Published in final edited form as:

NMR Biomed. 2021 March ; 34(3): e4464. doi:10.1002/nbm.4464.

Improved Preclinical Hyperpolarized ^{129}Xe Ventilation Imaging with Constant Flip Angle 3D Radial Golden Means Acquisition and Keyhole Reconstruction

Peter J. Niedbalski^{1,†}, Zackary I. Cleveland^{1,2,3,4,*}

¹Center for Pulmonary Imaging Research, Division of Pulmonary Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

²Department of Biomedical Engineering, University of Cincinnati, Cincinnati, OH 45221

³Imaging Research Center, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229

⁴Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

Abstract

Hyperpolarized (HP) ^{129}Xe MRI is increasingly used to non-invasively probe regional lung structure and function in the preclinical setting. As in human imaging, the primary barrier to quantitative imaging with HP gases is non-equilibrium magnetization, which is depleted by T_1 relaxation and radio frequency (RF) excitation. Preclinical HP gas imaging commonly involves mechanically ventilating small animals and encoding k-space over hundreds or thousands of breaths, with small subsets of k-space data collected within each breath. Breath-to-breath magnetization renewal enables the use of large flip angles, but the resulting magnetization decay generates large view-to-view differences in within-breath signal intensity, leading to artifacts and degraded image quality. This deleterious signal decay has motivated the use of variable flip angle (VFA) sampling schemes, in which flip angle is progressively increased to maintain constant view-to-view signal intensity. However, VFA imaging complicates data acquisition and provides only a global correction that fails to compensate for regional differences in signal dynamics. When constant flip angle (CFA) imaging is used alongside 3D radial golden means acquisition, the center of k-space is sampled with every excitation, thereby encoding signal dynamics alongside imaging data. Here, keyhole reconstruction is used to generate multiple images to capture in-breath HP ^{129}Xe signal dynamics in mice and thus provide flip angle maps to quantitatively correct images without extra data collection. These CFA images display SNR that is not significantly different from VFA images, and further, high-frequency k-space scaling can be used to mitigate decay-induced image artifacts. Results are supported by point spread function calculations and simulations of radial imaging with preclinical signal dynamics. Together, these results show that CFA, 3D radial golden means ventilation imaging provides comparable image quality to VFA in small animals and allows for keyhole reconstruction, which can be used to generate flip angle maps and correct images for signal depletion.

*Corresponding author: Zackary I. Cleveland, 3333 Burnet Ave., MLC-2021, Cincinnati, OH 45229, 513-803-7186, zackary.cleveland@cchmc.org.

[†]Current Address: Division of Pulmonary, Critical Care, and Sleep Medicine, University of Kansas Medical Center, Kansas City, KS 66160

Keywords

^{129}Xe ; MRI; Mouse; Preclinical; Radial; Hyperpolarized; Constant Flip Angle; Variable Flip Angle

Introduction:

Hyperpolarized (HP) gas MRI has become an established modality in humans to image regional ventilation.¹ Given its utility in measuring both lung structure and function at high resolution, HP gas MRI is likewise gaining traction as a preclinical tool for quantitative imaging of animal models of human disease.²⁻¹⁰ In contrast to human imaging, where images are usually acquired in a single breathhold, preclinical imaging in small animals is most commonly accomplished by mechanically ventilating animals with HP gas and encoding k-space over a large number of breaths.^{2, 3, 5} As a result, magnetization does not decay monotonically as it does in human HP gas imaging, but rather, it displays a repeated pattern of replenishment and decay over tens to hundreds of breaths.¹¹

As with human imaging, the primary challenge to perform quantitative HP gas MRI remains signal depletion due to radio frequency (RF) excitation and T_1 relaxation.¹ However, in mouse imaging, the duration of breath-holds (200-500 ms) is short compared to T_1 in vivo (15-30 s),¹² making in vivo T_1 relaxation negligible. However, T_1 relaxation in the HP gas reservoir from which animals are ventilated is a significant source of longitudinal magnetization loss. This longitudinal relaxation prior to inhalation is dominated by interactions between the gas and the reservoir surface (typically a fluoropolymer bag), meaning that T_1 varies based on the size and inflation of the reservoir, and the field strength and homogeneity in which it is located.¹³ For 2D slice selective imaging, images can most conveniently be acquired by sampling one phase encoding line per breath, mitigating RF depletion of HP gas signal,¹⁴ which could otherwise lead to significant image artifacts.¹⁵ This leaves reservoir T_1 effects as the dominant depletion effect, which can be corrected by scaling projections based on the fitted relaxation.¹³ However, 3D volumetric imaging, for example using radial sampling, is often preferable for a variety of reasons. Relative to slice-selective imaging, it can provide superior resolution in the slice dimension. Furthermore, using fast-encoding techniques such as 3D radial imaging avoids effects of T_2^* relaxation ($T_2^* \sim 5$ ms),¹⁶ which can degrade the signal intensity, true resolution, and quantitative accuracy of images. Images acquired with 3D radial are significantly oversampled at the k-space center (k_0), which makes them robust to motion and undersampling. This allows projections that are corrupted (e.g., by motion or low signal at the end of an acquisition) to be discarded prior to reconstruction with minimal impact on image quality. The repeated sampling of k_0 also allows for the tracking of hyperpolarized gas signal dynamics. Specifically, the k_0 intensity of the first projection acquired in each breath provides a means of tracking signal dynamics within the HP gas reservoir, and the pulse-to-pulse k_0 intensity within each held breath provides information about in vivo signal dynamics.

