New developments in attenuation and phase-contrast microtomography using synchrotron radiation with low and high photon energies

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ABSTRACT

Microtomography using synchrotron radiation is widely used in fields of e.g. medicine, biology and material science. Using attenuation contrast at photon energies in the range of 8 to 25 keV and phase contrast at photon energies of 12 keV, 20 keV and 24 keV the method of microtomography is applied to a large number of samples. A comparison of the two different contrast mechanism is presented. Feasibility, advantage and limits of these methods are shown in theory and by experiment. New developments in high-energy microtomography using synchrotron radiation in the energy range of 60 to 100 keV are described. Using attenuation contrast, several samples are investigated. For the investigation of larger specimens with diameters on the order of 1-2 cm, the use of a new μCT-technique based on scanning a 2-dim. X-ray detector is demonstrated. At 70 keV photon energy an X-ray LLL-interferometer is tested and used to measure phase projections. For the first time, phase-contrast microtomography could be applied to weakly and normally absorbing material at a high photon energy.

Keywords: Microtomography, tomography, phase contrast, attenuation contrast, high-energy interferometry

1. INTRODUCTION

The microtomography system at HASYLAB (DESY, Germany) using synchrotron radiation to reveal the 3-dim. structure of small samples consisting of normal and weakly absorbing elements is installed at beamline BW2. The system for attenuation-contrast microtomography (μCT) was recently extended to perform phase-contrast microtomography (PμCT). Section 2 deals with the basic components of the apparatus. The different contrast mechanisms are described and compared.

sample manipulator

X-ray camera

CCD camera lens fluorescent screen

interferometer

sample in liquid rotating phase shifter

monochromatic beam

Figure 1: Experimental setup for X-ray microtomography using phase contrast (with interferometer) and attenuation contrast (without interferometer).

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in theory and experiment in section 3. Structures of different samples measured at BW2 and the first use of the \( \mu \)CT-system using high photon energies at beamline BWS are presented in section 4.

2. EXPERIMENT

The experimental setup shown in Figure 1 is used for \( \mu \)CT. The apparatus consists of three main parts: the X-ray camera, the sample manipulator and the X-ray interferometer. To perform attenuation-contrast microtomography the interferometer unit can be removed.

2.1. X-ray camera

The 2-dim. X-ray detector uses a fluorescent screen to convert X-rays to visible light. The created image is then magnified by a lens onto a CCD camera. The present system consists of the following hardware components:

- **CCD camera:** KX2, Apogee Instruments, Inc.; 14 bit digitalization at 1.25 MHz
- **CCD chip:** Kodak KAF1600, grade 1, 1536 x 1024 pixels, each 9 x 9 \( \mu \)m
- **lens:** video-camera lens, Fa. Schneider-Kreuznach, Xenon 1:1.4, 25 mm focal length
- **fluorescent screen:** CdWO\(_4\) single crystal, thickness 200 \( \mu \)m

The optical magnification of the system can be altered to adapt the size of the sample to the given size of the CCD chip. To describe the spatial resolution of the X-ray detector the modulation transfer function (MTF) is calculated from the measured projection of a lead edge. For a magnification of 1.9 (5.5) a spatial resolution of 8 \( \mu \)m (3 \( \mu \)m), respectively, was obtained.

2.2. Sample manipulator

In addition to rotating the sample it can also be translated in z-direction to facilitate the measurement of reference images. Furthermore, in order to allow different scanning techniques for the measurement of more extended samples, the x- and z-position of the sample manipulator relative to the X-ray camera / interferometer can also be altered (see section 4).

2.3. Interferometer

The X-ray interferometer used for \( \mu \)CT features four Laue-case X-ray mirrors to split, mirror and combine the monochromatic beam. The specimen is contained in the upper beam of the so-called skew-symmetric LLL-interferometer. The rotating phase shifter located immediately behind the beam splitter crystal serves to scan the overall phase shift of the measured interference pattern. The liquid cell shown in Figure 1 serves to eliminate interference fringes stemming merely from the outer shape of the specimen and to reduce the maximum projected phase shift difference between interfering beams. A more detailed description of the system is given elsewhere.

3. MEASUREMENT OF PROJECTION

The use of synchrotron radiation (SR) for X-ray tomography yields several advantages. The high intensity of SR especially at wiggler beamlines allows for using a double crystal monochromator to get a highly monochromatic X-ray beam with low divergence. Employed photon energies can be matched to the composition and size of the sample. Due to the large distance between source and experiment and the low divergence of the SR, the sample is projected by a nearly parallel beam. To perform a tomographical scan the sample is rotated in equal steps of typically 0.1 to 1 degree. Over the range of 0 to \( \pi \) projections are recorded by the CCD. The tomographical reconstruction then yields the 3-dim. spatial description of the projected property of the sample. The measurement of parallel projection also allows for the applicability of scanning techniques presented in section 4. Next the different contrast mechanism of the projections will be discussed.

