Chapter 18
Animal Handling and Preparation for Imaging

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1 Introduction

Molecular Imaging refers to observations and measurements of molecular processes in living cells or animals [1]. Anything that alters physiological processes can change molecular pharmokinetics (PK) and pharmodynamics (PD), thus these factors are important to understand, control or eliminate wherever possible. Even with in-vitro or ex-vivo measurements, the environment during formation of the molecular target and conditions during exposure to the imaging probe may alter the experimental results. The capacity for biological systems to modify themselves, sometimes very quickly, means that the biological status is a potentially changeable condition.

In-vivo physiology can be influenced by a wide range of factors, including anesthetic agent, animal and room temperature, injection method and time of day [2]. These in turn can influence the specific accumulation and clearance of imaging agents and any endogenous background signals. In many cases it is possible to control these parameters, reducing or eliminating their effect on imaging experiments.

In addition to physiology, there are physical parameters that can be optimized to ensure the best possible data from the imaging systems. In most imaging devices there is a particular region where sensitivity or resolution may be highest, such as the center of a SPECT, PET or CT scanner. There are also times when sequential serial or longitudinal imaging may be useful, thus a fixed position for animals or cells can be quite useful. A positioning system ensures both optimal acquisition of image data, and also often aides in the subsequent data analysis.

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This chapter will discuss the various factors that can alter in vivo physiology and imaging probe signal, background and contrast between signal and background. Potential solutions for reducing physiological variability and the resulting impact on imaging experiments are described, along with the use of contrast agents for CT imaging.

2 Anesthesia

Molecular imaging systems are much like photographic cameras, in that they require a certain amount of time to collect data to form an image. It may take seconds or hours, and the systems may be tolerant or intolerant of motion during the imaging process. For example, current PET imaging systems have resolutions of 1–2 mm, so movements less than this distance might not seriously change the results. Some optical imaging systems can tolerate some amount of movement since light scatter substantially blurs the detected signal. Other systems such as MRI and CT, or high resolution SPECT, may not produce useful data at all if there is much movement of the animal. The tolerance for movement is set by the resolution, with high resolution imaging requiring little if any motion during data collection. In order to acquire high quality images, anesthesia is normally used to immobilize the animals. While anesthesia eliminates motion from the animals moving around, it does not stop cardiac or respiratory motion, which will be addressed in a later section.

Two types of anesthesia are used: injected and gas. Injected anesthetics such as pentobarbital, ketamine, midazolam and xylazine are controlled substances that require careful tracking, security and prescriptions, primarily because most are also drugs of abuse that alter the dopaminergic system. It is worth noting that if one wishes to study the dopaminergic system, it is important to understand the effects of anesthesia on the neuronal circuitry being investigated [3]. Injected anesthetics have a decided advantage, in that the only requirements are a syringe, needle and bottle of anesthetic. No other equipment is required to use injected anesthetics. This is particularly important for working with larger animals such as non-human primates, where in most cases gas cannot be safely used for the induction of anesthesia.

There are multiple disadvantages to injected anesthetics. Pentobarbital is very long lasting, which is both good and bad, since full recovery could take a long time, though this might be useful for long imaging sessions. Ketamine and xylazine are relatively short lasting, with some agents such as propofol very short lived, so these agents often require multiple injections or constant infusion. This can lead to a variable depth of anesthetic state, which also means variable physiology during imaging [4]. It may be difficult to inject additional anesthesia during imaging, and the right amount of additional dose may not be easy to determine. While it is possible to infuse injected anesthetics, it may be troublesome to titer the dose properly over time. Injected anesthetics also can easily lead to overdose, particularly with inexperienced personnel who are not at ease and adept with handling animals. The lethal dose is in most cases not much more than the amount required for deep
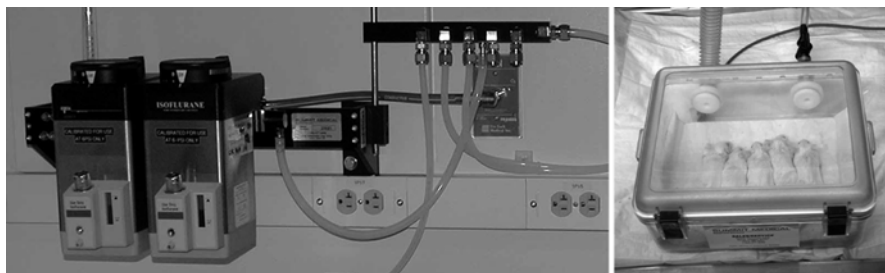
Fig. 18.1 *Left image*: wall mounted gas anesthesia system with manifold supplying isoflurane to multiple sites of use. *Right*: heated induction box with simple on/off gas control

anesthesia. There is also no way to recover from an accidental overdose if too much is injected. Despite these difficulties, injected anesthetics are commonly used and both safe and effective when used correctly.

Inhalant or gas anesthesia is the preferred method of keeping animals from moving for most molecular imaging research. Most common in use today is isoflurane, although there is increasing interest in using sevoflurane as it appears to have less effect on glucose levels in blood, which may be important for FDG imaging using PET [5]. Inhalant anesthetics have the advantage of creating a constant level of anesthesia that lasts as long as required, and it is less likely to kill animals by overdose. If the anesthesia setting is too high and the animal begins to have labored breathing, turning down the amount of anesthesia can quickly bring the animal back to a more appropriate respiratory rate. Both heart and respiratory rates are different between species and even between animal strains, so knowledge of what is normal is required to determine the optimal rates. Animals also recover very quickly from gas anesthesia, thus having less physiological impact, which may be important if animals are being imaged frequently. In addition to being safe, quick and effective, gas anesthetics are also readily available and are currently not controlled substances in the United States. Inhalant anesthetics can be used with induction boxes (Fig. 18.1), nose cones and through intubation either with our without ventilation. There is no need for analgesia and often the breathing rate remains much higher than with injected anesthetics.

Given that anesthetics alter animal physiology [5], there is considerable interest to image without anesthesia. In some cases this may mean restraining the conscious animals [6], or perhaps letting them roam free and tracking the movement [7]. One group has gone so far as building a miniature camera that is mounted on the animal [8]. A few groups have gone to great lengths to train animals to hold still during imaging, which provides interesting and valuable information, but at great cost in resources to train animals [9]. Other systems allow mice to roam freely and provide a video signal of optical signals [10]. Although the majority of molecular imaging in animals will continue to use anesthesia, conscious studies will always have an important role and may well expand as new ideas and imaging systems are developed.

