

Ultrastructural 3D investigations of cells and cell organelles

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Ultrastructural investigations by transmission electron microscopy (TEM) are commonly performed using a limited number of ultrathin sections. In many cases the obtained results will be sufficient and accurate in order to achieve a detailed characterization of cell structures on a high level of resolution. Due to the thickness of the used sections (50-80nm) only very small parts of cells and organelles can be investigated, leading to more or less two dimensional information of the investigated structures. In addition calculations of areas or volumes are neither possible nor accurate. Therefore three dimensional (3D) investigations are essential to get detailed information about the arrangement, association or the amount of subcellular structures inside a cell or organelle, respectively.

Here we present a method which is based on TEM serial sections and computer assisted reconstructions, which allows obtaining both morphometric data (areas and volumes) and 3D images on a high level of resolution thus providing new possibilities for the detection of organelle characteristics, associations or alterations inside cells or organelles. This 3D reconstruction method allows the visualization of a cell volume of about $3000\mu\text{m}^3$ enabling investigations of the 3D integrity of several organelles, including for instance chloroplasts with volumes of about $30\text{-}40\mu\text{m}^3$, or even complete small cells like yeast cells. Therefore this method is advantageous when compared to electron tomography [1,2] allowing 3D visualizations of cell volumes only up to $25\mu\text{m}^3$ but with the advantage of an excellent resolution in all three dimensions [3]. With the 3D reconstruction and quantification technique applied here both organelle organization and quantification of their associations, being highly relevant for the intracellular translocation and communication, were evaluated in whole yeast cells [4]. In another study covering total plant cell organelles significant quantitative differences were demonstrated for spinach chloroplasts and mitochondria when compared with drought stressed plants [5].

In the present study 3D morphological and quantitative ultrastructural features of selected plant cell organelles with main emphasis on chloroplasts are presented (Figure 1). Employing computer supported image analysis of serial sections we were able to show that chloroplasts of *Spinacia oleracea* differ from *Picea abies* regarding their relative content of internal fine structures. One example is the content of plastoglobules with a mean number of 439 in spinach and 1556 in spruce chloroplasts. This difference is also reflected in their absolute volume, being $0.4\mu\text{m}^3$ in spinach and $2.7\mu\text{m}^3$ in spruce chloroplasts. The obtained quantitative data will be discussed with respect to a precise evaluation of naturally occurring or stress induced ultrastructural organelle characteristics.

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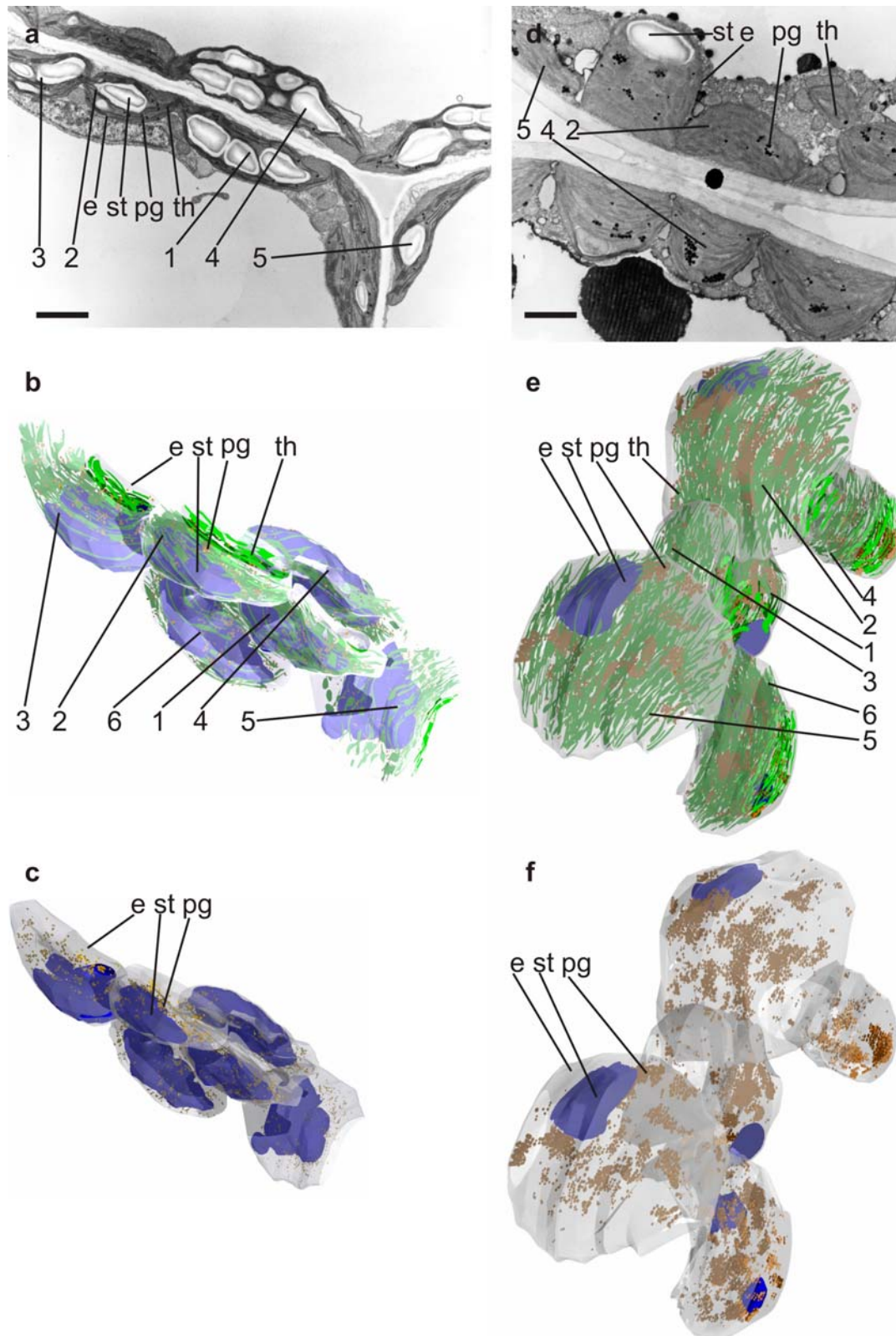


Figure 1. Parts of *Spinacia* (a-c) and *Picea* (d-f) leaf mesophyll cells showing the conventional TEM-images (a,d) and the corresponding 3D reconstructed chloroplasts with their internal fine structures (b,e) and after fading out the thylakoids (c,f). Bars= 2μm
Numbers (1-6): corresponding chloroplasts, chloroplast envelopes (e), plastoglobules (pg), starch grains (st), thylakoids (th)