Large Histological Serial Sections for Computational Tissue Volume Reconstruction

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Introduction The three-dimensional (3-D) characterization of invasion patterns of squamous cell carcinoma of the uterine cervix is a current clinical question and is matter of investigations with respect to prognosis. Available 3-D microscopic techniques for in-vivo or ex-vivo analyses, as e.g., miniaturized computed tomography (µCT), miniaturized nuclear magnetic resonance imaging (µMRI), or confocal LASER scanning microscopy (CLSM), etc. However, for reasonable specimen sizes of 100mm³ or more, and for some required degree of detail of about 10µm either the applicable penetration range, the usable contrast or the spatial resolution exhibits substantial limitations. The modality we therefore have decided to use is conventional bright-field transmitted-light microscopy as it is basically applied in clinical routine, but have virtually extended it to 3-D using large histological serial sections of several hundreds of slices. This in turn gives demand for high level digital image processing in order to do an appropriate 3-D tissue reconstruction mainly by sequentially accomplishing certain reference-free image registration procedures. With the obtained 3-D microscopic data, a new quality for the morphological assessment of the considered tumour's invasion fronts is achievable [1]. In this present work we demonstrate the potential of our technology for *alternating histological stainings* for spatial co-localisation using dedicated staining combinations without requiring to apply laborious dedicated multiple-staining techniques for large serial sections.

Reconstruction Procedure Resected specimens obtained from patients with cervical cancer (T1b1–T2b) which underwent radical hysterectomy were serially sliced (thickness typically 10µm), histologically stained and digitised (1300×1030 pixels, area 10.45mm × 8.28mm = 0.865cm², pixel size 8.04µm²). Slicing and staining, however, unavoidably induces severe artefacts, mainly different kinds of distortions. These can be algorithmically treated using our dedicated image processing chain consisting of a series of linear and non-linear automatic image registrations as well as an intermediate tumour segmentation step as was described in [1].

Now, for the registration of images of alternately stained consecutive slices a problem arises due to a partial loss of spatial correspondences between two respective images with possibly totally different distributions both in the colour as well as in the position space, unfortunately preventing the direct registration of the images. As solution we newly introduce a *consistent image segmentation* step prior to the above mentioned non-linear registration. We consider this step essential in order to obtain the optimum accuracy for the respective registration transformation. This consistent segmentation's identification of different tissue types does not only simply consider pixel colour differences to build up the segmentation vector for each pixel; statistical properties within the pixel neighbourhood may also be added to the vector, e.g. via sampling along an Archimedian spiral, starting at the respective pixel and analysing the frequency distribution of the one-dimensional Fourier transform. Further, the vector may additionally include such colour features taken from several Gaussian smoothed image instances. We basically apply a fuzzy c-means clustering method and estimate the parameters of an overall distribution (described as linear combination of normal distributions) by means of a variant of the expectation maximisation algorithm [2]. Using the estimated distribution we label every pixel with a class number to obtain the image segmentation, however for a pair of differently stained slices the segmentation results may still differ, e.g. with respect to the determined class number. This basically can occur if one of the specific stainings marks certain structures which remain invisible in the corresponding slice's image. For such case the class labels have to be merged to correspondingly describe the same regions with the same labels in order to achieve the best possible consistent segmentation. Moreover, merging may also be advised if more than a single normal distribution is required to segment a certain histological structure due to staining inhomogeneities.

Once the segmentation was done for an image pair, we take the respective two scalar images to compute the displacement vector field for the non-linear non-parametric curvature-based registration [3]. The latter requires the numerical solution of a coupled system of fourth-order partial differential equations. Finally, with the obtained displacement vector field to register one image onto its predecessor in the series, the original colour image is just transformed according to this transformation in order to eventually yield – after hundreds of registration steps along one serial section – a reconstructed volume data set of the original image modality.

