YAP-(S)PET Small Animal Scanner: Quantitative Results

G. Di Domenico, G. Zavattini, Member, IEEE, E. Moretti, A. Piffanelli, M. Giganti, A. Motta, N. Sabba, L. Uccelli, E. Benini, A. Duatti, C. Bolzati, A. Boschi, and A. Del Guerra, Senior Member, IEEE

Abstract—The University of Ferrara YAP-(S)PET scanner is currently being employed in small animal SPECT studies, with $^{99m}$Tc labeled radiotracers, with the goal of obtaining quantitative activity measurements from region of interest (ROI) analysis of reconstructed images of rats. The measurements will be compared directly with traditional ex vivo measurement of activity with gamma counters. To achieve this goal, the scanner was calibrated relative to a standard Isodose dose calibrator which was calibrated with various certified activity sources. The calibration factor K was defined as the ratio of the activity measured with the Isodose (MBq) and the image count rate (cps). We find $K = 0.1346 \pm 1.34 \times 10^{-4}$ MBq/cps, with good linearity over a wide range of activities (9–88 MBq). With the calibrated scanner we compared results of activity measurements from images of whole-rat heart acquisitions versus excised hearts. We found good agreement between the two measurements.

Index Terms—Calibration, cardiac radiotracers, image processing, quantitation, single photon emission computed tomography (SPECT), small animal imaging, tomography.

I. INTRODUCTION

The trend in modern molecular biology to study the molecular basis of organ functions and diseases can be facilitated by the use of imaging technologies that allow scientists to study in vivo molecular mechanisms by monitoring the pathways of molecular probes in living animals with dedicated scanners. Both positron emission tomography (PET) and single photon emission computed tomography (SPECT) are important tools that may assist biologists in developing and understanding of a particular molecular probe by being able to observe both its temporal and spatial biodistribution in vivo.

The importance of these devices, as well as the need for quantitative results, has been stressed in a number of papers [1]–[3]. In response to the needs of laboratories that use small animals, a number of groups have, recently, developed dedicated nuclear medicine imaging systems that have pushed the limits of spatial resolution [4]–[9]. Among these systems is a small animal PET system, based on four rotating planar detector heads [10], which has been built and tested at Ferrara University, Ferrara, Italy.

In this system each detector module is composed of a matrix of 400 YAP:Ce finger crystals ($2 \times 2 \times 30 \text{ mm}^3$ each) directly coupled to a position sensitive photomultiplier tube (Hamamatsu R2486-06).

By applying two high resolution parallel hole collimators to opposite detectors we have created a system that functions as both a PET and a SPECT scanner for small animals [11]. This dual functionality can be traced to two factors: 1) the YAP:Ce crystal has sufficient efficiency for detecting 511 keV radiation for PET, generates enough light to allow the detection of the low energy gammas for SPECT (140 keV for the isotope $^{99m}$Tc) and does not have an intrinsic background and 2) the planar geometry of the scanner detectors allows the use of more or less standard flat collimators, maintaining the same field of view (FOV) both in PET and in SPECT. The collimators are made of lead with circular holes that are 20 mm long, and 0.6 mm in diameter and with 0.15 mm septa. The readout and data acquisition systems are standard NIM/CAMAC electronics.

The FOV of the tomograph has a diameter of 4 cm and an axial length of 4 cm for both the PET and SPECT configurations. The spatial resolution and sensitivity are appropriate for mice and rat studies for both modalities. A summary of the YAP-(S)PET scanner performances is reported in Table I.

In studies using small animal tomographic scanners, in addition to obtaining high quality images, extraction of quantitative activity measurements is also highly desirable. To achieve this goal, the scanner was calibrated in reference to a Isodose dose calibrator based on Geiger–Muller counters which previously underwent an absolute calibration with several certified activity sources.

At present, the Department of Experimental and Clinical Medicine, University of Ferrara, the scanner is employed in small animal SPECT studies of myocardial perfusion agents labeled with $^{99m}$Tc.

The aim was to quantify the activity measured in a ROI within a reconstructed image of a rat and compare results with those derived from standard biodistribution measurements, which consist of sacrificing the rat and measuring the activity in the organ of interest with a calibrated gamma counter. A demonstration that traditional biodistributions could be replaced by SPECT quantitative measurements would reduce the number of animals sacrificed and therefore the costs of some experiments.

