Imaging using BM3

BL47XU@SPring-8
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1. Materials and Methods

BM3 is a "Beam Monitor 3" is a high-resolution x-ray imaging detector whose specs are described (http://www-bl20/detectors/prop.html). In this experiment, a ×20 lens was used with C4880-10-14A to obtain 0.5 μm × 0.5 μm pixels.

The x-ray energy was 20.0 keV. The undulator gap was changed between 14.6 and 15.5 mm. Thus, the 20 keV was around the second harmonic was the linear undulator (http://www.spring8.or.jp/e/facility/bl/insertion/IDtech/IDdata/BL47XUdat.html), providing a weak but large beam. The front-end slits were closed to 0.45 mm vertically and 0.09 mm horizontally. Since the apparent source size of a horizontal undulator is large, it is necessary to close the horizontal slit to secure the high horizontal coherence. Note that this condition is different experiments made at this beamline (Yagi et al., *Med. Phys.* **26**, 2190-2193 (1999)), in which the front-end slits were wide open to obtain a large field of view. The storage ring was run in "Top-Up" mode so that no correction is required for the change in the intensity (apart from the instability of the cryo-cooled monochromator).

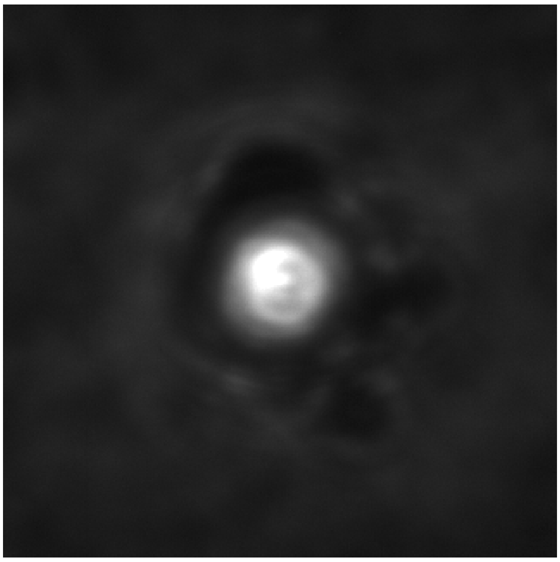
The distance from the specimen to the detector was 145 cm. When a pinhole was placed upstream to the specimen, the distance from the pinhole to the specimen was 12 cm.

One specimen was hollow glass spheres ("bubbles", SEL-STAR Z-27 from Tokai Kogyo Co., Ltd., http://www.tokai-kc.co.jp/english/works/works04.html#01, filtered using 45um and 75um sieves). They were either put between two cover slips or sprinkled on a cover slip (nominal thickness 0.12 to 0.17 mm). Another specimen was euthanized hairless mice.

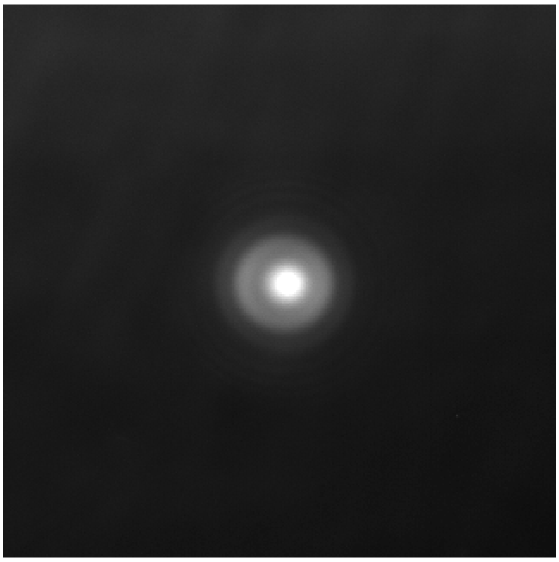
2. Comparison of pinholes

Pinholes from two different manufacturers were tried. The first one was made by an

American company with a nominal diameter of 5 μm. The price was several thousand yen. The second one was by a Japanese company (keV Corporation) with a nominal diameter of 2 μm (but the SEM image provided by the manufacturer showed it was actually about 4 μm). The price was a few hundred thousand yen. Both were made in a 50μm Ta plate. The beam images at 157 cm from the pinhole were compared. The left image was from the American pinhole and the right one was from the Japanese one.



(321×321 pixels, pinhole¥pinhole1.tif)