Despite its advantages, 3D radial imaging suffers from inefficient coverage of k-space. Thus, to acquire images in a reasonable time and with a reasonable amount of HP gas, multiple projections must be acquired per breath.² In standard human imaging, the need to fully acquire k-space within a single breath leads to the use of small RF flip angles. However, because data is acquired over many breaths with magnetization being replenished every breath, much larger flip angles (up to 90 degrees) are used in small animal imaging, and thus, greater consideration must be given selecting RF pulse parameters. The most obvious approach is to employ constant flip angle (CFA) RF excitation, but this approach causes view-to-view variations in signal intensity within a particular breath as hyperpolarized magnetization is consumed (Figure 1). This variable signal causes blurring and streaking artifacts in reconstructed images, and, while this can be compensated for via appropriate selection of projection ordering,¹⁵ it can still affect both qualitative and quantitative image analysis.¹⁵

To combat this loss of image quality, variable flip angle (VFA) schemes have been proposed.¹⁷⁻¹⁹ Rather than playing out a single, constant RF flip angle, VFA acquisitions apply progressively larger flip angle RF pulses—typically finishing the breath with a 90° pulse (Figure 1). If implemented correctly, this approach generates constant view-to-view transverse magnetization and makes maximal use of the available longitudinal magnetization. In multi-breath, small animal imaging, the flip angle pattern is repeated for each new bolus of gas delivered by the ventilator, keeping magnetization relatively constant from across all breaths, though reservoir T₁ depletion does cause a steady decline in signal over the course of the scan. Notably, this technique is a global correction, meaning that some regions of the lungs could experience greater or lesser flip angle, particularly when using inhomogeneous imaging coils. This “over-flipping” or “under-flipping” leads greater or lesser signal depletion in certain regions of the lungs, causing images to misrepresent the ventilation distribution.

Instead of prospective depletion corrections using VFA sampling, it is possible to make some corrections for signal depletion retrospectively. Such methods include global decay compensation and single breath flip angle mapping.^{15, 20} Additionally, it has recently been shown that for center-out k-space encoding, a Bloch-equations based model of HP magnetization dynamics during constant flip angle acquisitions can be combined with keyhole image reconstruction to map hyperpolarized signal decay during gas-phase human lung imaging.²¹ That is, using data from a single radially-acquired acquisition, multiple temporally resolved images can be generated and used to provide a regional map of HP magnetization decay. These regional signal maps can be used to quantitatively correct images, providing more accurate images with no additional HP gas or imaging time. Herein, we expand this method to the signal dynamics intrinsic to multi-breath preclinical imaging using HP ¹²⁹Xe ventilation imaging in mice. This allows flip angle maps to be produced when performing preclinical HP gas MRI, thereby providing a simple method of quantitatively correcting RF-induced signal decay in preclinical HP gas ventilation images.

Theory

Signal Dynamics in Small Animal Imaging

For preclinical HP gas imaging, fresh, highly polarized gas with non-equilibrium magnetization $M_z(j)$ is delivered to the animal following the j th inhalation.¹¹ For 3D radial ventilation imaging, this magnetization is sampled using n radial projections acquired each breath over n_b breaths, for a total of $N = n \times n_b$ radial projections. If we assume that no magnetization from the previous breath remains when the subsequent breath is complete and neglect the ~2% of xenon magnetization that is dissolved in tissue, the signal intensity resulting from the i th projection in the j th breath ($s_{i,j}$) is

$$s_{i,j} = M_z(j) \sin(\alpha) \left(\cos(\alpha) \exp\left(-\frac{TR}{T_1}\right) \right)^{i-1} \quad (1)$$

where α is the applied, constant flip angle, TR is the imaging repetition time, and T_1 is the longitudinal relaxation time. For preclinical imaging, HP gas is in the lungs for <500 ms while $T_1 \approx 15$ -30 s,^{12, 22} so $T_1 \gg TR$, and Eq. 1 simplifies to

$$s_{i,j} = M_z(j) \sin(\alpha) \cos(\alpha)^{i-1}. \quad (2)$$

Then, for center-out encoding strategies like radial, the overall signal intensity (S) for the total image is simply the average signal of all N projections

$$S = \frac{1}{N} \sum_{j=1}^{n_b} \sum_{i=1}^n M_z(j) \sin(\alpha) \cos(\alpha)^{i-1} \quad (3)$$

which can be simplified to

$$S = \frac{1}{N} \sin(\alpha) \frac{1 - \cos(\alpha)^n}{1 - \cos(\alpha)} \sum_{j=1}^{n_b} M_z(j) \quad (4)$$

Then, we can define the average magnetization delivered to the mouse over the course of the scan to be

$$\overline{M_z} = \frac{1}{n_b} \sum_{j=1}^{n_b} M_z(j) \quad (5)$$

so Equation 3 can be written as

$$S = \frac{\overline{M_z}}{n} \sin(\alpha) \frac{1 - \cos(\alpha)^n}{1 - \cos(\alpha)} \quad (6)$$

Flip angle Optimization

Having defined the signal intensity as a function of flip angle and number of projections per breath, we can calculate the optimal flip angle to use for a given number of projections per

breath. However, the definition of optimal depends on the desired goal; here we consider two cases. First, the total signal intensity (Equation 6) can be maximized. Second, the signal intensity difference between adjacent projections (Equation 2) can be minimized, thereby reducing decay-induced image artifacts. This is done by finding the points at which the first derivatives (with respect to α) are zero. For maximization of overall signal intensity, we have:

$$\frac{\partial S}{\partial \alpha} = \frac{\overline{M_z}}{n} \left[\cos(\alpha) \frac{1 - \cos^n(\alpha)}{1 - \cos(\alpha)} + \frac{n \sin^2(\alpha) \cos^{n-1}(\alpha)}{1 - \cos(\alpha)} - \frac{\sin^2(\alpha)(1 - \cos^n(\alpha))}{(1 - \cos(\alpha))^2} \right] = 0 \quad (7)$$

Unfortunately, there is no simple analytical solution to this equation, and thus it must be solved numerically. This was done using Matlab 2019b (Mathworks, Natick, MA) (Figure 2), and the solutions for 1-10 projections per breath are shown in Table 1.

For the case of minimizing image artifacts, the minimization equation becomes.

$$\frac{\partial s}{\partial \alpha} = M_z [n \cos^n(\alpha) - (n-1) \cos^{n-2}(\alpha)] = 0 \quad (8)$$

In this case, the zeros of this equation are found at (Figure 2)

$$\alpha = \cos^{-1} \left(\sqrt{\frac{n-1}{n}} \right) \quad (9)$$

The flip angles needed to minimize image artifacts for 1-10 projections per breath are also listed in Table 1.

