Published in final edited form as:

Phys Med Biol. 2008 October 21; 53(20): 5867–5882. doi:10.1088/0031-9155/53/20/021.

Accuracy and reproducibility of tumor positioning during prolonged and multi-modality animal imaging studies

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Abstract

Dedicated small-animal imaging devices, e.g. positron emission tomography (PET), computed tomography (CT) and magnetic resonance imaging (MRI) scanners, are being increasingly used for translational molecular imaging studies. The objective of this work was to determine the positional accuracy and precision with which tumors *in situ* can be reliably and reproducibly imaged on dedicated small-animal imaging equipment. We designed, fabricated and tested a custom rodent cradle with a stereotactic template to facilitate registration among image sets. To quantify tumor motion during our small-animal imaging protocols, ‘gold standard’ multi-modality point markers were inserted into tumor masses on the hind limbs of rats. Three types of imaging examination were then performed with the animals continuously anesthetized and immobilized: (i) consecutive microPET and MR images of tumor xenografts in which the animals remained in the same scanner for 2 h duration, (ii) multi-modality imaging studies in which the animals were transported between distant imaging devices and (iii) serial microPET scans in which the animals were repositioned in the same scanner for subsequent images. Our results showed that the animal tumor moved by less than 0.2–0.3 mm over a continuous 2 h microPET or MR imaging session. The process of transporting the animal between instruments introduced additional errors of ~0.2 mm. In serial animal imaging studies, the positioning reproducibility within ~0.8 mm could be obtained.

1. Introduction

Numerous dedicated small-animal imaging devices, e.g. microPET (Myers and Hume 2002), animal MRI (Bradley *et al* 2007) and microCT (Li *et al* 2006), have emerged in the past decade, and are becoming increasingly important for the evaluation of new molecular imaging agents and protocols undergoing preclinical investigation. Small-animal imaging enables noninvasive tracking of molecular and physiological events important for the biology of the disease processes and facilitates determination of the pharmacokinetics of prospective diagnostic and therapeutic agents. More versatile equipment, such as advanced multi-modality animal imaging devices, is currently under development (Catana *et al* 2006). One commercial device, the X-SPECT™ (Gamma Medica, Inc., Northridge, CA) comprises a microSPECT-microCT

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and optional PET combination in the same unit, thereby allowing multi-modality imaging of the animal whilst positioned on the same couch. However, most investigators still rely upon separate scanners and 'software' registration to generate multi-modality images (Deroose *et al* 2007). Accurate registration is crucial in multi-modality imaging studies and a number of methods have been developed to facilitate this (Fei *et al* 2006, Chow *et al* 2006, Beattie *et al* 2007). Although some detailed tumor registration studies have been performed, more typically the focus of such studies is on the biological content of the images with the assumption, either implicit or explicit, that tumors undergo negligible change in position over the course of experiments (Dandekar *et al* 2007). Papers addressing complex imaging procedures on tumor-bearing rodents rarely provide estimates of the amount of tumor motion. This is understandable due to the difficulty of performing these kinds of measurements.

The purpose of this study was to investigate the accuracy and precision of tumor positioning during animal imaging studies involving long scan durations with serial and/or multi-modality procedures. In particular, we wanted to evaluate the magnitude of the various registration errors associated with these studies. An example of this would be a microPET/MR imaging study of the type shown in figure 1 in which an attempt to measure and spatially correlate blood flow and hypoxia were performed in the same animal. Whereas the images of blood flow and hypoxia appear to be spatially well registered (figure 1(d)), the numerical accuracy of this registration was not known. Three generic classes of experiment were performed corresponding to typical types of molecular imaging procedures: (A) Sequential or prolonged scans using a single modality, e.g. dynamic PET scans (Chen *et al* 2004, Yuan *et al* 2006), performed in one anesthesia session; (B) cross-modality studies, e.g. PET and MRI on the same animal (Fei *et al* 2006, Rowland *et al* 2005) in which the animal remains anesthetized and immobilized but is imaged on two or more devices and transported between them; (C) serial studies repeated over longer time periods where the animal is returned to its cage between successive imaging sessions (Dandekar *et al* 2007, Zanzonico *et al* 2004).

