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Broad-Spectrum Multi-Modality Image Registration: From PET, CT, and MRI to Autoradiography, Microscopy, and Beyond

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Abstract—**Image registration and fusion are increasingly important components of both clinical and small-animal imaging and have lead to the development of a variety of pertinent hardware and software tools, including multi-modality, e.g. PET-CT, devices. At the same time, advances in microscopic imaging, including phosphor-plate digital autoradiography and immunohistochemistry, now allow ultra-high (sub-100 μm)-resolution molecular characterization of tissue sections. To date, however, in vivo imaging of intact subjects and ex vivo imaging of harvested tissues sections have remained separate and distinct, making it difficult to reliably inter-compare the former and the latter. The Department of Medical Physics and the Radiation Biophysics Laboratory at Memorial Sloan-Kettering Cancer Center, under the direction of Dr. Clifton Ling, has now designed, fabricated, and tested a stereotactic imaging system for so-called "broad-spectrum" image registration, from coarser-resolution in vivo imaging modalities such as PET, CT, and MRI to ultra-high-resolution ex vivo imaging techniques such as histology, autoradiography, and immunohistochemistry.**

I. Introduction

Information derived from structural and functional images is often complementary and integration (i.e. registration and fusion) of this information may be extremely useful, e.g. in localizing a "signal" focus to a specific normal or abnormal structure. Practical and reasonably reliable hardware and software tools, including multi-modality (e.g. PET-CT) devices, applicable to the various in vivo imaging modalities are now widely available[1,2,3]. At the same time, however, rigorous registration and fusion of in vivo images with ex vivo microscopy images has been largely ignored - despite the richness of specific molecular imaging probes now available for immunohistochemistry[4]. Among other considerations, the broad range of spatial resolution - from several millimeters for in vivo modalities to less than 100-μm for ex vivo techniques - makes this technically challenging. In addition, ex vivo microscopy images of two-dimensional (i.e. very-thin) sections of tissue are completely displaced from its host in vivo anatomy, further complicating registration of these disparate types of images.

As described in this paper, the Department of Medical Physics and the Radiation Biophysics Laboratory at Memorial Sloan-Kettering Cancer Center (MSKCC), under the direction of Dr. Clifton Ling, has now designed, fabricated, and tested a rather unique stereotactic imaging system for image registration of coarser-resolution in vivo imaging modalities such as PET, CT, and MRI to ultra-high-resolution ex vivo imaging techniques such as histology, autoradiography, and immunohistochemistry. In addition, this stereotactic system can be used to guide interstitial probe measurements of tissue parameters such as partial pressure of oxygen (pO_2) and to spatially index such measurements to allow spatial correlation between the parameter values and voxel values in in vivo (e.g. PET and/or MRI) images. Because of the unusually wide range of spatial resolution and the disparate image types to which this procedure is being applied, we have characterized it as "broad-spectrum" image registration.

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II. Stereotactic Template Design for Broad-Spectrum Image Registration

A. Scope of Work

The objective of this work was to design, fabricate, and test - for small laboratory *animals* (i.e. mice and rats) - a practical stereotactic template system for image acquisition that will provide a reference three-dimensional coordinate system for registration of multi-modality in vivo images (i.e. PET, SPECT, CT, and MRI) with each other and with tissue-sections ex vivo (i.e. histology, digital phosphor plate autoradiography, and immunohistochemistry) images. Such a system would allow, for example, direct comparison of serial microPET images acquired at different times or consecutively acquired multi-modality images (i.e. microPET and MRI images). In addition, the stereotactic template can be used to guide in situ measurements of pO_2 and spatially index such intra-tumoral measurements so that these data can be spatially correlated with the voxel values in microPET and/or NMR images.

There are two distinct components of our multi-modality image registration system: a) an immobilization device for reproducible positioning for in vivo imaging, i.e. for serial intra-modality (e.g. PET) imaging and/or sequential multi-modality (e.g. PET and MRI) imaging; and b) a stereotactic template for three-dimensional registration of sequential in vivo macroscopic images (e.g. PET and MRI) and ex vivo microscopic images (i.e. autoradiograms and/or histological- or immunohistochemical tissue-section images).

