

Histological validation of VERDICT cellularity map in a prostatectomy case

Joseph G Jacobs^{1,2}, Edward Johnston³, Alex Freeman⁴, Dominic Patel⁵, Manuel Rodriguez-Justo⁵, David Atkinson³, Shonit Punwani³, Gabriel Brostow², Daniel C Alexander^{1,2}, Eleftheria Panagiotaki^{1,2}

¹Centre for Medical Image Computing, University College London, London, United Kingdom, ²Department of Computer Science, University College London, London, United Kingdom, ³Centre for Medical Imaging, University College London, London, United Kingdom, ⁴Department of Histopathology, University College London Hospitals NHS Foundation Trust (UCLH), University College London, London, United Kingdom, ⁵Department of Research Pathology, UCL Cancer Institute, University College London, London, United Kingdom

SYNOPSIS

This study aims to validate the cellularity map produced by the VERDICT framework for prostate tissue with histology. The VERDICT cellularity map is an indication of the number of cells present in each voxel in a diffusion-weighted MR (DW-MR) image. We attempt to validate this measure by comparing it with a cellularity map produced from a corresponding prostatectomy histological section. We find that the VERDICT cellularity map is able to differentiate between areas of tumour and benign tissue with statistical significance. This result demonstrates the potential of VERDICT as a method for non-invasive quantification of tumours.

PURPOSE

This work uses histology to validate the microstructural map of cellularity produced by the VERDICT (Vascular Extracellular and Restricted Diffusion for Cytometry in Tumours)¹ framework for prostate. Previous diffusion MRI studies in the prostate show that the VERDICT cellularity map differentiates tumour from benign regions better than conventional methods such as ADC maps². Histological information from such an imaging method has great potential for cancer diagnosis and monitoring.

METHODS

We use the optimised VERDICT imaging protocol for prostate³ to image a patient with Gleason Score 7 (3+4, 4+3). Following imaging, the patient underwent radical prostatectomy. From the specimen we slice and digitise whole-mount (WM) hematoxylin and eosin (H&E) stained sections. We then automatically identify the cells in the histological slices to produce a histological cellularity map and compare this against the VERDICT cellularity map.

MRI: After multiparametric MRI, we acquire diffusion-weighted-MR (DW-MR) images with a Philips Achieva 3T MRI using a pulse-gradient spin-echo sequence and a 32 channel cardiac coil with b values of 90-3000s/mm² in 3 orthogonal directions (voxel size=1.3×1.3×5mm, matrix size=176×176, TR=2000ms). Table 1 shows the imaging parameters. We normalised the data with a b=0 image for every echo time (TE) to avoid T2 dependence.

VERDICT model and fitting procedure: We fit the VERDICT model³ to the DW-MRI to estimate parameters f_{EES} (extracellular/extravascular space volume fraction), f_{IC} (intracellular (IC) volume fraction), f_{VASC} (vascular volume fraction), cell radius R , diffusivity D and pseudo-diffusion P . We produce parametric maps with an iterative optimization procedure^{1,2} that accounts for local minima and Rician noise. The cellularity map is an estimate of cell density obtained by dividing the f_{IC} by the cube of the R estimate (cell volume).

Histology: Following prostatectomy, we fix the specimen in 10% buffered formalin for 48 hours. We then slice the prostate at 5mm intervals and cut 5 μ m thick sections for H&E staining and digitization at 20X magnification (pixel size 0.5×0.5 μ m).

MRI and histology correspondence: Two experienced radiologists (EJ, SP) and a histopathologist (AF) visually registered MR images to histologic specimens using anatomically defined areas.

Histological analysis: We define histological cellularity as the number of cells per unit area. Given a region of interest (ROI) on the WM slide (Figure 1), we estimate cellularity in $1.3 \times 1.3 \text{ mm}$ sub-windows by counting the nuclei in each window using a Regression Forest model⁴. We use a dataset of 68 $0.25 \times 0.25 \text{ mm}$ manually annotated prostate biopsy images (verified by AF) to train the model.

Table 1 Diffusion MRI acquisition details for prostate VERDICT analysis

b value s/mm^2	Δ/δ ms	TE ms	$ G $ T/m
3000	24.7/43.8	90	0.0439
2000	13.2/32.3	67	0.0758
1500	24.7/43.4	90	0.0311
500	12.2/31.3	65	0.0415
90	12.2/23.8	50	0.0506

