Quantifying the subharmonic mucosal wave in excised larynges via digital kymography

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Abstract:

Objective: In this paper, a nonlinear least-squares fitting method was proposed to quantify subharmonic mucosal waves.

Study Design and Methods: Subharmonic mucosal waves from ten excised canine larynges were recorded using digital kymography (DKG) and analyzed using nonlinear least-squares and linear least-squares methods. Amplitudes of fundamental and subharmonic mucosal wave components of right-upper, right-lower, left-upper, and left-lower vocal fold lips were calculated. Lastly, phase differences of fundamental and subharmonic components of the left and right vocal folds were compared.

Result: The results showed that the nonlinear least-squares analysis method provides a more effective complement to the linear fitting method for subharmonic mucosal wave extraction. There was a significant difference in amplitudes between the subharmonic and fundamental components of mucosal waves ($p < 0.05$). The phase differences of the fundamental and subharmonic components of the right and left vocal folds were not significantly different.

Conclusion: The application of the nonlinear least-squares analysis method in DKG is useful for the characterization of subharmonic mucosal waves.

Keywords: Subharmonic mucosal wave; Nonlinear least-squares method; Digital kymography; Excised larynx experiment

1. Introduction

Vocal fold vibration consists of medial and lateral movements of the vocal folds induced by airflow travelling through the trachea and larynx. This vibration pattern produces a mucosal wave as it travels from the lower to the upper vocal fold lips. The formation of a mucosal wave enables the transformation of pulmonary airflow into sound. This acoustic phonation produced by the larynx can be modulated by a time-varying vocal tract, which serves as a filter. Vocal tract filtering allows sound energy to pass through at certain frequencies but restrain other frequencies. Once the vocal-tract configuration changes, both the resonance and the vowel quality change as well. The shape, length, and volume of the vocal tract determines the format frequencies corresponding to high amplitude. Since vocal track filtering and glottal turbulence cannot provide direct information about vibratory dynamics of vocal fold, objective characterization of this wave is essential to clinically evaluate laryngeal disorders. Digital kymography (DKG) allows direct line-scan visualization of vocal fold vibrations, leading to easier measurement, a smaller amount of data, and a higher frame rate imaging of mucosal waves than traditional high-speed recording.¹⁻⁵ Also, DKG has been proven as an effective technique to investigate mucosal wave dynamics.⁶⁻⁹ Jiang et al. have employed DKG imaging of excised canine larynges from a supraglottic perspective to examine the effects of airflow, vocal fold elongation, and thyroarytenoid contraction on the mucosal wave.⁸⁻¹⁰ Previous studies have investigated mucosal wave characteristics of nearly periodic vocal fold vibrations based on the assumption that the mucosal wave moves in a sinusoidal fashion.⁶⁻¹⁰ Thus, a linear least-squares method has been proposed to quantify the amplitude, frequency, and phase differences of a periodic mucosal wave.^{6,7}

However, voice production is a result complex nonlinear of aerodynamic, physiological, and biomechanical properties of the larynx.¹¹ Nonlinearity is inherently involved in the laryngeal system, such as the nonlinear relation of glottal pressure to airflow, the nonlinear stress–strain characteristics of tissues, and nonlinear vocal fold collision. Bifurcation and chaos have been

widely observed in computational models of the vocal folds, excised larynx experiments, and human voices.¹²⁻¹⁵ In 1995, Titze suggested a three type classification scheme for acoustic voice signals: type 1 voice signals only containing the nearly periodic model, the type 2 signals containing strong modulating or subharmonic frequencies, and the aperiodic type 3 signals.¹⁶ These subharmonic patterns have also been observed in excised larynx vibrations using DKG imaging.⁶ However, the mucosal wave properties of subharmonic patterns have not yet been investigated or quantified.

