Int J CARS (2010) 5:471–487
DOI 10.1007/s11548-010-0405-z

REVIEW ARTICLE

Optimum slicing of radical prostatectomy specimens for correlation between histopathology and medical images

Li Hong Chen · Henry Ho · Richie Lazaro · Choon Hua Thng · John Yuen · Wan Sing Ng · Chris Cheng

Received: 6 November 2009 / Accepted: 15 January 2010 / Published online: 24 February 2010

Abstract
Purpose There is a need for methods which enable precise correlation of histologic sections with in vivo prostate images. Such methods would allow direct comparison between imaging features and functional or histopathological heterogeneity of tumors. Correlation would be particularly useful for validating the accuracy of imaging modalities, developing imaging techniques, assessing image-guided therapy, etc. An optimum prostate slicing method for accurate correlation between the histopathological and medical imaging planes in terms of section angle, thickness and level was sought.
Methods Literature review (51 references from 1986–2009 were cited) was done on the various sectioning apparatus or techniques used to slice the prostate specimen for accurate correlation between histopathological data and medical imaging. Technology evaluation was performed with review and discussion of various methods used to section other organs and their possible applications for sectioning prostatectomy specimens.
Results No consensus has been achieved on how the prostate should be dissected to achieve a good correlation. Various customized sectioning instruments and techniques working with different mechanism are used in different research institutes to improve the correlation. Some of the methods have convincingly shown significant potential for improving image-specimen correlation. However, the semisolid consistent property of prostate tissue and the lack of identifiable landmarks remain challenges to be overcome, especially for fresh prostate sectioning and microtomy without external fiducials.
Conclusions A standardized optimum protocol to dissect prostatectomy specimens is needed for the validation of medical imaging modalities by histologic correlation. These standards can enhance disease management by improving the comparability between different modalities.

Keywords Prostate slicing techniques · Medical image correlation · Pathological sections · Heterogeneity of prostate tumors

L. H. Chen (✉) · W. S. Ng
School of Mechanical and Aerospace Engineering,
Nanyang Technological University, 50 Nanyang Avenue,
Singapore 639798, Singapore
e-mail: chen0610@ntu.edu.sg

H. Ho · J. Yuen · C. Cheng
Urology Center, Singapore General Hospital, 11 Hospital Drive,
Singapore 169610, Singapore

R. Lazaro
Department of Pathology, Singapore General Hospital,
11 Hospital Drive, Singapore 169610, Singapore

C. H. Thng
Department of Oncologic Imaging, National Cancer Centre,
11 Hospital Drive, Singapore 169610, Singapore

Introduction

The histopathology information remains the gold standard for prostate cancer diagnosis. To date, it provides the most comprehensive information for therapy decision making. Although imaging modalities such as magnetic resonance imaging (MRI) and ultrasound scanning (US) can provide useful anatomic and physiological information in vivo, their value for disease diagnosis and prognosis is still limited in terms of specificity and sensitivity. Precise correlation (probably on a pixel-by-pixel basis) between histopathological sections and imaging sections is important since practitioners rely more and more on image-guided technologies.

Accurate correlation also allows the evaluation of the imaging techniques and promotes the development of new imaging modalities that allow more accurate cancer detection and localization. However, histopathology-medical image correlations are limited by surgical distortion, tissue shrinkage during fixation or processing, and difficulties in obtaining consistent section thicknesses and angles. Whilst surgical distortion and tissue shrinkage may be reduced by careful handling and correction factors [1–3], obtaining precise section thicknesses and angles remains a challenge.

To address the challenge of the latter, a number of new techniques have been proposed. The majority of these studies [4–12], still adopt the traditional free hand specimen dissection method. These mainly focus on the pre-processing steps before the histopathological and image plane comparisons, and can be categorized into two main streams of work: (a) based on registration techniques to correct the tissue distortion during image processing [9,13–17] and (b) based on customized sectioning apparatus to reduce the tissue distortion during the process of specimen orientation, embedment and sectioning [13,18–27]. Registration methods, especially three-dimensional (3D) registration, usually can correct the angle and thickness differences by translation and rotation. Some authors that perform 3D registration methods claim that these do not require precise correlation of the image section thickness and angle [28]. However, the registration methods are often laborious and a number of issues must be considered [16]. Firstly, the accuracy relies largely on the available landmarks due to the high complexity of deformation during the tissue preparation. Secondly, the inherent differences of the imaging characteristics between the histopathology and the imaging modalities further raise the difficulty of registration. Since the accuracy of these methods remains unsystematically quantified, the feasibility and reliability of the registration methods for clinical applications remain unclear. This accentuates the need for optimum tissue sectioning techniques and tissue processing methods that will result in minimal deformation and distortion.

In this paper, in order to investigate an optimum slicing method for radical prostatectomy specimens, a comprehensive review is included on the different methods used in research and clinical applications. Issues in achieving a good correlation between histopathology and medical images without compromising the diagnosis accuracy will also be discussed.

Comparison of pathologic sections and in vivo prostate images

From previous research, various issues regarding obtaining accurate correlation between histopathology and in vivo medical images of prostate have been raised. To summarize, these can be categorized into five key issues as discussed below:

ISSUE 1: Difficulty in obtaining good histopathology-image correlation whilst maintaining the consistency and reproducibility of diagnosis quality for clinical practice. Radical prostatectomy specimens give the most precise and comprehensive information about the histological grade, margin status, tumor extent and cancer stage. These pathological data are critical for determining the adjuvant therapy and predicting the patient out-come. In order to achieve consistent and reproducible tumor observations from prostatectomy specimens, a standardized routine for tissue sectioning and processing is required and the issue has been addressed with consensus by different groups of researchers and societies [29–33]. At present, however, there is still no consensus on how the prostate specimen should be sectioned and prepared for accurate correlation with medical images. This has precluded the effective comparison of the study results between different researchers and raises questions to the study outcomes. To maintain a balance between the research interest in acquiring accurate correlation and the diagnosis quality and work efficiency for routine pathological practice, an optimum tissue sectioning protocol is required. This method should be able to produce an acceptable quality for diagnosis in clinical applications, yet is cost effective and simple to use.

ISSUE 2: Should the prostate be sectioned in its fresh or fixated state? The fixation of the prostate specimens before sectioning is usually recommended in routine pathological practice. Adequate fixation before the sectioning does not only preserve the surgical margins but also enhances subtle characteristics of the tumor foci such as firmness and discoloration. The fixation, however, inevitably causes tissue shrinkage which leads to errors in section angle and thickness correlations. With increasing interest in obtaining fresh tissue for research, many pathologists also section the prostate before fixation [34]. The sampling of fresh prostate specimen remains challenging [32]. Firstly, the semisolid consistency of the fresh prostate gland makes it difficult to slice evenly with uniform thickness and this poses difficulties for embedding, processing and consistent histological sectioning. Secondly, as soon as a scalpel nicks the capsule of the fresh prostate to slice it, the tissue within the gland emerges through this cut because of the high pressure of any hypertrophic nodules. As a result, the prostate is deformed and its orientation is disturbed. Lastly, the time taken to store the fresh tissue appropriately may increase and this affects the maintenance of the molecular profile. With these concerns, an optimum method which could facilitate and regulate such correlations has yet to be unified.

ISSUE 3: Insufficient landmarks for orientation of the prostate during pathological dissection. The prostate is a donut-shaped gland that surrounds the curved urethra tract. It has a broad and flat base and a narrower apex. The seminal vesicles and vasa deferentia enter the gland at the posterior aspect of the base. The anterior surface of the gland is rounded and convex while the posterior surface is broad and flat. The position of the seminal vesicles, vasa deferentia and the contour of the gland may serve as landmarks for quick identification of the base, apex, posterior and anterior surface of the gland for gross orientation during routine pathological dissection. For precise orientation, the landmarks available are unfortunately insufficient as the surface of the gland is usually three-dimensionally irregular and the urethra usually retracts after the prostatectomy.

ISSUE 4: Dilemma between correlating the medical images with whole-mount prostate slide versus prostate biopsy. The multiple prostate biopsies serve as the gold standard for diagnosis of prostate cancer before the prostate is taken out of the body. Although this is one of the main streams of methods for correlating the medical image with histopathology, it is unable to provide accurate estimation of tumor sizes and the grade due to the sampling error [35]. It has also been reported that the prostate biopsy can miss up to 20-30% of cancers even with recent advances in biopsy techniques [36].

ISSUE 5: Is the use of medical images with 2D pathological slices or 3D reconstructed pathology better? The verification of radiological methods with cross-sectional histopathology is difficult due to the poor correlation of slice thickness and orientation. Two-dimensional (2D) data as determined in histological slices often differs from the non-invasive images which parameterize the information in volumetric units. This further reduces the significance of the correlation [37–39]. Therefore, several researchers [40–42] attempted three-dimensional (3D) approaches for a more accurate match slice geometry. The details of the methods used in 3D reconstruction of histology for image correlation is beyond the scope of this review. Even though the 3D approach is a more accurate representation, the accurate matching of histology and radiology nonetheless also highly dependent on the section registration technique, quality of tissue, effects of tissue processing and sectioning, as well as the large amount of data that needs to be generated [43].

Prostate sectioning techniques

Traditional surgical pathology prostate dissection

As early as the beginning of the 1980s, the researchers began to study prostate medical images by correlation with the pathology of the entire prostate gland. To date, the free hand whole-mount prostate specimen dissection remains the most common tissue dissection methods [4–12]. The traditional sectioning of the prostate specimen includes the following procedures: orientating the prostate, inking the prostate surface, trimming the margin, and dissecting the prostate main body. The prostate is orientated by locating the seminal vesicles and vasa deferentia which insert into the posterior aspect of the base of the gland, as well as distinguishing the rounded and convex anterior from the broad and flat posterior surface from the contour of the gland. After orientation, the prostate is inked with different colors on the surface for proper orientation and margin identification. Subsequently, the margin is trimmed for examination, then the prostate main body is manually sectioned transversely or sagittally at 3–5 mm intervals perpendicular to the rectal surface as illustrated in Fig. 1.

