Technical Note: Synchrotron-based high-energy x-ray phase sensitive microtomography for biomedical research

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(Received 15 January 2015; revised 5 August 2015; accepted for publication 9 August 2015; published 8 September 2015)

Purpose: Propagation-based phase-contrast CT (PPCT) utilizes highly sensitive phase-contrast technology applied to x-ray microtomography. Performing phase retrieval on the acquired angular projections can enhance image contrast and enable quantitative imaging. In this work, the authors demonstrate the validity and advantages of a novel technique for high-resolution PPCT by using the generalized phase-attenuation duality (PAD) method of phase retrieval.

Methods: A high-resolution angular projection data set of a fish head specimen was acquired with a monochromatic 60-keV x-ray beam. In one approach, the projection data were directly used for tomographic reconstruction. In two other approaches, the projection data were preprocessed by phase retrieval based on either the linearized PAD method or the generalized PAD method. The reconstructed images from all three approaches were then compared in terms of tissue contrast-to-noise ratio and spatial resolution.

Results: The authors' experimental results demonstrated the validity of the PPCT technique based on the generalized PAD-based method. In addition, the results show that the authors' technique is superior to the direct PPCT technique as well as the linearized PAD-based PPCT technique in terms of their relative capabilities for tissue discrimination and characterization.

Conclusions: This novel PPCT technique demonstrates great potential for biomedical imaging, especially for applications that require high spatial resolution and limited radiation exposure.

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Key words: X-ray imaging, phase contrast, phase retrieval, tomography, synchrotron radiation

1. INTRODUCTION

X-ray computed microtomography (μ -CT) is a well-established technique for 3D imaging of the internal structure of objects. Conventional μ -CT is based on the differential attenuation of transmitted x-rays by structures within an object, whose contrast mechanism is effective for distinguishing between elemental components with significant differences in atomic number or electron density. However, the difference in x-ray attenuation for different types of soft tissues is typically rather small, which results in poor image contrast between the tissues and therefore hampers diagnostics. It has been suggested that x-ray phase-contrast imaging can be utilized for improvement of the contrast in transmission images of noncrystalline objects consisting predominantly of light chemical elements. Currently, four methods are in use: interferometry, analyzer-based or diffraction enhanced imaging, grating interferometry, and propagation-based phase-contrast imaging.¹⁻⁴ Of these methods, the last two hold the distinct advantage of the ability to be used with broadband sources. The Talbot-Lau interferometry can also be performed with extended sources, as the demands on spatial coherence are lowered at the cost of losing a portion of the flux by using a source grating.

Propagation-based phase-contrast is the simplest arrangement and therefore the least sensitive to misalignments of the optical components. None of the x-ray phase-contrast methods yield direct measurements of the phase, thus further data processing is required. The most demanding is for propagation-based phase-contrast imaging, which requires the complicated process of phase retrieval (PR) to extract a sample's phase map from the measured intensity. Therefore, the experimental simplicity is offset by more complex data processing.

In the direct phase-contrast CT (PPCT) method, the angular projection data are directly used for tomographic reconstruction, as one first makes angular projections by using the propagation-based phase-contrast technique. From these angular projections, 3-D tomograms are then reconstructed using the standard filtered backprojection (FBP) algorithm. In each of the angular projections, the phase-contrast mechanism generates edge-enhancing interference fringes at the interfaces between different tissues, therefore the reconstructed PPCT tomograms exhibit edge-enhancement at the tissue interfaces and boundaries.⁵⁻⁷ However, these tomograms are not the maps of the linear attenuation coefficients (LACs) of the sample; rather, they may present anomalously large or even negative apparent LAC values at interfaces between different

tissues. These “artifacts” may cause faulty interpretation of sample structures and impede even qualitative characterizations for tissues and materials.

Another type of PPCT technique is the phase-retrieval-based PPCT, which can be performed as a two-stage process: first, the acquired projections are preprocessed with the phase retrieval algorithms, and then 3D images are reconstructed with the standard FBP algorithm. Performing phase retrieval on acquired angular projections can enhance image contrast and enable quantitative tomography.^{8–10}

In order to retrieve a phase map for a given angular view, one should decode the phase information from the projections in the view by using the x-ray transport equation, which is shown in Refs. 11 and 12 as

$$I(\vec{r}_D) = \frac{I_{in}}{M^2} \left\{ \cos\left(\frac{\lambda R_2}{4\pi M} \nabla^2\right) \left[A^2(\vec{r}) + \frac{\lambda R_2}{2\pi M} (\nabla \cdot (\varphi(\vec{r}) \nabla A^2(\vec{r}))) \right] - 2 \sin\left(\frac{\lambda R_2}{4\pi M} \nabla^2\right) [A^2(\vec{r}) \varphi(\vec{r})] \right\}, \quad (1)$$

