Title: Spatial Distribution of Airway Wall Displacements during Breathing and Bronchoconstriction measured by Ultrasound Elastography Using Finite Element Image Registration

Brian C. Harvey ^a, Kenneth R. Lutchen ^a, Paul E. Barbone ^b

Abstract

With every breath, the airways within the lungs are strained. This periodic stretching is thought to play an important role in determining airway caliber in health and disease. Particularly, deep breaths can mitigate excessive airway narrowing in healthy subjects, but this beneficial effect is absent in asthmatics, perhaps due to an inability to stretch the airway smooth muscle (ASM) embedded within an airway wall. The heterogeneous composition throughout an airway wall likely modulates the strain felt by the ASM but the magnitude of ASM strain is difficult to measure directly. In this study, we optimized a finite element image registration method to measure the spatial distribution of displacements and strains throughout an airway wall during pressure inflation within the physiological breathing range before and after induced narrowing with acetylcholine (ACh). The method was shown to be repeatable, and displacements estimated from different image sequences of the same deformation agreed to within 5.3 μm (0.77%). We found the magnitude and spatial distribution of displacements were radially and longitudinally heterogeneous. The region in the middle layer of the airway experienced the largest radial strain due to a transmural pressure (P_{tm}) increase simulating tidal breathing and a deep inspiration (DI), while the region containing the ASM (i.e., closest to the lumen) strained least. During induced narrowing with ACh, we observed temporal longitudinal heterogeneity of the airway wall. After constriction, the displacements and strain are much smaller than the relaxed airway and the pattern of strains changed, suggesting the airway stiffened heterogeneously.

Keywords: Elastography, finite element method, image registration, airway smooth muscle, deep inspiration, bronchodilation

1. Introduction

The lungs and branching network of airways are periodically stretched with every breath. The luminal diameter of an airway, which determines the resistance to flow, is believed to be determined as a dynamic equilibrium of the forces which favor narrowing and those that oppose narrowing [1]. Bronchoconstriction, as occurred during an asthma attack, is a result of active force generated by airway smooth muscle (ASM) embedded with an airway wall in response to acetylcholine (ACh) released from parasympathetic nerves. The forces of tidal breathing and deep inspirations (DIs) may work to mitigate this airway narrowing in healthy subjects through straining of the ASM [2]. This beneficial effect of a DI is absent in asthmatics [3–6] and it has been hypothesized that this is because asthmatics are unable to sufficiently strain their ASM during a DI which is indicative of airway wall properties that are stiffer and that can potentiate more reactivity.

While most researchers have focused on the effect of stretching of strips of isolated ASM, the airway wall is a construct comprised of several different components which together create a nonlinear, heterogeneous, thick-walled cylinder. In contrast to the results from isolated ASM strip experiments, recent studies performed on healthy airway segments suggest that transmural pressures simulating tidal breathing and DIs are ineffective at preventing future airway narrowing and only moderately effective at reversing induced constriction [7–10]. The heterogeneous composition and mechanical properties throughout an airway wall likely modulate the strain felt by the ASM. Therefore, understanding the spatial distribution of displacements and strains throughout the thickness of an airway wall could lead to better understanding of airway

narrowing by elucidating contributions of different tissue types to the mechanical behavior of the airway, and the role that breathing and DIs play in modulating airway narrowing.

Elastography is a technique to image tissue based on contrast in mechanical properties [11]. In quasistatic elastography, a tissue is imaged before and after a force is applied and the resulting images are compared to estimate tissue deformation. These deformations are then visualized directly (e.g., strain imaging) or used to infer the mechanical properties of the tissue via modulus reconstructions [11–13]. The technique has been applied in a variety of tissues using many different image modalities such as ultrasound [14], magnetic resonance [15], and optical coherence tomography [16]. In this study, ultrasound imaging was utilized due to its high axial resolution and phase information, high frame rate, and availability. Elastography has many different applications [17] such as detecting breast tumors [18] and predicting plaques vulnerable for rupture in atherosclerosis [19,20], but has never been used to study the spatial distribution of displacements throughout an airway wall.

Many different approaches have been developed to estimate tissue motion from ultrasound image sequences. Among the most common is block matching using normalized cross-correlation [11]. In this approach, the motion within each block of pixels is assumed to be uniform. While the block matching method is computationally inexpensive and easy to implement, it performs poorly at estimating displacements in regions of highly heterogeneous deformation or large strains [21,22], it has recognized biases when estimating sub-sample displacements [23,24], and incorporating prior information through regularization in a systematic way is cumbersome. In this study we utilized a finite element based image registration approach similar to the

deformable grid methods [25–28]. These methods easily accommodate arbitrary geometries, allow for large local strain within an element, and accommodate prior information through regularization in a natural way. They have the drawback that they are comparatively computationally costly, which for the present application was not a concern.

