Multiscale imaging and registration-driven model for pulmonary acinar mechanics in the mouse

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Abstract

Three-dimensional (3D) visualization and characterization of the 3D acinar structure and to study alveolar mechanics using image registration. Instead of relying only on in vivo imaging modalities, the approach involves imaging the mouse lung at varying resolutions. The objectives of this work are threefold. A realistic 3D representation of the acinus is first obtained in a mouse lung sample fixed at an inflation pressure of 20 cmH2O. The second objective of this paper is to obtain Microcomputed tomography (μCT) scans were obtained at multiple resolutions. Substructural morphometric analysis of a complete acinus was performed by computing a surface-to-volume (S/V) ratio directly from the 3D reconstruction of the acinar geometry. A geometric similarity is observed to exist in the acinus where S/V is not significantly altered. The developed method forms a useful tool in registration-driven fluid and solid mechanics studies as displacement of the alveolar wall becomes available in a discrete sense. Microcomputed tomography; high resolution; mouse lung; acinus; image registration

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lungs were extracted en bloc with the heart and dried while maintaining the same constant tracheal pressure. This fixation method preserves the lung structure in a near anatomical state, where airways, alveoli, blood vessels, and capillaries remain recruited (35).

For X-ray-based imaging of biological samples it is important to have a high contrast between the tissue and air spaces in lung samples. Therefore the fixed lungs require drying. This induces some minimal shrinkage that can be determined using whole lung segmentation. Our perfusate [modified Heitzman solution (11)] in conjunction with pressurized drying allows a preservation that shows limited shrinkage over very long periods of time. The lung sample used for this study showed a general shrinkage of 3% in the first 21 days postfixation and 10% after 109 days at the time point at which the acinar high-resolution scan was performed.

Low- and high-resolution scanning. Very high resolutions are required to image the alveolar structures of an acinus. A multiresolution scanner that uses different optical magnifications (MicroXCT 400, XRadia, Concorde, CA) was used in this study. The fixed lung is nondestructively imaged in two steps. First, an initial whole lung scan was acquired with a low magnification, which was used to determine the coordinates of an acinus to be imaged. The lowest magnification was used for the whole lung scan with a resolution of 12–28 μm/voxel depending on the overall lung size. At this resolution it is possible to clearly identify terminal bronchioles of the mouse lung and therefore it is possible to determine precise locations of individual acini. The 10× magnification provided both the necessary field of view (FOV) of 2 × 2 × 2 mm as well as the required resolution of 2 μm/voxel to allow visualization of the septal walls. The unique capability of this scanner to zoom in at specific locations within an object allowed us to preserve the spatial information of an acinus with the potential to correlate it back to the in vivo scan (33, 34).

Acinar segmentation. A novel framework of multiscale topomorphologic opening approach allowed us to segment the acinus in high-resolution images. Some initial manual seeding allowed the algorithm to decide which air spaces needed to be separated from each other (28, 33). Segmentation and separation of a specific acinus in the high-resolution images presented several challenges for simple image processing tools due to noise, image artifacts, and small wall inconsistencies. A cleanup of the mask is therefore necessary before converting it to a surface mesh. The mask from the high-resolution fixed lung scan is processed using an in-house developed software package [Pulmonary Analysis Software Suite, University of Iowa (10)]. Once the acinar segmentation is obtained, it is converted into a surface mesh utilizing a marching cubes algorithm (18), and further smoothing of the mesh is performed using a without-shrinkage smoothing technique (30). The final surface mesh is a triangulated acinar surface with 7.5 million surface elements and 3.75 million nodes required to capture the highly resolved features of the acinus.

Deforming acinar model registration. To build the deforming acinus model, we adopt an image registration technique. The flow chart for the entire registration framework is shown in Fig. 1. The process involves high-resolution and low-resolution scans of the lung at different lengths scales (i.e., multiresolution), resulting in a multiscale approach. As shown in Fig. 1, the procedure uses three different sets of images: low-resolution in vivo scans of the whole lung at 20 and 25 cmH2O, low-resolution ex vivo scan of the whole lung fixed at 20 cmH2O, and high-resolution ex vivo scan of the acinus. Note that only the acinus is scanned ex vivo at a high magnification. The whole lung scan is performed both in vivo and ex vivo, and a simple translation registers the acinus into the ex vivo whole lung domain.

