Research Article

Grating-based X-ray Dark-field Computed Tomography of Living Mice

CrossMark

A. Velroyen [a,*], A. Yaroshenko [a], D. Hahn [a], A. Fehringer [a], A. Tapfer [a], M. Müller [a], P.B. Noël [b], B. Pauwels [c],
A. Sasov [c], A.Ö. Yildirim [d,f], O. Eickelberg [d,f], K. Hellbach [e], S.D. Auweter [e], F.G. Meinel [e], M.F. Reiser [e,f],
M. Bech [a,g], F. Pfeiffer [a,*]

[a] Lehrstuhl für Biomedizinische Physik, Physik-Department & Institut für Medizintechnik, Technische Universität München, Garching, Germany
[b] Department of Radiology, Technische Universität München, Munich, Germany
[c] Bruker microCT, Kontich, Belgium
[d] Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München and Ludwig-Maximilians-University Hospital Munich, Munich, Germany
[e] Institute for Clinical Radiology, Ludwig-Maximilians-University Hospital, Munich, Germany
[f] German Center for Lung Research (DZL), Germany
[g] Medical Radiation Physics, Lund University, Lund, Sweden

ARTICLE INFO

ABSTRACT

Changes in x-ray attenuating tissue caused by lung disorders like emphysema or fibrosis are subtle and thus only resolved by high-resolution computed tomography (CT). The structural reorganization, however, is of strong influence for lung function. Dark-field CT (DFCT), based on small-angle scattering of x-rays, reveals such structural changes even at resolutions coarser than the pulmonary network and thus provides access to their anatomical distribution. In this proof-of-concept study we present x-ray *in vivo* DFCTs of lungs of a healthy, an emphysematous and a fibrotic mouse. The tomographies show excellent depiction of the distribution of structural – and thus indirectly functional – changes in lung parenchyma, on single-modality slices in dark field as well as on multimodal fusion images. Therefore, we anticipate numerous applications of DFCT in diagnostic lung imaging. We introduce a scatter-based Hounsfield Unit (sHU) scale to facilitate comparability of scans. In this newly defined sHU scale, the pathophysiological changes by emphysema and fibrosis cause a shift towards lower numbers, compared to healthy lung tissue.

1. Introduction

Lung diseases pose one of the leading causes of death worldwide (World Health Organization, 2011). A large fraction of those mortality numbers is represented by chronic obstructive pulmonary disease (Zvezdin et al., 2009), of which lung emphysema is a common component. The disease is associated with inflammatory processes in lung tissue caused most commonly by smoking. In consequence, the alveolar walls necessary for gas exchange in the blood are destroyed, resulting in enlarged distal air spaces and consequently deteriorated lung function and decreased quality of life (Ley-Zaporozhan et al., 2008). Pulmonary fibrosis can be idiopathic or caused by inhalation of pollutants, infection, or adverse reactions to drugs (Bourke, 2006). In the course of this interstitial lung disease normal parenchyma is gradually replaced by connective tissue (scarring), resulting in a restricted lung and impaired gas exchange (King, 2005). The sensitivity and specificity of

conventional x-ray projection radiography in diagnosing those disorders is low for mild to moderate stages (King, 2005; Washko, 2010), and lung function testing is still gold standard, while direct assessment of the microstructural changes and disease progression is only available *via* invasive biopsy. Attenuation-based high-resolution computed tomography (HRCT) serves for improved emphysema and fibrosis imaging, also providing information about the three-dimensional regional distribution of the disease necessary for lung volume reduction surgery (Washko et al., 2008; Criner and Mamary, 2010), but its use is limited due to the required substantial radiation dose (Washko, 2010).

