Published in final edited form as:

Appl Radiat Isot. 2008 December ; 66(12): 1861–1869. doi:10.1016/j.apradiso.2008.06.008.

Practical method for radioactivity distribution analysis in small-animal PET cancer studies

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Abstract

We present a practical method for radioactivity distribution analysis in small-animal tumors and organs using positron emission tomography imaging with a calibrated source of known activity and size in the field of view. We reconstruct the imaged mouse together with a source under the same conditions, using an iterative method, Maximum Likelihood Expectation-Maximization with System Modeling, capable of delivering high resolution images. Corrections for the ratios of geometrical efficiencies, radioisotope decay in time and photon attenuation are included in the algorithm. We demonstrate reconstruction results for the amount of radioactivity within the scanned mouse in a sample study of osteolytic and osteoblastic bone metastasis from prostate cancer xenografts. Data acquisition was performed on the small-animal PET system which was tested with different radioactive sources, phantoms and animals to achieve high sensitivity and spatial resolution. Our method uses high resolution images to determine the volume of organ or tumor and the amount of their radioactivity, has the possibility of saving time, effort and the necessity to sacrifice animals. This method has utility for prognosis and quantitative analysis in small-animal cancer studies, and will enhance the assessment of characteristics of tumor growth, identifying metastases, and potentially determining the effectiveness of cancer treatment. The possible application for this technique could be useful for the organ radioactivity dosimetry studies.

1. Introduction

Complex clinical decisions on treatment are often guided by Positron Emission Tomography (PET) imaging principally using ^{18}F FDG. PET is often used with other imaging techniques (by combining with CT or MRI) to obtain complementary information. Imaging with ^{18}F FDG or other agents often requires quantitative measurements associated with the imaging data.

The factors that affect PET quantitation are resolution, photon attenuation and scattering, random coincidence rate, detector normalization, dead time and noise. It is very difficult to account for these factors for quantitative analysis of three-dimensional reconstructed radioactivity in tumors or mouse organs.

In most PET studies the *Standardized Uptake Value* (SUV) method is used to quantify tumor radioactivity. As the most widely used semi-quantitative parameter for tumor diagnosis, SUV determination involves measuring activity at a target site, with correction for injected dose, plasma glucose level, uptake period, body weight and, more important, correction for reconstruction method (DiChiro et al 1988, Keyes 1996, Thie et al 2000, Huang 2000, Truong

et al 2004, Kok et al 2005, Popperl et al 2006). To eliminate the need for body-weight correction, SUV has been calculated on the basis of body weight: tissue concentration [MBq/g]/injected dose [MBq]/body weight [g] (see other SUV determinations in de Boer et al 2003). However, the SUV method does not correct for any inaccuracy in the measured dose, which may occur with injected dose extravasations, or with an elevated uptake elsewhere in the body. The accuracy of the SUV and the accuracy of relative change during treatment are not well documented and it might be a problem for diagnostic purposes in multicenter studies (Boellaard R et al 2004). Recent studies even find that SUV readings vary on different PET systems (Takahashi Y et al 2007) and *Regions of Interest (ROI)* can influence quantitative FDG-PET study results (Evilevich V et al 2007).

Thus, the microPET-R4 rodent scanner and ASIPro reconstruction software (both from CTI Concorde Microsystems Inc., Knoxville, TN) were used for semi-quantitative radioactivity analysis in two recent studies: to delineate the stages involved in the development of arthritis (Wipke et al 2004) and breast cancer metastasis (Liang et al 2005). In the first study quantitative analysis of *ROI* was performed over the selected mouse tissues and averaging the radioactive concentration over the contained voxels. In the second study a pixel *ROI* was outlined in the regions of increased FDG uptake, and after correction for radioactivity decay, the maximum SUV was semi-quantitatively calculated according to the method of Truong et al 2004.

A more sophisticated method to determine the maximum radioactivity concentration within a tumor or an organ was described in Zhang et al 2006. In this method, mean pixel values within the multiple *ROI* volumes were converted to $\mu\text{Ci}/\text{mL}/\text{min}$ by using a calibration constant (Wu et al 2005). Recently, an analytic semi-automated approach to calculate body distribution of PET tracers using co-registration a digital mouse phantom with small-animal images was proposed in Kesner et al 2006. The main goal in Domenico et al 2003 was to quantify the activity measured in a *ROI* within a reconstructed image of a small-animal and compare results with the ones derived from standard biodistribution methods: sacrificing the animal and putting each organ of interest in a calibrated gamma counter. Note that all PET devices are calibrated periodically for detector sensitivity using a calibrated source (generally a syringe filled with a known amount of radioisotope). From this scan, the number of counts per radioactivity detected by each detector pair is recorded. These numbers are used for subsequent scans to normalize the number of counts for detector efficiencies and to determine the amount of activity within the scanned object, but it not possible with high resolution to determine activity in small-animal tumors or organs.

We present a new practical method (Slavine and Antich ©2007) to determine radioactivity distribution in *ROI* from reconstructed PET images with a source of known activity and size in the field of view (*FOV*) in an example using osteolytic and osteoblastic bone metastasis from prostate cancer xenografts. Our method is different, more precise and yet simpler than that described above. It is based on a 3D reconstruction method capable of delivering high resolution images, has the possibility of saving time, effort and the necessity to sacrifice animals. The 3D high resolution reconstruction and radioactivity analysis can help to analyze the size and aggressiveness of the tumor, determine its growth in time, and the effectiveness of the treatment.

