

Small Animal PET Imaging for the Study of Diseases and Drug Development

Richard Laforest, Ph.D.

Mallinckrodt Institute of Radiology,
Washington University, St. Louis, MO, U.S.A.

Positron Emission Tomography (PET) has been used clinically for a few years already for staging and evaluation of malignancy of numerous forms of cancer. Its strength resides in the ability to accurately measure the amount of tracer accumulation in organs. The recent progress of scanner development has made possible the construction of imaging devices with less than 2 mm spatial resolution, sufficient for the imaging of radiotracers in small laboratory animals, such as mice or rats. This capability is very attractive as it permits the possibility to study diseases *in vivo*, to monitor the response to

novel therapy, and to evaluate the action of newly developed drugs. The wide availability of animal models, often from transgenic animals, allows for faster and lower cost development of more efficacious treatment strategies, the most successful of which will eventually be used in humans.

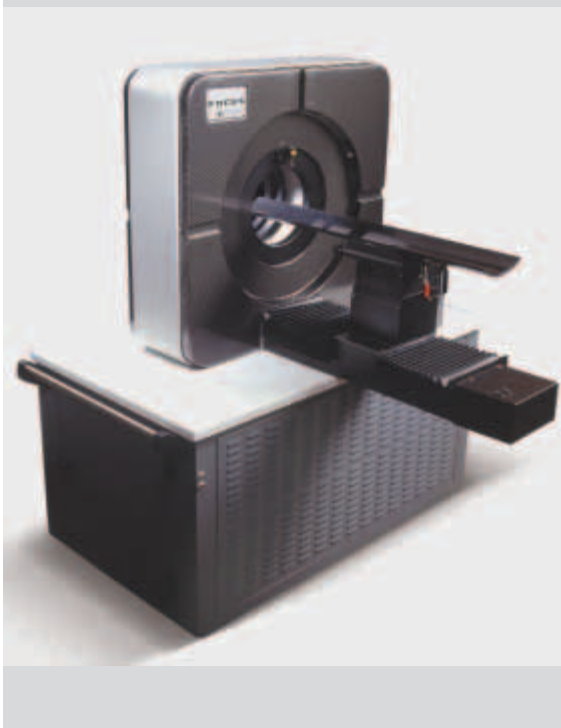
At the heart of nuclear medicine is the tracer principle. The recognition that the location and quantitation, *in vivo*, of a minute amount of tracer by mass can be imaged and can lead to a diagnosis that changes the outcome of a patient's treatment is the most fundamental concept that has resulted from the development of nuclear medicine imaging technologies. PET, by its design, merges the best combination of sensitivity and spatial resolution and makes this technology particularly well suited for drug development and the study of diseases. Over the last decade, several small animal imaging systems have been developed and now small animal PET imaging, namely in mice, rats, or small nonhuman primates, offers the added benefit that the animals can be their own control, reducing the interanimal variability, and making repeat studies on the same animal possible. Small animal PET is particularly useful for testing hypotheses during the study of diseases and in the development of new imaging and therapeutic drugs. It is thus not surprising that small animal PET now provides the best tool for the accurate measurement of the pharmacokinetics of imaging agents *in vivo*.

Overview of Technology

In PET imaging systems, images are formed by the detection of the two high-energy photons emitted by the annihilation of the positron emitted during the decay of an unstable radioactive nuclide via the beta-decay process. Positron-emitting nuclides are attached to biological molecules or analogs and can be monitored *in vivo* after injection into a living animal or human being. Projection view images are created by arrays of gamma ray detectors surrounding the subject. By combining multiple view angles from around the subject, volumetric maps of the activity distribution in the subject can be calculated by tomographic image reconstruction techniques. Since the two photons are emitted nearly back to back, there is no need for mechanical collimation. The line joining the two detectors determines that the radionuclide that emitted the positron is located along this line of response. Since no collimator is required, sensitivity can be maximized and is typically increased by at least two orders of magnitude, compared to Single Photon Emission Tomography (SPECT). PET cameras are characterized by their sensitivity and spatial resolution. Simultaneous optimization of these two parameters is generally

difficult and the required necessary resolution in small animal imaging must be of the order of 1–2 mm or less. High resolution is achieved by decreasing the sampling distance with the use of smaller detector elements. Because the crystals can be cut into small elements, high-atomic number (i.e., high detection efficiency) scintillators have been the preferred detection technology. Recently, scintillation materials such as LSO, LYSO, or GSO have replaced the traditionally used BGO, due to their more favorable properties of speed, light output, and detection efficiency. Scintillation light output is collected by photo-multiplier tubes (PMTs) and in some systems, the use of position-sensitive photomultiplier tubes has enabled the construction of highly compact systems. These faster detector crystals allow for improved suppression of random events and enhanced acquisition efficiency at high count rates.

