

ClusterViSu, a method for clustering of protein complexes by Voronoi tessellation in super-resolution microscopy

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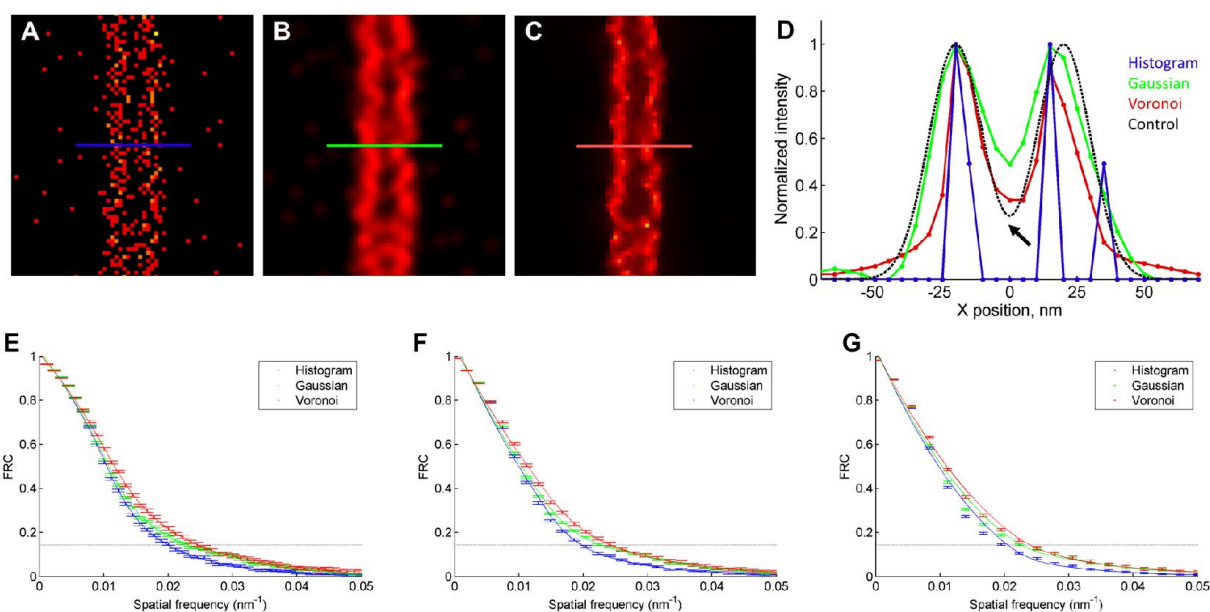


Figure S1 Evaluation of the quality of Voronoi image reconstruction

(A-C) Simulated images of two lines separated by 40 nm and composed of localization events with a standard deviation of 10 nm in histogram (A), Gaussian (B) and Voronoi-based interpolated (C) local density representations (same as **Figs. 1A-C**, but with line indicating the line profile). (D) One-pixel width profiles along the colored lines of images A-C. (E-G) FRC curves calculated from an image region in **Fig. 1E** in histogram (blue), Gaussian (green) and Voronoi local density (red) representations; pixel size of the image: 5 nm (E), 3 nm (F), 2nm (G). The graphs show only the frequency range from 0 to 0.05 nm⁻¹ for consistency with **Fig. 1F**.

