Tiny golden angle ultrashort echo-time lung imaging in mice

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Imaging the lung parenchyma with MRI is particularly difficult in small animals due to the high respiratory and heart rates, and ultrashort T2* at high magnetic field strength caused by the high susceptibilities induced by the air–tissue interfaces. In this study, a 2D ultrashort echo-time (UTE) technique was combined with tiny golden angle (tyGA) ordering. Data were acquired continuously at 11.7 T and retrospective center-of-k-space gating was applied to reconstruct respiratory multistage images. Lung (proton) density (fP), T2*, signal-to-noise ratio (SNR), fractional ventilation (FV) and perfusion (f) were quantified, and the application to dynamic contrast agent (CA)-enhanced (DCE) qualitative perfusion assessment tested. The interobserver and intraobserver and interstudy reproducibility of the quantitative parameters were investigated. High-quality images of the lung parenchyma could be acquired in all animals. Over all lung regions a mean T2* of 0.20 ± 0.05 ms was observed. FV resulted as 0.31 ± 0.13, and a trend towards lower SNR values during inspiration (EX: SNR = 12.48 ± 6.68, IN: SNR = 11.79 ± 5.86) and a significant (P < 0.001) decrease in lung density (EX: fP = 0.69 ± 0.13, IN: fP = 0.62 ± 0.13) were observed. Quantitative perfusion results as 34.63 ± 9.05 mL/cm³/min (systole) and 32.77 ± 8.55 mL/cm³/min (diastole) on average. The CA dynamics could be assessed and, because of the continuous nature of the data acquisition, reconstructed at different temporal resolutions. Where a good to excellent interobserver reproducibility and an excellent intraobserver reproducibility resulted, the interstudy reproducibility was only fair to good. In conclusion, the combination of tiny golden angles with UTE (2D tyGA UTE) resulted in a reliable imaging technique for lung morphology and function in mice, providing uniform k-space coverage and thus low-artefact images of the lung parenchyma after gating.

KEYWORDS
2D UTE, fractional ventilation, lung, lung density, MRI, perfusion, self-gating, T2*, tiny golden angle

Abbreviations: AIF, arterial input function; ASL, arterial spin labeling; BW, bandwidth; CA, contrast agent; DC, direct current component occurring at center of k-space; DCE, dynamic contrast-enhanced; EX, (end-)expiration; FA, flip angle; FD, Fourier decomposition; FOV, field of view; FV, fractional ventilation; ICC, intraclass correlation coefficient; igUTE, intragate UTE; IN, (end-)inspiration; k0, center of k-space; ROI, region of interest; sD, slice thickness; SD, standard deviation; SNR, signal-to-noise ratio; TACQ, acquisition time; TE, echo time; TR, repetition time; tyGA, tiny golden angle; UTE, ultrashort echo-time; μCT, X-ray microcomputed tomography.

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1 | INTRODUCTION

In preclinical research, small animal models of lung disease, especially transgenic mouse models, are frequently used to investigate the nature of the underlying mechanism of normal and abnormal lung function, and provide a unique opportunity to test potential therapeutic interventions. Lung diagnostics represents a field of increasing interest and quickly growing importance in preclinical practice. Noninvasive medical imaging is highly recommended due to its ability to spatially localize morphology and evaluate functional changes in a quantitative and noninvasive way. Where X-ray microcomputed tomography (μCT) mainly provides data on lung density, its relatively poor soft-tissue contrast limits its application in soft-tissue analysis. And the related rather high radiation dose in μCT may affect intrinsic or exogenous biological activity and, even though less relevant in small animal research, may render repeated examinations during longitudinal studies impossible. By contrast, magnetic resonance imaging (MRI) represents an attractive and versatile modality for in vivo longitudinal characterization of lung morphology, functionality and microstructure in a noninvasive and nonionizing way.

