



## Accuracy of automatic airway morphometry in computed tomography—Correlation of radiological–pathological findings<sup>☆,☆☆</sup>

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### ABSTRACT

**Objectives:** Airway morphology shows characteristic changes in different pathologies. This study assesses the accuracy of a current automatic airway assessment technique by correlating CT images of porcine airways to histological slices of the same localization.

**Materials and methods:** Four isolated and ventilated porcine lungs were frozen in a liquid nitrogen bath and examined with a CT scanner (MDCT). This technique both preserved normal radiomorphological appearance and made it possible to slice the specimens for histological examination for subsequent correlation. The parameters wall thickness (WT), wall percentage (WP), and total diameter (TD) were assessed by computer-aided measurement of the MDCT images using an integral-based method (IBM) and by manually measuring the histological slices with an electronic caliper.

**Results:** The radiological–pathological correlation could be performed for 16 localizations. Mean relative errors for WT, WP, and TD were 11%, 5.6%, and 8.5%, respectively. Correlation was very high with coefficients  $r$  of 0.951 for WT, 0.916 for WP, and 0.987 for TD.

**Conclusions:** Our results are comparable to previously described errors in phantom correlations but are the first proof of ex vivo feasibility. Thus, by applying this freezing technique to MDCT data of diseased, explanted lungs and by combination with the IBM, further experiments can be performed to explore the effects of airway pathology on radiological morphology.

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### 1. Introduction

For imaging of the tracheobronchial tree, computed tomography provides the highest spatial resolution among the noninvasive imaging techniques and thus has become the gold standard and most frequently used technique for diagnosing airway diseases. Different diseases are associated with changes in airway morphol-

ogy. Chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis (CF), or bronchiolitis obliterans (BO) can cause dilatation of the airway's lumen and thickening of the airway wall [1–3]. In special therapeutic settings, such as after lung transplantation or after allogeneic bone marrow transplantation, these changes can help to distinguish infectious disease from immunogenic causes of a decrease in pulmonary function [4,5]. While in daily clinical routine radiological evaluation still involves subjective rating or manual measurements, semi-automatic or automatic assessment of airway morphology has been described several times in the scientific literature [6–8]. Although systems for airway assessment are commercially available the accuracy of in vivo measurements is completely unknown. In previous studies we have demonstrated the accuracy of our software, particularly in airways with wall thicknesses smaller than 1 mm which are frequently overestimated with standard measurement protocols due to the blurring effect [9], by using an anthropomorphic phantom [10]. A few study groups also evaluated the accuracy of their technique, mostly using a more or less anthropomorphic phantom [11,12]. But the layered interface between an airway and the lung parenchyma has not been simulated in a phantom study correctly. Only one study correlated the results with pathological specimens [13]. However the applied

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**Fig. 1.** Intubated and ventilated porcine lung lowered into a bath of liquid nitrogen leading to immediate freezing and preserved anatomy while CT scanning.

assessment technique did not account for the blurring effect and lung fixation was performed by formalin steam a method that is known to cause density changes [14,15] and severe degradation of bronchial epithelium [16]. Thus the proof of adequate accuracy when applying the assessment techniques in vivo is still lacking. Measurements of airway wall thickness on CT images depend on aerated lung parenchyma surrounding the bronchus as airway wall density and density of surrounding atelectasis are too similar to detect the outer limits of the airway wall. Only two methods are known to preserve parenchymal aeration: the above mentioned formalin steam ventilation and a liquid nitrogen freezing technique that has not been applied to airway studies up to now. The aim of this experiment was to evaluate the accuracy of objective airway assessment in an organic, anatomical setting as a precondition for further use in clinical and scientific settings.

