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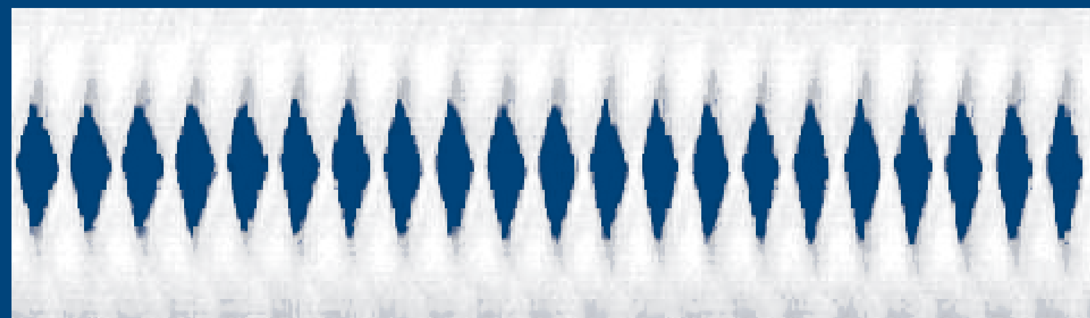

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STUDIES IN LOGOPEDICS AND PHONIATRICS, NO.14

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ABSTRACT

The overall aim of the thesis was to develop new methods for analysis of vocal fold vibrations and viscoelasticity and to test them in human subjects.

In Study I the onset of vibration and irregular vocal fold vibration was examined with laryngoscopy using a high-speed camera at a frame rate of about 2000 images/sec. A new software called High-Speed Tool Box, HSTB, was developed for automatic analysis. The HSTB can read the sound signal and present the sound exactly synchronized with the image. It can also trace the vocal fold edges during vibration and display the area as a graph. From the high-speed recording it is also possible to make a kymogram, which shows the vibration in one part of image, but for a longer period. Data from one subject with voice tremor and one with diplophonic phonation are presented.

When examining the vocal folds with a rigid or flexible endoscope the amplification of the image differs with the distance to the object. In study II we adapted a laser triangulation method for use in larynx to measure both horizontal distances and vertical movements in a mm-scale. The standard error of measurements in the horizontal plane was between 3-6% and in the vertical plane about 10%.

In study III twenty-seven professional opera singers were examined with laser triangulation to measure the vocal fold sizes related to voice category of the singers. The result showed that the bass group had significantly longer vocal folds than the sopranos and mezzos; also, the males had significantly longer vocal folds than females ($p<0.05$). Measured values for vocal fold width were significantly larger for males than for females and for the bass group as compared to the other categories ($p<0.05$).

In study IV a new method for measuring vocal fold viscoelasticity, called Air Pulse Elasticity Measure (APEM), was developed. The method can be used in local anesthesia on human subjects. By blowing controlled air pulses on the vocal folds and measure the resulting mucosal deflections with help of a laser it was possible to calculate a value reflecting vocal fold elasticity. Nine normal vocally healthy subjects were examined with air pulse stimulations on the vocal folds, on the skin (cheek and dorsum of the hand) and on the inside of the lip. The elasticity data showed no differences between the vocal folds, lips or cheeks. The hand data showed significant higher stiffness as compared to the other three measured tissues ($p<0.001$). The coefficient of variation was about 35% for all measurement points, but in ideal conditions on skin it was 9%. The results indicate that the technique allows automatic quantitative non-invasive vocal fold elasticity measurements on awake subjects even if some methodological development is needed before it can be used clinically.

In study V the APEM was used to measure the elasticity in scarred rabbit vocal folds. Ten scarred New Zealand rabbit vocal folds and 4 normal rabbit folds were measured directly after sacrifice. The elastic data were compared to histological sections from the scarred vocal folds analysed by a pathologist. The results showed significantly lower elasticity (higher stiffness) values for the more scarred vocal folds as compared to samples with minor damage ($p=0.03$).

In conclusion these new methods are reliable and can be used in practice for analysis of human vocal fold function. However some development is needed before they are clinically useful.

Key words: High-speed video imaging, kymography, acoustic analysis, vocal fold vibration, glottal edge, glottal area, laser, mucosal wave, triangulation, vocal fold, vocal fold length, vocal fold width, vocal fold strain, non-invasive, vocal fold scarring, air pulse stimulation

SAMMANFATTNING (SUMMARY IN SWEDISH)

Metoder för mätningar av stämbandsvibrationer och viskoelasticitet

Ljudkällan för den mänskliga rösten utgörs framför allt av de vibrerande stämbanden. De övre luftvägarna svalg, näs- och munhåla filtrerar ljudet så att meningsfullt och förståeligt tal uppstår. Ljudet genereras av att stämbanden sluter (adduceras) och återkommande pressas isär av luftströmmen från lungorna så att en vibrerande rörelse uppstår under röstbildning.

Stämbandens svängningar är mycket komplexa och nya metoder behövs för att analysera funktionen. Målsättningen med denna avhandling är att utveckla nya metoder för att analysera stämbandens vibrationer och dessutom mäta elasticiteten i stämbanden samt att testa detta i praktiken.

Undersökning av stämbandens vibrationer sker vanligen kliniskt med en stroboskopisk ljuskälla, som ger en skenbar bild med långsamt vibrerande stämband. Detta fungerar endast på stämband som vibrerar med relativt konstant frekvens. För att studera starten av en vibration (tonansatsen) eller oregelbundet vibrerande stämband kan istället en höghastighetskamera som tar 2000-4000 bilder/sekund användas. Denna kamera ger 10-40 bilder för varje vibrationscykel, vilket möjliggör en noggrannare analys. En nackdel är att en normal inspelning på 2-4 sekunder ger 4000-8000 bilder. För att analysera denna stora mängd bilder krävs ett speciellt datorprogram.
I studie I har en sådan mjukvara utvecklats, kallad High-Speed Tool Box (HSTB). Programmet har flera verktyg, det kan presentera alla 8000 bilder från 4 sekunders inspelning med önskad hastighet eller visa bild för bild för detaljerad analys. HSTB kan även spela in ljudet (rösten) och göra en exakt synkronisering med bilden. Stämbandens kanter kan markeras och öppningsytan visas automatiskt som en graf under vibration. Från inspelningen är det även möjligt att göra ett kymogram, som är ett sätt att visa vibrationerna i en del av stämbanden under en längre tidsperiod. Data från en patient med rösttremor samt av oregelbunden (sk diplofon) röstkvalitet presenteras i artikeln.

Vid undersökning av stämband används stelt eller flexibelt endoskop vilket ger varierande bildförstoringen med avståndet. Därför är det omöjligt att göra mätningar av stämbanden från bilden i absoluta enheter (mm). I studie II har vi anpassat en laser trianguleringsmetod för användning i larynx och undersökt nio friska försökspersoner. Med metoden är det möjligt att mäta stämbandsstorlek och även mäta vertikala rörelser under fonation. Metodens osäkerhet (standard error) är 3-6% vid mätningar av sträckor i horisontalled och cirka 10% i vertikalled.

I studie III har laser triangulering använts för att mäta stämbandsdimensioner (längd och bredd) på 27 professionella operasångare vilket jämförts med det röstfack som sångarna tillhör. Resultat visar signifikant längre stämband för män än kvinnor. Basarna hade signifikant längre stämband jämfört med sopranerna och mezzosopranerna. Även stämbandsbredden var signifikant större för män än kvinnor och bas/barytonsångarna hade signifikant bredare stämband än övriga grupper.

Stämbandens viskoelasticitet är mycket viktig för en fungerande röst och varierar för olika röststörningar, t.ex. polyper, knutor, cystor samt cancer och ärrskador. I studie IV har en ny metod för att mäta stämbandselasticitet utvecklats, kallad Air Pulse Elasticity Measure (APEM). Genom att blåsa små kontrollerade luftpulser på stämbanden och mäta resulterande slemhinnerörelser med hjälp av laser, är det möjligt att icke-invasivt få ett mått på stämbandens elasticitet. Nio friska försökspersoner ingick i studien där elasticiteten mättes på stämbanden och för att få referensvärden på insidan på läppen, på utsidan av kinden, samt på ovansidan av handen. Resultatet visade ingen skillnad mellan stämband, läpp och kind. Däremot var handens hud signifikant styvare jämfört med de andra tre mätpunkterna. Variationskoefficienten var 35% för alla mätpunkter men under ideala förhållanden för huden var detta 9%. Sammanfattningsvis visar metoden att det är möjligt att icke-invasivt mäta stämbands- elasticiteten på vakna patienter, även om viss metodutveckling behövs innan metoden är kliniskt användbar. I studie V har APEM metoden använts för att mäta elasticiteten på ärrskadade kaninstämband. Tio ärrskadade kaninstämband (New Zealand white) och 4 normala kaninstämband undersöktes direkt efter avlivning. Elasticitetsdata jämfördes med analys av histologiska vävnadssnitt utfört av en patolog. Resultatet visade signifikant lägre elasticitet (högre styvhet) för de stämband som har uttalad ärrighet jämfört med de med mindre ärrighet ($p=0.03$).

Sammanfattningsvis kan dessa nya metoderna var till stor hjälp vid analys av stämbandsfunktion. Tester visar också att de är användbara i praktiken även om ett visst utvecklingsarbete återstår innan de är kliniskt användbara.

