

Supplementary Method S2

Construction of histology images of cell density and blood vessel density.

Images of cell density (*CD*) and blood vessel density (*BVD*) in prostatectomy specimens were based on whole-mount sections stained for CD31 and counterstained with hematoxylin. Hematoxylin was used as blue nuclei stain, and CD31 positive endothelial cells were visualized by 3,3'-Diaminobenzidine (DAB). Figure 1 illustrates the image analyses performed in an in-house developed Matlab program. Images were recorded at a resolution of 1.4 $\mu\text{m}/\text{pixel}$ on AxioImager Z1 ApoTome microscope system (Carl Zeiss) equipped with a 5x/NA0.13 lens and a 1ccc1 ccd camera (Carl Zeiss). Analysis was performed on the entire tumor as a whole image (Fig. 1C), where the tumor was delineated based on the pathologist's outline in hematoxylin-eosin stained sections. For illustration purpose a small section of the tumor, equal to one field of view in the microscope (Fig. 1A), is shown in Figure 1B.

Cell nuclei and vascular masks were generated by the following procedure:

- I. Hematoxylin and DAB staining was separated by color deconvolution (Ruifrok and Johnston, 2001) into two images (Fig. 1B; I).
- II. Thresholding and filtering were applied to the images to segment out cell nuclei and blood vessels. First, the DAB images were manually thresholded to achieve the highest endothelial specificity and sensitivity. The same threshold was applied to all images. Objects smaller than 10 pixels were considered to be noise and removed. To ensure that blood vessels with open lumens were considered as one complete vessel, a dilate function adding 4 pixels to the edge of each object was applied, followed by a function to fill all holes in objects. Then, an erode filter was applied to remove 4 pixels from each object. Second, a threshold was applied to the hematoxylin images to include as many cell nuclei as possible, while avoiding non-nuclei objects

- from background staining. Objects smaller than 6 pixels were considered to be noise and were removed. The resultant hematoxylin and DAB binary images were used as cell nuclei and vascular masks respectively (Fig. 1B; II). Pixels defined as endothelial cells in the vascular mask, were removed from the cell nuclei mask.
- III. To verify the accuracy of the program and correct possible errors, the cell nuclei mask and the vascular mask were overlaid in an RGB image with green and red color, respectively, for visual inspection (Fig. 1B; III).

CD and *BVD* images of the entire tumor were created by the following procedure:

- I. Region of interest (ROI) was defined by manually outlining tumor in the RGB images, as indicated in green in Figure 1C; I. Areas with damaged tissue or biopsy holes, indicated in red in the figure, were removed by manual delineation and excluded from the analysis.
- II. The procedure described above for Figure 1B was performed on the ROI, leading to cell density and vascular masks of the entire tumor (Fig. 1C; II).
- III. *CD* and *BVD* images were created by calculating the positive area fraction in each square of a grid superimposed on the masks (Fig. 1C; III). Each square had a size of 500 x 500 pixels, corresponding to the in-plane-resolution of the DW-MR images (700 μ m/pixel). From the images, histograms of each parameter were generated (Fig. 1C; III). The median *CD* and *BVD* were used in analysis against DW-MR image parameters and pimonidazole staining.

Reference

Ruifrok AC and Johnston DA. Quantification of histochemical staining by color deconvolution. *Analytical and Quantitative Cytology and Histology* 2001; 23, 291–299.

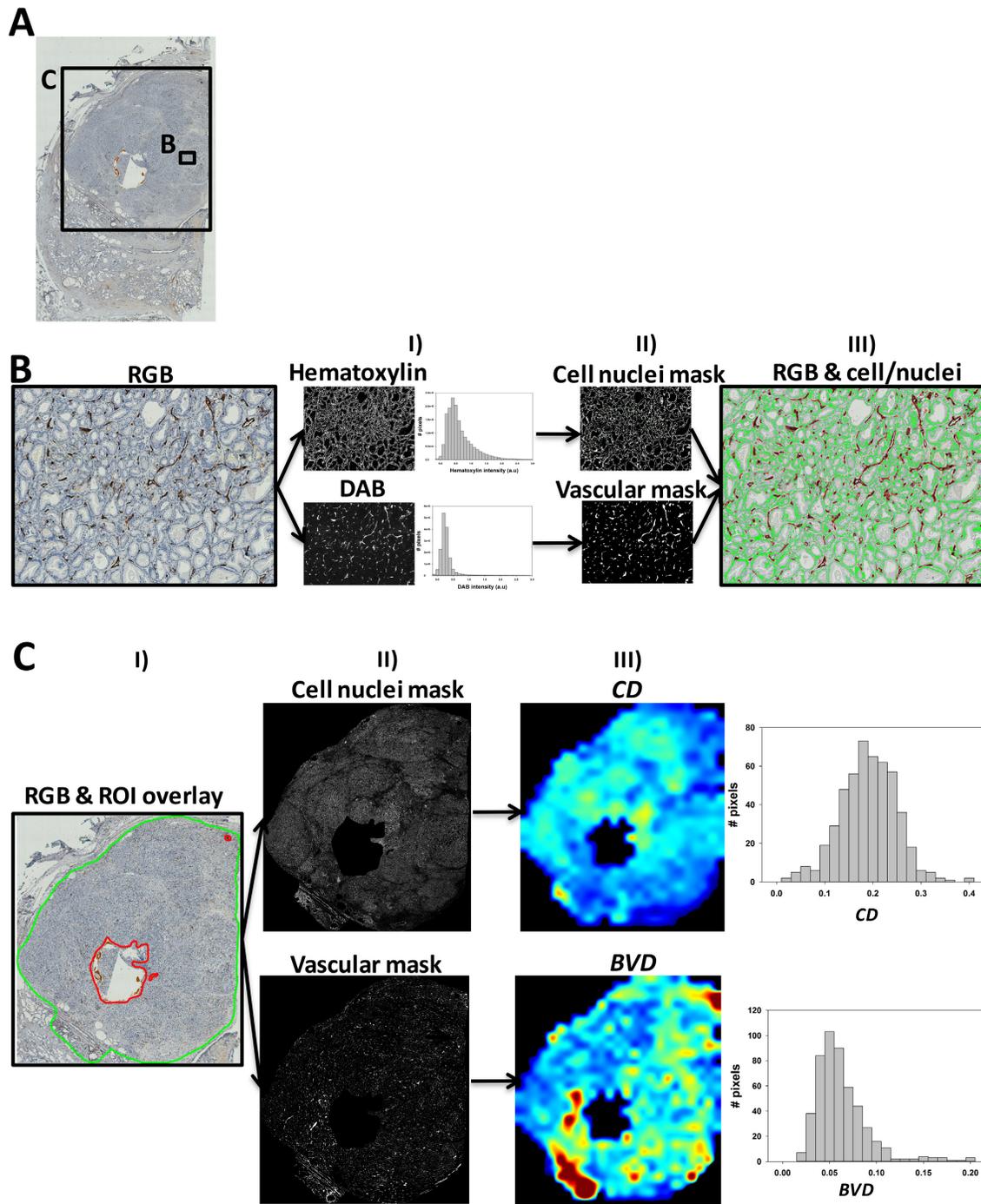


Figure 1. Procedures to construct histology images of cell density (CD) and blood vessel density (BVD) in whole-mount sections from prostatectomy specimens. A, Whole-mount section (half of prostate) stained for CD31 and counterstained with hematoxylin, which served as basis for the analyses. The tumor sections shown in B and C are indicated. B, Procedure to generate cell nuclei and vascular masks. C, Procedure to construct CD and BVD images of the entire tumor.