Three-dimensional endomicroscopy of the human colon using optical coherence tomography

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Abstract

Three-dimensional (3D) endomicroscopy imaging of the human gastrointestinal tract is demonstrated in vivo using a swept source optical coherence tomography (OCT) system. 3D datasets of normal and pathologic regions of the colon, rectum, and anal verge were obtained from seven volunteers undergoing diagnostic or therapeutic colonoscopy. 3D-OCT enables high resolution endomicroscopy examination through visualization of tissue architectural morphology using virtual cross-sectional images with arbitrary orientations as well as en face projection images. Axial image resolutions of 6 μm in tissue are obtained over a ~180 mm² field with an imaging range of 1.6 mm. A Fourier domain mode locked (FDML) laser providing a tuning range of 180 nm at a sweep rate of 62 kHz is used as the system light source. This clinical pilot study demonstrates the potential of 3D-OCT for distinguishing normal from pathologic colorectal tissue, assessing endoscopic therapies and healing progression.

1. Introduction

Optical coherence tomography (OCT) is a high-resolution biomedical imaging modality that produces depth-resolved images of tissue microstructure by interferometrically measuring the amplitude and echo time delay of light backscattered from a sample [1]. Since imaging can be performed using fiber optics and miniaturized lens systems, OCT is amenable to use inside the human body in conjunction with conventional video endoscopy. An OCT probe can be inserted down the working channel of a standard endoscope, enabling two-dimensional (2D) depth-resolved imaging of tissue microstructure over a linear dimension of several millimeters to several centimeters with micron-scale resolutions [2].

Gastroenterology is an especially relevant application for OCT due to the high incidence rates of gastrointestinal (GI) pathologies, the clinical benefits of early detection, and the need for pre- and post-treatment assessment of therapies. Endoscopic 2D-OCT has been applied extensively for GI studies [3-5] with particular emphasis on the early detection of cancer in Barrett’s esophagus patients [6-11]. Endoscopic 2D-OCT has also been used to study inflammatory bowel diseases in the colon [12]. These studies demonstrated that OCT can be
a valuable tool for gastroenterologists, with one major goal being the guidance of excisional biopsy to reduce sampling errors and false negative rates associated with the procedure. One significant drawback, however, is the restriction of 2D-OCT analysis to tissue features lying in the cross-sectional plane of the image. This precludes characterization of three-dimensional architectural morphology and makes registration of cross-sectional images with en face features difficult. Additionally, the collection of single cross-sectional images can make 2D-OCT prone to the same random sampling errors that affect excisional biopsy.

Recent advances in ultrahigh-speed OCT using Fourier domain detection techniques [13-15] have enabled in vivo 3D-OCT with imaging speeds of up to 370,000 axial lines per second [16]. By developing miniaturized probes that scan in two spatial dimensions, endoscopic 3D-OCT can be performed. 3D-OCT overcomes the limitations of 2D analysis by enabling comprehensive visualization of tissue microstructure over larger fields of view. For GI applications, endoscopic 3D-OCT has previously been demonstrated in the pig [17] and human esophagus [18] using a wavelength-swept polygon scanning laser and balloon catheter. This system operated at 5,000 – 40,000 axial lines per second (5 – 40 kHz) and achieved axial resolutions of 8 μm with a transverse spot size of 20 μm [18]. The human upper airway has been imaged using a commercial wavelength-swept laser and MEMS micro-motor catheter, acquiring axial lines at 20 kHz with axial resolutions of 8 μm and transverse spot sizes of 20 μm [19]. Using a Fourier domain mode locked (FDML) laser [16,20-22] and proximally actuated spiral scanning probe, the rabbit colon has been imaged at 100 kHz with axial resolutions of 7 μm and a transverse spot size of 9 μm [23].

