Phase contrast x-ray velocimetry of small animal lungs: optimising imaging rates

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Abstract: Chronic lung diseases affect a vast portion of the world’s population. One of the key difficulties in accurately diagnosing and treating chronic lung disease is our inability to measure dynamic motion of the lungs in vivo. Phase contrast x-ray imaging (PCXI) allows us to image the lungs in high resolution by exploiting the difference in refractive indices between tissue and air. Combining PCXI with x-ray velocimetry (XV) allows us to track the local motion of the lungs, improving our ability to locate small regions of disease under natural ventilation conditions. Via simulation, we investigate the optimal imaging speed and sequence to capture lung motion in vivo in small animals using XV on both synchrotron and laboratory x-ray sources, balancing the noise inherent in a short exposure with motion blur that results from a long exposure.

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1. Introduction

Imaging the soft tissues of the body, and in particular the lungs and airways, has traditionally been difficult in the x-ray regime due to the similarities in the x-ray absorption properties of muscle, tissues and liquids. This has made high-resolution respiratory imaging difficult, particularly in clinical diagnosis and treatment monitoring. Current techniques for imaging the lungs, such as magnetic resonance imaging (MRI), x-ray computed tomography (CT) or positron emission tomography (PET), lack either the spatial resolution to resolve airway and alveolar structures or the temporal resolution to image throughout ventilation, or both, and often require the use of contrast agents, radioisotopes or relatively high radiation doses [1]. Additionally, non-imaging methods used to provide quantitative measurements for lung function, including spirometry and plethysmography, lack the ability to measure regional lung function [2,3]. As such, our understanding of the progression and intricacies of chronic lung diseases such as asthma, emphysema and cystic fibrosis, and our ability to diagnose and treat these conditions earlier, can be significantly improved with the ability to image the structure of the lungs and airways at high resolution and the ability to perform regional lung function testing.

Propagation-based phase contrast x-ray imaging (PB-PCXI) has proven to be a forefront imaging modality in the advancement of high-resolution lung imaging [4–7]. Recent applications have combined PB-PCXI with a common engineering technique used to study fluid flows, namely velocimetry. These studies have shown that velocimetry can also be used to study the motion of the lungs during ventilation [3,8], and have shown that altered lung motion, as detected by this combined technique, can be a sensitive indicator of regional lung disease [2]. This technique, combining PB-PCXI and velocimetry, has come to be known as x-ray velocimetry (XV). Lung XV shows potential in reaching clinical application, and so work must be done to optimise laboratory x-ray source imaging systems that can provide the necessary flux and spatial coherence required. Whilst synchrotron facilities have been fundamental to the development of phase contrast lung imaging, they do not possess the financial practicality or convenience for widespread clinical medical imaging. To make such a technique widely available, it is...
crucial that a more compact, less expensive imaging setup be developed, with the ultimate goal of clinical implementation.

In this paper we present an optimisation of imaging rates for XV of the lungs on both a synchrotron, where XV research began and will continue to develop, and also on a laboratory x-ray system, with the translation of XV from the synchrotron to the laboratory being inevitable. We give a brief summary of both phase contrast x-ray imaging and x-ray velocimetry in §2.1 and §2.2 respectively, followed by the important imaging parameters to consider in combining these techniques (§2.3). We detail the methods used in this paper, describing imaging system parameters and how the simulated lung speckle was created (§3.1). We then outline the XV analysis methods (§3.2). The results of optimising the exposure time ratio and, separately, exposure time are discussed in §4.1 and §4.2 respectively, with important implications to future users to obtain the best quality XV from their systems, whether they be synchrotron- or laboratory-based. We conclude the results of this investigation in §5.

