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Imaging Techniques for Small Animal Models of Pulmonary Disease: MR Microscopy

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Abstract

In vivo magnetic resonance microscopy (MRM) of the small animal lung has become a valuable research tool, especially for preclinical studies. MRM offers a noninvasive and nondestructive tool for imaging small animals longitudinally and at high spatial resolution. We summarize some of the technical and biologic problems and solutions associated with imaging the small animal lung and describe several important pulmonary disease applications. A major advantage of MR is direct imaging of the gas spaces of the lung using breathable gases such as helium and xenon. When polarized, these gases become rich MR signal sources. In animals breathing hyperpolarized helium, the dynamics of gas distribution can be followed and airway constrictions and obstructions can be detected. Diffusion coefficients of helium can be calculated from diffusion-sensitive images, which can reveal micro-structural changes in the lungs associated with pathologies such as emphysema and fibrosis. Unlike helium, xenon in the lung is absorbed by blood and exhibits different frequencies in gas, tissue, or erythrocytes. Thus, with MR imaging, the movement of xenon gas can be tracked through pulmonary compartments to detect defects of gas transfer. MRM has become a valuable tool for studying morphologic and functional changes in small animal models of lung diseases.

Keywords

MRI; hyperpolarized; ^3He ; ^{129}Xe ; lung imaging; pulmonary function; rodent

Introduction

To study the function of rodent lungs, one might like to directly image the flow of gases into the lungs, the gas exchange across the blood–gas barrier, and to see gases carried away by the pulmonary circulation. Additionally, one might like to wrap this detailed functional picture in a high-quality anatomical image. Finally, these techniques should be noninvasive so that specific animals can be monitored over time as they either develop disease or respond to a specific course of treatment. With advances in magnetic resonance microscopy, the goal of morphologic imaging has been largely met (Hedlund and Johnson, 2005).

MR imaging methods and applications to small animal models of lung disease will be briefly reviewed here. Perhaps the most significant problem associated with imaging the lung is imaging the gas spaces that account for up to 80% of its volume and provide no intrinsic signal. Of any imaging modality, MR provides a unique solution to airway imaging by using special breathable gases that are MR signal sources. The development of hyperpolarized noble gases for MR imaging has been a major accomplishment in the past decade, making it possible to

image their distribution with exquisite resolution despite their 3000-fold lower density than tissue.

Gas imaging has been realized through an ongoing convergence of atomic physics research, biomedical engineering, and magnetic resonance imaging (MRI) research. Combined structural and functional MRI of the lung is thus poised to become a powerful tool in small animal research of the respiratory system. Thus, we will also provide a detailed overview of hyperpolarized gas MRI and the functional information that can be derived from this technology with a focus on application to small animal models of pulmonary disease.

MRI Basics

To appreciate the special challenges and opportunities of MR microscopy in small animals, it is instructive to briefly review the basic operating principles of MRI (Bushberg et al., 2001; Hornak, 2006). The signal source in conventional MRI is water; more specifically, the tiny magnetic moments of the protons that make up the nuclei of the hydrogen atoms in water. To derive a signal from protons requires them to oscillate, thereby changing the magnetic flux they present to a surrounding receiver coil (a loop of wire). A changing magnetic flux in a coil induces a voltage just as the rotating turbine of a power plant spins a large magnet that induces the familiar 60 Hz voltage coming out of our walls. Protons (or any magnetic moments) can be enticed to oscillate by placing them in a magnetic field and tickling them with a radio frequency pulse. In a magnetic field, protons can either line up with the magnetic field or against it.

A radiofrequency pulse applied to the protons via the same coil used for detection, torques them away from the magnetic field axis and causes them to wobble around this axis at a frequency that is precisely proportional to the strength of the magnetic field and their own magnetic moment. This wobbling is called precession. For example, in a clinical 1.5 Tesla MRI scanner, the ^1H precession frequency is about 64 MHz. The flux from the spinning nuclear moments is picked up by the coil, amplified, and digitized. In the case of water protons, after a few hundred milliseconds of precessing, the protons return back to being aligned with the magnetic field and they can be pulsed and detected again.

