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Characterization of Speckle in Lung Images Acquired with a Benchtop In-line X-ray Phase Contrast System

A.B. Garson III¹, E.W. Izaguirre^{1,2,3}, S.G. Price^{2,3}, and M.A. Anastasio¹

E.W. Izaguirre: eizaguirre@radonc.wustl.edu; M.A. Anastasio: anastasio@wustl.edu

¹Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO 63130

²Washington University School of Medicine, St. Louis, MO 63110

³Nuclear Science and Engineering Institute. University of Missouri, Columbia, MO, 65211

Abstract

We investigate the manifestation of speckle in propagation-based X-ray phase-contrast (XPC) imaging of mouse lungs *in situ* by use of a benchtop imager. The key contributions of the work are the demonstration that lung speckle can be observed by use of a benchtop imaging system employing a polychromatic tube-source and a systematic experimental investigation of how the texture of the speckle pattern depends on the parameters of the imaging system. Our analyses consists of image texture characterization based on the statistical properties of pixel intensity values.

1. Introduction

Due to the weak absorption of X-rays by soft tissues at diagnostic energies, many lung conditions are challenging to identify radiographically until substantial progression of the disease has occurred. Even with the significant structural changes to lung tissue associated with interstitial lung disease, lung radiographs can appear normal (Lynch, et al., 2000). Detecting pulmonary disease is important as lung cancer remains the leading cause of cancer-related death worldwide (Siegel, et al., 2012). In addition, there remains an important need for the development of improved imaging methods for the early detection of radiation-induced lung damage in animal models and humans.

X-ray phase-contrast (XPC) imaging exploits X-ray refractive index properties of weakly absorbing materials and offers improved image contrast from normally undetectable structures imaged with conventional radiographic techniques. It has been demonstrated that XPC imaging techniques have excellent potential for many medical applications (Lewis, 2004; Donath, et al., 2010). For example, XPC angiography may offer improved visualization of blood vessel structure without the need for contrast agents based on heavy elements, a major risk factor associated with conventional contrast agent angiography (Momose, et al., 2000; Takeda, et al., 2002; Laperle, et al., 2008). XPC imaging has also been shown to improve the visualization of cartilage and to detect early degenerative changes after damage or disease, a task not possible with conventional radiography (Mollenhauer, et al., 2002; Majumdar, et al., 2004). Increasing the effectiveness of digital mammography while minimizing dose has motivated numerous XPC imaging studies which

have demonstrated improved image quality over conventional mammograms with comparable dose (Arfelli, 2000; Arfelli, et al., 2000; Castelli, et al., 2011). XPC technology has been implemented in a mammography system developed by Konica Minolta Medical & Graphic (Tanaka, et al., 2005). These are just a few examples illustrating how XPC imaging is contributing to biomedical applications, for more extensive review see (Bravin, et al., 2013; Fouras, et al., 2009; Nugent, 2010) and references within.

There are several XPC imaging techniques: the simplest form, in-line XPC imaging, requires an X-ray beam with a high degree of spatial coherence, a detector with high spatial resolution, and an imaging system geometry with appreciable distance between the sample and detector (Wilkins, et al., 1996). By generating an X-ray beam using a small source spot size, the image formation process can be modeled using wave optics formalism. When a coherent wavefront interacts with an object, differences in the materials' projected indices of refraction will result in strong deflection of X-rays at interfaces between different media. If the wavefront is allowed to propagate through free space after exiting the object, constructive and destructive interference will occur which can be visualized with a detector having sufficient spatial resolution. The net result is the edge enhancement of the interfaces between different materials providing a method to image the boundaries of weakly absorbing materials. XPC imaging has largely been performed using synchrotron sources which provide highly coherent (spatially and temporally) X-ray beams, but the technique has also been demonstrated on benchtop systems in efforts to bring the method closer to clinical and applied biomedical applications e.g. (Engelhardt, et al., 2007; Pfeiffer, et al., 2006; Zysk, et al., 2012).

A number of preclinical investigations of XPC lung imaging have been conducted (Kitchen, et al., 2005a; Connor, et al., 2011; Jheon, et al., 2006; Yagi, et al., 1999). It has been demonstrated that, when the X-ray beam is sufficiently coherent and a high resolution camera is employed, a speckled intensity pattern can be recorded in projection images corresponding to lung regions. While the speckle pattern resembles a visualization of alveoli themselves, the effect has been attributed to multiple refractions of the X-ray beam as it travels through the lung (Suzuki, et al., 2002). Kitchen *et al.* (2004) numerically modeled the XPC lung image formation process and demonstrated that speckle can be explained in terms of focusing effects due to the air-filled alveoli.

In addition to propagation-based XPC imaging, analyzer based XPC systems (Zhong, et al., 2000; Lewis, et al., 2003; Kitchen, et al., 2010; Kitchen, et al., 2011; Fouras, et al., 2012) and grating-based systems (Schleede, et al., 2012) at synchrotron facilities have provided several instances of speckle visualization. The results from a comparison of XPC techniques in detecting and characterizing lung speckle (Kitchen et al., 2005a) indicate that differences in physical structure of the lung will affect the characteristics of speckle in images acquired with analyzer based phase contrast techniques. Applying phase-retrieval algorithms to propagation-based XPC (Snigirev, et al., 1995; Wilkins et al., 1996; Pogany, et al., 1997; Gureyev, et al., 2001; Mayo, et al., 2003) lung data has provided approximate projected lung thicknesses and demonstrated the potential for improved detection of some lung diseases over conventional chest radiography (Kitchen, et al., 2005b). In fact, *in vivo* imaging of newborn rabbit pups has demonstrated the ability to accurately measure air volume within

the lung at different time points in the breathing cycle (Kitchen, et al., 2008). To the best of our knowledge, all reported studies of XPC lung imaging have been conducted at synchrotron beamline facilities, which restricts the widespread application of the method. Accordingly, there remains an important need for additional investigations to determine the conditions under which lung-induced speckle can be realized in XPC employing tube-based X-ray sources.

