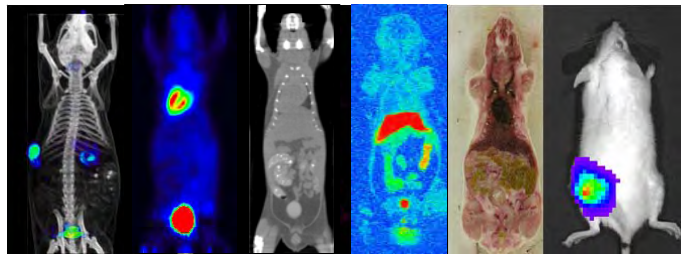
Part 2 Topics

- Experimental Design Considerations
- Creating Images & Archiving
- Imaging Display & Analysis

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

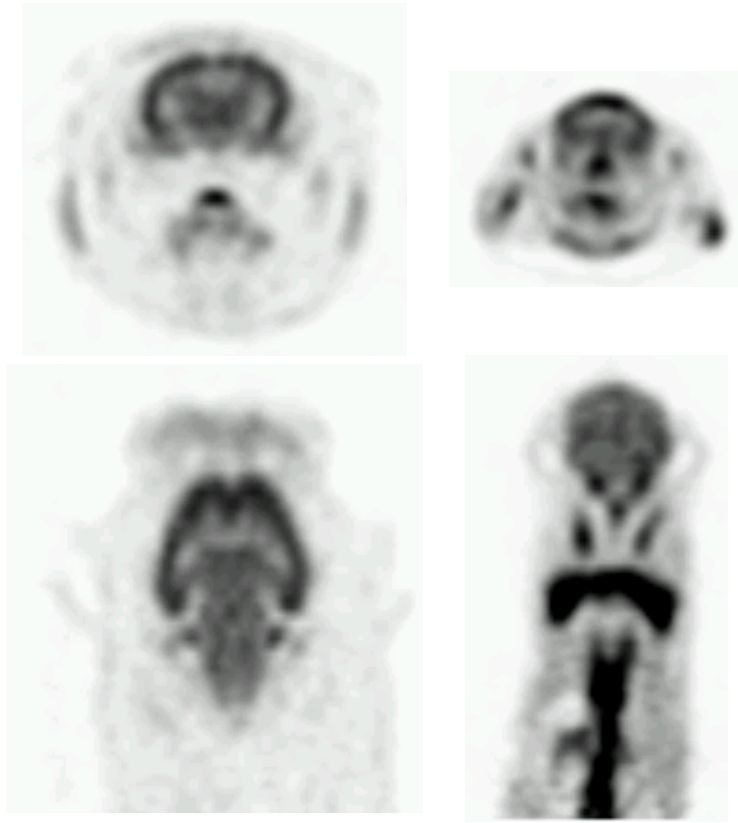
MR: movement, contrast agents, temperature

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

What Species to Use

- Structure size compared to imaging resolution
- Probe contrast, specific to non-specific
- Blood sampling
- Metabolite analysis
- Cost
- Suitable model for working with human diseases
- Whole body imaging, sequential imaging or region imaging
- Acceptance of human tissue implantation

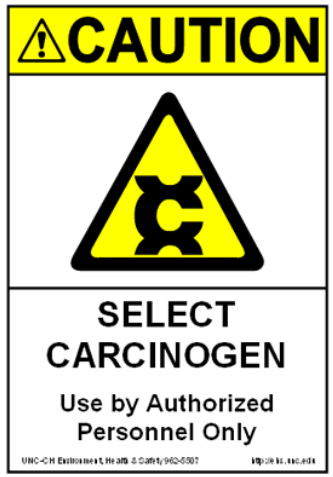


Rat Head Mouse

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.

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.

Vivarium

UCLA vivarium room contains shelves for housing rats, ventilated rack for mice, biosafety cabinet for cage changes and a radiation ‘hot’ rack for overnight decay.

Experimental Design Issues

- How to design an experiment that can be answered
- Critical elements to control or measure
- Quantitation: how far is necessary
- Dynamic or static imaging
- Uptake and clearance times
- Anesthesia issues
- Restraint and positioning
- Reproducibility
- Temperature
- Viral vectors, infectious agents
- Immunocompromised imaging

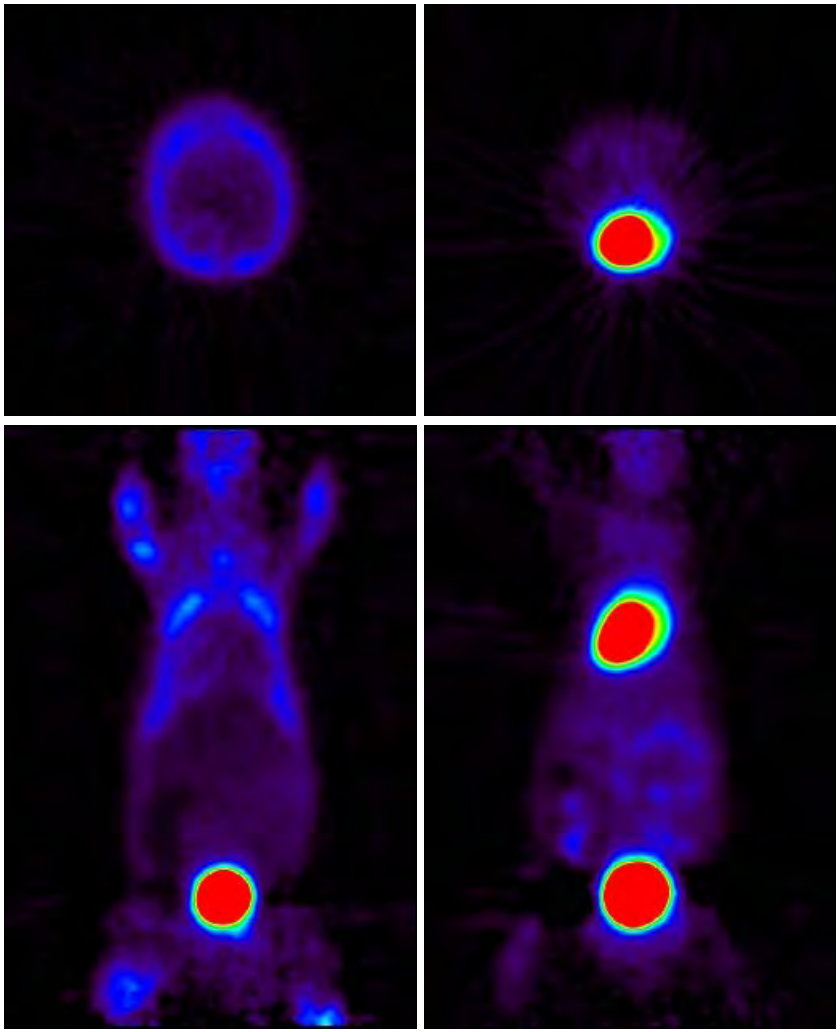
Calibrated Imaging

- Normalization
- Decay correction
- Deadtime correction
- Randoms correction
- Scatter correction
- Attenuation correction
- Blood input function
- Partial Volume Effect
- Probe metabolism
- Calibration Constant

- Percent Injected Dose, SUV
- Tissue time activity
- Graphical Kinetics
- Compartmental Models
- KIS: online modeling

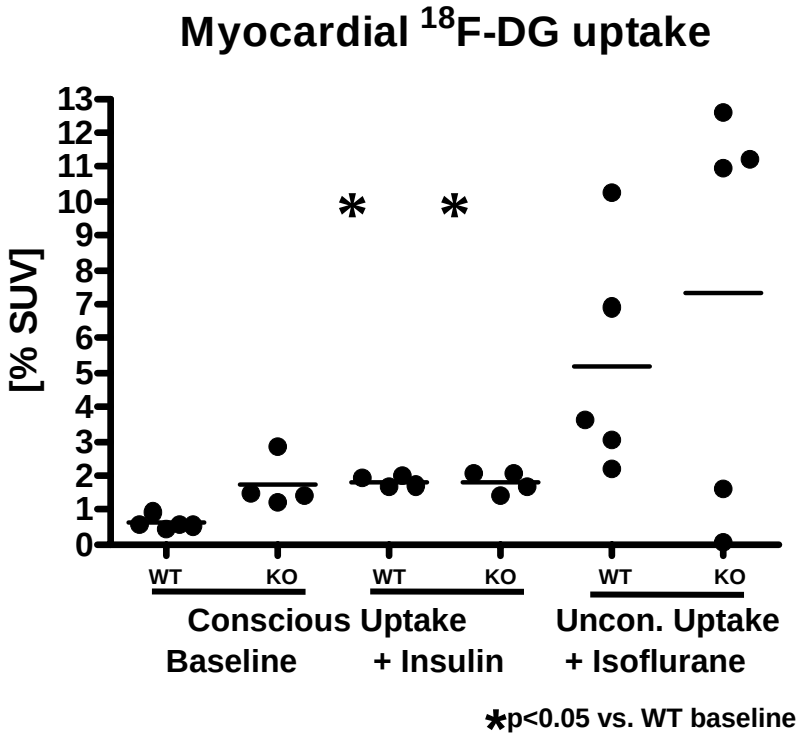
The endpoint measurement criteria will determine the acquisition conditions necessary for the experiment.

