

Small Animal Imaging

For Investigators at the Children's Cancer Research Institute (CCRI)

University of Texas Health Science Center at San Antonio

Small Animal Imaging Protocol

Animals with tumors will be assessed for tumor size and extent of metastases by small animal imaging using X-ray computed tomography¹, optical imaging¹, hi-frequency ultrasound¹, microMRI² and microPET².

X-ray Computed Tomography at CCRI

Introduction:

Computed Tomography (CT) is a radiological method of observing internal organs of animals in a non-invasive manner to obtain accurate and quantitative data. This method of visualization delivers high quality volumetric images. The animal is exposed to a small amount of radiation (3-45 cGy), depending upon the quality of the scan. CT is useful for a broad range of applications including assessing the development and progression of various bone diseases such as osteoporosis and arthritis, as well as evaluating the therapeutic efficacy by quantifying bone mineral density and architectural changes. It can also differentiate normal tissues from tumors to allow the measurement of solid tumor volumes to quantify angiogenesis and metastases. CT is also a very good tool for studying respiratory and cardiovascular diseases, allowing investigators to visualize and quantify airway structures and vasculature of the lungs, image stenosis in the arteries, and analyze the effects of therapies. And finally, CT imaging is useful for phenotyping and characterizing anatomical differences in tissue, organ, vascular and skeletal formation in normal or transgenic models.

Contrast administration:

To improve the information value of the scan, mice may receive clinical gastrointestinal or intravenous contrast agents (barium- or iodine-based). Administration of contrast agents would be performed by a

trained technician from the Principle Investigator's lab. Gastrointestinal tract agents such as iodine would be administered at a concentration of 0.425% organic iodine in food or water or by gavage bolus without anesthesia, as is done in clinical practice. Intravenous contrast agents such as iodine would be administered by bolus using peripheral (e.g. tail vein) injection using a 25-27 gauge needle without anesthesia, as is done in clinical practice. For visualization of vessels, live mice will be injected with 0.4 mL/25 g of a 50-mg iodine/mL 150-nm particle diameter iodinated triglyceride blood pool contrast agent (Fenestra VC; Alerion Biomedical, San Diego, CA) into the distal tail vein using a 25- or 27-gauge needle. For animals not originating at CCRI, contrast and subsequent anesthesia induction would be administered in the double-sided hood at the CCRI mouse receiving area.

Anesthesia:

After contrast administration, the animal would be deeply anesthetized using ketamine plus xylazine or Avertin intraperitoneally, or isoflurane by inhalation (see Table 1). Initial anesthesia to the animal will be administered by the Principle Investigator's technician and the animal will be brought into the facility in a HEPA-filtered container. Isoflurane may be used in conjunction with a mouse ventilator (MRI-1, CWE Inc., Ardmore, PA) when precise visualization of the heart and lungs is necessary. If a saphenous vein is used for peripheral vascular access prior to the scan, the animal will also receive intramuscular fentanyl for anesthesia prior to venipuncture. The animal will be restrained for safety in a specially designed 3 x 3 x 7" multimodality holder with micro-filtered free flowing air and will remain immobilized for the 5 – 60 minutes required for the computed tomography scan during which time anesthesia will be continued through isoflurane by inhalation administered by the facility manager or his trained technician. Both micro-isolator and transportation isolation mouse holders will be decontaminated appropriately following the LAR Decontamination Protocol, initially developed to address infection control at the CCRI barrier facility when using the Cobalt Irradiation Resource.

Scanning and visualization:

Volumetric CT of anesthetized mice will be performed at 27-93 μm^3 voxel resolution using the eXplore Locus Small Animal MicroCT Scanner (GE Healthcare, London, Ontario) available at CCRI's Small

Animal Imaging Facility. This volumetric scanner employs a 3500 x 1750 CCD detector for Feldkamp cone-beam reconstruction. Platform-independent parameters of current and voltage can be kept constant at 450 μ A and 80 kV, respectively. The exposure time can be varied between 100 and 2000 msec depending on the resolution of the scan. The number of views can be varied between 180 and 720 and the number of frames per view varied from 1 to 8. The animal will be observed by direct visualization for any signs of distress during the scan. CT images will be reconstructed with the manufacturer's proprietary EVSBeam software and visualized with the open-source MicroView and BioImage software.

