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Detection of Lung Cancer with Phase-Contrast X-ray Imaging Using Synchrotron Radiation

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***Abstract*—In the present study, the feasibility of applying synchrotron radiation for the detection of lung cancers was investigated. Lung cancer tissues grown in mice over different periods were observed with phase-contrast synchrotron X-ray chest imaging. Irregular and tortuous vessels appearing in early cancer angiogenesis were found in the periphery region of the implanted cancer. Structure difference was clearly shown between normal tissue and early stage solid cancer in micrometers. Results from this study indicate that synchrotron X-ray may open broad perspectives for imaging cancer development and progression noninvasively.**

I. Introduction

LUNG cancer is one of the most common and deadly diseases in the world. It is the leading cause of death from cancer worldwide [1], in both developed and developing countries, and its incidence is increasing globally.

Early detection of lung cancer is the key to its cure, but a difficult task to undertake. The five-year survival rate of patients with lung cancer is approximately 14 percent and has not changed over the past several decades [2]. Long-term survival rate would be improved by increasing the number of early stage diagnoses [3], [4].

Presently, X-ray chest films, pathological diagnosis, CT, MRI, isotope, bronchoscopy, etc. are used for lung cancer diagnosis. Specimens of needle biopsies can be analyzed through pathological studies. Although MRI is an ideal diagnostic method in determining the cancer character, size, configuration, and position, it usually requires contrast agent [9]. The theoretical advantage of CT for lung cancer detection is its ability to demonstrate small cancers, presumably at stage I [10,11]. However, neither CT nor MRI can be used to clearly detect the detailed structures on micron scale that is useful to the early diagnosis of cancers [12-14].

Conventional X-ray imaging utilizes absorption imaging, for cancer detection. It shows the presence of details in the sample by distinguishing different attenuation coefficients resulted from different tissues. Because there is nearly no difference in linear attenuation coefficient of biological objectives such as soft tissues, it is very difficult to discriminate cancer lesions from normal tissues [15]. Phase-contrast imaging, on the other hand, using the typical edge-enhancement mechanism could strongly enhance the differences among soft biological tissues. It has the potential to reveal the structures inside soft tissues without the aid of contrast agent and would apply to very challenging diagnostic examinations.

Synchrotron radiation (SR), which has recently been introduced as a substitute to conventional X-rays, has properties allowing high resolution imaging down to the micrometer scale [16]. The unmonochromatic synchrotron beam is perfectly suitable for the phase-contrast technique and obtaining higher quality images. The potentiality of SR has been exploited in diagnostic radiology and specially in medical imaging. Phase-contrast X-ray radiography with synchrotron radiation reveals cerebellar structures of a rat [17] and human metasatic cancer lesions [18]. This opens the door of SR X-ray imaging in biology and medicine, not only to high spatial resolution but also to high time resolution [19].

The large difference of refractive index between air and soft tissues makes the lung an ideal candidate for phase-contrast imaging [20, 21]. In this paper, phase-contrast X-ray with synchrotron radiation was used for the first time to investigate the structure characters of lung cancer in vivo in different days after the implantation in mice. Phase-contrast images were compared with absorption images both using synchrotron radiation to determine the features represented by them respectively. 3D volume-rendered images were reconstructed using the series projection images on a workstation. The resolution characterisitics and image quality using phase-contrast X-ray with synchrotron radiation were evaluated.

II. Materials and Methods

A. Animal Model

BALB/c nude mice (20±2g 5 week) were bought from the Animal Center, CAS, Shanghai, China. They were fed with sterile food, acidified water with the pH value kept at 2.5-2.8, and housed in the isolated cages with a 12-h light/dark cycle. Lewis lung cancer cells were cultured with medium1640 with 10% FBS. Cells were growing in a 5% CO_2 incubator until they were 90% confluent. Cells were harvested at a concentration of $1\text{-}2\times10^6$ cells ml^{-1}. 0.1ml lung cancer cells were surgically transplanted into nude mice by percutaneous inoculation methods. On the 7th day after inoculation, solitary pulmonary nodules can be observed in the nude mice. On the 7th, 9th, 11th day after Lewis lung cancer being implanted, the cancer nodules inside the lung was excised, fixed with 10% buffered formalin. The specimens fixed with 10% buffered formalin were cut into 2-mm-thick sections. After taking phase-contrast X-ray images with synchrotron radiation, correlative histological sections of 5μm thickness were prepared and stained with hematoxylin and eosin. Lung cancer tissues were investigated using a 100× oil objective.

