Published in final edited form as:

Acad Radiol. 2010 May ; 17(5): 614–623. doi:10.1016/j.acra.2010.01.003.

Validation of automatic target volume definition as demonstrated for ¹¹C-Choline PET/CT of human prostate cancer using multi-modality fusion techniques

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Abstract

Rationale and Objectives—PET is actively investigated to aid in target volume definition for radiation therapy (RT). Our objective was to apply an automatic computer algorithm to compute the target volume and validate the algorithm using histological data from real human prostate cancer.

Materials and Methods—Various modalities of prostate were collected. *In-vivo* imaging included T2 3T MRI and ¹¹C-Choline PET. *Ex-vivo* imaging included 3T MRI, histology, and block face photos of the prostate specimen. A novel registration method based on mutual information and thin-plate spline was applied to all modalities. Once PET is registered with histology, a voxel by voxel comparison between PET and histology is possible. A thresholding technique based on a various fractions of the maximum standardized uptake value (SUVmax) in the tumor was applied and the respective computed threshold PET volume was compared with histological truth.

Results—Sixteen patients whose primary tumor volumes ranged from 1.2 cm³ to 12.6 cm³ were tested. PET has low spatial resolution, thus only tumors larger than 4 cm³ were considered. Four cases met this criterion. A threshold value of 60% of the ¹¹C-Choline SUVmax resulted in highest volume overlap between threshold PET volume and histology. Medial axis distances between threshold PET volume and histology showed following errors, 7.7±5.2 mm (mean error ± SD).

Conclusions—This is a proof of concept paper demonstrating for the first time that histology-guided PET thresholding can delineate tumor volumes in real human prostate cancer.

Keywords

automatic target volume definition; image registration; prostate cancer; histology; thresholding; PET; MRI; multi-modality image fusion

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Introduction

Substantial technological progress involving intensity-modulated radiotherapy (IMRT) and three-dimensional planning of brachytherapy for prostate cancer has enabled the delivery of radiation treatment with high geometric precision (1). Radiation oncology refers to the volume including the entire tumor and its microscopic extensions as the clinical target volume (CTV). The general goal of RT is to maximize the dose to the target volume while minimizing damage to surrounding normal tissues. With advances in image guidance, accurate target volume definition is becoming even more important as highly differential radiation doses can only be justified when the planning target volume (PTV) is precisely matched with the “true” tumor volume.

Currently, target volume definition is based on anatomical imaging with magnetic resonance imaging (MRI) or computed tomography (CT). While anatomical imaging can convey structural information with high resolution, it suffers from a serious limitation. Anatomical imaging portrays the tumor volume using variation of tissue density (for CT) or relaxation properties (for MRI) using visualized macro-anatomic changes and contrast enhancement, which are not necessarily specific tumor characteristics, thus may not accurately differentiate tumor tissue from normal. As a result, the tumor volume is not properly conveyed on anatomical images when visual structural cues related to tumor tissue are lacking.

Because functional tissue properties may correlate better with the true extent of tumor tissue compared to anatomical imaging, the incorporation of positron emission tomography (PET) image data into radiotherapy planning has been considered for numerous malignancies including prostate cancer. Apparent biological target volumes (BTV) may substantially differ from the PTV based on anatomical imaging alone (1-4). In theory, 3-dimensional radiotherapy and IMRT would allow to increase the radiation dose to a given tumor lesion by decreasing the dose to non-malignant prostate tissues. However, there is considerable uncertainty among radiation oncologist how to define or integrate tumor volumes based on functional information into radiation treatment planning.

Several automatic methods for target volume definition based on PET have been developed in the past. Automatic methods are preferred to reduce the complexity of the target volume definition process and to minimize operator bias. Most of the existing algorithms derive a threshold value from the standardized uptake value (SUV) of the tumor tissue or a ratio between tumor and normal SUV values (5-9). Some algorithms integrate additional factors such as tumor size (8,9). However, these methodologies have not been validated as to how accurately the resulting BTV match the true histological extent of the tumor in humans.

Our goal here is to test a common automatic target volume definition algorithm on real prostate cancer patient data including histology. Functional image data are derived from ^{11}C -Choline, a PET radiotracer that is preferably taken up in proliferating aggressive primary prostate cancer (10,11). A computer algorithm computed a threshold PET volume which was later compared with expert drawn histology via an automatic image registration algorithm. The main novelty of this paper is that we have used PET thresholding based on registered histology derived from 4 human prostate cancer datasets. The core technology is the registration methodology (12), which enables us to adaptively threshold PET volumes to compare with expert drawn histology.

