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Invited Paper

In vivo x-ray imaging of the respiratory system using synchrotron sources and a compact light source

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ABSTRACT

Bright synchrotron x-ray sources enable the capture of high-speed image sequences, both in projection and in three-dimensions via computed tomography (CT). These x-ray movies can capture how biological samples change with time, giving vital information about function as well as structure. The use of a synchrotron x-ray source also provides high spatial coherence, which facilitates the capture of not only a conventional attenuation-based x-ray image, but also phase-contrast and dark-field signals. These signals are especially strong from air/tissue interfaces, which means that they are particularly useful for examining the respiratory system. We have performed a range of x-ray imaging studies that look at lung function, airway surface function, inhaled and instilled treatment delivery, and treatment effects in live small animal models. These have utilised a range of optical set-ups and phase-contrast imaging methods in order to be sensitive to the relevant sample features, and be compatible with high-speed imaging. For example, we have used a grating interferometer to measure how the air sacs in the lung inflate during inhalation, via changes in the dark-field signal; a single-exposure, single-grid set-up to capture changes in the liquid lining of the airways; and propagation-based phase contrast to image clearance of inhaled debris. Studies have also utilised a range of analysis methods to extract how the sample features change within a time-sequence of two-dimensional projections or three-dimensional volumes. While these imaging studies began at large-scale facilities such as the SPring-8 and Australian synchrotrons, we have also recently performed these kinds of studies at a compact synchrotron based on the inverse-Compton effect, at the Munich Compact Light Source (MuCLS).

Keywords: X-ray phase contrast, biomedical imaging, respiratory imaging, Talbot-Lau grating interferometry

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1. INTRODUCTION

X-ray imaging has been widely adopted as a non-invasive method for visualising the interior of a sample, in particular in the field of medical diagnostics. Conventional x-ray imaging is sensitive to highly-attenuating materials, such as metal or bone, but has limited sensitivity to biological structures that are weakly attenuating, such as the lungs, heart and other soft-tissue organs. Research in recent years has led to the development of two x-ray contrast generation mechanisms that exhibit far greater sensitivity to these soft-tissue features; phase-contrast imaging and dark-field imaging.[1–3] These approaches, described in Section 2, are of interest in respiratory imaging because they are particularly sensitive to structures composed of air and soft tissue.

X-ray phase and dark-field imaging were developed in large part using synchrotron x-ray sources (see Section 2), which provide high x-ray flux. This flux enables short exposure times, and hence high-speed image sequences. This is particularly useful when imaging the lungs and airways, both given that they are constantly moving in a live patient, and because their motion is an important descriptor of their function.[4] The ability to use a short exposure time is particularly important when imaging at high resolution, where even a small amount of motion can result in image blur. X-rays provide the distinct advantage of high spatial resolution when compared with other non-invasive medical imaging approaches. This means it is possible to image individual airways, and also evaluate local airway or lung health. The key disadvantage of x-ray imaging is the associated radiation dose, which increases when multiple exposures are collected for computed tomography (CT) or a time-sequence.

There are several possible applications of phase-contrast and dark-field x-ray imaging in the respiratory system. The first is diagnostic imaging, aiming to diagnose disease earlier, and better monitor the progression of disease.[5,6] The potential advantages in this field include the sensitivity of these developing techniques to airway structures, the ability to measure function via a time sequence, and the ability to measure locally. Current methods of assessing lung function—such as spirometry (to produce indices such as FEV1) or multiple-breath wash in/out methods (producing a lung clearance index)—are measured at the mouth, effectively averaging the health of the entire lung into a single global measurement. The ability to look at health locally via x-ray imaging means that a small, local change in lung condition may be more easily detected. This is particularly important for patchy lung diseases such as cystic fibrosis. However, achieving phase and dark-field imaging in the clinic is associated with a number of challenges, including acceptable radiation dose, the size and cost of appropriate x-ray sources, and the stability of imaging set-ups to vibrations and other environmental factors.