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Images taken under the trans-illumination were collected.

B. Phase-contrast X-ray Imaging with SR

For imaging of mice lung cancer in vitro, the International Consortium of Phase Contrast Imaging and Radiology (7B2) beam at the third generation synchrotron radiation facility Pohang Light Source (PLS) in Korea was used. The PLS is designed to provide synchrotron radiation with continuous wavelengths down to 1 A. It has been running at 2.5 GeV since 2000.

A key parameter in the phase-contrast radiology is the distance between specimens and detector. 20-40 cm can wash out other types of phase-contrast effects such as Fresnel diffraction fringes and optimize the refraction effect [19]. The experimental parameters for phase-contrast X-ray imaging with SR were as follows: an exposure time of 50 msec, 20×objective lens, and specimen-detector distance of 20cm. In order to compare the image quality,, absorption images were taken at the same time with the specimen-detector distance of 2 cm.

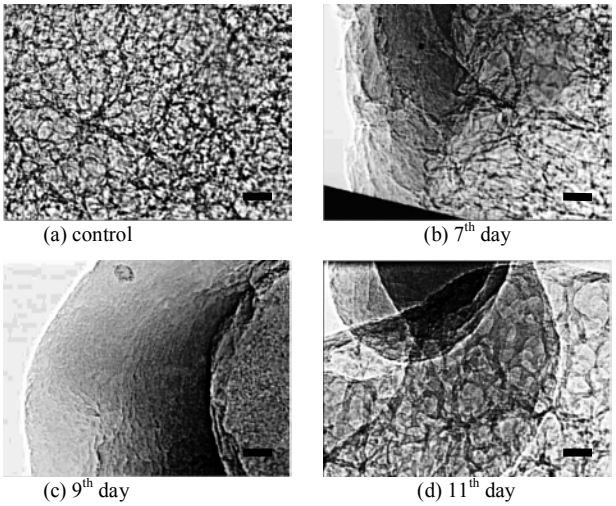
C. 3D Reconstruction process

The samples were mounted on a translation/rotation stage that was controlled by a step motor allowing precise positioning. For biological samples with intrinsically limited x-ray phase contrast, a single image (typically 1600×1200 pixel, horizontal field of view (FOV) 540 μm) could be taken within 50 ms. Four images were taken at each position and averaged. The final image was obtained by subtracting the background acquired at the same height. As the samples were usually 2~3 mm both in length and width, arrays of images should be projected accordingly and patched by a program via Matlab automatically. After that, the areas of interest, usually the edge of the cancer with plenty of angiogenesis, were chosen. 3D reconstruction was then performed. During the 3D reconstruction, the stage should be aligned horizontally to include the area of interest in the image field. Serial images were taken every 0.5 degree by the step motor automatically for reconstructing the 3D volume-rendered image. Through the standard filtered back projection algorithm process, the imaging results were input into the software Amira for the 3D display. The corresponding radiation dose did not produce any detectable damage to tissue.

III. RESULTS

A. SR imaging in vitro

The phase-contrast X-ray images with SR were shown in Figure 1a-d. Normal structures of the secondary pulmonary lobule, consisting of the pulmonary arteriole, terminal brochiole, air sacs, and venules in the interlobular septa continuing into the visceral pleura, are clearly illustrated in Figure 1a. The minimal diameters of the alveoli, alveolar duct, and venules in the lobule were detected on the SR image. The images obtained from the formalin fixed lung cancer tissue in different days with the synchrotron X-ray are shown in Figure 1b-d. In Figure 1b, there are significant differences found in structures between the cancerous and normal lung tissue regions. In the cancerous region, cells are closely packed to form dense and smooth cancer tissue, quite different from the normal lung tissue where pulmonary arteriole, terminal brochiole, and air sacs are clearly seen. Irregularly formed and tortuous early cancer angiogenesis can be observed in the periphery region of the implanted cancer. Figure 1c shows the densed smooth cancerous tissue on the 9th day. On the 11th day, the boundary between cancerous and normal tissues is distinguishable. Cancer cells are growing along the alveolar wall filled with air sacs that is mildly thickened (Figure 1d). Corresponding histopathological studies the lung cancer specimens were performed and the detailed structures observed with SR was confirmed (Figure 2).



