Chapter 3
Considerations for Preclinical Laboratory Animal Imaging Center Design, Setup, and Management Suitable for Biomedical Investigation for Drug Discovery

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Abstract In vivo imaging techniques are rapidly becoming routine procedures for biomedical research and drug/biologics development. Imaging is an outstanding tool for noninvasively testing the response to therapy by examining animals as whole organisms. Many kinds of imaging platforms are now commercially available, and many are optimized for smaller species. Before beginning construction for a centralized in vivo imaging facility, one must first define the requirements and limitations of the facility. The planning should involve laboratory animal veterinarians, investigators, imaging specialists, occupational health specialists, and administrators. Considerations include the animal models of interest, the scientific questions to be addressed, inclusion of radiochemistry (PET/SPECT), select agent use (BSL requirements), logistics and laboratory flow, and personnel safety within the imaging environment. Architects must incorporate functional design into the technical requirements and building aesthetics. The limitless variables prevent the production of a step-by-step imaging center design manual; however, we suggest a foundation of advice learned from our experiences with the National Institutes of Health Mouse Imaging Facility. Magnetic resonance imaging is an infrastructure-dependent platform and is a recommended base to start facility design. The importance of preplanning and clear communications for success cannot be overemphasized.

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Abbreviations

BAS	Building automation system
CT	Computed tomography
ECG	Electrocardiogram
EEG	Electroencephalogram
FDG	2-[^{18}F]fluoro-2-deoxy-D-glucose
HVAC	Heating, ventilating, and air conditioning
LAN	Local area network
MHz	Megahertz
MPW	Medical pathological waste
MRI	Magnetic resonance imaging
NHP	Nonhuman primate
PET	Positron emission tomography
PPE	Personnel protective clothing and equipment
RF	Radio frequency

3.1 Introduction

Biomedical research requires periodic glimpses of the inner workings of an organism. This may be accomplished by a variety of means to determine the effects of disease or treatment. Animal research plays a key role in the advancement of medicine and drug discovery. In the past, as researchers developed animal models of human diseases, dissection at important timepoints enhanced our understanding of those diseases and their treatments. Old experimental designs included cohorts of animals large enough to sacrifice subsets of subjects along the course of the timeline to observe effects. Each individual animal was considered part of the whole, and another set of animals served as untreated or sham controls. A modern approach to the same experiment now includes in vivo imaging. Imaging allows the researcher to observe biological processes in the animal in a noninvasive fashion and document those changes over time in an individual. The ability to use an animal as its own control and observe the effects of disease or treatment noninvasively results in the use of fewer animals. Imaging provides excellent anatomical, physiological and/or functional details in individuals because it more closely resembles the use of human models (who are not sacrificed during experimental timepoints). Additionally, the use of fewer animals results in a significant reduction in the cost of research, probably totaling in the millions of dollars overall.

Preclinical in vivo imaging modalities span a wide range of options from low-resolution anatomical scanners up to devices capable of measuring specific molecular functions on a cellular level. In the context of drug discovery, it may be prudent to include as many options as possible. This would allow for investigation of

unanticipated effects, both beneficial and detrimental. In fact, it may lead to new discoveries as occasionally occurs with drug development.

The Food and Drug Administration (FDA) requirements for new drug approval include safety and efficacy testing in two animal species prior to being used in clinical trials. An ideal design for a drug development imaging center would allow for imaging of multiple species of animal models. Unfortunately, imaging devices optimized for larger species may not generate acceptable data for smaller species, thus commanding the need for double the amount of equipment. But if money and space were unlimited, this would be the ideal situation. As budgets tend to be restricted, one should carefully consider what equipment could serve multiple species most efficiently. The services of an experienced imaging consultant may be money well spent.

The experimental animal varieties and types of imaging equipment will define the remainder of the imaging center's design. These are the two most important decisions and must be made first and before any other considerations. Thoughts for future expansion will allow for flexibility once researchers are familiar with imaging methods and its utility. Preclinical imaging technologies are continually advancing, and an ideal imaging facility will include sufficient design flexibility (and space) to expand and incorporate new techniques such as imaging of transgenic animals (Budinger et al. 1999). Species-specific housing designs may be found in any laboratory animal facility design and planning guide. We attempt to point out the species-specific issues worth consideration when designing an imaging facility.

Providing a step-by-step guide for designing an animal imaging center is nearly impossible; however, based upon our experiences with the National Institutes of Health Mouse Imaging Facility, we will offer advice for an ideal laboratory animal imaging center. This paper will be divided into five major sections: (1) facility design (including general considerations, animal support, MRI-specific considerations, and miscellaneous topics), (2) animal imaging support, (3) personnel, (4) imaging equipment, and (5) data management. MRI is heavily infrastructure dependent. MR imagers require particular structural specifications in the building and trained personnel to operate and maintain the equipment, and the MRI environment brings unique occupational safety hazards. Because of its restricting conditions, we use MRI as the limiting factor for the rest of the facility design. It is often easier to incorporate other imaging devices as the facility expands without the need for major construction, as is usually the case with MRI. We lend advice specific to various aspects of design as appropriate. Generic discussion of other topics leaves the facility planners to accommodate the unique and specific needs of the facility; however, we suggest that an ideal imaging facility for drug development includes research and technical support for imaging optimization, contrast enhancement and intellectual collaboration, technical expertise for animal procedures, and an informatics division. A successful facility design is the result of attentive forethought toward facility short- and long-term goals and future plans and collaborations with specialists in the various aspects of the center components.

3.2 Facility Design

Countless factors influence the function and design of an in vivo animal imaging facility. This paper will explore what we consider the four major topics in facility design. The first discusses general facility needs for employees involved in animal research. Much of this discussion applies to any types of workspace, office space, storage space, restrooms, break rooms, and conference rooms, building environmental parameters, and the pathways which animals and humans take to get to various locations. Animal imaging support, including procedure rooms, housing, and euthanasia, is discussed in the second section. The third major topic focuses on the design specifics associated with MRI. These include structural requirements, tools and magnet location, as well as MRI magnet room environmental concerns and cryogen gas usage and storage. Lastly we explore assorted ancillary equipment that is useful in the imaging and animal research areas (biological safety cabinets, chemical fume hoods, autoclaves) and various other safety issues (radiation, biological).

3.2.1 General Facility Considerations

When planning a preclinical imaging center, always remember the prime directive: to image numerous animals with high-quality data as easily and efficiently as possible. This includes minimizing stress to the experimental animals as well as the employees. Key decisions will determine several building design factors: the types of imaging animals and their proximity to the imaging facility; the traffic patterns relative to animal entry, room layouts, and loading docks; and the use of modules or blocks constituting repetitive design. Incorrect assumptions, inaccurate information transfer, and lack of communication may result in planning errors (Ruys 1990). Communication mistakes and erroneous professional judgment, including failure to ensure that all assumptions upon which decisions are made are correct assumptions, are more difficult to guard against and could prove catastrophic in accomplishing a successful design. Soliciting the opinions of knowledgeable and experienced consultants may prevent the need for costly corrections of design errors. Do not assume that the architect is familiar with all the functions of a research animal facility. Be sure to communicate functional design priorities with a clear explanation of the reasoning behind the design to ensure the architect's understanding. Aesthetics have no value if the building design does not support an efficient workflow, and project success depends on the architect's comprehension of the functional design criteria. Keep all communications clear and concise. Summarize all discussions in writing for all parties, emphasizing the functional goals and reiterating the rationale for the design layout (purpose, workflow) at the risk of redundancy.

The imaging center planning committee should include a variety of people with several areas of expertise. Laboratory animal veterinarians experienced with each of the desired imaging species and managing the required support staff will be knowledgeable in logistics of animal transportation, preparation areas, emergency support, and meeting the requirements of AAALAC accreditation (if desired). Specialists with expertise in citing the various desired imaging modalities can point out the specific building design requirements needed for the equipment and support areas. The input of an occupational health specialist will prove invaluable for a healthy work environment as well as a design to accommodate imaging pathogens with higher designations. Imaging scientists will offer insight into some of the ancillary support equipment that may enhance the imaging environment. Examples of supplementary equipment and space may include planning for an area to perform surgical procedures prior to imaging, perfusion fixation for post-imaging histology, a room for euthanasia and tissue harvest, and a clinical laboratory area for time-sensitive assays that may need to be run immediately prior to, during, or after imaging (blood gasses, ammonia levels, clotting factors, blood levels of drugs or metabolites). Inclusion of an administrator, human resource person, or someone otherwise nonaffiliated with imaging may offer a unique and valuable perspective for personnel requirements and visitors to the center. It is easy to get tunnel vision regarding the desired imaging goals and overlook "normal" requirements for the employees such as break rooms.