The second group of applications are research studies that seek to better understand respiratory physiology[7,8] and test treatment strategies,[9,10] typically in small animal models. As an advantage over studies in tissue cultures or using histology, x-ray imaging allows for *in vivo* imaging. The high spatial resolution that these x-ray setups can provide is particularly important for studies in anaesthetised small animals (e.g. mice) where the entire lungs are not much more than 10 mm wide. Biomedical research studies have the advantage over clinical imaging in that the requirement for them to be completed at a synchrotron source or other dedicated imaging facility is not a major barrier. Because of the longitudinal health risks associated with the high associated radiation dose, these small-animal experiments are typically non-recovery.

This paper focuses on respiratory research studies using x-ray phase-contrast and dark-field imaging, and describes some of our key challenges and latest results. This includes studies completed at large-scale synchrotron facilities such as the SPring-8 synchrotron and the Australian Synchrotron, as well as at the Munich Compact Light Source (MuCLS), a set-up at the Technical University of Munich (TUM) that uses a new source based on inverse-Compton scattering.[11]

2. X-RAY MODALITIES FOR RESPIRATORY IMAGING

Attenuation-based x-ray imaging set-ups are regularly used to image the lungs, but typically result in weak contrast. As shown in the first row of Fig. 1, attenuation by the sample decreases the total intensity of the x-ray radiation that passes through the sample to the detector, and hence reveals the bones of the skeletal system most clearly (Fig. 1, image outlined in green). To capture phase-contrast and dark-field x-ray images, additional optics must be introduced to convert sample-induced variations in x-ray phase and dark-field into detectable intensity variations. This is typically followed by the application of some kind of reconstruction process to the raw images.

