UW Small Animal Imaging and Radiotherapy Facility (SAIRF)

MISSION

The mission of the University of Wisconsin Carbone Cancer Center's (UWCCC) Small Animal Imaging and Radiotherapy Facility (SAIRF) is to provide innovative, state-of-the-art, affordable, noninvasive, high-resolution, *in-vivo* and *ex-vivo* imaging and radiotherapy support to UWCCC 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 are best suited to meet their research objectives. The SAIRF has a scientific advisory committee as a resource that provides expertise in the areas of molecular imaging, radiochemistry, ultrasound/photoacoustics, computed tomography (CT), magnetic resonance imaging, image processing, and radiotherapy. The SAIRF staff will advise regarding optimal parameters and considerations in order to answer specific scientific questions. As some examples, animal diet, temperature, anesthesia, imaging modality, contrast agent(s), drug administration, time point(s), experimental group numbers, scan duration, gating, temporal/spatial resolution, static vs dynamic, and image reconstruction are considered. Lastly, SAIRF staff will train users to accurately and efficiently analyze imaging data using our free post-processing workstations.

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 Wisconsin Institutes for Medical Research (WIMR) 1 tower, a 9-story research building attached to the hospital, and adjacent to the School of Pharmacy and the Waisman Research Institute. This facility houses the Siemens Inveon Hybrid microPET/CT, MILabs U-SPECT^{6}/CT^{UHR}, Perkin Elmer IVIS Spectrum, Fujifilm VisualSonics Vevo2100 LAZR photoacoustic/ultrasound, Faxitron Ultrafocus Digital X-ray, 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, RS225, and CIX3). We have a designated area with 4 computationally powerful workstations for image analysis. We boast two of our own animal holding rooms; one dedicated to housing imaging animals and the other for radiotherapy (external beam and systemic molecularly targeted radionuclide therapy) animals. Each animal housing facility has strictly regulated temperature, humidity, pressure, light/dark cycles, and contains Innovive HEPA-filter ventilated rodent housing racks, specifically for holding radioactive animals and those involved in longitudinal studies. The WIMR complex is strategically located adjacent to the animal vivarium where non-radioactive animals involved in imaging studies are housed. The SAIRF preclinical molecular imaging suite is designed with translational research in mind as supported by our lab neighboring the clinical research GE Discovery VCT, GE Discovery 710 PET/CT, and GE Signa PET/MRI scanners. Also, next to the SAIRF is 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 coordinate radionuclide and radiotracer synthesis with the Cyclotron Group led by Drs. Jon Engle and Todd Barnhart in the Medical Physics Department. Alternatively, agents may be acquired from commercial sources such as PETNET, IBA Molecular, or Sofie Biosciences.

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Anatomic microCT imaging

In December 2006, we received the first ever Inveon microPET/CT scanner from Siemens. The CT can achieve 50 micron spatial resolution for high resolution imaging, but it primarily serves as an anatomical reference for PET scans while enabling attenuation and scatter correction.

In November 2020, we received the MILabs U-SPECT^{6}CT$^{\text{UHR (ultra-high-resolution)}}$ system. The CT can achieve 10 micron spatial resolution and thus serves as our go-to system for ultra-high-resolution applications.

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.

Functional microSPECT and hybrid microSPECT/CT imaging

In December 2020, we received the MILabs U-SPECT^{6}CT$^{\text{UHR (ultra-high-resolution)}}$ which is the first and only microSPECT/CT system in Wisconsin. The U-SPECT from MILabs allows imaging of both standard and high-energy theranostic radioisotopes that are commonly used in brain, heart, bone and cancer applications, although many other uses exist as well. The MILabs CT$^{\text{UHR}}$ can achieve spatial resolution as good as ~15 microns. The SPECT system is equipped with 3 collimators trading off sensitivity and spatial resolution. One specific collimator allows for the detection of relatively high energy SPECT isotopes that is otherwise not feasible with other collimators. The anatomical CT and the functional SPECT images are automatically co-registered for easy analysis. An integrated physiologic monitoring system allows for cardiac and respiratory gating and animal monitoring. There is also a built in isoflurane anesthetic gas system and a heated bed to maintain the animal's temperature.

Gamma counting

The PerkinElmer Wizard^{2} 2480 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 (fluorescence & bioluminescence) imaging

The PerkinElmer IVIS Spectrum can detect both bioluminescence and fluorescence light. SAIRF users 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 into the near-infrared wavelength. The Spectrum can excite from the bottom (trans-illumination) for deep tissue or from the top (epi-illumination) to illuminate *in-vivo* fluorescent probes. The instrument is equipped with ten 30nm bandwidth excitation filters and eighteen 20nm bandwidth emission filters that significantly reduce auto-fluorescence signal by applying 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.

Optical (NIR fluorescence) imaging

The SAIRF is one of the first facilities in the US to obtain the Fluobeam™ (Fluoptics) real-time hand-held near-infrared fluorescence guided intraoperative system. 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 users 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**.** Users 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 (US) & photoacoustic (PA) imaging

In April 2015, the SAIRF acquired the VisualSonics Vevo2100 LAZR (FujiFilm) ultrasound/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

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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. 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

The SAIRF acquired our own dedicated complete blood count (CBC) and serum chemistry equipment in order to run radioactive blood samples.

The Abaxis VetScan HM5 complete blood count (CBC) heme analyzer was acquired in the fall of 2016. 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 50µl 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.