The microPET® System.



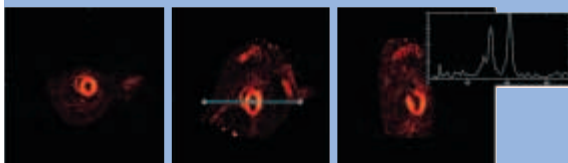
These two effects have direct influence on image quality and quantitative accuracy.

The optimization of sensitivity or resolution is usually mutually exclusive; high sensitivity is usually achieved at the cost of resolution. High resolution demands a fine sampling of the object, which is achieved with the use of a larger number of smaller detection elements. The sensitivity-per-detector element is thus reduced. In the last few years, maximization of sensitivity has been achieved by improving crystal-cutting technology and by reducing the gap separating the crystals by using reflective material. The depth at which the high-energy photons interact in the detector material often results in degradation of the spatial resolution, and a tradeoff therefore exists between sensitivity and resolution. Thicker detector material improves sensitivity, but inexorably leads to the deterioration of spatial resolution. A few imaging systems can determine the depth of interaction (DOI) and many are being developed. Only experimentation with devices that provide access

to the DOI information can determine the degree of improvement in PET resolution and sensitivity. Reviews of the DOI technology and applications can also be found in [1–3].

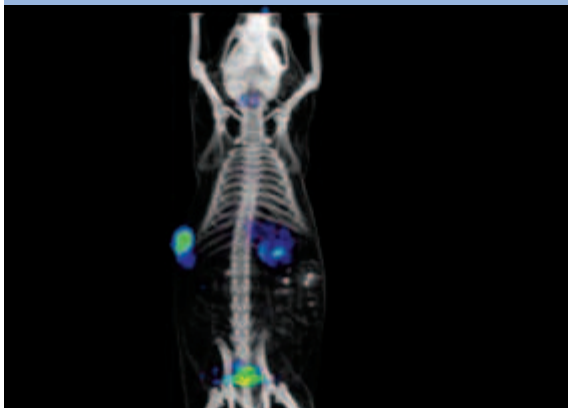
PET Applications

PET has the ability to quantify the activity concentration localized in living bodies. The use of small animals as imaging subjects can thus facilitate the development of new drugs for both diagnosis and therapy, but PET is also useful for the study of disease progression or the study of treatment efficacy. Applications can be found in many areas including oncology, cardiology, neurology, pulmonology and immunology. Extensive development has been done by various research institutions and it would be impossible to include them all in this short review. For this reason, only the few major areas of applicability of PET are illustrated here. Several excellent review articles include extensive reference lists that give a more detailed presentation of each subfield of PET imaging [1, 4–6].



Time activity curves can be formed from dynamic data sets and fit to user-defined compartmental models.

(Courtesy of Washington University, St. Louis, MO, U.S.A.)



Fused PET/CT human colon cancer xenograft, with I-124 labeled anti-CEA engineered antibody fragment (tunable pharmacokinetics). Screening CT scan (400 μm).

(Courtesy of Crump Institute for Molecular Imaging, Los Angeles, CA, U.S.A.)

Imaging Flow

A radiopharmaceutical is delivered to tumors or other target tissues by blood flow, and the simple mode of delivery is the one-pass extraction from the blood stream. Agents such as labeled $^{15}\text{O-H}_2\text{O}$ or $^{15}\text{O-O}_2$ are prime examples. Active transport occurs when the imaging agent is taken up by tumor cells by an active membrane transport mechanism, like cationic or lipophilic radiopharmaceuticals. For example, antitumor properties were discovered in bis-thiosemicarbazones or analogs labeled with Cu. These neutral lipophilic radiopharmaceuticals are rapidly taken up by cells where Cu is reduced, then dissociate and bind to intracellular proteins. Cu-PTSM is one analog that can be labeled with a radioactive copper isotope. Several positron-emitting isotopes of copper exist: ^{60}Cu , ^{61}Cu and ^{64}Cu are cyclotron produced [7], while ^{62}Cu can be produced using a generator [8]. This selection opens the possibility of selecting an isotope based on its half-life and the understanding of the time course of the agent's pharmacokinetics or perhaps other experimental needs or objectives.