Traffic Patterns. When planning corridors and access points, consider all people, animals, and equipment (corridor width) that will need to get from one place to another. Ideally the animal housing facility is incorporated within the imaging center or conveniently adjacent. Animal transportation can cause physiological stress (and therefore confound physiological imaging data) so it is best to keep travel time, distances, and environmental factors to a minimum. Plan for easy, direct routes from building entrances to preparation areas and imaging suites. Additionally, locate imaging areas such that people and animals do not pass through other imaging areas to arrive at the designated location. A perfect scenario would include an animal prep and recovery area adjoining each distinct imaging area. These imaging areas would have solid walls and doors to provide physical barriers to prevent any cross contamination when multiple imaging devices are being used simultaneously. Additionally, attention to study flow improves efficiency when there is adequate working space such that one animal can be prepped while one is being imaged and another is recovering.

Remember to place the personnel offices (with a dedicated human entrance) outside of the animal imaging areas. Working with laboratory animal species generally requires the use of personal protective equipment (PPE) to protect both the animals and employees from any cross contamination or allergens. It is not practical to have areas where PPE is required and areas where PPE is not required (offices) within the same sections of a building. Areas designated for animal work and people have different environmental requirements with regard to temperature, humidity, air exchange, and relative air pressure. Keep the animal imaging and procedural areas

physically separated from the human areas to maintain the most effective and convenient animal biosecurity and occupational health standards.

Designated corridors for animal transportation to and from imaging and procedure areas and restricted corridors for human traffic only are ideal. This separation will help to minimize the potential of one species posing an infectious disease threat to another species. Old world nonhuman primates (NHP) may carry endemic herpes viruses that can be deadly to new world NHP and humans (Coulibaly et al. 2004, Gay and Holden 1933; Loomis et al. 1981; Weigler 1992; Wilson et al. 1990). Additionally, humans are a significant risk to both old and new world NHP with regard to measles and tuberculosis. The risk of infectious pathogen exchange may be somewhat reduced between human and rodent species, but risk still exists. Additionally, it has been demonstrated that laboratory workers may develop allergies or asthma to rodents (Aoyama et al. 1992; Bardana 1992; Chan-Yeung and Malo 1994; Hollander et al. 1997) so it is best to prevent unnecessary occupational exposure to allergens.

Cross contamination of murine pathogens can be prevented through good practices and management strategies. A preclinical imaging center may serve rodents of varying health status. While a strict barrier facility tolerates no murine pathogens, it may be impractical to try to maintain barrier status for imaging animals unless the imaging equipment is located within the barrier. Conventional housing facilities offer some leniency with regard to pathogen tolerance and may facilitate movement of animals to and from imaging more easily. Animals of various levels of immunocompetency may be needed for cancer studies and other immunomodulatory drug research, as well as animals purposely infected with pathogenic organisms that require containment. Consider all these factors when planning corridors within the facility. An example of an imaging facility design is offered in Fig. 3.1.

Supply Storage Rooms. Storage space is often in short supply and comes at a premium price. When planning a new imaging facility, be sure to consider all aspects of the facility operations to ensure adequate storage space allocation. It may be beneficial to define these areas as “support space” instead of “storage space” to make certain its value is not depreciated. Each imaging suite should have designated areas for frequently used consumable items such as personal protective equipment (PPE), gloves, disposable drapes, syringes, and needles. The amount of space within each room may vary by imaging equipment type, as some devices require minimal supplies. Contrast agents, emergency support drugs, anesthetics, sterile eye lubricants, and other pharmaceuticals would also be ideally located within the imaging suite. Spare boxes of paper hand towels, gauze sponges, and tape could be stored in each room. These types of consumables could easily be stored under laboratory bench tops or within portable storage cabinets. Lockable storage is always desirable and may be required. Dedicated storage rooms are needed for larger quantities of consumables and general supplies since they can require quite a bit of space. It is likely not practical to order small weekly quantities

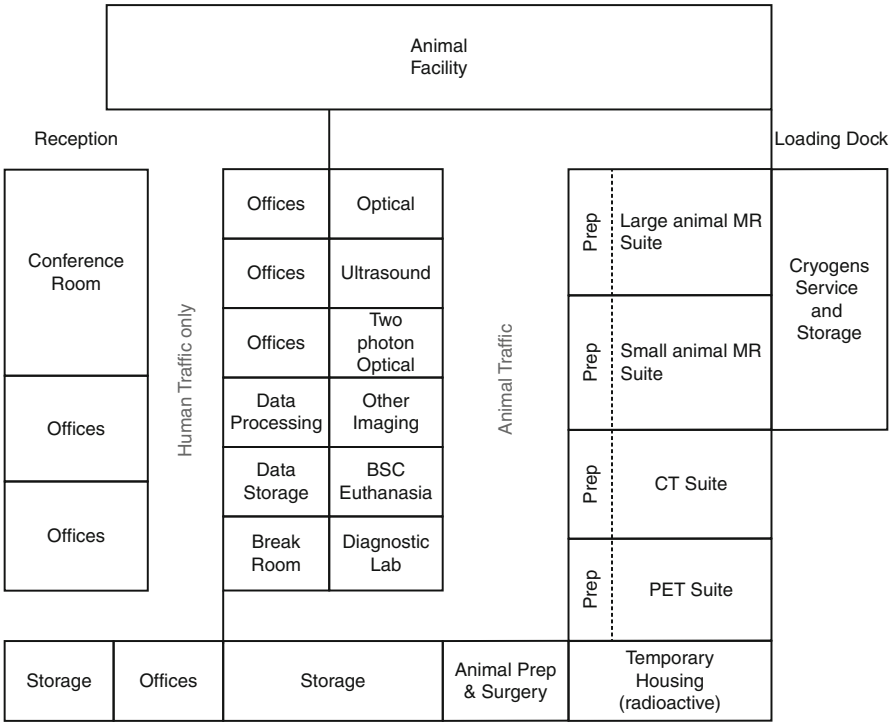

Fig. 3.1 An example layout for an in vivo imaging center, including suites for multiple imaging methods and support spaces. Note that the center separates human spaces from animal spaces and the access pathways between these areas. This is critical to maintain hygiene and pathogen transmission controls. Imaging suites should be dedicated to a single modality to reduce problems with dual scheduling. Small prep areas should be dedicated spaces in each suite to prep an animal for imaging studies independent of any particular preparation in the biomedical study

of provisions. Garbage bags, medical pathological waste (MPW) containers, empty sharps containers, clean mop heads, etc., will all need a larger storage area and may also require safety shielding for radiopharmaceutical waste, animal carcasses, and tissue that are being held for counting/processing. Remember to allow storage space for administrative supplies such as CDs and DVDs, printer paper, toner cartridges, pens, notepaper, and files.

Hazardous chemical storage (including cleaning solutions) will require special consideration such as fire and explosion proof cabinetry. OSHA and your Department of Occupational Health and Safety will determine the requirements. It may be useful to over-anticipate current needs to allow for greater flexibility for future experiments.

Each specific imager will have its own unique storage needs. Most MRI magnets are superconducting and thus necessitate allocated space to store large cylinders of

cryogens such as liquid helium and liquid nitrogen. This storage area requires easy access to a loading dock for cylinder exchanges and liquid gas refills. Various imaging accessories such as MRI gradients and coils should be conveniently stored near the magnets for easy access, including magnet safe tools and devices. Space within magnet rooms is often ideal so storage cabinets or shelves could be built in during construction. Remember to consider the best location for a loading dock to receive animals and supplies.

Administrative and Personnel Offices. An imaging facility requires skilled people to run the scanners and maintain daily operations of the facility. Each employee needs a space for desk work and their personal belongings and to eat lunch. Strategic locations of office space in close proximity to workspace will provide a pleasant work environment while meeting the demands of the center. Senior laboratory staff and scientists, principal investigators, and facility managers should all have private offices, and upper level personnel should be provided enough space to have collaborative or private discussions. Animal imaging technical support staff (veterinarians, technicians) may have double duties within the housing facilities and imaging suites. Depending on where they spend most of their time, office/desk space may be located within the imaging or housing facility. If animals are maintained in a barrier, it is more practical to have support staff office space adjacent to the barrier, but if personnel are dedicated to the imaging facility, then desks within the facility are more practical. Consider space allotment for visiting scientists and other temporary personnel such as postdoctoral fellows and graduate students. Remember to include storage space for common office supplies, fax/copy machines, and mailboxes. Reference manuals, standard operating procedures, and animal study proposals should be located in a secure, employee-accessible area.