where λ is the x-ray wavelength, $I(\vec{r}_D)$ denotes the projection x-ray intensity, I_{in} is the entrance x-ray intensity, and $\vec{r}_D$ is the position vector in the detector plane. Also in Eq. (1), $A^2(\vec{r})$ and $\varphi(\vec{r})$ are the attenuation map and phase-shift map of the sample, respectively, R_2 is the object-detector distance, and M is the magnification factor employed in the projection. Note that in this equation, $\sin(\lambda R_2 \nabla^2 / 4\pi M)$ and $\cos(\lambda R_2 \nabla^2 / 4\pi M)$ are the 2D pseudodifferential operators, in which the operator ∇^2 is the 2D transverse Laplacian operator $\nabla^2 \equiv (\partial^2 / \partial x^2 + \partial^2 / \partial y^2)$. In mathematics, the action on a function $g(\vec{r}_o)$ of a 2D pseudodifferential operator $O(c \nabla^2)$ is defined as

$$O(c \nabla^2) g(\vec{r}_o) \equiv \iint \exp(i2\pi(\vec{q} - \vec{r}_o) \cdot \vec{f}) \cdot O(-4\pi^2 c \vec{f}^2) g(\vec{q}) d^2 \vec{q} d^2 \vec{f}. \quad (2)$$

This general transport equation is the basis for developing phase retrieval methods. The x-ray transport equation [Eq. (1)] can be simplified in many applications where the maximal Fresnel propagator phase ($FPP_M \equiv \pi \lambda R_2 M / 4p^2$) is much less than 1,¹³ where p denotes the pixel size of the detector. If an imaging setup employs a detector pixel size on the order of tens of micrometers and a sample-detector distances of a few meters or less, then the corresponding maximal Fresnel propagator phase $FPP_M \ll 1$. Under this condition of $FPP_M \ll 1$, the $\sin(\lambda R_2 \nabla^2 / 4\pi M)$ and $\cos(\lambda R_2 \nabla^2 / 4\pi M)$ terms can both be linearized in terms of the transverse Laplacian operator ∇^2 , and general x-ray transport equation (1) can therefore be reduced to

$$I(\vec{r}_D) = \frac{I_{in}}{M^2} \left\{ A^2(\vec{r}) - \frac{\lambda R_2}{2\pi M} (\nabla \cdot (A^2(\vec{r}) \nabla \varphi(\vec{r}))) \right\}. \quad (3)$$

This simplified transport equation is the transport of intensity equation (TIE) detailed in the literature.^{14,15} Hence, the task of phase retrieval is to extract the phase map $\varphi(\vec{r})$ from phase contrast projections by using Eq. (1) or Eq. (3) if $FPP_M \ll 1$.

From the x-ray transport equations in both Eqs. (1) and (3), it is clear that the projection intensity $I(\vec{r}_D)$ is not simply the

attenuation-map $A^2(\vec{r})$, but it is encoded with the differentials of the phase map $\varphi(\vec{r})$. Hence, a single phase-contrast image is not enough for retrieving the phase map $\varphi(\vec{r})$. In general, phase retrieval requires at least two measurements of the intensity, taken at two different sample-to-detector distances (SDDs).^{12,16} This requirement for multiple image acquisitions adds complexity to the experiments and data-alignment processing, increases the radiation dose delivered to the biomedical samples, and also increases the measurement time. Thus, taking only a single image at each view angle is desirable for the PPCT implementation in biomedical research. A PPCT technique with such an implementation is also called a single-distance PPCT technique in the literature.^{10,16–25}

Investigating a single image based on the phase retrieval method, we realized that tissues' phase shifts could be uniquely related to their attenuations under certain conditions. For example, materials or tissues consisting of low- Z elements ($Z < 10$) will have their attenuation and phase shifts determined completely by their projected electron densities when illuminated with high-energy x-rays (60–500 keV). This is because Compton scattering dominates the interaction between the x-rays and the low- Z elements within this photon energy range. Under these conditions, the attenuation becomes $A^2 = \exp(-\sigma_{KN} \rho_{e,p})$, where σ_{KN} denotes the Klein–Nishina total cross section for x-ray Compton scattering and $\rho_{e,p}$ is the projected electron density, the ray integral of the sample's electron densities. On the other hand, the phase shift is given by $\varphi = -\lambda r_e \rho_{e,p}$, where $r_e = 2.818 \times 10^{-15}$ m is the classic electron radius. Hence, under the above conditions, a special relationship exists between the phase shift and attenuation $\varphi = (\lambda r_e / \sigma_{KN}) \cdot \ln A^2$, which we call the phase-attenuation duality (PAD).^{13,16} Since the composition of soft tissue is dominated by low- Z elements ($Z < 10$), the PAD relationship holds when utilizing x-rays of 60–500 keV. The associated inverse problem of phase retrieval is also solved under this condition. We have found that the x-ray transport equation [Eq. (1) or Eq. (3)] becomes greatly simplified with the PAD relationship, such that the robust phase retrieval can be achieved from a single projection.^{13,17,24} We first applied the PAD relationship to the simplified x-ray transport equation in Eq. (3) to develop a single image based phase retrieval method, which we call the linearized PAD method of phase retrieval.¹⁶ Extending the PAD-based algorithm to high-resolution imaging with the maximal Fresnel propagator phase $FPP_M > 1$, we later developed the generalized PAD method of phase retrieval by using the general x-ray transport equation in Eq. (1).^{11,13} These two PAD methods will be presented in Sec. 2.