Imaging Metabolism

The most widely used tracer in clinical PET is, without contest, FDG for imaging tumor diagnosis and for studying diseases of the brain and the heart. FDG, or 2-(^{18}F)fluoro-2-deoxy-D-glucose, is a sugar analog that is taken up by cells as any sugar would be. It is thus taken up more actively by cells with higher metabolism, such as those in tumors, in the brain, or in myocardial muscle. FDG metabolism differs from glucose in that FDG follows the glucose pathway, i. e. it is metabolized through the glycolytic system and phosphorylated by hexokinase to FDG-6-phosphate, but then catabolism is restricted by enzymatic action and ^{18}F -FDG gets trapped in the cell. The localization and quantitation of ^{18}F -FDG in tumors is a direct measurement of glucose metabolism, which is used clinically for detection, staging, and restaging of diseases, notably in oncology. An extensive review of FDG imaging and applications can be found in [9].

Cardiac metabolism can be studied in small animal PET with tracers such as ^{11}C -glucose (glucose consumption), ^{11}C -acetate (oxidation), and ^{11}C -palmitate (fatty acid metabolism). In particular, such studies are now being conducted in small animal models of the diabetic

heart. ^{11}C -acetate also finds application in tumor detection as an alternative to FDG, especially in brain, renal, and prostate malignancies. Choline is an essential substrate for cell membrane synthesis and ^{11}C -choline has shown potential use for tumor detection in cases where FDG lacks sensitivity. Cell proliferation can be monitored with an agent such as ^{18}F -FLT (fluorothymidine), an analog of thymidine, which can be directly incorporated into DNA. In addition, FLT may be more suitable than FDG in brain metastases because of its low incorporation into normal brain cells.

Imaging of Receptor-/Antigen-Specific Agents

Receptor-based imaging rests on the premise that specific receptors are overexpressed on the surface of some cells. The design of receptor ligands is aimed to optimize the high selectivity for a particular receptor, to minimize the nonspecific binding and to maximize the percentage of incorporation of the radionuclide. Designing agents with lower lipophilicity minimizes nonspecific binding. High contrast in images is obtained by a careful design of the probe to target-specific cell receptors with high affinity and with a low nonspecific affinity to other receptor sites. Receptor ligands are large molecules such as peptides or MoA fragments, or smaller organic molecules such as dopamine or folic acid. Small animal PET imaging permits rapid discrimination of biomolecules in small animal disease models and enables researchers to more efficiently focus on the best candidates in the development process of new imaging agents. For example, detailed understanding of the receptor systems for hormones and growth factors [10] derived from research in cancer biology can lead to imaging agents that give improved diagnostic accuracy and treatment effectiveness [10].

Considerable progress has been made in the study of somatostatin analogs as imaging and therapeutic agents for somatostatin receptor-positive tumors. Somatostatin analogs were among the first peptide-based tumor receptor imaging agents of the hormone somatostatin, which is a 14-amino acid peptide involved in the regulation and release of several hormones (e. g., growth hormones and hormones that stimulate the thyroid). The somatostatin analog, octreotide, has been used extensively due to its more favorable biological half-life compared with that of somatostatin

itself, which clears quickly. The 8-amino acid octreotide can be labeled with ^{111}In using DTPA and has been used clinically in SPECT (OctreoScan™, Mallinkrodt, Inc., St. Louis, MO). Octreotide can also be labeled with ^{68}Ga or ^{64}Cu to make it a PET marker. ^{64}Cu -TETA-octreotide has been labeled and is now being evaluated for neuroendocrine tumors. Other octreotide analogs have been labeled with ^{86}Y for PET imaging. For example, ^{86}Y -DOTA-Tyr3-octreotide demonstrated high-target uptake and rapid renal clearance.

