Supplementary Information

Analysis of sparse molecular distributions in fibrous arrangements based on the distance to the first neighbor in single molecule localization microscopy.

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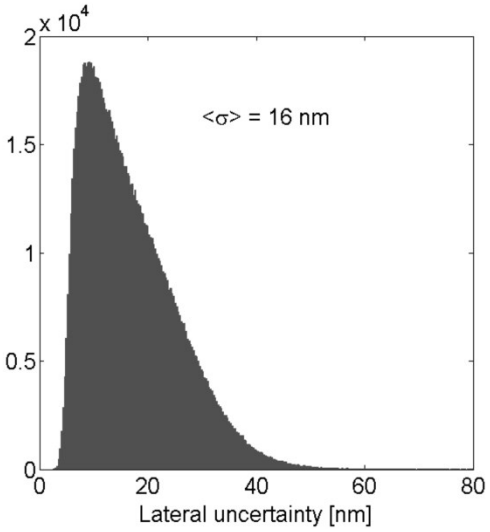


Figure S1: Distribution of the value of σ, the uncertainty in the determination of the center of the point spread function of each individual emitter

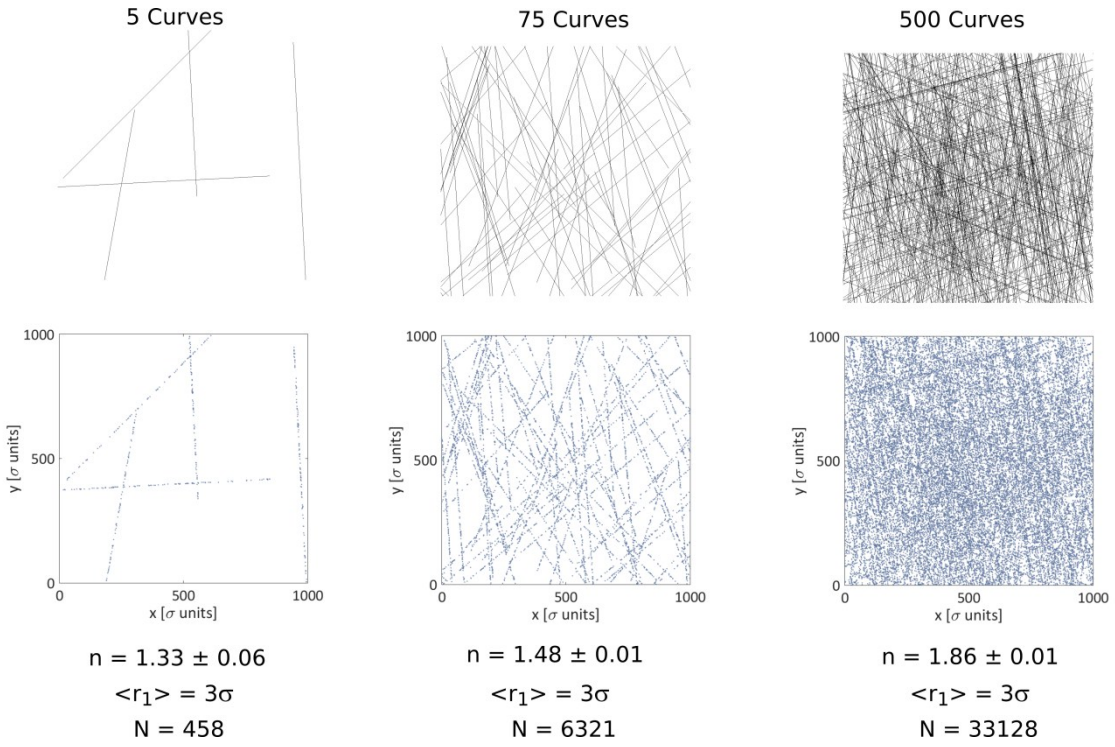


Figure S2: Simulations of lines with increasing crossing and its influence in the determination of the distance scaling exponent, *n*. Upper plots: Patterns for molecular locations. Lower plots: Random distribution of N points in the corresponding pattern. The value of n is indicated in each column.

