Magnetic Resonance Histology for Morphologic Phenotyping

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Magnetic resonance histology (MRH) images of the whole mouse have been acquired at 100-micron isotropic resolution at 2.0T with image arrays of 256 × 256 × 1024. Higher resolution (50 × 50 × 50 microns) of limited volumes has been acquired at 7.1T with image arrays of 512 × 512 × 512. Even higher resolution images (20 × 20 × 20 microns) of isolated organs have been acquired at 9.4T. The volume resolution represents an increase of 625,000× over conventional clinical MRI. The technological basis is summarized that will allow basic scientists to begin using MRH as a routine method for morphologic phenotyping of the mouse. MRH promises four unique attributes over conventional histology: 1) MRH is non-destructive; 2) MRH exploits the unique contrast mechanisms that have made MRI so successful clinically; 3) MRH is 3-dimensional; and 4) the data are inherently digital. We demonstrate the utility in morphologic phenotyping a whole C57BL/6J mouse.

Key Words: magnetic resonance microscopy; phenotyping; active stain; mouse; morphology


THE EXPLOSIVE INCREASE in mouse models for basic research over the last five years has spurred an extraordinary interest in new methods for phenotyping these models. New technologies for behavioral, functional, and morphologic phenotyping are rapidly maturing. One of the most promising new methods for morphologic phenotyping is the use of magnetic resonance histology (MRH) (1). The goals of this manuscript are to review the history of MRH, demonstrate the virtues of MRH for structural phenotyping, and present representative examples.

The founders of magnetic resonance imaging (MRI) envisioned its use in both clinical settings and for more basic applications. Both Lauterbur and Mansfield saw the potential for extension of the imaging method to microscopic resolution (2–3). MR microscopy (MRM) was first practiced in 1986 (4–6). Since that time, the technologies have matured to produce a steady increase in resolution. The definition of “microscopic” resolution is somewhat subjective. Since the human eye can barely resolve 300 microns at a normal viewing distance, it is common to use this as the starting point for “microscopic” resolution. Because MRI/MRM are tomographic imaging methods, the resolution is most appropriately defined in terms of the voxel volume. A typical clinical body image is acquired with 1 × 1 × 10 mm voxels (10 mm3). The mouse at roughly 25 grams is approximately 4000-times smaller than a 100-kg man. If one wishes to image a mouse with relative organ definition comparable to that in human studies, one must have voxels of ~0.0025 mm3 (i.e., resolution of 165 × 165 × 165 microns).

While founded on the same principles of MRI, MRM is subtly, but fundamentally different from MRI. While MR images of mice can be obtained on clinical MRI systems, they are generally of limited resolution. The gradients of most clinical systems are inadequate for the high resolution required. Since the voxels need to be 4000× smaller in the microscopic image, the signal will be 4000× weaker. Clinical systems are generally not sufficiently sensitive. Dedicated systems for MR microscopy have been built and are now commercially available. These systems typically employ encoding gradients that are at least 5×–100× higher than those of clinical systems. Sensitivity is enhanced by working at higher magnetic fields through use of specially constructed probes and specialized pulse sequences. This combination of instrumentation differences leads to the subtle but profound differences between MRI and MRM. Since the operational fields can be as high as 14T and the gradients can be 100× higher, the contrast mechanisms are substantially different in MR microscopy than in the clinical setting. One must be careful in mapping information from the large literature of clinical MRI to the application of MRM and MRH.

“Histology,” defined by Webster, is the structure of tissue. The term “magnetic resonance histology” (MRH) was coined in 1993 by Johnson et al (7). MRH refers to the use of MRM to characterize tissue structure. The

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non-invasive nature of MRM lends itself well to live animal studies and numerous examples exist in the literature. There is little doubt that there will be increasing use of MRM in screening and characterizing live animals. But motion will inevitably limit the spatial resolution in live animal studies. The highest resolution will be attained in fixed tissues—the specific application which draws our interest here. The focus of this paper is the collection of methods that we have developed to make MRH routine. We demonstrate the utility in a representative study of the C57BL/6J mouse.