Isoflurane Effects on FDG Uptake



Conscious Uptake

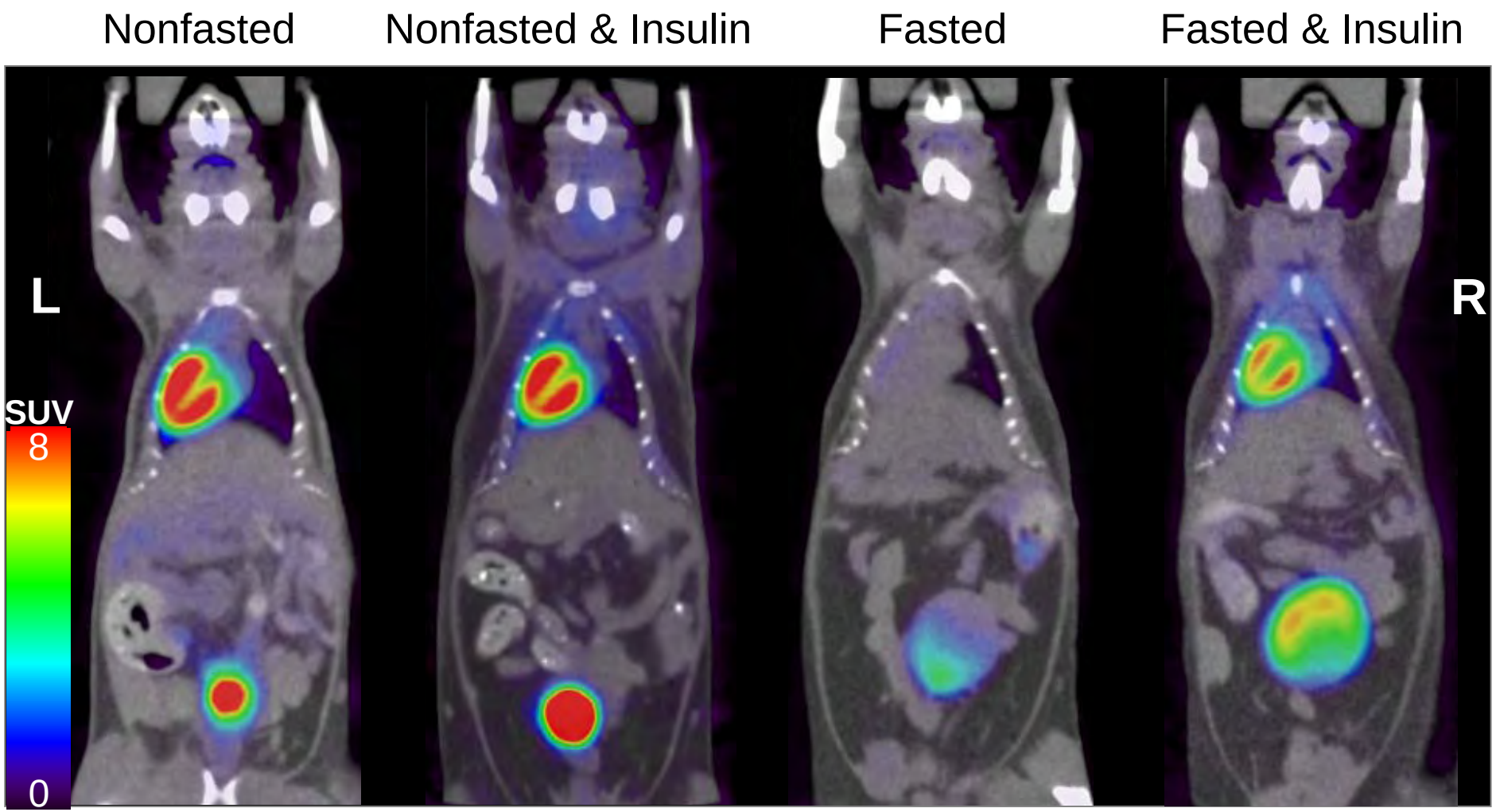
Unconscious Uptake with Isoflurane



Isoflurane increases heart uptake of FDG and can mask findings.

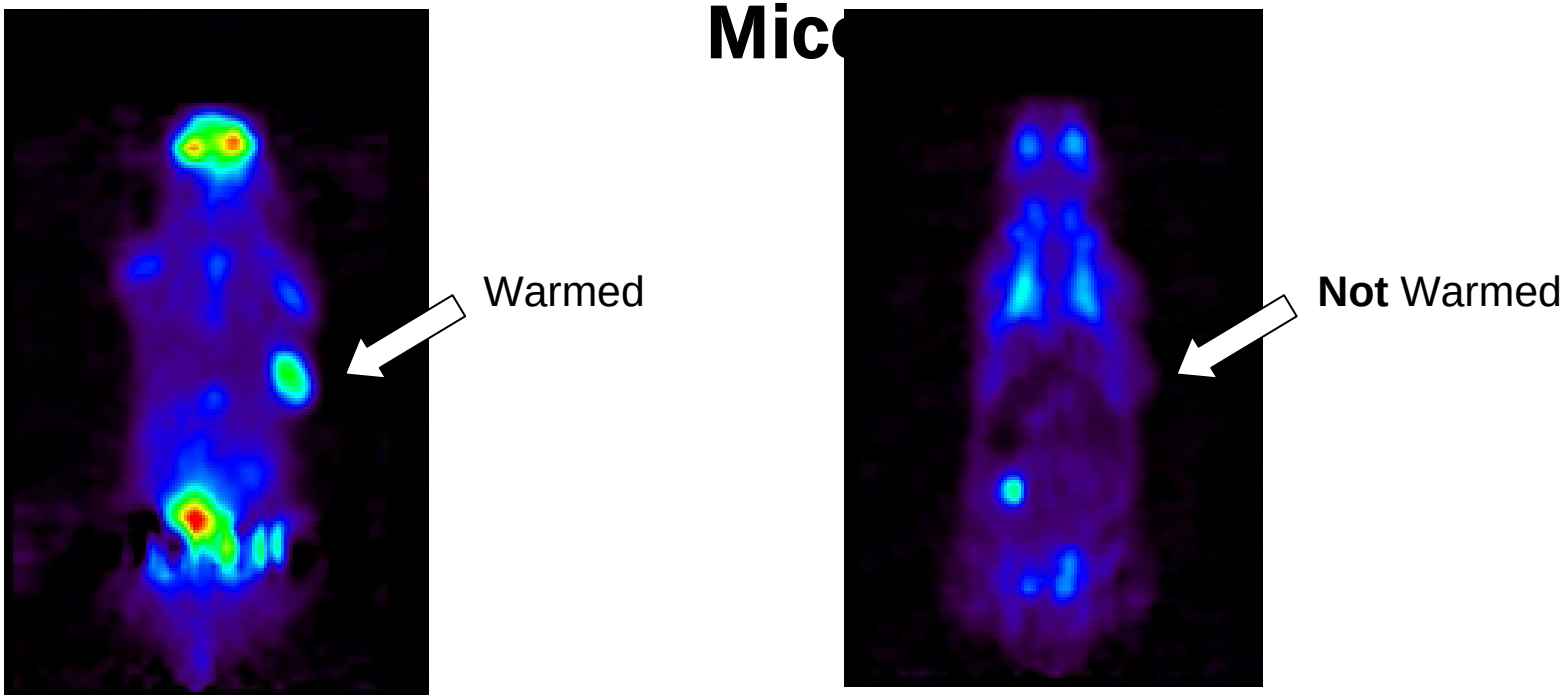
All anesthesia changes physiological measurements.

Glucose Effects on FDG



Example of how the inclusion of microCT images helps aide in the understanding of the metabolic microPET image data.

Temperature Effects in Tumor Bearing Mic

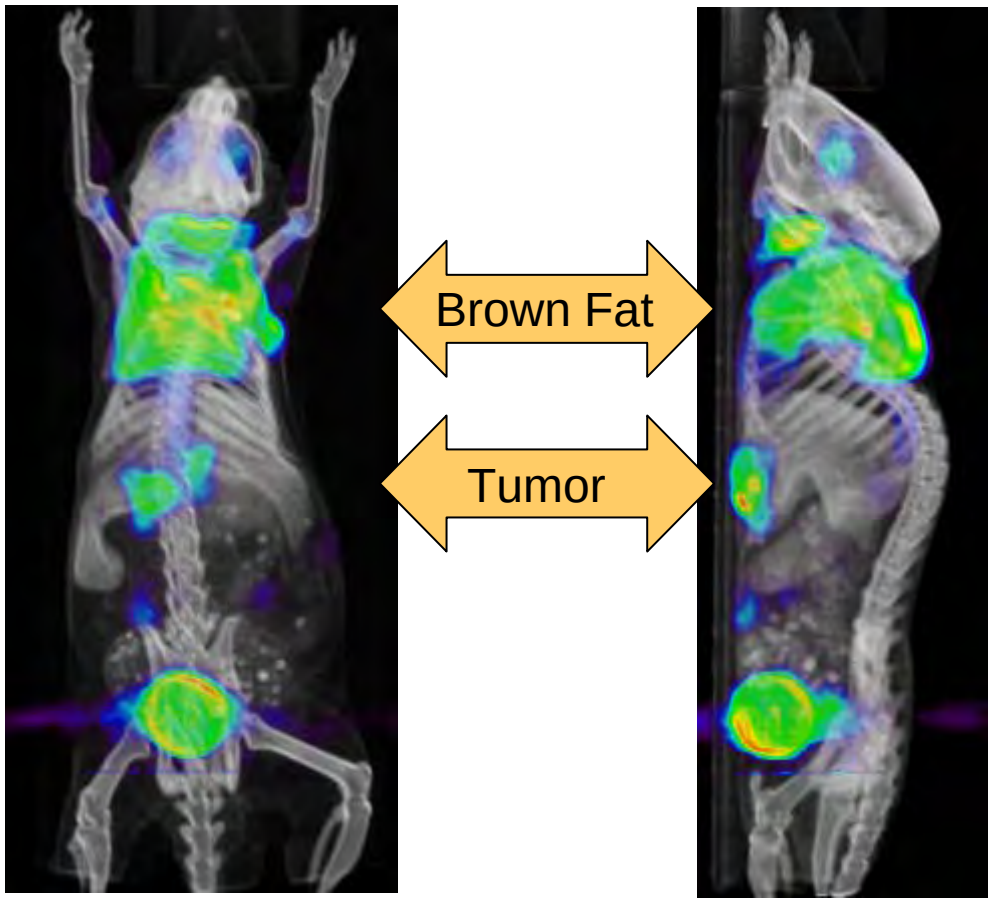


Same mouse scanned two days apart. Temperature is the primary reason for the substantial uptake difference in the tumor.