Here we show results of a clinical pilot study using endoscopic 3D-OCT in the human colon in vivo. An FDML laser operating at a sweep rate of 62 kHz is combined with a high-speed data acquisition and image processing system (LightLab Imaging Inc.) and spiral-scanning fiber optic probe to capture cross-sectional images at 30 – 60 frames/s. The speed advantage achieved by the system is used to acquire densely-sampled datasets, enabling 3D visualization of features as small as single colonic crypts. 3D endomicroscopy and the 3D evaluation of microstructural tissue features is therefore possible with this system. 3D data sets enable the generation of en face images, enabling precise registration of cross-sectional data with en face features. Projection techniques can be used to selectively display specific depths within the tissue, enhancing image contrast for specific architectural features. Cross-sectional images with arbitration orientation can also be generated from the 3D data. A range of tissue types are analyzed, including normal colon, normal anal verge, ulcerative colitis and radiation proctitis. In one case, the same volunteer was imaged at multiple time points following endoscopic therapy to assess the ability of OCT to track the healing process. Overall, this study demonstrates the potential of 3D-OCT endomicroscopy for distinguishing normal from pathologic colorectal tissue, assessing endoscopic therapies, and evaluating healing progression.

2. Imaging system

The 3D-OCT endomicroscopy system used in this study is similar to the setup previously described by our group [23]. The system was developed in collaboration with LightLab Imaging and uses swept source OCT detection to achieve fast imaging speeds and high sensitivity. An FDML laser with a center wavelength of 1310 nm, a total tuning range of 180 nm, a full-width-half-maximum bandwidth of 122 nm, and an average output power of 42 mW at a sweep rate of 62 kHz is used as the wavelength-swept light source. The laser sweep rate was decreased compared to our previous system [23] in order to improve sensitivity and imaging range for imaging in human tissue. The laser supports an axial resolution of 6.5 μm in air (4.7 μm in tissue). System sensitivity with 13 mW of power incident on the sample is 105 dB at short delays and drops by 7.8 dB at a ranging depth of 2 mm in air. This exposure

Opt Express. Author manuscript; available in PMC 2010 June 16.
level is within the guidelines established in ANSI standard Z136.1-2000 and is consistent with optical power levels used in commercial endoscopic and cardiovascular OCT instruments.

The system uses the interference fringes detected from a Mach-Zehnder interferometer with a fixed path imbalance as an optical frequency clock, triggering the data acquisition card at evenly-spaced optical frequency intervals and precluding the need for software recalibration of the OCT signal [23]. For each axial line, 720 samples are acquired followed by a 1024 point software Fourier transform and logarithmic conversion. The measured axial resolution of the system is ~6 μm in tissue following data acquisition and processing. The resolution loss compared to the value supported by the laser is predominantly due to phase jitter in the optical frequency clock. The maximum imaging measurement range supported by the system was 2.2 mm in air (1.6 mm in tissue) and was limited by the bandwidth of the 200 megasample/s digitizer card. In practice, this range is sufficient to reach the limit where multiple scattering events and optical attenuation prevent visualization of deeper structures. The system performance represents a 7 dB increase in sensitivity and a 200 μm increase in imaging range compared to the 100 kHz configuration previously reported by our group [23]. These improvements are due to the increased tissue exposure time and lower interference fringe frequencies associated with a slower sweep rate.

The imaging probe is similar to the one previously described by our group [23], except the sheath thickness was increased for better mechanical stability. This probe outer diameter was 2.2 mm and was sufficiently small to fit down the working channel of a standard gastroscopy or colonoscope to enable simultaneous video endoscopy and 3D-OCT endomicroscopy examination of the tissue. A fused fiberoptic lens system produces a ~12 μm spot and an angle polished lens tip is used to direct the beam out the side of the sheath. The probe is rotated rapidly at 30 – 60 Hz while being pulled back at 0.5 – 1.0 mm/s along the sheath, producing a spiral volumetric scan pattern. The rotational and pullback speeds can be varied to adjust the spatial sampling density and field of view of the system. Visualization of small features requires increased sampling density, so the system parameters were typically set to provide 740 to 1200 axial lines per rotational frame, corresponding to a transverse line spacing of 7 – 12 μm (measured ~200 μm beneath the tissue surface) and frame-to-frame spacing of 8 – 12 μm. Approximately 850 data points were acquired during each laser sweep. Zero-padding was used to increase the axial line record length to 1024 points, followed by a 1024 point software FFT. Following removal of the redundant FFT mirror image, the 512 remaining sample points gave an axial pixel spacing of 3 μm.

