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Phase Contrast Micro-Computed Tomography of Biological Sample at SSRF*

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Abstract: In line X-ray phase contrast micro-computed tomography (IL-XPCT), which can be implemented at third generation synchrotron radiation sources or by using a micro-focus X-ray tube, is a powerful technique for non-destructive, high-resolution investigations of a broad variety of materials. At the Shanghai Synchrotron Radiation Facility (SSRF), the X-ray Imaging and Biomedical Applications Beamline was built and started regular user operation in May 2009. Both qualitative (without phase retrieval) and quantitative (with phase retrieval) three-dimensional IL-XPCT experimental techniques have been established at the beamline. IL-XPCT experiments of a test sample (plastic pipes) used to evaluate the technique, and of a biological sample (locust) at the beamline are reported. Two series of images, qualitative and quantitative, including tomographic slices and three-dimensional rendering images were obtained. In qualitative images, there is a strong edge-enhancement which leads to very clear sample contours, while in quantitative images, the edge-enhancement fades but quantitative measurement of sample's phase information could be achieved. The experiments demonstrate that the combination of qualitative and quantitative images is useful for biological sample studies.

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

Introduction

X-ray absorption-based computed tomography (CT) is an essential imaging technique for numerous scientific and technological applications[1]. However, a well known problem in absorption-based CT is low sensitivity, which makes it difficult to image soft tissue, such as biological samples. On the other hand, biological samples which show very weak absorption contrast, produce significant phase shifts in the X-ray beam. The use of phase information for imaging purposes is therefore a suitable alternative method[2,3]. In particular, we focus here on in line X-ray phase contrast micro-computed tomography (IL-XPCT), also known as propagation-based phase contrast CT. Its set-up is extremely simple, identical to the one for absorption-based CT except for increasing sample-to-detector distance (SDD) and provided the beam is sufficiently spatially coherent.

The qualitative reconstruction of IL-XPCT is related to the three-dimensional Laplacian of the refractive index distribution, which usually can be seen as a form

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of edge enhancement, and can be used for qualitative analysis[4]. However, a quantitative relationship between the phase shift induced by the sample and the contrast recorded at an imaging plane does exist. Such phase contrast radiographs can be used to pose an inverse problem to retrieve the phase shift induced by the sample. Moreover, phase retrieval of radiographs can be coupled to CT algorithm. The phase shift induced by the sample is first retrieved for each projection angle image, and then fed into a CT reconstruction algorithm to yield a three-dimensional reconstruction of the refractive index, which can be used for quantitative analysis.

Different algorithms have been proposed to solve the phase retrieval problem. One is based on the transport of intensity equation (TIE), which requires images acquired at two different SDDs and is only valid for short propagation distances where the contrast is weak[5,6]. The other relies on the contrast transfer function (CTF), which is only valid for weak absorption, but allows the use of multi-SDDs data and is not restricted to short distances[7,8]. However the aforementioned methods require images acquired at least at two different SDDs, which will sharply increase the data collecting time (since typical data sets run into hundreds or thousands of projection angles) and consequently the radiation damage to samples, that is of particular importance for biological samples and for potential *in vivo* studies. Moreover the images at different SDDs have to be pre-processed to make up for misalignment and magnification that is a time consuming routine.

Alternatively, a phase retrieval method proposed by Gureyev, which is based on Born approximation, can be combined with the phase-attenuation duality of soft tissues. According to Gureyev's simulation study, phase shift retrieval from only one SDD phase-contrast radiograph is possible[9,10].

We established the qualitative (without phase retrieval) and the quantitative (with phase retrieval) three-dimensional IL-XPCT biological sample experimental method, in which only one single SDD of IL-XPCT projection images is needed, at the X-ray Imaging and Biomedical Application Beamline at the Shanghai Synchrotron Radiation Facility (SSRF). The preliminary IL-XPCT experiments of a test sample (plastic pipes) and a biological sample (locust) at the beamline are reported.

