A method for accurate spatial registration of PET images and histopathology slices

Positron emission tomography (PET) is an imaging technique used for the assessment of tissue of interest via administration of radiopharmaceutical commonly known as tracer. A newly developed tracer for PET imaging should be validated by comparison with the gold standard of histopathology imaging before it can add value for clinical purposes, for example, diagnosis, prognosis and response to treatment. The tracer validation studies may become more meaningful if the quantitative comparison between the PET and histopathology images comes from spatially corresponding regions.

An image processing technique called 'image registration' is used to establish spatial alignment between two image datasets. An image registration example between two datasets is shown in Figure 1. The registration between tomographic and three dimensional (3D) histopathology data have been previously shown to obtain satisfactory results [1,2]. However, the process involved in obtaining 3D histopathology data is expensive and not always feasible in routine pathology settings.

The registration between PET and histopathology slices become challenging when the sectioned specimen is non-parallel, non-contiguously cut and non-mega-block sized (i.e. standard sized) because systematic sectioning of a specimen provides thickness estimates of the sectioned slices, contiguous sectioning minimises the errors in reconstructing histology volume and mega-block sized slices provides tissue boundaries for matching with blockface/tomographic data. We present a registration methodology for an accurate spatial alignment between PET and histopathology data obtained in routine pathology settings such that the sectioned slices may be non-parallel, non-contiguously cut and of standard block sized.

Data from males with histo-pathologically proven advanced squamous cell carcinoma of the head and neck (SCCHN) cancer was used. Relevant patient permissions and regulatory approvals were obtained. Subjects underwent 64Cu-copper-II-diacetyl-bis (N4-methylthiosemicarbazone) 64Cu-ATSM PET-CT (computed tomography) scan a week before the surgery. Pimonidazole was administered a day before the laryngectomy. The spines of the black sea urchins were used as the fiducial markers which were inserted into the fresh specimen thereafter fixed in formalin and scanned CT *ex-vivo*. Specimen was sliced and blockface images of the tissue blocks were obtained. From these thick tissue blocks, a subsection of tissue from the tumour region was extracted for the preparation of histopathology slides and digitised using a light microscope (Figure 2).

The total registration errors between

Figure 1: An example of rigid registration where Image2 is translated and rotated to spatially align with Image1.

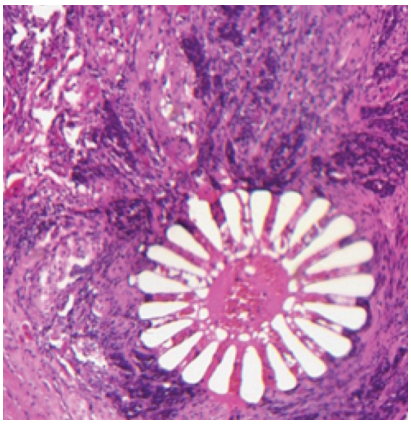
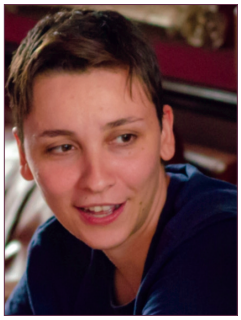
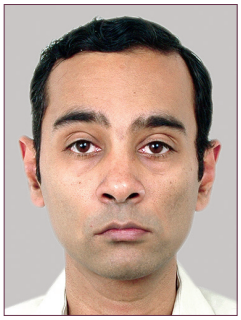


FIGURE 2: A 5-µm thin pimonidazole stained histopathology slice with spine of black sea urchin that was cut orthogonally and scanned under a light microscope at a resolution of 1 µm/pixel.

Authors


Tanuj Puri[1,2]


Anastasia Chalkidou[1]


Arunabha Roy[1]


Rhonda Henley-Smith[3]


Paul R Barber[4,5]

[1]Kings College London, Imaging Sciences and Biomedical Engineering, London, UK; [2]Current address: Department of Oncology, CRUK and MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford, UK, [3]Kings College London, Pathology department, Guy's Hospital, London, UK; [4]Department of Oncology, Cancer Research UK and Medical Research Council Oxford Institute for Radiation Oncology, University of Oxford, Oxford, UK; [5]Kings College London, Institute for Mathematical and Molecular Biomedicine, London, UK; [6]Department of Head & Neck Surgery, Guy's & St. Thomas' Hospital NHS Trust, London, UK.