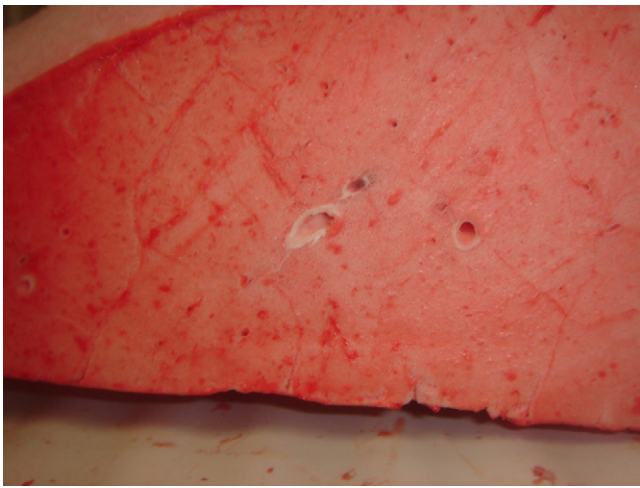
## 2. Materials and methods

After a different animal experiment was terminated (visualization of inert gas wash-out during high-frequency oscillatory ventilation without further lung manipulation), two porcine lungs were explanted immediately after euthanasia. Two other lungs (connected to the pharynx, tongue, larynx, heart, and liver) were obtained from the local abattoir immediately after slaughtering and were transported to the radiological preparation room. No animals were killed for the particular purpose of this study. The lungs were isolated from the appending liver and pharynx and larynx, and the trachea was intubated with a conventional tracheal tube (Willy R sch GmbH, Kernen, Germany). All lungs were ventilated with a mechanical ventilator (SV 900, Siemens-Elcoma AB, Sweden). For the first few minutes, a recruiting maneuver was performed with a positive end expiratory pressure (PEEP) of up to 20 mm Hg. When the lung had visually totally unfolded, inspiration was held or the tracheal tube was obturated with a clamp forceps. The ventilated lung was lowered into a liquid nitrogen bath (temperature  $-183^{\circ}\text{C}$ ), which immediately froze the specimens to total rigidity within a few seconds according to [14] (Fig. 1). After lateral resection by using an electric food cutter, the lungs were placed in a box partly filled with curd; the box was closed and lowered into the liquid nitrogen bath again immediately freezing the curd for optimal stabilization of the whole specimen for later cutting. The frozen lungs were then transported to the MDCT scanner in the nitrogen bath. CT scanning of the curd and box-embedded laterally diminished lungs simulated a routine chest CT examination

protocol (Brilliance 64, Philips Medical Solutions, Leiden, Netherlands, tube voltage – 120 kV, tube current – 100 mAs, collimation –  $64\text{ mm} \times 0.625\text{ mm}$ , slice thickness – 0.8 mm, increment – 0.4 mm, kernel L). Immediately after reconstructing the images, optimal localizations where bronchi crossed the image plane perpendicularly were marked with ink and marked mechanically (wire/metal ruler) with the aid of the CT gantry's laser light. These markers were used to macroscopically cut the specimens with an anatomical saw (Selekta 3, Mado, Dornhan/Blackforest, Germany). After this procedure, we first correlated (macroscopically) the slice surface of the specimens and the CT slice displayed on a mobile computer and only those that fitted well were selected for further study. They were transported under cooled conditions to the pathological institute. The selected bronchi were then isolated and placed in a cryomicrotome, where sections of  $5\text{ }\mu\text{m}$  thickness were cut. Subsequently, the slides were stained with hematoxylin and eosin and mounted according to standard methods. For the second correlation (microscopic), slides were excluded that could not be correlated or if tissue was destroyed due to microtome cutting, so that only exactly and perfectly preserved specimens were used for the measurements to minimize potential artifacts for CT data correlation. Slides were analyzed under an Olympus microscope (BX45, Olympus, Hamburg, Germany). The wall thickness (WT) was only measured histologically in well-correlating specimens by using digital quantification software (Cellb, Olympus, Hamburg, Germany). Wall percentage (WP) and total diameter (TD) were calculated with respect to the assessed wall thickness. At least 10 measurements were performed at different wall positions and we took special care of the centrifugal orientation of the measurement axis. The correlating CT measurement was performed with a system called YACTA (yet-another-CT-analyzer) as described previously [10]. A total of 256 centrifugal rays detected the gray scale profile across the airway wall and we used the integral-based method to quantify the median airway WT, WP, and TD. Localizations where the gray scale profile could not be reasonably assessed (e.g., when bronchial arteries accompanied the airway wall) were automatically excluded and the median WT was determined for the parts of the airway surrounded by parenchyma. Statistical analysis was performed with Microsoft-Excel and SPSS 17. Correlation coefficients (Pearson) were calculated.

