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Challenges in accurate registration of 3D medical imaging and histopathology in primary prostate cancer

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Abstract

Due to poor correlation of slice thickness and orientation, verification of medical imaging results with histology is difficult. Often validation of imaging findings of lesions suspicious for prostate cancer is driven by a subjective, visual approach to correlate *in vivo* images with histopathology. This manuscript describes fallacious assumptions for correlation of imaging findings with pathology and identifies the lack of accurate registration as a major obstacle in the validation of PET and PET/CT imaging in primary prostate cancer. Specific registration techniques that facilitate the most difficult part of the registration process—the mapping of pathology onto high-resolution imaging, preferably aided by the *ex vivo* prostate specimen—are discussed.

Keywords

Primary prostate cancer; registration of 3D medical imaging onto pathology; mutual information; computer-aided design

Introduction

The American Cancer Society estimated that in 2012 about 241740 men were newly diagnosed with prostate cancer in the United States [1]. Due to prostate-specific antigen (PSA) screening and more frequent prostate biopsies, a significant stage and grade migration has occurred. As a result, many newly diagnosed prostate cancers represent clinically insignificant (indolent) tumors. The identification of such disease poses the tremendous clinical, financial and ethical dilemma of possible overtreatment [2]. On the other hand, roughly 28170 men died of prostate cancer in the U.S. in 2012 [1], indicating that the risk of prostate cancer-related death remains a great concern. Accordingly, there is considerable uncertainty as to the “best” course of prostate cancer management, one that will significantly reduce morbidity and mortality resulting from tumor progression, yet avoid significant therapeutic side effects and costs related to overtreatment.

From a clinician’s perspective the most important imaging task in the primary setting is not necessarily the identification of prostate cancer itself, but accurate staging and the ability to predict the presence of clinically significant (or relevant) disease. Given currently accepted criteria for local significant disease (Gleason > 6, extra prostatic extension, seminal vesicle and nodal disease involvement, tumor volume > 0.5 ml [3, 4]), the identification of significant disease at an early stage is challenging for anatomic, functional and metabolic

Conflicts of interest

The authors declare that they have no conflict of interest.

imaging alike. In addition as highlighted in this article, assessing the accuracy of imaging in identifying prostate cancer is difficult in itself, especially when considering the multi-focality of primary prostate cancer [5]. As a result, there is considerable uncertainty about the performance of imaging in the setting of primary prostate cancer, particularly for PET and PET/CT.

In a recent review, the diagnostic efficacy of ^{18}F - and ^{11}C -choline for the diagnosis of primary prostate cancer is listed from 17 studies with over 500 patients [6]. Reported sensitivities and specificities for prostate cancer detection varied widely (sensitivity range 66 – 100%, specificity range 43 – 86%). Most studies use absolute uptake measures (SUV, SUV_{max}) in characterizing disease. Other studies investigating tumor-to-background ratios concluded that normalization of tumor uptake may improve correct disease classification [7–9]. However, normalization may be a source of additional bias. Moreover, the assessment of efficacy requires a comparison of imaging findings with pathology as gold standard. In this manuscript, we examine the current standard practice and emerging methods for comparing imaging findings obtained from the prostate gland with pathology.

Current standard practice

When examining methods of comparing PET imaging results with histological findings, 4 approaches with increasing complexity are presented in the medical imaging literature:

1. lesion-based comparison of radiotracer uptake findings for each prostate lobe,
2. sector-based (also called template-based) approach for which the prostate is divided into specific sectors on imaging and pathology,
3. visual comparison of the entire 3-dimensional (3D) prostate volume with multiple discrete 2-dimensional (2D) slices, which requires whole-mount sectioning of prostatectomy specimen, and
4. direct registration (or mapping) of the 3D prostate imaging volume with stacked 2D whole-mount hematoxylin-eosin (HE) stained histological slices.

The first approach simply assumes that any focally abnormal uptake on PET represents malignancy and that the identified focus represents disease identified at pathology in the same lobe. Due to the lack of sufficient spatial information on pathology, this precludes the ability to assess the performance of a given imaging test unless the cancer involves the entire prostate lobe.