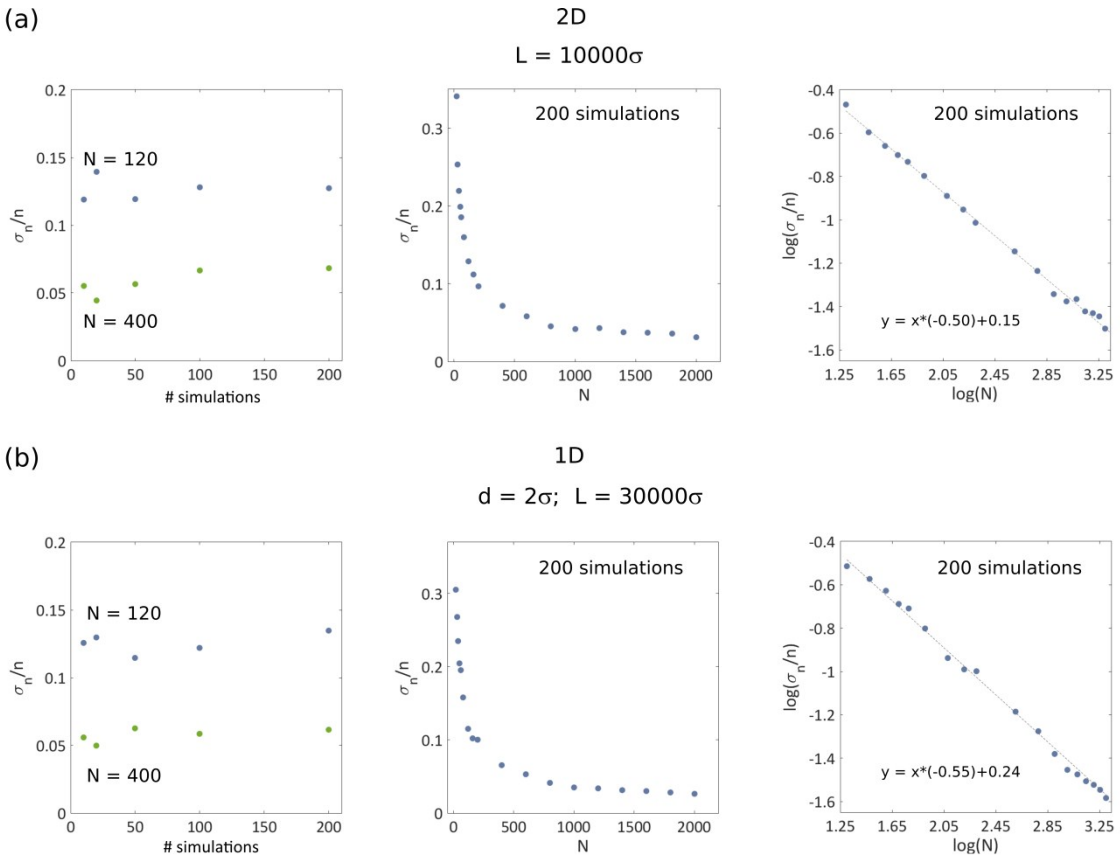


Figure S3: Analysis of σ_n, the uncertainty in the determination of the value of n in 2D and 1D simulations. (a) 2D simulations in an area of 10,000σ x 10,000σ. The left panel shows the relative dispersion, σ_n/n, for N = 120 and 400 molecular localizations, as a function of the number of simulation cycles. The central panel shows the dependence of σ_n/n, in 200 simulations, on the number of molecules. The right panel shows the linear dependence of $\log(\sigma_n/n)$ on log(N). (b) Same type of plots as in (a) for 1D simulations in a line of length L = 30,000σ, and width d = 2σ.

