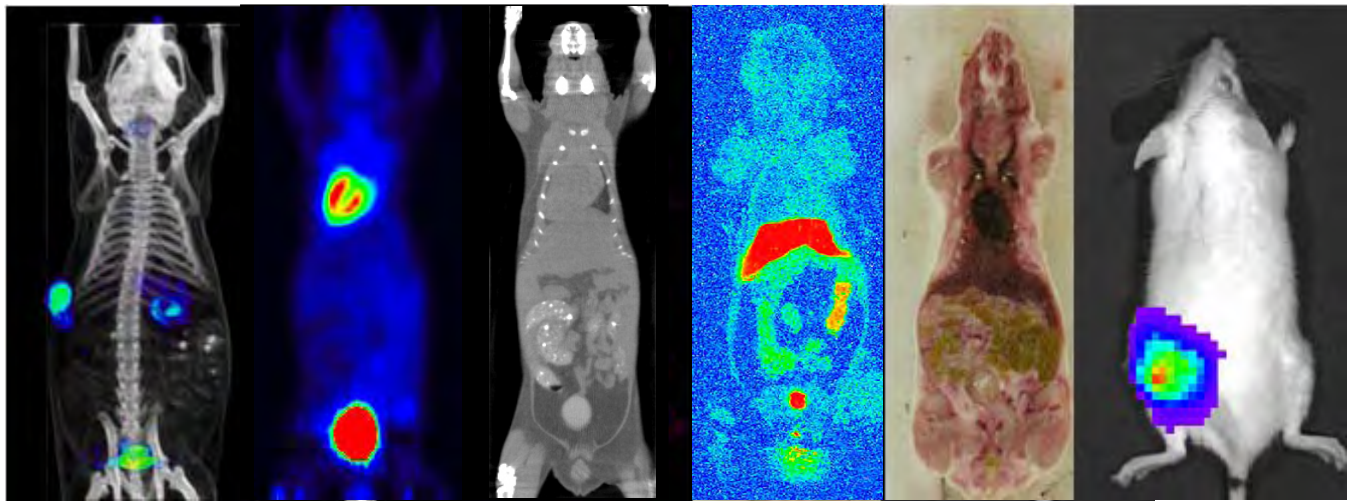
Preclinical Molecular Imaging
using microPET, microCT & Optical Methods



microPET-CT microPET microCT Autoradiograph Photograph Optical

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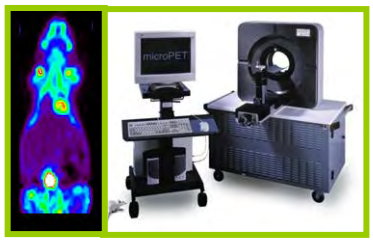
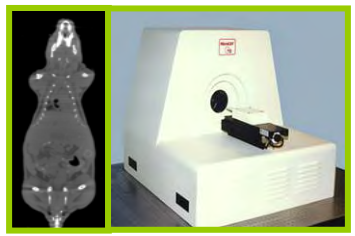
David Geffen School of Medicine

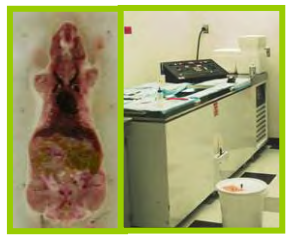
Topics

- Imaging Center Design
- Cyclotron & Radiochemisty
- Imaging Modalities & Examples
- Multimodality Imaging

Systems and Personnel Requirements







Staff Operated

Investigator Operated

Several regulatory agencies have oversight over the imaging systems and need to be consulted to ensure compliance with appropriate regulations:

-Radiation Safety
-Biosafety Committee
-Animal Research Committee (IACUC)
-Dept. Laboratory Animal Medicine (vivarium)

Planning a new facility

Plan out the facility requirements using manufacturers specifications. Critical specifications include:

-Power requirements

-Heat generation

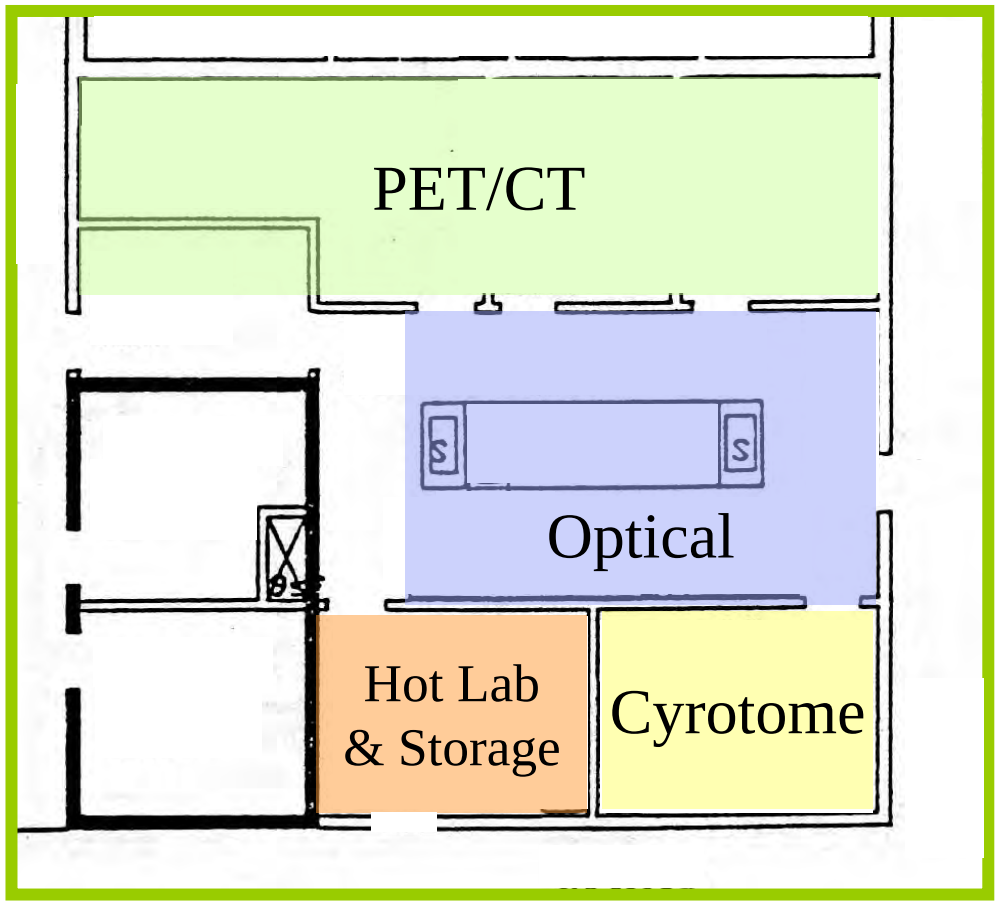
-Ventilation

-Working and servicing space

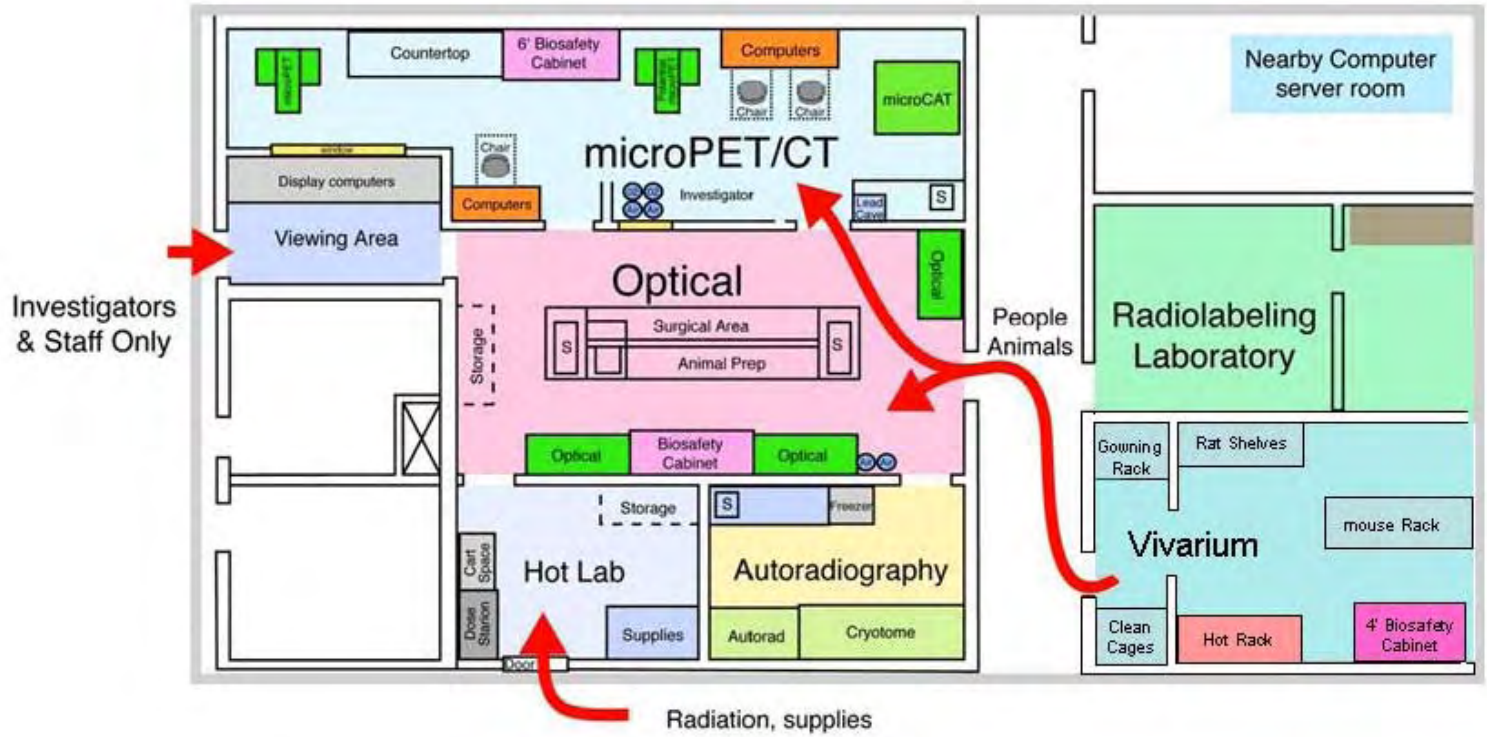
-Usage patterns

-Ancillary equipment

-Access requirements



Planning the Flow of the Workspace



Approximately 1860 sq. ft.
Badge activated door locks
Isolated radiation areas
Isolated break/viewing area
Built in anesthesia & scavenging
Storage cabinets for supplies

Where will people enter?
Where will animals enter?
Where will supplies go?
How will O2 tanks be delivered?
Radioactivity Routes
General versus restricted access

Anesthesia System

Wall mounted vaporizers

Integration with biosafety cabinet & induction boxes

Gas anesthesia provides a safe and effective method for immobilizing animals for imaging work. Consider both supply and exhaust of anesthesia.

The system above was created together with Summit Anesthesia (now part of MIP) in collaboration with the UCLA vivarium, biosafety and imaging center staff.

More on this later.

Equipment Layout

Optimize the imaging equipment to minimize people and animal movement.

Allow for easy traffic flow of carts, equipment and supplies.

Keep desk space clear using overhead storage for records and equipment.

Use two computers per imaging system, one for acquisition and one for database and web-based work.

