In Vivo Optical Coherence Tomography of the Human Larynx: Normative and Benign Pathology in 82 Patients

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**Objectives:** Optical coherence tomography (OCT) is an emerging imaging modality that combines low-coherence light with interferometry to produce cross-sectional images of tissue with resolution about 10 μm. Patients undergoing surgical head and neck endoscopy were examined using a fiberoptic OCT imaging probe to study and characterize microstructural anatomy and features of the larynx and benign laryngeal pathology in vivo. **Materials and Methods:** OCT imaging of the larynx was performed in 82 of 115 patients who underwent surgical endoscopy for various head and neck pathologies. The OCT device employs a 1.3 μm broadband light source (FWHM, 80 nm). The frame rate is 1 Hz. Imaging was performed using a handheld probe placed in near contact with the target site. The maximum axial and lateral dimensions for the region of interest imaged were 2.5 mm × 6 mm, with resolutions of 10 μm. Simultaneously, conventional endoscopic images were obtained to provide anatomic correlation with OCT images and histology. Optical micrometry was performed to measure the epithelium thickness. **Results:** Systematic OCT imaging of laryngeal structures and subsites provided information on the thickness of the epithelium, integrity of the basement membrane, and structure of the lamina propria. Microstructural features identified included glands, ducts, blood vessels, fluid collection/edema, and the transitions between pseudostratified columnar and stratified squamous epithelium. The mean epithelial thickness of laryngeal subsites was calculated: true vocal cord (129 μm), false vocal cords (124 μm), aryepiglottic fold (177 μm), subglottis (98 μm), and epiglottis (185 μm). True vocal cord pathology imaged included Reinke’s edema, papillomatosis, polyps, mucous cysts, and granulation tissue. Subglottic imaging identified boundaries between epithelium, lamina propria, and cartilage. The OCT images compared favorably with conventional histopathology. **Conclusion:** OCT has the unique ability to image laryngeal tissue microstructure and can detail microanatomic changes in benign, premalignant, and malignant laryngeal pathologies. OCT holds the potential to guide surgical biopsies, direct therapy, and monitor disease, particularly when office-based systems are developed. This is a promising imaging modality to study the larynx. **Key Words:** Optical coherence tomography, larynx, vocal cord, vocal cord polyps, laryngeal cancer, Reinke’s edema, endoscopy, optical biopsy, respiratory papillomas, hyperkeratosis, laryngology.

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**INTRODUCTION**

Optical coherence tomography (OCT) is an emerging imaging modality that uses advanced photonics to produce high-resolution images in living tissues. OCT works in a manner analogous to ultrasound but relies on light rather than sound to discern intrinsic differences in tissue structure and uses coherence gating instead of time-of-flight images.
Clinical applications of OCT have generated the most interest in the diagnostic evaluation of structures where tissue microstructural information is of paramount importance, but where biopsy carries significant morbidity. The greatest clinical utility to date of OCT has been in retinal imaging where OCT can diagnose subtle pathologic changes within the retina. Likewise, there is tremendous interest in developing OCT as a diagnostic tool for management of cardiovascular disease. In contrast, OCT applications in otolaryngology have been limited.

Biopsy of the true vocal cords (TVC) in the larynx may produce significant morbidity, albeit less than retinal or intraarterial procedures. Voice quality may change dramatically and permanently after vocal fold surgery regardless of whether the pathology is benign or malignant. The negative impact of TVC surgery on voice quality has been well documented, and there are a number of cases of malignant lesions, and benign disorders that clinically mimic cancer remains a challenge. Currently, no imaging technology is available to aid the guidance and targeting of biopsies or for that matter in the monitoring of disease progression.

Analogous to real-time CT, ultrasound, and magnetic resonance imaging (MRI) guided therapy, OCT has a potential role in the management of superficial disease where the pathologic and key structural information is at the histologic level. Even though ex vivo OCT imaging was first reported in 1991, the first clinical imaging of the larynx was not reported until 1997. This first clinical study was limited in case numbers and used a slow, low-resolution, first generation system to accomplish imaging. The present study is a comprehensive evaluation of 82 patients who underwent laryngeal OCT imaging during surgical endoscopy. The current series demonstrates the in vivo microstructural features of the larynx while providing quantitative epithelial information. We present the largest series of OCT head and neck pathology reported to date.

