

Endoscopic Techniques for Optical Imaging

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Introduction

Lung cancer is the leading cause of cancer death in the industrialized world (Landis et al., 1998). Despite recent advancements to reduce the mortality associated with this disease, patient prognosis remains poor, with the current 5-year survival rate under 16%, a rate that has seen no dramatic change over the past 30 years (Jemal et al., 2007; Proctor 2001). It is set to become a worldwide epidemic with the World Health Organization estimating 10 million deaths per year worldwide by the year 2030 (Proctor 2001). While fatal in most cases, early stage lung cancer can be cured. At the time of presentation, less than 15% of patients have localized disease that may be amenable to surgical resection and potential cure; of these patients, the 5-year survival is still a low 60–70%, indicating that even earlier diagnosis is important.

Multidetector Computed Tomography

We now have the ability to detect early, small lung cancer through multidetector computed tomography (MDCT) scanning, presenting as lung nodules less than 10 mm diameter (and often less than 5 mm diameter), but 60% of smokers have at least one such lung nodule, and less than 1% are cancer. Current recommendations are to follow these lung nodules over time to assess growth – thereby potentially missing the opportunity for cure. Hence a recently introduced term known as the ‘lung cancer paradox’ – early detection is possible, but early diagnosis is not. In addition, while MDCT scanning can now detect small peripheral lesions, central lesions are still often radiographically occult in the early stages of disease progression. Clearly, new methods for evaluating suspect areas in the human airways

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at a cellular and molecular level are needed and would provide important information for early diagnosis. The ability to characterize the cellular and molecular compositions of a tumor in vivo will allow diagnosis of the tissue, but also of equal importance, track the progress of proceeding therapies. This feedback mechanism will provide faster evaluation of therapeutic techniques and enable cellular characterization of tumors, which is also currently lacking. Eventually this may lead to a better understanding of lung cancer and in particular its path at an early stage.

Conventional Pulmonary Imaging

Conventional endoscopic imaging or macro-optical imaging techniques are routinely employed in pulmonary disease management. The most common is the white light color or monochromatic bronchoscope; here a flexible fiberscope or charge-coupled device (CCD) endoscope is utilized to image the inside of the airway lumen with a white light illumination source. Assessment of the airway is performed based on subjective color, texture, and structural visualization of the reflectance image obtained through the bronchoscope. Many common lung pathologies can be distinguished using this form of bronchoscopy through assessment of such alterations as airway wall thickening, mucosal topography, mucosal color changes, and vascular changes. Bronchoscopy also provides a means for biopsy sampling using forceps biopsy, brush cytology, or needle aspiration through an auxiliary channel.

Autofluorescent Bronchoscopy

Autofluorescent bronchoscopy, a form of bronchoscopy where endogenous fluorescence of the airway and tissues are visualized either individually or simultaneously alongside white light images, has recently become available. This form of bronchoscopy reveals autofluorescence principally from connective tissue such as collagen, but also from cellular chromophores such as flavins, NADH, and porphyrins (Richards-Kortum and Sevick-Muraca 1996; Feller-Kopman et al., 2005). As the bronchial mucosa becomes abnormal with cell proliferation, it becomes thicker, and it has been suggested that the autofluorescence signal becomes less detectable. The combined use of white light and autofluorescence bronchoscopy has been shown to increase the overall sensitivity of identifying lung pathologies in the airways when compared to white light bronchoscopy alone (Beamis et al., 2004; Feller-Kopman et al., 2005; Herth et al., 2006; Lam et al., 1993; 2000; Haussinger et al., 2005), although this increase came at the cost of specificity (Kennedy et al., 2001). The utility of autofluorescence bronchoscopy is increasingly seen as an important tool for the management of patients with early lung cancer.

Recently, several other bronchoscopy techniques have also emerged, including computer-aided color and textural analysis of the airway lumen, fluorescence microvascular imaging using exogenous sodium fluorescein (Suter et al., 2005b, c, 2004b, 2008b), and narrow-band imaging (Herth et al., 2006).

Micro-optical Imaging Techniques

Micro-optical imaging techniques have also generated interest from within the pulmonary field, for evaluation of lung pathology in vivo (McWilliams et al., 2002; Sutedja 2003). Micro-optical imaging devices that seem to have promise in diagnosing lung cancer based on their ability to visualize cellular and molecular markers and have the potential for endoscopic miniaturization for use within the airways are confocal microscopy (Aziz and Gmitro 1993; Delaney et al., 1993; Namati et al., 2008b; Sung et al., 2002; Kiesslich and Neurath 2005; Rouse et al., 2004; Flusberg et al., 2005a), optical coherence tomography (Fujimoto et al., 1995; 2000; Yang et al., 2004; Yun et al., 2006), various types of spectroscopy (Weissleder and Pittet 2008; Bard et al., 2005), and fluorescence or luminescence detection systems that rely on an administered compound.

Confocal Endo-microscopy

Confocal endo-microscopy systems are now commercially available for both the gastro-intestinal and pulmonary systems, with promising early results. Development of these techniques, including technical advancements in the imaging hardware and development of fluorescent biomarker are currently ongoing. These endeavors may ultimately enable the diagnosis of suspect lesions in vivo.