In clinical radiotherapy, there exists a considerable body of literature on the accuracy of patient positioning and repositioning (Ezzell *et al* 2007, Allison *et al* 2006, Murray *et al* 2007, Lovelock *et al* 2005). Based on these methodologies, we have developed a special cradle for animal positioning and atraumatic immobilization which includes a stereotactic template with fiducial markers to facilitate registration. The use of special frames for repositioning rodents has been described by other investigators (Rubins *et al* 2001, Schiffer *et al* 2006). The key advantage of an external fiducial marker-based approach is that it provides a reference coordinate system against which animal motion can be accurately determined. In order to visualize animal and, in particular, tumor motion with respect to external fiducial markers, there are two possibilities: anatomical-based landmarks or implanted markers. While the use of anatomical landmarks is atraumatic, it has the disadvantage that the markers may not be readily identifiable, especially for nuclear imaging or studies focusing on soft tissues such as tumors growing on the hind limb. The use of implanted reference markers has the potential disadvantage of marker displacement *in situ* due to trauma or other mechanisms. However, the main advantage of these is ease of visualization and, even if some displacement occurs, comparisons of external and internal markers enable the determination of an upper limit on tumor movement.

As our group has a particular interest in nuclear imaging, we employed the method of implanting reference markers to quantify tumor repositioning accuracy. This approach was initially devised in radiation therapy for this particular purpose (Lam *et al* 1993, Balter *et al* 1995, Xu *et al* 2006). It is important to note that the sole purpose of this study was to assess tumor repositioning accuracy and precision and not to derive biological information from images. Such knowledge will help in the interpretation of studies focused on the acquisition

of biological information from images where the use of implanted markers would not be appropriate.

Rats were selected for these studies due to the ability of growing tumors to sizes suitable for marker implantation. Animals were kept anesthetized for the duration of the imaging procedures and were euthanized immediately upon completion of the experiments. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

2. Methods and materials

2.1. Stereotactic rodent holder and tumor-implantable markers

It is crucial to employ animal-specific immobilization in order to ensure minimal animal motion during long and complex molecular imaging procedures. For the purpose of our investigations, we designed and fabricated a dedicated rodent holder system compatible with the different scanners, thereby allowing multi-modality imaging with the animals anesthetized in the same position. The essential components of the rodent holder system, shown in figure 2, include: (a) a semi-cylindrical plastic cradle (compatible with MR and resulting in low attenuation for PET or CT photons), (b) an animal-specific mold (Zanzonico *et al* 2006) made from SecureFoam™ (Bionix Radiation Therapy) to conform to the animal body contour within the cradle and (c) a stereotactic template to facilitate rigid-body image registration.

The semi-cylindrical cradle provides a platform for the animal mold and MRI coil. The stereotactic template is positioned on vertical plastic posts that screw into threaded holes in the cradle base. This was specifically designed for the study of tumors growing on the hind limbs of rats, a common location in many xenograft studies. The tumor-bearing leg is positioned between the rods, with the tumor directly beneath the template. Helmholtz coils are placed around the fiducial template when MR studies are performed (figure 2(d)).

The fiducial template design was developed from the multi-modality biological imaging method first described by Humm *et al* (2003). It consists of a Teflon template, with three holes 1 mm in diameter: two holes are parallel to the axis of the disk and the third is perpendicular to the first two. These holes provide the conduits into which multi-modality imaging agents (PET tracer and/or MR/CT contrast media) may be filled to form fiducial rod markers, with which the position and orientation of the rodent–cradle assembly are determined at each imaging session.

Our initial experiments designed to measure tumor location accuracy, after registration using the external stereotactic fiducial markers, resulted in ambiguities because of the reliance upon the subjective judgment of common anatomic landmarks among image sets. Therefore, in the current work, artificial landmarks were created in the form of small reference point markers inserted into the tumor via angiocatheter and visible by each of the intended imaging modalities. This approach to multi-modality marker design and point marker position detection was adapted in part from the method developed by Wang *et al* (1996). The point markers were made of 24-gauge PFA medical micro tubing (Scientific Commodities, Inc., Lake Havasu City, AZ). Pieces of micro tubing were filled with ^{18}F -FDG (^{18}F -fluorodeoxyglucose) solution (2–3 μCi each) and/or MR/CT contrast agents, and sealed at both ends with DOUBLE/BUBBLE® epoxy (Elementis Specialties, Inc., Hightstown, NJ). Less than 1:10 000 v/v Gadolinium-based Magnevist® Injection (Berlex Laboratories, Inc.) was used in the solution for MR imaging. The CT contrast media was Omnipaque™ (iohexol) Injection (Amersham Health, Cork, Ireland), containing 300 mg organic iodine per milliliter. The dimensions of all point markers were 1 mm in external diameter ranging in length from 4 to 5 mm and the aqueous volume contained within the marker tubing was on average 0.6 mm $\times$ 1.5 mm cylinder. All fiducial rod markers and point markers were prepared immediately before imaging studies.

