Assessment of local vocal fold deformation characteristics in an *in vitro* static tensile test

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Voice quality is strongly dependent on vocal fold dynamics, which in turn are dependent on lung pressure and vocal fold biomechanics. Numerical and physical models are often used to investigate the interactions of these different subsystems. However, the utility of numerical and physical models is limited unless appropriately validated with data from physiological models. Hence a method that enables analysis of local vocal fold deformations along the entire surface is presented. In static tensile tests, forces are applied to distinctive working points being located in cover and muscle, respectively, so that specific layer properties can be investigated. The forces are directed vertically upward and are applied along or above the vocal fold edge. The resulting deformations are analyzed using multiple perspectives and three-dimensional reconstruction. Deformation characteristics of four human vocal folds were investigated. Preliminary results showed two phases of deformation: a range with a small slope for small deformations fading into a significant nonlinear deformation trend with a high slope. An increase of tissue stiffness from posterior to anterior was detected. This trend is more significant for muscle and in the mid-anterior half of the vocal fold.

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I. INTRODUCTION

A healthy voice is characterized by symmetric and periodic vocal fold oscillations.¹ Both the geometry and the biomechanical tissue properties of the vocal folds influence voice quality, glottal airflow,² and the fundamental frequency of vibration.³ In general, dysphonias based on morphological alterations yield a disturbed acoustic voice signal (e.g., hoarseness) and can be easily detected in endoscopic examinations. On the contrary, functional dysphonias, which occur as a result of biomechanical disturbances, cannot be perceived as easily as they only present themselves by irregular vocal fold oscillations, e.g., asymmetric or non-periodical dynamics.⁴ For a comprehensive understanding of either pathologic or healthy vocal fold dynamics, the interactions of both tissue and airflow have to be investigated.

Different methods have been presented in literature to investigate the deformation properties of vocal fold tissue. Alipour and Titze analyzed dissected specimens of canine vocal fold tissue (muscle and cover) in longitudinal cyclic

tensile tests at low frequencies.⁵ They measured non-linear deformation behavior for both muscle and cover and noted that the cover had a more elastic nature. Similar tensile experiments were performed by Zhang *et al.*⁶ They also found a non-linear deformation trend as well as age- and gender-related elastic differences of human vocal fold cover. In contrast to these methods, which investigated dissected tissue specimens, other studies emphasized the measurements of intact vocal folds. Dailey *et al.*⁷ and Hess *et al.*⁸ employed a linear skin rheometer to measure the elastic properties of vocal folds in animal and human hemi-larynges and depicted them in two-dimensional surface maps. They measured differences in vocal fold stiffness regarding the posterior, middle and anterior region. In contrast to the experiments with tissue specimens^{5,6} that delivered global mean values, the methods that examined intact vocal folds^{7,8} were applied to investigate local properties.

The emphasis of the present work is the investigation of local deformation characteristics of human vocal folds in natural surroundings in hemi-larynges. Hence, we present a method that allows the determination of three-dimensional (3D) deformations of human vocal folds as consequence of locally applied forces. Micro-sutures on the vocal fold

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surface serve as position markers for distinctive flesh points. Forces are applied to five defined working points longitudinally distributed along the superior part of the vocal fold. The resulting deformations are detected by optical marker registration and 3D reconstruction of the position markers. The method allows for the investigation of local deformation characteristics as the forces are (a) applied at five distinctive working points and (b) either induced into the cover or muscle layer. Furthermore, in a preliminary study the deformations of four human vocal folds are investigated.