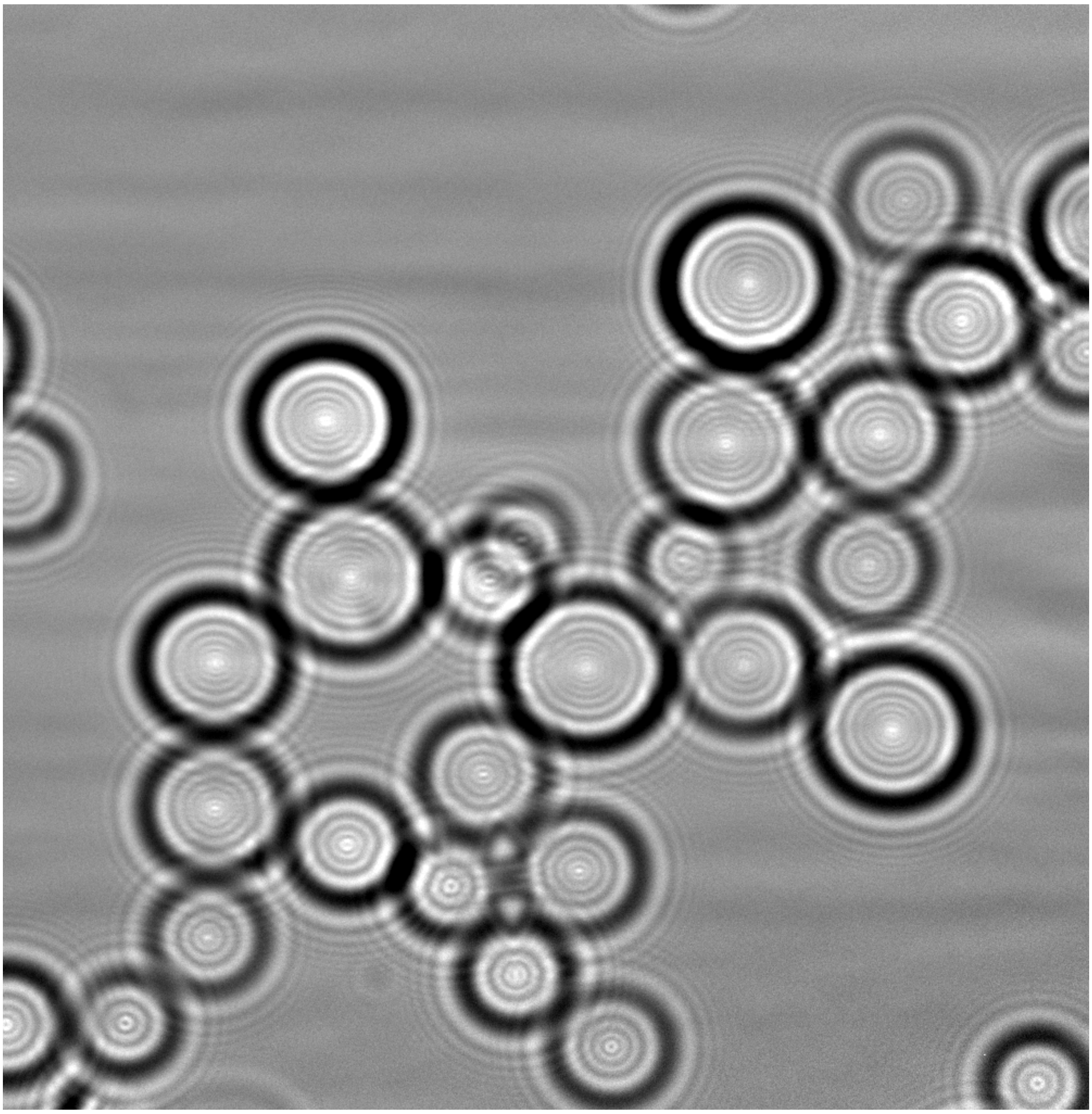
(321×321 pixels, pinhole¥pinhole2.tif)

The American pinhole has a very dirty profile. Although the peak FWHM of the beam may be smaller, the beam does not have a uniform profile. On the other hand, the Japanese pinhole gives an almost ideal Fraunhofer diffraction (actually not quite Fraunhofer, $\lambda L/d^2 = 2.5$). Thus, the Japanese pinhole was used.

Since the specimen was 15 cm downstream from the pinhole, the beam size at the specimen was estimated to be around 6 μm from its original size of 4 μm at the pinhole.

3. Observation of "bubbles" without a pinhole

Glass spheres were sprinkled on a cover slip. They were imaged and corrected with the direct beam (I_0 beam). This is a 1000×1018 image so that the field of view is about 0.5mm square.



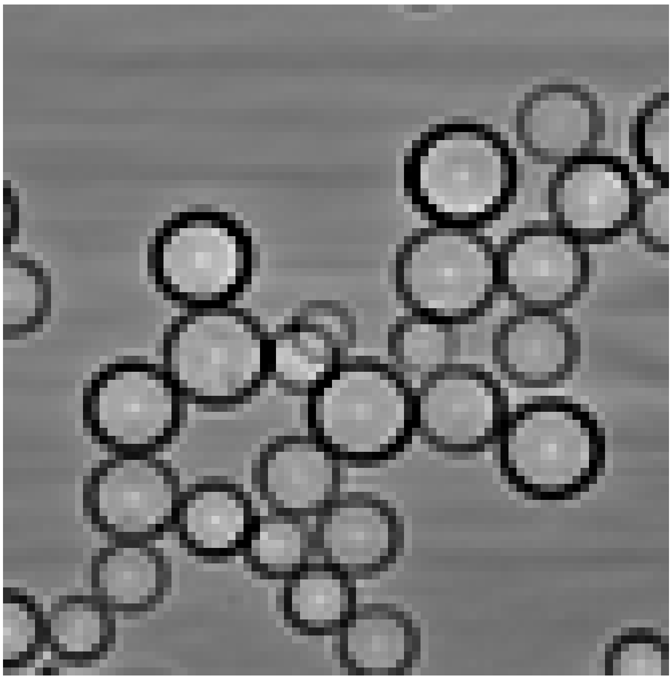
(1000×1018 pixels, coverslip¥sample2corrected.tif)

The fringes around the spheres are due to diffraction. There are interesting interferences where the spheres overlap. The broad vertical non-uniformity is due to instability of the monochromator. When the horizontal slit was wider, the horizontal fringes were either blurred or not observed.