Calculation of Flip Angle Maps

One of the advantages of radial sampling is that the center of k-space, which encodes image signal, is significantly oversampled relative to the edges, which encode fine structure. This oversampling of low-frequency k-space enables pseudo-dynamic imaging via keyhole image reconstruction.²³ Briefly, subsets of data from the central portions of k-space are combined with the entirety of the periphery of k-space, allowing reconstruction of multiple, time-resolved fully sampled images (or equally undersampled if the original data set was undersampled). This requires the assumption that high frequency components of the image are constant, a good assumption in the case of HP signal decay within a given breath.²¹

Based on these considerations, we can use keyhole reconstruction for HP ^{129}Xe 3D radial images in the preclinical setting. The logical splitting of data into keys is to generate 1 image for each projection per breath. That is, if an image is encoded using 5 radial projections per breath of ^{129}Xe gas, 5 images can be reconstructed (Figure 3). For visual clarity, Figure 3 shows a 2D-radial sampling pattern with signal intensity shown on the z axis, but the concept is generalizable to 3D sampling. The first key contains the low frequency data from the first projections of each breath, the second key contains the low frequency data of the second projections of each breath, and so on. These keys are then combined with all of the

high frequency data (the “keyhole”) to create several temporally resolved, equally sampled datasets. To mitigate artifacts from non-constant signal intensity, the keyhole is scaled such that each projection (at k_0) has the same signal intensity as the mean of the projections in a given key. The resulting signal intensity of key κ can be written as

$$S_{\kappa} = \frac{1}{n_b} \sum_{j=1}^{n_b} M_z(j) \sin(\alpha) \cos(\alpha)^{\kappa-1} = \overline{M_z} \sin(\alpha) \cos(\alpha)^{\kappa-1} \quad (10)$$

This expression for the signal of each key allows fitting of the reconstructed key images as a function of κ and subsequent generation of a voxel-by-voxel map of the RF flip angle, neglecting T_1 relaxation¹³ and xenon dissolved in tissues,²⁴ both of which should contribute minimally. Once the RF flip angle is known, images can be corrected for RF depletion on a voxel-by-voxel basis by rearranging equation 6.

$$\overline{M_z} = S \times n \frac{1}{\sin(\alpha)} \frac{1 - \cos(\alpha)}{1 - \cos(\alpha)^n} \quad (11)$$

Image Quality Optimization

While this process enables creation of a flip angle map when using 3D radial constant flip angle acquisition, the non-constant k_0 signal intensity can impact the quality of images by introducing reconstruction artifacts. One possible method of mitigating these artifacts is to scale the signal intensity of individual projections within a given breath such that the first projection of every breath or every projection has the same k_0 signal intensity.^{15, 16} However, such methods fail to correct regional flip angle variation and obscure the dynamic signal intensity information provided by the un-scaled k_0 that allows the calculation of flip angle maps via the proposed keyhole method. However, it has been demonstrated previously that scaling only high-frequency k-space data can improve image quality.²⁵ Therefore, in this work, data acquired using constant flip angle acquisition is scaled such that the high frequency data on a given projection (>50% toward the edge of k-space) corresponds to a signal intensity matching the mean of all k_0 points.

Methods

Simulation

All simulations were performed using Matlab version 2019b.

To examine the effect of hyperpolarized gas signal dynamics on real resolution for both the CFA and VFA case, point spread simulations were performed for four different cases. In the first three—VFA, CFA optimized for maximum signal intensity, and CFA optimized for minimum signal intensity difference between projections—the effect of unscaled signal dynamics are examined. In the fourth, the high frequency k-space data for the maximum signal intensity CFA case was normalized to the mean k_0 signal intensity, thereby testing whether scaling only high frequency data is capable of improving image quality. In all cases, reservoir and in vivo T_1 (~45 minutes and ~30 seconds, respectively) was ignored.

A digital phantom containing a single, central point was generated (Image Size = 64 x 64 x 64) and its k-space sampled using a 3D radial golden means strategy identical to that used on our animal imaging scanner.²⁶ For comparison, we also performed simulations using a sequential projection ordering. K-space was fully Nyquist sampled (12868 Projections). For the VFA case, the signal intensity of the phantom was held constant for all sampling. For the CFA cases, sampling was simulated with the effect of RF decay imposed to match the case of 5 RF excitations per breath. That is, signal intensity is depleted by $\cos(\alpha)$ for 5 consecutive projections before returning to its initial value and repeating the pattern. The flip angle was chosen based on table 1, with one simulation each being performed for flip angles of 38.1° and 26.6°.

Images were reconstructed using open source reconstruction software tools written for Matlab^{27, 28} and using iterative density compensation.²⁹ Subsequent to image reconstruction, images were visually inspected and examined in planes passing through the center point orientations to determine the effect to which CFA sampling induces image artifacts.

Simulations were also performed to gauge the ability of keyhole reconstruction to generate flip angle maps from CFA data. A simple 2D digital phantom was created with similar features to those seen in hyperpolarized gas imaging (i.e. regions of both smooth and sharp variation in signal intensity). K-space for this phantom was sampled using a 2D radial sampling scheme with a linear radial sampling pattern (i.e. radial spokes ordered in even steps from 0° to 360°). Sampling was performed using CFA acquisition with sharp spatial variation in the applied flip angle. Flip angles varied from 15°-50° over the image. Other simulation parameters: Image Size = 256 x 256, Radial Projections = 805 (100% Nyquist sampled), Simulated Projections per Breath = 5. In addition to this illustrative example, we also performed a 3D simulation using a modified 3D Shepp-Logan phantom, sharply varying flip angles from 20-50°, and radial golden means k-space trajectories. Other simulation parameters: Image Size = 128 x 128 x 128, Radial Projections = 51472 (100% Nyquist sampled), Simulated Projections per Breath = 5.

After radial sampling, the raw data was separated into 5 keyholed datasets (Figure 3). The first n_{key} points on each projection were emptied, leaving an empty “keyhole” at the center of k-space. Then, subsets of the center of k-space data (“keys”) based on the projection number are placed within the keyhole (i.e. high frequency k-space data). That is, there are keys composed of the first n_{key} points on the first projection of each breath, on the second projection of each breath, etc. The keyhole radius, n_{key} , was chosen such that the projections in each key provided 100% Nyquist sampling at the edge of the keyhole.^{21, 23} The high frequency data was scaled to match the signal intensity of the particular key and images reconstructed. After reconstruction, key images were fit pixel-by-pixel to Equation 10, generating a flip angle map. The mean extracted flip angle in regions with the same applied flip angle was plotted against the applied flip angle and fit using a least squares linear fit. Finally, an image reconstructed from the whole of the original k-space data was corrected using the generated flip angle map and Equation 11. We note that this “corrected” image is an image that has signal intensity corrected for HP signal depletion. This is distinct from the high-frequency-scaled image, which is an image with unaltered signal intensity

whose artifacts have been reduced by reconstructing the image with scaled high-frequency k-space data.