2.2. Animal preparation and marker insertion procedure

For this study, 6–10 week old NIH-rnu nude rats (NCI-Frederick Cancer Research, Frederick, MD) were injected subcutaneously with approximately 2×10^6 cultured R3327AT cells into the right hind limbs. The imaging studies were conducted 2 weeks later when the R3327AT Dunning syngeneic tumors had reached a diameter of 1.5–2 cm. The animal was anesthetized by inhalation of 1.5% v/v isoflurane (Baxter Healthcare Corporation, Deerfield, IL) in oxygen at a flow rate of 1.5 l min^{-1} , and placed prone in its freshly made mold. The animal was anesthetized during mold fabrication and then again from point marker insertion through to the completion of the imaging study. Two or three point markers were inserted into each tumor depending on tumor size and accessibility. For marker implantation, the skin and fascia over the tumor were perforated at selected points with a 16-gauge Introcan Safety® IV catheter (B. Braun Medical Inc.), through which the point markers were pushed and inserted into the tumor. These internal point markers served to allow systematic measurement of the relative motions of tumor xenografts during prolonged imaging studies and transports as well as evaluation of the reproducibility of animal repositioning. After bleeding (which was minimal) stopped, the animal was positioned in its mold and the fiducial template was placed just above the tumor and secured with nuts on the two vertical poles attached to the cradle (as described above). The height of the template was adjusted using spacers to ensure that the template did not touch the tumor. Immediately following the completion of the imaging study, the animal was euthanized by carbon dioxide asphyxiation.

The animals were maintained in accordance with the guidelines of the Research Animal Resource Center at Memorial Sloan-Kettering Cancer Center (MSKCC) and all experiments were conducted under the approval of an IACUC animal protocol.

2.3. Small-animal MR, PET and CT imaging protocols

All imaging sessions were performed with the animal immobilized and anesthetized in the rodent holder cradle, the design of which allowed the whole assembly to be moved among different imaging scanners. The dedicated animal imaging devices used in this study were the Focus 120 microPET™ (Concorde Microsystems, Knoxville, TN, now Siemens Preclinical Solutions), the Bruker 4.7 T Biospec MR (Bruker Biospin MRI, Inc., Billerica, MA) and the CT subsystem of the X-SPECT™ scanner.

A home-built Helmholtz RF coil circuit was used for MR detection. The Helmholtz coils had a diameter of 41 mm and spacing of 35 mm between the two coil rings. The coils were fitted through the foam animal mold and surrounded the tumor and the template. T2 scout images of the tumor and the template markers were first acquired. T2 RARE (Rapid Acquisition with Relaxation Enhancement) images in coronal orientation of the rat leg tumor and the template were then acquired. The imaging parameters were: RARE acceleration factor 8, FOV 40 mm, TR 3.5 s, TE 45 ms, slice thickness 0.5 mm, NEX 20, in-plane resolution $312 \mu\text{m} \times 312 \mu\text{m}$. There were a total of 25–30 slices depending on tumor size and the total acquisition time was 18 min for a single imaging study.

PET imaging was performed using a 350–750 keV energy window and a 6 ns timing window. The duration of the scans was typically 10 min, with at least 10 million coincidence events per acquisition. The resulting list-mode data were sorted into 2D histograms by Fourier rebinning and transverse images reconstructed by filtered back-projection into a $128 \times 128 \times 63$ matrix. The reconstructed spatial resolution for ^{18}F was 1.7 mm full-width half-maximum (FWHM) at the center of the field of view. The image data were corrected for nonuniformity of response of the microPET, deadtime count losses and radioactive decay but no attenuation, scatter or partial-volume averaging correction was applied.

Transmission CT imaging was performed using a tube voltage of 50 kVp and filament current of 600 μ A. Images were reconstructed into a $256 \times 256 \times 256$ image matrix using the Feldkamp conebeam reconstruction algorithm provided by the manufacturer. Total CT acquisition time was ~ 10 min and the spatial resolution was $\sim 300 \mu\text{m}$ FWHM.

2.4. Three types of imaging study

The experiments performed in this work comprise three distinct types, which will subsequently be referred to as types A, B and C, respectively.

Type A consisted of experiments designed to simulate long dynamic scans and to measure the effects of intra-scan animal tumor motion. In these experiments, rats remained continuously anesthetized and positioned within their respective customized molds for approximately 2 h, during which a series of individual images were acquired. These experiments provided baseline measurement data for the degree of tumor movement for rodents maintained under such fixed conditions.