Volumetric data sets were acquired with a real-time frame-by-frame preview, then stored to the computer hard drive and processed offline. During acquisition, images were displayed in a radial format consistent with the rotational motion of the imaging catheter. This is more intuitive for the endoscopist than displaying data in rectangular frames. Data post-processing consisted of several steps. First, each radial frame was converted to a rectangular frame by unwrapping the data at one transverse position. Next, the outer surface of the sheath was isolated using a Sobel edge detection algorithm. A polynomial was fit to the sheath surface and used to flatten each frame. This flattening step is necessary for accurate visualization of en face image features. Each frame is then stored as a grayscale JPEG file and exported to commercial 3D rendering software (ResolveRT, Mercury Computer Systems) for further analysis. Single frames oriented in arbitrary planes can then be extracted from the 3D set. Averaging the data over ~20 μm thick sections significantly reduces speckle noise while preserving relevant image features, as previously described [23], and this technique was used in the data presented below unless otherwise specified. In general, 3D-OCT analysis enables a range of powerful image processing techniques that can enhance visualization of tissue microstructure.

*Opt Express.* Author manuscript; available in PMC 2010 June 16.
3. Clinical pilot study results

The clinical pilot study was conducted at the Veterans Affairs Boston Healthcare System (VAHS), Jamaica Plain Campus. Informed consent was obtained from each volunteer and the study was conducted in accordance with protocols approved by the VAHS, Harvard Medical School, and the Massachusetts Institute of Technology.

3.1 Normal colon

For the first phase of the study, four healthy male volunteers who were undergoing routine screening colonoscopy were recruited. This recruitment was necessary to establish the 3D image properties of normal colorectal tissue. Representative data from one volunteer is shown in Figs. 1 and 2. Each volume is $7 \times 20 \times 1.6$ mm in dimension and was acquired in 20 s. To obtain maximum tissue coverage, air was removed from the colon using the colonoscope suction port immediately prior to 3D-OCT imaging. This collapsed the mucosa around the probe and ensured good tissue contact over the majority of the probe circumference. The same procedure was used throughout this study.

Figure 1(a) shows an en face image of normal glandular mucosal tissue formed by axial summation of the entire 3D logarithmic dataset. Summation over the full axial range of the volume maximally reduces speckle noise, while preserving only those image features that persist over significant depths. Macro-scale tissue folds are visible in the mucosal surface. A mottled texture is observed due to the presence of a regular, uniform crypt pattern in the epithelium. Crypts are the main glandular structures in the human colon, and changes in crypt size and appearance are associated with the earliest forms of colorectal cancer [24] and other diseases. The ability to assess the 3D structure of crypts is therefore of potential value for future applications in cancer detection and treatment. Figure 1(b) shows a cross-sectional image oriented along the XZ axis, which is the fast rotational axis during data acquisition. Consistent with previous results in ex vivo human tissue [25], optical transmission is increased through the crypt lumens. Figure 1(c) shows a cross-sectional YZ image, which is the slow pullback axis during data acquisition. Uniform crypt structures are also visible in this plane. Both cross-sections were formed by averaging consecutive slices over a 20 $\mu$m thickness to reduce speckle noise. Figure 1(d) shows an enlarged region of Fig. 1(a). The microstructural en face crypt pattern is clearly visualized and correlates well to the representative en face histology image shown in Fig. 1(e). Figure 1(f) shows a white light video endoscopy image taken near the OCT imaging site prior to removal of air from the colon. The mucosa appears regular and the crypt pattern cannot be distinguished by conventional endoscopy.