Animal Preparation

Nine C57BL/6J (4 male, 5 female, weight 24 ± 2 g, age 9 ± 2 weeks, Jackson Laboratory, Bar Harbor, Maine) were imaged following procedures approved by the Cincinnati Children's Hospital Institutional Animal Care and Use Committee. Animals were anesthetized using intraperitoneal (IP) injections of triple sedative (67/3.3/0.17 mg/kg Ketamine/ Xylazine/ Acepromazine). Anesthesia was maintained once per hour with IP injections of the same triple solution at a dose of 17/0.83/0.04 mg/kg.

Animals were intubated perorally using homebuilt, metal-free intubation cannulas made following the design of MacDonald et al.³⁰ Mice were then mechanically ventilated using a homebuilt HP gas compatible ventilator^{5, 16, 31} at a breathing rate of 80 breaths per minute with a tidal volume of 10 mL/kg. Breaths were composed of a gas mixture of 21% oxygen balanced with either nitrogen during normal breathing or hyperpolarized xenon during imaging. Breath timing consisted of a 200 ms inhalation, 200 ms breath-hold, and 350 ms passive exhalation. A pressure transducer incorporated in the ventilator monitored airway pressure at the mouth of the animal.

While mice were being imaged, the temperature in the bore of the MRI scanner was maintained at $\sim 37^\circ\text{C}$ using warm air flowing through the bore (Small-Animal Instruments monitoring system, SAI, Inc, Stony Brook, NY).

Gas Polarization and Delivery

Isotopically enriched xenon gas (83% ^{129}Xe , Line Specialty Gases, Stewartville, NJ) was polarized to 40-50% using a Polarean 9820 ^{129}Xe Hyperpolarizer (Polarean Imaging Plc., Durham, NC). Gas was dispensed into 300 mL Tedlar bags (Jensen Inert Products, Coral Springs, FL) which were then placed into a pressurized Nalgene cylinder (3.0 – 6.0 PSIG) for animal delivery. This cylinder was placed in the fringe field of the magnet such that T_1 was approximately 45 minutes.

Imaging

Animals were imaged using a 7T Bruker Biospec MRI (Bruker, Billerica, MA). Prior to each animal imaging session, the approximate ^{129}Xe center frequency and RF excitation flip angle were coarsely calibrated using non-localized spectroscopy on a thermally polarized cylindrical phantom containing 2.5 atmospheres (~ 400 mL) xenon and 2 atmospheres O_2 .

Mice were placed in the bore of the magnet and localized with their lungs at magnet isocenter using a gradient echo sequence with a 38 mm inner diameter proton imaging coil (Bruker, Billerica, MA). Once the mouse was localized, the proton coil was removed and replaced with a 50 mm inner diameter quadrature birdcage ^{129}Xe imaging coil, leaving the mouse at isocenter during the swap.

Once localized, mice were ventilated with a mixture of hyperpolarized xenon and oxygen (79% xenon, 21% oxygen), during which they were imaged. Images were acquired at

full inspiration, 250 ms after the beginning of the inhalation period (i.e. 50 ms into breath-hold). With each batch of hyperpolarized xenon, the first ~30 breaths of hyperpolarized xenon were used to calibrate the exact center frequency of the ^{129}Xe signal in vivo and to ensure the lungs were in the center of the magnet using a 2D coronal image over a thick slab. Additionally, for the first batch of HP ^{129}Xe delivered to an animal, a very highly undersampled image (~100-fold undersampled) using a variable flip angle radial imaging scheme was acquired. This was done to examine the k_0 point for each projection to determine whether the flip angle was properly calibrated. That is, if every projection within a breath had approximately equal k_0 intensity, the flip angle was properly calibrated. The flip angle calibration was then adjusted as necessary to ensure an accurate calibration.

After localizing, center frequency, and flip angle calibration was performed, animals were imaged twice with a 3D radial imaging sequence with golden means projection ordering²⁶: TR/TE = 9.0/0.92 ms, Bandwidth = 16025 Hz, Matrix Size = 64 x 64 x 64, Field of view (FOV) = 26 x 26 x 26 mm³, Slab Thickness = 26 mm, Number of Projections = 6435 (50% Sampling), Number of Projections per Breath = 5. For one scan, a constant RF flip angle of 30° was used, which was chosen to be intermediate between the optimization methods, thereby having signal and artifacts intermediate between the extremes provided by the optimal flip angles. For the other, a variable flip angle scheme with progressively increasing RF strength was used. For this case, the RF flip angle for the i th projection per breath was calculated according to¹⁷

$$\theta_i = \tan^{-1}\left(\frac{1}{\sqrt{n-i}}\right). \quad (12)$$

In our case, the number of excitations per breath, n , is 5, so the flip angles applied for the VFA image acquisition were 26.6°, 30.0°, 35.3°, 45.0°, and 90°. Each image took about 16 min to acquire and used about 250 mL of hyperpolarized gas. A fresh bag of hyperpolarized gas was used for each image.

Image Reconstruction and Analysis

All image reconstruction, fitting, and statistical analysis were performed in MATLAB R2018b (Mathworks, Natick, MA).

For images acquired with a constant 30° flip angle, raw data was separated into 5 subsets to generate 5 keyholed images as described above. The keyhole radius was set such that keys were 100% Nyquist sampled at the edge of the keyhole, which, for the imaging parameters set, was 10 points along the radius.

For the key images reconstructed from constant flip angle acquisitions, images were fit using a voxel-by-voxel non-linear least squares fit to Equation 10. From this fit, a flip angle map can be generated and a “corrected” image obtained. Flip angle maps and corrected images were only generated in regions with signal present, as determined by the masks generated in ITK-SNAP. Comparisons of mean flip angles between the pulmonary airspaces and the conducting airways were done using paired Wilcoxon Signed Rank Tests and comparisons

of mean angles between scaling types were done using repeated measures ANOVA with post-hoc Tukey tests. Results were considered significant for $p < 0.05$.

