Optical Coherence Tomography in Respiratory Science and Medicine: From Airways to Alveoli

Optical coherence tomography is a rapidly maturing optical imaging technology, enabling study of the in vivo structure of lung tissue at a scale of tens of micrometers. It has been used to assess the layered structure of airway walls, quantify both airway lumen caliber and compliance, and image individual alveoli. This article provides an overview of the technology and reviews its capability to provide new insights into respiratory disease.

Advances in a wide range of imaging technologies are providing clinicians and researchers with new insights into the structural and functional consequences of respiratory disease. When pathological changes are assessed, it is informative to view tissue at different scales, from gross changes in anatomical structures through to the cellular level, with each imaging technology offering a different trade-off between resolution and field of view.

On the coarsest scale, magnetic resonance imaging (MRI) provides excellent soft tissue differentiation over an entire organ, typically with millimeter resolution, enabling morphological measurements of airways (98) or entire lungs (8). In combination with idealized mathematical models, MRI has been used to estimate alveolar volume and surface area (32). High-resolution computed tomography (HRCT) offers a spatial resolution of hundreds of micrometers and has been used to image and measure airway wall thickness and lumen area (11, 57, 86, 88). Emphysematous lung regions and areas of gas trapping are routinely identified by CT (29, 91). X-ray microcomputed tomography has been used to acquire in vivo images at a resolution of tens of micrometers in small animal models of cancer (89), asthma (65), chronic pulmonary inflammation (4), and emphysema (26), and ex vivo images to a scale of ~5 μm in animal and human tissue (63, 70, 101, 126). More recently, high-energy X-ray synchrotron sources have been used to acquire rapid dynamic scans at 60 frames/s and to reconstruct 3D data volumes as fine as 20 μm/voxel (25).

In an endoscopic setting, endobronchial ultrasound provides localized guidance, in particular for fine-needle aspiration of pulmonary lesions (31), and white-light videobronchoscopy has been complemented with narrow-band imaging, in which selected narrow bands of optical wavelengths are used to highlight mucosal and submucosal vasculature (140).

At finer resolutions, optical techniques such as intravital (59) and confocal microscopy (27, 103, 118, 137) provide cellular-level imaging, aided by the use of intrinsic or exogenous fluorophores (59). However, such optical techniques provide insight only over very superficial surface layers and small fields of view. The optical properties of turbid tissue limit their imaging depth penetration to a few hundred micrometers at best (112).

Optical coherence tomography (OCT) is an optical imaging technique that fills the niche between the scales of gross and cellular imaging, offering resolutions in the range 1–20 μm, typically to a depth of 2–3 mm in tissue. It is used clinically in ophthalmology (28) and cardiology (116), and increasingly is finding applications in cancer imaging (123). In respiratory medicine, OCT is in its infancy but has already demonstrated the potential to address a range of preclinical and clinical questions (39), providing static and dynamic imaging of airway wall structure, down to the resolution of a single alveolus.

This review will describe the present role and capabilities of OCT in lung imaging, beginning with an overview of key aspects of the technology and then exploring the range of questions that OCT may address in both airways and alveoli.

**OCT**

First introduced in the early nineties (44), OCT is conceptually similar to ultrasound but uses reflections (backscatter) of near infrared light waves instead of sound waves. A tissue sample is illuminated by a weakly focused beam of near infrared light, and a small proportion of this light is reflected (or backscattered) from different depths in the sample and detected by the OCT scanner. This provides a one-dimensional “depth scan” of the tissue at a particular lateral location. Using terminology from ultrasound, the one-dimensional signal is referred to as an A-scan.

Light is backscattered from multiple depths in the tissue simultaneously. Much of the inner workings of
an OCT scanner serves to separate the components of backscattered light from different depths using a technique referred to as low-coherence interferometry. In brief, the beam from the light source is separated into two identical beams, one of which is used to illuminate the tissue sample, whereas the other remains internal to the scanner, guided through a similar path length and directed onto a fixed mirror instead of tissue. The backscatter from both light beams is collected and combined, and, from their constructive and destructive interference pattern, it is possible to quantify the amount of light backscattered from each depth in the tissue and form an A-scan (16, 23, 42, 44).