Imaging Experiments: Two Roles

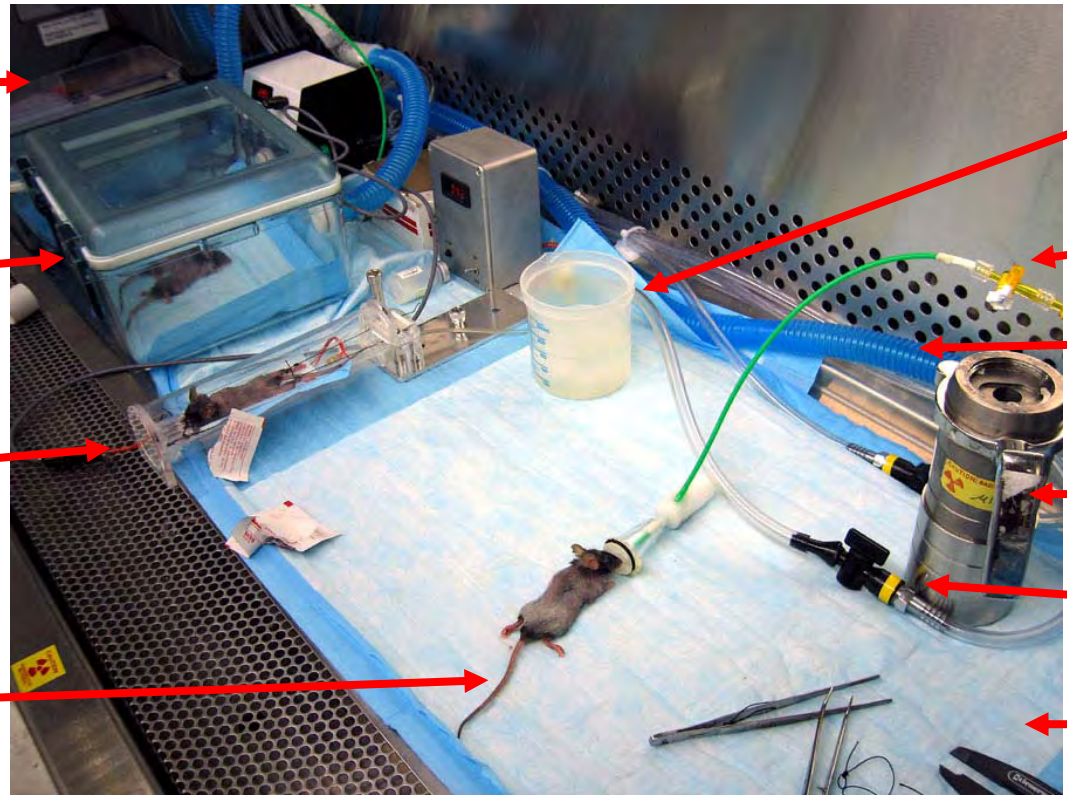
Staff Responsibilities:

- Imaging system QC
- Supplies & anesthesia
- Acquisition of image data
- Processing & archiving of images
- Training for animal handling
- Obtain radioactivity & dispense individual doses
- Database entries and recordkeeping
- Tracking usage & regulatory reports
- Scheduling
- Clean work environment

Investigator Responsibilities

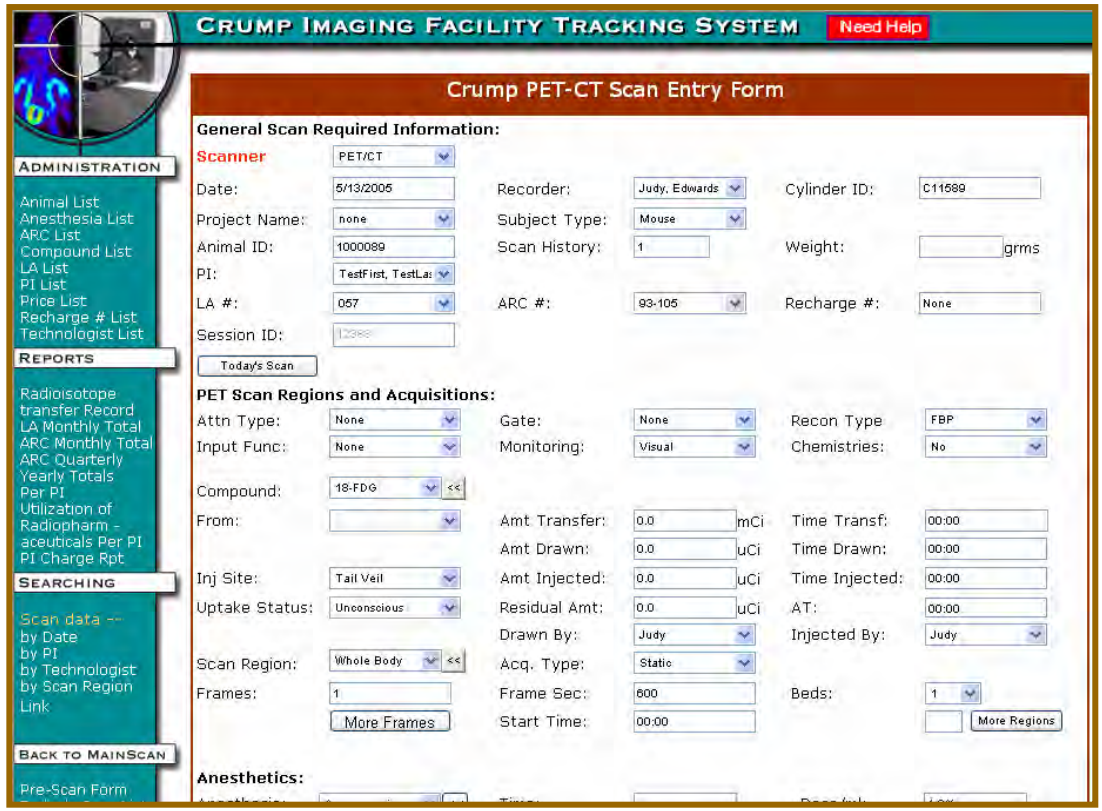
- ARC, RSO Biosafety authorizations
- Proper attire and ID
- Timely arrival & departure
- All animal care & anesthesia
- Injection of radiation
- Positioning and placement of animals in imaging chambers
- Recovery and clean-up
- Vivarium storage & transport
- Animal health, including heating and physiological monitoring

Staging Mice



Multi-mouse PET-CT imaging is a complex process with many details to track. Success comes with planning, training and experience, together with equipment and facilities that are optimally designed for the imaging methods.

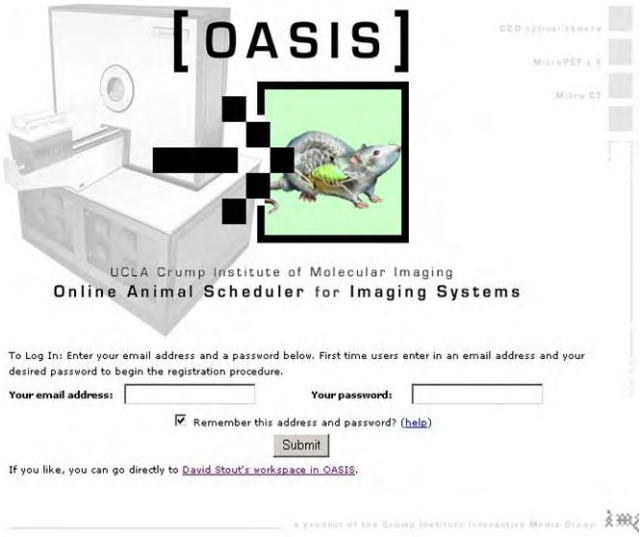
Database & Image Management

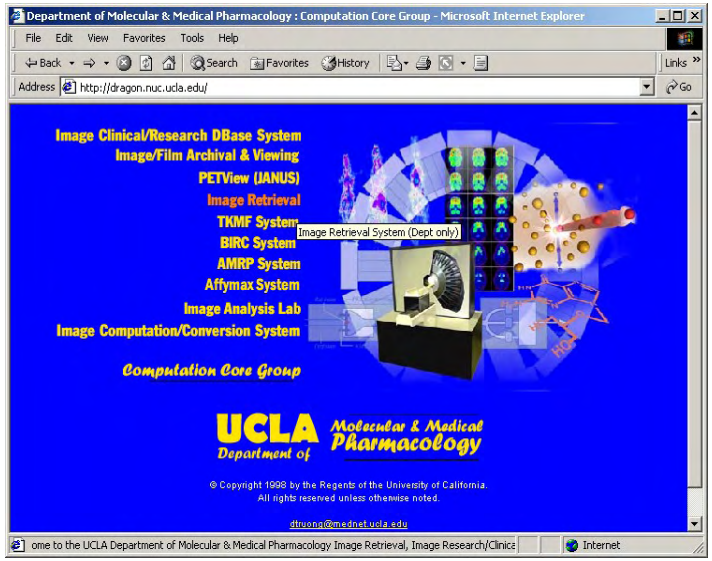


Database storage of all relevant information is essential. Data is required for analysis by investigators, reports by staff and billing by fund managers.

Filemaker Pro is an alternate option that works well, especially since data can easily be exported to spreadsheets and documents.

Scheduling Usage & Retrieving Data





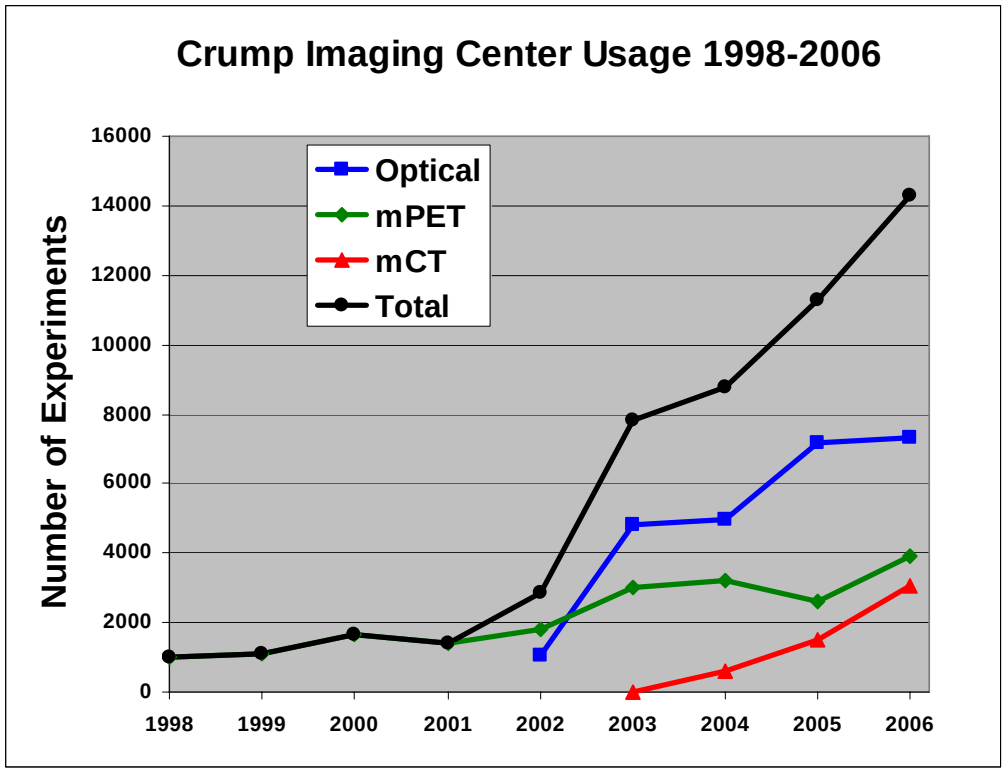
Scheduling website for user operated equipment (optical, autoradiography).

PET &CT scheduling with probe production and staffing time is better served via email requests. A resource allocation committee coordinates the priorities of research and clinical research with cyclotron production.

Image archiving and retrieval can be automated, with online access for requesting image data from a secure server to a network shared folder.

Our website incorporates online viewing and data analysis features such as kinetic modeling.