To examine methods of improving image SNR and fidelity, multiple images were generated using different scaling techniques. In the first case, images were un-altered. In the second, high frequency k-space data was scaled prior to reconstruction such that k-space data $>50\%$ to the edge of k-space corresponded to a k_0 signal intensity of the mean of all k_0 values. Constant flip angle data were also reconstructed using the keyhole steps described above. These scaling methods led to two images reconstructed for each VFA and CFA dataset (un-scaled, high-frequency-scaled), plus two keyhole reconstructions (un-scaled, high-frequency-scaled) for every CFA dataset.

Images were reconstructed using the same reconstruction tools as used in point-spread function calculations. To mitigate signal intensity differences between scans, every image was scaled by dividing by the mean signal of the top 1% of image voxels. VFA images were registered to the non-keyhole CFA images using affine image registration and difference maps were made using these co-registered images. Comparisons between image SNR were performed using paired Wilcoxon Signed Rank tests and repeated measures analysis of variance (ANOVA) with post-hoc Tukey tests.

Masks of both the airway volume and lung parenchyma volume were made for images using semi-automatic, threshold-based segmentation in ITK-SNAP.³² Manual intervention was used where necessary to improve image masks. Noise masks for each image were made by dilating image masks using a structuring element radius of 3 voxels and selecting all voxels not contained in this dilated mask. This dilation removes partial volume effects and blurring from the image volume. SNR was then calculated using the equation:

$$SNR = \frac{mean(Image) - mean(Noise)}{std(Noise)} \quad (13)$$

Results

Simulations

Point spread simulations showed only subtle differences between VFA and CFA images (Figure 4). In particular, the full width at half maximum of the image remained essentially unchanged (~ 1.05 voxels in all dimensions) between the cases of variable flip angle, constant flip angle optimized for total signal intensity, and constant flip angle optimized for signal difference between projections. In the periphery of images, there were some increased artifacts (off-center intensity spikes) in CFA images, which as expected, were reduced in the case of flip angle optimized for signal difference between projections. It was found that scaling the k-space data beginning at 50% of the maximum k-space radius maintained the same full width at half maximum, but removed reconstruction artifacts,²¹ leading to a point spread function nearly identical in shape to the VFA case while preserving the RF depletion dynamics at k_0 . Finally, images acquired with a sequential ordering of projections provided similar point spread functions to those using golden means ordering, though imperfections were cast further from the central point.

For keyhole simulations, the digital phantom (Figure 5a) was sampled using sharply varying flip angles (Figure 5b) and reconstructed (Figure 5c). In the reconstructed image, there is obvious signal variation due to flip angle inhomogeneity. Key images showed a monotonic decrease in signal intensity (Figure 5d). Fitting these key images pixel-by-pixel to Equation 10 generated a flip angle map (Figure 5e) that shows excellent visual agreement with the applied flip angle (Figure 5b). Moreover, using the flip angle map to correct the original reconstructed image (Figure 5f), mitigated the obvious signal intensity imperfections seen in Figure 5c. Spatial averages of the measured flip angle map compared against the applied flip angle shows excellent correlation for both the 2D ($R^2 = 0.99$, Figure 5g) and 3D ($R^2 = 0.98$, Figure 5h) simulations.

Animal Imaging

For each mouse, one image was acquired for each flip angle scheme (i.e. CFA and VFA). Animal compliance to the ventilator was generally good. In particular, the signal intensity of the first projection on a given breath was generally within 10% of the first projection on both the preceding and following breath for both VFA and CFA acquisitions (Figure 6). For seven out of nine mice, VFA raw data had less than 10% variation in k_0 intensity from first to last acquisition of each breath. Images were able to be reconstructed for both scaling methods for both VFA and CFA datasets.

For images reconstructed using unscaled data, SNR ranged from 11.9 to 20.4 for CFA images and from 12.3 to 30.8 for VFA images. Though there are confounding factors to SNR comparisons, such as different initial xenon polarization, the difference in SNR between the two types of acquisition does not reach significance ($p = 0.16$). SNR increased significantly between non-scaled images and the high-frequency-scaled images for CFA images ($p = 0.008$), and trended toward a significant increase for VFA images ($p = 0.054$).

Image quality was similar for both VFA and CFA images, though VFA images generally had higher signal intensity in airways than did CFA images. When brightness and contrast on images is enhanced, there are blurring and streaking artifacts visible in all images (Figure 7), which are more pronounced in CFA images (Figures 7 and 8). Whether using VFA or CFA acquisition, scaling high frequency k-space prior to reconstruction improved image quality and reduced coherent artifacts.

For CFA images, keyhole reconstruction successfully generated five distinct images for each subject (Figure 6). From these five images, a flip angle map was able to be created (Figure 9). Using the unscaled datasets, the mean flip angle for the different mice imaged ranged from 27.3° to 35.7° with a mean $\pm$ standard deviation of $29.8 \pm 2.6^\circ$ (Nominal applied flip angle was 30°). The spread of flip angles within an individual flip angle map was relatively small, but showed that there are some inhomogeneities, with an average standard deviation of 3.2° . The calculated flip angle in the airways was consistently slightly higher than within the lung parenchyma ($31.2 \pm 3.7^\circ$ vs $29.9 \pm 2.4^\circ$, $p = 0.027$). The mean flip angle values and standard deviations were not significantly different among the unscaled and scaled datasets ($p > 0.73$ in all cases). Alongside flip angle maps, a “corrected” image was able to be generated.

Discussion

Simulation

Point spread function simulations show that 3D radial images acquired with preclinical HP gas signal dynamics do not experience significant blurring or streaking due to non-constant signal intensity. That is, the broadening of a single point has essentially no change whether using constant or variable flip angles and whether using golden means or sequentially ordered projections. This is in contrast to what has been observed in Cartesian and 2D radial sequences^{15, 17} in cases more akin to human imaging where images are acquired using a single bolus of gas. Here, the combination of a small number of discrete levels of signal intensities that are repeated with each breath lead to RF depletion effects being evenly distributed throughout k-space, leading to negligible blurring. Importantly, we ignored reservoir T_1 relaxation for these point spread function calculations, which led to similar results for sequential and golden means projection ordering. In reality, reservoir T_1 combined with sequential projection ordering will lead to T_1 effects unevenly distributed through k-space, which will deleteriously affect image quality. Thus, golden means projection ordering (or some other adaptive k-space sampling method) is important for obtaining high quality images.

