V. 2. μ-CT Images of the Egg of Drosophila

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Introduction

The technology to observe cells in a living body such as a cloning technology and a gene therapy is in progress in the field of biology and medicine. It is very important to develop a drug for a gene therapy and the production of organs for transplant. To observe the cell, one needs a device that can enlarge a microscopic area in the cell (e. g., microscope). For this purpose, we are developing a 3 dimensional micro computed tomography (3D μ-CT) which enables to observe the interior of living small insects[1−5]. The 3D μ-CT uses monoenergetic low-energy X-rays produced by bombarding a target with proton microbeam. Therefore, the contrast of the image for small insects is superior to that of other CT systems which uses continuous X-rays. Furthermore, our system can readily change the X-ray energy to obtain a better contrast depending on the elements contained in the insects[1-4]. In this study, we applied the μ-CT to the egg of drosophila, since the drosophila is used to various basic studies, such as gene research.

μ-CT system using micro-PIXE

The 3D μ-CT comprises a monoenergetic μ-X-ray source, a rotating sample stage and a high-speed X-ray CCD camera. Its technical details were written in Ref. 1. In this system, a sample is encapsulated in a polyimide tube with atmospheric pressure and placed on the rotating stage. This tube is rotated by a stepping motor. An X-ray producing target is set at 30 ~ 45 degrees with respect to the horizontal axis and produces a cone beam of X-ray by microbeam bombardment. In this study, 2.4 MeV proton microbeams were used. The exposure of CCD is begun by an outside trigger and stops when a total beam

charge reaches a constant value; readout of data finishes while the target rotates. A 100 μm Mylar film is placed in front of CCD to prevent recoil protons from entering CCD. 2D transmission data is obtained by the cone beam. 3D image is reconstructed on the basis of an iteration method (Maximum Likelihood-Expectation Maximization method) [5].

Results and discussion

Figure 2 shows the photograph of imagoes and eggs of drosophila melanogaster (Oregon-R). The eggs of drosophila incubate in 24 hr. Considering breeding and measuring times, we used an egg, which was laid 12 hr ago, encapsulated in a polyimide tube (inside diameter of 500 μm and wall thickness of 25 μm). Figure 3 shows the photograph of the tube with the egg. To prevent the egg to dry out, the gelatin was inserted together. The inside is kept in atmospheric pressure with appropriate moisture. Under this treatment, we confirmed that the egg is alive and incubates in the tube[3].

To obtain CT images with a good contrast, The X-ray producing target should be selected in consideration of absorption coefficient of the egg and detection efficiency of the CCD camera. Figure 4 shows the comparison of projection images of an egg for various X-ray targets. It is apparent that the result of Sc target shows the highest contrast.

Figure 5 shows the cross sectional views of the egg; the left figure shows the longitudinal cross sectional view and the other is the vertical view. The experimental conditions are summarized in Table 1. The interior of the egg is uniform. It seems that the egg has grown during the measurement. To confirm this, the cross sectional images reconstructed from the first half and the latter half data in a series measurement are shown in Fig. 6. It is apparent that the shape of the egg changes because of the growth.

CT images of a dried egg were obtained. The egg which was laid about 22 hr before was encapsulated in the tube and was kept for 4 months to dry. Figure 7 shows the cross sectional view and the 3D image of the dried egg. The shell and the interior structure are observed. We recognize a large difference between the image of the dried egg and that of the living egg. It is considered that organs in the living egg have moved thus the interior of the living egg is uniformly seen.

In the previous study, the images of the ant's head fixed by formalin and the one of the living ant's head were compared[3]. There was a difference between them. In the case of the fixed ant, the brain shrank and something was washed out due to formalin-fixation. In the case of the living ant, the interior was uniform. The situation is similar to the

present result.

Conclusion

The CT images of the egg of drosophila were obtained by using the μ-CT. The interior of the living egg was uniform. In the case of the dried egg, the shell and the interior structure were confirmed. It is considered that the living egg has grown during the measurement. The measurement time of 3.5 hr is long enough to change the shape of the living egg because the eggs of drosophila incubate in 24 hr. Therefore, the intensity of the X-ray has to be increased.

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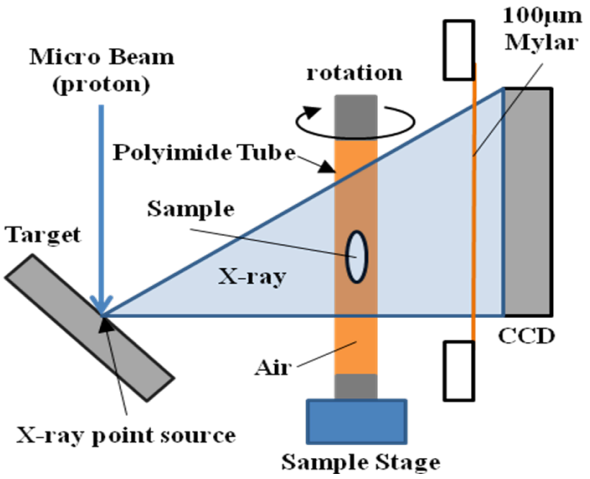
Table 1. Experiment condition (living egg).

X-ray target	Sc
Target angle with respect to the horizontal axis	45°
Beam particles	2.4 MeV protons
Beam spot size	1.33 × 1.00 μ^2m
The number of projection images	250 (250 images/rotation)
Measurement time	12660 sec (about 3.5 hours)

Table 2. Experiment condition (dried egg).