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I. Larsson H, Hertegård S, Lindestad PA, Hammarberg B: Vocal fold vibrations: high-speed imaging, kymography, and acoustic analysis: a preliminary report. Laryngoscope 2000;110:2117-2122

II. Larsson H, Hertegård S: Calibration of high-speed imaging by laser triangulation. Logoped Phoniatr Vocol 2004;29:154-161

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IV. Larsson H, Hertegård S, Lindestad PÅ: Air pulse elasticity measurement. A new method for non-invasive vocal fold analysis. Submitted manuscript

V. Hertegard S, Larsson H, Nagubothu SR,Tolf A, Svensson B: Elasticity measurements in scarred rabbit vocal folds using air pulse stimulation. Logoped Phoniatr Vocol: in press

CONTENTS

LIST OF ABBREVIATIONS

A/D	Analog digital converter
APEM	Air Pulse Elasticity Measure
CCD	Charge Coupled Device, Camera sensor
EGG	Electroglottography
EMG	Electromyography
HD	High definition video
HSP	High-speed photography
HSTB	High-Speed Tool Box
LP	Lamina propria
LSR	The Linear Skin Rheometer
NTCS	US video system
PAL	European video system
SFF	Speaking Fundamental Frequency
VKG	Videokymography

1 INTRODUCTION

The human voice is mainly generated by vocal fold vibrations. The larynx is a major, but not the only acoustic source in speech. Sound is created through rhythmic opening and closing of the vocal folds. During oscillation, the vocal folds are brought close together in such a way that air pressure builds up. The folds are then pushed apart by increased subglottal pressure, with the inferior part of each fold leading the superior part during the vibration. Under ideal conditions this oscillation is self sustaining. In essence, sound is generated in the larynx by the chopping up a steady flow of air into little puffs. The vocal fold oscillations generate a sound which is rich in harmonics. The rest of the upper airways, the pharynx, the oral cavity to the lips and nasal cavity filter the sound in such a way that speech is produced (Titze 1994).

The oscillating frequency is equivalent to the fundamental frequency and is on an average about 100 Hz for normal males and about 200 Hz for women. In children the frequency is over 300 Hz (Pegoraro Krook 1988).

A person's habitual fundamental frequency is influenced by many factors, including the length, size, and tension of the vocal folds. The tension is mainly controlled by the vocalis (thyroarytenoid) and cricothyroid muscles.

The vibration pattern of the vocal folds can vary in many different ways. In males, normal vibration at comfortable pitch and loudness is associated with a complete closure of the membranous vocal folds, but in normal female phonation the pattern can be different with incomplete glottal closure (Södersten et al 1990).

Voice problem can be caused by many factors like scars, chronic inflammation, vocal fold atrophy, sulcus glottidis, cysts and deposites. This will result in stiff vocal fold folds with deteriorated voice. Chronic edema and vocal fold paresis on the other hand may increase softness and elasticity which may also result in malfunctioning vocal folds (Hillman et al 1990).

1.1 ELASTICITY

The layered structures of the vocal folds are important to the vocal fold oscillatory capacity. The vibrating vocal fold consists of a surface epithelium, the lamina propria (LP) and the vocalis (thyroarytenoid) muscle. The LP of the vocal fold consists of three layers, the most superficial layer is characterized histologically by loose tissue with few collagen and elastin fibres. The middle layer is found to contain increasing numbers of elastin fibres while the deepest layer has an increased number of collagen fibres and also of hyaluronic acid (Hammond et al 1998; Gray et al 2000), see Fig 1.

The LP consists of two major classes of matrix proteins, including fibrous proteins (such as collagen and elastin) and interstitial proteins (such as decorin and fibromodulin). These matrix proteins are distributed in different orientations and densities in different layers of the lamina propria, with fibrous proteins being randomly oriented in the superficial layer and organized in parallel in the intermediate and deep layers. The superficial layer is the major vibratory portion of the vocal fold, particularly during small amplitude oscillations for phonation onset and offset. The intermediate

and deep layers are known as the vocal ligament, which is pertinent to the passive tensile stress generated during vocal fold elongation. Gray et al (2000) showed that the medial and superficial tissue on the top of the fold slides, glides and moves over a more rigid body of tissue.
It has also been shown by Chan et al (2007) that the fibrous proteins collagen and elastin contribute significantly to the tensile stress-strain characteristics of the vocal folds, while interstitial proteins such as proteoglycans and hyaluronic acid determine the viscoelastic shear properties of the vocal folds.

The layered structure is very important to achieve good vibration, moreover the surface epithelium has to be moist. The epithelium has a higher viscosity as compared to the superficial LP. Understanding of the viscoelastic behaviour of the LP is important for determining the biomechanics of fundamental frequency regulation, which is dictated by the quantitative relationship between vocal fold length, tension, and stiffness. The elasticity of the vocal folds and LP in particular is most commonly studied by stress-strain measurements.
LP has viscoelastics characteristic with a non linear stress-strain relationship (Alipour-Haghighi et al 1991; Gray et al 2000). The stress-strain curve shows hysteresis which indicates viscous energy loss during vibration.

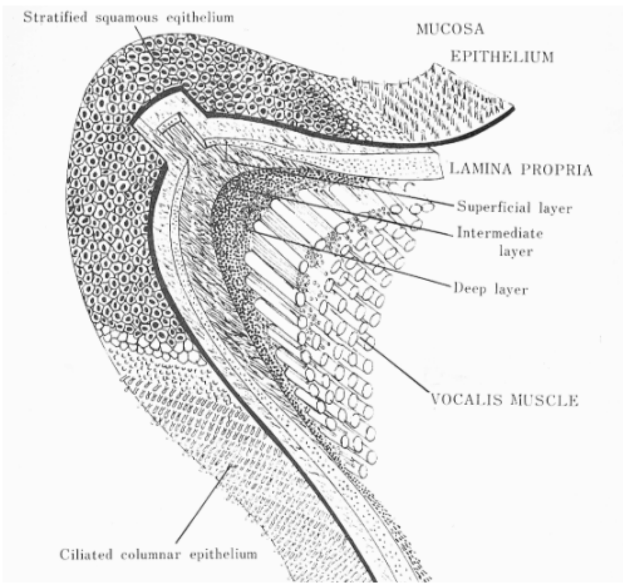

Figure 1. Morphology of a human vocal fold in the coronal plane. The view is slightly oblique, showing the flat surface of the epithelium.
From: Hirano M. Official Report on Phonosurgery. The 76th Annual Convention of the Oto-Rhino-Laryngological Soc. of Japan, 1975. Printed by courtesy of Professor Hirano.

1.2 VOCAL FOLD SCARRING

Stedman's Medical Dictionary defines scar as "the fibrous tissue replacement of normal tissue destroyed by injury or disease". A scar is therefore a natural late sequelae of injury and repair (Benninger et al 1996). In the larynx, and more specifically on the vocal folds, scarring results in a functional deficit because of the loss of mucosal pliability and the deviance in viscoelasticity (Hertegård et al 2004, 2006; Woo et al 1994). Scar tissue disrupts normal vibratory function by changing the physical properties mainly of LP (Thibeault et al 2002; Rousseau et al 2003). Increased effort during phonation to overcome the mucosal stiffness caused by the scar results in a poor voice, frequently hyperfunctional and often with glottal insufficiency.

A change in the properties of LP either by scarring or other disease disturbs the viscoelastic properties of the vocal fold. Voice is often breathy or aphonic and the phonation threshold pressure, which corresponds to "easiness of phonation" (Titze 1994), is elevated. The voice can also become strained, harsh or diplophonic.

1.3 CLINICAL METHODS FOR VOCAL FOLD EXAMINATION

1.3.1 Videostroboscopy

For examination of vocal fold function several clinical methods are available, e.g. stroboscopy, high-speed image recordings, and videokymography. Each method requires an endoscope, either a rigid endoscope or a flexible endoscope (Fig 2). A rigid endoscope uses a mirror and a lens system in order to obtain a sharp picture of the vocal folds. The traditional flexible endoscopes have a light fibre system with many fibres which gives a picture with lower resolution as compared with the rigid endoscope. During examination a rigid endoscope is inserted via the mouth and has a working distance of about 50-70 mm from the vocal folds. A flexible endoscope is inserted through the nose and placed about 20-30 mm from the vocal fold surface. During the actual stroboscopy it may be moved even closer to the folds. Both types of endoscopes have a light channel to illuminate the vocal folds. More recently, flexible endoscopes with a CCD sensor at the distal tip have been introduced. This type gives a much better resolution. Usually a video camera is connected to the optical endoscope and the image is displayed on a monitor and can be recorded digitally for later analysis.

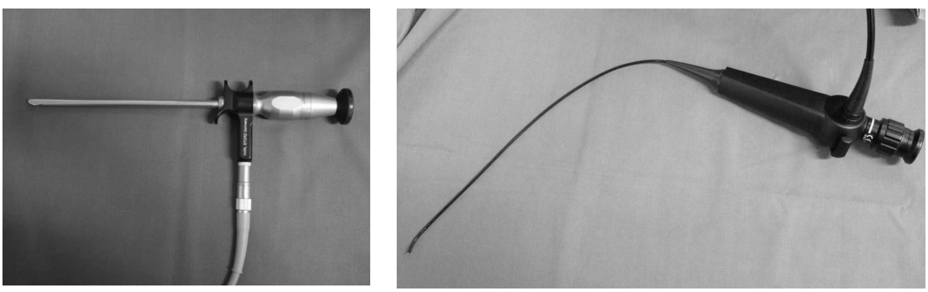

Figure 2. Rigid (left) and flexible (right) endoscopes for vocal fold examination

Stroboscopy is the clinically most used method for examination of the vocal folds. During examination, a microphone (airborne or a laryngeal contact microphone) is used to determine the fundamental frequency and the signal obtained triggers a light source flash with a frequency of about 1 Hz lower than the actual vocal fold vibration. In that way an imaginary slow motion image of vocal fold vibrations is obtained (Fig 3) (Kitzing 1985). Stroboscopy is only working well for vocal folds vibrating regularly and at a relatively constant frequency, which is indeed the case in many patients. However for examination of vibration onset or irregular vocal fold vibrations, a better method to use is a high-speed recording (HSP) (Hertegård et al 2003). With this technique up to 2000-4000 images/sec or higher can be recorded. This makes it possible to record and analyze 10-40 images of every vibrating vocal fold cycle.