Despite minimal blurring induced by the use of constant flip angles, streaking artifacts are induced by the use of CFA acquisition. These artifacts are relatively minor, with artifact spikes having intensity less than 1% of the image intensity. Artifacts are most intense when using a constant flip angle optimized for overall signal intensity, but they are mitigated by optimizing the flip angle for signal difference between adjacent projections. Artifacts can be almost entirely removed through post-acquisition scaling of the high frequency k-space data, even for image data acquired with signal-optimized flip angles.

Simulations using a 2D CFA radial sampling showed that keyhole reconstruction could be used to generate one image per projection per breath, providing a set of images with signal intensity changing according to the regional RF depletion experienced by spins. A flip angle map generated by fitting these key images has good visual agreement with the applied flip angle map and comparing regional flip angle means gives a linear plot with $R^2 = 0.99$ (3D case: $R^2 = 0.98$) and a slope close to 1.0 for both 2D and 3D simulation cases. When correcting images, some of the sharp signal intensity changes brought on by inhomogeneous flip angle seen in the original image reconstruction are smoothed, providing an image that more closely matches the original digital phantom. These point spread function and CFA simulation results provide confidence that constant flip angle acquisition can provide high quality images and that flip angle maps and corrected images can be accurately generated.

Animal Imaging

CFA and VFA acquisition schemes both resulted in good quality, 3D HP gas ventilation images in mice. As suggested by point spread function simulations, the real resolution of both CFA and VFA images were comparable, but there were more pronounced streaking artifacts in CFA images (Figures 7, 8). These artifacts were mitigated through the use of simple post-acquisition high-frequency k-space filtering, providing images with a

comparable level of image streaking artifacts to those acquired with VFA. Artifacts outside of those induced by non-constant k_0 signal intensity are likely caused by undersampling and by animal non-compliance to the ventilator. Even animals that have been anesthetized will occasionally attempt to breathe over the ventilator. When this occurs, the volume of xenon, and thus signal intensity, present in the lungs changes unexpectedly. The high frequency k-space scaling techniques used to correct raw imaging data effectively mitigated the extra-ventilator motion artifacts.

Beyond the extra-ventilator motion artifacts, our images also sometimes display bright airways and lower signal intensity within the lung parenchyma. This likely stems from pressure differentials at the mouth-ventilator connection. More specifically, the cannula used for intubation of the mice necessarily has a very small diameter in order to fit within the mouse's trachea (~1 mm diameter). As the gas passes from the larger diameter tubing of the ventilator to the narrow cannula, the flow is restricted, leaving excess pressure within the tubing after the conclusion of the inhalation. This excess pressure then diffuses into the mouse throughout the breath-hold period. Therefore, polarized gas that has not been depleted by RF excitation is introduced into the mouse, increasing the signal intensity measured within that region. For VFA images, flip angles increase throughout the breath, leading to a runaway effect where gas with no previous RF depletion is excited by very large flip angles. The effect is less pronounced in CFA images since the RF flip angle remains constant. This overemphasis of airway voxels is made clear in difference maps between CFA and VFA images, particularly in Figure 8a.

In addition to providing quality images, the use of CFA acquisition provides additional dynamic signal intensity information that can be extracted through the use of keyhole image reconstruction. With no extra data collection, flip angle maps, and hence corrected images, can be generated by reconstructing extra images using subsets of the low frequency raw data combined with the entirety of the high frequency data.

When performing this keyhole flip angle extraction using images acquired with constant flip angles, we found that the applied RF flip angle was typically close to the nominal 30° , with some small deviations. These deviations have little bearing on the quality of CFA images, but can drastically affect the utility of VFA sampling by prematurely depleting the magnetization within a breath or under-utilizing the magnetization. Furthermore, even in the small environment of mouse lungs in a highly homogenous birdcage coil, the standard deviation in the measured flip angle within the masked volume averaged about 3.2° (Figure 9). We note that any imperfections in coil homogeneity will impede the ability to use variable flip angle acquisition schemes effectively as it will lead to regional over-flipping or under-flipping, thereby leading to inaccurate images. Finally, the flip angle was often measured to be higher in the airways than in the lung parenchyma, likely due to non-depleted magnetization diffusing into airways from the ventilator dead volume. However, the airways are typically of minimal interest in preclinical imaging, so this is only a minor limitation.

Conclusion

When 3D radial imaging is used to produce high resolution ventilation images, constant flip angle sampling leads to view-to-view signal intensity changes within a breath that can be tracked and mitigated using keyhole image reconstruction. Key images can be fit voxel-by-voxel to generate flip angle maps with no additional data collection, and these maps can further be used to compensate images for RF-induced depletion. Finally, one of the primary concerns of CFA imaging, artifacts stemming from non-constant signal intensity, seem to be minimal when using 3D radial golden means imaging. Simple post-processing techniques such as high frequency k-space filtering prior to image reconstruction can yield comparable results to VFA imaging. Overall, 3D radial golden means imaging with constant flip angle acquisition is able to produce high quality preclinical hyperpolarized gas ventilation images while simplifying setup, capturing HP signal dynamics, and quantitatively correcting for RF signal depletion.

Acknowledgements

We would like to thank Joseph Plummer for preparing the hyperpolarized gas used in experiments, Randy Giaquinto for building the xenon imaging coil used in experiments, and Elizabeth M. Fugate for assistance with mouse preparation. This research was funded by the National Institutes of Health (R01HL143011, T32HL007752).