The purpose of this study is to investigate the use of a nonlinear least-squares fitting analysis method to quantify subharmonic patterns of mucosal waves. Unlike previous studies that were focused on first-order fundamental components of mucosal waves, this study quantifies the amplitudes of fundamental and subharmonic mucosal wave components of four vocal fold lip regions for statistical comparisons. The vocal folds are composed of bilateral folds on the right and left sides.² The vocal fold regions studied were comprised of the right-upper, right-lower, left-upper, and left-lower lip regions. The phase differences of fundamental and subharmonic components of left and right vocal folds were also compared. We hypothesized that a nonlinear least-squares analysis method would provide valuable insight into subharmonic mucosal wave dynamics.

2. Materials and methods

2.1 Excised Larynx Setup

The excised larynx experimental setup is shown in Fig. 1. Ten canine larynges harvested from healthy laboratory dogs were used in an experimental trial 12 to 36 hours after excision. Using a hose clamp, a segment of each trachea was secured to a pipe. A conventional air compressor, conditioned to 25-30°C, at 95% relative humidity and less than 45dB noise level, was used to generate airflow. The airflow was measured by an airflow meter. A bilateral three-pronged micrometer was used to approximate the arytenoid cartilages while subglottal pressure in the pseudo-lung was sufficiently increased to drive vocal fold vibrations. A manometer was used to

measure the subglottal pressure. For each trial, subglottal pressure and flow were held constant to ensure that stable phonation was maintained and high-speed measurement was performed accurately. The vocal fold vibratory images were recorded by a high-speed digital camera (Phantom MIRO M110, Ametek) at a sampling rate of 4000 frames/s with a pixel resolution of 512×256 pixels. 76800 pixels in each high-speed image correspond to the area of 255 mm². The videos were recorded at 30cm distance from each larynx.

2.2 Nonlinear Least-squares Mucosal Wave Extraction Method

The glottal edge curves for both left and right vocal folds segmented into upper and lower vocal fold lips were described as, $x_\alpha(t_i)$, $i=1,2,\dots$, where $\alpha = 1,2,3,4$ correspond to right-upper, right-lower, left-upper, and left-lower vocal fold lips, respectively. Based on the glottal edges of the DKG image series, four mucosal wave curves were derived by using the Fourier series fitting function:

$$x_\alpha = A_{\alpha,0} + \sum_{k=1}^N A_{\alpha,k} \sin\left(\frac{k\omega}{M}t + p_{\alpha,k}\right) \quad (N \geq M) \quad (1)$$

Where $A_{\alpha,0}$ denotes the direct component of mucosal wave amplitude and $A_{\alpha,k}$ denotes the k-th mucosal wave amplitude coefficient, ω is the fundamental frequency and ω/M represents the subharmonic frequency, and M is the integer. Also, $p_{\alpha,k}$ denotes the phase difference of each order component. Eq. (1) was further rewritten by using the nonlinear least-squares method as:

$$x'_\alpha(t_i) = f(\bar{\mathbf{b}}), \quad (2)$$

where $\bar{\mathbf{b}} = (A_{\alpha,0} \dots A_{\alpha,N}, \omega, p_{\alpha,1} \dots p_{\alpha,N})^T$ is the undetermined coefficient vector.

From the time series of the DKG image, as shown in Fig. 2(e), the mucosal wave edge points could be extracted as the original signal $x_\alpha(t_i)$ by image processing. Therefore, $\{x_\alpha(t_i), t_i\}$ was a known value. Next, the error e_i between the original signal $x_\alpha(t_i)$ and fitting function $x'_\alpha(t_i)$ was described as:

$$e_i = x_\alpha(t_i) - x'_\alpha(t_i) = x_\alpha(t_i) - f(\bar{\mathbf{b}})(t_i) \quad (3)$$

According to the principles of the nonlinear least-squares method, the objective function satisfied:

$$Q = \sum_{i=1}^N e_i^2 = \sum_{i=1}^N [x_\alpha(t_i) - f(t_i, \bar{b})]^2 \quad (4)$$