**MATERIALS AND METHODS**

**Patient Population and Endoscopy**

OCT imaging was performed in 115 patients undergoing laryngeal, esophageal, or tracheobronchial endoscopy under general anesthesia at the University of California, Irvine (UCI) Medical Center in accordance with guidelines of the Human Subjects Institutional Review Board at UCI. Eighty-two of these patients had at least one subsite of the larynx evaluated using OCT, whereas other regions of the head and neck were imaged in the remaining patients. OCT imaging of the larynx was performed with or without the use of laryngeal suspension depending on the clinical need. When possible, correlative digital images of the larynx were recorded using either a rigid telescope or operating microscope. When clinically indicated (e.g., for tumor diagnosis, polyp removal), biopsy or excision of suspicious mucosal lesions was performed. Information obtained from OCT imaging was purposely not used to alter clinical decision making in any way during this study. Imaging generally required approximately 5 minutes of additional operating room time.

**OCT Instrumentation**

The core OCT device has been described previously. The system used in this study used a low coherence light source (central wavelength $\lambda = 1310$ nm, FWHM $\Delta \lambda = 80$ nm, AFC BT 1310, JDS Uniphase, San Jose, CA). Raster scanned images were generated by controlled motion of the imaging fiber using a precision piezoelectric translation stage (Model 663.4pr, Physik Instrumente, Tustin, CA). The delicate fiber and optical elements are enclosed by a transparent plastic tube, which was, in turn, surrounded by a second metal tube (diameter 2 mm) for mechanical support.

The axial resolution of the system is 9 $\mu$m and is determined by the coherence length of the light source. The lateral resolution is diffraction limited (20 $\mu$m). Signals were obtained up to a depth of 2.6 mm, whereas the lateral extent of each image was determined by the length over which the fiber was translated by the stage, typically 6 mm.

**OCT Imaging**

Images were acquired during surgical endoscopy by inserting the probe through the laryngoscope and placing the tip either in near contact or gentle contact with the region of interest. OCT images were acquired with the left and right side of each image, respectively, representing proximal and distal tissues. The image plane is defined by the direction of light propagation, which is orthogonal to the long axis of the probe. OCT imaging was typically performed in tandem with a rigid endoscope, allowing simultaneous viewing of the video and the OCT image on adjacent monitors. OCT images were digitally captured and then catalogued in a database.

**Image Analysis**

Digital micrometry (Photoshop, Adobe, San Jose, CA) was performed to obtain normative measurements of epithelial thickness in each laryngeal subsite. In each image, measurements of epithelial thickness were obtained at five evenly spaced points separated by 1 mm. These data were averaged to obtain the epithelial thickness per subject. In turn, measurements for each patient were analyzed to obtain an overall average of our study group. Measurements were not taken from those images in which the demarcation between the epithelium and underlying lamina propria (LP) could not be clearly identified. Whereas 82 patients underwent laryngeal imaging, the TVCs were imaged only in 68. In the case of TVC imaging, many patients had abnormal vocal cords features (i.e., nodules, papillomas [P], tumors) and were not included in the normative analysis. Imaging of other laryngeal subsites was limited by the constraints of operative time and also the need to reposition the laryngoscope in those patients undergoing laryngeal suspension.

**Histopathology**

Sixty of the 82 patients underwent laryngeal biopsy, and vocal cord biopsies were obtained in 23. Specimens were fixed in 10% neutral buffered formalin, sectioned in 5-micron thickness, and then stained with hematoxylin-eosin followed by histologic examination under microscopy.