X-ray target	Sc
Target angle with respect to the horizontal axis	30°
Beam particles	2.4 MeV protons
Beam spot size	1.1 × 1.1 μ^2m
The number of projection images	500 (500 images/rotation)
Measurement time	9480 sec (about 2.6 hours)



Figue 1. Principle of μ-CT.

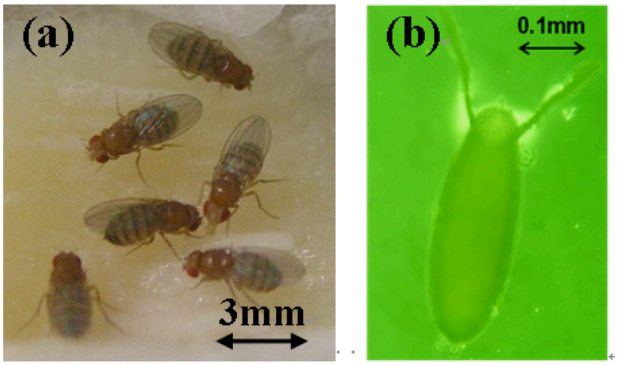


Figure 2. Imagoes of drosophila melanogaster (Oregon-R) (a) and, egg of drosophila (b) observed with an optical microscope.

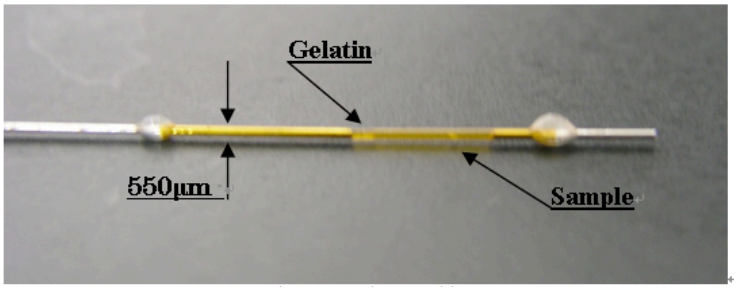


Figure 3. Sample assembly.

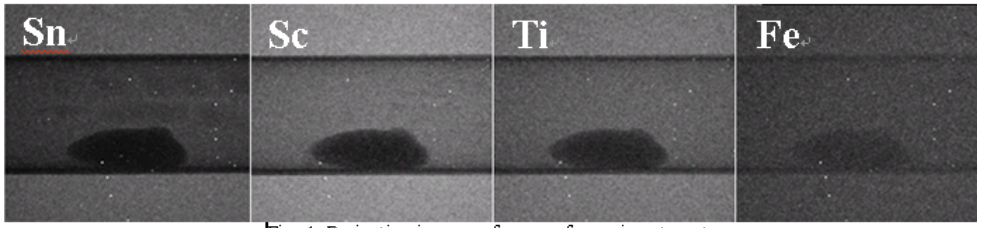


Figure 4. Projection images of an egg for various targets.

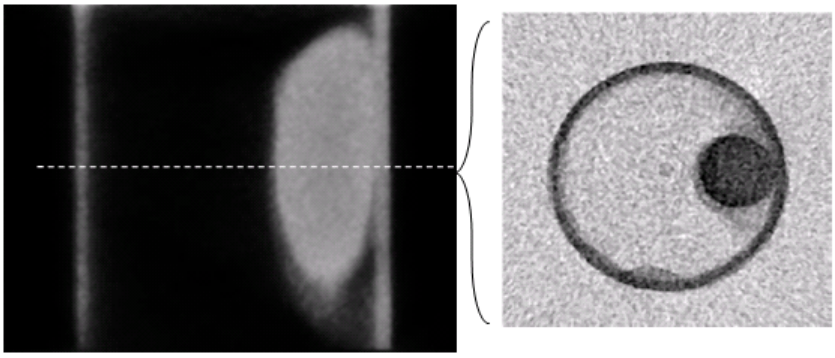


Figure 5. Cross sectional views of the egg (left : longitudinal view, right : vertical view).

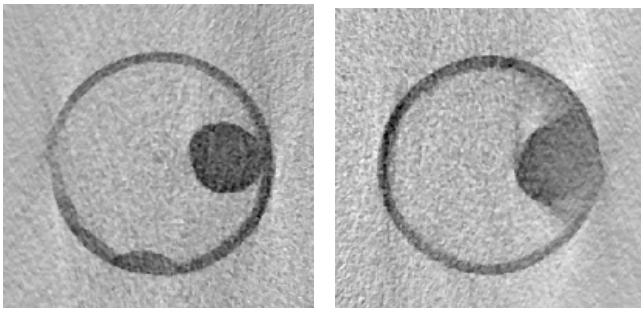
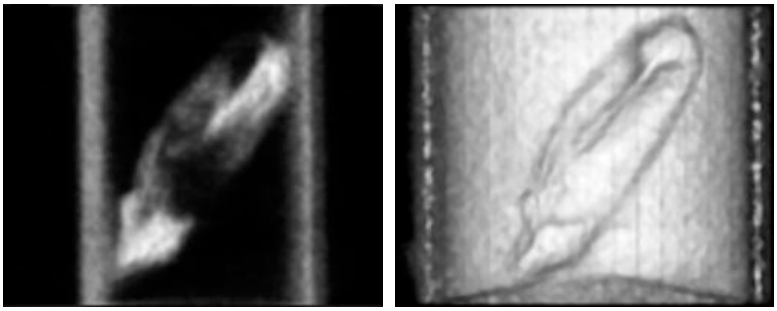


Figure 6. Vertical cross sectional view of the egg (left image is reconstructed from the first half date, right image is reconstructed from the latter half date).



Figyre 7. CT images of the dried egg (left : longitudinal cross sectional view, right : 3D image).