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Synchrotron Radiation Imaging of Internal Structures in Live Animals

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Ionizing radiation has long been used in medicine since the discovery of X-rays. Diagnostic imaging using synchrotron radiation has been under investigation since Rubenstein et al. reported dual-energy iodine-K-edge subtraction coronary angiography. Recently, computed tomography (CT) and magnetic resonance imaging (MRI) have provided better quality results than conventional radiology, providing important information on human internal structures. However, such techniques are unable to detect fine micron sized structures for the early diagnosis of tumors, vascular diseases and other medical objectives. Third generation synchrotron X-rays are well known for their superiority in coherence and energy tunability with respect to conventional X-rays. Consequently, new contrast mechanisms with a superior spatial resolution are becoming available. Here we present the extremely fine details of live animal internal structures using unmonochromatized synchrotron X-rays (white beam) and a simple detector system. Natural movements of the internal organs are also shown. The results indicate that this imaging technique can be applied to investigating microstructures and evaluating the function of the internal organs. Furthermore, this imaging system may be applied to humans as the next tool beyond CT and MRI.

Key Words: Synchrotron radiation, white beam, imaging technique, phase contrast, internal organ

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INTRODUCTION

According to the current trends, health care tends to be dominated by technology. As a result, many scientists have developed many imaging and therapeutic medical technologies such as radiography, CAT scanning, nuclear medicine, radiotherapy, magnetic resonance imaging (MRI), and ultrasound. Among them, most commonly used medical imaging and therapeutic techniques are based on ionizing radiation.[1] Ionizing radiation has long been used in medicine since the discovery of X-rays[2] and diagnostic imaging using synchrotron radiation has been investigated since Rubenstein et al. reported dual-energy iodine-K-edge subtraction coronary angiography for a safe intravenous angiography technique in 1981.[3]

Synchrotron radiation provides X-rays that are much more intense, polarized, and continuous over a wide energy range than conventional sources.[4] Even after the beam has undergone monochromatisation (filtering to allow only X-rays within a narrow bandwidth to pass), there is still an adequate number of photons for imaging purposes. A third generation of synchrotron radiation, characterized by a small electron-beam size, a low electron-beam emission, and long straight sections, have been virtually applied to the medical field as angiography, computed tomography, radiotherapy, X-ray microscopy, and structural biology.[1,5] For example, phase-contrast X-ray radiography with synchrotron radiation has revealed the cerebellar structures of rats[6] and human metastatic tumor lesions.[7] Phase-contrast X-ray computed tomography (CT) has also demonstrated rabbit cancer lesions,[8] the rat cerebrum,[9] and human cancer lesions.[10]

Although many techniques such as computed tomography (CT) and magnetic resonance imaging (MRI) have been developed for imaging the internal structures of live specimens, these techniques cannot detect the fine micron sized structures for the early diagnosis of tumors, vascular disease and other medical objectives.[11-13] Present in-vitro studies using micro-CT or MRI with phantoms and specimens such as bone and breast tissues can detect objects several ten microns in size.[11-13] The spatial resolution of cine-angiographic studies of the heart coronary arteries, hind limb and abdominal ileal arteries in animals using monochromatic synchrotron radiation with iodine K-edge contrast enhancement makes it possible to detect details as small as 20 μm.[14-23] In this study, an attempt was made to observe the internal structures of live specimens using unmonochromatized synchrotron X-rays (white beam) and a simple detector system.

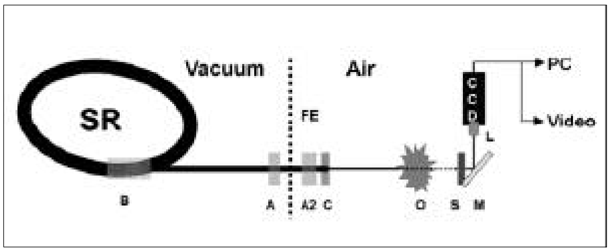

Fig. 1. Schematic drawing of the experimental set-up. This system is similar to an 'in-line phase sensitive imaging system'1 with a high resolution and high magnification. Unmonochromotized synchrotron X-rays (30 keV) arrive from the bending magnet device (B) installed in the storage ring (SR) of the PLS in Korea and passes two sets of attenuators (A1 & A2), which are composed of silicon wafers for optimizing the X-ray energy spectrum. Three pieces of silicon wafer (A1) are placed in series in the vacuum pipeline, and 4 silicon wafers (A2) in the air. By using manually controlled slits (C), the X-ray beam is reduced to a 10 × 10 mm beam size for matching the scintillator. The X-rays passing through the live object hit the scintillator (S), which is a thin plate of $CdWO_4$ (10×10× 0.1 mm, Bicron Co., USA). Visible-light images of the live object from the scintillator are reflected at 90 degrees by a gold-coated mirror (M) placed just behind the scintillator and enlarged by a magnification lens (L) and finally, the rays reach the CCD camera. Digital images from the CCD camera are fed into either a computer (PC) or a video recorder (Video). The distances from the bending magnet to the object and from the object to the scintillator were 28.53 - 28.83 m and 17 - 47 cm, respectively.