The measured data serve the following main purposes: First, the experimental setup allows for a direct analysis of local deformation characteristics of human vocal folds in natural surroundings that have not been fully explored. Second, the experimental data can be applied as input for a numerical material optimization problem solver.⁹ This, in turn, would yield material parameters for multi-layer silicone models of the larynx with human-like deformation characteristics and more realistic dynamics. Thus the data can be used to refine physical models of the larynx.^{2,10}

II. METHODS

To maintain tissue properties, human larynges were quick frozen in liquid nitrogen shortly after excision and stored in a freezer at -80°C .¹¹ The larynges were obtained from the autopsy unit of the UCLA Medical Center. They had no evidence of previous laryngeal diseases or injuries. Before preparation, the larynges were slowly thawed in a refrigerator for 12 h. For all larynges, the right vocal fold was removed by a sagittal-cut, carefully avoiding injury to the anterior vocal fold attachment to the thyroid cartilage. In this manner, visibility of the medial surface of the remaining (left) vocal fold was achieved, and a so-called *hemi-larynx*

was created. A grid of regularly arranged micro-sutures (6 rows, 5 columns) was sewn into the cover layer with the second top most row sewn along the vocal fold edge (Fig. 1, left). The first row was sewn above the edge and the remaining rows beneath the edge. The sutures served as position markers for distinctive flesh points. Depending on the experiment, the five sutures of the row above the edge or along the edge served as working points for induced forces. These five specified sutures will be subsequently denoted as *pulling sutures*. Depending on whether deformations of the cover or the muscle were to be measured, the pulling sutures were anchored into the cover or the muscle. After preparation the vocal folds were conditioned to room temperature.

The experimental setup is similar to the one presented by Döllinger and Berry for the investigation of 3D vocal fold dynamics in excised hemi-larynges¹² and *in vitro* canine experiments.¹³ The trachea was shortened to approximately 3 cm, mounted over a steel tube, and clamped to prevent vertical displacement of the hemi-larynx. A glass plate was placed vertically at the top end of the tube and the hemi-larynx pressed against it anteriorly and posteriorly through the use of adjustable length micrometers. This anchorage guaranteed fixed boundary conditions while applying forces to the vocal fold. For computation of 3D coordinates, a right angle prism was placed on the side of the glass plate opposite to the medial surface of the vocal fold to provide two effective camera views. For calibration purposes, a brass cube (side length: 5.0 mm) was glued to the glass plate directly above the vocal fold (Fig. 1, left). A schematic of the exact arrangement of the glass plate, the prism, the cube, and the hemi-larynx can be seen in the publication of Döllinger and Berry¹² on page 371, Fig. 2. The vocal fold was deformed by applying vertical directed forces via strands and pulleys to the working points in the fold. Ethilon 6.0

FIG. 1. Left: Split view by the prism of a vocal fold with sewn position markers and calibration cube. The pulling markers are sewn above the vocal fold edge and the first marker is pulled. The forces are induced via strands to the fold. Right: 3D reconstruction of the vocal fold surface on basis of the reconstructed position markers of the split view (left). The circle indicates the pulled marker (here M1), the arrow the induced force direction. The boundary points have been extrapolated and are necessary for post processing (Ref. 9). The marker and boundary points are connected by patches, thus forming the reconstructed vocal fold surface.

FIG. 2. Force vs normalized deformation for experiments E1 to E4. The symbols represent the deformation data of the pulled sutures M1 to M5. The graphs are fitted [$f(x) = a \cdot (e^{b \cdot x} - 1)$]. The vertical line marks the boundary between range I that is characterized by a small slope and range II that presents a nonlinear deformation characteristic with a high slope. All deformations are normalized to the maximum measured deformation relating to experiments E1 to E4.

sutures were used for the pulling markers and strands; the positioning sutures were sewn with Ethilon 9.0. The applied forces were realized by weights of 10 g, 20 g, 50 g, and 100 g. The surface deformations were recorded with a gray scale digital camera at a resolution of 1024×1024 pixels. The scene was lit by a 150 W light source.