By scanning the light beam across the tissue sample and acquiring a sequence of A-scans at adjacent locations, the OCT scanner creates a 2D image of the tissue, referred to as a B-scan. Acquiring a sequence of such B-scans, the system constructs a 3D data set that is referred to, intuitively, as a C-scan.

The choice of light wavelength used in OCT is dictated by the light propagation properties of tissue and the operational wavelength ranges of available optical components. Light propagation in tissue depends on the scattering and absorption properties of the tissue’s components: cells, cell organelles, and various fiber structures. The transparency of most tissue types reaches a maximum in the near infrared range (122). Depending on the tissues being imaged, the optimal operational wavelength is typically in the range of 600–1,600 nm, referred to as the diagnostic window, the upper limit of which is set by rapidly increasing absorption by water (43).

The axial resolution of the OCT system is set by the bandwidth of the near infrared light source, and the lateral resolution by the focusing optics. Typical OCT systems achieve resolutions in the range of 5–20 μm, although ultra-high-resolution systems have been reported, with resolutions of 1–3 μm (14, 24, 51). The development of such high-end systems has generally been driven by ophthalmological applications, but recent work has begun to explore applications in lung tissue, such as measuring the thickness of the periciliary liquid layer in airways (71).

Types of Optical Scanning Probes

Three strategies have been adopted to enable OCT imaging of pulmonary structures: external scanning, endoscopic imaging, and needle-based imaging. External imaging uses an optical configuration similar to a microscope, utilizing mirrors, typically mounted on galvanometers, to scan the illuminating light beam across the tissue sample. Several such systems are available commercially (5, 69).

The scanning setup can be miniaturized to fit within a hand-held probe, but the size of the scan head limits its application to imaging external to an organ. Such probes have been used to image lung parenchyma in vivo via an invasive thoracic window model (82), wherein an incision is made through the chest wall.

A second strategy is to access internal organ regions by way of an endoscopic OCT probe. Flexible endoscopic imaging probes that can be inserted into the lumen of a hollow organ (e.g., airway passage) typically consist of a length of optical fiber to carry the light beam to the distal focusing optics, with the entire assembly encased in a protective transparent plastic catheter. Radial scanning is typically achieved by rotating the entire probe and the connected fiber within the catheter in a similar fashion to that used in endoscopic ultrasound. Rotating only the focusing optics through the use of microelectromechanical (MEMS) motors placed in the probe head has also been demonstrated (115, 138). Each rotation produces a radial 2D B-scan, and the probe may be retracted while accumulating a sequence of B-scans to construct a 3D C-scan. Such probes can provide high-resolution lumen profiles of hollow structures. In practice, the endoscopic OCT probe is typically positioned through a bronchoscope, placing a lower limit on the caliber of airway that can be scanned, although the OCT probe may be advanced into smaller airways or beyond obstructions (130). Rigid endoscopic probes, similar in form to a thoracoscope, have also been proposed (134, 139).

We note that as the probe is retracted, the geometry of a 3D C-scan may not truly reflect the shape of the organ being scanned. The reconstructed images are acquired relative to the position and orientation of the probe head. However, flexible endoscopic OCT probes have no way to measure how the probe head is reoriented during scanning as it is withdrawn along a curved airway. Thus successive B-scans are simply stacked, producing a C-scan that depicts an artificially straight airway. Preliminary work has been published incorporating a magnetic tracking system into an endoscopic OCT probe to provide a more sophisticated and accurate reconstruction of the 3D lumen surface (64).

The third strategy is to encase highly miniaturized focusing optics within a hypodermic needle: an OCT needle probe. OCT needle probes can be inserted through tissue into the region of interest, with the considerable advantage of providing access to tissues that may be situated several centimeters below the surface of the organ. As with endoscopic probes, a length of optical fiber is used to couple the light between the scanner and the focusing optics in the probe head. Miniaturization
of the focusing optics so that they are accommodated within a needle presents considerable design and manufacturing challenges, but a range of solutions exists (34, 78, 110, 117, 133). The smallest reported scanning probes capable of acquiring a 3D scan have been encased within a 30-gauge needle (outer diameter 310 μm) (72).