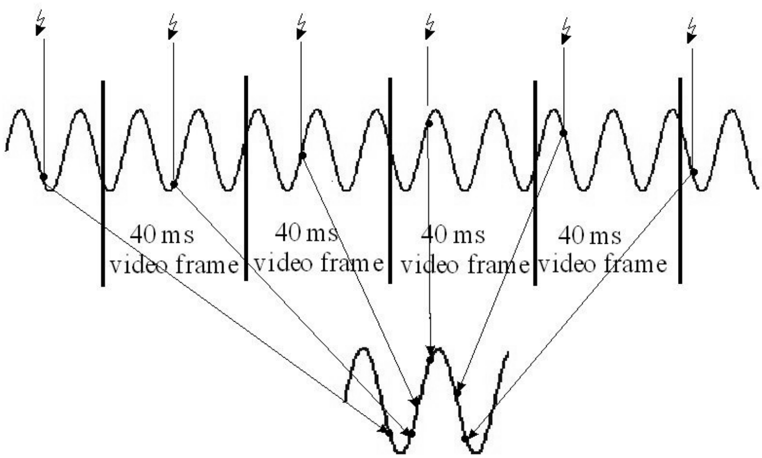

Figure 3. The stroboscopic principle for examining regular vocal fold vibrations.

1.3.2 Videokymography.

Videokymografy, VKG (Svec et al 1996) is another method to present the vocal fold movements. Here only one line of each image is used and added along a time scale to form a new image (Fig 4). It can be explained as a way to present the movements of a small part of the vibrating vocal folds, but for a longer period. Thus, only the part of the image which is marked along a line is collected for an extended time period. This collection forms a new image (Fig 4) with which it is possible to study the vibration pattern. A kymogram can be displayed by a special camera which has a time resolution corresponding to approximately 8000 images/sec. The VKG camera is much cheaper than a high-speed camera. The camera can work in two modes, either in normal video mode (PAL or NTSC) or in kymography mode. By changing between these two modes it is possible to make an examination relatively easy.

1.3.3 Kymography from high-speed recordings

It is also possible to create a kymogram from a recording made with a high-speed camera and a special software. This is extensively described in study 1. This creates a similar image as the VKG but here it is derived off-line from a pixel line selected in the

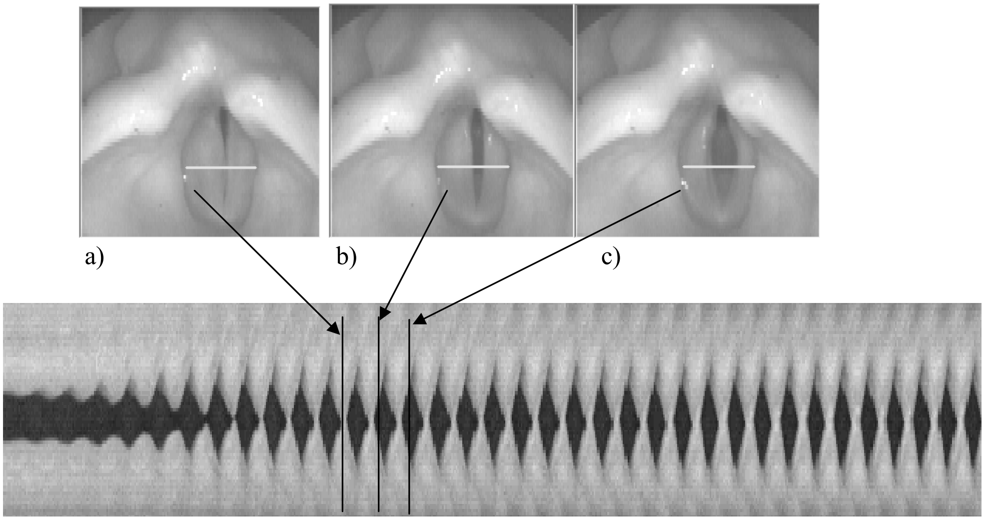

Figure 4. High-speed kymogram of a normal phonation in a male subject showing onset of phonation, a) closed phase, b) glottal opening c) maximum glottal opening.

overview picture. The overview high-speed picture is kept and any other line can be selected later for analysis. Most high-speed systems available give a lower resolution of the image as compared to a VKG camera.

1.3.4 EGG

Electroglottography, EGG, is an electrical non-invasive method for studying the vocal fold vibratory movements, or rather vocal fold collisions and the time relations within and between those (Baken 1992). A small current passes between electrode plates placed on the neck at the level of the larynx. The impedance change during the glottal cycle as the vocal folds open and close. The changes in impedance are measured with the electrodes and presented as a graph representing the vocal fold movements.

1.3.5 EMG

For studying the laryngeal muscle activity electromyography, EMG, can be used. With needle or wire electrodes inserted in the different muscles it is possible to study muscle activity qualitatively and quantitatively at rest and during phonation (Sataloff 2006). There are different methods for quantification of the amplitude and the frequency content of the signal.

1.3.6 Perceptual evaluation

For directly analysing voices clinically or from acoustic voice recordings several methods are available. The most common is perceptual evaluation where the examiner assesses defined perceptual parameters such as breathiness, roughness, hyperfunction and perceived pitch, which generates a voice profile (Hammarberg 2000; Imaizumi 1991).

1.4 ACOUSTIC ANALYSIS

Fundamental frequency (F0) analysis is the most commonly used acoustic method in the clinic. Different perturbation analyses, such as jitter and shimmer analysis, may give useful information on voice irregularity. Various computer programs are designed for this. The Soundswell software (Ternström 2000) is widely used in Sweden. Many other programs are available, like Multi-Speech from KayPentax. The Correlogram program developed by Granqvist (1998) has shown to be useful when analyzing irregular voices.

1.4.1 Phonetogram

The Phonetogram is also a useful and widely spread method. A graph is plotted with fundamental frequency on the x-axis and sound level on the y-axis (Schutte et al 1983; Gramming 1988). A phonetogram (or voice range profile) can be made online with special software. If the recording is made during normal speech the graph will show the normal speaking range. It is also possible to let the subject find the extreme limits for the voice range (Fig 5). Recently the method has been further developed for the singing voice (Lamarche 2009)

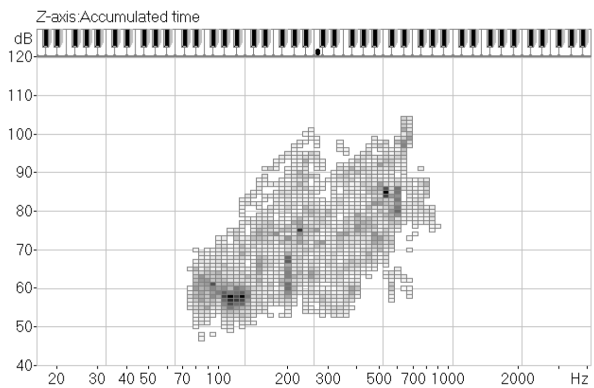

Figure 5 A phonetogram with frequency in the x-axis and sound level on the y-axis, showing the extreme voice range of a normal individual.

1.4.2 Inverse filtering

Inverse filtering is a technique for indirectly analyzing the glottal air flow (acoustic voice source) during vocal fold vibration (Rothenberg 1973, Hertegård 1994). Using a facemask combined with pressure and flow transducers it is possible to make an inverse filtering of the oral flow signal and thus remove the effects of the formants in the resonating tube. This makes it possible to calculate the transglottal airflow from the oral flow signal registered with the mask. Inverse filtering can also be made from the acoustic signal obtained from a microphone recording.

1.5 HIGH-SPEED IMAGE RECORDING, HSP

The first high-speed film of the vocal folds was produced in 1937 at Bell Telephone Laboratory. This made accurate measurements of the vocal fold vibrations possible. Other high-speed recordings followed (Farnsworth 1940). Due to technical difficulties, e.g. illumination problems and extremely time consuming film development and analysis procedures, the method has previously been used mainly for voice research purposes (Timcke 1958). Digital high-speed cameras were primary developed for the car industry, but since 1985 HSP has been used and further developed for the study of vocal fold vibrations in real time (Imagawa et al 1987; Hirose 1991; Hess 1993; Hammarberg 1995). This technique allows instant digital slow motion control and subsequent digital storage or documentation on a normal video recording system. Eysholdt et al (1996) have developed algorithms for analysis of high-speed filming. There is however no clear definition of high-speed recording. The normal video rate (PAL/NTSC) is 25 or 30 images/sec, but the vocal folds are vibrating at approximately 110 Hz for men and 200 Hz for females, with great variation. In order to get a good time resolution in a high-speed recording at least 10 images per cycle are required. Thus 2000 images/sec is the minimum camera speed for studying the vocal folds at work. For studies of singers who perform at high pitches a minimum of 4000 images/sec is needed, and even that is not enough when you record at higher frequencies than 4-500 Hz (Deliyski et al 2008). Commercial high-speed cameras normally deliver 2000 images/sec, but by decreasing the vertical spatial resolution of the image they can work at up to 4000 images/sec. The resulting image resolution is then 256x128. During the last years even colour high-speed cameras with a relatively high resolution and sensitivity have been made available, for example HResEndocam 5562, Richard Wolf GmbH, Germany.