In the conventional dissecting, the specimens are grossly orientated and sectioned perpendicular to the rectal surface. Although the quality of the sections obtained with this method is adequate for routine pathological diagnosis, it is not sufficient for precise correlation. Firstly, there is poor correlation of section angle between the pathological section and the medical image section. This is because the *posterior surface* of the prostate gland is usually irregular after prostatectomy. Thus, perpendicular cutting to the posterior surface actually is not achieved. The angle and location of the histopathologic sections can differ with MR imaging sections by 5–15° [5]. Besides, the transrectal ultrasound or the endorectal MR imaging planes are not obtained ideally transversely or sagittally as assumed [10,45–47]. The rectal filling of the transrectal ultrasound probe or the endorectal coil with endorectal balloon inflated with 40–100 ml air can unavoidably compress the prostate through the rectal and cause and distinct shape deformation. I.e. an increase at transverse dimension for whole gland, a decrease at anterior–posterior direction, and no statistically change at superior–inferior direction; and relatively greater decrease for peripheral zone than for central gland (including central zone and transition zone). Secondly, there is poor correlation of section thickness. The thickness of the section is usually measured with eye without calibration and the fresh prostate can be easily deformed with blades nicked on the surface during sectioning. This has added the difficulty to obtain the even thickness of the section. Thirdly, the shrinkage after fixation may have changed the size, the shape and the orientation of prostate; making it difficult to orientate the prostate exactly perpendicular to posterior surface and hence obtain an identical section thickness for correlation.

Due to the poor correlation in section angle and thickness, the extents of correlations vary in different studies and are limited to region basis [5–8,10,11,45] or large tumor size [12]. Although the image registration techniques

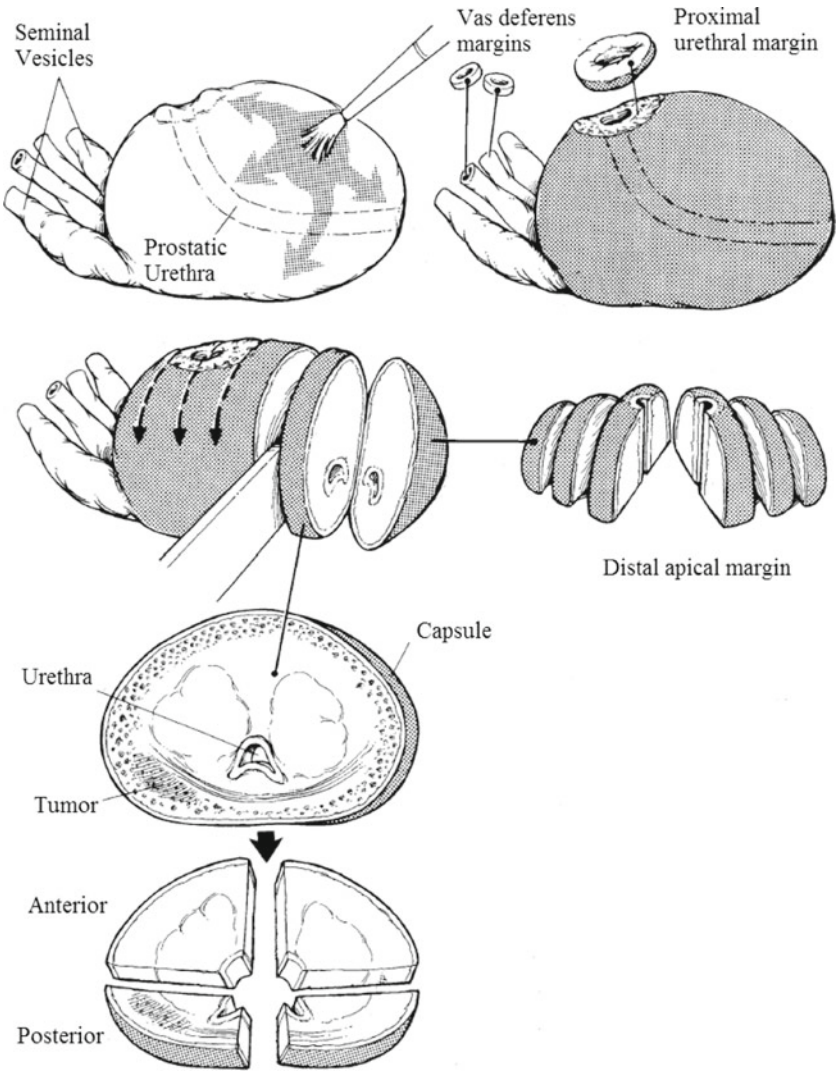

Fig. 1 Conventional prostate surgical pathology dissection [44]

or some other methods such as introducing a reference plane to predefine angle of the medical imaging plane, an accurate point to point correlation unfortunately is not available [8,9]. Besides, the accuracy of correlation is usually not systematically evaluated. At present, due to the inconsistence of the techniques and extents of image correlation, a valid comparison between different studies and a reliable validation of the imaging modalities are not achievable.

Customized sectioning device facilitated dissection

With the development of imaging techniques, as well as the application of image guided therapy for prostate cancer, an imaging modality with validated accuracy is required for accurate cancer localization. In order to improve the correlation between the histopathology and radiology (sometimes on a pixel-by-pixel basis), customized specimen dissection methods are investigated by different institutions and different slicing apparatus or methods are proposed recently. However, none of slicing devices has been widely accepted.

The CIMIL slicing apparatus

Ma et al. from the Computer Integrated Medical Intervention Laboratory (CIMIL) at the Nanyang Technological University (NTU) Singapore [18] developed a slicing apparatus that allows orientating and slicing the prostate specimens accurately according to the MRI plane. The section thickness was 3.5 mm as shown in Fig. 2. The slicing box consists of five main parts: (i) a chamber, (ii) a cutting slits, (iii) a disposable blade attached to a handle, (iv) two transparent linear scales at the ends, and (v) a moveable stopper. The chamber is for orientating placement of the prostate specimen. Cutting slits is placed at fixed distance of 3.5 mm apart, allowing a sharp blade to pass through to trim the tissue block. The sharp trimming blade is used to minimize tissue distortion. At the ends of the chamber, there are two linear scales sharing the

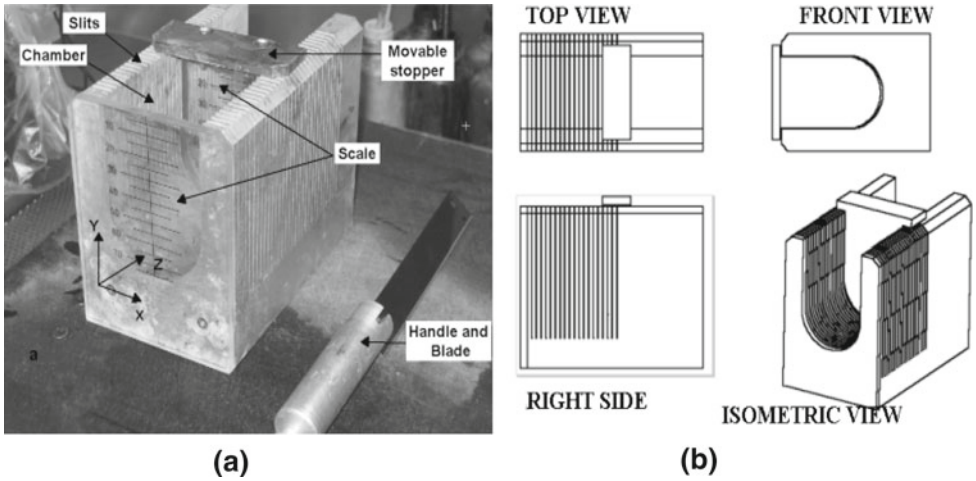

Fig. 2 **a** Prostate slicing box for sectioning the prostate accurately according to orientation to MRI, **b** technical drawing of the prostate slicing box [18]

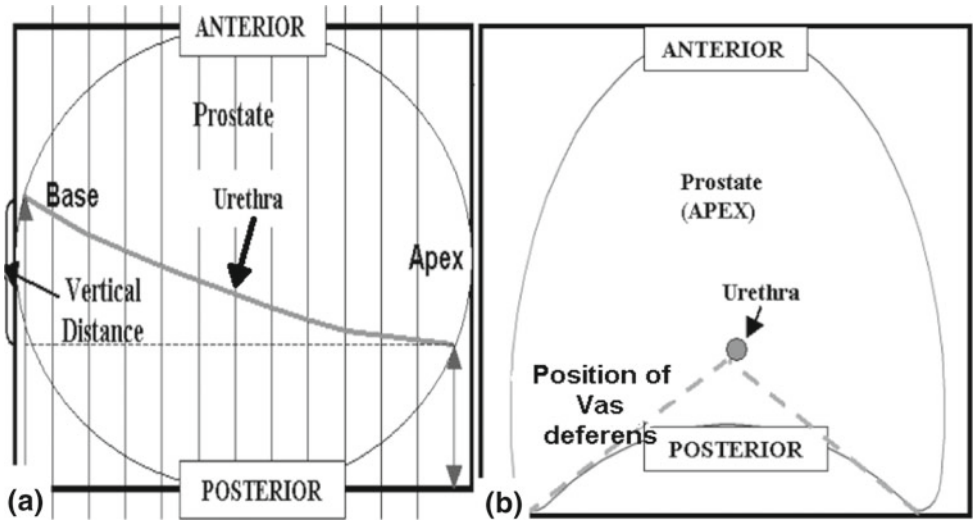

Fig. 3 **a** Sagittal view of the prostate inside the prostate slicing box; **b** transverse view of the prostate, with positioning of vas deferens to determine orientation inside the prostate slicing box [18]

common horizontal datum. The scales enable the pathologist to position the apex and base of the prostate according to the measurement of the vertical dimension of the urethra entry and exit sited.