When an isolated sphere is found, integrated intensity of the area of the sphere, including the fringes, was calculated. The value was the same as that in the area without a sphere. This shows that the spheres are acting as phase objects, with no absorption.

The above image was binned by 12×12 pixels to simulate an image obtained with a

6μm-pixel detector.



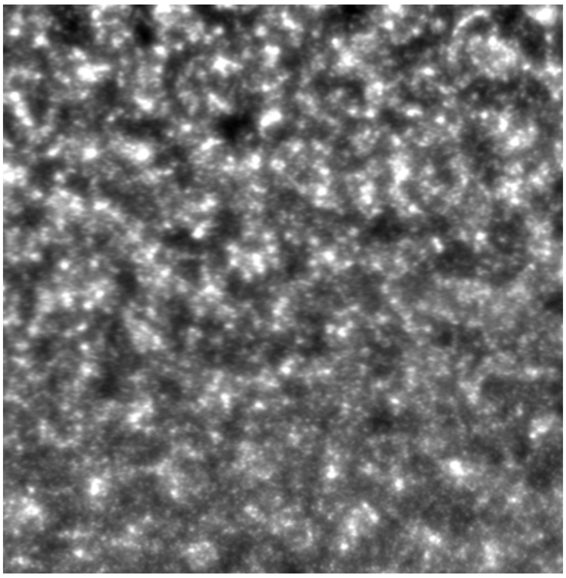
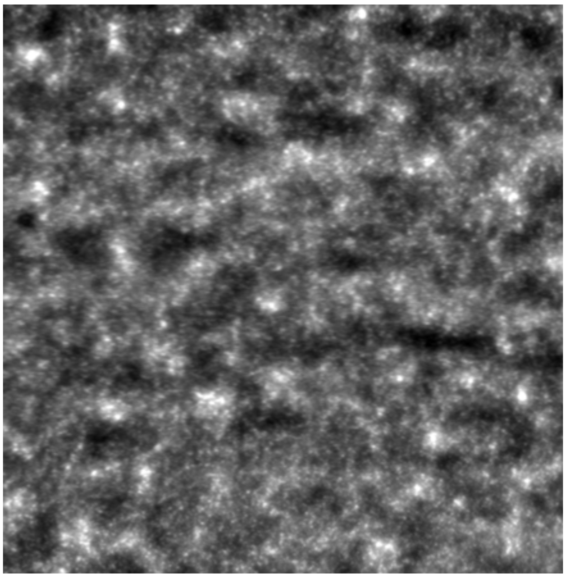
Most of the fringes are not observed. However, this is not an absorption image because, as shown above, the spheres do not absorb 20keV x-rays much.

4. Observation of layers of bubbles without a pinhole

Four samples were images: sample C (3 mm thick), sample D (nominally 1mm but actually about 1.2 mm), sample E (0.6 mm), sample F (0.3 mm). All images are corrected with the direct beam.

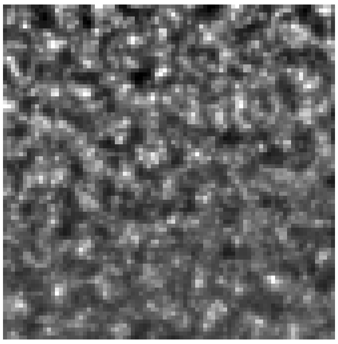
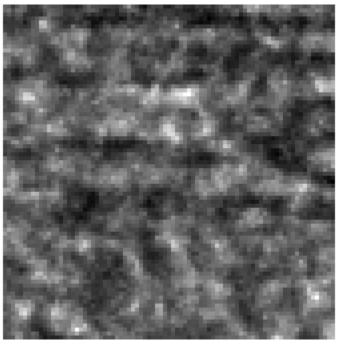
<u>Sample B (3 mm thickness)</u>

The image depends on the area of the sample (0.5 mm x 0.5 mm). The two images below were recorded from different parts of the sample. The packing of the spheres may be different.

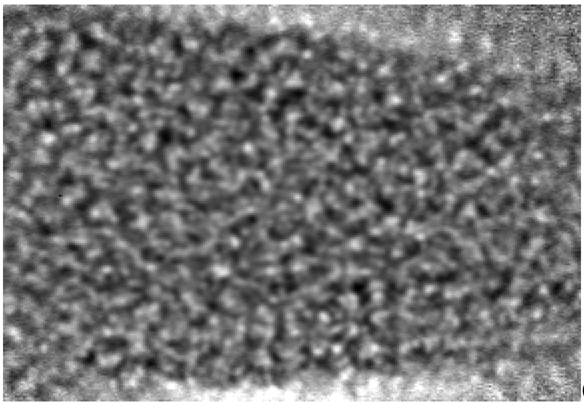
(1000×1018 pixels, sample-B¥sample1.tif) (1000×1018 pixels, sample-B¥sample2.tif)

These images were binned 12 times to simulate images recorded with a 6μm-pixel detector.

