Preclinical Multimodality Imaging in Vivo

David B. Stout, PhD[a], Habib Zaidi, PhD, PD[b,*]

KEYWORDS

• Small-animals • Multimodality imaging
• Molecular imaging • Image fusion • Biomedical research

WHY MULTIMODALITY IMAGING?

The field of diagnostic imaging encompasses a wealth of modalities that are fundamental for assessing and managing patients requiring medical care. Conventional radiologic imaging modalities, such as plain film radiography, and modern techniques, such as CT[1] and MR imaging,[2] can be used to evaluate a patient's anatomy with submillimeter spatial resolution to distinguish structural abnormalities and to evaluate the location and extent of disease. These techniques also offer reasonably fast scan times, precise statistical characteristics, and good tissue contrast, especially when contrast media are administered to a patient. MR imaging can be combined with functional MR imaging[3] or magnetic resonance spectroscopy[4] to measure regional biochemical content and to assess metabolic status or the presence of neoplasia and other disease conditions in specific tissue areas.

In contrast to the anatomic imaging techniques, functional imaging modalities, including conventional 2-D planar scintigraphy, single photon emission CT (SPECT),[5] and positron emission tomography (PET),[6] assess regional differences in the biochemical status of biologic tissues and organs. This is performed by administering a biologically active molecule or pharmaceutical that is radiolabeled and accumulated in response to its biochemical attributes. That is, these techniques rely on the tracer principle in which a minute amount of a radiotracer is administered to assess physiologic function or the biomolecular status of a tissue, tumor, or organ within a patient. Similar to other biologic imaging techniques, such as optical imaging (OI),[7] PET can be used to study the cellular and molecular processes associated with disease. The lower spatial resolution and high statistical noise inherent to the procedure compared with anatomic imaging persuaded clinicians to allude to this modality as "unclear medicine," although this is becoming less true with new advances in submillimeter SPECT imaging.

The common practice is that patients receiving medical diagnosis typically undergo anatomic and functional imaging from commonly available stand-alone medical imaging systems. The anatomic images usually are viewed side by side or fused using image registration software with the functional images when desired. Nevertheless, many practitioners witnessed fundamental potential for multimodality imaging in the sense that it offers essential features for diagnostic studies and patient management.[8,9] First, the anatomic and functional information are complementary and not redundant. As noted previously, anatomic imaging is performed with techniques, such as CT or MR imaging, that have excellent spatial resolution and signal-to-noise characteristics, but that may offer low specificity for differentiating disease from normal structures. In contrast, nuclear imaging generally targets a specific functional or metabolic signature in a way that can be

This work was supported by grant no. SNSF 3100A0-116547 from the Swiss National Foundation.
[a] Crump Institute for Molecular Imaging, Department of Molecular and Medical Pharmacology, The David Geffen School of Medicine at UCLA, 570 Westwood Plaza, CNSI Building, Room 2151, Los Angeles, CA 90095, USA
[b] Division of Nuclear Medicine, Geneva University Hospital, CH-1211 Geneva, Switzerland
* Corresponding author.
E-mail address: habib.zaidi@hcuge.ch (H. Zaidi).

PET Clin 3 (2009) 251–273
doi:10.1016/j.cpet.2009.03.001

highly specific but generally lacks spatial resolution and anatomic cues, which often are needed to localize or stage a disease or plan therapy.[10,11] Similarly, the availability of correlated functional and anatomic images improves the detection of disease by highlighting areas of increased radiotracer uptake on the anatomic images, whereas regions that look abnormal on the anatomic images can draw attention to a potential area of disease where radiopharmaceutical uptake may be low. The information from CT or MR imaging supplements that from nuclear imaging and vice versa; therefore, it generally is advantageous to view CT and nuclear images side by side during diagnostic interpretation. In other cases, it can be valuable to view fused dual-modality images in which the SPECT or PET data are presented as a color overlay on gray-scale CT or MR images. Multimodality imaging can be used to guide radiation treatment planning, for example, by providing anatomic and functional data that are important for defining the target volume and indicating normal regions that should avoid irradiation.[12] Similar roles are played when the dual-modality data are used to guide surgery, biopsy, or other interventional procedures.[13]

In addition, multimodality imaging provides complementary information that cannot be discerned easily from one type of image modality alone. This is best illustrated in oncologic applications where anatomic imaging often is needed to differentiate whether or not a radiopharmaceutical has localized in sites of disease (eg, in the primary tumor, lymphatic system, or metastatic site) or as part of a benign process (eg, in the gastrointestinal tract, urinary system, or in sites of inflammation).[14] An example of combining multiple imaging modalities to address a basic research question is shown in **Fig. 1**, where OI, PET, and CT are used together, each playing to its own strengths, to demonstrate the feasibility of using recombinant human adenoviral vectors to detect nodal metastases in a human prostate cancer model.[15]

CLINICAL HISTORY OF MULTIMODALITY IMAGING

Traditionally, multimodality imaging was achieved through the use of software-based image registration and fusion to correlate anatomic (CT and MR imaging) and functional (SPECT-PET) information in clinical and research settings.[16,17] Depending on the application and regions of the body involved, this has been performed using rigid or nonrigid registration approaches. Rigid-body registration basically involves simple geometric transformations, such as translation and rotation, to match the two image data sets. These techniques have been successfully applied to brain studies, where the skull provides a rigid structure that preserves the geometric relationship of regions within the brain, and have been routinely used worldwide since the 1990s in clinical and research settings.[18–20] The solution to the image registration problem becomes more complicated, however, when applied to other regions of the body (eg, thorax and abdomen) where the body can bend and flex. This is true particularly when the functional (SPECT-PET) and anatomic (CT–MR imaging) data are acquired in separate sessions on stand-alone systems, often in different locations and on different days. In this case, geometric relationships between different anatomic regions might be affected by the shape of the patient bed; the orientation of the body and limbs (up or down) during scanning, which is dictated by the modality; internal organ shift between the two procedures; and the respiratory state of patients. In these situations, the image registration process might result in good matching of only one particular region of a patient's anatomy, not necessarily the whole scanned region. Nonrigid registration (warping) has been introduced as a technique to improve registration accuracy over a larger region of a patient's body. Software-based image registration is challenging and time consuming in most cases, thus limiting its use to academic institutions with advanced technical support that can accommodate the requirements of these procedures (scanning on both modalities on the same day using carefully matched anatomic positioning and respiration protocols).[21,22]

Although the introduction of clinical, hardware-based, dual-modality imaging systems in the clinic is fairly new, the prospective advantages of combining anatomical and functional imaging has been recognized by radiological scientists and physicians since the inception of medical imaging.[23] Many of the pioneers of nuclear medicine acknowledged that the capabilities of a radionuclide imaging system could be augmented by adding an external radioisotope source to acquire transmission data for anatomic correlation of the emission image. The conceptual designs were never reduced to practice or implemented in an experimental or a clinical setting, however, until Hasegawa and colleagues (University of California, San Francisco) pioneered in the 1990s the development of dedicated SPECT-CT[24,25] and, later, Townsend and coworkers (University of Pittsburgh) pioneered in 1998 the development of combined PET-CT imaging systems, which have the capability to record emission and transmission x-ray CT data for correlated functional/structural

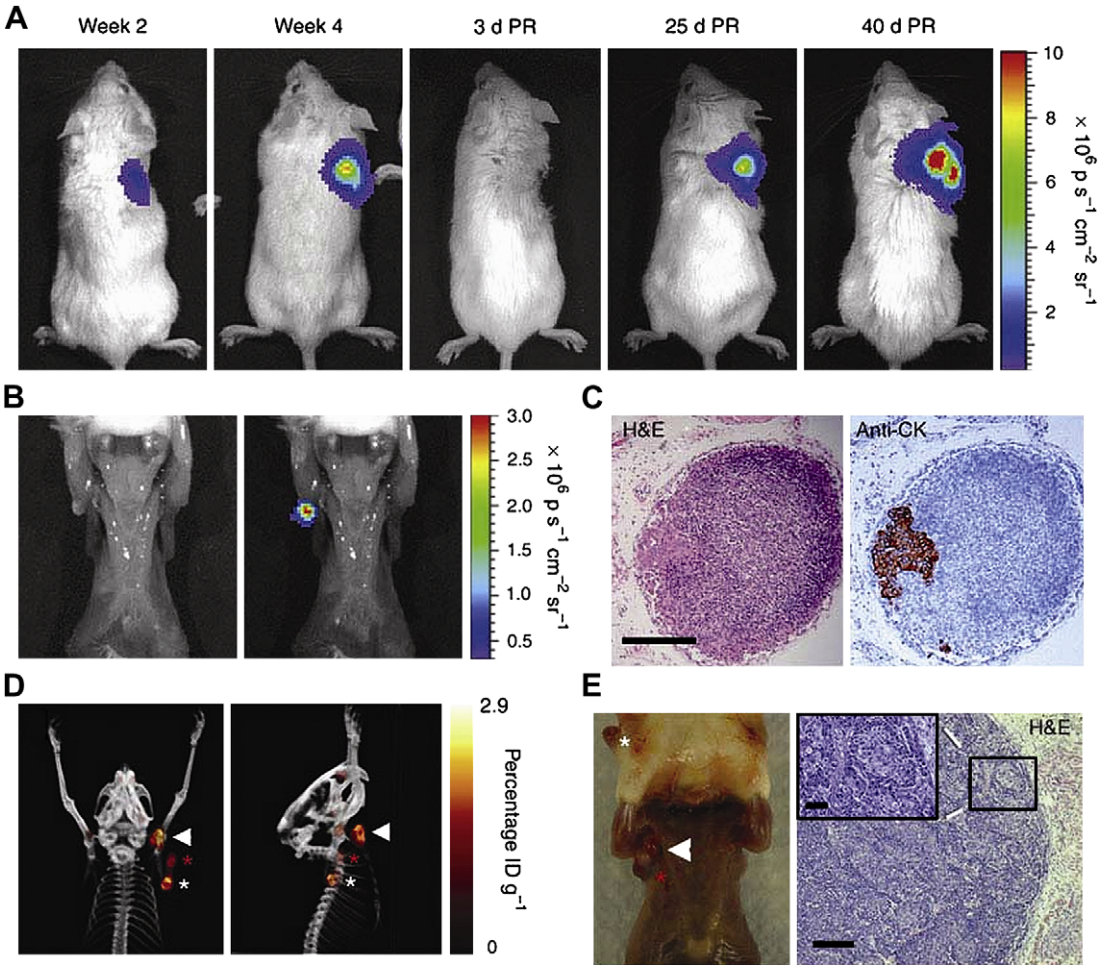

Fig. 1. (*A*) Representative images of mouse with LAPC-9–VEGF-C–GFP–RL tumor cells grafted on right shoulder to promote metastasis to brachial and axillary lymph nodes. Kinetics of tumor growth can be monitored by bioluminescence imaging of *Renilla* luciferase–expressing tumor cells. Color bar represents photons s^{-1} cm^{-2} sr^{-1} (p $s^{-1}cm^{-2}$ sr^{-1}); PR, post resection. (*B*) Bioluminescence (*Renilla* luciferase activity) in exposed ipsilateral axillary lymph node of mouse with tumor 4 weeks after implantation: (*left*) photo; (*right*) overlay. (*C*) Histologic analysis of photon-emitting lymph node (*B*) shows subcapsular microscopic lesion as visualized by staining with hematoxylin-eosin (*left*) and with antibody to human cytokeratin (anti-CK) (*right*). (*D*) PET-CT imaging with F18-fluorothymidine at 30 days after resection of primary tumor shows extensive metastases (color bar represents percentage injected dose per gram, ID g^{-1}). (*E*) Photograph (*left*) of this mouse shows extensive metastases in ipsilateral primary axillary (*white arrowhead*) and accessory axillary (*red asterisk*) lymph nodes. Primary tumor regrowth (*white asterisk*) also is present. Hematoxylin-eosin staining (*right*) shows extensive infiltration by tumor cells, distending axillary lymph node. Higher magnification inset (*inset*) of boxed region shows that tumor cells make up most of population. Scale bars, 200 μm (*C, E [right]*), 50 μm (*E [right (inset)]*). p s^{-1} cm^{-2} sr^{-1}, photons per second per centimeter squared per steridian. (*Reprinted from* Burton JB, Johnson M, Sato M, et al. Adenovirus-mediated gene expression imaging to directly detect sentinel lymph node metastasis of prostate cancer. Nat Med 2008;14:882–8; with permission.)

imaging.[14,26] Thereafter, SPECT-CT and PET-CT dual-modality imaging systems were introduced by the major scanner manufacturers for routine clinical use in 2001. Since that time, the number of combined PET-CT units sold annually has increased steadily owing to their wide clinical acceptance, leading to manufacturers completely stop the production of stand-alone PET scanners, replacing them with combined PET-CT units since 2006.