In our previous experiments, we utilized the linearized PAD method of phase retrieval in PPCT to demonstrate that a striking improvement of density resolution can be achieved for hybrid material samples. We also extended the validity of PAD to the cases involving strongly absorbing materials.¹⁸ However, we found that the linearized PAD-based PPCT falls short of expectations in its ability to discriminate tiny tissue structures in PPCT experiments requiring higher resolution on the order of a few microns. After a thorough analysis, we realized that it would be necessary to employ the generalized

PAD-based method of phase retrieval for high-resolution PPCT with maximal Fresnel propagator phase $FPP_M \geq 1$. Using the generalized PAD method of phase retrieval, in this work, we demonstrate the validity and advantages of this novel technique for high-resolution PPCT. We will show that it is superior to the direct PPCT technique as well as the linearized PAD-based PPCT technique, through comparisons of their relative tissue discrimination and characterization capabilities. The paper is organized as follows. After the Introduction, in Sec. 2, we discuss the three PPCT implementation approaches and their experimental setups, as well as the phase retrieval algorithms of the two PAD methods. In Sec. 3, the reconstructed images will be compared in terms of tissues contrast-to-noise ratio (CNR) and spatial resolution values. The advantages of the generalized PAD-based PPCT technique will be demonstrated. In Sec. 4, we briefly discuss the other single-distance PPCT techniques and present conclusions.

2. MATERIALS AND METHODS

2.A. Experiment setup

The experiment was performed in the X-ray Imaging and Biomedical Application Beamline (BL13W1) at the Shanghai Synchrotron Radiation Facility (SSRF, China). The experimental setup is depicted in Fig. 1. In the figure, the x-ray beam, which is produced by a 16-pole wiggler source on a 3.5 GeV storage ring (with ring current $I = 210$ mA), is monochromated with Si (111) and Si (311) double-crystal monochromators (DCM) and tuned into the energy range from 8 to 72.5 keV. In this experiment, the data sets were acquired with an x-ray energy of 60 keV by tuning DCM Si (311). The sample is placed approximately 34 m downstream from the wiggler source, which provides not only appropriate spatial coherence but also a nearly parallel beam (for plane waves the magnification factor $M = 1$) incident on the sample. Through adjusting the sizes of slit1 and slit2, the maximal beam size can reach 48 mm (horizontal) and 5 mm (vertical) with a divergence of 1.5 mrad (horizontal) and 0.2 mrad (vertical). The KOHZU 6D sample stage is fixed on the 3 m-long granite experimental platform installed at the end-station and intended for high stability and high-resolution imaging. Coupled with a 100 μm thick phosphor screen and visible-light optics for yielding various effective-pixel-sizes, the CCD detector is installed on a precise slide rail, along with remote control motors capable of providing an easily adjustable SDD. In our experiments, we employed the single-crystal YAG

($\text{Y}_3\text{A}_5\text{O}_{12}:\text{Ce}$) scintillator, which is a transparent nonscattering scintillator. In contrast to the conventional powder phosphor screen, which provides a spatial resolution approximately the same as the screen thickness, the resolution of the single-crystal YAG scintillator is primarily limited by the resolving power of the microscope optical objective. The resolving power of the microscope is mainly determined by the depth of focus of the optics. The effective pixel size of the detection system is 3.7 μm , while the spatial resolution of the detector system is higher than 9 μm , as demonstrated by the line spread function (LSF) measurement shown in Fig. 3 in Sec. 3.

In order to test the biomedical applications of PAD PR-based PPCT using a synchrotron source, the measurements utilized a sample which was a type of Chinese fish named *Schizothorax Grahmi*. The fish was sacrificed before the experiment, and the entire fish sample was then placed in an upright position in a 7-mm-diameter plastic tube to facilitate the inline experimental setup. The raw images for the PAD phase retrieval, which consisted of 900 tomographic projections over 180° of rotation, were acquired with an x-ray energy of 60 keV by tuning DCM Si (311) and using a sample-to-detector distance $R_2 = 100$ cm. The effective pixel size of the CCD detector was 3.7 μm . In addition, the x-ray phase-contrast angular projections were collected using the IMAGE PRO plus 6.0 software package. Prior to phase retrieval and image reconstruction, dark signal correction and flat field normalization were both applied to each raw data set. Note that for our experiment setting, the maximal Fresnel propagator phase $FPP_M = 1.19$. The acquired angular projection data set was then reconstructed using three different techniques, as discussed below.

