Evaluation of intrinsic respiratory signal determination methods for 4D CBCT adapted for mice

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Purpose: 4D CT imaging in mice is important in a variety of areas including studies of lung function and tumor motion. A necessary step in 4D imaging is obtaining a respiratory signal, which can be done through an external system or intrinsically through the projection images. A number of methods have been developed that can successfully determine the respiratory signal from cone-beam projection images of humans, however only a few have been utilized in a preclinical setting and most of these rely on step-and-shoot style imaging. The purpose of this work is to assess and make adaptations of several successful methods developed for humans for an image-guided preclinical radiation therapy system.

Methods: Respiratory signals were determined from the projection images of free-breathing mice scanned on the X-RAD system using four methods: the so-called Amsterdam shroud method, a method based on the phase of the Fourier transform, a pixel intensity method, and a center of mass method. The Amsterdam shroud method was modified so the sharp inspiration peaks associated with anesthetized mouse breathing could be detected. Respiratory signals were used to sort projections into phase bins and 4D images were reconstructed. Error and standard deviation in the assignment of phase bins for the four methods compared to a manual method considered to be ground truth were calculated for a range of region of interest (ROI) sizes. Qualitative comparisons were additionally made between the 4D images obtained using each of the methods and the manual method.

Results: 4D images were successfully created for all mice with each of the respiratory signal extraction methods. Only minimal qualitative differences were noted between each of the methods and the manual method. The average error (and standard deviation) in phase bin assignment was 0.24 ± 0.08 (0.49 ± 0.11) phase bins for the Fourier transform method, 0.09 ± 0.03 (0.31 ± 0.08) phase bins for the modified Amsterdam shroud method, 0.09 ± 0.02 (0.33 ± 0.07) phase bins for the intensity method, and 0.37 ± 0.10 (0.57 ± 0.08) phase bins for the center of mass method. Little dependence on ROI size was noted for the modified Amsterdam shroud and intensity methods while the Fourier transform and center of mass methods showed a noticeable dependence on the ROI size.

Conclusions: The modified Amsterdam shroud, Fourier transform, and intensity respiratory signal methods are sufficiently accurate to be used for 4D imaging on the X-RAD system and show improvement over the existing center of mass method. The intensity and modified Amsterdam shroud methods are recommended due to their high accuracy and low dependence on ROI size.

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Key words: respiratory signal, 4D CBCT, cone-beam CT, lung, image analysis

1. INTRODUCTION

Motion due to breathing presents challenges for the imaging of the thorax and abdomen. 4D imaging and gating have been developed in both human and preclinical settings to study lung motion and certain functions as well as eliminate motion artifacts for better visualization of structures. In either case, a respiratory signal that can be correlated to time during image acquisition is required. In 4D imaging, the respiratory signal is used to sort projections into bins based on their phase within respiration or amplitude and in gating it is used to either trigger acquisition during certain phases or retrospectively select a subset of the projections corresponding to certain phases. A number of groups have developed 4D imaging capabilities and gating on micro CT.^{1–6} One application of 4D imaging is the study of lung function and tumor motion before and after radiation treatments. Recently, high precision radiation delivery systems for small animals have become available that can mimic human treatments. One such system is the X-RAD 225Cx (Precision x-ray, North Branford, CT) which offers treatment to within 0.5 mm and has cone-beam (CB) CT image guidance. It is important to determine the amount of lung tumor motion in order to evaluate the best imaging and treatment strategy that will ensure coverage of the tumor and minimize normal tissue damage. Additionally, as with 4D CT in humans, treatment contours can be drawn based on the tumor motion as determined by 4D imaging. Retrospective gating can be used for better visualization of tumors since it decreases motion blurring. With these goals in mind, we sought to develop 4D and retrospective gating cone-beam CT imaging for the X-RAD system. The focus placed on the respiratory signal as errors in this process can result in errors in the motion amount and tumor trajectory determination. If phase bins are not adequately centered on peak inspirations or expirations, the full extent of motion may not be observed as the average position of the diaphragm within the phase bin shifts away from the maximum or minimum (see Sec. 3.F).

