

Investigation of the 3D Tomography Images of Marine Dinoflagellate (*Symbiodinium* spp.) by Synchrotron Radiation-based Nano-transmission X-ray Microscopy (SR-nTXM)

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Endosymbiosis in cnidaria-dinoflagellate association plays critical role in regulating productivity of corals and related marine ecosystems. Cellular endosymbiosis with dinoflagellate *Symbiodinium* spp. (i.e. the symbiont or “zooxanthellae” in generic name) in corals (i.e. the host) is initiated by the internalization of symbionts via phagocytic process into host endoderm cells (Fig. 1). However, the mechanism by which the symbiont is able to reside inside the host cell and establish an obligatory and mutualistic association remain unclear after four decades of investigation. Thus, we tried to use varied molecular and cellular technologies for exploring the molecular mechanism of the endosymbiosis within cnidaria-dinoflagellate. In this study, the non-destructive synchrotron-radiation-based microscopy in energy range of hard X-ray provides a solution for investigating the 3D tomography images of the dinoflagellate in symbiotic or aposymbiotic state.

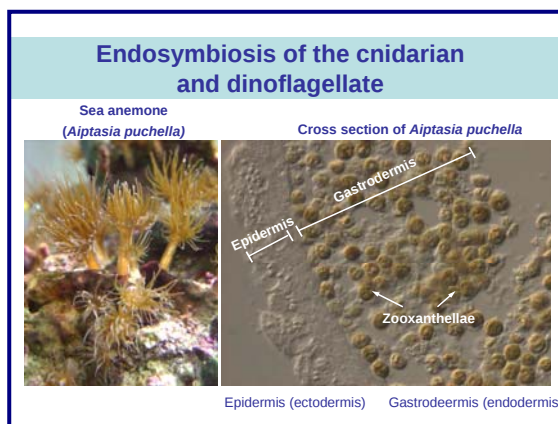


Fig. 1: Photograph of the sea anemone (*Aiptasia puchella*) and the symbiotic algae within the gastrodermal tissue.

Due to the limitation for obtaining lot's coral samples. We used sea anemone (*Aiptasia puchella*) to investigate the endosymbiosis as suggested by the pioneers of coral biologist [1]. We also established the culture system to culture dinoflagellate in vitro (aposymbiotic). So, we could compare the molecular and structural difference between symbiotic and aposymbiotic dinoflagellate.

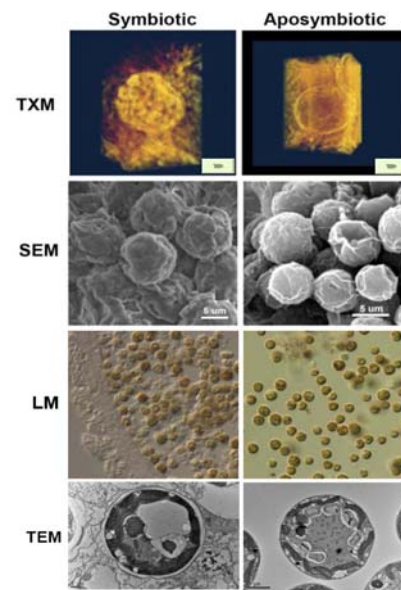


Fig. 2: 3D tomography images of the dinoflagellate in symbiotic or aposymbiotic state. The tentacles of *Aiptasia pulchella* were removed, paraffin-embedded, sectioned and subjected to uranyl acetate and lead citrate, then observed by SR-nTXM. The cultured dinoflagellate were also collected, fixed, stained by the same protocol, then observed by SR-nTXM. The representative images of the symbiotic and aposymbiotic dinoflagellate were also obtained by scanning electron microscopy (SEM), light microscopy (LM) and transmission electron microscopy (TEM).

The preliminary data (Fig. 2) demonstrated that the synchrotron radiation-based nano-transmission X-ray microscopy (SR-nTXM) could detect and reconstruct the 3D images of the dinoflagellate, and distinguish the structural changes of symbiotic and aposymbiotic dinoflagellate, especially on the surface area of the algae. It also revealed that the surface of dinoflagellate was modified and regulated specifically for establishing the mutual relationship of symbiosis.

- [1] V. M. Weis, S. K. Davy, O. Hoegh-Guldberg, M. Rodriguez-Lanetty, and J. R. Pringle, Trends in Ecology and Evolution. **23**(7), 369-376 (2008).