B. *A Small-Animal Immobilization Device for Reproducible Positioning for in vivo Imaging*

An inexpensive (~$6 per mouse or rat), rapid (less than 30 min per mouse or rat), and well-tolerated method for immobilization and reproducible positioning of small laboratory animals has been developed [6]. A commercial quick-setting mold kit known as Rapid-Foam™, used to fabricate patient-specific molds for radiation therapy, is used (Figure 1). The two Rapid-Foam™ reagents ("A" and "B") are mixed, poured into a plastic-lined container, covered

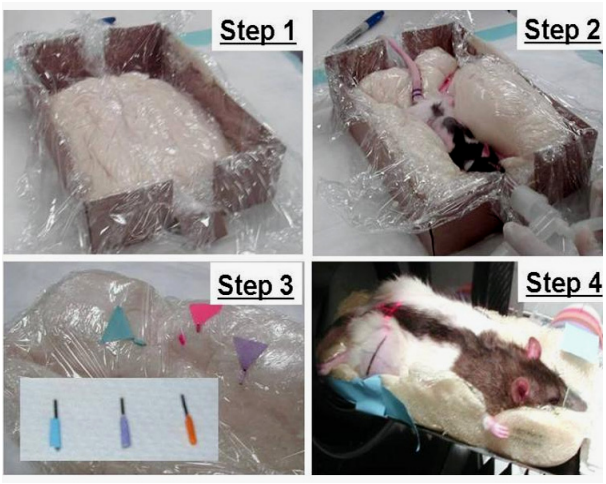


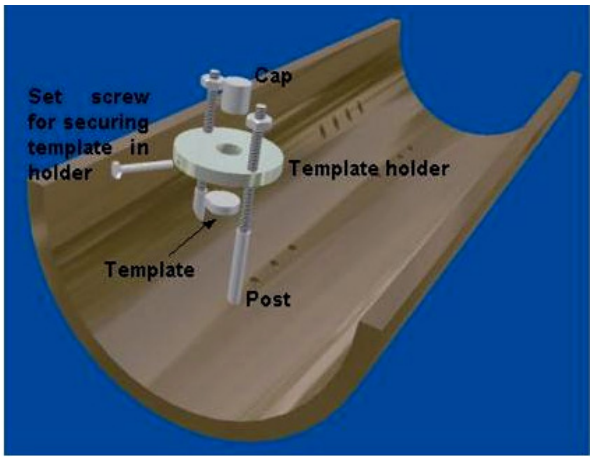
Figure 1. Step-by-step fabrication of animal-specific molds for immobilization and reproducible positioning for imaging of mice or rats.

with plastic and allow to cure (harden) for ~5 minutes (Step 1). The anesthetized mouse or rat, with its legs extended as for imaging, is gently pressed supine into the still-soft mixture and left in place for 15 minutes (Step 2). The mouse or rat is then removed and the hardened mold trimmed to fit into the imaging gantry. For each microPET™ image, for example, the mold is placed on the animal palette and fiduciary markers (e.g. 3 steel-encased germanium-68 (^{68}Ge) rods (370 kBq = 10 μCi each, 1x10 mm) are placed on or inserted into the hardened mold at specific positions (Step 3). Of course, by using "MRI-visible" (e.g. non-metallic gadolinium-filled) markers, this method is adaptable to MRI. The fiduciary markers allow for either manual or automatic registration of the image sets among the serial intra-modality or consecutive inter-modality image sets. The animal is then placed in its custom mold and imaged (Step 4).

C. *A Stereotactic Template for Spatial Registration of Macroscopic and Microscopic Images*

A practical stereotactic fiduciary marker-based method for immobilization and reproducible positioning of animals for sub-millimeter three-dimensional registration of multi-modality images from the in vivo macroscopic to the ex vivo microscopic scale has been developed by Dr. John Humm and colleagues at MSKCC[5]. This method has been developed specifically for registration of images of a tumor xenograft implanted in the hind limb of a mouse or rat. A specially designed jig (Figure 2) lies at the heart of this image registration method. The largest component of the jig is a semi-circular piece of Lucite. It serves as

Figure 2. Jig for multi-modality image registration. The jig



consists of semi-circular piece of Lucite (which serves as the animal palette and the Rapid-Foam™ "container"), two vertical posts and nuts which support the template holder, the stereotactic template, and the cap which (like the template) accommodates fiduciary markers and which nests in a recessed central portion of the template holder where the template is positioned. Except for the piece of Lucite, all components are made of Teflon. Courtesy of Dr. John Humm.