2. Background

2.1 Phase contrast x-ray imaging

Phase contrast x-ray imaging utilises the refractive properties of materials to produce high-resolution soft-tissue sensitive images. As an x-ray passes through a sample, changes to both the amplitude and phase of the wavefield are introduced based on the refractive indices, $n$, of the materials within the sample and the sample thickness [9]. The variations in density and atomic make-up of different biological tissues create differences in their refractive abilities, and it is these refractive properties that create the contrast seen in phase contrast imaging. In such a way, PCXI can prove to be significantly more sensitive than conventional x-ray imaging methods [10]. There are numerous ways to convert these phase variations to observable intensity variations, the simplest of which is propagation-based PCXI. This technique differs from the conventional x-ray imaging setup only by the increased distance from the sample to the detector, with no additional optical elements required. In PB-PCXL x-rays produced by a sufficiently coherent source pass through the sample and are refracted according to the refractive indices of the materials present within the object, as can be seen in Fig. 1. As the distorted wavefront propagates towards the detector the rays diverge and interfere, producing a Fresnel diffraction pattern at the imaging plane, resulting in intensity variations in the form of bright-dark fringes. These fringes enhance the visibility of material boundaries, thus allowing subtle features within the sample to have their visibility enhanced. With an increased sample-to-detector propagation distance, $R_2$, the fringes of the Fresnel diffraction pattern initially increase in both width and relative magnitude, thus creating tuneable increased edge contrast [11].

Fig. 1. A schematic of a propagation-based phase contrast x-ray imaging set-up of the lungs. The x-rays propagate over the distance $R_1$ from the source through the sample, where they undergo refraction based on the refractive indices of the material interfaces. The refracted and diffracted x-rays then propagate over distance $R_2$ to the detector, where a Fresnel diffraction pattern is recorded.
2.2 X-ray velocimetry

X-ray velocimetry is used to study the flow of air through the lungs and is a technique based on particle image velocimetry (PIV). PIV is a well-established technique used in fluid mechanics to measure the flow and movement of fluids [12,13]. Conventionally used to image optically transparent systems with lasers, PIV has been extended in recent years to optically opaque systems, such as biological objects, with the use of x-rays [1,14]. The advancement of ultra-fast detector systems and the development of phase-contrast imaging has increased the applicability of this technique to live imaging in vivo, namely to the speckle patterns created from the blood [15,16] and the lungs [2,3]. Thus, the application of PIV to biological systems using x-rays has been dubbed x-ray velocimetry (XV).

In PIV and XV, movement or flow of the sample is determined by statistically analysing two images separated by a known time interval. In phase-contrast XV of the lungs, the speckle pattern created from phase contrast imaging of the lungs provides the pattern through which the movement of the lungs can be traced. The image pairs are divided into many sampling windows, commonly known as ‘interrogation windows’, and analysed by cross-correlation to produce correlation peaks. In each cross-correlation, the distance from the centre to the maximum peak shows the inter-frame displacement for that interrogation window, as can be seen in Fig. 2. By iteratively applying this process from larger interrogation windows to progressively smaller windows, the accuracy and resolution of the speckle displacement between frames can be refined. This displacement can then be displayed as a velocity vector given a known time-lapse between image frames. Post-processing can then allow the user to map local lung expansion and airflow through the lungs [2,3].

![Fig. 2. Schematic diagram illustrating the technique of x-ray velocimetry, where the speckle images taken at two different time points are discretised into interrogation windows, the cross correlation calculated for each window, and the resultant vector field produced to show the motion of the sample.](image.png)

2.3 Important XV imaging parameters

When optimising an imaging system for XV, there are multiple parameters to consider. Optimisation of magnification, source size and pixel size for XV have previously been investigated by Ng et al. [17], and thus the present investigation focuses on optimising imaging rates, in particular exposure time and the time between exposures. Exposure time is a fundamental parameter to optimise when imaging dynamic samples. If the exposure time is too short then the image will be noisy. Whilst from a phase-contrast image quality point-of-view this is undesirable, noise is particularly problematic for XV analysis of the lungs. When noise distorts the already spatially fine lung speckle the correlation peak will have a small signal-to-noise ratio (SNR), resulting in noise in the motion vectors. However, motion blur becomes an issue when the exposure time is too long, relative to the speed of the lung motion. In lung XV, motion blur will broaden and decrease the
visibility of the lung speckle, reducing the correlation peak height, and hence the ability of XV to be able to accurately track the speckle movement in the presence of any noise. To better understand this, each smeared speckle can be approximated via convolution with a top-hat function. The cross-correlation peak of two top-hat functions is triangular, with the area under the triangle being fixed. The longer the exposure time and the higher the velocity, the more the speckle will suffer from motion blur and thus the shorter and wider the top hat function becomes. This in turn drives the cross correlation peak lower, thus reducing the SNR of the peak and reducing the accuracy of the XV. This concept is explored in more detail in Fouras et al. [18], along with the importance of the time between exposures in XV imaging. In their study of a particle-laced fluid moving through a cylinder, Fouras et al. found that in an image with multiple velocities present during a single exposure, motion blur affected the higher-velocity particles, and thus reduced the cross-correlation peak height for higher velocities. The iterative XV analysis method therefore biased results towards the lower displacement components of the field that had higher correlation peaks, leading to an underestimation of the velocity in the faster moving areas of the image. It was found that optimising the dead-time between exposures relative to the exposure time, henceforth described as the exposure time ratio (exposure time/dead-time between exposures), $\varepsilon_t$, is crucial in preventing this underestimation of velocity. In this study, we investigate the optimal exposure time ratio for XV imaging of the lungs of small animals.

