Imaging the Human Prostate Gland Using 1-μm-Resolution Optical Coherence Tomography

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Context.—The accuracy of needle biopsy for the detection of prostate cancer is limited by well-known sampling errors. Thus, there is an unmet need for a microscopic screening tool that can screen large regions of the prostate comprehensively for cancer. Previous prostate imaging by optical coherence tomography (OCT) has had insufficient resolution for imaging cellular features related to prostate cancer. We have recently developed micro-optical coherence tomography (μOCT) that generates depth-resolved tissue images at a high frame rate with an isotropic resolution of 1 μm.

Objective.—To demonstrate that optical images obtained with μOCT provide cellular-level contrast in prostate specimens that will enable differentiation and diagnosis of prostate pathologies.

A definitive diagnosis of prostate cancer is currently made by transrectal needle biopsy. Although this practice has been the standard of care for decades, the limitations of needle biopsy continue to be a major impediment to achieving effective management of prostate cancer. The most common biopsy protocol involves random excision of 12 distinct cores of tissue from the apex, mid, base, and lateral sides of the gland.1 Because those cores are very small (0.08 cm diameter by 1.2 cm long),2 only a small portion (approximately 0.1%) of the gland is sampled, resulting in many cancers being missed.3 Even for patients with biopsy-proven cancer, sampling error may lead to significant uncertainty about histopathologic grade (Gleason score), which is an important factor that governs prognosis, entry into active surveillance programs, and selection and timing of crossover to intervention.3–11 The rate of change of tumor grade and/or size may be an indicator of tumor aggressiveness and therefore a means for stratifying indolent from aggressive cancers. These rates are currently difficult to assess because the tissue under investigation is removed and rebiopsies are inherently excised at different locations from the original biopsy. Finally, there is increasing interest in focal therapy of neoplastic prostate lesions,12–15 where instead of treating the entire gland, the tumor is ablated locally via cryotherapy, radiofrequency, or high-frequency ultrasound, sparing the vessels and nerves that can be damaged during radical prostatectomy. Because biopsies and noninvasive imaging are unable to visualize the microscopic boundaries of those tumors, those processes leave open the possibility that residual disease will be left behind.15 These limitations of prostate needle biopsy underlie a critical barrier in this field: The diagnosis, management, and treatment of prostate cancer is hindered by our inability to comprehensively visualize the entire prostate gland at the microscopic level in vivo.

Optical coherence tomography (OCT) is an analog to ultrasound that uses light to obtain depth-resolved images of tissue with an axial resolution of 10 μm.16,17 The high-resolution, cross-sectional imaging capabilities of that technology make it a possible solution for whole prostate microscopy in vivo.18 Early reports of prostate imaging by OCT ex vivo suggested that prostate adenocarcinoma can be visualized at the microscopic scale in situ.19–21 These images were obtained using slower and less sensitive first-generation OCT technologies, providing penetration depths ranging from approximately 0.75–1.5 mm.19–21 More recent data using commercially-available second generation swept source OCT (SS-OCT) technology have shown the potential

Design.—Fresh prostate specimens obtained from surgical resections were scanned with μOCT ex vivo. Histologic features in the μOCT images were correlated to the corresponding conventional histology.

Results.—Findings indicate that μOCT is capable of resolving many of the architectural and cellular features associated with benign and neoplastic prostate.

Conclusions.—Because μOCT can be implemented in a small-diameter flexible probe, this study suggests that high-resolution μOCT imaging may be a useful tool for needle-based virtual biopsy of the prostate gland.

of OCT to obtain virtual biopsy cores through a needle. The image resolution (10 μm axial and 30 μm lateral) of OCT used in those reports was insufficient to visualize cells; therefore the investigators extracted the attenuation coefficient from the OCT image as a proxy for visualizing morphologic markers of cancer. Higher-resolution full-field OCT was shown to be capable of providing exquisitely detailed images of prostate architectural and cellular microscopic morphology but provided en face images at a rate that was too slow for practical in vivo imaging of large tissue volumes. In this article, we present prostate images using a new form of OCT termed micro-optical coherence tomography (μOCT).

Figure 1. A, Micro-optical coherence tomography (μOCT) imaging of benign prostate glands obtained from fresh tissue ex vivo. Epithelium is demarcated by red arrow. The μOCT image dimensions are 1 mm by 0.5 mm. B, Corresponding histopathology (hematoxylin-eosin, displayed magnification x15).

Figure 2. A, Micro-optical coherence tomography (μOCT) image of a benign prostate gland containing secretions (*) obtained from fresh tissue ex vivo. The epithelium can be clearly visualized (arrow). The μOCT image dimensions are 1 mm by 0.5 mm. B, Corresponding histopathology with the epithelium highlighted (arrow) (hematoxylin-eosin, displayed magnification x15).

