UW Small Animal Imaging and Radiotherapy Facility (SAIRF)

MISSION

The mission of the UW Small Animal Imaging and Radiotherapy Facility is to provide innovative, state-of-the-art, affordable, noninvasive, high-resolution, *in-vivo* and *ex-vivo* imaging and radiotherapy support to UW Carbone Cancer Center members, UW researchers, and industries that use small animal models in their research.

Consultation

The facility director and manager provide consultation to investigators to determine which imaging and radiotherapy methodologies that are best suited to meet their research objectives. The SAIRF has a scentific advisory committee as a resource that provides expertise in the areas of molecular imaging, radioproduction and radiotherapy, ultrasound/photoacoustics, computed tomography (CT), magnetic resonance imaging, image analysis, and radiotherapy. The SAIRF staff can provide guidance to your overall study design, for example, whether to include control groups or to determine the number of subjects for statistical significance. More specifically, the SAIRF will decide which of the following parameters are optimal in order to answer your specific questions: animal diet and temperature, anesthesia, imaging modality, contrast agent, imaging time points, scan duration, gating, temporal and spatial resolution, static or dynamic framing, and image reconstruction. Lastly, we can train you to accurately and efficiently analyze your data using our free-of-charge post-processing computers.

Micro-imaging & radiotherapy suite

We have enjoyed tremendous institutional support for our first class 2000 square foot facility that was specifically designed for small animal molecular imaging and radiotherapy in the WIMR (Wisconsin Institutes for Medical Research) 1 tower, a 9-story research building attached to the hospital, and adjacent to the School of Pharmacy and the Waisman Research Institute. This new facility houses the Siemens microCATII, Siemens Inveon Hybrid microPET/CT, Perkin Elmer IVIS, Fujifilm VisualSonics Vevo2100 LAZR, Perkin Elmer Wizard^2 Gamma Counter, Fluoptics Fluobeam, Abaxis HM5 Hematology complete blood count (CBC) Analyzer, Abaxis VS2 Blood Chemistry Analyzer, Agilent 4.7T MRI and associated hyperpolarization apparatus, and three X-ray radiators (Xstrahl SARRP, Xstrahl RS225, and Precision X-Ray X-Rad320). We have a designated area with computationally powerful workstations for image analysis. We boast two of our own animal holding rooms, one to house imaging animals and one to house radiotherapy (external beam and systemic targeted radionuclide therapy) animals, which have strictly regulated temperature, humidity, pressure, light cycles, and contain passively ventilated rodent housing racks, specifically for holding hradioactive animals and those involved in longitudinal studies. The new WIMR complex is strategically located adjacent to the new animal vivarium where non-radioactive animals involved in imaging studies are housed. This preclinical molecular imaging suite is designed with translational research in mind as supported by our lab neighboring the clinical research GE Discovery VCT and GE Discovery 710 PET/CT scanners, and a GE Signa PET/MRI scanner. Also, next to the small animal imaging and radiotherapy suite are the cyclotron, radiochemistry, and radiopharmacy facilities which provide expertise on PET agent production and synthesis in a collaborative or fee-for-service basis. The SAIRF director and manager can coordinate radionuclide and radiotracer synthesis with the Cyclotron Group led by Drs. Jon Engle and Todd Barnhart in the Medical Physics Department.

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Alternatively, agents may be acquired from commercial sources such as PETNET or IBA Molecular.

Anatomic microCT imaging

In December 2005, we acquired the microCATII from Siemens. This scanner has a large field-of-view for scanning large rodents (ie. rats up to 300 grams), and a variable source for high-resolution scanning (~20 microns). This scanner was funded entirely by an NCRR shared instrumentation grant awarded to Dr. Weichert. In late December 2006, we received the first Inveon microCT scanner from Siemens. This CT can achieve 50 micron spatial resolution, but it is primarily used to acquire anatomical images for functional PET scanning.

Functional microPET and hybrid microPET/CT imaging

In late December 2006, we received the first ever Inveon hybrid microPET/CT scanner from Siemens. Coupled with our own proprietary cell-selective imaging contrast agents, this scanner affords our investigators unique disease detection and evaluation which can only be provided at the UW. This scanner provides unsurpassed PET sensitivity (>10%), 1.2mm resolution, and a large axial field of view (13 cm). The anatomical CT and the functional PET images are automatically co-registered for easy analysis. An integrated isoflurane anesthetic gas system and physiologic monitoring system allow for image gating and animal monitoring during scanning.