2.B. Direct PPCT technique

In direct propagation-based phase-contrast tomography, tomograms are reconstructed directly from projection phase-contrast images using the standard FBP algorithm. The reconstructed image is a map of the apparent LACs. The image contrast mechanism of direct PPCT is therefore a mixture of attenuation contrast and phase contrast. It can be shown that the reconstructed apparent LAC $\mu_{\text{apparent}}(\vec{r})$ is a sum of three components,²³

$$\mu_{\text{apparent}}(\vec{r}) = \mu(\vec{r}) - \frac{R_2}{M} \cdot \left(\frac{\partial^2}{\partial x_1^2} + \frac{\partial^2}{\partial x_2^2} + \frac{\partial^2}{\partial x_3^2} \right) \cdot \delta_{\text{refr}}(\vec{r}) + \mu_{\text{mixed}}(\vec{r}), \quad (4)$$

where the first component $\mu(\vec{r})$ is the sample's actual LAC. The second component is proportional to the 3D Laplacian of the sample's refractive index decrement, $\delta_{\text{refr}}(\vec{r})$,^{24,26} and it is responsible for the anomalous values of apparent LAC and the strong edge-enhancement observed in such images. The third component denotes the artifacts related to the global distribution of the attenuation coefficients and refraction indices. The direct PPCT images are particularly suitable for observing the structural outlines of weak-attenuation tissues. However, the reconstructed apparent LAC (μ_{apparent}) is not sufficiently accurate and quantitative for analyzing biomedical

Fig. 1. Schematic of propagation-based imaging system.

samples, due to the edge oscillations producing overshooting of the apparent LAC at edges as well as negative LAC values. This phenomenon is not consistent with the true LAC values of real samples, but it is the result of the combination of the attenuation contrast and phase contrast during the projections. Hence, in order to overcome the problem with Direct PPCT, one must separate the attenuation and phase contrast that combined in the projections. Thus, one should preprocess the dataset of angular projections through applying phase retrieval before tomographic reconstruction.

2.C. Linearized PAD based PPCT technique

As mentioned in the Introduction, extracting the phase map of a sample for any given angular view typically requires at least two, but often four to five, projections for the angular view. In PPCT, multiple image acquisitions for each of the angular views will burden the experiment with setup complexity, long acquisition times, and increased radiation doses to the sample. However, in our experiments a good approximation of the PAD relationship holds, as the fish sample is composed mainly of low- Z elements ($Z < 10$) and the x-ray energy (60 keV) is high enough to ensure that incoherent scattering (Compton scattering) dominates the soft tissue attenuation. According to the linearized PAD method of phase retrieval,¹⁶ one applies the PAD relationship to Eq. (3) to determine the phase map. In this way we found that the phase map φ_θ of a given view angle θ can be retrieved from a single projection image I_θ ,

$$\varphi_\theta(\vec{r}) = \frac{\lambda r_e}{\sigma_{\text{KN}}} \cdot \ln \left\{ \left[1 - \left(\frac{\lambda^2 r_e R_2}{2\pi M \sigma_{\text{KN}}} \nabla^2 \right) \right]^{-1} \cdot \left(\frac{M^2 I_\theta(\vec{r}_D)}{I_{\text{IN}}} \right) \right\}. \quad (5)$$

In this equation, the phase retrieval operator $[1 - (\lambda^2 r_e R_2 / (2\pi M \sigma_{\text{KN}})) \nabla^2]^{-1}$ is a pseudodifferential operator, as defined in Eq. (2). Note also that σ_{KN} , the Klein–Nishina total cross section for x-ray photon Compton scattering from a single free electron, is x-ray energy dependent,

$$\sigma_{\text{KN}}(E) = 2\pi r_e^2 \left\{ \frac{1+\eta}{\eta^2} \left[\frac{2(1+\eta)}{1+2\eta} - \frac{1}{\eta} \log(1+2\eta) \right] + \frac{1}{2\eta} \log(1+2\eta) - \frac{1+3\eta}{(1+2\eta)^2} \right\}, \quad (6)$$

where $\eta = E_{\text{ph}}/m_e c^2$, E_{ph} represents x-ray photon energy, and $m_e c^2$ is the rest electron energy, which is equal to 511 keV. From the retrieved phase map φ_θ of an angular view, we can compute the projected electron densities $\rho_{e,p}(\theta) = -\varphi_\theta/\lambda r_e$. Applying the standard FBP algorithm to the retrieved projected electron densities from all the angular views, one can reconstruct a 3D map of the sample's electron densities. The accuracies of the reconstructed electron densities depend on the accuracies of this linearized PAD method of phase retrieval. As detailed earlier, the linearized PAD method is applicable only if the maximal Fresnel propagator phase $\text{FPP}_M \ll 1$. However, as described above, $\text{FPP}_M = 1.19$ for our experiments, thus errors in the reconstructed electron densities were expected when using this linearized PAD based PPCT technique.