For a closer study of the mucosal wave, even higher image rates than 4000 images/sec are needed. Deliyski et al (2008) have studied laryngeal function with such cameras, originally used for military purposes, and show interesting results. However, so far this type of camera has not been adapted for clinical use. An inherent problem is the amount of light that is required when you speed up the recordings, which easily makes the instruments and the mucosa of the patient overheated.

A necessity and at the same time a disadvantage with HSP is that a normal recorded sequence of 2-4 seconds generates 4000-8000 images. To analyse that amount of data some type of software is required. An example of such a program package is High-Speed Tool Box (HSTP) which was used in study 1 in this dissertation project.

1.6 CALIBRATION AND DIRECT MEASUREMENTS OF THE LARYNX

To measure real size and dimensions of the vocal folds most studies have been performed on excised larynges or by direct measurements during general anesthesia (Su et al 2002). Kakita et al (1991) used a stereo-laryngoscope but it was too complicated for clinical use. A new technique was presented by Schade et al (2002). He developed a two beam laser method, in which the two beams with a fixed distance between them were projected on the vocal fold. In that way it was possible to continuously project a marker in the image which represented a fixed distance. This allowed for absolute

measurements at least in the horizontal plane. Hertegård et al (1998) used laser triangulation to measure vertical distances (Mannerberg et al 2001).
This method has been developed to easy handling and horizontal calibration and is presented in study 2 of this dissertation project (Larsson et al 2004).

1.7 ELASTICITY MEASUREMENTS

Viscoelasticity is a property of (bio)materials that have both viscous and elastic characteristics when undergoing deformation. Viscous materials, like honey, resist shear flow and strain linearly with time during stress. Elastic materials strain instantaneously when stretched and quickly return to their original state once the stress is removed. Viscoelastic materials have elements of both of these properties and, as such, exhibit time dependent strain. Biological materials show a viscoelastic behaviour.
In solid mechanics, Young's modulus is a measure of the stiffness of an isotropic elastic material. It is defined as the ratio of the stress over the strain within the range of stress in which this load does not exceed the elastic limit of the material. This can be experimentally determined from the slope of a stress-strain curve created during a test of the material. The parameter is mostly used in material science but can be useful even for biological materials, e.g. tissue measurements (Tran et al 1993).

1.7.1 Parallel-plate rheometry

Parallel-plate rheometry is more or less the standard method in measurements of viscoelasticity of biological materials. However it is limited to in vitro measurements and has mainly been used for measuring the vocal fold elasticity on excised larynges (Chan et al 1998). In several studies stress-strain tests on vocal folds have been made in a specially designed measure chamber (Perlman et al 1988; Alipour-Haghighi et al 1991).
The rheometer consists of two parallel plates separated by a space of about 0.5 mm where the measured tissue sample is placed. The upper plate vibrates with small-amplitude oscillations at increasing frequency (from 0.01–15 Hz), and the forces are measured (Chan et al 1998). In more recent versions, the rheometer can be oscillated with higher frequencies (Chan et al 1999).

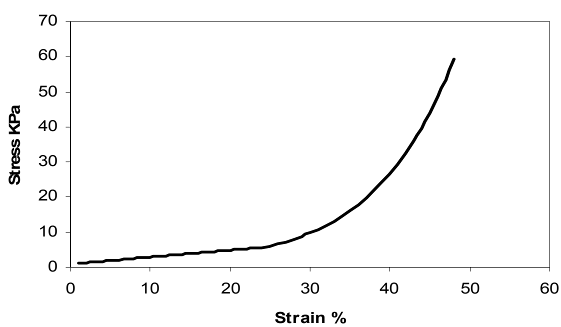

Figure 6. A schematic stress-strain curve for an excised vocal fold with a linear part and an exponential part.

1.7.2 Stress-strain measures

Another method which can be used in excited larynx is the stress-strain examinations were the samples are placed in a measurement chamber and it is possible to examine the relationship between stain and stress (Alipour-Haghighi et al 1991; Min et al 1995) Fig 6.

1.7.3 Linear Skin Rheometer

The Linear Skin Rheometer (LSR) is another method for measurements of tissue elasticity (Hess et al. 2006; Goodyer et al 2006; 2007). It is mainly suitable for in vitro measurements, but it has also been used for in vivo measurement in humans during general anesthesia.
LSR uses a long steel probe. The probe is held in contact with a tissue surface which is measured with the probe moving sinusoidal at 0.3 Hz and with a peak amplitude of about the equivalent of 1 g. The force/displacement data are recorded continuously. With this method it is possible to measure elasticity in different directions. With an extra long probe it also possible to measure in vivo during general anesthesia.

1.8 AIMS

The overall aims of this project was to develop new methods for analyzing vocal fold function.

1.8.1.1 Specific aims

To develop and test clinical methods for analyzing high-speed recordings of the vocal folds.

To develop methods for calibration and quantification of images from vocal fold examinations.

To analyze vocal fold dimensions in professional opera singers and relate these to voice category.

To develop a new method for examination of the vocal fold elasticity in vivo.

To relate the elasticity measurements in scarred rabbit vocal folds to histological data.

2 METHODS IN THIS PROJECT

2.1 HIGH-SPEED RECORDING

At Karolinska University Hospital Huddinge we have been using a high-speed camera since 1999 (Weinberger Speedcam+ 500) and at that time started to developed a user friendly software called High-Speed Tool Box (HSTB). This development is described in study 1. Figure 7 shows a block diagram of the high-speed recording system to the left and to the right it shows the control panel as it is displayed on the computer screen.

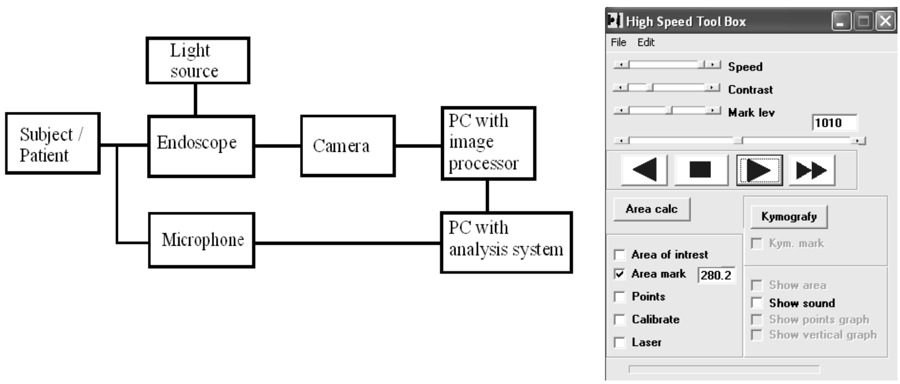

Figure 7. Left: a block diagram of the high-speed recording system. Right:The control panel.

The Weinberger Speedcam+ 500 camera can work in different modes, but in our studies we normally recorded with a pixel resolution of 256x64 at about 2000 images/sec and thus were able to store 8000 images in 4 sec. The camera uses “post triggering” which means that it is basically recording continuously and when the operator decides to start storing the images a trigger button (actually a foot pedal) is pressed. At that moment the recording is stopped. This results in storage of the 4 last seconds which can be displayed and analysed. The camera operates in black and white mode.

The HSTB has several options for analysis of the recorded material. It can present all 8000 images from the 4 sec recording at desired speed or show images frame by frame for detailed studies at adjustable image size. We always make a simultaneous sound recording by means of the software Soundswell (Ternström 2000). In order to synchronize between the sound signal and the high-speed images a trigger signal channel is recorded in the sound file. The HSTB can read the sound signal and present the sound wave form on the screen in a window exactly synchronized with the high-speed image. The delay caused by sound transit time from the vocal folds to the microphone is adjusted for. Thus, during replay it is possible to follow the cursor to see exactly what is produced in terms of sound in relation to the vibrations.

Figure 8. Vocal folds from a normal phonation, with traced edges at maximum amplitude.

During analysis of the vocal fold movements there is an option to track the glottal edges during vibration. This is first done by improving the contrast in the image and selecting an area of interest. Through a snake algorithm and a series of tests it is then possible to automatically identify the vocal fold edges and glottal border (Fig 8). When the edges are found it is possible to calculate the area of the glottis in pixels. The area can then be presented as a graph and be compared to the sound signal (Fig 9). As the edges are identified it is also possible to display a graph over how any selected point on the vocal fold is vibrating.