3.1. Attenuation projection

For \( \mu \)CT the interferometer unit in Figure 1 will be removed and the sample is directly projected by the monochromatic X-ray beam onto the fluorescent screen. By taking an image with (\( I(x, z) \)) and without (\( I_0(x, z) \)) a sample the attenuation projection (\( \int \mu(x, y, z) \, dy \)) can be calculated.
\begin{equation}
I(x,z) = I_0(x,z) \exp[-\int \mu(x,y,z) \, dy].
\end{equation}

Except close to absorption edges, for photon energies (E) used at RW2 in the range of 8 up to 25 keV the attenuation coefficient \( \mu \) at position \( (x,y,z) \) of the specimen (atomic number \( Z \), mass density \( \rho \)) can approximately be expressed by

\begin{equation}
\mu(x,y,z) \approx Z^4 E^3 \rho(x,y,z).
\end{equation}

As the maximal projected attenuation of the sample should be 2, the photon energy \( E \) has to be adapted to the sample composition (atomic number \( Z \)) and size. Due to relation (2) \( \mu \text{CT} \) is usually limited to investigate only the structure of the most absorbing element in the sample.

3.2. Phase projection

To obtain a phase projection (\( \Phi \)) interference patterns with (\( V_j \)) and without (\( W_j \)) a sample at different overall phase shift (\( \varphi_j \)) are measured:

\begin{equation}
V_j(x,z) = I(x,z) + K(x,z) \cos[f(x,z) + \varphi_j + \Phi(x,z)]
\end{equation}

\begin{equation}
W_j(x,z) = I(x,z) + K(x,z) \cos[f(x,z) + \varphi_j],
\end{equation}

with \( I = \) incoherent part, \( K = \) coherent part, \( f = \) built in phase structure and \( \varphi_j = 2\pi j/N, j=0,1,...N-1 \).

Taken \( N \geq 3 \) and calculating the weighted sums

\begin{equation}
F(x,z) = \sum J V_j(x,z) \exp[-i2\pi j/N]
\end{equation}

\begin{equation}
G(x,z) = \sum J W_j(x,z) \exp[-i2\pi j/N]
\end{equation}

the phase projection (\( \Phi \)) can be determined modulo \( 2\pi \) by evaluating

\begin{equation}
\ln[F(x,z)/G(x,z)] = i \Phi(x,z) + i 2\pi k(x,z) \text{ with } k(x,z) = (0,1,2,...).
\end{equation}

In a second step the \( 2\pi \) phase ambiguity (\( k(x,z) \)) has to be eliminated. In Figure 7 the measured interference pattern for one phase projection of a human bone sample embedded in polymethylmethacrylate (PMMA) is shown. The phase projection before and after the elimination of the \( 2\pi \) ambiguity obtained from measured interference patterns like those in Figure 7 is presented in Figure 6.

For photon energies (E) not close to absorption edges the phase shift \( \phi \) of a sample with the electron density (\( \sigma \)) at position \( (x,y,z) \) is given by

\begin{equation}
\Phi(x,y,z) \approx \sigma(x,y,z) E^{-1} \text{ with } \Phi(x,z) = \int \phi(x,y,z) \, dy.
\end{equation}

For P\( \mu \text{CT} \) there exist no optimal value for the maximum projected phase shift. The only limitation is given by the contrast of the interferometer and the spatial resolution of the detector which limits the maximal detectable phase ambiguity. To optimize the contrast of the interferometer and to reduce the number of phase jumps the fluid used in the liquid cell of the interferometer unit (Figure 1) is density matched to the specimen.
Figure 2: Reconstructed slice of mouse kidney embedded in PMMA obtained by μCT (left) and μCT (right) using 12 keV photon energy.

Figure 3: Reconstructed slice of a rat trigeminal nerve investigated by μCT using 12 keV (left) and 24 keV (right) photon energy. Note the halved phase scale at 24 keV compared to that at 12 keV.

Figure 4: A reconstructed slice (left) and a volume rendering (right) of a long-fiber MMC-pin.

Figure 5: Reconstructed slice of a rat trigeminal nerve sample using μCT at 12 keV photon energy.

Figure 6: Phase projection of human bone sample with (top) and without (bottom) the 2π phase ambiguity.

Figure 7: Interference patterns taken with (left) and without (right) the sample.
3.3. Comparison of μCT to PuCT

As seen in the last paragraph the contrast mechanism and the measurement procedure for μCT and PuCT are totally different. The projected property of the sample is given by equations (1) and (8).

\[
\mu = Z^d E^3 \quad \quad \quad \quad \phi = Z E^3.
\]

(9)

For a quantitative comparison we define the so-called 1%-thickness for each method. It represents the material thickness used to introduce a 1% signal change of the maximum detectable signal. It could be shown that phase contrast is much more sensitive than attenuation contrast not only for materials consisting of light elements (factor of 1000 for H₂O) but also for absorbing elements (factor of >100 for Ca, >10 for Au) using photon energies greater than 20 keV.

