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3D Reconstruction of Plant Leaf Cells Using TEM and FIB-SEM

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The three-dimensional (3D) reconstruction of individual plant cells is an important tool to extract volume data of organelles and is necessary to fully understand ultrastructural changes and adaptations of plants to their environment [1-3]. Current methods such as the 3D reconstruction of cells based on light microscopical images often lack the resolution necessary to clearly reconstruct all cell compartments within a cell [4,5]. The 3D reconstruction of cells through serial sectioning and transmission electron microscopy (TEM) is technically very challenging and therefore not widely used [6,7]. Here we present a method for the 3D reconstruction and volume extraction of plant cells based on focused ion beam (FIB) milling and scanning electron microscopy (SEM) and compare the results with 3D reconstructions obtained with TEM.

Leaves of *Nicotiana tabacum* and *Cucurbita pepo* were prepared for TEM and FIB-SEM as described previously [7]. For TEM polymerized samples were sectioned (80 nm) with an ultramicrotome (Leica Microsystems, Vienna, Austria). Sections were picked up with single slot grids. Digital images of parts of one tobacco cell from 71 sections were taken with a Zeiss EM 902 TEM (Zeiss, Oberkochen, Germany). For FIB-SEM resin blocks were remounted on aluminum-stubs and sputter coated with iridium. FIB was used to remove 80 nm of block surface while SEM was used to image the surface of 126 slices. Images derived from TEM and FIB-SEM were used for 3D reconstructions using TrakEM2 (Image J). Volumes of chloroplasts, mitochondria and peroxisomes were calculated by TrakEM2. Volumes of cell walls, vacuoles, and nuclei were extracted from semi-thin sections (1 µm) of embedded material.

3D reconstruction by FIB-SEM was faster and less sophisticated than 3D reconstruction by TEM and serial sectioning. Nevertheless, both methods delivered adequate results. Tobacco cells were larger (31410 μm^3) than pumpkin cells (20697 μm^3) and contained more chloroplasts (175 vs. 124), mitochondria (1317 vs. 291) and peroxisomes (745 vs. 79). While individual chloroplasts, mitochondria, peroxisomes were larger in pumpkin plants (25, 53, and 50%, respectively) they covered more total volume in tobacco plants (5390, 395, 374 μm^3, respectively) when compared to pumpkin plants (4762, 134, 59 μm^3, respectively).

Summing up, the data presented in this study demonstrated that 3D reconstructions based on FIB-SEM and TEM are well suited to extract volume data of whole plant cells. While these technologies are able to reconstruct whole organelles (including fine structures) and small plant cells they can only be used to reconstruct parts of larger cells (between 2000 - 4000 μm^3). However, combined with data from light microscopical images volumes of whole cells can be calculated and data from larger organelles (vacuoles, nuclei, and chloroplasts if necessary) can be determined.