The use of anesthesia is common in preclinical settings, yet rare in clinical settings. The impact on the physiology and probe signals may be profound and this

Fig. 18.2 Standardized
uptake values (SUV) for FDG
in mouse heart 60 min
post-injection in both a wild
type (wt) and insulin
sensitivity knockout (ko)
model. Right two columns
show the high variability and
increased heart uptake with
the use of unconscious uptake
under isoflurane. Stars
indicate significant difference
between mouse models (*left*)
and wt baseline and wt insulin
challenge (*right*)
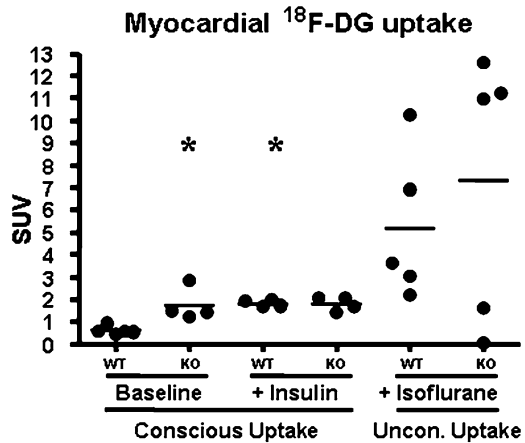
represents a clear distinction between what may be observed in humans versus that
seen in animals. One option is to use conscious injections and uptake time, followed
by induction of anesthesia and immediate imaging. This is often referred to as
conscious uptake, though it is followed by unconscious imaging. The idea is that
the probe is already sequestered and the background activity eliminated, so that the
signal and contrast levels are already set in place before the anesthetic agent is intro-
duced. In some cases this has proven to be very successful, allowing signals to be seen
that were otherwise masked by anesthetic effects, as shown in Fig. 18.2. The increased
uptake of FDG using isoflurane during unconscious uptake masked the significance
of the mouse knock-out model, which was observed by using conscious uptake
(unpublished results). Note that with conscious uptake, not only was the heart uptake
reduced, but the SUV variability was also substantially lower, making the differences
between the mouse models and insulin status significant. One consideration with con-
scious work is that there may be stress related changes in the animal, leading to altered
cortisone, glucose or other metabolic factors that can influence the experiment.

3   Temperature

Development of preclinical imaging systems with high resolution and sensitivity has
enabled a shift from large to small animals for molecular imaging research. For
example, the development of small animal PET systems such as microPET allowed
researchers to move from primates, pigs, dogs and other larger species into working
with rats and mice. There are tremendous advantages to using these rodents, includ-
ing lower cost, they are not endangered, it is easy to work with many animals per day,
they are easier to handle and housing larger numbers is possible in a small space.
Perhaps the most important is that we know the genome and many different genetic
knock out and knock in strains are available to study a wide range of diseases.

Animal supply vendors offer a huge range of mice with various genetic manipulations. One side effect of working with small animals is that temperature becomes a paramount issue to address since these small animals have little heat capacity.

With decreasing animal size, temperature control becomes increasingly important to regulate. Rodents are small, thus have relatively little thermal mass to maintain body temperature, especially when anesthetized. Anesthetized mice can quickly become hypothermic in as little as 5–6 min in a 20 °C room [11]. Larger species, such as primates or dogs, might not require heating during imaging, or might be sufficiently warmed using recirculating water baths or a heating blanket, though these systems provide little actual control over internal body temperature.

Biological systems are somewhat tolerant of low temperatures, with compensatory mechanisms to protect the animal's health. Surgeons sometimes take advantage of slowing physiological processes by chilling animals during surgery [12]. When cold, animals will divert blood flow from peripheral tissues to maintain core body temperature and this can cause the peripheral tumors to have significantly lower uptake, simply due to temperature [2]. Most work using tumors is focused on potential treatments and their effects, which is only confounded by variable uptake due to temperature changes. Frequently tumors are implanted in the shoulder region, which is well away from activity localizing in the bladder, GI and liver, however uptake of FDG in this region can be masked by high brown fat metabolism, as shown in Fig. 18.3. Given that mice thermoregulate by tail blood flow [13], it is especially important to keep animals warm if measurements or injections are going to make use of the tail vessels. In vivo metabolism is altered by hypothermia and can lead to variable or uncertain results depending on the conditions before and during uptake and imaging. There are also strain variations to consider, as different strains have differing responses to temperature, perhaps most obvious being nude mice, which do not have any insulating fur to help with temperature control [14].

Unlike lower temperatures, biological systems are rarely tolerant of high temperatures. Elevation of just 3–4 °C above normal is sufficient to denature proteins and cause heat shock [15]. For this reason, it is imperative that animals be carefully protected from overheating. Proper control requires monitoring of temperature to prevent overheating in any type of heating system. This is particularly important if the heating and sensing systems are separate, as would be the case if a thermocouple is used to sense the heat from a separate heating source. Thermocouples may work fine if the heating system is diffused before contact with the animal or well coupled to the heat source. One method that works well for certain cases is to use a resistive wire heating element, where the heat is applied and the temperature sensed by resistance from the same wire. This type of circuit enables precise control without overshooting the set point for the desired temperature. The precision and accuracy of control with resistive wire heating and quick equilibrium of animals to surrounding temperature also means that invasive thermocouple measurements are not necessary, saving cost, time and the potential to contaminate or damage the animals. Another heating method is the use of warmed air, often used with MRI systems. Air works well in a confined space such as the MRI gantry, however it does not carry much heat and can lead to dehydration over long usage times.
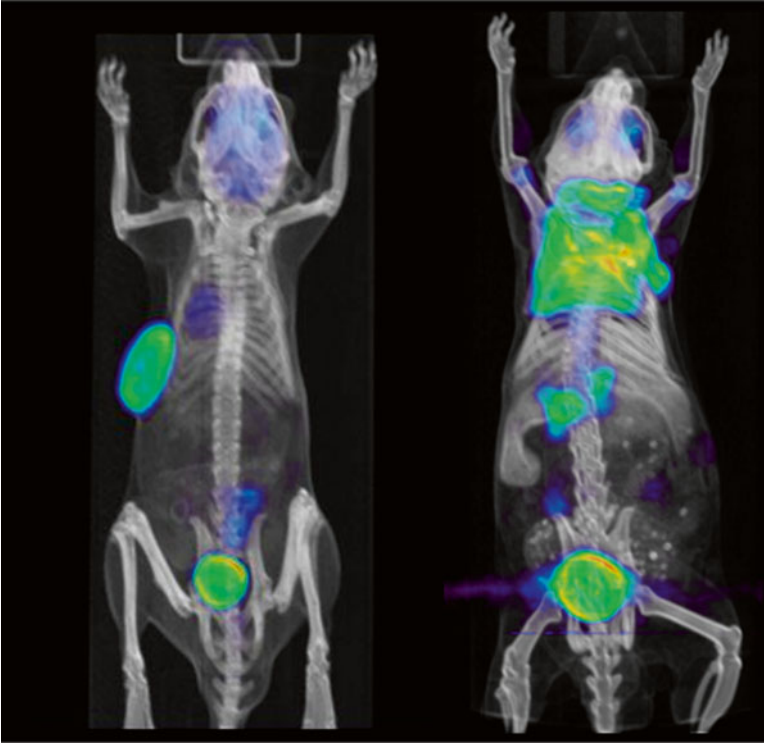
Fig. 18.3 PET/CT images of FDG uptake in a nude mouse. *Left* mouse is warmed prior to and during imaging, showing uptake in tumor and bladder. Mouse on *right* was not warmed and shows strong brown fat uptake in shoulder regions

