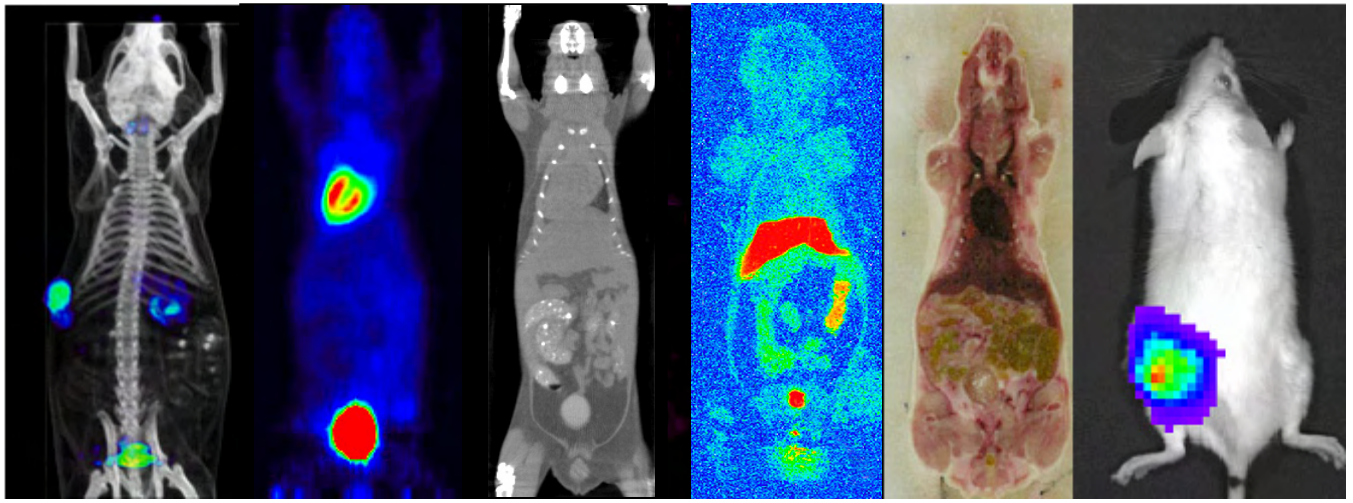
Preclinical Imaging Center

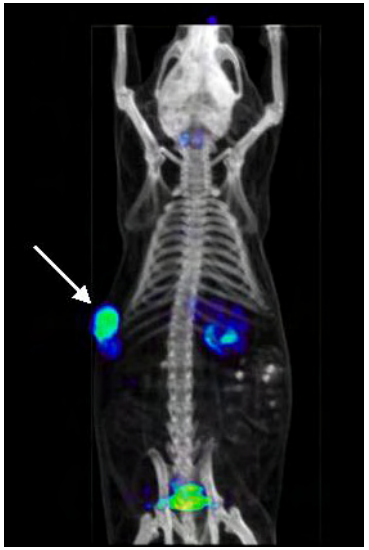


microPET-CT microPET microCT Autoradiograph Photograph Optical

- Physiology matters when imaging metabolism: temperature, fasting, stress, uptake time….
- Positioning and reproducibility important for long term studies
- Pathogen barrier for immunocompromised animals
- Anesthesia method: gas or injected
- Injection method may effect image probe distribution and timing

Convergent Domains

microPET



<u>Biology</u>
-Animals
-Genes
-Tumors
-Pharmacology

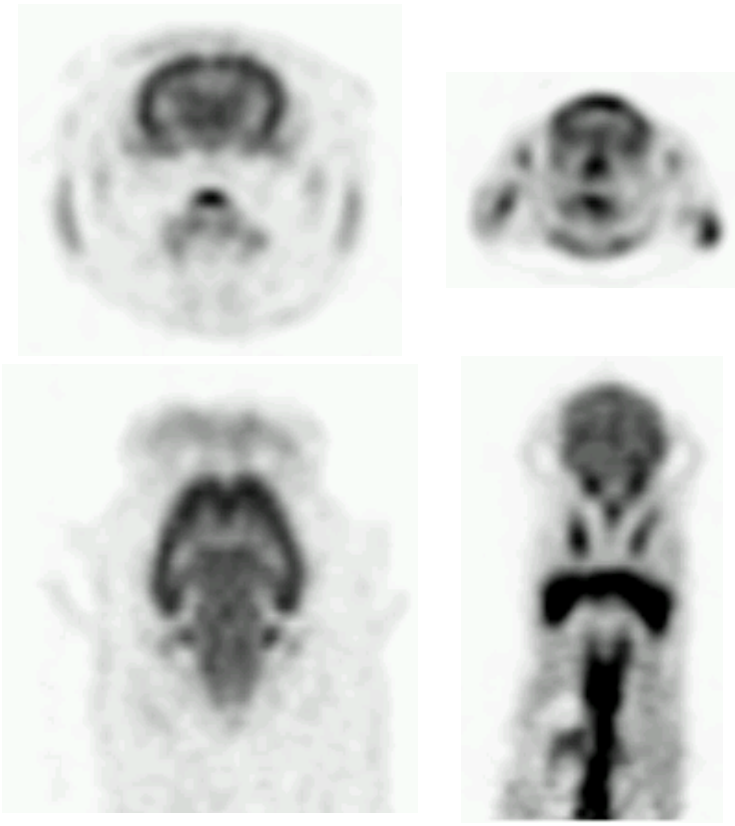
<u>Physics</u>
-Particle Accelerators
-Radiation detection
-Computation
-Image Generation

What Species to Use

Decision based on desired information:

- Disease model available
- Physiology matched to humans
- Structure size compared to resolution and sensitivity of imaging system
- Desired information (biochemical, behavioral, biodistribution)
- Number of measurements required (# animals)

Example showing mouse and rat brains imaged using microPET with FDG. Alzheimer's research would be extremely difficult in mice, but possible in rats.



Rat Head Mouse

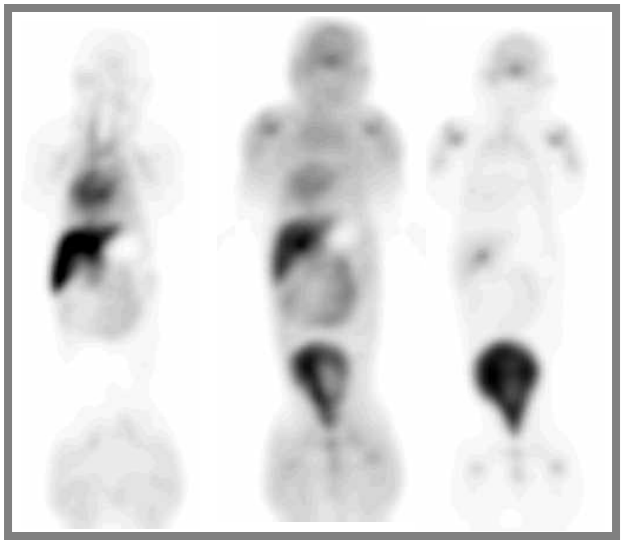
Larger Species

Advantages:

- Close to human behavior & physiology
- Dosimetry closely matched to humans
- Surgical interventions simpler
- Ability to draw larger blood samples
- Large structure size, good sampling

Disadvantages:

- Cost
- Handling
- Size (whole body only in steps)
- Safety (both from animals and protestors)
- Many species endangered



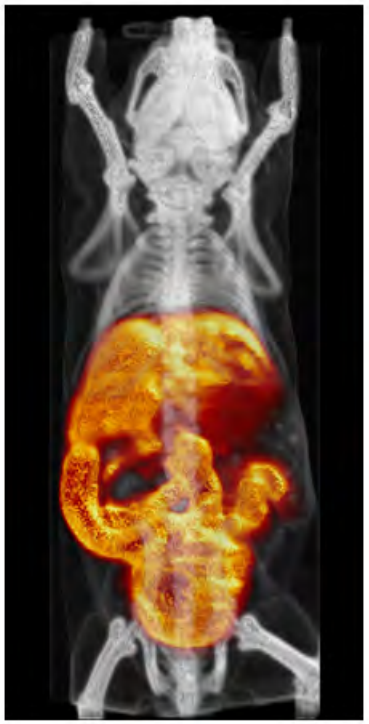
3 min 45 min 120 min

Monkey Dosimetry Experiment
Clinical PET system
New imaging probe followed over time

Smaller Species

Advantages:

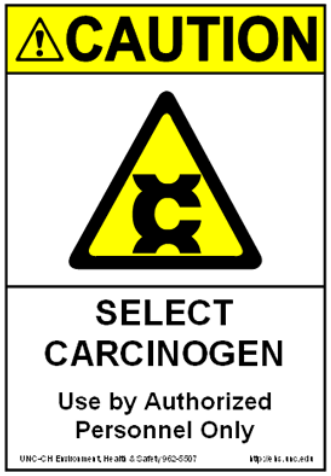
- Whole body imaging
- Inexpensive to purchase and house
- Easy to handle
- Low personnel radiation exposure
- Equipment required is small and easy to use
- Less scatter and attenuation
- Known genome
- Many genetically modified strains available
- Immune deficient strains for cancer research



Disadvantages:

- Blood sampling & Metabolite analysis
- Small size, hard to see structures
- Surgical intervention difficult
- May not match human physiology

Infectious & Carcinogenic Agents



- Need to observe early changes in immune system
- Research requiring repeated infections
- Use of chemotherapeutic agents for oncology research
- Confinement at procedure area, housing and imaging locations
- Infectious animal represent a threat to humans, thus we must protect ourselves from the animals. BSL 2, 2+ or 3 required.

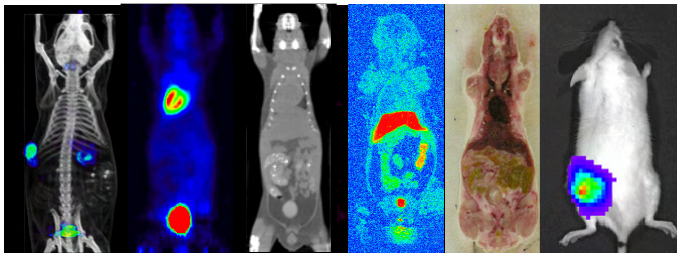
Immunocompromised Imaging

- Immunocompromised animals are missing part (nude) or all (SCID) of their immune system and must be protected from pathogen exposure. Requires BSL2-like conditions for imaging work.
- Used for oncology research, since they will not reject human tissue.
- Do not pose a risk to humans (injected substances may pose a threat). We pose a risk to the animals.

Tracer Detection

	Pros	*Cons*
Optical: Fluorescence Bioluminescence	Multiple photons/molecule (high sensitivity) Turns on/off	Not quantitative (in-vivo) Not applicable to humans Autofluorescence background with fluorescent imaging
PET, SPECT	Quantitative Human applications	Single signal/nucleus Radiation dose, Cost
CT	Anatomical information, bone density, PET attenuation coeff.	No metabolic information Poor soft tissue contrast, Radiation Dose
MR	No Radiation, High Resolution, anatomical information	little metabolic information, magnetic field, Cost,

What is being Imaged?