To generate a magnetic resonance image rather than a single-frequency signal, we apply a well-defined, linear magnetic field gradient during signal acquisition. The gradient reduces the magnetic field strength at one end of the sample and increases it at the other end. This makes the nuclei at one end of the sample precess more slowly than at the other because precession frequency is proportional to magnetic field strength. The detected signal is then broken down by its frequency components whose amplitudes represent a 1-dimensional image of the sample. This approach of using magnetic field gradients to encode the spatial distribution of nuclei can be readily extended to acquire images in 3 dimensions.

The final role of the magnetic field in imaging is to bring enough order to the system of nuclei to make them detectable. To see an MRI signal, an imbalance must exist in the number of nuclei starting out aligned versus anti-aligned with the magnetic field. These two populations have precession signals of opposite signs and would cancel each other exactly if present in equal numbers. Fortunately, the interaction of the nuclei with the magnetic field makes it slightly more energetically favorable for them to be aligned rather than anti-aligned with the field. This imbalance in the population of nuclei is called polarization and is defined as $P = |N_{\uparrow} - N_{\downarrow}| / (N_{\uparrow} + N_{\downarrow})$, where $N_{\uparrow}$ is the number of aligned nuclei and $N_{\downarrow}$ is the number of anti-aligned nuclei. However, even in large magnetic fields such as that of a 1.5 Tesla clinical MRI scanner, the tiny energy difference between states gives a polarization only 5 parts per million. Thus, most of the nuclei still cancel one another.

The strength of the MRI signal coming from a given three-dimensional pixel element (voxel) in the image is

$$S_{\text{MRI}} \propto \mu \times \omega \times P \times \rho \times V_{\text{vox}} \quad (1)$$

where μ is the nuclear magnetic moments, ω is their precession frequency around the magnetic field, P is their polarization, ρ is their density, and V_{vox} is the voxel volume. Both precession frequency ω and polarization P increase linearly with magnetic field strength that drives the never-ending quest for higher magnetic field strengths in MRI. The intuitive dependence on density also explains why water is the favored imaging source. The density of hydrogen nuclei [^1H] is 110 M, sufficient to provide ample signal despite relatively low nuclear polarization in even the largest magnetic fields.

Proton MR Microscopy for Morphology

As one translates proton MR imaging from clinical scales to the microscopic scales required for rodents, numerous additional challenges emerge. To image a human chest, we might take a field of view of 36×36 cm and image it in 10 mm-thick slices with an in-plane resolution of 256×256 . Thus, a typical voxel in such an image is $1.4 \times 1.4 \times 10$ mm³, comprising a volume of ~ 20 μL . To resolve comparable structures in a mouse chest, we employ a field of view of about 2 cm, and use 0.1 mm slices resulting in voxels of $78 \times 78 \times 100$ μm^3 , each with a volume of only 0.6 nL. Equation (1) tells us that the signal in a mouse voxel will be 33,000 times smaller than in the human. These signal deficits can be partially compensated by designing more efficient receiver coils, but the remainder must be made up through signal averaging. However, acquiring images over many respiratory and cardiac cycles introduces undesired motion artifacts, if not controlled.

Rodent respiratory and cardiac rates are up to an order of magnitude faster than in human subjects and animals cannot be instructed to hold their breath during imaging. Constant motion can degrade image quality with blurring and artifacts, so animals are placed on an MR-compatible ventilator (Hedlund et al., 2000; Mai et al., 2005) that controls the breathing cycle. Positioning of the lung is precisely reproduced from breath to breath (Mai et al., 2005) as the MR-compatible ventilator triggers the MR scanner to acquire data only during a specified phase of the breathing cycle, such as at end-expiration or at full inspiratory volume. Heart motion can also be frozen by providing a second image gating signal synchronized with the cardiac cycle (Brau et al., 2004). Additionally, to achieve resolution on the order of 100 μm requires large magnetic field gradients to create the needed frequency dispersion to distinguish neighboring voxels of such small dimensions. Thus, dedicated animal scanners with gradients an order of magnitude stronger than their clinical counterparts are a necessity for MRM.