**MATERIALS AND METHODS**

All work was performed at the Duke Center for In Vivo Microscopy, an NIH/NCRR National Resource dedicated to the development of the basic technology of MRM. Three different MR systems are available that operate at 2.0T, 7.1T, and 9.4T. Each system has shielded gradient coils with peak gradients of 180 mT/minute (2.0T), 650 mT/minute (7.1T), and 850 mT/minute (9.4T). All three systems are controlled by Signa consoles (Epic 5X, GE Medical Systems, Milwaukee, WI), which have been modified for operation at higher frequency through an intermediate up/down frequency converter.

Achieving the 4000× increase in sensitivity required for MRH is a non-trivial task. No single trick can yield the necessary gain. Rather we count on a collection of improvements. The specific gain for each of the approaches we employ is highly dependent on how the comparisons are made. Table 1 lists four approaches to increase the sensitivity and approximations of the gain in signal-to-noise relative to the clinical setting, in order to provide a gauge of the relative importance of each of the approaches.

The single largest gain in sensitivity is achieved through the use of optimized receiver coils. The exact gain is dependent on the quality of the coil, dielectric losses, and loading. We base our rough estimate on the relative volumes of a clinical body imaging coil and a specialized receiver for the whole mouse. A more detailed discussion of the improvement in sensitivity for rf coils for MRM can be found in the literature (8).

Three coils were used for the images included in these studies:

1. @ 2T for whole mouse studies with 25-mm-diameter solenoid, 100 mm long.
2. @ 7.1T for limited volumes of the whole mouse with 25-mm birdcage, 40 mm long.
3. @ 9.4T for isolated organs with 1-cm-diameter solenoid, 1.5 cm long.

The gain achieved from the increased polarizing field is the subject of frequent debate. From an engineering perspective, the signal-to-noise ratio (SNR) increase with frequency (field) scales as ω^n, where n ranges between 1 and 7/4 based on the relative loading of the coil and the noise sources (9). In the comparison we make here, the dependence of SNR in the resulting image is even more complex because the signal-to-noise depends explicitly on the T1 of the tissue and the T1 of most tissues is field dependent (10). For our comparison, we chose a conservative linear dependence.

An estimate of the SNR gain from the encoding method becomes even more difficult for similar reasons. The contrast is dependent on how the differential magnetization of two tissues might be exploited. Again we chose a conservative estimate, which compares the square of the total imaging time. All the MRH images shown in the figures were acquired using three-dimensional spin echo encoding. This method, which is particularly effective at signal averaging (11), has been extended to accommodate very large image arrays (1,12). All data were reconstructed as magnitude images. Volume rendering was performed using VoxelView, or Vitrea volume rendering software (Vital Images, St. Paul, MN). The whole mouse scanned @ 2T was acquired on a 256 × 256 × 1024 array. Limited volumes were acquired at 7.1T at 50-micron isotropic resolution and the isolated organs scanned at 9.4T were acquired on arrays as large as 512 × 512 × 1024.

The term “proton stain” has been used to help cross the cultural divide between those familiar with MRI and MRH and the biological end users, such as molecular biologists, pathologists, and toxicologists. The term proton stain refers in a broad sense to the many different methods of exploiting differences in tissue magnetization to provide soft tissue differentiation. Use of the spin lattice relaxation time difference can be referred to as a T1 “stain” by analogy to a hematoxylin and eosin stain in conventional optical histology. We have extended the analogy further through the use of the term “active stain,” by which we mean some process to selectively alter or enhance some of the properties of the tissue so that we improve the resulting MR histology images (1). In the example images shown here, we used a perfusion fixation method with solutions of 10% buffered formalin and Magnevist (gadopentetate dimeglu-
mine, Berlex, Inc.) or formalin and Prohance (gadoteridol, Bracco Diagnostics). The perfusion effectively reduces the T1 to less than 200 msec. The much shorter T1 allows us to effectively use three-dimensional spin echo encoding with short TR (less than 100 msec), in turn permitting acquisition of the very large arrays in reasonable scan times.

All studies were performed in accordance with the Duke University Institutional Animal Care and Use Committee. Male C57BL/6J animals were obtained from The Jackson Laboratory (Bar Harbor, ME).