## 3. Results

The lung could be ventilated very well without any remaining atelectasis or relevant pleural lacerations. The specimens were frozen within a few seconds and the cutting margins of the lungs were clear and did not show any damage macroscopically (Fig. 2). All airways were visible on the CT images as in an in vivo situation (Fig. 3). In all, 36 bronchi on different levels were identified that were surrounded by aerated lung parenchyma and that crossed the image plane perpendicularly, rendering them measurable by our software using the two-dimensional procedure. After the first (macroscopic) and the second (microscopic) correlation steps and after excluding bronchi that were destroyed during microtome cutting, 16 localizations could be used for measurement and further analysis. The main reason for a lack of correlation was slight angulation while the frozen lungs were being cut. The useable histologic images showed a very well preserved anatomy. Shock freezing and cutting did not cause any detectable damage at the microanatomical level. An example of a correlating microscopic and computed tomographic image is shown in Fig. 4. The results of the airway WT assessment are shown in Table 1. Microscopic measurement with Cellb of the WT gave a median of  $708\text{ }\mu\text{m}$  (minimum  $-354\text{ }\mu\text{m}$ , maximum  $1714\text{ }\mu\text{m}$ , mean  $779\text{ }\mu\text{m}$ ), corresponding to CT measurements with YACTA of  $775\text{ }\mu\text{m}$  (minimum  $-375\text{ }\mu\text{m}$ , maximum



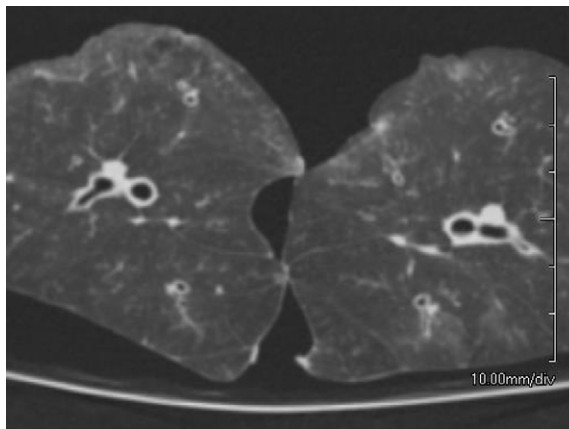
**Fig. 2.** Completely frozen lung after cutting showing clear margins.

1591  $\mu\text{m}$ , mean 791, 3  $\mu\text{m}$ ). The relative error ranged between  $-22.8\%$  and  $+44.5\%$  and the mean relative error was  $11\%$ . Calculation of WP from microscopic measurements with Cellb showed a median of  $52.3\%$  (minimum  $-39.5\%$ , maximum  $68.2\%$ , mean  $55\%$ ), corresponding to CT measurements with YACTA of  $51.3\%$  (min-

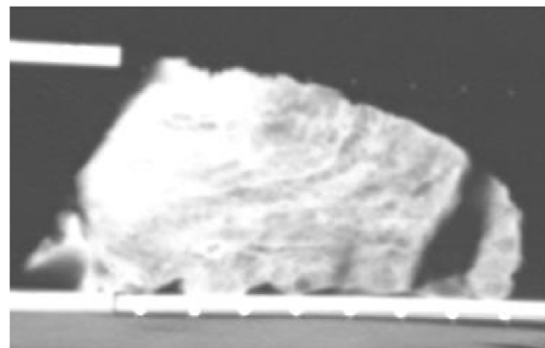
imum  $-37.4\%$ , maximum  $69.3\%$ , mean  $53.8\%$ ). The relative error ranged between  $-11.9\%$  and  $+9.9\%$  and the mean relative error was  $5.6\%$ . Microscopic measurement with Cellb of the total diameter gave a median of  $4.3\text{ mm}$  (minimum  $-2.5\text{ mm}$ , maximum  $10.7\text{ mm}$ , mean  $4.7\text{ mm}$ ), corresponding to CT measurements with YACTA of  $4.6\text{ mm}$  (minimum  $-3.2\text{ mm}$ , maximum  $11.4\text{ mm}$ , mean  $5\text{ mm}$ ). The relative error ranged between  $-9.8\%$  and  $+34.8\%$  and the mean relative error was  $8.5\%$ . For all parameters correlation was very high with coefficients  $r$  of  $0.951$  for WT,  $0.916$  for WP, and  $0.987$  for TD. The corresponding diagrams are presented in Fig. 5a–c. The mean relative errors are shown in Table 2. All correlations reached a level of significance of  $p < 0.001$ .