CT: Electron density

MR: Proton Density

Optical: Light making it out of the body

PET & SPECT: **Radioisotope** distribution

Things that can alter the signal pattern:

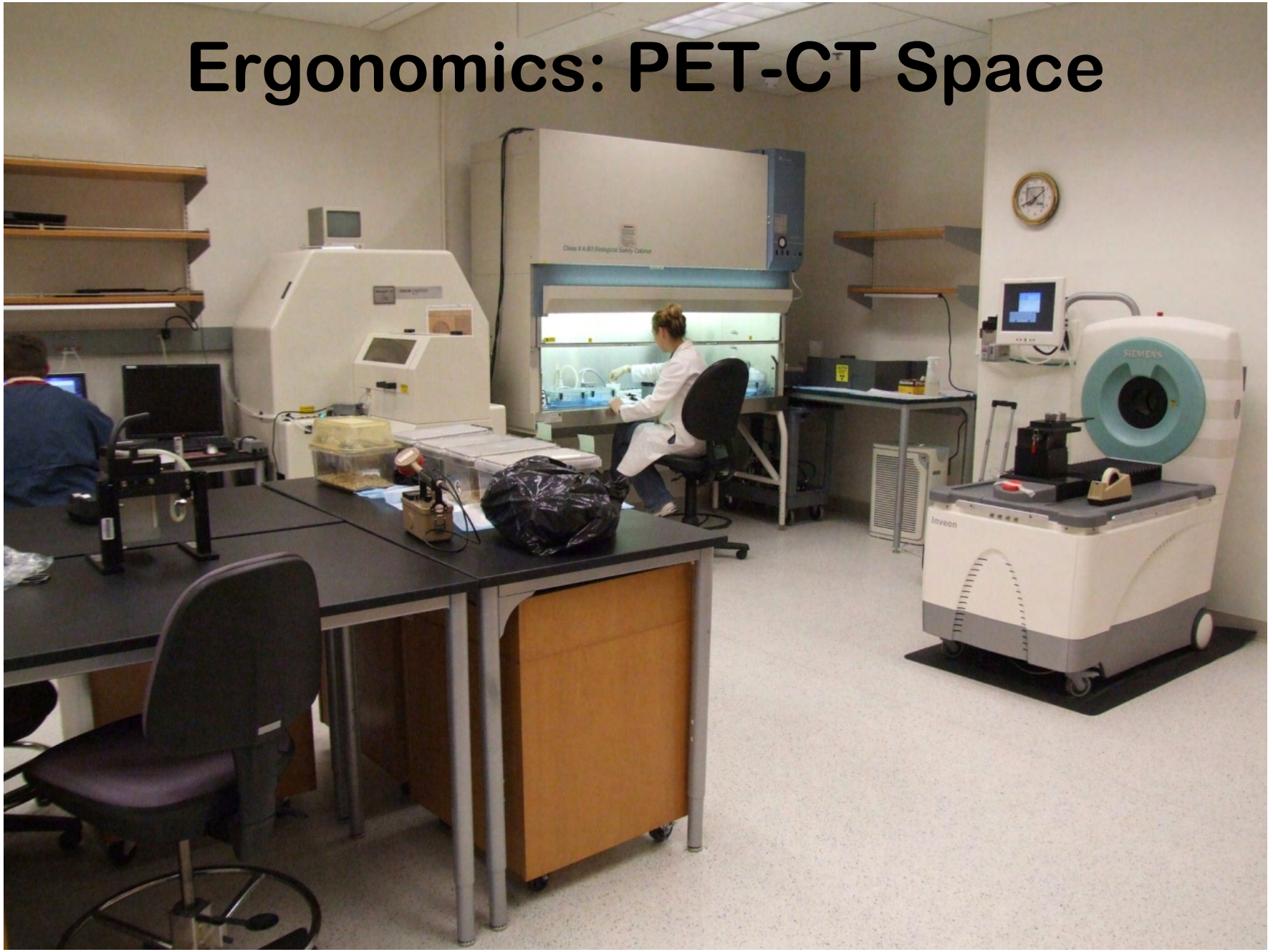
CT: movement, contrast agents, positioning, materials in the field of view (FOV), objects outside FOV

MR: movement, contrast agents, temperature

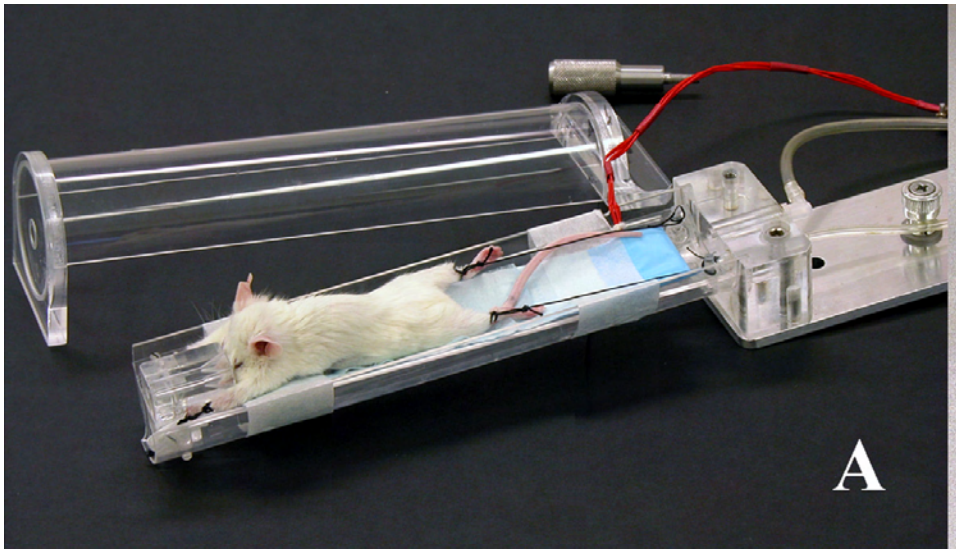
Optical: temperature, physiological state, time, metabolism, orientation of the animal, injection, time of day, anesthesia

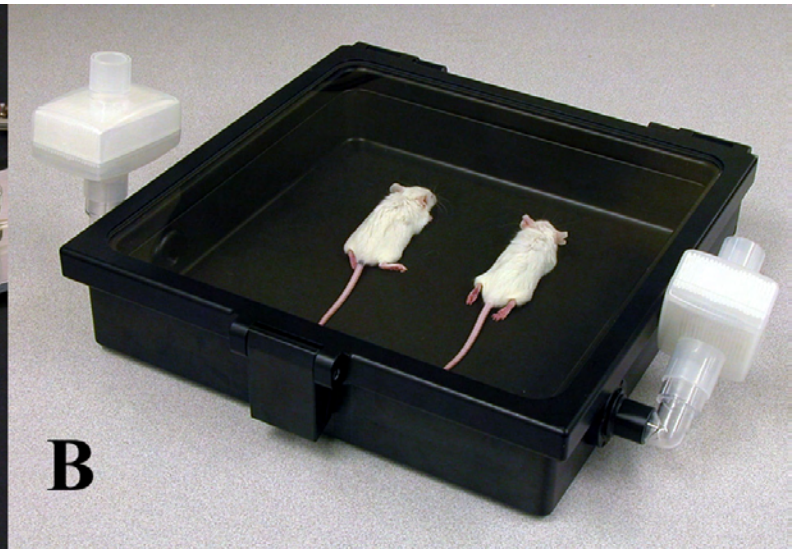
SPECT & PET: metabolism, elimination, time, movement, temperature, injection, anesthesia, positioning, endogenous competition, physiology

Ergonomics: PET-CT Space



Immunocompromised Imaging





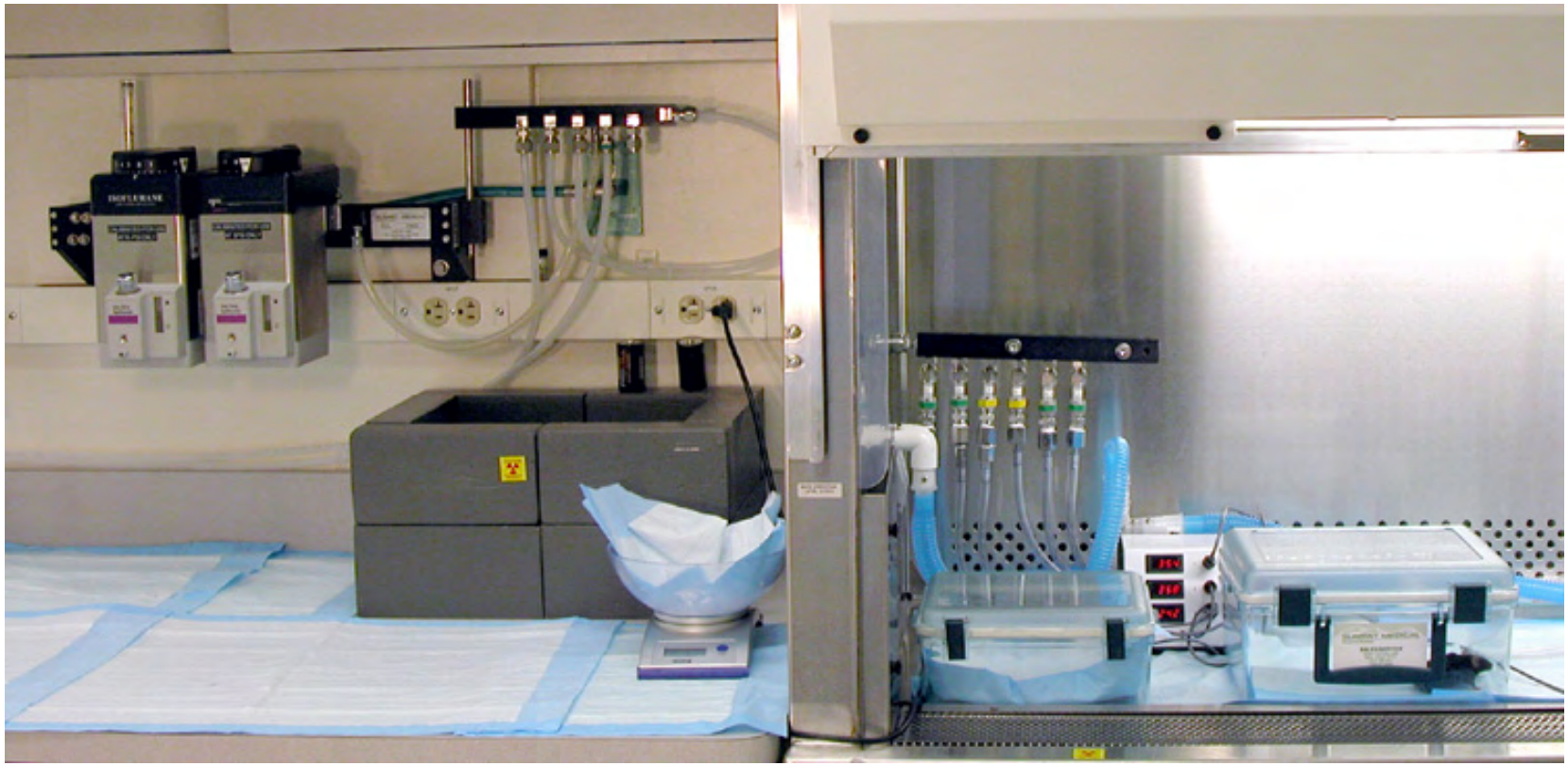
microPET-CT
Environmental Chamber

Optical Imaging
Environmental Chamber

The microPET-CT imaging chamber provides reproducible positioning, constant gas anesthesia, multi-modality imaging capability (PET, CT, MR), barrier conditions for immunocompromised mice and rats and temperature control.