Break rooms are a welcome addition to each floor of a busy facility. Personnel need space to safely consume food and beverages outside the imaging and animal areas. Sound buffers are desirable so that employees can enjoy a brief respite from loud imaging equipment and support areas. Since break rooms are gathering places, a white board, tack board, table, and chairs will provide a comfortable environment and an opportunity to discuss issues or post announcements. Since dining is the primary function of the break room space, the room is ideally equipped with a refrigerator, microwave, countertop, and a sink for washing hands and dishes. In the age of "green thinking," cabinets will provide space for reusable mugs, dishes, and silverware, and if space allows, a dishwasher is a welcome convenience. Remember to set aside space for waste and recycling containers of appropriate volumes.

Restrooms and Showers. Restrooms are required in any type of building and should meet Americans with Disabilities Act (ADA) requirements. Central locations for restrooms allow minimal disruption to the workday's activities. The size of the facility and number of employees will dictate the number and locations of restrooms. Showers may be necessary within the animal housing facility but are

optional for an imaging facility. Their necessity should be discussed with the laboratory animal veterinarian and occupational health specialist.

Conference rooms are valuable meeting rooms and can serve multiple functions. A conference room is an ideal location for invited speaker presentations, scientific discussions with investigators, sales representatives, journal club, employee training, and orientation and continuing education. The size of the imaging facility and number of researchers will suggest the number of conference rooms needed. The room should be equipped with state-of-the-art audiovisual equipment (adequate for large media file presentation), appropriate lighting control, and sound buffering for efficient communication. Access to the Internet via hardwire or wireless communication is necessary in the current world of cloud computing. Conveniently located electrical outlets are necessary to provide power to laptop computers or other devices such as vender equipment demonstrations. A dry erase board or blackboard is convenient for illustrating discussions.

A comfortable conference room is an ideal location for a laboratory animal imaging resource library. The bookshelves offer pleasant room aesthetics and the quiet room a refuge for study. The library's textbooks and manuals are also handy for quick reference sources during meetings. The conference room is ideally located in a quiet section of the building near personnel offices and outside of any restrictive areas that may contain magnet fringe fields or radiation hazards.

Environmental Considerations. Zoned control for temperature and humidity is desirable in the design of an animal imaging facility. Humans and animals have different environmental requirements than do equipment and computer areas. Both humans and animals will appreciate humidity levels between 30 and 70 %, but independent supplies and controls for human areas and animal areas are needed to meet the individual requirements of the experimental animals and personnel. Animal room temperatures range between 65 and 85 °F, but the animal species will dictate the desired ambient temperature so there may be different set points for different rooms. Most important is the ability to maintain the animal room temperature within 2 °F of the set point (Institute for Laboratory Animal Research 2011; Hessler and Leary 2002).

Computer rooms and many types of associated imaging equipment generate a lot of heat during daily operations. Stand-alone supplemental air conditioning is recommended for these areas to avoid overwhelming the building automation system (BAS). It is also a cost-effective alternative to overdesigning the BAS when these heat-generating areas are only a fraction of the entire building. The specific environmental requirements for specific imaging platform suites are defined in the manufacturer's siting recommendations.

Air exchange rates and relative air pressures are an important environmental control for air quality and biological security. Generally areas occupied by personnel maintain positive air pressures relative to corridors to prevent entrance of airborne hazards. By the same token, animal areas are usually adjusted to negative pressures

to contain animal odors, allergens, and potential pathogens. These parameters can certainly be set and adjusted after the building construction is finalized, but careful thought during the planning stages may help to avert any major HVAC renovations at a later date.

3.2.2 Animal Housing and Imaging Support

A preclinical imaging center is ideally located next to an animal holding facility. This ensures convenience for researchers and minimizes transport time for animals. Multiple species will be needed for drug development, and each has its own husbandry requirements. Regulated species necessitate an extra level of planning (such as outdoor exercise pens for canines). Guidelines for animal housing facility planning and construction can be found in many sources and will not be discussed in great detail here (Institute for Laboratory Animal Research 2011; Hessler and Leary 2002). Do consider the need to bring in animal models from outside sources. With numerous genetically manipulated mouse models available, it may be more appropriate for a study to use a model that is not available from a commercial source (i.e., from researchers in academia). The health status of outside sources may differ from that of the main colony, so quarantine areas and the ability to isolate populations are paramount and must be planned accordingly. The laboratory animal veterinarian can offer practical advice during the planning phase.

Housing and Holding Rooms. While animal housing and holding space is vital to a well-planned imaging facility, for the purposes of this document, we assume that the animal housing location is immediately adjacent to the imaging areas. Specific requirements and recommendations for each animal species can be found in several references (Institute for Laboratory Animal Research 2011; Hessler and Leary 2002) and are beyond the scope of this chapter. It may be appropriate to incorporate a small temporary housing area for smaller species like rats and mice if serial imaging will take place during a short period of time (6–24 h). Additionally, it may be beneficial to house radioactive animals from PET/SPECT studies within the PET/SPECT imaging area until the radiation emissions return to a safe level. It is sometimes permissible to house animals on bench tops for brief periods, but the environmental controls in imaging areas may not match the species requirements. If space allows the facility to provide "normal housing" identical to the home housing facility, it may help to minimize any anxieties the animals may have associated with the new imaging domain as well as maintain any microenvironmental controls. Additionally, if the home housing facility is a 10 min walk from the imaging suite, but the temporary housing space is within 1 min, its proximity adds to study/work efficiency. Temporary housing should use the same equipment as the home facility to facilitate interchangeable parts. A mouse or rat cage could be used for transporting the animal to the imaging suite in toto and then placed in the temporary housing rack without the need for a cage change. This temporary housing space could be located in a prep area adjacent to an imager or in a separate nearby location.

For both the home housing rooms and temporary rodent housing area, position the room away from loud and repetitive noise as it can produce deleterious effects on mice (Turner et al. 2005, 2007). If temporary housing areas exist within the imaging facility, appropriate accommodations for husbandry supply storage, cage changing, cage/wash washing, etc., all come into play. This may be avoided by utilizing the home housing facilities resources or use of disposable caging systems.

Procedure Rooms. General anesthesia is usually required to immobilize animals during imaging sessions, so every imager will need some type of animal procedure area. The complexity of the preparation space is defined by the imaging modality. For example, bioluminescence and fluorescence optical imaging is a relatively simple procedure compared to other modalities. An anesthetized animal is placed on an imaging platform (or under an imaging probe or microscope) and an image acquired over seconds to minutes. It may be necessary to inject a substrate (i.e., luciferin), but a small area where animals can be anesthetized, injected, and fur removed may suffice. On the other end of the complexity scale is MRI. After being anesthetized, animals are positioned on an imaging platform (or bed in a clinical scanner), an external heat source is positioned (if not built into the platform), sensors for physiological monitoring are applied, intravenous access established (if dynamic contrast study or blood draws for drug or biomarker kinetic analysis), and then the imaging coil is secured in place (or the animal is placed within a coil). For small animals such as rats and mice, this all may be done on a properly equipped bench top, but larger animals will need sufficient space for personnel to be able to properly prepare and have access to the animal for MR imaging. Larger animals are often intubated and ventilated, so allow space for stationary or portable large ventilators. Our facility performs a large number of NHP brain imaging procedures, and the animal is often positioned within a head stabilizer that is also used with stereotaxic coordinates for surgical procedures. Accurate animal positioning is critical to acquiring useful data and cannot be casually applied. Additionally, consider an emergency scenario: do personnel have enough room to perform cardiopulmonary resuscitation or rapid anesthetic induction, if needed? Ideally, the procedure room is located adjacent to each imaging room. It may be possible to design a shared space for several imagers, but this could hinder any simultaneous imaging on neighboring scanners. Cross contamination could also be a variable in a shared space.

Each procedure room/area should be equipped with the necessary tools for general anesthesia, imaging and associated procedures, and emergency situations. The basics for inhalation anesthesia include an induction chamber or induction drugs, precision vaporizer and vehicle gasses, intubation tubes or face masks, and ventilator (depending on species). Maintenance of normal physiological parameters is most important, so an external heat source and physiological monitoring equipment are also needed. All equipment used in proximity of scanners must be MRI compatible if preparing an animal for MRI, or low-density, low photon attenuation equipment for PET or SPECT. Emergency drugs and life support equipment and drugs should be conveniently located and readily accessible. An adequate method of handling waste anesthetic gasses is required to meet OSHA standards and assure personnel safety. Procedure rooms are a convenient location for eye wash stations

and NHP bite and scratch kits. Consultation with the laboratory animal veterinary staff during planning will ensure that the rooms are appropriately stocked and functionally designed.