Gamma counting

The PerkinElmer Wizard2 is a well-type, 10-detector gamma counter that collects signal from 3-dimensions of a radioactive sample versus 2-dimensions in autoradiography. There are consistent background readings and minimal crosstalk between samples making each measurement more precise. A built-in isotope library consisting of 45 radionuclides automatically adjusts window settings and half-lives, while any new isotopes can be added to the library manually. This suits the needs of any researcher interested in collecting biodistribution and relative concentration of radioligands in blood and tissues as a supplemental or surrogate measure to imaging and therapy.

Optical imaging

The IVIS Spectrum (Perkin Elmer) is capable of both bioluminescence and fluorescence scanning. We routinely use this system for non-invasive longitudinal monitoring of cancer progression, metastatic cell trafficking and gene expression and delivery in living animals. This system is also used to assess hypoxia, enzyme activity, angiogenesis, apoptosis, arthritis, neurological and infectious diseases, among many other applications. An optimized set of high efficiency filters and spectral un-mixing algorithms affords non-invasive imaging of bioluminescent and fluorescent reporters across the visible light spectrum up to the near-infrared wavelength. It also offers single-view 3D tomography for both fluorescent and bioluminescent reporters that can be analyzed in an anatomical context using a digital mouse atlas. The Spectrum can excite from the bottom (trans-illumination) for deep tissue or from the top (epi-illumination) to illuminate *in-vivo* fluorescent probes. 3D diffuse fluorescence tomography can be performed to determine source localization and concentration using the combination of structured light and trans-illumination fluorescent images. The instrument is equipped with ten 30nm bandwidth excitation filters and eighteen 20nm bandwidth emission filters that significantly reduce auto-fluorescence by the spectral scanning of filters and the use

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of spectral un-mixing algorithms. In addition, the spectral un-mixing tools allow the researcher to separate signals from multiple fluorescent reporters within the same animal.

Intraoperative real-time NIR imaging

The SAIRF is one of the first facilities in the US to obtain the Fluobeam™ (Fluoptics) hand-held imaging system which detects *in-vivo* near-infrared fluorescence in 2D. This system has a laser excitation dialed in at 780nm and a long pass emission filter at >820nm, and a crown of LEDs allowing one to work under white light in open space with a direct access to the animal. Focused on cancer surgery improvement, this technology will afford oncology surgeons a radically new efficiency in tumor resection. The success of this concept will largely depend on the ability of the optical agent to selectively localize in the tumor prior to surgery. Several UW investigators are currently developing tumor-specific NIR optical probes for intravenous administration that may potentially afford real-time intraoperative tumor margin illumination. Intraoperative margin illumination could have a significant impact in glioma resection and determining lymph node involvement during breast cancer resection, for example. This newly introduced unit is designed to be used in a surgical suite and therefore offers rapid clinical translation potential.

High resolution MRI

Installation of an Agilent 4.7T small animal scanner was completed in April of 2007. The horizontal bore imaging/spectroscopy system gives us the capability to scan rodents up to 600 grams with an in-plane resolution of 50 microns. The system is also equipped with a rodent isoflurane gas anesthesia system and physiologic monitoring system for image gating**.** We can scan broadband nuclei including 1H, 31P, 19F and 13C. T1 and T2 anatomical scans are possible as well as the creation of T1, T2 and T2* maps. The system is also capable of functional MRI (EPI), diffusion and diffusion tensor imaging, localized spectroscopy (STEAM and PRESS), chemical-shift imaging, and perfusion imaging with Gd-based contrast agents. These specifications allow investigators to visualize and quantify a variety of moieties and processes including metabolites (NMR spectroscopy), anatomical structures, tumor morphology, blood flow/vessels, fiber pathways, drug effects, brain activity, and heart motion. In early 2008, we became one of only 5 institutions in the US to receive a commercial dynamic nuclear polarization system from GE. This system allows rapid *in-vivo* investigation of biochemical events enhanced with carbon-13 labeled substrates at enhanced sensitivity levels.