Suppl. Fig. S1

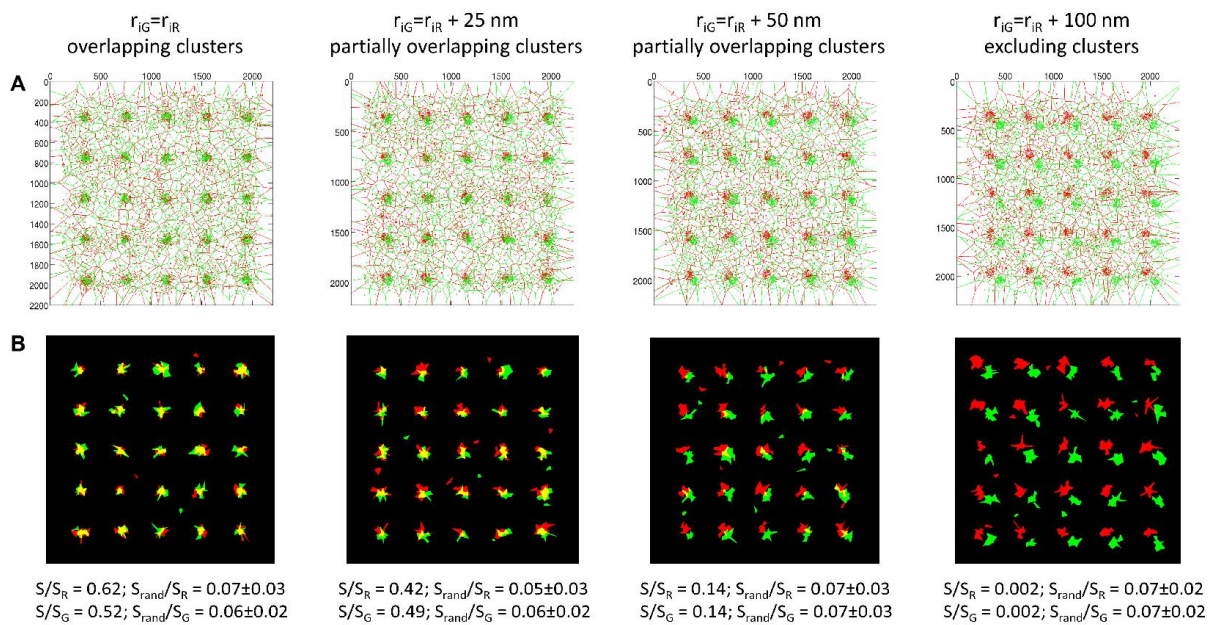


Figure S2 Simulations of different co-localization scenarios

(A) Voronoi diagrams of simulated double-color clusters. (B) Results of segmentation of the double-color clusters in ClusterViSu with the overlay area shown in yellow and the calculated co-localization values shown below. r_{IG} and r_{IR} are the Cartesian coordinates of the centers of the green and the red clusters, respectively. The simulated radius of clusters is 50 nm; density of events in clusters = $2.5 \cdot 10^{-3} \text{ nm}^{-2}$, in background = $1.25 \cdot 10^{-4} \text{ nm}^{-2}$. Localizations in the clusters and in the background are distributed randomly and independently for each color.