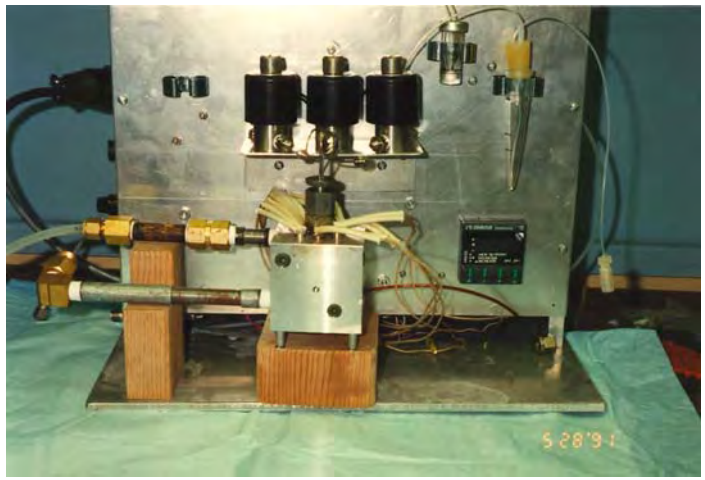
Usage Estimation: Now versus Future



Preclinical research has grown rapidly during the past 10 years. Many new imaging systems and techniques have focused on using mice in multiple imaging modalities.

Plan for future success, not just for current requirements.

Radioisotopes and Radiochemistry



The creation of imaging probes for PET imaging

Positron Emitting Isotopes

Isotope	Half-life	β+ fraction	Max. Energy	Positron Range
C-11	20.4 min	0.99	0.96 MeV	0.4 mm
N-13	9.96 min	1.00	1.20 MeV	0.7 mm
O-15	123 sec	1.00	1.74 MeV	1.1 mm
F-18	110 min	0.97	0.63 MeV	0.3 mm
Na-22	2.6 years	0.90	0.55 MeV	0.3 mm
Cu-62	9.74 min	0.98	2.93 MeV	2.7 mm
Cu-64	12.7 hours	0.19	0.65 MeV	0.3 mm
Ga-68	68.3 min	0.88	1.90 MeV	1.2 mm
Rb-82	78 sec	0.96	3.15 MeV	2.8 mm
I-124	4.18 days	0.22	3.16 MeV	2.8 mm

The most widely used isotopes for PET imaging are F-18, C-11, O-15 and N-13. Most produced in cyclotrons and used on-site or nearby due to the short half-life. Longer lived isotopes, such as I-124, Cu-64 and Na-22, are produced in reactors. Short lived isotopes like Rb-82 and Ga-68 are made using generators and must be used on site due to the very short half life.

Hot Cells & Radiochemistry Labs

Radioisotopes in either gas or liquid phase are delivered from the cyclotron to a shielded hot cell by delivery lines.

Prior to delivery, chemistry synthesis apparatus are configured, reagents loaded, HPLC systems set up and a final product vial located for easy removal.

Activity ranges are typically 100 mCi to several Curies of activity.

Time constraints of several hours or 3X the half life are imposed due to the short isotope half life.

Radiochemistry Considerations

Specific Activity (SA):

The activity concentration (in Bq/mol) of the isotope. Related to the ratio of radioactive atoms to non-radioactive atoms

Carrier Free:

Only the radioactive form of the isotope is present, without the corresponding non-radioactive isotope

Carrier Added:

The non-radioactive isotope is added or present during the preparation

Preparation Volume:

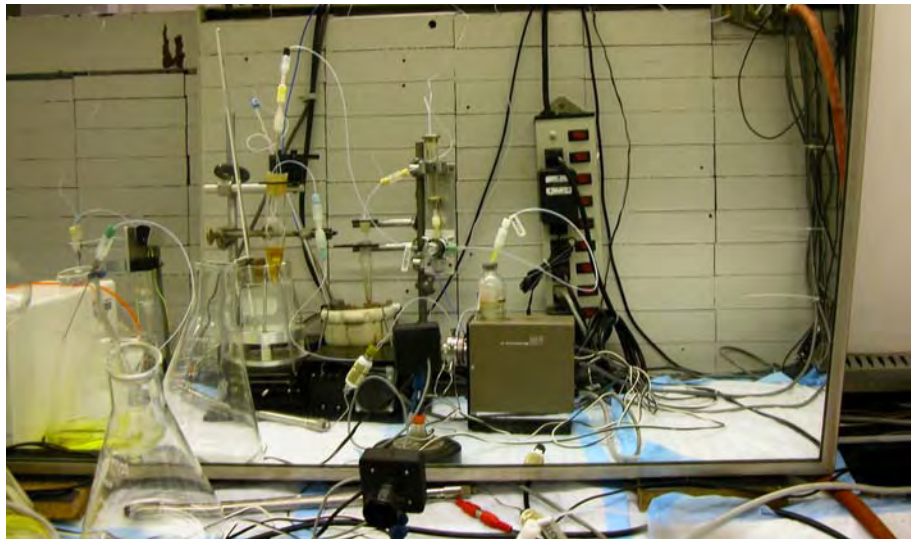
Mice have only ~2 ml of blood, only >250 uL can be injected

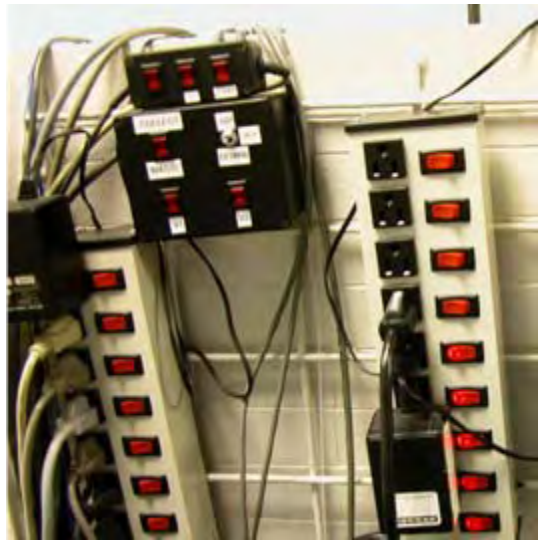
Radiochemistry Methods: Automated

Automated radiochemistry synthesis boxes are becoming increasingly prevalent and are now available from many companies.

These boxes are suitable for fast, reproducible and routine productions. In most cases, they can also be programmed to explore research chemistry methods for new imaging probe production.

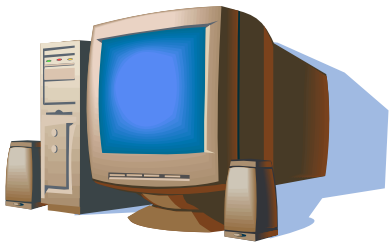
Radiochemistry Methods: Manual

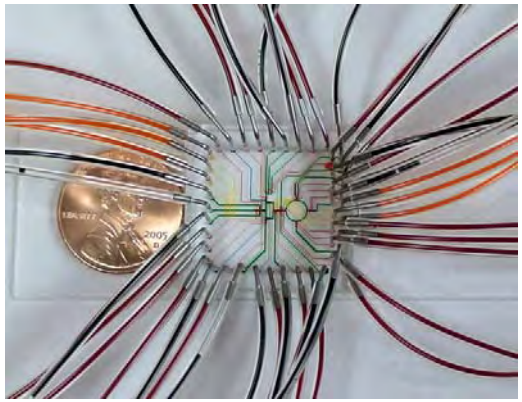




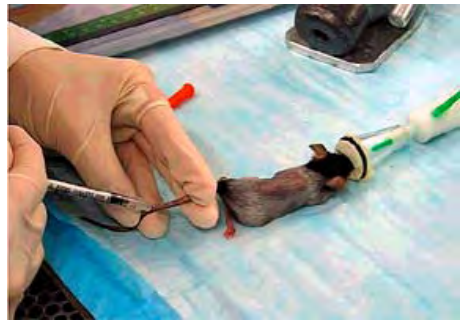
Manual methods are used for experimenting with new radiochemistry techniques. Syringes can be used to push or pull reactants, switches used to control valves, heating cooling and other equipment. Tongs and other remote manual handling devices might be used to transfer liquid transfer lines from waste to collection vials.

Radiochemistry of the Future





10 min?

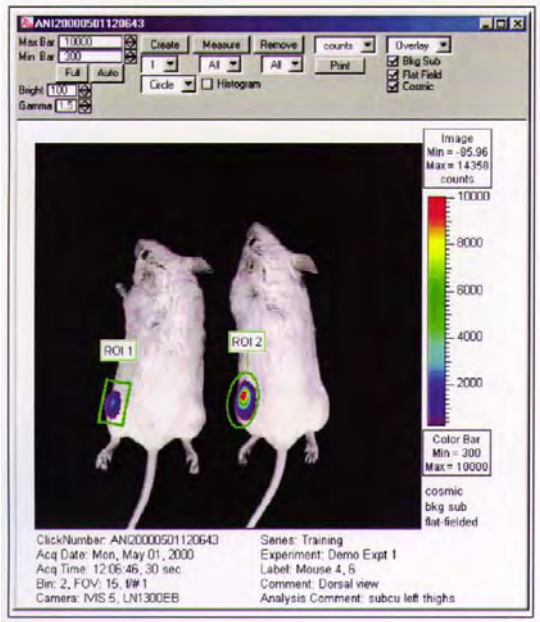


The goal is to move more towards probe synthesis and imaging on demand, to closer meet the needs of the biological community to image at the optimal times for the biological process under investigation.

Eventually the goal is to have multiple chip types with computer controlled synthesis to make a wide range of compounds as needed. This approach would require centralized F-18 production and delivery, but decentralized and site-specific synthesis of any compound desired.

Imaging Modalities and Examples

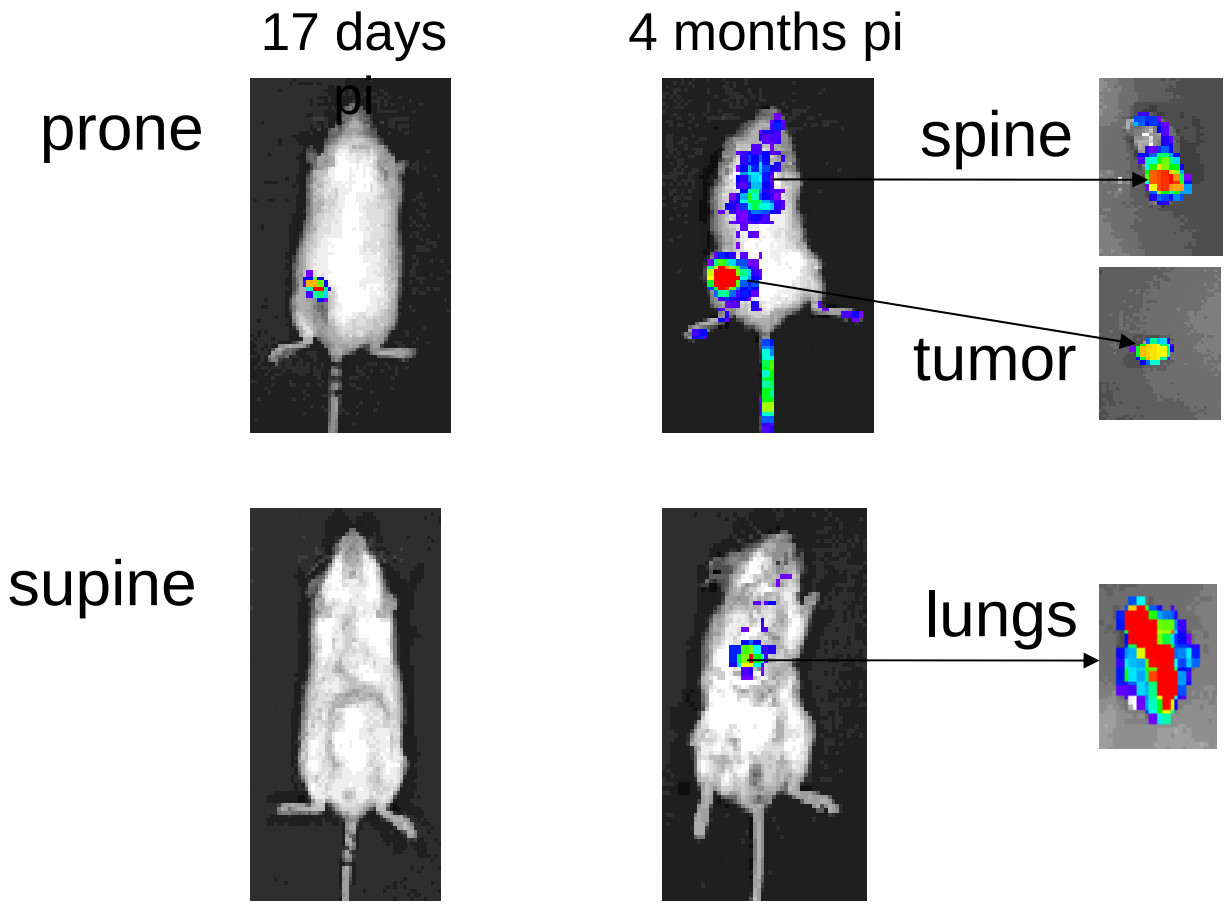
Optical Imaging



Two major types:

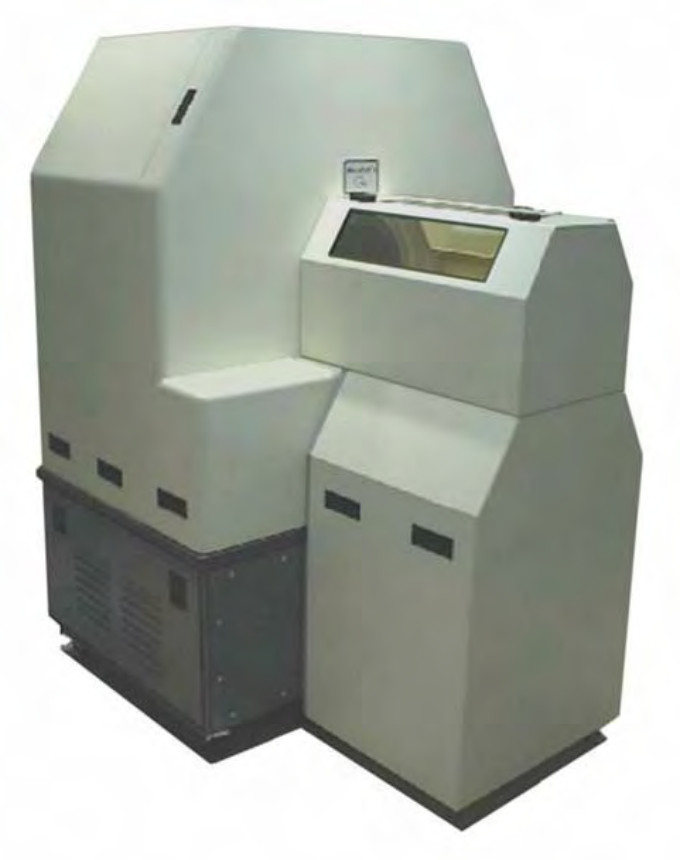
- **Bioluminescent Imaging**: Light generated within animal, emitted and detected
- **Fluorescent Imaging**: Light shined on animal, fluorophore wave shifts the light, light travels back out for detection

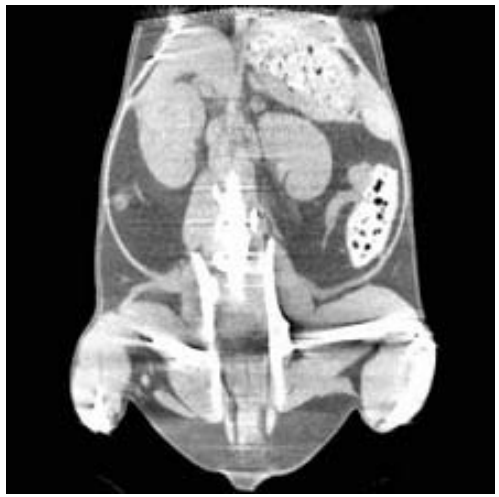
Optical Bioluminescence Example

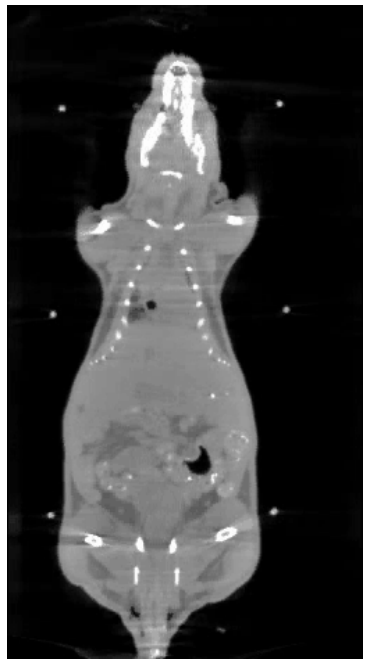


Optical Imaging of Prostate Tumor Metastasis in LAPC-4 Cell Line. Dr. Lily Wu. Unexpected prostate cancer metastasis in lung of mouse.

microCT small animal CT scanner

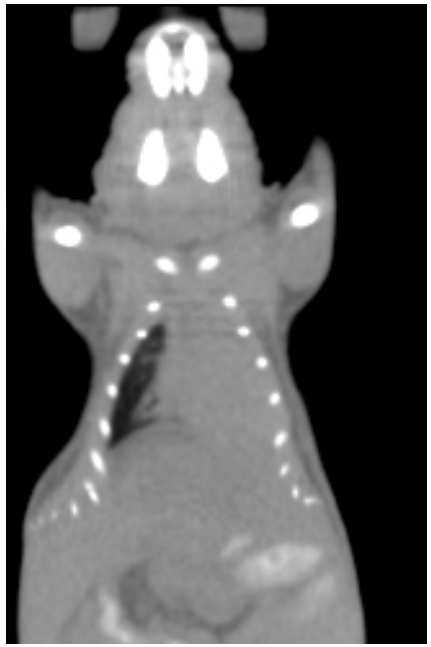


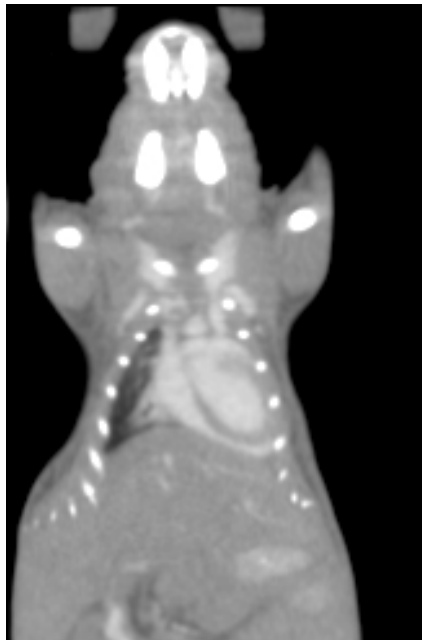




MicroCT system like the one shown above are capable of 50 micron resolution (~25 micron pixel size) or greater. High resolution is only possible in ex vivo imaging protocols, since heart and respirator movement will blur in vivo images.

Imaging with Fenestra VC



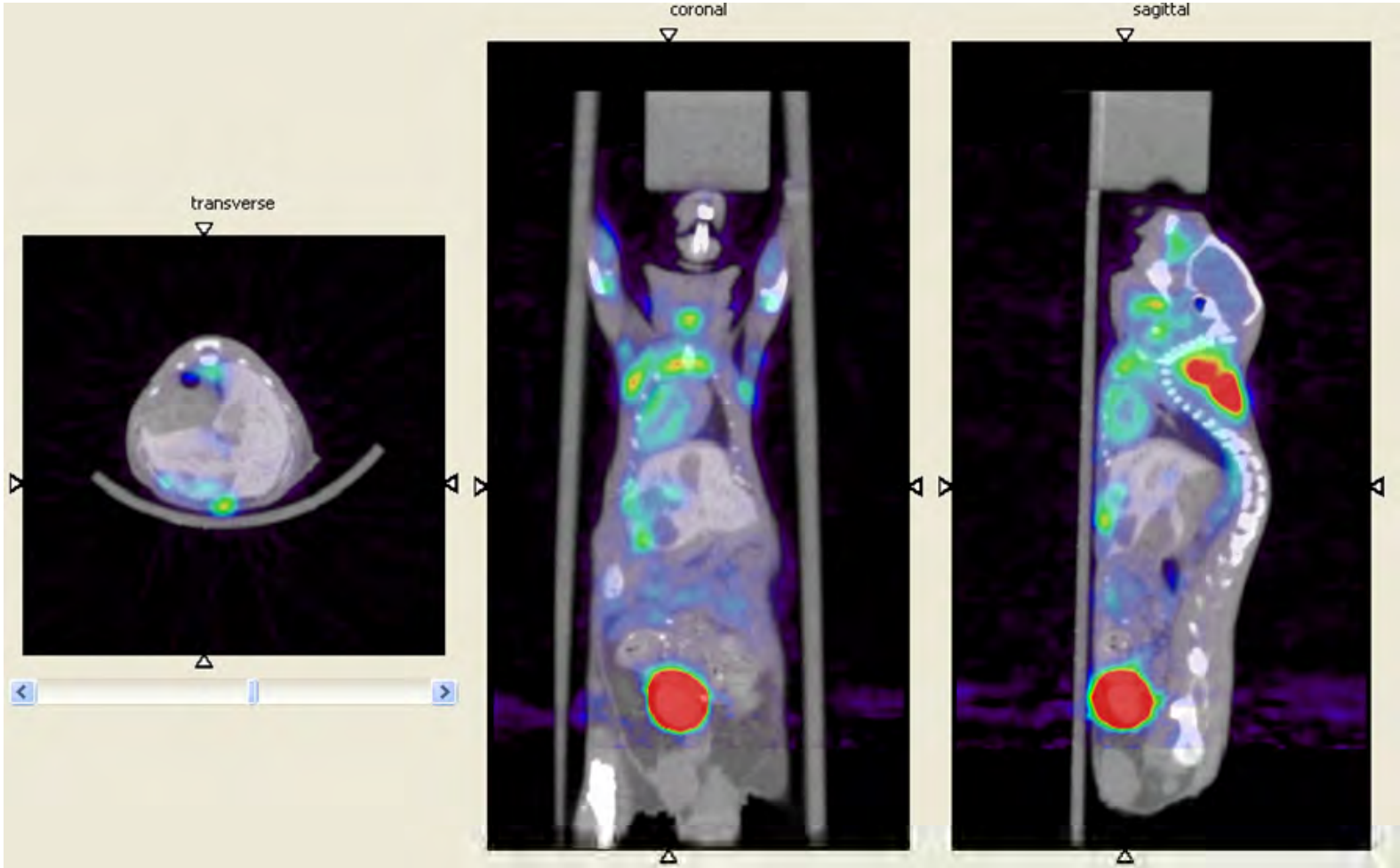


Unlike human CT scanners, most preclinical CT systems required several minutes to acquire data, thus conventional CT contrast agents that last 2-3 seconds are not suitable.

Recently longer lasting liver & vascular CT contrast agent Fenestra has been developed.

Courtesy of Dr. Kreissl

Liver Contrast Agent for microCT

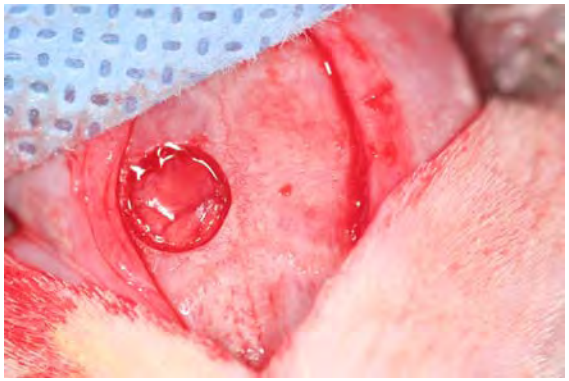


FDG PET with liver contrast CT showing implanted tumors.
Note high brown fat uptake due to insufficient heating during uptake.