#### 4. Discussion

We introduced a nitrogen freezing technique in order to correlate the pathological–radiological measurement of airway walls surrounded by aerated lung parenchyma in pigs and demonstrated a high accuracy of our dedicated software. The accuracy was comparable to our previous results in a phantom setting showing high correlation. WP and wall area are frequently used as relevant parameters in studies of airway morphometry and showed the lowest relative errors as compared to the WT in the present study. Airway WT is determined by a very small number of pixels on a CT image, which might be the reason for the higher rate of rel-

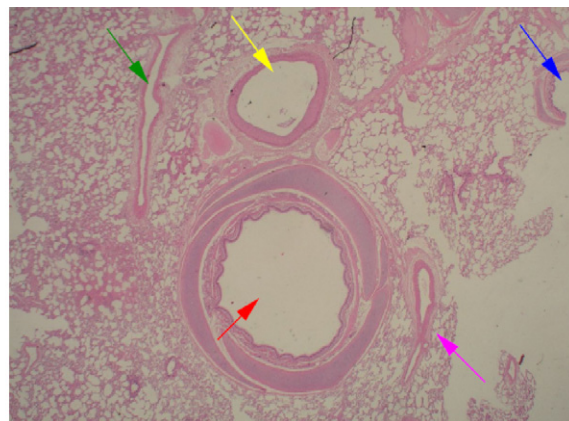
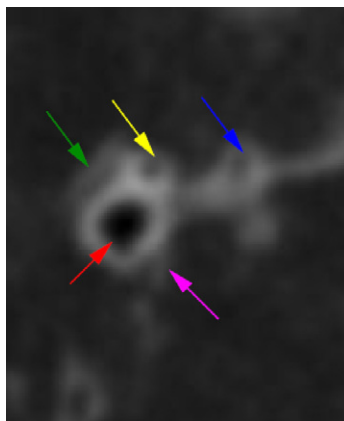


Ventilated and frozen lung



Non-ventilated lung specimen

**Fig. 3.** Comparison of CT images of a normal, not ventilated lung specimen (right) and the ventilated and frozen specimen (left). Note the preserved density difference between the airway walls and the lung parenchyma in the frozen specimen on the left side—mandatory for the automatic measurement of e.g. the airway wall thickness on CT images. The massive and ubiquitous atelectasis in the right image hinders airway morphometry.



**Fig. 4.** Correlation of the same bronchial localization by CT on the left side and the microscopic image on the right side. Colored arrows pointing on landmarks for a better orientation.



**Table 1**  
This table gives an overview of the general results for all 16 correlated airways (AW). Microscopic (MC) and computed tomography (CT) measurements are given for wall thickness (WT), wall percentage (WP), and total diameter (TD). Relative error (RE) in percent.

AW	Wall thickness–WT			Wall percentage–WP			Total diameter–TD		
	MC [ $\mu\text{m}$ ]	CT [ $\mu\text{m}$ ]	RE [%]	MC [%]	CT [%]	RE [%]	MC [mm]	CT [mm]	RE [%]
1	876	795	−9.2	58.6	54.8	−6.6	4.91	4.9	−1.1
2	838	929	+10.9	66.1	64.6	−2.3	4.01	4.6	+14.5
3	841	894	+6.3	65.6	66.2	+0.8	4.06	4.3	+5.2
4	733	759	+3.6	62.1	60.7	−2.2	3.81	4.1	+6.7
5	1156	892	−22.8	68.2	60.7	−11.0	5.31	4.8	−9.8
6	476	472	−0.8	51.1	49.5	−3.2	3.17	3.2	+2.2
7	865	989	+14.3	64.9	69.3	+6.8	4.24	4.4	+4.4
8	628	691	+10.0	48.5	50.4	+3.9	4.45	4.7	+5.1
9	694	791	+14.1	51.6	51.1	−1.0	4.56	5.3	+15.5
10	501	486	−2.9	53.0	51.6	−2.6	3.19	3.2	+0.2
11	1714	1591	−7.2	53.6	48.1	−10.3	10.74	11.4	+5.8
12	1060	974	−8.1	46.6	41.1	−11.9	7.88	8.4	+6.5
13	632	660	+4.4	53.1	50.1	−5.6	4.01	4.5	+12.0
14	354	512	+44.5	49.0	51.9	+5.9	2.48	3.3	+34.8
15	722	850	+17.8	48.0	52.7	+9.9	5.18	5.4	+5.1
16	371	375	+1.1	39.5	37.4	−5.3	3.34	3.6	+7.6

