Considerations When Setting Up a Preclinical *In Vivo* Imaging Laboratory

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Abbreviations

ALARA	As Low as Reasonably Achievable
BLI	Bioluminescence Imaging
CLI	Cherenkov Luminescence Imaging
CT	Computed Tomography
DRI	Direct Radioisotopic Imaging
ECG	Electrocardiogram
EMG	ElectroMagnetic Interference
HEPA	High-Efficiency Particle Air
MATS	Multimodal Animal Transport System
MMAB	Multimodal Animal Bed
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
RAID	Redundant Array of Inexpensive Disks
RSO	Radiation Safety Officer
SNR	Signal-to-Noise Ratio
SPECT	Single-Photon Emission Computed Tomography
SPF	Specific Pathogen Free
SUV	Standard Uptake Value
T	Tesla
USR	Ultra Shield Refrigerated
V	Voltage

Considerations for outfitting a preclinical imaging laboratory will depend partly on the specific imaging systems in the laboratory and anticipated workflow of studies that will be made. Additionally, some aspects of laboratory set-up are necessary to meet safety and regulatory requirements while others are intended to facilitate day-to-day laboratory productivity. This document provides an introduction to all such considerations, highlighting: general site requirements, system and software considerations, imaging accessory requirements, animal care solutions and training/personnel considerations for setting up single modality and multimodality preclinical imaging laboratories. Site requirements and recommendations for specific imaging systems can be further supported by your system specific support personnel. Details on system installations, clearance and weight tolerance requirements will be made available. For systems that require detailed considerations for room engineering, including heat sinks and shielding, etc., it is advisable that facilities and/or contract engineers be consulted at the earliest stages of the facility design. For systems that use or produce ionizing radiation, institutional, local, and national agency guidelines should be considered.

Considerations Common to All *In Vivo* Imaging Facilities

There are some considerations that may be common among all preclinical laboratories, regardless of the specific modalities present (see Table 1). The general need for solutions for animal transport, anesthesia delivery, animal preparation and monitoring, and data management will be common among most facilities. Solutions for these common areas can be tailored to the specific laboratory objectives.

Animal transport	Facility barriers
	SPF solutions
	Multimodal transport
Animal care	Anesthesia (injection/gas)
	Temperature control & monitoring
	General animal monitoring
Data	File management & backup
	Data reconstruction & analysis

Table 1: Common considerations for *in vivo* imaging facilities

Access and transport of animals in to an imaging facility is almost always a consideration for any imaging laboratory. For facilities where imaging equipment will be shared by multiple researchers this can be a particularly important consideration. Complications can arise when animals are to be transported form a dedicated animal housing facility that employs barrier and isolation policies. Many imaging facilities designed for institute-wide use are established within the facility's animal housing area. To some extent this can overcome some of the barrier and transport logistical issues for imaging. Solutions for isolating animals during transportation and imaging, even when animals are housed within an animal facility, may be required. SPF compatible accessories like the In-Vivo Xtreme™ II SPF animal chamber (SPFC), that is equipped with HEPA filters, are ideal for such animal handling requirements. Supporting infrastructure, including clean benches and sterile biosafety cabinets are mandatory to handle and prepare the animal(s) under sterile conditions and to transport them to the imaging system. For transport to and from staging areas, quick gas connectors to SPFCs are a prerequisite.

Optimum configurations for delivery of gas anesthesia for animal preparation and imaging, as well as end-user safety, should also be determined. Scavenging and ventilation solutions, storage and access of anesthetics supplies that conform to regulatory and legal requirements and proper mounting solutions for carrier gas (e.g., oxygen or medical air) cylinders should be included in the anesthesia setup design. Bruker supplies several solutions to facilitate functional and safe anesthesia delivery as well as waste gas exhaust with up

Figure 1

SPF Compatible Imaging Chamber in the In-Vivo Xtreme II. Chamber is equipped with HEPA filters and quick-connect gas tubing.

