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Flexible, high-resolution micro-optical coherence tomography endobronchial probe toward *in vivo* imaging of cilia

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Abstract

We report the design and fabrication of a flexible, longitudinally scanning high-resolution micro-optical coherence tomography (μ OCT) endobronchial probe, optimized for micro-anatomical imaging in airways. The 2.4 mm diameter and flexibility of the probe allows it to be inserted into the instrument channel of a standard bronchoscope, enabling real-time video guidance of probe placement. To generate a depth-of-focus enhancing annular beam, we utilized a new fabrication method, whereby a hollow glass ferrule was angle-polished and gold-coated to produce an elongated annular reflector. We present validation data that verifies the preservation of linear scanning, despite the use of flexible materials. When utilized on excised, cultured mouse trachea, the probe acquired images of comparable quality to those obtained by a benchtop μ OCT system.

Optical coherence tomography (OCT) is a biomedical imaging technology that obtains cross-sectional, real-time images [1,2] with significantly better resolution than other clinical imaging modalities such as ultrasound, computed tomography, and magnetic resonance

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imaging. Conventional OCT techniques produce images of tissue reflectance with resolutions of approximately 10 μm axially and 30 μm laterally, enabling visualization of microscopic architectural morphology [3]. The recent development of micro-optical coherence tomography (μOCT) has further improved resolution in both axial and lateral directions by an order of magnitude compared to traditional techniques, allowing cellular and sub-cellular microstructures of biological tissues to be resolved with applications in a variety of organ systems [4,5]. In particular, our group has applied μOCT to the dynamic imaging of respiratory cilia [6–8], the organelles on airway epithelia that are responsible for mucus clearance. Other OCT approaches to ciliary imaging and mucociliary transport have also been recently developed [9–11]. Clinical translation of μOCT so that it can be used for diagnostic imaging *in vivo* requires miniaturized probes that are small, stable, and flexible to reach a targeted area of interest. Much research has been devoted to high-resolution OCT probe development [12–15]. We recently reported the development of a rigid, 4 mm diameter μOCT probe and demonstrated its ability to capture high-resolution images and measure mucociliary function in swine *in vivo* [8]. However, the clinical utility of this probe was limited by its relatively large diameter and the fact that it was rigid. We also reported another μOCT probe design featuring a significantly reduced diameter and increased flexibility [16]. However, the longer working distance required for airway imaging creates a large optical path difference between the sample position and the glass reflection that acts as the reference, which would require an extremely high-resolution spectrometer to capture the resultant high-frequency fringes.

In this Letter, we describe a new μOCT probe design that is mechanically flexible and further miniaturized. The probe consisted of a flexible catheter enclosing a common-path beam-apodizing μOCT interferometer, designed to be inserted imaging of respiratory epithelium. The handle unit housed a motor to transduce rapid longitudinal scanning of the endoscope's optical components within the catheter's static outer sheath. The probe and handle were connected to an imaging platform that included the SD-OCT spectrometer, drive electronics, data acquisition hardware, and a computer system (Fig. 1).

The handle was designed and fabricated to aid in positioning the probe and to enclose the scanning motor. The handle unit contained the scan-driving motor (Fig. 2). The driveshaft was scanned inside the probe sheath by a small linear motor (Physik-Instrumente) housed in a waterproof and electrically isolating polymer grip housing. The grip housing held the outer sheath and reciprocating driveshaft together to allow insertion, retraction, and rotation by hand. A locking thumbscrew allowed fixation of the relative position of the catheter within the instrument channel of the bronchoscope. The flexible imaging end of the μOCT probe was designed for insertion into the bronchoscope channel for imaging, while allowing manual navigation via the ergonomic adapter assembly at the proximal end. The probe was also fully self-contained and was operable independently from the bronchoscope.

Two coaxial flexible tubes were needed to support longitudinal scanning, with the outer sheath remaining static relative to the tissue and bronchoscope, and the inner tube (driveshaft) transmitting the motion of the linear motor to the distal optics. Polyetheretherketone (PEEK) was chosen for both tubes for its biocompatibility, lateral flexibility, and compressive/tensile stiffness. The outer sheath and driveshaft PEEK tubes

were custom extruded (Zeus, Inc.). The dimensions of the outer sheath were 1.75 mm inner diameter (ID) and 2.4 mm outer diameter (OD), fitting into the 2.8 mm ID standard bronchoscope instrument channel. The driveshaft was 1.35 (OD) and 0.254 mm (ID) to admit a standard optical fiber. In addition, the outer surface of the driveshaft was engineered with a friction-reducing texture and coated with polytetrafluoroethylene (Teflon) to minimize friction during scanning.

