Chapter 40
Vocal folds vibration imaging with functional OCT

Gangjun Liu, Marc Rubinstein, Brian J.F. Wong & Zhongping Chen

Abstract

We describe here how OCT imaging can be applied to studying human vocal fold (VF) vibration. We show that OCT is earning a significant place within technological armamentarium for VF examination, especially as a tool for non-invasive VF “biopsy.” Doppler OCT is a functional extension of OCT, which can provide information beyond morphology. Doppler OCT is a promising technique for quantitative, non-contact imaging of VF vibration.

Keywords: OCT, ODT, SSOCT/ODT, VF, lesions, biopsy, cancer, F0

Introduction

Clinically, laryngeal videostroboscopy (LVS) and high-speed digital phonoscopy (HSDP) have been widely used to image VF vibration. These methods provide clinically relevant and important information on VF behavior in health and pathology. Functional information regarding VF vibration, such as vibrating frequency, velocity, and acceleration [1-3] can be obtained from these devices. However, LVS only provides information on the surface of the VF. Therefore, the condition of the VF underneath the surface remains unknown using these systems. There is a wide spectrum of diseases that can occur in the VF, including benign polyps and premalignant and malignant lesions. Differentiating these afflictions using only direct visualization can be difficult and a biopsy is often required.

The key element to differentiate these lesions has to do with visualizing the integrity of the basement membrane. A loss of the basement membrane integrity is a hallmark of cancers of the VF. Currently, there is no reliable noninvasive method to diagnose laryngeal cancer without introducing a biopsy. However, doing a biopsy of the VF can come with its own risk of creating permanent damage to the VF. Therefore, the importance of using a noninvasive imaging method that can visualize below the surface of the VF, such as ultrasound and OCT, is highly practical.

Ultrasound has also been used to image the vibrating VF [4-6]. Although functional information can be obtained from an ultrasound, color Doppler ultrasound images suffer from low resolution and low frame rate. Hence, there is immense value in being able to dynamically and in real-time image the structure and characteristics of the vibrating VF, as much pathology is below the thin subsurface of this organ. Recently, imaging vibrating VF using OCT has been demonstrated by several groups [7-9]. The vibration VF OCT image at an imaging speed of 10 f/s was demonstrated by Lüerßen et al. [7]. Our group has demonstrated in vivo imaging of human vibrating VF with a 1.3 µm, 20 kHz swept source OCT system and a hand-held probe [8]. Functional information such as vibrating frequency was obtained by analysis of the OCT structure images. Kober et al. used a trig-
gered 10 kHz swept source OCT system to image the excised half calf larynx [9]. With the help of the particle image analysis method, the authors obtained the velocity vector in the cross section images from the OCT structure images.

For humans, the actual voice fundamental frequency (F0) varies by gender. For habitual speaking voice, F0 in females is approximately 200 Hz and in males it is approximately 120 Hz. For imaging such high frequency movement, a high-speed imaging system is essential to provide high frame rate images for the analysis. In addition, Doppler OCT requires much more dense scanning between A lines. In order to cover a large enough field of view and obtain high quality Doppler images at the same time, a fast system is essential to provide high frame rate.

In this chapter, we show our recent work of functional imaging of vibrating pig VF ex vivo with a high-speed swept source (SS) OCT and optical Doppler tomography (ODT) system [10]. The SSOCT/ODT system has a maximum imaging speed of 100,000 A-line per second, a central wavelength of 1.05 µm, an imaging range of more than 2 mm, and a depth resolution of 7 µm. The functional information regarding the vibrating VF, such as vibrating frequency, vibrating amplitude, and speed, were obtained by fitting the surface curve of the vibrating VF. Our demonstration shows the great potential of functional OCT in the field of VF imaging.

Sample preparation

Fresh porcine larynges with an intact trachea were obtained from a local biological tissues supply company. Then, larynges were dissected with removal of supraglottic tissue exposing the VF, but leaving key structures (arytenoid cartilages, anterior commissure, thyroid cartilage) intact. A nylon suture was placed to approximate position of the arytenoid cartilages, and thus created adduction of the VF. Once the VF were exposed, the larynx was mounted on a custom made mount and air supply device. A cuffed endotracheal tube was placed from below into the trachea and the cuff was inflated to avoid air leakage. Then, warm air at different flow velocities was delivered through the endotracheal tube through the trachea and past the glottis to vibrate the VF. Figures 1 shows the side view and top view photographs of the larynges mounted on the holder.