ative errors compared to the airway WP as determined by a larger amount of CT image pixels. The relative error of WP was 5.6% for the anatomical correlation and 3.6% and 1.3%, respectively, for our previously described anthropomorphic phantom settings under similar circumstances [10,17]. Compared to other study groups, our software retains accuracy in airways with a WT below 1 mm. Objects of this size can be overestimated on CT images to a relevant degree [9,18,19]. Montaudon et al. [20] validated their measurement technique in a phantom study. However, it is complicated to compare their results to ours as the airway WT is not given but rather the wall area [ $\text{mm}^2$ ]. It ranged from 92.8 to 5.5  $\text{mm}^2$  for different silicon tubes. The overestimation of their CT measurements was approximately 40% for the smallest tube when scanned orthogonally to the scanner's z-axis and 62% when the tube was angulated 75° to the z-axis. For comparison purposes, the wall area of our smallest airway can be calculated by using the diameter (3.34 mm) and WP (39.5%) of the microscopic measurements at 3.46  $\text{mm}^2$ . A similar calculation of the CT parameters assessed accordingly gives a wall area of 3.8  $\text{mm}^2$  for the same bronchus. Although the calculation of these parameters has to be taken into account in this particular bronchus, the relative error would have been 9.9%. The first radiological–pathological correlation study to be conducted on airway morphometry known to us was published by King et al. in 2000 [13]. They used the formalin steam fixation technique described by Weibel and Vidone [21]. A lung is ventilated by formalin steam for a longer period of time (up to six weeks). The advantage compared to our technique is preservation of the lung while our specimens had to be kept permanently frozen until microtome cutting. The disadvantage is that bronchial epithelium in formalin-fixed lungs is severely altered and is not completely rigid, complicating the correlation. However the relative error was as high as 150% and showed high variability for wall areas smaller than 5%. In comparison our relative error was 5.6% for the WP. The measurement technique known as CTAM (computed tomographic airway morphometry) is responsible for the high variability in King et al.'s study. It was calibrated on an airway phantom by adjusting the window setting and the threshold to four different levels, thus rendering it prone to the blurring effect. As described previously, we compensated for the blurring effect by using the integral-based method, making it possible to measure airways with a WT below 1 mm accurately with our software. Saba et al. although integrated a technique to compensate for the blurring effect [22]. They showed absolute errors for radius estimations of between one third and one half of a 0.29 mm pixel validated in a phantom setting but failed to test the power of this method by recessing wall thicknesses below 1 mm.

Some drawbacks of our study should also be mentioned. First, although the freezing technique gave very good morphological results, it is unsatisfactory that only 16 bronchial localizations could be isolated. The main reason for this was the strict selection procedure. Only macroscopically well correlating bronchi were included for further preparation. Slight angulations during cutting of the frozen lungs were responsible for the differences between the CT image and the frozen cut surface. Another study by Namati et al. describes a potential solution for this problem: large image microscope array (LIMA). That system consists of an automated microtome for large-scale sectioning and minimizes unintentional angulations while cutting the specimen. However, it has not yet been applied to an airway quantification study [23]. We intend to use another method to compensate for this angulation problem: A slice-to-volume-registration that virtually adjusts the pathologic slice into the CT volume data by reconstructing the correctly corresponding slice could be helpful [24,25].