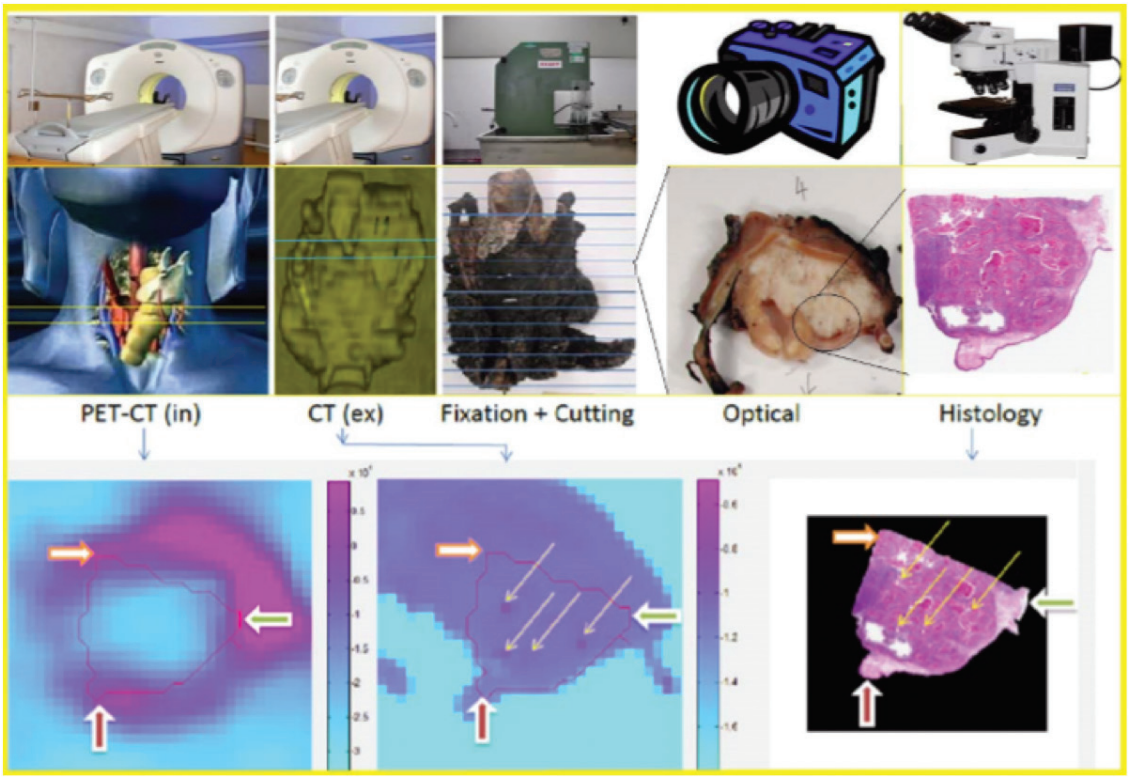


Figure 3: The top row shows (from left to right) a PET scanner, CT scanner, band-saw used to slice larynx, optical camera and a light microscope. The middle row shows images of PET-CT *in-vivo*, CT *ex-vivo*, images of *ex-vivo* specimen fixed and sliced images taken with a camera and histology sample digitised using a light microscope. The bottom row shows registered images of PET, CT *ex-vivo* and histology. Regions in PET and CT *ex-vivo* that correspond to histology are marked with red outline. Yellow markers show the sea urchin spine markers on CT *ex-vivo* and histology images.

PET and histopathology were reported as square root of the sum of the squares of the errors from the individual steps, namely, PET to CT *in-vivo*, CT *in-vivo* to CT *ex-vivo* and histopathology to CT *ex-vivo*. The registration results reported in Figure 3 were prepared using PMOD software (PMOD Technologies Ltd., Zurich, Switzerland), Matlab (The MathWorks, Inc, Natick, USA) and ImageJ open source software. A detailed methodology is presented in Puri et al [3]. The work was previously presented at the National Cancer Research Centre conference [4] and a Workshop on Imaging in Stratified Cancer Treatment at the Newcastle University [5].

