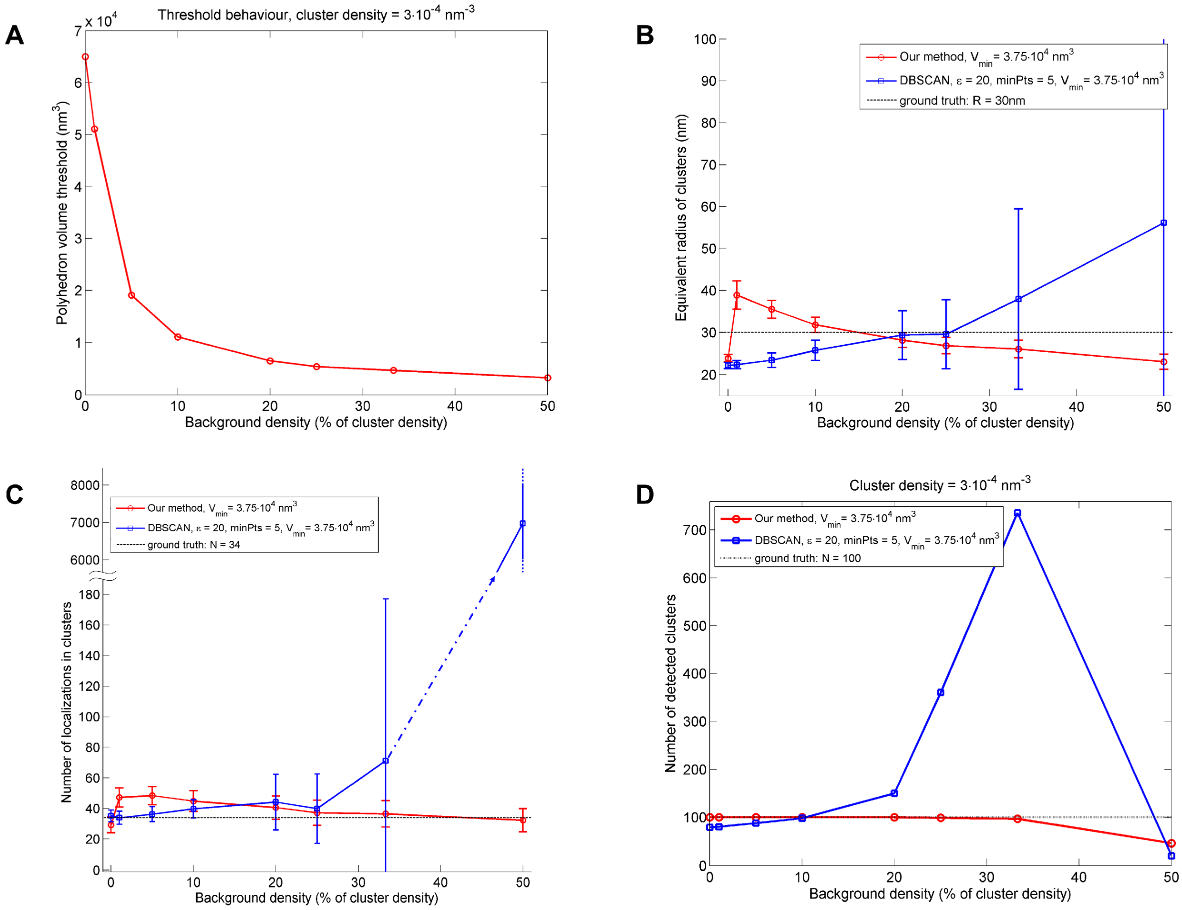

Figure S1. Evaluation of the performance of the 3D Voronoi tessellation method with synthetic data. **A** Behavior of the automatic segmentation threshold determination using Monte-Carlo simulations with varying background density. **B-D** Comparison of performance of our method with a 3D implementation of DBSCAN (with optimized parameters ε = 20, MinPts = 10) which behaves unstably with increasing background noise. Equivalent radius of clusters (**B**), number of localizations within clusters (**C**) and total number of clusters (**D**) detected from datasets with different amounts of noise. V_{min} is the minimal volume of an object to be considered as cluster (in order to remove spurious background clusters which are unavoidable with dense background noise). The data were generated with a constant density of clustered points ($3 \cdot 10^{-4}$ nm^{-3}) and a varying density of the background noise. Points in (**B-C**) indicate the mean values and the error bars indicate standard deviations.