In this study, we optimized our finite element image registration method to measure the spatial distribution of displacements and strains throughout an airway wall during pressure inflation within the physiological breathing range, as well as monitor tissue motions during induced narrowing. To measure large deformation over the entire physiological range of transmural pressure (P_{tm}), we incrementally match images captured over small changes in P_{tm} . We estimate the strain distributions throughout the airway walls which are used to gain insight into the relative moduli of the different airway wall components. We found the displacements and strain during inflation to be longitudinally and radially heterogeneous. The region in the middle layer of the airway experienced the largest radial strain due to a P_{tm} increase simulating tidal breathing and a DI, while the region containing the ASM (i.e., closest to the lumen) strained least. During induced narrowing with ACh, (10^{-3} mol/L), we observed temporal longitudinal heterogeneity of the airway wall. Equal pressure increments lead to much smaller strain and displacements in the constricting airway than in the relaxed airway. The spatial patterns of displacements and strains also changed, suggesting the components of the airway did stiffened heterogeneously.

2. Material and Methods

2.1. Intact Airway System

104

105 An intact airway (main stem bronchus, generations 4-10, 35 mm long) was dissected from a
106 fresh bovine lung (Research 87, Boylston, MA) and side branches were ligated to form a leak-
107 free airway. Cannulas were inserted into each end and the airway was mounted inside a tissue
108 bath with heated (37°C, 5% CO₂) Krebs solutions (Sigma, St. Louis, MO). The entire setup was
109 secured to a floating optical table (Newport I-2000/S-2000) to isolate the system from building
110 vibrations and increase displacement SNR. The airway was stretched to 120% of its resting
111 length to mimic airway lengthening during breathing and its length was held fixed throughout the
112 experiment [29]. The pressure inside the airway was controlled by modulating the height of fluid
113 in a pressure column in series with the airway [30]. Following 60 min in the heated bath, tissue
114 viability was confirmed with electric field stimulation (EFS) as previously described [8,30,31].

115

116 2.2. *Ultrasound Data Acquisition*

117

118 Ultrasound radio frequency (RF) data was collected and digitized to 16-bits at 40 MHz using an
119 ultrasound system (SonixTablet, Ultrasonix Medical Corporation, Richmond, BC, Canada). A
120 128 element linear array transducer (L40-8/12, 12 mm width) was partially submerged in the
121 tissue bath and mounted less than 7 mm above the outer edge of the airway and positioned
122 parallel to the long axis of the airway segment (Fig. 1). The imaging plane of the transducer cuts
123 through the middle of the airway along its length and the region was more than two diameters
124 away from the proximal and distal cannulas to minimize end effects. This transducer orientation
125 allowed for the dominant displacement component to be aligned with the direction of ultrasound
126 propagation. The field of view in the ultrasound axial direction (hereafter the “axial” direction)

and ultrasound lateral direction (hereafter the “lateral” direction) was 22 mm and 12 mm, respectively, and the axial and lateral pixel sizes were 18.7 μm and 46.9 μm , respectively. The resolution in the axial direction was approximately 103 μm . The transducer had a center frequency of 15 MHz and -6 dB bandwidth from 10.5-19.5 MHz. The RF data was upsampled by a factor of two using the fast Fourier transform interpolation method in order to improve the discrete integration of the image during processing.

In order to measure the displacement distributions throughout an airway wall, we collected ultrasound RF image data as the airway P_{tm} was incremented throughout the entire physiological range (i.e., -10 to 25 cmH₂O). Data was collected in increments of 0.5 cmH₂O, except in the range of P_{tm} where airways are most compliant (i.e. -5 to 10 cmH₂O), where data was collected in increments of 0.2 cmH₂O. The airway was held for approximate 5 s at each P_{tm} before data was captured. Two full cycles were recorded to verify measurement repeatability. Before data collection, the P_{tm} was slowly cycled between -10 and 25 cmH₂O at a constant rate of 1 cmH₂O to normalize for volume history. We repeated this process after the airway was constricted with an ASM agonist (ACh, 10⁻³ mol/L) in order to assess the changes that occur in displacement distributions of the airway walls due to ASM activation.

To induce airway narrowing akin to what occurs during an asthma attack, the airway was exposed to cumulative doses of ACh (10⁻⁵, 10⁻⁴, and 10⁻³ mol/L) and allowed to narrow against a constant P_{tm} of 5 cmH₂O for 10 min at each dose. During the constriction, ultrasound data was collected at a frame rate of 1.5 Hz in order to visualize narrowing dynamics.

During tidal breathing, P_{tm} fluctuate between approximately 5 cmH₂O at end-expiration and 10 cmH₂O at end-inspiration. During DIs, which people naturally take every 6 min [32], P_{tm} increases to approximately 25 cmH₂O.