MRI of the lung parenchyma as such is challenging. Respiratory and cardiac motion are prone to cause motion artefacts in the final image and need careful consideration. Additionally, the sparse signal source (gas spaces account for about 50% of its volume) results in an intrinsic low T2*relaxation signal decay. Over recent decades, major improvements in MRI methodology and instrumentation, advanced mathematical concepts for image reconstruction, and rapid data acquisition techniques, have enabled the use of various short-TE MR techniques on conventional small animal imaging systems. Beckmann et al. developed a TE-minimized gradient-recalled echo sequence (GRE) to image pulmonary edema in small rodents allowing for quantification of the lesion volume. However, the achievable milliseconds range of TEs was still longer than T2*, which particularly limits its application for lung imaging at ultrahigh field. Nonstandard center-out MRI techniques known as UTE imaging, zero echo-time imaging (ZTE), and sweep imaging with Fourier transformation (SWIFT) have recently gained attention in lung imaging and enabled visualization of lung tissues in 2D (UTE) and 3D (UTE, ZTE, SWIFT). Of these, UTE sequences are more straightforward to implement without the requirement for complex iterative reconstruction (ZTE) and specialized hardware (SWIFT), and hence appear to be a promising candidate for 2D imaging of lung function.

Due to the intrinsic oversampling of the k-space center, the center-out approach is relatively insensitive to motion artefacts. However, consideration of respiratory motion is still mandatory to avoid impairment of the image quality in high-resolution images and especially for providing data in different respiratory stages. The use of balloon pressure sensors has been reported to synchronize image acquisition to a specified phase of the breathing cycle. However, the resulting steady-state perturbation, varying TR, reduced scan efficiency and prolonged acquisition time, limit its application. As an alternative approach, retrospective gating with continuous data acquisition and subsequent reordering of the data according to navigator signals (e.g. derived from the data itself) has been introduced. One of the most commonly used retrospective gating strategies exploits the k-space center signal amplitude (DC) as a gating signal, as it is modulated by cardiac or respiratory motion. Image-based self-gating represents an alternative method using low-resolution images continuously reconstructed with high temporal resolution to extract respective gating signals. Because data are acquired over the whole motion cycle in the retrospective gating approach, this enables the reconstruction of images in different cardiac and respiratory motion stages, thus enabling assessment of functional parameters as ventilation and perfusion from signal intensity changes. Center-out techniques such as UTE and SWIFT are especially suited for self-gating, as they intrinsically sample k-space center during each acquisition, thus providing DC gating information without the need for additional measurements. In the case of a conventional angular increment, however, these techniques are prone to artefacts, because during each respiratory phase only a small angular segment is covered with high angular density. For rather short acquisitions in particular, this results in severely unevenly covered k-space causing distinct streaking artefacts (Figure S1). For supporting uniform k-space coverage after gating, center-out techniques can be combined with tiny golden angle (tyGA) angular spacing providing a nearly uniform angular distribution of radial spokes from almost any number of spokes, especially when chosen from the generalized Fibonacci series. Where on the one hand this property facilitates reduction of motion (streak) artefacts after gating, it also enables reconstruction of data at different spatial and temporal resolutions from a single continuously acquired dataset. With the intrinsic coverage of the k-space center with each readout, real-time as well as self-gated reconstructions can be derived from a single continuously acquired dataset (Figures S2 and S3).

It was the objective of this study to investigate the feasibility of applying 2D tyGA UTE for the assessment of lung function in freely breathing healthy mice at 11.7-T field strength. The technique was applied for the assessment of proton density, fractional ventilation (FV) and perfusion from self-gated data, as well as for real-time assessment of lung parenchyma intensity changes following intravenous administration of contrast agent (CA).
2  |  METHODS

2.1  |  Animals and ethics considerations

Seven female mice (mean age 12 weeks, mean weight $22.21 \pm 1.09$ g at the beginning of the study) were enrolled. The animals were housed in a temperature-controlled environment. The room temperature (23°C) and humidity (50% relative humidity) were constant, with a 12-h light/dark cycle. Access to food and tap water was ad libitum.

The experiments were approved by the local authorities and conducted according to the German law for animal welfare. All institutional and national regulations for care and usage of laboratory animals were followed.

