Differential diagnosis of human bladder mucosa pathologies *in vivo* with cross-polarization optical coherence tomography

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Abstract: Quantitative image analysis and parameter extraction using a specific implementation of polarization-sensitive optical coherence tomography (OCT) provides differential diagnosis of mucosal pathologies in *in-vivo* human bladders. We introduce a cross-polarization (CP) OCT image metric called Integral Depolarization Factor (IDF) to enable automatic diagnosis of bladder conditions (assessment the functional state of collagen fibers). IDF-based diagnostic accuracy of identification of the severe fibrosis of normal bladder mucosa is 79%; recurrence of carcinoma on the post-operative scar is 97%; and differentiation between neoplasia and acute inflammation is 75%. The promising potential of CP OCT combined with image analysis in human urology is thus demonstrated *in vivo*. © 2015 Optical Society of America

OCIS codes: (110.4500) Optical coherence tomography; (230.5440) Polarization-selective devices; (290.5855) Scattering, polarization; (100.2960) Image analysis; (170.1610) Clinical applications; (170.7230) Urology.

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1 Introduction

The most common pathological conditions of the bladder are inflammation (cystitis) and neoplasia. Chronic inflammation of the bladder and its associated microenvironment increase the risk of cancer and play a role in its progression. In fact, emerging evidence indicates that inflammation plays important roles at all stages of tumor development, including initiation, promotion, invasion, and metastasis [1]. For example, muscle-invasive bladder cancer, particularly invasive transitional cell carcinoma, is directly associated with the presence of chronic inflammatory urinary tract infection.

Bladder cancer is the sixth most common cancer in the United States (after lung, prostate, breast, colon cancers and lymphoma); it is approximately 4x more common in men than in women. ~75,000 new cases and ~16,000 deaths from bladder cancer are estimated to occur in the United States in 2014 [2]. This cancer is characterized by high recurrence rate of the aggressive forms of the disease (even if the primary was non-invasive), sometimes via local recurrence but often in different bladder wall locations away from the site of the initial tumor.

OCT is an actively researched emerging medical imaging modality with high spatial resolution (approaching microscopy) but limited penetration depth. This unique combination has already enabled its successful clinical translation in ophthalmology, with the current clinical frontier being intravascular imaging in cardiology. Much current OCT research is directed towards better imaging performance (e.g., speed, task-specific probes), multi-modality imaging (e.g., OCT + Raman spectroscopy), new clinical sites and scenarios (e.g., GI and urinary endoscopy, treatment monitoring in addition to early detection/diagnosis), novel sources of contrast (e.g., elastography, angiography), and image analysis / signal processing / feature extraction (e.g., segmentation of structural images, vessel tortuosity measures from microvascular studies). Note that pathological processes frequently lower the contrast between adjacent tissue regions. Thus, the last two OCT research themes (development of novel contrast mechanisms and development of robust image processing platforms) are being actively studied. This paper deals with polarization contrast in OCT and how to robustly detect and quantify the resultant CP images.

Polarization approaches such as polarization-sensitive (PS) and cross-polarization (CP) OCT detect changes in light polarization caused by asymmetric (anisotropic) tissue structures such as collagen fibers, cholesterol crystals, complexes of actin and myosin, myelin fibers and so on. By quantifying the phase delay between orthogonal polarization states (phase retardation or retardance), polarization OCT provides information about tissues birefringent properties (retardance depends on intrinsic tissue birefringence and effective light pathlength); this birefringence can in turn be related to the anisotropic tissue constituents alluded to above. Specifically, in CP OCT two co-registered images are recorded: parallel (conventional OCT image) and orthogonal (cross-OCT image) that detects tissue reflections with polarization state orthogonal to the incident one. Only orthogonally polarized backscattered light which is mutually coherent with the incident is contributing to the cross-polarized OCT image. The origin of such “coherent backscattering” includes random depolarization during light propagation in the media, depolarization during the backscattering process, and “regular” polarization changes associated with propagation back and forth in birefringent media [3].

As well, since the term “depolarization” is variously defined in the literature, we want to clarify our use of it. For the purposes of OCT imaging, we refer to depolarization as a process described by Schmitt et al as “cross-polarized backscatter” in [3]. This process was associated with single scattering from nonspherical (i.e., asymmetric) particles and multiple scattering by particles with sizes much larger than the OCT probing wavelength. Such scattering maintains a high degree of temporal coherence with the incident radiation and therefore can be detected by recombining it with a cross-polarized reference radiation, as it is done in cross-polarization OCT (CP OCT). Also note that as [3] described a phantom study, the emphasis was on
particle scattering; in our tissues samples with large extra-cellular matrix (ECM) compartment, fiber scattering as engendered by collagen is likely of more relevance.