A pair of volumetric in vivo images acquired at 20 and 25 cmH2O is matched with a mass preserving image registration algorithm. This registration method estimates local tissue fraction within the lung and minimizes the local tissue volume difference. A composite of six levels of B-splines was adopted to describe lung motion, and a sufficient condition was imposed to ensure a one-to-one mapping even for a registration pair with large volume difference. For complete details, please refer to Yin et al. (40, 41). The matching between the two in vivo scans provided the deformation. To apply such deformation into the acinus, we mapped the acinus into the in vivo image domain, which is achieved by the following two steps. First, a rigid mapping (translation and rotation) between the high-resolution ex vivo image and the low resolution ex vivo image is applied to transform the acinus into the low-resolution ex vivo image domain. The simple translation is sufficient to yield a perfect matching. Second, a registration is performed between the low-resolution ex vivo image and the 20 cmH2O in vivo image with mutual information as the similarity measure to account for intensity changes. A combination of rigid, affine, and four levels of B-splines with small node spacing were used in this step. The derived transformation could then be used to map the acinus from the ex vivo domain into the in vivo domain. Although the size of the apical lobe under investigation shrinks by as much as 14.7% in volume between in vivo and ex vivo lungs, the volume difference between in vivo and ex vivo lung after applying image registration is reduced to 4.8%.

RESULTS

Figure 2 shows the relative location of the acinus in the field of view (shown as a circle) of a slice in the apical lobe where the acinus is located. It also shows the terminal bronchiole (Fig. 2, marked airway, A) from which the acinus branched out as can be noticed from the entrance of the acinus marked “Entrance.” The acinus is located adjacent to a blood vessel marked “C.” The region outside lung is marked by “B.”

The acinus is a complex geometrical structure. The particular acinus studied here has 82 branch points. Such a complex structure makes further analysis challenging. To facilitate analysis, the acinus is split into substructures. The components are

![Fig. 1. Methodology flowchart describing the procedure to obtain registration driven wall motion for morphometry.](http://jap.physiology.org/doi/pdf/10.1152/japplphysiol.01136.2012)
shown in Figs. 3–5. The components are subtrees (S) of the acinus and are chosen such that they have increasing number of branch points: namely, S3 has 3, S7 has 7, and S10 has 10 branch points. Structure S3 spans 3 generations. The structures S7 and S10 span roughly last 5 generations. A segmented slice of the acinus is shown in Fig. 3. One peripheral alveolus, septal edges, boundary of the subtree S10, and the airway (AW) from which this particular acinus has branched out are indicated.

Figure 4 shows the reconstructed shapes of the entire acinus and its substructures from the ex vivo lung at 20 cmH2O. Individual alveoli are segmented out to document their shape and morphometry separately. Three different alveoli are randomly chosen for analysis. The alveoli are chosen from varying locations: one located peripherally as shown in Fig. 3, while the other two are more proximally located within the acinus. Figure 5 shows a virtual endoscopic view (view A-A) of component S3 that has only 3 branch points. The carinae at the first and second branch points are clearly visible. The alveoli in Fig. 4D have a mouth area of $\sim 0.0023 \text{ mm}^2$ and a corresponding hydraulic diameter $2\sqrt{\text{Area}/\pi}$ of $\sim 54 \mu\text{m}$. The hydraulic diameter at the entrance to the whole acinus marked “E” is roughly $160 \mu\text{m}$.