In this study, we focused our attention on heart studies conducted with $^{99m}$Tc–Sestamibi, $^{99m}$Tc–Myoview and with a new radiotracer called $^{99m}$Tc–NBODC5 [12], [13]. Our investigation deals with an ex vivo quantitative measurement of...
TABLE I
YAP-(S)PET Scanner Performances

<table>
<thead>
<tr>
<th>Mode</th>
<th>Number of Detector Heads</th>
<th>Energy Resolution FWHM @ 140 keV with Collimator</th>
<th>Spatial Resolution FWHM</th>
<th>Sensitivity cps/μCi</th>
<th>FOV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPECT</td>
<td>2</td>
<td>32%</td>
<td>Uniform 3.5 mm</td>
<td>0.55* constant over FOV</td>
<td>4 cm diameter, 4 cm axial</td>
</tr>
<tr>
<td>PET</td>
<td>4</td>
<td>27%</td>
<td>Uniform 1.8 mm</td>
<td>6.4/0 at center</td>
<td>4 cm diameter, 4 cm axial</td>
</tr>
</tbody>
</table>

Measured excluding photons with energy below 140 keV.

Fig. 1. Energy spectrum for a 10 mm, 99m Tc cylindrical source surrounded by a 11 mm lucite ring. Superimposed are the two gaussian functions deriving from the fit.

rat hearts that were obtained and imaged from rats that were sacrificed 1 h after the injection with each of the three radiotracers.

The acquired data were reconstructed by using an iterative EM algorithm reconstructions program (40 iterations) developed by the same group. With our program the images are reconstructed so as to represent count-rates within an energy window from 140 to 250 keV.

By applying such an energy cut we completely eliminate scatter contributions due to the animal. This reduces the number of reconstructed events but the efficiency corrections, necessary during reconstruction, are independent of the geometry of the object under study. In fact, the scatter contribution from 140 keV and above is negligible. This is shown in Fig. 1 which reports the energy spectrum for a 10 mm diameter cylindrical phantom, filled with 99m Tc, surrounded by a 11 mm thick lucite ring; observe how scatter tails are not present above 140 keV. With this choice of the energy window though, gain must be carefully monitored during reconstruction and drifts must be corrected for.

By calibrating the scanner with different size phantoms and by using it to compare the measured activity of the heart inside the rat body with that of the extracted heart, the contribution of attenuation is also estimated. Different papers about quantitation in small animal SPECT studies [14], [15] demonstrate that it is possible to obtain accurate results for both absolute activities in hand-drawn ROIs as well as dimensions of structures from reconstructed SPECT images, but no calibration procedure nor detailed ROI definition criteria are described.

The calibration method reported in this article, together with the criteria to define a user independent ROI, are intended to serve as an approach for obtaining quantitative results with other PET or SPECT scanners.

II. MATERIALS AND METHODS

A. ROI Definition Criteria

In order to measure the activity content of a region of a reconstructed image, we defined an absolute, user independent method to draw a ROI. To do this, we developed a procedure based on a simple model that simulates the distribution of activity of the organ of interest (the heart) and the surrounding activity. For simplicity we explain the model for one dimension, but our software was developed to work directly on 2-D transaxial images.

Fig. 2 represents an ideal 1-D profile of an activity region with a constant surrounding activity (full line). The square wave of height Y1 represents the heart with a mean surrounding activity (MSA) of value Y0. In absence of spread due to spatial resolution one would get the exact activity just by integrating the function from X1 to X2. The dashed line instead represents the effect of the finite spatial resolution of the tomograph on the original profile. The function due to convolution cuts the original (not convolved) function at height Y2 which, in absence of partial volume effects [16], as it is the case, result to be (signal + MSA)/2. So, if we take the integral of the activity from point X1 to X2 on the convolved profile we lose two times the activity corresponding to region indicated by the 1 directly above P1. We can recover this activity by integrating, instead, from point X0 to point X3; by doing so, we include region 2...
in the integral which is equal to region 1. But we also include the surrounding activity from point X0 to X1 and from X2 to X3 which must be subtracted. For simplicity we define as Small ROI (SR) the region from X1 to X2 and with Big ROI (BR) the one from X0 to X3. In conclusion, passing to 2-D image plots we define \( z = f(x, y) \) as the activity distribution function; determination of integral limits on 2-D image plots is performed as follows:

1) we estimate the MSA and the maximum of \( f(x, y) \), \( \max\{f(x, y)\} \), directly from the image;
2) we calculate the contour at half maximum \( \max\{f(x, y) + \text{MSA}\}/2 \) to obtain the SR;
3) we determine the BR by extending SR to where the value of \( z \) reaches MSA;
4) we use the following formula to calculate the total activity \( A \) in each slice:

\[
A = \left[ \int_{\text{BR}} f(x, y) \, dx \, dy \right] - (\text{BR} - \text{SR}) \cdot (\text{MSA})
\]

An example of the areas we find with our method is shown in Fig. 3 for an \textit{ex vivo} whole-rat heart imaging.

All counts we will refer to from now on, both for the calibration of the scanner and measurements of rat-heart activity, are obtained with the criteria outlined above.

**B. Activity Calibration of the Scanner**

For the calibration of the scanner all measurements were referenced to Isodose dose calibrator based on Geiger–Muller counters, previously tested with certified \( ^{57} \text{Co} \) calibration sources. We have also verified the counter’s linearity by following the activity decay of \( ^{99} \text{mTc} \). Several cylindrical phantoms and capillaries were filled with a solution of \( ^{99} \text{mTc} \), and their activities were measured with the dose calibrator. The phantoms were then placed in the YAP-(S)PET, acquisitions were performed and phantom images reconstructed (see Fig. 4). The cylinders and capillaries were placed in two different positions: at the center of the FOV of the scanner, and at about 1.5 cm from the center along the diagonal (see Table II). In the first column of Table II the phantom type and its dimensions are indicated (\( D \) is the diameter and L the length). The calibration factor K is chosen as the ratio between the activity value (MBq), obtained with the dose calibrator, and the image count rate (cps) associated to the whole phantom image. In Fig. 5 is shown the activity measured with the Isodose versus the count rate measured within the ROI. A good linear correlation was obtained (\( r = 0.998; p < 0.001 \)).

On the same graph are reported all measurements performed with the different phantoms listed in Table II. The slope of the graph gives the calibration factor K.

The error assigned to each measurement was taken as the standard deviation of the residuals of a fit with a straight line. This was done because the statistical error due to the Isodose did not give satisfactory \( \chi^2 \).

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**TABLE II**

<table>
<thead>
<tr>
<th>Phantom</th>
<th>Activity (MBq)</th>
<th>Position in FOV</th>
<th>Measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td>capillary</td>
<td>21.78±0.61</td>
<td>n.c.</td>
<td>4</td>
</tr>
<tr>
<td>cylinder</td>
<td>75.67±0.60</td>
<td>c.</td>
<td>5</td>
</tr>
<tr>
<td>cylinder</td>
<td>37.59±0.60</td>
<td>c.</td>
<td>3</td>
</tr>
<tr>
<td>cylinder</td>
<td>87.23±0.87</td>
<td>c.</td>
<td>4</td>
</tr>
<tr>
<td>cylinder</td>
<td>38.13±0.61</td>
<td>n.c.</td>
<td>4</td>
</tr>
</tbody>
</table>

**c. means centered; n. c. mans not centered.**
The calibration factor $K$ used in successive quantitative measurements, estimated by using the standard deviation of the residuals, is

$$K = 0.13\pm 0.0008 \text{ MBq/cps}.$$  

C. Quantitative Measurements in Rats

The precise calibration of the YAP-(S)PET scanner allows us to compare the activity measured in a ROI within a reconstructed image of a rat, with respect to the traditional measurement of activity obtained by sacrificing the rat and putting the organ of interest in a gamma counter. We considered the rat heart as the organ of interest, and we performed three measurements on each of the rats in the set. As already mentioned we injected three different heart radiotracers: $^{99m}$Tc – Sestamibi, $^{99m}$Tc – Myoview and $^{99m}$TcN – DBODC5 in a total of 12 rats; we are interested in both absolute value of activity in the heart and organ uptake fraction.

