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Cellular-level structure imaging in rat colon *ex vivo* and *in vivo* using micro-optical coherence tomography (μ OCT)

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Abstract: The feasibility of a constructed high-resolution OCT for cellular-level structures imaging in rat colon was evaluated for the first time. Imaging results demonstrated that OCT could be a viable tool for intestinal mucosa structures imaging.

OCIS codes: (110.4500) Optical coherence tomography; (170.3890) Medical optics instrumentation; (170.3880) Medical and biological imaging

1. Introduction

Optical coherence tomography (OCT) is a low-coherence interferometry based imaging tool [1]. Due to its noninvasive, high-resolution properties for cellular-/subcellular-level imaging, OCT is highly desired in clinical practice for early disease diagnoses, and has been established as a routine tool for eye and skin disease diagnoses [2], [3]. For OCT imaging of cellular-level microstructural details in intestinal tracts, however, there has been no report yet.

Intestinal crypts together with goblet cells are a key feature of large intestine in mucosal microanatomy. In clinical practice, goblet cells are a requirement for the diagnosis of intestinal metaplasia in large intestine, which is a sign of premalignant stage of gastric cancer [4]. So far, various imaging tools, such as line-scanning optical coherence microscopy (OCM), one-/two-photon fluorescence endomicroscopy, and multiphoton microscopy (MPM), etc., have been utilized for intestinal tract imaging. However, all those methods are associated with certain technical issues, which finally hinder their clinical use. As a noninvasive optical imaging modality that is able to provide cellular-/subcellular-level tissue imaging, μ OCT is believed to be a promising technology to overcome these technical issues.

In this work, we constructed a high-resolution μ OCT system, and evaluated its capability for detection of crypt structures and goblet cells in normal rats' colon *ex vivo* and *in vivo* for the first time. With a resolution of $2.0\ \mu\text{m}$ in both axial and lateral directions, such a OCT was utilized to acquire 3D images *ex vivo* and real-time cross-sectional images *in vivo* in rat colon. Such results were further validated as compared to the histology images.

2. Experimental setup

A fiber based μ OCT system was constructed for both crypts and goblet cells imaging in rat colon. A broadband laser source with a wavelength range of 480 nm-2200 nm and a repetition rate of 5MHz was employed as the light source. The laser output, which was measured to be 19.8 mW, was split by a broadband 75:25 fiber coupler. The beam output from the 75% port was directed to the sample arm optics that consists of a collimation lens, a galvo scanner and an objective lens. The illumination power on the sample was 10 mW. The reference arm optics has the same length as the sample arm. The interferometric signals generated by those two arms were finally collected by a spectrometer, which was comprised of a collimation lens (AC127-030-B-ML, Thorlabs Inc.), a diffraction grating (1200 l/mm 830nm, Wasatch Photonics Inc.), a camera lens (Nikon AF Nikkor 85mm f/1.8D), and a line scan CCD camera (E2V, AViiVA EM4). The detected signal was transferred to a computer through camera link cables and an image acquisition card (KBN-PCE-CL4-F, Bitflow Inc.) at 12-bit digital resolution. Fig. 1 illustrates that the resolving power of such constructed system was $\sim 2.0\ \mu\text{m}$ in both transverse and axial directions. The system sensitivity was measured to be $\sim 103.4\ \text{dB}$, which was slightly lower than the theoretical value of 105.6 dB.

Rats were anaesthetized using Ketamine (80 mg/kg bodyweight) and Xylazine (8 mg/kg bodyweight), and then surgical procedure was conducted on them. A 2-3 cm longitudinal incision were performed along the intestinal tract to fully expose the internal wall of colon (lumen side) for *in vivo* imaging. While for *ex vivo* study, those rats were sacrificed after the completion of *in vivo* experiments, and the colon tissues were harvested. Finally, those imaged areas were excised and then fixed using formalin for histological analysis. Those fixed sections were cut in both cross-sectional and *en face* directions relative to the luminal surface to allow comparison with OCT images.