Materials and Methods

In this section we cover the registration framework to compare PET volume with histology, detailed parameters of scan acquisition, and the method to compute the threshold PET volume.

The study was performed at the University of Michigan (Ann Arbor, Michigan, USA). Informed consent was obtained from all patients enrolled in this study, which was approved by the Internal Review Board (IRB) of this institution.

We previously published a methodology to register *in-vivo* scans including PET with histology (12). We adopt this registration methodology and compare thresholded ^{11}C -Choline PET with expert annotated histology. The registration methodology breaks up the difficult, direct registration of histology and *in-vivo* imaging into more accurate sub-registration tasks involving intermediate modalities (i.e., specimen MRI and block face photographs of the prostate). In the process we first register each histology section onto the block face photo taken before the histology section was microtomed. This 2D registration removes the deformations associated with microtoming. Then the collection of geometry-corrected block face photos are registered onto the *ex-vivo* specimen MRI, registers *ex-vivo* specimen MRI onto *in-vivo* MRI, and finally registers *in-vivo* MRI onto PET all in 3D. These sub-registration tasks can be performed more accurately since the deformations being modeled are far less complex and are supported by the information content of their associated image volumes. The methodology is automatic after the user's initialization and thus unbiased. One important thing to note in this registration methodology is that it allows a histology section to be registered onto a curved 2D baffle potentially intersecting multiple *in-vivo* MRI image slices. A histology section is likely to be mapped onto a 3D manifold spanning many MRI slices due to the complex deformation encountered during surgical extraction and histological sample preparation. Many of the existing literature on registering histology with *in-vivo* imaging makes the assumption that there is one particular plane in an *in-vivo* MRI slice that corresponds to one particular plane of histology section (13,14). The registration methodology we adopted here is free from such an assumption.

Registration Framework

Registration is a process of establishing a spatial correspondence between two scans so that both can be viewed in the same spatial frame. Registration methods have been well reviewed by Hill et al. (15). Five components need to be addressed for any registration algorithm: image feature, similarity function, class of admissible transforms, interpolation method, and optimization method. We used voxel intensity as the image feature to drive the registration. Mutual information (MI) was adopted as the similarity measure which measures degree of alignment between scans. The combination of voxel intensity and MI is well suited for driving multi-modal image registration as it assumes no linear relationship between voxel intensities (15). We adopted thin-plate splines (TPS) to generate the geometric transform. TPS is known to be maximally smooth among all geometric interpolants (16,17) thus is well suited for modeling smooth geometric transforms found in prostate deformations. The class of admissible transform is the non-linear transform space spanned by TPS. With TPS, there can be folding in the transform thus one-to-one mapping (also known as diffeomorphism) is not guaranteed. But for practical purposes, folding does not occur frequently as TPS driven registration are generally smooth.

Once the geometric transform was defined, we used tri-linear interpolation to interpolate the voxel intensities, which is the most commonly used voxel intensity interpolator. Lastly, we adopted simplex method to optimize the similarity measure (16). Computing the MI involves calculating probability density functions of grayscale value distributions. A simple histogram with fixed bin width is used to estimate the probability density function. The process of registration can be formulated as maximizing the chosen similarity measure (i.e., MI) under a hypothetical geometric transform,

$$\hat{T} = \arg \max_{T \in F} MI(A(\bullet), B(T(\bullet)))$$

$\hat{T}$; estimate of the transform
 F ; family of feasible transforms

One can also choose other combinations of similarity measures (e.g., normalized MI) and geometric transform (e.g., B-spline) (16). The degrees of freedom (DOF) of TPS are determined by the number of control points. Control points are different from manually picked landmarks or markers as they can be optimized automatically through a registration algorithm.