Although virtually all commercial clinical dual-modality systems have been configured in the form of PET-CT or SPECT-CT, several investigators have proposed and constructed prototype

preclinical systems that combine PET with MR imaging.[27–31] An overview of preclinical PET instrumentation is beyond the scope of this review. Readers are referred to the review by Levin and Zaidi.[32] Preclinical imaging entails difficult challenges for building systems with micrometer-level tolerances and sensitivity/resolution issues are not easily resolved. Much worthwhile research was performed to address the important challenges that must be overcome in implementing and operating combined PET–MR imaging or SPECT–MR imaging systems. One manufacturer developed a prototype PET–MR imaging system dedicated to high-resolution brain imaging,[33] and there are clear indications that several manufacturers are working toward the development of whole-body PET–MR imaging systems. In parallel, potential applications of this technology are being explored, as reported in the scientific literature.[33–35]

CHALLENGES IN PRECLINICAL SETTING

Animal Handling

Working with animals raises challenges not only in imaging system design and construction but also with related regulatory oversight and biosafety concerns. There are ongoing needs to train personnel in animal handling techniques, including surgical and injection skills needed to prepare and image animals. Space must be specifically configured for animal housing, with associated heating, light cycles, cage changing, and access control. In particular, biosafety control of carcinogenic or other hazardous materials may require considerable effort to address.

Several institutions have put the required training information online, including links to sites with information as to how training may be obtained.[36–38] Information about biosafety levels,[39–41] proper protection strategies, and garbing can be found online.[42,43] Advanced facilities have been designed with strong financial support by the National Institutes of Health[44] to establish a network of scientists active in collecting research data linked to small-animal models of human disease (eg, Mouse Models of Human Cancer Consortium[45]) to provide larger access to various mouse models to active investigators in the field.

Anesthesia and Heating

Most preclinical imaging systems require animals to remain motionless for several minutes to hours to obtain useful data; thus, some type of anesthesia is necessary. There is considerable interest in imaging without anesthesia, because conscious animals presumably have normal metabolic functions compared with those under anesthesia. Several groups have shown that this is feasible under some circumstances.[46–48] In particular, a group at Brookhaven National Laboratory (Upton, New York) has developed a rat conscious animal PET scanner (RatCAP), a complete 3-D tomograph designed to image the brain of an awake rat,[49] which incorporates the PET system into an integrated, compact arrangement of lutetium oxyorthosilicate/avalanche photodiode (LSO/APD) arrays with highly integrated electronics.[50] Nonetheless, most imaging work at present is conducted using anesthesia, and the type and injection route can play a significant role in the sedation and metabolic status of the animal.

Gas anesthesia is perhaps the most common method, offering safe, quick, and effective immobilization with quick recovery times. Isoflurane is commonly used; however, there also is increased interest in sevoflurane as it is becoming available as a generic brand at lower cost. There may be some advantage to using sevoflurane because it seems to have less effect on glucose levels compared with isoflurane.[51]

Injected anesthetics are common, although they need to be injected frequently or constantly infused to maintain a steady state of anesthesia. Commonly used injectibles include ketamine, midazolam, pentobarbital, and xylazine. These anesthetics are controlled substances and require a prescription. They must be kept under double lock and key and require careful tracking of their use. They are advantageous in that only a syringe and a vial of anesthetic are required for use. Any time animals are anesthetized, it is vital that proper heating be provided to maintain the core body temperature to prevent hypothermia. Heating is particularly important for peripheral tumors, where blood flow and, therefore, probe delivery are related to body temperature.[52]

Animal Access

Bringing detectors and collimators close to the animal offers certain advantages, including magnification, higher sensitivity, and possibly a smaller system size. Shielding for low-energy radiation, particularly with CT imaging, is necessary and requires an enclosed gantry. Unfortunately, this means that animals often are closely surrounded or sealed inside the system, leaving the animals hidden from view and access. This can be problematic for monitoring respiration and other motion problems and can make injection for probe and blood sampling difficult or impossible. Short-duration studies normally are not of concern, but longer-term studies lasting more than 30 minutes

often require adjustments to anesthesia levels. It is important to monitor respiration, because with time the breathing can become labored, requiring reduction of anesthesia to prevent large movements due to agonistic breathing motions. Respiration can be monitored by camera, ventilator settings, and noninvasive probes that can measure inhalation indirectly.[53] Monitoring systems suitable for mouse work, where heart rates can go up to 1000 beats per minute, only recently have become widely available and suitable for molecular imaging research.

Injecting probes for immediate dynamic imaging poses another challenge for imaging within confined systems. For these studies, it is necessary to place animals in a scanner and begin acquisition before injecting an imaging probe. Because of the limited blood volume of mice, this species normally is limited to injection volumes of 250 μL or less and requires an insertion of a catheter in the animal, most commonly in the tail vein or in some cases the jugular vein or femoral artery. Catheters have what is often called a dead volume, which is the amount left inside the tubing and is related to the length and interior diameter. Although it is possible to flush out this volume into an animal, this often leads to a double-pulsed injection and may require too much volume due to the probe/saline valve dead volume. If a syringe is removed from the catheter to place a saline syringe for flush, there is risk for the radioactive probe coming back through the tubing due to backpressure in the line from the animal's blood pressure, which can lead to spills or inaccurate injection activity measurements.

Physiologic Monitoring

In some cases, monitoring physiologic parameters, such as heart rate, temperature, respiration, or blood pressure, may be necessary. Fortunately, several options are available to measure these in small rodents. Although visual measurement is possible in some imaging systems, a measurement system, preferably one that can generate computerized output files, often is preferable for tracking and storing the physiologic data.

Heart rate can be measured by needle probes inserted into the skin; by leads placed on the skin surface, pressure cuffs; or by infrared probes. Respiration can be measured using optical probes, cameras, or pressure cuffs. Temperature usually is assessed using a rectal thermocouple, but this is somewhat invasive and can lead to perforated bowel if not inserted carefully. One option for temperature is to carefully control the environment. Because anesthetized mice have little heat capacity, they quickly equilibrate with ambient temperature at the ambient temperature. Often, monitoring systems suitable for one imaging modality are not ideal for other modalities, so that probes within the field of view must be appropriately selected to minimize artifacts and changes to the image data.

One area where respiration and heart rate measurements are essential is for gated acquisitions. Trigger signals for each breath or heart beat are sent to the imaging system, which trigger the acquisition or enable the image data to be divided up into various parts of the respiratory or heart rate cycles. These trigger signals can start or stop the image acquisition or can be put into the data stream for postprocessing.

Study Duration and Hydration

Modalities, such as PET and SPECT, allow static imaging and dynamic imaging over time. Static imaging, analogous to a snapshot at a given time, is useful for looking at the end-stage accumulation of probe after specific uptake and nonspecific clearance. Most common, perhaps, is F18-fluorodeoxyglucose (FDG) imaging approximately 1 hour after injection. This method allows many animals to be imaged in a short time period. For example a 10-minute imaging time allows 4 to 5 animals to be imaged per hour, making efficient use of the radioactive probe.

Dynamic imaging requires placing animals within a scanner and collecting data beginning from time of injection, typically for 60 to 90 minutes. Longer imaging times means fewer animals imaged per day and per radiochemistry synthesis. There also is the complication of injecting within the imaging system, where access to the animal likely is limited.

Despite the additional demands, dynamic imaging allows data to be obtained over time that can be used to estimate biodistribution, radiation dosimetry, and metabolic rate constants, which are true measures of biologic function rather than only endpoints. Whether or not using graphic methods (Patlak or Logan plots) or compartmental modeling, the blood and tissue time activity data are needed to estimate the rate constants.

In some instances, the endpoint may be the same in static and dynamic studies, but the process of getting there may differ and reveal insights to biologic processes. For example, intraperitoneal FDG injections compared with tail vein injections have different temporal and spatial probe distributions, but most organs have the same activity 1 hour after injection.[52] Another example is the tumor uptake of F18-fluorothymidine (FLT)

and FDG after radiation therapy, where both were the same at 1 hour although the uptake paths were different between control and irradiated tumors.[54]

Maintenance of near normal physiologic conditions is important when conducting dynamic studies, because changes in temperature, anesthetic state, hydration, breathing, and heart rate can have an impact on the biologic process under investigation. Hypothermia and hyperthermia are serious concerns, especially in small animals that have little heat capacity. Depth of anesthesia plays a role in heart rate and, perhaps most importantly, in breathing. Rodents require less gas anesthesia with time and, if not checked, agonistic breathing can result, causing large breathing motions that degrade the resulting images. For long studies, more than 90 minutes, in small rodents, dehydration can result, especially with the dry, moisture-free gases used with gas anesthesia. For these studies, subcutaneous injections of saline or slow saline infusions often are helpful.

Submillimeter Level Accuracy

Preclinical imaging often challenges the spatial resolution of imaging systems by attempting to look at small objects, for example, brain anatomy in rats or mice. In these cases, spatial resolution often is not sufficient, leading to problems with spillover activity and partial volume effects. The challenge for makers of these imaging devices lies in obtaining submillimeter resolution while maintaining reasonable signal-to-noise characteristics and measurement sensitivity. In addition to resolution, the imaging systems ideally need to reproducibly place the animal into a known position for coregistration with other modalities and to match previous experiments with the same animal. Achieving submillimeter reproducible positioning is a mechanical challenge and is not a trivial endeavor.

Lack of Image Format Standard Across Modalities

In clinical environments, the need for standardized image formats to enable medical personnel to evaluate multiple image information sources led to the development of Digital Imaging and Communications in Medicine (DICOM), a semi-standardized format for radiologic and other types of medical information. Unfortunately, there is not yet a standardized format for preclinical information, in part because of the wide range of image data sources (ultrasound, PET, CT, MR imaging, and OI). Each imaging modality often has its own unique image information; thus, a unified image format remains elusive. For example, optical data might require tracking light wavelengths, camera settings, stage height, and binning factors, whereas PET data require information about injected probe activity and type, dynamic framing sequences, voxel sizes, reconstruction parameters, and so forth. An image header format that attempted to include all divergent sources of information would quickly become far too large, and future information types will become necessary as new methods and modalities evolve.

Immunocompromised Animals, Biohazardous and Infectious Agents

One of the areas on which molecular imaging has had a huge impact is cancer imaging. Oncology research has greatly benefitted from the ability to image the same animals noninvasively over the course of a disease, treatment, or intervention. Understanding of rodent genomes and ability to create genetically modified strains has led to thousands of knockin and knockout deletions and insertions of specific genes or combinations of genes. Perhaps most fundamental are the nude and severe combined immunodeficiency mouse strains, which are missing the thymus and T cells or the thymus, T and B cells, and DNA repair mechanisms through a mutation in chromosome 16. These mice are extremely useful because they cannot reject implanted tumors, making it possible to study human xenografts over time and with various treatments. With suppression of the immune system comes the need to protect these animals from pathogens in the environment; thus, barrier facilities and imaging chambers are essential for maintaining the health of these animals.

Often investigators are interested in creating or treating animals using biohazardous or infectious agents. These might be viral vectors for gene insertion, chemotherapeutic agents, or perhaps bacteria or engineered cells. Certainly the activation and early response of the immune system is an interesting area of study, but this work is difficult to conduct given the necessity of protecting the researchers and other animals housed nearby. Use of these agents requires controlled environmental conditions, biosafety containment, and locating imaging systems within controlled areas or using sealed imaging chambers to isolate the hazardous agent.