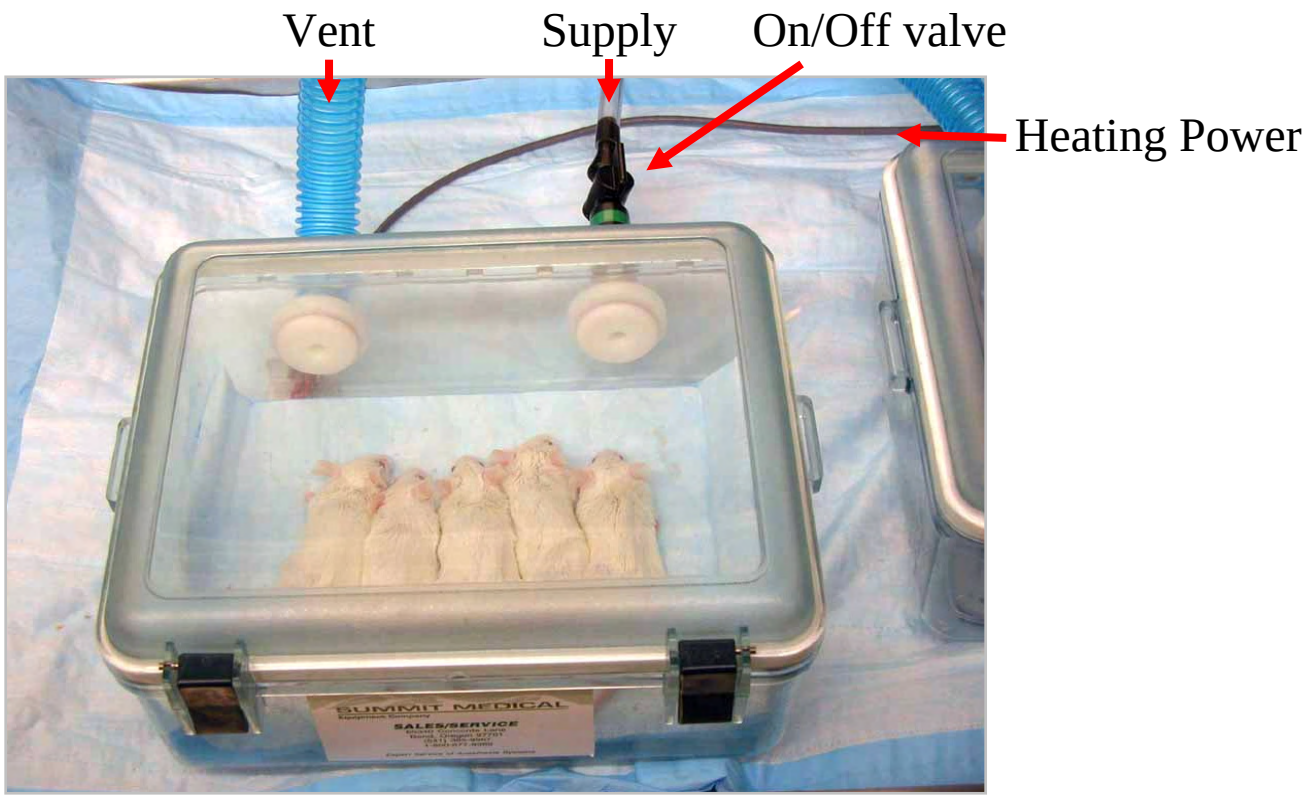
The optical chamber provides gas anesthesia and barrier conditions.

Anesthesia Support



Wall mounted gas anesthesia provides easy to use constant depth of anesthesia. Variable anesthesia can lead to movement, physiological changes altering blood flow, clearance and probe delivery.

Anesthesia Induction Boxes



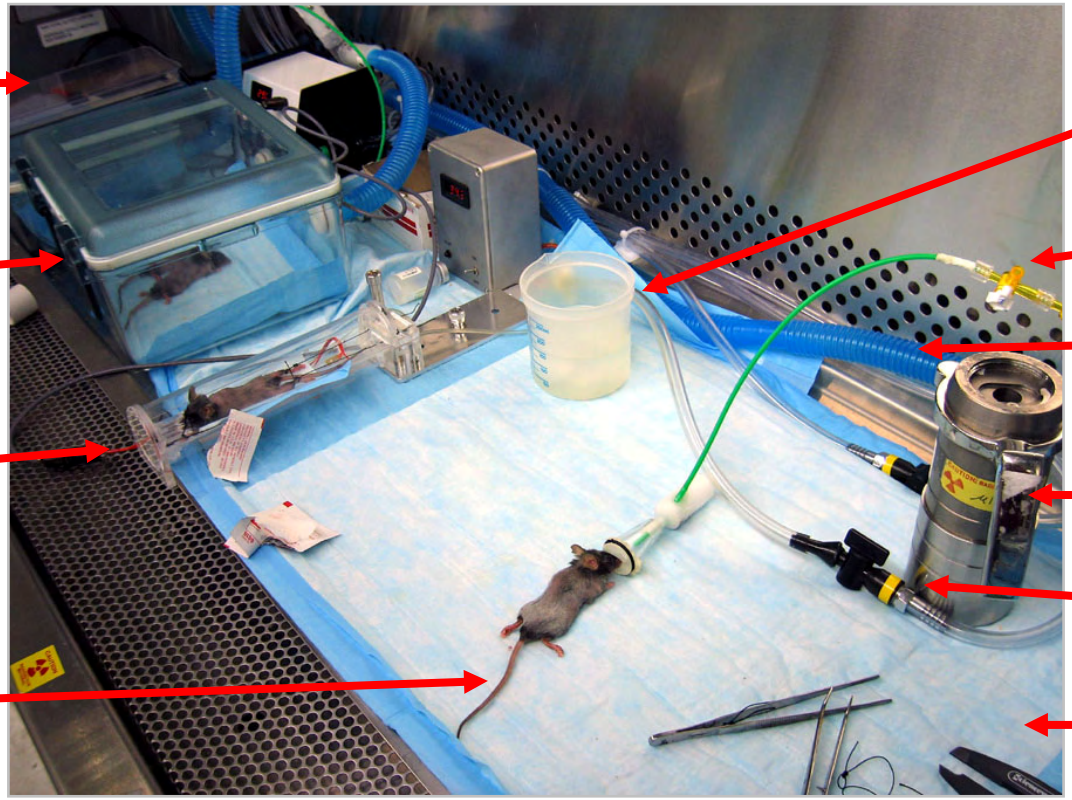
A fast, simple, easy to use box that can be used for multiple animals.

Anesthesia is delivered and removed via ports at the rear of the box.

Animals are kept warm by a heating plate located under the box.

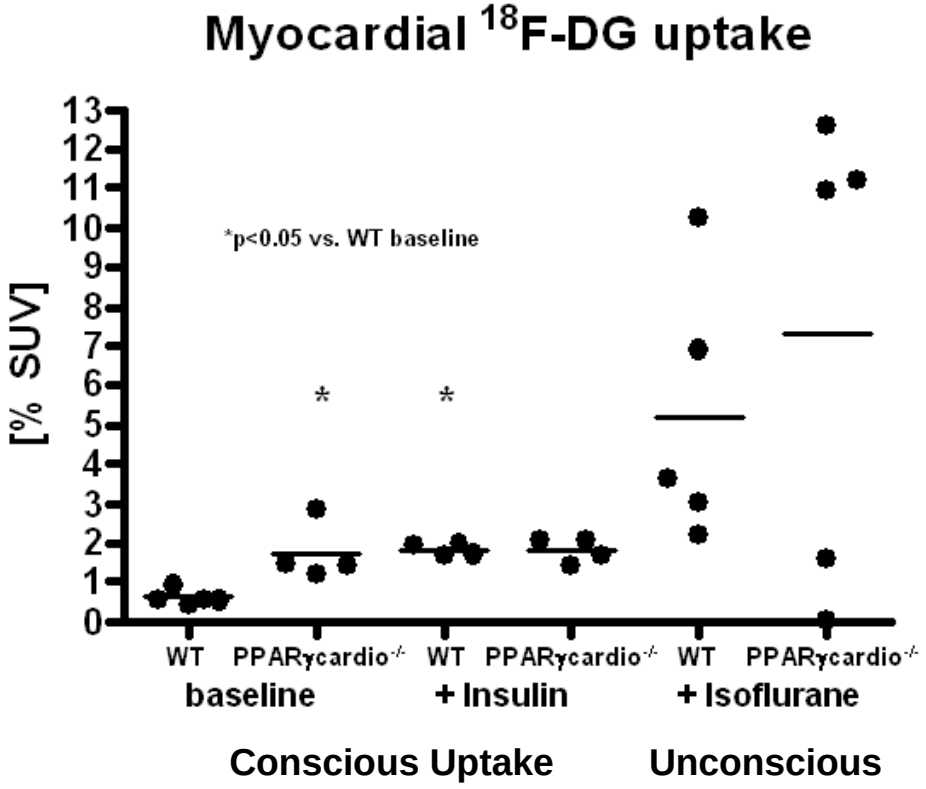
Easily sterilized using spray bottle containing Virkane of Trifectant.

Staging Mice

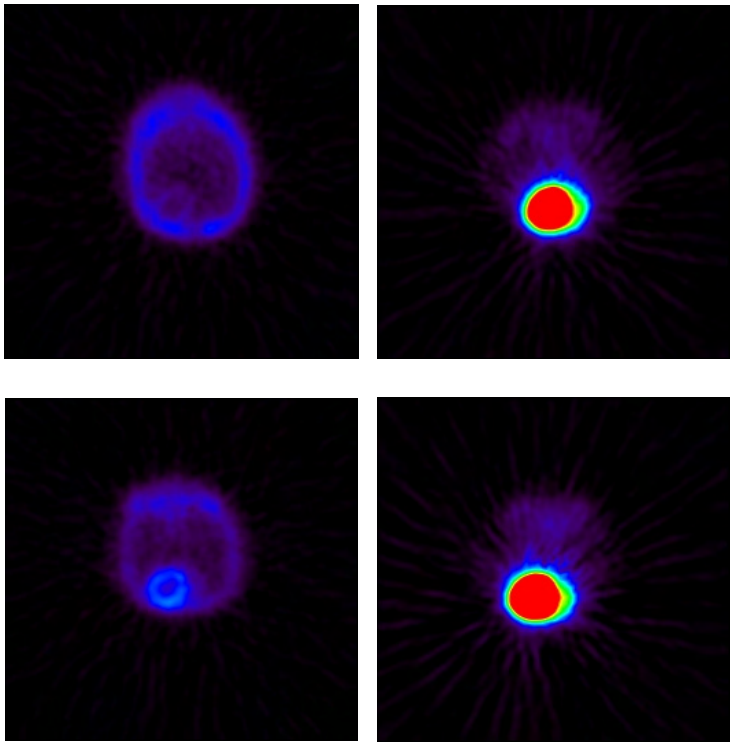


Usually several mice are undergoing injection, uptake and preparation for imaging at the same time. With the demands of knock-down, injection, chamber assembly, PET scanning, CT scanning and recovery happening in each 15 min block of time, the process needs to be simple and not require much interaction. Set-it and forget-it arrangements for heating and anesthesia are essential.