Combination Strategies

With early detection of suspicious nodules now possible with MDCT scanning, utilizing the three-dimensional (3D) data inherent to MDCT scans to aid bronchoscopy procedures has become possible. Several research and now commercial systems exist for identification of suspect lesions, visualization of the airways in three dimensions (virtual bronchoscopy), creation of a path to the suspect lesion (Kiraly et al., 2004; Ferguson and McLennan 2005; Tschirren et al., 2005; Negahdar et al., 2006; Baker et al., 2007; McLennan et al., 2007a; b) electromagnetic tracking of the bronchoscope for direct feedback of location with respect to the three-dimensional virtual airway image (Schwarz et al., 2003; Gildea et al., 2006; Schwarz et al., 2006; Eberhardt et al., 2007a, b; Seijo et al., 2007; Makris and Gourgoulialis 2008), and electromagnetic active guidance strategies (Riker et al., 2007).

It is envisioned that a combination of MDCT and macro-optical imaging systems will be used for localization of suspect lesions through virtual pathway finding, electromagnetic navigation, and electromagnetic active guidance, while micro-optical imaging systems will be utilized for tissue analysis and possible diagnosis.

Conventional Optical (Macro-optical) Imaging

Rigid bronchoscopy was first reported in 1897 by Gustav Killian (1898), where a rigid esophagoscope was used to remove a foreign body from the right main stem bronchus. Over the next century bronchoscopy continually advanced and slowly became adopted until the late 1960s where Ikeda et al. pioneered the use of the flexible fiber-optic bronchoscope opening an array of new possibilities (Ikeda et al., 1968). Flexible white light fiber-optic and digital CCD bronchoscopes are now a common and important tool for interventional pulmonology. These systems provide the ability to assess the central airways and enable tissue sampling through forceps biopsy, brush cytology, or needle aspiration. In addition, laser therapy, cryotherapy, electrocautery, and stenting are now possible and routinely performed using bronchoscopy (Herth et al., 2006).

Bronchoscopy systems can be either fiber-optic or CCD based, monochromatic or color, but in any case they are referred to as white light bronchoscopy (WLB), as the illumination source is white. WLB systems provide reflectance-based imaging of the airways, as light reflected from the surface of the lumen is detected and viewed either through the bronchoscope or on a video monitor.

Autofluorescence Bronchoscopy

In contrast to white light bronchoscopy, fluorescent or autofluorescent bronchoscopy, a developing form of bronchoscopy since the early 1990's, detects fluorescent emission of tissue using a high intensity light source. These systems generally excite the tissue within the 400–490 nm spectral range and detect light in the greater than 500 nm range. Several commercial autofluorescent bronchoscopy systems have been developed (LIFE, SAFE-1000, D-light) and their utility has been investigated. Autofluorescence in the airways as discussed already is a weak signal produced by chromophores (Feller-Kopman et al., 2005). The detection of its presence and/or absence can be difficult, and it is not surprising that there is observer and technology-induced variance in the assessment of the test results. The color changes reported by the bronchoscopist are false colors indicating a presence and/or absence of the autofluorescence signal likely due to the mucosal thickness and have no other biological specificity for cancer. These tests are not standardized by any measurable output such as fluorescence intensity or quantifiable spectral change. Therefore, they are not particularly reproducible, resulting in limited adoption of this technology.

From several different autofluorescence studies, an increase in the sensitivity of detecting pre-invasive bronchial lesions with respect to white light bronchoscopy was found (Feller-Kopman et al., 2005; Haussinger et al., 2005; Herth et al., 2006;

Lam et al., 1993; 2000). However, autofluorescence bronchoscopy of pre-invasive lesions is still problematic as the specificity is very low, and up to half of the abnormal fluorescence regions are false-positives (Feller-Kopman et al., 2005; Haussinger et al., 2005). In light of these results, further controlled clinical evaluation is necessary including development of techniques to differentiate between pre-invasive lesions and bronchitis, a major obstacle in autofluorescence bronchoscopy.

Narrow-Band Imaging

An alternative bronchoscopy technique that has recently become available is narrow-band imaging (NBI). NBI proposes to increase the contrast of blood vessels by filtering out the illumination source with the exception of two narrow bands centered at 415 and 540 nm. Incidentally these are the peak absorption spectra of oxyhemoglobin, resulting in pronounced blood vessel contrast in the narrow-band images. It is known that dysplastic airway lesions have abnormal capillary formation, both from gross observation and histopathology. It is proposed that with the increase in vessel contrast obtained in NBI, such abnormalities are more apparent than when using white light bronchoscopy alone.

Recent studies using NBI have shown that it is more specific for detecting dysplasia than white light bronchoscopy alone and in addition, more specific than white light bronchoscopy and autofluorescent bronchoscopy combined (Herth et al., 2006). However, these studies carry several limitations including observer bias and uncontrolled patient history and management (Vincent et al., 2007).

