En-face optical coherence tomography for the detection of cancer in prostatectomy specimens: Quantitative analysis in 20 patients

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Abstract
The increase histopathological evaluation of prostatectomy specimens rises the workload on pathologists. Automated histopathology systems, preferably directly on unstained specimens, would accelerate the pathology workflow. In this study, we investigate the potential of quantitative analysis of optical coherence tomography (OCT) to separate benign from malignant prostate tissue automatically. Twenty fixed prostates were cut, from which 54 slices were scanned by OCT. Quantitative OCT metrics (attenuation coefficient, residue, goodness-of-fit) were compared for different tissue types, annotated on the histology slides. To avoid misclassification, the poor-quality slides, and edges of annotations were excluded. Accurate registration of OCT data with histology was achieved in 31 slices. After removing outliers, 56% of the OCT data was compared with histopathology. The quantitative data could not separate malignant from benign tissue. Logistic regression resulted in malignant detection with a sensitivity of 0.80 and a specificity of 0.34. Quantitative OCT analysis should be improved before clinical use.

KEYWORDS
histopathology, one-to-one registration, optical coherence tomography, prostate

1 | INTRODUCTION

Prostate cancer accounted for almost 1 in 5 new cancer diagnoses in males in the United States in 2017, and 1 in 8 men will develop invasive cancer in the prostate [1]. Due to the screening for prostate-specific antigen (PSA), the incidence of low- and intermediate-risk prostate cancer diagnosis has increased [2]. For the intermediate-risk
patients, radical prostatectomy is one of the most common treatment options, comprising approximately 1330 radical prostatectomies per million men, 45 years and older, annually in the United States in the period 2010 to 2011 [3]. After removal of the prostate, the resected prostate is analyzed macroscopically and microscopically by a pathologist to assess disease stage, Gleason grade and surgical margins. Since the definition of clinically significant prostate cancer is a tumor size larger than 0.5 cm [4], the prostatectomy specimen is cut into slices with approximately 0.5 cm thickness. Of these slices, only the surface is microscopically assessed. In general, approximately 10 slices (depending on the size of the prostate), will be microscopically studied. It is essential for an accurate diagnosis that the pathologist can detect the presence of microscopic cancer within the whole slice with high sensitivity [5]. The pathologic analysis of one prostate is a time-consuming procedure. This is amplified by an increasing number of radical prostatectomies per year [6].

An automated method to aid the pathologist during this examination would facilitate this process and the prediction of microscopic cancer. Optical coherence tomography (OCT) is a noncontact real-time high-resolution imaging technique that images ex vivo and in vivo tissue specimens of up to approximately 1.5 mm in depth, with 15 μm resolution without the need for staining. OCT can detect small cancer lesions in several organs in vivo and ex vivo, based on morphological features and/or quantitative parameters as the measured attenuation coefficient [7–11].

Whereas OCT quantitative parameters have been proven to differentiate malignant from benign tissues in multiple organs, the OCT quantitative parameters have never been applied to distinguish different tissue types in the prostate automatically. Therefore, our goal is to evaluate the potential of quantitative OCT to distinguish benign from malignant prostate tissue automatically. One-to-one registration is challenging in heterogeneous tissue like prostatic tissue. In our previous study, we demonstrated our accurate method for one-to-one registration of 3D en-face OCT data with histology. Our aim here is to extend this study to evaluate the procedure in more patients and to improve classification statistics. For this purpose, we delineated tissue types on 54 fixed histology slides of 20 prostatectomy specimens and registered these with OCT of these same slices following the procedure described in our earlier work [12].

2 | METHODS

2.1 | Participants

Twenty patients, who were diagnosed with prostate cancer and selected for radical prostatectomy, were included at an outpatient clinic of the urology department at the Amsterdam UMC location VUMc in 2014. The ethical board of the medical center waived the need for evaluation.