Probe Uptake: Anesthesia effects



Transverse View of Mouse Heart

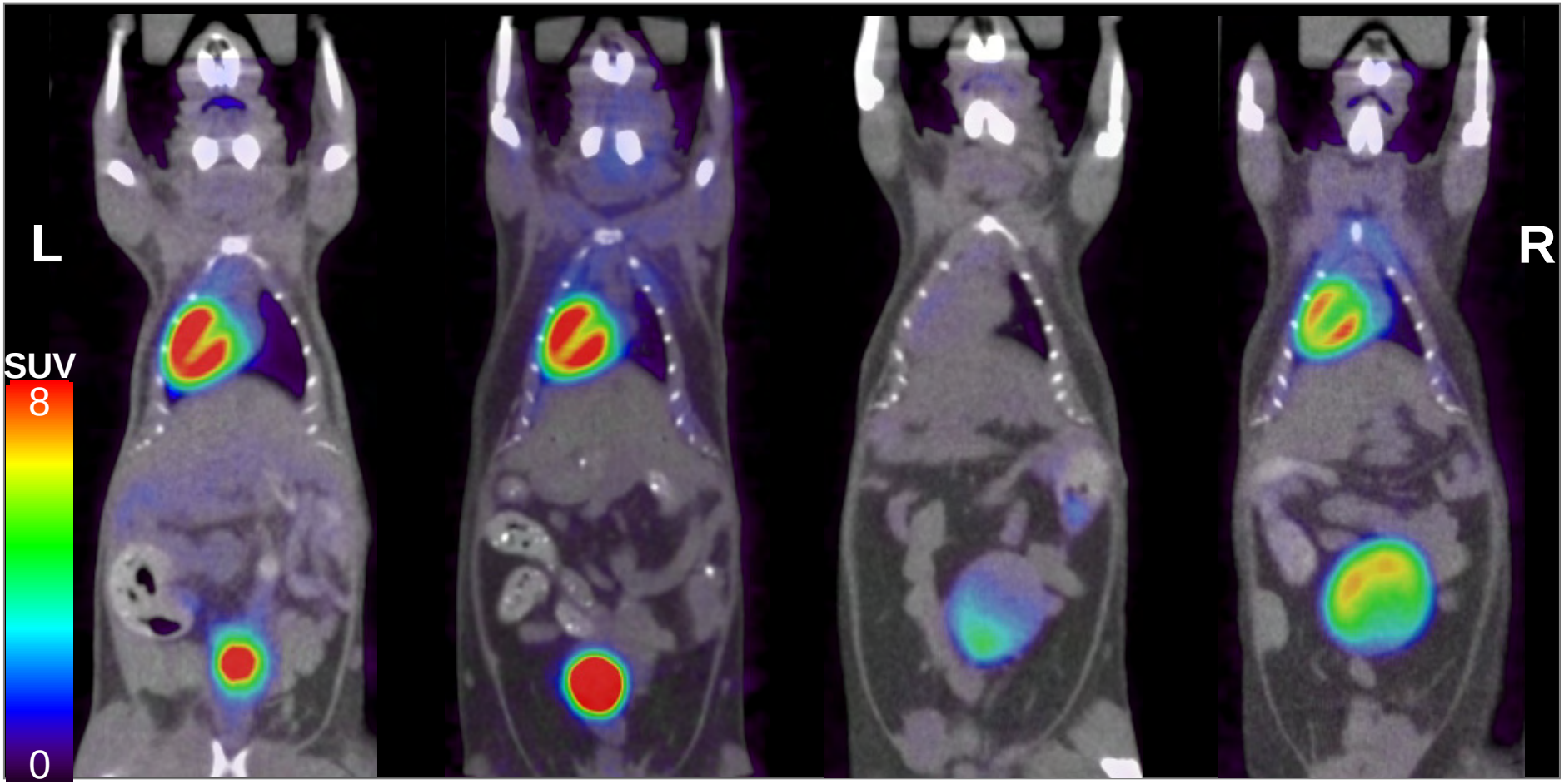


Conscious Uptake **Unconscious Uptake with isoflurane**

Anesthesia significantly alters physiology, such as heart rate and respiration. Animals need to be immobilized while imaging, however anesthesia can be given after injection. Keeping conditions reproducible and consistent helps ensure comparable data. In the example above, unconscious uptake resulted in variable SUV values.

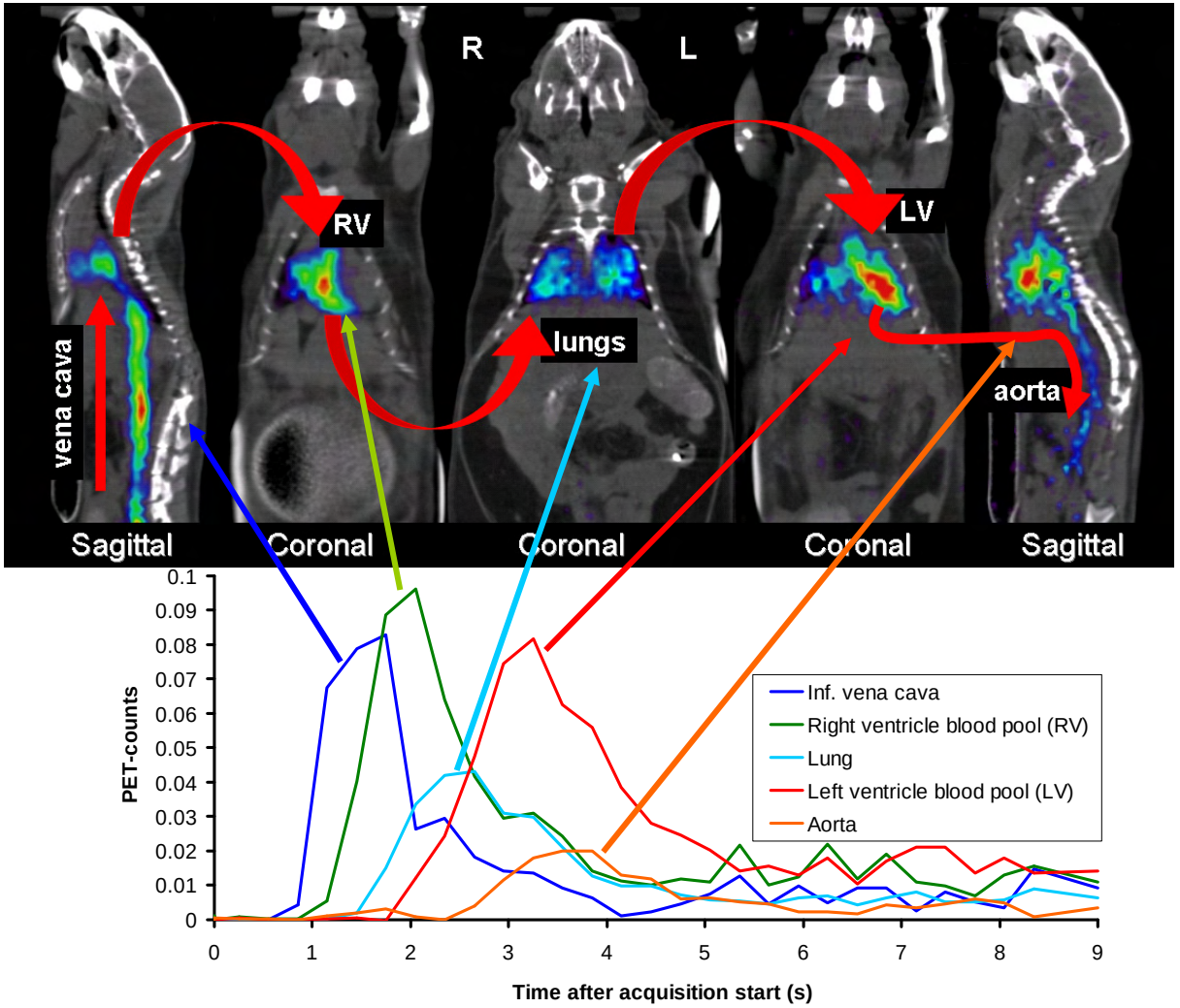
Glucose Effects on FDG

Nonfasted | Nonfasted & Insulin | Fasted | Fasted & Insulin



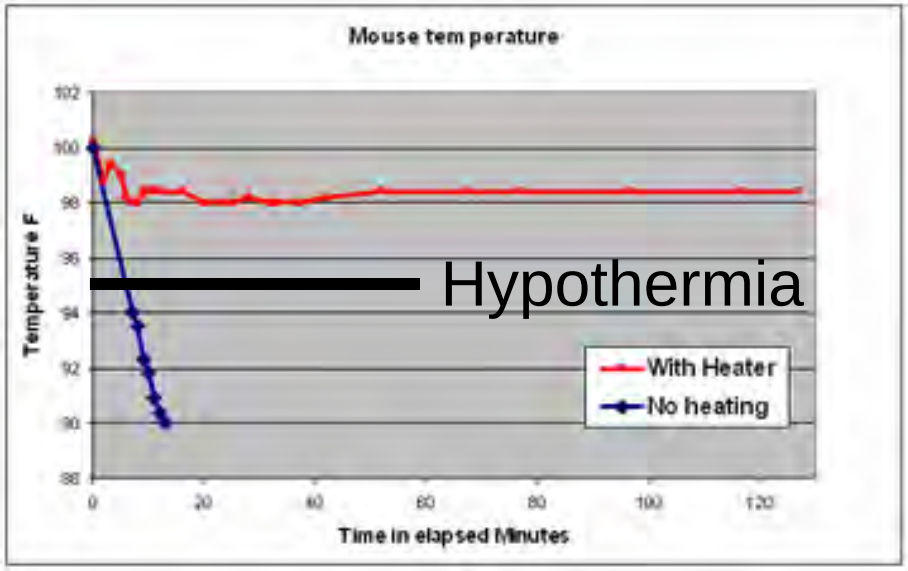
Endogenous glucose competes with FDG for tissue uptake. In the heart, and fatty acid versus glucose utilization plays a big role in myocardium uptake.

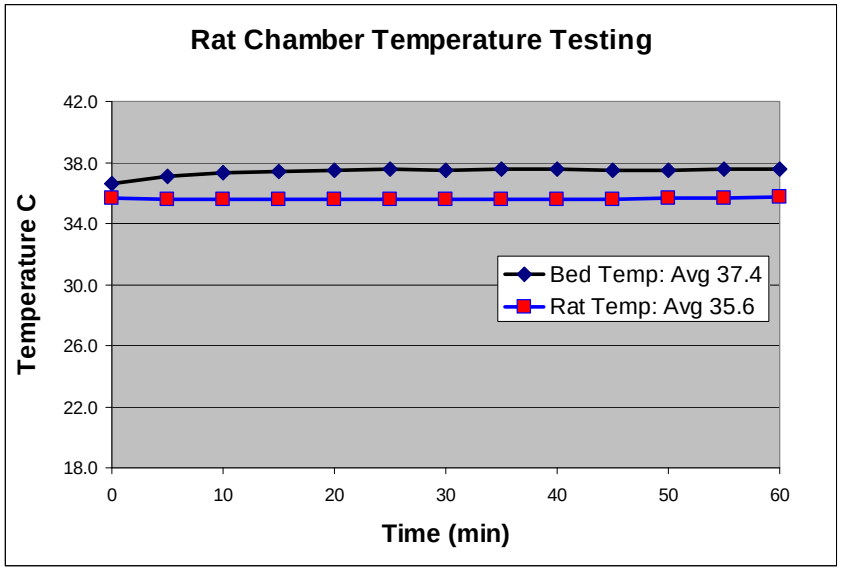
Fast Temporal Imaging: First past transit



First Pass transit of FDG Bolus in 35g Mouse (0.3 s/30frames)
Used to measure transit time and ejection fraction in live mouse

Temperature Control



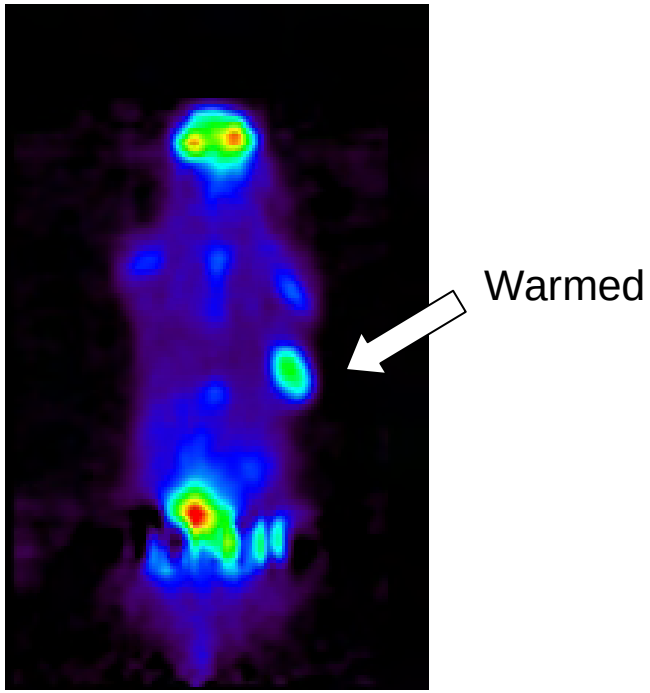


Mice and rats both quickly equilibrate to the sled temperature when under anesthesia. Regulated feedback control is essential to ensure that rodents are not overheated. Physiological tolerance for overheating is much less than colder conditions.

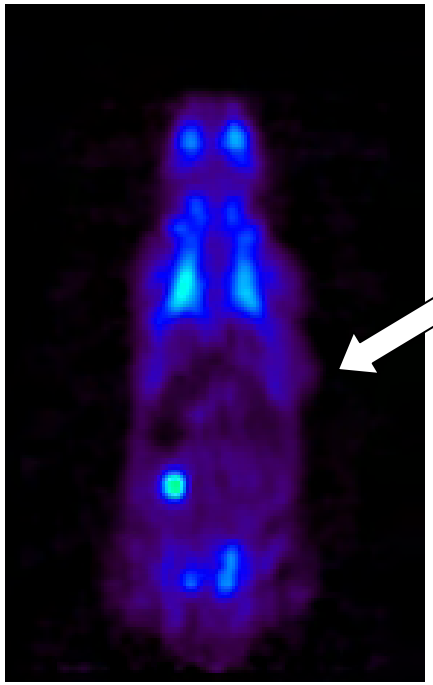
Heating is critical not just for brown fat uptake reduction. Animals quickly become hypothermic in the imaging center without heating and can easily die within minutes.