**Imaging Airway Wall Structure**

The ability of OCT to provide clear images of layered structures makes it well suited to assess the structural integrity of the airway wall. Remodeling of the airway wall is a prominent feature of airway diseases, including asthma and chronic obstructive pulmonary disease (COPD) (46). In asthma, inner and outer wall compartments are thicker, particularly the airway smooth muscle (ASM) layer (13, 45). Increased thickness of the ASM layer is believed to give rise to the primary functional abnormality characteristic of asthma “airway hyper-responsiveness” in which airways narrow excessively to bronchoconstrictor stimuli (62, 93). Airway remodeling is also present in COPD, although not to the extent seen in asthma, and preferentially in smaller compared with larger airways (60). The likely causal relationship between airway structure and function places great importance on the ability to assess airway structure in patients and research subjects.

Early animal studies focused on establishing the capabilities of OCT to visualize different layers of the airway wall. Preliminary studies in rabbit and pig airways showed that OCT could differentiate the epithelial layer, lamina propria, glandular tissue, and cartilage (33, 136). Demonstrating the potential for OCT to detect structural abnormalities, ex vivo animal work has measured changes in the submucosal layer in rabbit models: identifying changes in the trachea in the presence of inflammation (35, 50, 75) and in subglottic tissue in the presence of repeated intubation trauma (52). Our study using an OCT endoscopic probe to image isolated bronchial segments from pigs in vitro showed movement and bending of cartilage plates during induced bronchoconstriction (97).

OCT is well translated from animal models to the human airway wall. Ex vivo human studies, utilizing tissue obtained from pneumonectomies, lobectomies, and cadavers, show a similar discrimination between airway wall substructures when imaged by OCT (104, 121). In the presence of inflammation and malignancy, OCT images of bronchial tissue reveal a pathogenic loss of wall structure (127). Hariri et al. assessed OCT images of 22 ex vivo human lung specimens, with corresponding histology, in addition to in vivo scans of normal airway (36, 37). OCT imaging successfully distinguished normal airways from fibrotic tissue and those invaded by carcinomas. In FIGURE 1, a transverse section is shown of a normal airway from a 27-yr-old volunteer viewed by endoscopic OCT, illustrating contrast between the epithelial layer, lamina propria, and cartilage. Similar results have been demonstrated in vivo with endoscopic OCT probes showing layered structures in healthy tissue and a loss of normal structure in the presence of endobronchial carcinomas (85, 111, 121). In intubated newborns, endoscopic OCT identified vocal cords, cricoid cartilage, tracheal rings, ducts,

![FIGURE 1. In vivo endoscopic OCT of a normal airway](https://example.com/figure1.jpg)

A: radial B-scan image showing a cross-sectional view of the airway (imaging artifacts are marked by asterisks). B and C: high-magnification views of airway wall, showing airway wall layers. D and E: representative histology showing similar layers to those visible in the OCT. c, cartilage; e, epithelium; b, transition between epithelium and underlying basement membrane/lamina propria; lp, lamina propria; p, perichondrium; and c, cartilage. Scale bars in histology are 500 μm. Reproduced from Ref. 37 and used with permission.
glands, and vessels, supporting a role for the surveillance of neonatal airway in the context of stenosis produced by prolonged intubation (107).

One of the attractions of OCT imaging is the potential to quantify airway wall structures. Our earlier work showed that measurements of the absolute cross-sectional area of the inner wall of the porcine bronchus by OCT compared well with those determined in histological sections (97). Chen and colleagues have demonstrated that OCT is able to quantify changes in airway wall thickness in response to smoke inhalation injury (9, 138). Although HRCT imaging is also used to quantify airway wall structure, it is unable to distinguish inner and outer wall compartments (41), which is feasible with OCT. In an extensive in vivo study of 148 patients (61), epithelial thickness was calculated from OCT images of normal tissue and a range of malignant and premalignant pathologies. The study showed an increase in epithelial thickness between in situ and invasive carcinoma, and an increase in epithelial thickness in dysplasia compared with both metaplasia and hyperplasia.

There is early evidence to suggest that airway wall measurements by OCT relate to lung function. In a study of 44 current and former smokers, in vivo endoscopic OCT measurements of wall thickness in the smaller (5th generation) airways correlated with forced expiratory volume in 1 s (FEV1) (21). These findings are of relevance to the pathogenesis of COPD (19, 20), and this approach could potentially be extended to studies examining subjects with asthma.