To demonstrate the advantage of phase contrast for light elements we investigate a sample at the same photon energy with each method. The specimen chosen is a part of a mouse kidney embedded in PMMA and the experiment was performed at beamline BW2 using 12 keV photon energy. In Figure 2 a reconstructed slice using μCT (left) and PuCT (right) are shown. Though the best reconstructed slice for μCT is chosen, the kidney embedded in PMMA can only be guessed to be there. For PuCT a slice of a different region of the sample is presented. There the structure of the kidney can easily be distinguished from the embedding PMMA and the surrounding glucose solution. To increase the contrast for μCT one can also use a liquid cell to measure the attenuation difference to the surrounding liquid. But then the photon energy has to be decreased to obtain the optimal projected attenuation of 2. However, with this energy, the attenuation of the liquid cell and therefore the exposure time will increase dramatically.

Equation 9 also shows that for μCT the used photon energy has to match the sample characteristics fairly closely. If the photon energy is far away from the optimal value no useful information of the sample can be obtained. For PuCT the contrast is direct proportional to the atomic number and indirect proportional to the photon energy. Therefore the used photon energy can be chosen in a wide range without impairing the tomographical reconstruction. This advantage of PuCT is shown by the investigation of rat trigeminal nerve at different photon energies. At ID11 at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France, we perform PuCT using the experimental setup at 12 keV (220-reflection-Si) and 24 keV (440-reflection Si). Due to the use of the harmonic reflection, the experimental setup need not to be changed. Figure 3 presents the experimental results. It can easily be seen that the same structural information can be revealed from each slice. At the same time, phase sensitivity is halved at 25 keV when compared to 42 keV. Hence, at higher energies, the range of measured densities in the specimen is doubled.

Furthermore a great advantage of PuCT is that it can be used to reveal the 3-dim. structure of samples consisting of weakly and normal absorbing elements simultaneously. A more detailed comparison can be found elsewhere.

4. RESULTS

4.1 Experimental results at 8 – 25 keV X-rays

The wiggler beamline BW2 is used to perform μCT in the energy range of 8 to 25 keV and PuCT using photon energies of 12 and 20 keV. Some experimental results obtained in cooperation with several groups showing the feasibility of the methods will be presented.

4.1.1. Attenuation-contrast microtomography

Al-rod:

In cooperation with H.-A. Laueri, Department of Technical Mechanics, University of Bochum, Germany, several Al-rods, which were fractured by tension were investigated. The sample presented in Figure 8 was

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{fig8.png}
\caption{Volume rendering of an Al sample using μCT at 18 keV. The top surface is where the specimen fractured under tension. In the bulk, accumulation of voids close to the surface are of interest.}
\end{figure}
obtained using μCT at 18 keV photon energy. The 3-dim. view shows the surface of the crack.

Stapes of the human ear:

Tomographical scans were performed on several human bone samples. As part of the middle ear, the 3-dim. structure of the three ossicles (malleus, incus and stapes) and of the cochlea were determined in cooperation with U. Vogel from the Middle Ear Lab., Department of Oto-Rhino-Laryngology, University Hospital "Carl Gustav Carus", Technical University Dresden (Germany).

In Figure 9 the 3-dim. rendering of a stapes with the surrounding skull bones is shown. To be able to reveal this 3-dim. structure with a height of about 8 mm, three tomographical scans at different sample heights were performed. After reconstruction the three scans are combined to build one large data set.

Long-Fiber MMC-Pin:

In cooperation with P. Wyss, Eidgenössische Materialprüfungs- und Forschungsanstalt (EMPA), Switzerland, continuous-fiber metal-matrix composites were investigated. The sample shown in Figure 4 was taken from the beginning of the run-in of the casting machine. Its quality is therefore low. The investigation was performed to compare different analytical methods. 10. On the right side in Figure 4 a volume rendering of the sample is presented. The reconstructed slice on the left shows the feasibility of the method to reveal the fiber distribution and defects in the material.