The orientation of the specimen is taken in three dimensions. The rotation of the specimen about the X axis is correlated by matching the vertical distance between the entry site of the urethra at the base and the exit point of the urethra at the apex with MR images as shown in Fig. 3a. The rotation of the specimen about the Y axis is correlated by measuring the angle between the MR planes with the prostate vertical plane as shown in Fig. 4. The rotation about the Z axis is correlated by using the vas deferens as a reference as shown in Fig. 3b. After positioning the specimen in the chamber, the specimen is sectioned serially of equal thickness as shown in Fig. 5.

The accuracy of the slicing device was studied with three phantoms and 11 patients by calculating the overlap of the nodule areas between the MRI and the pathological slices. An overall accuracy of the prostate slicing box for section by section direct correlation is 79.6% combined with prostate boundaries and urethral as registration landmark during correlation. This is the first study with systematic evaluation of the slicing accuracy for prostate pathology MR imaging section correlation. The method differs with the conventional method by using the special relations of the limited landmarks to orientate the prostate and using parallel uniform slits to guide the sectioning. However, there are several drawbacks. Firstly, the rotation about the X, Y and Z axis are done manually without accurate calibration which are not stable and not accurate. Secondly, holding the prostate in position during sectioning is technical demanding. For one hand, there is a dilemma to hold the prostate in position with considerable pressure and to hold the prostate in its nature state without deformation which leads to inconsistent section thickness during sectioning. For another hand, the prostate specimen is held in place with one dimensional pressure acting the slidable stopper. It is possible that the prostate can

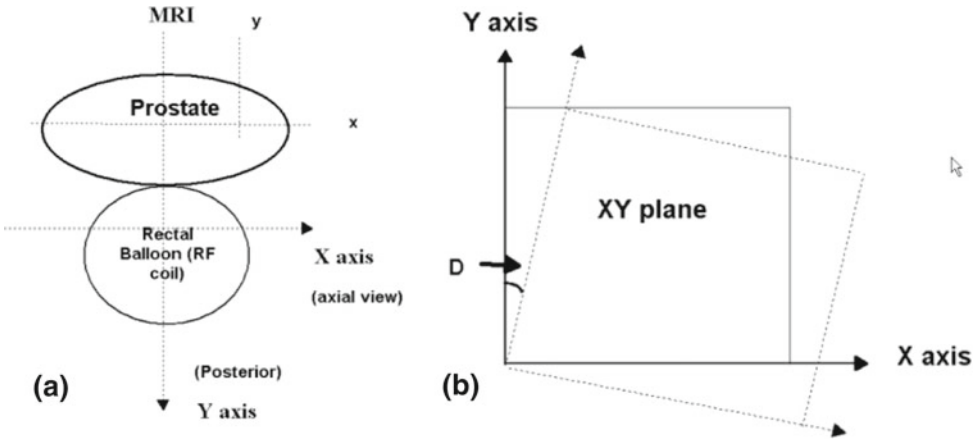

Fig. 4 **a** The axial view of $X - Y$ plane inside the MRI field; **b** the angular displacement between the prostate vertical plane and the MR image plane reported by radiologist [18]

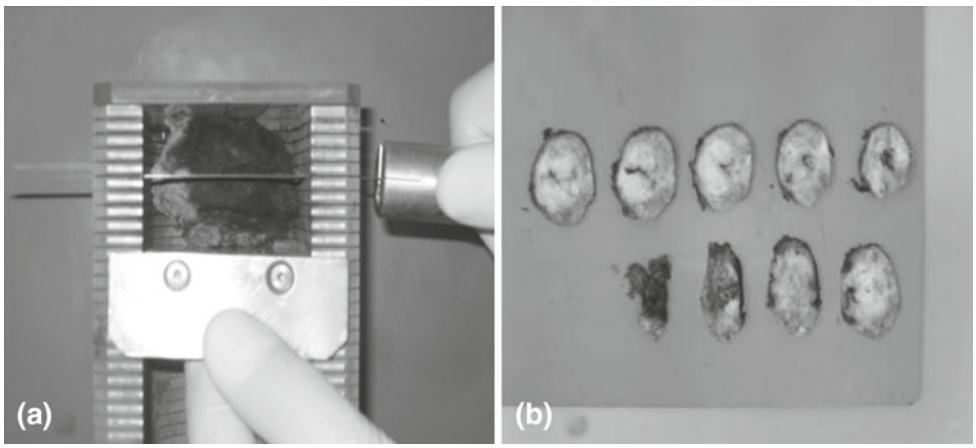

Fig. 5 **a** the prostate specimen is sliced with equal thickness; **b** equal thickness tissue slabs with some orientation as MR image scanning planes [18]

rotate during the sectioning and the angle of sectioning may have been changed. Thirdly, the section surface may be damaged due to the resistance and the heterogeneous property of the prostate. Improvements on the slicing box were proposed by the authors to use the gelatin to secure the specimen and to use multi-blades to minimize the damage to the tissue so as to improve the consistency and reproducibility of uniform sections. Nonetheless, further report with the improvement for this method is not found. Even with the improvement as mentioned above, how well the gelatin can be secure the prostate from movement and how well the multi-blades can section the prostate while it is held in gelatin remain as concern.

The UK patented slicing apparatus by Institute of Cancer Research, London

Jhavar et al. [19] in Institutes of Cancer Research, London, developed a prostate slicing apparatus to obtain fresh tissue for research and for correlating diagnostic and molecular results with preoperative imaging. The tissue slicer consists of a series of juxtaposed planar stainless steel blades linked to a support and a cradle adapted to grip the tissue sample and receive the blades as shown in Fig. 6. After the seminal vesicles are transected and the margins are shaved, the whole gland is held in the cradle and cut in a plane perpendicular to the posterior surface of the gland with the multi-blades of equal 4- mm intervals.

An alternative cradle of the slicing apparatus was also developed under the same patent as shown in Fig. 7. The cradle comprises a base having rows of holes therein, series of brass rods with grooves surrounding the surface and located in the holes at 4 mm interval and two end plates located between opposing end rods. The rods are to be located in the holes with straight or curved lines to account for the contours of the tissue sample and grip the specimen without deforming the specimen. The ending plate is located at a 4 mm interval to the ending rods that allows at least one end section of the prostate to be the same thickness with the middle sections.

There have been several studies [20,21,48] using this slicing apparatus to section the fresh prostate for pathology-medical image correlation. A systematic evaluation of the accuracy for the slicing protocol is not available. The way that the specimen is orientated notably is similar to the conventional prostate dissection which is inaccurate as discussed previously. Nonetheless, the slicing device differs from others by providing the cradle to hold the prostate in position during the dissection and using the multi-blade for sectioning which is obviously more efficient. This method

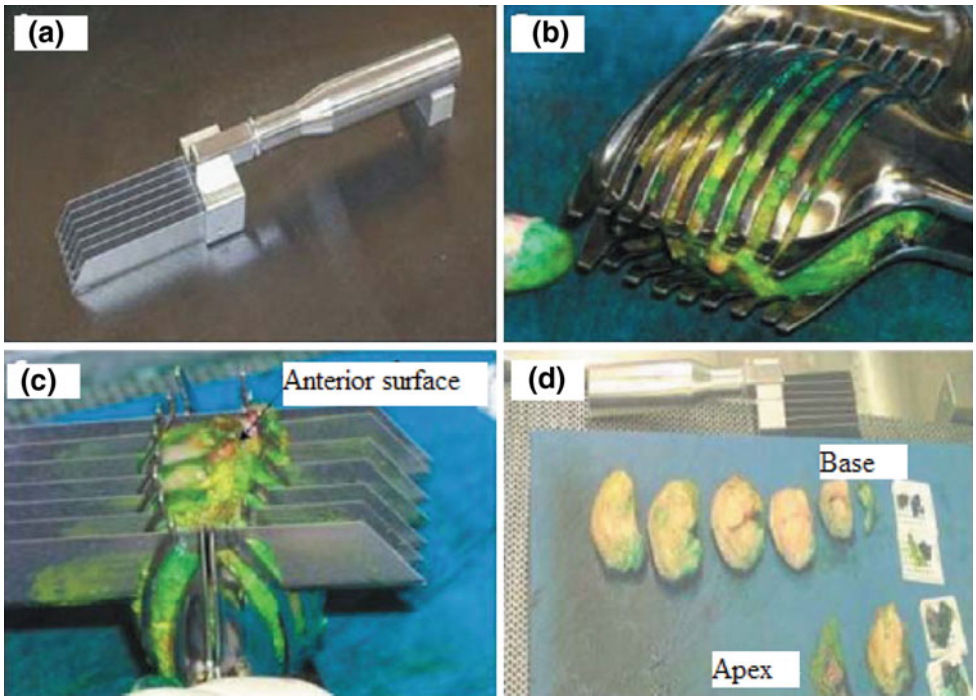

Fig. 6 Slicing of radical prostatectomy specimens [20]