Advanced image processing techniques have recently been used to extend the imaging capabilities of OCT to the assessment of the function of cilia lining the respiratory epithelium. With a length of \( \sim 7 \) \( \mu \)m and submicrometer width, cilia are below the imaging resolution of most OCT systems. However, time sequences of OCT measurements enable the analysis of the general direction of fluid flow in the mucus and periciliary layers. By assuming local homogeneity in the cilia beat direction, this was demonstrated to provide a gross indication of cilia movement on a scale of tens of micrometers in both an in vitro model of human bronchial epithelial cells and ex vivo mouse tracheal tissue (99). Alternative analysis techniques using exogenous particles have also been demonstrated in non-respiratory cilia, using OCT to track the movement of 5-\( \mu \)m-diameter polystyrene microspheres (49). Although this latter technique has potential to analyze more complex flows, imaging of the exogenous particles is anticipated to be challenging in the optically turbid mucus present in many respiratory diseases.

Quantification of Airway Lumen Caliber

There are number of physiological and clinical scenarios in which the caliber of the airway lumen may change, including lung inflation or deflation (12), encroachment into the lumen by a thickened/remodeled airway wall or loss of parenchymal tethering support in emphysema (102, 119), acute bronchoconstriction (76), airway collapse such as may occur in tracheobronchomalacia (66), physical obstruction by tumors or other lesions (109), or enlargement of the bronchial lumen characteristic of bronchiectasis (87). Such scenarios lend themselves to assessment by endoscopic OCT.

Unlike OCT systems used for the measurement of airway wall structure, which requires microscopic resolution over fields of view of a few millimeters, quantification of lumen diameter can require scanning over tens of millimeters. An offshoot of conventional OCT, known as anatomical OCT (2, 3, 18, 127a) or full-range OCT (47), provides this extended range, although with a reduced lateral resolution (typically 100–200 \( \mu \)m in the direction perpendicular to the light beam) due to the increased depth of focus required. A high resolution can be maintained in the axial direction (parallel to the light beam), with one recent system demonstrating an axial resolution (in air) of 25 \( \mu \)m over a range of 26 mm (30). FIGURE 2 shows a scan of a human upper pharynx acquired in vivo. Individual cross-sectional views are shown in FIGURE 2, A–D, each acquired from one full rotation of an endoscopic imaging probe. Because of the strength of the backscatter at the air-tissue interface, appearing here as a white contour, little subsurface depth penetration is achieved. Reconstructing a series of these cross-sectional B-scans allows the formation of a 3D volume showing the shape of the airway lumen, rendered in FIGURE 2E.

A challenge in acquiring long-range scans along the length of an airway in vivo is overcoming motion produced by the dynamic mechanical environment of the lung. Recent systems (47) have improved acquisition rates and are able to scan from the bottom of the larynx to the end of the nasal cavity within 40 s. Artifacts from respiratory movements during scanning can also be reduced by respiratory gating techniques (77). Using simultaneously acquired plethysmography measurements, the circumference of the airway can be reconstructed from image data acquired over multiple breath cycles, with each fragment of the airway circumference matched to a particular inspiratory/expiratory phase.

Anatomical OCT has been used in the assessment of the upper airway in patients with obstructive sleep apnea. Sleep studies have captured...
cross-sectional images of the collapse of the pharynx during an apneic event and the subsequent recovery (2, 67). Walsh et al. used anatomical OCT to quantify airway dimensions in awake patients, finding obstructive sleep apnea patients to have a similar pharyngeal shape but smaller velopharyngeal cross-sectional area than gender- and age-matched control subjects with comparable body mass index (124).

Anatomical OCT has also been used to image the lumen of lower airways (79), with measurements validated against CT in patients with a range of pathologies, including malignant airway obstruction, stenosis, tracheomalacia, and radiation fibrosis (131). Clinically, OCT scans are proposed as a method to obtain intra-operative measurements of the diameter and length of airway stenoses, and to guide the selection of stent size during respiratory interventions (130).

With respect to the functional assessment of airways, anatomical OCT provides an alternative approach to assess bronchoconstrictor responses to direct or indirect contractile stimuli. Our in vitro porcine model used OCT to measure airway narrowing induced by electrical field stimulation of cholinergic nerves (97). In a follow-up study, the heterogeneity of narrowing along an airway was demonstrated by administration of exogenous carbachol. Airway generation was positively correlated with the magnitude of airway narrowing along the length of the airway, i.e., more peripheral airways were more responsive to the same contractile stimulus (96). One limitation for the study of acute bronchoconstriction by OCT is that narrowing of the lumen encroaches on and around the OCT catheter, which is not an issue with techniques such as HRCT.