COREGISTRATION OF SEPARATE DATA VERSUS SAME GANTRY ACQUISITION

As discussed previously, several techniques have been developed to coregister clinical multimodality medical imaging data (see articles by Maintz

and Viergevera[55] and Pluim and colleagues[56] for review). Widely available image registration techniques developed specifically to address the needs of clinical imaging have yet to be translatable for small-animal imaging applications. Some investigators attempted to adapt popular image registration techniques using various combinations of functional and anatomic imaging data.[57,58] These studies reported various degrees of success when applying these algorithms in various scenarios.[59–66] Some of these techniques use external fiducial markers that are visible in the two-image datasets to be registered, which are attached to the animal body. Those techniques have been widely used for dual-modality image registration (eg, CT or MRI or, alternatively, SPECT or PET), although this is more challenging to accomplish for multimodality imaging where various modalities are involved. As PET and SPECT imaging probes become more targeted and nonspecific activity is eliminated, there may not be sufficient information contained in metabolic images to coregister with anatomic data. Inexpensive and easy-to-manufacture animal-specific molds also can be used for image registration of preclinical studies (accuracy within $\pm$1–2 mm for sequential PET images).[67] Other possible solutions for sequential imaging with combined PET-CT imaging include imaging chambers that can be rigidly and reproducibly mounted on single-modality preclinical scanners with submillimeter accuracy (**Figs. 2** and **3**).[68] More refined immobilization devices also were suggested, with registration accuracy of approximately 0.2 to 0.3 mm.[69] Other strategies for the design of dual-modality systems, including a rail-with-sliding-bed approach and various rail-based, docking, and click-over approaches for anatomic-molecular imaging fusion, also are being explored.[70]

More sophisticated techniques rely on unsupervised algorithms that do not involve user interaction. The simplest form of automated image registration techniques uses a rigid body transformation where an affine transformation model, which permits only global translations, rotations, scaling along each of the three axes, and shearing deformations, is determined and applied to the floating image.[71] In this case, the solution to the image registration task yields 12 parameters that are embedded in a transformation matrix. The drawback of such techniques is that they tend to ignore organ deformation owing to issues discussed previously (differences between shapes of the gantry bed, internal organ shift, respiratory motion, and so forth). To address these limitations, nonrigid registration algorithms that permit compensation for perceived organ deformation for different modalities, or that even spatially coregister images of different animals, have been developed.[22,57,58] Several companies have recently begun offering chamber-based solutions, including m2m Imaging Corp. (Cleveland, Ohio), ASI (Eugene, Oregon), and Bioscan (Washington, DC). Notwithstanding the significant advancements in the field, robust multimodality image registration for small-animal imaging remains challenging and likely will continue to be an active research field for years to come.

The availability of multimodality imaging systems facilitates the process of acquiring functional and anatomic data from animals in a consistent configuration and during a single study in a way that is faster and more cost efficient than attempting to register the images by software after they are acquired on separate imaging systems. In this regard, recently introduced dual-modality techniques consisting of two hard-wired systems (eg, SPECT or PET and CT or MR imaging) offer a critical advantage over separate anatomic and radiotracer imaging systems in correlating anatomic and functional images without moving animals. Only table translation is required for sequential systems (eg, PET-CT) whereas most recent technologies (PET-MR imaging) allow simultaneous scanning given that the PET insert

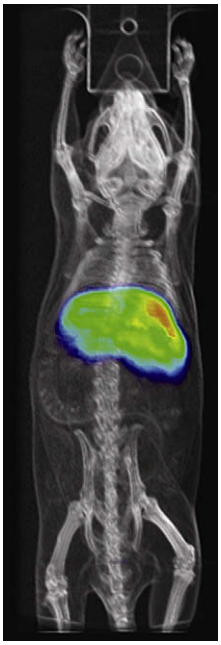

Fig. 2. Example of projection image showing all 3-D data for combined PET-CT scan using FDG in a tumor-bearing mouse. Using this view, all parts of data are visible as one image.

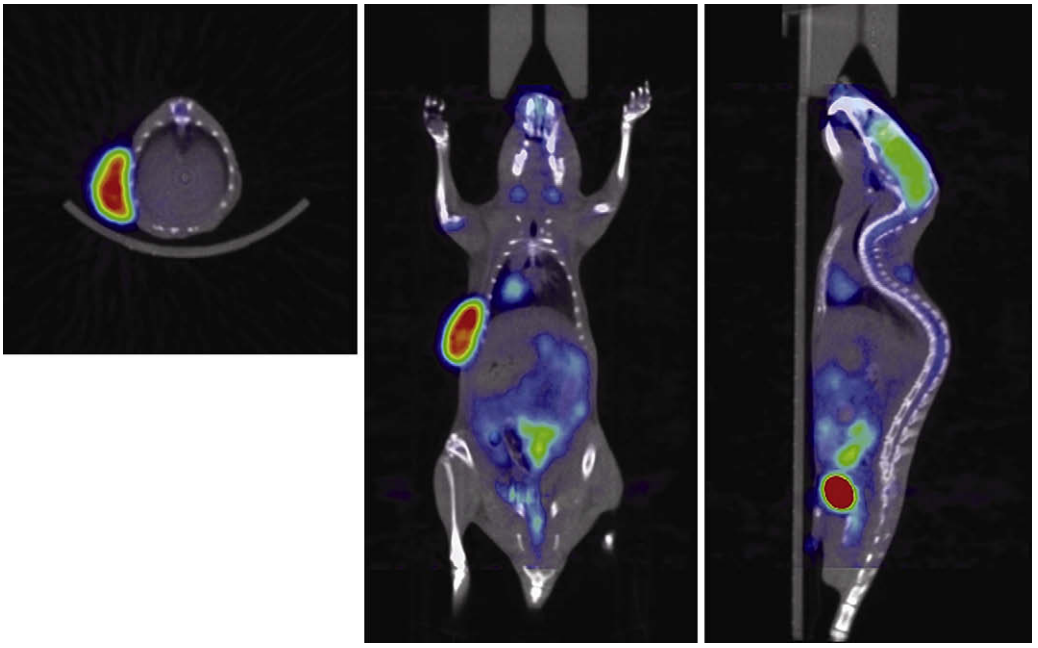

Fig. 3. Examples of transverse (*left*), coronal (*middle*), and sagittal (*right*) views of FDG uptake imaged in a mouse using PET and CT. These views enable detailed examination of all image data on slice-by-slice basis.

is introduced within the MR imaging scanner[30] (described later).

Multimodality imaging systems are designed to account consistently for differences in reconstruction diameter, offsets in isocenter, image reconstruction coordinates, and image format (eg, 512 × 512 versus 128 × 128) between the CT–MR and SPECT-PET image geometries to perform image coregistration and image fusion. Depending on the design of the system, image registration software may be needed to account for table sag or for misalignment when the animal moves between the CT-MR and SPECT-PET image scans. Generally, the coordinate systems implicit in the SPECT-PET and CT–MR image geometries are calibrated with respect to each other using fiducial markers that are scanned with both CT-MR and SPECT-PET imaging. The image registration must be confirmed to avoid misregistration errors in the multimodality images or in the SPECT-PET image reconstructed using CT- and, likely in the future, MR imaging–derived attenuation maps.

SIMULTANEOUS ACQUISITION VERSUS SEPARATE TEMPORAL DATA

Certain advantages exist with the acquisition of simultaneous data. As discussed previously, there would be no need to coregister separate datasets or worry about different experimental conditions. Metabolism (PET or SPECT), functional activity (MR imaging), and anatomic (CT or MR) information could be acquired with temporal and spatial matching. The drawbacks of this approach have, until recently, been problematic, requiring giving up the optimal imaging capabilities of one or more modalities. For example, Goertzen and colleagues designed a simultaneous PET-CT system,[72] although the partial ring design reduced the PET sensitivity to make space for the CT source and detector.

Current designs of PET–MR systems using an insert approach exhibit no change in MR imaging signals while maintaining a good PET imaging capability.[27–30,73–75] The PET currently is somewhat limited for axial extent compared with stand-alone PET systems; however, this is likely to improve in the future. Another approach was to cut the MR imaging magnet in half and insert a PET ring;[76] however, this proved difficult and expensive to produce and required a specialized magnet. Use of a PET insert enables using existing MR imaging systems for research and no specialized production or limitations on the MR imaging system.

Currently, the majority of multimodality research acquires sequential images by moving a bed from one location to another within a common gantry or by moving a bed or chamber from one system to another. Many preclinical imaging systems are sold with multimodality capability. For example, the Inveon (Siemens, Knoxville, Tennessee) and GE Healthcare/Gamma Medica-Ideas Flex Triumph

(Northridge, California) and Bioscan NanoSPECT/CT systems have a common CT and SPECT or PET gantry that allows sequential acquisition without moving the bed. In a docked or multimodality gantry configuration, PET imaging is acquired by moving the bed to another location. The sequential approach almost always is required when using PET imaging, because this modality requires a ring of detectors. Other methods, such as CT and SPECT, often image by rotating the detectors, so more than one modality is possible at the same time or at least mounted to the same gantry.

Sequential imaging in a preclinical setting has existed for many decades. Prior to high-resolution systems currently available, research typically used larger primates, canines, pigs, and other large species. These animals were imaged in clinical systems, often with rudimentary attempts to position the animals in the same position for both imaging systems, often PET and CT or MR imaging. In the late 1990s and early 2000s, combination PET-CT clinical systems quickly took over the market. It took nearly 7 years for those same combinations to become prevalent in preclinical systems, in part because of the complexity of the systems and the often micrometer-level tolerances for construction and positioning.

One solution to sequential imaging was developed at the University of California, Los Angeles (UCLA), for using PET and CT together through the use of imaging chambers.[68,77] This approach integrated heating, anesthesia, and positioning within a chamber having a common mounting plate for PET and CT beds. This hardware, when positioned in the same location every time for imaging, enabled a fixed translational offset to coregister the data, eliminating the often difficult task of software registration. Further refinements with software enable automated processing to create the fused images without user interaction, thus simplifying the creation of data and lessening the burden of the imaging scientist.

The drawbacks to sequential imaging include the need to move animals between positions in a gantry or between imaging systems. This requires changing connections for heating and anesthesia and perhaps physiologic monitoring systems. There always is a risk in moving animals in that they no longer are in the same orientation as in the previous imaging work. There also are anesthesia and temporal changes that may alter the orientation or physiology of the animal and make coregistration problematic. A single gantry system would have slower throughput, as animals are imaged first in one, then another, modality. Separate systems enable higher throughput, but this may increase the chance of movement and doubles the work for investigators because more animals are imaged at the same time.

DATA COLLECTION STRATEGIES (DYNAMIC VERSUS STATIC INFORMATION)

Routine PET scanning protocols used in clinics usually involve data acquisition in a static mode where collected events are stored in a scanner-specific predefined projection or sinogram format. An image reconstruction algorithm is then applied to the data set to yield a static or sum image over the whole acquisition duration of the study. The advantage of this protocol is simplicity of use and acceptable image quality produced as a result of the good statistics that can be acquired over the whole study duration. The major drawback is the absence of information of tracer kinetics that prevents the extraction of physiologically relevant parameters, thus limiting the analysis to straightforward semiquantitative parameters, such as the standardized uptake value in oncologic imaging.

One important aspect of nuclear medicine imaging, including PET, is the inherent capability to perform dynamic imaging, taking advantage of the high sensitivity offered by high-end multiring PET systems. This is a remarkable capability, allowing measurements of change in the biodistribution of radiotracers within the tissues or organs of interest over time. This in turn provides valuable information about the underlying physiologic or metabolic processes being investigated, allowing extraction of relevant parameters using kinetic modeling techniques.[78]

Dynamic PET data acquisition can be performed using one of two common approaches. In the first and standard approach, the desired framing sequence is prespecified before data acquisition and the detected events are binned online in the sinogram corresponding to each frame. In this case, static images also can be obtained by summing the data acquired in image or in sinogram space. In the second approach, available on modern clinical and almost all preclinical PET scanners, the detected events are stored in a so-called list-mode format,[79,80] where the parameters characterizing each coincidence event (time of detection, spatial coordinates of interaction points, and energy if required) are written on disk. Therefore, the list-mode acquisition capability permits the extra flexibility to users by allowing the specification of the framing sequence post acquisition to optimize the framing sequence based on the probe kinetics. Another advantage of list-mode data acquisition is the possibility of applying direct list-mode reconstruction, which

proved to have many added benefits compared with conventional reconstruction from binned projection data.[81] Alternatively, the standard approach for dynamic PET image reconstruction consists of independently reconstructing images corresponding to each dynamic frame. In either case, the result consists of a set of dynamic images containing information about tracer kinetics in the regions of interest.

OVERVIEW OF CURRENT SYSTEMS

Photograph Plus Data—Optical

A pseudomultimodality approach is used by several OI systems that use charge-coupled device (CCD) digital cameras to collect light information. Because these systems are suitable for low and high light flux uses, photographic images can be superimposed on top of the in vivo optical signals to provide a measure of spatial information. Despite using the same detector, the information obtained from the two methods comes from different sources. Photographs help with visual orientation of the subjects, whereas in vivo optical signals are related to the fluorophore or bioluminescent signal coming from within the animals.