Qualitative Color Analysis

White light bronchoscopy provides a visual link to bronchial mucosa topography and color; however, the subtle visual changes that indicate early stages in disease development may often be missed as a result of this highly subjective assessment. This is particularly apparent by the inexperienced bronchoscopist. The sensitivity of white light bronchoscopy for the detection of class III lesions has been reported to be as low as 10.6% with a corresponding specificity of 72.7% (Ernst et al., 2005). Recently, research focused on increasing the diagnostic yield of white light bronchoscopy while reducing user subjectivity has been performed using quantitative color analysis of the acquired white light bronchoscopy images (Gopalakrishnan 2003; Suter 2005; Suter et al., 2005d, 2004a, b). While the concept of color analysis of biological tissues is not new and has been used for a number of years in the field of dermatology, the utility in the pulmonary airways was limited until the introduction of color CCD chip bronchoscope (Aleva et al., 1998; Knyrim et al., 1987). A color analysis bronchoscope system with the primary objective of detecting color mucosal abnormalities was recently developed (Gopalakrishnan 2003; Suter 2005; Suter et al., 2005d, 2004a, b). This system was designed to provide real-time feedback to the treating physician by highlighting abnormal regions of interest on the WLB images adjacent to the live digital bronchoscope feed. Abnormal mucosal

regions of interest were identified based on comparative color analysis between the live digital bronchoscope feed and a library of mucosal colors generated from healthy non-smoking volunteers. Ideally this form of assessment will serve to guide the treating physician during the clinical bronchoscopy exam to regions of interest that may be consequently investigated further through optical or conventional biopsy.

Figure 1 depicts an example of a WLB image that has undergone quantitative color analysis. Regions of interest identified as being outside the determined range of healthy mucosal colors have been highlighted (Suter 2005).

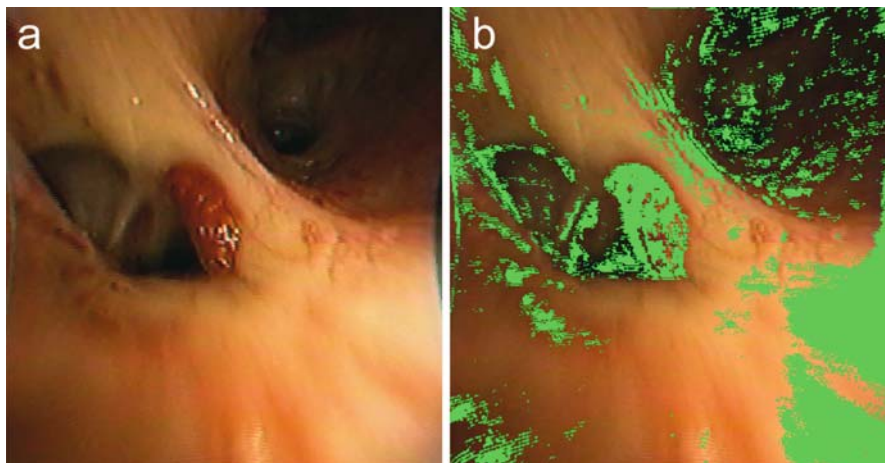


Fig. 1 This figure demonstrates the result of classifying the pulmonary mucosal colors based on comparison with a developed healthy database. (a) The original image of a pulmonary airway with known papillomatosis. (b) The result of comparing the hue and saturation values in the image to the developed normative database. This image was classified correctly as abnormal (Suter 2005)

In addition to the intrinsic utility as a biopsy guidance tool, quantitative color analysis of the bronchial mucosa may prove useful as a non-invasive diagnostic tool. A preliminary study on the use of color analysis together with automated neural network classification to categorize pulmonary pathology demonstrated that it was possible to accurately distinguish between healthy mucosa, carcinomas, granulation tissue, papillomatosis, and airway mucosa changes associated with idiopathic stenosis of the airways (Suter 2005).

Fluorescein Bronchoscopy

Fluorescein angiography is commonly used to qualitatively evaluate the circulation of the retina in order to detect and diagnose diseases including diabetes (Bjarnhall et al., 2002; Browning 1999). The process of fluorescein angiography involves injecting a bolus of fluorescein into the patient's circulatory system and observing

the time-based response in the retina (Flower 1973). Fluorescein is a highly fluorescent chemical compound that spontaneously fluoresces upon excitation with blue light. A large portion of the fluorescein injected into the blood stream binds to serum protein; however, it is the unbound fluorescein molecules that are responsible for the observed light emission (Boguta and Wrobel 2001). Recently bronchoscope-based fluorescence detection systems have been used in conjunction with fluorescein to assess the bronchial mucosal microvasculature (Suter et al., 2005a; Suter 2005). A 488 nm excitation source is delivered to the bronchial mucosa through a diffusing transmission fiber, and the fluorescein emission is detected by a fiber-optic bronchoscope connected to a CCD camera.

A preliminary study conducted on consenting volunteers from both a healthy non-smoking population and from patients with a significant smoking history revealed a statistically significant (p -value < 0.05) difference in the detected fluorescein emission (Suter 2005). The smoking population was found to have reduced fluorescein emission intensity when compared to the non-smoking population. While it is unclear whether the decrease in detected fluorescein emission was a direct result in a change in the bronchial mucosal microvasculature, a result of airway wall thickening, or some other mucosal alteration, future research in the assessment of the detectable superficial bronchial microvasculature may work toward enhancing our current knowledge and understanding of the airway microvasculature and its role in tissue remodeling in response to injury or disease.

Figure 2 (a) and (b) represents an example of a white light and fluorescein image acquired from a smoking individual with a significant obstructive cancer mass. In this example it is clear that the detected fluorescence emission is reduced over the identified mass.

