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A Simple Device to Convert a Small-Animal PET Scanner into a Multi-Sample Tissue and Injection Syringe Counter

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Abstract

Introduction—We describe a simple fixture that can be added to the imaging bed of a small-animal PET scanner that allows automated counting of multiple organ or tissue samples from mouse-sized animals and counting of injection syringes prior to administration of the radiotracer. The combination of imaging and counting capabilities in the same machine offers advantages in certain experimental settings.

Methods—A polyethylene block of plastic, sculpted to mate with the animal imaging bed of a small-animal PET scanner, is machined to receive twelve 5-ml containers, each capable of holding an entire organ from a mouse-sized animal. In addition, a triangular cross-section slot is machined down the centerline of the block to secure injection syringes from 1-ml to 3-ml in size. The sample holder is scanned in PET whole-body mode to image all samples or in one bed position to image a filled injection syringe. Total radioactivity in each sample or syringe is determined from the reconstructed images of these objects using volume re-projection of the coronal images and a single region-of-interest for each. We tested the accuracy of this method by comparing PET estimates of sample and syringe activity with well counter and dose calibrator estimates of these same activities.

Results—PET and well counting of the same samples gave near identical results (in MBq, $R^2 = 0.99$, slope = 0.99, intercept = 0.00-MBq). PET syringe and dose calibrator measurements of syringe activity in MBq were also similar ($R^2 = 0.99$, slope = 0.99, intercept = - 0.22-MBq).

Conclusion—A small-animal PET scanner can be easily converted into a multi-sample and syringe counting device by addition of a sample block constructed for that purpose. This capability, combined with live animal imaging, can improve efficiency and flexibility in certain experimental settings.

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Keywords

PET pre-clinical imaging; mouse bio-distribution studies; cancer drug development; PET imaging of excised mouse tissues

Introduction

Not surprisingly, small-animal PET scanners are often devoted exclusively to the task of imaging live small animals. However, such devices can also be used to image other positron-emitting radioactive objects and, in some cases, this capability can be of experimental value. In our laboratory, for example, certain mouse imaging studies require both PET imaging and well counting of post mortem organ and tissue samples, a process that for various technical reasons (see Discussion) substantially delays and complicates analysis of these data. In such studies, it would be helpful if only the PET scanner were needed to accomplish both imaging and counting tasks.

To this end, we drew on the whole-body imaging capability present in all small-animal PET scanners to automatically image multiple tissue samples and on the technical features of these machines that, in principle, allow accurate estimation of activities in these tissue specimens. This scheme requires only a multi-sample/syringe holder “block” that can be mated to the animal imaging bed of the scanner. The design details of this block, the sample containers and a quantitative comparison of sample and syringe activities determined with PET to well counter and dose calibrator measurements of these activities are described below.

Methods and Materials

Sample Block

The sample block is pictured in Figure 1. The dimensions of the block and sample container are shown in simplified form in Figure 2. Small additional machining details that allow the block to closely fit the curved inside edges of the imaging bed have been left out in Figure 2 since these details will differ from machine to machine.

The block is fabricated from low-density polyethylene stock. Twelve cylindrical holes (Figure 2) are machined part way through the block. The hole pitch and depth are the same for all holes. The diameter and depth of the holes were chosen such that a commercially available, disposable cylindrical plastic container with screw-on, watertight cap (5-ml CryoELITE™ Cryogenic Vials, Wheaton Science Products, Millville, NJ) could be inserted into each hole. These containers possess a shallow conical shaped bottom that acts as a self-centering mechanism to locate samples along the container axis. These 5-ml containers met the primary requirement of being able to hold any of the major organs in animals the size of 20 g mice (all < 5 g). Given this requirement and the maximum axial imaging length of the scanner (159.1 mm), 6 pairs of these sample containers could fit within this scanning field: 152.0 mm from the leading edge of containers in the first row to the trailing edge of the containers in the sixth row. Once the container size is set, the maximum axial traverse of the PET scanner bed determines the total number sample containers that can be placed in the

block. The number here (12) is comparable to the number of major body organs excised and counted in a typical mouse bio-distribution study so that a single mouse could be imaged followed shortly by sample counting of all the major body organs of that mouse.