Figure 2 shows 3D-OCT endomicroscopy images of glandular and squamous mucosa in the rectum and anal verge of the same volunteer as in Fig. 1. Squamous mucosa is smooth, crypt-free tissue lining the anal verge. Figure 2(a) shows an en face image formed by axial summation of the entire dataset. Squamous epithelial tissue can be readily identified on the right hand side of the image. Glandular epithelium is visible on the left hand side of the image. The squamocolumnar junction, or dentate line, is the location at which the tissue transitions from anal squamous epithelium to rectal glandular (columnar) epithelium. Figure 2(b) shows an XZ cross-section through the squamous region, illustrating regular layered architecture with subepithelial anal vessels. Figure 2(c) shows a YZ cross-section. Crypt-filled glandular tissue and layered squamous tissue are clearly distinguishable, in addition to a transition zone between the two regions. The region near the dentate line is shown under white light video endoscopy in Fig. 2(d). Histology images of normal glandular mucosa and normal squamous mucosa are shown in Fig. 2(e) and Fig. 2(f), respectively. Since biopsy is associated with a slight risk of complications, and since the normal volunteers did not present with any pathology, biopsy specimens were not obtained. The histology images in Fig. 1 and Fig. 2 were obtained from representative normal regions adjacent to pathologic tissue excised from other patients.
As shown in Figs. 1 and 2, 3D-OCT endomicroscopy is capable of differentiating glandular and squamous epithelium in vivo. 3D-OCT also enables co-registration of cross-sectional images to anatomic landmarks such as the dentate line. This ability could be important for applications such as assessment of endoscopic therapies at multiple time points, since it provides an inherent positional registration that is difficult to obtain with 2D-OCT.

3.2 Ulcerative colitis

Ulcerative colitis (UC) is a chronic inflammatory condition of the GI tract that produces abscesses, ulcerations, and bleeding. UC affects up to 780,000 individuals in the United States and Canada and is newly diagnosed in 7000 – 46,000 individuals per year [26]. UC is associated with a ~5x increase in risk of developing colorectal cancer compared to the general population, with colorectal cancer accounting for one sixth of all deaths in UC patients [27]. Unfortunately, early-stage dysplastic lesions are often flat, diffuse and multifocal in these individuals [28]. As a result dysplastic lesions are easily obscured by the gross inflammatory background of UC, making early detection extremely challenging. UC and Crohn's disease, a related condition, therefore represent diseases that can potentially benefit from 3D-OCT endomicroscopy examination for detection of abnormalities and assessment of therapies.

Figure 3(a) shows a white light video endoscopy image of a patient with active UC. The mucosa appears red, inflamed and ulcerative. The 3D-OCT catheter is also shown in position prior to imaging. Representative histology of UC in glandular mucosa [29] is shown in Fig. 3(b). Lymphocytic mucosal infiltration is present along with submucosal fibrosis. Ulceration results in the formation of a pseudo-polyp as the epithelium is stripped away to expose the submucosa. 3D-OCT endomicroscopy images acquired in the rectum near the anal verge are shown from one volunteer in Fig. 4. The volume is $8 \times 20 \times 1.6$ mm in dimension and was acquired in 20 s. Figure 4(a) shows an en face image formed by axial summation of a 20 μm thick section centered 350 μm beneath the luminal surface. Compared to normal squamous and columnar mucosa shown in Figs. 1 and 2, UC tissue appears highly irregular. Large subsurface voids and bands of hyperscattering tissue, possibly fibrotic, are apparent. A wedge of comparatively normal tissue is visible at the right of the image. Figure 4(b) shows an XZ cross-section through the region revealing a regular layered architecture consistent with anal squamous mucosa. The epithelium is thickened compared to healthy individuals, possibly as a result of healing in response to prior treatment with anti-inflammatory medications. The ulcerated region exhibits a loss of layered or columnar architecture, superficial edema, and a large subsurface abnormality consistent with submucosal fibrosis. Figure 4(c) shows a YZ cross-section with UC and normal squamous tissue visible on the left and right sides of the image, respectively. Figure 4(d) and Fig. 4(e) show enlarged regions of Fig. 4(c). A complete flythrough of the volume is available as a linked multimedia file.

3D-OCT endomicroscopy enables visualization of microstructural differences between the UC and normal regions. Furthermore, the extended field of view allows for evaluation of macro-scale features, such as subsurface voids, as well as microstructural details such as superficial edema and the layered architecture of the normal region.