1 Methods

1.1 Shanghai Synchrotron Radiation Facility

The Shanghai Synchrotron Radiation Facility is a third generation synchrotron radiation light source, and will be an invaluable tool for Chinese Scientific Research and Industry Community[11]. SSRF's electron energy is 3.5 GeV, which is fourth largest in the world, after Spring-8 in Japan (8 GeV), APS in USA (7 GeV), and ESRF of European Community (6 GeV). SSRF consists of a 150-MeV LINAC, a booster that can increase the electron energy from 150 MeV to 3.5 GeV in 0.5 s and a 3.5-GeV electron storage ring. The 20-cell, 4-fold structure storage ring is used to store an electron beam (average current 300 mA) with low emittance (minimum 4 nanoradian) and long lifetime (>10 h). By using advanced insertion devices, synchrotron radiation light with high flux and high brilliance will be produced. Along the ring, there are 40 bending magnets, 16 standard straight sections (6.5 m), and 4 long straight sections (12 m), which means that more than 60 beamlines could be installed in the storage ring.

1.2 X-ray Imaging and Biomedical Application Beamline

The X-ray Imaging and Biomedical Application Beamline is one of SSRF's seven initial beamlines. Figure 1 shows a schematic of the beamline. The radiation source is a 16-pole wiggler. The beam is monochromatized via a double silicon crystal monochromator, which makes available the beam energy range of 8 - 72.5 keV, with the energy resolution of about 0.5%. A filter system placed at the entrance of the experimental area is used to filter unwanted lower energy radiation.

The experimental station is located approximately at 34 m from the source. At 20 keV, the beam cross-section is 45 (horizontal)×5 (vertical) mm^2 and the photon flux on the sample is about 5×10^{10} photons/(mm^2·s). All experimental equipments, including the multi-dimensional stage, the precision slide rail, and digital detector, are placed on a marble table to reduce vibrations. Users can perform IL-XPCT projection images collection, IL-XPCT slices reconstruction, and

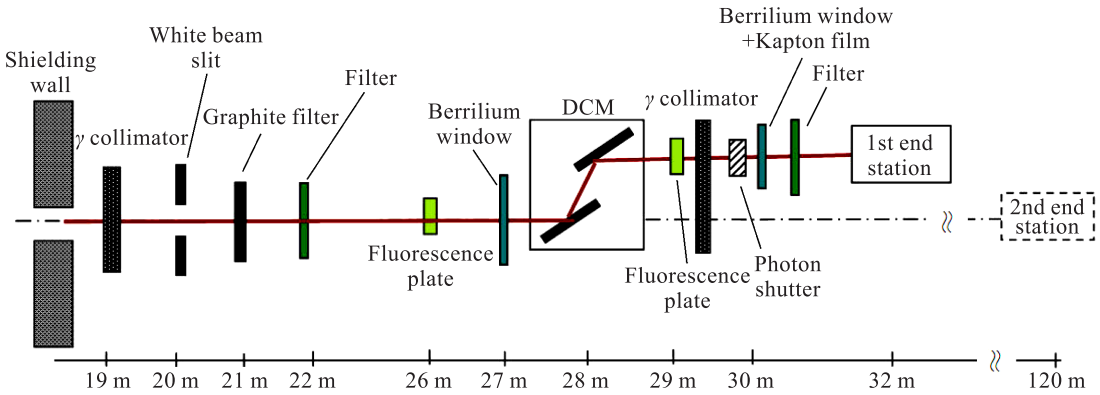


Fig. 1 Schematic of the X-ray Imaging and Biomedical Application Beamline at SSRF

three-dimensional rendering of the samples, which allows studying samples in a three-dimensional manner.

1.3 Inverse problem—phase retrieval

As shown in Fig. 2, the sample is illuminated by a parallel coherent X-ray of wavelength, and a position-sensitive detector is used to register the transmitted wave. The spatial distribution of the complex refractive index in the sample, $n(r)=1-\delta(r)+\mathrm{i}\beta(r)$, can be described by using Cartesian coordinates $r=(x,y,z)$.