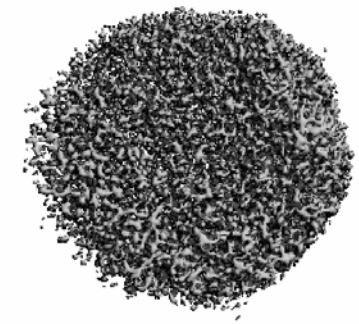
Courtesy of Dr. Herschman

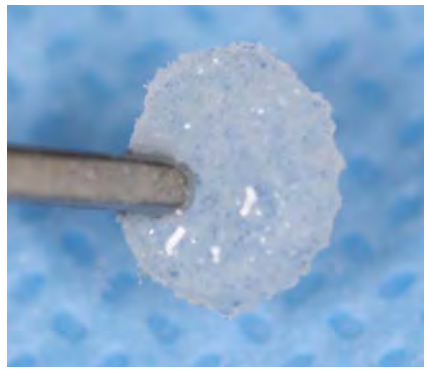
Optical-CT Multimodality imaging: Fluorescent Growth Factor on implanted scaffold

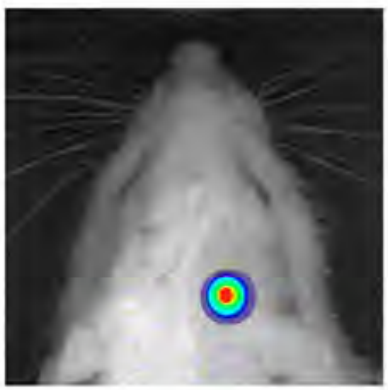
calvarial defect

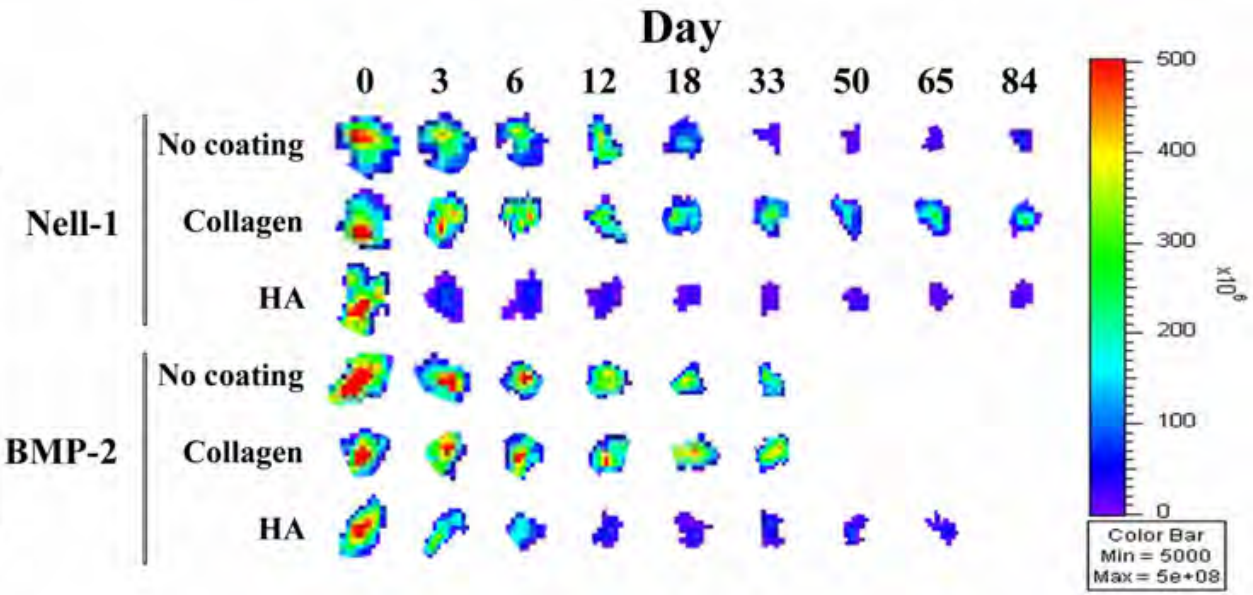


mCT of PLGA scaffold (5mm diameter)









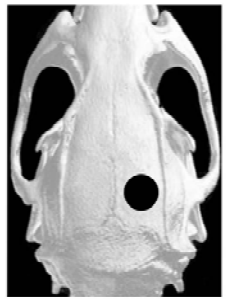
Courtesy of Dr. Cowan

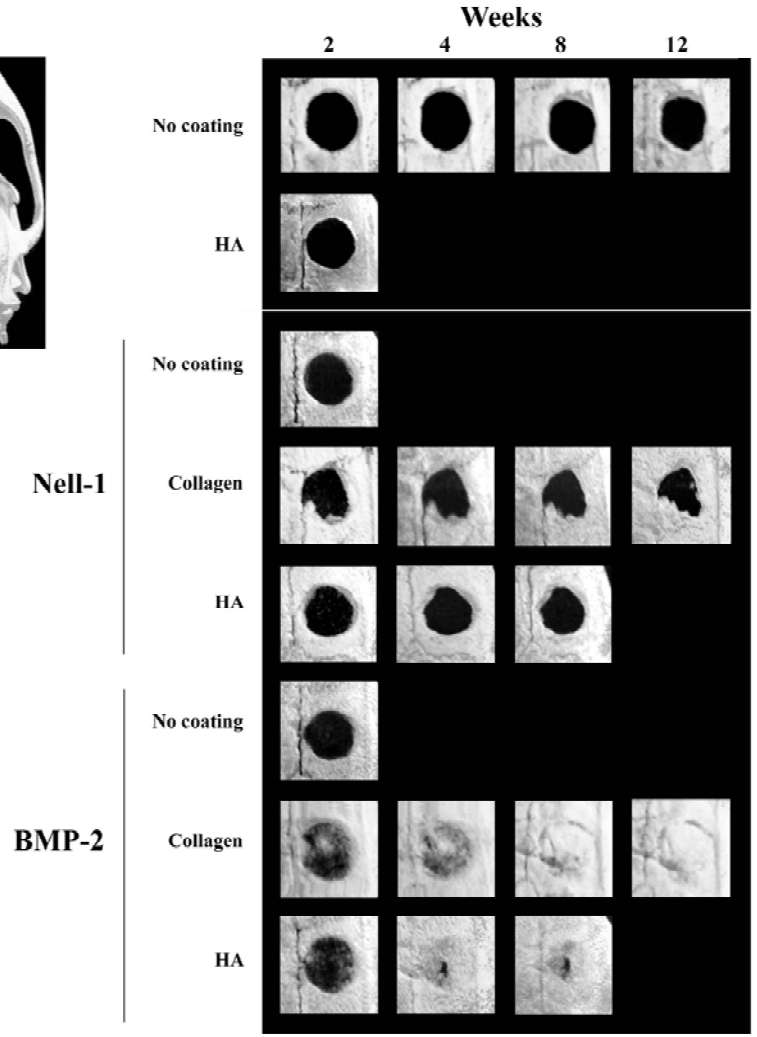
Optical-CT Multimodality imaging: Bone Formation Assessed using MicroCT

Optical fluorescent signal is linked to the growth factor, thus optical images show drug delivery over time.

CT images show bone growth over time.

The two imaging methods provide complementary information.

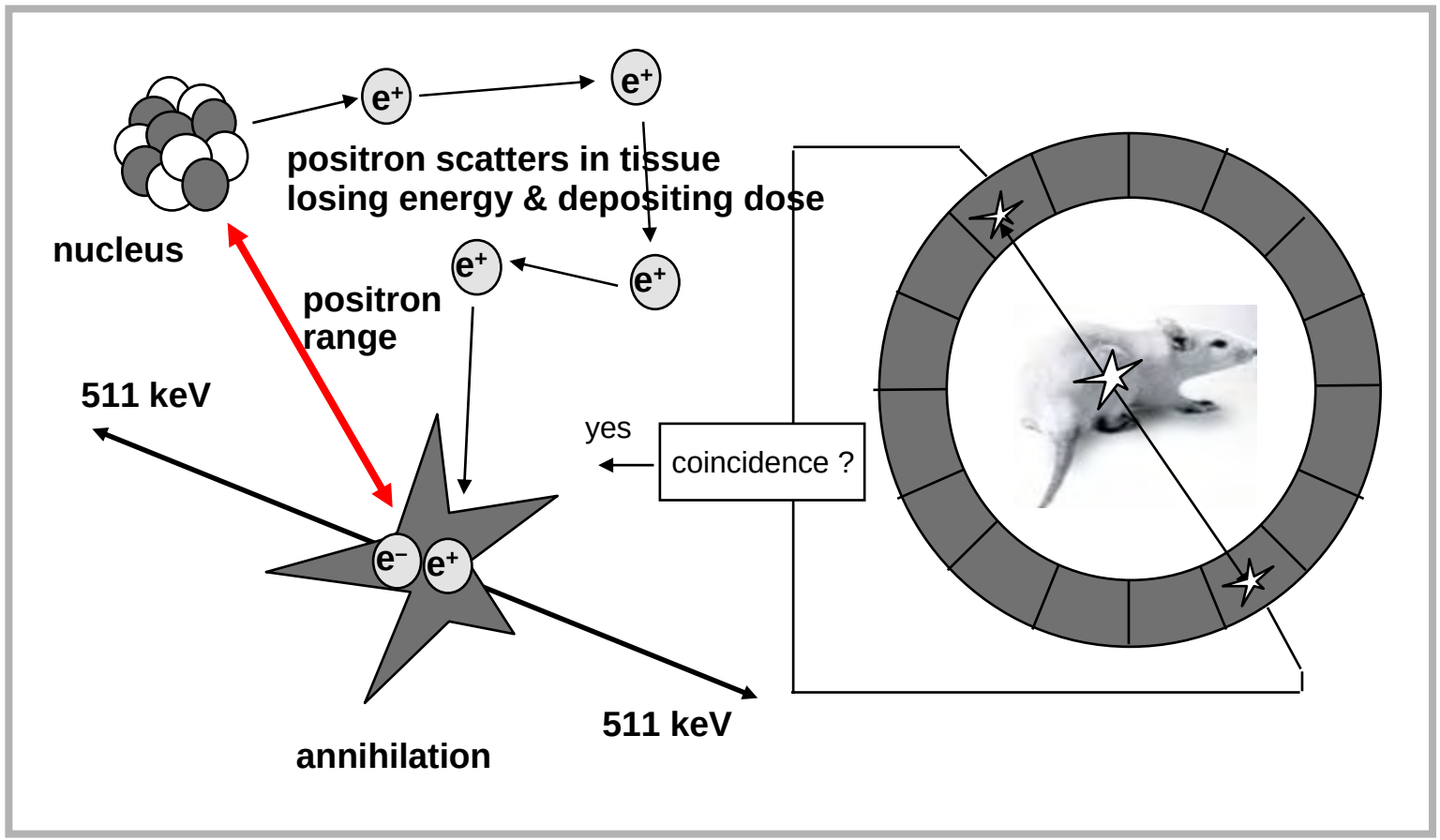




(some data missing since project is still underway)