2.D. Generalized PAD based PPCT technique

Since a good approximation of the PAD relationship holds for our experiments, we can apply the relationship to the general x-ray transport equation shown in Eq. (1) to solve for the phase map. The theoretical advantage of using Eq. (1) rather than Eq. (3) is that Eq. (1) is applicable as well to the cases with $\text{FPP}_M \geq 1$, as is the case in our experiment. Although Eq. (1) is more sophisticated than Eq. (3), we found that the phase map φ_θ of a given view angle θ can be retrieved as follows:¹³

$$\varphi_\theta(\vec{r}) = \frac{\lambda r_e}{\sigma_{\text{KN}}} \cdot \ln \left\{ \left[\cos \left(\frac{\lambda R_2}{4\pi M} \nabla^2 \right) - \left(\frac{2\lambda r_e}{\sigma_{\text{KN}}} - \frac{\lambda R_2}{4\pi M} \nabla^2 \right) \cdot \sin \left(\frac{\lambda R_2}{4\pi M} \nabla^2 \right) \right]^{-1} \cdot \left(\frac{M^2 I_\theta(\vec{r}_D)}{I_{\text{IN}}} \right) \right\}. \quad (7)$$

Comparing Eq. (7) to Eq. (5) of the linearized PAD method, the key difference is that the phase retrieval operator in Eq. (7) is a more convolved pseudodifferential operator $[\cos((\lambda R_2/4\pi M) \nabla^2) - ((2\lambda r_e/\sigma_{\text{KN}}) - ((\lambda R_2/4\pi M) \nabla^2)) \cdot \sin((\lambda R_2/4\pi M) \nabla^2)]^{-1}$. Note that the large value of the ratio $\lambda r_e/\sigma_{\text{KN}}$ reflects the high sensitivity of phase contrast imaging.^{16,26} Using the definition of a general pseudodifferential operator, we can rewrite Eq. (7) in a more convenient form for computation,

$$\varphi_\theta(\vec{r}) = \frac{\lambda r_e}{\sigma_{\text{KN}}} \cdot \ln \left\{ \hat{F}^{-1} \left\{ \frac{\hat{F} \left(\frac{M^2 I_\theta(\vec{r}_D)}{I_{\text{IN}}} \right)}{\left[\cos \left(\frac{\pi \lambda R_2 \vec{u}^2}{M} \right) + \left(2 \frac{\lambda r_e}{\sigma_{\text{KN}}} + \frac{\pi \lambda R_2 \vec{u}^2}{M} \right) \sin \left(\frac{\pi \lambda R_2 \vec{u}^2}{M} \right) \right]} \right\} \right\}, \quad (8)$$

where $\hat{F}$ denotes the 2D Fourier transform and $\hat{F}^{-1}$ is its inverse transform.

Computing the projected electron densities from $\rho_{e,p}(\theta) = -\varphi_\theta/\lambda r_e$ and applying the standard FBP algorithm to the

retrieved projected electron densities, one can reconstruct a 3D map of the sample's electron densities.

What are the physical and mathematical reasons for the linearized PAD method losing high frequency features

FIG. 2. Reconstructed images and their partial enlarged views of the fish head samples from slices of one pixel thickness ($3.7 \mu\text{m}$). (a), (c), and (e) are the same slices with different reconstruction methods as follows: direct PPCT, linearized PAD-PPCT, and generalized PAD-PPCT, respectively. (b), (d), and (f) are their partial enlarged views indicated by the yellow solid lines in (a), (c), and (e). The regions for calculating CNR are defined by the blue, red, and green solid-line rectangles denoting the background, soft tissue, and bone tissue in the enlarged views. The purple solid lines plotted along the edge in the three partial enlarged views were used for obtaining the LSFs to evaluate the SR. The length of the scale bar is $50 \mu\text{m}$.

when $\text{FPP}_M \geq 1$? The physical reason is that the linearized PAD method is derived from the simplified x-ray transport equation in Eq. (3), which is not applicable to cases with $\text{FPP}_M \geq 1$. In those cases, one should instead apply the general transport equation in Eq. (1). Mathematically, while both phase retrieval equations in Eqs. (5) and (7) contain the Laplacian operators, the Laplacian operator in Eq. (5)

can make the denominator very large for the cases where $\text{FPP}_M \geq 1$, thus Eq. (5) can unduly suppresses high-frequency components in the projection image and cause blurring. In contrast, in Eq. (7), the Laplacian operators are embedded into sinusoidal functions whose absolute values are bounded by 1, regardless of whether $\text{FPP}_M \geq 1$. In synchrotron-based high-resolution PPCT imaging, one may encounter a case of

$FPP_M \geq 1$, provided the sample-to-detector distance is 10 cm or larger. However, if the effective pixel sizes are relatively large (approximately 10 μm or larger) or the sample-to-detector distance is short such that the value of $FPP_M \ll 1$, then the linearized PAD method in Eq. (5) is still applicable.

3. RESULTS

Figure 2 provides three groups of fish head images that were reconstructed from the same acquired projection dataset by using the three different PPCT techniques as described earlier. Each group includes a slice image and an enlarged view of an inset from the slice. The slice images correspond to the same slice of the specimen, and the insets are from the same yellow-square-marked area of the sample slice. Figure 2(a) shows the slice image reconstructed with the direct PPCT technique. The different tissues of the fish head can be distinguished mainly through the edge-enhancements at the boundaries and interfaces. Figure 2(b) is the enlarged view of the inset from an area that is indicated by the solid line on Fig. 2(a). Figures 2(a) and 2(b) demonstrate weak contrast between different tissues in the bulk areas, which results in one being unable to clearly observe the gill arches and filaments. This is mainly caused by the small differences in linear attenuation coefficients between the tissues, as well as the interfering noise in the image. The slice in Fig. 2(c) was reconstructed with the linearized PAD-based PPCT technique by using Eq. (5). The image provides the reconstructed electron density distribution in the slice and depicts different tissues in the fish head specimen with greatly enhanced contrast-to-noise ratios. However, the enlarged view of the inset in Fig. 2(d) shows that the gill arch and filaments, pharynx, and tissue-air interfaces have been oversmoothed and blurred, which impedes the discrimination of tiny structures in the slice.