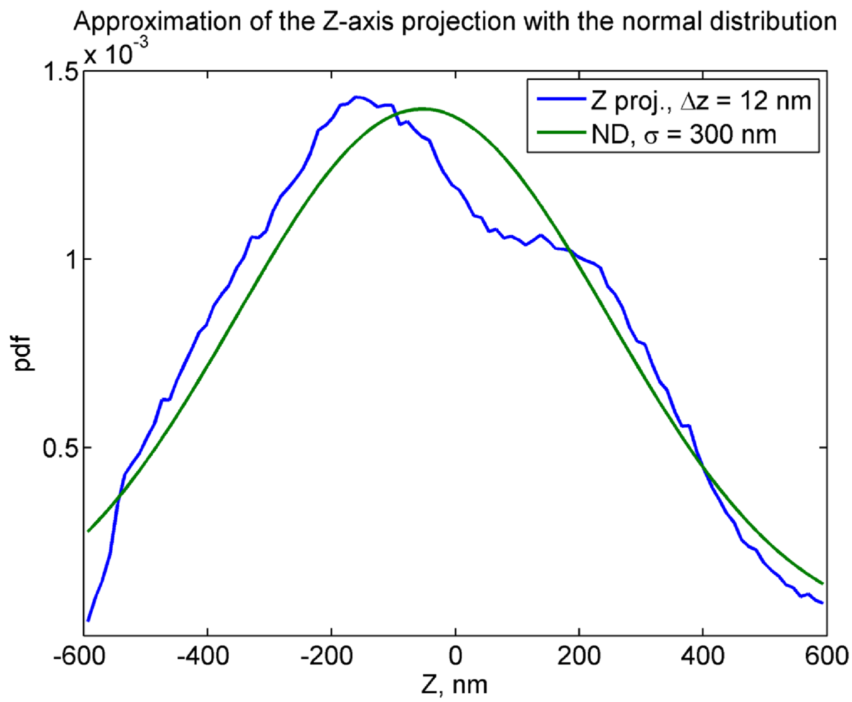

Figure S2. Approximation of the axial distribution of localizations in a 3D experiment acquired with astigmatism by a normal distribution (ND). σ, standard deviation; Δz, bin size of the histogram of the Z-positions of the dyes. Example of β-tubulin labelled with Alexa-647 conjugated antibodies (see methods).

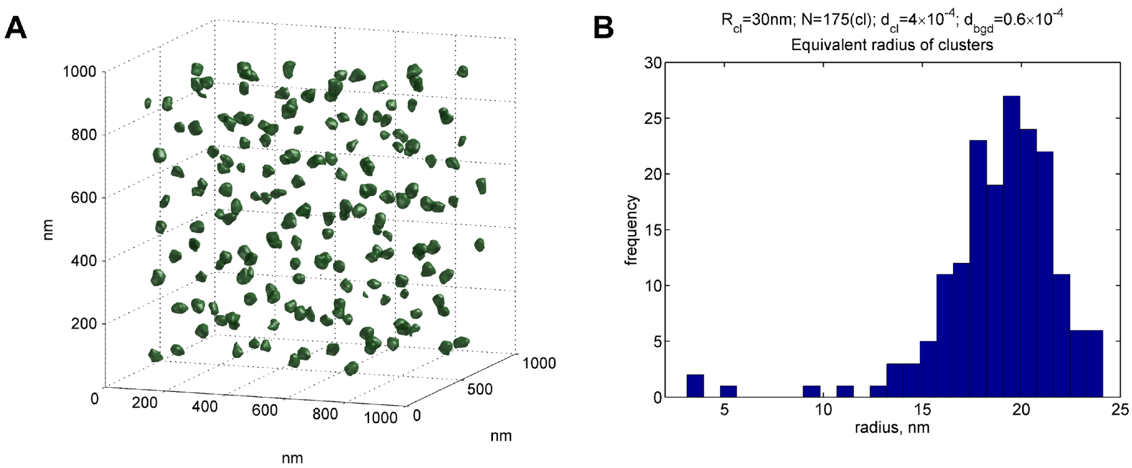

Figure S3. Segmentation of the dataset from **Fig. 2C** using first-rank neighbors for averaging the local densities using the 2D method described by Levet *et al.*, 2015, but implemented here in 3D for testing purposes. **A** General view of segmented clusters. **B** Histogram of the equivalent radius of the detected clusters: r=19+/-3nm, the simulated radius was 30 nm.