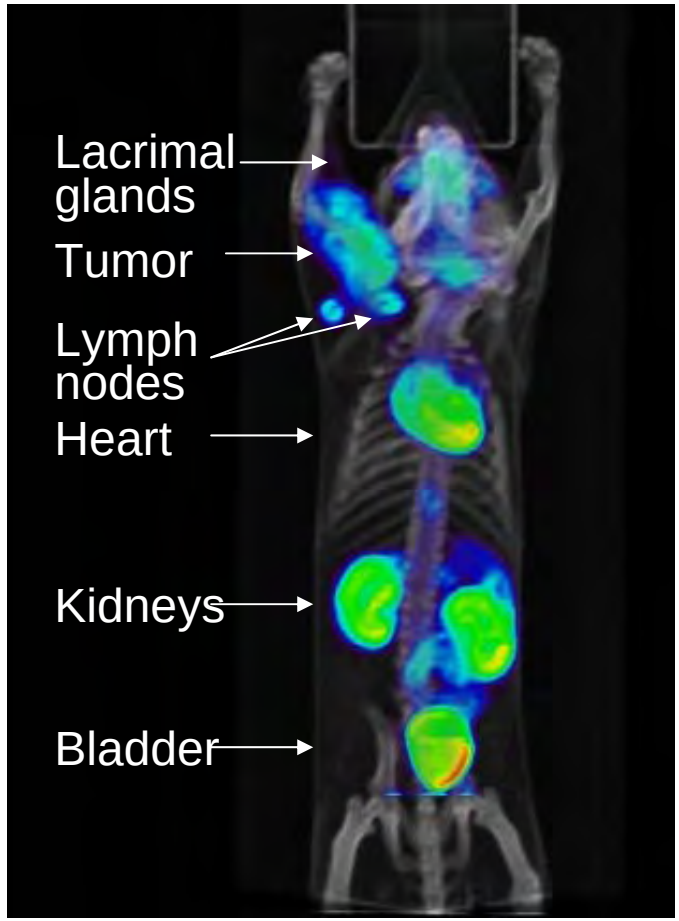
Variances in temperature between different imaging sessions could mask changes due to tumor growth or interventions.

Courtesy of B. Fueger & W. Weber

Brown Fat Uptake



Conscious uptake without heating in tumor bearing mouse

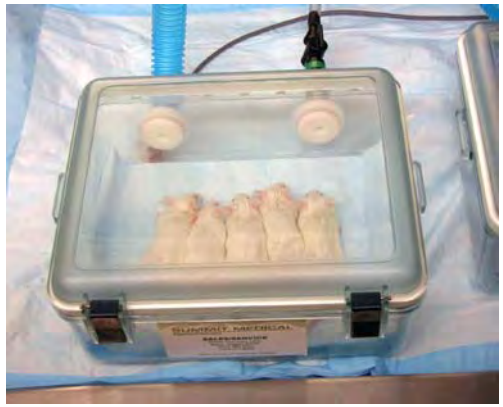


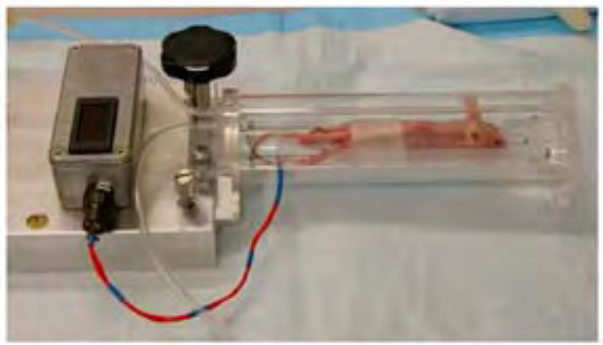
Unconscious uptake with heating

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



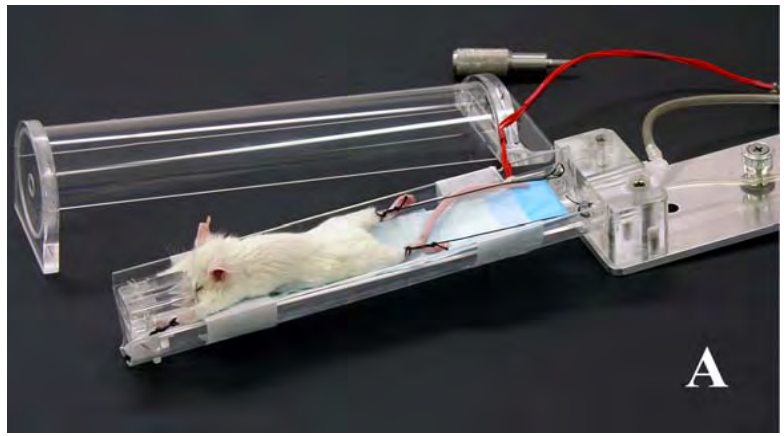


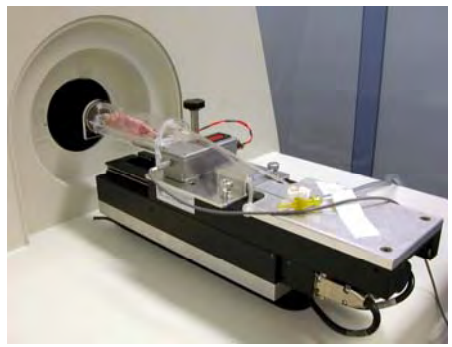
Various heating methods are employed to keep the animals warm during all steps of the imaging process.

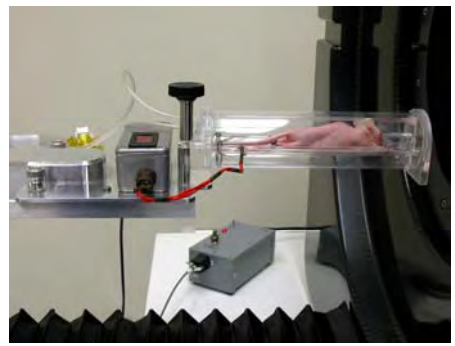
We have located heating plates wherever animal cages or animals are located, including the shielded recovery area.

microPET-CT-MR Chamber

- Designed for BLS2
- Completely sealed chamber coming soon, BSL3
- Constant gas anesthesia
- Heating
- Reproducible positioning
- Optimal placement within imaging systems
- Easy to use
- Designed for multiple imaging systems
- Commercially available