Data Availability

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

Abbreviations:

HP	Hyperpolarized
RF	Radio Frequency
CFA	Constant Flip Angle
VFA	Variable Flip Angle
ANOVA	Analysis of Variance

References:

1. Roos JE, McAdams HP, Kaushik SS, Driehuys B. Hyperpolarized gas MR imaging: Technique and applications. *Magn Reson Imaging Clin N Am*. 2015;23:217–229. [PubMed: 25952516]
2. Virgincar RS, Dahlke J, Robertson SH, et al. A portable ventilator with integrated physiologic monitoring for hyperpolarized ^{129}Xe MRI in rodents. *J Magn Reson*. 2018;295:63–71. [PubMed: 30125865]
3. Loza LA, Kadlecsek SJ, Pourfathi M, et al. Quantification of ventilation and gas uptake in free-breathing mice with hyperpolarized ^{129}Xe MRI. *IEEE Trans Med Imaging*. 2019;38:2081–2091. [PubMed: 30990426]
4. Ruppert K, Xin Y, Hamedani H, et al. Measurement of regional 2D gas transport efficiency in rabbit lung using hyperpolarized ^{129}Xe MRI. *Sci Rep*. 2019;9:2413. [PubMed: 30787357]

5. Nouis J, Fanarjian M, Hedlund L, Driehuys B. A constant-volume ventilator and gas recapture system for hyperpolarized gas MRI of mouse and rat lungs. *Concepts Magn Reson B*. 2011;39B:78–88.
6. Abdeen N, Cross A, Cron G, et al. Measurement of xenon diffusing capacity in the rat lung by hyperpolarized ^{129}Xe MRI and dynamic spectroscopy in a single breath-hold. *Magn Reson Med*. 2006;56:255–264. [PubMed: 16767751]
7. Boudreau M, Xu XJ, Santyr GE. Measurement of ^{129}Xe gas apparent diffusion coefficient anisotropy in an elastase-instilled rat model of emphysema. *Magn Reson Med*. 2013;69:211–220. [PubMed: 22378050]
8. Cleveland ZI, Moller HE, Hedlund LW, et al. In vivo MR imaging of pulmonary perfusion and gas exchange in rats via continuous extracorporeal infusion of hyperpolarized ^{129}Xe . *PLoS One*. 2012;7:e12192.
9. Driehuys B, Walker J, Pollaro J, et al. He-3 MRI in mouse models of asthma. *Magn Reson Med*. 2007;58:893–900. [PubMed: 17969115]
10. Ruan W, Zhong J, Wang K, et al. Detection of the mild emphysema by quantification of lung respiratory airways with hyperpolarized xenon diffusion MRI. *J Magn Reson Imaging*. 2017;45:879–888. [PubMed: 27472552]
11. Möller HE, Chen XJ, Chawla MS, Driehuys B, Hedlund LW, Johnson GA. Signal dynamics in magnetic resonance imaging of the lung with hyperpolarized noble gases. *J Magn Reson*. 1998;135:133–143. [PubMed: 9799687]
12. Möller HE, Hedlund LW, Chen XJ, et al. Measurements of hyperpolarized gas properties in the lung. Part III: ^3He T1. *Magn Reson Med*. 2001;45:421–430. [PubMed: 11241699]
13. Moller HE, Cleveland ZI, Driehuys B. Relaxation of hyperpolarized ^{129}Xe in a deflating polymer bag. *J Magn Reson*. 2011;212:109–115. [PubMed: 21752680]
14. Wang W, Nguyen NM, Yablonskiy DA, et al. Imaging lung microstructure in mice with hyperpolarized He-3 diffusion MRI. *Magn Reson Med*. 2011;65:620–626. [PubMed: 21337400]
15. Marshall H, Ajraoui S, Deppe MH, Parra-Robles J, Wild JM. K-space filter deconvolution and flip angle self-calibration in 2D radial hyperpolarised ^3He lung MRI. *NMR Biomed*. 2012;25:389–399. [PubMed: 21837646]
16. Niedbalski PJ, Cochran AS, Akinyi TG, et al. Preclinical hyperpolarized ^{129}Xe MRI: Ventilation and T2* mapping in mouse lungs at 7 T using multi-echo flyback UTE. *NMR Biomed*. 2020;33:e4302. [PubMed: 32285574]
17. Zhao L, Mulkern R, Tseng CH, et al. Gradient-echo imaging considerations for hyperpolarized Xe-129 MR. *J Magn Reson, Ser B*. 1996;113:179–183.
18. Zhong J, Ruan W, Han Y, Sun X, Ye C, Zhou X. Fast determination of flip angle and T1 in hyperpolarized gas MRI during a single breath-hold. *Article. Sci Rep* 2016;6:25854. [PubMed: 27169670]
19. Ouriadov AV, Santyr GE. High spatial resolution hyperpolarized ^3He MRI of the rodent lung using a single breath x-centric gradient-recalled echo approach. *Magn Reson Med*. 2017;78:2334–2341. [PubMed: 28112867]
20. Miller GW, Altes TA, Brookeman JR, de Lange EE, Mugler JP. Hyperpolarized He-3 lung ventilation imaging with B-1-inhomogeneity correction in a single breath-hold scan. *MAGMA*. 2004;16:218–226. [PubMed: 15108030]
21. Niedbalski PJ, Willmering MM, Robertson SH, et al. Mapping and correcting hyperpolarized magnetization decay with radial keyhole imaging. *Magn Reson Med*. 2019;82:367–376. [PubMed: 30847967]
22. Albert MS, Cates GD, Driehuys B, et al. Biological magnetic-resonance-imaging using laser polarized Xe-129 . *Nature*. 1994;370:199–201. [PubMed: 8028666]
23. Lethmate R, Wajer FTA, Crémillieux Y, van Ormondt D, Graveron-Demilly D. Dynamic MR-imaging with radial scanning, a post-acquisition keyhole approach. *journal article. EURASIP J Adv Signal Process* 2003;2003:794878.
24. Freeman MS, Cleveland ZI, Qi Y, Driehuys B. Enabling hyperpolarized ^{129}Xe MR spectroscopy and imaging of pulmonary gas transfer to the red blood cells in transgenic mice expressing human hemoglobin. *Magn Reson Med*. 2013;70:1192–1199. [PubMed: 24006177]

