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Title

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Permalink

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Journal

CHEST Journal, 130(3)

ISSN

0012-3692

Authors

Mahmood, Usama

Hanna, Nevine Mikhail

Han, Sugku

et al.

Publication Date

2006-09-01

DOI

10.1378/chest.130.3.863

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Peer reviewed

Evaluation of Rabbit Tracheal Inflammation Using Optical Coherence Tomography*

Usama Mahmood, BS; Nevine Mikhail Hanna, BS, MPH; Sugku Han, BS;
Woong-Gyu Jung, MS; Zhonping Chen, PhD; Bryan Jordan, RN;
Andrey Yershov, PhD; Ronald Walton, DVM; and Matthew Brenner, MD, FCCP

Background: Optical coherence tomography (OCT) is an evolving technology that is capable of delivering real-time, high-resolution images of tissues. The purpose of this study was to evaluate the feasibility of using OCT for detecting airway pathology in a septic animal model.
Methods: The tracheas of New Zealand white rabbits were inoculated endobronchially with various concentrations of live *Streptococcus pneumoniae* bacteria. After the development of pneumonia/sepsis, the animals were killed. OCT tracheal images and corresponding histologic specimens from these experimental animals were compared to control rabbit tracheas for morphologic features and quantitative tracheal mucosal thickness measurements.
Results: The results revealed significant airway mucosal thickening in the experimental group that was consistent with tracheal edema. Morphologic changes, including epithelial denuding and mucosal sloughing, were evident in regions of the experimental tracheas.
Conclusion: This study suggests that OCT is a potentially valuable imaging modality that is capable of evaluating superficial airway pathology with high-resolution *in vivo* images. Numerous applications of OCT can be envisioned in the realm of pulmonary medicine and thoracic surgery that may substantially increase the precision and accuracy of current bronchoscopic diagnostic and surgical techniques.   *(CHEST 2006; 130:863–868)*

Key words: bioengineering; pathology; trachea; tracheal injury; tracheal surgery

Abbreviation: OCT = optical coherence tomography

Optical coherence tomography (OCT) is a novel imaging modality that may help to improve the medical diagnostic modalities of pulmonary and airway diseases. Analogous to ultrasound, OCT uses the reflection of light waves to construct high-resolution structural representations of complex tissues.[1] Because OCT is based on light reflection rather than sound, it can be performed "noninvasively"; without requiring direct physical contact with the tissues being imaged. This property is of potential benefit in imaging structures such as the eyes or large airways. Second, because light has a substantially shorter wavelength than sound, OCT can produce images of considerably higher spatial resolution, in some cases up to one to two orders of magnitude higher, than current ultrasound capabilities.[2] Real-time images of tissues with resolutions of 1 to 15 μm can be obtained.[3–5] Since light is scattered and absorbed within most biological tissues, OCT suffers from the

disadvantage that it has a limited depth of penetration (approximately 2 to 3 mm with current technology). However, OCT can be performed with relatively inexpensive flexible fiberoptic imaging probes, enabling many clinically relevant tissue and organ surface and subsurface structures to be imaged.[6]

Applications of OCT have been investigated in a range of medical disciplines. However, translational work to pulmonary applications of OCT has been relatively lacking. Although research conducted within the past few years has described some aspects of normal airway structures as observed by OCT,[7,8] no studies have analyzed the ability to detect airway pathology. Because of the high tissue resolution capabilities and flexible fiberoptic bronchoscopic applicability of OCT, it has the potential to detect changes that are associated with various diseases that present in the superficial layers of the respiratory tract. It has even been suggested that OCT may eventually replace the need for biopsy, and become a method of "optical biopsy," though this is obviously highly speculative at this time.[9]

The purpose of this study was to investigate the potential utility of OCT in detecting airway pathology. In order to demonstrate this concept, an animal model of airway disease was employed in which the airways of live rabbits were inoculated with live *Streptococcus pneumoniae* bacteria. This bacterial lung infection and Pneumococcus induce an inflammatory process within the airways with resultant airway edema.[10] This study was designed to demonstrate the feasibility of using OCT imaging of tracheas of *S pneumoniae*-infected rabbits to detect pathologic changes indicative of airway edema and inflammation compared to controls.