4   Injection Methods

Animals can be injected using a variety of methods, from needles and catheters inserted into blood vessels, oral gavage, retro-orbital insertion and even by transdermal hypo spray [16]. Imaging agents are most commonly injected into the venous system via tail or leg veins (iv) or using intraperitoneal (ip) injections as shown in Fig. 18.3. Delivery can be as a single bolus pulse, or by infusion or sometimes is given as a bolus-infusion so as to reach a steady state in tissue [17]. The method used depends in the particular experiment and all have their advantages and disadvantages.

Tail vein injections are the most common with rodents, while other species such as rabbits have ear veins well suited for injections. Mouse blood vessels are very small, and many people struggle to inject imaging probes even in the readily accessible and visible tail vein. Venous injections are a quick and effective way to deliver imaging probes and contrast agents directly into the blood stream. Veins are often
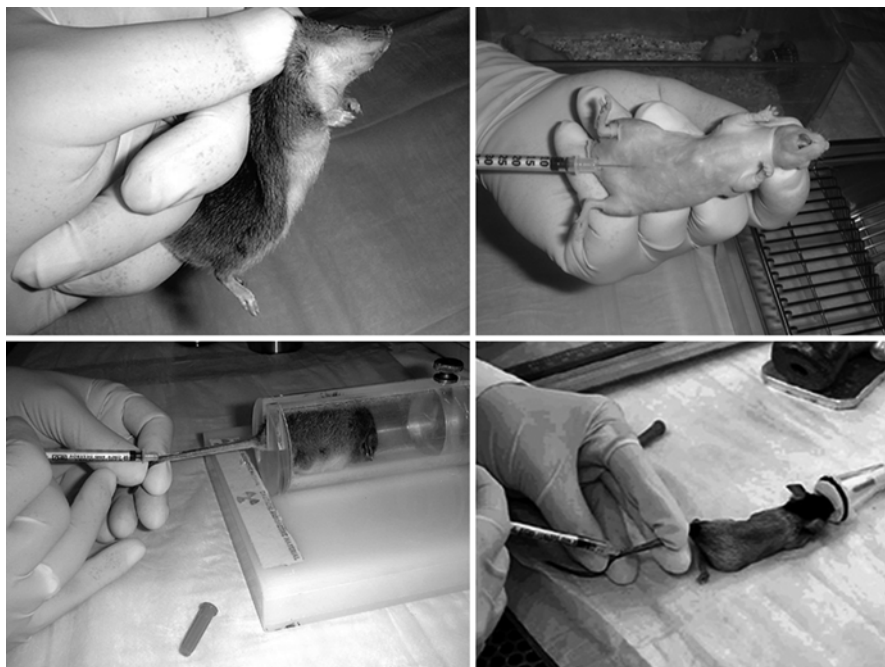
Fig. 18.4 Injection methods commonly used; *Top Left*: Proper grip for holding mice, *Top right*: intraperitoneal injection, *Bottom left*: conscious tail vein, *Bottom right*: unconscious tail vein

near the surface of the skin and have thin walls and lower blood pressure, making them capable of distension to fit a comparatively large needle or catheter inside the vessel. Removal of the needle or catheter is not likely to cause any significant blood loss, unlike arterial punctures. Rats pose a bit more of a challenge for injection in the tail since the tough skin is relatively thick, so a small incision can help for getting good access to the tail vein.

Tail vein injections can be done under either conscious or unconscious conditions, as shown in the lower images of Fig. 18.4. Conscious injections require restraint, either by holding the animals or using a mouse holder. Awake animals have higher blood pressures, so vessels are easier to find, however animals often flinch when needles are inserted and this can cause difficulties with injections. Conscious injections can also cause stress in both the animal and investigator if there are any difficulties, mainly due to lack of experience. Unconscious uptake is challenging due to the lower blood pressure and reduced flow, but the advantage is that there is less stress, a sense of more time available and the tail can be heated to improve blood flow. The choice whether to use anesthesia for injections also depends on whether conscious uptake is needed to minimize the physiological effects of anesthesia (see Fig. 18.2).

With any tail vein injection, there is always a certain amount of extravasation, where some injection solution is left in the tissues surrounding the injection site.

This remaining solution is not bio-available through the bloodstream and usually should not be included when considering how much activity was injected into the animal. The amount can be trivial for a good injection, or quite substantial if the injection was not done properly. For this reason, it is much more accurate to image the entire body and calculate the activity within the animal based on the image data, rather than use the amount of activity drawn into the syringe. Using the image-based activity also avoids needing to calculate and convert injected activity or image count rate data into the same unit of measurement. One must be careful however when using images to calculate injected activity, since if the animal eliminates some of the activity through urine or feces, the image data will not be accurate. Other studies involving longer lived isotopes imaged over several days will require using the syringe-based activity measurement, unless images are acquired immediately following injection.

One limitation with the use of small animal such as mice is the small blood volume. The safe limit for either injection or removal of blood is about 10 % of the total volume, which for mice can mean a restricted volume of ~200 μl [18]. Removing more than this volume can leave mice anemic and alter the physiology in a variety of ways. Injecting too much volume can cause fluid buildup in the lungs and cause temperature shock if the injection solution is not warmed. The volume limitation may mean that the concentration of imaging agent used for humans is too dilute, so it may be necessary to concentrate the activity in order to have sufficient signal in a small volume.

