

Small Animal Imaging with Hyperpolarized ^{129}Xe Magnetic Resonance

Hirohiko IMAI,^{*†} Atsuomi KIMURA,^{**} and Hideaki FUJIWARA^{**}

^{*}*Division of Systems Informatics, Department of Systems Science, Graduate School of Informatics, Kyoto University, Yoshida-honmachi, Sakyo, Kyoto 606-8501, Japan*

^{**}*Department of Medical Physics and Engineering, Division of Health Sciences, Graduate School of Medicine, Osaka University, 1-7 Yamadaoka, Suita, Osaka 565-0871, Japan*

High-sensitivity nuclear magnetic resonance (NMR) of gaseous atoms realized by using a hyperpolarization technique is an attractive research tool used in a wide range of areas, such as physics, chemistry, material science and biomedical imaging. One of the most promising applications of this technology is the use as a noninvasive diagnostic tool for pulmonary diseases, where hyperpolarized (HP) noble gases, ^3He and ^{129}Xe , play a role as gaseous (*i.e.* inhalable) contrast agents of magnetic resonance imaging (MRI). During the last two decades, lung MRI with HP gases has become widely applicable from mouse to human. In this review we present a brief overview of recent progress made by our group in the development of HP ^{129}Xe MR measurements, while focusing on the methodology for probing pulmonary dysfunctions in mice.

Keywords Hyperpolarization, lung MRI, pulmonary function, small animal imaging

(Received September 9, 2013; Accepted October 22, 2013; Published January 10, 2014)

1 Introduction	157	5-5 Fast imaging	
2 Hyperpolarization of Noble Gas Nuclei	158	5-6 Methodology for probing lung properties	
3 HP Gas Delivery to the Lung	160	6 Application to Mouse Models of Lung Diseases	161
4 Characteristics of HP ^{129}Xe MR in the Lung	160	6-1 Mouse models of emphysema	
5 Protocols for <i>in vivo</i> HP ^{129}Xe MR Experiments	160	6-2 Histologic analysis	
5-1 Animal preparation		6-3 HP ^{129}Xe MR in healthy and diseased lung	
5-2 ^{129}Xe polarization and gas delivery		7 Dissolved-phase ^{129}Xe MRI	163
5-3 Hardware		8 Conclusions	164
5-4 NMR spectroscopy		9 References	165

1 Introduction

In vivo imaging of the lungs of small rodents has value as a

preclinical research tool for a deeper understanding of respiratory diseases as well as drug discovery.¹ Since pulmonary diseases involve morphological and functional alterations with various distribution patterns in the lung, and these alterations relate to



Hirohiko IMAI received his M.S. degree in material science from Himeji Institute of Technology in 2004 and a Ph.D. in health science from Osaka University in 2008. He served as Specially Appointed Assistant Professor in the Graduate School of Pharmaceutical Sciences, Osaka University, and concurrently in the Graduate School of Medicine, Osaka University (2008 – 2010). He is currently Specially Appointed Assistant Professor in the Graduate School of Informatics, Kyoto University (2011 –). His current research interest is the development of

detection technology for biomedical information using magnetic resonance imaging and spectroscopy.



Atsuomi KIMURA, Associate Professor of Department of Medical Physics and Engineering, Graduate School of Medicine, Osaka University, received his Ph.D. of pharmaceutical sciences from Osaka University in 1996. After he served as a Research Associate, he was promoted to Associate Professor at Osaka University in 2003. His scientific interest is the development of novel medical applications of magnetic resonance imaging and spectroscopy (MRI/MRS), and is currently focused on pulmonary functional imaging by MRI with

hyperpolarized noble gases applicable to the preclinical evaluation.

[†] To whom correspondence should be addressed.
E-mail: imai@sys.i.kyoto-u.ac.jp

one another, it is important to comprehensively and quantitatively assess these aspects for diagnosing the diseases. Although X-ray computed tomography (CT) can provide lung images with excellent discrimination of the structural morphology, it is not suited for functional analysis, especially when early detection is essential. In addition, repeated use is limited due to perspective of radiation risks. Magnetic resonance imaging (MRI), on this contrary, is known to be a powerful modality for the non-invasive analysis of functions for different organs. The lung is, however, the most challenging organ to be imaged with MRI because the low density of lung tissues, about 1/3 of that for other parenchymal organs, means that much reduced number of ^1H nuclei are available for a signal source. Moreover, a fast disappearance of the signal, known as short T_2^* decay, makes imaging more difficult as the result of large susceptibility differences induced by the complex alveolar microstructure composed of a massive amount of air-tissue interfaces.

Hyperpolarization is an exciting technique that is yielding dramatically enhanced nuclear magnetic resonance (NMR) signals of noble gas nuclei, ^3He and ^{129}Xe , despite their low densities in the gaseous state.² Direct imaging of the gas makes it possible to visualize the full process of gas transfer in the lung, *i.e.* the distribution to alveoli by air flow, uptake by capillary blood, and transport to the heart by blood flow, which are the fundamental functions of the lung, known as ventilation, gas exchange, and perfusion, respectively.

Lung MRI with hyperpolarized (HP) gases has rapidly progressed in the last two decades after the first report on MRI of HP ^{129}Xe injected into extracted mouse lung in 1994.³ Ongoing studies on the developments of elemental technologies, such as high-performance polarizer,⁴⁻⁶ imaging strategy and analyzing methodology to sensitively detect disease sites as well as to derive physiological parameters,⁷⁻⁹ have been implemented. Owing to these efforts, lung MRI with HP gases has become widely applicable from small rodents to humans, and this technology is now recognized as a promising modality for diagnosing lung diseases noninvasively and robustly. In preclinical studies, mice have been commonly used because of the possibility of using genetically altered animals and the availability of wide ranges of diseased models.¹⁰ The mice, however, may be the most challenging species to be imaged with HP gas MRI because of the small lung size, small tidal volume, and high respiration rate.

In this review, we present a brief overview of the recent progress made by our group in the development of HP ^{129}Xe MR measurements that focus on studies in mice including some elemental techniques, such as ^{129}Xe polarizer, experimental protocols, fast imaging techniques, and several strategies to quantify multiple pulmonary microstructures and functions, and the applications to a mouse model of emphysema for detecting changes of important lung properties related to the disease.

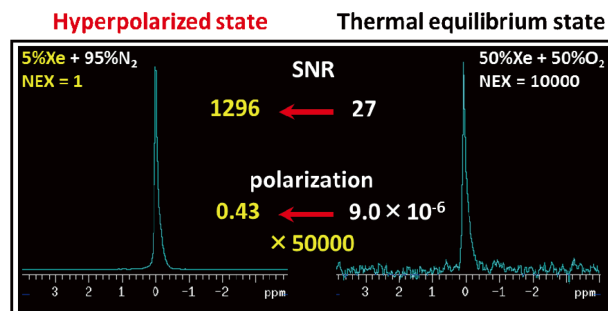
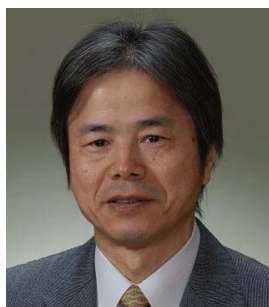


Fig. 1 NMR spectra of HP ^{129}Xe (left) and thermal equilibrium ^{129}Xe at 9.4T and room temperature (right). These spectra were acquired with a 10 ϕ NMR probe at 9.4T with a flip angle of 8°. The ^{129}Xe spectrum at thermal equilibrium state was measured for a sealed 10 ϕ glass tube including 0.5 atm of Xe gas mixed with 0.5 atm of O_2 to promote the efficiency of accumulation by reducing the longitudinal relaxation time, T_1 . The HP ^{129}Xe spectrum was measured for a 10 ϕ glass tube, while a HP gas mixture (0.05 atm of Xe and 0.95 atm of N_2) was flowed through the tube following hyperpolarization. The total acquisition time was ~ 300 ms (number of excitations: NEX = 1) for HP state and ~ 8 h (NEX = 10000) for the thermal equilibrium state.