2. Biological objectives and experimental methods

The prostate cancer (*CaP*) is the second leading cause of cancer-related death among men (Gao et al 1997, Ghosh et al 2004). Prostate-specific antigen testing has been widely adopted for screening prostate cancer, which have a propensity to metastasize to bone (Wu et al 1998, Hall et al 2005, Foss et al 2005 and Guise et al 2006). Prostate cancer may cause osteolysis or abnormal new bone formation (Roodman 2004). As shown by histologic examination

(Roudier et al 2003) the same prostate cancer patient often has evidence of osteolytic and osteoblastic disease. Therefore it is desirable to develop a noninvasive imaging technique to monitor individual tumors in individual patients.

To study both osteolytic and osteoblastic bone metastasis we used a mouse xenograft model (Wu et al 1998, Weerden 2000) and applied a multimodality imaging technique (Lin et al 2005) by combining our PET with CT, PET or MRI with our home built bioluminescent tomographic system (see details in Richer et al 2004, Dikmen et al 2005) or X-ray radiography. MR images were collected using a Varian Unity INOVA 4.7 T MR system equipped with actively shielded gradients. A spin echo sequence was used to obtain T1- and T2- weighted images of the control and tumor-bearing femurs. Note that these studies, in addition, have shown the feasibility and importance of multimodality imaging applied to investigations of tumor development. Each method can reveal different aspects of tumor development and together they have the potential to monitor tumor metastasis quantitatively, providing insight into tumor proliferation and differentiating the phenotypes of prostate cancer in mice.

We used the main results of these studies to confirm the ability of our method to achieve radioactivity distribution analysis in small-animal PET cancer studies.

3. Small-animal PET studies

3.1 Calibrated radioactivity source

In this practical method for radioactivity distribution analysis we used a ^{22}Na radioactive source (half life is 2.6003 years, average positron energy is 0.215 MeV, positron decay ratio of 89.8%, see National Nuclear Data Center, 2004) produced by Amersham Buchler GmbH & Co KG (Braunschweig, Germany) and certified by Lloyd's Register Quality Assurance (LRQA, Houston, TX). The active material is absorbed in a 1 mm diameter ion exchange bead, and sealed by ultrasonic welding between 0.5 mm polystyrene windows which are mounted in a plastic frame $23.5 \times 11.0 \times 2.0 \text{ mm}^3$ volume. The active bead is visible and at the geometric center of the radioactive source. The nominal activity of the source on reference day May, 1 1998 was $370.0 \pm 11.1 \text{ kBq}$. The reported calibration accuracy (overall uncertainty) of 3% was based on standard uncertainty multiplied by a coverage factor of 2, providing a level of confidence of approximately 95%. The photon emission and purity of the source were checked using a Ge (Li) detector. On the day of PET tumor imaging the source activity was about $67.0 \pm 2.0 \text{ kBq}$.

3.2 Small-animal PET device

Positron emission tomography of tumor-bearing mice was performed on our home built small-animal PET device based on two detectors with adjustable separations for optimizing system sensitivity and field of view (see more details in Tsyganov et al 2006). Our PET imager uses the two-fold coincident detection of a single event in two orthogonal fibers to detect the location and the energy transferred at a point within the detector. A single Position-Sensitive Photomultiplier Tube Hamamatsu R-2486 is used to perform a single-ended readout of all fiber arrays oriented in each direction. The scanner, with an effective field of view of $100 \times 100 \text{ mm}^2$, has vertical and horizontal bed motion. The point source sensitivity is about 12.6 cps/kBq at 90 mm inner diameter. The current data transfer rate is about 6 MB/s (~40 K events per second). The two detectors are mounted on a gantry with rotational and translational degrees of freedom which can be chosen to suit a particular imaging protocol. Measurement of spatial resolution - *Full-Width at Half-Maximum* (FWHM) - for the calibrated positron emitting source was done in this PET device and was found to be about 1.0 mm.

3.3 Animal preparation and measurements

All animal studies were conducted in compliance with guidelines set by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas. Male athymic nude mice at the age of 6–8 weeks weighing 20g – 25g (Harlan Inc. Indianapolis, IN), were housed in a barrier filter room in a sterilized environment and fed Purina rodent chow ad lib. About one week later, the mice were used for tumor implantation. Two human *CaP* cell lines, *PC-3* (osteolytic) and *C4-2* (osteoblastic), were stably transfected with luciferase gene. An intraosseous *CaP* mouse xenograft model was established by injection of a single-cell suspension (10^6 cells, $20 \mu l$) of *C4-2* or *PC-3* cells into the bone marrow of the right femur using a 23-gauge syringe. The left femur served as contralateral control. During the studies, prostate-specific antigen levels were measured every week in *C4-2* tumor-bearing mice.

Food and water were removed from the mouse cage 30 min before the PET study. The mice were injected with no-carrier-added ^{18}F FDG (was obtained from Petnet Solution, Knoxville, TN), then first anesthetized with 2% isoflurane and placed near the center of the *FOV* of the PET device where the highest sensitivity and image resolution are obtained. The total initial activity for *PC-3* injected mouse was 30.01 ± 0.60 MBq (mean $\pm$ SD) and 34.78 ± 0.70 MBq for *C4-2* injected mouse. One hour after injection, when data acquisition was started, the radioactivity was 20.57 ± 0.41 MBq and 23.83 ± 0.48 MBq respectively. The Capintec CRC-15R dose calibrator (Capintec Inc., NJ) was used to measure activity levels with accuracy better than 2%. The mouse was kept under anesthesia with 1.5% isoflurane via a nose cone during the scan. The mouse in the prone position and the ^{22}Na calibrated source were placed between two detectors at a separation of 206 mm.

