High-Resolution Revolution

Every so often, a technology emerges that revolutionizes a field of scientific study. Magnetic resonance microscopy (MRM), being developed by the Duke University Center for In Vivo Microscopy in conjunction with the NIEHS, promises to do just that for the field of toxicologic pathology. "This tool will allow us to explore the full potential of animal models," says G. Allan Johnson, director of the center. "It will bring us dramatically closer to identifying the environmental factors that contribute to human disease."

MRM is an evolution of magnetic resonance imaging (MRI), which has been used in clinical settings since the early 1980s. Both employ powerful magnets to beam radiofrequency signals through the body, causing hydrogen atoms in soft tissue to resonate. A computer detects this resonance, which varies according to factors such as water content, and produces an image that pathologists and radiologists can use to differentiate healthy versus diseased tissue. MRM differs from MRI in that it works with a much stronger magnetic field and delivers images at more than 250,000 times greater resolution. MRM is used exclusively to scan small animals, with a focus on rodents as an animal model for human disease.

Imaging Evolution

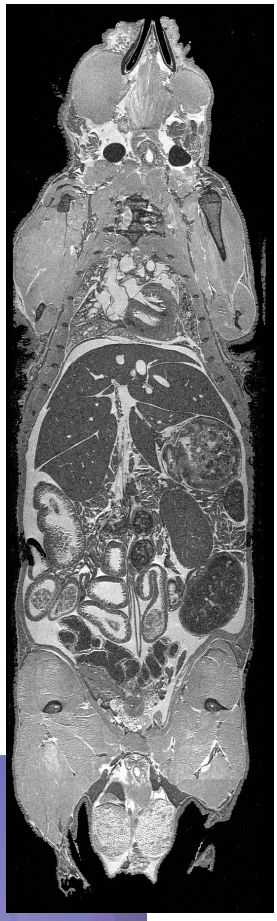
Researchers at Duke have been refining MRM since 1986, employing ever-stronger magnets, larger imaging arrays, and more sensitive radiofrequency receivers. The center can image a whole mouse at a resolution of 100 microns (the width of a human hair is about 200 microns) using a 2.0-T (tesla) magnet. (A tesla is a unit of strength of a magnet.) A 7-T magnet is used to image a part of the body at a resolution of 50 microns. Using their most powerful device, a 9.4-T magnet, the center can scan individual organs at a resolution of 25 microns.

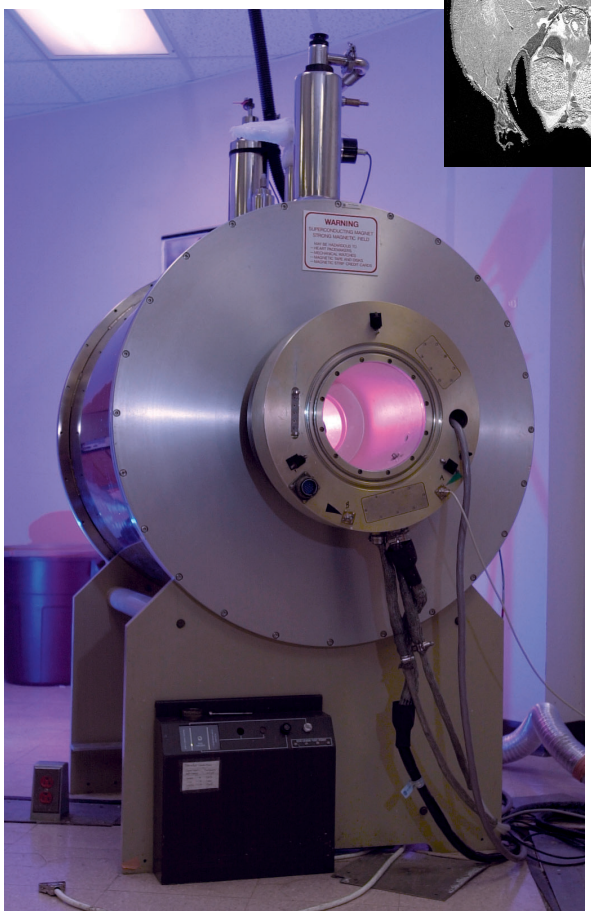
"The people at the center are doing really impressive work," says W. Thomas Dixon, physicist at General Electric's Global Research Center in Niskayuna, New York, which has helped Duke modify General Electric imaging consoles for use with MRM. "It was originally believed you couldn't get decent contrast at high fields of resolution, but they have proven that wrong."

Both live and dead animals can be imaged with MRM. Motion will degrade the signal, which limits the use of live animals for high-resolution scanning. Although live animals are anesthetized, they may make slight movements during scanning periods, which last anywhere from 20 minutes to more than an hour. Johnson and colleagues have been able to compensate for the motion of heart and lungs by synchronizing the signal with the animal's heartbeat and breathing.

During the imaging process, data are produced in digital form, transferred to a computer, and displayed on a conventional desktop monitor. The images are isotropic, that is, are of equal resolution in three dimensions and can be turned on any angle. Researchers can "slice" the image along any plane and proceed through hundreds of slices in a minute. Robert Maronpot, chief of the Laboratory of Experimental Pathology at the NIEHS and a collaborator with Johnson in the development of MR histology (the study of the structure and chemical composition of animal tissues as related to their function), says the ability to manipulate imagery through MRM is unprecedented.