Finally, of all organs, the lung presents the biggest challenge for MR imaging. First, its low tissue density compared to other organs makes it an unusually poor source of proton signal. Furthermore, the very structure of the lung with its myriad of gas/alveolar tissue interfaces causes the MR signal to decay more rapidly than occurs in solid tissues. This further reduces the already low signal intensity in the lung. To overcome this problem, specialized imaging sequences have been developed to capture signal more rapidly, within microseconds instead of the milliseconds used in conventional scans (Gewalt et al., 1993).

The apparatus used in a typical ventilation-controlled imaging session of a mouse is shown in Figure 1. The mouse is intubated with a 20-gauge endotracheal tube and ventilated with an MR-compatible constant volume ventilator at 100 breaths per minute with 0.2 ml tidal volume (Chen et al., 2003). The imaging coil is dual-tuned to support hyperpolarized ^3He and ^1H

imaging on a 2 T MR scanner. Airway pressure, temperature, and ECG are monitored continuously and body temperature is controlled by warm air circulating through the bore of the magnet using feedback from a rectal temperature probe.

The ventilator triggers the MRI scanner at end inspiration or end expiration to capture image data during this brief moment when lung motion is suspended. Images are acquired over multiple breaths to attain sufficient signal-to-noise in the nanoliter voxels, typically 20–40 acquisitions per breath, until the necessary number of image frames has been acquired. To achieve the short echo times necessary for lung imaging, we employ radial sampling of the image space, which requires us to acquire 800 “rays” of image data to adequately sample a single image “slice” with a 256×256 matrix. Thus, we require roughly 40 well-controlled breath-hold periods to acquire one image slice. Extending to high-resolution 3D imaging can easily expand the required number of image views to 100,000 or more, which can take a good fraction of an hour to acquire. Such calculations illustrate the critical interplay of image acquisition requirements with the need for precise ventilatory control over the animal. Figure 2 shows an example of a proton morphologic image of a rat lung acquired in this manner with an isotropic resolution of $117 \times 117 \times 117 \mu\text{m}^3$ (Johnson et al., 2001).

This exquisite capability of proton MR to reveal soft tissue differences and its sensitivity to tissue water has made MR a valuable tool to study pulmonary pathologies associated with edema, inflammation, fibrosis, and emphysema. For instance, in a model of edema and fibrosis lung injury from the herbicide paraquat, animals were followed with MR microscopy (MRM) from day 1 of exposure with development of extensive edema, to its resolution after 7 days, and development of fibrosis by day 14 in rats (Hedlund et al., 1996). Rats exposed to hyperoxia (85%) for 7 days exhibited on day 1 peribronchial inflammation, on day 4 peribronchial edema and severe pleural effusion, more severe edema by day 5, while effusions were slightly diminished, and by day 7, the edema and effusion were completely reabsorbed (Hedlund et al., 1996). Small animal MR imaging has also been used to study pulmonary edema resulting from allergen challenge in rats (Beckmann et al., 2001).

Hyperpolarized Gas MRI Basics

One of the most important features of pulmonary MRI is the capability to directly image the airway structure of the lung using inhaled gases. However, to image gases using MRI requires us to overcome substantial obstacles, since the low density would normally render the gas invisible. Hyperpolarization is the technical trick that makes it possible to image the gaseous stable isotopes ^3He and ^{129}Xe with the same sort of exquisite spatial resolution that we have come to expect from “conventional” proton MRI. Hyperpolarized ^3He and ^{129}Xe have their nuclei in nearly perfect alignment with the magnetic field as compared to the relatively weak alignment generated by thermal equilibrium dynamics. As can be seen in Equation (1) with polarization levels approaching 50% rather than 0.0005%, the signal enhancement of roughly 10^6 is more than sufficient to overcome the density deficit that gases suffer compared to solid tissues.

Hyperpolarization of ^3He and ^{129}Xe has been an active field of physics research for several decades, but the application of these gases to biological imaging has spurred renewed activity and fairly dramatic leaps in the technology over the past decade. Excellent introductory references are given by Leawoods et al. (2001), Moller et al. (2002), and Kadlecsek (2002). The essence of the hyperpolarization relies on angular momentum transfer from light. The difference between a nuclear moment precessing clockwise vs. counterclockwise around the magnetic field is exactly one quantum of angular momentum. By adding enough angular momentum to an ensemble of nuclei, the nuclei become polarized.