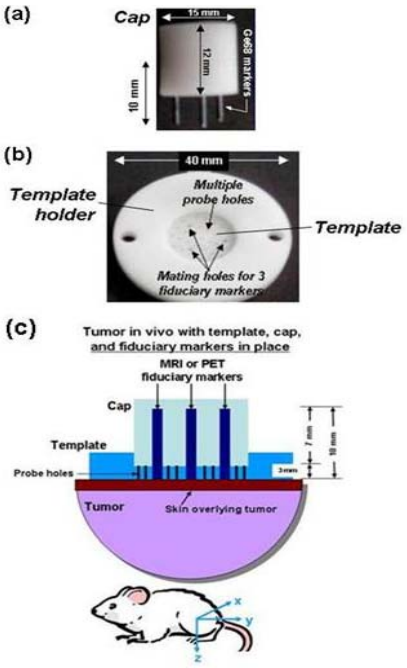


Figure 3. (a) Cylindrical cap (side view), with the three steel-encased ^{68}Ge fiduciary markers in place. Of the 10-mm total length of each fiduciary marker, 7 mm is inserted into the cap and the remaining 3 mm protrude from the end of the cap. (b) Template in place in the template holder (end view), with the three larger-diameter fiduciary marker holes

(at the vertices of a right triangle) among the multiple small-diameter holes. The ends of the fiduciary markers protruding from the cap mate with the three fiduciary marker holes in the template. (c) Diagram (lateral view) of the template and cap, with the three fiduciary markers in place, over a subcutaneous tumor xenograft in the hind limb of a rodent as positioned for in vivo (e.g, microPET or MRI) imaging. Note that when the cap, fiduciary markers, and template are in position for imaging, the proximal ends of the markers are flush with the proximal surface of the template and define a 3D (xyz) coordinate system, by identifying the xy-plane at z = 0, for registration of in vivo and in vitro images. Courtesy of Dr. John Humm.

an animal palette (or bed), as a platform for precise and stable positioning of a fiduciary marker/interstitial probe template relative to the tumor and as a "container" for the quick-setting mold material which securely, but atraumatically, immobilizes the animal in a fixed position for imaging and for transfer among multiple imaging devices (e.g. MRI to microPET). Incorporated into the jig are two threaded Teflon posts onto which a template holder, template, and cap are secured. The rat's tumor-bearing limb is positioned between the posts so that the template is centered over and just above (dorsal to) the tumor. Like the head frame used in stereotactic radiosurgery, the purpose of the template, about the size of a shirt button, is to define a 3D coordinate system for all image types. As indicated in Figures 3 (a) and (b), the cap and template have three holes for each of three fiduciary markers: for MRI, a gadolinium solution in sealed capillary tubes; for microPET™, three steel-encased 1x10-mm tubes each containing 370 kBq = 10 μCi of ^{68}Ge. The cylindrical Teflon cap (pictured in Figure 3 (a) with the ^{68}Ge fiduciary markers in place) has matching holes for the three markers and fits into the recessed central portion of the template (shown in Figure 3 (c)) to securely and reproducibly position the fiduciary markers in the template. As indicated in Figure 3 (c) and explained in more detail below, the details of the cap and template are critical for defining a 3D coordinate system and for 3D registration of the in vivo macroscopic and the ex vivo microscopic images - that is, for registration in the xy-plane parallel to the template (i.e. 2D registration) *plus* registration in the z direction (i.e. depth-wise perpendicular to the template).

The ends of the jig are open to allow access to the animal but are temporarily "sealed" with foil for preparation of the animal mold using the Rapid-Foam™ kit, pouring the mixture of reagents "A" and "B" into the sealed jig and covered with plastic. After waiting 5 min for the mold to cure, the anesthetized mouse or rat, with its tumor-bearing leg extended and positioned with the tumor centered between the two Teflon posts, is gently pressed supine into the still-soft mixture and left in place for an additional 15 min to cure completely. The animal, and the foil at the ends of the jig, are then removed from the mold and the excess foam material trimmed from the hardened mold so that the jig will fit into the imaging bore of the PET and MRI scanners. For MRI, a portion of the bottom of the mold must be cut away from the tail end of the jig and the two Teflon posts must be unscrewed from the threaded holes to allow positioning of the coil. Once in position, the Teflon posts are screwed back in place and the template, etc. positioned for imaging.

