Animal Imaging Equipment: Recent Advances

Small animal imaging is undergoing continuous advances, as researchers, in partnership with industry, explore not only enhancements to existing technologies but novel technologies and increasing numbers and types of hybrid imaging units. This comes at precisely the time when regulatory and funding bodies are looking to molecular medicine to provide new approaches to drug discovery and development, diagnosis, therapy monitoring, and prognostic abilities.

Small Animal PET

Until recently, nearly all commercial, small-animal PET systems used relatively small crystals (predominately bismuth germanate, germanium oxyorthosilicate, lutetium oxyorthosilicate, or cerium-doped lutetium yttrium orthosilicate of 1–2 mm in size) coupled to conventional photomultiplier tubes (PMTs). Slight departures from this scheme have involved the use of position-sensitive PMTs that can improve the intrinsic resolution of the detector by more accurately detecting the coordinates of the photon on the face of the detector. The state-of-the-art crystal/PMT–based scanners now achieve 1-mm resolution and 2%–3% sensitivity, which corresponds to detection limits of a few pM. One manufacturer has moved away from PMT–based designs, using solid state detectors (avalanche photodiodes) coupled directly to individual crystals. This overcomes some of the errors inherent in position decoding using PMTs. Some systems also incorporate depth encoding, generally using different crystal types, in order to reduce the parallax error associated with the depth of the photon interaction in the crystal. In addition, some of the physics limitations of positron-emitting isotopes, such as positron range and photon acolinearity, can be corrected during the reconstruction phase, provided these effects can be modeled accurately.

In theory, resolution could be improved further with smaller crystals. However, machining crystals smaller than about 0.3 mm is extremely difficult without damaging them. Consequently, many investigators are looking at solid state detectors that convert the photon energy without crystals. Cadmium-zinc-telluride (CdZnTe) strip detectors have shown promise in this application, although with reduced stopping power compared with conventional crystal detectors. However, stacking detectors together may improve their efficiency. Although improvements in spatial resolution are important, increasing sensitivity is also vital, particularly to reduce the injected dose and decrease the radiation burden to the animal under study. Submillimeter resolution and 10%–20% absolute sensitivities are a realistic goal for future small animal PET scanners.

Small Animal SPECT

Preclinical SPECT imaging has been the focus of an explosion of interest over the past few years and certainly has not been overshadowed by small animal PET scanners. The main advantage of SPECT imaging compared with PET is significantly higher spatial resolution, with submillimeter resolution relatively easy to obtain. Indeed, some applications have seen resolutions down to 200 μm.

To obtain high resolutions with SPECT, conventional parallel-hole collimators have been replaced by specialized pinholes that give a large magnification effect. The sensitivity, resolution, and field of view of a pinhole SPECT system are all interrelated, and the system can be optimized for a specific application by trading between these parameters. For example, moving the pinhole as close as possible to the subject gives the highest resolution and sensitivity but at a cost of reduced field of view.

The main disadvantage of small animal SPECT has been low sensitivity using single-pinhole collimators that require the injection of very large doses of radioactivity to provide reasonable image statistics. The solution to this problem has been the development of multiple-pinhole and multiple-detector systems to increase the detection sensitivity. Systems are now available with detectors surrounding the subject, rather like a PET scanner, with multiple pinholes providing excellent sensitivity (up to 1%) and submillimeter resolution. Solid state detectors are being used, similar to those used in PET, that will improve the performance of SPECT systems still further.

Small Animal CT

Small animal CT scanners provide detailed anatomic information at resolutions down to 20 μm. Most systems utilize a cone-beam x-ray source and a solid-state detector rotating around the subject that enables the acquisition of a whole mouse in a single scan. Applications include but are not limited to providing an anatomic reference for coregistered PET or SPECT images, detailed studies of bone and joint structure, measurement of tumor size and location, and visualization of airway structure in the lungs. The main disadvantages of the first generation of microCT scanners have been poor soft tissue contrast (even using contrast agents), high radiation dose, long scan times, and image artifacts, particularly at the highest resolutions.

The next generation of small animal CT scanners has started to utilize much of the technology from clinical scanners, including flat-panel detectors and more powerful x-ray tubes. These systems can now obtain volume images in less than a second, which opens up the possibility of

dynamic imaging of contrast agents and quantitative physiologic measurements.

Small Animal MR Imaging

Small animal MR imaging systems use very high-field-strength magnets (3–14T) to provide a higher signal-to-noise ratio (SNR) and microscopic resolution. However, the contrast characteristics of live tissue are different at these field strengths. As with most imaging modalities, the overall image quality of MR depends on a number of interdependent factors. High SNR can be obtained through sacrificing spatial resolution or with longer imaging times, whereas resolutions down to tens of microns can be achieved in living animals. The very high field strengths used in animal magnets can introduce susceptibility artifacts, particularly at tissue boundaries, although various methods of correction have been developed.