A triangular cross-section trough was also machined down the centerline of the block. The width and depth of the trough were chosen such that 1 – 3-ml syringes could be laid in the trough thereby centering themselves in the field of view and resisting movement during placement for imaging.

Quantitative Accuracy

We hypothesized that scanning the length of the sample block in whole body mode and imaging a syringe in a single bed position would yield estimates of total sample and syringe activities that were in close agreement with those obtained directly by well counting these same samples (the “gold standard”) and by comparison to dose calibrator measurements of syringe activities. We tested this hypothesis as described below.

A dose calibrator (CRC® 25W, Capintec, Inc., Florham Park, NJ) was used as the standard assay device for all syringe and sample measurements. The BioPET/CT [1] (Sedecal, Madrid, Spain) served as the PET/CT small-animal scanner and a 2480 Wizard 3 scintillation well counter system with automatic sample changer (Perkin Elmer, Shelton, CT) as the well counter.

--Syringe Measurements--Five 1-ml TB syringes (Terumo Medical Corp., Somerset, NJ) were filled sequentially with increasing volumes of a uniform concentration of F-18 in water. After drawing up the needle activity into the barrel of the syringe, the needle was removed (to avoid metal-induced artifacts in the subsequent CT scan) and each syringe was assayed in the dose calibrator set to F-18. The first syringe contained approximately 1.9-MBq of F-18, the second 2.9-MBq of F-18 and so on up to 5.9-MBq of F-18 in the fifth syringe. Each syringe was then imaged in a single bed position with the PET scanner with the syringe lying in the trough in the sample block and centered in the axial field of view. At the end of each of these imaging sessions, a CT scan of the block, syringe and bed combination was taken for subsequent correction of the emission data for attenuation.

--Sample Measurements--A stock solution of F-18 in water was prepared using an amount of F-18 determined by dose calibrator. This stock solution was then sequentially diluted to create six F-18 samples, each a factor of two lower in total activity than the previous dilution (that is, the last of the six samples was 32 times lower than the first). At each step, two sample containers were filled with these same activities and their total volumes adjusted to 1-ml. The activity in each of these samples was calculated from the known starting activity in the stock solution and from the progressive dilutions by micro-pipetting.

The twelve containers were then placed in the sample block (Figure 1) with one column arranged in order of decreasing sample activity and the other in order of increasing sample activity. The sample block was then imaged in PET whole body mode (250–700 keV energy window) using 4 bed positions at 10 minutes per position (40 minutes total imaging time)

and a 16-slice overlap between bed positions. At the end of each imaging session, a CT scan of the block was performed. This entire process was repeated four times to obtain sample count rates with decreasing activity due to radioactive decay. The first of these data sets was taken as the calibration data set from which the PET calibration factor (cps/MBq) would be determined for this machine.

At the end of the PET whole body imaging sessions, the sample containers were placed in the plastic containers designed for use with the automatic sample changer of the well counter (a near exact fit) and all twelve counted (400–1200 keV energy window) for one minute approximately 11 hours after PET imaging (to allow radioactive decay to bring the sample count rates into the linear counting range of the well counter). For reasons noted below, the well counter calibration factor (cps/MBq) was determined from the average calibration factor calculated from all twelve of these measurements.

Data Processing

Three data sets were available at the end of data acquisition, one from PET syringe imaging comprised of five PET single bed position scans, one from the PET studies comprised of four sequential PET/CT whole body scans of the sample block and one from direct well counting of all twelve sample containers. In addition, the activity present in each sample container was also known by calculation from sequential dilutions of the stock solution.