One way of getting the necessary respiratory signal for 4D CBCT or respiratory gating imaging in mice is through the use of an external respiratory monitoring device. A pneumatic cushion has been developed for mice for this purpose. Pressure measurements of a pressure chamber placed under the abdomen of a prone facing mouse were shown to correlate well with respiration-induced organ motion.⁵ The additional setup and cost can be avoided, however, by finding the respiratory signal directly from the cone-beam projections. Several groups have used cone-beam projections to obtain a respiratory signal for mice on other systems. Using a system with a step-and-shoot image acquisition, Chavarrías *et al.* subtracted images at each angle from an average image at that angle to enhance areas of motion.³ They then summed the pixel intensities in the image to get a respiratory signal for each angle. This method was a modification from a previous method by Hu *et al.* in which the pixel intensity values within a region of interest (ROI) were used to find a respiratory signal for each angle.⁶ However, the Chavarrías method cannot be applied as is to systems, like the X-RAD, which do not have the option to acquire data in a step-and-shoot fashion. Bartling *et al.*

tracked the center of mass (COM) in a ROI on a system that could achieve frame rates of up to 100 frames/s with a limited FOV (Ref. 7) and Kuntz automated this method on the same system.⁸ Ertel *et al.* modified the COM method in a system with a slower, 25 frames/s, frame rate.⁴

In addition to the methods mentioned that have been applied in mice, some methods have been tested in human CBCT imaging that may potentially be applicable to small animal imaging as well and provide improvement over existing methods used in mice. Kavanagh, like Hu, used the sum of pixel values within a ROI as the respiratory signal.⁹ In what has become known as the Amsterdam shroud (AS) method,^{10,11} 1D projections of edge-enhanced projection images are used to extract the respiratory signal. While this method was shown to work well in situations where the diaphragm is clearly visible in all of the projection images, the signal becomes difficult to pick out when the diaphragm is not included.^{9,10,12} Vergalasova *et al.* used a method that relied on the Fourier shift theorem^{13,14} to find the respiratory signal. Spatial shifts were monitored by tracking changes in the phase of the Fourier transform. This method showed some improvement over the AS method when the diaphragm was not visible, but showed dependence on the size of the region of interest.¹² They also used the amplitude of the Fourier transform to obtain a respiratory signal. However, this method is effectively the same as the intensity based method of Kavanagh. More recently, Yan *et al.* used feature extraction and principal component analysis on AS images.¹² This method improves upon the AS method by removing background information through the feature extraction and isolating oscillations caused by respirations from those caused by the rotation of the gantry through the principal component analysis.

These methods can potentially be applied to small animal imaging for respiratory signal extraction that does not rely on step-and-shoot image acquisition. Of the methods tested in small animals, currently only the COM method can be used without modification in systems without a step-and-shoot acquisition option. Methods, such as the AS and Fourier transform, that perform well in humans with the right regions scanned and selected for analysis can be expected to also perform well in mice since the entire mouse is in the scan field of view. Direct application of human techniques of respiratory signal extraction in mice is not guaranteed, however, due to the higher frequency of breathing and different breathing patterns. When mice are anesthetized, which is typically done for imaging, they tend to spend much of the breathing cycle in a nearly motionless exhalation, which is followed by an abrupt inhalation¹⁵ (as an example, the mice in this study spent approximately 60% of the time with no diaphragm motion and only 15% of the time in inspiration). The purpose of this work is to compare several of these methods for their applicability in small animal 4D CBCT with modifications being made as necessary to tailor the methods to mouse as opposed to human imaging. The Amsterdam shroud, intensity based, and Fourier transform methods were chosen for their effectiveness in humans. The method used by Yan *et al.* was excluded from this study since it requires an Amsterdam shroud image which was found to be qualitatively different in mice and humans.

These were compared to the existing method, center of mass, for non-step-and-shoot small animal systems to look for significant improvements in accuracy that can be gained by adapting established human methods to mice.

2. METHODS

2.A. CBCT scanning protocol

As part of a separate study, nine mice cone-beam CT scans at 60 kVp and 4 mA were obtained on the XRAD 225Cx (Precision x-ray, North Branford, CT). The five mice used in this study had a strong predisposition to lung cancer and tumors were visible in several of the scans. The mice were anesthetized with 1.5%–3% isoflurane and allowed to breathe freely during image acquisition. A sampling rate of 15 frames/s and a single gantry rotation at 0.5 rotations/min were chosen in order to adequately sample the respiratory signal. In total, roughly 1800 projections were obtained for each scan with about 100 breath periods for each scan. No pixel binning was performed giving a useable projection size of 975×975 pixels of size 0.2 mm.