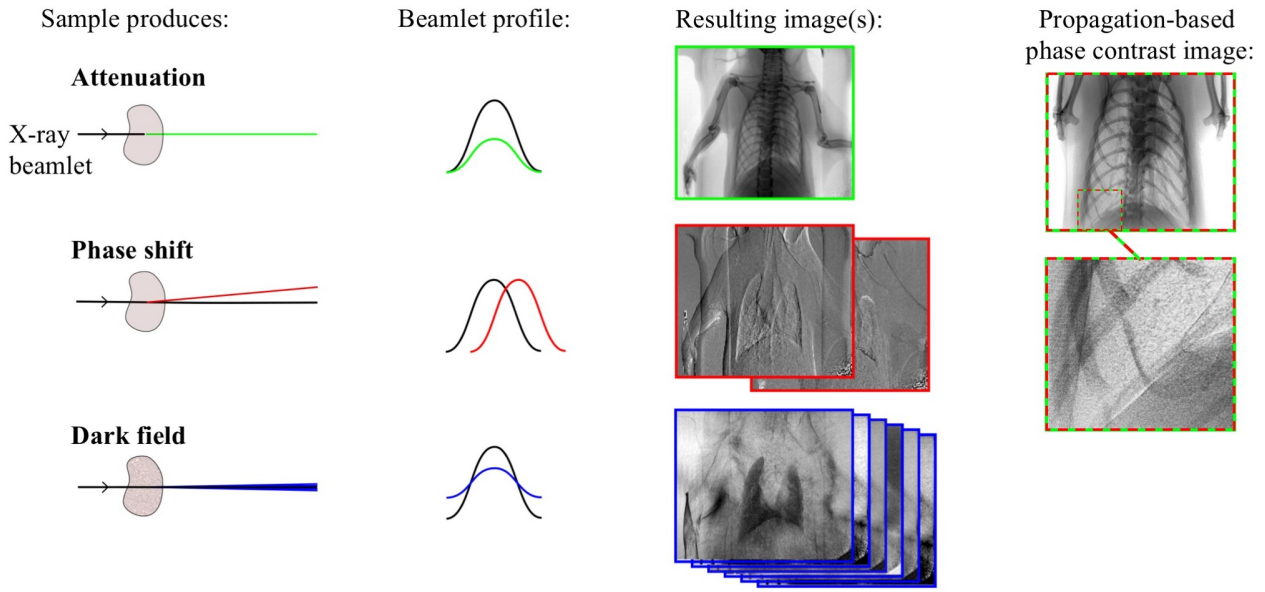

Figure 1. Emerging methods of x-ray imaging can capture three types of image contrast; Attenuation (first row), phase (second row), and dark-field (third row). This diagram shows how a beamlet would be altered by a sample for each of these situations (first column), how interaction with the sample changes the intensity profile of the beamlet seen downstream at a detector (centre column) and the resulting image if this information is collected at every pixel across the image (third column), shown here for the chest of a mouse. To the far right, a propagation-based image is shown, which includes contrast resulting from both attenuation and phase effects. An inset shows the periphery of the lung and the speckle pattern seen from the air sacs in the lung. The propagation-based image was captured at the Australian Synchrotron, and the three remaining images were captured at the Munich Compact Light Source using grating interferometry. Note that the secondary phase and dark-field images (indicating a perpendicular direction or complementary measure of the relevant signal) are simulated for illustrative purposes. Note also that the broadening of the dark-field signal in profile is shown as if there were additional beamlets on either side of this particular beamlet, as is typically the case.

Phase-contrast x-ray imaging is sensitive to subtle changes in the x-ray phase that are imprinted onto the x-ray wavefront by the sample or patient. This can be seen as a local change in the direction of propagation of the wavefield (Fig. 1, centre row), so that a narrow x-ray beamlet would move transversely across the image. Because there is a large difference in the phase shift introduced by soft tissue compared to air, the respiratory system shows up very clearly in phase-contrast imaging. If the local angle of wave propagation is captured, then a differential phase image is seen, as shown in Fig. 1 in the images outlined in red. The strongest contrast is seen from the airways and lungs, as well as the outline of the body sitting within the surrounding air. Depending on the technique, images can capture both the horizontal and vertical phase shifts (depicted as the front and back images respectively). Techniques designed to capture differential phase images include analyser-based imaging,[12] grating-interferometry,[13,14] and single-grid[15,16] or speckle-based[17,18] imaging. The experimentally-simplest approach to phase-contrast x-ray imaging is the propagation-based approach,[19,20] where the detector is simply shifted back by a distance (e.g. 1 metre) so that the wavefield self-interferes. This interference results in bright and dark intensity lines wherever there is a strong change in phase, typically around edges or material interfaces. The resulting images—an example of which is shown in Fig. 1 with the green/red dashed outline—contain both attenuation and phase properties, revealing the bones and also a detailed image of the lungs, with bright/dark lines showing up the edge of the airways and lungs. In addition, the air sacs in the lungs act like tiny x-ray lenses, focusing the x-ray light into a speckle pattern (inset of the aforementioned image).[21]

Dark-field x-ray imaging is not sensitive to specific materials, but to sub-pixel structures that scatter the x-ray wavefield, broadening an x-ray beamlet (see third row of Fig. 1). Capturing this broadening allows the presence of small sample features to be detected without having to directly resolve those features. This is particularly useful because large pixels can be used, which are typically more efficient, and hence can enable low-dose imaging.

The strength of the dark-field signal relates to the size and distribution of these sub-pixel structures, as well as the difference in electron density between the two or more materials that make up these structures. The many air sacs in the lungs, typically tens of microns in dimension, generate a strong dark-field signal. Figure 1 shows the strong lung signal in the image outlined in blue, with extra signal seen from the fur of the mouse (particularly where it is compacted under the arm), and the fibrous Micropore™ (3M Corporation) tape that holds the arm in position. Multiple dark-field images are shown to indicate that the direction and shape of the scattering can provide additional information.[22]

Both phase-contrast and dark-field x-ray imaging can be combined with computed tomography (CT) to provide three-dimensional mapping of the weakly-attenuating features of a sample,[23,24] and three-dimensional mapping of the presence and scattering strength of micro-structures.[25]

To be sensitive to these phase changes and dark-field effects, typically the x-ray source must have high spatial coherence, which is seen from a small or distant source. With the advent of synchrotron x-ray sources and micro-focus x-ray sources, high spatial coherence is readily available. While the high spatial and temporal coherence found at a synchrotron source will result in the highest-quality experimental images, some phase-contrast techniques are also robust to low temporal coherence.[26] In the last few years, Lyncean Technologies Inc. (Fremont, USA) has developed an x-ray source based on inverse-Compton scattering,[27] and installed the first machine at the Munich Compact Light Source (MuCLS). This source produces high spatial coherence and low divergence (4 mrad divergence with a 42 µm source size[11]), is close to monochromatic (3-4.3% bandwidth, depending on set energy), and provides high flux density for fast imaging. This makes the source well-suited to fast imaging of the respiratory system,[28–31] and the location of the source on a university campus makes longitudinal or repeat measures studies[32] more plausible than at heavily-booked large-scale synchrotron facilities. The present paper includes results captured using both this source and conventional synchrotrons.