MATERIALS AND METHODS

Description of Technology

A schematic representation of the OCT interferometry principles and design used in these studies is illustrated in Figure 1. Short-coherence-length broadband laser light is emitted from a light source toward a partially reflecting mirror that acts as an optical beam splitter.[1] One of the resulting light beams is directed toward a precisely controlled reference mirror at a specific distance from the beam splitter. The other beam is aimed toward the biological tissue to be examined. Light directed toward the reference mirror reflects back with a time delay (proportional to the distance traveled), while the reflected light beam from a biological tissue consists of multiple reflected "echoes" that are determined by the optical reflectivity of structures within the sample. These two reflected light beams (the returning reference and sample arms) are then recombined at the partially reflecting mirror creating an interference signal that is directed toward a photodetector for reading. Varying the position of the reference mirror allows investigation of different tissue depths. The pattern of interference created by the two recombined light beams is decoded and plotted on a logarithmic two-dimensional scale to reconstruct an image of the structures within the biological sample. Many successive axial measurements are obtained along a longitudinal surface, and the images are combined to create extremely high-resolution cross-sectional images of the tissues.

Tracheal Specimen Preparation

Structural OCT was used to obtain and compare images from the tracheas of four groups of New Zealand White rabbits. The four groups include the following: (1) normal control group; (2) saline solution control group; (3) intubated control group; and (4) pneumonia experimental group.

The normal control group (group 1) consisted of four rabbits from which 10 tracheal specimens were harvested. As part of an unrelated study to evaluate acute hemorrhagic shock hemodynamics, successive amounts of blood were removed and replaced with saline solution within a 3-h period. At the completion of these experiments, the rabbits were killed, and their tracheas were removed, maintained in isotonic saline solution, and imaged on site at the Beckman Laser Institute on the campus of the University of California at Irvine.

In the saline solution stored control group (group 2), the tracheas were harvested in the manner mentioned above. The tracheas were imaged immediately after resection, then stored in saline solution. Serial OCT images were obtained daily up to 4 to 5 days postresection to note the effects of saline solution on the mucosa and submucosa of the tracheas.

At the Brook Army Hospital Institute of Research in San Antonio, TX, two additional groups of New Zealand White rabbits (groups 3 and 4) were anesthetized, intubated with a 3.0-mm cuffed endotracheal tube, and mechanically ventilated for a period of several hours. The intubated control group (group 3; consisting of three animals from which six tracheal specimens were obtained) was allowed to recover immediately following the above-mentioned procedure. These animals subsequently underwent a panel of diagnostics tests that was similar to that which the experimental group of rabbits underwent (described in the next paragraph).

The experimental group (group IV; consisting of 12 animals from which 29 tracheal specimens were attained) was first inoculated with various quantities of *S pneumoniae* using a sterile pediatric suction catheter. This infected group of animals was monitored at the time of exposure and at 24, 48, 72, and 96 h postexposure via blood work, pulmonary function tests, vital sign data, CT scans, and flow cytometry; cultures of BAL fluid were used to confirm diagnosis of pneumonia. On the fourth day following inoculation, the surviving rabbits were killed, and their tracheas were excised and placed in isotonic saline solution packed in ice. These tissues were then sent via overnight delivery and were imaged at the University of California at Irvine.

Previous experience with OCT imaging demonstrated that the tracheal microstructure remained intact without evident change for a period of up to 4 days in cold isotonic saline solution. Nonetheless, an effort was made to image all specimens as soon as possible, usually within 2 days of excision. Furthermore, throughout their manipulation, care was taken to ensure that the architecture of the tracheas was not damaged.