2 Hyperpolarization of Noble Gas Nuclei

The hyperpolarization technique can overcome the difficulty of detecting the NMR signal at the gaseous state caused by its extremely low density compared to that of condensed (liquid or solid) matter. Figure 1 shows the ^{129}Xe NMR spectra at the HP state and the thermal equilibrium state. In this case, the polarization level of ^{129}Xe nuclei was enhanced by a factor of 50000. It should be noted that we take a fair amount of time for the ^{129}Xe spectrum at thermal equilibrium to be acquired (8 h in this spectrum) because a number of signal accumulations (number of excitations: NEX = 10000) are needed to obtain the spectrum with an acceptable signal-to-noise ratio (SNR), while the HP ^{129}Xe spectrum can be acquired by only a single shot (NEX = 1).

The system generating HP gases, called “polarizer”, is needed to implement HP gas MR measurements. Before MR experiments, noble gas nuclei are hyperpolarized by a polarizer, located outside of the MR scanner. The performance of the polarizer is one of the most crucial components for acquiring higher quality images. A brief outline of the principle for hyperpolarizing noble gases is as follows. The hyperpolarized state can be achieved based on the transfer of the angular momentum carried by photons. For this transfer process, alkali metal (typically rubidium, Rb) is used as an intermediate. One of the two spin states of the valence electron of a vaporized Rb atom is populated by absorbing the circularly polarized laser light having a wavelength of 795 nm (this process is called



Hideaki FUJIWARA, Invited Professor as well as Professor Emeritus at the Department of Medical Physics and Engineering, Division of Health Sciences, Graduate School of Medicine, Osaka University, was born in 1946 in Fukui Prefecture. After receiving a Bachelor (1969) degree from Nagoya Institute of Technology, he received Master (1971) and Doctor (1974) degrees from the Department of Chemistry, Faculty of Science, Graduate School of Tohoku University. He served as Educational Staff (1976 – 1977), Research Associate (1977 – 1980), Associate Professor (1980 – 1992) and Professor (1992 – 1995) in Faculty of Pharmaceutical Sciences, Osaka University, and then moved to the Department of Medical Physics and Engineering, Division of Health Sciences, Graduate School of Medicine, Osaka University (1995 – 2010). He retired from the University (2010), and is presently working as a Specially Appointed Professor or Invited Professor in the same Department (2010 –). His research interest has been nuclear magnetic resonance (NMR) from his bachelor study throughout, and now his main interest is magnetic resonance imaging (MRI), especially hyperpolarized noble gas MRI.

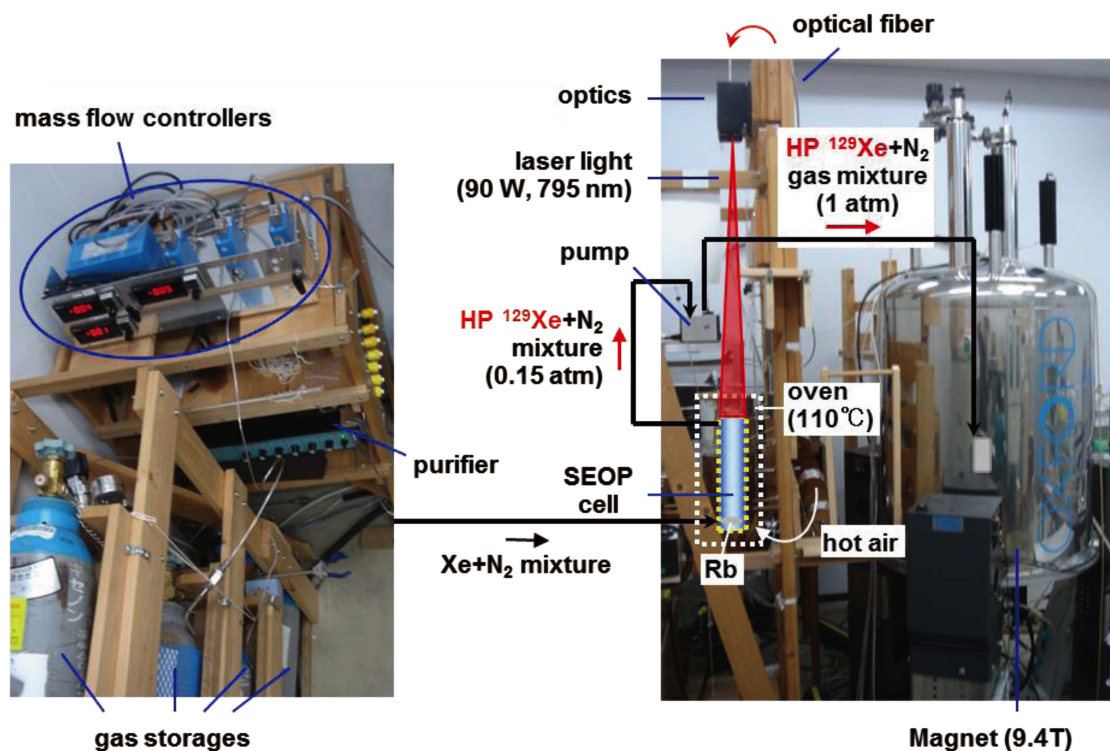


Fig. 2 Experimental setup for a low-pressure and flow-through ^{129}Xe polarizer. ^{129}Xe was polarized by Rb- ^{129}Xe spin-exchange optical pumping (SEOP) at 0.15 atm, compressed to 1 atm by a diaphragm pump, and then flowed into the MR scanner. This cycle runs continuously.

optical pumping). Subsequent collisions between spin-polarized Rb atoms and noble-gas atoms transfer the spin-polarization from the Rb electron to noble-gas nuclei (this process is called spin exchange). Although the physics of hyperpolarization is somewhat complicated, noble gases can be hyperpolarized without serious difficulty.

Here, we describe our home-built polarizer. As our first generation of the polarizer, we built a simplified and easy-to-use polarizer working at atmospheric pressure throughout the system,¹¹ allowing the continuous polarization and delivery of HP ^{129}Xe with physiologically acceptable pressure without additional components, which have a possibility to cause the destruction of polarization (depolarization). This polarizer consists of only a few components: gas storage, gas purifier, flow controller, spin-exchange optical pumping (SEOP) cell, which is a glass container enclosing Rb, an oven for heating the SEOP cell (*i.e.* increasing Rb vapor density), a high-power laser diode, and optics.

The NMR signal intensity is proportional to the number of ^{129}Xe atoms and their polarization level. Unfortunately, these two factors contradict each other, *i.e.* increasing partial pressure of Xe in the SEOP cell leads to a reduction of the ^{129}Xe polarization. To solve this problem, we built a second generation of our polarizer, in which the ^{129}Xe nuclei are polarized at 0.15 atm of the total pressure in the SEOP cell; then, polarized gas is compressed up to atmospheric pressure by a diaphragm pump without serious depolarization (Fig. 2).¹² This polarizer can generate HP ^{129}Xe with an enhanced SNR as a factor of 2 compared to the first generation, owing to a polarization enhancement without reducing the number of Xe atoms in the delivering gas.