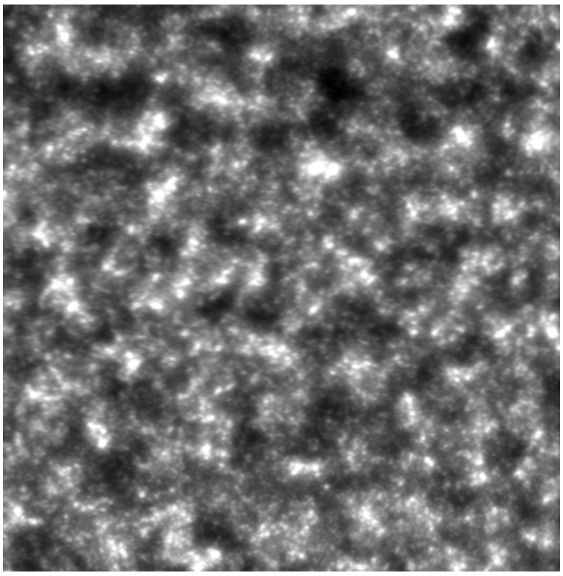
Although it is difficult to notice in a print, images obtained with the high-resolution detector have higher contrast than those obtained with the low-resolution detector.

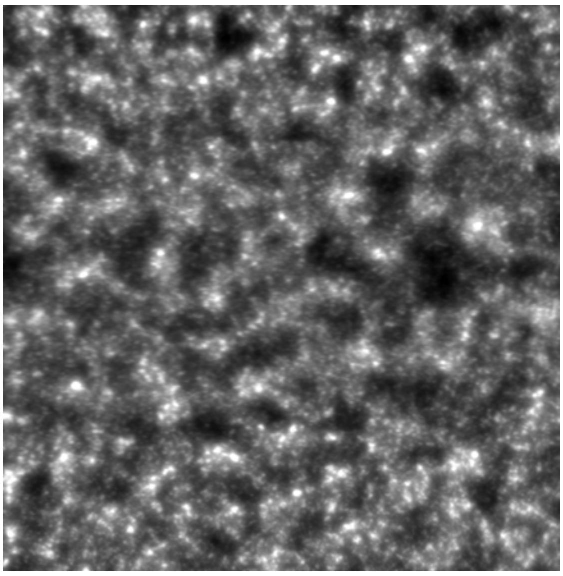
For comparison, this is an image obtained in the previous experiment with E=32keV, L=246cm from the same specimen.


(288×197 pixels)

Sample D (1.3 mm thickness)

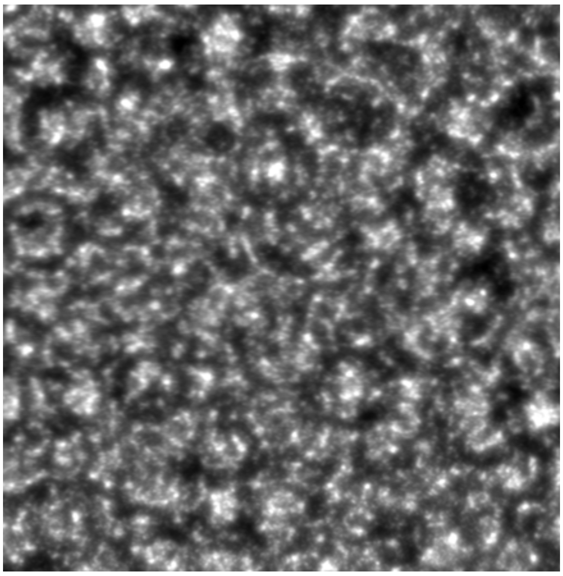
The two images below were recorded from different parts of the sample. There is considerable variation in the appearance, probably due to different packing densities.


(1000×1018 pixels, sample-D¥sample1.tif)


(1000×1018 pixels, sample-D¥sample2.tif)

Sample E (0.6 mm thickness)

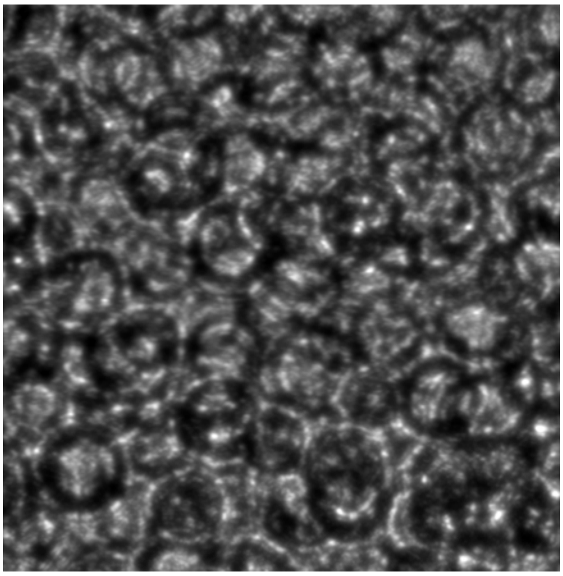
With this sample, you may have an impression that you can identify each bubble.