to 99.9 % scavenging of isoflurane. For example, the In-Vivo Xtreme II system may be equipped with various EquaFlow manifolds (for 1 rat, 3 mice or 5 mice) which are connected to a dedicated, activated carbon scavenging system. EquaFlow manifolds are designed to distribute gas anesthesia equally to each nose cone, securing not only equivalent anesthetic doses but also securing similar oxygen (or air) flow rates. Such uniformity in animal treatment is critical when imaging *in vivo* bio-distribution and/or kinetic reactions given that enzymatic reactions can be disrupted in hypoxic environments and variable anesthetic conditions. Additionally, Bruker offers anesthesia systems that use either oxygen or medical air as carrier gases. Some institutions prefer the use of medical air over oxygen as it is safer (oxygen is considered to be an explosive in some regions if it is used at above 21% v/v).

Increasingly, institutional imaging systems are installed in a centralized location with multiple platforms available in a close proximity. The Bruker Multimodal Animal Beds (MMABs) (see Figure 2) maximize imaging potential and facilitate cross-platform multimodal imaging. The MMABs can be transported between the Bruker MMAB compatible imaging systems like the In-Vivo Xtreme I & II, SkyScan microCT® 1176 and 1278, BioSpec MRI systems, ICON™ MRI system, Albira II and Si PET/SPECT/CT, and the PET/MR 3T.

Figure 2

Bruker MMAB and adapters. (A) In-Vivo Xtreme I & II adapter. (B) SkyScan microCT adapter. (C) Albira II & Si PET/SPECT/CT adapter. (D) MMAB chamber. (E) ICON MRI adapter.

Hardware solutions for animal monitoring, including respiratory, cardiac, body temperature monitoring, during sample preparation and imaging may also be relevant. (All of these considerations are supported by the Bruker MMABs). Additionally, laboratory space to perform animal procedures including injections and necropsies should be allotted. Compartments for waste (e.g. syringes and decontaminated materials) and other laboratory consumables should be included. Autoclaves, sterile hoods, interim animal cages including sterile ventilation and exhaust, cold storage of tracers (4° and -20 °C) as well as sample preparation materials and space (e.g. for tissue preparation and immunohistochemical tissue sampling) should be considered as well.

Support for data reconstruction solutions and data management/backup solutions should also be considered. Implementations of data storage solutions should provide reliable data retrieval, RAID multi-storage systems, dedicated server configurations, and/or cloud solutions. Finally, imaging analysis may be extensive for some applications and may occupy several hours of processing. Depending on the anticipated level of use for equipment, it may be advisable to configure separate workstations that are dedicated for analysis only. Workstations should be configured with sufficient processers to support image analyses which can be computationally demanding.

Optical Imaging Laboratories

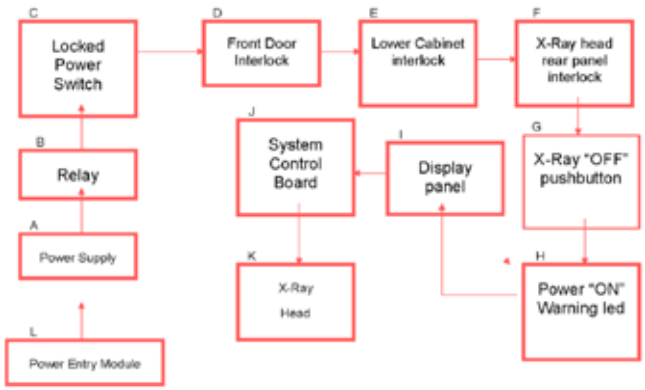
Optical imaging is generally considered to be the most accessible preclinical imaging modality due to relatively simple acquisition and analysis processing, and also because of the relatively low associated overhead cost, minimal system footprint and integration requirements. Preclinical optical imaging systems like the Bruker In-Vivo Xtreme (see Figure 3), are frequently installed without any need for significant modification to an existing laboratory environment. Specific system requirements for an optical system include power requirements (typically standard 120 or 240 V), system clearance requirements, and environmental working conditions, as well as peripheral requirements that support optical imaging studies. Optical imaging reagents commonly require refrigeration and/or light protection prior to use. Many optical imaging laboratories include a small refrigerator/freezer so that *in vivo* fluorescent and luminescent substrates can be stored in close proximity.