Scan Acquisition

Many imaging modalities are required for this study as the registration methodology requires additional intermediate modalities in addition to the *in-vivo* PET and histology. Note that T2 weighted MRI defines the prostate's in-vivo geometry:

- T2 weighted anatomical 3T MRI of pelvis using the regular body coil: axial orientation, high resolution sequence, TR = 3208 ms, TE = 86 ms, 720×720×24 image matrix, 0.31×0.31×4 mm³ voxel dimension.
- ¹¹C –Choline PET/CT was obtained on a Siemens BioGraph hybrid PET/CT system consisting of a Siemens HR+ PET scanner and a 2 slice CT scanner, where the intrinsic PET image resolution of the system varies between 4.1 mm FWHM at center and 7.8 mm at r = 20 cm in axial direction, 128×128×210 image matrix, 3.90×3.90×4 mm³ voxel dimension Choline PET: half life = 6586 s
- Abdominal CT : non-contrast, 512×512×²10 matrix, 0.98×0.98×4 mm³ voxel dimension

After the patient undergoes prostatectomy and the prostate specimen is removed from the body. The prostatectomy specimen is first fixed in 10% paraformaldehyde. After 48 hour fixation time, the prostate specimen is then imaged with a 3T MRI scanner surrounded by perfluorocarbon solution. The specimen is then grossly sectioned into ~3mm whole mount slices for further processing (i.e., alcohol dehydrated, stained, paraffin-embedded), and microtomed into 5-15µm thick slide-mounted sections. The slides are stained using the standard hematoxylin and eosin stain. In addition to Pathology's standard methodology of processing of slides, we acquired digital photographs of the stained and fixed whole mount tissue block (referred to in the text herein multiple times as “block face photo”) before each microscopic slide section is cut. We acquired the following *ex-vivo* image modalities: specimen MRI of the *en-block* excised prostate, block face photographs of the specimen, and photographs of their corresponding histology sections. Parameters for *ex-vivo* scan acquisition are as follows:

- Specimen MRI: field strength = 3T, T2 weighted spin echo sequence, TR = 1500 ms, TE = 80 ms, 512×512×60 matrix, 0.195×0.195×0.7 mm³ voxel dimension,
- Block face photograph: obtained from digital camera, 3648×2736 matrix, 0.3532 mm² pixel dimension, 6 or 7 slices in total, roughly 3 mm apart in apex-base direction,
- Histology: obtained from digital camera, 4256×2848 matrix, 0.0852 mm² pixel dimension, 6 or 7 slices in total, roughly 3 mm apart in apex-base direction.

Registration Schematic

The registration methodology we adopted establishes registration between histology sections of the prostate and *in-vivo* imaging including anatomical MRI and PET. Below is a brief description of the methodology used for registration.

In-vivo anatomical MRI was chosen as the reference space onto which all other scans will be registered. Registrations within *in-vivo* imaging modalities are relatively simple as the deformations involved are mild as the prostate is entirely contained in the abdomen before surgery. While the goal is to register histology sections and *in-vivo* MRI, all other *in-vivo* modalities including PET are also registered onto the same common reference. Once mapped all imaging modalities including *in-vivo* PET and *ex-vivo* histology can be compared. The overall registration schematic is given in Fig. 1 and details of individual sub-registration tasks are provided here in conjunction with Fig. 1.

1. Register each histology photo onto the corresponding block face photo using 12 DOF optimizer driven 2D TPS interpolant. This registration process is repeated for all available histology sections.
2. Stack block face photos to form a sparsely sampled 3D volume. Successive block face photos are registered in rigid (i.e., rotate-translate) fashion and then stacked. Rigidly registered photos are placed at the same spacing used to section the prostate (i.e., 3 mm) and zero valued slices are inserted in between so that individual slices have a slice thickness of 0.6 mm.
3. Perform a 3D registration between stacked block face photos and the *ex vivo* specimen MRI using 54 DOF optimizer driven 3D TPS. The second and third tasks described here account for 3D, non-linear deformation of the prostate specimen associated with excision, handling, dehydration, paraffin embedding and gross sectioning
4. Register *ex vivo* specimen MRI and *in vivo* anatomical MRI using 21 DOF optimizer driven 3D TPS. This registration process accounts for the deformation of prostate as it is taken out of the body.
5. Combine registration results from above four tasks and achieve registration between histology slides and *in vivo* anatomical MRI. The stacked block face photo volume is the key bridge between histology and *in vivo* MRI.
6. Assuming the patient didn't move register PET and CT using an identity transform. Registration of PET and CT is trivial as both CT and PET come from a PET/CT scanner where both scans reside in the same spatial framework assuming the patient doesn't move between scans. Then we register the CT and the anatomical MRI using 6 control points. Using the composition of the two registrations, we then map PET onto the reference space of the histology slide.