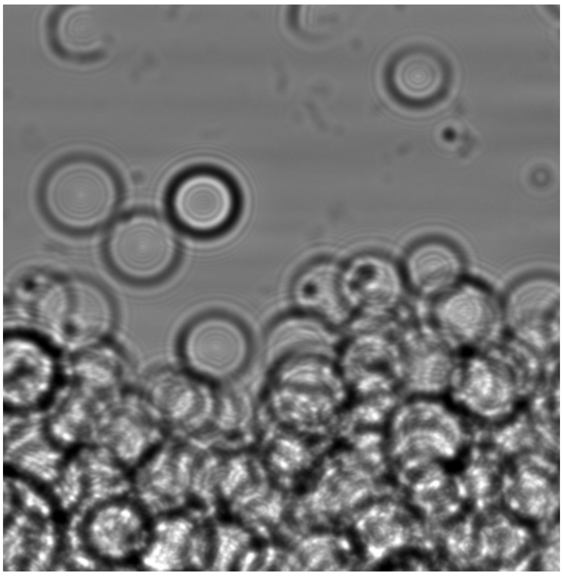
(1000×1018 pixels, sample-E¥sample1.tif)

Sample F (0.3 mm thickness)

Each bubble is identified, even when there are several layers of thickness. The right image is at the top of the bubbles.

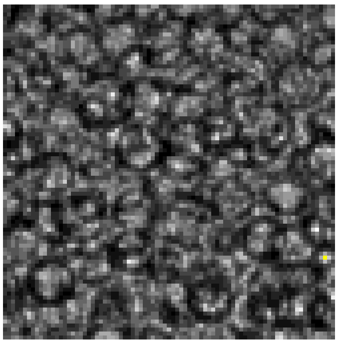


(1000×1018 pixels, sample-F¥sample1corrected.tif)



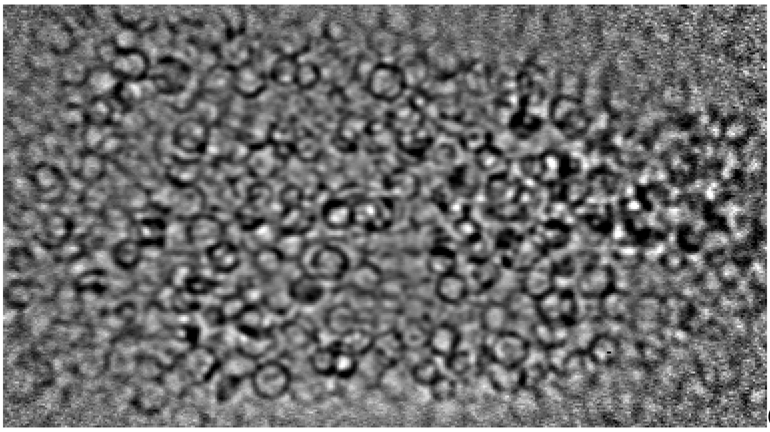
(1000×1018 pixels, sample-F¥sample4.tif)

The left image was binned by 16 times.



The contrast is lower, but you can still imagine each sphere.

For comparison, this is an image obtained in the previous experiment with E=32keV, L=246cm from the same specimen.

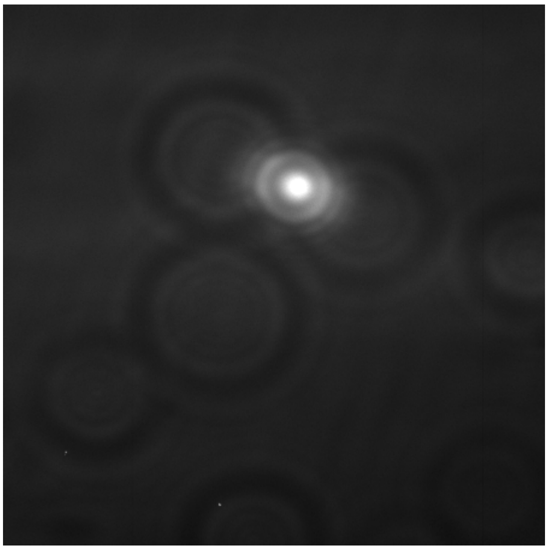
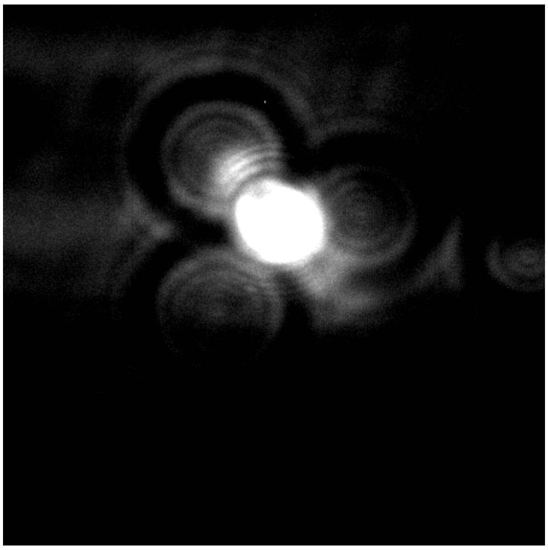


(382×211 pixels)

5. Pinhole scan of bubbles

In the first scan, the sample was stable and the pinhole was moved in the beam. Deviation of the beam was clearly observed. The scan was made in a raster mode with a step size of 4 μm in both vertical and horizontal directions. Since the x-ray beam from the pinhole had fringes, it was difficult to see an exact 2D profile of the spread of the beam. However, the deviation of the center of the beam was clear. Also, the beam sometimes reached a region where the pinhole beam had no intensity.