1) Prostate preparation
   - Fixation
   - Cut prostate into slices

2) OCT data acquisition
   - 1.2 cm x 1.2 cm per tile
   - 400x400x400 pixels per tile
   - 12-26 tiles per slice

3) Histological evaluation
   - Cut histology slides
   - Uro-Pathologist analysis
   - Demarcations
   - Overlapping region check

4) Stitching tiles to OCT mosaic
   - Downsizing tile to 200x200x200 pixels
   - Overlaying OCT-parts used for recognition

5) One-to-one registration
   - Histology overlay on OCT mosaic
   - Registration by hand
   - Registration precision measurement
   - Overlay & histology rating

6) Quantitative analysis
   - Averaging 21x21 a-lines
   - Attenuation coefficient $\mu_{OCT}$ calculations 51 pixels in depth
   - Residue and $R^2$ calculations

7) Masking and sorting
   - Masking pathology regions
   - Sorting $\mu_{OCT}$, Residue, $R^2$ per tissue type

8) Results per tissue type
   - $\mu_{OCT}$
   - Residue
   - $R^2$

FIGURE 1 Flowchart of OCT analyzing and comparison with histology, adapted from [12]. The extra steps compared to the original flowchart are overlapping region check at step 3 and overlay and histology rating at step 5 (both italicized). OCT, optical coherence tomography.

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2.2 Data analysis

The analysis of the prostatectomy specimens, as depicted in the flowchart in Figure 1, was carried out following eight previously described steps [13]. These steps are summarized as follows:

Step 1. First, all resected prostates were fixated, painted for orientation and cut into multiple slices of approximately 5 mm thickness.

Step 2. For practical reasons (limitations of data-processing and the workload of the pathologist), depending on the volume of the prostate, 2 or 3 slices were analyzed with en-face OCT, that is, one in three of the original slices. A tabletop OCT system (Santec OCT system IVS-2000, Santec USA Corporation, Hackensack) with a center wavelength of 1310 nm operating at 50 kHz was used to scan each prostate slice which was positioned on an articulating platform set at ~8° from horizontal, and on an XY-stage. The following settings were used: OCT acquisition 400 × 400 × 400 pixels; scanning area of 1.2 × 1.2 cm², with an optical ranging depth set at 7 mm, axial resolution 12 μm in tissue, the lateral resolution of 50 μm and beam waist 25 μm. Because the OCT scanning area was smaller than the area of the prostate slice, multiple OCT acquisitions (“tiles”) were taken with 0.2 cm overlap and later stitched together (step 4).

Step 3. The uropathologist identified and demarcated the following tissue types on the histology slides: benign glands, cystic atrophy, inflammation, stroma, Gleason 3, Gleason 4, Gleason 5, fat and regular atrophy. The outlines were filled using a flood fill algorithm using a custom-made Matlab script (Matlab R2017b, The MathWorks, Inc.) creating regions of homogeneous tissue type [13]. Importantly, for locations where tissue types overlap, the tissue type with the smallest region was kept.

Step 4. The OCT tiles were stitched in roof tile formation into one OCT mosaic using Worldmatch [12, 14].

Step 5. The OCT mosaics and the histology images were registered visually allowing affine transformations. During this step, the quality of each histology slide (noticeable artifacts, folded areas or missing pieces) and the quality of its registration with the OCT mosaics was visually rated (by AS). The histology slides and the registrations were categorized in good, medium and bad according to the criteria in Table 1. Only good quality histology slides (rated by AS) were accepted, all of which also had a good or medium quality of registration with the OCT mosaic.

Step 6. For each 3D OCT dataset, the optical attenuation coefficients (μOCT), residue and R² of the fit were automatically determined using a custom-written code in Matlab (Matlab 7.11.0 R2010b, The Mathworks Inc., Natick, Massachusetts) [13]. All data were automatically straightened to flatten the tissue edge. Next, the data were divided into blocks of 21 × 21 × 51 pixels and corrected for point spread function and sensitivity roll-off related signal losses. The attenuation coefficient was then determined over a depth of 0.32 mm, starting from 0.10 mm below the automatically detected tissue edge using a supervised automated exponential fit. The attenuation coefficients were multiplied with 1.4 to correct for the assumed refractive index of tissue. Finally, all μOCT, residue, and R² values were reconstructed into en-face maps for visualization.