Post-anesthesia recovery:

After the scan, the animal will be recovered on a heating pad in a normal mouse cage to maintain its body temperature at 37-37.2 °C for a 1 hour-period of post-anesthesia recovery. Since the ratio of body surface area to body mass is greater in mice than in larger animals, thermal support is crucial to the successful recovery of mice from anesthesia. It is also important to administer an ocular lubricating ointment because most anesthetic drugs inhibit blinking, which can result in drying of the cornea and increase the risk of corneal ulceration. After making sure the animals are stable and have returned to a safe level from anesthesia, they will then be placed in a biological safety hood in the LAR quarantine area for Principle Investigator or his technician to pickup.

Optical Imaging at CCRI

Introduction:

The Xenogen IVIS Imaging System 200 allows researchers to use real-time luminescence and fluorescence (400-900nm) imaging to non-invasively monitor and record cellular and genetic activity within a living organism.

Animal preparation, scanning, and visualization:

Animal preparation for optical imaging procedures will be essentially the same as microCT except that the activation of optical reporter genes will be either low energy laser scanning for fluorescence imaging or an intraperitoneal injection of 150 mg/kg luciferin, the substrate for firefly luciferase for luminescence

imaging. Anesthesia can be administered intraperitoneally using ketamine plus xylazine or Avertin, or by inhalation using isoflurane. If isoflurane was used to initially anesthetize the animal, it will be necessary to continue the administration of isoflurane along with oxygen using a nose cone during the scan to immobilize the animal until the scan is done. Living Image® Software from Xenogen will be used for image acquisition. Scan times vary between 0.5 second and 3 minutes.

Post-anesthesia recovery:

Similarly, a heating pad will be used to maintain the mouse's body temperature at 37-37.2 °C for a 1 hour-period of post-anesthesia recovery. And an ocular lubricating ointment will be administered until spontaneous blinking is resumed.

MRI at the Research Imaging Center

Introduction:

Magnetic resonance imaging (MRI) is available at the Research Imaging Center (RIC), UTHSCSA. Equipment includes a large-bore Siemens 3T TRIO MRI and an Elscint 2T MRI. Funding has been approved to purchase a new Siemens 3T TIM MRI and an animal-dedicated Bruker 7T ClinScan MRI. These multiple MRI scanners allow imaging of small (mice, rats) to large non-human primates (e.g., baboons). MRI can be used to visualize anatomical features (aMRI) down to <100microns using a scan time between 10 min to 1 hour for live animals. In addition, MRI can be used to observe functional brain activity (fMRI), MR spectroscopy of several anylates, including fat and muscle. Perfusion MRI measures blood flow and blood volume and can with performed using anterior spin labeling or with exogenous contrast agents such as gadolinium or ferodex. The field of view of the instrument is 3-70 cm using an array of custom coils.

Animal preparation, scanning, and visualization:

Before imaging tumor-bearing mice using MRI, the mice will be anesthetized by inhaled isoflurane anesthesia or by the intramuscular administration of a mixture of ketamine and xylazine (see Table 1). The contrast agents can then be injected intravenously at a dose of 0.1 mmol-Gd/kg to enhance contrast

in the blood pool for better visualization of tumors and angiogenic blood vessels connected to tumor tissues. For minimization of movement artifacts, mice can be placed in a custom-built, commercially available isolator with continuous airflow delivered by a generic fish tank pump. MR images will be acquired following the injection of the contrast agent on the Siemens Trio 3T scanner or Bruker 7T MRI using a 3D fast low-angle shot (FLASH) (FL3D) pulse sequence. In most cases, specialized RF coils will be used or developed to improve SNR and minimize artifacts. The imaging parameters can be set as follow: 2.47-ms echo time (TE), 7.36-ms repetition time (TR), 25° RF tip angle, 120-mm field of view (FOV), and 0.5-mm coronal slice thickness.

Post-anesthesia recovery:

Similarly, a heating pad will be used to maintain the mouse's body temperature at 37-37.2 °C for a 1 hour-period of post-anesthesia recovery. And an ocular lubricating ointment will be administered until spontaneous blinking is resumed.