![Figure 1](image-url)

**Figure 1.** a) Photograph of side view of the porcine larynx mounted on the stage and b) photograph of top view of the porcine larynx.
Experimental results and analysis

**M-mode imaging**

In M-mode imaging, the laser beam is not scanning and OCT images provide the depth profile of a single location at different times. In this mode, the vertical movement of the VF vibration is monitored. The location of the incident beam is shown as the red dot in Figure 1b. Figure 2 shows the M-mode OCT structure image. The oscillation pattern of the VF can be clearly seen from the images.

![Figure 2. M-mode OCT image of the vibrating VF.](image)

The parameters such as oscillation period, amplitude, and speed are important to analysis of the imaged sample. In order to get these parameters, the surface curve of the VF was found by an intensity threshold method. The surface is overlapped with the OCT image as depicted by the solid red line in Figure 2. A bilaterally fixed-ends vibrating string model was used to simulate the VF vibration [4]. At a fixed location, the surface curve can be described by a sinusoidal function:

\[
y(t) = y_0 + A \cdot \sin \left( \frac{\pi(t - t_c)}{w} \right)
\]

where \(y(t)\) is the surface location at time \(t\), \(A\) is the amplitude of the vibration, \(w\) is the period of the vibration, \(t_c\) and \(y_0\) are two parameters decided by the acquisition start time and initial surface location of the sample at rest, respectively. By fitting the surface curve in Figure 2 with Equation 1, these parameters can be obtained. Clearly, the sine function fits well with the curve of tissue surface movement, especially in the down slope of the vibration. The amplitude \(A\) was found to be 709 µm and \(w\) was 5.3 ms. The period is 10.6 ms and the vibration frequency is 94.3 Hz. From the equation, the velocity of the tissue surface movement can also be obtained. This can be obtained with the following equation:

\[
v(t) = \frac{d[y(t)]}{dt} = A \cdot \frac{\pi}{w} \cdot \cos \left( \frac{\pi(t - t_c)}{w} \right)
\]
where \( v(t) \) is the velocity at the tissue surface and the other parameters are the same as in Equation 1. The maximum velocity is shown in Equation 3, which is 0.415 m/s in this case.

\[
v_{\text{max}} = A \cdot \frac{\pi}{W} \quad (3)
\]

The velocity of the tissue surface was obtained with the above mentioned fitting method. In addition to the velocity of the tissue surface, the velocity distribution beneath the tissue surface is also valuable. Here, the phase resolved ODT was used to obtain the velocity distribution. Phase resolved ODT has been used to image blood vessels in tissue. In addition, it utilizes the phase difference between adjacent A-lines to estimate the velocity value along the incident light beam direction. The Doppler frequency caused by the sample movement in the axial direction can be obtained by the following equation:

\[
f_d = \frac{2v_z}{\lambda_c} = \frac{\Delta \theta}{2\pi T} \quad (4)
\]

where \( f_d \) is the Doppler frequency, \( v_z \) is the sample velocity along the light beam direction, \( \lambda_c \) is the central wavelength of the incident beam, \( \Delta \theta \) is the phase difference between adjacent A-lines, and \( T \) is the time difference between adjacent A-lines. Figure 3 shows the velocity distribution of a cross-sectional image obtained with the phase resolved Doppler method. In Figure 3, a quasi-periodic pattern was caused by phase wrapping and the phase difference is wrapped between \(-\pi \) and \(\pi \). However, wrapped phase images also give the qualitative information regarding the acceleration. The absolute value of the velocity can be obtained using the following simple method.

\[
v_z = \frac{\lambda_c \Delta \theta}{4\pi T} \quad (5)
\]
In this experiment $\lambda = 1.05 \, \mu m$, $T = 10 \, \mu s$, and $\Delta \theta = 2\pi$, which corresponds to a velocity difference of 0.0525 m/s. In Figure 3, the black striations, as indicated by the white arrows, correspond to a velocity value of $n \times 0.0525 \, m/s$, where $n$ is an integer. The regions with $n = 0$ are decided based upon the peak and valley location of the oscillation. The values for $n$ in the other regions can then be decided by their relative distance to the $n = 0$ region.