4.1.2. Phase-contrast microtomography

Using μCT at BW2 several different biological samples were investigated. 9,11 in cooperation with Prof. M. F. Rajewsky, Institute of Cell Biology [cancer research], University of Essen, Medical School. The aim was to reveal in rat trigeminal nerves the 3-dim. structure of the early state of tumor creation. 12,13

Rat trigeminal nerve 11:

The rat trigeminal nerve sample shown in Figure 5 and 10 is embedded in paraffin and was cut by a microtome before the investigation. For performing μCT at 12 keV photon energy linseed oil is used in the liquid cell of Figure 1. Figure 10 shows 3-dim. views of the whole data set built of two scans which were performed at different heights. On the left the surface resulting from preparing histological cuts is shown. The rotated view on the right represents the natural surface of the nerve. In Figure 5 a measured slice parallel to the surface of the upper scan is given. The boundary of nerve to brain (top) is clearly visible. Also fine nerve fibers (diameter of about 25 μm) can be followed crossing the nerve/brain boundary. The noticeable density change in the presented slice is about 1 mg/cm³.
4.2. Microtomography results using 70 keV X-rays

To extend the method of μCT and PμCT to samples consisting of higher-absorbing elements and to samples with greater diameter, higher photon energies are needed. Therefore the apparatus shown in Figure 1 was setup at the wiggler beamline BW5 at HASYLAB to use photon energies in the range of 60 keV up to 100 keV. The principal experimental setup used is illustrated in Figure 11.

![Experiment Diagram]

Figure 11: Principal experimental setup for X-ray microtomography at BW5.

The incident white beam is monochromatized by an controlled-imperfect 111-Si-Laue crystal and limited in its size by the use of two collimators to 6 x 6 mm². To protect the CCD-chip from the incident X-rays a lead glass is added between the fluorescent screen and the lens of the X-ray detector in Figure 1.

4.2.1. Attenuation-contrast microtomography

Figure 13 shows a reconstructed slice (top) and a 3-dim. view (bottom) of a steel sample which was fractured by tension. The scan was performed at 70 keV photon energy. Several defects could be recognized in the slice shown.

To demonstrate the feasibility of the investigation of larger samples, a scanning technique was applied to a specimen consisting of copper. To reveal the 3-dim. structure three scans at different lateral positions (x-direction) were performed. In a second step the three 2-dim. projections are combined. The tomographical reconstruction then yields the 3-dim. data volume. On the left in Figure 12 a reconstructed slice normal to the tomographical z-axis is shown. The artifacts in the middle are due to the borders of the different scans. In the slice on the right, which has been calculated at right angles to that shown on the left, fine structures inside the sample are clearly visible. The spatial resolution is of the order of 20 μm.

![Figure 12: Reconstructed slice (left) of a copper sample and a view (right) at right angles to the slice shown on the left.]

![Figure 13: Reconstructed slice (top) and a 3-dim. rendering (bottom) of a steel sample at 70 keV photon energy.]

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4.2.2. Phase-contrast microtomography

To perform PμCT at BW5 we installed an interferometer using 70 keV photon energy. In Figure 14 first results are given. On the left phase projections of a mouse kidney embedded in PMMA at different sample rotation are shown. The slice on the right is reconstructed from 90 projections with an angular increment of 2°. The surrounding liquid (water), the embedding material (PMMA) and the structure of the mouse kidney are clearly visible.

![Phase projections and reconstructed slice](image.png)

Figure 14: Phase projections at different sample rotations (left) and a reconstructed slice (right) of a mouse kidney embedded in PMMA. The surrounding liquid used is water.

To demonstrate the feasibility of PμCT of samples consisting of absorbing elements, projections of a human bone sample were measured. Figure 7 shows interference patterns with (left) and without (right) the human bone sample at different overall phase shift. By combining these 6 images the phase projection modulo 2π is determined (top in Figure 6). After the elimination of the 2π phase ambiguity one phase projection of the human bone sample is given (bottom in Figure 6). The feasibility of the method could thus be demonstrated.

5. PRESENT AND FUTURE

The use of μCT and PμCT using synchrotron radiation to investigate samples consisting of weakly and normal absorbing elements using photon energies of 8 to 25 keV at beamline BW2 and the feasibility of performing μCT and PμCT measurements at 70 keV at beamline BW5 could be shown. The present status of microtomography at beamline BW2 is:

<table>
<thead>
<tr>
<th></th>
<th>μCT</th>
<th>PμCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photon energies:</td>
<td>8 - 25 keV</td>
<td>12, 20 keV</td>
</tr>
<tr>
<td>Sample diameter:</td>
<td>up to 10 mm</td>
<td>up to 7 mm</td>
</tr>
<tr>
<td>Sample height:</td>
<td>up to 15 mm</td>
<td>up to 8 mm</td>
</tr>
<tr>
<td>Spatial resolution:</td>
<td>up to 2 μm</td>
<td>(x/y) 8μm / (z) 15μm *</td>
</tr>
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To investigate larger samples with high spatial resolution the scanning techniques presented in section 4 will be introduced to become a "normal" feature used for microtomography. Higher photon energies in the range of 70 to 120 keV at beamline BW5 will be used for standard tomographical investigations of higher absorbing materials.

*The spatial resolution of PμCT in z-direction is less than in xy-direction because the beam transmitted by the interferometer is widened in z-direction by a dynamic diffraction effect known as "Borrman fan".
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