Figure 3

In-Vivo Xtreme optical multimodal imaging system. System conforms to cabinet X-ray standards with interlocks and integrated shielding.

For multimodal optical imaging systems that offer X-ray and/or radioisotopic imaging, radiation safety regulations may be relevant. Laboratories that intend to use SPECT and/or PET radionuclides will be required to comply with relevant institutional radiation regulations. The multimodal In-Vivo Xtreme systems provides flexible BLI, FLI, CLI, DRI, and X-ray imaging. In compliance with TUV, NF C 74-100 and FDA regulation CFR 1020.40 for X-ray cabinet systems, the In-vivo Xtreme X-Ray imaging produces < 0.5 mR/hr at 5 cm from the cabinet and can be considered as fully shielded equipment. As such, the system can be operated in typical laboratory environments without the need for any additional external shielding. The system is equipped with fail-safe interlocks and other safety mechanisms (see Figure 4). Even so, some institutes require radiation safety training for system operators.

Figure 4



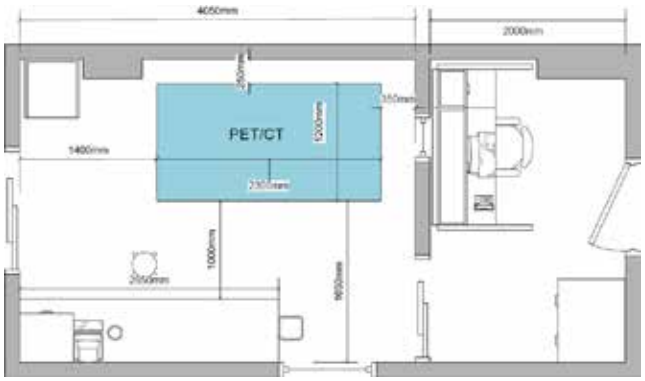
X-ray safety mechanism flow chart of a Bruker In-Vivo Xtreme optical multimodal imaging system.

Preclinical optical imaging is the most cost accessible modality. It provides valuable, high throughput and high sensitivity imaging capabilities for demanding cell tracking and disease model systems. In many laboratories, personnel that are not imaging specialists can be quickly trained to acquire and analyze optical imaging data. However, even though the learning curve for optical imaging is relatively short, solutions for training new personnel, on a regular basis, on operating optical imaging systems should be considered. Because optical imaging systems tend to be employed for a larger number of animals and by a large number of researchers, access to image analysis tools is critical. The In-Vivo Xtreme system is provided with a site license for the Bruker Molecular Imaging Software. This facilitates access to the software analysis features for optical imaging analysis by a large number of users. Finally, in image core facilities providing institution-wide use, it is common for new optical imaging applications to emerge over time as investigators explore new ways to address their study objectives through non-invasive optical imaging.

MicroPET/SPECT Laboratories

A moderate degree of planning and site preparation will be required to support preclinical PET imaging. Some considerations for µPET laboratories relate to the optimal layout for workflow. Many considerations for µPET are applicable for µSPECT laboratories. There are currently a few commercial benchtop µPET systems available that can fit in an existing space, though benchtop systems typically have significant limitations including low resolution imaging (results in high partial volume effect), high dead-time errors (in some cases severely limits the activity that can be used), and small axial FOVs (limits whole body dynamic imaging). More space will typically be required for µPET systems with good performance specifications (sub-millimeter resolution, > 9 % sensitivity, large FOV, and integrated anatomical modalities, and/or integrated SPECT). The Bruker Albira Si PET system offers leading performance specifications and is available with integrated CT and/or SPECT components all in a compact footprint. Integrated CT can provide anatomical localization and attenuation correction for PET. The Bruker PET/MR 3T employs the same high performance PET detector as the Albira Si PET. The Bruker PET design uses silicon PMTs that are not susceptible to MR interference obviating the need for extensive RF-shielding. The optimum layout for a preclinical laboratory varies depending on study and regulatory variables, but an example floor plan with space allotted for storage and a dedicated control room is shown in Figure 5.