Arterial blood is often preferred for sampling radioactivity over time, since it contains the activity presented to tissues for uptake. Venous blood has passed through the capillary bed and may contain less activity, metabolites or if the probe is first pass extracted, nothing at all. Arterial injections are sometimes useful, since the blood is directly delivered to any downstream tissues without first passing through the heart and lungs. The relatively thick muscle walls of arteries make it more difficult to penetrate without going through the far side of the vessel. The thicker walls also mean there is less distension of the vessel to accommodate a needle or catheter. One potential drawback of inserting an arterial catheter is that usually the artery needs to be cut and the catheter tied into place to prevent it slipping out over time. This means the artery is completely blocked and often tied off, thus there is no blood flow in the vessel except when sampling through the catheter. Cutting the artery is also usually considered a terminal experiment, so only a single use is possible for each animal.

Subcutaneous (subQ) and ip injections are very simple and relatively easy to do. If large numbers of animals are used, this is a quick efficient way to make sure animals are injected and makes it easy to stick to a schedule. Few probes are injected this way, in part because many may remain in place, or take considerable time for the lymphatic system to move the probe into the bloodstream. With ip injections, there is also the risk of puncturing the bowel or bladder, which may cause the probe to stay in the wrong region and can cause health problems for the animals. Nonetheless, if speed of delivery is not a crucial factor, these injection methods are the simplest and most reliable methods to inject animals, provided the imaging agent moves out of the skin or ip cavity and into the bloodstream.
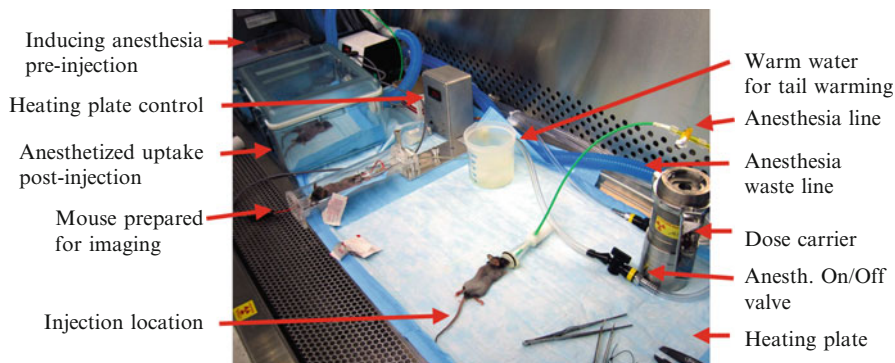
Fig. 18.5 Short imaging times following a period of uptake and clearance result in multiple mice at various stages of the injection and imaging process. This image shows mice in many stages, including anesthesia induction, injection, uptake and prepared for imaging

Retro-orbital injections or blood sampling is a method whereby the blood vessel rich area behind the eye is used to sample or insert imaging agents. While effective, this is hard to do inside an imaging system and often is restricted to only once every 2 weeks. The amount of volume that can be inserted or removed is very limited and blood sampling has the potential to cause infection and blindness. Similar to ip injections, in the hands of a skilled worker this technique can be done quickly and safely in large numbers of animals.

Oral gavage is a method where a syringe with a short tube is used to insert contrast agents, drugs and imaging probes directly to the stomach. Oral contrast delivery is needed for upper GI tract imaging using CT. Gavage subjects the drugs and probes to the digestive process, which may alter the molecules before they enter the bloodstream. In humans, taking drugs orally is the preferred method, primarily to avoid injections; therefore it is often useful to test out how well the compound gets into the bloodstream via the GI tract. There may also be a preference to use gavage for daily or frequent dosing, since it does not adversely damage the vascular system, which might be needed for injection of imaging agents.

Regardless of the injection method used, a well lit warm location is needed for injecting animals. Any support equipment required should be easily at hand, including alcohol wipes, needles and syringes, waste disposal containers, anesthesia control and a comfortable work environment. If immune compromised animals are in use (see section below), then the injection area is best located inside a biosafety cabinet, as shown in Fig. 18.5. This figure shows an experiment in process where animals are first anesthetized, then injected, then held under anesthesia for ~45 min, then placed into an imaging chamber for subsequent PET and CT imaging. At any one time, there may be up to eight animals in the process of being imaged, thus a well laid out and easy to use facility is essential given the challenging logistics of juggling so many tasks.

When mice are placed in an induction box and exposed to gas anesthesia, they tend to run around and get all mixed up, making it hard at times to keep track of

which animals have and have not been injected. Ear tags interfere with CT scans and animal marking tend to fade or may be hard to see, which can make it difficult to identify specific animals. This could easily lead to animals being injected twice or not injected at all. A simple solution is to have two induction boxes; one for animals being induced and a separate box for animals following injection. Since injected animals are already anesthetized, they do not move and can easily be laid out in the order in which they will be imaged. For conscious uptake studies, a separate clean cage for injected animals can be used to easily separate the animals; however careful marking will be needed to ensure that animals are imaged in the proper order. Simple, easy to follow solutions such as two induction boxes, simple on/off switches for heating and anesthesia, along with having all the necessary support equipment immediately on hand are the key to successful experiments.

5   Animal Access

During imaging experiments, usually animals are placed inside a space that is partially or completely enclosed. The enclosure may be present to keep out light (optical), contain radiation (CT) or the imaging detectors may be placed close to the animal to obtain the best possible image data (PET, SPECT, MRI). When imaging times are short, as with most optical methods, there may be little or no need to observe or otherwise have access to the animals. With longer imaging times, it is very important to observe the respiration and sometimes also heart rate to monitor the animal's physiological condition. These observations can be made by eye or by remote sensing systems (see section on gating below). Depth of anesthesia is something best checked every 10–15 min, both to ensure the animal is still alive and well, and to moderate the anesthesia dose. Typically the longer the animal is under anesthesia, the less anesthetic concentration is needed to keep the animal from moving. Depending on the heating system employed, monitoring of animal temperature may also be required.