The computation of the three-dimensional marker coordinates and the resultant surface deformations was realized by a linear approximation method.¹² In the setup, the prism simulated two camera views of different perspectives (Fig. 1, left). Three-dimensional points were estimated on the basis of a linear mapping by their two-dimensional marker coordinates in the two images of the split view (Fig. 1, right). The segmentation of the two-dimensional marker coordinates was performed by a semi-automatic procedure.¹⁴

The deformations of four vocal folds were determined in dependency on the applied force $F = m \cdot g$ ($g = 9.81$ mN/g, gravitational constant). Additionally the local (vertical) stiffness of the vocal fold was computed:

$$D = \frac{F}{x}, \quad [D] = \text{mN/mm}, \quad (1)$$

with x being the measured deformation. The subject information is summarized in Table I. The vocal folds were obtained

TABLE I. Subject information of the four analyzed vocal folds (E1–E4).

Experiment	Sex	Age	Pulling sutures	
E1	Male	65	Cover	Above edge
E2	Male	31	Cover	On edge
E3	Female	73	Cover	Above edge
E4	Male	72	Muscle	Above edge

from three male and one female subjects. In the following, the measurements of the four vocal folds are denoted as experiments E1 to E4, see Table I. Three experiments were performed to analyze the deformation characteristics while applying the forces into the cover (E1–E3), one while applying the forces into the muscle (E4). In one experiment (E2), the forces were induced exactly on the fold edge. On three folds the forces were applied above the edge (E1, E3, E4). Table II shows the information on the vocal fold lengths as well as the longitudinal distances between the five pulling markers in the unloaded states. The vocal fold length is defined as the distance between the vocal process (v.p.) and the anterior commissure (a.c.). As all experiments investigated the left vocal fold, the pulling markers were arranged from posterior (M1) to anterior (M5). To allow for a later comparison between the four larynges, the location markers were sewn approximately 2 mm apart, measured along the length of the vocal fold (see Table II).

TABLE II. Longitudinal marker distances between the pulled sutures in the unloaded state (approximately 2 mm). The total vocal fold length is defined as the distance between the vocal process (v.p.) and the anterior commissure (a.c.). The distance between the vocal process and M1 is smaller than between M5 and the anterior commissure for all folds. All values are given in mm.

Experiment	v.p.– M1	M1– M2	M2– M3	M3– M4	M4– M5	M5– a.c.	Vocal fold length
E1	0.8	1.8	2.0	1.6	1.9	1.3	9.4
E2	0.6	2.2	2.2	2.5	2.4	1.4	11.3
E3	0.7	1.4	1.9	2.0	2.2	1.5	9.7
E4	0.8	2.5	2.0	1.6	2.0	2.0	10.9

TABLE III. Measured absolute displacements (mm), displacements normalized to the maximal deflection of the particular fold (%) and stiffness (D) of the loaded pulling markers. Stiffness is given in mN/mm. The pulling markers M1 to M5 are arranged from posterior to anterior. The percentage values in each column heading show the longitudinal arrangement of the five pulling markers M1 to M5 from the vocal process with 100% corresponding to the anterior commissure. The maximum deformation and stiffness values of each fold are highlighted by bold printing.