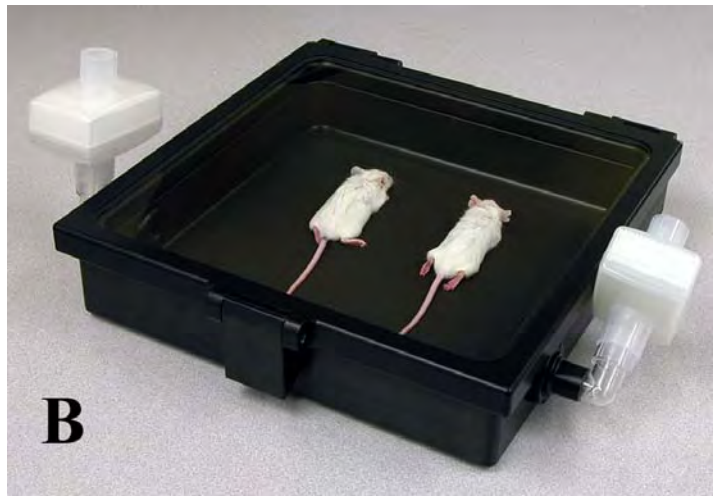




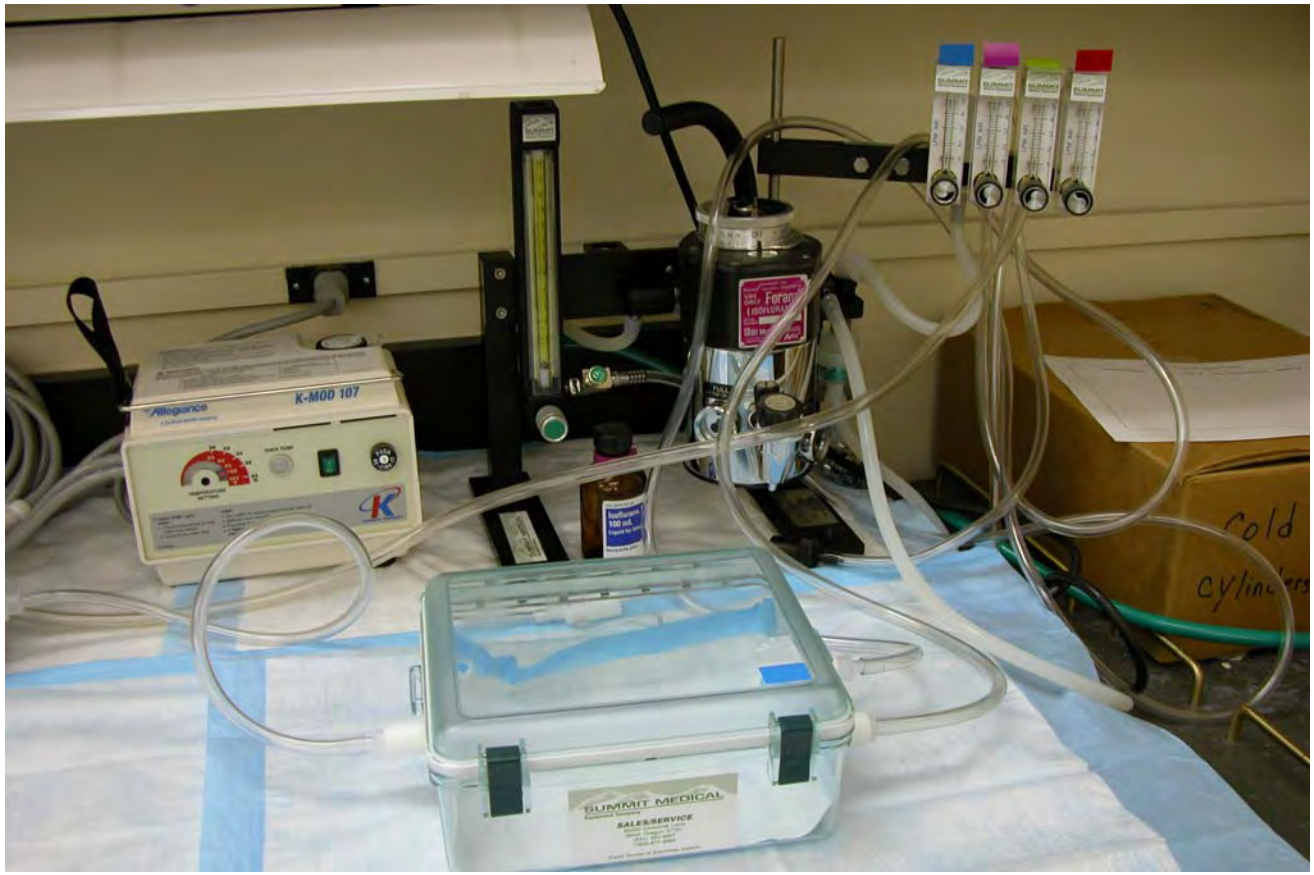


Optical Chamber

- Optimized for projection imaging in Xenogen (Caliper) or CRi Maestro optical systems
- Isolated barrier conditions, BSL2, perhaps BSL3
- No temperature control
- No positioning
- Gas anesthesia
- Commercially available
- Latest version has 5 nose cones and quick release sealed anesthesia line fittings

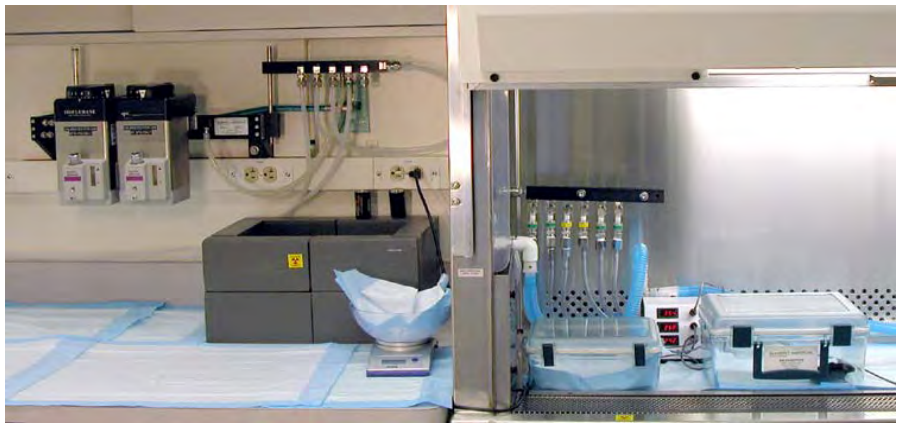


Conventional Anesthesia Configuration



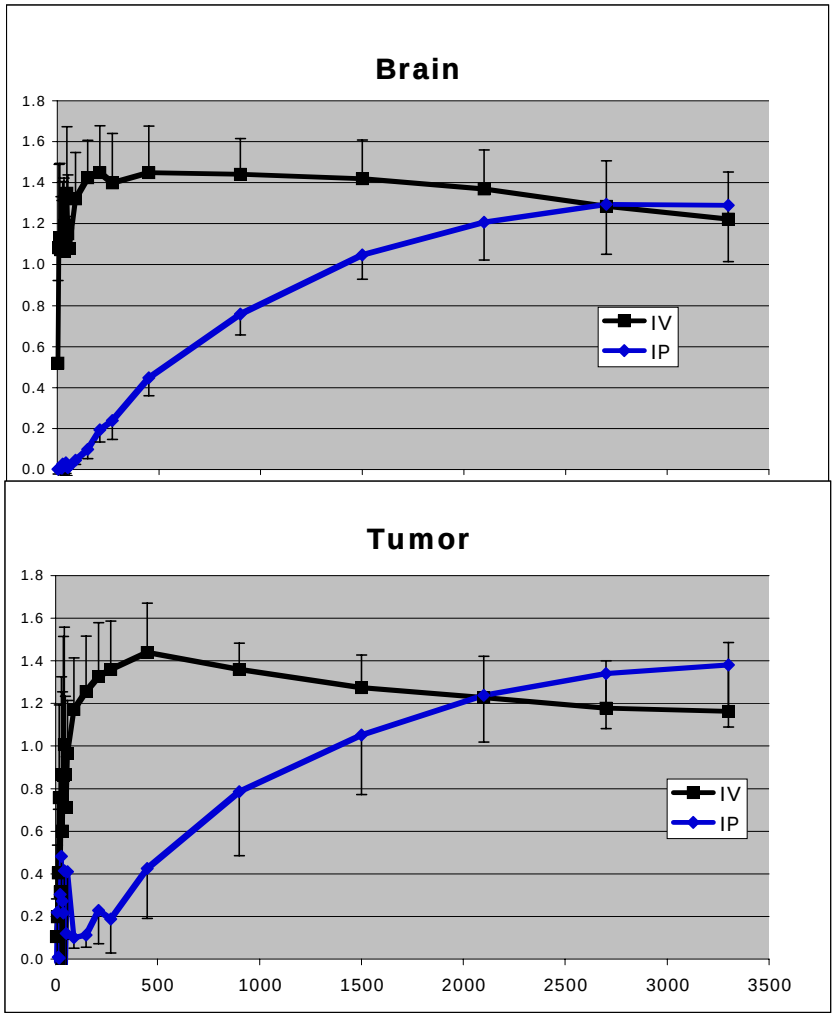
Conventional anesthetic user requires flow meters, which must be readjusted any time concurrent use is added or stopped. This arrangement proved unworkable with multiple points of use and multiple investigators requiring anesthesia at the same time.

Integrated Anesthesia System



- Optimized for multiple location usage
- Constant pressure, easy on/off valves, no flowmeters
- Expandable, simple, easy to use
- Only provides one level of anesthesia concentration
- Used for 4+ years, 15,000+ experiments without failure

IP versus IV FDG distribution



The injection route alters the dynamic delivery of radioactivity.
After 1 hour, FDG uptake is similar in most tissues except the heart.

Blood Sampling Options

Sample Based

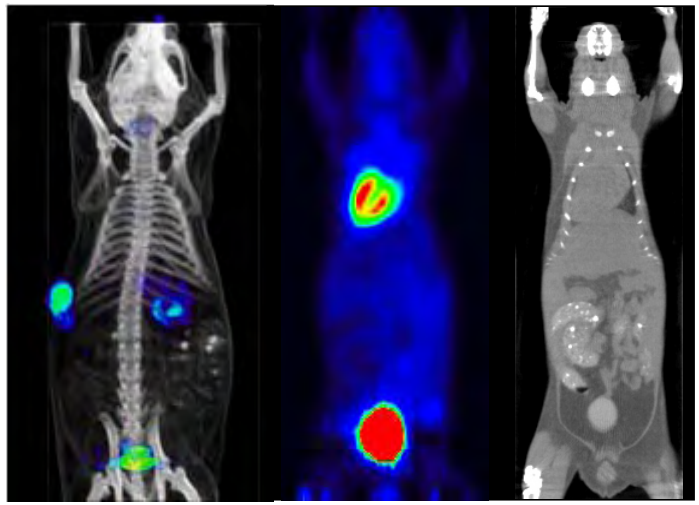
- Arterial Catheter
  - Usually terminal
  - Surgical procedure
- Tail Catheter
  - Low flow/sampling
  - venous
- Needle stick
  - Venous
  - Poor temporal sampling
  - Cardiac sticks often fatal

Image Based

- Left Ventricle
  - Largest blood pool
  - Reasonably cylindrical
  - Myocardium spillover
- Right Ventricle
  - Complex shape
  - One sided spillover
- Aorta
  - Partial Volume
  - Finding it

In Vivo Imaging Acquisition

What are the steps necessary to conduct imaging experiments



microPET-CT microPET microCT

Figure 5 Session & Animal ID's

5253	May 9, 2005	Anna Wu/Tova
5254	May 9, 2005	Anna Wu/Tova
5255	May 10, 2005	Czernin/Barrio/Huang
5256	May 11, 2005	Schelbert
5257	May 11, 2005	Schelbert
5258	May 11, 2005	Schelbert