MATERIALS AND METHODS

This study was performed using the 5C1 beam line (unmonochromatized, peak≈30keV) of the Pohang Light Source (PLS) in Korea, which has a third generation synchrotron radiation facility. The schematic diagram shown in Fig. 1. illustrates the experimental conditions used. This is a type of an 'in-line phase sensitive X-ray system'.[24-26] However, unmonochromatic synchrotron X-rays and a simple detector system were used.

The animals used were male hairless mice (SKH-2, Charles River Co., Maine, USA, body weight: 20 - 25 gm), mouse fetuses on the 15th day of pregnancy, and male 12 week old rat (Sprague Dawley, body weight: 150 - 250 gm). The experimental animals were positioned in an up right position using an animal holder in front of the detector. During the experimental procedures, the animals were generally anesthetized and monitored in the usual manner. This study was approved by The Committee for the Care and Use of Laboratory Animals in Yonsei University College of Medicine based on the "Guide for Animal Experiments".[27]

The fine structures of the rat cervical inter-vertebral region, the mouse femoral joint, and the mouse fetal bone were recorded using a Kodak CCD camera (Model 4.0/8bit, 2,112 × 2,112 pixels) with a 30 ms exposure time. Natural movements of the internal organs, that is, the esophagus, stomach, and the renal system were recorded by using a Toshiba CCD camera (model IK-536, 30 frames/sec) with a zoom lens and a Sony Digi-Cam (model 360X). Small amounts of iodine contrast dye (Imagopaque®, 300 mg I/ml) were used for clear visualization.

RESULTS

Fig. 2. shows several bony structures with a high resolution and in real time. They are the cervical inter-vertebral region (a) in a rat, the right

femoral joint (b) in a hairless mouse, the ribs and vertebrae (c), and the paw (d) in a mouse fetus on the 15th day of pregnancy. Note the extremely fine detail of each structure, which cannot be visualized by conventional or special X-ray imaging techniques and even by currently used CT or MRI.[11,13,28,29] Note that this superior performance is related to the differences in the refractive index (phase contrast or similar mechanisms), which enhance the conventional absorption contrast. Furthermore, the resolution limit has not yet been reached.

Fig. 3. shows one of the natural movements of the internal organs, peristalsis, which is a worm-like slow consecutive movement of the intestines, in real time and with a relatively high resolution. From the mid-esophageal region (a~c) and the pyloric portion of the distal stomach (d~f), peristaltic movement was clearly noted over approximately 10 seconds, which is comparable with the human intestinal peristaltic movement (5 - 6 times per min.). At the beginning of the ureter (urethra?) and at the distal pelvic region of the kidney, the period was approximately 2 seconds (Fig. 4. a - c), while in humans it depends on the volume of urine production in the kidney. Some urine, which was ejected abruptly to the distal urethral portion via an external sphincter, was also successfully detected in real time (Fig. 4. d - f). When compared with the images shown in fig. 2, these organs are barely visible without an iodine contrast dye, probably due to limited differences in the refractive index, which decreased the effectiveness of the phase contrast, thereby enhancing the role of absorption contrast.

DISCUSSION

A third generation of synchrotron radiation has been applied to medical imaging and for developing fine images of the internal structures. Generally, a monochromator and complex equipment are used to make contrast enhanced images in this imaging system.[1] The imaging technique used in this study is simpler. It uses unmonochromotized synchrotron X-ray with several inexpensive pieces of equipment including a scintillator, a magnification lens and a digital CCD camera. This system enables the bio-morpholo