Receptor sites for steroid hormones can be found in tens of thousands at the surface of prostate or breast cancer cells. Steroid ligands were labeled with radiohalogens for evaluation as imaging probes for hormone receptor-positive tumors. The ^{18}F -estrogen receptor ligand estradiol (FES) was one of the initial fluorinated estrogen analogs to be evaluated [11, 12]. Alternatively, prostate diseases can be studied with androgen receptor-specific radiopharmaceuticals; for example, ^{18}F -FDHT (fluoro-dihydrotestosterone), an analog of testosterone.

Imaging Response to Therapy

The focus of PET imaging traditionally has been on the imaging of tissue uptake with a tracer, yet PET can also be used as a marker for efficacy of therapeutic drugs. This is another application of FDG in which it is used to evaluate the recurrence of cancer after therapy. Another example, FES-PET, can be used to monitor the status of estrogen receptors in patients with primary and metastatic breast cancer. Studies have found that FDG-PET was most sensitive for staging, but FES-PET was the most accurate at predicting whether the patient would respond to hormonal therapy [11, 12]. The same concept has also been employed to determine whether prostate cancer would respond to androgen therapy.

Two major difficulties encountered in the treatment of cancer are multidrug resistance (MDR) and hypoxia (areas of low oxygenation). Multidrug resistance correlates with the overexpression of a family of associated MDR genes, which code for transport proteins that pump the drugs out of the cells. Imaging for MDR is performed with $^{99\text{m}}\text{Tc}$ -sestamibi in SPECT and a PET equivalent is now currently being evaluated using $^{94\text{m}}\text{Tc}$

(a positron emitter with $T_{1/2} = 52$ min). Hypoxic tumors have been shown to resist chemo- and radiotherapy, and a diagnostic test of hypoxia therefore would be useful clinically. The first radiopharmaceutical agents for hypoxia were based on nitromidazoles, and analogs have been labeled with ^{18}F for PET (^{18}F -MISO) and ^{123}I (azomycin) for SPECT. Another PET hypoxia agent can be found in ^{64}Cu -ATSM [13], which is a copper complex that was shown to delineate hypoxic tissue in *in vivo* tumors. Other agents exist and partly overcome the limitations of ^{18}F -MISO.

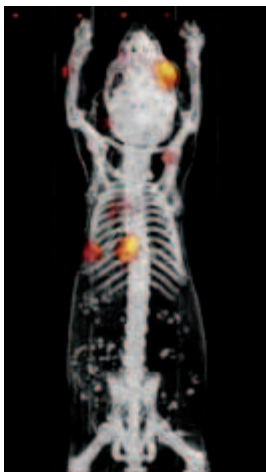
Imaging Gene Activity

There is presently an intense interest in molecular biology and molecular imaging in gene expression imaging, in which carefully designed radiolabeled molecular probes can target reporter genes in specific cells [3]. This area of research is very exciting because it allows, through imaging, the noninvasive tracking of biological changes resulting from the modification of genes in animals and, possibly, in humans. The basic concept is that a reporter gene is introduced into a target tissue using an appropriate vector (such as a virus). This reporter gene can produce enzymes that will be released under the presence of a PET reporter probe and these enzymes will trap the PET reporter probe inside the cell. Activity can thus be seen to gradually increase inside those cells that have expressed a particular gene as more enzymes are encoded/released, leading to signal amplification and thus improved sensitivity for detecting or monitoring a given disease. Two specific PET reporter genes are the herpes simplex type 1 virus thymidine kinase (HSV1-tk) and dopamine type 2 receptor (D2R). Various PET reporter probes can be phosphorylated by enzymes encoded by the HSV1-tk reporter gene. Notably, FHBG (^{18}F -labeled penciclovir) has been seen to be the most effective imaging probe. An important difficulty resides in selecting which tissue in the animal, either organ or tumor, receives the reporter gene. The technique to deliver the reporter genes can use either viruses or nonviral complexes. Also, transgenic mice can be bred to carry a specific reporter gene. In the former case, genes will be expressed only in cells in which the transcription was activated by a user-chosen promoter gene. These techniques have found applications for the monitoring of cell trafficking as cells metastasize in organs, ►

monitoring endogenous gene expression, studying the interaction of two populations of cells, and studying gene delivery and expression in living animals – with the goal of optimizing the delivery of a specific gene to a given target [5]. In a specific example, this technique has been applied for the study of quantitative evaluation of gene expression in pulmonary diseases [14].