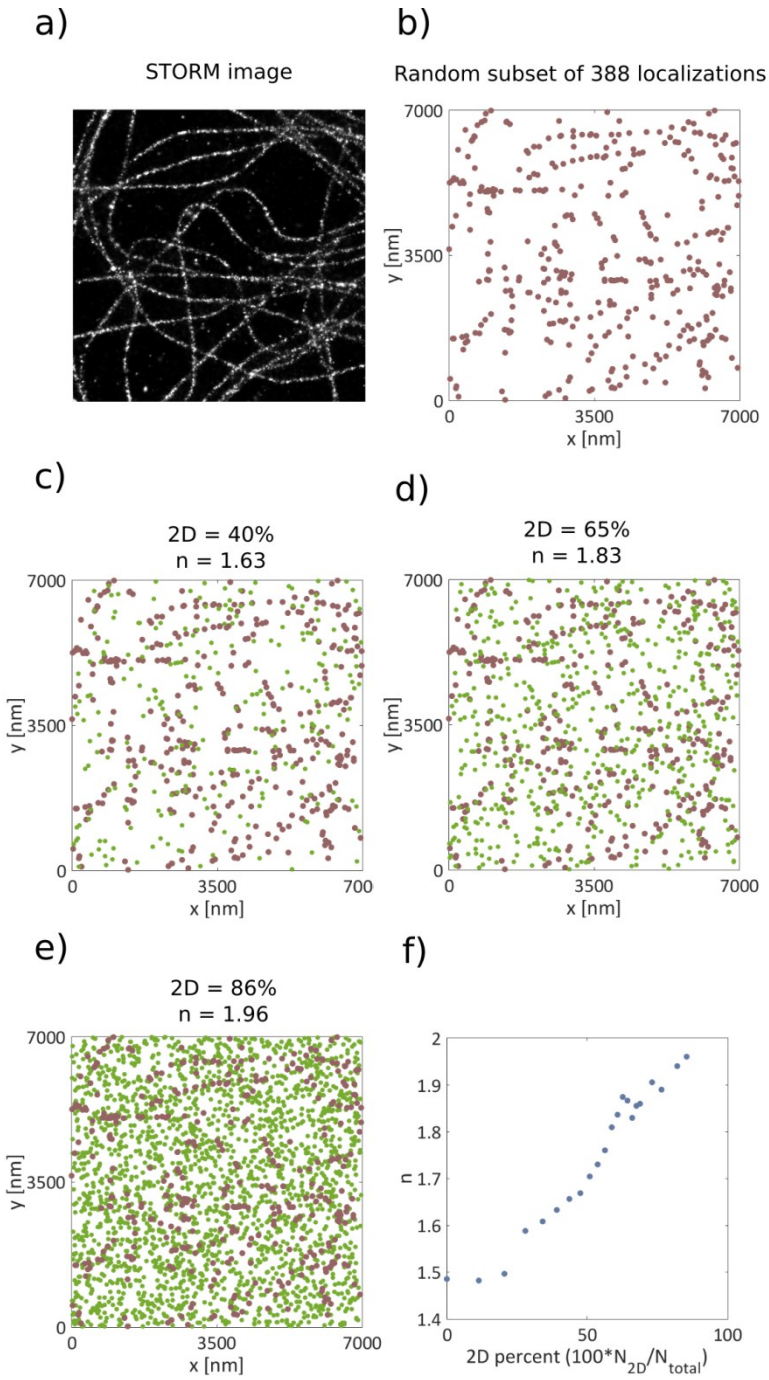
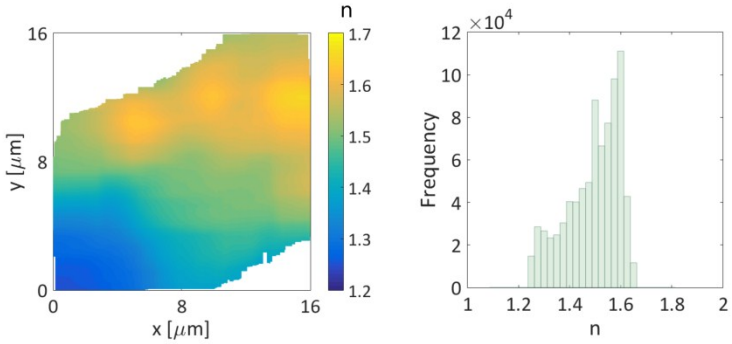


Figure S4: a) Same image as Figure 4a. b) Distribution of a random subset of 338 molecules from the whole image. c), d), e) images with the locations of panel b) (brown dots) and 40%, 65%, and 86% proportion of randomly distributed locations (green dots). f) Plot of the value of the distance scaling exponent as a function of the proportion of randomly distributed molecules