Several manufacturers offer optical-photographic systems, including Caliper Life Sciences, Hopkinton, MA (formerly Xenogen), Carestream, Rochester, NY (formerly Kodak), and Cambridge Research and Instrumentation (Woburn, MA). The IVIS 3-D system (Caliper) takes the photographic information one step further and creates a spatial map in 3-D using transillumination to estimate the source of the light coming from the animals. IVIS fluorescent systems decode spectral information using a series of back to back image acquisitions using different filter wheels to separate auto fluorescence from specific signal. This method enables acquisition of divergent information using the same imaging system to gain additional information from a subject. The Maestro system (Cambridge Research and Instrumentation) uses a tunable liquid crystal to obtain multispectral wavelength information, enabling decoding of multiple fluorophores with one scan, which are then overlaid on photographic images for spatial orientation. Timing information can be used to decode the optical signal, such as the eXplore Optix system from Advanced Research Technologies (Montréal, Quebec).

PET-CT and SPECT-CT

Similar to commercial clinical dual-modality systems, which have been configured in the form of SPECT-CT or PET-CT scanners, several investigators proposed and in many cases have implemented and tested prototype preclinical dual-modality systems that combine SPECT with CT and PET with CT.[82–84] The popularity of using animal models as models of human disease stimulated the growth of preclinical CT systems, which incorporate a low power x-ray tube and a phosphor-coupled CCD camera or similar 2-D x-ray imaging detector to achieve spatial resolutions as high as 25 μm or better.[85–88]

Taking advantage of the availability of high-resolution preclinical x-ray imaging systems, several investigators have developed and are continuing to develop dual-modality imaging systems specifically designed for imaging small animals. Cherry and coworkers have developed a combined preclinical PET-CT imaging system.[72] The PET detectors use an LSO scintillator coupled to a fiberoptic taper to a position-sensitive photomultiplier tube. These are placed on opposite sides of an animal with the annihilation photons from the positron emission detected in coincidence. The system includes a preclinical CT system having a microfocus x-ray tube and an amorphous selenium detector coupled to a flat panel thin film resistors readout array.[89] A second system was built later by the same group, who designed a novel preclinical CT scanner using photodiode detectors that have a flexible C-arm gantry design with adjustable detector positioning that was integrated with the microPET II scanner [2400].[90] A flexible design of a commercial system (Gamma Medica-Ideas) also can be configured as PET-CT (discussed later).

As an alternative to this design, the Sherbrooke group led by Lecomte is working toward a combined PET-CT system based on the LabPET scanner,[91] developed by the same group (now commercialized by Gamma Medica-Ideas), where PET and CT data are acquired using the same detector channels and electronics, thus allowing true simultaneous PET-CT scanning with the possibility to count and discriminate individual x-ray photons in CT mode.[92,93] This can be achieved by sampling the analog signal using high-speed analog-to-digital converters and digital processing in field-programmable gate arrays. The parallel architecture and fast digital processing electronics allow high count rates for PET and CT modes whereas the modularity of the system design allows extending the number of channels by 10^4 or more.

SPECT-CT systems designed specifically for small animal imaging are being developed by several investigators.[84,94–103] One of the first small animal SPECT-CT systems was developed by a consortium that included the Thomas Jefferson National Accelerator Facility (Jefferson

Laboratory), University of Virginia, and researchers at the College of William and Mary.[96–98] These systems use a compact scintillation camera that operates with multiple Hamamatsu R3292 position-sensitive photomultiplier tubes (PSPMTs) coupled to a pixelated array of cesium iodide crystals using pinhole and parallel-hole collimators. The x-ray data are acquired using a small fluoroscopic x-ray system (Lixi, Downers Grove, Illinois).[96]

Gamma Medica-Ideas has developed and introduced a small animal SPECT-CT system[95,100] with two compact scintillation cameras[104–106] and a high-resolution CT subsystem[100] for dual-modality imaging of rodents and other small animals. The SPECT camera can be operated with pinhole collimators that provide submillimeter spatial resolution in the reconstructed images or with parallel-hole collimators when higher detection sensitivity or whole body imaging is desired. The system includes a high-resolution preclinical CT subsystem[100] configured with a complementary metal-oxide semiconductor (CMOS) x-ray detector coupled to a gadolinium oxysulfide scintillator and a low-power x-ray tube. The preclinical CT system provides anatomic imaging with a spatial resolution of approximately 50 μm; the resulting x-ray data can be used for attenuation correction and for anatomic localization of the radionuclide data. In addition, the SPECT data can be acquired with respiratory and ECG gating for cardiovascular imaging applications where wall-motion abnormalities, ejection fraction calculations, or other assessments of ventricular function are necessary.

Another project, launched by Barrett from the Center for Gamma-Ray Imaging, University of Arizona, aimed to develop a high-resolution SPECT-CT system for small animal imaging.[99] High-resolution SPECT is performed with a modular semiconductor detector that consists of a 2.5 × 2.5 × 0.2–cm^3 slab of cadmium zinc telluride (CZT) operated with a continuous gold electrode to apply bias on one side, and a 64 × 64 array of pixelated gold electrodes on the opposite side connected to an application-specific integrated circuit (ASIC) for readout of the individual pixel signals. The detector has a 380-mm pixel pitch and 330-mm–wide pixels coupled to a 7-mm–thick parallel-hole collimator for radionuclide imaging. The x-ray and radionuclide imaging subsystems are mounted with their image axes perpendicular to one another with the animal rotated vertically within the common field of view. The x-ray and radionuclide projection data are acquired sequentially and corrected for distortions and nonuniformities introduced by each of the detectors, then reconstructed with statistical iterative algorithms (OSEM).

The Inveon system, sold by Siemens, can be manufactured in the form of PET-CT, SPECT-CT, or all three (discussed later) in a single gantry system or docked PET with CT and/or SPECT. The Inveon PET system[107] uses block modules comprising 12 × 12 arrays of 1.5 × 1.5 × 10 mm^3 LSO crystals, arranged in a 16.1-cm diameter ring, with a 12-cm diameter bore and 10-cm transaxial and 12.7-cm axial field of view.

Positron Emission Tomography–MR Imaging and SPECT–MR Imaging

The history of combined PET–MR imaging dates to the mid-1990s, when image coregistration between separately acquired data was used to create postacquisition combined data sets.[108] At approximately the same time, an improvement in PET spatial resolution was characterized when positron annihilation takes place in a strong magnetic field.[109,110] The group from the University of Minnesota pioneered the design of the first combined system.[111] A collaborative effort between UCLA and Guy's and St Thomas' National Health Service Foundation Trust, London, followed, leading to the design of an MR imaging–compatible preclinical PET system modified to optically couple the detector crystals coupled to an external array of PSPMTs through 3-m–long fiber optics.[27,112] In this way, the combined system could acquire simultaneously PET and MR imaging data without measurable mutual interaction effects. More recently, other groups have used similar design approaches[28,29,113,114] or adopted more complex magnet designs, including a split magnet[76] or field-cycled MR imaging.[115]

A more attractive approach consists in using PET inserts that can be operated within existing MR magnets by designing suitable MR imaging–compatible PET systems using solid-state detectors that are insensitive to magnetic fields.[30,50,73–75] This includes APDs[116] and silicon photomultiplier tubes,[117,118] the latter of which look more promising for this application as they allow a significant reduction in the electronics required inside the MR imaging.[119] Several academic sites are equipped with PET inserts built using this technology that can be operated within a high-field MR imaging magnet.

Alternatively, the motivations for developing combined SPECT–MR imaging systems were addressed recently.[120] The availability of semiconductor-based SPECT detectors, such as CZT coupled with high-density low noise ASIC electronics to read out the semiconductor detectors, which are insensitive to magnetic fields, are enabling the design of integrated SPECT–MR

imaging systems. The preclinical SPECT–MR imaging prototype constructed by Gamma Medica-Ideas consists of a polygonal ring of CZT detector with a field-of-view of 2.54 × 12.7 cm^2 fitted with a parallel-hole collimator. The materials used for fabrication (shielding, support, positioning, and cooling) were carefully selected to reduce their possible impact on magnetic field homogeneity. The design of the detector and front-end electronics were optimized for spectroscopic and timing performance, minimization of power dissipation, and low electromagnetic interference.[121]

Positron Emission Tomography–Optical Imaging

OI provides a sensitive method for examining gene expression due to the low background light levels. Unlike radioactivity imaging, where the radioactive signal is always "on," bioluminescent imaging creates light only where the inserted enzyme, substrate, oxygen, and ATP are present. This ability to see very small signals enables visualization of early expression and signal changes when compared with PET imaging. PET, however, is quantitative and can provide measurements of metabolic function with only minimal scatter and attenuation in rodents. The potential combination of these two modalities has been demonstrated by Chatziioannou (combined optical imaging and PET [OPET])[122,123] and Cherry, who used different instrumentation approaches. OPET uses the same detectors to image optical and radiation signals, whereas Cherry's system uses a conical mirror placed within a small animal PET scanner and a nearby separate optical detection camera.[124] A more recent design by the German Cancer Research Center (Heidelberg, Germany) uses a radial cylindric lattice of microlens arrays (115-mm diameter), which is mounted in front of PET detector blocks.[125] A network of optical fibers is allocated on a multihole plate such that the focal points of the individual microlenses correspond locally to single fiber–ending points.

Autoradiography Combined with other Modalities

The definitive method to determine the location of imaging probes remains autoradiography (AR), a modality capable of very high-resolution imaging, with localization possible at or below 10 μm. Given light scatter with OI and resolution limitations in SPECT, MR imaging, and PET systems, AR provides the best way of determining where the imaging probe is located within an animal. Unfortunately, this method requires freezing and slicing of the animals and thus is restricted to ex vivo single measurement points. Pairing AR with other nuclear medicine–based methods (PET or SPECT) and photographic images often is used to validate the findings in the nuclear based methods.

The AR images can provide additional information to determine the true location of PET probes in vivo. For example, recent work showing gut uptake of a newly labeled PET imaging probe was shown via AR to be located in the gastrointestinal lining, rather than in the gastrointestinal lumen (**Fig. 4**).[126] This was important information, because the lining required a bloodstream probe delivery rather than a gallbladder bile excretion route, which provided valuable insight concerning the metabolic fate of the imaging probe.

Optical Imaging–CT

The combination of OI with x-ray projection imaging pairs in vivo imaging information with anatomic information. This combination proves useful for imaging radiation exposure using gels, particularly in radiation therapy situations.[127] Carestream (formerly Kodak) markets an optical x-ray integrated system that uses the same detector system for x-ray photons and optical photons. Although both image sets are planar projections, the x-ray image provides some information about the location of lung, soft tissue, and bone that may be correlated to the optical signal source.[128] A more recent technique uses a time series of images acquired after injection of an inert dye where differences in the dye's in vivo biodistribution dynamics allow precise delineation and identification of major organs.[129]

MULTIPROBE IMAGING (MULTIMODALITY WITH DIFFERENT PROBES)

PET-SPECT

The demand for multiprobe molecular imaging of small animals using single-photon and positron-emitting radiotracers has stimulated the development of dedicated small-bore high-resolution systems for rodent imaging, allowing concurrent acquisition of SPECT and PET data. One system developed to match these needs is the yttrium aluminum perovskite (YAP)-(S)PET scanner.[130] The system consists of four rotating heads spaced 15 cm apart, each with an active area of 4 × 4 cm^2, containing a 20 × 20 array of 2 × 2 × 30 mm^3 optically isolated YAP crystals coupled to PSPMTs, forming a 4-cm transaxial and axial field of view. Multiprobe scanning can be performed through energy discrimination, allowing acquisition of SPECT and PET data in different energy windows.