3.3 Imaging the healing process following treatment for radiation proctitis

Radiation proctitis (RP) is another chronic inflammatory condition of the GI tract that causes bleeding, diarrhea, mucous discharge, rectal pain, and fecal incontinence. RP is a common side effect of pelvic radiation therapy, which is often used to treat prostate and cervical cancers. RP affects 5.0 – 7.5% of patients who undergo pelvic radiation therapy [30], and so is a significant cause of GI morbidity. There are several treatment options available for RP, including medical [31,32], surgical [33], and endoscopic [34-36]. Endoscopic therapies are currently preferred due to their shorter recovery time and reasonably efficacies. Endoscopic therapy is also gaining
popularity for a range of GI pathologies in addition to RP. 3D-OCT endomicroscopy examination can potentially enhance endoscopic therapies by providing an approach for selecting the optimal treatment plan based on the microstructural characteristics of the individual lesion. 3D-OCT could also be used to monitor therapy, assess healing, and check for recurrence. Finally, 3D-OCT analysis can provide depth-resolved tissue information when excisional biopsy is contraindicated, as is the case with conditions such as RP where the risk of bleeding is high.

In order to assess 3D-OCT as a tool for assisting in GI therapy, a volunteer with RP was imaged at two different time points following endoscopic therapy. The volunteer was previously treated with radiofrequency ablation (Halo 90 system, BARRX Medical, Inc.) to control bleeding from RP. Radiofrequency ablation produces a broad, superficial ablation pattern and has previously been used to successfully treat Barrett's esophagus [37]. Endoscopic 2D-OCT analysis has shown that some patients can experience recurrence of diseases such as Barrett's esophagus following this treatment [38], further motivating the need for 3D-OCT endomicroscopy over larger fields of view with complete visualization of 3D tissue microstructure. The volunteer was imaged with 3D-OCT at 12 months and 14 months following the initial radiofrequency ablation treatment. Figure 5(a-c) shows white light video endoscopy images prior to treatment and at 12 and 14 months post-treatment. Prior to treatment, bleeding vessels and ulcerations are clearly apparent. At 12 months post-treatment, the rectal mucosa shows some residual inflammation but bleeding has markedly decreased. At 14 months post-treatment, the rectum appears largely normal. Figure 5(d) shows representative reference histology of RP, with inflammatory infiltrates and large superficial vessels present. Fresh pinch biopsy samples were not obtained because it is contraindicated in subjects with RP due to the risk of bleeding.

Figure 6 shows 3D-OCT endomicroscopy images of the same RP volunteer 12 months after radiofrequency ablation therapy, acquired in the region of prior treatment. The volume is 7 × 11 × 1.6 mm in dimension and was acquired in 20 s. Figure 6(a) shows an en face image formed by axial summation of the entire volume, and Fig. 6(b) shows an axial summation of a 20 μm thick section centered at a depth of 350 μm beneath the luminal surface. The full thickness summation reveals a large hypointense feature, but the tissue appears relatively normal otherwise and is consistent with squamous mucosa. The 20 μm summation, on the other hand, reveals a series of smaller sub-surface cyst-like structures. En face scanning through the tissue volume reveals that the structures are interconnected, suggesting that they may be ectatic blood vessels or lymphatics. Figure 6(c) shows an XZ cross-section with one small vascular feature visible. Figure 6(d-f) shows a series of YZ cross-sections at different transverse locations in the tissue. A full flythrough of this dataset is available as a linked multimedia file. Figure 6(d) reveals a central region containing many small vascular structures surrounded by normal mucosa on either side. Figure 6(e) shows a region that appears entirely normal, while Fig. 6(f) illustrates that the large hypointense feature visible in Fig. 6(a) and Fig. 6(b) is actually focal, superficial fibrosis. This tissue region was previously treated with argon plasma coagulation (APC) to reduce bleeding associated with RP. The focal fibrosis is likely a result of APC treatment, while the small buried vessels are likely covered by neo-squamous tissue that regrows following radiofrequency ablation. These results point out that individual 2D-OCT frames are often not sufficient to assess the morphology of pathologic GI tissue. 3D-OCT endomicroscopy examination of this volunteer shows that healing at 12 months post-treatment has resulted in the appearance of some normal epithelium, but that sub-surface vascular abnormalities covered with squamous tissue still remain.