A ready supply of angular momentum is carried by photons, which when circularly polarized, each carry +1 quantum of angular momentum. Since nuclei cannot directly absorb light, an intermediary is needed to absorb the light (and the angular momentum). Circularly polarized laser light is absorbed by an alkali metal atom such as rubidium (Rb), causing the outer valence electron of each atom to precess in the same direction around the local magnetic field (electron spin polarization). Subsequent collisions between the polarized valence electrons and the noble gas nuclei slowly exchange the angular momentum stored in electron spins to angular momentum stored in nuclear spins of the gas. This method of three-step angular momentum transfer is known as optical pumping and spin exchange as illustrated in Figure 3.

While hyperpolarization physics is elegant and complex, the practical implementation of the method is more straightforward. To an outside observer, hyperpolarization appears merely as shining a bright laser on a glass container holding a blob of Rb alkali metal and a substantial density of ^3He or ^{129}Xe gas. The gas is contained under pressure (~ 10 atm) in an optical cell and heated to about 200°C to create a dilute vapor ($\sim 10^{-6}$ atm) of Rb to absorb the laser light. Once the nuclear polarization is saturated, it is stabilized in the ^3He or ^{129}Xe nuclei by cooling the system down so that all Rb atoms condense out of the vapor phase and spin exchange stops. A schematic of such a system is shown in Figure 4.

Typical production rates are ~ 0.5 –1 liter per hour of polarized ^3He or ^{129}Xe . Such quantities of gas are sufficient for imaging human lung function in a single breath, or imaging rodent lung function over many breaths. Moreover, if the hyperpolarized gas is maintained in an environment free of magnetic impurities, it retains its nuclear polarization for hours or even days, thus allowing the gases to be readily transported long distances from the polarizer to the MR scanner (van Beek et al., 2003). An important consequence of hyperpolarization versus thermal equilibrium polarization employed for ^1H MRI is that since ^1H is polarized by the magnetic field, it can be re-polarized by the same field, whereas ^3He and ^{129}Xe being polarized by light are not re-polarized in the magnetic field and become completely silent after imaging. Since the very act of imaging the gas causes it to lose its hyperpolarized state, scan protocols must be specially tailored to extract maximum information from a single dose of hyperpolarized gas. However, selective destruction of the hyperpolarized state of ^{129}Xe can also be used to advantage in following gas transfer in the lung (described in the next sections).

Airspace MRM with Hyperpolarized ^3He

Biological imaging of hyperpolarized gases dates back to the first visualization of ^{129}Xe in a mouse lung preparation by Albert and co-workers in 1994. Since that demonstration, the field moved quickly to in vivo small animal imaging (Black et al., 1996) and the first demonstrations in human subjects (Ebert et al., 1996; MacFall et al., 1996). In the clinical realm, hyperpolarized ^3He MRI of airspace ventilation has been applied to study asthma (Samee et al., 2001, 2003), chronic obstructive pulmonary disease (COPD) (Salerno et al., 2002; Fain et al., 2006), cystic fibrosis (Donnelly et al., 1999; Koumellis et al., 2005; Mentore et al., 2005), and bronchiolitis obliterans associated with lung transplant rejection (McAdams et al., 1999).

As with ^1H MR imaging where many forms of soft tissue contrast can be exploited such as T_1 , T_2 , or diffusion-weighted imaging, hyperpolarized gas imaging can be readily extended beyond simple “gas density” imaging. Additional MR contrast mechanisms for hyperpolarized ^3He include apparent diffusion coefficient (ADC) imaging for assessing alveolar microstructure (Chen et al., 2000; Salerno et al., 2002), dynamic imaging of gas inflow (Salerno et al., 2001a; Viallon et al., 2000), and regional measurement of oxygen partial pressure (Deninger et al., 1999; Kadlecik et al., 2005). Excellent reviews on the clinical exploration of hyperpolarized ^3He can be found in (Salerno et al., 2001b; van Beek et al., 2004).

