Seeing beyond the Bronchoscope to Increase the Diagnostic Yield of Bronchoscopic Biopsy

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The accurate diagnosis and molecular assessment of lung tumors are increasingly important in determining the optimal course of treatment for patients with lung cancer (1–3). This more personalized approach necessitates accurate tissue sampling to ensure the biopsy contains sufficient tumor for both morphologic diagnosis and molecular testing to determine the mutational status of the tumor (4–6). Tumor sampling may be accomplished by low-risk bronchoscopy techniques. However, the diagnostic yield of these approaches is variable because the biopsies can contain nontumor elements, such as areas of fibrotic tumor stroma and/or surrounding normal lung tissue (6). Diagnostic yields in transbronchial fine needle aspiration (TBNA) and transbronchial biopsy can significantly vary depending on the size and location of the nodule (7–10). The addition of endobronchial ultrasound guidance or electromagnetic navigation increases diagnostic yield, but the yields are still lower for lesions not more than 2.0 cm in diameter (56.3% for lesions $\leq$ 2.0 cm vs. 77.7% for lesions $>$ 2.0 cm) (7–13). *In vivo* real-time biopsy site assessment could aid in lung nodule targeting and provide more selective tissue acquisition to increase the diagnostic yield of bronchoscopic biopsy. Implementation of novel optical imaging techniques that are complementary to traditional bronchoscopy could provide physicians with a valuable tool to microscopically assess a lung nodule before biopsy and determine the optimal site for tissue acquisition. Some of the results of these studies have been previously reported in the form of abstracts (14, 15).

Optical coherence tomography (OCT) is a nondestructive imaging tool that rapidly generates high-resolution ($<10\ \mu\text{m}$) cross-sectional images of tissue with penetration depths approaching 2–3 mm (16–18). OCT is a time-of-flight imaging technology in which images are generated by measuring the intensity of backscattered near-infrared light using low-coherence interferometry. This is similar in principle to measuring sound echoes reflected back from tissue in ultrasound. There are a number of catheter-based OCT systems that are commercially

available, including both longitudinal and cross-sectional scanning catheters that range from 0.8 to 2.5 mm in diameter. OCT has been used to accurately detect and diagnose cardiovascular (19–21) and gastrointestinal (17, 22, 23) pathology, and more recently has been used to assess the tracheobronchial tree and parenchyma *in vivo* (24–33). OCT imaging of the normal bronchial wall enables visualization of the fine layered features of the airway, including epithelium, basement membrane, lamina propria, bronchial glands and ducts, vessels, and cartilage with surrounding perichondrium. Lung parenchyma appears as a lattice-like structure with signal-void alveoli (Figure 1A). OCT has been used to investigate airway-based lesions in the clinical setting, including dysplasia, carcinoma *in situ*, and invasive cancer (26–29). More recently, preliminary OCT assessments of peripheral tumors have been performed *ex vivo*, including benign lesions such as hamartomas (32). These studies demonstrate that OCT is able to identify many of the features of the normal and diseased lung and may have a future role in diagnostic imaging.