Figure 7 shows 3D-OCT endomicroscopy images of the same RP volunteer 14 months after radiofrequency ablation therapy, acquired over the region of previous treatment. The volume is 8 × 20 × 1.6 mm in dimension and was acquired in 20 s. Figure 7(a) shows an en face image formed by axial summation of a 20 μm thick section centered at a depth of 460 μm beneath
the luminal surface. This dataset was acquired over the dentate line, which is visible as the dividing region between crypt-laden columnar epithelium on the left side of the image and smooth squamous epithelium on the right side. Compared to Fig. 6(b) the tissue appears increasingly normal after an additional 2 months. There are no focal fibrotic regions. Figure 7(b) and 7(c) show XZ and YZ cross-sectional images, illustrating the transition from normal columnar to normal squamous mucosa. A number of large hypointense, weakly-scattering features buried beneath the epithelium are visible in the en face and cross-sectional images. En face sectioning through the tissue volume again reveals that these structures are connected and are vascular in nature. The structures likely represent former large ecstatic blood vessels that are covered in neo-squamous tissue following radiofrequency ablation.

One further benefit of 3D-OCT endomicroscopy is the ability to create cross-sectional image planes at arbitrary orientations. These planes can be co-registered to the en face surface view to enable full characterization of microstructural tissue features. Fig. 7(d) shows a virtual image plane oriented parallel to the long axis of one large sub-surface vessel. The structure can be easily located by comparing the cross-section to the en face view in Fig. 7(a). 3D-OCT endomicroscopy can enable measurement of pathologic structures, suggesting the possibility of quantitative assessment of disease progression, treatment, healing, and recurrence.

4. Discussion

The results shown here demonstrate in vivo 3D-OCT endomicroscopy analysis of normal and pathologic tissue in the human colon. Although larger-scale clinical studies are needed to definitively establish the utility of this technique for specific applications, the pilot study described here illustrates several potential avenues for future investigation. First, 3D-OCT can be used to distinguish normal from abnormal tissue for applications involving the detection of pathology. In addition, it can be used as an adjunct to endoscopic therapies by assisting in treatment planning, monitoring, and follow-up assessment.

The majority of previous endoscopic OCT studies in the GI tract have focused on detecting pathology, with special emphasis on detecting dysplasia in Barrett's esophagus [6-11]. Because 2D-OCT is constrained to image individual cross-sections of several mm to cm length, it remains prone to random sampling errors also common in pinch biopsy. Furthermore, subtle dysplastic lesions obscured by diffuse inflammatory processes have proven difficult to detect using single cross-sectional images. This may be partially due to a lack of contextual awareness from analyzing 2D views of 3D tissue microstructure. 3D-OCT endomicroscopy could enhance pathology detection by providing a larger field of view, 3D visualization of tissue structure, and higher dimensional information for use in automated tissue classification algorithms. 3D-OCT may also be valuable for detecting early-stage cancers in the colon, where inflammatory conditions such as UC and Crohn's disease can mask the subtle subsurface features associated with dysplasia. This is an analogous situation to the detection of dysplasia in BE, where 3D-OCT endomicroscopy could also find utility.

3D-OCT endomicroscopy can also be a valuable tool for use with endoscopic therapies. Previous investigations of OCT as a therapeutic monitoring tool have been relatively limited, although some studies have been performed using endoscopic 2D-OCT to monitor photodynamic therapy (PDT) [39,40]. OCT may be used in conjunction with a wide variety of other therapies including argon plasma coagulation, laser ablation, radiofrequency ablation, EMR, band ligation and snare resection. 3D-OCT has the potential to significantly expand and enhance applications such as this by enabling 3D microstructural analysis of the entire target site. Prior to treatment, the lesion can be assessed for transverse extent, axial penetration, vascularization and structural makeup. During a therapy such as PDT, radiofrequency ablation, or laser coagulation, 3D-OCT could monitor the treatment site to assess when complete
destruction of the lesion is achieved. This could be useful to prevent overexposure and unnecessary collateral damage to surrounding healthy tissue. The concept of monitoring the response of tissue to thermal therapy has been demonstrated ex vivo using pig tissue [41] and could be applied in vivo using 3D-OCT endomicroscopy. Following therapy, 3D-OCT can be used during follow-up visits to assess healing and check for disease recurrence. The ability to precisely co-register cross-sectional images to anatomic landmarks or other en face features could be valuable when imaging the same patient at multiple time points, since it enables the same tissue region to be imaged with greatly improved registration.