The detectors were rotated to obtain a complete 3D experimental raw data set. About 2.5 million valid list-mode PET events were collected in a 30 min acquisition. During all static imaging scans the ^{22}Na source was situated below the mouse at a distance of about 40 mm.

4. Iterative 3D image reconstruction method

Prior to performing the 3D image reconstruction, we calculated corrections for the ratios of geometrical efficiencies for different detector positions and took into account the radioisotope's decay at the time of imaging.

Our reconstruction algorithm consists of two main steps: 3D reconstruction of the imaged mouse together with a calibrated source under the same conditions used to determine the characteristics of calibrated source (size and activity), geometry and volume of each *ROI* - followed by calculation of the amount of radioactivity in each *ROI* using the radioactivity calculation method.

The advantages and disadvantages of using iterative reconstruction methods to produce accurate high resolution images were recently described in the literature (see, for example Leahy et al 2000, Vandenberghe et al 2001, Reader et al 2002 and Qi et al 2006). Our image reconstruction technique is based on the MLEM method (Shepp and Vardi 1982) which was improved for the One-Pass List-Mode high resolution algorithm with System Matrix modeling (MLSM) (Reader 2002 et al, Zinchenko et al 2003, Antich et al 2005) to take into account most of the finite resolution effects such as positron range, photon acollinearity, intrinsic detector resolution, scatter and random events. The method is generally applicable to any design geometry, to single (SPECT), or a double (PET) photon emission tomography.

The standard MLEM algorithm based on the measured list-mode data iterates the unknown activity value n_j^k of voxel j (image is assumed to be discretized into J voxels) for each iteration step k :

$$n_j^{k+1} = \frac{n_j^k}{\sum_{i=1}^I a_{ij}} \sum_{i=1}^M a_{ij} \frac{1}{q_i^k} \text{ where } q_i^k = \sum_{j=1}^J a_{ij} n_j^k \quad (1)$$

where n_j^{k+1} and n_j^k are the voxel values for the new and old image estimates, q_i^k is the expected count in *Line of Response (LOR)* i for the intensity estimate n_j^k , I is the number of all possible system LORs and a_{ij} represents the probability that an emission from voxel j will be detected along *LOR* i . The measured data now consist of a list of M *LOR* definitions with implicitly

equal to 1 for each acquired *LOR*. The normalization factor $\sum_{i=1}^I a_{ij}$ includes all possible measurable *LORs*. This algorithm is extendible to a data subset implementation to achieve significant acceleration and will generate a sequence of image estimates for each of N subsets with equal numbers (M/N) of list-mode events. The final image update is taken as the result of 3D reconstruction.

In order to take into account finite resolution effects we need to decompose the matrix of probabilities $A = (a_{ij})_{I \times J}$ into a product of three new matrices $A = WXH$. $W = (w_{ii})_{I \times I}$ is the diagonal matrix of weighted factors for geometric sensitivity correction accounting for detector rotation, and is necessary to compensate for a sensitivity loss with increasing perpendicular distance d_i from the axis of rotation (Clark et al 1984):

$$w_i = \left\{ 2 \left[\arctan\left(\frac{L}{S}\right) - \arcsin\left(\frac{d_i}{\sqrt{L^2/4 + S^2/4}}\right) \right] \right\}^{-1} \quad (2)$$

where L is the transverse length of the detector and S is the separation of the PET detectors.

$X = (x_{ij})_{I \times J}$ is a matrix whose elements correspond to the intersection length of *LOR* i with the voxel j and $H = (h_{ij})_{J \times J}$ is a square matrix which accounts for finite resolution effects. Attenuation estimate was carried out in backprojection space using attenuation correction factors for each voxel j (Reader 1999):

$$ac_j = \frac{\sum_{i=1}^I l_{ij} L_i}{\sum_{i=1}^I l_{ij} L_i \exp(-\mu L_i)} \quad (3)$$

where l_{ij} is the path length of *LOR* i through voxel j , L_i is the path length of *LOR* i through the attenuating object and the μ -linear attenuation coefficient.

Now, using the new expression for the matrix A , we can rewrite equation (1) in the form:

$$n_j^{k+1} = \frac{n_j^k}{ac_j \sum_{b=1}^J h_{bj} \sum_{i=1}^I w_{ii} x_{ib}} \sum_{b=1}^J h_{bj} \sum_{i=1}^M x_{ib} \frac{1}{\sum_{\alpha=1}^J x_{i\alpha} \sum_{\beta=1}^J h_{\alpha\beta} n_{\beta}^k} \quad (4)$$

The expression (4) can be rewritten in terms of J -dimensional vectors with an element-by-element multiplication between vectors:

$$n^{k+1} = n^k \times s \times c^k \quad (5)$$

here s is the sensitivity correction factors and c^k contains the multiplicative image correction value and can be given by:

$$c^k = H^T \left[\sum_{i=1}^I BP_i \left(\frac{1}{FP_i(Hn^k)} \right) \right] \quad (6)$$

where the matrix H is multiplied by a J -dimensional vector to give a new one, FP_i is an operator which forward projects vector Hn^k along LOR i to give a scalar value and, the operator BP_i back-projects the scalar value along LOR i into a three-dimensional image for k^{th} -iteration.