The optimal estimation of $\bar{b}$ was obtained by minimizing the objective function Q as

$$\frac{\partial Q}{\partial \bar{b}} = 0 \quad (5)$$

Because of the nonlinearity of Eq. (5), the Gauss-Newton iterative method was used to approach the solution of $\bar{b}$. Firstly, the initial value of $\bar{b}$ was given as $\bar{b}^{(0)} = (A_{\alpha,0}^{(0)} \dots A_{\alpha,M}^{(0)}, \omega_\alpha^{(0)}, p_{\alpha,1}^{(0)} \dots p_{\alpha,M}^{(0)})$, and the perturbation parameter Δ was $\Delta = \bar{b} - \bar{b}^{(0)} = (\delta A_{\alpha,0}^{(0)} \dots \delta A_{\alpha,M}^{(0)}, \delta \omega_\alpha^{(0)}, \delta p_{\alpha,1}^{(0)} \dots \delta p_{\alpha,M}^{(0)})^T$. For sufficiently small perturbation Δ , the linearized fitting function satisfied:

$$\begin{aligned} f(t, \bar{b}) \approx f(t, \bar{b}^{(0)}) &+ \frac{\partial f}{\partial A_{\alpha,0}^{(0)}} \delta A_{\alpha,0}^{(0)} + \dots + \frac{\partial f}{\partial A_{\alpha,M}^{(0)}} \delta A_{\alpha,M}^{(0)} \\ &+ \frac{\partial f}{\partial \omega_\alpha^{(0)}} \delta \omega_\alpha^{(0)} + \frac{\partial f}{\partial p_{\alpha,1}^{(0)}} \delta p_{\alpha,1}^{(0)} + \dots + \frac{\partial f}{\partial p_{\alpha,M}^{(0)}} \delta p_{\alpha,M}^{(0)} \end{aligned} \quad (6)$$

For the time series $x_\alpha(t_i)$, the objective function was obtained as

$$Q = \sum_{i=1}^N \left\{ x_\alpha(t_i) - f_i(t_i, \bar{b}^{(0)}) - \frac{\partial f_i}{\partial A_{\alpha,0}^{(0)}} \delta A_{\alpha,0}^{(0)} - \dots - \frac{\partial f_i}{\partial A_{\alpha,M}^{(0)}} \delta A_{\alpha,M}^{(0)} \right. \\ \left. - \frac{\partial f_i}{\partial \omega_\alpha^{(0)}} \delta \omega_\alpha^{(0)} - \frac{\partial f_i}{\partial p_{\alpha,1}^{(0)}} \delta p_{\alpha,1}^{(0)} - \dots - \frac{\partial f_i}{\partial p_{\alpha,M}^{(0)}} \delta p_{\alpha,M}^{(0)} \right\}^2 \quad (7)$$

where $\frac{\partial f_i}{\partial A_{\alpha,j}^{(0)}} = \frac{\partial f}{\partial A_{\alpha,j}^{(0)}} \Big|_{x=x_\alpha(t_i), \bar{b}=\bar{b}^{(0)}}$, $\frac{\partial f_i}{\partial \omega_\alpha^{(0)}} = \frac{\partial f}{\partial \omega_\alpha^{(0)}} \Big|_{x=x_\alpha(t_i), \bar{b}=\bar{b}^{(0)}}$, and $\frac{\partial f_i}{\partial p_{\alpha,j}^{(0)}} = \frac{\partial f}{\partial p_{\alpha,j}^{(0)}} \Big|_{x=x_\alpha(t_i), \bar{b}=\bar{b}^{(0)}}$. Then the condition

$$\frac{\partial Q}{\partial \bar{b}} = \frac{\partial Q}{\partial \Delta} = 0 \text{ led to}$$

