Vessel Imaging by Interferometric Phase-Contrast X-Ray Technique

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Background—Phase-contrast x-ray imaging using an x-ray interferometer has great potential to reveal the structures inside soft tissues, because the sensitivity of this method to hydrogen, carbon, nitrogen, and oxygen is \(1000\) times higher than that of the absorption-contrast x-ray method. Imaging of vessels is very important to understand the vascular distribution of organs and tumors, so the possibility of selective angiography based on phase contrast is examined with a physiological material composed of low-atomic-number elements.

Methods and Results—Phase-contrast x-ray imaging was performed with a synchrotron x-ray source. Differences in refractive index, \(d\), of physiological saline, lactated Ringer’s solution, 5% glucose, artificial blood such as pyridoxylated hemoglobin–polyoxyethylene conjugate, and perfluorotributylamine were measured. Because the \(d\) of physiological saline has highest contrast, it was used for the phase-contrast x-ray imaging of vessel, and this was compared with absorption-contrast x-ray images. Vessels \(>0.03\) mm in diameter of excised liver from rats and a rabbit were revealed clearly in phase-contrast x-ray imaging, whereas the vessel could not be revealed at all by the absorption-contrast x-ray image. Absorption-contrast x-ray images with iodine microspheres depicted only portal veins \(>0.1\) mm in diameter with nearly the same x-ray dose as the present phase-contrast x-ray imaging.

Conclusions—Phase-contrast x-ray imaging explored clear depiction of the vessels using physiological saline with small doses of x-rays. (Circulation. 2002;105:1708-1712.)

Key Words: imaging ■ vessels ■ liver ■ angiography ■ contrast media

Present clinical x-ray images are generated by the difference in x-ray absorption determined by the linear attenuation coefficient. The difference in the x-ray absorption of the blood against the vessel wall and surrounding tissue is very small; thus, the vessel structure must be imaged by filling the vessel with a contrast agent containing a heavy element such as iodine. Iodine contrast agent, however, has various disadvantages, such as high osmolarity, high viscosity, and allergic reaction.

X-rays have the nature of wave, so the wave shifts when x-rays pass through an object, and this is called x-ray phase shift. Phase-contrast x-ray imaging is advantageous because a cross section of the phase shift is \(1000\) times larger than that of absorption for low-atomic-number elements, such as hydrogen, carbon, nitrogen, and oxygen.\(^1\)\(^2\) Several x-ray imaging techniques for detecting the x-ray phase shift, such as an interferometric method\(^1\) using a crystal x-ray interferometer,\(^4\) a Schlieren-like method using crystal diffraction,\(^3\) and a holography-like method,\(^6\) are being developed. Among these methods, the interferometric method using a crystal x-ray interferometer is the most sensitive method for detecting the minute difference of refractive index in soft tissue. With the x-ray interferometer, slices of a rat cerebellum\(^1\) and a human metastatic liver tumor\(^2\) have been imaged without contrast agents. Furthermore, phase-contrast x-ray CT\(^3\) demonstrated a rabbit cancer lesion\(^2\) and rat brain\(^5\) and human cancer lesions.\(^9\)\(^11\)

By use of phase-contrast x-ray imaging with the x-ray interferometer, the possibility of demonstrating vessels by blood itself was suggested\(^12\) and the vessels in excised livers of a mouse and a rat were revealed.\(^13\) For clinical purposes, however, the investigation of particular vessels of interest is indispensable. Therefore, selective contrast enhancement with some new contrast agent is necessary even when the phase contrast is used. An important advantage is that one does not need to use contrast agents containing heavy elements; rather, the present new materials cause the x-ray phase to shift sufficiently.

The purpose of this study was to investigate the potential candidates for contrast agents in phase-contrast x-ray imaging and to image the vessels of rat and rabbit by one agent.
vessel images with phase contrast were compared with those with absorption contrast at nearly the same x-ray dose.