In this article, we investigate the manifestation of speckle in propagation-based XPC imaging of mouse lungs *in situ* by use of a benchtop imager. The key contributions of the work are the demonstration that lung speckle can be observed by use of a benchtop imaging system employing a polychromatic tube-source and a systematic experimental investigation of how the texture of the speckle pattern depends on the parameters of the imaging system. Our analyses consist of image texture characterization based on the statistical properties of pixel intensity values.

2. Methods

2.1. Imaging system

The XPC imaging system was supported on an optical table and utilized high-precision rails to allow the sample and the detector to be positioned at distances from the X-ray source ranging from ~20 cm to ~2 meters. X-ray generation was provided by a Kevex PSX10-65W microfocus source (Thermofisher) which has a variable spot size ranging from 7 microns to 100 microns and can operate at tube voltages between 45 kV and 130kV. For this X-ray source, the beam current setting determines the spot size for a given tube voltage setting with larger currents corresponding to larger spot sizes. The X-ray camera is a super-cooled QuadRO: 4096 (Princeton Instruments) which uses a 4096×4096 pixelated Si-based CCD detector with 15 micron pixel pitch, 33 micron effective FWHM resolution, and 36 cm^2 detection area. The CCD is illuminated by a Gd₂O₂S:Tb phosphor screen optimized for 17.5 keV X-rays which is coupled directly to the CCD via optical fibers with a 1:1 taper ratio. During image acquisition, samples were placed on computer-controlled X,Y linear translation stages (Thorlabs LTS150).

2.2. Data acquisition and animal description

This phase-contrast imaging system was operated in projection mode to acquire in-line data of two healthy Balb/cByj mice *in situ*. Both animals were five-months old and weight 20 grams prior to imaging. One animal (M_1) was imaged on May 5, 2012. The second animal (M_2) was imaged on Feb 19, 2013. The mice were euthanized according to the protocol approved by the Washington University in St. Louis Animal Studies Committee. During euthanasia we sustained a positive physiological pressure of CO₂ in the lungs using a plastic cannulation for 10 minutes. The CO₂ flow rate and pressure were then slowly reduced (30 min) to atmospheric values. During this process, a natural post mortem reduction of tissue elasticity occurs and the lungs maintain their natural physiological volume. Minimum or null lung tissue reduction was confirmed in pilot tests prior to these studies in which animals prepared using this procedure were imaged with cone beam CT and autopsied.

For M_1 , the fur was shaved from the chest area. Data acquisition commenced approximately 30 minutes post mortem and lasted six hours and five hours for M_1 and M_2 , respectively. The mice were supported vertically on the imaging platform by attaching the front legs to posts with tape while the bottom of the mouse rested on the platform. The mouse was positioned facing the X-ray source. Throughout the experiments, no stress was applied to the animal thoracic and abdominal region to avoid tissue compression, shifting, or deformation. During imaging sessions lungs were at atmospheric pressure. In future studies designed to correlate speckle texture measures with physical lung microstructure we will control the lung internal pressure (Bates & Irvin, 2003; Irvin, et al., 2003).

Radiation doses (absorbed) were measured with a micro ion chamber CC04 (Scanditronix & Wellhoffer) and a CNMC K602 electrometer (CNMC Co Inc.). A 1 mm aluminum filter was used to pre-harden the X-ray beam during acquisition. Exposure times were chosen so that the largest intensity values in an image corresponded to ~75% of the detector's average full well capacity. Three frames were acquired for each geometry. Corresponding brightfield and darkfield correction data were acquired for each data set.

2.2.1. System geometry study—In the first part of the study, we investigated the dependence of image texture characteristics on the imaging system geometry while keeping the other imaging system parameters constant. Specifically, the total imaging system length was varied while maintaining a magnification factor value of 2. The imaging system parameters employed in this portion of the study are summarized in Table 1 including exposure times, source-to-object distances (SOD), object-to-detector distance (ODD), and the measured doses. The X-ray source was operated with 55 kV bias and a 13-micron spot size. It is important to note that in maintaining a constant magnification factor ($M = (SOD + ODD) / SOD$) and effective spatial resolution in the geometries considered here, both the SOD and ODD become larger with an increase in total system length. The SOD and ODD will each influence the phase-contrast sensitivity of the imaging system for different reasons.

As we are employing a propagation-based XPC technique, increasing the propagation distance (ODD) of the system allows the quasi-coherent X-ray wavefront perturbed by the sample to undergo additional constructive and destructive interference as it travels from the object to the detector, which will in turn increase the strength of the phase contrast signal. Large propagation distances also have an inherent scatter rejection property: With increasing ODD, X-rays which Compton scatter in the object have an increased probability of escaping the system before reaching the detector. When these scattered photons are detected by a pixel that does not represent the original trajectory from the X-ray source, they become a source of noise and degrade image contrast.