2.1.1  |  Anesthesia of the animals

The animals were anesthetized with isoflurane (5% for induction, 1%-1.5% to maintain the respiratory frequency at 100–120 respiratory cycles per min) in medical air (0.1 L/min). A water blanket was used for maintaining the temperature of the animal.

2.2  |  MRI protocol

All measurements were performed with an 11.7-T small animal MR scanner (BioSpec 117/16; Bruker Biospin, Ettlingen, Germany). The system was equipped with a high-performance gradient system (maximal strength of 760 mT/m, maximal slew-rate of 6840 T/m/s). Excitation was performed with a 72-mm quadrature transmit/receive coil and the data were acquired with a dedicated four-channel thorax coil (RAPID Biomedical, Rimpar, Germany). The mice were in prone position, head first, and kept anesthetized during the entire measurement. Throughout the whole experiment, the respiratory rate was monitored with a respiratory sensor (balloon pressure sensor) and the temperature of the animal was measured with a rectal temperature probe.

3D UTE acquisitions with different TEs ($TE = 0.008, 0.015, 0.02, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0$ and $1.5$ ms) were performed for T2* quantification (see the supporting information for detailed information) and 2D tyGA UTE for deriving lung parenchymal density, ventilation and perfusion assessment. For each animal the actual trajectory (2D and 3D) was mapped individually from a calibration scan (PV6.01; Bruker Biospin, Ettlingen, Germany), as suggested by.26

2.2.1  |  Proton density, FV and perfusion

All mice were investigated with a 2D tyGA UTE protocol in axial (five slices, cranial to caudal) and coronal (three slices, anterior, middle and posterior) orientation (Figure 1). The acquisition parameters were: $TE = 0.253$ ms; $TR = 4$ ms; $T_{ACQ} = 6$ min per slice; number of
projections = 90, 190; BW = 250 kHz; flip angle (FA) = 8°; slice thickness (sD) = 1 mm; slice gap = 1 mm (coronal)/0.5 mm (axial); matrix = 150 x 150; FOV = 30 mm x 30 mm; resolution = 0.22 mm²; and tiny golden angle (Ψ) = 23.62°.

The data were reconstructed with an in-house reconstruction framework implemented in Matlab (MathWorks, Natick, MA, USA). Reconstructions were performed from respiratory or cardiac self-gated data using the k-space center (DC) intensities, derived from the coil showing the most prominent DC signal amplitude changes in the expected frequency range, as identified by Fourier analysis.

For proton density and FV assessment, the data were sorted into an end-inspiration (IN, upper 20%) and end-expiration (EX, bottom 20%) bin. Intermediate data were not considered further. For deriving ventilation and perfusion data as suggested by Fischer et al., multimotion stage datasets (40 frames per cycle, 50% temporal overlap) were generated independently for respiratory and cardiac motion cycles.

Final image reconstruction was performed by conventional gridding, under full consideration of the mapped trajectory and resulting sampling density. To avoid any impact of an erroneous coil sensitivity pattern resulting from the very low signal-to-noise ratio (SNR) in the lungs, reconstruction was performed independently for each coil and the final image was compiled as sum-of-squares.

2.2.2 | Contrast-enhanced perfusion

Acquisition was performed with a multislice 2D tyGA UTE sequence, measuring n = 8 interleaved slices within a repetition time of TR = 24 ms. Parameters were similar to the functional imaging part, but with FA = 10°. Data were continuously acquired for 5 min. After about 2 min of continuous scanning, a single bolus of around 35–50 μL with triple-dose Gd³⁺ (0.3 mmol/kg gadolinium) was injected. Injection was performed through a homemade catheter (30.5 G needle, 200 μm hose), preloaded with heparinized saline (0.05 mL heparin [1000 USP units/mL] in 1 mL of sterile saline), introduced into the tail vein.

Sliding window reconstructions were performed with a constant temporal increment of n_c = 25 projections yielding Δt = TR · n_c = 600 ms and different window widths of n_w = 100, 250, 500 and 1000 projections (t_w = 2, 4, 6, 12 and 24 s) with our in-house developed reconstruction framework. No parallel imaging or compressed sensing was performed. The reconstructed image series was filtered with a three-point median filter over time for outlier removal.