Specimen Imaging

The specimens from all four groups of animals underwent the same OCT imaging procedures. Tracheas were cut open longitudinally along their musculofibrous membranes and were divided into sections that were approximately 1 × 2 cm. Each tracheal specimen yielded two samples, representing the upper
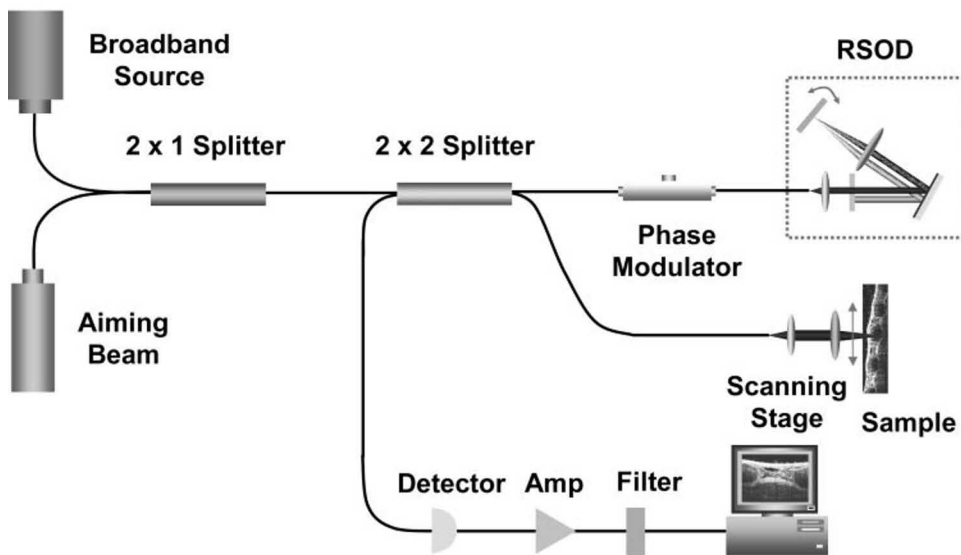
a.
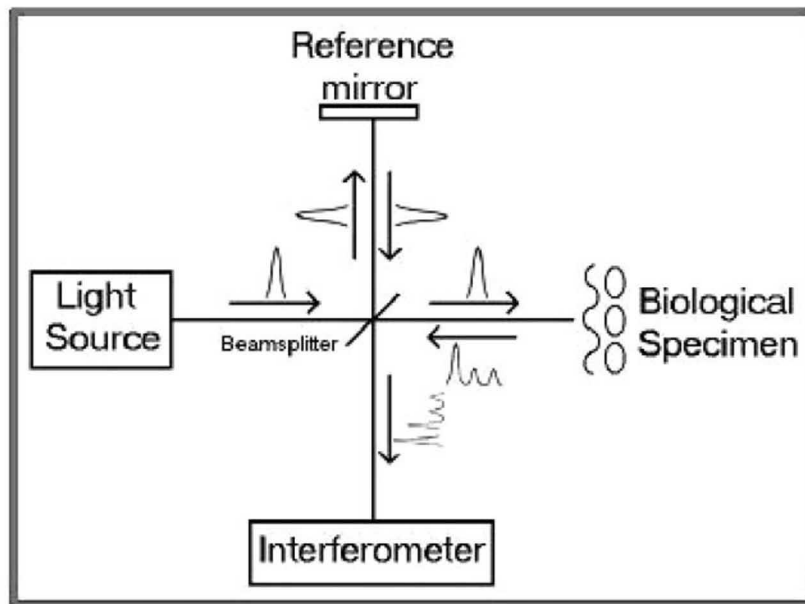
b.

FIGURE 1. *Top*, *a*: a schematic representation of the prototype OCT device used in these studies. *Bottom*, *b*: schematic of the OCT imaging system constructed in our laboratory. The light source was a 1,310-nm broadband superluminescent diode with a 70-nm bandwidth (full width at half maximum resolution), and a theoretical resolution of approximately 10 to 15 μm. The light source was coupled with an He-Ne laser guidance beam. RSOD = rapid scanning optical delay.