Once all sub-registration tasks are performed, then spatial correspondence between any combinations of modalities can be established by combining results from suitable sub-registration tasks. A sample registration result is given in Fig. 2. Anatomical MRI, diffusion MRI, and PET are mapped onto the histology space for quantitative comparison. This is feasible as any modality can be mapped onto any other modality by combining results from suitable sub-registration tasks. Note that the results shown in Fig. 2 are just one slice out of a 3D volume. Once mapped onto the histology space, we can observe how *in-vivo* imaging features are correlated with high resolution histological truth. In Fig. 2, slice 3 has a large tumor located at the left lobes of the peripheral zone. Registered anatomical MRI and diffusion MRI show signs of cancer (i.e., lower gray value) in the same area. Registered PET shows increased tracer uptake (i.e., higher SUV) in the same area.

PET Thresholding Algorithm

Many algorithms to automatically compute the tumor volume in PET determine the threshold value based on SUV value of normal and tumor tissues (5-8,18,19). An area whose SUV is above the threshold is computed as the tumor target volume. We chose the threshold value based on the SUV value of the tumor tissue, which in case of ^{11}C -Choline increases with

aggressiveness of prostate cancer compared to the non malignant prostatic tissues (11), which is by far the most frequently applied thresholding technique for PET (8). We optimized the threshold value to maximize the Dice overlap coefficient between threshold PET volume and histology (20). Overlap can be computed as both volumes reside in the same space via the registration algorithm. Overlap index refers to the amount of overlap between the two volumes and is computed as $2(A \cap B)/(A+B)$, where A and B are two volumes under comparison. Threshold values were set with respect to the maximum tumor SUV. Following is the procedure for PET thresholding:

1. Given registered histology and PET volumes
2. Set threshold values as between 20 and 80% of the maximum SUV (in 5% increments)
 - 2-a. Compute threshold PET volume as collection of voxels whose SUV uptake is larger than the defined threshold within the pelvic region
 - 2-b. Measure and observe Dice overlap between threshold volume and histology
3. Choose the threshold volume whose overlap is the largest

Registration Error

Here we briefly describe the registration error from the adopted registration methodology (12). Validation of a registration methodology for human scans almost always requires implanted markers or intrinsic landmarks that need to be identified for all modalities. Landmarks are difficult to identify and it is very difficult to find corresponding landmarks on *in-vivo* imaging. The accuracy of the registration algorithm is computed comparing the tumor boundary obtained from histology with a surrogate marker for tumor boundary in diffusion MRI indicated by the edge of low apparent diffusion coefficient (ADC) MRI values. This method of assessing the accuracy is quite stable as all scans in the study have primary prostate cancer. Tumor area identified in diffusion MRI may appear smaller or larger than the tumor area identified in histology depending on thresholding of the diffusion MRI as its spatial resolution is many orders of magnitude worse than histology. The 3D medial axis representation was used due to its inherent insensitivity to resolution and thresholding differences between diffusion MRI and histology; typical overlap measures such as the Dice coefficient would penalize for such resolution differences (20). The medial axis of a boundary is defined to be the locus of the centers of spheres that are tangent to the boundary in two or more points, which roughly translates to a curve that runs along the middle of a boundary. We adopt an error that will measure distance between medial axes of diffusion MRI and histology. This way we do not penalize a good registration if the boundary in diffusion MRI is a dilated or eroded version of the boundary found in histology. Medial axes of the tumor boundaries are computed for the lesion on the ADC MRI and histology slides and the distance between the medial axes are computed as the registration error. The registration error between the shortest central medial axes of diffusion MRI and histology was 3.74 mm and 2.26 mm (mean 3 mm). The final intended registration is between PET and histology thus contains an additional registration error term between diffusion MRI and PET which needs to be accounted for. Registration accuracy between *in-vivo* imaging modalities is well established and its accuracy is reported to be smaller than an *in-vivo* voxel (15) which for PET is on the order of 3-4 mm. As the variances of the independent errors are additive, the intended registration accuracy between PET and histology will not be significantly larger than the registration error between *in-vivo* MRI and PET.