If we neglect the scattering effects, the blurring component H of the system matrix can be represented as a set of shift-invariant kernels ρ . In this case resolution blurring will be invariant across the image. For resolution modeling we use a convolution technique, so that the MLSM algorithm becomes:

$$n^{k+1} = n^k \times s \times \left(\left[\sum_{i=1}^I BP_i \left(\frac{1}{F_i(n^k \otimes \rho)} \right) \right] \otimes \rho \right) \quad (7)$$

where ρ is the resolution kernel and can be chosen as a Gaussian function (or more complex if needed) with standard deviation σ as parameter, $\otimes$ - denotes convolution procedure.

The algorithm described above, even using subsets, and especially for high amounts of list-mode data, requires many more iterations to image converge. To significantly increase reconstruction time savings, the image deblurring procedure with double Compton scattering model was developed (see more details in Antich et al 2005).

This iterative reconstruction algorithm with system matrix modeling was tested on several phantoms and successfully used for 3D image reconstruction for mice and rats, either bearing tumors or affected by genetically induced bone diseases (Antich et al 2002, Zinchenko et al 2003, Jennewein et al 2004–2008, Antich et al 2005, Lin et al 2005, Lewis et al 2005, Tsyganov et al 2006).

5. Radioactivity distribution value calculation

In our PET imaging system two high sensitivity detectors simultaneously record data in list-mode format. The data for each of the eight (45° angle step) positions was combined for all rotation angles and used for reconstruction. The total field of view of $110 \times 130 \times 120$ mm³ in our 3D image reconstruction was subdivided into voxels of 0.5 mm³ in size. About 2.5 million

events were reconstructed into a $220 \times 260 \times 240$ array. Resolution was modeled with a Gaussian function. The resolution parameter σ was 1.2 mm during the 30 iterations. Results from our 3D reconstruction of the mice injected with ^{18}F FDG in the presence of a ^{22}Na source in the field of view for different bone metastasis from prostate cancer xenografts is shown on Figure 1– Figure 6. The unit of the histogram's colorbar is the voxel intensity after final iteration.

Figure 1–Figure 2 and Table 1 show our reconstruction results separately for source areas of the mouse. Calibrated source was situated below the mouse at a distance of about 40 mm at $Z=81.51\text{mm}$ for mouse with *PC-3* tumor (see Fig. 1a and Table 1) and at $Z=81.58\text{ mm}$ for the mouse with *C4-2* tumor (see Fig. 2a and Table 1). Figure 1b shows transversal (for Y and Z profiles see Table 1) profile of the central axial slices for ^{22}Na source after 3D MLSM reconstruction for the mouse with *PC-3* tumor. Figure 2b and Table 1 show the same for the mouse with *C4-2* tumor. Each profile was fitted with a Gaussian function, and the FWHM of the distribution is taken as the reconstructed source size. It can be seen that reconstructed spatial resolution (source size) for each calibrated source is in excellent agreement with the actual size. Correspondence of reconstructed position coordinates for both sources show agreement when close to each other (see Table 1). Peaks of the reconstructed source intensities also demonstrate close agreement with each other with a difference of less than 1.0% (see legend box in Fig. 1b and Fig. 2b). Accuracy of the source reconstruction characteristics is very important for the activity calculation procedure.

Once the animal with the calibrated ^{22}Na source in the field of view is reconstructed with high precision (see results on Fig. 3 and Fig. 4) we can calculate the tumor's or organ's volume. On the Figure 3 we indicate the *PC-3* tumor location and source position and on the Figure 4 same for the mouse with *C4-2* tumor. Figure 3 and Figure 4 also demonstrate the good quality of 3D MLSM organs reconstruction. For image manipulations (rotation, chosen volume determination size, etc.) we applied the interactive utility for visualizing data on a PC graphic display Physics Analysis Workstation (PAW 1995) based on a statistical analysis and histogram package (HBOOK 1998). Note, the algorithm based on PAW was successfully used for volume size quantification in our bioluminescence imaging investigations (Richer et al 2004, Slavine et al 2006).

Now, after we reconstruct the intensity and size of the source and determine the geometry of interested *ROI*, we can calculate a radioactivity distribution value (RDV) for tumor and mouse organs. First we determine the number of *LORs* i (reconstructed intensity) through each voxel j (volume) of the tumor (organ) and the calibrated source volume. Radioactivity value of the tumor (organ) will be determined as a result of comparing both these values for tumor (organ) and source volumes.

6. Results

Figure 5a and Figure 5b demonstrate the transverse slices of the right femur tumor through the 3D reconstruction and evidently confirm the particular characteristics of osteolytic and osteoblastic cancer disease-damaged femur is visible for the *PC-3* tumor (Fig. 5a) and abnormal new bone formation for the *C4-2* tumor (Fig. 5b). The reconstructed PET results (Fig. 5–Fig. 6 and Table 2) strongly indicated a significantly elevated ^{18}F FDG uptake in the right tumor-bearing femur compared to the left control in these mice. Our quantitative distribution activity analysis (see Table 2 and Fig. 6) also confirms that the *PC-3* tumor was of a more aggressive nature than the *C4-2* tumor and had a higher ratio of ^{18}F FDG uptake between the right femur and the left control femur in the *PC-3* tumor (2.61) than in the femur with *C4-2* one (1.85).