RESULTS

Figure 1a shows a representative volume image of a normal C57BL/6J mouse from the visible mouse project (1). The isotropic 256 × 256 × 1024 array allows interactive demonstration of any arbitrary plane at 100 × 100 × 100-micron resolution (1 × 10^{-5} mm^3). The oblique coronal plane, shown in blue on the volume image in Figure 1a, is shown in Figure 1b. This particular plane was chosen to slice through the right kidney in a coronal plane for comparison to the images that follow.

Since the data is isotropic, the resolution remains at 100 microns when viewing any plane. Figure 2 shows the axial plane, which is defined in red in Figure 1b. Since MR histology is non-destructive, one can perform repeated scans with variation in scanning system and/or parameters. Figure 2b shows the same animal scanned at 7.1T over a restricted 25-mm cube at an 8× increase in spatial resolution (50 × 50 × 50 microns, i.e., 1.25 × 10^{-4} mm^3). With 50-micron slices, it is trivial to page through the data to match the same plane acquired at the lower resolution. Note the excellent agreement in anatomic landmarks, such as papilla and renal vessels, vertebral body, and intestine. Note in particular how the increased resolution at 7T now allows differentiation of the villi in the intestine.

Figure 3a shows a coronal plane from the same 50-micron data set shown in Figure 2 for comparison to the 100-micron coronal image in Figure 1. Figure 3b shows the same plane through the same kidney after it was removed from the animal and imaged on the 9.4T system with another 8× increase in spatial resolution (25 × 25 × 25 microns, i.e., 1.56 × 10^{-5} mm^3). This higher resolution allows one to discern all of the major structures of the kidney.

Figure 4 demonstrates one of the most powerful advantages of MRH over more traditional methods for...
histologic phenotyping—the ability to survey a very large volume of the animal in three dimensions. In Figure 4a, the 50-micron resolution in the 50-micron-thick slice shows context for the anatomy. Closer examination of the liver in Figure 4b reveals subtle shading that we attribute to the liver lobules. In Figure 4c, the same level is viewed, but now with an effective slice of 0.5 mm. Volume rendering allows projection of the vascular detail in this thicker slice, while keeping the liver lobules in Figure 4a in context. Upon magnification by $2\times$ in Figure 4d, one can appreciate vessels that we estimate are 100 microns in diameter.

A reasonable question is to ask how high a resolution can we attain in tissue specimens. Figure 5 shows representative images from a C57BL/6J mouse brain. The resolution of the arrays @ $20 \times 20 \times 20$ microns ($8 \times 10^{-6}$ mm$^3$) is 625,000$\times$ higher than that of a routine clinical brain scan. Figure 5a was rendered as a 100-micron slice to accentuate the hippocampus, which is magnified in Figure 5b. The layer of cells shown by the arrows is only a few cells thick.

**Figure 2.** Since MRH is non-destructive, the same specimen can be scanned multiple times. The volumetric scans allow easy matching of slices for comparison. **a:** An axial slice from the 100-micron survey scan at 2.0T shows excellent renal detail. **b:** The same animal was scanned at $8\times$ increase in resolution at 7.1T. Note the villi (arrow) in the small intestine that are visible at the higher resolution.

**Figure 3.** Left - A coronal slice through the right kidney from the same data set (50 microns at 7.1T) displayed in Fig. 2b can be compared to the same level at 100 microns at 2.0T (in Fig. 1b), or right - the excised kidney scanned at $8\times$ higher resolution (25 microns) at 9.4T. Note the following gross structures on the left: **(a)** liver, **(b)** stomach, and **(c)** spleen. On the higher resolution image on the right, one can see the following: **(d)** medulla, **(e)** arcuate veins, **(f)** papilla, and **(g)** medullary rays.
DISCUSSION

The potential for using MR histology for phenotyping has been clear for some time (7). MRH has four attributes that distinguish it from conventional optical methods:

1. It is non-destructive.
2. It takes advantage of the unique soft tissue contrast that has made MRI so valuable in the clinical setting.
3. It is inherently three-dimensional.
4. It is inherently digital.

As demonstrated in Figures 1 and 2, the non-destructive nature of MRH allows one to scan the same specimen many different ways. The use of active stains for MRH is compatible with more conventional histology methods since the vehicle (10% buffered formalin) is the traditional first step in preparing tissues for optical histology. Once scanned, the specimen can be easily forwarded for more traditional physical sectioning. The results presented here only involve the use of buffered formalin and Magnevist, or Prohance.