Step 7. To combine the histology (step 5) and the quantitative outcomes (step 6), a Labview program was written (LabVIEW 2017, National Instruments, Austin, Texas). Only the attenuation coefficient fits with R² > 0.9, and attenuation coefficient values between 0.014 and 14 mm⁻¹ were included. Small potential misalignments between the histology and the OCT mosaic could lead to misregistration of OCT data with histological tissue type. To prevent misregistration at the edges of tissue type, areas were eroded using a “cross”-mask (allowing only blocks where adjoining horizontal and vertical blocks had the same histology classification).

Step 8. Based on previous results [13], only the data after “cross” erosion of the masks will be used for statistical analysis. A Kruskal Wallis test was performed (using MedCalc Software 15.8, Belgium) to compare the attenuation coefficients of all tissue types, a P-value of ≤0.05 stated significantly. The μOCT, residue, and R² of the selected slices were grouped and visualized per tissue type. Also, the “benign tissues” are combined (benign Glands, regular atrophy, cystic atrophy, and inflammation) in “group A”

<table>
<thead>
<tr>
<th>TABLE 1 The criteria for rating the quality of the histology slide and the registration of the histology slide with the OCT mosaic</th>
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<tbody>
<tr>
<td><strong>Histology</strong></td>
</tr>
<tr>
<td>Complete full mount histology slide</td>
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<tr>
<td>Registration Mismatch of</td>
</tr>
<tr>
<td>&lt; 1 mm</td>
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<tr>
<td>between histology and OCT mosaic</td>
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Abbreviation: OCT, optical coherence tomography.
and will be compared to all malignant tissue combined (Gleason 3, Gleason 4 and Gleason 5) in “group B.” Fat is only located outside the prostate and is not included in the group A nor group B.

We performed multivariate analysis for binary classification to leverage the information content of the parameter sets \( \{ \mu_{\text{OCT}}, \text{residue} \} \) and \( \{ \mu_{\text{OCT}}, \text{residue}, R^2 \} \). We used a logistic regression (LR). All analyses were implemented in LabVIEW 2017 (National Instruments) based on the LabVIEW Analytics and Machine Learning toolkit to classify measurement sets into “group A” = “benign tissues” or “group B” = “malignant” categories. Half of the dataset was used for training, and the other half was used for validation. The reported numbers are for the validation set.

LR finds the optimal linear combination of parameters \( l = \beta_0 + \sum \beta_i x_i \) where \( \beta_0 \) is an offset and the sum runs over the available parameters. The model output is the probability of belonging to the malignant group given input \( l \) by passing \( l \) to a sigmoid function, \( p( \text{group B} | l) = \frac{1}{1 + e^{-l}} \). The parameters \( \beta \) are optimized during training. Half of the dataset is evaluated using the fixed (optimized) parameters. A “confusion matrix” is returned from which sensitivity and specificity are calculated. To estimate the statistical uncertainty of the obtained sensitivity and specificity, we applied a bootstrapping procedure where the training and validation cycle was repeated 100 times, each time with a randomized input set (thus, the samples in training and validation set varied for each iteration). Metrics are reported as mean ± SD.

### RESULTS

#### 3.1 Patient characteristics

Twenty patients were included; their characteristics are stated in Table 2. Nineteen patients underwent prostate biopsies, and one patient was included based on benign prostate hyperplasia transurethral resection results. The Gleason scores in the biopsies (19 patients) ranged from 3 + 3 to 4 + 4. Gleason scores in the specimens (20 patients) were generally higher and ranged from 0 + 0 to 4 + 5.

#### 3.2 Histology processing

In total, 54 pathology slides were digitized and annotated. An example of the annotated slide can be seen in Figure 2A. The filled version can be seen in Figure 2B. These colored histology datasets are later used to sort and mask the OCT reading. The brown areas on the histology slide are artifacts that are mostly the result of cutting the whole mount histology slides (Figures 2 and 3B, at yellow star). The histologic evaluation provided one benign specimen and Gleason scores in the other prostates ranging from 3 + 4 to 4 + 5. Extracapsular invasion, pelvic lymph node dissection, seminal vesicle invasion and positive resection margins were present in six, one, two and five patients, respectively.