Narrow-diameter, flexible OCT catheters compatible with the working channel of standard bronchoscopes have been developed for airway-based imaging and, more recently, needle-based OCT catheters have also been developed (34–37) to facilitate imaging beyond the boundaries of the airway wall. These catheters include rigid needle catheters for transthoracic imaging (35–37), and flexible catheters compatible with standard TBNA needles for transbronchial imaging (34). We have developed a transbronchial OCT needle probe compatible with standard bronchoscopes that may be used to rapidly assess needle placement before TBNA specimen collection (described by Tan and colleagues [34]). An example of inflated lung parenchyma imaged by needle-based OCT is demonstrated in Figure 1A. On the basis of previously established image interpretation criteria, OCT can readily distinguish airway wall and lung parenchyma (Figure 1A) from lung nodules (Figures 1B–1D) (32). This ability suggests OCT may assist in assessing whether a lung nodule has been accurately targeted for biopsy. Even when lung tumors are accurately targeted, nondiagnostic tissue specimens consisting predominantly of dense tumor fibrosis and/or necrosis can still be obtained, rendering the biopsy insufficient for diagnosis and molecular profiling (6). Structural OCT is capable of identifying necrosis, which has a distinct appearance characterized by well-defined, signal-poor regions (demonstrated in an example of squamous cell carcinoma with necrosis; Figure 1C). However, structural OCT lacks the contrast to reliably differentiate tumor from organized fibrosis or tumor-associated, dense desmoplastic fibrosis, as is exemplified in Figure 1B (carcinoid tumor) and 1D (central scar in squamous cell carcinoma). In Figure 2, the structural OCT images of the tumors (Figures 2A, 2D, and 2G) appear similar despite having varying compositions of tumor and fibrosis. Although structural OCT provides a useful method for accurate targeting of peripheral nodules and identifying necrosis, the differentiation of tumor from

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Figure 1. Structural optical coherence tomography (OCT) of lung parenchyma and tumor in *ex vivo* lung. (A) Normal lung parenchyma obtained by needle-based OCT. (B) Carcinoid tumor presenting as a peripheral lung nodule. (C) Necrosis in squamous cell carcinoma appearing as signal-poor regions with ill-defined borders (arrows). (D) Tumor-associated, dense desmoplastic fibrosis within a squamous cell carcinoma. Scale bars: 1 mm. The vertical backscattering, shadow artifacts, indicated by the asterisks in C, are artifacts from ink marks used to register polarization-sensitive OCT and histology.

fibrosis is critical for effective biopsy guidance to maximize diagnostic yield.

Polarization-sensitive OCT (PS-OCT) generates microstructural images while simultaneously measuring tissue birefringence (38–46). When light travels through birefringent tissues, the orthogonal polarization components of the light will undergo a phase retardation with respect to one another. The magnitude of this phase retardation is dependent on the orientation of the polarized state of the light with respect to the organized linear structures within the tissue. Highly linearly organized connective tissues, such as collagen, are therefore associated with a strong birefringence signature (47–50). Birefringence detection is achieved in PS-OCT by modulating the polarization state of the source between distinct polarization states in successive A-lines (axial depth profiles) and then combining

A-line pairs by Stokes vector (39) or Jones matrix (40) analysis. PS-OCT may provide a significant enhancement over traditional OCT in differentiating tumor from tumor-associated fibrosis.

Figure 2 demonstrates the ability of PS-OCT to differentiate tumor from fibrosis by detecting a strong birefringence signature in dense desmoplastic fibrosis in *ex vivo* lung tissue. High birefringence is observed in areas of dense fibrosis in the illustrated squamous cell carcinoma (Figures 2A–2C), which correlates with regions of fibrosis on trichrome staining (seen as regions with deep blue staining; Figure 2C). In tumors with early desmoplastic response surrounding invasive adenocarcinoma (Figures 2D–2F), areas of dense collagen display high birefringence (Figure 2E, lower half), whereas areas with young, poorly organized collagen and elastic fibers admixed with malignant glands show lower birefringence (Figure 2E, upper half).

Figure 2. Polarization-sensitive optical coherence tomography (PS-OCT) of tumor and tumor-associated desmoplasia in *ex vivo* lung. (A) Structural OCT of squamous cell carcinoma with dense desmoplastic fibrosis. (B) PS-OCT of squamous cell carcinoma with dense desmoplastic fibrosis, with high birefringence in the fibrotic component. (C) Trichrome stain of squamous cell carcinoma with dense desmoplastic fibrosis, displaying deep blue staining in areas of dense fibrosis. (D) Structural OCT of adenocarcinoma with early desmoplasia. (E) PS-OCT of adenocarcinoma with early desmoplasia showing moderate birefringence in the lower half, where more organized collagen is present, and less birefringence in the upper half, where tumor is admixed with young, poorly organized collagen and elastic fibers. (F) Trichrome stain confirming more organized desmoplasia in the lower half staining deeper blue and less organized