Sprague–Dawley rats were injected with a known activity, and were sacrificed one hour after the injection. A first tomographic SPECT dataset was acquired (see Table III, Whole-Rat Heart Activity). Then the heart was removed and a data set was acquired with both the SPECT scanner and the Isodose (see Table III, Excised Heart Activity). SPECT images were obtained and measurements were made on a ROI centered on the heart for both tomographic SPECT images. The previously obtained calibration factor $K$ was then used to convert the image counts to absolute activity.

All acquisitions were performed with 180° rotation of the detectors over the chest of the rat with 32 angular views and by acquiring 30 000 counts per view for whole rat acquisitions and 10 000 counts per view for excised hearts.

III. RESULTS

A. Quantitative Measurements

The data reported in Table III and Table IV show the comparison between measurements with the YAP-(S)PET scanner and the traditional gamma counter technique.

<table>
<thead>
<tr>
<th>Radiotracer</th>
<th>Excised Heart Activity (MBq)</th>
<th>Whole-Rat Heart Activity (MBq)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isodose</td>
<td>YAP-(S)PET</td>
</tr>
<tr>
<td>DBODC5 (1)</td>
<td>1.98±0.14</td>
<td>1.94±0.01</td>
</tr>
<tr>
<td>DBODC5 (2)</td>
<td>1.89±0.14</td>
<td>1.71±0.01</td>
</tr>
<tr>
<td>DBODC5 (3)</td>
<td>2.31±0.15</td>
<td>1.96±0.01</td>
</tr>
<tr>
<td>Sestamibi (1)</td>
<td>2.28±0.15</td>
<td>2.09±0.01</td>
</tr>
<tr>
<td>Sestamibi (2)</td>
<td>2.18±0.15</td>
<td>2.32±0.01</td>
</tr>
<tr>
<td>Sestamibi (3)</td>
<td>1.89±0.14</td>
<td>1.68±0.01</td>
</tr>
<tr>
<td>Sestamibi (4)</td>
<td>2.82±0.16</td>
<td>2.52±0.02</td>
</tr>
<tr>
<td>Myoview (1)</td>
<td>1.87±0.14</td>
<td>1.82±0.01</td>
</tr>
<tr>
<td>Myoview (2)</td>
<td>1.70±0.13</td>
<td>1.63±0.01</td>
</tr>
<tr>
<td>Myoview (3)</td>
<td>1.43±0.12</td>
<td>1.63±0.01</td>
</tr>
<tr>
<td>Myoview (4)</td>
<td>1.47±0.12</td>
<td>1.51±0.01</td>
</tr>
</tbody>
</table>

The variability of the uptake fraction value (Table IV) is due to the different dimensions of the hearts considered.

In particular, note the Fig. 6 plots of Isodose versus YAP-(S)PET excited heart activities. Linearity is verified but the significant dispersion of the residuals causes an error in the angular coefficient of 2.8%; this is greater than the error in K (0.5%) and is due to poor counting statistics of the Isodose in this range of activities. So for low activities the YAP-(S)PET measurements would be preferred.

Fig. 6 can also be regarded as a confirmation of the value of calibration factor between the Isodose and the YAP-(S)PET for low activities.

Fig. 7 shows plots of the heart activities of whole rat versus excited heart activities, both from YAP-(S)PET acquisitions. Data fitting with a line shows an angular coefficient of 0.91; this means that activities of whole rat hearts are underestimated by
By extracting dimensions from the CT scan of a Sprague–Dawley rat (Fig. 8), of the same weight as the ones used for SPECT imaging, we find that the mean muscle thickness of the chest is about 6–7 mm.

This measurement, together with the assumption on the linear coefficient of muscles, demonstrate the consistency of our whole-rat analysis; this is not meant, of course, to quantify exactly attenuation from CT images (chest thickness is variable and bones are present).

Future work will complete the study by evaluating a priori attenuation maps with a dedicated CT acquisition.

V. CONCLUSION

We have successfully calibrated the YAP-(S)PET scanner for quantitative SPECT measurements. The activity of $^{99m}$Tc–Sestamibi, $^{99m}$Tc–MyoView and $^{99m}$Tc– DBODC5 measured in the rat heart was compared to the standard measurement performed using a gamma counter. The comparison showed that the method can be successfully used in quantitative in vivo activity determination.

REFERENCES