2.B. Respiratory signal determination

The respiratory signal necessary for 4D and retrospective gating reconstruction was obtained using five different methods as outlined below.

2.B.1. Modified Amsterdam shroud (mAS) method

The first method used was modeled after the so-called Amsterdam shroud method proposed by Zijp *et al.* for respiratory signal determination from human cone-beam CT projections¹¹ and will be referred to as the mAS method. Briefly, a region of interest was manually placed on the first projection such that it enclosed the entire diaphragm and width of the mouse in each of the projections. After log normalization, a Sobel filter (a derivative filter which enhances edges in a specific orientation) was used to enhance edges along the direction of the diaphragm. For each projection image, pixel intensities were summed to create a projection along the cranial–caudal axis, and these 1D projections were combined into a 2D “shroud” image. Previous implementations of this method in humans focused on extracting the sinusoidal variations in the shroud to get the respiratory signal. However, due to the difference

in anesthetized mouse breathing, the mice shrouds contained essentially vertical lines over a slowly varying background as shown in Fig. 1. The following modifications were made to the Amsterdam shroud method as part of this work so that it could be used in mice. An additional Sobel filter was used to enhance the vertical lines in the shroud, as opposed to horizontal enhancement in the original method, giving a set of high contrast lines for each peak inspiration and suppressing the background variations between the peaks. It is not necessary in this case to register each of the columns as was done in the original method. Instead, the columns were summed to give a signal such as the one shown in Fig. 2. This signal is not strictly speaking a respiratory signal since the peak inspirations are not peaks in the signal but a valley between the two peaks associated with the two high contrast lines after edge enhancement. However, these valleys can be automatically picked out of the signal as described in Sec. 2.C, allowing the peak inspirations to be found from this signal.

2.B.2. Fourier transform-phase (FT-p) method

Following the method of Vergalasova *et al.*, the Fourier shift theorem, which states that a spatial shift in a function manifests as a phase shift in the Fourier transform, was utilized to find the respiratory signal.^{13,14} This method will be referred to as the FT-p. Pixels outside of the ROI described above were set to zero and a 2D Fourier transform and its corresponding phase component were calculated. As in Vergalasova, the value of the pixel corresponding to the DC component in the left–right direction and lowest non-DC frequency in the cranial–caudal direction was tracked to obtain the respiratory signal like the one shown in Fig. 2. The zero-padding was done so that looking at the same pixel location gives the same frequency component regardless of ROI size.¹⁴ Without the zero-padding, this method would be more heavily influenced by higher frequencies, such as noise and cardiac motion, for small ROIs since the lowest non-DC frequency division includes a larger range of frequencies.

2.B.3. Intensity method

Based on the method of Kavanagh,⁹ pixel intensity values were summed within a region of interest for each projection and used as the respiratory signal as shown in Fig. 2. We chose not to use a high pass filter to remove the slowly varying component of the signal from the gantry rotation since the

FIG. 1. Diagram of the modified Amsterdam shroud method showing from left to right the region of interest in the original projection, edge-enhanced ROI, horizontal sum, Amsterdam shroud, edge-enhanced shroud, and signal.

FIG. 2. Sections of respiratory signals obtained using the mAS (top left), FT-p (top right), COM (bottom left), and intensity (bottom right) methods. The direction of inspiration and expiration is indicated next to the figures (peak inspiration for the mAS method is a valley between two positively oriented peaks).

algorithm we used to find the peaks in the signal was generally able to locate all peaks.

2.B.4. Center of mass method

The existing COM method tracks the location of the center of mass along the direction of motion of the diaphragm (the z axis). Pixel values in a ROI were projected onto the z axis, and a weighted sum based on the z position ($z = 0$ defined as the center of the ROI) of the pixels was performed.⁷ Mathematically this process is expressed as

$$\text{COM} = \frac{1}{M} \sum_z m_z Z, \quad \text{with } M = \sum_z m_z, \quad (1)$$

where m_z is the sum of pixels at a particular Z location. A representative signal is shown in Fig. 2.