Dynamic imaging is where animals are injected within the imaging system and imaged continuously for a period of time to observe changes in the probe biodistribution. This allows the fast temporal changes associated with delivery and early metabolism or elimination to be observed. Injecting within the imaging system is a task that may be simple or complex, depending on the ability to access the animal within the gantry. Direct injection using a needle is often extremely challenging in tight confined spaces, so most dynamic imaging is done using a short catheter inserted to a vein and connected to a syringe. The catheter has a certain volume based on the length and diameter of the tubing, termed the dead volume, which is not injected unless followed by a flush with saline. Often flushing is impractical, since it leads to a double-pulsed injection and it can be difficult to change syringes or have a switch that does not add its own considerable dead volume. In some cases a syringe pump can be used to inject the imaging agent; however it may be difficult

to have the pump close to the animal, leading to long catheter lines that may be filled with radioactivity. The dead volume in the syringe and tubing should be minimized to reduce radiation scatter and background, especially if they are left in place during imaging. Often it is best to leave the catheter in place after the injection; otherwise the pressure in the tubing and from the animal's blood pressure can cause blood and the injection fluid to drip or spill on the animal or surfaces within the imaging volume.

During the imaging time, it may be necessary to sample the blood to measure radioactivity, metabolites and blood concentrations of endogenous competitors to the imaging probes, such as glucose for FDG or thymidine for FLT. These endogenous molecules compete with the imaging probe for tissue uptake and may need to be measured to increase the accuracy of the measurements. As with injections, access to the animal for sampling can be challenging, especially in enclosed systems. If the animal is accessible, it may be possible to draw blood using catheters inserted before imaging or by nicking the tail vein and drawing off a small sample. A number of researchers have developed microfluidic sampling devices, which use a catheter to take small and sometimes frequent of continuous blood samples during the imaging process [19, 20].

When experiments last more than 60–90 min, it is advisable in mice to provide saline or Ringer's lactate to prevent dehydration. A simple method is to inject a small amount, ~100 µl, as a subQ injection between the shoulder blades just prior to imaging. Careful consideration is advised, since too much saline may lead to urination during imaging, which may cause artifacts or complicate the image analysis if the probe is excreted through the urine.

6   Circadian Rhythm Effects

It is well known that circadian rhythm alters the physiology of all animals [18], however the effect on imaging agents is not well understood. Rodents are nocturnal, thus imaging during the daytime when humans are active may have different results compared with imaging at night. Glucose levels, amino acid concentrations and even enzyme expression are known to follow a day/night cycle [18]. A simple way to avoid this is to conduct the experiments at approximately the same time of day when acquiring multiple measurements. Another alternative is to reverse the day/night cycle by changing the lighting sequence used within the vivarium.

In some cases, it appears that anesthesia may create an overriding physiological change, thus masking circadian effects [21]. While anesthesia may mask effects for FDG using PET, enzymatic expression cycles are less likely to be overridden since the time scale to change enzyme levels is longer than the glucose/insulin feedback cycle. This has implications for optical imaging using Luciferin/Luciferase, since work has shown substantial changes in enzyme expression based on time of day [22]. Research has shown that the cyclical nature of normal fluctuations of endogenous amino acids can alter PET probe uptake by competition for transport [23].

7 Reproducible Positioning and Multimodality Imaging

Most imaging systems have a central position within the field of view where resolution and/or sensitivity have the best values. The goal therefore is to put the area of interest to be imaged into this region, however there is often a trade-off if the goal is to survey the whole body or to examine two structures at once. One example is the desire to look at the brain for probe uptake, while at the same time imaging the heart to obtain image-based measurements of the left ventricular blood pool to use as an input function for kinetic modeling. For a mouse, the distance between brain and heart is small and it might not be a problem to see both regions, depending on the axial extent of the field of view. Often with brain imaging, the limited resolution in PET or SPECT requires the use of larger animals, such as a rats or small primates. Centering a rat between heart and brain may move both structures into positions of lower sensitivity and image quality, making the desired findings difficult to obtain. In this case, a series of multiple short acquisitions alternating over each structure might be a better option. Some systems also offer automated continuous bed motion, moving the animal back and forth between specified start and end positions, which can provide a more uniform sensitivity over the entire image [24].

For multimodality work involving two or more imaging methods, there are two types of positioning methods possible: single gantry and separate systems. A single gantry, or two systems rigidly connected together, requires only one positioning of the animal within an environmental support system. The animal is then imaged in the same location for both modalities or shuttled between imaging areas without removing the animal from the support or chamber. Shuttling of animals between positions is common for PET/CT, since the ring geometry of PET scanners does not easily allow space for the CT source and detectors, thus these two modalities are usually located adjacent to each other and require moving the animal from one location to another. The advantage with single gantry systems is that the animal is only placed in position once and in most cases the images can easily be co-registered from both systems. The best way to ensure that the animal position and physiological state is the same for both imaging modalities is to acquire simultaneous data, which is possible using SPECT/CT and PET/MRI systems. The disadvantage of a combined system is often little or no access to the animal for injections, blood sampling or monitoring of the animal's heart or breathing rate. This makes monitoring the health status of the animal difficult unless connected to heart and respiratory monitors. Although relatively easy to use monitoring systems are now available, their use adds to the complexity of the experiments and they may interfere with data acquisition. Electrical fields and more than tiny amounts of metal can cause artifacts in MRI and CT images.

The alternative option to an enclosed single gantry is the use of separate imaging systems, which requires the use of a common chamber or platform for animal support that can be used in both systems [11]. The advantage is that visibility and access can be very good for animals imaged in large bore PET systems, while the
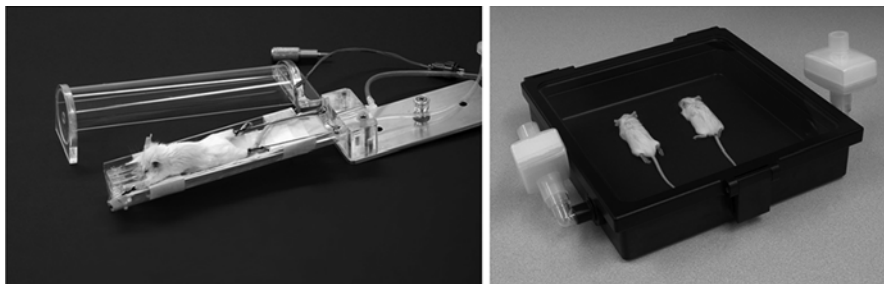
Fig. 18.6 Environmental chambers developed for PET/CT (*left*) and optical bioluminescence or fluorescent imaging (*right*)

limited access in an enclosed CT or MRI system may not be a problem if acquired after the PET scan. MRI and CT systems effectively isolate the animals from the investigators due to the long bore and shielding, however some PET and SPECT systems allow better access while imaging since they are not always completely enclosed. A trade off for PET and SPECT is that by bringing in the detectors closer to the animals, the sensitivity is improved and the system costs can be reduced. The choice of using a small or large bore PET scanner depends on the need for animal access or upon the availability of systems that enable monitoring and sampling using remote control.