2.3 | Data analysis

2.3.1 | Lung parenchyma SNR, density, ventilation and perfusion

The data analysis of the 2D tyGA UTE images was performed on the magnitude data. To minimize the impact of the noise floor on the low SNR data analysis, the mean noise S^noise derived from an artefact-free background region of interest (ROI) (identified by A.B.) was subtracted from the magnitude data before further analysis.

For quantitative analysis, in a first step, the lung parenchyma was segmented with the Image Segementer App (Matlab; MathWorks, Natick, MA, USA). Care was taken to exclude the pulmonary vessels.

For evaluation of the image quality, the SNR in the lung parenchyma was calculated for all reconstructed images. The SNR was estimated by calculating the local mean (S) and standard deviation (σ) of a 3 x 3 region for every parenchyma voxel according to:

\[ \text{SNR} = \frac{S}{\sigma}. \]

This approach reduces the impact of background artefacts and coil sensitivities.

For the lung density, the proton fraction (f_P) was calculated pixel-wise according to:

\[ f_P = \frac{S_{\text{lung}}}{S_{\text{muscle}}} \cdot \exp \left( \frac{\text{TE}}{T_2^*} \right), \]

with S_{lung} being the signal intensity of a lung parenchymal voxel, S_{muscle} the mean reference signal intensity of a ROI placed in the muscle (Figure S5), and T2* as derived in this study (T2* = 0.20 ± 0.05). Correction for T2* decay was limited to the lung parenchyma, as the signal from muscle (T2* = 4.6 ± 1.2 ms) showed negligible (<5%) signal decay for the used TE of 0.253 ms.

Prior to further analysis for all data in different respiratory stages, a nonrigid image registration was performed with the freeware Medical Image Registration Toolbox for Matlab to ensure proper matching of the lung voxels in different respiratory phases. No registration was performed for the perfusion analysis (cardiac gating), assuming that cardiac motion does not cause substantially deformations of the lung.
The FV\textsuperscript{32} was calculated according to:
\[
FV = \frac{SI_{EX} - SI_{IN}}{SI_{EX}},
\]
with \(SI_{EX}\) and \(SI_{IN}\) being the signal intensities of a lung voxel in EX and IN.

Ventilation and perfusion maps were derived from Fourier analysis of the registered respiratory motion-resolved and cardiac motion-resolved datasets. Ventilation and perfusion contributions were derived from the amplitude of the first frequency component next to DC in the respective spectra, as suggested by Fischer et al.\textsuperscript{23}

Quantitative perfusion data were derived as suggested by Kjørstad et al.,\textsuperscript{33} in which the partially blood-filled voxels in the lung parenchyma are compared with the fully blood-filled pixels in the aorta or heart. The quantitative perfusion \(f\) was calculated according to:
\[
f = \frac{SI_{lung}}{SI_{blood}} \cdot \frac{1}{2T_{RR}},
\]
with \(SI_{lung}\) and \(SI_{blood}\) being the signal intensity of lung parenchyma and blood, respectively, and \(T_{RR}\) the heart cycle duration.

2.3.2 | Contrast-enhanced perfusion

ROIs in the heart, lung, muscle and background were identified manually and the mean value of each ROI followed over time. For the lung, ROIs of pulmonary vessels were carefully avoided. The perfusion was assessed by calculation of the percentage signal change, \(\%_p\), after CA injection, according to\textsuperscript{34}:
\[
\%_p = 100 \cdot \frac{SI_{post} - SI_{pre}}{SI_{pre}}.
\]

2.4 | Reproducibility

Interobserver and intraobserver reproducibility as well as interstudy reproducibility were assessed. For the assessment of the interobserver reproducibility, analysis was performed independently by two experienced observers (A.B. and H.L.). Intraobserver reproducibility was assessed by repeated analysis of the data from the same acquisition (A.B.). The interstudy reproducibility was assessed from repeated measurements performed with the same MR protocol within 1 week (A.B.).