To measure normal metabolism, not metabolism under hypothermic conditions, the mouse must be warmed prior to, during and post injection.

Temperature Effects in Tumor Bearing Mice



Warmed



Not Warmed

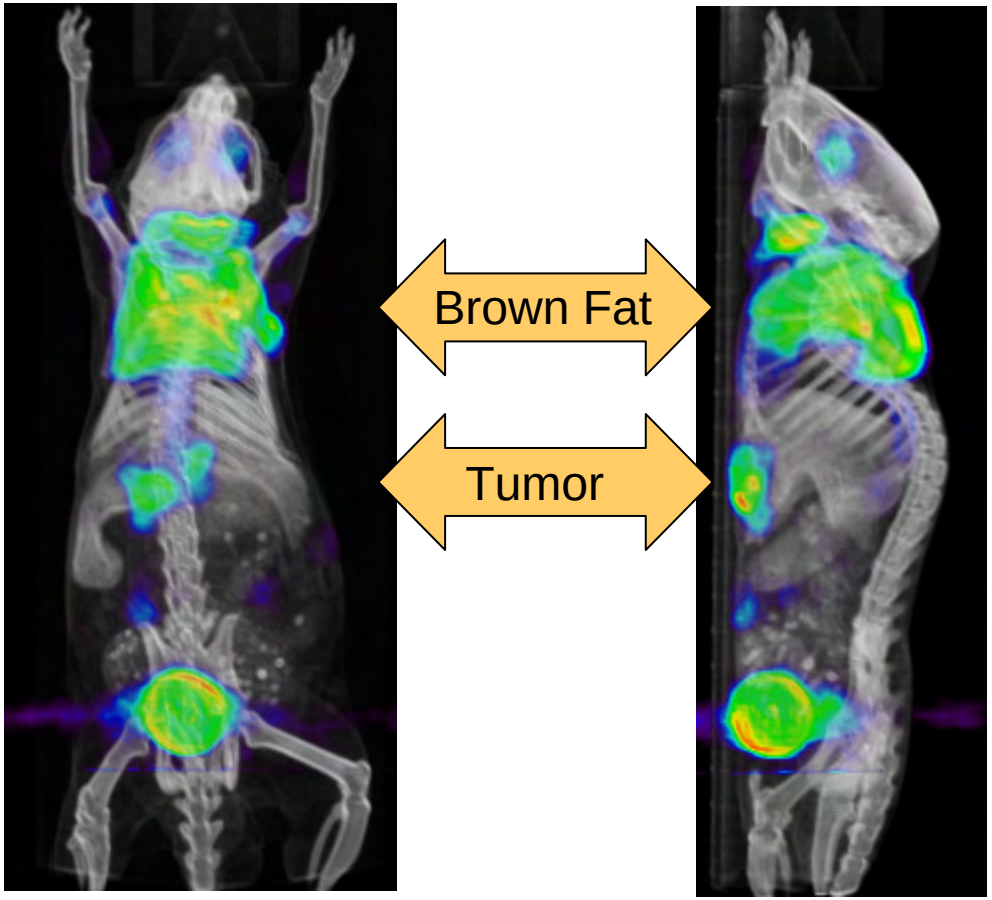
Temperature is the primary reason for the substantial tumor uptake difference.

Peripheral tumors are more likely to show temperature effects, since thermoregulation is in part controlled by blood flow to peripheral regions.

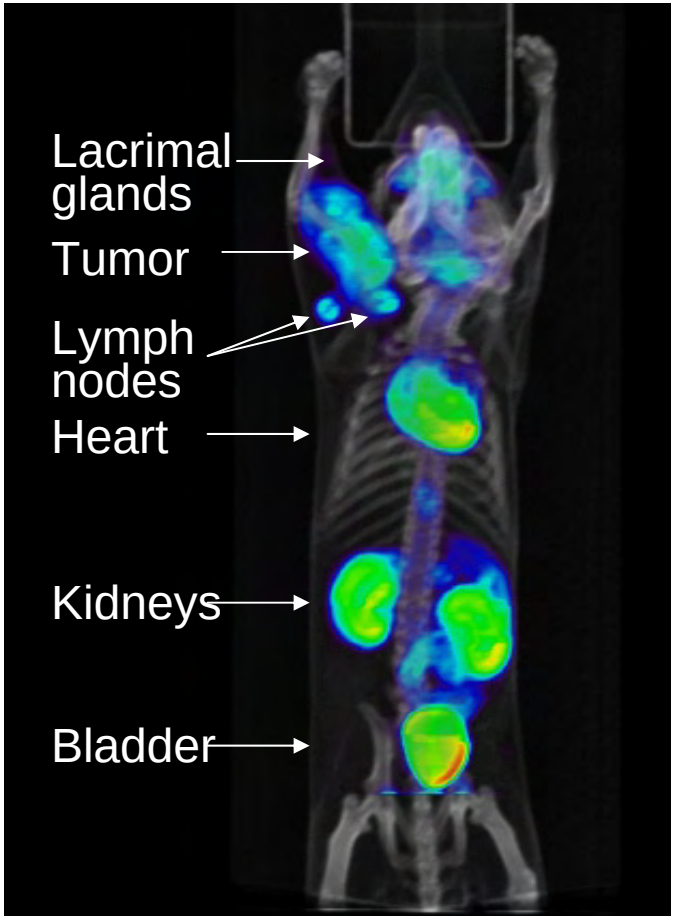
Variances in temperature between different imaging sessions could mask changes due to tumor growth or interventions.

Courtesy of B. Fueger & W. Weber

Brown Fat Uptake



Conscious uptake without heating in tumor bearing mouse

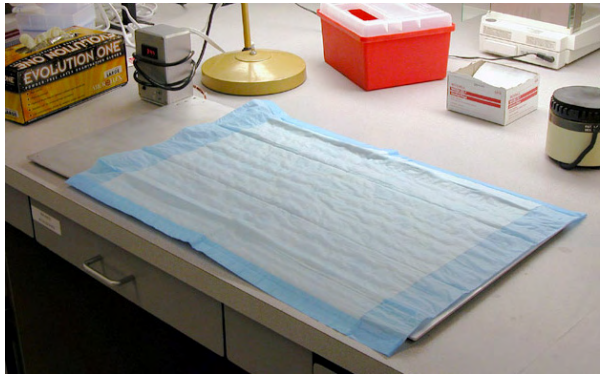


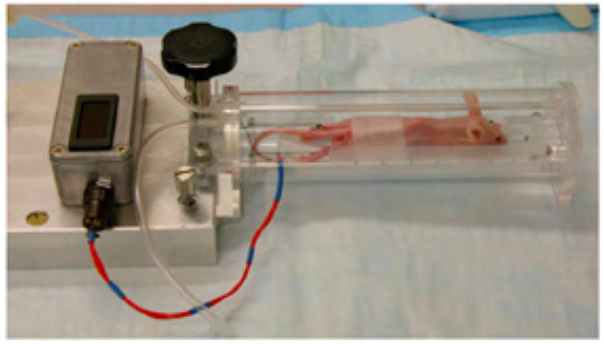
Unconscious uptake with heating

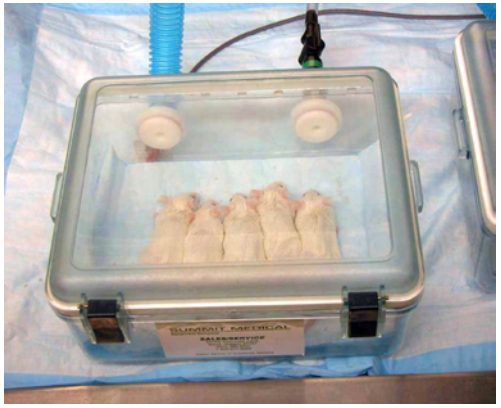
Cold animals compensate by activating brown fat, a highly metabolically active tissue that can mask nearby FDG signals.

Courtesy of H-J. Li & H. Herschman

Heating Options



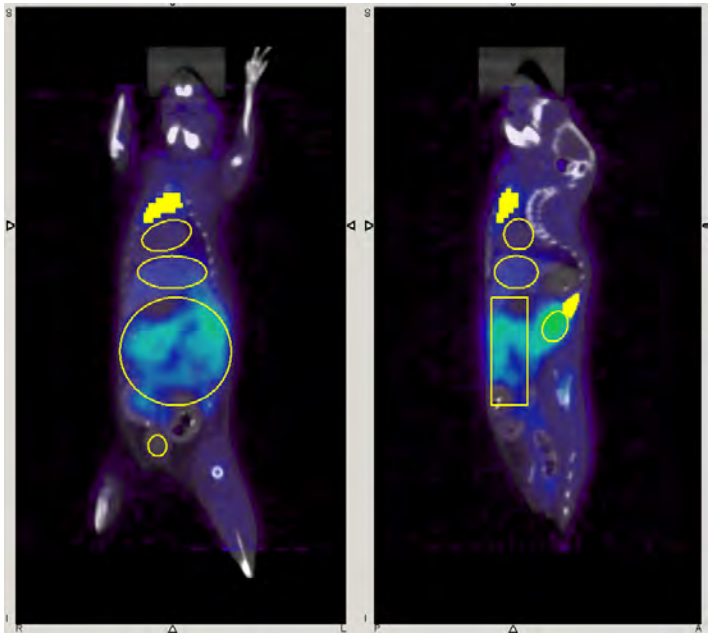


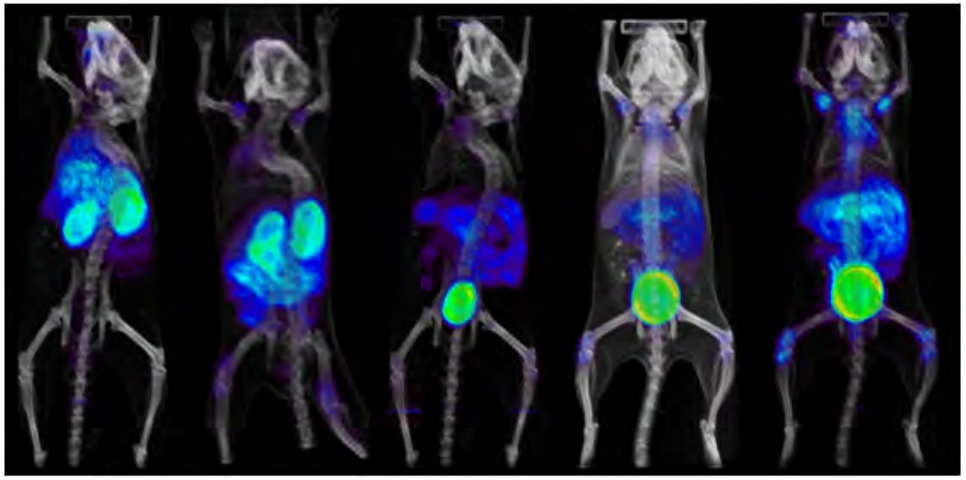


Various heating systems have been employed to keep the animals warm during all steps of the imaging process.

Recent work has shown the importance of preheating the animals prior to injection, therefore we have located heating plates wherever animal cages or animals are located, including the shielded recovery area.