To investigate the utility of our polarizer for *in vivo* study, ^{129}Xe spectra and images were acquired from mouse lung under

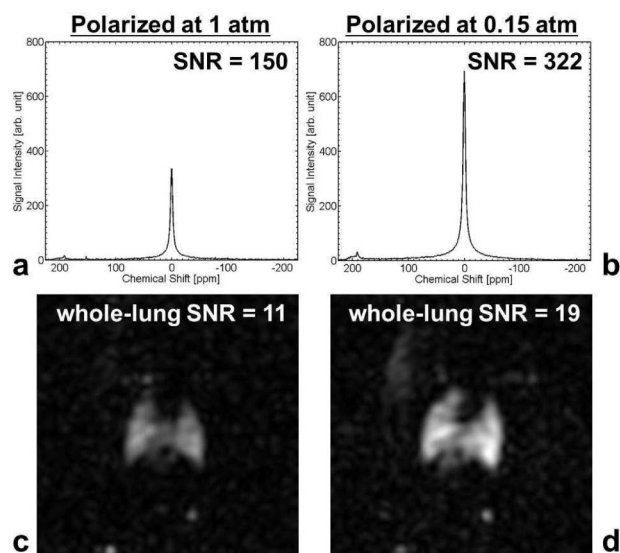


Fig. 3 HP ^{129}Xe spectra (upper row) and images (lower row) obtained from the lung of a single mouse. Xe gas was polarized at a pressure of 1 atm (left column) and 0.15 atm (right column). Other conditions, such as the gas flow rate and acquisition parameters, were the same in the experiments under both pressures. Eight spectra as well as sixteen images were averaged so that the oscillation in signal intensity caused by the effect of spontaneous breathing was averaged. Images were acquired using a 2D spoiled gradient echo sequence in a time of 2.6 min, field of view (FOV) = $50 \times 50 \text{ mm}^2$, matrix = 32×32 , repetition time (TR)/echo time (TE) = 300 ms/1.5 ms.

the two polarizing conditions; Xe gas was polarized at a total

pressure of 1 and 0.15 atm. The SNR enhancement by a factor of ~ 2 was observed in the spectra as well as images acquired using HP ^{129}Xe polarized at 0.15 atm (Fig. 3).

3 HP Gas Delivery to the Lung

In contrast to thermal equilibrium magnetization, for which longitudinal component recovers to the initial equilibrium state with a longitudinal relaxation time T_1 when some perturbation is applied that changes the magnetization, HP magnetization is impossible to be recovered to the initial HP state once taken out of the SEOP cell: its longitudinal component, which is extraordinary larger than the thermal equilibrium value, always decays toward the thermal equilibrium one when removed from the SEOP cell. Fortunately, the T_1 relaxation time of HP gas is quite long (many hours or more), and thus a loss of magnetization from T_1 decay during gas transport from the SEOP cell to the storage reservoir or subject is not substantial. However, it should be kept in mind that the T_1 relaxation time strongly depends on the environment where HP gas is placed.^{13,14} In the experiments needed to acquire data during prolonged gas delivery from the storage, one should consider the effect of depolarization of HP magnetization in storage. Also, paramagnetic oxygen leads to an increase in relaxation;¹⁵ for instance, the T_1 value drops to ~ 30 s for HP gas in a normoxic mixture (*i.e.* mixed gas containing 20% O_2).⁷

In animal studies, lung MRI with HP gases is usually performed on intubated and respirated animals with a respiratory device.^{16,17} This approach has advantages since respiratory-synchronized image acquisition with gas delivery is allowed, where respiratory parameters, such as the respiration period, tidal volume and peak inspiratory pressure, are controlled at constant levels, making high-quality image acquisition possible. In addition, the extra consumption of precious HP gases is restricted. On the negative side, the respiration is forced, and not under natural conditions, sometimes resulting in a side effect of ventilator-associated lung injury; thus, the lung structure and functions depend on the ventilator settings, leading to the possibility of missing information about the symptoms of the respiratory diseases of the individual animal. On the other hand, measurements under spontaneous breathing are fully noninvasive, making it possible to perform long-term and repeated measurements on the same individual under natural respiration.^{18,19}

Since a series of MR measurements in the same animal is needed to comprehensively assess multiple quantities related to the lung properties, and follow their time course, a stable and continuous supply of HP gases and noninvasiveness of the protocol are essential for relatively prolonged measurements. For this purpose, it is useful to adopt the protocol of spontaneous breathing of continuously delivered HP gases, unlike that of mechanically controlled ventilation with batch production used in the majority of conventional studies. The continuous delivery of HP gases can avoid considering the depolarization of HP magnetization in storage, making it possible to assess the lung properties quantitatively. The use of HP ^{129}Xe gas can realize a semi-permanent delivery of HP gas to the subject because a continuous polarization and delivery cycle is possible with the aid of its short polarization time ($\sim$ minutes), unlike ^3He ($>$ several hours).

In the delivery of Xe gas to the lung, the concentration of Xe in inhaled gas should be considered because Xe becomes a general anesthetic when its alveolar concentration is in the range of 70%.²⁰

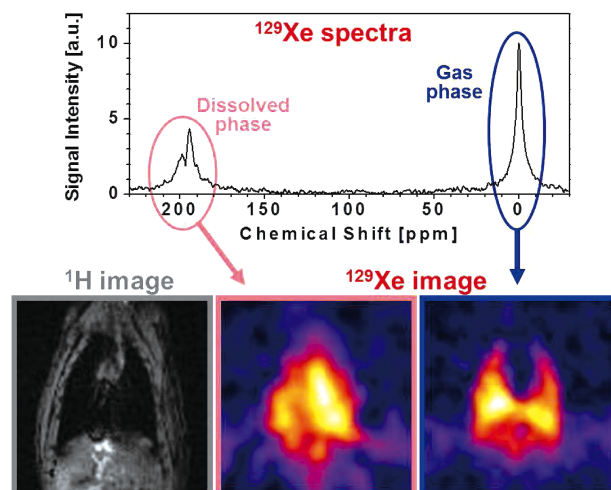


Fig. 4 HP ^{129}Xe MR spectra and images obtained from the mouse chest. The large peak at 0 ppm is the gas signal in lung airspace (gas phase), and peaks around 200 ppm arise from ^{129}Xe dissolved in lung tissue and blood (dissolved phase). The gas- and dissolved-phase ^{129}Xe images were acquired with a frequency-selective excitation centered at 0 and 197 ppm, respectively. A ^1H image is also shown. HP ^{129}Xe images were acquired using a 2D radial sequence in 32 min for gas phase and 41 min for dissolved phase, FOV = $50 \times 50 \text{ mm}^2$, matrix = 64×64 , TR/TE = 300 ms/0.3 ms (Reprinted from Ref. 22).

4 Characteristics of HP ^{129}Xe MR in the Lung

Although HP ^{129}Xe MRI suffers an inherent loss in signal compared to ^3He because of its low gyromagnetic ratio ($\gamma_{^{129}\text{Xe}}/\gamma_{^3\text{He}} = \sim 1/3$) and obtainable polarization, HP ^{129}Xe offers additional attractive characteristics over ^3He ; namely it is more soluble in a variety of media, and its NMR chemical shift exhibits a wide range, reflecting the physicochemical property of the medium.²¹ Once HP ^{129}Xe gas is inhaled into the lung airspace, it diffuses into the lung tissues and blood across the blood-gas barrier, and resonates at different frequencies ~ 200 ppm apart from the gas signal at 0 ppm, which allows HP ^{129}Xe residing in lung airspaces (gas-phase ^{129}Xe) and dissolved in lung parenchyma and blood (dissolved-phase ^{129}Xe) to be excited and detected selectively or simultaneously, as shown in Fig. 4.²²

5 Protocols for *in vivo* HP ^{129}Xe MR Experiments

In this chapter, we describe typical protocols for HP ^{129}Xe MR experiments in mice, which are routinely implemented by our group.