Using separate systems can be challenging, since the animals must be accurately and most importantly be precisely placed within the systems in order to match the locations for the fused images. There is always the risk of changing the animal position during the move between imaging systems. A blind co-registration without using the image data to align the two image sets is often essential, since the anatomical information in CT and MRI may not have much, if anything, in common with the metabolic information within the SPECT or PET images. Coregistering the two image volumes, then using a fixed or known bed position for every scan allows the use of fixed reslice parameters to fuse the two images [25]. Another alternative is to use fiducial markers to align the images, however this may add unwanted signal within the data, be expensive or difficult to use routinely and adds complexity that might be avoided by using a well-designed chamber system. Fiducial markers also need to be replaced as the radioactivity source decays.

Several companies make imaging chambers and two examples are shown in Fig. 18.6. For 2D optical imaging, a flat surface is needed for the camera to see the light with minimal distortion. PET, CT and MRI work are best served with a rotationally symmetrical chamber to minimize any artifacts within the images. Chamber materials must be suitable for the particular modalities, such as non-phosphorescent for optical, no metal for CT, non-magnetic for MRI and low density for PET. Environmental support requirements typically require heating, positioning, anesthesia and temperature control, plus the ability to sterilize the surfaces.

8   Respiratory and Cardiac Gating

For some experiments, measurements of respiratory or cardiac motion may be desirable. Numerous systems are now available that can accurately monitor the relatively high heart and breathing rates found in rodents. Typical resting rates in mice can run as high as 600–700 beats per minute (bpm) [26]. Human and primate pulse oxymeters typically have a maximum of 250 bpm, thus are not suited for rodent work.

Heart and respiratory rates can be measured using several different physical means. Needles inserted under the skin have been used historically to provide electrocardiogram data. While this method works well, the presence of metal needles and wires may not be suitable for some imaging methods, such as MRI or CT. Carbon electrodes are a non-ferrous alternative, which are not magnetic and do not cause significant image artifacts in PET, CT and MRI images. Tail cuffs use a pressurized ring around the tail, however they are not well accepted by many investigators, mainly because tail blood flow is directly linked to mouse temperature and thus can be highly variable [13]. Perhaps the easiest system to use is a red light emitting diode and detector, used commonly for humans and primates. The light and sensor can be attached using spring clips, adhesive gauze, tape or other methods. Occasionally there can be problems getting a signal, especially when there is dark skin or too much light shining on the sensor, but this type of sensor is often very easy to use and provides good results without any invasive requirements.

The close link between anesthetic depth and respiratory rate means that the breathing rate needs to be monitored frequently and the anesthesia concentration changed as needed. This means that the breathing rate is not constant during an experiment, so a measurement system is needed that creates a signal for each breath and be recorded along with the image data. Similarly, the heart rate also changes with anesthetic depth.

Many imaging systems have the ability to accept heart rate and breathing data through an electrical connection. The signal can trigger acquisition of data, or be included into the data stream for later processing. Images can be created where the respiratory and/or heart cycle can be divided into multiple parts. Similar to dividing up time into a series of frames, the cycles can be divided into a series of gates, hence the term gated imaging. Dividing the data into separate gates results in larger data sets, and reduces the data events in each image. This may be important, since too many partitions of the data can result in very noisy and very large datasets. Under complete anesthesia sedation, the inhalation phase is about 1/4th or less of the respiratory cycle; so eight gates can describe the respiration motion reasonably well [27]. Light anesthesia can result in much more rapid breathing, as much as 2–3 per second, thus might require a finer sampling period. With deep anesthesia and little time spent in the inhalation phase, many people do not bother with respiratory gating as it may only provide minimal improvement in image data. The motion associated with breathing can be quite large if the animal is over anesthetized and exhibiting agonistic breathing, which is the gasping or large inhalation of air. Proper depth of anesthesia is therefore called for to reduce respiratory motion.
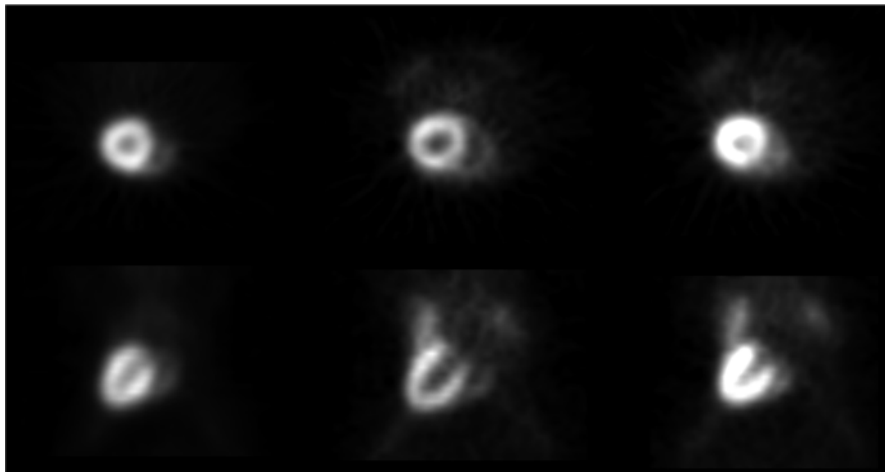
Fig. 18.7 Heart FDG images in a C57 mouse. *Top row*: transverse images of non-gated, diastole and systole. *Bottom row*: coronal view of the same data

Heart motion is fairly small, however gating can be used to separate systolic (contracted) from diastolic (relaxed) phases of the cardiac motion as seen in Fig. 18.7. Gating improves the visualization of both the cardiac wall and blood pools in the left and right ventricles and is particularly important for minimizing spillover and partial volume effect if the left ventricle blood pool is being used to derive the blood time activity data [28].

Unlike heart motion, respiratory motion can be controlled or eliminated by intubation and ventilation of the animals. A tube is inserted into the airway and a cuff inflated to block off any airflow around the tube. Using a pump, either air, oxygen or other gas mixtures can be forced in and out of the lungs. This approach enables controlled respiratory motion. The ventilation unit can trigger imaging, where data can be acquired during breath hold or at specific phases of the respiratory cycle. One interesting approach is high frequency oscillatory ventilation, where the equipment can bring in fresh gas and remove carbon dioxide without moving the animal at all [29]. This unique approach offers continuous ventilation without motion, which can be useful for long imaging times. The short tubing and magnetic pump does create some difficulties for use in certain imaging systems.