Although somewhat dependent on the tissue type and clinical site, most of the CP OCT contrast (OCT signal in the orthogonal polarization channel) comes from collagen. Collagen is the most abundant structural protein of the body’s ECM, and alterations in its amount, composition and micro-organization are sensitive markers of the tissue’s “health” and functional status. Its accurate imaging and quantification can thus enable early detection of inflammation and other abnormalities [4], provide clues for success or failure of tissue grafts, and report on the progress of regenerative medicine treatments [5]. In oncology, collagen condition can help to identify of early neoplastic processes [6]. There were published works describing the qualitative OCT criteria for different types of inflammation, severe dysplasia and cancer of the mouth, cervix uteri, and bladder [7, 8]. Undoubtedly then, high-resolution collagen detection and its robust quantification is important in a number of clinical sites and applications.

As mentioned, OCT image quantification is an important but challenging area of OCT research actively pursued by many research and industrial labs. Both ‘conventional’ structural and polarization OCT images are being analyzed. For the former, methods of measuring brightness histograms [9], mean-square deviation of OCT signal intensity [10], OCT signal attenuation with depth [11], signal normalization (for example, relative brightness) [12], or approaches based on reconstruction of optical characteristics of biotissues from OCT images [13] and segmentation of tissue types from the images that do not contain visible borders or layers or other obvious visible features [14] were proposed to calculate the diagnostic parameters. Other research groups use a complex approach to the quantitative processing of 3D OCT images: their segmentation, blurring of speckles, quick Fourier transform, assessment of standard deviation and histograms of brightness [15].

As for the polarization OCT images, it should be noted that the majority of research is performed on PS OCT devices and quantitative processing of PS OCT images is based on analyzing the phase retardation maps [16, 17], the rate of change of which characterizes orientation, the degree of structural organization of collagen on fiber and tissue levels, and it allows judging about the presence of pathology [18]. However these approaches have limited or no use to assess connective tissue of mucous membranes where collagen fibers are located randomly and do not show birefringence. Despite 10 years of implementation of CP OCT method in clinical research, the studies of quantitative assessment of CP OCT images of mucous membranes were very limited [4, 19].

This paper thus describes our efforts to increase the differential diagnosis accuracy of OCT in bladder pathologies (inflammation, fibrosis and neoplasia) using CP images and an optimized image analysis / quantification platform, based on the underlying alterations in the collagen compartment of interrogated tissue’s ECM.

2 Materials and methods

2.1. Patients

The work involved 68 patients (average age 45.8 ± 2.6 years) and 5 healthy volunteers (Group 1 – see Table 1). The indication to cystoscopic, histological and CP OCT tests for all patients was patients’ chronic cystitis or suspicion of bladder cancer.
Table 1. Relevant patient and imaging information for the CP OCT bladder study

| State of collagen | Study cohort and its mucosal process | N of patients | N of zones and analyzed CP OCT images / N of histological slices |
|-------------------|-------------------------------------|---------------|---------------------------------------------------------------|
| Normal            | 1. Normal                           | 5             | 10 / 10                                                       |
|                   |                                     |               |                                                               |
| Excessive         | 2. Severe fibrosis at chronic inflammation<sup>a</sup> | 18            | 18 / 30                                                       |
| accumulation      | 3. Scar<sup>b</sup>                 | 18            | 18 / 30                                                       |
|                   |                                     |               |                                                               |
| Degradation       | 4. Acute inflammation<sup>c</sup>  |               | 14 / 42                                                       |
|                   | 5. Carcinoma in situ<sup>d</sup>   | 18            | 8 / 16                                                        |
|                   | 6. Flat transitional cell carcinoma, stage I-IIa<sup>d</sup> |               | 14 / 18                                                       |
|                   | 7. Recurrence of carcinoma in scar  | 14            | 14 / 22                                                       |
|                   |                                     |               |                                                               |
| Totals:           |                                     | 73            | 96 / 168                                                      |

<sup>a</sup> - patients with chronic cystitis lasting 5-15 years at remission stage; <sup>b</sup> - patients with post-operation scar on bladder tissue without any symptoms of inflammation and without any suspicion of carcinoma recurrence on the scar; <sup>c</sup> - patients with acute inflammation because of exacerbation of chronic cystitis or presence of carcinoma; <sup>d</sup> - patients with flat suspicious area which has the following cystoscopic findings: inflamed suspicious of neoplasm, hyperemic, loose, ulcerated mucosa, with villous growth or with pathological changes in form of a white plaque.