The change in SOD associated with different system lengths will also influence the performance of the imaging system. The effective spot size at the object decreases with increasing SOD, and thus, the lateral spatial coherence of the beam ($l_{coh} = wavelength \times SOD / source\ spot\ size$) will be improved in addition to the introduction of a larger propagation distance to the imaging system. It should be recognized that variations in both beam coherence and propagation distance contribute to differences in phase contrast speckle characteristics for this part of the study.

2.2.2. Spot size study—In the second part of the study, we explored the dependence of image texture characteristics on beam coherence by varying the X-ray source spot size while keeping the other imaging system parameters constant. In addition to effects on the beam coherence level, the spot size also is a factor in geometric blur ($blur_{geo} = (SOD / ODD) \times (source\ spot\ size)$) of both the absorption and XPC components of the X-ray signal. The loss of resolution due to $blur_{geo}$ becomes more significant to detected intensity signals as its value approaches the characteristic spatial resolution of the detector. For this part of the study, $blur_{geo}$ is greater than the detector resolution for source spot sizes larger than 18.5 microns.

The mouse was placed 39.7 cm from the source and 110.3 cm in front of the detector resulting in a magnification factor of ~3.8. The X-ray tube was biased at 120 kV and the tube current was varied so that data was acquired for six different spot sizes. Spot size values were measured by the X-ray source manufacturer using the The British Standard Method (Standards, 1988). The imaging system parameters employed in this portion of the study are summarized in Table 2 including exposure times, spot sizes, and the measured doses.

2.2.3. Beam spectra study—To better understand the influence of the X-ray beam spectrum on lung speckle characteristics, we repeated the spot size investigation described above using the same system geometry with a lower X-ray tube voltage of 55 kV. The imaging system parameters employed in this portion of the study are also summarized in Table 2 including exposure times, spot sizes, and the measured doses.

2.3. Analysis of intensity speckle

All image data were corrected for system noise and non-uniformities by subtracting the frame-averaged background data from the image data and brightfield data and then dividing the image data by the brightfield data. Image data were further processed by thresholding intensities to within the top/bottom 2% of a 100-bin histogram. Pixel intensities were then normalized to values between 0 and 1 for each image prior to region extraction. These two processing steps were not required for texture analysis and general texture measure trends change little if the steps are omitted. Their inclusion in the analysis chain provides increased separation in texture measure space between results from different regions-of-interest (ROI) in an image. ROIs were selected for texture analysis from three lung regions and three non-lung regions from each data set. Intensity histograms were constructed with 100 bins for each ROI and used to calculate the statistical texture measures as described below.

A straightforward method to characterize image texture is to examine the statistical properties of intensity distributions. This is often accomplished by constructing and evaluating intensity histograms. Let $f[n,m]$ denote the recorded XPC image, where n and m are pixel indices. One can obtain the approximate probability density of occurrence of the intensity levels, $p(i)$, by dividing a given histogram, $h(i)$, by the total number of pixels

$$p(i)=h(i)/NM=\frac{1}{NM}\sum_{x=1}^N\sum_{y=1}^M\delta[f[n,m],i];i=1,2,\dots,G \quad (1)$$

where N and M are the number of pixels in the x and y directions, respectively, $\delta[j, i]$ is the Kronecker delta function, and G is the total number of quantization levels in the image. We use statistical properties of intensity histograms as a basis for identifying and measuring the texture quality of grayscale images. The six descriptive quantities we employ in this study are summarized in Table 3. These quantities include five statistical characteristics of image histograms (mean, standard deviation, entropy, uniformity, and relative smoothness) and visibility (V), which is based on the maximum and minimum pixel intensity values of a given ROI (i_{max} and i_{min}).

3. Results

Images representative of the system geometry study and the spot size study are shown in Figs. 1-(a) and (b), respectively. The rectangles in Fig. 1-(a) denote the 100×100 pixel² ROIs employed in the system geometry study, which had physical areas of 750×750 μm^2 . The rectangles in Fig. 1-(b) denote the 100×100 pixel² ROIs employed in the spot size study, which represent physical areas of 400×400 μm^2 . Figs. 1-(a) and (b) show M_1 while Figs 1-(c) and (d) show corresponding data of M_2 . Similar ROIs were selected from each animal's data, although differences in anatomy and position on the imaging platform prevented identical ROI selection. In these images, speckle features are observed in lung regions due to the high phase-contrast sensitivity resulting from the imaging system settings. The following sections describe how measures of the image texture change with the imaging system geometry, spot size, and beam spectrum. It is important to note that no separation of the lung tissue from the rib cage is observed in these images which confirms the absence of lung collapse during data acquisition.

3.1. System geometry dependence

The change in lung speckle appearance for different imaging system lengths is shown in Figure 2. This figure shows the left lung ROI (Figure 1-(a)) acquired at the five imaging system lengths considered in the study. The ROIs show a 750×750 μm^2 region of the lung. For the longest system length (Figure 2-(e)), the speckle pattern is prominent and intensity variations occur on short spatial scales. As the system length decreases, the speckle becomes less pronounced and the overall texture becomes smoother. We quantified this change in appearance by computing the texture summary measures defined in Section 2.3. The values are plotted in Figure 3 versus imaging system length for the six ROIs shown in Figure 1-(a) and (c). The first and third columns give results for M_1 and the second and fourth columns show results for M_2 . For each ROI and system geometry combination, the data point represents the average texture measure value from three frames and the error bar gives the standard deviation. Error bars smaller than data point size are not visible.