2.B.5. Manual method

Since an external monitoring device was not available for the study, a manual method was used to determine the true respiratory signal. The projection images were scrolled through and locations of the peak inspirations (when the dome of diaphragm was most inferior) were noted.

2.C. 4D image reconstruction

For each respiratory signal, the locations of the peak inspirations were automatically picked out using an algorithm that looked at both the sharpness (first derivative) and value at each point in the signal. Points that had derivatives above a specified sharpness threshold (predetermined fraction of highest first derivative present in the signal) were selected and the difference in projection number of each of these points to the next was determined. Selected points that were within a specified maximum distance from surrounding selected points were grouped together along with a specified number of points to each side of the region. Within each group, the highest valued point was considered a peak assuming that it had a value that surpassed the value threshold (determined in a similar manner as the sharpness threshold). Prior to peak extraction it was assured that the peaks had a positive polarity and that the minimum value of the signal was zero. Modifications to this algorithm were needed to find the peak inspirations in the distinctive mAS signal. Points were grouped together based on sharpness as before. The maximum distance allowed between points within the same group was increased such that both peaks surrounding the valley of interest are considered part of the same group. Each group was divided evenly in half, and the peak for each half was found as before. The lowest point between these two peaks was considered the peak inspiration. Parameters of the algorithm were adjusted for each method

FIG. 3. Peak expiration images obtained as part of an eight phase 4D image (a) and a 4 phase modified 4D image (b) are compared against a full 3D reconstruction (c). The only difference observed between (a) and (b) is that there are noticeably less noise and streak artifacts in the modified 4D image. We do not observe differences in motion blurring between the (b) and (c) even though the projections where the diaphragm are moving have been removed from (b).

but held constant for all testing for a particular method. We observed that, in general, when we were able to visually distinguish peaks from background noise, the algorithm was also able to, but it sometimes missed or added additional peaks when noise levels were high. Other peak finding algorithms can conceivably be used with similar overall results, assuming they can similarly identify peaks in the presence of background noise or slow variations in signal intensity (or these features have been reduced in the signal before peak finding). Each breathing period was evenly divided into eight phases and the projections were sorted into these phase bins. Each phase bin was reconstructed using a FDK algorithm and combined to form a 4D image with voxel sizes $0.1 \times 0.1 \times 0.3$ mm.

2.D. Modified 4D imaging

Since anesthetized mice spend much of their breathing cycle in motionless expiration, most of the 4D phases provide repetitive information. To better utilize the information available, we developed what is essentially a cross between 4D imaging and retrospective gating. Again each breathing period was divided evenly into eight phases with phase 1 as peak inspiration. Phases 3 through 7 were combined before reconstruction and phases 8, 1, and 2 were reconstructed separately. We are left with an image of peak inspiration, two images showing the transition into and out of peak inspiration, and a high quality image showing peak expiration. This method requires prior knowledge of the approximate proportion of time the diaphragm of the mouse moves. We measured the fraction of time the diaphragm moved and how much of that movement was due to the process of inspiration rather than expiration. We looked at the first 200 projections of each mouse and determined the fraction of projections where any movement and movement due specifically to inspiration were observed.

2.E. Region of interest size variation

In order to test the sensitivity of the respiratory signal extraction methods to the size and shape of the ROI, the height and width were varied for two mice for each of the methods. The width was varied from the full width to fully within the mouse (widths: 975, 775, 575, 475, 375, 275, 175, 125, and 75 pixels). For the height variations, the center of the ROI was fixed at the superior end of the dome of the diaphragm at peak expiration and the height increased from 10 to 120 pixels in

increments of 10 pixels (5 on either side of the diaphragm). For each ROI, the FT-p, mAS, intensity, and COM methods were used to find the respiratory signal and the projections were sorted based on these signals.

3. RESULTS

3.A. 4D images

4D images which demonstrated the expected breathing pattern were successfully created for each of the scans. Streak artifacts were observed, particularly in the axial and sagittal views, but did not prevent the study of lung motion in an in-house study using these images.¹⁶ Peak inspiration phase images showed blurring of the diaphragm due to the large amount of motion within a short period of time during inspiration.

3.B. Modified 4D images

Modified 4D images were successfully created for each of the mice. As shown in Fig. 3, the expiration image of the modified 4D image shows the same information as the peak expiration phase of the 4D image but with less noise and fewer streak artifacts. Additionally, there is little difference between the modified 4D peak expiration image and the full 3D image.