Methods

Phase-Contrast X-Ray Imaging System

The phase-contrast x-ray imaging system consisted of a silicon asymmetrical cut crystal to make a 2D beam, a monolithic x-ray interferometer, a phase shifter, an object cell, and an x-ray sensing pickup tube. To obtain phase-contrast x-ray images, a monolithic x-ray interferometer having a 25×25-mm field of view was made using a commercially available highly perfect single-crystal silicon ingot 10 cm in diameter. The size of the beam on the sample, however, was 25×15 mm, because the height (15 mm) was limited by the maximal beam height available. Because the pixel size of the x-ray–sensing pickup tube was 0.012×0.018 mm, the view size was 9.2×13.7 mm. A sample cell filled with water was inserted into a beam path between M1 and A of the interferometer (Figure 1). The synchrotron x-ray has excellent properties, such as natural beam collimation and high x-ray flux for obtaining phase-contrast x-ray images, so this system was installed at a vertical wiggler beam line of the Photon Factory in Tsukuba, Japan. The x-ray energy was set at 17.7 keV by the monochromator, and x-ray flux in front of the sample was ~7.5 mR/s at a beam current of 350 mA with 2.5-GeV energy.

Phase-Contrast X-Ray Projection Image (Phase Map)

The phase shift, $\Phi$, is given as $\Phi = \frac{2\pi\delta x}{\lambda}$, where $\delta$, $x$, and $\lambda$ are refractive index decrement of sample, position along x-ray beam, and x-ray wavelength, respectively. The phase map that describes spatial distribution of the phase shift within a sample cannot be obtained directly by use of the interferometer. The technique of phase-shifting interferometry is used to generate a phase map. The phase map of the sample was obtained from several interference patterns (5 in this experiment), which were measured sequentially by changing the phase of the reference beam. Each interference pattern was acquired at 3-second exposure, and the total exposure time to obtain for a phase map was 15 seconds. The thickness of sample cell was 12 mm and the total x-ray exposure was 113 mR to obtain a phase map. The spatial resolution of the phase map was ~0.03 mm because of the diffraction phenomenon within the crystal wafers in the analyzer.

Phase-Contrast X-Ray CT

Phase-contrast x-ray CT images mapped the variation of the difference of refractive index, $\delta$, with a voxel size of 0.018×0.018×0.012 mm. The $\delta$ of specimens was evaluated in 5 regions of the phase-contrast x-ray CT image.

Absorption-Contrast X-Ray Images

The absorption-contrast image was obtained by the x-ray–sensing pickup tube without the use of the interferometer at the same cell thickness as the phase map. The experimental setup of the phase-contrast x-ray system requires 1 day for installation. In view of this, samples were prepared individually to obtain a phase-contrast x-ray image and a corresponding absorption-contrast x-ray image. The entrance surface x-ray exposure was 122 mR and nearly comparable to the phase map.

In the imaging with iodine microspheres, the mass attenuation coefficient of iodine is 35 cm²/g at 17.7 keV. This value is almost the same as that just above the K-edge energy of 33.2 keV (34 cm²/g) used for synchrotron radiation angiography with iodine. The absorption-contrast image was obtained by the x-ray–sensing pickup tube without the use of the interferometer at the same cell thickness as the phase map.

Candidate Contrast Agents for Phase-Contrast X-Ray Imaging

The $\delta$ of physiological saline, lactated Ringer’s solution, 5% glucose, artificial blood such as 2% pyridoxylated hemoglobin-polyoxyethylene conjugate (Fe-C), and perfluorotributylamine (FC-43) was measured as the candidate contrast agent. The mass densities ($\rho$) of these liquid samples are 1.006, 1.006, 1.019, 1.020, and 1.105 g/mL, respectively. The wedge cell filled with various liquid samples was used to determine the difference of refractive index, $\delta$, against water.

Animal Preparations

Ten Wistar rats and a rabbit were anesthetized with pentobarbital, and cannulation to the portal vein was performed surgically. Among the liquid samples, the physiological saline was used for contrast enhancement. The physiological saline or iodine contrast agent was injected from the portal vein, and whole blood in the liver was replaced. After infusion, the portal vein, hepatic artery, and hepatic vein were ligated to prevent air contamination.

Phase maps (5 rats) and absorption-contrast x-ray images (3 rats) of the excised liver infused with physiological saline were obtained. The liver was placed in a square cell filled with physiological saline to reduce the effect of the thickness variation of the object.