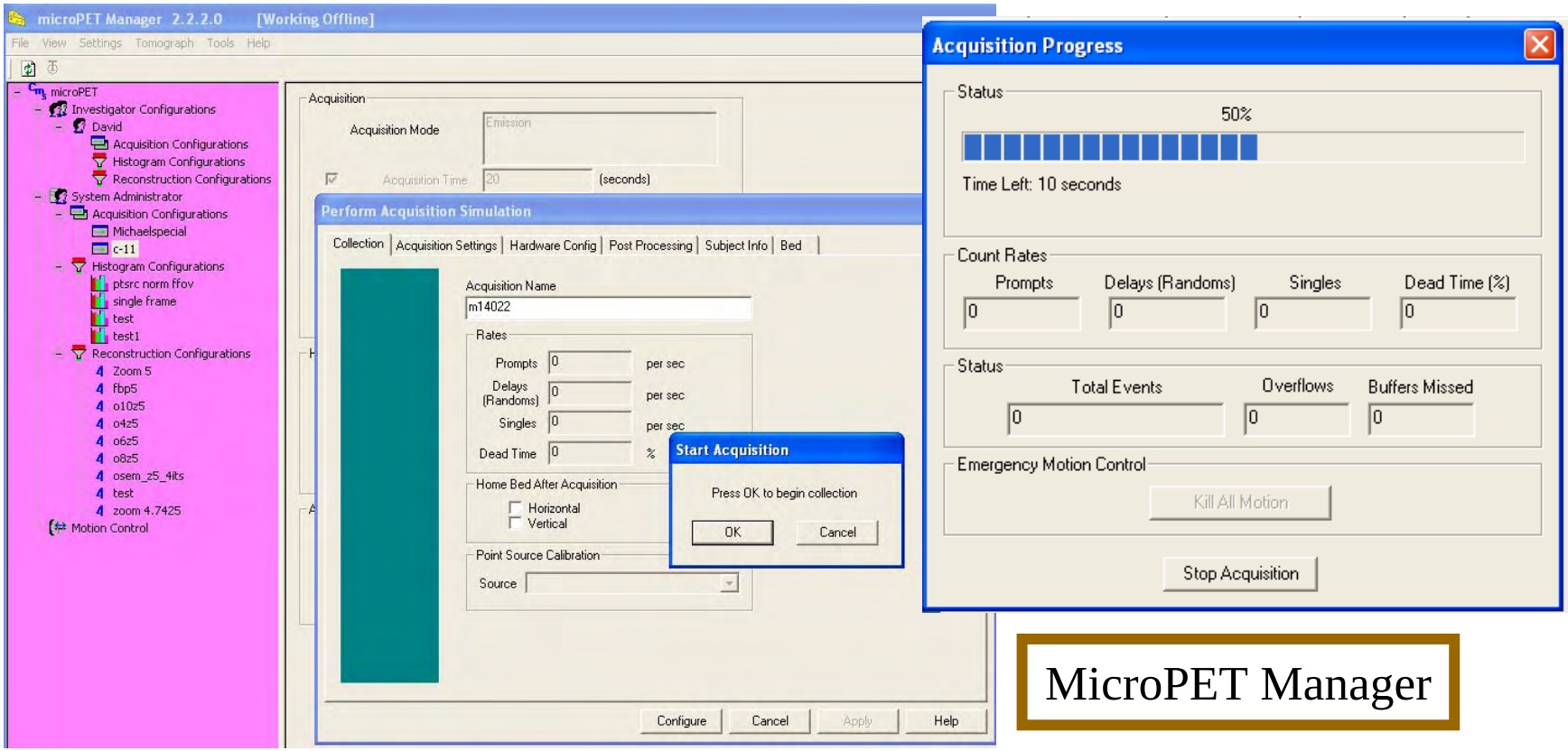
Mouse / Rat / Rabbit

A new microPET SID = m14285

Animal ID number; needed due to multiple imaging sessions

Session ID number; distinguish between serial experiments

Data Acquisition



MicroPET Manager

Vendor-based software is used to acquire and often process the data.

Protocols for acquisition and processing often are stored for repeat use.

Image Generation for microPET

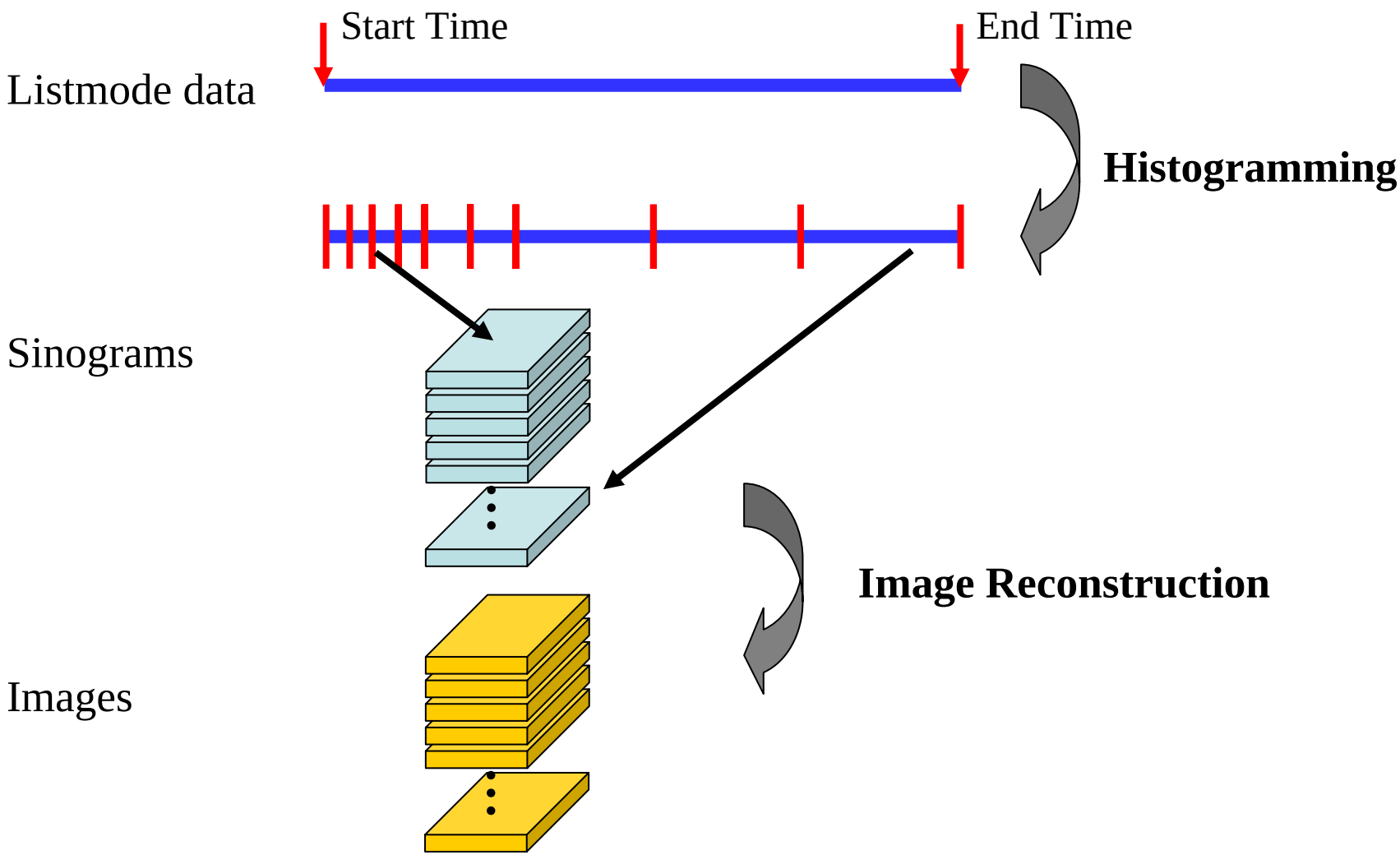
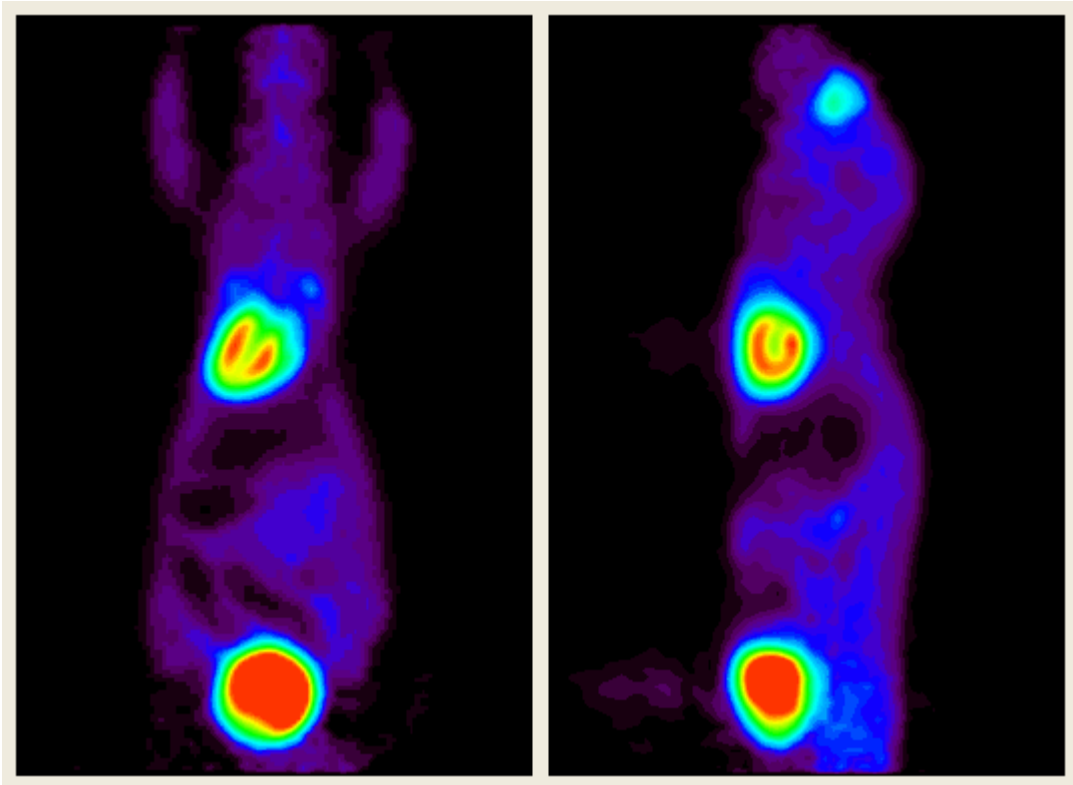