		E1 (male, cover, above edge)					E2 (male, cover, on edge)				
Force (mN)		M1 (9%)	M2 (28%)	M3 (49%)	M4 (66%)	M5 (86%)	M1 (5%)	M2 (25%)	M3 (44%)	M4 (66%)	M5 (88%)
98.1	mm	2.9	2.7	2.5	2.5	1.8	3.0	4.2	4.4	4.0	3.9
	%	48	44	41	41	30	41	58	60	55	53
	D	34	36	40	40	55	33	23	22	24	25
196.2	mm	4.3	3.7	3.4	3.3	2.4	5.0	5.0	4.3	4.8	4.8
	%	70	61	56	54	39	68	68	59	66	66
	D	46	53	57	60	83	39	39	46	41	41
490.5	mm	5.0	4.9	4.7	4.4	3.2	5.9	6.1	5.7	5.4	5.1
	%	82	80	77	72	52	81	84	78	74	70
	D	97	100	104	112	152	84	80	87	90	95
981	mm	6.1	6.0	6.0	5.6	4.8	7.0	7.3	7.0	6.5	5.8
	%	100	98	98	92	79	96	100	96	89	79
	D	160	163	165	175	203	139	134	141	151	169
		E3 (female, cover, above edge)					E4 (male, muscle, above edge)				
Force (mN)		M1 (7%)	M2 (22%)	M3 (41%)	M4 (62%)	M5 (85%)	M1 (7%)	M2 (30%)	M3 (49%)	M4 (63%)	M5 (82%)
98.1	mm	5.1	5.5	4.9	4.1	3.4	3.9	3.9	2.8	2.2	2.1
	%	66	71	64	53	44	65	65	47	37	35
	D	19	18	20	24	29	25	25	35	44	48
196.2	mm	6.1	6.2	6.1	5.0	3.5	4.6	4.9	3.5	3.2	2.5
	%	79	81	79	65	45	77	82	58	53	42
	D	32	31	32	39	57	43	40	57	62	79
490.5	mm	6.8	7.1	7.0	5.6	3.9	5.4	5.1	4.1	3.4	2.8
	%	88	92	91	73	51	90	85	68	57	47
	D	72	69	71	87	126	91	96	119	145	177
981	mm	7.2	7.7	7.4	6.2	4.2	6.0	5.9	4.7	3.8	3.1
	%	94	100	96	81	55	100	98	78	63	52
	D	135	127	133	158	234	164	166	210	257	316

The five pulling markers were consecutively loaded from M1 to M5 with the same weight with only one suture loaded at a time. At the beginning of each test series, the unloaded state was taken as reference. With five pulling sutures and four weights, this resulted in 20 deformation states per hemi-larynx. The resulting vocal fold deformations were determined as the difference between the marker's location in the loaded and in the unloaded state. The images were taken immediately after attaching the weight, and the masses were uncoupled right after having taken the images. All experiments were performed at room temperature, and the vocal folds were hydrated intermittently throughout the duration of the experiments by applying physiological NaCl solution.

III. RESULTS

The displacements of the five consecutively loaded pulling markers were analyzed for all four measured vocal folds. To compute the deformations, we considered the change of the markers' positions relative to their positions in the unloaded state. The measured displacements of the pulled markers are specified in Table III separated for the applied forces. The deformations are presented in two different ways: As absolute measured deformations in millimeters as

well as deformations normalized to the maximal measured deflection on the particular vocal fold in percentages. The rows titled with *D* present the computed local stiffness [see Eq. (1)]. The numbers in brackets in the cells of M1 to M5 denote the longitudinal distance between the specified marker and the vocal process, normalized to the particular vocal fold length (see Table II). The values show that the longitudinal marker positions M1 to M5 of the four different hemi-larynges represent approximately the same longitudinal positions. Averaging over the four larynges results in positions of $7\% \pm 2\%$ for M1, $26\% \pm 4\%$ for M2, $46\% \pm 4\%$ for M3, $64\% \pm 2\%$ for M4, and $85\% \pm 3\%$ for M5. The values in brackets are the standard deviations. For all vocal folds, M1 is located closer to the vocal process than M5 is to the anterior commissure (see also Table II). In E1, M3 is exactly in the middle of the fold; in E2 to E4, the middle of the fold is in between M3 and M4.

The measured deformations, with respect to all four vocal folds, ranged from 1.8 mm (98 mN, E1, M5) to 7.7 mm (981 mN, E3, M2). The smallest deformations in general occurred when the pulled marker was M5, i.e., the most anterior marker. The sutures with the maximum deformation of each vocal fold are highlighted in Table III by bold printing. These are the values that were used to normalize the deformations.

FIG. 3. Deformation vs marker position. The deformations are normalized to the maximum measured deformation relating to experiments E1 to E4. The marker positions are given in distance from the vocal process normalized to the vocal fold length, 100% match with the anterior commissure. The plots are separated for the applied forces. The symbols represent the data measured in experiments E1 to E4.