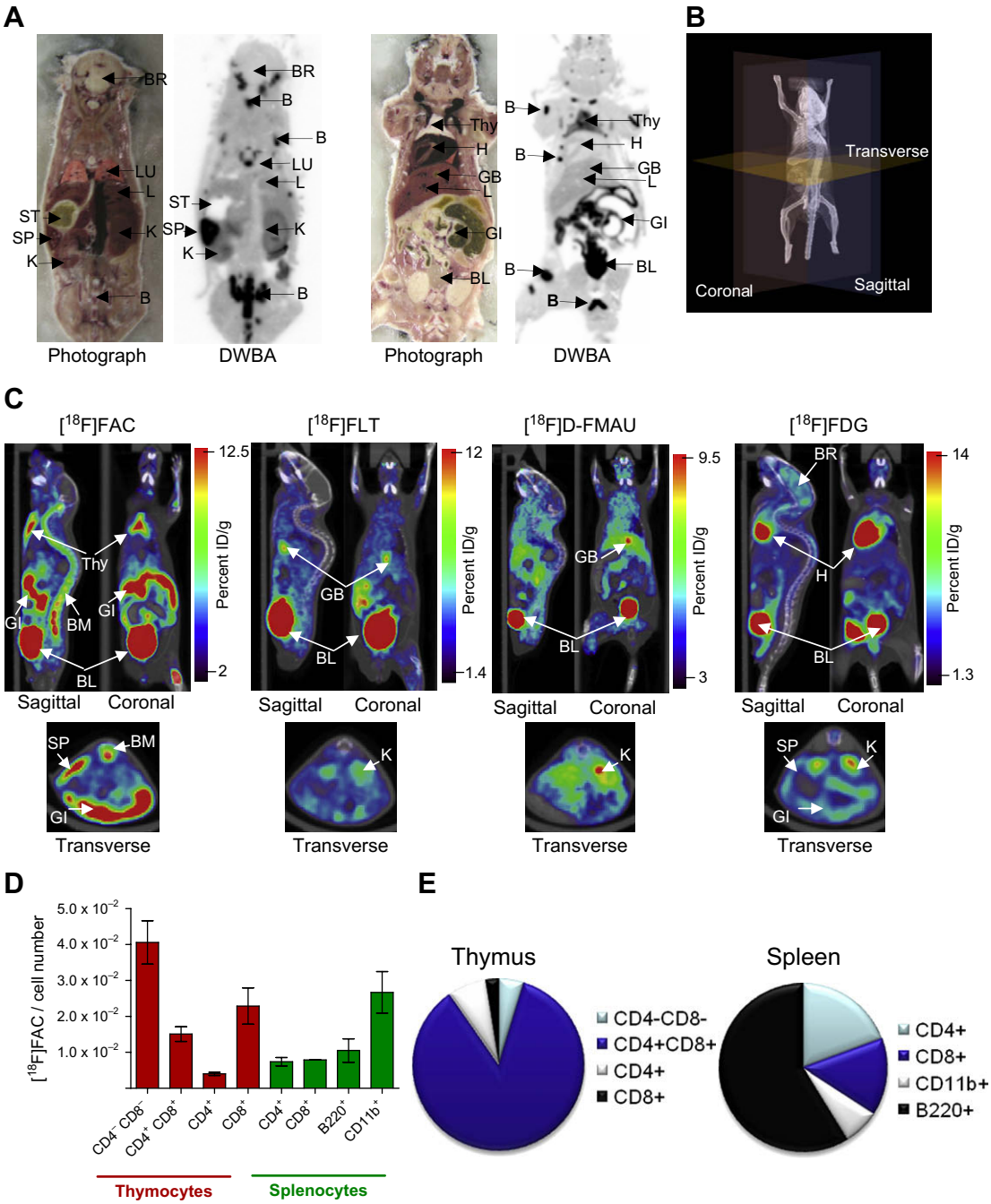

Fig. 4. (*A*) 18F-fluoroarabinofuranosyl cytosine (^{18}F-FAC) digital whole-body autoradiography shown along with corresponding tissue sections. B, bone; BL, bladder; BM, bone marrow; BR, brain; GB, gall bladder; GI, gastrointestinal tract; H, heart; K, kidney; L, liver; LU, lung; SP, spleen; ST, stomach; Thy, thymus. (*B*, *C*) C57/BL6 mice were scanned by microPET-CT using ^{18}F-FAC, F18-fluorothymidine (^{18}F-FLT), ^{18}F-D-FMAU, and ^{18}F-FDG. Mice were imaged 60 minutes after intravenous injection of probes. Orientation of sagittal, coronal, and transverse sections is depicted in 3-D microCT image in (*B*). Images are 1-mm thick. Percentage ID/g, percentage injected dose per gram of tissue. (*D*) ^{18}F-FAC retention/cell number in thymocytes and splenocytes. Error bars represent means ±SEM, and results are representative of two independent experiments. (*E*) Proportion of ^{18}F-FAC retention per cell lineage per lymphoid organ. (*Reprinted from* Radu CG, Shu CJ, Nair-Gill E, et al. Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new [18F]-labeled 2′-deoxycytidine analog. Nat Med 2008;14:783–8; with permission.)

SPECT with SPECT (Energy Discrimination)

Dual-tracer imaging using SPECT, where multiple energy windows are used for simultaneous imaging of radiotracers using radionuclides emitting γ-rays at different energies, is one of the unique advantages inherent to SPECT technology. Examples of this include (1) ^{99m}Tc (140 keV) sestamibi stress and ^{201}Tl (75 keV/167 keV) rest myocardial perfusion imaging and (2) simultaneous use of a ^{99m}Tc (140 keV)–labeled perfusion agent and an ^{123}I (159 keV)–labeled neurotransmitter agent (eg, in neurodegenerative diseases). The use of simultaneous acquisition reduces the overall acquisition time and, therefore, the duration of anesthesia to the animal. Another significant advantage is that the resulting images from the different radiotracers are perfectly registered in space and time.

A complication with dual-tracer imaging is the presence of crosstalk between the multiple energy windows. In the case of, for instance, imaging with ^{99m}Tc (140 keV) and ^{201}Tl (75 keV/167 keV), the lower-energy ^{201}Tl energy window is contaminated by ^{99m}Tc photons scattered in patients or collimator (referred to as down-scatter) and lead x-rays generated by scattered and unscattered ^{99m}Tc photons in the collimator. In addition, the ^{99m}Tc data are contaminated by scattered (approximately 135 keV) and unscattered (167 keV) ^{201}Tl photons. To address these difficulties, current research has focused on optimization of multiple energy-window acquisition parameters[131,132] and modeling of crosstalk effects (ie, down-scatter and collimator x-ray generation) in the reconstruction task.[133–135] Combinations of these methods and detailed clinical evaluation are still required to make dual-tracer SPECT imaging an acceptable protocol for small laboratory animal research.

Positron Emission Tomography–Positron Emission Tomography

Multiprobe imaging has been used for several decades[136] to look at multiple information sources using the same imaging modality. A prime example is in cardiac imaging with the use of radiolabeled ammonia (NH_3) for blood flow and FDG for energy use using PET imaging.[137] The short half-life of NH_3 (10 minutes) enables a scan to be followed up with a second (stress versus rest) or alternative (FDG) imaging probe. In the case of NH_3-FDG, this shows where cardiac blood is flowing versus viable tissue, which is of great value to cardiac surgeons who would like to know before surgery whether or not a bypass procedure would have any benefit to patients. More often, the same subject or patient undergoes imaging a day or two apart. With adequate control over reproducible positioning, this method can provide imaging information from any combination of probes. Recently, Wu and colleagues[138] have shown that signals from serially injected probes using the same F18 isotope can be temporally decoded in mice, enabling sequential imaging without moving the subject between the two probe injections. This reduces changes in position and potentially in biologic status. Similar efforts are under examination in clinical settings.[139]

The dual-tracer approach is not straightforward for use in PET imaging because the detected events emanating from all radiotracers used have the same energy (511 keV). Despite the difficulties, few groups are investigating the feasibility of scanning multiple PET radiotracers using dynamic imaging techniques, where the signals from each tracer are separated based on differences in tracer half-life, kinetics, and distribution.[140–142] The single tracer components then can be assessed through multivariate analysis tools, such as principal component analysis. This field is an area of active research and to be successful, the approach deserves further research and development efforts and additional evaluation for potential clinical use.[143,144]

Optical Fluorescence–Wavelength-specific Probes

Similar to SPECT probe discrimination by energy, multiple fluorophores can be imaged in vivo using wavelength separation. Several companies have systems with this feature, including the Maestro, IVIS Spectrum, and others. The fluorophores can be excited using different filters or excitation lasers. Separation of the resulting signals can be by a series of specific band pass filters or, in the case of the Maestro, by a tunable liquid crystal display that sweeps through the spectral range. The goal is to separate nonspecific autofluorescence background (hemoglobin, fur, chow, and collagen) from the specific fluorophore signals. Each fluorophore has its own heterogeneous background signal, which can make specific signal detection difficult. Because light has to go into and out of an animal and activates any endogenous fluorescent molecules, fluorescent imaging is inherently less sensitive due to background than bioluminescent imaging. Nonetheless, fluorescent imaging enables following the expression or signal movement of multiple sources at the

same time. In the biologic realm, where multiple factors are involved with any biologic process under investigation, the more information that can be obtained at the same time about various states, the better the system can be accurately characterized. A distinct advantage of fluorescence imaging is that the temporal movement of fluorophores can be followed, because there is no substrate injected that is delivered and consumed over time.[7,145]

TRIMODALITY OPTIONS

Positron Emission Tomography–SPECT–CT

In response to the need of integrated multimodality platforms for multiprobe imaging, it was conjectured that the availability of a trimodality imaging system, allowing combining three modalities and recording quasisimultaneously complementary information gathered from SPECT, PET, and CT, might offer many advantages in some situations. Currently available commercial trimodality systems include the Inveon (Siemens) and the FLEX Triumph platform (developed by Gamma Medica-Ideas).[146] The Inveon is the current generation system of Siemens microPET product line.[107] The Siemens preclinical SPECT-CT system has been out for several years and the Inveon now can be bought within the same gantry as the SPECT-CT. The FLEX system uses the SPECT and CT sys-tems (described previously) and can be configured with the X-PET[147] or the LabPET[148] as a PET subsystem. It also has been argued that the APD-based detector module proposed by Saoudi and Lecomte[149] is particularly attractive for the design of compact multimodality (PET-SPECT-CT) imaging systems.

MR Imaging–Functional MR Imaging–Positron Emission Tomography

Recent developments with insert-based PET scanners that can be placed within MR imaging magnets[30,31] enable the combination of PET imaging with MR imaging and functional MR imaging. Anatomic MR images provide excellent soft tissue contrast that can be used to correct PET images for attenuation.[150–152] Using functional MR imaging, it is possible to measure blood flow and brain activation, typically using nonradioactive gadolinium contrast agents. Spectroscopic magnetic resonance measurements can distinguish probes and their metabolites, potentially eliminating the need for blood sampling to establish a metabolism profile. Metabolite analysis, especially in mice, remains a challenge in small animals due to their limited blood pool and need for taking samples at multiple time points. Combined with radioactive PET probes, MR imaging and functional MR imaging can be used at the same time to observe a metabolic process (PET) separate from blood flow and anatomic measurements.

The primary advantage of combining these systems together is the ability to acquire simultaneous information from an animal in vivo. This eliminates the need to coregister divergent and often different image data, removes the need to move the animal between imaging systems, and ensures that the biologic state is the same for all measurements. The combination of nanomolar tracer metabolism measurements using PET with exquisite anatomic information from MR imaging makes a powerful combination for in vivo research, particularly when paired with functional MR imaging for metabolite analysis. PET and MR imaging have cost and safety concerns, which may limit the widespread use of this combination, although this approach remains the best option for neuroscience research.

SPECT–CT–Optical Imaging

A trimodality (SPECT-CT-OI) small animal imaging system is being developed at the German Cancer Research Center.[125,153] The SPECT component consists of a compact detector of a 2×2 array of PSPMTs, which are connected to a 66×66 array of optode-coupled $1.3 \times 1.3 \times 6$–mm^3 sodium iodide crystals. The optical subsystem consists of a high-resolution CCD camera containing a progressive scan interline CCD chip. Various laser sources, selected by wavelength and light power requirements, can be mounted on the gantry. The x-ray CT component uses an x-ray tube having a 35-μm focal spot size and a cone angle of 24° whereas the x-ray detector consists of a 49.2×98.6–mm^2 gadolinium oxysulfide scintillator screen placed in direct contact with a CMOS photodiode array with 48-μm sensor pixel size. The modular design allows mounting of the subsystems on a common gantry, enabling a wide range of applications to be performed.

CHALLENGES FACED

Maintaining Image Quality, Low Dose, and Quantitation

There are several challenges facing the use of preclinical multimodality imaging, which may represent inherent limitations in these techniques. These include appropriate selection of an imaging modality or combination of multiprobe imaging modalities and the design of optimal task–specific acquisition and processing protocols. In this respect, small-animal imaging poses many

challenges when it comes to maintaining image quality and improving quantitative accuracy compared with clinical studies. Image quality in preclinical studies should be carefully optimized taking into account the physical performance characteristics of the imaging system used and the purposes of the experiment.