The two figures below show such extreme cases. Since the 20keV x-ray pass through the Ta plate of the pinhole to some extent (0.6% transmission), it is also possible to observe the arrangement of the bubbles in the sample. This is not what we intended. We should have used lower energy!

(450×450 pixels, pinhole¥scan1¥a024.img) (450×450 pixels, pinhole¥scan1¥a071.img)

There is a movie in http://yagi.spring8.or.jp/movies/bubblescan1.avi (133 MB). A background-subtracted movie shows the movement of the beam better (http://yagi.spring8.or.jp/movies/bubblescan1d.avi (133 MB)).

In the second experiment, the pinhole was fixed and the sample was moved. The movie is http://yagi.spring8.or.jp/movies/bubblescan2.avi (133 MB). The broad stripes from the top-right to the bottom-left are due to uneven thickness of the Ta plate.

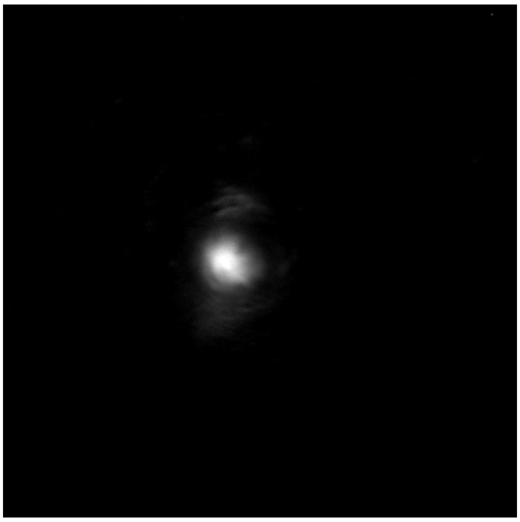
6. Pinhole scan of layers of bubbles

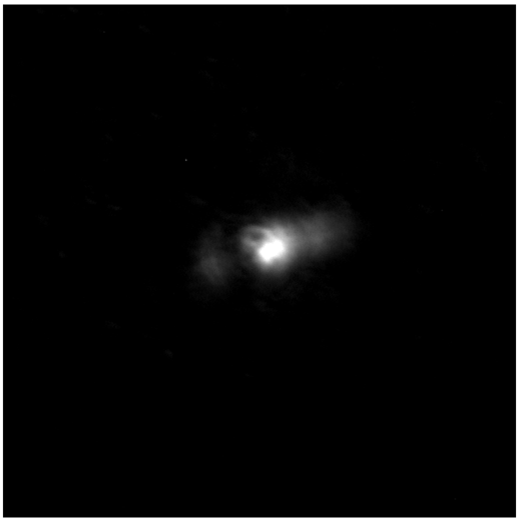
With the pinhole fixed, samples were moved with 4μm steps. The beam center without a specimen was at the center of the image.

<u>Sample F (0.3 mm thickness)</u>

Two movies were made. One with the weak image of the bubbles that came through the Ta plate (http://yagi.spring8.or.jp/movies/scanF.avi 176MB), the other with background subtracted (http://yagi.spring8.or.jp/movies/scanFd.avi 174MB, a frame around 51st and another around 94th are missing due to a software error). Background subtraction was done by scanning the sample without a pinhole. However, because of the instability of the monochromator, the background image still remains slightly. The images with background show that the beam moves mostly in the area where high intensity is observed in the image without a pinhole. This may give a clue to the formation of the "speckle" image.

In the following two images, the specimen was moved only by 4 μm but the beam goes to entirely different directions.


(512×512 pixels, scan5¥ad015.img)


(512×512 pixels, scan5¥ad016.img)

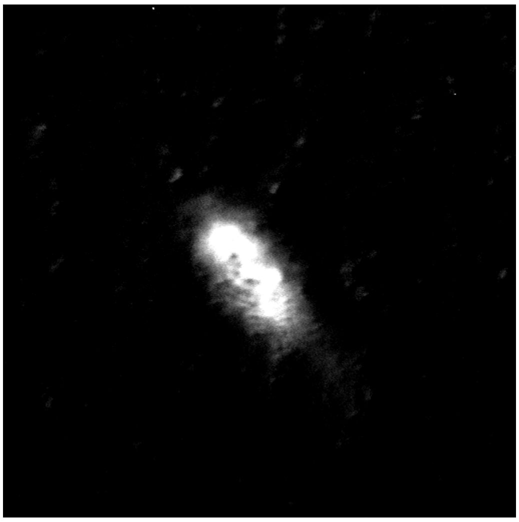
<u>Sample E (0.6 mm thickness)</u>

A movie with the weak image of the bubbles that came through the Ta plate (http://yagi.spring8.or.jp/movies/scanE.avi 707MB), one with background subtracted (http://yagi.spring8.or.jp/movies/scanBd.avi 707MB). The larger file size is due to the larger number of frames (900).

