Improved Detection Sensitivity of Line-Scanning Optical Coherence Microscopy

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Abstract—Optical coherence microscopy (OCM) is a promising technology for high-resolution cellular-level imaging in human tissues. Line-scanning OCM is a new form of OCM that utilizes line-field illumination for parallel detection. In this study, we demonstrate improved detection sensitivity by using an achromatic design for line-field generation. This system operates at 830-nm wavelength with 82-nm bandwidth. The measured axial resolution is 3.9 μm in air (corresponding to ~2.9 μm in tissue), and the transverse resolutions are 2.1 μm along the line-field illumination direction and 1.7 μm perpendicular to line illumination direction. The measured sensitivity is 98 dB with 25 line averages, resulting in an imaging speed of ~2 frames/s (516 lines/s). Real-time, cellular-level imaging of scattering tissues is demonstrated using human-colon specimens.

Index Terms—Confocal microscopy, medical and biological imaging, optical coherence microscopy (OCM), optical coherence tomography (OCT).

I. INTRODUCTION

OPTICAL coherence tomography (OCT) is an emerging medical imaging technology that enables imaging of tissue microstructure with resolution approaching that of histopathology but without the need to excise and process specimens [1], [2]. Optical coherence microscopy (OCM) improves the transverse resolution of OCT by using a high-numerical aperture (NA) objective to generate en face images with cellular-level resolution [3], [4]. Real-time, high-resolution OCM imaging of scattering tissues has been previously performed by raster scanning a tightly focused beam [5]–[7] [see Fig. 1(a)]. However, it is challenging to develop a robust two-axis miniaturized optical scanner for endoscopic cellular-resolution OCT applications [8]. Alternately, OCT and OCM can be implemented using full-field (FF) illumination and detection so that no scanning component is required [9]–[13] [see Fig. 1(c)]. FF-OCT and OCM are based on the Linnik interference microscope where two identical objectives are placed in both the sample and reference arms [9]. FF-OCT/OCM has the advantage of using incandescent light sources rather than lasers, reduced speckle, and low-cost. Previous studies have demonstrated excellent image quality with cellular level resolutions in a range of tissues [9]–[13]. Furthermore, FF-OCT/OCM is amenable to endoscopic applications delivered through imaging fiber bundles [14]. However, FF techniques have increased incoherent scattered light and pixel crosstalk, which results in lower sensitivity when compared with point-scanning OCT. FF detection also lengthens the pixel dwell time, and thus increases the susceptibility to phase/fringe averaging effects from tissue motion.

Line-field illumination and detection [see Fig. 1(b)] offers a potential solution to overcome those limitations. Line-scanning OCM (LS-OCM) has several advantages over FF-OCM. First, line-field illumination reduces pixel crosstalk because a line, rather than the full imaging field is illuminated. Second, the confocal gate helps to reject out-of-focus incoherent scattered light. Since incident light levels are typically limited by detector saturation from scattered light, this limits detection sensitivity. Depending on the tissue scattering, line scanning enables the...
sensitivity to be increased by more than an order of magnitude. Finally, line detection is less sensitive to sample motion compared with FF detection because phase-sensitive information is acquired more rapidly, before significant phase averaging effects occur. This is important for future in vivo imaging applications where sample motion can cause fringe averaging effects that reduce sensitivity.

We have previously developed an LS-OCM system with 93-dB detection sensitivity [15]. Table I summarizes several representative OCM systems previously published in literature and their reported sensitivities. For better comparison, the sensitivity is also normalized to the same image speed (frame rate) of 1 Hz. From Table I, point-scanning OCM system has the highest sensitivity, followed by LS-OCM, and then FF-OCM.

In our previously reported LS-OCM system, a femtosecond Ti:Sapphire laser was used as the light source and the line illumination was performed by the combination of a spherical focusing lens and a plano-concave cylindrical lens. This design has the advantage that it does not require focusing with a cylindrical singlet lens and hence the aberration is less severe. Nevertheless, the aberration is severe enough to increase the focused line width on the sample (∼5 μm) by about a factor of 3 from the theoretical value, which is calculated to be 1.5 μm using the Fraunhofer diffraction theory. This not only reduces the system sensitivity by increasing the incoherent light, but also lowers the light collection efficiency. Design of diffraction limited cylindrical lenses can help improve the system performance.

In this study, we improve the LS-OCM performance by using an achromatic cylindrical lens for line-field generation. The feasibility of imaging highly scattering biological tissues is demonstrated on human colon specimens ex vivo.