The mean intensity histogram values are constant for every ROI (Figure 3-(a) and (b)) regardless of imaging system length. This indicates that the different geometries may change the texture qualities of an image, but the intensity distributions gather around the same mean

value for a given ROI regardless of the system length. The other texture measures (Figures 3-(c-l)) show a dependence on imaging system length for lung regions but change little for non-lung regions. All of the ROIs are separated in the parameter space for all system geometries, but are more easily distinguishable as the system length increases.

In addition to calculating texture measures for specific ROIs, global maps of texture features were produced for overall comparisons of the in-line images. Using the central 3600×3600 pixels of an image, texture measures were determined for the 31×31 pixel² region surrounding a given pixel. The measure value was then assigned to that central pixel. The results for *relative smoothness* are shown in Figure 4 (M_1). The panels from left-to-right represent increasing imaging system length. These results demonstrate that the texture characteristics in the lung region, as reflected by the *relative smoothness* measure, vary significantly as SOD and ODD increased. The *relative smoothness* texture measure maps shown here are representative of the trends for all texture quantity maps: the lung regions' texture quantities have a stronger dependence on imaging system parameters affecting phase contrast sensitivity compared to non-lung regions.

3.2. Spot size dependence

The qualitative change in lung speckle appearance for different spot sizes is readily apparent in Figure 5. This figure shows region (A) from Figure 1-(b) for the six spot sizes considered in the study. For the smallest spot size (Figure 5-(a)), the speckle pattern is apparent with intensity variations occurring on short spatial scales. As the spot size increases, the overall speckle becomes less distinct, eventually disappearing to a relatively smooth texture for the largest spot size. We quantify this change in appearance using the texture characteristics defined in section 2.3. The values are plotted in Figure 6 versus spot size for the 6 ROIs shown in Figure 1-(b) and (d). The panels of Figure 6 are arranged as in Figure 3, with the first and third columns showing results from M_1 . For each ROI and spot size, the data point represents the average texture measure value from three frames and the error bar gives the standard deviation. Error bars smaller than data point size are not visible.

Similar to the system geometry study results, the mean intensity values are the only texture characteristic that shows little dependence on spot size for every ROI (Figure 6-(a)). For the other texture measures (Figures 6-(b)–(f)), lung regions show a clear dependence on spot size (*open symbols, dotted lines*), while non-lung regions (*filled symbols, solid lines*) remain fairly constant except for the smallest spot size. All of the ROIs are well-separated in the parameter space for small spot sizes, but approach the same values for larger spot sizes.

Maps of the speckle visibility were constructed from the M_1 XPC image data acquired with different spot sizes in the same manner described in Section 3.1 and are displayed in Figure 7. Differences between the visibility values within the lungs and surrounding regions are recognizable in the cases corresponding to the smaller spot size values. As the spot size increases towards the largest value (right subfigure), the visibility values are reduced such that only materials with largely different physical properties (e.g. rib vs. non-rib) are recognizable. The texture measure map of the visibility quantity shown here is representative of all texture quantity maps: the lung regions become more pronounced as

compared to non-lung regions as system parameters become more conducive for detecting phase contrast signals.

3.3. X-ray tube voltage dependence

We investigated the effects of using a lower tube bias voltage (55 kVp vs. 120 kVp) on image texture measure values. To better understand differences in beam spectral characteristics for the two tube bias voltages, we measured the beam spectrum at 98 cm from the source using a CdTe spectrometer (X-123: Amptek Inc.) and give the results in Figure 8. The spectral measurements were acquired using a 13 μm spot size and with 1mm aluminum filtration. For the 55 kVp and 120 kVp spectra, the (weighted) mean energies are ~ 29 keV and ~ 50 keV with bandwidths of ~ 27 keV and ~ 60 keV, respectively. The bandwidths represent the energy range in which 80% of photons were detected.

For the X-ray source employed in our studies, the spot size range for 55 kVp was smaller than the spot size range for 120 kVp (5 μm – 38 μm and 7 μm – 91 μm , respectively), and the lower kVp spot size study was restricted to spot sizes of 5 μm , 13 μm , 26 μm , and 38 μm . Texture measures were calculated for the ROIs shown in Figure 1-(b) and (d) and the results are plotted versus spot size in Figure 9. As with the 120 kVp results, the mean intensity is constant for all spot sizes. At first glance, the other texture measures appear to have weaker spot size dependence than those shown in Figure 6 for 120 kVp. However, when comparing results for spot sizes that are the same for both tube voltages (i.e. 13 μm , 26 μm , and 38 μm), the results are qualitatively similar. Figure 10 displays the standard deviation and visibility texture measures for these three spot sizes. Results for M_1 and M_2 are shown in the top and bottom rows, respectively. The measures were calculated from lung regions for both the 55 kVp (*dotted lines*) and 120 kVp (*solid lines*) tube settings. The results from the two kVp values display similar trends for increasing spot size.

4. Discussion

We have demonstrated the ability to acquire XPC images of mouse lungs using a benchtop system with sufficient sensitivity to reveal speckle features. We quantified the quality of the lung speckle texture using statistical measures derived from the intensity histograms extracted from the images and demonstrated that these lung speckle characteristics have a strong dependence on imaging system parameters that are important for XPC sensitivity.