Figure 5



Example floor plan for the Albira Si PET/SPECT/CT system.

Equipment layout should be designed to optimize the workflow of people, animals, and radioactive compounds. Consideration must be given to store, handle and manage isotopes including radioactive waste and animals. There are several commercially available turnkey solutions for end-user shielding for isotope storage, for shielded dose and animal preparation, and for shielded storage of active animals. Many aspects of radionuclide safety will be addressed through radiation safety programs managed by an institutional RSO. Typical components of radiation safety program include training to minimize exposure (e.g. ALARA), radiation surveys using wipes or counters where appropriate, and personal dosimetry hardware may also be employed to ensure that personal exposure limits are not exceeded.

Laboratory Configuration/ Preparation	Optimum workflow Power requirements Temperature and humidity requirements
Radiation Safety	Where relevant, shielding for isotopes Physical shielding solutions Training Monitoring Personal dosimeters (finger, lab coat)
Tracers	Access and proximity to PET tracers Radiochemist (if custom labeling applies)
Peripheral accessories	Dose calibrator Gating and monitoring hardware Injector and blood sampling hardware
Expertise	Requires moderate to high degree of imaging and analysis expertise

Table 2: Considerations for µPET facilities

Other considerations for µPET laboratories include proximity and access to radioisotopes for applications relevant to the laboratory. 18FDG is broadly relevant to many studies in oncology, cardiology, neurology and metabolics, and is probably the most common PET isotope used in clinical use and preclinical studies. 18F has a 110 minute half-life, and laboratories intending to use 18F compounds will need to be in a reasonable proximity to sources (i.e. cyclotron) that produce 18F. Other radionuclides (e.g. 11C and 89Zr) more suitable for compound labeling or studies of compounds with long half-lives are also used in preclinical PET imaging (Velikyan 2014, Van Dongen et al., 2015, Petrik et al., 2015). For custom radiochemistry and tracer development a skilled radiochemist will be necessary.

An adjacent location for animal housing and preparation is also of importance. This room should have a dedicated "hot" rack to store radioactive animals before and after PET acquisitions as well as a "cold" rack to allow investigators to store their animals for the duration of the study. A biosafety cabinet should also be available for the investigators to perform animal care, small surgery or other preparation steps before imaging.

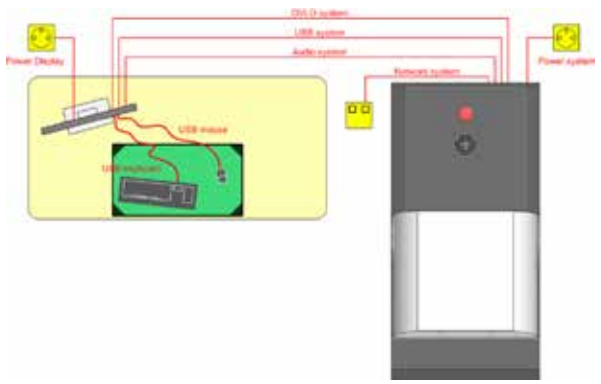
Peripheral devices and solutions are necessary for some µPET applications. For example, the SUV calculation is a standard analysis calculation in many µPET imaging studies. SUV calculations and other quantitative µPET calculations require a known and accurate starting dose activity measured with a dose calibrator at the study outset. Additionally, cardiac and respiratory studies can require ECG and respiratory monitoring and gating. For studies in drug and tracer kinetics, accessories and/or methods for precision tracer injection and blood sampling may also be required.