Average of 100 distributions for *n*



Examples of single distributions for *n*

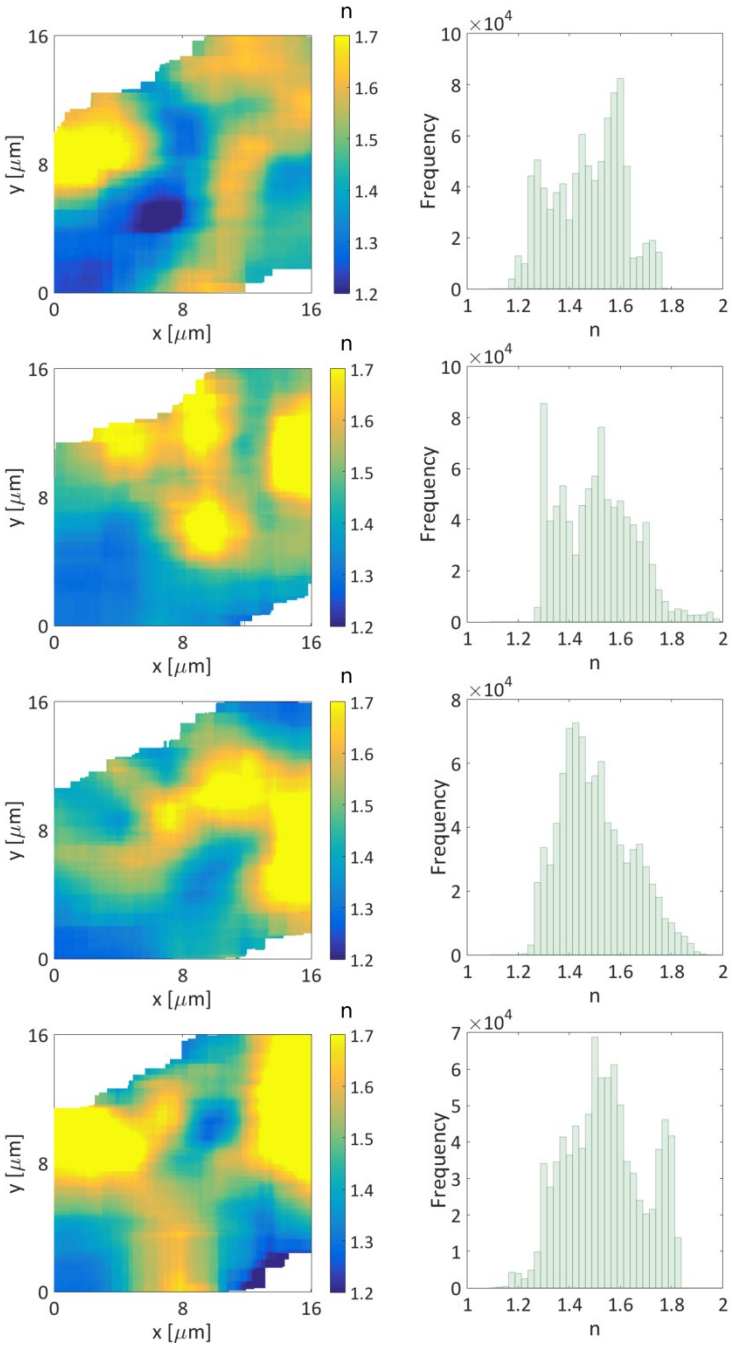


Figure S5: Comparison of four different realizations of the result of 1 determination on 2000 localizations and of the result of averaging 100 different random choices of 2000 localizations to build the color map of *n*. Left: Color map of the spatial distribution of n. Upper map is the same as Figure 5c, average of 100 simulations. All others are single distributions. Right: Histogram of the distribution of the local values of *n* in the corresponding color figure.