3.C. Mouse breathing patterns

We found an average of 39.4% with standard deviation; 10.0% of projections had noticeable diaphragm movement and $16.6 \pm 4.4\%$ of projections showed movement associated with the act of inspiration. We noticed that there was little variation in the number of projections (3.3 ± 0.5 projections) involved in inspiration even when the breathing period showed large variation. This suggests that the mice take a roughly fixed amount of time, approximately 0.2 s, to inhale regardless of their rate of breathing.

3.D. Qualitative comparison of respiratory signal extraction methods

4D images created using the mAS, FT-p, and COM methods were compared qualitatively to images created using the manual method. As demonstrated in Figs. 4 and 5, only very small differences are observed between the images created with each of the methods. From difference images it was

FIG. 4. Coronal slices at peak inspiration [(a)–(e)] and peak expiration [(f)–(j)] for the manual [(a) and (f)], mAS [(b) and (g)], FT-p [(c) and (h)], intensity [(d) and (i)], and COM [(e) and (j)] methods of respiratory signal extraction. Horizontal lines are added to emphasize the difference between inspiration and expiration and to make any differences in diaphragm position between the methods more obvious. No observable differences are noted between images produced by the different methods.

noted that except in a few isolated locations and phases, the difference between the tested methods and the manual method was in the location of the streak artifacts.

3.E. Error in projection sorting

The sorting of projections into phase bins done by the manual method was considered ground truth. The projection sorting for each scan was compared to the ground truth to determine an error associated with each method. For each projection, the absolute value of the difference between the phase bins (numbered 1–8) of the method in question and the manual method was calculated, with wrap-around included such that phase 1 and 8 differed by one. These differences were averaged across the projections, giving an error measurement where 0 signifies no difference in how the projections are sorted, 1 means that on average the projections are off

by one phase bin (12.5% of breathing cycle), and 4 is the highest possible error value. This metric was chosen over one comparing the location of the peaks since it more directly relates to the error in the images and includes the case of a missing or additional peak in a straightforward manner since there are large phase differences in these regions. However, this measurement does face the limitation that a phase shift can show up as having a high error despite the possibility of it only differing in the numbering of the phase bins. Therefore, a second metric was used. The difference between the manual and tested methods' bin assignments for each projection was again calculated, this time without taking an absolute value. A standard deviation of this quantity was taken and used as a metric to evaluate performance. Small standard deviation values would suggest that any errors in projection sorting are due to a phase shift, whereas larger values suggest errors that are more likely to result in errors in the images. However,

FIG. 5. Axial slices at peak inspiration [(a)–(e)] and peak expiration [(f)–(j)] for the manual [(a) and (f)], mAS [(b) and (g)], FT-p [(c) and (h)], intensity [(d) and (i)], and COM [(e) and (j)] methods of respiratory signal extraction. Minor differences are observed between the COM and manual methods and to a lesser extent the FT and manual methods in the peak inspiration images (e.g., white arrows). No observable differences are noted between images produced by the different methods for the peak expiration images or between the mAS and intensity and manual images.

TABLE I. Peak number errors. Difference in the number of peaks detected between each tested method and the manual method for each of the eight mice used in analysis.

mAS	FT-p	COM	Intensity
0	0	0	1
-1	1	1	1
0	0	0	0
0	0	-1	0
0	0	0	0
0	0	0	2
1	0	0	0
1	0	0	0

note that a phase shift of a fraction of a phase bin can limit the ability to fully detect motion (see Sec. 3.F). In addition to these metrics, the difference in the number of peaks detected between the tested and manual methods was quantified and reported in Table I. Figure 6 demonstrates the effect of the error metric on the images by showing images at peak inspiration of selected results which had a range of errors. With an error of 0.2, only minor differences are seen. Differences in the shape and size of structures and a noticeable increase in the blurring of the diaphragm are observed with an error of 0.5, which may have an impact on applications that use these images. Increased blurring of the diaphragm and the structures visible is seen with an error of 1.1. These differences are expected to be less in other phases where there is little difference between adjacent phases due to little motion. Across the eight scans considered, the average error was 0.24 ± 0.08 for the FT-p method, 0.09 ± 0.03 for the mAS method, 0.09 ± 0.02 for the intensity method, and 0.37 ± 0.10 for the COM method (one mouse was removed from quantitative analysis since it was substantially off centered in the projections). The average