5-1 Animal preparation

All experimental procedures and animal care conform to the guidelines of Osaka University. Typically, male ddY mice are used. The age and body weight are 6–8 weeks and around 30 g, respectively. Each mouse is anesthetized with 2% isoflurane, and a plastic mouth mask, to which three polyethylene tubes are connected for HP gas delivery, O_2 delivery and exhaust, is attached before being placed in the MR scanner. For respiratory gated imaging, a respiratory sensor is fixed to the abdomen. The body temperature is maintained with warm water circulating through a rubber tube placed on the abdomen.

5-2 ^{129}Xe polarization and gas delivery

^{129}Xe is polarized by Rb- ^{129}Xe SEOP with a home-built flow-through polarizer, as described in the previous chapter. Mixed gas of 70% Xe (natural abundance of 26.4% ^{129}Xe) + 30% N_2 is used as a source of the HP gas. The Xe gas mixture is polarized at 0.15 atm, and then compressed to atmospheric pressure by a diaphragm pump for delivery to the mask. This cycle is performed continuously with ^{129}Xe polarization of approximately 10%. A hyperpolarized ^{129}Xe gas mixture and O_2 gas is flowed into the mask at a rate of 35 and 9 mL/min, respectively. O_2 gas is mixed with a HP ^{129}Xe gas mixture in the mask just before the mice breathe these gases spontaneously. The percentage of O_2 and Xe in inhaled gas is 20 and 56%, respectively.

5-3 Hardware

All MR measurements are performed with an Agilent Unity INOVA 400WB (Agilent Technologies, Inc., Santa Clara, CA) installed with VNMR 6.1C software. A 9.4-T vertical magnet with a bore width of 89 mm is used. A self-shielded gradient probe with an inner diameter of 34 mm is used with Litz volume radiofrequency (RF) coils tunable to Larmor frequencies of ^{129}Xe (110.6 MHz) and ^1H (399.6 MHz) (Clear Bore DSI-1117; Doty Scientific, Inc., Columbia, SC).

5-4 NMR spectroscopy

Before each MR measurement, the ^{129}Xe NMR spectra from the mouse lung are confirmed for the appearance of gas-phase and dissolved-phase ^{129}Xe signals, together with their resonance frequency for setting the spectroscopy or imaging parameters. Also, the flip angle of the RF pulse is calibrated using NMR spectroscopy. Whether ^{129}Xe magnetization in both gas-phase and dissolved-phase compartments has reached the steady state is confirmed by applying RF pulses repetitively during spontaneous inhalation of the HP ^{129}Xe gas. When the mouse inhales HP ^{129}Xe gas by spontaneous respiration, ^{129}Xe signal intensities of both phases gradually increase. This wash-in process is completed approximately 10 s after the beginning of inhalation, followed by a steady-state process that continues until the supply of HP ^{129}Xe gas is stopped. A detailed description of this process was reported previously, including theoretical models, experimental results, and analysis.²³ MR measurements are performed after confirming the steady state in ^{129}Xe signals in both of the gas and dissolved phases.

5-5 Fast imaging

Gas-phase ^{129}Xe images of the lung are acquired with a fast imaging sequence of balanced steady-state free precession (bSSFP), which can give high-SNR images within a very short time.²⁴ Highly efficient data collection with bSSFP is useful for HP gas MRI, which is required to conserve the non-equilibrium and non-renewable HP magnetization during image acquisition.²⁵ By using bSSFP, ^{129}Xe lung images of end-inspiratory as well as end-expiratory phases was obtained from spontaneously breathing mice with a 105 ms per image (Figs. 5a and 5b), where the respiratory sensor is used to acquire images at a given respiratory phase. Furthermore, the compressed sensing (CS) approach can be combined with bSSFP. Compressed sensing is a novel nonlinear reconstruction paradigm, which is extremely useful for improving the temporal resolution of MRI by reconstruction from significantly undersampled datasets with high accuracy.²⁶ Using bSSFP combined with CS, ^{129}Xe lung images were successfully acquired with a 66 ms per image without any loss of SNR and spatial resolution (Figs. 5c and 5d). The acquisition time of 66 ms is a moderate time for

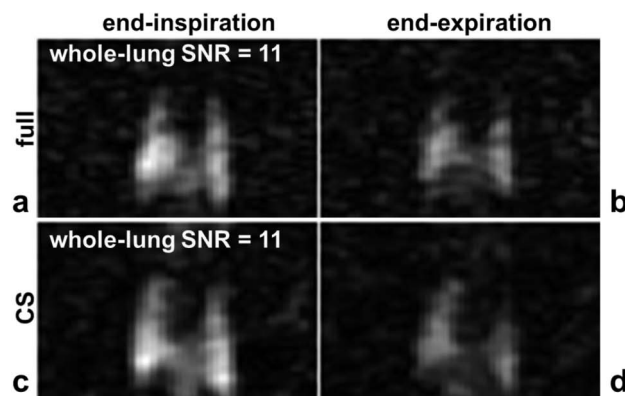


Fig. 5 HP ^{129}Xe images in end-inspiratory phase (left column) and end-expiratory phase (right column) reconstructed from acquired data of 32 full phase encode lines with conventional 2D fast Fourier transformation (upper row) and from acquired data of 20 sparse phase encode lines with CS algorithm (lower row). Images were acquired using a 2D single-shot balanced steady-state free precession (bSSFP) sequence in 104 ms (a, b) and 66 ms (c, d), FOV = $40 \times 25.6 \text{ mm}^2$, matrix = 32×32 , TR/TE = 3.2 ms/1.6 ms.

imaging certain respiratory phases in mice because a single respiratory cycle for mice is about 480 ms.²⁷

5-6 Methodology for probing lung properties

The chemical shift saturation recovery (CSSR) method²⁸ is used for a quantitative evaluation of the alveolar septal thickness, h , septum-to-alveolar volume ratio, V_s/V_a , and the transit time of blood through the gas-exchange region, τ . In this method, the recovery process of the NMR signal of ^{129}Xe dissolved in alveolar septa (dissolved-phase ^{129}Xe) is measured after selective saturation (Fig. 6a). This dynamics reflects the amount of ^{129}Xe diffused from the alveolar airspace to the septum during the delay time from saturation. The data are analyzed using a simple model of ^{129}Xe septal uptake²⁹ and h , V_s/V_a , and τ are derived. Further details regarding CSSR measurements and analysis have been provided previously.³⁰

For evaluating ventilation, two lung images of gas-phase ^{129}Xe in end-inspiratory and end-expiratory states are acquired separately (Fig. 6b). A ventilation parameter, r_s , which is defined as the fraction of the gas-phase signal that replaces with fresh HP ^{129}Xe gas within a single breath (difference between ^{129}Xe signal intensities of lung images at end-inspiration and end-expiration normalized by the signal at end-inspiration), is calculated pixel by pixel. Gas exchange is evaluated by the xenon polarization transfer contrast (XTC) strategy.³¹ As a quantitative parameter for the gas exchange, the depolarization, f_D , which is defined as the fraction of the gas-phase signal that disappears as a result of the inversion of dissolved-phase magnetization and subsequent exchange, is calculated pixel by pixel; f_D is evaluated from two images of gas-phase ^{129}Xe acquired at end-expiration with and without inversion pre-pulses (Fig. 6b). Further details regarding respiratory gated fast imaging for evaluating ventilation and gas exchange in spontaneous breathing mice were provided previously.³²