While applying the smallest force, the vocal folds reached deflections in the range of 30% to 53% of their maximal occurring deformations (E1–E4, M5). Table III shows a general trend of increasing deformation with increasing force (exception: E2 at M3 for $F = 98$ mN and $F = 169$ mN). The highest deformations were always measured at M1 or M2. Furthermore, the deformations at M1 are always higher than those at M5 for all forces and vocal folds (exception: E2 for $F = 98$ mN). The stiffness D takes values in the range of 19 mN/mm (98.1 mN, E3, M1) and 316 mN/mm (981 mN, E4, M5). The local stiffness increases with increasing force. The stiffest deformations for all forces were always measured at M5; exceptions occurred for E2. The stiffest deformations are highlighted in Table III by bold printing.

The deformation data of Table III is plotted in Fig. 2 as induced forces (in mN) against the normalized marker displacements (in percentage). The four graphics are the experiments E1 to E4; the symbols represent the deformations of the individually pulled markers M1 to M5. The graphs are fitted by nonlinear least square optimization following the equation $f(x) = a \cdot (e^{b \cdot x} - 1)$.⁵ All vocal folds show the same nonlinear deformation characteristics that can be divided into two phases, namely, a range with a small slope for small deformations fading over to a range with a high slope for increasing deflections. The boundary between the two ranges can be set by a visual analysis to 30% for E1 to E3. In E4, the boundary is a little lower at about 25%. The boundary is indicated in Fig. 2 by the vertical line, the two ranges by I and II. Within range I, the graphs of M1 to M5 lie near to each other. For deformations above the boundary, the graphs begin to spread. This observation is independent of the deformed tissue layer and the location of the induced forces (above or on the vocal fold edge) but is more significant in the experiments E3 and E4.

The graphs of E1 to E4 present a general trend of descending deflections from posterior (M1) to anterior (M5) when applying the same force (range II). This fact is emphasized in Fig. 3 where the deformations are plotted against the longitudinal marker position. The graphics are separated for the applied force, whereby the symbols represent the deformations at the markers M1 to M5 of the vocal folds E1 to E4. In general, the more anterior the marker (increasing value on the x axis), the smaller the resulting deformation for all applied forces. The decrease of deformation is particularly significant for the region M3 to M5, i.e., for the range between the vocal fold's mid and the a.c. Thus for each experiment the deflection difference between locally neighbored markers is larger for M3 to M5 than for M1 to M2. Figures 2 and 3 visualize that the decrease of deformation is larger in E3 and E4 than in E1 and E2, especially for forces higher than 491 mN. In the region of M1 to M2 (< 26% posterior distance), both E4 and E3 show the highest deformations; in the region of M3 to M5 (above 50%), E4 shows the smallest deformations. Thus E4 presents the highest decrease of deformation and thus the highest anterior-posterior difference. The constants of linear fits to the data of Fig. 3 are summarized in Table IV.

The plots in Fig. 4 show the local stiffness as function of the normalized deformations. The symbols represent values that are computed from the applied forces and measured deformations as presented in Table III. The lines represent the stiffness computed from the fitted values of Fig. 2. To avoid a division by zero [see Eq. (1)], the start value for the computation was $x = 0.001\%$. The stiffness behaves nonlinearly with increasing deformation and can be divided into a range with small slope and a range of higher slope, similar to the deformation trend in Fig. 2. The boundary is

TABLE IV. Constants of linear fits to the data of Fig. 3 following the equation $y = m \cdot x + t$.

Experiment	98 mN		169 mN		491 mN		981 mN	
	<i>m</i>	<i>t</i>	<i>m</i>	<i>t</i>	<i>m</i>	<i>t</i>	<i>m</i>	<i>t</i>
E1	−0.2037	50.49	−0.3294	70.62	−0.3616	88.10	−0.2550	105.20
E2	0.1116	48.09	−0.0402	67.03	−0.1520	83.80	−0.2365	103.10
E3	−0.3212	74.89	−0.4119	88.38	−0.5046	100.50	−0.5218	108.90
E4	−0.4587	71.64	−0.4869	84.40	−0.5870	94.48	−0.7027	111.10

approximately at 30%, too. The highest stiffness was measured for muscle tissue (E4, M5). Nevertheless, the measured values for E1 to E4 at M1 and M2 are all in the same order of magnitude. The stiffness distribution from M1 to M5 is bigger for E3 and E4 than for E1 and E2.

