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Comparison of CT-derived ventilation maps with deposition patterns of inhaled microspheres in rats

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Abstract

Purpose—Computer models for inhalation toxicology and drug-aerosol delivery studies rely on ventilation pattern inputs for predictions of particle deposition and vapor uptake. However, changes in lung mechanics due to disease can impact airflow dynamics and model results. It has been demonstrated that non-invasive, *in vivo*, 4DCT imaging (3D imaging at multiple time points in the breathing cycle) can be used to map heterogeneities in ventilation patterns under healthy and disease conditions. The purpose of this study was to validate ventilation patterns measured from CT imaging by exposing the same rats to an aerosol of fluorescent microspheres (FMS) and examining particle deposition patterns using cryomicrotome imaging.

Materials and Methods—Six male Sprague-Dawley rats were intratracheally instilled with elastase to a single lobe to induce a heterogeneous disease. After four weeks, rats were imaged over the breathing cycle by CT then immediately exposed to an aerosol of ~1µm FMS for ~5 minutes. After the exposure, the lungs were excised and prepared for cryomicrotome imaging, where a 3D image of FMS deposition was acquired using serial sectioning. Cryomicrotome images were spatially registered to match the live CT images to facilitate direct quantitative comparisons of FMS signal intensity with the CT-based ventilation maps.

Results—Comparisons of fractional ventilation in contiguous, non-overlapping, 3D regions between CT-based ventilation maps and FMS images showed strong correlations in fractional ventilation ($r=0.888$, $p<0.0001$).

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Declarations of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Conclusion—We conclude that ventilation maps derived from CT imaging are predictive of the $1\mu\text{m}$ aerosol deposition used in ventilation-perfusion heterogeneity inhalation studies.

Introduction

Measurement of *in vivo* ventilation patterns and ventilation-perfusion heterogeneity offer unique views of pulmonary function and response to inhaled aerosols and particles. Both are important boundary conditions for computational fluid dynamics models in inhalation toxicology and aerosolized drug delivery, focused on site-specific pulmonary particle deposition and vapor uptake in animal models and humans (1-3). Such models provide important insight into predictions of particle deposition and vapor uptake in a range of animal species and airway geometries (4-7).

Methods of measuring local ventilation non-invasively have been developed in recent years utilizing four-dimensional x-ray computed tomography (4D CT) imaging – 3D CT images acquired at multiple time points through the breathing cycle. One approach measures the voxel-by-voxel volume fractional change in air content by comparing the CT signal intensity between exhale and inhale images (8-11). Alternatively, nonlinear image registration, or image warping, can be used to determine a displacement field between pairs of images, and thereby a voxelwise Jacobian, which is a measure of tissue compression or expansion (12-15). Lastly, a combined approach jointly estimates local ventilation from signal intensity and the tissue Jacobian (16, 17).

There have been relatively few attempts to validate the ventilation maps calculated from CT images. Using the Jacobian-based approach, Reinhardt *et al.* (14) and Ding *et al.* (12) used xenon gas as a tracer for CT images of regional ventilation in ventilated sheep. In both studies, strong correlations were observed between the Jacobian-based ventilation and CT. In contrast, Kipritidis *et al.* (18) used PET (positron emission tomography) imaging of inhaled, ^{68}Ga -labeled nanoparticle aerosol in free-breathing humans and showed relatively poor correlations. Similarly, Yamamoto *et al.* (15) used SPECT (single photon emission computed tomography) imaging of an inhaled, $^{99\text{m}}\text{Tc}$ -labeled pentetic acid aerosol (www.medinuclear.com), which had a mass median aerodynamic diameter of $0.28\mu\text{m}$ with a broad range of particle sizes up to $1\mu\text{m}$, and also showed uneven voxel-to-voxel correlations, ranging from 0.03 to 0.85. These strong variations were attributed to air trapping and pendeluft, phenomena that could not be accounted for by the Jacobian alone, which is purely a measure of deformation.