Suppl. Fig. S2

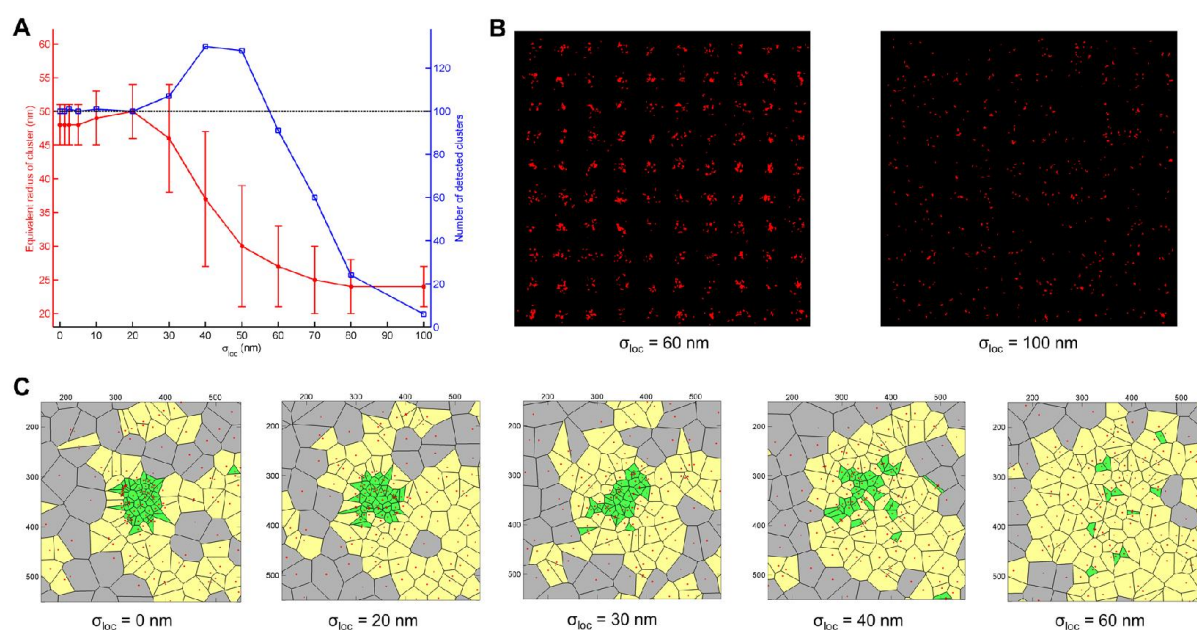


Figure S3 Effect of the localization error on Voronoi clustering

(A) Results of segmentation of a simulated cluster data with different amounts of localization error. σ_{loc} is the standard deviation of the normal distribution used for simulating the coordinates of the points. Clusters containing 5 or less molecules were removed. (B) Complete segmented FOVs of the simulated data with high localization errors σ_{loc} demonstrating remaining clusters at $\sigma_{loc} = 60$ nm and $\sigma_{loc} = 100$ nm. (C) The Voronoi diagrams of the upper-left corner of the simulated data with different localization errors. The density of clustered localizations is 0.01 nm^{-2} , the density of background is 0.002 nm^{-2} .

Suppl. Fig. S3

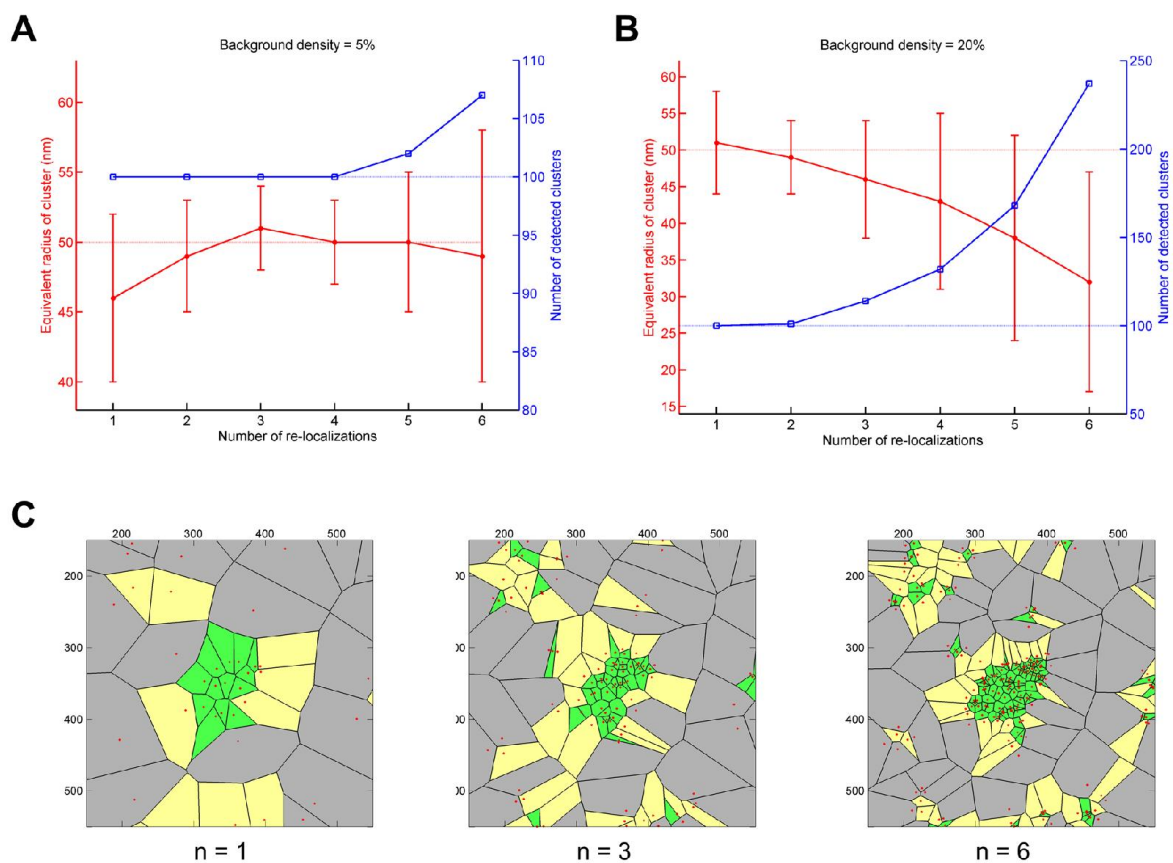


Figure S4 Effect of multiple localizations on Voronoi clustering

(A&B) Results of segmentation of simulated cluster data with different numbers of re-localizations. Clusters containing 5 or less molecules were removed. (C) Comparison of Voronoi diagrams with different numbers of re-localizations (n). The density of clustered localizations (at n = 1) is 0.0025 nm⁻², the density of background is 20% of the cluster density; the localization precision $\sigma_{loc} = 10$ nm.