We computed four types of performance measures to measure the registration error between PET and histology: centroid error, overlap index, medial axis error, and boundary error. Centroid error refers to the difference in centroid locations of threshold PET and histology. Overlap index refers to the amount of overlap between thresholded PET and histology, and

varies from 0 (i.e., no overlap) to 1 (i.e., perfect overlap). Medial axis error refers to the mean Euclidean distance between medial axes of histology boundary and threshold PET boundary. Boundary error refers to the mean Euclidean distance between histology boundary and threshold PET boundary. At a given boundary voxel in histology, the closest boundary voxel in threshold PET is located and the distance between two voxels is computed. The process is repeated for all voxel boundary voxels in histology and the mean is reported as the boundary error.

Results

The test population consisted of 16 patients whose largest primary prostate cancer tumor nodule had volumes between 1.2 and 12.6 cm³. Since PET suffers from relatively low resolution; we restricted the evaluation to tumors with volumes ≥ 4 cm³. Because any thresholding technique requires a differential (increased) lesion uptake, and since mostly only aggressive primary prostate cancers displayed increased ¹¹C-Choline uptake compared to benign prostate gland tissues, 4 tumors matched these criteria. For each case, threshold values were optimized for maximum Dice overlap and the associated errors were reported. Error values for a sample case with varying threshold are given in Fig. 4.

Once the optimal threshold was set, a planning tumor target volume was defined. In the next step, the thresholded PET volume was compared with the registered histology. With aid from the registration methodology threshold PET volume and histology reside in the same spatial framework thus can be compared on a voxel by voxel basis. In Fig. 3, we can compare histology, PET, threshold PET, and anatomical MRI all in one space. Note that the boundary of thresholded PET SUV image may not match the tumor boundary of histology since ¹¹C-Choline uptake in tumor tissues may vary. Thus, even if the registration process and thresholding algorithm are error free, the tumor boundaries of histology may not match those of threshold PET. At the best threshold PET volume will be entirely included in the tumor volume in histology (i.e., threshold PET is a scaled version of tumor volume in histology whose centroid locations coincide).

Centroid error is primarily sensitive to translation between two volumes and boundary error is sensitive to shape changes including rotation, translation, and scaling. Medial axis error is sensitive to rotation and translation but not sensitive to scaling, which is useful for comparing PET threshold volume and histology, since we prefer not to penalize when threshold PET volume is just a scaled version of histology volume. When the overlap index is highest, the medial axis error is considered as the one error value representing the case. The boundary error is the one that is clinically important as we set out to define the tumor volume automatically. The boundary error will be larger than the medial axis error since it is sensitive to scaling which exists between PET and histology volumes.

Error statistics for four cases are reported in Table 1. For the evaluated 4 cases, the most consequential error, the medial axis errors, was determined as 7.7 ± 5.2 mm when using an optimized threshold value of 60% of the maximum tumor SUV. The mean medial axis error is around 8 mm which is two times the typical point spread function (PSF) of the PET (i.e., 4 mm). The boundary errors varied between 6.2 and 17.7 mm (10.3 ± 5.1 mm). The mean boundary error is around 10 mm which is 2.5 times the PSF of PET. Recall that the registration of MR and histology has error of around 3 mm and that the registration of PET and MR has error of around 4 mm. Assuming additive nature of independent errors, the actual error of the threshold PET and histology might increase as high as 5 mm (i.e., $3^2 + 4^2 = 5^2$).

Discussion

Four out of 16 patients presented with tumor volumes $\geq 4 \text{ cm}^3$ which was set as our criterion for this analysis. Most patients undergoing prostatectomy have relatively small tumor volumes confined to the prostate. High volume disease may improve tumor lesion visualization on ^{11}C -Choline PET and MRI; however, such advanced local disease often presents with additional nodal metastatic disease, which – at our institution - are not considered for radical surgery. As histology information was only obtainable from prostatectomy specimen, suitable cases for analysis were limited.