Multimodality molecular imaging has a long tradition of incorporating quantitative analysis in research protocols. Until recently, the analysis was based on functional or metabolic images as the sole input although the importance of the complementary information available from other anatomic modalities or from earlier scans has long been recognized. In addition, the visual quality and quantitative accuracy of small-animal imaging can be improved using anatomic imaging techniques to guide the reconstruction procedure[154] and to correct the radionuclide data for physical errors contributed by photon attenuation,[155] scatter radiation,[156] and partial volume effects[157] due to the limited spatial resolution of the nuclear imaging system. CT-based attenuation correction usually requires x-ray CT scanning of a cylindric phantom containing cylindric holes filled with a mixed solution of potassim phosphate and water with varying concentrations to simulate biologic tissues with different densities (**Fig. 5**). The calibration curve obtained then can be used for conversion of CT images of the animal to an attenuation map that can be used for attenuation correction purposes (**Fig. 6**). Alternatively, absolute quantification using PET generally requires accurate measurement of activity concentrations in arterial blood, which provides the input function to the kinetic model used. Although many dedicated blood sampling devices have been designed specifically for this purpose (eg,[158,159]), it remains a challenging task in small-animal imaging.

The radiation dose delivered to animals is a critical issue in preclinical imaging and can be high depending on the experiments and should be carefully monitored as it might change tumor characteristics; induce significant biologic effects, thus changing the animal model being studied; or even cause lethality.[160,161] The same applies to other imaging modalities, such as CT,[162] particularly when performed on multimodality imaging systems where the resulting absorbed dose is the sum of the individual contributions of each modality. Although much worthwhile effort has been devoted toward the assessment of radiation dose delivered to human subjects, few research studies addressed this issue for small animals.[160,162–164] High-resolution CT implies high radiation dose to the animal. If only a low-resolution scan is acquired, then the majority of the radiation dose comes from the PET tracer. Thus, increasing the sensitivity of preclinical PET systems might allow injecting lower activities and thus reducing the absorbed dose in the animal.

Creating and Timing Protocols to Obtain Simultaneous Data

One of the challenges to simultaneous and independent imaging procedures is recognizing when imaging data are informative and interesting. In static imaging situations, where there are no fast temporal changes of signal, it is simple to acquire data such that precise timing of the acquisition rarely is essential. With dynamic imaging, signals may change rapidly, such as with the first pass of a probe or contrast agent through the tissue bloodstream. There may be difficulties in starting

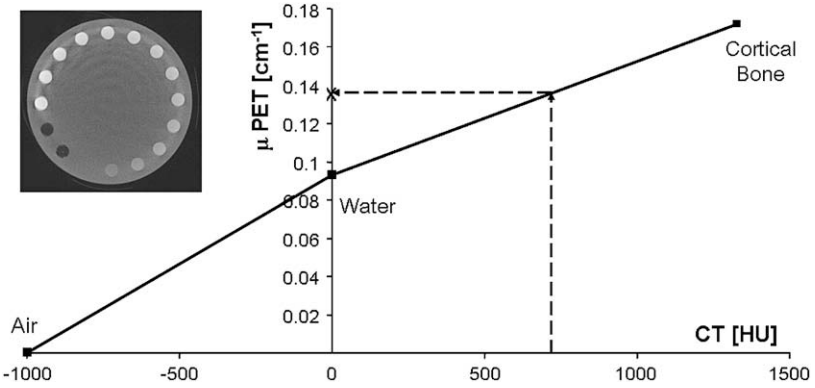

Fig. 5. Typical bilinear calibration curve for conversion of CT numbers (HU) into linear attenuation coefficients at 511 keV for preclinical PET-CT scanner. The in-house designed polyethylene cylindric phantom containing 16 cylindric holes is shown in upper left corner. Samples contained mixed solutions of K2HPO4 and water with concentrations varying between 80 mg/μL and 1000 mg/μL to simulate cortical bone with different densities.

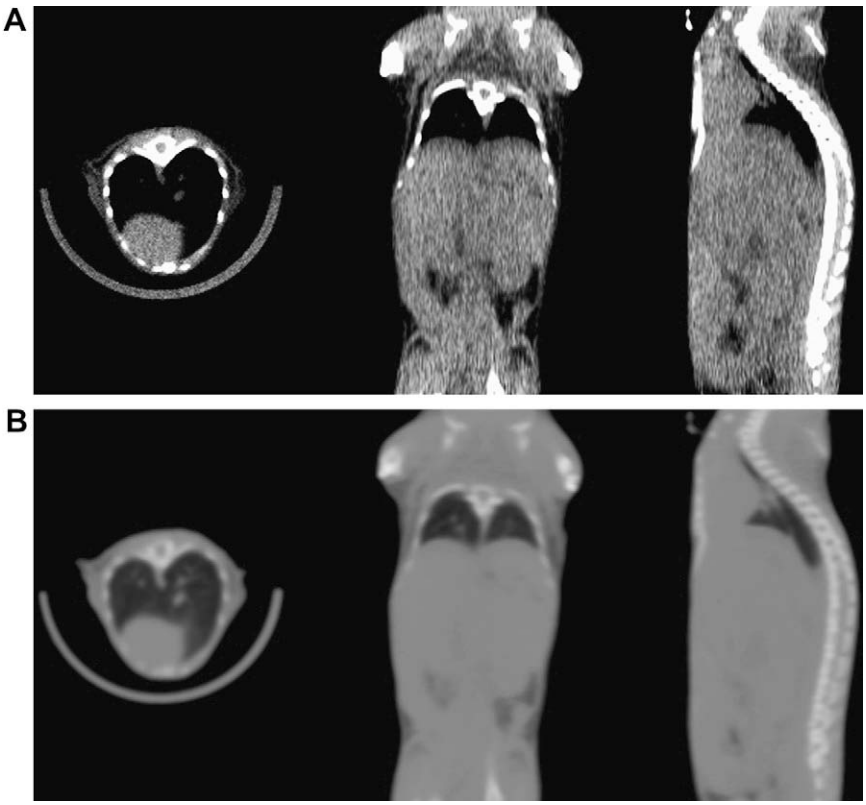

Fig. 6. Representative slices of original CT image in Hounsfield units (*A*) and generated attenuation map (*B*). Procedure involved the following steps: down-sampling from 512 × 512 to 256 × 256, followed by energy mapping by transforming CT numbers into linear attenuation coefficients at 511 keV using bilinear calibration curve shown in **Fig. 5**, and finally gaussian smoothing to match spatial resolution of preclinical PET scanner.

acquisitions at exact times, delays due to configuration of files, activation of injection systems, or simply having to start two separate computers at the same time. There also is a challenge to create images that have specific information from a certain period of time. An example might be an image showing optimal contrast or probe localization in one region of space that has a fast temporal change. This sort of optimization of the image might require considerable reprocessing of the data (or even specific timing of the acquisition) to get the best possible image. Often offline processing is desirable so that this optimization process does not tie up the imaging systems. Wherever possible, automation of the acquisition and processing can greatly facilitate optimal image timing and generation.

FUTURE TRENDS

The future of preclinical multimodality imaging lies in the creation of systems that make imaging simple, easy, and reproducible. The target information is not actually pretty images but rather the information content related to how much probe went to which specific location. Preclinical imaging systems will expand to incorporate systems for positioning; physiologic support, such as heating and anesthesia; enclosures for immunodeficient or infected animals; and operation and use by minimally trained personnel. The process of creating fused coregistered images from multiple sources will become increasingly automated and requires less user interaction. The images themselves will become increasingly less emphasized as the content becomes the focus, shifting from pictures to relevant data about timing and uptake information (parametric). It is possible that one day images will disappear, replaced by an automated process that maps uptake data onto standardized mouse or rat atlases. The future will likely see a shift toward the end user, typically a biologist, as the primary user with little or no support required to operate and analyze the imaging-based data.

One issue remains clear, which is that the more information that can be obtained, sequentially or simultaneously, the better a biologic system can

be understood. Often the imaging modalities are complementary, providing different pieces of information about the same animal; thus, the multimodality approach is likely to become the standard way that imaging-based research is conducted in the future.

REFERENCES

1. Kalender WA. X-ray computed tomography. Phys Med Biol 2006;51:R29–43.
2. Holdsworth SJ, Bammer R. Magnetic resonance imaging techniques: fMRI, DWI, and PWI. Semin Neurol 2008;28:395–406.
3. Matthews PM, Honey GD, Bullmore ET. Applications of fMRI in translational medicine and clinical practice. Nat Rev Neurosci 2006;7:732–44.
4. Prost RW. Magnetic resonance spectroscopy. Med Phys 2008;35:4530–44.
5. Madsen MT. Recent advances in SPECT imaging. J Nucl Med 2007;48:661–73.
6. Muehllehner G, Karp JS. Positron emission tomography. Phys Med Biol 2006;51:R117–37.
7. Ntziachristos V. Fluorescence molecular imaging. Annu Rev Biomed Eng 2006;8:1–33.
8. Phelps ME. PET: the merging of biology and imaging into molecular imaging. J Nucl Med 2000;41:661–81.
9. Bar-Shalom R, Yefemov N, Guralnik L, et al. Clinical performance of PET/CT in evaluation of cancer: additional value for diagnostic imaging and patient management. J Nucl Med 2003;44:1200–9.
10. Vogel WV, Oyen WJ, Barentsz JO, et al. PET/CT: panacea, redundancy, or something in between? J Nucl Med 2004;45(Suppl 1):15S–24S.
11. Macapinlac HA. Clinical applications of positron emission tomography/computed tomography treatment planning. Semin Nucl Med 2008;38:137–40.
12. Zaidi H, Vees H, Wissmeyer M. Molecular PET/CT imaging-guided radiation therapy treatment planning. Acad Radiol 2009, in press.
13. Schoder H, Ong SC. Fundamentals of molecular imaging: rationale and applications with relevance for radiation oncology. Semin Nucl Med 2008;38:119–28.
14. Townsend DW. Multimodality imaging of structure and function. Phys Med Biol 2008;53:R1–39.
15. Burton JB, Johnson M, Sato M, et al. Adenovirus-mediated gene expression imaging to directly detect sentinel lymph node metastasis of prostate cancer. Nat Med 2008;14:882–8.
16. Hutton BF, Braun M. Software for image registration: algorithms, accuracy, efficacy. Semin Nucl Med 2003;33:180–92.
17. Maes F, Vandermeulen D, Suetens P. Medical image registration using mutual information. Proceedings of the IEEE 2003;91:1699–722.
18. Pelizzari CA, Chen GT, Spelbring DR, et al. Accurate three-dimensional registration of CT, PET, and/or MR images of the brain. J Comput Assist Tomogr 1989;13:20–6.
19. Pietrzyk U, Herholz K, Fink G, et al. An interactive technique for three-dimensional image registration: validation for PET, SPECT, MRI and CT brain studies. J Nucl Med 1994;35:2011–8.
20. Woods RP, Grafton ST, Holmes CJ, et al. Automated image registration: I. General methods and intrasubject, intramodality validation. J Comput Assist Tomogr 1998;22:139–52.
21. Krishnasetty V, Fischman AJ, Halpern EL, et al. Comparison of alignment of computer-registered data sets: combined PET/CT versus independent PET and CT of the thorax. Radiology 2005;237:635–9.
22. Slomka PJ. Software approach to merging molecular with anatomic information. J Nucl Med 2004;1(45 Suppl):36S–45S.
23. Hasegawa B, Zaidi H. Dual-modality imaging: more than the sum of its components. In: Zaidi H, editor. Quantitative analysis in nuclear medicine imaging. New York: Springer; 2006. p. 35–81.
24. Hasegawa BH, Gingold EL, Reilly SM, et al. Description of a simultaneous emission-transmission CT system. Proc Soc Photo Opt Instrum Eng 1990;1231:50–60.
25. Hasegawa BH, Iwata K, Wong KH, et al. Dual-modality imaging of function and physiology. Acad Radiol 2002;9:1305–21.
26. Beyer T, Townsend D, Brun T, et al. A combined PET/CT scanner for clinical oncology. J Nucl Med 2000;41:1369–79.
27. Shao Y, Cherry SR, Farahani K, et al. Simultaneous PET and MR imaging. Phys Med Biol 1997;42:1965–70.
28. Mackewn JE, Strul D, Hallett WA, et al. Design and development of an MR-compatible PET scanner for imaging small animals. IEEE Trans Nucl Sci 2005;52:1376–80.
29. Raylman RR, Majewski S, Velan SS, et al. Simultaneous acquisition of magnetic resonance spectroscopy (MRS) data and positron emission tomography (PET) images with a prototype MR-compatible, small animal PET imager. J Magn Reson 2007;186:305–10.
30. Judenhofer MS, Wehrl HF, Newport DF, et al. Simultaneous PET-MRI: a new approach for functional and morphological imaging. Nat Med 2008;14:459–65.
31. Pichler BJ, Wehrl HF, Kolb A, et al. Positron emission tomography/magnetic resonance imaging: the next generation of multimodality imaging? Semin Nucl Med 2008;38:199–208.
32. Levin CS, Zaidi H. Current trends in preclinical PET system design. PET Clinics 2007;2:125–60.