Small animal imaging of hyperpolarized gases is predictably more challenging than clinical imaging. After all, the human patient can be asked to take a deep breath of gas from a bag and hold for 10 or 15 seconds while imaging is performed, but not so with a rodent. In addition to motion degradation of image quality previously discussed for ^1H MRM, blurring and attenuation can occur because of the high diffusivity of ^3He gas, creating an additional obstacle for resolving small airways and related structures. Thus, specialized MRI image acquisition strategies (pulse sequences) have been implemented specifically for high-quality imaging of hyperpolarized gases in small animals (Johnson et al., 1997).

These techniques have been used to study models of pulmonary pathology in the rat and when used with a dual-tuned imaging coil, both solid tissue (proton) and gas distribution (^3He) can be obtained in the same imaging session. Furthermore, imaging hyperpolarized helium at several different gradient strengths yields data that can be used to calculate an ADC, which can reveal the underlying microstructure of the gas spaces of the lungs. For instance, rats treated with porcine elastase develop panacinar emphysema that results in a larger helium ADC because its diffusion is less restricted than in the normal lung (Chen et al., 2000).

Conversely, rat lungs exposed to therapeutic radiation develop interstitial fibrosis that reduces alveolar volumes, greatly restricting helium diffusion and resulting in a lower ADC than in a normal lung (Ward et al., 2004). In these examples, concurrent proton images reveal the underlying structural changes and associated reduction in parenchymal signal intensity seen in the emphysema model and elevated signal intensities in the fibrotic lung.

Although imaging the rat lung with HP gas and proton MR is challenging, an even greater challenge is to image the mouse lung. However, the availability of genetically manipulated mouse models for research on pulmonary diseases like asthma and COPD has compelled researchers to develop techniques to image these creatures that are roughly one-tenth the size of a rat. Imaging allows us to better evaluate pulmonary function on a regional level in response to challenges and in response to therapeutic agents. The reduction of ^3He MRI to the scale of a mouse lung has proven difficult due to the small tidal volumes and the exquisite resolution needed to resolve airways of the mouse. At $\sim 200\ \mu\text{l}$, the tidal volume of the mouse is an order of magnitude smaller yet, than that of the rat. Hyperpolarized ^3He imaging in the mouse lung was first demonstrated by Dugas et al. (2004), who achieved a resolution of $0.25 \times 0.25 \times 3\text{mm}^3$. Chen et al. (2005) demonstrated rather spectacular resolution images of ^3He distribution in mouse lungs with a resolution of $70 \times 70 \times 800\ \mu\text{m}^3$, but to do so, the animal consumed 1.2 liters of ^3He in 25 minutes of image acquisition time. Here, we will discuss our recent progress in creating high-resolution images in mouse models of asthma using modest quantities of hyperpolarized ^3He and sufficiently rapid scan times to efficiently image the transient narrowing of the airways induced by methacholine. Recently, we were able to observe the dramatic airway narrowing and reduced ^3He ventilation in BALB/C mice that had been sensitized with ovalbumin and challenged with methacholine (Driehuys et al., 2006b).

Hyperpolarized gas imaging takes advantage of the same scan-synchronous ventilator technology developed for controlling respiratory motion in ^1H structural MRI (Figure 1). A critical requirement is the ability to deliver hyperpolarized ^3He to the animal in microliter amounts at specific phases of breathing and in such a way as to prevent depolarization before reaching the lungs. This is accomplished by rapid remote control switching of the breathing gas from air to a mixture of 75% HP ^3He mixed with 25% O_2 to achieve the 0.2 ml tidal volume. Again, images are acquired over multiple breaths to achieve sufficient signal-to-noise (SNR) for the extremely high resolution employed. To reduce the effects of diffusion blurring, we use a radial sampling of the image space. We acquire 800 “rays” of image data to adequately sample a single image “slice” with a 256×256 matrix. Thus, we require roughly 40 well-controlled breaths to acquire one image slice in this manner.