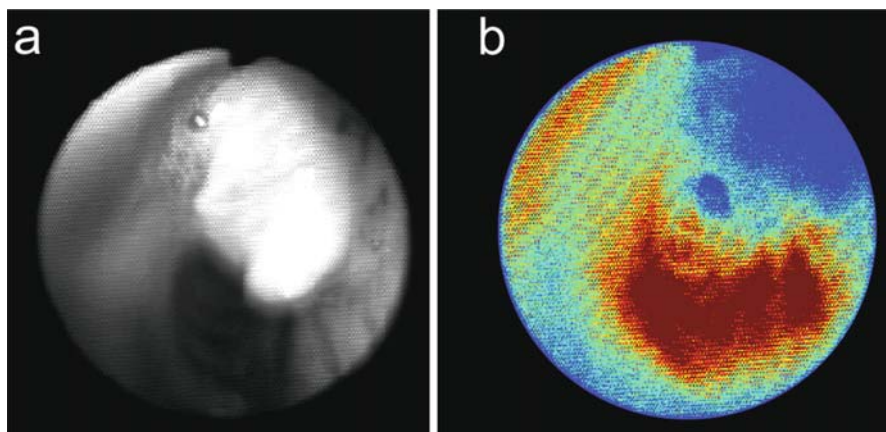


Fig. 2 Image of an airway with a significant obstructive mass (*top right quadrant*) from a smoking individual with signs of chronic bronchitis. (a) White light illumination bronchoscopy image and (b) a detected fluorescence emission image of the airway shown in (a) during laser excitation. The fluorescence image is pseudocolored with blue to red representing low to high intensity

Micro-optical Imaging

Micro-optical imaging techniques have recently generated great interest in the medical field, for their potential to translate into clinical in vivo biopsy systems (McWilliams et al., 2002; Sutedja 2003). Promising techniques include optical coherent tomography (OCT) (Fujimoto et al., 1995; 2000; Yang et al., 2004; Yun et al., 2006), fiber-optic spectroscopy (Weissleder and Pittet 2008; Bard et al., 2005), confocal microscopy (Aziz and Gmitro 1993; Delaney et al., 1993; Namati et al., 2008; Sung et al., 2002; Kiesslich and Neurath 2005; Rouse et al., 2004; Flusberg et al., 2005a), and two-photon microscopy (Jung and Schnitzer 2003; Kim et al., 2008; Gobel et al., 2004; Bird and Gu 2002).

Optical Coherence Tomography

Optical coherence tomography (OCT) is a non-contact optical imaging modality that provides tomographic images of tissue at resolutions comparable with architectural histology ($<10\text{ }\mu\text{m}$) (Bouma et al., 2000; Sergeev et al., 1997; Sivak et al., 2000; Tearney et al., 1997; Hsiung et al., 2005; Chen et al., 2007). The underlying concept of OCT is parallel to that of ultrasound, where measuring the delay of the source, as it is reflected off subsurface structures in biological tissues, generates depth information. Unlike ultrasound, however, a broadband light source is used in OCT, and due to the high speed of light propagation in tissue, optical reflectance is measured using low-coherence interferometry.

Several preliminary ex vivo studies have been conducted in recent years on the use of optical coherence tomography for the assessment of bronchial mucosa (Yang et al., 2004; Ikeda et al., 2007; Tsuboi et al., 2005; Whiteman et al., 2006). These studies have demonstrated that optical coherence tomography can indeed be used to visualize and evaluate the pulmonary tissue demonstrating good correspondence with histopathological sections. Endoscopic optical coherence tomography has also been used to interrogate the bronchial mucosa in a limited in vivo human proof of principle study (Tsuboi et al., 2005). Recently a large 138-patient in vivo study was conducted demonstrating that epithelial thickness measurements of invasive carcinoma, carcinoma in situ, and dysplasia could be used to differentiate between metaplasia and hyperplasia (Lam et al., 2008). To date these in vivo studies have generally been limited to point sampling techniques due to the inherent limitations of the optical coherence tomography imaging technique.

As lung cancer and its precursors are often multifocal and can arise anywhere within the airway tree (Kerr 2001), a diagnostic tool for evaluating this disease must be able to comprehensively investigate large areas in multiple bronchial segments during a clinical viable procedure time (1–5 min). Recently, a second-generation optical coherence tomography technology, termed optical frequency domain imaging (OFDI) or swept source optical coherence tomography, has been developed (Yun et al., 2003). While optical coherence tomography uses a broadband light source and

detects the optical reflectance using low coherence interferometry, OFDI is based on frequency domain interferometry and utilizes a rapidly tuned wavelength swept source (Brinkmeyer and Ulrich 1990; Chinn et al., 1997; Golubovic et al., 1997; Lexer et al., 1997; Yun et al., 2003; 2003). The key advantage of OFDI over optical coherence tomography is that it provides images at rates that are $100\times$ faster than conventional optical coherence tomography. Therefore OFDI, along with appropriate catheter designs, can be utilized to systematically image or screen the bronchial tree in a manner compatible with the temporal requirements of the bronchoscopy procedure. Figures 3 and 4 illustrate cross-sectional and volume-rendered images obtained in a recent study from ex vivo swine lungs in the central airways (Suter et al., 2008a). Layers of the bronchial wall including the epithelia, lamina propria, smooth muscle, perichondrium, and cartilage are clearly distinguishable.