Besides appropriate dose prescription and control throughout the treatment, successful 3-dimensional radiotherapy and IMRT requires adequate selection and delineation of target volumes including detailed knowledge of setup uncertainties (21). Current methods for BTV definition that go beyond a simple adaptation of the radiation field according to positive (or negative) findings on PET by either including (or excluding) specific areas are poorly validated. Many different methods have been suggested to threshold PET image data in order to maximize the overlap between the PET derived tumor volumes and true pathological volume (5-9,18). The main obstacle for the validation of such algorithms in-vivo remains the inability to reliably delineate the true tumor volume. Yaremko et al. determined an threshold value with respect to the maximum tumor SUV for ^{18}F -FDG tested on a lung cancer phantom (8). Brambilia et al. and Davis et al. used specific phantoms to evaluate a threshold based on a ratio between normal and tumor SUV (5,7). While phantoms are indispensable to assess effects of scanner performance, motion and partial volume effects on biological target volume definition, they only provide theoretical information about the accuracy of the overlap between the BTV and pathological tumor volume. An indirect validation of PET derived BTV can be obtained by maximizing the overlap between thresholded PET and anatomical tumor volumes based on CT (9,18). However, it is difficult to justify this approach recognizing that anatomical and biological target volumes may differ substantially.

This study is the first to actually evaluate the spatial accuracy of biological target volume definition derived from thresholded ^{11}C -Choline PET/CT with a “true” tumor volume derived from pathology in the same spatial framework. Our results show that there are errors in the order of PSF of PET between threshold PET and expert drawn histology.

Several important sources of discrepancies exist between tumor volumes derived from pathology compared with image derived tumor volumes: registration errors, partial volume effects and PET signal heterogeneity related to biology. The image registration process itself is an intrinsic source of error. Accuracy of the image registration is determined by the shared information content between images being registered. *In vivo* MRI simply does not have all the information found in histology, thus there will be image registration errors. Another source of error is related to unavoidable partial volume effects. While partial volume effects are present with both, high resolution anatomical imaging (CT, MRI) and PET, the comparatively low resolution of PET scanners will negatively influence lesion characterization (22). In this study, patients were imaged on a PET/CT scanner with an intrinsic axial resolution of approximately 5.2 mm full width at half maximum (FWHM) in the center of the field of view and a reconstructed resolution of approximately 9 mm (11,23). As this spatial resolution is finite, the image of a small lesion will appear larger and with less activity than it ultimately should have. In addition, images are sampled on a predefined voxel grid and the true tumor borders will likely not match the voxel borders. Therefore, at the tumor borders most voxels will contain a mixture of different types of tissues further limiting border detection due to blurring. Thus, even if the entire tumor volume would have homogeneously increased radiotracer uptake, a given threshold would underestimate the true tumor volume when low volume disease is present. Since the uptake differential found with ^{11}C -Choline is relatively low (2-4 times higher

in aggressive disease than benign prostate tissue), partial volume correction algorithms will likely not overcome this limitation. In addition, such algorithms assume homogeneous tracer uptake in both, the lesion and its vicinity (24), a precondition which would only rarely be fulfilled in prostate cancer. For instance, ^{11}C -Choline may cause intraluminal rectal uptake which might interfere with SUV measurements in the nearby prostate gland (see Fig. 2).

Discrepancies between pathology and PET image derived tumor volumes may also be related to the specific biological properties of PET radiotracer uptake. Such properties may limit increased tracer uptake to a certain subvolume of the entire pathological volume or cause more diffuse intralesional signal heterogeneity (25). In this study, increased ^{11}C -Choline uptake is preferentially seen with aggressive primary prostate cancers (11). Such differential biological properties are not uncommon. The most widely used PET tracer, ^{18}F -FDG, is also influenced by the level of tumor proliferation (26), metastatic potential and aggressiveness in a variety of malignancies (27). In addition, the ^{18}F -FDG uptake is also strongly affected by the intensity of inflammatory responses (28). Discrepancies with pathology are also expected when using radiotracers specific for amino acid metabolism (^{11}C -Methionine), cell proliferation (3'-deoxy-3'-[^{18}F]fluorothymidine) or hypoxia (^{18}F -Fluoroazomycin Arabinoside) (29-32). Thus, specific biological features are always expected to contribute to intratumoral heterogeneity of the PET signal. As a result, it is questionable whether a given threshold derived from PET imaging can sufficiently describe the biological feature (such as tumor cell proliferation) in question.

The overlap between threshold PET volume and histology volume is not as high compared to a standard registration/segmentation method as there are many sources of discrepancies such as registration errors, partial volume effects and PET signal heterogeneity related to biology. In our approach we defined a subsection of prostate as the target volume; this is more specific than defining the whole prostate as the target volume since we are interested in tumor target volume. We investigated what type of errors can be expected if a sub-volume of the prostate is defined as a potential target volume for radiation boost, while the remainder of the prostate would receive a standard treatment dose. Our results show what is possible with the state of the art registration and segmentation technology. It would be premature to draw further conclusions regarding the accuracy of automatic target volume definition based on this limited study population. In addition, it is entirely unknown whether such treatment modification would result in improved outcome. Ultimately, one needs to observe improved sparing of normal tissue and patient survival associated with the new target volume definition, which remains as a future work.