In comparable work, others have investigated ventilation maps derived using the CT ventilation images calculated based on signal intensity. Also using SPECT imaging and an inhaled $^{99\text{m}}\text{Tc}$ -labeled aerosol, Castillo *et al.* (19) showed that both Jacobian-based and intensity-based methods correlate well with global ventilation, but that on a region-to-region basis there was a “low correlation” between the intensity-based approach and SPECT, a difference primarily attributed to the known tendency of the aerosol to deposit on conducting airways. On the other hand, using hyperpolarized ^3He as an inhaled contrast agent for 2D magnetic resonance imaging (MRI), Mathew *et al.* (11) found a strong correlation between ^3He distribution and CT-based ventilation. Furthermore, ^3He MRI of

pulmonary airflow has also been shown to correlate strongly with computational fluid dynamics predictions in the rat (20).

Overall, prior studies suggest that tracer gases, such as ^3He for MRI and xenon for CT, provide the most representative view of ventilation, whereas inhaled aerosols labeled with a radiotracer may not be accurate surrogates for ventilation. This is particularly relevant since particulates in the nanometer range can be expected to approach the behavior of an inhaled gas with deep lung penetration but a large fraction of particles exhaled without deposition, while particles in the 1-3 μm range are expected to deposit deep into the alveolar regions with some deposition in the conducting airways (21).

In this study, we quantitatively compare CT-based ventilation maps in live rats to images derived from serial cryosections of the same rat lungs after exposure to an aerosol of 1 μm fluorescent microspheres (FMS). By measuring the spatial correlation of fractional FMS deposition to image-based volume change, we demonstrate that the CT approach is predictive of actual FMS deposition patterns on the mm length scale.

Methods

Animal Treatment

All animal work was approved by the Institutional Animal Care and Use Committee of Pacific Northwest National Laboratory prior to initiation of the study.

Male Sprague-Dawley rats weighing 234 ± 6 g were used. To generate a heterogeneous disease, six rats were dosed in a single lobe with 45 U/kg porcine pancreatic elastase (EMD Chemicals, Inc., Cat# 324682) dissolved in 200 μL saline. Dosing was accomplished by orally intubating the anesthetized rats and gently feeding a narrow, flexible tube deep into the lung. Following dosing, rats were revived and allowed access to food and water *ad libitum*.

CT Imaging

Four weeks after dosing, rats were prepared for 4D CT imaging, as described previously (22-24). Briefly, rats were anesthetized with isoflurane, surgically intubated, and attached to a pressure-limited mechanical ventilator using time-cycled, volume-targeted breaths. Rats were ventilated with a target tidal volume (T_v) of 2.0 mL (with an average peak inspiratory pressure (PIP) of 5.9 cmH_2O) at 54 breaths per minute, with an inspiration time (T_i) of 500 ms and an exhalation time of 610 ms. A set of eleven 3D CT images was acquired with 100 ms temporal resolution; however, only two images, one at end expiratory volume (EEV) and one at PIP, were used in this analysis. Imaging parameters were: 80 kV peak tube voltage, 32 mA tube current, 16 ms exposure time, and 360 projections with 1° separation. Total imaging time per rat was approximately 29 minutes. Images were reconstructed to 200 μm isotropic resolution.

Particle Exposure

Immediately following imaging, rats were removed from isoflurane, injected intraperitoneally with ketamine/xylazine, and transferred to a volume-controlled, piston-

driven ventilator within a fume hood for particle exposure. The ventilator was set to 60 breaths per minute, with $T_i = 500$ ms, $T_v = 2.0$ mL, and $PIP \approx 6.0$ cmH₂O – the same settings as the imaging ventilator. An aerosol of 1 μ m red FMS (Molecular Probes, Eugene, OR) was generated by an in-line ultrasonic aerosol generating system (Microstat Ultrasonic Nebulizer, Mountain Medical Equipment, Littleton, CO) that passed the aerosol through a drying column before entry into the trachea (25). Rats were exposed to the aerosol for 5-7 minutes. Body temperature, blood-oxygen saturation, and heart rate were continuously monitored. We note that alveolar clearance mechanisms are of a much longer time scale than the exposures in this study (days vs. minutes) (26).