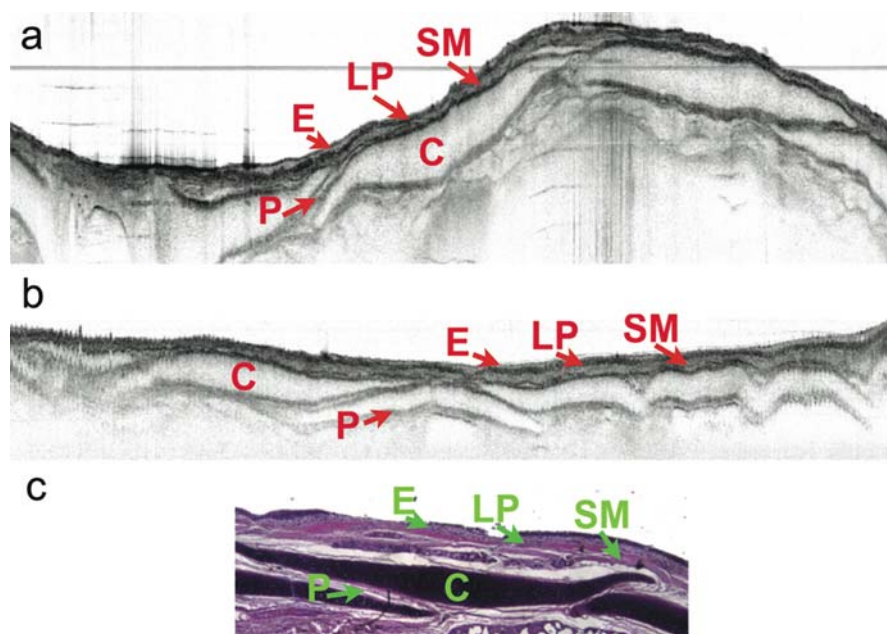


Fig. 3 (a) Transverse and (b) longitudinal cross-sectional OFDI images of the bronchial wall with the corresponding histology (c). E, epithelia; LP, lamina propria; SM, smooth muscle; P, perichondrium; and C, cartilage

Confocal Endo-microscopy

Confocal endo-microscopy, defined as confocal microscopy designed for endoscopic applications, has in particular gained attention for their miniaturization and high-resolution imaging potential. Their ability to non-invasively resolve cellular structures down to the micron level provides a high sensitivity and specificity

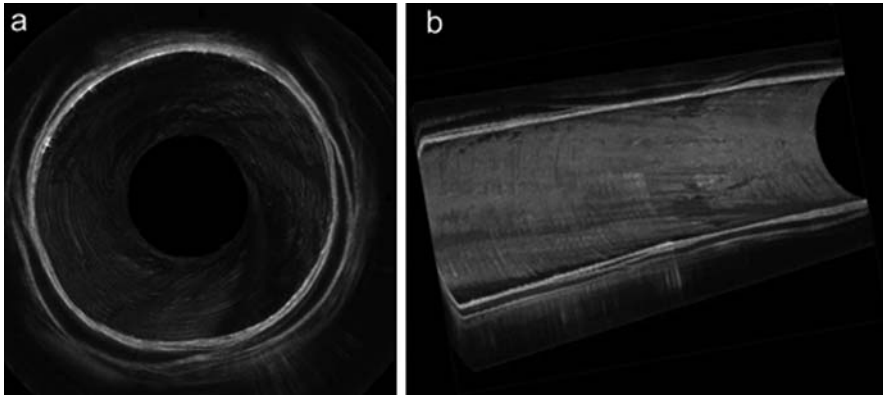


Fig. 4 This figure shows a volume rendering of the comprehensive OFDI data set depicted in Fig. 3. Rings of cartilage are clearly identifiable in the three-dimensional rendering

for assessment of disease states in vivo. This has led to the development and commercialization of such systems.

Confocal microscopy requires the scanning of a focused point source within the medium of interest, in this case a suspicious region inside the human body, with a high-powered light source and detection of fluorescent emission or reflectance via a sensitive photodetector. An image is obtained by reconstructing the raster-scanned detected light onto a grid (Minsky 1961, 1988). An array of confocal endomicroscopy systems currently exist. They generally fall under two groups: one that performs the laser scanning at the distal tip and a second that performs the scanning at the proximal tip (Flusberg et al., 2005a).

The most common technique includes scanning a flexible fiber bundle, containing thousands of coherently placed fibers, at the proximal end that relays the laser scan to the distal tip (Aziz and Gmitro 1993, 1994; Collier et al., 2002; Drezek et al., 2000; MacAulay et al., 2004; Rouse and Gmitro 2000; Rouse et al., 2004; Sabharwal et al., 1999; Carlson et al., 2007a, b; Collier et al., 2007; Maitland et al., 2008; Smithpeter et al., 1998). This is technically the simplest approach; however, the resolution and field of view are ultimately limited by the fiber-to-fiber distance and number of fibers in the bundle. An increase in the resolution can be obtained by mounting an objective lens to the distal tip; however, any increase in resolution also results in a decrease in the field of view (Carlson et al., 2005; Chidley et al., 2006; Sung et al., 2002; Rouse et al., 2004; Jung and Schnitzer 2003).