(a) control (b) 7^{th} day

(c) 9^{th} day (d) 11^{th} day

Figure 1 The lung cancer growth on the 7^{th}, 9^{th}, 11^{th} day viewed phase contrast image with SR.

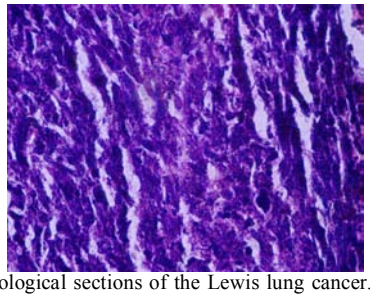
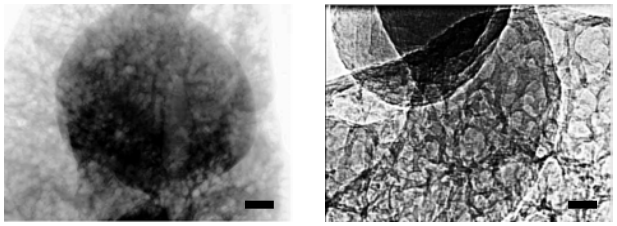


Figure 2 Histological sections of the Lewis lung cancer. Tumor cells growing along the alveolar walls. The alveolar walls were mildly thickened.

The lung cancer absorption image and phase-contrast image with SR are shown in Figure 3 a, b, respectively. In comparison, the phase-contrast image with SR shows much higher spatial resolution, distinguishable boundary between cancerous and normal tissues, as well as mildly thickened alveolar walls and tortuous angiogenesis.



(a) Absorption image (b) Phase contrast image

Figure 3 The Lewis lung cancer viewed with absorption image and phase contrast image with SR. (a) The absorption image of lung cancer with SR couldn't provide greater details and clear boundaries in the tissue; (b) The phase contrast image with SR show much higher spatial resolution, mildly thickened alveolar walls and tortuous angiogenesis could be observed clearly. (bar: 40um)

B. 3D Reconstruction

Figure 4 shows the patched image, tomographic reconstruction, and volume rendered 3D structure of the normal lung specimen, respectively. The injector's tip, which is visible in the middle of Figure 4a, was used to fix the sample to the holder. As identified by the arrow, alveolus can be easily found on the edge of the specimen, while in the middle part of the image, it is somewhat blur because of the thickness. Also the trachea or the bronchia can be seen across the specimen. The white box in this image is the area of interest, which was tomographic reconstructed in Figure 4b. The reconstruction was based on 360 projections within 2 min. It clearly reveals the alveolus wall. Note that only the area in the box was reconstructed due to the limited field of view as large amount of image acquisition required for tomography reconstruction [22]. Quality of the reconstruction was not optimized due to the slight movement of the sample during the measurement. The reconstruction was also blurred by the "local tomography" effects [23, 24] due to the large dimension of the sample. Figure 4c shows the 3D volume-rendered from the tomographic reconstructed data as exampled in Figure 4b. One can found that not only the alveolus is clearly imaged, but also the bronchia.

Figure 5a shows the lung cancer imaged on the 11th day after the implantation. Figure 5b presents its tomographic reconstructed image. The entire region is pretty uniform without much structure observed. But, the wall of the alveolus is thickened. In Figure 5c, the 3D volume-rendered image is shown to further reveal the smooth surface of lung cancer .

The above mentioned preliminary results have shown that angiogenesis of early stage lung cancer is detectable in the periphery region, while the center is uniform using phase contrast SR imaging. These findings are similar to those previously reported in [25].

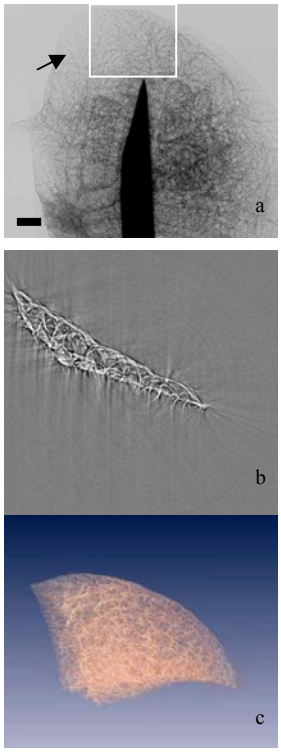


Figure4 Images of normal lung specimen. a, patched phase-contrast micrograph of the whole specimen, an injector's tip was used to fix the specimen to the holder. The white box in this image is the area of interest (AOI). Bar: 200um; b, tomographic reconstruction of part of the same specimen; c, The volume rendered 3D structure of the same specimen.

