Methods for quantitative analysis electron tomographic reconstructions of structures in the wall of hazel pollen grains

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Three-dimensional reconstructions of structures in the wall of negatively stained Epon-embedded hazel pollen grains (Fig. 1), the channels and the bacula cavities, were quantitatively analyzed in order to estimate their role in the release of allergen proteins from the pollen grains [1]. In order to perform reliable quantitative analysis, the irradiation-induced movements of resin sections were eliminated by splitting of large areas of interest into smaller portions that partially overlapped. Each of the sub-tomograms was separately aligned by conventional sequential cross-correlation followed by several cycles of matching of projections with re-projections of the reconstructed volumes. Typically three to six cycles of this iterative projection matching were necessary until all detected displacements between the positions of the original projections and the computed re-projections were smaller than 1 pixel (~1.3 nm). The iterative projection matching method used is a simplified version of the method described in [2] but was more suitable for our specimen due to the distribution of stained material within the sections. It significantly improved the quality of the three-dimensional reconstructions because both the number and the magnitude of the arc-like features emerging from the electron-dense structures observable in planes perpendicular to the tilting axis decreased, while the shape of the channels in the cross-sections approximately perpendicular to the channel axis became more compact (Fig. 2).

Due to the complexity of the spatial arrangement of the bacula cavities, segmentation was performed by thresholding. A correct thresholding level could be found by comparison of the thresholded original untilted projection with the untilted re-projections of the reconstructed volume thresholded at increasing intensity levels in overlay images (Fig. 3).

After the alignment refinement and thresholding, the individual reconstructed sub-volumes were finally assembled back into a large volume.

The amino-acid sequence of the molecule of the hazel allergen protein Cor a 1 is highly similar to the amino-acid sequence of the birch protein Bet v 1, whose structure is known [3]. Due to its compact structure, the Bet v 1 protein could be approximated by a hard sphere. Based on the similarity the hard-sphere approximation was used also for the protein Cor a 1. The assembled thresholded volume was then searched for positions, in which the whole hard sphere was fully embedded within the segmented structures. The spatial continuity of the segmented structures as well as of the detected position of the hard sphere was investigated by routines for object identification in 3D space in 18-neighborhood, which are analogous to the standard algorithms for object identification in images by 4- and 8-neighborhood analysis.

The adopted methods allowed the estimation of the potential pathways of allergen proteins within the reconstructed structures. The results clearly showed that the allergens may

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easily traverse through the channels and that the dimensions and the architecture of the bacula cavities support their movement towards the surface of the pollen grains.

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3. M. Gajhede et al., Nat. Struct. Mol. Biol. **3**(12) (1996) p.1040.
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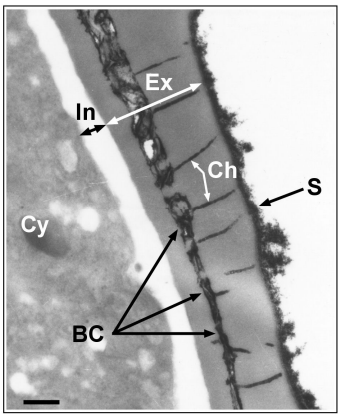

Figure 1. Transmission electron micrograph of a 80 nm thick section of a resin-embedded hazel pollen grain, showing part of the wall with channels and bacula cavities, obtained by an EM 410 (Philips, Eindhoven, The Netherlands) at 80 kV. Ex - exine, In - intine, BC - bacula cavities, Ch - channels, Cy - cytoplasm, and S - surface. Scale bar: 200 nm.

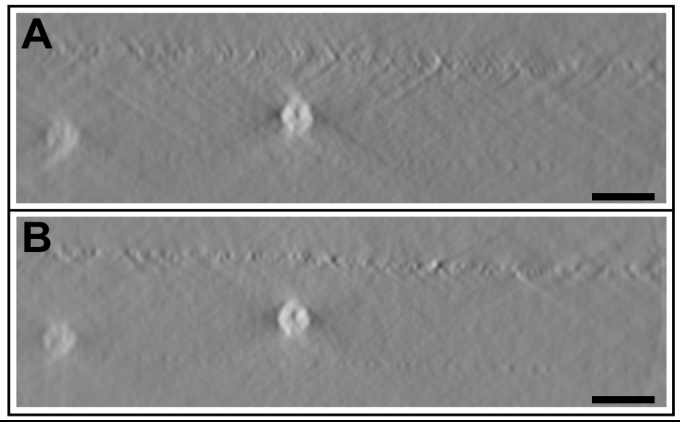

Figure 2. Improvement of the quality of a three-dimensional reconstruction achieved by iterative projection matching. A selected x-z- cross-section (perpendicular to the tilting axis) through a contrast-inverted sub-volume, which cuts the channels approximately perpendicularly, is shown in (A) after the initial cross-correlation alignment, and in (B) after three loops of the iterative projection matching. Scale bars: 50 nm.

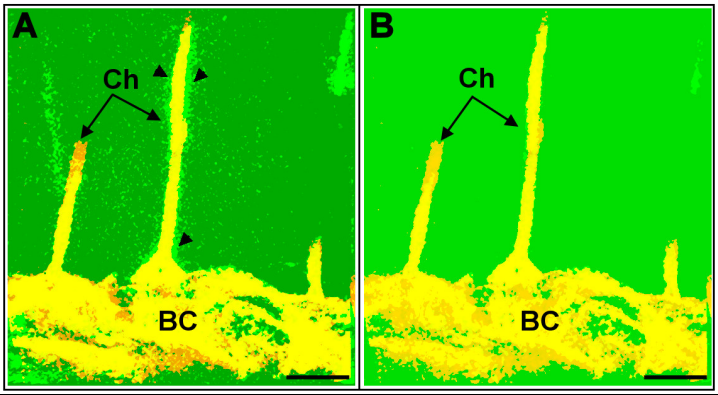

Figure 3. Estimation of the correct thresholding intensity level in red-green overlay images. The original untilted projection was thresholded according to its bimodal histogram (red image). The reconstructed volume was thresholded at increasing intensity levels and re-projected in the direction of the untilted projection (green image). (A) The threshold level was set too low because the side-arcs around the channels are clearly visible (arrowheads). (B) Re-projection of a correctly thresholded volume contains channels of the same diameter as in the original projection. Scale bars:100 nm. BC - bacula cavities, Ch – channels.

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