Suppl. Fig. S4

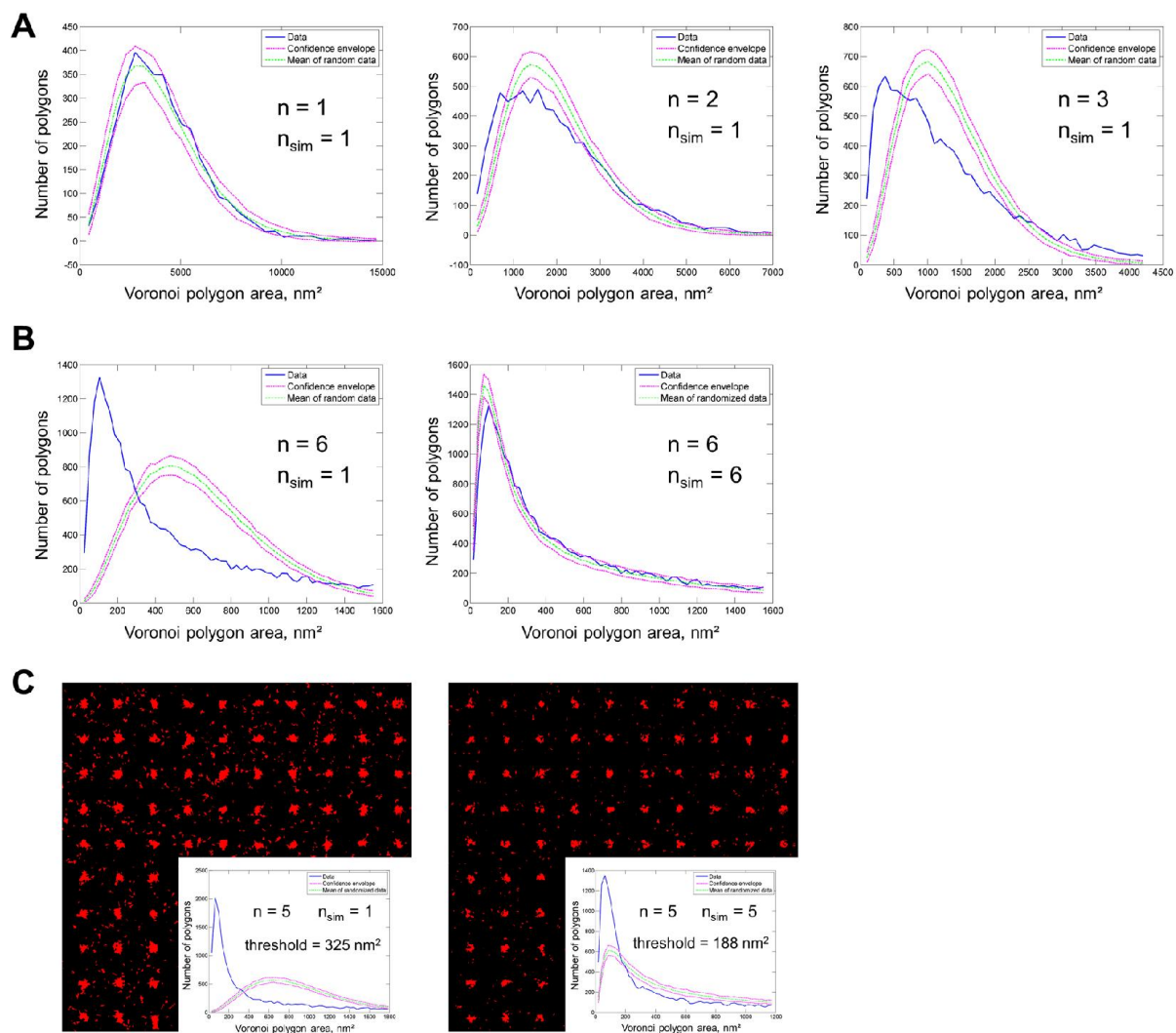


Figure S5 Effect of multiple localizations on Monte-Carlo simulations

(A&B) Voronoi polygon distributions of spatially random datasets with different numbers of re-localizations (n) and results of Monte-Carlo simulations taking into account multiple localizations (n_{sim}). (C) Simulated clustered datasets with re-localizations ($n = 5$) segmented by thresholds determined by simulations without ($n_{\text{sim}} = 1$) and with ($n_{\text{sim}} = 5$) multiple localizations. The density of localizations (at $n = 1$) is 0.001 nm^{-2} (A&B); the density of clustered localizations (at $n = 1$) is 0.0025 nm^{-2} , the density of background is 20% of the cluster density (C); the localization precision $\sigma_{\text{loc}} = 10 \text{ nm}$ (A-C).