Table 1 reports measurements of the acinus at 20 cmH2O. The ex vivo acinus has a surface area of 6.9 mm$^2$ and occupies...
a volume of 0.145 mm³. Three alveolar spaces were separately segmented (manually) as shown in Fig. 4D. A mean alveolar volume of 0.148 nl was observed. Parameswaran et al. (24) measured a mean alveolar airspace volume of 0.12 nl (measured by averaging 65 or more alveoli identified manually) in healthy mice that falls in the range of our measured alveolar volume. Vasilescu et al. (34) determined in a recent study using stereological principles applied to μCT images that the average alveolar volume in the C57Bl/6J mice is 0.25 nl. Note that the smallest alveolus in the present study measured was ~0.11 nl in volume at the fixation pressure of 20 cmH₂O.

The parameter of interest chosen in this study to compare the shape and size of an acinus at 20 and 25 cmH₂O is the S/V. The alveoli measured in this study have an average S/V of 78 mm⁻¹. Osmanagic et al. (23) presented S/V measurements (of single alveoli) in a mouse lung and compared their results with

Fig. 4. Acinus and its components and branches. Shapes shown correspond to 20 cmH₂O ex vivo scan. A: acinus shown in Fig. 2 with substructures shown in color. B, Structure S10; C, structure S7; D, 3 alveolar spaces.

Fig. 5. Virtual endoscopy of S3 showing the carinae at first branch point (C0) and at 2 daughter branches (C1). C0 and C1 are also marked in the figure. “0” is used to indicate the carinae (C) for parent generation while index “1” is used for the daughter generation.
previous microscopic studies. They noted that the overall range of S/V observed by various researchers typically falls in a wide range of 50–100 mm\(^{-1}\) for this strain of mice and is in agreement with our observation.

The second step in our multi-scale approach (Fig. 1) is the mapping of the high-resolution acinus to the in vivo scans of the apical lobe. The result of this transformation is shown in Fig. 6. Figure 6A shows the various subcomponents S3, S7, and S10 and its relative location in the acinus. Jacobian (26, 27) is defined as the ratio of the voxel volume after transformation at 25 cmH\(_2\)O over the volume of the corresponding voxel at 20 cmH\(_2\)O. If Jacobian equals unity, there is no local volume change. If Jacobian is greater (or less) than unity, local volume undergoes expansion (or contraction). Figure 6B plots the Jacobian map at one of the intermediate slices. It clearly shows the nonuniform deformation within the lobe. Note that the relative size of the acinus in relation to the size of the lobe validates the multiscale modeling strategy. The Jacobian values (average in the whole subregion) are consistent as the values listed in the Table 2. The Jacobian values reflect the local volume changes. A part of the acinus is located on the boundary of a high Jacobian-value region.

Once the transformation (rigid and nonrigid) is performed, the displacement on every point on the wall of the acinus becomes available. The result is the predicted shape of the in vivo acinus at 25 cmH\(_2\)O. It is our objective to understand the area and volume changes due to lung inflation in the acinus. The surface area (S), volume (V), and S/V (expressed in mm\(^{-1}\)) for the in vivo acinus at 20 and 25 cmH\(_2\)O are reported in Table 1 and Table 2, respectively. In Table 1, there are two columns showing the acinar surface area and volume at 20 cmH\(_2\)O. Both ex vivo and in vivo states are presented. Due to the reasons mentioned in MATERIALS AND METHODS, the surface area and volume of the acinus and its constituent subcomponents under consideration are also smaller in the ex vivo lung, but by a smaller percentage. For example, the acinus suffers a ~6% reduction in surface area and ~6% reduction in volume. S/V values from the estimated surface area and volume are also reported in the last two columns of Table 1 for ex vivo and in vivo acinus respectively. Table 2 presents the surface area, volume, percentage increase in surface area and volume between 20 and 25 cmH\(_2\)O. The volume change for the substructures is not uniform. For example, the volume of S10 increases by 10% accompanied by a 9.5% increase in surface area while for S7, the percentages are smaller. This nonuniform behavior of the alveolar volume and surface area change in the different subcomponents of the acinus could be related back to the distribution of Jacobian map as the acinus spans a region of the displacement map with varying Jacobian values. The last column in Table 2 presents the S/V for in vivo 25 cmH\(_2\)O geometry. During inflation, if both surface area and volume show an increase (although by different amounts for various components) S/V would change proportionally. As seen from Table 2, S/V changes marginally from 20 to 25 cmH\(_2\)O. The acinus increases 6.2% by volume and 5.4% in area, but the S/V remains almost same. The S/V of alveolus decreases due to expansion. For example, the S/V of alveolus 3 at 20 cmH\(_2\)O is 82.6 mm\(^{-1}\) and at 25 cmH\(_2\)O it is 80.3 mm\(^{-1}\). This can be compared with an expansion of a hemispherical surface. In a spherical approximation of alveoli, S/V is inversely proportional to radius (exactly equal to \(3/R\), R being the radius). Hence, a decrease in S/V with higher inflation pressure is understandable. In this analysis and those that follow in the