2.3. Displacement Estimation

2.3.1 Edge detection and mesh generation

An automated segmentation algorithm developed in MATLAB (MathWorks, Natick, MA, USA) was used to determine the location of the airway walls, as previously described [7]. RF data was first converted to B-Mode [33] through FIR filtering, envelope detection, non-linear compression, and scan conversion. The airway walls were then segmented from the Krebs solution by thresholding the image [34], followed by morphological operations (e.g. smoothing) to form contiguous walls with smooth boundaries.

An unstructured, quadrilateral finite element mesh was then generated from approximately equally spaced (in real space) nodes on the identified airway boundary for both airway walls [35].

2.3.2 Image registration using finite elements

The recorded RF ultrasound data, $RF_t(t, n)$, is a function of echo transit time, t , and the line number, n . This data is scan converted to physical Cartesian coordinates, based on the speed of

172 sound, c , and the distance between RF lines, dy
 173

$$RF(x, y) = RF_t\left(\frac{t}{c}, n \cdot dy\right). \quad (1)$$

174

175 Spatial deformation distributions through the airway walls were estimated using a finite element
 176 image registration technique adapted from the method described by Richards *et al.* [26]. The
 177 deformation required to map the reference image of the tissue, I_l , and the image after a
 178 mechanical perturbation (i.e., a small change in Ptm) is assumed to result directly from
 179 underlying tissue motion, $u(X)$. For clarity, we define $X(x, y)$ as a two dimensional space
 180 position ($u(X) = u_x(x, y)\hat{i} + u_y(x, y)\hat{j}$):

$$I_1(\mathbf{X}) \approx I_2(\mathbf{X} + \mathbf{u}(\mathbf{X})). \quad (2)$$

181

182 To find $u(X)$, the displacement vector of the tissue originally at spatial location X , the following
 183 functional was minimized using the Gauss-Newton method

$$\Pi[u(X)] = \frac{1}{2} \int_{\Omega} [I_2(x + u(X)) - I_1(X)]^2 d\Omega + \frac{\alpha}{2} \int_{\Omega} (\nabla u - \varepsilon_t)^2 d\Omega. \quad (3)$$

184

185 The first term of the functional, $F_{mismatch}$, minimizes the difference between motion compensated
 186 image pairs over the spatial domain of interest. The second term, F_{reg} , is a H_1 -seminorm
 187 regularization term which penalizes large gradients in $u(X)$. The target strain in the axial
 188 direction, ε_t , is a constant used to correct for the expected gradient of $u(X)$. ε_t is estimated for
 189 each pair of images as the mean difference between the displacements of the outer and inner
 190 walls, divided by the mean wall thickness. The strength of regularization is determined by the
 191 parameter, α ; the selection of α is described below.

192

193 The function $u(X)$ was discretized using bi-linear finite element interpolation on quadrilateral
194 finite elements. Though $u(X)$ varies linearly over the element, the RF-image intensities may
195 oscillate significantly over an element. Therefore, rather than standard low-order Gauss
196 quadrature over each finite-element, the parent domain of each element was divided into sub-
197 domains. Integration over each sub-domain was performed using 3x3 point Gaussian quadrature.
198 RF images were evaluated at each Gauss integration point in the subdomains using cubic
199 Lagrange polynomial interpolation of the image intensity. The number of element sub-domains
200 was chosen to yield roughly one Gauss point per pixel and exact evaluation of the cubic
201 interpolated image polynomials.

202 2.3.3 *Initial Guess*

203 Since the Gauss-Newton method is an iterative method, an initial guess is required. When
204 matching RF images, we found the results significantly depended on the choice of the initial
205 guess. We found the following two-step procedure gives reliably repeatable results for an image
206 pair. For the first step, an initial guess for $u(X)$ was chosen as the spatial linear interpolation of
207 the mean displacements of the inner and outer edge detected walls. This displacement estimate is
208 then refined by registering the envelope detected image data (i.e., $I(X) = \text{abs}(\text{hilbert}(RF(X)))$,
209 where hilbert refers to the Hilbert Transform) according to equation (3). Matching the envelope
210 detected data first helps to avoid local minima when matching RF data. The output of this
211 process was then used as the initial guess for matching of $RF(X)$.

212 2.3.4 *Regularization Strength*

213 The correct value for α depends on the images, SNR, and the magnitude of the displacements.
214 We therefore used the L-curve method to determine the best value of α for our data [36,37].

Specifically, F_{mismatch} was plotted vs F_{reg} on a log-log plot. The optimal α was chosen at the corner of the resulting L-curve. This value of α represents a balance between the image matching error and regularization (i.e., smoothing) error [38]. The image matching component of the functional was calculated after each iteration to ensure the algorithm converged.