For phase-contrast x-ray CT, a rabbit liver perfused with physiological saline was fixed in formalin, because the data acquisition time of phase-contrast x-ray CT required >6 hours. A column specimen 10 mm in diameter was cut from rabbit liver, because the effective size of the detector was 13.8 mm.

Absorption-contrast x-ray vessel images of excised liver of 2 rats were obtained by use of iodine microspheres. These images were compared with phase-contrast x-ray images with physiological saline under the same x-ray dose. The iodine microspheres, with a mean diameter of 0.015 mm and an iodine concentration of 37% (1 to 2×10⁸), were injected into the portal vein. Then portal...
veins > 0.015 mm were filled with the radiopaque microspheres retrogradely.

The present experiment was approved by the Medical Committee for the Use of Animals in Research of the University of Tsukuba, and it conformed to the guidelines of the American Physiological Society.

**Statistical Analysis**

Data of the $d_0$ were expressed as mean ± SD. A 1-sample $t$-test was used to compare the measured mean values of the sample to theoretical values. Statistical significance was defined at the level of $P < 0.05$.

**Results**

**Measured Values of the Candidate Contrast Agents**

The $d_0$ values of the liquid samples are shown in Figure 2. The values of $d_0$ were $0.44 \times 10^{-6} \pm 0.12 \times 10^{-6}$, $0.46 \times 10^{-6} \pm 0.10 \times 10^{-6}$, $1.29 \times 10^{-6} \pm 0.13 \times 10^{-6}$, $1.28 \times 10^{-6} \pm 0.17 \times 10^{-6}$, and $5.86 \times 10^{-6} \pm 0.18 \times 10^{-6}$ for physiological saline, lactated Ringer's solution, 5% glucose, artificial blood such as Fe-C, and FC-43, respectively. The measured $d_0$ values, except for that of FC-43, correspond to theoretical $d_0$ values calculated from mass density (see Appendix 1).

**Phase Map and Absorption-Contrast X-Ray Image**

Vessels in the rat liver were clearly shown as dark gray in the phase map (Figure 3A), because physiological saline has a low refractive index compared with that of normal liver tissue. The vessel ~0.03 mm in diameter was imaged clearly. The image obtained by absorption contrast, however, could not reveal the vessel structure at all (Figure 3B). Absorption-contrast x-ray images with iodine-loaded acrylic microspheres demonstrated only large portal veins 0.1 mm in diameter at nearly the same x-ray dose as the phase map (Figure 3C).

**Phase-Contrast X-Ray CT Images**

The vessels in the rabbit liver were visualized as voids on the phase-contrast x-ray CT image shown in Figure 4A. The $d_0$ of liver tissue and vessel filled with physiological saline was $3.83 \times 10^{-6} \pm 0.21 \times 10^{-8}$ and $0.10 \times 10^{-6} \pm 0.23 \times 10^{-8}$, respec-
Discussion

Various liquid samples for phase-contrast x-ray imaging were investigated quantitatively, and good correspondence was observed between the measured values of $d_0$ and theoretical values. Liquid samples, except for FC-43, had lower $d_0$ values compared with the $d_0$ of liver tissue ($3.5 \times 10^{-6}$). The measured $d_0$ of FC-43 was lower than the theoretical value. We speculate that the density of FC-43 decreased during stabilization of the interferometer ($\approx 5$ to 10 minutes) because of the nature of its rapid precipitation. Because the difference in $d_0$ between the physiological saline and the liver was highest among the liquid samples, the physiological saline was used as a contrast agent for phase-contrast vessel imaging.

The vessel of liver filled with physiological saline was revealed clearly both in phase-map and phase-contrast x-ray CT images, whereas the absorption-contrast x-ray image could not depict the vessel structure at all. Although the vessel 0.05 mm in diameter was depicted by the blood itself, the vessel 0.03 mm in diameter was revealed clearly by physiological saline. This significant image contrast produced by physiological saline will enable us to select the vascular branches. Conversely, the absorption-contrast x-ray image with iodine-loaded acrylic microspheres demonstrated only large vessels 0.1 mm in diameter at almost the same x-ray dose as the phase map. To depict the vessel 0.03 mm in diameter by the absorption-contrast method, an x-ray exposure 17 times higher than the present phase map (113 mR) was required (see Appendix 2).

