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Title of experiment: Phase-contrast X-ray imaging of pulmonary fibrosis

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Beamline used: BL20B2

Research purpose and background

We have shown that phase contrast X-ray imaging greatly enhances the visibility of the air-filled lung over attenuation contrast alone. During previous visits to SPring-8, we used propagation-based (PBI) and analyser-based (ABI) phase contrast X-ray imaging to observe and measure the rate and spatial pattern of lung aeration, in real-time, from birth. This imaging technique is able to resolve the very smallest of terminal respiratory units (alveoli; <100 micrometers) as they fill with air.

The high sensitivity of phase contrast to density variations led us to hypothesise that it may be sensitive to pathological changes occurring with lung disease. We aim to determine the potential for phase contrast X-ray imaging as a diagnostic tool for detecting early lung disease. To do this we propose to image a mouse model of pulmonary fibrosis which is characterized by the excessive deposition of fibrous connective tissue in the lung.

Pulmonary fibrosis is a major consequence of ventilator-induced lung injury in very preterm infants which is the most significant problem facing neonatal medicine. Pulmonary fibrosis is also a major cause of pulmonary related morbidity and mortality in adults, but the ability to detect this disease in its early stages, when treatments may be possible, is very limited.

We aim to find the optimal phase contrast imaging modality for detecting pulmonary fibrosis using a variety of techniques. The primary imaging technique will be propagation-based phase contrast imaging (PBI), for which we have extensive experience. However, we have also developed an analytical technique for measuring expansion/deflation velocities within different regions of the lung, throughout each breath, using particle image velocimetry (PIV). By tracking the motion of the speckled intensity patterns of the lung created using PBI, velocity fields within specific regions of the lung can be generated throughout a breath.

As lung pathologies such as pulmonary fibrosis inherently affect lung tissue compliance, we intend to determine whether PIV can detect lung tissue movement abnormalities caused by pulmonary fibrosis. Thus, we will compare the potential of phase contrast imaging and PIV for detecting lung disease using the well described model of pulmonary fibrosis in adult mice.

Since we have previously developed phase retrieval algorithms that can accurately calculate lung gas volumes within specific regions of the lung, the combination of this analysis with PIV analysis will enable us to obtain, for the first time, relative measures of regional lung compliance. However, the segmentation of the images into specific lung regions that can be tracked as the lung inflates and deflates during a breath is greatly limited by overlapping structures, particularly bony structures such as the ribcage. Many methods have been proposed to segment chest images to separate the bones from the soft tissues. Arguably the most successful technique has required two images to be recorded at different X-ray energies and the bones are then isolated based on their relative intensity differences between the recordings [1, 2]. As an extension to our planned studies, we aim to test a method for segmenting soft tissue and bone from images of the thorax. We will acquire two different phase contrast images of the same object and use phase retrieval methods to isolate the hard and soft tissues. This idea was first proposed by Gureyev et al [3] but failed because the images could not be acquired simultaneously, which caused strong artifacts in the reconstructed images. We will acquire two images by performing analyser-based imaging in the Laue geometry, whereby two spatially separated phase contrast images can be recorded simultaneously on a large area detector.

Switching between modalities will only take a few minutes once they are set up. Adult nude mice will be used primarily to reduce the phase contrast associated with fur.

Experimental/analytical method

Mice will be divided into four groups (n=6 for each) and pulmonary fibrosis will be induced in 3 groups by the intratracheal administration of bleomycin (50 μL of 0.1U); the 4th group will act as a control and will receive a saline bolus. The bleomycin treatment takes ~3 days to induce significant lung pathologies. Mice will be imaged at 48h (Group 1), 72h (Group 2; when pathology is first detectable physiologically) and 96h (Group 3) after treatment. All mice will be anaesthetised and ventilated at a pre-imaging rate of 120 bpm using a tidal volume of 7.5ml/kg. During imaging, the inspiratory time will be increased to 2 secs and the expiratory time to 1 sec for 10 consecutive breaths. During this time, images will be rapidly acquired (using 20 ms exposures, with a frame rate of 20 fps for PIV studies), triggered by the ventilator to synchronize image acquisition with the stage of breath. Immediately following imaging, the mice will be humanely killed (using an anaesthetic overdose). We will then image one mouse from each group using high resolution Computed Tomography to compare the sensitivity of our technique with the current, "state-of-the art"

technique for detecting lung pathologies in situ. Finally, the lungs of each mouse will be removed and fixed with formalin for histological analysis. The lungs will then be stained with a Masons trichrome stain to determine histologically whether disease is present yet. The results of the imagining and histology will then be compared.

Research results

To date we have completed imaging and collected the lungs of control as well as mice exposed to 12hr, 24hr, 36hr 2, 5 and 6 days of bleomycin (which induces changes in lung structure). We are currently beginning analysis on all specimens to determine the degree of injury present.

Current and future issues/challenges

Currently this project is facing no challanges.

References

1. Suzuki, K., H. Abe, H. MacMahon, *et al. IEEE T. Med. Imaging*, 2006. **25**(4): p. 406-16.
2. Ishigaki, T., S. Sakuma, Y. Horikawa, *et al. Radiology*, 1986. **161**: p. 271-3.
3. Gureyev, T.E., A.W. Stevenson, D.M. Paganin, *et al. J. Synchrotron Rad.*, 2002. **9**(3): p. 148-53.

Status of publication and patent

The data from these experiments are currently being analysed and a manuscript is being written for publication based on these results