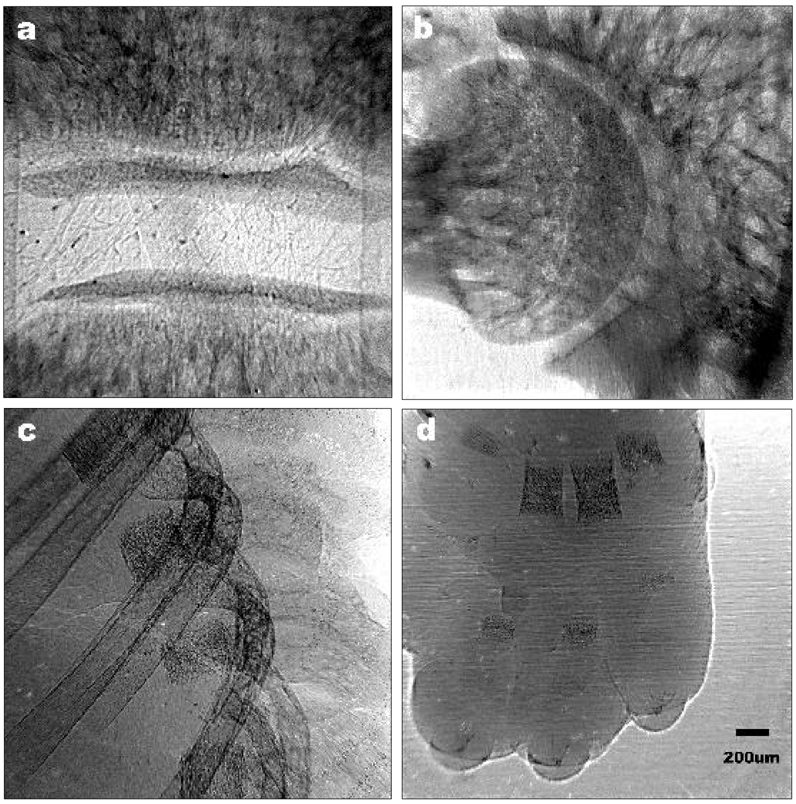

Fig. 2. Images showing extremely fine details of the internal structures in a rat, a mouse and a mouse fetus. Significant differences in the refractive index produce a phase contrast, thus enhance the conventional absorption contrast mechanism. Such a contrast specifically occurs between bony tissue surrounding the soft tissues in a (rat cervical inter-vertebral region), b (mouse femoral joint), c and d (fetal mouse - 15days after mating, ribs, vertebral bodies and paw). Extra fine details of a bony trabeculation are also well visualized (a, b, c & d), as well as the muscular fascicles in c. A Kodak CCD camera (Model 4.0/8 bit, 2,112 × 2,112 pixels) was used for image detecting. The exposure time for each photo was 30 ms. Scale bar: 200 μm.

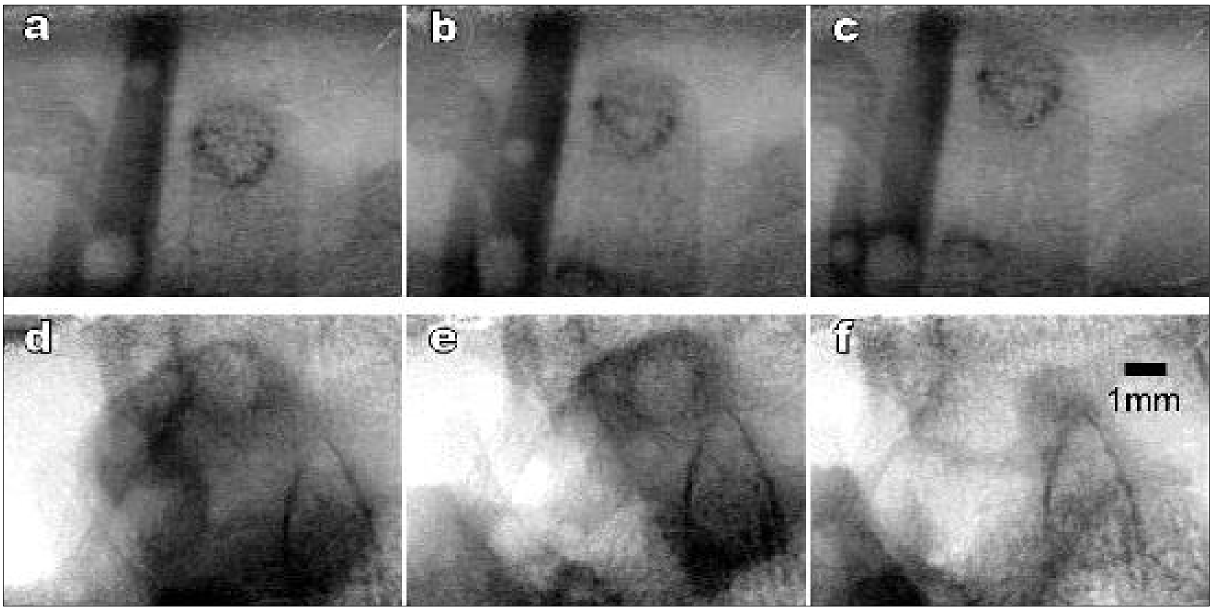

Fig. 3. Real time fine images of natural movements of the esophagus and the stomach in live rats. The natural movement directed in the oro-rectal direction at the gastro-intestinal tract is known as peristalsis, and is a worm-like slow and repeating movement resulting in food transportation. An ingested iodine contrast dye (Imagopaque®, 300 mg l/ml) with minute air bubbles traveled progressively down to the stomach in a, b and c due to the peristaltic movement. It then reached the distal stomach, pyloric region, where the same movement was well visualized in d, e, and f. These peristaltic movements occurred repeatedly about every 10-second.