9   Immune Compromised Animals

Oncology research has greatly benefited from the creation of mice missing part or all of their immune system, which enables human tumors to be implanted and grown without rejection by the mouse immune system [30]. Nude hairless mice and rats are missing the thymus and ability to produce T cells. Severely compromised

immune deficient or SCID mice also do not have a thymus and additionally are unable to create B cells and have a defect in chromosome 16 that inactivates the DNA repair mechanism. Given the partial or complete lack of immune function, these animals are subject to illness unless protected under barrier conditions from environmental pathogens. These animals pose a special challenge to molecular imaging, since it is advisable to maintain the barrier conditions throughout all steps of the imaging process.

One option to control pathogen exposure is to have all the imaging work conducted in a clean environment, such as biosafety level 3 (BSL3) conditions or a clean room. This poses a challenge since humans have to carefully garb and contain themselves, plus it is difficult to disinfect the imaging systems between groups of animals. Service contract costs can become extremely expensive in BSL3 conditions and access for system repairs may be a problem for service personnel who visit multiple sites. BSL3 conditions may be required for infective and dangerous agents; however immune compromised animals need only be protected from exposure to pathogens if they do not contain biohazardous or carcinogenic agents.

Recently the author's university changed policy to reclassify human tumor xenografts from BSL1 to BSL2, which now requires additional safety requirements for housing and handling of these animals. The potential presence of biohazards such as herpes, AIDS or other blood borne pathogens, while perhaps not a threat to rodents, does represent a hazard to personnel handling these tissues. There is no widespread consistent interpretation about how xenograft tissues are treated, thus the requirements may differ concerning acceptable handling and environmental controls required. In some cases, the requirement of BSL2 conditions may require considerable and impractical changes to how things might have been done under BSL1. There are also AAALAC accreditation requirements that specify separate storage of BSL1 and BSL2 animals, which depending on the housing situation might be simple or very difficult to implement.

For immunocompromised work, a simpler solution is to contain the animals within imaging chambers (see Fig. 18.5) [11]. Animals can be handled, injected and prepared within biosafety cabinets, then imaged using sealed or positive pressure chambers. There are additional advantages of using chambers beyond pathogen control, including the ability to control heating, reproducibly position animals, ability to provide a constant level of gas anesthesia and providing a platform for imaging in multiple modalities.

10   Biohazardous and Infective Work

Unlike immune compromised research, where positive pressure is necessary to keep animals safe from the environment, animals containing biohazardous, carcinogenic or infectious agents must be isolated to protect people. These procedures require sealed or negative pressure containment. If the chambers are sealed and a BSL3 room is located nearby, it may be possible to assemble animals containing infectious,

carcinogenic or other biohazardous materials into chambers, disinfect the outside of the chamber, and conduct the imaging experiment in a BSL1 or BSL2 imaging facility. This approach allows the imaging systems to remain in an open environment and removes the need to disinfect the systems between different types of use.

Animals of different biosafety levels might not be allowed to be mixed together in the same space, which can cause some difficulty with housing, procedure areas and imaging. Chambers and separate preparation rooms may allow for multiple types of use; however the facility design and protocols for imaging work must be carefully determined to ensure this type of mixed use is allowed. It may be necessary to have separate housing and procedure spaces to enable the flexibility of imaging multiple types of biohazardous agents. Alternately, BSL1 animals might be allowed in a BSL2 environment if treated as if they were BSL2 level animals.

The creation of genetically altered animals often makes use of viral vectors for inserting genetic material [31]. These vectors are usually replicate incompetent; however they still require isolation and treatment as if they were infective for several days, depending on the viral agent. Many of the chemotherapeutic agents used to treat cancer are actually carcinogenic, thus are biohazardous agents and require special handling for injections and animal storage. Even though the amount of drug used is miniscule and large quantities are put into patients every day, protection strategies are required to keep personnel safe from exposure. In some cases microisolator cages can be used to house carcinogenic and biohazardous animals in a common vivarium with uninfected animals. More often however there is a requirement that these animals be kept isolated in a separate space, necessitating an additional room for housing. The procedures area where the viral vectors and biohazardous materials are used will require a ducted fume hood or biosafety cabinet, controlled access to the room and usage requirements equal to or greater than biosafety level 2 containment requirements.

11   Contrast Agents

In a broad sense, any imaging agent can be considered a contrast agent, since it is the difference between a specific signal and the background or reference tissue that provides the measured value. More commonly, contrast agents are defined as the agents used to distinguish anatomical structures when imaging with an anatomical system such as MRI or CT. For MRI, Gadolinium, Manganese and Iron are frequently used to give positive contrast in T1 images, while superparamagnetic iron oxides are used to create negative contrast. In CT, Iodine or Barium are used to create a higher density to visualize blood vessels and blood flow rates.

Preclinical imaging using CT poses a challenge when contrast agents are needed, as imaging can take from many seconds up to hours to acquire the data. Human CT systems and angiography studies are very rapid, requiring times of only a few seconds. In humans, contrast agents are injected rapidly at high concentrations and last only a few seconds. There is little persistence of the agents as they are quickly
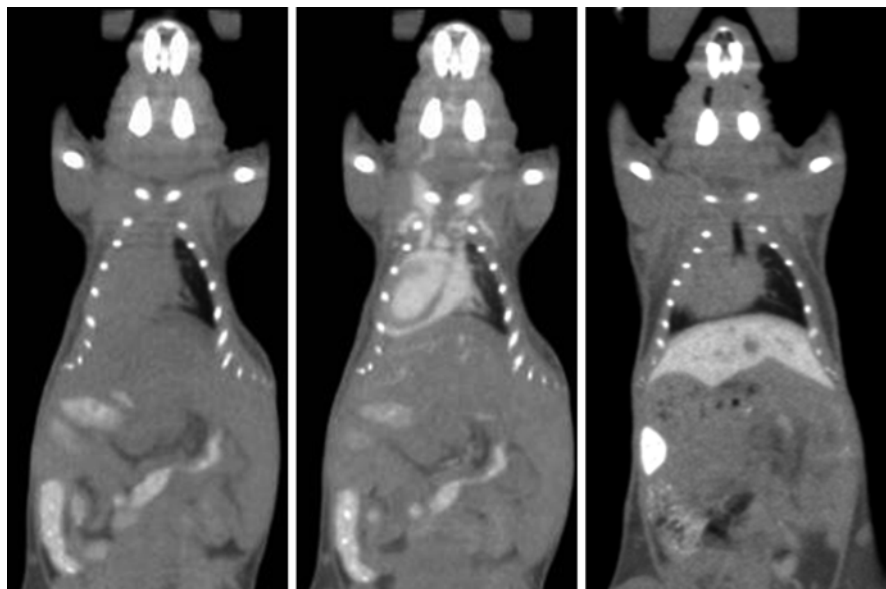
Fig. 18.8 Mouse CT images with no contrast (*left*), vascular Fenestra VC (*middle*) 10 min after a 100 µl injection and Fenestra LC showing liver and spleen (*right*) at 10 mg/kg after 2 h uptake