33. Schlemmer HP, Pichler BJ, Schmand M, et al. Simultaneous MR/PET imaging of the human brain: feasibility study. Radiology 2008;248:1028–35.
34. Gaa J, Rummeny EJ, Seemann MD. Whole-body imaging with PET/MRI. Eur J Med Res 2004;30: 309–12.
35. Seemann MD. Whole-body PET/MRI: the future in oncological imaging. Technol Cancer Res Treat 2005;4:577–82.
36. Small Animal Imaging Workshop at Stanford. Available at: http://radiologycme.stanford.edu/2008smallanimal/. Accessed November 30th, 2008.
37. Small Animal Imaging Workshop at Tuebingen. Available at: http://89.110.144.29:8080/4th-small-animal-imaging-workshop-2009. Accessed November 30th, 2008.
38. In vivo small animal imaging at UCDavis. In vivo small animal imaging. Available at: http://imaging.bme.ucdavis.edu/events.html. Accessed November 30th, 2008.
39. Richmond JY, Hill RH, Weyant RS, et al. What's hot in animal biosafety? ILAR J 2003;44:20–7.
40. Pritt S, Hankenson FC, Wagner T, et al. The basics of animal biosafety and biocontainment training. Lab Anim (NY) 2007;36:31–8.
41. Richmond JY. The 1, 2, 3's of biosafety levels. Available at: http://www.cdc.gov/OD/ohs/symp5/jyrtext.htm. Accessed November 30th, 2008.
42. Johns Hopkins Animal Care and Use. Animal care and use training. Available at: http://www.jhu.edu/animalcare/training3.html. Accessed November 30th, 2008.
43. Howard Hughes Medical Institute. Lab safety training materials. Available at: http://www.hhmi.org/about/research/training.html. Accessed November 30th, 2008.
44. Peters LL, Robledo RF, Bult CJ, et al. The mouse as a model for human biology: a resource guide for complex trait analysis. Nat Rev Genet 2007;8:58–69.
45. Mouse Models of Human Cancer Consortium. Available at: http://mouse.ncifcrf.gov/. Accessed November 30th, 2008.
46. Wang YX, Betton G, Floettmann E, et al. Imaging kidney in conscious rats with high-frequency ultrasound and detection of two cases of unilateral congenital hydronephrosis. Ultrasound Med Biol 2007;33:483–6.
47. Ferris CF, Febo M, Luo F, et al. Functional magnetic resonance imaging in conscious animals: a new tool in behavioural neuroscience research. J Neuroendocrinol 2006;18:307–18.
48. Kyme AZ, Zhou VW, Meikle SR, et al. Real-time 3D motion tracking for small animal brain PET. Phys Med Biol 2008;53:2651–66.
49. Vaska P, Woody CL, Schlyer DJ, et al. RatCAP: miniaturized head-mounted PET for conscious rodent brain imaging. IEEE Trans Nucl Sci 2004; 51:2718–22.
50. Woody C, Schlyer D, Vaska P, et al. Preliminary studies of a simultaneous PET/MRI scanner based on the RatCAP small animal tomograph. Nucl Instr Meth A 2007;571:102–5.
51. Flores JE, McFarland LM, Vanderbilt A, et al. The effects of anesthetic agent and carrier gas on blood glucose and tissue uptake in mice undergoing dynamic FDG-PET imaging: sevoflurane and isoflurane compared in air and in oxygen. Mol Imaging Biol 2008;10:192–200.
52. Fueger BJ, Czernin J, Hildebrandt I, et al. Impact of animal handling on the results of 18F-FDG PET studies in mice. J Nucl Med 2006;47:999–1006.
53. Star Life Sciences probe. White paper. 2008?
54. Pan MH, Huang SC, Liao YP, et al. FLT-PET imaging of radiation responses in murine tumors. Mol Imaging Biol 2008;10:325–34.
55. Maintz JB, Viergever MA. A survey of medical image registration. Med Image Anal 1998;2:1–36.
56. Pluim JP, Maintz JB, Viergever MA. Mutual-information-based registration of medical images: a survey. IEEE Trans Med Imaging 2003;22:986–1004.
57. Zanzonico PB. Broad-spectrum multi-modality image registration: from PET, CT, and MRI to autoradiography, microscopy, and beyond. Conf Proc IEEE Eng Med Biol Soc 2006;1:1584–8.
58. Zanzonico PB, Nehmeh SA. Introduction to clinical and laboratory (small-animal) image registration and fusion. Conf Proc IEEE Eng Med Biol Soc 2006;1:1580–3.
59. Cross DJ, Minoshima S, Nishimura S, et al. Three-dimensional stereotactic surface projection analysis of macaque brain PET: development and initial applications. J Nucl Med 2000;41:1879–87.
60. Shimada Y, Uemura K, Ardekani BA, et al. Application of PET-MRI registration techniques to cat brain imaging. J Neurosci Methods 2000;101:1–7.
61. Vaquero JJ, Desco M, Pascau J, et al. PET, CT, and MR image registration of the rat brain and skull. IEEE Trans Nucl Sci 2001;48:1440–5.
62. Humm JL, Ballon D, Hu YC, et al. A stereotactic method for the three-dimensional registration of multi-modality biologic images in animals: NMR, PET, histology, and autoradiography. Med Phys 2003;30:2303–14.
63. Jan M-L, Chuang K-S, Chen G-W, et al. A three-dimensional registration method for automated fusion of micro PET-CT-SPECT whole-body images. IEEE Trans Med Imaging 2005;24:886–93.
64. Fei B, Wang H, Muzic J, et al. Deformable and rigid registration of MRI and microPET images for photodynamic therapy of cancer in mice. Med Phys 2006;33:753–60.
65. Deroose CM, De A, Loening AM, et al. Multimodality imaging of tumor xenografts and metastases in

mice with combined small-animal PET, small-animal CT, and bioluminescence imaging. J Nucl Med 2007;48:295–303.
66. Pascau J, Gispert JD, Michaelides M, et al. Automated method for small-animal PET image registration with intrinsic validation. Mol Imaging Biol 2009; 11(2):107–13.
67. Zanzonico P, Campa J, Polycarpe-Holman D, et al. Animal-specific positioning molds for registration of repeat imaging studies: comparative microPET imaging of F18-labeled fluoro-deoxyglucose and fluoro-misonidazole in rodent tumors. Nucl Med Biol 2006;33:65–70.
68. Chow PL, Stout DB, Komisopoulou E, et al. A method of image registration for small animal, multi-modality imaging. Phys Med Biol 2006;51: 379–90.
69. Christian N, Lee JA, Bol A, et al. Immobilization device for in vivo and in vitro multimodality image registration of rodent tumors. Radiother Oncol 2008;87:147–51.
70. Beekman F, Hutton B. Multi-modality imaging on track. Eur J Nucl Med Mol Imaging 2007;34:1410–4.
71. Collins DL, Neelin P, Peters TM, et al. Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 1994;18:192–205.
72. Goertzen AL, Meadors AK, Silverman RW, et al. Simultaneous molecular and anatomical imaging of the mouse in vivo. Phys Med Biol 2002;21:4315–28.
73. Catana C, Wu Y, Judenhofer MS, et al. Simultaneous acquisition of multislice PET and MR images: Initial results with a MR-compatible PET scanner. J Nucl Med 2006;47:1968–76.
74. Pichler BJ, Judenhofer MS, Catana C, et al. Performance test of an LSO-APD detector in a 7-T MRI scanner for simultaneous PET/MRI. J Nucl Med 2006;47:639–47.
75. Judenhofer MS, Catana C, Swann BK, et al. Simultaneous PET/MR images, acquired with a compact MRI compatible PET detector in a 7 Tesla magnet. Radiology 2007;244:807–14.
76. Lucas AJ, Hawkes RC, Ansorge RE, et al. Development of a combined microPET-MR system. Technol Cancer Res Treat 2006;5:337–41.
77. Suckow C, Kuntner C, Chow P, et al. Multimodality rodent imaging chambers for use under barrier conditions with gas anesthesia. Mol Imaging Biol 2008;10(2):114–20.
78. Bentourkia MH, Zaidi H. Tracer kinetic modeling in PET. PET Clinics 2007;2:267–77.
79. Snyder DL. Parameter estimation for dynamic studies in emission-tomography systems having list-mode data. IEEE Trans Nucl Sci 1984;31: 925–31.
80. Rahmim A, Lenox M, Reader AJ, et al. Statistical list-mode image reconstruction for the high resolution research tomograph. Phys Med Biol 2004;49:4239–58.
81. Reader AJ, Zaidi H. Advances in PET image reconstruction. PET Clinics 2007;2:173–90.
82. Cherry SR. Multimodality in vivo imaging systems: twice the power or double the trouble? Annu Rev Biomed Eng 2006;8:35–62.
83. Del Guerra A, Belcari N. State-of-the-art of PET, SPECT and CT for small animal imaging. Nucl Instr Meth A 2007;583:119–24.
84. Franc BL, Acton PD, Mari C, et al. Small-animal SPECT and SPECT/CT: Important tools for preclinical investigation. J Nucl Med 2008;49:1651–63.
85. Paulus MJ, Gleason SS, Kennel SJ, et al. High resolution X-ray computed tomography: an emerging tool for small animal cancer research. Neoplasia 2000;2:62–70.
86. Ritman EL. Micro-computed tomography-current status and developments. Annu Rev Biomed Eng 2004;6:185–208.
87. Kiessling F, Greschus S, Lichy MP, et al. Volumetric computed tomography (VCT): a new technology for noninvasive, high-resolution monitoring of tumor angiogenesis. Nat Med 2004;10:1133–8.
88. Vaquero JJ, Redondo S, Lage E, et al. Assessment of a new high-performance small-animal X-ray tomograph. IEEE Trans Nucl Sci 2008;55: 898–905.
89. Andre MP, Spivey BA, Martin PJ, et al. Integrated CMOS-selenium x-ray detector for digital mammography. Proc Soc Photo Opt Instrum Eng 1998;3336:204–9.
90. Liang H, Yang Y, Yang K, et al. A microPET/CT system for in vivo small animal imaging. Phys Med Biol 2007;52:3881–94.
91. Dumouchel T, Bergeron M, Cadorette J, et al. Initial performance assessment of the LabPET™ APD-based digital PET scanner [abstract]. J Nucl Med 2007;48:39P.
92. Fontaine R, Belanger F, Cadorette J, et al. Architecture of a dual-modality, high-resolution, fully digital positron emission tomography/computed tomography (PET/CT) scanner for small animal imaging. IEEE Trans Nucl Sci 2005;52:691–6.
93. Bérard P, Riendeau J, Pepin C, et al. Investigation of the LabPETTM detector and electronics for photon-counting CT imaging. Nucl Instr Meth A 2007;571:114–7.
94. Iwata K, Wu MC, Hasegawa BH. Design of combined x-ray CT and SPECT systems for small animals. IEEE Nuclear Science Symposium and Medical Imaging Conference Record 1999;3: 1608–12.
95. Iwata K, Hwang AB, Wu MC, et al. Design and utility of a small animal CT/SPECT system. IEEE Nuclear Science Symposium and Medical Imaging Conference Record 2002;3:1849–52.