IV. DISCUSSION

We presented an experimental approach for measuring local vocal fold deformations in natural surroundings. By means of a static tensile, test forces were applied to distinctive working points along or above the vocal fold edge. The resulting deformations were measured on the basis of suture displacements using 3D reconstruction techniques. Forces were directly induced into the different tissue layers cover and muscle, respectively. Hence local deformations and stiffness of both tissue layers were analyzed. Preliminary application of this methodology was performed on four human vocal folds. The experiments yielded the following preliminary results:

(1) All vocal folds exhibited a nonlinear deformation trend when examining applied force as a function of resultant deformation (Fig. 2).

- (2) The deformation trend can be divided into two parts (Fig. 2): A range with a small slope for small deformations (range I) fading over to a range with a high slope (range II). The boundary between the two ranges is approximately at 25% to 30% normalized deformation.
- (3) Within region I, the fitted deformations behave quite similarly; in range II, they spread out (Fig. 2).
- (4) A decrease of deformation from posterior to anterior while applying the same force is observed (Figs. 2 and 3).
- (5) The decrease of deformation was observed irrespective if applying the forces along or above the vocal fold edge (Fig. 3).
- (6) The decrease of deformation is more significant for the markers M3 to M5 than for M1 to M3 (Fig. 3).
- (7) The decrease of deformation is more significant for the muscle (E4) than for the cover (E1 to E3) (Fig. 3).

The decrease of deformation from posterior to anterior is equivalent to an increase of stiffness from posterior to anterior of human vocal fold deformations. This, and the fact that we measured the highest stiffness in the muscle, is in accordance to Hirano’s cover-body model.¹⁵ The stiffness of

FIG. 4. Stiffness vs normalized deformation for experiments E1 to E4. The symbols represent the local stiffness computed by the measured data at M1 to M5. The graphs show the stiffness computed by the values of the fitted lines in Fig. 2. All deformations are normalized to the maximum measured deformations in the particular experiments E1 to E4.

the vocal fold tissue increases from cover toward the deep layers of the lamina propria. Thus deformations of the muscle are smaller than those of the cover. The thickness of the cover decreases from the mid toward the posterior and anterior end of the vocal fold at which it is posterior a little thicker than anterior. Under the assumption that we deform always the same amount of tissue, more pliable tissue is deformed in the posterior part of the fold than anterior where the proportion of stiffer tissue is higher. This might explain the drop of deformation from posterior to anterior for muscle and cover. Investigations on local stiffness were also performed by Dailey *et al.*⁷ and Hess *et al.*,⁸ who examined local vocal fold deformations (cover) of intact human larynges and hemi-larynges by means of a linear skin rheometer (LSR). In agreement with our data, both groups observed a posterior-anterior difference. But in disagreement, they located the highest stiffness in the posterior region. This fact is the opposite of our results. Additionally, they measured a decreasing stiffness toward the middle of the fold and an increase from the middle toward the a.c. This trend was not seen in our experiments although it would be due to the cover-body theory of Hirano.¹⁵ We measured a stepless increase of stiffness from posterior to anterior (Table III). Hess *et al.*⁸ found the increase from middle to anterior commissure was significant only in porcine and human hemi-larynges but not in intact larynges. Dailey *et al.*⁷ also investigated a hemi-larynx. Additionally, the data of both groups are derived from only one hemi-larynx in each case. Unlike their studies, we observed the increasing stiffness from posterior to anterior in all four investigated hemi-larynges. Dailey *et al.*⁷ as well as Hess *et al.*⁸ applied a force equivalent to 1 g and measured stiffness values of 27 mN/mm and 83 mN/mm, respectively. Although the applied force is only 10% of our smallest applied force (equivalent to 10 g), the stiffness values measured by Dailey are in the same order of magnitude as ours; those of Hess are even higher. Hess *et al.*⁸ measured a factor between the stiffness of the posterior end (highest measured stiffness) and the middle of the fold of approximately 9. A factor of such magnitude was not observed in our measurements. Dailey *et al.*⁷ explain their measured stiffness distribution (smallest stiffness in the middle fold) as due to the thinning of the superficial lamina propria from the midpoint toward both ends of the vocal fold.⁷ This might be true as the LSR measures tissue properties rather than deformation properties. Thus they measure the highest deformations in the middle fold where the thickest lamina propria is located. The present method is configured to investigate the deformation characteristics of the complete fold in natural surroundings. Thus the measured deformation are a result of the interactions of all vocal fold layers and not only the local superficial lamina propria.