and lower trachea. Triangular notches were cut into opposite ends of each specimen to delineate the intended line of image acquisition, perpendicular to the cartilage rings. The tracheas were secured to pieces of cork using metal pins placed along their perimeter and covered liberally by a layer of water-based lubricant (K-Y Jelly; McNeil PPC; Fort Washington, PA) in order to prevent desiccation during imaging (Fig 2). The tracheas were then placed on a moveable sample platform and a visible-light guiding beam was used to match the line of image acquisition with the triangle notches already present. Images of various size and resolution were obtained using the prototype 1,310-nm broadband ($\Delta\lambda$ = 80 nm), superluminescent diode laser-based

OCT device that was constructed in our laboratory. The images constructed were displayed using a logarithmic intensity scale with the most backscattering areas represented in white and the least backscattering areas represented in black.

After OCT images were obtained, the tracheas were processed with routine histologic preparation. The tissues were placed in formalin for a period of 2 days followed by standard paraffin embedding. The tissues were cut into 6-μm sections along the line of OCT image acquisition, and were stained with hematoxylin-eosin. Images of these histologic specimens were captured using a light microscope (model BH2; Olympus; Tokyo, Japan) and an attached digital camera.
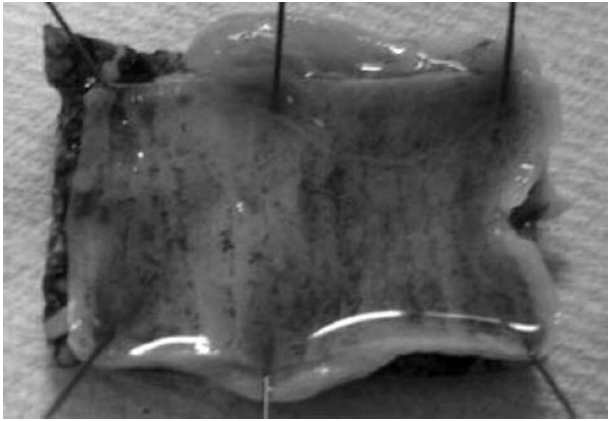
FIGURE 2. Tissue setup for OCT imaging. The trachea was opened longitudinally, pinned to a cork backboard, and imaged.

OCT/Histology Matching and Statistical Analysis

The OCT images and corresponding histologic preparations of the control group (group 1), the saline solution stored control group (group 2), the intubated control group (group 3), and the infected tracheal specimens group (group 4) were compared on the basis of the morphology of the structures present. The tracheal mucosal thickness was measured as the average distance between all the apices of the cartilage rings and the tracheal lumen attained from each OCT image. This was performed for every image. Measurements were made using digital imaging software and image editing software (Photoshop; Adobe; San Jose, CA). The values obtained from the animal groups were then compared using analysis of variance statistical analysis (SYSTAT, version 10.0; Systat Software Inc; Richmond, CA) comparing the combined control groups (groups 1 to 3) against the infected group (group 4) [after it was shown that the control subgroups were statistically similar to each other].

RESULTS

High-resolution images of the normal control group (group 1), the saline solution stored control group (group 2), the intubated control group (group

3), and the experimental infected tracheal specimens group (group 4) were obtained using OCT and revealed the ability to detect tissue structures that had been seen previously in other studies.[7,8] These included the epithelium, lamina propria, submucosa with glandular tissue, and various layers of tracheal cartilage and trachealis muscle (Fig 3, 4).

Though there was noticeable variability within the samples from each of the four groups, marked differences among the tracheas of the infected group (group 4) compared to the other three combined control groups (groups 1 to 3) were clearly visualized using OCT. Most pronounced among these changes was a swelling of the mucosal and submucosal layers found in the infected specimens, which was suggestive of severe tracheal edema and congestion (Fig 3). The infected tracheas displayed characteristic morphologic changes, prominently including epithelial denuding and mucosal sloughing (Fig 4).

The mean ($\pm$ SD) tracheal mucosal thickness above the cartilage rings for the combined control groups (groups 1 to 3) was 150 $\pm$ 2.5 μm, whereas that for the pneumonia-infected group (group 4) was 228 $\pm$ 1.7 μm. There is a significant difference ($p < 0.005$) [Fig 5] between the infected mucosal thicknesses and the thicknesses from the other two control groups. There were no differences among the values obtained from the three control subgroups ($p = 0.50$). There were no significant differences between upper vs lower tracheal specimens, and storing the tracheal sample in saline solution for up to 4 days had no impact on image appearance or the thickness of the mucosal and submucosal layers.