The fact that beam coherence level and propagation distance affect the image quality for propagation-based XPC imaging systems is well-expected. By quantifying the texture of lung images, we gained insight into the relative importance of these system settings in providing a means to distinguish regions in lung images that possess similar appearance but with slight variations that represent small differences in physical structure. Comparing the plots in Figures 3 and 6, we see that the spot size dependence of texture measures is strongest for small spot sizes and changes relatively little for spot sizes in the range between 36 and 91 microns. However, the imaging system length dependence is rather linear over the entire range of distances considered in the study. With larger imaging system lengths comes an increased separation in texture measure space between values from lung and non-lung regions. It is reasonable to expect that a change in the trend will occur if the propagation

distance approaches the *near-field Fresnel* regime ($ODD_{Fresnel} \approx (feature\ size)^2 / wavelength$) which is located at ODD 36 m for 30-micron diameter alveoli and 50 keV mean beam energy. Images acquired in the *near-field Fresnel* regime will bear less resemblance to the original object than those acquired in the *near-field near-Fresnel* regime ($0 < ODD < ODD_{Fresnel}$) as the constructive and destructive interference of the perturbed X-ray wavefront begins to dominate the intensity signal. This disparity between images and the object will continue to grow as ODD surpasses the *near-field Fresnel* regime into the *diffractive far-field* regime where images can be described as the Fourier transform of the objects transmission function.

To facilitate comparison of the plots in Figures 3, 6, and 9, the y-axes for a given texture measure cover the same range. When comparing the figures between different portions of the study, one should be aware that the results correspond not only to different ROIs (Figure 1), but the ROIs represent regions of different physical size. The 100×100 pixel² ROIs correspond to a $\sim 400 \times \sim 400$ μm^2 physical area for the spot size study and a 750×750 μm^2 area in the imaging system geometry data. The lung ROIs for the spot size study are in close proximity to each other in the central region of one lung, while the lung ROIs for the system geometry study are spaced relatively farther apart and sample regions in both lungs.

An important result of this study is found by comparing the dose measurements for the two beam spectra used in the spot size study (Table 2). It is not unreasonable to expect that data acquired at higher kVp settings are accompanied with lower doses based on the physics of x-ray interactions in biological materials. The small difference in texture measure values presented in Figure 10 for the two source settings suggests that the lower dose corresponding to the higher kVp data does not come at a sacrifice to texture measure quantities or trends. However further investigation, such as a dedicated study of dose dependence on speckle features, is required to draw more conclusive results. It is worthwhile to note that shorter exposure times also accompany the higher kVp data.

As visibility is the only texture measure used here that is not derived from an intensity histogram in our analysis, it offers an alternative view of image characteristics. One noticeable difference in the visibility plots is the larger range of values in the results for three frames acquired for a given system setting, especially for lung ROIs. As visibility is calculated from the maximum and minimum pixel intensities in an ROI, fluctuations in noise and beam stability are a larger factor than when using intensity histogram statistics that consider the distribution of all pixel intensity values in an ROI. While visibility values follow the general trend of the other texture measures, the values for different ROIs are not as well-separated as for the other texture measures in the spot size study. This is not the case in the system geometry study: different lung ROIs give similar visibility values which indicates a limited ability to use visibility for mapping characteristics within the lung.

The texture maps shown in Figures 4 and 7 are representative of the resulting maps of all texture quantities considered in the study. They reveal how the appearance of the lungs changes with varying imaging system parameters. The lung is prominent for small spot size and large imaging system lengths. While the appearance of the skeletal and muscle structures remains relatively constant as system settings change, the lung speckle becomes

less visible. The shape of the heart is pronounced for largest imaging system lengths in Figure 4 while it is barely perceptible in the original intensity data (Figure 1-(b)). The central and lower lobe regions of the lungs have relatively strong texture measure signals compared to those from the apex.

It is conceivable to attribute the texture measure trends presented here to physiological changes to the mouse over time during the imaging session. To better understand the time-dependence of the texture measures, data were acquired at six different time intervals over the course of the entire experiment using constant imaging system parameters (55 kVp, 13 μ m spot size, 150 cm system length). Texture measures were calculated for two lung ROIs and two non-lung ROIs extracted from each data set. The results for mean intensity and standard deviation are shown in Figure 11. These two quantities form the basis for the majority of texture measures presented in this study. It is evident that the measures show no time-dependence for any of the ROIs, indicating that the change in texture measure values for different system parameters is unlikely to be the result of physical changes to the mouse (e.g. the amount of air in the lungs).

It is difficult to use the results presented here to evaluate the texture measures' effectiveness as indicators of physical structure, as this initial study's goal is to show how the texture measures depend on the imaging system settings, not the properties of the object. It is important to note that the presented results are unique to the imaging system and objects used in this study as XPC sensitivity depends largely on the specification of imaging system components. To conclusively draw a line connecting a texture measure's value and a physical quantity (e.g. density, projected thickness, alveoli sizes) an in-depth investigation with numerous well-characterized lung phantoms in tandem with mathematical modeling is necessary. Such a study is beyond the scope of this work, although we are developing an investigational framework for that exact purpose. Nonetheless, it remains interesting to compare the results between the two mice. In the system geometry study, we find that the texture measure values are slightly larger for M_1 than M_2 while resulting texture quantities are more comparable in the spot size study. The animals were the same age and weight at the time of imaging and the differences between Figures 1-(a) and (c) indicate that the mice were not positioned identically during data acquisition. Overall, there is remarkable agreement between the texture measure trends from the two animals. This indicates that our analysis methods are robust with respect to mouse positioning. While the texture measure values may be different for similar ROIs and imaging system settings between the two mice, these values change in the same manner as imaging system parameters are varied.