FIG. 6. Visual effect of the error in projection sorting on coronal images of the peak inspiration phase. The manual method displayed in (a) is considered ground truth, arrows point to areas where differences were observed in some images, and the horizontal line indicates the top of the diaphragm for the manual method. The effect of increasing the error is shown in the remaining images [errors of 0.2 (b), 0.5 (c), and 1.1 (d)].

standard deviation was 0.49 ± 0.11 for FT-p, 0.31 ± 0.08 for mAS, 0.33 ± 0.07 for intensity, and 0.57 ± 0.08 for COM. The relatively high standard deviation values compared to the mean errors suggest that errors were not generally due to an overall phase shift, although a few isolated cases of phase shifts were observed. The FT-p, mAS, and intensity methods all showed a statistically significant decrease in sorting error and standard deviation over the existing COM method ($p < 0.05$) and mAS and intensity methods showed a statistically significant improvement over the FT-p method ($p < 0.005$). The mAS and intensity methods showed no statistically significant difference in performance ($p = 0.8$ for mean and $p = 0.06$ for standard deviation).

3.F. Effect of error on detected tumor motion

Since these methods may be used to evaluate tumor motion in mice, the effect on such a measurement was evaluated for a tumor in one of the mice (mouse 2 from Sec. 3.G). Rigid registration of each phase to the peak inspiration phase in a ROI surrounding the tumor was done for images obtained using a subset of respiratory signals with a variety of errors. The signals used to create these images include a manual one, one with low error (0.1), one with high error (1.1) but no observed phase shift in the 4D image, and one with medium error (0.5), and an observed phase shift in the 4D image of a partial phase bin. Tracking of the tumor in the image with low error nearly matched that observed in the manual method image (Fig. 7). However, both the phase shifted and high error images underestimated the total tumor motion. This underestimation was primarily due to a blurring of the tumor at peak inspiration with data that were not at peak inspiration.

FIG. 7. Graph of tumor position (a) for images with associated sorting errors of 0 (manual), 0.1 (small error), 0.5 (phase shift), and 1.1 (large error). Images at peak inspiration (left) and expiration (right) of the tumor are shown for the manual method (b), phase shift (c), and large error (d).

FIG. 8. Dependence of the error in projection sorting on ROI height (left) and width (right) for two mice (top and bottom). Error is defined as the average across the projections of the absolute value of the difference between phase bins for the method in question and the manual method. For width dependence, error is displayed relative to the error for the full width of a well performing ROI height such that an error of 1 means there is no change in error between that width and the full width. It is observed that while the error for the mAS decreases gradually with ROI height and the error for the intensity method remains essentially constant, the error for the FT-p and COM methods have relatively high error for small ROIs. Additionally, the mAS and COM show no dependence on width while the error of the FT-p method decreases with decreasing width and the intensity method shows some dependency on width.

3.G. Dependence on ROI size

The error and standard deviation in projection sorting as a function of region of interest height are shown in Figs. 8 and 9 and the difference in number of detected peaks is shown

in Tables II and III. The error and standard deviation have essentially the same height dependence. The intensity method shows the least dependence on ROI height, essentially remaining flat as the height is increased. The mAS method shows a gradual decrease in the error and standard deviation as the

FIG. 9. Dependence of the standard deviation in projection sorting on ROI height (left) and width (right) for two mice (top and bottom). Standard deviation was calculated by finding the difference in phase bin number between the method in question and the manual method and finding the standard deviation of this quantity for all projections. The height and width dependences of the standard deviation are similar to that of the error in Fig. 8.

TABLE II. Height dependence of the number of detected peaks. Difference in the number of peaks detected between each tested method and the manual method.