In Figure 3, the maximum $n$ is 7 and the maximum velocity is between the velocities 0.3675 m/s and 0.42 m/s, which correspond to $n = 7$ and $n = 8$, respectively. Therefore, this value is close to the maximum velocity value obtained with the previous fitting method, which shows the value is 0.415 m/s. For Figure 3, we can find that the velocity distribution in the down slope region is different from that in the up slope region. In the down slope, the velocity distribution pattern of the tissue surface is more like a sine function. The velocity changes fast at the peak and valley regions, and it changes slower at the waist region. However, in the up slope, the velocity distribution pattern cannot be seen clearly.

**B-Mode imaging**

In M-mode imaging, the laser beam is scanned laterally and OCT images provide the cross-sectional image of the VF. The ODT images will provide cross-sectional velocity information when the VF vibrates. The B-mode OCT and ODT images are shown in Figures 4a and 4b. The solid green line in Figure 4b shows the location of the scanning trace. When analyzing B-mode images, we should pay attention that the B-mode images are not “snap-shot” images of the vibration sample because each A line is obtained at a different time. Taking the B-mode image as a “snap-shot” image may cause misleading results, especially when the frame rate of the system is close to or slower than the vibration frequency of the sample. A sliding window covering 50-100 A lines may be used to analyze these images. The information provided in this window can be considered as an “instant” or “snap-shot” image. Using the same analysis method proposed in the previous section, we can obtain the velocity distribution in the B-mode cross-sectional images. Similarly, from the ODT image in Figure 4b, the velocity distribution in the up slope and that of the down slope are different. As mentioned, this image is not a “snap-shot” image and the slopes mentioned here are different from the actual “instant” slopes of vibrating VF.

**Figure 4.** OCT images of a vibrating VF with frequency of around 94.3 Hz. a) B-mode OCT structure image; b) B-mode color Doppler OCT image; and c) B-mode Doppler variance OCT image. Scale bar: 500 μm.

The velocity distribution pattern of the B-mode image is similar to that of the M-mode image. In the down slope, the velocity distribution is more like a sine function. In addition, the velocity changes faster at the peak and valley regions and slower at the waist region. Consequently, the acceleration is larger at the peak and valley regions and
smaller at the waist region. However, the acceleration in the up slope is more uniform than that in the down slope and the acceleration at the peak region is larger than that at the valley region. On the other hand, from Figure 5 we are able to see that the sine function fitting of the vibrating tissue surface works better during the down slope than during the up slope. Clearly, the mechanics of VF vibration are very complex. The vibration takes place not only in the vertical direction but also in the horizontal direction. In the setup used here, phase resolved color Doppler can only detect the velocity in the vertical direction. Doppler variance is an extension of the Doppler imaging technique; it uses the bandwidth of the Doppler spectrum to quantify the transverse speed of the imaging sample [11]. The transverse velocity of the vibrating VF with the Doppler variance image is quantified in Figure 4c. We can see that the horizontal velocity is high at the waist region and low at the peak and valley region.

![Figure 5. OCT Movies of a vibrating VF with frequency of around 1.1 Hz. a) B-mode OCT structure image; b) B-mode color Doppler OCT image; and c) B-mode Doppler variance OCT image. Scale bar: 500 μm.](image)

Due to the limited penetration depth of the OCT, the velocity distribution at greater depths inside the tissue cannot be resolved with the current technique. Slower vibrations may ease the requirement for deeper penetration and some mechanics properties may be found from the superficial layers of the VF. By controlling the volume of the airflow rate, we are able to control the frequency and amplitude of the VF vibration.