Figure 5 shows an example of a 3D ^3He image acquisition in a control mouse. The resolution of this image is $125 \times 125 \times 1000 \mu\text{m}^3$, which required 5.8 minutes to acquire 11520 rays and consumed 92 ml of ^3He . This scan time is fast enough to capture the short duration airway responses from an intravenous injection of a muscarinic receptor agonist such as methacholine (Driehuys et al., 2006b). The use of ^3He MRI in imaging of mouse models of asthma provides a level of regional specificity that has never before been possible. The 3D images can be nicely represented as a maximum intensity projection (MIP) images to highlight the structure of the major airways, as shown in Figure 6. It is possible to acquire images even faster by acquiring a 2-dimensional projection of the whole lung, as also shown in Figure 6c. While the detail in this image is not as exquisite as that of the 3D composite, the image was acquired in only 12 seconds and consumed only 2 ml of ^3He . Thus, we see a wide range of possibilities for ^3He imaging to answer many biological questions. Although exquisite resolution requires significant time and gas consumption, it enables us to observe subtle functional changes not detectable by other means.

Functional MRM with Hyperpolarized ^{129}Xe

While hyperpolarized ^3He has ushered in a capability to trace the morphology of the lung airspaces, hyperpolarized ^{129}Xe is poised to provide an even more complete picture of lung function by supplying information about the gas exchange process at the alveolar level. Unlike ^3He , which is nearly insoluble in most tissues and fluids, ^{129}Xe has significant solubility in blood and all tissues in the body. Thus, inhaled ^{129}Xe is readily distributed throughout the body. More importantly, ^{129}Xe shifts its resonant frequency dramatically upon experiencing a new molecular environment. So, not only can one image ^{129}Xe distribution throughout all perfused organs, but one can discriminate the type of tissue environment in which it resides. This sensitivity to molecular environment results from the transient binding that xenon undergoes with almost all proteins (Cherubini and Bifone, 2003). Such interactions perturb its large electron cloud, which slightly alters the magnetic field experienced by the nucleus. This potentially powerful aspect of hyperpolarized ^{129}Xe has been overshadowed by its weaker MRI signal and less mature state of hyperpolarization technology than ^3He .

Recent technical advancements have greatly improved ^{129}Xe imaging. Figure 7 shows a progression of small animal ^{129}Xe ventilation images from this laboratory, starting with the earliest work in 1998 (Chen et al., 1999) to images acquired today. Through a combined focus on the physics of hyperpolarization and MR imaging strategies, the image resolution we attain has improved by a factor of 7, while signal-to-noise has improved 3-fold. With these steps, ^{129}Xe is poised to start providing airspace images that are competitive with ^3He . Moreover, such signal improvements now put us in a position to exploit the properties that make ^{129}Xe so interesting in the first place—solubility and chemical frequency shift.

A good example of exploiting the solubility and frequency shift of ^{129}Xe for deriving unique pulmonary function information can be seen in our study of pulmonary fibrosis (Driehuys et al., 2006a). When ^{129}Xe is inhaled into the lung and enters into the alveolar air-spaces, a small fraction is absorbed into the moist epithelial surface. ^{129}Xe dissolved in the alveolar epithelium, interstitium, capillary endothelium, and plasma resonates at a frequency that is shifted 197 parts per million (ppm) (4.64 kHz in a 2 T field) from the 23.64 MHz gas reference frequency 0 ppm (Sakai et al., 1996). The atoms diffuse across the tissue barrier and their concentration equilibrates in the red blood cells (RBCs) where the resonant frequency shifts again to 211 ppm away from the gas resonance (Albert et al., 1995). Figure 8 illustrates this simple three-compartment model of the lung and the associated ^{129}Xe frequency spectrum. The ability to discriminate ^{129}Xe in these three compartments allows us to image gas transfer dynamics with unprecedented precision. In healthy lung, the barrier between airspace and RBC is only about $1 \mu\text{m}$, a distance ^{129}Xe can diffuse across in just over a millisecond. However, if inflammation

or fibrosis causes a thickening of this barrier to even 5 μm , the time for ^{129}Xe to diffuse across it and reach an RBC increases to 40 ms. By imaging ^{129}Xe in the barrier and RBC with a radiofrequency pulse repetition rate on this time scale, dissolved ^{129}Xe is continually depolarized and becomes silent, only to be replenished by diffusion of fresh ^{129}Xe from the airspaces. Barrier thickening is readily identified by an absence of RBC signal in the images, which results from the inability of ^{129}Xe to diffusively traverse the barrier on the repetition timescale of the image.