High resolution ultrasound & photoacoustic imaging

In April of 2015, the SAIRF acquired the VisualSonics Vevo2100 LAZR (FujiFilm) ultrasound and photoacoustic imaging system. The system is a high frequency array-based ultrasound system with center frequencies in the 20-70 MHz range, designed specifically for the depth and resolution needed for scanning small animals. In turn, photoacoustic imaging uses non-ionizing laser pulses which are non-invasively delivered into biological tissues and/or contrast agents creating a momentary thermoelastic expansion at the ultrasonic emission wavelength, and detected by traditional ultrasound transducers. Measuring this thermoelastic data allows for analysis of functional parameters such as oxygen saturation, total hemoglobin and the microdistribution of biomarkers in real-time. The system can be used as a stand-alone ultrasound or in conjunction with photoacoustic imaging. When used simultaneously, there is an automatic co-registration of photoacoustic signal to the anatomic ultrasound image. Included with the system is an integrated rail mount that allows for easy setup and adjustment of the ultrasound probe as well as a heated animal positioning platform with physiological monitoring.

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A motorized probe driver allows for real-time 3D volumetric imaging, and a mounted micro-injection system is available for precise, image-guided injections.

Hematology analyzing

In the Fall of 2016, the SAIRF acquired the Abaxis VetScan HM5. This system is a fully automated hematology analyzer that uses impedance technology to distinguish blood cell types based on the pulse generated as each cell passes through an electrically charged aperture. The volume of each cell is directly proportional to the magnitude of the electrical pulse generated. This size determination, along with susceptibility to various lysing agents, provides the basis for blood differentials on as little as 25µl of blood (validated for mouse, rabbit, rat, ferret, pig, goat, monkey, sheep and guinea pig). This analyzer allows investigators the ability to monitor up to 22 heme parameters, including platelet (thrombocyte) counts, mean volume, hematocrit, and distribution width, on radioactive animals during tumor treatment. Other heme parameters include red blood cell (RBC) count and indices, RBC hemoglobin, hematocrit, lymphocytes and lymphocyte percentage, monocytes and monocyte percentage, neutrophil and neutrophil percentage, eosinophil and eosinophil percentage, basophil and basophil percentage, mean cell volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and red cell distribution width.

Image Analysis Workstations

We provide three powerful PCs equipped with up to 64GB of RAM, dual 3.1 GHz processors, NVIDIA Quadro 4000 graphics cards, on a 64-bit Windows 7 OS to facilitate 2-D and 3-D image viewing, manipulation, and quantitative analysis of large files. The workstations are furnished with several imaging software packages including Siemens Inveon Research Workplace, Image J, GIMP, Amira, VivoQuant, and IVIS's Living Image. We can easily convert imaging data to a universal DICOM format if you choose to perform analysis on your own workstation.

Radiotherapy (External Beam Therapy (XRT) & Targeted Radionuclide Therapy (TRT))

The SAIRF manages three x-ray source irradiators (Xstrahl SARRP, Xstrahl RS225, and Precision X-Ray XRad320)

Xstrahl Small Animal Radiation Research Platform (SARRP): The SARRP was newly installed in the SAIRF in June, 2018. The SARRP delivers targeted external beam radiation to pre-clinical animal models with great accuracy, conforming to a tissue of interest as is done in clinical radiotherapy. The system is equipped with a computed tomography (CT), allowing the user to contour a tissue of interest (ie. tumor) to evaluate the dose and to allow for animalized (akin to "personalized") therapy. The University of Wisconsin Radiation Calibration Laboratory, which is an Accredited Dosimetry Calibration Laboratory (ADCL) by the American Association of Physicists in Medicine (AAPM) and through the American Association for Laboratory Accreditation (A2LA), completes biannual commissioning and monthly quality management, and Radiation Oncologist, Dr. Randall Kimple, and Medical Physicist, Dr. Bryan Bednarz, provide expertise on treatment planning and dosimetry, respectively.