If imaging will occur immediately prior to or after a surgical procedure, it may be useful to provide a surgical area. Larger animals require a properly designed dedicated surgical area. If imaging facility space is not available for a dedicated surgery suite, then surgical procedures are best done in the home housing facility. For smaller animals, transportation under anesthesia is much more challenging, so the ability to perform surgery and imaging in neighboring rooms is advantageous. If possible, follow the same guidelines for designing a small animal surgical suite as would be used for regulated species. This not only assures a suitable surgical environment for small animals but allows for future flexibility should a surgical area be needed for a regulated species.

Euthanasia. Euthanasia techniques should be done as painless and stress-free as possible and performed in such a way to minimize any animal distress and anxiety prior to loss of consciousness. Stressed animals emit alarm pheromones that can affect other animals within a room, thus propagating the distress and anxiety (Brechbühl et al. 2008). If euthanasia will be performed in awake animals, an isolated room should be dedicated for euthanasia procedures to comply with international standards (AVMA 2013; Institute for Laboratory Animal Research 2011). The home housing facility will surely be equipped for these procedures so it may not be needed in an imaging facility. Animals under general anesthesia for imaging can easily be euthanized under the same anesthesia to avoid the necessity of a dedicated area. Additionally, it is aesthetically better than recovering the animal from anesthesia and then performing euthanasia. Space to perform perfusion fixation and tissue harvests at the time of necropsy are discussed in other sections. A cold room or freezer for carcass disposal may be convenient to investigators in the imaging facility, and its size should be coordinated with the species undergoing the imaging studies as well as the rate of animals needing these disposal sites.

3.2.3 Magnet (MRI)-Specific Facility Designs

Location. MRI magnets have specialized building and environmental requirements. The architect or engineer needs to work closely with the end users and the MRI manufacturers to determine the structural details required for the instruments. Instrument makers provide detailed installation documentation to end users and the facility engineers to assure a successful installation and operation. The magnetic field in and around the MRI magnet can inactivate or alter life support devices such as pacemakers, neurostimulators, and insulin pumps. The location of MRI scanners must be carefully considered and planned such that the magnetic fields surrounding these instruments (fringe fields) do not interfere with other equipment or present a health hazard to personnel. Fringe fields of neighboring instruments may overlap,

but the manufacturing engineers should be consulted before finalizing plans. A magnetic field of 5 gauss is the FDA limit for the general public including people with pacemakers or other internal devices (Erdogan 2002; Faris and Shein 2006; Shinbane et al. 2007; Expert Panel on MR Safety 2013). The 5 gauss (G) fringe (peripheral to the magnet core) field lines should be well marked and protected from inadvertent access. Areas that personnel or visitors may use (hallways, offices, restrooms) must be located outside of all 5G contours. In addition to electromagnetic instruments, static MR imaging magnets must be isolated from large moving metal masses such as elevators. The latest American College of Radiology (ACR) MR safety guidelines should be consulted prior to start of any major MRI project. Though written for scanning of human patients, most of the major points also apply for preclinical imaging subjects (Expert Panel on MR Safety 2013).

Structural Considerations. Depending on the equipment's specifications, MRI magnets may be located within electromagnetically shielded rooms. This forms a physical isolation of the magnet that isolates and protects the magnet and personnel from safety hazards. The RF shielded room is specifically to isolate the MRI platform from the surrounding electromagnetic spectrum in the RF frequency range. MRI uses RF to generate images, and RF from other devices (such as physiological monitoring equipment) can interfere with imaging. The shielded room also serves to decrease the level of ambient noise generated by the operation of the MRI to operators outside the room. Special acoustical design features may be required to mitigate the transfer of sound and vibration through the structure to adjacent areas. Pits may be needed for the larger pieces of equipment. Many MRI scanners are located on basement or ground levels due to the weight of the instruments. The floors of scanner rooms may require reinforcement to support weights from 200 to 20,000 kg (44,200 lbs). Access and clearance, both vertical and horizontal, around the equipment must be carefully planned for equipment requirements, maintenance, and initial delivery and setup. The weight and size of these instruments may require that they be lowered into their resting places by a crane through a specially designed roof hatch opening. This is another reason that it may be practical to locate the imaging equipment outside the main footprint of the building. In all cases, it should be considered to have an installation access pathway for the magnet into the shielded room or the MRI suite outside of a standard or wide standard door. It is most likely that end users will want to exchange or upgrade the MRI as technological developments and user sophistication makes a newer instrument or instrument modifications an attractive possibility.

Cryogen Gasses. The large majority of MRI magnets are superconducting, so special cooling requirements will be determined by the specifications of the instrument. Consideration must be given to the access and storage of liquid gas Dewar flasks with the clearance needed to add cryogens to the devices. Typical cryogens are liquid helium and liquid nitrogen. These inert gasses pose a threat of asphyxiation if released in an enclosed space with poor ventilation, so each magnet room must include an oxygen sensor at standard head height that alarms when oxygen levels fall below normal limits (usually 18 %). If possible a method of

increasing ventilation in the MRI room during cryogen service operation should be included. All superconducting MRIs include manufacturer-mandated emergency ventilation from the magnet in case of a failure. The design engineer or architect must comply with these safety requirements when installing the emergency vent system.

Environmental Considerations. MRI suites require stable temperature and humidity control in the vicinity of the magnets and the supporting electrical equipment room. The design of the control systems must be coordinated with the mechanical systems design such as HVAC, plumbing, and other support services to minimize environmental condition fluctuation across magnets. The typical MRI room will require constant monitoring of temperature, humidity, and oxygen levels. Humidity requirements must be coordinated with the equipment manufacturer but will usually be around 20–25 % with minimal fluctuation. Requirements of the dehumidification system requirements should be listed with the system used.

Work Areas and Tools. Besides the magnet room itself, several operational spaces are needed. The ancillary equipment such as power supplies, RF amplifiers, gradient power supplies, and magnet cryogenic refrigerators are located close to the magnet room itself. The requirements for this operational equipment can be as large and specific as the magnet itself. Depending on the type of MRI system, the location can be within the publically precluded fringe field which allows a more efficient use of space. The operator console area should be able to support the scanner console and observer space. The console area is where the MRI scans are configured, initiated, and monitored. It is ideal to have additional workspace adjacent to the console area for laptops or analytical workstations. Animal preparation workspace must also be considered. Simple surgical instruments such as scissors, scalpels, and needles can become life-threatening projectiles if taken too close to the magnetic field. Additionally, repairs and periodic maintenance within the magnet room will require use of nonmagnetic tools (including screwdrivers, wrenches, scissors). Nonmagnetic tools (e.g., beryllium-copper alloy or titanium) are an additional expense that cannot be dismissed as unnecessary because standard instruments made of stainless steel and iron are rendered useless in the magnet room if not outright dangerous depending on proximity to magnet. Nonmagnetic light sources (such as flashlights) and cleaning supplies (brooms, mops, and buckets) are another necessity. Due to the magnetic field, there are significant safety hazards associated with working in the MRI environment. We recommend required safety training for all personnel and facility users.

3.2.4 Additional Facility Design Considerations (PET/SPECT, etc.)

Radiation Safety. Ionizing radiation is a useful tool for imaging functional processes with positron emission tomography (PET) or single photon emission

computed tomography (SPECT) and targeted tissue studies using autoradiography. Its use warrants special consideration during building planning phases and should involve a radiation safety specialist. The management of radioactive reagents, animals, and radioactive waste must satisfy nuclear regulatory requirements as well as any local and institutional policies. This includes restricted areas to prevent unauthorized entry and lockable storage containers. Reagent preparation areas, space for a scintillation counter, waste storage, record keeping, and decontamination chemicals (laboratory surfaces and skin contamination) must all be considered.

The half-lives of many commonly used PET radionuclides are short lived (some only minutes to hours) so commercially available imaging probes are often limited (e.g., 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG); half-life of 109 min (Vijayakumar et al. 2006). These short-lived isotopes require space for a nearby radiochemistry (radiosynthesis) laboratory, and a cyclotron to produce custom radionuclides is an ideal laboratory situation. Delivery routes of the radioactively labeled probes, whether custom or commercially produced, must be designed to minimize personnel exposure and transportation time and facilitate emergency procedures in the event of a spill.

Animals given radiolabeled probe may require special housing which can include metabolic caging to capture urine and fecal excretions for metabolic or kinetic analyses. It may be useful to incorporate temporary rodent housing within the imaging areas to allow decay to safe levels before returning animals to home facilities. For larger species, it may be prudent to plan for housing radioactive animals away from the main facility to avoid exposure to non-treated animals and to staff while an animal may await multiple images to follow the biodistribution. Animal waste, bedding, etc., may require special handling and must be taken into account during the planning phase.