Contrary to conventional medical x-ray imaging, grating-based phase-contrast and dark-field imaging (PCI/DFI) generate contrast from perturbations in the x-ray wave-front caused by refraction and ultra-small angle scattering in tissue (Weitkamp et al., 2005). Its proven feasibility with conventional polychromatic x-ray sources (Pfeiffer et al., 2008; Pfeiffer et al., 2006) has recently triggered developments towards preclinical and clinical translation, such as *in vivo* radiographic projection imaging (Bech et al., 2013) at a dedicated preclinical table-top scanner (Tapfer et al., 2012). Earlier *in vivo* studies using different PCI/DFI techniques were restricted to synchrotron radiation or very small field of view (Bravin et al., 2013). However, the benefit of exploiting

* Corresponding authors.
E-mail addresses: astrid.velroyen@ph.tum.de (A. Velroyen), franz.pfeiffer@tum.de (F. Pfeiffer).

refractive index differences in x-ray imaging of weakly absorbing airways tissue could already be demonstrated *in vivo* at the synchrotron (Lewis et al., 2005). In preceding studies, we introduced emphysema diagnosis on living mice based on x-ray scatter-based dark-field projection imaging (Meinel et al., 2014; Hellbach et al., 2015).

In this work, we present the first successful x-ray dark-field (DF) computed tomography (CT) scans of *in vivo* mice with a dedicated micro-CT scanner (Tapfer et al., 2012). To exploit the DF signal's ability to depict sub-resolution microstructures, thoracic tomographies were acquired of a healthy control mouse, a mouse with pulmonary emphysema and a mouse with pulmonary fibrosis. The scope of this study is to highlight the feasibility and demonstrate the potential diagnostic benefit of the novel contrast modality in three-dimensional imaging. Complementary to the quantitative Hounsfield scale in attenuation-based CT, we also introduce a scatter-based Hounsfield scale for quantitative DFCT of lung tissue.

2. Materials & Methods

2.1. Murine Model

Animals were housed in SPF rooms maintained at constant temperature and humidity with a 12-h light cycle and were allowed food and water *ad libitum*. All experiments were conducted under strict governmental (GV-SOLAS) and international guidelines (FELASA) and were approved under number 2532-77-12 by the local government for the administrative region of Upper Bavaria. For this study 6 to 8 week old pathogen-free female C57BL/6N (Charles River Laboratories, Sulzfeld, Germany) mice were used. Pulmonary emphysema was introduced by orotracheal application of an 80 μl solution of porcine pancreatic elastase (80 U per kilogram bodyweight; Sigma-Aldrich, Munich, Germany) in sterile phosphate-buffered saline (PBS). Pulmonary fibrosis was provoked by orotracheal insertion of 2.5 U bleomycin per kg bodyweight. The control animal was treated with 80 μl PBS only. Treatments were performed under anesthesia by intraperitoneal injection of medetomidine (500 μg/kg), midazolam (5 mg/kg) and fentanyl (50 μg/kg). The anesthesia was then antagonized with a subcutaneous injection of atipamezole (50 mg/ml), fulimazenil (0,1 mg/ml) and naloxone (0,4 mg/ml). Control and emphysema mouse were scanned 21 days, fibrosis mouse 14 days after application.

2.2. Grating-based Dark-field and Phase-contrast Imaging

To measure perturbations of the x-ray wave front other than the mere attenuation and to subsequently extract the dark-field and phase-contrast signals, it is necessary to place optical elements, in our case grating structures, into the x-ray beam path between source and detector. The three gratings as displayed in the sketch in Fig. 1 a comprise the so-called Talbot–Lau interferometer: The source grating G0 counteracts the incoherence of the extended and polychromatic conventional x-ray source by creating an array of smaller line sources that exhibit sufficient coherence. The phase grating G1 acts as a beam splitter generating an interference pattern downstream. Relative to an empty beam, placing an object into the setup influences the interference pattern in three different ways: First, the overall intensity drops due to *attenuation*. Second, the pattern is laterally shifted if the x-rays undergo a resolvable refraction in the material, which is exploited as the (differential) *phase contrast* (for the sake of simplicity not shown in the sketch). Third, scattering of the x-rays at microstructures that cannot be spatially resolved by the imaging system causes the minima and maxima of the interference pattern to be less pronounced, which is translated into the *dark-field* signal. As the pattern cannot be resolved directly by the detector pixel size, the analyzer grating G2 is used to sample the intensity variation: In a process called 'phase stepping' (Weitkamp et al., 2005), several images are acquired at different lateral positions of the three gratings relative to one another over one period of the pattern. The intensity thereby recorded in one detector pixel over the grating position can be described by a sinusoidal curve that changes its parameters offset, phase, and amplitude between an empty beam (reference) and an object in the beam (see Fig. 1 b). Using sinusoidal fits or Fourier analysis, attenuation, differential phase and dark-field signal are extracted respectively from the parameters of the two curves. Thus three different modalities per projection angle are acquired. As in conventional CT, a three dimensional representation of the object can be calculated also for dark-field and phase-contrast imaging, using tomographic image reconstruction algorithms.