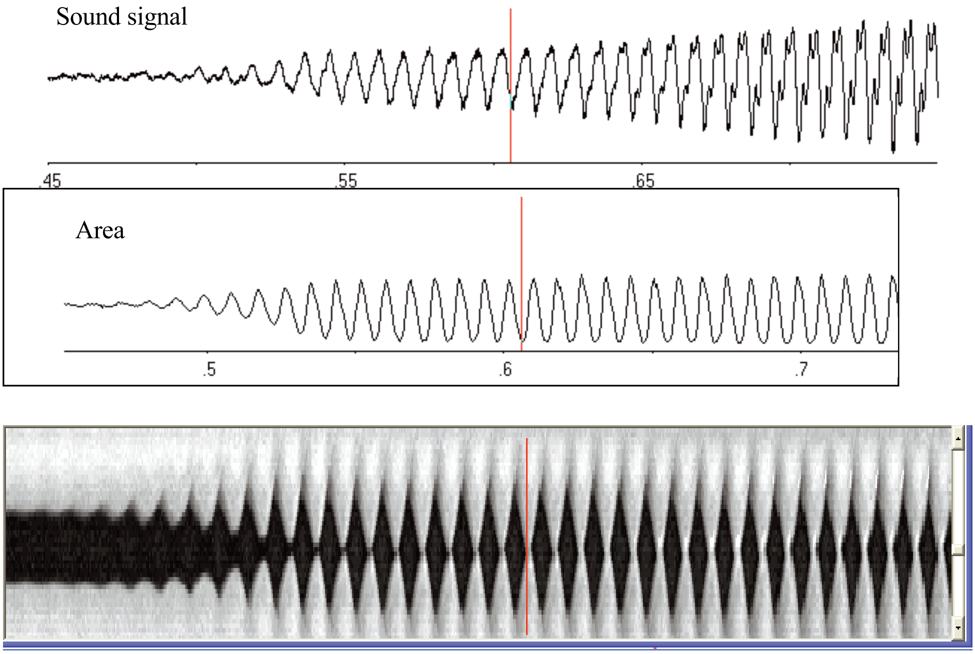

Figure 9. The sound signal (top), the glottal area variations (middle), and the kymogram (bottom) displayed simultaneously for a sustained phonation produced by a normal male subject. All three signals show regular vibrations. The glottal area signal and the kymogram indicate complete glottal closure. The kymogram also shows symmetrical left and right vocal fold vibrations.

From the high-speed recording it is also possible to make a kymogram, which shows the vibration in one part of image, but for a longer period (see page 5). By combining several types of graphs like the area display, the sound signal and the kymogram on the same time scale, details of phonation can be analyzed (Fig 9).

2.2 LASER TRIANGULATION

When examining the vocal folds with a rigid or a flexible endoscope the amplification of the image varies substantially with the distance to the object. For this reason is it more or less impossible to measure calibrated distances from an image. One way to solve that problem is to use triangulation, which is a well known technique to measure distances. By knowing the distance to the object, the magnification factor of the endoscope is also known. We made use of this fact to make it working also for laryngoscopic purposes. The principle requires an angle difference between the optical axis and the laser beam. A laser was developed which fitted onto a rigid endoscope with an angle of 8 degrees between the optical axis of the endoscope and the laser. The beam is projected to a point on the vocal folds (Fig 10). By varying the distance to the endoscope, the laser spot moves along a line in the image. The distance to the object can be calculated from the position of the laser spot on the line. After a calibration procedure of the laser system to a prototype with known dimensions the distance can be calibrated in absolute units (mm). The magnification of the endoscope can be calibrated in relation to the distance between the endoscope and the object with a second procedure. From these two calibrations absolute measurements of the image as well as of movements and distances in the vertical direction can be calculated.

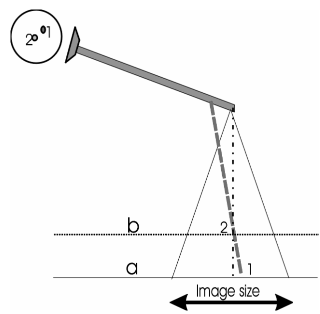

Figure 10. Triangulation: Measurement principle. A laser attached to an endoscope projects a beam (slashed). The laser hits the surface (a) at (1) and results in a point in the ocular (1). When the spot on the surface has moved to a position closer to the instrument, position (b), the laser spot in the ocular has moved to position (2).

2.2.1 Technical equipment

A 1 mW laser was built into a specially designed house (Fig 11, left), and attached to a 70-degree rigid endoscope, Richard Wolf GmbH, Germany. The laser beam was fed through a 4 mm tube to a mirror at the end. The mirror reflected the beam with an angle of 8 degrees in relation to the optical line of the endoscope. The end of the tube with the mirror was placed approximately 9 mm from the optical lens of the endoscope

(Fig 11, right). The laser was adapted to the endoscope so that the laser spot projected to the center of the endoscopic image at the normal working distance during laryngoscopy (about 60-70 mm).

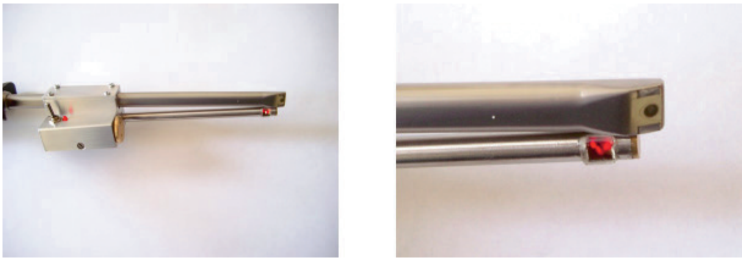

Figure 11. The laser attached to the endoscope (left).The tip of the endoscope and the mirror which reflects the laser beam (right).

2.2.2 Software and calibration

The software for measuring area and movements is part of our software package High-Speed Tool Box. Prior to or after every measurement session the system must be calibrated. The calibration is done by recording a dot pattern with 5 mm distance between dots at two known distances from the endoscope. Both the position of the laser spot and the distance between the dots in the pattern is measured. We use a custom made device with a known horizontal dot pattern and vertical level differences.
The calibration program identifies the laser spot and all points in the dot pattern. From those values, all parameters in the calibration algorithm can be determined. During the measurements the exact position of the laser spot is determined in two steps. As the laser is moving along a line in the image, the first step is to find the maximum intensity. From that position a scan over the laser spot is performed and the weighted average of the laser light intensity calculated.

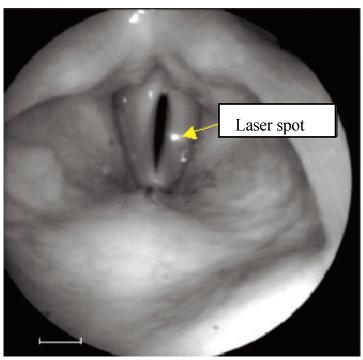

Figure 12. A high-speed image with the laser spot displayed. Below a scale showing a 5 mm distance.

The position on the line gives the vertical distance to the endoscope. From that value the magnification factor of the endoscope can be determined. A measuring stick in the High-Speed Tool Box is then automatically calibrated, and a line equivalent to a 5 mm distance displayed at the bottom of the image (Fig 12). It is also easy to check the calibration by recording the dot pattern and measuring the 5 mm distance at different levels from the endoscope. The accuracy of the laser method depends on several factors, such as the angle between the optical axis and the laser, the size of the laser spot and the pixel resolution of the camera.

2.3 AIR PULSE METHOD

A new method was developed with which elasticity measurements were performed by the use of air pulses. The method was called Air Pulse Elasticity Measure (APEM). The hypothesis was that normal vocal fold vibration is initiated as a result of an air stream, in analogy, the new device would blow air on the vocal folds. The measured resulting mucosal cavity would reflect the elasticity. As it is easy to blow air in a tubing it was also possible to administer the air steam through the working channel of a flexible endoscope. The resulting cavity was measured with the help of a laser.

2.3.1 Equipment

The air pulse stimulation unit consisted of 3 main parts, a stimulation catheter, a controlling unit and a computer (Fig 13). The catheter was a plastic tubing with a diameter of 2 mm and 1 m length containing 3 optic fibres, each with a diameter of 0.2 mm. One of the laser fibres was adapted to a 10 mW solid state laser using an optic system .

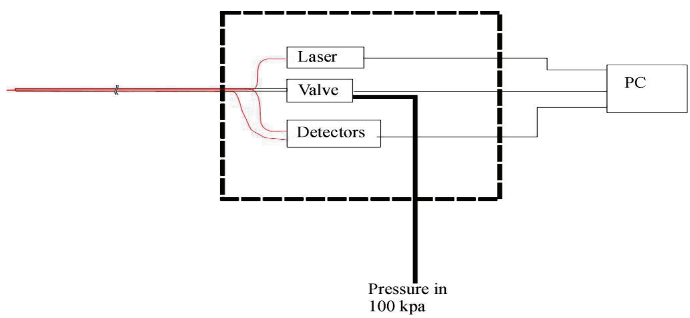

Figure 13. Block diagram of the APEM unit.

The two other optic fibres were connected to two photo detectors and amplifiers in a custom-made construction. The laser was controlled by an output signal by the A/D converter. The detector signals were read by the same A/D converter. The A/D converter was also controlling a small pneumatic valve which in turns controlled the air pulses. All electronic and pneumatic devices were mounted in a box (Fig 14). From the control box a catheter was connected which lead the air pulses to the tip. The pneumatic pulses were fed with compressed air of 100 kpa from a small gas bottle with

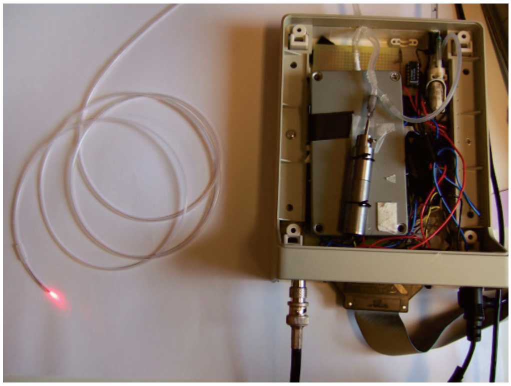

Figure 14. Picture of the control unit with the measuring catheter.

a gas regulator. The whole system was controlled by a PC and specially developed software. The software displayed a graph of the reflected laser pulse intensity and another graph of calculated tip to tissue distance during pulse stimulation.
The tip of the catheter had a custom-made design (Fig 15). The 3 fibres were fitted to the tubing wall by a smaller tubing which was used to prevent any movement of the optical fibres in relation to the tubing. At the tip of the tubing there was also a smaller tip tubing “probe” which focused the air stream. The three fibres were mounted in the centre of the probe. The fibres were also painted black and glued together in a way that one detector fibre and the laser light fibre were approximately 1 mm in front of the tip tubing and the second detector fibre 0.5 mm behind the others.