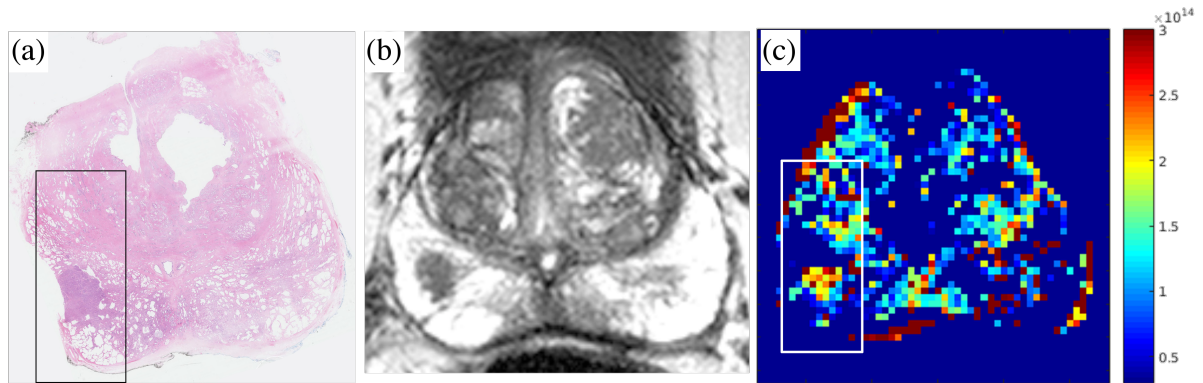


Figure 1 (a) H&E prostatectomy section, (b) T2 MRI and (c) VERDICT cellularity map. The prostatectomy and VERDICT cellularity map figures also show the ROI that was analysed.

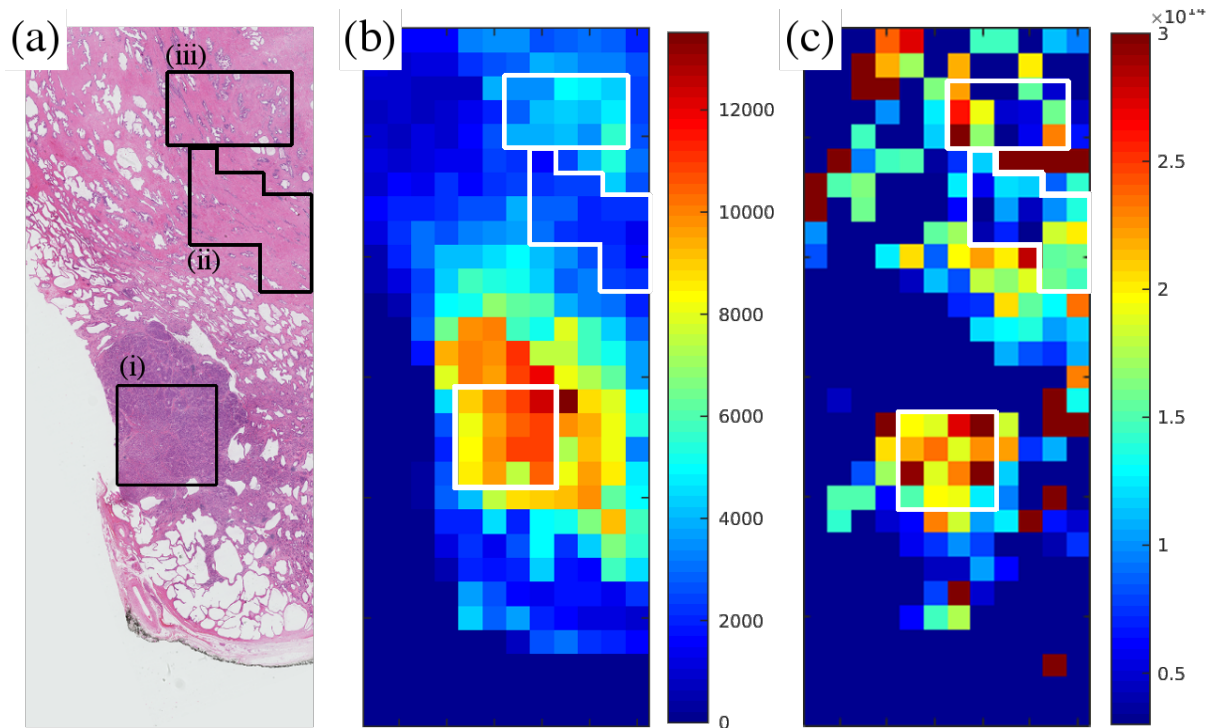


Figure 2 (a) Histology of ROI, (b) histology cellularity map for ROI and (c) VERDICT cellularity map for ROI. Highlighted in the images are regions of (i) tumour, (ii) stroma and (iii) HGPIN & inflammation.