2.5 | Statistical analysis

All reconstructed images were normalized before the analysis. All continuous data are presented as mean ± standard deviation (SD). Comparisons of SNR, \(f_P\), FV and \(f\) between different slices were performed independently for inspiration and expiration (SNR, \(f_P\)) or diastole and systole (\(f\)) applying a Kruskal–Wallis test with pairwise post hoc analysis. Comparisons of SNR, \(f_P\) and \(f\) between inspiration and expiration (SNR, \(f_P\)), and diastole and systole (\(f\)), were performed based on the mean value of all slices applying a Mann–Whitney U test. Comparisons between inspiration and expiration on a slice basis were performed independently for each slice by applying a Mann–Whitney U test. Normal distribution of the data was checked by applying a Kolmogorow–Smirnow test. The interstudy, interobserver and intraobserver reproducibility was assessed by Bland–Altman analysis and the intraclass correlation coefficient (ICC).

The analysis of the data was performed with the Excel plug-in Real Statistics (real-statistics.com). Differences were considered significant for \(P\) less than 0.05.

3 | RESULTS

\(T_2^*\) mapping by 3D UTE imaging was successful in all cases. The self-gated 2D tyGA UTE technique yielded high-quality images of the lungs in all animals with no apparent motion artefacts (Figure 1). The derived self-gating signal could successfully be applied for multiphase reconstruction of the respiratory and cardiac cycle (Figures 2 and S2). Real-time imaging yielded sufficient image quality to provide CA dynamics at flexible temporal resolution.
3.1 | Lung parenchyma SNR, density and FV

A significant ($P < 0.05$) difference between the normalized parenchymal signal intensities in expiration and inspiration was observed. The calculation of FV, proton fraction and SNR was successful in all 2D tyGA UTE datasets.

3.1.1 | Signal-to-noise ratio

In axial slice orientation, there was a significantly ($P < 0.001$) higher mean SNR in EX than in IN. A similar, but not yet significant ($P = 0.32$), trend was observed in the coronal slices. Where for the axial slices an increasing mean SNR ($P < 0.001$) from caudal to cranial could be observed, in coronal orientation the mean SNR was independent on the slice position (Figure 3A).

3.1.2 | Lung density ($f_P$)

In all animals a significant difference ($P < 0.001$) between EX and IN was observed for the proton fraction values ($f_P$). The mean value over all coronal slices resulted as $0.69 \pm 0.13$ for EX and $0.62 \pm 0.13$ for IN, and as $0.78 \pm 0.09$ (EX) and $0.71 \pm 0.10$ (IN) in axial orientation. Density increased from anterior to posterior ($P < 0.05$) and from caudal to cranial ($P < 0.001$ between cranial and caudal). Further, a higher proton fraction could be appreciated in the right sections of the lung (Figures 3B and 4).

3.1.3 | Fractional ventilation

FV maps could be successfully obtained from the analysis of the signal intensity changes between EX and IN (Figures 2 and 3C). The mean value over all axial slices was $0.19 \pm 0.09$, with increasing values ($P < 0.01$) from cranial to caudal. In the coronal slices, an almost constant FV with a mean value of $0.31 \pm 0.13$ was observed. Detailed values are summarized in Table S2.

3.2 | Fourier analysis of ventilation and perfusion

In all cases, high-quality multiphase reconstruction of the cardiac and respiratory cycle with 40 frames and 50% overlapping was achieved. Even although cardiac motion was not considered during reconstruction of the respiratory cycle and vice versa, no apparent streak artefacts were
observed in the respective images and a clear delineation of the lung anatomy was achieved. For both cases, Fourier analysis provided a clear signal related to intensity changes occurring with the frequency of the respective motion. In cases of respiratory motion, some higher frequency components were observed (Figures 5 and S6).

3.3 | Quantitative perfusion (f)

Perfusion could be successfully quantified according to Kjørstad et al. in all animals. Perfusion was in the range of 30–40 mL/cm³/min, with significant differences from anterior to posterior and from cranial to caudal. Not yet significant differences were observed over the cardiac cycle with reduced perfusion during diastole (Figures 3D, 6 and S7).