Following the particle exposure, rats were humanely euthanized with CO₂ asphyxiation then imaged again at total lung capacity (TLC). For this, rats were attached via the trachea tube to a constant air pressure source set to 25 cmH₂O and imaged with the following parameters: 90 kV peak tube voltage, 40 mA tube current, 16 ms exposure time, and 900 projections with 0.4° separation. Total imaging time was about 5 minutes. Images were reconstructed to 50 μ m isotropic resolution.

Cryomicrotome Imaging

Next, the lungs were removed from the thorax and filled with ~9 mL of clear OCT (Tissue-Tek® Optimal Cutting Temperature media). Based on prior experiments in our lab in the same strain and gender rats of similar weight, ~9 ml of OCT was adequate to achieve TLC. Lungs were immediately frozen and embedded in black OCT (clear OCT treated with carbon black powder) in preparation for cryomicrotome sectioning. The Imaging Cryomicrotome (Barlow Scientific, Inc., Olympia, WA, USA) sections the frozen lung and photographs the tissue autofluorescence and FMS distribution at high resolution. The instrument has been previously described (27) but has been improved through several recent modifications (28). Briefly, it consists of a Redlake MegaPlus II ES 3200 digital camera (San Diego, CA) with a resolution of 2184×1472 pixels, a metal halide lamp (PE300BF Cermex, Excelitas Technologies, Fremont, CA), and filters limiting the excitation and emission wavelengths. Fluorescence images are acquired with a 180 mm Micro-Nikkor lens (Nikon, Corp., Tokyo, Japan). A custom-built LabVIEW (version 8.2, National Instruments Inc., Austin, TX, USA) application controls the motor, emission and excitation filter wheels, fine-focus, and image capture and display. The slice thickness was 24 μ m, and the camera was set at a distance from the sample surface to give an isotropic in-plane resolution of 24 μ m.

Image Registration

To calculate maps of regional ventilation from the dynamic CT images, we followed the methods described in detail in (17). In brief, non-lung regions of the images were masked, and nonlinear registration was used to warp the PIP image to the EEV image. The deformation, or Jacobian, of the resulting 3D vector displacement field together with the CT signal intensity values were used to calculate the volume change, or ventilation, between the EEV and PIP images on a voxel-by-voxel basis.

The excised lung undergoes considerable geometric distortion due to the combination of excision and filling with OCT. Specifically, the parenchyma experienced large non-uniform deformations, the vasculature was collapsed, and the lobes were separated, leading to a registration problem wherein both the geometry (the relative orientation of internal features) and, more importantly, the topology (the gross external shape) differed from the *in situ* TLC CT image. However, to make a quantitative comparison between the CT-based ventilation and FMS distribution images, the geometric and topologic mismatches must be minimized. To do so, we pursued a multi-step nonlinear registration approach. First, a cryomicrotome image acquired under white-light illumination was registered to the TLC image. Second, the TLC image was registered to the PIP image. Subsequently, the two computed transformations were applied to the FMS image to bring it into the same space as the PIP image. Finally, the FMS image was registered to the EEV image using the same transformation used to calculate the CT-based ventilation image.

To affect the first step, the white-light microtome image (Figure 1B) was downsampled to the size of the TLC image (Figure 1A) and its look up table (LUT) was inverted (Figure 1C). Inversion of the LUT ensured that signal intensities of features in the CT image better corresponded to the same structures in the cryomicrotome image. Conversely, to affect the registration from TLC to the PIP image, the latter was upsampled to the TLC resolution and no LUT inversion was applied. Additionally, there were anomalous bright spots, or “stars,” in the FMS images due to particle agglomeration of unknown origin. These stars were bright enough to saturate the digital camera and cause blooming (spillover of signal into adjacent pixels) and, as a result, were much brighter than the surrounding signal. To minimize their influence on subsequent fractional ventilation measurements, these stars were masked. Once images were pre-processed, the transformations were computed using ANTs open-source registration software (Advanced Normalization Tools, www.picsl.upenn.edu/ANTS). Both a rigid and affine transformation were computed using a mutual information metric. The deformable registration step was computed using the b-spline variant of the symmetric normalization diffeomorphism described in (29) as a transformation model and a local cross-correlation similarity metric (30, 31).