MicroPET imaging requires a moderate level of expertise for basic imaging applications. Applications related to tracer development, kinetic imaging and kinetic modeling, and detailed functional cardiac applications require a high degree of expertise. Specialized analysis software packages for µPET image analysis for brain studies, kinetic modeling, and specific disease models are available, but can require specialized training. Frequently, µPET laboratories have designated system operators to facilitate imaging and provide expertise for study design and analysis.

MicroCT Laboratories

Modern preclinical µCT systems can frequently be incorporated in a preclinical imaging suite with relative ease. The footprint of modern µCT systems (e.g. SkyScan 1176 or 1278) is small, with minimal requirement for laboratory modifications. An example layout for a commercial µCT system is shown in Figure 6. Modern scanners are self-shielded cabinet systems, and have multiple layers of safety interlocks, and emergency stops to prevent accidental radiation exposure to the personnel. These safety features can allow for active system operation with operators and laboratory personnel in the direct area and obviate the necessity for dedicated remote operator rooms. Still, there are typically some basic environmental requirements for µCT systems. MicroCT systems use high precision movements during acquisitions and have sensitive electronics. Therefore systems should operate in recommend humidity and temperature ranges and be installed on a stable surface.

Figure 6



Example floor plan for the SkyScan 1176 preclinical µCT system. MicroCT system shown at right and workstation shown at left.

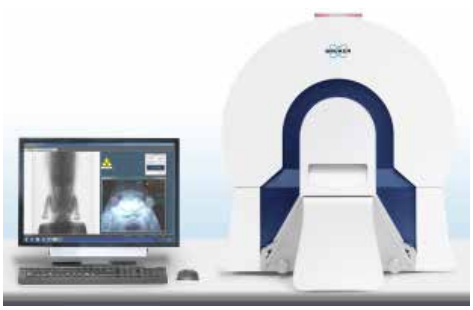
Expertise requirements for µCT imaging/analysis can vary depending on the application. Frequently, *in vivo* µCT systems are employed for imaging and analyzing bone disease, pulmonary disease, obesity models, and models of cardiac/disease function. Much of the analysis is application specific and varies in complexity from basic image segmentation (i.e. Grayscale or Hounsfield image selection) for applications including adipose quantification to cardiac measurements, bone feature analysis (e.g. bone density and measurements of bone morphometry), to complex cardiac function analysis. Frequently, individual µCT laboratories are experts in analysis of their dedicated field of research.

Bone, fat, and lungs have innate contrast, but many soft tissue applications can be enhanced using appropriate (e.g. gold nanoparticles, alkaline earth metals, iodine based compounds) contrast agents. Additional expertise is required using contrast imaging. Many useful agents are now commercially available.

Some cardiac and pulmonary applications may require animal monitoring (i.e. ECG and respiratory monitoring) and gated imaging. Bruker *in vivo* µCT systems are equipped with a robust physiological monitoring system that measures the breathing rate, heart rate, and ambient temperature. A heated fan is also available to keep the ambient temperature inside the imaging bore stable. In addition, the systems have the capability of communicating with external devices that can be used to ventilate or monitor the physiological condition of the animal.

Bruker's SkyScan 1176 and 1278 (Figure 7) *in vivo* µCT systems offer high resolution and high speed imaging, respectively. For certain bone applications, high resolution imaging is required. These systems conform to FDA regulation CFR 1020.40 for X-ray cabinet systems and provide modern shielding and safety designs with compact footprints. Reconstruction and analysis of high resolution µCT datasets can take considerable time and computer resources. Bruker offers a comprehensive software package to address this high demand data processing requirement of µCT imaging with industry leading software for fast reconstruction and analysis with a user friendly GUI interface that has many advanced features such as automated segmentation, batch processing of multiple datasets, calculating morphometric as well as bone mineral density analysis. The software is provided as a site license to enable easy and convenient scheduling of analysis for the users. All computers supplied with the system use a Windows based operating system.

