Four-dimensional optical coherence tomography imaging of subpleural alveoli in mice

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Abstract

The development of protective ventilation strategies for patients suffering from life-threatening lung diseases and the promotion of numerical simulation of lung tissue mechanics requires detailed knowledge about the three-dimensional alveolar micro-structure, their dynamics and elastic properties. Subpleural lung tissue can be observed in animal models utilizing optical coherence tomography (OCT) with a high spatial resolution. We present four-dimensional high-speed OCT imaging of single alveoli as a suitable technique for the visualization of the temporal changes of the three-dimensional structure during the ventilation with a temporal resolution of 17 stacks per ventilation cycle. The acquired four-dimensional information allows a quantitative evaluation revealing the volume-pressure curve and the compliance for single alveoli.

1 Introduction

High-speed optical coherence tomography imaging of rodent subpleural alveolar tissue with high temporal resolution as well as with high spatial resolution can provide information about the structural dynamics of single alveoli and about their elastic properties. The acquired data about subpleural alveoli can promote the development of numerical simulations of the lung with the clinical objective to develop protective ventilation strategies for patients suffering from urgent lung diseases, as acute respiratory distress syndrome (ARDS), acute lung injury (ALI) or ventilator induced lung injury (VILI).

Optical coherence tomography (OCT) [1] is an interferometric and contact-free optical imaging technique providing cross-sections and stacks of scattering tissue with a spatial resolution of typically 10 µm. The image formation is comparable to sonography. Cross-sections consist of many depth scans at adjacent lateral positions, each calculated by applying a Fourier-transformation from one interference spectrum, acquired with a Michelson-interferometer setup.

In previous studies, OCT has been utilized successfully for the visualization of the subpleural alveolar structure [2, 3]. Achieving a good temporal resolution is challenging due to the short time scales; for instance, the breathing cycle of mice is in the range of one second. In order to visualize the dynamically time-varying three-dimensional structure during the ventilation, it has been necessary to trigger the acquisition of single cross-sections on pressure levels and extend the image acquisition of stacks over several ventilation cycles while changing the lateral position. The entire stacks are composed after image acquisition using registration. Changing the pressure levels reveals a virtual four-dimensional image [3]. The influence of the jitter and a possibly non-reproducible alveolar dynamic over the long acquisition period may hinder the stack composition.

In this proof-of-principle study, we are using a high-speed OCT system to perform real four-dimensional imaging. The objective is to acquire three-dimensional stacks with a sufficient high temporal resolution in order to visualize the structural change of single alveoli directly during one single ventilation cycle.

2 Methods

A fast image acquisition is crucial for achieving a high temporal resolution and reducing motion artifacts, which could be caused by the fast moving lung tissue and the rapidly changing pressure during the ventilation. In the utilized swept-source OCT setup, the interference spectra are acquired in time multiplex using a tunable laser as a light source. One very efficient way providing high repetition rates of wavelength sweeps is Fourier-domain mode locking (FDML) [4, 5]. The principle setup of the FDML laser is comparable to conventional in-fiber ring lasers. A broadband semiconductor optical amplifier operating around 1300 nm is used as a laser medium and the instantaneous wavelength is sinusoidally swept using a tunable Fabry-Perot filter. Additionally, a long single-mode fiber (in the order of km) is inserted into the ring resonator providing optical round trip times of a few µs. By matching the sweep frequency of the Fabry-Perot filter with the optical round trip frequency, the laser is kept active on the complete emission wavelength range, simultaneously. Thus, the repetition rate is physically not limited but predicted by the feasible driving frequencies of the Fabry-Perot filter. During one period, a forward-sweep (increasing wavelength) and a backward sweep (decreasing wavelength) are generated, but only the former was used for OCT imaging due to better sensitivity of 94 dB and a higher spectral width resulting in a depth resolution up to 9 µm. The sweep rate was further duplicated to 122.6 kHz utilizing the buffered FDML principle [6]. The emitted wave-
length sweeps are nonlinear in wave number space. Hence, for rescaling the interference spectra into wave number space, a second interference spectrum of a Mach-Zehnder interferometer with fixed spectral range has to be used. Both interference spectra were acquired simultaneously using a two-channel high-speed digitizer (M3i.4142, spectrum GmbH). Details about the high-speed OCT setup have been previously published in [7, 8].