--PET Processing--Each of the four whole body scans and five syringe scans was reconstructed using all corrections available on the scanner, i.e. attenuation, scatter, random coincidences, dead time, radioactive decay to the start time of the scan. These data were reconstructed with a 2D OSEM resolution recovery algorithm (16 subsets, 2 iterations) and the resulting tomographic data set rendered in coronal projection. The coronal slices were then volume summed to yield a single image of the twelve samples and each syringe (not shown). A circular region of interest (ROI) was drawn around each sample container and an irregular ROI drawn around each syringe that matched the contour of the projected shape of the syringe. The total count rate within each of these ROI was then determined. A representative volume (summed) projection image of the sample array is shown in Figure 3.

Instrument Calibration

The count rates from each of the imaged samples in the first PET sample block collection were divided by the dilution-calculated values for these same samples at the start time of the PET data collection to obtain the calibration factor for this machine configuration (1616.2 ± 70.3 cps/MBq ($\pm 4.3\%$)). This factor was then applied to the PET sample count rates for the first and the other three PET sample collections to calculate sample activity in MBq for each.

The PET count rates from the five syringes lying in the sample block were decay corrected to the time of dose calibration of these syringes. A calibration factor relating PET sample block syringe count rate to dose calibrator activity was determined independently by imaging a sixth syringe in the sample block after filling with approximately 9-MBq of F-18. The count rate from this syringe, decay corrected to the dose calibration time, yielded a

calibrator factor of 1361.1 cps/MBq. This factor was then applied to each of the 5 individual syringe measurements to express each syringe count rate in MBq for comparison with their corresponding dose calibrator activities. (Note that each of these calibration factors, sample and syringe, are corrected for the positron branching fraction of F-18, 0.97)

The count rates from each of the well counter samples were divided by the dilution-determined activities at the start time of well counting (the time to which the well counter values are corrected) and the average value of the ratio of sample count rate to sample activity determined. The calibration factor for the well counter determined this way was $5.938 \times 10^5 \pm 8.51 \times 10^4$ cps/MBq ($\pm 1.4\%$). The well counter sample measurements were converted to MBq with this factor and decay corrected back to the start time of each of the PET scans of the sample block. Note that although this method does not give an “independent” estimate of this calibration factor, the very small variation around the mean calibration factor indicates that, as a practical matter, a single or the average of any small subgroup of estimates would yield PET sample activities nearly identical regardless of choice.

Results

Total sample activities obtained by PET imaging of the sample block at four different times (48 measurements) are compared to the well counter estimates of these same activities at these same times in Figure 4A. The regression relation to these data (inset in figure) indicates a near unity slope and near zero intercept suggesting that the PET measurements are an unbiased estimate of the absolute well counter values. It is noteworthy that the lowest activity and highest activity shown in this plot differ by more than a factor of 80.

It is also noteworthy that the syringe and dose calibrator measurements of syringe activity compared in Figure 4B span a three-fold range in activity (and volume), a range that more than includes typical activities (and volumes) used in “real” 20–25 g mouse studies in our laboratory (3.70 – 5.55-MBq, 0.1–0.2-ml).

Discussion

It is not uncommon in rodent drug development studies to use several instruments to obtain the desired experimental result. In our laboratory, for example, we sought to determine the accuracy of PET imaging in estimating the total radioactivity content of mouse tumor xenografts exposed to putative F-18-labeled diagnostic agents. This preliminary study was undertaken to verify that PET imaging would, in fact, yield accurate estimates of tumor activities before being applied to a much larger group of animals and a wider range of experimental drugs.