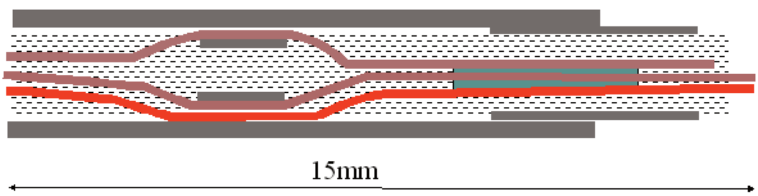

Figure 15. The tip of the catheter probe with fixation of the laser fibres.

The laser light was feed to the probe and illuminated the area in front of the probe. It was pulsed at 80 Hz in such a way that it was possible to measure the difference between the reflected light and the background light. The two other optical fibres were used to measure the reflected light. From the distance difference of 1 mm it was possible to compensate for the distance difference to the measured object.
The size of the catheter (2mm) was small enough to fit in the lumen of a flexible endoscope. Figure 16 shows an image from the endoscope during examination of the vocal folds.

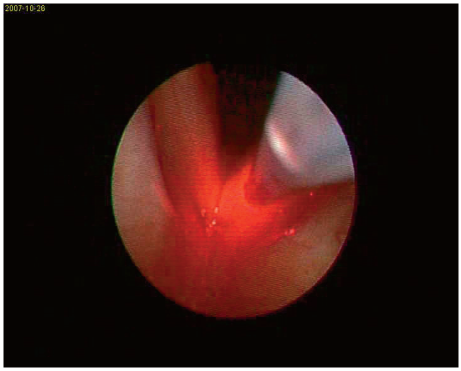

Figure 16. Endoscopic image of the tip of the tubing (right) during examination of a vocal fold.

2.4 SUBJECTS

Papers 1, 2 and 4 are methodological studies. In study 1 the subjects were one healthy male speaker (age 48) and a female patient with essential voice tremor (70 years old). In study 2 the subjects were 3 vocally healthy male speakers (age between 44-50) and 7 females (between 30 and 47 years old). In study 4 there were 5 healthy males (age 38-55, mean age 45) and 4 females (32-60 years old, mean age 47).
Paper 3 was a study designed to measure vocal fold dimensions in professional opera singers. Singers in different voice categories were studied. Twenty-seven professional opera singers participated, 14 females (mean age 44 years, range 31–54 years) and 13 males (mean age 41 years, range 26–49 years). Twenty-four of the subjects were full-time singers at the Royal Opera in Stockholm, either in the choir or as soloists, and three subjects were full-time freelance singers. There were nine sopranos, five mezzos, eight bass/baritones, and five tenors. There were no significant age differences between the female and male subject groups. However, there was a significant age difference between the groups bass/baritones and tenors, the basses/baritones were significantly younger than the tenors ($P < 0.05$). Inclusion criteria were that the subject should tolerate the use of a rigid endoscope and that it was possible to place the laser spot on the vocal folds during phonation. All singers were healthy and had no voice complaints. A video laryngoscopic recording was performed to confirm normal vocal fold status. The singers reported to which voice category (soprano, mezzo, tenor, or baritone/bass) they belonged and also their musical singing voice range (i.e. not the total physiological voice range). They also reported their body length.

In study 5 the objects were 12 New Zealand rabbits of about 3kg weight, 10 with scarred vocal folds and 2 with normal vocal folds.

3 EXPERIMENTS AND RESULTS

3.1 STUDY 1

The aim of the study was to develop methods to analyze high-speed recordings. This resulted in a software “High-Speed Tool Box”, HSTB, which has many options for studying high-speed recordings.
The automatic kymogram and glottal area analyses allow for multidimensional study of phonatory mechanisms and can easily be compared with the sound signal. The system is particularly useful for studying aperiodicity, e.g. diplophonic voices, voice tremor, and related voice qualities which are common characteristics of pathological voices. This combined high-speed-acoustic-kymographic analysis package is a promising tool for separating and specifying different voice qualities and also for studying phonation onset. Besides the clinical use, this technique may be of help for increasing the accuracy of the terminology that describes different voice qualities.
The results show that HSTB can be useful in the analysis of irregular phonations. Figure 17 shows the glottal area and the sound signal for a patient with voice tremor.

Original sound wave

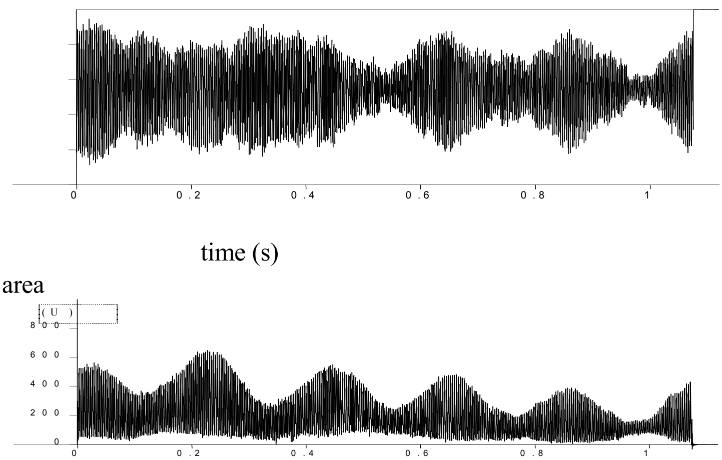

Figure. 17. The sound signal (top) and the glottal area waveform (bottom) for a patient with essential voice tremor sustaining an */i/*-like vowel.

3.2 STUDY 2

The aim of this study was to develop a method for calibration of high-speed recordings in order to measure vocal fold dimensions and movements in absolute units. A new method using laser triangulation was developed. With a laser triangulation device attached to a rigid endoscope it was possible to make measurements in the horizontal plane of the image with an accuracy of 0,06 mm and measurements in the vertical direction with an accuracy of 0.14 mm.

Vocal fold length (membranous part) and vertical amplitude of vibration was measured in 7 normal subjects (4 males and 3 females) during phonation close to habitual fundamental frequency of speech. The result showed a length of 9-12.5 mm, and a vertical amplitude of 0.3-1.5 mm (Table 1).

A second test was performed in which 4 normal subjects (3 females and one male) phonated at one low and one high pitch. The laryngeal vertical height difference varied from 2-23.5 mm between the different pitches.

It was concluded that the triangulation technique produced reliable absolute measurements of vocal fold dimensions during vibration.

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Study 2. Table 1. Results from experiment 1
Vertical mucosal amplitude 1.5 mm from the vocal fold edge at the mid membranous part, sound level at 30 cm, fundamental frequency (F0), vocal fold length (membranous part) and closing speed for 7 subjects (- =missing data)

Subject	Sex	Age	Vertical amplitude		lev (dB)	F0	Vocal fold length	Closing speed
			Right (mm)	Left (mm)		Hz	mm	m/sec
A	m	44	1.1	1.1	70	118	12.5	0.38
C	m	50	0.9	1.5	68	103	11.2	1.3
G	m	47	1.2	-	66	170	14.4	-
B	f	37	0.8	0.5	69	210	9	0.71
D	f	30	1.0	0.9	65	211	-	0.43
E	f	43	0.5	0.3	70	185	9.4	-
F	f	40	1.2	1.5	73	224	11.8	0.5

3.3 STUDY 3

The main aim of this study was to measure the vocal fold dimensions by means of laser triangulation on professional opera singers and to relate these observations to their respective voice category. We tried to measure the vocal fold sizes at habitual speaking frequency (SFF), but in 50% of the singers the laser spot was hidden by epiglottis at this pitch level. However, for 14 subjects the vocal length value at SFF was significantly larger ($P < 0.05$) for males (13.4 ± 1.1 mm) than for females (10.3 ± 0.4 mm). At double SFF, vocal fold length values were 13.4 ± 0.9 mm for males and 11.4 ± 0.5 mm for females ($P < 0.05$).

When comparing voice categories, only the bass group showed significantly larger values concerning vocal fold length and glottal width as compared to the soprano and the mezzo group at double SFF ($P < 0.05$), Fig 18.

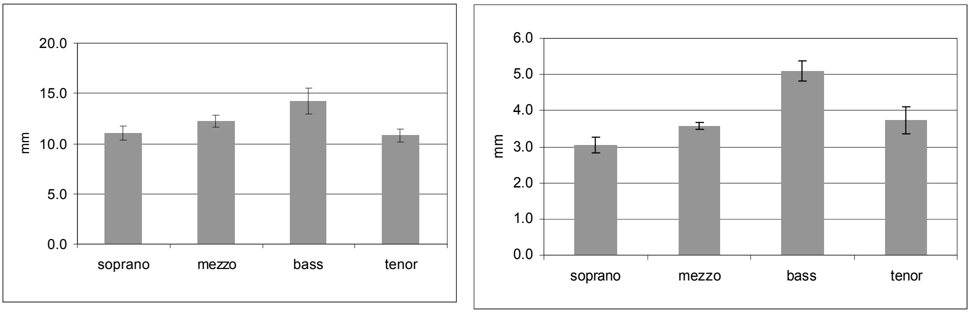

FIGURE 18. Vocal fold length (left) and width (right) at double the habitual fundamental frequency.