A nonlinear deformation trend was also measured by Min *et al.*,¹⁶ who examined deformations of dissected specimens of human vocal ligament in longitudinal direction while applying sinusoidal forces. In accordance with the results presented here, their measurements showed a nonlinear deformation trend. Because of the high similarity, we utilized the same exponential equation to fit our data (see Fig. 2). Min *et al.* divided their measured deformations into two

parts: A linear region for small deformations, fading into a nonlinear range for higher deformations. Our results are in agreement with these findings. Range I in Fig. 2 presents on the one hand a small slope but on the other hand a small curvature, too. Thus range I could be linearly approximated. This conclusion is based solely on the fitted graphs as there are no measured data points within range I. Alipour and Titze⁵ performed longitudinal tensile experiments to discover the elasticity of the tissue but using canine tissue specimens. They measured a linear range reaching up to roughly 30% of the maximum deformation for muscle and roughly 20% for cover tissue. In our measurements, the boundary was at 30% and thus in the same order of magnitude. Alipour and Titze⁵ identified the cover tissue to be stiffer than the muscle. For small deformations, i.e., within the linear region, their measurements indicated that the cover was twice as stiff as muscle tissue. The offsets of E1 and E2 in Fig. 4 are significantly higher than those of E4. For E3, this is not the case. As we have no measured data points within that region we cannot prove cover to be twice as stiff as muscle. Within the nonlinear region we did not measure a stiffer cover compared to muscle. However, Alipour and Titze⁵ did not consider static forces on the tissue similar to the present study, but sinusoidal forces, which may induce distinct responses from the two different tissue layers. Furthermore, dissected specimens were examined in isolation from other tissue layers. In the study presented, the deformation characteristics of the cover were influenced by that of the muscle layer and vice versa. That may be an explanation for the differences measured in the nonlinear deformation range.

Zhang *et al.*⁶ performed a study to investigate dependencies of age, gender, and stiffness. In contrast to our method, they applied the forces in longitudinal direction to dissected human cover specimens. They discovered an increasing stiffness with increasing age. We measured stiffer deformations on E1 (65 yr) than on E2 (31 yr); this is in accordance with the findings of Zhang *et al.*⁶ The experiments of Zhang *et al.*⁶ also examined differences in male and female specimens of cover tissue. They reported double the stiffness for a male cover as compared to the cover for a female subject of the same age. Similarly, in our study we measured the highest deformations in the female tissue (E3: Table III, Figs. 2 and 3). For small forces (98.1 mN), we also observed a stiffness factor of approximately two between E1 and E3 (Table III). Nevertheless, the present study as well as those of Zhang *et al.* have few samples and so must be considered preliminary. Thus, to deliver significant dependencies a larger number of vocal folds will have to be investigated.