6 Application to Mouse Models of Lung Diseases

Mouse models of pulmonary diseases, such as emphysema,^{33,34} asthma,^{35,36} inflammation,³⁷ and cancer,^{33,38} have been employed

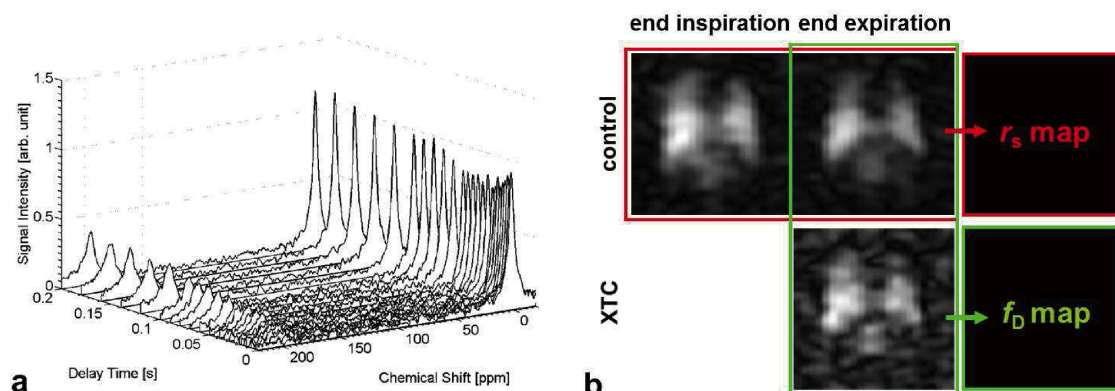


Fig. 6 a. Example of a series of HP ^{129}Xe spectra obtained from the mouse chest on ^{129}Xe dynamic spectroscopy. Two RF pulses (90° flip angle, centered at dissolved-phase frequency (197 ppm)) separated by variable delay times are applied to destroy ^{129}Xe magnetization in the septa and to generate free induction decay (FID) signals from ^{129}Xe diffused from the alveolar airspace to the septum during the delay time (Reprinted from Ref. 30). b. Examples of HP ^{129}Xe control images in end-inspiratory and end-expiratory phases, and XTC image in end-expiratory phase. A ventilation parameter, r_s , which is defined as $r_s = (1 - S_{\text{end-inspiration}}/S_{\text{end-expiration}}) \times 100$, was calculated pixel by pixel, where $S_{\text{end-expiration}}$ and $S_{\text{end-inspiration}}$ are the signal intensities of ^{129}Xe lung images at end-expiration and end-inspiration, respectively. In the XTC measurements, four dissolved-phase selective inversion pulses ($N = 4$) were repetitively applied with a delay time of 20 ms for labeling dissolved-phase magnetization before gas-phase ^{129}Xe imaging. The gas-exchange parameter, f_D , was calculated from two images of gas-phase ^{129}Xe acquired at end-expiration with (control image) and without (XTC image) inversion pre-pulses by the following equation: $f_D = (1 - \sqrt[N]{S_{\text{XTC}}/S_{\text{control}}}) \times 100$, where S_{XTC} and S_{control} are the signal intensities of XTC and control images, respectively.

to assess functional or structural changes using HP ^3He MRI, where the ventilation property and apparent diffusion coefficient are measured. On the other hand, the application of HP ^{129}Xe to mouse models has been limited to our studies of emphysema^{30,32,39–41} and cancer.²³ In this chapter, our studies on elastase-induced emphysema models are described.

6.1 Mouse models of emphysema

Mouse models of emphysema are prepared by endotracheal administration of elastase (porcine pancreatic; activity, 140 units/mg). Mice are instilled a solution of elastase in saline at a dosage of 300 units/kg body weight; for example, mice received 0.15 mL at 60 units/mL elastase. MR measurements are typically performed 2 weeks after the administration. During this period, emphysema-like alveolar wall destruction is induced.

6.2 Histologic analysis

Although the pulmonary function cannot be derived from histological assessments, some parameters related to the morphological aspects of the lung can be compared between the histology and HP gas MR for assessing the validation of the HP gas MR methodology. After the MR experiments, mice are killed with a lethal dose of sodium pentobarbital. Lungs are instilled *in situ* with 10% formalin at 25 cm H_2O , extracted and then immersed in 10% formalin. The lungs are processed for conventional histology and stained hematoxylin and eosin (H&E). The mean linear intercepts (L_m), which is the gold standard for evaluating the degree of airspace enlargement caused by emphysematous alveolar tissue destruction, is calculated. In addition, alveolar septal thickness ($h_{\text{histology}}$) and the septum-to-alveolar volume ratio ($V_s/V_{a,\text{histology}}$) are assessed.^{30,32}

6.3 HP ^{129}Xe MR in healthy and diseased lung

A series of MR measurements were performed in the same animal to evaluate emphysema using the following parameters: the alveolar septal thickness, h , and the septum-to-alveolar volume ratio, V_s/V_a , as pulmonary structures, and ventilation, r_s , gas exchange, f_D , and perfusion, τ , as pulmonary functions, and then compared with healthy and elastase-induced emphysematous lungs.

Figure 7 shows representative results of HP ^{129}Xe MR measurements from control and elastase-treated mice. Figure 7a shows a plot of $F(t)$ and the fitted curve observed from CSSR measurements. The dynamics of the recovery curve of $F(t)$ varied by mouse. The initial buildup of the recovery curve for the elastase-treated mouse was slower than that of the control mouse, reflecting the increase of the septal thickness (h). Furthermore, the values of $F(t)$ for the elastase-treated mouse were significantly lower than that of the control mouse throughout the delay time, reflecting a reduction of the volume ratio (V_s/V_a). Figures 7b and 7c show ventilation (r_s) and gas exchange (f_D) maps, respectively. The regions of lower r_s and f_D for the elastase-treated mouse were clearly found in the r_s map and the f_D map, respectively. The histology of the lungs from two mice is shown in Fig. 7d. A breakdown of the alveolar walls was clearly seen in the elastase-treated mouse. These tendencies were consistent for all mice that we measured.^{30,32,41} A comparison of all parameters between control and elastase-treated groups is summarized in Fig. 8. When the body weight was compared between the two groups, significant differences were found during 2 weeks after elastase administration. The septal thickness in the elastase-treated group was significantly thicker than that of the control group, reflecting the development of lung inflammation. The volume ratio in the elastase-treated group was significantly lower than that of the control group, reflecting the destruction of the alveolar wall. On the other