This preliminary comparison required PET imaging in groups of tumor-bearing mice followed by well counting of the excised tumors, a lengthy process since PET imaging and well counting possess very different sensitivities: the sensitivity of the well counter used here is more than 400 times greater than the PET scanner so that tissues labeled with relatively large amounts of F-18 for imaging cannot be counted in the well counter until 10–12 hours later when typical tumor or organ activities are within the linear counting range of

the well counter. Although waiting yields the desired result (the total activity content of the tumors), these data are only available after overnight counting of the tumor samples. In addition, use of the well counter creates a separate data processing pathway that further complicates and lengthens the overall data analysis process. PET imaging followed shortly by PET counting of excised tissues and organs from the same animal avoids this problem and consolidates data analysis into a single data processing pathway. Other applications can also be envisioned that depend on the experimental environment, for example, a laboratory without a well counter could use the PET scanner (without imaging) for organ/tissue assays in experiments where direct tissue counting was deemed necessary.

While offering novel opportunities in certain experimental situations, this scheme also possesses several significant practical and technical limitations. For example, PET/CT imaging, image reconstruction and image analysis of the sample block or syringe data are, by themselves, time consuming and while imaging a mouse, the scanner cannot be used for sample counting (and vice versa). These factors conspire to limit the number of animals that could be studied to completion (imaging and sample counting) in any given day. In addition, the performance characteristics of the PET scanner can also limit use of this scheme. For example, count rate is a linear function of activity within the field of view of the PET scanner used here for activities up to approximately 14.80-MBq so that the sum of activities in any four adjacent samples (roughly the number of samples visible to the scanner in a single bed position) cannot exceed this amount. For typical 20 g mouse studies in our laboratory, total injected activities never exceed 5.55-MBq, so this 4-sample maximum can never be exceeded but could be in a general sample counting experiment where injected doses and organ activities might be much higher.

Finally, the present study was intended to test the accuracy of the proposed scheme and was not optimized for actual mouse imaging/counting experiments. The dwell time per bed position was arbitrarily chosen at 10 minutes to minimize statistical errors in the lowest activity samples counted at the latest imaging times. In a “real” experiment, dwell time could be adjusted downward (say to 5 minutes per bed position) to reduce the data collection interval and be shortened further if fewer tissue samples were required (by reducing the number of bed positions). We further reduced data processing time by volume projecting the coronal image stack to create a single image of the sample block that requires at most only 12 regions of interest (ROIs) to measure the count rate in each sample rather than the hundreds of ROIs that would be needed if these same data were analyzed slice by slice by transverse tomographic slice. Given the fixed geometry associated with the sample block measurements, it should be possible to automate all, or portions of, these data processing tasks, for example, apply a pre-defined set of 12 ROIs to the sample block image that automatically measures the sample count rates and, given the calibration factor, directly reports the total activity of each sample (or syringe) to the user. It should also be noted that the sample block need not be created by machining, but could instead be easily fabricated from low density plastic by 3D printing [2].

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Figure 1.
PET sample block mated to mouse imaging bed. A sample container is held at the lower right. A triangular groove for injection syringes is just visible down the centerline of the block. The distance between the leading edge of the first pair of sample containers (upper left) and the trailing edge of the last pair of containers (lower right) is slightly less than the maximum axial scanning field of the PET scanner when using a 16 slice overlap between each of 4 bed positions.

Figure 2.

Design details of the sample block. The size and number of the compartments can be tailored to the available PET scanner constrained only by the diameter of the scanner transverse field of view and by the maximum axial traverse of the scanning bed. The pictured block was designed to hold enough sample containers to count all of the major body organs of 20 g mice.

Figure 3.

Coronal volume projection image of the sample block, i.e. (sum of coronal slices spanning the vertical thickness of the sample containers, where all samples have the same volume (1-ml) but differ from their (left/right) neighbor by a factor of two in total activity and by a 32-fold range in total activity from one end of the block to the other. The lowest two activity samples are barely visible because of this wide range. The center of each sample appears brighter than the surrounding fluid since the containers have a shallow conical bottom and the sample thickness is greatest at the center. A faint reconstruction artifact is also visible on the interior side of each high activity sample (left and right ends of the block).

Figure 4. (A) Sample activity measured with the PET scanner compared to sample activity measured with the well counter (N = 48). (B) Syringe activity measured with the PET scanner compared to syringe activity measured with the dose calibrator.