<table>
<thead>
<tr>
<th>Structure</th>
<th>S(_{20}), In Vivo Surface Area, mm(^2)</th>
<th>V(_{20}), In Vivo Volume, mm(^3)</th>
<th>S(<em>{20}/V</em>{20}), mm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolus-1</td>
<td>0.0120</td>
<td>1.45 × 10(^{-4})</td>
<td>82.76</td>
</tr>
<tr>
<td>Alveolus-2</td>
<td>0.0142</td>
<td>1.95 × 10(^{-4})</td>
<td>72.82</td>
</tr>
<tr>
<td>Alveolus-3</td>
<td>0.0082</td>
<td>1.04 × 10(^{-4})</td>
<td>78.85</td>
</tr>
<tr>
<td>S3</td>
<td>0.2979</td>
<td>0.00624</td>
<td>47.74</td>
</tr>
<tr>
<td>S7</td>
<td>0.5608</td>
<td>0.01201</td>
<td>46.69</td>
</tr>
<tr>
<td>S10</td>
<td>0.7780</td>
<td>0.01654</td>
<td>47.04</td>
</tr>
<tr>
<td>Acinus</td>
<td>6.9154</td>
<td>0.14553</td>
<td>47.52</td>
</tr>
</tbody>
</table>

Fig. 6. A: deformed whole acinar geometry (gray) and substructures (pink: S3; purple: S7; brown: S10); B: Jacobian map of the apical lobe viewed from the base to apex. Acinus is overlaid on the lobe. A part of the acinus is located on the boundary of a high Jacobian-value region.
DISCUSSION, trends in $S/V$ are used to point toward the nature of acinar expansion.

**DISCUSSION**

Understanding $S/V$ ratio. The motivation behind the computation of $S/V$ is to gain insight into the nature of alveolar expansion with inflation pressure. There are many works in literature that compute alveolar surface area and volume from serial sectioning, light, and electron microscopy (1, 9, 13, 20, 21, 31). For example, Bachofen et al. (1) showed that the alveolar surface area increases by as much 80% as volume increases from 40 to 80% total lung capacity (TLC) in rabbit lungs. Knudsen et al. (13) measured an intercept chord length in perfusion fixed rabbit lungs by means of stereology. They found that the mean linear intercept, changed from 64 to 105 μm between 40 and 80% TLC. They argued that much of the volume change could occur only in the ducts. $S/V$ in the present work is measured from the 3D reconstructions, which rely on the multiresolution imaging and image registration.

Various inferences can be drawn from Tables 1 and 2. The acinus and its substructure within its volume were observed to have a similar $S/V$ irrespective of the number of acinar generations within the structure. This behavior may arise due to the presence of an inherent geometric similarity. It is also reflective of the space-filling character of the pulmonary acinus because for a given volume unit the net surface area would be a result of how alveolar surfaces are spatially distributed for efficient gas exchange. For example, S10 is only 1/9th the volume of the acinus but has almost the same $S/V$ as the acinus. Similarly, S7 has almost twice the surface area of S3, yet $S/V$ decreased only by ~4%. The data in Table 1 further show that the $S/V$ of an alveolus is much larger than components with branches. Since the ductal or airway lumen is formed by the surrounding alveoli, there is less nonalveolated portion of the duct. Hence, $S/V$ of the acinus, S10, S7, and S3 is much smaller than that of an alveolus. Finally, it is observed that the $S/V$ seems to remain approximately the same, although the geometry undergoes an increase in area and volume from 20 to 25 cmH$_2$O.