Image Analysis

Due to the large geometrical deformations and topological differences between the *in situ* and excised lungs, it was not possible to enhance the intensity-based similarity metric with feature information, such as segmented vessels or lobes, as has been successfully used to improve pulmonary registration accuracy (32). Thus, a meaningful voxel-by-voxel comparison of ventilation fraction between the FMS image and ventilation map was not feasible, and it became necessary to compare fractional ventilation within cubes consisting of multiple voxels.

In general, selection of the ideal cube size can be completely arbitrary while profoundly affecting the correlation results; therefore, we implemented a semi-empirical approach to minimize bias. First, we implemented (in the Python programming language) an automated, image-to-image comparison using non-overlapping cubic blocks of voxels. The fractional signal intensity – the total signal intensity within a cube divided by the total signal intensity

of the lung – within each cube in the FMS image was measured and compared to the fractional signal of the corresponding cube at the same location in the CT ventilation map. All non-lung background voxels, as defined by a 3D connected threshold binary mask implemented prior to ventilation map calculation (17), were ignored. Next, we calculated the Pearson correlation coefficient and slope of a linear least squares fit of the FMS versus ventilation map data over a $\sim 10^4$ range of cube sizes (in voxels): 2^3 , 5^3 , 10^3 , 15^3 , 20^3 , 25^3 , 30^3 , and 40^3 (i.e. ranging in size from 8 voxels to 64,000 voxels). Then we determined the “ideal” cube size by finding the smallest cube for which there was no significant change in the slope (of the six-rat ensemble) between it and the next largest cube; i.e. the point at which the slope stopped changing significantly. To test for significance, a one-way ANOVA with a Holm post hoc test ($\alpha = 0.05$) was performed.

Results

Figure 2 shows an example of typical axial slices from: A) CT image at EEV, B) cryomicrotome white light image, C) CT-based ventilation map, and D) inhaled FMS image. Panels A and C are the same slice, and panels B and D are the same slice; however, due to deformation of the lung after removal from the thoracic cavity, it was impossible to exactly match the CT slice with a cryomicrotome slice. In Figure 2, comparably located slices were found by matching the approximate relative positions of the major conducting airways (black arrows in A and B). The consequence of the dissimilar geometries is that any meaningful voxel-to-voxel, or even region-to-region, comparisons of ventilation fraction between the ventilation maps and the FMS images are impossible without first performing a nonlinear registration of the FMS image to the ventilation map. Also shown in the FMS images (panel D) are examples of stars caused by particle agglomeration (white arrows) that resulted in detector saturation.

Figure 3 shows an example of a comparison of the ventilation map with the final warped FMS image. The top row shows a coronal slice and the bottom row is an axial slice. After registration, the geometry of the lung in the FMS image (B and D) more closely matches that of the ventilation map (A and C), allowing for an image-to-image comparison of fractional ventilation. However, the FMS images illustrate the blurring that resulted from the warping process, visible as smearing or swirling in the image, which confounded voxel-to-voxel comparison of ventilation fraction and, as a result, necessitated the use of the multi-voxel cubes to compare fractional ventilation. Also visible in Figure 3 are examples of agglomerated particles, or stars, that were masked prior to warping (white arrows).

To determine the cube size to be used in the final analysis, fractional ventilation comparisons were made between the CT ventilation maps and warped FMS images using a range of cube sizes. Figure 4 shows how the slope of the linear least squares fit (A) and correlation coefficient (B) changed as a function of the cube size. The average slope of the least squares fit (Figure 4A) was observed to reach 1 and stop increasing – indicating a 1:1 correspondence in fractional ventilation – as expected, since larger cubes contain an ever-increasing fraction of the lung (a cube containing the entire lung would have a fractional ventilation of 1 for both images). The point at which the slope effectively stopped increasing was at the $15 \times 15 \times 15$ cube size, which had a slope that was significantly higher than the

10×10×10 cubes yet was not significantly different than the 20×20×20 cubes. Thus, we chose the 15×15×15 cube for the quantitative comparison between images; we note that this cube size has a volume of 3×3×3 mm³. For scale, Figure 5 shows a 15×15×15 cube next to a 3D representation of the CT image. The typical number of 15×15×15 cubes containing any fraction of lung was ~590 per rat. We further observed that the correlation coefficient (Figure 4B) continued to asymptotically approach 1 with increasing cube size, indicating expected stronger correlations with cubes containing larger fractions of the total lung.

