A method for obtaining serial ultrathin sections in transmission electron microscopy

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Observing cells and cell components in three dimensions at high magnification in transmission electron microscopy requires preparing serial ultrathin sections of the specimen. Although preparing serial ultrathin sections is considered to be very difficult, it is rather easy if the proper method is used [1]. In this paper, we show a step-by-step procedure for safely obtaining serial ultrathin sections of microorganisms. The key points of this method are: 1) to use the large part of the specimen and adjust the specimen surface and knife edge so that they are parallel to each other; 2) to cut serial sections in groups and avoid difficulty in separating sections using a pair of hair strands when retrieving a group of serial sections onto the slit grids; 3) to use a 'Section-holding loop' and avoid mixing up the order of the section groups; 4) to use a 'Water-surface-raising loop' and make sure the sections are positioned on the apex of the water and that they touch the grid first, in order to place them in the desired position on the grids; 5) to use the support film on an aluminum rack and make it easier to recover the sections on the grids and to avoid wrinkling of the support film; and 6) to use a staining tube and avoid accidentally breaking the support films with tweezers. This new method enables obtaining serial ultrathin sections without difficulty. The method makes it possible to analyze cell structures of microorganisms at high resolution in 3D, which cannot be achieved by using the automatic tape-collecting ultramicrotome method and serial block-face or focused ion beam scanning electron microscopy.

[1] Yamaguchi M, Chibana H: A method for obtaining serial ultrathin sections of microorganisms in transmission electron microscopy. J Vis Exp, 131, e56235, doi:10.3791/56235, 2018.

Fig. 1. Ultrathin sections obtained by this protocol. Note five groups of serial sections about 1.8 mm long are already separated after cutting.

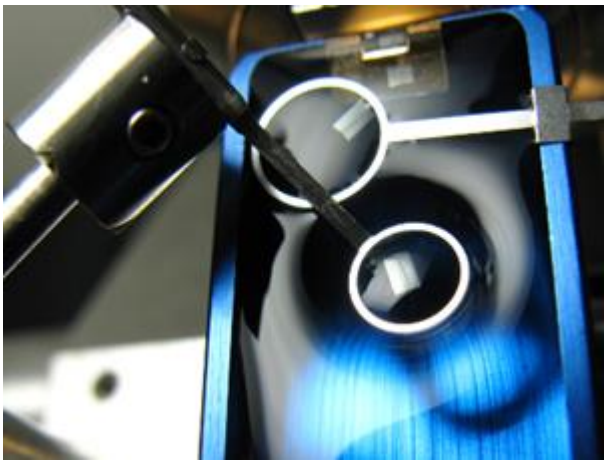

Fig. 2. Retrieval of serial sections.

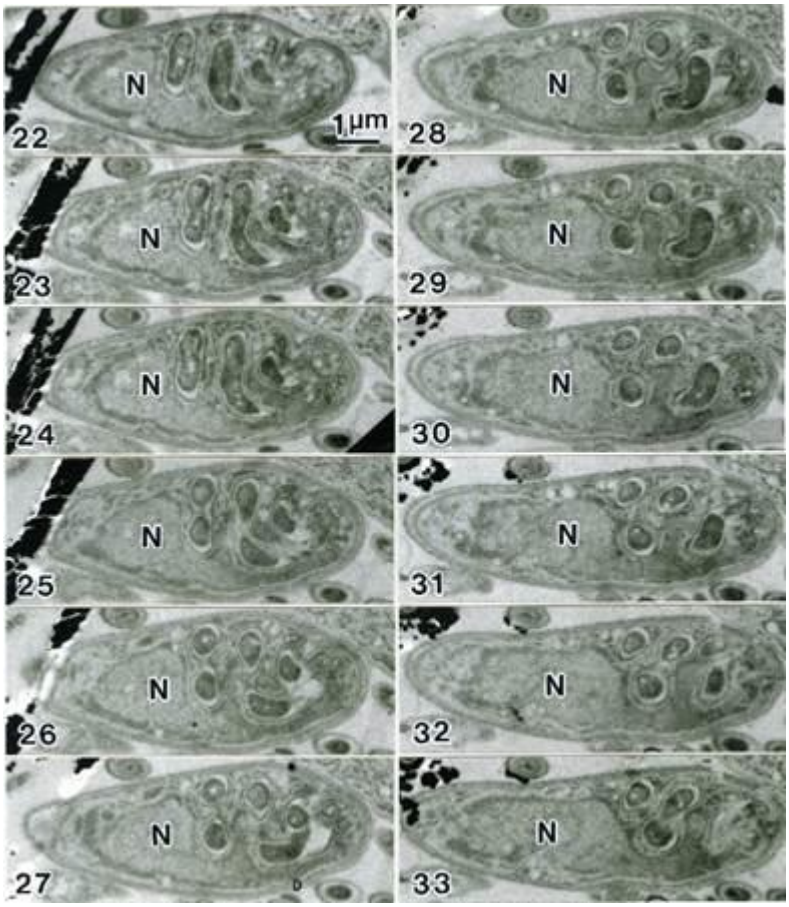

Fig. 3. Relationship between the loop, water surface, sections, and grid (side view).

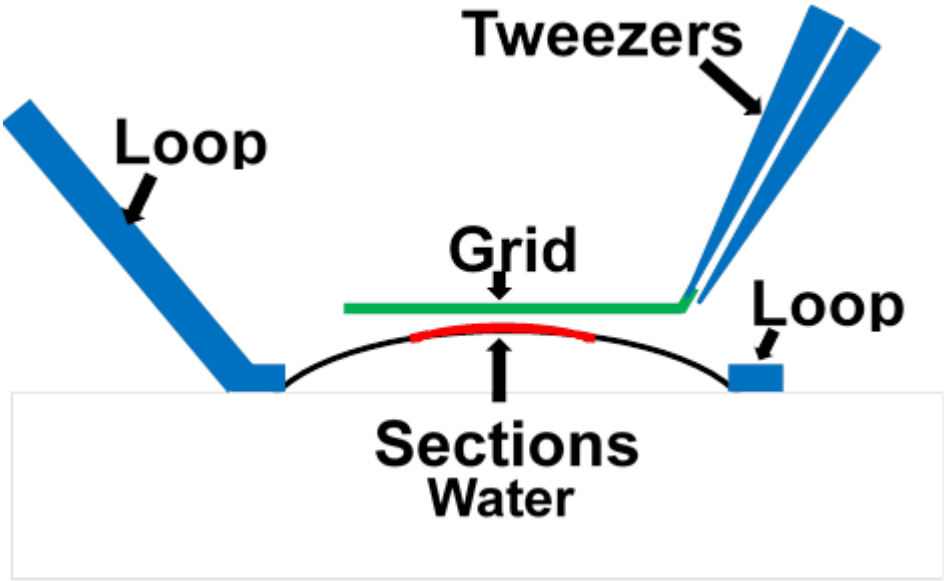

Fig. 4. Serial sections of *Parakaryon myojinensis*. The figure shows 12 out of 67 complete sections. This cell was found to have a large nucleoid consisting of naked DNA fibers, with a single nucleoid membrane, and endosymbionts that resemble bacteria, but no mitochondria. Thus, this organism appears to be an intermediate life form evolving from prokaryote to eukaryote.

(Yamaguchi M, et al.: Prokaryote or eukaryote? A unique microorganism from the deep-sea. J Electron Microsc, 61: 423-431, 2012.)