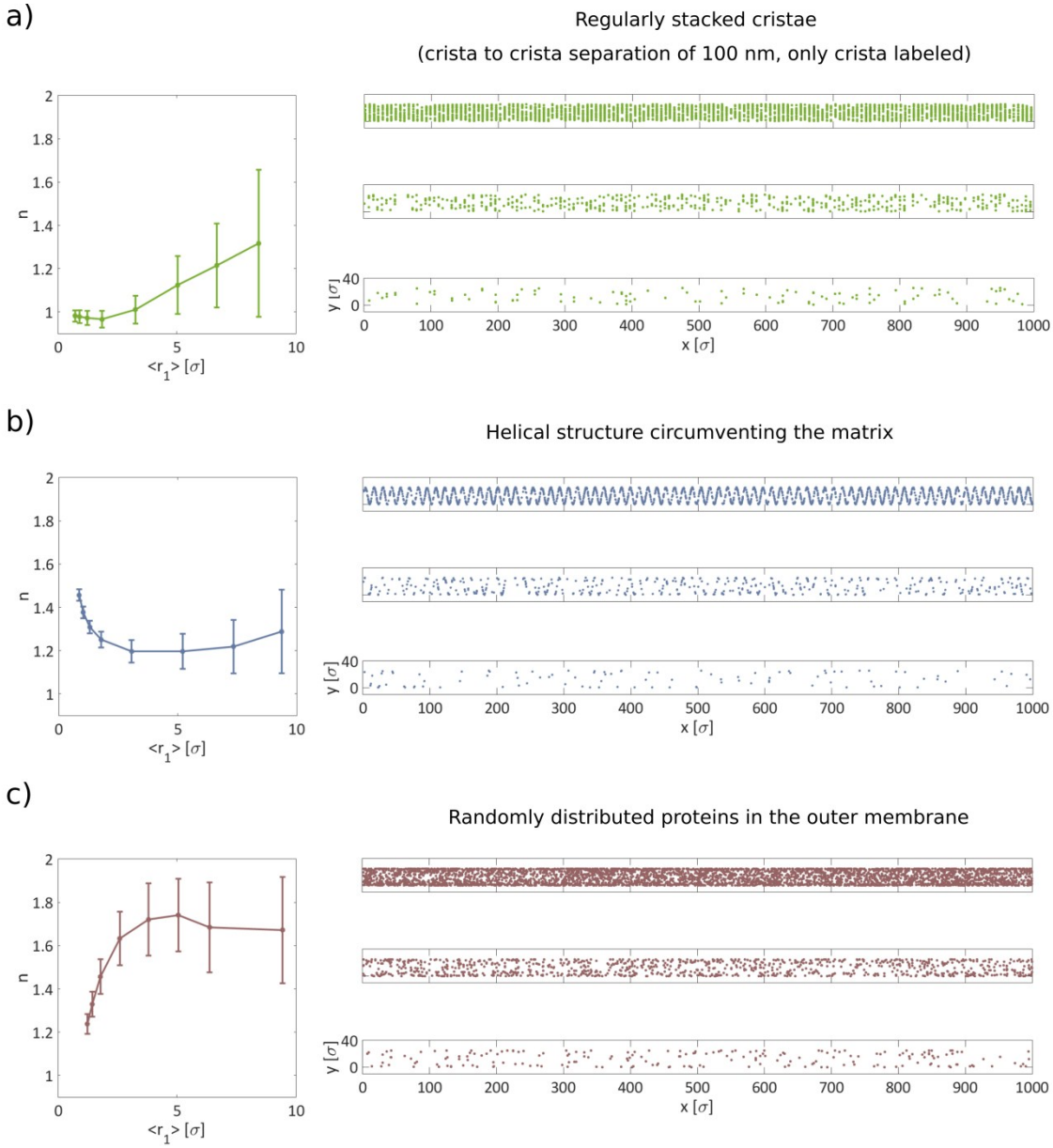


Figure S6: Simulations of random distributions in three different environments expected for mitochondria. Panels a), b), and c) show at the right distributions of different density and at the left the dependence of the distance scaling coefficient with the average distance to the first neighbor. Labels associated to a) regularly stacked cristae, b) helical structure circumventing the matrix, c) the outer membrane.