**RESULTS**

OCT imaging of at least one laryngeal subsite was performed in 82 patients during surgical endoscopy. The
The majority of the subjects demonstrated normal vocal cords (n = 43); however, the larynx was imaged during operative endoscopy for pathology in other regions of the head and neck. The laryngeal diseases and lesions studied are listed in Table I by subsite. Some patients underwent endoscopy for injection TVC medialization to improve dysphonia associated with vocal cord paralysis. Some of the subjects evaluated in this study were diagnosed with laryngeal cancer and are excluded from the present analysis and will be reported in greater detail in the future. The present results primarily focuses on normative laryngeal structure, benign pathology, and limited examples of invasive laryngeal cancer.

The following images are representative illustrations of the features, disorders, and pathology that were imaged and selected to emphasize the potential of OCT as a tool in laryngology. It must be emphasized that OCT imaging, similar to ultrasound, results in the acquisition of time-series imaging in registry and can be viewed as a “video,” albeit at slower frame rates. The difference between individual static images and an OCT “video” examination is significant, and representative video clips are archived on the internet-based companion to this journal.

Figure 1 is a montage of OCT images of the true (A) and false (B) vocal cords and the gland-bearing aryepiglottic fold (c). The junction between the stratified squamous epithelium (SS) and lamina propria (LP) in each of these three images is very well defined, and arrows indicate the location of the basement membrane (BM) in each image.

The epithelium is identified as a thin structure at the surface demonstrating low signal intensity. Low signal intensity is a reflection of less optical scattering and is consistent clinically with the translucent appearance of the epithelium. In contrast, higher signal intensity is produced by the optically dense structures in the LP such as blood vessels, adjacent lymphatics, and the dense layers of collagen and elastin fibers. The heterogeneity of the texture and appearance of tissue within the LP is demonstrated by the variegated regions of high (white) and low (black) signal intensity within the images. The signal diminishes near a depth of 2 mm. At the lateral extent of these images (and images in subsequent figures), motion artifacts (produced by direction changes in the translation stage) occur and are indicated by the white open brackets in Figure 1. All subsequent optical cross sections are 6 mm long and 2.6 mm deep, except when otherwise note. A 1 mm measurement bar is included for reference.

The transition zone between the SS of the TVC and the gland-bearing ciliated pseudostratified columnar epithelium (PS) of the subglottis is shown in Figure 2A. Notably, structures consistent with seromucinous glands (SG) and seromucinous ducts (SD) were identified and indicated by the arrows, although because correlative biopsies were not obtained, this information is presumptive. In the central region of the image, what appears to be a...
duct-like structure terminates on the surface of the cord. The epiglottis (Fig. 2B) is also known to be highly glandular, and similar glandular features were identified. A sharp demarcation exists between the LP and what is likely the smooth surface of the epiglottic cartilage (EC).

Gland-like structures and soft tissue-cartilage borders are also seen in the subglottis (Fig. 3). In the proximal subglottis (Fig. 3A), low signal intensity structures extending longitudinally from proximal to distal were identified and may be consistent with glandular tissues. At the left side, the high signal intensity at the epithelial surface is caused by specular reflection and is an artifact. At the far right, the arc shaped border between the lamina propria and the cricoid cartilage (CC) is seen. Distally (B), the CC is seen as well. The high signal intensity line at the top of the image is caused by serosanguinous debris collecting on the plastic surface of the OCT probe.

Pathologic conditions were imaged as well using OCT. The microstructure of the vocal cord in Reinke’s edema has been primarily studied during limited postmortem examinations. Little is known about the true in vivo changes in TVC structure accompanying this disorder. Five patients with a clinical diagnosis of Reinke’s edema were examined. The image in Figure 4A shows numerous well-demarcated fusiform low-signal intensity regions within the LP, which we believe are the regions where clear fluid has collected (E). Homogenous regions with low signal intensity are produced by a low optical scattering in clear rather than turbid media. At the upper left of the image, two arrows point toward circular low-signal intensity regions, which may represent capillaries (CP) as vessels, were directly visualized during endoscopy in this general vicinity. The LP in the image has greater signal intensity, likely because of the increased density of matrix proteins such as collagen and elastin. Numerous linear structures are seen as well, suggesting a layered structure of the TVC LP in Reinke’s edema. Of interesting note is...
the accumulation of fluid collections in discrete locations within the vocal cord, rather than as a diffuse swelling throughout the LP. The dimensions of Figure 4A are only 1.275 x 3 mm, which was selected to provide a more detailed image.