Height	Mouse 1				Mouse 2			
	mAS	FT-p	COM	Intensity	mAS	FT-p	COM	Intensity
10	0	3	0	0	4	37	0	0
20	0	0	5	0	0	17	0	0
30	0	0	3	0	0	6	0	0
40	0	0	0	0	0	2	4	0
50	0	0	0	0	0	-1	-1	-1
60	0	0	0	0	0	-1	-1	-1
70	0	0	0	0	0	-1	-1	-1
80	0	0	0	0	0	-1	-1	-1
90	0	0	0	0	0	-1	-1	-1
100	0	0	0	0	0	-1	-1	-1
110	0	0	0	0	0	-1	-1	-1
120	0	0	0	0	0	-1	-1	-1

ROI height increases. This decrease is more prominent in the second mouse than in the first. Large errors for small ROIs are observed for the FT-p and COM methods, with the FT-p method only noticeably showing this behavior for one mouse and the COM method having low error for the smallest ROI before peaking. It was observed that this peak was associated with a reversal of the polarity of the peak inspirations. With the same height, the projection sorting error relative to the full width remains essentially constant for the mAS and COM methods. However, for the FT-p method, the error decreases by about 60%–80% from the full width to fully within the mouse. The intensity method shows some width dependence (up to a 45% change from highest to lowest error was observed). However, no clear pattern in this dependence was noted other than that the error was near its maximum for the full width and for ROIs that do not include the full width of the mouse.

4. DISCUSSION

Each of the methods for respiratory signal extraction, the Fourier transform, modified Amsterdam shroud, intensity, and

center of mass methods, were able to be used to create 4D images that had only minor differences from those created using the standard manual method. However, differences were observed in the error and standard deviation in projection sorting and in the sensitivity of these metrics to the ROI size. The mAS method showed little dependence on the ROI size or shape, only a gradual decrease in error as the amount of lung coverage was increased. This method largely picks up on the movement of the diaphragm, so as long as it is fully included, adding extra moving tissue does little to improve the signal. While increasing the ROI beyond the useful area adds extra background, the signal is strong enough after edge enhancements to still be observed and the background usually does not change the location of the peak. This relative insensitivity is useful in attempts to automate the entire process, including ROI selection, as slight errors in ROI selection are not expected to have a noticeable impact on the resulting signal. If careful selection of a ROI is feasible, however, it is recommended for best results to include the most inferior portion of the lungs up to mid-lung or higher and extend from just to the left of the mouse to just to the right.

The COM method similarly showed no dependence on the width of the ROI. Like the mAS method, increasing the width

TABLE III. Width dependence of the number of detected peaks. Difference in the number of peaks detected between each tested method and the manual method.

Width	Mouse 1				Mouse 2			
	mAS	FT-p	COM	Intensity	mAS	FT-p	COM	Intensity
75	0	0	0	1	0	0	0	-1
125	0	0	0	1	0	0	0	0
175	0	0	0	1	0	0	0	0
275	0	0	0	1	0	0	0	0
375	0	0	0	0	0	0	0	0
475	0	0	0	0	0	0	0	0
575	0	0	0	0	0	0	0	0
775	0	0	0	0	0	0	0	0
975	0	0	0	1	0	0	0	0

of the ROI increases the amount of background averaged into the pixel sum and with a strong enough signal does not affect the location of the peaks. The COM method did however show a greater sensitivity to the ROI height. An interesting feature of the height dependence is a prominent peak in the error as the height increases for small ROI heights. This peak corresponds to a change of the peak inspirations being at the valleys of the respiratory signal to them being at the peaks as demonstrated in Fig. 10 (a change in polarity). In the regions of transition of polarity, the height of the peaks decreased making them difficult to distinguish from background noise. In addition to the possibility of peaks becoming lost in the background, the unknown polarity of the signal makes a fully automated peak inspiration determination with this method difficult. To use this method, careful manual selection of the ROI, or sufficiently sophisticated automatic ROI selection algorithm, should be used to ensure the polarity of the signal is known and not in the transitional region. The ideal ROI should reach from the most

inferior portion of the lung to near the most superior portion and from just to the left of the mouse to just to the right.