Xstrahl RS225: The RS225 was acquired by the UW Medical School in 2017. The SAIRF took over the management of this irradiator in October 2018. This system is a self-contained X-ray irradiator that delivers an accurate and precise radiation dose to cellular in vitro studies. The system is "turn-key" and can be operated by novice users. The X-ray tube produces a highly homogeneous beam that is dialed in at 195kVp and 10mA, and a 3mm filter is used to mitigate

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beam hardening. The software interface allows multiple lab username logins with programmable and customizable protocols. The Department of Medical Physics' Radiation Calibration Laboratory performs monthly quality control to ensure system stability and annual commissioning for dose verification.

Precision X-Ray XRad320: The XRad320 was acquired by the UW Medical School in 2012. The SAIRF took over the management of this irradiator in October 2018. This system is a self-contained X-ray irradiator that delivers an accurate and precise radiation dose to rodents and other biological specimens. The system is "turn-key" and can be operated by novice users. The software interface allows multiple lab username logins with programmable and customizable protocols. The X-ray tube produces a highly homogeneous beam that is dialed in at 320kVp and 12.5mA, specifically for radiation therapy applications. The Department of Medical Physics' Radiation Calibration Laboratory performs monthly quality control to ensure system stability and annual commissioning for dose verification.

Targeted Radionuclide Therapy (TRT): The SAIRF performs tail-vein injections for systemically delivered targeted radionuclide therapy agents. Following administration, radioactive animals can be housed in our animal housing room in BRMS which is exclusively dedicated to TRT experiments. Animals needing to be monitored after radioactive decay can be transferred to a neighboring non-radioactive containment room. See more details about animal housing below.

Animal housing

In order to ensure and preserve the pathological integrity of the Biomedical Research Model Services (BRMS) facilities, investigators are required to transfer their animals to our general microimaging protocol (M005532) by completing an animal transfer form. Only after approval from BRMS can animals be transported to our facility in WIMR.

The dedicated SAIRF animal housing facility utilizes Innovive ventilated rodent housing systems, in rooms with automatic 12hr dark/light cycle, and strictly regulated temperature, pressure, and humidity controls to ensure a healthy, stable environment. Animal health, food, water, and bedding are monitored twice per day and maintained as necessary by the SAIRF and BRMS staff. Apart from the cost of disposable cages, no additional housing costs are applied if the animals are part of an imaging experiment, including longitudinal studies.

If the animals are *not* included in an imaging experiment but are housed in the SAIRF facility for radiotherapy or biodistribution studies, a housing fee will be applied. A dedicated radiotherapy room was added to the SAIRF space within the WIMR vivarium in January 2019. Radioactive animals will remain in our facility until the experiment is completed and background levels of radiation have been reached. Radioactive materials can only leave the imaging facility if approved by radiation safety. Under no circumstances are radioactive animals allowed to return to their original housing facility. In approved cases, non-radioactive animals can be rehoused in an approved containment suite.

Contrast agent development

Our investigators are well-versed in the development of cell-selective contrast imaging agents useful for CT, MRI, optical/NIR, and nuclear medicine. Two agents developed in our labs, Fenestra™ VC and LC, are now commercially available to the research community from

MediLumine (Montreal, QC, Canada). Imaging examples and results from our lab can be viewed on the Small Animal Imaging and Radiotherapy Facility Image and Video Gallery.

Oversight committee

The policies of the facility are established and governed by an oversight advisory board committee comprised of imaging scientists, physicists, cancer biologists, and veterinarians. This committee meets every 6 months. At least one member of the committee will always be a current member of the Medical School animal care committee. Administrative support is provided by UWCCC management and personnel.

The small animal imaging and radiotherapy facility oversight committee consists of the following people:

- Frank Korosec - Chief of the Imaging Sciences Section of the Department of Radiology, Director of Research Resources, & Director of Clinical MRI Physics
- Jamey Weichert - Associate Professor of Radiology, Medical Physics and Pharmaceutics
- Weibo Cai - Associate Professor & Director of the UW Molecular Imaging and Nanotechnology Lab
- Amy Fowler – Assistant Professor, Department of Radiology
- Amy Moser - Associate Professor of Human Oncology
- Rich Halberg – Assistant Professor of Gastroenterology & Hepatology and LAR Director
- Tomy Varghese -
- Brigitte Raabe – DVM, DACLAM
- Meghan Hessler – DVM
- Ellen Leiferman - DVM
- Justin Jeffery – SAIRF manager
- Ashley Weichmann - SAIRF staff