Occasionally, radioactive reagents may be used during other types of imaging for later validation of imaging methods (e.g., autoradiography, longer-lived tracers, i.e., C-14, S-35, I-125). These methods require similar consideration for reagent preparation areas, space for a scintillation counter, autoradiography filming (potential need for a darkroom for film or liquid photographic emulsions) or phosphorimager methods, isotope accounting, study record keeping, dosimetry, and decontamination chemicals. The reader is encouraged to also see the chapter on autoradiography techniques included in this volume.

Animal Biosafety. The option to perform research involving the use of infectious pathogens and biohazardous reagents should be determined in the planning stages of the facility design. Specific environmental controls must be incorporated in the building design in order to achieve adequate biosecurity. Additionally, the specific pathogen status of the experimental animals must be considered. With the explosion of genetically manipulated mouse models (and other species), researchers may want to bring animals from noncommercial sources into the facility for their research. Containment (quarantine facilities) and transportation routes should be considered

during planning phases to minimize contamination potentials. Biosafety works in two directions: preventing animals or people from being exposed to a known research-associated biohazard (viral vectors, toxic metabolites) and preventing research animals from inadvertent exposure to environmental hazards (employees with influenza, immunocompromised animals and opportunistic pathogens). When allocating appropriate workspaces and storage for biohazardous materials during planning phases, give special consideration for decontamination procedures in the event of a biosecurity breach. This could include facilities for autoclaving of bedding and waste in the case of infectious disease imaging. The reader is encouraged to read the chapter in this volume on BSL-3 and BSL-4 nuclear and MR imaging.

Laminar flow hoods provide a unidirectional air flow at a fixed velocity that creates a protective "air curtain" within the hood. Several different types of biological safety cabinets (BSC) exist, each with varying levels of protection. Class I BSCs offer protection to personnel and the environment, but not the object within the cabinet. These are often used for procedures that have potential to create hazardous aerosols or equipment enclosure such as centrifuges. All class II BSCs are designed to protect the contents within the hood from contamination, as well as the personnel and environment (Chosewood and Wilson 2009).

3.3 Animal Imaging Support

The use of laboratory animals in drug discovery cannot be entirely avoided. In order to collect useful imaging data, it is imperative to minimize or prevent movement during acquisition for many in vivo imaging devices. Although it is possible to train some animals to accept restraint during certain imaging sessions through reward training, most animals are imaged under general anesthesia. Anesthesia allows the researcher to position a relaxed animal for optimal imaging data, minimizes motion artifact, and eliminates any animal stress or fear during restraint. This "animal normalization" tends to promote more uniform image data, especially in neurotransmitter imaging or functional MRI (blood flow; blood oxygen level-dependent imaging, BOLD). The facility's laboratory animal veterinarian will serve an important role in determining the best anesthetic protocols for each type of experiment. The following section will examine anesthesia equipment in the imaging environment, physiological monitoring equipment, and useful ancillary resources for imaging studies.

3.3.1 Anesthesia Equipment

We are currently fortunate to have choices of many safe, effective, and reasonably priced anesthetic options for the laboratory animal. There are many injectable

agents to achieve various levels of restraint or a surgical plane of anesthesia, and some are even rapidly reversible. Additionally, there are also several approved inhalational anesthetics. Every drug has a desired effect as well as potentially undesirable secondary effects. Researchers should discuss the experiments in detail with the laboratory animal veterinarian so the veterinarian can design an anesthetic protocol to minimize undesired effects that could impact results.

Inhalation anesthesia has many advantages over an injectable agent. Small animals such as rodents can be anesthetized with minimal stress of handling. They are gently placed in an anesthesia induction chamber, and the anesthetic agent is delivered by a gas vehicle, such as 100 % oxygen or oxygen-enhanced gas mixtures. Once the rodent is unconscious and unresponsive, they can be maintained under anesthesia with the aid of a nosecone. It is possible to intubate rodents, but aside from the technical challenges of correctly placing a rodent intubation tube, we found it more problematic due to respiratory secretions clogging the airways. Details about rodent anesthesia can be found in many sources (Fish et al. 2008).

For larger species, general anesthesia is usually achieved by administering an injectable induction agent, but we still prefer using an inhalant anesthesia during in vivo imaging. The single most important advantage of inhalant anesthesia in the imaging environment is the ability to rapidly adjust the level of anesthesia remotely (preferred design is outside the imaging room). Once an animal is positioned within a scanner for optimal imaging, it is not efficient to stop a scan in order to administer another dose of anesthetic agent. Several additional advantages of inhalational anesthesia include the following: it has physiological properties of minimal metabolism and rapid clearance which make it relatively safe to use in healthy and compromised animals, it provides an ability to titrate to effect, and it is not a controlled substance. Disadvantages of inhalational anesthetics include the required use of expensive precision delivery vaporizers that must be cleaned and calibrated annually, accessible sources of delivery gasses (oxygen, medical air, nitrogen, nitrous oxide), potential need for a species-related ventilators (larger species), and management of waste anesthetic gasses (WAG).

Injectable anesthetic agents offer many conveniences. The drugs are portable, and some can be administered by a variety of routes (intravenous, intraperitoneal, subcutaneous, oral). For brief periods of restraint during short imaging sessions (with no painful procedures), injectable anesthetics may be suitable. Disadvantages of some injectable anesthetics are that once given, the dose cannot be adjusted; the resultant effects are sometimes unpredictable and variable in individuals; and the drug must be metabolized by the body making any impairment to metabolism (renal, hepatic, or circulatory systems) prolong its clearance. Poor clearance can lead to toxic exposures over time, and some of these anesthetic drugs are schedule II controlled substances and require special handling and accounting. An exception to the disadvantages of injectable class of anesthetics is propofol. Propofol is ultrarapidly metabolized and easily titratable to effect, and its cost has come down in recent years. The use of propofol could prove to be just as safe as an inhalant anesthesia under certain circumstances except that it requires a continuous intravenous

infusion to maintain general anesthesia. While this may be easily achieved in larger species, it is challenging in rodents.

We recommend that all imaging areas be designed to incorporate use of inhalant anesthesia. Access to sources of anesthetic delivery gasses (oxygen, medical air, nitrogen, nitrous oxide) is required. Central sources of gas piped through the facility will provide convenience to users. Consider planning for an area to generate house oxygen. Waste anesthetic gas (WAG) is an occupational hazard so methods for its management must be considered during planning (US Dept of Labor 2013). A centralized vacuum that vents to the rooftop after passing through some filtration is an excellent way to handle WAG. Depending on the species to be imaged, the facility may need several different ventilators and associated equipment. Facility plans for storage areas when these devices are not in use are advised. Before purchasing any laboratory equipment, remember that some items may need to be MRI compatible.

3.3.2 Physiological Monitoring

Several modes of in vivo imaging prevent direct visualization of animals while in the device for scanning. In order to ensure that the animal is alive, physiologically stable, and at the proper level of anesthesia, it is important to utilize physiological monitoring equipment. The animal species will determine the type of monitoring equipment that can be used. Human and veterinary devices work well for larger species, but rodents and animals with heart rates above 300 beats per minute (and breathing rates above 60 breaths per minute) require specialized equipment. Technology has finally caught up to the demand so that now there are several physiological monitoring devices that can reliably measure heart rate, respiratory rate, body temperature, and pulse oximetry in rodents and they can be easily found by key word web searches. Other physiological parameters that may be measured include electrocardiogram (ECG), electroencephalogram (EEG), and respiratory wave patterns. Blood pressure, end-tidal carbon dioxide ($ETCO_2$) levels, anesthetic gas levels, and blood gasses (O_2, CO_2) can be measured in larger species noninvasively, but such measures are currently challenging for rodents. Fiber optic pressure monitors exist for measuring arterial/venous blood pressures in mice. The challenge always lies in catheter placement in the smaller rodent species.

Anesthesia is known to interfere with the brain's homeostatic control of core body temperature. It is important to plan for the use of supplemental heat to keep animals warm during imaging. Several options are available for patient warming such as warmed air devices or warmed circulating water pads. Before installing any warming or physiological monitoring devices, be sure to consult with the imaging specialist. It is important to determine if the device may introduce noise into the image data; additionally, it must be safe to use in the imaging environment (MRI). Wires and tubes attached to the animal may need to be connected to a central unit outside the imager so their route must be considered. Electronics are often

connected through a patch panel for MRI. Detailed anesthetic records should be maintained for every animal regardless of the species to help with image interpretations or to understand anomalous results.