$$A^T A \Delta = A^T (X - F) \quad (8)$$

where

$$A_{n \times M} = \begin{bmatrix} \frac{\partial f_1}{\partial A_{\alpha,0}^{(0)}} & \dots & \frac{\partial f_1}{\partial A_{\alpha,M}^{(0)}} & \frac{\partial f_1}{\partial \omega_{\alpha}^{(0)}} & \frac{\partial f_1}{\partial p_{\alpha,1}^{(0)}} & \dots & \frac{\partial f_1}{\partial p_{\alpha,M}^{(0)}} \\ \frac{\partial f_2}{\partial A_{\alpha,0}^{(0)}} & \dots & \frac{\partial f_2}{\partial A_{\alpha,M}^{(0)}} & \frac{\partial f_2}{\partial \omega_{\alpha}^{(0)}} & \frac{\partial f_2}{\partial p_{\alpha,1}^{(0)}} & \dots & \frac{\partial f_2}{\partial p_{\alpha,M}^{(0)}} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ \frac{\partial f_n}{\partial A_{\alpha,0}^{(0)}} & \dots & \frac{\partial f_n}{\partial A_{\alpha,M}^{(0)}} & \frac{\partial f_n}{\partial \omega_{\alpha}^{(0)}} & \frac{\partial f_n}{\partial p_{\alpha,1}^{(0)}} & \dots & \frac{\partial f_n}{\partial p_{\alpha,M}^{(0)}} \end{bmatrix},$$

$\mathbf{X} = [x_{\alpha}(t_1), x_{\alpha}(t_2), \dots, x_{\alpha}(t_n)]^T$, and $F = [f(t_1, \bar{b}^{(0)}), f(t_2, \bar{b}^{(0)}), \dots, f(t_n, \bar{b}^{(0)})]^T$. In order to ensure that Eq. (8) has solution, $A^T A$ should not be a singular matrix. Then, the perturbation parameter $\Delta^{(0)}$ could be estimated from the given $\bar{b}^{(0)}$.

Furthermore, the fitting coefficient vector was adjusted as $\bar{b}^{(1)} = \bar{b}^{(0)} + \Delta^{(0)}$ after the first iteration. Repeating the above steps Eqs. (6)-(8) with the parameter $\bar{b}^{(k)}$, $\Delta^{(k)}$ was obtained iteratively. For sufficiently large k , the perturbation parameter $\Delta^{(k)}$ at the k -time iteration satisfied $\|\Delta^{(k)}\| < \varepsilon$, where $\varepsilon = 10^{-6}$ is a given value. When $\|\Delta^{(k)}\| < \varepsilon$ was reached, the fitting parameter $\bar{b}^{(k)}$ was estimated and then the fitting curves of mucosal waves were derived as:

$$x'_{\alpha}(t_i) = f(t_i, \bar{b}^{(k)}) \quad (9)$$

In order to verify the fitting result, *R-square* (the coefficient of determination) was introduced. *R-square* is a method to quantify how well a curve represents the data. It produces a value between 0 and 1. If the *R-square* value is close to 1, this indicates that the curve fits the data. Contrarily, if the *R-square* value is close to 0, this indicates that the fitting curve does not effectively model the data. *R-square* was given as:

$$R\text{-square} = 1 - \frac{SSE}{SST} \quad (10)$$

where $SSE = \sum_{i=1}^n w_i (x'_{\alpha}(t_i) - x_{\alpha}(t_i))^2$ and $SST = \sum_{i=1}^n w_i (x_{\alpha}(t_i) - \bar{x}_{\alpha}(t_i))^2$.

2.3 Statistical analysis

For ten subharmonic vibratory DKG patterns of excised larynx, the mucosal wave parameters (amplitude and phase difference) of the four vocal fold lip regions were obtained using the nonlinear least-squares mucosal wave extraction analysis method. The Mann-Whitney rank sum test was utilized with amplitude and phase difference as dependent variables. The fundamental and subharmonic components were independent variables in this calculation. Statistical significance was set at 0.05 for all tests. Box plots were used to graphically display the data, with the box boundaries representing the 25th and 75th percentiles, the error bars representing the 10th and 90th percentiles, the line through the box representing the median, and the dots representing outliers. SigmaStat 3.0 software was used for statistical analysis and graphing.