An example of PET and histology registered to CT *ex-vivo* are shown in Figure 3. The total registration error between PET and histology slices was approximately 3±0.7mm assuming 1mm alignment accuracy between PET and CT. The registration error between CT *in-vivo* and CT *ex-vivo* was 2.66±0.66mm where the registration was performed using anatomical landmarks. The registration error between CT *in-vivo* and CT *ex-vivo* was 1.41±0.05mm where the same step was repeated using segmented larynx in three datasets only. The registration error between histology and CT *ex-vivo* was 0.86±0.41mm using fiducial markers.

When the specimen is systematically cut in parallel consecutive slices, the thicknesses are known and the identification of the z-axis level between the 2D histology and the 3D image can be performed by counting [6]. However, not all pathology departments have equipment required to perform parallel sectioning and would require deviation from the local pathology laboratory protocol. The proposed method affected the routine pathology workflow with a simple extra step, i.e. the insertion of the fiducial markers, which was done by the specimen handling staff. The optical images and recorded anatomical information from the pathology records were used to identify the


Richard Oakley[6]


Ricard Simo[6]


Jean-Pierre Jeannon[6]


Mark McGurk[6]


Paul Marsden[1]

Correspondence to: Dr Tanuj Puri, Department of Oncology, CRUK and MRC Oxford Institute for Radiation Oncology, University of Oxford, Oxford, UK.
E: tanujpuri82@gmail.com

Acknowledgements: We would like to acknowledge King's College London and UCL Comprehensive Cancer Imaging Centre, funded by CRUK and EPSRC in association with MRC and DoH (England). The authors thank the staff at the KCL Oral Pathology laboratory and King's Health Partner's Biobank at Guys' Hospital and the PET Imaging Centre at St. Thomas' Hospital for their excellent technical support. This research was supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the author(s) and not necessarily those of KCL, the NHS, the NIHR or the Department of Health

approximate level of each tissue blocks on the *ex-vivo* CT before the inter marker distances were used to choose the final CT *ex-vivo* slice for registration.

The blockface images are mainly used for shrinkage correction [7], our choice of rigid (only) registration obviated the exclusion of histology-to-blockface step. Excluding the use of blockface images avoided the errors from three different steps namely, (1) reconstructing blockface volume, (2) CT *ex-vivo* to blockface registration and (3) histology-to-blockface registration. It was anticipated that the combined registration error from these three steps may be similar to the one obtained from not correcting for shrinkage.

The use of PET-CT scanner was a major advantage. This is because an intermediate high resolution CT *ex-vivo* of the larynx specimen served as the reference dataset that corresponded well with the CT *in-vivo* data (from PET-CT scanner) and also with the histopathology slice that included boundaries, edges and fiducial markers.

The *in-vivo* and *ex-vivo* CT alignment was performed using point-based registration and also with bone segmentation based registration which obtained lower registration errors. This was probably due to the large pre-registration errors of 2.54±0.42mm in manually identifying the corresponding pair of landmarks. Consequently, manual point based registration should be avoided in each of the registration step whenever possible. The segmentation-based registration may allow an automated implementation of this methodology with a potential to facilitate radiotherapy planning studies.

This study suffers from a number of limitations. This work is a specific case where tumour is surrounded by the larynx cartilage that may prevent any unexpected deformation of the tumour during surgery, fixation and slicing procedure. Another limitation is that the quantitative comparison between the two