#### 3.3 One-to-one registration of OCT and histology

In total, 54 slices were digitized into OCT mosaics following our OCT data acquisition protocol. These 54 OCT mosaics are a combination of 1350 OCT scans of 400 × 400 × 400 pixels. All OCT tiles of the slices were successfully stitched together: interfaces between different tiles are hardly visible, see Figure 3A,B. One tile was missing off one mosaic (Figure 3B), the corresponding

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Patient characteristics</th>
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<tbody>
<tr>
<td>Patients</td>
<td>20</td>
</tr>
<tr>
<td>Age at time of operation (years)</td>
<td>Mean 64.5 (range 55-76)</td>
</tr>
<tr>
<td>PSA prior to operation (ng/ml)</td>
<td>Mean 11.5 (range: 2.9-36)*</td>
</tr>
<tr>
<td>Biopsies (Gleason) 3 + 3</td>
<td>5</td>
</tr>
<tr>
<td>3 + 4</td>
<td>11</td>
</tr>
<tr>
<td>4 + 3</td>
<td>2</td>
</tr>
<tr>
<td>4 + 4</td>
<td>1</td>
</tr>
<tr>
<td>Total number of cores</td>
<td>Mean 9.6 (range 6-15)</td>
</tr>
<tr>
<td>Number of positive cores</td>
<td>Mean 4 (range 1-7)*</td>
</tr>
<tr>
<td>Specimen (Gleason) 0 + 0</td>
<td>1</td>
</tr>
<tr>
<td>3 + 4</td>
<td>7</td>
</tr>
<tr>
<td>4 + 3</td>
<td>11</td>
</tr>
<tr>
<td>4 + 5</td>
<td>1</td>
</tr>
<tr>
<td>Extracapsular invasion</td>
<td>6</td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td>2</td>
</tr>
<tr>
<td>Pelvic lymph node dissection</td>
<td>7</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>1</td>
</tr>
<tr>
<td>Resection margins positive</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviation: PSA, prostate-specific antigen.

*The patient with PSA 2.9 received Combodart (medication for the treatment of symptomatic benign prostatic hyperplasia).

*One patient underwent a radical prostatectomy based on a histopathological Gleason score 4 + 4 after transurethral resection of the prostate.
**FIGURE 2** A, The whole mount histology slide with the delineated areas, performed by an uropathologist. The black dots indicate cysts. B, The areas colored by the custom made Matlab program. The missing data (brown) is probably the results of the cutting. Cutting the slices is the most challenging preparation step of the whole mount histology slide.

**FIGURE 3** Two examples of the one-to-one registration of the OCT mosaic (gray image) and the H&E stained histology slide. Image A shows proper registration of OCT with a histology slide with limited artifacts. Image B shows the best possible registration of the OCT mosaic with a profoundly damaged and deformed histology slide. Cutting artifacts are visible around the yellow star; missing histology areas are indicated as “x” and obvious misalignments are encircled in yellow. The yellow square OCT tile, this only happened once, in this slice. The example in image A was included for further analysis (good histology and good registration); the example in image B was excluded for further analysis (bad histology and bad registration). OCT, optical coherence tomography.
3.4 | Quantitative analysis

All $\mu_{\text{OCT}}$, residue and $R^2$ were determined successfully per block of $21 \times 21 \times 51$ pixels ($\sim 1 \times 1 \times 1$ mm$^3$). Figure 5A shows an example of a $\mu_{\text{OCT}}$-map. Some outliers (mainly located outside of the prostate or at its edge) are shown in yellow with $\mu_{\text{OCT}} > 14$. Please note that cutting artifacts (*) in the histology slice (Figure 3B) are not present in the attenuation coefficient map (Figure 5A).