Conclusion

We tested an algorithm for automatic target volume definition against real histological data. Our data indicate that automated threshold PET and histology volumes do not align perfectly. Even when considering that our summed boundary errors are in part related to registration errors not present in a real radiation treatment planning environment, the true tumor border will almost never be clearly delineated on PET image data due to unavoidable resolution restrictions, partial volume effects and potential additional heterogeneity of the PET uptake data. Thus, such automated algorithms should only be used with great caution keeping in mind uncertainties of boundary errors. This paper demonstrates the possibilities associated with utilizing state of art registration and segmentation algorithms.

Acknowledgments

Supported by NIH grants 1P01CA87634 and P50CA069568

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Figure 1. Registration schematic

All registration sub-tasks are 3D registrations except for histology onto block face registration. Solid arrows indicate each sub-registration task. Dotted arrow indicates the difficult direction registration between anatomical MRI and histology. Pictures of anatomical MRI (reference space), *ex-vivo* specimen MRI, and histology section are provided for better visualization. Note that histology section is made into grayscale. Note that registration within *in-vivo* imaging allows PET and histology to be compared.

Figure 2. Sample registration results of anatomical MRI, diffusion MRI, and PET onto histology
 Registration results for histology slices 3 out of 7 slices are presented. Columns of images are histology (grayscale converted), registered anatomical MRI, registered diffusion MRI, and registered PET, respectively from left to right. Cancerous tissue is enclosed in dotted lines on histology. Middle row images are colored overlay of registered anatomical MRI, registered diffusion MRI, and registered PET all in grayscale with histology in a green hue. Bottom row images are alternating checkerboard fusion of registered anatomical MRI, registered diffusion MRI, and registered PET with histology. On histology slides, R, L, A, and P denotes right, left, anterior, and posterior respectively. Case#2 (in Table 1) was used to generate this figure.

Figure 3. Visualization of threshold PET and histology in the same spatial framework

Registration results for slice 4 (top figure) and slice 5 (bottom figure) for one patient. Only two slices out of a total of 7 slices are presented here. Columns of images are histology (grayscale converted), registered PET, registered threshold PET, and registered anatomical MRI, respectively from left to right. A threshold of 60% with respect to the maximum tumor SUV was used to produce the threshold PET volume. Cancerous tissue is enclosed in dotted lines on histology. Middle row images are colored overlay of registered PET, registered threshold PET, and registered anatomical MRI, all in grayscale with histology in a green hue. Bottom row images are alternating checkerboard fusion of registered PET, registered threshold PET, and registered anatomical MRI with histology. Case#1 (in Table 1) was used to generate this figure

Figure 4. Error values for one patient

Centroid error in millimeters is plotted in the top left figure. Overlap index is given in the top right figure. Boundary error in millimeters is given in the bottom left figure. Medial axis error is given in the bottom right figure. The x axis is the threshold values used and the y axis is the error values of choice. The black arrows in the figures indicate the error values at the chosen threshold level (i.e., 0.6) which maximizes the overlap index.

Table 1
Error values between threshold PET and histology

Four types of error and the corresponding threshold values are given. The error types are medial axis error (row 4), boundary error (row 5), centroid error (row 6), and overlap index (row 7). Corresponding threshold value and tumor volume are also given in rows 2 and 3 respectively. Mean and standard deviation for the each row are given in the right most column. The most representative errors, boundary error and medial axis error, are highlighted in gray.

	Case1	Case2	Case3	Case4	mean/std
Independent variables	PET SUVmax threshold [unitless]	0.6	0.6	0.6	0.6/0.0
	Tumor volume [cm ³]	12.6	9.9	7.2	6.8
Dependent variables	Medial axis error [mm]	14.1	7.3	8.0	1.3
	Boundary error [mm]	8.4	17.7	6.2	9.0
	Centroid error [mm]	8.1	9.0	12.2	6.9
	Overlap index [unitless]	0.5	0.4	0.1	0.6