2.3.5 Successive Image Matching and Lagrangian Displacement Estimation

A large deformation can be computed by matching successive pairs of images in a long sequence of images. If in the process of matching these images in the sequence, one uses the same finite element mesh (i.e., one keeps the finite element nodes fixed in space), then the resulting displacement field represents an Eulerian description of the displacement field. We sought to measure the Lagrangian description of the displacement distribution, however, over the full range of P_{tm} . To do so, we incrementally matched images captured at increasing P_{tm} . For each displacement increment, the finite element nodes were moved from their original positions to updated positions determined by the estimated displacement. The next two images were then matched on the updated mesh. Thus the finite element mesh followed individual tissue regions throughout the pressure increments. We then calculated the total displacement of each node on the original mesh by simply summing the incremental displacements nodally to thus obtain the Lagrangian description of the total displacement field. In a similar manner, airway narrowing was visualized by matching successive frames after the airway was activated.

2.3.6 Sign Convention and Strain Calculation

Radial outward displacement is defined as positive, while radial inward displacement is defined as negative. In order to gain insight into the mechanical property distribution throughout the airway wall, strain fields in the radial (i.e., y) direction were calculated as $\partial u_r / \partial R$ using the *GradientOfUnstructuredDataSet* filter in ParaView [39]. Here, u_r is the displacement in the

radial direction and R is the radial distance from the axis of the airway. Circumferential strain was calculated as u_r/R . We defined compressive strain as negative and tensile strain as positive.

2.3.7 Quality Assurance

Two different metrics of quality assurance were applied to ensure the results were internally consistent. One is a metric that measures the quality of the image registration process which assures that the images are well matched. Having well matched images is necessary, but not sufficient, to having a good displacement field. Therefore, another test was performed to measure displacement accuracy and consistency.

To measure how well the images were matched, the quality of the image registration itself was quantified within each element. To do so, the quantity s was computed within each element, e , as the normalized L^2 norm of the difference in the motion compensated image pairs

$$s_e = \frac{\int_{\Omega_e} [I_2(x+u(x)) - I_1(x)]^2 d\Omega}{\sqrt{\int_{\Omega_e} (I_1(x))^2 d\Omega \int_{\Omega_e} (I_2(x+u(x)))^2 d\Omega}}. \quad (4)$$

A value of s_e of 0 corresponds to a perfect matching of the data within an element while values of greater than 1 correspond to poorly matching data. The mean value of s_e over all elements was confirmed to converge.

A second check is on self-consistency of the measured displacements. To test this, the displacements from matching data at all Ptm increments (i.e. increments of 0.2 cmH₂O) were compared to matching every other Ptm increment (i.e. increments of 0.4 cmH₂O) in the tidal

breathing range. This test assures that registering different images in a sequence gives the same displacement fields.

2.4. Comparison to homogeneous thick-walled cylinder approximation

A stress-strain relationship was estimated from the inflation Ptm -diameter data assuming a homogeneous, thick-walled and incompressible cylinder, and following the technique described in [40]. The Cauchy shear stress (τ) was calculated as

$$\tau = \frac{Ptm}{2 \ln(r_o/r_i)}, \quad (5)$$

where r_i and r_o are the inner and outer radii, respectively, at a given Ptm . The Cauchy shear strain (B) was defined as

$$B = \frac{1}{2 \ln(r_o/r_i)} \left(\frac{1}{2\lambda_z^2} \left(\frac{R_o^2}{r_o^2} \right) + \frac{1}{\lambda_z} \ln \left(\frac{r_i R_o}{r_o R_i} \right) \right), \quad (6)$$

where r_i and r_o are the inner and outer radii, respectively, at $Ptm = 0$, and R_i and R_o are the deformed inner and outer radii, respectively. The stretch ratio in the longitudinal direction, λ_z , for an isotropic, incompressible material, is given as $\lambda_z = (R_o^2 - R_i^2)/(r_o^2 - r_i^2)$. Finally, an effective shear modulus was defined as the ratio of Cauchy shear stress to shear strain

$$\mu_{\text{eff}} = \frac{\tau}{B} = Ptm \left[\frac{1}{2\lambda_z^2} \left(\frac{R_o^2}{r_o^2} \right) + \frac{1}{\lambda_z} \ln \left(\frac{r_i R_o}{r_o R_i} \right) \right]^{-1}. \quad (7)$$

After combining equations (6) and (7), we numerically solve for r_i and calculate the radial displacement throughout the thickness of the airway wall, u_r , as

$$u_r = r - R = \sqrt{r_i^2 + \frac{(R^2 - R_i^2)}{\lambda_z}} - R, \quad (8)$$

where $R_i \leq R \leq R_o$.

To help visualize how the displacement varied throughout the thickness of the walls, each wall was binned into equally spaced distance from the inner wall to the outer wall. The radial displacements in each layer were averaged and compared to the approximations of a homogeneous, thick-walled cylinder.