The strong phase-contrast and dark-field seen from air/tissue structures provide a strong motivation to apply these techniques for both clinical imaging of the lung and biomedical respiratory research.[33]

3. FEATURES OF INTEREST IN THE RESPIRATORY SYSTEM

X-ray projection imaging or CT can capture a number of structural changes in the respiratory system that are relevant to respiratory conditions and disease. Imaging could capture either a detrimental change due to the disease, or an improvement in response to an effective treatment. Such measures can therefore provide feedback when testing new respiratory therapies. Note that the references included in the text below provide a few examples of x-ray phase-contrast and dark-field work that has studied the diseases mentioned, however this is not a comprehensive list.

As seen in Fig. 2a, a simple change to capture is a narrowing of the airways, as seen in reactive airway diseases including asthma,[34] Chronic Obstructive Pulmonary Disease (COPD), upper respiratory infections, and bronchitis. The edges of the airways are easily seen in propagation-based phase-contrast imaging, showing up as a bright/dark line, making these kind of measurements possible in projection or in three-dimensions via phase-contrast CT.

Figure 2b shows the detail of the airway surface, relevant to conditions including Cystic Fibrosis (CF) and Primary Ciliary Dyskinesia (PCD). Normal healthy airways are lined with a liquid of 8 - 30 µm in depth, filling the space around tiny hair-like structures called cilia. These cilia move back and forth to propel any inhaled debris or pathogens away from the lungs and towards the mouth, an essential cleaning mechanism for maintaining healthy lungs, known as mucociliary clearance. In CF the airway surface liquid depth is decreased, and in PCD the cilia do not function properly, both of which result in compromised mucociliary clearance. With sufficient spatial and temporal resolution, x-ray imaging can capture the depth of the liquid[9] and the rate of clearance.[35] In order to differentiate the very-similar soft tissue and airway surface liquid, imaging must utilise a differential method of phase contrast (preferably single-exposure[36]),[37] or some kind of marker/contrast agent.[38]

The complex structure of the lungs—including local or global alterations—is particularly well captured by phase-contrast computed tomography. Examples of local changes include the presence of a tumour,[39] or CF-related mucus obstructions.[40] Lung diseases that can result in changes across the lung include emphysema[41] and fibrosis,[42] where the air sacs are increased or decreased in size, respectively, as depicted in Fig. 2c. Changes in