This behavior of $S/V$ raises two questions. What can be inferred about a geometrical structure which expands volumetrically resulting in negligible change in $S/V$ although the increase in volume is as much as 10%? How does the alveolar duct expand compared with the alveoli around it? Consider the expansion of a hemispherical cavity attached to a cylindrical duct as a simple alternative model. Three possible modes of expansion may occur. First, when the volumetric expansion is only due to alveolar duct with increase in the radius of the cylinder, the $S/V$ of alveolus remains same and the net $S/V$ reduces. As an example, consider an alveolar duct with radius 50 μm, length 150 μm, attached to a hemispherical cavity of radius 60 μm. For a 10% increase in volume (uniform radial expansion) of the duct, it can be verified that $S/V$ decreases by ~4%. In a second scenario, when the entire volumetric increase (of 10% assumed) is due only to an isotropic expansion of hemispherical cavity while alveolar duct remains rigid, $S/V$ reduces by ~9%. The third scenario is when both hemispherical cavity and the duct expand during inflation. In all three scenarios, a nonnegligible decrease in $S/V$ is observed due to increase in volume of the acinus. From this simplistic model, some idea may be derived on how the acinus may deform in vivo. The last columns of Tables 1 and 2 give values of $S/V$ at 20 and 25 cmH$_2$O, respectively. A general trend is that $S/V$ shows a negligible increase or decrease (by as much as 1%) at 25 cmH$_2$O compared with 20 cmH$_2$O. This is not consistent with the simplistic model above. From the discussion above and the results in Table 2, it may be speculated that the acinus does not deform isotropically in this pressure range. It expands unlike how a hemisphere (for alveoli)-cylinder (for alveolar duct) representation would uniformly expand. This is understandable in the range of 20 to 25 cmH$_2$O given that lungs are stiffer at roughly 60% TLC and tidal breathing in mice occurs at 2–10 cmH$_2$O. A further deduction requires a much more detailed analysis and is beyond the scope of this paper. However, knowledge on how alveolar duct and alveoli expand is useful for example in acinar fluid dynamic simulations.

**Fixation and shrinkage.** One limitation of the current framework is the shrinkage between the in vivo and ex vivo lungs due to issues related to the proximity of the high-resolution scanning facility. As discussed in the MATERIALS AND METHODS, the percentage shrinkage is still within acceptable limits compared with earlier works in literature and provides a time stable fixation method. In addition, a mass-preserving image registration can further reduce the volume difference between in vivo and ex vivo lung.

**Use in tissue mechanical and fluid dynamical studies.** Imaging the 3D acinar structure is very useful for tissue mechanics. Tissue mechanical studies on alveolar wall (6, 8) is difficult due to modeling and boundary condition limitations. Availability of wall deformation will help compute heterogeneity in tissue mechanical properties. For example, alveolus and acinar bulk modulus or compliance could be estimated. Heterogeneity in compliance is related to heterogeneity in alveolar ventilation. For our acinus, compliance ($\Delta V/\Delta P$) is estimated as $\sim 0.0019$ mm$^3$/cmH$_2$O. For one alveolus, compliance is estimated as $\sim 2.3 \times 10^{-6}$ mm$^3$/cmH$_2$O. Assuming the

### Table 2. Morphometry of acinus and its components at 25 cmH$_2$O using registration between 20 and 25 cmH$_2$O scan using methodology in Fig. 1