3. Results

3.1. Quasistatic inflation

Figure 2a shows the mean luminal diameter calculated using the detected edges along the length of the airway as a function of P_{tm} in both the relaxed (black) and constricted (gray) state. In the relaxed curve, the solid symbols are data from the first recorded cycle while the open symbols are from the second cycle. The solid lines show averaged and smoothed curves. The relaxed airway is most compliant between P_{tm} of -5 and 10 cmH₂O and then becomes very stiff. The constricted airway is much stiffer than the relaxed state between P_{tm} of -5 and 10 cmH₂O, but continues to dilate above a P_{tm} of 10 cmH₂O. Interestingly, the increased stiffness of the constricted airways prevents full closure even at negative P_{tm}. Fig. 2b shows the Cauchy shear stress as a function of the Cauchy-Green shear strain. The relaxed airway displays a nonlinear stress-strain relationship at high strains, while the constricted airway is stiffer and nearly linear over the range of strains studied. The airway is subjected to the same range of P_{tm} (i.e., -10 to 25 cmH₂O) in both the relaxed and constricted states, but the shear stress (i.e., the non-pressure component of the stress) is much higher in the more compliant, relaxed state than in the stiffer, constricted state.

299

300 Figure 3a-d depicts B-mode ultrasound data collected at four notable P_{tm} : a. 0 cmH₂O
301 (undeformed state), b. 5 cmH₂O (P_{tm} corresponding to functional residual capacity (FRC)), b. 10
302 cmH₂O (P_{tm} corresponding to end inspiration during tidal breathing), and d. 15 cmH₂O (at end
303 inspiration of exaggerated breathing, after the plateau of P_{tm} -diameter relationship). Videos of
304 B-mode images showing the inflation of the airway throughout the entire P_{tm} range in the
305 relaxed and constricted states are available as supplementary content. The inner and outer wall
306 edges are detected and overlaid. As the P_{tm} increases, the luminal diameter increases while the
307 wall thickness decreases since the tissue is nearly incompressible.

308

309 Figure 3e shows the initial mesh on the airway walls at $P_{tm} = 0$ cmH₂O. The mesh used
310 consisted of 250 quadrilateral finite elements in each wall. Each element contained between 50-
311 250 RF pixels. Figure 3f-h show the Lagrangian radial displacement of the airway due to an
312 increase in P_{tm} from 0 up to 5, 10, and 15 cmH₂O, respectively. Overlaid on each image are the
313 deformed meshes with each node moved by its estimated displacement, $u(X)$. The displacements
314 are longitudinally and radially heterogeneous, with the deformation greatest near the inner lumen
315 and decreasing throughout the thickness of the wall. The pattern of deformation is qualitatively
316 similar for the three magnitudes of P_{tm} .

317

318 Figure 4a-d show the radial strain maps as P_{tm} changes from 0 (Fig. 4a,e) up to 5 (Fig. 4b), 10
319 (Fig. 4c), and 15 (Fig. 4d). Interestingly, the middle layer of the airway wall appears to compress
320 significantly more than the regions closer to the inner and outer walls, implying a more compliant
321 and highly compressible material. The pattern of strains remains relatively constant at the three

different P_{tm} levels. In the bottom wall, areas of low strain are visible which might correspond to cartilage plates within the wall. Fig. 4e-h show the circumferential strain resulting from the same increases in P_{tm} from 0 cmH₂O. The circumferential strain is highest near the lumen and decreases toward the outside of the wall.

Figure 5 quantifies the radial distribution of displacements throughout the top (black) and bottom (gray) airway walls and compares the results to those predicted by the thick-walled cylinder approximation (equation (8)) for a cylinder with $\mu_{eff} = 0.8$ kPa. As expected, the radial displacement decreases with $1/r$ from the inner to outer wall.

3.2. Displacements and Strains During Breathing

In section 3.1 we calculated the Lagrangian displacements throughout the airway wall relative to the unstressed condition at $P_{tm} = 0$ cmH₂O. In vivo, the airways are prestressed and breathing occurs above FRC which is typically assumed to be at $P_{tm} = 5$ cmH₂O. We calculated the displacement and strains in the tidal breathing range from 5 to 10 cmH₂O (Fig. 6a,c) and due to an increase in P_{tm} from 5 to 25 cmH₂O simulating a DI (Fig. 6b,d). A video showing the cumulative displacement distributions with deformed meshes (green) overlaid as the P_{tm} is increased from 5 to 15 cmH₂O is available as supplement content.

Figure 7 shows the corresponding displacements and strains of the airway following constriction with ACh (10^{-3} mol/L). After narrowing, the displacements are much smaller than the relaxed airway and the pattern of displacements has changed, suggesting the airway did not stiffen homogeneously.