Last, Fig. 2(e) and its enlarged inset view Fig. 2(f) were reconstructed with the generalized PAD-based PPCT technique by using Eq. (8). Compared to Figs. 2(c) and 2(d) reconstructed with the linear PAD method, where the fine structures of gill filaments and pharynx are hardly recognizable, Figs. 2(e) and 2(f) are clearly superior, as these fine structures are easily depicted. In addition, compared to Figs. 2(a) and 2(b) reconstructed with the direct PPCT technique, Figs. 2(e) and 2(f) demonstrate greatly enhanced tissue contrast, especially for the tissues in the bulk of the sample.

The reconstructed tomographic slices were examined by the metrics of CNR and spatial resolution. The following formula was used to calculate the CNR,

$$\text{CNR} = \frac{|S_{\text{obj}} - S_{\text{bg}}|}{\sqrt{\sigma_{\text{obj}}^2 + \sigma_{\text{bg}}^2}}, \quad (9)$$

where S_i and σ_i represent mean pixel values and standard deviations, respectively, of a manually defined object and background regions of interest (ROI). As shown in Figs. 2(b), 2(d), and 2(e), these regions are defined by blue, red, and green rectangles, which denote the background, soft tissue, and bone tissue, respectively. Table I lists the measured CNRs

TABLE I. The calculated CNRs of the three tomographic images in Figs. 2(b), 2(d), and 2(f).

RIO	Direct PPCT	Linearized PAD-PPCT	Generalized-PAD-PPCT
Soft tissue	9.4	81.5	78.6
Bone tissue	6.1	60.7	57.8

from the three tomograms in Figs. 2(b), 2(d), and 2(e), which were reconstructed with the three different PPCT techniques. Table I shows that, for soft tissues as well as bone tissues, both the PAD-based techniques achieved almost 8 times higher CNR values than that with the direct-PPCT technique. The CNR of the bone tissue is lower than that of the soft tissue, which is because the dominating low-Z elements in soft tissues meet the PAD assumption better than the elements in bone tissues. Table I also shows that the linearized PAD-based PPCT technique images have slightly higher CNR values than that with the generalized PAD technique. This increase of CNR values with the linearized PAD method is accompanied by losses in spatial resolution as demonstrated in Figs. 2(c) and 2(d). While the linearized PAD method unduly suppresses high-frequency components in the projection image and causes blurring, it simultaneously suppresses the high-frequency noise to improve the CNR values.

The image resolution can generally be evaluated as the spatial resolution and the density resolution. The image spatial resolution can be estimated from a criterion based on the full widths at half maximum (FWHM) of the line spread function (LSF) curves. For a reconstructed slice, the LSF can be derived from a line profile taken along a bright-and-dark edge in the slice. The line profile is then fitted with an error function by means of a least-squares fit to obtain a line spread function of the test image. Utilizing this method, the image spatial resolutions can be determined by the FWHM of the LSF function. In order to quantitatively compare the estimated spatial resolutions for the three different techniques, the LSF curves associated with the three techniques were measured as shown in Fig. 3. From these LSF curves in Fig. 3, the FWHMs were determined to be 17.28, 27.95, and 9.25 μm , corresponding to the direct PPCT, linearized PAD-based PPCT, and generalized PAD-based PPCT techniques, respectively. Compared in terms of these FWHM values, the resolution of the generalized PAD-based technique is more than triple that of the linearized PAD-based technique and almost double that of the direct PPCT technique. This result explains the observation that among the three circular regions marked by the white dashed lines in Figs. 2(b), 2(d), and 2(f), the round region in Fig. 2(f) is the sharpest in exhibiting the fine structures. Hence the generalized PAD-PPCT technique has demonstrated not only high density resolution with high tissue CNR values, but also high spatial resolution for fine structure discrimination.

Moreover, the density resolutions with the three techniques were also evaluated by generating histograms based on the reconstructed pixel values of each of the three tomograms in Fig. 2. The density resolution as demonstrated by a histogram could play an important role in determining how

FIG. 3. Calculations of line spread functions (LSF), as determined from the SRFs. The spatial resolutions of the three tomographic slices can be estimated from the FWHM of the LSFs. The FWHMs of the LSFs of direct PPCT, linearized PAD-PPCT, and generalized PAD-PPCT are 17.28, 27.95, and 9.25 μm , respectively.

well a method provides the ability to discriminate biological tissues with varying density. The histograms of the normalized reconstructed values of tissues, corresponding to the enlarged views shown in Figs. 2(b), 2(d), and 2(f), are shown in Fig. 4. It can be seen clearly that the histogram in Fig. 4(a), which is associated with the direct PPCT, has a wide merged peak, which indicates the inability to distinguish different tissue types in the tomogram. In contrast, both the linearized PAD-based PPCT and generalized PAD-based PPCT techniques as shown in Figs. 4(b) and 4(c), respectively, demonstrate better density resolution capability for distinguishing bone tissues, soft tissues, and the background, which is expected from the earlier discussion on the tissue CNR-enhancement achieved with the two PAD-based PPCT techniques.