Similar to the methods of Dailey *et al.*⁷ and Hess *et al.*⁸ and in contrast to the methods of Alipour and Titze⁵ and Zhang *et al.*,⁶ the method presented here has the advantage that the vocal fold remains inside the excised larynx and thus in its natural environment. The presence of cartilages, natural surrounding tissue and muscles promote a more functional and real-life deformation. The sagittal-cut was executed in such a manner that it does not affect the functionality of the cartilages and the anchorage of the anterior

vocal fold to the thyroid cartilage, thus conserving the physiological conditions.

The accuracy of the applied linear approximation method for the 3D reconstruction was extensively discussed in the study of Döllinger and Berry.¹² The vertical calibration error is about 8% to 17% depending on the distance to the glass plate. The effects caused by viscoelastic creep due to the applying of loads can be neglected as the magnitudes of the occurring deformations are by far larger than those due to the creep effects. Furthermore, we paid attention to keep the duration of force application quite small. The period between applying the load and removing it was less than 4 s.

In the present setup, the anchorage of the pulling markers is sewn in the respective tissue layer to measure the deformations of the muscle and the cover. Hence, the deformations of the muscle are also affected by the elasticity properties of the cover as both tissue layers have to be deformed in that case. Thus we measure the deformation behavior of the complete vocal fold, i.e., the cooperation of muscle and cover. The induced forces were directed vertically upward because this is the propagation direction of the mucosal wave and thus of particular interest.

In summary, the method presented allows for the determination of local deformation characteristics. This is of particular interest for scarred tissue. Scars stiffen the surrounding tissue¹⁷ and as a result increase the necessary subglottal pressure for phonation onset.¹⁸ Thus the dynamic properties of the vocal folds are significantly influenced by the passive deformation properties of the folds. Measuring the local properties may thus help to investigate the influence of tissue alterations on the dynamic behavior of the vocal folds. The present setup also enables the investigation of deformation characteristics of different tissue layers. The experimental data may be used to improve physical vocal fold models.⁹ Here, an optimization method computes model parameter values to adapt the model deformations to those measured in the excised human larynges. Such optimized parameter values may subsequently serve as a guide for producing multi-layer physical models of the vocal folds.² The agreement between our measured data and the results of other groups^{5,7,8} demonstrates the applicability of the proposed method in measuring local deformation properties of human vocal fold tissue in different layers. The different deformation behaviors of muscle and cover highlight the importance of multi-layered vocal fold models³ as well as the fact that local differences have to be considered when using models to simulate vocal fold dynamics.^{7,8} An advantage of the method presented compared to others is that it is easily expandable to perform dynamic experiments. This is because the present setup is similar to that of Döllinger and Berry,¹⁹ who investigated vibration patterns of the vocal fold surface in hemi-larynx experiments. Thus a combination of static and dynamic investigations can be performed on the same vocal fold by cutting the pulling strands after the static tensile tests.

Finally, some limiting factors have to be mentioned. Currently, the measurements are performed without any longitudinal tension of the vocal folds. In future experiments, an initial and variable pre-tension will be applied to the vocal fold so that its dependency on the local deformation charac-

teristics can be examined. Currently, the smallest weight applied is 10 g, which already results in normalized deformation values of at least 30% (Table III: E1, M5). Consequently, future measurements will start with smaller weights. More data points in the first range (see Fig. 2) will lead to more detailed information about the linear approximation and the transition to the nonlinear phase. A further consideration is the replacement of the prism by two digital cameras. Mounting them in different angles would allow for the reconstruction of the marker points with the procedure of stereo-triangulation.²⁰ Thus additional directions for inducing forces are possible. Right now only vertical (1D) deformations are considered. In future experiments, 3D deformations will be analyzed by inducing arbitrarily directed forces.

The present results are preliminary as they were collected from four larynges that differed in age, gender, anchorage of the marker points, and deformed tissue layer. To get more reliable and statistically significant results, additional experiments will be performed to investigate uniformly prepared vocal folds across the different variables of interest. Finally, the tissue deformations along the complete medial vocal fold surface will be investigated by analyzing the displacements of all the sewn suture markers. This would result in a surface map to displays the local deformations, similar to Dailey *et al.*⁷

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