A second image of Reinke’s edema (Fig. 4B) is shown alongside an inset endoscopic image of the patient’s larynx. Arrows in the image point to regions of what is likely fluid collection. Given the location (TVC), it is unlikely that these structures are glandular. They are not blood vessels because the erythrocytes absorb light and cast an “optical shadow,” obfuscating structures distal to the direction of light propagation.

OCT images of vocal cord polyps (Fig. 5, A to C) were also obtained, where A is an endoscopic image of an organized polyp, B is the corresponding OCT image, and C is the corresponding histopathology after excision. There is good correlation between the in vivo OCT images and histopathology, although differences result from registration mismatches because of orientation, sectioning, and artifacts produced by fixation. A photograph of a simple supraglottic respiratory epithelial lined mucous retention cyst (Fig. 5D) and the corresponding OCT image of the cyst (Fig. 5E) are also illustrated. Correlative histopathology (not shown) demonstrated the cyst was lined with respiratory epithelium.

Several examples of histologically confirmed laryngeal hyperkeratosis (Fig. 6A) and respiratory papillomatosis (Fig. 6B) were imaged during the study. In hyperkeratotic lesions of the larynx, the epithelial layer is markedly thickened up to 300 μm, whereas the BM was clearly demonstrated to remain intact. Similarly, with laryngeal papilloma (P), illustrated in the lower image, the BM was also shown to be intact. The double-headed arrow indicates the extent of projection of the P. Normal SS is seen to the right of the lesion in this image.

BM disruption was seen in several cases in the study. Loss of a BM on OCT was seen in cases of microinvasive squamous cell carcinoma of the larynx and in vocal process granulomas. Early microinvasive squamous cell carcinoma was imaged (Fig. 7A), which clearly demonstrated the loss of the BM and confluence of structural detail between superficial epithelium and the LP. The diagnosis was confirmed histologically. This demonstrates the potential value of OCT as a means to guide or direct biopsies into regions of the cord where the diagnostic yield would be greater. Likewise, granulomas (Fig. 7B) also demonstrate loss of BM integrity on OCT just as they do on Fig. 7A (the length of the image has been cropped to 4 mm). Although clinically vocal process granulomas have a distinctive history and appearance, there can be clinical concern for the possibility of malignancy. OCT was able to demonstrate loss of BM integrity in both processes, but the resolution is not yet adequate to provide the cellular detail needed to distinguish benign from malignant cellular processes. In this case, the diagnosis can only be made using microscopy as structural detail below the OCT resolution limit of 10 μm is required.

Epithelial thickness was measured at several laryngeal subsites (Table II). The epithelium was thinnest in the subglottis and thickest along the lingual surface of the epiglottis. TVC and false vocal cord thickness were both about 125 μm, which corresponds to approximately 10 cell layers in thickness. Measurements were made only in those subjects who had normal larynges and were undergoing endoscopy for other abnormalities of the head and neck.

**Discussion**

We present our experience in imaging normal larynges as well as benign pathologic conditions in 82 adult subjects and report the first comprehensive study to systematically examine vocal cord microstructure using OCT. Present image guided therapies relies on the relatively coarse imaging resolution of CT, MRI, or ultrasound to diagnose or treat disease with tissue resolution at very best of 1 mm. Contact endoscopy, a form of microscopy, requires extensive training, the use of dyes to stain tissue for contrast, and can only provide extremely superficial
en-face image date. In contrast, OCT is a high-resolution (approximately 10 μm) imaging modality, which is optimally suited to evaluate thin-layered structures such as those that line the surface of the body, airway, and gastrointestinal tract. Many of the laryngeal pathologies are identified within the first 1 to 2 mm of the mucosa and make OCT an ideal diagnostic imaging modality. Current noninvasive diagnostic tests rely on either two dimensional imaging or global physiologic measures of cord function such as those acquired during videostroboscopy or acoustic analysis. With the exception of contact endoscopy, which is not widely practiced, no other noninvasive imaging technique has been applied to provide detailed microanatomic information of the larynx.