Figure 7



SkyScan 1278 µCT system and workstation.

All systems include on-site training and support from for application specific related questions. Bruker also conducts annual microCT user meetings and workshops at various locations around the world to cover the wide installation user base and serve as a venue to introduce new hardware/ software features and applications.

MRI Laboratories

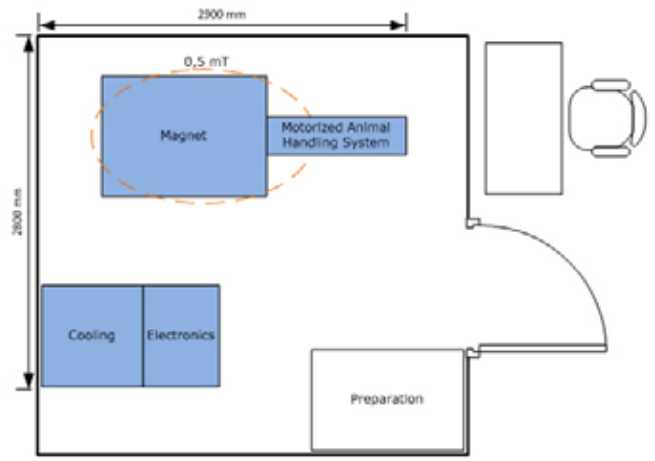
The planning and infrastructure required for preclinical MRI systems can range from moderate to extensive depending on the location of the system, magnetic field strength, and the system's configuration. Low field systems (i.e. 3T and lower) have been designed to be convenient solutions for users with low to moderate technical expertise. Higher field systems (4.7T or higher) generally require moderate to high levels of expertise due to the infrastructure requirements and the complexity of these instruments.

There are some considerations for selecting the appropriate MRI field strength for a facility. Generally, the system magnet field strength and system bore size determine the maximum sample size for imaging and the relationship between field strength and bore size is generally inversely proportional. Rat or guinea pig imaging requires a larger bore size and is as a result typically limited to imaging in MR systems with fields less than about 11.7 T while primates require even larger bore sizes limiting the field strength to 4.7 or 7 T.

With an installation footprint of 1.5m X 1.5m and weight of 1200 kg, the 1T ICON system has the fewest infrastructure and maintenance requirements. The ICON requires a 208 V 50/60 Hz 2-phase AC power plug with 15 amp fuse to operate. The typical lab layout for the 1T ICON is similar to the new BioSpec 3T (see Figure 8), though minus additional floor space for a cooling unit and the electronics. With the permanent magnet design employed with the 1T ICON the magnet will remain charged even after a power outage, minimizing the system downtime in the event of a power outage. The self-shielded scanner design eliminates ElectroMagnetic Interference (EMI) from nearby lab equipment and a Faraday cage is unnecessary. Minimal expertise is required to operate the ICON system.

The newest cryogen-free 3T Biospec magnet system is designed to have slightly low to moderate maintenance and infrastructure requirements. The cryogen-free magnet design obviates the need for liquid helium or nitrogen fills and no quench exhaust is needed for venting the cryogens. The latest in MRI magnet technology ensures the magnet remains on field during power outage and/or cold water failure for up to 4 hours in the event of a power failure. The system is self-shielded so a Faraday cage is unnecessary. The BioSpec 3T comes with a motorized animal handling and positioning system, including touchscreen operation for a simplified, precise workflow. The new Bruker PET/MR 3T system offers integrated inline PET using the same advanced silicon PET detectors employed in the Albira Si PET system (see above).