"Using conventional pathology, it would take weeks of sectioning to get 10 micron slices of an entire mouse brain," he says. "That would produce approximately 2,000 slides that would then have to be examined by a pathologist. Even then, you would only be dealing with two dimensions. It would take at least a year to reconstruct the brain in three dimensions from the 2,000 slides. MRM can do all this in an hour-long scan at a fraction of the cost." Johnson estimates the cost to recreate a mouse brain in three dimensions using conventional pathology at roughly $60,000; with MRM it would be $2,000. However, he says a strict comparison is not possible because "you could never match up the slides using conventional pathology as you can with MRM."





Gazing inward. A magnetic resonance microscope, developed by the Duke University Center for In Vivo Microscopy in conjunction with the NIEHS, allows scientists a clear picture of the inner workings of small animals, particularly rodents.

Top to bottom: Duke University; Arnold Greenwell/EHP

Maronpot says MRM will not replace conventional pathology, but rather complement it. "MRM will lead us to prudently select sample sites for traditional pathology," he says. "The latter is still needed to look at individual cells and characterize lesions."

Advances in the field of MR histology are being made possible not just through the development of better hardware, but also by improved stains (MR contrast agents). Before an animal is scanned for MRM, it is perfused with a fixative containing a stain that selectively alters or enhances certain properties of the tissue in a way that improves the scanned images. Different paramagnetic compounds are used as stains, depending upon what properties researchers want to highlight. Johnson and colleagues

have had particular success using formalin laced with gadolinium—a highly magnetic rare earth element—for imaging soft tissue.

Coincident with the improvement in hardware and fixation/perfusion methods has been the development of computing power needed to process the vast amount of data produced by MRM. Imaging of a single mouse produces eight gigabytes of data. Acquiring the computing power to scan and store data on the millions of rodents being produced for research is one of the major challenges of MRM. Johnson and colleague Robert Lontz have founded a company, MRPath, Inc., to offer MRM scanning services and viewing software to the larger research community.

Targeting Tissues

According to Maronpot, MRM has immediate application in the field of developmental biology and toxicologic research, allowing researchers to follow the progression and regression of toxic and carcinogenic processes in animal models more thoroughly and more easily than with conventional pathology. Maronpot says the arrival of this technology is particularly timely, given the number of genetically engineered mice being produced for research. "It would be totally impractical to employ conventional histopathology to phenotype all these mice," he says. "With MRM, we have a better chance."

Maronpot says MRM may allow researchers to use fewer animals, given that one can more thoroughly analyze each animal, and when imaging live animals, the same animal can be followed over the progression of the study without the need to kill it. "You can even use the same animal as its own control," he says, because with this technology the animal can also be examined for preexisting tumors before it is exposed to test substances.

Eric Wisner, a professor of surgical and radiologic sciences at the University of California at Davis, sees other advantages to MRM. "In addition to examining tissues, MRM is very good at functional imagery, things like blood flow and fluid perfusion and diffusion," he says. "I think you will see these techniques growing along with the phenotyping of genetically engineered animals."

Currently, the NIEHS is involved in three studies using MRM on fixed specimens. Robert Sills, head of Molecular Pathology at the NIEHS, is using MRM to study the effects of carbonyl sulfide in rats. Carbonyl sulfide is a by-product of many industrial processes and is listed as a chemical of concern under the Clean Air Act. The U.S. Environmental Protection Agency (EPA) has nominated the chemical for study regarding three possible effects: on the nervous system, on reproductive and developmental processes, and on cancer development. Rats have been exposed to the compound and scanned at the Center for In Vivo Microscopy. Using MRM to scan the whole brain, researchers have found lesions where they never expected to find them.

Arnold Greenwell/IHP

The inside story. Robert Maronpot of the NIEHS (left) and Allan Johnson of Duke University (right) examine the results of a mouse scanned using magnetic resonance microscopy. Such scans will offer insights into the mechanisms by which environmental agents cause cancer tumors and other adverse effects.

"MRM has greatly increased what we can do in this study," Sills says. "Because the brain is still intact, we can go back and make assessments as to how large an area of the brain is involved, differences in terms of exposure, and how the lesions progressed over time. We can look at the brain not only in cross sections, but also longitudinal sections, which might reveal other areas involved. It's a very exciting tool."

Maronpot is principal investigator of a second study examining birth defects in rats exposed to high levels of vitamin A. This pilot study examined the use of MRM in teratology, rather than investigating the toxicity of vitamin A. In the study, pregnant rats were exposed to the compound, and their pups were delivered by caesarian section. The pups were processed using fixative containing paramagnetic gadolinium and then examined by MRM. "This pilot study has enabled us to work out the best imaging parameters," Maronpot says. "We've now refined the MRM procedures and are ready to do a complete study."

Maronpot is also involved in a study with the EPA on the health effects of ammonium perchlorate. This chemical is a primary ingredient in solid propellant for rockets, missiles, and fireworks. Perchlorate is exceedingly mobile in water systems and can persist for many decades under typical groundwater and surface water conditions. It is manufactured in 44 states, and 14 states currently have confirmed releases in ground and surface waters. Although there is no national primary drinking water regulation for perchlorate, the chemical is known to be toxic at some level, inhibiting the uptake of iodide in the thyroid. In this study, breeding pairs of rats are exposed to perchlorate via drinking water, and their pups were exposed through nursing. Maronpot and Johnson are scanning the pups' brains for neurotoxicologic defects using MRM. The EPA will then do an evaluation of the images. "With MR histology, we can examine the whole brain and get a much more accurate assessment of the effects than we could with conventional pathology," Maronpot says.

Asked where MRM technology will be in five years, Johnson says, "I think we can get down to 10-micron isotropic resolution. We are working with a company to build more sensitive radio receivers. We are also looking at intelligent ways to use all the data generated by this technology."

Johnson continues, "The imaging game is just starting to catch on for small animal research. We are going to see some dramatic advances in the coming years."

John Manuel