Cystoscopy, CP OCT and biopsy of all regions were performed during a single patient examination session. The final diagnosis in each cite of interest and in reference cites were the histology with several stains, including polarization microscopy with picrosirius red (PSR).

The patient cohorts were grouped according to the pathological diagnosis at histology, and included 18 patients with chronic cystitis (Group 2), 18 patients with post-operation scar on bladder tissue (Group 3), 18 patients with acute inflammation (Group 4) and flat transitional cell carcinoma I-IIa, who have local non-exophytic pathological foci (Groups 5 and 6) and 14 patients with recurrence of carcinoma in scars (Group 7). Patients with suspicion of bladder carcinoma had several flat suspicious areas in which biopsy were taken thus the total number of investigated zones is more than number of patients. The total number of analyzed images equals 96 (Table 1).

The clinical study was approved by the Ethics Committee of Nizhny Novgorod State Medical Academy for scientific research involving human subjects. All patients voluntarily signed informed consent for the study.

2.2. CP OCT system

The work was performed with the CP OCT imager "OCT1300-U" developed and built at the Nizhny Novgorod’s Institute of Applied Physics of the Russian Academy of Sciences. The system has a common-path optical layout and two signal acquisition channels: one for scattered light that maintained initial polarization (“co-polarized”), and one that now exhibits orthogonal polarization (“cross-polarized”). Performance characteristics of the time-domain OCT imager operating at a central wavelength of 1315 nm are: tissue incident power ~4 mW,
2.3 CP OCT bladder examination procedure

The CP OCT probe was inserted into a bladder cavity through a working channel of a rigid cystoscope (outer diameter = 2.7 mm, working channel diameter = 8 mm). After selection of the imaging site by visual inspection, the forward-looking probe was pressed onto the tissue surface for 2-3 sec. From each site, two CP OCT images were acquired (to ensure repeatability). A biopsy for histological analysis was then taken from the same site. The landmark for targeted biopsy was a round indentation left by the OCT probe. The CP OCT images of normal and pathologically changed tissues were compared with the corresponding histological slides and the features of pathological processes reported in the literature [22–24]. After a thorough visual inspection of CP OCT images (to exclude ones with motion artifacts or bad probe contact with tissue surface), quantitative processing was performed on the remaining 96 CP OCT image sites of bladder mucosa.

2.4. Histology

Histology, including morphological study of collagen, involved microscopic analysis of tissue slices using brightfield and polarization darkfield microscopy. The standard methods of histological preparations were applied. Paraffin sections 5–7 µm thick were stained with hematoxylin-eosin (H&E) for traditional histological evaluation. Histological slides were viewed and photographed with a microscope equipped with a digital camera (Leica DM 2500, DFC 245C) in transmitted light. Picosirius red (PSR) staining and polarized light transmission microscopy were used for evaluation of structural and spatial organization of collagen fibers (CF).

Two histopathologists independently evaluated the pathological slides stained with H&E and PSR. The diagnosis coincided in 98% of cases. It is known that collagen type stained with PSR and examined with polarized light reveals its birefringent properties, visualized as bright colors against the background of dark surrounding tissue elements (reference). Collagen type I has red and red-yellow color (fiber thickness ~2-5 µm and higher (thick fibers)) and collagen type III – green and green-white color (fiber thickness ~1 µm (thin fibers)). Red-orange color of thick CF is also associated with densely packed CF indicative of their maturity [25]. Here, PSR preparations were assessed semi-quantitatively based on color, thickness and density of packing of CF. Domination of red-yellow thick or dense packed CF...
corresponds to fibrosis whereas thin greenish fibers, its loose location or lack of luminescence evidences about degradation of ECM cause of inflammation or carcinoma.