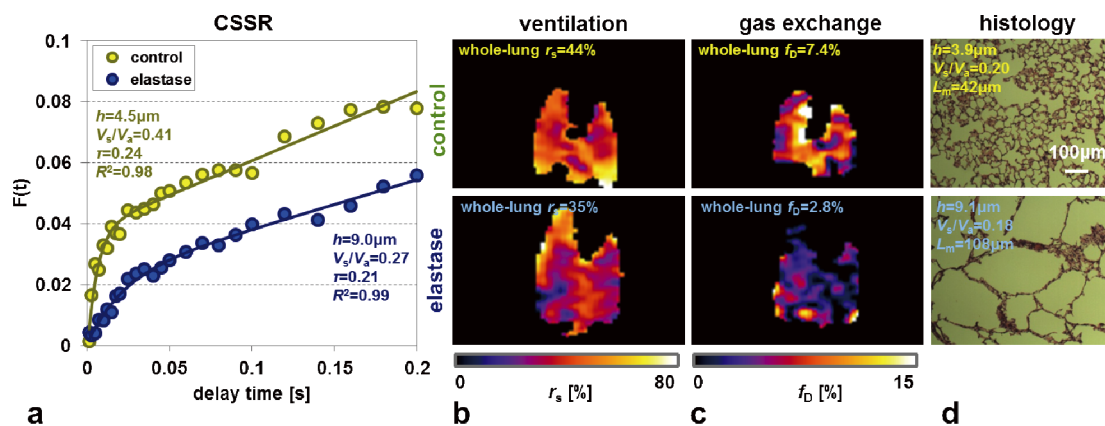


Fig. 7 Examples of CSSR results (a) and parametric maps of ventilation (b) and gas-exchange maps (c) for control (upper row) and elastase-treated mice (lower row). H&E-stained histology is also shown (d) (Reprinted from Ref. 32). In the CSSR measurements, the ratio of HP ^{129}Xe magnetization in the dissolved phase, M_{diss} , at time t to that in the gas phase, M_{gas} , at time $t = 0$, $F(t)$, was calculated from HP ^{129}Xe signal intensity by the following equation: $F(t) = M_{\text{diss}}(t)/M_{\text{gas}}(0) = s_{\text{diss}}(t)/s_{\text{gas}}(0) \cdot \sin \alpha_{\text{gas}} / \sin \alpha_{\text{diss}}$, where s_{diss} , s_{gas} , α_{diss} , and α_{gas} are the signal intensity for dissolved phase and gas phase, and the flip angle for dissolved phase and gas phase, respectively. In this measurement, α_{diss} and α_{gas} were 90° and 4.9° , respectively. s_{diss} and s_{gas} were obtained from the integrals of gas-phase and dissolved-phase ^{129}Xe spectra in the dynamic study. The data were analyzed by least-squares fitting to the simple model for ^{129}Xe uptake to derive physiological quantities.

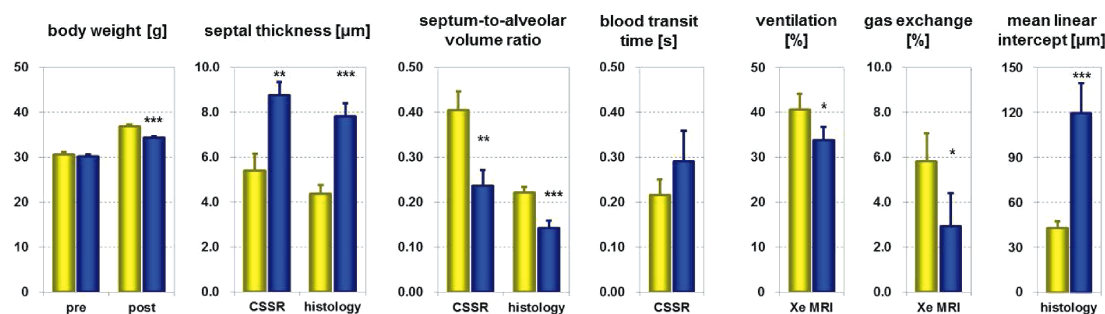


Fig. 8 Comparison of all parameters between the control (yellow bar) and elastase-treated (blue bar) groups. From left to right: body weight, septal thickness, h , septum-to-alveolar volume ratio, V_s/V_a , blood transit time, τ , ventilation parameter, r_s , gas-exchange parameter, f_D , and mean linear intercept, L_m . Data are the mean values for all mice from the control ($n = 4$) and elastase-treated ($n = 3$) groups, with the standard deviation (SD) displayed as an error bar. Statistical significance of the values for all parameters between control and elastase-treated groups was determined by Student's t test. p values of less than 0.05 were considered to be significant. Asterisk indicates significant difference: * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$.

hand, the transit time of blood was not significant between the two groups. Statistically significant differences between the two groups were found for L_m , $h_{\text{histology}}$, and $V_s/V_{a_{\text{histology}}}$. These structural parameters derived from histological analysis were very consistent with those from HP ^{129}Xe -based methods. From imaging studies, significant differences were found for the ventilation parameter, r_s , and gas-exchange parameter, f_D , reflecting the ventilation and gas-exchange dysfunctions, respectively.

7 Dissolved-phase ^{129}Xe MRI

The appearance of the dissolved-phase ^{129}Xe signal is a unique aspect that has been utilized for assessing pulmonary gas exchange and perfusion processes. It has been reported that

several physiological quantities related to these processes were globally evaluated from measurements of alveolar gas uptake dynamics using CSSR in animals^{30,42-45} and humans.^{39,46,47} Whole-lung spectroscopy, however, limits the assessments to be accepted as global measures without any information about the spatial distribution in the lung. An extension of spectroscopy-based methods to imaging-based methods is an important step toward identifying a region with impaired function with higher sensitivity and evaluation of its variation in the whole lung. Imaging the dissolved-phase ^{129}Xe is difficult, because its signal intensity is very weak due to the small number of dissolved ^{129}Xe spins; that is, the low density of lung tissues and solubility of Xe in lung tissues give only a few percent of the signal from gaseous ^{129}Xe . Despite the difficulty, recently, dissolved-phase ^{129}Xe MRI has become progressively feasible from mouse to human.^{40,45,48-53}

Here, we introduce our studies on dissolved-phase ^{129}Xe MRI in mice. Figure 9 shows the imaging of alveolar gas uptake dynamics. Although the delay time (repetition time, TR, in this imaging case) is only three points, this is a successful example of the extension of CSSR spectroscopy to CSSR imaging. The signal intensity of the dissolved-phase image was increased with increasing TR, reflecting the Xe uptake process due to diffusion from airspace to tissue and blood at short TR, followed by transfer by blood flow at long TR. At a TR of 200 ms, the ^{129}Xe signal in the upper region outside the left lobe of lung (arrow), which might originate from ^{129}Xe dissolved in the pulmonary vein, as confirmed from the ^1H image in this region, can be seen, while the signal in this region is not observable at a TR of 50 ms. Figures 10a and 10b show examples of dissolved- and gas-phase ^{129}Xe images obtained from control and elastase-treated mice. It is noted that the dissolved-phase ^{129}Xe image in elastase-treated mouse shows a low signal intensity, whereas that of the gas-phase image is slightly higher than that in control

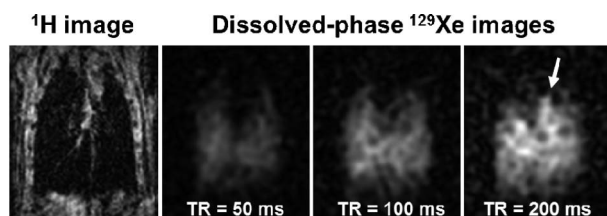


Fig. 9 Imaging of ^{129}Xe uptake dynamics acquired using an ultra-short echo time (UTE) sequence⁵⁴ with variable repetition times (TRs) and 90° flip angle. Left to right: ^1H image, ^{129}Xe images acquired with TR of 50, 100, 200 ms. HP ^{129}Xe Images were acquired in 11 min (TR = 50 ms), 21 min (TR = 100 ms) and 43 min (TR = 200 ms), FOV = $40 \times 40 \text{ mm}^2$, matrix = 64×64 , TE = 0.2 ms (Reprinted from Ref. 40).

mice, reflecting the reduced uptake efficiency due to alveolar tissue destruction. To quantitatively assess the uptake efficiency, we defined the parameter F as the relative amount of dissolved-phase magnetization of ^{129}Xe diffused into septa within 50 ms. The F map corresponds to mapping the value of F (50 ms) in CSSR measurements. The distribution of the uptake efficiency in the lung was successfully visualized, as shown in Fig. 10c. The mean F value was significantly reduced in elastase-treated mice, and exhibits a significant correlation with the histologically derived alveolar surface-to-volume ratio.⁴⁰

Unfortunately, in mice, the two types of ^{129}Xe dissolved-phase signals for red blood cells (RBCs) and plasma/parenchymal tissue are not separated, although they are clearly separated by 10–20 ppm from each other in animals other than mice. Separate observation of the two dissolved-phase peaks in mice, if possible, would surely be useful to characterize gas exchange over a wide range of pulmonary diseases. More recently, a HP ^{129}Xe study using transgenic mice expressing human hemoglobin appeared,⁴⁵ where the transgenic mice displayed two dissolved-phase peaks as being similar to that observed in humans, and the separate dissolved ^{129}Xe imaging were demonstrated, depicting gas uptake by plasma/parenchymal tissue and RBCs were demonstrated.