3. Results

The DKG measurement line at the midpoint of the vocal fold was selected to create a DKG image, as shown in Fig.2. At 20 cmH₂O of subglottal pressure (P_s), periodic vibrations of larynx no.7 in high-speed imaging produced a sinusoidal mucosal wave in DKG traveling from the bottom to top lips with a phase difference, as shown in Fig. 2(a) and Fig. 2(b), where the time interval of successive frames in two-cycle high-speed images is 0.94ms. The fundamental frequency of the periodic glottal area from high-speed imaging can be determined in the spectrum, as shown in Fig. 2(c). However, for high subglottal pressure of 40 cmH₂O, the excised larynx displayed a subharmonic vibratory pattern that was double the normal glottal opening and closure rate in high-speed imaging, as shown in Fig. 2(d) and Fig. 2(e), where the time interval of successive frames in two-cycle high-speed images is 0.61 ms. The fundamental frequency and subharmonic component can be seen in the frequency spectrum, as shown in Fig. 2(f). In DKG, the mucosal wave alternated between large and small amplitudes. Subharmonic vibrations were usually produced at higher subglottal pressures than normal vibrations. Berry *et al.*¹⁹ reported this subharmonic pattern at high subglottal pressure in excised larynx experiments previously.

Following Titze's¹⁶ classification scheme, this has been defined as a subharmonic mucosal wave. All other nine larynges showed qualitatively similar subharmonic mucosal wave patterns within the pressure range of 30~40 cmH₂O, and thus their mucosal wave parameters would be more accurately quantified using the nonlinear least-squares Fourier-based estimations.

Fig. 3 compares the mucosal wave fitting results of the linear (a) and nonlinear least-squares (b) analysis method for larynx 7. The first-order linear least-squares method gave the fitting mucosal wave functions of the subharmonic pattern and R-square of fitting curves as:

$$x_1(t) = 49.23 + 23.18 \sin(283.20t) \text{ of the right-upper lip, } R\text{-square}=0.4514$$

$$x_2(t) = 73.92 + 13.48 \sin(299.00t) \text{ of the right-lower lip, } R\text{-square}=0.4307$$

$$x_3(t) = 53.00 + 18.19 \sin(295.60t) \text{ of the left-upper lip, } R\text{-square}=0.4360$$

and $x_4(t) = 64.41 + 14.47 \sin(438.10 * 2\pi t + 4.17) \text{ of the left-lower lip. } R\text{-square}=0.4941$

As shown, the R-square values when using the first-order linear fitting method were all less than 0.5. Thus, this method cannot accurately describe subharmonic mucosal waves. Next, the nonlinear least-squares method derived the fitting function and R-square of fitting curves as:

$$x_1(t) = 5.97 + 7.94 \sin(162.70t) + 11.25 \sin(325.40t) \text{ of the right-upper lip, } R\text{-square}=0.9064$$

$$x_2(t) = 60.85 + 4.34 \sin(173.6t) + 9.72 \sin(273.6t) \text{ of the right-lower lip, } R\text{-square}=0.9596$$

$$x_3(t) = 61.89 + 5.72 \sin(168.6t) + 10.55 \sin(337.2t) \text{ of the left-upper lip, } R\text{-square}=0.9295$$

and $x_4(t) = 60.24 + 5.01 \sin(173.3t) + 9.84 \sin(266.4t) \text{ of the left-lower lip. } R\text{-square}=0.9383$

The R-square values of the nonlinear least-squares method were all greater than 0.9. Therefore, the nonlinear least-squares method could effectively quantify the subharmonic mucosal waves of the nonlinear DKG pattern.

For ten canine larynges, Figs. 4 and 5 show the amplitude and phase difference results of mucosal wave analysis at middle line-scan positions of four vocal fold lips. In these figures, $\frac{1}{2}$ -order represents the subharmonic component and 1-order represents the fundamental component of the mucosal waves. A summary of the mucosal wave amplitude, frequency, and phase difference results were given in Table 1. For the right vocal fold, a significant amplitude difference between the subharmonic and fundamental components was found in the upper lip ($p = 0.009$) and lower lip ($p = 0.006$). The left vocal fold showed that subharmonic amplitudes were significantly lower than those of fundamental components in the upper lip ($p = 0.002$) and lower lip ($p < 0.001$). The fundamental frequency was twice as large as the frequency of subharmonic components. No significant difference in phase difference was found in either the left ($p = 0.845$) or right ($p = 0.932$) vocal folds.