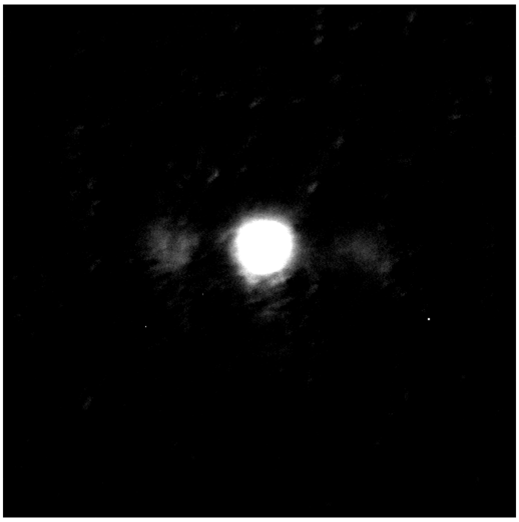
<u>Sample B (3 mm thickness)</u>

A movie with the weak image of the bubbles that came through the Ta plate (http://yagi.spring8.or.jp/movies/scanB.avi 172MB), one with background subtracted (http://yagi.spring8.or.jp/movies/scanBd.avi 170MB, this has higher background to show the spread of the beam better, a frame around 85th is missing).

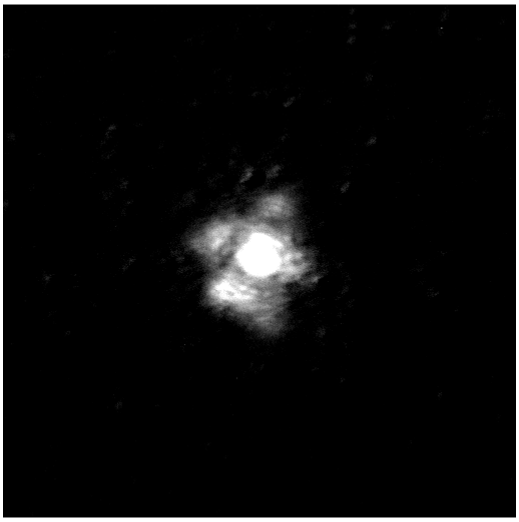
Here are some examples of how the beam spreads in the sample.



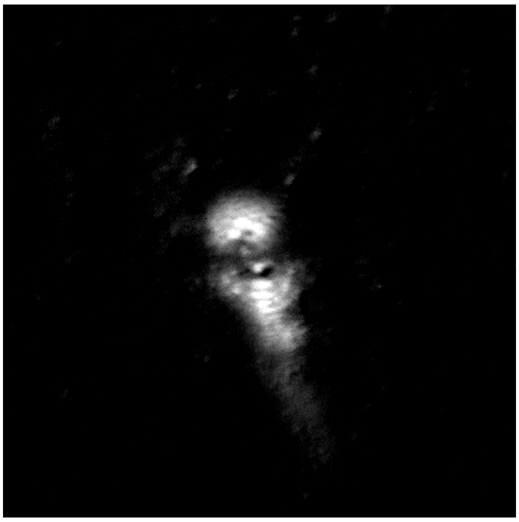
(512×512 pixels, scan7¥ad51.tif)



(512×512 pixels, scan7¥ad56.tif)



(512×512 pixels, scan7¥ad60.tif)

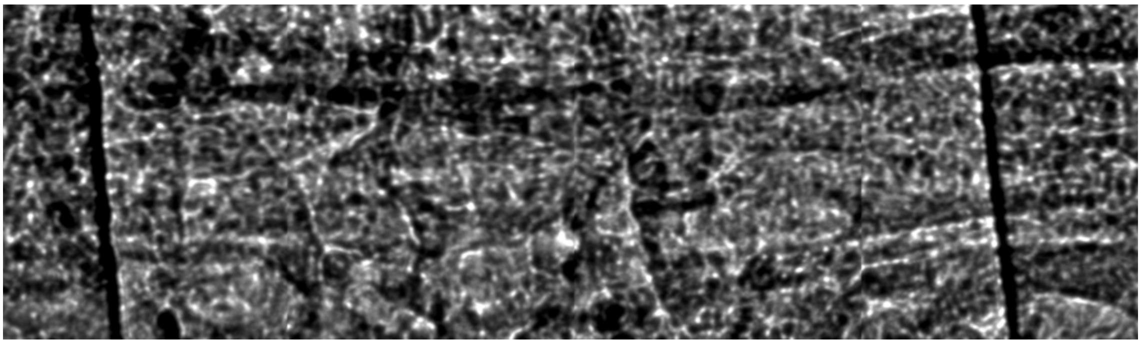


(512×512 pixels, scan7¥ad68.tif)

7. Observation of a mouse airway

A hairless mouse was used. X-ray energy was increased to 30 keV using the 3rd harmonic of the undulator radiation. Scanning a mouse at this high resolution was not easy because the mouse moves throughout the experiment. The mouse was fixed with a thread attached to its front teeth, but the thread was gradually stretched. With a field of view of 0.5mm square, it is also difficult to find which part of the mouse we are looking at. Therefore, scanning was necessary.