A significant advantage of endoscopic OCT is the potential to acquire multiple in vivo measurements over an extended time period without exposure to ionizing radiation. The near infrared light sources used in OCT typically have an average output power on the order of tens of milliwatts, which, as scanned across the tissue, produce negligible heating. Armstrong et al. (2) demonstrated the feasibility to use such a probe in an overnight in vivo study of a patient with obstructive sleep apnea.

Evaluating Airway Compliance

Airway remodeling may alter the mechanical properties of the airways. For example, several studies suggest that the airway wall is less distensible during lung inflation in subjects with asthma (10, 48, 125, 132). A reduction in airway distensibility in asthma may reflect reduced compliance of the airway wall as a result of remodeling (125) but is also influenced by parenchymal tethering forces, which may be decoupled in asthma (74). The level of airway smooth muscle activation further impacts airway compliance, whereby contraction of the smooth muscle produces wall stiffening (94, 95). Conversely, beta-agonists producing airway smooth muscle relaxation can increase airway compliance (1, 53).

Airway compliance may be characterized by measuring local changes in airway lumen size as a function of airway pressure, and anatomical OCT provides an effective endoscopic technique to perform such measurements. Williamson et al. (128) performed multiple OCT scans over a range of transmural pressures in an excised porcine airway segment. The resulting compliance curves were quantified by their Colebatch shape factor, which is indicative of the nonlinearity of the area-pressure curve, and provides an index of distensibility independent of airway size (128). The same approach has been used to perform in vivo measurements in humans in both the upper (124) and lower airway (130). In control subjects and subjects with asthma, COPD, and bronchiectasis, it was observed using anatomical OCT that lower airways become maximally distended at lower airway pressures in asthma or bronchiectasis but not in COPD (129).

Computational models of airway compliance have also been derived from 3D anatomical OCT scans, providing insight into the pressure distribution in the pharynx of sleep apnea patients (17, 73). Extending on from these gross measurements of lumen size, Robertson et al. demonstrated that a rapid-scanning endoscopic OCT probe may be used to calculate a radial map of regional airway compliance, using image-processing techniques to track movement in the airway wall (108).
Alveolar Imaging

The high spatial resolution of OCT accommodates assessment of lung parenchyma, with imaging systems able to resolve individual alveoli. Early work utilized external scanning probes to establish the capabilities of OCT with ex vivo human (7) and animal parenchyma (105), with the latter study allowing visualization of the 3D structure of the alveolar network. Such probes have subsequently been used in vivo through a thoracic window model, where upper intercostal muscle layers are resected between the ribs to allow external imaging access to the lung (82). The thoracic window approach has been applied in both mouse and rabbit models, and used to explore alveolar size over a range of pressures in healthy and acid-injured lungs (6, 84). This work has also been extended to dynamic imaging, adopting the previously discussed respiratory-gated imaging protocol. Acquisition of multiple OCT scans from different points across the respiratory cycle allowed tracking of individual subpleural alveoli during inspiration (83). Dynamic, nonrespiratory-gated OCT imaging has also been demonstrated with a lightweight probe attached to the pleural surface of an in vivo porcine lung (90).

A limitation of conventional OCT is its shallow tissue penetration depth of at best only 2–3 mm. This limitation has been addressed with the development of 3D scanning OCT needle probes (106), which may be inserted deep within the tissue. Extending beyond the endoscopic limits of imaging bronchial wall structure, OCT needle probes have been proposed to aid in guidance of transbronchial needle aspiration of solitary pulmonary lesions (117), using the presence of alveoli to help differentiate regions of normal parenchyma from pulmonary lesions (38).