Note, in addition, our multimodality investigation results (Lin et al 2005) clearly showed that the *PC-3* tumor grew aggressively over time. Both T1- and T2-weighted MRI images were

used to differentiate bone and tumor from other soft tissues. The T2-weighted images exhibited the osteolytic nature of the *PC-3* tumor and revealed that both *PC-3* and *C4-2* tumors progressed to the whole femur and disseminated to the surrounding tissues after five weeks p.c.i. X-ray radiography further demonstrated the damaged femur in mice bearing *PC-3* tumors and new bone branching from the femur containing *C4-2* tumor in close agreement with our 3D reconstruction results (Fig. 5).

7. Conclusion

In Table 2 and Figure 6 we compare our quantitative results for mice femurs and other organs calculated by using the iterative MLSM algorithm and RDV calculation method. The results of this example study of osteolytic and osteoblastic bone metastasis from prostate cancer xenografts have provided quantitative data, show the tomographic capabilities of our small-animal PET imaging system and clearly show perfect practice ability of this method for radioactivity distribution calculations in tumors and small-animal organs.

The radioactivity distribution value calculation method is applicable to complex geometries, can be used with any 3D high resolution reconstruction algorithm (Liu et al 2001 McColl et al 1990), and is amenable to fast imaging calculations (depending on number of voxels, iterations and amount of list-mode data from PET system). The average processing time per image update for MLSM reconstruction procedure was a few min (time dependent upon 3D convolution algorithm). The imaging procedure and reconstruction software were verified using a single source and several phantoms (Antich et al 2005). The number of iterations (image updates) in the 3D reconstruction procedure was chosen to obtain close agreement between calibrated source actual dimensions with reconstructed values - thus saving time, and effort and providing the opportunity for the reconstruction procedure to be semi-automated. By using this method we don't need to sacrifice animals to calculate the radioactivity in mice organs.

The method to determine the radioactivity distribution value in tumors or organs proposed here has utility for prognosis and quantitation in small-animal cancer studies to better understand the effects of human disease in animal models (Lin et al 2005, Mason 2007, Jennewein et al 2008). As shown in Table 2 and Figure 6 this method could be used as an additional useful tool to the semi-quantitative SUV method or to any other method (Kesner et al 2006, Zhong et al 2008) to estimate radioactivity value in tumor or any mouse organs. The possible application for this technique could be useful for the organ radioactivity dosimetry studies (Smith T 1992, Virta et al 2008) that required before mass balance study (Argenti et al 2000, Beumer et al 2005) may be performed in humans.

In case of the future improvements we will extend the method to our SPECT small-animal studies (Soesbe et al 2007). The quantitative accuracy in PET/SPECT reconstruction is particularly poor for small tumors because of *partial volume effect* (Quarantelli 2004, Wells 2007). This effect arises because of the blurring induced by the imaging system's point spread function (Barrett and Myers 2004), producing intra-voxel mixing of the activity arising from different functional tissue classes. In this case we have to include partial volume correction techniques in our image quantification method.

Acknowledgments

We are very grateful to Drs. Xiankai Sun, R. P. Mason, G. Arbiq, J. Anderson, Jer-Tsong Hsieh, V. D. Kodibagkar, M.A. Lewis, R. McColl, O. Oz, R. Parkey, Zhengwang Zhang, A. Zinchenko and our student Mai Lin for their advice and the valuable discussions. We would like to thank Mr. S. Seliounine and Dr. E. Tsyganov for experimental assistance with small-animal PET measurements.

This work was supported in part by the NIH/National Cancer Institute U24 CA126608-SAIRP