Comment

These studies substantiate OCT as a high-resolution imaging modality that is capable of detecting and evaluating superficial airway pathology. Not only
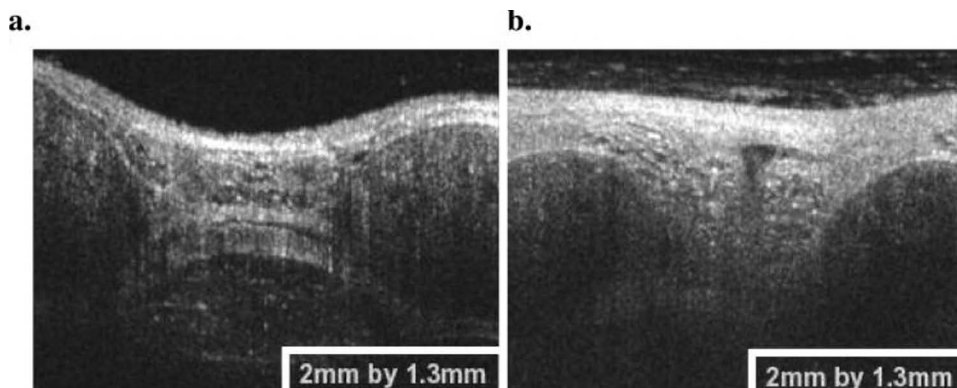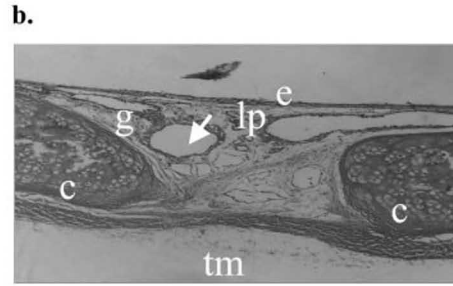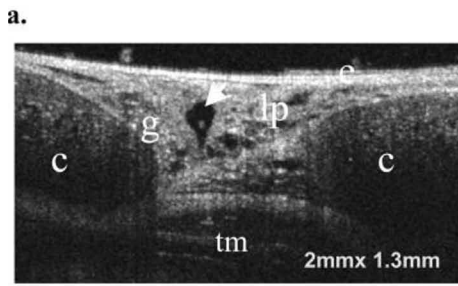

FIGURE 3. OCT images of a control trachea (*left, a*) and an experimental trachea (*right, b*). The edematous nature of the experimental tissue is evident from the marked increase in the thickness of the submucosal layer above the cartilage, as well as from the swelling and crowding of the submucosal tissue.
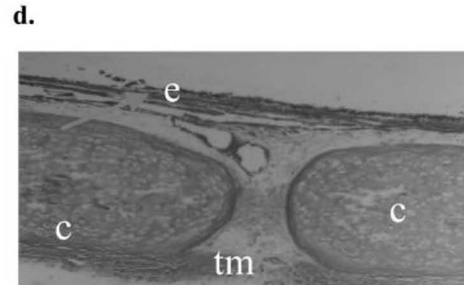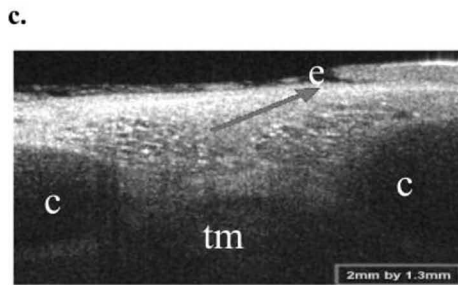
Normal:

Septic:

FIGURE 4. Comparison between OCT (*top left*, *a*, and *bottom left*, *c*) and hematoxylin-eosin stained sections (*top right*, *b*, and *bottom right*, *d*) of a rabbit trachea. Cartilage (c), epithelium (e), lamina propia (lp), and tunica muscularis (tm) are clearly differentiated as well as a number of glandular tissues (g) and epithelial sloughing (arrow in *bottom left*, *c*, and *bottom right*, *d*) [original ×4].

was OCT able to detect a significant difference in the tracheal mucosal thickening between the infected and other control groups of animals, it also reliably identified morphologic changes such as epithelial denuding and mucosal sloughing that are associated with airway inflammation.