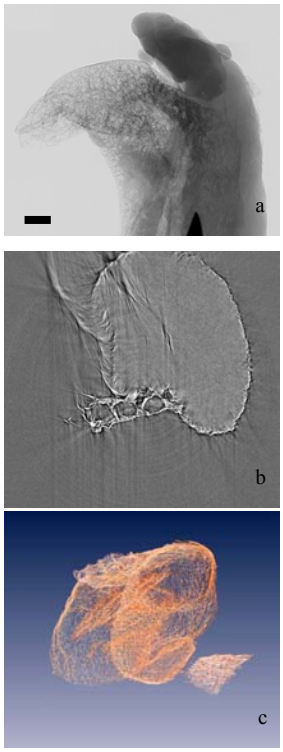


Figure 5 Images of 11th day lung cancer specimen. a, patched phase-contrast micrograph of the whole specimen, an injector's tip was used to fix the specimen to the holder. The white box in this image is the area of interest (AOI). Bar: 200um; b, tomographic reconstruction of part of the same specimen; c, The volume rendered 3D structure of the same specimen.

IV. DISCUSSION

Although conventional x-rays have been widely used to diagnostic imaging based on the absorption characteristics, the high flux and brightness of SR beam provide an ideal X-ray source for many applications in medical science. It is considered that SR could be applied to study in vitro such as X-ray microscopy, structural biology, and cell biology [25]. Because the refraction imaging with SR can emphasize the edge effect of the structures of an object on the image, it has extremely high intensity to observe the very fine structures of the tissue. In our study, the SR images depicted the structures of 2mm-thick sections of normal and cancerous lung tissues. Compared with conventional absorption image, phase contrast image with SR X-ray showed much higher spatial resolution and better image contrast. Fine internal structures of the lung cancer tissues were visualized with resolutions of a few micrometers and enhanced edge contrast. In thick slices, numerous pulmonary structures overlapped each other. These overlapping structures were mostly recognized on SR images, including cancer region, normal tissue and new blood vessels. The aim of this study was to clarify the radiological and pathological characteristics of small lung cancer. In this paper, pathologic findings of the each resected cancer were compared to SR findings. The phase contrast image with SR

showed cancer cell invasion and vascular invasion, the results are consistent with pathologic findings. Angiogenesis is a complex process that may determine the growth and metastasis of a malignant cancer. It is very important to the early and accurate detection of cancers and newly developed vessels in cancer diagnosis. Thus, phase-contrast image with SR providing information about angiogenesis in the periphery region would be useful for distinguishing the boundary between normal and cancerous tissues.

This was the first step of using 3D synchrotron radiation X-ray imaging for cancer detection. The tomographic reconstruction based on the unmonochromatic synchrotron X-ray phase-contrast imaging can clearly produce 3D volume-rendered images of the lung cancer samples. It had already fulfilled our objectives. However, there were still some problems existing during experiments. The most significant one was the sample stability on the stage. Because of the rotation during the image acquisition, the sample would shake slightly on the stage. As the resolution of the image was a few micrometers, any slight motion could not be omitted. This is why the 3D reconstructed images are a bit blur. The thickness was another factor why the cancers could not be detected. The images on the thinner edges of the sample were much clearer than those of the center. All of these should be improved in future experiments.

In mice with small cancer implanted, SR proved to be a fast and noninvasive imaging device for the detection of lung cancers. The diseased lung tissue could be discriminated from normal soft tissue in the SR images both at an early stage and a more mature stage of cancer development. A good correlation was found between data from SR and visual inspection of the excised lungs as well as the microscopic observation of histological sections afterwards. But presently, large radiation dose limits the use of SR imaging system in clinical application. The SR x-ray exposure dose should be substantially reduced while providing better images before it can be used in vivo.

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