3. Methods

To make PCXI techniques widely available, it is crucial that a compatible synchrotron-independent x-ray imaging setup be developed. However, biomedical research utilising XV continues to advance on synchrotron sources as well. Thus, this study investigates the optimal imaging rate for XV of small animals on both laboratory and synchrotron sources. Simulations are based on existing experimental imaging setups. The laboratory images are modelled on an imaging system based around a liquid-metal jet source available at Monash University, Australia. The source is the Excillum MetalJet D2 70 kV [19], with an In/Ga alloy (65% In, 35% Ga) jet, and images are collected by a Varian PaxScan 2020 + flat panel detector [20]. Synchrotron images are based on in vivo lung experiments performed on the Imaging and Medical Beam Line (IMBL) of the Australian Synchrotron with the ‘Ruby’ beam monitor [21,22].

3.1 Creating the simulated lung speckle

The image generation process is detailed below, however a summary of the parameters used for both the synchrotron and laboratory setups can be found in Table 1. Alveolar diameters in mice are typically in the region of 38-80 µm, with a density of 5500 alveoli/mm$^3$ [23–25]. However with an effective pixel size of 10 µm, used for both imaging systems, and associated source and detector blurring, the resulting speckle features cannot be individually resolved. However, clusters of alveoli can be resolved in both simulation and the experimental trials we performed on the laboratory setup imaging the lungs in vivo. It was thus concluded that larger scale lung structures were responsible for the speckle pattern produced, and hence we modelled our simulated lung speckle images on alveolar clusters of approximately 5-6 alveoli per cluster. As each alveolus within the cluster would not be resolved with a pixel size of 10 µm, each cluster was represented by a 3D model of a single air-filled sphere of 200 µm in diameter, surrounded by lung tissue with a complex refractive index, $n = 1 - \delta + i\beta$ ($\delta = 4.15 \times 10^{-7}$, $\beta = 2.42 \times 10^{-10}$ [26]). This model was then propagated through free space to create a high-resolution (1 µm pixels) 2D phase contrast image (Fig. 3(a)). This process used an algorithm based on the projection approximation and transport-of-intensity equation [27] as shown in [17], incorporating the source size and energy spectrum of the respective synchrotron or laboratory source (Table 1), with the polychromatic laboratory x-ray source images being created by summing images created at energy steps of 2 keV, weighted according to the spectrum of the source.
In order to model how different regions of the lungs expand and contract at different velocities, image pairs were generated that contained a range of different velocities. This was done by rotating a 2D speckle image around the central point, so that all possible directions of transverse movement relative to the pixel grid were present for a range of speeds. The first step in creating this image was to generate a position map for a large number of spheres, which could then be convolved with a sphere image to simulate an experimental speckle image. The position map was generated using a base image of zero intensity and then setting a given number of randomly positioned pixels to intensity = 1.

In order to simulate image blur within an exposure, 150 position images were summed, each a version of the initial position map, but rotated (in degrees) by a fraction of the overall rotation that would take place during an entire exposure. The summed image contained rotating streaks of length specified by the length of the exposure time and speed of rotation. It was observed that if the 150 images were each rotated by exactly 1/150th of the full rotation, moiré fringes appeared. To overcome this, the $n^{th}$ image in the sum of 150 images used a rotation of:

$$\text{rotation of } n^{th} \text{ image} = \left( n \pm \sum \right) \frac{\text{full rotation angle}}{150}$$  \hspace{1cm} (1)

where $\sum$ is a normal random variable of mean 0 and standard deviation 0.2. This resulted in a much more realistic, smooth blurring of the speckle image as a result of motion (Fig. 3(b)).

