsoid* volume, which fits very well to the shape of the kidney.

6. Adjust the geometry of the double-oblique shim volume, such that the volume tightly encloses the kidney in the "coronal" view and that it is about twice as thick as the image slice

package. Figure 10 shows the ellipsoid shim volume with a thickness of 4 mm for one image slice of 1.5 mm).

3.4.2 Shimming (Bruker ParaVision)

1. Finish all other changes you may want to make to the scan protocol.

2. In the *Adjustment Platform* select the *B0 map* and in the protocol parameters enable the respiratory triggering. It is usually OK to use the default FOV, but check that all the visible part of the animal lies within in (*see* **Note 5**).

3. Start the acquisition of the B_0 map, this usually takes a couple of minutes. When the acquisition has finished, exit the *Adjustment Platform* and return to the scan protocol.

4. In the *Adjustment > Automatic Shim* tab select *Mapshim* (you probably did that already during the shim volume planning).

5. In the *Adjustment > Automatic Shim* tab enable *Iterative Correction* of the first order shims.

6. It should be displayed that a B_0 map is available (if it does not, go back to acquiring the B_0 map).

7. *Apply* the changes to the protocol and run the scan—shimming will be performed as the first step of the data acquisition, followed by a frequency adjustment (necessary after shimming).

8. For repeating the same scan later in the experiment, copy this protocol and make sure to change the selection of *Mapshim* to *Current Shim* in the *Adjustment > Automatic Shim* tab.

9. To use the new shim for subsequent measurements, select *Current Shim* in the *Adjustment > Automatic Shim* tab of each of these protocols.

3.4.3 Shimming on a Clinical MR System (Siemens Syngo)

1. Load into the viewer localizer images acquired in the *coronal-to-the-kidney* orientation, and the *sagittal-to-the-kidney* orientation.

2. Open the scan protocol for which the shimming should be performed.

3. Click on the icon for the *Position Toolbar* to open it.

4. In the *Position Toolbar* click on the *Adjust Volume on/off* button. The default shim volume geometry that is identical to the slice geometry is now displayed as a green frame (Fig. 11).

5. Adjust the geometry of the shim volume, such that the volume encloses the kidney in the "coronal" view and that it is about twice as thick as the image slice package. Figure 11 shows the cuboid shim volume with a thickness of 4 mm.
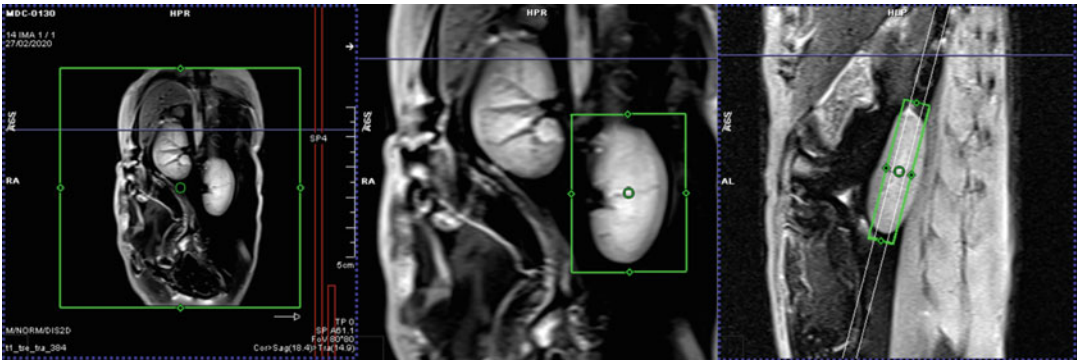
Fig. 11 Shimming on a rat kidney on a clinical MR system (here 3 T Siemens Skyra with Syngo). After clicking on the *Adjust Volume on/off* button in the *Position Toolbar* the default shim volume geometry is displayed as a green frame (left). Then the geometry of the shim volume was adjusted, such that it encloses the kidney in the coronal-to-the-kidney view (center) and that it is about twice as thick as the image slice, as can be seen in the *sagittal-to-the-kidney* scan (right). Here the size of the shim volume was (20 $\times$ 16 $\times$ 4) mm^3

6. In the scan protocol parameter setup click on the *System* tab and then the *Adjustments* tab. Select as the *B0 Shim Mode = Standard* or *Abdominal*.

7. *Apply* the changes to the protocol and start the scan—B_0 mapping (if not already done) and shimming will be performed as the first step of the data acquisition, followed by a frequency adjustment (necessary after shimming).