3.4 | CA-enhanced perfusion

The inflow of the CA was clearly visible in the heart (Figure 7A,B), lung and muscles. Rapid uptake in the left (LV) and right (RV) ventricle and the lungs (L1–4) was observed, while uptake in the muscle appeared slower. Shortly after peak enhancement, slight but steady signal decrease was
observed in the LV and RV, while enhancement remained constant for L1–4 and showed some tiny but steady enhancement in the muscles (Figure 7D). Even although increasingly noisy, the difference in the CA dynamics was more obvious in sliding window reconstructions with higher temporal resolution (Figure 8). The percentage change in the signal intensity preinjection and postinjection was 24% ± 12% (RV) and 41% ± 15% (lung), as summarized in Table S3 (Figure 7C).

3.5 | Reproducibility analysis

Good to excellent interobserver reproducibility could be observed for all investigated parameters (FV, proton fraction and SNR) for axial and coronal slice orientation (an ICC range of 0.70–0.98), with only fair to good (an ICC range of 0.55–0.69) interstudy reproducibility (Table S4). The intraobserver study showed excellent results (an ICC range of 0.90–0.99), except for SNR in coronal orientation (ICC 0.75). Bland–Altman analysis (Figure 9) demonstrates good reproducibility of the mean values, but for the proton fraction and SNR in particular, rather high interstudy variation.

4 | DISCUSSION

The lungs are vital organs to enable the gas exchange between blood and airspaces. Therefore, it is of primary interest to obtain spatially resolved information about lung function and enable the study of mechanisms in preclinical research. In this work, we have shown that the properties of 2D tyGA UTE allow derivation of relevant lung function parameters from a single continuous scan at 11.7-T field strength. In combination with DC self-gating, high-quality low-noise images of the parenchyma could be derived for multiple respiratory and cardiac phases. The suggested approach could be applied successfully to obtain quantitative and qualitative regional parameters of the lung parenchyma density, ventilation and perfusion.

In recent decades, lung MRI has been widely used to detect and track lung tumors in mice noninvasively.35–37 However, the characterization of gas exchange and evaluation of lung function with MRI is challenging due to the lung’s intrinsically low spin density. One approach is based on inhalation of polarized or fluorinated gases (e.g. ³He, ¹²⁹Xe, sulphur hexafluoride [SF₆] and hexafluoroethane [C₂F₆]).38–41 A major limitation of this technique arises from the requirement for complex and expensive hyperpolarization hardware and gases (³He, ¹²⁹Xe) and the requirement for...
multinuclei hardware. Another technique is oxygen-enhanced MRI.\textsuperscript{42,43} Due to its paramagnetic characteristics, oxygen modulates the MRI signal upon inhalation by inducing changes in tissue relaxation. However, the expected changes may be limited in high field strengths. As an alternative, ventilation-weighted proton MRI investigates regional ventilation by analyzing proton density changes of the lung parenchyma over the respiratory cycle. In our approach, different respiratory phases were reconstructed from data continuously acquired during free-breathing. The regional lung FV as well as perfusion could be reliably quantified from the signal intensity differences over the respiratory and cardiac cycles. For accurate

**FIGURE 5**  Ventilation (middle, left) and perfusion (middle, right) as resulting from the Fourier analysis of signal intensity changes in the respective multiphase images (top) of the respiratory and cardiac cycle. Respective spectra (bottom) reveal a clear peak at the first frequency component next to direct current (DC) (respective video data are provided in Figure S7)

**FIGURE 6**  Quantitative perfusion analysis shows higher perfusion in systole (right) compared with diastole (left). (Multicardiac phase reconstructions are provided in Figure S7)
FIGURE 7  Analysis locations of the regions of interest (ROIs) in the heart (red), lung (green), muscle (yellow) and background (orange) superimposed onto a precontrast agent (pre-CA) image (A). After CA injection, an obvious enhancement can be noticed in the heart (B). Percentage change of signal intensity (SI) (C) and time-intensity curves (D; LV, left ventricle; RV, right ventricle; L1–4, lung; M, muscle; N1–3, noise) clearly show postcontrast enhancement in the heart, lung, and (at a different pace) the muscle. No enhancement was observed in the background ROIs. (D) Sliding window reconstruction was performed from 1000 projections (24 s) with a step size of 25 projections (0.6 s). The gray lines indicate the time points of the pre- and post-CA image shown (respective video data are provided in Figure S8).