4. Discussions

Based on the previous studies, quantitative research on subharmonic mucosal waves has unique clinical value. Voice production is a complex, nonlinear result of a combination of aerodynamic, physiological, and biomechanical properties of the larynx. In addition, subharmonic pattern of vocal folds might be been found in laryngeal pathologies, such as laryngeal paralysis, vocal nodules and polyps. Some common diseased vocal folds often produce the nonlinear vibratory patterns, which have been divided into periodic, subharmonic and chaotic signals by Titze.¹⁶ Subharmonic mucosal waves have been observed in previous studies and this paper is a continuation of that research⁶. The purpose of this study is to observe the changes of parameters by quantifying the subharmonic mucosal waves and to provide a scientific reference for laryngeal pathology and clinical treatment.

Commonly used acoustic voice recording methods are comprised of vocal fold vibratory information and other external factors such as vocal tract filtering and aerodynamic noise. DKG is a powerful imaging tool for intuitive visualization of vocal fold vibrations and quantification of

mucosal wave movement.^{8,9,18} When used in combination with DKG, excised larynx experiments facilitate observation and measurement of laryngeal activities. Physical similarities between canine and human larynges allow the use of the canine larynx as an organic model to investigate mucosal wave mechanisms.¹⁹ In this study, subharmonic mucosal waves were measured using DKG in excised canine larynges. A subharmonic pattern of vocal fold vibration was found at high subglottal pressure, as shown in Fig. 2(b), supporting findings from previous studies that sufficiently high subglottal pressure produces nonlinear vocal fold vibrations with large tissue deformations and strong vocal fold collisions.^{17,20} Thus, our excised larynx experimental study shows qualitative agreement with previous modeling findings.

With a sinusoidal assumption of mucosal waves, previous linear methods may be applicable for the first-order fundamental components of periodic vocal fold vibrations.⁶ However, the subharmonic mucosal waves could not be reliably obtained by using the first-order linear least-squares method, as shown in Fig. 3(a). The nonlinear least-squares method represents a more effective method to quantify subharmonic mucosal waves, as shown in Fig. 3(b). The Gauss-Newton iterative method was applied in this study as the core algorithm for nonlinear least-squares fitting. This method searches the minimum under systems with undetermined parameters and solves the optimal solution of undetermined parameters. It also has the advantages of fast convergence and high precision. This nonlinear algorithm allowed more precision and reliability for analysis of multiple mucosal wave parameters. Titze²¹, Zhang²²⁻²⁴ and Karnell²⁵, etc. have suggested that perturbation methods based on time-frequency analysis, such as jitter and shimmer, are appropriate only for nearly periodic voice signals. Nonlinear dynamic methods may be a significant resource to quantify characteristics of nonlinear subharmonic and chaotic signals.

In this study, the mucosal wave amplitudes of subharmonic components were significantly lower than those of fundamental components of right-upper, right-lower, left-upper, and left-lower vocal fold lips, as shown in Figs. 4 and 5. However, no significant phase differences between

subharmonic and fundamental components were found in either the left or right vocal folds. The estimated fundamental frequency was twice as large as the subharmonic frequency, as seen in Table I. From the extracted phase difference and frequency, the mucosal wave velocities (c) of the subharmonic and fundamental components could be estimated according to Titze et al.²⁶, $c = 360z/\phi T$, where z is distance between the upper and lower vocal fold lips, ϕ is the phase difference in degrees, and T is the vibratory period. For a certain value of z , the mucosal wave velocity is the function of phase difference and vibratory period. Phase differences of subharmonic components were close to those of fundamental components. The subharmonic frequency was one half of fundamental frequency, meaning that subharmonic wave velocity was one-half of the fundamental wave velocity. Thus, the subharmonic mucosal wave traveled much slower than fundamental mucosal wave, suggesting that a subharmonic component is created by fundamental components.