The blocks of the histology (Figure 5B) and the erosion map (Figure 5C) correspond with the $\mu_{\text{OCT}}$, residue and $R^2$ maps (eg, the $\mu_{\text{OCT}}$ in Figure 5A). The quantitative data from the selected 31 slices were sorted per tissue type for “cross”-mask erosion. In total 63 136 blocks were analyzed, from which 59 013 blocks met our requirements of $R^2 > 0.9$, and $\mu_{\text{OCT}}$-values between 0.014 and 14 mm$^{-1}$. The “cross”-mask erosion effect reduced the total number to 31 779 blocks (53.9%). The number of slices with the tissue type present after “cross” masking reduced as follows: Gleason 3 (13 to 11 slices), Gleason 4 (20 to 17 slices), Gleason 5 (3 to 1 slice), inflammation (13 to 7 slices), cystous atrophy (17 to 16 slices), regular atrophy (6 to 4 slices) and fat (22 to 18 slices). The resulting attenuation coefficients, residues, and $R^2$ after...
FIGURE 6  The attenuation coefficient, residue and $R^2$ per tissue type after “cross” mask erosion. Boxplots represent medians and interquartile ranges. The means are indicated by the square in the middle of each boxplot.

FIGURE 7  Boxplots of the attenuation coefficients of stroma vs benign tissue (cystous atrophy, regular atrophy, inflammation, benign glands and stroma) vs malignant tissue (Gleason 3, Gleason 4 and Gleason 5). Fat is located outside of the prostate and therefore not included as benign tissue. Visible is a big overlap between all three boxplots. Boxplots represent medians and interquartile ranges. The means are indicated by the square in the middle of each boxplot.
“cross” mask erosion are presented in Figure 6 per tissue type in boxplots. Figure 7 shows the boxplots of the attenuation coefficients of stroma vs benign tissue (cystous atrophy, regular atrophy, inflammation, benign glands and stroma) vs malignant tissue (Gleason 3, Gleason 4 and Gleason 5).

Classification between the benign and malignant tissue based on the $\mu_{\text{OCT}}$ alone provided a sensitivity of 63% and a specificity of 54% with an area under the curve of 0.62. The multivariate analysis for binary classification using the information content of the parameter sets $\{\mu_{\text{OCT}}, \text{residue}\}$ provided a sensitivity of 72% and a specificity of 43%. Using the parameter set $\{\mu_{\text{OCT}}, \text{residue}, R^2\}$ resulted in a sensitivity of 80% and a specificity of 34%.

4 | DISCUSSION

In this study, we evaluated the potential of quantitative OCT to distinguish benign from malignant prostate tissue applying an automated data-analysis pipeline in order to aid the pathologist during the examination of the prostatectomy specimen. We scanned 54 slices of 20 fixed prostatectomy specimens with OCT and registered the quantitative parameters to the histopathology proven tissue types using a demonstrated one-to-one registration method [13]. By selecting the slices with good quality histology and medium/good registration, the number of slices reduced to 31. The automatic quantitative OCT analysis, which is essential during research and clinical use, was capable of processing all data where the histology had sufficient quality. In order to prevent artifacts due to incorrect edge detection or multiple layers of different tissue types, the use of cutoff values for the attenuation coefficients and $R^2$ were needed. These cut-off values reduced the number of blocks available for analysis by approximately 7%. In order to have high certainty in the classification of the OCT data with histology, we used cross-masking, which reduced the available data by another 46%. Still, a large number, 31 779, blocks were available for the final analysis (see Appendix). Our analysis showed a large spread and overlap among the attenuation coefficients, residues, and the $R^2$ of the different tissue types. This spread and overlap hampers classification of a tissue type or malignant from benign tissue using the studied OCT quantitative parameters on fixated prostatic tissue.

Combining all quantitative parameters—attenuation coefficient, residue, and $R^2$—with LR resulted in a malignant tissue detection with a sensitivity of 0.80 and a specificity of 0.34. Given the achieved sensitivity and specificity, this technique should be further improved before it is suitable for clinical use.

Muller et al. showed the possibilities of high accuracy registration of histology with OCT images obtained by 106 pullbacks with a needle-based rotating side-firing probe OCT in fresh prostatic tissue [7, 15, 16]. With the rotating side-firing probe, the sampling volume was limited. In our study, we, therefore, imaged en-face the whole cross-section of the same 20 prostates. Although in our study, the tissue was fixated, still, the values for the measured attenuation coefficients were very similar [7]. The mean attenuation coefficient of stroma for fixated tissue we found is just above 5 mm$^{-1}$ and while Muller et al. found a mean attenuation coefficient of just under 5 mm$^{-1}$ for fresh tissue. The spreads of the attenuation coefficients for stroma and malignant tissue were substantial, and both categories overlap in both studies, with slightly higher attenuation coefficients in malignant tissue compared to stroma. Because we both corrected for the point spread function and sensitivity roll-off, quantitative comparison of results is possible. Consequently, the mean attenuation coefficients for fixated and the fresh prostatic tissue seem to be similar.