3.5 Fast and Simple Test of Renal Blood Flow (Optional)

Time-of-flight (TOF) angiography is a simple technique to check the perfusion in the large renal blood vessels. Acquiring a TOF angiogram only requires a few seconds and it can provide very valuable information in experiments that involve major changes in renal blood flow, such as short-time reversible occlusions of the renal artery, renal vein, or aorta as well as ischemia–reperfusion. One can confirm that the vessel occlusion and reperfusion are successful, or otherwise repeat the procedure. TOF angiography is also essential in MRI experiments that include implanted invasive probes (e.g., fiber-optical pO_2 probes, ultrasound flow probes, near-infrared probes) or devices for physiological interventions, such as inflatable balloons that serve as remotely controllable blood-vessel occluders. The reason is that these devices are relatively large and if they are placed in proximity to a blood vessel they may apply too much pressure on it, hindering or even stopping the blood flow within it. TOF angiography can help to detect such hypoperfusion early in the experiment, when a surgical correction may still be possible.

3.5.1 Measurement Concept

For correct interpretation of the TOF angiogram it is important to understand the measurement concept. The measurement uses a conventional gradient echo (GRE) pulse sequence with a high flip angle (FA) and very short repetition time (TR). After a few

excitations this results in saturation, that is, a suppression of the signal (from stationary protons), because there is too little time for T_1 recovery from the previous excitation leading to almost no signal being available for the next excitation. However, the fast-moving spins of blood flowing in large vessels that cross the image slice are not stationary—under normal blood flow conditions the blood present in the image slice during one excitation has been entirely replaced with new blood (from outside of the slice) before the next excitation. That means that the water protons of the blood are still fully "relaxed" when the excitation RF pulse is applied—these spins have never seen an RF pulse before and hence give maximum signal. The signal of the blood is not suppressed, because the effective TR is infinitely long—unlike for stationary protons in tissue to which the very short nominal TR applies.

The TOF contrast is <u>not</u> a quantitative measure. While in theory there is a window of flow velocities that will create intermediate blood signal intensities (between maximum signal from fresh blood to almost no signal from stationary blood), in practice the TOF contrast has an approximately binary character: you see signal or you do not. What flow velocity is needed to see the maximum blood signal can be roughly estimated from the distance that blood needs to flow (i.e., the image slice/slab thickness) and the available time (i.e., TR). However, the vessel orientation and flow profile in the vessel also play a role, so one cannot draw conclusions about the actual blood flow velocity; it rather serves as a simple tool for qualitative comparison of blood flow between (patho)physiological states.

3.5.2 Protocol

Here we describe how to perform a TOF angiography to check for (ab)normal perfusion in the large renal blood vessels.

Please note that this protocol is not designed for creating a 3D depiction of the renal vascular architecture via maximum-intensity projections (MPIs)—this would require whole kidney coverage, a higher spatial resolution, and hence a longer acquisition time.

1. In the viewer open the images obtained from the *axial pilot* scan, *coronal-to-the-kidney* scan, and the *sagittal pilot* scan (Fig. 12).

2. Load the *kidney TOF-angiography* protocol.

3. If you have acquired a *sagittal pilot scan* at the beginning of the experiment, then copy the slice orientation from that scan (*see* **Note 3**). Otherwise start with a default sagittal slice orientation.