3.3. Airway narrowing

Figure 8a shows the mean luminal diameter of the airway while exposed to increasing doses of ACh. Figure 8b is a detailed view of the airway's luminal diameter in response to 10^{-5} mol/L ACh where displacements were tracked. The white circles show frames which were matched which were chosen when the displacement of the luminal walls had moved by more than a chosen threshold (> 2 pixels). The red open circles highlight five time points of interest shown. Figure 9 displays the B-mode (a-e), initial mesh (f), and displacements (g-j) at each of these points of interest. The narrowing is heterogeneous along the length of the airway. In time point II (b,g), the proximal portion begins to narrow. Shortly after, at time point III (c,h), the distal portion of the airway narrows. As the narrowing continues, the constriction proceeds (IV, d,i) as a wave from the distal to proximal side and by time point V, the entire length of the airway narrowed.

3.4. Regularization tuning

Figure 10 shows the L-curve comparing $F_{mismatch}$ and F_{reg} when the data at 0 cmH₂O was matched to 0.2 cmH₂O for values of α ranging over three decades. The optimal value of α was chosen as 5.6×10^7 . This value was found consistently to be the optimal value for matches at different Ptm and was used for all matches. The displacements calculated when using these values of α were also consistent with the optimal value determined by subjective visual inspection of the displacement maps. The regularization strength in the direction perpendicular to the ultrasound propagation (α_x) was set to be 100 times larger than the regularization strength in the direction of propagation (α_y) in order to suppress spurious noise in the lateral displacement estimates.

3.5. Quality Assurance

Figure 11 displays the progression of our method. In Fig. 11a, an initial guess based on the displacements of the detected edges is shown. The corresponding map of mean match quality, s_e

(equation (4)), is shown in Fig. 11c, which is averaged for each element over all the matches from P_{tm} 5 to 10 cmH₂O. The final estimate of displacements due to an increase in P_{tm} from 5 to 10 cmH₂O is shown in Fig. 11b, with the corresponding map of s_e shown in Fig. 11d.

Two tests were performed to ensure our measurements were repeatable. First, two full inflation-deflation loops were recorded for the relaxed airway and consistent results were obtained, as shown in Fig. 2.

Next, in the tidal breathing range of P_{tm} (5 to 10 cmH₂O), ultrasound images were collected in increments of 0.2 cmH₂O (25 images total, mean nodal displacement: 780 ± 377 μ m). Total displacements were calculated separately by matching all frames (i.e. 5.0, 5.2, 5.4, ..., 10.0 cmH₂O), matching just the odd frames (i.e., 5.0, 5.4, 5.8, ..., 10.0 cmH₂O), and matching just the even frames (i.e., 5.0, 5.2, 5.6, ..., 10.0 cmH₂O). The median absolute difference in total displacements calculated using only odd frames from those calculated using only even frames was 2.8 μ m (0.43%) with a range of 0.02 to 79.2 μ m (0.001% to 11.0%). Similar results were found comparing the total displacements from all frames to only odd frames (median: 3.8 μ m (0.49%)) and all frames to only even frames (median: 5.3 μ m (0.77%)). The largest differences tended to be in the regions of lowest signal intensity.

4. Discussion

In this study, we optimized a finite element image registration technique to estimate tissue deformation within an airway wall. By incrementally matching ultrasound data collected over the physiological range of P_{tm}, the spatial distribution of displacements during normal tidal

breathing and during a DI were estimated. The displacements calculated were heterogeneous, both longitudinally and radially through the thickness of the wall. Further, deformations during active airway narrowing were tracked which showed a large amount of temporal longitudinal heterogeneous deformation.

This study aimed to develop a robust approach for investigating of the complex way changes in P_{tm} imposed to a heterogeneous wall structure translate into strain of the individual components of the airway wall. Subsequent studies will examine the consistency of how airway wall constituents strain locally before and after induced narrowing for similar sized airways. These displacement distributions can then be used as an input to an inverse problem in order to estimate the spatial distribution of material parameters throughout an airway wall.

Of particular interest is the strain experienced by the ASM layer embedded within the wall, since stretching of an isolated strip of ASM causes a dramatic decrease in the amount of force it can generate. For our airway, we found that the region of the wall closest to the inner lumen displaced the most; the displacement magnitude gradually decreased toward the outer wall. This is qualitatively consistent with the prediction of a homogeneous thick walled cylinder (Fig. 5). We found that as P_{tm} increased, different regions and components of the airway wall (i.e., epithelium, ASM, cartilage plates) experienced different strains. Specifically, we found that a region in the middle layer experienced greater radial strain during inflation than the regions near the inner and outer walls, implying that part of the airway was more compliant than others. The ASM layer is located very close to the inner lumen, directly behind the basement membrane, so is likely not contained in this compliant region of the wall. After induced narrowing (ACh, 10^{-3}

mol/L) the displacements and strains decreased non-uniformly, suggesting the airway stiffened heterogeneously.