FIGURE 8  Signal intensity changes during contrast agent injection. The time frames in the graphs were reconstructed with different sliding window width (A) 1000, (B) 500, (C) 250 and (D) 100 projections. The sliding window step was chosen as 25 projections (respective video data are provided in Figure S8).
quantification, reliable lung segmentation and registration between different respiratory stages were performed to avoid falsified results by partial volume effect or blood vessels and comparison of nonmatching tissues.22

The observed proton density in the range of 70% to 80% appears overestimated and is not perfectly in line with the 50% air-filled space, as reported by Bell et al.6 However, it is well in line with previously reported values by Tibiletti et al.,44 and even slightly lower than the lung parenchyma fraction of 84% derived by μCT and light microscopy analysis in excised mouse lungs, as reported by Vasilescu et al.45 The differences may be explained by the different pressure conditions and the much lower spatial resolution of in vivo MRI not allowing for perfect exclusion of small blood vessels. Further, small errors in the proton density estimation may rise in our study from not compensating for the coil sensitivity pattern. Even although the applied four-element wrap-around could provide quite homogenous sensitivity over the imaged regions, slight mispositioning of the animal might cause slight signal reduction in the muscle tissue, mimicking increased lung density values. The increasing lung density from anterior to posterior observed in our study is not in line with previously CT-derived data.46 A possible explanation may be the rather narrow coil applying some force on the back of the animal, but further studies are needed to clarify this point.

In preclinical practice, pulmonary perfusion quantification is mainly based on dynamic contrast-enhanced (DCE) MRI with systemic injection of CA or arterial spin labeling (ASL) techniques.11,47 DCE-MRI requires injection of multiple CA boli, and due to the inherent noise signal of the MR images, advanced regularization techniques are required for proper deconvolution of the signal.48 As an alternative, Fourier decomposition (FD) has been proposed to quantify perfusion and ventilation from signal variations over continuously acquired images during free-breathing by analysis of signal intensity changes in the respective frequency range,33,49 which reported the possibility for perfusion quantification.50 Even

![Bland–Altman plots with limits of agreement (1.96 SD) demonstrate the reproducibility between two different observers (A) and the interstudy reproducibility of the 2D ultrashort echo-time (UTE) protocol (B) and the intraobserver (C). The values of the axial and coronal measurements are combined. SNR, signal-to-noise ratio](image-url)
although FD-MRI has been proven to compare well with DCE-MRI, its translation to rodent imaging is severely limited by the only poor signal intensities in the lungs, especially at high field strengths and high respiration and cardiac rates. Therefore, lung perfusion quantification in small rodents has only been reported in rats with ASL or DCE techniques. In the current study, we combined the tyGA technique with a SENCEFUL approach for deriving qualitative and quantitative perfusion data as well as with single bolus Gd injection. Even although quantitative perfusion data in mice are rare, the resulting values compared well with the reported data of Tibiletti et al.11 derived from ASL. Further differences over the cardiac cycle were observed, indicating the sensitivity of the approach. From the CA-enhanced data, the upslopes clearly revealed the characteristic differences between different tissues.

In comparison with the established methods, the main advantage of the proposed tyGA UTE approach results from its capabilities to provide a highly flexible choice regarding the number of spokes used for a single image with inherent coverage of a k0 self-gating signal, thus enabling trading spatial versus temporal resolution, as well as allowing self-gated reconstructions, which facilitates application-tailored image quality. In comparison with acquisitions with conventional angular ordering, tyGA UTE shows less susceptibility to aliasing artefacts (streak artefacts).