In addition to modeling and imaging phantoms, continued *in situ* imaging combined with histological results could allow for improved determination of the relationship between an image's speckle properties and physical lung structure. Investigating lung texture measures *in vivo* would allow for characterizing lung speckle at different times in the breathing cycle associated with differing volumes of air in the lung. While the implementation of such an experiment with a benchtop system will be challenging due to long exposure times, short respiration time scales, and movement due to breathing, instances of *in-vivo* small mammal lung imaging have been accomplished using a gating technique to monitor the breathing cycle and trigger acquisition (Kitchen et al., 2008). It may be necessary to sacrifice some

XPC sensitivity by reducing propagation distance and increasing spot size in order to implement reasonable exposure times. However, carbon nanotube field emission X-ray sources offer extremely short yet bright X-ray pulses and have been used to provide X-ray images with minimal motion artifacts e.g. (Cao, et al., 2009). We believe the results presented in this article will provide guidance for the preclinical implementation of *in-vivo* XPC lung imaging.

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References

- Arfelli F. Synchrotron light and imaging systems for medical radiology. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. 2000; 454(1):11–25.
- Arfelli F, et al. Mammography with Synchrotron Radiation: Phase-Detection Techniques I. *Radiology*. 2000; 215(1):286–293. [PubMed: 10751500]
- Bates JH, Irvin CG. Measuring lung function in mice: the phenotyping uncertainty principle. *Journal of Applied Physiology*. 2003; 94(4):1297–1306. [PubMed: 12626466]
- Bravin A, et al. X-ray phase-contrast imaging: from pre-clinical applications towards clinics. *Physics in medicine and biology*. 2013; 58(1):R1. [PubMed: 23220766]
- Cao G, et al. A dynamic micro-CT scanner based on a carbon nanotube field emission x-ray source. *Physics in Medicine and Biology*. 2009; 54:2323. [PubMed: 19321922]
- Castelli E, et al. Mammography with synchrotron radiation: first clinical experience with phase-detection technique. *Radiology*. 2011; 259(3):684–694. [PubMed: 21436089]
- Connor D, et al. Diffraction enhanced imaging of a rat model of gastric acid aspiration pneumonitis. *Academic Radiology*. 2011; 18(12):1515–1521. [PubMed: 21958600]
- Donath T, et al. Toward clinical X-ray phase-contrast CT: demonstration of enhanced soft-tissue contrast in human specimen. *Investigative Radiology*. 2010; 45(7):445–452. [PubMed: 20498610]
- Engelhardt M, et al. High-resolution differential phase contrast imaging using a magnifying projection geometry with a microfocus x-ray source. *Applied Physics Letters*. 2007; 90(22):224101–224101.
- Fouras A, et al. Altered lung motion is a sensitive indicator of regional lung disease. *Annals of biomedical engineering*. 2012; 1–10. [PubMed: 22012081]
- Fouras A, et al. The past, present, and future of x-ray technology for in vivo imaging of function and form. *Journal of Applied Physics*. 2009; 105(10):102009–102009.
- Gureyev TE, et al. Quantitative In-Line Phase-Contrast Imaging with Multienergy X Rays. *Phys. Rev. Lett*. 2001; 86:5827–5830. [PubMed: 11415368]
- Irvin CG, et al. Measuring the lung function in the mouse: the challenge of size. *Respir Res*. 2003; 4(4):1–9. [PubMed: 12617755]
- Jheon S, et al. High-resolution X-ray refraction imaging of rat lung and histological correlations. *Microscopy Research and Technique*. 2006; 69(8):656–659. [PubMed: 16770790]
- Kitchen M, et al. Dynamic measures of regional lung air volume using phase contrast x-ray imaging. *Physics in Medicine and Biology*. 2008; 53:6065. [PubMed: 18843172]
- Kitchen M, et al. Phase contrast X-ray imaging of mice and rabbit lungs: a comparative study. *British Journal of Radiology*. 2005a; 78(935):1018–1027. [PubMed: 16249603]
- Kitchen M, et al. Analysis of speckle patterns in phase-contrast images of lung tissue. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. 2005b; 548(1):240–246.

