Phase-contrast X-ray microtomography of mouse fetus

Mouse is one of the important animals used in many kinds of experiments. In particular, transgenic mice are now regarded as a common tool for investigating the functions of proteins *in vivo*. Mouse fetuses are extensively studied to determine the roles of genes in development and congenital abnormalities. With the increase in new types of transgenic animals, effective methods of identifying novel phenotypes in these fetuses are crucial. However, up to now, the detailed examination of a mouse fetus has involved thin sectioning for observation under a microscope. Here, we applied phase-contrast X-ray microtomography (CT) to visualize the development of mouse fetuses without the need for sectioning or treatments other than fixation by formalin [1].

In the synchrotron radiation facilities, phase-contrast X-ray CT using an X-ray interferometer has been under development since the 1990s [2]. In general, the compound refractive index in X-rays is represented as $n = 1-\delta + i\beta$, where δ and β represent the phase and absorption factors, respectively. In biological soft tissues composed of low-Z elements such as hydrogen, carbon, nitrogen, and oxygen, the absorption factor β is too small to use for the conventional X-ray absorption radiogram. On the other hand, the phase factor δ is about 1000 times larger than β. Therefore, phase-contrast X-ray CT using δ enables us to visualize the three-dimensional structures in biological soft tissues with much higher contrast than by conventional X-ray CT. From the tomographic reconstruction, a three-dimensional map of $\Delta\delta$ is obtained. Since δ is roughly proportional to the density of the material when it contains only low-Z elements, it is also possible to estimate a three-dimensional map of the density.

To measure phase shift for imaging, a grating-based interferometer, which is called as Talbot interferometer, is used [3]. The Talbot interferometer is typically composed of two transmission gratings: a phase grating (G1) and an absorption grating (G2). Since the Talbot interferometer is a robust technique against disturbances of the system, compared with a crystal-based interferometer, it has been used for many applications. In SPring-8, this technique has been used in structural studies of biological soft tissues such as brain and eye lenses [4]. Since an embryo and a fetus before the calcification of bones are regarded as soft tissues, this technique is suitable for these samples.

The visualization of mouse fetuses by phase-contrast X-ray CT was conducted at bending magnet beamline **BL20B2**. Grating parameters and materials are varied depending on the size of the fetus samples. Embryos of 6 and 9 days were measured at the upstream experimental hutch located 44 m from the source. For these small samples, both gratings are made of tantalum and the pattern thicknesses of G1 and G2 are 0.96 μm and 4.75 μm, respectively. The pitch of both gratings is 5 μm and the grating area size is 5 mm (width) by 10 mm (height). G2 is inclined 60 degrees towards the beam so as to increase the effective X-ray absorption by the grating. The X-ray imaging detector consists of a visible light conversion unit, "beam-monitor," and a charge-coupled device (CCD) camera. The beam-monitor is composed of a 10-μm-thick P43 ($Gd_2O_2S:Tb^+$) phosphor screen and a f = 50 mm camera lens. The CCD camera is a fast read-out one (C9100-02, Hamamatsu Photonics) equipped with a f = 85 mm camera lens. The effective pixel size is 4.9 μm. On the other hand, embryos of 10, 11, 13, and 15 days were measured at the downstream hutch located 200 m from the source. In this case, G1 is made of tantalum and G2 is made of gold with pattern thicknesses of 2.1 μm and 16.6 μm, respectively. The pitch of both gratings is 10 μm and the grating area size is 25 mm (width) by 25 mm (height). The inclination angle of G2 is 45 degrees. The beam-monitor is composed of a 20-μm-thick P43 phosphor screen and an f = 105 mm camera lens. The CCD camera (C4880-41S, Hamamatsu Photonics)

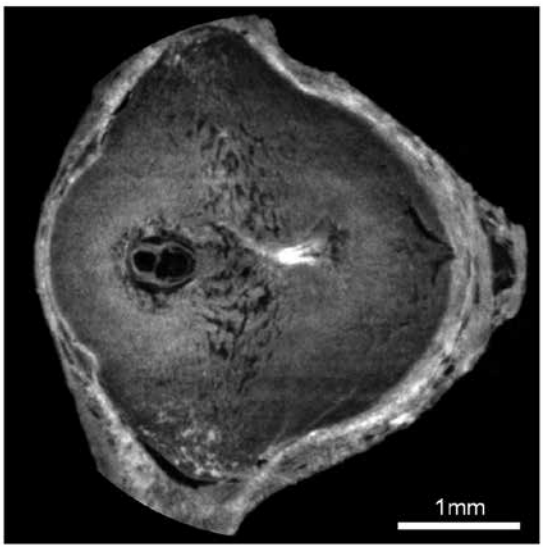

Fig. 1. Phase-contrast X-ray CT image of a mouse fetus at gestational day 6.