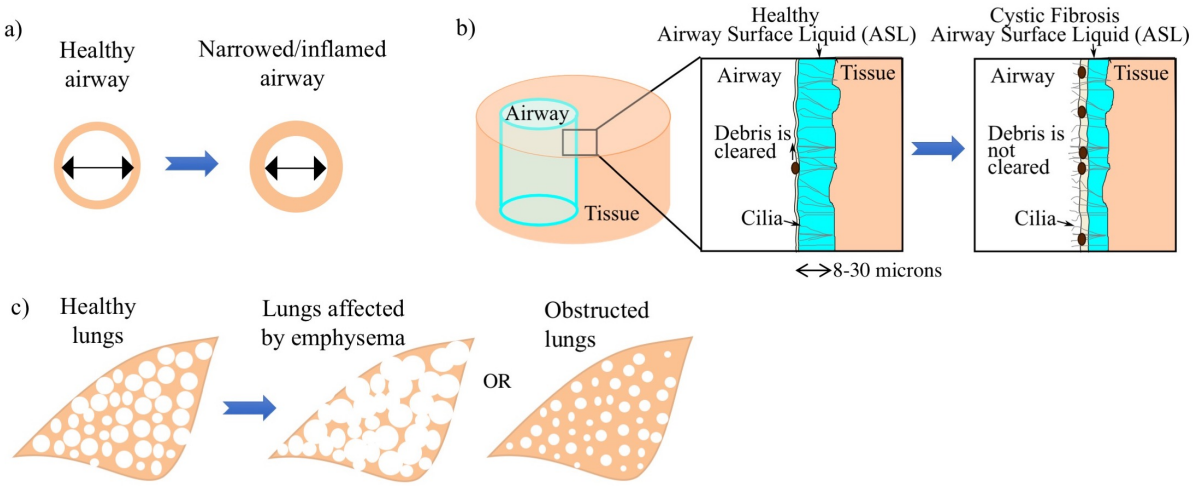

Figure 2. X-ray imaging can be used to capture a) changes in airway diameter, b) changes in airway surface hydration and clearance of inhaled debris along the airway surface, and c) changes in the structure of the lungs. Changes in lung function can be captured via time-sequence imaging.

the size of the air sacs alter the strength of the x-ray dark-field signal,[41,42] or the characteristics of the speckle in propagation-based phase-contrast x-ray imaging,[43] providing opportunities for quantitative measures of air sac health without having to resolve and isolate each air sac via CT. Changes in the structure of the lung over time, as a patient inhales and exhales, will provide functional information.[44] For example, airflow measurements can be inferred by measuring the inflation of local volumes of the lung.[45]

4. CHALLENGES ASSOCIATED WITH *IN VIVO* RESPIRATORY IMAGING

The non-invasive capture of changes in a living respiratory system is associated with a number of experimental challenges.[46,47] The first is sufficient time resolution to capture images without motion blur. The lungs move continuously through the breath, and the airway surfaces move as air passes from the mouth or nose into the lungs and back out again. This means that a short exposure time is required, which is possible using x-ray imaging provided there is sufficiently high flux. High x-ray flux can be found alongside high spatial coherence at synchrotron sources, or in high-flux small-spot-size sources like liquid-metal-jet sources produced by Excillum,[48,49] or at the MuCLS inverse-Compton source.[11] This flux requirement is particularly strict for high-resolution imaging, so the studies described in Section 5 that examine the airway surface (using a field of view of around 1 mm, see Fig. 2b) all utilise the BL20XU beamline at the SPring-8 synchrotron,[50] where the illuminating beam is around 1 mm across and extremely bright. The whole-lung studies described here have utilised the BL20B2 beamline at the SPring-8 synchrotron, the Imaging and Medical Beamline (IMBL) at the Australian Synchrotron, the MuCLS, or Excillum's liquid metal jet source.

The next challenge—sufficient sensitivity to resolve airway structures—is provided by phase-contrast x-ray imaging and dark-field imaging. As mentioned in Section 3, the requirements of a particular study may restrict which phase-contrast experimental set-ups are applicable. Spatial resolution is a third requirement, where x-ray imaging also has an advantage. With sufficiently coherent and intense sources, we have been able to image changes in airway surface liquid depth of only a few microns.

Overlying anatomy can be a problem in *in vivo* imaging, increasing the total attenuation, and hence the required exposure times, while also obscuring the anatomy of interest. While CT can help to isolate a feature, in the case of treatment effects the anatomy may change irreversibly over timescales that are too short to capture a high-resolution CT dataset, and only projection imaging may be possible. However, if the changes are periodic, like the respiratory cycle, multiple opportunities to capture an exposure with the anatomy in the 'same' position are available. While the imaging at the magnification required to visualise the airway surface will typically see movement breath-to-breath, on the whole-lung scale, the differences are typically close to or smaller than

the pixel size. The consistency of position and the available time for an exposure can be maximised by using a ventilator to precisely control the breathing of the animal and to trigger image capture.[46] If necessary, a breath-hold can also be inserted in the ventilation protocol to keep the lungs at a certain pressure for longer image captures.[29] The other factor to address is overlying anatomy. Because of the many air interfaces in the fur of a small animal, strong phase-contrast effects are seen from every hair, and these can obscure the airway surface, particularly when imaging at high magnification. One solution to this is local removal of the fur from the imaging region using hair removal cream.[46]

5. RESPIRATORY RESEARCH RESULTS

This section presents an overview of how our studies have utilised the advantages of phase-contrast x-ray imaging in respiratory research, focusing on some of our most recent results. Note that this section is not meant to be a review of all work in respiratory phase-contrast and dark-field x-ray imaging, and so includes only those references most relevant to the particular studies performed and presented here by the authors of this paper.