For interstitial measurements, e.g. of pO_2, the hole through which the probe is inserted determines the xy position and the depth of insertion of the probe tip (measured using a micromanipulator calibrated in sub-millimeter increments) determines the z position of the measurement. Each measurement can thus be spatially correlated with the voxel value at that xyz position in the PET image sets.

Multi-modality images may be registered either manually or automatically. *Manually*, using one image set (e.g. the MRI image set) as the reference, the operator rigidly transforms (translates and rotates) the other image set (the microPET image set) until the fiduciary markers in both image sets are precisely registered in all three orthogonal images. By rendering the reference image as semi-transparent, the concordance of the two overlaid images can be visualized during the image manipulation step. Iteratively, by minimizing the sum of the Euclidean distances (ie $_{i=1}\Sigma^{n}\,[(x_{ij}-x_{ik})^2 + (y_{ij}-y_{jk})^2 + (z_{ij}-z_{jk})^2]^{1/2}$) between the respective fiduciary markers i on multi-modality images j and k, the images may be *automatically* registered.

An additional key feature of the stereotactic system described is its unique capability to register ex vivo microscopic images with in vivo macroscopic images. This is accomplished as follows. After the final in vivo image has been acquired, the animal is quickly sacrificed by CO_2 asphyxiation in place in the jig and an angiocatheter then passed through each of the three fiduciary marker holes in the template and through the entire depth of the tumor (Figure 4(a)). The animal is then removed from the jig and

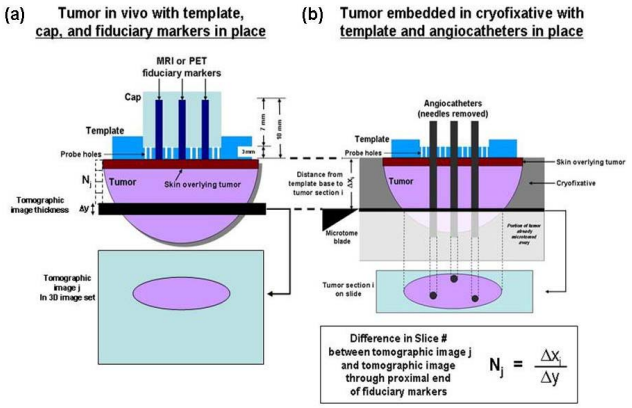


Figure 4. Registration of ex vivo microscopic images of tissue sections in the z (tumor-depth) direction with in vivo macroscopic images. The distance N_j, in number of in vivo

tomographic images (slices) from the proximal surface (base) of the template to the tomographic image j (a), corresponds to the in vitro section i at the same distance Δx_i divided by the tomographic image thickness Δy. The distance Δx_i is directly measured with a finely graduated ruler as the distance from the base of the template to the position of the microtome blade when about to cut section i. Courtesy of Dr. John Humm.

the tumor quickly harvested with the template, catheters, catheter needles and underlying skin still in place - the catheter needles securing the template in position over the tumor - and the combination of the harvested tumor, catheters, and template is frozen in cryofixative on dry ice. Only then are the angiocatheter needles retracted (removed), leaving the catheters in place in the frozen tumor. The catheter hubs are then cut off and sectioned in a cryostatic microtome with the template and catheters in place. Selected 10-μm-thick sections are mounted on glass slides for phosphor-plate digital autoradiography or staining. The catheters in the tumor sections are plainly visible in both H&E-stained sections and the frozen tissue block (as indicated diagrammatically in Figure 4 (b)). The tumor is positioned and sectioned parallel to the template (i.e. with the angiocatheters perpendicular to the template), yielding tissue sections parallel to the coronal aspect of the tumor. The resulting H&E and other histological sections are digitized by scanning with a microscope equipped with a computer-controlled digital camera and motorized slide stage.