The principal value of OCT in laryngology is that it stands alone in its ability to provide in vivo images of vocal cord microstructure to a depth not attainable with other imaging modalities. The images presented in Figures 1 to 7 are static representations of tissue structure and unfortunately do not demonstrate the full capabilities of the near real-time in vivo capability of OCT imaging. The electronic supplement to this article on the Internet contains several such “video” OCT image clips that correspond to the single image figures, which will be described below. In our study, an OCT image was acquired every second during endoscopic study. Similar to clinical ultrasonography, a great deal more information can be gleaned in these near real-time “movies” compared with images. Structures can be gently palpated using the probe to assess the degree of compressibility, the angle or image plane can be reoriented to obtain other views or projections, and larger regions of interest can be scanned in a sweeping panorama. As high-speed, high-resolution OCT systems evolve, the use of this technology will likely be more similar to dynamic imaging modalities such as ultrasound rather than static imaging like CT or MRI. Although the present system can acquire images at up to 12 frames/second, a high-speed endoscopic probe that uses micro-electromechanical system (MEMS) technology is currently under development in our laboratories.

The second objective of this study was to obtain quantitative measurements of the thickness of the epithelium in the different subsites of the larynx (Table II) because this variable may be important in aiding in the diagnosis of numerous processes such as epithelial hyperkeratosis. A comprehensive analysis of the epithelial thickness of

<table>
<thead>
<tr>
<th>Laryngeal Subsite</th>
<th>Number of Patients</th>
<th>Average number of Images per patient</th>
<th>Epithelial Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Vocal Cord</td>
<td>46</td>
<td>2.96</td>
<td>129 ± 58</td>
</tr>
<tr>
<td>False Vocal Cord</td>
<td>25</td>
<td>1.68</td>
<td>124 ± 47</td>
</tr>
<tr>
<td>Aryepiglottic fold</td>
<td>6</td>
<td>1.67</td>
<td>177 ± 70</td>
</tr>
<tr>
<td>Subglottis</td>
<td>15</td>
<td>1.87</td>
<td>98 ± 44</td>
</tr>
<tr>
<td>Laryngeal epiglottis</td>
<td>15</td>
<td>1.93</td>
<td>185 ± 82</td>
</tr>
<tr>
<td>Lingual epiglottis</td>
<td>4</td>
<td>1.50</td>
<td>271 ± 26</td>
</tr>
</tbody>
</table>

Table II. Laryngeal Epithelium Thickness.
each laryngeal subsite in vivo has not been previously reported. The benefits of in vivo real-time tissue analysis include avoidance of formalin fixation artifacts. The data in Table II provides the first set of data on the normative in vivo microstructural anatomy of the larynx.

Only one very limited study has previously imaged the human larynx in vivo using OCT and reported findings on 15 patients, all with vocal cord pathology (primarily cancer).8,12 Although this initial study did demonstrate the potential value of OCT, there were a number of shortcomings, which the present study addresses. From a technical standpoint, Sergeev et al.8 used an 830 nm light source with a narrower bandwidth, yielding a shallower depth of penetration (approximately 1 mm) and lower resolution (approximately 20 μm) in comparison with the 2 to 3 mm depth of penetration and 10 μm resolution in our present study. Their mechanical scanning was nonlinear, resulting in image distortion, and required 1.5 seconds to image a 2 mm wide region of interest. Our relatively rapid acquisition of images in this study required only 1 second to image a 6 mm frame and provided a substantial increase in image size (510 × 1,200 pixels vs. 200 × 200 pixels).