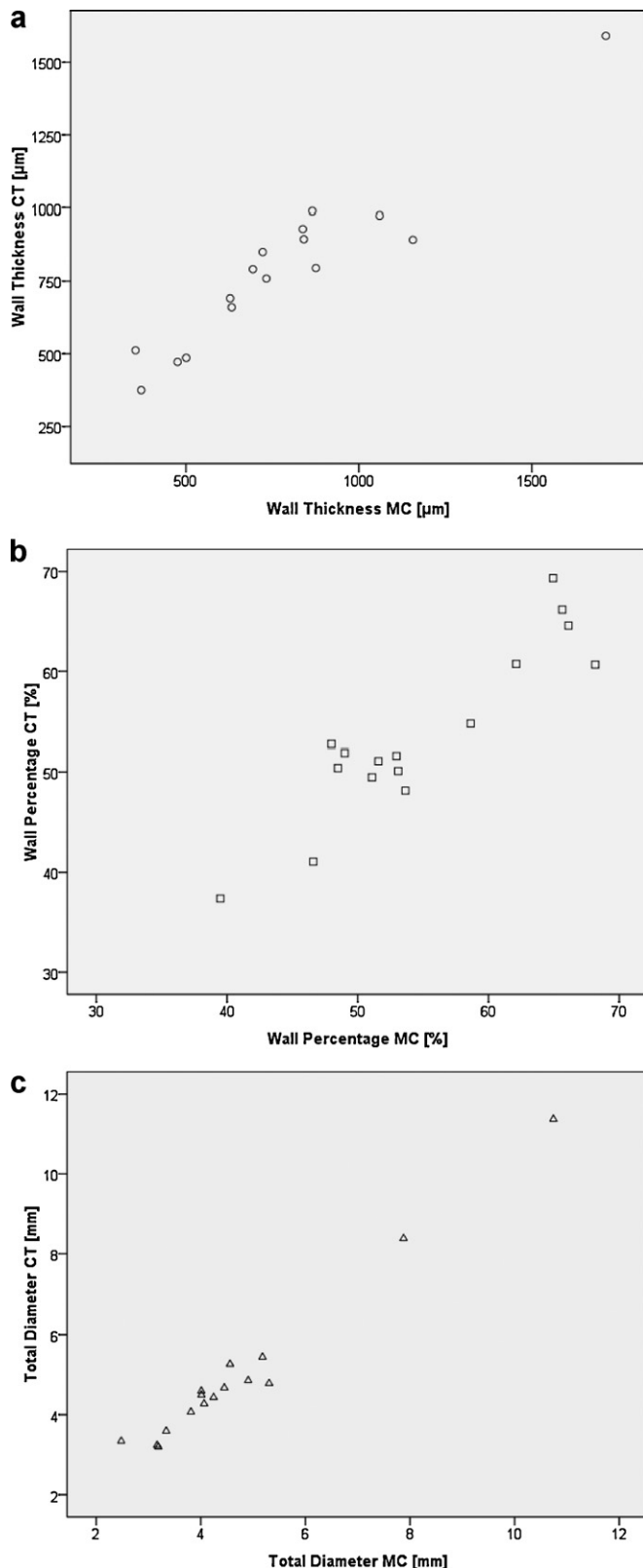
Secondly, we only applied a two-dimensional quantification approach although YACTA provides a full three-dimensional quantification algorithm with segmentation, skeletonization, reconstruction of an orthogonally angulated slice, and measurement within this reconstructed slice at multiple localizations. However, for microscopic evaluation along these reconstructed image planes, macroscopic cutting needs to be oriented first and we do not know of any technique that marks the longitudinal axis of a nonvisible airway to the surface of the frozen lung.

Thirdly the optimised imaging situation has to be taken into account. It was devoid of respiratory and cardiac motion resulting in a completely rigid interstitium. Artefacts caused by soft-tissue and bones were completely missing. Although a slightly lowered tube current was applied to compensate for the missing chest wall, the image quality might have been higher and thus the correlation could have been better than in a real in vivo situation.

Looking into the future a combination of the two methods, the freezing technique and the YACTA measurement, might represent a potential tool to gain deeper insight into pathological conditions of the airways, their surroundings and the impact on MDCT morphology. Especially the detection of early inflammatory, immunogenic,

**Table 2**  
Relative error (RE) of the automatic airway assessment.

	RE wall thickness [%]	RE wall percentage [%]	RE total diameter [%]
Range	−22.8 to +44.5	−11.9 to +9.9	−9.8 to +34.8
Mean	11	5.6	8.5



**Fig. 5.** Correlation diagrams of the CT derived measurements and the microscopically assessed measurements for the parameters wall thickness (a), wall percentage (b) and total diameter (c) showing high correlation.

or toxic alterations of the airway WT could be investigated by this method.

## 5. Conclusion

Our study gives an important fundament for future application of automated, objective airway assessment in MDCT data in scientific and clinical settings as previous phantom studies could not definitely prove adequate accuracy due to the suspected morphological differences between an anthropomorphic phantom and an organic situation. Objective airway assessment can be applied to ex vivo MDCT data with high accuracy even in airways with wall thicknesses below 1 mm if adequate compensation of the blurring effect, e.g. the IBM, has been proved on anthropomorphic phantoms. Furthermore the presented combination of objective airway assessment and the freezing technique for pathological correlation allows future investigation of the influence of airway disease on CT morphology.

## Conflict of interest

None.

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