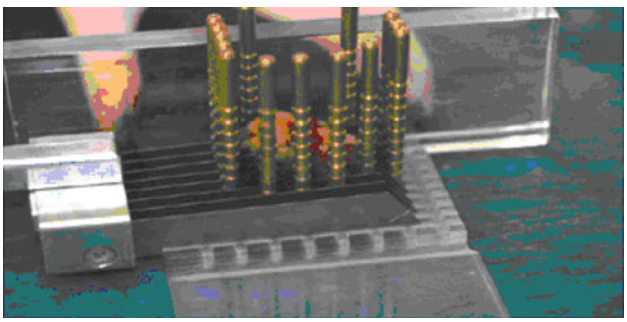

Fig. 7 An alternative cradle holding the specimen in house and sectioned by multi-bladed knife [21]

although is promising, has some deficiencies too. Firstly, it does not allow image correlation with any orientation. The images taken are required to be perpendicular to the posterior surface. Secondly, the two versions of cradle as presented actually denote the conflicts between holding the specimen firmly in position and holding it without deformation. The tong shape cradle can hold the specimen firmly (tong shape cradle) for even sectioning. It is not adaptable for any size of prostate specimen and the gripping force may inevitably distort the specimen and lead to a different section thickness and level compared with the medical imaging planes. The alternative version of the slicing device is adjustable to the size and shape of the prostate, causes less deformation to tissue and allows acquisition of identical section thickness and level of end sections with medical imaging plane. However, it cannot hold the specimen in place without movement or rotation during sectioning. In summary, these two slicing apparatuses may be used to section the prostate with same thickness and possibly with the same section level with medical imaging planes, but it does not provide a good way to orientate the prostate and it is not applicable for any imaging planes taken at any angle.

The PlaneFinder device by Mayo Clinic US

Rouvière et al. [22] in Mayo Clinic US developed a method to cut the histological specimens along the MR imaging plane by using the same system of coordinates used on MR scanners. The method included two major steps. The first is to use three acrylic paint markers to define the MR imaging plane. The second is to use a device called "PlaneFinder device" (PFD) to rotate the specimen in three dimensions under the guidance of the MR scanner to find the predefined imaging plane for dissection as shown in Fig. 8. The PFD is an 18.5-cm mobile polycarbonate and acrylic platform that can rotate in the horizontal plane and tilted along two axes. An acrylic cylinder measuring 55 mm in height and 82 mm in diameter is sealed onto the center of the platform to hold the specimen. The device is not visible on MR images. A small cylindrical vial measuring 5 cm in diameter and 2 cm in height contains a 1 g/l $CuSO_4$ solution to mark the bottom of the surface. This serves as an internal reference for distance calculation.

The principle of the method is illustrated in Fig. 9. Three MR-visible and macroscopically visible fiducial markers are injected in the tissue ex vivo to define the imaging plane.

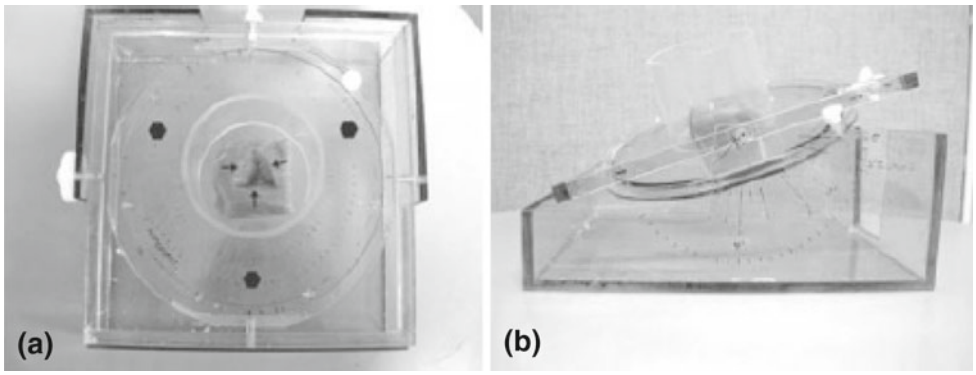

Fig. 8 The top view and side view of the PlaneFinder Device [22]

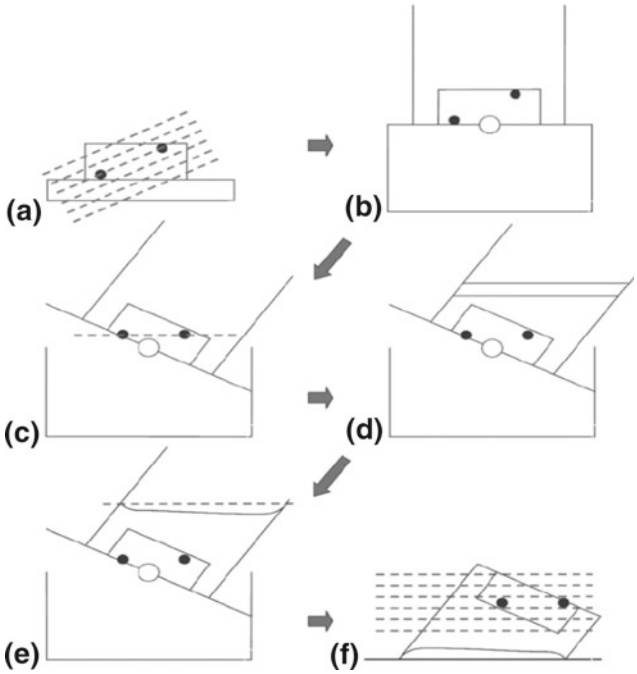

Fig. 9 Schematic diagrams illustrating the working principle of the method [22]

The MR images are then taken parallel to the defined plane. After the MRI study, the specimen is fixed in formalin. After fixation, the specimen is placed in the PFD within the central cylinder for MR scanning again to identify the same orientation for sectioning. After the specimen is tilted in the cylinder with the predefined plane horizontally, the specimen is then embedded with double layer wax to make sure the top surface is horizontal. The wax cylinder is then removed and cut with 5–10 mm thickness paralleling to the top surface.

The accuracy of the method was evaluated by assessing the pyramidal central-hole-area in the markers' plane of the pork muscle. The error of angle was estimated to be $\leq$3 degree in 88–95% of the cases. A recent application of the method was done by Kimm et al. [23] in Stanford University in the study to correlate the ex vivo MR to human prostate specimens with the PFD to orientate the specimen for sectioning. In this study, an improvement was made by using small rods to anchor the prostate to the desired position during MR scanning. After the fixation in formalin, the prostate was orientated at identical position under MR guidance. The accuracy of the correlation was determined by the degree of the alignment of 30 registration points including injected fiducials, anchoring rods, and the internal anatomic structures. A final result of an average displacement of 0.86 ± 0.19 mm (mean $\pm$ SD) was obtained.

This method improves the correlation of angle between the histopathological and MR imaging sections by introducing a predefined plane and using the ex vivo MRI to facilitate the orientation. This method promises to provide good correlation between the histopathological and imaging planes, however, it has some limitations too. Firstly, the fiducial markers are needed to define the imaging plane. The application of fiducial markers is invasive and technically demanding in vivo. The acrylic fiducial markers are useful when used in animal or ex vivo experiments, but their safety for use in in vivo is unclear [28]. Thus, without a suitable marker, the application of the method for in vivo study is limited. Secondly, the method uses MRI to guide the orientation of the specimen which requires the application of MR compatible materials that may be technically demanding and costly. Thirdly, orientation, embedding and sectioning of the specimen are taken after the specimen is fixed. The tissue shrinkage again presented as a distortion which may reduce the reliability of the section level correlation. Fourthly, the sectioning of the prostate when it is fresh was not discussed in the study. The sectioning while the prostate is fresh remains unclear.

Other sectioning methods

The customized apparatus by Case Western Reserve University Cleveland

Breen et al. [13,24,25] in Case Western Reserve University Cleveland developed a 3D method which requires both computer registration methods and tissue handling techniques to

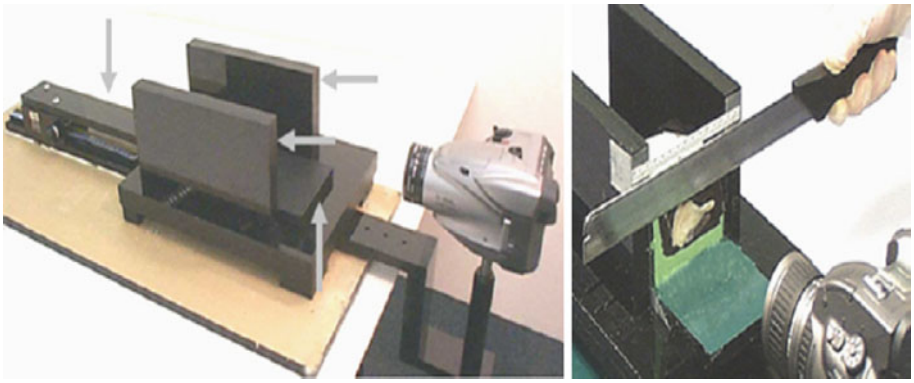

Fig. 10 Tissue-slicing apparatus and process [13]

align medical scanner data with histological sections in animal experiments. To obtain a volume of tissue section images, a customized apparatus was used to slice and photograph the tissue as shown in Fig. 10. The tissue-slicing apparatus contains a digital camera, a sliding tissue platform (indicate by the upward arrow), and a linear displacement device (indicate by the downward arrow) to advance the platform in a precise stepped fashion.