This image sum was then divided by the total number of images (150) to normalise the background intensity. The result was taken as the alveoli position map for the first simulated lung image in the image pair, referred to as T1. The second time-point image, T2, was then generated by rotating the T1 position map by a set number of degrees, $\theta$, where:

$$\theta = \text{speed of rotation} \left( \text{exposure time} + \text{dead-time between exposures} \right) \hspace{1cm} (2)$$

The motion-blurred position maps (Fig. 3(b)) were then convolved with the image of the phase-contrast sphere (Fig. 3(a)) to generate the speckle images (Fig. 3(e)) at different time points. Images rotating at 10 revolutions per minute (rpm) were created. The average number of pixel counts in each image was adjusted based on exposure time and imaging source (i.e. synchrotron vs. lab), in line with calibration experiments performed on both the IMBL and Excillum setups. Images were then binned to 10 µm square pixels to simulate a realistic imaging setup, creating images 1296 × 1296 px in size. The blurring effects of both the finite source size and the point-spread function of the detector were then added to the speckle images using Gaussian based convolutions. Noise was then added to the images based on a Poisson distribution of the number of photons, which was calculated relative to the number of pixel counts in the images based on experimental calibration. The final simulated images represented an experimental image with the experimental dark field already subtracted, which is a standard experimental post-processing step.

3.2 XV analysis

For the XV analysis to determine the vector map of our rotating lung speckle images, an iterative approach was used. Initial vectors from 256 px² windows, placed every 16 px, gave an estimate of the displacement of the lung tissue. A further four passes then refined the vector lengths and directions, down to final interrogation windows of 16 px², every 16 px. Vectors were rejected where the calculated velocity deviated by greater than 1.5 px from the predicted velocity, based on the trends of the surrounding vectors. For the lab source images, an 8 px median filter was first applied to the simulated experimental images to decrease high frequency noise, as can be seen in Fig. 4, a step that is commonly applied in XV to high-noise images.

To evaluate the quality of the XV, a perfect displacement vector field for each exposure time was generated for the given rotation speed. This enabled the effects of detector, source, and most importantly, motion blur, on the accuracy of the velocity
vectors to be assessed. Error vectors were produced by subtracting the retrieved experimental vector field from the perfect vector field.

Fig. 3. Generation of simulated lung speckle images with a rotational motion centered at the middle of the image. (a) A high-resolution (1 µm pixel size) phase contrast x-ray image of air-filled sphere surrounded by lung tissue, simulating a single alveolus, generated using a polychromatic spectrum based on the Excillum source. (b) Single white pixels build the sphere position map for generating the motion-blurred speckle image. The sphere image from a) is convolved with the motion-blur position map from b) and the intensity is adjusted dependent on exposure time, with (c-d) showing close up images of how the speckle is generated by increasing the number of overlying spheres. (e) Shows the speckle image generated, with motion-blur evident toward the outer edges of the image, and (f) shows the final image with noise, source and detector blurring added.
Table 1. Summary of parameters used in both producing the lung speckle images based on both a synchrotron and laboratory x-ray source, and the XV parameters analysed

| Parameter                                      | Synchrotron                | Laboratory                                      |
|-----------------------------------------------|-----------------------------|-------------------------------------------------|
| Spectrum                                      | Monochromatic 30 keV        | Polychromatic                                  |
| Peak x-ray energies (keV)                     | 30                          | Excillum source at 70 kVp², with a 0.1 mm thick |
|                                               | 8 & 24                      | Aluminium filter                               |
| R₁ (source to sample distance) (m)            | 135                         | 0.2                                             |
| R₂ (sample to detector distance) (m)          | 2                           | 2.27                                            |
| Magnification                                 | 1.01                        | 12.35                                           |
| Effective source size (FWHM)                  | 5 x 1 mm (width x height)   | 46 x 20 μm (width x height)                     |
| Pixel size                                    | 10 μm                       |                                                 |
| Detector point spread function (FWHM)         | 2.2 pixels                  |                                                 |
| Rotation speed                                | 10 revolutions per minute   |                                                 |
| Sphere size (representative of alveolar cluster) | 200 μm                     |                                                 |
| Exposure times (ms)                           | 2.5-80 ms in 2.5 ms increments |                                               |
| Exposure time ratios (exposure time: dead-time between exposures) | 1:1, 1:2, 1:3, 1:4, 2:3, 3:4 |                                               |