2.5. Statistical processing

Statistical processing was performed with MS Excel 2003 with a software package for statistical analysis Real Statistics and GraphPad Prism 6. The primary variable for statistical comparison between different bladder pathologies was the Integral Depolarization Factor (IDF, see below) calculated for each CP OCT image pair. We calculated descriptive statistics (mean value M and standard deviation SD). The level of significance for the differences between the groups was calculated with a nonparametric Mann-Whitney test. In all cases, differences were considered statistically significant with a p-value of <0.05. Additionally, the multiple comparisons with the Bonferroni correction were used when it was needed to compare 3 or 4 groups. Then differences were considered statistically significant with a p-values of <0.025 for comparison of 3 groups, and <0.017 for comparison of 4 groups. Confidence intervals were expressed as M ± 2SD.

Sensitivity, specificity, and diagnostic accuracy parameters for the comparison of different groups were also calculated. For detection of «weak fibrosis vs normal» true positive (TP) (i.e., prone to fibrosis) cases were considered if the IDF was greater than the lower limit of the 95% CI in «weak fibrosis». The threshold level was 0.18 (see Table 2). Sensitivity, specificity, diagnostic accuracy were calculated using this level to assess 28 CP OCT images. For detection of «neoplasia vs acute inflammation», TP (i.e., malignant) cases were considered if the IDF was below the upper limit of the 95% CI in pooled two groups «carcinoma in situ + transitional cell carcinoma I-IIa» (interval 0.03-0.05). The threshold level was 0.05 (Table 2). Sensitivity, specificity, diagnostic accuracy were calculated using this level to assess 36 CP OCT images. For detection of «recurrence of carcinoma on the scar vs scar» TP (i.e., malignant on the postsurgical scar) cases were considered if the IDF was below the upper limit of the 95% CI in «recurrence of carcinoma on the scar». The threshold level was 0.12. Sensitivity, specificity, diagnostic accuracy were calculated using this level to assess 32 CP OCT images (Table 2).

2.6 Integral depolarization factor

A ratiometric approach obtained by dividing the orthogonal by initial polarization images yielded robust data largely independent of optical power fluctuations and other system noise sources, and was thus used for quantitative CP OCT data analysis. Specifically, we calculated an integral depolarization factor (IDF), a ratio of the OCT signal in orthogonal polarization to the analogous value calculated in the initial polarization [26], both averaged over the transverse coordinates (B-scan direction). This was done in all areas of the image where the orthogonal signal exceeds average noise level by doubled noise standard deviation. It was calculated according to the formula:

$$\text{IDF} = \frac{1}{N} \sum_{i=1}^{N} \frac{P_{i}^{\perp} - \langle P_{\text{noise}} \rangle}{P_{i}^{\|}},$$

where $P_{i}^{\perp}$ and $P_{i}^{\|}$ are the raw OCT signals in the orthogonal and initial polarizations in $i^{th}$ pixel after averaging over the transverse coordinate, and $N$ is the number of transverse pixels in the orthogonal polarization image for which the OCT signal exceeds the average value of noise $\langle P_{\text{noise}} \rangle$ + doubled standard deviation $2\sigma_{\text{noise}}$. The system noise was calculated from the OCT signal of the areas where the useful signal is absent. Such areas were selected at the top or at the bottom of the orthogonal OCT image; the area width was selected equal to the whole width of the image while its height varied from 20 to 50 pixels depending on the image.
origin. Average noise value and its standard deviation were calculated from the OCT signal values within this area.

3 Results

3.1. Visual assessment of CP OCT images and PSR preparations

Figure 2 shows the CP OCT and corresponding picrosirius red histology results for the normal (Group 1) and the six pathology groups, highlighting the collagen state in the connective tissue stroma and in urothelium: different degree of excessive accumulation of CF (fibrosis) as a result of inflammation (severe fibrosis, Group 2), wound healing (scar tissue, Group 3), CF degradation at acute inflammation (Group 4), carcinoma in situ (Group 5), transitional cell carcinoma I-IIa (Group 6), and recurrence of carcinoma on the scar (Group 7).

Orthogonal polarization OCT image of the normal bladder mucosa indicates depolarization of incident radiation, which we believe is caused mainly by CF. The signal, is bright and heterogeneous, visualized as thin horizontal layers (Fig. 2, Group 1, upper image). On PSR preparations, these appear as bright red, consistent with their structural integrity and predominant amount of type I collagen. The fibers are located loosely and randomly (Fig. 2, Group 1, lower image). The average thickness of single fiber CF was 3.9 ± 0.8 µm according to the higher magnification view (not shown).