Based on the distribution of the gray values for each pixel in the reconstructed tomograms, Fig. 5 presents the pseudocolored maps of the tomograms from Fig. 2. A pseudocolored map can generally facilitate a more sensitive visual inspection using the color variation instead of the gray scale variation. A comparison between the pseudocolored tomographic images reconstructed with the three PPCT techniques demonstrates that the generalized PAD-

FIG. 4. Histograms of the normalized electron density measured in the Figs. 2(b), 2(d), and 2(f). (a)–(c) are, respectively, based on the direct PPCT, linearized PAD-PPCT, and generalized PAD-PPCT.

based technique not only distinguishes the detailed biological structures effectively but also exhibits the variation of tissue densities in high-resolution imaging.

In summary, the results from this experiment show that the generalized PAD-based PPCT technique is superior to the direct PPCT technique as well as the linearized PAD-based

FIG. 5. The pseudocolored tomographic images of Figs. 2(b), 2(d), and 2(f). The images in (a)–(c) are, respectively, based on the direct PPCT, linearized PAD-PPCT, and generalized PAD-PPCT. The length of the scale bar is 50 μm .

PPCT technique in terms of both tissue density resolution and fine structure discrimination.

4. DISCUSSIONS AND CONCLUSION

The capability of quantitative tissue characterization is also a desirable feature for a PPCT technique. Direct PPCT cannot provide quantitative characterization for tissue structures at the edges and internal boundaries, where the refractive index of tissue varies. As shown in Eq. (4), the variation in the refractive indices contributes to the reconstructed apparent LAC through a scaled Laplacian differential, which is difficult to be quantified. Hence, the LAC value could only be deemed equal to the reconstructed apparent LAC in a uniform region of the sample. On the other hand, as detailed previously, the reconstructed tomograms using both PAD-based PPCT techniques represent the electron density distribution of the sample. In order to evaluate the accuracies of the reconstructed electron densities, we have checked the average reconstructed values of the ROIs in an area of uniform soft tissue. These ROIs are indicated by the red squares in Figs. 2(b), 2(d), and 2(f). From the ROI in Fig. 2(b) we found that the average LAC $\mu_{\text{ave}} = 0.215 \text{ cm}^{-1}$ and from the ROIs in Fig. 2(d) for the linearized PAD-PPCT and Fig. 2(f) for the generalized PAD-PPCT, we found that the average electron densities were $\rho_{e,\text{ave}} = 4.56 \times 10^{23} \text{ cm}^{-3}$ and $\rho_{e,\text{ave}} = 3.78 \times 10^{23} \text{ cm}^{-3}$, respectively. In these measurements, we were careful to select the ROIs away from the edges to strive for measurement accuracy. For example, the apparent LAC become negative values (down to -0.12 cm^{-1}) at the edge area in Fig. 2(b) due to the refraction effect of Eq. (1). Ideally, the relationship $\sigma_{\text{KN}} \cdot \rho_{e,\text{ave}} = \mu_{\text{ave}}$ should be satisfied if x-ray Compton scattering dominates. Using $\sigma_{\text{KN}} = 5.45 \times 10^{-25} \text{ cm}^2$ for 60 keV x-ray and the measured average electron density values from the two ROIs in Figs. 2(d) and 2(f), we calculated the values for both PAD-PPCT techniques. For the ROI from the generalized PAD-PPCT technique in Fig. 2(f), we found the value of $\sigma_{\text{KN}} \cdot \rho_{e,\text{ave}} = 0.206 \text{ cm}^{-1}$, and the relative error $\text{RE} = |(\sigma_{\text{KN}} \cdot \rho_{e,\text{ave}} - \mu_{\text{ave}}) / \mu_{\text{ave}}| \times 100\% = 4.18\%$, which is close to the value of 4.2% measured using standard test samples with hybrid components in our previous work.²⁶ However, for the ROI in Fig. 2(d) reconstructed by using the linearized PAD-PPCT technique, the value of relative error reaches as high as 15.60%, which is almost 4 times larger than that with the generalized PAD-PPCT technique.