The FT-p method shows sensitivity to both ROI height and width. This observation is consistent with the assessments of Yan¹² in human patients as well as Vergalasova's^{13,14} own observations. The more dramatic observations in this case compared to human cases are likely the result of the inclusion in the projections of the whole mouse and large margins around the mouse in the projection data. This difference makes for more ways for ROIs to include different sections of lung and background. The FT-p method shows a similar increase in error with changes in height due to a change in polarity as the COM method (although we do not observe the opposite polarity before the transition in this case). The similarity in behavior is expected when we consider that changes in the phase of the Fourier transform are related to the shift in the center of mass of the image by the Fourier shift theorem. However, the FT-p method does not give us exactly the center of mass change, as it only tracks one pixel in the phase image, corresponding to one frequency component of the image. The lower error of the FT-p method may be explained when we consider that the FT-p method is only tracking center of mass shifts of low frequency components of the image while the center of mass method tracks shifts from all frequency components, leading to a noisier signal. The ideal ROI method is the same as that for the COM method, with care being required to avoid large margins to the side of the mouse or small ROIs in the height direction.

The intensity method shows the least dependence on ROI height. However, it shows somewhat unpredictable dependence on the width of the ROI. While the ROI width analysis did not give a clear ideal ROI width, it is recommended to extend the ROI to include small margins to the sides of the mouse. This configuration was used for the overall accuracy measurements and showed relatively little variation. ROIs with widths that are smaller than the mouse or are much larger than it result in the highest errors for this method. When the full width of the mouse is not included in the ROI, we can expect some degradation of accuracy since the volume and density of tissue being imaged (and therefore intensity values) changes for different projection angles independent of any respiratory motion.⁹ In human imaging, the whole width of the patient is not generally included in the scan, reducing the accuracy of the intensity method relative to other methods in human cases¹² compared to in mice. If ROIs that are too wide or narrow can be reliably avoided, the use of either the intensity or mAS method is recommended over other methods. However, when large margins to the sides of the mouse are included in the ROI, the use of the modified Amsterdam shroud method is recommended. One potential issue with the intensity method is the dependence of the pixel values on the position of the gantry, which introduces a slowly varying component to the signal (Fig. 2). The effect of gantry position is less than in humans due to the inclusion of the full width of the mouse. However, there is still a positional dependence due to different scattering properties at different angles.⁹ A high pass filter can be used to lessen this component of the signal. However, with a sophisticated enough algorithm for

FIG. 10. Sections of COM method respiratory signals for different height ROIs: 10 (0.7 mm) (a), 20 (1.4 mm) (b), and 50 (3.7 mm) (c) pixels. The black arrows indicate the location of the first peak inspiration which switches from a peak (a) to valleys [(b), (c)] as the ROI size increases. In the transition (b), some peaks become lost to background noise (e.g., white arrow).

automatically finding the peaks in the signal, this step may not be necessary.

The respiratory signals obtained from these methods can be used for a number of imaging tasks including 4D imaging, retrospective gating, and the modified 4D imaging proposed here. 4D imaging can be used to assess respiratory motion for radiation treatment planning or other basic science applications and does not require prior knowledge of the proportion of time spent in each phase. The modified 4D imaging improves the quality of the peak expiration image without sacrificing motion information. The peak expiration image can be used to more accurately identify tumors without having to separately reconstruct a 3D image. Like retrospective gating, this image also removes motion blurring, theoretically improving the ability to identify tumors and other structures over 3D imaging. However, the effect of the reduction in motion blurring was observed to be minimal in this study. As mentioned in Sec. 2, the modified 4D imaging requires some prior knowledge about the fraction of time spent in motion. While variation between the mice in this study in this area was small enough not to impact these images, there may be differences between different strains of mice, the type of anesthetic used, or the health of the lungs (notably, the mice in this study had induced lung tumors which may affect their breathing patterns to some degree). Large differences in breathing patterns may also impact the respiratory signal extraction methods, particularly the modified Amsterdam shroud method, suggesting that care should be used if these methods are used for mice with qualitatively different breathing patterns to ensure they are performing as expected.

5. CONCLUSION

Three methods for respiratory signal determination for 4D CBCT, the Amsterdam shroud, Fourier transform phase, and intensity were successfully adapted from their original use in humans to use in mice for the first time and compared against the existing method for non-step-and-shoot small animal 4D CBCT, the center of mass method. In the Amsterdam shroud method, modifications were made so that the respiratory signal could be found from the shroud image, which was qualitatively different from that of humans. With appropriate ROI selection, all methods sufficiently determined the locations of peak inspirations better than the existing center of mass method. The intensity and Amsterdam shroud methods had the lowest errors in projection sorting as well as the least sensitivity to the size and shape of the ROI.

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