II. METHODS

A. LS-OCM Imaging System

Fig. 2(a) shows the diagram of the LS-OCM system. The system was based on a Linnik-type interference microscope [15] with two identical objectives (Zeiss, 10x, 0.3 NA, water immersion, working distance 3.1 mm, infinity corrected) placed in the sample and reference arms, respectively. A compact broadband Ti:Sapphire laser (Femtolaser Produktions GmbH) was used to generate a spectral bandwidth of 82 nm at a center wavelength of 820 nm. The output light was coupled by a single-mode fiber; ACL: achromatic cylindrical lens; BS: beam splitter; MO: microscope objectives; M: mirror; ND: neutral density filter; GP: glass plate for dispersion compensation; PZT: piezoelectric transducer; LSCCD: line-scan CCD camera.
The interferometer was constructed using a broadband cube beam splitter which balanced the dispersion in the sample and reference paths. The beamsplitter cube was rotated slightly off axis in order to avoid parasitic reflections from the surfaces from saturating the camera. The images from the sample and reference arm objectives were focused onto the camera using a 75-mm achromatic lens, creating a 25× magnification. The line illumination field of view was 500 μm and was limited by the Gaussian beam shape of the illuminating beam along the direction of the line. Fig. 2(b) shows a photo of the system. The light path is indicated by the red lines. The incident beam was tilted at an angle to minimize the light reflection from the surfaces of the beam splitter. In addition, an area-scan CMOS camera was used to facilitate the beam alignment.

The interference signal was modulated by actuating the reference mirror with a piezoelectric transducer (PZT, PZ-14, Burleigh), using four integrating-bucket technique [16] with sinusoidally phase modulation to extract the interference (ac) component. The interference signal was integrated successively over the four quarters of the modulation period. Four interferograms were then obtained for each pixel of the image, and the reflectivity of the pixel can be reconstructed by algebraic operations of the four interferograms [16]. En face images were generated by transversely scanning the sample, orthogonal to the illumination line with a precision translation stage. A series of en face images at different imaging depths were acquired by translating the sample in the axial direction. The line-scan camera was read at ~52 Mpixel/s, corresponding to a line acquisition rate \( f = 51.6 \) kHz. The PZT was actuated at \( f/4 = 12.9 \) kHz and synchronized to the camera frame grabber. Typically, 25 line scans from each of the four-quadrant integrating-buckets (100 line scans in total) were averaged to increase the signal-to-noise ratio, resulting in an image acquisition speed of 516 lines/s.

Imaging was performed by translating the sample using stepping motor stage with a step size of 0.1 μm at a rate of 0.4 mm/s. Each en face image consisted of 300 lines and was acquired in 0.58 s.

In OCM, it is important to coordinate the confocal gate with the coherent gate to achieve the optimal optical sectioning. In this study, gate coordination is performed by first setting both the reference and sample mirrors to the focal point of the objectives using the area-scan CMOS camera. Then, while the sample arm is fixed, the delay between sample and reference arms is adjusted by moving the reference arm objective and mirror in tandem to reach maximum interference signal.

**B. Design of Achromatic Cylindrical Lens**

Line-field illumination was achieved previously [15] by a cylindrical singlet lens and the chromatic aberration was severe enough to increase the focused line width on the sample (∼5 μm) by a factor of ∼3 from the theoretical value, which is calculated to be 1.5 μm using the Fraunhofer diffraction theory. This not only reduces the system sensitivity by increasing the incoherent light, but also lowers the light collection efficiency. Design of diffraction limited cylindrical lenses can significantly improve the system performance. Fig. 3 shows the design (a) and the calculated performance of the focusing cylindrical lens (b). Over the full spectral bandwidth of the laser (750–900 nm), the chromatic focal shift of the achromatic design cylindrical lens is less than half of the Rayleigh range of the focused spot, with a more than fivefold improvement compared to a cylindrical singlet lens used in the previous experiment. The diffraction-limited focusing of <2 μm was verified by relay imaging the focused line onto the area-scan CMOS camera. The improved focusing leads to a reduced illumination power on the sample as well as an improved sensitivity.

**III. Results**

**A. Characterization of the LS-OCM System**

Fig. 4(a) shows the spectrum from the Ti:Sapphire laser after fiber coupling and Fig. 4(b) shows the axial point spread function measured by translating a mirror in the sample arm. The measured axial resolution (full-width at half-maximum of the point spread function) was 3.9 μm in air (corresponding to ∼2.9 μm in tissue assuming an index of refraction of 1.33).

