Three-dimensional analysis of the intermediate filament network using SEM-tomography

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Pancreatic cancer is characterized by its early infiltration of adjacent tissue. In a previous paper we verified a correlation between keratin filament network structure and the migration ability of cells [1]. This was corroborated by quantitatively analysing two-dimensional electron microscopical images of the three-dimensional keratin network [2].

In our ongoing research we are expanding these studies to visualize the true three-dimensional structure of the intermediate filament network. For this purpose we investigated and compared different preparation and imaging protocols for preservation and recording of the three-dimensional filament network, including high-pressure freezing, freeze substitution, embedding and thin sectioning. We analyzed these samples with regular TEM, 300 kV STEM and 30 kV STEM. It turned out that it was difficult to track the individual filaments in the very complex context of all the retained cellular structures of unextracted cells. We analyzed, therefore, intermediate filament networks from detergent-extracted cells. Most of the cellular structures are, thereby, removed and only the finely woven cytoskeleton remains, with the filaments surrounded by vacuum. (Possible artifacts of this preparation protocol have also been discussed [3, 4].) When imaged with a 300 kV STEM and a 30 kV STEM (**Fig. 1a**), the signal of the filaments was insufficient compared to the strong signal of unextracted agglomerates. So, we used the secondary electron signal, which is surface-dependent and therefore relatively strong from thin filaments (**Fig. 1b**). The tomographical datasets were recorded in a S-5200 in-lens SEM (Hitachi, Tokyo) at an accelerating voltage of 5 kV in different areas within cells (**Fig. 2**) by tilting the sample with 2° step size to a maximum tilt of -60° to +60° by using a specially designed pretilted holder (**Fig. 3a**). For reconstruction the iMod software [5] was used (**Fig. 3b**). Statistical analysis of network morphology requires the extraction of the network graphs from the tomograms. For this purpose after binarization by thresholding (**Fig. 3c)** the tomograms were skeletonized by an algorithm supplied by the Avizo software package [6]. Artefacts arising from skeletonization, errors in continuity tracking and tomographic reconstruction were removed by algorithms designed individually for the specific type of data and implemented within the framework of the Geostoch software library [7] (**Fig. 3d**). *(Supported by the DFG Sonderforschungsbereich 518, Project B21)*

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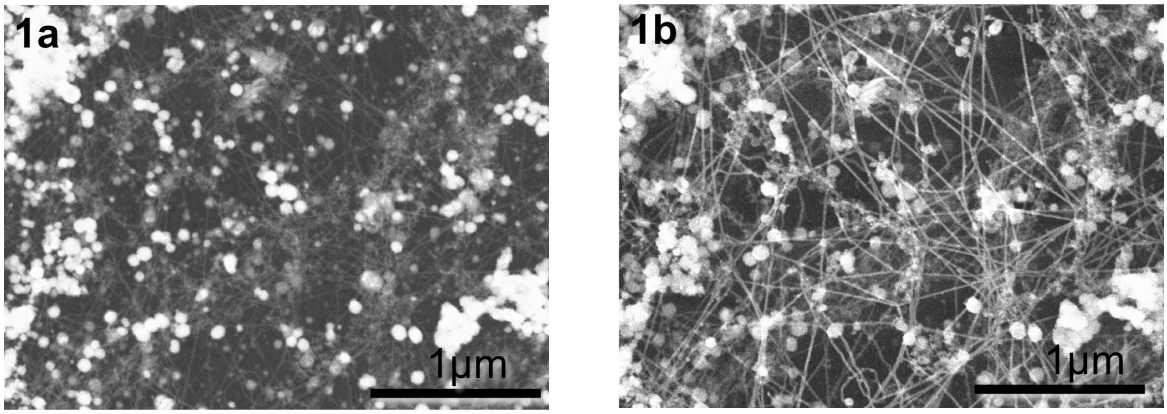

Figure 1. Detail images of an extracted Panc1 cell, same area in a.) STEM dark field image at 30kV b.) SEM secondary electron image at 30kV.

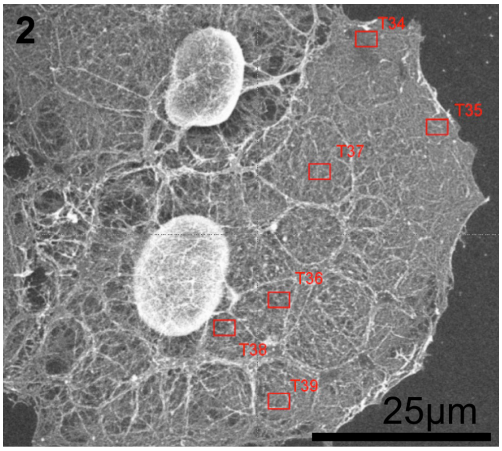

Figure 2. Overview of extracted Panc1 cells; tomograms T34-T39 were recorded in different areas within one cell.

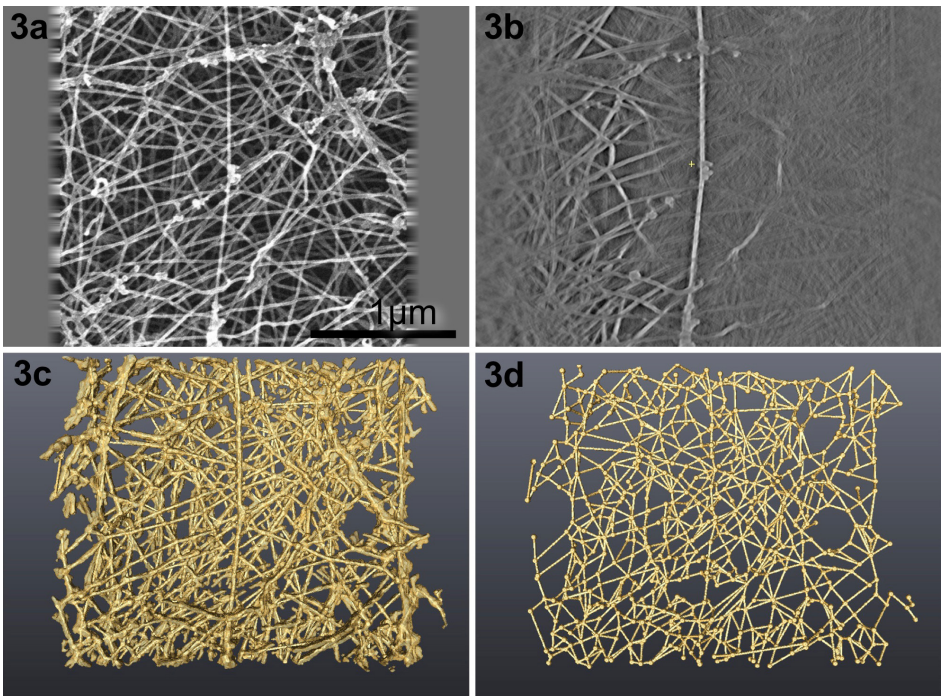

Figure 3. Tomogram of area T34. a.) one SEM image of the tilt series b.) one layer of the reconstructed tomogram c.) tomogram after thresholding d.) network graph after several processing steps

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