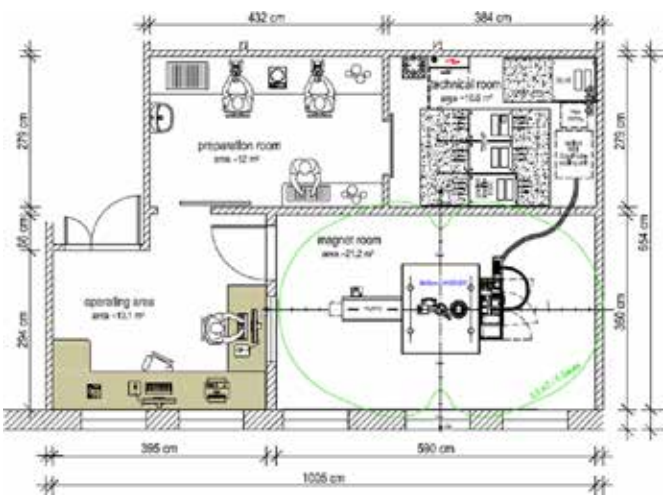
Figure 8



Example floor plan for a BioSpec 3T USR MR.

Higher field BioSpec magnet systems (4.7T or higher) can require moderate to extensive site planning and preparation. An example floor plan for a high field BioSpec 94/20 USR (9.4 T, 20 cm bore size) system is shown in Figure 9. These systems require a quench line to vent cryogenic gases and in some cases a Faraday cage is recommended to minimize EMI. Due to the higher field strength, these systems capture images with higher SNR which can be used to create high resolution images. High field systems with a MRI Cryoprobe™ can achieve in-plane resolutions of 20µm. The selection of gradient coils and amplifiers will also limit the achievable resolution of the scanner and Bruker offers gradient coils and amplifiers that permit gradient strengths up to 1,000 mT/m. Spectroscopic applications also benefit from the higher field because the individual line shapes have a greater degree of separation; making it easier to resolve and quantify features in spectroscopic data.

Figure 9



Example floor plan for a BioSpec 94/20 USR MR.

Bruker also offers a MRI CryoprobeTM which can further enhance SNR levels by a factor of 2.5-4 depending on the field strength of the scanner. This SNR gain can be used to capture ultra-high resolution MR images (up to 20µm on high field systems) or this additional gain in SNR can be used to reduce scan times by the square of the SNR gain. The lower scan times then can be used to increase scanner throughput. Additional site planning and infrastructure are required.

The level of imaging expertise for personnel in preclinical MR laboratories frequently varies from MR physicists to general biologists. Additional expertise for development and use of contrast agents and use of hyperpolarization methods would be necessary if required. Animal monitoring and cardiac and respiratory gating is necessary for some MR applications. Unique to the MRI product line is the ability to use intragate which is a self gated, steady-state cardiac imaging method that does not require external sensors, hardware and triggering devices. All Bruker MRI systems are configured with the ParaVision and TopSpin software that support all levels of MR operation including presets for standard anatomical applications and custom coding options for advanced sequence programming. In addition to onsite training, Bruker offers application courses for advanced and re-training purposes.

Conclusion

There are several considerations for outfitting and establishing preclinical imaging facilities. Some considerations are common regardless of modality. All modalities require special attention to animal care and animal handling. This is partially related to safe and precise anesthesia delivery and animal transport.

Individual modalities have specific requirements for successful imaging, and functional modalities have specific considerations for reporter/tracers access, chemistry and radiation storage/waste solutions. Additionally, some modalities require peripheral equipment (e.g. dose calibrator) for successful imaging.

Modality specific performance characteristics should be considered. Typical performance characteristics vary depending on the modality, but can include sensitivity, resolution, imaging speed, and multimodal potential. Solutions for analysis and application expertise can be overlooked, but are important for successful execution of complex studies.

Bruker offers a full range of preclinical *in vivo* imaging systems including multimodal optical systems (In-Vivo Xtreme II), PET (Albira Si PET and PET/MR 3T) and SPECT systems, MRI systems (BioSpec and ICON), and microCT systems (SkyScan 1176 and 1278). These systems supply leading performance characteristics and are supported by dedicated applications personnel.

Acknowledgements

We would like to thank Dr. David Viertl (Centre Hospitalier Universitaire Vaudois) for reviewing the manuscript.

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