The majority of the conventional histologic methods require tissue dehydration early in the specimen preparation. But clinical MR has made it abundantly clear that the proton stains, for example T1, T2, and diffusion, can be very sensitive detectors of pathology ranging from necrosis and inflammation to ischemia. The potential for active stains to enhance the value of MRH is even greater. We used Prohance or Magnevist to reduce the T1, thereby enhancing the signal. Many variations on both fixatives and contrast agents show potential. More specific staining methods are already in development (13–14).

MRH is inherently three-dimensional, particularly when practiced with the isotropic encoding methods described here. As is evident in Figures 1, 2, and 3, one can interactively section the volume along any plane to understand complex three-dimensional structures. Since the specimen is completely intact, very accurate morphologic measurements are possible without the shrinkage or sectioning artifacts that accompany traditional methods of physical sectioning. Equally important to the molecular biologist, geneticist, or pathologist using MRH, morphometric measurements can be normalized. For example, the absolute size of the ventricular system can be measured in the brain and normalized to the total brain volume, a feat that would be very challenging with more traditional methods. For the scientist attempting to structurally phenotype a mouse, this normalization can help remove variability arising from size variations due to simple total weight differences.

Figure 4. The unique ability to survey the entire volume is shown in a 512 × 512 × 512 array acquired through the thorax and abdomen at 7.1T. a: A single 50-micron axial image shows liver, stomach, and intestine. b: A magnified view of the upper right quadrant allows one to appreciate the individual liver lobules. c: The same level volume rendered with a 500-micron slice highlights the vascular detail. d: The magnified view shows vessels estimated to be ~100 microns.
Finally, MRH is digital, with all the benefits we see of digital images in the clinical arena, plus one particularly important benefit to the basic scientist seeking to use the tool in phenotyping. Since the data is digital, it will be possible to assemble web-based atlases for reference. Indeed, such an effort is already under way through the mouse brain imaging research network (MBIRN) (15).

The data shown here are part of the visible mouse project, an effort to provide the broadest scientific community access to the same data. With established, web-accessible data sets such as these, and standardized methods for scanning, it will become possible for scientists throughout the world to share and compare their mouse models via the worldwide web. As new mutants are scanned, the quantitative morphometry made possible by MRH will provide critical methods for connecting genetic changes to specific morphologic and pathologic changes.

But what resolution will be required? Is the resolution available sufficient or even useful? The volume comparisons above and the substantial experience available for clinical MRI can provide some guidance. Few would question the value of clinical MRI. The typical body scan acquired with $1/1000 \times 10 \text{ mm}$ voxels is sufficient to detect and characterize a broad range of clinical conditions. If one scales by the body weight from man to mouse (100 kgm/25 gm), a factor of at least 4000$^\times$ is required. The scan of the whole mouse shown in Figure 1 is acquired at 10,000$^\times$ that of the typical clinical body MRI exam. The whole mouse body images shown in Figures 2 and 4 are acquired at a resolution increase of 80,000$^\times$ over the clinical setting. The brain image in Figure 5 is acquired at 625,000$^\times$ over that of a typical clinical brain MRI.

This scaling exercise, from man to mouse, must also take into consideration some of the structures under study. While the mouse volume is $1/4000$th of a man, many of the basic biological units are not that much smaller. For example, the liver lobule of the mouse seen in Figure 4 is roughly the same size as the liver lobule in a man. The capillaries in the mouse are roughly the same size as those in a man. Thus, as we scale the resolution to support MRH in the mouse, we come closer to the basic biological building blocks.

In conclusion, the combined use of higher magnetic fields, specialized rf coils and encoding methods, and the use of active stains has allowed us to increase the spatial resolution in MRH studies of the whole mouse by 80,000$^\times$ and 625,000$^\times$ for an isolated organ relative to routine clinical MRI. There seems little doubt that these methods will prove beneficial for the molecular biologist, pathologist, geneticist, and toxicologist. The technology will continue to improve. But we find ourselves at this point in much the same place we found ourselves in the clinical arena in the early 1980s. At that point MRI was a very new tool for the radiologist and clinician. Some of the potential was clear, but most of it was not. By analogy, the potential for MRH is now clear, but the full potential has yet to be realized.

REFERENCES