8 Conclusions

This article has reviewed our recent progress in HP ^{129}Xe MR measurements in mouse lung. By applying several methodologies, a wider variety of physiological parameters were evaluated in the same animal: ventilation, gas exchange, perfusion, septal thickness, and septum-to-alveolar volume ratio. In addition, it was shown that the MRI of HP ^{129}Xe dissolved in pulmonary tissues and blood is useful for the regional evaluation of pulmonary gas exchange. The combination of dissolved- and gas-phase ^{129}Xe MRI will provide

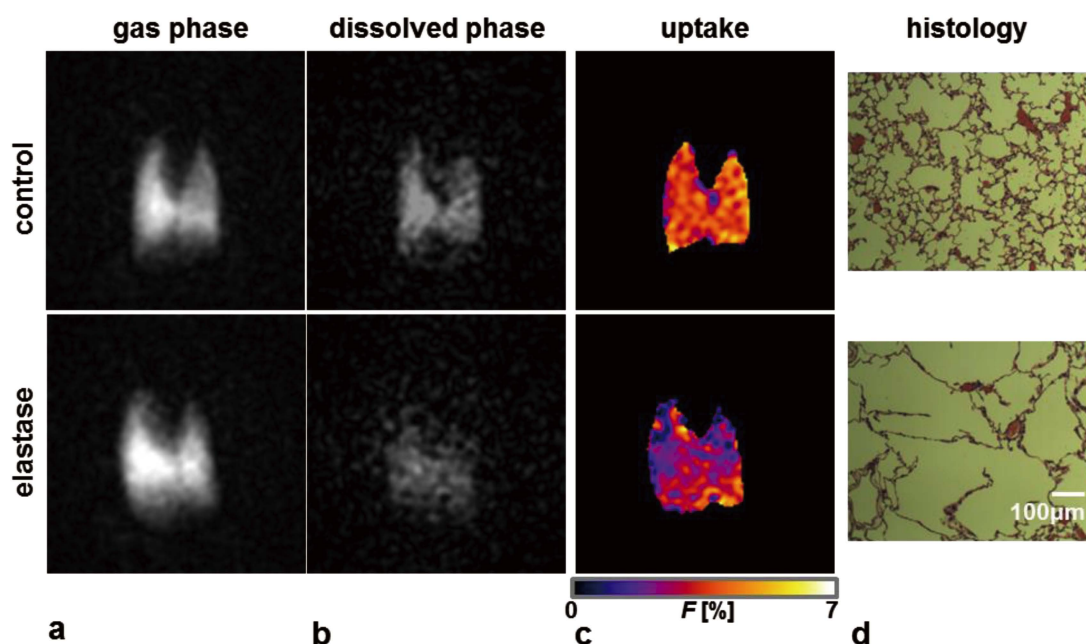


Fig. 10 Examples of gas-phase (a) and dissolved-phase (b) ^{129}Xe images acquired from control (upper row) and elastase-treated mice (lower row), and F maps calculated from gas- and dissolved-phase images. H&E-stained lung histology is also shown (Reprinted from Ref. 40).

new opportunities to comprehensively evaluate the pulmonary gas transport process quantitatively in terms of the three essential components of ventilation, gas-exchange, and perfusion, leading this technology to a prospective powerful modality for evaluating, monitoring, and understanding lung diseases.

The above-mentioned methodologies are also applicable to porous materials with nano-size pores that resemble the lung in microstructure.²⁸ Further, a ¹²⁹Xe NMR study of porous materials, such as reported previously,^{55,56} can be greatly extended by incorporating the hyperpolarized spins.