The advantages of phase-contrast CT were utilised in our earliest work to visualise the intricate three-dimensional structure of the nasal airways, of particular interest when placing electrophysiological recording probes to target specific airway surfaces where cilia are present.[51] This type of structural imaging has been applied at scales ranging from the mouse nose[52] to the more 'human-sized' lung conducting airways in pigs.[53]

Phase-contrast CT is also useful in capturing the distribution of treatments delivered to the respiratory system. This can be useful in designing treatment delivery devices or methods[29,54] or monitoring treatment effects relative to treatment dose/deposition. As shown in Fig. 3, we recently found that if particulates delivered to the lung are sufficiently large to be directly resolved, then their three-dimensional distribution within the airway tree can be captured. Alternatively, we have shown that a highly-attenuating contrast agent can be added to a liquid delivery to provide stronger CT contrast in those parts of the lung where the treatment has deposited.[31] In addition to post-delivery CT image acquisition, this MuCLS-based project utilised high-speed projection image sequences to capture the dynamics of treatment delivery. Due to the consistency of the breath cycle, a 'pre-treatment' lung image can be subtracted from each image in the sequence to maximise the visibility of the treatment itself. These image sequences revealed the mechanism by which an instilled liquid can be evenly distributed through the lung, seen as an accumulation of liquid in the trachea, followed by a 'secondary aerosolisation' caused by subsequent breaths.

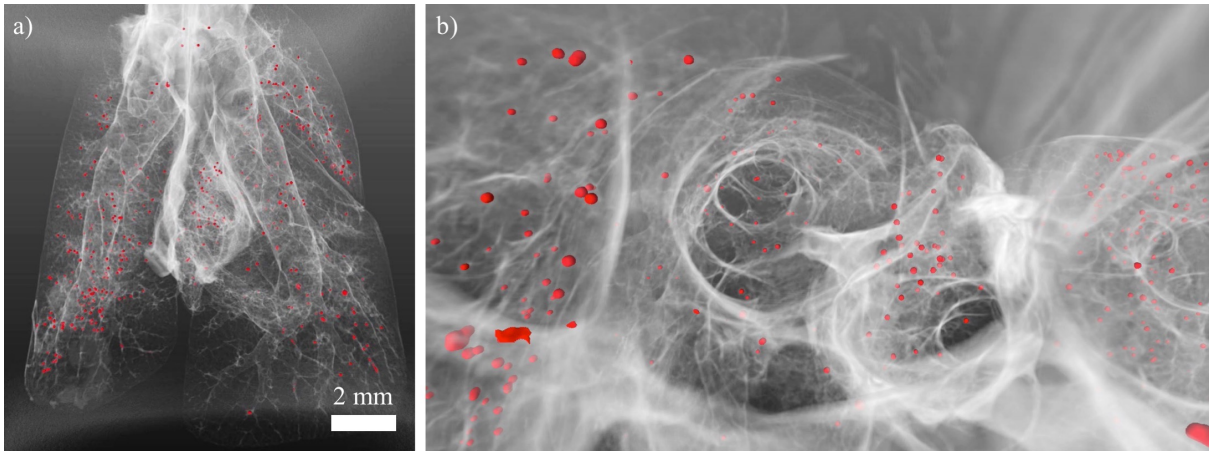

Figure 3. The three-dimensional spatial distribution of glass particles delivered to the lungs is visualised using propagation-based phase-contrast x-ray tomography at the Munich Compact Light Source. Renderings visualise the CT dataset a) from outside the CT volume, and b) looking down into the CT volume from the trachea (top of the image in panel a). The particles were segmented and are shown here in red, and the lungs were rendered using a palette that reaches from translucent for low-density voxels to white for high-density voxels. The lungs have been excised and dried as described in Gradl et al.,[31] and imaged at 25 keV.