The second approach uses a sector-based comparison of PET imaging results with pathology by dividing the prostate volume into 6 (sextant) or more (usually 12) sectors taking into account anatomical regions (such as apex, mid, base, peripheral and central/transitional zones). This method is current standard practice and commonly applied for comparisons of pathology with imaging. The method mimics the pathologist's approach for a systematic evaluation from both biopsies and prostatectomy specimens. For this comparison of imaging with pathology, a number of assumptions are made that are considered invalid.

Combining the sector-based analysis with whole-mount histology of the prostate specimen (third approach) improves spatial information content at pathology and improves comparability [10]. This approach however has still severe limitations as the correct classification into specific regions may be arbitrary due to an intrinsic lack of anatomical features (landmarks) that would clearly separate these zones, which is particularly true for low-resolution (PET) and low-contrast (CT) imaging. In addition, any subsequent deformations of the prostate specimen due to fixation and sectioning are not taken into account.

Furthermore, imaging studies that derive their pathological correlate from prostate biopsies are limited due to the inherent uncertainty of the biopsy location, even when using template-based biopsies. Also due to significant sampling errors, Gleason grades obtained from prostate biopsies may over- or under-estimate the true Gleason grade obtained from a prostatectomy specimen [11], limiting the ability to accurately assess Gleason grading capabilities of *in vivo* imaging.

In this review, we examine the registration process and review recent progress in registration techniques between *in vivo* image volumes (MRI, PET) with multiple whole mounted radical prostatectomy specimens (fourth approach).

Examination of fallacious assumptions for registration

The following is a list of fallacious assumptions that compromise the registration accuracy of histology and *in vivo* imaging modality combinations in many prior publications that ultimately all result from assuming the registration or mapping problem in this context is fundamentally less than a fully 3D problem, i.e. 3D histology “mapped” (alternatively “registered”) to 3D medical imaging (3D $\leftrightarrow$ 3D).

Assuming that the registration of just the outer 2D manifolds (3D organ surfaces) between differing modalities such as histology and *in vivo* imaging, accurately matches internal structures is fallacious. An associated 2D corollary, i.e. assuming registration of 2D internal structures of multimodality slices from 1D manifolds (outer perimeters) is equally erroneous.

Finding correct outline correspondences requires more than implementing arbitrary and biased user assumptions of matching outline segments, such as overlaying evenly arc-segmented radial lines on both data sets each centered at some arbitrary point and forcing so called “homologous” points that intersect at the outlines to be registered. Matching outlines involves finding truly matching tangential homologous line segments along the outlines, not just 2D points which define a point-to-point homology with no regard to local tangents. Even so, the warping of the interior is not defined by matching points on the outline. Matching the outlines and expecting the rest of the registration to be correct requires that all deformations are proportionately radial about some unknown center point, clearly something that rarely exists in nature, if at all. Accurate internal registration by matching outlines would require the use of a 2D finite element model (FEM) which assumes prior segmentation knowledge of the slice’s internal features and each segment’s elastic properties. But if the loci of such parameters were known, it would be possible to register them by geometric methods without using a FEM in the first place. However, neither option is typically feasible due to the missing joint spatial and elasticity information for FEM segments. Likewise, registration of just the outer 3D surface shell (2D manifold) cannot correct local volumetric deformations such as local nonzero divergences and curls that may occur within tissues due to fixation-related changes. Even if the outer 3D shells were registered, the registration error would generally increase with distance inward away from the registered outer shell.

Prostate deformation

Assuming that the 3D *ex vivo* radical prostatectomy specimen that has been formalin-fixed for histology sectioning has the same 3D geometry as the 3D *in vivo* medical images, such that the two can be registered at most by a simple 3D rotate, translate, and isotropic scaling transformation is also fallacious. An associated, fallacious 2D corollary assumes that a slice in 3D *in vivo* imaging can be found, possibly by reformation of the 3D medical imaging data, which matches a slice of histology such that the 2D histology slice only needs a 2D

transformation to map its geometry onto the 2D geometry of the corresponding “found” *in vivo* slice.