Suppl. Fig. S5

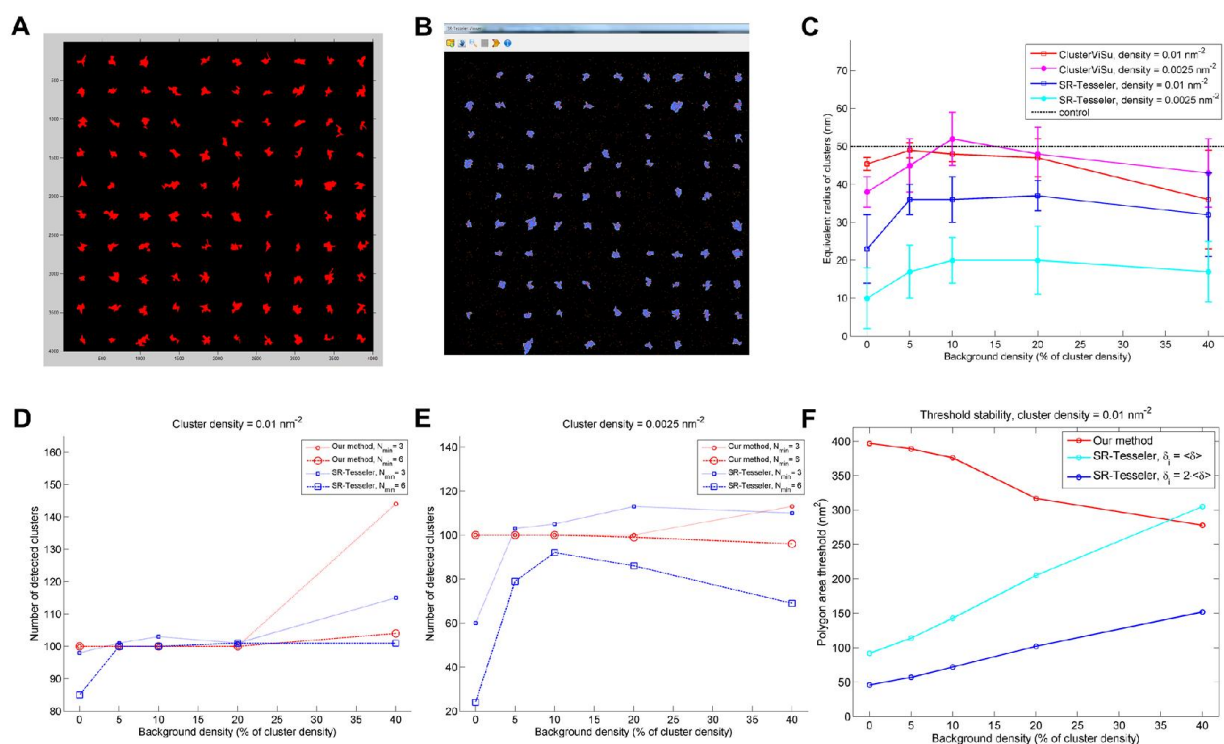


Figure S6 Comparison of ClusterViSu with SR-Tesseler.

(A&B) Clustered dataset segmented in ClusterViSu with threshold determined by Monte-Carlo simulations **(A)** and in SR-Tesseler with threshold determined by average localization density¹⁷ $\delta_1^i = 2 \cdot \langle \delta \rangle$ **(B)**. Density of clustered localization is 0.0025 nm^{-2} , background density is 0.01 nm^{-2} . Clusters containing five or less molecules were removed. **(C)** Retrieval of the equivalent radius of clusters in ClusterViSu and SR-Tesseler. Clusters containing two or less molecules were removed. **(D&E)** Number of clusters detected by ClusterViSu (red curves) and SR-Tesseler (blue curves) as function of background density. N_{\min} , minimal number of events in clusters kept for quantification. **(F)** Variation of segmentation threshold determined by Monte-Carlo simulations in ClusterViSu (red curve) or by average localization density in SR-Tesseler¹⁷ (cyan and blue curves), depending on background density.