Type B consisted of experiments to determine the positional accuracy of multi-modality molecular tumor imaging studies. As in type-A studies, animals remained positioned and anesthetized within their molds, but were transported between separate facilities (microPET and MR) for the individual scans. The results from these animals reflected the motion incurred by transportation and differences in the spatial uniformity between the different imaging devices as well as the effects of animal motion within the mold.

Type C consisted of experiments simulating prolonged serial studies in which animals are returned to their cages and allowed to awaken between successive scans. Since animals needed to remain anesthetized due to the implantation of the reference point markers, these studies were conducted in a single 2 h session in which the animal was removed from and then repositioned in the mold between successive scans.

2.5. Experimental imaging study design

Because the point marker insertion was an invasive procedure, we attempted (and were required by the IACUC) to use as few animals as possible. The final results were obtained from 11 rats, divided among the type-A, -B and -C groups.

Group A consisted of five rats, numbered A1 through A5. Three animals (Rats A1 to A3) were positioned on the microPET scanner and consecutively imaged for up to 10 times. The further two (Rats A4 and A5) were scanned likewise on the MR scanner for six times. The entire study lasted about 2 h per animal to simulate a dynamic scan or other long-duration scan.

Group B included a total of four rats, numbered B1 through B4. The first two (Rats B1 and B2) underwent serial microPET imaging to provide information regarding the effects of holder removal, transfer and repeat setup on the same scanner between successive scans (a process performed eight times for each animal), separate from the registration errors associated with different imaging devices. The rodent holder assembly was removed from the scanner, placed on a cart carrying an isoflurane anesthesia unit, and transported within the imaging facility for about 5 min before the next scan. The second two (Rats B3 and B4) in this group underwent microPET/MRI cross-modality imaging studies to determine the accuracy with which multi-modality microPET and MR imaging studies can be performed. After microPET studies, these animals were transported using the above-mentioned cart for at least 15 min to the MR lab in a nearby building across street.

Group C consisted of two rats, numbered C1 and C2, which were used to simulate serial scans during which the animals must be returned to cages. Each animal underwent 10 separate

microPET scans. Between successive scans the animal was removed from the cradle for a sham placement in cage, and then immediately returned the scanner under continuous anesthesia. This study simulated experiments requiring animal repositioning within the cradle but without the long duration between successive scans necessitating allowing the animal to awaken from anesthesia between scans. The study performed in this way also removed possible additional error associated with tumor growth.

In all of the above imaging studies, the anesthetized rats were positioned unrestrained in their respective molds, and were not touched in the type-A and -B studies. The results of the foregoing experiments thus reflected the overall movement of subcutaneous hind-limb tumors when subject to the imaging procedures described above.

2.6. Detection of point markers and fiducial rods

Due to its definite dimensions, each intra-tumoral point marker was imaged as a 3D object occupying several voxels in each of the image sets. The position of a point marker was therefore equated with a set of intensity-weighted average coordinates, i.e. the centroid (Wang *et al* 1996), which defined a point in space that was used to measure the positional accuracy of an intra-tumoral location between scans.

The position of each point marker could be detected at subvoxel accuracy as its centroid. Our computer simulations showed that, if the point markers were imaged in the microPET and MRI scanners as described above, and a proper threshold of image intensity was selected in the centroid computation, the standard deviation of measurement errors in centroid coordinates would be ~0.1 voxels, with a maximum error of ~0.2 voxels for both scanners. In principle, the smaller voxel size of MRI (0.3 mm vs. 0.8 mm of microPET) is advantageous in terms of the centroid detection accuracy. In order to evaluate the actual accuracy of determining the marker positions, we proposed observing the measured variance in the distance between two point markers bound to a rigid body. From fundamental definitions in statistics, we obtained equation (1) which relates the standard deviation σ_d of the distance between two bound point markers measured from consecutive images, during which the bound markers are randomly replaced within the field of view of the imager:

$$\sigma_d^2 = 2\sigma_{x,y,z}^2 \quad (1)$$

where $\sigma_{x,y,z}$ is the standard deviation of the measurement errors of individual coordinate x , y or z in the image, which is considered same in the three directions for cubic voxels. The σ_d values measured on microPET using multiple point markers indicated that the above-mentioned computer simulations in fact derived the upper limits for the scanners.

The template fiducial rod markers served as a frame of reference with which to measure the relative motion of the intra-tumor point markers. The fiducial rod markers, employed to define the coordinate system in the stereotactic template, were detected in cross-section in the appropriate (i.e. axial, coronal or sagittal) projections. The centroid was calculated for the cross-section of each rod, in the same manner as for the point markers for each image slice; thus, a rod was converted into a 'string' of points.