9 References

1. C. J. A. van Echteld and N. Beckmann, *J. Pharmacol. Exp. Ther.*, **2011**, 337, 335.
2. T. G. Walker and W. Happer, *Rev. Mod. Phys.*, **1997**, 69, 629.
3. M. S. Albert, G. D. Cates, B. Driehuys, W. Happer, B. Saam, C. S. Springer Jr., and A. Wishnia, *Nature*, **1994**, 370, 199.
4. B. Driehuys, G. D. Cates, E. Miron, K. Sauer, D. K. Walter, and W. Happer, *Appl. Phys. Lett.*, **1996**, 69, 1668.
5. I. C. Ruset, S. Ketel, and F. W. Hersman, *Phys. Rev. Lett.*, **2006**, 96, 053002.
6. P. Nikolaou, A. M. Coffey, L. L. Walkup, B. M. Gust, N. Whiting, H. Newton, S. Barcus, I. Muradyan, M. Dabaghyan, G. D. Moroz, M. S. Rosen, S. Patz, M. J. Barlow, E. Y. Chekmenev, and B. M. Goodson, *Proc. Natl. Acad. Sci. U. S. A.*, **2013**, 110, 14150.
7. S. B. Fain, F. R. Korosec, J. H. Holmes, R. O'Halloran, R. L. Sorkness, and T. M. Grist, *J. Magn. Reson. Imaging*, **2007**, 25, 910.
8. J. P. Mugler III and T. A. Altes, *J. Magn. Reson. Imaging*, **2013**, 37, 313.
9. D. M. L. Lilburn, G. E. Pavlovskaya, and T. Meersmann, *J. Magn. Reson.*, **2013**, 229, 173.
10. N. Beckmann, R. Kneuer, H-U. Gremlich, H. Karmouty-Quintana, F. X. Blé, and M. Müller, *NMR Biomed.*, **2007**, 20, 154.
11. J. Fukutomi, E. Suzuki, T. Shimizu, A. Kimura, and H. Fujiwara, *J. Magn. Reson.*, **2003**, 160, 26.
12. H. Imai, J. Fukutomi, A. Kimura, and H. Fujiwara, *Concepts Magn. Reson., Part B*, **2008**, 33B, 192.
13. B. C. Anger, G. Schrank, A. Schoeck, K. A. Butler, M. S. Solum, R. J. Pugmire, and B. Saam, *Phys. Rev. A*, **2008**, 78, 043406.
14. H. E. Möller, Z. I. Cleveland, and B. Driehuys, *J. Magn. Reson.*, **2011**, 212, 109.
15. C. J. Jameson, A. K. Jameson, and J. K. Hwang, *J. Chem. Phys.*, **1988**, 89, 4074.
16. B. T. Chen, A. T. Yordanov, and G. A. Johnson, *Magn. Reson. Med.*, **2005**, 53, 69.
17. J. Nouls, M. Fanarjian, L. Hedlund, and B. Driehuys, *Concepts Magn. Reson., Part B*, **2011**, 39B, 78.
18. M. Zurek and Y. Crémillieux, in "In vivo NMR Imaging, Methods in Molecular Biology", ed. L. Schroder and C. Faber, **2011**, Chap. 4, Springer Science+Business Media, LLC, New York, 459.
19. K. Mosbah, V. Stupar, Y. Berthezène, N. Beckmann, and Y. Crémillieux, *Magn. Reson. Med.*, **2010**, 63, 1669.
20. S. C. Cullen and E. G. Gross, *Science*, **1951**, 113, 580.
21. B. M. Goodson, *J. Magn. Reson.*, **2002**, 155, 157.
22. M. Narazaki, A. Kimura, T. Wakayama, H. Imai, and H. Fujiwara, *Magn. Reson. Med. Sci.*, **2011**, 10, 149.
23. H. Imai, A. Kimura, T. Ito, and H. Fujiwara, *Eur. J. Radiol.*, **2010**, 73, 196.
24. K. Scheffler and S. Lehnhardt, *Eur. Radiol.*, **2003**, 13, 2409.
25. F. Imai, R. Kashiwagi, H. Imai, S. Iguchi, A. Kimura, and H. Fujiwara, *Magn. Reson. Med. Sci.*, **2011**, 10, 33.
26. M. Lustig, D. Donoho, and J. M. Pauly, *Magn. Reson. Med.*, **2007**, 58, 1182.
27. M. Narazaki, T. Wakayama, H. Imai, A. Kimura, and H. Fujiwara, *Magn. Reson. Med. Sci.*, **2006**, 5, 119.
28. J. P. Butler, R. W. Mair, D. Hoffmann, M. I. Hrovat, R. A. Rogers, G. P. Topulos, R. L. Walsworth, and S. Patz, *J. Phys.: Condens. Matter*, **2002**, 14, L297.
29. S. Patz, I. Muradyan, M. I. Hrovat, M. Dabaghyan, G. R. Washko, H. Hatabu, and J. P. Butler, *New J. Phys.*, **2011**, 13, 015009.
30. H. Imai, A. Kimura, S. Iguchi, Y. Hori, S. Masuda, and H. Fujiwara, *Magn. Reson. Med.*, **2010**, 64, 929.
31. K. Ruppert, J. F. Mata, J. R. Brookeman, K. D. Hagspiel, and J. P. Mugler III, *Magn. Reson. Med.*, **2004**, 51, 676.
32. H. Imai, A. Kimura, Y. Hori, S. Iguchi, T. Kitao, E. Okubo, T. Ito, T. Matsuzaki, and H. Fujiwara, *NMR Biomed.*, **2011**, 24, 1343.
33. J. P. Dugas, J. R. Garbow, D. K. Kobayashi, and M. S. Conradi, *Magn. Reson. Med.*, **2004**, 52, 1310.
34. M. Ishii, K. Emami, Y. Xin, A. Barulic, C. J. Kotzer, G. A. Logan, E. Chia, J. P. MacDuffine-Woodburn, J. Zhu, S. Pickup, N. Kuzma, S. Kadlecck, P. L. Podolin, and R. Rizi, *J. Appl. Physiol.*, **2012**, 112, 135.
35. B. Driehuys, J. Walker, J. Pollaro, G. P. Cofer, N. Mistry, D. Schwartz, and G. A. Johnson, *Magn. Reson. Med.*, **2007**, 58, 893.
36. A. C. Thomas, S. S. Kaushik, J. Nouns, E. N. Potts, D. M. Slipetz, W. M. Foster, and B. Driehuys, *J. Appl. Physiol.*, **2012**, 112, 1437.
37. L. E. Olsson, A. Smailagic, P-O. Önnervik, and P. D. Hockings, *J. Magn. Reson. Imaging*, **2009**, 29, 977.
38. R. T. Branca, Z. I. Cleveland, B. Fubara, C. S. Kumar, R. R. Maronpot, C. Leuschner, W. S. Warren, and B. Brieuhs, *Proc. Natl. Acad. Sci. U. S. A.*, **2010**, 107, 3693.
39. Y. Hori, A. Kimura, T. Wakayama, M. Kitamoto, F. Imai, H. Imai, and H. Fujiwara, *Magn. Reson. Med. Sci.*, **2009**, 8, 73.
40. S. Iguchi, H. Imai, Y. Hori, J. Nakajima, A. Kimura, and H. Fujiwara, *Magn. Reson. Med.*, **2013**, 70, 207.
41. S. Tetsumoto, Y. Takeda, H. Imai, A. Kimura, Y. Jin, K. Nakanishi, Y. Maeda, H. Kuhara, K. Tsujino, T. Iwasaki, H. Shigeta, Y. Kondo, M. Ito, T. Minami, H. Hirata, R. Takahashi, S. Kohmo, I. Nagatomo, K. Inoue, H. Kida, T. Kijima, I. Tachibana, N. Maeda, T. Funahashi, I. Shimomura, H. Fujiwara, and A. Kumanogoh, *Am. J. Respir. Cell Mol. Biol.*, **2013**, 49, 592.
42. K. Ruppert, J. R. Brookeman, K. D. Hagspiel, B. Driehuys, and J. P. Mugler III, *NMR Biomed.*, **2000**, 13, 220.
43. S. Månsson, J. Wolber, B. Driehuys, P. Wollmer, and K. Golman, *Magn. Reson. Med.*, **2003**, 50, 1170.
44. N. Abdeen, A. Cross, G. Cron, S. White, T. Rand, D. Miller, and G. Santyr, *Magn. Reson. Med.*, **2003**, 50, 1170.
45. M. S. Freeman, Z. I. Cleveland, Y. Qi, and B. Driehuys, *Magn. Reson. Med.*, **2013**, 70, 1192.
46. S. Patz, W. Hersman, I. Muradyan, M. I. Hrovat, I. C. Ruset, S. Ketel, F. Jacobson, G. P. Topulos, H. Hatabu, and J. P. Butler, *Eur. J. Radiol.*, **2007**, 64, 335.
47. S. Patz, I. Muradyan, M. I. Hrovat, I. C. Ruset, G. P. Topulos, S. D. Covrig, E. Federick, H. Hatabu, W. Hersman,

- and J. P. Butler, *Acad. Radiol.*, **2008**, *15*, 713.
48. B. Driehuys, G. P. Cofer, J. Pollaro, J. B. Mackel, L. W. Hedlund, and G. A. Johnson, *Proc. Natl. Acad. Sci. U. S. A.*, **2006**, *103*, 18278.
49. T. Wakayama, M. Narazaki, A. Kimura, and H. Fujiwara, *Magn. Reson. Med. Sci.*, **2008**, *7*, 65.
50. T. Wakayama, M. Kitamoto, T. Ueyama, H. Imai, M. Narazaki, A. Kimura, and H. Fujiwara, *J. Magn. Reson. Imaging*, **2008**, *27*, 777.
51. Z. I. Cleveland, G. P. Cofer, G. Metz, D. Beaver, J. Nouis, S. S. Kaushik, M. Kraft, J. Wolber, K. T. Kelly, H. P. McAdams, and B. Driehuys, *PLoS One*, **2010**, *5*, e12192.
52. J. P. Mugler III, T. A. Altes, I. C. Ruset, I. M. Dregely, J. F. Mata, G. W. Miller, S. Ketel, J. Ketel, F. W. Hersman, and K. Ruppert, *Proc. Natl. Acad. Sci. U. S. A.*, **2010**, *107*, 21707.
53. K. Qing, K. Ruppert, Y. Jiang, J. F. Mata, G. W. Miller, Y. M. Shim, C. Wang, I. C. Ruset, F. W. Hersman, T. A. Altes, and J. P. Mugler III, *J. Magn. Reson. Imaging*, **2013**, in press.
54. J. E. Holmes and G. M. Bydder, *Radiography*, **2005**, *11*, 163.
55. K. Saito, A. Kimura, and H. Fujiwara, *Magn. Reson. Imaging*, **2003**, *21*, 401.
56. Y. Kawata, Y. Adachi, S. Haga, J. Fukutomi, H. Imai, A. Kimura, and H. Fujiwara, *Anal. Sci.*, **2007**, *23*, 1397.
-