In this study, the in vivo MR data and histological measurement of the thermal ablation in rabbit thighs was correlated. After performing the radio-frequency (RF) ablation procedure under MR guidance, two MR-compatible fiducial needles were inserted in the lesion vicinity with one fiducial approximately parallel and a second fiducial at an angle of approximately 45° to the RF electrode with the guidance of MR again. Following the placement of the fiducial needles, a MR scan perpendicular to the RF electrode was taken. The rabbit was sacrificed after imaging and the tissue was fixed in formalin. The sectioning of the tissue was then done using the special cutting apparatus. To fix the tissue, the tissue was embedded in wax and positioned in styrofoam block which was secured to the platform. After the tissue position was fixed, a brain autopsy knife was used to slice the tissue perpendicular to the fiducial needle which was oriented parallel to the RF electrode. The section thickness was controlled at 3-mm intervals as it was advanced by the linear displacement device. The tips of the fiducial needles were exposed at the tissue block face and highlighted by ink and photographed with digital camera. This process was repeated until the entire sample is sliced. To calibrate the macroscopic tissue images, a ruler was placed in the plane of the tissue slice as seen above the knife.

The histological and the 3D medical images were correlated using a semiautomatic registration algorithms and the registration accuracy was validated by an ellipsoid model which however is out of the scope of the review. In this study, a registration accuracy of 0.96 ± 0.13 mm was obtained. This method as claimed by the authors can be used for accurate tissue typing to relate the 3D medical images to tissue features including tumor. The methodology nonetheless has several limitations. Firstly, the corrections of the tissue movement tearing and shrinkage during dissection and processing rely largely on the registration techniques which are also technically challenging for prostate medical-histopathological images correlation as discussed previously. Secondly, the placement of the fiducial needles is error-prone and the sectioning perpendicular to the needles is difficult in case of inadvertent displacement of the needles. Thirdly, this dissection taken after the specimen was fixed although facilitates the dissection, it raises problems for obtaining identical section thickness and level due to the tissue shrinkage of the tissue especially for prostate of heterogeneous property. Lastly, this method requires the input of fiducial needles to orientate the specimen and guide the sectioning which however are too invasive and not applicable for practicing in vivo human prostate.

The slicing machine for oriented sectioning of irregular tissue block by Denmark

Sørensen et al. [26] in University of Aarhus Denmark designed a method that allows direct comparison between present digital scanning modalities with histological data. The method involves orientating the specimen with the aid of the angle protractor, embedding of the specimen with alginate, and cutting with a special slicing machine as illustrated in Fig. 11. The experimental subject was a pig brain which was fixed for 14 days before the embedding and the sectioning. Alginate polymer from HistO-tech ApS (Århus, Denmark) was used as the embedding medium. During the embedding, the angle protractor as shown in Fig. 12a was used to orientate the specimen via the anatomical landmarks of the pig brain. An alginate mould on the top and bottom of the tissue was used to support the tissue during the cutting procedure and avoid the tissue deformation as Fig. 11b. The tissue and alginate bottom was then placed on a suction pad of the tissue slicer and centered in relation to the knife frame with the aid of a laser pointer as Fig. 11c. With

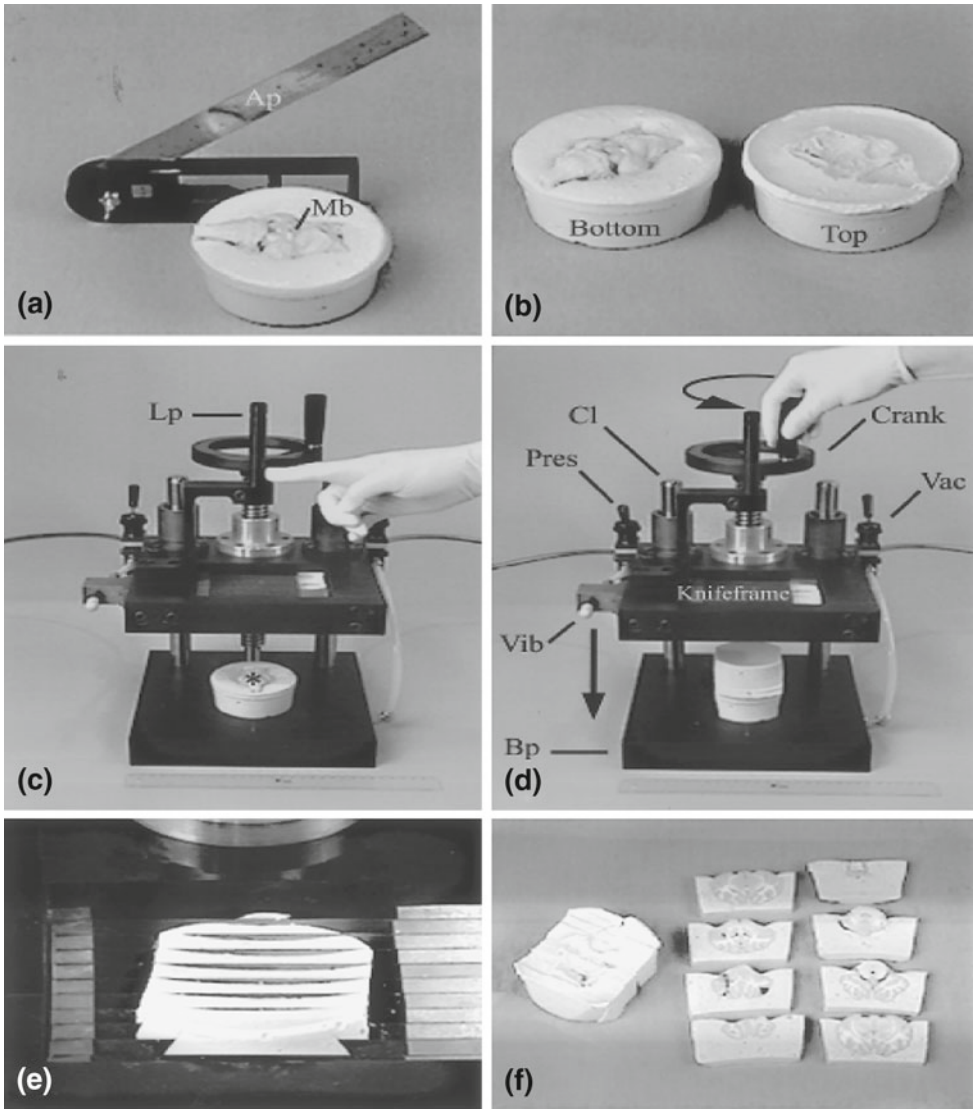

Fig. 11 Illustration of the method to orientate, embed and section the specimen [26]

the alginate and tissue block fixed by activating a vacuum valve, parallel oriented knives positioned in a frame with equal intervals were steady lowered through the alginate and tissue block by vibrating the frame. A set of alginate and tissue slabs which were of equal section thickness and oriented to corresponding pre-operation medical images was obtained.

This method presents a systemic way to improve the correlation of pathology-medical imaging by improving the orientation, securing the position during sectioning, and better sectioning procedures. It varies from other studies by using alginate polymer as an embedding medium and a pneumatic vibrator to secure the sectioning direction. This alginate polymer is soft and deformable to begin with while cutting and allows orientation of the specimen during embedding. The vibration of the knife frame set diminishes friction as the knives move through the alginate polymer and the tissue. The special embedding and dissection techniques allow the acquisition of uniform regular sections from irregular tissue blocks which fulfils the requirements for quantitative stereological analysis. Nonetheless, the method is primarily designed for mammalian brains which has many landmarks for orientation and may not applicable for the human prostate. Firstly, the prostate does not have sufficient identifiable landmarks for orientation such that the angle protractor is not applicable for the prostate gland. Secondly, the measuring of the angle is taken manually and is error prone. This further prevents the accurate orientation of the specimen for correlation. Thirdly, although the embedding medium can secure the brain at a certain position; however, the semisolid consistency of the prostate specimen differs largely from the brain, so that the embedding medium may not be able to secure the prostate as well during sectioning.

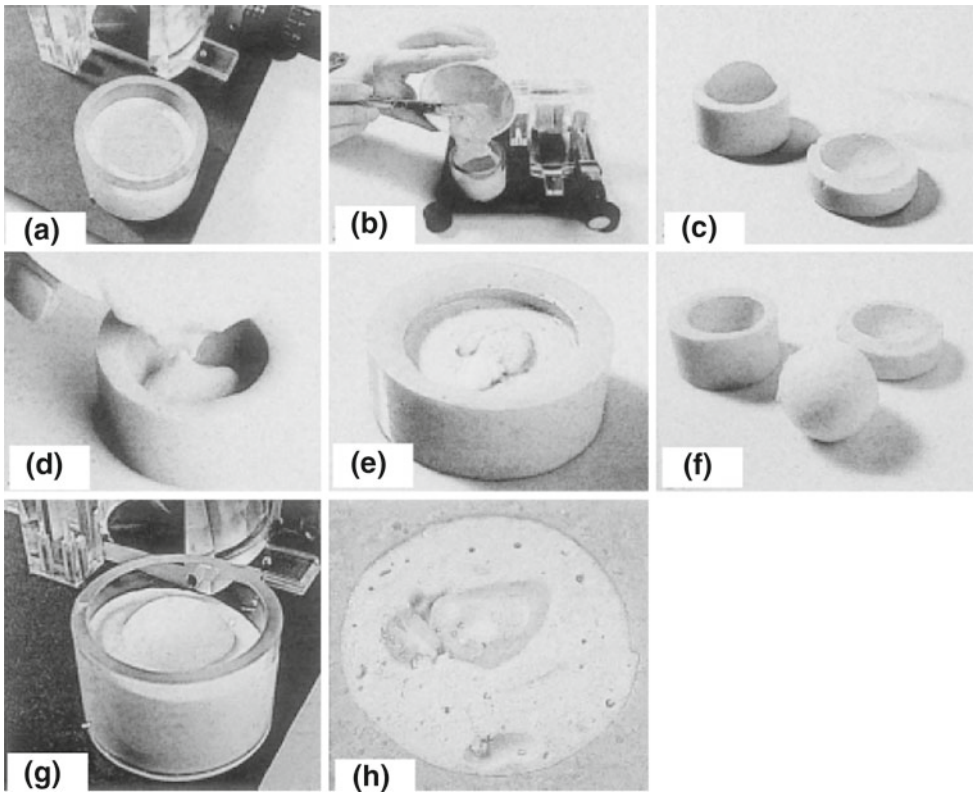

Fig. 12 Techniques to obtain the isotropic uniform random brain sections [27]