In addition, the use of physiological saline for vessel imaging has significant merits, because the side effects of the iodine contrast agent used here, such as high osmolarity, high viscosity, and allergic reaction, will be completely eliminated. The osmolarity and viscosity will be reduced by one half to one third and one thirteenth to one fourth, respectively, by use of physiological saline, so a more physiological examination of the vessel would be possible.

Present Limitations and Future Plans

Field of View

With the use of a monolithic x-ray interferometer made from a silicon ingot, the maximal image size achievable is limited by its diameter. Therefore, another type of interferometer, a nonmonolithic one, is being developed to obtain a large field of view, $> 25 \times 25$ mm. In addition, the monolithic x-ray interferometer has another problem: The heat from live objects causes its instability. The nonmonolithic configuration can solve the heat problem, because an object can be placed apart from the half-mirror, and it will be possible to perform phase-contrast x-ray imaging of live animals.

High X-Ray Energy

The x-ray energy in this study was 17.7 keV. The use of higher x-ray energy is suitable, however, in phase-contrast x-ray imaging of thick objects. Then, x-ray energy of 30 to 40 keV is planned to be used for imaging objects 4 cm thick.

Cinematic Image Acquisition

In this study, phase-contrast x-ray images were obtained under stable conditions, because the x-ray flux was insufficient to acquire the image at video rates. The flow condition would be revealed cinematically, however, by use of the
high-flux-density x-ray beam available at the most advanced synchrotron x-ray facilities.

We are planning to perform live-animal experiments for visualizing tumor vessels using physiological saline dynamically.

Conclusions

The hepatic vessels of rats and a rabbit could be revealed clearly by physiological saline in phase-contrast x-ray imaging. This study suggests that physiological imaging of vessels might be obtained without the side effects of the currently used iodine contrast agents and with small doses of x-rays.

Appendix 1

Calculation of Refractive Index

The x-ray complex refractive index, \( n \), is described as

\[
\delta = \frac{\pi \lambda}{2} \sum N_k \left( Z_k + f_k \right) \exp\left\{ \frac{4.49 \times 10^{-16}}{\lambda} \delta N_e \right\},
\]

where \( N_k, Z_k, f_k \) are atomic density, atomic number, and real part of the anomalous atomic scattering factor of element \( k \), respectively. The \( N_e, \lambda, \varepsilon \) are electron density, wavelength, and classic electron radius, respectively. The above equation suggests that the value for \( \delta \) is approximately proportional to the mass density (\( \rho \)), and \( \delta \) is estimated with the formula

\[
\Delta \delta = 1 - \rho \delta_0 = 7.4 \times 10^{-7} \text{ at } 17.7 \text{ keV}.
\]

Appendix 2

Estimation of Photon Number in Absorption-Contrast X-Ray Image

The signal-to-noise ratio (S/N) is defined as the ratio of the image signal to the statistical noise associated with the detection of the photon number in front of the object. The S/N of the image, the required photon number, \( F \), before the object is calculated as

\[
F = \frac{\{1 + \exp\left[ -d (\mu_r^* - \mu_0) \right]\} \times (S/N)^2}{\{1 + \exp\left[ -d (\mu_r^* - \mu_0) \right]\} \times (S/N)^2 + \left[ \exp\left(-b \mu_0 \right) \right]^2},
\]

where \( a \) is the area of each pixel (0.012 \times 0.018 mm), \( b \) is the detection efficiency of the pickup tube (70%), \( \mu_r^* \) is the x-ray linear attenuation coefficient of iodine contrast agent at 17.7 keV (109 cm\(^{-1}\)), \( b \) is the square cell thickness (12 mm), and \( d \) is the diameter of the vessel (0.03 mm). When we defined the S/N ratio as 3/0.5, the photon number in front of the object is calculated as 6.9 \times 10^1 photons/mm\(^2\).

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References

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