Normalized vocal fold width values around SFF as well as at 2* SFF were significantly higher for the males than for the females ($p<0.01$). Comparing vocal fold width values for all voice categories, the bass/baritone group had significantly higher values ($p<0.05$) than all other groups (Fig18).

In 9 female and 5 male subjects it was possible to measure the membranous vocal fold length close to SFF (within 2-3 semitones), and also to measure the size changes during a glissando towards high pitch. A pitch increase of one octave resulted in an increase of vocal fold length with about 20% for both males and females. However, inter-subject variation was large (Fig19).

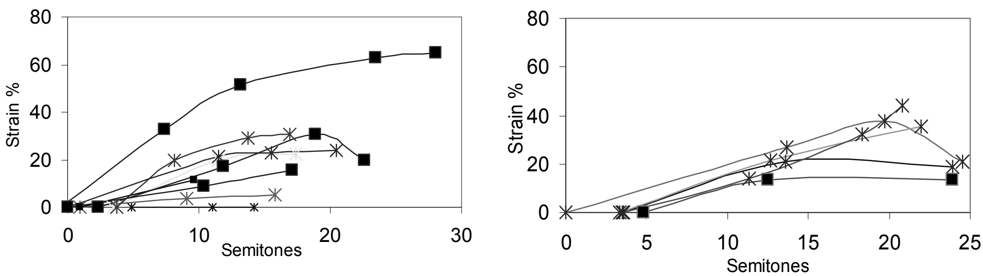

Figure 19. Relative stretch (strain percentage) of the vocal folds for left**,** sopranos (*) and mezzo (■)), right , bass (*) and tenor (■) when performing a glissando phonation from habitual speaking pitch. Only the subjects for which it was possible to measure close to habitual speaking pitch were included (n=14).

For one mezzo, vocal fold length increased with 70% during ascending glissando from her habitual speaking fundamental frequency up to 5 SFF. For another mezzo, however, vocal fold length did not change at all during glissando phonations up to 3*SFF (Fig 19).

The conclusion was that vibrating length of the vocal folds is not sufficient as the sole predictor for determination of voice category for singers. However, it was found to differ significantly between males and females.

3.4 STUDY 4

The purpose of this study was to develop a method to measure the vocal fold elasticity in vivo. A new method was designed called Air Pulse Elasticity Measure (APEM). Compressed air pulses were blown onto the mucosa of the vocal folds and other structures through the working channel of a flexible endoscope and the resulting tissue deformation was measured with a laser. The method was tested both in vitro and in vivo. The in vitro test showed a good correlation between the measured value and the depth of the cavity, which in turn was hypothesized to reflect the tissue elasticity. An in vitro test utilizing a loudspeaker to simulate the pulse effect showed a variation of 5% in the measurement distance range of 1-5 mm from the tissue.
The in vivo test was performed on 9 normal subjects. For each subject the pulse effect was measured on the vocal folds, on the inside of the lower lip, on the cheek and on the dorsum of the hand. About 30 measurements were made from each site.
The results for in vivo tests are presented as the cavity depth in mm (Table 2). There was an average in cavity depth of 0.58±0.11 mm for vocal folds, 0.56±0.16 for lips, 0.57±0.25 for cheeks and 0.33±0.11 for hands. There was a significant difference between the vocal fold and the hand data ($p<0.001$ at t-test). The differences between cheek and hand and between lip and hand were also significant ($p<0.001$).

The conclusions were that the new method APEM allows for measurements of tissue deformation on air pulse stimulation, and it was stipulated that these measures could be regarded as a reflection of tissue elasticity. The method can be used on the awake patient as an office procedure. Local anesthesia is required. Due to a relatively high variation, especially when measuring on mucous membranes, some further development is needed, both concerning the technical aspects of the method and the examination procedure as such.

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Study 4 Table 2. Cavity depth and variation of measurements on the vocal folds, lips, cheeks and hands for 9 subjects

	Cavity depth (mm)				Variation (standard deviation/mean %)			
Subj	**Vocal fold**	**Lip**	**Cheek**	**Hand**	**Vocal fold**	**Lip**	**Cheek**	**Hand**
A	0.68	0.56	0.56	0.20	20	20	13	44
C	0.52	0.58	0.48	0.25	38	15	30	20
E	0.45	0.82	0.96	0.49	41	23	15	13
F	0.49	0.37	0.90	0.36	37	53	14	35
G	0.57	0.72	0.57	0.37	31	41	15	42
H	0.79	0.58	0.69	0.48	45	33	30	40
I	0.57	0.37	0.37	0.23	35	39	40	45
J	0.65	0.39	0.36	0.37	17	50	27	14
K	0.48	0.66	0.20	0.20	52	19	74	56
Mean	0.58	0.56	0.57	0.33	35	33	29	34

3.5 STUDY 5

In this study the APEM method from study 4 was used to test the elasticity of scarred rabbit vocal folds (Hertegård et al 2003; 2006). Ten rabbit vocal folds with scarring and 2 animals with normal vocal folds were selected after sacrifice of the animal and measured with the air pulse method. The results were compared to the histological analysis. Figure 20 shows the correlations between the elastic data and the histological gradings of scarring. The data indicate that the more scarring present in the vocal folds, the higher the degree of stiffness found the air pulse stimulation data. Although the number of samples analyzed were small, a significant correlation was found between the histologically rated degree of scarring and the elasticity values from the air pulse stimulations. The comparison of the elastic data between 10 scarred vocal folds and the normal non-scarred folds showed no significant difference. However, reliable data was only available for 2 normal rabbit vocal folds.

The results indicate that the air pulse stimulations may be used to quantify scarring. There are however a number of questions remaining. A significant difference was only found between clear vs. severe scarring and normal vs. minor scarring.

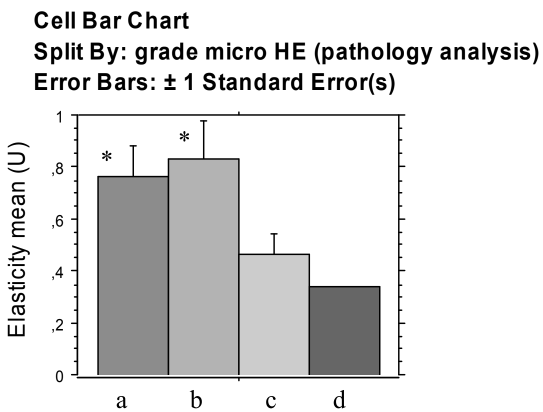

Figure 20. Elasticity data from vocal folds with different degrees of scarring shown in hematoxyline-eosine stainings and judged by a histopathologist. Grade a: None or minimal scarring, grade b: Less or focal scarring in lamina propria and superficial vocal muscle, grade c: More compact scarring in lamina propria and superficial muscle with chronic inflammation, grade d: Compact fibrosis in both lamina propria and superficial muscle, as well as fibrosis in deeper muscle with chronic inflammation. The * marks significant difference at Mann-Whitney test between the combined groups a-b and c-d (p=0.03).

4 GENERAL DISCUSSION

4.1 HIGH-SPEED IMAGE RECORDING

Digital High-Speed Image Recording of vocal folds has been used since more than 10 years and during the last years camera devices for recordings in colour have been developed and made commercially available i.e. KayPentax and Richard Wolf GmbH. The recording speed has increased and it is now possible to make recordings at 2000 images/sec in colour with a resolution of 512x512 pixels. With a decreased vertical resolution, higher speed can be achieved. The software has also been improved, a kymogram option has been added, but still high-speed photography is not as user friendly as videostroboscopy. Most high-speed cameras are still used mainly for research and are by many clinicians considered to be too complicated or time consuming to be used in clinical practice. There is a need for even better resolution and improved software. New faster and more light sensitive colour HSP cameras are currently being developed (Deliyski et al 2008) but they are still too heavy and too expensive to use clinically in a larger scale.

The High-Speed Tool Box (HSTB) was developed in close cooperation with Phoniatric/ENT specialists. However, the computer and the camera were not fast enough to create a software with all options. HSTB has instead been used in several research studies e.g. by Lindestad et al (1999, 2004), who examined ventricular fold vibration, or by Laukkanen et al (2007) who studied the glottal area variation during artificial lengthening of the vocal tract. Granqvist et al (2003) presented an interesting study of the relationship between transglottal airflow and the opening area from a simultaneous high-speed recording. The flow was measured using a face mask and an inverse filter. The glottal area was analyzed with HSTB. The result showed a non-linear phase shifted relationship between area and flow. There was also a small airflow during closed phase which can be explained by a glottal piston effect during the closed phase (Hertegård 1994).

As analysis of high-speed recordings is time consuming, kymograms are a fast shortcut to get an overview of the vibration. Kymograms, however, show only the vibration in one selected point or rather along a transversal line in the vibrating glottis. Eysholdt et al. (2008) and Lohscheller et al. (2008) have developed new methods for analysing high-speed recordings where new types of images called phonovibrograms are created. One image type is the function of deflection from the midline, another shows vocal fold velocities during vibration, and a third displays the acceleration of the vocal folds. These types of images can be helpful, but they require a lot of new experience to interpret and the connection to the real image is not yet clarified.