4. Plan the slice geometry sagittal with regard to the kidney, as shown in Fig. 12.

5. Start the scan. Figure 13 shows an example image in which six major vessels are clearly visible (in Wistar rat kidneys typically 6 or 8 vessels are seen). During temporary occlusions of the
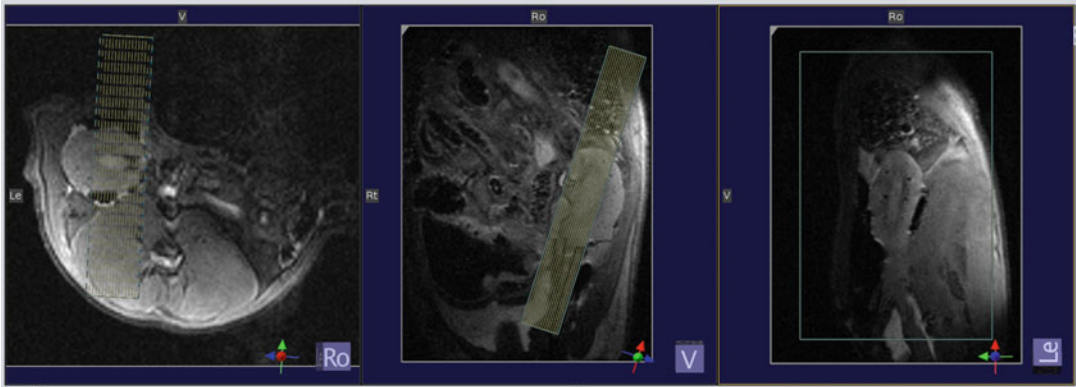
Fig. 12 Basic TOF angiography of a rat kidney on a small animal MR system (here 9.4 T Bruker Biospec with ParaVision 6). Images obtained from the *axial pilot* scan (left), *coronal-to-the-kidney* scan (center), and an *sagittal pilot* scan (right) showing the planning of the *kidney TOF-angiography*. The slice package is positioned sagittal with regard to the kidney
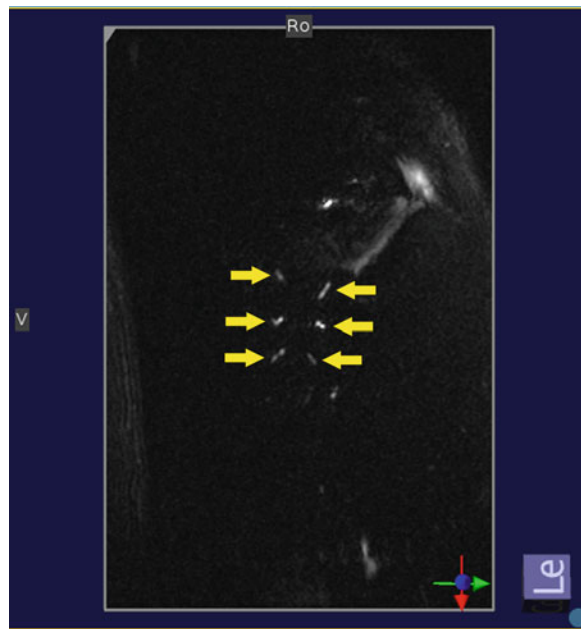

Fig. 13 Selected images slice obtained from the *kidney TOF-angiography*. Six major vessels can be clearly seen. In experiments where a temporary occlusion of the renal artery, vein, or aorta via a remote controlled hydraulic occluder is performed, this scan is very useful to confirm vessel occlusion was successful (vessels are not visible anymore) and reperfusion was successful (vessels are visible again like above)

renal artery, vein, or aorta (via a remote controlled hydraulic occluder) the vessels will disappear and reappear after successful reperfusion.

4 Notes

1. The saturation effect is an inevitable artifact that occurs when several crossing slices are acquired in an interleaved manner, that is, excitation of all slices during one TR. This effect can be avoided be acquiring all slices consecutively, however, that significantly increases the scan time. For the initial scan this artifact is acceptable because it only serves to localize the kidney, so speed is given preference.

2. If you use a RARE protocol like described here, the TR needs to be relatively long to allow for sufficient T_1-recovery and hence acceptable signal-to-noise ratio (SNR). Due to the long TR it is advisable to have only one excitation per breath. To ensure that there will be only one triggered acquisition per breath, adjust the TR so that it is longer than the respiratory trigger window but shorter than the current respiration period. Alternatively, you could adjust the trigger window in the monitoring software to be very short (e.g., 10 ms), but then you will have to reverse this change for every gradient echo scan. You must monitor the respiration continuously throughout the entire experiment and, if necessary, adapt the TR accordingly.

3. With "copying the slice orientation" we mean copying the spatial location of the center of the slice package as well as copying its spatial orientation, that is, the angulation in all three directions x, y, and z (or left–right, anterior–posterior, rostral–caudal). On a system running Bruker ParaVision this is achieved by copying the slice orientation; in contrast, copying the slice geometry includes the FOV, slice thickness, and number of slices.

4. Considering the small size of a rat and mouse kidney and the limited capabilities of shim coils, it is unrealistic to expect that a "perfect" shim on the kidney can be achieved. In practice, one aims to remove a linear B_0 gradient in the kidney. Nonlinear B_0 gradients within the kidney are very challenging to compensate for, because they are only a few millimeters in size. This would require a high-end gradient set with very high-order shims.

5. For rats the default shim volume has a reasonable size, but you must check for your particular setup (RF coil, rat size) that it is large enough so that there will be no aliasing artifacts in the two phase encoding directions. If the default shim volume is much larger than the object (possible for small mice) one can reduce the size of the shim volume. As this will reduce the signal-to-

noise ratio of the B_0 map and hence it may be useful to increase the number of averages. When you modify the shim volume FOV try to maintain the *isotropic* voxel size, as this permits capturing the B_0 inhomogeneities in all spatial directions with equal accuracy.

Acknowledgments