3.3.3 Ancillary Support

During drug development, all aspects of efficacy and safety will likely be explored as best as possible. Equipment used to examine various physiological parameters may be located in laboratory space or the housing facility. It may also be convenient to consider having a diagnostic laboratory with microscopes, serum chemistry machines, hematocrit centrifuges, and complete blood count analyzers located near the imaging suites. While the animal is under anesthesia for imaging, the potential exists to collect tissue samples (such as blood), and rapid processing is usually beneficial if not required. Noninvasive blood pressure monitors and electrocardiograms may be useful to compliment the imaging data.

When imaging is at the experimental endpoint, it is possible to perform the euthanasia before recovery from imaging anesthesia. This is aesthetically more pleasant for animal care staff. A chemical fume hood conveniently located near the imaging suite will also facilitate perfusion fixation procedures without the need to recover the animals and transport them to another location.

3.4 Personnel

A successful laboratory animal imaging center requires a team approach to personnel which have a variety of skill levels and skill sets to efficiently and successfully master the tasks at hand. An ideal imaging laboratory will be self-contained in terms of critical core personnel such that any problem or opportunity can be addressed in a timely fashion to minimize downtime. The ideal facility would employ imaging specialists, animal support technicians, computer information technologists, and building staff appropriate for the installation. These different positions are discussed below in piece.

Imaging Specialists. Generally each imaging modality needs some imaging specialist to lead operations, planning, and technical developments on each modality. Depending on the modality, the level of training will vary. In an imaging facility that does technical development and research in addition to providing routine imaging services, additional expertise is required. To facilitate the scientific collaborations and nurture the advancement of the animal imaging technologies, doctorate-level researchers are necessary. The physics, chemistry, and biology peculiar to each imaging modality can be uniquely singular from the other imaging methods in the facility. Specialized training will be required to be

proficient at any imaging method so staffing and regular trainings are expected to maintain proficiency and reproducibility across studies. For MRI, the specialist should be skilled in the physics of the imaging process in order to develop new methodologies as required. For CT, PET, or SPECT, the specialist will need training regarding the physics of the imaging process, radiation safety and measurement, and knowledge of chemistry and physiology to develop new methods and application of radiopharmaceuticals and contrast agents. Specially trained personnel are needed to maintain the imaging magnets, CT systems, optical platforms, autoradiographic equipment, and PET/SPECT scanners. All modern imaging modalities are heavily dependent on cutting edge computer technology and data handling/storage to operate the scanners, reconstruct the data into useful images, and process the images into physiological relevant information. This can be accomplished via service and maintenance contracts with the vendors or other providers at the expense of imaging time delays (and disruption to imaging studies) that may occur if personnel are not in house. If funding will allow, specialists employed in each of the imaging modalities are ideal, but the reality is that motivated and skilled personnel may also perform adequately on more than one imaging method.

Animal support personnel are a critical element in the success of any imaging center. Technicians are needed to perform imaging procedures, anesthesia, catheterization, and other assorted surgical procedures, as defined by the study needs and the center SOPs. Technicians may be trained to run specific or routine imaging procedures in order to free up intellectual time for the imaging physicists. Husbandry personnel and support staff are required to maintain the animal housing facility and are a key element in maintaining the colony veterinary care. A small facility may require some cooperative technical staff for husbandry tasks, but a larger facility should have dedicated teams to handle each workload area. Veterinarians and veterinary technicians are vital to maintaining the health of the animal colonies and imaging subjects. Again, the number of personnel needed will be dictated by the number and variety of animals housed within the center, and a properly designed facility will aid in reducing staffing costs due to redundancies, gown changings, supply maintenance, etc.

Computer Information Technology (CIT). All imaging methods covered in this chapter create a digital record of the image, and a number of computers are required in the generation, recording, and interpretation of the imaging data. In addition to running the operational software for imaging devices, computers are needed for data management and personnel needs (email, ordering supplies, record keeping). Our experience suggests creation of an in-house or local network facilitates data storage and manipulation, so it is necessary to retain dedicated personnel capable of maintaining the network computer equipment. Although computer downtime will inevitably occur, this time should be kept to a minimum with the presence of dedicated CIT staff. Specific tasks of data processing personnel are discussed in another section.

Housekeeping and building maintenance services are necessary for a fully functioning facility employing numerous personnel. This includes waste removal, cleaning of imaging facilities, and animal preparation rooms. This is not a substitute for the standard animal hygiene and biohazard preparation of a bench space prior to and post performing an animal procedure. High traffic areas of animal movement or mixed functions need to be disinfected routinely to control disease vectors and transmission. Environmental conditions such as room temperature and humidity must be carefully controlled for all operational spaces such as scanner rooms, computer rooms, and animal housing rooms. Fluctuations outside of normal ranges should be corrected as quickly as possible, so building maintenance personnel should be available at any time. It is imperative that all support personnel be trained in safety procedures around the imaging equipment, including contract personnel.

Unique MRI Personnel Safety Considerations. The hazards associated with working around a high magnetic field are due to the difficulty of containment and the interaction of the concomitant field with items that are used in the normal course of a modern laboratory. Because of these hazards, employees should be carefully screened for contraindications to the MRI environment. The American College of Radiology (ACR) has very strict guidelines on who may be allowed to undergo an MRI scan. These guidelines should be considered with all personnel operating in and around the MRI magnet. The same contraindications the ACR is concerned about apply to MRI staff due to their exposure to the high magnetic field environment. Personnel with cardiac pacemakers, neurostimulators, aneurysm clips, stents, cochlear implants, drug pumps, or other metallic implants, including shrapnel, should not work in close proximity to the magnets unless they are cleared by direct consultation with appropriate MRI safety personnel. Metallic implants can shift within tissue if too close to the magnetic field. Working implanted devices may be inactivated in the magnetic fields which could result in a fatal accident (Erdogan 2002; Faris and Shein 2006; Shinbane et al. 2007). Many newer surgical devices and tools are MRI compatible, and personnel can safely work in the magnetic field, but this needs to be cleared by the appropriate MRI safety official (Shellock 2007). Warning signs should be posted in highly visible areas all around the facility to warn people of the magnetic environment. As stated earlier, magnets are best located in isolated areas where people may not inadvertently wander into the magnetic fields.

3.5 Imaging Equipment

A brief overview of several in vivo imaging methods follows, but details about each technique are beyond the scope of this paper. We advise the facility planners to further educate themselves on each of the techniques or consult with a specific imaging platform expert before making final decisions. Included in the following

brief in vivo imaging introduction are magnetic resonance imaging (MRI), X-ray computed tomography (CT), positron emission tomography (PET), ultrasound (US), and optical (OP) imaging. Other chapters in this volume will describe these and other modalities, lending nuances which each author brings on their respective discipline.

Magnetic resonance imaging (MRI) is a powerful, three-dimensional imaging modality that uses the property of nuclear spin in certain isotopes of elements to form anatomical images (Haake et al. 2000). Due to the expense of the MRI instrumentation (usually on the order of 0.5–1.5 million US dollars) and the environmental design requirements, the modality is usually placed in a shared imaging facility to maximize use. MR images are most structurally sensitive to soft tissue (e.g., nerves, muscle, blood) and can detect a large range of physiological conditions beyond static anatomy such as blood flow, perfusion, functional brain activity, or white matter orientation in the CNS or musculature (Kwong et al. 1992; Tseng et al. 1999; Mori and Zhang 2006) with minimal changes to imaging conditions for the preclinical subject. In general, MRI does not require contrast agents for images, but many contrast agents have been developed and are available clinically per specific FDA-approved indications but may be used experimentally to exploit a new drug or biological mechanism of action in the regulatory path of drug development.

As with all imaging techniques, MRI is sensitive to motion during the scan interval (acquisition period). The length of this scan interval—from seconds to minutes—makes accounting for animal motion such as respiration or cardiac motility an imperative. Periodic motion such as these can be mitigated using a form of synchronized acquisition or "gating" to time the movements during the scan such that the animal is always in the same position relative to the time of actual scanning interval. These gating methods can be either prospective, timing the scan only for a certain phase in the respiratory and/or cardiac cycle, or retrospective, whereby image data is selected for reconstruction based on the place in the cycle that it was acquired. Such gating techniques can provide stop motion cine loops of cardiac phases to provide direct measurements of cardiac wall motion, ejection fraction, and heart muscle perfusion (de Crespigny et al. 1991; Rose et al. 1994). Similarly to cardiac gating, respiratory gating is used to reduce or eliminate motion triggered by lung inflation/expiration and diaphragm movement. Respiratory gating is useful for mitigating motion transmitted within the abdomen and thoracic cavity, though most motion can be suppressed if scanning during the end-tidal respiration interval which is similar to a "breath-hold."