The relaxation time T2* may reflect pathological changes in lung tissues. Bianchi et al.14 reported significantly reduced T2* values in the emphysema rat model and the results were excellently correlated with histology and μCT. As the apparent transverse relaxation time was directly linked to the pulmonary microstructure, a significant difference T2* was observed in the left lungs of COPD animals. This indicates a potential relevance of T2* as a sensitive biomarker for characterizing early pathological alterations in lungs. Noticeably, no data for mouse lung T2* were previously reported at 11.7 T. Togao et al.53 reported a T2* value of 0.91 ± 0.10 ms at 3 T, which decreased as the positive end-expiratory pressure level became higher. Lower T2* values were reported at 4.7 T (0.46 ± 0.05 ms)54 and 7 T (0.395 ± 0.033 ms),55 respectively. At 11.7 T we could derive a T2* in the lung parenchyma of 0.20 ± 0.05 ms, which is well in line with the expected inverse relationship between the T2* values and field strength.

Additionally, the FV evaluation showed that the lung tissue is ventilated differently at different positions in the lung, with higher values towards the caudal areas.

In small animal imaging, it is particularly important to ensure high reproducibility within and between observers and between different acquisitions to ensure sensitive quantification of in vivo data. In our study, with the high spatial and temporal resolution images, a good to excellent intraobserver reproducibility was observed. However, only poor to fair interstudy reproducibility was achieved. In contrast to human studies, achieving a high interstudy reproducibility in small animal imaging appears more challenging. As anesthesia is generally required in small animal imaging to provide immobility, the effects on cardiac and respiratory function need to be considered. Moreover, circadian rhythms and body temperature also show impacts. In our experiments, inhalation anesthesia with isoflurane was used because it provides greater safety, lesser cardiovascular and respiratory depression, and rapid recovery and convenient adjustments and maintenance during scans. Further, the body temperature and respiratory rate of the animal as well as the entire scan duration may impact the derived functional parameters.

A general limitation of using signal intensities for quantifying changes over the respiratory and/or cardiac cycle results from likely different T2* values between inspiration and expiration, as shown by Olsson and Hockings for T2.56 Even although the intensity changes are still due to changes in the air/tissue fraction, a strictly linear relation cannot be expected due to the nonlinear contribution of the T2* contribution and needs further investigation.

A major limitation of the proposed technique results from its current restriction to 2D imaging. Encoding schemes providing similar sampling distributions in 3D have been reported57,58 and have partly been applied to lung imaging.13 Where in principle a translation of the proposed techniques to 3D is feasible, the resulting long acquisition times required for high-fidelity reconstruction of different respiratory and/or cardiac motion stages will pose challenges, and transfer to dynamic perfusion imaging appears to be impossible, even if advanced parallel of compressed sensing reconstruction or even more advanced deep learning59,60 approaches are applied. The T2* values of the lung parenchyma showed quite large variations over the different sections of the lung (especially when comparing the cranial with the caudal sections). Respective impact on the lung density calculation has not been considered in this study due to the mean T2* value used during data analysis. Whether usage of regional T2* values and an individual mapping of T2* values will further increase sensitivity remains to be investigated. Further, this study only proves the principal use of the tyGA technique for lung function imaging in a small cohort of healthy animals. Further studies need to be performed to assess the sensitivity in animal disease models.

5 | CONCLUSION

The 2D tyGA UTE approach in mice appears feasible with sufficient image quality for the quantification of lung function parameters. With retrospective gating, multiphase images of the respiratory and, if required, cardiac cycle, can be derived without any cardiac or respiratory synchronization, also yielding lung density details. In principle, lung density, FV and perfusion can be derived from a single continuous scan with minimal motion artefacts due to the intrinsic properties of the center-out technique. Additionally, the technique can be applied to real-time imaging for following an intravenously administered CA bolus as an alternative method of lung perfusion assessment. In summary, the current study reveals strong evidence about the potential role of 2D tyGA for functional lung imaging in rodents.
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