*Excillum full spectrum available at www.excillum.com/products-and-services/metaljet-x-ray-sources/metaljet-d2-70kv.html, 25/05/2015*

Fig. 4. Rotationally averaged power spectrum of the lung speckle images at different exposure times, demonstrating that the application of an 8px median filter to the laboratory images, a common experimental post-processing technique, reduces the noise floor of the images, but does not remove the important spatial frequency data associated with the lung speckle.
4. Results and discussion

4.1 Exposure time ratio

Our investigations found that the most accurate reconstruction of vectors occurred when the exposure time ratio (exposure time/dead-time between exposures), $\varepsilon_t$, was set to 0.5, as can be seen in Fig. 5. This means that the most accurate XV was performed when the dead-time between exposures was twice as long as the exposure time. When plotting these points, a ’nearest point’ interpolation showed that the minimum error occurred at $\varepsilon_t = 0.5 \pm 0.1$ consistently for all velocities sampled. This result is consistent with that found in Fouras et al. [18]. Thus a ratio of 1:2 (exposure time:dead-time between exposures) was utilised for the remainder of the simulated studies into optimal exposure time.

As can be seen in Fig. 5, the optimum $\varepsilon_t$ is consistent over the full range of velocities investigated, proving convenient experimentally as this suggests that optimum exposure time ratio is not dependent upon velocity, at least across the range of velocities investigated here. This is particularly beneficial for XV imaging of the lungs as there is a wide range of lung velocities present during ventilation, and using $\varepsilon_t = 0.5$ will consistently provide the most accurate XV analysis.

4.2 Exposure time, as determined by velocity

The optimal exposure time was determined using the optimal exposure time ratio of 1:2. Fundamentally, the exposure time, $t_e$, is the crucial parameter in limiting motion blur and noise. Figure 6 shows the retrieved velocity obtained from the simulated images representing experimental conditions (blue) compared to the theoretical vectors (red).
From Fig. 6 the general trend observed for both the laboratory and synchrotron data is an increase in percentage error observed at the shorter exposure times (i.e. <10 ms) due to the increased noise in the image, which makes it difficult for the XV analysis to accurately track the speckle movement. For this reason, the blue experimental points can be seen more clearly than when compared to the 30 ms data, for instance. A greater number of blue experimental points are visible both above and below the red theoretical vectors, due to the larger standard deviation present in this exposure time. Additionally, particularly at the small velocities near the centre of the image, the small (sub-pixel) displacement makes tracking the displacement of the speckle difficult. As the exposure time increases toward the optimal exposure time for each system respectively, the percentage error in the velocity vectors decreases, and as such so does the standard deviation of the experimental points. For this reason, the blue experimental points seem to ‘blend’ with the red theoretical vectors, and become less visible, because they are closer to the same value. This indicates a higher accuracy XV analysis, and hence a more optimal exposure time. As we continue to increase \( t_e \), the percentage error once again begins to increase as motion blur decreases the speckle visibility and begins to impact the ability of the XV to accurately track the speckle pattern. As expected, when comparing
the laboratory data to the synchrotron data, the laboratory results show increased errors due to the comparatively low photon flux, and hence increased noise level. The laboratory data also results in a reduced number of resulting vectors, with more vectors rejected during the iterative analysis, due to deviations greater than 1.5 px from the local shift. This results in the sparser distribution of blue data points in the laboratory results shown in Fig. 6 than the synchrotron results.

It is interesting to note that both the laboratory and synchrotron XV analysis begin to break down at approximately the same exposure time for a given rotational speed. This was investigated in more detail by plotting the percentage error associated with each velocity and exposure time (Fig. 7), and determining the corresponding rotation and shift of the speckle pattern between exposures. Calculating the percentage error allowed us to (a) visualise the optimal exposure time for a given velocity sample movement for the two experimental set-ups, and (b) explore the limitations of XV in imaging lung speckle and the limitations of our model.