The optical core of the probe was a common-path, circular-obscuration apodization design, similar to [8]. The common-path design minimized material dispersion, and the beam apodization extended DOF as reported in [5,8] [Fig. 3(c)]. The following describes the illumination path through the probe optics, with collection occurring in reverse. Laser light was transmitted to the probe through a SM600 fiber (Thorlabs, Inc.), expanded within a borosilicate spacer (Fisher Scientific, 1 mm Dia), and focused by a 1.0 mm GRIN lens (Edmund Optics, 1.0 mm diameter, 810 nm DWL, 0.0 mm WD, NIR Coated). The converging light then encountered the 41° polished face of a hollow glass cylinder (1 mm OD, 129 μm ID). The polished ferrule face was gold-coated to promote reflectivity, forming an elongated annular reflector. A portion of the beam reflected by the annular coating became the OCT sample beam and was directed out of the imaging window to the sample. The slight acute angle of reflection from the ferrule served to reduce specular reflections from the window and tissue surfaces. A portion of the beam entering the hollow ferrule center became the OCT reference, reflected from a cleaved fiber acting as a reference reflector [Fig. 3(a)]. The cleaved fiber was not coated; the relatively low 4% reflectance from the surface returned sufficient power to act as the OCT reference while maintaining high sensitivity. Index-matching UV-curing optical adhesive (Norland, NOA 86) was used to bond all optical components. Compared to our previous beam apodization method [8], involving manual removal of reflective coating from a reflective prism, the ferrule-based method greatly improved beam shape accuracy and centration, simplifying fabrication, and significantly lowering the cost.

A stainless steel hypotube (McMaster-Carr, 18 Gauge) connected the ferrule reflector to the GRIN lens [Fig. 3(d)]. The total rigid length of the 1 mm diameter distal optics was approximately 9 mm [Fig. 3(d)]. The entire optical subsystem was protected by a stainless steel hypotube (VitaNeedle, 1/16 OD X.003) with a slot milled into the side to allow light transmission. The probe optics were affixed to the driveshaft via the protective hypotube. The outer sheath, which isolated the inner probe from the tissue environment, consisted of an in-house modified PEEK catheter (Zeus, Inc., customized, 1.75 mm ID, 2.4 mm OD), transparent Iridium polyolefin heat shrink tube (Cobalt Polymers, U2-110-CLR), and an in-house fabricated PEEK cap [Fig. 3(e)]. The catheter was slotted at the side and machined to reduce its distal diameter by ~150 μm to make a step for the transparent heat shrink imaging window [Fig. 3(e)]. The cap was rounded to prevent tissue injury during insertion [Fig. 3(e)]. The transparent heat shrink material served as the imaging window, allowing optical transmission while acting as a moisture barrier.

A probe scanning test was conducted with the complete handle assembly to determine the linearity, hysteresis and backlash of the scan. The driveshaft was connected to a linear piezo actuator (PI, P-602.5SL) at the proximal end via a custom fabricated brass adaptor, and the

linear piezo actuator was affixed to the housing by a set screw. A 10 V peak-to-peak sinusoidal signal at 40 Hz was used as the command signal for the actuator in closed-loop operation. The unloaded actuator traveled over a range of ~400 μm . The beam output at the distal end of the fully assembled device was traced using a line-scan camera (SPL8192, Basler, Germany) with a 10 μm pixel size. Linear regression was conducted for the first rising edge. The coefficient of determination R^2 was calculated for all rising edges in one second.

To verify the axial resolution and depth of focus (DOF) of the probe's optics, we imaged a solid bead phantom composed of a mixture of 30–50 μm diameter poly-dispersed glass spheres, 10 μm polystyrene mono dispersed polystyrene spheres, and diatomaceous earth mixed in the range 1–3 μm in size with various forms in a matrix of a Sylgard 184 elastomer. Standard SDOCT post-processing was employed [17], and A-lines were remapped from a sinusoidal scan pattern to a linear scan pattern [8].

The scan test demonstrated ~200 μm scanning distance after passing through the full assembly of a bronchoscope, together with an in-house fabricated airway phantom, which was used to mimic the curves in the human upper airway. The mean R^2 was 0.9875 over 40 cycles with standard deviation of 0.0026, which indicates good linearity and repeatability. As the linear motor reversed direction, the driveshaft transitioned between compression and tension, resulting in a constant-position hysteresis period at the crests and troughs of the scan waveform [Fig. 4(b)]. The duty cycle was approximately 30%.