Several techniques have been proposed and built based on the second approach where scanning is performed at the distal tip. Most of these techniques utilize one or two single fibers for transmission and detection of light. Many different scanning apparatus mounted to the tip of the endoscope have been proposed and presented including a piezoelectric scanner (Rector et al., 2003; Myaing et al., 2006), a Micro-electromechanical Systems (MEMS) single (Flusberg et al., 2005b; Dickensheets and Kino 1996) or dual axis scanner (Liu et al., 2006; 2007; Wang et al., 2003a,

b, 2004), or a resonant tuning fork scanner (Delaney et al., 1993; 1994; King and Delaney 1994; Polglase et al., 2006). In either case, the resolution and field of view of the laser scanning can be adjusted or designed with varying specifications, and is not limited to the constraints discussed with the fiber bundle approach. Also, these techniques only require one or two single fibers to the distal tip, enabling highly flexible probe designs.

Confocal endo-microscopy systems are now commercially available for the gastro-intestinal (GI) tract, skin, and pulmonary systems (CellVizio Inc, Optiscan Pty. Ltd., Lucid Inc.). Recently an increasing number of research studies have been undertaken using confocal endo-microscopy systems in the GI tract with some early studies in the cervical and pulmonary regions. The initial results from these studies indicate a significant increase in sensitivity and specificity of disease assessment either directly with confocal microscopy imaging or as an aid for accurate biopsy sampling (Anandasabapathy 2008; Becker et al., 2008; Collier et al., 2002; Deiner et al., 2007; Drezek et al., 2000; Goetz et al., 2006; Goetz and Kiesslich 2008; Hendee 2002; Hoffman et al., 2006; Hurlstone et al., 2008; Hurlstone and Sanders 2006; Hurlstone et al., 2008; Kakeji et al., 2006; Kiesslich et al., 2007a, b, 2005, 2006a, b; Kiesslich and Neurath 2005, 2006, 2007; Kitabatake et al., 2006; Liu et al., 2008; MacAulay et al., 2004; Miehke et al., 2007; Pech et al., 2008; Polglase et al., 2005; Tan et al., 2007; Thong et al., 2007; Zheng et al., 2004). White light endoscopes for the GI tract are generally larger (>6 mm) than that used in the pulmonary system (<6 mm), hence deployment of confocal endo-microscopy systems for the lung has been limited. However, recently fabrication of small catheter-based systems that can be fed through the standard auxiliary port of a bronchoscope has been achieved.

Catheter-Based Confocal Microscopy (CBCM)

Catheter-based confocal microscopy (CBCM), defined as systems small enough to be fed through small catheters (<3 mm), has recently been investigated in the pulmonary field, for characterization of normal and diseased lung tissue in mouse models (Namati et al., 2007a; b; Namati et al., 2008a; b, c), pigs (Rogers et al., 2008), and humans (Thiberville et al., 2007). Many of the developed catheter-based confocal microscopy systems have been designed to fit through the auxiliary channel of a standard bronchoscope, and therefore can be used to investigate lung tissue from the airway epithelium via the subtending airways (Namati et al., 2007a; b; Namati et al., 2008; Thiberville et al., 2007). In some cases the catheter-based confocal microscopy probes are small enough to reach the peripheral alveoli. Figure 5 (a)–(c) represents three images acquired using catheter-based confocal microscopy systems on a mouse, pig, and human lung, respectively. The mouse in this example was administered with sodium fluorescein (Namati et al., 2008), while the pig and human lung images represent the endogenous autofluorescence of the collagen and elastin network.

A recent study using a commercial catheter-based confocal microscopy system (CellVizio, France) successfully demonstrated specific basement membrane

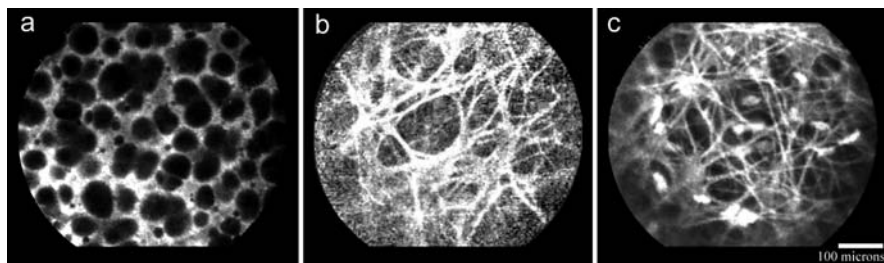


Fig. 5 Catheter-based confocal microscopy image from a (a) mouse, (b) pig, and (c) human lung

alterations associated with pre-malignant bronchial lesions. In this study, five distinct microscopic patterns were initially identified in normal regions from the proximal airways down to distal respiratory bronchi. In 29 patients with a high risk of lung cancer, disorganization of the elastin network associated with pre-invasive lesions, altered autofluorescent microstructure representing metaplasia or dysplasia, carcinoma in situ and invasive lesions were identified. In addition, spectral analysis was also simultaneously performed using the same device, and provided a quantitative adjunct for analysis of the connective tissue microstructure of the airways (Thiberville et al., 2007). This study provides positive initial results for assessment of lung cancer using a catheter-based confocal microscopy system in humans.

A further series of alveolar-based studies have also been recently described, with alveoli from non-smoking and smoking patients being imaged. Clear depiction of the alveolar elastin matrix and alveolar macrophages has been shown using autofluorescence. The results of these studies indicate a significant increase in the number and motility of autofluorescent macrophages in smoking patients versus non-smoking patients (Heckly et al., 2008; Thiberville et al., 2006).

In addition, clinical trials are now underway using this commercial catheter-based confocal microscopy system for assessment of alveolar structure and function in normal and diseased patients, as well as evaluation of lung pathologies such as lung cancer (MaunaKeaTechnologies 2008).