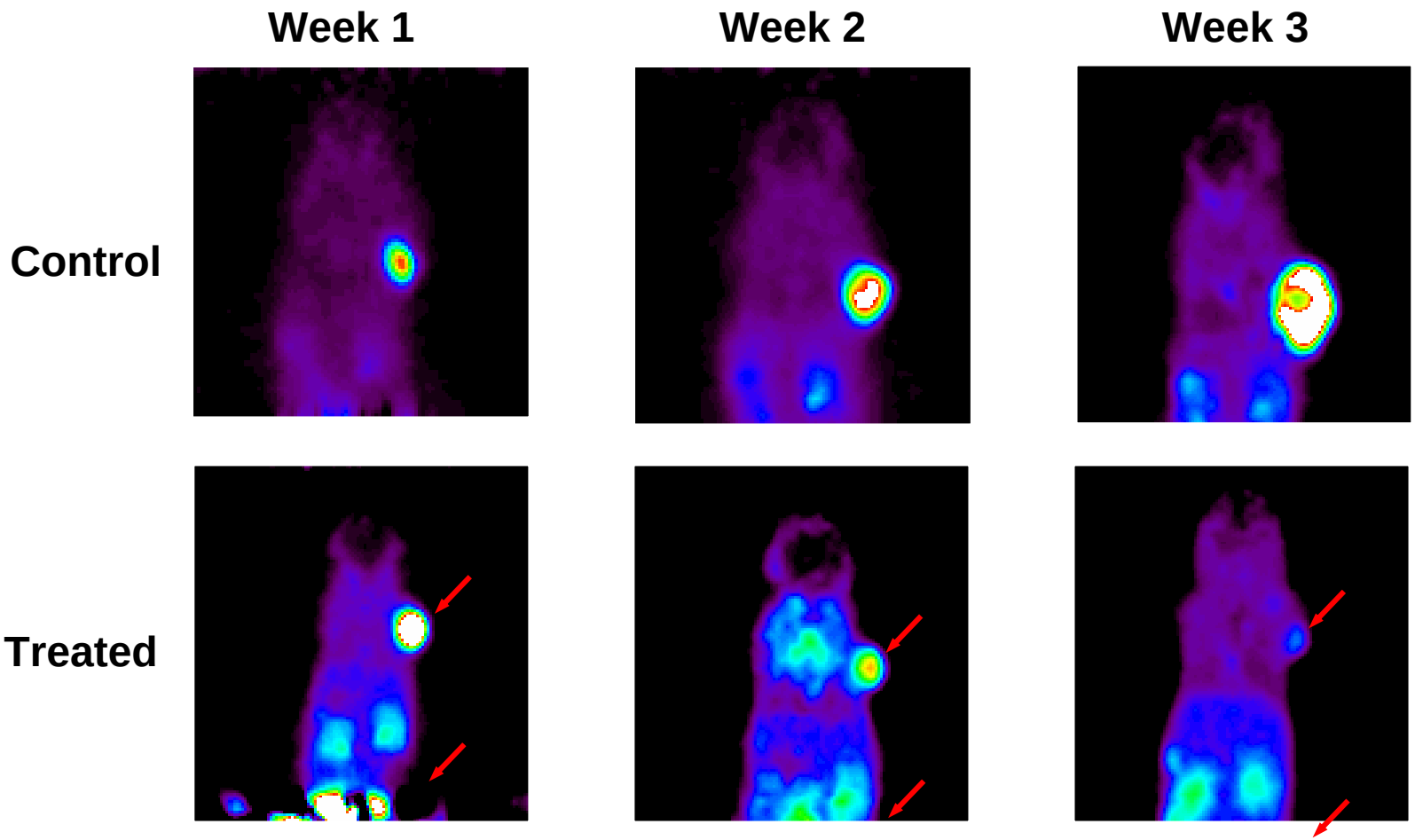
Courtesy of Dr. Cowan

Positron Emission Tomography



A positron is ejected from the nucleus with a characteristic kinetic energy, which is locally deposited as radiation dose and is related to the distance the positron travels before annihilation (positron range). Annihilation with an electron creates two 511 keV gamma rays emitted in opposite directions. By detecting these gamma rays, an image can be determined.

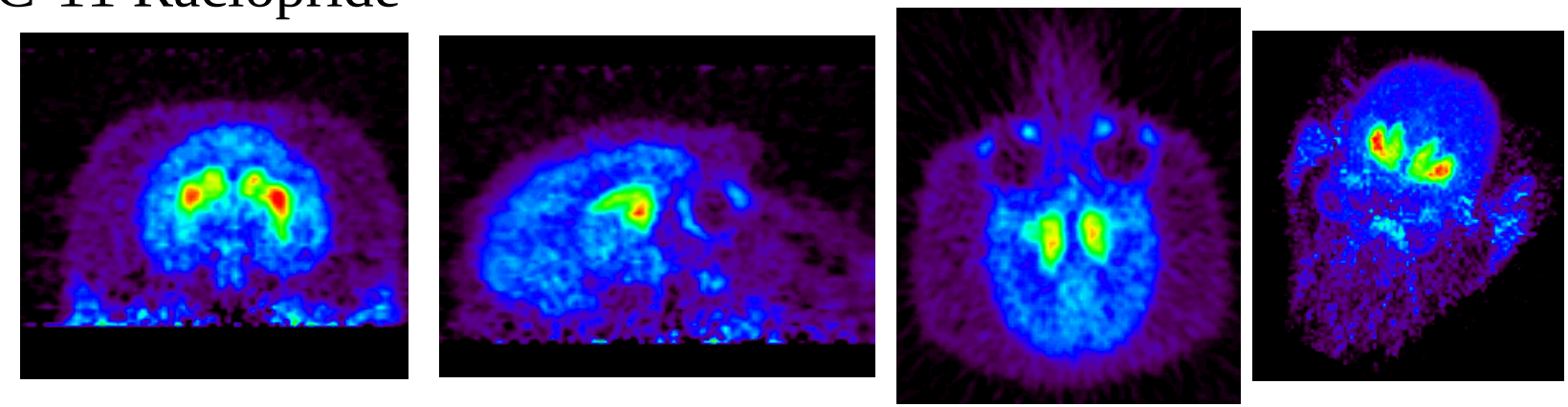
Oncology Imaging:
FLT Measurements of DNA Synthesis



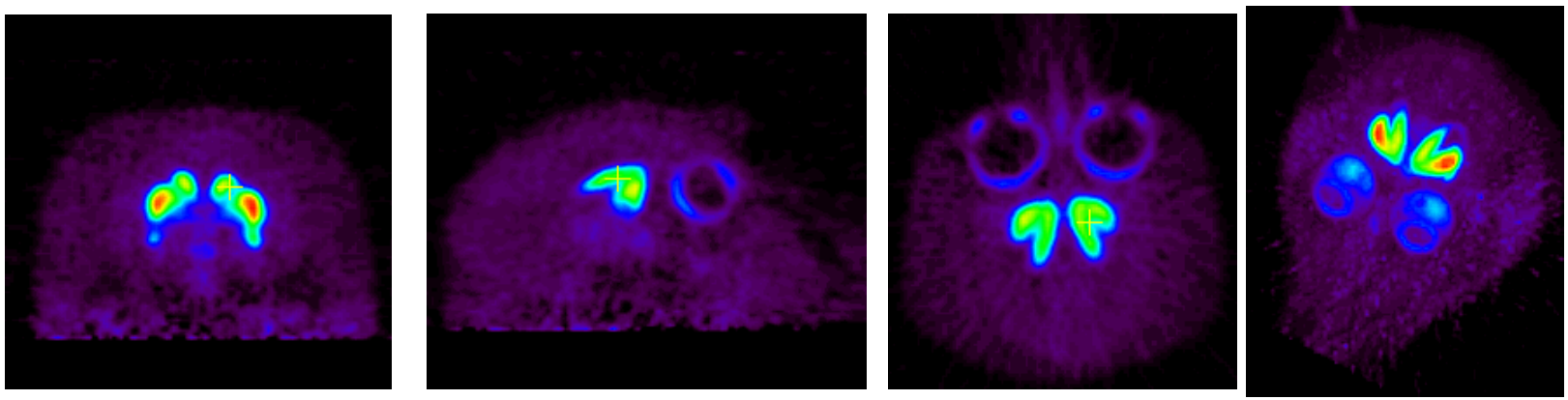
Courtesy of Drs. C. Waldherr & J. Czernin

D2 Receptor Ligand Imaging of vervet monkey in P4 microPET

C-11 Raclopride



F-18 Fallypride



Coronal Sagittal Transverse Projection View

Courtesy of Drs. B. Lee & E. London

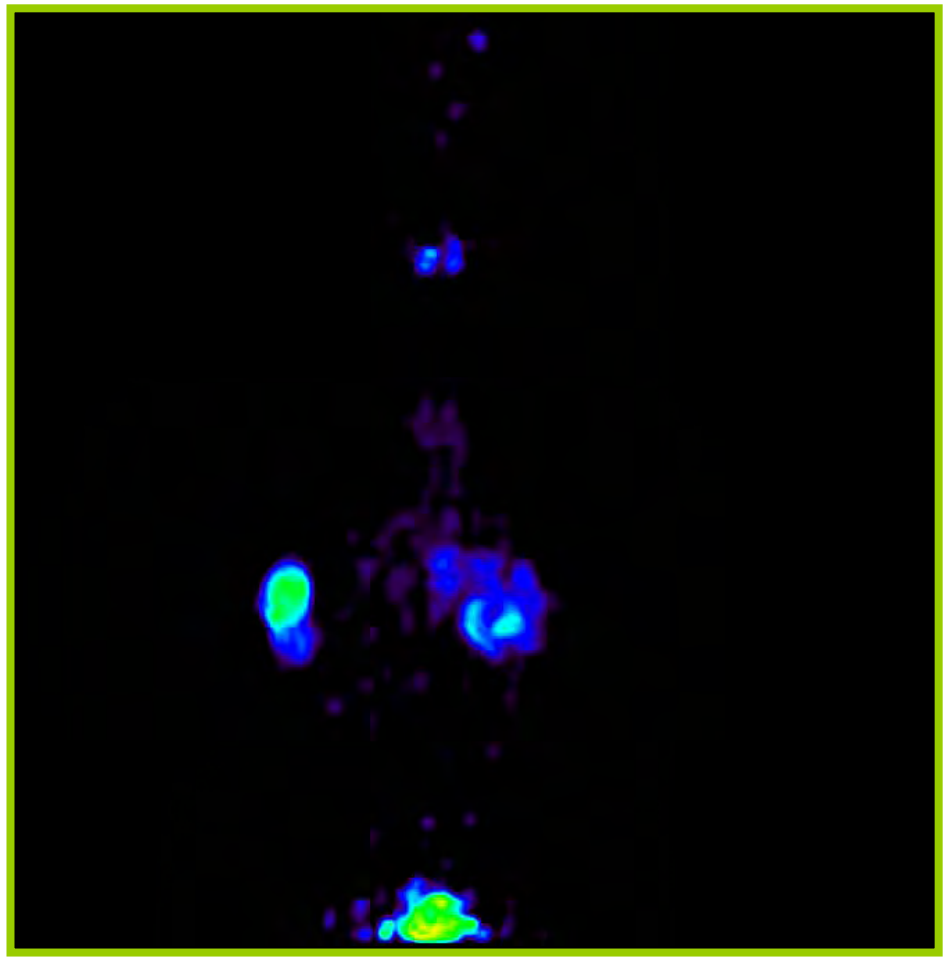
microPET: Oncology
PET-Only: What is it?

Human colon cancer xenograft

100 uCi I-124

Labeled anti-CEA engineered antibody fragment (tunable pharmacokinetics)

Where is the activity located?
What are we looking at?