Example A serial section with 84 consecutive slices of a cervical carcinoma was alternately stained with haematoxylin/eosin (H&E) as routine reference stain, the immuno-histochemical stain $p16^{INK4a}$ labelling cervical tumour cells, and additionally with CD3 in order to specifically detect T-lymphocytes. Applying our automated reconstruction procedure (**Fehler! Verweisquelle konnte nicht gefunden werden.**), this example may illustrate the achieved accuracy of the 3-D reconstructed volume data set with different alternating histological stainings. The combined reconstructed tissue was automatically segmented with respect to the tumour ($p16^{INK4a}$) and the T-cells (CD3) and resulting segments were visualised in 3-D. Now, the spatial relationship of the tumour invasion and the inflammatory response can be visually inspected interactively and further assessed quantitatively. Within an overall reconstructed tissue volume of 60.9mm³ the tumour within this volume of interest (VOI) fills 11.6mm³ and T-lymphocytes have been detected for another 1.1mm³. Measures both plausibly and illustratively describing the spatial relationship between tumour and inflammation are currently under development.

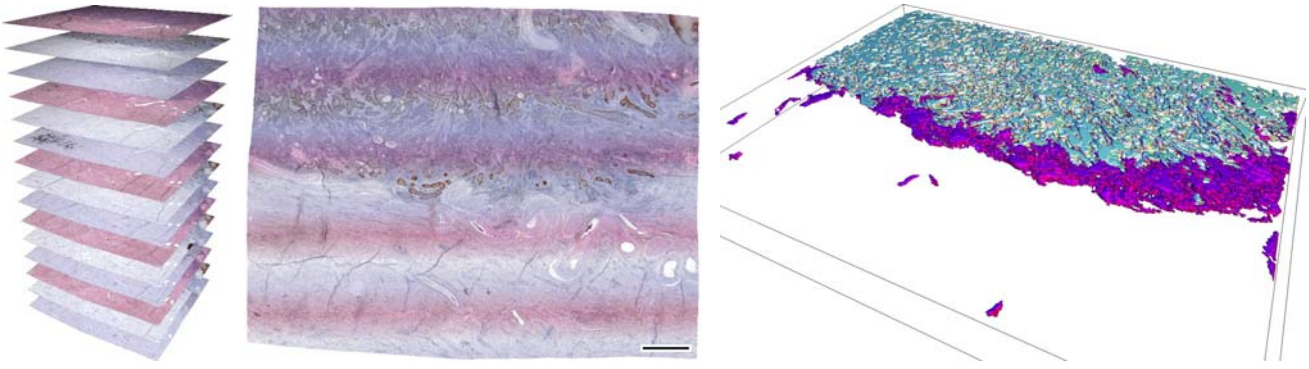

Fig. 0 The registered alternately stained histological serial section (left: five selected triple-sequences H&E–$p16^{INK4a}$–CD3 from top). Within the 3-D reconstructed tissue volume any arbitrary virtual slicing plane can be computed (middle). This image shows a plane with 1 degree tilt by this passing the same 15 consecutive sections (scale bar: 1mm, the irregular outline is due to the applied registration procedures). The upper half mainly consists of tumour invading into the stroma. While the tumour is more or less visible in all three stainings, it is best delimitable at the $p16^{INK4a}$ slices as saturated brown structures. Due to the high accuracy of the obtained reconstruction, tumour structures as well as vessels and many other details smoothly continue in the adjacent slices. The spatial localisation of the inflammatory response between the tumour invasion front and the stroma as can be seen in the middle of the virtual plane is of particular tumour-biological interest. In the 3-D surface rendering (right) both the automatically segmented tumour and those tissue regions exhibiting T-lymphocytes (purple) are simultaneously shown. Distant small segments are due to staining artefacts. The overall height of the 3-D reconstructed tissue volume is 0.84mm.

Discussion The present work exemplifies the potential for serial section based 3-D tissue volume reconstructions for histological analyses. Even though the serial section based procedure may not be considered for clinical routine, it provides a practicable alternative for histology research. Going

beyond our previous work [1] focussing on the 3-D pattern of invasion for cervical tumours, we now could successfully demonstrate an important expandability of our technology for computational tissue volume reconstruction. Our work will continue with the application of this algorithm for larges VOIs and the inclusion of further stainings, e.g. CD34 to analyse the vascularisation with respect to the tumour invasion front.

References

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