References

- Antich PP, Constantinescu A, et al. FDG Imaging of Lung Metastases in Copenhagen Rats on a Small-Animal PET System with Positron-Encoding Fiber Optic Detectors. *J Nuclear Medicine* 2002;43(5):216.
- Antich PP, Parkey R, et al. Application of Expectation Maximization Algorithms for Image Resolution Improvement in a Small-Animal PET System. *IEEE Trans on Nuclear Science* 2005;53(3):684–690.
- Argenti D, Jensen B, et al. A Mass Balance Study to evaluate the Biotransformation and Excretion of [^{14}C]-Triamcinolone Acetonide following oral administration. *J Clinical Pharmacology* 2000;40:770–780.
- Barrett, HH.; Myers, KJ. *Foundations of Image Science*. Noboken, New Jersey: Wiley&Sons Inc; 2004.
- Beumer JH, Rademaker-Lakhai JM, et al. Trabectedin (YondelisTM, formerly ET-743), a Mass Balance Study in patients with Advanced Cancer. *Inv New Drugs* 2005;23(5):429–436.
- Boellaard R, Nanda C, et al. Effects of Noise, Image Resolution, and ROI Definition on the Accuracy of Standard Uptake Values: a Simulation Study. *J Nuclear Med* 2004;45(9):1519–1527.
- de Boer JR, Pruim J, et al. 1- ^{11}C -Tyrosine PET in Patients with Laryngeal Carcinomas: Comparison of Standardized Uptake Value and Protein Synthesis Rate. *J. Nuclear Medicine* 2002;44(3):341–346.
- Clack R, Townsend D, Jevons A. Increased sensitivity and field of view for a rotating Positron Camera. *Phys. Med. Biol* 1984;29(11):1421–1431. [PubMed: 6334321]
- Di Chiro G, Brooks R. RPET quantitation: blessing and curse. *J. Nuclear Med* 1988;29:1603–1604.
- Di Domenico G, Zavattini G, et al. YAP-(S) Small Animal Scanner: Quantitative Results. *IEEE Trans on Nuclear Science* 2003;50(5):1351–1356.
- Dikmen ZG, Gellert G, Dogan P, et al. A new diagnostic system in cancer research: Bioluminescent Imaging. *Turk. J Medical Science* 2005;35:65–70.
- Evilevich V, Eilber F, et al. Influence of ROI definition on the results of FDG-PET studies in patients with sarcomas. *J Nuclear Medicine* 2007;48:145P.
- Foss CA, Mease RC, et al. Radio labeled Small-Molecule Ligands for Prostate Specific Membrane Antigen: *In vivo* Imaging in Experimental Models of Prostate Cancer. *Clinical Cancer Research* 2005;11(11):4022–4028. [PubMed: 15930336]
- Gao X, Porter AT, Grignon DJ, et al. Diagnostic and Prognostic Markers for Human Prostate. *Cancer Prostate* 1997;31:264–681.
- Ghosh A, Heston WD. Tumor target Prostate Specific Membrane Antigen (PSMA) and its regulation in Prostate Cancer. *J Cell Biochem* 2004;91:528–539. [PubMed: 14755683]
- Guisse TA, Mohammad KS, et al. Basic mechanisms responsible for Osteolytic and Osteoblastic Bone Metastases. *Clinical Cancer Research* 2006;12:6213s–6216s. [PubMed: 17062703]
- Hall CL, Bafico A, et al. Prostate Cancer Cells Promote Osteoblastic Bone Metastases through Wnts. *Cancer Research* 2005;65(17):7554–7560. [PubMed: 16140917]
- Sung-Cheng, Huang. *Anatomy of SUV*. *J Nuclear Medicine & Biology* 2000;27:643–646.
- HBOOK. *Statistical Analysis and Histogramming*, CERN Program Library Y250. Geneva Switzerland: CERN; 1998. (www.cern.ch/cernlib).
- Jennewein M, Constantinescu A, et al. Molecular imaging of the vascular targeting antibody Vatumab[®] in rat Prostate Cancer. *Europ J Nucl Med & Molec Imaging* 2004;31:264.
- Jennewein M, Constantinescu A, Bergner O, Slavine NV, et al. Radioactive Arsenic Isotopes: New Tools for the Imaging of Tumor Targeting Antibodies. *J Molec Imaging & Biology* 2004;16(150):108–117.
- Jennewein M, Qaim SM, et al. A New Method for Radiochemical separation of Arsenic from Irradiated Germanium Oxide. *Applied Radiation & Isotopes* 2005;63:343–351. [PubMed: 15955705]
- Jennewein M, Lewis MA, Dhao Dawen, et al. Vascular Imaging of Solid tumors in rats with a Radioactive Arsenic-Labeled Antibody that binds exposed Phosphatidylserine. *Clinical Cancer Research* 2008;14:1377–1385. [PubMed: 18316558]
- Kesner AL, Dahlbom M, et al. Semiautomated analysis of Small-Animal PET data. *J. Nuclear Medicine* 2006;47(7):1181–1186.
- Keyes JW Jr. SUV: standard uptake or silly useless value? *J. Nuclear Medicine* 1996;36(10):1836–1839.