Courtesy of Drs. V. Kenanova & A. Wu

microPET: Oncology

Dual Modality imaging: PET & CT

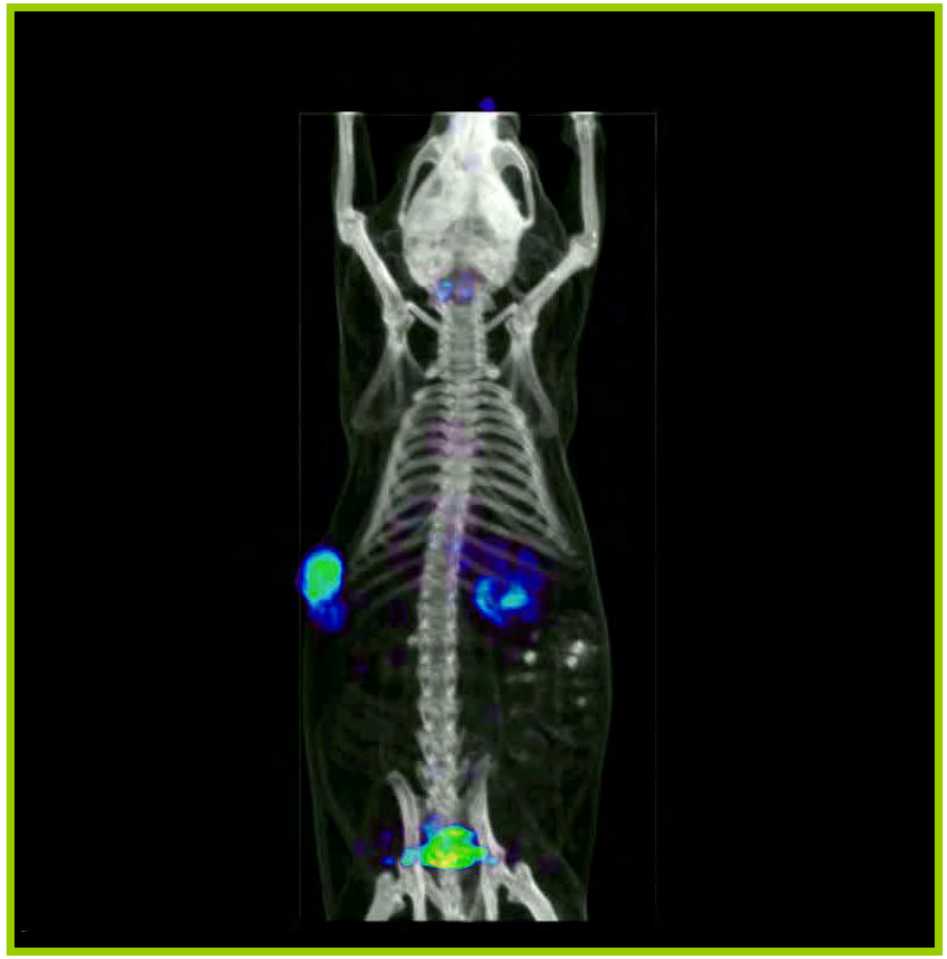
Human colon cancer xenograft

100 uCi I-124

Labeled anti-CEA engineered antibody fragment (tunable pharmacokinetics)

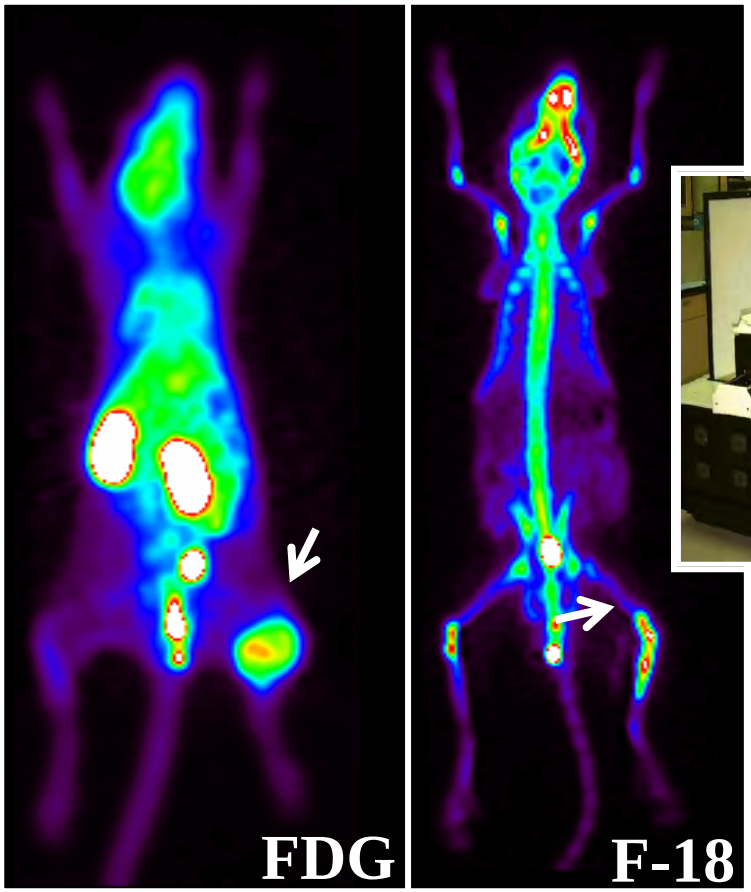
Screening CT scan (200 um)

Adding the anatomical information makes the image understandable.



Courtesy of Drs. V. Kenanova & A. Wu

Prostate Cancer: Functional & Morphological Imaging of a Tibia Lesion



µPET

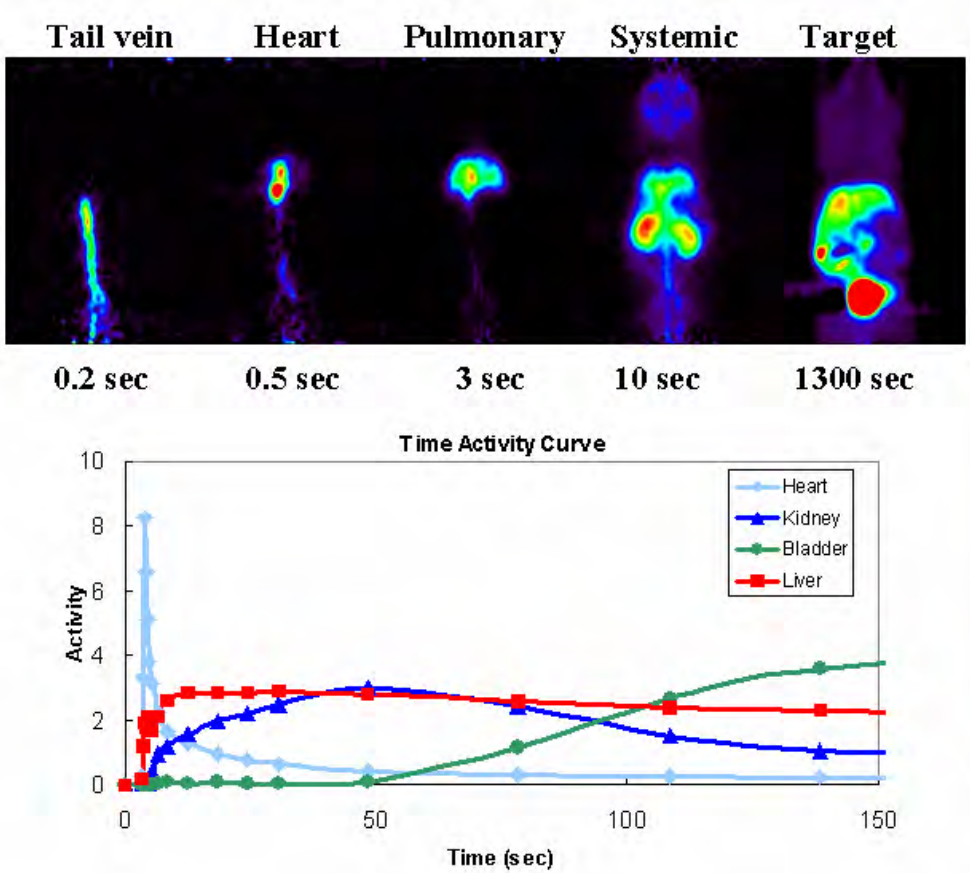
&

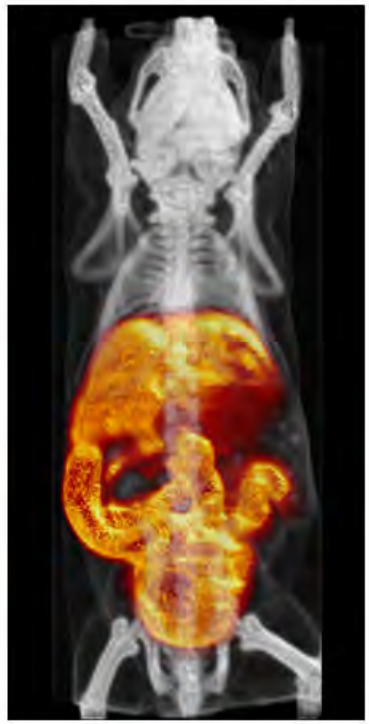
µCAT

These 3 images of the same mouse show tumor inflammation (FDG), bone formation (Fluoride Ion) and bone degradation (microCT).

Courtesy of Drs. F. Berger & S. Gambhir

Dynamic Imaging of FHBG

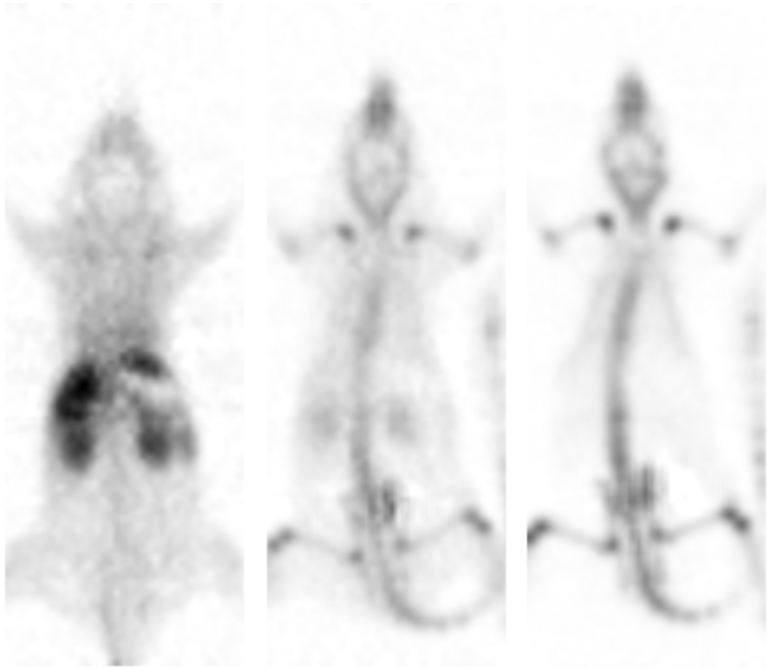




PET imaging is capable of capturing images over time, showing the movement of imaging probe throughout the animal.

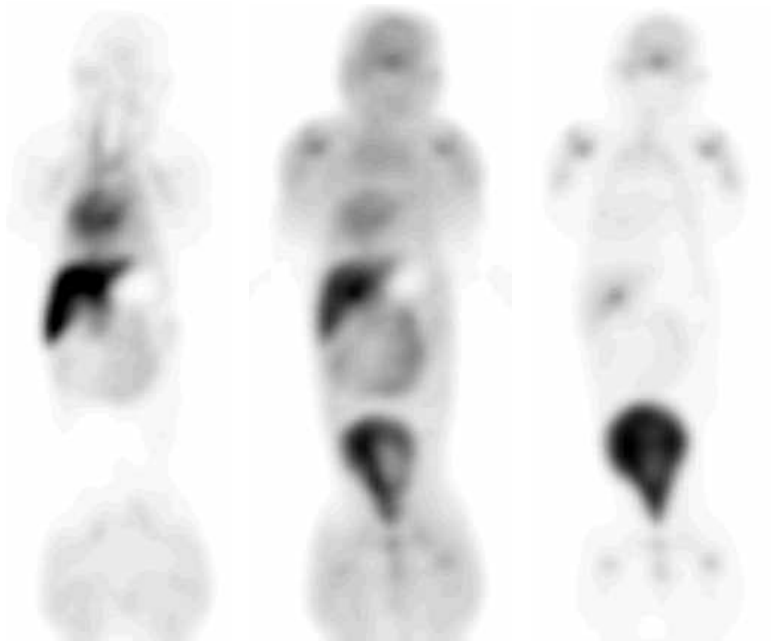
Courtesy of Drs. D. Stout & A. Chatziioannou