Soon after, in early 2017, the SAIRF added the Abaxis VetScan VS2 for serum chemistry analysis. Capable of reading serum, plasma or whole blood, the VS2 can be used to evaluate multiple analytes using only 100µl of blood. With a single use rotor, up to 15 of the following analytes can be read on a single sample: ALB, ALP, ALT, AMY, AST, BA, BUN, Ca, CHOL, CHW, CK, Cl- CRE, GGT, GLOB*, GLU K+, Mg, Na+, PHB, PHOS, T4, TBIL, tCO2, TP, UA -- depending on the chemistry profile you choose.

Image Analysis Workstations

We provide four powerful Windows 10 Analysis Workstations equipped with at least 64GB of RAM and high-end processors and graphics cards to facilitate 2-D and 3-D image viewing, manipulation, and quantitative analysis of large datasets. These workstations are furnished with several imaging software packages including Siemens Inveon Research Workplace, Image J, GIMP, Bioptics, Amira, PMOD, Imalytics and Living Image. We can convert imaging data to various formats (DICOM, NifTI, tif, etc) if users prefer to perform analysis on their own workstations.

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

The SAIRF manages three x-ray source irradiators (Xstrahl's SARRP, RS225, and CIX3).

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 for *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 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.

Xstrahl CIX3: In February 2021, the SAIRF acquired the Xstrahl CIX3 cabinet irradiator. 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 300kVp and 10mA, 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 TRT agents. Following administration, radioactive animals can be housed in the SAIRF animal housing facility within Biomedical Research Model Services (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 BRMS facilities, investigators are required to transfer their animals to the general SAIRF microimaging protocol (M005532) by completing the animal transfer form. Only after approval from BRMS can animals be transported to our facility in WIMR.

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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. A daily per diem rate per cage, in addition to the cost of disposable cages, is applied to all studies requiring animal housing in the SAIRF.

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 used 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 SAIRF 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 SAIRF 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 – Associate Professor of Medical Physics
- Brigitte Raabe – DVM, DACLAM
- Ellen Leiferman - DVM
- Ashley Weichmann - SAIRF staff
- Justin Jeffery – SAIRF manager

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UWCCC Small Animal Imaging and Radiotherapy Facility Major Equipment

Siemens Inveon microPET/CT

Faxitron Ultrafocus Digital X-Ray

Agilent 4.7T MRI & HyperSense C-13/N-15 Hyperpolarizer

Perkin Elmer IVIS

Fluoptics Fluobeam (NIR)

Fujifilm VisualSonics Vevo2100 LAZR

Perkin Elmer Wizard Gamma Counter

Abaxis VS2 Blood Chem Analyzer

Abaxis HM5 CBC Analyzer

MILabs SPECT^{6}CT^{UHR}

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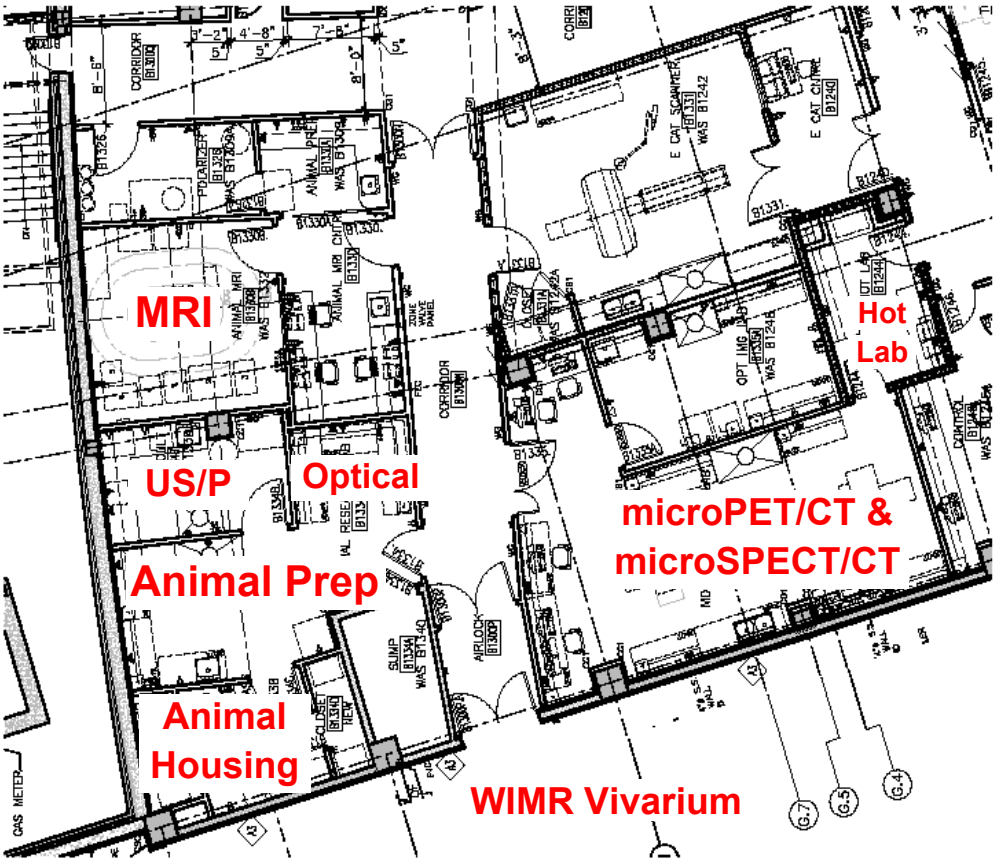
Xstrahl SARRP
(Precision Image-Guided Animal Irradiator)

Xstrahl CIX3

Xstrahl RS225 (Cell Irradiator)

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 Workstations: Four image analysis work stations.
MicroPET/CT: Siemens Inveon Hybrid microPET/CT
MicroSPECT/CT: MILabs U-SPECT^{6}/CT^{UHR} (ultra-high-resolution)
Optical: Perkin Elmer IVIS and Fluoptics 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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