Presented in this way, it becomes apparent that the speckle rotation becomes a significant factor in the accuracy of the XV analysis. Rotation of the entire speckle image results in both translational and rotational motion at a local XV analysis window. If either the rotation or translation is large enough, the XV analysis will not be able to correlate the speckle patterns and will break down. The rotational component becomes problematic when the speckle pattern in each interrogation window has rotated to a degree to which the cross-correlation (which does not consider rotation) can no longer match the respective speckle patterns, and thus a weaker correlation signal results. To this extent, the upper exposure limit of an image rotation of approximately 13° is a limitation when considering rotational flow, as used in the model, rather than a general experimental limitation. Even though the model used here conveys motion that is locally linear (i.e. over small displacements), the affect of the rotation becomes evident in larger displacements, which correlated with longer exposure times. As local lung motion is generally a translation only, this rotational limit is likely not encountered in experimental conditions, particularly if the speckle displacement is within the optimum range. However it is important to also note that the amount of translational motion undertaken during each frame acquisition can also contribute to XV error, and arguably this is the most important contributing factor to XV error in most cases of dynamic imaging. The distance the speckle moves between frames imposes certain limitations on imaging speeds, relative to the size and speed of the speckle, as can be seen by the solid white lines in Fig. 7. It is particularly important to point out that in Fig. 7 the speckle undergoing displacement (i.e. being tracked) is considered as a whole – that is, if the speckle is blurred due to motion within each image, the size of the blurred speckle is considered, where the characteristic size of the blurred speckle may be much larger than the un-blurred speckle. This means speckle moving at different velocities can actually be displaced by the same amount if one considers displacement in the units of blurred speckle widths moved. This is also why the absolute upper limit for blurred speckle displacement in both our lab and synchrotron results is 3, as within our optimised imaging ratio of 1:2 (exposure time:dead-time between frames) the speckle cannot move more than 3 exposure times worth of movement. If a different imaging ratio were to be used, different limits for displacement would apply, however we found this exposure time ratio to be optimal.

Thus, for a 1:2 exposure time ratio, the optimum XV results were obtained when the displacement, measured in blurred speckle widths (bSW), was $2 < bSW < 2.7$ for the laboratory imaging setup, and $1 < bSW < 2.5$ for the synchrotron imaging setup. When $bSW > 2.8$ for the lab system and $bSW > 2.7$ for the synchrotron source, the percentage error of the XV analysis was, for the most part, greater than 50%. Differences in this upper limit can be attributed to differences in effective image resolution, as even though both sets of simulations were run with 10 µm pixels and a detector PSF of 2.2 px, the different effective source sizes of the lab and synchrotron sources will impact the characteristic speckle sizes for each imaging setup, and thus the characteristic size of the blurred speckle. Conversely, when $bSW < 1$ for the lab system and $bSW < 0.5$ for the...
synchrotron setup, again the percentage error is most often >50%, with the effect of noise on the raw image becoming more visually apparent. The presence of noise effectively introduces differences to the speckle between images, which introduces noise to the cross correlation, making small displacements harder to accurately track, and thus reducing the accuracy of the cross-correlation. As such, the lab system encounters a lower limit earlier, due to the decreased flux and increased noise, compared to a synchrotron source.

In principle, a biological object can undergo successful XV analysis, so long as the exposure time and interrogation window size are appropriately selected, and there is sufficient flux and contrast. If the displacement between frames is too great, even with a short exposure time, a solution is to reduce the camera zoom (increase the effective pixel size). In this way the displacement within interrogation windows between frames is reduced, increasing the accuracy of the XV analysis, though at the loss of imaging resolution. The results presented in this paper can be applied to a broad range of imaging systems, provided the characteristic size of the speckle and flux in relation to exposure time is taken into consideration. For these results, simulating small-animal (namely, mouse) lung imaging at common experimental resolutions (taking into account both detector and source size) with current flux capabilities, the velocity of the speckle in px ms$^{-1}$ is also given for quantitative comparison, and it is important to note that the velocities explored in this paper are comparable to the velocities observed of lung tissue in vivo during the breathing cycle of live mice. In Dubsky et al. [3], the majority of velocities measured were ≤ 1 px ms$^{-1}$, and in Dubsky et al. [8] the maximum velocity measured was 7.2 mm/s, which equates to 0.72 px ms$^{-1}$ with 10 µm pixels, less than our maximum velocity of 1 px ms$^{-1}$.