The effect of a treatment can also be measured, either on the surface of the airways or within the lungs. Our ongoing studies look at the surface of murine airways to measure changes in airway surface liquid depth[9] and mucociliary clearance rate[35,46] in response to treatments designed for Cystic Fibrosis. These measurements provide real-time, *in vivo* feedback on airway health, and have shown significant differences in airway hydration and mucus clearance between control and active treatments. While it is possible to capture spontaneous clearance of inhaled debris,[51] in order to ensure good statistics, we have introduced tracking particles that are visible using phase contrast to the trachea (e.g. glass beads of diameter in the tens of microns), then imaged the clearance of those particles along the airway surface. Most recently, these clearance measurements have been transitioned to porcine airways[53] at the Australian Synchrotron. In addition, the Munich Compact Light Source has enabled larger-scale imaging that captures particles emerging from the lungs along the bronchi and lower airways. Our other airway clearance studies have examined the types of particles that are most easily cleared from the lung, and those that remain in the airways. Our early work looked at conventional pollutants like asbestos,[55] and more recently we have shown that marker particles of identical size and shape will clear at different rates depending on their surface coating.[56] A key advance in accelerating these airway clearance projects has been the development of automated detection and tracking algorithms to quantify the behaviour of the deposited particles.[57,58]

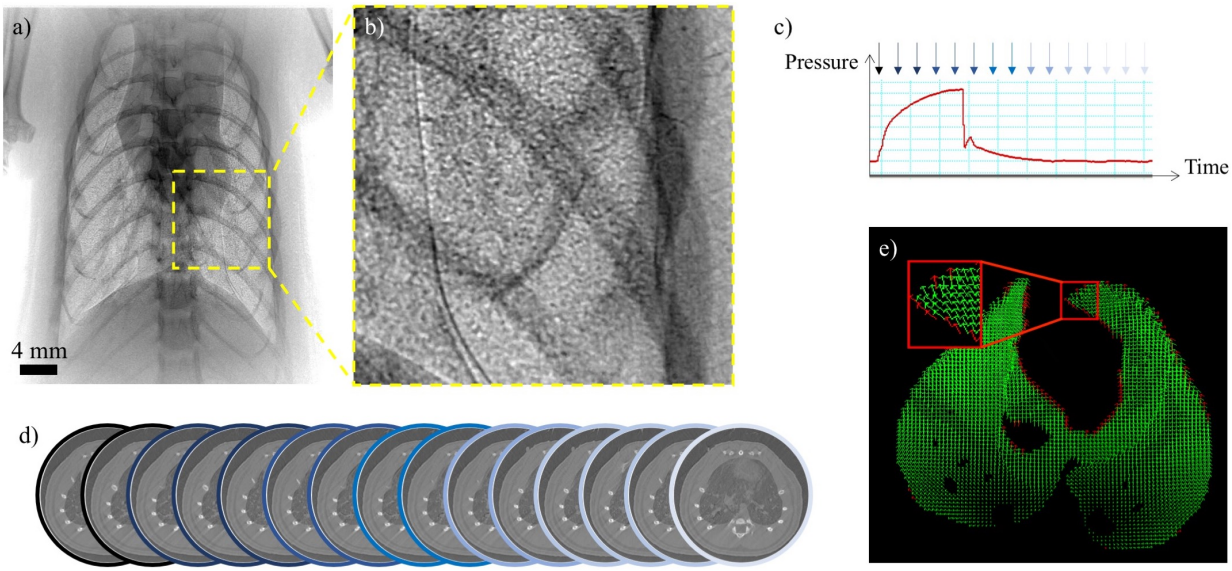

Figure 4. Phase-contrast x-ray CT of rat lungs are captured throughout the breath cycle at 30 keV, using the IMBL at the Australian Synchrotron. a) A projection image of the lungs shows b) the speckle pattern produced by phase contrast imaging of the lung tissue. This pattern can be locally tracked during the breath, to measure the movement of the lung. To capture these dynamics in three dimensions, the rat can be ventilated so that c) 15 images are collected per breath cycle. The next 15 projections are acquired over another breath cycle at the next angle in the CT sequence, with images collected over a total of 180° to enable d) CT reconstructions of the lung volume at 15 breath points. e) Utilising XV, the movement of the lungs can be extracted and plotted as vectors (one axial slice shown here, with red arrows indicating a high-uncertainty measurement and green a low-uncertainty measurement).