Conclusion and Outlook

The use of small animal PET imaging systems has led to multiple advances in drug discovery and the study of diseases. Drug discovery can be expedited in multiple ways as a single test can demonstrate the stability and pharmacokinetics in vivo of a given drug and thus enabling a faster decision process from a pharmaceutical company regarding continued investment in a given family of tracer. As small animal PET cameras are now commercially available, additional biotechnology laboratories and pharmaceutical companies are likely to adopt the technology. It is likely these labs will discover new applications of PET in animal research. On the technological side, electronic and computer advances will bring us better and more performing scanners. The next leap is likely to be in the area of multilayer systems with depth of interaction for both improved sensitivity and resolution. The combination of PET with CT is currently being offered, and PET combined with MRI is on the horizon. In short, PET provides the best opportunity for disease study, due in part to its intrinsic sensitivity and resolution and also to the recognized sensitivity and specificity of the radiotracers that have been developed through the years.



*PET/CT image.
(Courtesy of
Crump Institute for
Molecular Imaging,
Los Angeles, CA, U.S.A.)*

E/E

For more information, please contact
laforestr@mir.wustl.edu

References:

- [1] Lewis JS, AS, Garbow JR, Laforest R, Welch MJ, Small animal imaging. Current technology and perspectives for oncological imaging. *Eur. J. Cancer*, 2002 (2173-88).
- [2] Chatziioannou AF, Molecular imaging of small animals with dedicated PET tomographs. *Eur. J. of Nucl. Med.*, 2002. 29 (1): p. 98-114.
- [3] Cherry SR and Gambhir SS, Use of positron emission tomography in animal research. *ILAR Journal*, 2001. 42 (3): p. 219-232.
- [4] Cutler CS, Lewis JS, and Anderson CJ, Utilization of metabolic, transport and receptor-mediated processes to deliver agents for cancer diagnosis. *Adv. Drug Del. Reviews*, 1999. 37: p. 189-211.
- [5] Massoud TF and Gambhir SS, Molecular imaging in living subjects. *Genes Dev.*, 2003. 17: p. 545-580.
- [6] Floyd E and Mcshane TM, Development and use of biomarkers in oncology drug development. *Toxicologie Pathology*, 2004. 32 (1): p. 106-115.
- [7] McCarthy DW, Bass LA, Cutler PD, Shefer RE, Klinkowstein RE, Herrero P, Lewis JS, Cutler CS, Anderson CJ, Welch MJ, High purity production and potential applications of copper-60 and copper-61. *Nucl. Med. Biol.*, 1999. 26: p. 351-358.
- [8] Fujibayashi Y, Matsumoto K, YY, Konishi J, Yokoyama A, A new zinc-62/copper-62 generator as copper-62 source for PET radiopharmaceuticals. *Jour. Nucl. Med.*, 1989. 30 (11): p. 1838-1842.
- [9] Gambhir SS, A tabulated summary of the FDG PET literature. *Jour. Nucl. Med.*, 2001. 42: p. 15-925.
- [10] Katzenellenbogen JA, Coleman RE, and Hawkins RA, Tumor receptor imaging: proceedings of the National Cancer Institute Workshop, review of current work, and prospective for future investigations. *Clin. Can. Res.*, 1995. 1 (921-932).
- [11] Dehdashti F, et al., Positron tomographic assessment of estrogen receptors in breast cancer: comparison with FDG-PET and in vitro receptor assays. *Jour. Nucl. Med.*, 1995. 36: p. 1766-1774.
- [12] Mortimer JE, et al., Positron emission tomography with 2-[18F]fluoro-2-deoxy-glucose and 16a-[18F]-fluoro-17b-estradiol in breast cancer: correlation with estrogen receptor status and response to systemic therapy. *Clin. Can. Res.*, 1996. 2: p. 933-939.
- [13] Fujibayashi Y, et al., Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential. *J Nucl Med*, 1997. 38 (7): p. 1155-60.
- [14] Schuster D, Pulmonary transgene expression imaging, in *Molecular Imaging of the Lungs*, Schuster DP and Blackwell TS, Editors. 2005, Taylor & Francis: FL. p. 210-236.