The results of this paper allow clinicians to observe the changes in objective parameters by quantifying the subharmonic mucosal waves and provide a scientific reference for laryngeal pathology and clinical treatment. Since pathological vocal folds often produce nonlinear vibratory patterns, traditional linear analysis methods are not reliable or valid. This is because the vibratory patterns of the vocal fold are a direct reflection upon the health of the larynx. Thus, a nonlinear analysis method would provide more accurate information about a diseased subharmonic mucosal wave. This information could be utilized by clinicians to investigate the pathophysiology behind certain laryngeal disorders by more accurately describing the subharmonic mucosal wave. Additionally, the nonlinear analysis method derived from this study could allow for the expansion of evidence-based practices in management of laryngeal disorders. Future research can utilize this approach to examine the efficacy of a specific intervention by tracking how the nonlinear parameters change during a treatment period.

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References

- [1] Yan Y, Chen X, Bless D, Automatic tracing of vocal fold motion from high-speed digital images, *Journal of Biomedical Engineering* 2006; 53:1394-1400.
- [2] Tao C, Zhang Y, Jiang JJ, Extracting physiologically relevant parameters of vocal folds from high-speed video image series, *Journal of Biomedical Engineering* 2007; 54:794-801.
- [3] Svec JG, Schutte HK, Videokymography: high-speed line scanning of vocal fold vibration, *Journal of Voice* 1996; 10:201 – 205.
- [4] Svec JG, Schutte HK, Sram F, Videokymography: A new high-speed method for the examination of vocal-fold vibrations, *Otorinolaryngol a Foniatrie* 1999; 48:155 – 162.
- [5] Schutte HK, Svec JG, Sram F, First results of clinical application of videokymography, *The Laryngoscope* 1998; 108:1206-1210.
- [6] Zhang Y, Krausert CR, Kelly MP, Jiang JJ, Typing vocal fold vibratory patterns in excised larynx experiments via digital kymography, *Ann Otol Rhinol Laryngol* 2009; 118:598-605.
- [7] Witt RE, Taylor LN, Regner MF, BS, Jiang JJ, Effects of surface dehydration on mucosal wave amplitude and frequency in excised canine larynges, *Otolaryngol Head Neck Surgery* 2011; 144:108-113.
- [8] Jiang JJ, Zhang Y, Kelly MP, Bieging ET, Hoffman MR, An automatic method to quantify mucosal waves via videokymography, *The Laryngoscope* 2008; 118:1504-1510.
- [9] Jiang JJ, Chang CIB, Raviv JR, Gupta S, Banzali FM, Jr, Hanson DG, Quantitative study of mucosal wave via video-kymography in canine larynges, *The Laryngoscope* 2000; 110:1567-1573.
- [10] Jiang JJ, Yumoto E, Lin SJ, Kadota Y, Kurokawa H, Hanson DG, Quantitative measurement of mucosal wave by high-speed photography in excised larynges, *Ann Otol Rhinol Laryngol* 1998; 107: 98-103.
- [11] I. R. Titze, R. Baken, H. Herzel, Evidence of chaos in vocal fold vibration, In Titze IR eds. *Vocal Fold Physiology: Frontiers in Basic Science*. Singular, San Diego; 1993:143-188.

- [12] Jiang JJ, Zhang Y, Sterm J, Modeling of chaotic vibrations in symmetric vocal folds, *Journal of Acoustic Society of America* 2001; 110:2120-2128.
- [13] Jiang JJ, Zhang Y, Ford CN, Nonlinear dynamics of phonations in excised larynx experiments, *Journal of Acoustic Society of America* 2003; 114:2198-2205.
- [14] Zhang Y, Sprecher AJ, Zhao ZX, Jiang JJ, Nonlinear detection of disordered voice production from short time series based on a Volterra-Wienrra-Korenberg model, *Chaos, Solitions & Fractals* 2011; 44:751-758.
- [15]Zhang Y, Shao J, Krausert CR, Zhang S, Jiang JJ, High-speed image analysis reveals chaotic vibratory behaviors of pathological vocal folds, *Chaos, Solitions & Fractals* 2011; 44:169-177.
- [16] Titze IR, Workshop on acoustic voice analysis: summary statement, National Center for Voice and Speech, 1995.
- [17] Jiang JJ, Zhang Y, Nonlinear dynamic analysis of speech from pathologic subjects, *Electronics Letter* 2002; 38:294-295.
- [18] Qiu Q, Schutte HK, Gu L, Yu Q, An automatic method to quantify the vibration properties of human vocal folds via videokymography, *Folia Phoniatica et Logopaedica* 2003; 55:128-136.
- [19] Berry DA, Herzel H, Titze IR, Story BH, Bifurcations in excised larynx experiments, *Journal of Voice* 1996; 10:129-138.
- [20] Svec JG, Schutte HK, Miller DG, A subharmonic vibratory pattern in normal vocal folds, *Journal of Speech, Language and Hearing Research* 1996; 39:135-143.
- [21] Titze IR, Horii Y, Scherer RC, Some technical considerations in voice perturbation measurements, *Journal of Speech, Language and Hearing Research* 1987; 30:252-2601.
- [22] Zhang Y, Jiang JJ, Wallace SM, Zhou L, Comparison of nonlinear dynamic methods and perturbation methods for voice analysis, *Journal of Acoustic Society of America* 2005; 118:2551 – 2560.
- [23] Zhang Y, McGilligan C, Zhou L, Vig M, Jiang JJ, Nonlinear dynamic analysis of voices before