Prior to any actual animal experiments, a series of validation tests were conducted to determine the robustness of the fiducial rod and reference point marker centroid determination in phantom experiments in which the point markers were attached to an empty cradle and for which there was zero displacement of the markers relative to the fiducial rods among serial scans. These data are presented in the Results section.

2.7. Image processing

Tomographic images provide an intrinsic coordinate system in which each voxel defines a location within the acquisition device. The fiduciary rods, which are rigidly incorporated into the stereotactic template affixed to the animal immobilization cradle, establish a second independent coordinate system that travels with the rodent holder assembly. As stated above, there are two possible scenarios in terms of the position of the animal holder: (1) in some imaging studies, the holder remained in the same position during the series of scans. In this case, the tumor position (represented by the centroids of the point markers) was measured relative to the imager, i.e. each image voxel represents a defined point inside the scanner; (2) in studies where the holder assembly moved relative to the scanner, only the holder with its fiduciary template can be used as the frame of reference for determining tumor position.

If x, y, z denote the coordinates of a point in the image coordinate system, and u, v, w its coordinates in the template system, the transformation of the two coordinate systems follows

$$\begin{pmatrix} u \\ v \\ w \end{pmatrix} = M \cdot \begin{pmatrix} x \\ y \\ z \end{pmatrix} + \begin{pmatrix} u_0 \\ v_0 \\ w_0 \end{pmatrix} \quad (2)$$

where u_0, v_0, w_0 are the coordinate offsets of the image in the template system, and the elements of the transformation matrix M are functions of rotation angles around the x, y, z axes. More detailed discussions on 3D coordinate transformation can be found in previous publication (Zhang *et al* 2004, Gong *et al* 2005).

For the current studies, each animal was positioned so as to align its body central axis parallel to the dedicated rodent cradle which in turn docked parallel to the scanner axis. The template was designed to attach to the rodent holder in such a way that u, v, w axes were approximately parallel to x, y, z axes, respectively, of the imaging devices (microPET and MRI). Positive coordinates were defined with respect to the animal as follows: for x (or u), from right to left; for y (or v), from ventral to dorsal or upward; for z (or w), from tail to head. The transformation matrix M in equation (2) was determined by a rigid registration algorithm in which the square of 'fiducial registration error (FRE)' (Fitzpatrick *et al* 1998) or the quantity FRE^2 , was minimized. The fiducial rod-based registration used in this project deviated from conventional point marker-based registration (Arun *et al* 1987, Maurer *et al* 1997) in that three rods replaced the commonly used point markers as registration fiducials, thus the definition of FRE^2 was also slightly modified. If d_i, d_j and d_k are the distances between the detected centroids of the fiducial rods and their corresponding axes defined in the template coordinate system, then a modified FRE^2 can be expressed as

$$\text{FRE}^2 = \sum_i d_i^2 + \sum_j d_j^2 + \sum_k d_k^2. \quad (3)$$

A MatLab® (The MathWorks, Inc.) program was written that iteratively computed the image offsets and rotation angles based on minimization of FRE^2 . Compared with point marker-based registration, in a rod-based image registration approach each fiduciary rod source provides a direction for alignment. The centroid coordinates (x, y, z) of an intra-tumoral point marker in the image system were transformed to coordinates (u, v, w) in the fiducial template system using the derived transformation parameters.

3. Results

All of the results of the animal studies in this paper are presented in terms of a shift in the locations of the intra-tumoral reference point markers relative to the imager or to the stereotactic reference frame. This measure is used as a surrogate estimate of the magnitude of the tumor motion occurring during the different imaging procedures. We denote the animal groups by A, B and C, the individual animals by 1, 2, ... (as indicated above), and the point markers by lower case Roman numerals (i, ii, ...). The combinations of these symbols are used where appropriate. The tabulated data are rounded to 0.01 mm, one order of magnitude smaller than the uncertainties associated with the centroid measurement (see section 2.6).

3.1. Baseline validation of fiducial rods and point markers alignment using a phantom setup

Surface plots of the signal intensity profiles of the fiduciary rods as visualized by microPET, MR and microCT are shown in figure 3. The multi-modality images of a tumor implanted with three point markers are shown in figure 4. The error analysis for the reproducibility of tumor position/location in this study was based upon the accuracy and precision in identifying the centroid coordinates extracted from the profiles across the fiduciary rods and the reference point markers. Before applying the methodology to animals, preliminary tests of the robustness of the fiducial template and holder assembly were performed using point markers glued to an empty animal mold attached to the cradle. The results of two sets of baseline experiments are shown in table 1. The first study provides the intrinsic error (standard deviation) resulting from image intensity fluctuations arising from Poisson and any other statistical noise combined with the error associated with centroid computation for four individual point markers (numbered P-i through P-iv) glued to the mold from 10 individual microPET scans. The results also provide the standard deviation for the centroids of the same point markers as determined from the fiducial coordinate system by transformation using equation (2).