Figure 7 confirms the need for shorter exposure times at higher velocities. The average grey level counts provided in Fig. 7 allow readers utilising sources of higher or lower flux outputs and/or a different detector to utilise our results to identify the most appropriate exposure time for their own system. Additionally, the synchrotron imaging setup provides greater flexibility in exposure times than the laboratory setup, with an increased range of velocities reconstructing successfully at a given exposure time. This is to be expected due to the increased flux available at synchrotron sources, minimising relative noise levels, particularly at low exposure times (i.e. <10 ms). However, that is not to say that laboratory sources cannot achieve useful XV results at imaging rates suitable for live small animal imaging. This is particularly true when using a divergent x-ray source, as this allows for large detector pixels and a thicker scintillator to be used, thus collecting more light per exposure than from an equivalent power low-divergence source. For the Excillum setup modelled here, the optimal exposure time for the range of velocities tested was 35 ms, yet results of less than 20% error were still produced with exposure times in the range of 20-45 ms for most of the velocities tested. For a 3 GeV, 30 keV synchrotron imaging setup, the optimal exposure times can be found from 15 to 40 ms, however a range of 5-55 ms will still give an XV accuracy of <15% for most velocities up to 1 px ms$^{-1}$. This shows that whilst greater experimental flexibility is provided by a synchrotron setup, XV of small animal lungs in vivo is achievable using a laboratory imaging system.

Within these optimal exposure time ranges for high accuracy XV of the lungs on each respective system, it is important to consider the radiation dose rate in relation to in vivo imaging. In the interest of minimising the radiation dose as much as possible, the shortest exposure time within the optimal range should be selected. Experiments performed on the IMBL by Murrie et al. [22], with similar parameters to those simulated here, reported an air Kerma dose rate of 24 mGy.s$^{-1}$. During a 15 ms exposure, the smallest optimal exposure time in range reported for the synchrotron data, this equates to a radiation dose of 0.36 mGy per image, thus resulting in a combined dose of 0.72 mGy per image pair acquired (assuming the source is shuttered for the dead-time between exposures and not taking into account the time required for the shutter to close). The air Kerma dose rate experimentally measured on the Excillum imaging system was found to be 5 mGy.s$^{-1}$, resulting in a radiation dose of 0.175 mGy per 35 ms optimal exposure on the laboratory system, and thus 0.35 mGy per image pair at the optimal exposure time.
Fig. 7. The XV percentage error as a function of exposure time and speckle velocity for laboratory (top) and synchrotron (bottom) generated images. Dotted lines show the degree of rotation between images (i.e. rotation at start of T2 from 0°). Solid lines show the blurred speckle displacement between images. As the total image cycle is made up of 3x exposure times (1 exposure and 2 times the exposure time between exposures), the total blurred speckle displacement cannot be greater than 3 blurred speckle widths.
5. Conclusions

The optimal imaging rates for performing x-ray velocimetry on live small animal lungs, *in vivo*, were determined via simulation for a given laboratory and synchrotron imaging setup. This optimisation focused on the balance required between short exposure times for non-blurred high-speed live animal imaging and longer exposure times for higher SNR. We investigated this relationship using a simulated lung speckle model undergoing rotational motion, allowing us to explore a range of velocities up to 1 px ms\(^{-1}\), a range comparable to lung velocities observed *in vivo* in mice. The use of px ms\(^{-1}\) allows the results presented to be expanded beyond the specific pixel and speckle sizes investigated here. It was found that, independent of experimental setup or lung velocity, the exposure time ratio that achieved optimal XV analysis was consistently 1:2, meaning that the dead-time in between exposures should be twice as long as the exposure time. The optimal exposure time for an Excillum source laboratory setup for the range of speckle displacements/velocities tested was 35 ms, with a range of 20-45 ms still giving results with <20% error for most of the velocities analysed. For a 3 GeV synchrotron imaging setup at 30 keV, the optimal exposure times for the range of velocities can be found from 15 to 40 ms, however a range of 5-55 ms will still give an XV accuracy of <15% for most velocities up to 1 px ms\(^{-1}\). However, the average grey level counts can be used to guide appropriate exposure times for other imaging systems.

It is important to reiterate that the velocity of the object itself is not the limiting factor in XV, but it is the displacement between frames that should guide the selection of imaging speeds. This cannot be understated, and it is this fact that truly allows our results to be expanded and utilised beyond the current experimental parameter ranges presented within this study.

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