Fig. 1. Spatial resolutions of the constructed μ OCT system. (a) Lateral resolution: group 7 element 6 ($2.19 \mu\text{m}$) were resolved. (b) Axial PSF ($2.0 \mu\text{m}$ at FWHM) measured.

3. Results

Figure 2(a) shows a cross-sectional OCT image of the colon mucosa *ex vivo*, which consists of 1024×566 pixels covering an imaging area of $872 \mu\text{m} \times 435 \mu\text{m}$. Results show that the detailed microstructures were clearly resolved. The crypt lumens have very good contrast relative to the epithelium, while the goblet cells distributed around the crypt lumens were clear and appeared to be empty with the translucent mucin contained. Fig. 2(b) shows a cross-sectional histology of the rat colon tissue. The OCT image have very good correspondence to the histology image, yet show relatively low image penetration.

Fig. 2. *Ex vivo* cross-sectional rat colon image. (a) Cross-sectional OCT image ($0.872 \text{ mm} \times 0.435 \text{ mm}$). (b) Histology, hematoxylin and eosin, $100\times$. The crypt lumen (red arrows) structures, individual goblet cells (yellow arrows) and lamina propria (green arrows) were clearly identified.

Figs. 3(a) and 3(b) present 3D reconstructed *en face* images at a respective imaging depth of $80 \mu\text{m}$ and $120 \mu\text{m}$, which further show the structures of both crypt lumens and individual goblet cells. Both images were comprised of 1024×1024 pixels, covering an imaging area of $872 \mu\text{m} \times 872 \mu\text{m}$. Imaging results showed that the microstructures, such as crypt lumen, goblet cells and the lamina propria, etc., could be clearly identified, and matched well with the representative *en face* histology image of the colon tissue. Specifically, when comparing Fig. 3(a) and Fig. 3(b), it was also interesting to note that as the imaging depth increases, the diameters of crypt lumens become narrower, i.e., the average sizes of the black dots as indicated by the red arrows become smaller.

Experiments were also conducted to test such constructed μ OCT's capability for cellular-level microstructures imaging *in vivo*. The system scanning speed was set to be 60 fps, and each cross-sectional image consists of 1024×767 pixels, covering an imaging area of $872 \mu\text{m}$ (W) $\times$ $610 \mu\text{m}$ (D). Figure ?? depicts an OCT cross-sectional image of the colon tissue *in vivo*. As can be seen, both the crypt lumen delineation along the axial direction as well as the goblet cells that were around the crypt lumens were clearly identified. Specifically, with the transparent mucins contained in, those goblet cells appeared to be low scattering structures. Such results were consistent with those in Fig. 2(a). Due to the heart beat of the rat, clearer identifications for both crypt lumen and goblet cell structures could be observed with a sequential series of images *in vivo*.

Fig. 3. *En face* image of rat colon *ex vivo*. (a) *En face* OCT image at imaging depth of 80 μm . (b) *En face* OCT image at imaging depth of 120 μm . The structures of crypt lumen (red arrows), individual goblet cells (yellow arrows) as well as lamina propria (green arrows) could be identified. (c) An *en face* histology image with hematoxylin and eosin, 100 $\times$.

Fig. 4. Cross-sectional image of rat colon *in vivo*. Microstructures like crypt lumen (red arrows) and goblet cells (yellow arrows) were identified.

4. Conclusion

In conclusion, we constructed a high-resolution μOCT system and evaluated its feasibility for cellular-level structure imaging in rat colon both *ex vivo* and *in vivo* for the first time. Imaging results showed that μOCT is able to visualize the cellular-level anatomic structures clearly, and those results were further confirmed by the histology images. This study demonstrates that μOCT could be a viable tool to perform “optical biopsy” in intestinal tracts, which thus lays a preliminary foundation for our future *in vivo* endoscopic studies.

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