Figure 3. A, Micro-optical coherence tomography (μOCT) image of benign prostate gland containing corpora amylacea (arrows). The μOCT image dimensions are 1 mm by 0.5 mm. B, Corresponding histopathology with the epithelium highlighted (arrows) (hematoxylin-eosin, displayed magnification x15).

Figure 4. A, Micro-optical coherence tomography (μOCT) image of benign prostate gland showing faint evidence of cell membranes (arrows). The μOCT image dimensions are 1 mm by 0.5 mm. B, Corresponding histopathology (hematoxylin-eosin, displayed magnification x15).
tomography (µOCT), which, with a high frame rate and image resolution of 1 to 2 µm, is potentially capable of being used to acquire images of the prostate through a needle at cellular-level resolution.

**METHODS**

**µOCT System**

The benchtop µOCT system used in this study has been previously described. Briefly, µOCT is conducted with a spectral-domain OCT system with a broad bandwidth supercontinuum light source spanning from 650 to 950 nm, which provides an axial resolution of less than 1 µm in tissue. Light from the broadband source enters a common path interferometer and is split into a light source spanning from 650 to 950 nm, which provides an axial-domain OCT system with a broad bandwidth supercontinuum to create depth-resolved reflectivity profiles (A-scan). A 3-dimensional µOCT data set is created by recording multiple A-scans as the sample beam is scanned along 2 lateral dimensions.

**Prostate Acquisition**

Prostate specimens were acquired from either the Pathology Service at Massachusetts General Hospital (Boston) or the National Disease Research Interchange (Philadelphia, Pennsylvania). Prostate specimens acquired from the Massachusetts General Hospital Pathology Service were collected from patients undergoing whole or partial surgical resections of the prostate. After review of the organ, discarded deidentified surgical specimens were collected for this study, placed in phosphate-buffered saline solution, and imaged within 24 hours of resection. This ex vivo imaging study was approved by the Partners HealthCare-Somerville Institutional review board (No. 2010P0000845). Whole prostate organs received from National Disease Research Interchange were recovered at autopsy from men older than 70 years who did not have a history of prostate surgery, prostate implants, or other prostatic surgical procedures. Specimens were recovered within 24 hours after death, placed in phosphate-buffered saline solution, and shipped on wet ice. Specimens were imaged in less than 48 hours after death to limit tissue degradation. This National Disease Research Interchange study was approved under a separate protocol (No. 2013P001310).

Before imaging, whole prostate specimens were “breadloafed”: orthogonal to the urethra in approximately 1-cm sections. Sections were visualized grossly to identify diseased features such as nodules or discoloration of the parenchyma. Regions of interest were cut from the involved tissue. All specimens were trimmed to provide a clean front-imaging plane and a second orthogonal cut was made on the left edge as a means for registering the tissue between µOCT imaging and subsequent histology. After tissue preparation, the prostate specimens were placed on phosphate-buffered saline-soaked gauze before imaging.

**µOCT Imaging**

Each prostate specimen was placed on a 5-axis stage with the front edge of the specimen aligned with the fast-scanning axis and imaged at room temperature. Because of the depth range of µOCT (approximately 300–500 µm), the scanning beam was advanced approximately 1 mm into tissue from the front edge and the first scan was acquired on the side with subsequent scans moved to the left. Using galvanometer beam scanners, the µOCT system acquired 512 frames over a 1-mm by 1-mm area. All µOCT image stacks were frame averaged (n = 3) and saved using a logarithmic gray-scale look-up tables.

**Histology**

After imaging, a small ink spot was placed on the back left corner of each specimen. To preserve the orientation during histologic processing, both the bottom side and left edge were painted with tissue-marking ink. Specimens were placed in 10% buffered formalin for a period of not less than 48 hours and submitted for routine paraffin-embedded histology. During embedding, processed specimens were oriented such that the histology sections were in the same plane as the images. Tissue sections (5 µm thick) were captured for hematoxylin–eosin histology at level spacing of 100 µm for up to 20 sections. All histology slides were digitized with a whole slide scanner (Nanozoomer, Hamamatsu Photonics, Hamamatsu, Japan) at a native resolution of ×40.

**µOCT-Histology Matching**

Mosaic images of digitized histology sections and µOCT image stacks from different sites on the same tissue were created in ImageJ (US National Institutes of Health, Bethesda, Maryland), which allowed all histology images be viewed and compared with µOCT image stacks. For each specimen, a diagnosis was rendered by a pathologist. Then, the mosaic of µOCT image stacks was reviewed to identify landmark features, which could then be searched for within the histology mosaic. When matches were not exact, the match was termed representative. Once corresponding features were identified, the appropriate histology section was determined and opened with the Nanozoomer NDPVIEW (Hamamatsu Photonics) application for visualizing the digitized histology at high resolution, so that the scale was approximately the same as that of the µOCT data. High-resolution images of both the matching µOCT and digitized histology frames were selected as matches. Features in the matching data sets were identified and annotated on the histology and matching µOCT image data.