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UWCCC Small Animal Imaging and Radiotherapy Facility Scanners

Siemens Inveon microPET/CT

Siemens MicroCATII

Agilent 4.7T MRI

Perkin Elmer IVIS

Fluoptics Fluobeam (NIR)

FujiFilm VisualSonics Vevo2100 LAZR

Perkin Elmer Wizard Gamma Counter

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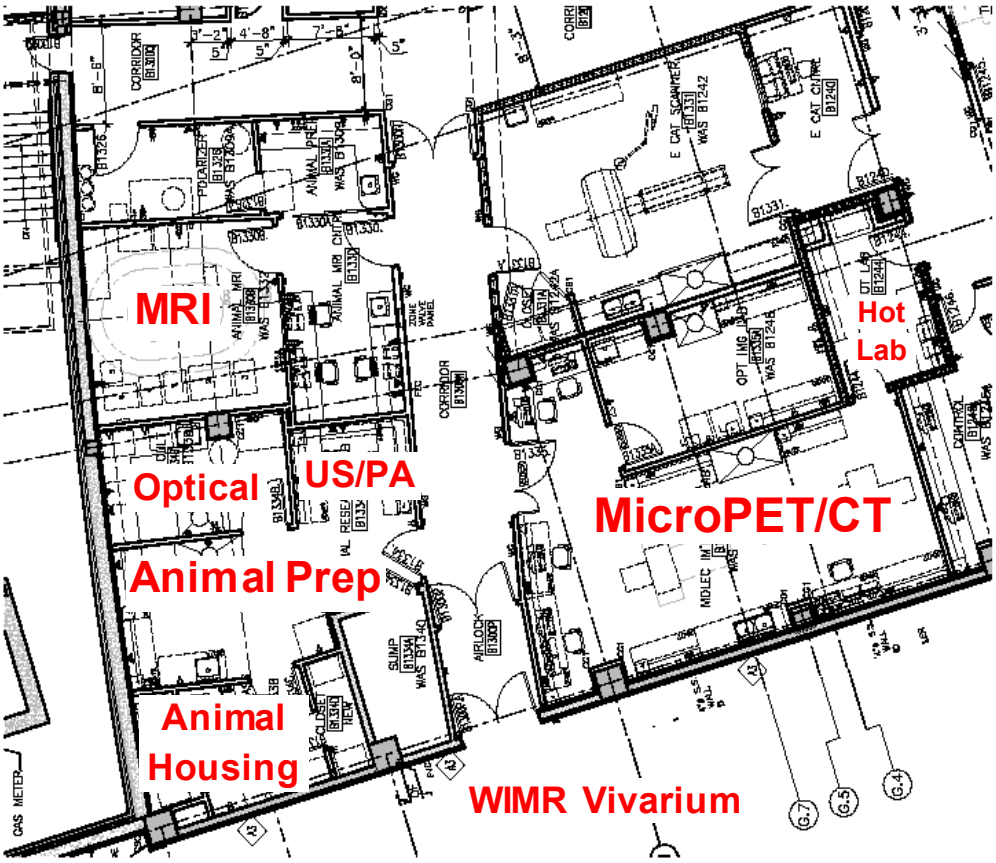
Abaxis HM5 Hematology Analyzer

UWCCC Small Animal Imaging and Radiotherapy Facility Floor Plan



MRI: 4.7T Agilent and GE Hyperpolarizer system
Animal Prep: Cell culture, radioactive animal holding (rats and mice), gamma counter, and hematology.
Analysis: Three Siemens IRW imaging analysis work stations.
MicroPET/CT: Siemens Inveon Hybrid microPET/CT and Siemens microCATII
Optical: Perkin Elmer IVIS and Fluoptic Fluobeam (NIR)
Ultrasound/photoacoustic (US/PA): Fujifilm VisualSonics Vevo2100 LAZR
Hot lab: Radioactive doses stored, drawn, and assayed in this shielded room.
WIMR Vivarium: Houses rodents (B-level) and non-human primates (Level 1).

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