4.2 CALIBRATION OF ENDOSCOPIC IMAGES

Calibration of endoscopic images is a very interesting research issue but has so far not been used clinically. There are two main methods. One is the triangulation of a single laser beam developed by our group (study 2) and a second is the two beam laser method (Schade et al 2002, Schuberth et el 2002). Both methods have their advantages. The single beam method can also measure vertical movements but has the

disadvantage that it requires a calibration procedure and that automatic identification of the laser spot is needed for calibration. With the two beam method the calibration distance between the two laser spots is directly visible in the recorded image. However, automatic identification could be helpful. Both methods have the disadvantage that it sometimes can be difficult to identify which is the laser spot and which is some other reflection in the image. The triangulation method includes an automatic search function along a line in the image. Recently Wurzbacher et al (2008) developed the two beam laser device to a two beam laser line method. With this new laser it is easier to identify the laser line and they have also created an algorithm to detect the line in the image. The single laser method has also been developed to a line search by George et al (2008). With this setup it is possible to display a 3D-imaging of the endoscopic vocal fold images with options to study the mucosal wave more exactly.
In study 2 we measured the vertical amplitude which is correlated to the mucosal wave. George et al reported the same magnitude of amplitude (0.7-1.2 mm) as in our study.
In study 3 we used the laser triangulation method from study 2 to measure the vocal fold dimensions in opera singers and compared this with voice category. There are other studies on laryngeal dimensions but most are performed on excised larynges or in vivo during general anaesthesia (Nishizawa 1988; Su et al 2002; Schuster et al 2005). Schuster et al. (2005) measured vocal fold dimensions with the two laser method, but the subjects were not singers. The largest study in this field was published by Roers (2005; 2009) who analyzed old X-ray images from singers at a musical school in former East Germany. Also Sonninen et al (1998) did radiographic studies on singers. Unfortunately, the length of the membranous part of the vocal fold could not be accurately determined from the X-ray profiles in all of the subjects, because the laryngeal cartilages were not calcified enough to detect the X-ray shadow, but in some subjects it was indeed possible. Alternative measurement points were introduced, such as the diameter of the subglottal tracheal contour. Some of these parameters would include the lower part of the vocal tract. The results showed a clear covariance between predicted vocal length and voice classification, the sopranos had measurements indicating shortest vocal folds followed by altos, tenors and basses. This is grossly in accordance with our findings, except for the tenor group for which the values were of the same magnitude as for the sopranos in our study 3. For some subjects in study 3 there were problems to measure at habitual fundamental frequency as some part of the vocal folds were hidden by the ventricular folds or the laser beam by the epiglottis. For that reason we also compared the voice categories at double fundamental frequency. The fact that there were rather few subjects in each group may explain why significant differences were not found between all groups.

4.3 ELASTICITY MEASUREMENTS

To measure the vocal fold elasticity is not a straight forward task. The first question is how to define elasticity. Sometimes it is called stiffness or Young modulus equivalent. Chan et al. (2007) have a definition where they state “ The somewhat more intuitive and laymen understanding of elasticity as describing the springy or bounce nature of tissues or material demonstrating elasticity energy storage and geometric recover (as in strings and rubber bands) is not an acceptable scientific definition”(page 1479). Most elasticity measurements are performed on excised larynges using a parallel-plate rheometer which has more or less become a standard method. An alternative method is the stress-strain tests where a tissue sample is placed in a measure chamber in which it is possible to measure the relationship between strain and stress (Chan et al 1998, Chan et al 2007). Of course, these methods represent an exact way to test the vocal fold tissue and by this way it is possible to get basic data from the vocal folds. However the tests cannot be made in vivo.

Hess et al (2006) state that "Measurements on intact vocal folds have been technically difficult but are clearly essential for many clinical applications. In vivo measurement could potentially help identify abnormal regions, provide feedback during augmentation surgery and aid in the objective assessment of surgical procedures designed to manipulate vocal fold material properties" (page 214).

Several methods have been proposed for in vivo elasticity measurements. Tanaka et al (1990) have described a method in which they applied a negative pressure to a small plastic catheter. The catheter was inserted via an endoscope near the vocal fold to a distance from the surface at which the vocal fold was sucked into the opening. The force at which the mucosa was sucked in should reflect the elasticity. They were able to show that the stiffness for carcinoma and sulcus vocalis was significantly larger than for normals.

Berke et al (1992) did a test with a prototype which could be attached to the vocal fold during general anesthesia. They performed a vocal fold lateral deflection and measured the force. The device was completely mechanical with springs and scales. Tran et al (1993) reported an interesting study where the device was used in humans during general anaesthesia and applied through a laryngoscope. A nerve stimulator was then applied with needle electrodes to the recurrent laryngeal nerve. By applying different levels of electrical current to the nerve a relationship between stiffness (Young's modulus) and stimulation level could be shown.

More recently the Linear skin rheometer, LSR, was presented (Goodyer et el 2006, Hess et al 2006). It uses the same principle as the method by Berke and Tran but the LSR is electrically driven and has a computer interface. The probe can be attached to a vocal fold or to other tissue, which is examined. The probe is moving with a sinusoidal movement of 0.3 Hz and a peak amplitude of about the equivalent of 1 g. The force/displacement data are recorded. With this method it is possible to measure the lateral elasticity in different directions and in one point. Also, this method is best adapted for in vitro use or for examination during general anesthesia. A study on 8 subjects showed elasticity values of similar magnitude as data from an excised larynx, but with high variation up to 27% (Goodyer et al 2007). Hess et al (2006) showed that the elasticity was highest close to the vocal process, lowest at the mid membranous part and had a mid value close to the anterior commissure.

No previously described device can be used in vivo on awake subjects. Therefore our goal was a try to develop such a method. As air from the lung sets the vocal folds in vibration, the idea was that it would be natural to blow air on the vocal folds, register the deformation and measure the result. It ended up in the Air Pulse Elasticity Measure (APEM), described in study 4. As the vocal folds are not touched the principle is quite different from those of the previously mentioned methods and our data are not directly comparable. We measured the depth of the cavity. The width of cavity is difficult to determine exactly. The device used by Tran et al (1993) compressed the tissue with a plate over a defined area. Goodyer et al (2006) and Dailey et al (2007) compressed the vocal fold tissue with a needle or rod which moves laterally. In the APEM method the compression with air can only make an orthogonal pressure to the tissue. As the probe also is placed in position with help of an endoscope it is difficult to know if the angle is indeed exactly orthogonal which to some extent can influence the result. The depth is measured with laser light and is depending on how and where the light is reflected. On skin which has a distinctly reflecting surface it is working without problems, but on mucosal membranes it is much more difficult to decide where the reflection takes place. Although we have tried to compensate for this by using three laser fibres and also tested several types of algorithms, this is still a problem which probably explains the relatively high variation in our results. Occasionally, the concavely compressed mucosa can act as a parabol and increase the reflecting light which may also increase the variation in measurement data.

The APEM method has limitations but on the other hand it is currently the only method which can be used on awake subjects. Moreover, the experiment on scarred rabbit vocal folds in study 5 showed a significant correlation between the degree of scarring (severe and minor scarring) and the elasticity data. This corroborates our assumption that the APEM elasticity parameter is correlated to the degree of tissue damage which is known to increase stiffness.

The APEM method can be further improved to allow more advanced measurements. For example, with some further development it could be possible to measure elasticity during phonation on some parts of the vocal folds.

4.4 FUTURE POSSIBILITIES

In the research field, there are many methods which are more or less clinically useful for measuring vocal fold function. In the high-speed area, new colour cameras are under development with higher speed (Deliyski et al 2008). This will make it possible to study the vibration pattern in detail, especially the mucosal wave. Also with better software it should be easier to use high-speed recordings clinically.

HD (high definition) video is a new area with far better image resolution which may be combined with high-speed recording to add information in vocal fold research.

Ultrasound has not been used for vocal fold analyses to a significant extent. The technical development is relatively fast in this area and the amount of images/sec are increasing. New algorithms have a big potential in characterizing the movements and also the elasticity in an easy way.

4.5 CONCLUSIONS

This thesis focused on the development of new methods for examination of vocal fold function. The High-Speed Tool Box together with laser triangulation give new possibilities to quantify the area of the open glottis, vocal fold sizes, as well as the magnitude of the mucosal wave (study 1 and 2). When comparing these parameters to the sound signal and kymography, complex vocal fold movements during phonation can be analysed in detail in a relatively easy way. Examination of aperiodic phonation, such as diplophonia and voice tremor, is particularly suited for the analysis. An investigation of professional opera singers showed significant differences in vocal fold length and width between males and females and between different voice categories (study 3). However, vibrating vocal fold length data were not sufficient as the sole predictor for determining voice category in classical opera singers.

The newly developed method Air Pulse Elasticity Measure (APEM) can give further information about vocal fold elasticity, which is an important factor for vocal fold function. The APEM is at present the only available method for examination of vocal fold elasticity in local anesthesia on human subjects. Examinations from 9 normal subjects showed that it is possible to get reliable vocal fold elasticity data (study 4). The vocal fold elasticity values did not differ significantly from data obtained from the subject's lip mucosa and cheek skin. Measurements from the skin of the hand dorsum, however, showed significantly lower elasticity (indicating higher stiffness). In study 5, APEM elasticity measurements from scarred rabbit vocal folds were compared to the grade of scarring from histopathological analysis of tissue stainings. The histological severity of scarring correlated well to the elasticity data.

The data variability of the APEM is at present high (in ideal conditions, e.g. at the skin below 10% and up to 40% at the vocal folds). Remaining methodological problems and some further development is needed, both on technical aspects of the method and the examination as such.

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