Image Verification

Potential problems:
-No injection
-Positioning error
-Movement
-Animal not in FOV
-Urination
-Scanner error

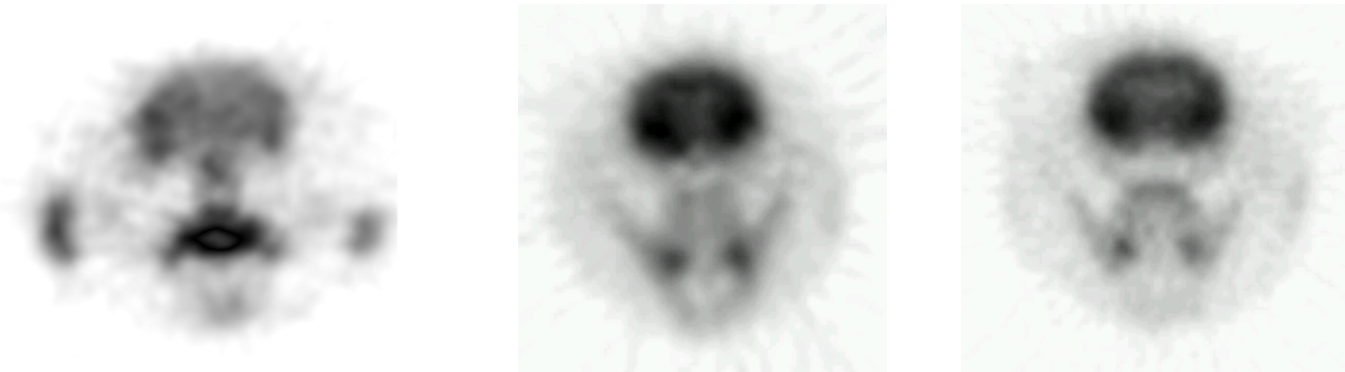


Every image ideally should be checked, preferably immediately after acquisition, even if this means reconstructing again with different settings at a later time.

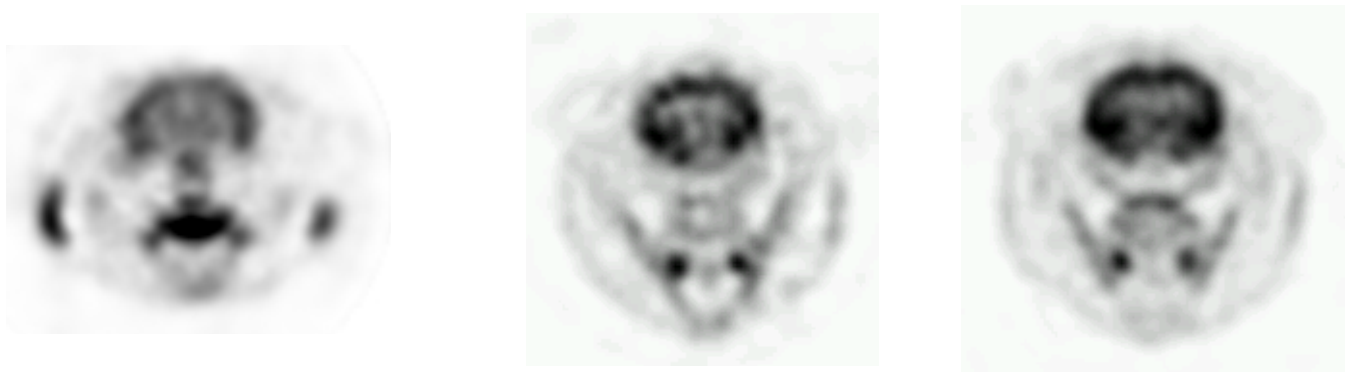
Image Reconstruction

Prototype 1997 P4 2000 Focus 2003

Filtered Back Projection



MAP



Some imaging systems offer a choice of image creation options, such as above for MAP or FBP with microPET data.

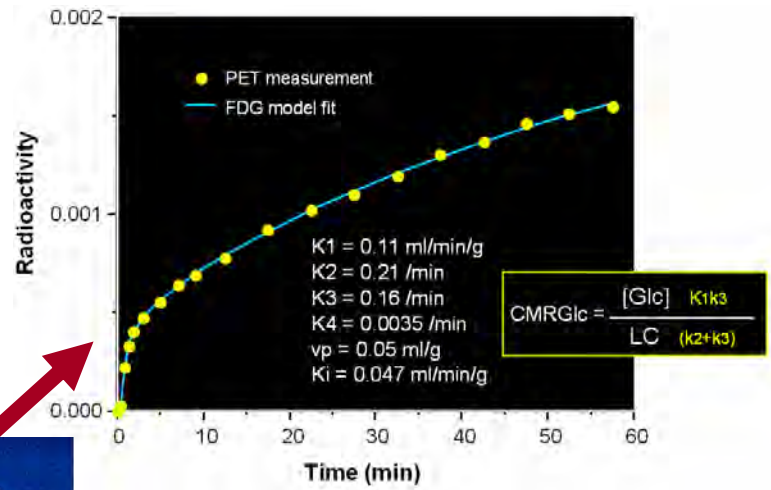
Image Archiving

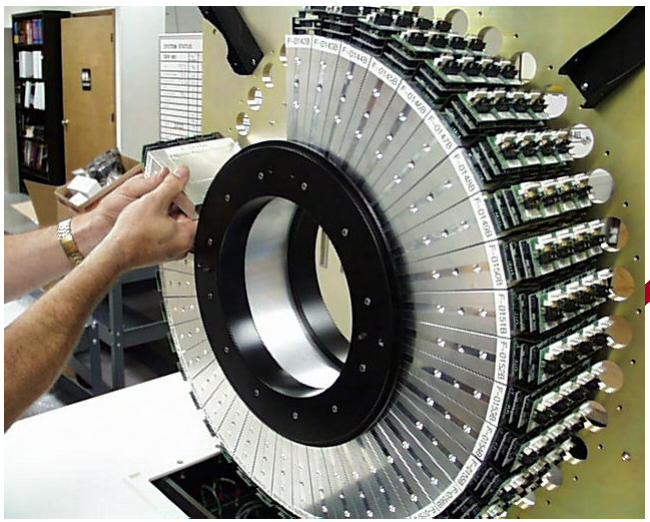
Archiving all the data is not always necessary, since some data can be recreated from raw data.

At UCLA, for microPET data we archive only sinograms and images for short static studies. For dynamic experiments, listmode and images are saved.

Image Analysis & Quantitation

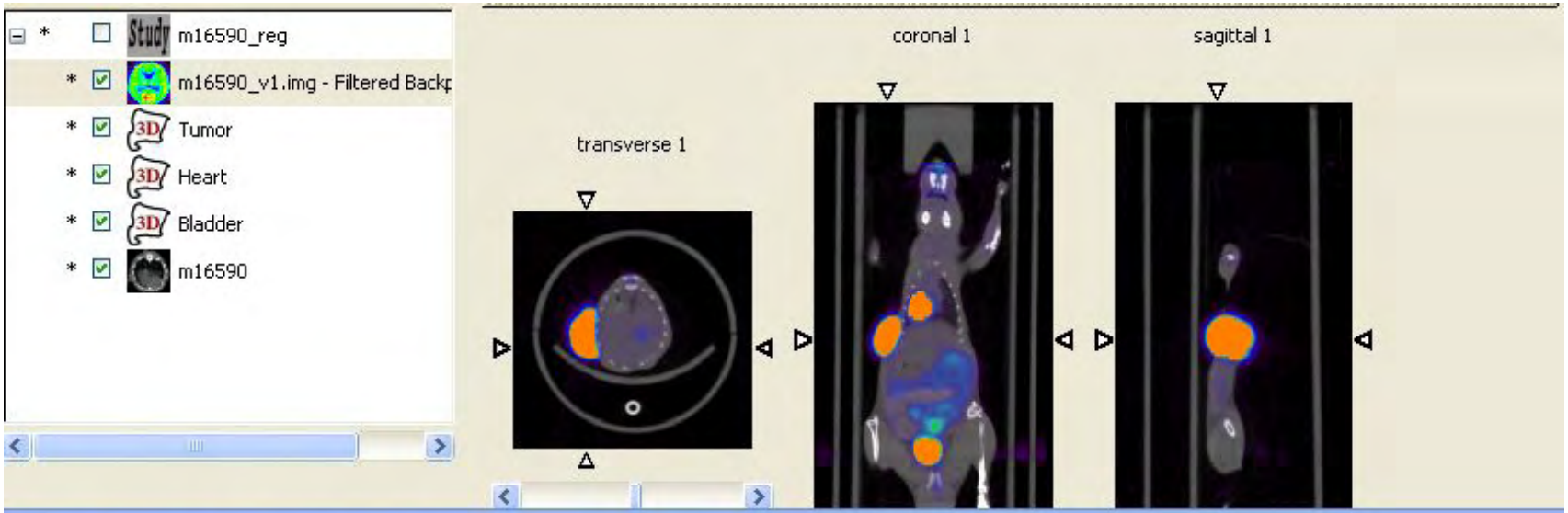
Qualitative & Quantitative Imaging
% Injected dose, SUV
Tracer Kinetic Modeling
Software Demonstrations