The results of the second study provide similar data for the accuracy of another four point markers (numbered P-v through P-viii) glued to the mold, but under conditions of template disassembly and reassembly between successive microPET scans. This error measure thus reflects the stability of the template assembly in addition to the effects of image statistics and fiduciary rod and point marker centroid detection. Error data in the form of standard deviations are also shown in table 1 as calculated from 10 repeat microPET scans. This study showed a small increase in the error, presumably resulting from the slight deformability of the plastic components of the rodent holder system when the stereotactic template was removed and reattached. However, the errors in table 1 are subvoxel in magnitude on the microPET scanner (the modality with the coarsest spatial resolution), and thus demonstrate not only the stability of the rodent holder system but also its ability to provide useful data over the range of tumor motion among successive scans.

3.2. Tumor motion during continuous imaging

Group A animal experiments involved repeat microPET or MR imaging studies in which the animals remained immobilized and anesthetized during the entire procedure, simulating a long-duration dynamic scan. Results are presented for two sets of studies. In the first three animals (rats A1, A2 and A3), the tumor was implanted with two or three point markers and repeatedly imaged on the microPET over approximately 2 h. Because the animal holder remained at the same position during each 2 h study, the coordinates of point markers relative to both the microPET image and the fiduciary rod frames of reference were a measure of the 'best-achievable' stability of the whole holder assembly. Figure 5 shows graphically the changes in coordinates (x , y , z) of the three point markers against their values in the first image for rat A1. In these three animals, the largest deviation for any coordinate was less than 0.29 mm over the 2 h duration of the study (more results are shown in table 2, rows A1 to A3). This result is a

cumulative measure comprising of tumor tissue movement and marker movement and serves as an important baseline of the technique representing a dynamic microPET study when the animal is immobilized in an animal-specific support mold and not moved for the duration of the experiment.

The same experimental design was also employed for two additional animals (rats A4 and A5) but with serial MR imaging. The standard deviation of the location error for each of the three reference markers for six repeat MR image sets per animal is shown in rows A4 and A5 of table 2. The spatial resolution of MR is finer than that of microPET, and therefore the error range and the standard deviation for each point marker are presumably more certain than those measured by microPET. The microPET and the MR results for the tumor motion over the total 2 h imaging session were, however, in close agreement, suggesting that the degrees of animal movement when immobilized in each scanner are comparable. In these five animals, the maximal 3D point marker displacement was less than 0.4 mm.

3.3. Tumor motion resulting from animal transport between scans and scanners

In group B experiments, the effect of animal transport between scans was evaluated. The position of an implanted point marker was computed for and expressed as its coordinates in the fiduciary marker coordinate system. Figure 6 shows the variations in the position coordinates of the three markers in one of the rat tumors (B1) over a 2 h microPET study. In these animal transports, the additional 3D displacement in all point markers had a median of 0.17 mm and a maximum of 0.43 mm. The deviations in the locations of the intra-tumoral markers were larger, as expected, but still no more than half a microPET voxel (table 2, rows B1 and B2).

Actual multi-modality imaging studies were performed in which two animals (rats B3 and B4) underwent a microPET scan followed by an MRI scan (15-min transport). Measurements included the tumor motion error associated with animal transport as well as the error associated with the centroid determination of the reference point markers and fiduciary rods by the different modalities. Figure 7 shows the microPET and MR images of the same tumor registered using the fiduciary rods, demonstrating the perceivable displacement caused by transportation between these devices. The results for the positional accuracy of the intra-tumoral point markers are shown in table 3. On average, the position of a point marker changed by about 0.5 mm between scanners, with the direction of the greatest magnitude approximately parallel to the long axis of the extended tumor-bearing limb. These data reflect the displacement of the tumor relative to the stereotactic template and rodent–mold–cradle assembly.

3.4. Tumor location reproducibility from animal repositioning

In the group C experiments, the precision of animal repositioning was measured for those studies necessitating return of the animal to its cage between scans. Two animals (C1 and C2) that each underwent 10 repeated microPET scans were removed from their respective custom molds and then immediately repositioned in their molds—as accurately as possible—and rescanned. Table 2 (rows C1 and C2) presents the standard deviations of the measured intra-tumoral reference point marker locations based upon measurements against the imager (C1) and transformation of the tumor images from the registered fiduciary rods (C2). Figure 8 shows the results for the relative change in marker coordinates (x , y , z) of each intra-tumoral point marker from rat C1. The 3D deviations in point marker coordinates had a median of 0.35 mm, with a maximum of 1.15 mm between consecutive scans. Mostly, the deviations were no more than 0.8 mm (voxel size of microPET) in any coordinate relative to the initial value.