The most important observation in this study is the consistent identification of the demarcation between the laryngeal epithelium and the underlying LP in normal patients and most benign pathologic processes, including cysts, polyps, hyperkeratotic lesions, and swelling within the LP (Fig. 4 to 6). This sharp border likely represents the junction between the epithelial BM and LP. The BM separates the translucent epithelium largely devoid of blood vessels from the vascularized LP, which is also composed of relatively dense layers of collagen and elastin. Even in benign disorders, such as that illustrated in Figure 4 (Reinke’s edema) and Figure 5 (cysts), this demarcation remains distinct. The potential clinical impact of OCT’s ability to delineate the BM cannot be overemphasized because it is the disruption of BM integrity, which defines invasive cancer. Most likely the single greatest utility of OCT will be its ability to identify subtle disruptions in BM integrity. This ability can be used to characterize lesions, identify areas for biopsy, and perhaps aid earlier detection of malignant lesions by decreasing the likelihood of missing a cancer because of sampling error during biopsy. Figure 7A illustrates the potential of OCT in early cancer management; the image depicts both the thickening of the epithelium and the loss of a clear and sharp transition between this layer and the LP. Identifying the location of these sites could facilitate more accurate targeting of biopsies and improve the diagnostic yield while reducing the number of procedures required.

The presumptive identification of glandular structures and ducts in the supraglottic and subglottic larynx is a novel in vivo observation that has not been previously demonstrated using any imaging modality. The images acquired in the transition zones between the TVC and either the gland-bearing false vocal cords (Fig. 2) or subglottis (Fig. 3) identified glandular structures in a complex ductal system terminating at the epithelial surface.

The OCT findings in Reinke’s edema (Fig. 4) are striking because discrete regions of low signal intensity (presumably clear serous fluid) were readily identified. Reinke’s edema is essentially a clinical entity, with little known about its true cross-sectional histology. Reinke’s edema presumably results from the paucity of lymphatics in the TVC and build up of gelatinous edema. Whereas removal of chronic irritation such as reflux or smoking are the first options, surgical treatment involves microsurgery and removal of the fluid or gel. Currently, laryngologists operate without knowledge of where or how deep this fluid is sequestered. The present OCT images demonstrate the depth and location of these fluid/gel filled regions, and imaging Reinke’s edema during surgery in the future can aid or guide surgery, particularly if high-resolution robot-assisted microsurgery is adopted by surgeons. In the future, office-based laryngeal OCT imaging may provide insight into the pathophysiology as well because laryngeal abnormalities can be imaged as they evolve over time without the need for anesthesia.

OCT remains a technology in continual evolution and broader clinical adoption will be driven by reduction in component costs, further commercialization, and advances broadband light sources. The technology related factors, in our opinion, are most important as the cost of the components for OCT systems continues to drop. The OCT system used in this study is a relatively advanced device with respect to clinical implementation and has limit of 9 μm resolution. However, it does require the patient to be relatively immobile to eliminate severe motion artifacts. Our next generation instruments using broadband fiber laser sources should be able to obtain images with resolution approaching 1 μm, which would facilitate the identification of individual cells at faster scanning rates13,14 but at present remains a research device and cannot be transported to the operating room.

Our future work is focused on evaluating the efficacy in detecting BM integrity in patients with visible lesions suspicious for early laryngeal cancer. We are focused on the development of a better portable high-speed and high-resolution OCT system, which will allow imaging of cells and subcellular level structures. In addition, office-based OCT system is currently in development as well and is currently being evaluated on patients during office videostroboscopy.

CONCLUSIONS
1. We present the largest study of OCT imaging published to date examining its utility in evaluating laryngeal pathologies.
2. We have found considerable utility for OCT in clinically assessing laryngeal pathology.
3. OCT images the laryngeal tissues to a resolution and depth of penetration not obtainable with any currently available imaging modalities in a noninvasive fashion.
4. OCT has the potential to be a valuable complementary tool in laryngeal diagnostics, to provide more targeted biopsy of suspicious lesions, and to allow more objective, noninvasive monitoring of disease progression and response to therapeutic interventions.
5. The importance of the ability of OCT to demonstrate the integrity or disruption of the BM cannot be overemphasized.

Acknowledgments

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