Biodistribution, Dosimetry & Toxicology



5 min 15 min 60 min

Mouse in microPET



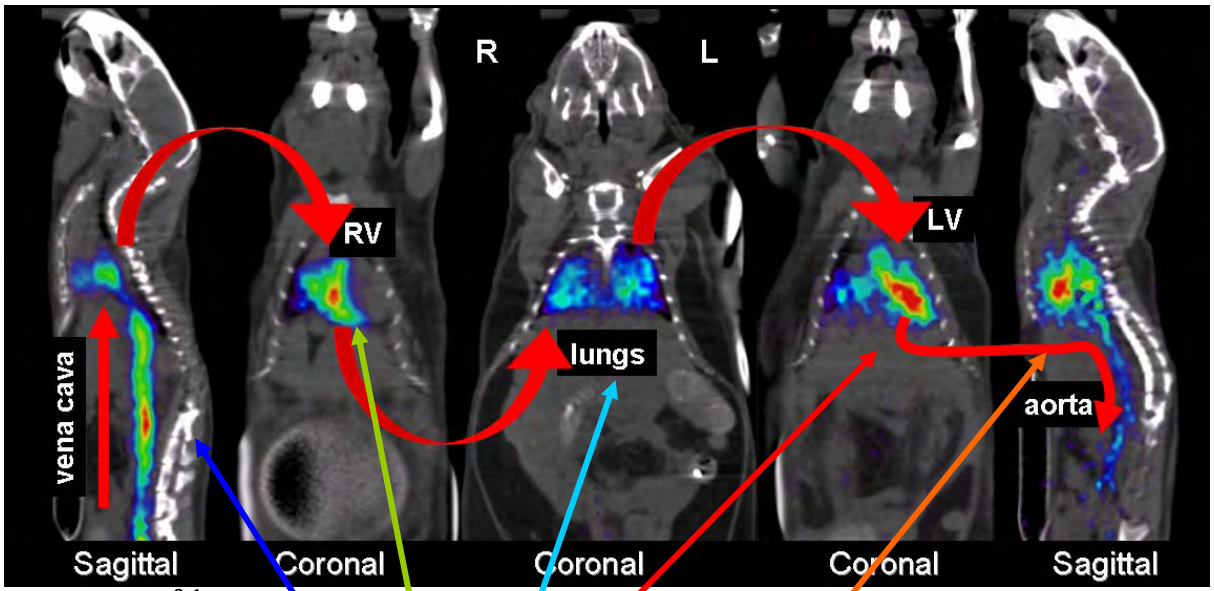
3 min 45 min 120 min

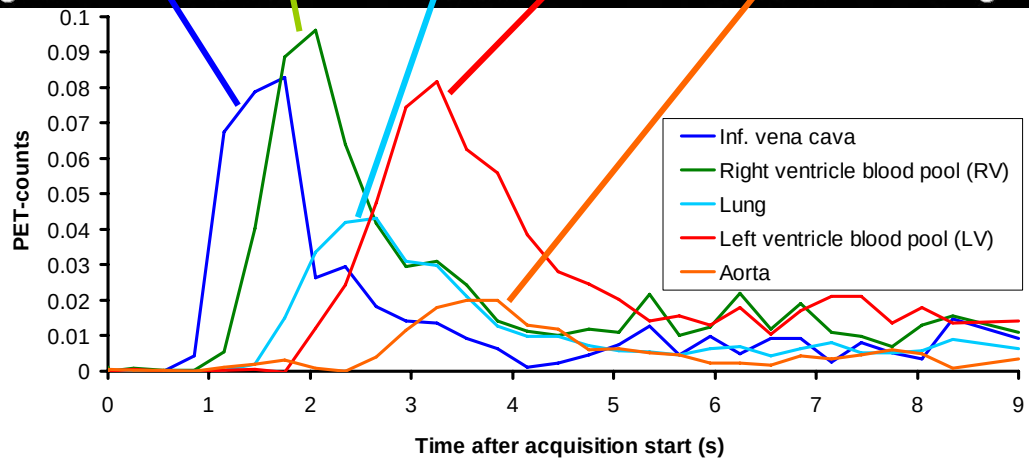
Primate in Clinical PET system

Biodistribution studies are simple to do with dynamic PET imaging.
This data also can provide radiation dosimetry estimates.

Courtesy of Drs. D. Stout & J. Czernin

Fast Temporal Imaging: First past transit





Courtesy of Dr. Kreissl

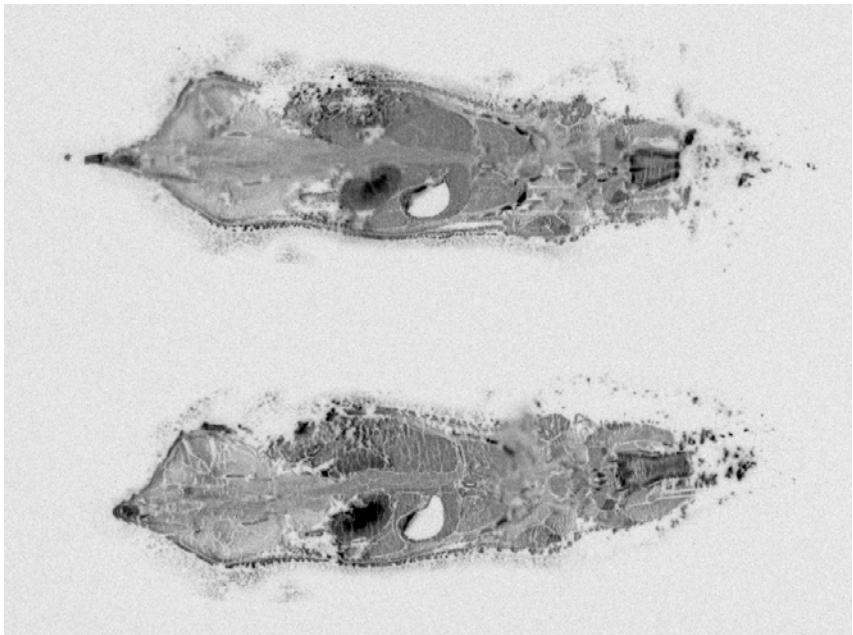
Autoradiography

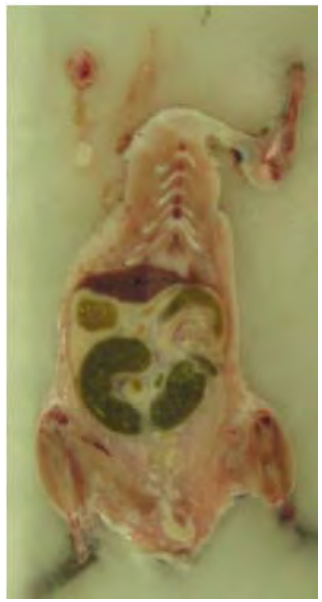
Autoradiography and tissue dissections can produce 25-200 micron resolution images and sensitive assay measurements, but this technique requires sacrifice of the animals and only provide information from a single point in time.

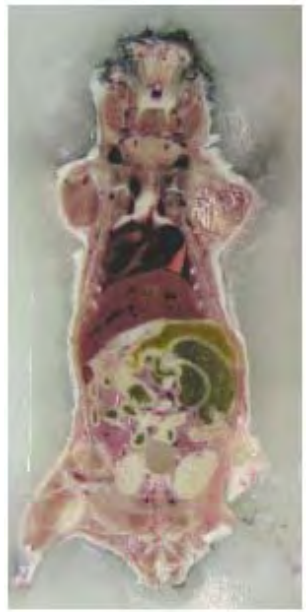
Autorad Results

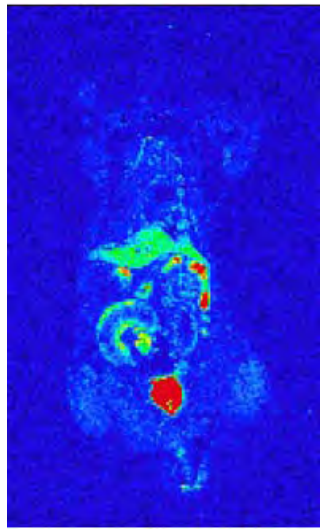
Photographic and autoradiographic images are often used together to help localize where probes go in tissue.

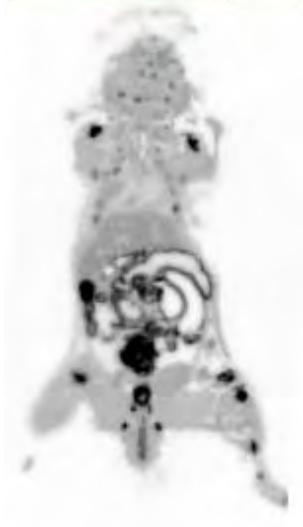
This method is good for high resolution imaging work to more closely identify where the probe is take up, but provides only a single point in time.



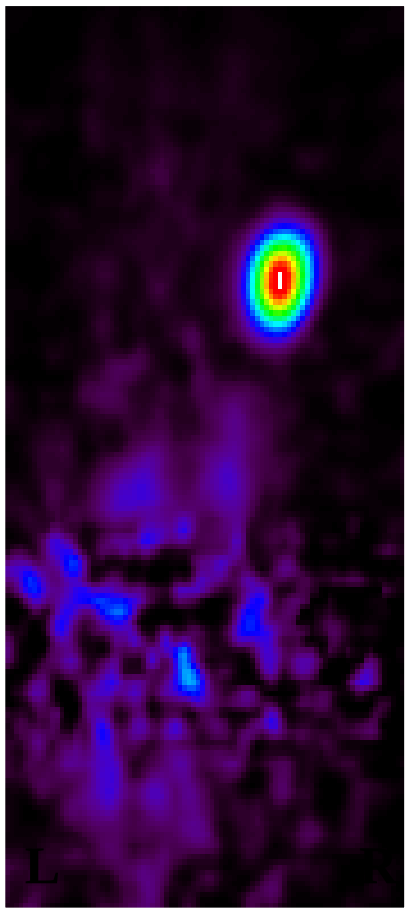




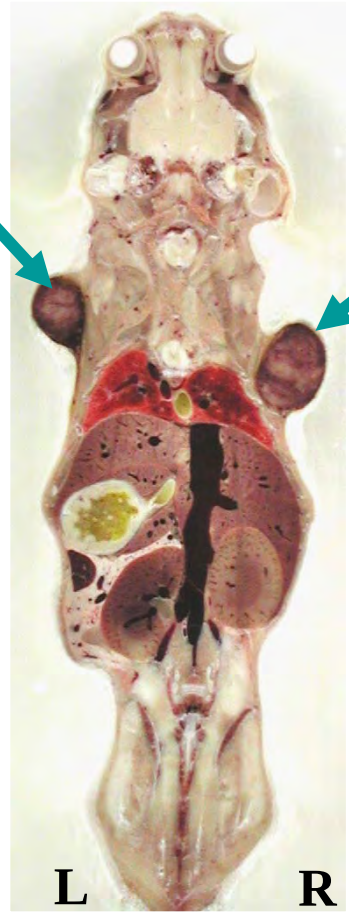




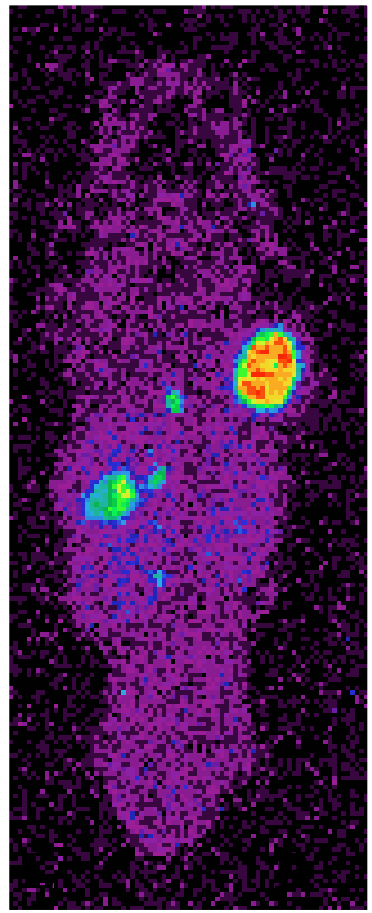
Imaging of Prostate Cancer Using PET-Reporter Gene and FHBG



microPET



Photo



Autorad

Courtesy of Dr. Sam Gambhir

Summary

- Several in vivo imaging methods exist to follow the same animals over time
- Preclinical research increasingly uses more than one imaging modality
- Preclinical imaging is a rapidly growing field
- Facilities planning requires knowledge of systems, usage patterns and data flow to create an optimal plan