Figure 6 illustrates why the slope and correlation change with increasing cube size, with a comparison of two of the linear regressions: the 10×10×10 and 30×30×30 cube sizes from one rat. The larger cubes each contain a much greater fraction of the lung (i.e. $10^3 = 1000$ and $30^3 = 27000$), resulting in fewer lung-containing cubes and fractional ventilation values that are spread over a much broader range. This increased spread has the effect of inducing a stronger correlation.

Figure 7A is a scatter plot of the fraction of total signal in each 15×15×15 cube for the FMS images and the corresponding ventilation maps for all six rats. For the group, the FMS images and ventilation maps were strongly correlated ($r=0.888$, $p<0.0001$). A least squares fit resulted in a slope of 0.946 ± 0.008 , indicating a nearly 1:1 spatial correspondence of ventilation fraction. Correlation and fit results for individual rats are shown in Table 1. To check for any fixed bias in the data, we constructed a Bland-Altman plot (Figure 7B). A 1-sample t-test showed that the mean difference does not vary significantly from 0 ($p=0.92$), confirming a lack of bias.

Discussion

The goal of this study was to validate the ventilation distribution measured by CT – which utilizes images acquired at multiple inflation levels and nonlinear registration to calculate a Jacobian-based volume change on a voxel-by-voxel basis – by using a “ground-truth” approach of inhaled particle distribution. We used 1 μm aerosolized particles, within the size range that is optimal for achieving deposition into the deep lung (21). In spite of two important limitations (see below) that precluded us from making voxel-by-voxel comparisons between the CT ventilation maps and the FMS distribution, we showed in six rats with heterogeneous ventilation patterns that there is a strong correlation ($r=0.888$) of spatial fractional ventilation between the two approaches (see Figure 7).

Similar FMS aerosols have been used in studies of ventilation and ventilation-perfusion heterogeneity (25, 28). Although the analysis of FMS data can only be performed on excised lungs and is therefore limited to non-clinical research, as a benchmark for non-invasive measures of ventilation heterogeneity it has the dual advantages that the particle deposition – and thereby ventilation – can be imaged in 3D and at extremely high resolution. The latter enables an investigation of the scale at which registration-based estimates of regional ventilation remain accurate – an important datum for multiscale computational models of respiration and inhalation exposure, since previous studies have shown that ventilation heterogeneity persists down to the subacinar scale (28). Similarly, the scale at which these two measures correspond – ~3 mm in this study – would also indicate the scale at which

registration-based measures of ventilation are predictive of aerosol deposition of particles at or below 1 μm diameter. Lastly, when paired with biochemical imaging, such as mass spectrometry imaging or immunohistochemistry, an aerosol-based approach may be adopted to co-locate ventilation defects with cellular response.

For this work, we wanted to assure a heterogeneous ventilation pattern in order to correlate CT-based ventilation with FMS distribution over a spatially varying range of ventilation within each animal. To achieve this, we utilized an animal model with a unilateral instillation of elastase, which results in tissue destruction and air trapping that physiologically imitates human emphysema (33). It is well known that heterogeneous emphysema results in inhomogeneous ventilation, as has been demonstrated with hyperpolarized ^3He MRI both in humans (34, 35) and unilaterally treated rats (36). It has also been shown that emphysema affects particle deposition patterns in small animal models (37, 38). As illustrated in Figures 2 and 3 by the large voids of signal in the ventilation maps and FMS imgs, we also realized ventilation heterogeneity using this model.