Taking advantage of the fluorescence approach, where light of one wavelength is used to excite a specimen emitting light at a higher wavelength, highly sensitive biomarker tagging strategies can also be deployed. Two distinct optical biopsy strategies may unfold, one that uses specific fluorescent biomarker tagging of important genetic, molecular, and cellular structures and a second that assesses general information regarding the tumor micro-environment, such as pH, oxygen, glucose, and specific amino acids.

An example of catheter-based confocal microscopy imaging on a mouse model of lung cancer is shown in Fig. 6 (a–c). Here three regions have been imaged in a living mouse lung: (a) ‘normal’ parenchyma, (b) suspicious parenchyma, and (c) tumor region. In this example, sodium fluorescein has been systemically administered for visualization of general tissue structure (green) while acridine orange has been applied topically over the region of interest for identification of nuclei (yellow-blue).

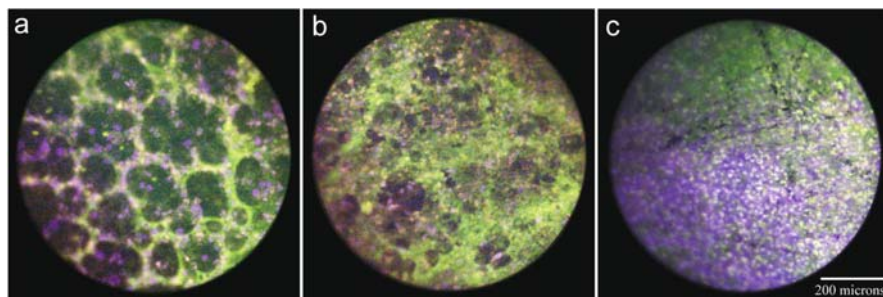


Fig. 6 Catheter-based confocal microscopy image of a mouse model of lung cancer, (a) ‘normal’ parenchyma, (b) suspicious region, and (c) tumor region

Catheter Navigation and Guidance

In the case of lung cancer, the majority of suspicious nodules identified in MDCT scans are in the peripheral region of the lung. However, using standard bronchoscopy systems only 10 out of the 26 generations of airways can be navigated due to size restrictions. The benefit of utilizing such micro-optical imaging systems discussed above for characterization and diagnosis of early peripheral lung nodules thus becomes important and requires new approaches.

Accurate identification and biopsy of suspicious regions of interest (nodules and lymph nodes) in the central lung is a difficult task for even the well-trained bronchoscopist. The utility of a pathway finding system with the ability to track the tip of the bronchoscope and relate back to a virtual bronchoscopy image generated from a MDCT scan has recently become apparent. Virtual bronchoscopy systems have been investigated since the mid-1990s (Ferretti et al., 1995; Summers et al., 1996; Vining et al., 1996; Ferretti et al., 1997; Higgins et al., 1998); however, only recently has their integration with electromagnetic guidance systems provided a practical utility during clinical procedures (Schwarz et al., 2003; Gildea et al., 2006; Schwarz et al., 2006; Eberhardt et al., 2007a; b; Seijo et al., 2007; Makris and Gourgoulialis 2008). Commercial systems are now available that provide virtual bronchoscopy and electromagnetic navigation for real-time tracking of the bronchoscope during a clinical bronchoscopy procedure.

Virtual bronchoscopy systems can be used to create a path to the region of interest using automated or manual routines as shown in Fig. 7 (Kiraly et al., 2004; Ferguson and McLennan 2005; Tschirren et al., 2005; Negahdar et al., 2006; Baker et al., 2007; McLennan et al., 2007a, b).

This path can then be followed during the live bronchoscopy procedure. However, there is an inherent problem associated with such a technique; virtual bronchoscopy is based on the MDCT scan, and hence the quality and depth of the airway segmentation is limited by the resolution of MDCT technology (Higgins et al., 1998). Currently with advanced airway segmentation algorithms up to 6–8 generations can be accurately identified as seen in Fig. 7 (Kiraly et al., 2002; 2004;