As can be expected, the *in vivo* configuration of the prostate will change with bladder and rectal filling. In addition, depending on technique, the use of a balloon for endorectal coil MRI can significantly change the configuration of the prostate gland. The mechanical deformation of the *in vivo* specimen in the process of surgical extraction alone is sufficient to understand the fallacy of the assumption. Surgical extraction of the prostate begins with transecting periprostatic tissues including vessels, urethra and bladder, resulting in significant deformation of the prostate. The surgeon uses manual or robotic probing to tear the prostate away from the supporting surrounding tissue, and finally the prostate is firmly grasped and extracted from the body cavity. At this point the geometry of the prostate is already altered far beyond a rigid body deformation due to the vascular and urethral pressure collapse, manual compression and tissue destruction. Orczyk et al. calculated an average 10 % loss in volume due to surgical resection alone as measured by *in vivo* and *ex vivo* MRI [12]. Further the dehydration of a tissue sample during formalin fixation yields differential shrinkage of internal tissue components (fat, muscle, collagen, calcifications, etc.) due to their initial differential water hydration states [13, 14].

While the previous paragraph describes 3D deformations to the intact prostate, recall that preparation of 2D histology slices requires the mechanical cutting of the fixed tissue volume into 2D slices with a microtome, mounting the slice on a glass slide and staining the tissue. If a frozen sample is cut via cryomicrotome, the result is referred to as the “butter” transform, where the cryostat is set at a temperature to keep the tissue sample sufficiently solid to cut nearly parallel slices without breaking the blade. If instead the sample is cut into slices after formalin fixation followed by paraffin embedding, the sample is still non-uniformly deformed due to the differential tensile strengths of the structures cut [13]. While torn slices are usually discarded, such slides may still be selected for the study because of the pathology they uniquely represent. The presence of these often random effects strongly suggests that it is important to map/register each 2D slice back onto the geometry of the (frozen or paraffin-fixed) tissue block before it was microtomed, which can be captured in a picture taken of the block face ideally just before the section is cut (or even afterwards if the sections are very thin and thus very similar). Otherwise the alternative is to try to compensate for all of these random, fundamentally 2D deformations after the slices have been bound into a volume where no geometrical truth exists. While several published papers justify ignoring these 2D tasks while citing the need for laboriously assigning manually chosen point correspondences on each of the slices, it is the case that automatic registration algorithms possessing sufficiently large capture ranges can be successful with little/no manual intervention [15]. If torn tissue slides are discarded, their location needs to be preserved with null, non-registration biasing entries so as to maintain the relatively correct spacing of the stack of histology slices to limit the required degrees of freedom (DOF) of the next registration step.

Prostatic motion

Assuming that *in vivo* acquisition motion artifacts can be safely ignored is problematic when performing MRI and PET due to extended volume collection durations. CT acquisitions in late generation machines are now sufficiently fast that motion artifacts can be avoided, but the same is not true for MRI and PET. It is worth noting that maximal cranial-caudal motion of an adult prostate has been measured at ~1 cm between full inspiration and full expiration [16–18]. However, under normal breathing conditions in a comfortable supine position, respiratory motion of the prostate appears to be less than 4 mm [19]. Immobilization in thermoplastic shells (for radiation treatment) may increase prostate motion synchronously with respiration, rather than preventing it [18]. These data suggest that respiratory gated PET

acquisitions in list mode may improve prostate registration with other modalities. However, preventing patient motion by optimizing patient comfort during imaging is most relevant. This is particularly true for endorectal coil MRI, as rectal and pelvic muscular contractions can significantly alter the position of the prostate [17].

Advances in registration techniques

Specialized registration techniques are needed to facilitate the registration of histology onto *in vivo* imaging. Histology information content is multiple levels of scale higher than that of MRI, which is typically the *in vivo* imaging modality obtained with the greatest contrast resolution to discern intra- prostatic anatomy. Therefore, the limiting factor for registration accuracy is primarily the local information content of the *in vivo* imaging, independent of modality. CT has high spatiotemporal resolution but poor soft-tissue contrast resolution; thus the information content of its resulting imaging volume for soft tissues is limited primarily by the radiation dose and attenuation characteristics of the image volume. MRI has high intrinsic soft-tissue contrast resolution, but lesser spatiotemporal resolution, which can reduce the contrast resolution achieved due to tissue motion during acquisition. The soft-tissue contrast resolution of PET is dependent on the achievable target-to-background contrast of the selected tracer, but their spatiotemporal resolutions are generally much worse than CT or MRI. Thus, in general we assume for imaging stationary soft tissue the following ordering of image volume information content (I) from each of the imaging modalities, i.e.

$$I_{\text{Histology}} \gg I_{\text{MRI}} > I_{\text{CT}} > I_{\text{PET}},$$

where the ordering of the average information content of *in vivo* imaging modalities may be altered under different imaging conditions than those described above.