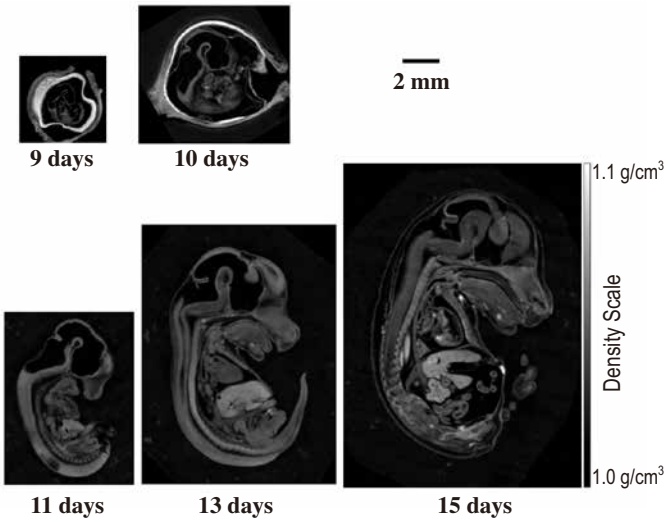

Fig. 2. Phase-contrast X-ray CT images of mouse fetuses at gestational days 9 to 15.

is equipped with an f = 105 mm camera lens. In this case, the effective pixel size is 5.87 μm per pixel. In the actual measurements, the CCD camera is used in the binning mode (4×4 binning with 23.5 μm pixel size).

A cross section of mouse uterus at gestational day 6 is shown in Fig. 1. Only the egg cylinder and remains of the uterine lumen (with higher density) can be observed. Approximately sagittal tomographic sections of mouse fetuses at gestational days 9, 10, 11, 13, and 15 are shown in Fig. 2. The gray scale represents density estimated from the results of phase-contrast X-ray CT. At day 9, the fetus is strongly flexed in a dorsally convex direction. At the center of the embryo is the heart in the early development stage. Other organs are not identifiable, but it is clear that the present technique allows us to visualize the details of early development. At day 11, 32 somites can be counted, and most of the major organs, such as brain tube, heart, liver, stomach, and intestine, are visible, among which liver has the highest density. At 13 days, compared with adult mouse the head is disproportionately large. However, the brain is still undeveloped and large ventricles occupy the major part of the head. On the other hand, the tongue and palatal process are well developed and Meckel's cartilage is present. Chondrification of the vertebrate column is still in progress at 15 days. All these features are well correlated with the microscopic observations of stained sections [5]. In phase-contrast X-ray CT, three-dimensional analysis is also possible, as shown in Fig. 3.

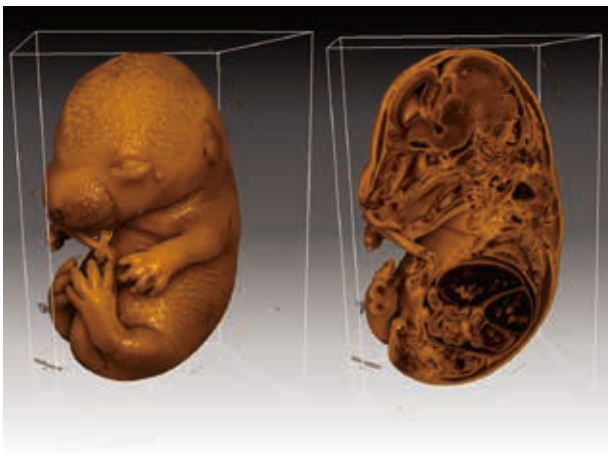

Fig. 3. Three-dimensional rendering of a mouse fetus at gestation day 15.

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