Needle insertion will result in some local tissue trauma, which may be naturally reduced by minimizing the outer diameter of the encasing needle. Our own work utilized a 30-gauge OCT needle probe (outer diameter of 310 μm) to acquire 3D images of rat and lamb lungs at a depth of several centimeters below the lung surface (80). Figure 3 shows representative OCT images, paired with matching histology, acquired on saline-filled rat lungs. A radial image, constructed from one full rotation of the OCT needle probe, is shown in Figure 3A. The needle hole (labeled n) is located at the center of the image, surrounded by alveoli and a small blood vessel (labeled v1) to the left of the image. Each alveolar space presents as a small region of low backscatter (dark gray), incompletely delineated by the more highly backscattering alveoli walls (light gray). Figure 3B is a longitudinal OCT image extracted from the same data volume, orientated perpendicular to the radial image and with its horizontal axis aligned along the direction of the needle retraction. A bronchiole (labeled b) and three vessels (labeled v1, v2, and v3) are visible, showing the correspondence with the matching hematoxylin and eosin (H&E) stained histology section in Figure 3C.

Through the use of 3D visualization techniques, OCT needle probes have the potential to explore the network of bronchioles, vessels, and alveoli present in small sections of lung parenchyma. The bottom row of Figure 3 shows 3D volumetric scans of a fetal lamb lung and a rat lung, both acquired with a 30-gauge OCT needle probe. Videos of these 3D-rendered volumes are available as supplementary videos 1 and 2, respectively, available at the Physiology website. To provide a sense of scale, we note that the length of the cylindrical data sets shown here is 2 mm. The fetal lamb lung volume has been cropped so as to reveal the bifurcation of two bronchioles. Recent work has seen the application of automated quantification algorithms to analyze such volumetric data and compute stereological measures of the air space dimensions (100). The result of one such algorithm is shown in Figure 3E, with the air spaces delineated in green. Estimates of the median chord length (58) were found to be within 5 μm of manual measurements.

In contrast to such static 3D data volumes, “dynamic” OCT needle probes have been used to acquire rapid sequences of 2D images visualizing alveolar dynamics. One design consists of an inner “imaging” probe that rapidly translates back and forth multiple times per second, enclosed within a stationary outer needle. The light beam from the inner probe is emitted through a long slit window in the outer needle, with each stroke of the inner probe acquiring a 2D rectangular image of the adjacent tissue (80). Supplementary video 3 (available at the Physiology website) shows changes in parenchyma during simulated deflation and inflation of an excised, saline-filled pig lung. Selected images from the sequence, showing maximum deflation and maximum inflation, are presented in Figure 4.

Air-filled lungs present significant challenges for OCT and optical imaging in general because of the large refractive-index mismatch between alveoli walls and the enclosed air-filled region. At each air-tissue interface, the light is strongly backscattered, such that image penetration is typically limited to only one or two alveoli. Previous work has either been restricted to only imaging the superficial layer of alveoli (37, 83, 90) or has reduced the refractive index mismatch by displacing the air with a saline lavage (80, 106) or perfusion with ethanol (81). Although these latter approaches greatly
improve image penetration to five to six alveoli in depth, they are unsuitable for translation to in vivo imaging. We have recently proposed the use of an optofluidic needle probe, combining an OCT needle probe with an integrated channel to inject minute quantities of fluid directly to the region of lung parenchyma being imaged (106a). This has the significant advantage of delivering fluid focally to the area being imaged, while leaving the remainder of the lung undisturbed. Such probes also have potential to deliver other fluids during imaging, both for diagnostic and therapeutic purposes.

Limitations of OCT

OCT has demonstrated the potential to address a wide range of clinical and preclinical questions, yet it has not become a mainstream imaging modality, with most research emerging from a relatively small number of centers. One reason is the lack of pulmonary OCT imaging systems available for use by researchers without specialist optics expertise. Although some companies are developing products for the pulmonary market (92, 113, 120) and a small number of studies have made use of commercially available systems (21, 61, 121), success in implementation of the technology in respiratory science is generally through close collaboration between optical engineers and physiologists or medical health professionals.

There are also some specific limitations of OCT that require consideration. Reliable characterization of an entire organ, such as the lungs, is impacted by how much of the tissue may be feasibly imaged. Whilst endoscopic and needle probes allow imaging deep within the body, scattering and absorption of near infrared light in turbid tissue restricts the image penetration depth of OCT to a few millimeters. This can make accurate characterization difficult given the highly heterogeneous nature of many lung diseases. With respect to proposed needle-based scanning of lung parenchyma, this approach will result in some local trauma, both due to insertion and rotation of the needle. The latter may