The clinical aims of this study were to find ways of using CP OCT to differentiate pathological collagen accumulation in bladder connective tissues (systemic fibrosis), and to quantify scar imaging (including its carcinoma progression). Analysis of the structural organization of CF at fiber and tissue levels in the ECM fibrosis groups (2 and 3) showed that high CP OCT signal is caused by excessive synthesis and accumulation of CF (its assembly / bunching in the background of reduced degradation) (Fig. 2, Groups 2 and 3, upper images). Scar tissue in orthogonal polarization appears bright (Fig. 2, Group 3, upper image) cause the size and abundance of CF bundles has increased significantly in scar (Fig. 2, Group 3, lower image) whereas the brightness from diffuse fibrosis tissue formed as the beginning of chronic inflammation are not so expressed because of presence thinner and not so densely packed CF and collagen bunches (Fig. 2, Group 2).

Analysis of the acute inflammation (Group 4), carcinoma in situ (Group 5), transitional cell carcinoma I-IIa (Group 6) and recurrence of carcinoma on a post-operation scar (Group 7) revealed states different degrees of CF degradation (Fig. 2, Group 4-7, upper images). PSR
Polarized microscopy showed that at acute inflammation and neoplasia the samples look dark, and CFs are thin of pale-green color. The majority of CF are destroyed or they are in the state of mucoid and fibrinoid swelling (Fig. 2, Group 4-7, lower images). These processes lower the brightness of orthogonal OCT signal compared to the normal structure (Fig. 2, Group 4-7, upper images). In case of invasion of transitional cell carcinoma into the connective tissue stroma (Group 6), nearly complete loss of OCT signal in orthogonal polarization is observed (Fig. 2, Group 6, upper image).

Based on the qualitative considerations above, it appears that morphological and polarization changes of CF properties in various pathological states are complex and interrelated. However for a more robust and objective assessment, a rigorous quantitative approach is needed as potentially offered by IDF analysis.

3.2. Quantitative assessment of CP OCT images with IDF

Average IDF values from the CP OCT results for the seven cohort groups are presented in Table 2 and in Fig. 3.

IDF analysis revealed statistically significant differences of CF increase at severe fibrosis which is typical of chronic cystitis (Group 2), as well as excessive accumulation of CF in scar tissue (Group 3) compared to normal (Group 1) (p<0.05) (Fig. 3(a)). The three groups of CP OCT images corresponding to histological states of acute inflammation (Group 4), carcinoma in situ (Group 5) and transitional cell carcinoma I-IIa (Group 6) also exhibit IDF values which significantly different from normal. It is important that IDF values between groups of acute inflammation and carcinoma and between group of severe dysplasia of epithelium and carcinoma have statistically significant difference (Table 2, Fig. 3(b)). The threshold value of IDF for diagnosing neoplasms in suspicious bladder cites was 0.05 (interval 0.04-0.06). It thus appears that IDF analysis of CP OCT images can detect transitional cell carcinomas including its carcinoma in situ stage.

### Table 2. Results of IDF calculation (M ± SD: mean ± standard deviation) and their confidence intervals (95% CI = M ± 2S) for CP OCT images of bladder mucosa in the normal and the six pathology groups. N refers to the number of analyzed OCT image sets in each group

| Group | IDF in normal (n = 10) | IDF in case of excessive accumulation of CF (n = 18) | IDF in cases of CF degradation (n = 14) |
|-------|-----------------------|-----------------------------------------------|------------------------------------|
| 1     | 0.14 ± 0.02          | 0.19 ± 0.02*                                  | 0.05 ± 0.02*                       |
| 2     | 0.03 ± 0.03          | 0.27 ± 0.05*                                  | 0.02 ± 0.01*                       |
| 3     | 0.08 ± 0.03*         | 0.03 ± 0.03*                                  | 0.03 ± 0.01*                       |
| 4     | 0.05 ± 0.02*         | 0.04-0.06                                      | 0.03-0.04                          |
| 5     | 0.02 ± 0.01*         | 0.06-0.12                                      |                                    |
| 6     | 0.03 ± 0.01*         | 0.05 ± 0.05*                                  |                                    |
| 7     | 0.09 ± 0.05*         | 0.06-0.12                                      |                                    |

Group 1 – normal, Group 2 – severe fibrosis, Group 3 – scar tissue, Group 4 – acute inflammation, Group 5 – carcinoma in situ, Group 6 – transitional cell carcinoma I-IIa, Group 7– recurrence of carcinoma on the post-operation scar.