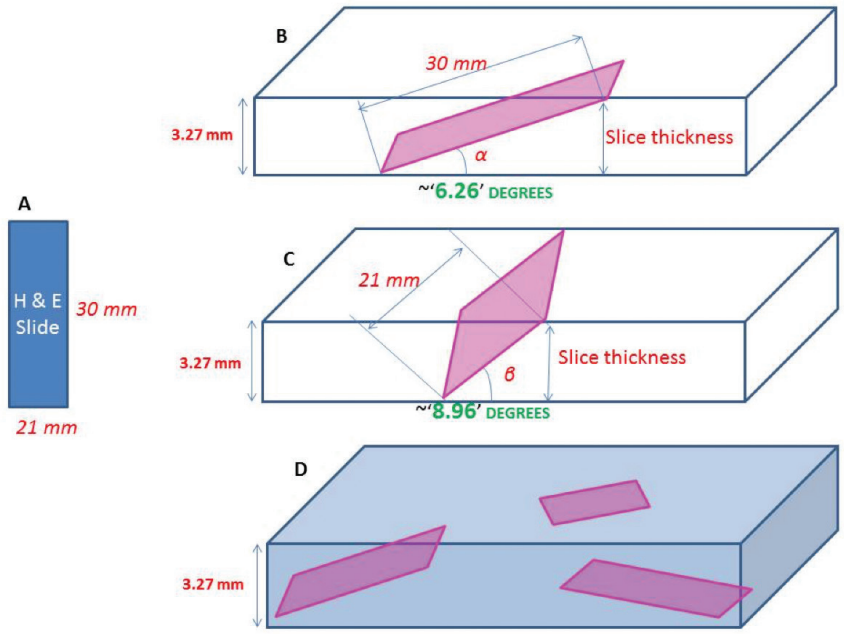


FIGURE 4 (A): Shows the dimensions of a histopathology slide used in routine pathology settings and in our study. (B) Shows the calculation of maximum angle a slide can make within a single PET slice along its long side. (C) Shows the calculation of maximum angle a slide can make within a single PET slice along its short side. (D) The few possible ways in which the histo-pathology may truly correspond to a corresponding spatial location in a PET image. However, since PET image slice anyways represent an average of 3.27mm thick spatial space, correction within this space of single PET slice would be meaningless. The inclination correction of the histopathology slides would be more meaningful if the inclination is greater than 6.26 degrees considering the spatial resolution of the PET scanner is greater than the thickness a PET slice.

datasets may be largely dictated by partial volume errors as the ratio between PET and histopathology slice thickness is 654 (i.e. 3.27mm/5μm). Therefore, obtaining contiguous histopathology slices from each tissue block may be more appropriate for radiotracer validation studies albeit controversial due to the logistics and cost involved. Another limitation was the assumption about inclination between histology and CT *ex-vivo* which was considered less than 8.96 degrees (Figure 4). The Figure 4 shows the difficulty in correcting histopathology for inclination if It belongs to only one CT slice since each slice represent an average over 3.27mm space with a loss of true information within that cuboid like region representing a single PET slice (Figure 4B and 4C). A true inclination correction may be appreciable only when one histopathology slice belongs to two or more PET-CT slices.

Due to the differences in true inter-marker distances on CT *ex-vivo* and histology, it may be more appropriate to first correct the histology for shrinkage using blockface images and then measure the inclination. However, a simple alternative would be to acquire CT images of the tissue blocks to provide an accurate thickness estimates and to reconstruct a 3D volume from sliced tissue blocks.

In conclusion, we have developed and assessed a method for aligning PET and histopathology slices obtained in routine pathology settings such that the slices may be non-parallel, non-contiguously cut and non-mega-block sized in male larynx with advanced SCCHN cancer. The average registration error between PET and histopathology was 3.0(SD:0.7)mm which is better than the 6.00mm full-width half maximum spatial resolution of the PET scanner.

References

1. Ceritoglu C, Wang L, Selemon LD, et al. *Large deformation diffeomorphic metric mapping registration of reconstructed 3D histological section images and in vivo MR images.* Frontiers in human neuroscience 2010;vol. 4, 20.
2. Dauguet J, Delzescaux T, Condé F, et al. *Three-dimensional reconstruction of stained histological slices and 3D non-linear registration with in-vivo MRI for whole baboon brain.* Journal of neuroscience methods 2007; 164:191-204.
3. Puri T, Chalkidou A, Henley-Smith R, et al. *A method for accurate spatial registration of PET images and histopathology slices.* EJNMMI Res 2015;5:64.
4. Puri T, Chalkidou A, Henley-Smith R, et al. *A method for accurate spatial registration of PET images and histopathology slices.* In 9th National Cancer Research Institute (NCRI) Cancer Conference, Liverpool, UK, Nov 2013.
5. Puri T, Chalkidou A, Henley-Smith R, et al. *A method for accurate spatial registration of PET images and histopathology slices.* In Workshop on Imaging in Stratified Cancer Treatment – Methodology, pre-clinical discovery and clinical trials, Newcastle University, Newcastle, UK, Nov 2013.
6. Raleigh JA, Calkins-Adams DP, Rinker LH, et al. *Hypoxia and vascular endothelial growth factor expression in human squamous cell carcinomas using pimonidazole as a hypoxia marker.* CancerRes. 1998;58(17):3765–8.
7. Caldas-Magalhaes J, Kasperts N, Kooij N, et al. *Validation of Imaging with Pathology in Laryngeal Cancer: Accuracy of the Registration Methodology.* International journal of radiation oncology, biology, physics 2012; 82:e289-e98.