- Kitchen M, et al. On the origin of speckle in x-ray phase contrast images of lung tissue. *Physics in Medicine and Biology*. 2004; 49:4335. [PubMed: 15509069]
- Kitchen MJ, et al. X-ray phase, absorption and scatter retrieval using two or more phase contrast images. *Optics express*. 2010; 18(19):19994–20012. [PubMed: 20940891]
- Kitchen MJ, et al. Phase contrast image segmentation using a Laue analyser crystal. *Physics in medicine and biology*. 2011; 56(3):515. [PubMed: 21212473]
- Laperle CM, et al. Low density contrast agents for x-ray phase contrast imaging: the use of ambient air for x-ray angiography of excised murine liver tissue. *Physics in medicine and biology*. 2008; 53(23):6911. [PubMed: 19001700]
- Lewis R. Medical phase contrast x-ray imaging: current status and future prospects. *Physics in Medicine and Biology*. 2004; 49(16):3573. [PubMed: 15446788]
- Lewis R, et al. X-ray refraction effects: application to the imaging of biological tissues. *British Journal of Radiology*. 2003; 76(905):301–308. [PubMed: 12763945]
- Lynch, D., et al. *Imaging of diffuse lung disease*. B.C. Decker Inc.: 2000.
- Majumdar S, et al. Diffraction enhanced imaging of articular cartilage and comparison with micro-computed tomography of the underlying bone structure. *European radiology*. 2004; 14(8):1440–1448. [PubMed: 15232709]
- Mayo S, et al. X-ray phase-contrast microscopy and microtomography. *Optics Express*. 2003; 11(19):2289–2302. [PubMed: 19471337]
- Mollenhauer J, et al. Diffraction-enhanced X-ray imaging of articular cartilage. *Osteoarthritis and cartilage*. 2002; 10(3):163–171. [PubMed: 11869076]
- Momose A, et al. Blood Vessels: Depiction at Phase-Contrast X-ray Imaging without Contrast Agents in the Mouse and Rat Feasibility Study 1. *Radiology*. 2000; 217(2):593–596. [PubMed: 11058666]
- Nugent KA. Coherent methods in the X-ray sciences. *Advances in Physics*. 2010; 59(1):1–99.
- Pfeiffer F, et al. Phase retrieval and differential phase-contrast imaging with low-brilliance X-ray sources. *Nature Physics*. 2006; 2(4):258–261.
- Pogany A, et al. Contrast and resolution in imaging with a microfocus x-ray source. *Review of Scientific Instruments*. 1997; 68(7):2774–2782.
- Schleede S, et al. Emphysema diagnosis using X-ray dark-field imaging at a laser-driven compact synchrotron light source. *Proceedings of the National Academy of Sciences*. 2012; 109(44):17880–17885.
- Siegel R, et al. *Cancer statistics, 2012*. CA: A Cancer Journal for Clinicians. 2012
- Snigirev A, et al. On the possibilities of x-ray phase contrast microimaging by coherent high-energy synchrotron radiation. *Review of Scientific Instruments*. 1995; 66(12):5486–5492.
- Standards B. Method for measurement of the effective focal spot size of mini-focus and micro-focus x-ray tubes used for industrial radiography. *BS*. 1988; 6932(1988)
- Suzuki Y, et al. X-ray refraction-enhanced imaging and a method for phase retrieval for a simple object. *Journal of Synchrotron Radiation*. 2002; 9(3):160–165. [PubMed: 11972371]
- Takeda T, et al. Vessel imaging by interferometric phase-contrast X-ray technique. *Circulation*. 2002; 105(14):1708–1712. [PubMed: 11940551]
- Tanaka T, et al. The first trial of phase contrast imaging for digital full-field mammography using a practical molybdenum x-ray tube. *Investigative radiology*. 2005; 40(7):385–396. [PubMed: 15973129]
- Wilkins S, et al. Phase-contrast imaging using polychromatic hard X-rays. *Nature*. 1996; 384(6607):335–338.
- Yagi N, et al. Refraction-enhanced x-ray imaging of mouse lung using synchrotron radiation source. *Medical Physics*. 1999; 26:2190. [PubMed: 10535637]
- Zhong Z, et al. Implementation of diffraction-enhanced imaging experiments: at the NSLS and APS. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*. 2000; 450(2):556–567.
- Zysk A, et al. Nondestructive volumetric imaging of tissue microstructure with benchtop x-ray phase-contrast tomography and critical point drying. *Biomedical Optics Express*. 2012; 3(8):1924. [PubMed: 22876355]

Figure 1.

Example in-line image data for the system geometry (*left*) and spot size (*right*) studies, acquired using 55 kVp and 120 kVp, respectively. Rectangles indicate 100×100 pixel² regions used for texture analysis. The magnification factor is smaller for the system geometry study than that used for the spot size study resulting in a larger effective FOV (9 cm^2 vs. 2.6 cm^2). The lung regions of both images exhibit speckle texture patterns not observed in other regions. Both images are shown in the same greyscale.

Figure 2. Lung ROI Intensity Appearance for Imaging System Lengths

An extracted lung ROI from Figure 1-(a) is shown for different imaging system geometries used during data acquisition (55 kVp, 13-micron spot size). A speckle pattern is readily recognized for largest system length (*subfigure a*). The overall texture appears smoother as the imaging system length decreases. All images are shown with the same greyscale window which ranges from zero to one. The images represent a $750 \times 750 \mu\text{m}^2$ region of the lung.

Figure 3. Texture Measures vs. Imaging System Length for Six ROIs-55 kVp

Texture characteristics and their dependence on imaging system length are given for the six ROIs shown in Figure 1-(a). Results for the two mice are shown with the first and third columns corresponding to M_1 and the second and fourth columns corresponding to M_2 .

Data points represent the average texture measure value from three frames and error bars represents the standard deviation. While the mean intensity remains constant for all system geometries (*subfigure a*), the remaining texture characteristics show a dependence on spot size for ROIs selected from lung regions. Texture measures calculated for non-lung ROIs are relatively constant over the range of imaging system lengths investigated in the study. Lines are drawn between the data points for each ROI/system geometry combination and are provided to aid the reader in distinguishing results for different ROIs.

Figure 4.

Mapping Image Texture Characteristics: Relative Smoothness vs. Imaging System Length

The texture characteristic relative smoothness is calculated using a 31×31 pixel² ROI surrounding each pixel in the 55 kVp intensity images produced for the imaging system geometry study (e.g. Figure 1-(a)). The maps reveal how image texture quantities associated with lung regions change dramatically with increasing system length (*left-to-right*) compared to texture quantities from non-lung regions. All images are shown with the same greyscale window that ranges from 0.0 to 0.002.

