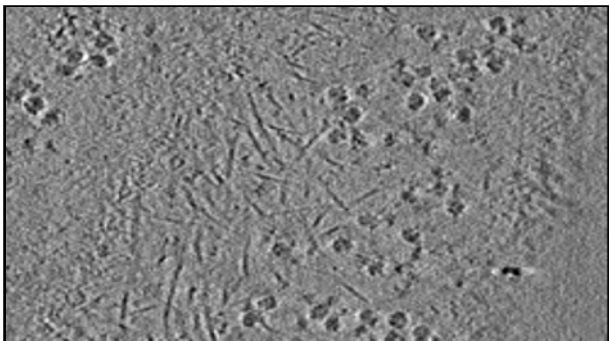
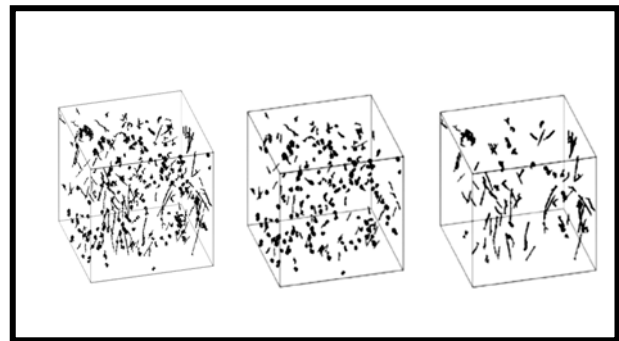


A major challenge in tomographic imaging of sub-cellular structures is the segmentation of the image data with respect to identification macro-molecular structures such as the actin-cytoskeleton or cell organelles. The Minkowski Functionals [1] in 3D - a set of global topological descriptors corresponding to volume, surface, mean integral curvature, and Euler-Poincaré characteristic - have been introduced to medical image processing in the context of structural analysis of bone architecture [2, 3]. In this work we provide a morphological filtering algorithm based on the Minkowski Functionals in 3D for the segmentation of macro-molecular structures in intact eukaryotic cells depicted by cryo-electron tomography.

We analyse 3D image data of an ice-embedded *Dictyostelium* amoeba directly grown on an electron microscopy grid (Figure 1) [4]. The specimen is scanned by low dose transmission electron microscopy employing a CM 300 FEG TEM with a single axis tilt series of  $-50^\circ$  to  $+41.5^\circ$  at an increment of  $1.5^\circ$ . The reconstructed 3D representation of the dataset contains  $128 \times 255 \times 255$  voxels. Image pre-processing involves binarization and noise reduction by removal of objects with less than 50 connected voxels.



**Figure 1:** Microscopic image slice reconstructed from cryo-electron tomography of the *Dictyostelium* amoeba.



**Figure 2:** The binarized image data comprises 201 convex image components (left), which are then separated by Minkowski-based thresholding into spherical (middle) and filamentary (right) objects.

The resulting sub-population of 201 image components have volumes of 50 to 1338 voxels, surface areas of 90 to 2616, mean integral curvatures of 11 to 207 and Euler-Numbers of 1 to 3.

By inspection, 86 of these are classified as filamentary structures, 78 as spherical and the remaining 37 as complex objects (Figure 2). Significant MF-based segmentation results are achieved when considering the 3rd functional (mean integral curvature). Filamentary image components tend to be characterized by high curvatures whereas spherical objects have lower values. When choosing a cut-off value for mean integral curvature at 30, the group of 111 objects with curvature  $\leq 30$  consist of 61 spherical, 28 filamentary and 22 complex structures. The set of image components with curvature  $>30$  comprises 58 filamentary, 17 spherical and 15 complex objects. The area-under-the-curve for mean integral curvature was 0.77 for correct detection of spherical image components and 0.78 for filamentary structures.

In conclusion, the morphological properties of binarized tomographic image data expressed in terms of the Minkowski Functionals in 3D can successfully be employed for segmentation of filamentary and spherical image components. This new approach may serve as a tool for reducing the time-consuming process of segmenting macro-molecular structures as depicted by cryo-electron tomography of intact eukaryotic cells which today still involves a substantial amount of human interaction. In future, considerable effort will be put into finding an automatic binarization procedure. We also hope to improve segmentation / classification results further by consideration of additional morphological properties.

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