With recent advancements in image quality and ^{129}Xe polarization and a novel imaging sequence, we have been able to directly image this process of alveolar-capillary gas transfer (Driehuys et al., 2006a). Figure 9 shows an image of ^{129}Xe in the airspace, barrier space, and red blood cells in a control rat and one with left-lung pulmonary fibrosis induced by intratracheal instillation of bleomycin. Note that in the left fibrotic lung, virtually no RBC signal can be detected, while the barrier image matches the ventilation pattern. The absence of signal in the RBCs is the result of the increased time it takes for ^{129}Xe to diffuse across the thickened blood-gas barrier, as predicted. Indeed, examination of the left lung via conventional histology after imaging showed significant thickening of the alveolar septa. This example illustrates how the ability to discriminate ^{129}Xe in different tissue compartments can lead to very powerful markers of function, in this case, gas transfer.

Further development of hyperpolarized ^{129}Xe polarization technology and imaging strategies should lead to greatly improved images of both the airspace and ^{129}Xe -containing tissue compartments. Combining the signal of hyperpolarized ^{129}Xe with additional agents such as cryptophane cages, which can be designed to bind to receptors of choice (Driehuys, 2006; Schroder et al., 2006), may in fact open up a brand new approach to molecular imaging of cellular processes.

Discussion and Conclusion

In the last few years, small animal imaging has become an increasingly important research tool for studying disease models (Schuster et al., 2004) and for preclinical testing (Beckmann et al., 2003). This increase in popularity of small animal imaging has been fostered partly by the ready availability of commercial imaging systems and the demonstration of the value of in vivo, nondestructive, and longitudinal monitoring capability of these imaging systems. MR microscopy, an outgrowth of clinical MRI, has a more than 20-year history in small animal imaging research. We have shown here examples of the value of MRM in studying the normal lung and disease models.

Traditionally proton imaging has been the mainstay of MR, but in the past 10 years, high-resolution imaging of gas distribution dynamics using hyperpolarized gas MRI has opened up a new window into functional imaging of the lung. The front-runner in this technology is hyperpolarized ^3He , which is ideally suited to making exquisite resolution images of gas distribution in the airspaces. ^3He MRI is now being routinely applied to functional lung imaging of biological systems ranging from mouse to humans. Hyperpolarized ^{129}Xe , which lags technically behind ^3He , may yet exceed the capabilities of ^3He through a powerful combination of its solubility and extraordinary sensitivity to molecular environment. A continued multidisciplinary effort spanning atomic physics, MR physics, spectroscopy, and the involvement of clinicians and biologists could lead this technology to play a central role in non-invasive functional assessment of pulmonary pathologies.

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Abbreviations

3D	Three-dimensional
ADC	Apparent diffusion coefficient
COPD	Chronic obstructive pulmonary disease
Gd	Gadolinium
H	Hydrogen
He	Helium
HP	Hyperpolarized
M	Molar (mol/liter)
MIP	Maximum intensity projection
MR	

	Magnetic resonance
MRI	Magnetic resonance imaging
MRM	Magnetic resonance microscopy
O₂	Oxygen
ppm	Parts per million
Rb	Rubidium
RBC	Red blood cell
SNR	Signal-to-noise ratio
T	Tesla
μL	Micro liter (mm ³)
Xe	Xenon

Figure 1.

A. Apparatus for in vivo imaging showing a 23-gram mouse in an imaging cradle with ECG and temperature leads attached, with a 3-cm inner diameter, dual-frequency imaging (^1H - ^3He) MR imaging coil in the background. B. Ventilation system near the bore of the 2 T MR small animal imaging system, after the mouse and coil have been inserted into the magnet.

Figure 2.