MR imaging has the capability to study live organisms without exposing them to potentially harmful ionizing radiation. Depending on the technique used, slice-based as well as true 3D image sets can be obtained. MR images can be acquired in several ways, and the different pulse sequences used are key determinants of tissue contrast. In addition to anatomical imaging, MR is capable of providing physiologic information about several important aspects of biological processes, including circulation and cerebrospinal fluid flow, cerebral blood volume distribution, activity mapping with functional MR imaging, metabolite distribution with chemical shift imaging, or in vivo MR spectroscopy. Contrast agents greatly enhance the SNR, with recent studies of cell tracking using superparamagnetic iron oxide particles detecting single cells.

Small Animal Optical Imaging

A plethora of optical imaging technologies are currently under development, including fluorescence imaging, in which a light signal activates fluorophores that, in turn, emit detectable light; and bioluminescence, in which light is emitted from luminescent centers without activation. Optical imaging has exceptionally high sensitivity, in the sub-pM range. However, its main drawback is the rapid scattering and attenuation of the light signal by the overlying material; this limits its practical use to small animals and sources near the surface.

Optical bioluminescence imaging equipment can be as simple as a black box containing a highly sensitive cooled charge-coupled device camera that obtains images of the light distribution on the surface of the animal. Fluorescence imaging requires the addition of a light source, and many modern systems include multiple filters to obtain a spectrum of the emitted light.

One of the most difficult problems in optical imaging is the reconstruction of fully 3-dimensional (3D) tomographic images from surface projections acquired in the imaging device. This is a highly ill-posed inverse problem, because a particular pattern of light on the surface of an animal could have arisen from a large source close to the surface or a smaller source deeper inside the animal. Photon transport equations, which accurately model the inhomogeneous structure of the animal, are used to reconstruct the images. Gathering multiple views of the light emitted over the entire surface of the animal provides tomographic information that can be supplemented by recording the spectrum of the light, giving information on the depth of the source. These multispectral or hyperspectral data can result in 3D reconstructed images of spatial resolution <2 mm.

Time domain fluorescence imaging uses short pulses of laser light to illuminate the specimen and then detects the emitted photons according to their time of flight in the tissue. This additional timing information, like the spectral data acquired in some bioluminescence imagers, provides depth information that can be used to reconstruct 3D data. Further information is provided on the nature of the scattering medium that can be used to distinguish between tissue types, such as cancerous and healthy cells.

Small Animal Ultrasound

Ultrasound research based on small animal models currently focuses on 3 broad areas: cancer, cardiovascular applications, and embryology. The majority of work to date has been carried out using ultrasound scanners developed for human use. This work involves imaging frequencies from 6 to 17 MHz with resolution up to 200 μm at frame rates of 30 Hz and above (i.e., real time) and real-time blood flow imaging using color or power Doppler imaging as well as pulsed Doppler for more quantitative measurements in smaller regions. However, over the last 5 years, a system dedicated to ultrasonic microimaging and small animal imaging research has been developed. This scanner operates at frequencies up to 82.5 MHz, which provides resolution down to 30 μm (at a penetration of some 15 mm) and allows mouse embryos to be imaged in real time from 5.5 days onward.

Moreover, with the use of encapsulated, gas-filled micro- or nanobubbles as ultrasound contrast agents, it is possible to significantly increase the sensitivity of ultrasound imaging and measure flow in vessels 30–40 μm in diameter. Because of the interaction between the acoustic pulses and the contrast microbubbles (which can be destroyed at higher acoustic powers) users can have complete control over the presence of the contrast within the imaging field. Interestingly, individual microbubbles can be directly visualized with current technologies and act as true blood flow tracers, outlining small angiogenic neovessels in murine xenografts. However, it is also possible to attach ligands or antibodies to the bubble shell, making it a targeted contrast agent that will selectively bind to specific cells or processes (e.g., angiogenesis or inflammation). Researchers and industry are currently working on expanding the many contrast-specific imaging techniques developed for use in humans to the field of small animal imaging and to transfer transducer technologies from the human application range

(i.e., for frequencies <20 MHz) to the high-frequency range (>40 MHz).

Multimodality Systems

A number of manufacturers have begun integrating functional and anatomic imaging modalities into a single system. Small animal PET and SPECT systems have been coupled to microCT scanners in a manner similar to clinical hybrid scanners. This provides complementary information from a single unit. Because the images from the 2 systems are in near-perfect alignment (within the constraints of the movement of the animal), the CT data can be used to provide anatomic references for the functional radionuclide images and have been used to perform attenuation correction of the PET and SPECT data. One manufacturer now provides a triple-modality system that contains PET, SPECT, and CT within a single gantry.

Although CT images can provide useful information, combining PET and SPECT with MR imaging could be of greater importance because of the improved soft tissue contrast and because MR imaging requires no additional radiation dose. A number of groups are developing PET/MR systems although significant difficulties remain to be overcome, such as radiofrequency interference between the 2 systems, disruptions in magnetic field uniformity from the PET components, and operating PET detectors in high magnetic fields.