diluted and stop being useful very quickly. The long imaging times for preclinical systems mean that commercially available CT contrast agents for humans are unlikely to work for preclinical experiments. Fortunately, a number of long lasting agents are now available, which include Fenestra [32] and Exia [33], both of which have been characterized in mice. These agents are liposome encapsulated iodine agents, which depending on the surface treatment can target liver and spleen or remain in the vascular system, as seen in Fig. 18.8.

Care is required in the use of these CT agents, as the temperature and rate of injection can adversely affect the animals. At high concentrations these agents can be toxic, thus it is advisable to use only enough to achieve the desired minimum contrast in the images, typically 100 µl or at most 200 µl. Given that these agents last hours or days, and may require some time to reach peak effectiveness, a slow injection over a minute or two is much safer than a fast bolus injection. CT contrast agents have rather limited applications and are not currently in widespread use.

12 Diet

What animals are fed and when they eat can alter the images obtained using various modalities. With CT, there are often small bits of metal in the chow pellets, which come from the grinding process of the feed stock processing. These particles may also

affect MRI studies. The chlorophyll that makes rodent chow green is also a natural fluorophore, thus adds a signal when imaged using fluorescence optical methods. Switching to a chlorophyll free diet has been shown to eliminate the signal associated with chow, leaving the GI tract and abdominal area with a lower background signal and improving the ability to see fluorescent signals in these regions [34].

Fluorodeoxyglucose, FDG, is a frequently used PET imaging probe that is taken up by glucose transporters and phosphorylated by the hexokinase enzyme. Endogenous glucose competes with FDG for uptake, thus the amount of FDG signals is inversely proportional to glucose levels in blood [35]. Glucose levels are in turn linked to both time of day and feeding cycles [18], thus the fasting state of the animals will alter glucose and FDG uptake measurements. A special case is present with cardiac imaging, where the heart preferentially uses glucose, but under fasting conditions can switch to fatty acids as an energy source. Fasting the animals can actually turn off the heart FDG uptake [36], and also lower tumor signals [2, 37]. This may be a benefit for pulmonary work, but could be a detriment for cardiac imaging. For this reason, it is important to measure glucose levels in animals when imaging with FDG, so the image data can be normalized for endogenous glucose levels to remove some of the measurement variability. Mouse plasma glucose concentrations average about 100 mmol/l [18], however stress and other factors can easily triple this concentration, leading to substantial changes in FDG uptake.

Many imaging probes are taken up by transporter systems and are subsequently phosphorylated or metabolized into a molecule that is trapped in tissue, creating a signal as the uptake occurs and the background activity is washed away. Any endogenous molecules that would normally use those transporters and enzyme pathways would compete for the probe, thus changes in their concentrations would inversely affect probe uptake. One example is the competition of FDOPA for uptake into the brain with the plasma large neutral amino acid concentrations [23]. These amino acids vary based on diet and time of day. Another PET imaging probe subject to this effect is fluoro-L-thymidine (FLT), which is an amino acid analog subject to the same large neutral amino acid transport system as FDOPA.

13 Database and Archival Strategies

Images are only pretty pictures, unless provided with information about what they contain. Details of the imaging process need to be recorded and archived, such as the probe used, injection time and amount, processing details, anesthesia parameters, glucose levels and other associated meta data. By including information such as the people involved, time required, investigator name, recharge numbers, animal and radiation use authorizations, the database can be used to track usage and generate reports for radiation and animal use, billing statements and allows tracking of the operations within an imaging center. If web based, then investigators can be given password protected access to this data, enabling them to call up records from their lab on any computer. Web-based systems are also useful for viewing and modeling kinetic rate constants [38].

Tracking image data can be fairly simple by using a unique session identification number, which can be generated by many methods, from a simple table in a spreadsheet to a web-based database system. It may also be helpful to create animal identification numbers, since each animal may be imaged multiple times. The creation of a data record to be filled out as the imaging experiment progresses is often one of the first steps in starting an experiment.

An archival strategy is essential for an imaging center, regardless of how the data is stored. Imaging systems typically have a single hard drive for collecting data that does not have any redundancy for data preservation if the drive fails. Computer systems can also slow down considerably as the hard drive becomes full. Best transfer times and least chance of data loss can be achieved by archiving data and emptying the acquisition computer drives each day.

Several options exist for storing and retrieving data, which include a web-based retrieval system, network storage drives, DVDs, USB drives, tape and external hard drives. However the data is stored, a process to track, create, store and retrieve the data is necessary. Using the web or network via ftp server is a convenient way to enable the users of the center to obtain their data from any location for local processing. It also prevents any loss of data or physical archival media, since nothing physical is exchanged. The archival site can also be used to make software for image analysis easily available to investigators.

14 Summary

How animals are injected, handled and imaged has a profound effect on the resulting data, thus it becomes imperative that care is taken to minimize, measure or control these factors. While the array of factors and options might appear overwhelming, once these issues are understood, it is relatively straightforward to create an environment and procedures optimized to support the creation of the best possible data. Most imaging work is fairly routine, with only minor changes or the addition or subtraction of certain measurements. The ability to have various procedures ready to use and pre-approved helps greatly in many ways, from obtaining oversight approvals to writing methods in publications. Certain measures can be taken for all studies, such as heating and anesthesia, whereas others can be a toolbox of options available as resources to investigators. Wherever possible, solutions are best integrated into the procedures in ways that make it easy and simple for the investigator to carry out their research. Careful attention to detail will result in data with less variability, therefore capable of finding significant changes using fewer animals and with less work. The savings in cost and time are well worth the effort.

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