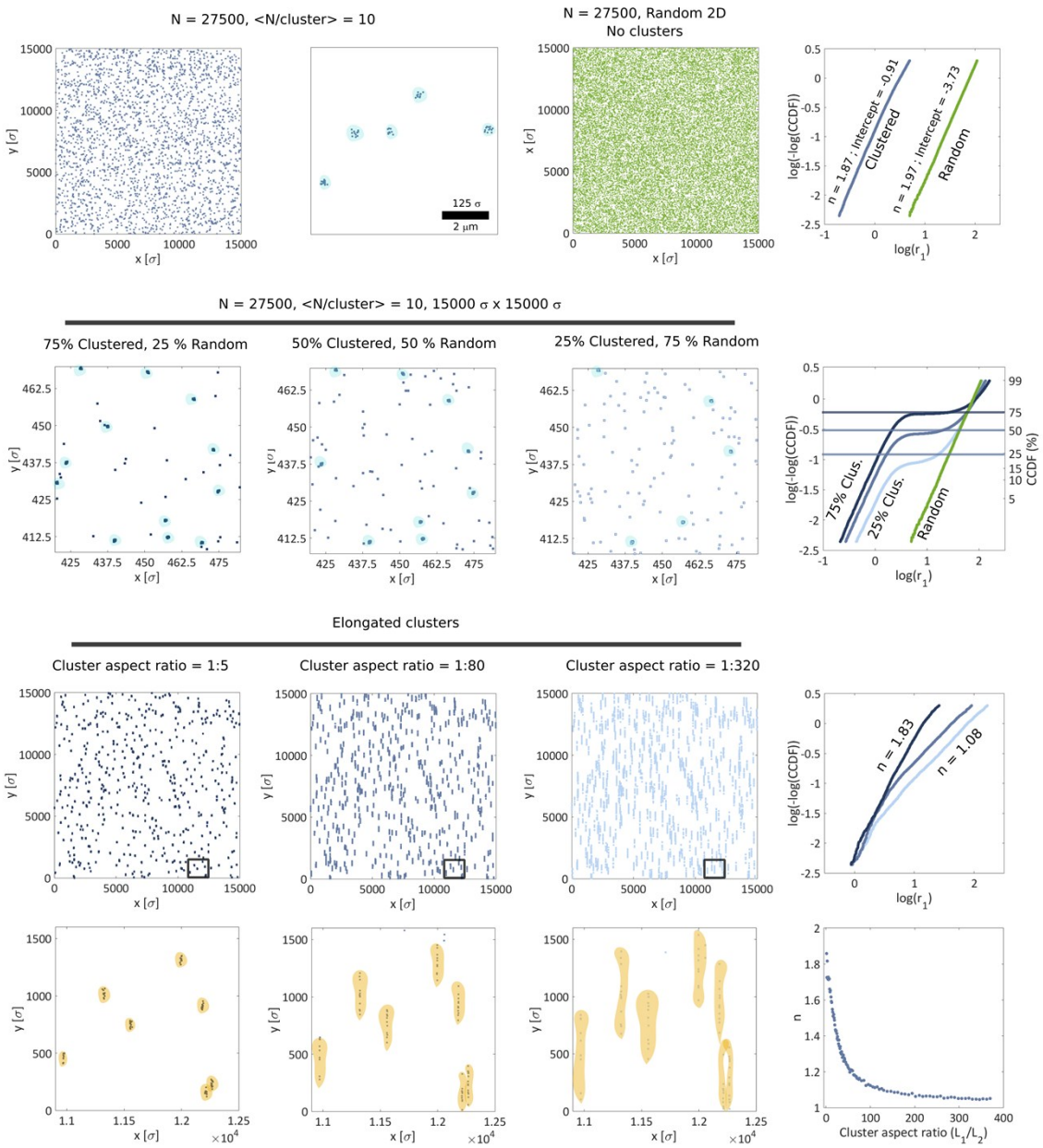


Figure S7: Mixed clustered and random distributions.
Upper two rows: Mixed clustered and random distributions with the same average density: 27500 localizations in a (15000 σ)^2 area. In all cases, the clusters have an average of 10 points, distributed randomly within a circle of radius s. First row, from left to right, the panels show a 100% clustered distribution, a detail of this clustered distribution, a 100% random distribution, and the corresponding plots for the distance scaling exponent. Second row: three mixed random and clustered distribution with different proportions of clustered localizations. The right most panel displays the corresponding distance scaling plots of these three distributions compared to the completely random case. Horizontal lines show the value of CCDF corresponding to the proportion of total clustered localizations.
Lower two rows display three clustered distributions with different aspect ratio of the cluster, with its corresponding detail of the marked area. Aspect ratio is defined as the quotient between the bigger and the smaller dimension of the clusters. The distance scaling plots of the three distributions are displayed together with a plot of the scaling coefficient as a function of the aspect ratio.

In Figure S7, the random 2D distribution is compared to a clustered distribution of the same average density. Clusters have an average of 10 localizations, themselves randomly distributed in a circle. Cluster centers are randomly distributed in 2D.
The scaling exponent is two for the two distributions, in agreement with their bidimensional random character, while the different intercepts of the lines reflect the different local densities around each point.
When mixed clustered and random distributions are simulated with different proportion of total localizations clustered localizations, two features are evident in the plots of the scaling exponent. One is their sigmoideal shape, arising in the different local and average density. The other is the position of the inflection point of the plot that reflects the proportion of total localizations present in the clusters.
Finally, clusters of different aspect ratios are simulated and analyzed. The trend in the distance scaling exponent with increasing value of the aspect ratio reflects the tendency of the distribution to approach a linear array.
The differences and main features of each distribution are neatly displayed by the analysis method.