The main limitation of this study was the inability to directly compare the unaltered FMS images to the CT images. This was due to deformation of the lung from its *in situ* shape once it was removed from the thoracic cavity. As a result of this, a multi-step nonlinear registration had to be employed to best match the FMS image to the CT image at EEV. Such registration was first complicated by stark differences in the cryomicrotome and CT images. For example, the bright, prominent features in the CT images are the lung vasculature, whereas in the cryomicrotome images the vasculature appear dark and are largely missing or misshapen due to compression by the OCT in the adjacent conducting airways (compare Figures 2A to 2B). With a lack of common internal landmarks, the registration process must rely more heavily on external landmarks, primarily the boundary of the lung. However, the lobes of the excised lung had separated, resulting in an altered topology with respect to the *in situ* lung, meaning that even a registration driven simply by the boundary of the two objects was problematic. Registering the inverted cryomicrotome image to the TLC image helped mitigate this problem by more closely matching the *ex situ* and *in situ* lung boundaries (see Figure 1). However, large deformations, such as from TLC to EEV, remain computationally challenging. This problem was minimized to the extent possible by using the PIP image as an intermediary between the TLC image and the EEV image, although this was also complicated by considerable differences in lung signal intensity between TLC and PIP. Importantly, the fact that we obtain a reasonable correlation in spite of these challenges, albeit with a cube size larger than a typical rat lung acinus, suggests that improvements to our approach could in the future enable higher resolution, even voxel-to-voxel, comparisons. For example, filling the lung with OCT *in situ* combined with whole body cryosectioning without removing the lungs would result in lung sections that more closely match the *in vivo* CT images.

A second important limitation was the FMS agglomeration, which resulted in bright spots, or “stars,” dispersed throughout the lungs. Such stars were not observed in our previous work (25, 28), and we are uncertain of their origin. We cannot accurately estimate their actual size, because the light they generated saturated the cryomicrotome camera’s CCD sensor making them appear larger due to blooming. Nevertheless, the results suggest that

these agglomerates were not large enough to be responsible for any airflow obstructions. This is because they were observed to occur throughout the lungs in both unventilated and well-ventilated regions, and the same gross regions were observed to be unventilated in the CT images, which were acquired first. We measured the distribution of FMS signal in the masked images, and we found that the signal intensity of the stars was typically ~20 standard deviations above the mean FMS signal. Because the stars were so much brighter than the typical FMS signal, we were concerned that they would bias the fractional ventilation measurements toward unrealistically high ventilation. Therefore, to minimize this potential bias, we elected to eliminate the stars by applying a threshold mask, the results of which are visible in Figure 4.

To determine the origin of these particle agglomerations, we investigated whether agglomerations had formed in the aerosolizer or whether they resulted from impaction loci at the bifurcations of major airways and were subsequently translocated to the deep lung by the delivery of the OCT. By reexamining the aerosolizer system, we readily confirmed that it was indeed creating single FMS particles, with occasional doublets, and thus was not responsible for agglomerations.

To investigate the potential role of the delivery of OCT in anomalous particle agglomerations, we carried out two follow-up studies. First, we aerosolized an additional untreated rat but filled the lung vasculature with OCT rather than the airways. After cryomicrotome sectioning, we observed: 1) that FMS show up on the tracheal wall particularly at the end of the trachea tube, but generally not further down the tracheal wall and conducting airway tree, 2) that there is indeed some FMS concentration at bifurcations in the airway system, and 3) that most of the FMS actually do deposit beyond the airway tree further out into the parenchyma, as expected. Importantly, particle agglomerations were again found in the deep lung even though no OCT had been pushed into the airways.

Secondly, to test if the OCT was translocating FMS agglomerations from the major airways or bifurcations and depositing deeper in the distal lung, we exposed another untreated rat, filled the airways with OCT, and examined multiple slices of tissue under a microscope. This was to determine whether FMS were attached to the walls or were mixed with the OCT in the lumen away from the walls of the airways. Specifically, multiple 5 μm slices were cut from the tissue block, mounted on cold microscope slide, and freeze dried. In a very limited sampling of only 3-4 slices we found only 3 out of the 163 total FMS counted (< 2%) were not in direct contact with tissue, suggesting the OCT was not appreciably removing them from the walls. Indeed, Geiser et al. (39) showed that polystyrene particles similar in size to those used in our research quickly became embedded into the liquid lining of the lung. We speculate that the immiscibility of OCT with the aqueous lining layer will considerably hinder movement of the particles into the OCT – with the caveat that we have no evidence of what happens with a large particle agglomerates. Furthermore, while microspheres are likely able to roll along the airway wall, we expect non-laminar flow of the OCT at bifurcations to have moved displaced particles away from the walls and into the center streams of the airway (i.e. deep into the OCT). Tsuda et al. demonstrated that such mixing of high viscosity fluids (~15 cP) occurs within conducting airways at low flow rates (40). However, as we did not observe particles mixed within the OCT, we interpreted this as