Animal Handling and Anesthesia

A number of recent studies have demonstrated the impact of animal handling and various anesthetic agents on the results of imaging studies. To maintain a consistent physiology between animals and across studies, the preparation of the animal must be uniform and the imaging protocol identical. This involves maintaining body temperature and monitoring vital signs throughout the study. Many commercial systems now recognize the importance of physiologic monitoring and control and include these systems as part of the imaging equipment. Indeed, many manufacturers add physiologic measurements to the imaging data stream, so the data can be included as part of the analysis.

Multimodality imaging systems offer an advantage in the consistent handling of experimental animals because they do not require the subject to be moved between scans. Although some systems offer a common animal palette that can be moved between scanners, the simple act of moving the subject could interfere with the results.

Many of the small animal imaging systems replicate the types of information available in clinical scanners. This ability to translate imaging results from small animals to human subjects with little or no modification to the protocol is a key reason behind the success of molecular imaging. However, important confounds are introduced in small animal imaging that could make translation to humans more difficult. One of the most significant factors is the use of anesthesia in animals. Anesthetics are known to alter animal physiology dramatically, causing changes in respiration, heart rate, blood pressure, and temperature. Of particular concern are the effects of anesthesia on the brain, including significant changes in cerebral blood flow and metabolism and direct interaction with certain binding sites. In addition, the various types of anesthetics cause different effects that are highly dose dependent. As a consequence, any interpretation of imaging results in small animals must include an analysis of the effects of the anesthetic before it can be translated to humans.

Questions and Controversial Issues

(1) **Small animal PET.** The radiation dose to the animal, particularly from the positron, is substantial. Is this dose interfering with the results, particularly in longitudinal studies where animals receive multiple scans? How high can the sensitivity be increased in order to reduce the radiation dose to the animal? Assuming the engineering problems can be resolved, what spatial resolution do we need to achieve for routine biomedical applications? Is 1 mm sufficient, or is there value in going smaller?

(2) **Small animal SPECT.** The issue of radiation damage to the animal is similar to the radiation issues found in PET: Are results being compromised by radiation damage to the animal? Small animal SPECT has an advantage over PET in that the system can be tuned for a specific application. Given that resolutions far superior to PET are achievable, and with multiple-pinhole systems providing much better sensitivity, should SPECT replace PET as the standard radionuclide imaging tool?

(3) **Small animal CT.** Soft tissue contrast remains poor, even on the more recent systems. Is there a role for CT, given the superior performance and greater flexibility of small animal MR imaging? Is the whole-body radiation dose to the subject from small animal CT scanners still too large? As in MR imaging, CT scanners produce huge quantities of image data in a short period of time (a single scan of a mouse may require more than 1 GB of storage space). The reconstruction, processing, and analysis of these vast quantities of data demand dedicated computing power and personnel.

(4) **Small animal MR imaging.** How high can field strength go? Increasing field strength clearly improves SNR, but how does this affect the tissue or animal under study? Is it worthwhile going to better spatial resolution? Physiologic movement of the animal and tissues resulting from respiration, circulation, and fluid flow make higher resolutions unrealistic. Perhaps more effort should be put into improving image contrast, particularly using new contrast agents, which moves MR imaging more into the "molecular imaging" field. The cost of establishing a small animal MR imaging facility can be prohibitive—magnets alone can cost up

to $2 million and require considerable support structure, including physicists, technicians, and programmers. MR imaging systems generate huge quantities of imaging data in a short period of time that may overwhelm investigators and available computing power. Data processing and analysis introduce another level of cost and complexity to MR studies.

(5) Small animal optical imaging. Optical imaging will always be limited to a depth resolution of a few centimeters, which limits its application solely to small animals. Consequently, it is not useful in translational research, because it will never be applied to humans.

(6) Small animal ultrasound. Can even higher frequencies (>100 MHz) be utilized for small animal imaging without a corresponding reduction in penetration (e.g., by using pulse coding)? Can the operator dependency of ultrasound imaging be eliminated or at least significantly reduced by using 3D and 4D (i.e., real-time 3D) imaging? Can successful contrast-specific imaging techniques for high-frequency applications be developed?

(7) Multimodality systems. Is the additional expense of a dual-modality system worthwhile? It ties up 2 scanners when, in general, only 1 is being used at a time. Separate systems offer more flexibility, because they can be used simultaneously, and perfectly good image registration algorithms are available to join the images in postprocessing. Will MR/PET systems require compromises to both systems, making them perform poorly compared with standalone devices? How strong can the magnetic field be in a combined system, and will it be strong enough for useful research?

(8) Animal handling and anesthesia. Given the dramatic effects of anesthesia on animals, how can small animal imaging results be translated to humans? Effort should be focused on developing technologies that allow the imaging of unanesthetized animals.

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