We also observed and quantified the temporal longitudinal heterogeneity of the airway wall during induced narrowing (Figure 9). This behavior could be explained by variation in the amount of ASM along the length of the airway. Alternatively, the ASM might be equally distributed along the length but the mechanical properties and relative amounts of other airway components could vary along the length and account for this observation. It is also possible that the ACh is arriving at the ASM at different times along the length, although this is unlikely given the way the agonist is added to the bath.

As with any image registration technique, there are several sources of error which limit the accuracy of our displacement estimates. First, we assume in Equation (2) that the deformation required to map an undeformed image to the deformed image results from in plane tissue motion alone. Out of plane tissue motion and acoustic artifacts such as reverberations could violate this assumption. The steps taken to minimize these effects include proper orientation of the transducer such that most of the movement occurred in the plane, and by collecting data over many small increments of Ptm. The quality assurance results in section 3.5 demonstrate that these steps were adequate.

Next, the highly oscillatory nature of the RF ultrasound data makes finding local minima likely. To help avoid this, we used the movements of the inner and outer wall to aid in the initial guess. We then used the displacements estimated by matching the envelope-detected image data as an

initial guess for matching the RF data. Low values of s_e for all elements in the domain confirmed that our solutions avoided local minima.

The accuracy of our results depends on both the noise inherent in the imaging and measurement system, and error introduced by the image registration algorithm. Specifically, the ultrasound system is limited by its resolution and the digitization of the RF data to 16 bit integers. A high resolution transducer was utilized and ultrasound imaging parameters such as line density (256 lines) and foci location (two, one located near each wall) were optimized. We orientated the transducer such that the majority of displacement was in the direction of ultrasound propagation since the axial displacement resolution of ultrasound is significantly better than the lateral resolution. Another source of noise is environmental vibrations which we minimized by collecting data on a floating optical table.

The finite element based image registration technique utilized here is advantageous in that it allows for arbitrary mesh geometries and distortion of elements. The regularization term was necessary to make the optimization problem more well-posed and to help ensure more meaningful displacement estimates. H^1 semi-norm regularization typically forces boundary displacement toward a zero strain condition, resulting in errors near the boundaries [41]. We mitigated this error by modifying the regularization term in Equation (3) to include an estimate of the target strain, ε_t .

A unique method we implemented in this study was calculating the Lagrangian displacements over a large range of P_{tm} by matching the ultrasound data over many small P_{tm} increments and

moving the finite element nodes by the estimated displacements of the previous match. Calculations of these large displacements over many small increments significantly increased the SNR of results and may allow for the estimation of nonlinear mechanical properties in the future.

We ensured the quality of our displacement estimates using two metrics. First, we verified that, given the calculated displacement field, the RF data within each element was well-matched between the undeformed and deformed images (see Fig. 11). In addition, we calculated nearly identical results when matching only odd frames compared to matching only even frames over a wide range of P_{tm} (i.e., 5 to 10 cmH₂O). Comparing odd to even frames is a particularly challenging test because almost no data is shared between the two calculations. In particular, only the end frames (i.e., $P_{tm} = 5.0$ and 10.0 cmH₂O), out of the total of 26 frames are common between the two datasets.

While this study advances a powerful technique to measure the displacements throughout an airway wall, there are limitations that should be noted. First, the airway was slowly inflated in small increments while capturing the ultrasound data. In vivo, however, tidal breathing and DIs occur at a faster rate (i.e., 0.2-0.3 Hz). This different loading rate might result in different deformations due to the tissue's viscoelastic and active properties. The frame rate of ultrasound is sufficiently high to quantify displacements of an airway during physiological strain rates in a future study.

In order to control for the viscoelastic effects of the tissue, ultrasound images were captured at fixed intervals of 5 s during the inflation of the airway. However, the airway is an active, living

tissue. The discontinuities observed in Fig. 2 during the inflation limb of the P_{tm} -diameter curve are potentially attributable to the intrinsic tone of the ASM. This region of the curve potentially corresponds to the peak of the ASM's force-length relationship [42].

We oriented the ultrasound transducer parallel to the long axis such that the majority of the displacement during inflation and narrowing was in the direction of ultrasound propagation. Over the large range of P_{tm} examined, however, some out of plane tissue motion occurred. The elevation focus of our transducer (i.e., in the out of plane direction) is fixed at a depth of 6 mm. We found that positioning the top edge of the airway at a depth slightly below this focus resulted in the best results by capturing a slightly thicker slice without compromising lateral resolution. To further address this limitation, this method can be extended naturally to three dimensions in order to track out of plane motion while also enforcing tissue incompressibility [26].