FIGURE 3. OCT images of rat lungs and 3D visualization of fetal lamb lung

Top: OCT images of rat lungs. A: radial OCT image acquired from one rotation of the OCT needle probe. Dashed green vertical line shows location of the longitudinal image shown in B. B: longitudinal reconstructed OCT image showing a bronchiole and vessels. Dashed blue line shows location of radial image in A. C: corresponding H&E section. Image reproduced from Ref. 80. Bottom: 3D visualization of fetal lamb and rat lung. D: 3D visualization of fetal lamb lung, showing alveoli and bifurcation of bronchioles. See supplementary video 1 available at Physiology website. Image and video are reproduced from Ref. 80 and used with permission. E: automated identification of air spaces (green) in an OCT needle scan of a rat lung. See supplementary video 2 available at Physiology website. Image and video are reproduced from Ref. 100 and used with permission. n, Needle hole; b, bronchiole; v1, v2, v3, blood vessels. Scale bars are 200 μm. Length of both 3D cylindrical data volumes is 2 mm.
be reduced by enclosing the rotating needle in a stationary transparent catheter (68, 114, 117), although this will increase the outer diameter of the probe, which itself may restrict the utility of the technique.

A final consideration when assessing the appropriateness of OCT for a specific imaging function is whether there will be sufficient contrast between the relevant tissue types. Image contrast is dictated by the optical properties of the tissue in the near infrared spectrum, and tissues with very similar optical properties are unlikely to be distinguishable. Under these circumstances, application of OCT will not provide useful information. Conversely, light is strongly backscattered at locations where the optical properties change rapidly, such as in the layered structures found in the airway walls. For this reason, OCT lends itself to imaging of the integrity of layered structures, in particular in the scenario of distinguishing invasive and in situ malignant lesions (37). Further validation using new and refined OCT systems is required to fully determine the research and clinical questions that can be effectively addressed.

**Outlook**

Emerging research in OCT is extending the modality beyond structural imaging based simply on the amount of backscattered light, to provide improved contrast between different tissue types. A variant of OCT, referred to as polarization-sensitive OCT (PS-OCT) (22), allows calculation of the optical birefringence of tissue. Birefringence results from the regular arrangement of tissue ultrastructure, such as is found in the highly linearly organized arrangement of collagen in fibrotic tissue but is often absent in pathological tissue. This provides additional contrast between tissue types that is indicative of structure below the spatial resolution of normal OCT. Hariri et al. (40) presented images of lung tumor and fibrotic tissue, which appeared very similar under normal OCT but were clearly distinguished by the degree of birefringence when

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**FIGURE 4. Images acquired with a dynamic OCT needle probe**

Images acquired with a dynamic OCT needle probe showing simulated deflation (A) and inflation (B) in an ex vivo pig lung. See supplementary video 3 available at Physiology website to view the dynamic sequence. C: overlaid image of deflated (green) and inflated (orange) parenchyma. Scale bars are 1 mm. Video is reproduced and images are adapted from Ref. 80 and used with permission.
imaged with PS-OCT. Automated quantification algorithms have been used to calculate absolute measures of birefringence (15), which in other (non-lung) applications has been used to provide improved delineation between healthy and pathological tissue (135).

Another emerging area in OCT is the localized measurement of tissue compliance. Referred to as optical coherence elastography (OCE) (54), it uses the high spatial resolution of OCT to measure small-scale deformations of the tissue in response to an applied mechanical force. Analysis of force-induced deformations in tissue allows the calculation of local elastic properties at a spatial resolution of tens to hundreds of micrometers. The first results in airways have demonstrated differentiation of tissue layers in ex vivo porcine samples based on OCE (55, 56).

Conclusions

OCT occupies a useful niche among imaging modalities, offering superior image resolution to techniques such as HRCT, ultrasound, or MRI, and greater image penetration than optical techniques such as confocal microscopy. Use of low-power, near-infrared light avoids the risks associated with ionizing radiation, making it appropriate for longitudinal studies. Work to date has demonstrated the potential for different OCT systems to assess physiologically meaningful parameters in respiratory organs by visualizing alveoli populations and quantifying airway wall structure, compliance, and lumen caliber. Of most relevance are applications requiring near histology scale imaging, but where removal of tissue is unacceptable, such as in vivo assessment of airway wall structure. OCT and its emerging variants provide a powerful tool for physiologists exploring the function and pathology of the lung. Although OCT is unlikely to reach the level of detail provided by histology, the capacity for “dynamic morphology” is particularly enticing. ■

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