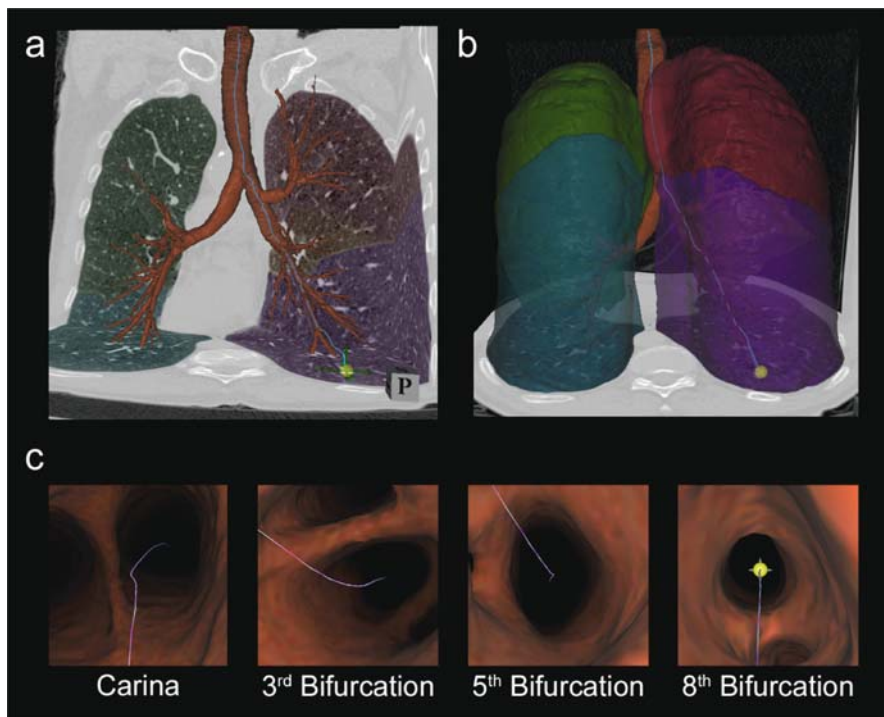


Fig. 7 (a–c) Automated virtual path finding to a nodule of interest. The path is indicated in *blue* and the nodule is indicated by the *yellow sphere*. (a) Represents the automated segmented airways and direction path (*blue*), with the original CT data color coded for individual lobes. (b) Represents a volume reconstruction of the airways and the lung into its individual lobes. (c) Represents the Carina, 3rd, 5th, and 8th bifurcation leading toward the nodule indicated by the *yellow sphere*. Images courtesy of VIDA diagnostics

Tschirren et al., 2005a, b). Several strategies exist for extending the path of interest, including manual segmentation of a single path (Graham et al., 2008), and further development in this area is ongoing.

Navigation of catheter-based micro-optical imaging systems can now be implemented using such electromagnetic navigation systems. However, as indicated above, with limited visual feedback from the virtual bronchoscopy view in the peripheral regions, and the lack of steerability in current micro-optical imaging systems, new approaches for guidance are also needed. Guidance of catheters can be performed using 360° steerable catheter sheaths, as implemented by SuperDimension Inc.; however, such systems are again limited by the diameter of the sheath. A technique recently proposed (Riker et al., 2007) is the use of two large external magnets to steer a ferromagnetic-tipped catheter. A commercial system currently exists for steering catheters into coronary arteries (Grady et al., 1989, 1990; Ernst et al., 2004; McPherson and Warnick 2004; Chun et al., 2007; Kiemeneij et al., 2008; Mehta et al., 2008), and development of pulmonary applications is currently

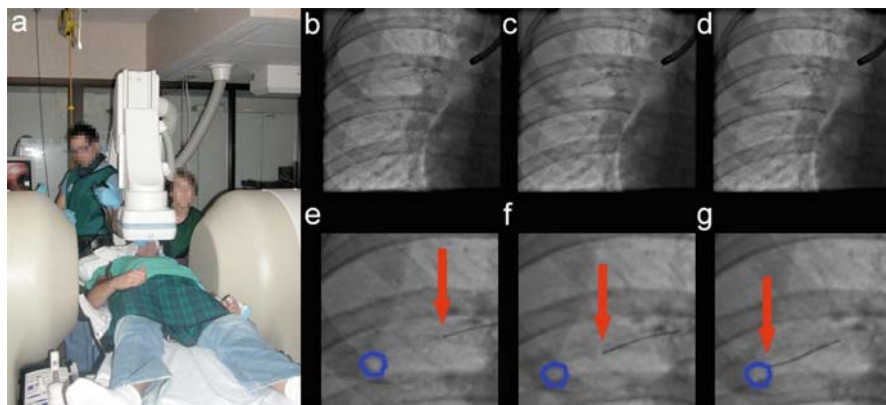


Fig. 8 (a) Bronchoscopy procedure utilizing a Niobe Stereotaxis magnetic guidance system; (b–d) a series of chest X-rays depicting a ferromagnetic guide wire steered toward a peripheral nodule; (e–g) magnified series of X-rays shown in (b–d) with the guide wire tip indicated by a *arrow* and the boundary of the peripheral nodule circled

underway (Di Biase et al., 2007) (Riker et al., 2007). Figure 8 represents a recent study, where a Niobe magnetic guidance system (Stereotaxis Inc, St. Louis) was used to direct a ferromagnetic-tipped guide wire to small peripheral lung nodules. This was part of a proof of concept study investigating the ability of such a technique for steering micro guide wires to peripheral nodules that would otherwise be very difficult to reach using standard bronchoscopy.

Summary and Future Direction

In future, the combination of macro-optical imaging techniques such as white light, autofluorescent, and fluorescein bronchoscopy will be utilized for the initial identification of suspicious regions, while micro-optical techniques such as the optical coherence tomography or catheter-based confocal microscopy system will analyze and classify the regions of interest, enabling live pathologic assessment. In addition, with the integration of navigation and guidance systems, micro-optical catheters will be directed to regions of interest well past the limited bronchoscope range, enabling diagnosis of distant peripheral nodules.

The digital imaging revolution has greatly advanced methods such as macro- and micro-optical endoscopy and X-ray imaging techniques such as CT scanning. By merging these technologies together, with CT providing detection, some characterization, and pathway guidance (together with electromagnetic strategies) for the macro- and micro-optical bronchoscopic techniques, almost any lung nodule can be sampled within the lung. Nodules that are small and may be pre-cancerous can in the future be ablated at the same bronchoscopic procedure. This process can solve

the lung cancer paradox, in much the same way as a dermatologist will ablate small lesions on sun-exposed skin at risk for skin cancers.

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