25. Cooper KJ, Lee KJ, Teh K, Wild J. Retrospective adaptive k-space filtering for improved image quality in hyperpolarized gas MRI. In: Proc. Intl. Soc. Mag. Reson. Med16. 2007:1821.
26. Chan RW, Ramsay EA, Cunningham CH, Plewes DB. Temporal stability of adaptive 3D radial MRI using multidimensional golden means. Magn Reson Med. 2009;61:354–363. [PubMed: 19165897]
27. Robertson SH, Virgincar RS, He M, Freeman MS, Kaushik SS, Driehuys B. Optimizing 3D noncartesian gridding reconstruction for hyperpolarized ^{129}Xe MRI—focus on preclinical applications. Concept Magn Reson A. 2015;44:190–202.
28. Fessler JA, Sutton BP. Nonuniform fast Fourier transforms using min-max interpolation. IEEE Trans Signal Process. 2003;51:560–574.
29. Pipe JG, Menon P. Sampling density compensation in MRI: Rationale and an iterative numerical solution. Magn Reson Med. 1999;41:179–186. [PubMed: 10025627]
30. MacDonald KD, Chang HYS, Mitzner W. An improved simple method of mouse lung intubation. J Appl Physiol. 2009;106:984–987. [PubMed: 19150857]
31. Akinyi TG. An affordable open-source small animal MR and hyperpolarized gas compatible ventilator: Feasibility in preclinical imaging. Master's Thesis. University of Cincinnati; 2017. http://rave.ohiolink.edu/etdc/view?acc_num=ucin1490354672385997
32. Yushkevich PA, Piven J, Hazlett HC, et al. User-guided 3D active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. NeuroImage. 2006;31:1116–1128. [PubMed: 16545965]

Figure 1.

Applied RF flip angle and associated k_0 signal intensity for constant flip angle (top) and variable flip angle (bottom) sampling schemes. In CFA, RF intensity remains constant, resulting in depleted signal intensity over the course of the breath-hold. In VFA, on the other hand, RF intensity increases, keeping k_0 signal intensity constant throughout the scan.

Figure 2.

Optimization of RF flip angle for the case of preclinical HP gas imaging, where freshly polarized gas is introduced with each breath. **(a)** Optimizing the total signal intensity by finding the 0 point of the first derivative the equation for total signal intensity as a function of projections per breath and flip angle. **(b)** Minimizing the difference between adjacent projections, thereby reducing imaging artifacts. This is done by finding the zeros of the first derivative of the equation for signal within each breath as a function of flip angle and projections per breath. **(c)** Total Image signal intensity as a function of projections per breath and flip angle. The solid line shows the flip angle to be used when optimizing for total signal intensity, and the dashed line shows the flip angle for minimizing imaging artifacts.

Figure 3.

Workflow for keyhole reconstruction of CFA datasets. 2D-radial sampling is shown for visual clarity, but the concepts are generalizable to 3D-radial sampling. (a) The original object. (b) Neglecting reservoir T_1 relaxation, radial projections are acquired with at n discrete signal intensities due to RF depletion within a breath hold. (c) Low frequency and high frequency data is separated, grouping low frequency data (keys) according to their location within the breath (i.e. signal intensity). (d) Individual keys are combined with the entirety of the high frequency data, which is scaled to match the signal intensity of that particular key. (e) Images are reconstructed, providing images encoding RF depletion effects. (f) Images are fit to Equation 10, providing a flip angle map.

Figure 4.

Surface plots showing a center slice of 3D point spread function. (a) Point spread function for VFA acquisition showing narrow center point with no artifact spikes. (b) Point spread function for CFA acquisition optimized for maximum signal intensity has the same full width at half maximum as VFA acquisition but has reconstruction artifacts (red arrows). (c) For a CFA acquisition using flip angles that minimize the difference between adjacent projections artifacts are noticeably reduced. (d) Using raw data from a CFA acquisition optimized for signal intensity and scaling the high frequency k-space to the mean k_0 totally removes artifact spikes. (e, f) Using sequential projection ordering instead of golden means ordering provides minimal artifacts (black arrows) in the case of optimizing signal intensity (e), that are mostly removed when minimizing the difference between adjacent projections (f).

Figure 5.

CFA Imaging and Flip Angle Mapping Simulation. (a) Original Digital Phantom. (b) Applied flip angle map. (c) Reconstructed image with no corrections. (d) Key images showing RF depletion. (e) Flip angle map generated by fitting the key images. Visual agreement with the applied flip angle is excellent. (f) Reconstructed image is corrected using the measured flip angle map, showing greater fidelity to the original digital phantom. (g, h) Regional means of measured flip angle correlate highly with the applied flip angle for both 2D (g) and 3D (h) simulations.

Figure 6. k_0 signal dynamics in vivo. (a) Representative k_0 dynamics for VFA image acquisition showing relatively constant signal intensity view to view. (b) k_0 dynamics for CFA image acquisition showing decreasing signal intensity throughout each breath. (c) Representative maximum intensity projections for key images generated from CFA data, showing reduction in overall signal intensity.

Figure 7.

Representative slice of CFA and VFA images showing how image characteristics change with k-space corrections. For both CFA and VFA images, the unscaled images and images resulting from different types of k-space scaling are shown alongside a difference map showing the difference between the scaled and unscaled images. Images are brightened to emphasize streaking artifacts, and artifacts that are mitigated through k-space scaling are highlighted by arrows.

Figure 8.

Representative image slices from CFA and VFA images measured back to back on the same mice. Images largely show the same features. Difference maps show the differences between the unscaled and high-frequency-scaled CFA images and the unscaled VFA images. With high-frequency k-space corrections, the difference maps within the lungs become more homogeneous and coherent noise features are reduced, bringing the quality of CFA images near to that of VFA images.

Figure 9.

Flip angle mapping and correcting images in vivo. Representative image slices from CFA images with scaled high frequency k-space are shown alongside their associated flip angle maps and corrected images. Corrected images show only minimal differences from original images but should provide a more quantitative measure of regional ventilation. Note that for mouse 1, flip angles are measured higher in the main airways suggesting diffusion of fully polarized gas from the ventilator dead volume during the breath hold.

Table 1.

Optimal flip angles for 3D radial preclinical imaging when using a given number of projections per breath.

Projections per breath	1	2	3	4	5	6	7	8	9	10
Maximizing Signal	90.0°	60.0°	49.1°	42.6°	38.1°	34.8°	32.2°	30.1°	28.4°	26.9°
Minimizing Artifacts	90.0°	45.0°	35.3°	30.0°	26.6°	24.1°	22.2°	20.7°	19.5°	18.4°