MicroPET at the Research Imaging Center

Introduction:

MicroPET offers the unique opportunity to image small animal models of diseases, including genetically engineered animals. It is a functional imaging modality at molecular level and provides valuable insights into biochemical, physiological, pathological or pharmacological processes *in vivo*. Data can be obtained noninvasively, repeatedly, and quantitatively in the same animal. Current applications include a diverse field such as perfusion, metabolism, protein targets and substrate utilization in various vital organs including heart and brain, gene expression and stem cell tracking, neurotransmitter and receptors, neural activation and plasticity, targeting tumor antigens and elucidating tumor biology such as angiogenesis, hypoxia and apoptosis. Recent research efforts find application in a wide area ranging from basic insights into the normal physiology and disease processes to drug development and early response to anticancer and gene therapy.

A microPET system from Concorde Microsystems (microPET Rodent R4) with an axial field of view of 8cm also available at RIC can be used for PET imaging. The system demonstrates a spatial resolution of 1.85 mm full-width at half-maximum (FWHM) in the axial direction and 1.66 mm FWHM in the transaxial direction measured from the center with a 1 mm diameter sodium-22 point source. Within the inner 20 mm of the FOV the linear resolution is better than 2.5 mm in all three directions. The use of radioisotopes are required for PET imaging, most of which are generated, onsite, using a 21 MeV cyclotron. Commonly used radioisotopes are fluoro-2-deoxy-D-glucose, FDG (F^{18}) and oxygen-15-labeled water [O^{15}]- H_2O .

Animal preparation and scanning:

Before imaging using the microPET, animals are first given a dose equivalent to 0.25-4 mCi of FDG (F^{18}) through an intravenous/intraperitoneal injection and placed in an isolated environment for 20-30 minutes. During this uptake period FDG enters the brain and is converted to FDG-6-phosphate, which is metabolically trapped and reflects regional rates of glucose consumption. Animals are anesthetized with ketamine/xylazine (see Table 1) and then scanned in the microPET for 10-40 minutes. For shorter-lived isotopes, such as ^{15}O - base tracers, scans can be repeated within 7 minutes.

Post-anesthesia recovery and quarantine/euthanasia-disposal of radioactive animal:

Similarly, a heating pad will be used to maintain the mouse's body temperature at 37-37.2 °C for a 1 hour-period of post-anesthesia recovery. And an ocular lubricating ointment will be administered until spontaneous blinking is resumed.

If the animal does not recover or if PET imaging is a terminal experiment, all animal carcasses and tissues containing radioactive waste must be bagged in strong plastic bags and securely fastened. A tag with the date of disposal, responsible investigator, nuclide, and its activity must be attached to the bag. The labeled carcasses are then stored in a designated freezer until picked up by Radiation Safety personnel for proper disposal.

A summary of various anesthetics and contrast agents, their dosage information, route of administration, and duration in the body are listed below in Table 1.

Table 1:

Anesthetic	Dose	Route	Duration
Avertin	0.3mL/25g of 2.5%	Intraperitoneal	20-30 min
Ketamine/Xylazine	80-100mg/kg + 10-12mg/kg	Intraperitoneal	20-30 min
Isoflurane	2% per 2.5L oxygen	Inhaled (nose cone)	20-60 min
+/- Fentanyl	0.25mg/kg	Intramuscular	20-30 min

A summary of various imaging modalities and their imaging requirements are listed below in Table 2.

note: "MRI", implies "anatomical MRI", "functional MRI (fMRI)" and MR spectroscopy.

Table 2:

Modality	Contrast Agent	Dose	Route of Administration	Scan Time
microCT	Fenestra LC	0.4ml/25g of animal	i.v.	5-60min
	Fenestra VC	0.4ml/25g of animal	i.v.	5-60min
	Iodine water	1 bottle of water with 0.425% organic iodine	feed for 2 days	5-60min
Florescence	ICG	15µg/animal	i.v. or i.p.	0.5s-3min
Luminescence	Luciferin	0.1ml/10g of animal	i.p.	0.5s-3min (After 15 min wait)
microPET	[F ¹⁸]-FDG, [O ¹⁵]-H ₂ O, [O ¹⁵]-CO, [O ¹⁵]-O, Cu64	1-4 mCi	i.v. or i.p. or intra-tracheal	[O ¹⁵]- every 7 min; FDG After 20 min wait)
MRI	Gadolinium (Ferodex®)	0.1 mmol/kg of animal	i.v.	10-60min (After 5 min wait)

¹ Instrument located in CCRI imaging facility

² Instrument located in Research Imaging Center