Figure 5 shows a B-mode OCT structure, color Doppler, and Doppler variance images of the VF vibrating at slow frequency and small amplitude. The images are acquired at 100 f/s. The cross-sectional velocity distribution at different time points can be seen. Figure 6 shows four ODT images extracted from a sequence of vibration VF OCT images and the time difference between adjacent images is 12 ms. The velocity distribution in the cross-section of the VF can be obtained by the color Doppler images. An interesting phenomenon is that the change in velocity is in the radial direction as pointed out by the blue arrows in Figure 6.

![Figure 6. B-mode color Doppler images of a slow vibrating VF. Scale bar: 500 μm.](image)
Although this phenomenon has been found in the waist region of the fast vibration case, as shown in Figure 5, this phenomenon is more evident in the case of slower vibration like that in Figure 6. Furthermore, the radial directed velocity in this area is changing when the wave travels from right to the left, as shown in the progression of Figure 6 a-d. For these images, we can also quantify the wave traveling speed in the horizontal direction. The distance between the two yellow vertical lines in Figures 6a and 6c is 224 μm. Since the time difference between Figures 6a and 6c is two frame separations (12 ms × 2 = 24 ms), the velocity for the transverse wave is 9.3 mm/s (assuming a constant velocity for the wave).

\textit{B-mode imaging of imaging both VF}

Human VF are paired structures and they vibrate and are brought in contact during phonation. Sound is generated through the rhythmic opening and closing of the VF. The relative movement between the two-paired VF can also be revealed when the OCT scanning beam covers both the paired VF. The solid green line in Figure 7 shows the location of the scanning beam trace.

The B-mode OCT images are shown in Figure 8. The dynamics of both vibration VF can be identified simultaneously. The information regarding the vibration in both lateral and vertical directions can be obtained. In Figure 8, several OCT images are obtained by repeat scanning the same location and they show the relative locations of both VF at different stages of a vibration cycle. The time difference between adjacent OCT images is 50 ms. The moment of each VF as well as the relative movement between the two VF are clearly visible from these images.

\textbf{Figure 7.} Photograph of top view of the porcine larynx with red line showing the OCT beam scanning location.

\textbf{Figure 8.} B-Mode OCT images of vibration VF at different stages of a vibration cycle. The time difference between adjacent images is 50 ms.
Challenges and future work for *in vivo* human functional OCT imaging

Although bulk motion is not applicable in our current experiment, the human bulk motion will affect the results of the Doppler OCT for awaken patient imaging. The bulk motion will introduce bulk phase and usually the structural images will not be affected. The vibration frequency, amplitude, and period are extracted from the structure images and we estimate that the bulk motion will not affect the extraction of these parameters. However, the velocity information is obtained with the phase-revolved method and will be affected by bulk motion. The bulk motion artifacts may not be able to be eliminated with the histogram based statistic method usually adopted in ophthalmology application. A way to minimize this effect is to increase the speed of the system so that the imaging time is reduced.

With current system setup, the imaging speed is 100 f/s. This imaging speed has hindered us from imaging fast vibrations. Kilo-f/s systems will enable us to image VF vibrations at normal vocal F0. A Mega-A-line per second system will help us to realize that. With the development of swept source laser technique, these fast systems have been demonstrated by several groups.

Human VF vibration imaging must be performed on awake people. A suitable imaging probe is necessary for this purpose. The probe can image the vibration VF through either the oral or nasal cavity. We have shown a handheld OCT probe, which can image the VF in awake patient through oral cavity [8]. A tiny, flexible probe is necessary for imaging the VF through the nasal cavity. A forward-view probe based on a fiber-cantilever piezotube scanner is suitable for this purpose [12]. However, further minimization and shortening of rigid parts is necessary for VF imaging through nasal cavity.

Summary

Functional imaging of vibrating *ex vivo* porcine VF was demonstrated with a high speed swept source OCT and ODT system. The functional information regarding the vibrating VF was obtained with this high-speed system. The tissue surface of the vibrating VF was extracted to obtain functional information such as vibration amplitude, vibration frequency, velocity, and acceleration. Color Doppler and Doppler variance methods were used to obtain the velocity distribution characteristics in the cross sections. Essentially, the use of this system, or one similar to it, in laryngology could show how laryngeal carcinomas and other afflictions differ from baseline characteristics because factors such as velocity are monitored to show how the inertial movement of the tissue is affected.

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References