Perhaps the most significant current limitation in 3D-OCT is the availability and performance of scanning imaging probes suitable for endoscopic use. The ability to resolve microstructural features in three dimensions requires precise and reproducible two dimensional beam scanning at the distal end of an endoscope. In this study, we used a proximally actuated spiral-scanning probe because it can be readily introduced into the working port of an endoscope and enables long regions of the lumen to be imaged. However the ability to resolve very small features in three dimensions was limited by rotational uniformity of the distal optics. Although frame-to-frame positional jitter was < 10 μm for the majority of frames in a typical 3D dataset, approximately 20% of the frames showed visible motion artifacts when viewed at extremely high magnification. For these frames, sequential rotational images had a maximum jitter in position on the order of 10 – 18 milliradians, corresponding to a 15 – 25 um maximum rotational frame variation measured ~200 μm beneath the tissue surface. Rotational uniformity degrades as the probe length is extended, rotational speed is increased, or if sharp bends are present in the catheter. There were also occasional larger discontinuities which produced artifacts in the 3D data sets and en face views. The rotational uniformity can be improved in the future by redesigning the torque cable and sheath material used in the imaging probe.

Other groups have performed studies using spiral-scanning probes with balloon stabilization in the pig [17] and human esophagus [18] and using a spiral-scanning MEMS micro-motor probe in the human airway [19]. The balloon design is well-suited for use in the esophagus, where heartbeat- and respiration-induced motion artifacts can significantly degrade image quality. The balloon design centers the optical fiber in the middle of the esophageal lumen, which increases the working distance and limits the minimum spot size to ~15 μm [17,42]. The design has the advantage of enabling large areas to be imaged, however variation between sequential rotational frames is expected to be high, owing to the large circumference of the balloon. The highest precision two dimensional scanning approaches will probably require the use of distal actuators. However, the design and packaging of these micro-scanning systems is challenging due to the miniaturization required for use in the working channels of standard endoscopes. The MEMS design reported in [19] uses a micro-motor at the distal tip of the probe to produce high speed rotational motion and an external linear stage to produce a slow pullback. The MEMS device can be miniaturized to an outer diameter of 2.2 mm, making it suitable for use in the working channel of an endoscope. However the rigid length of the probe must also be minimized in order to enable it to be inserted into the curved introducer portion of endoscopes.

From the viewpoint of OCT technology itself, as data acquisition and signal processing continues to improve, 3D-OCT endomicroscopy should be possible at even higher axial line rates of 250 – 500 kHz supported by advanced FDML lasers. This will enable increased spatial sampling densities for improved resolution, decreased motion artifacts for improved image quality, and still-larger fields of view for reduced sampling errors. All of these advances will improve the ability of 3D-OCT to detect subtle changes associated with early GI disease and to assist in endoscopic therapies, potentially leading to more effective interventions and decreased morbidity and mortality.
5. Conclusions

We have demonstrated 3D-OCT endomicroscopy examination of the human colon in vivo. Normal colorectal mucosa, ulcerative colitis, and radiation proctitis following treatment were imaged in a clinical pilot study of six volunteers. Averaging of thin 20 μm tissue sections was used to produce cross-sectional images with low noise levels. These cross-sections could be oriented in arbitrary planes and co-registered to anatomic or other en face surface features. Normal tissue could be clearly differentiated from pathologic tissue. Clear differences were observed between normal glandular epithelium, normal squamous epithelium, chronic inflammation from ulcerative colitis, and treated mucosa at 12 and 14 months following therapy for radiation proctitis. The high spatial sampling density and large field of view enabled both macroscopic and microscopic analysis of tissue structure. With further development and larger-scale clinical studies, 3D-OCT endomicroscopy could have a significant impact on disease detection and endoscopic therapy.