The HistOtech Vario slicer and tissue orientator for isotropic random orientated dissection of small brain specimen by Denmark

Other than the slicing machine discussed above, Bjarkam and Sørensen et al. [27] also developed a slicing machine and technique for fresh or fixed brain tissue from small laboratory animals. This method allows orientation of brain tissue in any given position with optimal thickness between 0.5 and 20 mm. The technique includes the application of a specific embedding medium HistOmer, a tissue orientator and a Vario Slicer. The HistOmer is actually an alginate cold polymer that can polymerize into a "rubber-like solid' and has buoyancy that can hold the brain specimen in place after positioning. The tissue orientator actually includes making two hemispheric embedding chambers to embed the specimen and making the embedding block in a ball shape for isotropic orientation during sectioning as shown in Fig. 12. The Vario Slicer is composed of a cylindrical embedding chamber for oriented HistOmer embedding of the tissue and a cutting stage where the embedded block can be placed, orientated and sectioned uniformly with the guidance of parallel guiding plates as illustrated in Fig. 13.

The method is quite similar with previous method by Sørensen et al. for sectioning large irregular tissue. Both of the studies used the special embedding media HistOmer. The method designed for isotropic orientation of brain tissue from small laboratory animals was able to slice the tissue serially with identical section thickness and is capable of isotropic orientation. However, it may not be applicable for sectioning and orientating the human prostate. There is a lack of discussion regarding the accuracy of the method in the given studies. It requires identifiable landmarks for orientation which are not available for the prostate as previously discussed. Although the method may obtain a desired uniform section thickness, sectioning at similar section levels as in medical imaging remains problematic. Further improvement for sectioning at the same section level is needed. Unfortunately, there are no further studies addressing this issue.

The commercially available rotary slicer with agar-embedding

In the study by Dahele et al. [49] to correlate PET-CT images with histopathology of lung cancer and the study by Zarow et al. [50] to correlate brain MRI to pathology, the specimens were both embedded in agar and sliced by commercial rotary slicers. In the study done by Zarow et al, the brain was embedded in the agar and the fiducial rods were fitted into the agar to make holes for registration as shown in Fig. 14. It was claimed by the authors that the agar embedding methods can minimize the distortion during the handling and slicing of the specimens, allow tinting to enhance the contrast

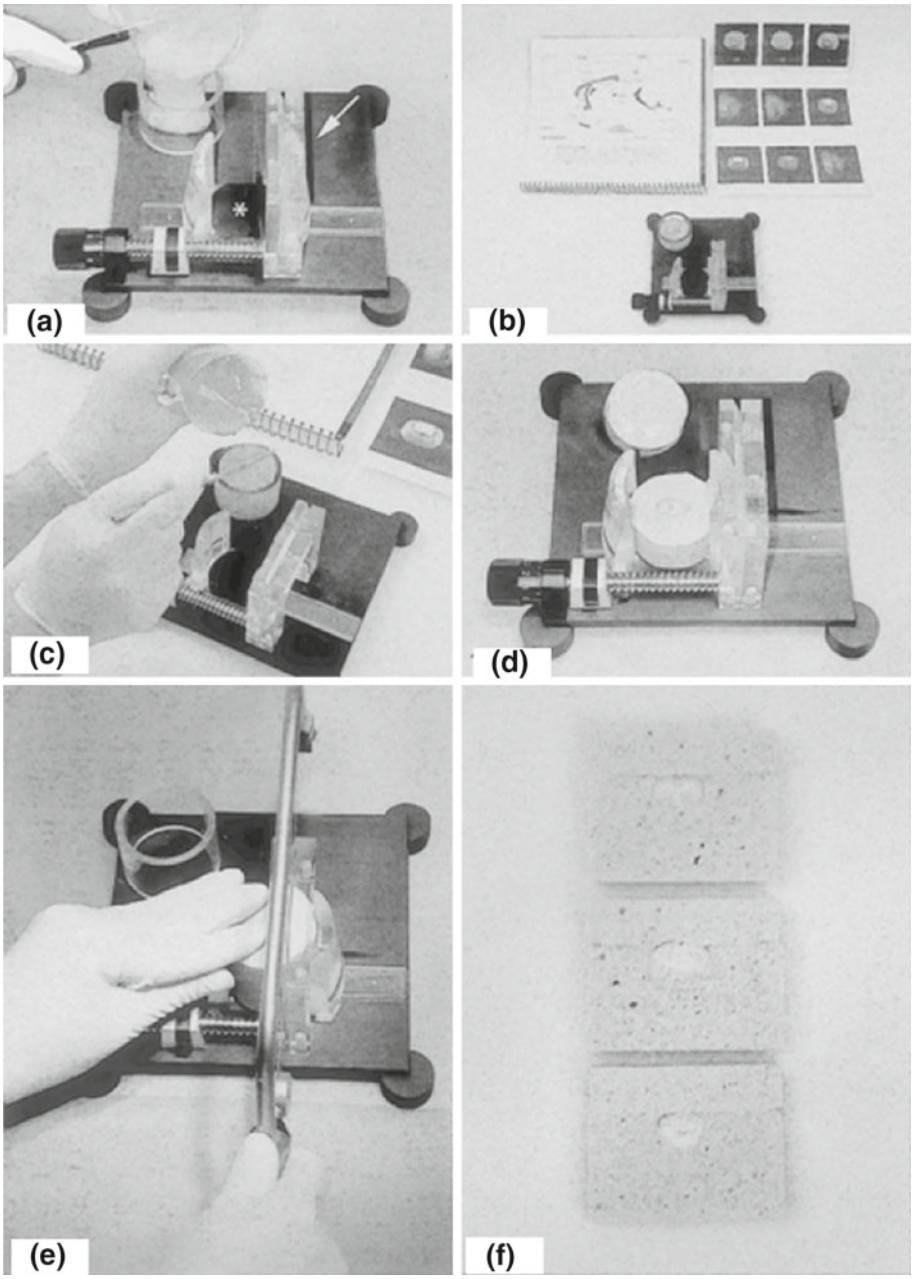

Fig. 13 The use of HistOtech Vario Slicer and the embedding medium (HistOmer) to obtain the oriented rat brain sections [27]

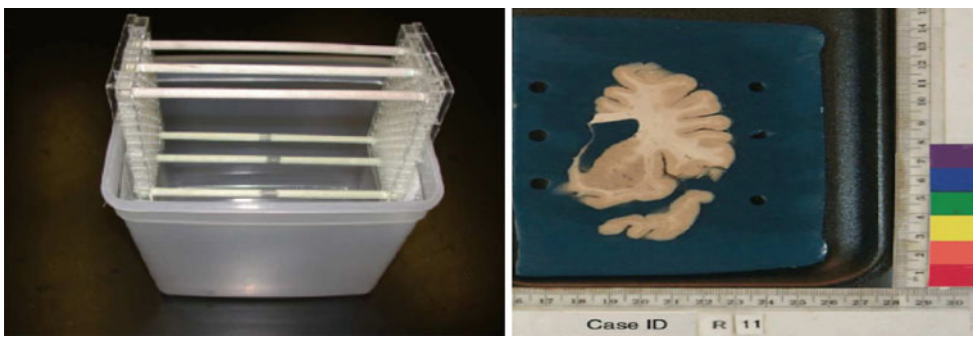

Fig. 14 An example of brain embedded in the agar with fiducial rods fitted into the agar that makes holes for registrations [50]

Table 1 A summary and comparison of different methods for orientating the specimen for correlation

Methods	Studies	Advantage	Disadvantage
Gross orientation by identifiable anatomic landmarks (seminal vesicles and vasa deferentia contour of prostate)	Jhavar et al. [20] Jackson et al. [21] Other various studies using traditional prostate dissection [4–12]	Easy to perform in routine practice	Limited accuracy: up to 5–15° of difference in section angle
Matching measurements of the spatial distance or angle between identifiable anatomical landmarks with measurements taken during in vivo imaging	Ma et al. [18] Sørensen et al. [26] Bjarkam et al. [27]	Allow 3D orientation; Achieve accuracy of 79.6% matching area under ROC curve	Require special measurement during the in vivo image scanning; Technically demanding and error prone to perform
External landmark as orientation reference			
Needle	Breen et al. [13]	Facilitate visible landmarks for orientation and registration	Invasive and technically demanding to perform;
Air-filled Teflon rods	Humm et al. [51]	More convenient to inject the fiducial marker;	Error prone due to inadvertent movement; Cannot shrink and distort with adjacent tissue
Acrylic fiducial markers	Rouvière et al. [22]	Stable and no inflammatory reaction; Act as internal markers that can shrink and distort with adjacent tissue	The toxicity of the markers is not clear for applying in human study, this precludes its application in in vivo human study
Combination of using acrylic fiducial markers to predefine imaging plane and orientate specimen under the guidance of imaging modality	Rouvière et al. [22] Kimm et al. [23]	Achieve a mean maximum angle error of ≤3 degree in 88–95% of the cases	Costly, invasive, and technically demanding to perform; Need to define the imaging plane with fiducial markers; No suitable fiducial markers for in vivo human study yet

between tissue and matrix, and facilitate image registration by incorporating external fiducial markers in the agar. The commercial rotary slicers were used to produce consistent regular space slices. In the study by Dahele et al. [49], the combination of electric rotary cutter and agar embedding was compared to the freehand knife and customized sectioning box for sectioning to find a best way to cut the lung tissue for correlation. It was suggested that the combination of agar embedding and electric rotary provided the most consistent sections. In these two studies, the agar may help to minimize the geometrical distortion during the handling and slicing the fatty specimen (brain and lung) and may facilitate the input of external reference "landmarks" to align the serial images. However, it is not applicable for the prostate as the prostate presents as a semi-solid consistency gland that requires certain force for sectioning while the agar is soft and fragile and may not provide good support to the specimen. In addition, the agar needs to be heated to 50–60°C which may cause damage to the fresh tissue. Although the commercial rotary slicer can possibly produce consistent regular section for correlation, the possibility of orientating the prostate at a desired angle remains unclear.