An exciting application in MRI is tracking of individually labeled cell populations in vivo. This has a number of uses in disease and injury processes particularly in the novel stem cell sciences. Stem cells are trackable in deep tissues or optically opaque tissues in the living animal over time (Epstein et al. 2002; Frank et al. 2003; Shapiro et al. 2004). The reader is encouraged to refer to the appropriate chapters in this volume on cell labeling and tracking.

X-ray computed tomography (CT) uses a series of radiographic images, acquired at different angles around the animal, to mathematically reconstruct a three-dimensional image of the subject (Paulus et al. 2000). The method of operation for most in vivo small animal imaging X-ray tomography scanners is to have the X-ray source and detector rotate around the animal synchronously. These images can be 2D or 3D giving the area of the detector, with individual slices, overlapping volumes or an entire volume being reconstructed and processed to form a 3D image of the preclinical animal from head to toe. These high-resolution CT systems are called "micro-CT" to describe the resolution of the images which range from 10 to 95 μm isotropic. The scanners are much smaller in size and in voxel volume than human clinical CT scanners (Jiang et al. 2000). Due to differences in absorptivity of X-rays, CT excels at visualization of bone structures when in proximity of soft tissue (muscle, connective tissue, etc.) or air. The fundamental physical interaction in X-ray imaging is the absorption or scattering of the X-ray by the electrons of the nucleus. The denser the tissue or the higher the number of electrons, then the greater the absorption, for example, calcium in bone absorbs more than carbon in fat. This property can be effectively used to visualize bony structures, fat tissues, or air spaces due the very high contrast between these materials. For materials that have little intrinsic contrast, i.e., the liver, the use of contrast agents can be quite useful in producing image contrast that is biologically meaningful (i.e., cysts or tumor locations). For X-ray CT, these contrast agents have a high atomic number element, usually iodine, attached to a molecule with the useful osmotic properties and penetration in tissue. Most contrast media available in human practice can be adapted for small animals when taking into account changes in blood volume, renal clearance rate, and other relevant factors (see the chapter in this volume on allometrics). Many other preclinical contrast media are available that give a much larger range of studies than the media adapted from medical practice. Many of these preclinical contrasts are based on a variety of nanoparticle technologies that can include therapeutic drug loads in addition to imaging contrasts. Such applications can include localization of tumors, vascular tree imaging, renal clearance, and hepatic structure (Bakan et al. 2002; Vera and Mattrey 2002; Weber et al. 2004).

Positron emission tomography (PET) forms an image based on radioisotope decay of a compound administered to the animal before initiating scanning (Cherry 2004). Radioisotope imaging methods can be very specific due to the low natural background radiation. PET isotopes emit a positron, which forms two opposed gamma rays (photons) upon annihilation with a local electron. This physical 180° oppositional detection provides a physical collimation which increases the statistical certainty of localization and a high signal-to-noise ratio (SNR) from otherwise scatter photons reaching the detectors. Limitations to radiation exposure require that the PET tracer agents be of a low concentration (i.e., high specific radioactivity and low mass for nonphysiological actions). Consequently, due to this low mass requirement and the inherent physics of positron annihilation source location, PET image resolution is lower than some of the other main volumetric imaging methods

like MRI and CT (Cherry 2006). A commonly used PET tracer is 2-[18F]fluoro-2-deoxy-glucose (FDG) for monitoring glucose metabolism and locating areas of high glycolytic activity. This is used in applications such as localizing metastatic tumor load and exceptional brain activity (and glucose consumption) such as during seizures. As glucose is metabolized throughout the body at some level, FDG PET provides an image with some relevant anatomy visualized. More target-specific PET agents can target cell surface binding sites or specific gene-expression products that are more generally sparse, and due to high efficiency, targeting the agent does not provide "anatomic" positioning and thus requires an additional imaging method for useful anatomical references such as MRI and CT (Beyer et al. 2000; Yaghoubi and Gambhir 2006). Current preclinical or "micro-PET" scanners have an image resolution of below 1 mm which is still much larger than competing scanner resolutions from MRI or CT which are submillimeter (Shao et al. 1997; Catana et al. 2006). PET agents have highly specific requirements to accurately and reproducibly synthesize products with cyclotron-produced isotopes and a very short shelf life. Commercial imaging products are available for certain widely used compounds (e.g., FDG). Often a laboratory is limited to production of specific agents such as FDG and a few others, which can travel well upon production to remote imaging facilities that do not have synthetic capabilities. Laboratory preparation of PET agents requires access to a particle accelerator to prepare the PET isotope prior to reacting with the target pharmaceutical for use as a radiotracer.

Single photon emission computed tomography (SPECT) estimates the distribution of radioactivity from an injected radiotracer (non-positron; single photon emission isotopes) injected typically into the bloodstream. Like a PET tracer, the radiotracer distributes in the body based on differences in perfusion and affinity of the tracer compound to the local microenvironment. The SPECT camera acquires a number of radioactivity maps, or projections, from a series of angular views. The spatial radioactivity distributions serve as the input for a mathematical transformation that produces a three-dimensional distribution of the radiotracer. Radiation dosage is not an intrinsic limit in preclinical animal imaging: high-resolution SPECT scanners use extreme collimation techniques to create higher-resolution images. Recently, combined SPECT-CT scanners or SPECT-MRI scans have been developed to reduce the demands of the SPECT system to produce an anatomically detailed map (low-dose CT radiographs, essentially, using an external rotating source for a photon attenuation scheme to correct for tissue density and photon scatter), utilizing the anatomical information from the co-registered imaging technique (Ji et al. 2010; Goetz et al. 2008). Applications for SPECT include preclinical models to recreate clinical conditions of stroke by using Tc-99 sestamibi, a cationic isonitrile that locks into mitochondria of intact uninjured cells, where the animal is imaged following coronary artery ligation to investigate infarct recovery. This example is being used for novel agents to facilitate recovery from strokes or reperfusion injury (Liu et al. 2002, 2004).

Ultrasound (US) imaging is produced from sound waves, and the resultant echoes at tissue interfaces (organ to organ, tissue to blood, etc.) to generate

two-dimensional images in real time. The frequency of the sound wave produced has a direct relation to the image resolution formed. Clinical ultrasound (up to ~14 MHz) is an excellent modality for observing moving organs and tissues such as cardiac wall motion, blood flow in major vessels, and certain anatomical structures such as fetuses. Preclinical US has advanced to higher frequencies (up to ~55 MHz) to produce near microscopic resolution images (~50 μm) of mice noninvasively for highly visualized structures near the surface (Foster et al. 2000, 2002; Zhou et al. 2004). Interactive applications of ultrasound imaging can include image-guided cardiac inoculations of cells and injections of mouse embryos (Slevin et al. 2006).

Volumetric ultrasound is possible by combining multiple 2D images with a registration algorithm to form a 3D image (Solberg et al. 2007). Using additional constraints, it is possible to evolve a 3D image in time to produce a 4D (3 spatial, 1 time) image (Yagel et al. 2007). Preclinical uses of 3D ultrasound can include such applications as cardiac evaluation and cancer diagnosis (van den Bosch et al. 2006; Badea et al. 2007; Correale et al. 2007; Mitterberger et al. 2007). The immediacy and lack of special facility requirements for ultrasound imaging is a strong support for including it in any imaging suite.

Optical Imaging. A plethora of optical imaging scanners, projectors, and spectrometers are available for laboratory animals. Optical imaging capitalizes on the physical properties of light (generated by various mechanisms) and at various wavelengths to generate two-dimensional images or in limited cases three-dimensional images or tomograms.

Laser Doppler imaging (LDI) is a simple and useful tool for assessing blood flow in patients and animals (Bohling et al. 2006; Humeau et al. 2007). LDI utilizes the Doppler shift in the reflectance of hemoglobin to produce images of blood flow below the tissue surface. LDI is limited due to the shallow depth of penetration, the exiting light, and the time required to raster scan over a surface to produce an image. The technique is useful to evaluate perfusion during healing, surgery, and other circumstances. Though limited in their capabilities, LDI scanners are inexpensive, produce short scan times, and have no special environmental requirements.

Fluorescence imaging uses a fluorescent chemical moiety or fluorophore and excitation light source and a wavelength sensitive detector. The method leverages the rich background of light microscopy and the labels, techniques, and specific fluorophores that have been built over decades of investigation and have recently been adapted to in vivo preclinical imaging (Graves et al. 2004; Hassan and Klaunberg 2004; Montet et al. 2007). The numbers of applications are too numerous for this chapter; however, a few examples include cell trafficking, tumor diagnosis, and staging. The technique does have to contend with many of the naturally fluorescent compounds within the animal's body, and this can interfere with signal location and quantitation (Hoffman and Yang 2006; Zacharakis et al. 2006). A number of in vivo fluorescence imaging devices are commercially available. Unlike radioactive techniques, fluorescence imaging devices require minimal environmental conditions and have been engineered to be easy to operate. The limits to the technique can be stretched to allow placement of a minimally invasive fiber optic within an animal

to observe a fluorescent signal in vivo at cellular resolution (Al-Gubory and Houdebine 2006; Pelled et al. 2006; Snedeker et al. 2006).