Fig. 5(a) shows an en face OCM image of a USAF test target. The smallest group 7, element 6 bars were resolved, indicating the transverse resolution is less than 2.2 μm. By plotting the
intensity profiles along both vertical (Y) (line illumination direction) and horizontal (X) dimensions and measure the 20–80% transition across a sharp edge [17], the respective transverse resolutions along the line illumination and perpendicular to the illumination are 2.1 and 1.7 μm, respectively [see Fig. 5(b) and (c)]. The sensitivity was quantified by placing a mirror in the sample arm with a calibrated attenuator. By summation of the signal-to-noise ratio (in decibels) of the attenuated mirror image with twice the calibrated attenuation (accounting for the double-pass of the light), 98-dB detection sensitivity was measured with an imaging speed of 1.7 frames/s (with 25 line averages). Each image frame contained 300 × 500 pixels.

B. Imaging Human Tissues

The feasibility of imaging high-scattering biological tissues was demonstrated on human colon specimens ex vivo. The specimens were preserved in Formalin and imaged within a few hours after excision. During imaging, phosphate-buffered saline was used to immerse the microscope objective and maintain tissue hydration. En face images at 40, 100, and 150 μm depths are shown in Fig. 6. Detailed structures such as the crypt lumens, the epithelium, and lamina propria are clearly visualized. Translucent mucin-containing goblet cells are abundant within the epithelium. Deeper images of crypt lumen show narrower size than those at shallower depth. Representative histology images at the corresponding depth are also presented, which show good correlation with LS-OCM images. These results demonstrate the high-resolution, cellular-level imaging capability of the LS-OCM system.

Three-dimensional imaging was also performed by acquiring a stack of en face images while translating the sample in depth. A total of 100 en face images from 30 to 175 μm were acquired in 50 s with a frame rate of 1.7 Hz. Supplemental Movie 1 shows the movie of en face LS-OCM images as the imaging plane is stepped into the tissue. The lumen diameter decreases as the imaging plane is moved deeper into the mucosa. The continuous changing of goblet cell distribution can be visualized as the imaging plane is moved deeper into the lumen. Fig. 7 shows the 3-D isosurface view of two central colonic crypt lumens and the adjacent goblet cells. Supplemental Movie 2 further reveals the 3-D morphology of these two crypts and their adjacent goblet cells from different perspectives. The characteristic cylindrical shape of goblet cells can be visualized clearly. Those cells contain a narrow base and expanded apical portion that sometimes extends into the crypt lumen. The main function of goblet cells is to secrete mucus, which serves many functions including protection against shear stress and chemical damage [18]. The ability of LS-OCM to visualize the crypt morphology and goblet cells in 3-D could potentially be utilized in revealing certain pathologies of the colon.
achieved at the imaging speed of 516 lines/s. Normalized to the same image speed as shown in Table I (250 lines/s), it is equivalent to 101 dB, or 6 dB higher than our previously reported LS-OCM system. In addition, the illumination power on the sample is reduced from 25 to 15 mW. The improved sensitivity and the reduced illumination power are due to the narrower line focusing of achromatic lens (hence the improved power density per pixel) and the reduced incoherent scattered photons. Higher sensitivity is important because this enables a corresponding increase in imaging speeds.

Another factor determining the sensitivity is the camera’s FWC. The camera we used for this study has only 180 ke FWC, typical of a silicon CCD camera, and averaging of 25 frames is necessary to achieve 98-dB sensitivity with imaging speed of 1.7 frames/s. Imaging speeds can be increased up to ~7 frames/s by using two integrating-bucket demodulation [20] and higher speed, 512 pixel CCD cameras. InGaAs cameras, in general, have an FWC an order of magnitude higher than silicon CCD cameras and previous studies have shown that FF-OCM using InGaAs cameras has superior performance [13]. Use of InGaAs cameras also enables operation at longer wavelengths to reduce scattering and achieve deeper imaging depth. Line-scan InGaAs cameras have the advantage that there are significantly lower cost than area InGaAs cameras.

V. CONCLUSION

This paper demonstrates LS-OCM using achromatic cylindrical lens to improve the line-field generation and detection sensitivity. A sensitivity of 98 dB was achieved at an imaging speed of 1.7 frames/s. Real-time cellular-resolution OCM imaging of scattering tissues was demonstrated using human colon specimens ex vivo. LS-OCM promises to enable in vivo imaging by reducing sensitivity to sample motion when compared with FF camera detection because phase-sensitive information is acquired rapidly before phase averaging effects occur. With future technology development, LS-OCM has the potential to be miniaturized for endomicroscopy imaging because only 1-D scanning is required. Compared with FF-OCM, LS-OCM has the advantage that line-field illumination reduces saturation effects from incoherent scattered light in biological tissues, enabling higher sensitivity and faster imaging speeds.

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REFERENCES

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