Additionally, the generalized PAD-PPCT results were cross-checked by estimating the tissue mass densities from their reconstructed electron densities. These estimated mass density values are compared to the “standard values” of mass densities listed in the standard literature.^{14,15} Using the generalized PAD-based PPCT technique, we obtained the electron densities of different tissues from Fig. 2(f), in which the blue, red, and green ROIs depict the liquid, muscle-like soft tissue and bone in the fish head slice. From these ROIs, we found the following electron density values: for the liquid, $\rho_{e\text{-liquid}} = 3.26 \times 10^{23} / \text{cm}^3$, for the muscle-like tissue, $\rho_{e\text{-soft tissue}} = 3.85 \times 10^{23} / \text{cm}^3$, and for bone, $\rho_{e\text{-bone}} = 4.39 \times 10^{23} / \text{cm}^3$. The mass density ρ of a material is related to

its the electron density ρ_e by $\rho = \rho_e / (N_a \sum w_i Z_i / A_i)$, where N_a denotes the Avogadro's number ($6.022 \times 10^{23} / \text{mol}$), Z_i and A_i denote the atomic number and atomic weight of an element of the material, respectively, and W_i is the weight fraction of the element. Hence, we cannot get the mass density of a material from its electron density without knowing the material's chemical composition. However, for the purposes of estimation, we can assume that the effective Z/A -ratios of these three tissues are equal to the mean Z/A -ratios given in the standard literature for similar tissue types. The mean Z/A -ratios of water, muscle and bone, referring to Refs. 14 and 15 are given as 0.555 08, 0.549 96, and 0.514 78, and the corresponding mass densities are 1.0, 1.06, and 1.2 g/cm^3 , respectively. Note that the mass densities for different bones vary in a large range from 1.12 to 1.92 g/cm^3 ,¹² and fish bone is quite flexible and has a relatively low mass density. Using the mean Z/A -ratios and the reconstructed electron densities, we calculated the mass density estimates for liquid, soft tissue, and bone as 0.98, 1.16, and 1.42 g/cm^3 , respectively. Compared to the standard mass densities, the relative differences in the estimated mass densities of the liquid, muscle-like soft tissue, and bone were -2% , 9.4% , and 18.3% , respectively. The relative large discrepancy between the estimated value and the standard value of fish bone mass density is expected, as our phase retrieval is based on the principle of phase-attenuation duality, which is valid only for low- Z ($Z < 10$) elements. The calcium in the bone has an atomic number of $Z = 20$, thus the PAD does not hold. As a result, the retrieved phase values for high- Z components include errors, but the accuracy in the retrieved phase values for the low- Z components is not affected, since the PAD equation is a differential equation and its solution is unique in its locality. In order to determine whether our generalized PAD-based PPCT technique provides accurate tissue electron densities, we should conduct a future study using samples with known electron densities, which will facilitate comparison of the reconstructed electron density values with the known electron densities.

Finally, the techniques described in this work can be categorized as single-distance PPCT techniques, which reconstruct tomograms using only a single angular projection dataset. There are several other single-distance PPCT techniques reported in the literature, thus we will briefly comment on the applicability of these different single-distance PPCT techniques. For the imaging tasks with the maximal Fresnel propagator phase $\text{FPP}_M \ll 1$, one could employ the Paganin single-material method and its variation known as the modified Brannikov method.^{20,22,27–29} The Paganin single-material method and the modified Brannikov method are very similar, but the single-material method requires the known material constants, and the modified Brannikov method involves a constant which is normally determined through trial and error. However, these methods can only be used for samples with position-independent constant ratios of δ/μ , that is, these methods can only be applied to homogeneous or quasihomogeneous samples. This constraint severely limits the utility of these two methods. Hence, one typically only considers these two methods if a relatively low-energy x-ray

is employed, as one can apply the linearized PAD-based PPCT techniques to any inhomogeneous light material and soft tissues when high-energy x-rays (60–500 keV) are utilized. For imaging tasks with the maximal Fresnel propagator phase $FPP_M \geq 1$, the contrast transfer function (CTF) method or Fourier methods using either the Born or Rytov approximation can be chosen.^{30–33} Similar to the single material method, the utility of these methods in single-distance PPCT is limited to the cases of homogeneous or quasihomogeneous samples, and one only considers these methods if relatively low-energy x-ray is employed. For imaging tasks with high-energy x-ray (60–500 keV) and any inhomogeneous light material and soft tissues, one can simply apply the generalized PAD-based PPCT technique.

In summary, in this work, we investigated the validity and advantages of the generalized PAD-based PPCT technique. For high-resolution imaging tasks such as those with $FPP_M \geq 1$, our experimental results demonstrated that this novel technique is superior to the linearized PAD-based PPCT technique in fine structure discrimination and characterization. In comparison to the direct PPCT technique, our experiment demonstrated a striking CNR-enhancement with this novel technique. Hence, the generalized PAD-based PPCT technique has a good potential for biomedical imaging, especially for those applications that require high spatial resolution but simultaneously are subject to stringent radiation dose constraints.

ACKNOWLEDGMENTS

The experiment was performed at the BL13W1 of the Shanghai Synchrotron Radiation Facility (SSRF), and this work was supported by the External Cooperation Program of Chinese Academy of Science (Grant No. GJHZ09058) and the National Natural Science Foundation of China (Grant No. 11105213). X. Wu was supported in part by the NIH Grant Nos. R01CA142587 and R01CA193378. The authors are grateful to Dr. Molly Wong for her meticulous help in editing the paper.

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