

# Anatomical Observation of a Lancelet by a Full-Field Imaging Soft X-ray Microscopy

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Lancelets, classified as chordata, are marine animals, which distribute in the Torrid and the Temperate zone widely and live in sandy seafloor (a photograph is shown in Fig. 1). Though lancelets are invertebrate animals, they have notochord, a rod-shaped structure in dorsal side of a body, instead. Vertebrates also have notochords in their early stages of development and notochords disappear in later stage to have vertebrae. Lancelets are only animals that

hold notochord throughout their lifetime. According to this remarkable feature, lancelets are regarded as invertebrate animals which are closely related to vertebrate animals in phylogenetic position and are also regarded as the one of the modern survivors of ancient animals. Therefore, study of lancelets can be a key to complement the gap of evolution between vertebrate and invertebrate animals. In recent study, genome analysis revealed that lancelets have nearly 60% of homologous gene with human in the specific segments [1]. Anatomical observation of organs of lancelets is the alternative strategy of further investigations because lancelets have organs which are assumed to be prototypes of those of higher animals, such as a notochord (prototype of a vertebra), an endostyle (a thyroid gland), a Hatschek's pit (a pituitary gland), a cerebral vesicle (a brain), photoreceptor cells (an eye), and so on. Then, a full-field imaging soft x-ray microscope (SXRIM) installed at BL-12 is a suitable technique for this purpose with advantages of relatively high resolution (about 70 nm at wavelength of 2.4 nm [2, 3]), high transmittance and performing elemental analysis by using x-ray absorption

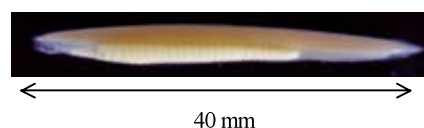


Fig. 1: a photograph of a lancelet, *Branchiostoma belcheri*. The head is on the left side and the tail on the right side

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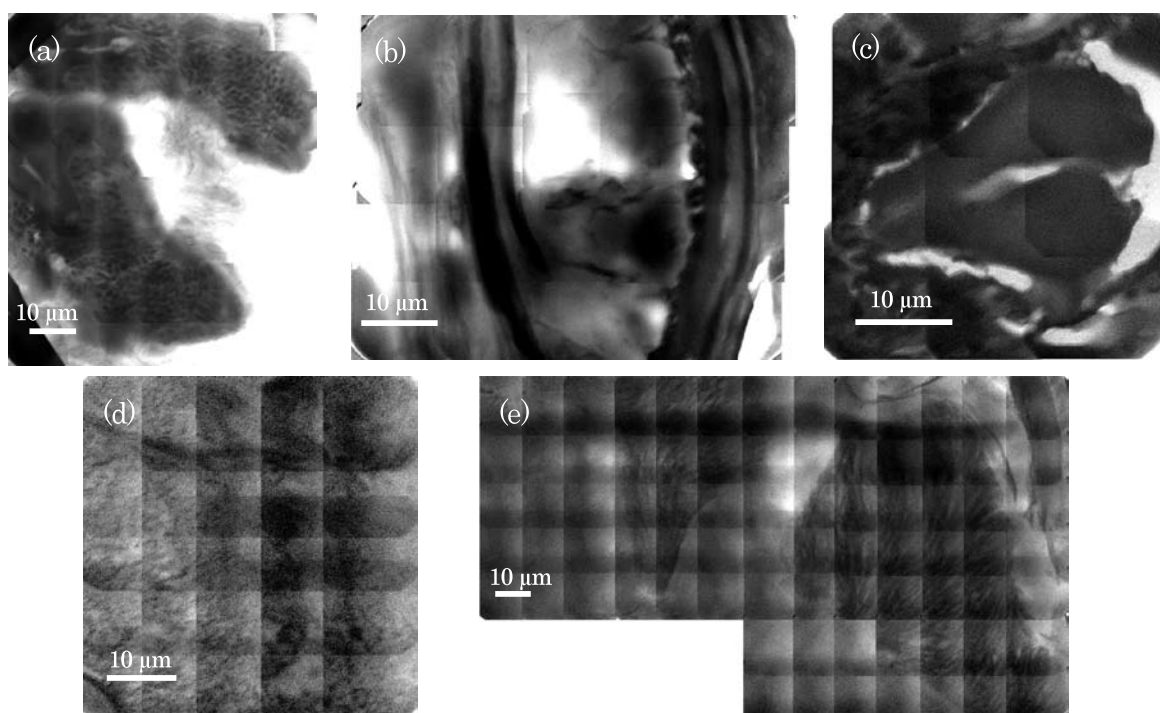
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edge. Especially, the high transmittance enables to observe sample in the atmosphere without an evacuated chamber and to observe relatively thick specimen. This feature makes sample preparation easy.

As a sample preparation, a lancelet (*Branchiostoma belcheri*) was fixed with Bouin's solution and embedded in paraffin. A thin specimen was cut as 2  $\mu\text{m}$  thick by a microtome and was set on a polyimide film of  $\sim 1 \mu\text{m}$  thick. In the process of the sample preparation, the specimen was not dyed at all. In the observation, wavelength of 1.87 nm was used and magnification of the optical system was 810. X-ray microscopic images of internal organs of a lancelet, gills, a notochord, a nerve cord and an endostyle, are shown in Figs. (2). In Fig. 2(a), many grain-like structures of high absorption (i.e. shown in darker color in the image) are seen on all over the gills. They are considered as cross sections of cilium or those of blood vessels. These structures cannot be observed by an optical microscope without dyeing. In Fig. 2(e), zone 1 to 3 of the endostyle was observed and shapes of single cells were distinguished barely with low contrast. For improving the image quality, the x-ray of longer wavelength should be used to gain the higher contrast.

As perspective of this study, the internal organs of the lancelet which was bred in different environment (e.g. iodine rich environment) is due to be observed. Then M absorption edge of iodine (1.97 nm) is useful to obtain high contrast and elemental distribution. In particular, the endostyle is



Figs. 2: X-ray microscopic images of internal organs of a lancelet, (a) gills, (b) a notochord, (c) a gills, (d) a nerve cord and (e) an endostyle. These images are composite images of individual x-ray microscopic images. Their measurement times were (a) 3 min  $\times$  56, (b) 1 min  $\times$  20, (c) 30 sec  $\times$  9, (d) 1 min  $\times$  25 and (e) 1 min  $\times$  79.

assumed as a prototype of the thyroid gland of higher animals, which accumulates iodine for thyroid hormone. These observations have possibility to clarify not only the adaptation of internal organs to the environment but also some process of evolution by comparing the internal organs with a control.

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