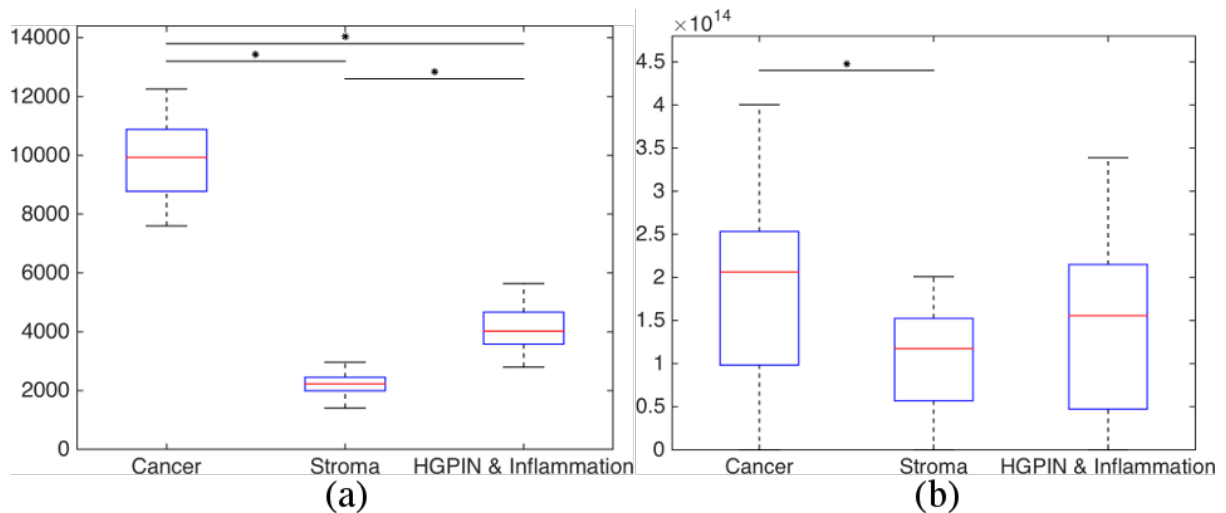


Figure 3 Boxplots showing (a) the histological cellularity and (b) VERDICT cellularity in the three different tissue regions highlighted in Figure 2. The horizontal bar and asterisk across two regions indicate a statistically significant difference between the cellularity values in those two regions ($p < 0.05$, Mann-Whitney U test).

RESULTS

Figure 2 compares the cellularity maps from histology (2b) and VERDICT (2c) for the ROIs (2a). The mean cross-validation nuclei counting error is 20.16 nuclei per mm^2 . The tumour identified in histology corresponds to regions of high cellularity in both cellularity maps while there is lower cellularity in the surrounding benign tissue. Surrounding the tumour there is cystic atrophy. In the region of cystic atrophy beneath the tumour there is low cellularity in both maps. However, in VERDICT the region above the tumour is less cellular than it is in histology.

The boxplots in Figure 3 compare the histology and VERDICT cellularity in the ROIs (Figure 2a). In both, cellularity is highest in tumour, lowest in stroma and elevated in high-grade prostatic intraepithelial neoplasia (HGPIN) and inflammation. In histology, there is a statistically significant difference between cellularity in all regions, while with VERDICT there is only significant difference between cellularity in cancer and stromal tissue ($p < 0.05$, Mann-Whitney U test).

DISCUSSION & CONCLUSION

Results show similar trends in the histology and VERDICT cellularity maps, providing some validation of the VERDICT cellularity estimates. The VERDICT model appears to be able to detect the diffusion signals from proliferated epithelial cells in the tumour and differentiate this from the surrounding cystic atrophy and stromal tissue with statistical significance.

The main limitation of VERDICT this analysis reveals is its inability to distinguish between tumour and HGPIN. While VERDICT detects lower cellularity in HGPIN compared to the tumour, the difference is not statistically significant. This is not surprising as HGPIN is also characterised by proliferation of epithelial cells, and perhaps a more complex model or pulse sequence is needed to capture the subtle difference between these pathologies.

One drawback of this study is the registration method between MRI and histology. An automated registration technique could account for variations in slicing orientation and other deformations caused by tissue preparation. Another limitation is the error in estimated nuclei counts. While the model is able to detect nuclei fairly accurately, it is sensitive to noise, artefacts and the quality of digitisation.

REFERENCES

1. Panagiotaki E. et al. Noninvasive Quantification of Solid Tumor Microstructure Using VERDICT MRI. *Cancer Research* 2014; 74(7), 1902-1912.
2. Panagiotaki E. et al. Microstructural Characterization of Normal and Malignant Human Prostate Tissue With Vascular, Extracellular, and Restricted Diffusion for Cytometry in Tumours Magnetic Resonance Imaging. *Investigative Radiology* 2015; 50(4): 218–227.
3. Panagiotaki E et al. Optimised VERDICT MRI protocol for prostate cancer characterisation. *ISMRM* 2015.
4. Fiaschi L. et al. Learning to count with regression forest and structured labels. *ICPR* 2012; 2685–2688.