- Kok PJ, van Eerd JE, et al. Biodistribution and Imaging of FDG in rats with LS174T Carcinoma Xenografts and focal Escherichia coli Infection. *J. Cancer Biotherapy & Radiopharmaceuticals* 2005;20(3):310–315.
- Leahy RM, Qi J. Statistical approaches in quantitative positron emission Tomography. *Statistic and Computing* 2000;10:147–165.
- Lewis, M.; Arbique, G.; Richer, E.; Slavine, NV., et al. Small-Animal SPECT Imaging. Springer Science Media Inc.; 2005. Projection and Pinhole based data Acquisition for Small-Animal SPECT using Storage Phosphor Technology. Chapter 24; p. 279-285.
- Liang Z, Yoon Y, Votaw J, et al. Silencing of *CXCR4* blocks breast cancer Metastasis. *Cancer Research* 2005;65(3):967–971. [PubMed: 15705897]
- Lin, M.; Wang, Z.; Kodibagkar, VD.; Slavine, NV., et al. Multimodality Imaging Studies of Bone Metastasis in an Intraosseous Prostate Cancer Xenograft Mouse Model. Soc. of Nuclear Medicine; 52nd Annual Meeting; Puerto-Rico. 2005. N651850
- Lin X, Combat C, et al. Comparison of 3D Reconstruction with 3D OSEM and with FORE+OSEM for PET. *IEEE Trans on Medical Imaging* 2001;20(8):804–814.
- Mason, R. Radiology. UT Southwestern at Dallas; 2007. UT Southwestern In Vivo Cancer Cellular and Imaging Program. <http://cip.swmed.edu/ICMIC>
- McColl, R.; Faber, T.; Opperman, R., et al. *TR3: A Simple Multimodality Image Handling Environment*; 10th conf. on Computer Applications to assist Radiology; 1990. p. 457-462.
- National Nuclear Data Center. Brookhaven National Laboratory; 2004. www.nndc.bnl.gov/nudat2
- PAW - Physics Analysis Workstation. Switzerland: CERN Program Library Q121 CERN Geneva; 1995. (www.cern.ch/cernlib).
- Popperl G, Kreth FW, Herms J, et al. Analysis of ¹⁸F-FET PET for grading of recurrent gliomas: is evaluation of uptake kinetics superior to standard methods. *J Nuclear Medicine* 2006;47(3):393–403.
- Qi J, Leahy RM. Iterative reconstruction techniques in emission computed Tomography. *Physics in Medicine & Biology* 2006;51:R541–R574. [PubMed: 16861768]
- Quarantelli M, Berkouk K, et al. Integrated Software for the Analysis of Brain PET/SPECT Studies with Partial Volume Effect Correction. *J Nuclear Medicine* 2004;45:192–201.
- Reader AJ, Erlandsson K, et al. Attenuation and scatter correction of List-Mode Data Iterative and Analytic Image Reconstruction Algorithm for 3D PET systems. *IEEE Trans on Nuclear Science* 1999;46(6):2218–2226.
- Reader AJ, Ally S, Bakatselos F, et al. One-Pass List-Mode EM Algorithm for High Resolution 3D PET Image Reconstruction into Large Arrays. *IEEE Trans on Nuclear Science* 2002;49(3):693–699.
- Richer E, Slavine NV, Lewis M, et al. Three Dimensional Light Emission Tomography using multiple rotating CCD cameras. *J. Molec. Imaging* 2004;3(3):229–230.
- Shepp LA, Vardi Y. Maximum Likelihood reconstruction for emission tomography. *IEEE Trans on Medical Imaging* 1982;1:113–122.
- Roodman GD. Mechanisms of Bone Metastasis. *N Engl J Medicine* 2004;350:1655–1664.
- Roudier MP, Vesselle H, et al. Bone Histology at Autopsy and matched Bone Scintigraphy findings in Patients with Hormone Refractory Prostate Cancer: the effect of Bisphosphonate Therapy on Bone Scintigraphy Results. *Clinic Exp Metastasis* 2003;20:171–180.
- Soesbe T, Lewis M, Richer E, Slavine NV, Antich PP. Development and Evaluation of an EMCCD based Gamma Camera. *IEEE Trans on Nuclear Science* 2007;54(5):1516–1524.
- Slavine, NV.; Lewis, MA.; Richer, PP. Medical Physics. Vol. 33. 2006. Iterative reconstruction method for light emitting sources based on diffusion equation; p. 61-68.
- Slavine, NV.; Antich, PP. Patent disclosure filed with University of Texas Southwestern Medical Center at Dallas in July 2007. 2007. Practical Method for Radioactivity Distribution Analysis in Small-animal PET Cancer Studies©. #UTSD-1999
- Smith T. Comparison of two methods of quantitation in Human studies of Biodistribution and Radiation Dosimetry. *Phys in Medicine and Biology* 1992;37:1065–1076.
- Takahashi Y, Otake H, et al. Variety of SUVs among different PET systems in 18F-FDG study. *J. Nuclear Medicine* 2007;48:462P.

- Thie JA, Hubner KF, Smith GT. The diagnostic utility of the lognormal behavior of PET Standardized Uptake Values in Tumors. *J Nuclear Medicine* 2000;41(10):1664–1672.
- Truong MT, Erasmus JJ, Munden RF. Focal FDG Uptake in mediastinal brown fat mimicking malignancy: a potential pitfall resolved on PET/CT. *Am. J Roentgenol* 2004;183:1127–1132. [PubMed: 15385319]
- Tsyganov E, Anderson J, Arbique G, Constantinescu A, et al. UT SWMC Small-Animal Positron Emission Imager. *IEEE Trans on Nuclear Science* 2006;53(5):2591–2600.
- Vandenberghe S, Asseler YD, et al. Iterative Reconstruction Algorithms in Nuclear Medicine. *Computerized Medical Imaging and Graphics* 2001;25:105–111. [PubMed: 11137786]
- Weerden van WM, Romijn JC. Use of Nude Mouse Xenograft Models in Prostate Cancer Research. *The Prostate* 2000;43:263–271. [PubMed: 10861745]
- Wells K, Chiverton J, et al. Quantifying the Partial Volume Effect in PET using Benford's Law. *IEEE Trans on Nuclear Science* 2007;54(5):1616–1625.
- Virta JR, Tovanen T, et al. $1\text{-}^{11}\text{C}$ -Methyl-4-Piperidiny-N-Butyrate Radiation Dosimetry in Humans by dynamic Organ Specific Evaluation. *J Nuclear Medicine* 2008;49(3):347–352.
- Wipke BT, Zheng Wang, et al. Staging the Initiation of Autoantibody-Induced Arthritis: a Critical Role for Immune Complexes. *J Immunology* 2004;7694–7702. [PubMed: 15187152]
- Wu TT, Sikes RA, et al. Establishing Human Prostate Cancer Cell Xenografts in Bone: Induction of Osteoblastic Reaction by Prostate-Specific Antigen producing Tumors in Athymic and SCID/bg Mice using LNCaP and Lineage-derived Metastatic Sublines. *Int. J. Cancer* 1998;77:887–894. [PubMed: 9714059]
- Wu Y, Zhang X, et al. Micro PET Imaging of Glioma Integrin $\alpha_v\beta_3$ Expression using ^{64}Cu - labeled Tetrameric RGD Peptide. *J Nuclear Medicine* 2005;46(10):1707–1718.
- Zhang X, Xiong Z, Wu Y, Cai W, et al. Quantitative PET imaging of tumor Integrin $\alpha_v\beta_3$ Expression with ^{18}F -FRGD2. *J Nuclear Medicine* 2006;47(1):113–121.
- Zhong H, Weiss E, Siebers JV. Assessment of Dose Reconstruction Errors in Image-guided Radiation therapy. *Phys in Medicine & Biology* 2008;53:719–736.
- Zinchenko A, Tsyganov E, Slavine NV, et al. Expectation Maximization Algorithm with Resolution Deconvolution for 3D Image Reconstruction in Small-Animal PET Imager. *J Nuclear Medicine* 2003;44(5):165–166.