For performing OCT imaging of subpleural alveoli, an in vivo mouse model [9] was used under post mortem conditions. Access to the subpleural lung tissue is achieved by dissecting a chest window. After inserting an intra-thoracic catheter, the window is sealed with a transparent wrapping film glued to the ribcage. The physiological situation is achieved by removing the excess air in the thorax. Additionally, a tracheal tube is inserted for artificial ventilation which was performed using a self-built animal ventilation device [10]. The ventilation parameter was set to 20 cmH$_2$O inspiratory plateau pressure, 0 cmH$_2$O endexpiratory pressure and 50 bpm ventilation rate. The pressure curve, which is shown in image 1, was measured simultaneously to the OCT imaging.

![Image 1](image1.png)  
**Image 1** The course of the applied pressure over a time period of one ventilation cycle (1.2 s) was measured simultaneously to OCT imaging. The green box represents the 70 ms acquisition time of an exemplary three-dimensional stack. The stack number allows comparison with image 3.

### 3 Results

For the visualization of the dynamics of subpleural alveolar structure, three-dimensional stacks were acquired with a temporal resolution of 14 stacks per second which corresponds to 17 stacks per ventilation cycle. Every stack consists of 64 cross-sections, each composed of 128 depth scans, and was acquired within 70 ms. Due to the high cross-section frequency, the deflection of the used high-speed galvanometric scanners for the beam deflection over the sample deviated from the saw-tooth driving signal. Thus, only the part of the cross-sections with linear galvanometer movement is shown in the following images and was used for further investigations. The single stacks were directly composed of the cross-sections without registration. To facilitate an appropriate evaluation and visualization, the vertical and horizontal movement of the entire lung tissue, visible between subsequent stacks and caused by the ventilation itself, was corrected by means of aligning the pleura and eliminating the lateral shift. Additionally, a median filter with one pixel radius was applied to all presented images. Image 2 presents typical stacks and cross-sections of subpleural alveoli. The pleura appears as a bright horizontal surface at the top of the tissue, visible in image 2a and 2c, whereas the alveolar space appears dark or transparent in the three-dimensional views.

![Image 2](image2.png)  
**Image 2** The stack (A), composed of 50x64 depth scans with lateral extension of 200 µm times 256 µm, shows subpleural lung tissue after alignment of the pleura, which appears as a bright surface in (A) and also in the corresponding green marked cross section (C). Hiding the pleura permits a view into the three-dimensional alveolar structure. The en face image (D) is extracted from the stack at a depth of 40 µm beneath the pleura (red marked position). The changes in the three-dimensional alveolar structure during the ventilation cycle are visible in similar en face images presented in image 3. An increasing and decreasing alveolar area can be observed during the inspiration or expiration, respectively. Due to the fast image acquisition, the pressure changes during the capture of one stack are small, typically 2 cmH$_2$O, and thus the reconstructed alveolar structure appears undistorted with widely reduced motion artifacts. In order to determine the volume of a cluster of three alveoli, labeled with a blue circle in image 3, a segmentation of the alveolar space in each stack was per-
formed using a manually set threshold. The simultaneously acquired pressure allowed the assignment to the ventilation cycle and the determination of a volume-pressure curve for this cluster of alveoli, presented in image 4, which reflected the expected hysteresis behavior. This course was reproducible within a second ventilation cycle, which is not shown here for clarity. Fitting a sigmoid function in the inspiratory slope provided the alveolar compliance of 5.1 pl/cmH\textsubscript{2}O.

**Image 3** The *en face* images at a depth of 40 µm beneath the pleura with a lateral extension of 200 µm times 256 µm are labeled with the stack number for comparison with the pressure curve in image 1. The air filled alveolar space appears dark and exhibits an increasing area during the inspiration (stack 1 to 5) and a decreasing area during the expiration (stack 7 to 15). The cluster of three alveoli (blue circle) was chosen for a three-dimensional segmentation and a quantitative evaluation.

**Image 4** The volume-pressure curve was determined from the stack series by segmenting the alveolar volume of the blue marked cluster of alveoli in image 3. Fitting a sigmoid function (gray dashed line) allowed the determination of the alveolar compliance.

### 2 Conclusion

Within this study, four-dimensional OCT imaging has been presented as a promising technique for the visualization and quantitative investigation of the dynamics of subpleural alveoli. Utilizing a high-speed OCT system and FDML technology provided high depth scan rates of 122.6 kHz. Due to the fast image acquisition, a temporal resolution of 17 stacks per ventilation cycle was achieved without perceptible image artifacts. The quantitative evaluation revealed the alveolar volume-pressure curve and the alveolar compliance, emphasizing that the entire four-dimensional information can be detected within a single ventilation cycle which suggests high-speed OCT imaging with the presented imaging protocol as a suitable tool in longitudinal animal studies for instance.

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### 4 References