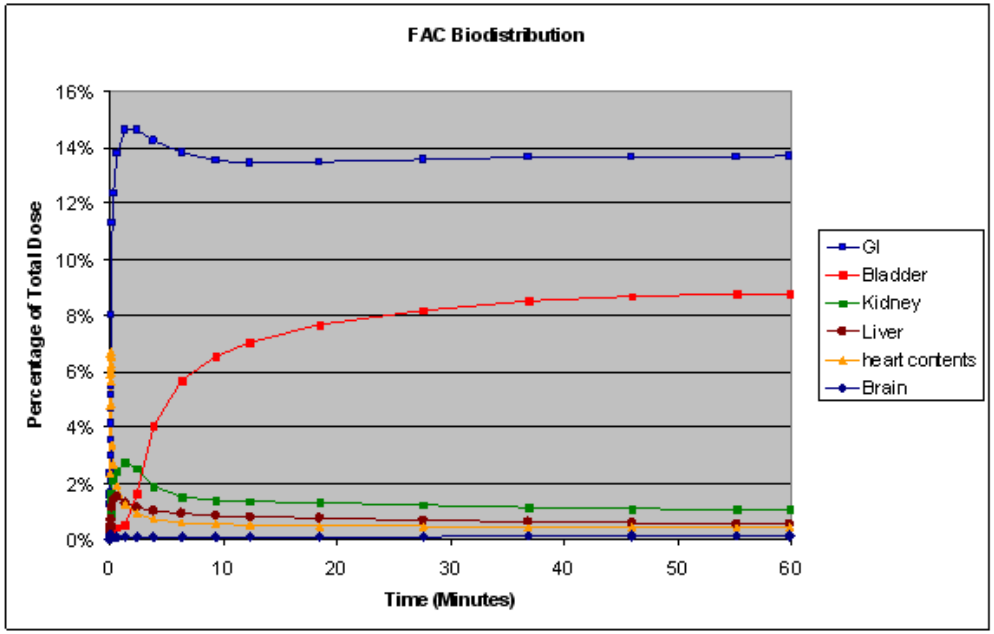
Biodistribution



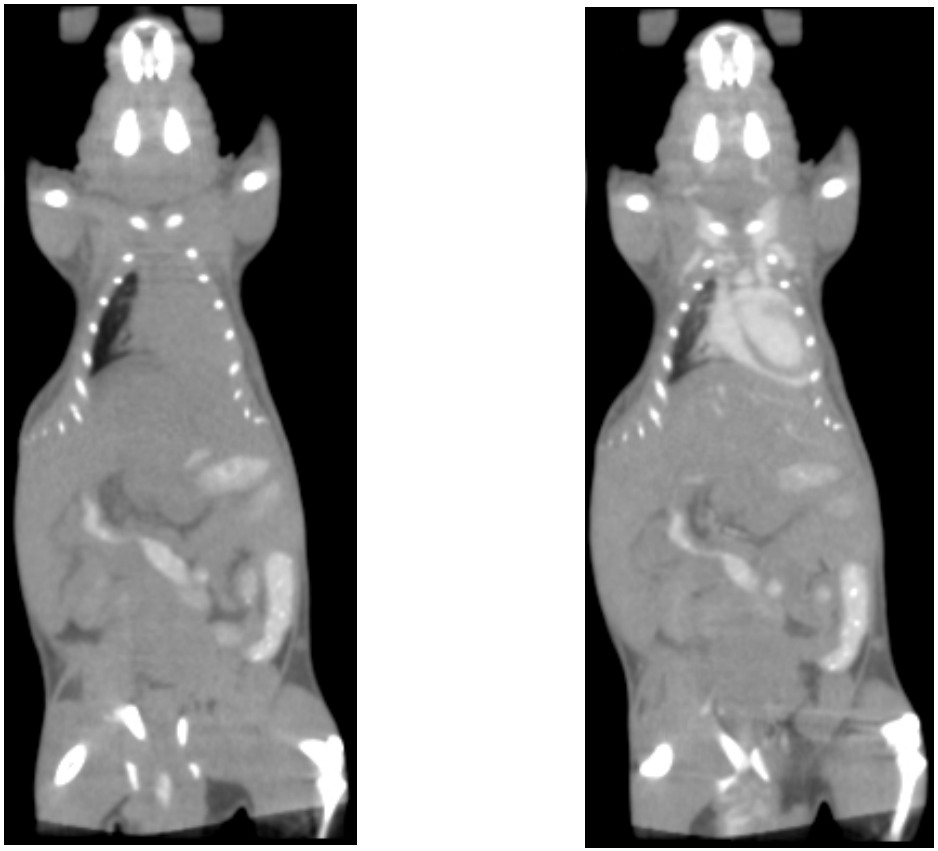


Biodistribution data can be altered by changes in blood flow, temperature, diet, positioning, anesthesia and other factors.

Experimental design should take these factors into account and optimize conditions and measure or track variables, such as glucose.



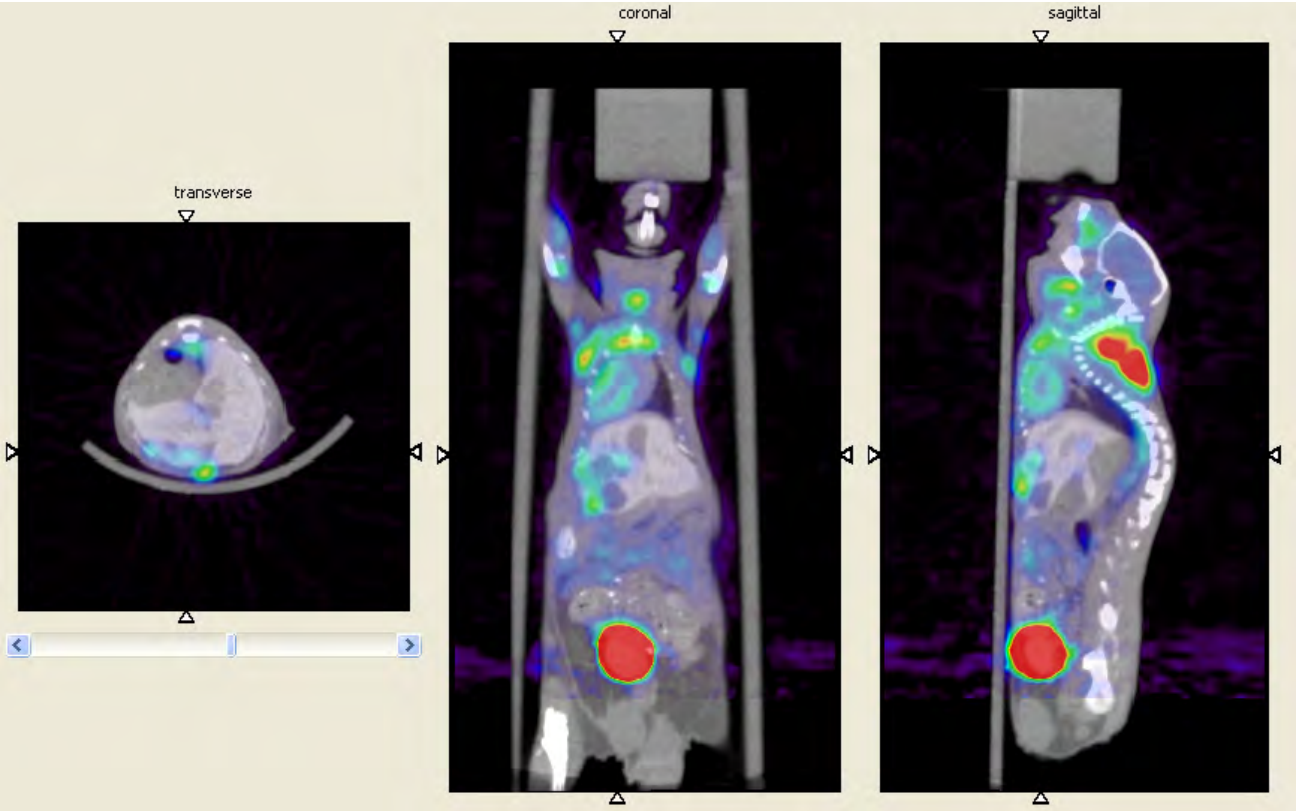
Imaging with Fenestra VC



This vascular contrast agent stays in the blood for 3-4 hours.
Although no longer available, another similar compound is now on the market called Exia.

Courtesy of Dr. Kreissl

Imaging with Fenestra LC: Liver Contrast



Liver and spleen would have the same image intensity as soft tissue without contrast and could not be separately distinguished. In this example, liver and spleen are visible, along with implanted tumors in the liver. Note the heterogeneous FDG uptake in the tumors, which are only clearly visible in the CT images.

Courtesy of H-J. Li & Herschman

Circadian Effects

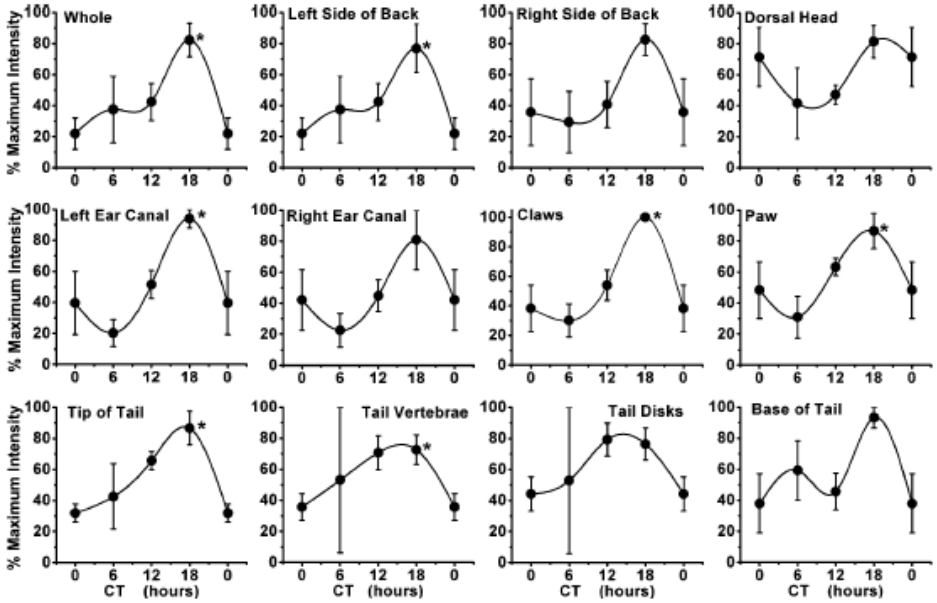


Fig. 3. Circadian bioluminescence rhythms in Hr-CMV mice maintained on running wheels. Average intensity of the 12 brightest body areas during four quadrants of the circadian cycle after normalizing intensity to the maximum in each of five mice. Error bars are standard error. *Significant by Friedman's test ($p < 0.05$).

MOLECULAR IMAGING AND BIOLOGY

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ORIGINAL ARTICLE

Circadian Regulation of a Viral Gene Promoter in Live Transgenic Mice Expressing Firefly Luciferase

Anne M. Collaco, MS,[1] Sima Rahman, BS,[1] Edward J. Dougherty, MS,[1] Brett B. Williams, BS,[1] Michael E. Geusz, PhD[1,2]

Time of day effects Luciferase expression.