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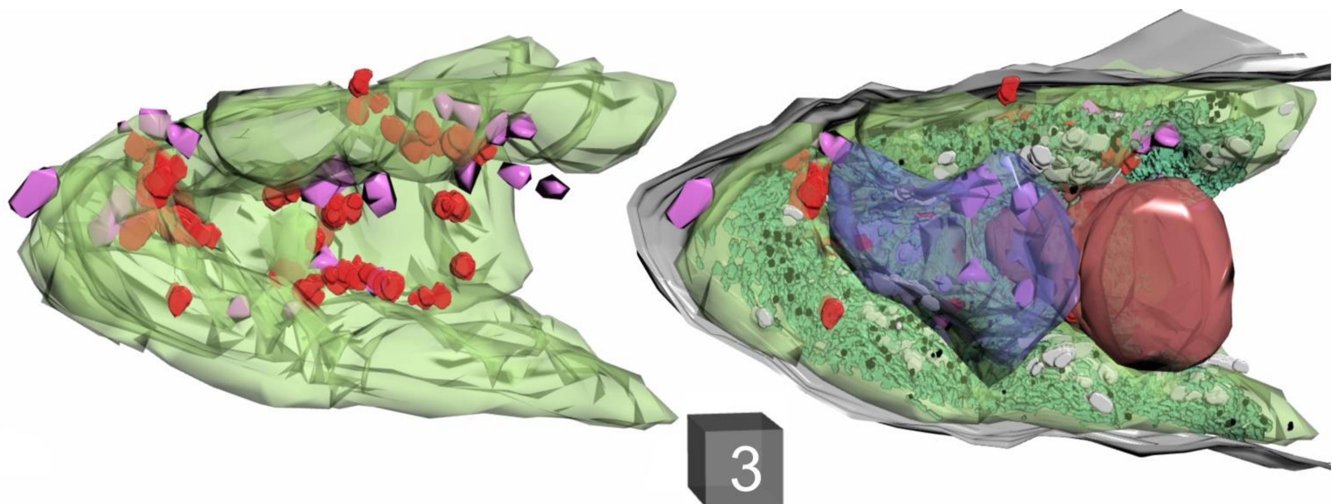

Figure 1. 3D reconstruction of tobacco plant leaf cell based on TEM and serial sectioning according to Zechmann et al. [7]. Reconstruction is based on 71 sections. Cell wall (gray), chloroplasts (green), mitochondria (red), nucleus, (brown) peroxisomes (purple), and vacuole (blue). Cube = 3 μm^3.

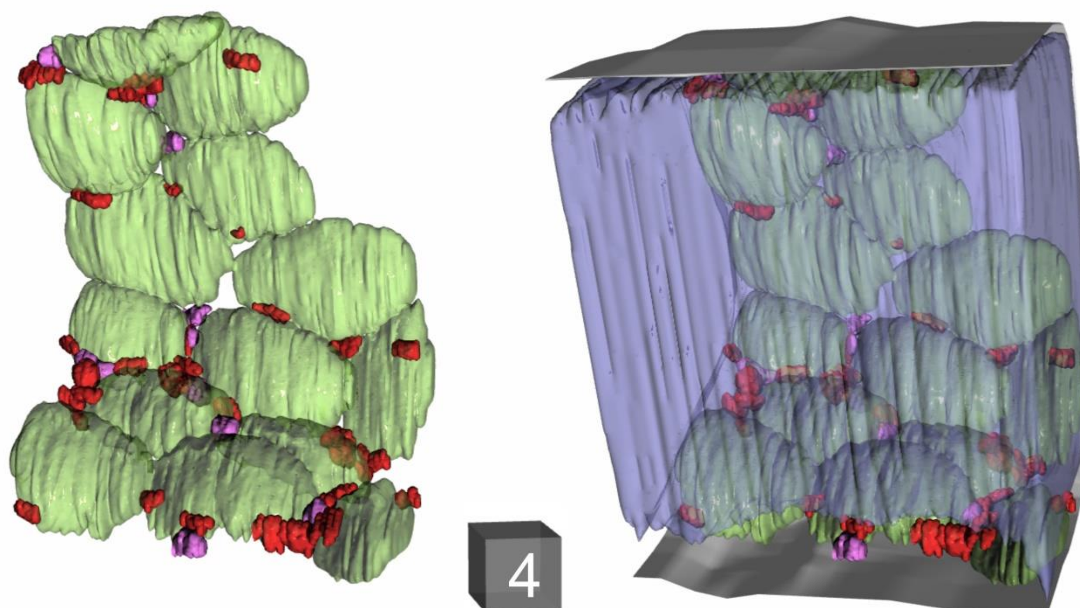

Figure 2. 3D reconstruction of pumpkin plant leaf cell based on FIB-SEM. Reconstruction is based on 126 sections according to Zechmann et al. [7]. Cell wall (gray), chloroplasts (green), mitochondria (red), peroxisomes (purple), and vacuole (blue). Cube = 4 μm^3.

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