Figure 1.

Sagittal profile of all axial slices after 3D reconstruction for ^{18}F FDG injected mouse with PC-3 tumor combined with calibrated ^{22}Na source (a) and transversal profile of the central axial slice for ^{22}Na source (b). Source was situated below the mouse at a distance of about 40 mm at $Z=81.51$ mm. The voxel size is 0.5 mm^3 .

Figure 2.

Sagittal profile of all axial slices after 3D reconstruction for ^{18}F FDG injected mouse with C4-2 tumor combined with calibrated ^{22}Na source (a) and transversal profile of the central axial slice for ^{22}Na source (b). Source was situated below the mouse at a distance of about 40 mm at $Z=81.58$ mm. The voxel size is 0.5 mm^3 .

Figure 3.

Coronal (a) and sagittal (b) slices through the 3D reconstruction for ^{18}F FDG injected mouse with PC-3 tumor combined with calibrated ^{22}Na source ($X=72.98$ mm, $Y=114.31$ mm, $Z=81.51$ mm) in the field of view (about of 30 MBq of ^{18}F FDG, 20 g nude mouse, 30 min acquisition time for 2.5×10^6 list-mode PET events). The voxel size is 0.5 mm^3 .

Figure 4.

Coronal (a) and sagittal (b) slices through the 3D reconstruction for ^{18}F FDG injected mouse with C4-2 tumor combined with calibrated ^{22}Na source ($X=73.07$ mm, $Y=114.73$ mm, $Z=81.58$ mm) in the field of view (about of 35 MBq of ^{18}F FDG, 25 g nude mouse, 30 min acquisition time for 2.5×10^6 list-mode PET events). The voxel size is 0.5 mm^3 .

Figure 5.

Transverse slices of the right femur tumor through the 3D reconstruction of the ^{18}F FDG injected nude mouse and ^{22}Na calibrated source in the field of view. Transaxial images demonstrated the damaged femur in the mouse with *PC-3* tumor (a) and new bone branching for the femur with *C4-2* tumor (b). Left femurs were left as controls. The voxel size is 0.5 mm^3 . Resolution was modeled with a 1.2 mm resolution kernel.

Figure 6. Number of voxels as a function of its reconstructed intensity for ¹⁸F DG injected tumor-bearing mice of right femur with *PC-3* tumor (a), *C4-2* tumor (b) and for comparison of the two types of tumors (c). The intensities curves are normalized to the maximum intensity of the right femur.

Table 1

Reconstructed source positions and FWHM for X, Y and Z profiles in ^{18}F FDG scan with *PC-3* and *C4-2* tumor-bearing right mice femurs. Each profile was fitted with a Gaussian function, and the FWHM of the distribution is taken as the reconstructed source size.

Source Coordinate	<i>PC-3</i>		<i>C4-2</i>	
	position [mm]	FWHM [mm]	position [mm]	FWHM [mm]
X	72.98	1.009	73.07	0.998
Y	114.31	0.999	114.73	1.006
Z	81.51	1.010	81.58	1.012

Table 2

Reconstructed radioactivity in mice femurs with and without tumors and in other mouse organs calculated by using iterative 3D reconstruction algorithm and method for RDV calculation. On the day of PET tumor imaging the source activity was about 67.0 ± 2.0 kBq.

Femur/Organ	Radioactivity value [MBq]	
	<i>PC-3 tumor</i>	<i>C4-2 tumor</i>
Total (t=0 h)	30.01 ± 0.60	34.78 ± 0.70
Total (t=1 h)	20.57 ± 0.41	23.83 ± 0.48
Total (recon)	20.36 ± 0.65	23.47 ± 0.75
Femur tumor	0.60 ± 0.02	0.37 ± 0.01
Femur Left	0.23 ± 0.01	0.20 ± 0.01
Heart	1.16 ± 0.04	1.26 ± 0.04
Tail	2.55 ± 0.08	2.93 ± 0.18
Head	4.29 ± 0.14	4.49 ± 0.09
Bladder *	0.72 ± 0.02	0.76 ± 0.02
Kidney Right	0.22 ± 0.01	0.28 ± 0.01
Kidney Left	0.21 ± 0.01	0.27 ± 0.01
Back	2.08 ± 0.07	1.82 ± 0.06
Other	8.34 ± 0.27	11.09 ± 0.35

* Relatively low activity in the bladder because we forced a decreased urine level in the bladder for these measurements