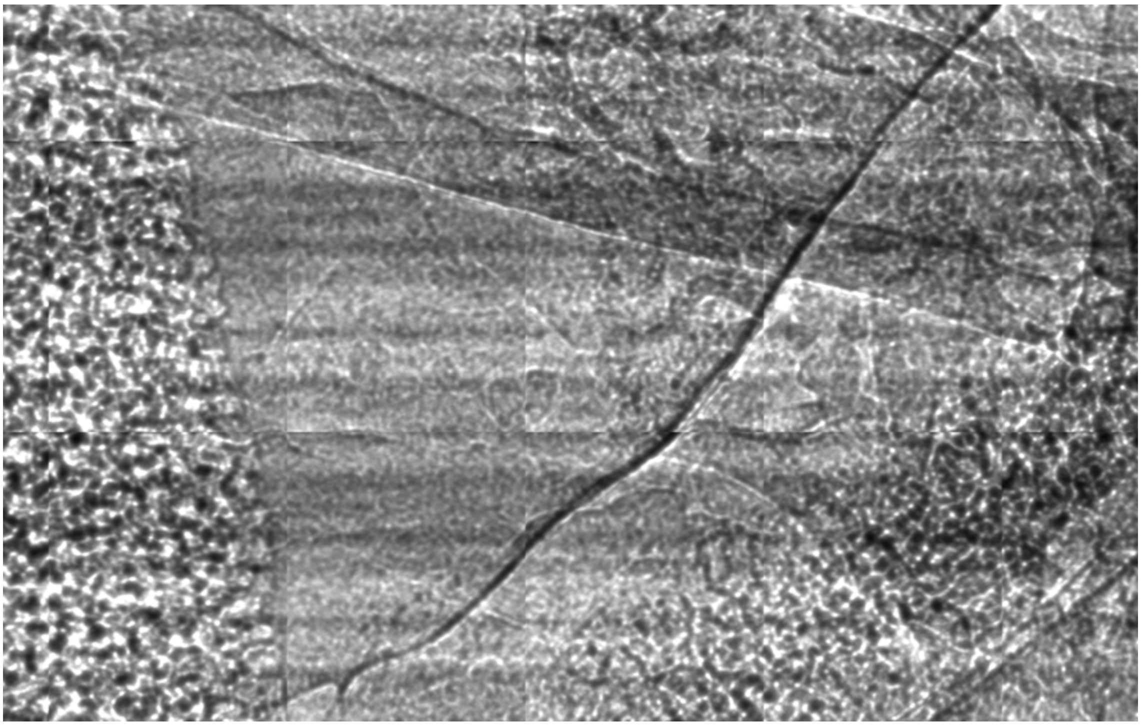
In the figure below, the two vertical lines are inner walls of trachea. They do not look smooth because there are a lot of noise from other overlapping tissues. The interface between the air and the wall is delineated clearly by fringes. However, the details of the wall are not seen at all.



(2680×792 pixels, mouse1¥scan15a.img)

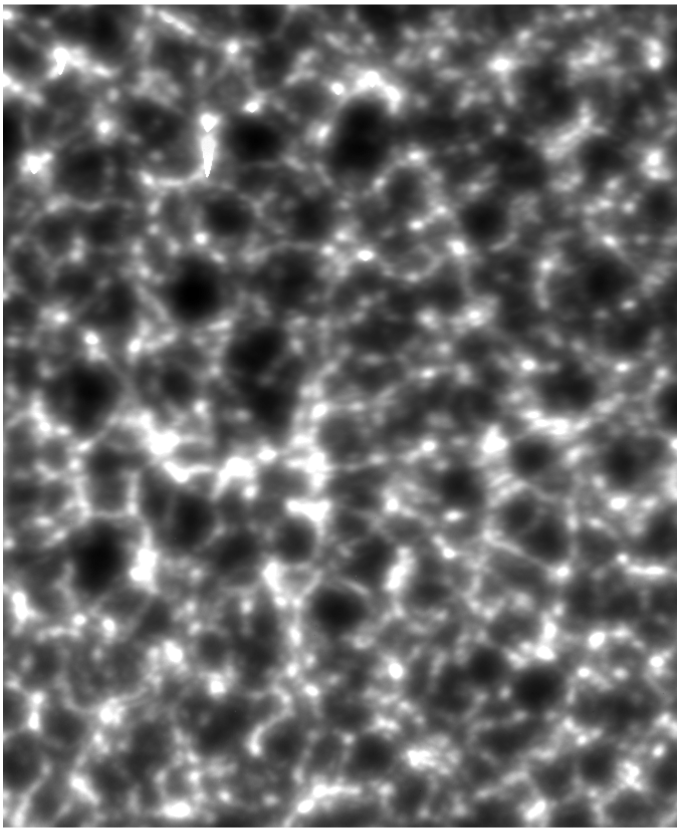
For the highest spatial resolution, the distance between the specimen and the detector should be smaller. However, even under that condition, it would be difficult to observe the surface structure.

The figure below shows a bronchus (from top-right to bottom-left). On the left is lung. The speckle-like pattern in the bronchus is of an unknown origin.



(3229×2039 pixels, mouse1¥scan15b.img)

The lung looks like this. Some regions were saturated.



(671×820 pixels, mouse2¥scan20a.img)

The black disks are with a diameter of 20-40 μm, similar to the size of aliveoli.

This is an image of nose.　There must be airway somewhere but it is hard to see.