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Current status of X-ray phase imaging at SPring-8: Toward 4D X-ray phase tomography for biological samples

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Abstract. Measurement conditions in X-ray phase tomography using a grating interferometer were evaluated to achieve fast measurements toward 4D X-ray phase tomography for biological samples. X-ray phase tomographic images obtained from different exposure time and the number of projections were examined to determine required statistics for high density resolution while keeping a fast measurement. Application of a high-efficiency fiber-coupled X-ray imaging detector to X-ray phase tomography was also discussed from a comparison between the fiber-coupled and lens-coupled detectors.

1. Introduction
X-ray phase contrast imaging enables us to measure weak absorption materials such as a biological sample with much higher image contrast compared to the absorption-based imaging. In particular, use of an X-ray interferometer is helpful for the quantitative phase measurement. A grating interferometer is one of the X-ray interferometer, which has been used at not only synchrotron radiation facility but also the laboratory-based X-ray source [1-3].

At the bending magnet beamline BL20B2 in SPring-8, X-ray phase tomography using the grating interferometer has been routinely applied to quantitative measurements of biological samples. Recently, fast and high-throughput X-ray phase tomography has been developed to realize quantitative dynamic measurements. 4D X-ray phase tomography based on the simple uniaxial stretch-and-release cyclic scan was demonstrated by optimizing the measurement procedure [4]. To achieve such a fast and high-throughput measurement, some optimizations in the measurement condition and apparatus were carried out. In this paper, their results and discussions to achieve fast and high-throughput X-ray phase tomography using a grating interferometer are presented. In addition, application of a high-efficiency fiber-coupled X-ray imaging detector to the X-ray phase tomographic measurement is also discussed.

2. Measurement setup for X-ray phase tomography
An X-ray phase tomographic system using a grating interferometer was developed at the bending magnet beamline BL20B2 in SPring-8. An upstream experimental hutch located at 43m from the source was used. The X-ray energy was set to 18.8keV (3 × 10^9 photons/sec/mm²). The grating interferometer is composed of a phase grating (G1) and an absorption grating (G2). G1 is made of Ta and the pattern thickness is 2.1µm, which produces a phase shift of π/2 at the X-ray energy of 18.8keV. G2 is made of Au and the pattern thickness is 16.6µm. The grating pitch is 10µm in both gratings. The
distance between G1 and G2 was set to 1\textsuperscript{st} order fractional Talbot distance. G2 was set with an inclined condition to increase the effective absorption at the grating pattern. The detail of the inclined condition is described elsewhere [5]. A sample was measured in a specially designed water bath filled with normal saline. In our measurement system, the biological sample is suspended in the water bath and rotated for the tomographic measurement. The thickness of the water bath is 15mm in which more than 70\% X-ray photon are absorbed. A phase stepping method was used for the phase retrieval. G2 set on the Piezo stage was translated with a triangle-shaped analog signal from a waveform generator. Detail of the fast phase stepping method used in the measurement is described in our recent work [4].

3. Results and discussion

3.1. Application of high-efficiency X-ray imaging detector to X-ray phase tomography

To achieve an efficient measurement, we have introduced a fiber-coupled X-ray imaging detector (C12849-101U, Hamamatsu Photonics). Here, the performance of the fiber-coupled detector in X-ray phase tomography was evaluated by comparing it with a conventional lens-coupled detector. The fiber-coupled detector is composed of P43 scintillator with 10µm in thickness, a straight fiber and a scientific CMOS sensor. On the other hand, the lens-coupled detector is composed of a beam-monitor (AA40, Hamamatsu Photonics) with P43 scintillator with 10µm in thickness and a scientific CMOS camera (ORCA Flash4.0 ver.2, Hamamatsu Photonics). AA40 has a lens with the focal length of 50mm (Nikon, AI AF Nikkor 50mm f/1.4D). Another lens with the focal length of 50mm (Nikon AI Nikkor 50mm f/1.2S) is installed in front of the camera to realize a 1:1 optical system. This optical configuration in the lens-coupled detector has been typically employed in the X-ray micro-imaging at BL20B2. The effective pixel size and field of view in both detectors are 6.5µm and 13.3mm (H)×13.3mm (V), respectively. Measured conversion gains from a single X-ray photon to the digitalized counts in the fiber-coupled and lens-coupled detectors were 40 counts/photon and 8.8 counts/photon, respectively, while both sensors had a 16bit analog-digital convertor resolution. Although two different sCMOS sensors were used in this comparison, more than 4 times efficiency could be confirmed in the fiber-coupled detector at the same pixel size.