Discussion

Among all the studies of pathology-medical imaging correlation, the improvements to the tissue dissection methods can be summarized from three different aspects: how the specimen may be orientated before sectioning; how the specimen may be secured from movement during the sectioning; and how the specimen may be sectioned to obtain the tissue sections of equal thickness and desired section level. The ways that the specimens are orientated includes the gross identification of the anatomical landmarks of the prostate [4–12,20,21], the matching of the measurements of the spatial distance or angle between identifiable anatomical landmarks [18,26], the introduction of external landmarks such as needles or air-filled Teflon rods to guide medical image scanning and specimen orientation[13,51], as well as the

Table 2 A summary and comparison of different methods for securing the position of the specimen for during sectioning

Methods	Studies	Advantage	Disadvantage
Free hand holding	Various studies using traditional prostate dissection [4–12]	Easy to perform	The prostate, especially fresh prostate may be deformed and the orientation is distorted; Technically challenging to obtain even sections; The section surface may be damaged
Secured by embedding media			
Agar	Zarow et al. [50] Dahele et al. [49]	Secure specimen especially the fatty tissue in position during sectioning;	No quantitative studies comparing embedding media (in terms of composition, toxicity, PH, temperature, oxygenation, and lubricating properties) are available yet;
Wax	Rouviere et al. [22] Kimm et al. 2009 [23] Breen et al. [13]	Allow tinting the embedding medium to provide contrast between tissue and matrix;	A suitable embedding medium for embedding the fresh prostate is not available
Alginate	Sørensen et al. [26] Bjarkam et al. [27]	Facilitate the introduction of fiducial markers for registration	
Cradle			
Tong-shape cradle	Jhavar et al. [20]	The position of the prostate is well fixed from three dimensions during sectioning;	The prostate may be deformed due to the gripping force;
		Can obtain identical section thickness for middle sections	Section quality may vary between different users; Correlation of section level is not achieved; Not adjustable for different size of prostate; The thickness of end section is not secured
Rows of brass rods	Jackson et al. [21]	Less gripping force cause less deformation; Can obtain identical section thickness for end sections; Can cope with different size of prostate; May obtain identical section level	The prostate is secured only in two dimensions, rotation may still occur during sectioning
Positioned in the slicing box with force acting on one sliceable wall	Ma et al. [18]	Easy to perform	The force acting on the specimen during sectioning may deform the prostate and lead to different thickness of section; Technically demanding to hold the prostate in position, the prostate may rotate during the sectioning

application of fiducial landmarks such as acrylic paint markers to define the medical imaging plane and orientate under the guidance of the medical scanner [22,23] as listed and compared in Table 1. The ways that the specimen are held in position during sectioning can be categorized into four types: (i) to hold it with free hand [4–12], (ii) to hold it in a box with stress acting on one side of the wall [18], (iii) to use the embedding medium (wax, agar, alginate polymer) [13,22,23,26,27,49,50], and finally, (iv) to use a holding cradle (cradle resembles tongs, cradle with rods of brass and side

Table 3 A summary and comparison of different methods for sectioning the specimen with correlated section thickness, section angle

Methods	Studies	Advantage	Disadvantage
Cutting slits of equal space to guide the sectioning	Ma et al. [18] Jhavar et al. [20] Jackson et al. [21]	The direction of cutting blade is guided, the error due to manual handing is reduced	Gap between slits cannot be adjusted
Parallel multi-blade with equal thickness for one time sectioning	Jhavar et al. [20] Sørensen et al. [26]	Quicker and easier than sectioning with single blade	Need to adjust the size of the multi-blades for different interval dissection
Linearly displacement device	Breen et al. [13] Bjarkam et al. [27]	Can obtain desired section thickness more easily	The specimen needs to be embedded before sectioning
Commercial rotary meat slicer	Dahele et al. [49] Zarow et al. [50]	Easy to perform; May obtain equal thickness section	Not applicable for accurate orientation; Section angle and section level are not correlated

walls) to grip the specimen while sectioning [20,21] as listed and compared in Table 2. The ways to section the specimen with equal thickness and section level involves using a series of cutting slits of equal interval to guide the cutting [18], parallel multi-blades of equal intervals [20,26], and linear displacement devices [13,27] as illustrated and compared in Table 3.

Conclusions

This paper summarizes the various techniques used to correlate histopathological data and medical images. The pros and cons of different methods have been presented and discussed. To summarize, the lack of standardization of the sampling and sectioning methods induces unknown variables between different studies on histopathological medical image correlation for medical image validation or studies, etc. This precludes the reliable comparison between different studies and an exact evaluation of a specific imaging modality. Unfortunately, a unifying agreement on an optimum specimen sectioning technique which allows correlation of either section thickness, section angle or section level has not been obtained. To satisfy the increasing need to quantify and qualify the ability and accuracy of the emerging imaging modalities for better disease management, as well as to promote the comparability between different studies, a standardized optimum protocol to dissect the specimen is demanded and is achievable in the near future.

References

1. Schmid HP, McNeal JE (1992) An abbreviated standard procedure for accurate tumor volume estimation in prostate cancer. Am J Surg Pathol 16(2):184–191
2. Schned AR, Wheeler KJ, Hodorowski CA, Heaney JA, Ernstoff MS, Amdur RJ, Harris RD (1996) Tissue-shrinkage correction factor in the calculation of prostate cancer volume. Am J Surg Pathol 20(12):1501–1506
3. Lemaitre L, Puech P, Poncelet E, Bouyé S, Leroy X, Biserte J, Villers A (2009) Dynamic contrast-enhanced MRI of anterior prostate cancer: morphometric assessment and correlation with radical prostatectomy findings. Eur Radiol 19(2):470–480
4. Dähnert WF, Hamper UM, Eggleston JC, Walsh PC, Sanders RC (1986) Prostatic evaluation by transrectal sonography with histopathologic correlation: the echopenic appearance of early carcinoma. Radiology 158(1):97–102
5. Jager GJ, Ruijter ET, van de Kaa CA, de la Rosette JJ, Oosterhof GO, Thornbury JR, Barentsz JO (1996) Local staging of prostate cancer with endorectal MR imaging: correlation with histopathology. Am J Roentgenol 166(4):845–852
6. Pucar D, Shukla-Dave A, Hricak H, Moskowitz CS, Kuroiwa K, Olgac S, Ebora LE, Scardino PT, Koutcher JA, Zakian KL (2005) Prostate cancer: correlation of MR imaging and MR spectroscopy with pathologic findings after radiation therapy-initial experience. Radiology 236(2):545–553
7. Salomon G, Köllerman J, Thederan I, Chun FK, Budäus L, Schlomm T, Isbarn H, Heinzer H, Huland H, Graefen M (2008) Evaluation of prostate cancer detection with ultrasound real-time elastography: a comparison with step section pathological analysis after radical prostatectomy. Eur Urol 54(6):1354–1362
8. Villers A, Puech P, Mouton D, Leroy X, Ballereau C, Lemaitre L (2006) Dynamic contrast enhanced, pelvic phased array magnetic resonance imaging of localized prostate cancer for predicting tumor volume: correlation with radical prostatectomy findings. J Urol 176(6):2432–2437
9. Xu J, Humphrey PA, Kibel AS, Snyder AZ, Narra VR, Ackerman JJ, Song SK (2009) Magnetic resonance diffusion characteristics of histologically defined prostate cancer in humans. Magn Reson Med 61(4):842–850
10. Haider MA, van der Kwast TH, Tanguay J, Evans AJ, Hashmi AT, Lockwood G, Trachtenberg J (2007) Combined T2-weighted and diffusion-weighted MRI for localization of prostate cancer. Am J Roentgenol 189(2):323–328
11. Scheidler J, Hricak H, Vigneron DB, Yu KK, Sokolov DL, Huang LR, Zaloudek CJ, Nelson SJ, Carroll PR, Kurhanewicz J (1999) Prostate cancer: localization with three-dimensional proton MR spectroscopic imaging-clinicopathologic study. Radiology 213(2):473–480