supportive evidence that microspheres do not move an appreciable distance. In summary, these preliminary studies suggest that agglomeration was not occurring in the aerosolizer, nor were agglomerated particles being pushed deeper into the lung from branch points by the OCT, although follow-up work is necessary to verify these findings.

We note that in previous FMS studies performed by our group that no particle agglomerates were observed (25, 28). Unlike those earlier studies, in the present study FMS exposure was preceded ~30 min of ventilation during CT acquisition, which may have resulted in increased mucous production or in the concentration of existing mucous. This notion is supported by a separate cryomicrotome study of a rat (unpublished data) in which we superimposed FMS signal onto a segmentation of the conducting airways and observed what appeared to be droplets of mucus secretions containing FMS. Future, more systematic tests will be required to confirm these observations.

Conclusion

We conclude that ventilation maps derived from 4D CT images of the breathing rat correlate well with images of inhalation-deposited fluorescent microspheres in the same rat. However, we have identified several limitations of the experiment that may be overcome to allow a more robust, voxel-by-voxel comparison of ventilation heterogeneity. Nevertheless, these results are important for strengthening the credibility of predictive inhalation toxicology models of particle deposition in the rodent respiratory tract.

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Figure 1.

Images used for nonlinear registration of the cryomicrotome images to the CT images. A) *In situ* TLC image acquired post mortem. Vasculature are bright structures and conducting airways are dark. B) White light cryomicrotome image downsampled to match the size of A. C) Color-inverted version of B to better match the feature intensities of A. Note the general absence of vasculature compared to A.

Figure 2.

Example of axial slices of the CT and cryomicrotome images. A) *In vivo* CT image at EEV. B) White light cryomicrotome image of approximately the same slice as A. The major airways in A and B (black arrows) were used as landmarks to select comparable slices. C) *In vivo* CT-based ventilation image. D) Cryomicrotome image of FMS distribution. White arrows indicate receiver saturation due to particle agglomerations.

Figure 3.

A visual comparison of the CT-based ventilation map with the nonlinearly registered FMS image. Coronal and axial views of a CT-based ventilation map (A and C) are shown side-by-side with comparable views of the FMS images (B and D). Arrows indicate FMS particle agglomerations that were erased prior to nonlinear registration.

Figure 4.

Plots of the least squares fit linear slope (A) and correlation coefficient (B) versus cube size in voxels for the spatial comparison of ventilation maps to FMS images.

Figure 5.

A 3D representation of a rat lung at EEV shown with a $15 \times 15 \times 15$ voxel cube for relative size comparison. The $15 \times 15 \times 15$ cube is 3 mm on a side.

Figure 6.

Scatter plot showing a comparison of the fractional ventilation (in percent) measured by two different cube sizes (10×10×10 and 30×30×30) for a single rat to illustrate the effect of cube size on the resulting slope and correlation. The solid lines represent linear least-squares fits.

Figure 7.

A) Scatter plot of the fractional signal (in percent) contained within each $15 \times 15 \times 15$ cube for all six rats. The black line is a linear least-squares fit, with slope of 0.946 ± 0.008 . B) Bland-Altman plot of data for all rats. Dashed lines show ± 1.96 standard deviations of the difference.

Table 1

Correlation coefficient (R-value) and least squares fit slope of fractional signal intensity of CT-based ventilation maps and cryomicrotome FMS images for the 15×15×15 cube size.

Rat	R-value	Slope	Number of boxes
1	0.868	0.895 ± 0.022	553
2	0.888	0.943 ± 0.019	633
3	0.892	0.961 ± 0.020	593
4	0.908	0.908 ± 0.017	593
5	0.900	0.982 ± 0.020	590
6	0.881	1.01 ± 0.02	574

All correlations are statistically significant with $p < 0.0001$.