The effect of a treatment or disease can be measured throughout the lung using x-ray phase-contrast or dark-field imaging. A diseased lung will not only have altered structure, but also dynamics.[59] In order to capture these dynamics, we have been imaging the lungs with CT at 15 points through the breath-cycle, and locally measuring the lung tissue expansion. This work utilises techniques developed by Fouras, Dubsky et al.[44,45] that apply x-ray particle image velocimetry (XV) to track the motion of the speckles seen in phase-contrast x-ray images of the lungs (Fig. 4a and b). This technique is being developed commercially by Australian Company 4Dx[60] for clinical application, primarily in projection, and we are utilising the approach in biomedical research to evaluate lung health in three dimensions. In these lung XV studies, the small animal is ventilated, and images are acquired continuously as the animal is rotated by 180° (since the x-ray source is fixed and the beam has negligible divergence). A trigger signal is sent from the ventilator to the detector via our Arduino-based timing

box,[47] configured to send exactly 15 trigger points per breath cycle (see Fig. 4c, where each arrow indicates a trigger for image capture). After acquisition the projections are sorted into breath points, and a 3D CT volume is reconstructed for each breath point, as shown in Fig. 4d. XV algorithms can then track the motion of the lung tissue to produce a vector plot in three dimensions over 15 timepoints (one slice is shown in Fig. 4e). This dataset can then be used to calculate the local expansion of the lung. The local measures of health can be used in a number of ways, for example, to understand the presentation of disease,[40] to look at changes in lung health in response to treatment, to study how motion of the heart affects the lungs,[61] or how high frequency ventilation can oscillate the lung tissue.[8] Our ongoing studies at the Australian Synchrotron and the Monash liquid-metal-jet source[49] are looking at the lung disease associated with Cystic Fibrosis, and lung injury associated with artificial ventilation.[62]

The health of the lung can also be revealed via dark-field imaging. Studies have been published showing that the dark-field signal is locally reduced in the presence of lung cancer,[39] fibrosis,[42] emphysema[41,63–65] or a pneumothorax.[66] This Munich-based research program is now looking at translating this diagnostic capability to the clinic.[5,6,67,68] The dark-field images in these studies have usually been captured over multiple breaths[63–65] or while the lungs are stationary using a breath-hold,[67] since around 7 exposures are used to extract a dark-field signal when using a grating interferometer. In our recent small-animal work at the Munich Compact Light Source, we captured the dark-field signal generated by the lung at time points spread throughout the breath.[30] This was possible using a similar approach to the lung XV work, utilising the repeated motion of the lungs to provide additional opportunities to collect the multiple exposures required to extract the dark-field signal. In this case, the set of images through the breath cycle shown in Fig. 4c was repeated for a number of interferometer grating positions, instead of a number of angular projections. The raw interferometer images could then be sorted into breathpoints to extract a dark-field image at each point in the breath cycle. The resulting images showed a decrease in the dark-field signal as the air sacs of the lungs inflated,[30] while using large, dose-efficient pixels. These time-resolved images can provide deeper insight into how the lung tissue locally expands and identify the most diagnostically useful point in the breath for single-projection clinical imaging.

6. CONCLUSION

The sensitivity and speed of phase-contrast and dark-field x-ray imaging provide unique advantages in respiratory diagnostics and research. These imaging modalities can provide local measures of health non-invasively and at high spatial resolution. In addition, the repeated motion of the lungs presents the opportunity to capture multiple exposures with the lungs in approximately the same position. If these multiple exposures are captured at different angular projections, then CT can be captured, or if the exposures are at different optical configurations, additional image contrast mechanisms can be extracted, such as dark-field. High resolution imaging of the airway surface presents some challenges clinically, including a large local radiation dose and demanding spatial resolution requirements that restrict compatible sources and detectors. Nevertheless, this high-resolution imaging can benefit research studies that seek to better understand and treat respiratory disease. Whole-lung imaging can be performed inside and outside the synchrotron to benefit both research and clinical diagnostics, and is indeed already progressing in the clinical direction via the work of 4Dx[60] and the Munich-based research program.[5,6]

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