12. Braeckman J, Autier P, Garbar C, Marichal MP, Soviany C, Nir R, Nir D, Michielsen D, Bleiberg H, Egevad L, Emberton M (2008) Computer-aided ultrasonography (histoscanning): a novel technology for locating and characterizing prostate cancer. BJU Int 101(3):293–298
13. Breen MS, Lazebnik RS, Wilson DL (2005) Three-dimensional registration of magnetic resonance image data to histological sections with model-based evaluation. Ann Biomed Eng 33(8):1100–1112
14. Hyunjin P, Kwee S, Thibault GP, Stack R, Sesterhenn IA, Potter K, Meyer CR (2007) Registration methods for histological slides and ex vivo MRI of prostate. In: Nuclear science symposium conference record. NSS '07. IEEE 2007
15. Park H, Piert MR, Khan A, Shah R, Hussain H, Siddiqui J, Chenevert TL, Meyer CR (2008) Registration methodology for histological sections and in vivo imaging of human prostate. Acad Radiol 15(8):1027–1039
16. Ou Y, Shen D, Feldman M, Tomaszewski J, Davatzikos C (2009) Non-rigid registration between histological and mr images of the prostate: a joint segmentation and registration framework. In: IEEE conference on computer vision and pattern recognition, CVPR 2009
17. Zhan Y, Ou Y, Feldman M, Tomaszeweski J, Davatzikos C, Shen D (2007) Registering histologic and MR images of prostate for image-based cancer detection. Acad Radiol 14(11): 1367–1381
18. Ma HY, Phee SJ, Thng CH, Tan PH, Yuen J, Ng WS (2004) A novel device for sectioning prostate in correlation to MRI. In: Second IASTED international conference on BIOMACHANICS, Honolulu Hawaii, USA
19. Jhavar SG, Reinsberg S, Jackson A, Cummings C, Eeles R, Falconer A, Leach M (2005) Apparatus and methods for tissue preparation. US patent US 2005/0095666 A1
20. Jhavar SG, Fisher C, Jackson A, Reinsberg SA, Dennis N, Falconer A, Dearnaley D, Edwards SE, Edwards SM, Leach MO, Cummings C, Christmas T, Thompson A, Woodhouse C, Sandhu S, Cooper CS, Eeles RA (2005) Processing of radical prostatectomy specimens for correlation of data from histopathological, molecular biological, and radiological studies: a new whole organ technique. J Clin Pathol 58(5):504–508
21. Jackson ASN, Reinsberg SA, Sohaib SA, Charles-Edwards EM, Jhavar S, Christmas TJ, Thompson AC, Bailey MJ, Corbishley CM, Fisher C, Leach MO, Dearnaley DP (2009) Dynamic contrast-enhanced MRI for prostate cancer localization. Br J Radiol 82(974):148–156
22. Rouvière O, Reynolds C, Hulshizer T, Rossman P, Le Y, Felmlee JP, Ehman RL (2006) MR histological correlation: a method for cutting specimens along the imaging plane in animal or ex vivo experiments. J Magn Reson Imaging 23(1):60–69
23. Kimm SY, Lee JH, Nishimura DG, Tarin TV, Hu BS, Jensen K, Brooks JD (2009) MR-histology correlation in ex-vivo human prostate specimens. In: ISMRM 17th Scientific meeting & exhibition, Honolulu Hawaii, USA
24. Breen MS, Lazebnik RS, Fitzmaurice M, Nour SG, Lewin JS, Wilson DL (2004) Radiofrequency thermal ablation: correlation of hyperacute mr lesion images with tissue response. J Magn Reson Imaging 20(3):475–486
25. Breen MS, Lancaster TL, Lazebnik RS, Nour SG, Lewin JS, Wilson DL (2003) Three-dimensional method for comparing in vivo interventional MR images of thermally ablated tissue with tissue response. J Magn Reson Imaging 18(1):90–102
26. Sørensen JC, Bjarkam CR, Danielsen EH, Simonsen CZ, Geneser FA (2000) Oriented sectioning of irregular tissue blocks in relation to computerized scanning modalities: results from the domestic pig brain. J Neurosci Methods 104(1):93–98
27. Bjarkam CR, Pedersen M, Sørensen JC (2001) New strategies for embedding, orientation and sectioning of small brain specimens enable direct correlation to MR-images, brain atlases, or use of unbiased stereology. J Neurosci Methods 108(2):153–159
28. Rouvière O, Reynolds C, Le Y, Lai J, Roberts LR, Felmlee JP, Ehman RL (2006) Fiducial markers for MR histological correlation in ex vivo or short-term in vivo animal experiments: a screening study. J Magn Reson Imaging 23(1):50–59
29. Sung M-T, Davidson DD, Montironi R, Lopez-Beltran A, Cheng L (2007) Radical prostatectomy specimen processing: a critical appraisal of sampling methods. Curr Diagn Pathol 13(6):490–498
30. Srigley John R. (2006) Key issues in handling and reporting radical prostatectomy specimens. Arch Pathol Lab Med 130(3):303–317
31. Bostwick DG, Montironi R (1997) Evaluating radical prostatectomy specimens: therapeutic and prognostic importance. Virchows Archiv 430(1):1–16
32. Sakr WA, Grignon DJ (1999) Prostate. Practice parameters, pathologic staging, and handling radical prostatectomy specimens. Urol Clin North Am 26(3):453–463
33. Montironi R, Mazzucchelli R, Kwast T (2003) Morphological assessment of radical prostatectomy specimens. A protocol with clinical relevance. Virchows Archiv 442(3):211–217
34. True LD (1994) Surgical pathology examination of the prostate gland: Practice Survey by American Society of Clinical Pathologists. Am J Clin Pathol 102(5):572–579
35. Kirkham APS, Emberton M, Allen C (2006) How good is MRI at detecting and characterising cancer within the prostate. Eur Urol 50(6):1163–1175
36. Silletti JP, Gordon GJ, Bueno R, Jaklitsch M, Loughlin KR (2007) Prostate biopsy: past, present, and future. Urology 69(3):413–416
37. Pathak AP, Schmainda KM, Ward BD, Linderman JR, Rebro KJ, Greene AS (2001) MR-derived cerebral blood volume maps: issues regarding histological validation and assessment of tumor angiogenesis. Magn Reson Med 46(4):735–747
38. Jacobs MA, Windham JP, Soltanian-Zadeh H, Peck DJ, Knight RA (1999) Registration and warping of magnetic resonance images to histological sections. Med Phys 26(8):1568–1578
39. Kiessling F, Krix M, Heilmann M, Vosseler S, Lichy M, Fink C, Farhan N, Kleinschmidt K, Schad L, Fusenig NE, Delorme S (2003) Dynamic parameters of tumor vascularization in nude mice revealed by magnetic resonance imaging and contrast-enhanced intermittent power doppler sonography. Invest Radiol 38(8):516–524
40. Kiessling F, Le-Huu M, Kunert T, Thorn M, Vosseler S, Schmidt K, Hoffend J, Meinzer H-P, Fusenig NE, Semmler W (2005) Improved correlation of histological data with dce MRI parameter maps by 3D reconstruction, reslicing and parameterization of the histological images. Eur Radiol 15(6):1079–1086
41. Ourselin S, Roche A, Subsol G, Pennec X, Ayache N (2001) Reconstructing a 3D structure from serial histological sections. Image Vis Comput 19(1–2):25–31
42. Simonetti AW, Elezi VA, Farion R, Malandain G, Segebarth C, Rémy C, Barbier EL (2006) A low temperature embedding and section registration strategy for 3D image reconstruction of the rat brain from autoradiographic sections. J Neurosci Methods 158(2):242–250
43. Yagi Y, Sohani AR, Mino-Kenudson M, Gilbertson JR. (2009) 3D microscopic anatomy using whole slide imaging (WSI). In: 2009 Annual Meeting of United States and Canadian Academy of Pathology, Boston, Massachusetts
44. Westra WH, Hruban RH, Phelps TH, Isacson C (2003) Prostate. In: Surgical pathology dissection: an illustrated guide, 2nd edn. Springer, New York, pp 176–179

45. Halpern EJ, McCue PA, Aksnes AK, Hagen EK, Ferdinand Frauscher F, Gomella LG (2002) Contrast-enhanced US of the prostate with sonazoid: comparison with whole-mount prostatectomy specimens in 12 patients. Radiology 222(2):361–366
46. Hirose M, Bharatha A, Hata N, Zou KH, Warfield SK, Cormack RA, D'Amico A, Kikinis R, Jolesz FA, Tempany CMC (2002) Quantitative MR imaging assessment of prostate gland deformation before and during MR imaging-guided brachytherapy. Acad Radiol 9(8):906–912
47. Ten Haken RK, Forman JD, Heimburger DK, Gerhardsson A, McShan DL, Perez-Tamayo C, Schoeppel SL, Lichter AS (1991) Treatment planning issues related to prostate movement in response to differential filling of the rectum and bladder. Int J Radiat Oncol Biol Phys 20(6):1317–1324
48. Van As N, Charles-Edwards E, Jackson A, Jhavar S, Reinsberg S, Desouza N, Dearnaley D, Bailey M, Thompson A, Christmas T, Fisher C, Corbishley C, Sohaib C (2008) Correlation of diffusion-weighted MRI with whole mount radical prostatectomy specimens. Br J Radiol 81(966):456–462
49. Dahele M, Hwang D, Peressotti C, Sun L, Kusano M, Okhai S, Darling G, Yaffe M, Caldwell C, Mah K, Hornby J, Ehrlich L, Raphael S, Tsao M, Behzadi A, Weigensberg C, Ung YC (2008) Developing a methodology for 3-D correlation of PET-CT images and whole-mount histopathology in non-small cell lung cancer. Curr Oncol 15(5):62–69
50. Zarow C, Kim TS, Sigh M, Chui HC (2004) A standardized method for brain-cutting suitable for both stereology and MRI-brain co-registration. J Neurosci Methods 139(2):209–215
51. Humm JL, Ballon D, Hu YC, Ruan S, Chui C, Tulipano PK, Erdi A, Koutcher J, Zakian K, Urano M, Zanzonico P, Mattis C, Dyke J, Chen Y, Harrington P, O'Donoghue JA, Ling CC (2003) A stereotactic method for the three-dimensional registration of multimodality biologic images in animals: NMR, PET, histology, and autoradiography. Med Phys 30(9):2303–2314