* — statistically significant difference compared to the CF state in normal, p<0.05 (Mann-Whitney test)

° — statistically significant difference between Groups 6 and 4, 6 and 5, 7 and 3, p<0.05 (Mann-Whitney test)

We also used multiple comparisons with the Bonferroni correction for 3 and 4 groups clustered for different clinical tasks (as illustrated in Fig. 3(a), 3(b)), and estimated statistically significant differences between groups.
The results of comparing IDF in two groups of carcinoma recurrence on the scar (Group 7) and the normal scar tissue (Group 3) are represented in Table 2 and in Fig. 3(c). A statistically significant difference in IDF values between the two groups is seen (\(p<0.0001\)), which testifies to the possibility of effective cystoscopic recognition of carcinoma on the post-operation scar with the IDF / CP OCT platform. It is known that CF are destroyed under the influence of tumor cell enzymes [6]; this likely decreased the resultant orthogonal-polarization OCT signal. The quantitative assessment of CP OCT images obtained from the suspicious zones of post-operation scars can thus help distinguish between scar-only and scar + tumor regions.

Sensitivity, specificity and diagnostic accuracy of quantitative assessment of CP OCT images with IDF in separating clinically relevant pathological processes in the bladder are presented in Table 3.

Table 3. Diagnostic effectiveness of the quantitative assessment of CP OCT images with IDF for separating clinically relevant pathological states in bladder mucosa

| States of CF                                      | Threshold level of IDF | Sensitivity (%) | Specificity (%) | Diagnostic accuracy (%) |
|--------------------------------------------------|------------------------|-----------------|-----------------|-------------------------|
| Benign states together (severity of fibrosis)     | weak fibrosis vs normal (Group 2 vs Group 1)  | 0.18            | 72              | 90                      | 79                      |
| Benign and malignant states of the bladder       | neoplasia vs acute inflammation (Groups 5 + 6 vs Group 4) | 0.05            | 86              | 68                      | 75                      |
|                                                  | recurrence of carcinoma on the scar vs scar (Group 7 vs Group 3)  | 0.12            | 93              | 99                      | 97                      |

IDF values for all pathological states were compared to the value for normal. The diagram of the IDF values is represented in Fig. 4. We note that with excessive accumulation of CF, the IDF increases by 29% (Group 2 – severe fibrosis), by 86% (Group 3 – scar). In the opposite case, with degradation of CF there is IDF decrease by 48% (Group 4 – acute inflammation), by 63% (Group 5 – carcinoma in situ), by 76% (Group 6 – carcinoma with initial invasion), by 38% (Group 7 – recurrence of carcinoma on the scar).
It thus appears that the quantitative assessment of the state of the connective tissue stroma of bladder mucosa in CP OCT images yields objective diagnostic criteria of neoplasia (compared to the qualitative and subjective visual assessment). The integral depolarization factor (IDF) is a sensitive metric for quantifying mucosal CP OCT images, and specifically reflects the tissue collagen compartment. Differential diagnosis of clinically relevant pathological states of bladder mucosa may become possible.

4. Discussion

Tissue fibrosis is an excessive accumulation of collagen and other connective tissue components, resulting in imbalance of normal ECM synthesis and degradation processes. Further, fibrosis is morphologically manifest by pathological thickening of CF and their assembly into bunches [27–30]. Evidently, intensity of cross-polarized coherent backscatter as detected by CP OCT depends on the degree of CF organization as influenced by physical processes of packing, thickening and orientation/ordering.

The IDF values demonstrate increase in case of fibrosis development; this agrees with recent results that show abnormal deposition of connective tissue during bladder fibrosis, including an increase in total collagen [27]. Observation of fibrosis development as indicated by the current CP OCT study is of great clinical significance and represents a presently-unmet clinical need. This is because clinical studies show that long-term chronic bladder inflammation can cause interstitial cystitis and total fibrosis of the whole organ, with decrease in its volume and functional morbidity, which require surgical interference [31].

The detection of post-radiation reactions, which commonly included bladder fibrosis, is another clinical scenario where quantitative CP OCT may play a role. The danger of post-radiation fibrosis lies in the corresponding high probability of neoplastic processes, in addition to the decrease in patients’ life quality and the necessity of long-term management / treatment [32]. Radiation fibrosis in humans is associated with increased collagen synthesis, altered remodeling and sequential activation of key fibrogenic growth factors and cytokines, including TGFβ1 and CTGF [33]. Unregulated expression of collagens at mRNA and protein levels was reported in human gastro-intestinal tract 1–75 months post-radiotherapy in 22 patients [34]. Timely diagnosed progressing fibrosis of the bladder stroma can help adjust / guide the therapy and avoid undesirable consequences. CP OCT with IDF calculation can be a
minimally invasive method of monitoring fibrosis development and treatment-induced regression.