Chrono-tolerance

Circadian Rhythms of the Mouse

Burghart Jilge and Emanuela Kunz
Laboratory Animal Research Unit, University of Ulm, Germany

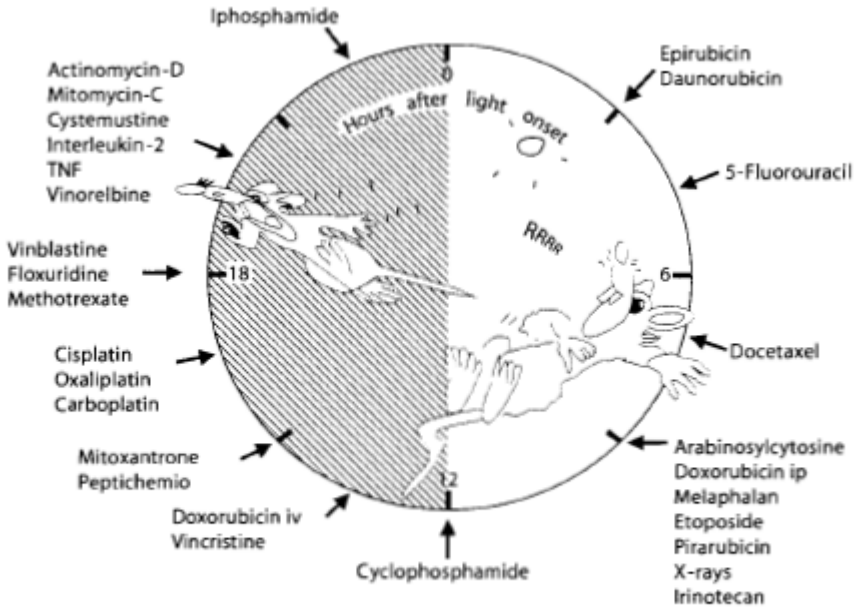


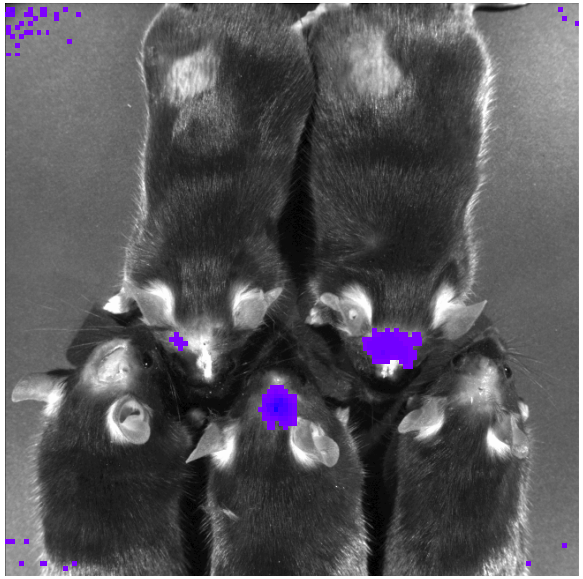
Figure 20.8 Circadian chronotolerance for some cytostatic drugs in rats and mice living in a regular LD 12 : 12. The time of highest tolerance (lowest mortality) is indicated by an arrow (reproduced from Lévi, 2002).

Radio-tolerance

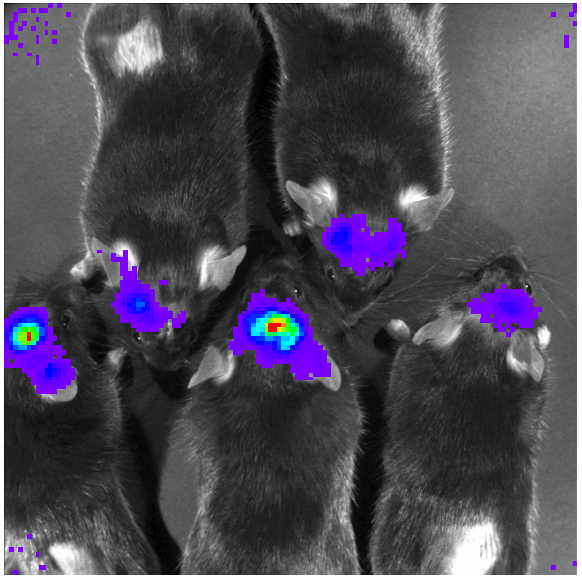
A single dose of 5.5 Gy radiation at mid dark cycle is 100% lethal in BalbC mice
Same dose at end of light cycle cause no mortality

Imaging of B16-fLuc in the CNS

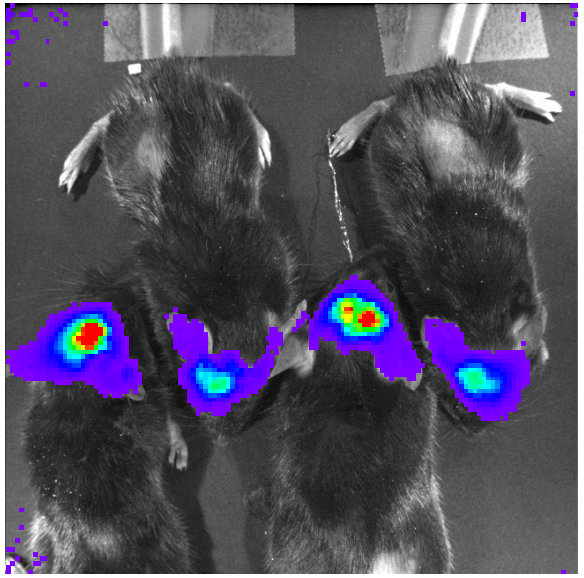
Day 8



Day 14



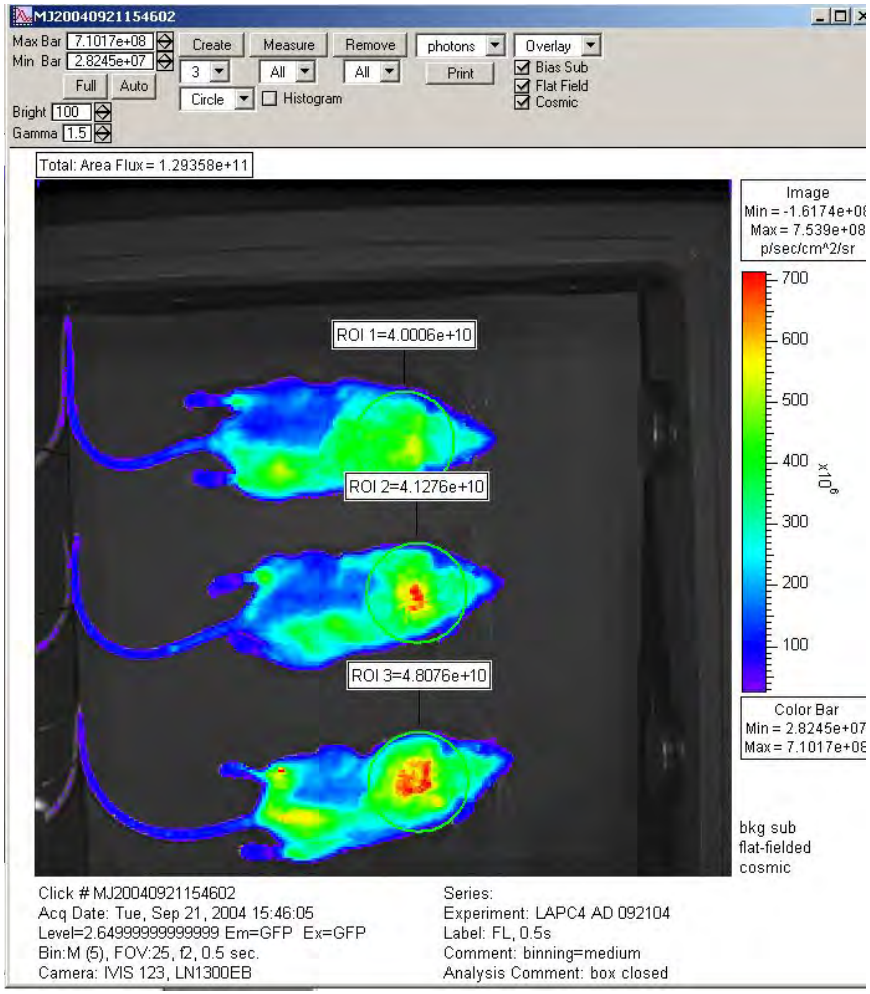
Day 20



Yes, you can image the brain using bioluminescence optical imaging in mice

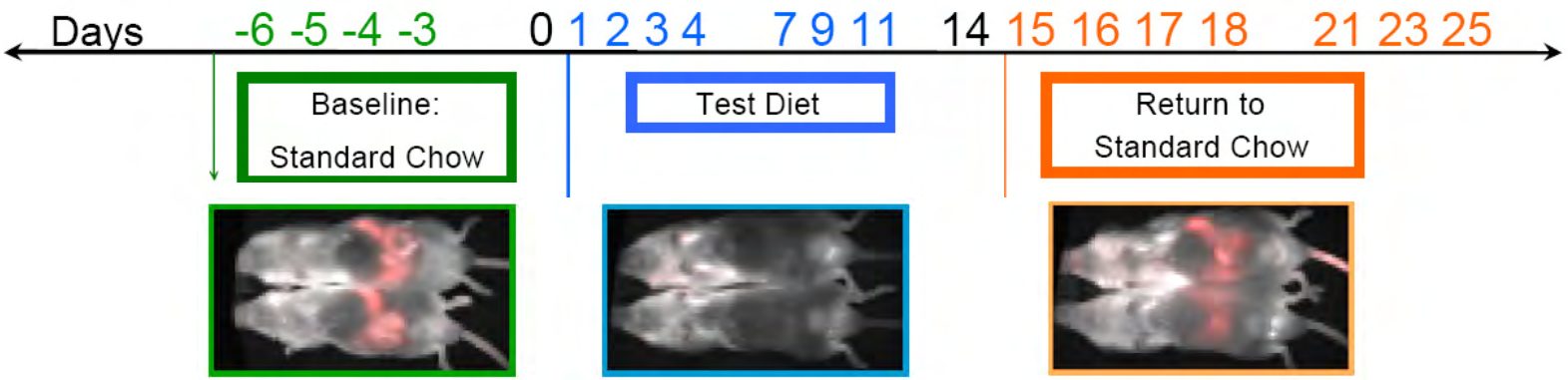
Bioluminescence

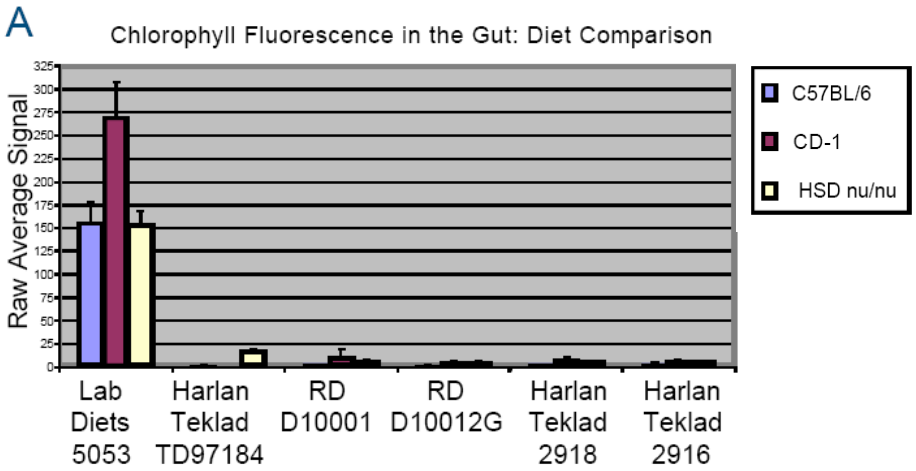
Fluorescence

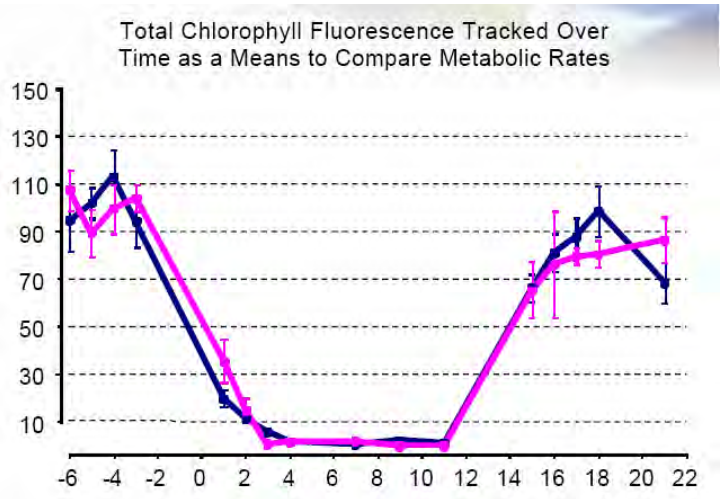


Effects of hemoglobin, fur and skin

Effect of Diet
Tracking Skin and Food Autofluorescence:

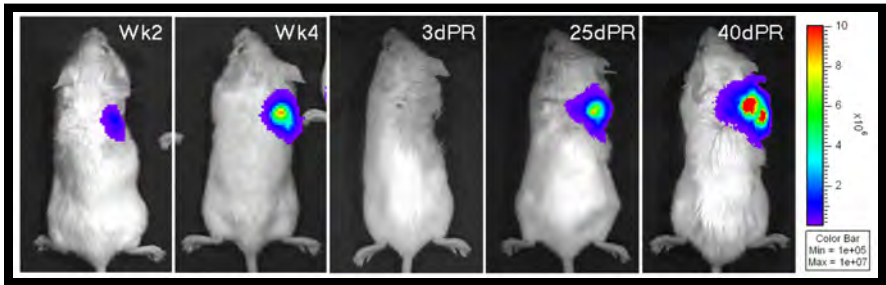






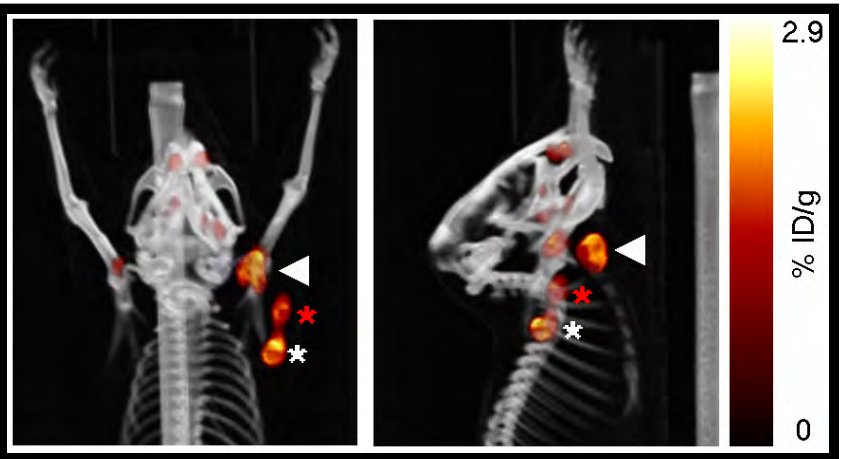
Reduction of Skin and Food Autofluorescence in Different Mouse Strains through Diet Changes
Sarah A. MacLaurin, Matthew Bouchard, Peter Dwyer, Richard Levenson, James Mansfield and Thomas Krucker
Novartis Institutes for BioMedical Research, Discovery Technologies, Cambridge MA, USA; CRi Inc. Woburn, MA, USA

Adenovirus Validation in Lymph Node Metastasis

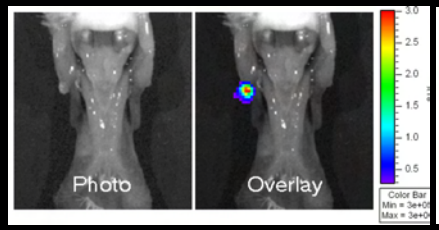


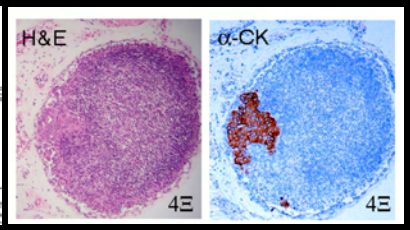
Pre-Resection | Post-Resection

A: Bioluminescent images of primary tumor and metastasis following resection

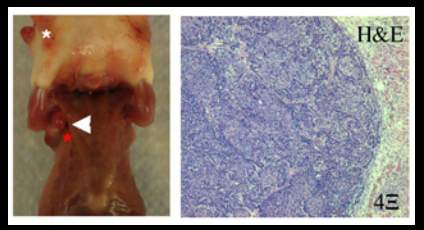


D: FLT microPET-CT images of lymph node tumor metastasis



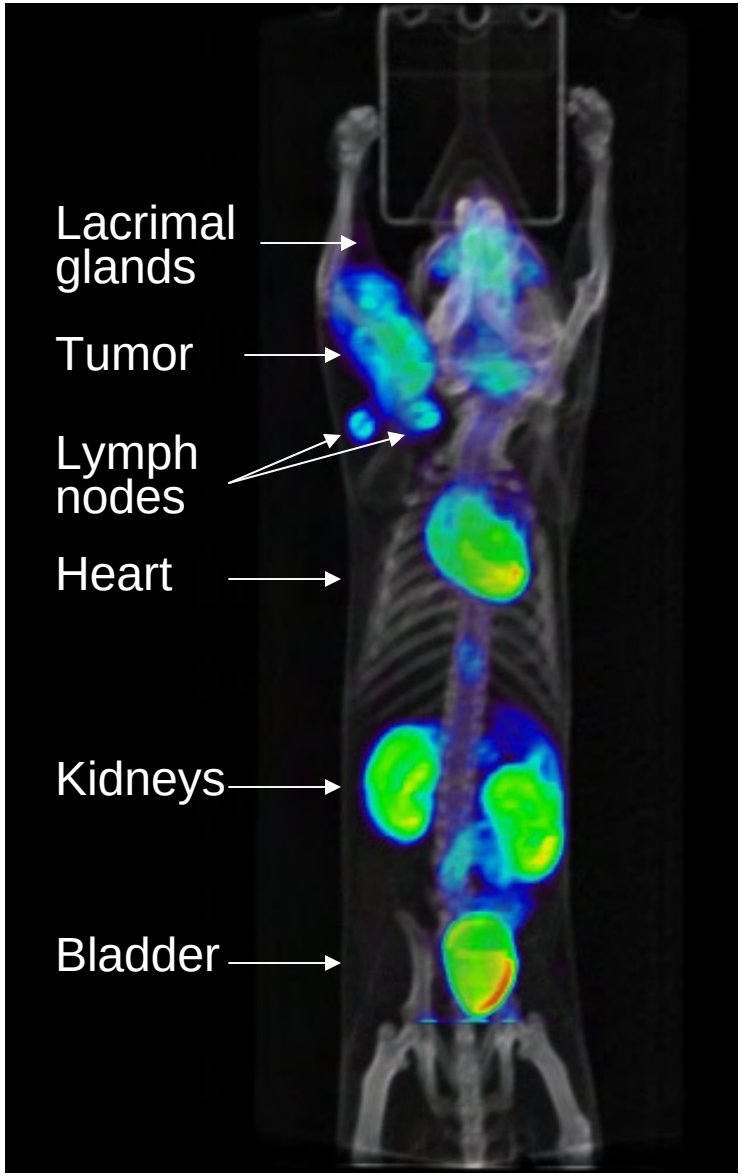


B: Optical ex vivo imaging C: Histology



E: Histology staining after PET imaging

Optical bioluminescence imaging followed primary tumor, then lymph node metastasis following resection. FLT microPET imaging shows localization of DNA synthesis, with histological follow-up.



microPET-CT FDG projection image of tumor bearing mouse

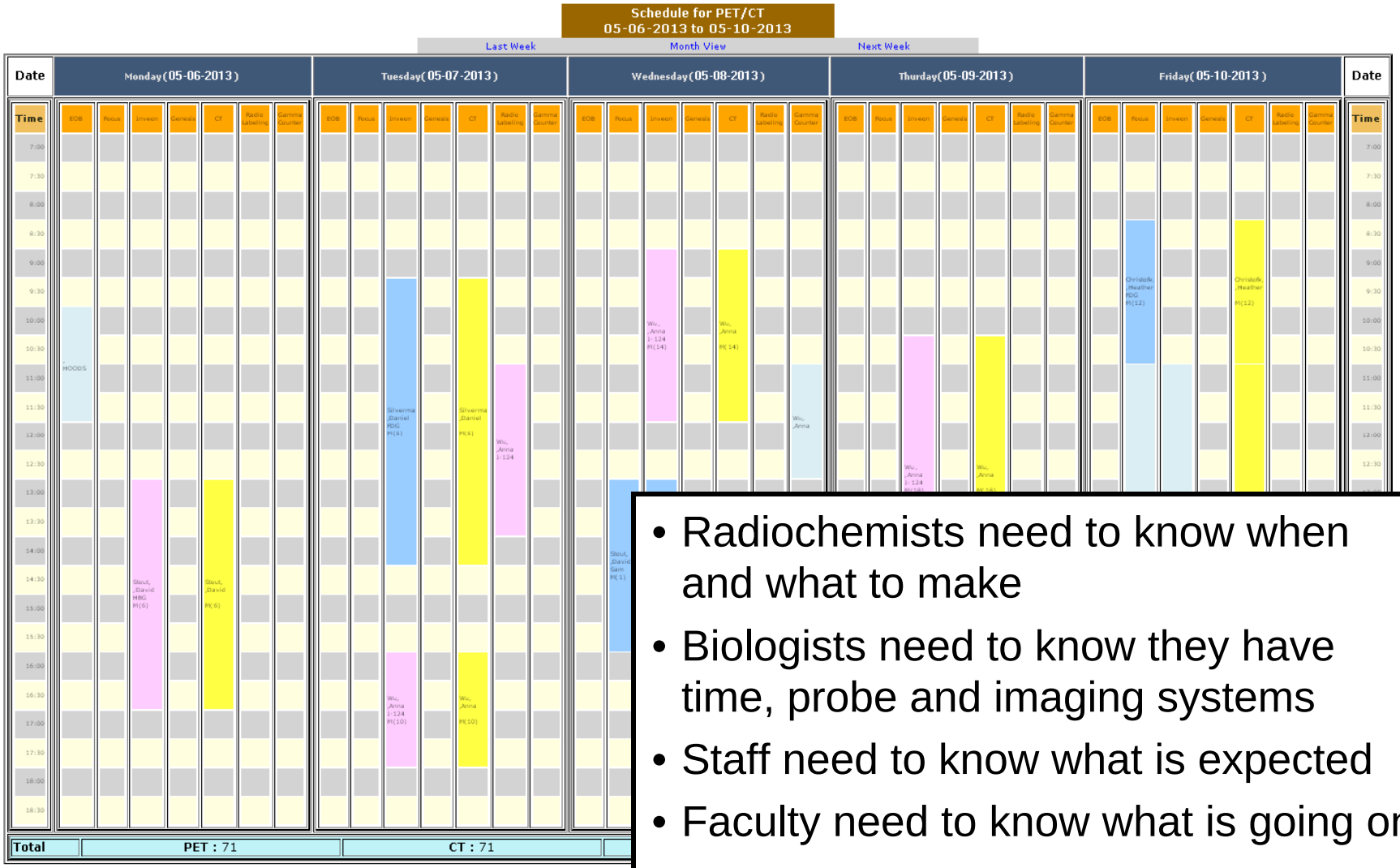
Getting it right

- To make the best use of animals, time and resources, it is important to control, measure and understand parameters that effect the distribution of the probe.
- Animals should be kept as close as possible to normal physiological conditions.
- Brown fat and hypothermia can be controlled by proper heating.
- Reproducible positioning can help produce comparable images and aides data analysis.
- Maintaining a barrier around immunocompromised animals ensures good health of the animals.
- Gas anesthesia is a safe, effective method that lasts as long as needed, has quick recovery and rarely results in loss of the animals.

Database & Image Management

An easy to use image archiving strategy is essential. Database must include all relevant data related to the experiment, both for the investigator's subsequent image analysis and for reports generated for various regulatory agencies and grant reviews.

Scheduling



- Radiochemists need to know when and what to make
- Biologists need to know they have time, probe and imaging systems
- Staff need to know what is expected
- Faculty need to know what is going on

Scheduling

- Currently one cyclotron facility supports two research areas and the nuclear medicine clinic
- Coordination of radiochemistry essential
- Weekly meeting in chairman’s office Tuesday morning to set the schedule
- Time on schedule is guaranteed, additions or changes discouraged
- Sometimes biology and radiochemistry do not cooperate

Scheduling

- Biologists want probes mid morning, since imaging and post imaging work can exceed 8 hours
- Uptake and non-specific clearance times range from 1-4 hours for most F-18 probes; potential long wait
- Circadian rhythm effects can alter uptake, so imaging should be at a reproducible time
- Delivered dose needs to be safe for injection and in a small volume, typically 1-3 ml
- Radiochemists & cyclotron operators need time to make isotopes and probes
- Need to run QC tests before probe is used, would like to use larger volumes, DMSO, MeOH, EtOH, etc. which are not safe to inject

Crump Preclinical Imaging Technology Center

- Cyclotron
- Radiochemistry
- Radiolabeling
- Vivarium
- PET/CT Imaging
- Optical Imaging
- Autoradiography
- Research Space

Let’s go see the area and what is happening today