According to the Born approximation and combined with CT theory, the intensity distribution at SDD $z=d$ and rotation angle θ is approximated by the following equation[10,12,13],

$$\begin{aligned}\mathrm{F}[(I^{\theta}_{z=d}/I^{\theta}_{z=0}-1)/2]=\\ \cos[\pi\lambda d(\xi^2+\eta^2)]\mathrm{F}\{-k[\boldsymbol{P}_{\theta}\beta(r)](x,y)\}+\\ \sin[\pi\lambda d(\xi^2+\eta^2)]\mathrm{F}\{-k[\boldsymbol{P}_{\theta}\delta(r)](x,y)\}\end{aligned}\quad(1)$$

where F indicates the Fourier transform, $\boldsymbol{P}_{\theta}$ means projection along z of rotation angle θ, (ξ,η) is the Fourier coordinate of (x,y), and $k=2\pi/\lambda$.

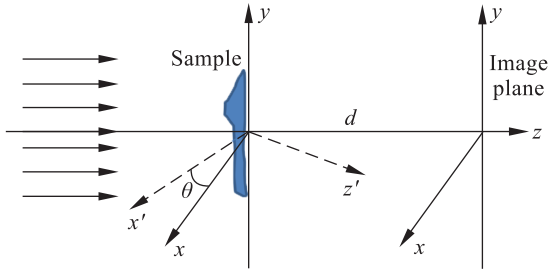


Fig. 2 Coordinate system for sample and image plane of IL-XPCT

Equation (1) can be used to reconstruct refractive index δ and absorptive index β from IL-XPCT projection images. However, by evaluating the equation, it is easy to find that Eq. (1) needs two different SDDs of IL-XPCT projection images, which increases the data collecting time and complicates data processing. Fortunately, for a single material sample, the distributions of the refractive and absorptive index are proportional to each other[14], i.e.,

$$\delta(r)=\varepsilon\beta(r)\quad(2)$$

where ε is the proportional value between refractive index and absorptive index that do not depend on r. Moreover, consider the case of a sample with uniform absorption, which means $I^{\theta}_{z=0}\approx 1$. The following equation will be obtained by combing Eqs. (1) and (2) and abovementioned condition:

$$\begin{aligned}[\boldsymbol{P}_{\theta}\delta(r)](x,y)=\\ -k^{-1}\mathrm{F}^{-1}\left\{\frac{\mathrm{F}[(I^{\theta}_{z=d}-1)/2]}{\varepsilon^{-1}\cos[\pi\lambda d(\xi^2+\eta^2)]+\sin[\pi\lambda d(\xi^2+\eta^2)]}\right\}\end{aligned}\quad(3)$$

where F^{-1} indicates the inverse Fourier transform. Equation (3) needs only one SDD IL-XPCT projection image to reconstruct the refractive index δ. The absorptive index β can be reconstructed via Eq. (2). The quantitative three-dimensional distribution of δ can be reconstructed by feeding the $\boldsymbol{P}_{\theta}\delta(r)(0\leqslant\theta\leqslant\pi)$ into a CT reconstruction algorithm. A qualitative three-dimensional reconstruction of the sample is by constructed feeding $I^{\theta}_{z=d}(0\leqslant\theta\leqslant\pi)$ into the CT reconstruction algorithm.