and after surgical excision of vocal polyps, Journal of Acoustic Society of America 2004; 115:2270 – 2277.

[24] Jiang JJ, Zhang Y, Ford CN, Nonlinear dynamics of phonations in excised larynx experiments. Journal of Acoustic Society of America 2003; 114:2198 – 2205.

[25] Karnell MP, Chang A, Smith A, Hoffman H, Impact of signal type of validity of voice perturbation measures, NCVS Status and Progress Report 1997; 11:91-94.

[26] Titze IR, Jiang JJ, Hsiao TY, Measurement of mucosal wave propagation an vertical phase difference in vocal fold vibration, Ann Otol Rhinol Laryngol 1993; 102:58-63.

Table 1. Fundamental and subharmonic mucosal wave parameters of four vocal fold lips

Parameters	Fundamental	Subharmonic
Amplitude(pixels)		
Right-upper lip	13.99 (4.98)	8.54 (3.37)
Right-lower lip	11.10 (4.39)	5.24 (1.55)
Left-upper lip	15.13 (5.03)	7.70 (2.03)
Left-lower lip	13.54 (0.24)	5.71 (1.85)
Frequency(Hz)		
Right-upper lip	334.00 (127.88)	167.00 (63.94)
Right-lower lip	315.95 (94.55)	157.97 (47.27)
Left-upper lip	327.47 (125.56)	163.73 (62.78)
Left-lower lip	316.37 (111.06)	158.19 (55.53)
Phase Difference (degree)		
Right vocal fold	-1.92 (61.45)	-4.84 (80.73)
Left vocal fold	9.54 (65.66)	15.94 (71.11)

The numbers in parentheses represent standard deviations.

Figures Caption

Figure.1 A schematic diagram of an excised larynx experimental set-up.

Figure.2 (a) Two-cycle high-speed images of periodic vibratory patterns of an excised larynx. (b) DKG image sequences of periodic vibratory patterns of excised larynx. (c) FFT of periodic vibratory patterns of an excised larynx. (d) Two-cycle high-speed images of subharmonic vibratory patterns. (e) DKG image sequences of subharmonic vibratory patterns. (f) FFT of subharmonic vibratory patterns. The red lines in (a) and (d) represent the single line at the midpoint of vocal fold. The two-cycle mucosal waves marked by the blue box in (b) and (e) DKG image sequences were produced from corresponding two-cycle high-speed images.

Figure.3 Subharmonic mucosal wave curve fitting using (a) linear least-squares method and (b) nonlinear least-squares method for larynx #7, where the blue, green, red, and light blue curves correspond to the right-upper, right-lower, left-upper, and left-lower vocal fold lips, respectively.

Figure.4 Fundamental and subharmonic mucosal wave amplitudes of (a) right-upper and right-lower vocal fold lips and (b) left-upper and left-lower vocal fold lips.

Figure.5 Fundamental and subharmonic mucosal wave phase difference of left (a) and right (b) vocal folds.

Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5