<table>
<thead>
<tr>
<th>Component</th>
<th>$S_{25}$, Area, mm$^2$</th>
<th>% Area Increase = 100$(S_{25}-S_{20})/S_{20}$</th>
<th>Volume, $V_{25}$, mm$^3$</th>
<th>%Volume Increase 100$V_{25}/V_{20}-1$</th>
<th>$S_{25}/V_{25}$ Ratio, mm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alveolus-1</td>
<td>0.0132</td>
<td>6.45%</td>
<td>$1.62 \times 10^{-4}$</td>
<td>8.00%</td>
<td>81.48</td>
</tr>
<tr>
<td>Alveolus-2</td>
<td>0.0164</td>
<td>3.80%</td>
<td>$2.28 \times 10^{-4}$</td>
<td>5.81%</td>
<td>72.09</td>
</tr>
<tr>
<td>Alveolus-3</td>
<td>0.0096</td>
<td>7.60%</td>
<td>$1.20 \times 10^{-4}$</td>
<td>10.10%</td>
<td>80.33</td>
</tr>
<tr>
<td>S3</td>
<td>0.3438</td>
<td>7.14%</td>
<td>0.00697</td>
<td>8.02%</td>
<td>49.35</td>
</tr>
<tr>
<td>S7</td>
<td>0.6654</td>
<td>9.82%</td>
<td>0.01405</td>
<td>8.66%</td>
<td>47.36</td>
</tr>
<tr>
<td>S10</td>
<td>0.8759</td>
<td>9.49%</td>
<td>0.01845</td>
<td>10.87%</td>
<td>47.47</td>
</tr>
<tr>
<td>Acinus</td>
<td>7.7524</td>
<td>5.40%</td>
<td>0.16421</td>
<td>6.20%</td>
<td>47.21</td>
</tr>
</tbody>
</table>
acinus has about 500 alveoli, the acinar compliance is ~0.0012 mm³/cmH₂O of same order as the actual acinar compliance. Not surprisingly, the %volume increase in Table 2 shows a similar trend.

Another potential use of the acinar expansion information is in fluid dynamical studies (CFD) studies. Two important alveolar flow modeling aspects are the choice of a geometric representation of the acinus and the details of alveolar wall and septal movement. Conventional geometric representations of alveolar ducts and alveoli in CFD calculations are the torus (12), spherical cavity (29), azimuthally divided cylinder (5), or honeycomb-like structure (14, 15). To date, the studies focusing on acinar transport have used simple sinusoidal isotropic expansion (12) or a rigid wall assumption. Darquenne et al. (5) demonstrated a significant difference in deposition in the presence and absence of isotropic wall motion. For 1-μm particles, as much as a 150% or higher difference in relative deposition was observed. More recently, Chhabra et al. (3, 4) performed particle image velocimetry measurements using a balloon model in which the alveolar duct walls were rigid and the alveolus expanded uniformly. The resulting flow structure was shown to be significantly different from previous findings. Given such marked differences and varying assumptions on acinar expansion, novel methods that could provide improved understanding of the mechanics at the microstructural level will be useful.

Limitations and room for improvement. The use of registration to compute the deformation and its fidelity relies on the resolution at which displacement information is available and on the process of transfer of deformation from the lobe to the acinus. An alveolus has a typical characteristic dimension of 40–50 μm. The resolution of the large field of view scan is 14 μm. Although the current method captures the gross deformation of one alveolus, specific details such as expansion of the alveolar mouth and interalveolar septa cannot be accurately computed from image registration due to this limitation. An alveolar mouth and interalveolar septa cannot be accurately determined using scans at more than two pressures and hence provide more accurate reproduction of acinar deformation. The authors would like to thank Mr. Abhilash Kizhakke Puliyakote for his assistance in processing some of the image data, and acknowledge Dr. Leizlei Yin, from the Beckman Institute, Urbana/Champaign at Illinois, where the high resolution images were acquired. We also thank Dr. Weibel for some of his valuable comments after reading the paper.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: H.K., D.M.V., Y.Y., and C.-L.L. conception and design of research; H.K. analyzed data; H.K., D.M.V., and C.-L.L. interpreted results of experiments; H.K., D.M.V., and Y.Y. performed experiments; D.M.V., Y.Y., E.A.H., M.H.T., and C.-L.L. edited and revised manuscript; E.A.H., M.H.T., and C.-L.L. approved final version of manuscript.

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