From solid phantom images, the axial resolution was 1.5 μm , determined by measuring the full width at half-maximum (FWHM) of the axial point spread function with a DOF of ~170 μm at a distance of 1.2 mm from the center of the probe. The measured focal distance was 1 mm shorter than the Matlab simulated result, indicating that the effective NA of the fabricated probe was larger than designed. This larger NA also caused the measured DOF to be 130 μm shorter than that of the Matlab simulated result.

The Matlab simulation predicted a 2.6 μm FWHM diameter spot at focus. The lateral resolution was measured to be 4 μm using a solid bead phantom.

We performed *ex vivo* μOCT imaging of mouse trachea using both the benchtop μOCT instrument we utilized in previous studies [16] and the flexible probe attached to a typical SD-OCT platform (Fig. 1). Figure 5 compares two images of excised trachea from mice, one obtained from a benchtop μOCT system (top) and the other from the miniaturized flexible μOCT probe (bottom). The image in Fig. 5(b) was obtained by translating the sample at 35 mm/s. A 40 fps video of excised mouse trachea obtained using the μOCT probe is also presented in Visualization 1, demonstrating beating cilia. In both cases, microanatomical features such as cilia on the apical surface were clearly distinguishable, which indicates that the flexible bronchial probe has comparable imaging performance to that of the benchtop system. This speed and resolution will enable quantitative metrics of functional microanatomy to be determined such as measures of the thickness of the airway surface liquid and periciliary liquid, ciliary beat frequency, and mucociliary transport.

In conclusion, we have developed a flexible high-resolution μ OCT endobronchial probe that can be inserted into a standard bronchoscope instrument channel. Compared to our previous μ OCT linear scanning probe, this version is more navigable due to its flexibility, reduced size, and compatibility with flexible bronchoscopy. The flexibility of the probe was enabled by the reduction in size conferred by the 1 mm ferrule-based apodization (our previous design utilized a 2 mm manually apodized prism), as well as the manufacture of custom PEEK tubing that maximized lateral flexibility while minimizing longitudinal deformability and friction.

This instrument may be utilized to image cilia and mucociliary transport in small distal airways *in vivo*, where the pathogenesis of lung diseases such as cystic fibrosis (CF) and chronic obstructive pulmonary disease are believed to originate [6], allowing the underlying mechanisms of these pathologies to be studied. The roles and relationships of mucus volume, mucus clearance, and cilia in the CF airway remain controversial, in part due to the lack of tools capable of measuring these actors *in vivo*, a deficiency that can be addressed with the probe described here. Further, patient-specific imaging of a mucociliary function would allow personalized monitoring and tailoring of interventional treatments, which would be particularly valuable in diseases such as CF that frequently require cocktail pharmacologic therapies.

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References

1. Huang D, Swanson EA, Lin CP, Schuman JS, Stinson WG, Chang W, Hee MR, Flotte T, Gregory K, Puliafito CA, Fujimoto JG. Science. 1991; 254:1178. [PubMed: 1957169]
2. Tearney GJ, Brezinski ME, Bouma BE, Boppart SA, Pitris C, Southern JF, Fujimoto JG. Science. 1997; 276:2037. [PubMed: 9197265]
3. Brezinski ME, Tearney GJ, Bouma BE, Boppart SA, Hee MR, Swanson EA, Southern JF, Fujimoto JG. Am. J. Cardiol. 1996; 77:92. [PubMed: 8540467]
4. Yu X, Luo Y, Liu X, Chen S, Wang X, Chen S, Liu L. IEEE Photon. J. 2016; 8:1.
5. Liu L, Gardecki JA, Nadkarni SK, Toussaint JD, Yagi Y, Bouma BE, Tearney GJ. Nat. Med. 2011; 17:1010. [PubMed: 21743452]
6. Birket SE, Chu KK, Liu L, Houser GH, Diephuis BJ, Wilsterman EJ, Dierksen G, Mazur M, Shastry S, Li Y, Watson JD, Smith AT, Schuster BS, Hanes J, Grizzle WE, Sorscher EJ, Tearney GJ, Rowe SM. Am. J. Respir. Crit. Care Med. 2014; 190:421. [PubMed: 25029666]
7. Liu L, Chu KK, Houser GH, Diephuis BJ, Li Y, Wilsterman EJ, Shastry S, Dierksen G, Birket SE, Mazur M, Byan-Parker S, Grizzle WE, Sorscher EJ, Rowe SM, Tearney GJ. PLoS ONE. 2013; 8:e54473. [PubMed: 23372732]
8. Chu KK, Unglert C, Ford TN, Cui D, Carruth RW, Singh K, Liu L, Birket SE, Solomon GM, Rowe SM, Tearney GJ. Biomed. Opt. Express. 2016; 7:2494. [PubMed: 27446685]
9. Oldenburg AL, Chhetri RK, Hill DB, Button B. Biomed. Opt. Express. 2012; 3:1978. [PubMed: 23024894]
10. Zhou KC, Huang BK, Gamm UA, Bhandari V, Khokha MK, Choma MA. Biomed. Opt. Express. 2016; 7:1590. [PubMed: 27375926]