2.3. Scanner Setup

The images were acquired using a dedicated small-animal x-ray dark-field and phase-contrast CT scanner developed in collaboration with *Bruker microCT* (Tapfer et al., 2012). The scanner comprises a setup consisting of detector (flatpanel by Hamamatsu C9312SK-6), source (tungsten-target x-ray tube RTW, MCBM 65B-50 W, approx. 50 μm focal spot) and Talbot–Lau interferometer on a rotating gantry and facilities to monitor heart beat, breathing motion and temperature of the animal. The gratings of the interferometer are specified by the following bar materials, heights, and periods, respectively: G0 — gold, 35 μm, 10 μm; G1 — nickel, 4 μm, 3.2 μm; G2 — gold 45 μm, 4.8 μm.
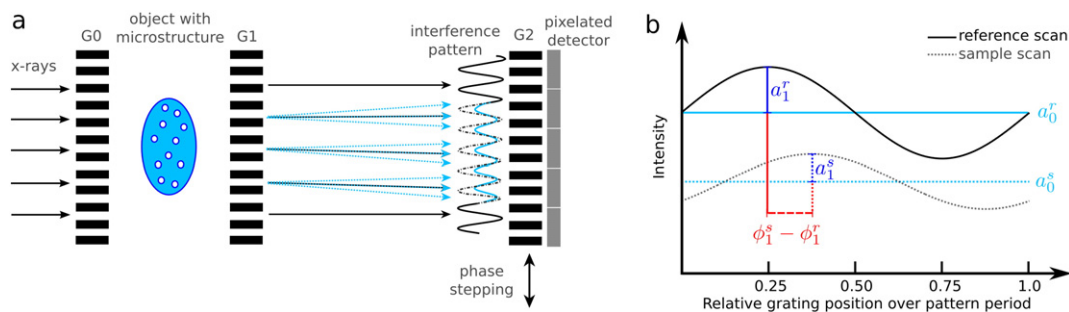

Fig. 1. Methodology of grating-based dark-field and phase-contrast imaging. **a**: Sketch of a Talbot–Lau interferometry setup. By the use of three optical grating structures (source grating G0, phase grating G1, analyzer grating G2) the perturbations introduced by the sample to the x-ray wave front are translated into intensity variations that can be measured by the x-ray detector. The original (reference) interference pattern created by G1 (dashed and solid black line) is changed by the presence of the object in terms of overall intensity (decreased offset of blue line) due to attenuation, and in terms of smoothing of the extrema (reduced peaks and valleys of the blue line) due to scattering at microstructures. Resolvable refraction of the x-rays in the sample would result in a lateral shift of the blue relative to the black dashed line (not shown here for the sake of simplicity). By recording images with the detector at different grating positions over one period of the interference pattern ('phase stepping'), the pattern is sampled. **b**: Measured intensity in one detector pixel for a reference (empty beam, superscript r) and a sample scan (superscript s) over one phase-stepping cycle. By determining the change of the parameters offset a_0, amplitude a_1, and relative phase ϕ_1, the three signals attenuation, dark-field, and phase are extracted.