Example of a high-resolution proton morphologic image of a rat lung using the 2T system shown in Figure 1. The parenchyma signal intensity has been enhanced by an infusion of gadolinium chelate (Gd) contrast agent (Magnevist Berlex Imaging, Wayne, NJ). These 6 slices are taken from an isotropic dataset with resolution of $117 \times 117 \times 117 \mu\text{m}^3$. Reprinted with permission from (Johnson et al., 2001).

Figure 3.

Schematic representation of hyperpolarization by optical pumping and spin exchange. ^3He and ^{129}Xe nuclei acquire a high degree of nuclear magnetic alignment through angular momentum transfer from laser photons to vaporized Rubidium (Rb) and then spin exchange with $^3\text{He}/^{129}\text{Xe}$. In this hyperpolarized state, these gases enhance the MRI signal by roughly 10^5 , more than enough to overcome the low density and enable the gases to be imaged with exquisite resolution.

Figure 4.

A schematic of a gas polarizer. Laser light is circularly polarized so that all photons carry +1 quantum of angular momentum. The laser light is absorbed by an Rb vapor in the optical gas cell, thus aligning the valence electrons of all the Rb atoms with the weak magnetic field provided by the Helmholtz coils. Collisions between polarized Rb atoms and ^3He or ^{129}Xe atoms transfer the alignment to the ^3He or ^{129}Xe nuclei, as illustrated in Figure 3. The oven elevates the gas cell temperature to about 200°C to vaporize the Rb. The sense coil monitors the extent of gas polarization. (Reprinted with permission from (Kadlecek et al., 2002).

Figure 5.
3D ^3He MR images of a 25-gram mouse. The resolution is $125 \times 125 \times 1000 \mu\text{m}$. The image required 5.8 minutes to acquire and consumed 92 ml of hyperpolarized ^3He .

Figure 6.

These ³He images illustrate different representations and image acquisition strategies. (A) Maximum intensity projection (MIP) of all coronal slices in the three-dimensional dataset shown in Figure 5. This MIP representation acquired in 5.8 minutes more clearly shows major lobar airways. (B) A single central slice from the same dataset as in Figure 4. (C) A full dorso-ventral projection image from a different mouse required only 12 seconds to acquire and consumed only 2 ml of HP ³He. However, this image shows less detail of the airways. These images illustrate the trade-offs that are made in image acquisition and temporal resolution to derive the desired information.

Figure 7.

The progress in xenon ventilation imaging in the rat. (A) One of the first ^{129}Xe images made in this laboratory in 1998 with $0.84 \times 0.84 \text{ mm}^2$ resolution and SNR of about 3. (B–D) show progressively better image quality as polarization, gas delivery technology, and MR acquisition strategies have been improved. Our current standard ^{129}Xe image (D) with resolution of $0.31 \times 0.31 \text{ mm}^2$ and an SNR of about 20. Continued progress in these areas should lead to even further improvement in image quality.

Figure 8.

(A) A schematic representation of the different molecular environments experienced by ^{129}Xe in the lung (left) and (right) schematic summary of functional compartments: air space (0), barrier (197) (alveolar epithelium, interstitium, capillary endothelium, plasma), and red blood cells (RBCs) (211). (B) A spectrum of ^{129}Xe in the rat lung. The spectrum exhibits 3 distinct resonances at 211 ppm (RBC), 197 ppm (barrier space), and 0 ppm (air space). The ability to discriminate ^{129}Xe in these distinct environments creates a unique tool to measure the transfer dynamics of ^{129}Xe among these various compartments, thereby supporting measurement of micron-scale thickening of the alveolar-capillary barrier, such as may be present in inflammation or fibrosis.

Figure 9.

^{129}Xe images in three different pulmonary compartments—gas space (A, D), barrier (B, E), and RBC (C, F). The top panels (A–C) depict a control rat. The bottom panels (D–F) show a rat with left lung fibrosis from a unilateral instillation of bleomycin. In the fibrotic lung, there is full absorption into the “barrier” tissue space (E) and a dramatic lack of ^{129}Xe signal in the RBC compartment (arrows) (F). We hypothesize that the lack of RBC signal is due to the increased diffusion time required for ^{129}Xe to traverse the thickened blood–gas barrier.