Other commonly used imaging modalities, such as CT scanning, MRI, and ultrasound, have the ability

FIGURE 5. There is a significant difference (p < 0.005) between the mucosal thicknesses of the infected tracheas and the mucosal thicknesses from the tracheas taken from the other control groups.

to image subsurface architectures, yet none offer the soft-tissue resolution of 1 to 15 μm that OCT does. Hence, those other modalities cannot be used to reliably depict mucosal changes in the superficial airways. Novel endoscopic confocal imaging techniques, on the other hand, have remarkable soft-tissue resolution (< 10 μm); however, they suffer from very shallow depth of penetration (generally, < 800 μm).[11] Of the current imaging modalities, only OCT offers a unique combination of high resolution (1 to 15 μm) and a depth of penetration (2 to 3 mm) that is adequate for imaging superficial airway anatomy and pathology. Moreover, using modern fiberoptic technology, OCT can and has been used with endoscopic devices, allowing for real-time, *in vivo* imaging.[12]

Given the real-time tissue-resolution capabilities of OCT, its flexible fiberoptic bronchoscope compatibility, and its relatively inexpensive optical components, OCT has the potential to some day become a useful tool in pulmonary diagnostic medicine.[13,14] This study was designed to demonstrate the feasibility of high-resolution OCT in detecting disease-induced airway changes and some of the capabilities of OCT imaging of complex airway tissue. A variety of possible pulmonary applications for this technology can be envisioned, including bronchogenic carcinoma detection. OCT may be studied to screen for
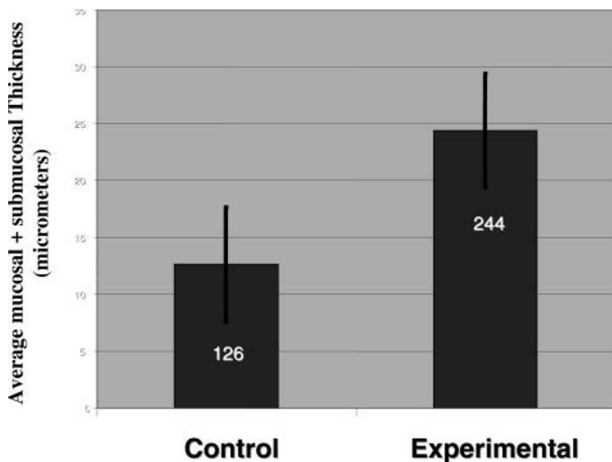
early neoplastic changes and to assess the submucosal spread or degree of dysplasia in endobronchial masses. OCT might also have a role in the treatment of airway malignancy by increasing the efficacy of tumor resection and by evaluating the effects of therapy.

More investigation needs to be conducted before OCT can be used in routine thoracic and pulmonary clinical settings. Research needs to be carried out to evaluate the ability of OCT to recognize alterations in models of respiratory pathologies, such as bronchogenic carcinoma and inhalation injury. Imaging of human airway specimens displaying a range of pathologic processes will be an important step toward clinical application validation. Parallel efforts need to be made toward the further development of OCT technology. Faster image acquisition rates are needed before OCT can consistently make high-resolution, larger area images from a moving respiratory tract. Better resolution and contrast will also be needed to increase the sensitivity and specificity with which OCT can detect airway pathology.[15] Specific probes and delivery methods need to be optimized for bronchoscopic approaches.

