Prospects of TEM tomography for ultrastructural studies of pollen and spores of the higher plants

Polevova, S.[1] and Zavialova, N.[2]

[1] Lomonosov Moscow State University, Russian Federation, [2] A.A.Borissiak Palaeontological Institute, Russian Academy of Sciences, Russian Federation

Among currently existing techniques of visualization of microscopical objects, TEM provides the most detailed information on the inner structure of palynological objects. However, it produces 2-D images, whereas 3-D reconstructions of constituting elements and their co-arrangement are needed. TEM-tomography can possibly be useful in this relation.

We have tested it on palynological objects and compared with the routine TEM. The infratectum of pollen grains of *Ginkgo biloba* L. was studied in series of ultrathin sections. By tracing outlines of individual structural elements from section to section, we concluded that the infratectum is pseudocolumellate. For TEM tomography, a semithin section 200 nm thick was repeatedly photographed in an analytical TEM at an accelerating voltage of 200 kV; the section was tilted at angles from -70° to +70°, with a step of 1°. A final file was generated from these images, which can be viewed as a video and used for preliminary estimations; and a 3-D model was made. Before observations, the section was carbon-coated to diminish the impact of irradiation and thermal drift, since several hours were needed to obtain a series of images. We found a region of the section that was situated close to the center of the grid and in the central area of a mesh, more or less perpendicularly to the sporoderm. It contained two elements, which we hoped to discern as 3-D elements. The section was situated more or less perpendicular to the axis of rotation of the specimen holder. The obtained results confirmed the routine TEM conclusions, but the obtained images were of poor quality.

Prospects of TEM-tomography were better seen on a more elaborated exine of members of the Compositae: the double columellate layer in such exines is connected via a reticulate intermediate zone. Columellae of the inner layer appear branching if observed in solitary sections, and columellae of the outer layer seem to be continuations of these branches. However, oblique sections and tomograms show that the net of fine elements contact with inner columellae and bases of the outer columellae (Figure).

In sum, arguments for the application of TEM-tomography to our objects are the suitable range of magnification for the majority of coats of microspores and pollen grains and ideal resolution; whereas arguments against the application are the destructiveness of the method, the low contrast of obtained images, and the necessity to increase the contrast. The dimensions of objects are also a problem: the larger the object, the higher should be penetration capability, but the higher the penetration capability, the stronger the organic matter is destroyed. The presence of an embedding medium strongly diminishes the electron transparency of the section at tilting angles that are greater than 60° and that makes focusing virtually impossible. TEM-tomography perhaps will become more useful for pollen morphology, if a possibility is found to dissolve the embedding medium and preserve the sections on grids.

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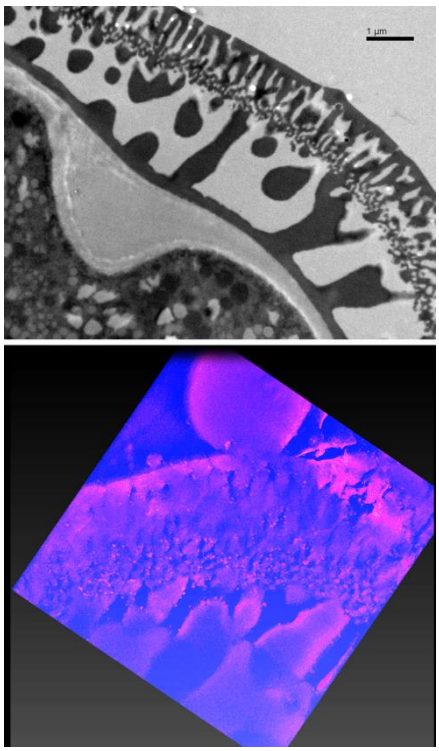

Figure. Ultrathin section and a tomogram of pollen grain of modern *Cyanus segetum* (L.) Hill, 1762.