By combining appropriate modality pairings, it is possible to match the needed DOF of warping with the appropriate mutual information (MI) content to improve the overall registration accuracy between histology and *in vivo* imaging. For example, when high DOF warping is needed for registration, appropriate modality pairings are required whose MI supports such DOF. A one-step registration performed by mapping an accurately formed histology volume to the *in vivo* MRI volume, which requires large DOF due to the large bending energy differences between the two datasets, is not supported by the MI of the combination, specifically due the low information content of the *in vivo* MRI. Thus the use of intermediate stages of high information content imaging is required to provide support for the needed DOF necessary to ultimately map the two datasets. For example a high resolution block face photo taken just before the slice of interest is cut with the microtome will provide sufficient information for the resulting histology slice to be deformed back into its *en bloc* geometry [20]. Note that the largely random 2D deformations (including tearing) that occur during microtoming the tissue block cannot be corrected later by 3D deformations unless the deformations persist systematically across many slices, i.e. are truly correlated in 3D (which is extremely unlikely). Finally it is clear that inclusion of more whole mount sections/slices in the representation of the histology volume is beneficial due to the increased information content of the resulting histology volume. While pathologists typically sample and annotate between 5–7 sections (each section is usually 0.4–0.5 cm in thickness) for clinical diagnosis, the inclusion of additional non-annotated slices will improve the following joint volumetric registration tasks.

The resulting stack of 2D geometry-corrected histology slices, which represents a sampling of the prostatic volume *en bloc*, can be more accurately mapped to its *in vivo* geometry through the use of higher DOF warping. This can be achieved by first mapping the *en bloc*

geometry to its *ex vivo* state as acquired by motionless, thin slice, multiple signal-averaged anatomical T2-weighted MRI imaging begun immediately after the prostate is surgically removed. This imaging should be performed while the prostate specimen is supported ideally in a neutrally-buoyant, formalin-containing solution so as to prevent further deformation of the *ex vivo* organ from its *in vivo* geometry [20]. If the start of *ex vivo* imaging begins immediately after the fresh prostate specimen is placed in formalin, then only the surface of the prostate will be fixed within in the first hour, and the remainder of the prostate will not be cross-linked. If this is not possible (as in Figure 1), then the imaging protocol must be changed to accommodate the imaging of the fixed prostate using a proton density weighted acquisition (long TR/short TE of 1000–2000/20–30) at higher field strengths, i.e. 7-Tesla. Either way the signal-to-noise ratio (SNR) and therefore information content of the *ex vivo*, thin slice MRI acquisition can be made far higher than that of the *in vivo* anatomical MRI due to repeated signal averaging supported by motionless imaging where the averaging time for the fixed specimen is on the order of 8–10 times that of the unfixed specimen [21].

The last step of mapping the histology volume now deformed to approximately the same geometry of the *ex vivo* prostate back to its *in vivo* geometry will only support low DOF due to the low SNR of the *in vivo* image. Warping methodologies that only support high DOF must choose appropriate penalty terms to penalize for higher local bending energies and/or are applied at sufficiently low spatial scales such that the resulting local bending energies are limited to prevent folding singularities in the resulting deformation.

The registration method described by Park et al. [20] allowed registration without the use of extrinsic fiducial markers or unsubstantiated assumptions regarding geometric relationships between *in vivo* and *ex vivo* image sets and was applied successfully in several clinical studies [22–25]. The method, however, suffered from uncertainties related to variable geometry (slice thickness) of individual gross specimen sections regarding their location in the *ex vivo* specimen MRI.