David Stout PhD
UCLA Crump Institute for Molecular Imaging

Generating Image Data



amide Roi Analysis: Study m16590_reg

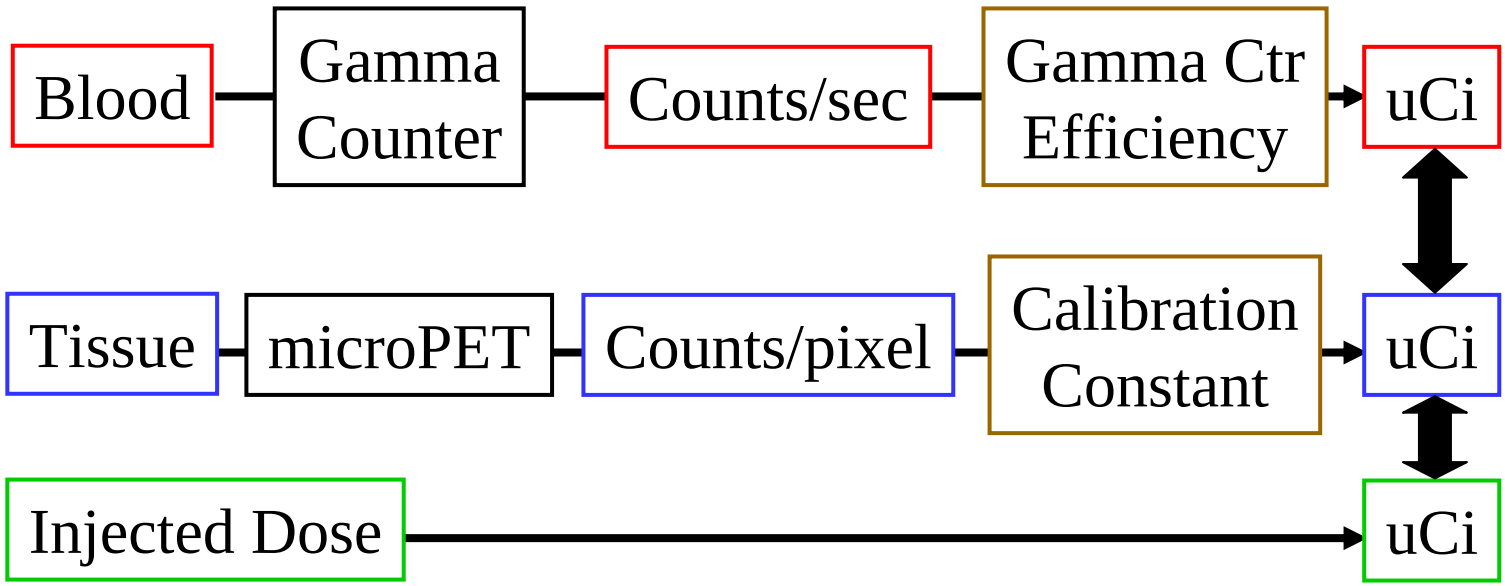
ROI Statistics

ROI	Data Set	Median	Mean	Var	Std Dev	Min	Max	Size (mm^3)	Frac. Voxels	Voxels
Tumor	m16590_v1.img - Filtered Backprojection	0.00259029	0.00253273	2.8109e-07	0.000530179	0.00156405	0.0034441	334.702	2628	2628
Heart	m16590_v1.img - Filtered Backprojection	0.00157011	0.00157053	3.33857e-08	0.000182717	0.0012528	0.0019746	87.8784	690	690
Bladder	m16590_v1.img - Filtered Backprojection	0.00572742	0.0090515	6.3676e-05	0.00797972	0.00207049	0.0366431	117.171	920	920

Regions of interest (ROI) are drawn on images to get uptake information.

Example above using AMIDE for display of microPET-CT data.

Calibration Constant



Measurements are made using a mouse sized cylinder to obtain both the calibration constant and gamma counter efficiency.
This is the best quality control method, since it mimics the animal imaging conditions.
Used to convert measurements to a common reference unit.

%Injected Dose per gram

$$\%ID/g = C_t \cdot \frac{V_t}{W_t} \cdot \frac{1}{D_{inj}} \cdot 100\left(\%/g\right)$$

$C_t = tissue\_concentration = activity/volume$

$V_t = tissue\_volume$

$W_t = tissue\_weight$

$D_{inj} = Dose\_injected$

Example 1: Skinny mouse

V_t=10cc

C_t=1mCi/cc

D_{inj}=10mCi

%ID/g = 10(%/g)

Example 2: Average mouse

V_t=20cc

C_t=0.5mCi/cc

D_{inj}=10mCi

%ID/g = 5(%/g)

Standardized Uptake Value: SUV

(the animal weight is accounted for in this calculation)

$$SUV = (\%ID/g) \cdot W_p / 100$$

$$W_p = patient\_weight$$

Example 1: Skinny mouse

V_t=10cc

%ID/g = 10%

D_{inj}=10mCi

SUV = 1

Example 2: Average mouse

V_t=20cc

%ID/g = 5%

D_{inj}=10mCi

SUV = 1

Graphical analysis methods: Patlak & Logan Plots

Patlak plot:

For measuring irreversible reaction rates.

Slope is equal to Ki=K1k3/(k2+k3) .

$$\frac{C_i(T)}{C_p(T)} = K_i \frac{\int_0^T C_p(\tau)\,d\tau}{C_p(T)} + \frac{k_2 k_3}{(k_2 + k_3)^2}$$

Logan plot:

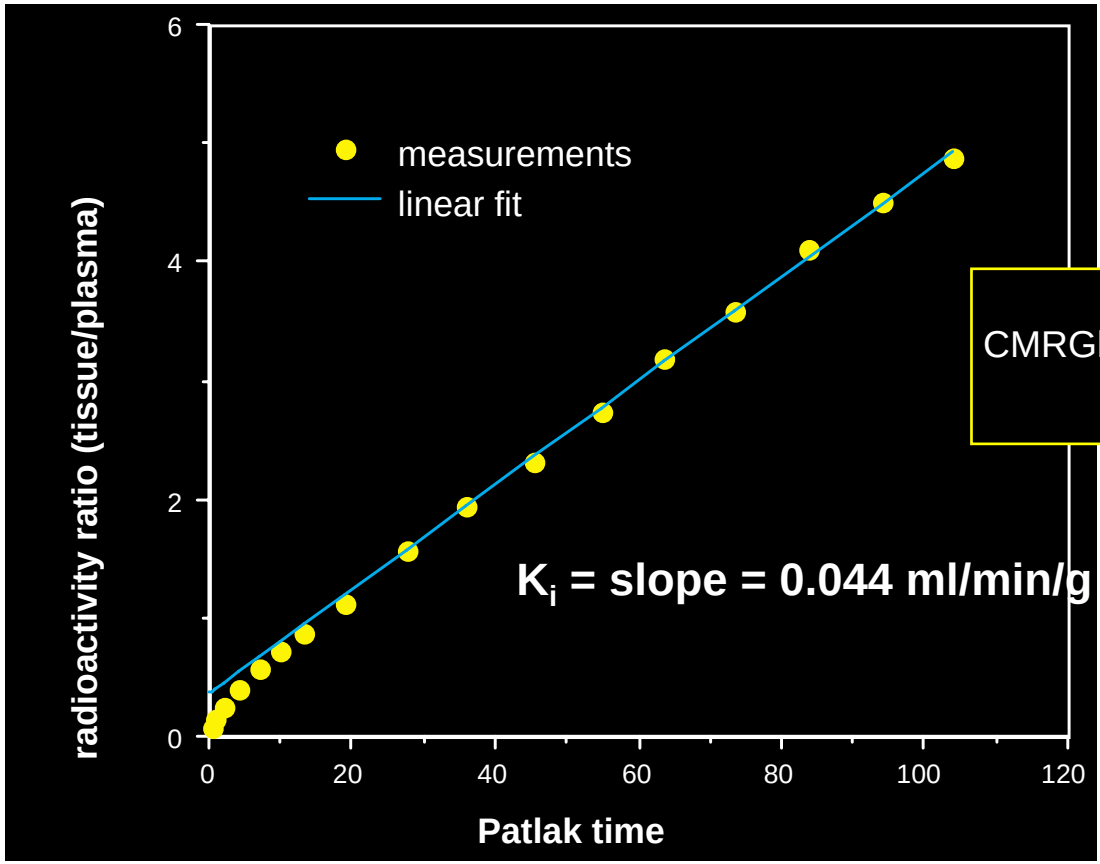
For measuring reversible ligand-receptor bindings.

Slope is equal to Vd=K1/k2 (1+(k3/k4)),

where k3/k4 = Bmax/KD .