4. Discussions and conclusions

In continuous (e.g. dynamic) small-animal imaging studies, it is implicitly assumed that the animal does not move over the duration of the scan. However, for such studies—and even for those studies (e.g. serial imaging) where this assumption is clearly not valid, estimates of the magnitude of intra- and/or inter-scan motion are rarely, if ever, provided. Our initial efforts to quantitate animal motion for various imaging protocols were conducted without any internally inserted extrinsic markers. However, it was difficult in practice to unambiguously quantify the magnitude of the positional errors for subjectively identified intrinsic (i.e. anatomic) landmarks. Therefore, the intent of this study was to perform an analysis of the accuracy and precision of the reproducibility of tumor positioning for a number of common small-animal imaging protocols by implanting directly into tumors point markers visible by each imaging modality as ‘gold standard’ landmarks.

This study provides new data on the positional accuracy of hind-limb xenografts achievable in small-animal imaging. Using the rodent cradle and immobilization mold, this study demonstrated that for experiments based on a single, continuous anesthesia session up to 2 h in duration, registration accuracy of less than 0.4 mm can be achieved. For multi-modalities imaging protocols, image registration accuracy of less than 0.6 mm can be achieved. For those studies which require longer imaging times, i.e. when the animal must be returned to its cage and re-imaged at a later time, repositioning accuracy of less than 1.2 mm can be obtained using an animal-specific immobilization assembly. The standard deviations of the locations of intra-tumoral reference point markers were determined from repeat experiments performed, because of ethical considerations, on only a small number (a total of 11) of marker-implanted rats. Our ability to determine the accuracy of intra-tumoral locations over time and after different manipulative procedures common in small-animal imaging studies was made possible by the design of a dedicated stereotactic template and inserted point markers. However, the intra-tumoral markers served the sole purpose to evaluate positional accuracy in this particular work, and our results provide some essential data for various types of animal imaging studies in which the tumors are generally intact. To reproduce the animal setup accuracies and precisions reported in this study, only reliable rodent positioning and immobilization are required.

Acknowledgments

We thank the staff of the machine shop of Medical Physics Department, MSKCC for the construction of the rodent holder system and stereotactic template, the staff of Nuclear Medicine, MSKCC for preparing ^{18}F -FDG doses, and Dr Joseph O'Donoghue for revising the manuscript. We gratefully acknowledge funding support from the National Cancer Institute P01 CA115675-01 (P.I. C. Clifton Ling), and the technical services provided by the MSKCC Small-Animal Imaging Core Facility, supported in part by NIH Small-Animal Imaging Research Program (SAIRP) Grant No R24 CA83084 (PI Jason A Koutcher) and NIH Cancer Center Support Grant No P30 CA08748.

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Figure 1.

Cross-modality molecular imaging. (a) Photograph of the R3327-AT tumor grown in a nude rat after sacrifice and with the skin removed. (b) Parametric image of blood flow obtained by dynamic contrast enhanced MRI. (c) Hypoxia marker ^{18}F -MISO microPET image performed on the same animal whilst immobilized in the rat cradle. (d) Fusion of the two images using a green (gray-) scale for the MR image intensity and blue for the PET image intensity. This figure illustrates the ability to acquire good image registration between studies, but without allowing the accuracy of tumor registration to be determined.

Figure 2.

Rat setup devices. (a) Perspex rodent cradle with stereotactic template and Helmholtz coils. (b) Rodent cradle showing rat immobilized in SecureFoam™ mold. (c) Detail of the stereotactic template showing two of the fiduciary rods (arrows) containing radioactive and contrast materials. The third rod (not seen) is inserted into the side of the template and is used to define an origin for the y-axis. (d) Setup with the tumor-bearing leg positioned between the rods, with the tumor directly beneath the template. Helmholtz coils are in place when MR studies are performed.

Figure 3.

2D intensity profiles through a cross-section of a fiduciary rod orthogonal to the rod axis for each of the three imaging modalities: MRI, microPET and microCT. The size of each profile is about $10\text{ mm} \times 10\text{ mm}$.

Figure 4.

Sample images of one subcutaneous xenograft with inserted multi-modality point markers. Left: section of an MRI image showing three point markers in tumor. Middle: section of a microPET image showing three point markers in tumor but surrounding tissue is completely dark. Right: section of a microCT image showing three point markers in the central region of tumor. The scale bar is 5 mm.