There are a number of specific limitations to this study. All specimens were imaged *in vitro*. Even though care was taken to maintain the original tissue architecture throughout the preparation of the sample, some changes inevitably occur during preparation. These limitations may be overcome once *in vivo* measurements can be readily performed. There are limitations in quantitatively describing some of the differences between images obtained from the four animal groups. Our method of comparing mucosal thickness measurements and qualitatively describing morphologic changes overcomes some of the limitations in objectively describing image comparisons but cannot fully define the changes seen.

The current "first-generation" OCT prototype device for pulmonary applications has limitations as well. The current resolution falls short of the "gold standard" of tissue biopsy preparation and hematoxylin-eosin staining. This gap will improve as OCT technology advances in the future. Some of the discrepancies between OCT imaging and light microscopy are due to the principle that the two methodologies examine different tissue properties and optical reflectivity in OCT vs those in dye absorption in hematoxylin-eosin staining. One of the advantages of OCT is its ability to avoid the changes in tissue that occur as a result of histologic processing, which alters the tissue due to the excision, fixation, and microtoming processes.[16]

OCT represents a promising new imaging technology for pulmonary diagnostics. In addition to structural imaging, future OCT technology will also be capable of providing functional information such as localized blood flow and tissue birefringence. Improved resolution, acquisition time, and probes will enable real-time fiberoptic airway imaging at near histologic levels of resolution.

REFERENCES

1 Bouma BE, Tearney GJ. Handbook of optical coherence. New York, NY: Marcel Dekker, 2002
2 Hrynchak P, Simpson T. Optical coherence tomography: an introduction to the technique and its use. Optom Vis Sci 2000; 77:347–356
3 Boppart SA, Herrmann JM, Pitris C, et al. Interventional optical coherence tomography for surgical guidance. Presented at Conference on Lasers and Electro-Optics CLEO'98, San Francisco, CA, May 3–8, 1998
4 Brezinski ME, Tearney GJ, Bouma B, et al. Optical biopsy with optical coherence tomography. Ann N Y Acad Sci 1998; 838:68–74
5 Brezinski ME, Fujimoto JG. Optical coherence tomography: high-resolution imaging in nontransparent tissue. IEEE J Quantum Electronics 1999; 5:1185–1192
6 Tearney GJ, Brezinski ME, Bouma BE, et al. *In vivo* endoscopic optical biopsy with optical coherence tomography. Science 1997; 276:2037–2039
7 Yang Y, Whiteman S, van Pittius DG, et al. Use of optical coherence tomography in delineating airways microstructure: comparison of OCT images to histopathological sections. Phys Med Biol 2004; 49:1247–1255
8 Pitris C, Brezinski ME, Bouma BE, et al. High resolution imaging of the upper respiratory tract with optical coherence tomography: a feasibility study. Am J Respir Crit Care Med 1998; 157:1640–1644
9 Fujimoto JG, Brezinski ME, Tearney GJ, et al. Optical biopsy and imaging using optical coherence tomography. Nat Med 1995; 1:970–972
10 Xu H, Xiong M, Huang Q. The study on COPD rat model produced by bacterial infection. Zhonghua Jie He He Hu Xi Za Zhi 1999; 22:739–742
11 American Society for Gastrointestinal Endoscopy. Technology status evaluation report: high resolution and high magnification endoscopy. Gastrointest Endosc 2000; 52:864
12 Tran PH, Mukai DS, Brenner M, et al. *In vivo* endoscopic optical coherence tomography by use of a rotational microelectromechanical system probe. Opt Lett 2004; 29:1236–1238
13 Fujimoto JG. Optical coherence tomography. Comptes Rendus de l'Academie des Sciences, Series IV Physics, 2001; 2:1099–1111
14 Fujimoto JG. Biomedical imaging using optical coherence tomography. SPIE 1999; 3749:402–403
15 Knuttel A, Bonev S, Knaak W. New methods for evaluation of *in vivo* scattering and refractive index properties obtained with optical coherence tomography. J Biomed Opt 2004; 9:265–273
16 Herz PR, Chen Y, Aguirre A, et al. Ultrahigh resolution optical biopsy with endoscopic optical coherence tomography. Optics Express 2004; 12:3532–3542