III. Examples of Registered Multi-modality Imaging Studies

To illustrate the application and utility of registered multi-modality imaging studies in tumor xenografts in rodents, examples are presented in Figures 5 and 6.

In the Figure-5 study, a male Copenhagen rat with an R3327-AT anaplastic Dunning prostate tumor xenograft (1.7 gm) in its right hind leg (12 days after subcutaneous injection of $2x10^6$ cells) was studied by dynamic contrast (gadolinium (Gd))-enhanced MRI (DCE-MRI) and then ^{18}F-fluoromisonodazole (FMiso) microPET™ using the multi-modality image registration algorithm just described. FMiso is used an hypoxia imaging agent[7,8]. Isoflurane gas anesthesia (~1.5% with air, not oxygen, as the carrier gas) was used throughout the experiment. DCE-MRI was initiated immediately before a tail vein bolus injection of Gd (0.2 mmol/kg body mass), acquiring, through the mid-depth of the tumor, five 1.5-mm-thick images. Approximately 2 hours later, with the anesthetized rat remaining in place in its custom mold in the jig, microPET™ imaging was initiated immediately before a tail vein bolus injection of ^{18}F-FMiso (37 MBq = 1 mCi), acquiring list-mode data up to 20 minutes post-injection. At 2 hours post-injection of the FMiso, the rat was re-imaged on the microPET™. In Figure 5, all images represent *registered* coronal sections, with the tumor (arrow) at the right and the animal's head towards the top of the figure. Figure 5 (a) shows the microPET™ image acquired during the first 20 min after ^{18}F-FMiso injection and thus predominantly reflects blow flow (perfusion), with the brighter periphery of the tumor indicating higher blood flow and the darker center of the tumor poorer blood flow. Figure 8 (b) shows the microPET™ image acquired 2 hours after ^{18}F-FMiso injection and thus presumably reflecting hypoxia, with the brighter center of the tumor being more hypoxic than the periphery. Figure 5 (c) shows one of the DCE-MRI images, with the high signal intensity (brighter area) identifying the well-perfused periphery of the tumor and the lower signal intensity the poorly perfused center of the tumor. Figure 5 (d) is an overlay of the registered ^{18}F-FMiso image in Figure 5 (b) and the DCE-MRI image in Figure 5 (c). This is consistent with the expectation that ^{18}F-FMiso is trapped in tissue regions which are poorly perfused and therefore hypoxic, that is, in regions where DCE-MRI shows low initial signal intensity.

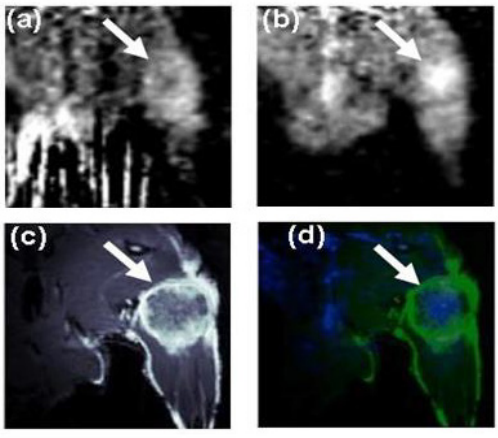


Figure 5. ^{18}F-fluoromisonodazole (FMiso) microPET™ ((a) and (b)), dynamic contrast-enhanced MRI (DCE-MRI) (c), and overlayed FMiso microPET™ and DCE-MRI (d) coronal images of a Copenhagen rat with an R3327-AT anaplastic Dunning prostate tumor xenograft (arrows) in its right hind leg. The animal was imaged in the registration jig (Figure 2) and remained anesthetized and in position in the jig from the start to the completion of imaging. The images were subsequently registered using a fiduciary marker-based rigid transform as described. Overlayed images, although shown in gray scale in (d), overlayed images are best viewed in color. Courtesy of Dr. Jason Koutcher.