Figure 5.
Relative changes in the coordinates of the centroid for each reference point marker during continuous microPET imaging.

Figure 6.
Relative changes in point marker coordinates caused by animal transportation.

Figure 7.

Overlay of MR and microPET images allowing two point markers to be observed upon fiduciary rod-based image registration only. (a) One MR image section with two inserted markers surrounded by dark circles. (b) Registered microPET image section of the same level showing two inserted markers. (c) Fused MR and microPET images in which the slight mismatch between the markers illustrates 0.5 mm displacements due to the transport from the microPET to MR scanner.

Figure 8.
Relative changes in point marker coordinates resulting from animal repositioning.

Table 1

Standard deviation of point marker coordinates calculated from serial images during which the markers remained stationary within the cradle mold (markers P-i through P-iv), and from serial images between which the fiducial template was disassembled and re-assembled (markers P-v through P-viii).

Reference system	Marker #	$\sigma_x(\sigma_u)$ (mm)	$\sigma_y(\sigma_v)$ (mm)	$\sigma_z(\sigma_w)$ (mm)
Imager	P-i	0.01	0.01	0.04
	P-ii	0.02	0.03	0.03
	P-iii	0.02	0.01	0.01
	P-iv	0.04	0.01	0.02
Template	P-i	0.02	0.01	0.04
	P-ii	0.02	0.03	0.02
	P-iii	0.04	0.01	0.01
	P-iv	0.04	0.01	0.01
Template	P-v	0.09	0.03	0.03
	P-vi	0.08	0.01	0.02
	P-vii	0.09	0.07	0.02
	P-viii	0.12	0.04	0.02

Table 2 Statistics of all point markers in the longitudinal microPET and MRI studies. The point markers are identified by their respective group, animal and marker designations. Listed in the table are standard deviations of the measured coordinates, and the median d_{median} and maximum d_{max} of 3D marker motion, where d is the displacement between two successive images for each marker. Coordinates (x , y , z) are used for group A and rat C1 and (u , v , w) for group B and rat C2.

Rat group	Marker #	$\sigma_x(\sigma_u)$ (mm)	$\sigma_y(\sigma_v)$ (mm)	$\sigma_z(\sigma_w)$ (mm)	d_{median} (mm)	d_{max} (mm)
Group A	A1-i	0.03	0.05	0.07	0.05	0.07
	A1-ii	0.01	0.09	0.04	0.02	0.08
	A1-iii	0.06	0.06	0.02	0.08	0.12
	A2-i	0.02	0.07	0.03	0.04	0.08
	A2-ii	0.04	0.09	0.02	0.07	0.10
	A3-i	0.07	0.05	0.09	0.07	0.12
	A3-ii	0.09	0.08	0.05	0.05	0.15
	A4-i	0.02	0.01	0.01	0.02	0.04
	A4-ii	0.04	0.05	0.04	0.07	0.11
	A4-iii	0.04	0.06	0.03	0.05	0.09
Group B	A5-i	0.05	0.04	0.04	0.12	0.17
	A5-ii	0.01	0.02	0.05	0.04	0.14
	A5-iii	0.03	0.03	0.03	0.06	0.08
	B1-i	0.14	0.16	0.09	0.17	0.31
	B1-ii	0.16	0.17	0.09	0.19	0.33
	B1-iii	0.14	0.12	0.08	0.18	0.43
	B2-i	0.13	0.07	0.08	0.22	0.28
	B2-ii	0.06	0.06	0.09	0.13	0.22
	B2-iii	0.09	0.07	0.07	0.14	0.25
	C1-i	0.12	0.21	0.13	0.23	0.53
Group C	C1-ii	0.11	0.16	0.23	0.29	0.58
	C1-iii	0.16	0.15	0.27	0.35	0.78
	C2-i	0.33	0.26	0.24	0.36	1.06
	C2-ii	0.31	0.26	0.25	0.39	1.15

Table 3

Comparison of marker coordinates in microPET and MRI images of two rats in group B that underwent cross-modality imaging studies. For each marker, the relative changes in (u , v , w) coordinates and the 3D displacement d are listed.

Marker	Δu (mm)	Δv (mm)	Δw (mm)	d (mm)
B3-i	0.0	-0.07	0.34	0.35
B3-ii	-0.11	0.13	0.51	0.54
B3-iii	-0.19	-0.07	0.42	0.46
B4-i	0.15	-0.05	0.54	0.57
B4-ii	-0.11	-0.12	0.43	0.46
B4-iii	0.05	-0.30	0.50	0.58