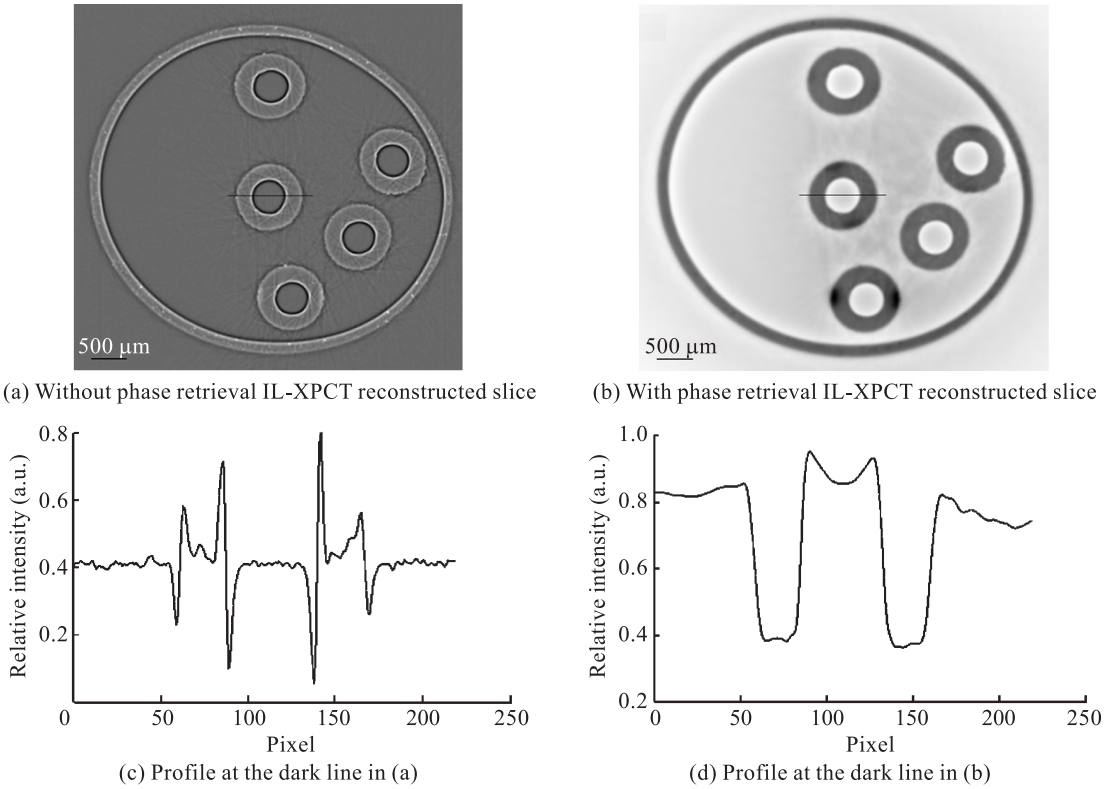
2 Results

The IL-XPCT experiment setup at the X-ray Imaging and Biomedical Application Beamline is identical to the one for absorption-based CT, except for the increase of SDD to 1.2 m in this experiment. After traversing the sample, the X-rays are recorded by a CCD camera with an effective pixel size of 9 μm. The photon energy used for the experiment is 15 keV and

10 keV for the test and locust samples respectively.

A test sample (plastic pipes), which consists of one plastic drinking straw (diameter 5.5 mm) and five wiring looms (diameter 0.9 mm) was first used to test the technique.

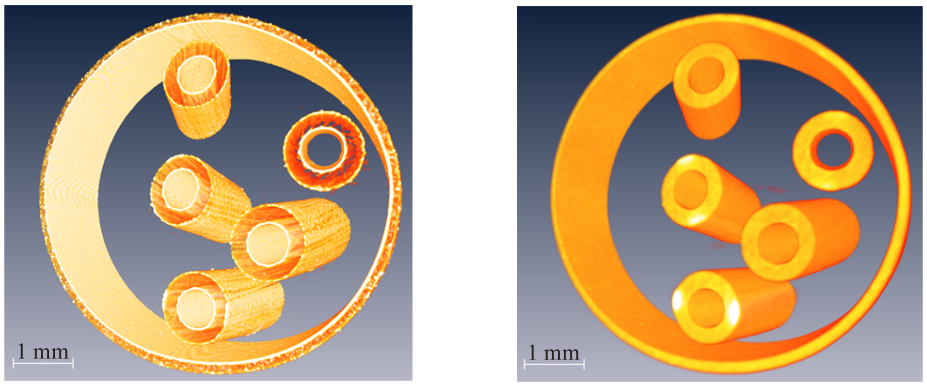
Figures 3a and 3b show IL-XPCT reconstructed slices of test samples without and with phase retrieval. The contours of the different pipes are clearly reconstructed in both images. Figures 3c and 3d, which are the profiles at the dark line position of Figs. 3a and 3b respectively, show that there is a high degree of edge enhancement in Fig. 3a but the reconstructed value of the pipe is not uniform. On the contrary, in Fig. 3b, the edge enhancement is almost removed and the reconstructed value of pipes is well retrieved and the quantitative measure of the phase of the sample could be achieved. Figures 4a and 4b are the three-dimensional rendering images of test samples without and with phase retrieval, respectively.