Figure 6 compares coronal in vivo MRI images (Figures 6 (a)-(c)) and in vitro H&E, pimonidazole and Hoechst tumor-section images (Figures 6 (d)-(f), respectively)) for the previously described R3327-AT anaplastic Dunning prostate tumor study (Figure 5). Pimonidazole, a nitroimidazole-based hypoxia-localizing immunohistochemical stain, was co-injected with the ^{18}F-FMiso and Hoechst, a fluorescent

stain distributed in relation to perfusion, was intravenously injected 30 seconds prior to sacrificing the animal. Figure 6 (a) is a T2-weighted MRI image that reveals a central region of low signal intensity consistent with compromised cell viability (dashed-line region of interest (ROI)) near the center of the tumor and also identified in the H&E section (Figure 6 (d)). Figure 6 (b) is a T1-weighted MRI image acquired 30 minutes after administration of the gadolinium-based contrast agent, the high signal intensity corresponding to "delayed" washout of contrast from the poorly perfused central region (ROI) of the tumor. Figure 6 (c) shows a DCE-MRI image acquired 120 sec after contrast administration, with high signal intensity in the well-perfused regions of the tumor and low signal intensity in the poorly perfused central portion (ROI) of the tumor. The three NMR images appear internally consistent, with poor perfusion in the tumor center identified in all three. The H&E-stained section (Figure 6 (d)) shows a central compromised zone grossly in the same location as that identified in the NMR images in Figures 6 (a) and (b). Note, further, in the H&E tissue section (Figure 6 (d)) the clear visualization of the three angiocatheters. The most intense pimonidazole staining (Figure 6 (e)), presumably identifying hypoxic tumor tissue, appears to be localized in the poorly perfused portion of the tumor identified by the ROI. And finally, the Hoechst distribution (Figure 6 (f)), with the most intense staining generally localized in the periphery of the tumor, is largely consistent with the patterns of perfusion manifested by the other images. Note, in particular, the general absence of Hoechst staining (Figure 6 (f)) and therefore of perfusion in the hypoxic portion of the tumor, that is, the portion most intensely stained with pimonidazole in Figure 6 (e).

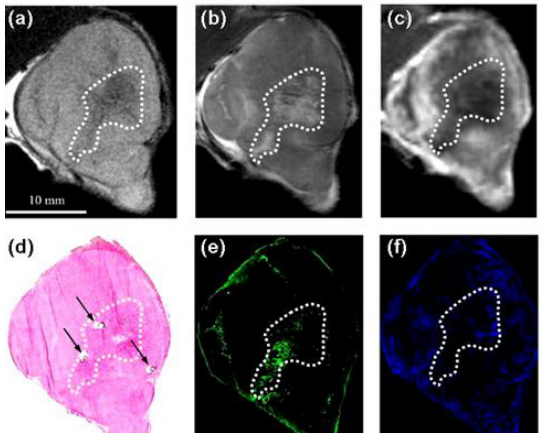


Figure 6. Registered in vivo MRI coronal images and a corresponding tissue section through a R3327-AT anaplastic Dunning prostate tumor xenograft in the hind limb of a rat. (a) T2-weighted MRI image. (b) T1-weighted Gd contrast-enhanced MRI image. (c) Contrast-enhanced MRI image 120 seconds after intravenous injection of the Gd contrast agent. (d) H&E, (e) pimonidazole, and (f) Hoechst staining of the corresponding tumor section. The dotted ROI identifies a portion of the tumor with the expected spatial correlation between areas of lower blood flow and associated structural changes, (b), (c), and (f) and (a) and (d), respectively, and hypoxia, (e). In the H&E section (d), the arrows indicate the three angiocatheters. Courtesy of Dr. Jason Koutcher.

IV. Concluding Remarks

Image registration and fusion have rapidly emerged as an invaluable component of both clinical and small-animal imaging. However, rigorous registration and fusion of in vivo images with ex vivo microscopy images has been remains particularly challenging and has been largely ignored - despite the detailed structural information discernible with histology and the richness of specific molecular imaging probes now available for immunohistochemistry. As described, our laboratory has now designed, fabricated, and tested a unique stereotactic imaging system for image registration of coarser-resolution in vivo imaging modalities such as PET, CT, and MRI to ultra-high-resolution ex vivo imaging techniques such as histology, autoradiography, and immunohistochemistry. This stereotactic system can also be used to guide interstitial probe measurements of tissue parameters such as partial pressure of oxygen (pO_2) and to spatially index such measurements to allow correlation between the parameter values and n vivo-images voxel values.

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