desmoplasia in the upper half staining a lighter blue-gray. (G) Structural OCT of carcinoid tumor and (H) PS-OCT of carcinoid tumor showing little to no birefringence. In both the structural and PS-OCT images of the carcinoid, there is a high-signal intensity imaging artifact across the surface of the tissue sample that is commonly found in OCT images at air–tissue interfaces. (I) Trichrome stain confirming lack of significant collagen with the carcinoid. Scale bars: 1 mm.

Figure 3. Polarization-sensitive optical coherence tomography (PS-OCT) of poorly differentiated carcinoma with surrounding fibrosis in *ex vivo* lung. (A) Structural OCT does not clearly show a distinction between solid carcinoma and adjacent fibrosis. Calcifications within the fibrosis can be seen as signal-poor structures (C). (B) PS-OCT shows a clear delineation between solid carcinoma (left of line) and fibrosis (right of line), with no birefringence signal in the regions of carcinoma and high birefringence signal in the regions of fibrosis. (C) Matched histology confirming the demarcation between carcinoma and fibrosis. Scale bars: 1 mm.

Tumors with little intervening connective tissue, such as carcinoid tumors (Figures 2G–2I), show little to no birefringence signal. Trichrome staining confirms the presence of both early desmoplasia with poorly organized collagen fibers within the adenocarcinoma (regions of light blue-gray staining; Figure 2F) and the absence of prominent connective tissue in the carcinoid (Figure 2I).

The advantage of polarization sensitive imaging is further highlighted in Figure 3, where a solid poorly differentiated carcinoma is surrounded by dense desmoplastic fibrosis in an *ex vivo* lung specimen. Structural OCT (Figure 3A) lacks the ability to clearly distinguish the areas of solid carcinoma from fibrosis. However, PS-OCT (Figure 3B) visibly delineates the fibrosis from carcinoma, with high birefringence signal in the regions of fibrosis and lack of birefringence in the adjacent solid carcinoma. The boundary between the solid carcinoma and fibrosis clearly visualized with PS-OCT is confirmed with matched histology (Figure 3C). These results suggest that PS-OCT could serve as a powerful imaging technology for assessing tissue acquisition sites within lung nodules by drastically enhancing differentiation between tumor and fibrosis.

PS-OCT shows significant potential for biopsy guidance. However, this work was performed in *ex vivo* lung resection specimens and may potentially have some limitations in translation to *in vivo* imaging, such as motion artifacts, blood contamination, and the development of appropriate catheter designs. All of these potential limitations also apply to structural OCT, which has been successfully performed endobronchially in pilot clinical studies with similar image quality (24–33), including imaging of airway-based masses and alveolar attachments. Catheter-based PS-OCT systems are not currently commercially available, but may be implemented by modulating the polarization state of a structural OCT system light source without any modification to the imaging catheters (41–46). We therefore do not anticipate any difficulties in translating PS-OCT into the clinical setting.

Accurate diagnosis and molecular profiling are central to targeted therapy in lung cancer. However, standard approaches for obtaining tissue specimens often result in the collection of specimens containing insufficient tumor. Structural OCT may be useful for targeting nodules and identifying necrosis, but it lacks the ability to reliably differentiate fibrosis from tumor. Our results demonstrate that PS-OCT provides complementary information that may be leveraged to ensure that the collected specimens have high tumor content by avoiding nondiagnostic tissues including fibrosis. Together, structural and polarization-sensitive OCT provide a synergistic depiction of both tissue microstructure and composition that may be useful in guiding biopsy site selection and optimizing tumor content for both diagnosis and molecular profiling. Although these results are promising, this work is still in the clinical research stage and further large-scale *in vivo* studies are needed to fully assess the potential sensitivity and specificity of PS-OCT in guiding lung biopsy before widespread clinical adoption.

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