Figure 5. Lung ROI Intensity Appearance With Different Spot Sizes

An example ROI from Figure 1-(b) is shown for different spot sizes during data acquisition (120 kVp). A speckle pattern is readily recognized for the smallest spot size (*subfigure a*). The overall texture appears smoother as the spot size increases. All subfigures are shown with the same intensity greyscale window that ranges from 0.0 to 1.0.

Figure 6. Texture Characteristics vs. Spot Size for Six ROIs-120 kVp

Texture characteristics and their dependence on spot size are given for the six ROIs shown in Figure 1. Results for the two mice are shown with the first and third columns corresponding to M_1 and the second and fourth columns corresponding to M_2 . Data points represent the average texture measure value from three frames and error bars represent the standard deviation. Lines are drawn between the data points for each ROI/spot size combination to aid the reader in distinguishing results for different ROIs. Mean intensities do not change for different spot sizes (*subfigures a and b*). The other texture measures (*subfigures c–l*) show a strong dependence on spot size for lung regions (*open symbols, dashed lines*) while non-lung regions show little change except for the smallest spot sizes (*filled symbols, solid lines*).

Figure 7. Mapping Image Texture Characteristics: Visibility vs. Spot Size

The visibility texture measure is calculated using a 31×31 pixel² ROI surrounding each pixel in the 120 kVp intensity data (e.g. Figure 1-(b)). Different spot sizes are represented by subfigure with the smallest spot size at the left and largest spot size at the right. The lung region is prominent for small spot sizes and becomes less pronounced as spot size increases. All of the subfigures are shown with the same greyscale window that ranges from 0.0 to 1.0.

Figure 8. Measured beam spectra for two X-ray source settings used in the study
Beam spectra for the 55 kVp (*left*) and 120 kVp (*right*) settings. The source spot size was 13 μm and a 1 mm aluminum filter was in place during both measurements.

Figure 9. Texture Characteristics vs. Spot Size for Six ROIs-55 kVp

Texture characteristics and their dependence on spot size are given for the six ROIs shown in Figure 1-(a). Results for the two mice are shown with the first and third columns corresponding to M_1 and the second and fourth columns corresponding to M_2 . Data points represent the average texture measure value from three frames and error bars represent the standard deviation. Lines are drawn between the average values of three data points for each ROI/spot size combination to aid the reader in distinguishing results for different ROIs. Mean intensities do not change for different spot sizes (*subfigures a,b*). The other texture measures (*subfigures c-l*) show a strong dependence on spot size for lung regions (*open symbols, dashed lines*) while non-lung regions show little change except for the smallest spot sizes (*filled symbols, solid lines*).

Figure 10.

The texture measures standard deviation (*top row*) and uniformity (*bottom panels*) are shown versus spot size for data extracted from lung ROIs acquired using 55 kV and 120 kV X-ray tube potentials (*dotted lines and solid lines, respectively*). Results for M_1 and M_2 are given in the left and right columns, respectively. The data point values are the mean of values from three frames and the error bars give the standard deviation. The lines are added to aid the eye and do not represent a fit to the data. The results for the two kVp values show similar trends with the 55 kVp values covering a slightly larger range.

(a) Mean: M_1 (b) Standard Deviation: $[M_1]$ **Figure 11.**

The time dependence of statistical texture measures are shown for a constant imaging system configuration (55 kVp, 13 μm spot size, 150 cm system length). The small changes in the mean intensity and standard deviation values are negligible compared to the results shown in Figures 3 and 6 for lung ROIs. The consistency of the values over time suggests that physical structure of the lungs did not change significantly over the course of the experiment.

The imaging system parameters employed in the system geometry aspect of the study are summarized. The measured absorbed dose is given for each geometry.

Table 1

System Geometry Distance Study						
kVp	Spot Size	M	SOD [cm]	ODD [cm]	Exposure Time	Dose [cGy]
55	13 μm	2	97.1	97.1	160 sec	0.479
			78.2	78.3	90 sec	0.492
			50.8	50.8	44 sec	0.409
			33.1	33.2	20 sec	0.556
			15.5	15.5	4.2 sec	0.578

Table 2

The definitions of the image texture quantities used in the spot size study. The measured absorbed dose is given for each system setting.

Spot Size and Beam Spectra Studies						
kVp	Spot Size	M	SOD [cm]	ODD [cm]	Exposure Time	Dose [cGy]
120	7 μm	3.8	39.7	110.3	140 sec	1.923
	13 μm				62 ¹ sec	1.854
	26 μm				31 sec	1.530
	38 μm				20 sec	1.365
	50 μm				15 sec	1.287
	91 μm				9.5 sec	1.356
55	5 μm	3.8	39.7	110.3	600 sec	2.022
	13 μm				120 sec	2.045
	26 μm				62 sec	1.770
	38 μm				44 sec	1.684

¹M1 exposure time was 72 sec with a corresponding dose of 2.137 cGy

Table 3

The definitions of the image texture quantities used in this study

Mean	$\mu = \sum_{i=1}^G ip(i)$
Standard Deviation	$\sigma = \left\{ \sum_{i=1}^G (i - \mu)^2 p(i) \right\}^{\frac{1}{2}}$
Entropy	$H = - \sum_{i=1}^G p(i) \log_2[p(i)]$
Uniformity	$U = \sum_{i=1}^G p^2(i)$
Relative Smoothness	$R = 1 - \frac{1}{1 + \sigma^2}$
Visibility	$V = \frac{i_{max} - i_{min}}{i_{max} + i_{min}}$