11. Ansari R, Buj C, Pieper M, König P, Schweikard A, Hüttmann G. *Opt. Express*. 2015; 23:23217. [PubMed: 26368424]
12. Yuan W, Mavadia-Shukla J, Xi J, Liang W, Yu X, Yu S, Li X. *Opt. Lett.* 2016; 41:250. [PubMed: 26766686]
13. Tumlinson AR, Barton JK, Považay B, Sattman H, Unterhuber A, Leitgeb RA, Drexler W. *Opt. Express*. 2006; 14:1878. [PubMed: 19503517]
14. Fujimoto JG. *Nat. Biotechnol.* 2003; 21:1361. [PubMed: 14595364]
15. Sivak MV Jr, Kobayashi K, Izatt JA, Rollins AM, Ungrunyawee R, Chak A, Wong RCK, Isenberg GA, Willis J. *Gastrointest. Endosc.* 2000; 51:474. [PubMed: 10744825]
16. Yin B, Chu KK, Liang C-P, Singh K, Reddy R, Tearney GJ. *Opt. Express*. 2016; 24:5555.
17. Wojtkowski M, Srinivasan VJ, Ko TH, Fujimoto JG, Kowalczyk A, Duker JS. *Opt. Express*. 2004; 12:2404. [PubMed: 19475077]

Fig. 1.

Schematic diagram for the μ OCT system and flexible endobronchial probe. Supercontinuum laser (SCL, SuperK Extreme OCT, NKT Photonics, Birkerød, Denmark) light was filtered by a dichroic mirror (DM), a spectral filter (SF), and a 90:10 broadband beam splitter before being directed toward the probe via a collimating lens (L) and single-mode fiber (SMF), with a resultant 18 mW optical power delivered to the sample. The interferometric signal returning from the probe was directed toward the focusing and collimation lens through the 90:10 (R: T) beam splitter (BS). The collimated beam was dispersed by a diffraction grating (G, Wasatch 1200 lp/mm at 830 nm) and focused by a customized focusing lens to a line-scan camera (LSC, SPL8192, Basler, Germany). The spectrogram was transferred to an image acquisition board (IMAQ) through a Camera Link (CL) cable. Imaging was conducted at a 35 kHz A-line rate (27 μ s integration time).

Fig. 2. Bronchoscope with μ OCT and probe insertion assembly. (a) Bronchoscope hand control segment with probe insertion assembly installed. (b) Fabricated assembly. B, bronchoscope; IT, insertion tube; LA, Lock adapter; TS, thumb screw; FS, foot strap; MH, motor housing; PT, probe tube; ST, strain relief tube. The maximum extension is 70 mm.

Fig. 3.

Illustration of μ OCT endoscopic probe distal end design and fabrication. Probe optics schematic (a), (b), simulated DOF without and with apodization (c) and fabricated probe (d), (e). (a) Beam propagation illustration and apodized beam in the far field. (b) 1, outer sheath; 2, driveshaft; 3, fiber; 4, ferrule; 5, spacer; 6, GRIN lens; 7, sample reflector; 8, reference reflector. (c) x-z beam profile of unapodized beam (left) and apodized beam (right). (d) Optics 1 mm in diameter and less than 9 mm in length. (e) Outer sheath OD 2.4 mm, rounded cap, and flat imaging window.

Fig. 4. 10-V sinusoidal driven signal scanning test for the full ergonomics assembly at 40 Hz. (a) Beam position recorded by the line-scan camera over time. (b) Zoomed in scanning pattern showing two complete cycles to demonstrate repeatability and linearity.

Fig. 5. Comparison of mouse trachea tissue images (*ex vivo*, tissue culture) obtained from a benchtop μ OCT system (top) and from the flexible bronchial probe (bottom). EP, epithelium. Arrows, cilia; image size, 315 μ m (lateral) $\times$ 243 μ m (axial). Visualization 1: video of excised mouse trachea acquired with the probe, demonstrating beating cilia.