96. Williams MB, Zhang G, More MJ, et al. Integrated CT-SPECT system for small animal imaging. Proc Soc Photo Opt Instrum Eng 2000;4142:265–74.
97. Welsh RE, Brewer P, Bradley EL, et al. An economical dual-modality small animal imaging system with application to studies of diabetes. IEEE Nuclear Science Symposium and Medical Imaging Conference Record 2002;3:1845–8.
98. Weisenberger AG, Wojcik R, Bradley EL, et al. SPECT-CT system for small animal imaging. IEEE Trans Nucl Sci 2003;50:74–9.
99. Kastis GA, Furenlid LR, Wilson DW, et al. Compact CT/SPECT small-animal imaging system. IEEE Trans Nucl Sci 2004;51:63–7.
100. MacDonald LR, Iwata K, Patt BE, et al. Evaluation of x-ray detectors for dual-modality CT-SPECT animal imaging. Proc Soc Photo Opt Instrum Eng 2002;4786:91–102.
101. Song X, Frey EC, Tsui BMW. Development and evaluation of a microCT system for small animal imaging. IEEE Nuclear Science Symposium and Medical Imaging Conference Record 2002;3: 1600–4.
102. Hwang AB, Iwata K, Sakdinawat AE, et al. Gantry specifications for a dual modality imaging system for small animals. IEEE Nuclear Science Symposium and Medical Imaging Conference Record 2003;2:1303–7.
103. Hasegawa BH, Wu MC, Iwata K, et al. Applications of penetrating radiation for small animal imaging. Proc Soc Photo Opt Instrum Eng 2002; 4786:80–90.
104. MacDonald LR, Patt BE, Iwanczyk JS, et al. Pinhole SPECT of mice using the LumaGEM gamma camera. IEEE Trans Nucl Sci 2001;48:830–6.
105. MacDonald LR, Iwanczyk JS, Patt BE, et al. Development of new high resolution detectors for small animal SPECT imaging. IEEE Nuclear Science Symposium and Medical Imaging Conference Record 2002;3:21/75.
106. McElroy DP, MacDonald LR, Beekman FJ, et al. Performance evaluation of A-SPECT: a high resolution desktop pinhole SPECT system for imaging small animals. IEEE Trans Nucl Sci 2002;49:2139–47.
107. Visser EP, Disselhorst JA, Brom M, et al. Spatial resolution and sensitivity of the Inveon small-animal PET scanner. J Nucl Med 2009;50:139–47.
108. Lin K-P, Sung-Cheng H, Baxter LR, et al. A general technique for interstudy registration of multifunction and multimodality images. IEEE Trans Nucl Sci 1994;41:2850–5.
109. Rickey D, Gordon R, Huda W. On lifting the inherent limitations of positron emission tomography by using magnetic fields (MagPET). Automedica 1992;14:355–69.
110. Hammer BE, Christensen NL, Heil BG. Use of a magnetic field to increase the spatial resolution of positron emission tomography. Med Phys 1994; 21:1917–20.
111. Christensen NL, Hammer BE, Heil BG, et al. Positron emission tomography within a magnetic field using photomultiplier tubes and light-guides. Phys Med Biol 1995;40:691–7.
112. Slates R, Cherry SR, Boutefnouchet A, et al. Design of a small animal MR compatible PET scanner. IEEE Trans Nucl Sci 1999;46:565–70.
113. Yamamoto S, Takamatsu S, Murayama H, et al. A block detector for a multislice, depth-of-interaction MR-compatible PET. IEEE Trans Nucl Sci 2005;52: 33–7.
114. Raylman RR, Majewski S, Lemieux SK, et al. Simultaneous MRI and PET imaging of a rat brain. Phys Med Biol 2006;51:6371–9.
115. Handler WB, Gilbert KM, Peng H, et al. Simulation of scattering and attenuation of 511 keV photons in a combined PET/field-cycled MRI system. Phys Med Biol 2006;51:2479–91.
116. Renker D. Geiger-mode avalanche photodiodes, history, properties and problems. Nucl Instr Meth A 2006;567:48–56.
117. Dolgoshein B, Balagura V, Buzhan P, et al. Status report on silicon photomultiplier development and its applications. Nucl Instr Meth A 2006;563: 368–76.
118. McElroy DP, Saveliev V, Reznik A, et al. Evaluation of silicon photomultipliers: a promising new detector for MR compatible PET. Nucl Instr Meth A 2007;571:106–9.
119. Moehrs S, Del Guerra A, Herbert DJ, et al. A detector head design for small-animal PET with silicon photomultipliers (SiPM). Phys Med Biol 2006;51:1113–27.
120. Wagenaar DJ, Kapusta M, Li J, et al. Rationale for the combination of nuclear medicine with magnetic resonance for pre-clinical imaging. Technol Cancer Res Treat 2006;5:343–50.
121. Wagenaar D, Nalcioglu O, Muftuler L, et al. A multiring small animal CZT system for simultaneous SPECT/MRI imaging. J Nucl Med 2007;48:89P, –c-.
122. Prout DL, Silverman RW, Chatziioannou A. Detector concept for OPET-A combined PET and optical imaging system. IEEE Trans Nucl Sci 2004;51: 752–6.
123. Douraghy A, Rannou FR, Silverman RW, et al. FPGA electronics for OPET: a dual-modality optical and positron emission tomograph. IEEE Trans Nucl Sci 2008;55:2541–5.
124. Li C, Mitchell GS, Yang Y, et al. Simultaneous PET and multispectral three-dimensional fluorescence optical tomography imaging system for small animals. World Molecular Imaging Conference (WMIC), Nice, France, 10–13, Sept 2008.
125. Peter J, Semmler W. vECTlab-A fully integrated multi-modality Monte Carlo simulation framework

for the radiological imaging sciences. Nucl Instr Meth A 2007;580:955–9.
126. Radu CG, Shu CJ, Nair-Gill E, et al. Molecular imaging of lymphoid organs and immune activation by positron emission tomography with a new [18F]-labeled 2′-deoxycytidine analog. Nat Med 2008;14: 783–8.
127. Oldham M, Kim L, Hugo G. Optical-CT imaging of complex 3D dose distributions. J Phys 2005;5745: 138–46.
128. McLaughlin W, Douglas V. Kodak in vivo imaging system: precise co registration of molecular imaging with anatomical X-ray imaging in animals. Available at: http://www.carestreamhealth.com/WorkArea/showcontent.aspx?id=360874. Accessed November 30th, 2008.
129. Hillman EMC, Moore A. All-optical anatomical co-registration for molecular imaging of small animals using dynamic contrast. Nat Photonics 2007;1: 526–30.
130. Del Guerra A, Bartoli A, Belcari N, et al. Performance evaluation of the fully engineered YAP-(S)PET scanner for small animal imaging. IEEE Trans Nucl Sci 2006;53:1078–83.
131. Du Y, Frey E, Wang W, et al. Optimization of acquisition energy windows in simultaneous 99mTc/123I brain SPECT. IEEE Trans Nucl Sci 2003;50: 1556–61.
132. Wang W, Tsui B, Lalush D, et al. Optimization of acquisition parameters for simultaneous 201Tl and 99mTc dual-isotope myocardial imaging. IEEE Trans Nucl Sci 2005;52:1227–35.
133. de Jong HW, Beekman FJ, Viergever MA, et al. Simultaneous (99m)Tc/(201)Tl dual-isotope SPET with Monte Carlo-based down-scatter correction. Eur J Nucl Med Mol Imaging 2002;29:1063–71.
134. Song X, Frey E, Wang W, et al. Validation and evaluation of model based crosstalk compensation method in simultaneous 99mTc stress and 201Tl rest myocardial perfusion SPECT. IEEE Trans Nucl Sci 2004;51:72–9.
135. Ouyang J, El Fakhri G, Moore SC. Fast Monte Carlo based joint iterative reconstruction for simultaneous 99mTc/123I SPECT imaging. Med Phys 2007;34:3263–72.
136. Phelps M, Huang S, Hoffman E, et al. Cerebral extraction of N-13 ammonia: its dependence on cerebral blood flow and capillary permeability—surface area product. Stroke 1981;12:607–19.
137. Schelbert HR, Henze E, Phelps ME, et al. Assessment of regional myocardial ischemia by positron-emission computed tomography. Am Heart J 1982;103.
138. Wu H-M, Yu AS, Lin H-D, et al. The feasibility of performing longitudinal measurements in mice using small animal PET imaging and a microfluidic blood sampling device. IEEE Nucl Sci Symp Conf Rec NSS '07, Honolulu, Hawai, Oct. 26-Nov. 3 2007, 2007;6:4174–5.
139. Tian J, Yang X, Yu L, et al. A multicenter clinical trial on the diagnostic value of dual-tracer PET/CT in pulmonary lesions using 3'-Deoxy-3'-18F-Fluorothymidine and 18F-FDG. J Nucl Med 2008;49: 186–94.
140. Kadrmas DJ, Rust TC. Feasibility of rapid multitracer PET tumor imaging. IEEE Trans Nucl Sci 2005;52:1341–7.
141. Rust TC, DiBella EVR, McGann CJ, et al. Rapid dual-injection single-scan 13N-ammonia PET for quantification of rest and stress myocardial blood flows. Phys Med Biol 2006;51:5347–62.
142. Black NF, McJames S, Rust TC, et al. Evaluation of rapid dual-tracer (62)Cu-PTSM + (62)Cu-ATSM PET in dogs with spontaneously occurring tumors. Phys Med Biol 2008;53:217–32.
143. Verhaeghe J, D'Asseler Y, De Winter O, et al. Simultaneous dual tracer NH3/FDG cardiac PET imaging: a simulation study [abstract]. J Nucl Med 2005;46:56P.
144. El Fakhri G, Sitek A, Guérin B. Simultaneous dual tracer PET using generalized factor analysis of dynamic sequences. Proc. IEEE Nuclear Science Symposium and Medical Imaging Conference, San Diego, CA, 2006:2128–30.
145. Park JM, Gambhir SS. Multimodality radionuclide, fluorescence, and bioluminescence small-animal imaging. Proceedings of the IEEE 2005;93: 771–83.
146. Parnham KB, Chowdhury S, Li J, et al. Second-generation, tri-modality pre-clinical imaging system. IEEE Nucl Sci Symp Conf Rec 2006;3:1802–5.
147. Xie S, Ramirez R, Liu Y, et al. A pentagon photomultiplier-quadrant-sharing BGO detector for a rodent research PET (RRPET). IEEE Trans Nucl Sci 2005;52:210–6.
148. Bergeron M, Cadorette J, Beaudoin JF, et al. Performance evaluation of the LabPET™; APD-based digital PET scanner. IEEE Nucl Sci Symp Conf Rec 2007;6:4185–91.
149. Saoudi A, Lecomte R. A novel APD-based detector module for multi-modality PET/SPECT/CT scanners. IEEE Trans Nucl Sci 1999;46:479–84.
150. Zaidi H, Montandon M-L, Slosman DO. Magnetic resonance imaging-guided attenuation and scatter corrections in three-dimensional brain positron emission tomography. Med Phys 2003; 30:937–48.
151. Zaidi H. Is MRI-guided attenuation correction a viable option for dual-modality PET/MR imaging? Radiology 2007;244:639–42.
152. Hofmann M, Steinke F, Scheel V, et al. MRI-based attenuation correction for PET/MRI: a novel approach combining pattern recognition and Atlas registration. J Nucl Med 2008;49:1875–83.

153. Peter J, Semmler W. A modular design triple-modality SPECT-CT-ODT small animal imager [abstract]. Eur J Nucl Med Mol Imaging 2007;34: S158.
154. Baete K, Nuyts J, Van Paesschen W, et al. Anatomical-based FDG-PET reconstruction for the detection of hypo-metabolic regions in epilepsy. IEEE Trans Med Imaging 2004;23:510–9.
155. Zaidi H, Montandon M-L, Alavi A. Advances in attenuation correction techniques in PET. PET Clinics 2007;2:191–217.
156. Zaidi H, Montandon M-L. Scatter compensation techniques in PET. PET Clinics 2007;2:219–34.
157. Rousset O, Rahmim A, Alavi A, et al. Partial volume correction strategies in PET. PET Clinics 2007;2: 235–49.
158. Convert L, Morin-Brassard G, Cadorette J, et al. A new tool for molecular imaging: the microvolumetric {beta} blood counter. J Nucl Med 2007;48: 1197–206.
159. Wu HM, Sui G, Lee CC, et al. In vivo quantitation of glucose metabolism in mice using small-animal PET and a microfluidic device. J Nucl Med 2007; 48:837–45.
160. Funk T, Sun M, Hasegawa BH. Radiation dose estimate in small animal SPECT and PET. Med Phys 2004;31:2680–6.
161. Taschereau R, Chatziioannou AF. Monte Carlo simulations of absorbed dose in a mouse phantom from 18-fluorine compounds. Med Phys 2007;34:1026–36.
162. Taschereau R, Chow PL, Chatziioannou AF. Monte Carlo simulations of dose from microCT imaging procedures in a realistic mouse phantom. Med Phys 2006;33:216–24.
163. Stabin MG, Peterson TE, Holburn GE, et al. Voxel-based mouse and rat models for internal dose calculations. J Nucl Med 2006;47:655–9.
164. Hindorf C, Ljungberg M, Strand SE. Evaluation of parameters influencing S values in mouse dosimetry. J Nucl Med 2004;45:1960–5.