(a) Without phase retrieval IL-XPCT reconstructed slice (b) With phase retrieval IL-XPCT reconstructed slice

(c) Profile at the dark line in (a) (d) Profile at the dark line in (b)

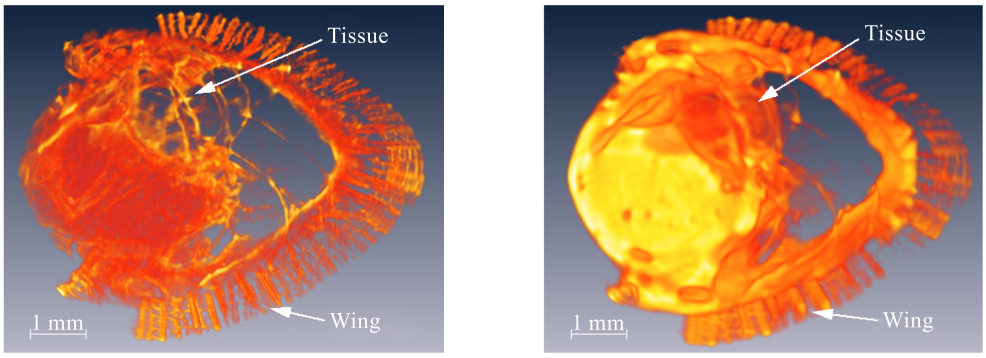
Fig. 3 Test samples without and with phase retrieval IL-XPCT reconstructed slice



(a) Without phase retrieval (b) With phase retrieval

Fig. 4 Three-dimensional rendering image of test sample without and with phase retrieval

A fresh biological sample (locust), without any pretreatment, was then used for the IL-XPCT experiment. Figures 5a and 5b show the three-dimensional rendering images obtained without and with phase retrieval, respectively. The locust's wing and internal tissue distribution are clearly visible in both images. Figure 5a conveys more detailed information and sharper contour of the locust than Fig. 5b due to the edge enhancement in the qualitative reconstruction. On the other hand, in Fig. 5b the reconstructed value of the different parts of the sample is more uniform than in Fig. 5a, which is closer to the actual form of the sample and may allow a more intuitive approach for research study. This comparison suggests the complementary nature of the two approaches, which underline either the detail (qualitatively) or the overall structure (quantitatively) of the sample.



(a) Without phase retrieval (b) With phase retrieval

Fig. 5 Three-dimensional rendering image of locust sample without and with phase retrieval

3 Discussion

We have demonstrated that the qualitative and the quantitative three-dimensional distribution of the biological sample can be reconstructed from a single SDD IL-XPCT projection image. In the quantitative three-dimensional phase information (refractive index) reconstruction, depending on the sample structure and composition, the approximations of a uniform absorption over the entire object and proportional distributions of the refractive index and absorptive index (Eq. (2)) may not be well satisfied and may induce some artifacts, as the white halos inside the pipes in Fig. 3b. However, as well shown from Fig. 3 to Fig. 5, the quality of the reconstruction is sufficient for further study. On the other hand, the qualitative three-dimensional reconstruction has sharper contour than the quantitatively reconstructed images due to the edge enhancement, and can therefore provide more detail.

In general, the two methods are complementary. The IL-XPCT is experimentally simple, very fast, and ideally suited for small samples, especially for studies of biological samples.

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