X-ray phase tomographic images of a formalin fixed mouse fetus measured with the fiber-coupled and lens-coupled detectors are shown in Fig. 1(a). The measurement condition is as follows: the number of projection 900, 5-step fringe scan for the phase retrieval and the exposure time 60ms. Total measurement time was less than 8min. The fiber-coupled detector provides a sharper image compared to that obtained from the lens-coupled detector. Magnified images at the rectangles indicated in Fig. 1(a) are shown in Fig. 1(b) and 1(c). Small features are clearly depicted in the fiber-coupled detector. On the other hand, standard deviation values at the background were measured to evaluate the density resolution. In this case, phase shift values obtained from tomography were converted into the mass density. Standard deviation values obtained from the fiber-coupled and lens-coupled detectors are 2.4mg/cm\textsuperscript{3} and 1.9mg/cm\textsuperscript{3}, respectively.

To discuss the result shown above, a point spread function (PSF) of each detector was measured using a pinhole with 5µm in diameter. Each PSF is shown in Fig. 2. The PSF in the lens-coupled detector has higher background around the main peak as shown in the magnified profile while the full width at half maximum (FWHM) is almost equal between fiber-coupled (FWHM: 17µm) and lens-coupled (FWHM: 16µm) detectors. The higher background observed in PSF may be due to the lens system and influence the image quality as some sort of smoothing filter.

From these results and discussion, a fiber-coupled X-ray imaging detector is useful to realize high-efficiency detection, and it provides a sharper image in the current X–ray phase tomographic system. In contrast, the density resolution in the lens-coupled detector may be improved by the smoothing effect compared to the fiber-coupled detector. Although a spatial resolution in the grating interferometer is ultimately limited by the grating period, the optical system in the visible light conversion type X-ray detector also affect the image quality in the phase tomogram.
Figure 1. (a) X-ray phase tomographic images of a mouse fetus (gestation day 14) obtained from a fiber-coupled detector (left) and a lens-coupled detector (right). (b), (c) Magnified images at the rectangles indicated in 1(a).

Figure 2. Point spread functions measured with fiber-coupled and lens-coupled detectors.

3.2. Evaluation of measurement condition in fast X-ray phase tomography
X-ray phase tomographic measurements of a standard sample were carried out to evaluate the measurement condition in fast X-ray phase tomography. Here, the fiber-coupled X-ray imaging detector was used. The standard sample was composed of three kinds of water solution. Each water solution was set in the pipette tube made of polypropylene. One of the X-ray phase tomographic images of the standard sample is shown in Fig. 3(a). Since the sample was measured in normal saline, the difference of the density between pure water and the background was approximately 7 mg/cm³. The standard deviation at the background was measured to evaluate the density resolution. The results obtained from five kinds of measurement conditions are summarized in Fig. 3(b). Insets shown in Fig. 3(b) are X-ray phase tomographic images of pure water in the pipette tube. As a result, standard deviation values at the background were drastically improved by increasing the exposure time from 10 ms to 60 ms. Line profiles measured at inset images in conditions 1 and 3 are shown in Fig. 3(c). In the exposure time of 10 ms (estimated contribution of X-ray photons in single pixel of the detector: 237 photons/pixel/10 ms), it is difficult to find a clear distinction between pure water and normal saline. On the other hand, the difference of the density is recognized in the exposure time of 60 ms (estimated contribution of X-ray photons in single pixel of the detector: 1423 photons/pixel/60 ms). In the current system, a binning pixel mode will be useful to shorten the exposure time while keeping high density resolution. It will be helpful to ensure X-ray photon statistic. Furthermore, the density resolution may be improved by degrading the spatial resolution.
Figure 3. (a) X-ray phase tomographic image of three kinds of water solution in the pipette tubes. The gray-scale represents the mass density converted from phase shift values. (b) Standard deviation values measured at the background of the tomographic images obtained under five different measurement conditions. Insets show sectional images of pure water in the pipette tube under five different conditions. Difference of the density between pure water and background is approximately 7mg/cm$^3$.
(c) Line profiles measured across the sectional images of pure water in conditions 1 and 3.

4. Conclusion
Measurement conditions in fast X-ray phase tomography with a grating interferometer when using a high-efficiency fiber-coupled X-ray imaging detector was evaluated toward 4D X-ray phase tomography. Although it was possible to acquire X-ray phase images in the photon limited condition, a certain level of photon statistic was required to ensure high density resolution. 4D X-ray phase tomography based on the knowledge obtained from this evaluation has already been employed in user applications.

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