Finally, while the stiffness of the material can be inferred from strain maps (i.e., Fig. 4), solving the inverse problem with proper boundary conditions and constitutive model is thought to yield more meaningful and accurate results [12]. In a future study, we will formulate and solve this inverse problem in order to reconstruct the distribution of material parameters throughout the airway wall.

5. Conclusions

In this study, we successfully optimized a finite element image registration method to quantify the deformation of airway walls. We found the deformation to be heterogeneous, with the medial layer undergoing significantly higher strains than neighboring layers. The ASM layer, which is

506 close to the lumen, strains less than other components of the airway wall. We found that
507 narrowing of the airway wall with an ASM agonist leads to heterogeneous stiffening of the wall
508 components. More broadly, this study demonstrates that spatial heterogeneity in airway
509 deformation means that different tissue types in the airway experience different mechanical
510 environments.

511

512 **Acknowledgements**

513 This project was funded by the National Institutes of Health Grants No. R01-CA-140271 and

514 R01-HL-096797 as well as the National Science Foundation Grant No. 1148124.

Figure Legends

Figure 1: Intact airway mounted in the tissue bath with high frequency ultrasound transducer mounted above.

Figure 2: (a) Quasistatic P_{tm}-diameter relationship of an intact airway segment. P_{tm} is slowly increased from -10 to 25 cmH₂O and back to -10 cmH₂O twice. Closed circles represent the first recorded loop, open circles the second loop, Gray circles depict the P_{tm}-diameter relationship after induced narrowing with 10⁻³ mol/L ACh. Red lines show binned average of the curves (b) Cauchy shear stress-strain relationship computed from the data in (a) for the relaxed (black) and constricted (gray) airway.

Figure 3: Top: B-mode image of an isolated airway segment at 0 (a), 5 (b), 10 (c), and 15 (d) cmH₂O. Middle: Estimated Lagrangian radial displacement of the airway due to an increase in P_{tm} from 0 up to 5 (f), 10 (g), and 15 (h) cmH₂O with deformed meshes (green) overlaid. Panel (e) depicts the initial finite element mesh at 0 cmH₂O.

Figure 4: Radial (top) and circumferential (bottom) strain fields for the undeformed airway (a, e) and due to an increase in P_{tm} from 0 to 5 (b, f), 10 (c, g), 15 (d, l) cmH₂O.

Figure 5: Binned and averaged radial distribution of displacements throughout the top (black) and bottom (gray) airway walls compared to distribution predicted by a homogeneous thick-walled cylinder with the same μ_{eff} (red).

Figure 6: Radial displacement fields of a relaxed airway during tidal breathing (i.e., increase in P_{tm} from 5 to 10 cmH₂O) (a) and during a DI (i.e., increase in P_{tm} from 5 to 25 cmH₂O) (b). Corresponding radial strain maps are shown in (c) and (d), respectively.

Figure 7: Radial displacement fields of an airway which has been previously constricted with ACh (10⁻³ mol/L). Displacement distributions resulting from an increase in P_{tm} corresponding to

tidal breathing (i.e., 5 to 10 cmH₂O) (a) and due to an increase in P_{tm} simulating a DI (i.e., 5 to 25 cmH₂O) (b). Corresponding radial strain maps are shown in (c) and (d), respectively.

Figure 8: (a) Mean luminal diameter of an airway after exposed to three increasing doses of ACh. The gray section in (a) is expanded in (b). Frames that were used for matching are indicated by white circles and red open circles indicate time points of interest shown in Fig. 9.

Figure 9: B-mode (a-e), initial mesh (f), and displacements (g-j) at each of the points of interest from Fig. 8. The narrowing is heterogeneous along the length of the airway as the constriction proceeds.

Figure 10: L-curve analysis used to choose the optimal regularization strength, α . The value of the mismatch term of the function $F_{mismatch}$ is compared to the regularization term F_{reg} a log-log scale for a wide range of α . The optimal α (red) corresponds to the corner of the resulting curve. Note the reverse direction of the x-axis.

Figure 11: Progression of image registration process of airway inflated from a P_{tm} of 5 to 10 cmH₂O. The initial guess based on the detected edges (a) results in a very poor image match (i.e., high s_e) (c). The final displacement estimate field (b) matches the images very well (i.e., low s_e) (d).

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Figure1

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Figure 9

(a) $t = 0.88 \text{ min}$ (b) $t = 1.12 \text{ min}$ (c) $t = 1.31 \text{ min}$ (d) $t = 2.12 \text{ min}$ (e) $t = 2.99 \text{ min}$

(f) $t = 0.88 \text{ min}$ (g) $t = 1.12 \text{ min}$ (h) $t = 1.31 \text{ min}$ (i) $t = 2.12 \text{ min}$ (j) $t = 2.99 \text{ min}$

Figure 10

Figure11