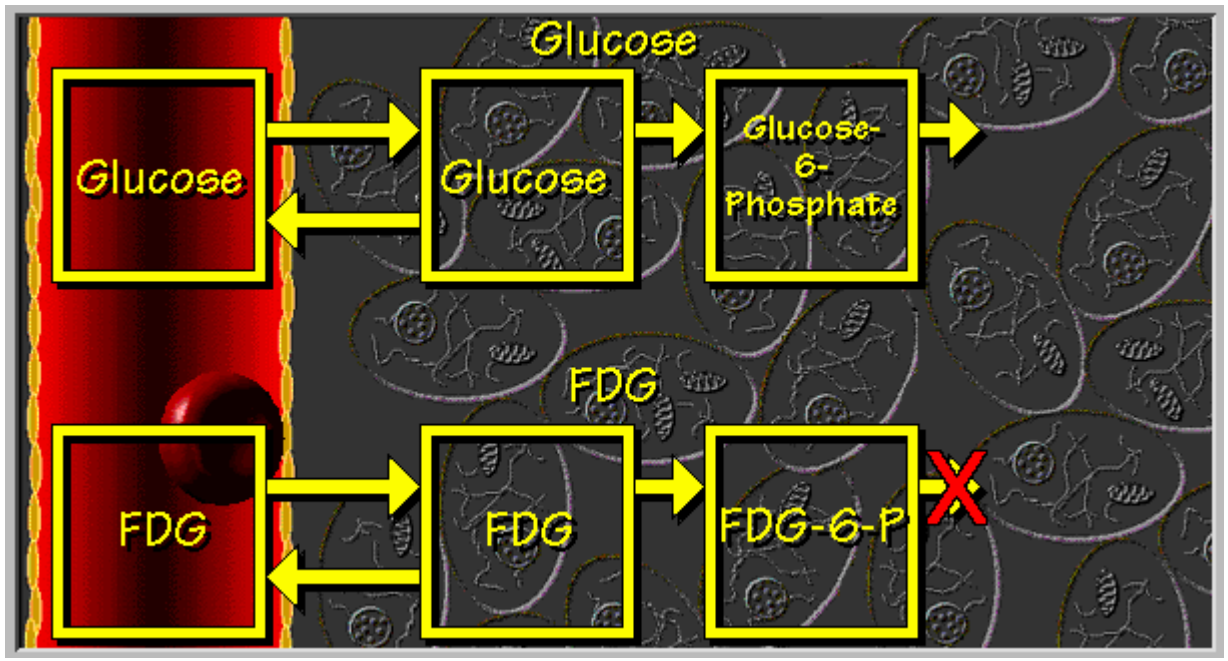
$$\frac{\int_0^T C_t(\tau)d\tau}{C_t(T)} = \frac{K_1}{k_2}\left(1+\frac{k_3}{k_4}\right)\frac{\int_0^T C_p(\tau)d\tau}{C_t(T)} - \frac{1}{k_2}\left(1+\frac{k_3}{k_4}\right)$$

Patlak Analysis of FDG Data in Brain Tissue



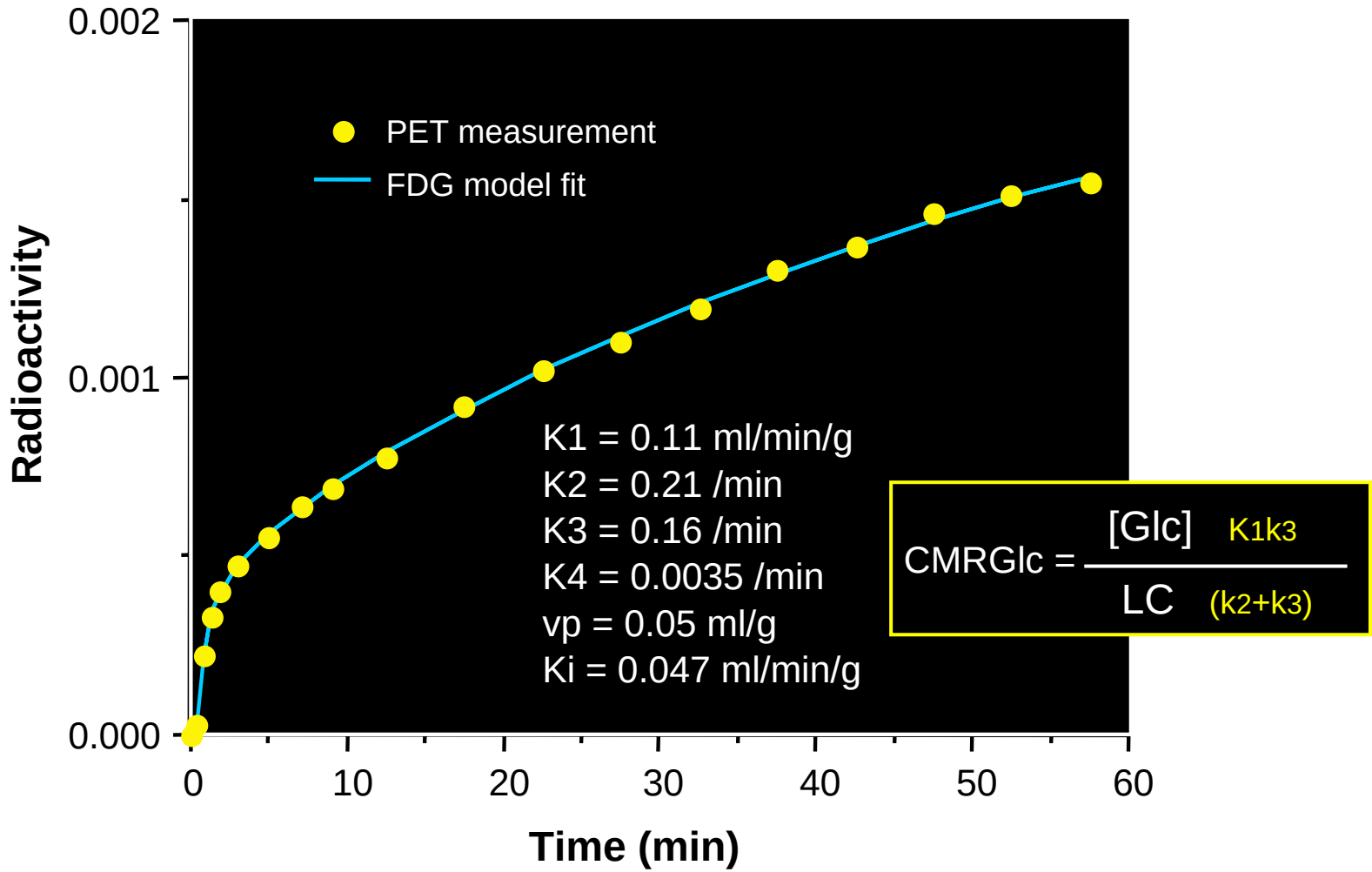
$$CMRGlc = \frac{[Glc]}{LC} slope$$

Compartmental Models

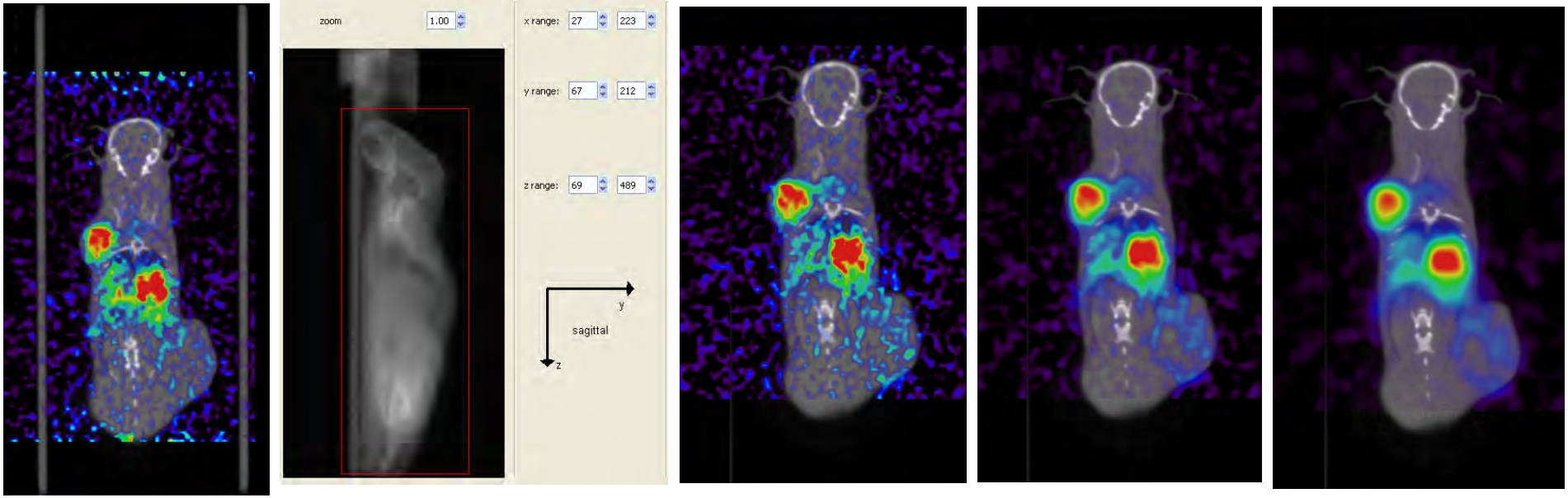


Compartmental modeling requires temporal sampling of the input function. Provides more complete parameter measurements for in vivo processes.

Model Fitting: FDG Kinetics



Blurring & Cropping for Display



For image display (not quantitation), noisy data can be cropped and blurred to create an image more suitable for display. The idea is to get the picture to tell the story, not have the noisy and unrelated information hinder the visual impression.

Summary

- Immunocompromised animal work requires BSL2-like level conditions in order to keep animals safe from human pathogens.
- Infective or carcinogenic agents require containment to protect humans, which may be BSL2 or BSL3 depending on the agent.
- The level of quantitation will determine the conditions required for imaging protocols.
- Handling, anesthesia, fasting and temperature can substantially alter the uptake of metabolic imaging probes.
- Each imaging system has its own set of unique advantages and disadvantages. Proper design of experiments is essential to obtain appropriate and accurate data.