To overcome this limitation, various dedicated sectioning devices have been developed allowing gross sectioning in defined fixed intervals and orientation [26, 27]. With the introduction of rapid prototyping technology, further improvements utilizing custom-made molds for the prostate specimen are now feasible [12, 26, 28–30]. Rapid prototyping allows conversion of a computer generated 3D model into a physical model. Acrylonitrile butadiene styrene plastics (ABS) are common materials that can be used to print molds capable of holding the specimen in a defined position and shape for *ex vivo* imaging and gross sectioning. Mold creation is usually based on the 3D representation of the prostate from *in vivo* high-resolution anatomical MRI [28, 29]. The design requires segmentation of the prostate surface and is usually performed on a stereo lithography computer-aided design (CAD) workstation. An advantage of this technique is that the mold can be manufactured prior to prostatectomy, with evenly spaced slits encouraging nearly parallel gross sectioning in a pre-defined orientation and direction [29]. In addition, the technique can be applied to sectioning of fresh (non-fixated) prostate specimens. Nevertheless due to the differential compression of the *ex vivo* prostate during gross sectioning, the resulting sections have neither planar nor co-planar faces. Thus the exclusive use of such devices without additional nonlinear (warping) registration will not yield sufficient results because the *in vivo* MRI and *ex vivo* sectioned 2D manifold (identified as such because their shape may look more like that of a thick potato chip) still do not match, and only the appropriate use of non-linear registration can account for the above mentioned prostate deformations.

Additional *ex vivo* MRI of the prostate specimen has several advantages. First, image data can be acquired from a non-moving object in exquisite resolution. Second, such image data

allow independent verification of *in vivo* imaging features. Using this approach, Fan et al. were able to demonstrate that *ex vivo* MR imaging of the excised prostate specimen with a high-field-strength magnet (9.4-Tesla) can have similarities to *in vivo* endorectal coil MR imaging at 1.5-Tesla in both the peripheral and central zone, but their series only studied nine prostates and they did not assess changes in image contrast which can occur within the prostate related to dehydration and/or fixation following prostatectomy [30]. Figure 1 displays the registration process of *ex vivo* T2-weighted MRI of a prostatectomy specimen obtained at 3-Tesla onto whole mount pathology. Mold design was based on *in vivo* MRI. Figure 2 illustrates the entire registration schematic.

Once registration (mapping) of the *in vivo* 3D MRI volume with a stack of warped 2D histopathology slices is achieved, image information about any part of the gland—such as foci of cancer, benign hyperplastic nodules (BPH), or inflammation—can be transferred across all *in vivo* modalities, thus providing researchers with valuable spatial information about the significance of imaging features. Previously, we reported registration errors between pathology and *in vivo* MRI using MI registration techniques and *ex vivo* MRI (but without the use of custom molds) in the range of 2.3 – 3.7 mm [20].

Many iterative and semi-automatic deformable registration algorithms have been developed to facilitate the comparison of imaging and histology, but credible estimates for registration errors for many methods have not been published. Since these methods do not rely on the intermediate data provided by *ex vivo* imaging, they appear to possess incomplete information to drive all the necessary deformations for accurate registration. However with sufficient image information provided by both *ex* and *in vivo* imaging, and volumetric histopathology, automated registration techniques can reduce the laborious and time-consuming registration task, and can improve the accuracy and applicability of registration techniques [31] in mapping histopathology back into *in vivo* imaging.

Conclusions

The ability to unequivocally link specific *in vivo* prostate cancer imaging features to pathology is of great importance. This is especially true as clinical trials investigating the performance of imaging in primary prostate cancer are time-consuming and costly. The additional expenses related to advanced registration techniques between *in vivo* imaging and pathology which require *ex vivo* imaging of the prostate specimen and possibly custom mold production are small in comparison to the overall costs of clinical trials. Employing advanced registration techniques will improve the impact of such research. One can also speculate that the total number of subjects needed to assess efficacy of imaging in primary prostate cancer could be reduced as a result of decreased bias and measurement errors. Employing advanced registration techniques may well be cost effective and is recommended for research applications. This is particularly true for primary prostate cancer, which often presents with multi-focal disease of varying clinical significance.

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Figure 1.

Formalin fixated specimen (A) underwent *ex vivo* MRI (B) in a custom-made prostate mold (C). Gross sectioning was done in identical position with evenly spaced distances (D). Tissue block face photos were obtained from these 3 mm sections (E). After HE staining, the pathologist outlined prostate cancer lesions (blue dotted hand writings) on whole-mount HE slices (F). To demonstrate the process, the T2-weighted specimen MRI (TE: 325 and TR: 1733) volume obtained at 0.15 mm slice thickness was registered to a single 2D HE slice. The registration result is shown in G. As demonstrated in the fusion image H (50 % MRI and 50 % HE), registration between pathology and *ex vivo* MRI is excellent.

Figure 2.
Registration schematic.