Acknowledgments

We gratefully acknowledge the technical contributions of Dr. Robert Huber, Dr. Yu Chen, and Robert Shearer. We acknowledge the clinical support of Dr. Laren Becker and Marisa Figueiredo. This research was sponsored in part by the National Institutes of Health R01-CA75289-12 and R01-EY011289-22; the Air Force Office of Scientific Research FA9550-07-1-0014 and Medical Free Electron Laser Program FA9550-07-1-0101; the National Science Foundation BES-0522845. Mr. Adler acknowledges support from the Natural Sciences and Engineering Research Council of Canada and the Heritage Scholarship Fund of the Province of Alberta. Mr. Tsai acknowledges support from the Taiwan Merit Scholarship from the National Science Council of Taiwan. Dr. Fujimoto receives royalties from intellectual property owned by MIT and licensed to LightLabs Imaging and Carl Zeiss Meditec.

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Fig. 1. 3D-OCT images of columnar epithelial tissue in the human colon. a, *En face* image constructed by axial summation of the entire dataset. Dashed lines show locations of cross-sections. b, XZ cross-section showing typical columnar structure. c, YZ cross-section. d, Enlarged view of a, showing *en face* crypt pattern. e, Representative *en face* histology of human colon. f, White light video endoscopy image of region analyzed with 3D-OCT.
Fig. 2.
3D-OCT images near the dentate line. a, En face image constructed by axial summation of entire dataset. Dashed lines show locations of cross-sections. b, XZ cross-section showing typical squamous structure. c, YZ cross-section showing shift from columnar C to squamous S epithelium over a transition zone T. d, White light video endoscopy image of region analyzed with 3D-OCT. e, Representative cross-sectional histology of columnar epithelium. f, Representative cross-sectional histology of squamous epithelium. Arrows in b and f indicate normal anal vessels.
Fig. 3.
Conventional examination of UC. a, White light video endoscopy image of UC showing the 3D-OCT probe in position. The tissue surface is inflamed with ulcerations and bleeding. b, Representative cross-sectional histology of UC showing an ulcerative pseudo-polyp.
Fig. 4.
3D-OCT images of ulcerative colitis. a, *En face* image constructed by summation of 20 um axial section. Large subsurface voids and ulcerations are present, while regular crypt pattern is absent. Dashed lines show locations of cross-sectional images. b, XZ cross-section containing normal squamous epithelium S and ulcerative colitis U. c, YZ cross-section showing similar structure. d, Enlarged view of left portion of c, showing disorganized structure and superficial voids. e, Enlarged view of right portion of c, showing regular squamous epithelium. (Media 1)
Fig. 5.
Conventional examination of radiation proctitis. a, White light video endoscopy image of radiation proctitis prior to treatment with radiofrequency ablation. Arrows indicate regions of bleeding and ulceration. b, Image 12 months after treatment. c, Image 14 months after treatment. d, Representative cross-sectional histology image of radiation proctitis. Arrow indicates a large superficial vessel.
Fig. 6.
3D-OCT images of radiation proctitis 12 months after treatment. a, Full thickness *en face* summation. Arrow indicates a large abnormality. b, 20 um *en face* summation. Subsurface vessels and voids are present. Dashed lines show locations of cross-sectional images. c, XZ cross-section showing irregular epithelium with vascular structures V. Dashed line shows depth of the *en face* image in b. d-f, YZ cross-sections showing similar features, including vascular structures V and superficial focal fibrosis F. (Media 2)
Fig. 7.
3D-OCT images of radiation proctitis 14 months after treatment. a, 20 um en face summation. Columnar epithelium proximal of the dentate line appears normal. Squamous epithelium is largely normal, with likely former ectatic vessels shown by arrows. Dashed lines show locations of cross-sectional images. b, XZ cross-section showing regular squamous S and columnar C epithelium. c, YZ cross-sections showing similar features. d, Arbitrary cross-section through the long axis of an ectatic vessel remnant R. (Media 3)