**RESULTS**

A total of 18 samples were excised from 11 specimens from the human prostate resections. Three whole prostate specimens were obtained at autopsy, generating an additional 32 samples. Per histopathology assessment, 7 (14%) of the 50 samples contained prostate cancer, and 43 samples (86%) were benign.

**Benign Prostatic Parenchyma**

Benign prostate imaging by µOCT was characterized by features that include prostatic glands that were separated by large amounts of prostate stroma (Figure 1, A and B), the presence of large amounts of prostatic secrections (Figure 2, A and B), and corpora amylacea (Figure 3, A and B). The epithelium of the prostate was frequently seen as a moderately backscattering layer lining the glands (arrows in Figures 1, A and B; 2, A and B). Sometimes, cell membranes could be faintly visualized (Figure 4, A and B), but this feature was not commonly identified. It was not possible to clearly differentiate basal from luminal epithelial cells.

**Prostate Cancer**

Figure 5, A depicts a µOCT image of high-grade (Gleason score 4 + 4) invasive prostate cancer adjacent to corresponding histology (Figure 5, B). Individual cancer cells can be visualized in the µOCT image as clear open spaces with little µOCT signal. When compared with histology, those cells exhibit clear cell changes, which is an appearance that is seen for some prostate cancers. The µOCT and histology images also show an excellent example of corpora amylacea (red arrow) which serves as a fiducial marker for both µOCT and histology images.

A µOCT image of a Gleason grade 3 + 4 nodule of prostate cancer is shown in Figure 6, A, with corresponding histology (Figure 6, B and C). The cancer can be seen by µOCT as a nodule within surrounding uninvolved prostatic
Figure 5. A, Micro-optical coherence tomography (μOCT) image of prostate cancer reformatted from a 3-dimensional data set in an oblique plane obtained from fresh tissue ex vivo. The μOCT image dimensions are 1.5 mm by 1.0 mm. B, Corresponding histopathology. Red arrowheads in each image correspond to the corpora amylacea (hematoxylin-eosin, displayed magnification ×15).

Figure 6. A, Micro-optical coherence tomography (μOCT) image of prostate cancer. The μOCT image dimensions are 1 mm by 0.5 mm. B, Corresponding histopathology. C, High-power corresponding histology. Red arrows point to the prostate cancer. Yellow arrows (A) and blue arrows (C) correspond to structures consistent with crystalloids that are commonly found in prostate cancer (hematoxylin-eosin, original magnifications ×15 [B] and ×35 [C]).
parenchyma (red arrows in Figure 6, A and B). Linear structures in the μOCT image (yellow arrows in Figure 6, A) are likely crystalloid structures that are frequently seen in prostate cancer, as also seen in the corresponding histology (blue arrows in Figure 6, C).

DISCUSSION

The preliminary results of this study provide information on the potential for use of μOCT to diagnose prostate cancer. High-quality image data were obtained at a penetration depth up to 500 μm. Invasive cancer, clear cell changes, cancer-related crystalloids, and corpora amylacea were evident by μOCT and were confirmed by the corresponding histopathology. Our findings show that the micrometer-scale resolution of this technology does indeed provide cellular-level detail that is diagnostically relevant. The improvements afforded by the high resolution and relatively high imaging speed of this technology make it a promising candidate for large area needle-based prostate diagnosis.

Even though the results presented here are encouraging, there are many cellular features seen by conventional brightfield histopathology that are not distinguishable by μOCT. Nuclei are not clearly discernable from cytoplasm and basal cells cannot be differentiated from luminal cells. These limitations of μOCT as currently configured could make it difficult to discriminate certain benign from malignant prostate glands based on standard histomorphometric patterns. As a result, clearer visualization of architectural morphology and associated findings (eg, crystals) seen uniquely by the higher-resolution technology will govern the differences in diagnostic performance between μOCT and conventional OCT.

The μOCT-imaging penetration depth found in this study ranged from 300 to 500 μm. Although that penetration depth is greater than that seen with other in vivo microscopy modalities (eg, confocal microscopy), it is insufficient for imaging the entire prostate with a realistic number of needle insertions, nominally 24. It is well known that OCT imaging at longer wavelengths increases penetration depth significantly and imaging within the 1.3 μm or 1.6 μm spectral windows will likely double or triple that value. These considerations suggest that the development of μOCT at longer wavelengths is merited.

The true potential of μOCT can only be investigated when this technology is implemented in a small-diameter probe that is capable of being inserted into a prostate biopsy needle. By providing in vivo microscopy images through a needle for large regions of the prostate in real time, this advance could significantly decrease biopsy sampling error with or without ultrasound or magnetic resonance imaging-based28 image guidance. Recently, methods for creating such high-resolution probes have been reported,27,29 making the translation of needle-based μOCT possible. Once μOCT is implemented in vivo, the findings in this study can be confirmed, and the potential utility of this imaging modality for comprehensive in situ prostate diagnosis can be validated clinically.

References


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