Inflammation processes are characterized by degradation of collagen fibers. The crucial contribution is provided by matrix metalloproteinase. In its degraded / disorganized state, collagen is not capable of effective depolarization and becomes less visible in orthogonal OCT images; then the CP OCT signal brightness becomes a sensitive tool for quantifying the presence of the preserved or newly synthesized organized collagen.

We posit that in case of tumor invasion, a sharp decrease in OCT signal in orthogonal polarization is caused by overgrown carcinoma cells and active degradation of collagens of basal membrane and sub-epithelial stroma under the effect of collagenase (produced by carcinoma cells) (Fig. 2) that facilitates the process of tumor infiltration. It is known that tumor often appears at the background of recurrent inflammation, therefore it is clinically important to differentiate severe dysplasia of epithelium and a developed transitional cell carcinoma from acute inflammation.

Previous OCT quantification studies in the bladder deserve a brief mention. In the study by Gladkova et al [19], quantitative processing of CP OCT images with calculation of standard deviation index (SD index) was performed. When the threshold level of CP OCT SD index was used (4.3 dB), differentiation of malignant and benign lesions achieved sensitivity of 96.4%, specificity of 92.1%, diagnostic accuracy of 93.6%. Quantitative analysis of CP OCT SD index increased the sensitivity by better identification of carcinoma in situ and reduction of false negative cases. However, manual selection of the region of interest was required, a subjective procedure that decreased the result objectivity compared to the current study. In the study by Ren [35] quantitative processing of 3D OCT images was used to recognize carcinoma in situ using segmentation, blurring of speckles, fast Fourier transforms, and assessment of standard deviation and histograms of brightness. It was revealed that on progressing of neoplasia there is increase in architectural heterogeneity of urothelium. Sensitivity and specificity for diagnosing carcinoma in situ in 2-dimension OCT images turned to be considerably lower (56.5% and 61.5%, respectively) than the same parameters on quantitative assessment of 3-dimensional OCT images (95.7% and 92.3% respectively, p < 0.031). However, these data were obtained on bladders of transgenic mice ex vivo with the use of non-endoscopic OCT system. In contrast, our CP OCT + IDF quantification platform suitable for in vivo human cystoscopy yields comparable / better robust results and is well positioned for routine clinical deployment.

In this paper, we propose IDF for evaluation of collagen content / organization in connective tissue, where the presence of increasing amounts of collagen leads to increased levels of depolarization and thus changes IDF. Conversely, lower collagen content (e.g., adipose tissue, blood compartment), and / or tissues with high well-ordered collagen content that exhibit strong birefrigence (tendon, ligaments, sclera) do not cause extensive depolarization. Overall then, the IDF parameter is useful for evaluating tissue depolarization properties, and its changes may be linked to collagen content and organization.

5. Conclusion

Analysis and quantitative assessment of CP OCT images confirmed the presence of depolarizing properties of connective tissue stroma in human mucosa that are determined by well-defined spatial and structural organization of collagen matrix. Collagen fibers undergo significant changes as a result of pathological processes which affect their depolarizing properties as detected and quantified in vivo with CP OCT.

An approach to quantitative, robust and potentially automated assessment of CP OCT images reflecting the state of bladder collagen fibers was developed. It was shown that IDF can be applied to in vivo detected clinically relevant pathological states in urology. The initial clinical results indicate IDF diagnostic accuracy in severe fibrosis identification of bladder mucosa (relative to normal) is 79%, while differentiation between neoplasia of the bladder...
and acute inflammation with IDF yields a diagnostic accuracy of 75%. Differentiating recurrence of carcinoma on a post-operation scar from tumor-free scar resulted in a diagnostic accuracy of 97%. We thus show the promising potential of CP OCT and IDF quantification to visualize and quantify the structural and spatial organization of CF in bladder tissues for clinical disease assessment including differential diagnosis applications.

Acknowledgments

The authors acknowledge support of the Russian Federation Government contract No 14.B25.31.0015 for Leading Scientists to Russian Educational Institutions.