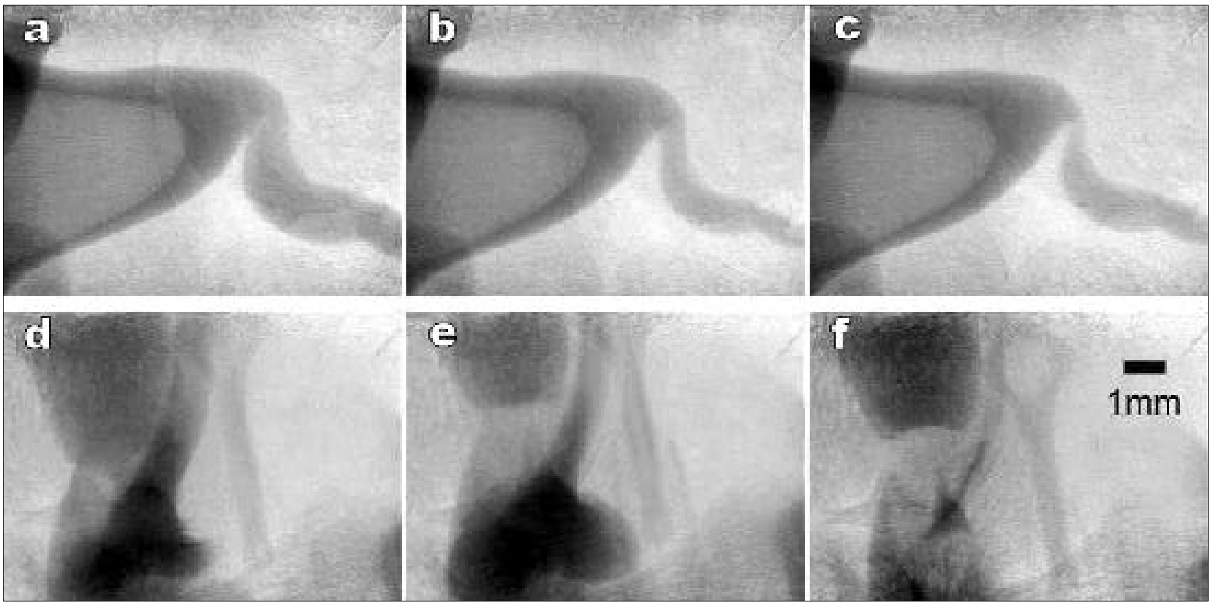

Fig. 4. Real time fine images of natural movements of the renal system in live rats. a through c show natural movement of the ureter (urethra?) in the renal system for urine transportation, repeating about every 2 seconds. The urine ejection at the urethral portion was detected successfully in d through f. To visualize the renal system, small amounts of an iodine contrast dye (Imagopaque®, 300 mg l/ml) were injected through the femoral vein where a fine polyethylene tube (0.8 mm OD) was introduced during animal preparation. A Toshiba CCD camera (model IK-536, 30 frames/sec) with a zoom lens and a Sony DigiCam (model 360X) were used. Scale bar: 1.0 mm.

gical changes of extra fine internal structures in live objects to be studied at micron resolution, regardless of the specific (absorption and/or phase) contrast mechanisms. Higher resolution imaging is possible if a higher magnification lens is used, although the field of view would be narrowed. This means that this system could act as a micro-probe for viewing the fine internal structures of live subjects and in a similar way as with light microscopy, various magnification lenses can visualize the microstructures in very thin tissue slices.

Thus, it is possible to observe extremely fine micron-size structures in live specimens by simply using a higher magnification lens without sophisticated and expensive optical systems including monochromators, interferometers and analyzers, etc.[30-33] This would allow more fundamental studies concerning angiogenesis (vessel formation), osteogenesis (bone formation) and oncogenesis (tumor development) to be done. Clinical applications to human patients for early and accurate detection of tumors, bone and vascular problems, and abnormal changes in the inherent movement of vital organs might have a substantial impact on human health care. For these objectives, further research on the safety aspects, and on the source and detection of X-rays, extending the present limited medical applications of synchrotron radiation are recommended.[34-39]

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