Ex vivo OCT measurements can be registered to histology with very high precision, but the ex vivo tissue lacks perfusion and in our case was fixated for cutting purposes. Clearly, in vivo OCT measurements are a better representative for future in vivo usage for tissue classification. Indeed, multiple in-vivo studies showed the use of OCT quantitative parameters to discriminate malignant from benign tissues, for example, in the skin [17], vulva [18], urinary tract [9, 19], kidneys [20] and brain [21].

Future studies, in which OCT pull-back data obtained in vivo in the prostate are registered with the histopathology, are needed. First, proof-of-principle measurement in two patients showed the feasibility of OCT in the prostate [22].

This study does have a number of limitations. First, in this study, one uropathologist annotated the slides, for a more accurate annotation a consensus between multiple uropathologists has to be achieved. Next, we carefully registered the OCT data to a tissue type by overlaying the annotated histology slide. We opted for a rigid registration instead of a deformable registration because we did not a priori know what landmarks would be visible in both modalities, which is a requirement for deformable registration. However, miscorrelations could occur due to deformation during histology slide preparation or incorrect interpretation. By reducing the area of the annotated area at the edges, referred in the article as masking, we reduced the probability of miscorrelated OCT data providing quantitative values that belong to the right tissue type [13]. The downside of masking is the reduction of the number of quantified blocks. Despite the careful registration process, this study showed that quantitative
OCT data, attenuation coefficient, residue, or the $R^2$, will not support the pathologist in tissue classification.

Our results show that it is not possible to distinguish malignant from benign tissue on fixed prostate tissue with our tested OCT quantitative parameters. Other steps must be taken to make OCT a successful addition to prostate cancer care. The extensive OCT database that is now annotated by tissue type makes it ideal also to try other possibilities to differentiate prostate cancer from benign tissue types automatically. One option could be image recognition by finding comparable visual characteristics by texture analysis [23–25]. Another possibility is to use tissue recognition using similar deep learning algorithms now used in histology [26]. OCT images with a higher resolution [27] could improve these two options, allowing more subtle differences to be detected.

If en-face OCT can detect tumors, it could be used during prostatectomy. After the prostate has been removed, it can be immediately scanned to establish whether the prostatectomy is successful or not support the pathologist in tissue classification. OCT analysis would further help the pathologist by pointing out points of interest, just like the CAD systems support radiologists with mammograms. An alternative method to assess the prostatectomy specimen for free cutting edges would MRI—dedicated point-of-care MRI systems exist [28]. However, the question is whether the MRI resolution is high enough, and the lack of blood flow makes the MRI assessment possible.

5 CONCLUSIONS

In this study, we demonstrated accurate registration of OCT data with histology proven tissue type (annotated on histopathology) after excluding unregistrable, heavily morphed, or damaged histology slides. The results of the one to one registration with gold standard histopathology showed that the OCT quantitative parameters attenuation coefficient, $R^2$, and residue could not differentiate the different tissue types or malignant from benign tissue in fixed prostate tissue. Attenuation coefficients of stroma and malignant tissue are similar to data previous quantitative OCT study of fresh ex vivo prostatic tissue.

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APPENDIX

"Cross masking" affects the number of quantitative blocks. The total number is reduced from 59,013 to 31,779 blocks. Because of the nature of the prostate, the different tissue types are not evenly distributed and come in other shapes and quantities. As a result, the effect of masking is also different per tissue type. In Figure A1, the masking effect is illustrated by the reduction of the mean and changes of the median number of quantitative blocks per slice.

FIGURE A1 The figures show the effect of "cross masking" on the mean quantitative blocks per slice per tissue type (left, orange and green) and the median quantitative blocks per slice per tissue type (right, yellow and blue). The figure left shows the mean of the quantitative blocks always reduces after "cross masking." The median also reduces after "cross masking," with an exception for Gleason 5 and inflammation.