Bioluminescence imaging (BLI) produces a light signal via a biochemical reaction (Sato et al. 2004; Zhao et al. 2005; Shinde et al. 2006). This method is intrinsically preferred in a reporter gene study for tumor growth and metastasis, cell trafficking, or intracellular function. As no external light source is required to produce a signal, quantitation can be more strictly used in this technique. All visible light methods suffer from photon diffusion and local variable tissue absorbance. BLI has the advantage that known anatomical absorbance and light path information can be used to overcome these limitations to some degree. The device is similar to the in vivo fluorescence imager (black box with camera) and is routinely easy to operate. The reader is encouraged to read the chapter on BLI included in this volume.

3.6 Data Management

A well-managed imaging facility may have available scanner time booked in excess of 70 % over the operating day. It is great to have plenty of business, but this starts to reduce available time. Time is required to perform system maintenance, quality assurance checks, cleanup, and data analysis for scans in the center. Most imaging studies have a number of data points to be taken from each scan, and most of those have to be interpreted by a trained observer. A clinical radiologist spends all day looking at hundreds of images and dictates out the results that are rarely more than a simple linear measurement or a perceived hyper- or hypo-intensity at a certain location in an image. The resolution of most preclinical imaging methods means that it generates 10- to 100-fold more data than a standard clinical scan, with increased demand for more intensive processing. Most MRI scans create hundreds of megabytes of image data to gigabytes. Almost all micro-CT scans create tens of gigabytes of data. This puts an increased demand for moving data off the limited storage space of scanners and into the hands of the researchers and technician who are interpreting this information often using remote data analysis stations. Unless the local imaging facility is a service, where animals are submitted and reports are delivered, much of the data analysis and interpretation will be in the hands of the researchers whose animals are the experimental model. It is important to make the movement of image data as seamless and simple as possible while maintaining good backup discipline. It is also imperative that other supporting data, i.e., animal anesthesia and drug use and timing and handling, also be collected and reported with the image data.

The acquisition of the imaging data is actually a fraction of the time necessary to process, analyze, and interpret the data. Therefore, it is important to have the capacity to absorb the inflow from the imaging devices and hold the data for processing until the project is completed when the data can be safely archived for future retrieval. Otherwise, it is quite possible for the nonclinical investigator or the analyzing staff to find themselves virtually overwhelmed in data, and this can lead to

processing and interpretive errors and loss of study integrity which is needed for regulatory filings.

Data processing and management need to be included at initial planning stages for an imaging center. In vivo imaging can produce enormous volumes of digital data in short time. A whole body mouse CT scan of 35 μm resolution produces 5 Gb of radiographic projection data and 2 Gb of reconstructed image data in the course of a 20 min experiment. A micro-MRI of a whole brain at 35 μm creates a 1.05 Gb data file with a 0.5 Gb reconstructed image. These examples illustrate the need for the ability to control the flow of information from scanners. The imaging data is collected from each scanner in a usually proprietary format that is designed for the most efficient use by the scanner software and requires a subsequent reconstruction operation to transform the collected data into a usable image. The reconstruction requirements vary based on the type of imaging modality, the complexity of the acquisition, and the scalability of the algorithm processing the data. Some processes are handled well with personal computers, and others require an array of parallel processors or recently designed graphic processor units (GPUs). Data transfer over fast network connections eliminates the need to produce multiple disk copies for transport and provides for faster data reconstruction and analysis. Robust networks also ease the task of keeping robust and up-to-date backups in case of accidental data deletion or scanner failure.

Data analysis in the ideal imaging facility will use data management technicians and aid investigators with data processing and analysis. Generally the data analysis can require more time to analyze and evaluate than collection of said data. A busy facility may find it difficult for imaging specialists to find the time away from data collection to provide in-depth instruction and help with analysis. An image analysis specialist or team is a valuable asset in these cases where taking time away from data collection is a detriment. Additional prudent assets to the imaging facility would include a veterinary radiologist to assist with interpreting image data in a veterinary clinical context, and robust statistical analysis would be aided with the addition of a biostatistician. In our experience, most facility users have no or limited experience interpreting anatomical images, analyzing three-dimensional data sets, or using the analysis software which in many cases have a steep learning curve. Generally, facility users fall into one of three classes: (1) just give me the results, (2) help me with the hard analysis, or (3) teach me how to do it myself. The first class of investigators has no interest in the analysis and prefers just to have the processed results given to them. In this case, the imaging facility is operating as a service and as such should be sufficiently and expertly staffed to produce a reasonable flow of reliable information. In the second class, the imaging specialists or data analysts teach the investigators the portion of the analysis that is quick to pickup and performs the difficult part out of expediency. The third class is the most confident in their abilities and wants to fully control the flow of information into their experiments. If the imaging facility offers analysis as part of the imaging service, then such recognition as coauthorship on any publications may not be necessary. It is strongly advised that these arrangements be settled before any

scanning study is commenced as a good management practice. For investigators, that perform the analysis and processing independently, it is necessary to provide computer workstations for the express purpose of independent analysis as these computers usually have extreme processing, memory, and storage demands placed upon them by the analysis software. Another incentive is the cost of many software packages may exceed the budget of investigators. Avoiding duplication of software helps to reduce pressure on research laboratory as well as biotech/corporate laboratory budgets. Also the analysis team can use these workstations for training and collaboration with investigators and aid in the production of publication quality images and presentations.

Data Storage. As pointed out earlier, the amount of data that can be generated in a busy facility can be very large. The storage and management of the image data for investigators should be decided early in the planning process. Data discipline should be adhered to throughout the facility to avoid a backlog on scanners with insufficient space for new experiments. Investigators need to be provided with a copy of their image data in the format that is most useful (CD, DVD, portable device). A large facility should forgo this approach and have a central storage facility. Clinical radiology departments utilize centralized PACS (Picture Archiving and Communications Systems) for storage and local distribution. Most preclinical software has very limited export capability for these types of systems, and usually there is a loss of image information in terms of image modality details. For this reason, the ideal imaging facility would have an independent and imaging modality neutral data center to store and distribute imaging data. This can be as simple as a centralized server with adequate disk space or as complex as an array of storage systems for each imaging modality.

3.7 Summary

Planning, designing, and implementing a preclinical animal in vivo imaging facility are no small undertaking. The team of designers and key investigators need to understand all aspects of the functional facility and use the knowledge to guide the architects and builders. It is necessary that experienced personnel be included in the planning stages to avoid costly mistakes in later phases. A smooth workflow will be easy to maintain in a well-designed imaging center. A shared facility is the most common implementation of a multimodal imaging center as the large capital costs of equipment, personnel, and overhead make this the most efficient use of resources. Added benefits of a shared facility will be fostering cross-disciplinary collaborative projects, conservation of resources, reduced duplication of effort and equipment, and production of competitively outstanding data. An ideal imaging facility needs to provide state-of-the-art in vivo imaging equipment and have animal housing resources in the nearby area and preparation areas with surgical capabilities to reduce transportation trauma to and from imaging sessions. The imaging center

design needs to include the following elements: trained personnel, staff workspaces and offices, animal short-term housing and imaging support areas, adequate building requirements to accommodate specific imaging types such as MRI, and allowances to incorporate good safety practices for biological and radiation agents.

The constituent imaging devices in the center need to reflect the goals of the user community and satisfy the needs for the ongoing research studies. In the case of a limited budget, facility planners should choose the most essential modalities for the facility operation based on highest anticipated need. For the lucky few with a large or unlimited budget, the facility should reflect the most versatility to use imaging technology to attack a research problem with a wide area of imaging devices. Up-to-date anesthesia and physiological monitoring equipment are critical to maximize animal safety and validity of research results. Imaging technical development requires specialized staff to produce high-quality data beyond canned manufacturer protocols. Additional support staff, including housekeeping services (training in safety and hazards is required) and veterinary care, is necessary for successful facility to maintain focus. Data management must be accounted for in planning to have a successful flow of information to the completion of imaging projects. Through the use of deliberative planning with facility designers and experienced imaging experts, the current and future requirements of facility users, with good continuing management, will produce a highly utilized and ultimately successful preclinical in vivo imaging facility.

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